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AAC-11 derived peptides antagonize the survival and infection of HIV-1 susceptible CD4+ T cells and macrophages

par *Anastassia Mikhailova*

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Dirigée par Dr. Asier Sáez-Cirión

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Président du jury : Hivroz, Claire / PhD, HDR, DR / INSERM, Institut Curie, Université Paris Descartes

Rapporteur : Lambotte, Olivier / Professeur, PhD, MD, DR / INSERM, Université Paris Sud

Rapporteur : Cimarelli, Andrea / PhD, HDR, DR / INSERM, Ecole Normale Supérieure de Lyon

Examinateur : Sauvonnet, Nathalie / PhD, HDR, DR / INSERM, Institut Pasteur, Université Paris-Saclay

Examinateur : Perfettini, Jean Luc / PhD, HDR, DR / INSERM, Université Paris-Saclay

Directeur de thèse : Sáez-Cirión, Asier / PhD, HDR, DR / Institut Pasteur, Université Paris Diderot

ABSTRACT

Despite its discovery more than 30 years ago, HIV epidemic remains one of the global health challenges of today. Among many important questions remaining is how does HIV-1 successfully establish infection and persist in its reservoirs despite viral cytotoxic effect observed both *in vitro* and *in vivo*? This work shows that HIV-1 relies on AAC-11 anti-apoptotic pathway for the establishment of productive infection in CD4+ T cells and macrophages, primary targets of HIV-1. We observed the expression of AAC-11 to increase with progressive CD4+ memory T cell differentiation and to associate with the expression of cell cycle, activation and metabolism genes, known factors for HIV-1 susceptibility. Antagonism of AAC-11 survival pathway with the peptides derived from its sequence lead to cell death of HIV-1 susceptible target cells and resistance of surviving cells to infection. The peptides caused preferential elimination of effector and transitional CD4+ T cell memory subsets as well as highly activated and metabolically active cells, and their activity was at least in part dependent on caspase 2 activation. Thus, our results provide a proof of concept that the selective targeting of the survival pathways used by HIV-1 is a possible approach to antagonize the seeding of HIV-1 CD4+ T cell and macrophage reservoirs.

Keywords:

Anti-apoptotic clone 11, human immunodeficiency virus, CD4+ T cell, cell death, immuno-metabolism, HIV-1 dependency factors

RÉSUMÉ

Malgré plus de 30 ans de recherche intensive depuis le début de l'épidémie, le virus de l'immunodéficience humaine (VIH) reste l'un des plus gros problème de santé publique. L'une des problématiques les plus importantes empêchant le progrès vers la résolution de combat avec cette maladie reste de comprendre comment le VIH de type 1 établie l'infection et la persistance dans ses réservoirs. Et ce, malgré l'effet cytotoxique du virus qui est observé in vitro et in vivo. Ce travail montre que le VIH-1 dépend de la voie de signalisation anti-apoptotique d'AAC-11 pour établir l'infection dans les lymphocytes CD4+ T et les macrophages qui sont les cibles importantes du VIH-1. Nous observons que l'expression d'AAC-11 augmente avec la différentiation des lymphocytes CD4+ T mémoires. Nous avons également remarqué que l'expression d'AAC-11 est associée avec l'expression des gènes du cycle cellulaire, de l'activation et du métabolisme, des facteurs qui jouent un rôle dans la susceptibilité du VIH-1. Nous avons modulé la voie de signalisation d'AAC-11 avec l'antagonisme provenant des peptides dérivés de sa séquence et observé la mort cellulaire des cellules susceptibles à l'infection. Nous avons également observé que les cellules restantes étaient résistantes à l'infection. Les peptides ont éliminé préférablement les cellules effectrices et transitionnelles mémoires CD4+ T, ainsi que les cellules activées et métaboliquement actives. Leur activité était en partie dépendant de l'activation de caspase-2. Par conséquent, nos résultats présentent une preuve de concept que le ciblage des voies de survie cellulaires utilisées par le VIH est une approche plausible pour impacter négativement l'ensemencement du réservoir de VIH, dont font parties les cellules CD4+ T et macrophages.

Mots-clés:

Virus de l'immunodéficience humaine, mort cellulaire, lymphocyte CD4+ T, macrophage

Human immunodeficiency virus, cell death, CD4+ T lymphocyte, macrophage

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AAC-11	anti-apoptotic clone 11
Ag	antigen
AMPK	5'-AMP activated protein kinase
ASC	apoptosis-associated speck-like protein containing C-terminal CARD
ATM	*a*taxia *t*elangiectasia *m*utated
ATP1A1	Na^+/K^+ ATPase subunit α isoform 1
ATP1A2	Na^+/K^+ ATPase subunit α isoform 2
ATP1A3	Na^+/K^+ ATPase subunit α isoform 3
ATP1A4	Na^+/K^+ ATPase subunit α isoform 4
ATP1B1	Na^+/K^+ ATPase subunit β isoform 1
ATP1B2	Na^+/K^+ ATPase subunit β isoform 2
ATP1B3	Na^+/K^+ ATPase subunit β isoform 3
ATP5B	ATP synthase subunit β
BAD	BCL2 antagonist of cell death
BAK	BCL2 antagonist/killer
BAX	BCL2 associated X protein
BID	truncated BH3-interacting domain death
BIM	BCL2 interacting mediator of cell death
CA	capsid
CAMKII	calcium/calmodulin dependent kinase II
CARD	caspase activation and recruitment domain
cART	combination anti-retroviral therapy
CCR7	C-C chemokine receptor type 7

CD27	cluster of differentiation 27
CD4	cluster of differentiation 4
CD45R0	cluster of differentiation 45 R0
CD45RA	cluster of differentiation 45 RA
CDK2	cyclin dependent kinase 2
CDK9	cyclin dependent kinase 9
CG	cardiac glycoside
CNS	central nervous system
CPSF6	cleavage and polyadenulation specificity factor 6
CRAC	Ca^{2+} release-activated Ca^{2+} channel
CTL	cytotoxic T cell response
CycT1	cyclin T1
CypA	cyclophilin A
DAMP	damage-associated molecular pattern
DC	dendritic cell
DNA-PK	DNA-dependent protein kinase
EDTA	ethylenediaminetetraacetic acid
ENO1	α-enolase
Env	envelope protein
ER	endoplasmic reticulum
ESCRT	endosomal sorting complex required for transport
FAO	fatty acid oxidation
FAS	fatty acid synthesis
FMO	fluorescence minus one
HDF	HIV-1 denepdency factor

HDF	HIV-1 dependency factor
HMGA1	High mobility group protein A1
IFN	interferon
IKK	IkB kinase
IMM	inner mitochnodrial membrane
IN	integrase
LDHA	L-lactate dehydrogenase α chain
LEDGF/p75	Lens epithelium derived growth factor
LTR	long-terminal repeat
LZ	leucine zipper
MA	matrix
Mdm2	mouse double minute 2
MFI	mean fluoresecence intensity
MLKL	mixed lineage kinase domain-like
MOMP	mitochondrial outer membrane permeabilization
MPT	mitochondrial permeability transition
MPTP	mitochondrial permeability transition pore
mTOR	mechanistic target of rapamycin
mTORC1	mTOR complex 1
mTORC2	mTOR complex 2
NC	nucleocapsid
NFAT	nuclear factor of activated T cells
NFkB	nuclear factor κ-light-chain-enhancer of activated B cells
NKA	Na^+/K^+ ATPase
NPC	nuclear pore complex

NRTI	nucleoside analog reverse transcriptase inhibitor
OMM	outer mitochondrial membrane
OXPHOS	oxidative phosphorylation
P-TEFb	Positive transcriptional elongation factor b
PAK1	serine/threonine-protein kinase PAK1
PAMP	pathogen-associated molecular pattern
PARP	Poly(ADP-ribose) polymerase
PBMCs	peripheral blood mononuclear cells
PIC	pre-integration complex
PIDD	p53-induced protein with a death domain DD
PM	plasma membrane
PPP	pentose phosphate pathway
PRR	pattern recognition receptor
PtdIns(4,5)P_2	phosphatidylinositol-4,5-bisphosphate
PTPC	permeability transition pore complex
PUMA	p53-upregulated mediator of apoptosis
RAG	recombination activating gene
RAIDD	receptor-interacting protein-associated ICH-1/CED-3 homologous protein with a DD
Rev	regulator of expression of viral proteins
RF	restriction factor
RF	restriction factor
RIPK1	receptor interacting protein kinase 1
RIPK3	receptor interacting protein kinase 3
RT	reverse transcription/reverse transcriptase
Sp1	specificity protein 1

$t_{1/2}$	half-life
TAR	transactivation response element
tBID	truncated BID
TBP	TATA binding protein
Tcm	central memory CD4+ T cells
TCR	T cell receptor
Te	effector CD4+ T cells
Tem	effector memory CD4+ T cells
TF	transcription factor
Tfh	T follicular helper cell
Tn	naïve CD4+ T cells
TRPM4	transient receptor potential cation channel subfamily M member 4
Tscm	stem cell-like memory CD4+ T cells
Ttm	transitional memory CD4+ T cells
Vpr	viral protein R

INTRODUCTION

PREFACE

Worldwide Human immunodeficiency virus (HIV) epidemic still remains a major global health challenge, despite the existence of combination anti-retroviral therapy (cART). In 2017, the global population of people living with HIV was estimated to be 36.9 million, with 0.94 million new infections and 1 million acquired immunodeficiency syndrome (AIDS)-related deaths that year (UNAIDS). The number of new infections is steadily declining in some areas of the world (strongest decrease seen in Eastern, southern Africa, Western and central Europe as well as North America), while remaining steady (Latin America) or even increasing (Eastern Europe) in others (UNAIDS). Socio-economic barriers undoubtedly present a major challenge to control of the epidemic. It is, therefore, imperative to continue research on the immuno-pathology of HIV infection with the goal to design vaccine and curative strategies, which do not exist today.

HIV has two subtypes, HIV-1 and HIV-2, both of which cause AIDS. HIV-2 causes milder symptoms with most infected individuals not progressing to AIDS. HIV-2 virus remains largely restricted to West Africa and its prevalence rates are declining due to lower transmission rates (Sharp and Hahn, 2011). Infection with HIV-2 will not be considered here. HIV-1 consists of four genetically distinct groups: M (major), O (outlier), N (non-M, non-O), and P (Garcia-Tellez et al., 2016). Group O represents less than 1% of global infections and is restricted to Central West Africa (Cameroon, Gabon and neighbouring countries). Cases from groups N and P are even more restricted with only few cases identified for each of these groups (Sharp and Hahn, 2011). The pandemic group M is further subdivided into many subtypes (A, B, C, D, F, G, H, J, K) with distinct geographical distribution. Dominant subtype in Americas, Western Europe and Australia is subtype B. Subtype C is prevalent in Southern Africa and India. In addition, great number of recominant circulating forms that are a mix of different subtypes exist.

BRIEF NATURAL HISTORY OF HIV-1 INFECTION

HIV spreads by exposure of mucosal surfaces (sexual transmission, breast feeding) or through direct blood contact (intravenous drug use, transfusion, mother to child transmission during pregnancy and delivery). During sexual transmission, HIV-1 encounters its first target cells in the genital and rectal mucosa (dendritic cells, macrophages, resident CD4+ T cells), which then carry the virus to peripheral lymph nodes and gut associated lymphoid structures for further dissemination to CD4+ T cells in these tissues (Hladik and McElrath, 2008). This process is accompanied by the onset of systemic inflammation that never resolves and serves as a prognostic marker of AIDS.

The first wave of CD4+ T cell infection and massive depletion of this population in lymphoid tissues leads to high viremia associated with flu-like symptoms (fever, headache, rash) and is termed the acute stage of the disease (Figure 1). The risk of HIV transmission is high during this period ("The Stages of HIV Infection Understanding HIV/AIDS," 2018). HIV-1 specific cytotoxic CD8+ T cell (CTL) response develops during this stage of infection somewhat limiting viral replication. This leads to decline of viremia to the viral set point and stabilization of CD4+ T cell count (Borrow et al., 1994; Ndhlovu et al., 2015), which occurs within 3-6 months of infection (McMichael et al., 2010). This stage is termed the chronic phase, is typically asymptomatic and, in the absence of treatment, leads to AIDS in 10 years or less. Risk of transmission still remains at this stage of infection. Viral escape mutations and other viral evasion mechanisms, renders CTL arm of immunity largely ineffective in the control of infection during chronic disease (Borrow et al., 1997; Deng et al., 2015). Natural killer (NK) cell and cytotoxic CD4+ T cell responses also fail to control the virus in majority of patients, although a genetic association between efficient NK cell and CTL responses and HIV control has been reported (McMichael et al., 2010). Similarly, B cell responses are impaired during HIV-1 infection. Production of immune complexes followed by non-neutralizing antibodies is seen early in infection (McMichael et al., 2010). HIV-1 neutralizing antibody responses take years to develop and do not control infection in majority of cases.

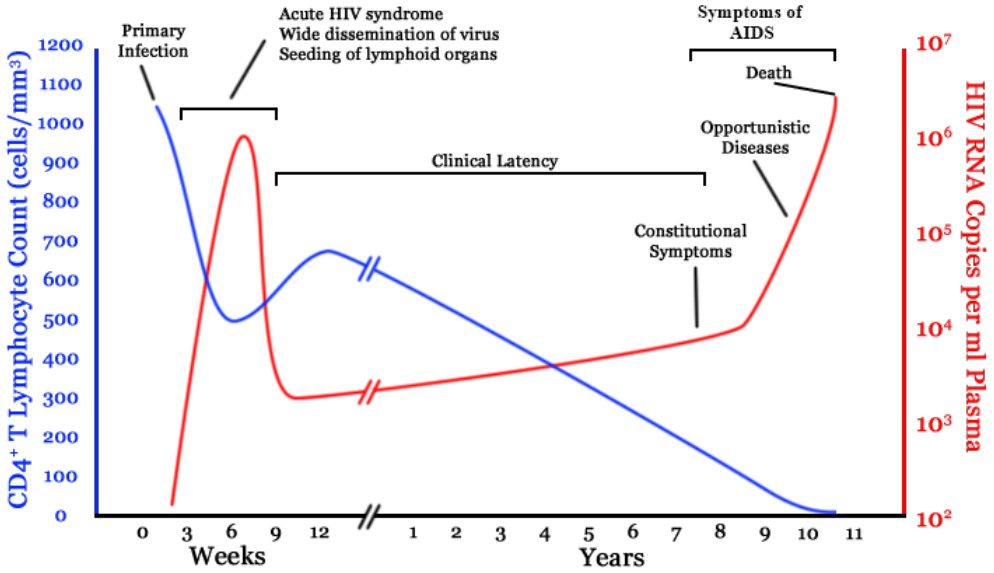

Figure 1. Clinical stages of HIV-1 infection.

At the end of the chronic phase, CD4+ T cell counts decline further and the viral load drastically rises again marking the onset of AIDS. This stage is marked by the drop of blood CD4+ T cell count to below 200 cells/mm^3 and the onset of opportunistic infections. The transmission chance at this stage is high. The survival rate in the absence of treatment is less than 3 years ("About HIV/AIDS | HIV Basics | Act Against AIDS | CDC," 2018).

ORIGIN OF HIV AND NON-HUMAN PRIMATE MODELS OF HIV-1 INFECTION

HIV-1 and HIV-2 infections of humans have resulted from cross-species transmission events of simian immunodeficiency viruses (SIV) originating from several African primate species (Figure 2). Each of the HIV-1 groups resulted from an independent transmission event. For example, HIV-1 groups M and N originated from West-Central Africa chimpanzees *(Pan troglodytes troglodytes)*, in which SIV_{cpz} virus causes redued life span (Garcia-Tellez et al., 2016). These primates, in turn, were infected by simian immunodeficiency virus (SIV) from red-capped mangabese (*Cercocebus torquatus*) and several *Cercopithecus* species. Group P originated from a gorilla SIV (SIV_{gor}) transmission event and the origin of Group O is unknown (Sharp and Hahn, 2011). HIV-2 origninated from sooty mangabey SIV virus (SIV_{smm}), which is non-pathogenic in this natural host (Sharp and Hahn, 2011).

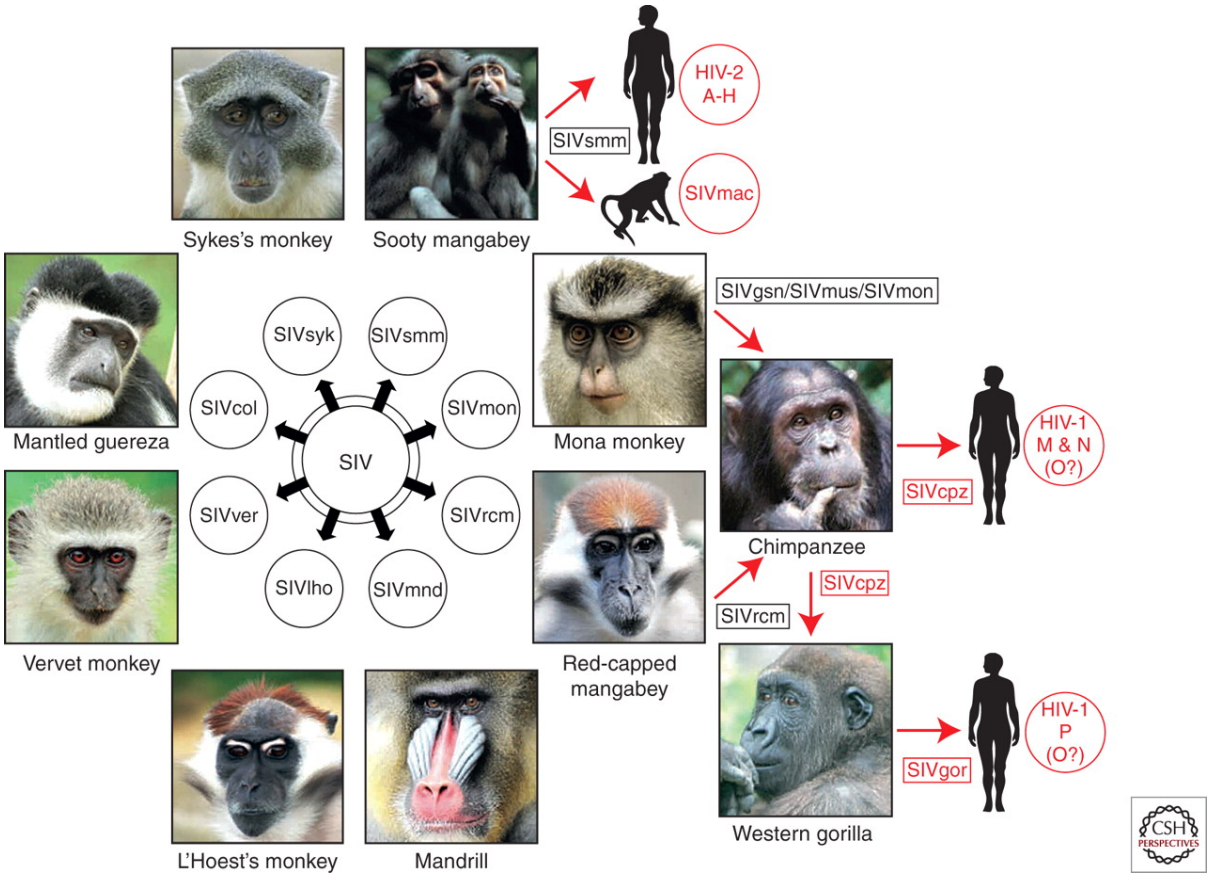

Figure 2. Origins of human AIDS viruses and diversity of simian immunodeficiency viruses. Adapted from (Sharp and Hahn, 2011)

It is not surprising, therefore, that non-human primates (NHP) are widely used as a disease model for HIV-1 infection and AIDS. Importantly, infection of different NHP species of different genetic background with SIV strains of various virulence allows to recapitulate all disease progression profiles found in humans (Figure 3) (Saez-Cirion et al., 2014, p.) (Garcia-Tellez et al., 2016). Three macaque species are mainly used to model disease progression (rapid or slow) to AIDS: cynomolgus macaque *(Macaca nemestrina)*, pig-tailed macaque *(Macaca fascicularis)*, and rhesus macaque *(Macaca mulatta)* (Figure 3). SIV_{mac} viral strains infecting macaques recapitulates HIV-1 infection pathophysiology and mirrors clinical stages of the disease found in humans (Garcia-Tellez et al., 2016) (Sui et al., 2013). Some of the similarities between human and simian disease include: mucosal transmission, tropism for CD4+ T cells and macrophages, clinical stages of the disease and immune activation (Sui et al., 2013).

Fastest progression to AIDS is generally observed upon infection of Indian rhesus macaque with SIV_{mac239} strain (Garcia-Tellez et al., 2016). On the other hand, spontaneous viral control resembling natural control of HIV-1 in humans has been demonstrated in rhesus and cynomolgus macaques with specific MHC alleles, similarly to genetic protective associations found in controller humans (Saez-Cirion et al., 2014, p.). Finally, natural SIV infection models in species where the virus does not cause a disease exist: SIV_{agm}-infected African green monkeys, SIV_{smm}-infected sooty mangabeys and SIV_{mnd}-infected mandrills (Jacquelin et al., 2012). The major goal of studying infection of such natural hosts is to identify correlates of protection against disease in these animals and try to induce these mechanisms in animal pre-clinical models and during pathogenesis in humans.

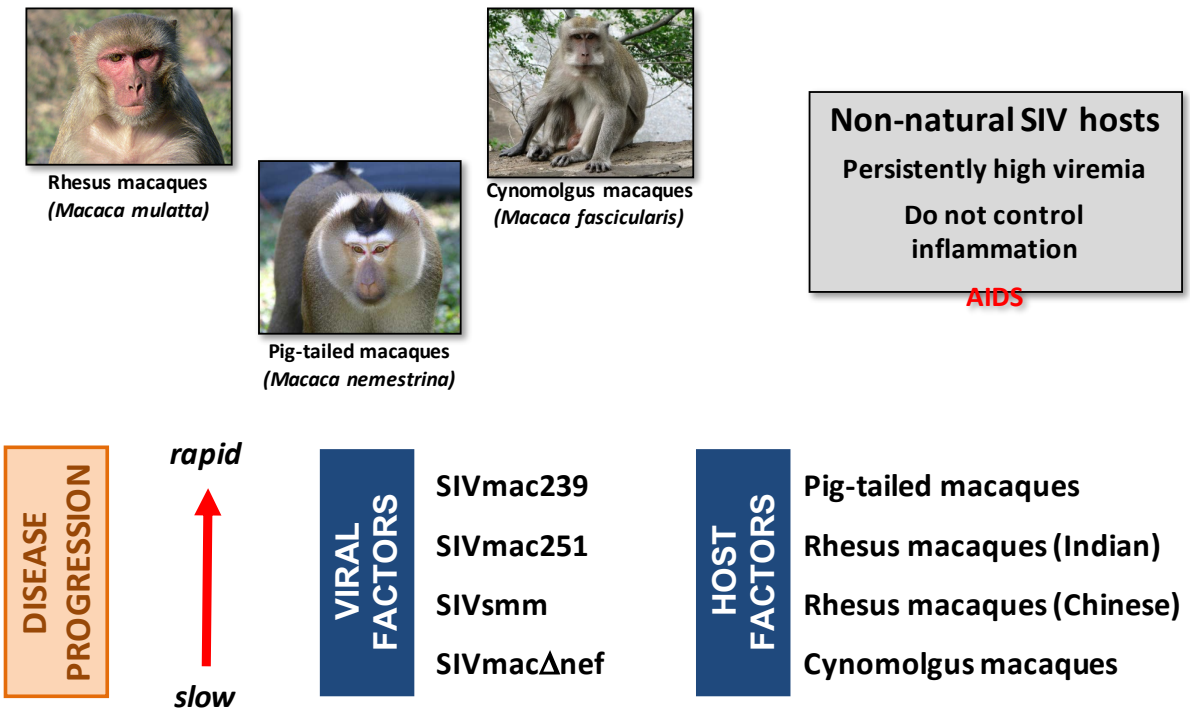

Figure 3. Non-human primate models of HIV-1 caused disease progression found in humans. Courtesy of Passaes CP.

HUMAN IMMUNODEFICIENCY VIRUS-1

VIRAL PARTICLE

HIV-1 viral particle is enveloped by a lipid bilayer supported by the matrix (MA) protein and has 5-15 trimers of envelope (Env) glycoproteins embedded into it (Brandenberg et al., 2015). The membrane and MA enclose the viral capsid core, composed of a polymer of capsid protein (CA) arranged in a conical shape (Yamashita and Engelman, 2017). The capsid core contains 2 copies of the 9,230nt long RNA genome, which is decorated with nucleocapsid (NC) proteins. Viral reverse transcriptase (RT), integrase (IN) and other proteins (both viral and host cellular) are also found in the capsid (Yamashita and Engelman, 2017) (Figure 4).

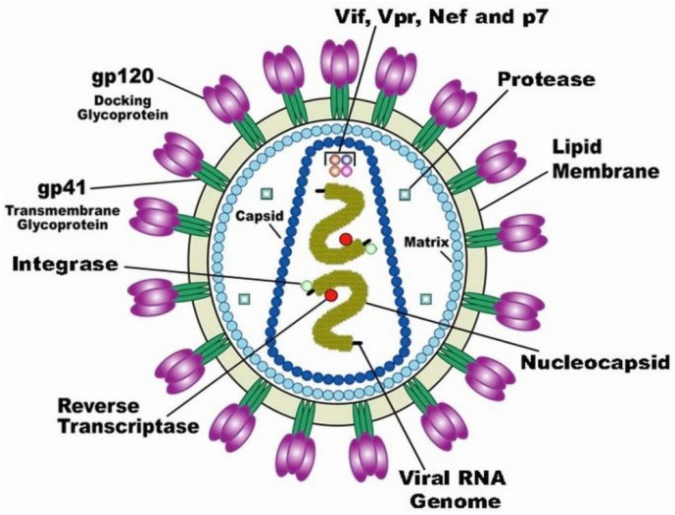

Figure 4. HIV-1 viral particle

In addition to structural proteins, HIV-1 particle carries an array of accessory proteins that are important for viral infectivity due to their antagonism of cellular restriction factors (RFs) (Figure 5) and interaction with cellular processes that are necessary for HIV infection. Some of these proteins are carried inside the cell by the viral particle, while others are synthesized de-novo upon viral transcription and translation. These proteins include: Vif, Vpr, Vpu, Nef, Tat and Rev. All of these proteins have multiple functions, which are described throughout

the text. Briefly, however, the most known functions of some of these proteins are outlined here. For example, Nef boosts the activity of transcription factor (TF) NF-kB early during infection to induce viral promoter transcription (see below) and downregulates CD3, CD4, CD8, CD28 and MHC-I expression to suppress adaptive immunity (Sauter and Kirchhoff, 2018). Vpu, on the other hand, inhibits NF-kB during later phases of infection to prevent transcription of anti-viral factors (Sauter and Kirchhoff, 2018). Vpu has also been shown to suppress the expression of RF tetherin and HLA-B, important for adaptive immunity. Vpr blocks the cell cycle of dividing cells in G2 phases thus ensuring maximal activity of LTR promoter. In non-dividing cell, Vpr ensures nuclear import of viral pre-integration complex (González, 2017). Vif counteracts host cell RF APOBEC3 (Nakano et al., 2017). Tat activates viral transcription and regulates viral mRNA splicing (Jablonski et al., 2010) and Rev promotes splicing and nuclear export of nascent viral mRNA (Freed, 2015).

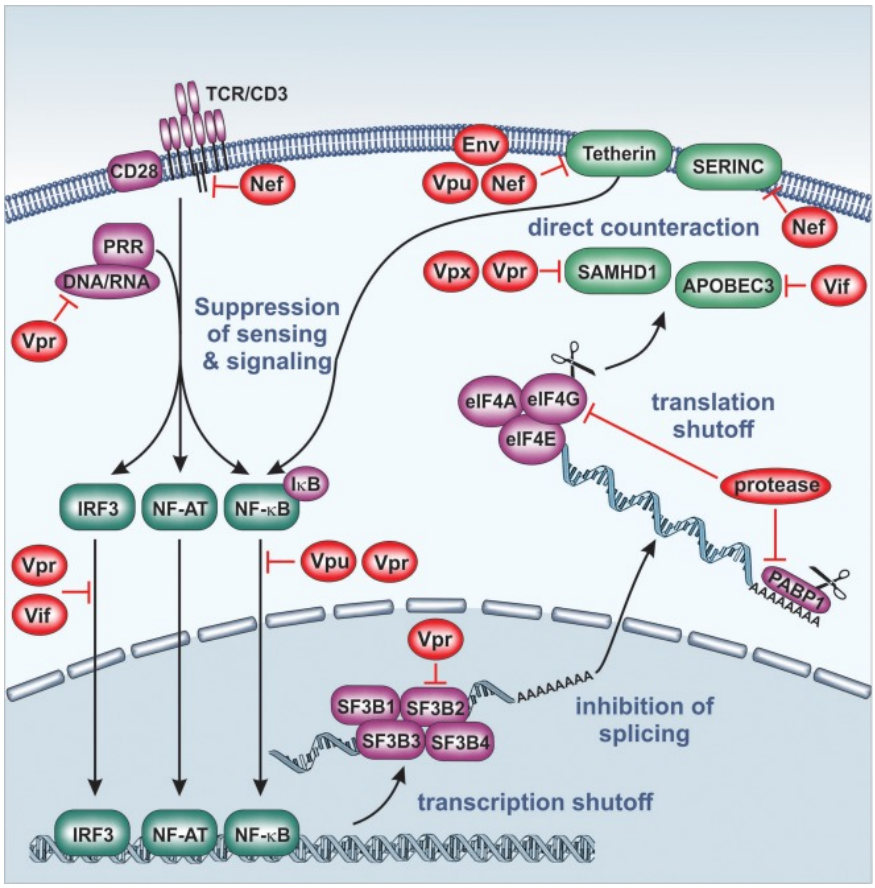

Figure 5. Interaction of HIV-1 accessory proteins with cellular factors. Adapted from (Sauter and Kirchhoff, 2018)

VIRAL LIFE CYCLE

HIV-1 life cycle begins with the entry of the viral particle mediated by the attachment to its receptor and co-receptors into the cell. After the attachment, the viral particle membrane fuses with the cellular plasma membrane to inject the viral capsid into the cytoplasm. Alternatively, HIV-1 has been reported to be able to enter the cell through fusion event in the endosome (de la Vega et al., 2011)(Fackler and Peterlin, 2000)(Carter et al., 2011). However, this remains controversial (Gregory B Melikyan, 2014). Once inside the cell, the viral capsid travels to the nuclear envelop while the genomic RNA is reverse transcribed into DNA. The viral DNA then integrates into the host chromosome. From here, the virus follows one of two fates: active transcription of viral gene products or latency. Latency is a state of transcriptional silencing of the virus. Transcription and subsequent translation of viral mRNAs forms structural and non-structural viral proteins followed by their trafficking and assembly of the viral particle at the plasma membrane. The viral particle buds at the plasma membrane, matures and re-infects another cell to initiate a new cycle of infection (Figure 6).

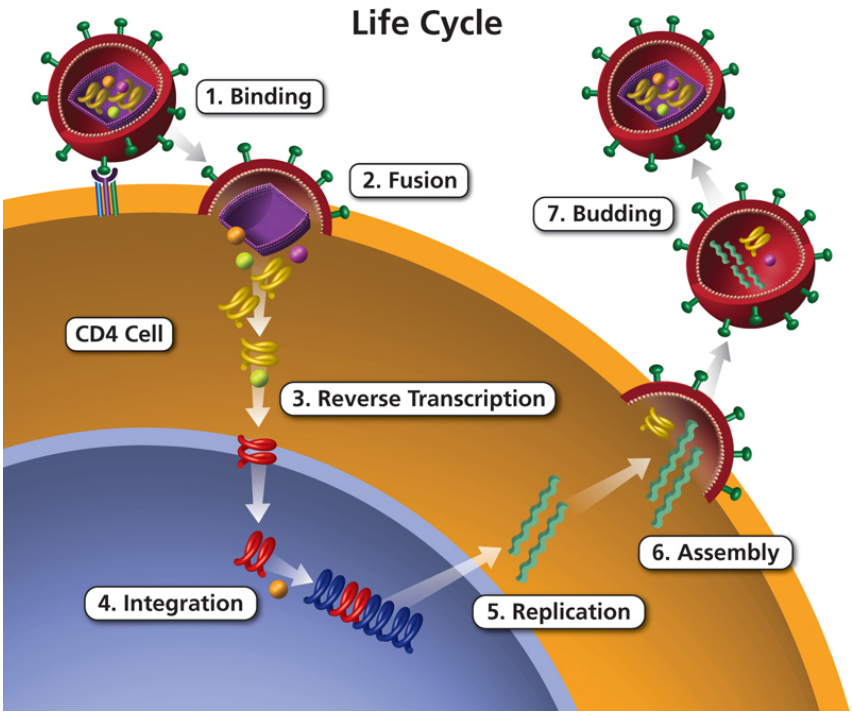

Figure 6. HIV-1 life cycle. Adapted from AIDSinfo.nih.gov

Entry

The entry of the HIV-1 viral particle into the cell is mediated via the attachment of the Env glycoprotein to its receptor CD4 and co-receptor CCR5 or CXCR4. Env is a glycosylated trimer of non-covalently attached gp120 and gp41 heterodimers. Env interacts with CD4 and its co-receptor via gp120. Viruses that use CCR5 co-receptor are called R5 viruses and viruses that use CXCR4 co-receptor are called X4 viruses. R5X4 viruses also exist.

Co-receptor binding on the cell surface exposes gp41 fusion peptide, which inserts itself into the cell membrane and, via the structural rearrangement, brings viral and cell membranes in close proximity resulting in the fusion of the viral capsid into the cytoplasm (Wilen et al., 2012).

Reverse Transcription

After viral core fusion into the cytoplasm, reverse transcription is initiated. Viral capsid surrounds genomic RNA and RT and protects viral genome from the recognition by the innate pattern recognition sensors in the cytoplasm. However, it is permeable to small molecules such as dNTPs, which are delivered inside the capsid to serve as reverse transcription substrates (Sleiman et al., 2012). Viral RT has a high affinity for dNTPs (Skasko et al., 2005), permitting reverse transcription to take place even in the limiting concentrations of dNTPs, which is the case for non-replicating cells such as macrophages (Diamond et al., 2004, p. 200).

Reverse transcription starts with the binding of $tRNA^{Lys}_3$ primer (carried in the viral particle from the previous host cell) to the primer binding site located close to the 5'-end of the genomic RNA (Sleiman et al., 2012). The DNA minus strand is synthesized by the RT using genomic RNA as a template. Nascent RNA-DNA duplex is recognized by the RT's RNaseH domain and RNA is cleaved at numerous sites. Only short RNA fragments (polypurine tracts) remain hybridized to the nascent DNA strand. These fragments serve as primers for the synthesis of the DNA plus strand and get subsequently degraded by the RNaseH activity of the RT (Amie et al., 2013).

Integration

Lentiviruses such as HIV-1 can infect both diving and terminally differentiated non-dividing cells due to their ability to traverse the nuclear envelope (Fassati, 2006). Viral core enclosed by the CA travels through the cytoplasm to the nuclear pore intact, uncoated, or partially uncoating in the process (a matter of debate) while reverse transcription is taking place (Yamashita and Engelman, 2017)(Hilditch and Towers, 2014). The viral core docks at the nuclear pore via its interaction with the CA (Lusic and Siliciano, 2017). Pre-integration complex (PIC), which consists of viral DNA in complex with an IN multimer (an intrasome), CA, MA, Virus protein R (Vpr) and cellular proteins, traverses the nuclear pore through the nuclear pore complex (PNC) to enter the nucleus periphery. Vpr is a key regulator of viral nuclear import and is indispensable for the infection of non-dividing cells such as macrophages (Hattori et al., 1990)(Popov et al., 1998).

After the nuclear import the intrasome binds to the target cellular DNA where IN mediates viral DNA integration. IN cleaves 2 nucleotides from each 3' end of the DNA strand, which exposes chemically reactive -OH group, thereby creating a double strand break. IN then mediates strand transfer, a process by which viral DNA is recombined with the cellular DNA. The cellular machinery that normally repairs chromosomal DNA damage repairs single-stranded gaps that remain after the recombination event. The integration generally favors gene-dense, active transcription sites at the nuclear periphery (Marini et al., 2015) and was correlated with the level of splicing inside those sites (Singh et al., 2015) (Lusic and Siliciano, 2017).

Transcription

After integration, HIV-1 genome can either enter into the state of latency or be transcribed under the control of the 5' long terminal repeat (LTR) promoter. LTR promoter is a promiscuous promoter that mediates gene transcription under the control of cellular transcription factors (SP1, NFkB, NFAT and TBP are the most recognized, although others are known) (Pereira et al., 2000). LTR contains binding sites for these promoters as well as

additional functional domains such as the TATA box and the transactivation response element (TAR) element (Figure 7). Sp1 binding site and the TATA box are essential for transcription initiation and basal promoter activity (Ne et al., 2018). Binding of these transcription factors promotes the recruitment of histone-modifying enzymes that allow for transcriptionally permissive chromatin environment. This, in turn, facilitates the installation of transcriptional machinery on the LTR promoter (Ne et al., 2018).

Upon the first rounds of transcription from the HIV-1 LTR, low levels of the Transcriptional activator (Tat) is produced. Tat binds to the TAR element, a short transcript with the hairpin structure (Asamitsu et al., 2018). Tat initiates high levels of viral transcription via recruitment of Cdk9, which phosphorylates and activates RNA Polymerase II (Salerno, 2007) (Asamitsu et al., 2018). Tat also mediates transcriptional elongation and stabilizes viral mRNA (Asamitsu et al., 2018). Viral genome is first transcribed as a full-length precursor mRNA molecule, which can then undergo extensive alternative splicing to generate mature mRNAs for structural and non-structural proteins of the virus or serve as a genomic RNA molecule (Sertznig et al., 2018).

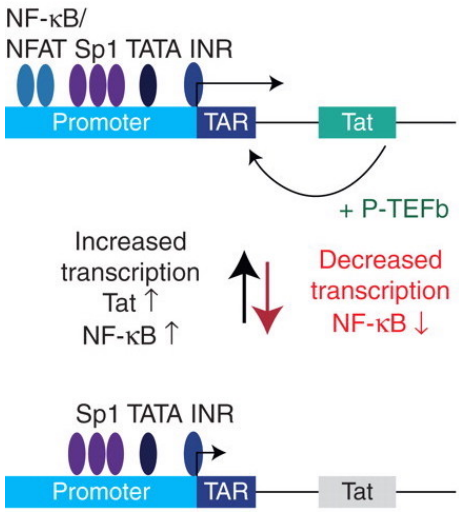

Figure 7. 5' LTR promoter domains. Low levels of Tat are produced due to low level transcription from the LTR promoter. Tat binds to nascent TAR RNA transcript and recruits p-TEFb and other elongation factors to the transcription complex thus boosting transcription from the LTR promoter. The presence of the NF-kB, TATA, Sp1, NFAT and other transcription factors promotes HIV-1 transcription and their relative absence drives HIV-1 into latency by inhibiting transcription. Adapted from (Karn and Stoltzfus, 2012)

Latency

After integration, HIV-1 can enter the state of latency through multiple mechanisms. These mechanisms inhibit the production of Tat, which normally stimulates transcription from viral LTR promoter. In general, the state of latency strongly depends on the transcriptional activity of the host gene containing the integration site. However, even in actively transcribed genes, several molecular mechanisms can inhibit viral transcription. These mechanisms act to remove TF or inhibit the activation of LTR promoter. For example, steric hindrance occurs when HIV-1 integrates downstream and in the same orientation as the host gene. In that case, RNA polymerase transcribing the host gene displaces the key TFs from the viral promoter that are necessary for transcription initiation (Darcis et al., 2017a).

Epigenetic mechanisms also play a key role in latency establishment (Figure 8). During latency, the integrated provirus exhibits inhibitory heterochromatin marks thus silencing active transcription. In particular, during latency, two nucleosomes (nuc-0 and nuc-1) are situated at the viral promoter irrespective of its integration site (Darcis et al., 2017a). Histone acetyl transferases (HATs) and histone deacetylases (HDACs) can acetylate or deacetylate respectively lysine residues in histone tails thus acting to remodel the chromatin and making the viral promoter more or less respectively accessible to transcription. Similarly, histone methyltransferases can induce transcriptional repression (H3K9 and H3K27 trimethylation) thus contributing to latency induction (Friedman et al., 2011; Imai et al., 2010). In addition to histone modifications, DNA methylation contributes to latency. During latency, HIV-1 promoter is hypermethylated at two CpG islands, which directly blocks access of TFs to the promoter, or induces repressive heterochromatin environment (Darcis et al., 2017a).

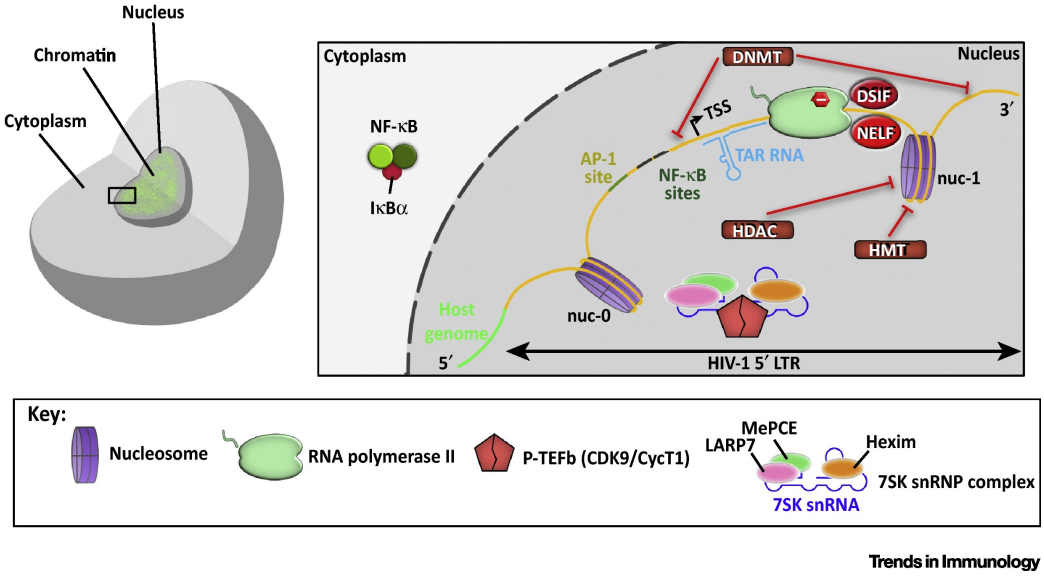

Figure 8. Mechanisms of HIV-1 latency induction. HIV-1 integrates in the periphery of the nucleus. During latency, transcription elongation by RNA polymerase II is blocked by nucleosome nuc-1 present after transcription start site (TSS). Repressive epigenetic environment consisting of DNA methylation (mediated by DNMT – DNA methyltransferase), histone deacetylation (mediated by HDACs – histone deacetylases) and methylation (mediated by HMT – histone methyltransferase) as well as sequesteration of NF-kB in the cytoplasm and p-TEFb in an inactive complex induces viral latency. Other molecular factors such as DSIF - 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) sensitivity-inducing factor and NELF – negative elongation factor also negatively regulate HIV-1 transcription. Adapted from (Darcis et al., 2017b)

Activation status of the infected cell also plays a critical role in latency establishment. Resting cells provide low supply of TFs and other molecules needed for efficient transcription. For example, cyclin T1 (a subunit of P-TEFb, HIV dependency factor important for viral transcription) is found in extremely low quantities in resting CD4+ T cells and the rest of the P-TEFb is sequestered to prevent its activity. Similarly, NF-kB is sequestered in the cytoplasm of unstimulated cells and is thus unavailable for transcription activation (Darcis et al., 2017a).

Viral particle assembly, budding and maturation

Once the viral mRNA is synthesized, it is transported from the nucleus to the cytoplasm where the viral proteins are translated. Gag polyprotein is synthesized as a precursor that is cleaved inside the viral particle by the protease (PR) into MA, CA, NC, p6 peptides as well as other small peptides (Freed, 2015). MA domain of the Gag polyprotein targets it to the plasma membrane, where it promotes incorporation of Env into the budding virion (Ono et al., 2004). CA domain drives Gag multimerization during particle assembly. NC contains two zinc finger-like domains that mediate its interaction with the viral genomic RNA. Unspliced viral RNA that serves as the genome is exported from the nucleus via its interaction with the Rev protein (Freed, 2015). NC acts as a nucleic acid chaperone and mediates RNA recruitment to the plasma membrane and its incorporation into the viral particle. Lastly, P6 peptide of the Gag polyprotein ensures Vpr incorporation and recruits endosomal sorting complex required for transport (ESCRT) which drives membrane fission during particle budding (Freed, 2015).

Env glycoprotein is also synthesized as a polyprotein precursor (gp160) that contains gp120 and gp41 proteins. Gp160 monomer trimerizes in the ER and is cleaved into gp120 and gp41 in the Golgi, after which it traffics through the secretory pathway to the plasma membrane (Checkley et al., 2011). Following cleavage, gp120 and gp41 remain associated by non-covalent interaction. In part to prevent the premature interaction of Env with its receptor CD4, viral Vpu protein binds to CD4 and mediates its proteosomal degradation. At the plasma membrane, gp120/gp41 are continuously recycled via the endosomal recycling pathway, which ensures low recognition by the immune system and low incorporation into the viral particle (Brandenberg et al., 2015).

GagPol is another precursor polyprotein that is synthesized and contains PR, RT and IN. The budded viral particle is immature and non-infectious with spherical capsid composed of Gag polyproteins. After budding, the viral particle matures, via the cleavage of Gag and GagPol polyproteins by the viral protease (PR) (Briggs et al., 2003). Following the cleavage of Gag by

PR, CA protein reassembles to form a conical shaped core around the viral genome and associated NC, RT and IN (Yamashita and Engelman, 2017).

CELLULAR DETERMINANTS OF HIV-1 INFECTION

The ability of the HIV-1 to establish infection depends on the tight balance of intracellular restriction vs viral dependency factors that allow for efficient viral replication. Although several of these factors are known, this area remains under intense investigation today.

I start my discussion of HIV restriction factors with interferons, a family of immunomodulatory cytokines, which induce many known HIV restriction factors.

INTERFERON

Interferons (IFNs) (type I, II and III) are a family of cytokines that are induced during viral infection. Type I IFNs (IFN-I) (IFNα, IFNβ, IFNω, IFNε and IFNκ) are most known for their anti-viral function. Upon binding to their cell surface receptors type I IFNs induce, among other functions, an anti-viral state through the induction of multiple interferon stimulated genes (ISGs) (Goodbourn et al., 2000). Some of these genes serve as restriction factors for HIV-1 infection (see below) (Doyle et al., 2015) (Goodbourn et al., 2000).

Upon HIV-1 infection, various pattern recognition molecules [ex. Interferon gamma inducible protein 16 (IFI16) and cyclic GMP-AMP synthase (cGAS) in the cytoplasm and toll-like receptor 7 (TLR7) in the endosome] recognize viral nucleic acid material and stimulate IFN-I production (Doyle et al., 2015). As a result, high levels of IFN-α is seen in the plasma of HIV-1 infected individuals during acute infection (Stacey et al., 2009), which has been postulated to be important in controlling initial phase of infection (Harris et al., 2010)(Sandler et al., 2014)(Soper et al., 2018). During chronic infection however, sustained IFN levels were shown to be associated with disease progression by driving chronic inflammation, immune activation and CD4+ T cell depletion (Jacquelin et al., 2009)(Harris et al., 2010)(Fraietta et al., 2013) (Soper et al., 2018). Animal and human trials of IFN treatment and IFN receptor block showed results consistent with the known role of IFN during the course of the disease. Treatment of macaques with IFN-α2a before and during acute infection transiently prevented systemic infection but exacerbated infection during chronic treatment (Sandler et al., 2014).

Administration of IFN-α2 to infected people resulted in transient decrease in plasma viremia but no long term benefit and even further decrease in CD4+ T cell counts (Utay and Douek, 2016).

HIV-1 RESTRICTION FACTORS

Several HIV-1 restriction factors are known today. However, most of them are ineffective against the virus due to their efficient neutralization by viral factors (Figure 9).

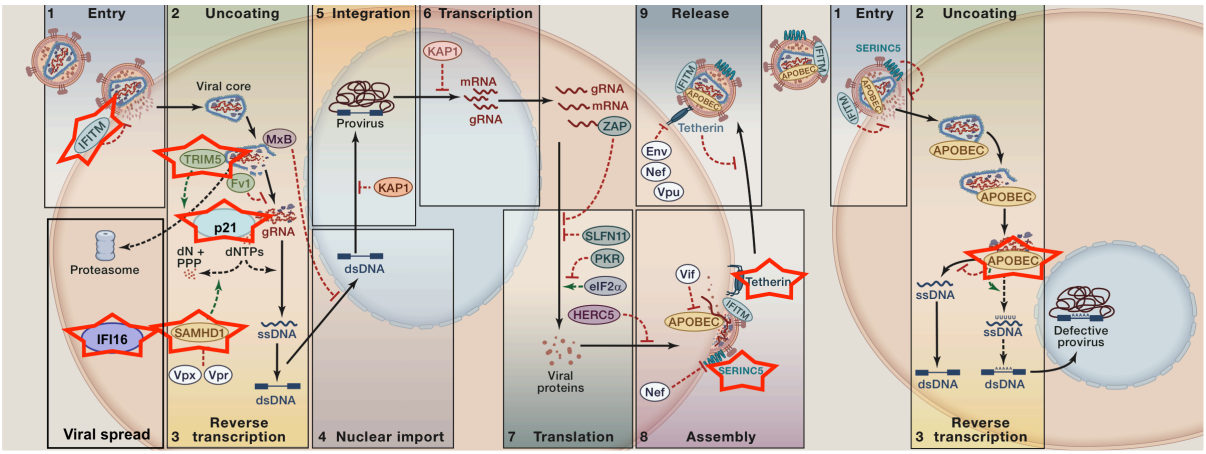

Figure 9. HIV-1 restriction factors and their known role in the viral life cycle. The restriction factors mentioned in the text are marked with red stars. SERINC and IFITM proteins prevent viral particle fusion; SAMHD1, p21 and APOBEC proteins inhibit reverse transcription; Tetherin inhibits budding and IFI16 prevents viral spread. Adapted from (Kluge et al., 2015)

Entry

Serine Incorporator (SERINC) proteins incorporate serine into cell membranes and facilitate synthesis of phosphatidylserine and sphingolipids (Ghimire et al., 2018). Of these, SERNIC3 and 5 were shown to restrict HIV-1 but are counteracted by viral protein Nef (Rosa et al., 2015)(Usami et al., 2015). In the absence of Nef, SERINC3/5 incorporate into the viral particle

plasma membrane and prevent their fusion (Rosa et al., 2015)(Usami et al., 2015)(Sood et al., 2017). Nef, by contrast, targets SERINC into late endosomal compartment and thus inhibits its incorporation into budding virions (Rosa et al., 2015).

Interferon-induced transmembrane (IFITM) proteins are a family of transmembrane proteins that have broad antiviral activity against many enveloped viruses (Shi et al., 2017). In HIV-1, IFITMs were shown to be effective against free virus infection when expressed on target cells (Lu et al., 2011) and against cell-associated viral infection when expressed on donor cells (Compton et al., 2014)(Tartour et al., 2014). They were also shown to be incorporated into viral particles (Compton et al., 2014) (Tartour et al., 2014). IFITM1-3 proteins restrict viral entry by poorly understood mechanisms, which may include alteration of membrane biophysical properties, cholesterol content (Shi et al., 2017) and inhibition of hemifusion states (Yu et al., 2015).

Reverse transcription and integration

Sterile alpha motif and histidine-asparatate domain containing protein 1 (SAMHD1) has several known functions. Its triphosphohydrolase (dNTPase) enzymatic activity converts deoxynucleotide triphosphates (dNTPs) into deoxynucleosides (Powell et al., 2011), thus depleting dNTP substrates for viral RT (Lahouassa et al., 2012)(Goldstone et al., 2011). It also restricts HIV-1 infection via its 3'-5'-exoribonuclease (RNase) activity thus degrading HIV-1 genomic RNA (Ryoo et al., 2014)(Choi et al., 2015). The relative importance of these two functions for HIV-1 restriction is debated (Ryoo et al., 2014).

SAMHD1 is critical for HIV-1 restriction in myeloid cells (Hrecka et al., 2011)(Laguette et al., 2011) and in resting CD4+ T cells (Baldauf et al., 2012), where levels of active SAMHD1 are abundant. SAMHD1 is, however, ineffective at restricting HIV-1 in activated, cycling CD4+ T cells and cycling macrophages due to its lower levels and cell cycle dependent inhibitory phosphorylation at Thr592 (Ryoo et al., 2014)(Mlcochova et al., 2018) (Lindgren et al., 2018). It is also ineffective in SIV and HIV-2, where viral protein Vpx targets it for proteosomal degradation (Hrecka et al., 2011)(Laguette et al., 2011). Interestingly, induction of DNA

double strand breaks (DSB) was shown to decrease the levels of phosphorylated SAMHD1 and enhance its anti-viral function (Jáuregui and Landau, 2018) (Mlcochova et al., 2018).

P21 is a cell cycle inhibitor, which, together with p53, is required to sustain G2 cycle cycle arrest after DNA damage through its inhibitory interaction with cyclin-dependent kinase 2 (CDK2) (Asada et al., 1999) (Bunz et al., 1998). Intriguingly, viral protein Vpr was reported to mediate cell cycle arrest in G2 phase via the induction of p21 expression, presumably to enhance LTR-driven transcription (Chowdhury et al., 2003). On the other hand, however, p21 inhibits CDK2 dependent phosphorylation of viral reverse transcriptase, which is necessary for efficient viral reverse transcription (Leng et al., 2014). P21, therefore, could have a complex role in HIV-1 infection depending on the viral infection stage and the host cell type.

In intravenous Ig (IVIG)-stimulated macrophages and mature DCs, p21 has been found, similarly to SAMHD1, to inhibit reverse transcription of HIV-1 (Bergamaschi et al., 2009) (Allouch et al., 2013) (Valle-Casuso et al., 2017). In contrast to SAMHD1, p21 does not directly hydrolyse dNTPs but rather inhibits their biosynthetic pathway via inhibition of dNTP biosynthetic enzymes RNR2, TYMs and TK1 (Allouch et al., 2013) (Valle-Casuso et al., 2017, p.). P21 has also been shown to increase phosphorylation of SAMHD1 (Allouch et al., 2014; Pauls et al., 2014) (Badia et al., 2016)(Valle-Casuso et al., 2017) in macrophages and to be induced in response to DNA damage (Mlcochova et al., 2018).

The role of p21 in HIV-1 infection of CD4+ T cells, however, is less clear. p21 mRNA was found to be upregulated in productively infected CD4+ T cells and was associated with reduction in p21-specific micro RNA (miR) expression (Guha et al., 2016). However, when p21 was induced by CD4+ T cell treatment with a cholesterol lowering drug (atorvastatin), this lead to p21-mediated inhibition of infection (Elahi et al., 2016). The p21 expression levels *in vivo* are also not always consistent with protection. The levels of p21 mRNA were shown to be higher in CD4+ T cells of HIV-1 infected patients who can spontaneously control infection (HIV controllers) in some reports (Sáez-Cirión et al., 2011)(Chen et al., 2011) but not in others (de

Pablo et al., 2015). Furthermore, even in controller cohorts where p21 expression was higher, it is unclear that p21 exerts a direct control of viral replication in these cells (Sáez-Cirión et al., 2011)(Chen et al., 2011).

Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3 (APOBEC3) proteins belong to the family of single-stranded DNA deaminases (Simon et al., 2015, p. 20). Out of all known APOBEC3s, APOBEC3B, D, F, G and H are active against HIV-1, although APOBEC3G is the most potent (Holmes et al., 2007b). Despite their activity, HIV-1 has evolved an efficient way of counteracting APOBEC3 via its proteosomal degradation mediated by viral protein Vif thus drastically decreasing its incorporation into virions (Marin et al., 2003). In the absence of Vif, however, APOBEC3 proteins are packaged into the viral particles via the interaction with the genomic viral RNA and Gag (Zennou et al., 2004) where they mediate cytidine deamination of the viral genome (Mangeat et al., 2003)(Apolonia et al., 2015) (Sheehy et al., 2002). The edited viral genomes are aberrantly or not at all reverse transcribed during the next infectious cycle thus greatly reducing the amount of RT products (Mangeat et al., 2003).

Evidence exists, however, that antiviral action of APOBEC3 proteins may be partially independent of its deaminase activity and that it is able to inhibit RT directly (Bishop et al., 2008; Holmes et al., 2007b)(Bishop et al., 2006, p. 2006)(Holmes et al., 2007a). Various mechanisms by which this is accomplished have been proposed including interference with RT by steric hindrance (Bishop et al., 2008), inhibition of tRNA primer binding (Guo et al., 2006), defects in plus-strand DNA transfer (Mbisa et al., 2007) .

Even in the presence of Vif, some APOBEC3 manages to get incorporated in the viral particles, where it has been suggested to contribute to viral evolution (Holmes et al., 2007b). APOBEC3-created mutations have been shown to contribute to viral adaptation in natural infection by creating immune system escape mutations (Kim et al., 2014). To add further complexity, APOBEC3C and 3G have been shown to be upregulated in CD4+ T cells upon activation

(Refsland et al., 2010) and was recently found by us to correlate with HIV-1 infection in CD4+ T cells (Valle-Casuso, submitted; See Annex 2).

TRIM5α binds to viral capsid upon entry accelerating its uncoating and thus exposing reverse transcription to the cellular environment of the cytoplasm (Malim and Bieniasz, 2012). This could have dual effect: it disrupts reverse transcription complex architecture and exposes viral genome to the recognition by PRRs thus blocking reverse transcription process. However, human TRIM5α is only active against certain strains of mouse retrovirus but not HIV-1 or SIV (Malim and Bieniasz, 2012).

Cyclic GMP-AMP synthase (cGAS) is a cytosolic DNA recognition molecule (Altfeld and Gale Jr, 2015), which detects HIV and SIV DNA and triggers a signaling cascade that mediates interferon production (Gao et al., 2013). However, this PRR was not reported to directly restrict viral replication itself.

Viral spread

Tetherin also termed BST2 or CD317 is a transmembrane protein that has also GPI-membrane anchor (Kupzig et al., 2003). It is thus inserted into the cell plasma membrane and anchors onto the viral particle lipid bilayer therefore 'tethering' released viral particles onto the cell surface. Clustered viral particles are then re-endocytosed back into the cell (Neil et al., 2007). Tetherin is an IFNα inducible factor (Neil et al., 2006) that is effectively counteracted by viral Vpu of HIV-1 (Van Damme et al., 2008)(Neil et al., 2008) or Nef of SIV (Zhang et al., 2009)(Jia et al., 2009). The cytoplasmic tail of tetherin also induces NFkB proinflammatory signaling (Tokarev et al., 2013)(Galão et al., 2012). Thus, tetherin also acts as a viral sensor molecule.

IFN-inducible protein 16 (IFI16) is an interferon stimulated factor (ISG) whose localization is both cytosolic and nuclear (Altfeld and Gale Jr, 2015). IFI16 recognizes DNA products of HIV-1 RT and effectively recognizes HIV-1 DNA in macrophages, where it restricts HIV-1 replication

and stimulates production of interferons (Jakobsen et al., 2013). IFI16 also recognizes HIV-1 DNA in resting lymphoid tissue CD4+ T cells, where it mediates pyroptosis in abortively infected cells through the activation of caspase-1-dependent cell death pathway (Monroe et al., 2014). Additionally, IFI16 has now been characterized as direct transcriptional regulator of HIV-1 and other viruses (Jakobsen and Paludan, 2014). IFI16 has been reported to interact with Sp1 thus preventing its recruitment to promoters (Jakobsen and Paludan, 2014). In HIV-1, IFI16 is implicated in the reduction of transcription through interfering with LTR promoter activity and Gag processing (Telenti, 2014). This antiviral activity is counteracted by viral Vpr protein (Telenti, 2014).

HIV-1 DEPENDENCY FACTORS (HDFs)

HDFs are intracellular factors that are important or essential for establishment of productive viral infection. Generally, HDFs are identified using genome-wide RNAi or CRISPR/Cas9 knockdown/knockout approaches. Although generating a lot of hits, such reports published by different groups rarely identify the same factors thus questioning the power of these approaches (Schott and König, 2017)(Pache et al., 2011)(Bushman et al., 2009). Moreover, most studies are carried out in cell lines, often of non-lymphoid origin thus limiting the extrapolation power to primary CD4+ T cells and myeloid cells. However, despite these limitations, factors that have been identified by 2 or more reports yield more confidence (Bushman et al., 2009). Many other HDFs were identified serendipitously and have been since confirmed by others. Categorization of HDFs made it clear that HIV-1 relies heavily on several cellular pathways: cell activation, cell cycle progression, proliferation and metabolism. The role of cell survival pathways and apoptosis is also discussed.

ENTRY AND TRAFFICKING

In addition to entry through its receptor and co-receptors, HIV was shown to use other surface molecules to target cells. Env has been reported to bind to heparan sulfate proteoglycans on macrophages (Saphire et al., 2001). DCs were also shown to capture HIV via scavenger receptors such as DC-SIGN (Geijtenbeek et al., 2000), Siglec 1 (Izquierdo-Useros et al., 2014), Syndecan 3 (de Witte et al., 2007) and others. $\alpha 4\beta 7$ integrin on peripheral T cells was also implicated in HIV entry (Arthos et al., 2008).

A whole cascade of actin polymerizing proteins gets activated upon the viral engagement of CD4 and the co-receptors. This leads to actin-dependent clustering of CD4 and CCR5/CXCR4 on the cell surface hence promoting viral entry (Ospina Stella and Turville, 2018). Fused viral capsid also depends on cellular cytoskeleton (actin, microtubules and associated proteins) for travelling through the cytoplasm towards the nucleus (Yamashita and Engelman, 2017).

REVERSE TRANSCRIPTION

CA protein that forms the viral capsid interacts with **cyclophilin A (CypA)** in the cytoplasm. CypA is a peptidyl prolyl isomerase, and has been reported to either enhance or inhibit viral infectivity depending on CA sequence and cell host type (Hilditch and Towers, 2014). For example, in DCs CypA has been shown to sense CA, promote DC maturation, launch type I IFN response and inhibit DC-mediated trans-infection of T cells (Manel et al., 2010). On the other hand, CypA was shown to be incorporated into viral particles via its interaction with Gag and to enhance viral infectivity (Thali et al., 1994)(Franke et al., 1994). In some cell types, it promotes capsid stability by delaying uncoating and promotes RT and integration (Yamashita and Engelman, 2017).

High level of dNTPs in cycling, activated cells allows the virus to establish infection more efficiently. For example, high levels of dNTPs in activated CD4+ T cells (2-5 μM) allows for efficient HIV-1 reverse transcription upon entry (Diamond et al., 2004). On the other hand, dNTP levels are low in resting CD4+ T cells and myeloid cells (Triques and Stevenson, 2004) (Diamond et al., 2004), which reduces but not blocks efficient viral RT and replication.

INTEGRATION

Several cellular factors play an important role in import of PIC into the nucleus through the NPC. Some of the known factors include cyclophilin A, RANBP2, transportin 3 (TNPO3), nucleoporin 153 (NUP153) and nucleoporin 358 (NUP358) (Lusic and Siliciano, 2017). Barrier-to-integration (BAF) (Chen and Engelman, 1998) and High mobility group protein A1 (HMGA1) proteins have also been reported to be integration co-factors (Farnet and Bushman, 1997).

Lens epithelium derived growth factor (LEDGF/p75) is probably the most known among a few specific integration HDFs described. This protein is a transcriptional activator although its precise function is not clear. It interacts with integrase of all lentiviruses (Poeschla, 2008),

targets it to the nucleus (Poeschla, 2008) and tethers it to the chromatin at active transcription unit sites (Meehan et al., 2009). Additionally, LEDGF/p75 was shown to interact with splicing factors and thus direct HIV-1 integration to transcription units that are highly spliced (Singh et al., 2015). Furthermore, it protects integrase from proteosomal degradation in the cytoplasm (Llano et al., 2004, p. 2).

TRANSCRIPTION

Cellular transcription factors

The presence of the activation stimuli ensures the supply of the right transcription factors at the LTR that allows intiation of viral transcription. The most known example of activation-dependent HIV-1 dependency factor is NFkB transcription factor. NF-kB is induced by cell activation (in T cells by TCR stimulation), among other stimuli, and induces transcription of many effector genes involved in the cell cycle, DNA repair, cell differentiation, anti-viral immunity (Heusinger and Kirchhoff, 2017). In the context of HIV-1, NF-kB is well known to bind to the viral LTR promoter where it initiates first lentiviral gene expression (Nabel and Baltimore, 1987) (Williams et al., 2007). On the other hand, it was shown that NF-kB can also promote HIV-1 latency (Williams et al., 2006). NF-kB can also initiate transcription of anti-viral gene effectors (Heusinger and Kirchhoff, 2017). Thus, HIV-1 modulates NFkB activity depending on the stage of the viral cycle and signaling context (Sauter and Kirchhoff, 2018). The activity of NFkB is boosted by viral Nef and Env during the early stages of infection to initiate viral transcription from the LTR promoter (Sauter et al., 2015), but is suppressed in a viral Vpu-dependent manner during the later stages to prevent transcription of anti-viral factors (Hotter et al., 2017; Sauter et al., 2015). Vpu prevents translocation of NFkB from the cytoplasm to the nucleus via stabilization of IkB, which is associated with the decrease in the interferon stimulated gene (ISG) transcription (Sauter et al., 2015).

BUDDING

Gag polyprotein interacts with PtdIns(4,5)P_2 during its recruitment to the plasma membrane and induces the recruitment of cholesterol and sphingolipids, which serve as the viral budding platforms (Freed, 2015). These lipids, therefore, serve as HDFs for viral budding.

The ESCRT machinery is indispensable for the fission of the budding viral particle from the cell surface. Gag has been found to interact with tumor susceptibility gene 101 (TSG101), part of the ESCRT-I machinery, and ALG2-interacting protein X (ALIX), part of the ESCRT-II and III (Freed, 2015).

CONTROL OF HIV-1 WITH ANTI-RETROVIRAL THERAPY

Infection with HIV-1 in the absence of anti-retroviral therapy leads to the progression towards AIDS within 10 years of infection ("WHO | HIV/AIDS," 2018). The rapid development of combination anti-retroviral therapy (cART) saved millions of lives around the globe. The treatment effectively lowers the blood viral load to undetectable levels (below 50 RNA copies/ml), drastically lowers chronic immune activation and inflammation associated with the disease. The life expectancy of virologically suppressed patients now approaches that of the general population ("Survival of HIV-positive patients starting antiretroviral therapy between 1996 and 2013," 2017). It, hence, represents one of the major success stories of modern global health.

The first cases of AIDS were described in United States in the early 1980s. The first anti-retroviral medication, 3'-azido-3'-deoxythymidine (AZT) or zidovudine (ZDV) appeared on the market in 1987 (Lange and Ananworanich, 2014). AZT belongs to a nucleoside analog reverse transcriptase inhibitor (NRTI) drug class, which inhibit reverse transcriptase activity of the virus. Other NRTI compounds together with protease inhibitors (PIs) were developed in the subsequent years (Flexner, 1998). But It was not until 1996 that the triple combination therapy using 1 PI and 2 NRTIs was introduced that achieved durable long lasting control of the virus (Hammer et al., 1997)(Lange and Ananworanich, 2014). Today, there exist 23 antiretroviral drugs of various classes (Table 1) (including non-nucleoside reverse transcriptase inhibitors (NNRTIs), fusion inhibitors, integrase inhibitors and CCR5-blockers) that are used in cART (Palmisano and Vella, 2011), with the goal to reach durable suppression of the blood viral load below the standard limit of detection (50 copies/ml).

However, viral load rebound is observed in the blood upon cART interruption. This is due to reactivation of the replication competent virus found in persistantly infected cells (see below). Moreover, detectable virus replication is found in gastrointestinal tract (Yukl et al., 2010) (Horiike et al., 2012) (Belmonte et al., 2007), lymph nodes (Horiike et al., 2012) (Chun et al., 2008), spleen (Horiike et al., 2012), genital tract and the central nervous system (Cory

et al., 2013). All of these compartments, hence, represent virus sanctuaries that harbor cells containing replication competent virus – *viral reservoirs*.

Table 1. Anti-retroviral drugs approved by the US FDA between 1987-2014. Adopted from Eriksson et al, 2013

Antiretroviral (abbreviation)	Drug class	Year of US FDA approval
Zidovudine (ZDV)	NRTI, nucleoside	1987
Didanosine (ddI)	NRTI, nucleoside	1991
Zalcitabine (ddC)	NRTI, nucleoside	1992
Stavudine (d4T)	NRTI, nucleoside	1994
Lamivudine (3TC)	NRTI, nucleoside	1995
Abacavir (ABC)	NRTI, nucleoside	1998
Tenofovir disoproxil fumarate (TDF)	NRTI, nucleotide	2001
Emtricitabine (FTC)	NRTI, nucleoside	2003
Saquinavir (SQV)	PI	1995
Ritonavir (RTV or r)	PI	1996
Indinavir (IDV)	PI	1996
Nelfinavir (NFV)	PI	1997
Amprenavir (APV)	PI	1999
Fosamprenavir (fos-APV)	PI	2003
Lopinavir (LVP)	PI	2000
Tipranavir (TPV)	PI	2005
Darunavir (DRV)	PI	2006
Nevirapine (NVP)	NNRTI	1996
Delavirdine (DLV)	NNRTI	1997
Efavirenz (EFV)	NNRTI	1998
Etravirine (ETV)	NNRTI	2008
Rilpivirine (RPV)	NNRTI	2011
Enfuvirtide (T20)	Fusion inhibitor	2003
Maraviroc (MVC)	CCR5-blocker	2007
Raltegravir (RAL)	Integrase inhibitor	2007
Elvitegravir (EVT)	Integrase inhibitor	2012
Dolutegravir (DTG)	Integrase inhibitor	2013

NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; US FDA, United States Food and Drug Administration.

BARRIERS TO ELIMINATION OF HIV-1 RESERVOIRS

Early seeding of reservoirs

The first reservoirs are seeded in mucosal tissues within hours of infection from where the virus rapidly spreads to the local draining lymph nodes (Hu et al., 2000). At these and other tissues sites, the reservoirs are seeded within hours-days of infection (Stieh et al., 2016) (Haase, 2010)(Whitney et al., 2014). Because the reservoir seeding precedes clinical signs of disease, in most cases the initiation of therapy misses the critical window of opportunity to prevent reservoir establishment. However, early cART initiation has been shown to limit the size of the seeded reservoir that could lead to the spontaneous control of infection upon therapy interruption (Ananworanich et al., 2015)(Sáez-Cirión et al., 2013). The determinants of such control are currently being explored.

Viral latency

Indeed, cART does not cure HIV but only effectively prevents new rounds of infection from taking place. However, integrated, replication competent virus persists in latently infected cells (Dahabieh et al., 2015). Virus reactivation and new rounds of infection from this source happen during therapy interruption. CD4+ T cell activation due to antigen recognition and cytokine stimulation during cART may also play a role (Ruelas and Greene, 2013). The most accepted model is that latency is established after productive infection of activated CD4+ T cells that revert back to the resting state or of effector CD4+ T cells that become resting memory CD4+ T cells (Sengupta and Siliciano, 2018; Shan et al., 2017). However, an alternative model suggests that latency can be established directly in activated and resting CD4+ T cells (Chavez et al., 2015).

Persistence of infected cell and ongoing re-seeding of reservoirs

Long half-life of persistently infected cells is thought to be one of the major contributing factors to persistance of HIV-1 reservoirs. Half-life of infected resting memory CD4+ T cells on cART was proposed to be 3.7 years (Siliciano et al., 2003)(Crooks et al., 2015), although could be much longer in tissues. It is thus doubtful that even a life time of treatment would suffice

to eradicate this reservoir. Some CD4+ T cell subsets contribute more to the blood reservoir due to their long $t_{1/2}$: central memory CD4+ T (Cm) and Tscm cells with half life of months to years, while Tm and Tem subsets are more short lived (Mikhailova, 2018). Tm and Tem, however, were shown to maintain the reservoir by low level ongoing proliferation (Chomont et al., 2009a)(Hiener et al., 2017). Moreover, infected tissue macrophages and microglial cells could also greatly contribute to reservoir persistance due to their long half-life. Macrophages and microglial cells were reported to survive for months to years in different body compartments, although their $t_{1/2}$ and infection status on cART is poorly known (Mikhailova, 2018).

Tissue penetration of anti-retroviral drugs and residual viral replication

Another barrier to clearance of viral reservoirs on cART is unequal and sometimes inefficient drug penetration into tissues. Multiple studies showed reduced penetration (2-50 times lower) of anti-retroviral drugs into CNS, lymph nodes and genital organs when compared to drug concentrations in the plasma (Solas et al., 2003) (Mascio et al., 2009)(Fletcher et al., 2014)(Sawchuk and Yang, 1999)(Gray et al., 2013)(Estes et al., 2017). CNS is especially affected (Sawchuk and Yang, 1999), hence representing a distinct compartment where the virus evolves separately and represents a genetically distinct reservoir (Dahl et al., 2014)(Gianella et al., 2016)(Edén et al., 2010)(Canestri et al., 2010)(Gama et al., 2017). Moreover, it is now known that cART is less efficient against cell-to-cell viral transmission as in the case of viral spread from dendritic cells to T cells (Kim et al., 2018).

Although debated, ongoing viral replication in tissue sanctuary sites relatively inaccessible by cART and immune system is also thought to contribute to reservoir persistence (Lorenzo-Redondo et al., 2016). Low levels of ongoing viral replication was observed in lymphoid tissue compartment despite effective suppression of blood viremia (Estes et al., 2017)(Lorenzo-Redondo et al., 2016). The virus has been shown to persist in female genital tract and is detected in genital secretions despite cART (Launay et al., 2011). Similarly, male reproductive

tract was identified as a viral reservoir where, similarly to the CNS, HIV-1 has been shown to evolve independently (Tortorec and Dejucq-Rainsford, 2010), support ongoing viral replication despite therapy (Tortorec and Dejucq-Rainsford, 2010) and to be intermittently shed in the semen (Svicher et al., 2014). In these tissues, resident macrophages and CD4+ T cells have been identified as viral carriers (Tortorec and Dejucq-Rainsford, 2010).

WHICH CELL POPULATIONS CONSTITUTE THE HIV-1 RESERVOIR?

CD4+ T CELLS

CD4+ T cell subset diversity

CD4+ T cells are major targets of HIV-1, where the virus replicates efficiently ultimately resulting in the depletion of these cells during the progression of infection. Despite the expression of CD4 and CCR5 – receptor and co-receptor of HIV – on their surface, different subsets of CD4+ T cells support productive infection at different rates. For example, resting and naive CD4+ T cells are highly restrictive to infection, while activated and memory subsets are permissive to infection. Here, I first outline the state of knowledge about the diversity of CD4+ T cells. I then present what is known about relative contribution of different CD4+ T cell subsets to HIV-1 reservoir.

Current model of T cell development indicates that after positive and negative selection in the thymus, CD4+ T cells enter blood as mature naïve cells (Tn). Each of the cell clones contains a given T cell receptor (TCR) Ag specificity. T cells recirculate through secondary lymphoid organs where they are primed by Ag-bearing dendritic cells (DCs), differentiate and proliferate. Primed T cells then migrate to target tissues where they further proliferate and differentiate into different types of effector cells that mount an effective immune response (Mahnke et al., 2013)(Mueller et al., 2013). A combination of different activation signals (cytokines, co-stimulation molecules, TCR stimulation, etc) and the type of Ag dictates the subtype of effector cell and the tissue localization of the primed T cell. Several effector CD4+

T cell subtypes are known: Th1, Th2, Th17, Th22, Tfh, Tregs and cytotoxic CD4+ T cells (Gagliani and Huber, 2017; Vella et al., 2017). Briefly and simplistically, Th1 cells provide CD8+ T cell help; Th2 cells control parasitic infections and mediate tissue repair; Th17 control bacterial infections in the GI tract; Tfh cells mount B cell help in the lymphoid nodes; and Tregs downmodulate immune responses (Gagliani and Huber, 2017).

Following Ag clearance, more than 95% of effector cells die, leaving behind a pool of effector cells that will differentiate into resting memory cells. These cells retain a long-term Ag memory, meaning that they have high potential to quickly and effectively mount a secondary immune response upon Ag rechallenge (measured by proliferation, cytokine secretion, cytotoxicity and enhanced tissue migratory capacity) (Mahnke et al., 2013)(Mueller et al., 2013). Human T cell memory subsets follow a differentiation continuum from Tn cells through stem cell like memory (Tscm), central memory (Cm), transitional memory (Tm), effector memory (Em) to effector cells (Figure 10) (Mahnke et al., 2013). Tn, Tscm and Cm express lymph node homing receptors CD62L and CD27, which permits them to recirculate through the lymphoid tissues, be primed by DCs and initate an adaptive immune response. Generally, memory T cell subsets are distinguished from their naïve counterparts by the expression of CD45R0 and the lack of expression of CD45RA surface markers (Mahnke et al., 2013). CCR7 was further shown to divide Em from Cm cells. CCR7+ Cm cells have high proliferative capacity, lack immediate effector functions (rapid secretion of effector cytokines such as IFNγ, IL-4 and IL-5) and secrete high levels of IL-2 (homeostatic proliferation cytokine) (Sallusto et al., 1999). Cm, together with Tscm cells, are thought to maintain long-lived T cell memory pool. CCR7- Em cells, on the contrary, express receptors required for homing to inflamed peripheral tissues, rapidly secrete effector cytokines in response to Ag challenge and secreate low levels of IL-2 (Sallusto et al., 1999). CD27 marker, in combination with CCR7 is used to distinguish a fourth memory population, Tm cells. These cells are CD27+ CCR7- and are thought to represent an intermediate stage of differentiation between Cm and Em subsets. In addition, tissue resident memory T (Trm) cells, intially defined by high expression of CD69 and CD103, are now known (Mueller and Mackay, 2016). These cells have a distinct transcriptional profile, and thus are different, from Cm and Em cells (although also derived from naïve precursors). They do not appear to recirculate through the blood and lymph, have

minimal turnover and high longevity and mount local tissue immune responses (Mueller et al., 2013)(Mueller and Mackay, 2016).

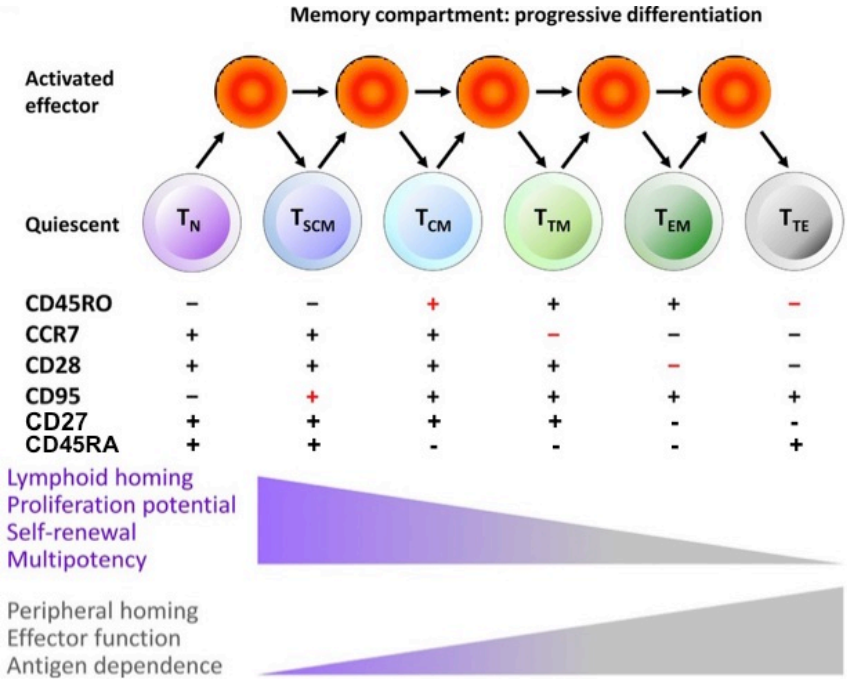

Figure 10. CD4+ T cell subsets. Adapted from (Mahnke et al., 2013)

CD4+ T cell reservoir in blood

Initiation of cART is accompanied by the rapid drop of blood viremia due to the clearance of free viral particle and productively infected cells (Perelson et al., 1997). However, the cell reservoir carrying virus remains. This reservoir is thought to be mostly composed of resting memory CD4+ T cells (Siliciano et al., 2003)(Finzi et al., 1997). Indeed, replication competent virus can be produced upon stimulation (with PHA or anti-CD3/anti-CD28 Ab) of resting CD4+ T cells from patients receiving long-term (years) cART (Chun et al., 1997)(Finzi et al., 1997)(Wong et al., 1997). This resting CD4+ T cell reservoir has been determined to consist mostly of central and transition memory CD4+ T cells with lesser contribution of effector memory CD4+ T cells (Chomont et al., 2009a). Recently, however, the importace of Em cell subset is beginning to be underscored. First, Em cells are best able to support productive viral infection *in vitro* (Valle-Casuso, submitted; see Annex 2). They are now thought to be the

source of latent Cm reservoir due to the differentiation of Em into Cm cells (Cheung et al., 2018; Shan et al., 2017). Moreover, they were recently found to contain highest proportion of full length viral genomes in virally suppressed patients (Hiener et al., 2017). HIV-specific memory CD4+ T cells were also shown to contain higher proportion of viral DNA than other subsets although replication competence of these viruses is unknown (Douek et al., 2002). Stem cell like memory CD4+ T cells (Tscm) may also have an important contribution to HIV-1 persistance due to their pluripotency potential and long half-life (Buzon et al., 2014).

CD4+ T cell reservoir in the tissues

Mucosal and gut CCR6+ Th17 cells are preferentially infected (Stieh et al., 2016), depleted first from the gut mucosa and are not fully recovered during cART (Brenchley et al., 2008). These cells were shown to have more favourable intracellular HIV-1 dependency factor (HDF) environment than other CD4+ T cell subsets (Cleret-Buhot et al., 2015, p.). During cART, lymphoid tissues, including lymph nodes and gut associated lymphoid structures, have been found to carry infected CD4+ T cells (Chun et al., 2008) (Perreau et al., 2013) (Banga et al., 2016). For example, T follicular helper (Tfh) in germinal centers are relatively inaccessible to cytotoxic CD8+ T cell activity and are thus deemed an important reservoir component (Perreau et al., 2013) (Banga et al., 2016). Tissue resident and other long-lived memory CD4+ T cell populations undoubtedly contribute to latent HIV-1 reservoir in lymphoid and non-lymphoid tissues but the precise identity of these cells remains to be characterized. In other tissues such as adipose, memory CD4+ T cells have also been shown to carry viral nucleic acid material (Couturier et al., 2015) and replication competent virus (Damouche et al., 2015). High proportion of infected Tfh cells have been detected in SIV infected macaques in cervical lymph nodes (Dave et al., 2018) indicating that CD4+ T cells infiltrating the CNS could be an important component reservoir (Kivisäkk et al., 2003).

BLOOD AND TISSUE MYELOID CELLS

Genetic studies on viral diversity during viral rebound upon treatment interruption have demonstrated that resting CD4+ T cells are not the only HIV-1 reservoir (Chun et al., 2000) (Dybul et al., 2003). Despite difficulty of infecting monocytes *in vitro* due to their low CD4 and CCR5 expression and resistance to viral replication, infected monocytes were found to persist during cART (Zhu et al., 2002) (Lambotte et al., 2000). These monocytes were shown to be capable of migration into tissues where they differentiate into macrophages, which provide more favorable intracellular viral replication environment (Palmisano and Vella, 2011).

Although they are able to be infected, macrophages express lower CD4, CCR5 and CXCR4 levels and provide more hostile intracellular environment to the virus than activated CD4+ T cells (Jayakumar et al., 2005)(Lee et al., 1999). Tissue macrophages were shown to carry viral nucleic acids (Jambo et al., 2014) and persist in multiple tissues despite cART treatment (Araínga et al., 2017)(Matusali et al., 2015)(Cribbs et al., 2014)(Zalar et al., 2010). Importantly, macrophages from different tissue sources appear to have different susceptibility to productive infection. For example, alveolar (Jambo et al., 2014) and vaginal (Shen et al., 2009) macrophages are highly susceptible but not intestinal macrophages (Shen et al., 2011, 2009). Macrophages probably have an important contribution to HIV reservoirs due to their long $t_{1/2}$.

Dendritic cells are thought to be relatively resistant to productive infection (Manel et al., 2010), with mature DCs being more resistant than immature DCs (Valle-Casuso et al., 2017). In addition to being infected, DCs capture viral particles and transport them to the lymph nodes where they mediate CD4+ T cell trans-infection (Kwon et al., 2002). Follicular dendritic cells were shown to capture and hold infectious virus for at least months even during cART (Fletcher et al., 2014) (Smith et al., 2001).

CELLS OF THE CENTRAL NERVOUS SYSTEM

CNS as a replication-competent HIV-1 reservoir has been disputed due to difficulties, until recently (Avalos et al., 2017), of isolating infectious virus from this compartment (Gray et al., 2014). However, several cell populations in the CNS have been clearly shown to be susceptible to infection and to carry proviruses.

Microglial cells are of embryonic macrophage origin and serve as phagocytic and antigen presenting cells of the CNS (Lannes et al., 2017). They express CD4, CXCR4 and CCR5 (He et al., 1997, p. 3)(He et al., 1997, p. 3)(Lavi et al., 1997) and are thus susceptible to infection. Infected microglial cells were detected *in vivo* in untreated people and monkeys (Churchill et al., 2006) (Cosenza; et al., 2002) (Llewellyn et al., 2018). Because they are extremely long-lived (years) (Gray et al., 2014) and are relatively isolated with regards to drug and immunity penetration, it is possible that these cells retain replication-competent virus on cART.

Perivascular macrophages, are derived from blood circulating monocytes. They have been described to be the primary targets of SIV infection during peak viremia in acute phase of infection (Williams et al., 2001). HIV-1 DNA has also been detected in perivascular macrophages of humans (Thompson et al., 2011). However, their moderate turnover of 2-3 months (Williams et al., 2001) could mean that they make a lesser contribution to reservoirs under cART than other populations.

Astrocytes positive for viral antigens and DNA were detected in infected brain tissue sections (Churchill et al., 2009) (Churchill et al., 2006)(K. A. Thompson et al., 2001) although they do not express CD4 or CCR5 (Russell et al., 2017) (Sabri et al., 1999). It has been recently shown, however, that astrocytes phagocytose viral particles and even infected cells and hence could be an important viral reservoir (Russell et al., 2017).

IMMUNO-METABOLISM OF CD4+ T CELLS AND HIV-1 INFECTION

GENERAL OVERVIEW OF IMMUNO-METABOLISM IN CD4+ T CELLS

Mounting an effective immune response requires drastic changes in CD4+ T cell activation, differentiation and proliferation. These processes are accompanied by changes in the cell's metabolic requirements. Thus, different stages of CD4+ T cell differentation and activation are supported by distinct metabolic programs that allow the cell to support its function but also respond to local tissue environmental pressures (Dumitru et al., 2018).

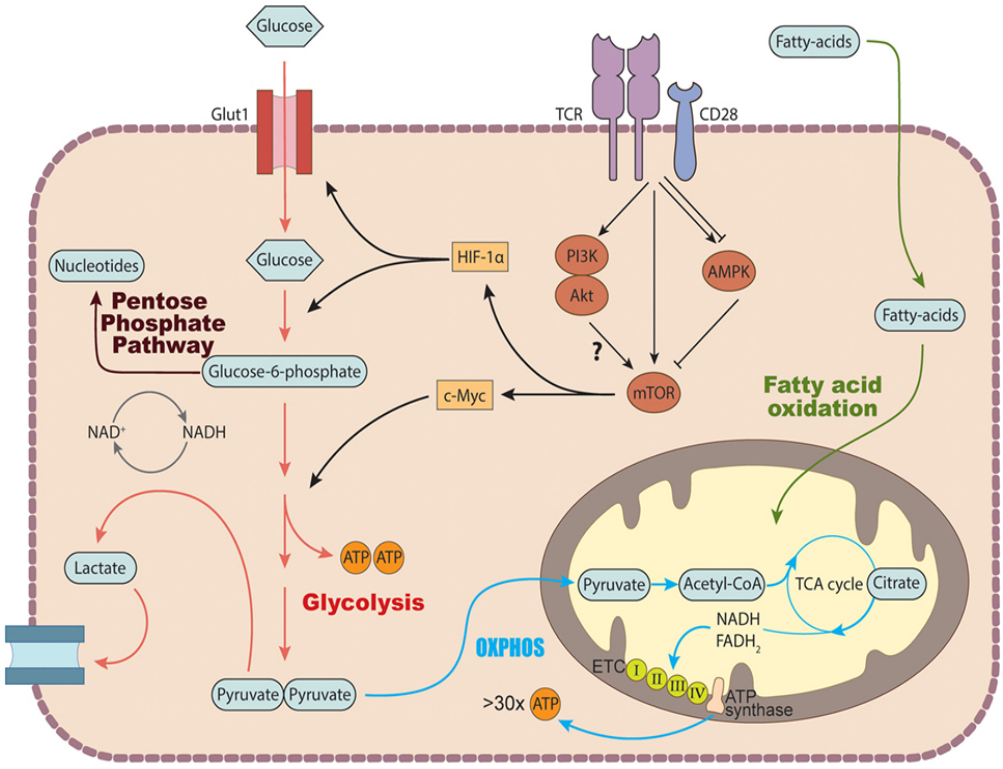

Figure 11. Metabolic pathways in CD4+ T cells. Adapted from (Dumitru et al., 2018)

Quiescent Tn cells rely predominantly on mitochondrial respiration to fuel energy production (Figure 11 and Figure 12) (Pearce et al., 2013). During this process, the available nutrients (glucose, fatty and amino acids) are broken down and their metabolic intermediates are fed into the tricarboxylic acid (TCA) cycle in the mitochondria. NADH and $FADH_2$ produced in the TCA cycle feed their electrons through the electron transport chain located in the inner mitochondrial membrane to drive the generation of the proton gradient across the

membrane, which fuels ATP production by ATP synthase (a process called oxidative phosphorylation (OXPHOS)). OXPHOS generates high number of ATP molecules (about 30 ATP per 1 glucose molecule) and is thus extremely energy efficient. While both OXPHOS and glycolysis are upregulated during T cell activation, relative contribution of OXPHOS to cellular metabolism is decreased and aerobic glycolysis becomes a dominant pathway (Figure 12). Glycolysis is less energetically efficient that OXPHOS (2 molecules of ATP per molecule of glucose) but provides biosynthetic intermediates more readily. Glycolysis (together with TCA cycle), a breakdown pathway of glucose in the cytoplasm, generates biosynthetic intermediates that are funneled through different metabolic pathways such as pentose phosphate (PPP), serine biosynthesis, hexosamine and fatty acid synthesis pathways (Almeida et al., 2016). These pathways serve to eventually sythesize molecular building blocks of the cell such as nucleotides (PPP pathway), amino acids (serine biosynthesis pathway) and fatty acids. Lactate that is generated as a final product of aerobic glycolysis, is exported from the cell.

Effector and regulatory T cells rely on different metabolic pathway for their function. For example, effector cells such as Th1, Th2 and Th17 utilize high rates of glycolysis while Tregs rely preferentially on fatty acid oxidation (FAO) pathways (Dumitru et al., 2018). As effector cells differentiate into memory cells, their metabolism reverts back from high reliance on glycolysis to OXPHOS and FAO (Figure 12).

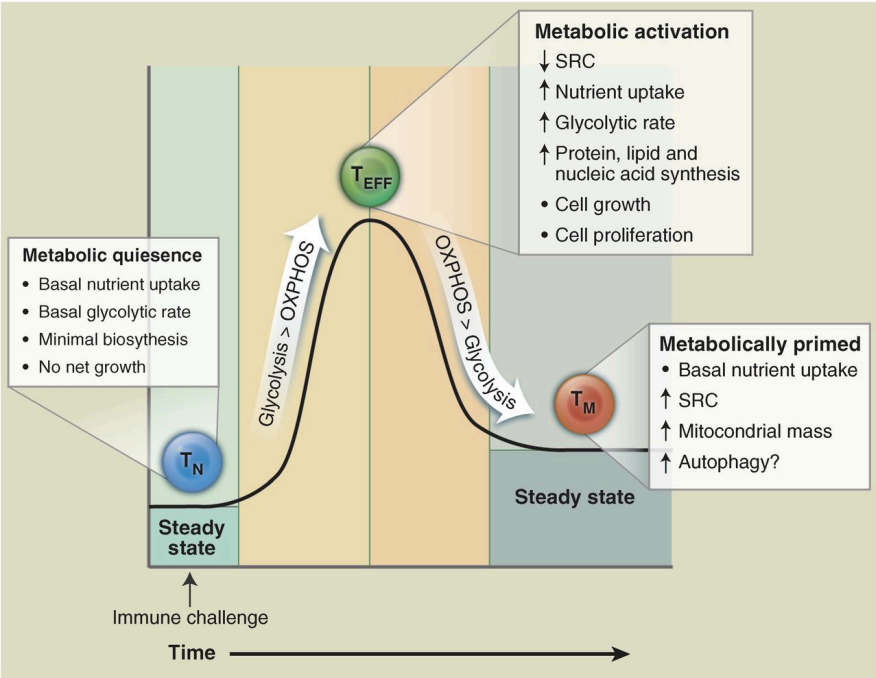

Figure 12. Changes in T cell metabolism according to differentiation stage. Adapted from (Pearce et al., 2013)

THE ROLE OF CD4+ T CELL METABOLISM IN HIV-1 INFECTION

In parallel to the activation and cell cycle status, the cell's metabolic state plays a key role in efficient HIV-1 replication. Upregulation of Glut1 expression on CD4+ T cell surface has been directly linked to susceptibility of the cells to HIV-1 infection (Loisel-Meyer et al., 2012a). Moreover, Glut1 levels have been shown to be increased on CD4+ T cells during chronic HIV-1 infection and not completely return to baseline upon treatment (Palmer et al., 2012). Dependence of HIV-1 infection on Glut1 expression is thought to be linked to its role as a glucose transporter. Consequentially, successful HIV-1 replication has been shown to rely on glycolysis (Hegedus et al., 2014a)(Hollenbaugh et al., 2011a)(Valle-Casuso, submitted; see Annex 2), which has been shown to suppply metabolic intermediates for the viral cycle (Valle-Casuso, submitted; see Annex 2). More recently, activation of PI3K metabolic pathway, and

its surrogate marker OX40 were proposed to be a better predictor to viral infection susceptibility in combination with Glut1 expression than Glut1 expression alone (Palmer et al., 2017).

As mentioned before, activated CD4+ T cells reply heavily on glycolysis for their function. TCR stimulation alone is sufficient to upregulate Glut1 glucose transporter as well as other glutamine and amino acid transporters on the cell surface (Almeida et al., 2016)(Pearce et al., 2013). Moreover, CD28 co-stimulation was directly linked to upregulation of glycolytic pathway (Jacobs et al., 2008)(Frauwirth et al., 2002). T cell activation also leads to the induction of mammalian target of rapamycin (mTOR) pathways. mTOR is a serine/threonine kinase that can exist as part of two protein complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), both of which integrate different environmental and T cell activation signals to mount an appropriate metabolic and differentiation responses (Jones and Pearce, 2017). mTORC1, for example, induces the expression of HIF-1α transcription factor, which upregulates glycolytic pathways and glucose transporters. mTORC1 has been shown to be important for Th1 differentiation , while mTORC2 seems to be important for Th2 differentiation and survival (Almeida et al., 2016). Other anabolic pathways such as glutaminolysis are upregulated upon T cell activation as well.

A central regulator of metabolic processes, mTOR was shown to have potential for therapeautic intervention during HIV-1 infection. mTORC1 inhibitor rapamycin was shown to potentiate anti-viral effect of CCR5 antagonist vicriviroc (Heredia et al., 2008) and to inhibit viral particle production and viral transcription (Roy et al., 2002)(Rai et al., 2013). INK128 compound, which blocks the activity of both mTORC1 and mTORC2 was shown to inhibit entry, reverse transcription and integration of R5 but not X4 viruses and be effective at lowering viral load by several logs in a humanized mouse model (Heredia et al., 2015). Moreover, viral infection was identified to activate mTOR in a Tat dependent manner (Kumar et al., 2016). Additionally, mTOR was demonstrated to control HIV-1 latency through phosphorylation of p-TEFb subunit CDK9. Inhibition of mTOR by Torin1 and pp242 inhibited

latent HIV-1 reactivation and prevented Tat-dependent and Tat-independent HIV-1 transcription (Besnard et al., 2016).

In light of this knowledge, and the failure of “shock and kill” approaches to clear viral reservoirs, “block and lock” model now proposes to limit the reactivation of latent HIV-1 reservoir at least partially through inhibition of mTOR activity (Darcis et al., 2017b; Palmer et al., 2018). Alternatively, the “starve” model proposes limiting the metabolic activity of reservoir cells to limit their homeostatic proliferation (Palmer et al., 2018; Palmer and Crowe, 2016).

THE ROLE OF CELL SURVIVAL VS CELL DEATH IN HIV-1 INFECTION

Cell death clearly plays an important role in the pathogenesis of HIV-1 infection. Drastic depletion of CD4+ T cells is well documented both during acute and chronic stages of infection. However, precise molecular reasons for the depletion of CD4+ T cells during HIV-1 pathology are still poorely understood. Moreover, despite high levels of mortality in both infected and non-infected 'bystander' cell populations, the virus persists in surviving cells. Therefore, how some cells succumb to virus induced cytotoxicity while other survive remains an important open question in the field. Below, I present some of the general cell death pathways that are most relevant in the context of HIV-1 infection, and describe the current stage of knowledge of the role of the viral components in molecular pathways of cell death.

GENERAL OVERVIEW OF CELL DEATH MECHANISMS

Twelve forms of regulated cell death are currently recognized by the Nomenclature Committee on Cell Death (Galluzzi et al., 2018). Morphologically, cell death is classified into three forms. 1) Apoptosis exibits characteristic cell shrinkage, chromatin condensation, nuclear fragmentation, plasma membrane blebbing and the formation of apoptotic bodies (small vesicles). 2) Atophagy is characterized by extensive cytoplasmic vacuolization. 3) Necroptosis involves swelling of the mitochnodria and rupture of the plasma membrane. However, molecular pathways governing cell death processes are highly diverse and go beyond morphological characterization (Galluzzi et al., 2018). Here I review only three cell death pathways: apoptosis, pyroptosis and necroptosis - pathways most recognized in the context of HIV-1.

Apoptosis

Intrinsic apoptosis is intiated by various environmental perturbations such as growth factor withdrawal, DNA damage, endoplasmic reticulum stress, etc. BH3-only proteins are initiators of apototic pathway (Figure 13). Some of the examples of this family include BCL2 associated death promoter (BAD), BCL2 interacting mediator of cell death (BIM), p53-upregulated

mediator of apoptosis (PUMA), NOXA and truncated BH3-interacting domain death (tBID). They are induced either transcriptionally or post-translationally by cytotoxic stress signals. They either neutralize the pro-survival BCL2 family proteins (ex. MCL1, BLC2, BCL-W, etc) or directly activate apoptotic initiators BCL2 associated X protein (BAX) and BCL2 antagonist/killer (BAK) (Czabotar et al., 2014).

In healthy cells, BAK is a transmembrane protein residing in the outer mitochondrial membrane (OMM) and BAX shuttles between OMM and the cytoplasm. During activation of apoptotic program, BAX and BAK change their conformation to form homo-oligomers that form pores on the OMM, which mediates irreversible mitochondrial outer membrane permeabilization (MOMP). MOMP releases mitochondrial apoptogenic factors like cytochrome c and apoptosis-inducing factor (AIF) into the cytosol, which promotes activation of caspase 9, which, in turn, mediates activation of effector caspases (caspase 3, caspase 7 and caspase 6) (Czabotar et al., 2014)(Galluzzi et al., 2018). Effector caspases, in turn, mediate apoptotic such as DNA fagmentation, phosphatidylserine exposure and the formation of apoptotic bodies.

Extrinsic apoptosis is initiated when members of the tumor necrosis factor receptor (TNFR) family (ex. FAS or TNF receptor) are ligated (for example by FasL, TNF or TRAIL) at the plasma membrane. Ligation of the death receptors leads to the formation of multiprotein complex at the receptor's cytoplasmic tail callsed death-inducing singlaing complex (DISC). DISC operates like a molecular platform mediating the activation of caspase 8. Caspase 8 activates executioner caspases and proteolytic cleavage of Bid protein to generate truncated Bid (tBid), which engages the mitochondrial apoptotic pathway by activating BAK and BAX (Czabotar et al., 2014)(Galluzzi et al., 2018).

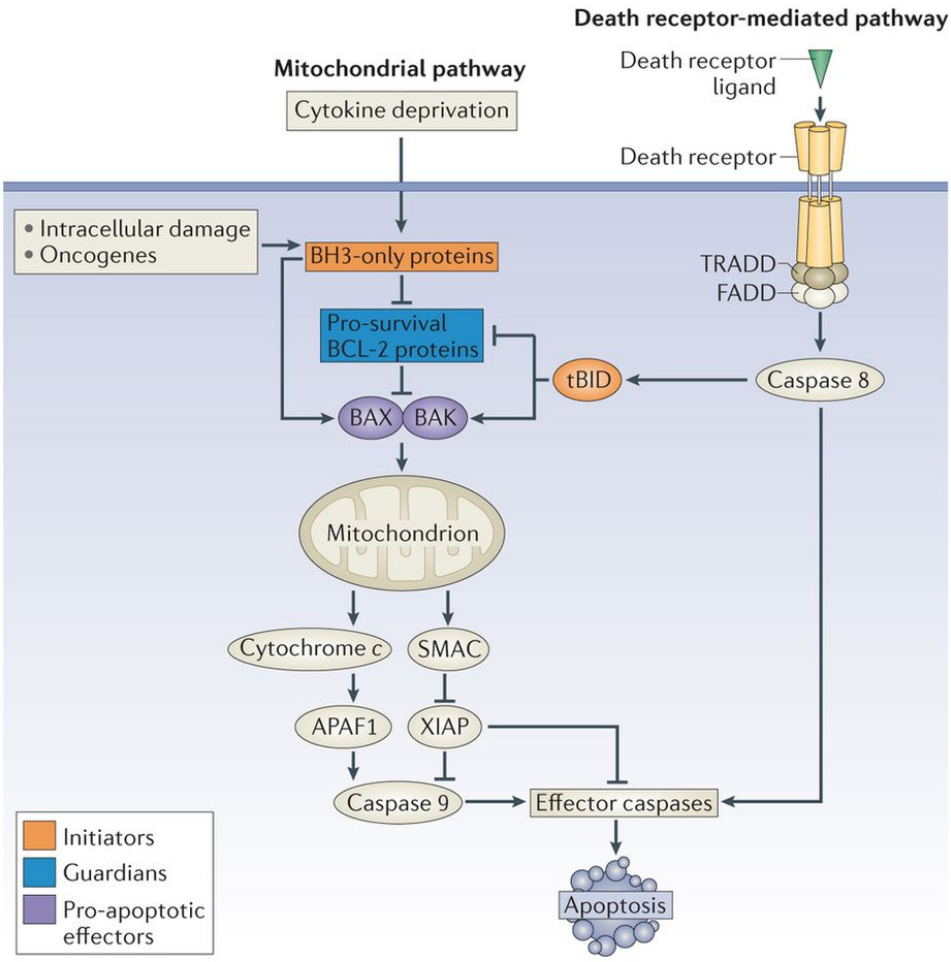

Figure 13. Basic apoptosis molecular pathways. Adapted from (Czabotar et al., 2014)

Pyroptosis

In contrast to apoptosis, pyroptosis is a highly immunogenic form of cell death that takes place in response to recognition of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) (such as K^+ efflux) by the pattern recognition receptors (PRRs) (Kunzelmann, 2016). PRRs then assemble into multiprotein signaling complexes, called inflammasomes, and recruit adaptor protein apoptosis-associated speck-like protein containing C-terminal CARD (ASC), which then recruits and activates caspase-1 or

8 via their CARD domain (Songane et al., 2018) (see below) (Figure 14). These caspases cleave IL-18 and IL-1β to their mature forms. They also cleave and activate gasdermin D, which translocates to the inner leaflet of the PM generating a pore that leads to the secretion of mature IL-18 and IL-1β, K^+ efflux (Gutierrez et al., 2017), PM permeabilization and cell lysis (Galluzzi et al., 2018)(Jorgensen and Miao, 2015). The rapid rupture of the PM releases the cytoplasmic contents into the environments. DNA damage accompanies pyroptosis, similarly to apoptosis. However, there is little DNA fragmentation and, altought chromatin condensation is observed, the nucleus remains intact (Table 2) (Jorgensen and Miao, 2015).

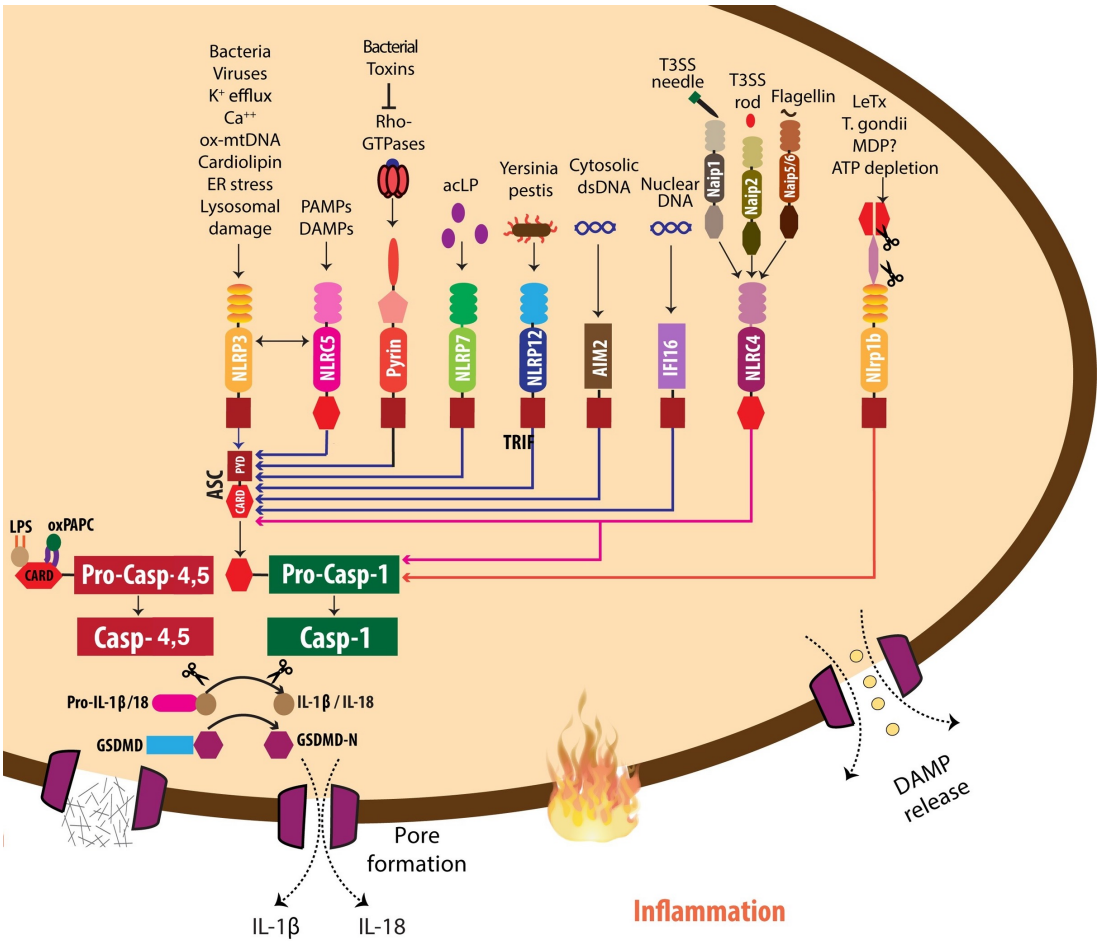

Figure 14. Diversity of inflammasomes and their known activating signals. Adapted from (Songane et al., 2018)

Table 2. Morphological and molecular determinants of pyroptosis vs apoptosis. Adapted from (Jorgensen and Miao, 2015)

Characteristic	Pyroptosis	Apoptosis
Inflammatory versus non-inflammatory	Inflammatory	Non-inflammatory
Lytic versus non-lytic	Lytic	Non-lytic
Initiator caspase	Caspase-1/4/5/11	Caspase-2, 8, 9, 10
Effector caspase	None	Caspase-3, 6, 7
DNA damage		
Laddering	No	Yes
TUNEL stain	Yes	Yes
ICAD cleavage	No	Yes
Chromatin condensation	Yes	Yes
Nucleus intact	Yes	No
Plasma membrane pore formation	Yes	No
PARP cleavage	No	Yes
AnnexinV staining	Yes	Yes

Necroptosis

Necroptosis is a regulated form of cell death that resembles necrosis. Therefore, morphological features of necroptosis are: cell swelling, rapid PM permeabilization, intact nuclei and the absence of caspase activation (Moreno-Gonzalez et al., 2016, p.)(Kunzelmann, 2016). Necroptosis can occur in response to signaling through the TNF receptor family, to triggering of PRR receptors and in response to IFN signaling (Figure 15). Key molecular events of necroptosis are association and autophosphorylation and transphorylation of kinases RIPK1 and RIPK3 and phosphorylation of MLKL (Tait et al., 2014) (Moreno-Gonzalez et al., 2016, p.)(Berghe et al., 2014). These events are accompanied by ROS production by the mitochondria (Berghe et al., 2014). Activated RIPK3 and MLKL are targeted to the PM (Moreno-Gonzalez et al., 2016, p.). MLKL oligomerizes and either form PM pores directly leading to Ca^{2+} influx-dependent cell lysis or engages ion channels leading to influx of Na^{+} or Ca^{2+} ions triggering cell death (Moreno-Gonzalez et al., 2016, p.)(Berghe et al., 2014).

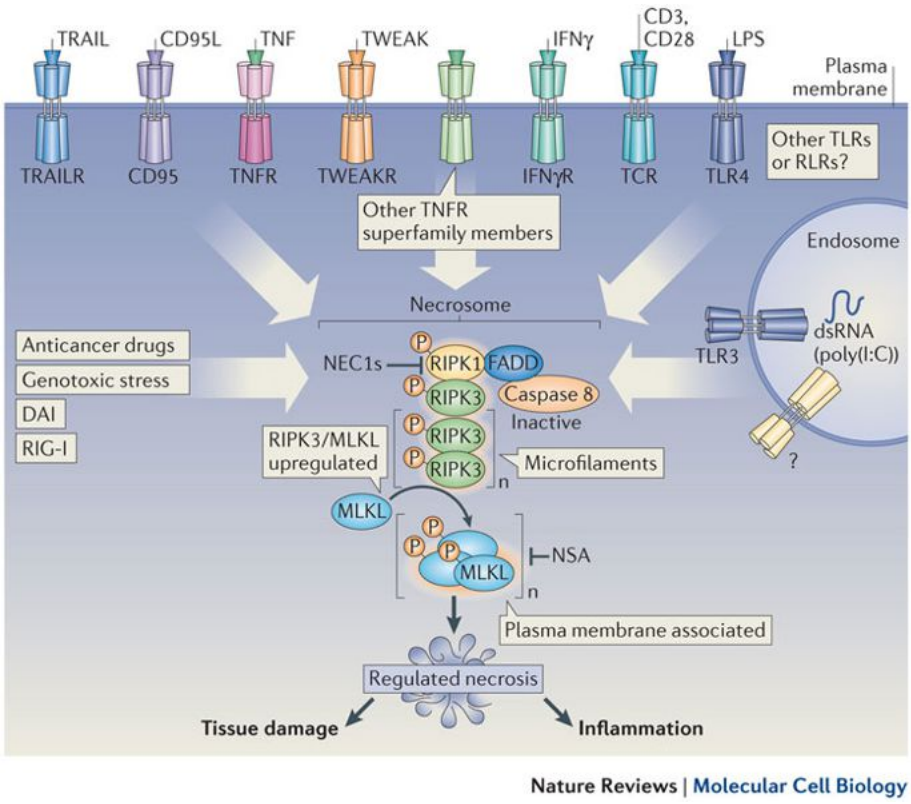

Figure 15. Triggers and central mechanism of necroptosis. Adapted from (Berghe et al., 2014)

MOLECULAR PLAYERS OF CELL DEATH

Caspases: initiators and effectors of cell death

Caspases are a cysteine-dependent aspartate-specific proteases. They are produced as inactive zymogens and are activated in response to inflammatory and death signals. All caspases contain a C-terminal effector protease domain and some (caspases 1, 2, 4, 5, 9, 12) also contain an N-terminal caspase activation and recruitment domain (CARD) (Figure 16). Caspases 8 and 10, on the other hand, contain death effector domain (DED) at the N-terminus. Caspases are generally classified into initiator (caspase-2, 8, 9, 10) and effector classes (caspase-3, 6, 7) (Table 2). Intiator caspases are able to auto-activate and mediate, via cleavage, the activation of effector caspases, which then activate mediators of apoptosis (Man and Kanneganti, 2016).

Zymogens of caspases 8 and 10 are inert monomers, which are recruited to the high molecular weight oligomeric molecular complexes such as death-inducing signaling complex (DISC) via their DED domain, where they are activated by dimerization and auto-cleavage (Songane et al., 2018). Similar mechanism is thought to operate for the activation of CARD-containing caspases. These caspases are also recruited into oligomeric protein complex via the interaction of their CARD domain with the CARD domain of the recruitment proteins. For example, caspase-9 is recruited via its CARD to interact with the CARD of Apaf-1 polymers in the apoptosome (Figure 17) (Ramirez and Salvesen, 2018). Induced proximinty of caspase-9 monomers results in their dimerization and auto-cleavage between large and small subunits of the catalytic domain, which results in its activation. Caspase-1 is activated by its recruitment via the CARD domain to the inflammasome macromolecular complex. Similarly, caspase-2 interacts via its CARD domain with the CARD of RAIDD protein in the PIDDosome (Tinel and Tschopp, 2004) (Figure 18). Caspases 4 and 5 oligomerize and auto-activate via the CARD domain upon binding to bacterial lipopolysaccharide (LPS) (Ramirez and Salvesen, 2018). Zymogens of the effector caspases-3, 6 and 7 are dimers and their activity is inhibited by a linker separating small and large subunits of the catalytic domain (Figure 16). Activation of these caspases proceeds via their proteolytic cleavage by the initiator caspases through the cleavage of the linker to separate large and small subunits of the catalytic domain, which then assemble for full activity (Shi, 2004).

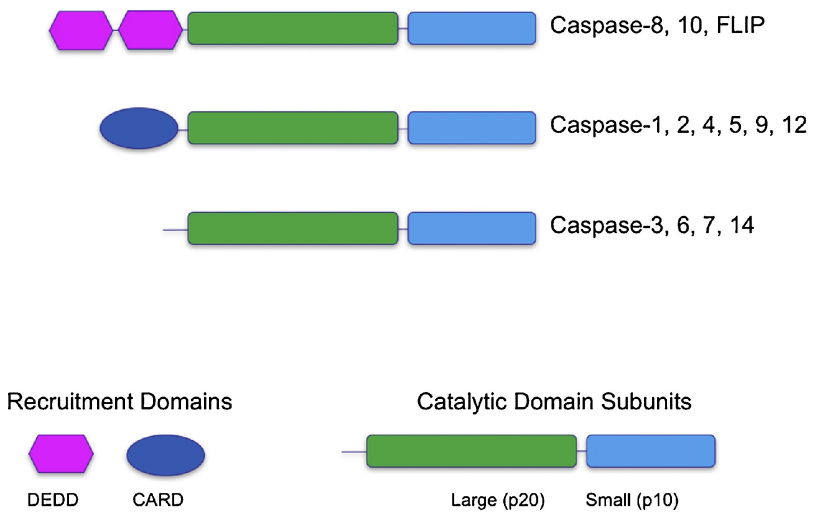

Figure 16. Functional domains of caspases. Adapted from (Ramirez and Salvesen, 2018).

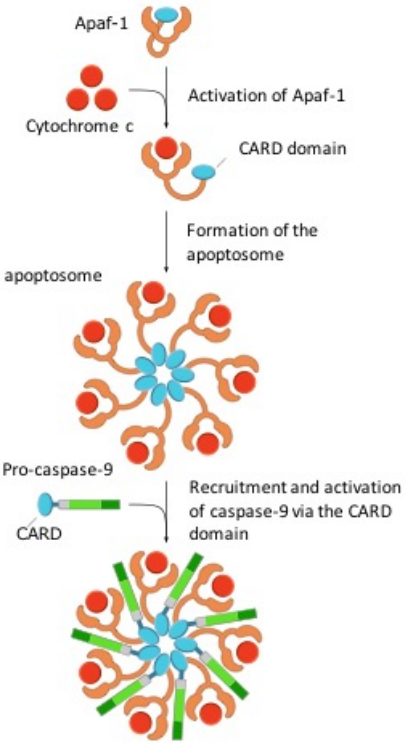

Figure 17. Recruitment and activation of caspase-9 via the CARD domain in the apoptosome. Adapted from Wikipedia Commons.

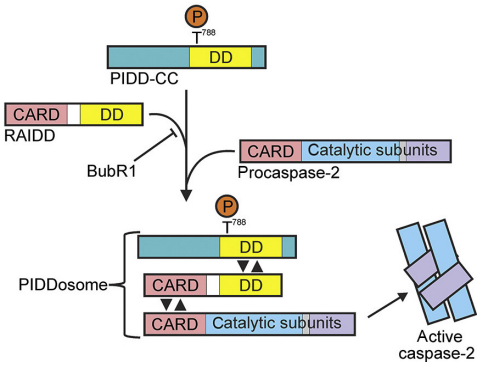

Figure 18. Activation of caspase 2 in the PIDDosome. Adapted from (Miles et al., 2017)

The function of each caspase seems to be multidimentional albeight being not well characterized. Caspases-2, 3 and 6-10 are thought to activate apoptosis death pathways. Caspase-1 is thought to be activated primarily in the association with inflammasomes. This caspase mediates cleavage of IL-1β and IL-18 cytokines into their mature forms, although caspase-1 independent cleavage of these cytokines via is now known (Man and Kanneganti, 2016). Caspases-4 and 5 are also implicated in the non-classical inflammasome activation and intitiation of pyroptosis pathway (Man and Kanneganti, 2016) by direct recognition of microbial products, activation and cleavage of IL-1β and Gasdermin D (Songane et al., 2018). Caspase-2 was also recently reported to be activated downstream of the inflammasome (Bronner et al., 2015) although it is most known to be activated in response to DNA damage to mediate apoptosis or cell cycle arrest (Olsson et al., 2015). Caspase-8, together with caspase-10 initiates extrinsic apoptosis pathway (Songane et al., 2018). It is implicated in the activation of the NFkB signaling patwhay and, similarly to caspases 4 an 5, the cleaveage of IL-1β and IL-18. It has also been reported to block the inflammasome activity. Caspase-12 function in humans remains enigmatic because of high prevalence of inactive short form of caspase-12 in human population (Man and Kanneganti, 2016). So far, the active form of this caspase has been proposed to limit the activation of the inflammasome and caspase-1. Additionally, caspase-14, most enigmatic of all, so far has not been implicated in inflammasome activation or apoptosis and seems to be specialized in keratinization of the skin (Ramirez and Salvesen, 2018).

The role of ion channels in cell death

The cell tightly controls the intracellular levels of mono- and divalent ions (Figure 19). During health, Ca^{2+}, Na^{+}, K^{+}, Cl^{-} and other ions regulate variety of cellular processes. Ca^{2+}, for example, signals T cell activation, K^{+} and Cl^{-} regulate cell volume. It is not surprising that perturbation of intracellular ion homeostasis, therefore, was shown to accompay the process of cell death. Intracellular Ca^{2+} homeostasis is controlled by Ca^{2+} channels and pumps located in the plasma membrane (PM) and in the membranes of various organelles (Kondratskyi et al., 2015) (Kunzelmann, 2016). Ca^{2+} overload of the cytoplasm stimulates mitochondrial Ca^{2+} uptake which triggers opening of mitochondrial permeability transition pore (MPTP), activating

intrinsic apoptotic pathway. Cytoplasmic Ca^{2+} can also activate Ca^{2+}-dependent cystein proteases, caplains, which cleave BID, BCL-2 and BCL-XL and trigger MOMP.

Cellular K^+ and Na^+ homeostasis are directly linked. While the cell ensures high intracellular K^+ concentration (around 130mM), Na^+ is continuously exported to results in 15mM intracellular concentration. This is achieved by the action of Na^+/K^+ ATPase. In addition, multiple Na^+ channels (similarly to Ca^{2+} channels) mediate rapid intracellular Na^+ increase during membrane depolarization. An increase in intracellular Na^+ occurs early in apoptosis (Kondratskyi et al., 2015). However, how Na^+ is involved in molecular pathways of cell death is poorly understood.

Apoptotic volume decrease (AVD) is a hallmark and the initial step of apoptosis and is caused by K^+ and Cl^- efflux. K^+ leaves the cell through the activation of K^+ channels (ex. Voltage-gated Kv, ATP-regulated K_{ATP}, Ca^{2+}-dependent K^+ channels, etc) (Kunzelmann, 2016). Similarly, Cl^- leaves the cell through Cl^- channels (voltage gated, Ca^{2+}-activated, volume regulated anion, ligand gated and other channels). Loss of intracellular K^+ is thought to favor activation of caspases and nucleases (Kondratskyi et al., 2015), although it was found not to be obligatory for apoptosis to take place (Börjesson et al., 2011). Moreover, increase in extracellular K^+ and Cl^- concentration was reported to inhibit apoptosis (G. J. Thompson et al., 2001).

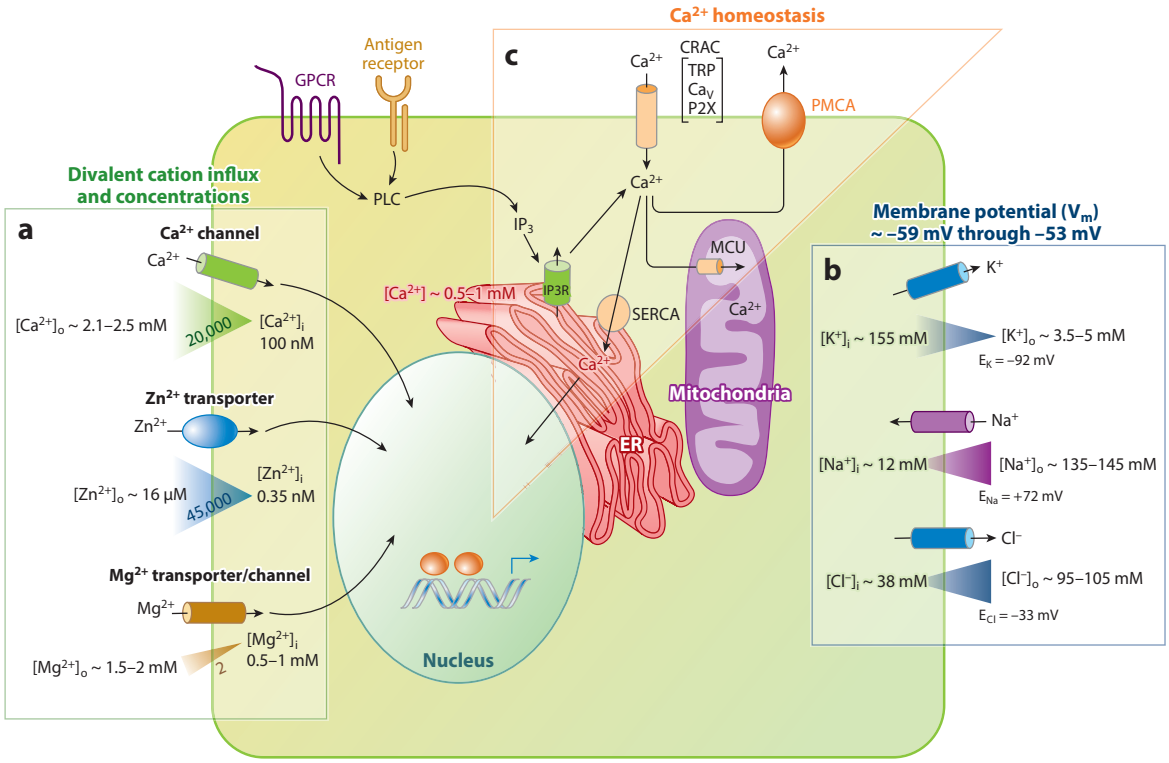

Figure 19. Intracellular homeostasis of various ions. Adapted from (Feske et al., 2015a)

CELL DEATH IN HIV-1 INFECTION

The hallmark of HIV-1 infection is the depletion of CD4+ T cells. Hence, the dogma in the field was, until recently, that the virus promotes cell death of infected cells. Indeed, this may be the case during acute phase of infection, when rapid viral replication leads to massive CD4+ T cell depletion. During chronic infection, however, the contribution of direct viral cytotoxicity to cell death is limited as the percentage of cells actively producing the virus is lower (Finzi et al., 1997)(Embretson et al., 1993). This also supported by the lack of appreciable effect of latency reversal agents, that have not been shown to eliminate infected cells due to virus reactivation (Lehrman et al., 2005; Rasmussen et al., 2014; Rasmussen and Lewin, 2016; Routy et al., 2012). Additionally, apoptosis has been shown to occur predominantly in 'bystander' cells in lymphoid tissues and not in productively infected cells (Finkel et al., 1995). Viral persistance in the infected cell that survive even during cART tells us that the cell's life/death balance during HIV-1 infection may be more complex than once appreciated.

Overall, it seems that the activation status of cells plays a role in the susceptibility to cell death during HIV infection. Like so, highly activated effector memory CD4+ T cells are depleted fastest and first from gut mucosal sites (Grossman et al., 2006) and naïve T cells displaying resting phenotype are resistant to depletion in lymphoid tissues (Veazey et al., 2000). Additionally, it was shown that blood-derived CD4+ T cell that display deeper resting state than lymphoid tissue derived cells are more resistant to pyroptosis despite carrying viral genetic material (Muñoz-Arias et al., 2015). Thus, infected naïve, central memory and stem cell like memory T cells displaying less activated phenotype could be less prone to these mechanisms of virally induced cell death than highly activated effector memory T cells, and thus presist longest hence supplying the reservoirs (Olvera-García et al., 2016)(Buzon et al., 2014).

On the molecular level, multiple mechanisms have been described to either favour death or survival of infected cells but how exactly the virus modulates these is unknown. *In vitro* activated, infected CD4+ T cells have been reported to undergo integration-dependent cell death, which has been liked to the recruitment of DNA-PK (Cooper et al., 2013a). Activated productively infected T cells have also been shown to express activated capsase-3 (Doitsh et al., 2010) and undergo caspase-3 mediated cell death (Jekle et al., 2003). On the other hand, death of non-productively infected CD4+ T cells have been reported to result from the detection of viral reverse transcription products by DNA sensor IFI16, which leads to caspase-1 activation thereby triggering pyroptosis (Doitsh et al., 2014, 2010; Monroe et al., 2014, p. 2).

Viral proteins could have a dual role in modulating pro- and anti-survival signals. Productively infected cells were shown to be protected from Fas-mediated apoptosis by expression of Nef protein, which inhibits Fas signaling (Geleziunas et al., 2001)(Ohnimus et al., 1997). Nef was also shown to inactivate pro-apoptotic BAD protein hence rendering infected T cells more resistant to apoptosis (Wolf et al., 2001a). Env was observed to induce resistance to TRAIL-

induced apoptosis in macrophages (Swingler et al., 2007). On the other hand, viral proteins Tat (Westendorp et al., 1995), Env (Cicala et al., 2000), Vpr (Muthumani et al., 2002) and Nef (Muthumani et al., 2005; Xu et al., 1999) were shown to have an apoptotic effect *in vitro*. Thus, how the virus modulates cell death pathways remains an open question.

HYPOTHESIS AND OBJECTIVES

CONTEXT

In recent years, the lab of Dr. Saez-Cirion focused part of its efforts in understanding how the HIV virus establishes productive infection in CD4+ T cells as well as macrophages and DCs. One of the important questions that was adressed was how does intracellular environment of different CD4+ T cell memory subsets, which are known to contribute differently to the HIV reservoir, dictate their relative susceptibility to infection. Naïve CD4+ T cells are known to be resistant to the establishment of productive viral cycle, while memory subsets are permissive. It is well established that the activation plays a key role in cellular permissiveness to infection. Additionally, the role of CD4+ T cell metabolism is now beginning to be appreciated. Therefore, JC Valle-Casuso et al investigated activation and metabolic factors that could play a role in the establishment of productive infection in four CD4+ memory subsets: Tn, Cm, Tm and Em. The expression of metabolism-related genes was correlated to the degree of infection seen in different CD4+ T cell subsets. This work (see Annex 2) clearly showed the dependence of the viral replication on metabolic activity, especially glycolysis (but also OXPHOS), of each cell subset. The relative contribution of each memory subset (as compared to non-activated cells) directly mimicked the metabolic profile of that cell subset (Figure 20). This work further showed that the virus preferentially infects metabolically active cells within each subset and this was independent of cell activation and differentiation status (Figure 21A). Finally, inhibition of glycolysis by glucose starvation of glucose analog 2-deoxyglucose (2 DG) reduced viral infection via suppression of reverse transcription (Figure 21B).

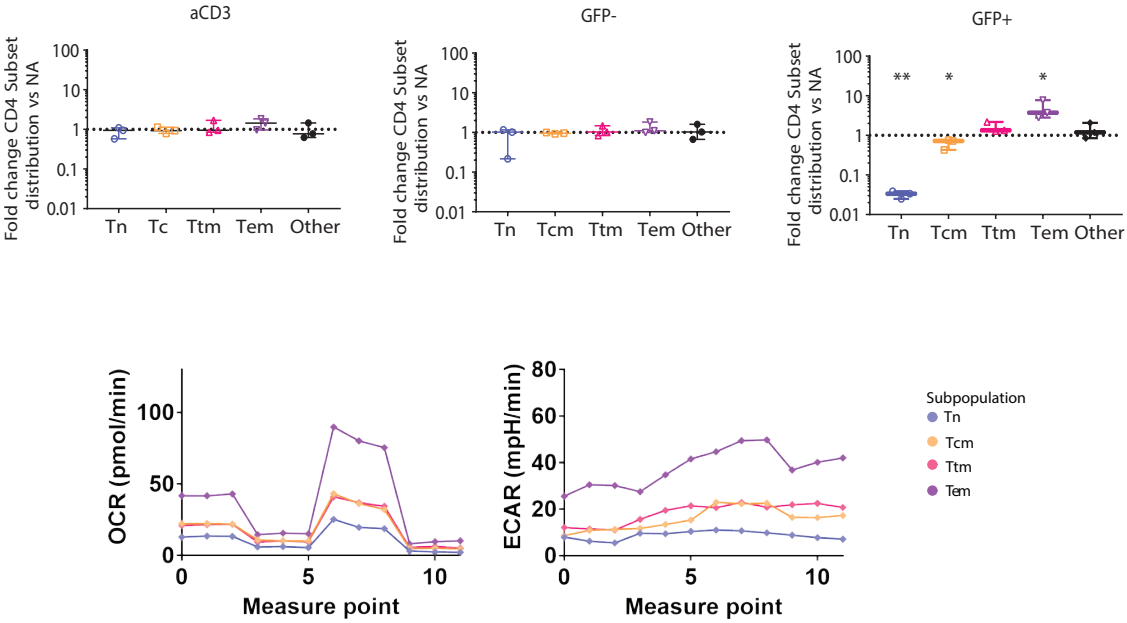

Figure 20. Relative contribution of CD4+ T cell memory subsets to infected cell pool and their respective metabolic profiles. Adapted from Valle-Casuso, submitted (Annex 2)

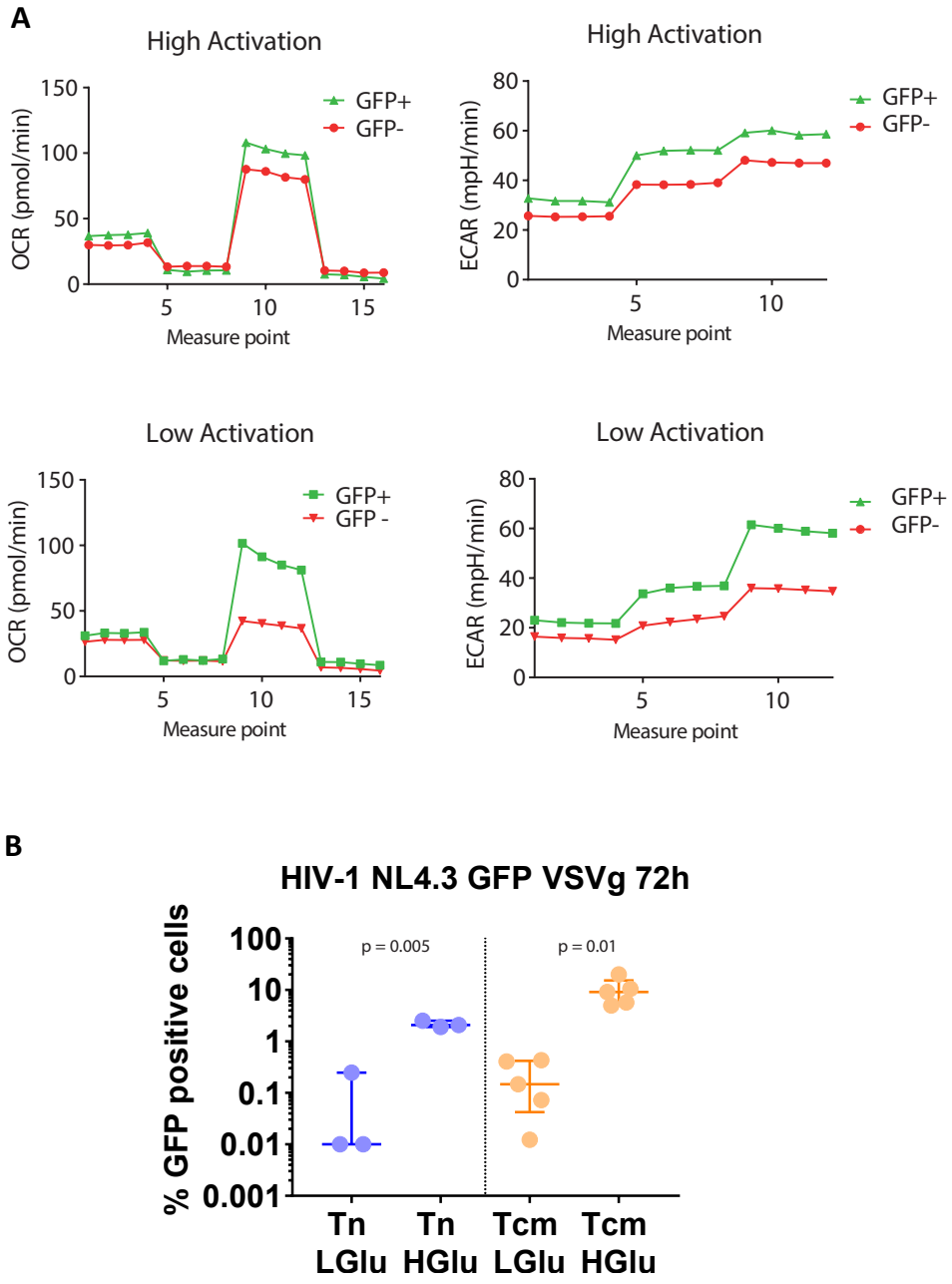

Figure 21. HIV-1 preferentially infects metabolically active cells irrespective of their activation status and differentiation stage. A) HIV-1 infected cells (GFP+) display higher metabolic activity (maximal respiratory capacity) and glycolysis than exposed non-infected (GFP-) cells. B) HIV-1 infected cells are preferentially contained within high glucose consuming cell population (HGlu) vs within low glucose consuming cells (LGlu)

This work indentified a clear relationship between the ability of different CD4+ T cell memory subsets to be infected and their metabolic status. Yet another important question is how HIV-1 susceptible cells survive *in vitro* cytopathic effect of the virus and other cell death-triggering mechanisms operating *in vivo* to establish productive viral infection leading to the formation of the reservoir? CD4+ T cell subsets vary in their half-life *in vivo* (Gattinoni et al., 2011)(Lugli et al., 2013)(Mahnke et al., 2013) and it is interesting that HIV-1 persists in permissive subsets that display long-term survival such as Cm cells (Olvera-García et al., 2016). We thus wondered if the intrinsic anti-apoptotic pathways expressed by CD4+ memory T cells (Gattinoni et al., 2011)(Lugli et al., 2013)(Mahnke et al., 2013) could be responsible for the survival of these cells upon infection leading to their dominant contribution to HIV-1 reservoirs (Chomont et al., 2009b). If so, what would be these pathways and is it possible to counteract them to prevent survival of HIV-1 target cells?

JC Valle-Casuso et al analyzed gene expression of factors implicated in activation, metabolism and cell death and correlated their expression to the proportion of infection of CD4+ T cells. Among factors implicated in cell death pathways (ex. FAS, E2F1, IFI16, CASP3, MTOR, CASP8, BCL11B, IL17R), AAC-11 expression increased with the state of differentiation of memory CD4+ T cell subsets (Cm<Tm<Em) and correlated significantly with infection in each subset (Figure 22A and B).

AAC-11 attracted our attention due to its described role in the protection of cancer cells against apoptosis in the conditions of metabolic stress. HIV-1 targets highly glycolytic cells for productive infection (Loisel-Meyer et al., 2012b)(Hegedus et al., 2014b; Hollenbaugh et al., 2011b)(Palmer and Crowe, 2016)(Valle-Casuso, submitted; see Annex 2), as these cells provide the right combination of metabolic substrates and cell factors for the progression of its life cycle. At the same time, long-term persistance of HIV-1 infected cells *in vivo* indicates that HIV-1 could also be targeting cells with upregulated survival pathways, as was already shown for Cm cells (Olvera-García et al., 2016). These two aspects parallel the hallmarks of cancer cells, which are known to be, on one hand, high level of glycolysis (Warburg effect), and on the other hand, resistance to apoptosis.

AAC-11 was first identified in mice and was shown to protect cells from apoptosis in serum deprivation grown conditions (Tewari et al., 1997). It is overexpressed in many cancers (Koci et al., 2012) and has been shown to support cancer cell survival in the conditions of growth factor withdrawal (Tewari et al., 1997). Its expression is associated with poor prognosis in non-small lung and cervical cancers (Cho et al., 2014; Sasaki et al., 2001; Wang et al., 2010). AAC-11 contains several protein interaction domains, including leucine zipper (LZ) domain (Han et al., 2012) and was shown to inhibit apoptotic effectors such as E2F1 (Morris et al., 2006), Acinus (Rigou et al., 2009) and caspase-2 (Imre et al., 2017). AAC-11 prevents apoptotic Acinus-mediated DNA fragmentation by interacting with it via its LZ domain (Rigou et al., 2009). It has also been reported to inhibit caspase-2 activity via its direct binding to caspase recruitment domain (CARD) and prevention of caspase-2 dimerization and activation (Imre et al., 2017). Given AAC-11's anti-apoptotic function, we wondered if HIV-1 targets cells that rely on AAC-11 pathway for survival.

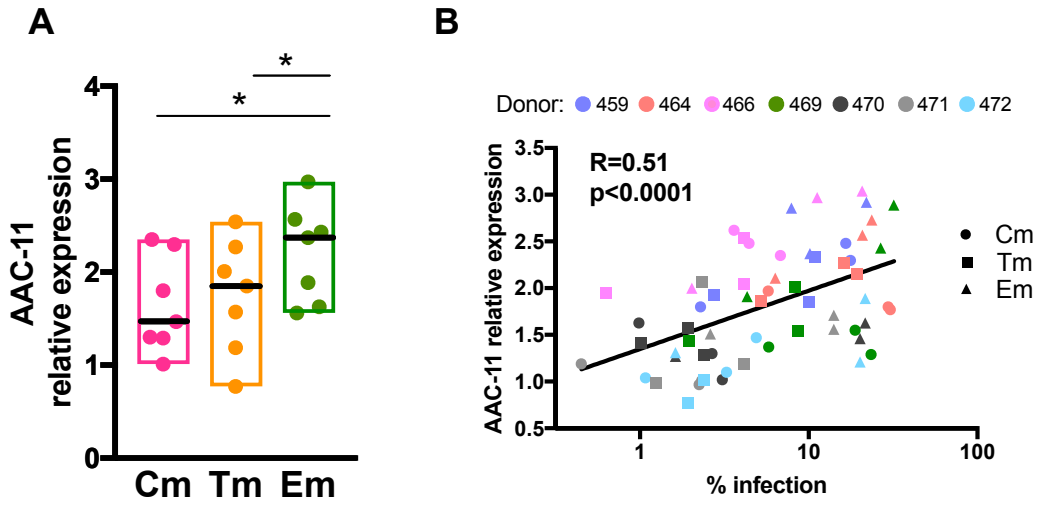

Figure 22. AAC-11 gene expression in CD4+ T cell memory subsets and its correlation with infection. A. AAC-11 gene expression increases with CD4+ T cell memory differentiation (Cm<Tm<Em) B. Correlation of AAC-11 gene expression at the time of infection in different CD4+ T cell memory subpopulations with the percent infected cell in that subset at day 3 of infection.

HYPOTHESIS

HIV-1 preferentially targets cells programmed for survival via the high expression of AAC-11. This factor, therefore, could represent a target of vulnerability of HIV infection, which can be exploited by counteracting its anti-apoptotic activity to selectively eliminate cells supporting the establishment of HIV-1 infection.

APPROACH

In order to counteract AAC-11 activity we used a panel of peptides designed and kindly provided to us by the team of Dr. Jean-Luc Poyet at Hôpital Saint Louis. The design of these peptides was based on the LZ domain of AAC-11 fused to penetratin peptide of *Drosophila melanogaster* (here designated as RK16) (Figure 23). Penetratin was shown to spontaneously cross cellular membranes and is used as an intracellular delivery method for various molecules (Derossi et al., 1998). AAC-11-derived peptides were designed to competitively inhibit AAC-11 interaction with its partners via its LZ domain (Jagot-Lacoussiere 2016). Dr. Poyet's team already successfully used RT53, the peptide that spans the complete length of the LZ domain and is fused to penetratin (Figure 23), to antagonize the survival of cancer cells both *in vitro* and *in vivo* in a murine model of melanoma (Jagot-Lacoussiere et al., 2016).

We thus tested if AAC-11 derived peptides would counteract the survival of HIV-1 target cells. To this end, we treated infected and non-infected cells with these peptides and determined their effect on the survival of HIV-1 infected cells.

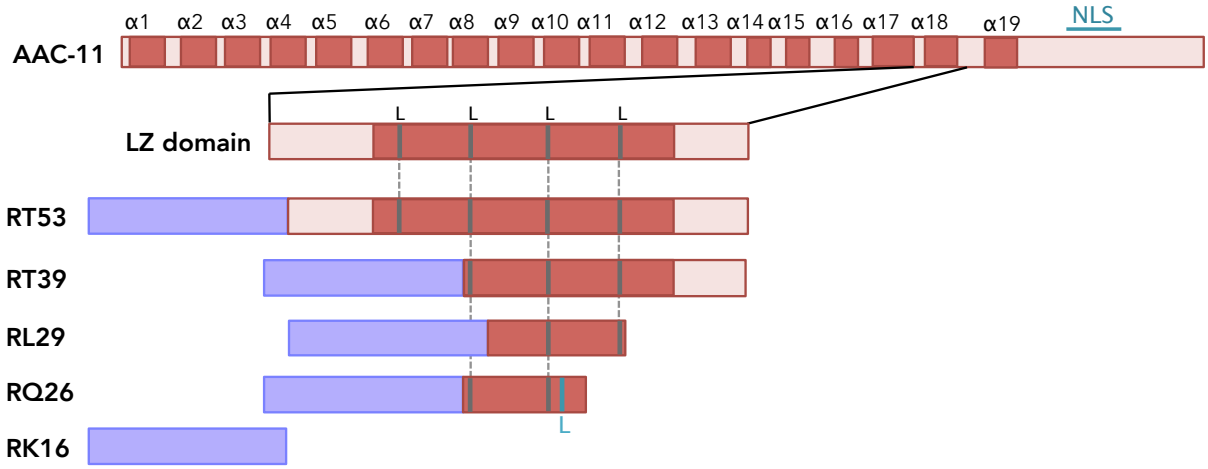

Figure 23. Schematic of AAC-11 derived peptides.

OBJECTIVES

1. To determine if AAC-11-derived peptides are effective at eliminating cells susceptible to productive HIV-1 infection or inhibiting infection
2. To determine how the viral life cycle is impacted
3. To establish the characteristics of the cell subset affected by AAC-11-derived peptides
4. To determine the mechanism of action of AAC-11-derived peptides

CHAPTER 1:

AAC-11 DERIVED PEPTIDES INDUCE DEATH OF CD4+ T CELLS SUSCEPTIBLE TO HIV-1 INFECTION

Primary article, submitted

AAC11 derived peptides induce death of CD4+ T cells susceptible to HIV-1 infection

Anastassia Mikhailova[1,4], Annie David[1], José Carlos Valle-Casuso[1], Valérie Monceaux[1], Stevenn Volant[2], Caroline Passaes[1], Michaela Müller-Trutwin[1,4], Jean-Luc Poyet[3], Asier Sáez-Cirión[1,4]

[1]Institut Pasteur, Unité HIV Inflammation et Persistance, Paris, France

[2]Institut Pasteur, Hub Bioinformatique et Biostatistique – C3BI, USR 3756 IP CNRS – Paris, France

[3]INSERM UMRS1160, Institut Universitaire d'Hématologie, Hôpital Saint-Louis, Paris, France.

[4]Université Paris Diderot, Sorbonne Paris Cité, Paris, France

Corresponding Author:

Asier Sáez-Cirión: Unité HIV Inflammation et Persistance, Institut Pasteur, 28 rue du Docteur Roux, 75015 Paris, France. Tel: 33-145-688-768. Fax: 33-145-688-957

Email: asier.saez-cirion@pasteur.fr

ABSTRACT

Despite viral cytotoxic effect classically associated with *in vitro* infection and massive depletion of viral target cells during infection *in vivo*, HIV-1 successfully establishes infection and persists in its reservoirs. However, how productively infected cell survive and persist remains to be understood. Here we propose that HIV-1 relies on Anti-apoptotic clone 11 (AAC11) anti-apoptotic pathway for the establishment of productive infection in CD4+ T cells. We observed the expression of AAC11 to increase with progressive CD4+ T cell memory differentiation in association with the expression of cell cycle, activation and metabolism genes and to correlate with susceptibility to HIV infection. Antagonism of AAC11 survival pathway with peptides derived from its sequence lead to cell death of HIV-1 susceptible target cells and selection of cells resistant to infection. The peptides caused preferential elimination of activated and metabolically active cells, in particular effector and transitional CD4+ T cell memory subsets, and their activity was at least in part dependent on caspase 2 activation. Thus, our results provide a proof of concept that the selective targeting of the survival pathways used by HIV-1 to persist is a possible approach to antagonize seeding of HIV-1 CD4+ T cell reservoirs.

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) is a persistent viral infection that has claimed millions of lives around the globe. The era of combination anti-retroviral therapy (cART) has extended life expectancy of people living with HIV and improved their quality of life. However, persistence of viral reservoirs remains a major challenge as they are at the origin of viral rebound if therapy is interrupted (Davey et al., 1999; García et al., 1999; Harrigana et al., 1999). Therefore, recent efforts of the scientific community have been focused on the precise characterization of HIV-1 reservoirs in the hope of designing new treatment strategies for their prevention and/or elimination.

Memory CD4+ T cell subsets are thought to be the major contributor to the persistent HIV-1 reservoir under cART (Barton et al., 2016). Among these, central memory CD4+ T cells were shown to be important due to their self-renewal ability and long-term survival, while transitional and effector memory cells were shown to sustain the reservoir by homeostatic proliferation (Chomont, Nat Med, 2009) (Heiner, 2017). The seeding of the HIV reservoir in different CD4+ T cell subsets is first determined by their relative susceptibility to HIV-1 infection. Naïve CD4+ T cells are relatively resistant to infection and susceptibility to HIV increases with T cell differentiation and activation. We have recently described how the infectability of CD4+ T cell subsets is related to their metabolic activity (Valle-Casuso, submitted). Numerous reports have shown that immunometabolism is a critical element in the regulation of T cell differentiation, survival and function (Almeida et al., 2016). Upon antigenic stimulation T cells upregulate metabolic fluxes to provide the energy necessary to support cellular processes and to increase the pool of substrates necessary for building proteins, lipids, nucleic acids and carbohydrates. We and others have shown that this metabolically rich environment is necessary for the establishment of both productive and latent HIV infection (Valle-Casuso, submitted) (Hegedus et al., 2014; Hollenbaugh et al., 2011), as it is also the case for other infections (Heaton et al., 2010; Manel et al., 2003; McArdle et al., 2012).

The survival of infected cells, including to the cytopathogenic effects of the virus, is another critical characteristic for the persistence of the HIV reservoir that is not well understood. In our previous study (Valle Casuso, submitted) we found that, together with several other genes associated with cell metabolism, the anti-apoptosis clone 11 (AAC11) [also known as apoptosis inhibitor 5, API5] was significantly correlated with infection in different subsets of memory CD4+ T cells. AAC11 is overexpressed in many cancers (Koci, 2012) and allows cancer cell survival in conditions of metabolic stress (Kim et al., 2000). Its expression is associated with poor prognosis in non-small lung and cervical cancers (Wang, 2010; Sasaki, 2001; Cho, 2014). Although the mechanisms associated with its antiapoptotic activity have not been clearly elucidated, AAC11 contains several protein interaction

65 domains, including a leucine zipper (LZ) domain (Han, 2012), and has been proposed to repress
66 apoptotic effectors such as E2F1 (Morris, 2006), Acinus (Rigou, 2009) and caspase-2 (Imre, 2017).
67 We hypothesized that, similarly to its role in cancer, the antiapoptotic activity of AAC11 could
68 contribute to the survival of metabolically active cells that are preferential targets of HIV, hence
69 representing a potential target of vulnerability in infection.
70
71 In order to antagonize the activity of AAC11 we used a panel of synthetic peptides based on the LZ
72 domain sequence of AAC11 to impair the interaction of AAC11 with potential pro-apoptotic
73 partners. Some of these peptides were previously shown to be cytotoxic to cancer cells both *in vitro*
74 and *in vivo* in a mouse model of melanoma (Jagot-Lacoussiere, 2016). We found that AAC11
75 peptides blocked HIV infection through the preferential killing of HIV-1 'infectable' cells thus serving
76 as a proof of concept that selective elimination of HIV-1 targets is a possible therapeutic strategy in
77 tackling infection of CD4+ T cells.
78
79

RESULTS

AAC11 derived peptides display anti-viral activity against diverse HIV-1 viral strains and SIV

We previously analyzed the susceptibility of memory CD4+ T cell subpopulations (central (Cm), transitional (Tm) and effector (Em) memory) to infection with single cycle HIV NL4.3 Δ Nef Δ env GFP particles pseudotyped with VSVG envelope. We also analyzed the expression, at the time of infection, of a panel of 96 genes related to T cell differentiation, function and survival as well as restriction and HIV facilitating factors (Valle-Casuso, submitted). Among other genes, we found the expression of anti-apoptotic factor AAC11 to increase progressively with the stage of differentiation of memory CD4+ T cells (Cm<Tm<Em) and to correlate with infection in these subsets (Fig 1A,B). The expression of AAC11 was strongly correlated with the expression of multiple genes related to cell metabolism (e.g. SLC1A5, MTOR, HIF1A, GUSB, GAPDH), cell cycle (e.g. RHOA, RB1, MAPK1, MKI67) and genes well known to be necessary for different steps of the HIV replication cycle (RRM2, SAMRCB1, DDB1, CFL1, ACTB, CDK9, NFKB1) (Fig 1C). We hypothesized that the AAC11 antiapoptotic pathway is upregulated in highly metabolically active cells, which offer an optimal environment for HIV replication (Valle-Casuso, submitted), and may facilitate HIV persistence by allowing the survival of infected cells.

To explore this possibility we sought to antagonize the action of AAC11 using a panel of synthetic peptides derived from the LZ domain located in the alpha helix 18 of AAC11 (Han, 2012) fused to a cell penetrating sequence of antennapedia protein of *Drosophila melanogaster* [also commonly known as penetratin] (RK16 here) (Derossi, 1998) (Fig 2A). These peptides act as competitive inhibitors of protein-protein interactions, can prevent AAC11 antiapoptotic activities and have shown anti-tumor activities *in vitro* and *in vivo* (Jagot-Lacoussiere, 2016). We applied these peptides to activated primary human CD4+ T cells (Sáez-Cirión et al., 2011) after spinoculation with HIV-1 and measured infection 3 days later by flow cytometry (Fig 2B). AAC11-derived peptides reduced HIV infection to various degrees in a concentration-dependent manner (Fig 2C). Shorter peptides,

106 constructed from progressively smaller regions of AAC11 LZ domain were less effective at inhibiting
107 infection (Fig 2C, D). As expected, RK16 did not show any anti-viral effect even at the highest
108 concentration tested. We observed a rapid drop in the proportion of infected cells at 6μM peptide
109 concentration for RT53 and RT39, peptides exhibiting the strongest anti-viral effect. At this
110 concentration, RT53 was most potent at suppressing infection (Fig 2D), indicating that the entire
111 length of the LZ domain of AAC11 was necessary for full peptide potency.
112
113 Moreover, we saw that RT53 was able to inhibit infection of diverse HIV-1 isolates (Fig 3). Both R5
114 (Bal) and X4 (NL4.3) viruses as well as primary isolates (BX08, DH12, 132w) and single cycle VSVG-
115 pseudotyped HIV-1 particles were inhibited, albeit less efficiently for the latter. RT53 was also able
116 to inhibit *in vitro* infection of human CD4+ T cells with simian immunodeficiency virus SIV_{mac251} and
117 viral spread from splenic CD4+ T cells of Cynomologus macaques previously infected with SIV_{mac251}.
118 This is coherent with the high conservation of AAC11 across species (Morris et al., 2006) and 100%
119 homology between humans and macaques. In contrast, RK16 alone had no inhibitory effect. Overall,
120 our results show that AAC11 derived peptides were able to impair HIV-1 and SIV infection of CD4+ T
121 cells *in vitro* or spread from *in vivo* infected CD4+ T cells.
122
123 **Blockage of HIV infection by AAC11-derived peptides is associated with cell death**
124 Since our goal was to use the peptides to antagonize the anti-apoptotic activity of AAC11, we next
125 evaluated the amount of cell death in our cultures. Indeed, we saw that all peptides caused various
126 rates of cell death (Fig S1A). Moreover, we found that the proportion of cell death caused by
127 different peptides in different donors was negatively correlated with the proportion of HIV infected
128 cells in the cultures (Fig S1B). As before, RT53 was most potent at causing cell death. Interestingly,
129 RT53 induced similar death rates in cells infected with various HIV-1 strains and in non-infected cells
130 (Fig 3B). This suggested that the peptides caused cytotoxicity of cells with particular intrinsic anti-
131 apoptotic program that were also targeted by HIV for infection. Overall, these results supported the

idea that HIV-1 preferentially infected cells that rely on AAC11-survival pathway. Hence, antagonizing this survival pathway may lead to selective elimination of HIV-1 targets.

Cell death induced by AAC11-derived peptides is associated with the activation of caspase 2

We then decided to investigate the mechanism of RT53-mediated effect. We started by evaluating the kinetics of RT53 action. We used "real time" flow cytometry to read out changes in several molecular indicators associated with cell death as a function of time (Elliott et al., 2006) (Fig 4A). We found that treatment of activated CD4+ T cells with RT53 induced a sharp and rapid increase in the surface levels of phosphatidylserine (PS), as determined by binding to annexin V-FITC. In a subset of RT53-treated CD4+ T cells, we also observed a decrease in the intracellular levels of K^+ ion, the efflux of which has been linked to the activation of the cell death machinery (Bortner JBC 1997; McCarthy Cell Death Different 1997; Yu, 2003). These events were accompanied by the increase in DNA labelling with 7-Aminoactinomycin D (7-AAD), a classical marker for dead cells. Cell death peaked at 30 minutes after treatment with the peptides in both non-infected and infected cells, with no significant further increase at later time points (Fig 4B). This rapid action indicated that RT53 could act as a molecular switch for a repressed cell death-triggering cellular factor that was strongly enriched in a subset of cells.

We have found that the expression levels of AAC11 correlated with gene expression of caspase 3 (Fig 1B). We thus evaluated the activation of caspase 3 and other caspases, known activators and executioners of cell death. Although we did not see significant activation of caspase 1 or caspases 3 and 7, we saw a strong activation of caspase 2 in cells treated with RT53 (Fig 4C). Activation of caspase 2 was maximal within the first hour after treatment and decreased afterwards (Fig 4D), in agreement with the kinetics of cell death triggered by the peptides. To further link cell death and viral inhibition we sought to revert peptide-induced cytotoxicity by antagonizing intracellular K^+ efflux with increasing KCl concentrations in the culture medium (Thompson et al., 2001). Non-

158 infected CD4+ T cells or cells that had been pre-infected with VSVG-pseudotyped HIV particles were
159 incubated with RT53 or RK16 for 5 hours in presence of increasing extracellular K^+ concentration,
160 from around 5mM in control condition up to 100 mM upon addition of increasing amounts of KCl
161 (Fig 4E). We observed a KCl concentration-dependent decrease in RT53-mediated cell death, relative
162 to RK16 or control condition, which was accompanied by a progressive increase in the proportion of
163 infected CD4+ T cells recovered at the end of the culture. No differences were observed in the rates
164 of cell death and the percentage of infected cells between RT53 and RK16 or control when the
165 cultures were done in the presence of 100 mM of KCl, although we should notice that at this
166 concentration the rates of infection were overall lower than in CD4+ T cells cultured in the absence
167 of extra KCl (Fig 4E). Overall, our results confirmed that RT53 induced cell death in a subset of CD4+
168 T cells and that this cytotoxic effect was directly related to the antiviral effect of the peptide.
169
170 **RT53 preferentially depletes effector and transitional memory CD4+ T cell subpopulations**
171 We next assessed the characteristics of the CD4+ T cells that were sensitive to the cytotoxic action of
172 RT53. Our results suggest that the AAC11 survival pathway was upregulated with CD4+ T cell
173 differentiation (Fig 1A). We thus explored if RT53 treatment changed the distribution of CD4+ T cell
174 subsets. When compared to non-treated or RK16-treated conditions, RT53 treatment changed the
175 relative contribution of CD4+ T cell subsets to the total pool of CD4+ T cells (Fig 5A, Fig S2). Naïve
176 CD4+ T cells were enriched upon treatment with RT53 while the more differentiated Tm and Em
177 CD4+ T cells were depleted. Although activation *in vitro* changed somewhat the relative contribution
178 of CD4+ T cell subsets to the pool of CD4+ T cells, RT53 had a similar impact on CD4+ T cell subsets
179 when we used non-activated or *in vitro* activated cells (Fig 5A). These results show that the different
180 susceptibility of the CD4+ T cell subsets to the action of the peptides was related to the intrinsic
181 characteristics of each subset rather than provoked by cell activation. In agreement with other and
182 our own previous results (Valle-Casuso, submitted)(Roederer et al., 1997; Schnittman et al., 1990;
183 Spina et al., 1997), naïve CD4+ T cells were highly resistant to infection with both VSVG pseudotyped

184 HIV-1 particles and HIV-1 Bal. Susceptibility to infection increased with T cell differentiation, with Em
185 and Tm cells showing the highest infection levels (Fig 5B). Thus, our results confirmed that RT53
186 preferentially targeted differentiated memory cells with heightened susceptibility to HIV infection.
187
188 **RT53 targets metabolically active cells**
189 Although AAC11-derived peptides preferentially targeted differentiated T cells, the effect was not
190 absolute and not all Em and Tm cells were depleted after RT53 treatment. On the other hand we
191 observed some extent of peptide-induced cell death in all CD4+ T cell subsets, including naïve cells.
192 This suggested that AAC11-derived peptides targeted cells with specific characteristics that were
193 strongly enriched, but not exclusively found, within more differentiated subsets. AAC11 was
194 previously shown to increase the survival of cancer cells during metabolic stress. Cancer cells rely on
195 high level of glycolysis for adequate supply of biosynthetic intermediates for their life cycle. We
196 found that the gene expression level of AAC11 was strongly correlated with the expression of
197 numerous genes associated with cell metabolism (Fig 1B). We thus analyzed if the differential
198 sensitivity of CD4+ T cells to RT53 treatment was related to differences in cellular metabolism.
199 Following treatment of CD4+ T cells with RT53 but not RK16, we observed disappearance of larger
200 CD4+ T cells (determined by FCS in flow cytometry) (Fig S3) and a decrease in the proportion of
201 $CD25^{high}$ and HLA-DR^{high} cells (Fig 6A). We next analyzed the metabolic activity [glycolysis (measured
202 by extracellular acidification rate (ECAR) and oxidative phosphorylation (OXPHOS) (measured by
203 oxygen consumption rate (OCR)] of living CD4+ T cells in non-treated or cells treated with RT53/RK16
204 peptides (Fig 6B). We found that the cells that remained after RT53 treatment had lower
205 mitochondrial respiration (basal and spare capacity) and glycolysis (basal ECAR and glycolytic reserve
206 (ECAR after FCCP)) than cells that had not been treated or treated with RK16. We thus concluded
207 that CD4+ T cells with the highest metabolic activity were more sensitive to the action of AAC11-
208 derived peptides.
209

RT53-surviving cells are non-permissive to HIV-1 replication

To further confirm that RT53 was killing HIV-1 susceptible target cells and selecting for cells resistant to infection, we treated activated CD4+ T cells with RT53 or RK16 for 24 hours, sorted live cells from dead cells and infected sorted live cells (Fig 7A). We found that the cells that survived RT53 treatment were resistant to infection with both HIV-1 Bal and VSVG-pseudotyped HIV-1 particles (Fig 7A), although the inhibition was more marked for the replication competent virus. Importantly, we did not see any further increase in cell death in sorted living cells. We next sought to determine which stage of viral infection was blocked in cells surviving RT53 treatment. RT53 induced a slight decrease in the proportion of $CCR5^{high}$ cells (Fig S4), although this difference did not appear sufficient to explain the level of HIV inhibition found. We next measured the levels of total (as a measure of reverse transcription) and integrated viral DNA at various time points post-infection in RT53/RK16 treated or untreated cells (Fig. 7B). We could detect viral DNA at 4 hours post-infection in all treatment conditions suggesting that the virus is able to enter RT53 treated cells. Levels of both total and integrated HIV-1 DNA drastically increased as a function of time in RK16-treated and untreated cells but not in RT53-treated cells. These results indicate that although the virus is able to enter live cells after RT53 treatment, these cells are non-permissive to viral replication. Thus, RT53 treatment is killing virally susceptible targets while allowing the survival of cells where HIV-1 cannot complete the first post-entry steps of its replication cycle.

DISCUSSION

In this study we showed that peptides antagonizing the antiapoptotic action of AAC11 inhibit HIV-1 infection of primary CD4+ T cells. In particular, we found that RT53 was able to block infection with a large panel of lab adapted and primary viral strains by inducing apoptosis of CD4+ T cells that offered the best conditions to sustain HIV replication. Our results also suggest that the AAC11 survival pathway is involved in the regulation of T cell differentiation by facilitating the survival of subsets of cells with high metabolic activity.

We found that gene expression of the apoptosis inhibitory protein AAC11 increased with differentiation of memory CD4+ T cells. Furthermore we found a strong correlation between the expression of AAC11 and multiple genes involved in the regulation of cell metabolism. Upon antigenic stimulation CD4+ T cells upregulate their metabolic activities, in particular glucose metabolism, to cope with the bioenergetics demands necessary to exert their functions. Metabolic arrangements are also necessary to sustain long-term memory cells. Cellular metabolism and apoptosis are deeply entangled. External signals provided by TCR activation or growth factors such as IL-2 and IL-7 promote an anti-apoptotic state of the cell by increasing the levels of metabolite transporters (e.g. Glut1) that ensure the supply of nutrients necessary to sustain the bioenergetic demands of the cell. The absence of these signals provokes a limitation in the influx of nutrients that results in metabolic stress, the activation of apoptotic pathways and cell death. We show that RT53 antagonized the anti-apoptotic activity of AAC11 and induced death in CD4+ T cells, and that sensitivity to RT53 increased with T cell differentiation and metabolic activity of the cells. Our results suggest that the AAC11 survival pathway may be involved in regulation of T cell immunity, a function that has not been previously described and deserves further detailed exploration.

Although the antiapoptotic action of AAC11 is well documented, the molecular mechanisms underlying its activity are still unclear. AAC11 possesses several domains predicted to be responsible

257 for protein-protein interactions (Han et al., 2012), and its expression may interfere with different
258 mechanisms of cell death. The peptides that we tested here were designed to mimic the heptad
259 leucine repeat region of AAC11 and to be used as competitive inhibitors that abrogate the interaction
260 of ACC11 with some of its partners. Along these lines RT53 has been shown to exert its function
261 without directly affecting AAC11 itself (Jagot-Lacoussiere et al., 2016). We found that AAC11
262 expression, both on the mRNA and protein level, was unchanged with RT53 treatment (Fig S5). This
263 indicates that de-repression of pro-apoptotic AAC11 partner(s) rather than changes in AAC11
264 expression itself is responsible for cell death differences observed in our cells upon treatment with
265 the peptides. Moreover, the relationship between the HIV-1 inhibition and AAC11 may not be direct
266 but rather involves a molecular pathway with complex interplay of signaling factors the relative
267 contribution of which to HIV-1 infection is worth investigating further. We found that among the
268 peptides tested, those spanning the whole LZ domain were more potent inducers of cell death and
269 blocked HIV infection more efficiently. Additionally, the fast kinetics of their activity points to an action
270 through rearrangement of pre-existing cellular factors. In tumor cells physical interaction between the
271 LZ domain of AAC11 and Acinus prevents Acinus pro-apoptotic cleavage by caspase 3. In our study we
272 did not observe significant changes in the expression of Acinus upon treatment with AAC11-derived
273 peptides (not shown) and caspase 3 activity was not significantly detected in our experimental
274 conditions, suggesting that this pathway was not the major contributor to T cell death observed in our
275 experiments. In contrast we found that the treatment with RT53 induced a strong increase in caspase
276 2 activity. AAC11 has been recently shown to physically bind to the caspase recruitment domain of
277 caspase 2 preventing its activation (Imre et al., 2017). Our results suggest that RT53 may impair this
278 interaction and facilitate caspase 2 dimerization and initiation of apoptosis. However, RT53-induced
279 caspase 2 activation did not prompt cell death in all cells suggesting that additional factors intervened
280 downstream in this process and were not equally expressed by all cells.
281

The main goal of this study was to explore if interfering with AAC11 activity could affect HIV infection. We show that AAC11 peptides, particularly RT53, strongly impaired infection with different HIV-1 strains and SIV_{mac251}. There was a close association between peptide-induced cell death and inhibition of HIV infection. Not only the rates of cell death induced by AAC11 peptides correlated with the extent of HIV inhibition, but precluding apoptosis by increasing extracellular levels of K^+ also preserved the infectability of CD4+ T cells. Moreover, pre-treatment of CD4+ T cells with RT53 selected for cells that were strongly resistant to HIV-1 infection (for instance reverse transcription was severely impaired in these cells). This and the fact that RT53 depleted highly metabolic cells are in agreement with the observation that a rich metabolic environment is necessary for HIV replication (Valle-Casuso, submitted). It is interesting to notice that AAC11 gene expression was correlated with the expression of RRM2, an enzyme that is critical for the de novo synthesis of dNTPs, and the depletion of which blocks HIV-1 infection in macrophages and dendritic cells (Allouch et al., 2013; Valle-Casuso et al., 2017). AAC11 mRNA levels also correlated with the expression of other genes that have been associated with the HIV replication cycle in particular trafficking (i.e. CFL1, DYNC1H1, ACTB) and transcription (i.e. CDK9, NFKB1) suggesting that additional steps of the HIV-1 cycle may be impaired in cells resisting RT53-induced cell death. Of note, although we observed a very consistent inhibition of infection with VSVG-pseudotyped NL4.3 single cycle particles upon treatment with AAC11 peptides, the inhibition was more important when we used wild type viruses, either R5 or X4. There are several possible explanations to this. First, the additional block in HIV infection observed when we used WT viruses could be due to a cumulative effect of multiple infection cycles. Second, cells selected upon treatment with AAC11 may offer an extra restriction to WT virus. For instance cells expressing high levels of CCR5 were depleted upon treatment with RT53. In contrast RT53 sharply increased surface levels of phosphatidylserine, which may facilitate entry mediated by VSVG (Carneiro et al., 2002). We cannot exclude that treatment with AAC11-derived peptides not only depleted the CD4+ T cells that were most susceptible to HIV-1 infection but also induced viral restriction in the cells that survived. For instance, AAC11 has been shown to regulate the cell cycle, and interference with AAC11 activity

might affect G1 into S transition (Navarro et al., 2013) and HIV infection (Wang et al., 2011)(Clark et al., 2000).

Our results show that AAC11 antiapoptotic pathway is active in CD4+ T cells that are particularly susceptible to HIV-1 infection. It remains to be established if this pathway may contribute to persistence of infected cells on the long-term. The mechanisms by which an infected cell survives and persists over time are not completely clear, as HIV has cytopathic effects that lead to the death of most infected CD4+ T cells. It has been recently reported that persistent latently infected CD4+ T cells are intrinsically resistant to killing by HIV-specific CD8+ T cells (Huang et al., 2018), which may indicate that the persistent reservoir may be seeded in cells programmed to resist cell death. Like the mechanisms associated with HIV persistence, it is also unclear how some T cells survive to become long-lived memory cells. While most T cells undergo apoptosis at the end of the immune response when environmental signals wane, a few cells survive even in the absence of growth factors (Sallusto et al., 2010). AAC11 prevents apoptosis of tumor cells in conditions of nutrient deprivation and in the absence of growth factors. We can therefore speculate that the AAC11 survival pathway might play a similar role in promoting persistence of memory CD4+ T cells and HIV infection.

Although the differences between infection with WT viruses and single-cycle VSVG pseudotyped particles were visible both when cells were treated with RT53 hours before infection or at the time of infection, we cannot exclude that infection with WT HIV may induce cellular changes that can further increased the susceptibility of infected cells to the action of AAC11-derived peptides. HIV has been shown to induce metabolic changes in infected cells and in particular an increase in the expression of Glut1-3 (Sorbara et al., 1996). It has been recently shown that BIRC5 (a.k.a. Api4 or survivin), a member of the inhibitor of apoptosis protein family, is upregulated in CD4+ T cells during HIV-1 infection and contributes to the persistence of infected cells (Kuo et al., 2018). Upregulation of BIRC5 on infected CD4+ T cells was triggered by OX40, a costimulatory receptor that promotes T cell

differentiation and survival (Huddleston et al., 2006; Redmond et al., 2009) and contributes to the metabolic boost necessary for T cell activation (Pacella et al., 2018). It is thus possible that HIV-1 exploits/activates several cellular survival programs associated with T cell activation and metabolic activity to ensure its persistence.

MATERIAL AND METHODS

Peptides, antibodies and probes

Peptides (Proteogenix) were received as dry powder and reconstituted with water for use. Peptide sequences are as follows.

RT53: RQIKIWFQNRRMKWKKAKLNAEKLKDFKIRLQYFARGLQVYIRQLRLALQGKT,

RT39: RQIKIWFQNRRMKWKKLQYFARGLQVYIRQLRLALQGKT,

RL29: RQIKIWFQNRRMKWKKYFARGLQVYIRQL,

RQ26: RQIKIWFQNRRMKWKKLQYFARGLLQ,

RK16: RQIKIWFQNRRMKWKK.

Antibodies and dyes used for flow cytometry and FACS sorting are as follows: LIVE/DEAD™ Violet Viability dye (ThermoFisher), CD3-PE (clone SK7, Biolegend), CD4-A700 (clone OKT4, eBioscience), CD45RA-APC-Cy7 (clone HI100, Biolegend), CCR7-Pe-Cy7 (clone GO43H7, Biolegend), CD27-APC (clone M-T271, Myltenyi), CD25-PE-Dazzle594 (clone M-A251, Biolegend), HLA-DR-PerCP-Cy5.5 (clone G46-6, Biolegend), CCR5-PE (clone 3A9, BD), p24-FITC (clone KC57, Coulter). Caspase activity was assayed with the following probes according to the manufacturer's instructions: caspase 1 660-YVAD-FMK probe (FLICA 660 Caspase-1 Assay Kit, ImmunoChemistry Technologies^{LLC}), caspase 2 FAM-VDVAD-FMK probe (FAM-FLICA Caspase-2 Assay Kit, ImmunoChemistry Technologies^{LLC}), caspase 3/7 SR-DEVD-FMK probe (SR-FLICA® Caspase 3/7 Assay Kit, ImmunoChemistry Technologies^{LLC}). FACS sorting was performed on BD Aria and flow cytometry acquisition on BD LSRII.

Isolation and culture of primary human CD4+ T cells

Healthy donor blood prepared as a buffy coat was obtained from *Etablissement Français du Sang (EFS)* (agreement with Institut Pasteur C CPSL UNT, 15/EFS/023). Blood was overlayed on Ficol (EuroBio) at a ratio of 2:1 v/v blood to Ficol and centrifuged at 1,800 rpm for 30 minutes at a minimum acceleration/deceleration to obtain peripheral blood mononuclear cells (PBMCs). CD4+ T cells were then purified from PBMCs by negative selection using StemCell EasySep™ Human CD4+ T cell Isolation

Kit. Cells were counted and cultured in RPMI-1640 containing Glutamax (ThermoFisher), 10% fetal bovine serum (FBS), penicillin-streptomycin (ThermoFisher) (100 U/ml) and IL-2 (Myltenyi) (100U/ml) (thereafter referred to as culture medium) at 10^6 cells/ml in 37° degree, 5% CO_2 humidified incubator. Cells were suboptimally activated with soluble anti-CD3 (clone UCHT-1) (Biolegend) for 5 days prior to infection or analysis as previously described (Sáez-Cirión et al., 2011).

Isolation and culture of primary infected macaque CD4+ T cells

Macaque splenic CD4+ T cells were obtained from Cynomologus macaques (*Macaca fascicularis)* that were imported from Mauritius island and housed at *Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA)* at Fontenay-aux-Roses in France under the compliance with the Standards for Human Care and Use of Laboratory Animals (Assurance number A5826-01). These animals were part of the pVISCONTI study, which received ethics approval under the number APAFIS#2453-2015102713323361 v2. They were infected intravenously with 1000 AID_{50} of $SIVmac_{251}$ and sacrificed at a study end point to obtain spleen samples. Splenic macaque CD4+ T cells were purified by mechanical disruption of a spleen sample in RPMI media using GengleMACS™ dissociator (Milntenyi) followed by cell overlay over Ficol (EuroBio) (diluted with PBS to 90% prior to use) and centrifugation at 350g for 20min. Cells were then subjected to red cell lysis and then to CD4+ T cell negative selection using CD4+ T cell negative selection kit (Myltenyi). Cells were cultured in the culture media overnight prior to incubation with peptides. Viral spread from in vivo infected cells was monitored by ELISA quantification of p27 (Expressbio) levels on culture supernatants.

Infection and peptide treatment of primary human CD4+ T cells

Activated CD4+ T cells were infected with either: Bal (2.9 ng/ml p24), BX08 (21 ng/ml p24), DH12 (3 ng/ml p24), 132w (6.3 ng/ml p24), NL4.3 (7 ng/ml p24), vesicular stomatitis virus glycoprotein (VSVG) pseudotyped-Δenv-Δnef-GFP (7 ng/ml p24) or SIV_{mac251} (36.7 ng/ml p27) viruses by

centrifuging at 1,200g for 1 hour at room temperature and then incubating for 1 hour at 37° degrees in a humidified 5% CO_2 incubator (spinoculation in text). Cells were then washed once with PBS, incubated in the culture medium and treated with peptides at 6 μM concentration unless otherwise indicated. Cell death and infection were measured on day 3 post-infection unless otherwise stated. Cell death was evaluated using flow cytometry (LIVE/DEAD^{TM} Violet Viability dye (ThermoFisher)) and infection was evaluated by either flow cytometry (intracellular p24 staining) or p24/p27 ELISA (XpressBio).

Real-time flow cytometry

Cells were washed once with PBS and incubated in Annexin buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4) in the presence of Annexin V-FITC (Biolegend) at a concentration of 10^6 cells/ml for 15 minutes. 7-AAD (Biolegend) was added for the last 5 minutes of incubation. Cells were then directly passed through the flow cytometer to acquire fluorescence vs time without washing. Alternatively, cells were stained with asanate potassium green 2 (APG-2) AM (Abcam), a fluorescent K^+ indicator, at a final concentration of 1 μM in plain RPMI-1640 medium for 30 minutes at room temperature, washed twice with plain RPMI-1640, resuspended in PBS at 10^6 cells/ml and incubated with 7-AAD (Biolegend) for 5 minutes before acquisition. 6 μM peptides were added after 2 minutes of baseline acquisition and acquisition continued for additional 28 minutes.

Measurements of cellular metabolism

Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured on Seahorse XF96 analyzer using Seahorse XF Cell Mito Stress Test Kit (Agilent). Briefly, activated CD4+ T cells were incubated with 6 μM RT53 or RK16 for 4 hours at 37°C. Cells were then counted, washed in a Seahorse XF medium (Agilent Seahorse XF base medium with 2mM Glutamax (Agilent, 102365-100) containing 10mM glucose (Sigma), 2mM sodium pyruvate (LifeTechnologies) and adjusted to pH 7.4). Equal number of live cells was seeded at $2*10^5$ cells per well in XF96 V3 PS plates (Seahorse

Bioscience) precoated with 0.5 mg/ml Corning® Cell-Tack™ Cell and Tissue Adhesive (Corning, 354240), and incubated for a minimum of 30 minutes in a CO_2-free, 37°C incubator prior to acquisition. The following drugs were placed at injection ports: Seahorse XF medium (port A), 2.5 μM Oligomycin (port B), 0.9 μM FCCP (port C), 1 μM rotenone and 1 μM antimycin A (port D).

Quantitative RT-PCR

Evaluating the expression of AAC11 and other genes in CD4+ T cell memory subsets

The expression levels of AAC11 and other genes on CD4+ T cell subsets were analyzed in a previous study (Valle-Casuso, submitted). Briefly, the total RNA from $5*10^4$ cells was extracted using RNA Trace Kit (Macherey Nagel), treated with DNase, reverse transcribed using Reverse Transcription Master Mix (Fluidigm), pre-amplified using PreAmp Master Mix (Fluidigm) and treated with exonuclease I (New England Biolabs). Samples were then mixed with SsoFast EvaGreen Supermix with Low ROX (Biorad), DNA Binding Dye (Fluidigm), and assay mix (assay loading reagent (Fluidigm) and Delta Gene primers (Fluidigm)). The expression levels were read on Biomark HQ system (Fluidigm). The expression levels of BECN1 were used for normalization. Gene expression values are plotted as $2^{-\Delta\Delta Ct}$.

Evaluating the expression of viral gene products

Cells were collected by centrifugation and dry pellet was stored at -80°C until DNA extraction. DNA was extracted using NucleoSpin® Tissue Kit (Macherey Nagel). Real time PCR (RT-PCR) to quantify total and integrated HIV-1 DNA was performed as described previously (David et al., 2006) using TaqMan™ Universal PCR Master Mix (ThermoFisher). Briefly, total viral DNA was quantified using the following primers and probe: CTT TCG CTT TCA AGT CCC TGT T (forward), AGA TCC CTC AGA CCC TTT TAG TCA (reverse), (FAM)-TGG AAA ATC TCT AGC AGT GGC GCC C-(BHQ1) (probe). 8E5 cell line containing a single viral copy per cell was used as a standard. Integrated viral DNA was quantified by first pre-amplifying the DNA using AccuTaq™ LA DNA Polymerase (Sigma) using the following

primers: AGC CTC CCG AGT AGC TGG GA (FirstAluF); TTA CAG GCA TGA GCC ACC G (FirstAluR); CAA TAT CAT ACG CCG AGA GTG CGC GCT TCA GCA AG (NY1R). Second DNA amplification round was performed with TaqMan™ Universal PCR Master Mix (ThermoFisher) using the following primers: AAT AAA GCT TGC CTT GAG TGC TC (NY2F); CAA TAT CAT ACG CCG AGA GTG C (NY2R); FAM-AGT GTG TGC CCG TCT GTT GTG TGA CTC-TAMRA (NY2ALU probe). HeLa cells line containing HIV-1 integrated DNA was used as a standard (David et al., 2006). Results were normalized to ng actin using Human DNA standard (Sigma). Primers used for quantification of actin were: TGC ATG AGA AAA CGC CAG TAA (forward); ATG GTC GCC TGT TCA CCA A (reverse); (FAM)-TGA CAG AGT CAC CAA ATG CTG CAC AGA A-(TAMRA) (probe).

Statistical analysis

Statistical analysis was performed using GraphPad Prism software. Linked parametric or non-parametric one-way ANOVA or two-way ANOVA were used depending on the experiment. * denotes $p \leq 0.05$, ** denotes $p \leq 0.01$, *** denotes $p \leq 0.001$, **** denotes $p \leq 0.0001$. Dunnett's, Dunn's or Holm-Sidak's multiple comparisons tests were used for post-hoc analysis.

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Author Contributions

AM, AD, JCV-C, VM performed experiments; AM, AD, JCV-C, VM, SV and AS-C analyzed the data; JLP and CP provided key reagents; JLP and AS-C conceived the study; AM, AD, JCV-C and AS-C designed the experiments; AS-C supervised the study; AM and AS-C drafted the article; and all authors critically reviewed the manuscript.

Bibliography

Allouch, A., David, A., Amie, S.M., Lahouassa, H., Chartier, L., Margottin-Goguet, F., Barré-Sinoussi, F., Kim, B., Sáez-Cirión, A., Pancino, G., 2013. p21-mediated RNR2 repression restricts HIV-1 replication in macrophages by inhibiting dNTP biosynthesis pathway. PNAS 110, E3997–E4006. https://doi.org/10.1073/pnas.1306719110

Almeida, L., Lochner, M., Berod, L., Sparwasser, T., 2016. Metabolic pathways in T cell activation and lineage differentiation. Seminars in Immunology 28, 514–524. https://doi.org/10.1016/j.smim.2016.10.009

Barton, K., Winckelmann, A., Palmer, S., 2016. HIV-1 Reservoirs During Suppressive Therapy. Trends in Microbiology 24, 345–355. https://doi.org/10.1016/j.tim.2016.01.006

Carneiro, F.A., Bianconi, M.L., Weissmüller, G., Stauffer, F., Da Poian, A.T., 2002. Membrane recognition by vesicular stomatitis virus involves enthalpy-driven protein-lipid interactions. J. Virol. 76, 3756–3764.

Clark, E., Santiago, F., Deng, L., Chong, S. yen, Fuente, C. de la, Wang, L., Fu, P., Stein, D., Denny, T., Lanka, V., Mozafari, F., Okamoto, T., Kashanchi, F., 2000. Loss of G1/S Checkpoint in Human Immunodeficiency Virus Type 1-Infected Cells Is Associated with a Lack of Cyclin-Dependent Kinase Inhibitor p21/Waf1. J. Virol. 74, 5040–5052. https://doi.org/10.1128/JVI.74.11.5040-5052.2000

Davey, R.T., Bhat, N., Yoder, C., Chun, T.-W., Metcalf, J.A., Dewar, R., Natarajan, V., Lempicki, R.A., Adelsberger, J.W., Miller, K.D., Kovacs, J.A., Polis, M.A., Walker, R.E., Falloon, J., Masur, H., Gee, D., Baseler, M., Dimitrov, D.S., Fauci, A.S., Lane, H.C., 1999. HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. PNAS 96, 15109–15114. https://doi.org/10.1073/pnas.96.26.15109

David, A., Sáez-Cirión, A., Versmisse, P., Malbec, O., Iannascoli, B., Herschke, F., Lucas, M., Barré-Sinoussi, F., Mouscadet, J.-F., Daëron, M., Pancino, G., 2006. The Engagement of Activating

FcγRs Inhibits Primate Lentivirus Replication in Human Macrophages. The Journal of Immunology 177, 6291–6300. https://doi.org/10.4049/jimmunol.177.9.6291

Elliott, J.I., Sardini, A., Cooper, J.C., Alexander, D.R., Davanture, S., Chimini, G., Higgins, C.F., 2006. Phosphatidylserine exposure in B lymphocytes: a role for lipid packing. Blood 108, 1611–1617. https://doi.org/10.1182/blood-2005-11-012328

García, F., Plana, M., Vidal, C., Cruceta, A., O,Brien, W.A., Pantaleo, G., Pumarola, T., Gallart, T., Miró, J.M., Gatell, J.M., 1999. Dynamics of viral load rebound and immunological changes after stopping effective antiretroviral therapy. AIDS 13, F79.

Han, B.-G., Kim, K.H., Lee, S.J., Jeong, K.-C., Cho, J.-W., Noh, K.H., Kim, T.W., Kim, S.-J., Yoon, H.-J., Suh, S.W., Lee, S., Lee, B.I., 2012. Helical Repeat Structure of Apoptosis Inhibitor 5 Reveals Protein-Protein Interaction Modules. J. Biol. Chem. 287, 10727–10737. https://doi.org/10.1074/jbc.M111.317594

Harrigana, P.R., Whaley, M., Montaner, J.S.G., 1999. Rate of HIV-1 RNA rebound upon stopping antiretroviral therapy. AIDS 13, F59.

Heaton, N.S., Perera, R., Berger, K.L., Khadka, S., LaCount, D.J., Kuhn, R.J., Randall, G., 2010. Dengue virus nonstructural protein 3 redistributes fatty acid synthase to sites of viral replication and increases cellular fatty acid synthesis. PNAS 107, 17345–17350. https://doi.org/10.1073/pnas.1010811107

Hegedus, A., Kavanagh Williamson, M., Huthoff, H., 2014. HIV-1 pathogenicity and virion production are dependent on the metabolic phenotype of activated CD4+ T cells. Retrovirology 11, 98. https://doi.org/10.1186/s12977-014-0098-4

Hollenbaugh, J.A., Munger, J., Kim, B., 2011. Metabolite profiles of human immunodeficiency virus infected CD4+ T cells and macrophages using LC–MS/MS analysis. Virology 415, 153–159. https://doi.org/10.1016/j.virol.2011.04.007

Huang, S.-H., Ren, Y., Thomas, A.S., Chan, D., Mueller, S., Ward, A.R., Patel, S., Bollard, C.M., Cruz, C.R., Karandish, S., Truong, R., Macedo, A.B., Bosque, A., Kovacs, C., Benko, E., Piechocka-

Trocha, A., Wong, H., Jeng, E., Nixon, D.F., Ho, Y.-C., Siliciano, R.F., Walker, B.D., Jones, R.B., 2018. Latent HIV reservoirs exhibit inherent resistance to elimination by $CD8^+$ T cells. J Clin Invest 128, 876–889. https://doi.org/10.1172/JCI97555

Huddleston, C.A., Weinberg, A.D., Parker, D.C., 2006. OX40 (CD134) engagement drives differentiationof CD4+ T cells to effector cells. European Journal of Immunology 36, 1093–1103. https://doi.org/10.1002/eji.200535637

Imre, G., Berthelet, J., Heering, J., Kehrloesser, S., Melzer, I.M., Lee, B.I., Thiede, B., Dötsch, V., Rajalingam, K., 2017. Apoptosis inhibitor 5 is an endogenous inhibitor of caspase-2. EMBO reports 18, 733–744. https://doi.org/10.15252/embr.201643744

Jagot-Lacoussiere, L., Kotula, E., Villoutreix, B.O., Bruzzoni-Giovanelli, H., Poyet, J.-L., 2016. A Cell-Penetrating Peptide Targeting AAC-11 Specifically Induces Cancer Cells Death. Cancer Res 76, 5479–5490. https://doi.org/10.1158/0008-5472.CAN-16-0302

Kim, J.W., Cho, H.S., Kim, J.H., Hur, S.Y., Kim, T.E., Lee, J.M., Kim, I.-K., Namkoong, S.E., 2000. AAC-11 Overexpression Induces Invasion and Protects Cervical Cancer Cells from Apoptosis. Lab Invest 80, 587–594. https://doi.org/10.1038/labinvest.3780063

Kuo, H.-H., Ahmad, R., Lee, G.Q., Gao, C., Chen, H.-R., Ouyang, Z., Szucs, M.J., Kim, D., Tsibris, A., Chun, T.-W., Battivelli, E., Verdin, E., Rosenberg, E.S., Carr, S.A., Yu, X.G., Lichterfeld, M., 2018. Anti-apoptotic Protein BIRC5 Maintains Survival of HIV-1-Infected CD4+ T Cells. Immunity 48, 1183-1194.e5. https://doi.org/10.1016/j.immuni.2018.04.004

Manel, N., Kim, F.J., Kinet, S., Taylor, N., Sitbon, M., Battini, J.-L., 2003. The ubiquitous glucose transporter GLUT-1 is a receptor for HTLV. Cell 115, 449–459.

McArdle, J., Moorman, N.J., Munger, J., 2012. HCMV Targets the Metabolic Stress Response through Activation of AMPK Whose Activity Is Important for Viral Replication. PLOS Pathogens 8, e1002502. https://doi.org/10.1371/journal.ppat.1002502

554 Morris, E.J., Michaud, W.A., Ji, J.-Y., Moon, N.-S., Rocco, J.W., Dyson, N.J., 2006. Functional
555 Identification of Api5 as a Suppressor of E2F-Dependent Apoptosis In Vivo. PLOS Genet 2,
556 e196. https://doi.org/10.1371/journal.pgen.0020196
557 Navarro, M.G.-J., Basset, C., Arcondéguy, T., Touriol, C., Perez, G., Prats, H., Lacazette, E., 2013. Api5
558 Contributes to E2F1 Control of the G1/S Cell Cycle Phase Transition. PLOS ONE 8, e71443.
559 https://doi.org/10.1371/journal.pone.0071443
560 Pacella, I., Procaccini, C., Focaccetti, C., Miacci, S., Timperi, E., Faicchia, D., Severa, M., Rizzo, F.,
561 Coccia, E.M., Bonacina, F., Mitro, N., Norata, G.D., Rossetti, G., Ranzani, V., Pagani, M.,
562 Giorda, E., Wei, Y., Matarese, G., Barnaba, V., Piconese, S., 2018. Fatty acid metabolism
563 complements glycolysis in the selective regulatory T cell expansion during tumor growth.
564 PNAS 201720113. https://doi.org/10.1073/pnas.1720113115
565 Redmond, W.L., Ruby, C.E., Weinberg, A.D., 2009. The role of OX40-mediated co-stimulation in T-cell
566 activation and survival. Crit. Rev. Immunol. 29, 187–201.
567 Sáez-Cirión, A., Hamimi, C., Bergamaschi, A., David, A., Versmisse, P., Mélard, A., Boufassa, F., Barré-
568 Sinoussi, F., Lambotte, O., Rouzioux, C., Pancino, G., Cohort, for the A.C., 2011. Restriction
569 of HIV-1 replication in macrophages and CD4+ T cells from HIV controllers. Blood 118, 955–
570 964. https://doi.org/10.1182/blood-2010-12-327106
571 Sallusto, F., Lanzavecchia, A., Araki, K., Ahmed, R., 2010. From vaccines to memory and back.
572 Immunity 33, 451–463. https://doi.org/10.1016/j.immuni.2010.10.008
573 Sorbara, L.R., Maldarelli, F., Chamoun, G., Schilling, B., Chokekijcahi, S., Staudt, L., Mitsuya, H.,
574 Simpson, I.A., Zeichner, S.L., 1996. Human immunodeficiency virus type 1 infection of H9
575 cells induces increased glucose transporter expression. J. Virol. 70, 7275–7279.
576 Thompson, G.J., Langlais, C., Cain, K., Conley, E.C., Cohen, G.M., 2001. Elevated extracellular [K+]
577 inhibits death-receptor- and chemical-mediated apoptosis prior to caspase activation and
578 cytochrome c release. Biochemical Journal 357, 137–145.
579 https://doi.org/10.1042/bj3570137

Valle-Casuso, J.C., Allouch, A., David, A., Lenzi, G.M., Studdard, L., Barré-Sinoussi, F., Müller-Trutwin, M., Kim, B., Pancino, G., Sáez-Cirión, A., 2017. p21 Restricts HIV-1 in Monocyte-Derived Dendritic Cells through the Reduction of Deoxynucleoside Triphosphate Biosynthesis and Regulation of SAMHD1 Antiviral Activity. J. Virol. 91, e01324-17. https://doi.org/10.1128/JVI.01324-17

Wang, J., Reuschel, E.L., Shackelford, J.M., Jeang, L., Shivers, D.K., Diehl, J.A., Yu, X.-F., Finkel, T.H., 2011. HIV-1 Vif promotes the G_1- to S-phase cell-cycle transition. Blood 117, 1260–1269. https://doi.org/10.1182/blood-2010-06-289215

FIGURES AND FIGURE LEGENDS

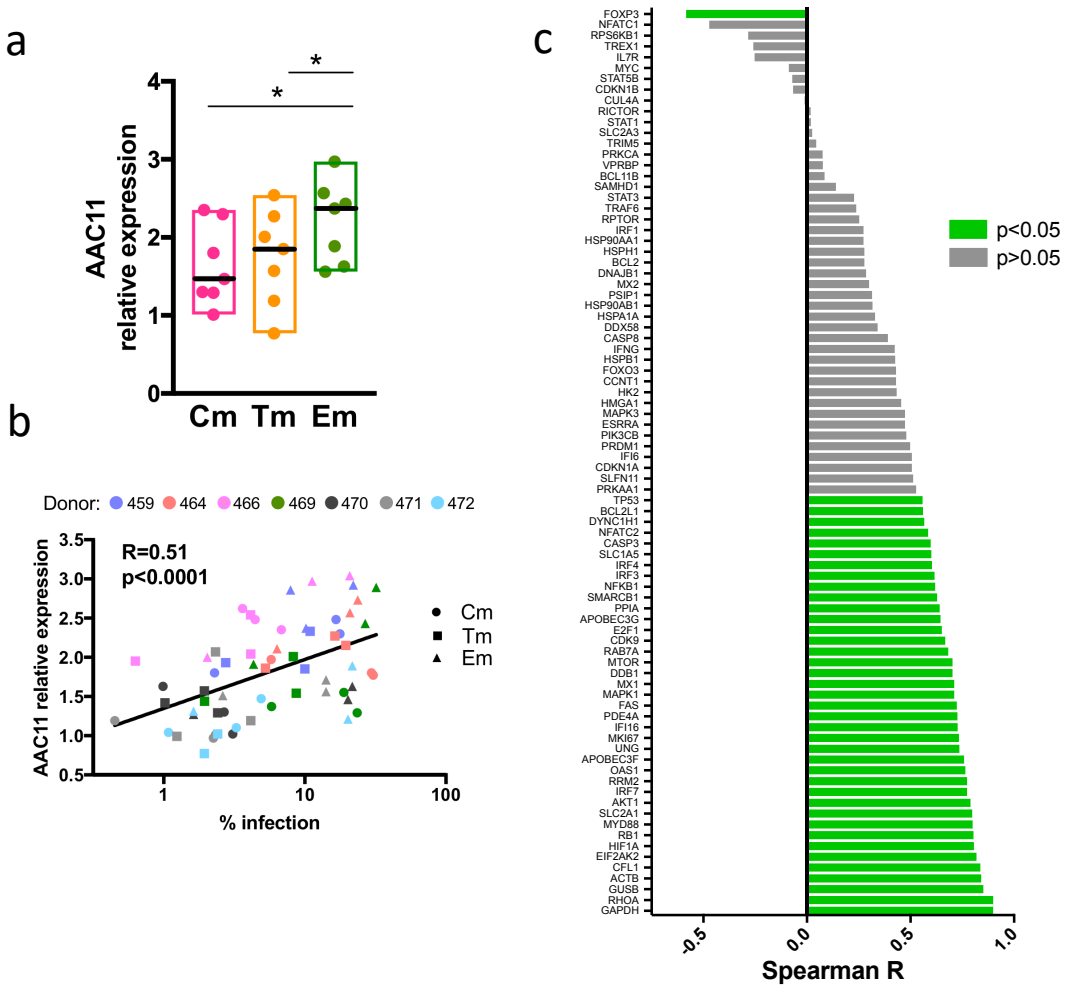

Figure 1. AAC11 gene expression correlates with the proportion of infection in each memory CD4+ T cell subset and is associated with expression of various cell cycle and metabolism genes. A. Suboptimally activated CD4+ T cell memory subsets (Cm, Tm, Em) were analyzed for the expression of AAC11 at the time of infection. **B.** Suboptimally activated (day 3 and 5) or non-activated Cm, Tm and Em subsets were analyzed for the levels of AAC11 gene expression and correlated with % of infected cells in each subset at day 3 post-infection **C.** The correlation of AAC11 gene expression with other genes in a 96-gene array.

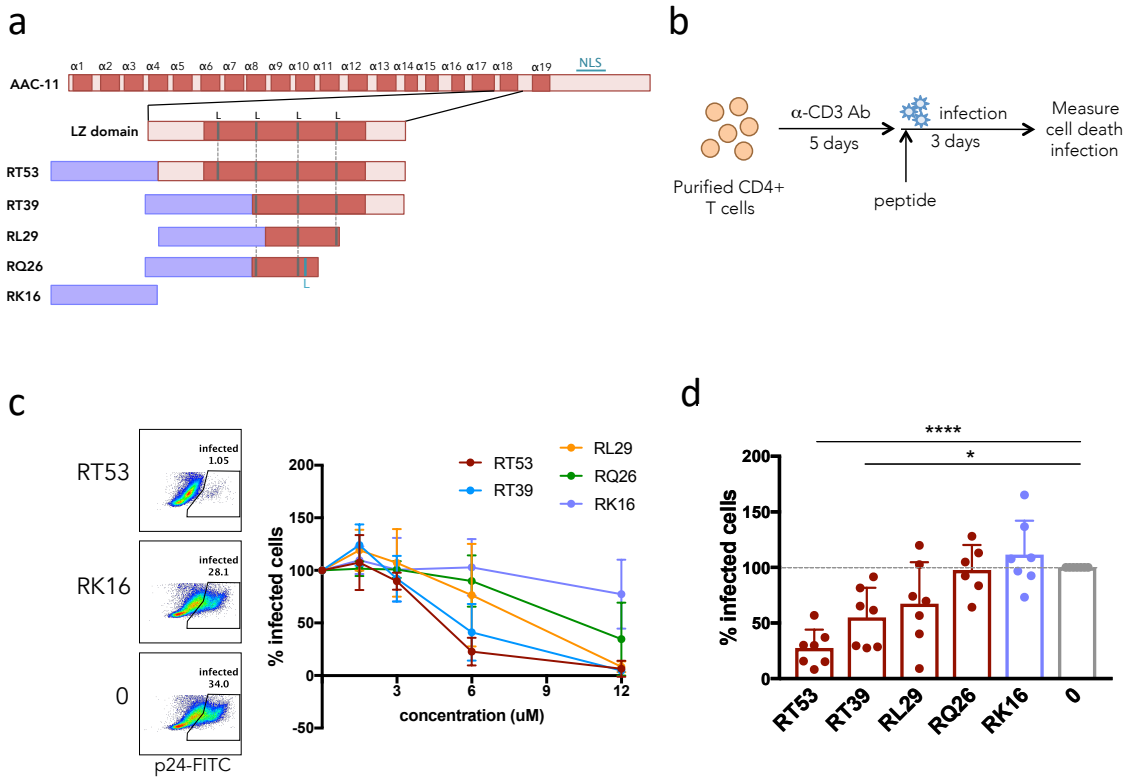

Figure 2. AAC11-derived peptides inhibit HIV-1 infection. A. Schematic representation of AAC11-derived peptides indicating LZ domain **B.** Experimental scheme of treatment of infected cells with AAC11-derived peptides **C.** Representative flow cytometry plot of p24 staining in live, untreated or cell treated with 6 μM RT53 or RK16. A dose-response curve of the effect of AAC11-derived peptides on HIV-1 infection in living cells **D.** The proportion of infection as compared to non-treated control among living cells infected with HIV-1 BaL and treated with 6 μM of indicated peptides.

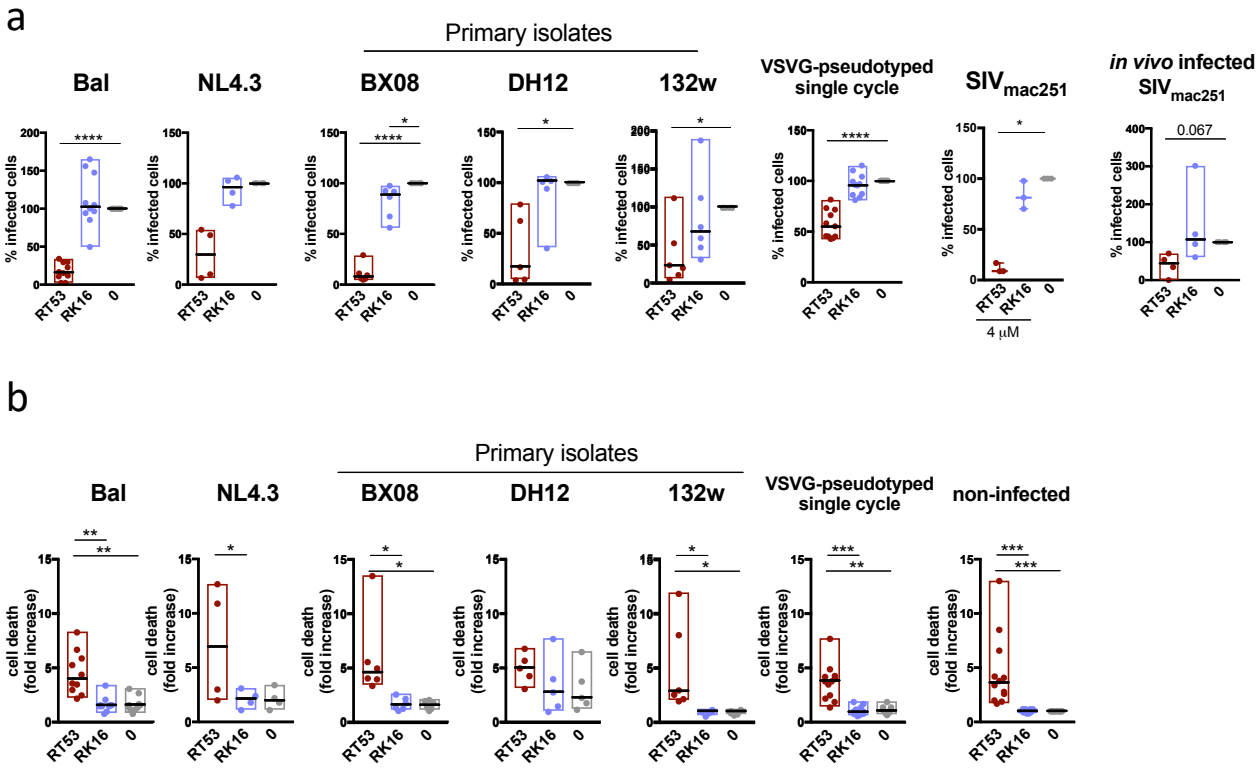

Figure 3. RT53 inhibits infection of diverse HIV-1 strains and SIV. Activated primary CD4+ T cells were spinoculated with different HIV-1 viral strains and incubated with 6 µM RT53 or RK16 for 3 days

A. Infection was measured by flow cytometry (intracellular p24) or ELISA (p24/p27) in supernatants

B. Cell death was measured by flow cytometry (LIVE/DEAD™ Violet Viability dye). Values are expressed as a range (min-max) with median indicated. Each symbol represents experiments with cells from a different donor.

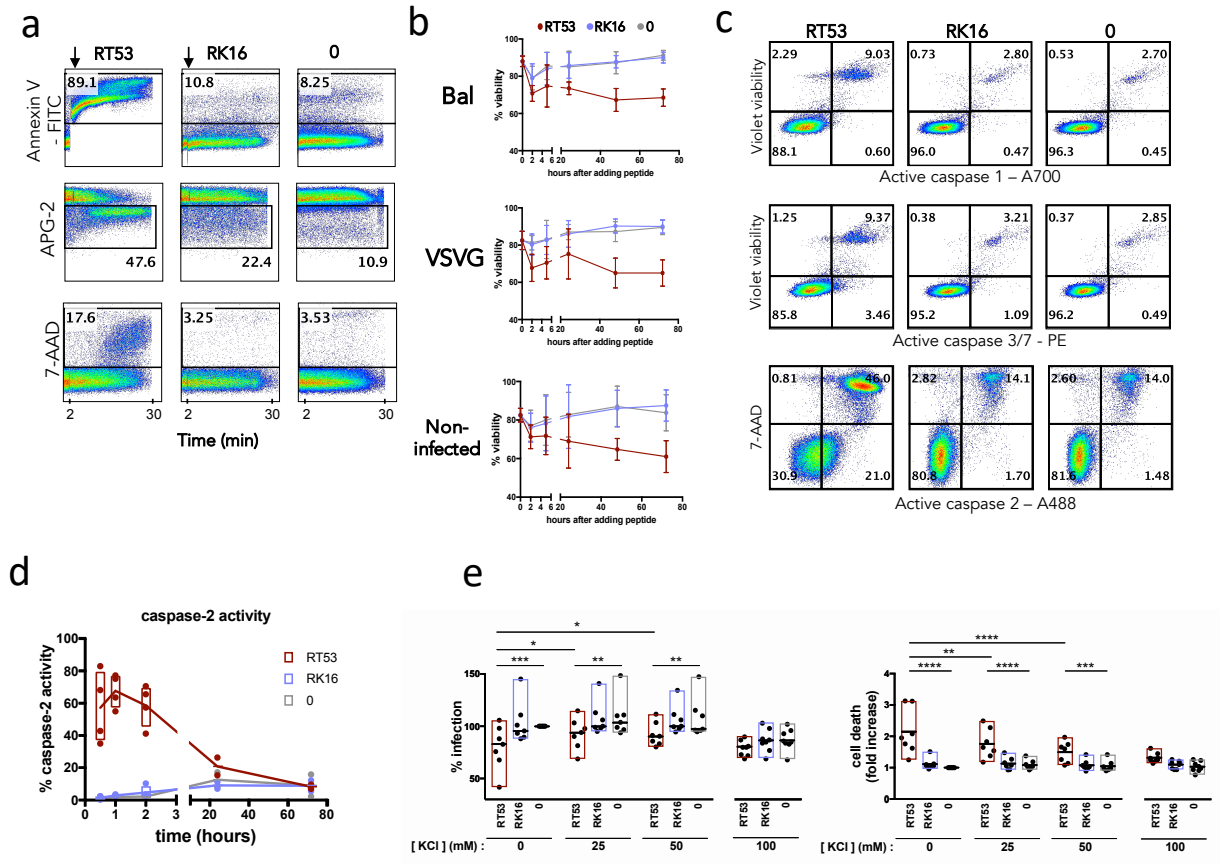

Figure 4. RT53-mediated cell death is rapid and is associated with caspase 2 activity. A. Cells were placed in Annexin V-FITC and 7-AAD staining buffer or loaded with fluorescent K^+ indicator (APG-2) and run on a flow cytometer to acquire fluorescence over time. Time point of peptide addition (after 2 minutes of acquisition) is indicated with an arrow **B**. Cells infected by spinoculation were incubated with 6 μM RT53 or RK16 and viability determined by tryptan blue **C.** Cells were incubated with 6 μM RT53 or RK16 for 2 hours and stained with probes for active caspases **D.** Peptide treated cells were stained with caspase 2 activity probe at various time points post peptide treatment **E.** Cells pre-infected with VSVG-pseudotyped HIV-1 particles (day 3 of infection) were incubated with 6 μM RT53 or RK16 in the presence of various concentrations of KCl for 5 hours. Infection and cell death were then evaluated by flow cytometry. Symbols represent experiments with cells from different donors. Bars represent min-max range with medians indicated.

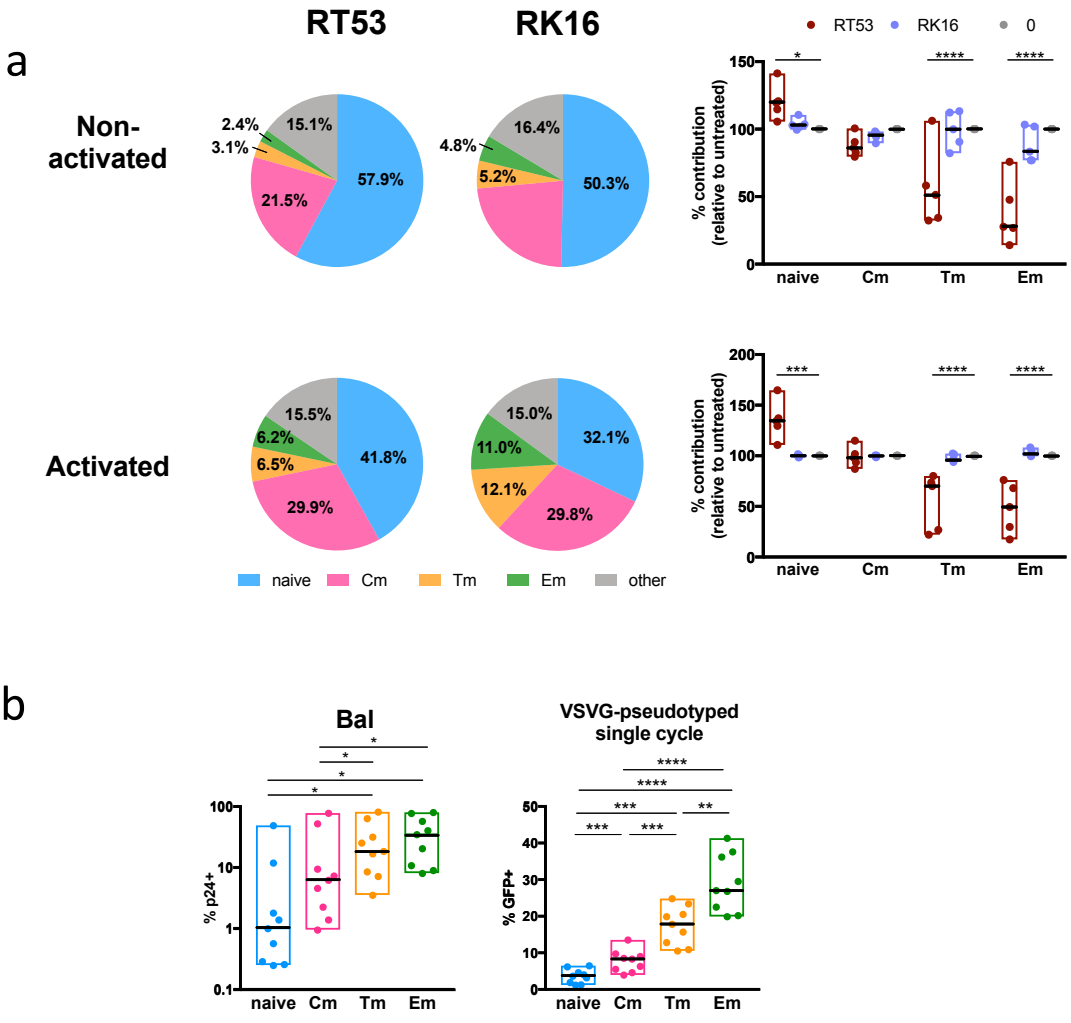

Figure 5. RT53 preferentially targets Tm and Em cells to cell death. A. *In vitro* activated or non-activated CD4+ T cells were pulsed with 6 μM RT53 or RK16 for 5 hours, harvested and stained for markers of CD4+ T cell memory subsets. **B.** *In vitro* activated CD4+ T cells were infected with HIV-1 Bal or VSVG-pseudotyped HIV-1 viral particles. On day 3 of infection, cells were phenotyped for CD4+ T cell memory subpopulations, infection (intracellular p24) and cell death by flow cytometry. % of p24 or GFP+ cells in each subset is indicated. Values are expressed as min-max range with median indicated.

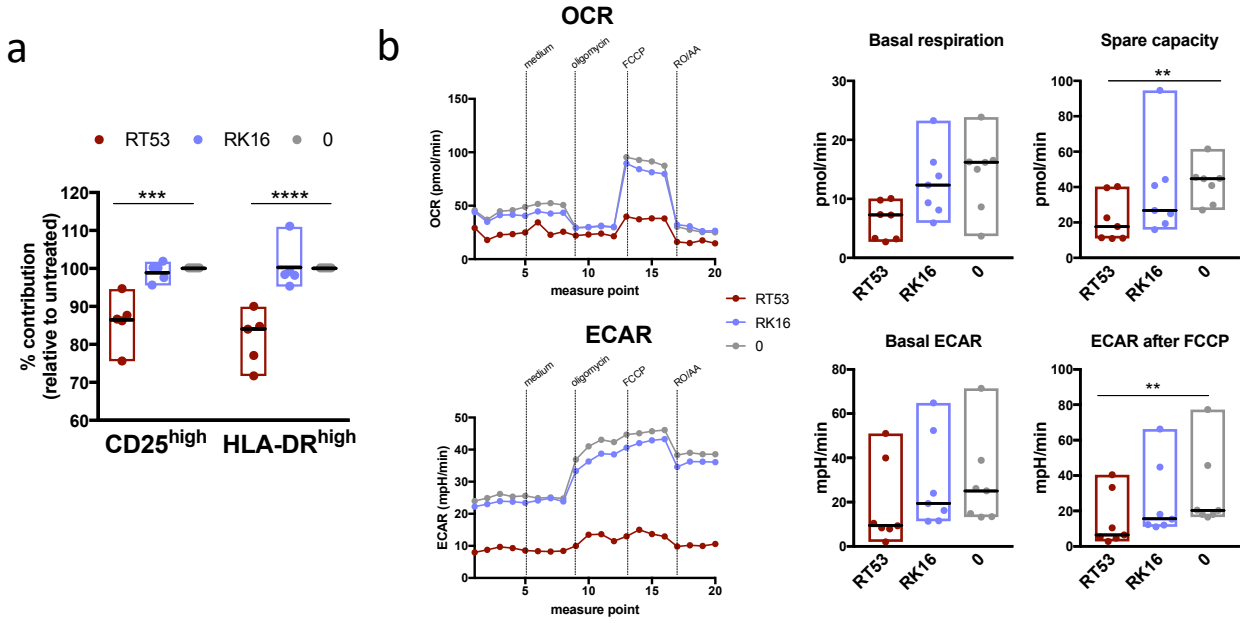

Figure 6. RT53 preferentially targets activated and most metabolically active cells. **A**. *In vitro* activated cells were pulsed with 6 μM RT53 or RK16 for 5 hours. Surface expression of CD25 and HLA-DR was evaluated by flow cytometry **B**. OCR and ECAR values of CD4+ T cells from one representative donor (left). Basal mitochondrial respiration (Basal respiration), spare mitochondrial capacity (Spare capacity), basal glycolysis (basal ECAR) and glycolytic reserve (ECAR after FCCP) obtained with cells from 7 donors (right). Values are expressed as min-max range. Median is indicated.

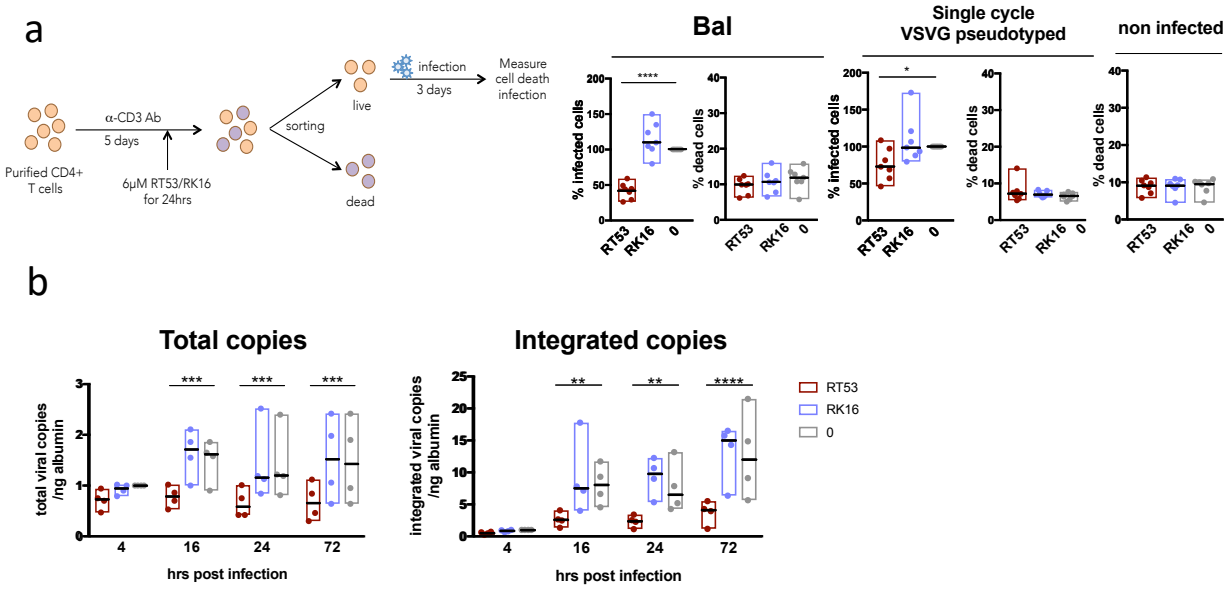

Figure 7. RT53-surviving cells are resistant to HIV infection. A. *In vitro* activated CD4+ T cells were incubated with 6 μM RT53 or RK16 for 24 hours. Live cells were then sorted from dead cells using fluorescently activated cell sorting (FACS) and live cells were infected with indicated HIV-1 strains. Cell death and infection were evaluated by flow cytometry 3 days later. **B.** Total and integrated viral copies evaluated at various time points post-infection in cells infected with VSVG-pseudotyped HIV-1 viral particles and treated with 6 μM RT53 or RK16. Values are normalized to untreated 4 hours post-infection condition and expressed as min-max range with median indicated.

SUPPLEMENTARY INFORMATION

SUPPLEMENTARY METHODS

Immunofluorescence microscopy

Activated CD4+ T cells were incubated with 6 µM RT53 or RK16 (Proteogenix) for 2 hours. Cells were then washed twice with PBS, immobilized on poly-lysine coated coverslips, fixed with 4% paraformaldehyde for 10 minutes at room temperature (RT), neutralized with 50mM NH_4Cl for 10min at room temperature and washed twice with PBS. All antibody incubations and washes were performed in 1% bovine serum albumin (BSA) in PBS. Cells were incubated with primary mouse anti-CD4 antibody (clone OKT4, Tonbo Biosciences, 70-0048-U100) and rabbit polyclonal anti-CCR5 (abcam, ab7346) at 1/100 dilution for 1 hour at RT, washed and incubated with secondary antibodies anti-mouse IgG-A488 (LifeTechnologies, A11029) and anti-rabbit IgG-A647 (LifeTechnologies, A31573) at 1/1000 dilution for 30 minutes at RT. Coverslips were then washed once in PBS and mounted using Fluoromont G (ThermoFisher) mounting medium.

AAC11 Western blot

10^6 cells was pelleted and stored as dry pellet in -80°C until use. The cell pellet was lysed in a lysis buffer (150mM NaCl, 50mM pH 7.4 Tris, 1mM pH 8 EDTA, 1% Triton X-100, containing 1 tablet of complete EDTA-free protease inhibitor cocktail (Sigma) per 10ml in water) for 20min on ice with occasional vortexing. 30 µg total protein, previously denatured in the presence of 1% v/v β-mercaptoethanol, was ran on a 4-15% gradient SDS-PAGE gel (Mini Protean TGX Stain-Free gel, Biorad). Samples were then transferred on PVDF membrane (Dutscher Dominique), blocked in 5% BSA in Tris-Buffered Saline 0.1% tween-20 (TBST) and blotted with anti-AAC11 Ab (abcam, ab99307) at 1/2,500 dilution overnight at 4°C in TBST, washed, and blotted by anti-rabbit HRP (Sigma, A0545) at 1/1,000 dilution in TBST for 45 minutes at RT. Membranes were also blotted with anti-β-actin (Sigma, A5316) at 1/1,000 dilution for 45 minutes, followed by goat anti-mouse HRP (Sigma) as controls. Membrane was developed with ECL reagent (Thermo Scientific).

AAC11 RT-PCR

Cells were collected by centrifugation, lysed in RA1 buffer (Macherey-Nagel) and stored at -80°C until RNA extraction. RNA was purified using RNA isolation kit (Macherey-Nagel) and converted to cDNA using SuperScript II (Thermo Fisher). RT-PCR was performed on 7500 Real Time PCR System (Applied Biosystems) using TaqMan Universal Master Mix II, with UNG (Thermo Fisher) with the AAC11 (Hs00362482_g1) and BECN1(Hs01007018_m1) FAM-MGB-primer-probe pairs (Thermo Fisher). ΔCt was calculated using BECN1 and ΔΔCt was calculated using reference *ex vivo* purified CD4+ T cell sample.

SUPPLEMENTARY FIGURES

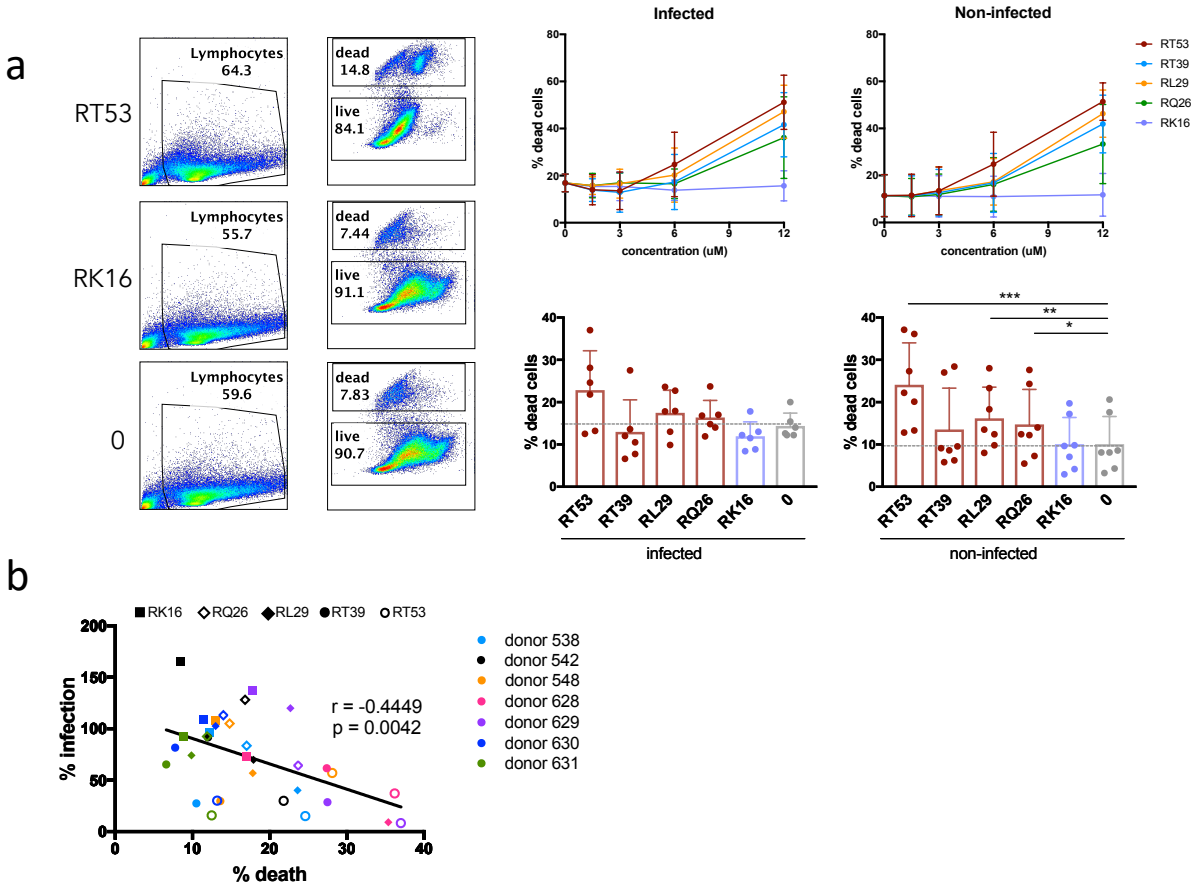

Figure S1. Decrease in HIV-1 infection is associated with cell death

A. Example of flow cytometry gating strategy to evaluate cell death in cultures. *In vitro* activated primary CD4+ T cells were incubated with various concentrations of AAC11-derived peptides after spinoculation with HIV-1 Bal and cell death was evaluated by flow cytometry on day 3 of infection **B**. Proportion of infected cells among live cells vs cell death in different donors' CD4+ T cells incubated with 6 µM indicated peptides.

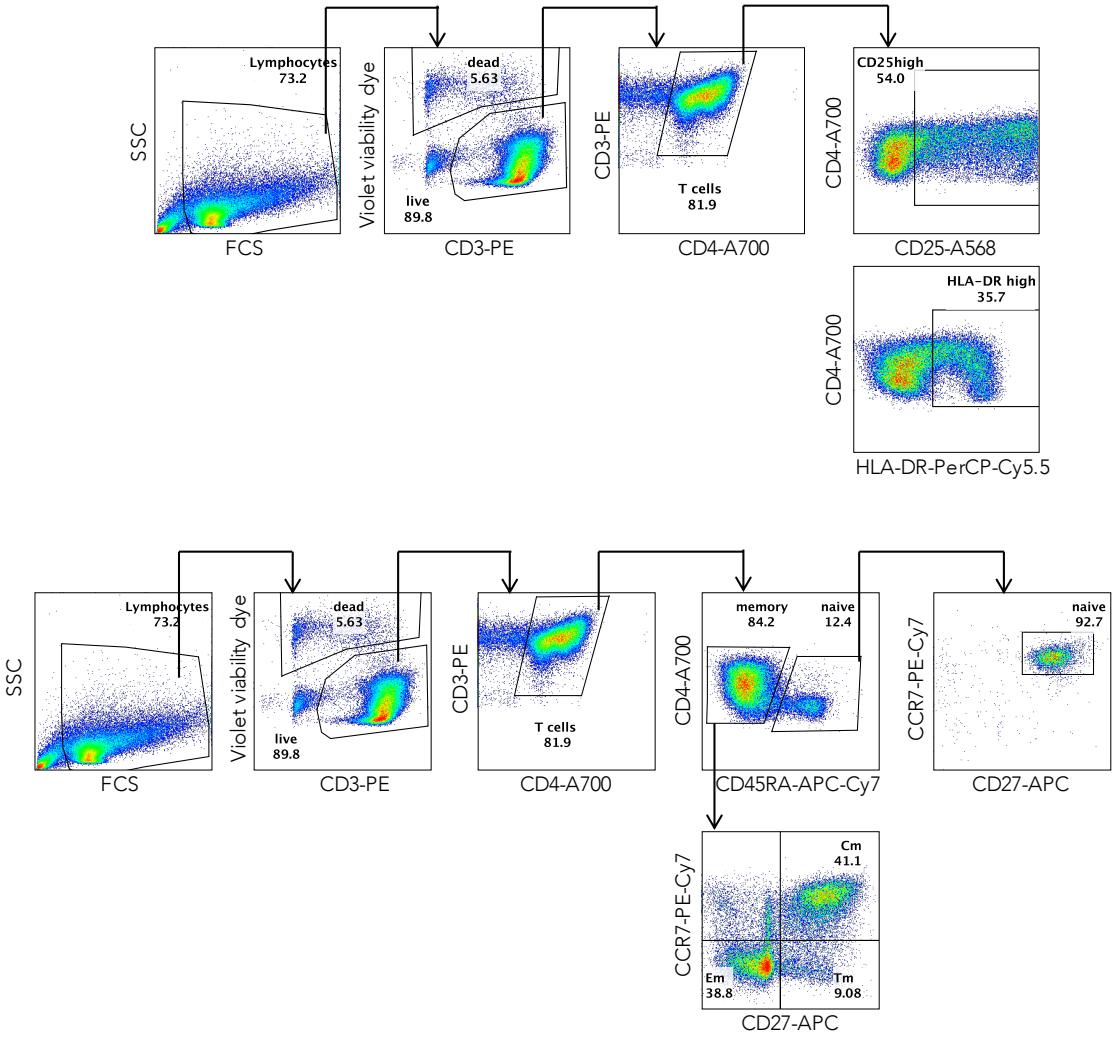

Figure S2. Example of the flow cytometry gating strategy for CD25 and HLA-DR activation markers as well as naïve, Cm, Tm and Em CD4+ T cell subsets.

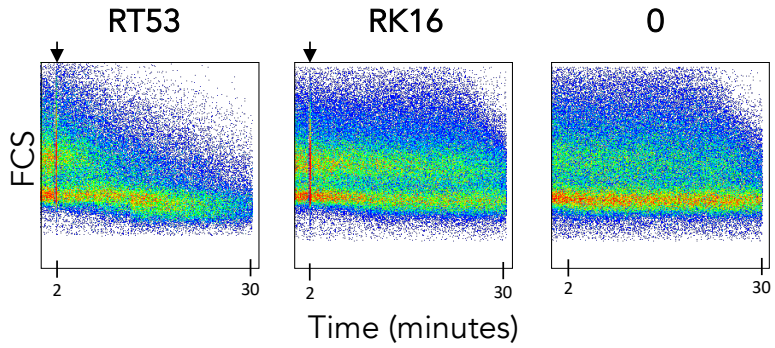

Figure S3. RT53 treatment preferentially targets large cells (evaluated by FSC parameter by real-time flow cytometry).

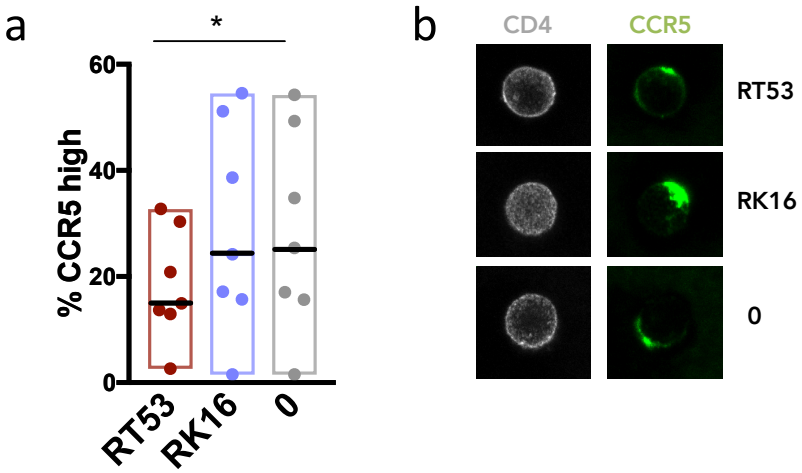

Figure S4. CCR5 expression and distribution upon RT53/RK16 treatment A. Proportion of CCR5^{high} expressing cells and **B**. CCR5 cell surface distribution among *in vitro* activated cells after 5 hours of treatment with 6 µM RT53 or RK16.

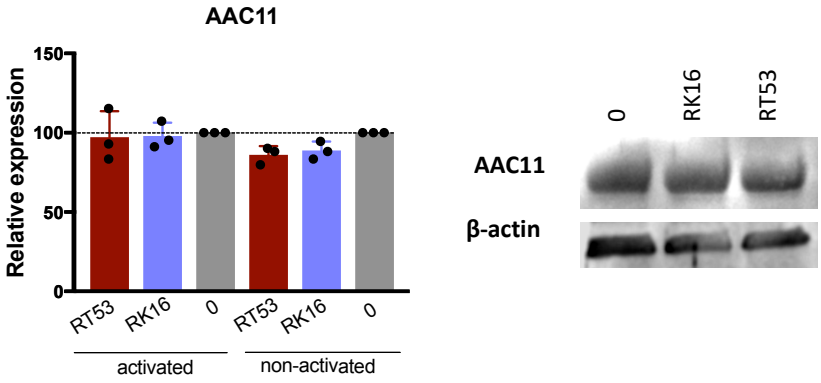

Figure S5. AAC11 gene (*in vitro* activated or non-activated CD4+ T cells) and protein expression (*in vitro* activated CD4+ cells) upon treatment with 6 μM RT53/RK16 for 5 hours.

CHAPTER 2:

INVESTIGATION OF RT53 SPECIFICITY AND MECHANISM OF ACTION

In Chapter 1 of this thesis manuscript, I described that AAC-11-derived peptides preferentially targeted HIV-1 susceptible CD4+ T cell population to cell death and left surviving cells resistant to productive infection. We observed that RT53, the most potent peptide in terms of cell death and anti-viral activity, displayed selectivity for highly differentiated, activated and metabolically active CD4+ T cell subsets. These characteristics have already been described as factors associated with permissibility of cells to HIV-1 infection. We also saw that RT53-mediated cell death was associated with caspase-2 activation. In this chapter, I attempt to further characterize RT53 cell type specificity and the mechanism of action.

Extended insight into the enhanced sensitivity of wild type HIV-1 to the action of RT53

We have shown that AAC-11 derived peptides induced cell death of CD4+ T cell subpopulations that are particularly susceptible to HIV-1 infection. However, infection with WT viruses was more sensitive to the action of the peptides than single cycle VSVG pseudotypes (Chapter 1, Fig 3). We have shown that treatment with RT53 depleted more differentiated cells while enriched the cultures in naïve CD4+ T cells (Chapter 1, Figure 5). We observed similar results when RT53 was added to cultures upon infection with VSVG-pseudotyped particles, showing that such infection did not alter the survival pattern of CD4+ T cell subsets. Indeed, infection with VSVG-pseudotyped particles did not alter the relative distribution of CD4+ T cells when compared to non-infected cultures (Fig 1A). In contrast, Bal infection, in the absence of peptide treatment, already resulted in the some depletion of Em and Tm subsets relative to non-infected control (Fig 1B), presumably due to HIV cytopathic effect. Moreover, we could not observe further change in the relative distribution of CD4+ T cell subsets upon treatment with RT53 in cells that had been exposed to Bal (Fig 1A). These results suggest that infection with wild-type HIV-1 may be modifying the pro-/anti-apoptotic intracellular balance of exposed cells. This is in agreement with previous observations that signaling through HIV-1 Env and Nef (absent in VSVG-pseudotyped HIV-1 particles) induces changes in key molecular regulators of apoptosis (Perfettini et al., 2004)(Perfettini et al., 2005)(Castedo et al., 2002)(Genini et al., 2000)(Cooper et al., 2013b)(Wolf et al., 2001b).

We next investigated cell death and HIV inhibitory capacity of RT53 when added at various time points post-infection with HIV-1 Bal (Fig 1C). RT53 potently blocked infection when added at 0, 24 or 72 hours post-infection. However while the amount of cell death induced by the peptide did not change (when compared to control cells at the same time points), RT53 was most effective in blocking Bal when added at the earlier time points. This indicated to us that RT53 1) may induce an extra block to infection with Bal in cells that survive treatment with the peptide and that this effect may be partially lost once the virus is integrated, and/or 2) Bal infection could be modifying infected cell survival to RT53 treatment, as hypothesized above, and this effect may wane overtime.

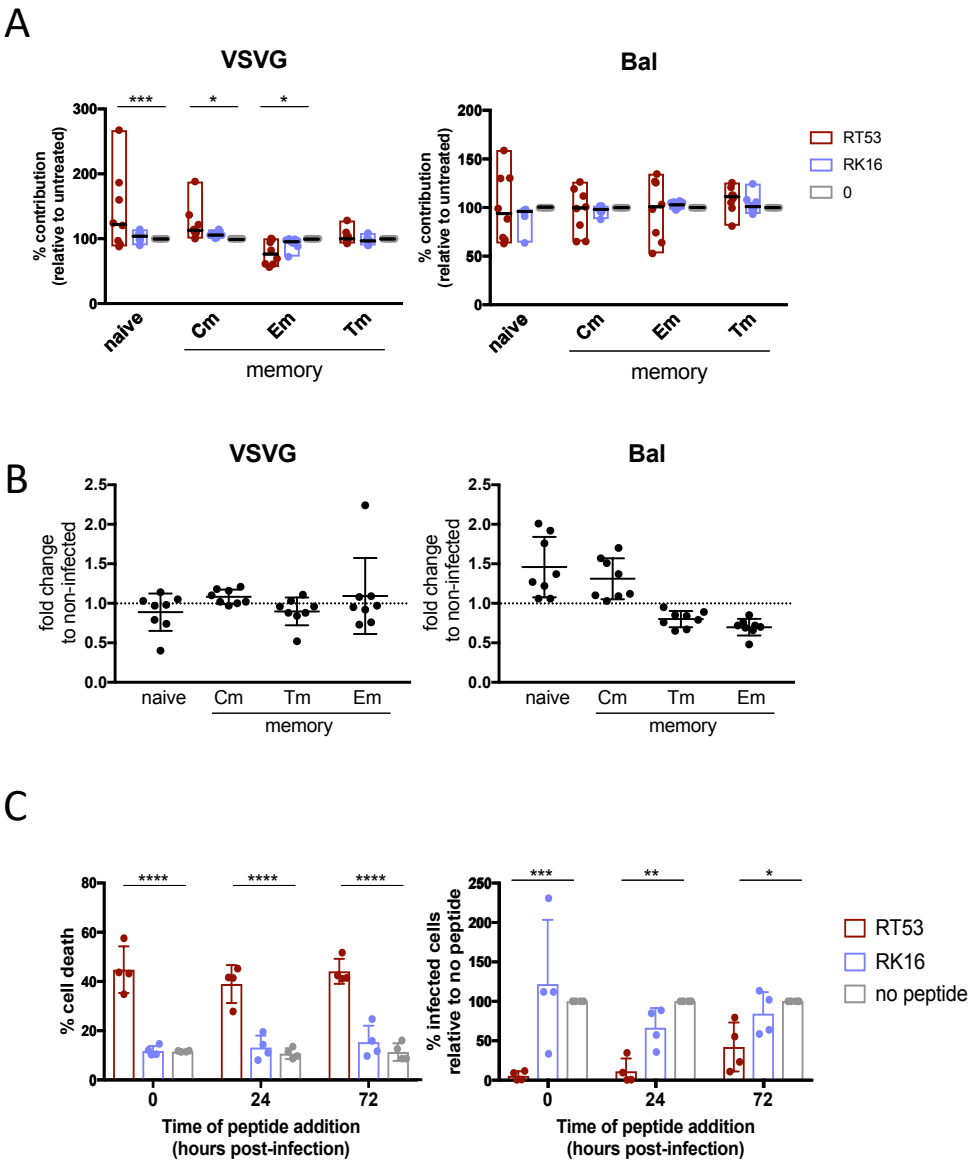

Figure 1. **Bal infection modifies cell survival to RT53 treatment. A.** Suboptimally activated CD4+ T cells were infected with indicated HIV-1 viral strains and pulsed with 6 µM RT53 or RK16. Cells were collected and phenotyped by flow cytometry on day 3 of infection. **B.** Relative contribution of CD4+ T cell memory subsets to the total CD4+ T cell pool on day 3 post-infection with indicated viral strains in the absence of peptide treatment. **C.** Activated CD4+ T cells were infected with HIV-1 Bal and 6 µM RT53 or RK16 were added at indicated hours post-infection. The amount of cell death and infection were quantified by flow cytometry on day 3 post-infection.

Investigation of RT53 cell type specificity

RT53-induced mortality is not specific to CD4+ T cells

Our previous results suggest that AAC-11 might be involved in the regulation of the CD4+ T cell response, and we hypothesize that the AAC-11 survival pathway may be also active in other blood cell fractions. We thus analyzed the effect of RT53-treatment on different subpopulations of peripheral blood mononuclear cells (PBMCs) *ex vivo*. We evaluated the levels of cell death in B cells, CD8+ and CD4+ T cells, NK cells and monocytes after 5 hour treatment with RT53 or RK16 (Fig 2A). RT53 caused mortality in B cells, CD4+ and CD8+ T cells but not in NK cells or monocytes (Fig 2B). Therefore, AAC-11 survival pathway targeted by RT53 may be important/specific for small lymphocyte development, maturation and survival but not for maintenance of other subsets.

Interestingly, RT53 treatment was not cytotoxic to CD4+ T cell lines Jurkat and SupT1 at 6μM peptide concentration, at significant cytotoxicity was observed in primary CD4+ T cells (Chapter 1, Fig S1), and only produced some cell death at 8μM. The impact on infection was, at best, very modest even at 8μM (Fig 2C). Thus, cancer cell lines are less sensitive to the action of RT53 than primary T cells, which is consistent with known overexpression of AAC-11 survival pathway in cancer cells. In addition the absence of significant effect of RT53 on infection levels in these cell lines is likely linked to the overall enhanced susceptibility of these cells to HIV-1 infection, perhaps related to their heightened metabolism.

The absence of dramatic cytotoxic effects of RT53 on monocytes made us wonder whether RT53 may cause mortality and inhibition of infection in macrophages, one of the major targets for HIV infection that could play an important role in HIV-1 persistence due to their longevity. To evaluate this, CD14+ monocytes were purified from human PBMCs and cultured for 6 days for differentiation into macrophages (Fig 2D). Cells were then infected with Bal or VSVG-pseudotyped single cycle HIV-1 and pulsed with 6 μM RT53 or RK16. Infection and cell death were evaluated 3 days later. Similarly to the results observed in CD4+ T cells, RT53 resulted in

a drastic decrease in Bal infection of primary human macrophages but the effect on VSVG-pseudotyped viral particle infection was heterogeneous (Fig 2D). Interestingly, variable levels of cell death were observed. Therefore, RT53 is able to inhibit wild-type multi-cycle HIV-1 infection in primary macrophages. However, the sensitivity of monocytes and macrophages to RT53-induced cell death needs to be investigated in more detail.

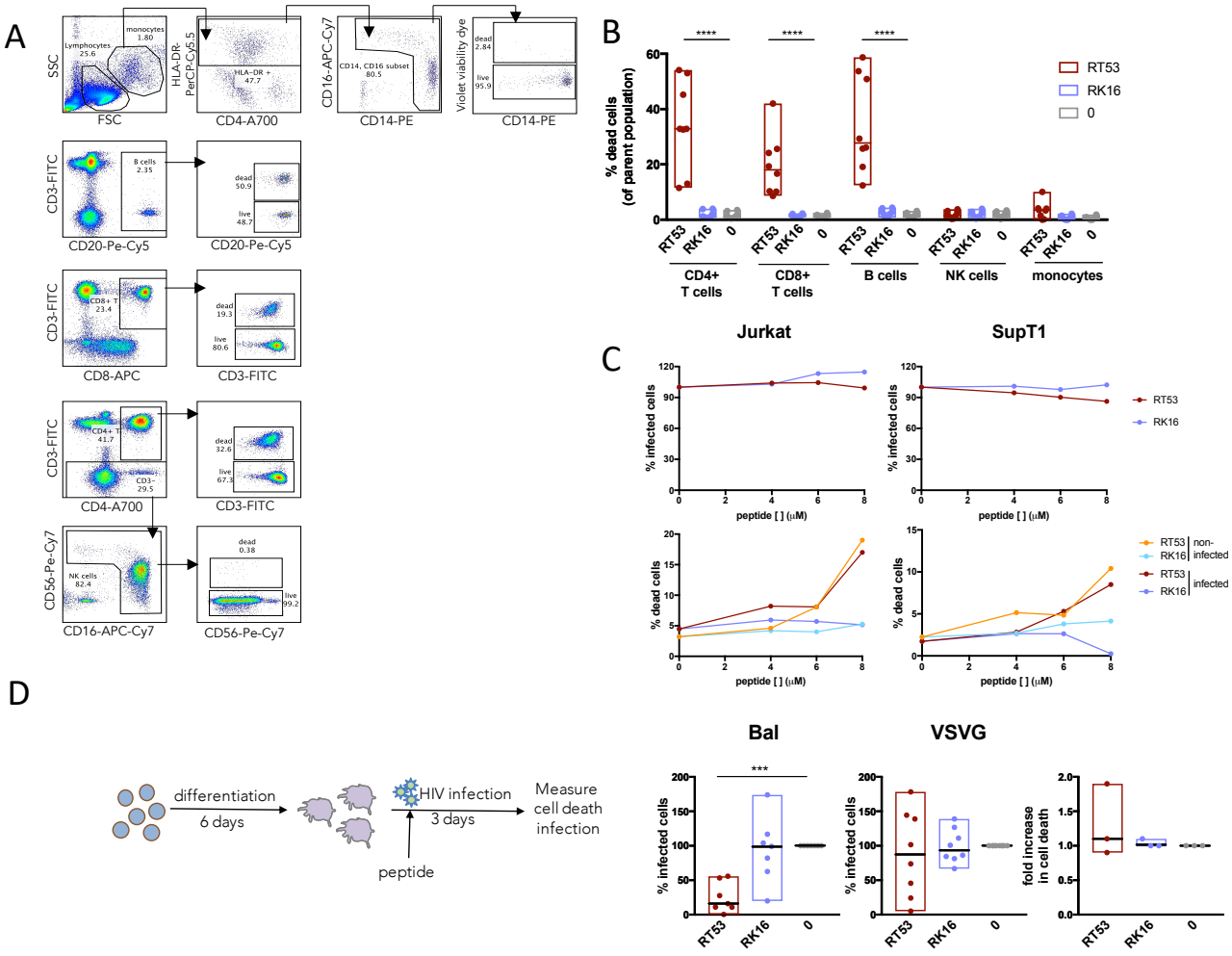

Figure 2. RT53 selectively targets small lymphocytes to cell death and inhibits HIV-1 infection in macrophages. A. Gating strategy for PBMC subsets. **B.** *Ex vivo* purified PBMCs were treated with 6 µM RT53 or RK16 for 5 hours prior to flow cytometry staining **C.** Jurkat and SupT1 CD4+ T cell lines were infected with VSVG-pseudotyped HIV-1 particles and incubated with 6 µM RT53 or RK16 for 3 days. % infected cells is normalized to infected cells not treated with peptides **D.** The effect of RT53 and RK16 on primary human macrophages infection and cell death. Min-max range and medians are indicated.

DNA double strand breaks (DSB) as a marker of RT53-susceptible cell population

Earlier in our experiments we observed a strong activation of caspase-2 in cells treated with RT53 (Chapter 1, Fig 4D). Caspase-2 activation is most known to trigger apoptosis in response to DNA damage (Miles et al., 2017). In this setting, *a*taxia *t*elangiectasia *m*utated (ATM) is recruited to DSB, where it induces the formation of the PIDDosome and activation of caspase-2 (Tinel and Tschopp, 2004). Upon activation, caspase-2 cleaves and activates various mediators of apoptosis. The presence of DSB in cells in non-cytotoxic conditions is linked to their cell cycle phase (ie DSB during DNA replication). Different CD4+ T cell subsets are known to exibit different cycling states in the blood. Naïve cells are mostly non-cycling, followed by intermediate cycling in Cm and high cycling in Em cells (Thome et al., 2014). On the other hand, the presence of DSB in memory T cell subsets has also been linked to telomere shortening and predisposition to cell senesence in more differentiated memory subsets (Mitri et al., 2011). During HIV infection, DSBs are created during integration and are repaired by cellular DNA damage repair machinery. We, therefore, wondered if AAC-11 survival pathway might repress caspase-2 induced apoptosis in CD4+ T cell memory subsets with DSB acquired during DNA replication. We thus evaluated the proportion of cells with DSB in CD4+ T cells. We measured DSB by flow cytometric detection of H2AX histone phosphorylated at Ser139 (also known as γH2AX), a common marker for DSB (Kuo and Yang, 2008). We reasoned that if RT53 triggers cell death mechanism in cells with DSB, we would see a selective depletion of a cell population expressing γH2AX. Similarly to previous reports (Mitri et al., 2011), we observed higher expression of γH2AX in Em and Tm cells followed by Cm and finally only a low level of γH2AX in naïve cells (Fig 3A). We saw that CD4+ T cells with high expression of γH2AX were preferentially depleted, whereas γH2AX-low cells were enriched by RT53 treatment (Fig 3B) This correlated well with the preferential targeting of Em and Tm cells by RT53. $\gamma H2AX^{high}$ population was depleted in all subsets, even in naïve cells (Fig 3C). However, RT53 treatment also depleted Em and Tm cells with low levels of γH2AX (Fig 3A). Thus, although cells with high levels of DSB appeared to be more sensitive to the action of RT53, DSB level was not an absolute marker for RT53 susceptibility.

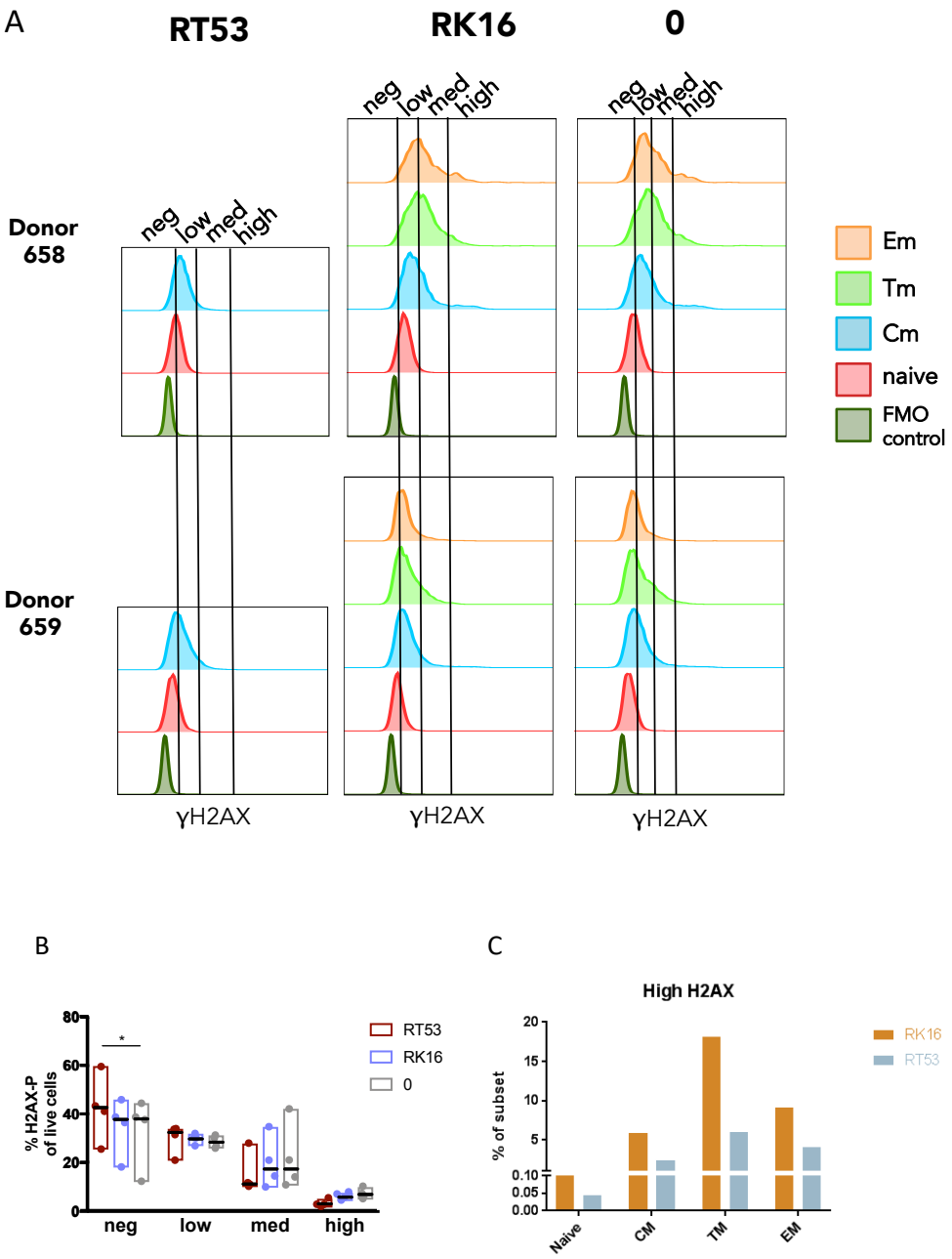

Figure 3. RT53 preferentially targets cells with high proportion of DSB A. The expression of γH2AX across CD4+ T cell memory subsets upon treatment with RT53/RK16. Gating to identify positive population was performed using fluorescence minus one (FMO) control **B.** The amount of DSB in remaining total live cells after peptide treatment according to the levels of expression of γH2AX **C**. Percent of γH2AX^{high} population among CD4+ T cell memory subsets after treatment with RT53 or RK16.

Identification of cellular partners of RT53

RT53 partially localizes to the same cell compartment as AAC-11

Although we have found that the transcript levels of AAC-11 in the different subpopulations of memory CD4+ T cells at the time of infection correlated with infection levels on day 3 post-infection in (Chapter 1, Fig 1), we did not observe any change in AAC-11 expression upon treatment with RT53, at least as measured by qPCR or western blot on bulk CD4+ T cells (Chapter 1, Fig S5). This is consistent with previously published observation that RT53's mechanism of action did not affect AAC-11 expression (Jagot-Lacoussiere et al., 2016) and it is probably related to interaction of RT53 with an AAC-11 partner that is expressed only in some of the cells upregulating AAC-11. In order to identify putative partners for RT53 we used fluorescently labelled peptides to determine their cellular localization. As expected RK16 penetratin was found mostly intracellularly (Fig 4A). In contrast, RT53 predominantly localized at the cell surface, clustered in what looked like a micro-domain or a membrane patch, probably retained there via interaction of AAC-11 LZ domain with a membrane partner. It is important to notice that RT53 was also found intracellularly, although at lower levels, where AAC-11 was localized itself. Infection or RT53 did not appear to change the cellular distribution of AAC-11, which remained mostly nuclear and some cytoplasmic (Fig 4A and B). These results suggest that one main RT53 interactor is located at the plasma membrane, although it is unclear whether the cytotoxic action of the peptides was related to the fraction retained at the membrane or the molecules that reached the intracellular compartments.

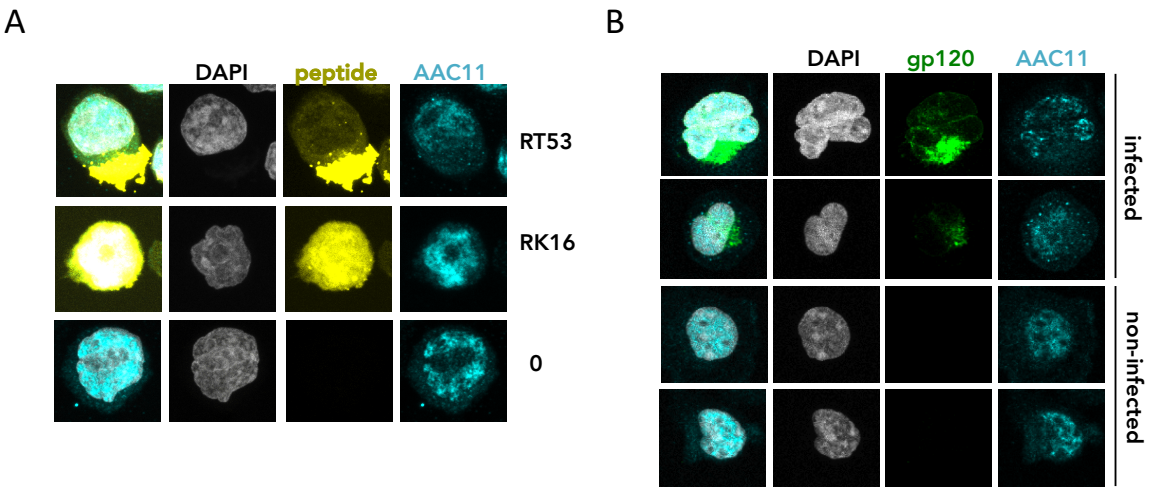

Figure 4. Cellular distribution of RT53/RK16 and AAC-11 after peptide treatment and infection A. Immunofluorescence of intracellular AAC-11 upon 2 hour treatment with 6 µM RT53-Rd or RK16-Rd B. Immunofluorescence of intracellular AAC-11 in Bal infected (gp120 positive) and non-infected CD4+ T cells.

Investigation of potential RT53 molecular partners

RT53 is expected to interact with an AAC-11 repressed partner thus unleashing a cell death cascade (Jagot-Lacoussiere et al., 2016). We, therefore, attempted to identify such a molecule. We first turned to Acinus, a known AAC-11 partner (Rigou et al., 2009), which mediates chromatin condensation (Sahara et al., 1999)(Hu et al., 2005) and nuclear DNA fragmentation (Joselin et al., 2006) during apoptosis. Acinus was also proposed to play a role in HIV-1 splicing (Rodor et al., 2016; Wojcechowskyj et al., 2013). We evaluated Acinus expression and localization upon infection and peptide treatment. We did not see any drastic changes in Acinus expression and localization upon cell activation or infection (Fig 5A, B). There were also no changes in Acinus expression or localization after cell treatment with RT53/RK16 (Fig 5C, D). These data suggest that RT53 does not act directly act through Acinus to induce cell death.

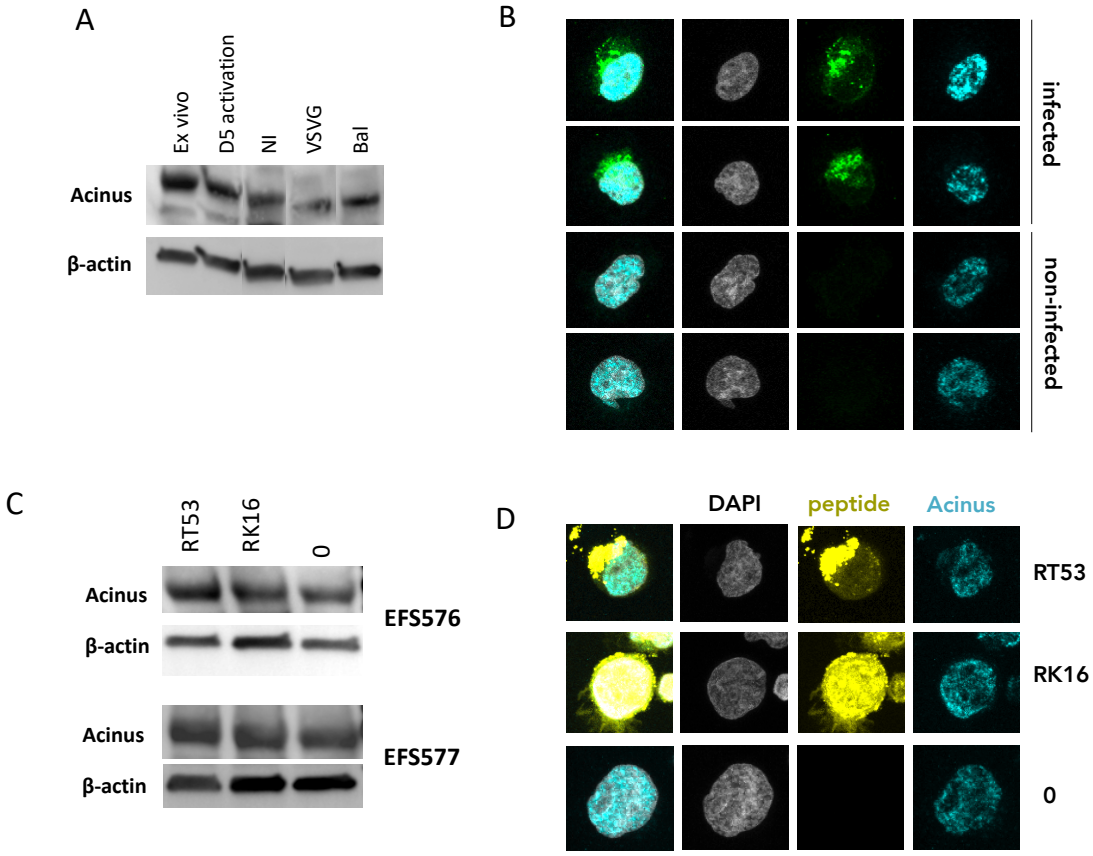

Figure 5. Impact of infection and RT53/RK16 treatment on Acinus in CD4+ T cells A. Expression of Acinus in *ex vivo* purified, activated (day 5), non-infected (NI) or infected (VSVG and Bal) (day 7 of infection) CD4+ T cells **B.** Acinus expression by immunofluorescence in infected (gp120 positive) and non-infected CD4+ T cells **C.** Expression of Acinus by Western blot upon 6 µM RT53 or RK16 treatment in CD4+ T cells from two donors (EFS576 and EFS577) **D.** Expression of Acinus in RT53-Rd or RK16-Rd (6 µM) treated CD4+ T cells.

Dr. Jean-Luc Poyet and his team used HuT78 T cell line to perform pull-down experiments followed by Mass Spectrometry to identify potential cellular partners of AAC-11-derived peptides. These experiments identified ATP synthase subunit β (ATP5B), L-lactate dehydrogenase α chain (LDHA), α-enolase (ENO1) and serine/threonine-protein kinase PAK1 (PAK1), among others. We thus set out to explore if any of these proteins could be the interacting partners of RT53 in our system. As before, we reasoned that if RT53 targets a cell

population expressing high levels of these proteins, this population would be preferentially depleted leaving surviving cells expressing lower levels of the protein of interest. We, therefore, evaluated the expression of these candidates first by PCR (Fig 6A) and then by flow cytometry (Fig 6B). We could not observe any consistent difference in the expression (at the gene or protein level) of these factors upon treatment with RT53 when compare to RK16 in experiments performed with cells from three donors. These results contrasted with the increased in RT53 induced cell mortality that was always observed in the same experiments. Like in the case of Acinus, our results, however, do not definitively exclude these candidates as potential interacting partners of RT53. It is possible that RT53 interacts with some or all of these molecules but only causes an effect in the downstream signaling pathway that is triggered only in a subset of cells. The cell surface and cytoplasm expression (and not total expression) of these proteins needs to be evaluated separately in the cell subsets that we know are more susceptible to the action of RT53.

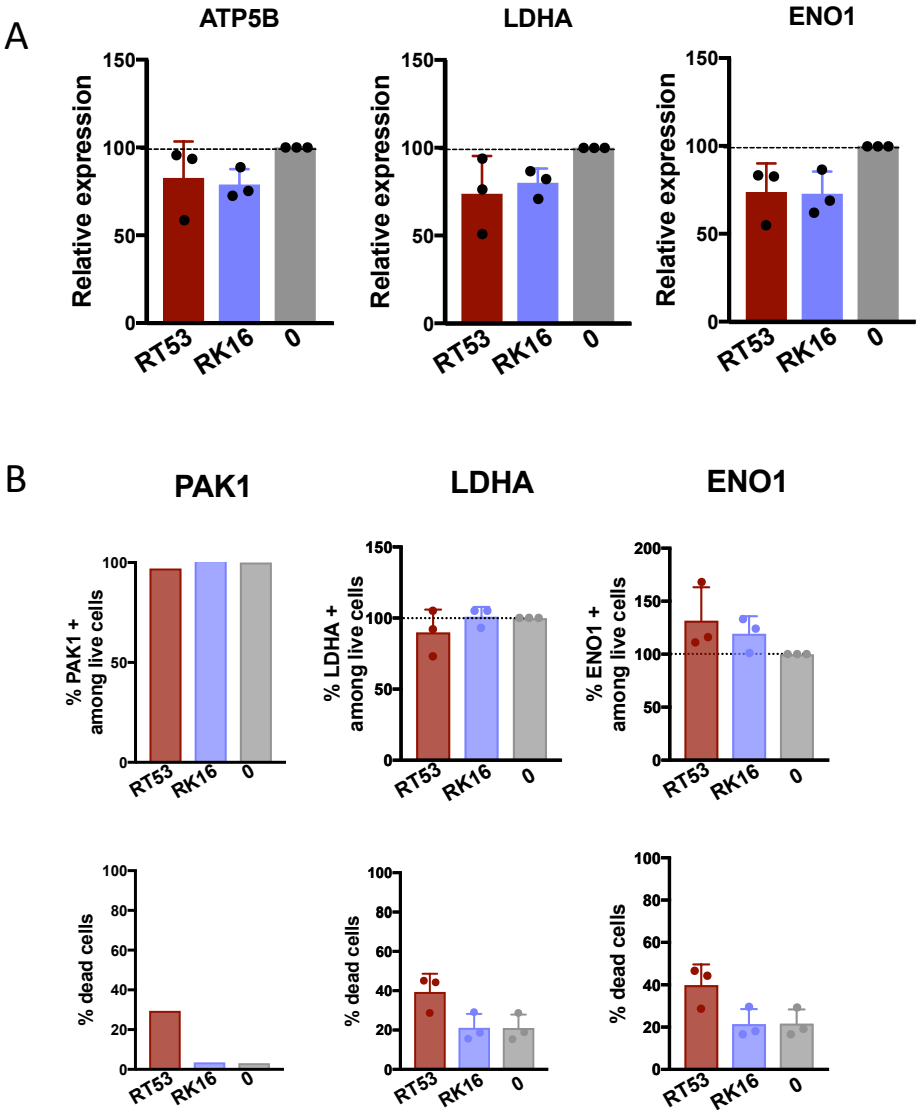

Figure 6. Changes in mRNA and protein expression of potential partners of RT53 peptide A. mRNA expression of ATP5B, LDHA and ENO1 upon 5 hours treatment with 6 µM RT53/RK16 **B.** Expression of indicated proteins evaluated flow cytometry upon 5 hours treatment with 6 µM RT53/RK16.

Role of ion homeostasis and cation channels in the action of RT53

Ca^{2+} homeostasis plays a role in RT53 mechanism of action

We had observed that K^+ efflux due to RT53 treatment in our cells and high concentrations of extracellular K^+ prevented RT53-induced cell death and preserved infectability of the cells (Chapter 1, Fig 4E). Several types of ion channels exist in T lymphocytes. Voltage-gated K^+ channels ($K_{v1.3}$) are activated in response to PM depolarization (inside of the cell becoming more positive) due to Ca^{2+} influx in response to TCR stimulation, which leads to K^+ efflux from the cell to restore resting membrane potential. $K_{Ca3.1}$ channels also mediate K^+ efflux, but are activated by the rise in cytoplasmic Ca^{2+}. Ca^{2+} release-activated Ca^{2+} (CRAC) channels are activated in response to depletion of ER Ca^{2+} stores and lead to Ca^{2+} influx (Cahalan and Chandy, 2009). Transient receptor potential cation channel subfamily M member 4 (TPRM4) is a cation channel that is more permeable to Na^+ than Ca^{2+}. It is activated by Ca^{2+} influx following TCR stimulation and results in Na^+ influx thus depolarizing the membrane and limiting Ca^{2+} influx (Feske et al., 2015b).

We previously demonstrated that RT53 causes a decrease in intracellular K^+ concentration (Chapter 1, Fig 4A), which we linked to RT53-mediated cell death. Ca^{2+} ions were also reported to activate molecular pathways of apoptosis. For example, cytoplasmic Ca^{2+} overload was reported to stimulate apoptosis via opening of MPTP (Kondratskyi et al., 2015). To determine if Ca^{2+} could also be linked to RT53-activated cell death pathway, we incubated cells for 5 hours in the presence of 6μM RT53 or RK16 ± 1mM ethylenediaminetetraacetic acid (EDTA) to chelate Ca^{2+} ions from the extracellular medium. The presence of EDTA in the extracellular medium further increased susceptibility of cells to RT53-mediated cell death (Fig 7A). We next tested if elevated extracellular Ca^{2+} could protect cells from RT53-triggered cell death. Indeed, similarly to what we observed with KCl, the addition of exogenous $CaCl_2$ decreased cell death and preserved susceptibility of CD4+ T cells to HIV-1 BaL and VSVG-pseudotyped HIV-1 infected particles (Fig 7B). The effect of $CaCl_2$ was dose dependent and cell death reached baseline at 50mM $CaCl_2$ (half of the molar ratio that was needed to suppress cell death with

KCl). Interestingly, we observed that cell death directly induced by infection with HIV-1 Bal, but not with VSVG-pseudotyped particles, increased with $CaCl_2$ concentrations (Fig 7B). This was accompanied by a decrease in infection with HIV-1 Bal at higher (50mM) $CaCl_2$ concentration in the absence of peptides. These results may reflect previously described changes in ion homeostasis induced by HIV Env and Nef proteins to enhance HIV replication (Séror et al., 2011)(Voss et al., 1996).

An inter-connected network of ion channels in the cell's membranes tightly regulates signaling and membrane potential (Bose et al., 2015). Cell hyperpolarization (the inside of the cell becoming more negative) by K^+ efflux is known to trigger influx of Ca^{2+} or Na^+ through TPRM channel to restore resting membrane potential of around -70mV (Bose et al., 2015). It is thus possible that activation of inward rectifying cation current of Ca^{2+} or Na^+ (not tested here) could act as a mechanism to protect the cell against hyperpolarization-driven cell death. RT53 could thus be interacting with K^+ ion channels to drive them into open conformation to induce K^+ efflux or K^+ efflux may be the result of RT53 directly inducing membrane pores upon interaction with another membrane partner (as already suggested in (Jagot-Lacoussiere et al., 2016)).

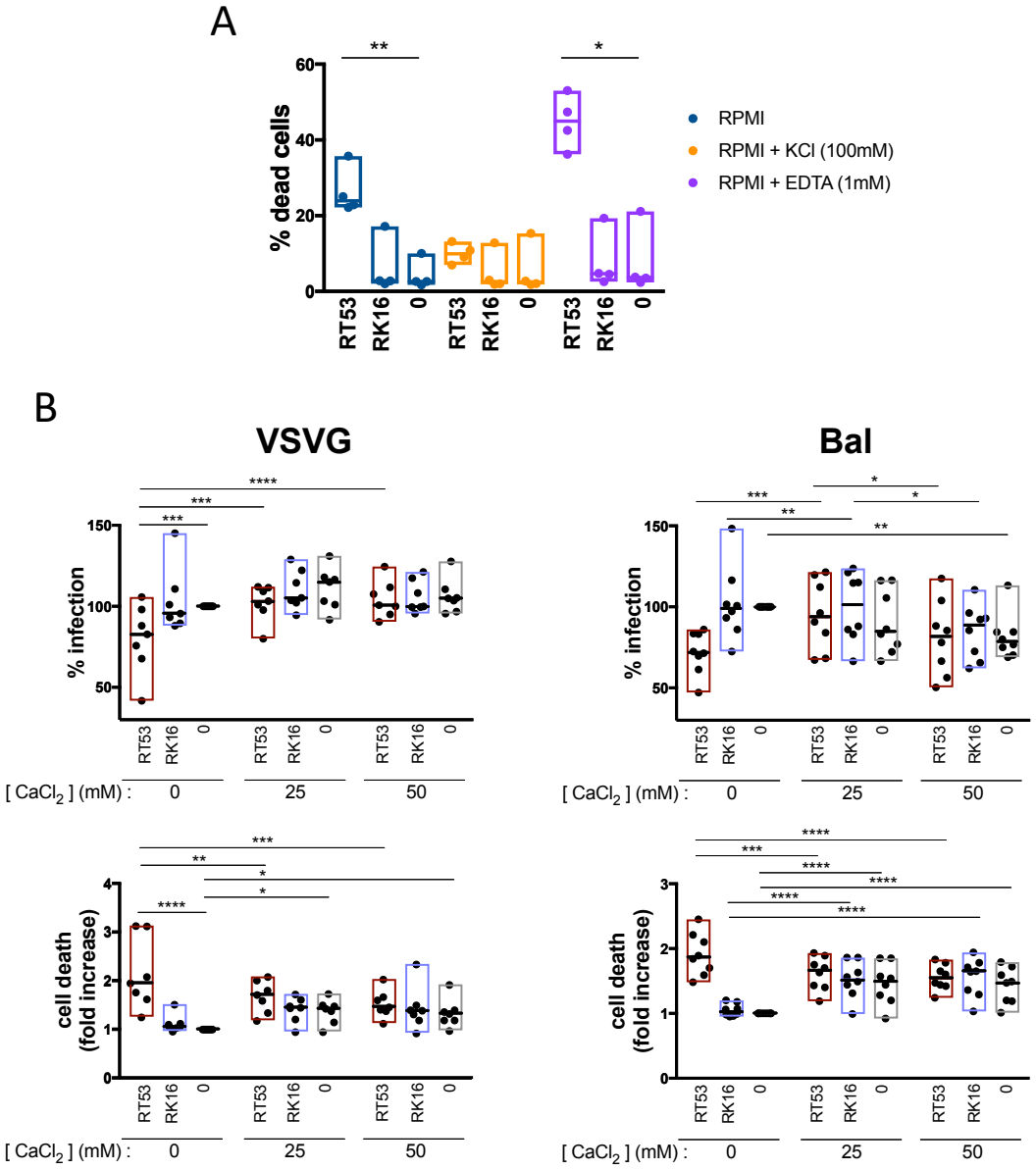

Figure 7. Ca^{2+} homeostasis plays a role in cell susceptibility to RT53-mediated cell death and infection A. CD4+ T cells were incubated with 6 µM RT53 or RK16 for 5 hours in the presence of 100mM KCl or 1mM EDTA. Cell death was evaluated by flow cytometry **B**. Elevated extracellular Ca^{2+} rescues cells from RT53-mediated cell death and inhibition of infection. Activated CD4+ T cells were infected with VSVG-HIV-1 or Bal for 3 days and then incubated with 6 µM RT53 or RK16 for 5 hours in the presence of the cell medium containing various concentrations of $CaCl_2$. Cell death and infection were quantified by flow cytometry.

Investigation of Na^+/K^+ ATPase (NKA) subunit expression upon peptide treatment

NKA ensures high intracellular K^+ and low intracellular Na^+ concentrations by pumping 2 K^+ ions inside and 3 Na^+ ions outside the cell. This is accomplished via energy derived from the hydrolysis of an ATP molecule (Banerjee and Xie, 2016) (Fig 8A). Thus, alternatively to RT53-mediated opening of K^+ channels, AAC-11-derived peptides could inhibit NKA function ultimately reducing intracellular K^+ concentration. NKA is a transmembrane heterodimer composed of 1 α and 1 β subunit. All subunits have transmembrane topology. The α subunit has a large cytoplasmic domain, which contains an ATP binding pocket. The β subunit, on the other hand, contains a large extracellular domain. A small transmembrane protein called the γ subunit is also found associated with NKA but will not be considered here because most of it is embedded in the membrane (Fig 8A). There are 4 known isotypes of the α subunit (ATP1A1-4) and 3 of the β subunit (ATP1B1-3). NKA inhibition by cardiac glycosides such as digitoxin, digoxin or ouabain was already implicated in the inhibition of several alpha viruses including Chikungunya, Ross River virus and Sindbis virus at post-entry level (Ashbrook et al., 2016). Recently, NKA also became implicated in HIV-1 infection. Expression of CD298, isoform 3 of NKA β subunit was strongly correlated with permissibility of primary CD4+ T cells to infection (Rato et al., 2017), although if CD298 expression in HIV-1 susceptibility is related to its function as a subunit of NKA is unclear.

Interestingly preliminary results by JL Poyet lab point to ATP1A4 as one possible interactor with AAC-11-derived peptides. We thus analyzed the expression of various isoforms of NKA α and β subunits in primary CD4+ T cells (Fig 8B). As before, we reasoned that if RT53 is targeting one of the NKA subunits, we would see a decrease in its expression due to selective death of cells expressing high levels of this subunit. The mRNA expression of isoforms 1, 3 and 4 (ATP1A1, ATP1A3 and ATP1A4 respectively) but not 2 (ATP1A2) of the α subunit was detected in CD4+ T cells. The mRNA expression of all three isoforms of the β subunit was detected (Fig 8C). The expression of ATP1A3 and ATP1A4 decreased with CD4+ T cell activation while the expression of ATP1B3 increased (Fig 8B and C). However, none of the subunits mRNA expression decreased consistently across donors upon RT53 treatment. This

provided us with limited information on the regulation of NKA upon RT53 treatment, however, as NKA is known to have a complex post-translational regulation. Therefore, we next proceeded to evaluate the expression levels of ATP1A1 on a protein level by flow cytometry. We saw some decrease in the expression of ATP1A1 protein (Fig 8D) although as before more thorough analyses of the expression of ATP1A1 at the CD4+ T cell subset level will be necessary to understand the implication of this protein in the action of RT53. The expression of the rest of the subunits is ongoing. Additionally, to identify if RT53 interacts with any of the NKA subunits on the cell surface, subunit-specific immunofluorescence experiments to see potential RT53 and NKA subunit co-localization need to be performed.

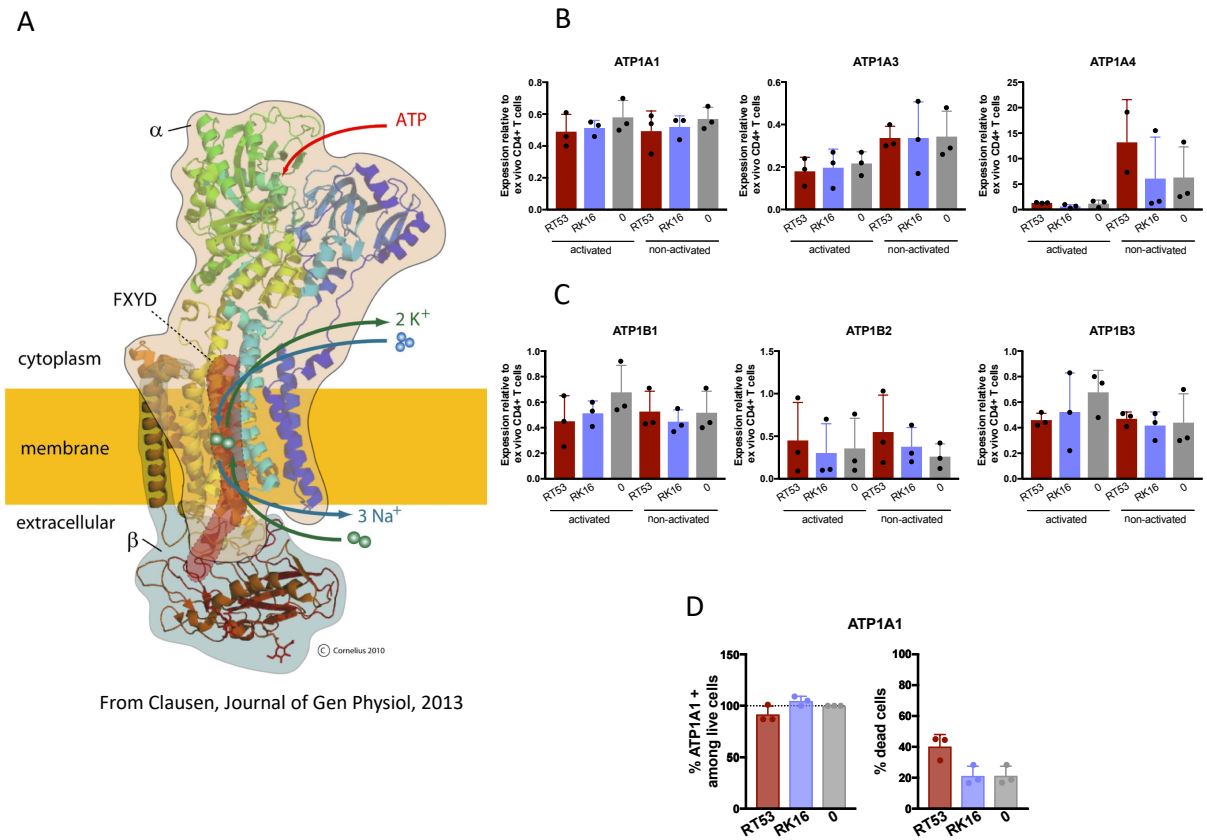

Figure 8. Expression of Na^+/K^+ ATPase subunits with CD4+ T cell activation and treatment with RT53 or RK16 A. Structure of NKA subunits and their associated function **B**. Cells were subjected to 6 μM RT53/RK16 for 5 hours and mRNA/protein levels were then evaluated by PCR or flow cytometry respectively. Changes in NKA α subunit and **C**. in NKA β subunit mRNA expression upon CD4+ T cell activation and peptide treatment **D**. Expression α subunit isoform 1 upon peptide treatment evaluated by flow cytometry.

Materials and Methods

Peptides, antibodies and probes

Peptides (Proteogenix) were received as dry powder and reconstituted with water for use. Peptide sequences are as follows.

RT53: RQIKIWFQNRRMKWKKAKLNAEKLKDFKIRLQYFARGLQVYIRQLRLALQGKT,

RT39: RQIKIWFQNRRMKWKKLQYFARGLQVYIRQLRLALQGKT,

RL29: RQIKIWFQNRRMKWKKYFARGLQVYIRQL,

RQ26: RQIKIWFQNRRMKWKKLQYFARGLLQ,

RK16: RQIKIWFQNRRMKWKK.

Antibodies and dyes used for flow cytometry and FACS sorting are as follows: LIVE/DEAD™ Violet Viability dye (ThermoFisher), CD3-PE (clone SK7, Biolegend), CD4-A700 (clone OKT4, eBioscience), CD45RA-APC-Cy7 (clone HI100, Biolegend), CCR7-Pe-Cy7 (clone GO43H7, Biolegend), CD27-APC (clone M-T271, Myltenyi), CD25-PE-Dazzle594 (clone M-A251, Biolegend), HLA-DR-PerCP-Cy5.5 (clone G46-6, Biolegend), CCR5-PE (clone 3A9, BD), p24-FITC (clone KC57, Coulter), CD14-PE (clone TUK4, Mylteniy), CD3-FITC (clone SK7, Biolegend), CD56-PE-Cy7 (clone N901, Beckman Coulter), HLA-DR-PerCP-Cy5.5 (clone G46-6, Biolegend), CD25-PC5 (clone B9E9, Beckman Coulter), CD16-APC-H7 (clone 3G8, BD), CD4-A700 (clone OKT4, eBioscience), CD8-V500 (clone RPA-T8, BD), γH2AX-A647 (clone 20E3, Cell Signaling Technology®), PAK1-A488 (clone EP795Y, abcam). The following primary and fluorochrome conjugated secondary antibody pairs were used for flow cytometry: rabbit anti-ATP1A1 IgG (Genscript, Cat A01483), rabbit anti-ENO1 (abcam, Cat ab52488) and rabbit anti-LDHA (abcam, Cat ab52488) all followed by anti-rabbit IgG-A647 (LifeTechnologies, Cat A31573). Flow cytometry acquisition was performed on BD LSRII.

Cell lines

Jurkat and SupT1 cell lines were cultured in RPMI-1640 containing Glutamax (ThermoFisher), 10% fetal bovine serum (FBS) and penicillin-streptomycin (ThermoFisher) (100 U/ml). Cells were split twice a week and used at a concentration of 10^6 cells/ml for peptide assays.

Isolation and culture of primary human CD4+ T cells

Healthy donor blood prepared as a buffy coat was obtained from *Etablissement Français du Sang (EFS)* (agreement with Institut Pasteur C CPSL UNT, 15/EFS/023). Blood was overlayed on Ficol (EuroBio) at a ratio of 2:1 v/v blood to Ficol and centrifuged at 1,800 rpm for 30 minutes at a minimum acceleration/deceleration to obtain peripheral blood mononuclear cells (PBMCs). CD4+ T cells were then purified from PBMCs by negative selection using StemCell EasySep™ Human CD4+ T cell Isolation Kit. Cells were counted and cultured in RPMI-1640 containing Glutamax (ThermoFisher), 10% fetal bovine serum (FBS), penicillin-streptomycin (ThermoFisher) (100 U/ml) and IL-2 (Myltenyi) (100U/ml) (thereafter referred to as culture medium) at 10^6 cells/ml in 37° degree, 5% CO_2 humidified incubator. Cells were suboptimally activated with soluble anti-CD3 (clone UCHT-1) (Biolegend) for 5 days prior to infection or analysis as previously described (Sáez-Cirión et al., 2011).

Generation of human monocyte-derived macrophages

CD14+ monocytes were purified from human PBMCs by EasySep™ Human CD14 Positive Selection Kit (StemCell) and differentiated as previously described (David et al., 2006). Briefly, CD14+ monocytes were placed on Lumox Teflon bottom plates (Starstedt, Cat 94.6077.305) at 10^6 cells/ml in RPMI-1640 medium containing 2 mM L-glutamine (LifeTechnologies), 100U/ml Penicillin, 100 µg/ml Streptomycin (LifeTechnologies), 10mM sodium pyruvate (LifeTechnologies), 10 mM HEPES (LifeTechnologies), 1% MEM vitamins (LifeTechnologies), 1% non-essential amino acids (LifeTechnologies), 50 µM β-mercaptoethanol (LifeTechnologies) (MDM medium) and 15% human AB serum and cultured for 6 days. Differentiated macrophages were harvested by gentle scraping, counted and resuspended at 10^6 cells/ml in MDM medium supplemented with 10% FBS for further experiments.

Western Blot

10^6 cells was pelleted and stored as dry pellet in -80°C until use. The cell pellet was lysed in a lysis buffer (150mM NaCl, 50mM pH 7.4 Tris, 1mM pH 8 EDTA, 1% Triton X-100, containing 1

tablet of complete EDTA-free protease inhibitor cocktail (Sigma) per 10ml in water) for 20min on ice with occasional vortexing. 30 µg total protein was loaded on a 4-15% gradient SDS-PAGE gel (Mini Protean TGX Stain-Free gel, Biorad) previously denatured in the presence of 20% v/v loading buffer (LifeTechnologies) and 1% v/v β-mercaptoethanol for 10min at 95°C, and run at 120V for 1 hour. Samples were then transferred on PVDF membrane (Dutscher Dominique) previously activated in 100% ethanol for 1 minute in a transfer buffer (25 mM Tris, 192 mM glycine, 20% ethanol, pH 8.3) at 95V (300mA) for 40 min. The membrane was blocked in 5% BSA in Tris-Buffered Saline 0.1% tween-20 (TBST) over night at 4°C. The membrane was blotted with anti-Acinus (Cell Signaling, 4934) at 1/2,500 dilution overnight at 4°C in TBST, washed 3 times with TBST and incubated with anti-rabbit HRP (Sigma, A0545) at 1/1,000 dilution in TBST for 45 minutes at RT. Membrane was developed with ECL reagent (Thermo Scientific).

Immunofluorescence microscopy

Activated CD4+ T cells were incubated with 6uM RT53-Rhodamine [Rhodamine-RQIKIWFQNRRMKWKKAKLNAEKLKDFKIRLQYFARGLQVYIRQLRLALQGKT] or RK16-Rhodamine [Rhodamine-RQIKIWFQNRRMKWKK] (Proteogenix) for 2 hours. Cells were then washed twice with PBS, immobilized on poly-lysine coated coverslips, fixed with 4% paraformaldehyde for 10 minutes at room temperature (RT), neutralized with 50mM NH_4Cl for 10min at room temperature and washed twice with PBS. Cells were then permeabilized with 0.1% Triton-X 100 in PBS for 5 min at RT. All antibody incubations were performed in 1% bovine serum albumin (BSA) in PBS. Intracellular staining for AAC-11 was performed with anti-AAC-11 antibody (Abcam, ab65836) 1/200 dilution for 1hr at RT followed by secondary Ab (Invitrogen, A31573) 1/400 dilution for 45min at RT. Intracellular staining for gp120 was performed with anti-gp120 (clone 2G12, NIH AIDS Reagent Program, Cat 1476) 1/100 dilution for 1hr at RT followed by anti-human IgG-A488 (Jackson, Cat 709-545-149) 1/400 for 45min at RT. Intracellular staining for Acinus was performed using anti-Acinus Ab (Cell Signaling, Cat 4934) 1/100 dilution for 1hr at RT followed by anti-rabbit IgG-A647 (LifeTechnologies, A31573) 1/400 dilution for 45 min at RT. Coverslips were then washed, stained with DAPI for 15min at RT and mounted using Fluoromont G (ThermoFisher) mounting medium.

RT-PCR

Cells were collected by centrifugation, lysed in RA1 buffer (Macherey-Nagel) and stored at -80°C until RNA extraction. RNA was purified using RNA isolation kit (Macherey-Nagel) as per manufacturer's instructions. RNA was then converted to cDNA using SuperScript II (Thermo Fisher) as per manufacturer's instructions. RT-PCR was performed on 7500 Real Time PCR System (Applied Biosystems) using TaqMan Universal Master Mix II, with UNG (Thermo Fisher) with the following probe (FAM-MGB)-primer pairs: ATP1A1 (Hs00167556_m1), ATP1A2 (Hs00265131_m1), ATP1A3 (Hs00958036_m1), ATP1A4 (Hs00380134_m1), ATP1B1 (Hs00426868_g1), ATP1B2 (Hs01020302_g1), ATP1B3 (Hs00740857_mH), ENO1 (Hs00361415_m1), LDHA (Hs01378790_g1), ATP5B (Hs00969569_m1), AAC-11 (Hs00362482_g1). ΔCt was calculated using BECN1 (Hs01007018_m1) and ΔΔCt was calculated using reference *ex vivo* purified CD4+ T cell sample.

DISCUSSION

The search for intracellular factors that allow the establishment of efficient HIV viral infection is an active area of investigation today. Molecular pathways responsible for cell activation, cell cycle progression and metabolism are now clearly shown to be used by HIV for its replication. These pathways provide favorable intracellular environment for all stages of HIV infection with particular impact on RT, integration and transcription. Cell activation is accompanied by increase in mitochondrial respiration and glycolysis, which together provide biosynthetic subsrates, such as nucleotides, amino acids and carbohydrates for most stages of viral replication (RT, transcription and translation, protein glycosylation, etc). Open chromatin conformation and active gene transcription associated with activated and cycling cells allow efficient viral integration and transcription.

Although poorly understood, cell survival pathways are equally important for HIV propagation and persistence. Despite viral cytotoxicity seen *in vitro*, productively infected cells persist in culture due to mostly uncharacterized survival mechanisms. Additionally, infected cells with long half-life (particularly Tscm, Cm CD4+ T cells and macrophages) survive *in vivo*, despite massive cell death observed in primary infection, and contribute to the HIV reservoir. Today, several key questions remain unresolved: how do HIV infected cells survive? Does the virus induce survival pathways and if so which ones? Which pre-existing cellular survival mechanisms are used by the virus? And how the survival pathways used by HIV interact with other known intracellular HIV dependency factors?

In this work, we identified AAC-11-dependent anti-apoptotic pathway to be targeted by HIV to establish productive infection. Disruption of this pathway via the competitive antagonism of AAC-11 with the peptides derived from its α18 helix containing the LZ domain caused selective death of cells that are preferentially targeted by HIV-1 for productive infection. These results provide, therefore, a proof of concept that selective targeting of intracellular survival factors can lead to elimination of HIV target cells. This work also revealed the interaction of AAC-11 survival pathway with cellular differentiation, activation and metabolism, a combination of which allowed for efficient viral replication, and elimination of which blocked productive infection.

In this work, we observed the infectability of CD4+ T cell memory subsets at various differentiation stages (naïve<Cm<Tm<Em) to correlate with the proportion of cell death seen in each subset due to the AAC-11-derived peptide treatment. The susceptiblity of each subset to cell death could be explained by intrinsic balance of intracellular pro- and anti-apoptotic factors characteristic of this subset. Indeed, memory T cells are thought to differ in their regenerative potential and longevity. Naïve T cells are known to be longer-lived than their memory counterparts (Macallan et al., 2017). Tscm and Cm cells are thought to be superior to other memory subsets in their ability to self-renew and sustain long-term survival (Gattinoni et al., 2011)(Lugli et al., 2013) (Mahnke et al., 2013). Em and Tm cells, on the other hand, are programmed for efficient effector responses and are relatively primed for apoptosis (Macallan et al., 2017). Therefore, the precarious viability of Em and Tm cells could dependend on several anti-apoptotic factors such as AAC-11 among the overall pro-apoptotic intracellular millieu. Hence, the inhibition of these factors by treatments such as RT53 could easily launch cell death program in these subsets.

In this and our previous work (Valle-Casuso, submitted, See Annex 2) we observed that Em and Tm subsets were preferentially infected with both VSVG-pseudotyped HIV-1 particles and wild-type Bal HIV-1 (Chapter 1, Fig 5B). We previously showed (Valle Casuso, submitted, Annex 2) that this preference is at least in part related to the enhanced metabolic activity of this subset relative to other CD4+ T cell memory cells. In the current work, we identify that a particular combination of anti-apoptotic pathways (we identified one node of this network, AAC-11, but other components remain to be explored) in some cells of this subpopulation could also be contributing to the favourable intracellular milieu for HIV. On the other hand, Em and Tm cells are also most prone to HIV-1 induced cytoxicity (Chapter 2, Figure 1B). Therefore, how some Em and Tm cells carrying the virus succeed to survive is an interesting and important question. *In vivo*, however, the relative contribution of Cm cells to the HIV reservoir is currently thought to be dominant over other CD4+ T cell subsets (Chomont et al., 2009b). Indeed, Cm subset is known to have higher survival and self-renewal capacity over other memory subsets. Therefore, while Em and Tm could be preferentially infected, long

term survival of Cm cells could contribute to its higher contribution to the HIV reservoir *in vivo*. Interestingly, it has been recently suggested that Em differentiate into Cm cells (Youngblood et al., 2017). Thus, AAC-11 dependent anti-apoptotic program of Em cells could give rise to long lived Cm cells harborning HIV-1 reservoir *in vivo*.

Our work further underlines the parallelism between cells susceptible to HIV infection and cancer cells, as well as demonstrates the potential usefulness of anti-cancer approaches as an anti-HIV strategy. The major hallmarks of cancer closely refect well-established intracellular requirements for efficient HIV infection. These hallmarks include chronic proliferation (ie constant progression through the cell cycle and cell growth) accompanied by increasing metabolic demands, evasion of apoptosis and genomic instability (Hanahan and Weinberg, 2011). AAC-11 is known to be important for cancer cell survival and was already used as a proof of concept treatment for the elimination of malignant cells *in vitro* and *in vivo* (Jagot-Lacoussiere et al., 2016). In our study, we observed that counteracting AAC-11 survival pathway lead to the elimination of cells with characteristics most closely resembling those of cancer hallmarks (highly activated, metabolic cells with high degree of DSB), despite being non-malignant.

Cancer cells are known to upregulate Glut-1 transporter and aerobic glycolysis known as the Warburg effect (Hanahan and Weinberg, 2011). Indeed, high levels of radiolabelled glucose utilization is now used to non-invasively visualize tumors *in vivo* using positron emission tomography. HIV-1 also preferentially infects CD4+ T cells with high level of glycolysis and the block in infection is observed with inhibition of glucose utilization (Valle Casuso, 2018). In our experiments, we observed that RT53 preferentially targeted highly metabolically active cells. This indicates that AAC-11 survival pathway may be most active in cells with most active metabolism. Indeed, the link between metabolic and cell survival processes is well known (Green et al., 2014). Metabolic checkpoints are crucial in cell fate decisions and can tip the balance towards life or death during noxious stimuli such as RT53 by activation/suppression of cell death or survival effectors. Nutrient availability is sensed by central regulator of metabolism and cell survival, mTOR complex. During starvation, inhibition of mTORC1

promotes apoptosis (Green et al., 2014). On the other hand, active mTORC1 drives metabolic processes and promotes cell survival (Galluzzi et al., 2013). Interestingly, it has been reported that cells undergoing enhanced rates of glycolysis are able to survive intrinsic apoptosis due to the release of the oxidized form of cytochrome c from the mitochodria, which is unable to activate caspases (Vaughn and Deshmukh, 2008) (Green et al., 2014). Could highly glycolytic cells targeted by HIV-1 use survival pathways induced in such cells, and if so, which are these pathways? Intriguintly, activation of caspase 2 has already been shown to be linked to cellular metabolism. Suppression of caspase 2 activity was observed by the intermediates of pentose phosphate pathway (PPP) in *Xenopus laevis* oocyte through its inhibitory phosphorylation (McCoy et al., 2013) (Nutt et al., 2005). Therefore, AAC-11 pathway could perhaps suppress apoptosis in highly glycolytic cells by repressing caspase 2 and other apoptotic effectors and this repression could be lifted upon RT53 treatment.

Chronic replication and avoidance of cell death are other hallmarks of cancer, which closely parallel HIV intracellular requirements. Many tumor suppressor molecules implicated in cell cycle and death decisions are now also identified as HIV-1 restriction facotrs. For example, p53 is a major tumor suppressor, which is stabilized by the action of caspase 2 in response to compromised genomic integrity (Oliver et al., 2011). It acts as a central decision point towards cell cycle arrest or apoptosis during genomic or metabolic insults (Kruiswijk et al., 2015)(Simabuco et al., 2018) and is inhibited in many cancers (Kruiswijk et al., 2015). Similarly to its role in suppressing cancer, p53 was reported to be an HIV-1 restriction factor through inhibition of its transcription (Mukerjee et al., 2010). Additionally, p53 downstream target p21, also strongly implicated in cancer through its regulation of cell cycle checkpoints (Georgakilas et al., 2017), has been characterized by us and others to be an HIV-1 restriction factor (Allouch et al., 2014; Pauls et al., 2014) (Badia et al., 2016)(Valle-Casuso et al., 2017).

P53 and its downstream targets act as genome guardians (Georgakilas et al., 2017), which, as mentioned above, are often disabled in cancer cells allowing cell survival and proliferation despite genomic instability, another key hallmark of cancer. Cancer provoking mutations often stem from the telomere shortening and associated DNA damage and chromosome

rearrangement events (Hanahan and Weinberg, 2011). Interestingly, subsets of memory T cells follow a gradient of telomere shortening and senescence-related proliferative defects that reflects their differentiation status (Mitri et al., 2011). Em and Tm cells are most primed for senesence and apoptosis due to their high expression of DSB related to telomere shortening, to which DNA repair machinery is rapidly recruited (Mitri et al., 2011). In our work, we observed that Em and Tm subsets that displayed the highest proportion of DSB were preferentially depleted by RT53 (Chapter 2, Fig 3). However, we cannot distinguish if the disappearance of cells enriched in DSB upon RT53 treatment is a cause or only a consequence of cell death in our experiments. Similarly to cancer, DSB are important for HIV life cycle. Viral integration critically relies on DNA repair machinery that is recruited to DSBs created by viral integrase (Daniel, 2006) (Daniel et al., 2004). Despite this, however, a subset of cells was reported to undergo apoptosis in response to HIV-1 integration (Cooper et al., 2013b). Therefore, successful viral integration and persistence critically relie not only on cellular DNA repair machinery but also on anti-apoptotic survival pathways in cells that are preferentially targeted by the virus.

We also saw that RT53 inhibited VSVG-pseudotyped HIV-1 with less potency than wild-type Bal (Chapter 1, Fig 3). This could be due to entry of these two viral strains to different subsets of CD4+ T cells. VSVG-pseudotyped HIV-1 particles are tought to enter equally into different T cell subsets (Dai et al., 2009). Wild-type HIV-1, on the other hand, enters preferentially into memory than naïve cells (Dai et al., 2009) due to their higher expression of HIV-1 co-receptor. Indeed, we did observe a decrease in $CCR5^{high}$ cells in our cultures upon peptide treatment, but this did fully not account for the observed effect. Multiplicity of viral cycles of wild-type Bal HIV-1 could also be responsible for stronger RT53-mediated inhibition. In this case, inhibition of Bal at early stages of replication in RT53 treated cells would produce higher differences with RK16 treated and untreated cells, which accumulated many viral re-infection cycles. VSVG-pseudotyped virus, on the other hand, would only be able to replicate once in its permissive cells.

We previously observed that RT53 elicited signaling in all cells (reflected by cell surface PS expression) (Chapter 1, Fig 4A). Additionally, we saw caspase 2 activation also in cells surviving RT53 treatment (Chapter 1, Fig 4D). However, as hypothesized above, only cells with a particular combination of pro-apoptotic intracellular environment die in response to this signaling. Although caspase 2 is mostly known for its pro-apoptotic function, it has also been implicated in the induction of cell cycle arrest while promoting survival in live cells (Miles et al., 2017). We, therefore, cannot exclude that in the cells surviving treatment RT53 could induce factors that negatively regulate HIV-1 life cycle (for example activation of a PRR or a restriction factor). Curiously, treatment of cells with 4 μM RT53 concentration, at which no significant increase in cell death was observed (and also no correlation between cell death and infection), already significantly inhibited Bal infection (Figure 1).

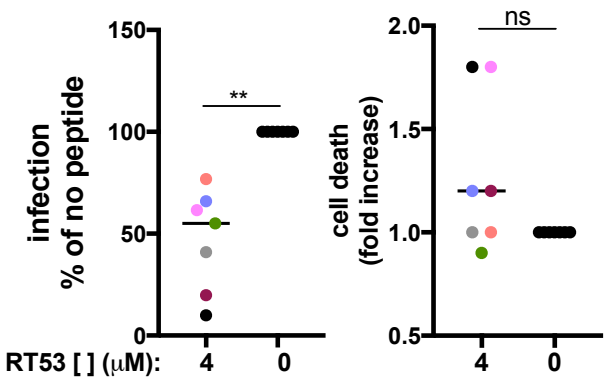

Figure 1. RT53 displays anti-viral effect before cytotoxicity. Median values are indicated with a line. Statistical significance was evaluated using Dunnett's multiple comparisons test

Given our promising *in vitro* results in elimination of HIV-1 target cells with AAC-11-derived peptides, it is tempting to speculate that this approach could be useful as a potential adjuct therapy during cART or 'shock and kill' approaches. The peptides were already successfully used in a murine tumor xenograph model thus passing first safety barrier (Jagot-Lacoussiere et al., 2016). Despite this, several aspects of our results warant caution when considering this therapy in monkeys or humans. First, RT53 induced cytotoxicity not only of CD4+ T cells but also of other lymphocyte populations (CD8+ T cells and B cells). This suggests that common factors responsible for the maintenance/survival of the lymphocyte lineage may be implicated in the response to RT53. This also indicates that AAC-11-derived peptides may trigger adverse effects due to their targeting of non-CD4+ T cells *in vivo*. Additionally, the

precise molecular mechanism of peptide action and associated cell death still remains to be identified. It is possible that the peptides are triggering the inflammatory types of cell death (pyroptosis or necroptosis), which was not formally evaluated in this work. In this case, they could trigger strong inflammation reaction *in vivo*, which is a known co-factor for HIV propagation. Additionally, complex network of signaling *in vivo* may change the response of cells to AAC-11-derived peptides and the response of tissues to peptide-induced death signals. Therefore, the outcome of AAC-11-derived peptide *in vivo* therapy can only be evaluated through animal trials.

ANNEX 1:

CELLULAR DETERMINANTS OF HIV PERSISTANCE ON ANTIRETROVIRAL THERAPY

Book chapter, published

Chapter 9
Cellular Determinants of HIV Persistence on Antiretroviral Therapy

Anastassia Mikhailova, Jose Carlos Valle-Casuso, and Asier Sáez-Cirión

Abstract The era of antiretroviral therapy has made HIV-1 infection a manageable chronic disease for those with access to treatment. Despite treatment, virus persists in tissue reservoirs seeded with long-lived infected cells that are resistant to cell death and immune recognition. Which cells contribute to this reservoir and which factors determine their persistence are central questions that need to be answered to achieve viral eradication. In this chapter, we describe how cell susceptibility to infection, resistance to cell death, and immune-mediated killing as well as natural cell life span and turnover potential are central components that allow persistence of different lymphoid and myeloid cell subsets that were recently identified as key players in harboring latent and actively replicating virus. The relative contribution of these subsets to persistence of viral reservoir is described, and the open questions are highlighted.

Keywords HIV reservoirs · CD4+ T-cell subsets · Macrophages · Dendritic cells · HIV susceptibility · Cell survival · Turnover potential

9.1 Introduction

Antiretroviral therapy (ART) has achieved impressive success in preventing progression of HIV-1 infection. However, ART is lifelong and is not a cure. HIV-1 persists in viral reservoirs (Barton et al. 2016) that lead to viral rebound if ART is discontinued. A better understanding of HIV reservoirs is fundamental in the search for an HIV cure (Deeks et al. 2016; Passaes and Saez-Cirion 2014). HIV-1 persistence under ART may be due to (1) low-level viral replication, in particular in tissues where concentration of antiretrovirals (ARVs) may not always reach optimal levels (Fletcher et al. 2014; Lorenzo-Redondo et al. 2016; Tobin et al. 2005), and (2)

Author contributed equally with all other contributors.Anastassia Mikhailova and Jose Carlos Valle-Casuso

A. Mikhailova · J. C. Valle-Casuso · A. Sáez-Cirión (✉)
Unité HIV Inflammation and Persistence, Institut Pasteur, Paris Cedex 15, France
e-mail: asier.saez-cirion@pasteur.fr

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the long half-life and self-renewal of latently infected cells (Chomont et al. 2009). Although it is likely that HIV-1 persistence is a consequence of the combination of these two processes, we will focus here on intrinsic cell properties that determine the maintenance of infected cells.

HIV-1 infects cells from the myeloid and T-cell lineages, but not all cell populations contribute equally to HIV persistence (Barton et al. 2016). HIV persistence on ART is largely secondary to infected CD4+ T cells (Chomont et al. 2009; Chun et al. 1998). Macrophages and dendritic cells play a critical role as early targets for HIV infection and as vehicles for HIV-1 dissemination throughout the body, but their role in long-term persistence is still unclear (Abbas et al. 2015; Coleman and Wu 2009). There are multiple subsets of CD4+ T cells that follow a gradient of maturation stages including naïve (TNA), stem cell memory (TSCM), central memory (TCM), transitional memory (TTM), effector memory (TEM), and terminally differentiated (TTD) cells (Mahnke et al. 2013). In HIV-infected individuals on ART, HIV DNA is most frequently detected in memory CD4+ T cells and in particular TCM and TTM (Chomont et al. 2009). However, the relative contribution of these subpopulations to the HIV reservoir may vary depending on whether the treatment was initiated in primary or chronic infection, and after long periods of ART, there is a greater contribution of very long-lived infected cells such as TSCM (Ananworanich et al. 2015; Buzon et al. 2014; Jaafoura et al. 2014; Cheret et al. 2015).

The establishment and maintenance of HIV-1 reservoirs is a multifaceted process that depends (1) on the relative cell susceptibility to HIV infection, (2) the capacity of the infected cell to resist HIV-induced apoptosis and escape immune surveillance, and (3) the infected cell's life span and turnover potential (Fig. 9.1). All these processes are determined by each cell type's program and regulated by tissue location, activation, and differentiation state of the cells in response to environmental conditions and stress signals. This chapter analyzes each of these processes with respect to how they contribute to HIV persistence on ART.

9.2 Being a Good or a Bad Host to the Virus

HIV-1 cell tropism is determined by the expression on the cell surface of the main HIV-1 receptor CD4 and at least one additional co-receptor, mainly CCR5 or CXCR4 (Wilen et al. 2012). CD4+ T cells, monocytes/macrophages, and dendritic cells are the major targets of HIV-1 (Kandathil et al. 2016; Kumar et al. 2014). HIV-1 replicates preferentially in activated CD4+ T cells and less efficiently in macrophages and immature dendritic cells, while resting CD4+ T cells, monocytes, and mature dendritic cells are relatively resistant to HIV-1 infection (Steinman et al. 2003; Wu and KewalRamani 2006; Izquierdo-Useros et al. 2010; Descours et al. 2012; Diamond et al. 2004). These differences are mostly explained by the relative abundance of cellular factors that participate in the virus life cycle, either facilitating or interrupting viral replication. Several studies have identified hundreds of cellular

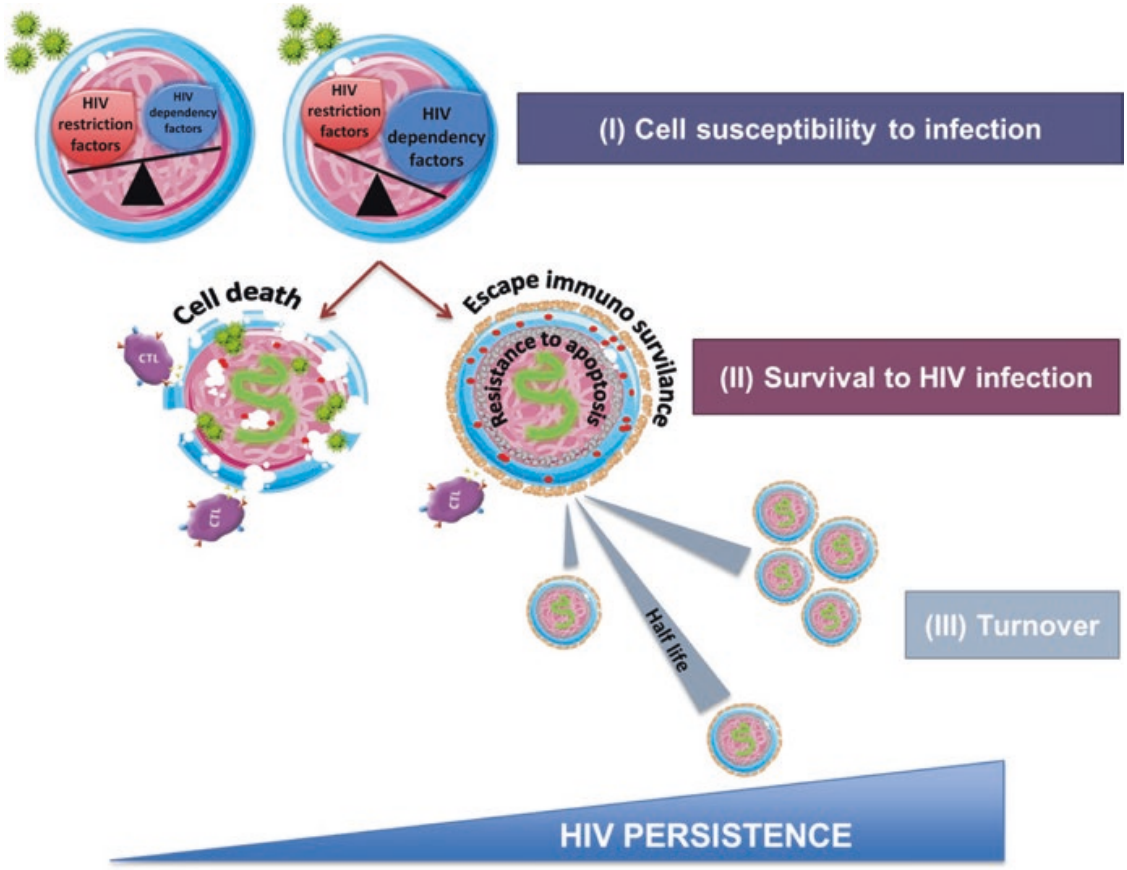

Fig. 9.1 Cellular determinants for the establishment and persistence of HIV on ART. HIV persistence is first determined by the susceptibility of different cells to infection (*i*), which is regulated by the balance of HIV host dependency factors and viral restriction factors present in the cells. In order to persist, infected cells need to resist apoptotic signals induced by viral infection and avoid immune surveillance (*ii*). These resistant infected cells will persist for variable periods of time depending on their specific life span and capacity to proliferate without enhancing HIV-dependent cell death signals (*iii*)

factors potentially required for HIV-1 to complete each step of its replication cycle, called HIV dependency factors (HDF) (Brass et al. 2008; König et al. 2008; Zhou et al. 2008; Chinn et al. 2010; Cleret-Buhot et al. 2015).

The expression of chemokine receptors varies with T-cell differentiation, impacting the susceptibility of the cells to HIV-1 (Sallusto et al. 1998; Veazey et al. 2000). Among CD4+ T helper lineages, Th2 cells are relatively resistant to HIV-1 infection, in particular to CCR5-using (R5) viruses due to low CCR5 expression) (Gosselin et al. 2010; Sun et al. 2015) although these cells might be targeted by CXCR4-using (X4) viruses late in infection (Maggi et al. 1994). Th1 cells are susceptible to both R5 and X4 viruses but to a lower extent than Th1/Th17 or Th17 cells that express CCR6 (El Hed et al. 2010; Gosselin et al. 2010; Sun et al. 2015). The enhanced susceptibility of CCR6+ CD4+ T cells to HIV-1 infection was linked to an enhanced expression of HDF in these cells (Cleret-Buhot et al. 2015).

In addition to HIV entry receptors, some of the best known HDF include cyclophilin, which binds to HIV-1 capsid and facilitates decapsidation/reverse

transcription through an unknown mechanism (De Iaco and Luban 2014); cytoskeleton, which is required for intracellular trafficking of the virus (Menager and Littman 2016; Stolp and Fackler 2011); lens epithelium-derived growth factor (EDGF)/p75, which interacts with integrase and is responsible for the tethering and selective integration of HIV into active transcription units of the chromatin and possibly also in the regulation of HIV latency (Gerard et al. 2015; Engelman and Cherepanov 2008); positive transcription elongation factor (P-TEFb, composed of cyclin-dependent kinase 9 (CDK9) and of cyclin T1 or T2 (CycT1/T2)) and nuclear factor of kappa light polypeptide gene enhancer in B-cells (NFκb) and nuclear factor of activated T cells (NAFT) transcription factors, which are required for HIV-1 transcription (Karn and Stoltzfus 2012); and endosomal sorting complex required for transport (ESCRT), which participates in HIV-1 budding (Usami et al. 2009).

While relying on numerous cellular factors, HIV-1 needs to overcome restriction factors that have evolved as part of our innate immune response to counteract infections (Arhel and Kirchhoff 2010). Although several cellular factors have been suggested to potentially inhibit HIV-1 infection (Liu et al. 2011), to date only a handful of restriction factors have been clearly validated. Serine incorporators 3 and 5 (SERINC3 and SERINC5) interfere with the delivery of viral particles to target cells (Rosa et al. 2015; Usami et al. 2015); apolipoprotein B mRNA editing enzyme catalytic subunit 3G (APOBEC3G), tripartite motif containing 5 (TRIM5alpha), and SAM domain- and HD domain-containing protein 1 (SAMHD1) impair reverse transcription (Santa-Marta et al. 2013; Simon et al. 2015); MX dynamin-like GTPase 2 (Mx2) hinders nuclear accumulation and integration of proviral DNA into the host chromatin (Goujon et al. 2013); bone marrow stromal cell antigen 2 (BST-2) retains newly produced viral particles at the surface of infected cells (Perez-Caballero et al. 2009). The strong expression of HDF present in immune cells suggests that HIV-1 needed to evolve to replicate in these cells, which contain critical factors for its replication cycle, and adapted to circumvent the action of restriction factors (Brass et al. 2008; Cleret-Buhot et al. 2015). Thus, the susceptibility of host cells to HIV-1 infection is largely dictated by the availability of HDF rather than the expression of restriction factors, with the exception of SAMHD1.

SAMHD1 possesses dNTPase and nuclease activities and can potentially interfere with HIV-1 reverse transcription by reducing the pool of intracellular deoxynucleoside triphosphate (dNTP) and by degrading incoming viral nucleic acids (Beloglazova et al. 2013; Laguette et al. 2011; Lahouassa et al. 2012), although the relative contribution of each of these activities is still unclear. This may vary as a function of cell type and cell cycle. SAMHD1 efficiently blocks HIV-1 infection in quiescent CD4+ T cells and monocytes and strongly decreases HIV-1 dynamics in differentiated myeloid cells, but it is inefficient in cycling CD4+ T cells (Laguette et al. 2011; Cribier et al. 2013).

The differences in the antiviral activity of SAMHD1 between these cell types are not related to its relative expression, but SAMHD1 antiviral activity is ablated by the phosphorylation of its threonine 592 (T592) residue (Cribier et al. 2013; White et al. 2013). Phosphorylation of SAMHD1 is regulated by cyclin-dependent kinases (CDK) 1/2, which coordinate T-cell division and differentiation in response to

antigen recognition (Wells and Morawski 2014). A recent report shows that tissue resident macrophages susceptible to HIV-1 infection such as microglial cells, which are responsible for HIV-1 persistence and compartmentalization in the brain (Schnell et al. 2011), are in a G1-like status and express high levels of phosphorylated (inactive) SAMHD1 (Mlcochova et al. 2017).

The influence of the cell cycle on HIV-1 replication is also well known (Goh et al. 1998). $P21^{cip1/waf1}$, a CDK inhibitor which regulates cell cycle arrest and is involved in monocyte differentiation (Asada et al. 1999; Xiong et al. 1993), is a potent inhibitor of HIV-1 infection (Bergamaschi et al. 2009). On the one hand, p21 controls the de novo synthesis of dNTPs by regulating the expression of the main enzymes involved in this process (Allouch et al. 2013; Valle-Casuso et al. 2017). Through its CDK inhibitor activity, p21 also controls the phosphorylation state of SAMHD1 (Allouch et al. 2014; Pauls et al. 2014b). p21 has also been shown to interfere with HIV-1 replication in hematopoietic stem cells (Zhang et al. 2007) and with CDK9-dependent transcription of HIV-1 in CD4+ T cells (Chen et al. 2011).

The differentiation state of the cells also influences the relative capacity of HIV-1 to replicate. HIV-1 replicates less well in naïve CD4+ T cells and monocytes than in memory CD4+ T cells and macrophages, and this is not only related to differential expression of HIV-1 co-receptors. Successful HIV infection requires stable integration of viral cDNA into the host cell genome. HIV integration occurs preferentially within transcription units of transcriptionally active genes (Han et al. 2004; Schroder et al. 2002). However, several mechanisms including epigenetic gene silencing, transcription gene silencing, and posttranscriptional gene silencing have been described to explain the establishment and maintenance of latency in target cells (reviewed in Van Lint et al. (2013)). Although these mechanisms are described in detail elsewhere in this book, it is interesting to note here that HIV gene expression is heavily dependent on the presence of several transcription factors, such as NFAT and NFκB. These factors are critical regulators of T-cell activation and differentiation, and their expression is necessary for rapid production of cytokines or effector molecules (Macian 2005; Oh and Ghosh 2013). Accordingly, NFAT and NFκB are expressed at very low levels in naïve and resting T cells and strongly expressed in activated and differentiated T cells.

In vitro studies suggest that latency can be established in both resting and activated CD4+ T cells (Chavez et al. 2015). However, it is reasonable to think that HIV-1 latency per integration event may be achieved more frequently in less-differentiated CD4+ T cells subsets than in effector cells, but this remains to be proven. The mammal target of rapamycin (mTOR) is a pivotal regulator of cell differentiation, cell cycle, proliferation, and survival (Chi 2012). mTOR directly regulates many HDF (e.g., cytoskeleton, NFAT) and it has been shown to control HIV-1 latency (Besnard et al. 2016). mTOR also regulates the metabolic activity of the cells (Powell and Delgoffe 2010), and expression of the glucose transporter Glut1 is required for HIV-1 replication (Loisel-Meyer et al. 2012). Thus, it is likely that mTOR has an important part in the regulation of HIV-1 infection in different cell subsets.

Much less evidence is available about the establishment of HIV latency in infected macrophages. As in CD4+ T cells, in macrophages HIV preferentially integrates into the transcriptionally active region of the chromatin (Kumar et al. 2014). However, it is not clear whether the mechanisms driving latency in CD4+ T cells are similar in macrophages. Latency can be established in macrophages in vitro (Brown et al. 2006), and HIV transcription is regulated in response to external signals and macrophage activation (Saez-Cirion et al. 2006). Moreover, the transcription factor B-cell CLL/lymphoma 11B (BCL11B/CTIP2), which is involved in multiple cellular processes including cell proliferation and survival, has been shown to repress HIV gene transcription in microglial cells by inhibiting the elongation factor P-TEFb and by inducing a compact, transcriptionally inactive, heterochromatic environment at the HIV promoter (Le Douce et al. 2010).

Although HIV-1 has evolved mechanisms to avoid the action of restriction factors such as APOBEC3G, this factor is expressed at very high levels in the cells that are more susceptible to HIV-1 infection, and this may come at a cost for the virus in terms of replication capacity in more differentiated cell subsets (Vetter et al. 2009). Finally, some cells, such as dendritic cells, are poorly or not susceptible to HIV-1 replication but can internalize free virions in non-cytolytic vesicles and transfer them at high concentrations to CD4 T cells upon interaction (Manches et al. 2014). Along these lines, follicular DCs have been shown to trap and retain infective HIV-1 particles for extended periods of time (Heesters et al. 2015).

9.3 Dodging Cell Death Upon Infection

9.3.1 Apoptosis

Although apoptosis is a major mechanism of defense against infection, viruses have evolved means to influence the balance of death and survival of the host cell in order to promote efficient virus replication and persistence of infection. Progressive CD4+ T-cell loss is a defining characteristic of uncontrolled HIV-1 infection. HIV-1 can provoke direct cytotoxicity on target cells. However, it is now well accepted that decline of CD4+ T cells in vivo is not solely due to direct viral cytotoxicity but to a multifactorial process that also includes apoptosis of "bystander" cells (Doitsh et al. 2010; Doitsh et al. 2014; Finkel et al. 1995) and killing of productively infected cells by immune effectors. Yet persistence of HIV-1 infected cells for long periods of time requires avoiding all these forms of cell death. Studies performed in vitro and ex vivo have shown the contribution of many different apoptotic molecules in CD4+ T cells and other HIV-1 susceptible cell subsets. The exact molecular mechanisms of HIV-1-induced cytotoxic or anti-apoptotic effects on infected cells that lead to long-lived HIV persistence are still not well understood.

Viral proteins such as tat (Li et al. 1995; Westendorp et al. 1995), env (Cicala et al. 2000), vpr (Muthumani et al. 2002), and nef (Muthumani et al. 2005; Xu et al. 1999) have been shown to have a pro-apoptotic effect in vitro. However, the action

of these proteins at physiological concentrations and in a complex immunological setting remains unclear. Moreover, the regulation of apoptosis depends on the interaction of viral factors with cell pathways, and the equilibrium between pro-apoptotic and anti-apoptotic signals may vary as a function of the stage of viral replication, nature, and state of the target/bystander cell and external signals. Nef has been shown to prevent apoptosis in productively infected cells upon Fas and tumor necrosis factor alpha (TNFalpha) ligation (Ohnimus et al. 1997) by inhibiting Fas signaling (Geleziunas et al. 2001). Nef was also shown to inactivate pro-apoptotic BCL2-associated agonist of cell death (Bad) protein, hence rendering infected T cells more resistant to apoptosis (Wolf et al. 2001).

On the other hand, myeloid lineage cells including monocytes and macrophages appear less sensitive to the cytopathic effect of HIV replication than T cells, suggesting that intrinsic properties of myeloid cells may render them selectively resistant to HIV-induced apoptosis (Kumar et al. 2014; Le Douce et al. 2010). Along these lines, telomerase activity increases in macrophages upon infection, rendering them more resistant to DNA damage or oxidative stress (Ojeda et al. 2014). Macrophages were observed to be more apoptosis resistant at least in part due to env (Swingler et al. 2007)- and nef (Olivetta and Federico 2006)-dependent alterations in apoptotic pathways.

Following ART, the contribution of direct viral cytotoxicity to cell death is likely minimal. Latently infected cells remain largely ignored by HIV-specific T cells due to the lack of expression of viral proteins, which favors persistence. However, infected CD4+ T cells have been reported to undergo integration-dependent cell death, linked to the recruitment of DNA-dependent protein kinase (DNA-PK) (Cooper et al. 2013). It is unlikely that latency is an “all or nothing” phenomenon, and it is possible that episodes of viral reactivation occur episodically in vivo even in the presence of effective cART (Nettles et al. 2005; Graf et al. 2013a). Recent studies have shown that reactivation of latently infected cells in ART-suppressed HIV-infected individuals with histone deacetylases (HDAC) inhibitors or other latency reversal agents was insufficient to promote cell death ex vivo or to decrease the frequency of integrated viral DNA or infectious units in vivo (Lehrman et al. 2005; Rasmussen and Lewin 2016; Rasmussen et al. 2014; Routy et al. 2012; Shan et al. 2012). Moreover, cells carrying latent replication competent viruses might be particularly resilient to CD8+ T cell-mediated killing even after viral reactivation (Huang and Jones 2017). Therefore, long-term HIV-1 persistence may be caused by cells that are particularly resistant to cell death, either due to their intrinsic properties or because of an anti-apoptotic state, supported by HIV factors.

Different studies using in vitro models of HIV-1 latency have described that establishment of latent HIV-1 infection is accompanied by the induction of anti-apoptotic proteins (e.g., B-cell CLL/lymphoma 2 (BCL2), cellular FLICE-like inhibitory protein (cFLIP), myeloid cell leukemia sequence 1 (Mcl-1) (Aillet et al. 1998; Berro et al. 2007; Tan et al. 2013) or the downregulation of pro-apoptotic proteins (e.g., BCL2-associated X protein (BAX), Fas-associating death domain-containing protein (FADD)) (Wang et al. 2011; Badley et al. 2013). Interestingly, in vivo, cells that support virus persistence such as TCM (Olvera-García et al. 2016)

and monocytes (Giri et al. 2009) had an anti-apoptotic gene signature in HIV-infected individuals compared to noninfected controls.

Death of nonproductively infected nonactivated T cells that do not express viral antigen has been reported to result from the detection of viral reverse transcription products by DNA sensor interferon gamma inducible protein 16 (IFI16), which leads to caspase-1 activation, thereby triggering pyroptosis (Doitsh et al. 2010; Monroe et al. 2014). However, the contribution of this form of cell death is probably limited once ART is initiated (Cai et al. 2016).

Apoptosis of bystander cells has also been linked to persistent immune activation seen in chronic infection via signaling by TNF family members (TNF-related apoptosis-inducing ligand (TRAIL), Fas ligand (FasL), and TNFalpha) (Herbeuval et al. 2005, 2006; Katsikis et al. 1995; Sloand et al. 1997). Death signals delivered via Fas ligation (Katsikis et al. 1995; Sloand et al. 1997) were shown to have an important contribution to bystander cell depletion in HIV-infected individuals (Badley et al. 1997; Badley et al. 1996, 1998). However, these signals were observed to be counteracted, at least in part, by viral proteins expressed by infected cells. For example, Env was observed to induce resistance to TRAIL-induced apoptosis in macrophages (Swingler et al. 2007).

Overall, it seems that the activation status of cells plays a role in the susceptibility to cell death during HIV infection, which is also reflected by differential loss of cell populations from different tissues, where more or less activated cell phenotypes are found. For example, in the lymph nodes of HIV-infected individuals, the degree of apoptosis has been correlated with virus- and microbial-driven immune activation observed in infection and not the viral load (Muro-Cacho et al. 1995). It has been further shown that highly activated effector memory CD4+ T cells are depleted faster and first from gut mucosal sites (Grossman et al. 2006) and naïve T cells displaying a resting phenotype are resistant to depletion in lymphoid tissues (Veazey et al. 2000). Additionally, blood-derived CD4+ T cells that display a deeper resting state than lymphoid tissue-derived cells are more resistant to pyroptosis despite carrying viral genetic material (Muñoz-Arias et al. 2015). Thus, infected naïve, central memory, and stem cell-like memory T cells displaying a less activated phenotype could be less prone to these mechanisms of induced cell death than highly activated effector memory T cells. These differences could contribute to shaping where HIV persists, even before treatment initiation.

The relevance of these mechanisms during ART is unknown. ART undoubtedly halts loss of CD4+ T cells, but some level of abnormal chronic inflammation persists (Paiardini and Muller-Trutwin 2013), and it is likely that this may contribute to selective elimination during treatment of cells that have the highest susceptibility to apoptosis. The susceptibility of infected cells to cell death may also vary in tissues depending on the cytokine milieu. For example, interleukin (IL)-7 protected resting CD4+ T cells from death during in vitro HIV infection (Trinité et al. 2016), whereas IL-12 protected while IL-10 augmented Fas-mediated cell death of CD4 T cells from HIV-infected individuals (Estaquier et al. 1995).

9.3.2 Immune Clearance

HIV-1-specific cytotoxic CD8+ T cells and natural killer (NK) cells are able to eliminate infected cells, and these responses have been linked to protection against HIV transmission or natural control of infection (Betts et al. 2006; Graf et al. 2013b; Jennes et al. 2006; Martin et al. 2002; Ravet et al. 2007; Sáez-Cirión et al. 2007; Sáez-Cirión et al. 2009). However, during progressive infection, selection in vivo of viral variants that escape this immune pressure occurs progressively diminishing the capacity of these cells to counteract infection (Alter et al. 2011; Borrow et al. 1997; Deng et al. 2015; Mailliard et al. 2013). In elite controllers, HIV-1 persists for many years even in the presence of highly efficient CD8+ T-cell responses (Noel et al. 2016), suggesting that persistent infected cells are able to avoid immune surveillance. Latently infected resting CD4+ T cells that do not actively express viral epitopes escape immune surveillance although transient expression of viral antigens may trigger their killing (Graf et al. 2013a). Viral proteins such as Nef (Cohen et al. 1999) and Vpu (Apps et al. 2016) downregulate major histocompatibility complex (MHC) class I and may contribute to protect infected CD4+ cells from the CD8+ T-cell response (Collins et al. 1998; Xu et al. 1997) (although this could make these targets susceptible to NK mediated killing (Cohen et al. 1999)).

Infected macrophages might be more resistant than CD4+ T cells to killing by CD8+ T cells in vitro, independently of nef (Rainho et al. 2015; Vojnov et al. 2012). Infected macrophage can be eliminated by HIV-specific cytotoxic CD4+ T cells (Sacha et al. 2009), which have been found to increase during acute infection (Soghoian et al. 2012) and in elite controllers (Johnson et al. 2015). In addition, in macrophages, HIV-1 particle assembly in intracellular virus-containing compartments (VCCs) (Jouve et al. 2007; Welsch et al. 2011) may provide protection from immune recognition (Tan and Sattentau 2013).

In addition to escape mutations and latency, effective immune responses are also curtailed by the physical separation of effector cells from their targets residing in tissue sanctuaries. The central nervous system (CNS) has long been considered an "immune-privileged" site where infected macrophages, astrocytes, and microglial cells are relatively inaccessible to antiviral immune responses and variably accessible to ART (Letendre et al. 2008; Joseph et al. 2015), thus constituting an important viral reservoir.

Cerebrospinal fluid (CSF) from noninfected individuals contains CD4+ T cells with an activated central memory phenotype (Kivisäkk et al. 2003), which are a preferential target of HIV-1. Compared to the blood, the ratio of CD8/CD4 T cells is much lower in the CNS, and it has been suggested that antigen-specific CD8+ T cells found in the CNS do not provide durable immune surveillance in the absence of antigen (Young et al. 2011). Resident memory CD8+ T cells can be found in the CNS in the context of viral infection (Wakim et al. 2010), and it is now recognized that functional HIV-1-specific CD8+ T cells infiltrate CNS during acute (Kessing et al. 2017) and chronic (Ganesh et al. 2016) HIV infection. Remarkably, CD8+ T-cell responses in the CNS are detected in elite controllers with undetectable viral

load in the CSF and blood (Sadagopal et al. 2008) and during ART (Lescure et al. 2013; Miller et al. 2004) and contribute to the control of infection in the CNS (Marcondes et al. 2015).

Several reports have shown accumulation of virus bearing Tfh cells in germinal centers of lymphoid follicles (Banga et al. 2016; Perreau et al. 2013) where CD8+ T cells are found in low frequencies as compared to T-cell zones (Connick et al. 2007; Folkvord et al. 2005; Fukazawa et al. 2015; Hong et al. 2012). However, recently identified follicular cytotoxic T cells (Tfc) were shown to enter B-cell follicles of HIV-infected individuals and to have cytotoxic potential (Leong et al. 2016; Petrovas et al. 2017). Cytotoxic CD8+ T cells displaying viral target lysis are also detected in the lamina propria (Murphey-Corb et al. 1999) as well as vaginal epithelium and submucosa (Lohman et al. 1995) of SIV-infected rhesus macaques. Activated CD8+ T cells with cytotoxic potential were also located in adipose tissue (which carried infected CD4+ T cells and macrophages) of SIV-infected monkeys (Damouche et al. 2015). It is, therefore, probable that HIV-specific CD8+ T-cell responses in tissue contribute to the elimination of infected cells in nonlymphoid tissues and even the CNS, although more studies will be needed to define their characteristics.

9.4 Endurance and Renovation

The number of cells containing HIV DNA decreases sharply during the first months following initiation of ART (Finzi and Siliciano 1998; Avettand-Fenoel et al. 2016). A steady state appears to be reached by 2 years on ART, although DNA decay continues for longer periods of time when ART is initiated during primary infection (Avettand-Fenoel et al. 2016). Modeling the dynamics of decline in HIV DNA showed that this decay occurs in several phases that have been attributed to the sequential loss of infected cells of varying half-life (Finzi and Siliciano 1998; Perelson 2002). In the context of ART efficiently blocking systemic HIV replication, cells with active viral replication are expected to be eliminated within a few days due to cytopathic effects or immune clearance (see above). However, some cells like macrophages can produce infectious viral particles for long periods of time without being killed and resting CD4+ T cells carrying latent provirus persist despite multiple decades of treatment.

The maintenance of infected CD4+ T cells under ART is driven by survival of long-lived cells and homeostatic proliferation (Chomont et al. 2009). The life span of quiescent CD4+ T cell progressively decreases with differentiation. Naïve CD4+ T cells are much longer lived than memory cells, and early differentiated memory CD4+ T cells have a longer half-life than terminally differentiated cells. TNA, TSCM, and TCM upregulate genes associated with survival and are less prone to undergo apoptosis, at least in vitro (Lugli et al. 2013; Mahnke et al. 2013). It has been estimated that one TNA has a half-life of 1 to several years, a TCM a few months to 1 year, while a TEM would only be a few weeks (Macallan et al. 2004;

Vrisekoop et al. 2008). TSCM have the highest survival capacity among memory T cells in the absence of cognate antigen (Lugli et al. 2013; Gattinoni et al. 2011). However, these estimations, largely based on the in vivo analysis of incorporation of deuterated glucose or water on CD4+ T cells, are limited by the lack of resolution on cells that migrate to the tissues. The recently described resident memory T cells are programmed to persist locally and not recirculate even in the absence of antigen (Clark 2015). The role of these cells in the context of HIV-1 infection has not been clarified yet but their potential contribution to the persistence of HIV on ART deserves analysis.

The contribution of infected macrophages to HIV persistence on ART is debated (DiNapoli et al. 2017). Resting CD4+ T cells constitute the bulk of persisting infected cells, but the potential implication of infected macrophages as source of rebounding virus if treatment is interrupted should not be overlooked (Crowe et al. 2003). Although it is accepted that viral decay in monocytes/macrophages is slower than in activated CD4+ T cells, it is often assumed that the half-life of infected monocytes/macrophages is shorter than that of quiescent CD4+ T cells (Finzi and Siliciano 1998; Stevenson 2003; Van Lint et al. 2013). However, the life span of macrophages, as for CD4+ T cells, also varies greatly. Depending on their tissue location, macrophages can live from a few months to several years. Alveolar macrophages, which can be infected by HIV (Jambo et al. 2014), have been found to persist for over 3 years in analyses performed after lung transplant (Eguiluz-Gracia et al. 2016; Nayak et al. 2016), and microglial cells persist for years in the CNS (Tay et al. 2017). Moreover, macrophages may be better prepared than CD4+ T cells to resist apoptosis under conditions of metabolic stress (Carter and Ehrlich 2008; Jones and Power 2006; McNelis and Olefsky 2014).

It was previously assumed that activation of HIV-infected CD4+ T cells driving them to proliferation would reverse viral latency and decrease the half-life of cells carrying replication competent virus. However, recent phylogenetic studies using ultra deep whole genome sequencing have shown the presence of proviruses with identical sequences in clonally expanded infected CD4+ T cells (Chomont et al. 2009; Cohn et al. 2015; Maldarelli et al. 2014; Simonetti et al. 2016; Wagner et al. 2014; Boritz et al. 2016). Moreover, it is now clear that these expanded infected CD4+ T cells can also harbor intact proviruses able to spread infection (Hosmane et al. 2017; Simonetti et al. 2016). Several reports have shown that proliferation of infected CD4+ T cells could be at least partially driven by the selective integration of HIV-1 into genes that have been associated with cell growth, division, and cancer (Maldarelli et al. 2014; Wagner et al. 2014).

In addition, CD4+ T cells can divide in response to antigenic stimulation or to homeostatic signaling to balance cell numbers although capacity of self-renewal is lost with progressive differentiation of memory CD4+ T cells (Berard and Tough 2002; Mahnke et al. 2013). Antigenic stimulation through the T-cell receptor entails the activation of the cell and triggers cell differentiation. However, naïve and early differentiated cells require a higher signaling threshold and prolonged contact with antigen-presenting cells, and they also depend more on co-stimulatory signals than more differentiated cells to respond to antigens. Thus, low levels of antigen during

treated infection might provide a suboptimal signal allowing some degree of activation of these cells. It is however unknown whether, in vivo, some transiently activated cells might escape cell death despite some degree of viral production to later regain a quiescent state. In contrast, in vitro studies have confirmed that infected CD4+ T cells can undergo homeostatic proliferation without significant viral production or cell death (Bosque et al. 2011).

Homeostatic proliferation is governed by members of the common gamma chain family of cytokines in the absence of antigenic stimulation (Boyman et al. 2007; Seddon et al. 2003; Surh and Sprent 2008). In particular, IL-7 plays a central role in CD4+ T-cell homeostasis and survival. In the case of naïve CD4+ T cells, IL-7 signaling and contact with self-MHC-peptides complexes promotes cell survival without inducing proliferation. In contrast, IL-7 signaling can promote proliferation of memory CD4+ T cells independently of TCR activation. Responsiveness to IL-7 is not equal among all CD4+ T-cell memory subsets. TSCM and TCM express high levels of the IL-7 receptor (CD127) and have strong proliferative potential, while TEM express lower levels of CD127 and have a limited proliferative potential (Mahnke et al. 2013). Other common gamma chain cytokines (such as IL-2 or IL-15) also influence the survival and turnover of T cells in their inflammatory environment (Pennock et al. 2013). Overall, once established, memory CD4+ T cells can persist for decades in the absence of antigen (Hammarlund et al. 2003).

Macrophages and dendritic cells are terminally differentiated cell populations that cannot be propagated in vitro. However, macrophage subsets that are susceptible to HIV-1 infection are not in a quiescent state and share some characteristics of cycling cells (Badia et al. 2016; Mlcochova et al. 2017; Pauls et al. 2014a). Although infected macrophages did not show evidence of division in vitro, it is now clear that in vivo some macrophages can proliferate locally in tissues in response to inflammatory signals (Jenkins et al. 2011; Robbins et al. 2013; Zamarron et al. 2016). Thus, the possible persistence of some infected macrophages through cell division cannot be discarded.

9.5 Conclusion

The persistence of HIV-1-infected cells on ART depends on a combination of cell intrinsic characteristics including the susceptibility of cells to infection and their capacity to survive and proliferate (Fig. 9.2). However, it is also influenced by the responsiveness of these cells to external signals (such as inflammatory cytokines or contact with antigen-presenting cells) or their localization and capacity to circulate. For instance, HIV-specific CD4+ T cells are preferentially infected by HIV during treatment interruption when compared to other antigen-specific memory CD4+ T cells, and this is likely due to the selective localization of activated HIV-specific cells to the sites of viral replication (Douek et al. 2002). HIV-1 can thus reside in multiple cell subsets in multiple tissues, while the main mechanisms for persistence are diverse. It is of the outmost importance to define which specific infected cell

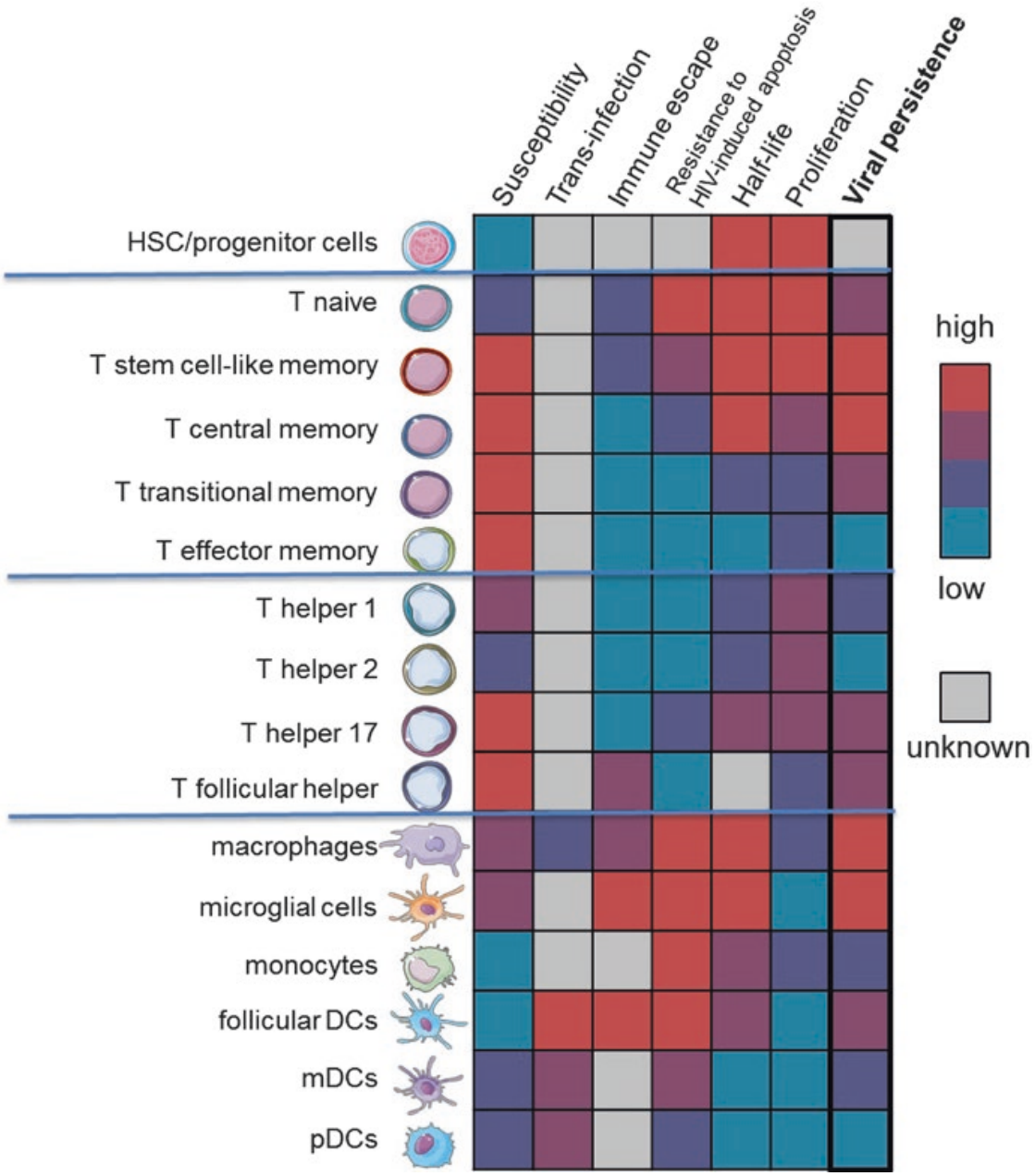

Fig. 9.2 Heatmap showing multiple features of different cell subsets that lead to HIV persistence on ART. Color codes were inferred on the basis of the literature discussed in this chapter. Among T-cell subsets, naïve, central memory, and stem cell-like memory T cells have a major contribution to long-term HIV persistence on ART, while effector memory and terminally differentiated helper T-cell subsets are depleted first during infection and have limited proliferative potential. Myeloid cells, despite their relatively low susceptibility to infection, are now increasingly recognized as important contributors to HIV persistence on ART in both lymphoid and non-lymphoid tissues due to their long half-life and resistance to apoptosis and immune-mediated killing. Although not shown here, tissue localization and activation status influence various parameters of persistence, with actively infected cells being more susceptible to cell death, thus reducing their contribution to long-term persistence

subset leads to viral rebound off ART and to determine if some of these subsets should be preferentially targeted. Irrespective, tackling HIV persistence will require multiple diverse strategies that target these difference mechanisms leading to cell survival.

References

Abbas W, Tariq M, Iqbal M, Kumar A, Herbein G (2015) Eradication of HIV-1 from the macrophage reservoir: an uncertain goal? Viruses 7:1578–1598. https://doi.org/10.3390/v7041578

Aillet F, Masutani H, Elbim C, Raoul H, Chêne L, Nugeyre MT, Paya C, Barré-Sinoussi F, Gougerot-Pocidalo MA, Israël N (1998) Human immunodeficiency virus induces a dual regulation of Bcl-2, resulting in persistent infection of CD4(+) T- or monocytic cell lines. J Virol 72:9698–9705

Allouch A, David A, Amie SM, Lahouassa H, Chartier L, Margottin-Goguet F, Barre-Sinoussi F, Kim B, Saez-Cirion A, Pancino G (2013) p21-mediated RNR2 repression restricts HIV-1 replication in macrophages by inhibiting dNTP biosynthesis pathway. Proc Natl Acad Sci U S A 110(42):E3997–E4006. https://doi.org/10.1073/pnas.1306719110

Allouch A, David A, Amie SM, Lahouassa H, Chartier L, Margottin-Goguet F, Barre-Sinoussi F, Kim B, Saez-Cirion A, Pancino G (2014) Reply to Pauls et al.: p21 is a master regulator of HIV replication in macrophages through dNTP synthesis block. Proc Natl Acad Sci U S A 111(14):E1325–E1326

Alter G, Heckerman D, Schneidewind A, Fadda L, Kadie CM, Carlson JM, Oniangue-Ndza C, Martin M, Li B, Khakoo SI, Carrington M, Allen TM, Altfeld M (2011) HIV-1 adaptation to NK-cell-mediated immune pressure. Nature 476:96–100. https://doi.org/10.1038/nature10237

Ananworanich J, Dube K, Chomont N (2015) How does the timing of antiretroviral therapy initiation in acute infection affect HIV reservoirs? Curr Opin HIV AIDS 10(1):18–28. https://doi.org/10.1097/COH.0000000000000122

Apps R, Del Prete GQ, Chatterjee P, Lara A, Brumme ZL, Brockman MA, Neil S, Pickering S, Schneider DK, Piechocka-Trocha A, Walker BD, Thomas R, Shaw GM, Hahn BH, Keele BF, Lifson JD, Carrington M (2016) HIV-1 Vpu mediates HLA-C Downregulation. Cell Host Microbe 19:686–695. https://doi.org/10.1016/j.chom.2016.04.005

Arhel N, Kirchhoff F (2010) Host proteins involved in HIV infection: new therapeutic targets. Biochim Biophys Acta (BBA) – Mol Basis Dis 1802:313–321. https://doi.org/10.1016/j.bbadis.2009.12.003

Asada M, Yamada T, Ichijo H, Delia D, Miyazono K, Fukumuro K, Mizutani S (1999) Apoptosis inhibitory activity of cytoplasmic p21(Cip1/WAF1) in monocytic differentiation. EMBO J 18(5):1223–1234. https://doi.org/10.1093/emboj/18.5.1223

Avettand-Fenoel V, Hocqueloux L, Ghosn J, Cheret A, Frange P, Melard A, Viard JP, Rouzioux C (2016) Total HIV-1 DNA, a marker of viral reservoir dynamics with clinical implications. Clin Microbiol Rev 29(4):859–880. https://doi.org/10.1128/CMR.00015-16

Badia R, Pujantell M, Riveira-Munoz E, Puig T, Torres-Torronteras J, Marti R, Clotet B, Ampudia RM, Vives-Pi M, Este JA, Ballana E (2016) The G1/S Specific Cyclin D2 Is a Regulator of HIV-1 Restriction in Non-proliferating Cells. Plos Pathogens 12(8). doi:ARTN e1005829 https://doi.org/10.1371/journal.ppat.1005829

Badley AD, McElhinny JA, Leibson PJ, Lynch DH, Alderson MR, Paya CV (1996) Upregulation of Fas ligand expression by human immunodeficiency virus in human macrophages mediates apoptosis of uninfected T lymphocytes. J Virol 70:199–206

Badley AD, Dockrell D, Simpson M, Schut R, Lynch DH, Leibson P, Paya CV (1997) Macrophage-dependent apoptosis of CD4+ T lymphocytes from HIV-infected individuals is mediated by FasL and tumor necrosis factor. J Exp Med 185:55–64. https://doi.org/10.1084/jem.185.1.55

Badley AD, Dockrell DH, Algeciras A, Ziesmer S, Landay A, Lederman MM, Connick E, Kessler H, Kuritzkes D, Lynch DH, Roche P, Yagita H, Paya CV (1998) In vivo analysis of Fas/FasL interactions in HIV-infected patients. J Clin Investig 102:79–87

Badley AD, Sainski A, Wightman F, Lewin SR (2013) Altering cell death pathways as an approach to cure HIV infection. Cell Death Dis 4:e718. https://doi.org/10.1038/cddis.2013.248

Banga R, Procopio FA, Noto A, Pollakis G, Cavassini M, Ohmiti K, Corpataux J-M, de Leval L, Pantaleo G, Perreau M (2016) PD-1+ and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals. Nat Med 22:754–761. https://doi.org/10.1038/nm.4113

Barton K, Winckelmann A, Palmer S (2016) HIV-1 reservoirs during suppressive therapy. Trends Microbiol 24(5):345–355. https://doi.org/10.1016/j.tim.2016.01.006

Beloglazova N, Flick R, Tchigvintsev A, Brown G, Popovic A, Nocek B, Yakunin AF (2013) Nuclease activity of the human SAMHD1 protein implicated in the Aicardi-Goutieres syndrome and HIV-1 restriction. J Biol Chem 288(12):8101–8110. https://doi.org/10.1074/jbc.M112.431148

Berard M, Tough DF (2002) Qualitative differences between naive and memory T cells. Immunology 106(2):127–138

Bergamaschi A, David A, Le Rouzic E, Nisole S, Barre-Sinoussi F, Pancino G (2009) The CDK inhibitor p21Cip1/WAF1 is induced by FcgammaR activation and restricts the replication of human immunodeficiency virus type 1 and related primate lentiviruses in human macrophages. J Virol 83(23):12253–12265. https://doi.org/10.1128/JVI.01395-09

Berro R, Cdl F, Klase Z, Kehn K, Parvin L, Pumfery A, Agbottah E, Vertes A, Nekhai S, Kashanchi F (2007) Identifying the membrane proteome of HIV-1 latently infected cells. J Biol Chem 282:8207–8218. https://doi.org/10.1074/jbc.M606324200

Besnard E, Hakre S, Kampmann M, Lim HW, Hosmane NN, Martin A, Bassik MC, Verschueren E, Battivelli E, Chan J, Svensson JP, Gramatica A, Conrad RJ, Ott M, Greene WC, Krogan NJ, Siliciano RF, Weissman JS, Verdin E (2016) The mTOR complex controls HIV latency. Cell Host Microbe 20(6):785–797. https://doi.org/10.1016/j.chom.2016.11.001

Betts MR, Nason MC, West SM, Rosa SCD, Migueles SA, Abraham J, Lederman MM, Benito JM, Goepfert PA, Connors M, Roederer M, Koup RA (2006) HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. Blood 107:4781–4789. https://doi.org/10.1182/blood-2005-12-4818

Boritz EA, Darko S, Swaszek L, Wolf G, Wells D, Wu X, Henry AR, Laboune F, Hu J, Ambrozak D, Hughes MS, Hoh R, Casazza JP, Vostal A, Bunis D, Nganou-Makamdop K, Lee JS, Migueles SA, Koup RA, Connors M, Moir S, Schacker T, Maldarelli F, Hughes SH, Deeks SG, Douek DC (2016) Multiple origins of virus persistence during natural control of HIV infection. Cell 166(4):1004–1015. https://doi.org/10.1016/j.cell.2016.06.039

Borrow P, Lewicki H, Wei X, Horwitz MS, Peffer N, Meyers H, Nelson JA, Gairin JE, Hahn BH, Oldstone MB, Shaw GM (1997) Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. Nat Med 3:205–211

Bosque A, Famiglietti M, Weyrich AS, Goulston C, Planelles V (2011) Homeostatic proliferation fails to efficiently reactivate HIV-1 latently infected central memory CD4+ T cells. PLoS Pathog 7:e1002288. https://doi.org/10.1371/journal.ppat.1002288

Boyman O, Purton JF, Surh CD, Sprent J (2007) Cytokines and T-cell homeostasis. Curr Opin Immunol 19:320–326. https://doi.org/10.1016/j.coi.2007.04.015

Brass AL, Dykxhoorn DM, Benita Y, Yan N, Engelman A, Xavier RJ, Lieberman J, Elledge SJ (2008) Identification of host proteins required for HIV infection through a functional genomic screen. Science 319(5865):921–926. https://doi.org/10.1126/science.1152725

Brown A, Zhang H, Lopez P, Pardo CA, Gartner S (2006) In vitro modeling of the HIV-macrophage reservoir. J Leukoc Biol 80(5):1127–1135. https://doi.org/10.1189/jlb.0206126

Buzon MJ, Sun H, Li C, Shaw A, Seiss K, Ouyang Z, Martin-Gayo E, Leng J, Henrich TJ, Li JZ, Pereyra F, Zurakowski R, Walker BD, Rosenberg ES, Yu XG, Lichterfeld M (2014) HIV-1

persistence in CD4+ T cells with stem cell-like properties. Nat Med 20(2):139–142. https://doi.org/10.1038/nm.3445

Cai R, Liu L, Luo B, Wang J, Shen J, Shen Y, Zhang R, Chen J, Lu H (2016) Caspase-1 activity in CD4 T cells is Downregulated following antiretroviral therapy for HIV-1 infection. AIDS Res Hum Retrovir 33:164–171. https://doi.org/10.1089/aid.2016.0234

Carter CA, Ehrlich LS (2008) Cell biology of HIV-1 infection of macrophages. Annu Rev Microbiol 62:425–443. https://doi.org/10.1146/annurev.micro.62.081307.162758

Chavez L, Calvanese V, Verdin E (2015) HIV latency is established directly and early in both resting and activated primary CD4 T cells. PLoS Pathog 11(6):e1004955. https://doi.org/10.1371/journal.ppat.1004955

Chen H, Li C, Huang J, Cung T, Seiss K, Beamon J, Carrington MF, Porter LC, Burke PS, Yang Y, Ryan BJ, Liu R, Weiss RH, Pereyra F, Cress WD, Brass AL, Rosenberg ES, Walker BD, Yu XG, Lichterfeld M (2011) CD4+ T cells from elite controllers resist HIV-1 infection by selective upregulation of p21. J Clin Invest 121(4):1549–1560. https://doi.org/10.1172/JCI44539

Cheret A, Bacchus-Souffan C, Avettand-Fenoel V, Melard A, Nembot G, Blanc C, Samri A, Saez-Cirion A, Hocqueloux L, Lascoux-Combe C, Allavena C, Goujard C, Valantin MA, Leplatois A, Meyer L, Rouzioux C, Autran B, Group OA-S (2015) Combined ART started during acute HIV infection protects central memory CD4+ T cells and can induce remission. J Antimicrob Chemother 70(7):2108–2120. https://doi.org/10.1093/jac/dkv084

Chi H (2012) Regulation and function of mTOR signalling in T cell fate decisions. Nat Rev Immunol 12(5):325–338. https://doi.org/10.1038/nri3198

Chinn LW, Tang M, Kessing BD, Lautenberger JA, Troyer JL, Malasky MJ, McIntosh C, Kirk GD, Wolinsky SM, Buchbinder SP, Gomperts ED, Goedert JJ, O'Brien SJ (2010) Genetic associations of variants in genes encoding HIV-dependency factors required for HIV-1 infection. J Infect Dis 202:1836–1845. https://doi.org/10.1086/657322

Chomont N, El-Far M, Ancuta P, Trautmann L, Procopio FA, Yassine-Diab B, Boucher G, Boulassel MR, Ghattas G, Brenchley JM, Schacker TW, Hill BJ, Douek DC, Routy JP, Haddad EK, Sekaly RP (2009) HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. Nat Med 15(8):893–900. https://doi.org/10.1038/nm.1972

Chun TW, Engel D, Berrey MM, Shea T, Corey L, Fauci AS (1998) Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. Proc Natl Acad Sci U S A 95(15):8869–8873

Cicala C, Arthos J, Rubbert A, Selig S, Wildt K, Cohen OJ, Fauci AS (2000) HIV-1 envelope induces activation of caspase-3 and cleavage of focal adhesion kinase in primary human CD4(+) T cells. Proc Natl Acad Sci U S A 97:1178–1183

Clark RA (2015) Resident memory T cells in human health and disease. Sci Transl Med 7:269rv261. https://doi.org/10.1126/scitranslmed.3010641

Cleret-Buhot A, Zhang Y, Planas D, Goulet J-P, Monteiro P, Gosselin A, Wacleche VS, Tremblay CL, Jenabian M-A, Routy J-P, El-Far M, Chomont N, Haddad EK, Sekaly R-P, Ancuta P (2015) Identification of novel HIV-1 dependency factors in primary CCR4+CCR6+Th17 cells via a genome-wide transcriptional approach. Retrovirology 12:102. https://doi.org/10.1186/s12977-015-0226-9

Cohen GB, Gandhi RT, Davis DM, Mandelboim O, Chen BK, Strominger JL, Baltimore D (1999) The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. Immunity 10:661–671. https://doi.org/10.1016/S1074-7613(00)80065-5

Cohn LB, Silva IT, Oliveira TY, Rosales RA, Parrish EH, Learn GH, Hahn BH, Czartoski JL, McElrath MJ, Lehmann C, Klein F, Caskey M, Walker BD, Siliciano JD, Siliciano RF, Jankovic M, Nussenzweig MC (2015) HIV-1 integration landscape during latent and active infection. Cell 160(3):420–432. https://doi.org/10.1016/j.cell.2015.01.020

Coleman CM, Wu L (2009) HIV interactions with monocytes and dendritic cells: viral latency and reservoirs. Retrovirology 6:51. https://doi.org/10.1186/1742-4690-6-51

Collins KL, Chen BK, Kalams SA, Walker BD, Baltimore D (1998) HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. Nature 391:397–401. https://doi.org/10.1038/34929

Connick E, Mattila T, Folkvord JM, Schlichtemeier R, Meditz AL, Ray MG, McCarter MD, MaWhinney S, Hage A, White C, Skinner PJ (2007) CTL fail to accumulate at sites of HIV-1 replication in lymphoid tissue. J Immunol 178:6975–6983. https://doi.org/10.4049/jimmunol.178.11.6975

Cooper A, García M, Petrovas C, Yamamoto T, Koup RA, Nabel GJ (2013) HIV-1 causes CD4 cell death through DNA-dependent protein kinase during viral integration. Nature 498:376–379. https://doi.org/10.1038/nature12274

Cribier A, Descours B, Valadao AL, Laguette N, Benkirane M (2013) Phosphorylation of SAMHD1 by cyclin A2/CDK1 regulates its restriction activity toward HIV-1. Cell Rep 3(4):1036–1043. https://doi.org/10.1016/j.celrep.2013.03.017

Crowe S, Zhu T, Muller WA (2003) The contribution of monocyte infection and trafficking to viral persistence, and maintenance of the viral reservoir in HIV infection. J Leukoc Biol 74:635–641. https://doi.org/10.1189/jlb.0503204

Damouche A, Lazure T, Avettand-Fènoël V, Huot N, Dejucq-Rainsford N, Satie A-P, Mélard A, David L, Gommet C, Ghosn J, Noel N, Pourcher G, Martinez V, Benoist S, Béréziat V, Cosma A, Favier B, Vaslin B, Rouzioux C, Capeau J, Müller-Trutwin M, Dereuddre-Bosquet N, Grand RL, Lambotte O, Bourgeois C (2015) Adipose tissue is a neglected viral reservoir and an inflammatory site during chronic HIV and SIV infection. PLoS Pathog 11:e1005153. https://doi.org/10.1371/journal.ppat.1005153

De Iaco A, Luban J (2014) Cyclophilin a promotes HIV-1 reverse transcription but its effect on transduction correlates best with its effect on nuclear entry of viral cDNA. Retrovirology 11:11. https://doi.org/10.1186/1742-4690-11-11

Deeks SG, Lewin SR, Ross AL, Ananworanich J, Benkirane M, Cannon P, Chomont N, Douek D, Lifson JD, Lo YR, Kuritzkes D, Margolis D, Mellors J, Persaud D, Tucker JD, Barre-Sinoussi F, International ASTaCWG, Alter G, Auerbach J, Autran B, Barouch DH, Behrens G, Cavazzana M, Chen Z, Cohen EA, Corbelli GM, Eholie S, Eyal N, Fidler S, Garcia L, Grossman C, Henderson G, Henrich TJ, Jefferys R, Kiem HP, McCune J, Moodley K, Newman PA, Nijhuis M, Nsubuga MS, Ott M, Palmer S, Richman D, Saez-Cirion A, Sharp M, Siliciano J, Silvestri G, Singh J, Spire B, Taylor J, Tolstrup M, Valente S, van Lunzen J, Walensky R, Wilson I, Zack J (2016) International AIDS society global scientific strategy: towards an HIV cure 2016. Nat Med 22(8):839–850. https://doi.org/10.1038/nm.4108

Deng K, Pertea M, Rongvaux A, Wang L, Durand CM, Ghiaur G, Lai J, McHugh HL, Hao H, Zhang H, Margolick JB, Gurer C, Murphy AJ, Valenzuela DM, Yancopoulos GD, Deeks SG, Strowig T, Kumar P, Siliciano JD, Salzberg SL, Flavell RA, Shan L, Siliciano RF (2015) Broad CTL response is required to clear latent HIV-1 due to dominance of escape mutations. Nature 517:381–385. https://doi.org/10.1038/nature14053

Descours B, Cribier A, Chable-Bessia C, Ayinde D, Rice G, Crow Y, Yatim A, Schwartz O, Laguette N, Benkirane M (2012) SAMHD1 restricts HIV-1 reverse transcription in quiescent CD4(+) T-cells. Retrovirology 9:87. https://doi.org/10.1186/1742-4690-9-87

Diamond TL, Roshal M, Jamburuthugoda VK, Reynolds HM, Merriam AR, Lee KY, Balakrishnan M, Bambara RA, Planelles V, Dewhurst S, Kim B (2004) Macrophage tropism of HIV-1 depends on efficient cellular dNTP utilization by reverse transcriptase. J Biol Chem 279(49):51545–51553. https://doi.org/10.1074/jbc.M408573200

DiNapoli SR, Ortiz AM, Wu F, Matsuda K, Twigg HL, Hirsch VM, Knox K, Brenchley JM (2017) Tissue-resident macrophages can contain replication-competent virus in antiretroviral-naive, SIV-infected Asian macaques. JCI Insight 2. https://doi.org/10.1172/jci.insight.91214

Doitsh G, Cavrois M, Lassen KG, Zepeda O, Yang Z, Santiago ML, Hebbeler AM, Greene WC (2010) Abortive HIV infection mediates CD4 T cell depletion and inflammation in human lymphoid tissue. Cell 143:789–801. https://doi.org/10.1016/j.cell.2010.11.001

Doitsh G, Galloway NLK, Geng X, Yang Z, Monroe KM, Zepeda O, Hunt PW, Hatano H, Sowinski S, Muñoz-Arias I, Greene WC (2014) Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. Nature 505:509–514. https://doi.org/10.1038/nature12940

Douek DC, Brenchley JM, Betts MR, Ambrozak DR, Hill BJ, Okamoto Y, Casazza JP, Kuruppu J, Kunstman K, Wolinsky S, Grossman Z, Dybul M, Oxenius A, Price DA, Connors M, Koup RA (2002) HIV preferentially infects HIV-specific CD4+ T cells. Nature 417(6884):95–98. https://doi.org/10.1038/417095a

Eguiluz-Gracia I, Schultz HH, Sikkeland LI, Danilova E, Holm AM, Pronk CJ, Agace WW, Iversen M, Andersen C, Jahnsen FL, Baekkevold ES (2016) Long-term persistence of human donor alveolar macrophages in lung transplant recipients. Thorax 71(11):1006–1011. https://doi.org/10.1136/thoraxjnl-2016-208292

El Hed A, Khaitan A, Kozhaya L, Manel N, Daskalakis D, Borkowsky W, Valentine F, Littman DR, Unutmaz D (2010) Susceptibility of human Th17 cells to human immunodeficiency virus and their perturbation during infection. J Infect Dis 201:843–854. https://doi.org/10.1086/651021

Engelman A, Cherepanov P (2008) The lentiviral integrase binding protein LEDGF/p75 and HIV-1 replication. PLoS Pathog 4(3):e1000046. https://doi.org/10.1371/journal.ppat.1000046

Estaquier J, Idziorek T, Zou W, Emilie D, Farber CM, Bourez JM, Ameisen JC (1995) T helper type 1/T helper type 2 cytokines and T cell death: preventive effect of interleukin 12 on activation-induced and CD95 (FAS/APO-1)-mediated apoptosis of CD4+ T cells from human immunodeficiency virus-infected persons. J Exp Med 182:1759–1767. https://doi.org/10.1084/jem.182.6.1759

Finkel TH, Tudor-Williams G, Banda NK, Cotton MF, Curiel T, Monks C, Baba TW, Ruprecht RM, Kupfer A (1995) Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. Nat Med 1(2):129–134

Finzi D, Siliciano RF (1998) Viral dynamics in HIV-1 infection. Cell 93:665–671. https://doi.org/10.1016/S0092-8674(00)81427-0

Fletcher CV, Staskus K, Wietgrefe SW, Rothenberger M, Reilly C, Chipman JG, Beilman GJ, Khoruts A, Thorkelson A, Schmidt TE, Anderson J, Perkey K, Stevenson M, Perelson AS, Douek DC, Haase AT, Schacker TW (2014) Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. Proc Natl Acad Sci U S A 111(6):2307–2312. https://doi.org/10.1073/pnas.1318249111

Folkvord JM, Armon C, Connick E (2005) Lymphoid follicles are sites of heightened human immunodeficiency virus type 1 (HIV-1) replication and reduced antiretroviral effector mechanisms. AIDS Res Hum Retrovir 21:363–370. https://doi.org/10.1089/aid.2005.21.363

Fukazawa Y, Lum R, Okoye AA, Park H, Matsuda K, Bae JY, Hagen SI, Shoemaker R, Deleage C, Lucero C, Morcock D, Swanson T, Legasse AW, Axthelm MK, Hesselgesser J, Geleziunas R, Hirsch VM, Edlefsen PT, Piatak M, Estes JD, Lifson JD, Picker LJ (2015) B cell follicle sanctuary permits persistent productive simian immunodeficiency virus infection in elite controllers. Nat Med 21:132–139. https://doi.org/10.1038/nm.3781

Ganesh A, Lemongello D, Lee E, Peterson J, McLaughlin BE, Ferre AL, Gillespie GM, Fuchs D, Deeks SG, Hunt PW, Price RW, Spudich SS, Shacklett BL (2016) Immune activation and HIV-specific CD8(+) T cells in cerebrospinal fluid of HIV controllers and noncontrollers. AIDS Res Hum Retrovir 32:791–800. https://doi.org/10.1089/AID.2015.0313

Gattinoni L, Lugli E, Ji Y, Pos Z, Paulos CM, Quigley MF, Almeida JR, Gostick E, Yu Z, Carpenito C, Wang E, Douek DC, Price DA, June CH, Marincola FM, Roederer M, Restifo NP (2011) A human memory T cell subset with stem cell-like properties. Nat Med 17(10):1290–1297. https://doi.org/10.1038/nm.2446

Geleziunas R, Xu W, Takeda K, Ichijo H, Greene WC (2001) HIV-1 Nef inhibits ASK1-dependent death signalling providing a potential mechanism for protecting the infected host cell. Nature 410:834–838. https://doi.org/10.1038/35071111

Gerard A, Segeral E, Naughtin M, Abdouni A, Charmeteau B, Cheynier R, Rain JC, Emiliani S (2015) The integrase cofactor LEDGF/p75 associates with Iws1 and Spt6 for postintegration

silencing of HIV-1 gene expression in latently infected cells. Cell Host Microbe 17(1):107–117. https://doi.org/10.1016/j.chom.2014.12.002

Giri MS, Nebozyhn M, Raymond A, Gekonge B, Hancock A, Creer S, Nicols C, Yousef M, Foulkes AS, Mounzer K, Shull J, Silvestri G, Kostman J, Collman RG, Showe L, Montaner LJ (2009) Circulating monocytes in HIV-1-infected Viremic subjects exhibit an Antiapoptosis gene signature and virus- and host-mediated apoptosis resistance. J Immunol 182:4459–4470. https://doi.org/10.4049/jimmunol.0801450

Goh WC, Rogel ME, Kinsey CM, Michael SF, Fultz PN, Nowak MA, Hahn BH, Emerman M (1998) HIV-1 Vpr increases viral expression by manipulation of the cell cycle: a mechanism for selection of Vpr in vivo. Nat Med 4(1):65–71

Gosselin A, Monteiro P, Chomont N, Diaz-Griffero F, Said EA, Fonseca S, Wacleche V, El-Far M, Boulassel M-R, Routy J-P, Sekaly R-P, Ancuta P (2010) Peripheral blood CCR4+CCR6+ and CXCR3+CCR6+ CD4+ T cells are highly permissive to HIV-1 infection. J Immunol 184:1604–1616. https://doi.org/10.4049/jimmunol.0903058

Goujon C, Moncorge O, Bauby H, Doyle T, Ward CC, Schaller T, Hue S, Barclay WS, Schulz R, Malim MH (2013) Human MX2 is an interferon-induced post-entry inhibitor of HIV-1 infection. Nature 502(7472):559–562. https://doi.org/10.1038/nature12542

Graf EH, Pace MJ, Peterson BA, Lynch LJ, Chukwulebe SB, Mexas AM, Shaheen F, Martin JN, Deeks SG, Connors M, Migueles SA, O'Doherty U (2013a) Gag-positive reservoir cells are susceptible to HIV-specific cytotoxic T lymphocyte mediated clearance in vitro and can be detected in vivo [corrected]. PLoS One 8:e71879. https://doi.org/10.1371/journal.pone.0071879

Graf EH, Pace MJ, Peterson BA, Lynch LJ, Chukwulebe SB, Mexas AM, Shaheen F, Martin JN, Deeks SG, Connors M, Migueles SA, O'Doherty U (2013b) Gag-positive reservoir cells are susceptible to HIV-specific cytotoxic T lymphocyte mediated clearance in vitro and can be detected in vivo. PLoS One 8:e71879. https://doi.org/10.1371/journal.pone.0071879

Grossman Z, Meier-Schellersheim M, Paul WE, Picker LJ (2006) Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. Nat Med 12:289–295. https://doi.org/10.1038/nm1380

Hammarlund E, Lewis MW, Hansen SG, Strelow LI, Nelson JA, Sexton GJ, Hanifin JM, Slifka MK (2003) Duration of antiviral immunity after smallpox vaccination. Nat Med 9:1131–1137. https://doi.org/10.1038/nm917

Han Y, Lassen K, Monie D, Sedaghat AR, Shimoji S, Liu X, Pierson TC, Margolick JB, Siliciano RF, Siliciano JD (2004) Resting CD4+ T cells from human immunodeficiency virus type 1 (HIV-1)-infected individuals carry integrated HIV-1 genomes within actively transcribed host genes. J Virol 78(12):6122–6133. https://doi.org/10.1128/JVI.78.12.6122-6133.2004

Heesters BA, Lindqvist M, Vagefi PA, Scully EP, Schildberg FA, Altfeld M, Walker BD, Kaufmann DE, Carroll MC (2015) Follicular dendritic cells retain infectious HIV in cycling endosomes. PLoS Pathog 11(12):e1005285. https://doi.org/10.1371/journal.ppat.1005285

Herbeuval J-P, Boasso A, Grivel J-C, Hardy AW, Anderson SA, Dolan MJ, Chougnet C, Lifson JD, Shearer GM (2005) TNF-related apoptosis-inducing ligand (TRAIL) in HIV-1–infected patients and its in vitro production by antigen-presenting cells. Blood 105:2458–2464. https://doi.org/10.1182/blood-2004-08-3058

Herbeuval J-P, Nilsson J, Boasso A, Hardy AW, Kruhlak MJ, Anderson SA, Dolan MJ, Dy M, Andersson J, Shearer GM (2006) Differential expression of IFN-α and TRAIL/DR5 in lymphoid tissue of progressor versus nonprogressor HIV-1-infected patients. Proc Natl Acad Sci 103:7000–7005. https://doi.org/10.1073/pnas.0600363103

Hong JJ, Amancha PK, Rogers K, Ansari AA, Villinger F (2012) Spatial alterations between CD4+ T follicular helper, B, and CD8+ T cells during simian immunodeficiency virus infection: T/B cell homeostasis, activation, and potential mechanism for viral escape. J Immunol 188:3247–3256. https://doi.org/10.4049/jimmunol.1103138

Hosmane NN, Kwon KJ, Bruner KM, Capoferri AA, Beg S, Rosenbloom DIS, Keele BF, Ho Y-C, Siliciano JD, Siliciano RF (2017) Proliferation of latently infected CD4+ T cells car-

rying replication-competent HIV-1: Potential role in latent reservoir dynamics. J Experiment Med:jem.20170193. https://doi.org/10.1084/jem.20170193

Huang S, Jones RB (2017) CTLs pare defective HIV proviruses without impacting infectious latent reservoirs. CROI, San Francisco

Izquierdo-Useros N, Naranjo-Gomez M, Erkizia I, Puertas MC, Borras FE, Blanco J, Martinez-Picado J (2010) HIV and mature dendritic cells: Trojan exosomes riding the Trojan horse? PLoS Pathog 6(3):e1000740. https://doi.org/10.1371/journal.ppat.1000740

Jaafoura S, de Goer de Herve MG, Hernandez-Vargas EA, Hendel-Chavez H, Abdoh M, Mateo MC, Krzysiek R, Merad M, Seng R, Tardieu M, Delfraissy JF, Goujard C, Taoufik Y (2014) Progressive contraction of the latent HIV reservoir around a core of less-differentiated CD4(+) memory T cells. Nat Commun 5:5407. https://doi.org/10.1038/ncomms6407

Jambo KC, Banda DH, Kankwatira AM, Sukumar N, Allain TJ, Heyderman RS, Russell DG, Mwandumba HC (2014) Small alveolar macrophages are infected preferentially by HIV and exhibit impaired phagocytic function. Mucosal Immunol 7(5):1116–1126. https://doi.org/10.1038/mi.2013.127

Jenkins SJ, Ruckerl D, Cook PC, Jones LH, Finkelman FD, Nv R, MacDonald AS, Allen JE (2011) Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. Science 332:1284–1288. https://doi.org/10.1126/science.1204351

Jennes W, Verheyden S, Demanet C, Adjé-Touré CA, Vuylsteke B, Nkengasong JN, Kestens L (2006) Cutting edge: resistance to HIV-1 infection among African female sex workers is associated with inhibitory KIR in the absence of their HLA ligands. J Immunol (Baltimore, MD: 1950) 177:6588–6592

Johnson S, Eller M, Teigler JE, Maloveste SM, Schultz BT, Soghoian DZ, Lu R, Oster AF, Chenine A-L, Alter G, Dittmer U, Marovich M, Robb ML, Michael NL, Bolton D, Streeck H (2015) Cooperativity of HIV-specific Cytolytic CD4 T cells and CD8 T cells in control of HIV Viremia. J Virol 89:7494–7505. https://doi.org/10.1128/JVI.00438-15

Jones G, Power C (2006) Regulation of neural cell survival by HIV-1 infection. Neurobiol Dis 21(1):1–17. https://doi.org/10.1016/j.nbd.2005.07.018

Joseph SB, Arrildt KT, Sturdevant CB, Swanstrom R (2015) HIV-1 target cells in the CNS. J Neurovirol 21:276–289. https://doi.org/10.1007/s13365-014-0287-x

Jouve M, Sol-Foulon N, Watson S, Schwartz O, Benaroch P (2007) HIV-1 buds and accumulates in "nonacidic" endosomes of macrophages. Cell Host Microbe 2(2):85–95. https://doi.org/10.1016/j.chom.2007.06.011

Kandathil AJ, Sugawara S, Balagopal A (2016) Are T cells the only HIV-1 reservoir? Retrovirology 13(1):86. https://doi.org/10.1186/s12977-016-0323-4

Karn J, Stoltzfus CM (2012) Transcriptional and posttranscriptional regulation of HIV-1 gene expression. Cold Spring Harb Perspect Med 2(2):a006916. https://doi.org/10.1101/cshperspect.a006916

Katsikis PD, Wunderlich ES, Smith CA, Herzenberg LA, Herzenberg LA (1995) Fas antigen stimulation induces marked apoptosis of T lymphocytes in human immunodeficiency virus-infected individuals. J Exp Med 181:2029–2036. https://doi.org/10.1084/jem.181.6.2029

Kessing CF, Spudich S, Valcour V, Cartwright P, Chalermchai T, Fletcher JLK, Nichols C, Josey BJ, Slike B, Krebs SJ, Sailsuta N, Lerdlum S, Jagodzinski L, Tipsuk S, Suttichom D, Rattanamanee S, Zetterberg H, Hellmuth J, Phanuphak N, Robb ML, Michael NL, Ananworanich J, Trautmann L (2017, 1999) High number of activated CD8+ T cells targeting HIV antigens are present in cerebrospinal fluid in acute HIV infection. J Acquir Immune Defic Syndr. https://doi.org/10.1097/QAI.0000000000001301

Kivisäkk P, Mahad DJ, Callahan MK, Trebst C, Tucky B, Wei T, Wu L, Baekkevold ES, Lassmann H, Staugaitis SM, Campbell JJ, Ransohoff RM (2003) Human cerebrospinal fluid central memory CD4+ T cells: evidence for trafficking through choroid plexus and meninges via P-selectin. Proc Natl Acad Sci 100:8389–8394. https://doi.org/10.1073/pnas.1433000100

König R, Zhou Y, Elleder D, Diamond TL, Bonamy GMC, Irelan JT, C-y C, Tu BP, De Jesus PD, Lilley CE, Seidel S, Opaluch AM, Caldwell JS, Weitzman MD, Kuhen KL, Bandyopadhyay S,

Ideker T, Orth AP, Miraglia LJ, Bushman FD, Young JA, Chanda SK (2008) Global analysis of host-pathogen interactions that regulate early-stage HIV-1 replication. Cell 135:49–60. https://doi.org/10.1016/j.cell.2008.07.032

Kumar A, Abbas W, Herbein G (2014) HIV-1 latency in monocytes/macrophages. Viruses 6(4):1837–1860. https://doi.org/10.3390/v6041837

Laguette N, Sobhian B, Casartelli N, Ringeard M, Chable-Bessia C, Segeral E, Yatim A, Emiliani S, Schwartz O, Benkirane M (2011) SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature 474(7353):654–657. https://doi.org/10.1038/nature10117

Lahouassa H, Daddacha W, Hofmann H, Ayinde D, Logue EC, Dragin L, Bloch N, Maudet C, Bertrand M, Gramberg T, Pancino G, Priet S, Canard B, Laguette N, Benkirane M, Transy C, Landau NR, Kim B, Margottin-Goguet F (2012) SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates. Nat Immunol 13(3):223–228. https://doi.org/10.1038/ni.2236

Le Douce V, Herbein G, Rohr O, Schwartz C (2010) Molecular mechanisms of HIV-1 persistence in the monocyte-macrophage lineage. Retrovirology 7:32. https://doi.org/10.1186/1742-4690-7-32

Lehrman G, Hogue IB, Palmer S, Jennings C, Spina CA, Wiegand A, Landay AL, Coombs RW, Richman DD, Mellors JW, Coffin JM, Bosch RJ, Margolis DM (2005) Depletion of latent HIV-1 infection in vivo: a proof-of-concept study. Lancet 366:549–555. https://doi.org/10.1016/S0140-6736(05)67098-5

Leong YA, Chen Y, Ong HS, Wu D, Man K, Deleage C, Minnich M, Meckiff BJ, Wei Y, Hou Z, Zotos D, Fenix KA, Atnerkar A, Preston S, Chipman JG, Beilman GJ, Allison CC, Sun L, Wang P, Xu J, Toe JG, Lu HK, Tao Y, Palendira U, Dent AL, Landay AL, Pellegrini M, Comerford I, McColl SR, Schacker TW, Long HM, Estes JD, Busslinger M, Belz GT, Lewin SR, Kallies A, Yu D (2016) CXCR5+ follicular cytotoxic T cells control viral infection in B cell follicles. Nat Immunol 17:1187–1196. https://doi.org/10.1038/ni.3543

Lescure F-X, Moulignier A, Savatovsky J, Amiel C, Carcelain G, Molina J-M, Gallien S, Pacanovski J, Pialoux G, Adle-Biassette H, Gray F (2013) CD8 encephalitis in HIV-infected patients receiving cART: a treatable entity. Clin Infect Dis 57:101–108. https://doi.org/10.1093/cid/cit175

Letendre S, Marquie-Beck J, Capparelli E, Best B, Clifford D, Collier AC, Gelman BB, McArthur JC, McCutchan JA, Morgello S, Simpson D, Grant I, Ellis RJ (2008) Validation of the CNS penetration-effectiveness rank for quantifying antiretroviral penetration into the central nervous system. Arch Neurol 65:65–70. https://doi.org/10.1001/archneurol.2007.31

Li CJ, Friedman DJ, Wang C, Metelev V, Pardee AB (1995) Induction of apoptosis in uninfected lymphocytes by HIV-1 Tat protein. Science 268:429–431. https://doi.org/10.1126/science.7716549

Liu L, Oliveira NM, Cheney KM, Pade C, Dreja H, Bergin AM, Borgdorff V, Beach DH, Bishop CL, Dittmar MT, McKnight A (2011) A whole genome screen for HIV restriction factors. Retrovirology 8:94. https://doi.org/10.1186/1742-4690-8-94

Lohman BL, Miller CJ, McChesney MB (1995) Antiviral cytotoxic T lymphocytes in vaginal mucosa of simian immunodeficiency virus-infected rhesus macaques. J Immunol 155:5855–5860

Loisel-Meyer S, Swainson L, Craveiro M, Oburoglu L, Mongellaz C, Costa C, Martinez M, Cosset FL, Battini JL, Herzenberg LA, Herzenberg LA, Atkuri KR, Sitbon M, Kinet S, Verhoeyen E, Taylor N (2012) Glut1-mediated glucose transport regulates HIV infection. Proc Natl Acad Sci U S A 109(7):2549–2554. https://doi.org/10.1073/pnas.1121427109

Lorenzo-Redondo R, Fryer HR, Bedford T, Kim EY, Archer J, Kosakovsky Pond SL, Chung YS, Penugonda S, Chipman JG, Fletcher CV, Schacker TW, Malim MH, Rambaut A, Haase AT, McLean AR, Wolinsky SM (2016) Persistent HIV-1 replication maintains the tissue reservoir during therapy. Nature 530(7588):51–56. https://doi.org/10.1038/nature16933

Lugli E, Dominguez MH, Gattinoni L, Chattopadhyay PK, Bolton DL, Song K, Klatt NR, Brenchley JM, Vaccari M, Gostick E, Price DA, Waldmann TA, Restifo NP, Franchini G, Roederer M (2013) Superior T memory stem cell persistence supports long-lived T cell memory. J Clin Invest 123:594–599. https://doi.org/10.1172/JCI66327

Macallan DC, Wallace D, Zhang Y, De Lara C, Worth AT, Ghattas H, Griffin GE, Beverley PC, Tough DF (2004) Rapid turnover of effector-memory CD4(+) T cells in healthy humans. J Exp Med 200(2):255–260. https://doi.org/10.1084/jem.20040341

Macian F (2005) NFAT proteins: key regulators of T-cell development and function. Nat Rev Immunol 5(6):472–484. https://doi.org/10.1038/nri1632

Maggi E, Mazzetti M, Ravina A, Annunziato F, de Carli M, Piccinni MP, Manetti R, Carbonari M, Pesce AM, del Prete G et al (1994) Ability of HIV to promote a TH1 to TH0 shift and to replicate preferentially in TH2 and TH0 cells. Science 265(5169):244–248

Mahnke YD, Brodie TM, Sallusto F, Roederer M, Lugli E (2013) The who's who of T-cell differentiation: human memory T-cell subsets. Eur J Immunol 43:2797–2809. https://doi.org/10.1002/eji.201343751

Mailliard RB, Smith KN, Fecek RJ, Rappocciolo G, Nascimento EJM, Marques ET, Watkins SC, Mullins JI, Rinaldo CR (2013) Selective induction of CTL helper rather than killer activity by natural epitope variants promotes dendritic cell-mediated HIV-1 dissemination. J Immunol (Baltimore, MD: 1950) 191:2570–2580. https://doi.org/10.4049/jimmunol.1300373

Maldarelli F, Wu X, Su L, Simonetti FR, Shao W, Hill S, Spindler J, Ferris AL, Mellors JW, Kearney MF, Coffin JM, Hughes SH (2014) Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. Science 345:179–183. https://doi.org/10.1126/science.1254194

Manches O, Frleta D, Bhardwaj N (2014) Dendritic cells in progression and pathology of HIV infection. Trends Immunol 35(3):114–122. https://doi.org/10.1016/j.it.2013.10.003

Marcondes MCG, Morsey B, Emanuel K, Lamberty BG, Flynn CT, Fox HS (2015) CD8+ T cells maintain suppression of simian immunodeficiency virus in the central nervous system. J Infect Dis 211:40–44. https://doi.org/10.1093/infdis/jiu401

Martin MP, Gao X, Lee J-H, Nelson GW, Detels R, Goedert JJ, Buchbinder S, Hoots K, Vlahov D, Trowsdale J, Wilson M, O'Brien SJ, Carrington M (2002) Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. Nat Genet 31:429–434. https://doi.org/10.1038/ng934

McNelis JC, Olefsky JM (2014) Macrophages, immunity, and metabolic disease. Immunity 41(1):36–48. https://doi.org/10.1016/j.immuni.2014.05.010

Menager MM, Littman DR (2016) Actin dynamics regulates dendritic cell-mediated transfer of HIV-1 to T cells. Cell 164(4):695–709. https://doi.org/10.1016/j.cell.2015.12.036

Miller RF, Isaacson PG, Hall-Craggs M, Lucas S, Gray F, Scaravilli F, An SF (2004) Cerebral CD8+ lymphocytosis in HIV-1 infected patients with immune restoration induced by HAART. Acta Neuropathol 108:17–23. https://doi.org/10.1007/s00401-004-0852-0

Mlcochova P, Sutherland KA, Watters SA, Bertoli C, de Bruin RA, Rehwinkel J, Neil SJ, Lenzi GM, Kim B, Khwaja A, Gage MC, Georgiou C, Chittka A, Yona S, Noursadeghi M, Towers GJ, Gupta RK (2017) A G1-like state allows HIV-1 to bypass SAMHD1 restriction in macrophages. EMBO J 36(5):604–616. https://doi.org/10.15252/embj.201696025

Monroe KM, Yang Z, Johnson JR, Geng X, Doitsh G, Krogan NJ, Greene WC (2014) IFI16 DNA sensor is required for death of lymphoid CD4 T cells abortively infected with HIV. Science 343:428–432. https://doi.org/10.1126/science.1243640

Muñoz-Arias I, Doitsh G, Yang Z, Sowinski S, Ruelas D, Greene WC (2015) Blood-derived CD4 T cells naturally resist Pyroptosis during abortive HIV-1 infection. Cell Host Microbe 18:463–470. https://doi.org/10.1016/j.chom.2015.09.010

Muro-Cacho CA, Pantaleo G, Fauci AS (1995) Analysis of apoptosis in lymph nodes of HIV-infected persons. Intensity of apoptosis correlates with the general state of activation of the lymphoid tissue and not with stage of disease or viral burden. J Immunol 154:5555–5566

Murphey-Corb M, Wilson LA, Trichel AM, Roberts DE, Xu K, Ohkawa S, Woodson B, Bohm R, Blanchard J (1999) Selective induction of protective MHC class I-restricted CTL in the intestinal Lamina Propria of rhesus monkeys by transient SIV infection of the colonic mucosa. J Immunol 162:540–549

Muthumani K, Hwang DS, Desai BM, Zhang D, Dayes N, Green DR, Weiner DB (2002) HIV-1 Vpr induces apoptosis through Caspase 9 in T cells and peripheral blood mononuclear cells. J Biol Chem 277:37820–37831

Muthumani K, Choo AY, Hwang DS, Premkumar A, Dayes NS, Harris C, Green DR, Wadsworth SA, Siekierka JJ, Weiner DB (2005) HIV-1 Nef-induced FasL induction and bystander killing requires p38 MAPK activation. Blood 106:2059–2068. https://doi.org/10.1182/blood-2005-03-0932

Nayak DK, Zhou F, Xu M, Huang J, Tsuji M, Hachem R, Mohanakumar T (2016) Long-term persistence of donor alveolar macrophages in human lung transplant recipients that influences donor-specific immune responses. Am J Transplant 16(8):2300–2311. https://doi.org/10.1111/ajt.13819

Nettles RE, Kieffer TL, Kwon P, Monie D, Han Y, Parsons T, Cofrancesco J, Gallant JE, Quinn TC, Jackson B, Flexner C, Carson K, Ray S, Persaud D, Siliciano RF (2005) Intermittent HIV-1 viremia (blips) and drug resistance in patients receiving HAART. JAMA 293:817–829. https://doi.org/10.1001/jama.293.7.817

Noel N, Pena R, David A, Avettand-Fenoel V, Erkizia I, Jimenez E, Lecuroux C, Rouzioux C, Boufassa F, Pancino G, Venet A, Van Lint C, Martinez-Picado J, Lambotte O, Saez-Cirion A, Prado JG (2016) Long-term spontaneous control of HIV-1 is related to low frequency of infected cells and inefficient viral reactivation. J Virol 90(13):6148–6158. https://doi.org/10.1128/JVI.00419-16

Oh H, Ghosh S (2013) NF-kappaB: roles and regulation in different CD4(+) T-cell subsets. Immunol Rev 252(1):41–51. https://doi.org/10.1111/imr.12033

Ohnimus H, Heinkelein M, Jassoy C (1997) Apoptotic cell death upon contact of CD4+ T lymphocytes with HIV glycoprotein-expressing cells is mediated by caspases but bypasses CD95 (Fas/Apo-1) and TNF receptor 1. J Immunol 159:5246–5252

Ojeda D, Lopez-Costa JJ, Sede M, Lopez EM, Berria MI, Quarleri J (2014) Increased in vitro glial fibrillary acidic protein expression, telomerase activity, and telomere length after productive human immunodeficiency virus-1 infection in murine astrocytes. J Neurosci Res 92(2):267–274. https://doi.org/10.1002/jnr.23294

Olivetta E, Federico M (2006) HIV-1 Nef protects human-monocyte-derived macrophages from HIV-1-induced apoptosis. Exp Cell Res 312:890–900. https://doi.org/10.1016/j.yexcr.2005.12.003

Olvera-García G, Aguilar-García T, Gutiérrez-Jasso F, Imaz-Rosshandler I, Rangel-Escareño C, Orozco L, Aguilar-Delfín I, Vázquez-Pérez JA, Zúñiga J, Pérez-Patrigeon S, Espinosa E (2016) A transcriptome-based model of central memory CD4 T cell death in HIV infection. BMC Genomics 17:956. https://doi.org/10.1186/s12864-016-3308-8

Paiardini M, Muller-Trutwin M (2013) HIV-associated chronic immune activation. Immunol Rev 254(1):78–101. https://doi.org/10.1111/imr.12079

Passaes CP, Saez-Cirion A (2014) HIV cure research: advances and prospects. Virology 454–455:340–352. https://doi.org/10.1016/j.virol.2014.02.021

Pauls E, Ruiz A, Badia R, Permanyer M, Gubern A, Riveira-Muñoz E, Torres-Torronteras J, Álvarez M, Mothe B, Brander C, Crespo M, Menéndez-Arias L, Clotet B, Keppler OT, Martí R, Posas F, Ballana E, Esté JA (2014a) Cell cycle control and HIV-1 susceptibility are linked by CDK6-dependent CDK2 phosphorylation of SAMHD1 in myeloid and lymphoid cells. J Immunol 193:1988–1997. https://doi.org/10.4049/jimmunol.1400873

Pauls E, Ruiz A, Riveira-Munoz E, Permanyer M, Badia R, Clotet B, Keppler OT, Ballana E, Este JA (2014b) p21 regulates the HIV-1 restriction factor SAMHD1. Proc Natl Acad Sci U S A 111(14):E1322–E1324. https://doi.org/10.1073/pnas.1322059111

Pennock ND, White JT, Cross EW, Cheney EE, Tamburini BA, Kedl RM (2013) T cell responses: naïve to memory and everything in between. Adv Physiol Educ 37:273–283. https://doi.org/10.1152/advan.00066.2013

Perelson AS (2002) Modelling viral and immune system dynamics. Nat Rev Immunol 2(1):28–36. https://doi.org/10.1038/nri700

Perez-Caballero D, Zang T, Ebrahimi A, McNatt MW, Gregory DA, Johnson MC, Bieniasz PD (2009) Tetherin inhibits HIV-1 release by directly tethering virions to cells. Cell 139(3):499–511. https://doi.org/10.1016/j.cell.2009.08.039

Perreau M, Savoye A-L, Crignis ED, Corpataux J-M, Cubas R, Haddad EK, Leval LD, Graziosi C, Pantaleo G (2013) Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. J Exp Med 210:143–156. https://doi.org/10.1084/jem.20121932

Petrovas C, Ferrando-Martinez S, Gerner MY, Casazza JP, Pegu A, Deleage C, Cooper A, Hataye J, Andrews S, Ambrozak D, Estrada PMDR, Boritz E, Paris R, Moysi E, Boswell KL, Ruiz-Mateos E, Vagios I, Leal M, Ablanedo-Terrazas Y, Rivero A, Gonzalez-Hernandez LA, McDermott AB, Moir S, Reyes-Terán G, Docobo F, Pantaleo G, Douek DC, Betts MR, Estes JD, Germain RN, Mascola JR, Koup RA (2017) Follicular CD8 T cells accumulate in HIV infection and can kill infected cells in vitro via bispecific antibodies. Sci Transl Med 9:eaag2285. https://doi.org/10.1126/scitranslmed.aag2285

Powell JD, Delgoffe GM (2010) The mammalian target of rapamycin: linking T cell differentiation, function, and metabolism. Immunity 33(3):301–311. https://doi.org/10.1016/j.immuni.2010.09.002

Rainho JN, Martins MA, Cunyat F, Watkins IT, Watkins DI, Stevenson M (2015) Nef is dispensable for resistance of simian immunodeficiency virus-infected macrophages to CD8+ T cell killing. J Virol 89:10625–10636. https://doi.org/10.1128/JVI.01699-15

Rasmussen TA, Lewin SR (2016) Shocking HIV out of hiding: where are we with clinical trials of latency reversing agents? Curr Opin HIV AIDS 11:394–401. https://doi.org/10.1097/COH.0000000000000279

Rasmussen TA, Tolstrup M, Brinkmann CR, Olesen R, Erikstrup C, Solomon A, Winckelmann A, Palmer S, Dinarello C, Buzon M, Lichterfeld M, Lewin SR, Østergaard L, Søgaard OS (2014) Panobinostat, a histone deacetylase inhibitor, for latent-virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase 1/2, single group, clinical trial. Lancet HIV 1:e13–e21. https://doi.org/10.1016/S2352-3018(14)70014-1

Ravet S, Scott-Algara D, Bonnet E, Tran HK, Tran T, Nguyen N, Truong LX, Theodorou I, Barré-Sinoussi F, Pancino G, Paul P (2007) Distinctive NK-cell receptor repertoires sustain high-level constitutive NK-cell activation in HIV-exposed uninfected individuals. Blood 109:4296–4305. https://doi.org/10.1182/blood-2006-08-040238

Robbins CS, Hilgendorf I, Weber GF, Theurl I, Iwamoto Y, Figueiredo J-L, Gorbatov R, Sukhova GK, Gerhardt LMS, Smyth D, Zavitz CCJ, Shikatani EA, Parsons M, van Rooijen N, Lin HY, Husain M, Libby P, Nahrendorf M, Weissleder R, Swirski FK (2013) Local proliferation dominates lesional macrophage accumulation in atherosclerosis. Nat Med 19:1166–1172. https://doi.org/10.1038/nm.3258

Rosa A, Chande A, Ziglio S, De Sanctis V, Bertorelli R, Goh SL, McCauley SM, Nowosielska A, Antonarakis SE, Luban J, Santoni FA, Pizzato M (2015) HIV-1 Nef promotes infection by excluding SERINC5 from virion incorporation. Nature 526(7572):212–217. https://doi.org/10.1038/nature15399

Routy J, Tremblay C, Angel J, Trottier B, Rouleau D, Baril J, Harris M, Trottier S, Singer J, Chomont N, Sékaly R, Boulassel M (2012) Valproic acid in association with highly active antiretroviral therapy for reducing systemic HIV-1 reservoirs: results from a multicentre randomized clinical study. HIV Med 13:291–296. https://doi.org/10.1111/j.1468-1293.2011.00975.x

Sacha JB, Giraldo-Vela JP, Buechler MB, Martins MA, Maness NJ, Chung C, Wallace LT, León EJ, Friedrich TC, Wilson NA, Hiraoka A, Watkins DI (2009) Gag- and Nef-specific CD4+ T

cells recognize and inhibit SIV replication in infected macrophages early after infection. Proc Natl Acad Sci U S A 106:9791–9796. https://doi.org/10.1073/pnas.0813106106

Sadagopal S, Lorey SL, Barnett L, Basham R, Lebo L, Erdem H, Haman K, Avison M, Waddell K, Haas DW, Kalams SA (2008) Enhancement of human immunodeficiency virus (HIV)-specific CD8+ T cells in cerebrospinal fluid compared to those in blood among antiretroviral therapy-Naïve HIV-positive subjects. J Virol 82:10418–10428. https://doi.org/10.1128/JVI.01190-08

Saez-Cirion A, Nicola MA, Pancino G, Shorte SL (2006) Quantitative real-time analysis of HIV-1 gene expression dynamics in single living primary cells. Biotechnol J 1(6):682–689. https://doi.org/10.1002/biot.200600045

Sáez-Cirión A, Lacabaratz C, Lambotte O, Versmisse P, Urrutia A, Boufassa F, Barré-Sinoussi F, Delfraissy J-F, Sinet M, Pancino G, Venet A, Group ANdRslSEHCS (2007) HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype. Proc Natl Acad Sci U S A 104:6776–6781. https://doi.org/10.1073/pnas.0611244104

Sáez-Cirión A, Sinet M, Shin SY, Urrutia A, Versmisse P, Lacabaratz C, Boufassa F, Avettand-Fènoël V, Rouzioux C, Delfraissy J-F, Barré-Sinoussi F, Lambotte O, Venet A, Pancino G, Group AEHCS (2009) Heterogeneity in HIV suppression by CD8 T cells from HIV controllers: association with Gag-specific CD8 T cell responses. J Immunol (Baltimore, MD: 1950) 182:7828–7837. doi:https://doi.org/10.4049/jimmunol.0803928

Sallusto F, Lenig D, Mackay CR, Lanzavecchia A (1998) Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. J Exp Med 187(6):875–883

Santa-Marta M, de Brito PM, Godinho-Santos A, Goncalves J (2013) Host factors and HIV-1 replication: clinical evidence and potential therapeutic approaches. Front Immunol 4:343. https://doi.org/10.3389/fimmu.2013.00343

Schnell G, Joseph S, Spudich S, Price RW, Swanstrom R (2011) HIV-1 replication in the central nervous system occurs in two distinct cell types. PLoS Pathog 7(10):e1002286. https://doi.org/10.1371/journal.ppat.1002286

Schroder AR, Shinn P, Chen H, Berry C, Ecker JR, Bushman F (2002) HIV-1 integration in the human genome favors active genes and local hotspots. Cell 110(4):521–529

Seddon B, Tomlinson P, Zamoyska R (2003) Interleukin 7 and T cell receptor signals regulate homeostasis of CD4 memory cells. Nat Immunol 4:680–686. https://doi.org/10.1038/ni946

Shan L, Deng K, Shroff NS, Durand CM, Rabi SA, Yang HC, Zhang H, Margolick JB, Blankson JN, Siliciano RF (2012) Stimulation of HIV-1-specific cytolytic T lymphocytes facilitates elimination of latent viral reservoir after virus reactivation. Immunity 36(3):491–501. https://doi.org/10.1016/j.immuni.2012.01.014

Simon V, Bloch N, Landau NR (2015) Intrinsic host restrictions to HIV-1 and mechanisms of viral escape. Nat Immunol 16(6):546–553. https://doi.org/10.1038/ni.3156

Simonetti FR, Sobolewski MD, Fyne E, Shao W, Spindler J, Hattori J, Anderson EM, Watters SA, Hill S, Wu X, Wells D, Su L, Luke BT, Halvas EK, Besson G, Penrose KJ, Yang Z, Kwan RW, Waes CV, Uldrick T, Citrin DE, Kovacs J, Polis MA, Rehm CA, Gorelick R, Piatak M, Keele BF, Kearney MF, Coffin JM, Hughes SH, Mellors JW, Maldarelli F (2016) Clonally expanded CD4+ T cells can produce infectious HIV-1 in vivo. Proc Natl Acad Sci 113:1883–1888. https://doi.org/10.1073/pnas.1522675113

Sloand EM, Young NS, Kumar P, Weichold FF, Sato T, Maciejewski JP (1997) Role of Fas ligand and receptor in the mechanism of T-cell depletion in acquired immunodeficiency syndrome: effect on CD4+ lymphocyte depletion and human immunodeficiency virus replication. Blood 89:1357–1363

Soghoian DZ, Jessen H, Flanders M, Sierra-Davidson K, Cutler S, Pertel T, Ranasinghe S, Lindqvist M, Davis I, Lane K, Rychert J, Rosenberg ES, Piechocka-Trocha A, Brass AL, Brenchley JM, Walker BD, Streeck H (2012) HIV-specific Cytolytic CD4 T cell responses during acute HIV infection predict disease outcome. Sci Transl Med 4:123ra125-123ra125. doi:https://doi.org/10.1126/scitranslmed.3003165

Steinman RM, Granelli-Piperno A, Pope M, Trumpfheller C, Ignatius R, Arrode G, Racz P, Tenner-Racz K (2003) The interaction of immunodeficiency viruses with dendritic cells. Curr Top Microbiol Immunol 276:1–30

Stevenson M (2003) HIV-1 pathogenesis. Nat Med 9(7):853–860. https://doi.org/10.1038/nm0703-853

Stolp B, Fackler OT (2011) How HIV takes advantage of the cytoskeleton in entry and replication. Viruses 3(4):293–311. https://doi.org/10.3390/v3040293

Sun H, Kim D, Li X, Kiselinova M, Ouyang Z, Vandekerckhove L, Shang H, Rosenberg ES, Yu XG, Lichterfeld M (2015) Th1/17 polarization of CD4 T cells supports HIV-1 persistence during antiretroviral therapy. J Virol 89(22):11284–11293. https://doi.org/10.1128/JVI.01595-15

Surh CD, Sprent J (2008) Homeostasis of naive and memory T cells. Immunity 29:848–862. https://doi.org/10.1016/j.immuni.2008.11.002

Swingler S, Mann AM, Zhou J, Swingler C, Stevenson M (2007) Apoptotic killing of HIV-1–infected macrophages is subverted by the viral envelope glycoprotein. PLoS Pathog 3:e134. https://doi.org/10.1371/journal.ppat.0030134

Tan J, Sattentau QJ (2013) The HIV-1-containing macrophage compartment: a perfect cellular niche? Trends Microbiol 21(8):405–412. https://doi.org/10.1016/j.tim.2013.05.001

Tan J, Wang X, Devadas K, Zhao J, Zhang P, Hewlett I (2013) Some mechanisms of FLIP expression in inhibition of HIV-1 replication in Jurkat cells, CD4+ T cells and PBMCs. J Cell Physiol 228:2305–2313. https://doi.org/10.1002/jcp.24397

Tay TL, Savage JC, Hui CW, Bisht K, Tremblay ME (2017) Microglia across the lifespan: from origin to function in brain development, plasticity and cognition. J Physiol-London 595(6):1929–1945. https://doi.org/10.1113/Jp272134

Tobin NH, Learn GH, Holte SE, Wang Y, Melvin AJ, McKernan JL, Pawluk DM, Mohan KM, Lewis PF, Mullins JI, Frenkel LM (2005) Evidence that low-level Viremias during effective highly active antiretroviral therapy result from two processes: expression of archival virus and replication of virus. J Virol 79:9625–9634. https://doi.org/10.1128/JVI.79.15.9625-9634.2005

Trinité B, Chan CN, Lee CS, Levy DN (2016) HIV-1 Vpr- and reverse transcription-induced apoptosis in resting peripheral blood CD4 T cells and protection by common gamma-chain cytokines. J Virol 90:904–916. https://doi.org/10.1128/JVI.01770-15

Usami Y, Popov S, Popova E, Inoue M, Weissenhorn W, Göttlinger H (2009) The ESCRT pathway and HIV-1 budding. Biochem Soc Trans 37:181–184. https://doi.org/10.1042/BST0370181

Usami Y, Wu Y, Gottlinger HG (2015) SERINC3 and SERINC5 restrict HIV-1 infectivity and are counteracted by Nef. Nature 526(7572):218–223. https://doi.org/10.1038/nature15400

Valle-Casuso JC, Allouch A, David A, Lenzi GM, Studdard L, Barre-Sinoussi F, Muller-Trutwin M, Kim B, Pancino G, Saez-Cirion A (2017) p21 restricts HIV-1 in monocyte-derived dendritic cells through the reduction of dNTP biosynthesis and regulation of SAMHD1 antiviral activity. J Virol. https://doi.org/10.1128/JVI.01324-17

Van Lint C, Bouchat S, Marcello A (2013) HIV-1 transcription and latency: an update. Retrovirology 10:67. https://doi.org/10.1186/1742-4690-10-67

Veazey RS, Mansfield KG, Tham IC, Carville AC, Shvetz DE, Forand AE, Lackner AA (2000) Dynamics of CCR5 expression by CD4+ T cells in lymphoid tissues during simian immunodeficiency virus infection. J Virol 74:11001–11007. https://doi.org/10.1128/JVI.74.23.11001-11007.2000

Vetter ML, Johnson ME, Antons AK, Unutmaz D, D'Aquila RT (2009) Differences in APOBEC3G expression in CD4+ T helper lymphocyte subtypes modulate HIV-1 infectivity. PLoS Pathog 5(2):e1000292. https://doi.org/10.1371/journal.ppat.1000292

Vojnov L, Martins MA, Bean AT, Veloso de Santana MG, Sacha JB, Wilson NA, Bonaldo MC, Galler R, Stevenson M, Watkins DI (2012) The majority of freshly sorted simian immunodeficiency virus (SIV)-specific CD8(+) T cells cannot suppress viral replication in SIV-infected macrophages. J Virol 86(8):4682–4687. https://doi.org/10.1128/JVI.06324-11

Vrisekoop N, den Braber I, de Boer AB, Ruiter AF, Ackermans MT, van der Crabben SN, Schrijver EH, Spierenburg G, Sauerwein HP, Hazenberg MD, de Boer RJ, Miedema F, Borghans JA,

Tesselaar K (2008) Sparse production but preferential incorporation of recently produced naive T cells in the human peripheral pool. Proc Natl Acad Sci U S A 105(16):6115–6120. https://doi.org/10.1073/pnas.0709713105

Wagner TA, McLaughlin S, Garg K, Cheung CYK, Larsen BB, Styrchak S, Huang HC, Edlefsen PT, Mullins JI, Frenkel LM (2014) Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. Science 345:570–573. https://doi.org/10.1126/science.1256304

Wakim LM, Woodward-Davis A, Bevan MJ (2010) Memory T cells persisting within the brain after local infection show functional adaptations to their tissue of residence. Proc Natl Acad Sci 107:17872–17879. https://doi.org/10.1073/pnas.1010201107

Wang X, Ragupathy V, Zhao J, Hewlett I (2011) Molecules from apoptotic pathways modulate HIV-1 replication in Jurkat cells. Biochem Biophys Res Commun 414:20–24. https://doi.org/10.1016/j.bbrc.2011.09.007

Wells AD, Morawski PA (2014) New roles for cyclin-dependent kinases in T cell biology: linking cell division and differentiation. Nat Rev Immunol 14(4):261–270. https://doi.org/10.1038/nri3625

Welsch S, Groot F, Krausslich HG, Keppler OT, Sattentau QJ (2011) Architecture and regulation of the HIV-1 assembly and holding compartment in macrophages. J Virol 85(15):7922–7927. https://doi.org/10.1128/JVI.00834-11

Westendorp MO, Frank R, Ochsenbauer C, Stricker K, Dhein J, Walczak H, Debating K-M, Krammer PH (1995) Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. Nature 375:497–500. https://doi.org/10.1038/375497a0

White TE, Brandariz-Nunez A, Valle-Casuso JC, Amie S, Nguyen LA, Kim B, Tuzova M, Diaz-Griffero F (2013) The retroviral restriction ability of SAMHD1, but not its deoxynucleotide triphosphohydrolase activity, is regulated by phosphorylation. Cell Host Microbe 13(4):441–451. https://doi.org/10.1016/j.chom.2013.03.005

Wilen CB, Tilton JC, Doms RW (2012) HIV: cell binding and entry. Cold Spring Harb Perspect Med 2(8):a006866. https://doi.org/10.1101/cshperspect.a006866

Wolf D, Witte V, Laffert B, Blume K, Stromer E, Trapp S, d'Aloja P, Schürmann A, Baur AS (2001) HIV-1 Nef associated PAK and PI3-kinases stimulate Akt-independent Bad-phosphorylation to induce anti-apoptotic signals. Nat Med 7:1217–1224. https://doi.org/10.1038/nm1101-1217

Wu L, KewalRamani VN (2006) Dendritic-cell interactions with HIV: infection and viral dissemination. Nat Rev Immunol 6(11):859–868. https://doi.org/10.1038/nri1960

Xiong Y, Hannon GJ, Zhang H, Casso D, Kobayashi R, Beach D (1993) p21 is a universal inhibitor of cyclin kinases. Nature 366(6456):701–704. https://doi.org/10.1038/366701a0

Xu XN, Screaton GR, Gotch FM, Dong T, Tan R, Almond N, Walker B, Stebbings R, Kent K, Nagata S, Stott JE, McMichael AJ (1997) Evasion of cytotoxic T lymphocyte (CTL) responses by nef-dependent induction of Fas ligand (CD95L) expression on simian immunodeficiency virus-infected cells. J Exp Med 186(1):7–16

Xu XN, Laffert B, Screaton GR, Kraft M, Wolf D, Kolanus W, Mongkolsapay J, McMichael AJ, Baur AS (1999) Induction of Fas ligand expression by HIV involves the interaction of Nef with the T cell receptor zeta chain. J Exp Med 189:1489–1496

Young KG, MacLean S, Dudani R, Krishnan L, Sad S (2011) CD8+ T cells primed in the periphery provide time-bound immune-surveillance to the central nervous system. J Immunol 187:1192–1200. https://doi.org/10.4049/jimmunol.1100695

Zamarron BF, Mergian TA, Cho KW, Martinez-Santibanez G, Luan D, Singer K, DelProposto JL, Geletka LM, Muir LA, Lumeng CN (2016) Macrophage proliferation sustains adipose tissue inflammation in formerly obese mice. Diabetes:db160500. https://doi.org/10.2337/db16-0500

Zhang J, Scadden DT, Crumpacker CS (2007) Primitive hematopoietic cells resist HIV-1 infection via p21. J Clin Invest 117(2):473–481. https://doi.org/10.1172/JCI28971

Zhou H, Xu M, Huang Q, Gates AT, Zhang XD, Castle JC, Stec E, Ferrer M, Strulovici B, Hazuda DJ, Espeseth AS (2008) Genome-scale RNAi screen for host factors required for HIV replication. Cell Host Microbe 4:495–504. https://doi.org/10.1016/j.chom.2008.10.004

ANNEX 2:

CELLULAR METABOLISM IS A MAJOR DETERMINANT OF HIV-1 RESERVOIR SEEDING IN CD4+ T CELLS AND OFFERS A UNIQUE OPPORTUNITY TO TACKLE INFECTION

Valle-Casuso et al, submitted

Cellular metabolism is a major determinant of HIV-1 reservoir seeding in CD4+ T cells and offers an opportunity to tackle infection

José Carlos Valle-Casuso[1], Mathieu Angin[1], Stevenn Volant[2], Caroline Passaes[1], Valérie Monceaux[1], Anastassia Mikhailova[1], Katia Bourdic[3], Veronique Avettand-Fenoel[4,5], Faroudy Boufassa[6], Marc Sitbon[7], Olivier Lambotte[3,8], Maria-Isabel Thoulouze[9], Michaela Müller-Trutwin[1], Nicolas Chomont[10] and Asier Sáez-Cirión[1]

[1]Institut Pasteur, Unité HIV Inflammation et Persistance, Paris, France

[2]Institut Pasteur, Hub Bioinformatique et Biostatistique – C3BI, USR 3756 IP CNRS – Paris, France

[3]Assistance Publique Hôpitaux de Paris, Hôpital Bicêtre, Service de Médecine Interne et Immunologie clinique, 94275 Le Kremlin-Bicêtre, France

[4] Université Paris Descartes, Sorbonne Paris Cité, EA7327, Paris, France.

[5] Assistance Publique Hôpitaux de Paris, CHU Necker-Enfants, Laboratoire de Virologie, Malades, Paris, France

[6]INSERM U1018, Centre de recherche en Epidémiologie et Santé des Populations, Université Paris Sud, Le Kremlin Bicêtre, France

[7] Institut de Génétique Moléculaire de Montpellier UMR 5535 CNRS, Montpellier, France

[8]CEA, Université Paris Sud, INSERM U1184, Immunology of Viral Infections and Autoimmune Diseases (IMVA), IDMIT Department / IBFJ, Fontenay-aux-Roses, France

[9]Institut Pasteur, Unité Virologie Structurale, Paris, France

[10]Centre de Recherche du CHUM and Department of microbiology, infectiology and immunology, Université de Montréal, H2X0A9, Canada

Corresponding author:

Asier Sáez-Cirión: Unité HIV Inflammation et Persistance, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France. Tel.: 33-145-688-768. Fax: 33-145-688-957.

E-mail: asier.saez-cirion@pasteur.fr

ABSTRACT

HIV persists in pools of long-lived infected cells that are not susceptible to current antiretroviral treatments. Targeting these HIV reservoirs is one of the main priorities in the HIV field. HIV reservoirs are mainly located in CD4+ T cells, but their distribution is variable in the different T cell subsets. CD4+ T cell susceptibility to HIV-1 infection varies with T cell differentiation, with naïve T cells strongly resistant to infection and effector memory cells highly susceptible. We evaluated whether the metabolic programming that supports the differentiation and function of CD4+ T cells affected the susceptibility of CD4+ T cell subsets to HIV infection. We found that differences in HIV susceptibility between naïve and more differentiated CD4+ T cell subsets were associated with the metabolic activity of the cells. We further found that HIV-1 selectively infected CD4+ T cells with high levels of oxidative phosphorylation and glycolysis, independent of their activation phenotype. Moreover, partial inhibition of glycolysis was sufficient to (i) impair productive and latent HIV-1 infection in vitro in all CD4+ T cell subsets, (ii) decrease the viability of pre-infected cells, and (iii) preclude HIV-1 reactivation in CD4+ T cells from HIV-infected individuals undergoing cART. Our results elucidate the link between cell metabolism and HIV-1 infection and identify a vulnerability that can be exploited to tackle HIV reservoirs.

INTRODUCTION

Current combination antiretroviral treatment (cART) efficiently blocks HIV-1 replication but does not eliminate infected cells. Replication competent HIV-1 persists for decades in cellular reservoirs that are the origin of rapid viral rebound when treatment is interrupted (Finzi et al., 1997). Identifying the factors underlying the seeding and survival of HIV-infected cells may guide the development of novel therapies against HIV and is a priority in the search for an HIV cure (Deeks et al., 2016; Passaes and Saez-Cirion, 2014). CD4+ T cells are the major target for HIV-1 infection and are thought to constitute most of the HIV-1 reservoir. However, not all CD4+ T cells contribute equally to the pool of persistently infected cells during cART. The composition of CD4+ T cells that remain infected is mainly determined by the relative susceptibility of CD4+ T cell subsets to HIV infection, their resistance to HIV-induced apoptosis and their life span and turnover potential (Barton et al., 2016). Naïve CD4+ T cells are highly resistant to HIV-1 infection, while HIV-1 susceptibility increases in more differentiated cell subsets (Roederer et al., 1997; Schnittman et al., 1990; Spina et al., 1997). Accordingly, there is a minimal contribution of naïve CD4+ T cells to the HIV reservoir during cART, which is mainly restricted to the memory cell subsets (Chomont et al., 2009). The susceptibility of CD4+ T cells to HIV-1 infection depends on the relative abundance of cell factors required by the virus to complete its replication cycle and of cellular restriction factors that counteract infection (Lever and Jeang, 2011). T cell activation sharply increases the expression of HIV dependency factors and thereby cell susceptibility to HIV-1 infection (Pan et al., 2013; Stevenson et al., 1990), despite the concomitant presence of some restriction factors that the virus can most often circumvent. However, responsiveness to TCR activation (Byrne et al., 1988; Roederer et al., 1997) and susceptibility to HIV infection are not homogeneous across or within CD4+ T cell subsets. This discrepancy in infection efficacy suggests that HIV-1 has adapted to infect CD4+ T cells with a specific cellular program (Cleret-Buhot et al., 2015). The cellular processes orchestrating the optimal conditions for the establishment of HIV-1 infection remain unclear.

Numerous studies have recently demonstrated the critical role of cellular metabolism in T cell immunity (Pearce et al., 2013; Waickman and Powell, 2012). Naïve T cells circulate in a quiescent state, relying essentially on oxidative phosphorylation (OXPHOS). However, upon T cell activation and after receiving appropriate cues (costimulation, cytokines), naïve T cells undergo metabolic reprogramming, strongly increasing OXPHOS and, especially, glycolysis, to cope with the energy demands of immune function and rapid proliferation (Pearce et al., 2013). The biomass accumulation that accompanies enhanced cellular metabolism may provide viruses with the abundance of factors that are necessary for their replication. It is worth noting that several retroviruses have evolved to use metabolite transporters as cellular receptors. The glucose transporter 1 (Glut-1) is the main receptor for HTLV-1 (Manel et al., 2003); phosphate transporters Pit1 and Pit2 have been reported as surface receptors for koala retrovirus, feline leukemia virus and murine leukemia viruses (Oliveira et al., 2006; Shojima et al., 2013; Takeuchi et al., 1992; von Laer et al., 1998); and the amino acid transporters ASCT1 and ASCT2 are the receptors for the feline RD-114 endogenous retrovirus (Shimode et al., 2013). Although HIV-1 does not use metabolite transporters as its main receptors, Glut-1 expression is necessary for the postentry steps of HIV-1 replication in CD4+ T cells (Loisel-Meyer et al., 2012). Moreover, the metabolism of nucleotides, which is controlled by glycolysis through the pentose phosphate pathway (Lane and Fan, 2015), is critical for HIV-1 reverse transcription (Amie et al., 2013).

In the present study, we undertook the analysis of the conditions determining the intracellular susceptibility of CD4+ T cell subsets to HIV-1 infection. In particular, we analyzed whether the metabolic program is distinct according to the differentiation of CD4+ T cell subsets and if this determines their susceptibility to HIV-1 infection. We show that cellular metabolism is a central factor driving the HIV-1 infection of CD4+ T cells and that it may be an important target for new therapies against HIV-1.

RESULTS

CD4+ T cell subsets have heterogeneous susceptibility to HIV-1 infection

We first assessed the relative intrinsic susceptibility of primary CD4+ T cell subsets (naïve, Tn; central memory, Tcm; transitional memory, Ttm; and effector memory, Tem) to HIV-1 infection. We used single-cycle NL4.3ΔenvGFP particles pseudotyped with VSV-G envelope protein to circumvent differences in the surface expression of CCR5 across CD4+ T cell subsets. We activated CD4+ T cells with soluble anti-CD3. This 'suboptimal' activation protocol has allowed us to expose differences in the susceptibility to HIV-1 of CD4+ T cells from different individuals, in particular resistance in cells from individuals naturally controlling infection, that were masked using more potent stimulation protocols (Saez-Cirion et al., 2011). In the present study, our activation protocol allowed the enhancement of the susceptibility to HIV-1 infection without the modification of the relative proportion of the CD4+ T cell subsets (Figure 1A, B). After infection, the relative frequencies of Tn, Tcm, Ttm and Tem cells among GFP-negative (GFP-) cells was identical to that among noninfected CD4+ T cells (Figure 1B). In contrast, the composition of HIV-infected GFP-positive (GFP+) cells was different from that of noninfected cells, with a significant exclusion of Tn cells and strong enrichment of Tem cells. Tcm cells were also slightly underrepresented, and Ttm cells were overrepresented among GFP+ CD4+ T cells when compared to the control condition (Figure 1B). These results suggested different susceptibilities to HIV-1 infection of CD4+ T cell subsets, with Tem cells being the most susceptible, followed by Ttm and Tcm cells, and with Tn cells being strongly resistant to infection.

To study if these differences were related to the inherent program of each CD4+ T cell subset, we isolated quiescent (CD25-, CD69-, HLA-DR-) CD4+ Tn, Tcm, Ttm and Tem cells (n=6 donors, Figure S1) using flow-based cell sorting, and we analyzed their susceptibility to HIV-1 with or without anti-CD3 activation. Activation enhanced the susceptibility of all CD4+ T cell subsets to HIV-1 infection (Figure 1C). However, this effect was variable according to the subset. There was a tendency for Tem cells to

be more susceptible than other subsets (p=0.06) in the absence of activation, and this difference became more pronounced after three (p=0.0004, all comparisons) or five days of activation (p=0.012, Tem vs Tn and Ttm and Tcm vs Tn and Ttm). Overall, our results recapitulated previous observations showing an inherent hierarchy in the susceptibility of CD4+ T cell subsets to HIV-1 infection (Buzon et al., 2014; Tabler et al., 2014).

Levels of HIV infection are related to the molecular program of CD4+ T cell subsets

To determine if a molecular program was associated with the susceptibility of CD4+ T cell subsets to HIV infection, we analyzed the expression of a panel of 96 genes (related to T cell activation, survival, differentiation and function as well as known viral restriction or HIV facilitating factors, Table S1) in each CD4+ T cell subpopulation at the time of infection. Nonactivated CD4+ T cell subsets showed distinct transcriptional profiles that were further enhanced after activation (e.g., 34 genes and 49 genes differently expressed between CD4+ T cell subsets without activation and after 3 days of anti-CD3 treatment, respectively, Figure 2A). These genes were mostly related to signal transduction and the response to stimulus, which could be related to the previously described different susceptibility to CD3 activation of the CD4+ T cell subsets (Croft et al., 1994; Kumar et al., 2011). We found that the level of HIV-infected cells correlated with the expression of several genes at the time of infection in the different conditions studied (Figure 2B and Figure S2). SAMHD1 showed a negative association with infection. In contrast, positive correlations were observed between infection levels and other antiviral factors (such as APOBEC3G or SLFN11)(Li et al., 2012; Sheehy et al., 2002) as well as several genes involved in the interferon response (IFI6, IFI16, EIF2AK2, and OAS1) (Kane et al., 2016). Significant positive correlations were also observed between the level of HIV infection and the gene expression levels of transcription factors (STAT3, E2F1, and PRDM1), genes that have been proposed to facilitate HIV-1 infection (RRM2, HSP90AA, CFL1, and DYNC1H1)(Allouch et al., 2013; Franke and Luban, 1996; Lukic et al., 2014; Roesch et al., 2012) and multiple genes involved in T cell metabolism (SLC2A3, SLC2A1, SLC2A5, CASP3, FAS, GAPDH, and GUSB). Taken together, these results suggest

153 that, with the exception of SAMHD1, the antiviral restriction factors analyzed did not decisively
154 influence the cell susceptibility to HIV-1, which is in line with the results of previous reports (Jia et al.,
155 2015). Interestingly, our data indicate that metabolically active cells may offer favorable conditions
156 for HIV infection.
157
158 **Hierarchy of susceptibility to HIV infection matches metabolic activity of CD4+ T cell subsets**
159 To explore the possible association between HIV infection and cell metabolism, we first determined
160 the metabolic activity of the CD4+ T cell subsets at the time of infection. We used a cell flux analyzer
161 to measure, in different conditions, the oxygen consumption rate (OCR) and the extracellular
162 acidification rate (ECAR) as indicators of oxidative phosphorylation (OXPHOS) and glycolysis,
163 respectively (Zhang et al., 2012). In the absence of activation in vitro and in agreement with their
164 quiescent nature, all sorted CD4+ T cell subsets had low levels of metabolic activity (Figure 3A).
165 Nonetheless, small differences between subsets were noted; basal metabolism and metabolic
166 potential were highest in Tem cells and lowest in Tn cells, while Ttm and Tcm cells presented similar
167 intermediate levels (Figures 3A, B). These differences were more pronounced after activation, with
168 all memory cell subsets increasing mitochondrial function and glycolysis to different extents and with
169 different kinetics. The highest metabolic activity was measured in Tem cells, peaking on day 3 after
170 activation and decreasing on day 5. The metabolism of Ttm and Tcm cells increased after 3 days of
171 activation and then remained stable in Ttm cells while continuing to increase in Tcm cells. In contrast,
172 Tn cells showed a modest increase only in mitochondrial function and not in glycolysis and only after
173 5 days of activation, when their metabolism was heavily relying on OXPHOS (Figure 3C). Accordingly,
174 important differences were also found between CD4+ T cell subsets regarding their capacity to
175 uptake glucose and their levels of the surface expression of the Glut1 receptor as analyzed by flow
176 cytometry, which were lowest in Tn cells and highest in Tem cells (Figures S3 and S4). The relative
177 metabolic activity levels of the different cell subsets matched their relative susceptibility to HIV-1
178 infection (Figure 1C), and we found positive correlations between HIV infection levels and multiple

metabolic functions in cells that had been activated (Figure 3D, Figure S5). These results further point to an influence of the metabolic activity of CD4+ T cells on their susceptibility to HIV-1.

HIV-infected CD4+ T cells are characterized by higher levels of metabolic activity independent of cell activation levels

To analyze if there was a direct link between cell metabolism and HIV-1 infection, we challenged 5-day activated bulk CD4+ T cells with VSV-G pseudotyped NL4.3ΔenvGFP particles, and we sorted noninfected GFP- and infected GFP+ cells three days later to analyze their metabolic activity levels. HIV-infected CD4+ T cells had much higher levels of basal metabolism and metabolic potential and, overall, a more energetic profile than noninfected cells (Figures 4A and S6). Although we could detect a significant fraction of infected cells among cells with low activation levels (Figure 4B), we found higher proportions of GFP+ cells among CD4+ T cells expressing activation markers. We therefore evaluated whether differences in the metabolic activity of infected and noninfected CD4+ T cells were just a consequence of the selective infection of CD4+ T cells with higher activation levels. We sorted CD4+ T cells first based on their expression of either high or low levels of both HLA-DR and CD25 and then based on whether they were GFP+ or GFP- (Figures 4B and S7). After 5 days of stimulation, the CD4+ T cell subsets expressed different levels of activation markers (Figure S7A), which were highest in Tem cells and lowest in Tn cells. This was translated to different contributions of CD4+ T cell subpopulations in the high- and low-activation sorted cell fractions (Figure 4B). Nevertheless, Tn cells were more frequently found in the GFP- fraction, both in high and low-activated cell populations, whereas the GFP+ fraction was enriched with Tem cells. These results matched the hierarchy of infection that we observed before (Figure 1) and further supported that the susceptibility of CD4+ T cell subsets to HIV-1 depends on the intrinsic characteristics of these cells independent of their activation status. In this regard, we found that infected GFP+ cells in both the high- and low-activation fractions, had higher basal metabolisms (OCR and ECAR) than noninfected

GFP- cells (Figure 4C). These results demonstrated that HIV-infected CD4+ T-cells were characterized by higher metabolic activity levels.

HIV-1 infection is preferentially established in CD4+ T cells with high metabolic activity levels

Our results suggest that HIV-1 infection is favored in the environment provided by CD4+ T cells with high metabolic activity levels. We analyzed if this was due to a selective infection of CD4+ T cells with the highest metabolic activity levels or if it was HIV-infection that increased the metabolic activity of the cells. We activated CD4+ T cells and sorted Tn and Tcm cells based on their capacity to uptake high or low levels of the fluorescent glucose analogue 2NBDG (Figure S8A), which corresponded to weakly and strongly glycolytic cells (Figure S8B). We infected these purified cell fractions with VSV-G pseudotyped NL4.3ΔenvGFP particles. Three days after infection, infected GFP+ CD4+ T cells were only observed among highly glycolytic Tn and Tcm cells, while weakly glycolytic cells were strongly resistant to infection (65x [41x-206x], median [IQR] fold increase in the proportion of GFP+ cells in HGlu vs LGlu cell subsets, p=0.008) (Figure 5). Overall these results confirmed that, in our conditions, the high metabolic activity of infected CD4+ T cells was one of the causes rather than a consequence of HIV infection.

Suboptimal inhibition of glucose metabolism blocks HIV-1 replication in CD4+ T cells

The above results indicate that HIV-1 infection of CD4+ T cells required high levels of metabolic activity. Therefore, we analyzed if HIV-1 replication could be blocked with metabolic inhibitors. We infected activated CD4+ T cells with VSV-G pseudotyped NL4.3ΔenvGFP particles in the presence of increasing amounts of etomoxir, an inhibitor of fatty acid oxidation (FAO), 6-diazo-5-oxo-l-norleucine (DON), a glutamine antagonist, or 2-deoxy glucose (2-DG), a competitive inhibitor of glycolysis (Figure S9). Etomoxir was able to reduce HIV infection but only at high concentrations, well above the levels needed to reduce mitochondrial respiration without inducing cell death and at concentrations known to induce off target effects (O'Connor et al., 2018; Yao et al., 2018)(Figure 6A and Figure S9). DON

reduced HIV infection without inducing cell death, although the extent of the inhibition was heterogeneous. Suboptimal amounts of 2-DG (e.g., 5 mM), which were enough to significantly reduce glycolysis (Figure S9), decreased HIV-1 infection of CD4+ T cells with minimal cell toxicity (Figure 6A). These results suggested a higher impact of glucose and glutamine metabolism than FAO on HIV-1 replication. The role of glucose metabolism was further confirmed in different sets of experiments in which the frequency of HIV-1-infected CD4+ T cells was reduced when the infections were performed in conditions of glucose starvation or in presence of UK5099, a molecule that inhibits the transport of pyruvate, an end product of glycolysis, to the mitochondria (Figure 6B). The presence of 2-DG impaired the accumulation of HIV-1 reverse transcribed products overtime pointing to an early block of viral replication (Figure 6C). Of note, 2-DG was able to reduce infection and reverse transcript levels to a similar extent whether it was added to the culture at the time of the challenge or up to 8h later (Figure 6D), indicating that 2-DG was affecting post-entry steps of viral replication. Overall, these results show that a glycolytic environment was necessary for HIV-1 to complete reverse transcription.

2-DG blocked HIV-1 infection in all CD4+ T cell subsets, although the differences were more pronounced in more differentiated (more glycolytic) cells (Figure 6E). Interestingly, Etomoxir slightly reduced viral replication in Tem cells but not in other T cell subsets, which could be related to the overall highly energetic nature of these cells. We then used VSV-G pseudotyped NL4.3Δenv Duo-Fluo I particles that allow HIV-1 latently and productively infected cells to be distinguished from each other (Calvanese et al., 2013; Chavez et al., 2015) (Figure S10A). Interestingly, latent infection was more prominent among Tn and Tcm CD4+ T cells, while productive infection was predominantly observed among Tem cells (Figure S10B). Overall, the presence of 2-DG significantly reduced the global number of both latently and productively infected CD4+ T cells (Figure 6F), which agreed with the need for a glycolytic environment for HIV-1 to complete the preintegration steps of its replication cycle.

Although our results indicated that the effect of 2-DG was not related to the entry of the VSV-G pseudotyped viral particles through the endocytic pathway, we nevertheless analyzed the impact of inhibition of glycolysis on the infection of CD4+ T cells with a R5 wild-type replication competent virus (HIV-1 Bal). We first confirmed that the hierarchy of infection of CD4+ T cell subsets that we observed with VSV-G single cycle particles (Tn<Tcm<Ttm<Tem) coincided with the hierarchy of infection when we used replication competent HIV-1 Bal (Figure S11A). Next, we found that 2-DG was also able to efficiently blocked infection of CD4+ T cells with HIV-1 Bal (Figure 6G), independently of whether it was added at the time of challenge or 4h/8h after challenge (Figure S11B). All together, these results show that the inhibition of metabolic activity blocked HIV-1 replication, corroborating that CD4+ T cell metabolism is an important determinant of HIV-1 infection.

Suboptimal inhibition of glycolysis eliminates HIV-1 infected cells and impairs HIV amplification from CD4+ T cell reservoirs

We next studied if the preferential establishment of HIV-1 infection in highly glycolytic cells could be used to target HIV-1 reservoirs. First, we analyzed if suboptimal inhibition of glycolysis could selectively eliminate CD4+ T cells that had been preinfected in vitro. We infected CD4+ T cells with VSV-G pseudotyped NL4.3ΔenvGFP particles for three days and sorted infected GFP+ from noninfected GFP- cells (Figure S6) and cultured them in the absence or presence of 2-DG to inhibit glycolysis. The presence of 2-DG induced much higher levels of cell death among infected GFP+ cells than among GFP- cells (Figure 7A and B), affecting all memory T cell subpopulations (Figure 7C).

As 2-DG was able to both block infection and eliminate infected cells, we wondered whether 2-DG could block HIV spread upon activation of CD4+ T cells from HIV-infected individuals receiving cART. We isolated CD4+ T cells from 6 individuals receiving cART (Table S2) and activated the cells with PHA in the absence or presence of 2-DG. In all cases, 2-DG potently blocked HIV-1 amplification, as measured by ultrasensitive analyses of p24 production in the culture supernatants (Figure 7D).

Therefore, the need of HIV for highly glycolytic cells reveals a vulnerability that can be exploited to tackle infection.

DISCUSSION

We show here that susceptibility to HIV-1 infection is affected by the different metabolic programming of CD4+ T cell subsets. In particular, we have found that HIV-1 selectively completes its replication cycle in those CD4+ T cells with the highest levels of metabolism. Consequently, suboptimal inhibition of glycolysis both impaired infection in vitro and selectively eliminated infected cells. Moreover, mild inhibition of glycolysis potently blocked HIV reactivation in CD4+ T cell reservoirs from HIV-infected individuals on antiretroviral therapy.

In this study, we performed a detailed characterization of the bioenergetics of CD4+ Tn, Tcm, Ttm and Tem cells. Upon potent TCR activation, naïve and memory cells have been shown to strongly upregulate their metabolism and acquire effector functions (van der Windt et al., 2013). Here, we show important metabolic differences among the three memory cell populations studied, even in the absence of stimulation. Upon anti-CD3 activation, all CD4+ T cell subsets enhanced their metabolic activity but essentially maintained their distinctive metabolic programs, which matched the requirements for their expected rapid reaction to antigenic stimulation (Tem>>Ttm>Tcm>>Tn). The metabolic activity of the T cell subsets overlapped with their susceptibility to HIV-1 infection (Figures 1C and 3B), supporting that the extent of HIV-1 infection in CD4+ T cell subsets was affected by the metabolic environment within the target cells.

Transcript profiling at the time of infection showed that among the CD4+ T cell subsets, there were positive correlations between the frequencies of HIV-infected cells and the expression levels of multiple genes related to cell metabolism. Negative correlations were found between the susceptibility of CD4+ T cells to HIV-1 infection and the expression of SAMHD1, an efficient HIV-1 restriction factor that also plays an important role in the regulation of cell metabolism (Descours et al., 2012; Laguette et al., 2011; Mathews, 2015). Surprisingly, strong positive correlations were found between the levels of HIV-infected cells and the expression of a cluster of genes related to the

311 interferon response. Although this point was not specifically explored in the present study, increasing
312 evidence has revealed the interrelationships between cell metabolism and the interferon response
313 (Ahmed and Cassol, 2017; Burke et al., 2014; Zhao et al., 2015). Some type 1 interferons might
314 enhance glycolysis (Fritsch and Weichhart, 2016), and interferon regulatory factors play a key role
315 during the metabolic reprogramming that follows TCR-mediated activation of T cells (Man et al.,
316 2013). The interaction between the interferon response and cell metabolism may somewhat explain
317 the dichotomy between antiviral and viral-enhancing interferon-stimulated genes (Schoggins and
318 Rice, 2011; Seo et al., 2011). Tem cells, which were the most susceptible to HIV infection in our assay,
319 expressed the strongest levels of several restriction factors such as SLFN11 or APOBEC3G. Our results
320 thus indicate that HIV-1 exploits the metabolic environment that most favors the completion of its
321 replication cycle, and this might be one of the factors underlying the adaptation of HIV-1 to evade
322 some restriction factors.
323
324 We further confirmed the association between T cell metabolism and HIV infection in a series of
325 functional analyses. First, we showed that HIV-infected CD4+ T cells had higher levels of metabolic
326 activity and metabolic potential than HIV-exposed but noninfected cells. This was not solely the
327 consequence of the preferential infection of cells with higher activation levels; when we sorted CD4+
328 T cells that were matched for the expression of common activation markers, we still found that HIV-
329 infected cells had higher metabolic activity levels than noninfected CD4+ T cells. Although there are
330 well-established links between T cell activation and cellular metabolism, it is increasingly clear that T
331 cell functions, including proliferation, the secretion of cytokines and cell survival, are supported
332 through different engagements of the various metabolic pathways (Jones and Bianchi, 2015). This
333 may explain the partial dichotomy between T cell activation and cell metabolism in HIV infection that
334 we observed in our experiments. Additionally, we found Tn cells expressing high levels of activation
335 markers upon anti-CD3 stimulation, but these cells remained mostly resistant to HIV-1 infection. In
336 contrast, the frequency of infected Tn cells sharply increased when we challenged highly glycolytic Tn

cells. This is in agreement with previous results that showed that expression of Glut-1 is necessary for HIV-1 infection of CD4+ T cells (Loisel-Meyer et al., 2012) and that, in vitro, HIV preferentially infects CD4+ T cells expressing Glut-1 and OX40 (Palmer et al.). Overall, our results demonstrate that cells that had higher metabolic activity levels were more susceptible to HIV infection.

In our experimental conditions, we could detect virtually no infected cells when we challenged cells with low metabolic activity levels. Thus, any potential change in cell metabolism that might have been induced directly by HIV particles was not sufficient to promote infection in cells that had low metabolic activity levels at the time of viral challenge. However, it is important to note that because we were interested in understanding the factors modulating HIV infection beyond the expression of HIV receptors, we used single-cycle particles devoid of HIV envelope and pseudotyped with VSV-G in this set of experiments. It is possible that fully replication-competent viruses have a stronger effect on modulating CD4+ T cell metabolism. CCL5 engagement with CCR5 has been described as increasing glycolysis in T cells (Chan et al., 2012), and it is possible that gp120 triggers a similar effect. Moreover, HIV infection has been shown to induce increased expression of several glucose transporters in in vitro experiments (Kavanagh Williamson et al., 2018; Sorbara et al., 1996). HIV-infected individuals carry higher frequencies of Glut1+ CD4+ T cells, and these levels are not normalized after the initiation of antiretroviral therapy (Palmer et al., 2014). Overall, viruses appear to possess different mechanisms to enhance cell metabolism to favor viral replication (Goodwin et al., 2015; Sanchez and Lagunoff, 2015), and this deserves additional exploration in the context of HIV infection.

Suboptimal inhibition of glycolysis impaired HIV replication, and this was observed with single-cycle VSVG pseudotyped particles and replication-competent HIV-1 Bal and for all CD4+ T cell subsets, although the effects were more pronounced in more energetic cells. Inhibition of glycolysis, including several hours after viral entry, severely reduced the accumulation of HIV reverse transcripts and

363 impaired the establishment of both productive and latent infections. Our results thus point to critical
364 steps early during the viral replication cycle (in particular reverse transcription) that are influenced by
365 glycolysis, which agrees with a previous report (Loisel-Meyer et al., 2012). Along these lines, the
366 synthesis of deoxynucleotides, the level of which is a limiting factor for HIV reverse transcription, is
367 very energy demanding and requires substrates that are provided by different metabolic pathways,
368 such as the pentose phosphate pathway that is parallel to glycolysis (Lane and Fan, 2015; Mathews,
369 2015). Although, unfortunately, genes involved in the PPP were not included in our gene expression
370 panel, we found important differences between CD4+ T cell subsets and strong correlations with
371 infection levels for several genes such as TP53, ESF1 and RRM2, which play critical roles in the de
372 novo synthesis of dNTPs. In particular we have recently shown that changes in the expression of
373 RRM2 impact HIV-1 replication in macrophages and dendritic cells by modifying the pools of dNTPS
374 (Allouch et al., 2013; Valle-Casuso et al., 2017). Moreover, SAMHD1, the expression levels of which
375 were negatively correlated with infection in our analysis, is a deoxynucleoside triphosphohydrolase
376 that contributes to control the intracellular dNTP concentration during cell-cycle (Mathews, 2015).
377 Our results therefore suggest that metabolically active cells offer an environment with positive
378 synthesis (RRM2) vs degradation (SAMHD1) of dNTP pools that favors HIV-1 reverse transcription.
379 However, other steps of the viral replication cycle may also depend on cell metabolism. The
380 inhibition of glycolysis has been shown to decrease the production of HIV-1 particles (Hegedus et al.,
381 2014), and mTOR, a key regulator of cellular metabolism (Waickman and Powell, 2012), appears to
382 be involved in the establishment of HIV-1 latency in CD4+ T cells (Besnard et al., 2016).
383
384 In our functional experiments we mostly focused on assessing the impact of glycolysis on HIV
385 infection. Our results showing that inhibition of pyruvate transport to the mitochondria with UK5099
386 blocked HIV infection suggests that glucose oxidation is important for HIV-1 infection. However the
387 relative contribution of aerobic vs oxidative glycolysis remains to be determined. It is likely that other
388 metabolic functions are also important for HIV-1 infection. The inhibition of fatty acid oxidation with

Etomoxir had a limited effect on HIV replication in suboptimal conditions, mostly in Tem cells, but it strongly inhibited infection at higher concentrations. However, caution is needed when interpreting results obtained with Etomoxir as it has been shown to produce off target effects at such high concentrations (O'Connor et al., 2018; Yao et al., 2018). A recent report suggested that fatty acid metabolism may also participate in the late steps of viral replication (Kulkarni et al., 2017). Our results with the glutamine antagonist DON suggest that glutamine metabolism may also be necessary for the optimal infection of CD4+ T cells. In general, the association between HIV infection and cell metabolism can be exploited to impair HIV-1 replication.

Cell survival is another process regulated by cell metabolism that could be critically relevant for the persistence of infected cells. We found that suboptimal inhibition of glycolysis induced the selective death of cells that had been preinfected in vitro, and this affected all CD4+ T cell memory subsets. It remains to be determined if HIV-infected cells sustain enhanced metabolic activity levels over time, even in the absence of active viral replication and if this could serve to identify cells carrying proviruses. CD4+ T cells expressing PD-1 and other immune checkpoints are enriched in HIV in HIV-infected individuals receiving cART (Banga et al., 2016; Chomont et al., 2009; Fromentin et al., 2016). Interestingly, these immune checkpoints appear to mediate their inhibitory activities through the metabolic reprogramming of the cells (Herbel et al., 2016; Lim et al., 2017; Patsoukis et al., 2015). We show here that the partial inhibition of glycolysis in CD4+ T cells from HIV-infected individuals on cART potently blocked viral reactivation and spread. Based on our results, this could be the result of a combination of both the elimination of infected cells and the blocking of new cycles of viral amplification by 2-DG. Overall our results point to the potential modulation of cell metabolism as a strategy to combat HIV infection.

Therapies targeting cellular metabolism are gaining interest in the cancer field (Hanahan and Weinberg, 2011; Zhao et al., 2013). Of note, metabolic reprogramming observed in tumor cells

closely resembles the metabolic profile of HIV-infected T cells that we describe here. In the context of the physiopathology of HIV infection, high glucose consumption by infected CD4+ T cells could have additional implications for immune responses. We recently found that while HIV-specific CD8+ T cells from rare individuals naturally controlling HIV infection are characterized by metabolic plasticity, HIV-specific CD8+ T cells from most HIV-infected subjects heavily rely on glycolysis to exert their functions (Angin *et al*, submitted). High levels of glucose consumption by CD4+ T cells at the sites of viral replication might severely limit glucose availability for these CD8+ T cells and impair their effector function. In addition, lactic acid, which is a product of glycolysis, inhibits effector functions in cytotoxic T cells (Mendler et al., 2012). Therefore, the metabolic characteristics of HIV-infected CD4+ T cells may provide the virus with additional mechanisms to mediate immune evasion, as has also been described for tumors (Sugiura and Rathmell, 2018). Because exploiting the host cell metabolic machinery appears to be a common strategy for invading pathogens, including viruses, bacteria and parasites, therapies targeting cell metabolism could affect a large spectrum of infections. Obviously, cell metabolism regulates critical physiological events, including immune responses, and it is necessary to develop a better understanding the links between cell metabolism and acute and chronic infections. Overall, our study shows that cellular metabolism is a central factor that drives the HIV-1 infection of CD4+ T cells more strongly than does the state of differentiation and/or activation, and cellular metabolism may be an important target for new therapies against HIV-1.

MATERIALS AND METHODS

Subjects

Blood samples from non-HIV-infected donors were obtained from the French blood bank (Etablissement Français du Sang) as part of an agreement with the Institut Pasteur (C CPSL UNT, number 15/EFS/023). Fifty-milliliter blood samples were obtained from six HIV-infected individuals on antiretroviral therapy who had HIV plasma viral loads <50 RNA copies/mL from the ANRS TRANSbioHIV study after obtaining written informed consent in accordance with the Declaration of Helsinki (Table S2). The TRANSbioHIV study was approved by the Ethics Review Committee (Comité de protection des personnes) of Île-de-France VII.

Isolation and culture of CD4+ T cells

CD4+ T cells were purified (>90%) from freshly isolated PBMCs by negative selection with antibody-coated magnetic beads (EasySep™ Human CD4+ T Cell Enrichment Kit Ref.19052) in a Robosep instrument (Stem Cell Technology).

Purified CD4+ cells (10^6 cell/mL) were cultured in RPMI 1640 containing GlutaMAX, 10% FCS, penicillin (10 IU/mL) and streptomycin (10 µg/mL) in the presence of IL-2 (Miltenyi) at 50 IU/mL (Culture media). Depending on the experiment, cells were left unstimulated or were stimulated for 3 or 5 days with 0.5 µg/mL soluble antiCD3 (BioLegend, Ref.300414, Clone UCHT1) in the absence of CD28 co-stimulation as previously described (Saez-Cirion et al., 2011). Different compounds that target metabolic pathways [2-deoxy-glucose, 2-DG (Seahorse Biotechnologies); (+)-Etomoxir sodium salt hydrate (Sigma, Ref. E1905); UK5099 (Sigma, Ref. PZ0160); 6-diazo-5-oxo-l-norleucine (DON)(Sigma, Ref. D2141); glucose (Seahorse Biotechnologies); oligomycin (Seahorse Biotechnologies) or carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone FCCP (Seahorse Biotechnologies)] were added to the culture media at different times and concentrations depending on the protocol conditions. A glucose-free culture media was used in some infection experiments and

460 is described in the results section [RPMI non-glucose, GlutaMAX, containing 10% FCS, penicillin (10
461 IU/mL) and streptomycin (10 µg/mL) in the presence of IL-2 (Miltenyi) at 50 IU/mL (culture media)].
462 After culture, living cells were counted with an automatic Countess cell counter (Invitrogen) based on
463 size and non-staining with trypan blue. The number of living cells was then normalized before
464 analysis.
465
466 **HIV infection in vitro**
467 Single-round infections were performed with HIV-1 NL4.3ΔenvΔnef/GFP (Amara et al., 2003) and
468 HIV-1-DuoFluoΔenv(R7GEmC) (provided by Professor Eric Verdin and Dr. Calvanese, NIH AIDS
469 Reagent Program, Division of AIDS, NIAID, NIH: Cat# 12595 DuoFluo (R7GEmC)) (Calvanese et al.,
470 2013). Both viruses were pseudotyped with the VSV-G envelope protein as previously described
471 (David et al., 2006). Nonactivated or activated CD4 + T cells were infected in triplicate ($5x10^4$
472 cells/well, 200 µL) with 35 ng/$1x10^6$ HIV-1 NL4.3Δnef/GFP/VSV-G and with 70 ng of HIV-1-
473 DuoFluo(R7GEmC)/VSVg per million cells. Active HIV-1 infection was estimated by flow cytometry
474 (BD LSRII, BD bioscience) as the percentage of GFP-expressing CD4+ T cells 72 h after infection. Latent
475 HIV infection was estimated by flow cytometry as the percentage of mCherry+GFP-CD4+ T cells 72 h
476 after infection with HIV-1-DuoFluo(R7GEmC) particles.
477
478 HIV-1 reverse transcripts (U5-Gag) were quantified by real-time PCR with an Applied Biosystems 7500
479 Real-Time PCR System (as described in (David et al., 2006)) 6, 16 and 72 h after infection of CD4+ T
480 cells with VSV-G–pseudotyped HIV-1 particles. Total DNA was extracted with the NucleoSpin 8/96
481 Tissue Core kit (Macherey-Nagel, Ref. 740453.4). DNA loading was controlled by concurrently
482 amplifying the albumin gene by real-time PCR and quantifying with reference to a control human
483 genomic DNA (Roche).
484

Productive HIV-1 infection in vitro was studied in suboptimally activated CD4+ T cells (10^6 cells/mL in triplicate) exposed to the HIV-1 BaL strain (R5) (10 ng p24/ml). The cells were cultured in 96-U-well plates for 14 days in the presence or absence of 2-DG (5 mM). Every 3-4 days, the culture supernatants were removed and replaced with fresh culture medium with or without 2-DG. Viral replication was monitored in the supernatants by p24 enzyme-linked immunosorbent assay (ELISA) (XpressBio) or at day 3 by intracellular p24 staining (p24-FITC (clone KC57, Coulter) (Saez-Cirion et al., 2010).

Flow-assisted sorting of CD4 + T cell subsets

Cells were first selected based on size and structure to eliminate cellular debris. Then cell singlets and living cells (not stained with LIVE/DEAD Fixable Aqua Dead Cell Stain Kit, Thermofisher) are gated before proceeding with further selection based on phenotypical or functional markers (Figure S8).

Resting (CD25-, CD69-, HLA-DR-) CD4+ T cell subsets [naïve (**Tn**; CD3+, CD4+, CD45RA+, CCR7+, CD27+, CD95-), central memory (**Tcm**; CD3+, CD4+, CD45RA-, CCR7+, CD27+), transitional memory (**Ttm**; CD3+, CD4+, CD45RA-, CCR7-, CD27+) or effector memory (**Tem**; CD3+, CD4+, CD45RA-, CCR7-, CD27-)] were sorted on a FACS ARIA III cell sorter (BD) using the following antibody panel: CD3-eFLuor450 (eBioscience), CD4-alexaFluor700 (BD), CD45RA-ECD (BC), CCR7-PE_Cy7 (BioLegend), CD27-APC (Miltenyi), CD95-PE (Miltenyi), CD25-FITC (BD), CD69-FITC (eBioscience) and HLA-DR-FITC (BD). The gating strategy is depicted in Figure S1. The number of sorted cells varied from 0.5 to 5 million cells depending on the CD4+ T cell subset and the donor. The purity of the sorted subset was greater than 98%.

GFP+ and GFP- CD4+ T cells were sorted 72 h after infection with VSV-G pseudotyped NL4.3ΔenvΔnef/GFP particles (Figure S6). For some experiments, GFP+ and GFP- cells were also sorted into the following categories based on their expression of activation markers (CD25-ECD, HLA-

DR_PerCyP5.5) (Figure 4 and Figure S7): high activation GFP+ [**H/+** (GFP+, CD25+,HLA-DR+)]; high activation GFP- [**H/-** (GFP-, CD25+, HLA-DR+)]; low activation GFP+ [**L/+**, (GFP+, CD25-,HLA-DR-)]; and low activation GFP- [**L/-** (GFP-, CD25-,HLA-DR-)].

For some experiments, CD4+ Tn and Tcm cells were sorted based on their level of glucose uptake after 5 days of stimulation with anti-CD3 (Figure S8). The cells were washed and incubated with 2-NBDG (2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose) (Thermo Fisher, Ref. N13195) at 75 µM in PBS for 30 min at 37°C. After 3 washes of 10 min each with fresh PBS, the cells were stained with antibodies (CD3-eFLuor450 (eBioscience), CD4-alexaFluor700 (BD), CD45RA-ECD (BC), CCR7-PE_Cy7 (BioLegend), and CD27-APC (Miltenyi)) and sorted as follows: **Tn HGlu** (CD3+, CD4+, CD45RA+, CCR7+, CD27+, 2NBDG+); **Tn LGlu** (CD3+, CD4+, CD45RA+, CCR7+, CD27+, 2NBDG-); **Tcm HGlu** (CD3+, CD4+, CD45RA-, CCR7+, CD27+, 2NBDG+); and **Tcm LGlu** (CD3+, CD4+, CD45RA-, CCR7+, CD27+, 2NBDG-).

Quantitative RT-PCR arrays

The expression levels of an array of 96 genes in the CD4+ T cell subsets were quantified by RT-qPCR with a Biomark HQ system. Total RNA was extracted from $5x10^4$ CD4+ T cells with an RNA trace kit (Macherey-Nagel, Ref. 740731.4) and treated with DNase, following the manufacturer's instructions. Twenty microliters of RNA (> 10 ng) was reverse transcribed with Reverse Transcription Master Mix (Fluidigm, 100-6298) (5 minutes at 25°C, 30 minutes at 42°C, and 5 minutes at 85°C). A specific target preamplification (STA) was performed by adding PreAmp Master Mix, 96 Primers Mix and EDTA to the cDNA, followed by STA cycling (95°C: 2 min, 18 cycles of [96°C: 5 s, 60°C 4 min]). The sample was then treated with exonuclease I (New England Biolabs) (37°C: 30 min, 80°C: 15 min). Sample premix (SsoFast EvaGreen Supermix with Low ROX (Biorad), DNA Binding Dye (Fluidigm), preamplified Exo 1-treated sample) and assay mix (assay loading reagent (Fluidigm), Delta Gene primers (Fluidigm)) were then loaded on primed 96.96 Dynamic Array chips (Fluidigm). The chips were transferred into a

Biomark HQ device (Fluidigm) for thermocycling, and fluorescence was acquired with the GE 96×96 PCR+Melt v2 program. Linear derivative mode baseline correction was applied. We used the Normfinder algorithm (Aarhus University Hospital, Denmark) (Andersen et al., 2004) to identify the optimal normalization gene among the assayed candidates for our experimental conditions. BENC1 was thus identified as the optimal normalization gene based on expression stability in the analyzed samples (Table S3), and the gene expression values were plotted as $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = \Delta Ct_{SAMPLE} - \Delta Ct_{CONTROL}$, and $\Delta Ct = Ct_{TARGET\ GENE} - Ct_{BENC1}$.

Measurement of oxygen consumption and extracellular acidification rates

The oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured using a Seahorse XF96 metabolic analyzer following the procedure recommended by the manufacturer. Briefly, for all the experiments, different CD4+ T cell populations were seeded at a concentration of 2×10^5 cells per well on XF96 plates (Seahorse Bioscience) precoated with 0.5 mg/ml Cell Tack (Corning, Ref. 354240) immediately before adding Seahorse XF culture media to each well. Cells were incubated for 50 min in a CO_2-free incubator at 37°C before loading the plate in the Seahorse analyzer. Different programs were run on the Seahorse analyzer depending on the assay. Drug Panel A (1) XFmedia 2) oligomycin (2.5 µM), 3) FCCP (0.9 µM) and 4) rotenone (1 µM) and antimycin A (1 µM)) was injected through ports A, B, C and D, respectively, for the mitochondrial stress test. Drug Panel B (1) XFmedia 2) glucose (10 mM) 3) oligomycin (2.65 µM), and 4) 2-DG (100 mM)) was used for the glycolysis stress test.

Phenotyping after sorting

In some experiments, sorted GFP+/- CD4+ T cells subset or CD4+ T bulk cells previously infected with NL4.3Δnef/GFP/VSV-G with or without 2-DG or Etomoxir were incubated with CD3-eFLuor450 (eBioscience), CD4-alexaFluor700 (BD Biosciences), CD45RA-ECD (BC), CCR7-PE_Cy7 (BioLegend) and CD27-APC (Miltenyi) to determine the CD4+ T cell subset distribution. In addition, the activation

levels of sorted CD4+ T cell subsets were assessed with CD25-ECD (BD Biosciences) and HLA-DR-FITC (BD Biosciences). For both protocols, cells were incubated with the antibodies for 25 minutes and then washed in PBS plus 1% FCS and fixed in 4% paraformaldehyde for flow cytometry on an LSRII device (BD Biosciences). The data were analyzed with Kaluza software (Beckman Coulter).

HIV-1 reactivation in CD4+ T cells from HIV-1-infected individuals.

Freshly isolated CD4+T cells (negative selection kit, Stem Cell) from HIV-individuals undergoing successful cART were seeded in 48-well plates ($1x10^6$ cells/well, in triplicate) and stimulated with phytohemagglutinin-L (PHA-L, Roche, 1 µg/mL) and IL-2 (Miltenyi) 100UI with or without 2-DG (5 mM). The culture supernatants were collected every 3 to 4 days, and fresh medium +/- 2-DG was added to the cultures. Supernatants were stored at -80°C, and HIV-1 p24 was analyzed later by ultrasensitive digital ELISA (Simoa, Quanterix) (Passaes et al., 2017).

Statistical analyses

Differential gene expression

For each gene, we implemented a mixed effects model to detect differential expression between cell types (Tn, Tcm, Ttm and Tem). We defined a model that included the type of cells as a fixed effect and the patient as a random effect. A p-value was then obtained by implementing a likelihood ratio test between the full model and a reduced model without the fixed effect.

Correlation between gene expression, metabolic parameters and HIV-1 susceptibility

We computed Spearman's correlation coefficient and tested for significance.

Linear discriminant analysis (LDA)

We implemented an LDA for each condition (control, 3d and 5d) aimed at separating the cell subsets by using the metabolic variables analyzed. The 2 first axes are represented in Figure 3.

Other analyses

Values are presented in the graphs as medians and interquartile ranges. Statistical analyses were performed using SigmaPlot (Systat Software). Differences between CD4+ T cell subsets in different conditions were analyzed with nonparametric signed ANOVA and the multiple comparison Student-Newman-Keuls method. Differences between GFP+ and GFP- CD4+ T cells or control vs treatment culture conditions were analyzed with paired t-tests. When multiple treatment conditions were tested, ANOVA analyses and the Holm-Sidak method for multiple comparisons versus control group were used. Differences were considered significant when $p < 0.05$.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

JCV-C, VM, MA, CP, AM, VA-F performed experiments; JCV-C, SV and AS-C analyzed the data; KB, FB, MS, M-IT and OL provided key reagents or contributed to the inclusion of study participants and the obtaining and validation of clinical information; JCV-C, NT, MS, MM-T, OL, NC and AS-C contributed to the conception and discussion of the study; JCV-C and AS-C designed the study; AS-C supervised the study; JCV-C and AS-C drafted the article; and all authors critically reviewed the manuscript.

620 **REFERENCES**

621 Ahmed, D., and Cassol, E. (2017). Role of cellular metabolism in regulating type I interferon
622 responses: Implications for tumour immunology and treatment. Cancer Letters *409*, 20-29.

623 Allouch, A., David, A., Amie, S.M., Lahouassa, H., Chartier, L., Margottin-Goguet, F., Barre-Sinoussi, F.,
624 Kim, B., Saez-Cirion, A., and Pancino, G. (2013). p21-mediated RNR2 repression restricts HIV-1
625 replication in macrophages by inhibiting dNTP biosynthesis pathway. Proc Natl Acad Sci U S A *110*,
626 E3997-4006.

627 Amara, A., Vidy, A., Boulla, G., Mollier, K., Garcia-Perez, J., Alcami, J., Blanpain, C., Parmentier, M.,
628 Virelizier, J.L., Charneau, P.*, et al.* (2003). G protein-dependent CCR5 signaling is not required for
629 efficient infection of primary T lymphocytes and macrophages by R5 human immunodeficiency virus
630 type 1 isolates. J Virol *77*, 2550-2558.

631 Amie, S.M., Noble, E., and Kim, B. (2013). Intracellular nucleotide levels and the control of retroviral
632 infections. Virology *436*, 247-254.

633 Andersen, C.L., Jensen, J.L., and Orntoft, T.F. (2004). Normalization of real-time quantitative reverse
634 transcription-PCR data: a model-based variance estimation approach to identify genes suited for
635 normalization, applied to bladder and colon cancer data sets. Cancer Res *64*, 5245-5250.

636 Banga, R., Procopio, F.A., Noto, A., Pollakis, G., Cavassini, M., Ohmiti, K., Corpataux, J.M., de Leval, L.,
637 Pantaleo, G., and Perreau, M. (2016). PD-1(+) and follicular helper T cells are responsible for
638 persistent HIV-1 transcription in treated aviremic individuals. Nat Med *22*, 754-761.

639 Barton, K., Winckelmann, A., and Palmer, S. (2016). HIV-1 Reservoirs During Suppressive Therapy.
640 Trends Microbiol *24*, 345-355.

641 Besnard, E., Hakre, S., Kampmann, M., Lim, H.W., Hosmane, N.N., Martin, A., Bassik, M.C.,
642 Verschueren, E., Battivelli, E., Chan, J.*, et al.* (2016). The mTOR Complex Controls HIV Latency. Cell
643 Host & Microbe *20*, 785-797.

644 Burke, J.D., Platanias, L.C., and Fish, E.N. (2014). Beta Interferon Regulation of Glucose Metabolism Is
645 PI3K/Akt Dependent and Important for Antiviral Activity against Coxsackievirus B3. J Virol *88*, 3485-
646 3495.
647 Buzon, M.J., Sun, H., Li, C., Shaw, A., Seiss, K., Ouyang, Z., Martin-Gayo, E., Leng, J., Henrich, T.J., Li,
648 J.Z*., et al.* (2014). HIV-1 persistence in CD4+ T cells with stem cell-like properties. Nature medicine *20*,
649 139-142.
650 Byrne, J.A., Butler, J.L., and Cooper, M.D. (1988). Differential activation requirements for virgin and
651 memory T cells. J Immunol *141*, 3249-3257.
652 Calvanese, V., Chavez, L., Laurent, T., Ding, S., and Verdin, E. (2013). Dual-color HIV reporters trace a
653 population of latently infected cells and enable their purification. Virology *446*, 283-292.
654 Chan, O., Burke, J.D., Gao, D.F., and Fish, E.N. (2012). The Chemokine CCL5 Regulates Glucose Uptake
655 and AMP Kinase Signaling in Activated T Cells to Facilitate Chemotaxis. J Biol Chem *287*, 29406-
656 29416.
657 Chavez, L., Calvanese, V., and Verdin, E. (2015). HIV Latency Is Established Directly and Early in Both
658 Resting and Activated Primary CD4 T Cells. PLoS Pathog *11*, e1004955.
659 Chomont, N., El-Far, M., Ancuta, P., Trautmann, L., Procopio, F.A., Yassine-Diab, B., Boucher, G.,
660 Boulassel, M.R., Ghattas, G., Brenchley, J.M*., et al.* (2009). HIV reservoir size and persistence are
661 driven by T cell survival and homeostatic proliferation. Nat Med *15*, 893-900.
662 Cleret-Buhot, A., Zhang, Y., Planas, D., Goulet, J.-P., Monteiro, P., Gosselin, A., Wacleche, V.S.,
663 Tremblay, C.L., Jenabian, M.-A., Routy, J.-P*., et al.* (2015). Identification of novel HIV-1 dependency
664 factors in primary CCR4+CCR6+Th17 cells via a genome-wide transcriptional approach. Retrovirology
665 *12*, 102.
666 Croft, M., Bradley, L.M., and Swain, S.L. (1994). Naive versus memory CD4 T cell response to antigen.
667 Memory cells are less dependent on accessory cell costimulation and can respond to many antigen-
668 presenting cell types including resting B cells. J Immunol *152*, 2675-2685.

David, A., Saez-Cirion, A., Versmisse, P., Malbec, O., Iannascoli, B., Herschke, F., Lucas, M., Barre-Sinoussi, F., Mouscadet, J.F., Daeron, M.*, et al.* (2006). The engagement of activating FcgammaRs inhibits primate lentivirus replication in human macrophages. J Immunol *177*, 6291-6300.

Deeks, S.G., Lewin, S.R., Ross, A.L., Ananworanich, J., Benkirane, M., Cannon, P., Chomont, N., Douek, D., Lifson, J.D., Lo, Y.R.*, et al.* (2016). International AIDS Society global scientific strategy: towards an HIV cure 2016. Nat Med *22*, 839-850.

Descours, B., Cribier, A., Chable-Bessia, C., Ayinde, D., Rice, G., Crow, Y., Yatim, A., Schwartz, O., Laguette, N., and Benkirane, M. (2012). SAMHD1 restricts HIV-1 reverse transcription in quiescent CD4(+) T-cells. Retrovirology *9*, 87.

Finzi, D., Hermankova, M., Pierson, T., Carruth, L.M., Buck, C., Chaisson, R.E., Quinn, T.C., Chadwick, K., Margolick, J., Brookmeyer, R.*, et al.* (1997). Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science *278*, 1295-1300.

Franke, E.K., and Luban, J. (1996). Inhibition of HIV-1 replication by cyclosporine A or related compounds correlates with the ability to disrupt the Gag-cyclophilin A interaction. Virology *222*, 279-282.

Fritsch, S.D., and Weichhart, T. (2016). Effects of Interferons and Viruses on Metabolism. Front Immunol *7*.

Fromentin, R., Bakeman, W., Lawani, M.B., Khoury, G., Hartogensis, W., DaFonseca, S., Killian, M., Epling, L., Hoh, R., Sinclair, E.*, et al.* (2016). CD4+ T Cells Expressing PD-1, TIGIT and LAG-3 Contribute to HIV Persistence during ART. PLoS Pathog *12*, e1005761.

Goodwin, C.M., Xu, S., and Munger, J. (2015). Stealing the Keys to the Kitchen: Viral Manipulation of the Host Cell Metabolic Network. Trends Microbiol *23*, 789-798.

Hanahan, D., and Weinberg, Robert A. (2011). Hallmarks of Cancer: The Next Generation. Cell *144*, 646-674.

693 Hegedus, A., Kavanagh Williamson, M., and Huthoff, H. (2014). HIV-1 pathogenicity and virion
694 production are dependent on the metabolic phenotype of activated CD4+ T cells. Retrovirology *11*,
695 98.
696 Herbel, C., Patsoukis, N., Bardhan, K., Seth, P., Weaver, J.D., and Boussiotis, V.A. (2016). Clinical
697 significance of T cell metabolic reprogramming in cancer. Clinical and Translational Medicine *5*, 29.
698 Jia, X., Zhao, Q., and Xiong, Y. (2015). HIV suppression by host restriction factors and viral immune
699 evasion. Current opinion in structural biology *31*, 106-114.
700 Jones, W., and Bianchi, K. (2015). Aerobic Glycolysis: Beyond Proliferation. Front Immunol *6*.
701 Kane, M., Zang, T.M., Rihn, S.J., Zhang, F., Kueck, T., Alim, M., Schoggins, J., Rice, C.M., Wilson, S.J.,
702 and Bieniasz, P.D. (2016). Identification of Interferon-Stimulated Genes with Antiretroviral Activity.
703 Cell Host Microbe *20*, 392-405.
704 Kavanagh Williamson, M., Coombes, N., Juszczak, F., Athanasopoulos, M., Khan, M.B., Eykyn, T.R.,
705 Srenathan, U., Taams, L.S., Dias Zeidler, J., Da Poian, A.T.*, et al.* (2018). Upregulation of Glucose
706 Uptake and Hexokinase Activity of Primary Human CD4+ T Cells in Response to Infection with HIV-1.
707 Viruses *10*.
708 Kulkarni, M.M., Ratcliff, A.N., Bhat, M., Alwarawrah, Y., Hughes, P., Arcos, J., Loiselle, D., Torrelles,
709 J.B., Funderburg, N.T., Haystead, T.A.*, et al.* (2017). Cellular fatty acid synthase is required for late
710 stages of HIV-1 replication. Retrovirology *14*, 45.
711 Kumar, R., Ferez, M., Swamy, M., Arechaga, I., Rejas, M.T., Valpuesta, J.M., Schamel, W.W., Alarcon,
712 B., and van Santen, H.M. (2011). Increased sensitivity of antigen-experienced T cells through the
713 enrichment of oligomeric T cell receptor complexes. Immunity *35*, 375-387.
714 Laguette, N., Sobhian, B., Casartelli, N., Ringeard, M., Chable-Bessia, C., Segeral, E., Yatim, A.,
715 Emiliani, S., Schwartz, O., and Benkirane, M. (2011). SAMHD1 is the dendritic- and myeloid-cell-
716 specific HIV-1 restriction factor counteracted by Vpx. Nature *474*, 654-657.
717 Lane, A.N., and Fan, T.W.M. (2015). Regulation of mammalian nucleotide metabolism and
718 biosynthesis. Nucleic Acids Res *43*, 2466-2485.

719 Lever, A.M., and Jeang, K.T. (2011). Insights into cellular factors that regulate HIV-1 replication in
720 human cells. Biochemistry *50*, 920-931.
721 Li, M., Kao, E., Gao, X., Sandig, H., Limmer, K., Pavon-Eternod, M., Jones, T.E., Landry, S., Pan, T.,
722 Weitzman, M.D*., et al.* (2012). Codon-usage-based inhibition of HIV protein synthesis by human
723 schlafen 11. Nature *491*, 125-128.
724 Lim, S., Phillips, J.B., Silva, L.M.d., Zhou, M., Fodstad, O., Owen, L.B., and Tan, M. (2017). Interplay
725 between Immune Checkpoint Proteins and Cellular Metabolism. Cancer Res *77*, 1245-1249.
726 Loisel-Meyer, S., Swainson, L., Craveiro, M., Oburoglu, L., Mongellaz, C., Costa, C., Martinez, M.,
727 Cosset, F.-L., Battini, J.-L., Herzenberg, L.A*., et al.* (2012). Glut1-mediated glucose transport regulates
728 HIV infection. PNAS *109*, 2549-2554.
729 Lukic, Z., Dharan, A., Fricke, T., Diaz-Griffero, F., and Campbell, E.M. (2014). HIV-1 uncoating is
730 facilitated by dynein and kinesin 1. J Virol *88*, 13613-13625.
731 Man, K., Miasari, M., Shi, W., Xin, A., Henstridge, D.C., Preston, S., Pellegrini, M., Belz, G.T., Smyth,
732 G.K., Febbraio, M.A*., et al.* (2013). The transcription factor IRF4 is essential for TCR affinity–mediated
733 metabolic programming and clonal expansion of T cells. Nature Immunology *14*, 1155-1165.
734 Manel, N., Kim, F.J., Kinet, S., Taylor, N., Sitbon, M., and Battini, J.L. (2003). The ubiquitous glucose
735 transporter GLUT-1 is a receptor for HTLV. Cell *115*, 449-459.
736 Mathews, C.K. (2015). Deoxyribonucleotide metabolism, mutagenesis and cancer. Nat Rev Cancer *15*,
737 528-539.
738 Mendler, A.N., Hu, B., Prinz, P.U., Kreutz, M., Gottfried, E., and Noessner, E. (2012). Tumor lactic
739 acidosis suppresses CTL function by inhibition of p38 and JNK/c-Jun activation. Int J Cancer *131*, 633-
740 640.
741 O'Connor, R.S., Guo, L., Ghassemi, S., Snyder, N.W., Worth, A.J., Weng, L., Kam, Y., Philipson, B.,
742 Trefely, S., Nunez-Cruz, S*., et al.* (2018). The CPT1a inhibitor, etomoxir induces severe oxidative stress
743 at commonly used concentrations. Sci Rep *8*, 6289.

744 Oliveira, N.M., Farrell, K.B., and Eiden, M.V. (2006). In Vitro Characterization of a Koala Retrovirus. J
745 Virol *80*, 3104-3107.
746 Palmer, C.S., Duette, G.A., Wagner, M.C.E., Henstridge, D.C., Saleh, S., Pereira, C., Zhou, J., Simar, D.,
747 Lewin, S.R., Ostrowski, M.*, et al.* Metabolically active CD4+ T cells expressing Glut1 and OX40
748 preferentially harbor HIV during in vitro infection. FEBS Lett, n/a-n/a.
749 Palmer, C.S., Ostrowski, M., Gouillou, M., Tsai, L., Yu, D., Zhou, J., Henstridge, D.C., Maisa, A., Hearps,
750 A.C., Lewin, S.R.*, et al.* (2014). Increased glucose metabolic activity is associated with Cd4+ T-cell
751 activation and depletion during chronic Hiv infection. Aids *28*, 297-309.
752 Pan, X., Baldauf, H.M., Keppler, O.T., and Fackler, O.T. (2013). Restrictions to HIV-1 replication in
753 resting CD4+ T lymphocytes. Cell Res *23*, 876-885.
754 Passaes, C.P., Bruel, T., Decalf, J., David, A., Angin, M., Monceaux, V., Muller-Trutwin, M., Noel, N.,
755 Bourdic, K., Lambotte, O.*, et al.* (2017). Ultrasensitive HIV-1 p24 Assay Detects Single Infected Cells
756 and Differences in Reservoir Induction by Latency Reversal Agents. J Virol *91*.
757 Passaes, C.P., and Saez-Cirion, A. (2014). HIV cure research: advances and prospects. Virology *454-*
758 *455*, 340-352.
759 Patsoukis, N., Bardhan, K., Chatterjee, P., Sari, D., Liu, B., Bell, L.N., Karoly, E.D., Freeman, G.J.,
760 Petkova, V., Seth, P.*, et al.* (2015). PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis
761 and promoting lipolysis and fatty acid oxidation. Nature Communications *6*, 6692.
762 Pearce, E.L., Poffenberger, M.C., Chang, C.H., and Jones, R.G. (2013). Fueling immunity: insights into
763 metabolism and lymphocyte function. Science *342*, 1242454.
764 Roederer, M., Raju, P.A., Mitra, D.K., Herzenberg, L.A., and Herzenberg, L.A. (1997). HIV does not
765 replicate in naive CD4 T cells stimulated with CD3/CD28. J Clin Invest *99*, 1555-1564.
766 Roesch, F., Meziane, O., Kula, A., Nisole, S., Porrot, F., Anderson, I., Mammano, F., Fassati, A.,
767 Marcello, A., Benkirane, M.*, et al.* (2012). Hyperthermia stimulates HIV-1 replication. PLoS Pathog *8*,
768 e1002792.

769 Saez-Cirion, A., Hamimi, C., Bergamaschi, A., David, A., Versmisse, P., Melard, A., Boufassa, F., Barre-
770 Sinoussi, F., Lambotte, O., Rouzioux, C*., et al.* (2011). Restriction of HIV-1 replication in macrophages
771 and CD4+ T cells from HIV controllers. Blood *118*, 955-964.
772 Saez-Cirion, A., Shin, S.Y., Versmisse, P., Barre-Sinoussi, F., and Pancino, G. (2010). Ex vivo T cell-
773 based HIV suppression assay to evaluate HIV-specific CD8+ T-cell responses. Nat Protoc *5*, 1033-1041.
774 Sanchez, E.L., and Lagunoff, M. (2015). Viral activation of cellular metabolism. Virology *479-480*, 609-
775 618.
776 Schnittman, S.M., Lane, H.C., Greenhouse, J., Justement, J.S., Baseler, M., and Fauci, A.S. (1990).
777 Preferential infection of CD4+ memory T cells by human immunodeficiency virus type 1: evidence for
778 a role in the selective T-cell functional defects observed in infected individuals. Proc Natl Acad Sci U S
779 A *87*, 6058-6062.
780 Schoggins, J.W., and Rice, C.M. (2011). Interferon-stimulated genes and their antiviral effector
781 functions. Current Opinion in Virology *1*, 519-525.
782 Seo, J.-Y., Yaneva, R., Hinson, E.R., and Cresswell, P. (2011). Human Cytomegalovirus Directly Induces
783 the Antiviral Protein Viperin to Enhance Infectivity. Science *332*, 1093-1097.
784 Sheehy, A.M., Gaddis, N.C., Choi, J.D., and Malim, M.H. (2002). Isolation of a human gene that
785 inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature *418*, 646-650.
786 Shimode, S., Nakaoka, R., Shogen, H., and Miyazawa, T. (2013). Characterization of feline ASCT1 and
787 ASCT2 as RD-114 virus receptor. Journal of General Virology *94*, 1608-1612.
788 Shojima, T., Yoshikawa, R., Hoshino, S., Shimode, S., Nakagawa, S., Ohata, T., Nakaoka, R., and
789 Miyazawa, T. (2013). Identification of a Novel Subgroup of Koala Retrovirus from Koalas in Japanese
790 Zoos. J Virol *87*, 9943-9948.
791 Sorbara, L.R., Maldarelli, F., Chamoun, G., Schilling, B., Chokekijcahi, S., Staudt, L., Mitsuya, H.,
792 Simpson, I.A., and Zeichner, S.L. (1996). Human immunodeficiency virus type 1 infection of H9 cells
793 induces increased glucose transporter expression. J Virol *70*, 7275-7279.

794 Spina, C.A., Prince, H.E., and Richman, D.D. (1997). Preferential replication of HIV-1 in the CD45RO
795 memory cell subset of primary CD4 lymphocytes in vitro. J Clin Invest *99*, 1774-1785.
796 Stevenson, M., Stanwick, T.L., Dempsey, M.P., and Lamonica, C.A. (1990). HIV-1 replication is
797 controlled at the level of T cell activation and proviral integration. EMBO J *9*, 1551-1560.
798 Sugiura, A., and Rathmell, J.C. (2018). Metabolic Barriers to T Cell Function in Tumors. J Immunol *200*,
799 400-407.
800 Tabler, C.O., Lucera, M.B., Haqqani, A.A., McDonald, D.J., Migueles, S.A., Connors, M., and Tilton, J.C.
801 (2014). CD4+ memory stem cells are infected by HIV-1 in a manner regulated in part by SAMHD1
802 expression. J Virol *88*, 4976-4986.
803 Takeuchi, Y., Vile, R.G., Simpson, G., O'Hara, B., Collins, M.K., and Weiss, R.A. (1992). Feline leukemia
804 virus subgroup B uses the same cell surface receptor as gibbon ape leukemia virus. J Virol *66*, 1219-
805 1222.
806 Valle-Casuso, J.C., Allouch, A., David, A., Lenzi, G.M., Studdard, L., Barre-Sinoussi, F., Muller-Trutwin,
807 M., Kim, B., Pancino, G., and Saez-Cirion, A. (2017). p21 Restricts HIV-1 in Monocyte-Derived
808 Dendritic Cells through the Reduction of Deoxynucleoside Triphosphate Biosynthesis and Regulation
809 of SAMHD1 Antiviral Activity. J Virol *91*.
810 van der Windt, G.J., O'Sullivan, D., Everts, B., Huang, S.C., Buck, M.D., Curtis, J.D., Chang, C.H., Smith,
811 A.M., Ai, T., Faubert, B.*, et al.* (2013). CD8 memory T cells have a bioenergetic advantage that
812 underlies their rapid recall ability. Proc Natl Acad Sci U S A *110*, 14336-14341.
813 von Laer, D., Thomsen, S., Vogt, B., Donath, M., Kruppa, J., Rein, A., Ostertag, W., and Stocking, C.
814 (1998). Entry of Amphotropic and 10A1 Pseudotyped Murine Retroviruses Is Restricted in
815 Hematopoietic Stem Cell Lines. J Virol *72*, 1424-1430.
816 Waickman, A.T., and Powell, J.D. (2012). mTOR, metabolism, and the regulation of T-cell
817 differentiation and function. Immunol Rev *249*, 43-58.

Yao, C.H., Liu, G.Y., Wang, R., Moon, S.H., Gross, R.W., and Patti, G.J. (2018). Identifying off-target effects of etomoxir reveals that carnitine palmitoyltransferase I is essential for cancer cell proliferation independent of beta-oxidation. PLoS Biol *16*, e2003782.

Zhang, J., Nuebel, E., Wisidagama, D.R., Setoguchi, K., Hong, J.S., Van Horn, C.M., Imam, S.S., Vergnes, L., Malone, C.S., Koehler, C.M.*, et al.* (2012). Measuring energy metabolism in cultured cells, including human pluripotent stem cells and differentiated cells. Nat Protoc *7*, 1068-1085.

Zhao, G.-N., Jiang, D.-S., and Li, H. (2015). Interferon regulatory factors: at the crossroads of immunity, metabolism, and disease. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease *1852*, 365-378.

Zhao, Y., Butler, E.B., and Tan, M. (2013). Targeting cellular metabolism to improve cancer therapeutics. Cell Death & Disease *4*, e532.

FIGURE LEGENDS

Figure 1. CD4+ T cells subsets have different susceptibilities to HIV-1 infection

Bulk CD4+ T cells from noninfected donors were infected with single-cycle HIV-1 NL4.3Δenv VSV-G particles carrying the GFP reporter gene after 5 days of suboptimal activation. A) Representative example of the proportion of GFP-expressing cells in the absence of infection (top) or 72 h after the challenge (bottom). B) Relative distribution of CD4+ T cell subsets (determined by flow cytometry) in nonactivated (NA) and activated (aCD3 5d) cells before HIV challenge and in activated cells not expressing GFP (aCD3 5d GFP-) or expressing GFP (aCD3 5d GFP+) 72 h post challenge. The pie charts (top) represent the median values (n=3 donors). The bottom panels represent the fold change in the CD4+ T cells subset contribution relative to the nonactivated condition (NA). The asterisks represent statistically significant differences (*$p<0.05$; ** $p<0.01$). In a different set of experiments, CD4+ T cell subsets were flow sorted ex vivo and then cultured under nonactivated (NA) or suboptimally activated conditions for 3 (3d) or 5 days (5d) and challenged with HIV-1 NL4.3ΔenvGFP VSV-G for 72 h. Flow cytometry was used to determine the % of GFP-positive cells. C) Representative example of infection levels in Tn, Tcm, Ttm and Tem cells from a donor in the different conditions analyzed. D) Medians and IQR values for experiments with cells from 6 donors. Blue bars represent Tn cells, orange bars represent Tcm cells, pink bars represent Ttm cells and violet bars represent Tem cells. Symbols represent the individual data points. Significant differences between experimental conditions are shown for each T cell subset as horizontal lines. The median infection level in NA Tn cells is displayed as a reference dashed line to facilitate comparison between T cell subsets.

Figure 2. HIV-1 infection levels in CD4+ T cell subsets correlate with the expression levels of genes related to cell metabolism

A) Heat maps displaying the genes differentially expressed ($p<0.05$) between the CD4+ T cell subsets (Tn, Tcm, Ttm and Tem) (n= 6 donors) in the absence of activation or after 3 or 5 days of suboptimal

activation with soluble anti-CD3 (i.e., at the time of HIV challenge). Heat maps were generated by K-means clustering (green = downregulation, red = upregulation). Data were filtered by variance ($\delta/\delta max=0.2$) to reduce background noise. Gene expression data were centered to a mean value of zero and scaled to unit variance. Variables are ordered by hierarchical clustering and samples by CD4+ T cell subsets. B) Spearman's correlation between the levels of gene expression at the time of HIV-1 challenge and HIV-1 infection levels 72 h after challenge. Only significant correlations ($p<0.05$) are represented in the graphs (green bars). Genes highlighted in red show the group of genes that correlated with infection levels in all conditions.

Figure 3. CD4+ T cell subsets have different metabolic profiles that coincide with their susceptibility to HIV-1 infection

Oxygen consumption (OCR) and extracellular acidification rates (ECAR) measured before and after the addition of oligomycin, carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP) and rotenone/antimycin A in nonactivated (NA), 3-day activation (3d) and 5-day activation (5d) CD4+ T cell subsets. A) Median values of the metabolic variables obtained for the CD4+ T cell subsets from the 6 donors in the different conditions analyzed. B) Median and IQR basal OCR (left panel) and ECAR (right panel) and C) basal ECAR/OCR ratio for CD4+ T cell subsets in different activation states (0d, 3d, 5d). Symbols represent the individual data points (n=6). Significant differences between experimental conditions are shown for each T cell subset as horizontal lines. Median values in NA Tn cells are indicated by dashed lines as a reference. Symbols represent independent experiments (n=6). D) Summary of correlations between metabolic parameters at the time of infection in NA, 3d and 5d activated CD4+ T cell subsets and the % of infected cells 72 h post infection. The green color indicates $p<0.05$. The size of the circle represents Spearman's coefficients.

Figure 4. HIV-1-infected CD4+ T cells are characterized by higher metabolic activity levels

A) HIV-infected GFP+ (green line/symbols) and noninfected GFP- (red line/symbols) bulk CD4+ T cells from donors (n=3) were flow sorted 72 h after being challenged with HIV-1 NL4.3ΔenvGFP VSV-G. Their metabolic activity levels were quantified with a Seahorse analyzer. Mitochondrial activity (as determined by OCR) and glycolysis (as determined by ECAR) were analyzed before and after the addition of oligomycin, FCCP and rotenone/antimycin A. The bioenergetic (XF) phenotypes of GFP+ and GFP- cells (right panel) were determined by the $OCR_{(basal)}$ and $ECAR_{(basal)}$ values. The symbols represent independent experiments (n=3 donors). B) In a different set of experiments, CD4+ T cells were flow sorted 72 h after HIV challenge based first on their activation levels (high activation, CD25+/HLA-DR+ or low activation, CD25-/HLA-DR-) and then on the level of GFP expression (GFP- or GFP+ cells). The gating strategy is shown on the left panels. Pie charts (right) represent the median (n=4 donors) distribution of the CD4+ T cell subsets (determined by flow cytometry) for each sorted cell fraction as follows: high activation and GFP+, high activation and GFP-, low activation and GFP+ and low activation and GFP- (n=4). C) Representative analyses of OCR and ECAR (measured as above) for each cell fraction (left) and the median and IQR basal OCR and ECAR for 6 (high activation) and 4 (low activation) donors (right).

Figure 5. Rate of glucose uptake by CD4+ T cell subsets is associated with their susceptibility to HIV-1 infection

CD4+ T cells activated for 5 days were incubated for 30 minutes with the glucose fluorescent analogue 2NBDG. Cells were then flow sorted based on their differentiation status (Tn or Tcm) and their rate of 2NBDG uptake. Sorted cells were then challenged with HIV-1 NL4.3ΔenvGFP VSV-G particles, and the rate of infected cells was determined as the % of cells expressing GFP 72 h post infection. A) Representative example of 2NBDG content after sorting (top panels) and the levels of GFP expression 72 h later in CD4+ T cell fractions exposed (HIV-1) or not (control) to HIV-1 NL4.3ΔenvGFP VSV-G particles. B) Percentage of GFP-positive cells among CD4+ T cell fractions (Tn and Tcm sorted depending on their preinfection 2-NBDG uptake). Symbols represent individual

values for experiments with cells from three (Tn) and five (Tcm) donors. Medians and IQR values are represented by horizontal lines.

Figure 6. Inhibition of cell metabolic pathways blocks HIV-1 infection of CD4+ T cells

A) CD4+ T cells activated for five days were infected for 72 h with HIV-1 NL4.3ΔenvGFP VSV-G particles in the absence or presence of increasing amounts of Etomoxir (left panel), 6-diazo-5-oxo-l-norleucine (DON) (middle panel), or 2-deoxy glucose (2-DG) (right panel). Blue bars represent the relative level of infection compared to the control conditions (median and IQR, n=3 donors). Purple bars represent the number of dead cells per million cells. B) Similar experiments in which CD4+ T cells were exposed to HIV-1 particles and cultured for 72 h in glucose-containing medium in the absence or presence of 2-DG (5 mM) or in culture medium without glucose (starvation) (left) or in the absence or presence of 2-Cyano-3-(1-phenyl-1H-indol-3-yl)-2-propenoic acid (UK5099) (25 μM) (right). C) Relative number of U5-Gag copies quantified by RT-qPCR in CD4+ T cells at 6 h, 15 h or 72 h after infection with single cycle HIV-1 particles in the absence or presence of 2-DG. Individual values (symbols), medians and IQRs (horizontal lines) for five different donors are shown. D) Infection levels and number of U5-Gag copies quantified by RT-qPCR 72 h after challenge with single cycle HIV-1 particles in the absence or presence of 2-DG added at the time of challenge, 4h or 8h post challenge. Values represent the relative levels of infection compared to the control condition (median and IQR, n=3 donors). E) Changes in HIV-1 infection levels in CD4+ T cell subsets as determined by flow cytometry 72 h after the infection of bulk CD4+ T cells in the absence or in presence of 2-DG (orange symbols) or Etomoxir (beige symbols). Medians (n=7 donors) are shown. F) Percentage of HIV-1 productively (left panel) or latently (right panel) infected cells 72 h after the infection of CD4+ T cells with single-round HIV-1 NL4.3HIV-1DuoFluo VSVG particles in the presence of 2-DG or etomoxir. Median and IQR values from experiments with 6 donors are shown. G) p24 production in supernatants from CD4+T cell cultures 3 and 7 days after infection with HIV-1 BaL in the absence (blue bars) or presence of 2-DG (5 mM) (orange bars). Means and standard deviations for three

934 replicates are shown at each time point for experiments done with cells from three different donors.
935 (A-F) Horizontal lines above the graphs denote statistically significant differences in the treatment vs
936 control conditions.
937
938 **Figure 7. Suboptimal inhibition of glucose metabolism selectively eliminates preinfected CD4+ T**
939 **cells and inhibits HIV-1 amplification from reservoirs**
940 A) Five days after suboptimal activation, CD4+ T cells were challenged with HIV-1 NL4.3ΔenvGFP VSV-
941 G, and 3 days later, infected GFP+ (green) or noninfected GFP- (red) cells were sorted and cultured
942 for 48 h in the absence or presence of suboptimal amounts of 2-DG. Cell viability after the treatment
943 was measured by flow cytometry. A) One representative example is shown. B) Relative survival of 2-
944 DG treated cells (circles) was compared to that of nontreated cells (squares) at 24 h and 48 h. C)
945 Changes in the CD4+ T cell subset distribution 48 h after the treatment of infected bulk CD4+ T cells
946 with 2-DG when compared with the distribution in the control condition. Median values and IQR for
947 experiments with cells from three donors are shown. Asterisks represent statistically significant
948 differences (*$p<0.05$; **$p<0.01$). D) CD4+ T cells freshly isolated from HIV-infected individuals with
949 suppressed viremia due to cART (n=6) were stimulated with PHA/IL-2 in the absence (blue
950 line/symbols) or presence of 2-DG (5 mM) (orange line/symbols). HIV-1 reactivation from CD4+ T
951 cells was evaluated by ultrasensitive quantification of p24 in culture supernatants. The reactivation
952 kinetics are shown for each individual (left panels) (mean and SD, 3 replicates). Comparison of mean
953 p24 values in the absence or presence of 2-DG on day 14 post stimulation is shown for all six
954 experiments (right panel).
955
956

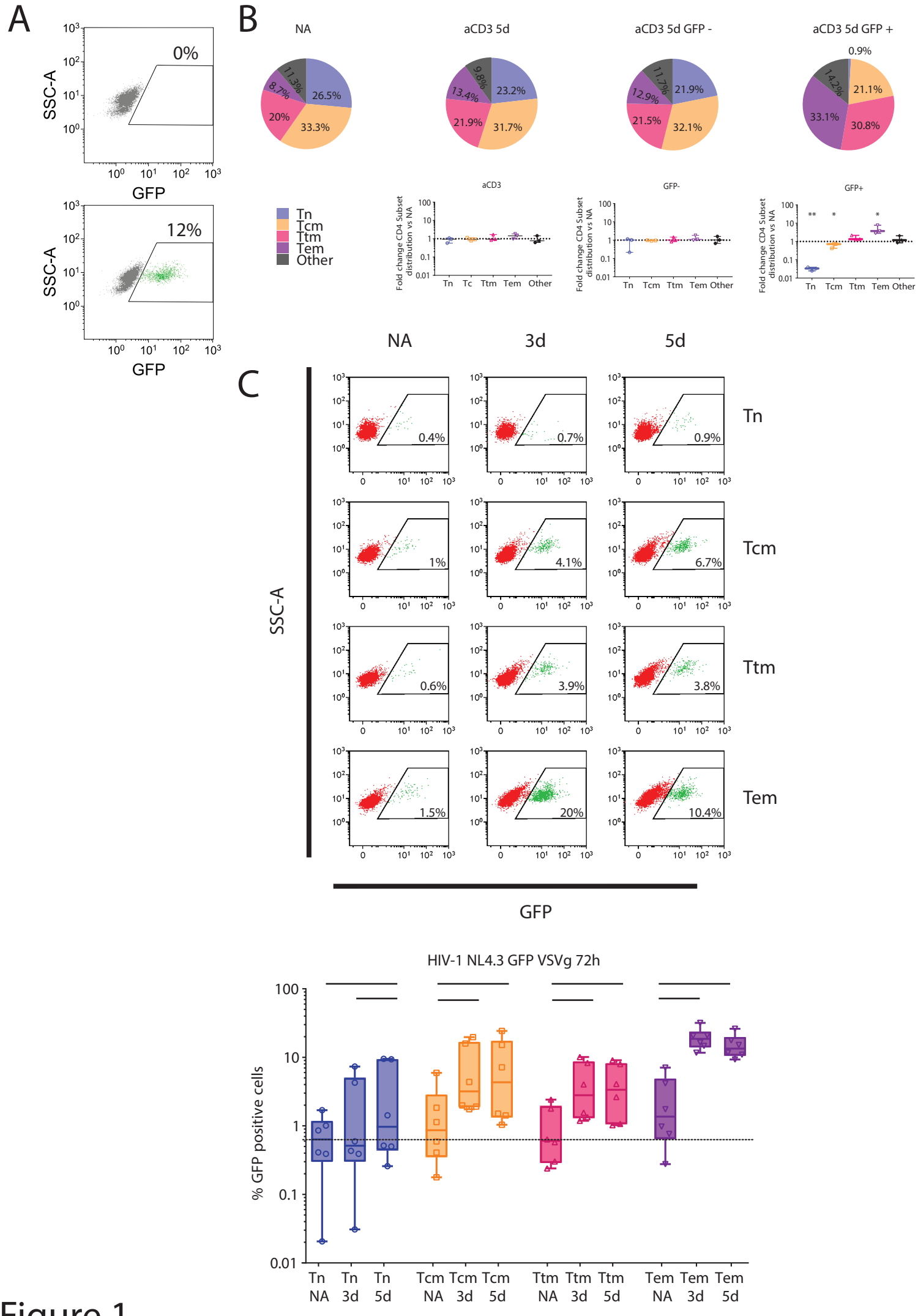
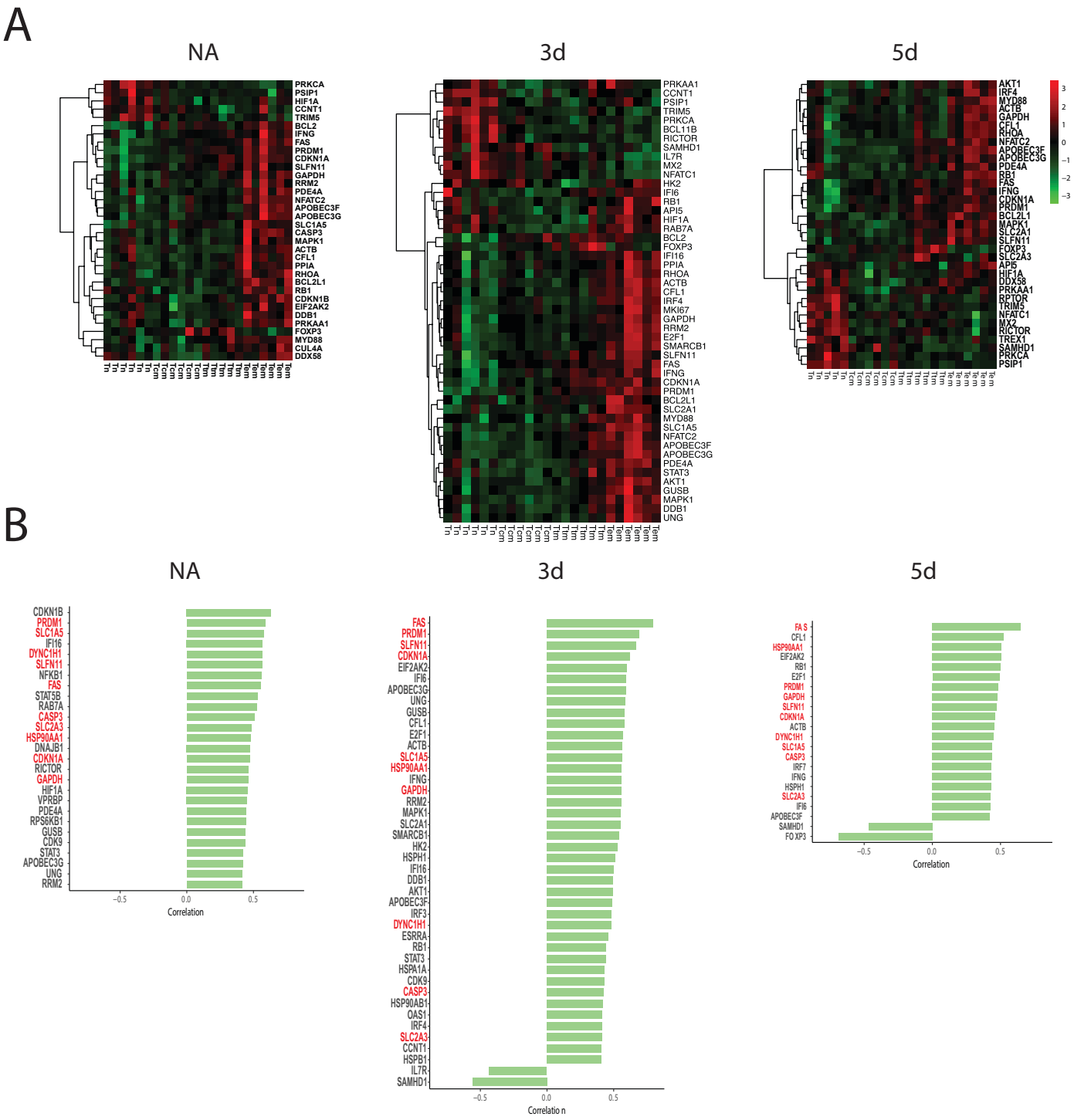

A
SSC-A
GFP
0%
12%
B
NA
aCD3 5d
aCD3 5d GFP -
aCD3 5d GFP +
26.5%
33.3%
20%
11.3%
8.7%
23.2%
31.7%
21.9%
13.4%
9.8%
21.9%
32.1%
21.5%
12.9%
11.7%
0.9%
21.1%
30.8%
33.1%
14.2%
Tn
Tcm
Ttm
Tem
Other
aCD3
GFP-
GFP+
Fold change CD4 Subset distribution vs NA
C
NA
3d
5d
0.4%
0.7%
0.9%
1%
4.1%
6.7%
0.6%
3.9%
3.8%
1.5%
20%
10.4%
SSC-A
GFP
HIV-1 NL4.3 GFP VSVg 72h
% GFP positive cells
Tn NA
Tn 3d
Tn 5d
Tcm NA
Tcm 3d
Tcm 5d
Ttm NA
Ttm 3d
Ttm 5d
Tem NA
Tem 3d
Tem 5d

Figure 1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NA
3d
5d
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PSIP1
HIF1A
CCNT1
TRIM5
BCL2
IFNG
FAS
PRDM1
CDKN1A
SLFN11
GAPDH
RRM2
PDE4A
NFATC2
APOBEC3F
APOBEC3G
SLC1A5
CASP3
MAPK1
ACTB
CFL1
PPIA
RHOA
BCL2L1
RB1
CDKN1B
EIF2AK2
DDB1
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FOXP3
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CUL4A
DDX58
PRKAA1
CCNT1
PSIP1
TRIM5
PRKCA
BCL11B
RICTOR
SAMHD1
IL7R
MX2
NFATC1
HK2
IFI6
RB1
API5
HIF1A
RAB7A
BCL2
FOXP3
IFI16
PPIA
RHOA
ACTB
CFL1
IRF4
MKI67
GAPDH
RRM2
E2F1
SMARCB1
SLFN11
FAS
IFNG
CDKN1A
PRDM1
BCL2L1
SLC2A1
MYD88
SLC1A5
NFATC2
APOBEC3F
APOBEC3G
PDE4A
STAT3
AKT1
GUSB
MAPK1
DDB1
UNG
AKT1
IRF4
MYD88
ACTB
GAPDH
CFL1
RHOA
NFATC2
APOBEC3F
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PDE4A
RB1
FAS
IFNG
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PRDM1
BCL2L1
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FOXP3
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PRKAA1
RPTOR
TRIM5
NFATC1
MX2
RICTOR
TREX1
SAMHD1
PRKCA
PSIP1
3
2
1
0
−1
−2
−3
B
NA
3d
5d
CDKN1B
PRDM1
SLC1A5
IFI16
DYNC1H1
SLFN11
NFKB1
FAS
STAT5B
RAB7A
CASP3
SLC2A3
HSP90AA1
DNAJB1
CDKN1A
RICTOR
GAPDH
HIF1A
VPRBP
PDE4A
RPS6KB1
GUSB
CDK9
STAT3
APOBEC3G
UNG
RRM2
−0.5
0.0
0.5
Correlation
FAS
PRDM1
SLFN11
CDKN1A
EIF2AK2
IFI6
APOBEC3G
UNG
GUSB
CFL1
E2F1
ACTB
SLC1A5
HSP90AA1
IFNG
GAPDH
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SLC2A1
SMARCB1
HK2
HSPH1
IFI16
DDB1
AKT1
APOBEC3F
IRF3
DYNC1H1
ESRRA
RB1
STAT3
HSPA1A
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CASP3
HSP90AB1
OAS1
IRF4
SLC2A3
CCNT1
HSPB1
IL7R
SAMHD1
−0.5
0.0
0.5
Correlatio n
FA S
CFL1
HSP90AA1
EIF2AK2
RB1
E2F1
PRDM1
GAPDH
SLFN11
CDKN1A
ACTB
DYNC1H1
SLC1A5
CASP3
IRF7
IFNG
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FO XP3
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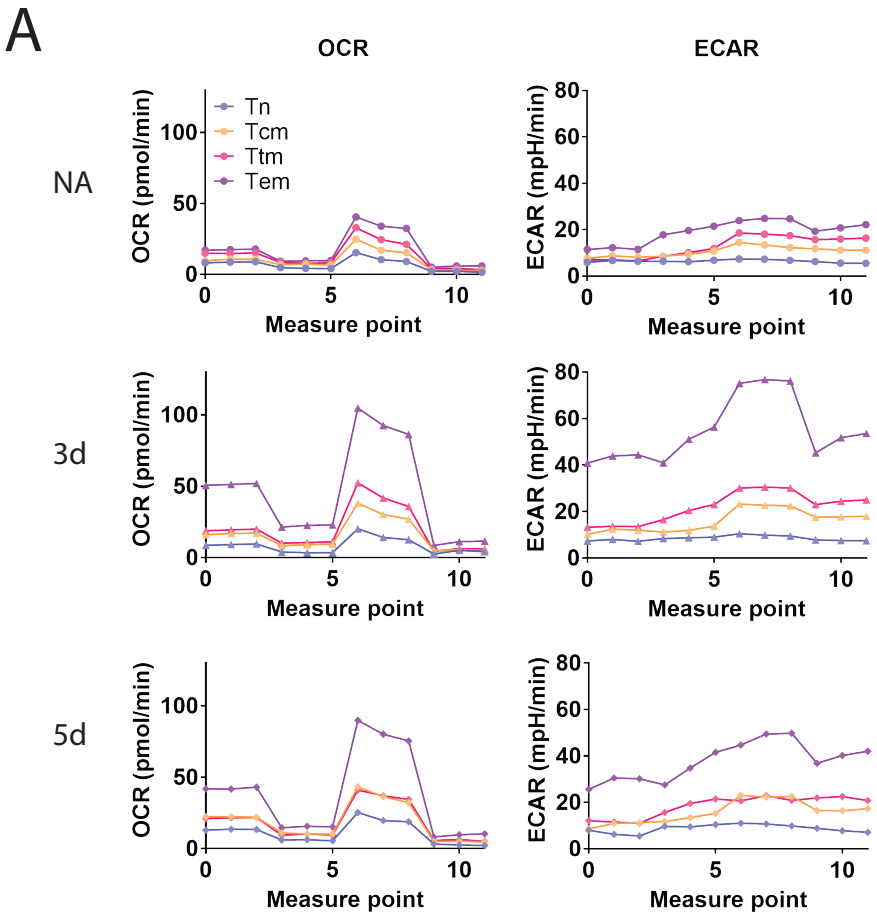
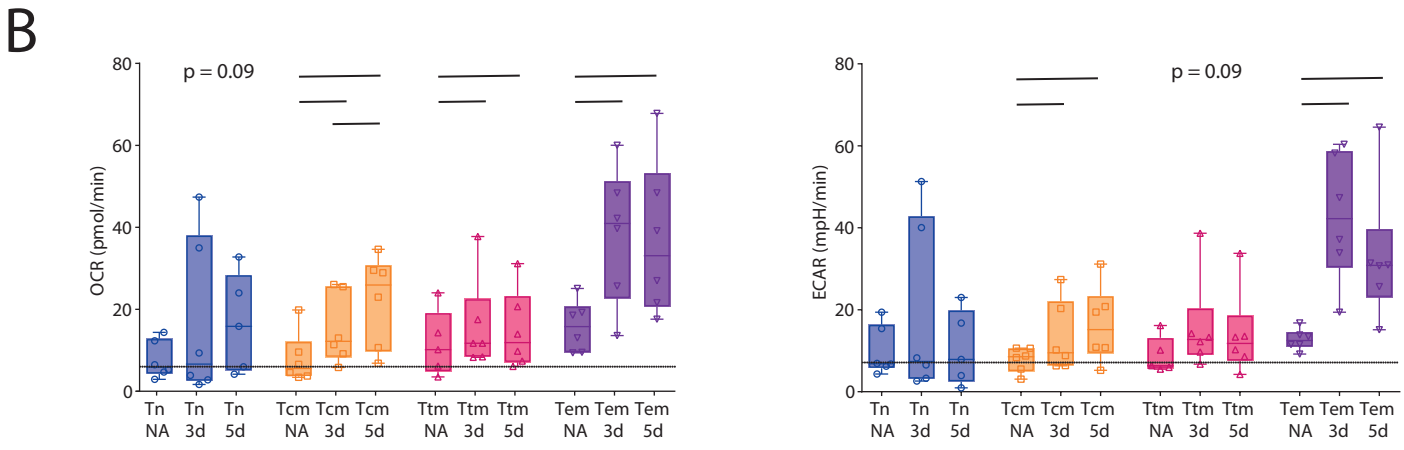
0.0
0.5
Correlation

Figure 2


A
OCR
ECAR
NA
3d
5d
Tn
Tcm
Ttm
Tem
OCR (pmol/min)
ECAR (mpH/min)
Measure point


B
p = 0.09
OCR (pmol/min)
ECAR (mpH/min)
Tn NA
Tn 3d
Tn 5d
Tcm NA
Tcm 3d
Tcm 5d
Ttm NA
Ttm 3d
Ttm 5d
Tem NA
Tem 3d
Tem 5d

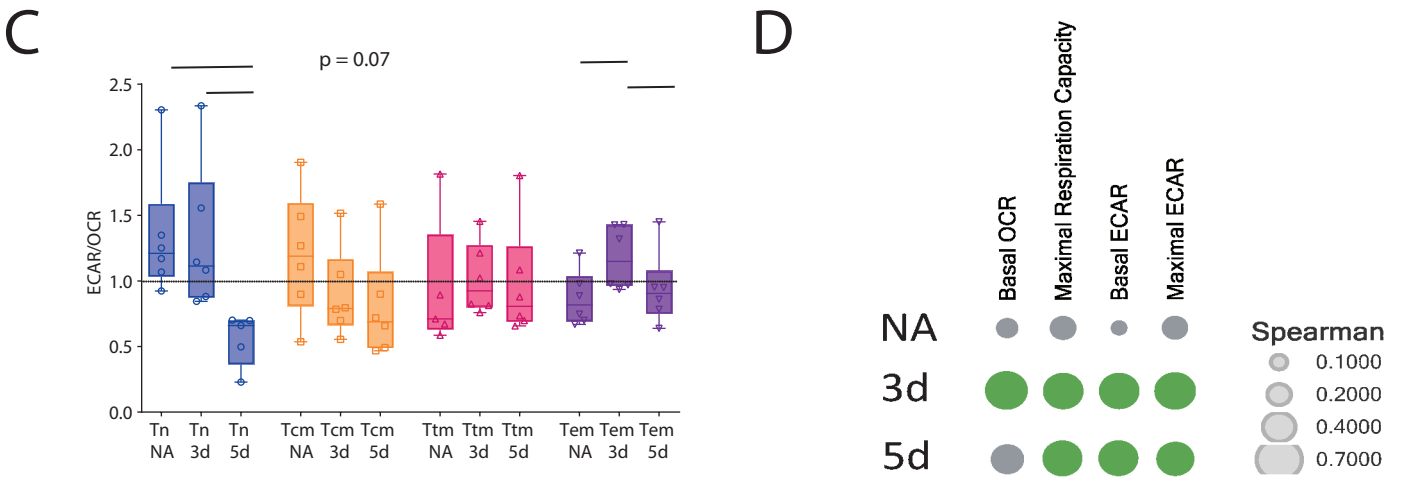

C
p = 0.07
ECAR/OCR
D
Basal OCR
Maximal Respiration Capacity
Basal ECAR
Maximal ECAR
NA
3d
5d
Spearman
0.1000
0.2000
0.4000
0.7000

Figure 3

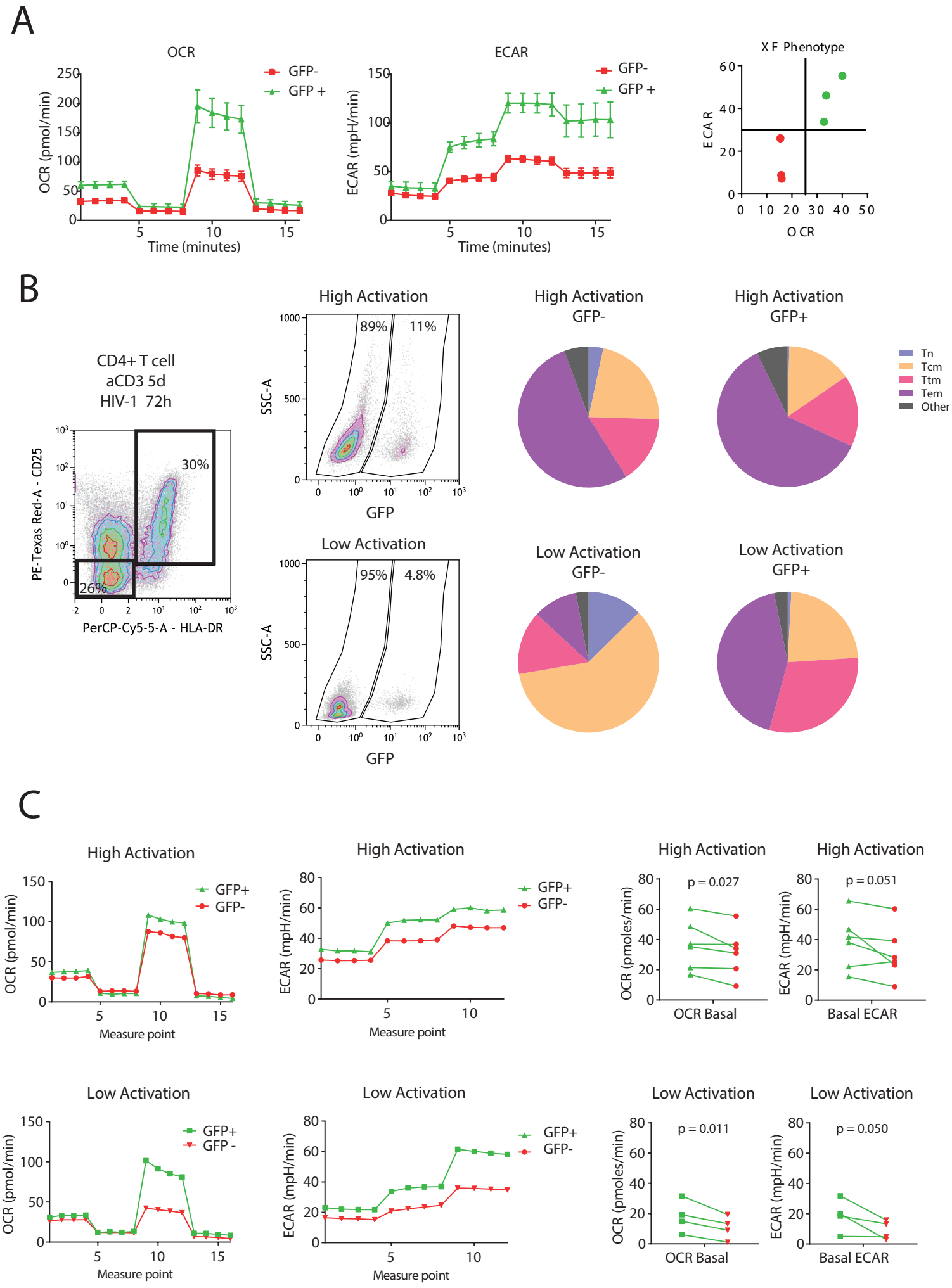
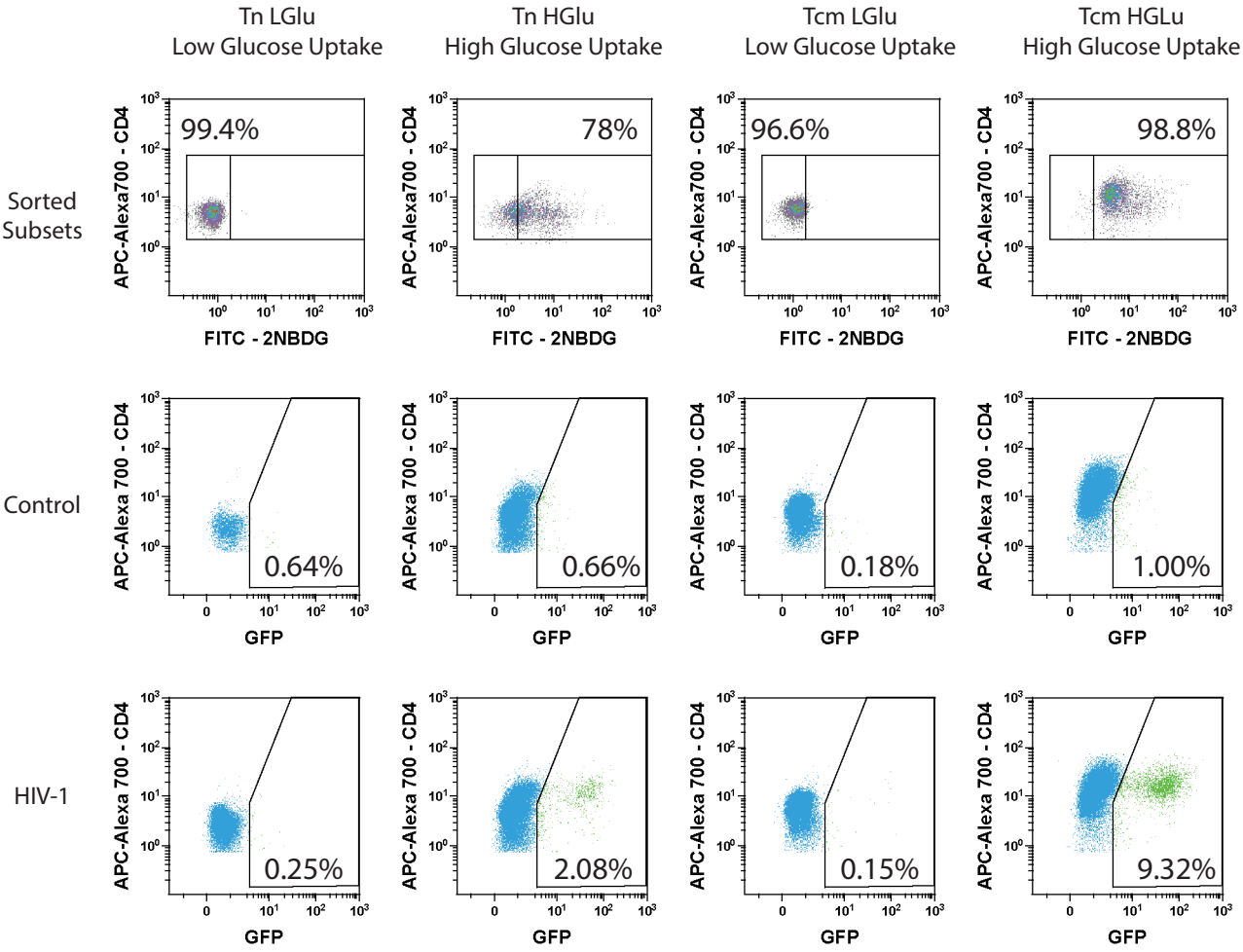

A
OCR
GFP-
GFP +
OCR (pmol/min)
Time (minutes)
ECAR
ECAR (mpH/min)
XF Phenotype
ECAR
OCR
B
CD4+ T cell
aCD3 5d
HIV-1 72h
PE-Texas Red-A - CD25
PerCP-Cy5-5-A - HLA-DR
30%
26%
High Activation
89%
11%
SSC-A
GFP
Low Activation
95%
4.8%
High Activation GFP-
High Activation GFP+
Low Activation GFP-
Low Activation GFP+
Tn
Tcm
Ttm
Tem
Other
C
High Activation
GFP+
GFP-
OCR (pmol/min)
Measure point
ECAR (mpH/min)
OCR (pmoles/min)
p = 0.027
OCR Basal
p = 0.051
Basal ECAR
Low Activation
GFP -
p = 0.011
p = 0.050

Figure 4

A


Tn LGlu
Low Glucose Uptake
Tn HGlu
High Glucose Uptake
Tcm LGlu
Low Glucose Uptake
Tcm HGLu
High Glucose Uptake
Sorted Subsets
Control
HIV-1
APC-Alexa700 - CD4
FITC - 2NBDG
APC-Alexa 700 - CD4
GFP
99.4%
78%
96.6%
98.8%
0.64%
0.66%
0.18%
1.00%
0.25%
2.08%
0.15%
9.32%

B

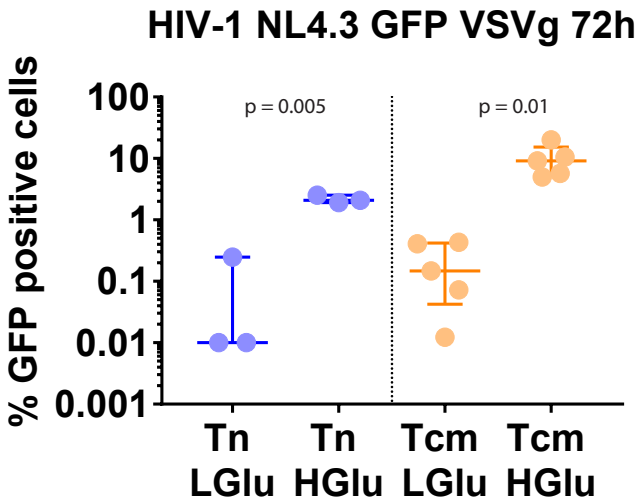

HIV-1 NL4.3 GFP VSVg 72h
% GFP positive cells
100
10
1
0.1
0.01
0.001
p = 0.005
p = 0.01
Tn LGlu
Tn HGlu
Tcm LGlu
Tcm HGlu

Figure 5

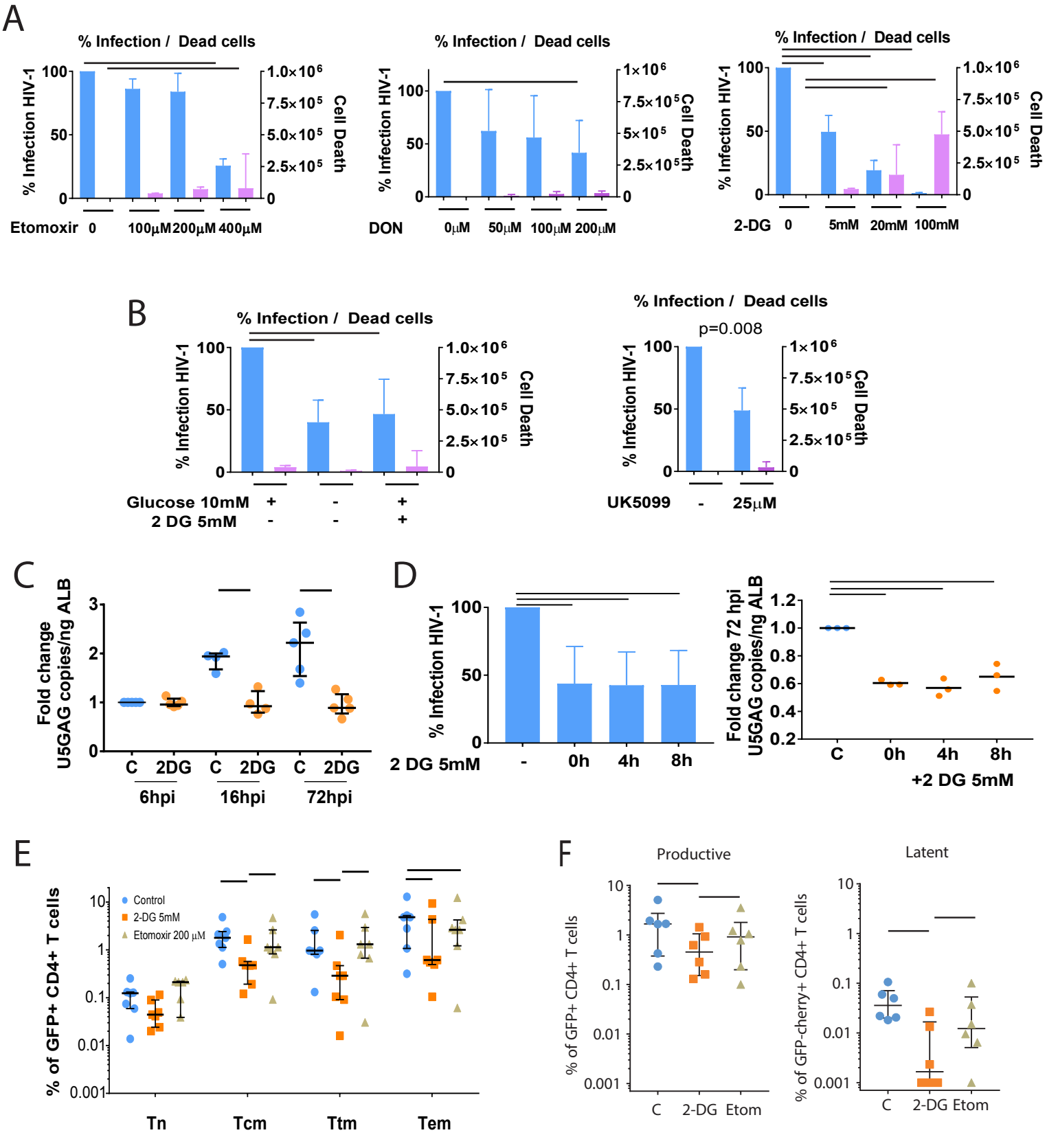

A
% Infection / Dead cells
% Infection HIV-1
Cell Death
Etomoxir 0 100µM 200µM 400µM
DON 0µM 50µM 100µM 200µM
2-DG 0 5mM 20mM 100mM
B
Glucose 10mM + - +
2 DG 5mM - - +
p=0.008
UK5099 - 25µM
C
Fold change U5GAG copies/ng ALB
C 2DG C 2DG C 2DG
6hpi 16hpi 72hpi
D
2 DG 5mM - 0h 4h 8h
Fold change 72 hpi U5GAG copies/ng ALB
C 0h 4h 8h
+2 DG 5mM
E
% of GFP+ CD4+ T cells
Control
2-DG 5mM
Etomoxir 200 µM
Tn Tcm Ttm Tem
F
Productive
Latent
% of GFP+ CD4+ T cells
% of GFP-cherry+ CD4+ T cells
C 2-DG Etom
G
EFS 543
EFS 559
EFS 560
p24 (pg/mL)
d3 d3 2-DG d7 d7 2-DG

Figure 6

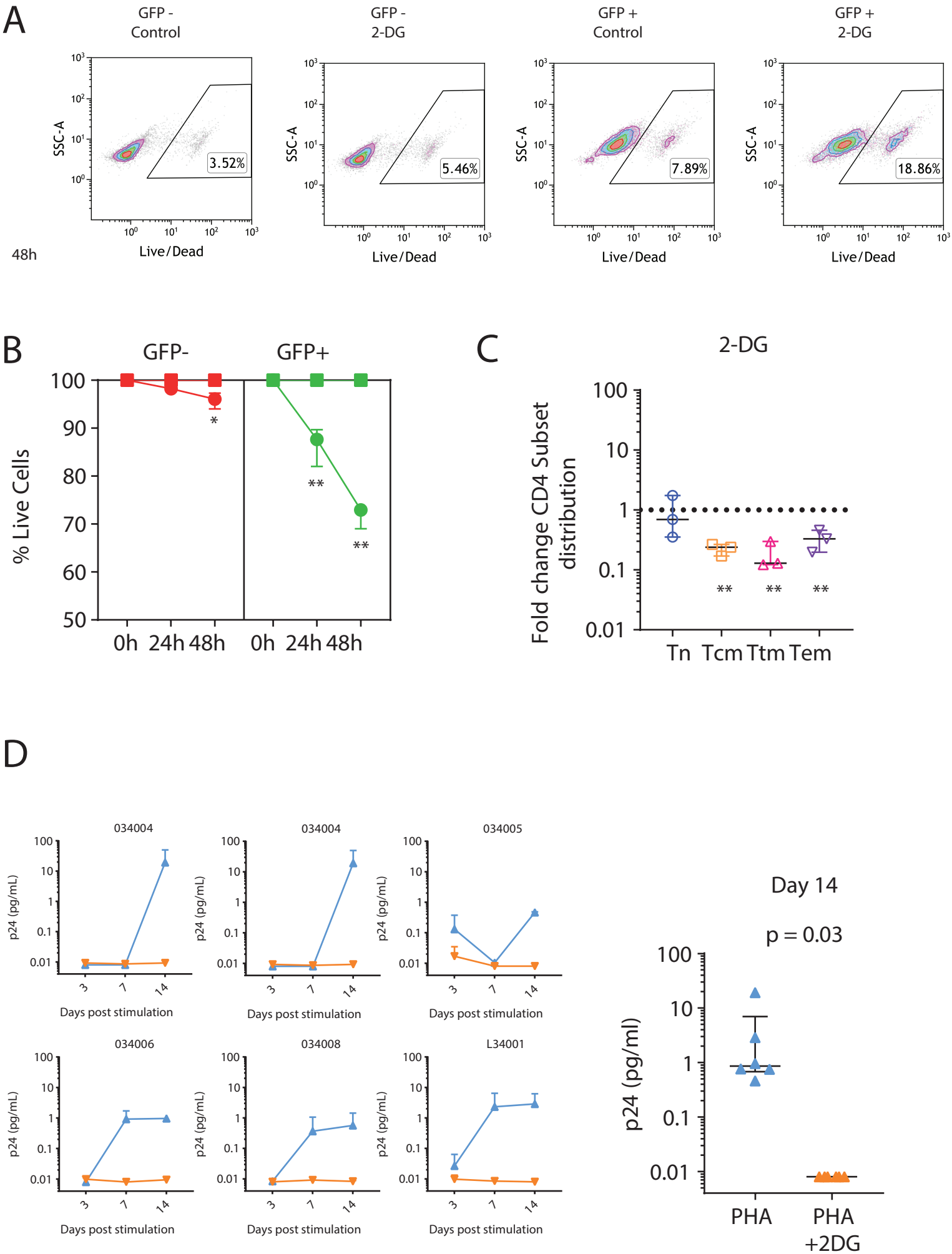

A
GFP -
Control
GFP -
2-DG
GFP +
Control
GFP +
2-DG
SSC-A
Live/Dead
3.52%
5.46%
7.89%
18.86%
48h
B
GFP-
GFP+
% Live Cells
0h 24h 48h
0h 24h 48h
C
2-DG
Fold change CD4 Subset distribution
Tn Tcm Ttm Tem
D
034004
034004
034005
034006
034008
L34001
p24 (pg/mL)
Days post stimulation
Day 14
p = 0.03
p24 (pg/ml)
PHA
PHA +2DG

Figure 7

Supplemental Material Legends

Figure S1

A representative example of the flow cytometry gating strategy used to sort CD4+ T cell subsets. Naïve (Tn; CD4-CD45RA+, CCR7+, CD27+, CD95-), central memory (Tcm; CD4-CD45RA-, CCR7+, CD27+), transitional memory (Ttm; CD4-CD45RA-, CCR7-, CD27+) or effector memory (Tem; CD4-CD45RA-, CCR7-, CD27-) displayed as standard pseudocolor dot plots. These sorted CD4+ T cell subsets were used in the gene expression analyses, metabolic profile assays and HIV-1 susceptibility assays. Table with gender, age and relative CD4+ T cell subset distribution ex vivo for each of the donors studied in the gene expression analyses, metabolic profile assays and HIV-1 susceptibility assays.

Figure S2

Spearman's correlation between the levels of expression of all genes analyzed at the time of HIV-1 challenge and HIV-1 infection levels 72 h after challenge for nonactivated (NA), 3-day activation (3d) or 5-day activation (5d) CD4+ T cells. Significant correlations ($p<0.05$) are shown in green. Venn diagram and table showing the genes correlating with infection in the different activation conditions.

Figure S3

Representative example of flow cytometry results at the end of the glucose uptake assay showing levels of 2-NBDG in nonactivated and 5-day activation CD4+ T cells. We defined the following 3 gates based on 2-NBDG capture levels: low, medium and high (upper panels). The levels of 2-NDBG captured by the different CD4+ T cell subsets are shown in the overlapping histogram (middle panels). The relative contributions of CD4+ T cell subsets to the low, medium and high 2-NBDG cell fractions are displayed in the cumulative bar chart (lower panels).

Figure S4

A representative example of GLUT-1 surface expression (left panel) and glucose uptake (right panel) (2-NBDG assay) in the CD4+ T cell subsets studied here after 5 days of suboptimal activation. CD4+ T cells were incubated with 2-NBDG and stained with a recombinant protein that recognizes GLUT-1 and set of antibodies to determine the CD4+T cell subsets.

Figure S5

Correlation between metabolic parameters (basal OCR, maximal respiratory capacity, basal and maximal ECAR measure with a Seahorse analyzer) at the time of infection in NA, 3d and 5d activated CD4+ T cell subsets and the % of infected cells 72 h post infection. Each symbol represents values for values for one CD4+ T cell subset from one individual. The p values of Spearman correlation analyses are shown. Lines depict linear regression of analyzed data.

Figure S6

A) Representative example of the flow cytometry gating strategy used to sort noninfected GFP- and infected GFP+ CD4+ T cells 72 h after challenge with single-cycle HIV-1 GFP particles and GFP expression in the purified populations. Representations are displayed as standard pseudocolor dot plots. B) Representative example of the glycolytic activity of GFP+ and GFP- sorted cells analyzed before and after the addition of glucose, oligomycin and 2-DG.

Figure S7

A) Percentage of cells among each CD4+ T cell subset expressing HLA-DR and CD25 five days after suboptimal activation with aCD3 (upper panel). Black horizontal lines above the symbols denote statistically significant differences between subsets. B) Representative example of expression levels of CD25 and HLA-DR for CD4+ T cell fractions sorted as high activation and GFP+, high activation and GFP-, low activation and GFP+ and low activation and GFP-. C) Changes in the relative contribution of

CD4+ T cell subsets to the GFP+ fractions when compared to their GFP- counterparts. Asterisks represent statistically significant differences (*p<0.05; ** p<0.01).

Figure S8

A) Flow cytometry gating strategy used to sort CD4+ Tn and Tcm cells according to their glucose (2-NBDG) uptake levels and the 2-NBDG content of the CD4+ T cell fractions after sorting. Representations are displayed as standard pseudocolor dot plots. Sorted cells were used for HIV-1 susceptibility assays. B) Representative example (left) and summary (median and IQR, n=3 donors) (right) of the glycolytic activity of sorted Tcm cells with high glucose uptake (TcmHGlu) and low glucose uptake (TcmLGlu).

Figure S9

Representative example of the metabolic profile of five-day activation CD4+ T cells after being treated with increased concentrations of the metabolic inhibitors Etomoxir (top panel) and 2-DG (middle panel). OCR/ECAR basal ratio after the administration of 2-DG and Etomoxir (Lower panel).

Figure S10

A representative example of GFP and mCherry expression in noninfected CD4+ T cells or in CD4+ T cells 72 h post infection with the HIV Duo-Fluo I virus. As described in (Calvanese et al., 2013; Chavez et al., 2015), cells that expressed GFP alone or in combination with mCherry were considered productively infected (I), cells that expressed only mCherry were considered latently infected (L), and cells that lacked expression of both fluorescent markers were considered noninfected (NI). Pie chart diagrams show the median (n=5 donors) contribution of CD4+ T cell subsets to the pool of HIV-1 productively infected (upper panel) or latently infected cells (lower panel) 72 h after infection of nonactivated (NA), 3-day activation (3d) or 5-day activation (5d) CD4+ T cells challenged with the HIV-1 DuoFluo I virus.

Figure S11

A) Relative infection levels (with respect to naive cells) in 5 days-activated CD4+ T cells from donors (n=9) 72h after challenge with HIV Bal or VSVG pseudotyped NL4.3ΔenvGFP particles. Infection levels were determined by flow cytometry quantification of intracellular p24 and GFP respectively (top panels). Changes in the distribution of cell subsets in HIV+ cells in relation to non-infected cells (bottom panels). The asterisks represent statistically significant differences (*p<0.05; ** p<0.01; *** p<0.001). B) Infection levels in 5 days-activated CD4+ T cells 72 h post challenge with HIV-1 Bal in the absence or presence of 2-DG added at the time of challenge, 4h or 8h post challenge. Blue bars represent the relative level of infection (determined by intracellular p24 staining) compared to the control conditions (median and IQR, n=3 donors). Horizontal lines indicate statistical significant differences.

Table S1

Gene symbols, RefSeq, full gene names and primers used in the transcriptomic q-PCR analysis

Table S2

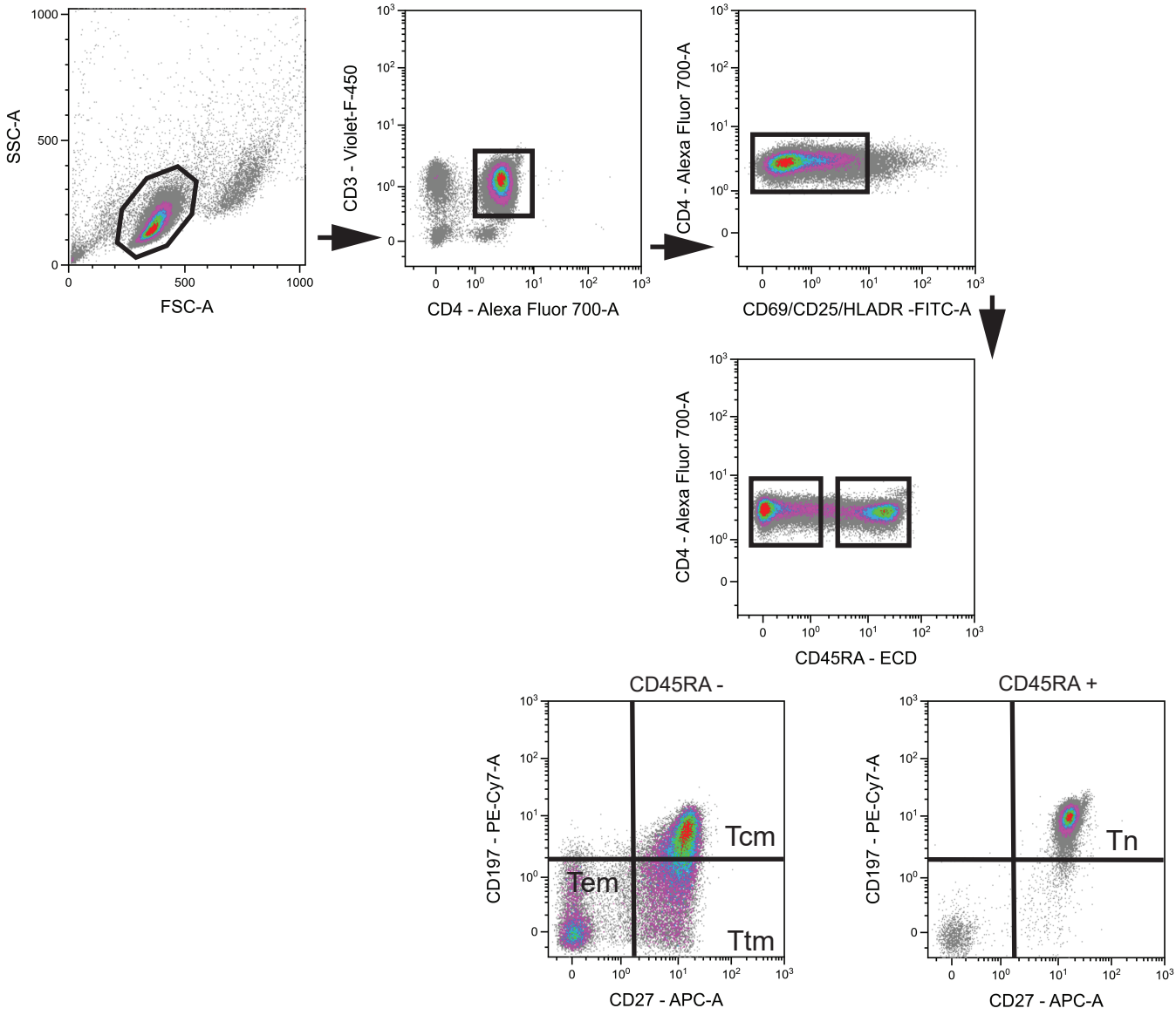
Clinical characteristics of HIV infected individuals whose CD4+ T cells were used in the study.

Table S3

Ranking of studied genes after analysis with the Normfinder algorithm (Aarhus University Hospital, Denmark). BECN1 was the most stable gene present in our panel and was used to normalize our RT-qPCR data.

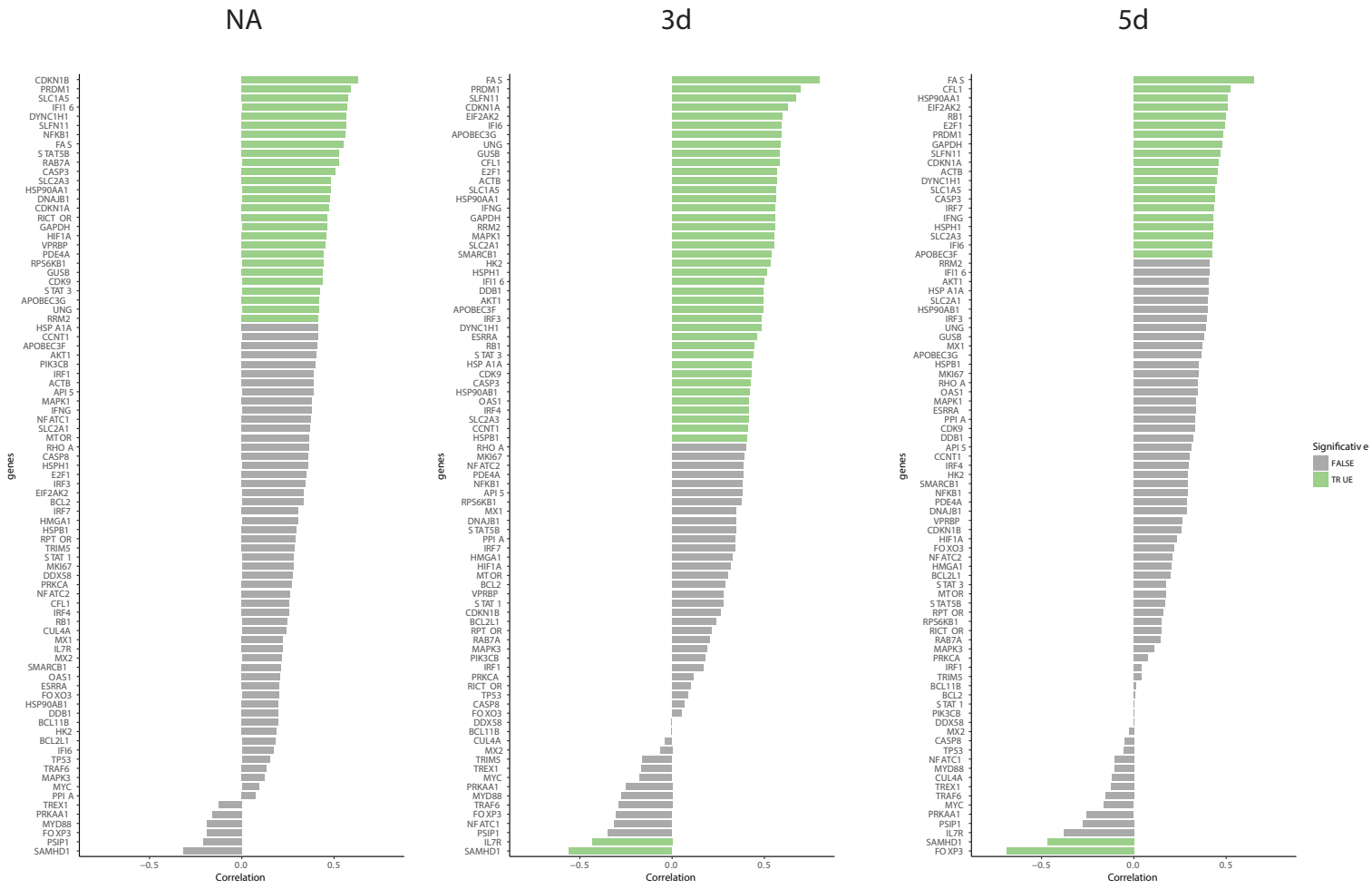
Calvanese, V., Chavez, L., Laurent, T., Ding, S., and Verdin, E. (2013). Dual-color HIV reporters trace a population of latently infected cells and enable their purification. Virology *446*, 283-292.

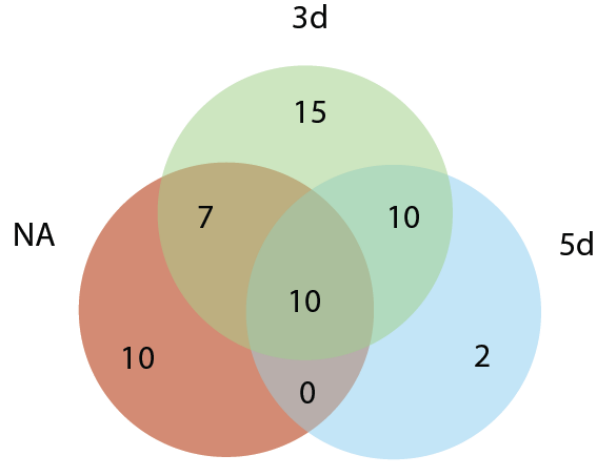
Chavez, L., Calvanese, V., and Verdin, E. (2015). HIV Latency Is Established Directly and Early in Both Resting and Activated Primary CD4 T Cells. PLoS Pathog *11*, e1004955.



EFS#	459	466	469	470	471	472
Gender	Female	Male	Female	Female	Male	Male
Age (years)	24	18	28	51	52	36
% Tn	41.7	50.7	37	41	43.5	15.5
% Tcm	21.8	17.2	20	24.1	18.9	30.2
% Ttm	15.9	16.8	18.3	14.7	13.4	28.5
% Tem	6.89	3.04	8.11	4.79	3.42	12.9
% Others	13.71	12.26	16.59	15.41	20.78	12.9

Figure S1





NA	3d	5d	NA & 3d	3d & 5d	NA & 5d	NA & 3d & 5d
RRM2	SAMHD1	FOXP3	RRM2	SAMHD1	GAPDH	GAPDH
UNG	IL7R	SAMHD1	UNG	SLC2A3	CDKN1A	CDKN1A
APOBEC3G	HSPB1	APOBEC3F	APOBEC3G	CASP3	HSP90AA1	HSP90AA1
STAT3	CCNT1	IFI6	STAT3	RB1	SLC2A3	SLC2A3
CDK9	SLC2A3	SLC2A3	CDK9	DYNC1H1	CASP3	CASP3
GUSB	IRF4	HSPH1	GUSB	APOBEC3F	FAS	FAS
RPS6KB1	OAS1	IFNG	GAPDH	HSPH1	SLFN11	SLFN11
PDE4A	HSP90AB1	IRF7	CDKN1A	GAPDH	DYNC1H1	DYNC1H1
VPRBP	CASP3	CASP3	HSP90AA1	IFNG	SLC1A5	SLC1A5
HIF1A	CDK9	SLC1A5	SLC2A3	HSP90AA1	PRDM1	PRDM1
GAPDH	HSPA1A	DYNC1H1	CASP3	SLC1A5		
RICTOR	STAT3	ACTB	FAS	ACTB		
CDKN1A	RB1	CDKN1A	SLFN11	E2F1		
DJB1	ESRRA	SLFN11	DYNC1H1	CFL1		
HSP90AA1	DYNC1H1	GAPDH	IFI16	IFI6		
SLC2A3	IRF3	PRDM1	SLC1A5	EIF2AK2		
CASP3	APOBEC3F	E2F1	PRDM1	CDKN1A		
RAB7A	AKT1	RB1		SLFN11		
STAT5B	DDB1	EIF2AK2		PRDM1		
FAS	IFI16	HSP90AA1		FAS		
NFKB1	HSPH1	CFL1				
SLFN11	HK2	FAS				
DYNC1H1	SMARCB1					
IFI16	SLC2A1					
SLC1A5	MAPK1					
PRDM1	RRM2					
CDKN1B	GAPDH					
	IFNG					
	HSP90AA1					
	SLC1A5					
	ACTB					
	E2F1					
	CFL1					
	GUSB					
	UNG					
	APOBEC3G					
	IFI6					
	EIF2AK2					
	CDKN1A					
	SLFN11					
	PRDM1					
	FAS					
27	42	22	17	20	10	10

Figure S2

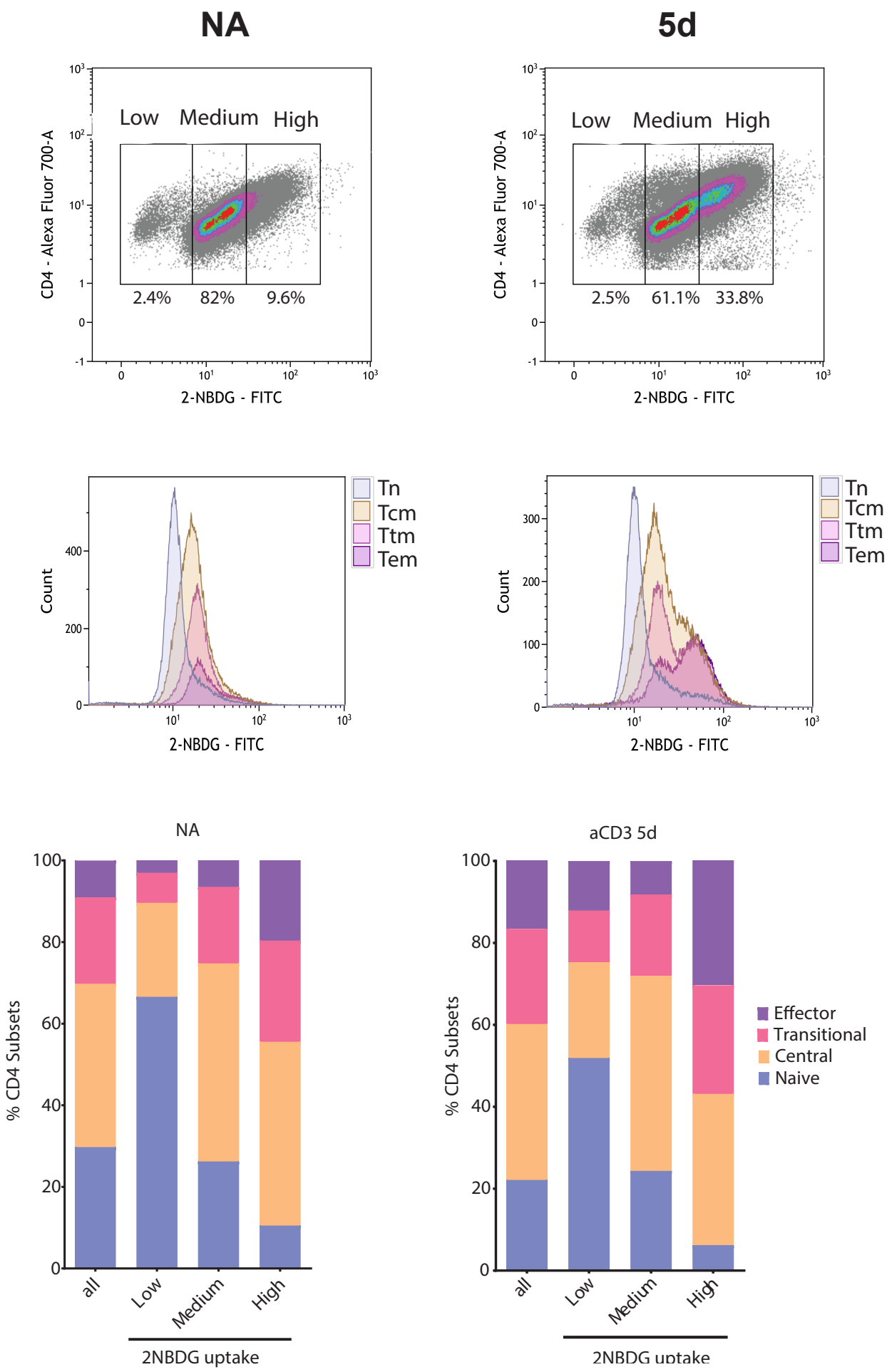

NA
5d
Low
Medium
High
2.4%
82%
9.6%
2.5%
61.1%
33.8%
CD4 - Alexa Fluor 700-A
2-NBDG - FITC
Count
Tn
Tcm
Ttm
Tem
NA
aCD3 5d
% CD4 Subsets
all
Low
Medium
High
2NBDG uptake
Effector
Transitional
Central
Naive

Figure S3

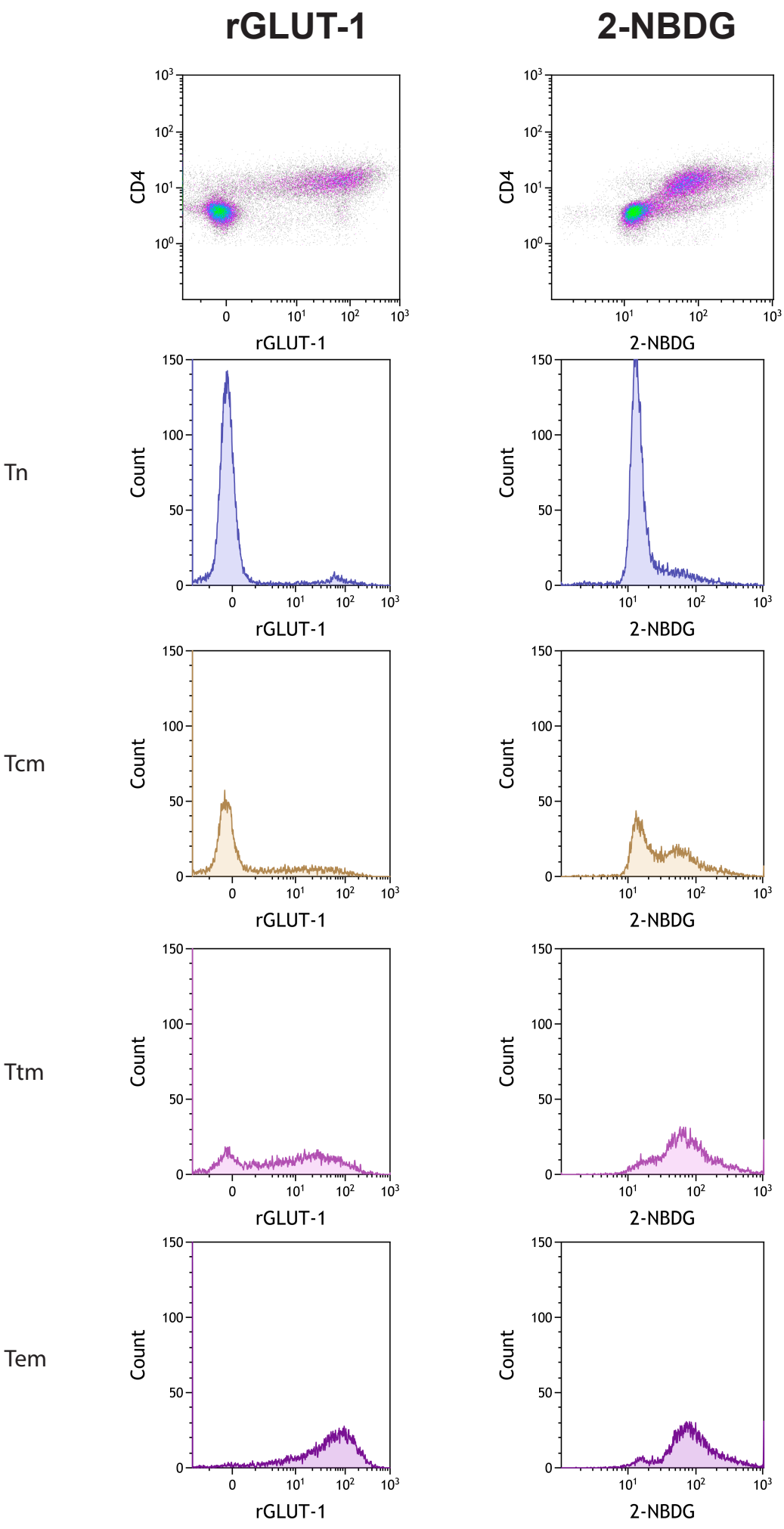


Figure S4

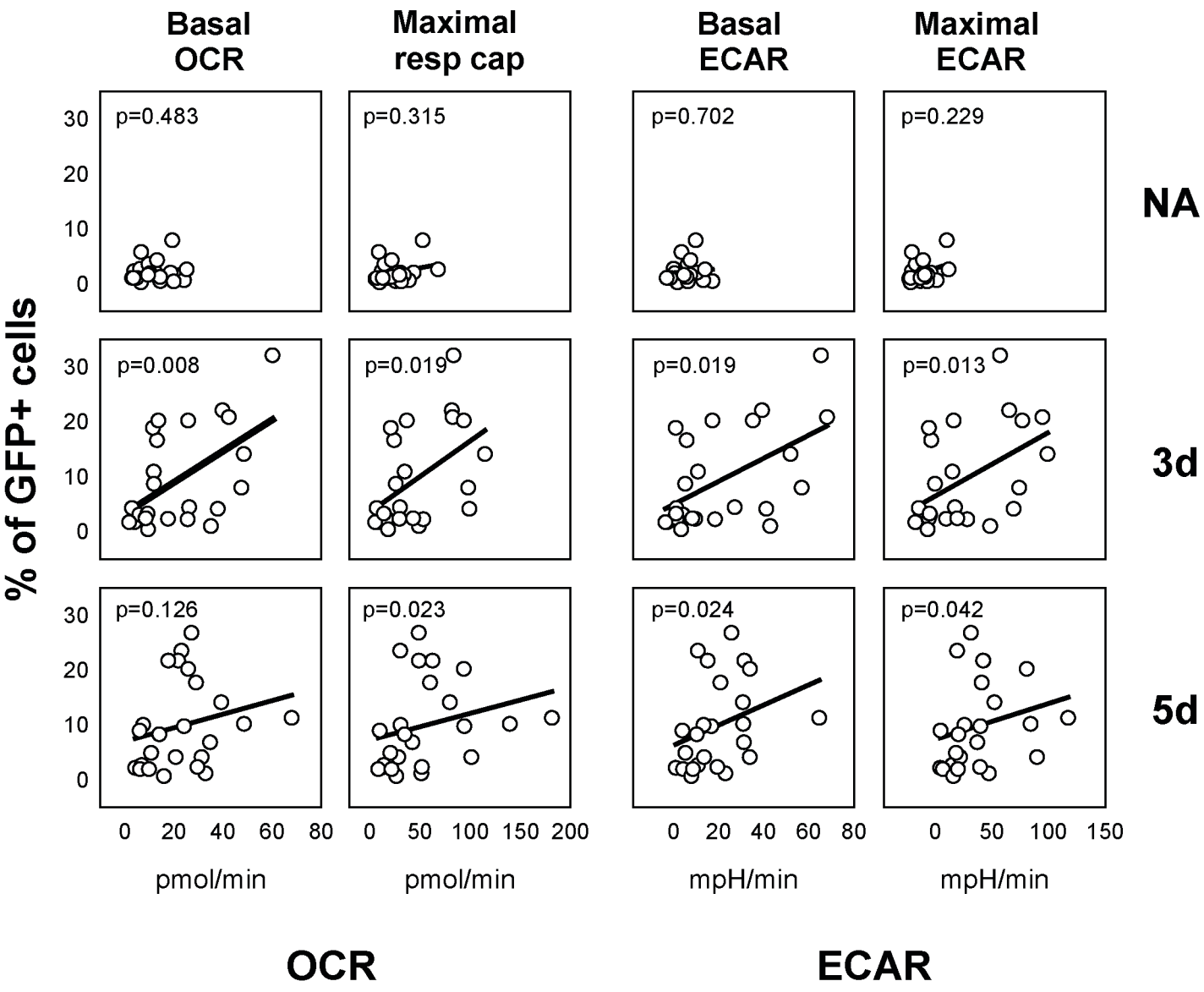
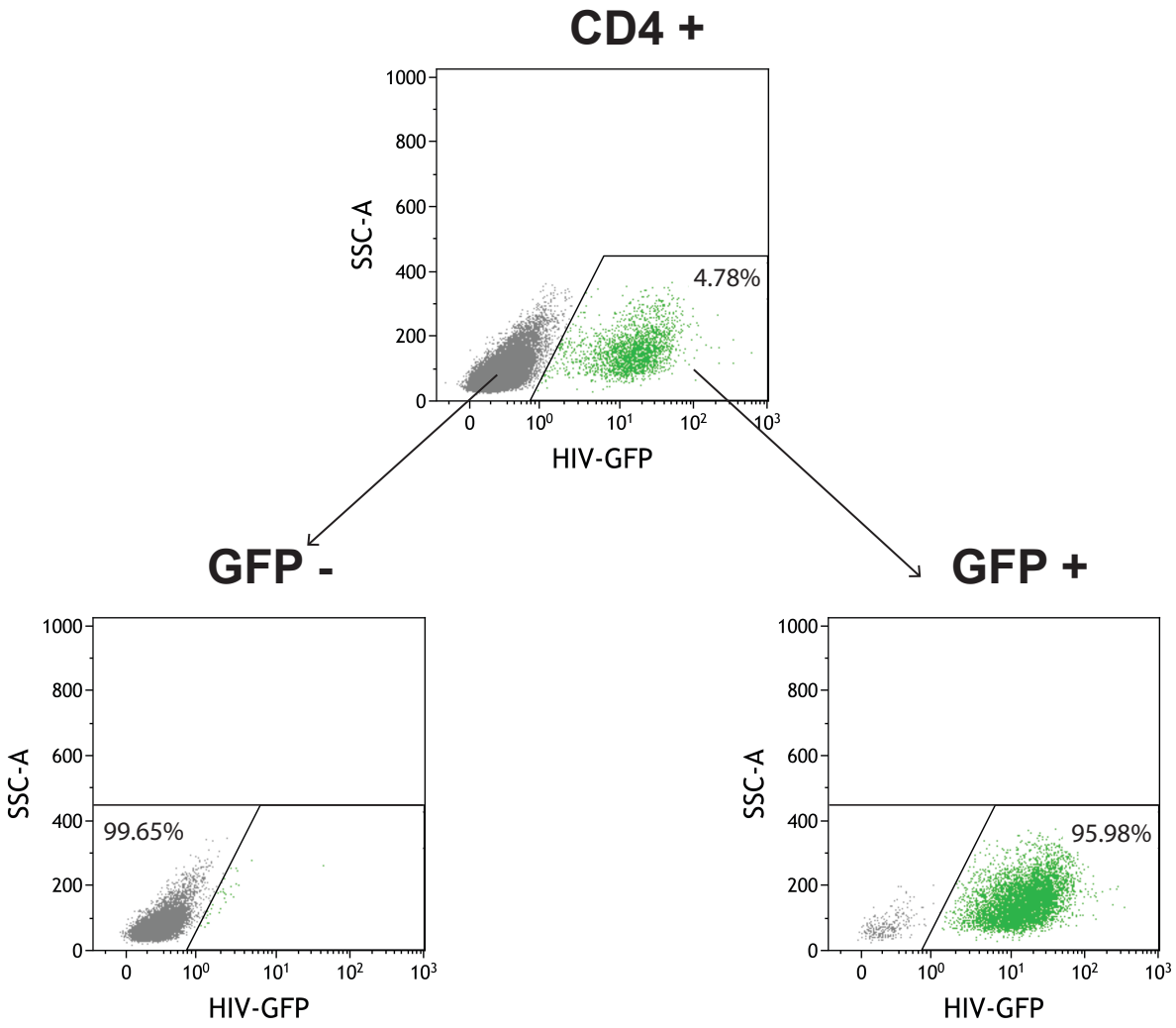

Basal OCR
Maximal resp cap
Basal ECAR
Maximal ECAR
% of GFP+ cells
NA
3d
5d
p=0.483
p=0.315
p=0.702
p=0.229
p=0.008
p=0.019
p=0.019
p=0.013
p=0.126
p=0.023
p=0.024
p=0.042
0 20 40 60 80
0 50 100 150 200
0 20 40 60 80
0 50 100 150
pmol/min
pmol/min
mpH/min
mpH/min
OCR
ECAR

Figure S5


CD4 +
SSC-A
HIV-GFP
4.78%
GFP -
99.65%
GFP +
95.98%

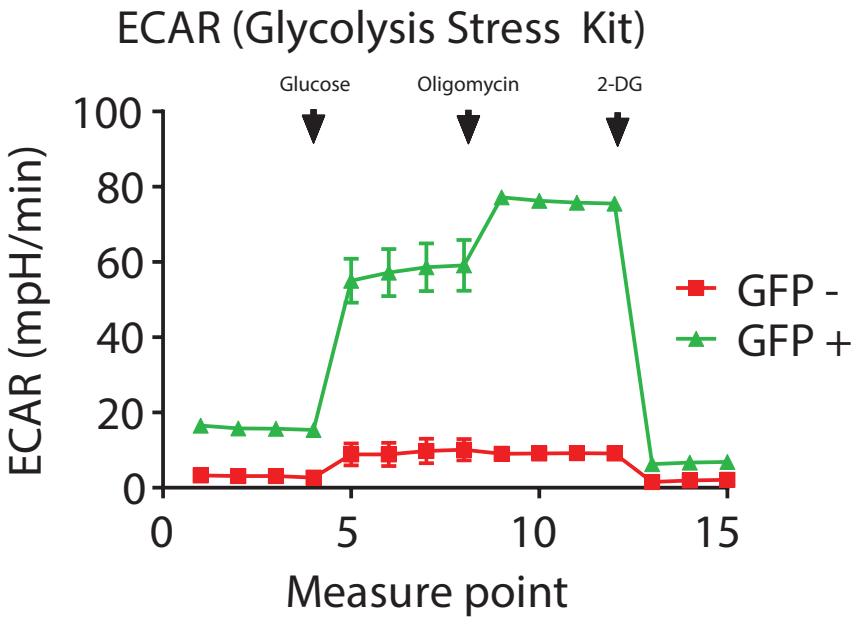

ECAR (Glycolysis Stress Kit)
Glucose
Oligomycin
2-DG
ECAR (mpH/min)
Measure point
GFP -
GFP +

Figure S6

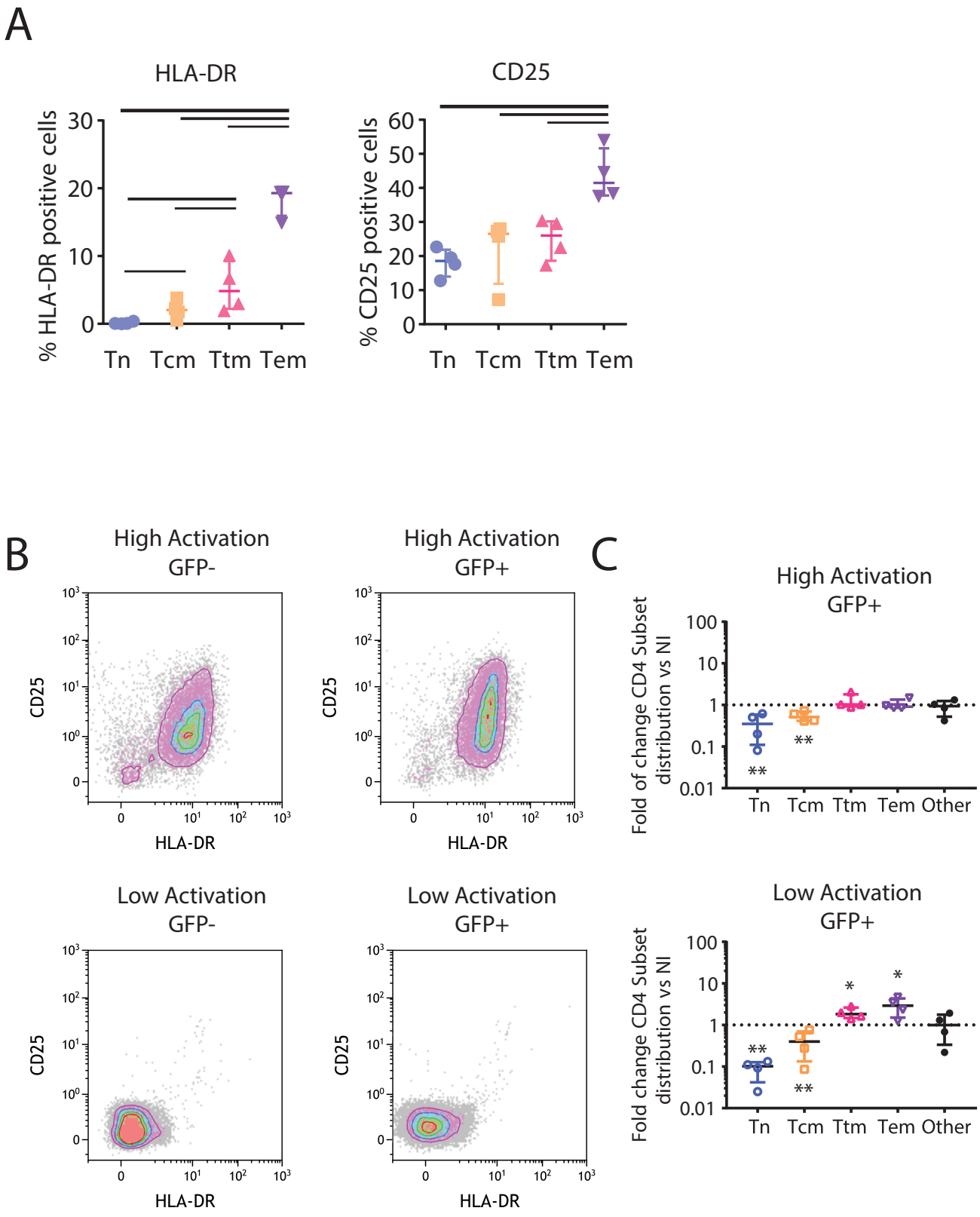
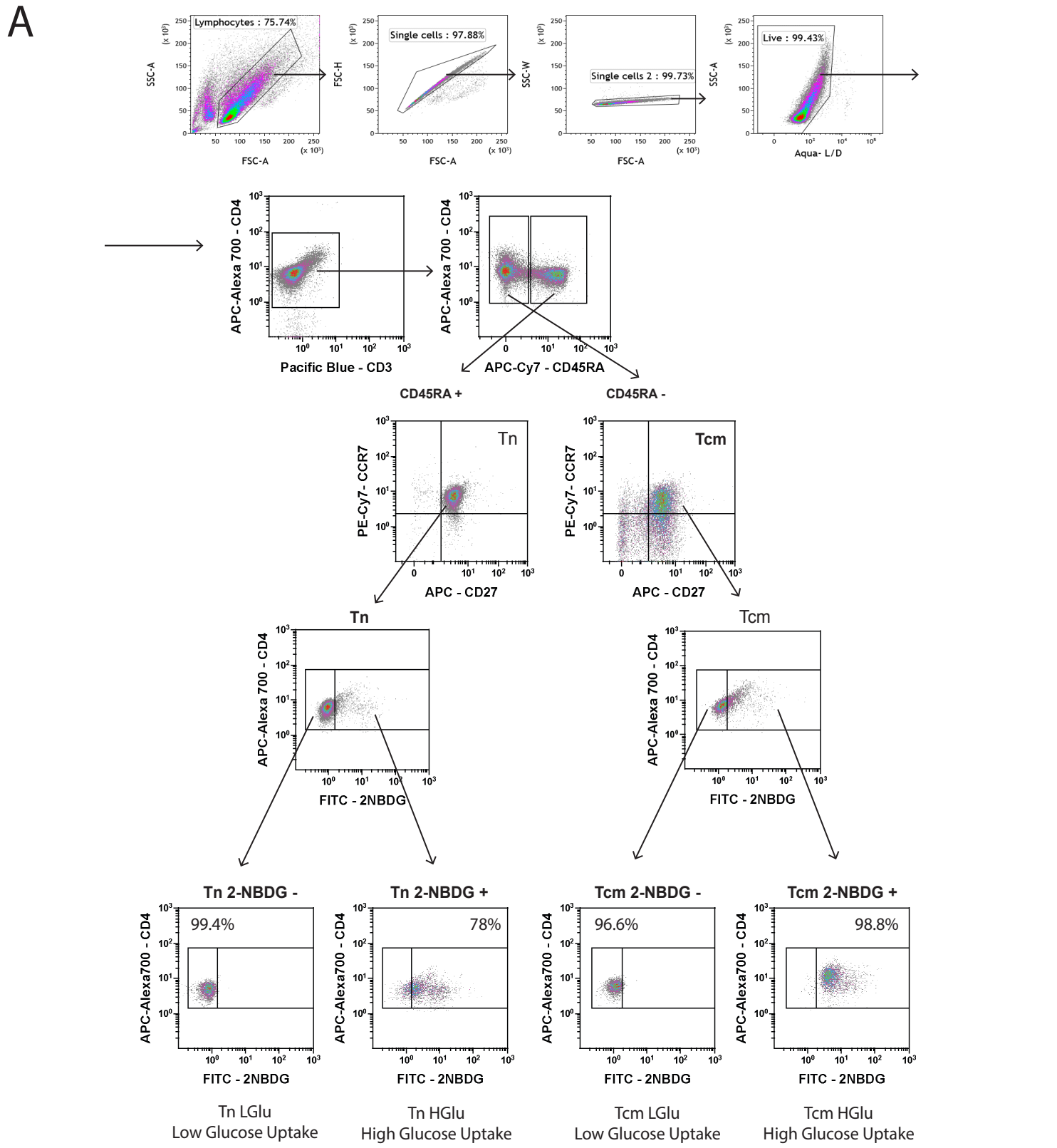

A
HLA-DR
% HLA-DR positive cells
30
20
10
0
Tn
Tcm
Ttm
Tem
CD25
% CD25 positive cells
60
50
40
30
20
10
0
Tn
Tcm
Ttm
Tem
B
High Activation
GFP-
High Activation
GFP+
CD25
HLA-DR
Low Activation
GFP-
Low Activation
GFP+
C
High Activation
GFP+
Fold of change CD4 Subset distribution vs NI
100
10
1
0.1
0.01
**
**
Tn
Tcm
Ttm
Tem
Other
Low Activation
GFP+
Fold change CD4 Subset distribution vs NI
*
*
**
**
Tn
Tcm
Ttm
Tem
Other

Figure S7


A
Lymphocytes : 75.74%
SSC-A
FSC-A
Single cells : 97.88%
FSC-H
FSC-A
Single cells 2 : 99.73%
SSC-W
FSC-A
Live : 99.43%
SSC-A
Aqua- L/D
APC-Alexa 700 - CD4
Pacific Blue - CD3
APC-Alexa 700 - CD4
APC-Cy7 - CD45RA
CD45RA +
CD45RA -
PE-Cy7- CCR7
APC - CD27
Tn
PE-Cy7- CCR7
APC - CD27
Tcm
Tn
APC-Alexa 700 - CD4
FITC - 2NBDG
Tcm
APC-Alexa 700 - CD4
FITC - 2NBDG
Tn 2-NBDG -
99.4%
APC-Alexa700 - CD4
FITC - 2NBDG
Tn 2-NBDG +
78%
APC-Alexa700 - CD4
FITC - 2NBDG
Tcm 2-NBDG -
96.6%
APC-Alexa700 - CD4
FITC - 2NBDG
Tcm 2-NBDG +
98.8%
APC-Alexa700 - CD4
FITC - 2NBDG
Tn LGlu
Low Glucose Uptake
Tn HGlu
High Glucose Uptake
Tcm LGlu
Low Glucose Uptake
Tcm HGlu
High Glucose Uptake

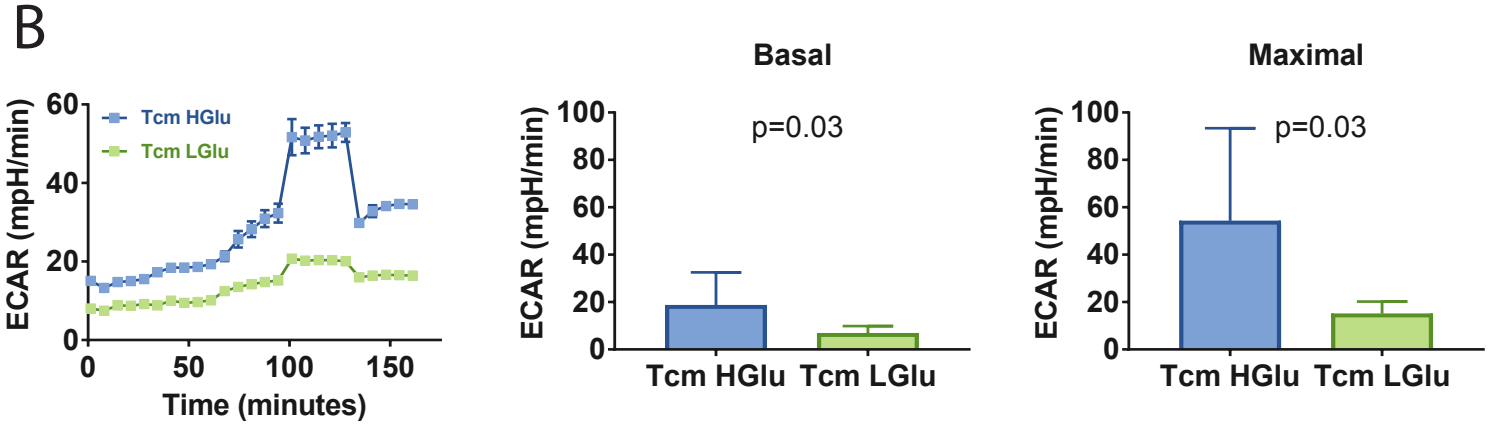
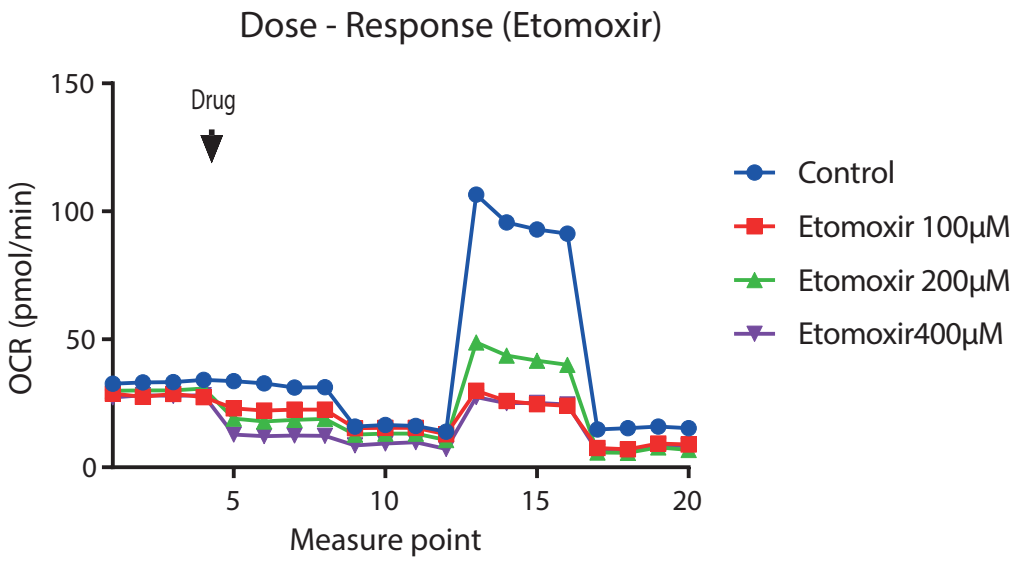
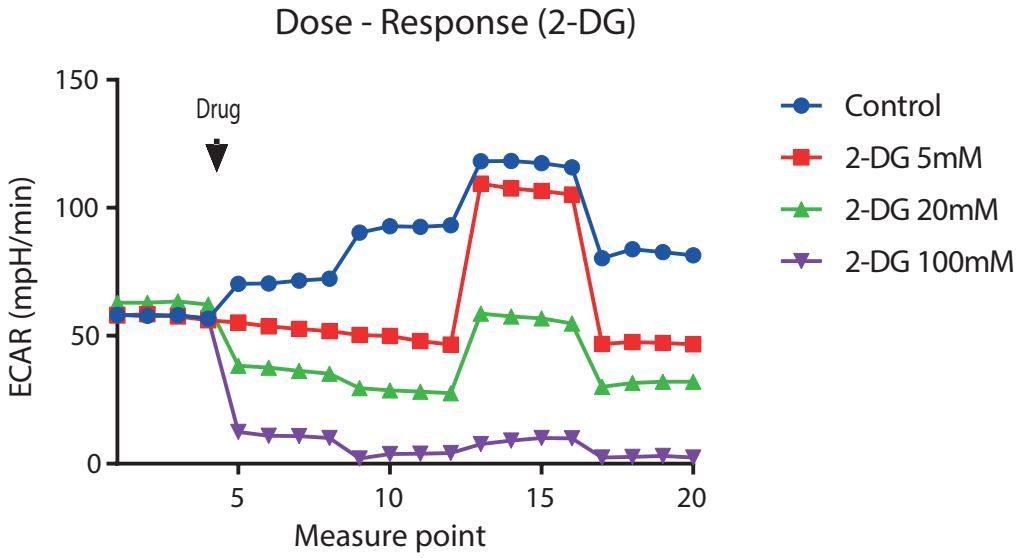

B
Tcm HGlu
Tcm LGlu
ECAR (mpH/min)
Time (minutes)
Basal
p=0.03
ECAR (mpH/min)
Tcm HGlu
Tcm LGlu
Maximal
p=0.03
ECAR (mpH/min)
Tcm HGlu
Tcm LGlu

Figure S8


Dose - Response (Etomoxir)
Drug
OCR (pmol/min)
150
100
50
0
5
10
15
20
Measure point
Control
Etomoxir 100µM
Etomoxir 200µM
Etomoxir400µM


Dose - Response (2-DG)
Drug
ECAR (mpH/min)
150
100
50
0
5
10
15
20
Measure point
Control
2-DG 5mM
2-DG 20mM
2-DG 100mM

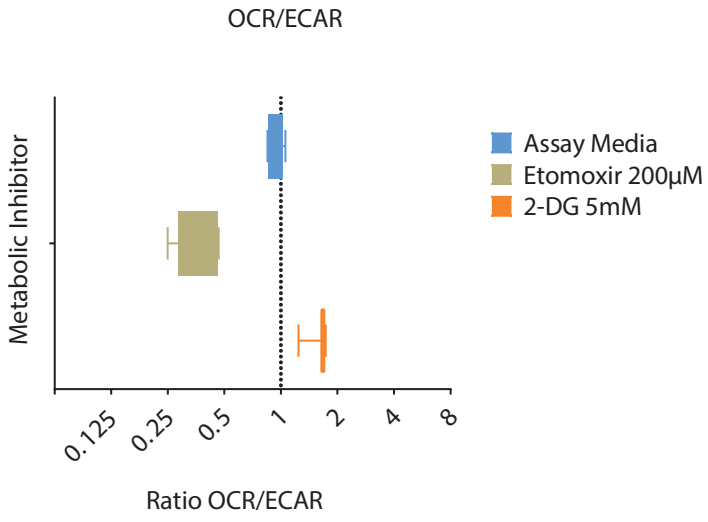
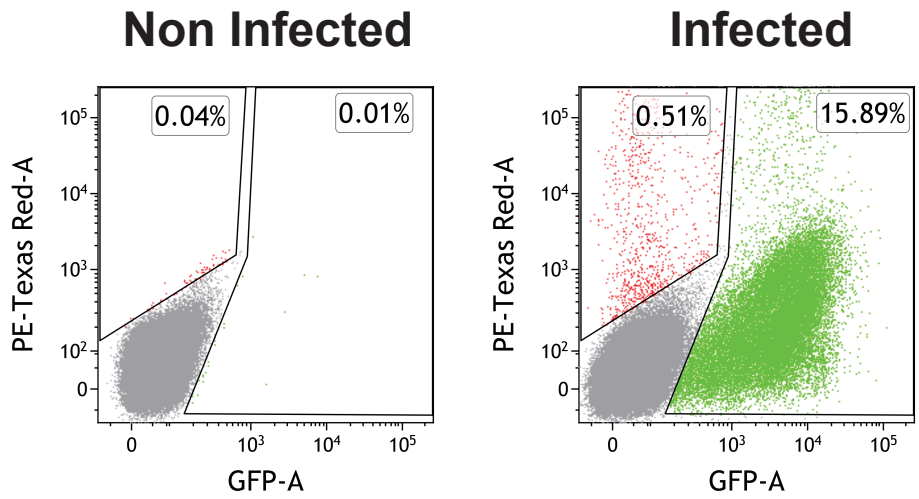

OCR/ECAR
Metabolic Inhibitor
0.125
0.25
0.5
1
2
4
8
Ratio OCR/ECAR
Assay Media
Etomoxir 200µM
2-DG 5mM

Figure S9

A



B

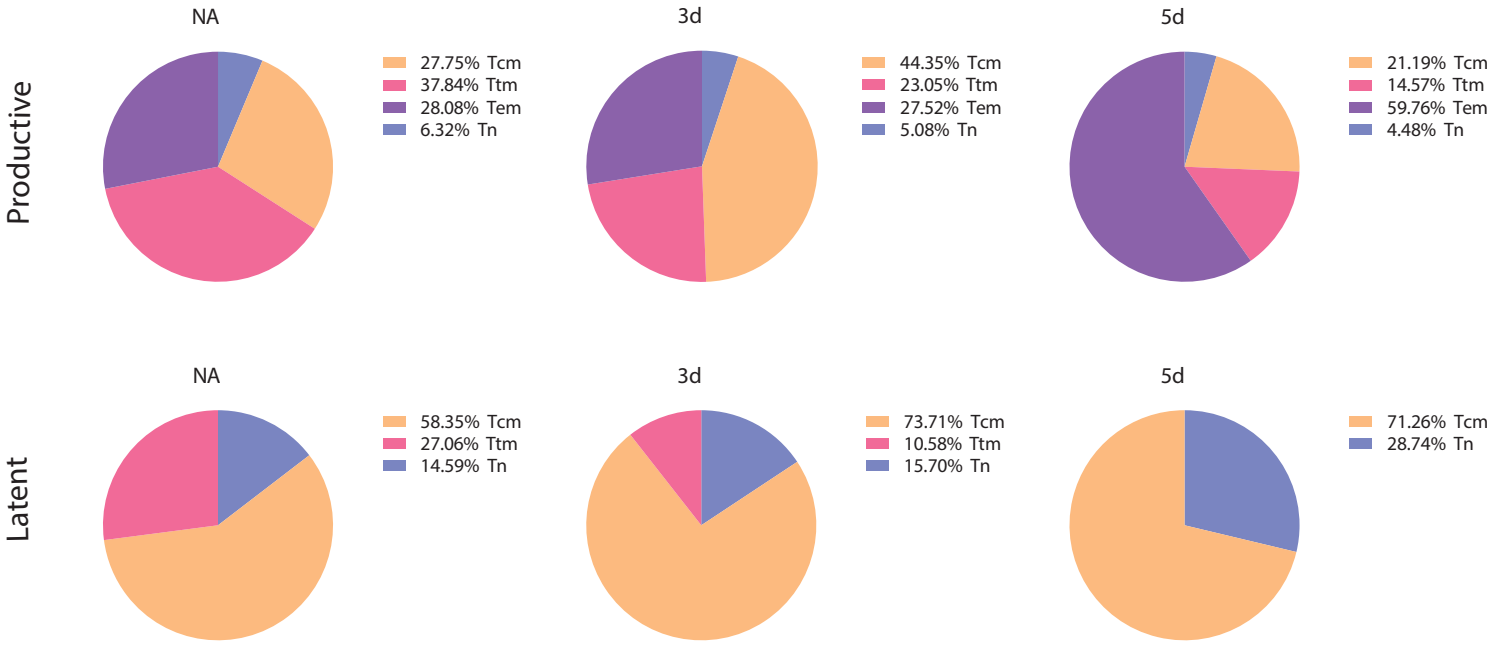
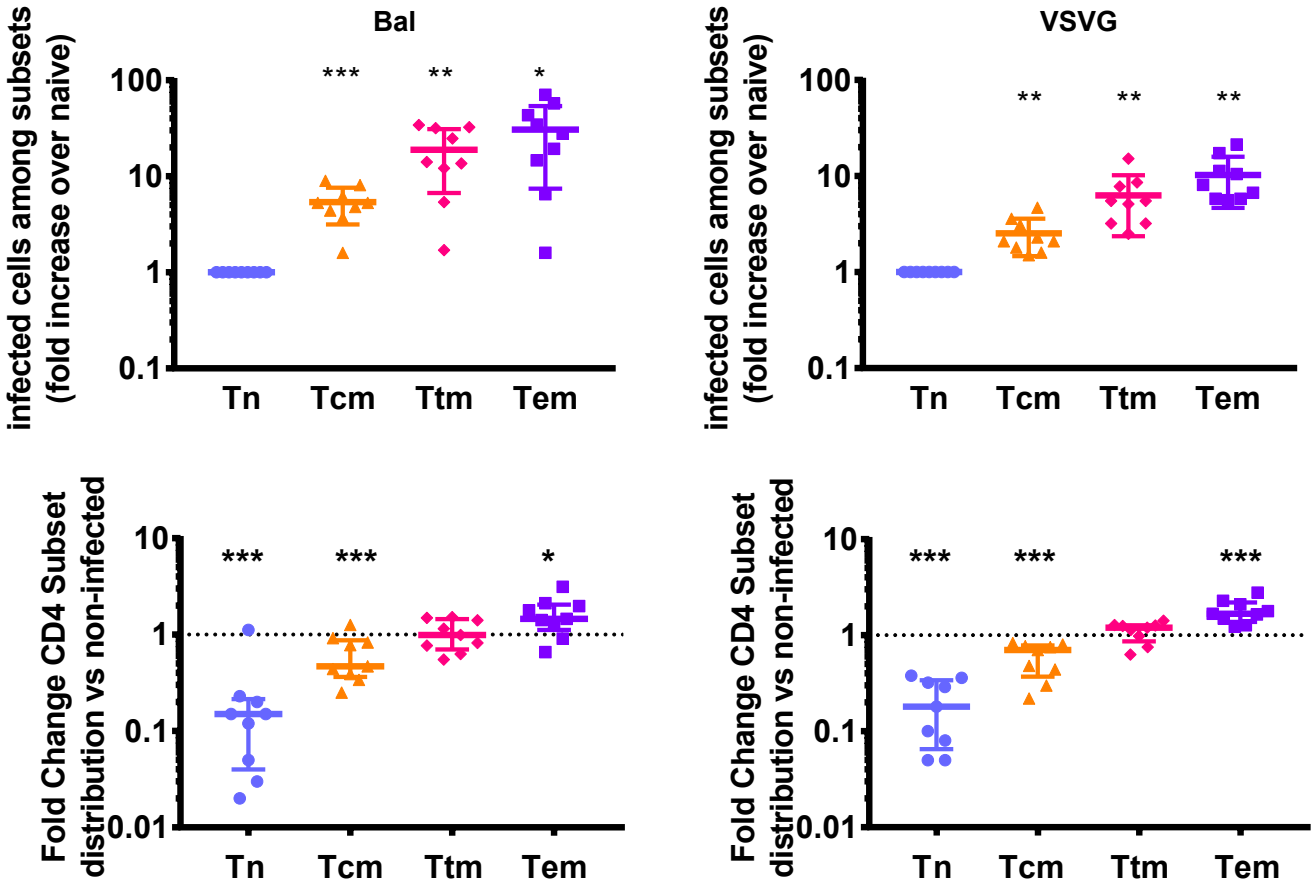


Figure S10

A



B

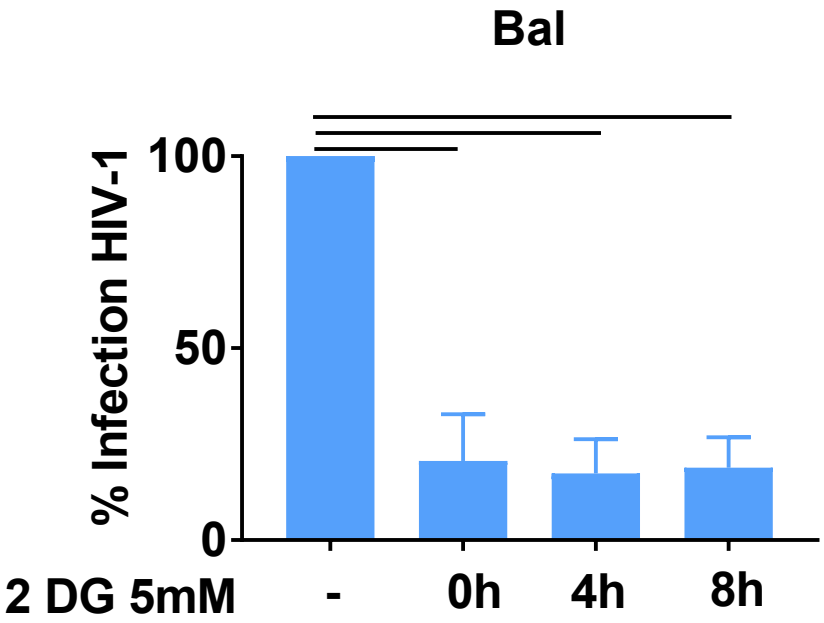


Figure S11

Gene	Design RefSeq	FP	RP	Gene Full Name
ACTB	NM_001101.3	CCAACCGCGAGAAGATGAC	TAGCACAGCCTGGATAGCAA	actin, beta
AKT1	NM_005163.2	CACACACTCACCGAGAACC	TCGTGGGTCTGGAAAGAGTA	v-akt murine thymoma viral oncogene homolog 1
API5	NM_006595.3	AGTTGGGCCGAAAACTTCCA	AGGCCCCGTGCAAAGTAC	apoptosis inhibitor 5
BCL11B	NM_138576.2	CAACCCGCAGCACTTGTC	CCTCGTCTTCTTCGAGGATGG	B-cell CLL/lymphoma 11B (zinc finger protein)
BCL2	NM_000633.2	ATGTGTGTGGAGAGCGTCAA	GTGCCGGTTCAGGTACTCA	B-cell CLL/lymphoma 2
BCL2L1	NM_138578.1	CTGCCTGCCTTTGCCTAA	CCAAAACACCTGCTCACTCA	BCL2-like 1
BECN1	NM_003766.3	GGTTGAGAAAGGCGAGACAC	ACTGCCTCCTGTGTCTTCAA	beclin 1, autophagy related
CASP3	NM_004346.3	AGGCCGACTTCTTGTATGCA	AACCAGGAGCCATCCTTTGAA	caspase 3, apoptosis-related cysteine peptidase
CASP8	NM_001080124.1	GGAAATCTCCAAATGCAAACTGG	CAGGATGACCCTCTTCTCCAT	caspase 8, apoptosis-related cysteine peptidase
CCNT1	NM_001240.3	GATTCAGTCCTTCACACAGTTCC	TTTTGGGCTGCTCCTCCA	cyclin T1
CDK9	NM_001261.3	GCCAGAAGGTGGCTCTGAA	GATCTCCCGCAAGGCTGTAA	cyclin-dependent kinase 9
CDKN1A	NM_078467.1	TGGAGACTCTCAGGGTCGAAAA	CGGCGTTTGGAGTGGTAGAA	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
CDKN1B	NM_004064.3	GCAATGCGCAGGAATAAGGAA	TTGGGGAACCGTCTGAAACA	cyclin-dependent kinase inhibitor 1B (p27, Kip1)
CFL1	NM_005507.2	GCTCCAAGGACGCCATCA	TCCTTGACCTCCTCGTAGCA	cofilin 1 (non-muscle)
CUL4A	NM_003589.2	TGCCGAAGGCCAAAGGTTAA	CTCCCTCTTCCTCTAAGCGTTTAC	cullin 4A
DDB1	NM_001923.3	TTGCCTACAAGCCCATGGAA	CAAGATTTCCACAGCACTCATCC	damage-specific DNA binding protein 1, 127kDa
DDX58	NM_014314.3	GACTGGACGTGGCAAAACA	CTCCACTGGCTTTGAATGCA	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58
DNAJB1	NM_006145.1	AAGGAGATCGCTGAGGCCTA	TGGGGCCACTCCCCTTTA	DnaJ (Hsp40) homolog, subfamily B, member 1
DYNC1H1	NM_001376.4	GTTCCGCTGGTGCTGTTTAA	CAAGTGGCCTTGAGGTTGAC	dynein, cytoplasmic 1, heavy chain 1
E2F1	NM_005225.2	AGCTCATTGCCAAGAAGTCCAA	TCCTGGGTCAACCCCTCAA	E2F transcription factor 1
EIF2AK2	NM_002759.2	TGCCTAATTCAGGACCTCCA	TCACCTTCTGGAAATTCTCTTCC	eukaryotic translation initiation factor 2-alpha kinase 2
ESRRA	NM_004451.3	GGAGCGAGAGGAGTATGTTCTAC	CTTCTCGCAGCTGCTCCA	estrogen-related receptor alpha
FAS	NM_000043.4	GCATCTGGACCCTCCTACC	CCTTGGAGTTGATGTCAGTCAC	Fas cell surface death receptor
FOXO3	NM_001455.3	CGCTCTCTCCGCTCGAA	TTTGCAGGGGCCACGTA	forkhead box O3
FOXP3	NM_014009.3	TGTGGGGTAGCCATGGAAA	GGGTCGCATGTTGTGGAA	forkhead box P3
GAPDH	NM_002046.4	GAACGGGAAGCTTGTCATCAA	ATCGCCCCACTTGATTTTGG	glyceraldehyde-3-phosphate dehydrogenase
HIF1A	NM_001530.3	CAGTCGACACAGCCTGGATA	TTCTTCTGGCTCATATCCCATCAA	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)
HK2	NM_000189.4	CTGCTGAAGGAAGCGATCCA	TAGTTCCGACTGTGTCGTTCAC	hexokinase 2
HMGA1	NM_002131.3	CCGACCAAAGGGAAGCAAAA	GTCTGCCCCTTGGTTTCC	high mobility group AT-hook 1
HSP90AA1	NM_001017963.2	ACTGCACCAGAATGAAGGAGAA	CCATGTTTCCGAAGACGTTCC	heat shock protein 90kDa alpha (cytosolic), class A member 1
HSP90AB1	NM_007355.2	TCTCGCATGAAGGAGACACA	CGCACTCGCTCCACAAAA	heat shock protein 90kDa alpha (cytosolic), class B member 1
HSPA1A	NM_005345.5	AGGCTTCCCAGAGCGAAC	GAGAAGAGCTCGGTCCTTCC	heat shock 70kDa protein 1A
HSPA1L	NM_005527.3	TGCGTAATCTGGACGTTTCCA	GGCGATTCCCTTGGCAGTA	heat shock 70kDa protein 1-like
HSPB1	NM_001540.3	AGCTGACGGTCAAGACCAA	GTGTATTTCCGCGTGAAGCA	heat shock 27kDa protein 1
HSPH1	NM_006644.2	TCGGAGTTGCAGCCAAAA	TGAATGCTCGGCCATGAAA	heat shock 105kDa/110kDa protein 1
IFI16	NM_005531.2	GTGAATGGGGTGTTTGAGGTAC	CACCACTTCCATCTTCCCTGTA	interferon, gamma-inducible protein 16
IFI6	NM_002038.3	TGCTACCTGCTGCTCTTCA	TCAGGGCCTTCCAGAACC	interferon, alpha-inducible protein 6
IFNA1	NM_024013.2	TGACTCATACACCAGGTCAC	CAGGGGTGAGAGTCTTTGAA	interferon, alpha 1
IFNB1	NM_002176.2	ATGAGCAGTCTGCACCTGAA	GACTGTACTCCTTGGCCTTCA	interferon, beta 1, fibroblast
IFNG	NM_000619.2	ACTGCCAGGACCCATATGTAA	GTTCCATTATCCGCTACATCTGAA	interferon, gamma
IL7R	NM_002185.2	GGAGAAAGTGGCTATGCTCAA	CTGCGATCCATTCACTTCCA	interleukin 7 receptor
IRF1	NM_002198.2	AACAAGGATGCCTGTTTGTTCC	TGGGATCTGGCTCCTTTTCC	interferon regulatory factor 1
IRF3	NM_001197123.1	ACCAATGGTGGAGGCAGTAC	TGGGGCCAACACCATGTTA	interferon regulatory factor 3
IRF4	NM_002460.3	CACCATGACAACGCCTTACC	CGAGGGGTGGCATCATGTA	interferon regulatory factor 4
IRF7	NM_004031.2	GGCAGAGCCGTACCTGTCA	ACCGTGCGGCCCTTGTA	interferon regulatory factor 7
KLF2	NM_016270.2	ATCCTGCCGTCCTTCTCCA	CCATGGACAGGATGAAGTCC	Kruppel-like factor 2
MAPK1	NM_002745.4	TTGGTACAGGGCTCCAGAAA	TCTGCCAGAATGCAGCCTA	mitogen-activated protein kinase 1
MAPK3	NM_001040056.1	GCTACACGCAGTTGCAGTACA	GTCTTGCGCACGTGGTCATA	mitogen-activated protein kinase 3
MKI67	NM_001145966.1	AGAGTAACGCGGAGTGTCA	CTTGACACACACATTGTCCTCA	marker of proliferation Ki-67
MTOR	NM_004958.3	CCAAACCCAGGTGTGATCAA	TCCTCATTTCCAGGCCACTA	mechanistic target of rapamycin (serine/threonine kinase)
MX1	NM_001144925.1	ATGCTACTGTGGCCCAGAAA	GGCGCACCTTCTCCTCATA	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)
MX2	NM_002463.1	CGACTGGCAGAAAGACTTACC	CTTCTGGTGGCTCTCCCTTA	myxovirus (influenza virus) resistance 2 (mouse)
MYC	NM_002467.4	CCTGGTGCTCCATGAGGA	CCTGCCTCTTTTCCACAGAAA	v-myc avian myelocytomatosis viral oncogene homolog
MYD88	NM_001172567.1	CTGCAGAGCAAGGAATGTGAC	TGCTGGGGAACTCTTTCTTCA	myeloid differentiation primary response 88
NFATC1	NM_172390.1	TCCTCTCCAACACCAAAGTCC	AGGATTCCGGCACAGTCAA	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1
NFATC2	NM_012340.3	TGGAAGCCACGGTGGATAA	TGTGCGGATATGCTTGTTCC	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2
NFKB1	NM_001165412.1	CTACCTGGTGCCTCTAGTGAAA	ACCTTTGCTGGTCCCACATA	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
OAS1	NM_016816.2	TACCCTGTGTGTGTCCAA	AGAGGACTGAGGAAGACAACC	2'-5'-oligoadenylate synthetase 1, 40/46kDa
PDE4A	NM_001111307.1	ATCACCCTGGGGTCTCCAA	GAGCACCGACTCATCGTTGTA	phosphodiesterase 4A, cAMP-specific
PIK3CB	NM_006219.1	GCCCTTCTGAACTGGCTTAA	CCAGCACAGGACAGTGTAAA	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit beta
PML	NM_033238.2	CCAACAACATCTTCTGCTCCA	TTGGAACATCCTCGGCAGTA	promyelocytic leukemia
PPIA	NM_021130.3	TCGTGCCGTTTTGCAGAC	ACGGCAATGTCGAAGAACAC	peptidylprolyl isomerase A (cyclophilin A)
PRDM1	NM_001198.3	CCTGGTACACACGGGAGAAAA	TTGAGATTGCTGGTGCTGCTA	PR domain containing 1, with ZNF domain
PRKAA1	NM_206907.3	CCAACTATGCTGCACCAGAA	AGAATAACCCCACTGCTCCA	protein kinase, AMP-activated, alpha 1 catalytic subunit
PRKCA	NM_002737.2	ACCATCCGCTCCACACTAAA	AGTCGTCGGTCTTTGTCTGAA	protein kinase C, alpha
PSIP1	NM_021144.3	GGTTTATGGGAGATAGATAACAATCCA	CATGGTCGGTATCTTCCTTTGAA	PC4 and SFRS1 interacting protein 1
RAB7A	NM_004637.5	GTGGATGACAGGCTAGTCACA	ACCTCTGTAGAAGGCCACAC	RAB7A, member RAS oncogene family
RB1	NM_000321.2	TGCGCTCTTGAGGTTGTAA	TCAGAATCCATGGGAAAGACAA	retinoblastoma 1
RHOA	NM_001664.2	GTGCCCACAGTGTTTGAGAA	TGTGTCCCACAAAGCCAAC	ras homolog family member A
RICTOR	NM_152756.3	CTTCCGTGTCGGAGGTTCATA	ACACAGCCTCTGCTTCTTCA	RPTOR independent companion of MTOR, complex 2
RPS6KB1	NM_003161.3	AGACCTGAAGCCGGAGAATA	AGTCCAAAGTCTGTTAGTTTCACA	ribosomal protein S6 kinase, 70kDa, polypeptide 1
RPTOR	NM_020761.2	GCTCAGAGCTGGAGGATGAA	AGGGTCCACACCAACATTCA	regulatory associated protein of MTOR, complex 1
RRM2	NM_001165931.1	GCAGCAAGCGATGGCATA	GAAACAGCGGGCTTCTGTAA	ribonucleotide reductase M2
SLC1A5	NM_001145145.1	CTCTACCACCTATGAAGAGAGGAA	CAGGATGTTCATCCCCTCCA	solute carrier family 1 (neutral amino acid transporter), member 5
SLC2A1	NM_006516.2	ATTGTGGGCATGTGCTTCC	AGAACCAGGAGCACAGTGAA	solute carrier family 2 (facilitated glucose transporter), member 1
SLFN11	NM_152270.3	TCCAAGAAAGGCCTGGAACA	TCTGAGATCAGGTCCCTCCA	schlafen family member 11
SMARCB1	NM_003073.3	CTCCACAACCATCAACAGGAAC	AGCTGGGTCATGGTCATCAA	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1
STAT1	NM_007315.3	ATGCTGGCACCAGAACGAA	GCTGGCACAATTGGGTTTCAA	signal transducer and activator of transcription 1, 91kDa
STAT3	NM_003150.3	GGAAATAATGGTGAAGGTGCTGAAC	CCGAGGTCAACTCCATGTCAAA	signal transducer and activator of transcription 3 (acute-phase response factor)
STAT5B	NM_012448.3	AACAGAGGTTGGTCCGAGAA	GTTTCTGGGACATGGCATCA	signal transducer and activator of transcription 5B
TLR3	NM_003265.2	TCTCATGTCCAACTCAATCCA	CAGCTGAACCTGAGTTCCTA	toll-like receptor 3
TLR7	NM_016562.3	TCTTCAACCAGACCTCTACATTCC	AGCCCCAAGGAGTTTGGAAA	toll-like receptor 7
TLR8	NM_138636.4	CTGCTGCAAGTTACGGAATGAA	GCGCATAACTCACAGGAACC	toll-like receptor 8
TLR9	NM_017442.3	TGCAACTGGCTGTTCCTGAA	ACAAGGAAAGGCTGGTGACA	toll-like receptor 9
TP53	NM_000546.5	GACTGTACCACCATCCACTACA	AAAGCTGTTCCGTCCCAGTA	tumor protein p53
TRAF6	NM_004620.2	TGGCAAATGTCATCTGTGAATAC	GCTGTAGGGCAGTCTAGATCA	TNF receptor-associated factor 6, E3 ubiquitin protein ligase
UNG	NM_080911.2	TGGACCCAGATGTGTGACATAA	AGAGCCCGTGAGCTTGATTA	uracil-DNA glycosylase
VPRBP	NM_014703.2	TGGATGGCGGATGCTTTGATA	TTCATTGGCTTCCCGGAACA	Vpr (HIV-1) binding protein
APOBEC3F	NM_145298.5	AGCCTGGAGCAGAAAGTGAA	ATCCTTGGCCGGCTAGTCC	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3F
APOBEC3G	NM_021822.3	CGCAGCCTGTGTCAGAAAA	TTGCTCCAACAGTGCTGAAA	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G
GUSB	NM_000181.3	CATCGATGACATCACCGTCAC	ACAGGTTACTGCCCTTGACA	glucuronidase, beta
KLF4	NM_004235.4	CTGCGGCAAAACCTACACAA	CGTCCCAGTCACAGTGGTAA	Kruppel-like factor 4 (gut)
SAMHD1	NM_015474.3	CCCGTGTCTGTGAAGTAGACAA	TGCGAGTGTGGAACATGTCA	SAM domain and HD domain 1
SLC2A3	NM_006931.2	CCGTCGGACTCTTCGTCAA	TAAAGCAGCCACCAGTGACA	solute carrier family 2 (facilitated glucose transporter), member 3
TREX1	NM_033629.3	ACCATCTGCTGTCACAACCA	TCTCTCCAAGGCTGGGACTA	three prime repair exonuclease 1
TRIM5	NM_033092.2	AGAAGCAGCAGGAAGCTGAA	GCCAAGACGTTGGTTTTGTCA	tripartite motif containing 5

Table S1

Code	Gender	Age	Viral load (RNA copies/ml plasma)	Months with undetectable viral load on ART	CD4 T cell count (cells/µl blood)	Cell-associated HIV DNA (copies/ 10^6 CD4)
034001	M	57	<40	77	858	3805
034004	F	66	<40	110	593	3605
034005	M	50	<40	39	971	2797
034006	M	61	<40	77	750	8715
034008	M	33	<40	36	1119	4891
L34001	M	55	<40	195	693	NA

M, male; F, female; NA, not available.

Table S2

Gene name	Stability value
BECN1	0,250549233
PRKAA1	0,288508569
RAB7A	0,30946967
TRAF6	0,319334695
TP53	0,328343002
VPRBP	0,365991082
API5	0,367327819
STAT3	0,370608212
EIF2AK2	0,384103082
HIF1A	0,391907917
TREX1	0,39253953
AKT1	0,394023857
RICTOR	0,395377783
MAPK1	0,396649613
PSIP1	0,408881136
CDKN1B	0,413957015
RPS6KB1	0,416878613
CCNT1	0,417659018
CDK9	0,426303822
MTOR	0,434251173
HMGA1	0,436534261
CUL4A	0,454108452
MYC	0,457005393
MYD88	0,461562789
NFATC1	0,463277429
SAMHD1	0,467104121
CASP8	0,467443456
NFKB1	0,472349996
NFATC2	0,476133255
PRKCA	0,47821775
DDB1	0,482170786
PIK3CB	0,487302561
DDX58	0,496549071
BCL11B	0,498962754
PPIA	0,499844178
BCL2	0,502606509
MAPK3	0,51179285
HSPB1	0,511905741
RHOA	0,515493522
STAT5B	0,516400376
CASP3	0,536630196
HSP90AB1	0,538067061
IRF3	0,546799681
SMARCB1	0,552440933
APOBEC3F	0,55660686
IFI16	0,570759333
IRF1	0,572196628
HSPH1	0,587874332
MX2	0,590279919
SLC2A3	0,601232687
IFI6	0,602522338
IRF4	0,610056472
MX1	0,613977716
CFL1	0,620028452
IL7R	0,64055187
HSP90AA1	0,662468904
HSPA1A	0,665415349
SLFN11	0,689147876
RB1	0,697664041
STAT1	0,702260041
FAS	0,703247399
DNAJB1	0,708604559
TRIM5	0,725969121
IRF7	0,734412588
FOXP3	0,739563525
SLC2A1	0,746229633
RPTOR	0,747416766
ACTB	0,759726907
GAPDH	0,768315184
DYNC1H1	0,77543431
APOBEC3G	0,776517129
HK2	0,794980726
GUSB	0,821595116
PRDM1	0,844574596
BCL2L1	0,867143178
UNG	0,89218868
CDKN1A	0,909445507
FOXO3	0,936183284
IFNB1	0,945277953
E2F1	0,993140533
RRM2	0,993363254
SLC1A5	1,128540312
ESRRA	1,228239862
MKI67	1,236841231
TLR9	1,23828716
IFNG	1,417604718
OAS1	1,614650004
KLF2	1,622314129
PDE4A	1,772367033
TLR8	1,903574476
IFNA1	2,132211213
TLR3	2,263468774
PML	2,39732875
TLR7	2,584686641
KLF4	2,652965521
HSPA1L	3,063617743

Better stability Value
(Best House Keeping Gene)

Worse stability Value
(Worse House Keeping Gene)

Table S3

BIBLIOGRAPHY

About HIV/AIDS | HIV Basics | Act Against AIDS | CDC [WWW Document], 2018. URL https://www.cdc.gov/actagainstaids/basics/whatishiv.html (accessed 5.24.18).

Allouch, A., David, A., Amie, S.M., Lahouassa, H., Chartier, L., Margottin-Goguet, F., Barré-Sinoussi, F., Kim, B., Sáez-Cirión, A., Pancino, G., 2014. Reply to Pauls et al.: p21 is a master regulator of HIV replication in macrophages through dNTP synthesis block. Proc. Natl. Acad. Sci. 111, E1325–E1326. https://doi.org/10.1073/pnas.1322699111

Allouch, A., David, A., Amie, S.M., Lahouassa, H., Chartier, L., Margottin-Goguet, F., Barré-Sinoussi, F., Kim, B., Sáez-Cirión, A., Pancino, G., 2013. p21-mediated RNR2 repression restricts HIV-1 replication in macrophages by inhibiting dNTP biosynthesis pathway. Proc. Natl. Acad. Sci. 110, E3997–E4006. https://doi.org/10.1073/pnas.1306719110

Almeida, L., Lochner, M., Berod, L., Sparwasser, T., 2016. Metabolic pathways in T cell activation and lineage differentiation. Semin. Immunol. 28, 514–524. https://doi.org/10.1016/j.smim.2016.10.009

Altfeld, M., Gale Jr, M., 2015. Innate immunity against HIV-1 infection. Nat. Immunol. 16, 554–562. https://doi.org/10.1038/ni.3157

Amie, S.M., Noble, E., Kim, B., 2013. Intracellular nucleotide levels and the control of retroviral infections. Virology 436, 247–254. https://doi.org/10.1016/j.virol.2012.11.010

Ananworanich, J., Dubé, K., Chomont, N., 2015. How does the timing of antiretroviral therapy initiation in acute infection affect HIV reservoirs? Curr. Opin. HIV AIDS 10, 18–28. https://doi.org/10.1097/COH.0000000000000122

Apolonia, L., Schulz, R., Curk, T., Rocha, P., Swanson, C.M., Schaller, T., Ule, J., Malim, M.H., 2015. Promiscuous RNA Binding Ensures Effective Encapsidation of APOBEC3 Proteins by HIV-1. PLOS Pathog. 11, e1004609. https://doi.org/10.1371/journal.ppat.1004609

Araínga, M., Edagwa, B., Mosley, R.L., Poluektova, L.Y., Gorantla, S., Gendelman, H.E., 2017. A mature macrophage is a principal HIV-1 cellular reservoir in humanized mice after treatment with long acting antiretroviral therapy. Retrovirology 14, 17. https://doi.org/10.1186/s12977-017-0344-7

Arthos, J., Cicala, C., Martinelli, E., Macleod, K., Van Ryk, D., Wei, D., Xiao, Z., Veenstra, T.D., Conrad, T.P., Lempicki, R.A., McLaughlin, S., Pascuccio, M., Gopaul, R., McNally, J., Cruz, C.C., Censoplano, N., Chung, E., Reitano, K.N., Kottilil, S., Goode, D.J., Fauci, A.S., 2008. HIV-1 envelope protein binds to and signals through integrin alpha4beta7, the gut mucosal homing receptor for peripheral T cells. Nat. Immunol. 9, 301–309. https://doi.org/10.1038/ni1566

Asada, M., Yamada, T., Ichijo, H., Delia, D., Miyazono, K., Fukumuro, K., Mizutani, S., 1999. Apoptosis inhibitory activity of cytoplasmic p21Cip1/WAF1 in monocytic differentiation. EMBO J. 18, 1223–1234. https://doi.org/10.1093/emboj/18.5.1223

Asamitsu, K., Fujinaga, K., Okamoto, T., 2018. HIV Tat/P-TEFb Interaction: A Potential Target for Novel Anti-HIV Therapies. Molecules 23, 933. https://doi.org/10.3390/molecules23040933

Ashbrook, A.W., Lentscher, A.J., Zamora, P.F., Silva, L.A., May, N.A., Bauer, J.A., Morrison, T.E., Dermody, T.S., 2016. Antagonism of the Sodium-Potassium ATPase Impairs

Chikungunya Virus Infection. mBio 7, e00693-16. https://doi.org/10.1128/mBio.00693-16

Avalos, C.R., Abreu, C.M., Queen, S.E., Li, M., Price, S., Shirk, E.N., Engle, E.L., Forsyth, E., Bullock, B.T., Mac Gabhann, F., Wietgrefe, S.W., Haase, A.T., Zink, M.C., Mankowski, J.L., Clements, J.E., Gama, L., 2017. Brain Macrophages in Simian Immunodeficiency Virus-Infected, Antiretroviral-Suppressed Macaques: a Functional Latent Reservoir. mBio 8. https://doi.org/10.1128/mBio.01186-17

Badia, R., Pujantell, M., Riveira-Muñoz, E., Puig, T., Torres-Torronteras, J., Martí, R., Clotet, B., Ampudia, R.M., Vives-Pi, M., Esté, J.A., Ballana, E., 2016. The G1/S Specific Cyclin D2 Is a Regulator of HIV-1 Restriction in Non-proliferating Cells. PLOS Pathog. 12, e1005829. https://doi.org/10.1371/journal.ppat.1005829

Baldauf, H.-M., Pan, X., Erikson, E., Schmidt, S., Daddacha, W., Burggraf, M., Schenkova, K., Ambiel, I., Wabnitz, G., Gramberg, T., Panitz, S., Flory, E., Landau, N.R., Sertel, S., Rutsch, F., Lasitschka, F., Kim, B., König, R., Fackler, O.T., Keppler, O.T., 2012. SAMHD1 restricts HIV-1 infection in resting $CD4^+$ T cells. Nat. Med. 18, 1682–1689. https://doi.org/10.1038/nm.2964

Banerjee, M., Xie, Z., 2016. Na/K-ATPase and Its Role in Signal Transduction, in: Regulation of Membrane Na+-K+ ATPase, Advances in Biochemistry in Health and Disease. Springer, Cham, pp. 31–50. https://doi.org/10.1007/978-3-319-24750-2_2

Banga, R., Procopio, F.A., Noto, A., Pollakis, G., Cavassini, M., Ohmiti, K., Corpataux, J.-M., Leval, L. de, Pantaleo, G., Perreau, M., 2016. PD-1^+ and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals. Nat. Med. 22, 754–761. https://doi.org/10.1038/nm.4113

Belmonte, L., Olmos, M., Fanin, A., Parodi, C., Baré, P., Concetti, H., Pérez, H., de Bracco, M.M.E., Cahn, P., 2007. The intestinal mucosa as a reservoir of HIV-1 infection after successful HAART: AIDS 21, 2106–2108. https://doi.org/10.1097/QAD.0b013e3282efb74b

Bergamaschi, A., David, A., Rouzic, E.L., Nisole, S., Barré-Sinoussi, F., Pancino, G., 2009. The CDK Inhibitor p21Cip1/WAF1 Is Induced by FcγR Activation and Restricts the Replication of Human Immunodeficiency Virus Type 1 and Related Primate Lentiviruses in Human Macrophages. J. Virol. 83, 12253–12265. https://doi.org/10.1128/JVI.01395-09

Berghe, T.V., Linkermann, A., Jouan-Lanhouet, S., Walczak, H., Vandenabeele, P., 2014. Regulated necrosis: the expanding network of non-apoptotic cell death pathways. Nat. Rev. Mol. Cell Biol. 15, 135–147. https://doi.org/10.1038/nrm3737

Besnard, E., Hakre, S., Kampmann, M., Lim, H.W., Hosmane, N.N., Martin, A., Bassik, M.C., Verschueren, E., Battivelli, E., Chan, J., Svensson, J.P., Gramatica, A., Conrad, R.J., Ott, M., Greene, W.C., Krogan, N.J., Siliciano, R.F., Weissman, J.S., Verdin, E., 2016. The mTOR Complex Controls HIV Latency. Cell Host Microbe 20, 785–797. https://doi.org/10.1016/j.chom.2016.11.001

Bishop, K.N., Holmes, R.K., Malim, M.H., 2006. Antiviral Potency of APOBEC Proteins Does Not Correlate with Cytidine Deamination. J. Virol. 80, 8450–8458. https://doi.org/10.1128/JVI.00839-06

Bishop, K.N., Verma, M., Kim, E.-Y., Wolinsky, S.M., Malim, M.H., 2008. APOBEC3G Inhibits Elongation of HIV-1 Reverse Transcripts. PLOS Pathog. 4, e1000231. https://doi.org/10.1371/journal.ppat.1000231

Börjesson, S.I., Englund, U.H., Asif, M.H., Willander, M., Elinder, F., 2011. Intracellular K+ Concentration Decrease Is Not Obligatory for Apoptosis. J. Biol. Chem. 286, 39823–39828. https://doi.org/10.1074/jbc.M111.262725

Borrow, P., Lewicki, H., Hahn, B.H., Shaw, G.M., Oldstone, M.B., 1994. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. J. Virol. 68, 6103–6110.

Borrow, P., Lewicki, H., Wei, X., Horwitz, M.S., Peffer, N., Meyers, H., Nelson, J.A., Gairin, J.E., Hahn, B.H., Oldstone, M.B.A., Shaw, G.M., 1997. Antiviral pressure exerted by HIV-l-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. Nat. Med. 3, 205–211. https://doi.org/10.1038/nm0297-205

Bose, T., Cieślar-Pobuda, A., Wiechec, E., 2015. Role of ion channels in regulating Ca2+ homeostasis during the interplay between immune and cancer cells. Cell Death Dis. 6, e1648. https://doi.org/10.1038/cddis.2015.23

Brandenberg, O.F., Magnus, C., Regoes, R.R., Trkola, A., 2015. The HIV-1 Entry Process: A Stoichiometric View. Trends Microbiol. 23, 763–774. https://doi.org/10.1016/j.tim.2015.09.003

Brenchley, J.M., Paiardini, M., Knox, K.S., Asher, A.I., Cervasi, B., Asher, T.E., Scheinberg, P., Price, D.A., Hage, C.A., Kholi, L.M., Khoruts, A., Frank, I., Else, J., Schacker, T., Silvestri, G., Douek, D.C., 2008. Differential Th17 CD4 T-cell depletion in pathogenic and nonpathogenic lentiviral infections. Blood 112, 2826–2835. https://doi.org/10.1182/blood-2008-05-159301

Briggs, J.A.G., Wilk, T., Welker, R., Kräusslich, H.-G., Fuller, S.D., 2003. Structural organization of authentic, mature HIV-1 virions and cores. EMBO J. 22, 1707–1715. https://doi.org/10.1093/emboj/cdg143

Bronner, D.N., Abuaita, B.H., Chen, X., Fitzgerald, K.A., Nuñez, G., He, Y., Yin, X.-M., O'Riordan, M.X.D., 2015. Endoplasmic Reticulum Stress Activates the Inflammasome via NLRP3- and Caspase-2-Driven Mitochondrial Damage. Immunity 43, 451–462. https://doi.org/10.1016/j.immuni.2015.08.008

Bunz, F., Dutriaux, A., Lengauer, C., Waldman, T., Zhou, S., Brown, J.P., Sedivy, J.M., Kinzler, K.W., Vogelstein, B., 1998. Requirement for p53 and p21 to Sustain G2 Arrest After DNA Damage. Science 282, 1497–1501. https://doi.org/10.1126/science.282.5393.1497

Bushman, F.D., Malani, N., Fernandes, J., D'Orso, I., Cagney, G., Diamond, T.L., Zhou, H., Hazuda, D.J., Espeseth, A.S., König, R., Bandyopadhyay, S., Ideker, T., Goff, S.P., Krogan, N.J., Frankel, A.D., Young, J.A.T., Chanda, S.K., 2009. Host Cell Factors in HIV Replication: Meta-Analysis of Genome-Wide Studies. PLOS Pathog. 5, e1000437. https://doi.org/10.1371/journal.ppat.1000437

Buzon, M.J., Sun, H., Li, C., Shaw, A., Seiss, K., Ouyang, Z., Martin-Gayo, E., Leng, J., Henrich, T.J., Li, J.Z., Pereyra, F., Zurakowski, R., Walker, B.D., Rosenberg, E.S., Yu, X.G., Lichterfeld, M., 2014. HIV-1 persistence in CD4+ T cells with stem cell-like properties. Nat. Med. 20, 139–142. https://doi.org/10.1038/nm.3445

Cahalan, M.D., Chandy, K.G., 2009. The functional network of ion channels in T lymphocytes. Immunol. Rev. 231, 59–87. https://doi.org/10.1111/j.1600-065X.2009.00816.x

Canestri, A., Lescure, F.-X., Jaureguiberry, S., Moulignier, A., Amiel, C., Marcelin, A., Peytavin, G., Tubiana, R., Pialoux, G., Katlama, C., 2010. Discordance Between Cerebral Spinal Fluid and Plasma HIV Replication in Patients with Neurological

Symptoms Who Are Receiving Suppressive Antiretroviral Therapy. Clin. Infect. Dis. 50, 773–778. https://doi.org/10.1086/650538
Carter, G.C., Bernstone, L., Baskaran, D., James, W., 2011. HIV-1 infects macrophages by exploiting an endocytic route dependent on dynamin, Rac1 and Pak1. Virology 409, 234–250. https://doi.org/10.1016/j.virol.2010.10.018
Castedo, M., Roumier, T., Blanco, J., Ferri, K.F., Barretina, J., Tintignac, L.A., Andreau, K., Perfettini, J.-L., Amendola, A., Nardacci, R., Leduc, P., Ingber, D.E., Druillennec, S., Roques, B., Leibovitch, S.A., Vilella-Bach, M., Chen, J., Este, J.A., Modjtahedi, N., Piacentini, M., Kroemer, G., 2002. Sequential involvement of Cdk1, mTOR and p53 in apoptosis induced by the HIV-1 envelope. EMBO J. 21, 4070–4080. https://doi.org/10.1093/emboj/cdf391
Chavez, L., Calvanese, V., Verdin, E., 2015. HIV Latency Is Established Directly and Early in Both Resting and Activated Primary CD4 T Cells. PLOS Pathog 11, e1004955. https://doi.org/10.1371/journal.ppat.1004955
Checkley, M.A., Luttge, B.G., Freed, E.O., 2011. HIV-1 Envelope Glycoprotein Biosynthesis, Trafficking, and Incorporation. J. Mol. Biol., Structural and Molecular Biology of HIV 410, 582–608. https://doi.org/10.1016/j.jmb.2011.04.042
Chen, H., Engelman, A., 1998. The barrier-to-autointegration protein is a host factor for HIV type 1 integration. Proc. Natl. Acad. Sci. 95, 15270–15274. https://doi.org/10.1073/pnas.95.26.15270
Chen, H., Li, C., Huang, J., Cung, T., Seiss, K., Beamon, J., Carrington, M.F., Porter, L.C., Burke, P.S., Yang, Y., Ryan, B.J., Liu, R., Weiss, R.H., Pereyra, F., Cress, W.D., Brass, A.L., Rosenberg, E.S., Walker, B.D., Yu, X.G., Lichterfeld, M., 2011. $CD4^+$ T cells from elite controllers resist HIV-1 infection by selective upregulation of p21. J. Clin. Invest. 121, 1549–1560. https://doi.org/10.1172/JCI44539
Cheung, K.-W., Wu, T., Ho, S.F., Wong, Y.C., Liu, L., Wang, H., Chen, Z., 2018. α4β7+ CD4+ effector/effector memory T cells differentiate into productively and latently infected central memory T cells by TGFβ1 during HIV-1 infection. J. Virol. JVI.01510-17. https://doi.org/10.1128/JVI.01510-17
Cho, H., Chung, J.-Y., Song, K.-H., Noh, K.H., Kim, B.W., Chung, E.J., Ylaya, K., Kim, J.H., Kim, T.W., Hewitt, S.M., Kim, J.-H., 2014. Apoptosis inhibitor-5 overexpression is associated with tumor progression and poor prognosis in patients with cervical cancer. BMC Cancer 14, 545. https://doi.org/10.1186/1471-2407-14-545
Choi, J., Ryoo, J., Oh, C., Hwang, S., Ahn, K., 2015. SAMHD1 specifically restricts retroviruses through its RNase activity. Retrovirology 12, 46. https://doi.org/10.1186/s12977-015-0174-4
Chomont, N., El-Far, M., Ancuta, P., Trautmann, L., Procopio, F.A., Yassine-Diab, B., Boucher, G., Boulassel, M.-R., Ghattas, G., Brenchley, J.M., Schacker, T.W., Hill, B.J., Douek, D.C., Routy, J.-P., Haddad, E.K., Sékaly, R.-P., 2009a. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. Nat. Med. 15, 893–900. https://doi.org/10.1038/nm.1972
Chomont, N., El-Far, M., Ancuta, P., Trautmann, L., Procopio, F.A., Yassine-Diab, B., Boucher, G., Boulassel, M.-R., Ghattas, G., Brenchley, J.M., Schacker, T.W., Hill, B.J., Douek, D.C., Routy, J.-P., Haddad, E.K., Sékaly, R.-P., 2009b. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. Nat. Med. 15, 893–900. https://doi.org/10.1038/nm.1972

Chowdhury, I.H., Wang, X.-F., Landau, N.R., Robb, M.L., Polonis, V.R., Birx, D.L., Kim, J.H., 2003. HIV-1 Vpr Activates Cell Cycle Inhibitor p21/Waf1/Cip1: A Potential Mechanism of G2/M Cell Cycle Arrest. Virology 305, 371–377. https://doi.org/10.1006/viro.2002.1777

Chun, T.-W., Jr, R.T.D., Ostrowski, M., Justement, J.S., Engel, D., Mullins, J.I., Fauci, A.S., 2000. Relationship between pre-existing viral reservoirs and the re-emergence of plasma viremia after discontinuation of highly active anti-retroviral therapy. Nat. Med. 6, 757–761. https://doi.org/10.1038/77481

Chun, T.-W., Nickle, D.C., Justement, J.S., Meyers, J.H., Roby, G., Hallahan, C.W., Kottilil, S., Moir, S., Mican, J.M., Mullins, J.I., Ward, D.J., Joseph A., K., Mannon, P.J., Fauci, A.S., 2008. Persistence of HIV in Gut-Associated Lymphoid Tissue despite Long-Term Antiretroviral Therapy. J. Infect. Dis. 197, 714–720. https://doi.org/10.1086/527324

Chun, T.-W., Stuyver, L., Mizell, S.B., Ehler, L.A., Mican, J.A.M., Baseler, M., Lloyd, A.L., Nowak, M.A., Fauci, A.S., 1997. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc. Natl. Acad. Sci. 94, 13193–13197.

Churchill, M.J., Gorry, P.R., Cowley, D., Lal, L., Sonza, S., Purcell, D.F., Thompson, K.A., Gabuzda, D., McArthur, J.C., Pardo, C.A., Wesselingh, S.L., 2006. Use of laser capture microdissection to detect integrated HIV-1 DNA in macrophages and astrocytes from autopsy brain tissues. J. Neurovirol. 12, 146–152. https://doi.org/10.1080/13550280600748946

Churchill, M.J., Wesselingh, S.L., Cowley, D., Pardo, C.A., McArthur, J.C., Brew, B.J., Gorry, P.R., 2009. Extensive astrocyte infection is prominent in human immunodeficiency virus–associated dementia. Ann. Neurol. 66, 253–258. https://doi.org/10.1002/ana.21697

Cicala, C., Arthos, J., Rubbert, A., Selig, S., Wildt, K., Cohen, O.J., Fauci, A.S., 2000. HIV-1 envelope induces activation of caspase-3 and cleavage of focal adhesion kinase in primary human CD4+ T cells. Proc. Natl. Acad. Sci. 97, 1178–1183. https://doi.org/10.1073/pnas.97.3.1178

Cleret-Buhot, A., Zhang, Y., Planas, D., Goulet, J.-P., Monteiro, P., Gosselin, A., Wacleche, V.S., Tremblay, C.L., Jenabian, M.-A., Routy, J.-P., El-Far, M., Chomont, N., Haddad, E.K., Sekaly, R.-P., Ancuta, P., 2015. Identification of novel HIV-1 dependency factors in primary CCR4(+)CCR6(+)Th17 cells via a genome-wide transcriptional approach. Retrovirology 12, 102. https://doi.org/10.1186/s12977-015-0226-9

Compton, A.A., Bruel, T., Porrot, F., Mallet, A., Sachse, M., Euvrard, M., Liang, C., Casartelli, N., Schwartz, O., 2014. IFITM proteins incorporated into HIV-1 virions impair viral fusion and spread. Cell Host Microbe 16, 736–747. https://doi.org/10.1016/j.chom.2014.11.001

Cooper, A., García, M., Petrovas, C., Yamamoto, T., Koup, R.A., Nabel, G.J., 2013a. HIV-1 causes CD4 cell death through DNA-dependent protein kinase during viral integration. Nature 498, 376–379. https://doi.org/10.1038/nature12274

Cooper, A., García, M., Petrovas, C., Yamamoto, T., Koup, R.A., Nabel, G.J., 2013b. HIV-1 causes CD4 cell death through DNA-dependent protein kinase during viral integration. Nature 498, 376–379. https://doi.org/10.1038/nature12274

Cory, T.J., Schacker, T.W., Stevenson, M., Fletcher, C.V., 2013. Overcoming pharmacologic sanctuaries: Curr. Opin. HIV AIDS 8, 190–195. https://doi.org/10.1097/COH.0b013e32835fc68a

Cosenza;, M.A., Zhao;, M.-L., Si;, Q., Lee, S.C., 2002. Human Brain Parenchymal Microglia Express CD14 and CD45 and are Productively Infected by HIV-1 in HIV-1 Encephalitis. Brain Pathol. 12, 442–455. https://doi.org/10.1111/j.1750-3639.2002.tb00461.x

Couturier, J., Suliburk, J.W., Brown, J.M., Luke, D.J., Agarwal, N., Yu, X., Nguyen, C., Iyer, D., Kozinetz, C.A., Overbeek, P.A., Metzker, M.L., Balasubramanyam, A., Lewis, D.E., 2015. Human adipose tissue as a reservoir for memory CD4+ T cells and HIV. AIDS Lond. Engl. 29, 667–674. https://doi.org/10.1097/QAD.0000000000000599

Cribbs, S.K., Lennox, J., Caliendo, A.M., Brown, L.A., Guidot, D.M., 2014. Healthy HIV-1-Infected Individuals on Highly Active Antiretroviral Therapy Harbor HIV-1 in Their Alveolar Macrophages. AIDS Res. Hum. Retroviruses 31, 64–70. https://doi.org/10.1089/aid.2014.0133

Crooks, A.M., Bateson, R., Cope, A.B., Dahl, N.P., Griggs, M.K., Kuruc, J.D., Gay, C.L., Eron, J.J., Margolis, D.M., Bosch, R.J., Archin, N.M., 2015. Precise Quantitation of the Latent HIV-1 Reservoir: Implications for Eradication Strategies. J. Infect. Dis. 212, 1361–1365. https://doi.org/10.1093/infdis/jiv218

Czabotar, P.E., Lessene, G., Strasser, A., Adams, J.M., 2014. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. Nat. Rev. Mol. Cell Biol. 15, 49–63. https://doi.org/10.1038/nrm3722

Dahabieh, M.S., Battivelli, E., Verdin, E., 2015. Understanding HIV Latency: The Road to an HIV Cure. Annu. Rev. Med. 66, 407–421. https://doi.org/10.1146/annurev-med-092112-152941

Dahl, V., Gisslen, M., Hagberg, L., Peterson, J., Shao, W., Spudich, S., Price, R.W., Palmer, S., 2014. An Example of Genetically Distinct HIV Type 1 Variants in Cerebrospinal Fluid and Plasma During Suppressive Therapy. J. Infect. Dis. 209, 1618–1622. https://doi.org/10.1093/infdis/jit805

Dai, J., Agosto, L.M., Baytop, C., Yu, J.J., Pace, M.J., Liszewski, M.K., O'Doherty, U., 2009. Human Immunodeficiency Virus Integrates Directly into Naïve Resting CD4+ T Cells but Enters Naïve Cells Less Efficiently than Memory Cells. J. Virol. 83, 4528–4537. https://doi.org/10.1128/JVI.01910-08

Damouche, A., Lazure, T., Avettand-Fènoël, V., Huot, N., Dejucq-Rainsford, N., Satie, A.-P., Mélard, A., David, L., Gommet, C., Ghosn, J., Noel, N., Pourcher, G., Martinez, V., Benoist, S., Béréziat, V., Cosma, A., Favier, B., Vaslin, B., Rouzioux, C., Capeau, J., Müller-Trutwin, M., Dereuddre-Bosquet, N., Grand, R.L., Lambotte, O., Bourgeois, C., 2015. Adipose Tissue Is a Neglected Viral Reservoir and an Inflammatory Site during Chronic HIV and SIV Infection. PLOS Pathog. 11, e1005153. https://doi.org/10.1371/journal.ppat.1005153

Daniel, R., 2006. DNA repair in HIV-1 infection: a case for inhibitors of cellular co-factors? Curr. HIV Res. 4, 411–421.

Daniel, R., Ramcharan, J., Rogakou, E., Taganov, K.D., Greger, J.G., Bonner, W., Nussenzweig, A., Katz, R.A., Skalka, A.M., 2004. Histone H2AX is phosphorylated at sites of retroviral DNA integration but is dispensable for postintegration repair. J. Biol. Chem. 279, 45810–45814. https://doi.org/10.1074/jbc.M407886200

Darcis, G., Driessche, B.V., Bouchat, S., Kirchhoff, F., Lint, C.V., 2017a. Molecular Control of HIV and SIV Latency, in: SpringerLink, Current Topics in Microbiology and Immunology. Springer, Berlin, Heidelberg, pp. 1–22. https://doi.org/10.1007/82_2017_74

Darcis, G., Van Driessche, B., Van Lint, C., 2017b. HIV Latency: Should We Shock or Lock? Trends Immunol. 38, 217–228. https://doi.org/10.1016/j.it.2016.12.003

Dave, R.S., Sharma, R.K., Muir, R.R., Haddad, E., Gumber, S., Villinger, F., Nehra, A.P., Khan, Z.K., Wigdahl, B., Ansari, A.A., Byrareddy, S.N., Jain, P., 2018. FDC:TFH Interactions within Cervical Lymph Nodes of SIV-Infected Rhesus Macaques. J. Neuroimmune Pharmacol. 13, 204–218. https://doi.org/10.1007/s11481-017-9775-0

David, A., Sáez-Cirión, A., Versmisse, P., Malbec, O., Iannascoli, B., Herschke, F., Lucas, M., Barré-Sinoussi, F., Mouscadet, J.-F., Daëron, M., Pancino, G., 2006. The Engagement of Activating FcγRs Inhibits Primate Lentivirus Replication in Human Macrophages. J. Immunol. 177, 6291–6300. https://doi.org/10.4049/jimmunol.177.9.6291

de la Vega, M., Marin, M., Kondo, N., Miyauchi, K., Kim, Y., Epand, R.F., Epand, R.M., Melikyan, G.B., 2011. Inhibition of HIV-1 endocytosis allows lipid mixing at the plasma membrane, but not complete fusion. Retrovirology 8, 99. https://doi.org/10.1186/1742-4690-8-99

de Pablo, A., Bogoi, R., Bejarano, I., Toro, C., Valencia, E., Moreno, V., Martín-Carbonero, L., Gómez-Hernando, C., Rodés, B., 2015. Short Communication: p21/CDKN1A Expression Shows Broad Interindividual Diversity in a Subset of HIV-1 Elite Controllers. AIDS Res. Hum. Retroviruses 32, 232–236. https://doi.org/10.1089/aid.2015.0137

de Witte, L., Bobardt, M., Chatterji, U., Degeest, G., David, G., Geijtenbeek, T.B.H., Gallay, P., 2007. Syndecan-3 is a dendritic cell-specific attachment receptor for HIV-1. Proc. Natl. Acad. Sci. U. S. A. 104, 19464–19469. https://doi.org/10.1073/pnas.0703747104

Deng, K., Pertea, M., Rongvaux, A., Wang, L., Durand, C.M., Ghiaur, G., Lai, J., McHugh, H.L., Hao, H., Zhang, H., Margolick, J.B., Gurer, C., Murphy, A.J., Valenzuela, D.M., Yancopoulos, G.D., Deeks, S.G., Strowig, T., Kumar, P., Siliciano, J.D., Salzberg, S.L., Flavell, R.A., Shan, L., Siliciano, R.F., 2015. Broad CTL response is required to clear latent HIV-1 due to dominance of escape mutations. Nature 517, 381–385. https://doi.org/10.1038/nature14053

Derossi, D., Chassaing, G., Prochiantz, A., 1998. Trojan peptides: the penetratin system for intracellular delivery. Trends Cell Biol. 8, 84–87.

Diamond, T.L., Roshal, M., Jamburuthugoda, V.K., Reynolds, H.M., Merriam, A.R., Lee, K.Y., Balakrishnan, M., Bambara, R.A., Planelles, V., Dewhurst, S., Kim, B., 2004. Macrophage Tropism of HIV-1 Depends on Efficient Cellular dNTP Utilization by Reverse Transcriptase. J. Biol. Chem. 279, 51545–51553. https://doi.org/10.1074/jbc.M408573200

Doitsh, G., Cavrois, M., Lassen, K.G., Zepeda, O., Yang, Z., Santiago, M.L., Hebbeler, A.M., Greene, W.C., 2010. Abortive HIV Infection Mediates CD4 T Cell Depletion and Inflammation in Human Lymphoid Tissue. Cell 143, 789–801. https://doi.org/10.1016/j.cell.2010.11.001

Doitsh, G., Galloway, N.L.K., Geng, X., Yang, Z., Monroe, K.M., Zepeda, O., Hunt, P.W., Hatano, H., Sowinski, S., Muñoz-Arias, I., Greene, W.C., 2014. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. Nature 505, 509–514. https://doi.org/10.1038/nature12940

Douek, D.C., Brenchley, J.M., Betts, M.R., Ambrozak, D.R., Hill, B.J., Okamoto, Y., Casazza, J.P., Kuruppu, J., Kunstman, K., Wolinsky, S., Grossman, Z., Dybul, M., Oxenius, A.,

Price, D.A., Connors, M., Koup, R.A., 2002. HIV preferentially infects HIV-specific CD4+ T cells. Nature 417, 95–98. https://doi.org/10.1038/417095a
Doyle, T., Goujon, C., Malim, M.H., 2015. HIV-1 and interferons: who's interfering with whom? Nat. Rev. Microbiol. 13, 403–413. https://doi.org/10.1038/nrmicro3449
Dumitru, C., Kabat, A.M., Maloy, K.J., 2018. Metabolic Adaptations of CD4+ T Cells in Inflammatory Disease. Front. Immunol. 9. https://doi.org/10.3389/fimmu.2018.00540
Dybul, M., Daucher, M., Jensen, M.A., Hallahan, C.W., Chun, T.-W., Belson, M., Hidalgo, B., Nickle, D.C., Yoder, C., Metcalf, J.A., Davey, R.T., Ehler, L., Kress-Rock, D., Nies-Kraske, E., Liu, S., Mullins, J.I., Fauci, A.S., 2003. Genetic Characterization of Rebounding Human Immunodeficiency Virus Type 1 in Plasma during Multiple Interruptions of Highly Active Antiretroviral Therapy. J. Virol. 77, 3229–3237. https://doi.org/10.1128/JVI.77.5.3229-3237.2003
Edén, A., Fuchs, D., Hagberg, L., Nilsson, S., Spudich, S., Svennerholm, B., Price, R.W., Gisslén, M., 2010. HIV-1 Viral Escape in Cerebrospinal Fluid of Subjects on Suppressive Antiretroviral Treatment. J. Infect. Dis. 202, 1819–1825. https://doi.org/10.1086/657342
Elahi, S., Weiss, R.H., Merani, S., 2016. Atorvastatin restricts HIV replication in CD4+ T cells by upregulation of p21. AIDS Lond. Engl. 30, 171–183. https://doi.org/10.1097/QAD.0000000000000917
Embretson, J., Zupancic, M., Ribas, J.L., Burke, A., Racz, P., Tenner-Racz, K., Haase, A.T., 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature 362, 359–362. https://doi.org/10.1038/362359a0
Estes, J.D., Kityo, C., Ssali, F., Swainson, L., Makamdop, K.N., Prete, G.Q.D., Deeks, S.G., Luciw, P.A., Chipman, J.G., Beilman, G.J., Hoskuldsson, T., Khoruts, A., Anderson, J., Deleage, C., Jasurda, J., Schmidt, T.E., Hafertepe, M., Callisto, S.P., Pearson, H., Reimann, T., Schuster, J., Schoephoerster, J., Southern, P., Perkey, K., Shang, L., Wietgrefe, S.W., Fletcher, C.V., Lifson, J.D., Douek, D.C., McCune, J.M., Haase, A.T., Schacker, T.W., 2017. Defining total-body AIDS-virus burden with implications for curative strategies. Nat. Med. 23, 1271–1276. https://doi.org/10.1038/nm.4411
Fackler, O.T., Peterlin, B.M., 2000. Endocytic entry of HIV-1. Curr. Biol. 10, 1005–1008. https://doi.org/10.1016/S0960-9822(00)00654-0
Farnet, C.M., Bushman, F.D., 1997. HIV-1 cDNA Integration: Requirement of HMG I(Y) Protein for Function of Preintegration Complexes In Vitro. Cell 88, 483–492. https://doi.org/10.1016/S0092-8674(00)81888-7
Fassati, A., 2006. HIV infection of non-dividing cells: a divisive problem. Retrovirology 3, 74. https://doi.org/10.1186/1742-4690-3-74
Feske, S., Wulff, H., Skolnik, E.Y., 2015a. Ion Channels in Innate and Adaptive Immunity. Annu. Rev. Immunol. 33, 291–353. https://doi.org/10.1146/annurev-immunol-032414-112212
Feske, S., Wulff, H., Skolnik, E.Y., 2015b. Ion Channels in Innate and Adaptive Immunity. Annu. Rev. Immunol. 33, 291–353. https://doi.org/10.1146/annurev-immunol-032414-112212
Finkel, T.H., Tudor-Williams, G., Banda, N.K., Cotton, M.F., Curiel, T., Monks, C., Baba, T.W., Ruprecht, R.M., Kupfer, A., 1995. Apoptosis occurs predominantly in bystander cells

and not in productively infected cells of HIV- and SIV-infected lymph nodes. Nat. Med. 1, 129–134.
Finzi, D., Hermankova, M., Pierson, T., Carruth, L.M., Buck, C., Chaisson, R.E., Quinn, T.C., Chadwick, K., Margolick, J., Brookmeyer, R., Gallant, J., Markowitz, M., Ho, D.D., Richman, D.D., Siliciano, R.F., 1997. Identification of a Reservoir for HIV-1 in Patients on Highly Active Antiretroviral Therapy. Science 278, 1295–1300. https://doi.org/10.1126/science.278.5341.1295
Fletcher, C.V., Staskus, K., Wietgrefe, S.W., Rothenberger, M., Reilly, C., Chipman, J.G., Beilman, G.J., Khoruts, A., Thorkelson, A., Schmidt, T.E., Anderson, J., Perkey, K., Stevenson, M., Perelson, A.S., Douek, D.C., Haase, A.T., Schacker, T.W., 2014. Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. Proc. Natl. Acad. Sci. 111, 2307–2312. https://doi.org/10.1073/pnas.1318249111
Flexner, C., 1998. HIV-Protease Inhibitors. N. Engl. J. Med. 338, 1281–1293. https://doi.org/10.1056/NEJM199804303381808
Foster, T.L., Wilson, H., Iyer, S.S., Coss, K., Doores, K., Smith, S., Kellam, P., Finzi, A., Borrow, P., Hahn, B.H., Neil, S.J.D., 2016. Resistance of Transmitted Founder HIV-1 to IFITM-Mediated Restriction. Cell Host Microbe 20, 429–442. https://doi.org/10.1016/j.chom.2016.08.006
Fraietta, J.A., Mueller, Y.M., Yang, G., Boesteanu, A.C., Gracias, D.T., Do, D.H., Hope, J.L., Kathuria, N., McGettigan, S.E., Lewis, M.G., Giavedoni, L.D., Jacobson, J.M., Katsikis, P.D., 2013. Type I Interferon Upregulates Bak and Contributes to T Cell Loss during Human Immunodeficiency Virus (HIV) Infection. PLOS Pathog. 9, e1003658. https://doi.org/10.1371/journal.ppat.1003658
Franke, E.K., Yuan, H.E.H., Luban, J., 1994. Specific incorporation of cyclophilin A into HIV-1 virions. Nature 372, 359–362. https://doi.org/10.1038/372359a0
Frauwirth, K.A., Riley, J.L., Harris, M.H., Parry, R.V., Rathmell, J.C., Plas, D.R., Elstrom, R.L., June, C.H., Thompson, C.B., 2002. The CD28 Signaling Pathway Regulates Glucose Metabolism. Immunity 16, 769–777. https://doi.org/10.1016/S1074-7613(02)00323-0
Freed, E.O., 2015. HIV-1 assembly, release and maturation. Nat. Rev. Microbiol. 13, 484–496. https://doi.org/10.1038/nrmicro3490
Friedman, J., Cho, W.-K., Chu, C.K., Keedy, K.S., Archin, N.M., Margolis, D.M., Karn, J., 2011. Epigenetic Silencing of HIV-1 by the Histone H3 Lysine 27 Methyltransferase Enhancer of Zeste 2. J. Virol. 85, 9078–9089. https://doi.org/10.1128/JVI.00836-11
Gagliani, N., Huber, S., 2017. Basic Aspects of T Helper Cell Differentiation, in: T-Cell Differentiation, Methods in Molecular Biology. Humana Press, New York, NY, pp. 19–30. https://doi.org/10.1007/978-1-4939-6548-9_2
Galão, R.P., Le Tortorec, A., Pickering, S., Kueck, T., Neil, S.J.D., 2012. Innate Sensing of HIV-1 Assembly by Tetherin Induces NFκB-Dependent Proinflammatory Responses. Cell Host Microbe 12, 633–644. https://doi.org/10.1016/j.chom.2012.10.007
Galluzzi, L., Kepp, O., Heiden, M.G.V., Kroemer, G., 2013. Metabolic targets for cancer therapy. Nat. Rev. Drug Discov. 12, 829–846. https://doi.org/10.1038/nrd4145
Galluzzi, L., Vitale, I., Aaronson, S.A., Abrams, J.M., Adam, D., Agostinis, P., Alnemri, E.S., Altucci, L., Amelio, I., Andrews, D.W., Annicchiarico-Petruzzelli, M., Antonov, A.V., Arama, E., Baehrecke, E.H., Barlev, N.A., Bazan, N.G., Bernassola, F., Bertrand, M.J.M., Bianchi, K., Blagosklonny, M.V., Blomgren, K., Borner, C., Boya, P., Brenner,

C., Campanella, M., Candi, E., Carmona-Gutierrez, D., Cecconi, F., Chan, F.K.-M., Chandel, N.S., Cheng, E.H., Chipuk, J.E., Cidlowski, J.A., Ciechanover, A., Cohen, G.M., Conrad, M., Cubillos-Ruiz, J.R., Czabotar, P.E., D'Angiolella, V., Dawson, T.M., Dawson, V.L., Laurenzi, V.D., Maria, R.D., Debatin, K.-M., DeBerardinis, R.J., Deshmukh, M., Daniele, N.D., Virgilio, F.D., Dixit, V.M., Dixon, S.J., Duckett, C.S., Dynlacht, B.D., El-Deiry, W.S., Elrod, J.W., Fimia, G.M., Fulda, S., García-Sáez, A.J., Garg, A.D., Garrido, C., Gavathiotis, E., Golstein, P., Gottlieb, E., Green, D.R., Greene, L.A., Gronemeyer, H., Gross, A., Hajnoczky, G., Hardwick, J.M., Harris, I.S., Hengartner, M.O., Hetz, C., Ichijo, H., Jäättelä, M., Joseph, B., Jost, P.J., Juin, P.P., Kaiser, W.J., Karin, M., Kaufmann, T., Kepp, O., Kimchi, A., Kitsis, R.N., Klionsky, D.J., Knight, R.A., Kumar, S., Lee, S.W., Lemasters, J.J., Levine, B., Linkermann, A., Lipton, S.A., Lockshin, R.A., López-Otín, C., Lowe, S.W., Luedde, T., Lugli, E., MacFarlane, M., Madeo, F., Malewicz, M., Malorni, W., Manic, G., Marine, J.-C., Martin, S.J., Martinou, J.-C., Medema, J.P., Mehlen, P., Meier, P., Melino, S., Miao, E.A., Molkentin, J.D., Moll, U.M., Muñoz-Pinedo, C., Nagata, S., Nuñez, G., Oberst, A., Oren, M., Overholtzer, M., Pagano, M., Panaretakis, T., Pasparakis, M., Penninger, J.M., Pereira, D.M., Pervaiz, S., Peter, M.E., Piacentini, M., Pinton, P., Prehn, J.H.M., Puthalakath, H., Rabinovich, G.A., Rehm, M., Rizzuto, R., Rodrigues, C.M.P., Rubinsztein, D.C., Rudel, T., Ryan, K.M., Sayan, E., Scorrano, L., Shao, F., Shi, Y., Silke, J., Simon, H.-U., Sistigu, A., Stockwell, B.R., Strasser, A., Szabadkai, G., Tait, S.W.G., Tang, D., Tavernarakis, N., Thorburn, A., Tsujimoto, Y., Turk, B., Berghe, T.V., Vandenabeele, P., Heiden, M.G.V., Villunger, A., Virgin, H.W., Vousden, K.H., Vucic, D., Wagner, E.F., Walczak, H., Wallach, D., Wang, Y., Wells, J.A., Wood, W., Yuan, J., Zakeri, Z., Zhivotovsky, B., Zitvogel, L., Melino, G., Kroemer, G., 2018. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ. 25, 486–541. https://doi.org/10.1038/s41418-017-0012-4

Gama, L., Abreu, C.M., Shirk, E.N., Price, S.L., Li, M., Laird, G.M., Pate, K.A.M., Wietgrefe, S.W., O'Connor, S.L., Pianowski, L., Haase, A.T., Van Lint, C., Siliciano, R.F., Clements, J.E., 2017. Reactivation of simian immunodeficiency virus reservoirs in the brain of virally suppressed macaques. AIDS Lond. Engl. 31, 5–14. https://doi.org/10.1097/QAD.0000000000001267

Gao, D., Wu, J., Wu, Y.-T., Du, F., Aroh, C., Yan, N., Sun, L., Chen, Z.J., 2013. Cyclic GMP-AMP Synthase Is an Innate Immune Sensor of HIV and Other Retroviruses. Science 341, 903–906. https://doi.org/10.1126/science.1240933

Garcia-Tellez, T., Huot, N., Ploquin, M.J., Rascle, P., Jacquelin, B., Müller-Trutwin, M., 2016. Non-human primates in HIV research: Achievements, limits and alternatives. Infect. Genet. Evol. 46, 324–332. https://doi.org/10.1016/j.meegid.2016.07.012

Gattinoni, L., Lugli, E., Ji, Y., Pos, Z., Paulos, C.M., Quigley, M.F., Almeida, J.R., Gostick, E., Yu, Z., Carpenito, C., Wang, E., Douek, D.C., Price, D.A., June, C.H., Marincola, F.M., Roederer, M., Restifo, N.P., 2011. A human memory T cell subset with stem cell–like properties. Nat. Med. 17, 1290–1297. https://doi.org/10.1038/nm.2446

Geijtenbeek, T.B.H., Kwon, D.S., Torensma, R., van Vliet, S.J., van Duijnhoven, G.C.F., Middel, J., Cornelissen, I.L.M.H.A., Nottet, H.S.L.M., KewalRamani, V.N., Littman, D.R., Figdor, C.G., van Kooyk, Y., 2000. DC-SIGN, a Dendritic Cell–Specific HIV-1-Binding Protein that Enhances trans-Infection of T Cells. Cell 100, 587–597. https://doi.org/10.1016/S0092-8674(00)80694-7

Geleziunas, R., Xu, W., Takeda, K., Ichijo, H., Greene, W.C., 2001. HIV-1 Nef inhibits ASK1-dependent death signalling providing a potential mechanism for protecting the infected host cell. Nature 410, 834–838. https://doi.org/10.1038/35071111

Genini, D., Sheeter, D., Rought, S., Zaunders, J.J., Susin, S.A., Kroemer, G., Richman, D.D., Carson, D.A., Corbeil, J., Leoni, L.M., 2000. HIV induces lymphocyte apoptosis by a p53-initiated, mitochondrial-mediated mechanism. FASEB J. 15, 5–6. https://doi.org/10.1096/fj.00-0336fje

Georgakilas, A.G., Martin, O.A., Bonner, W.M., 2017. p21: A Two-Faced Genome Guardian. Trends Mol. Med. 23, 310–319. https://doi.org/10.1016/j.molmed.2017.02.001

Ghimire, D., Rai, M., Gaur, R., 2018. Novel host restriction factors implicated in HIV-1 replication. J. Gen. Virol. 99, 435–446. https://doi.org/10.1099/jgv.0.001026

Gianella, S., Kosakovsky Pond, S.L., Oliveira, M.F., Scheffler, K., Strain, M.C., De la Torre, A., Letendre, S., Smith, D.M., Ellis, R.J., 2016. Compartmentalized HIV rebound in the central nervous system after interruption of antiretroviral therapy. Virus Evol. 2, vew020. https://doi.org/10.1093/ve/vew020

Goldstone, D.C., Ennis-Adeniran, V., Hedden, J.J., Groom, H.C.T., Rice, G.I., Christodoulou, E., Walker, P.A., Kelly, G., Haire, L.F., Yap, M.W., Carvalho, L.P.S. de, Stoye, J.P., Crow, Y.J., Taylor, I.A., Webb, M., 2011. HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. Nature 480, 379–382. https://doi.org/10.1038/nature10623

González, M.E., 2017. The HIV-1 Vpr Protein: A Multifaceted Target for Therapeutic Intervention. Int. J. Mol. Sci. 18, 126. https://doi.org/10.3390/ijms18010126

Goodbourn, S., Didcock, L., Randall, R.E., 2000. Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. J. Gen. Virol. 81, 2341–2364. https://doi.org/10.1099/0022-1317-81-10-2341

Gray, L.R., Roche, M., Flynn, J.K., Wesselingh, S.L., Gorry, P.R., Churchill, M.J., 2014. Is the central nervous system a reservoir of HIV-1? Curr. Opin. HIV AIDS 9, 552–558. https://doi.org/10.1097/COH.0000000000000108

Gray, L.R., Tachedjian, G., Ellett, A.M., Roche, M.J., Cheng, W.-J., Guillemin, G.J., Brew, B.J., Turville, S.G., Wesselingh, S.L., Gorry, P.R., Churchill, M.J., 2013. The NRTIs lamivudine, stavudine and zidovudine have reduced HIV-1 inhibitory activity in astrocytes. PloS One 8, e62196. https://doi.org/10.1371/journal.pone.0062196

Green, D.R., Galluzzi, L., Kroemer, G., 2014. Metabolic control of cell death. Science 345, 1250256. https://doi.org/10.1126/science.1250256

Gregory B Melikyan, 2014. HIV entry: a game of hide-and-fuse? - ScienceDirect [WWW Document]. URL https://www.sciencedirect.com/science/article/pii/S187962571300179X?via%3Dihub (accessed 7.31.18).

Grossman, Z., Meier-Schellersheim, M., Paul, W.E., Picker, L.J., 2006. Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. Nat. Med. 12, 289–295. https://doi.org/10.1038/nm1380

Guha, D., Mancini, A., Sparks, J., Ayyavoo, V., 2016. HIV-1 Infection Dysregulates Cell Cycle Regulatory Protein p21 in CD4+ T Cells Through miR-20a and miR-106b Regulation. J. Cell. Biochem. 117, 1902–1912. https://doi.org/10.1002/jcb.25489

Guo, F., Cen, S., Niu, M., Saadatmand, J., Kleiman, L., 2006. Inhibition of -Primed Reverse Transcription by Human APOBEC3G during Human Immunodeficiency Virus Type 1 Replication. J. Virol. 80, 11710–11722. https://doi.org/10.1128/JVI.01038-06

Gutierrez, K.D., Davis, M.A., Daniels, B.P., Olsen, T.M., Ralli-Jain, P., Tait, S.W.G., Gale, M., Oberst, A., 2017. MLKL Activation Triggers NLRP3-Mediated Processing and Release of IL-1β Independently of Gasdermin-D. J. Immunol. Baltim. Md 1950 198, 2156–2164. https://doi.org/10.4049/jimmunol.1601757

Haase, A.T., 2010. Targeting early infection to prevent HIV-1 mucosal transmission. Nature 464, 217–223. https://doi.org/10.1038/nature08757

Hammer, S.M., Squires, K.E., Hughes, M.D., Grimes, J.M., Demeter, L.M., Currier, J.S., Eron, J.J., Feinberg, J.E., Balfour, H.H., Deyton, L.R., Chodakewitz, J.A., Fischl, M.A., Phair, J.P., Pedneault, L., Nguyen, B.-Y., Cook, J.C., 1997. A Controlled Trial of Two Nucleoside Analogues plus Indinavir in Persons with Human Immunodeficiency Virus Infection and CD4 Cell Counts of 200 per Cubic Millimeter or Less. N. Engl. J. Med. 337, 725–733. https://doi.org/10.1056/NEJM199709113371101

Han, B.-G., Kim, K.H., Lee, S.J., Jeong, K.-C., Cho, J.-W., Noh, K.H., Kim, T.W., Kim, S.-J., Yoon, H.-J., Suh, S.W., Lee, S., Lee, B.I., 2012. Helical Repeat Structure of Apoptosis Inhibitor 5 Reveals Protein-Protein Interaction Modules. J. Biol. Chem. 287, 10727–10737. https://doi.org/10.1074/jbc.M111.317594

Hanahan, D., Weinberg, R.A., 2011. Hallmarks of Cancer: The Next Generation. Cell 144, 646–674. https://doi.org/10.1016/j.cell.2011.02.013

Harris, L.D., Tabb, B., Sodora, D.L., Paiardini, M., Klatt, N.R., Douek, D.C., Silvestri, G., Müller-Trutwin, M., Vasile-Pandrea, I., Apetrei, C., Hirsch, V., Lifson, J., Brenchley, J.M., Estes, J.D., 2010. Downregulation of Robust Acute Type I Interferon Responses Distinguishes Nonpathogenic Simian Immunodeficiency Virus (SIV) Infection of Natural Hosts from Pathogenic SIV Infection of Rhesus Macaques. J. Virol. 84, 7886–7891. https://doi.org/10.1128/JVI.02612-09

Hattori, N., Michaels, F., Fargnoli, K., Marcon, L., Gallo, R.C., Franchini, G., 1990. The human immunodeficiency virus type 2 vpr gene is essential for productive infection of human macrophages. Proc. Natl. Acad. Sci. 87, 8080–8084. https://doi.org/10.1073/pnas.87.20.8080

He, J., Chen, Y., Farzan, M., Choe, H., Ohagen, A., Gartner, S., Busciglio, J., Yang, X., Hofmann, W., Newman, W., Mackay, C.R., Sodroski, J., Gabuzda, D., 1997. CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. Nature 385, 645–649. https://doi.org/10.1038/385645a0

Hegedus, A., Kavanagh Williamson, M., Huthoff, H., 2014a. HIV-1 pathogenicity and virion production are dependent on the metabolic phenotype of activated CD4+ T cells. Retrovirology 11, 98. https://doi.org/10.1186/s12977-014-0098-4

Hegedus, A., Kavanagh Williamson, M., Huthoff, H., 2014b. HIV-1 pathogenicity and virion production are dependent on the metabolic phenotype of activated CD4+ T cells. Retrovirology 11, 98. https://doi.org/10.1186/s12977-014-0098-4

Heredia, A., Latinovic, O., Gallo, R.C., Melikyan, G., Reitz, M., Le, N., Redfield, R.R., 2008. Reduction of CCR5 with low-dose rapamycin enhances the antiviral activity of vicriviroc against both sensitive and drug-resistant HIV-1. Proc. Natl. Acad. Sci. 105, 20476–20481. https://doi.org/10.1073/pnas.0810843106

Heredia, A., Le, N., Gartenhaus, R.B., Sausville, E., Medina-Moreno, S., Zapata, J.C., Davis, C., Gallo, R.C., Redfield, R.R., 2015. Targeting of mTOR catalytic site inhibits multiple steps of the HIV-1 lifecycle and suppresses HIV-1 viremia in humanized mice. Proc. Natl. Acad. Sci. 112, 9412–9417. https://doi.org/10.1073/pnas.1511144112

Heusinger, E., Kirchhoff, F., 2017. Primate Lentiviruses Modulate NF-κB Activity by Multiple Mechanisms to Fine-Tune Viral and Cellular Gene Expression. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.00198

Hiener, B., Horsburgh, B.A., Eden, J.-S., Barton, K., Schlub, T.E., Lee, E., von Stockenstrom, S., Odevall, L., Milush, J.M., Liegler, T., Sinclair, E., Hoh, R., Boritz, E.A., Douek, D., Fromentin, R., Chomont, N., Deeks, S.G., Hecht, F.M., Palmer, S., 2017. Identification of Genetically Intact HIV-1 Proviruses in Specific CD4+ T Cells from Effectively Treated Participants. Cell Rep. 21, 813–822. https://doi.org/10.1016/j.celrep.2017.09.081

Hilditch, L., Towers, G.J., 2014. A model for cofactor use during HIV-1 reverse transcription and nuclear entry. Curr. Opin. Virol., Virus entry / Environmental virology 4, 32–36. https://doi.org/10.1016/j.coviro.2013.11.003

Hladik, F., McElrath, M.J., 2008. Setting the stage: host invasion by HIV. Nat. Rev. Immunol. 8, 447–457. https://doi.org/10.1038/nri2302

Hollenbaugh, J.A., Munger, J., Kim, B., 2011a. Metabolite profiles of human immunodeficiency virus infected CD4+ T cells and macrophages using LC–MS/MS analysis. Virology 415, 153–159. https://doi.org/10.1016/j.virol.2011.04.007

Hollenbaugh, J.A., Munger, J., Kim, B., 2011b. Metabolite profiles of human immunodeficiency virus infected CD4+ T cells and macrophages using LC–MS/MS analysis. Virology 415, 153–159. https://doi.org/10.1016/j.virol.2011.04.007

Holmes, R.K., Koning, F.A., Bishop, K.N., Malim, M.H., 2007a. APOBEC3F can inhibit the accumulation of HIV-1 reverse transcription products in the absence of hypermutation. Comparisons with APOBEC3G. J. Biol. Chem. 282, 2587–2595. https://doi.org/10.1074/jbc.M607298200

Holmes, R.K., Malim, M.H., Bishop, K.N., 2007b. APOBEC-mediated viral restriction: not simply editing? Trends Biochem. Sci. 32, 118–128. https://doi.org/10.1016/j.tibs.2007.01.004

Horiike, M., Iwami, S., Kodama, M., Sato, A., Watanabe, Y., Yasui, M., Ishida, Y., Kobayashi, T., Miura, T., Igarashi, T., 2012. Lymph nodes harbor viral reservoirs that cause rebound of plasma viremia in SIV-infected macaques upon cessation of combined antiretroviral therapy. Virology 423, 107–118. https://doi.org/10.1016/j.virol.2011.11.024

Hotter, D., Krabbe, T., Reith, E., Gawanbacht, A., Rahm, N., Ayouba, A., Driessche, B.V., Lint, C.V., Peeters, M., Kirchhoff, F., Sauter, D., 2017. Primate lentiviruses use at least three alternative strategies to suppress NF-κB-mediated immune activation. PLOS Pathog. 13, e1006598. https://doi.org/10.1371/journal.ppat.1006598

Hrecka, K., Hao, C., Gierszewska, M., Swanson, S.K., Kesik-Brodacka, M., Srivastava, S., Florens, L., Washburn, M.P., Skowronski, J., 2011. Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. Nature 474, 658–661. https://doi.org/10.1038/nature10195

Hu, J., Gardner, M.B., Miller, C.J., 2000. Simian Immunodeficiency Virus Rapidly Penetrates the Cervicovaginal Mucosa after Intravaginal Inoculation and Infects Intraepithelial Dendritic Cells. J. Virol. 74, 6087–6095. https://doi.org/10.1128/JVI.74.13.6087-6095.2000

Hu, Y., Yao, J., Liu, Z., Liu, X., Fu, H., Ye, K., 2005. Akt phosphorylates acinus and inhibits its proteolytic cleavage, preventing chromatin condensation. EMBO J. 24, 3543–3554. https://doi.org/10.1038/sj.emboj.7600823

Imai, K., Togami, H., Okamoto, T., 2010. Involvement of Histone H3 Lysine 9 (H3K9) Methyltransferase G9a in the Maintenance of HIV-1 Latency and Its Reactivation by BIX01294. J. Biol. Chem. 285, 16538–16545. https://doi.org/10.1074/jbc.M110.103531

Imre, G., Berthelet, J., Heering, J., Kehrloesser, S., Melzer, I.M., Lee, B.I., Thiede, B., Dötsch, V., Rajalingam, K., 2017. Apoptosis inhibitor 5 is an endogenous inhibitor of caspase-2. EMBO Rep. 18, 733–744. https://doi.org/10.15252/embr.201643744

Izquierdo-Useros, N., Lorizate, M., McLaren, P.J., Telenti, A., Kräusslich, H.-G., Martinez-Picado, J., 2014. HIV-1 Capture and Transmission by Dendritic Cells: The Role of Viral Glycolipids and the Cellular Receptor Siglec-1. PLoS Pathog. 10. https://doi.org/10.1371/journal.ppat.1004146

Jablonski, J.A., Amelio, A.L., Giacca, M., Caputi, M., 2010. The transcriptional transactivator Tat selectively regulates viral splicing. Nucleic Acids Res. 38, 1249–1260. https://doi.org/10.1093/nar/gkp1105

Jacobs, S.R., Herman, C.E., MacIver, N.J., Wofford, J.A., Wieman, H.L., Hammen, J.J., Rathmell, J.C., 2008. Glucose Uptake Is Limiting in T Cell Activation and Requires CD28-Mediated Akt-Dependent and Independent Pathways. J. Immunol. 180, 4476–4486. https://doi.org/10.4049/jimmunol.180.7.4476

Jacquelin, B., Mayau, V., Targat, B., Liovat, A.-S., Kunkel, D., Petitjean, G., Dillies, M.-A., Roques, P., Butor, C., Silvestri, G., Giavedoni, L.D., Lebon, P., Barré-Sinoussi, F., Benecke, A., Müller-Trutwin, M.C., 2009. Nonpathogenic SIV infection of African green monkeys induces a strong but rapidly controlled type I IFN response. J. Clin. Invest. 119, 3544–3555. https://doi.org/10.1172/JCI40093

Jacquelin, B., Zahn, R.C., Barré-Sinoussi, F., Schmitz, J.E., Kaur, A., Müller-Trutwin, M.C., 2012. Chapter 2 - Natural SIV Infection: Immunological Aspects, in: Models of Protection Against HIV/SIV. Academic Press, Boston, pp. 47–79. https://doi.org/10.1016/B978-0-12-387715-4.00002-2

Jagot-Lacoussiere, L., Kotula, E., Villoutreix, B.O., Bruzzoni-Giovanelli, H., Poyet, J.-L., 2016. A Cell-Penetrating Peptide Targeting AAC-11 Specifically Induces Cancer Cells Death. Cancer Res. 76, 5479–5490. https://doi.org/10.1158/0008-5472.CAN-16-0302

Jakobsen, M.R., Bak, R.O., Andersen, A., Berg, R.K., Jensen, S.B., Jin, T., Laustsen, A., Hansen, K., Østergaard, L., Fitzgerald, K.A., Xiao, T.S., Mikkelsen, J.G., Mogensen, T.H., Paludan, S.R., 2013. IFI16 senses DNA forms of the lentiviral replication cycle and controls HIV-1 replication. Proc. Natl. Acad. Sci. 110, E4571–E4580. https://doi.org/10.1073/pnas.1311669110

Jakobsen, M.R., Paludan, S.R., 2014. IFI16: At the interphase between innate DNA sensing and genome regulation. Cytokine Growth Factor Rev., Special issue: Innate Sensing and Response to Pathogens 25, 649–655. https://doi.org/10.1016/j.cytogfr.2014.06.004

Jambo, K.C., Banda, D.H., Kankwatira, A.M., Sukumar, N., Allain, T.J., Heyderman, R.S., Russell, D.G., Mwandumba, H.C., 2014. Small alveolar macrophages are infected preferentially by HIV and exhibit impaired phagocytic function. Mucosal Immunol. 7, 1116–1126. https://doi.org/10.1038/mi.2013.127

Jáuregui, P., Landau, N.R., 2018. DNA damage induces a SAMHD1-mediated block to the infection of macrophages by HIV-1. Sci. Rep. 8, 4153. https://doi.org/10.1038/s41598-018-22432-4

Jayakumar, P., Berger, I., Autschbach, F., Weinstein, M., Funke, B., Verdin, E., Goldsmith, M.A., Keppler, O.T., 2005. Tissue-Resident Macrophages Are Productively Infected Ex Vivo by Primary X4 Isolates of Human Immunodeficiency Virus Type 1. J. Virol. 79, 5220–5226. https://doi.org/10.1128/JVI.79.8.5220-5226.2005

Jekle, A., Keppler, O.T., Clercq, E.D., Schols, D., Weinstein, M., Goldsmith, M.A., 2003. In Vivo Evolution of Human Immunodeficiency Virus Type 1 toward Increased Pathogenicity through CXCR4-Mediated Killing of Uninfected CD4 T Cells. J. Virol. 77, 5846–5854. https://doi.org/10.1128/JVI.77.10.5846-5854.2003

Jia, B., Serra-Moreno, R., Jr, W.N., Rahmberg, A., Mackey, J., Fofana, I.B., Johnson, W.E., Westmoreland, S., Evans, D.T., 2009. Species-Specific Activity of SIV Nef and HIV-1 Vpu in Overcoming Restriction by Tetherin/BST2. PLOS Pathog. 5, e1000429. https://doi.org/10.1371/journal.ppat.1000429

Jones, R.G., Pearce, E.J., 2017. MenTORing Immunity: mTOR Signaling in the Development and Function of Tissue-Resident Immune Cells. Immunity 46, 730–742. https://doi.org/10.1016/j.immuni.2017.04.028

Jorgensen, I., Miao, E.A., 2015. Pyroptotic cell death defends against intracellular pathogens. Immunol. Rev. 265, 130–142. https://doi.org/10.1111/imr.12287

Joselin, A.P., Schulze-Osthoff, K., Schwerk, C., 2006. Loss of Acinus Inhibits Oligonucleosomal DNA Fragmentation but Not Chromatin Condensation during Apoptosis. J. Biol. Chem. 281, 12475–12484. https://doi.org/10.1074/jbc.M509859200

Karn, J., Stoltzfus, C.M., 2012. Transcriptional and Posttranscriptional Regulation of HIV-1 Gene Expression. Cold Spring Harb. Perspect. Med. 2, a006916. https://doi.org/10.1101/cshperspect.a006916

Kim, E.-Y., Lorenzo-Redondo, R., Little, S.J., Chung, Y.-S., Phalora, P.K., Berry, I.M., Archer, J., Penugonda, S., Fischer, W., Richman, D.D., Bhattacharya, T., Malim, M.H., Wolinsky, S.M., 2014. Human APOBEC3 Induced Mutation of Human Immunodeficiency Virus Type-1 Contributes to Adaptation and Evolution in Natural Infection. PLOS Pathog. 10, e1004281. https://doi.org/10.1371/journal.ppat.1004281

Kim, J.T., Chang, E., Sigal, A., Baltimore, D., 2018. Dendritic cells efficiently transmit HIV to T Cells in a tenofovir and raltegravir insensitive manner. PloS One 13, e0189945. https://doi.org/10.1371/journal.pone.0189945

Kivisäkk, P., Mahad, D.J., Callahan, M.K., Trebst, C., Tucky, B., Wei, T., Wu, L., Baekkevold, E.S., Lassmann, H., Staugaitis, S.M., Campbell, J.J., Ransohoff, R.M., 2003. Human cerebrospinal fluid central memory CD4+ T cells: Evidence for trafficking through choroid plexus and meninges via P-selectin. Proc. Natl. Acad. Sci. 100, 8389–8394. https://doi.org/10.1073/pnas.1433000100

Kluge, S.F., Sauter, D., Kirchhoff, F., 2015. SnapShot: Antiviral Restriction Factors. Cell 163, 774-774.e1. https://doi.org/10.1016/j.cell.2015.10.019

Koci, L., Chlebova, K., Hyzdalova, M., Hofmanova, J., Jira, M., Kysela, P., Kozubik, A., Kala, Z., Krejci, P., 2012. Apoptosis inhibitor 5 (API-5; AAC-11; FIF) is upregulated in human carcinomas in vivo. Oncol. Lett. 3, 913–916. https://doi.org/10.3892/ol.2012.593

Kondratskyi, A., Kondratska, K., Skryma, R., Prevarskaya, N., 2015. Ion channels in the regulation of apoptosis. Biochim. Biophys. Acta BBA - Biomembr., Membrane Channels and Transporters in Cancers 1848, 2532–2546. https://doi.org/10.1016/j.bbamem.2014.10.030

Kruiswijk, F., Labuschagne, C.F., Vousden, K.H., 2015. p53 in survival, death and metabolic health: a lifeguard with a licence to kill. Nat. Rev. Mol. Cell Biol. 16, 393–405. https://doi.org/10.1038/nrm4007

Kumar, B., Arora, S., Ahmed, S., Banerjea, A.C., 2016. Hyperactivation of mammalian target of rapamycin complex 1 by HIV-1 is necessary for virion production and latent viral reactivation. FASEB J. 31, 180–191. https://doi.org/10.1096/fj.201600813R

Kunzelmann, K., 2016. Ion channels in regulated cell death. Cell. Mol. Life Sci. 73, 2387–2403. https://doi.org/10.1007/s00018-016-2208-z

Kuo, L.J., Yang, L.-X., 2008. Gamma-H2AX - a novel biomarker for DNA double-strand breaks. Vivo Athens Greece 22, 305–309.

Kupzig, S., Korolchuk, V., Rollason, R., Sugden, A., Wilde, A., Banting, G., 2003. Bst-2/HM1.24 Is a Raft-Associated Apical Membrane Protein with an Unusual Topology. Traffic 4, 694–709. https://doi.org/10.1034/j.1600-0854.2003.00129.x

Kwon, D.S., Gregorio, G., Bitton, N., Hendrickson, W.A., Littman, D.R., 2002. DC-SIGN-Mediated Internalization of HIV Is Required for Trans-Enhancement of T Cell Infection. Immunity 16, 135–144. https://doi.org/10.1016/S1074-7613(02)00259-5

Laguette, N., Sobhian, B., Casartelli, N., Ringeard, M., Chable-Bessia, C., Ségéral, E., Yatim, A., Emiliani, S., Schwartz, O., Benkirane, M., 2011. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature 474, 654–657. https://doi.org/10.1038/nature10117

Lahouassa, H., Daddacha, W., Hofmann, H., Ayinde, D., Logue, E.C., Dragin, L., Bloch, N., Maudet, C., Bertrand, M., Gramberg, T., Pancino, G., Priet, S., Canard, B., Laguette, N., Benkirane, M., Transy, C., Landau, N.R., Kim, B., Margottin-Goguet, F., 2012. SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates. Nat. Immunol. 13, 223–228. https://doi.org/10.1038/ni.2236

Lambotte, O., Taoufik, Y., De, M.G., Wallon, C., Goujard, C., Delfraissy, J.F., 2000. Detection of infectious HIV in circulating monocytes from patients on prolonged highly active antiretroviral therapy. J. Acquir. Immune Defic. Syndr. 1999 23, 114–119. https://doi.org/10.1097/00126334-200002010-00002

Lange, J.M., Ananworanich, J., 2014. The discovery and development of antiretroviral agents. Antivir. Ther. 19, 5–14. https://doi.org/10.3851/IMP2896

Lannes, N., Eppler, E., Etemad, S., Yotovski, P., Filgueira, L., 2017. Microglia at center stage: a comprehensive review about the versatile and unique residential macrophages of the central nervous system. Oncotarget 8, 114393–114413. https://doi.org/10.18632/oncotarget.23106

Lavi, E., Strizki, J.M., Ulrich, A.M., Zhang, W., Fu, L., Wang, Q., O'Connor, M., Hoxie, J.A., González-Scarano, F., 1997. CXCR-4 (Fusin), a co-receptor for the type 1 human immunodeficiency virus (HIV-1), is expressed in the human brain in a variety of cell types, including microglia and neurons. Am. J. Pathol. 151, 1035–1042.

Lee, B., Sharron, M., Montaner, L.J., Weissman, D., Doms, R.W., 1999. Quantification of CD4, CCR5, and CXCR4 levels on lymphocyte subsets, dendritic cells, and differentially conditioned monocyte-derived macrophages. Proc. Natl. Acad. Sci. 96, 5215–5220. https://doi.org/10.1073/pnas.96.9.5215

Lehrman, G., Hogue, I.B., Palmer, S., Jennings, C., Spina, C.A., Wiegand, A., Landay, A.L., Coombs, R.W., Richman, D.D., Mellors, J.W., Coffin, J.M., Bosch, R.J., Margolis, D.M.,

2005. Depletion of latent HIV-1 infection in vivo: a proof-of-concept study. The Lancet 366, 549–555. https://doi.org/10.1016/S0140-6736(05)67098-5

Leng, J., Ho, H.-P., Buzon, M.J., Pereyra, F., Walker, B.D., Yu, X.G., Chang, E.J., Lichterfeld, M., 2014. A Cell-Intrinsic Inhibitor of HIV-1 Reverse Transcription in CD4+ T Cells from Elite Controllers. Cell Host Microbe 15, 717–728. https://doi.org/10.1016/j.chom.2014.05.011

Lindgren, A.A., Filipowicz, A.R., Hattler, J.B., Kim, S.O., Chung, H.K., Kuroda, M.J., Johnson, E.M., Kim, W., 2018. Lentiviral infection of proliferating brain macrophages in Hiv and simian immunodeficiency virus encephalitis despite sterile alpha motif and histidine-aspartate domain-containing protein 1 expression. Aids 32, 965–974. https://doi.org/10.1097/QAD.0000000000001793

Llano, M., Delgado, S., Vanegas, M., Poeschla, E.M., 2004. Lens Epithelium-derived Growth Factor/p75 Prevents Proteasomal Degradation of HIV-1 Integrase. J. Biol. Chem. 279, 55570–55577. https://doi.org/10.1074/jbc.M408508200

Llewellyn, G.N., Alvarez-Carbonell, D., Chateau, M., Karn, J., Cannon, P.M., 2018. HIV-1 infection of microglial cells in a reconstituted humanized mouse model and identification of compounds that selectively reverse HIV latency. J. Neurovirol. 24, 192–203. https://doi.org/10.1007/s13365-017-0604-2

Loisel-Meyer, S., Swainson, L., Craveiro, M., Oburoglu, L., Mongellaz, C., Costa, C., Martinez, M., Cosset, F.-L., Battini, J.-L., Herzenberg, Leonard A., Herzenberg, Leonore A., Atkuri, K.R., Sitbon, M., Kinet, S., Verhoeyen, E., Taylor, N., 2012a. Glut1-mediated glucose transport regulates HIV infection. Proc. Natl. Acad. Sci. 109, 2549–2554. https://doi.org/10.1073/pnas.1121427109

Loisel-Meyer, S., Swainson, L., Craveiro, M., Oburoglu, L., Mongellaz, C., Costa, C., Martinez, M., Cosset, F.-L., Battini, J.-L., Herzenberg, Leonard A., Herzenberg, Leonore A., Atkuri, K.R., Sitbon, M., Kinet, S., Verhoeyen, E., Taylor, N., 2012b. Glut1-mediated glucose transport regulates HIV infection. Proc. Natl. Acad. Sci. 109, 2549–2554. https://doi.org/10.1073/pnas.1121427109

Lorenzo-Redondo, R., Fryer, H.R., Bedford, T., Kim, E.-Y., Archer, J., Kosakovsky Pond, S.L., Chung, Y.-S., Penugonda, S., Chipman, J.G., Fletcher, C.V., Schacker, T.W., Malim, M.H., Rambaut, A., Haase, A.T., McLean, A.R., Wolinsky, S.M., 2016. Persistent HIV-1 replication maintains the tissue reservoir during therapy. Nature 530, 51–56. https://doi.org/10.1038/nature16933

Lu, J., Pan, Q., Rong, L., Liu, S.-L., Liang, C., 2011. The IFITM Proteins Inhibit HIV-1 Infection. J. Virol. 85, 2126–2137. https://doi.org/10.1128/JVI.01531-10

Lugli, E., Dominguez, M.H., Gattinoni, L., Chattopadhyay, P.K., Bolton, D.L., Song, K., Klatt, N.R., Brenchley, J.M., Vaccari, M., Gostick, E., Price, D.A., Waldmann, T.A., Restifo, N.P., Franchini, G., Roederer, M., 2013. Superior T memory stem cell persistence supports long-lived T cell memory. J. Clin. Invest. 123. https://doi.org/10.1172/JCI66327

Lusic, M., Siliciano, R.F., 2017. Nuclear landscape of HIV-1 infection and integration. Nat. Rev. Microbiol. 15, 69–82. https://doi.org/10.1038/nrmicro.2016.162

Macallan, D.C., Borghans, J.A.M., Asquith, B., 2017. Human T Cell Memory: A Dynamic View. Vaccines 5. https://doi.org/10.3390/vaccines5010005

Mahnke, Y.D., Brodie, T.M., Sallusto, F., Roederer, M., Lugli, E., 2013. The who's who of T-cell differentiation: Human memory T-cell subsets. Eur. J. Immunol. 43, 2797–2809. https://doi.org/10.1002/eji.201343751

Malim, M.H., Bieniasz, P.D., 2012. HIV Restriction Factors and Mechanisms of Evasion. Cold Spring Harb. Perspect. Med. 2, a006940. https://doi.org/10.1101/cshperspect.a006940

Man, S.M., Kanneganti, T.-D., 2016. Converging roles of caspases in inflammasome activation, cell death and innate immunity. Nat. Rev. Immunol. 16, 7–21. https://doi.org/10.1038/nri.2015.7

Manel, N., Hogstad, B., Wang, Y., Levy, D.E., Unutmaz, D., Littman, D.R., 2010. A cryptic sensor for HIV-1 activates antiviral innate immunity in dendritic cells. Nature 467, 214–217. https://doi.org/10.1038/nature09337

Mangeat, B., Turelli, P., Caron, G., Friedli, M., Perrin, L., Trono, D., 2003. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. Nature 424, 99–103. https://doi.org/10.1038/nature01709

Marin, M., Rose, K.M., Kozak, S.L., Kabat, D., 2003. HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation. Nat. Med. 9, 1398–1403. https://doi.org/10.1038/nm946

Marini, B., Kertesz-Farkas, A., Ali, H., Lucic, B., Lisek, K., Manganaro, L., Pongor, S., Luzzati, R., Recchia, A., Mavilio, F., Giacca, M., Lusic, M., 2015. Nuclear architecture dictates HIV-1 integration site selection. Nature 521, 227–231. https://doi.org/10.1038/nature14226

Mascio, M.D., Srinivasula, S., Bhattacharjee, A., Cheng, L., Martiniova, L., Herscovitch, P., Lertora, J., Kiesewetter, D., 2009. Antiretroviral Tissue Kinetics: In Vivo Imaging Using Positron Emission Tomography. Antimicrob. Agents Chemother. 53, 4086–4095. https://doi.org/10.1128/AAC.00419-09

Matusali, G., Dereuddre-Bosquet, N., Tortorec, A.L., Moreau, M., Satie, A.-P., Mahé, D., Roumaud, P., Bourry, O., Sylla, N., Bernard-Stoecklin, S., Pruvost, A., Grand, R.L., Dejucq-Rainsford, N., 2015. Detection of Simian Immunodeficiency Virus in Semen, Urethra, and Male Reproductive Organs during Efficient Highly Active Antiretroviral Therapy. J. Virol. 89, 5772–5787. https://doi.org/10.1128/JVI.03628-14

Mbisa, J.L., Barr, R., Thomas, J.A., Vandegraaff, N., Dorweiler, I.J., Svarovskaia, E.S., Brown, W.L., Mansky, L.M., Gorelick, R.J., Harris, R.S., Engelman, A., Pathak, V.K., 2007. Human Immunodeficiency Virus Type 1 cDNAs Produced in the Presence of APOBEC3G Exhibit Defects in Plus-Strand DNA Transfer and Integration. J. Virol. 81, 7099–7110. https://doi.org/10.1128/JVI.00272-07

McMichael, A.J., Borrow, P., Tomaras, G.D., Goonetilleke, N., Haynes, B.F., 2010. The immune response during acute HIV-1 infection: clues for vaccine development. Nat. Rev. Immunol. 10, 11–23. https://doi.org/10.1038/nri2674

Meehan, A.M., Saenz, D.T., Morrison, J.H., Garcia-Rivera, J.A., Peretz, M., Llano, M., Poeschla, E.M., 2009. LEDGF/p75 Proteins with Alternative Chromatin Tethers Are Functional HIV-1 Cofactors. PLOS Pathog. 5, e1000522. https://doi.org/10.1371/journal.ppat.1000522

Miles, M.A., Kitevska-Ilioski, T., Hawkins, C.J., 2017. Chapter Three - Old and Novel Functions of Caspase-2, in: Galluzzi, L. (Ed.), International Review of Cell and Molecular Biology. Academic Press, pp. 155–212. https://doi.org/10.1016/bs.ircmb.2016.12.002

Mitri, D.D., Azevedo, R.I., Henson, S.M., Libri, V., Riddell, N.E., Macaulay, R., Kipling, D., Soares, M.V.D., Battistini, L., Akbar, A.N., 2011. Reversible Senescence in Human CD4+CD45RA+CD27− Memory T Cells. J. Immunol. 187, 2093–2100. https://doi.org/10.4049/jimmunol.1100978

Mlcochova, P., Caswell, S.J., Taylor, I.A., Towers, G.J., Gupta, R.K., 2018. DNA damage induced by topoisomerase inhibitors activates SAMHD1 and blocks HIV-1 infection of macrophages. EMBO J. 37, 50–62. https://doi.org/10.15252/embj.201796880

Monroe, K.M., Yang, Z., Johnson, J.R., Geng, X., Doitsh, G., Krogan, N.J., Greene, W.C., 2014. IFI16 DNA Sensor Is Required for Death of Lymphoid CD4 T Cells Abortively Infected with HIV. Science 343, 428–432. https://doi.org/10.1126/science.1243640

Moreno-Gonzalez, G., Vandenabeele, P., Krysko, D.V., 2016. Necroptosis: A Novel Cell Death Modality and Its Potential Relevance for Critical Care Medicine. Am. J. Respir. Crit. Care Med. 194, 415–428. https://doi.org/10.1164/rccm.201510-2106CI

Morris, E.J., Michaud, W.A., Ji, J.-Y., Moon, N.-S., Rocco, J.W., Dyson, N.J., 2006. Functional Identification of Api5 as a Suppressor of E2F-Dependent Apoptosis In Vivo. PLOS Genet 2, e196. https://doi.org/10.1371/journal.pgen.0020196

Mueller, S.N., Gebhardt, T., Carbone, F.R., Heath, W.R., 2013. Memory T Cell Subsets, Migration Patterns, and Tissue Residence. Annu. Rev. Immunol. 31, 137–161. https://doi.org/10.1146/annurev-immunol-032712-095954

Mueller, S.N., Mackay, L.K., 2016. Tissue-resident memory T cells: local specialists in immune defence. Nat. Rev. Immunol. 16, 79–89. https://doi.org/10.1038/nri.2015.3

Mukerjee, R., Claudio, P.P., Chang, J.R., Del Valle, L., Sawaya, B.E., 2010. Transcriptional regulation of HIV-1 gene expression by p53. Cell Cycle Georget. Tex 9, 4569–4578. https://doi.org/10.4161/cc.9.22.13836

Muñoz-Arias, I., Doitsh, G., Yang, Z., Sowinski, S., Ruelas, D., Greene, W.C., 2015. Blood-Derived CD4 T Cells Naturally Resist Pyroptosis during Abortive HIV-1 Infection. Cell Host Microbe 18, 463–470. https://doi.org/10.1016/j.chom.2015.09.010

Muthumani, K., Choo, A.Y., Hwang, D.S., Premkumar, A., Dayes, N.S., Harris, C., Green, D.R., Wadsworth, S.A., Siekierka, J.J., Weiner, D.B., 2005. HIV-1 Nef-induced FasL induction and bystander killing requires p38 MAPK activation. Blood 106, 2059–2068. https://doi.org/10.1182/blood-2005-03-0932

Muthumani, K., Hwang, D.S., Desai, B.M., Zhang, D., Dayes, N., Green, D.R., Weiner, D.B., 2002. HIV-1 Vpr Induces Apoptosis through Caspase 9 in T Cells and Peripheral Blood Mononuclear Cells. J. Biol. Chem. 277, 37820–37831.

Nabel, G., Baltimore, D., 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature 326, 711–713. https://doi.org/10.1038/326711a0

Nakano, Y., Aso, H., Soper, A., Yamada, E., Moriwaki, M., Juarez-Fernandez, G., Koyanagi, Y., Sato, K., 2017. A conflict of interest: the evolutionary arms race between mammalian APOBEC3 and lentiviral Vif. Retrovirology 14, 31. https://doi.org/10.1186/s12977-017-0355-4

Ndhlovu, Z.M., Kamya, P., Mewalal, N., Kløverpris, H.N., Nkosi, T., Pretorius, K., Laher, F., Ogunshola, F., Chopera, D., Shekhar, K., Ghebremichael, M., Ismail, N., Moodley, A., Malik, A., Leslie, A., Goulder, P.J.R., Buus, S., Chakraborty, A., Dong, K., Ndung'u, T., Walker, B.D., 2015. Magnitude and Kinetics of CD8+ T Cell Activation during Hyperacute HIV Infection Impact Viral Set Point. Immunity 43, 591–604. https://doi.org/10.1016/j.immuni.2015.08.012

Ne, E., Palstra, R.-J., Mahmoudi, T., 2018. Chapter Six - Transcription: Insights From the HIV-1 Promoter, in: Loos, F. (Ed.), International Review of Cell and Molecular Biology, Transcriptional Gene Regulation in Health and Disease. Academic Press, pp. 191–243. https://doi.org/10.1016/bs.ircmb.2017.07.011

Neil, S.J.D., Eastman, S.W., Jouvenet, N., Bieniasz, P.D., 2006. HIV-1 Vpu Promotes Release and Prevents Endocytosis of Nascent Retrovirus Particles from the Plasma Membrane. PLoS Pathog 2, e39. https://doi.org/10.1371/journal.ppat.0020039

Neil, S.J.D., Sandrin, V., Sundquist, W.I., Bieniasz, P.D., 2007. An Interferon-α-Induced Tethering Mechanism Inhibits HIV-1 and Ebola Virus Particle Release but Is Counteracted by the HIV-1 Vpu Protein. Cell Host Microbe 2, 193–203. https://doi.org/10.1016/j.chom.2007.08.001

Neil, S.J.D., Zang, T., Bieniasz, P.D., 2008. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. Nature 451, 425–430. https://doi.org/10.1038/nature06553

Ohnimus, H., Heinkelein, M., Jassoy, C., 1997. Apoptotic cell death upon contact of CD4+ T lymphocytes with HIV glycoprotein-expressing cells is mediated by caspases but bypasses CD95 (Fas/Apo-1) and TNF receptor 1. J. Immunol. 159, 5246–5252.

Oliver, T.G., Meylan, E., Chang, G.P., Xue, W., Burke, J.R., Humpton, T.J., Hubbard, D., Bhutkar, A., Jacks, T., 2011. Caspase-2-Mediated Cleavage of Mdm2 Creates a p53-Induced Positive Feedback Loop. Mol. Cell 43, 57–71. https://doi.org/10.1016/j.molcel.2011.06.012

Olsson, M., Forsberg, J., Zhivotovsky, B., 2015. Caspase-2: the reinvented enzyme. Oncogene 34, 1877–1882. https://doi.org/10.1038/onc.2014.139

Olvera-García, G., Aguilar-García, T., Gutiérrez-Jasso, F., Imaz-Rosshandler, I., Rangel-Escareño, C., Orozco, L., Aguilar-Delfín, I., Vázquez-Pérez, J.A., Zúñiga, J., Pérez-Patrigeon, S., Espinosa, E., 2016. A transcriptome-based model of central memory CD4 T cell death in HIV infection. BMC Genomics 17, 956. https://doi.org/10.1186/s12864-016-3308-8

Ono, A., Ablan, S.D., Lockett, S.J., Nagashima, K., Freed, E.O., 2004. Phosphatidylinositol (4,5) bisphosphate regulates HIV-1 Gag targeting to the plasma membrane. Proc. Natl. Acad. Sci. U. S. A. 101, 14889–14894. https://doi.org/10.1073/pnas.0405596101

Ospina Stella, A., Turville, S., 2018. All-Round Manipulation of the Actin Cytoskeleton by HIV. Viruses 10, 63. https://doi.org/10.3390/v10020063

Pache, L., König, R., Chanda, S.K., 2011. Identifying HIV-1 host cell factors by genome-scale RNAi screening. Methods, Methods Related to the Molecular Virology of HIV 53, 3–12. https://doi.org/10.1016/j.ymeth.2010.07.009

Palmer, C., Jaworowski, A., McCune, J., Crowe, S., 2012. Glut1: establishing a new paradigm for HIV-1 infection by regulating glucose metabolism and activation in CD4+ T cells in HIV-1-positive subjects. J Int AIDS Soc 15, 0104.

Palmer, C.S., Crowe, S.M., 2016. Immunometabolism may provide new insights into novel mechanisms of HIV reservoir persistence: AIDS 30, 2895–2896. https://doi.org/10.1097/QAD.0000000000001114

Palmer, C.S., Duette, G.A., Wagner, M.C.E., Henstridge, D.C., Saleh, S., Pereira, C., Zhou, J., Simar, D., Lewin, S.R., Ostrowski, M., McCune, J.M., Crowe, S.M., 2017. Metabolically active CD4+ T cells expressing Glut1 and OX40 preferentially harbor HIV during in vitro infection. FEBS Lett. 591, 3319–3332. https://doi.org/10.1002/1873-3468.12843

Palmer, C.S., Palchaudhuri, R., Albargy, H., Abdel-Mohsen, M., Crowe, S.M., 2018. Exploiting immune cell metabolic machinery for functional HIV cure and the prevention of

inflammaging. F1000Research 7, 125. https://doi.org/10.12688/f1000research.11881.1
Palmisano, L., Vella, S., 2011. A brief history of antiretroviral therapy of HIV infection: success and challenges. Ann Ist Super Sanita 47, 44–48.
Pauls, E., Ruiz, A., Riveira-Muñoz, E., Permanyer, M., Badia, R., Clotet, B., Keppler, O.T., Ballana, E., Este, J.A., 2014. p21 regulates the HIV-1 restriction factor SAMHD1. Proc. Natl. Acad. Sci. 111, E1322–E1324. https://doi.org/10.1073/pnas.1322059111
Pearce, E.L., Poffenberger, M.C., Chang, C.-H., Jones, R.G., 2013. Fueling Immunity: Insights into Metabolism and Lymphocyte Function. Science 342, 1242454. https://doi.org/10.1126/science.1242454
Pereira, L.A., Bentley, K., Peeters, A., Churchill, M.J., Deacon, N.J., 2000. SURVEY AND SUMMARY A compilation of cellular transcription factor interactions with the HIV-1 LTR promoter. Nucleic Acids Res. 28, 663–668. https://doi.org/10.1093/nar/28.3.663
Perelson, A.S., Essunger, P., Cao, Y., Vesanen, M., Hurley, A., Saksela, K., Markowitz, M., Ho, D.D., 1997. Decay characteristics of HIV-1-infected compartments during combination therapy. Nature 387, 188–191. https://doi.org/10.1038/387188a0
Perfettini, J.-L., Castedo, M., Nardacci, R., Ciccosanti, F., Boya, P., Roumier, T., Larochette, N., Piacentini, M., Kroemer, G., 2005. Essential role of p53 phosphorylation by p38 MAPK in apoptosis induction by the HIV-1 envelope. J. Exp. Med. 201, 279–289. https://doi.org/10.1084/jem.20041502
Perfettini, J.-L., Roumier, T., Castedo, M., Larochette, N., Boya, P., Raynal, B., Lazar, V., Ciccosanti, F., Nardacci, R., Penninger, J., Piacentini, M., Kroemer, G., 2004. NF-κB and p53 Are the Dominant Apoptosis-inducing Transcription Factors Elicited by the HIV-1 Envelope. J. Exp. Med. 199, 629–640. https://doi.org/10.1084/jem.20031216
Perreau, M., Savoye, A.-L., Crignis, E.D., Corpataux, J.-M., Cubas, R., Haddad, E.K., Leval, L.D., Graziosi, C., Pantaleo, G., 2013. Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. J. Exp. Med. 210, 143–156. https://doi.org/10.1084/jem.20121932
Poeschla, E.M., 2008. Integrase, LEDGF/p75 and HIV Replication. Cell. Mol. Life Sci. CMLS 65, 1403–1424. https://doi.org/10.1007/s00018-008-7540-5
Popov, S., Rexach, M., Zybarth, G., Reiling, N., Lee, M.-A., Ratner, L., Lane, C.M., Moore, M.S., Blobel, G., Bukrinsky, M., 1998. Viral protein R regulates nuclear import of the HIV-1 pre-integration complex. EMBO J. 17, 909–917. https://doi.org/10.1093/emboj/17.4.909
Powell, R.D., Holland, P.J., Hollis, T., Perrino, F.W., 2011. Aicardi-Goutières Syndrome Gene and HIV-1 Restriction Factor SAMHD1 Is a dGTP-regulated Deoxynucleotide Triphosphohydrolase. J. Biol. Chem. 286, 43596–43600. https://doi.org/10.1074/jbc.C111.317628
Rai, P., Plagov, A., Kumar, D., Pathak, S., Ayasolla, K.R., Chawla, A.K., Mathieson, P.W., Saleem, M.A., Husain, M., Malhotra, A., Singhal, P.C., 2013. Rapamycin-induced modulation of HIV gene transcription attenuates progression of HIVAN. Exp. Mol. Pathol. 94, 255–261. https://doi.org/10.1016/j.yexmp.2012.09.009
Ramirez, M.L.G., Salvesen, G.S., 2018. A primer on caspase mechanisms. Semin. Cell Dev. Biol. https://doi.org/10.1016/j.semcdb.2018.01.002
Rasmussen, T.A., Lewin, S.R., 2016. Shocking HIV out of hiding: where are we with clinical trials of latency reversing agents? Curr. Opin. HIV AIDS 11, 394–401. https://doi.org/10.1097/COH.0000000000000279

Rasmussen, T.A., Tolstrup, M., Brinkmann, C.R., Olesen, R., Erikstrup, C., Solomon, A., Winckelmann, A., Palmer, S., Dinarello, C., Buzon, M., Lichterfeld, M., Lewin, S.R., Østergaard, L., Søgaard, O.S., 2014. Panobinostat, a histone deacetylase inhibitor, for latent-virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase 1/2, single group, clinical trial. Lancet HIV 1, e13–e21. https://doi.org/10.1016/S2352-3018(14)70014-1

Rato, S., Rausell, A., Muñoz, M., Telenti, A., Ciuffi, A., 2017. Single-cell analysis identifies cellular markers of the HIV permissive cell. PLOS Pathog. 13, e1006678. https://doi.org/10.1371/journal.ppat.1006678

Refsland, E.W., Stenglein, M.D., Shindo, K., Albin, J.S., Brown, W.L., Harris, R.S., 2010. Quantitative profiling of the full APOBEC3 mRNA repertoire in lymphocytes and tissues: implications for HIV-1 restriction. Nucleic Acids Res. 38, 4274–4284. https://doi.org/10.1093/nar/gkq174

Rigou, P., Piddubnyak, V., Faye, A., Rain, J.-C., Michel, L., Calvo, F., Poyet, J.-L., 2009. The antiapoptotic protein AAC-11 interacts with and regulates Acinus-mediated DNA fragmentation. EMBO J. 28, 1576–1588. https://doi.org/10.1038/emboj.2009.106

Rodor, J., Pan, Q., Blencowe, B.J., Eyras, E., Cáceres, J.F., 2016. The RNA-binding profile of Acinus, a peripheral component of the exon junction complex, reveals its role in splicing regulation. RNA 22, 1411–1426. https://doi.org/10.1261/rna.057158.116

Rosa, A., Chande, A., Ziglio, S., Sanctis, V.D., Bertorelli, R., Goh, S.L., McCauley, S.M., Nowosielska, A., Antonarakis, S.E., Luban, J., Santoni, F.A., Pizzato, M., 2015. HIV-1 Nef promotes infection by excluding SERINC5 from virion incorporation. Nature 526, 212–217. https://doi.org/10.1038/nature15399

Routy, J., Tremblay, C., Angel, J., Trottier, B., Rouleau, D., Baril, J., Harris, M., Trottier, S., Singer, J., Chomont, N., Sékaly, R., Boulassel, M., 2012. Valproic acid in association with highly active antiretroviral therapy for reducing systemic HIV-1 reservoirs: results from a multicentre randomized clinical study. HIV Med. 13, 291–296. https://doi.org/10.1111/j.1468-1293.2011.00975.x

Roy, J., Paquette, J.-S., Fortin, J.-F., Tremblay, M.J., 2002. The Immunosuppressant Rapamycin Represses Human Immunodeficiency Virus Type 1 Replication. Antimicrob. Agents Chemother. 46, 3447–3455. https://doi.org/10.1128/AAC.46.11.3447-3455.2002

Ruelas, D.S., Greene, W.C., 2013. An Integrated Overview of HIV-1 Latency. Cell 155, 519–529. https://doi.org/10.1016/j.cell.2013.09.044

Russell, R.A., Chojnacki, J., Jones, D.M., Johnson, E., Do, T., Eggeling, C., Padilla-Parra, S., Sattentau, Q.J., 2017. Astrocytes Resist HIV-1 Fusion but Engulf Infected Macrophage Material. Cell Rep. 18, 1473–1483. https://doi.org/10.1016/j.celrep.2017.01.027

Ryoo, J., Choi, J., Oh, C., Kim, S., Seo, M., Kim, S.-Y., Seo, D., Kim, J., White, T.E., Brandariz-Nuñez, A., Diaz-Griffero, F., Yun, C.-H., Hollenbaugh, J.A., Kim, B., Baek, D., Ahn, K., 2014. The ribonuclease activity of SAMHD1 is required for HIV-1 restriction. Nat. Med. 20, 936–941. https://doi.org/10.1038/nm.3626

Sabri, F., Tresoldi, E., Di Stefano, M., Polo, S., Monaco, M.C., Verani, A., Fiore, J.R., Lusso, P., Major, E., Chiodi, F., Scarlatti, G., 1999. Nonproductive Human Immunodeficiency Virus Type 1 Infection of Human Fetal Astrocytes: Independence from CD4 and Major Chemokine Receptors. Virology 264, 370–384. https://doi.org/10.1006/viro.1999.9998

Sáez-Cirión, A., Bacchus, C., Hocqueloux, L., Avettand-Fenoel, V., Girault, I., Lecuroux, C., Potard, V., Versmisse, P., Melard, A., Prazuck, T., Descours, B., Guergnon, J., Viard, J.-P., Boufassa, F., Lambotte, O., Goujard, C., Meyer, L., Costagliola, D., Venet, A., Pancino, G., Autran, B., Rouzioux, C., Group, the A.V.S., 2013. Post-Treatment HIV-1 Controllers with a Long-Term Virological Remission after the Interruption of Early Initiated Antiretroviral Therapy ANRS VISCONTI Study. PLOS Pathog. 9, e1003211. https://doi.org/10.1371/journal.ppat.1003211

Sáez-Cirión, A., Hamimi, C., Bergamaschi, A., David, A., Versmisse, P., Mélard, A., Boufassa, F., Barré-Sinoussi, F., Lambotte, O., Rouzioux, C., Pancino, G., Cohort, for the A.C., 2011. Restriction of HIV-1 replication in macrophages and CD4+ T cells from HIV controllers. Blood 118, 955–964. https://doi.org/10.1182/blood-2010-12-327106

Saez-Cirion, A., Jacquelin, B., Barré-Sinoussi, F., Müller-Trutwin, M., 2014. Immune responses during spontaneous control of HIV and AIDS: what is the hope for a cure? Phil Trans R Soc B 369, 20130436. https://doi.org/10.1098/rstb.2013.0436

Sahara, S., Aoto, M., Eguchi, Y., Imamoto, N., Yoneda, Y., Tsujimoto, Y., 1999. Acinus is a caspase-3-activated protein required for apoptotic chromatin condensation. Nature 401, 168–173. https://doi.org/10.1038/43678

Sallusto, F., Lenig, D., Förster, R., Lipp, M., Lanzavecchia, A., 1999. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 401, 708–712. https://doi.org/10.1038/44385

Sandler, N.G., Bosinger, S.E., Estes, J.D., Zhu, R.T.R., Tharp, G.K., Boritz, E., Levin, D., Wijeyesinghe, S., Makamdop, K.N., Prete, G.Q. del, Hill, B.J., Timmer, J.K., Reiss, E., Yarden, G., Darko, S., Contijoch, E., Todd, J.P., Silvestri, G., Nason, M., Jr, R.B.N., Keele, B.F., Rao, S., Langer, J.A., Lifson, J.D., Schreiber, G., Douek, D.C., 2014. Type I interferon responses in rhesus macaques prevent SIV infection and slow disease progression. Nature 511, 601–605. https://doi.org/10.1038/nature13554

Saphire, A.C., Bobardt, M.D., Zhang, Z., David, G., Gallay, P.A., 2001. Syndecans serve as attachment receptors for human immunodeficiency virus type 1 on macrophages. J. Virol. 75, 9187–9200. https://doi.org/10.1128/JVI.75.19.9187-9200.2001

Sasaki, H., Moriyama, S., Yukiue, H., Kobayashi, Y., Nakashima, Y., Kaji, M., Fukai, I., Kiriyama, M., Yamakawa, Y., Fujii, Y., 2001. Expression of the antiapoptosis gene, AAC-11, as a prognosis marker in non-small cell lung cancer. Lung Cancer 34, 53–57. https://doi.org/10.1016/S0169-5002(01)00213-6

Sauter, D., Hotter, D., Van Driessche, B., Stürzel, C.M., Kluge, S.F., Wildum, S., Yu, H., Baumann, B., Wirth, T., Plantier, J.-C., Leoz, M., Hahn, B.H., Van Lint, C., Kirchhoff, F., 2015. Differential Regulation of NF-κB-Mediated Proviral and Antiviral Host Gene Expression by Primate Lentiviral Nef and Vpu Proteins. Cell Rep. 10, 586–599. https://doi.org/10.1016/j.celrep.2014.12.047

Sauter, D., Kirchhoff, F., 2018. Multilayered and versatile inhibition of cellular antiviral factors by HIV and SIV accessory proteins. Cytokine Growth Factor Rev. https://doi.org/10.1016/j.cytogfr.2018.02.005

Sawchuk, R.J., Yang, Z., 1999. Investigation of distribution, transport and uptake of anti-HIV drugs to the central nervous system. Adv. Drug Deliv. Rev., Delivery of Anti-HIV Drugs 39, 5–31. https://doi.org/10.1016/S0169-409X(99)00017-4

Schott, K., König, R., 2017. Picking the Survivor! CRISPR Reveals HIV Dependency Factors. Trends Microbiol. 25, 243–245. https://doi.org/10.1016/j.tim.2017.02.004

Sengupta, S., Siliciano, R.F., 2018. Targeting the Latent Reservoir for HIV-1. Immunity 48, 872–895. https://doi.org/10.1016/j.immuni.2018.04.030

Séror, C., Melki, M.-T., Subra, F., Raza, S.Q., Bras, M., Saïdi, H., Nardacci, R., Voisin, L., Paoletti, A., Law, F., Martins, I., Amendola, A., Abdul-Sater, A.A., Ciccosanti, F., Delelis, O., Niedergang, F., Thierry, S., Said-Sadier, N., Lamaze, C., Métivier, D., Estaquier, J., Fimia, G.M., Falasca, L., Casetti, R., Modjtahedi, N., Kanellopoulos, J., Mouscadet, J.-F., Ojcius, D.M., Piacentini, M., Gougeon, M.-L., Kroemer, G., Perfettini, J.-L., 2011. Extracellular ATP acts on P2Y2 purinergic receptors to facilitate HIV-1 infection. J. Exp. Med. 208, 1823–1834. https://doi.org/10.1084/jem.20101805

Sertznig, H., Hillebrand, F., Erkelenz, S., Schaal, H., Widera, M., 2018. Behind the scenes of HIV-1 replication: Alternative splicing as the dependency factor on the quiet. Virology 516, 176–188. https://doi.org/10.1016/j.virol.2018.01.011

Shan, L., Deng, K., Gao, H., Xing, S., Capoferri, A.A., Durand, C.M., Rabi, S.A., Laird, G.M., Kim, M., Hosmane, N.N., Yang, H.-C., Zhang, H., Margolick, J.B., Li, L., Cai, W., Ke, R., Flavell, R.A., Siliciano, J.D., Siliciano, R.F., 2017. Transcriptional Reprogramming during Effector-to-Memory Transition Renders CD4+ T Cells Permissive for Latent HIV-1 Infection. Immunity 47, 766-775.e3. https://doi.org/10.1016/j.immuni.2017.09.014

Sharp, P.M., Hahn, B.H., 2011. Origins of HIV and the AIDS Pandemic. Cold Spring Harb. Perspect. Med. 1. https://doi.org/10.1101/cshperspect.a006841

Sheehy, A.M., Gaddis, N.C., Choi, J.D., Malim, M.H., 2002. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature 418, 646–650. https://doi.org/10.1038/nature00939

Shen, R., Meng, G., Ochsenbauer, C., Clapham, P.R., Grams, J., Novak, L., Kappes, J.C., Smythies, L.E., Smith, P.D., 2011. Stromal Down-Regulation of Macrophage CD4/CCR5 Expression and NF-κB Activation Mediates HIV-1 Non-Permissiveness in Intestinal Macrophages. PLOS Pathog. 7, e1002060. https://doi.org/10.1371/journal.ppat.1002060

Shen, R., Richter, H.E., Clements, R.H., Novak, L., Huff, K., Bimczok, D., Sankaran-Walters, S., Dandekar, S., Clapham, P.R., Smythies, L.E., Smith, P.D., 2009. Macrophages in Vaginal but Not Intestinal Mucosa Are Monocyte-Like and Permissive to Human Immunodeficiency Virus Type 1 Infection. J. Virol. 83, 3258–3267. https://doi.org/10.1128/JVI.01796-08

Shi, G., Schwartz, O., Compton, A.A., 2017. More than meets the I: the diverse antiviral and cellular functions of interferon-induced transmembrane proteins. Retrovirology 14, 53. https://doi.org/10.1186/s12977-017-0377-y

Shi, Y., 2004. Caspase activation, inhibition, and reactivation: A mechanistic view. Protein Sci. Publ. Protein Soc. 13, 1979–1987. https://doi.org/10.1110/ps.04789804

Siliciano, J.D., Kajdas, J., Finzi, D., Quinn, T.C., Chadwick, K., Margolick, J.B., Kovacs, C., Gange, S.J., Siliciano, R.F., 2003. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. Nat. Med. 9, 727–728. https://doi.org/10.1038/nm880

Simabuco, F.M., Morale, M.G., Pavan, I.C.B., Morelli, A.P., Silva, F.R., Tamura, R.E., 2018. p53 and metabolism: from mechanism to therapeutics. Oncotarget 9, 23780–23823. https://doi.org/10.18632/oncotarget.25267

Simon, V., Bloch, N., Landau, N.R., 2015. Intrinsic host restrictions to HIV-1 and mechanisms of viral escape. Nat. Immunol. 16, 546–553. https://doi.org/10.1038/ni.3156
Singh, P.K., Plumb, M.R., Ferris, A.L., Iben, J.R., Wu, X., Fadel, H.J., Luke, B.T., Esnault, C., Poeschla, E.M., Hughes, S.H., Kvaratskhelia, M., Levin, H.L., 2015. LEDGF/p75 interacts with mRNA splicing factors and targets HIV-1 integration to highly spliced genes. Genes Dev. 29, 2287–2297. https://doi.org/10.1101/gad.267609.115
Skasko, M., Weiss, K.K., Reynolds, H.M., Jamburuthugoda, V., Lee, K., Kim, B., 2005. Mechanistic Differences in RNA-dependent DNA Polymerization and Fidelity between Murine Leukemia Virus and HIV-1 Reverse Transcriptases. J. Biol. Chem. 280, 12190–12200. https://doi.org/10.1074/jbc.M412859200
Sleiman, D., Goldschmidt, V., Barraud, P., Marquet, R., Paillart, J.-C., Tisné, C., 2012. Initiation of HIV-1 reverse transcription and functional role of nucleocapsid-mediated tRNA/viral genome interactions. Virus Res., Retroviral RNA, protein co-factors and chaperones 169, 324–339. https://doi.org/10.1016/j.virusres.2012.06.006
Smith, B.A., Gartner, S., Liu, Y., Perelson, A.S., Stilianakis, N.I., Keele, B.F., Kerkering, T.M., Ferreira-Gonzalez, A., Szakal, A.K., Tew, J.G., Burton, G.F., 2001. Persistence of Infectious HIV on Follicular Dendritic Cells. J. Immunol. 166, 690–696. https://doi.org/10.4049/jimmunol.166.1.690
Solas, C., Lafeuillade, A., Halfon, P., Chadapaud, S., Hittinger, G., Lacarelle, B., 2003. Discrepancies between Protease Inhibitor Concentrations and Viral Load in Reservoirs and Sanctuary Sites in Human Immunodeficiency Virus-Infected Patients. Antimicrob. Agents Chemother. 47, 238–243. https://doi.org/10.1128/AAC.47.1.238-243.2003
Songane, M., Khair, M., Saleh, M., 2018. An updated view on the functions of caspases in inflammation and immunity. Semin. Cell Dev. Biol. https://doi.org/10.1016/j.semcdb.2018.01.001
Sood, C., Marin, M., Chande, A., Pizzato, M., Melikyan, G.B., 2017. SERINC5 protein inhibits HIV-1 fusion pore formation by promoting functional inactivation of envelope glycoproteins. J. Biol. Chem. 292, 6014–6026. https://doi.org/10.1074/jbc.M117.777714
Soper, A., Kimura, I., Nagaoka, S., Konno, Y., Yamamoto, K., Koyanagi, Y., Sato, K., 2018. Type I Interferon Responses by HIV-1 Infection: Association with Disease Progression and Control. Front. Immunol. 8. https://doi.org/10.3389/fimmu.2017.01823
Stacey, A.R., Norris, P.J., Qin, L., Haygreen, E.A., Taylor, E., Heitman, J., Lebedeva, M., DeCamp, A., Li, D., Grove, D., Self, S.G., Borrow, P., 2009. Induction of a Striking Systemic Cytokine Cascade prior to Peak Viremia in Acute Human Immunodeficiency Virus Type 1 Infection, in Contrast to More Modest and Delayed Responses in Acute Hepatitis B and C Virus Infections. J. Virol. 83, 3719–3733. https://doi.org/10.1128/JVI.01844-08
Stieh, D.J., Matias, E., Xu, H., Fought, A.J., Blanchard, J.L., Marx, P.A., Veazey, R.S., Hope, T.J., 2016. Th17 Cells Are Preferentially Infected Very Early after Vaginal Transmission of SIV in Macaques. Cell Host Microbe 19, 529–540. https://doi.org/10.1016/j.chom.2016.03.005
Sui, Y., Gordon, S., Franchini, G., Berzofsky, J.A., 2013. Non-human primate models for HIV/AIDS vaccine development. Curr. Protoc. Immunol. Ed. John E Coligan Al 102, 12.14.1-12.14.30. https://doi.org/10.1002/0471142735.im1214s102

Survival of HIV-positive patients starting antiretroviral therapy between 1996 and 2013: a collaborative analysis of cohort studies, 2017. . Lancet HIV 4, e349–e356. https://doi.org/10.1016/S2352-3018(17)30066-8

Swingler, S., Mann, A.M., Zhou, J., Swingler, C., Stevenson, M., 2007. Apoptotic Killing of HIV-1–Infected Macrophages Is Subverted by the Viral Envelope Glycoprotein. PLOS Pathog 3, e134. https://doi.org/10.1371/journal.ppat.0030134

Tait, S.W.G., Ichim, G., Green, D.R., 2014. Die another way – non-apoptotic mechanisms of cell death. J Cell Sci 127, 2135–2144. https://doi.org/10.1242/jcs.093575

Tartour, K., Appourchaux, R., Gaillard, J., Nguyen, X.-N., Durand, S., Turpin, J., Beaumont, E., Roch, E., Berger, G., Mahieux, R., Brand, D., Roingeard, P., Cimarelli, A., 2014. IFITM proteins are incorporated onto HIV-1 virion particles and negatively imprint their infectivity. Retrovirology 11, 103. https://doi.org/10.1186/s12977-014-0103-y

Telenti, A., 2014. Identification of IFI16 as HIV Restriction Factor | CROI Conference [WWW Document]. URL http://www.croiconference.org/sessions/identification-ifi16-hiv-restriction-factor (accessed 6.13.18).

Tewari, M., Yu, M., Ross, B., Dean, C., Giordano, A., Rubin, R., 1997. AAC-11, a Novel cDNA That Inhibits Apoptosis after Growth Factor Withdrawal. Cancer Res. 57, 4063–4069.

Thali, M., Bukovsky, A., Kondo, E., Rosenwlrth, B., Walsh, C.T., Sodroski, J., Göttlinger, H.G., 1994. Functional association of cyclophilin A with HIV-1 virions. Nature 372, 363–365. https://doi.org/10.1038/372363a0

The Stages of HIV Infection Understanding HIV/AIDS [WWW Document], 2018. . AIDSinfo. URL https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/19/46/the-stages-of-hiv-infection (accessed 5.24.18).

Thome, J.J.C., Yudanin, N., Ohmura, Y., Kubota, M., Grinshpun, B., Sathaliyawala, T., Kato, T., Lerner, H., Shen, Y., Farber, D.L., 2014. Spatial Map of Human T Cell Compartmentalization and Maintenance over Decades of Life. Cell 159, 814–828. https://doi.org/10.1016/j.cell.2014.10.026

Thompson, G.J., Langlais, C., Cain, K., Conley, E.C., Cohen, G.M., 2001. Elevated extracellular [K+] inhibits death-receptor- and chemical-mediated apoptosis prior to caspase activation and cytochrome c release. Biochem. J. 357, 137–145. https://doi.org/10.1042/bj3570137

Thompson, K.A., Cherry, C.L., Bell, J.E., McLean, C.A., 2011. Brain cell reservoirs of latent virus in presymptomatic HIV-infected individuals. Am. J. Pathol. 179, 1623–1629. https://doi.org/10.1016/j.ajpath.2011.06.039

Thompson, K.A., McArthur, J.C., Wesselingh, S.L., 2001. Correlation between neurological progression and astrocyte apoptosis in HIV-associated dementia. Ann. Neurol. 49, 745–752. https://doi.org/10.1002/ana.1011

Tinel, A., Tschopp, J., 2004. The PIDDosome, a Protein Complex Implicated in Activation of Caspase-2 in Response to Genotoxic Stress. Science 304, 843–846. https://doi.org/10.1126/science.1095432

Tokarev, A., Suarez, M., Kwan, W., Fitzpatrick, K., Singh, R., Guatelli, J., 2013. Stimulation of NF-κB Activity by the HIV Restriction Factor BST2. J. Virol. 87, 2046–2057. https://doi.org/10.1128/JVI.02272-12

Triques, K., Stevenson, M., 2004. Characterization of Restrictions to Human Immunodeficiency Virus Type 1 Infection of Monocytes. J. Virol. 78, 5523–5527. https://doi.org/10.1128/JVI.78.10.5523-5527.2004

Usami, Y., Wu, Y., Göttlinger, H.G., 2015. SERINC3 and SERINC5 restrict HIV-1 infectivity and are counteracted by Nef. Nature 526, 218–223. https://doi.org/10.1038/nature15400

Utay, N.S., Douek, D.C., 2016. Interferons and HIV Infection: The Good, the Bad, and the Ugly. Pathog. Immun. 1, 107–116.

Valle-Casuso, J.C., Allouch, A., David, A., Lenzi, G.M., Studdard, L., Barré-Sinoussi, F., Müller-Trutwin, M., Kim, B., Pancino, G., Sáez-Cirión, A., 2017. p21 Restricts HIV-1 in Monocyte-Derived Dendritic Cells through the Reduction of Deoxynucleoside Triphosphate Biosynthesis and Regulation of SAMHD1 Antiviral Activity. J. Virol. 91, e01324-17. https://doi.org/10.1128/JVI.01324-17

Van Damme, N., Goff, D., Katsura, C., Jorgenson, R.L., Mitchell, R., Johnson, M.C., Stephens, E.B., Guatelli, J., 2008. The Interferon-Induced Protein BST-2 Restricts HIV-1 Release and Is Downregulated from the Cell Surface by the Viral Vpu Protein. Cell Host Microbe 3, 245–252. https://doi.org/10.1016/j.chom.2008.03.001

Vaughn, A.E., Deshmukh, M., 2008. Glucose metabolism inhibits apoptosis in neurons and cancer cells by redox inactivation of cytochrome *c*. Nat. Cell Biol. 10, 1477–1483. https://doi.org/10.1038/ncb1807

Veazey, R.S., Mansfield, K.G., Tham, I.C., Carville, A.C., Shvetz, D.E., Forand, A.E., Lackner, A.A., 2000. Dynamics of CCR5 Expression by CD4+ T Cells in Lymphoid Tissues during Simian Immunodeficiency Virus Infection. J. Virol. 74, 11001–11007. https://doi.org/10.1128/JVI.74.23.11001-11007.2000

Vella, L.A., Herati, R.S., Wherry, E.J., 2017. CD4+ T Cell Differentiation in Chronic Viral Infections: The Tfh Perspective. Trends Mol. Med. 23, 1072–1087. https://doi.org/10.1016/j.molmed.2017.10.001

Voss, T.G., Fermin, C.D., Levy, J.A., Vigh, S., Choi, B., Garry, R.F., 1996. Alteration of intracellular potassium and sodium concentrations correlates with induction of cytopathic effects by human immunodeficiency virus. J. Virol. 70, 5447–5454.

Wang, Z., Liu, H., Liu, B., Ma, W., Xue, X., Chen, J., Zhou, Q., 2010. Gene expression levels of CSNK1A1 and AAC-11, but not NME1, in tumor tissues as prognostic factors in NSCLC patients. Med. Sci. Monit. Int. Med. J. Exp. Clin. Res. 16, CR357-364.

Westendorp, M.O., Frank, R., Ochsenbauer, C., Stricker, K., Dhein, J., Walczak, H., Debating, K.-M., Krammer, P.H., 1995. Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. Nature 375, 497–500. https://doi.org/10.1038/375497a0

Whitney, J.B., Hill, A.L., Sanisetty, S., Penaloza-MacMaster, P., Liu, J., Shetty, M., Parenteau, L., Cabral, C., Shields, J., Blackmore, S., Smith, J.Y., Brinkman, A.L., Peter, L.E., Mathew, S.I., Smith, K.M., Borducchi, E.N., Rosenbloom, D.I.S., Lewis, M.G., Hattersley, J., Li, B., Hesselgesser, J., Geleziunas, R., Robb, M.L., Kim, J.H., Michael, N.L., Barouch, D.H., 2014. Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. Nature 512, 74–77. https://doi.org/10.1038/nature13594

WHO | HIV/AIDS [WWW Document], 2018. . WHO. URL http://www.who.int/features/qa/71/en/ (accessed 5.24.18).

Wilen, C.B., Tilton, J.C., Doms, R.W., 2012. HIV: Cell Binding and Entry. Cold Spring Harb. Perspect. Med. 2. https://doi.org/10.1101/cshperspect.a006866

Williams, K.C., Corey, S., Westmoreland, S.V., Pauley, D., Knight, H., deBakker, C., Alvarez, X., Lackner, A.A., 2001. Perivascular Macrophages Are the Primary Cell Type Productively Infected by Simian Immunodeficiency Virus in the Brains of Macaques:

Implications for the Neuropathogenesis of AIDS. J. Exp. Med. 193, 905–916. https://doi.org/10.1084/jem.193.8.905
Williams, S.A., Chen, L.-F., Kwon, H., Ruiz-Jarabo, C.M., Verdin, E., Greene, W.C., 2006. NF-κB p50 promotes HIV latency through HDAC recruitment and repression of transcriptional initiation. EMBO J. 25, 139–149. https://doi.org/10.1038/sj.emboj.7600900
Williams, S.A., Kwon, H., Chen, L.-F., Greene, W.C., 2007. Sustained Induction of NF-κB Is Required for Efficient Expression of Latent Human Immunodeficiency Virus Type 1. J. Virol. 81, 6043–6056. https://doi.org/10.1128/JVI.02074-06
Wojcechowskyj, J.A., Didigu, C.A., Lee, J.Y., Parrish, N.F., Sinha, R., Hahn, B.H., Bushman, F.D., Jensen, S.T., Seeholzer, S.H., Doms, R.W., 2013. Quantitative phosphoproteomics reveals extensive cellular reprogramming during HIV-1 entry. Cell Host Microbe 13, 613–623. https://doi.org/10.1016/j.chom.2013.04.011
Wolf, D., Witte, V., Laffert, B., Blume, K., Stromer, E., Trapp, S., Paola, Schürmann, A., Baur, A.S., 2001a. HIV-1 Nef associated PAK and PI3-Kinases stimulate Akt-independent Bad-phosphorylation to induce anti-apoptotic signals. Nat. Med. 7, 1217–1224. https://doi.org/10.1038/nm1101-1217
Wolf, D., Witte, V., Laffert, B., Blume, K., Stromer, E., Trapp, S., Paola, Schürmann, A., Baur, A.S., 2001b. HIV-1 Nef associated PAK and PI3-Kinases stimulate Akt-independent Bad-phosphorylation to induce anti-apoptotic signals. Nat. Med. 7, 1217–1224. https://doi.org/10.1038/nm1101-1217
Wong, J.K., Hezareh, M., Günthard, H.F., Havlir, D.V., Ignacio, C.C., Spina, C.A., Richman, D.D., 1997. Recovery of Replication-Competent HIV Despite Prolonged Suppression of Plasma Viremia. Science 278, 1291–1295. https://doi.org/10.1126/science.278.5341.1291
Xu, X.-N., Laffert, B., Screaton, G.R., Kraft, M., Wolf, D., Kolanus, W., Mongkolsapay, J., McMichael, A.J., Baur, A.S., 1999. Induction of Fas Ligand Expression by HIV Involves the Interaction of Nef with the T Cell Receptor ζ Chain. J. Exp. Med. 189, 1489–1496. https://doi.org/10.1084/jem.189.9.1489
Yamashita, M., Engelman, A.N., 2017. Capsid-Dependent Host Factors in HIV-1 Infection. Trends Microbiol. 25, 741–755. https://doi.org/10.1016/j.tim.2017.04.004
Youngblood, B., Hale, J.S., Kissick, H.T., Ahn, E., Xu, X., Wieland, A., Araki, K., West, E.E., Ghoneim, H.E., Fan, Y., Dogra, P., Davis, C.W., Konieczny, B.T., Antia, R., Cheng, X., Ahmed, R., 2017. Effector CD8 T cells dedifferentiate into long-lived memory cells. Nature 552, 404–409. https://doi.org/10.1038/nature25144
Yu, J., Li, M., Wilkins, J., Ding, S., Swartz, T.H., Esposito, A.M., Zheng, Y.-M., Freed, E.O., Liang, C., Chen, B.K., Liu, S.-L., 2015. IFITM Proteins Restrict HIV-1 Infection by Antagonizing the Envelope Glycoprotein. Cell Rep. 13, 145–156. https://doi.org/10.1016/j.celrep.2015.08.055
Yukl, S.A., Gianella, S., Sinclair, E., Epling, L., Li, Q., Duan, L., Choi, A.L.M., Girling, V., Ho, T., Li, P., Fujimoto, K., Lampiris, H., Bradley Hare, C., Pandori, M., Haase, A.T., Günthard, H.F., Fischer, M., Shergill, A.K., McQuaid, K., Havlir, D.V., Wong, J.K., 2010. Differences in HIV Burden and Immune Activation within the Gut of HIV-Positive Patients Receiving Suppressive Antiretroviral Therapy. J. Infect. Dis. 202, 1553–1561. https://doi.org/10.1086/656722
Zalar, A., Figueroa, M.I., Ruibal-Ares, B., Baré, P., Cahn, P., de Bracco, M.M. de E., Belmonte, L., 2010. Macrophage HIV-1 infection in duodenal tissue of patients on long term

HAART. Antiviral Res., The VIZIER project: deciphering the world of RNA viruses 87, 269–271. https://doi.org/10.1016/j.antiviral.2010.05.005

Zennou, V., Perez-Caballero, D., Göttlinger, H., Bieniasz, P.D., 2004. APOBEC3G Incorporation into Human Immunodeficiency Virus Type 1 Particles. J. Virol. 78, 12058–12061. https://doi.org/10.1128/JVI.78.21.12058-12061.2004

Zhang, F., Wilson, S.J., Landford, W.C., Virgen, B., Gregory, D., Johnson, M.C., Munch, J., Kirchhoff, F., Bieniasz, P.D., Hatziioannou, T., 2009. Nef Proteins from Simian Immunodeficiency Viruses Are Tetherin Antagonists. Cell Host Microbe 6, 54–67. https://doi.org/10.1016/j.chom.2009.05.008

Zhu, T., Muthui, D., Holte, S., Nickle, D., Feng, F., Brodie, S., Hwangbo, Y., Mullins, J.I., Corey, L., 2002. Evidence for Human Immunodeficiency Virus Type 1 Replication In Vivo in CD14+ Monocytes and Its Potential Role as a Source of Virus in Patients on Highly Active Antiretroviral Therapy. J. Virol. 76, 707–716. https://doi.org/10.1128/JVI.76.2.707-716.2002