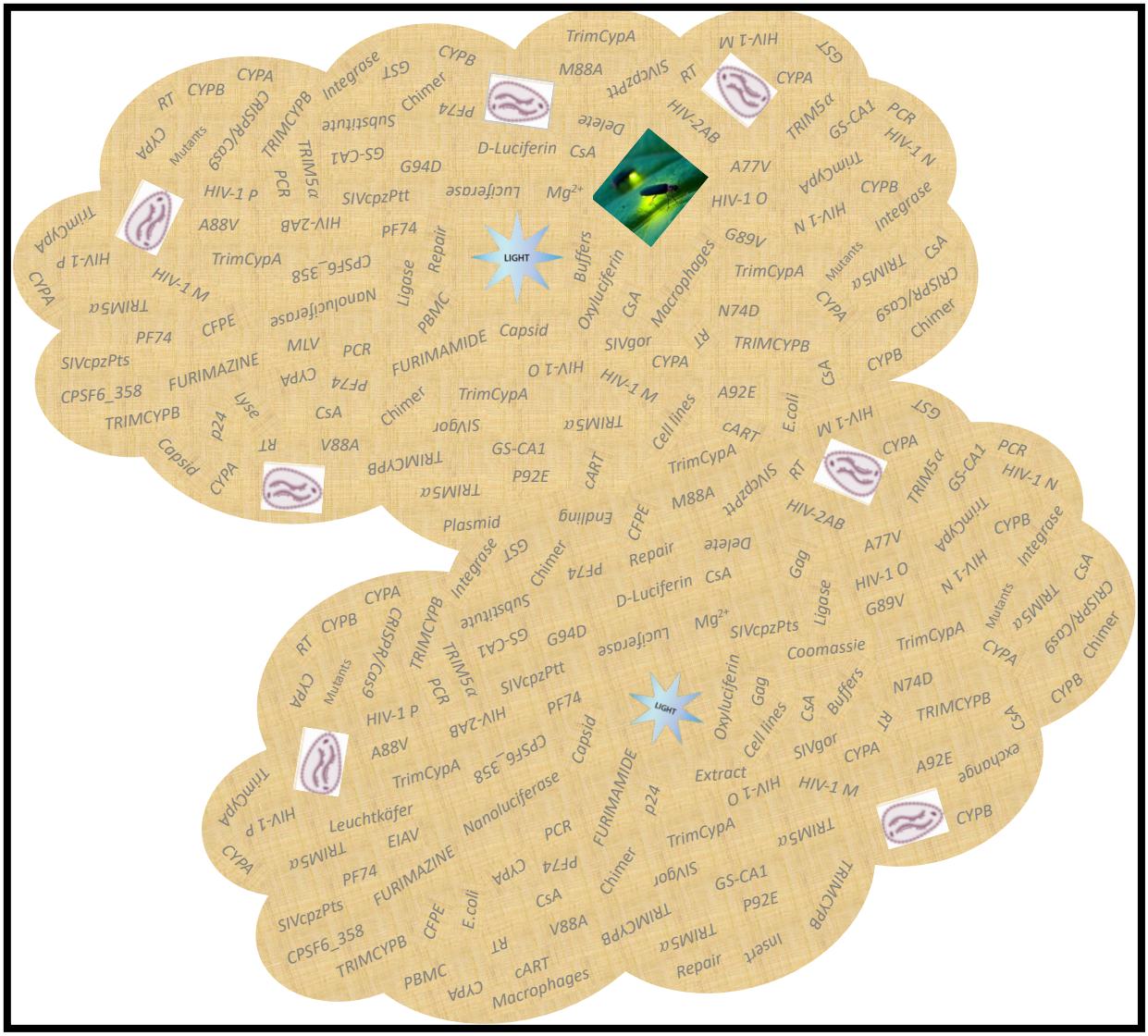

HIV and SIV capsid: A determinant for host and pharmacological factors

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HIV and SIV capsid: A determinant for host and pharmacological factors

Inaugural dissertation

for the attainment of the title of doctor
in the Faculty of Mathematics and Natural Sciences
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presented by

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Missing context always truncates reality, social and personality definitions.

Augustin Penda Twizerimana

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Summary

Following the retroviral-cell membrane fusion, the viral core is released in the cytosol. This triggers mobilization of capsid interactors that either shield the core, mediate its transport, or prematurely disassemble it. The Human Immunodeficiency Virus 1 (HIV-1) and its simian relatives Simian Immunodeficiency viruses (SIV) have specific interactions with factors of infected cells. In case of HIV-1, these include host proteins such as cyclophilin A or B (CYPA or CYPB), Tripartite motif-containing proteins (TRIM5α, TRIM34, TrimCYP) and Cleavage and Polyadenylation Specificity Factor 6 (CPSF6). The HIV capsid is also a binding site for many pharmacological factors such as PF74 and Lenacapavir, known to halt HIV replication cycle. Under this thesis are findings from three projects I worked on, to explore host or pharmacological interactions with viruses from the four groups of HIV-1, HIV-2, SIVgor and SIVcpz, in a way to decipher more on SIV molecular evolution towards HIVs as well as infection biology differences between HIVs.

In the first project, I focused on understanding molecular evolution of SIVcpz towards HIV-1. I investigated sensitivity of SIVcpzPtt (the origin of HIV-1 M and HIV-1 N) and SIVcpzPts (which did not spread to humans) to capsid inhibitors PF74, PF57 and GS-CA1 in human and non-human cells. I also explored their CYPA usage and sensitivity to truncated, cytoplasmic form of CPSF6, the CPSF6_358, which shares a binding site with PF74; all this in comparison with HIV-1 M. Findings show that SIVcpzPts can use CYPA to escape inhibition by capsid inhibitors and resisted inhibition by the antiviral CPSF6_358. The two SIVcpz differed in how they use CYPA as revealed by findings of CYPA blocking with CsA or CYPA depletion with CRISPR/Cas9 system. In this chapter I also present a review on post-entry restriction of SIVcpz.

In my second project, I explored why group N, group O, group P HIV-1 and HIV-2 did not set pandemics as opposed to HIV-1 M. I found that one of the possible several answers to this question is the sensitivity of non-M group HIVs capsids to human TRIM5α and a different use of CYPA. While HIV-1 M capsid binds CYPA as a protection against TRIM5α, HIV-1 N, HIV-1 O, HIV-1 P and HIV-2 can be inhibited by TRIM5α even when their cores bind CYPA. I also identified capsid residue 88 in the capsid CYPA binding loop, as an important regulator of CYPA activity for such differential sensitivity to TRIM5α.

In my last project, I present an anti-HIV role of CYPB, a CYPA closely related cyclophilin, which I found to use many residues in the capsid CYPA binding loop, to interact with viral Gag. I also used fusion proteins of CYPB and TRIM5 proteins (TrimCYPB) to make strong anti-HIV proteins which inhibited viruses from the four groups of HIV-1, as opposed to TrimCYP which is known to selectively inhibit these viruses. Finally, I used a range of assays to show that CYPB inhibits the early phase of HIV-1 infection.

My findings on SIVcpzPts open a door towards understanding why only SIVcpzPtt but not SIVcpzPts, managed to adapt to human cellular environment, to evolve as HIV-1, but more is needed to fully answer such question. To fully understand why non-M HIVs did not spread in form of pandemics, more work on possible contribution of host factors but also viral determinants, will be needed. Further investigations on the role of CYPB are needed to see if binding of this isomerase to HIV capsid also impairs or help other host or pharmacological players.

Summary in German (Zusammenfassung)

Nach der Fusion von Retrovirus und Zellmembran wird der Virus Kapsid im Zytosol freigesetzt. Dies löst die Mobilisierung von Kapsid-Interaktoren aus, die das Kapsid entweder abschirmen, seinen Transport vermitteln oder ihn vorzeitig abbauen. Das Humane Immundefizienzvirus 1 (HIV-1) und seine Verwandten, die Simianen Immundefizienzviren (SIV), haben spezifische Interaktionen mit Faktoren infizierter Zellen. Im Falle von HIV-1 handelt es sich dabei um Wirtsproteine wie Cyclophilin A oder B (CYPA oder CYPB), Tripartite motif-containing Proteins (TRIM5α, TRIM34, TrimCYP) und Cleavage and Polyadenylation Specificity Factor 6 (CPSF6). Das HIV-Kapsid ist auch eine Bindungsstelle für viele pharmakologische Faktoren wie PF74 und Lenacapavir, von denen bekannt ist, dass sie den HIV-Replikationszyklus stoppen. In dieser Arbeit werden die Ergebnisse von drei Projekten vorgestellt, an denen ich gearbeitet habe, um Wirts- oder pharmakologische Interaktionen mit Viren aus den vier Gruppen HIV-1, HIV-2, SIVgor und SIVcpz zu erforschen, um mehr über die molekulare Evolution von SIV in Richtung HIV sowie über die infektionsbiologischen Unterschiede zwischen den HIVs zu erfahren.

Im ersten Projekt konzentrierte ich mich auf das Verständnis der molekularen Evolution von SIVcpz gegenüber HIV-1. Ich untersuchte die Empfindlichkeit von SIVcpzPtt (dem Ursprung von HIV-1 M und HIV-1 N) und SIVcpzPts (das sich nicht auf den Menschen ausbreitete) gegenüber den Kapsid-Inhibitoren PF74, PF57 und GS-CA1 in menschlichen und nicht-menschlichen Zellen. Außerdem habe ich ihre CYPA-Nutzung und ihre Empfindlichkeit gegenüber der verkürzten, zytoplasmatischen Form von CPSF6, CPSF6_358, untersucht, die sich eine Bindungsstelle mit PF74 teilt; all dies im Vergleich zu HIV-1 M. Die Ergebnisse zeigen, dass SIVcpzPts CYPA nutzen, um der Hemmung durch Kapsid-Inhibitoren zu entgehen, und dass sie der Hemmung durch das antivirale CPSF6_358 widerstehen. Die beiden

SIVcpz unterschieden sich in der Art und Weise, wie sie CYPA nutzen, wie die Ergebnisse der CYPA-Blockierung mit CsA oder der CYPA-Depletion mit dem CRISPR/Cas9-System zeigen. In diesem Kapitel gebe ich auch einen Überblick über die Restriktion von SIVcpz nach dem Eintritt in die Zellen.

In meinem zweiten Projekt untersuchte ich, warum HIV-1 der Gruppen N, O, P und HIV-2 im Gegensatz zu HIV-1 M keine Pandemien auslösen. Ich fand heraus, dass eine der möglichen Antworten auf diese Frage die Empfindlichkeit der Kapside von Nicht-M-HIVs gegenüber dem Menschen TRIM5α und eine andere Verwendung von CYPA ist. Während HIV-1 M CYPA als Schutz gegen TRIM5α verwendet, können HIV-1 N, HIV-1 O, HIV-1 P und HIV-2 durch TRIM5α auch in Gegenwart von CYPA gehemmt werden. Ich habe auch den Capsid-Rest 88 in der CYPA-Bindungsschleife des Kapsids als einen wichtigen Faktor für diese unterschiedliche Empfindlichkeit gegenüber TRIM5α identifiziert.

In meinem letzten Projekt präsentierte ich eine neue Anti-HIV-Rolle von CYPB, einem CYPA-nahen Cyclophilin, von dem ich herausfand, dass es viele Reste in der CYPA-Bindungsschleife des Kapsids nutzt, um mit dem viralen Gag zu interagieren. Außerdem habe ich Fusionsproteine aus CYPB und TRIM5-Proteinen (TrimCYPB) verwendet, um starke Anti-HIV-Proteine herzustellen, die Viren aus den vier HIV-1-Gruppen hemmen, im Gegensatz zu TrimCYP, dass bekanntermaßen selektiv diese Viren hemmt. Schließlich habe ich mit einer Reihe von Tests gezeigt, dass CYPB die frühe Phase der HIV-1-Infektion hemmt.

Meine Erkenntnisse über SIVcpzPts öffnen eine Tür zum Verständnis, warum es nur SIVcpzPtt, nicht aber SIVcpzPts gelang, sich an die menschliche Zellumgebung anzupassen und sich als HIV-1 zu entwickeln, aber es sind noch weitere Untersuchungen erforderlich, um diese Frage vollständig zu beantworten. Um vollständig zu verstehen, warum sich nicht-M-HIVs nicht in Form von Pandemien ausgebreitet haben, sind weitere Arbeiten zum möglichen Beitrag von Wirtsfaktoren, aber auch von viralen Determinanten erforderlich. Weitere

Untersuchungen zur Rolle von CYPB sind erforderlich, um festzustellen, ob die Bindung dieser Cis-Trans-Isomerase an das HIV-Kapsid auch andere Wirtsfaktoren oder pharmakologische Faktoren beeinträchtigt oder unterstützt.

Publications

This work is based on the publications below:

1. Twizerimana AP, Scheck R, Becker D, et al. Cell Type-Dependent Escape of Capsid Inhibitors by Simian Immunodeficiency Virus SIVcpz. J Virol. 2020;94(23). doi:10.1128/JVI.01338-20
2. Twizerimana AP, Scheck R, Häussinger D, Münk C. Post-entry restriction factors of SIVcpz. *Future Virol*. 2018;13(10):727-745. doi:10.2217/fvl-2018-0093
3. Augustin P. Twizerimana[1], Daniel Becker[2], Shenglin Zhu[1], Holger Gohlke[2,3,4,5], Tom Luedde[1], Carsten Münk[1]. The CYPA binding loop of capsid regulates the human TRIM5α sensitivity of non-pandemic HIV-1
4. Augustin Penda Twizerimana[1], Daniel Becker[2], Marianne Wammers[1], Tom Luedde[1], Holger Gohlke[2,3,4,5], Carsten Münk[1]. Anti-HIV-1 activity of Cyclophilin B is mediated by various residues of the capsid cyclophilin A binding loop.

Other publications

1. Lucía Cano-Ortiz; Qinyong Gu; Patricia de Sousa-Pereira; Zeli Zhang; Catherina Chiapella; Augustin Penda Twizerimana; Chaohui Lin; Ana Cláudia Franco; Sue VandeWoude; Tom Luedde; Hanna-Mari Baldauf; Carsten Muenk. Feline Leukemia Virus-B Envelope Together With its GlycoGag and Human Immunodeficiency Virus-1 Nef Mediate Resistance to Feline SERINC5. Journal of Molecular Biology. 2022 Mar;434(6):167421. DOI: 10.1016/j.jmb.2021.167421. PMID: 34954236.

1. Introduction

Human being but also other living organisms live in a perpetual conflict with their surroundings including macroscopic, microscopic, and chemical injurious agents[1–3]. Among them are different classes of viruses including but not limited to retroviruses and it is clear today that, vertebrate genomes, particularly apes have been hosting retroviruses since several years ago[4,5]. This constant contact has not only resulted into host evolution, which led to specific host susceptibility or resistance phenotypes[6–8], but has also shaped retroviral strategies to adapt to current and future hosts[9–11], for intra- and inter-species colonization. In their fight to withstand viral infection, primates, including humans, use their innate and adaptive immune systems, comprising of organs, tissues, cells, and molecules in a highly complex but somehow controlled way that results in what is today known as immune response[12]. The retrovirus Human Immunodeficiency Virus (HIV) is the cause for the acquired immunodeficiency syndrome (AIDS) pandemic[13]. HIV is a product of the spread of Simian Immunodeficiency Viruses (SIV) and adaptations to human and human cellular factors[14,15].

1.1 Simians: a brief overview

Primate lentiviruses are hosted by many African simians and this colonization has been detected mainly through sequencing and antibody-antigen tests[16,17]. Simians are classified as either apes or monkeys and their several species and subspecies are differently distributed over many geographical areas of the globe[16]. Apes can be classified as great or small/lesser apes. Great apes, also known as hominids, include one species of humans (*Homo sapiens)*; two species of panins or chimps which include the female-dominated bonobo species or *Pan paniscus* and the male-dominated common chimpanzee species, the *Pan Troglodytes*. Under hominids, Gorillas and Orangutans species are also classified[16,18–21]. There is a single species of *Pan paniscus*, four subspecies under *Pan troglodytes* chimpanzee *(Pan troglodytes*

troglodytes, Pan troglodytes schweinfurthii, Pan troglodytes verus and *Pan troglodytes ellioti*[22]. Gorillas have two extant species, each with two subspecies (the western Gorilla or *Gorilla Gorilla* with *Gorilla gorilla gorilla and Gorilla gorilla diehli subspecies and the eastern Gorilla or Gorilla beringei with Gorilla beringei beringei and Gorilla beringei graueri as subspecies),* there are three existing subspecies under Orangutans[18,20,23]. Pan troglodytes and western Gorillas are hosts to SIVcpz and SIVgor, respectively[24].

Lesser apes or *Hylobatidae* also commonly known as gibbons include four genera namely *Symphalangus*, *Nomascus*, *Hoolock*, *Hylobates* and have around 20 different species[25,26].
Monkeys on the other hand, are classified as New or Old World monkeys. The New World monkeys spread over five families but with the broad-nosed monkeys, also known as platyrrhines, being the only extant family, they mainly occupy the America territory to which they are immigrants[27,28]. Old World monkeys, the Cercopithecidae, are classified into more than 100 species and mainly localizes in Africa but their presence extends to Asia[27,29]. SIV naturally-infected monkeys include many species of Old World monkeys such as the African green monkey or *Cercopithecus* which has different species and has the SIVagm[30,31]. Some Old World monkeys in captivity (e.g. in USA) are also known to harbour SIVs, it was recently shown that rhesus macaque monkeys in captivity in the USA got their SIV through accidental cross-species spill over of SIV of sooty mangabeys (SIVsmm)[30,32,33].

1.2 HIV and SIV are retroviruses

The family *Retroviridae* comprises several viruses grouped across seven genera and these include alpharetroviruses, betaretroviruses, gammaretroviruses, deltaretroviruses, epsilonretroviruses, spumaviruses and lentiviruses[34,35]. HIV and SIV are lentiviruses and possess, like other retroviruses, a two-copy positive single-stranded RNA genome, and their

replication requires reverse transcription of their genomic RNA into a double-stranded cDNA by one of the viral key enzymes, the reverse transcriptase[35,36]. The retroviral genome has key genes for the group specific antigen or Gag, polymerase or Pol, and envelope or Env proteins; edged by long terminal repeats (LTR) at both genomic ends[37]. Variable number of additional genes are found in lentiviruses and some other genera, *e.g.,* in the prototype primate lentivirus HIV-1, the genome contains sequences encoding viral accessory proteins such as the viral infectivity factor (Vif), the viral protein R (Vpr), the viral protein U (Vpu), the trans-activator of transcription (Tat), the regulator of expression of virion proteins (Rev) and the negative regulator factor (Nef)[38,39]. HIV-2 and different SIVs contain a *vpx* gene for viral protein X (Vpx), related to HIV-1 and some other SIV Vpr[39].

1.3 HIV-1 and HIV-2: Differences in origins

There are two types of HIV found in humans, namely HIV-1 and HIV-2, with different groups inside each type[40]. Work done to understand the origin of HIVs has shown that the different groups of HIVs are independent evolutionary offsprings of simian immunodeficiency viruses (SIV) from apes and monkeys (chimpanzees, Gorillas or Sooty mangabeys), following a few successful cross-spreads to humans, believed to have happened early in the 20th or end of 19th centuries[41,42]. Through transmission of many monkey SIVs and their recombination events inside *Pan troglodytes schweinfurthii* species of chimpanzees, the SIVcpzPts resulted and this, on its turn, jumped and adapted to Chimpanzees of the subspecies *Pan troglodytes troglodytes*, as SIVcpzPtt[43,44]. Two of the four groups of HIV-1 (HIV-1 group M or Major and HIV-1 group N or also known as New, Non-M, Non O) originated from this SIVcpzPtt, following adaptation to human cellular factors such as tetherin (BST-2) and APOBEC3 proteins[45]. Several clades or subtypes exist under HIV-1 group M[46]. On the other hand, sequence analysis has shown that HIV-1 group O (Outlier) and HIV-1 group P viruses have their origin in gorillas, from SIVgor,

a virus which also, has its origin from SIVcpzPtt[44]. At several times, SIV from sooty mangabeys (SIVsmm) transmitted to humans, adapted in the form of different viruses, which are today known as HIV-2[47]. Genetic recombination inside human host has been documented among both HIV-1 and HIV-2 viruses and they are the origin of circulating recombinant forms of HIVs (CRFs)[48]. Though recently molecularly characterized, HIV-1 M and HIV-1 O viruses are believed to have been circulating among humans as early as end of 19th to early 20th century (1890-1940), as compared to HIV-1 N and HIV-1 P which likely established among humans later[49,50]. Similarly, to HIV-1 O, other non-M group HIV-1 viruses predominate in Cameroon, Gabon and Equatorial Guinea[49,51,52], and despite that they all cause AIDS, they differ in their phylogenies, virological and epidemiological patterns[50,53,54]. Several more Old-World simians are natural hosts to many SIVs, where these viruses actively replicate in a non-pathogenic manner. Except the described SIVs from sooty mangabeys, gorillas, and chimpanzees, all other SIVs did not successfully spread to humans, despite possible contacts. Thus, such natural hosts and SIVs are good tools to understand why HIV leads to AIDS in humans[55].

1.4 HIV and SIV: Genome structure and function

1.4.1 The group specific antigen, *Gag* gene and the Gag polyprotein

The viral group specific antigen *Gag* gene codes for the Gag polyprotein from which the matrix protein (p17), capsid protein (p24), nucleocapsid (p7), protein 6 (p6) and two spacer peptides SP1 and SP2 are produced, upon a proteolytic cleavage by the viral protease[56,57].

Matrix p17 is in the N-terminus part of Gag and contains a myristic acid group, important for Gag polyprotein targeting to the plasma membrane of infected cell[58,59]. P17 also mediates viral envelope incorporation during viral assembly and budding[59], and constitutes a protective shell around the viral capsid[59]

Viral protein p24 is the building block for viral capsid. In addition to enclosing and protecting the viral genome and associated key enzymes, viral capsid is a key site for virus-cellular/pharmacological factors interactions[60]. The binding of such factors to capsid is crucial for a successful viral cytoplasmic transit and nuclear import but also cellular defense[61,62]. Capsid mutations have been shown to be important for adaptation to novel host species[63]. Nucleocapsid p7 works as viral RNA chaperon protein and participates to viral genomic RNA encapsidation for new virion formation[64]. SP2 domain has been described as a player for Gag maturation[65]. The p6 protein mediates Vpr incorporation into nascent virions and facilitates viral budding and release, through recruitment of the Endosomal Sorting Complex Required for Transport or ESCRT[66].

1.4.2 The viral *pol* gene is the source of many enzymes

From the *pol* gene, are viral protease (PR), reverse transcriptase (RT) and integrase (IN) enzymes synthesized[67]. Protease enzyme regulates viral protein production by several proteolytic cleavages, and it belongs to aspartate class of proteases[68]. Retroviral reverse transcriptase is essential for converting viral genomic RNA into double stranded DNA, an important step for viral integration into host DNA[69]. HIV-1 reverse transcriptase has two subunits, also called domains, the enzymatic 560 amino acids or p66 and the structural 440 amino acids or p51, produced by protease reaction on Gag-Pol polyprotein[69]. Both RT enzymatic parts, the polymerase and RNase H are found in the p66 subunit, the polymerase part is responsible for the viral cDNA synthesis while the RNase H part is involved in degradation of the template RNA during cDNA synthesis[70]. Integrase enzyme is mainly important for viral cDNA integration into host cell DNA[71,72].

1.4.3 HIV/SIV accessory proteins

Vif (virion infectivity factor) is the viral antidote to the DNA hypermutating cellular deaminases APOBEC3[73]. Vif functions as a substrate receptor for APOBEC3s by using a cellular E3 ligase of Cullin 5, Elongin BC and RING-box protein 2 (RBX2) for APOBEC3 degradation by the proteasomal system[73,74]. Furthermore, Vif has been shown to have many other functions aiming at blocking cellular anti-viral replication strategies[74,75]. Vpr or viral protein R is a 15 kilodalton viral accessory protein mainly used for nuclear entry and it is important for infection of non-dividing cells and for cell cycle arrest. Vpr has been shown to be a host DNA damaging factor at the same time preventing DNA repair by host machinery[76]. Vpu or viral protein U targets the cellular CD4 glycoprotein and the antiviral tetherin (BST-2) for degradation by proteasomal system[77]. It reduces intracellular accumulation of viral cDNA but also causes cell apoptosis through a pathway that culminates into NF-kappa B down-regulation[78,79]. The viral protein X also known as X ORF protein, Vpx, is an HIV-2 and some SIVs accessory protein. Vpx is a counteractor of host SAM domain and HD domain-containing protein 1 (SAMHD1), which inhibits viral replication by blocking reverse transcription, through hydrolysis and then depletion of dNTPs[80]. To mediate SAMHD1 degradation in infected cells, Vpx interacts with cellular VPRPB/DCAF-1, which recruits and allows association with cellular CUL4A-DDB1 E3 ligase complex[81]. This complex targets SAMHD1 to proteolysis by proteasomes, allowing infection in resting myeloid cells[81,82]. Both Vpx and Vpr are packaged by their respective virions, as they are required early during infection[83,84].

The trans-activating regulatory element (Tat) regulates viral genome transcription, through its binding to viral trans-activating response element or TAR, located inside LTR[85]. This complex regulation involves many cellular proteins such as RNA polymerase II, CDK9 and cyclin T1 but also chromosome remodeling[85–87]. Tat absence results in defective, truncated fragments of viral genomic RNA, which are not fit for next round of infection[85].

Rev is the regulator of expression of viral proteins, a transactivating protein which mediates nuclear export of lentiviral genomic and mRNAs, from which most of lentiviral viral proteins are translated[88,89]. Rev export of RNA involves its binding to RNA *Rev* responsive elements (*RRE*) and requires the presence of cellular proteins Ran-GTP, chromosomal region maintenance 1 also called exportin 1 (XPO1/CRM1) and likely other proteins[90,91]. Rev nuclear localization and nuclear export signals allows it to continuously shuttle between cytosol and nucleus for a successful viral protein synthesis[92]. Defective or absent Rev results in viral pre-mRNAs accumulation in the host cell nucleus, which affects viral protein synthesis and assembly phase[93,94].

HIV negative regulatory factor of infectivity (Nef) protein plays a major role in the viral replication and pathogenesis[95]. Nef from HIVs and SIVs downregulates key cellular factors including CD4, BST-2, molecules of major histocompatibility complex MHC-I and MHC-II, serine incorporator proteins 3 and 5 (SERINC3 and SERINC5), CCR5 and CXCR4 coreceptors, CD28 and many other proteins; important for counteraction of viral entry and release[96,97]. Nef cytopathic effects are known to impair the quality of immune response by negatively affecting viral antigen processing and presentation, and cytotoxic function of T lymphocytes on infected cells[98,99].

1.4.4 The viral envelope protein

Lentiviral envelope Env is a 160 kilodalton glycoprotein (gp160) which is proteolytically cut into two key sub-units, the gp120, located at N-terminus and the gp41 at the C-terminal[100]. The gp120 mediates viral docking to CD4 cell membrane receptor and coreceptors CCR5 or CXCR4, and gp41 is important for fusion step[101]. Since Env is the viral outer component, it is also susceptible to host defense strategies, particularly neutralizing antibodies and antiretroviral drugs which target Env are currently part of viral suppression strategies for HIV

infected people. Env undergoes mutations to escape host defense, but it also has conserved domains[100,102].

1.4.5 Lentiviral long terminal repeats

HIVs and SIVs have two identical long terminal repeats (LTR), one at the 5' and one at the 3'genomic end. LTR has binding sites for host and viral determinants important for viral genome transcription. It also mediates viral cDNA integration into host cell DNA[37]. On the DNA level, the LTR has the U3-R-U5 composition. On the genomic RNA level, the 5' sequence is composed of region R, U5 (untranslated), PBS (primer binding site) and L (leader), the genome 3' end has a polypurine tract (PPT), U3 and an R region[103,104]. Both R (repeat) regions are important for precise and full genomic RNA reverse transcription. The PBS sequence primes reverse transcription through binding of cellular tRNA (lys3), which also binds U5 sequences called PAS or primer activation signal[105]. The L region contains the packaging site sequences important for genomic RNA packaging during assembly phase of replication[106]. Proviral transcription is driven by the cellular RNA Pol II from the 5' U3 element and the RNA transcript starts with the R sequence. Tat protein binds in 5' region called Tar, to recruit the P-TEFb (CDK9/cyclin T1) and this results in the phosphorylation of RNA Pol II, to ensure optimal transcription[107]. The 3' end PPT sequence mediates the synthesis of plus DNA strand during reverse transcription[108].

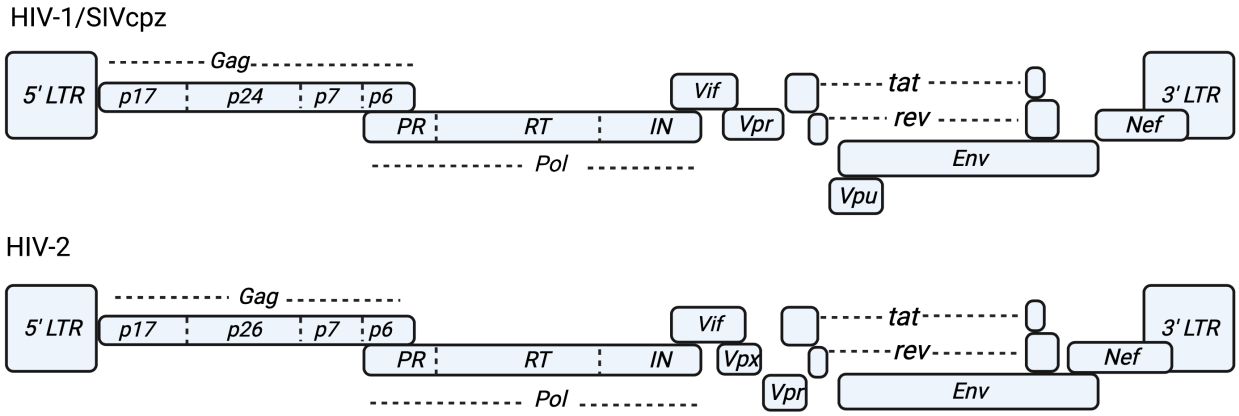

Fig. 1. Genome organization for HIV-1, SIVcpz and HIV-2. HIV and SIV genomes contain common and accessory retroviral genes in an overlapping arrangement. The genome contains two similar long terminal repeats, each at each end, Created with biorender.com.

1.5 HIV: Statistics and routes of transmission

According to WHO reports on HIV/AIDS, in 2021, nearly 38.4 million humans were living with HIV infection and 650 000 HIV-related deaths as well as 1.5 million new infections were recorded in the same year[109]. HIV has caused approximately 40 million deaths since its discovery in early eighties[110,111]. HIV can be transmitted through unprotected sexual contact with an infected partner, mother to fetus/infant across placenta or during breastfeeding but also through blood and blood products in medical transfusions or by HIV positive blood-contaminated sharp objects[112,113]. Most HIV infections are by HIV-1 group M, followed by HIV-2 viruses and HIV-1 group O, and only a few cases of HIV-1 group N and P infections have been reported[114–116]. Low numbers of non-M group HIVs may also be underestimated due gaps in testing, with more tests focusing on HIV-1 M. However, non-M group HIV infections progression is slow. One of the factors to explain the small number of cases from non-M group HIV-1 and HIV-2 is likely their limited adaptability to host factors[117].

The genomes of HIV-1 and HIV-2 have a similarity of less than 50% based on *env* gene sequences[50] but differences in other parts of the genome have also been highlighted[118]. HIV-2 differs from HIV-1 in pathogenesis[119], HIV-2 is associated with low viral RNA copy number,

slow CD4 positive cell depletion, a long asymptomatic phase, low mortality, and a reduced transmissibility[53,120].

1.6 HIV Replication cycle

1.6.1 Viral fusion with cell membrane

HIV infects human cells expressing the CD4 receptor. Cells expressing CD4 are key components of immune system and include many subsets of T lymphocytes like $CD4^+$ helper T lymphocytes but also macrophages and dendritic cells[121]. The early phase of HIV replication starts with virus-cell membrane interaction for entry and the late phase starts after viral cDNA integration into host cell genome[122] until release of new virions (Fig. 2). Virus binding to cell membrane, reverse transcription, pre-integration complex (PIC) formation, nuclear import and uncoating, are early phase steps of replication. Viral entry into the target cells is mediated by both cellular and viral factors, CD4 receptor, CXCR4 or CCR5 coreceptors from the cell and envelope glycoprotein 120 (gp120) and glycoprotein 41 (gp41) from the virus[123,124].

1.6.2 Capsid disassembly or uncoating

The term uncoating describes the disassembly of the viral core to free the viral genome for integration and it is one of the poorly understood steps of HIV replication. Recent findings suggest that uncoating occurs inside the nucleus[125,126], as opposed to old conclusions that this key step takes place at the nuclear envelope[127] or in the cytosol, immediately after entry and even before reverse transcription[128–130]. It is now emerging that HIV uncoats in the nucleus, where even reverse transcription of viral RNA to cDNA may complete[131] for integration. The transit of capsid through the small and highly selective nuclear pores is facilitated by host factors, mainly the cleavage and polyadenylation specificity factor 6 (CPSF6)[132] as well as nuclear envelope pore proteins particularly nucleoporins (NUP153 and NUP358 also called

RanBP) and transportin 3 (TNPO3). In fact, CPSF6 binding defective capsid mutants N74D and A77V differ in their interaction with nuclear pore complex and integration behavior, compared to wild type HIV-1[131]. Viral matrix protein p17, capsid p24, Vpr and integrase are also required in the nuclear import step[129].

The viral core has several key functions such as shielding the genetic material from cellular sensors and DNA degrading proteins, providing a reaction container for reverse transcription of the viral RNA, and transport of the viral genome into the nucleus[133]. Several cytosolic factors have been found to interact with HIV/SIV capsids and their specific binding sites have been mapped[134,135]. Furthermore, crucial capsid interactions with components of nuclear membrane pores for subsequent steps of HIV/SIV were identified[126].

1.6.3 HIV/SIV cDNA integration and production of new virions

Once viral cDNA or provirus is released in the nucleus, it integrates into the host DNA to ensure transcription[136]. Viral cDNA is joined to host DNA in two major steps, the processing of its 3' end and the transfer of the 3' processed viral cDNA to cellular DNA, also known as strand transfer. These two steps are mainly viral integrase dependent; though also require proteins from the host cell[137] such as lens epithelium-derived growth factor (LEDGF)[138] Following integration, HIV/SIV proviral DNA undergoes transcription and translation to produce both viral genomic RNA and viral proteins[139]. Viral envelope glycoproteins gp120 and gp41 are produced by a host furin protease cleavage on the glycosylated Env polyprotein gp160, which is also a result of a translation on a spliced *Vpu/Env* mRNA on the surface of rough endoplasmic reticulum[140]. Env proteins are then secreted by the Golgi apparatus to the plasma membrane, where a few molecules (less than 10 spikes) are used by each budding virion[140,141]. Gag and Gag-Pol polyproteins targeting to the plasma membrane of infected cells (Fig. 2), their assembly around viral genetic material, viral budding and maturation results into

a mature and infectious virion[142]. Viral components assembly and virion release are well-coordinated processes, which also involve cellular proteins such as proteins of the Endosomal Sorting Complex Required for Transport (ESCRT)[142–145]. Assembly step takes place at specific plasma membrane regions, rich in key lipids[146], cholesterol and sphingolipids. This step is also associated with interaction but also packaging of all viral (both structural and enzymatic) and cellular components (*e.g* $tRNA^{Lys3}$), important for next round of infection[147]. Virion maturation after virus budding involves structural changes of viral Gag polyprotein, which is cleaved by viral protease, and this leads to the availability of proteins like capsid p24 that assembles in pentamers and hexamers to produce the mature cone-shaped viral core with two similar viral positive single stranded RNA and associated enzymes inside[148]. The HIV conical capsid is composed of between 1500 and 2000 molecules of the capsid protein 24 (p24) forming hexamers and a few pentamers, produced from proteolytic action of the viral protease on Gag polyprotein of ~55 Kda[143,149]. Capsid p24 is composed of two parts, an N-terminal domain (NTD), spanning over around first 146 residues and a C-terminal domain (CTD), the two domains are joined by a short linker[150]. HIV capsid has been studied and several alpha helices have been highlighted over the entire p24 molecule. In addition, the N-terminal has a proline rich region[151,152].

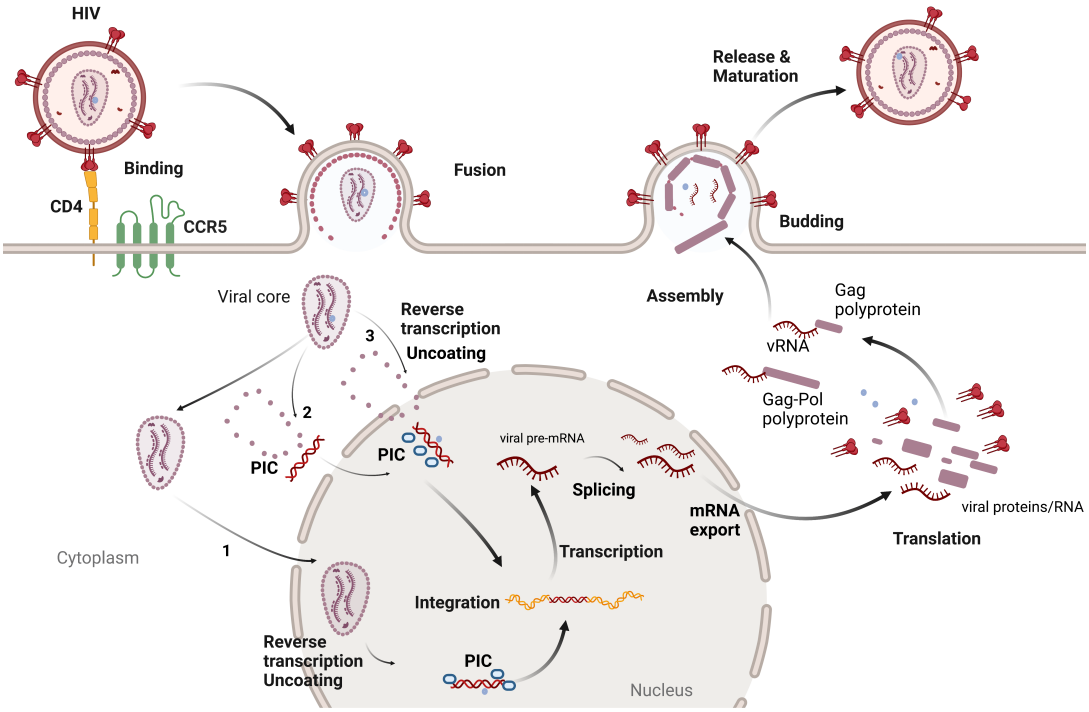

Fig 2. HIV replication cycle. HIV replication starts with viral interaction with cell membrane receptors and co-receptors. This is followed by release of viral capsid in the cell cytosol where it is suggested to follow one of the three uncoating pathways. Following reverse transcription, viral cDNA integrates cellular chromosomal DNA and this provirus is the origin for mRNA for both proteins and genome. The assembly of viral components at the plasma membrane is followed by viral budding, release, and maturation, before a new cycle is initiated. Created with biorender.com.

1.7 Dependency and restriction factors of HIV/SIV

1.7.1 Cyclophilins

Cellular proteins cyclophilin A (CYPA) and cyclophilin B (CYPB) are prolyl-peptidyl isomerases which have been shown to be involved in HIV replication[153–156].

CYPA has been shown to bind with HIV-1 Gag protein and be packaged into nascent virions[157]. HIV Gag residues important for CYPA interactions have been mapped at position 89 (glycine or G89) and 90 (Proline or P90) on the capsid p24, termed CYPA binding loop[156]. CYPA-Gag binding can be abolished using the cyclophilin binding drug cyclosporin A (CsA) which binds and inhibits CYPA, by CYPA knockout or by mutating CYPA binding loop key residues[158]. Human cyclophilin A, is required for HIV infection in many cell lines and human primary cells[159]. On the other hand, my unpublished work suggests an anti-HIV signature of CYPB, despite an existing report, which highlighted its HIV-infection-enhancing side[155] through

increase of cDNA import to the nucleus, in an overexpression approach. CYPA is also important for viral sensitivity/resistance to antiviral drugs[160]. My recent findings show that CYPA can protect SIVcpzPts against capsid inhibitors such as PF74 and PF57[161].

1.7.2 Tripartite Motif-containing proteins

Tripartite motif-containing proteins (TRIM) are characterized by their structure in three domains: The N-terminal RING, the single or double B-box and the coiled-coil domains. Some TRIM proteins have in addition, a C-terminal domain termed B30.2 or SPRY domain[162,163]. While the RING, B-box and the Coiled-coil domains mediate ubiquitylation and protein multimerization during TRIM protein function, the B30.2 domain specifically recognizes, binds and affects retroviral capsid structure[164]. More than 70 TRIM proteins have been identified from the human genome, some of them with a restrictive attribute against HIV, in addition to their roles in regulation of cell cycle, embryogenesis and cell signaling[165]. The alpha isoform of TRIM5 protein (TRIM5α) has a B30.2 domain and has been of particular interest to scientists due to its selective anti-retroviral activity among primates. For instance, human TRIM5α (huTRIM5α) does not block HIV-1 M infection, due to protection offered by CYPA, as recently deciphered[166,167]. On the other hand, HIV-1 and HIV-2 are very sensitive to African green and rhesus monkeys' TRIM5α (agmTRIM5α and rhTRIM5α). Additionally, huTRIM5α does not inhibit SIVcpz and SIVsmm, the believed nearest evolutionary ancestors of HIV-1 and HIV-2[44]. Apart from binding retroviral capsids and causing their premature disassembly or uncoating, TRIM5α block infection through autophagy a process in which the cell contains and degrades unwanted intracellular content[168]. In the context of HIV restriction, TRIM5α not only acts as a receptor of autophagy cargo, but also initiates the autophagy process for example, by mediating the formation of ULK1 and BECN1 protein complex. TRIM5α engages with autophagy regulator 8 (Atg8)[169,170]. With its E3-ubiquitin ligase activity, TRIM5α promotes

HIV sensing by innate immunity and this involves many other cellular factors such as UBC13, TAK1, AP-1 and TRIM5α works as a pattern recognition receptor[171,172].

In some primates, the B30.2 domain in TRIM5 protein has been replaced by CYPA through a retrotransposition process, which generated TRIMCyp fusion proteins[173]. The *Aotus* night monkey, a New World monkey and the Old World monkey rhesus macaque express omTRIMCyp and rhTRIMCyp, respectively. RhTRIMCyp blocks HIV-2 and many non-M HIV-1s, but not HIV-1 M. OmTRIMCyp is active against HIV-1 viruses but inactive against HIV-2[53]. Such differences in susceptibility to TRIMCyp by HIVs, is attributed to capsid sequence differences, particularly in the CYPA loop but variations at residue 66 and 69 in CYPA domain of TRIMCyp also determine which virus is inhibited by or resists TRIMCyp[54]. For instance, lentiviral capsid residue at position 88 (A88) is known to mediate engagement with host CYPA domain of rhTRIMCyp, with Alanine (A88) conferring resistance to rhTrimCyp, as in case of HIV-1 M, while valine (V88) or methionine (M88) expose non-M viruses to rhTRIMCyp inhibition[57]. Inside the TRIM family, many other members such as TRIM34 and TRIM11 (another B30.2-containing member) exhibit anti-viral properties, particularly against HIV[174].

1.7.3 APOBEC3 enzymes

Inside an invaded cell, retroviruses are also on spot of sophisticated and deadly DNA editing/mutating enzymes, the Apolipoprotein B mRNA Editing Catalytic polypeptides 3 or APOBEC3[175]. These deaminases have a basic structure with the zinc domain "H-x-E-x23-28-P-C-x2-4-C"[176]. These proteins carry out cytosine to uridine deamination of single stranded DNA, causing non-favoring mutations[177]. APOBEC3s interact with HIV/SIV Gag and incorporate in the viral core during assembly and mutate viral cDNA following next round of

cell entry[178]. Such DNA editing activities result in a replication defective provirus and hence halts viral infection and are believed to have played a key anti-retroviral cross-species spread role[179]. The viral Vif protein is the viral counteractor that destroys A3 in the viral producer cells thereby allowing the generation of A3-free virions[8]. The Vif-A3 interaction is in most cases species-specific[180].

1.7.4 The Bone marrow stromal antigen-2 (BST-2)

The bone marrow stromal antigen-2, BST-2 or Tetherin is one of the factors restricting the late phase of viral replication- [181]. It opposes viral release form infected cell membranes, and thereby stopping further viral spread[182]. Tetherin is counteracted by either viral Nef, Vpu or Envelope proteins, depending on the virus[182,183].

1.7.5 SERine INCorporator proteins, the SERINCs

Serine incorporators are protein, which are thought to mediate incorporation of SERINE in lipid bilayers, particularly in the cell membrane phospholipids[184]. Two members of SERINC protein family are involved in lentiviral restriction, SERINC3 and SERINC5[185]. In the absence of a counteracting viral Nef or Envelope, SERINCs impair new virion infectivity, following their inclusion into the viral membrane, by reducing an ill-defined step in fusion of the viral with the -cell membrane in the beginning of cell invasion[186].

1.8 Antiretroviral drugs: Capsid inhibitors, integrase inhibitors, RT inhibitors

While considerable effort has been invested to develop a curative treatment or vaccine, control of HIV infection still relies on antiviral drugs that aim at suppressing viral replication [187,188]. This approach has tremendously reduced HIV related mortality and increased life expectancy[189]. Around 43 anti-HIV molecules, also called antiretroviral drugs (ARVs) have

been given a green light for clinical use. These drugs target several key steps of the HIV replication chain[190] and many more are under development, particularly to target Gag polyprotein and its products, as key players of viral replication, assembly, and release[191]. Among the current ARVs in use are inhibitors of viral integrase, reverse transcriptase inhibitors, inhibitors of viral protease, and viral entry inhibitors, used in combined Antiretroviral Treatment (cART)[192]. Despite their efficacy, HIV and particularly non-M groups HIVs oppose resistance to routinely used anti-retroviral drugs and more work will be needed to understand such viral resistance towards drugs[193]. Several small capsid binding molecules have been identified and synthesized and their use has boosted our understanding of capsid-host factor complex interactions, their possible clinical use is under investigation[134,194]. The small molecules like PF-3450074 (PF74), PF-3759857 (PF57), the Gilead Lenacapavir or GS-6027 target capsid through binding to the interface between N- and C-terminal domains (NTD and CTD) of two capsid monomers, a binding site shared with the CPSF6, and near binding sites for NUP153 and NUP358[195,196]. Capsid inhibitors docking affects capsid structure depending on the dose and type, low doses of PF74 stabilize the capsid while higher doses result in early capsid disintegration, both outcomes negatively affect subsequent steps of viral replication[197]. Another class of capsid binding molecules include the viral maturation inhibitors like BI-3257[198].

1.8 Aims of the project

This doctoral project's main goal was to study HIV and SIV capsid interactions with host but also pharmacological inhibitors, to decipher more on the SIV evolutionary journey which culminated into HIVs. In addition, the project also tried to investigate why some HIVs (the non-M group HIVs, also known as non-pandemic HIVs) did get only a limited spread among us.

The aim of **chapter I** was to assess SIVcpzPts infection biology in human cell lines but also primary leucocytes, to understand why this virus did not directly cross-spread to humans. I also explored SIVcpzPts and SIVcpzPtt susceptibility to capsid inhibitors PF74, PF57 and GS-CA1, which share binding sites with or bind near binding sites for some host factors such as CPS6, NUP153 and NUP358. Since capsid binding host factors have been shown to affect each other during HIV-1 infection, I also assessed their role, particularly CYPA, in the activity of capsid inhibitors against SIVcpz. CPSF6 is known to bind and licence HIV capsid for a nuclear entry. Its short form, the CPS6_358 is, on the other hand antiretroviral and cytoplasmic, due to lack of the arginine-serine rich motif, required for CPSF6 nuclear translocation[199–201]. To answer the question on whether this truncated CPSF6 can also bind and inhibit SIVcpz, I assessed SIVcpzPts and SIVcpzPtt sensitivity to CPSF6_358. My findings show that SIVcpzPts can use CYPA to escape inhibition by pharmacological capsid destabilisers. In addition, this virus, as opposed to SIVcpzPtt and HIV, is not inhibited by the experimental cytoplasmic CPSF6-358. I also a reviewed available literature with the aim to highlight knowledge on post-entry restriction patterns against SIVcpz.

Despite my key findings which show differences between the human non-adapted SIVcpzPts and the human adapted SIVcpzPtt, in terms of cytoplasmic events during infection, more work will be needed to understand why SIVcpzPts was not able to colonize human cellular environment, more post-entry events may provide more light on such differences.

Chapter II had a main goal to answer the question on why are some HIVs (HIV-1 N, HIV-1 O, HIV-1 P and HIV-2) rare and less pathogenic, in comparison to the pandemic HIV-1 M. To answer this question, I explored early infection biology phase events of such viruses in the context of how they engage their capsids with human TRIM5α and CYPA. Capsid CYPA binding loop is diverse between these viruses. To understand possible contribution of such diversity to differences in infection biology, I assessed the role of capsid CYPA binding loop

for the activity of TRIM5α. I show that HIV-1 N, HIV-1 O, HIV-1 P, HIV-2 and SIVgor but not HIV-1 M are inhibited by human TRIM5α. More investigation will be needed to see if there are other host factors such as those involved in nuclear import and export of HIV and SIV viral components, which can explain why non-M HIVs did not manage to extensively spread among humans.

Under **chapter III**, the goal was to use different approaches such as gene knockout, protein overexpression and fusion proteins (TRIMCYPB), to detail the role of CYPB during HIV infection in a cell type manner. The project also aimed at answering questions on which capsid residues mediate CYPB-viral Gag interactions for CYPB antiviral activity, as described in my findings. The question on when does CYPB exhibit its antiviral activity during HIV infection was answered using a viral uncoating assay and 2-circle LTR measurement. Such approaches showed that CYPB anti-HIV effect starts early during infection. it will be interesting to investigate on whether CYPB binding to capsid also interferes with other host and pharmacological capsid inhibitors of HIV and SIV such TRIM5α, PF74, as does CYPA. CYPA has been highlighted as a factor influencing the nuclear import of HIV, in the presence of CPSF6[117]. Does CYPB do the same?

Bibliography

1. Marchese S, Polo A, Ariano A, Velotto S, Costantini S, Severino L. Aflatoxin B1 and M1: Biological Properties and Their Involvement in Cancer Development. *Toxins (Basel)*. 2018;10(6):214. doi:10.3390/toxins10060214
2. Sarfo FS, Eberhardt KA, Dompreh A, et al. Helicobacter pylori Infection Is Associated with Higher CD4 T Cell Counts and Lower HIV-1 Viral Loads in ART-Naïve HIV-Positive Patients in Ghana. Apetrei C, ed. *PLoS One*. 2015;10(11):e0143388. doi:10.1371/journal.pone.0143388
3. Rook G, Bäckhed F, Levin BR, McFall-Ngai MJ, McLean AR. Evolution, human-microbe interactions, and life history plasticity. *Lancet*. 2017;390(10093):521-530. doi:10.1016/S0140-6736(17)30566-4
4. McCarthy KR, Kirmaier A, Autissier P, Johnson WE. Evolutionary and Functional Analysis of Old World Primate TRIM5 Reveals the Ancient Emergence of Primate Lentiviruses and Convergent Evolution Targeting a Conserved Capsid Interface. Emerman M, ed. *PLoS Pathog*. 2015;11(8):e1005085. doi:10.1371/journal.ppat.1005085
5. Souquière S, Onanga R, Makuwa M, et al. Simian immunodeficiency virus types 1 and 2 (SIV mnd 1 and 2) have different pathogenic potentials in rhesus macaques upon experimental cross-species transmission. *Journal of General Virology*. 2009;90(2):488-499. doi:10.1099/vir.0.005181-0
6. Osei Kuffour E, Schott K, Jaguva Vasudevan AA, et al. USP18 (UBP43) Abrogates p21-Mediated Inhibition of HIV-1. Sundquist WI, ed. *J Virol*. 2018;92(20). doi:10.1128/JVI.00592-18
7. Meyerson NR, Warren CJ, Vieira DASA, et al. Correction: Species-specific vulnerability of RanBP2 shaped the evolution of SIV as it transmitted in African apes. Swanstrom R, ed. *PLoS Pathog*. 2018;14(4):e1006983. doi:10.1371/journal.ppat.1006983
8. Zhang Z, Gu Q, de Manuel Montero M, et al. Stably expressed APOBEC3H forms a barrier for cross-species transmission of simian immunodeficiency virus of chimpanzee to humans. Ross SR, ed. *PLoS Pathog*. 2017;13(12):e1006746. doi:10.1371/journal.ppat.1006746

9. Mariani R, Chen D, Schröfelbauer B, et al. Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. *Cell*. 2003;114(1):21-31. doi:10.1016/S0092-8674(03)00515-4
10. Meyerson NR, Warren CJ, Vieira DASA, Diaz-Griferro F, Sawyer SL. Species-specific vulnerability of RanBP2 shaped the evolution of SIV as it transmitted in African apes. Swanstrom R, ed. *PLoS Pathog*. 2018;14(3):e1006906. doi:10.1371/journal.ppat.1006906
11. Hasenkrug KJ, Chesebro B, Lynch C, Stoye JP. Immunity to retroviral infection: the Friend virus model. *Proc Natl Acad Sci U S A*. 1997;94(15):7811-7816. doi:10.1073/pnas.94.15.7811
12. Conway JM, Ribeiro RM. Modeling the immune response to HIV infection. *Curr Opin Syst Biol*. 2018;12:61-69. doi:10.1016/J.COISB.2018.10.006
13. Grossman Z. Immunological Paradigms, Mechanisms, and Models: Conceptual Understanding Is a Prerequisite to Effective Modeling. *Front Immunol*. 2019;10:2522. doi:10.3389/fimmu.2019.02522
14. Sharp PM, Hahn BH. Origins of HIV and the AIDS Pandemic. doi:10.1101/cshperspect.a006841
15. Papathanasopoulos MA, Hunt GM, Tiemessen CT. Evolution and Diversity of HIV-1 in Africa – a Review. *Virus Genes*. 2003;26(2):151-163. doi:10.1023/A:1023435429841
16. D'arc M, Ayouba A, Esteban A, et al. Origin of the HIV-1 group O epidemic in western lowland gorillas. *Proc Natl Acad Sci U S A*. 2015;112(11):E1343-52. doi:10.1073/pnas.1502022112
17. Sharp PM, Hahn BH. The evolution of HIV-1 and the origin of AIDS. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2010;365(1552):2487-2494. doi:10.1098/rstb.2010.0031
18. Pontzer H, Raichlen DA, Gordon AD, et al. Primate energy expenditure and life history. *Proc Natl Acad Sci U S A*. 2014;111(4):1433-1437. doi:10.1073/pnas.1316940111
19. Prado-Martinez J, Sudmant PH, Kidd JM, et al. Great ape genetic diversity and population history. *Nature*. 2013;499(7459):471-475. doi:10.1038/nature12228

20. Lowenstine LJ, McManamon R, Terio KA. Comparative Pathology of Aging Great Apes: Bonobos, Chimpanzees, Gorillas, and Orangutans. *Vet Pathol*. 2016;53(2):250-276. doi:10.1177/0300985815612154
21. Shimada MK, Hayakawa S, Humle T, et al. Mitochondrial DNA genealogy of chimpanzees in the Nimba mountains and Bossou, West Africa. *Am J Primatol*. 2004;64(3):261-275. doi:10.1002/ajp.20077
22. Gonder MK, Oates JF, Disotell TR, Forstner MRJ, Morales JC, Melnick DJ. A new west African chimpanzee subspecies? *Nature*. 1997;388(6640):337-337. doi:10.1038/41005
23. Suddendorf T, Collier-Baker E. The evolution of primate visual self-recognition: evidence of absence in lesser apes. *Proceedings of the Royal Society B: Biological Sciences*. 2009;276(1662):1671-1677. doi:10.1098/rspb.2008.1754
24. Li Y, Ndjango JB, Learn GH, et al. Eastern chimpanzees, but not bonobos, represent a simian immunodeficiency virus reservoir. *J Virol*. 2012;86(19):10776-10791. doi:10.1128/JVI.01498-12
25. Hill A, Collier-Baker E, Suddendorf T. Inferential reasoning by exclusion in great apes, lesser apes, and spider monkeys. *J Comp Psychol*. 2011;125(1):91-103. doi:10.1037/a0020867
26. Keita MB, Hamad I, Bittar F. Looking in apes as a source of human pathogens. *Microb Pathog*. 2014;77:149-154. doi:10.1016/J.MICPATH.2014.09.003
27. Püschel TA, Gladman JT, Bobe R, Sellers WI. The evolution of the platyrrhine talus: A comparative analysis of the phenetic affinities of the Miocene platyrrhines with their modern relatives. *J Hum Evol*. 2017;111:179-201. doi:10.1016/j.jhevol.2017.07.015
28. Bond M, Tejedor MF, Campbell KE, Chornogubsky L, Novo N, Goin F. Eocene primates of South America and the African origins of New World monkeys. *Nature*. 2015;520(7548):538-541. doi:10.1038/nature14120
29. Don E. Wilson And DeeAnn M. Reeder. *Mammal Species of the World | Hopkins Press*. Accessed February 20, 2022. https://www.press.jhu.edu/books/title/8864/mammal-species-world

30. Hirsch VM, McGann C, Dapolito G, et al. Identification of a New Subgroup of SIVagm in Tantalus Monkeys. *Virology*. 1993;197(1):426-430. doi:10.1006/VIRO.1993.1606
31. Filippone C, de Oliveira F, Betsem E, et al. Simian Immunodeficiency Virus seroreactivity in inhabitants from rural Cameroon frequently in contact with non-human primates. *Virology*. 2017;503:76-82. doi:10.1016/J.VIROL.2016.12.026
32. Menezes AN, Bonvicino CR, Seuánez HN. Identification, classification and evolution of Owl Monkeys (Aotus, Illiger 1811). *BMC Evol Biol*. 2010;10(1):248. doi:10.1186/1471-2148-10-248
33. Fujita K. Species recognition by five macaque monkeys. *Primates*. 1987;28(3):353-366. doi:10.1007/BF02381018
34. German Advisory Committee Blood (Arbeitskreis Blut), Subgroup 'Assessment of Pathogens Transmissible by Blood' S 'Assessment of PT by B. Human Immunodeficiency Virus (HIV). *Transfus Med Hemother*. 2016;43(3):203-222. doi:10.1159/000445852
35. Animal models for HIV infection and AIDS: memorandum from a WHO meeting. *Bull World Health Organ*. 1988;66(5):561-574.
36. Kuhn JH. Virus Taxonomy. *Encyclopedia of Virology*. Published online January 1, 2021:28-37. doi:10.1016/B978-0-12-809633-8.21231-4
37. Shah S, Alexaki A, Pirrone V, Dahiya S, Nonnemacher MR, Wigdahl B. Functional properties of the HIV-1 long terminal repeat containing single-nucleotide polymorphisms in Sp site III and CCAAT/enhancer binding protein site I. *Virol J*. 2014;11(1):92. doi:10.1186/1743-422X-11-92
38. Santiago ML, Bibollet-Ruche F, Bailes E, et al. Amplification of a complete simian immunodeficiency virus genome from fecal RNA of a wild chimpanzee. *J Virol*. 2003;77(3):2233-2242. doi:10.1128/JVI.77.3.2233-2242.2003
39. Guyader M, Emerman M, Sonigo P, Clavel F, Montagnier L, Alizon M. Genome organization and transactivation of the human immunodeficiency virus type 2. *Nature*. 1987;326(6114):662. doi:10.1038/326662a0
40. Nyamweya S, Hegedus A, Jaye A, Rowland-Jones S, Flanagan KL, Macallan DC. Comparing HIV-1 and HIV-2 infection: Lessons for viral

immunopathogenesis. *Rev Med Virol*. 2013;23(4):221-240. doi:10.1002/rmv.1739

41. Apetrei C, Kaur A, Lerche NW, et al. Molecular Epidemiology of Simian Immunodeficiency Virus SIVsm in U.S. Primate Centers Unravels the Origin of SIVmac and SIVstm. *J Virol*. 2005;79(14):8991-9005. doi:10.1128/JVI.79.14.8991-9005.2005
42. Hirsch VM, Olmsted RA, Murphey-Corbt M, Purcell RH, Johnson PR. *An African Primate Lentivirus (SIV Sm> Closely Related to HIV-2*.; 1989.
43. Gürtler L. SIV als Quelle von HIV. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz*. 2004;47(7):680-684. doi:10.1007/s00103-004-0862-z
44. Twizerimana AP, Scheck R, Häussinger D, Münk C. Post-entry restriction factors of SIVcpz. *Future Virol*. 2018;13(10):727-745. doi:10.2217/fvl-2018-0093
45. Vigan R, Neil SJD. Determinants of Tetherin Antagonism in the Transmembrane Domain of the Human Immunodeficiency Virus Type 1 Vpu Protein. *J Virol*. 2010;84(24):12958-12970. doi:10.1128/JVI.01699-10
46. Taylor BS, Sobieszczyk ME, McCutchan FE, Hammer SM. The challenge of HIV-1 subtype diversity. *N Engl J Med*. 2008;358(15):1590-1602. doi:10.1056/NEJMra0706737
47. Clavel F, Guétard D, Brun-Vézinet F, et al. Isolation of a new human retrovirus from West African patients with AIDS. *Science*. 1986;233(4761):343-346. doi:10.1126/science.2425430
48. Ibe S, Yokomaku Y, Shiino T, et al. HIV-2 CRF01_AB: First Circulating Recombinant Form of HIV-2. *JAIDS Journal of Acquired Immune Deficiency Syndromes*. 2010;54(3):241-247. doi:10.1097/QAI.0b013e3181dc98c1
49. Tebit DM, Patel H, Ratcliff A, et al. HIV-1 Group O Genotypes and Phenotypes: Relationship to Fitness and Susceptibility to Antiretroviral Drugs. *AIDS Res Hum Retroviruses*. 2016;32(7):676-688. doi:10.1089/AID.2015.0318

50. Mourez T, Simon F, Plantier JC. Non-M Variants of Human Immunodeficiency Virus Type 1. *Clin Microbiol Rev*. 2013;26(3):448-461. doi:10.1128/CMR.00012-13
51. Li G, Piampongsant S, Faria NR, et al. An integrated map of HIV genome-wide variation from a population perspective. *Retrovirology*. 2015;12(1):18. doi:10.1186/s12977-015-0148-6
52. Heuverswyn F Van, Li Y, Bailes E, et al. Genetic diversity and phylogeographic clustering of SIVcpzPtt in wild chimpanzees in Cameroon. *Virology*. 2007;368(1):155-171. doi:10.1016/J.VIROL.2007.06.018
53. Nakayama EE, Shioda T. TRIM5α and species tropism of HIV/SIV. *Front Microbiol*. 2012;3(JAN):1-12. doi:10.3389/fmicb.2012.00013
54. Price AJ, Marzetta F, Lammers M, et al. Active site remodeling switches HIV specificity of antiretroviral TRIMCyp. *Nat Struct Mol Biol*. 2009;16(10):1036-1042. doi:10.1038/nsmb.1667
55. Gao F, Vue L, Whitet AT, et al. *Human Infection by Genetically Diverse SIV SM·related HIV-2 in West Africa*.; 1992.
56. Su CTT, Koh DWS, Gan SKE. Reviewing HIV-1 Gag Mutations in Protease Inhibitors Resistance: Insights for Possible Novel Gag Inhibitor Designs. *Molecules*. 2019;24(18):3243. doi:10.3390/molecules24183243
57. Dick A, Cocklin S. Recent Advances in HIV-1 Gag Inhibitor Design and Development. *Molecules 2020, Vol 25, Page 1687*. 2020;25(7):1687. doi:10.3390/MOLECULES25071687
58. Cannon PM, Matthews S, Clark N, et al. Structure-function studies of the human immunodeficiency virus type 1 matrix protein, p17. *J Virol*. 1997;71(5):3474-3483. doi:10.1128/jvi.71.5.3474-3483.1997
59. Dolcetti R, Giagulli C, He W, et al. Role of HIV-1 matrix protein p17 variants in lymphoma pathogenesis. *Proceedings of the National Academy of Sciences*. 2015;112(46):14331-14336. doi:10.1073/pnas.1514748112
60. Zhuang S, Torbett BE. Interactions of HIV-1 Capsid with Host Factors and Their Implications for Developing Novel Therapeutics. *Viruses*. 2021;13(3). doi:10.3390/v13030417
61. Tang S, Zhao J, Wang A, et al. Characterization of Immune Responses to Capsid Protein p24 of Human Immunodeficiency Virus Type 1 and

Implications for Detection. *Clinical and Vaccine Immunology*. 2010;17(8):1244-1251. doi:10.1128/CVI.00066-10

62. Yamashita M, Engelman AN. Capsid-Dependent Host Factors in HIV-1 Infection. *Trends Microbiol*. 2017;25(9):741-755. doi:10.1016/j.tim.2017.04.004
63. Saito A, Yamashita M. HIV-1 capsid variability: viral exploitation and evasion of capsid-binding molecules. *Retrovirology*. 2021;18(1):32. doi:10.1186/s12977-021-00577-x
64. Mattei S, Flemming A, Anders-Össwein M, Kräusslich HG, Briggs JAG, Müller B. RNA and Nucleocapsid Are Dispensable for Mature HIV-1 Capsid Assembly. Sundquist WI, ed. *J Virol*. 2015;89(19):9739-9747. doi:10.1128/JVI.00750-15
65. de Marco A, Heuser AM, Glass B, Krausslich HG, Muller B, Briggs JAG. Role of the SP2 Domain and Its Proteolytic Cleavage in HIV-1 Structural Maturation and Infectivity. *J Virol*. 2012;86(24):13708-13716. doi:10.1128/JVI.01704-12
66. Wanaguru M, Bishop KN. HIV-1 Gag Recruits Oligomeric Vpr via Two Binding Sites in p6, but Both Mature p6 and Vpr Are Rapidly Lost upon Target Cell Entry. Kirchhoff F, ed. *J Virol*. 2021;95(17). doi:10.1128/JVI.00554-21
67. Hill M, Tachedjian G, Mak J. The Packaging and Maturation of the HIV-1 Pol Proteins. *Curr HIV Res*. 2005;3(1):73-85. doi:10.2174/1570162052772942
68. Gulnik S, Erickson JW, Xie D. HIV protease: Enzyme function and drug resistance. *Vitam Horm*. 2000;58:213-256. doi:10.1016/S0083-6729(00)58026-1
69. Sarafianos SG, Marchand B, Das K, et al. Structure and function of HIV-1 reverse transcriptase: molecular mechanisms of polymerization and inhibition. Published online 2008. doi:10.1016/j.jmb.2008.10.071
70. Mahboubi-Rabbani M, Hajimahdi Z, Abbasi M, Zarghi A. HIV-1 Reverse Transcriptase/Integrase Dual Inhibitors: A Review of Recent Advances and Structure-activity Relationship Studies. *Iranian Journal of Pharmaceutical Research*. 2021;20(2):333-369. doi:10.22037/IJPR.2021.115446.15370

71. Craigie R. The molecular biology of HIV integrase. *Future Virol*. 2012;7(7):679-686. doi:10.2217/fvl.12.56
72. Das K, Martinez SE, DeStefano JJ, Arnold E. Structure of HIV-1 RT/dsRNA initiation complex prior to nucleotide incorporation. *Proc Natl Acad Sci U S A*. 2019;116(15):7308-7313. doi:10.1073/pnas.1814170116
73. Stopak K, de Noronha C, Yonemoto W, Greene WC. HIV-1 Vif blocks the antiviral activity of APOBEC3G by impairing both its translation and intracellular stability. *Mol Cell*. 2003;12(3):591-601. doi:10.1016/s1097-2765(03)00353-8
74. Salamango DJ, Harris RS. Dual Functionality of HIV-1 Vif in APOBEC3 Counteraction and Cell Cycle Arrest. *Front Microbiol*. 2021;11:3424. doi:10.3389/fmicb.2020.622012
75. Kao S, Khan MA, Miyagi E, Plishka R, Buckler-White A, Strebel K. The Human Immunodeficiency Virus Type 1 Vif Protein Reduces Intracellular Expression and Inhibits Packaging of APOBEC3G (CEM15), a Cellular Inhibitor of Virus Infectivity. *J Virol*. 2003;77(21):11398-11407. doi:10.1128/JVI.77.21.11398-11407.2003
76. Cohen EA, Dehni G, Sodroski JG, Haseltine WA. Human immunodeficiency virus vpr product is a virion-associated regulatory protein. *J Virol*. 1990;64(6):3097-3099. doi:10.1128/JVI.64.6.3097-3099.1990
77. Montal M. Structure-function correlates of Vpu, a membrane protein of HIV-1. *FEBS Lett*. 2003;552(1):47-53. doi:10.1016/S0014-5793(03)00849-4
78. Akari H, Bour S, Kao S, Adachi A, Strebel K. The Human Immunodeficiency Virus Type 1 Accessory Protein Vpu Induces Apoptosis by Suppressing the Nuclear Factor κB–dependent Expression of Antiapoptotic Factors. *Journal of Experimental Medicine*. 2001;194(9):1299-1312. doi:10.1084/jem.194.9.1299
79. Volcic M, Sparrer KMJ, Koepke L, et al. Vpu modulates DNA repair to suppress innate sensing and hyper-integration of HIV-1. *Nat Microbiol*. 2020;5(10):1247-1261. doi:10.1038/s41564-020-0753-6

80. Ayinde D, Casartelli N, Schwartz O. Restricting HIV the SAMHD1 way: through nucleotide starvation. *Nat Rev Microbiol*. 2012;10(10):675-680. doi:10.1038/nrmicro2862
81. Schüle S, Kloke BP, Kaiser JK, et al. Restriction of HIV-1 Replication in Monocytes Is Abolished by Vpx of SIVsmmPBj. Harris RS, ed. *PLoS One*. 2009;4(9):e7098. doi:10.1371/journal.pone.0007098
82. Wang H, Guo H, Su J, et al. Inhibition of Vpx-Mediated SAMHD1 and Vpr-Mediated Host Helicase Transcription Factor Degradation by Selective Disruption of Viral CRL4 (DCAF1) E3 Ubiquitin Ligase Assembly. Ross SR, ed. *J Virol*. 2017;91(9). doi:10.1128/JVI.00225-17
83. Baldauf HM, Stegmann L, Schwarz SM, et al. Vpx overcomes a SAMHD1-independent block to HIV reverse transcription that is specific to resting CD4 T cells. doi:10.1073/pnas.1613635114
84. Planelles V, Barker E. Roles of Vpr and Vpx in modulating the virus-host cell relationship. *Mol Aspects Med*. 2010;31(5):398-406. doi:10.1016/j.mam.2010.05.002
85. Clark E, Nava B, Caputi M. Tat is a multifunctional viral protein that modulates cellular gene expression and functions. *Oncotarget*. 2017;8(16):27569-27581. doi:10.18632/oncotarget.15174
86. Marciniak RA, Sharp PA. HIV-1 Tat protein promotes formation of more-processive elongation complexes. *EMBO J*. 1991;10(13):4189-4196. doi:10.1002/j.1460-2075.1991.tb04997.x
87. Rice AP. The HIV-1 Tat Protein: Mechanism of Action and Target for HIV-1 Cure Strategies. *Curr Pharm Des*. 2017;23(28). doi:10.2174/1381612823666170704130635
88. Erkelenz S, Hillebrand F, Widera M, et al. Balanced splicing at the Tat-specific HIV-1 3′ss A3 is critical for HIV-1 replication. *Retrovirology*. 2015;12(1):29. doi:10.1186/s12977-015-0154-8
89. Brillen AL, Schöneweis K, Walotka L, et al. Succession of splicing regulatory elements determines cryptic 5′ss functionality. *Nucleic Acids Res*. 2017;45(7):4202-4216. doi:10.1093/nar/gkw1317
90. Blissenbach M, Grewe B, Hoffmann B, Brandt S, Überla K. Nuclear RNA Export and Packaging Functions of HIV-1 Rev Revisited. *J Virol*. 2010;84(13):6598-6604. doi:10.1128/JVI.02264-09

91. Arrigo SJ, Chen IS. Rev is necessary for translation but not cytoplasmic accumulation of HIV-1 vif, vpr, and env/vpu 2 RNAs. *Genes Dev*. 1991;5(5):808-819. doi:10.1101/gad.5.5.808

92. Pocock GM, Becker JT, Swanson CM, Ahlquist P, Sherer NM. HIV-1 and M-PMV RNA Nuclear Export Elements Program Viral Genomes for Distinct Cytoplasmic Trafficking Behaviors. Cullen BR, ed. *PLoS Pathog*. 2016;12(4):e1005565. doi:10.1371/journal.ppat.1005565

93. Schwartz S, Felber BK, Pavlakis GN. Expression of human immunodeficiency virus type 1 vif and vpr mRNAs is rev-dependent and regulated by splicing. *Virology*. 1991;183(2):677-686. doi:10.1016/0042-6822(91)90996-O

94. Hadzopoulou-Cladaras M, Felber BK, Cladaras C, Athanassopoulos A, Tse A, Pavlakis GN. The rev (trs/art) protein of human immunodeficiency virus type 1 affects viral mRNA and protein expression via a cis-acting sequence in the env region. *J Virol*. 1989;63(3):1265-1274. doi:10.1128/JVI.63.3.1265-1274.1989

95. Aqil M, Naqvi AR, Bano AS, Jameel S. The HIV-1 Nef Protein Binds Argonaute-2 and Functions as a Viral Suppressor of RNA Interference. Kashanchi F, ed. *PLoS One*. 2013;8(9):e74472. doi:10.1371/journal.pone.0074472

96. Rose JJ, Janvier K, Chandrasekhar S, Sekaly RP, Bonifacino JS, Venkatesan S. CD4 down-regulation by HIV-1 and simian immunodeficiency virus (SIV) Nef proteins involves both internalization and intracellular retention mechanisms. *J Biol Chem*. 2005;280(9):7413-7426. doi:10.1074/jbc.M409420200

97. Arias JF, Colomer-Lluch M, von Bredow B, et al. Tetherin Antagonism by HIV-1 Group M Nef Proteins. Kirchhoff F, ed. *J Virol*. 2016;90(23):10701-10714. doi:10.1128/JVI.01465-16

98. Lai RPJ, Yan J, Heeney J, et al. Nef Decreases HIV-1 Sensitivity to Neutralizing Antibodies that Target the Membrane-proximal External Region of TMgp41. Cullen BR, ed. *PLoS Pathog*. 2011;7(12):e1002442. doi:10.1371/journal.ppat.1002442

99. Collins KL, Chen BK, Kalams SA, Walker BD, Baltimore D. HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. *Nature*. 1998;391(6665):397-401. doi:10.1038/34929
100. Merk A, Subramaniam S. HIV-1 envelope glycoprotein structure. *Curr Opin Struct Biol*. 2013;23(2):268-276. doi:10.1016/j.sbi.2013.03.007
101. Arrildt KT, Joseph SB, Swanstrom R. The HIV-1 Env Protein: A Coat of Many Colors. *Curr HIV/AIDS Rep*. 2012;9(1):52-63. doi:10.1007/s11904-011-0107-3
102. Prabakaran P, Dimitrov AS, Fouts TR, Dimitrov DS. Structure and function of the HIV envelope glycoprotein as entry mediator, vaccine immunogen, and target for inhibitors. *Adv Pharmacol*. 2007;55:33-97. doi:10.1016/S1054-3589(07)55002-7
103. Gianechini FA, Méndez AH, Filippi LS, Paulina-Carabajal A, Juárez-Valieri RD, Garrido AC. A new furileusaurian abelisaurid from La Invernada (Upper Cretaceous, Santonian, Bajo de la Carpa Formation), northern Patagonia, Argentina. *J Vertebr Paleontol*. 2020;40(6):e1877151. doi:10.1080/02724634.2020.1877151
104. Charbonneau J, Gendron K, Ferbeyre G, Brakier-Gingras L. The 5' UTR of HIV-1 full-length mRNA and the Tat viral protein modulate the programmed -1 ribosomal frameshift that generates HIV-1 enzymes. *RNA*. 2012;18(3):519-529. doi:10.1261/rna.030346.111
105. Beerens N, Groot F, Berkhout B. Initiation of HIV-1 reverse transcription is regulated by a primer activation signal. *J Biol Chem*. 2001;276(33):31247-31256. doi:10.1074/jbc.M102441200
106. BEERENS N, BERKHOUT B. Switching the in vitro tRNA usage of HIV-1 by simultaneous adaptation of the PBS and PAS. *RNA*. 2002;8(3):S1355838202028194. doi:10.1017/S1355838202028194
107. Zhou M, Deng L, Kashanchi F, Brady JN, Shatkin AJ, Kumar A. The Tat/TAR-dependent phosphorylation of RNA polymerase II C-terminal domain stimulates cotranscriptional capping of HIV-1 mRNA. *Proc Natl Acad Sci U S A*. 2003;100(22):12666. doi:10.1073/PNAS.1835726100
108. Mbondji-wonje C, Dong M, Wang X, et al. Distinctive variation in the U3R region of the 5' Long Terminal Repeat from diverse HIV-1 strains.

Meng Z, ed. *PLoS One*. 2018;13(4):e0195661. doi:10.1371/journal.pone.0195661

109. Martial NT, Mubarik S, Yu C. The Trend of HIV/AIDS Incidence and Risks Associated with Age, Period, and Birth Cohort in Four Central African Countries. *Int J Environ Res Public Health*. 2021;18(5):2564. doi:10.3390/ijerph18052564
110. McLaren PJ, Fellay J. HIV-1 and human genetic variation. *Nat Rev Genet*. 2021;22(10):645-657. doi:10.1038/s41576-021-00378-0
111. Twizerimana AP, Mwatha J, Musabyimana JP, et al. IMMUNOLOGICAL PROFILES IN HIV POSITIVE PATIENTS FOLLOWING HAART INITIATION IN KIGALI, RWANDA. *East Afr Med J*. 2014;91(8):261-266.
112. Shaw GM, Hunter E. HIV transmission. *Cold Spring Harb Perspect Med*. 2012;2(11):a006965. doi:10.1101/cshperspect.a006965
113. Berzow D, Descamps D, Obermeier M, et al. Human Immunodeficiency Virus–2 (HIV-2): A Summary of the Present Standard of Care and Treatment Options for Individuals Living with HIV-2 in Western Europe. *Clinical Infectious Diseases*. 2021;72(3):503-509. doi:10.1093/cid/ciaa275
114. Kapoor AK, Padival S. *HIV-2*. StatPearls Publishing; 2021.
115. Mack K, Starz K, Sauter D, et al. Efficient Vpu-Mediated Tetherin Antagonism by an HIV-1 Group O Strain. Silvestri G, ed. *J Virol*. 2017;91(6):e02177-16. doi:10.1128/JVI.02177-16
116. Sauter D, Kirchhoff F. Key Viral Adaptations Preceding the AIDS Pandemic. *Cell Host Microbe*. 2019;25:27-38. doi:10.1016/j.chom.2018.12.002
117. Price AJ, Marzetta F, Lammers M, et al. Active site remodeling switches HIV specificity of antiretroviral TRIMCyp. *Nat Struct Mol Biol*. 2009;16(10):1036-1042. doi:10.1038/nsmb.1667
118. MacNeil A, Sarr AD, Sankalé JL, Meloni ST, Mboup S, Kanki P. Direct Evidence of Lower Viral Replication Rates In Vivo in Human Immunodeficiency Virus Type 2 (HIV-2) Infection than in HIV-1 Infection. *J Virol*. 2007;81(10):5325-5330. doi:10.1128/JVI.02625-06

119. Marlink R, Kanki P, Thior I, et al. Reduced Rate of Disease Development After HIV-2 Infection as Compared to HIV-1. *Science (1979)*. 1994;265(5178):1587-1590. doi:10.1126/science.7915856
120. Kirchhoff F. HIV Life Cycle: Overview. In: *Encyclopedia of AIDS*. Springer New York; 2013:1-9. doi:10.1007/978-1-4614-9610-6_60-1
121. Schröder ARW, Shinn P, Chen H, Berry C, Ecker JR, Bushman F. HIV-1 integration in the human genome favors active genes and local hotspots. *Cell*. 2002;110(4):521-529. doi:10.1016/s0092-8674(02)00864-4
122. Arhel N, Kirchhoff F. Host proteins involved in HIV infection: New therapeutic targets. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 2010;1802(3):313-321. doi:10.1016/J.BBADIS.2009.12.003
123. Shukla E, Chauhan R. Host-HIV-1 Interactome: A Quest for Novel Therapeutic Intervention. *Cells*. 2019;8(10):1155. doi:10.3390/cells8101155
124. Dharan A, Bachmann N, Talley S, Zwikelmaier V, Campbell EM. Nuclear pore blockade reveals that HIV-1 completes reverse transcription and uncoating in the nucleus. *Nat Microbiol*. Published online June 1, 2020:1-8. doi:10.1038/s41564-020-0735-8
125. Zila V, Margiotta E, Turoňová B, et al. Cone-shaped HIV-1 capsids are transported through intact nuclear pores. *Cell*. 2021;184(4):1032-1046.e18. doi:10.1016/j.cell.2021.01.025
126. Francis AC, Melikyan GB. Single HIV-1 Imaging Reveals Progression of Infection through CA-Dependent Steps of Docking at the Nuclear Pore, Uncoating, and Nuclear Transport. *Cell Host Microbe*. 2018;23(4):536-548.e6. doi:10.1016/j.chom.2018.03.009
127. Mamede JI, Cianci GC, Anderson MR, Hope TJ. Early cytoplasmic uncoating is associated with infectivity of HIV-1. *Proc Natl Acad Sci U S A*. 2017;114(34):E7169-E7178. doi:10.1073/pnas.1706245114
128. Dismuke DJ, Aiken C. Evidence for a Functional Link between Uncoating of the Human Immunodeficiency Virus Type 1 Core and Nuclear Import of the Viral Preintegration Complex. *J Virol*. 2006;80(8):3712-3720. doi:10.1128/JVI.80.8.3712-3720.2006

129. Rawle DJ, Harrich D. Toward the “unravelling” of HIV: Host cell factors involved in HIV-1 core uncoating. Evans MJ, ed. *PLoS Pathog*. 2018;14(10):e1007270. doi:10.1371/journal.ppat.1007270
130. Burdick RC, Li C, Munshi M, et al. HIV-1 uncoats in the nucleus near sites of integration. *Proc Natl Acad Sci U S A*. 2020;117(10):5486-5493. doi:10.1073/pnas.1920631117
131. Saito A, Henning MS, Serrao E, et al. Capsid-CPSF6 Interaction Is Dispensable for HIV-1 Replication in Primary Cells but Is Selected during Virus Passage *In Vivo*. Sundquist WI, ed. *J Virol*. 2016;90(15):6918-6935. doi:10.1128/JVI.00019-16
132. Rossi E, Meuser ME, Cunanan CJ, Cocklin S. Structure, Function, and Interactions of the HIV-1 Capsid Protein. *Life (Basel)*. 2021;11(2). doi:10.3390/life11020100
133. Kirmaier A, Wu F, Newman RM, et al. TRIM5 Suppresses Cross-Species Transmission of a Primate Immunodeficiency Virus and Selects for Emergence of Resistant Variants in the New Species. Emerman M, ed. *PLoS Biol*. 2010;8(8):e1000462. doi:10.1371/journal.pbio.1000462
134. Bush S, Tebit DM. HIV-1 group O origin, evolution, pathogenesis, and treatment: Unraveling the complexity of an outlier 25 years later. *AIDS Rev*. 2015;17(3):147-158.
135. Delelis O, Parissi V, Leh H, et al. Efficient and Specific Internal Cleavage of a Retroviral Palindromic DNA Sequence by Tetrameric HIV-1 Integrase. Schwartz O, ed. *PLoS One*. 2007;2(7):e608. doi:10.1371/journal.pone.0000608
136. Delelis O, Carayon K, Saïb A, Deprez E, Mouscadet JF. Integrase and integration: biochemical activities of HIV-1 integrase. *Retrovirology*. 2008;5(1):114. doi:10.1186/1742-4690-5-114
137. Vandekerckhove L, Christ F, van Maele B, et al. Transient and Stable Knockdown of the Integrase Cofactor LEDGF/p75 Reveals Its Role in the Replication Cycle of Human Immunodeficiency Virus. *J Virol*. 2006;80(4):1886-1896. doi:10.1128/JVI.80.4.1886-1896.2006
138. Sundquist WI, Kräusslich HG. HIV-1 assembly, budding, and maturation. *Cold Spring Harb Perspect Med*. 2012;2(7):a006924. doi:10.1101/cshperspect.a006924

139. Checkley MA, Luttge BG, Freed EO. HIV-1 envelope glycoprotein biosynthesis, trafficking, and incorporation. *J Mol Biol*. 2011;410(4):582-608. doi:10.1016/j.jmb.2011.04.042

140. Egan MA, Carruth LM, Rowell JF, Yu X, Siliciano RF. Human immunodeficiency virus type 1 envelope protein endocytosis mediated by a highly conserved intrinsic internalization signal in the cytoplasmic domain of gp41 is suppressed in the presence of the Pr55gag precursor protein. *J Virol*. 1996;70(10):6547-6556. doi:10.1128/JVI.70.10.6547-6556.1996

141. Janvier K, Pelchen-Matthews A, Renaud JB, et al. The ESCRT-0 Component HRS is Required for HIV-1 Vpu-Mediated BST-2/Tetherin Down-Regulation. doi:10.1371/journal.ppat.1001265

142. Henderson LE, Bowers MA, Sowder RC, et al. Gag proteins of the highly replicative MN strain of human immunodeficiency virus type 1: posttranslational modifications, proteolytic processings, and complete amino acid sequences. *J Virol*. 1992;66(4):1856-1865. doi:10.1128/JVI.66.4.1856-1865.1992

143. Votteler J, Sundquist WI. Virus budding and the ESCRT pathway. *Cell Host Microbe*. 2013;14(3):232-241. doi:10.1016/j.chom.2013.08.012

144. Sundquist WI, Kräusslich HG. HIV-1 assembly, budding, and maturation. *Cold Spring Harb Perspect Med*. 2012;2(7):a006924. doi:10.1101/cshperspect.a006924

145. Ono A, Freed EO. Plasma membrane rafts play a critical role in HIV-1 assembly and release. *Proc Natl Acad Sci U S A*. 2001;98(24):13925-13930. doi:10.1073/pnas.241320298

146. Miller JT, Khvorova A, Scaringe SA, Le Grice SFJ. Synthetic tRNALys,3 as the replication primer for the HIV-1HXB2 and HIV-1Mal genomes. *Nucleic Acids Res*. 2004;32(15):4687-4695. doi:10.1093/nar/gkh813

147. Ding L, Derdowski A, Wang JJ, Spearman P. Independent Segregation of Human Immunodeficiency Virus Type 1 Gag Protein Complexes and Lipid Rafts. *J Virol*. 2003;77(3):1916-1926. doi:10.1128/JVI.77.3.1916-1926.2003

148. Toccafondi E, Lener D, Negroni M. HIV-1 Capsid Core: A Bullet to the Heart of the Target Cell. *Front Microbiol*. 2021;12:755. doi:10.3389/fmicb.2021.652486

149. Zhao G, Perilla JR, Yufenyuy EL, et al. Mature HIV-1 capsid structure by cryo-electron microscopy and all-atom molecular dynamics. *Nature*. 2013;497(7451):643-646. doi:10.1038/nature12162

150. Barrera FN, Hurtado-Gómez E, Lidón-Moya MC, Neira JL. Binding of the C-terminal domain of the HIV-1 capsid protein to lipid membranes: a biophysical characterization. *Biochemical Journal*. 2006;394(1):345-353. doi:10.1042/BJ20051487

151. Fitzon T, Leschonsky B, Bieler K, et al. Proline Residues in the HIV-1 NH2-Terminal Capsid Domain: Structure Determinants for Proper Core Assembly and Subsequent Steps of Early Replication. *Virology*. 2000;268(2):294-307.

152. Kim K, Dauphin A, Komurlu S, et al. Cyclophilin A protects HIV-1 from restriction by human TRIM5α. *Nat Microbiol*. 2019;4(12):2044-2051. doi:10.1038/s41564-019-0592-5

153. Sokolskaja E, Sayah DM, Luban J. Target Cell Cyclophilin A Modulates Human Immunodeficiency Virus Type 1 Infectivity CA-CypA interaction. *J Virol*. 2004;78(23):12800-12808. doi:10.1128/JVI.78.23.12800-12808.2004

154. DeBoer J, Madson CJ, Belshan M, J D, CJ M, M B. Cyclophilin B enhances HIV-1 infection. *Virology*. 2016;489:282-291. doi:10.1016/J.VIROL.2015.12.015

155. Luban J, Bossolt KL, Franke EK, Kalpana G v., Goff SP. Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B. *Cell*. 1993;73(6):1067-1078. doi:10.1016/0092-8674(93)90637-6

156. Lin TY, Emerman M. Cyclophilin A interacts with diverse lentiviral capsids. *Retrovirology*. 2006;3:1-12. doi:10.1186/1742-4690-3-70

157. Braaten D, Aberham C, Franke EK, Yin L, Phares W, Luban J. Cyclosporine A-resistant human immunodeficiency virus type 1 mutants demonstrate that Gag encodes the functional target of cyclophilin A. *J Virol*. 1996;70(8):5170-5176. doi:10.1128/jvi.70.8.5170-5176.1996

158. Selyutina A, Persaud M, Simons LM, et al. Cyclophilin A Prevents HIV-1 Restriction in Lymphocytes by Blocking Human TRIM5α Binding to the Viral Core. *Cell Rep*. 2020;30(11):3766-3777.e6. Accessed June 1, 2020. https://doi.org/10.1016/j.celrep.2020.02.100

159. Zhou J, Price AJ, Halambage UD, James LC, Aiken C. HIV-1 Resistance to the Capsid-Targeting Inhibitor PF74 Results in Altered Dependence on Host Factors Required for Virus Nuclear Entry. *J Virol*. 2015;89(17):9068-9079. doi:10.1128/JVI.00340-15

160. Twizerimana AP, Scheck R, Becker D, et al. Cell Type-Dependent Escape of Capsid Inhibitors by Simian Immunodeficiency Virus SIVcpz. Kirchhoff F, ed. *J Virol*. 2020;94(23). doi:10.1128/JVI.01338-20

161. Imam S, Talley S, Nelson RS, et al. TRIM5α Degradation via Autophagy Is Not Required for Retroviral Restriction. Beemon KL, ed. *J Virol*. 2016;90(7):3400-3410. doi:10.1128/JVI.03033-15

162. D'Cruz AA, Babon JJ, Norton RS, Nicola NA, Nicholson SE. Structure and function of the SPRY/B30.2 domain proteins involved in innate immunity. *Protein Science*. 2013;22(1):1-10. doi:10.1002/pro.2185

163. Song B, Gold B, O'Huigin C, et al. The B30.2(SPRY) domain of the retroviral restriction factor TRIM5alpha exhibits lineage-specific length and sequence variation in primates. *J Virol*. 2005;79(10):6111-6121. doi:10.1128/JVI.79.10.6111-6121.2005

164. Cloherty APM, Rader AG, Compeer B, Ribeiro CMS. Human TRIM5α: Autophagy Connects Cell-Intrinsic HIV-1 Restriction and Innate Immune Sensor Functioning. *Viruses*. 2021;13(2). doi:10.3390/v13020320

165. Yu A, Skorupka KA, Pak AJ, Ganser-Pornillos BK, Pornillos O, Voth GA. TRIM5α self-assembly and compartmentalization of the HIV-1 viral capsid. *Nat Commun*. 2020;11(1):1307. doi:10.1038/s41467-020-15106-1

166. Chatterji U, Bobardt MD, Stanfield R, et al. Naturally occurring capsid substitutions render HIV-1 cyclophilin A independent in human cells and TRIM-cyclophilin-resistant in Owl monkey cells. *J Biol Chem*. 2005;280(48):40293-40300. doi:10.1074/jbc.M506314200

167. Mandell MA, Kimura T, Jain A, Johansen T, Deretic V. TRIM proteins regulate autophagy: TRIM5 is a selective autophagy receptor mediating

HIV-1 restriction. *Autophagy*. 2014;10(12):2387-2388. doi:10.4161/15548627.2014.984278

168. Rahm N, Telenti A. The role of tripartite motif family members in mediating susceptibility to HIV-1 infection. *Curr Opin HIV AIDS*. 2012;7(2):180-186. doi:10.1097/COH.0b013e32835048e1
169. Mandell MA, Kimura T, Jain A, Johansen T, Deretic V. TRIM proteins regulate autophagy: TRIM5 is a selective autophagy receptor mediating HIV-1 restriction. *Autophagy*. 2014;10(12). doi:10.4161/15548627.2014.984278
170. Fletcher AJ, Vaysburd M, Maslen S, et al. Trivalent RING Assembly on Retroviral Capsids Activates TRIM5 Ubiquitination and Innate Immune Signaling. *Cell Host Microbe*. 2018;24(6):761-775.e6. doi:10.1016/j.chom.2018.10.007
171. Pertel T, Hausmann S, Morger D, et al. TRIM5 is an innate immune sensor for the retrovirus capsid lattice. *Nature*. 2011;472(7343):361-365. doi:10.1038/nature09976
172. Wilson SJ, Webb BLJ, Ylinen LMJ, Verschoor E, Heeney JL, Towers GJ. Independent evolution of an antiviral TRIMCyp in rhesus macaques. *Proc Natl Acad Sci U S A*. 2008;105(9):3557-3562. doi:10.1073/pnas.0709003105
173. Ohainle M, Kim K, Komurlu Keceli S, et al. TRIM34 restricts HIV-1 and SIV capsids in a TRIM5α-dependent manner. Evans DT, ed. *PLoS Pathog*. 2020;16(4):e1008507. doi:10.1371/journal.ppat.1008507
174. Harris RS, Dudley JP. APOBECs and virus restriction. *Virology*. 2015;479-480:131-145. doi:10.1016/j.virol.2015.03.012
175. Salter JD, Bennett RP, Smith HC. The APOBEC Protein Family: United by Structure, Divergent in Function. *Trends Biochem Sci*. 2016;41(7):578-594. doi:10.1016/j.tibs.2016.05.001
176. Harris RS, Bishop KN, Sheehy AM, et al. DNA Deamination Mediates Innate Immunity to Retroviral Infection. *Cell*. 2003;113(6):803-809. doi:10.1016/S0092-8674(03)00423-9
177. Malim MH. Natural resistance to HIV infection: The Vif-APOBEC interaction. *C R Biol*. 2006;329(11):871-875. doi:10.1016/j.crvi.2006.01.012

178. Jaguva Vasudevan AA, Balakrishnan K, Gertzen CGW, et al. Loop 1 of APOBEC3C Regulates its Antiviral Activity against HIV-1. *J Mol Biol*. 2020;432(23):6200-6227. doi:10.1016/j.jmb.2020.10.014
179. Zhang Z, Gu Q, Jaguva Vasudevan AA, et al. Determinants of FIV and HIV Vif sensitivity of feline APOBEC3 restriction factors. *Retrovirology*. 2016;13(1):46. doi:10.1186/s12977-016-0274-9
180. Mack K, Starz K, Sauter D, et al. Efficient Vpu-Mediated Tetherin Antagonism by an HIV-1 Group O Strain. Silvestri G, ed. *J Virol*. 2017;91(6):e02177-16. doi:10.1128/JVI.02177-16
181. Bampi C, Rasga L, Roux L. Antagonism to human BST-2/tetherin by Sendai virus glycoproteins. *Journal of General Virology*. 2013;94(6):1211-1219. doi:10.1099/vir.0.051771-0
182. Kelly JT, Human S, Alderman J, et al. BST2/Tetherin Overexpression Modulates Morbillivirus Glycoprotein Production to Inhibit Cell–Cell Fusion. *Viruses*. 2019;11(8):692. doi:10.3390/v11080692
183. Inuzuka M, Hayakawa M, Ingi T. Serinc, an Activity-regulated Protein Family, Incorporates Serine into Membrane Lipid Synthesis. *Journal of Biological Chemistry*. 2005;280(42):35776-35783. doi:10.1074/jbc.M505712200
184. Usami Y, Wu Y, Göttlinger HG. SERINC3 and SERINC5 restrict HIV-1 infectivity and are counteracted by Nef. *Nature*. 2015;526(7572):218-223. doi:10.1038/nature15400
185. Heigele A, Kmiec D, Regensburger K, et al. The Potency of Nef-Mediated SERINC5 Antagonism Correlates with the Prevalence of Primate Lentiviruses in the Wild. *Cell Host Microbe*. 2016;20(3):381-391. doi:10.1016/j.chom.2016.08.004
186. Scriven YA, Mulinge MM, Saleri N, et al. Prevalence and factors associated with HIV-1 drug resistance mutations in treatment-experienced patients in Nairobi, Kenya. *Medicine*. 2021;100(40):e27460. doi:10.1097/MD.0000000000027460
187. Merci NM, Emerence U, Augustin N, et al. CD4+ cells recovery in HIV positive patients with severe immunosuppression at HAART initiation at Centre Medico-Social Cor-Unum, Kigali. *Pan African Medical Journal*. 2017;26(14). doi:10.11604/pamj.2017.26.14.10488

188. Arts EJ, Hazuda DJ. HIV-1 antiretroviral drug therapy. *Cold Spring Harb Perspect Med*. 2012;2(4):a007161. doi:10.1101/cshperspect.a007161
189. Jóźwik IK, Passos DO, Lyumkis D. Structural Biology of HIV Integrase Strand Transfer Inhibitors. *Trends Pharmacol Sci*. 2020;41(9):611-626. doi:10.1016/j.tips.2020.06.003
190. Singh K, Gallazzi F, Hill KJ, et al. GS-CA Compounds: First-In-Class HIV-1 Capsid Inhibitors Covering Multiple Grounds. *Front Microbiol*. 2019;10:1227. doi:10.3389/fmicb.2019.01227
191. Eggleton JS, Nagalli S. *Highly Active Antiretroviral Therapy (HAART)*. StatPearls Publishing; 2022. Accessed February 4, 2023. http://www.ncbi.nlm.nih.gov/pubmed/10337461
192. Zdanowicz MM. The pharmacology of HIV drug resistance. *Am J Pharm Educ*. 2006;70(5):100. doi:10.5688/aj7005100
193. Franke EK, Yuan HEH, Luban J. Specific incorporation of cyclophilin a into HIV-1 virions. *Nature*. 1994;372(6504):359-362. doi:10.1038/372359a0
194. Saito A, Ferhadian D, Sowd GA, et al. Roles of Capsid-Interacting Host Factors in Multimodal Inhibition of HIV-1 by PF74. Kirchhoff F, ed. *J Virol*. 2016;90(12):5808-5823. doi:10.1128/JVI.03116-15
195. Bhattacharya A, Alam SL, Fricke T, et al. Structural basis of HIV-1 capsid recognition by PF74 and CPSF6. *Proc Natl Acad Sci U S A*. 2014;111(52):18625-18630. doi:10.1073/pnas.1419945112
196. Peng K, Muranyi W, Glass B, et al. Quantitative microscopy of functional HIV post-entry complexes reveals association of replication with the viral capsid. *Elife*. 2014;3. doi:10.7554/eLife.04114
197. Lemke CT, Titolo S, von Schwedler U, et al. Distinct Effects of Two HIV-1 Capsid Assembly Inhibitor Families That Bind the Same Site within the N-Terminal Domain of the Viral CA Protein. *J Virol*. 2012;86(12):6643-6655. doi:10.1128/JVI.00493-12
198. Ning J, Zhong Z, Fischer DK, et al. Truncated CPSF6 Forms Higher-Order Complexes That Bind and Disrupt HIV-1 Capsid. Published online 2018. doi:10.1128/JVI.00368-18

199. Zhong Z, Ning J, Boggs EA, et al. Cytoplasmic CPSF6 Regulates HIV-1 Capsid Trafficking and Infection in a Cyclophilin A-Dependent Manner. *mBio*. 2021;12(2). doi:10.1128/mBio.03142-20

200. Hori T, Takeuchi H, Saito H, Sakuma R, Inagaki Y, Yamaoka S. A carboxy-terminally truncated human CPSF6 lacking residues encoded by exon 6 inhibits HIV-1 cDNA synthesis and promotes capsid disassembly. *J Virol*. 2013;87(13):7726-7736. doi:10.1128/JVI.00124-13

CHAPTER I

Cell Type-Dependent Escape of Capsid Inhibitors by Simian Immunodeficiency Virus SIVcpz.

1. Twizerimana AP, Scheck R, Becker D, et al. Cell Type-Dependent Escape of Capsid Inhibitors by Simian Immunodeficiency Virus SIVcpz. J Virol. 2020;94(23). doi:10.1128/JVI.01338-20

Twizerimana's contribution to this work:

a) Performed experiments for Fig. 1D-F, Fig. 4B-C, Fig. 6, Fig. 7, Fig. 10E-G, Fig. 11, Fig. 13 and Fig. 14
b) Wrote the original draft of the manuscript

Post-entry restriction factors of SIVcpz

2. Twizerimana AP, Scheck R, Häussinger D, Münk C. Post-entry restriction factors of SIVcpz. *Future Virol*. 2018;13(10):727-745. doi:10.2217/fvl-2018-0093

Twizerimana's contribution to this work:

a) Produced all figures and tables
b) Wrote the original draft of the review except section on SAMHD1

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VIRUS-CELL INTERACTIONS

Cell Type-Dependent Escape of Capsid Inhibitors by Simian Immunodeficiency Virus SIVcpz

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Augustin Penda Twizerimana and Rachel Scheck made equal contribution to the execution and analysis of experiments. Author order was determined by additional contribution to drafting of the manuscript by A.P.T.

ABSTRACT Pandemic human immunodeficiency virus type 1 (HIV-1) is the result of the zoonotic transmission of simian immunodeficiency virus (SIV) from the chimpanzee subspecies *Pan troglodytes troglodytes* (SIVcpzPtt). The related subspecies *Pan troglodytes schweinfurthii* is the host of a similar virus, SIVcpzPts, which did not spread to humans. We tested these viruses with small-molecule capsid inhibitors (PF57, PF74, and GS-CA1) that interact with a binding groove in the capsid that is also used by CPSF6. While HIV-1 was sensitive to capsid inhibitors in cell lines, human macrophages, and peripheral blood mononuclear cells (PBMCs), SIVcpzPtt was resistant in rhesus FRhL-2 cells and human PBMCs but was sensitive to PF74 in human HOS and HeLa cells. SIVcpzPts was insensitive to PF74 in FRhL-2 cells, HeLa cells, PBMCs, and macrophages but was inhibited by PF74 in HOS cells. A truncated version of CPSF6 (CPSF6-358) inhibited SIVcpzPtt and HIV-1, while in contrast, SIVcpzPts was resistant to CPSF6-358. Homology modeling of HIV-1, SIVcpzPtt, and SIVcpzPts capsids and binding energy estimates suggest that these three viruses bind similarly to the host proteins cyclophilin A (CYPA) and CPSF6 as well as the capsid inhibitor PF74. Cyclosporine treatment, mutation of the CYPA-binding loop in the capsid, or CYPA knockout eliminated the resistance of SIVcpzPts to PF74 in HeLa cells. These experiments revealed that the antiviral capacity of PF74 is controlled by CYPA in a virus- and cell type-specific manner. Our data indicate that SIVcpz viruses can use infection pathways that escape the antiviral activity of PF74. We further suggest that the antiviral activity of PF74 capsid inhibitors depends on cellular cofactors.

IMPORTANCE HIV-1 originated from SIVcpzPtt but not from the related virus SIVcpzPts, and thus, it is important to describe molecular infection by SIVcpzPts in human cells to understand the zoonosis of SIVs. Pharmacological HIV-1 capsid inhibitors (e.g., PF74) bind a capsid groove that is also a binding site for the cellular protein CPSF6. SIVcpzPts was resistant to PF74 in HeLa cells but sensitive in HOS cells, thus indicating cell line-specific resistance. Both SIVcpz viruses showed resistance to PF74 in human PBMCs. Modulating the presence of cyclophilin A or its binding to capsid in HeLa cells overcame SIVcpzPts resistance to PF74. These results indicate that early cytoplasmic infection events of SIVcpzPts may differ between cell types and affect, in an unknown manner, the antiviral activity of capsid inhibitors. Thus, capsid inhibitors depend on the activity or interaction of currently uncharacterized cellular factors.

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After receptor interaction, human immunodeficiency virus type 1 (HIV-1) infects cells by membrane fusion followed by reverse transcription of its viral genomic RNA into double-stranded DNA, which integrates into the host's chromosomal DNA in the nucleus. The capsid (CA) protein builds a cone-shaped core that delivers viral RNAs and enzymes into the cell. This core undergoes structural changes in a poorly described process called uncoating that takes place either in the cytoplasm, at the nuclear pore, or in the nucleus (1–7), where the CA protein interacts with diverse cellular proteins. To inhibit early capsid-dependent steps in viral infection, small compounds that target the CA protein have been developed (8, 9). Prominent examples of cellular human capsid interactors include cyclophilin A (CYPA) (PPIA [peptidylprolyl isomerase A]), TRIM5α (splice variant α tripartite motif-containing protein 5), MX2 (MX dynamin-like GTPase 2), karyopherin TNPO3 (transportin 3), CPSF6 (cleavage and polyadenylation specificity factor 6), and the nuclear pore proteins NUP358 (nucleoporin 358) (also called RANBP2 [RAN binding protein 2]) and NUP153 (nucleoporin 153) (10–17). NUP153, NUP358, CPSF6, and TNPO3 promote HIV-1 nuclear entry and positively influence integration (1, 17–19). Distantly related simian immunodeficiency viruses (SIVs) from African green monkeys (SIVagm) or rhesus macaques (SIVmac) also bind to NUP153 (19). Thus, it is likely that all primate lentiviruses use similar nuclear entry pathways, but NUP153 may not be required for all SIVs (20). HIV-1 is sensitive to human TRIM5α and MX2 as well as to an experimentally expressed truncated version of CPSF6 (CPSF6-358) (13, 14, 17, 21–23). TNPO3 protects HIV-1 from CPSF6-mediated capsid stabilization in the host cell cytoplasm and prevents CPSF6 from accumulating in the cytoplasm. The mislocalization of CPSF6 to the cytoplasm, as in the case of truncated CPSF6-358, results in the inhibition of HIV-1 (24). Oligomeric CPSF6-358 binds and disrupts the core of HIV-1 (25). Mutations in the CA protein can generate HIV-1 variants that are resistant to these restriction factors (17, 23, 24, 26–28).

One of the best-characterized CA inhibitors is PF74 (also called PF-3450074), which binds to CA at the N-terminal domain–C-terminal domain interface between two monomers of the CA hexamer in a pocket that overlaps the CPSF6 interface and is close to the NUP153 binding site (8, 19, 29–31). Treatment of cells with PF74 blocks HIV-1 via cooperative mechanisms of binding to the capsid and perturbs uncoating and reverse transcription at higher concentrations (5 to 10 μM); lower concentrations (up to 2 μM) block HIV-1 after reverse transcription and before nuclear entry but also inhibit in the nucleus (8, 29, 32–35). Capsid inhibitors with a similar binding location are in development for clinical use (36–38).

Pandemic HIV-1 is the result of the zoonotic transmission of simian immunodeficiency virus from the chimpanzee subspecies *Pan troglodytes troglodytes* (SIVcpzPtt). The related chimpanzee subspecies *Pan troglodytes schweinfurthii* is the host of a similar virus, SIVcpzPts, which did not successfully spread to humans (39–41). Like HIV-1, SIVcpzPtt is sensitive to human TRIM5α and MX2 but evolved in humans to counteract restriction by human BST2 (tetherin) and APOBEC3H (39, 42–45). In this study, we compared the sensitivities of HIV-1 and SIVcpz viruses to early cytoplasmic interactors such as CYPA, CPSF6-358, and capsid inhibitors.

RESULTS

Human cell lines are differentially permissive to SIVcpz viruses. We hypothesized that human-adapted HIV-1 and chimpanzee-adapted SIVcpz viruses differ in their capacities to interact with cytoplasmic cellular factors. We decided to study the capsid-interacting proteins CYPA and CPSF6-358 and capsid inhibitors binding in the CPSF6 groove of capsid. To identify suitable cell systems for our experiments, we tested commonly used cell lines with SIVcpzPtt, SIVcpzPts, and HIV-1 vesicular stomatitis virus G (VSV-G)-pseudotyped luciferase reporter viruses. We infected two human cell lines (HeLa and HOS), three simian cell lines (CV-1 from African green monkeys, FRhL-2 from

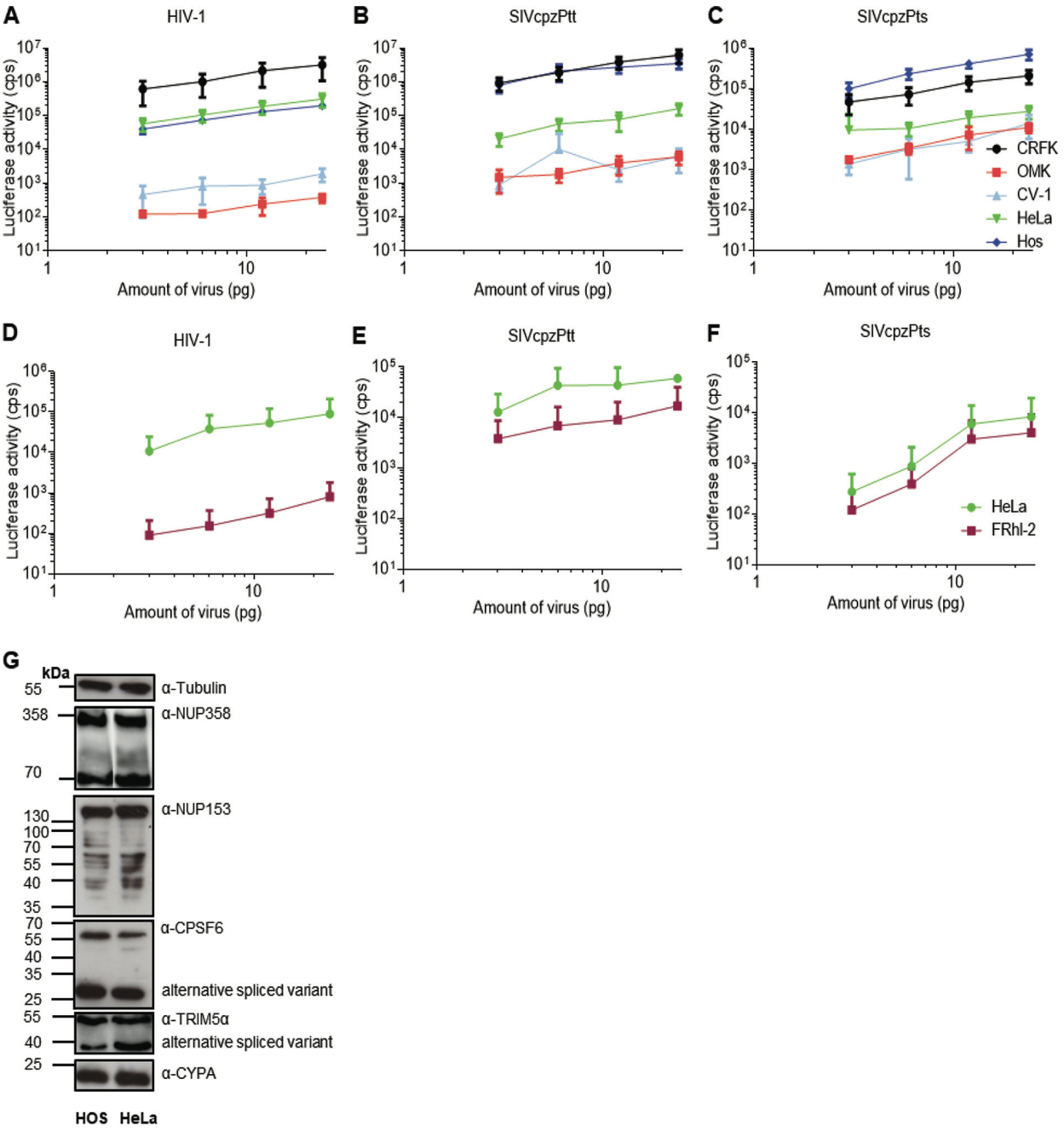

FIG 1 Infection of human and nonhuman cells by HIV-1, SIVcpzPtt, or SIVcpzPts. (A and D) HIV-1 reporter viruses were used to infect CRFK, FRhL-2, OMK, CV-1, HeLa, and HOS cells using increasing amounts of the virus-containing cell supernatant. Firefly luciferase was measured 2 days later. (B, C, E, and F) SIVcpzPtt (B and E) and SIVcpzPts (C and F) reporter viruses were used to infect CRFK, FRhL-2, OMK, CV-1, HeLa, and HOS cells with increasing amounts of the virus-containing cell supernatant. At 2 days postinfection, the activity of nanoluciferase was measured. Values are means with standard deviations (SD). Each experiment was performed at least three times and in triplicates. (G) Expression of HIV-1 host factors in HOS and HeLa cells. Shown are immunoblots of cell lysates of HOS and HeLa cells. Tubulin, NUP358, NUP153, CPSF6, TRIM5α, and CYPA were detected with their specific antibodies. α, anti.

rhesus macaques, and OMK from owl monkeys), and one feline cell line (CRFK) and measured luciferase activity at 2 days postinfection. Feline cells do not express a restricting TRIM5α protein (46), while CV-1 and FRhL-2 cells express TRIM5α proteins and OMK cells express TRIM5Cyp, which inhibit HIV-1 (47). Titration-infection experiments confirmed that both human cell lines were equally permissive to HIV-1, while CRFK cells were 10-fold more susceptible than HeLa or HOS cells, and that HIV-1 was restricted in simian cells (48–50) (Fig. 1A). The difference in infectivity between per-

missive CRFK and restrictive OMK cells was up to 10,000-fold. HIV-1 was slightly more restricted in OMK than in CV-1 cells, but both SIVcpzPtt and SIVcpzPts were equally inhibited in these simian cells (Fig. 1B and C). In further contrast to HIV-1, human HOS cells were more permissive than or equally as permissive as CRFK cells to SIVcpzPtt and SIVcpzPts. For HIV-1 infections, HOS and HeLa cells displayed comparable permissiveness; however, HeLa cells were 33- and 20-fold less infectible by SIVcpzPtt and SIVcpzPts, respectively, than HOS cells. Infection by HIV-1, SIVcpzPtt, and SIVcpzPts was lower in FRhL-2 cells than in HeLa cells, and the differences were the greatest with HIV-1 (Fig. 1D to F). We analyzed the presence of important host proteins in HOS and HeLa cells by immunoblotting and found that the two cell lines express similar amounts of TRIM5α, CYPA, CPSF6, NUP153, and NUP358 (Fig. 1G). Thus, the expression levels of these proteins cannot explain the different sensitivities of HOS and HeLa cells to the two SIVcpz viruses.

SIVcpz viruses show cell type-specific sensitivity to cyclosporine. Cyclophilin A is a cellular protein that has several postulated functions in HIV-1 infection (18, 32, 51–61). CYPA interacts with the capsid of HIV-1 (62) and is incorporated into nascent viral particles (10, 11). In the HIV-1 capsid, CYPA targets a proline-rich loop (also called the CYPA binding loop) with the key interacting residues G89 and P90 (63). Cyclosporine (CsA) is a natural product from a fungus that binds to CYPA and other cyclophilins, and CsA treatment of cells disrupts CYPA incorporation into budding HIV-1 virions (62, 64). HIV-1 mutation of the CYPA binding loop, depletion of CYPA in cells, or inhibition by the use of CsA was instrumental in identifying cell types in which HIV-1 depends on CYPA, such as HOS and primary $CD4^+$ T cells, and cell types in which HIV-1 replicates independently of CYPA, such as HeLa cells (56, 61, 65–71). We aligned the capsid residues of HIV-1 and SIVcpz viruses that are involved in the interaction with CYPA, CPSF6, and capsid inhibitors (Fig. 2A and B; see also Fig. S1 and S2 in the supplemental material). Despite similarities in capsid G89 and P90, which are residues known to be important for the interaction between HIV-1 capsid and CYPA, the alignment highlights differences in the CYPA binding loop on the capsids of HIV-1 and SIVcpz viruses (Fig. 2A and B). Most residues are highly or absolutely conserved within HIV-1 or SIVcpz sequences, respectively, which predominantly also applies to residues interacting with a ligand (marked by stars in Fig. 2A and B). Only residues 91 to 93 that interact with CYPA (blue stars in Fig. 2A and B) show higher sequence variability, also containing nonconservative substitutions within one lineage as well as between lineages. Binding mode models of human CYPA binding to HIV-1, SIVcpzPts, and SIVcpzPtt CA proteins confirmed that the interacting residues differ in 3 positions between HIV-1 CA and SIVcpzPts CA: ILE91 versus GLN91, ALA92 versus GLN92, and PRO93 versus ALA93. The interacting residues differ in 1 position between HIV-1 CA and SIVcpzPtt CA (ALA92 versus PRO92) (Fig. 3A and B).

To identify whether viral particles of SIVcpz and HIV-1 package CYPA in similar manners, immunoblots of virions were analyzed. In virions of SIVcpzPtt and SIVcpzPts, the levels of CYPA were similar to those in HIV-1 particles, and treatment of virus-producing cells with CsA blocked CYPA packaging by all three viruses (Fig. 4A). Glutathione *S*-transferase (GST) pulldown experiments were performed to test the binding of CYPA to SIVcpz GAG. HEK293T cells were cotransfected with plasmids for SIVcpz or HIV-1 and CYPA-GST in the presence or absence of CsA. Following transfection, cells and viruses were lysed, and the lysates were used for pulldown experiments using GST-Sepharose beads. Immunoblotting of the precipitated complexes demonstrated that CYPA-GST interacted with GAG proteins of HIV-1, SIVcpzPtt, and SIVcpzPts (Fig. 4B). Importantly, GST alone did not precipitate GAG, and the administration of 10 μM CsA prevented the binding of CYPA to GAG equally for all three viruses; in contrast, viruses carrying the G89V mutation in the presumed CYPA binding loop of the capsid (Fig. 2A) did not bind CYPA (Fig. 4C).

Since the activity of CYPA in HIV-1 target cells, and not virus-producing cells, regulates HIV-1 replication (56, 68), we treated human HOS and HeLa and rhesus

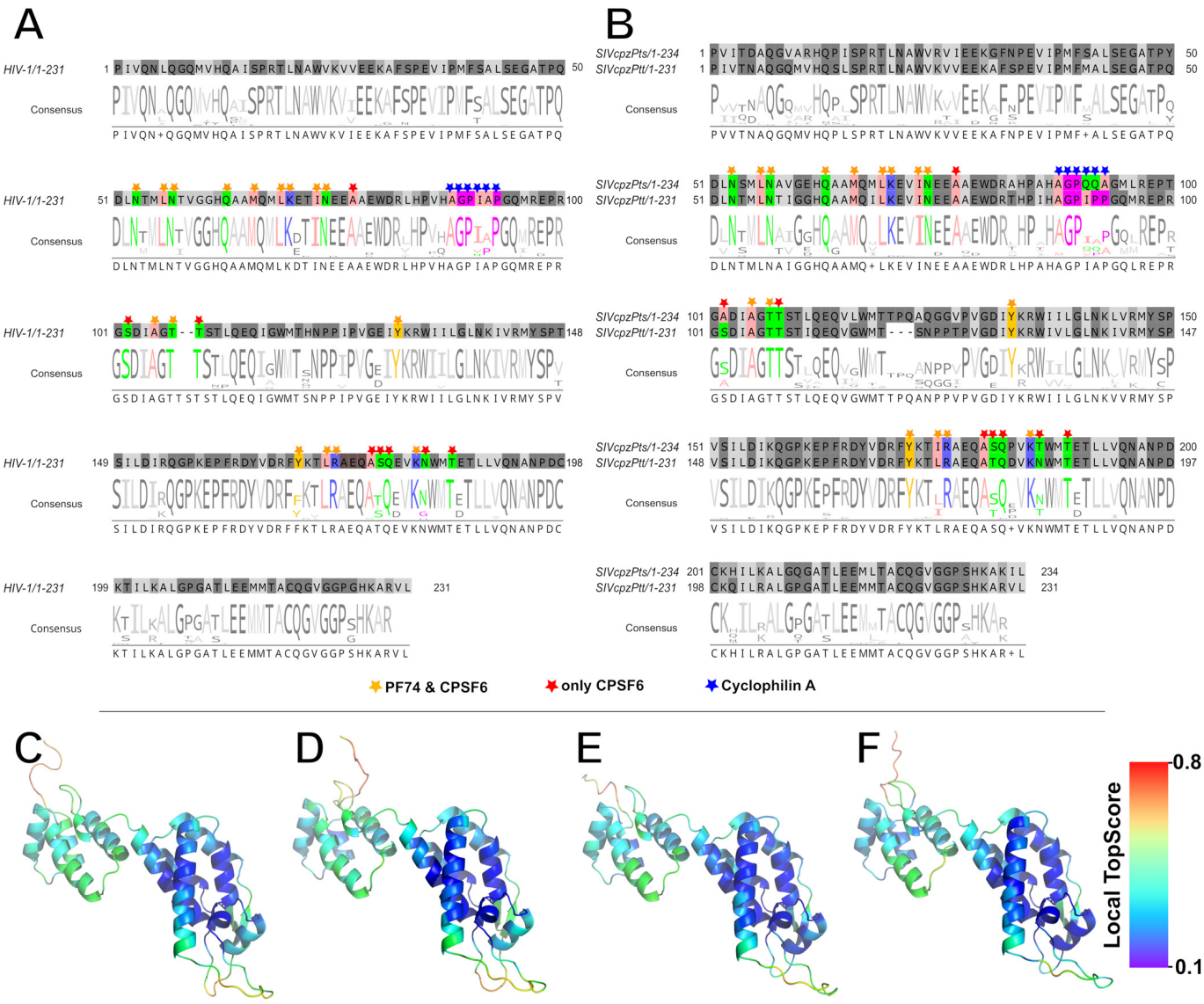

FIG 2 Alignment of CA protein sequences of HIV-1, SIVcpzPts, and SIVcpzPtt and residues engaged in interactions with PF74, CYPA, or CPSF6. (A) HIV-1 CA sequence with the position weight matrix represented as a Weblogo. The HIV-1 CA sequence was taken from the pMDLg/pRRE plasmid. The Weblogo was generated from randomly selected HIV-1 M sequences of different subgroups using MAFFT (108) and Jalview (109) (see also Fig. S1 in the supplemental material). Residues interacting with a ligand are marked with a star. (B) SIVcpz CA sequences (GenBank accession numbers JN835461 and AF447763) with the position weight matrix represented as a Weblogo. The randomly picked SIVcpz CA sequences were retrieved from the Los Alamos HIV and SIV sequence database. The Weblogo was generated from HIV sequences using MAFFT (108) and Jalview (109) (see also Fig. S2 in the supplemental material). Residues interacting with a ligand are marked with a star. For HIV-1 versus SIVcpzPts, the identity was 78.63% and the similarity was 87.18%. For HIV-1 versus SIVcpzPtt, the identity was 89.61% and the similarity was 94.81%. For SIVcpzPts versus SIVcpzPtt, the identity was 78.63% and the similarity was 87.18%. (C to F) Quality assessment of homology models on a per-residue level by TopScore (73). Blue, TopScore of 0.1 (high structural quality); red, TopScore of 0.8 (low structural quality). (C) Homology model of the SIVcpzPts CA monomer based on the structures of HIV-1 CA crystallized with CPSF6 used as the templates. (D) Homology model of the SIVcpzPts CA monomer based on the structures of HIV-1 CA crystallized with PF74 used as the templates. (E) Homology model of the SIVcpzPtt CA monomer based on the structures of HIV-1 CA crystallized with CPSF6 used as the templates. (F) Homology model of the SIVcpzPtt CA monomer based on the structures of HIV-1 CA crystallized with PF74 used as the templates.

macaque FRhL-2 cells with increasing amounts of CsA before infection. Cyclosporine treatment made HOS cells up to 6-fold more resistant to HIV-1 but increased the infection in HeLa and FRhL-2 cells (Fig. 5A). Infections with both SIVcpz viruses demonstrated that CsA treatment of either cell line had no inhibitory consequences and, in fact, stimulated infection (Fig. 5B and C). However, in HOS cells, CsA did not enhance SIVcpzPtt infection (Fig. 5B). To determine whether these observations were related to the CYPA protein, we tried to generate HOS and HeLa *CYPA* knockout (KO) cells (Fig. 6A and B). In HOS cells, the population of cells showed only a weak loss of CYPA protein in immunoblots, and thus, we established and used clonal cell lines that

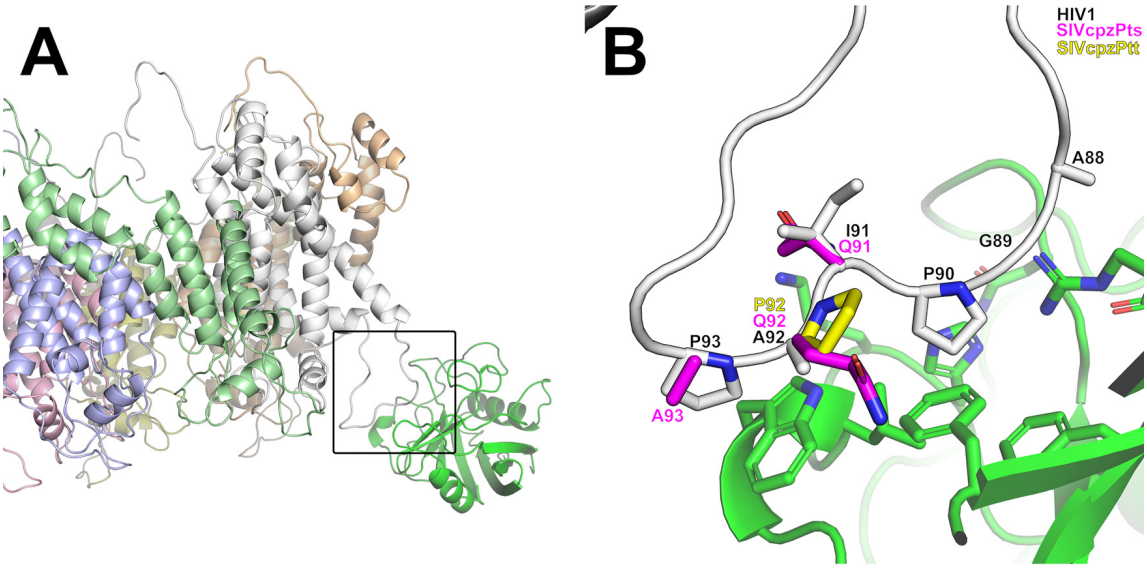

FIG 3 Structural models of binding of human CYPA to HIV-1, SIVcpzPts, and SIVcpzPtt CA proteins. For SIVcpzPts and SIVcpzPtt, CYPA coordinates were taken after superimposing the proteins onto HIV-1 CA bound to CYPA (PDB accession number 5FJB). Monomers are colored differently; the region in the black box in panel A is shown as a blowup in panel B, and side chains of interacting residues are shown as sticks. The interacting residues differ in 3 positions between HIV-1 CA and SIVcpzPts CA: ILE91 versus GLN91, ALA92 versus GLN92, and PRO93 versus ALA93. The interacting residues differ in 1 position between HIV-1 CA and SIVcpzPtt CA (ALA92 versus PRO92).

had a large deficiency in CYPA (Fig. 6A). HOS.CYPA KO clones 1, 3, and 5 showed reduced susceptibility to HIV-1 and SIVcpzPtt but not to SIVcpzPts (Fig. 6C to E). Treatment of HOS.CYPA KO cells with 5 μM CsA during infection did not significantly further increase the restriction of HIV-1 but, surprisingly, reverted the inhibition of SIVcpzPtt (Fig. 6D) and enhanced infections by SIVcpzPts in wild-type (WT) HOS cells and HOS.CYPA KO cells (Fig. 6E). In HeLa cells, we found a significant knockdown (KD) of CYPA in this population of cells, and these HeLa.CYPA KD cells were used for infections (Fig. 6B). CYPA-deficient HeLa cells showed a mild increase in permissiveness to HIV-1 (less than 2-fold) (Fig. 6F) and were 5- to 6-fold more permissive to SIVcpzPtt and SIVcpzPts (Fig. 6G and H). To test the effect of CsA on infection by these viruses in CYPA-deficient HeLa cells, cells were pretreated with 5 μM CsA and infected. Experiments in WT HeLa cells in the presence of CsA revealed data similar to those shown in Fig. 5, and infections by SIVcpzPtt and SIVcpzPts were more enhanced than infection by HIV-1 (Fig. 6F to H). Interestingly, HIV-1 infection was not increased by CsA treatment in HeLa.CYPA KD cells, and its infectivity was reduced by 30% (Fig. 6F). On the other hand, in HeLa.CYPA KD cells, SIVcpzPtt and SIVcpzPts infected 5- and 2-fold more in the presence of CsA, respectively (Fig. 6G and H). Remarkably, the combination of CYPA deficiency and CsA treatment enhanced the permissiveness of HeLa cells to SIVcpzPtt and SIVcpzPts by 30- and 11-fold, respectively. Taken together, these data indicate that CYPA is involved in SIVcpzPtt and SIVcpzPts infection, and for SIVcpzPts, blocking or removing CYPA enhances infection in human HOS and HeLa cells, while SIVcpzPtt enhancement is seen only in HeLa cells. Our data also suggest that CsA treatment influences CYPA-independent pathways of SIVcpz infection.

SIVcpzPts is insensitive to CPSF6-358. To test the effect of CPSF6-358, we generated HOS cells that expressed CPSF6-358 (HOS.CPSF6-358) (Fig. 7A). In HOS.CPSF6-358 cells, infection by HIV-1 was reduced compared to that in HOS cells with the empty vector, while HIV-1 with CA mutants in the CPSF6 binding site (N74D or A77V) (Fig. 2A) (72) escaped this restriction (Fig. 7B to D). CPSF6-358 also inhibited HIV-1 in HOS.CYPA KO cells (Fig. 7B). SIVcpzPtt showed a pattern of restriction in these cell lines similar to that of WT HIV-1 (Fig. 7E). In contrast, CPSF6-358 did not inhibit SIVcpzPts in WT HOS or HOS.CYPA KO cells (Fig. 7F).

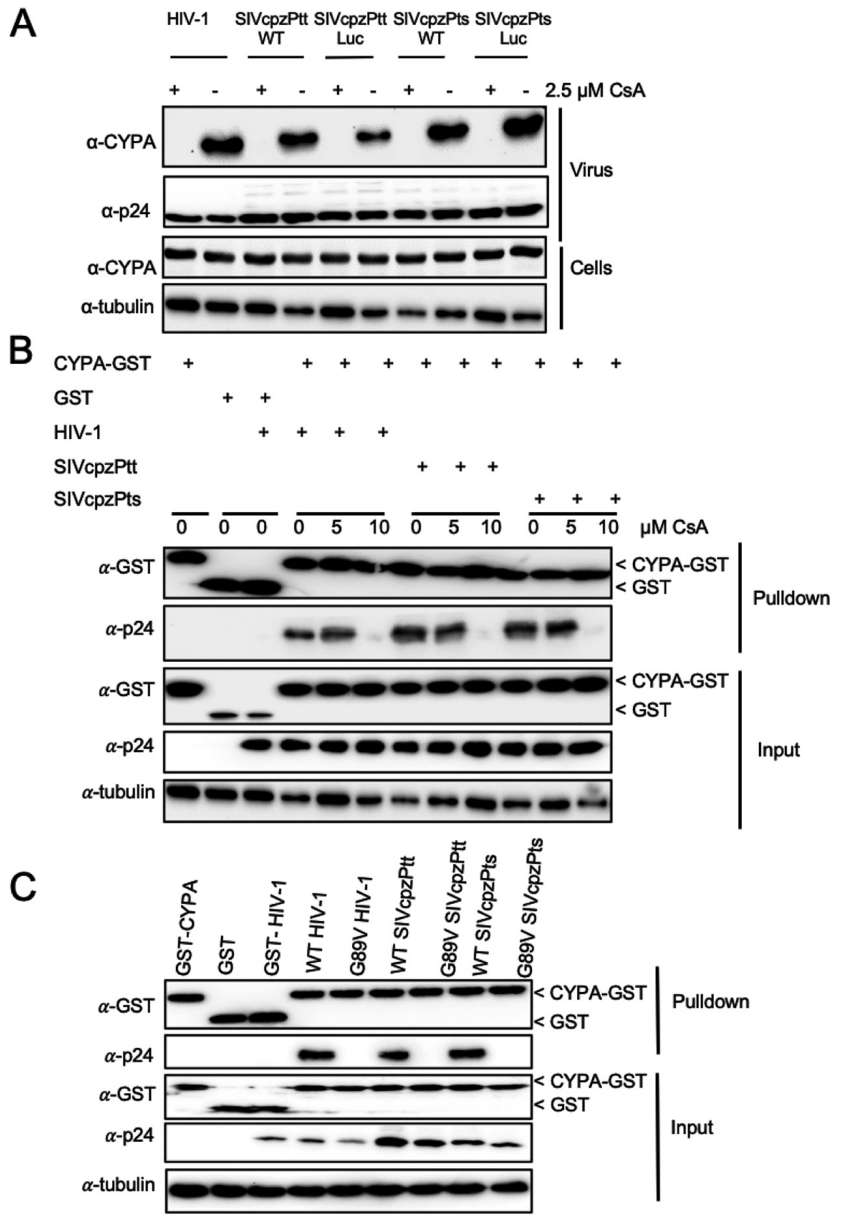

FIG 4 SIVcpz interacts with CYPA. (A) HIV-1, SIVcpzPtt, and SIVcpzPts encapsidate CYPA. Viruses were generated by the transfection of expression plasmids in HEK293T cells in the absence (−) or presence (+) of 2.5 μM CsA. SIVcpzPtt and SIVcpzPts wild-type (WT) and luciferase (Luc) reporter viruses were analyzed. Protein lysates of purified virions and HEK293T producer cells were subjected to immunoblotting. p24 (capsid) and CYPA were detected with specific antibodies. α, anti. (B) HIV-1, SIVcpzPtt, and SIVcpzPts GAGs interact with CYPA. HEK293T cells were cotransfected with HIV-1, SIVcpzPtt, or SIVcpzPts and CYPA-GST in the presence (+) or absence (−) of 5 or 10 μM CsA. Forty-eight hours later, cells and virions were lysed, and the lysate was used for pulldown experiments using GST-Sepharose beads. Proteins of cells were subjected to immunoblotting. p24 (capsid) and CYPA were detected with specific antibodies. (C) G89V in the CYPA binding loops of HIV-1, SIVcpzPtt, and SIVcpzPts capsids is important for interaction with CYPA. Similar to panel B, CYPA-GST pulldown of WT and G89V mutated viruses was performed.

Although no crystal structure information for a CPSF6/SIVcpz CA complex is available, CPSF6 likely binds at the same site in SIVcpzPts or SIVcpzPtt CA as in HIV-1 CA because of the high sequence identity between the proteins (HIV-1 CA versus SIVcpzPts CA, 79%; HIV-1 CA versus SIVcpzPtt CA, 90%) (Fig. 2A and B). The binding sites are located at the interface between two monomers, which are part of the hexameric CA structure. Although the binding sites for CPSF6 are highly similar for HIV-1 CA versus SIVcpzPtt CA (identity of 24 residues involved in CPSF6 binding, 96%), the binding sites

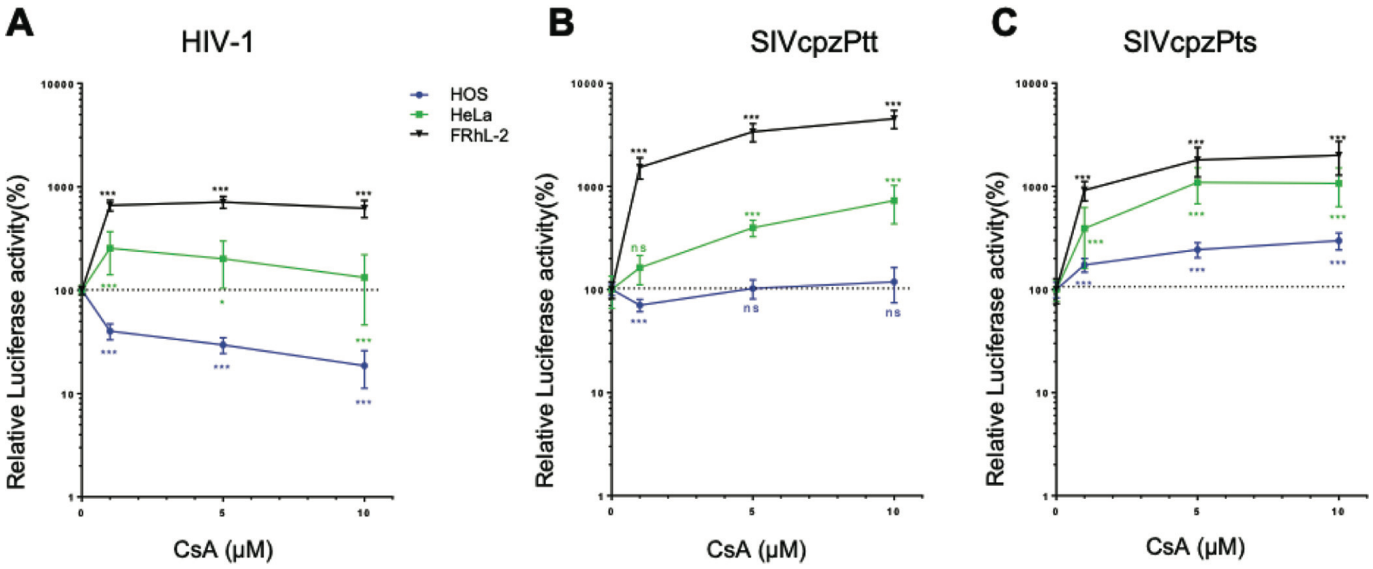

FIG 5 Infection of cells by HIV-1 or SIVcpz viruses in the presence of increasing amounts of CsA. HOS, HeLa, or FRhL-2 cells were treated with DMSO or CsA (1, 5, or 10 μM) 2 h before infection. (A) Infection by HIV-1 luciferase reporter virus. (B) Infection by SIVcpzPtt luciferase reporter virus. (C) Infection by SIVcpzPts luciferase reporter virus. Luciferase activity was measured at 2 days postinfection. Results were normalized to the DMSO control. Means and SD (error bars) are shown, and a Mann-Whitney U test was performed. Each experiment was performed three times in triplicates. ns, not significant; *, $P < 0.05$; ***, $P < 0.001$.

for HIV-1 CA versus SIVcpzPts CA are more distinct (88%) (Fig. 8A and B). To gain further insights, we generated structural models of CPFS6/CA proteins, using in all cases homology models of the CA proteins to improve their comparability. All homology models are of high structural quality, as indicated by the model quality assessment program TopScore (73) (Fig. 2C to F).

The binding site of SIVcpzPtt differs in 1 residue from HIV-1: SER178′ versus THR178′ (Fig. 8A to E). As in this case, the closest residue of CPFS6, GLN319, interacts with its N_δ with the backbone O of either serine or threonine, no impact of this substitution on CPSF6 binding is expected.

The binding site of SIVcpzPts CA differs in 3 residues from HIV-1 CA: SER102 versus ALA102, LEU172′ versus ILE175′, and ASN183′ versus THR186′ (dashed residues indicate a residue location on the second monomer; Fig. 8A to E). As residue 102 is more than 4.4 Å away from CPSF6, no direct impact on CPSF6 binding due to this substitution is expected. Residue 102 also differs within SIVcpz viruses: ALA102 and SER102 appear at equally frequencies in all investigated SIVcpz sequences (Fig. 2B). The replacement of leucine by isoleucine is a conservative one with respect to both the size of the residue and the potential interactions with CPSF6 and, hence, is not expected to influence CPSF6 binding either. Yet the replacement of asparagine by threonine may have an impact. Besides the difference in the sizes of the residues, C_γ of threonine and the phenyl ring of PHE316 of CPSF6 form a close contact that is not present in the case of asparagine. To conclude, the structural analysis of the binding mode models of CPSF6 in all three CA proteins suggests that CPSF6 binding to HIV-1 CA and SIVcpzPtt CA is similar, whereas differential binding may be expected in the case of SIVcpzPts CA. These results parallel the ones described above on the sensitivity of HIV-1 or SIV in the presence of CPSF6-358.

SIVcpz viruses, but not HIV-1, show cell type-specific sensitivity to capsid inhibitors. The unexpected observation that the nonzoonotically transmitted virus SIVcpzPts was insensitive to CYPA KO or the expression of CPSF6-358 in HOS cells motivated us to test capsid inhibitors that bind in the same capsid pocket as CPSF6 (Fig. 9A). We tested three cell lines (human HOS and HeLa and rhesus macaque FRhL-2) and compared the strong inhibitor PF74 with the less-potent variant PF57 (PF-3759857) (8) from 0.5 μM to 8 μM. HIV-1 was inhibited in all three cell lines by both inhibitors in a dose-dependent manner up to 100-fold by PF74 and up to 10-fold by PF57 (Fig. 9B and

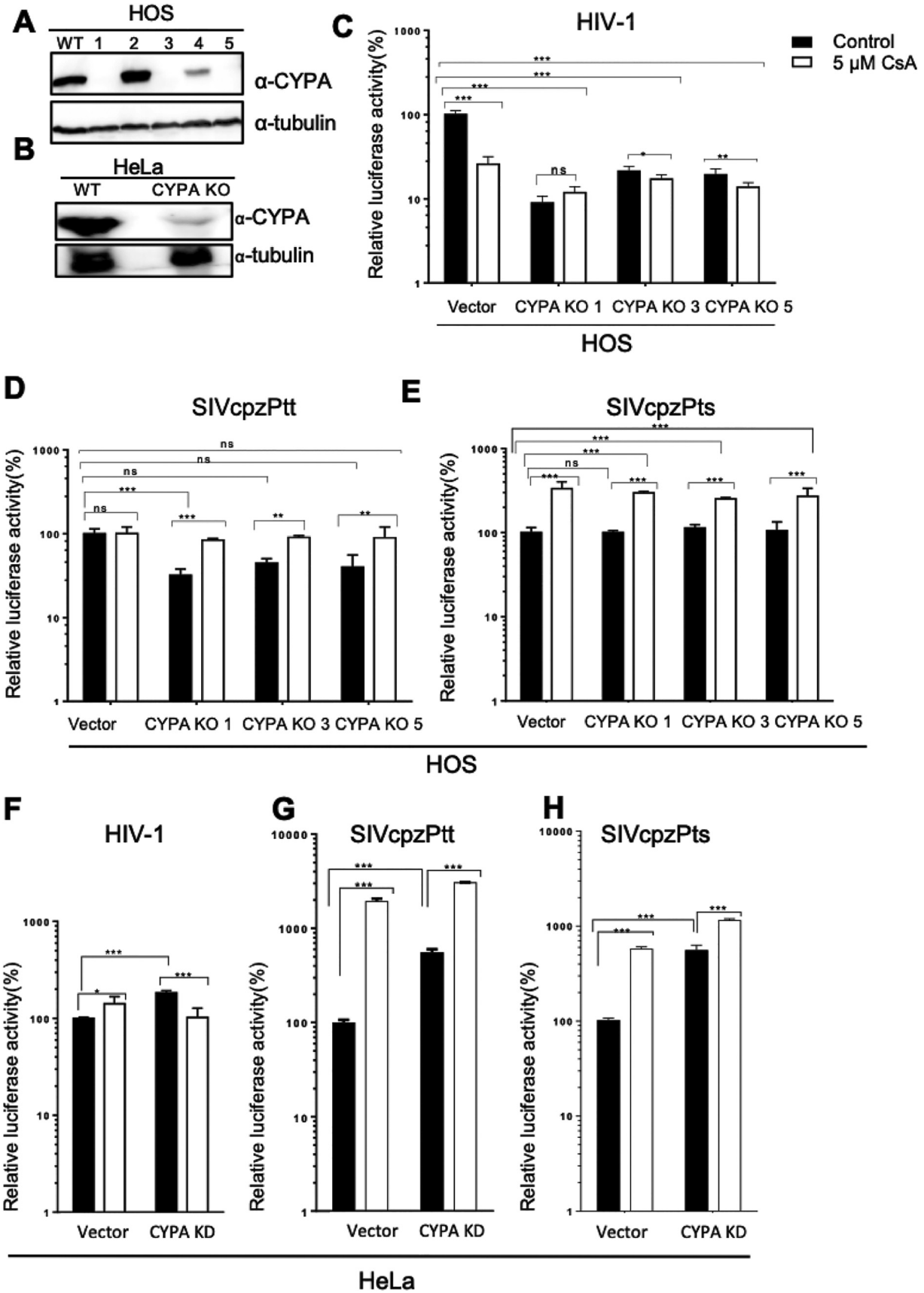

FIG 6 Infection of CYPA knockout cells by HIV-1, SIVcpzPtt, or SIVcpzPts. (A) Immunoblot of protein lysates of HOS cell clones with a CYPA knockout (KO). CYPA and tubulin were detected using specific antibodies. α, anti. (B) Immunoblot for CYPA knockdown (KD) in HeLa cells (cell

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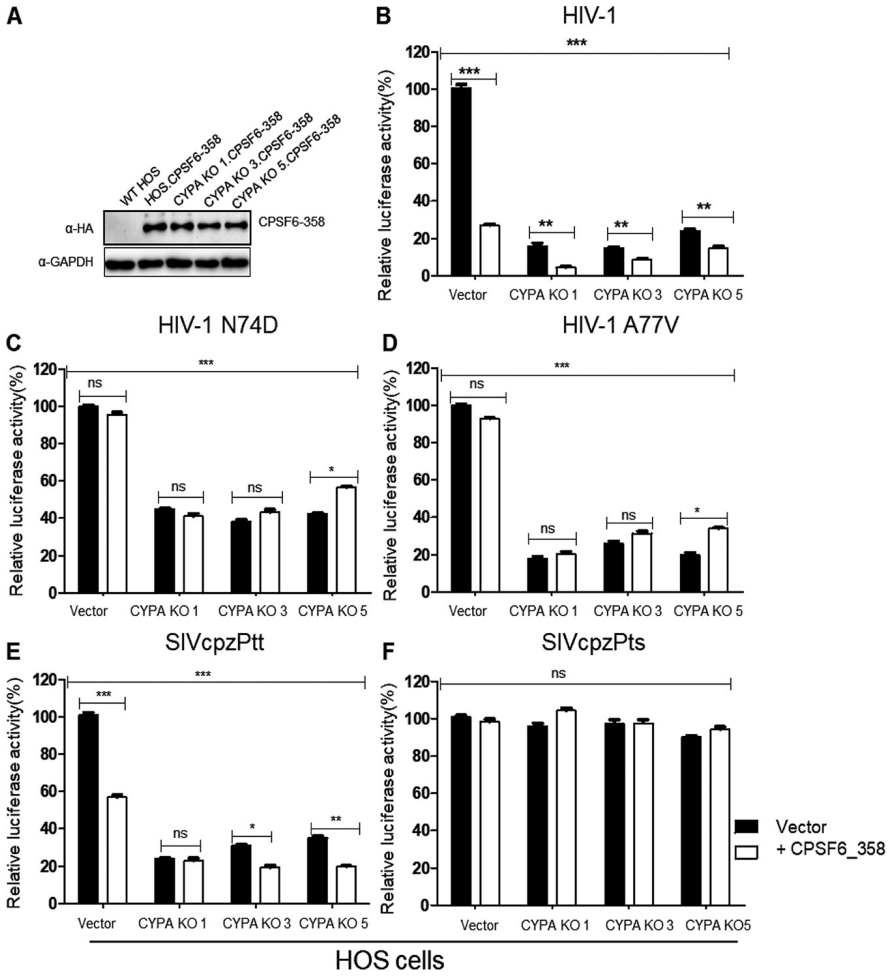

FIG 7 Infection of cells expressing CPSF6-358 by HIV-1, SIVcpzPtt, or SIVcpzPts. (A) Immunoblot of protein lysates of HOS cells for CPSF6-358 expression. HOS WT (vector pLNCX2), HOS.CPSF6-358, and HOS.CYPA KO.CPSF6-358 (clones 1, 3, and 5) cells are shown. HA-tagged CPSF6-358 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were detected using their specific antibodies. α, anti. (B to F) Luciferase reporter viruses of HIV-1 (B), the HIV-1 A77V capsid mutant (C), the HIV-1 N74D capsid mutant (D), SIVcpzPtt (E), and SIVcpzPts (F) were used to infect HOS cells with an empty vector, HOS cells expressing CPSF6-358, CYPA KO HOS cells, or CYPA KO HOS cells expressing CPSF6-358. Luciferase activity was measured at 2 days postinfection. Results were normalized to infection in cells with the empty vector. Means and SD (error bars) are shown, and a Mann-Whitney U test was performed. Each experiment was performed three times in triplicates. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

C). Each cell line showed a slightly different dose-response curve, and the inhibitory effect of both inhibitors was strongest in FRhL-2 cells. Infection by SIVcpzPtt and SIVcpzPts was different from that by HIV-1. PF57 and PF74 inhibited both SIVcpz viruses in human HOS cells in a dose-dependent manner to levels that were comparable to those for the inhibition of HIV-1 (Fig. 9D to G). PF74 reached saturating inhibition at the lowest tested concentration for SIVcpzPtt in HeLa cells, and this inhibition was 10-fold less than that in HOS cells (Fig. 9D). PF57 at 8 μM restricted SIVcpzPtt in HeLa cells similarly to that in HOS cells, but the dose-response curves differed (Fig. 9E). Neither

FIG 6 Legend (Continued)
population). (C to E) Infection of HOS cell clones 1, 3, and 5 and HOS cells carrying an empty vector with luciferase reporter viruses of either HIV-1 (C), SIVcpzPtt (D), or SIVcpzPts (E) in the absence (DMSO control) or presence of 5 μM CsA. Luciferase activity was measured at 2 days postinfection. (F to H) Infection of HeLa cells carrying an empty vector and CYPA knockdown HeLa cells with HIV-1 (F), SIVcpzPtt (G), or SIVcpzPts (H) in the absence (DMSO control) or presence of 5 μM CsA. Results were normalized to the DMSO control in cells carrying the empty vector. Means and SD (error bars) are shown, and a Mann-Whitney U test was performed. Each experiment was performed three times in triplicates. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

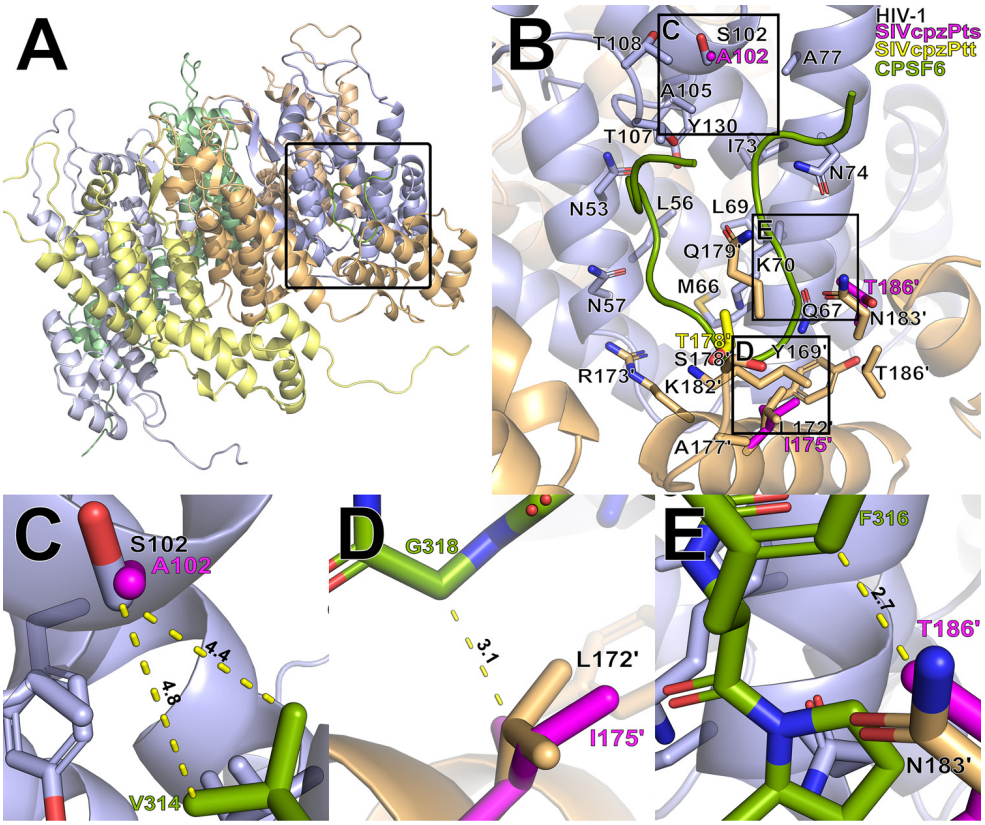

FIG 8 Binding of the CPSF6 fragment to CA hexamers. (A) Modes of binding of human CPSF6 to homology models of HIV-1 CA and SIVcpzPts/Ptt CA. For the latter two, CPSF6 coordinates were taken after superimposing the proteins onto HIV-1 CA bound to CPSF6 (PDB accession number 4WYM). Monomers are colored differently; the region in the black box is shown as a blowup in panel B, side chains of interacting residues are shown as sticks, and residues marked with dashed lines belong to a different chain. (B) The binding pockets differ in 3 residues between HIV-1 CA and SIVcpzPts CA: SER102 versus ALA102, LEU172′ versus ILE175′, and ASN183′ versus THR186′. The binding pockets differ in 1 residue between HIV-1 CA and SIVcpzPtt CA: SER178′ versus THR178′. Helix 179′–195′ is not shown. (C to E) Blowups of the regions in the black boxes in panel B. Side chains of interacting residues and the CPSF6 fragment are shown as sticks. Numbers indicate distances in angstroms.

inhibitor exhibited antiviral activity against SIVcpzPtt in the rhesus macaque FRhL-2 cell line (Fig. 9D and E). More unexpected results were obtained when testing SIVcpzPts. This virus was sensitive to capsid inhibitors in human HOS cells only but showed resistance in human HeLa and simian FRhL-2 cells (Fig. 9F and G). To rule out an effect of TRIM5α, which may be a factor that regulates viral susceptibility to capsid inhibitors, we infected feline CRFK cells that naturally lack the expression of a restricting TRIM5α protein with HIV-1 and both SIVcpz viruses (46). In CRFK cells, all three viruses were sensitive to PF74 and PF57, but SIVcpzPts was the least inhibited (Fig. 10A to C).

The recently described small-molecule HIV CA inhibitor GS-CA1 (Fig. 10D) has an antiviral activity that is >5,000-fold more potent than that of PF74 against HIV-1; both inhibitors use the same binding groove in CA (38). The use of 10 nM GS-CA1 was sufficient to inhibit HIV-1 infection of HeLa cells more than 400-fold. In comparison, SIVcpzPtt was inhibited only 16-fold and SIVcpzPts was inhibited only 3-fold by this concentration of GS-CA1 in HeLa cells (Fig. 10E to G). Despite the structural differences of PF74 and GS-CA1, SIVcpz viruses are 25- to 130-fold less sensitive to GS-CA1 than HIV-1.

To test if resistance to capsid inhibitors is also seen in primary human cells, we infected macrophages and peripheral blood mononuclear cells (PBMCs) in the presence of PF74. HIV-1 was inhibited up to 10-fold by PF74 in PMBCs and macrophages (Fig. 11A to D), while PF74 could not block infection by SIVcpzPtt and SIVcpzPts in PBMCs (Fig. 11A and B). However, in macrophages, PF74 showed some activity against SIVcpzPtt (up

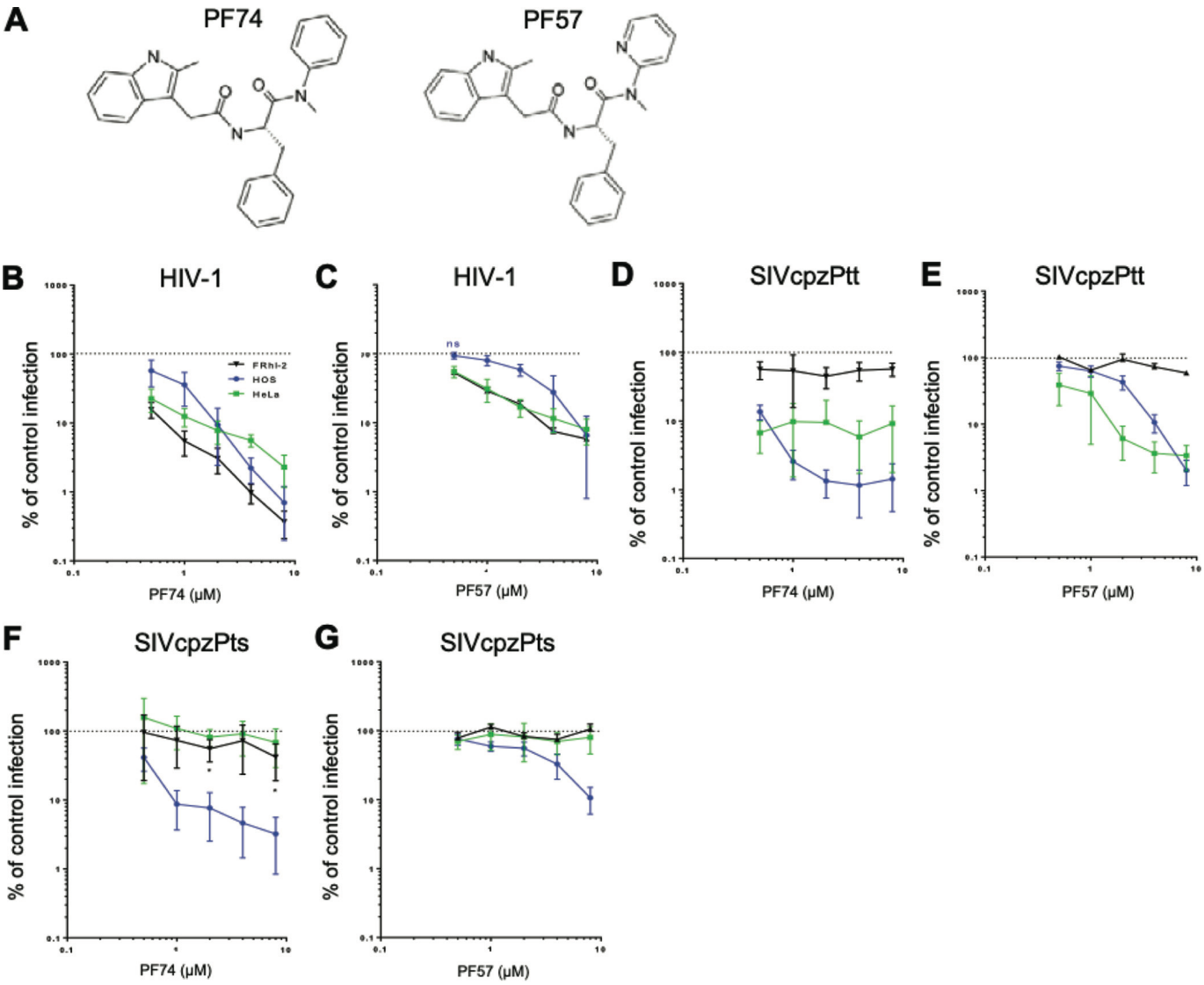

FIG 9 Infection of HOS, HeLa, or FRhL-2 cells with HIV-1, SIVcpzPtt, or SIVcpzPts in the presence of the capsid inhibitor PF57 or PF74. (A) Chemical structures of PF74 and PF57. Cells were incubated with increasing amounts of capsid inhibitors (PF57 or PF74) or DMSO for control infections. (B to G) Two hours later, cells were infected with luciferase reporter viruses of HIV-1 (B and C), SIVcpzPtt (D and E), or SIVcpzPts (F and G), and luciferase activity was measured at 2 days postinfection. Differences between control and PF74 or PF57 treatments are significant for HIV-1 in all cells ($P < 0.05$). For SIVcpzPts, differences are significant in HOS cells but not in HeLa or FRhL-2 cells; exceptions are marked with * for significant ($P < 0.05$). For SIVcpzPtt, inhibition is significant in HeLa and HOS cells. The infectivity of SIVcpzPts is significantly reduced only in HOS cells. Means and SD (error bars) are shown, and a Mann-Whitney U test was performed. Each experiment was performed at least three times in triplicates. ns, not significant.

to 2-fold inhibition) but not SIVcpzPts (Fig. 11C and D). Together, these results demonstrate that SIVcpz viruses in principle are sensitive to capsid inhibitors and that cellular factors may regulate whether these compounds can target these viruses.

Binding sites for PF74 are highly similar in the three CA proteins. In HIV-1 CA, the binding site for PF74 is also located at the interface between two monomers, as for CPSF6 (31) (Fig. 12A and B). Yet PF74 is small and interacts with only 16 residues compared to CPSF6 (Fig. 2A). Although no crystal structure information on a PF74/SIVcpz CA complex is available, PF74 likely binds at the same site in SIVcpzPts or SIVcpzPtt CA as in HIV-1 CA because of the high sequence identity between the proteins (Fig. 2A). In particular, the sites for binding or putative binding of PF74 are identical for HIV-1 CA versus SIVcpzPtt CA and highly similar for HIV-1 CA versus SIVcpzPts CA (identity of residues involved in PF74 binding, 94%) (Fig. 2A, B, D, and F and Fig. 12A and B). The putative binding site of SIVcpzPts differs in 1 residue from HIV-1: LEU172′ versus ILE175′ (Fig. 2A and B and Fig. 12A and B). As discussed above,

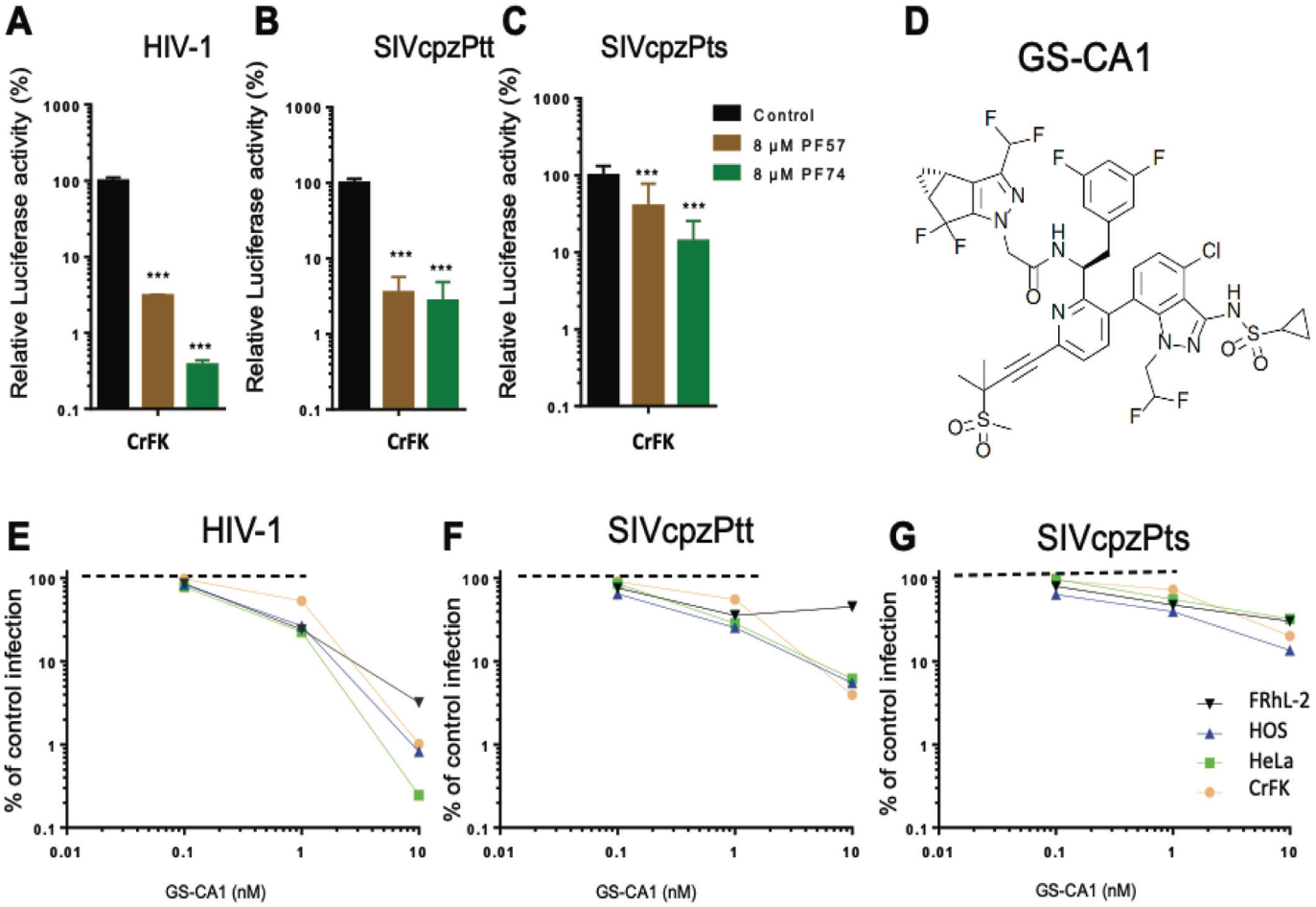

FIG 10 Infection of CRFK cells with HIV-1, SIVcpzPtt, or SIVcpzPts in the presence of the capsid inhibitor PF57 or PF74 and test of the antiviral activity of GS-CA1. (A to C) CRFK cells were incubated with capsid inhibitors (PF57 or PF74) or DMSO for control infections. Two hours later, cells were infected with luciferase reporter viruses of HIV-1 (A), SIVcpzPtt (B), or SIVcpzPts (C), and luciferase activity was measured at 2 days postinfection. Means and SD (error bars) are shown, and a Mann-Whitney U test was performed for significance. (D) Chemical structure of GS-CA1. (E to G) CRFK, HeLa, HOS, and FRhL-2 cells were incubated with the capsid inhibitor GS-CA1 or DMSO. Two hours later, cells were infected with luciferase reporter viruses of HIV-1 (E), SIVcpzPtt (F), or SIVcpzPts (G), and luciferase activity was measured at 2 days postinfection. Each experiment was performed more than three times in triplicates. ***, $P < 0.001$.

this is a conservative substitution with likely no impact on PF74 binding. To conclude, the structural analysis of the binding mode models of PF74 in all three CA proteins suggests that PF74 binding is highly similar in the three proteins.

Effective binding energies of PF74 are indistinguishable for HIV-1 and SIVcpz-Pts/Ptt CA proteins. To validate the data from the structural analysis of PF74 binding to HIV-1 and SIVcpzPts/Ptt CA proteins, we performed endpoint free energy computations using the molecular mechanics Poisson-Boltzmann (MM-PBSA) method (74) and the single-trajectory approach (75) based on five replicates of molecular dynamics (MD) simulations for each PF74/hexameric CA protein complex of 200 ns each (Fig. 12C). In all MD simulations, PF74 remained in the initial binding pose (no-fit PF74 root mean square deviations [RMSDs] of <3 Å in general, except for one binding site each in one replica each for SIVcpzPts and SIVcpzPtt [data not shown]). Furthermore, the C_α atom RMSD of binding-site residues is mostly around 1 Å (data not shown). Both results indicate that the initial starting structures of PF74 in homology models of CA proteins are plausible. The computed effective binding energies are generally stable over the simulation times and form Gaussian-shaped cumulative distributions (Fig. 12C). They mutually differ by less than 1 kcal mol^{-1} for the three PF74/CA protein complexes. As chemical accuracy is ~1 kcal mol^{-1}, which is the expected accuracy in an optimal case (76), the values are thus indistinguishable from each other (Fig. 12C).

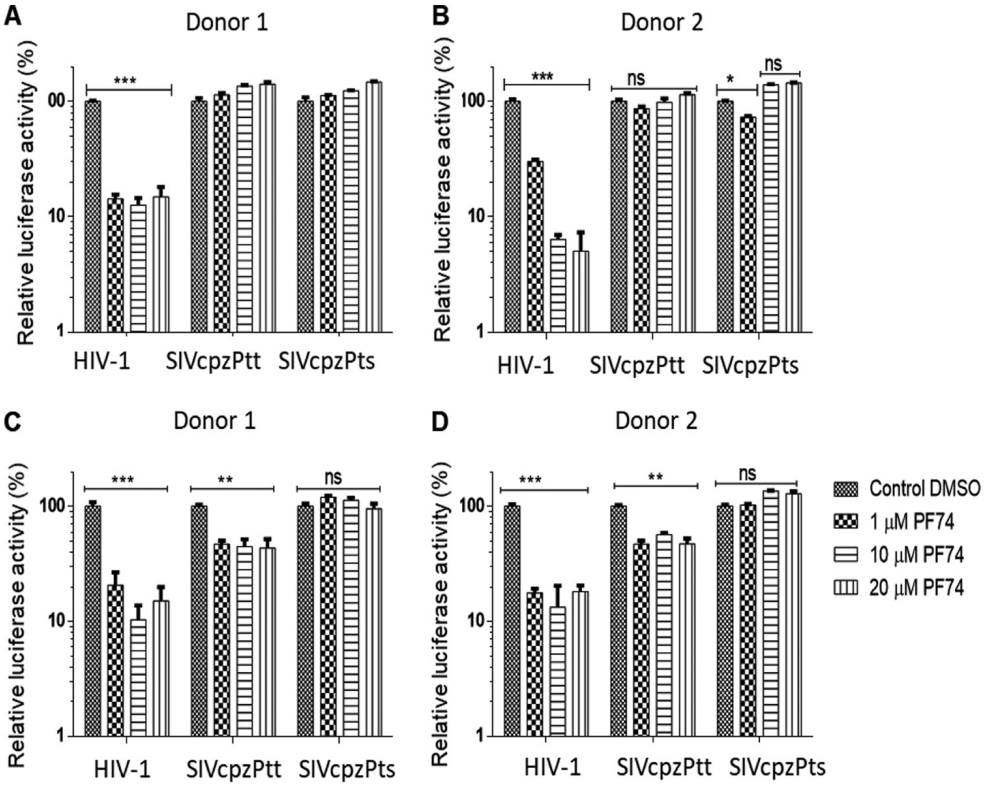

FIG 11 Infection of human PBMCs and macrophages with HIV-1, SIVcpzPtt, or SIVcpzPts in the presence of the capsid inhibitor PF74. PBMCs (A and B) and macrophages (C and D) were incubated with PF74 or DMSO and infected with luciferase reporter viruses of HIV-1, SIVcpzPtt, or SIVcpzPts. Luciferase activity was measured at 2 days postinfection. Means and SD (error bars) are shown, and a Mann-Whitney U test was performed for significance. Each experiment was performed more than three times in triplicates. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

To conclude, effective binding energy computations on PF74/CA protein complexes corroborate that PF74 binding is highly similar for the three proteins.

Cyclosporine treatment or mutations in the CYPA binding loop rescue PF74 activity against SIVcpzPts in HeLa cells. To investigate whether CsA treatment affects viral sensitivity to capsid inhibitors, drug combinations of CsA and PF74 were tested. We used PF74 either alone (1, 4, or 8 μM) or in combination with CsA (1 or 10 μM) in HeLa cells. Infections were normalized to those of cells treated with either dimethyl sulfoxide (DMSO) or CsA only to measure the inhibitory capacity of PF74. In HIV-1-infected cells, the addition of 10 μM CsA enhanced the antiviral activity of PF74 2- to 10-fold; 1 μM CsA did not change viral sensitivity to 1 μM PF74 but led to more inhibition at higher PF74 concentrations (Fig. 13A). A similar result was found in SIVcpzPtt infections, although 1 μM CsA slightly reduced inhibition by 1 μM PF74, and 10 μM CsA enhanced inhibition by 8 μM PF74 by 18-fold (Fig. 13B). Interestingly, CsA treatment changed the resistance of SIVcpzPts to PF74, and both 1 μM and 10 μM CsA enhanced the antiviral activity of higher concentrations of PF74 by 14-fold (Fig. 13C). Similar results were also obtained using PF57 (data not shown).

The finding that treatment of HeLa cells with CsA reverses the PF74 resistance of SIVcpzPts motivated us to ask if capsid binding to CYPA is important for this phenotype. For these experiments, we used viruses that carry the G89V mutation in the capsid loop that interacts with CYPA. The HIV-1 G89V mutant together with a low concentration of PF74 had 2-fold-higher sensitivity, but PF74 concentrations above 1 μM inhibited HIV-1 WT and G89V equally (Fig. 13D). PF74 had 5- to10-fold-increased antiviral activity against SIVcpzPtt G89V compared to the WT virus (Fig. 13E). Strikingly, the mostly PF74-resistant virus, SIVcpzPts, was completely sensitive to the capsid inhibitor, with up to 50-fold inhibition, when mutated to G89V in CA (Fig. 13F). Infections by HIV-1 and

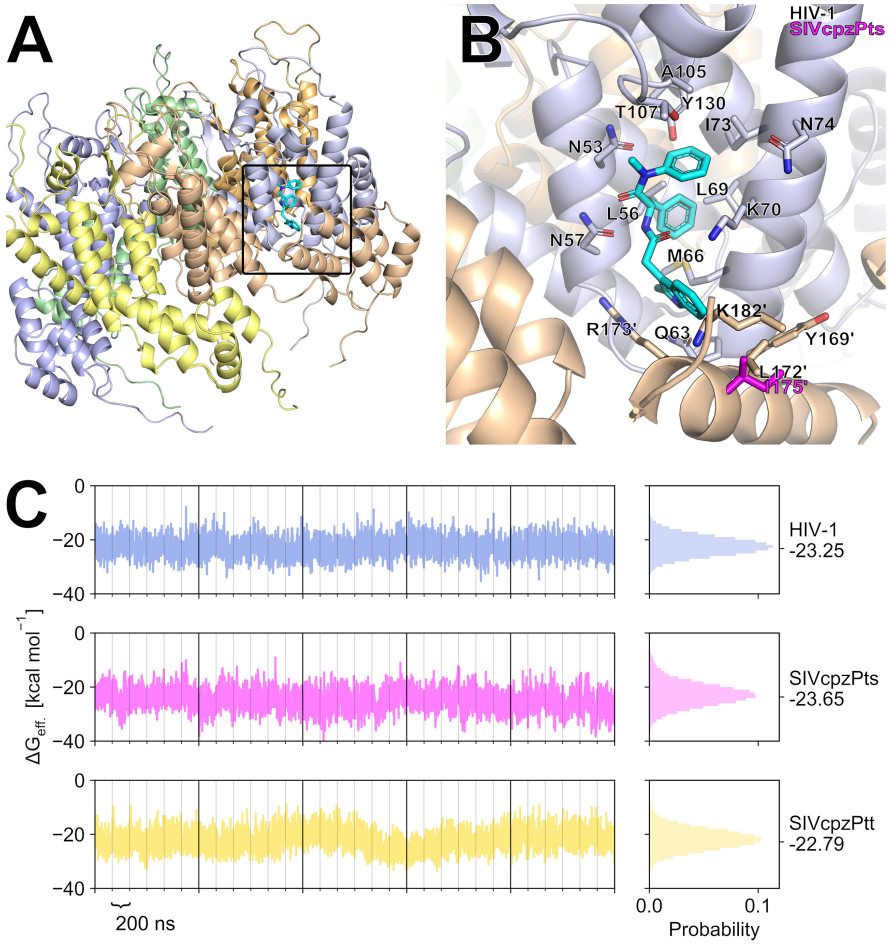

FIG 12 Binding of PF74 to CA hexamers. (A and B) Modes of binding of PF74 to homology models of HIV-1 CA and SIVcpzPts/Ptt CA proteins. For the latter two, PF74 coordinates were taken after superimposing the proteins onto HIV-1 CA bound to PF74 (PDB accession number 4XFZ). Monomers are colored differently; the region in the black box in panel A is shown as a blowup in panel B, side chains of interacting residues are shown as sticks, and residues marked with dashed lines belong to a different chain. The binding pockets differ in 1 residue between HIV-1 CA and SIVcpzPts CA: LEU172′ versus ILE175′. The binding pockets of HIV-1 CA and SIVcpzPtt CA do not differ. Helix 179′–195′ is not shown in panel B. (C) Effective binding energies computed by the molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) method. Shown are the effective binding energies of PF74 binding to HIV-1 (blue), SIVcpzPts (pink), and SIVcpzPtt (yellow) CA proteins as a function of the simulation time (left). Values per trajectory are separated by black vertical lines, and gray lines separate values computed for each binding site of a CA hexamer. On the right, the probability functions of the effective energies across all frames are shown. The average effective binding energy for each isoform is marked on the right axis. The standard error of the mean (see the equation in Materials and Methods) is <0.015 kcal mol^{-1} in all cases.

SIVcpz WT and G89V viruses in the presence of GS-CA1 generated similar results for HIV-1 and SIVcpzPtt, but the moderate inhibition of SIVcpzPts was enhanced only 5-fold by the G89V mutation (Fig. 13G to I). Our findings suggest that an important regulator of the antiviral activity of PF74-like capsid inhibitors is a CsA-sensitive protein, which is likely the SIVcpz capsid-interacting protein CYPA.

Cyclophilin A knockout overcomes the resistance of SIVcpzPts to capsid inhibitors in HeLa cells. To test whether the presence of CYPA in viral target cells protects SIVcpzPts against capsid inhibitors, HeLa.CYPA KD and HOS.CYPA KO (clone 3) cells were infected in the presence of increasing amounts of PF74 or PF57. For HeLa.CYPA KD cells compared to WT cells, HIV-1 was less inhibited by lower concentrations of PF74 and similarly inhibited at higher concentrations (Fig. 14A), which is similar to previously described results (33). In contrast, PF57 showed reduced activity against HIV-1 in HeLa.CYPA KD cells compared to WT cells at all drug concentrations (Fig. 14B). This

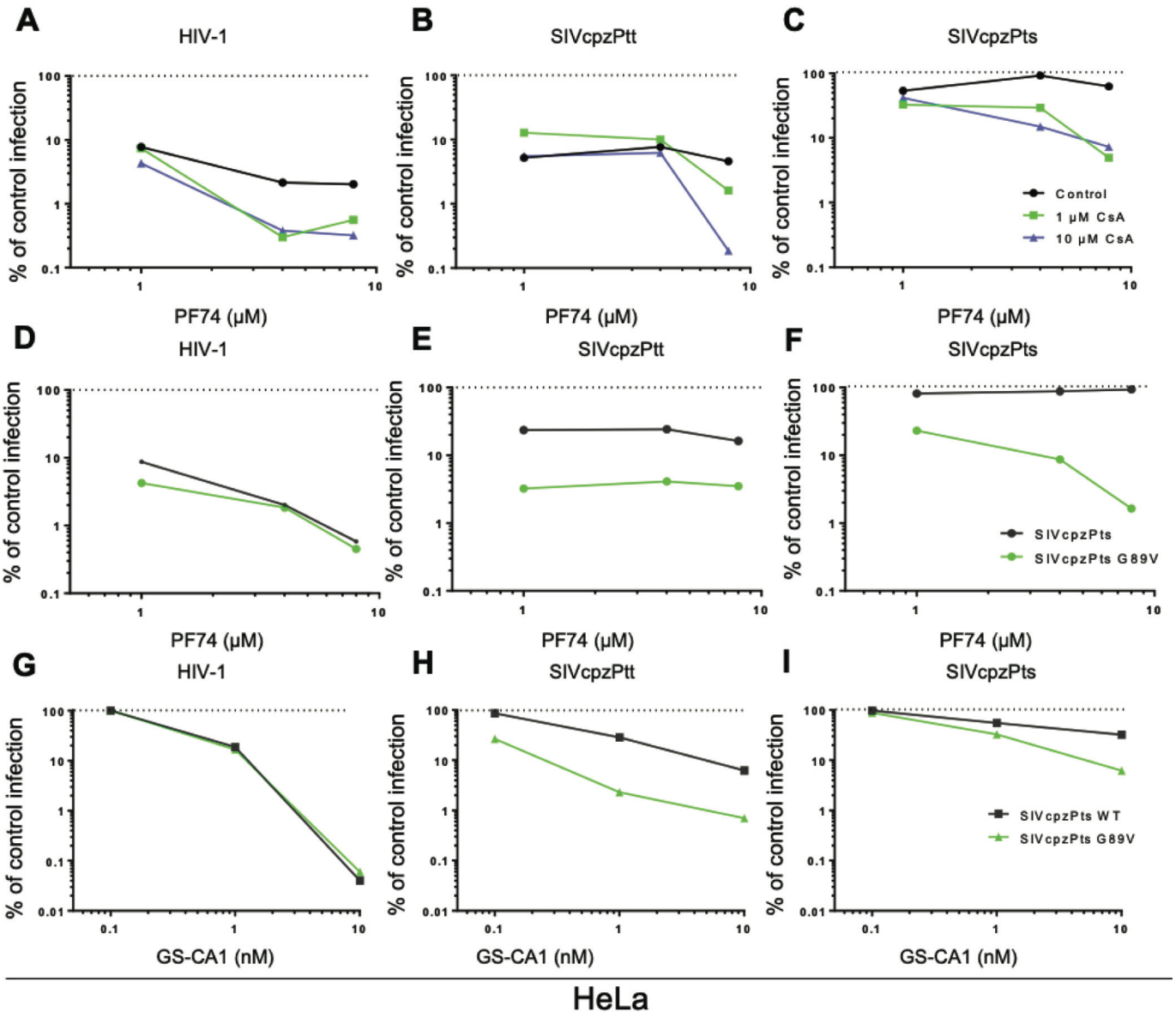

FIG 13 Infection of HeLa cells in the presence of CsA or infection with CYPA loop mutants (G89V) in the presence of capsid inhibitors. (A to C) HeLa cells were incubated with 1, 4, or 8 μM the capsid inhibitor PF74, DMSO, or the capsid inhibitor together with CsA (1 or 10 μM). Two hours later, cells were infected with luciferase reporter viruses of HIV-1 (A), SIVcpzPtt (B), or SIVcpzPts (C), and luciferase activity was measured at 2 days postinfection. Results were normalized to infection of cells treated with DMSO (control) or cells treated with CsA only. (D to I) Infection of HeLa cells with CYPA loop mutants (G89V) of HIV-1, SIVcpzPtt, or SIVcpzPts in the presence of capsid inhibitors. Cells were incubated with increasing amounts of the capsid inhibitor PF74 (D to F) or GS-CA1 (G to I) or with DMSO for control infections. Two hours later, cells were infected with luciferase reporter viruses of HIV-1 and HIV-1 G89V (D and G), SIVcpzPtt and SIVcpzPtt G89V (E and H), and SIVcpzPts and SIVcpzPts G89V (F and I). Luciferase activity was measured at 2 days postinfection. Results were normalized to infection of cells treated with DMSO. Each experiment was performed three times in triplicates.

pattern of inhibition was also seen in HOS.CYPA KO cells for HIV-1 (Fig. 14C and D) and SIVcpzPtt (Fig. 14G and H) treated with PF74 and PF57 and for SIVcpzPts treated with PF57 (Fig. 14L). PF74 inhibited SIVcpzPts in HOS.CYPA KO cells compared to WT cells, with reduced activity at low concentrations and higher inhibition at increased drug concentrations (Fig. 14K).

HeLa.CYPA KD cells infected with the SIVcpz viruses displayed two further inhibition curves. CYPA KD in HeLa cells minimally (<2-fold) enhanced PF74 activity but did not change PF57 activity against SIVcpzPtt (Fig. 14E and F). SIVcpzPts demonstrated the expected resistance to both capsid inhibitors in WT HeLa cells, but this resistance was completely lost in HeLa.CYPA KD cells (Fig. 14I and J), suggesting that in HeLa cells, CYPA is an antagonist of capsid inhibitors for SIVcpzPts.

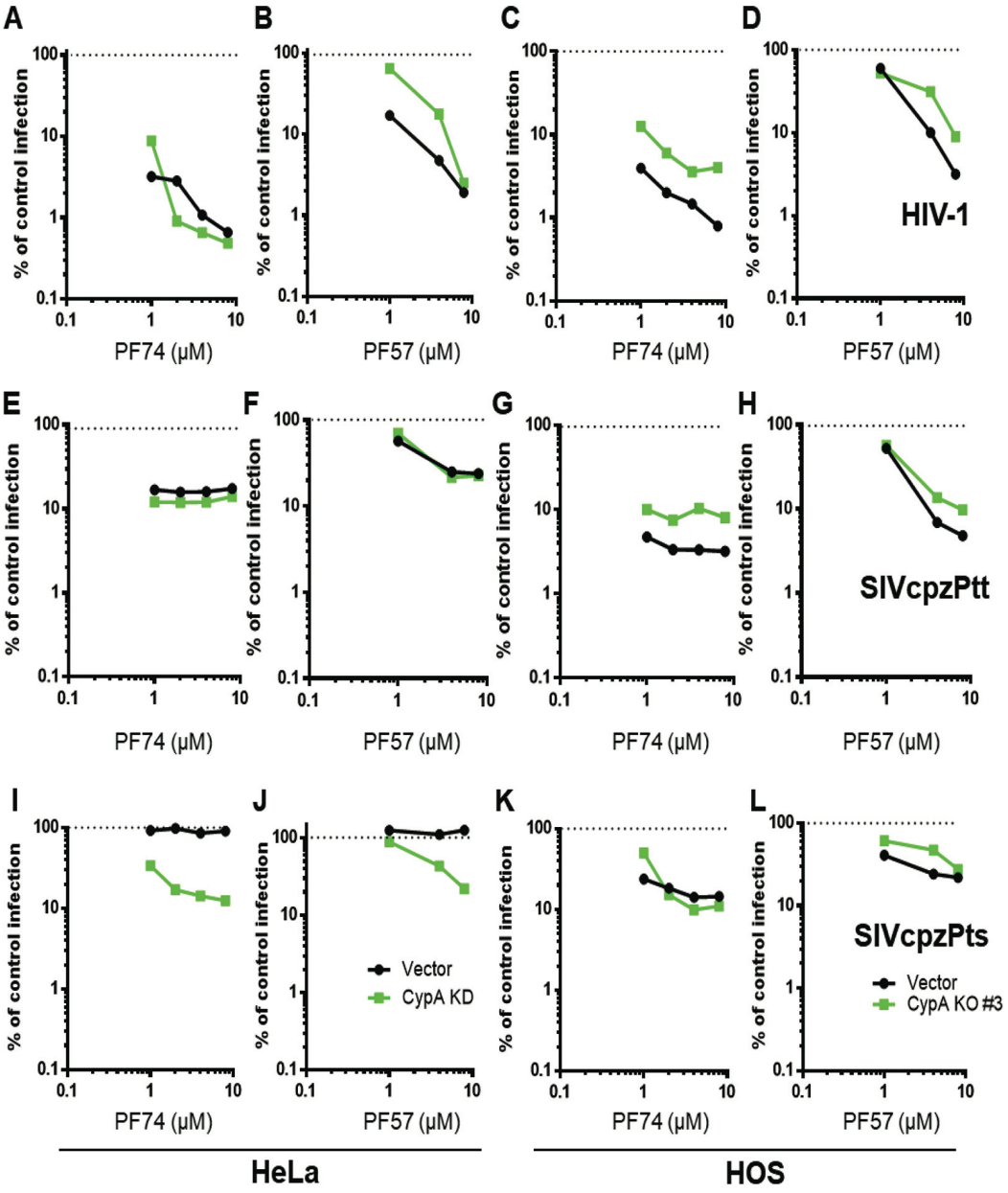

FIG 14 Infection of CYPA-KO cells with HIV-1, SIVcpzPtt, or SIVcpzPts in the presence of capsid inhibitors. HOS.CYPA KO and HeLa.CYPA KD cells were incubated with increasing amounts of capsid inhibitors (PF57 or PF74) or DMSO for control infections. Two hours later, cells were infected with luciferase reporter viruses of HIV-1 (A to D), SIVcpzPtt (E to H), or SIVcpzPts (I to L), and luciferase activity was measured at 2 days postinfection. Results were normalized to infection of cells treated with DMSO. Each experiment was performed three times in triplicates.

DISCUSSION

During the early phase of HIV-1 infection, capsid inhibitors such as PF74 either stabilize or disintegrate the core, depending on the concentrations applied (31, 32, 77, 78). The mechanism of inhibition by capsid inhibitors is currently poorly defined. Cyclophilin A was found to enhance the antiviral activity of PF74 but also to protect HIV-1 against high concentrations of PF74 (32, 33). The CYPA binding loop in the capsid does not overlap the PF74 binding site (Fig. 2A and Fig. 3), and thus, a model of how CYPA regulates PF74 activity is not straightforward. However, the binding site of PF74 in the assembled capsid hexamers and pentamers overlaps the binding sites of the cellular cofactors CPSF6 and NUP153, implicating a competition of PF74 and these cellular cofactors for the same binding groove (19, 29, 30, 33, 35, 78–80).

While the CYPA interaction with HIV-1 affects many different aspects of HIV-1 biology (18, 32, 51–60), recent studies concluded that the pathway most affected by CYPA inhibition in cell lines is viral nuclear entry (61, 81). By comparing the permissivities of human cells to SIVs of chimpanzees, we found that HeLa cells are much less permissive to these viruses than HOS cells. Treating HeLa cells with CsA or using CYPA KD HeLa cells reverted the reduced permissiveness to SIVcpz. Our data on the CYPA-capsid interaction collectively suggest that HIV-1 and SIVcpz GAG proteins interact with CYPA similarly despite differences in their CYPA binding loops. In addition to CYPA, we tested the sensitivities of the two SIVcpz viruses to the anti-HIV-1 factors CPSF6-358, PF74, and PF57 as well as the potent capsid inhibitor GS-CA1. The expression of truncated CPSF6 in the form of CPSF6-358 in human HOS cells repressed HIV-1 infections, which is consistent with previous observations (17, 33, 54, 82–84). Similarly, but more moderately, CPSF6-358 reduced the infectivity of SIVcpzPtt. However, SIVcpzPts escaped restriction by CPSF6-358 in HOS WT and in CYPA KO cells. Employing binding mode models of CPSF6 bound to all three CA proteins revealed that CPSF6 binding to HIV-1 CA and SIVcpzPtt CA is similar, whereas differential binding may be expected in the case of SIVcpzPts CA. These results parallel experimental ones in that HIV-1 and SIVcpzPtt, but not SIVcpzPts, were inhibited by CPSF6-358 expression.

The investigation of PF74 and related capsid inhibitors (PF57 and GS-CA1) confirmed that SIVcpz and HIV-1 have intrinsic biological differences that have not been observed previously. These three viruses respond differently to capsid inhibitors in a cell type-dependent manner. HIV-1 was sensitive to PF57, PF74, and GS-CA1 and was inhibited 250- to 500-fold in HOS, HeLa, FRhL-2, and CRFK cells. SIVcpz viruses showed similar sensitivities only in HOS and CRFK cells; in addition, SIVcpzPtt was sensitive to capsid inhibitors in HeLa cells. However, both SIVcpz viruses showed resistance in FRhL-2 cells and were inhibited only 2-fold at the maximum drug concentration; in addition, SIVcpzPts was resistant in HeLa cells. Importantly, the capsid inhibitors were also inactive against SIVcpzPts in human macrophages and PBMCs, and against SIVcpzPtt in PBMCs, while SIVcpzPtt was partially (2-fold) sensitive in macrophages.

Because SIVcpz viruses are not generally resistant to this type of inhibitor, we concluded that the antiviral activity of the capsid inhibitors depends on cellular infection pathways or the specific binding of a cellular protein. In particular, the finding that PF74 cannot interfere with SIVcpz infection in human PBMCs suggests that the results obtained with the cell lines are of biological relevance. Structural modeling of the PF74 interaction with CA proteins revealed that the binding sites are identical in HIV-1 CA versus SIVcpzPtt CA and highly similar for HIV-1 CA versus SIVcpzPts, suggesting that binding is highly similar for the three proteins. This suggestion was corroborated by effective binding energy computations, which are indistinguishable within chemical accuracy. These results are consistent with the similar sensitivities of all three viruses to PF74 in human HOS cells.

The PF74 inhibition curve for HIV-1 in HeLa cells was described as being triphasic, with two inhibitory phases flanking a plateau phase (19, 29, 33, 60). Here, we identified that the PF74 inhibition curve for HIV-1 in HeLa cells is only one of several different inhibition curves that capsid inhibitors can generate. Moreover, by testing capsid inhibitors against HIV-1, SIVcpzPtt, and SIVcpzPts in CsA-treated cells or in cells with CYPA KO or deficiency, we obtained a complex interaction pattern that suggests that in some cell types, and specifically for SIVcpzPts, CYPA is an important antagonist of PF74-type capsid inhibitors. Our results in HeLa cells demonstrate that the functional inactivation of CYPA by CsA at low PF74 concentrations differentially affects the antiviral activity of the capsid inhibitors. However, at high PF74 levels, CsA treatment reverted the resistance of SIVcpzPts to PF74 and further enhanced the sensitivities of HIV-1 and SIVcpzPtt. We also tested how SIVcpz and HIV-1 reacted to capsid inhibitors in HOS.CYPA KO and HeLa.CYPA KD cells. The virus inhibition curves of PF57 or PF74 in HOS or HeLa cells lacking CYPA showed either no change, 2- to 4-fold-lower antiviral activity, or slightly enhanced inhibition. The most striking observation was the total loss of resistance of SIVcpzPts to capsid inhibitors in HeLa cells due to CYPA KD. SIVcpzPts

also lost its resistance to PF74 in HeLa cells when the virus was mutated in the CYPA binding loop. This G89V mutation did not affect the sensitivity of HIV-1 to PF74 but further increased PF74's antiviral activity against SIVcpzPtt. These data suggest a diverse and elaborate pattern of regulation of antiviral capsid inhibitor activity by CYPA. However, in HeLa cells, CYPA has a clear protective role for SIVcpzPts against PF74 and PF57 at all drug concentrations.

The activity of PF74 and related compounds likely depends on the nuclear import of HIV-1 via NUP153, NUP358, and CPSF6 (1, 17–19, 33). HIV-1 variants that are more resistant to PF74's antiviral activity, but still bind the drug, are less dependent on NUP153 utilization (32, 33, 80). Knockdown of NUP153 in HeLa and HOS cells caused a 10-fold-reduced capacity of PF74 to inhibit HIV-1, with residual antiviral activity at high drug concentrations (19, 33). The knockdown of CPSF6 did not affect antiviral activity at high concentrations of PF74 but reduced the antiviral activity of the capsid inhibitor when applied at lower concentrations (33). These data, together with our findings, lead us to postulate that SIVcpz viruses, in particular SIVcpzPts, infect at least some cell types via a pathway that is independent of NUP153, NUP358, and CPSF6, consistent with recent findings describing a heterogeneity of nuclear pore complexes influencing HIV-1 infection (6, 85). We further speculate that during SIVcpz adaptation to humans, the capacity to use alternative nuclear entry pathways was lost for unknown reasons. Whether the nuclear entry pathway of SIVcpz viruses that can escape PF74 inhibition involves CYPA or CPSF6 requires further study.

MATERIALS AND METHODS

Plasmids. The murine leukemia virus (MLV) packaging construct pHIT60, which carries *gag-pol* of Moloney MLV, was provided by Jonathan Stoye (86). SIVcpzPts clone TAN1.910 (87) (GenBank accession number AF447763) was obtained from the NIH AIDS Reagent Program (Germantown, MD, USA). SIVcpzPtt clone MB897 (88) (GenBank accession number JN835461) was kindly provided by Frank Kirchhoff. The HIV-1 vector pSIN.PPT.CMV.Luc.IRES.GFP expresses firefly luciferase and green fluorescent protein (GFP) (89). The HIV-1 construct psPAX2 was obtained from the NIH AIDS Reagent Program (catalog number 11348), and pRSV-Rev, pMDLg/pRRE, and pMD.G (VSV-G) were described previously (90). HIV-1 capsid mutants were produced as follows. Using fusion PCR, the region flanking Eco72I (PmlI) and MunI (MfeI) in pMDLg/pRRE was amplified at the same time, introducing a G89V (56), N74D (54), or A77V (72) mutation in the capsid, and cloned into the pMDLg/pRRE plasmid digested by Eco72I (PmlI) and MunI (MfeI) (Thermo Fischer Scientific, Langenselbold, Germany). Using fusion PCR, the SIVcpzPtt region flanking SacII and AleI (Thermo Fischer Scientific) and the SIVcpzPts region between the NruI and BoxI (Thermo Fischer Scientific) restriction sites were amplified with the introduction of the G89V mutation into the capsids. Sequencing confirmed the desired mutations. This approach generated pMDLg/pRRE.G89V, pMDLg/pRRE.N74D, and pMDLg/pRRE.A77V HIV-1 but also SIVcpzPtt.nanoluciferase.G89V and SIVcpzPts.nanoluciferase.G89V CA mutants. To generate GST-tagged human CYPA, CYPA cDNA with HindIII and BamHI sites was cloned into the GST-containing pkMyc vector digested with the same enzymes (Thermo Fisher Scientific) to generate pkMyc.CYPA.GST. SIVcpzPtt MB897-nanoluciferase and SIVcpzPts TAN1-nanoluciferase were described previously (42). To generate pLNCX2.CPSF6-358, NotI- and XhoI-digested hemagglutinin (HA)-tagged CPSF6-358 cDNA (from plasmid LPCK-CPSF6-358.HA [17], a gift of Thomas Gramberg) was cloned into pLNCX2 (TaKaRa Bio Europe, Saint-Germain-en-Laye, France) digested with same restriction enzymes, and positive clones were confirmed by sequencing.

Cells. Wild-type HOS (ATCC CRL-1543; LGC Standards GmbH, Wesel, Germany), HeLa (ATCC CCL-2), HEK293T (ATCC CRL3216), OMK (637-69; ECACC, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), CV-1 (ATCC CCL-70), CRFK (ATCC CCL-94), and FRhL-2 (ATCCR CL-160) cells were maintained in Dulbecco's modified Eagle's medium (Pan-Biotech, Aidenbach, Germany) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 100 U/ml penicillin-streptomycin at 37°C in a humidified atmosphere of 5% CO_2. For HOS and HeLa CYPA knockout cells, 2 μg/ml puromycin was added to the culture medium for selection. HOS cells expressing CPSF6-358 were selected under 300 μg/ml Geneticin (Biochrom GmbH, Berlin, Germany). Human PBMCs and macrophages were isolated from whole blood, obtained from the university hospital of Heinrich Heine University Düsseldorf (ethical approval study number 3180). PBMCs were cultured in RPMI 1640 supplemented with 10 μg phytohemagglutinin (PHA) and later with 30 U/ml interleukin-2, and macrophages were maintained in RPMI 1640 containing 1,000 U/ml monocyte colony-stimulating factor (M-CSF).

HIV-1 and SIVcpz reporter viral particle production. HIV-1 VSV-G-pseudotyped viral particles were produced in a six-well plate by the transfection of HEK293T cells with 200 ng of pMD.G, 800 ng of pSIN.PPT.CMV.Luc.IRES.GFP, 350 ng of pRSV-Rev, and 800 ng of pMDLg/pRRE wild type, pMDLg/pRRE.N74D, or pMDLg/pRRE.A77V. To produce SIVcpzPtt-nanoluciferase or SIVcpzPts-nanoluciferase viruses, 2,250 ng of either reporter plasmid plus 200 ng pMD.G were used to transfect HEK293T cells. Forty-eight hours following transfection, the supernatants were collected and centrifuged for 5 min at 5,000 rpm at 4°C to pellet possible cells. Where needed, the reverse transcriptase (RT) activity of viruses was quantified using the Cavidi HS lenti RT kit (Cavidi Tech, Uppsala, Sweden).

Generation of CYPA knockout cells. The pLentiCRISPRv2 plasmid construct for CYPA knockout was constructed according to previously described protocols (91–93). Complementary oligonucleotides (5′-CACCGTTCTTCGACATTGCCGTCGA-3′ and 5′-AAACTCGACGGCAATGTCGAAGAA-3′ for HeLa cells; 5′-CACCGGACAAGGTCCCAAAGACAGC-3′ and 5′-AAACGCTGTCTTTGGGACCTTGTCC-3′ for HOS cells) containing the specific human CYPA single guide RNA (sgRNA) sequences were ligated into BsmBI-digested pLentiCRISPRv2 to generate the functional transfer vector. The LentiCRISPRv2 plasmid lacking an sgRNA sequence was used as the empty vector control. Transfection of HEK293T cells with the pLentiCRISPRv2 transfer vector containing CYPA knockout sgRNA, packaging plasmid psPAX2, and a VSV-G plasmid generated VSV-G-pseudotyped viral particles, which were used to transduce HeLa or HOS cells for 3 days. Transduced cells were then selected under 2 μg/ml puromycin. While this process almost completely removed CYPA in HeLa cells, it only reduced the protein in HOS cells. Using flow cytometry (FACSAria III sorter; BD, Heidelberg, Germany), single HOS cells with anti-CYPA constructs were isolated in wells of a 96-well plate for single clones. Gene knockout or knockdown in cell pools and clones was confirmed by immunoblotting.

Generation of CPSF6-358-expressing cells. A total of 1,150 ng of the pLNCX2.CPSF6-358 construct together with 1,150 ng of pHIT60 and 200 ng of pMD.G were used to produce gammaretroviral particles in HEK293T cells, and these particles were used to transduce wild-type or CYPA knockout HOS cells for the expression of CPSF6-358.HA. HOS cells expressing CPSF6-358 were selected using 300 μg/ml Geneticin (Biochrom GmbH). CPSF6-358 protein expression was analyzed by immunoblotting. Cells expressing the empty pLNCX2 or LentiCRISPRv2 vector were used as controls.

Single-round infection. A total of 10×10^3 cells were seeded into 96-well plates, and infection was performed the following day. PF-3450074 (PF74) (8) (Aobious Biotrend, Cologne, Germany), GS-CA1 (a gift of Gilead Sciences, Foster City, CA, USA), cyclosporine (CsA; Sigma-Aldrich), or PF-3759857 (PF57) (8) was added to cells 2 h before infection, and control infections were performed with DMSO. PF57 was obtained in two steps starting from *N*-Boc (butoxycarbonyl)-L-phenylalanine. After 48 to 72 h, firefly luciferase activity was measured with the Steady-Glo luciferase system (Promega, Mannheim, Germany), according to the manufacturer's instructions, on a MicroLumat Plus luminometer (Berthold Detection Systems, Pforzheim, Germany). For SIVcpz-nanoluciferase, cells were washed with phosphate-buffered saline (PBS) three times 48 h after infection, before lysis and luciferase measurement, in addition to a medium change 24 h following infection, to eliminate the effect of extracellular nanoluciferase on the supernatant. Nanoluciferase activity was measured with the Nano-Glo luciferase system (Promega) on a MicroLumat Plus luminometer (Berthold Detection Systems). Each experiment was performed in triplicates and at least three times.

Cyclophilin A encapsidation. HEK293T cells were seeded into 6-well plates (10^6 cells per well) 1 day before transfection. Two hours before transfection, 2.5 μM CsA or DMSO for controls was added to cells. Transfection was done as follows. Lipofectamine 2000 (Thermo Fisher Scientific) (according to the manufacturer's instructions) was used to complex a total of 2,500 ng of plasmid DNA for delivery into cells. For SIVcpz, 2,300 ng of the viral reporter plasmid and 200 ng pMD.G VSV-G were used. In the case of HIV-1, 200 ng of pMD.G, 975 ng of pSIN.PPT.CMV.Luc.IRES.GFP, 350 ng of pRSV-Rev, and 975 ng of pMDLg/pRRE were used for transfection. At 48 h posttransfection, the supernatant was collected, viral concentration was done through 20% sucrose for 4 h at 4°C, and viral pellets were lysed using Western blot lysis buffer.

Pulldown experiments. HEK293T cells were seeded into 6-well plates (10^6 cells per well) 1 day before transfection. Transfection was done as follows. Lipofectamine 2000 (Thermo Fisher Scientific) was used to complex a total of 2,600 ng of plasmid DNA for delivery into cells. For SIVcpz, 1,700 ng of the viral reporter plasmid, 200 ng of pMD.G VSV-G, and 700 ng of pcMyc.CYPA.GST constituted a plasmid complex for transfection. For HIV-1, 200 ng of pMD.G, 700 ng of pSIN.PPT.CMV.Luc.IRES.GFP, 300 ng of pRSV-Rev, 700 ng of pMDLg/pRRE, and 700 ng of pcMyc.CYPA.GST or 700 ng pk.GST, for the control, were used. A total of 700 ng of pk.GST or CYPA.GST together with 1,900 ng of pcDNA3.1 were used for the control. At 6 h posttransfection, 5 or 10 μM CsA or control DMSO was added to cells. At 48 h posttransfection, cells were lysed for 20 min on ice, followed by a 20-min centrifugation step at maximum speed in a benchtop centrifuge at 4°C. The supernatant of the cell lysate was added to GST beads together with viral lysates (obtained after 4 h of centrifugation under 20% sucrose at 4°C) and incubated according to the bead manufacturer's instructions (GE Healthcare, Solingen, Germany). Protein complexes were eluted from the beads and subjected to immunoblotting.

Immunoblotting. Cells were lysed for 20 min on ice using radioimmunoprecipitation assay (RIPA) buffer (25 mM Tris-HCl [pH 8.0], 137 mM NaCl, 1% NP-40, 1% glycerol, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 2 mM EDTA, and protease inhibitor cocktail set III [Calbiochem, Darmstadt, Germany]). The cell lysate was centrifuged at 14,800 rpm for 20 min at 4°C. Lysate protein denaturation was done for 5 min at 95°C in RotiLoad loading buffer (Roth, Karlsruhe, Germany), followed by Western blotting. CPSF6-358.HA was detected with mouse anti-HA (catalog number MMS-101P; Covance, Münster, Germany) (1:7,500 dilution). Tubulin was detected using an antitubulin antibody (clone DM1A, mouse monoclonal; Sigma-Aldrich) (1:20,000 dilution). CPSF6 was detected using an anti-CPSF6 antibody (rabbit polyclonal; Proteintech, Manchester, UK) (1:500 dilution). For TRIM5α detection, an anti-TRIM5 antibody was used (rabbit monoclonal, catalog number 143265; Cell Signaling Technology Europe BV, Frankfurt, Germany) (1:500 dilution). For NUP153 detection, an anti-NUP153 antibody was used (mouse monoclonal; Santa Cruz Biotechnology, Heidelberg, Germany) (1:500 dilution). An anti-NUP358 antibody (mouse monoclonal; Abcam, Cambridge, UK) (1:500 dilution) was used for NUP358 detection. For CYPA detection, an anti-CYPA antibody was used (mouse monoclonal; Santa Cruz Biotechnology) (1:500 dilution). CYPA.GST was detected using mouse anti-GST (kindly donated by Reza Ahmadian), and

TABLE 1 Homology modeling: templates used, sequence identities and similarities, and TopScore assessments

Model	Template	Sequence identity (%)	Sequence similarity (%)	TopScore[a]	TopScore Single[a]
PF74					
HIV1_PF74	4XFZ_A	92.2	92.2	0.17	0.17
	4QNB_A	92.2	92.2		
	4XRO_A	88.7	89.6		
	4U0E_A	89.6	89.6		
	6AXV_A	91.8	92.2		
SIVcpzPts_PF74	4XFZ_A	74.8	81.2	0.19	0.20
	4QNB_A	73.1	80.8		
	4XRO_A	71.0	78.6		
	4U0E_A	71.0	78.2		
	6AXV_A	74.6	81.6		
SIVcpzPtt_PF74	4XFZ_A	83.1	87.9	0.17	0.15
	4QNB_A	82.7	87.9		
	4XRO_A	80.5	85.7		
	4U0E_A	80.5	85.3		
	6AXV_A	82.7	87.9		
CPSF6					
HIV1_CPSF6	4WYM_A	90.9	90.9	0.19	0.17
	4U0A_A	88.3	88.3		
	6AY9_A	94.4	94.4		
	4B4N_A	58.9	58.9		
SIVcpzPts_CPSF6	4WYM_A	63.3	70.5	0.21	0.20
	4U0A_A	70.1	77.4		
	6AY9_A	75.2	81.6		
	4B4N_A	44.9	51.3		
SIVcpzPtt_CPSF6	4WYM_A	82.3	86.6	0.18	0.16
	4U0A_A	79.7	84.0		
	6AY9_A	84.9	89.6		
	4B4N_A	52.0	55.4		
Cyclophilin A					
HIV1_CYPA	5FJB_A	92.6	94.0	0.31	0.23
	1FGL_B	4.3	4.3		
	1M9D_C	60.2	60.6		
SIVcpzPts_CYPA	5FJB_A	74.0	81.6	0.33	0.28
	1FGL_B	2.1	2.6		
	1M9D_C	46.1	52.1		
SIVcpzPtt_CYPA	5FJB_A	84.0	89.6	0.31	0.23
	1FGL_B	3.5	3.9		
	1M9D_C	55.0	58.4		
Cyclophilin A	5FJB_C	99.4	99.4	0.09	0.17
	1FGL_A	100.0	100.0		
	1M9D_B	98.8	98.8		

[a]Lower TopScore or TopScore Single values indicate better structural quality. The values are bounded between 0 and 1.

p24/p27 monoclonal antibody (MAb) AG3.0 (1:250 dilution) was used to detect capsid p24. Anti-mouse antibody conjugated to horseradish peroxidase (catalog number NA931V; GE Healthcare) (1:10,000 dilution) and anti-rabbit antibody conjugated to horseradish peroxidase (catalog number NA9340V; GE Healthcare) (1:10,000 dilution) were used as secondary antibodies. Signals were visualized using ECL prime reagent (GE Healthcare).

Modeling of HIV-1, SIVcpzPts, and SIVcpzPtt CA protein-ligand structures. For each CA protein, three monomeric structures were built with TopModel (94) and validated with TopScore (73), one for each ligand. In addition, CYPA was modeled with TopModel (see Table 1 for the templates used). Hexameric structures were then constructed by alignment to the crystal structure of the HIV-1 CA protein (PDB accession number 4WYM) (31) using PyMOL 1.8. Finally, to generate three complex structures for each protein, ligands were inserted into the respective hexameric structures, using the position of PF74 from PDB accession number 4XFZ (95), the position of CPSF6 from PDB accession number 4WYM (31), and the position of CYPA from PDB accession number 1FGL (96) after superimposing the protein parts. For PF74, which was subsequently investigated in complex with the CA protein by molecular dynamics (MD) simulations and effective binding energy computations, superimposing was performed six times, to place PF74 in every binding pocket of the hexamer. For investigating CPSF6 and CYPA, for which only static complex structures were analyzed, this procedure was performed for one binding site only. Residues within 6 Å around the ligands were considered part of the binding pocket.

Molecular dynamics simulations of PF74 bound to HIV-1, SIVcpzPts, and SIVcpzPtt CA proteins. **(i) System setup.** For five replicas per hexameric CA protein-PF74 structure, MD simulations were performed with the Amber18 software package, resulting in a total of 15 MD simulations. Protonation states of protein residues and ligands were adjusted according to pH 7.4 using HTMD 1.12 (97). Atomic point charges of PF74 were generated according to the restrained electrostatic potential (RESP) procedure (98, 99), using antechamber (100) and GAUSSIAN16 (Rev. A.03) (101) at the HF/6-31G* level of theory. For the protein part, ff14SB force field parameters were used (102), and for the ligand, gaff2 force field parameters were used. Using tLEaP, the systems were solvated by an octahedral box of TIP3P water (103) such that the minimal distance between the box edge and any solute atom is 17 Å, and potassium ions were added to neutralize the charge of the systems (103).

(ii) Thermalization and production. Initially, the simulation systems (including the solute and solvent) were energy minimized using 10,000 steps of steepest-descent minimization, followed by 10,000 steps of conjugate gradient minimization, applying positional restraints on protein and ligand atoms with a force constant of 20 kcal mol^{-1} $Å^{-2}$. Second, the same minimization scheme was applied, now using positional restraints with a force constant of 20 kcal mol^{-1} $Å^{-2}$ on the protein atoms only. Finally, the systems were minimized using 20,000 steps of steepest-descent minimization, followed by 20,000 steps of conjugate gradient minimization, applying no restraints. Using NVT (constant number of particles, volume, and temperature) MD simulations, the systems were heated to 300 K using a constant heating rate over 210 ps and simulated for a further 70 ps at this temperature, with positional restraints with a force constant of 10.0 kcal mol^{-1} $Å^{-2}$ on protein and PF74 atoms. Subsequently, the density of the systems was adjusted using NPT (constant number of particles, pressure, and temperature) MD simulations and applying the Berendsen barostat for 1,300 ps, with positional restraints with a force constant of 2.0 kcal mol^{-1} $Å^{-2}$ on protein and PF74 atoms, followed by a final equilibration step of 1,720 ps in the NVT ensemble. For production, NVT MD simulations were performed for 200 ns, yielding an aggregate simulation time of $3 \times 5 \times 200$ ns $= 3$ μs, and coordinates were stored every 100 ps. For all simulations, the Langevin thermostat with a collision frequency of 2 ps^{-1} was used. Minimization and heating were performed on CPUs, and equilibration and production were performed on GPUs with PMEMD (104).

(iii) Calculation of effective binding energies. Effective binding energies were calculated using MMPBSA.py (74) from AmberTools19. For the calculation, the hexamers were split into six dimers containing one PF74 molecule each in the corresponding binding interface. Hence, 30 dimers per isoform were analyzed, corresponding to a total of 60,000 frames per isoform. We applied the 1-trajectory MM-PBSA approach, in which the snapshots of the complex, protein, and ligand are extracted from a single MD simulation of the complex (75). The polar part of the solvation free energy was computed with the linearized Poisson-Boltzmann equation using an internal dielectric constant of 4 and an external one of 80. The nonpolar part was computed using a surface tension of 0.0378 kcal mol^{-1} $Å^{-2}$ (105). To avoid any additional uncertainty in our calculations, we neglected contributions due to changes in the configurational entropy upon complex formation (106, 107).

The effective binding energies ($\Delta G_{\text{effective}}$) were averaged over five replicas, each containing six binding pockets ($\overline{\Delta G}_{\text{effective}}$). The distribution of $\Delta G_{\text{effective}}$ values is Gaussian, and no particular trends were observed over the simulation times. The SEM (standard error of the mean) over the 30 independent calculations for a system (SEM_{all}) was calculated by error propagation according to the following equation:

$$\text{SEM}_{\text{all}} = \frac{1}{5 \times 6} \sqrt{\sum_{i=1}^{5} \sum_{j=1}^{6} \text{SEM}_{i,j}^{2}}$$

In all cases, the statistical uncertainty (SEM_{all}) is <0.015 kcal mol^{-1} and, hence, below the chemical accuracy of 1 kcal mol^{-1}, which is the expected accuracy in an optimal case (76).

Protein sequence alignment. The alignment was generated with MAFFT (108) in Jalview (109). Jalview was also used to generate the Weblogos. The following randomly picked sequences were included in the analyses: HIV-1 M diverse subtypes (GenBank accession numbers A04321, AB023804, AB032740, AB049811, AB097865, AB098330, AB221005, AB221005, AB253421, AB485636, AB485638, AB485643, AB485646, AB485648, AB485656, AB485660, AB485662, AB703607, AB731663, AF110963, and AF190127, in addition to the sequence of the plasmid pMDLg/pRRE), SIVcpzPtt (GenBank accession numbers AF382828, AF103818.1, AF115393, AJ271369, AY169968, DQ373063, DQ373064, DQ373065, DQ373066, EF535993, FR686510, FR686511, GQ217539, JN091690, JN835461, JN835462, JX178450, and X52154), and SIVcpzPts (GenBank accession numbers AF447763, DQ374657, DQ374658, EF394356, EF394357, EF394358, JN091691, JN835460, JQ768416, JQ866001, and SIU42720).

Statistics. Statistical tests for significance (P value of <0.05) were performed with GraphPad Prism 5.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.6 MB.

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REFERENCES

1. Bejarano DA, Peng K, Laketa V, Borner K, Jost KL, Lucic B, Glass B, Lusic M, Muller B, Krausslich H-G. 2019. HIV-1 nuclear import in macrophages is regulated by CPSF6-capsid interactions at the nuclear pore complex. Elife 8:e41800. https://doi.org/10.7554/eLife.41800.
2. Arhel NJ, Souquere-Besse S, Munier S, Souque P, Guadagnini S, Rutherford S, Prevost MC, Allen TD, Charneau P. 2007. HIV-1 DNA Flap formation promotes uncoating of the pre-integration complex at the nuclear pore. EMBO J 26:3025–3037. https://doi.org/10.1038/sj.emboj.7601740.
3. Francis AC, Melikyan GB. 2018. Single HIV-1 imaging reveals progression of infection through CA-dependent steps of docking at the nuclear pore, uncoating, and nuclear transport. Cell Host Microbe 23:536–548.e6. https://doi.org/10.1016/j.chom.2018.03.009.
4. Mamede JI, Cianci GC, Anderson MR, Hope TJ. 2017. Early cytoplasmic uncoating is associated with infectivity of HIV-1. Proc Natl Acad Sci U S A 114:E7169–E7178. https://doi.org/10.1073/pnas.1706245114.
5. Burdick RC, Li C, Munshi M, Rawson JMO, Nagashima K, Hu WS, Pathak VK. 2020. HIV-1 uncoats in the nucleus near sites of integration. Proc Natl Acad Sci U S A 117:5486–5493. https://doi.org/10.1073/pnas.1920631117.
6. Dharan A, Bachmann N, Talley S, Zwikelmaier V, Campbell EM. 2020. Nuclear pore blockade reveals that HIV-1 completes reverse transcription and uncoating in the nucleus. Nat Microbiol 5:1088–1095. https://doi.org/10.1038/s41564-020-0735-8.
7. Zurnic Bönisch I, Dirix L, Lemmens V, Borrenberghs D, De Wit F, Vernaillen F, Rocha S, Christ F, Hendrix J, Hofkens J, Debyser Z. 2020. Capsid-labelled HIV to investigate the role of capsid during nuclear import and integration. J Virol 94:e01024-19. https://doi.org/10.1128/JVI.01024-19.
8. Blair WS, Pickford C, Irving SL, Brown DG, Anderson M, Bazin R, Cao J, Ciaramella G, Isaacson J, Jackson L, Hunt R, Kjerrstrom A, Nieman JA, Patick AK, Perros M, Scott AD, Whitby K, Wu H, Butler SL. 2010. HIV capsid is a tractable target for small molecule therapeutic intervention. PLoS Pathog 6:e1001220. https://doi.org/10.1371/journal.ppat.1001220.
9. Lamorte L, Titolo S, Lemke CT, Goudreau N, Mercier JF, Wardrop E, Shah VB, von Schwedler UK, Langelier C, Banik SS, Aiken C, Sundquist WI, Mason SW. 2013. Discovery of novel small-molecule HIV-1 replication inhibitors that stabilize capsid complexes. Antimicrob Agents Chemother 57:4622–4631. https://doi.org/10.1128/AAC.00985-13.
10. Franke EK, Yuan HE, Luban J. 1994. Specific incorporation of cyclophilin A into HIV-1 virions. Nature 372:359–362. https://doi.org/10.1038/372359a0.
11. Thali M, Bukovsky A, Kondo E, Rosenwirth B, Walsh CT, Sodroski J, Gottlinger HG. 1994. Functional association of cyclophilin A with HIV-1 virions. Nature 372:363–365. https://doi.org/10.1038/372363a0.
12. Stremlau M, Owens CM, Perron MJ, Kiessling M, Autissier P, Sodroski J. 2004. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. Nature 427:848–853. https://doi.org/10.1038/nature02343.
13. Kane M, Yadav SS, Bitzegeio J, Kutluay SB, Zang T, Wilson SJ, Schoggins JW, Rice CM, Yamashita M, Hatziioannou T, Bieniasz PD. 2013. MX2 is an interferon-induced inhibitor of HIV-1 infection. Nature 502:563–566. https://doi.org/10.1038/nature12653.
14. 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 502:559–562. https://doi.org/10.1038/nature12542.
15. 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 319:921–926. https://doi.org/10.1126/science.1152725.
16. König R, Zhou Y, Elleder D, Diamond TL, Bonamy GM, Irelan JT, Chiang CY, 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.
17. Lee K, Ambrose Z, Martin TD, Oztop I, Mulky A, Julias JG, Vandegraaff N, Baumann JG, Wang R, Yuen W, Takemura T, Shelton K, Taniuchi I, Li Y, Sodroski J, Littman DR, Coffin JM, Hughes SH, Unutmaz D, Engelman A, KewalRamani VN. 2010. Flexible use of nuclear import pathways by HIV-1. Cell Host Microbe 7:221–233. https://doi.org/10.1016/j.chom.2010.02.007.
18. Schaller T, Ocwieja KE, Rasaiyaah J, Price AJ, Brady TL, Roth SL, Hue S, Fletcher AJ, Lee K, KewalRamani VN, Noursadeghi M, Jenner RG, James LC, Bushman FD, Towers GJ. 2011. HIV-1 capsid-cyclophilin interactions determine nuclear import pathway, integration targeting and replication efficiency. PLoS Pathog 7:e1002439. https://doi.org/10.1371/journal.ppat.1002439.
19. Matreyek KA, Yucel SS, Li X, Engelman A. 2013. Nucleoporin NUP153 phenylalanine-glycine motifs engage a common binding pocket within the HIV-1 capsid protein to mediate lentiviral infectivity. PLoS Pathog 9:e1003693. https://doi.org/10.1371/journal.ppat.1003693.
20. Mamede JI, Sitbon M, Battini JL, Courgnaud V. 2013. Heterogeneous susceptibility of circulating SIV isolate capsids to HIV-interacting factors. Retrovirology 10:77. https://doi.org/10.1186/1742-4690-10-77.
21. Jimenez-Guardeno JM, Apolonia L, Betancor G, Malim MH. 2019. Immunoproteasome activation enables human TRIM5alpha restriction of HIV-1. Nat Microbiol 4:933–940. https://doi.org/10.1038/s41564-019-0402-0.
22. Ohainle M, Kim K, Komurlu Keceli S, Felton A, Campbell E, Luban J, Emerman M. 2020. TRIM34 restricts HIV-1 and SIV capsids in a TRIM5alpha-dependent manner. PLoS Pathog 16:e1008507. https://doi.org/10.1371/journal.ppat.1008507.
23. Selyutina A, Persaud M, Simons LM, Bulnes-Ramos A, Buffone C, Martinez-Lopez A, Scoca V, Di Nunzio F, Hiatt J, Marson A, Krogan NJ, Hultquist JF, Diaz-Griffero F. 2020. Cyclophilin A prevents HIV-1 restriction in lymphocytes by blocking human TRIM5alpha binding to the viral core. Cell Rep 30:3766–3777.e6. https://doi.org/10.1016/j.celrep.2020.02.100.
24. De Iaco A, Luban J. 2011. Inhibition of HIV-1 infection by TNPO3 depletion is determined by capsid and detectable after viral cDNA enters the nucleus. Retrovirology 8:98. https://doi.org/10.1186/1742-4690-8-98.
25. Ning J, Zhong Z, Fischer DK, Harris G, Watkins SC, Ambrose Z, Zhang P. 2018. Truncated CPSF6 forms higher-order complexes that bind and disrupt HIV-1 capsid. J Virol 92:e00368-18. https://doi.org/10.1128/JVI.00368-18.

26. Merindol N, El-Far M, Sylla M, Masroori N, Dufour C, Li JX, Cherry P, Plourde MB, Tremblay C, Berthoux L. 2018. HIV-1 capsids from B27/B57+ elite controllers escape Mx2 but are targeted by TRIM5alpha, leading to the induction of an antiviral state. PLoS Pathog 14:e1007398. https://doi.org/10.1371/journal.ppat.1007398.
27. Maillard PV, Zoete V, Michielin O, Trono D. 2011. Homology-based identification of capsid determinants that protect HIV1 from human TRIM5alpha restriction. J Biol Chem 286:8128–8140. https://doi.org/10.1074/jbc.M110.187609.
28. Busnadiego I, Kane M, Rihn SJ, Preugschas HF, Hughes J, Blanco-Melo D, Strouvelle VP, Zang TM, Willett BJ, Boutell C, Bieniasz PD, Wilson SJ. 2014. Host and viral determinants of Mx2 antiretroviral activity. J Virol 88:7738–7752. https://doi.org/10.1128/JVI.00214-14.
29. Price AJ, Jacques DA, McEwan WA, Fletcher AJ, Essig S, Chin JW, Halambage UD, Aiken C, James LC. 2014. Host cofactors and pharmacologic ligands share an essential interface in HIV-1 capsid that is lost upon disassembly. PLoS Pathog 10:e1004459. https://doi.org/10.1371/journal.ppat.1004459.
30. Price AJ, Fletcher AJ, Schaller T, Elliott T, Lee K, KewalRamani VN, Chin JW, Towers GJ, James LC. 2012. CPSF6 defines a conserved capsid interface that modulates HIV-1 replication. PLoS Pathog 8:e1002896. https://doi.org/10.1371/journal.ppat.1002896.
31. Bhattacharya A, Alam SL, Fricke T, Zadrozny K, Sedzicki J, Taylor AB, Demeler B, Pornillos O, Ganser-Pornillos BK, Diaz-Griffero F, Ivanov DN, Yeager M. 2014. Structural basis of HIV-1 capsid recognition by PF74 and CPSF6. Proc Natl Acad Sci U S A 111:18625–18630. https://doi.org/10.1073/pnas.1419945112.
32. Shi J, Zhou J, Shah VB, Aiken C, Whitby K. 2011. Small-molecule inhibition of human immunodeficiency virus type 1 infection by virus capsid destabilization. J Virol 85:542–549. https://doi.org/10.1128/JVI.01406-10.
33. Saito A, Ferhadian D, Sowd GA, Serrao E, Shi J, Halambage UD, Teng S, Soto J, Siddiqui MA, Engelman AN, Aiken C, Yamashita M. 2016. Roles of capsid-interacting host factors in multimodal inhibition of HIV-1 by PF74. J Virol 90:5808–5823. https://doi.org/10.1128/JVI.03116-15.
34. Hulme AE, Kelley Z, Foley D, Hope TJ. 2015. Complementary assays reveal a low level of CA associated with viral complexes in the nuclei of HIV-1-infected cells. J Virol 89:5350–5361. https://doi.org/10.1128/JVI.00476-15.
35. Balasubramaniam M, Zhou J, Addai A, Martinez P, Pandhare J, Aiken C, Dash C. 2019. PF74 inhibits HIV-1 integration by altering the composition of the preintegration complex. J Virol 93:e01741-18. https://doi.org/10.1128/JVI.01741-18.
36. Carnes SK, Sheehan JH, Aiken C. 2018. Inhibitors of the HIV-1 capsid, a target of opportunity. Curr Opin HIV AIDS 13:359–365. https://doi.org/10.1097/COH.0000000000000472.
37. Singh K, Gallazzi F, Hill KJ, Burke DH, Lange MJ, Quinn TP, Neogi U, Sonnerborg A. 2019. GS-CA compounds: first-in-class HIV-1 capsid inhibitors covering multiple grounds. Front Microbiol 10:1227. https://doi.org/10.3389/fmicb.2019.01227.
38. Yant SR, Mulato A, Hansen D, Tse WC, Niedziela-Majka A, Zhang JR, Stepan GJ, Jin D, Wong MH, Perreira JM, Singer E, Papalia GA, Hu EY, Zheng J, Lu B, Schroeder SD, Chou K, Ahmadyar S, Liclican A, Yu H, Novikov N, Paoli E, Gonik D, Ram RR, Hung M, McDougall WM, Brass AL, Sundquist WI, Cihlar T, Link JO. 2019. A highly potent long-acting small-molecule HIV-1 capsid inhibitor with efficacy in a humanized mouse model. Nat Med 25:1377–1384. https://doi.org/10.1038/s41591-019-0560-x.
39. Twizerimana AP, Scheck R, Häussinger D, Münk C. 2018. Post-entry restriction factors of SIVcpz. Future Virol 13:727–745. https://doi.org/10.2217/fvl-2018-0093.
40. Sharp PM, Hahn BH. 2010. The evolution of HIV-1 and the origin of AIDS. Philos Trans R Soc Lond B Biol Sci 365:2487–2494. https://doi.org/10.1098/rstb.2010.0031.
41. Gao F, Bailes E, Robertson DL, Chen Y, Rodenburg CM, Michael SF, Cummins LB, Arthur LO, Peeters M, Shaw GM, Sharp PM, Hahn BH. 1999. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. Nature 397:436–441. https://doi.org/10.1038/17130.
42. Zhang Z, Gu Q, de Manuel Montero M, Bravo IG, Marques-Bonet T, Häussinger D, Münk C. 2017. Stably expressed APOBEC3H forms a barrier for cross-species transmission of simian immunodeficiency virus of chimpanzee to humans. PLoS Pathog 13:e1006746. https://doi.org/10.1371/journal.ppat.1006746.
43. Sauter D, Schindler M, Specht A, Landford WN, Munch J, Kim KA, Votteler J, Schubert U, Bibollet-Ruche F, Keele BF, Takehisa J, Ogando Y, Ochsenbauer C, Kappes JC, Ayouba A, Peeters M, Learn GH, Shaw G, Sharp PM, Bieniasz P, Hahn BH, Hatziioannou T, Kirchhoff F. 2009. Tetherin-driven adaptation of Vpu and Nef function and the evolution of pandemic and nonpandemic HIV-1 strains. Cell Host Microbe 6:409–421. https://doi.org/10.1016/j.chom.2009.10.004.
44. Kratovac Z, Virgen CA, Bibollet-Ruche F, Hahn BH, Bieniasz PD, Hatziioannou T. 2008. Primate lentivirus capsid sensitivity to TRIM5 proteins. J Virol 82:6772–6777. https://doi.org/10.1128/JVI.00410-08.
45. Meier K, Jaguva Vasudevan AA, Zhang Z, Bahr A, Kochs G, Häussinger D, Münk C. 2018. Equine MX2 is a restriction factor of equine infectious anemia virus (EIAV). Virology 523:52–63. https://doi.org/10.1016/j.virol.2018.07.024.
46. McEwan WA, Schaller T, Ylinen LM, Hosie MJ, Towers GJ, Willett BJ. 2009. Truncation of TRIM5 in the Feliformia explains the absence of retroviral restriction in cells of the domestic cat. J Virol 83:8270–8275. https://doi.org/10.1128/JVI.00670-09.
47. Bieniasz PD. 2004. Intrinsic immunity: a front-line defense against viral attack. Nat Immunol 5:1109–1115. https://doi.org/10.1038/ni1125.
48. Münk C, Brandt SM, Lucero G, Landau NR. 2002. A dominant block to HIV-1 replication at reverse transcription in simian cells. Proc Natl Acad Sci U S A 99:13843–13848. https://doi.org/10.1073/pnas.212400099.
49. Cowan S, Hatziioannou T, Cunningham T, Muesing MA, Gottlinger HG, Bieniasz PD. 2002. Cellular inhibitors with Fv1-like activity restrict human and simian immunodeficiency virus tropism. Proc Natl Acad Sci U S A 99:11914–11919. https://doi.org/10.1073/pnas.162299499.
50. Besnier C, Takeuchi Y, Towers G. 2002. Restriction of lentivirus in monkeys. Proc Natl Acad Sci U S A 99:11920–11925. https://doi.org/10.1073/pnas.172384599.
51. Lu M, Hou G, Zhang H, Suiter CL, Ahn J, Byeon IJ, Perilla JR, Langmead CJ, Hung I, Gor'kov PL, Gan Z, Brey W, Aiken C, Zhang P, Schulten K, Gronenborn AM, Polenova T. 2015. Dynamic allostery governs cyclophilin A-HIV capsid interplay. Proc Natl Acad Sci U S A 112:14617–14622. https://doi.org/10.1073/pnas.1516920112.
52. Liu Z, Pan Q, Ding S, Qian J, Xu F, Zhou J, Cen S, Guo F, Liang C. 2013. The interferon-inducible MxB protein inhibits HIV-1 infection. Cell Host Microbe 14:398–410. https://doi.org/10.1016/j.chom.2013.08.015.
53. Rasaiyaah J, Tan CP, Fletcher AJ, Price AJ, Blondeau C, Hilditch L, Jacques DA, Selwood DL, James LC, Noursadeghi M, Towers GJ. 2013. HIV-1 evades innate immune recognition through specific cofactor recruitment. Nature 503:402–405. https://doi.org/10.1038/nature12769.
54. Ambrose Z, Lee K, Ndjomou J, Xu H, Oztop I, Matous J, Takemura T, Unutmaz D, Engelman A, Hughes SH, KewalRamani VN. 2012. Human immunodeficiency virus type 1 capsid mutation N74D alters cyclophilin A dependence and impairs macrophage infection. J Virol 86:4708–4714. https://doi.org/10.1128/JVI.05887-11.
55. Qi M, Yang R, Aiken C. 2008. Cyclophilin A-dependent restriction of human immunodeficiency virus type 1 capsid mutants for infection of nondividing cells. J Virol 82:12001–12008. https://doi.org/10.1128/JVI.01518-08.
56. Sokolskaja E, Sayah DM, Luban J. 2004. Target cell cyclophilin A modulates human immunodeficiency virus type 1 infectivity. J Virol 78:12800–12808. https://doi.org/10.1128/JVI.78.23.12800-12808.2004.
57. Burse M, Shi J, Aiken C. 2017. Cyclophilin A potentiates TRIM5alpha inhibition of HIV-1 nuclear import without promoting TRIM5alpha binding to the viral capsid. PLoS One 12:e0182298. https://doi.org/10.1371/journal.pone.0182298.
58. Sokolskaja E, Luban J. 2006. Cyclophilin, TRIM5, and innate immunity to HIV-1. Curr Opin Microbiol 9:404–408. https://doi.org/10.1016/j.mib.2006.06.011.
59. Kootstra NA, Münk C, Tonnu N, Landau NR, Verma IM. 2003. Abrogation of postentry restriction of HIV-1-based lentiviral vector transduction in simian cells. Proc Natl Acad Sci U S A 100:1298–1303. https://doi.org/10.1073/pnas.0337541100.
60. Shah VB, Shi J, Hout DR, Oztop I, Krishnan L, Ahn J, Shotwell MS, Engelman A, Aiken C. 2013. The host proteins transportin SR2/TNPO3 and cyclophilin A exert opposing effects on HIV-1 uncoating. J Virol 87:422–432. https://doi.org/10.1128/JVI.07177-11.
61. 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.
62. Luban J, Bossolt KL, Franke EK, Kalpana GV, Goff SP. 1993. Human

immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B. Cell 73:1067–1078. https://doi.org/10.1016/0092-8674(93)90637-6.
63. Gamble TR, Vajdos FF, Yoo S, Worthylake DK, Houseweart M, Sundquist WI, Hill CP. 1996. Crystal structure of human cyclophilin A bound to the amino-terminal domain of HIV-1 capsid. Cell 87:1285–1294. https://doi.org/10.1016/s0092-8674(00)81823-1.
64. Franke EK, 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. https://doi.org/10.1006/viro.1996.0421.
65. Yin L, Braaten D, Luban J. 1998. Human immunodeficiency virus type 1 replication is modulated by host cyclophilin A expression levels. J Virol 72:6430–6436. https://doi.org/10.1128/JVI.72.8.6430-6436.1998.
66. Braaten D, Franke EK, Luban J. 1996. Cyclophilin A is required for an early step in the life cycle of human immunodeficiency virus type 1 before the initiation of reverse transcription. J Virol 70:3551–3560. https://doi.org/10.1128/JVI.70.6.3551-3560.1996.
67. Braaten D, Franke EK, Luban J. 1996. Cyclophilin A is required for the replication of group M human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus SIV(CPZ)GAB but not group O HIV-1 or other primate immunodeficiency viruses. J Virol 70:4220–4227. https://doi.org/10.1128/JVI.70.7.4220-4227.1996.
68. Hatziioannou T, Perez-Caballero D, Cowan S, Bieniasz PD. 2005. Cyclophilin interactions with incoming human immunodeficiency virus type 1 capsids with opposing effects on infectivity in human cells. J Virol 79:176–183. https://doi.org/10.1128/JVI.79.1.176-183.2005.
69. Mlynar E, Bevec D, Billich A, Rosenwirth B, Steinkasserer A. 1997. The non-immunosuppressive cyclosporin A analogue SDZ NIM 811 inhibits cyclophilin A incorporation into virions and virus replication in human immunodeficiency virus type 1-infected primary and growth-arrested T cells. J Gen Virol 78(Part 4):825–835. https://doi.org/10.1099/0022-1317-78-4-825.
70. Saini M, Potash MJ. 2006. Novel activities of cyclophilin A and cyclosporin A during HIV-1 infection of primary lymphocytes and macrophages. J Immunol 177:443–449. https://doi.org/10.4049/jimmunol.177.1.443.
71. Towers GJ, Hatziioannou T, Cowan S, Goff SP, Luban J, Bieniasz PD. 2003. Cyclophilin A modulates the sensitivity of HIV-1 to host restriction factors. Nat Med 9:1138–1143. https://doi.org/10.1038/nm910.
72. Saito A, Henning MS, Serrao E, Dubose BN, Teng S, Huang J, Li X, Saito N, Roy SP, Siddiqui MA, Ahn J, Tsuji M, Hatziioannou T, Engelman AN, Yamashita M. 2016. Capsid-CPSF6 interaction is dispensable for HIV-1 replication in primary cells but is selected during virus passage in vivo. J Virol 90:6918–6935. https://doi.org/10.1128/JVI.00019-16.
73. Mulnaes D, Gohlke H. 2018. TopScore: using deep neural networks and large diverse data sets for accurate protein model quality assessment. J Chem Theory Comput 14:6117–6126. https://doi.org/10.1021/acs.jctc.8b00690.
74. Miller BR, McGee TD, Swails JM, Homeyer N, Gohlke H, Roitberg AE. 2012. MMPBSA.py: an efficient program for end-state free energy calculations. J Chem Theory Comput 8:3314–3321. https://doi.org/10.1021/ct300418h.
75. Homeyer N, Gohlke H. 2012. Free energy calculations by the molecular mechanics Poisson-Boltzmann surface area method. Mol Inform 31:114–122. https://doi.org/10.1002/minf.201100135.
76. Michel J, Essex JW. 2010. Prediction of protein-ligand binding affinity by free energy simulations: assumptions, pitfalls and expectations. J Comput Aided Mol Des 24:639–658. https://doi.org/10.1007/s10822-010-9363-3.
77. Rankovic S, Ramalho R, Aiken C, Rousso I. 2018. PF74 reinforces the HIV-1 capsid to impair reverse transcription-induced uncoating. J Virol 92:e00845-18. https://doi.org/10.1128/JVI.00845-18.
78. Fricke T, Buffone C, Opp S, Valle-Casuso J, Diaz-Griffero F. 2014. Bl-2 destabilizes HIV-1 cores during infection and prevents binding of CPSF6 to the HIV-1 capsid. Retrovirology 11:120. https://doi.org/10.1186/s12977-014-0120-x.
79. Buffone C, Martinez-Lopez A, Fricke T, Opp S, Severgnini M, Cifola I, Petiti L, Frabetti S, Skorupka K, Zadrozny KK, Ganser-Pornillos BK, Pornillos O, Di Nunzio F, Diaz-Griffero F. 2018. Nup153 unlocks the nuclear pore complex for HIV-1 nuclear translocation in nondividing cells. J Virol 92:e00648-18. https://doi.org/10.1128/JVI.00648-18.
80. Zhou J, Price AJ, Halambage UD, James LC, Aiken C. 2015. HIV-1 resistance to the capsid-targeting inhibitor PF74 results in altered dependence on host factors required for virus nuclear entry. J Virol 89:9068–9079. https://doi.org/10.1128/JVI.00340-15.
81. Lahaye X, Satoh T, Gentili M, Cerboni S, Silvin A, Conrad C, Ahmed-Belkacem A, Rodriguez EC, Guichou JF, Bosquet N, Piel M, Le Grand R, King MC, Pawlotsky JM, Manel N. 2016. Nuclear envelope protein SUN2 promotes cyclophilin-A-dependent steps of HIV replication. Cell Rep 15:879–892. https://doi.org/10.1016/j.celrep.2016.03.074.
82. De Iaco A, Santoni F, Vannier A, Guipponi M, Antonarakis S, Luban J. 2013. TNPO3 protects HIV-1 replication from CPSF6-mediated capsid stabilization in the host cell cytoplasm. Retrovirology 10:20. https://doi.org/10.1186/1742-4690-10-20.
83. Hori T, Takeuchi H, Saito H, Sakuma R, Inagaki Y, Yamaoka S. 2013. A carboxy-terminally truncated human CPSF6 lacking residues encoded by exon 6 inhibits HIV-1 cDNA synthesis and promotes capsid disassembly. J Virol 87:7726–7736. https://doi.org/10.1128/JVI.00124-13.
84. Lee K, Mulky A, Yuen W, Martin TD, Meyerson NR, Choi L, Yu H, Sawyer SL, KewalRamani VN. 2012. HIV-1 capsid-targeting domain of cleavage and polyadenylation specificity factor 6. J Virol 86:3851–3860. https://doi.org/10.1128/JVI.06607-11.
85. Kane M, Rebensburg SV, Takata MA, Zang TM, Yamashita M, Kvaratskhelia M, Bieniasz PD. 2018. Nuclear pore heterogeneity influences HIV-1 infection and the antiviral activity of MX2. Elife 7:e35738. https://doi.org/10.7554/eLife.35738.
86. Bock M, Bishop KN, Towers G, Stoye JP. 2000. Use of a transient assay for studying the genetic determinants of Fv1 restriction. J Virol 74:7422–7430. https://doi.org/10.1128/jvi.74.16.7422-7430.2000.
87. Takehisa J, Kraus MH, Decker JM, Li Y, Keele BF, Bibollet-Ruche F, Zammit KP, Weng Z, Santiago ML, Kamenya S, Wilson ML, Pusey AE, Bailes E, Sharp PM, Shaw GM, Hahn BH. 2007. Generation of infectious molecular clones of simian immunodeficiency virus from fecal consensus sequences of wild chimpanzees. J Virol 81:7463–7475. https://doi.org/10.1128/JVI.00551-07.
88. Bibollet-Ruche F, Heigele A, Keele BF, Easlick JL, Decker JM, Takehisa J, Learn G, Sharp PM, Hahn BH, Kirchhoff F. 2012. Efficient SIVcpz replication in human lymphoid tissue requires viral matrix protein adaptation. J Clin Invest 122:1644–1652. https://doi.org/10.1172/JCI61429.
89. Bähr A, Singer A, Hain A, Vasudevan AA, Schilling M, Reh J, Riess M, Panitz S, Serrano V, Schweizer M, Konig R, Chanda S, Häussinger D, Kochs G, Lindemann D, Münk C. 2016. Interferon but not MxB inhibits foamy retroviruses. Virology 488:51–60. https://doi.org/10.1016/j.virol.2015.10.034.
90. Dull T, Zufferey R, Kelly M, Mandel RJ, Nguyen M, Trono D, Naldini L. 1998. A third-generation lentivirus vector with a conditional packaging system. J Virol 72:8463–8471. https://doi.org/10.1128/JVI.72.11.8463-8471.1998.
91. Osei Kuffour E, Schott K, Jaguva Vasudevan AA, Holler J, Schulz WA, Lang PA, Lang KS, Kim B, Häussinger D, König R, Münk C. 2018. USP18 (UBP43) abrogates p21-mediated inhibition of HIV-1. J Virol 92:e00592-18. https://doi.org/10.1128/JVI.00592-18.
92. Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelson T, Heckl D, Ebert BL, Root DE, Doench JG, Zhang F. 2014. Genome-scale CRISPR-Cas9 knockout screening in human cells. Science 343:84–87. https://doi.org/10.1126/science.1247005.
93. Sanjana NE, Shalem O, Zhang F. 2014. Improved vectors and genome-wide libraries for CRISPR screening. Nat Methods 11:783–784. https://doi.org/10.1038/nmeth.3047.
94. Mulnaes D, Porta N, Clemens R, Apanasenko I, Reiners J, Gremer L, Neudecker P, Smits SHJ, Gohlke H. 2020. TopModel: template-based protein structure prediction at low sequence identity using top-down consensus and deep neural networks. J Chem Theory Comput 16:1953–1967. https://doi.org/10.1021/acs.jctc.9b00825.
95. Gres AT, Kirby KA, KewalRamani VN, Tanner JJ, Pornillos O, Sarafianos SG. 2015. Structural virology. X-ray crystal structures of native HIV-1 capsid protein reveal conformational variability. Science 349:99–103. https://doi.org/10.1126/science.aaa5936.
96. Zhao Y, Chen Y, Schutkowski M, Fischer G, Ke H. 1997. Cyclophilin A complexed with a fragment of HIV-1 Gag protein: insights into HIV-1 infectious activity. Structure 5:139–146. https://doi.org/10.1016/s0969-2126(97)00172-x.
97. Doerr S, Harvey MJ, Noé F, De Fabritiis G. 2016. HTMD: high-throughput molecular dynamics for molecular discovery. J Chem Theory Comput 12:1845–1852. https://doi.org/10.1021/acs.jctc.6b00049.
98. Bayly CI, Cieplak P, Cornell W, Kollman PA. 1993. A well-behaved electrostatic potential based method using charge restraints for deriv-

ing atomic charges: the RESP model. J Phys Chem 97:10269–10280. https://doi.org/10.1021/j100142a004.
99. Wang J, Cieplak P, Kollman PA. 2000. How well does a restrained electrostatic potential (RESP) model perform in calculating conformational energies of organic and biological molecules? J Comput Chem 21:1049–1074. https://doi.org/10.1002/1096-987X(200009)21:12<1049::AID-JCC3>3.0.CO;2-F.
100. Wang J, Wang W, Kollman PA, Case DA. 2006. Automatic atom type and bond type perception in molecular mechanical calculations. J Mol Graph Model 25:247–260. https://doi.org/10.1016/j.jmgm.2005.12.005.
101. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Petersson GA, Nakatsuji H, Li X, Caricato M, Marenich AV, Bloino J, Janesko BG, Gomperts R, Mennucci B, Hratchian HP, Ortiz JV, Izmaylov AF, Sonnenberg JL, Williams-Young D, Ding F, Lipparini F, Egidi F, Goings J, Peng B, Petrone A, Henderson T, Ranasinghe D, Zakrzewski VG, Gao J, Rega N, Zheng G, Liang W, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, Throssell K, Montgomery JA, Jr, Peralta JE, Ogliaro F, et al. 2016. Gaussian 16 Rev. C.01. Gaussian Inc, Wallingford, CT.
102. Maier JA, Martinez C, Kasavajhala K, Wickstrom L, Hauser KE, Simmerling C. 2015. ff14SB: improving the accuracy of protein side chain and backbone parameters from ff99SB. J Chem Theory Comput 11:3696–3713. https://doi.org/10.1021/acs.jctc.5b00255.
103. Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML. 1983. Comparison of simple potential functions for simulating liquid water. J Chem Phys 79:926–935. https://doi.org/10.1063/1.445869.
104. Salomon-Ferrer R, Götz AW, Poole D, Le Grand S, Walker RC. 2013. Routine microsecond molecular dynamics simulations with AMBER on GPUs. 2. Explicit solvent particle mesh Ewald. J Chem Theory Comput 9:3878–3888. https://doi.org/10.1021/ct400314y.
105. Tan C, Tan Y-H, Luo R. 2007. Implicit nonpolar solvent models. J Phys Chem B 111:12263–12274. https://doi.org/10.1021/jp073399n.
106. Gohlke H, Case DA. 2004. Converging free energy estimates: MM-PB(GB)SA studies on the protein-protein complex Ras-Raf. J Comput Chem 25:238–250. https://doi.org/10.1002/jcc.10379.
107. Hou T, Wang J, Li Y, Wang W. 2011. Assessing the performance of the MM/PBSA and MM/GBSA methods. 1. The accuracy of binding free energy calculations based on molecular dynamics simulations. J Chem Inf Model 51:69–82. https://doi.org/10.1021/ci100275a.
108. Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. https://doi.org/10.1093/molbev/mst010.
109. Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ. 2009. Jalview version 2—a multiple sequence alignment editor and analysis workbench. Bioinformatics 25:1189–1191. https://doi.org/10.1093/bioinformatics/btp033.

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Post-entry restriction factors of SIVcpz

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Pandemic HIV-1, a human lentivirus, is the result of zoonotic transmission of SIV from chimpanzees (SIVcpz). How SIVcpz established spread in humans after spillover is an outstanding question. Lentiviral cross-species transmissions are exceptionally rare events. Nevertheless, the chimpanzee and the gorilla were part of the transmission chains that resulted in sustained infections that evolved into HIV-1. Although many restriction factors can repress the early stages of lentiviral replication, others target replication during the late phases. In some cases, viruses incorporate host proteins that interfere with subsequent rounds of replication. Though limited and small, HIVs and SIVs, including SIVcpz can use their genome products to modulate and escape some of these barriers and thus establish a chronic infection.

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Keywords • APOBEC3 • chimpanzee • gorilla • HIV-1 • MXB • restriction factor • SAMHD1 • SERINC5 • tetherin • zoonosis

Lentiviruses are a subgroup of mammalian retroviruses that have spread among other species in Old World primates [1,2]. These SIVs have jumped from nonhuman primates to humans on several different occasions. Successful crossing to humans gave rise to different HIVs that cause the acquired immunodeficiency syndrome or AIDS [3], one of the worst pandemics of recent centuries. Pressure by retroviruses to colonize their hosts has forced animals to evolve restriction approaches, including but not limited to the production of restriction factors, to target key replication steps.

To survive this restrictive environment, retroviruses and specifically primate lentiviruses, have come up with a number of strategies:

- Mutating their genome for diversity in their phenotype;
- Antagonizing cellular antiviral factors using counteracting proteins;
- Escaping recognition by the host's sensing machinery;
- Hijacking cellular factors.

There are more than 45 Old World simian species living with lentiviruses [2], most of them in nonpathogenic virus–host relationships. In the family of Hominidae, chimpanzees, gorillas and humans can be infected by species-specific lentiviruses. The SIV of the central African chimpanzee *Pan troglodytes troglodytes* (SIVcpzPtt) transmitted zoonotically to humans and evolved to HIV type 1 (HIV-1) groups M and N [4–6] retroviruses. The western lowland gorilla (*Gorilla gorilla gorilla*) lentivirus (SIVgor) [7] adapted to humans to become the origin of HIV-1 groups P and O [8,9]. The pandemic HIV-1 M infected at least 45 million people, and the HIV-1 type O virus is found in around 100,000 humans while only single cases of infections with HIV-1 strains N and P have been reported. In contrast, the HIV type 2 (HIV-2) virus is a result of multiple cross-species transmissions of SIV of the sooty mangabey monkey (SIVsmm) (Figure 1) [10–12]. Here, we discuss the SIV from chimpanzee with regard to host resistance factors and viral strategies to overcome these factors in humans. The transmission of SIVcpz to humans that resulted in the evolution of the pandemic HIV-1 M virus likely occurred between 1853 and 1908 and other successful SIV transmissions to humans presumably happened thereafter [13–15].

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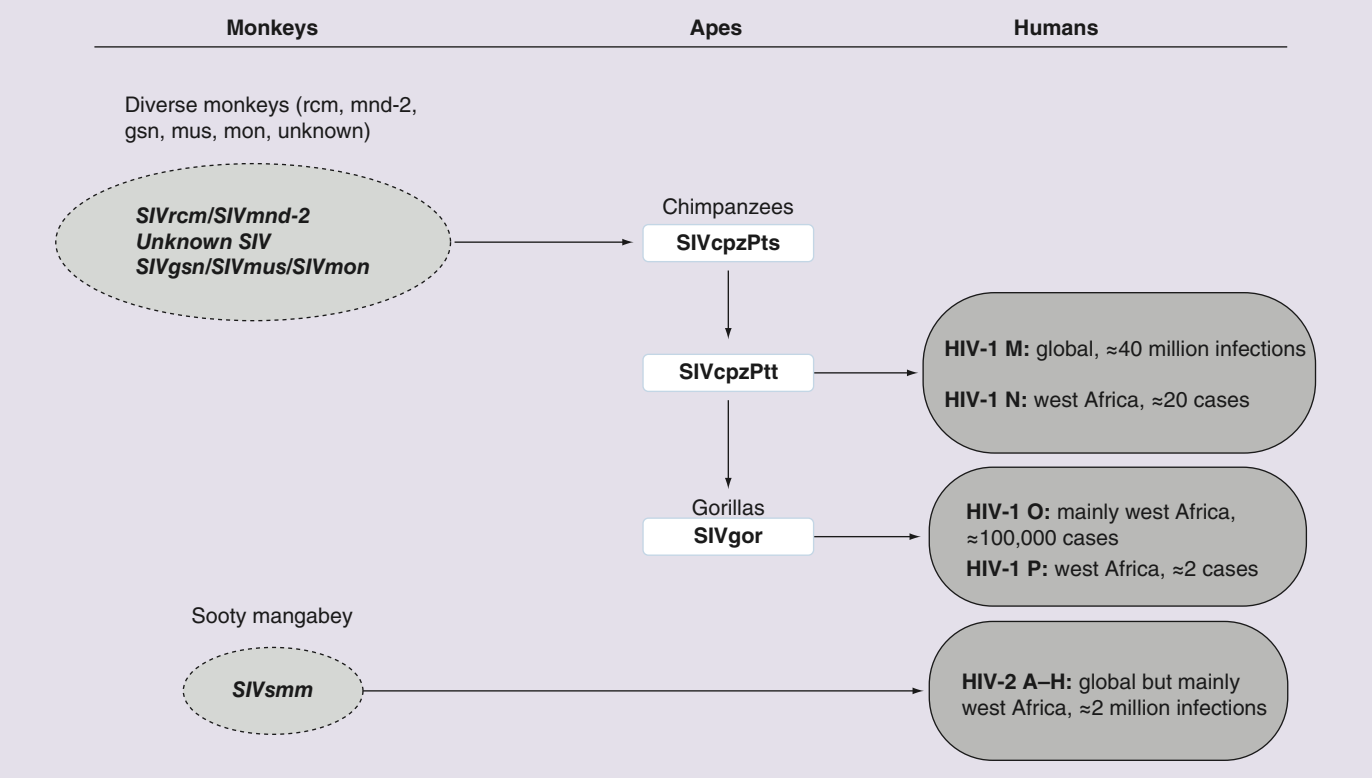

Figure 1. Cross-species transmission of SIVs to human resulted in the formation of HIVs. Chimpanzee acquired lentivirus infection from preyed monkeys. The chimpanzee lentivirus SIVcpzPts from eastern chimpanzee transmitted to western chimpanzee and evolved to SIVcpzPtt. SIVcpzPtt was transmitted to humans to become the global HIV-1 group M and the rare HIV-1 group N. SIVcpzPtt also spread to gorillas and evolved to SIVgor. SIVgor invaded humans and evolved to epidemic HIV-1 group O and rare cases of HIV-1 group P. SIVsmm of sooty mangabey made several independent cross-species transmissions and evolved in humans to HIV-2 strains.

SIVcpz: a result of lentiviral recombination

Of the four subspecies of the chimpanzees (*Pan troglodytes verus* [Ptv] or western chimpanzee, *Pan troglodytes ellioti* [Pte], *P. t. troglodytes* [Ptt] or central chimpanzee and the eastern chimpanzee, also known as *P. t. schweinfurthii* [Pts]), the central and eastern species are known to be naturally infected by SIVs [16–19]. Most infections do not appear to be highly pathogenic [20]. Eastern chimpanzee acquired their lentivirus infection (SIVcpzPts) from preyed monkeys. SIVcpzPts transmitted to western chimpanzee and evolved to SIVcpzPtt. SIVcpzPtt was transmitted to humans to become HIV-1 group M and group N and also spread to gorillas to evolve to SIVgor. SIVgor invaded humans and evolved to HIV-1 groups O and P (Figure 1).

All retroviruses encode three genes: *gag*, *pol* and *env,* as well as a long terminal repeats (LTRs) at each end of the genome. The *gag* gene encodes the structural matrix and capsid proteins, *pol* encodes reverse transcriptase, protease and integrase proteins, whereas *env* encodes the viral glycoprotein. In addition, the SIVcpz genome contains genes unique to lentiviruses, *vif, vpr*, *tat*, *rev* and *nef*. Moreover, SIVcpz has a *vpu* gene that is characteristic of its lineage (Figure 2). The genome of SIVcpz has been extensively analyzed and the data indicate that this virus arose from a recombination event between at least three viruses: a lentivirus of the collared mangabey (also known as red-capped mangabey) or *Mandrillus sphinx*, abbreviated as SIVrcm and SIVmnd-2, respectively, providing *integrase*, *vif* and *vpr* genes and, a virus of the greater spot-nosed/mustached guenon/mona monkey (SIVgsn/SIVmus/SIVmon) clade (providing *vpu*, *env*, *nef*), in addition to a currently unidentified SIV that donated the 5′ part of the genome including the LTR and the gag and reverse transcriptase genes [2,4,21–23].

Human TRIM5α does not inhibit SIVcpz

The large family of TRIM proteins shares a tripartite motif characteristic that includes a ring box, a B-box and a coiled-coil domain [24]. Although these domains are crucial for the antiviral activity of its member, the TRIM5α

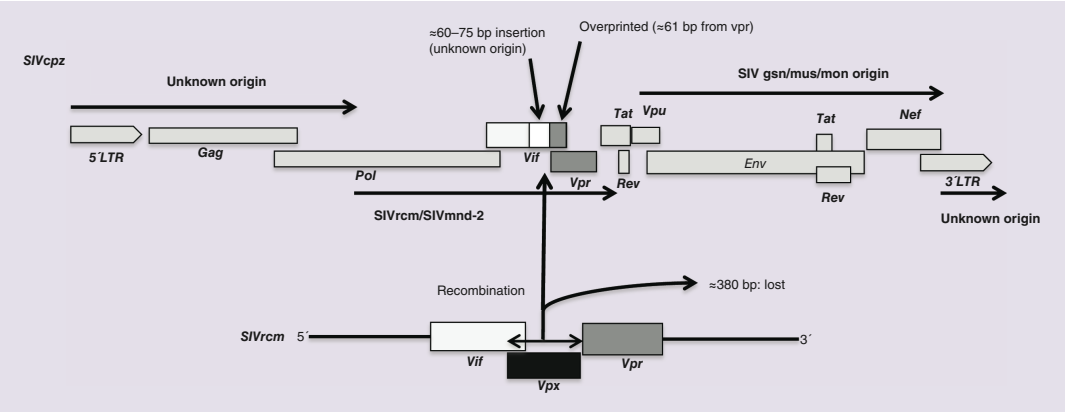

Figure 2. Structure of the multi-origin SIVcpz genome. SIVcpz is the result of recombination events between at least three different SIVs that chimpanzee obtained from monkey preys. 3′ and 5′ LTR, *gag* as well as half of *pol* are from a currently unidentified SIV. Remaining half of *pol* up to 5′ end of *tat* are believed to be from SIVrcm/SIVmnd-2 while the rest of the genome has been donated by an SIV from greater spot-nosed/mustached guenon/mona monkey, as indicated by 'origin' arrows on the figure. During this recombination process that generated SIVcpz, around 380 bp (a fragment that comprised the entire SIVrcm *vpx* gene, in addition to 3′ part of *vif*) were lost leaving a 'truncated' *vif*. An overprinting process at 5′ end of *vpr* (around 61 bp) generated a new 3′ end of *vif* including *vif* stop codon. Furthermore, there was an insertion of a 60–75 bp fragment (with unknown origin) upstream *vif-vpr* overlap (overprint). This insertion is also present in HIV-1 and SIVgor and crucial for lentiviral spread as it helps these viruses to deal with host APOBEC3 restriction.
LTR: Long terminal repeat.

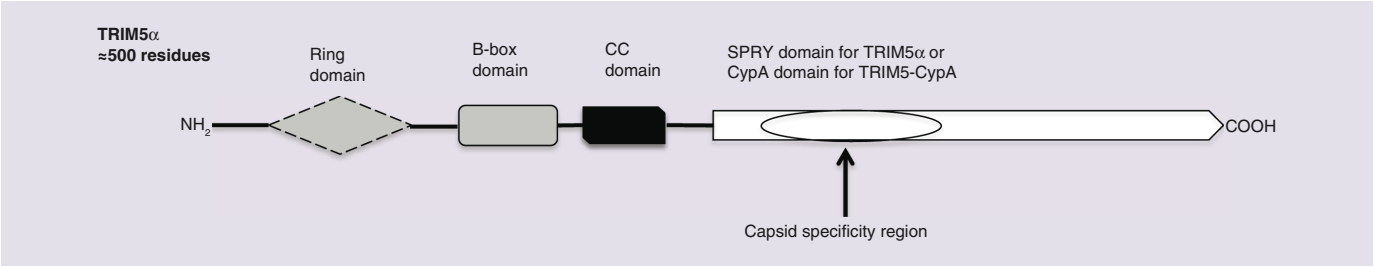

Figure 3. The structure of human TRIM5α with domains highlighted. Really Interesting New Gene (RING finger) domain mediates ubiquitylation, B-box and CC regulate the avidity of the TRIM5α protein for viral capsid through protein dimerization and higher order association, whereas the PRYSPRY/CypA domain is responsible for retroviral capsid binding.
CC: Coiled-coil.

protein, the residues that make this protein a powerful viral restrictor are located at the C-terminus. This domain is referred to as SPRY, PRYSPRY or B30.2 and can bind to the retroviral CA protein that builds the viral core (Figure 3). The SPRY domain is a hot spot of positive selection, insertions and deletions [25] and has evolutionarily been replaced by retrotransposition of cyclophilin (CypA) in some macaques and the New World primate owl monkey [26–28]. TRIM5α of the viral target cells can inhibit sensitive retroviruses by binding to the core during the early phase of infection in the cytoplasm. It forms a scaffold around the viral core causing enhanced and irregular uncoating thereby preventing successful reverse transcription [29–31]. In addition, TRIM5α is involved in virus sensing, which results in an antiviral innate immune response, and autophagy [32–35]. Since the discovery that TRIM5α proteins could restrict retroviruses [36], many other TRIM proteins have been studied for their potential role in antiviral immunity (Figure 4).

As highlighted in Table 1, the insensitivity of the capsid from SIVgsn, which is one of the precursors of SIVcpz, to chimpanzee TRIM5α provides clues as to the SIVcpz adaptation into its host chimpanzee. Furthermore, human TRIM5α is ineffective against the two types of SIVcpz (SIVcpzPtt and SIVcpzPts) and their precursor SIVgsn. This finding explains, in part, the human sensitivity to SIVcpz and how viral spillover to humans could have established a reservoir of virus-producing cells [37].

Inhibition of SIVs by TRIM proteins or other restriction factors have likely impaired the lentiviral movement among simians [38,39]. For instance, human TRIM5α is a poor restrictor for HIV-1, but HIV-1 is considerably

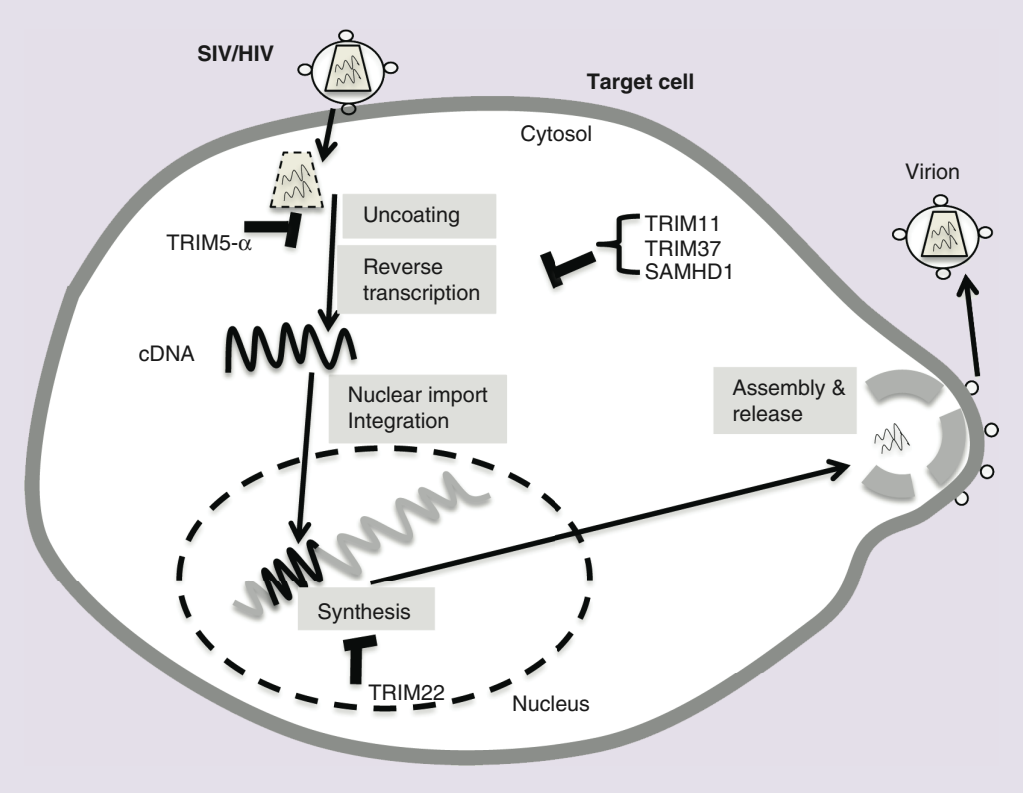

Figure 4. Restriction factors for SIV/HIV. TRIM5α promotes early uncoating to prevent successful reverse transcription. TRIM11 and TRIM37 impair reverse transcription. TRIM22 interferes with viral synthesis through targeting the LTR-driven transcription. Also shown in this figure is SAMHD1, which interferes with reverse transcription through decreasing the dNTP pool in the cytoplasm. SAMHD1 is not a member of the TRIM family of proteins.
LTR: Long terminal repeat.

Table 1. Restriction of HIV-1 and SIVs by simian TRIM5α proteins.

TRIM5α ortholog	HIV-1	SIVcpzPtt	SIVcpzPts	SIVmac	SIVagm	SIVgsn	SIVdeb
Human TRIM5α	-	-	-	-	-	-	-
CpzTRIM5α	-	-	-	-	-	-	-
GorTRIM5α	-	-	-	+	-	-	-
RhTRIM5α	+	+	+	-	+	+/-	+
AgmTRIM5α	+	+	-	-	-	+	-
PgtTRIM5α	-	-	-	-	-	-	-

Sensitivity of different retroviral capsids (from HIV, SIV from chimpanzees, SIV from rhesus macaques [SIVmac], African green monkey SIV [SIVagm], greater spot-nosed monkey [SIVgsn] and De Brazza's monkey [SIVdeb]) to primate TRIM5-α proteins. (-) indicates 'no effect' of TRIM5-α, (+) means TRIM5-α is antiviral [37].
agm: African green monkey; cpz: Chimpanzee; deb: De Brazza's monkey; gor: Gorilla; gsn: Greater spot-nosed monkey; pgt: Pigtailed macaques; rh: Rhesus macaques.

inhibited by rhesus monkey TRIM5α [36,40,41]. Since the viral capsid is the determinant of TRIM5α restriction, mutating the CA protein sequence by adaptation is a retroviral strategy to escape the barrier by antiviral TRIM5α orthologs [39,42].

Tetherin: a late acting restriction factor that is a repressor of SIVcpz in humans

In addition to targeting the early cellular stages of lentiviral infection, invaded cells also limit the release of newly formed virions as a way to contain the infection. Tetherin (or BST2), is a key cellular factor against virion release [43,44]. The *BST2* gene is interferon inducible and such genes have been extensively studied, particularly concerning restriction of retroviral replication [45,46].

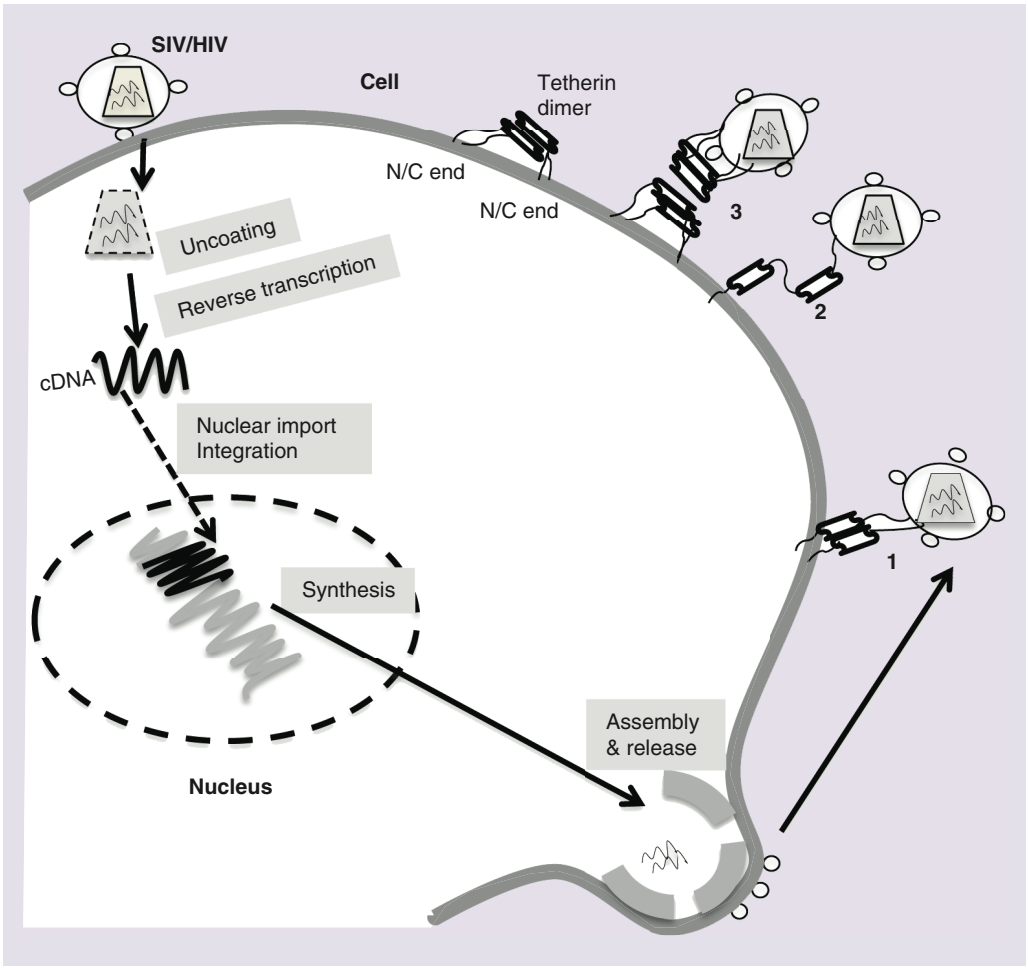

Figure 5. Tetherin and its suggested mechanisms of viral retention at the plasma membrane. Tetherin inhibits virus release if not counteracted by SIV/HIV proteins, for example, Vpu, Nef or Env. Of the three hypothesized mechanisms of viral to cell membrane retention by tetherin including **(1)** plasma membrane-attached dimeric tetherin inserting N- or C-termini into virions to oppose viral release, **(2)** only one monomer from a cell membrane dimeric tetherin binding to the virus to retain it at the cell surface or **(3)** non covalently interacting tetherin dimers (one in the virion envelope; the second in the plasma membrane) being used for virion-to-cell surface retention, scenario one **(1)** was found plausible.

Tetherin is an unusual membrane-associated protein with an N-terminal transmembrane domain and a C-terminal glycophosphatidylinositol lipid anchor [47,48]. However, the C-terminal region of BST2 may function as a second transmembrane domain as Andrew *et al.* suggested, opposing the glycophosphatidylinositol anchor model [49]. The mechanism by which tetherin blocks the release of enveloped viruses depends on both the N- and C-terminal domains, and disulfide bonding that is important for protein dimerization [50,51]; the impact of glycosylation is controversial [52]. Restricted lentiviral particles are tethered by tetherin to the plasma membrane of infected cells, which leads to endocytosis and lysosomal degradation of these virions, causing a net reduction in the number of released virions [43]. Tetherin acts in its dimeric form and while different possible configurations have been proposed, configuration one ((**1**) in Figure 5) was found to be most plausible [53].

To resist pressure from the host's cellular tetherin, SIVcpz and many other primate lentiviruses, including SIVmac, SIVsmm, and SIVgor, use their Nef proteins to remove tetherin from the cell surface [54–57]. The ability of Nef to oppose chimpanzee tetherin and to promote viral release varies among different SIVcpz strains [57]. Nef forms a physical contact with tetherin involving residues from its N-terminal globular core and flexible loop domains to cause a clathrin-mediated endocytosis of tetherin [58]. The Nef protein of SIVcpz does not bind human tetherin and it does not counteract human tetherin, because of evolutionary deletion events in the Nef binding site of human tetherin [56,57,59,60]. Hence, primate lentiviruses that were zoonotically transmitted to humans were forced to adapt

to the human tetherin protein to maintain high levels of replication. The cross-species transmissions of SIVcpz have resulted in the neo-functional adaptation of the Vpu protein and generated an adapted Nef protein [55,61]. Thus, HIV-1 utilizes its Vpu instead of Nef to inhibit human tetherin. Vpu is a phosphoprotein with a single transmembrane helix and a small cytoplasmic domain and is found only in HIV-1 and in its ancestor SIVs, including SIVcpz [62,63]. This anti-tetherin counteraction mechanism by Nef is not well understood but researches have highlighted cell surface tetherin-downregulation, proteasomal degradation but also retention at trans-Golgi network [64,65].

The *in vivo* replication of SIVcpz and HIV-1 and the impact to counteract human tetherin has been tested using mouse models transplanted with human blood cells (e.g., hu-BLT mice or NOG-hCD34 mice) in which the virus replicates only in the human cells [66–68]. In hu-BLT mice, SIVcpz strains are less or nonpathogenic compared to HIV-1 (group M), manifesting in lower degrees of $CD4^+$ T-cell depletion and immune activation; however, all tested SIVcpz strains replicated at high levels and had plasma viral load kinetics similar to HIV-1 in an observation period of 16 weeks [66,67]. These data suggest that SIVcpz is not restricted for spreading replication in human blood cells. To specifically address the impact of tetherin counteraction for SIVcpz *in vivo* replication, Sato *et al.* applied a different humanized mouse model (NOG-hCD34) [68–70]. In such *in vivo* experiments, HIV-1 Vpu was moderately associated with cytotoxicity, in which the wild-type (WT) virus caused a more severe decline of peripheral $CD4^+$ T cells than HIV-1Δ*vpu* while Vpu downregulated BST2 and was critically involved in early viral replication kinetics [69,70]. SIVcpz strain MB897, which is closely related to HIV-1 M, replicated *in vivo* more comparable to HIV-1 M than less related SIVcpz strains. Also, this strain showed increased replication when it was mutated in *vpu* to counteract human tetherin [68]. Using *in vivo* competition experiments, the SIVcpz with the gain-of-function *vpu* gene replicated more efficiently than the WT virus [68].

Similar experiments using SIVcpz strain EK505, which is related to the nonpandemic HIV-1 N, and a variant with altered Vpu residues that generated a Vpu that gained anti human tetherin activity showed, that the variant with the HIV-like Vpu competed out the WT virus, demonstrating an *in vivo* selection advantage of viral strains that evolved Vpu-mediated anti-tetherin activity [70].

Although HIV-1 M is a superior tetherin blocker, tetherin-antagonizing effects of other HIV-1 groups are variable. Poor adaptation to human tetherin may be one rreason for the limited spread of HIV-1 group N, O and P in the human population [56,71–74]. The HIV-1 group O tetherin blocking activity is strain-specific, with some O-type viruses being strong tetherin antagonists via Vpu [75] while others use their Nef to target a region adjacent to the human-specific deletion in tetherin to inhibit the transport of human tetherin to the cell surface [76]. Research on HIV-1 N has shown that Vpus are in a process of adaption and these proteins have acquired amino acid substitutions that allowed for interaction with human tetherin. However, these Vpus generally antagonize human tetherin only poorly [77] while HIV-1 P is unable to antagonize human tetherin by Vpu, Nef or Env [71].

HIV-2, the evolutionary progeny of SIVsmm, can also overcome human tetherin restriction. As the virus evolved as *vpu* negative, the anti tetherin activity of HIV-2 is determined by the viral envelope protein and this induces downregulation of BST2 and non-degradative sequestration into the trans Golgi network [78–81].

SERINC proteins allow virus release but impair infectivity

Virions that escape tetherin restriction face other challenges, including those from SERINC proteins [82,83]. These molecules were initially described as being involved in the synthesis of cell membrane lipids, phosphatidylserine and sphingolipids through incorporation of the hydrophilic amino acid serine [84], but this function has been questioned [85]. Among the SERINC family, antiviral activity has been identified in SERINC3 and SERINC5. This class of restricting factors affects lentiviral infectivity by incorporating into virions and impairing the next round of cell infection (Figure 6). Virions that have SERINC5 packaged into their envelope show a reduction in the virus–cell membrane fusion and are blocked to infect cells. By selectively inactivating sensitive viral glycoproteins, SERINC5 likely inhibits a step prior to the formation of the small fusion pores between HIV-1 and the target cell, which prevents the delivery of viral cores to the cytosol [82,83,86,87].

To bypass the SERINC restriction, primate lentiviruses use their multipurpose Nef protein and this mechanism is found across diverse SIVs and HIVs [82,83,88]. In addition to Nef, some HIV-1 Env glycoproteins contribute to viral resistance to SERINC5 [82,83,89]. Nef limits SERINC incorporation in newly formed virions by downregulation of SERINC5 from the cell surface via the endosome/lysosome system [90]. In addition, Nef can also antagonize the virion-incorporated SERINC [91]. Nef protein from HIVs and many SIVs including SIVcpz antagonize SERINC5 using a dileucine motif in their C-terminal loop; however, different mechanisms in other SIVs have

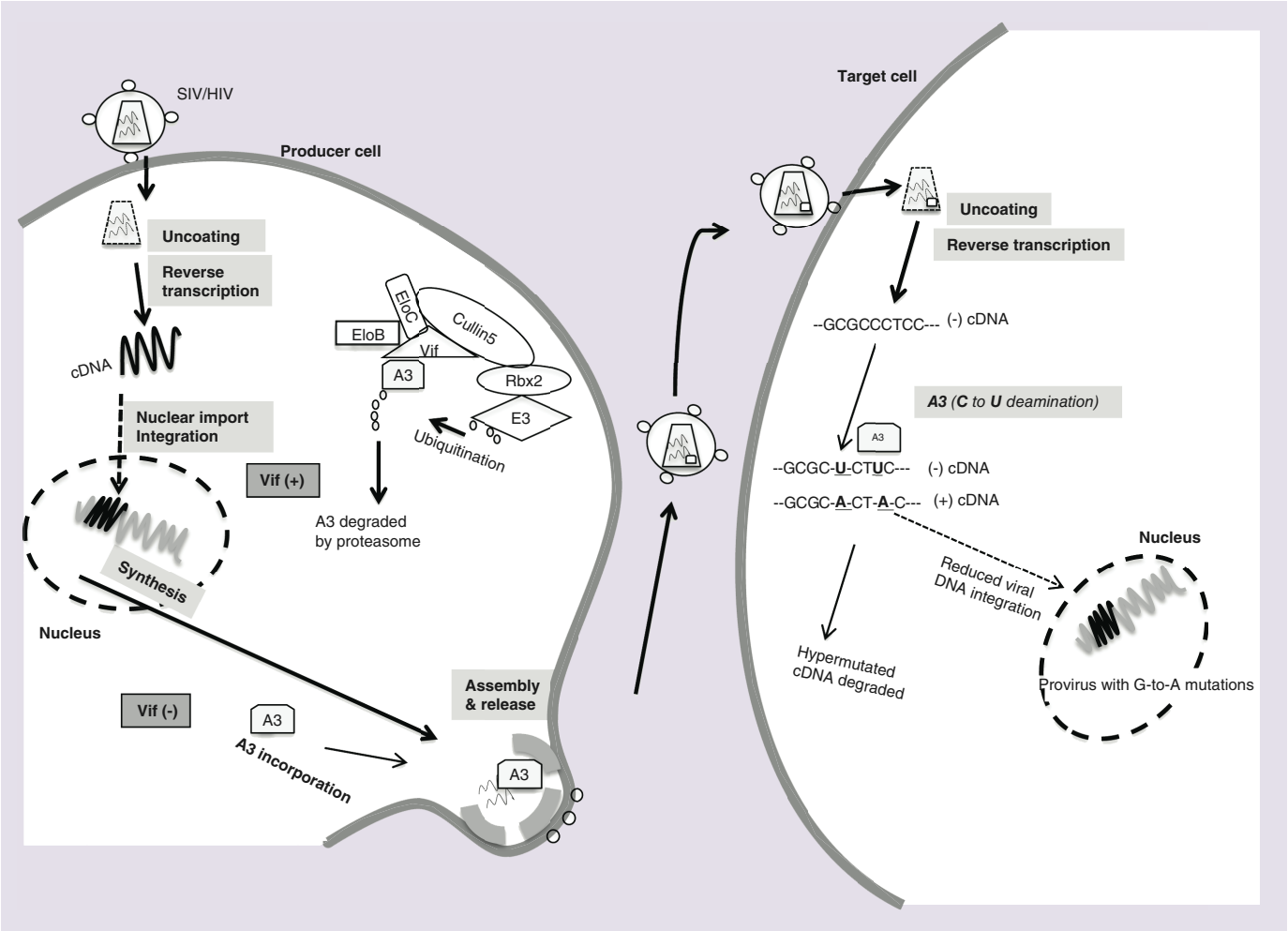

Figure 6. APOBEC3 mechanism of viralrestriction. During viral assembly, A3s areencapsidated in the virion. Following a new round of cell infection, A3 deaminate cytidine to uridine in the viral cDNA strand and these results into guanidine to adenine mutations in the positive strand of viral cDNA. Hypermutated viral DNAs are subject to enhanced degradation and form only reduced numbers of mutated proviruses. Primate lentiviruses prevent A3 encapsidation by using their Vif protein that binds A3 and mainly fates it for proteasomal degradation through a complex of many cellular proteins.

been suggested [88]. The Nefs of HIV-1 are more active against SERINC5 than HIV-2 Nefs, and Nefs of SIVcpz are more efficient than those of their monkey precursors [88]. Because the SIV prevalence correlates with the SERINC5 counteractivity by Nefs, it is speculated that the Nef-mediated SERINC5 antagonism impacts lentiviral spread [88]. Thus, human SERINC5 appears not to prevent SIVcpz transmission to humans.

APOBEC3: a deadly enzyme that the viral Vif can destroy

The APOBEC3 (A3) family of mammalian proteins contains functionally and structurally diverse members with innate antiviral activity. Humans encode 11 APOBECs, namely APOBEC1 (A1), AID, APOBEC2 (A2), APOBEC3A (A3A), A3B, A3C, A3D, A3F, A3G, A3H and APOBEC4 (A4) (Table 2) [92–95]. In contrast to A3s, A4 enhances HIV-1 infection [96]. Common in the structure of these proteins is at least one zinc-binding catalytic domain with conserved residues in the form of H-x-E-x_{23-28}-P-C-x_{2-4}-C (x for any amino acid).

Cytidine deamination of single-stranded polynucleotides is the key function of A3 proteins, AID and A1. A3 mediates a cytosine-to-uridine edit of single-stranded DNA during the reverse transcription of retroviral RNA genomes [92,100–107]. Lentiviral replication can be severely restricted by A3 proteins once they are encapsidated into the nascent viral particle (Figure 7). However, not all human A3s inhibit HIV-1 and SIVcpz and only A3D, A3F, A3G and A3H are antiviral in T cells and macrophages [97,100,108]. The incorporated A3 proteins survive in the viral core and find their substrate once the virus enters a new cell and starts reverse transcribing its viral RNA

Table 2. Anti SIVcpz activity of human APOBEC proteins.

APOBEC	Genomic locations	Protein size (residues)	Zinc/catalytic domains	Associated function	Antiviral against SIVcpzΔ *vif*	Antiviral against SIVcpz	Ref.
AID (AICDA)	Chr. 12	198	1	Immunoglobulin diversity	Not tested	Not tested	
APOBEC1	Chr. 12	229	1	Host mRNA editing	Not tested	Not tested	
APOBEC2	Chr. 6	224	1	Muscle development	Not tested	Not tested	
APOBEC3A	Chr. 22	199	1	Innate immunity	No	No	[97]
APOBEC3B	Chr. 22	362	2		Yes	Yes	[97]
APOBEC3C	Chr. 22	190	1		No	No	[97]
APOBEC3D	Chr. 22	368	2		Yes	No	[97]
APOBEC3F	Chr. 22	373	2		Yes	No	[68,97]
APOBEC3G	Chr. 22	384	2		Yes	No	[68,97–99]
APOBEC3H	Chr. 22	182	1		Yes	Yes	[97,99]
APOBEC4	Chr. 1	367	1	Enhancer of retroviral infection	Not tested	Not tested	

Chr: Chromosome

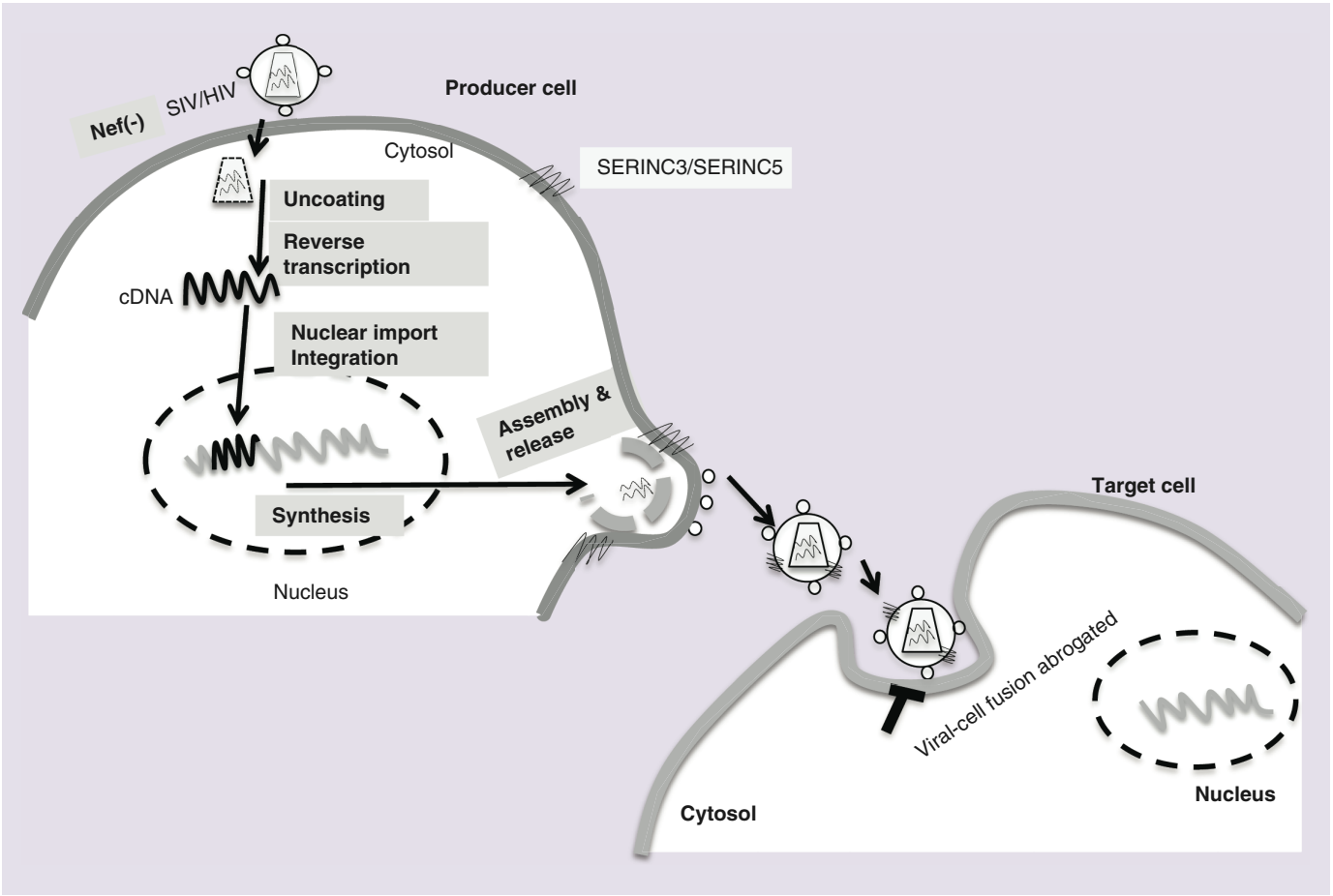

Figure 7. Restriction of viral replicationby SERINC3/5 proteins. In *nef* negative viruses, thecell proteins SERINC3 or SERINC5 will incorporate into newly formed virions into the viral envelope. In the next round of infection, viral particle incorporated SERINCs will block virus–cell membrane fusion, affecting viral entry. Nef counteracts the SERINC incorporation.

into single-stranded DNA. The virus-associated A3 proteins deaminate the deoxycytosines to deoxyuridines and cause hypermutation. Cytidine deamination in viral single-stranded DNA causes G-to-A nucleotide changes in the coding viral plus-strand DNA. Hypermutated viral DNAs are damaged and directed toward degradation and rarely integrate, and thus the A3-mediated mutations stop the replication process [100,109,110]. However, sublethal levels of editing might help the virus to diversify its genetic makeup, giving rise to resistant viral mutants. In addition, deamination-independent mechanisms to inhibit the virus exist in which binding of A3 to the viral genomic RNA, tRNA, reverse transcriptase or integrase is involved [111–123].

Lentiviruses prevent the lethal activity of host A3 proteins by their viral Vif protein that mediates the inactivation of A3s mainly by inducing proteasomal degradation of the A3s [124–130]; however, nondegrading mechanisms that sequester A3s away from budding virions were also observed [131,132]. Primate lentiviral Vifs bind species-own A3 proteins and attach them to an E3 ubiquitin ligase complex by interacting with the cellular proteins Cullin5, ElonginB, ElonginC, RBX2 and CBF-β [100,109,111,126,130,133–135]. The Vif-E3 complex adds poly-ubiquitin to the bound A3, which causes its degradation by the cellular proteasome. Thereby, Vif depletes A3 proteins from the virus-producing cell to generate virions without co-packaged A3s. All primate lentiviruses form a similar E3 ubiquitin ligase complex [130,136]; however, Vifs of non-primate lentiviruses evolved to hijack other host factors for their E3 complexes [137,138]. There is evidence that Vif is not the only way lentiviruses use to prevent inhibition by A3s, as HIV-2 and its ancestor SIVsmm can use Vpx to at least partially protect themselves against the antiviral A3A [139,140] and alternative mechanisms against A3G involving increased virus production have been suggested [141–143].

Although cognate A3s are sufficiently counteracted by SIVs, non-host A3s may be a potential barrier for cross-species transmissions. A3 proteins are under positive selection, as expected if involved in the virus arms race, a battle that has driven a dramatic diversification of viruses and host immune factors [94,144–148]. Primate species that are closely related to each other have A3 proteins that show less interspecies diversity than A3s of simian species in which the last common ancestor is older [94,149].

Vif proteins are adapted to bind a specific patch on the surface of an A3. HIV-1 Vif fails to counteract A3G of African green monkey, because Vif does not bind to this simian A3G [150–152]. Human A3F and A3G, however, are sensitive to SIVcpz and SIVgor Vifs [22,68,98]. Interestingly, gorilla A3G is resistant to SIVcpz Vif providing a possible explanation for why SIVcpz has not crossed between chimpanzees and gorillas more often [9]. However, a currently unidentified A3G polymorphism in gorilla could render certain gorilla individuals more susceptible to transmissions of SIVcpz, thus allowing the virus to establish infections in some gorillas [98]. Thus, variability in viral proteins and host gene polymorphisms are key factors that impact the fate of the virus after spillover. In humans, the variability in the A3 proteins is rather limited and only A3H has been identified as being polymorphic [153–157]. A3H has seven haplotypes and additional splice-variants. Only the *A3H* genes of haplotype II, V and VII form stable amounts of protein while the other haplotype A3H proteins are very low or undetectably expressed. SIVcpz Vif can counteract chimpanzee A3s and most human A3s [97]. The exception is human A3H, which is resistant to all tested Vifs from SIVcpz and SIVgor [97]. SIVcpzPtt Vif requires a minimum of two changed residues to gain the function to inhibit human A3H. In Africa, around 50% of people carry A3H genes, which encode stable A3H proteins while outside Africa the stable A3H haplotypes are minor alleles [153,156,157]. Thus, human A3H forms a strong barrier against SIVcpz cross-species transmission and individuals who lack A3H protein expression were likely the first humans to spread the SIVcpz zoonosis [97].

GTPases: halting the retroviral invasion

Interferon-inducible molecules that belong to the GTPases family [158] encompass four subfamilies including guanylate-binding proteins (GBPs) [159], immunity-related GTPases [160], very large inducible GTPases [161] and myxovirus resistance (MX) proteins [162]. GBPs have antiviral activity among other immune functions [163]. Of the seven human genome-encoded GBPs [164], GBP1 and GBP5 are the most studied antiviral proteins of this group of proteins. GBP5 has antiretroviral activity, as highlighted by its effects on HIV-1 and SIVs by affecting the processing and virion incorporation of the viral envelope protein during the formation of new virions [165]. Naturally occurring mutations in the start codon of the *vpu* gene that enhance Env expression might help HIV-1 to attenuate the effects of GBP5 at the cost of increased susceptibility to tetherin [165,166]. Currently, it is unknown whether human GBP5 also decreases the infectivity of SIVcpz [70]. In the group of MX proteins, MXB (MX2) proteins of human and nonhuman origin have also been shown to be antiretroviral, and to inhibit retroviral replication through targeting uncoating, nuclear entry and nuclear events [167–171]. SIVcpz shows sensitivity to human MXB similar to that of HIV-1 with a trend that the HIV-1 M subtype and SIVcpzPtt are slightly more inhibited than SIVcpzPts [172].

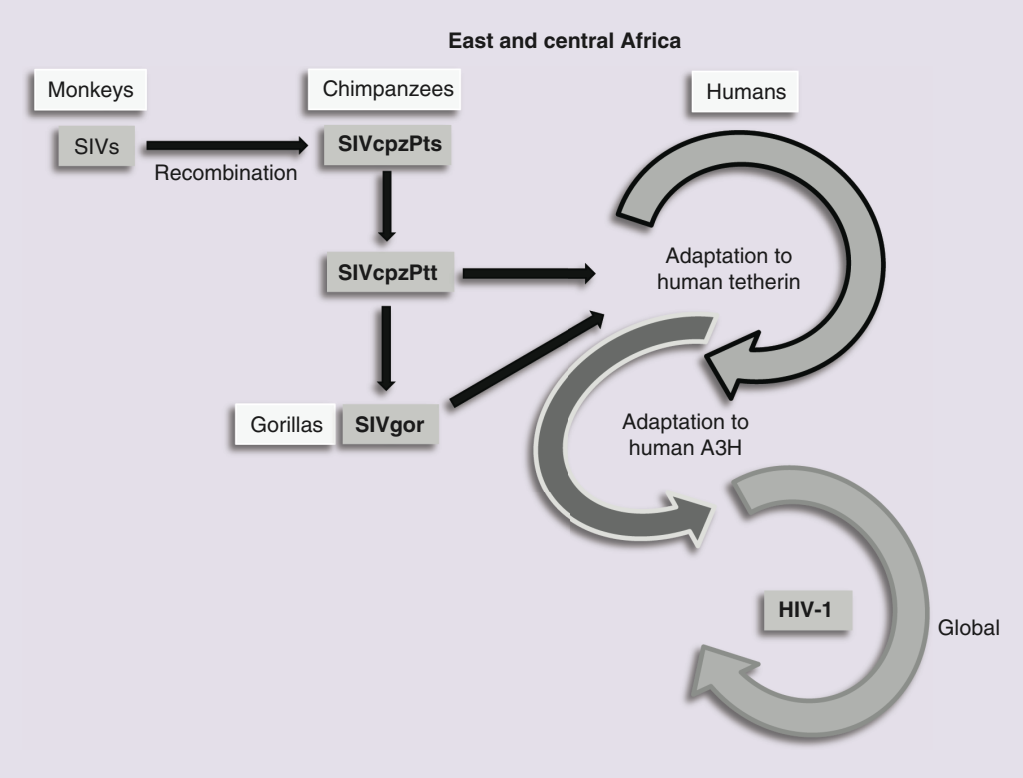

Figure 8. A model of SIVcpz's evolutionary path to HIV. SIVcpz to human cross-species transmission likely happened 100 years ago in central Africa. After spillover, SIV adapted to human cells. For high-level replication, SIVcpz had to adapt to human tetherin and evolve a Vif protein able to counteract human A3H. Only viruses that were able to adapt to these and possible other host-factors were able to spread globally.

SAMHD1: an important restriction for SIVcpz/HIV-1?

SAMHD1 is an IFNγ-inducible deoxynucleoside triphosphate triphosphohydrolase enzyme that inhibits reverse transcription of retroviruses by suppressing cellular dNTP pools [173–175]. SAMHD1 inhibits HIV-1 replication in non-dividing cells that have low dNTP pools, such as resting T-cells, macrophages and dendritic cells [176–178]. SAMHD1 is an important player in the replication stress response and promotes degradation of nascent DNA at stalled replication forks in human cell lines by stimulating the exonuclease activity of MRE11 [179]. In SAMHD1-depleted cells, single-stranded DNA fragments are released from stalled forks and accumulate in the cytosol, where they activate the cGAS–STING pathway to induce expression of pro-inflammatory type I interferons [179].

Some lentiviruses, such as HIV-2 or its ancestor SIVsmm, have evolved a protein called Vpx, which is capable of degrading SAMHD1. This degradation is often host specific, which indicates that SAMHD1 represents a barrier for cross-species transmissions [180]. Vpx induces proteasomal degradation of SAMHD1 by recruiting an ubiquitin CRL4-DCAF1-E3-ligase complex [176,177]. Other primate lentiviruses encode a paralogous gene *vpr*, but not all of the Vpr proteins are capable of binding and antagonizing SAMHD1 and Vprs that can degrade SAMHD1 are phylogenetically closer to Vpx than those that are incapable of inducing the SAMHD1 degradation [180]. The Vprs of HIV-1/SIVcpz do not counteract SAMHD1 [180]. Interestingly, SIVrcm, one of the ancestors of SIVcpz carries a gene for Vpx that was lost during the transmission to chimpanzees (Figure 2) [22]. SIVrcm Vpx is capable of degrading SAMHD1 of several simian species (rhesus, De Brazza's, African green monkeys, mandrills and its natural host red-capped mangabeys), but is unable to degrade SAMHD1 from humans or sooty mangabeys [180].

In the genome of SIVrcm, the *vpx* gene is located between *vif* and *vpr*, with overlap of the vpx 5′ end with the 3′ end of *vif* (Figure 2). During the transmission from red-capped mangabey to chimpanzees, the *vpx* gene was lost and *vif* was generated by partial overprinting with the 5′ end of *vpr* [22]. Overprinting refers to a type of overlap in which all or part of the sequence of one gene is read in an alternate reading frame from another gene at the same locus. It was concluded that the gain of function of Vif is more important than the Vpx function, suggesting that A3s form a stronger species barrier than SAMHD1 [22]. Why some primate lentiviruses kept the

SAMHD1-degradation function and others, for example, SIVcpz/HIV-1, evolved to replicate without SAMHD1 degradation is unknown. Alternative mechanisms to Vpx may involve adaptations in the reverse transcriptases to execute faster nucleotide incorporation (k_{pol} step), to partially balance the limited dNTP concentrations found in some non-dividing cells [181]. Thus, it is likely that primate lentiviruses evolved multiple strategies to prevent extinction by the host innate immune system to form proviruses in resting cells to keep a reservoir of latently infected cells.

Conclusion & future perspective

HIV-1 M and HIV-1 O strains form the pandemic or endemic HIV-1s. It is believed that each of these human viruses originated from a single successful spillover from chimpanzee (for HIV-1 M) or gorilla (for HIV-1 O) (Figures 1 & 8). Based on the evolutionary relationship of all three species, in retrospect it is not a surprise that human lentiviruses could evolve after zoonotic infections. It is more surprising that it happened efficiently only twice and two more times with less efficiency (for HIV-1 N and P). This implicates a level of cellular differences in dependency and restriction factors that form a barrier to viral spread after spillover (Figure 8). Hence, we predict the existence of unknown factors that protect humans from lentivirus infections. Indications that SIVcpz is forced to adapt to other cellular co-factors to maintain a high level of replication in human cells have been reported [67,68,182]. Since there is an ongoing risk for zoonotic transmissions of other SIVs, regular SIV screenings by PCR using generic HIV/SIV primers and antibody responses of bush-meat hunters and butchers in Africa

Executive summary

Background
- Chimpanzees acquired their SIVs from monkeys.
- In a direct transmission to humans, chimpanzees SIV (SIVcpz) evolved into HIV-1 M and HIV-1 N.
- SIVcpz also invaded gorillas, SIVgor developed.
- SIVgor spread to humans and evolved into HIV-1 O and HIV-1 P.
- HIV-2 is an evolutionary offspring of SIV from sooty mangabey.

Chimpanzee SIV: a result of lentiviral recombination
- SIVcpz is a result of genetic recombination between SIVs from more than two primates.

Human TRIM5α does not inhibit SIVcpz
- The antiretroviral activity of TRIM5α proteins is virus-specific among primates (e.g., human TRIM5α does not inhibit SIVcpz).
- TRIM5α is not the only antiretroviral protein in the TRIM protein family. Other TRIM proteins protect against retroviruses as well.

Tetherin: a late acting restriction factor that is a repressor of SIVcpz in humans
- Despite the viral escape from early phase restriction factors, cells can further target viral release using BST2 (also called tetherin).
- Retroviruses use their anti tetherin factors like Vpu or Nef proteins to ensure virion release.
- SIVcpz Nef counteracts chimpanzee tetherin but is inactive against human tetherin.
- During adaptation to humans, SIVcpz evolved its Vpu protein to target human tetherin.

SERINC proteins allow virus release but impair infectivity
- SERINC3 and SERINC5 can incorporate into virions to prevent the formation the of membrane fusion between a lentivirus and its target cell during next round of infection.
- SIVs/HIVs overcome SERINCs using their Nef protein.
- Human SERINCs are not inhibitors of SIVcpz infections.

APOBEC3: a deadly enzyme that the viral Vif can destroy
- Several APOBEC3 (A3) cytidine deaminases exist with antiretroviral activity following encapsidation into virions.
- Lentiviruses oppose A3 antiviral pathway using their Vif proteins, which target A3s for proteasomal degradation.
- Human A3H is a strong restriction factor of SIVcpz, which forms a barrier for zoonotic transmissions.

GTPases: halting the retroviral invasion
- The GTPase GBP5 interferes with processing and virion incorporation of envelope protein.
- MX proteins block lentiviral replication at different steps including uncoating, nuclear entry and beyond.
- Human GTPases show similar antiviral activities against HIV-1 and SIVcpz.

SAMHD1: an important restriction for SIVcpz/HIV-1?
- SAMHD1 depletes nucleotides and thereby represses reverse transcription of SIV/HIV RNA in resting cells.
- HIV-2 and related SIVs use their Vpx to degrade and remove SAMHD1 early during infection.
- HIV-1 and SIVcpz are not able to counteract SAMHD1.

may be important to monitor the formation of new human lentiviruses. SIVs in most African primates evolved into apathogenic viruses. The future perspective of SIV pathogenicity in chimpanzees is unclear [20,183,184] and some animals with AIDS-like symptoms have been described [185–187]. The impact of SIV infections on the health of gorillas is unknown. In case SIVcpz and SIVgor evolve to more aggressive, HIV-1-like, viruses, measurements beyond pharmaceutical antiretroviral therapy are needed to preserve these already endangered species.

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References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Bibollet-Ruche F, Galat-Luong A, Cuny G *et al.* Simian immunodeficiency virus infection in a patas monkey (*Erythrocebus patas*): evidence for cross-species transmission from African green monkeys (*Cercopithecus aethiops sabaeus*) in the wild. *J. Gen. Virol.* 77(Pt 4), 773–781 (1996).
2. Bell SM, Bedford T. Modern-day SIV viral diversity generated by extensive recombination and cross-species transmission. *PLoS Pathog.* 13(7), e1006466 (2017).

•• **This paper brings new insights on the origin of SIVcpz as well as cross-species transmission of SIVs among primates.**

3. Korber B, Muldoon M, Theiler J *et al.* Timing the ancestor of the HIV-1 pandemic strains. *Science* 288(5472), 1789–1796 (2000).
4. Sharp PM, Hahn BH. The evolution of HIV-1 and the origin of AIDS. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 365(1552), 2487–2494 (2010).
5. Gao F, Bailes E, Robertson DL *et al.* Origin of HIV-1 in the chimpanzee *Pan troglodytes troglodytes*. *Nature* 397(6718), 436–441 (1999).

•• **Identifies chimpanzee *Pan troglodytes troglodytes* as the primary reservoir for HIV-1.**

6. Keele BF, Van Heuverswyn F, Li Y *et al.* Chimpanzee reservoirs of pandemic and nonpandemic HIV-1. *Science* 313(5786), 523–526 (2006).
7. Takehisa J, Kraus MH, Ayouba A *et al.* Origin and biology of simian immunodeficiency virus in wild-living western gorillas. *J. Virol.* 83(4), 1635–1648 (2009).
8. Van Heuverswyn F, Li Y, Neel C *et al.* Human immunodeficiency viruses: SIV infection in wild gorillas. *Nature* 444(7116), 164 (2006).
9. D'Arc M, Ayouba A, Esteban A *et al.* Origin of the HIV-1 group O epidemic in western lowland gorillas. *Proc. Natl Acad. Sci. USA* 112(11), E1343–E1352 (2015).
10. Hirsch VM, Olmsted RA, Murphey-Corb M, Purcell RH, Johnson PR. An African primate lentivirus (SIVsm) closely related to HIV-2. *Nature* 339(6223), 389–392 (1989).
11. Gao F, Yue L, White AT *et al.* Human infection by genetically diverse SIVSM-related HIV-2 in west Africa. *Nature* 358(6386), 495–499 (1992).
12. Chen Z, Telfier P, Gettie A *et al.* Genetic characterization of new west African simian immunodeficiency virus SIVsm: geographic clustering of household-derived SIV strains with human immunodeficiency virus type 2 subtypes and genetically diverse viruses from a single feral sooty mangabey troop. *J. Virol.* 70(6), 3617–3627 (1996).
13. Worobey M, Gemmel M, Teuwen DE *et al.* Direct evidence of extensive diversity of HIV-1 in Kinshasa by 1960. *Nature* 455(7213), 661–664 (2008).
14. Wertheim JO, Worobey M. Dating the age of the SIV lineages that gave rise to HIV-1 and HIV-2. *PLoS Comput. Biol.* 5(5), e1000377 (2009).
15. de Sousa JD, Muller V, Lemey P, Vandamme AM. High GUD incidence in the early 20 century created a particularly permissive time window for the origin and initial spread of epidemic HIV strains. *PLoS ONE* 5(4), e9936 (2010).
16. Huet T, Cheynier R, Meyerhans A, Roelants G, Wain-Hobson S. Genetic organization of a chimpanzee lentivirus related to HIV-1. *Nature* 345(6273), 356–359 (1990).

17. Peeters M, Fransen K, Delaporte E *et al.* Isolation and characterization of a new chimpanzee lentivirus (simian immunodeficiency virus isolate cpz-ant) from a wild-captured chimpanzee. *AIDS* 6(5), 447–451 (1992).
18. Peeters M, Honore C, Huet T *et al.* Isolation and partial characterization of an HIV-related virus occurring naturally in chimpanzees in Gabon. *AIDS* 3(10), 625–630 (1989).
19. Santiago ML, Rodenburg CM, Kamenya S *et al.* SIVcpz in wild chimpanzees. *Science* 295(5554), 465 (2002).
20. Greenwood EJ, Schmidt F, Kondova I *et al.* Simian immunodeficiency virus infection of chimpanzees (*Pan troglodytes*) shares features of both pathogenic and non-pathogenic lentiviral infections. *PLoS Pathog.* 11(9), e1005146 (2015).
21. Courgnaud V, Salemi M, Pourrut X *et al.* Characterization of a novel simian immunodeficiency virus with a vpu gene from greater spot-nosed monkeys (*Cercopithecus nictitans*) provides new insights into simian/human immunodeficiency virus phylogeny. *J. Virol.* 76(16), 8298–8309 (2002).
22. Etienne L, Hahn BH, Sharp PM, Matsen FA, Emerman M. Gene loss and adaptation to hominids underlie the ancient origin of HIV-1. *Cell Host Microbe* 14(1), 85–92 (2013).

•• **This paper brings new insights on the origin of HIV-1 and shows that vpx, which in other lentiviruses antagonizes SAMHD1, was deleted during the creation of SIVcpz.**

23. Bailes E, Gao F, Bibollet-Ruche F *et al.* Hybrid origin of SIV in chimpanzees. *Science* 300(5626), 1713 (2003).
24. Reymond A, Meroni G, Fantozzi A *et al.* The tripartite motif family identifies cell compartments. *EMBO J.* 20(9), 2140–2151 (2001).
25. Sawyer SL, Wu LI, Emerman M, Malik HS. Positive selection of primate TRIM5alpha identifies a critical species-specific retroviral restriction domain. *Proc. Natl Acad. Sci. USA* 102(8), 2832–2837 (2005).
26. Nisole S, Lynch C, Stoye JP, Yap MW. A Trim5-cyclophilin A fusion protein found in owl monkey kidney cells can restrict HIV-1. *Proc. Natl Acad. Sci. USA* 101(36), 13324–13328 (2004).
27. Sayah DM, Sokolskaja E, Berthoux L, Luban J. Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1. *Nature* 430(6999), 569–573 (2004).
28. Wilson SJ, Webb BL, Ylinen LM, Verschoor E, Heeney JL, Towers GJ. Independent evolution of an antiviral TRIMCyp in rhesus macaques. *Proc. Natl Acad. Sci. USA* 105(9), 3557–3562 (2008).
29. Sastri J, Campbell EM. Recent insights into the mechanism and consequences of TRIM5alpha retroviral restriction. *AIDS Res. Hum. Retroviruses* 27(3), 231–238 (2011).
30. Sanz-Ramos M, Stoye JP. Capsid-binding retrovirus restriction factors: discovery, restriction specificity and implications for the development of novel therapeutics. *J. Gen. Virol.* 94(Pt 12), 2587–2598 (2013).
31. Nakayama EE, Shioda T. Impact of TRIM5alpha *in vivo*. *AIDS* 29(14), 1733–1743 (2015).
32. Pertel T, Hausmann S, Morger D *et al.* TRIM5 is an innate immune sensor for the retrovirus capsid lattice. *Nature* 472(7343), 361–365 (2011).
33. Mandell MA, Jain A, Arko-Mensah J *et al.* TRIM proteins regulate autophagy and can target autophagic substrates by direct recognition. *Dev. Cell.* 30(4), 394–409 (2014).
34. Mandell MA, Kimura T, Jain A, Johansen T, Deretic V. TRIM proteins regulate autophagy: TRIM5 is a selective autophagy receptor mediating HIV-1 restriction. *Autophagy* 10(12), 2387–2388 (2014).
35. Sparrer KMJ, Gack MU. TRIM proteins: new players in virus-induced autophagy. *PLoS Pathog.* 14(2), e1006787 (2018).
36. Stremlau M, Owens CM, Perron MJ, Kiessling M, Autissier P, Sodroski J. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature* 427(6977), 848–853 (2004).
37. Kratovac Z, Virgen CA, Bibollet-Ruche F, Hahn BH, Bieniasz PD, Hatziioannou T. Primate lentivirus capsid sensitivity to TRIM5 proteins. *J. Virol.* 82(13), 6772–6777 (2008).

• **This work describes differential sensitivity of SIVcpz capsids to TRIM5α proteins.**

38. Kirmaier A, Wu F, Newman RM *et al.* TRIM5 suppresses cross-species transmission of a primate immunodeficiency virus and selects for emergence of resistant variants in the new species. *PLoS Biol.* 8(8), e1000462 (2010).
39. Wu F, Kirmaier A, Goeken R *et al.* TRIM5 alpha drives SIVsmm evolution in rhesus macaques. *PLoS Pathog.* 9(8), e1003577 (2013).
40. Yap MW, Nisole S, Lynch C, Stoye JP. Trim5alpha protein restricts both HIV-1 and murine leukemia virus. *Proc. Natl Acad. Sci. USA* 101(29), 10786–10791 (2004).
41. Hatziioannou T, Perez-Caballero D, Yang A, Cowan S, Bieniasz PD. Retrovirus resistance factors Ref1 and Lv1 are species-specific variants of TRIM5alpha. *Proc. Natl. Acad. Sci. USA* 101(29), 10774–10779 (2004).
42. Wu F, Kirmaier A, White E *et al.* TRIM5alpha resistance escape mutations in the capsid are transferable between simian immunodeficiency virus strains. *J. Virol.* 90(24), 11087–11095 (2016).
43. Neil SJ, Zang T, Bieniasz PD. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature* 451(7177), 425–430 (2008).
44. Van Damme N, Goff D, Katsura C *et al.* 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(4), 245–252 (2008).

45. Kane M, Zang TM, Rihn SJ *et al.* Identification of interferon-stimulated genes with antiretroviral activity. *Cell Host Microbe* 20(3), 392–405 (2016).
46. Schoggins JW, Wilson SJ, Panis M *et al.* A diverse range of gene products are effectors of the type I interferon antiviral response. *Nature* 472(7344), 481–485 (2011).
47. Kupzig S, Korolchuk V, Rollason R, Sugden A, Wilde A, Banting G. Bst-2/HM1.24 is a raft-associated apical membrane protein with an unusual topology. *Traffic* 4(10), 694–709 (2003).
48. Billcliff PG, Gorleku OA, Chamberlain LH, Banting G. The cytosolic N-terminus of CD317/tetherin is a membrane microdomain exclusion motif. *Biol. Open* 2(11), 1253–1263 (2013).
49. Andrew AJ, Kao S, Strebel K. The C-terminal hydrophobic region in human BST-2/tetherin functions as a second transmembrane motif. *J. Biol. Chem.* 286 (46), 39967–39981 (2011).
50. Perez-Caballero D, Zang T, Ebrahimi A *et al.* Tetherin inhibits HIV-1 release by directly tethering virions to cells. *Cell* 139(3), 499–511 (2009).
51. Schubert HL, Zhai Q, Sandrin V *et al.* Structural and functional studies on the extracellular domain of BST2/tetherin in reduced and oxidized conformations. *Proc. Natl Acad. Sci. USA* 107(42), 17951–17956 (2010).
52. Andrew AJ, Miyagi E, Kao S, Strebel K. The formation of cysteine-linked dimers of BST-2/tetherin is important for inhibition of HIV-1 virus release but not for sensitivity to Vpu. *Retrovirology* 6, 80 (2009).
53. Venkatesh S, Bieniasz PD. Mechanism of HIV-1 virion entrapment by tetherin. *PLoS Pathog.* 9(7), e1003483 (2013).
54. Zhang F, Wilson SJ, Landford WC *et al.* Nef proteins from simian immunodeficiency viruses are tetherin antagonists. *Cell Host Microbe* 6(1), 54–67 (2009).
55. Sauter D, Specht A, Kirchhoff F. Tetherin: holding on and letting go. *Cell.* 141(3), 392–398 (2010).
56. Sauter D, Schindler M, Specht A *et al.* Tetherin-driven adaptation of Vpu and Nef function and the evolution of pandemic and nonpandemic HIV-1 strains. *Cell Host Microbe* 6(5), 409–421 (2009).

•• **Demonstrates that SIVcpz uses Nef rather than Vpu to counteract chimpanzee tetherin and that human tetherin is resistant to SIVcpz Nef and Vpu.**

57. Lim ES, Malik HS, Emerman M. Ancient adaptive evolution of tetherin shaped the functions of Vpu and Nef in human immunodeficiency virus and primate lentiviruses. *J. Virol.* 84(14), 7124–7134 (2010).
58. Serra-Moreno R, Zimmermann K, Stern LJ, Evans DT. Tetherin/BST-2 antagonism by Nef depends on a direct physical interaction between Nef and tetherin, and on clathrin-mediated endocytosis. *PLoS Pathog.* 9(7), e1003487 (2013).
59. Yang SJ, Lopez LA, Hauser H, Exline CM, Haworth KG, Cannon PM. Anti-tetherin activities in Vpu-expressing primate lentiviruses. *Retrovirology* 7, 13 (2010).
60. Sauter D, Vogl M, Kirchhoff F. Ancient origin of a deletion in human BST2/Tetherin that confers protection against viral zoonoses. *Hum. Mutat.* 32(11), 1243–1245 (2011).
61. Gotz N, Sauter D, Usmani SM *et al.* Reacquisition of Nef-mediated tetherin antagonism in a single *in vivo* passage of HIV-1 through its original chimpanzee host. *Cell Host Microbe* 12(3), 373–380 (2012).
62. Göttlinger HG, Dorfman T, Cohen EA, Haseltine WA. Vpu protein of human immunodeficiency virus type 1 enhances the release of capsids produced by *gag* gene constructs of widely divergent retroviruses. *Proc. Natl Acad. Sci. USA* 90(15), 7381–7385 (1993).
63. Greiner T, Bolduan S, Hertel B *et al.* Ion channel activity of Vpu proteins is conserved throughout evolution of HIV-1 and SIV. *Viruses* 8(12), 325 (2016).
64. Douglas JL, Viswanathan K, McCarroll MN, Gustin JK, Fruh K, Moses AV. Vpu directs the degradation of the human immunodeficiency virus restriction factor BST-2/tetherin via a {beta}TrCP-dependent mechanism. *J. Virol.* 83(16), 7931–7947 (2009).
65. Caillet M, Janvier K, Pelchen-Matthews A *et al.* Rab7A is required for efficient production of infectious HIV-1. *PLoS Pathog.* 7(11), e1002347 (2011).
66. Yuan Z, Kang G, Daharsh L, Fan W, Li Q. SIVcpz closely related to the ancestral HIV-1 is less or non-pathogenic to humans in a hu-BLT mouse model. *Emerg. Microbes Infect.* 7(1), 59 (2018).
67. Yuan Z, Kang G, Ma F *et al.* Recapitulating cross-species transmission of simian immunodeficiency virus SIVcpz to humans by using humanized BLT mice. *J. Virol.* 90(17), 7728–7739 (2016).
68. Sato K, Misawa N, Takeuchi JS *et al.* experimental adaptive evolution of simian immunodeficiency virus SIVcpz to pandemic human immunodeficiency virus type 1 by using a humanized mouse model. *J. Virol.* 92(4), e01905–e01917 (2018).
69. Sato K, Misawa N, Fukuhara M *et al.* Vpu augments the initial burst phase of HIV-1 propagation and downregulates BST2 and CD4 in humanized mice. *J. Virol.* 86(9), 5000–5013 (2012).
70. Yamada E, Nakaoka S, Klein L *et al.* Human-specific adaptations in Vpu conferring anti-tetherin activity are critical for efficient early HIV-1 replication *in vivo*. *Cell Host Microbe* 23(1), 110–120; e117 (2018).

• **Study uses a humanized mouse model and HIV-1 and SIVcpz strains with selective mutations in Vpu to demonstrate that the anti tetherin activity of Vpu is a prerequisite for efficient viral spread during the early phase of infection.**

71. Sauter D, Hue S, Petit SJ *et al.* HIV-1 Group P is unable to antagonize human tetherin by Vpu, Env or Nef. *Retrovirology* 8, 103 (2011).
72. Petit SJ, Blondeau C, Towers GJ. Analysis of the human immunodeficiency virus type 1 M group Vpu domains involved in antagonizing tetherin. *J. Gen. Virol.* 92(Pt 12), 2937–2948 (2011).
73. Vigan R, Neil SJ. Separable determinants of subcellular localization and interaction account for the inability of group O HIV-1 Vpu to counteract tetherin. *J. Virol.* 85(19), 9737–9748 (2011).
74. Yang SJ, Lopez LA, Exline CM, Haworth KG, Cannon PM. Lack of adaptation to human tetherin in HIV-1 group O and P. *Retrovirology* 8, 78 (2011).
75. Mack K, Starz K, Sauter D *et al.* Efficient Vpu-mediated tetherin antagonism by an HIV-1 group O strain. *J. Virol.* 91(6), e02177-16 (2017).
76. Kluge SF, Mack K, Iyer SS *et al.* Nef proteins of epidemic HIV-1 group O strains antagonize human tetherin. *Cell Host Microbe* 16(5), 639–650 (2014).
77. Sauter D, Unterweger D, Vogl M *et al.* Human tetherin exerts strong selection pressure on the HIV-1 group N Vpu protein. *PLoS Pathog.* 8(12), e1003093 (2012).
78. Chen CY, Shingai M, Welbourn S *et al.* Antagonism of BST-2/tetherin is a conserved function of the Env glycoprotein of primary HIV-2 isolates. *J. Virol.* 90(24), 11062–11074 (2016).
79. Hauser H, Lopez LA, Yang SJ *et al.* HIV-1 Vpu and HIV-2 Env counteract BST-2/tetherin by sequestration in a perinuclear compartment. *Retrovirology* 7, 51 (2010).
80. Le Tortorec A, Neil SJ. Antagonism to and intracellular sequestration of human tetherin by the human immunodeficiency virus type 2 envelope glycoprotein. *J. Virol.* 83(22), 11966–11978 (2009).
81. Exline CM, Yang SJ, Haworth KG *et al.* Determinants in HIV-2 Env and tetherin required for functional interaction. *Retrovirology* 12, 67 (2015).
82. Usami Y, Wu Y, Gottlinger HG. SERINC3 and SERINC5 restrict HIV-1 infectivity and are counteracted by Nef. *Nature* 526(7572), 218–223 (2015).
83. Rosa A, Chande A, Ziglio S *et al.* HIV-1 Nef promotes infection by excluding SERINC5 from virion incorporation. *Nature* 526(7572), 212–217 (2015).
84. Inuzuka M, Hayakawa M, Ingi T. Serinc an activity-regulated protein family, incorporates serine into membrane lipid synthesis. *J. Biol. Chem.* 280(42), 35776–35783 (2005).
85. Trautz B, Wiedemann H, Luchtenborg C *et al.* The host-cell restriction factor SERINC5 restricts HIV-1 infectivity without altering the lipid composition and organization of viral particles. *J. Biol. Chem.* 292(33), 13702–13713 (2017).
86. Sood C, Marin M, Chande A, Pizzato M, Melikyan GB. SERINC5 protein inhibits HIV-1 fusion pore formation by promoting functional inactivation of envelope glycoproteins. *J. Biol. Chem.* 292(14), 6014–6026 (2017).
87. Schulte B, Selyutina A, Opp S *et al.* Localization to detergent-resistant membranes and HIV-1 core entry inhibition correlate with HIV-1 restriction by SERINC5. *Virology* 515, 52–65 (2018).
88. Heigele A, Kmiec D, Regensburger K *et al.* The potency of Nef-mediated SERINC5 antagonism correlates with the prevalence of primate lentiviruses in the wild. *Cell Host Microbe* 20(3), 381–391 (2016).
89. Beitari S, Ding S, Pan Q, Finzi A, Liang C. Effect of HIV-1 Env on SERINC5 antagonism. *J. Virol.* 91(4), e02214-16 (2017).
90. Shi J, Xiong R, Zhou T *et al.* HIV-1 Nef antagonizes SERINC5 restriction by downregulation of SERINC5 via the endosome/lysosome system. *J. Virol.* 92 (23), e00196-18 (2018).
91. Trautz B, Pierini V, Wombacher R *et al.* The antagonism of HIV-1 Nef to SERINC5 particle infectivity restriction involves the counteraction of virion-associated pools of the restriction factor. *J. Virol.* 90(23), 10915–10927 (2016).
92. Salter JD, Bennett RP, Smith HC. The APOBEC protein family: united by structure, divergent in function. *Trends Biochem. Sci.* 41(7), 578–594 (2016).
93. Jarmuz A, Chester A, Bayliss J *et al.* An anthropoid-specific locus of orphan C to U RNA-editing enzymes on chromosome 22. *Genomics* 79(3), 285–296 (2002).
94. Münk C, Willemsen A, Bravo IG. An ancient history of gene duplications, fusions and losses in the evolution of APOBEC3 mutators in mammals. *BMC Evol. Biol.* 12, 71 (2012).

• **This paper depicts the evolutionary history of APOBEC3s among mammals.**

95. Conticello SG. The AID/APOBEC family of nucleic acid mutators. *Genome Biol.* 9(6), 229 (2008).
96. Marino D, Perkovic M, Hain A *et al.* APOBEC4 enhances the replication of HIV-1. *PLoS ONE* 11(6), e0155422 (2016).
97. Zhang Z, Gu Q, de Manuel Montero M *et al.* Stably expressed APOBEC3H forms a barrier for cross-species transmission of simian immunodeficiency virus of chimpanzee to humans. *PLoS Pathog.* 13(12), e1006746 (2017).

•• **This study identifies human APOBEC3H as a restriction factor of SIVcpz.**

98. Letko M, Silvestri G, Hahn BH *et al.* Vif proteins from diverse primate lentiviral lineages use the same binding site in APOBEC3G. *J. Virol.* 87(21), 11861–11871 (2013).
99. Li MM, Wu LI, Emerman M. The range of human APOBEC3H sensitivity to lentiviral Vif proteins. *J. Virol.* 84(1), 88–95 (2010).
100. Simon V, Bloch N, Landau NR. Intrinsic host restrictions to HIV-1 and mechanisms of viral escape. *Nat. Immunol.* 16(6), 546–553 (2015).
101. Mariani R, Chen D, Schröfelbauer B *et al.* Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. *Cell* 114(1), 21–31 (2003).
102. Harris RS, Bishop KN, Sheehy AM *et al.* DNA deamination mediates innate immunity to retroviral infection. *Cell* 113(6), 803–809 (2003).
103. Mangeat B, Turelli P, Caron G, Friedli M, Perrin L, Trono D. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. *Nature* 424(6944), 99–103 (2003).
104. Zhang H, Yang B, Pomerantz RJ, Zhang C, Arunachalam SC, Gao L. The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. *Nature* 424(6944), 94–98 (2003).
105. Lecossier D, Bouchonnet F, Clavel F, Hance AJ. Hypermutation of HIV-1 DNA in the absence of the Vif protein. *Science* 300(5622), 1112 (2003).
106. Suspene R, Sommer P, Henry M *et al.* APOBEC3G is a single-stranded DNA cytidine deaminase and functions independently of HIV reverse transcriptase. *Nucleic Acids Res.* 32(8), 2421–2429 (2004).
107. Yu Q, Konig R, Pillai S *et al.* Single-strand specificity of APOBEC3G accounts for minus-strand deamination of the HIV genome. *Nat. Struct. Mol. Biol.* 11(5), 435–442 (2004).
108. Hultquist JF, Lengyel JA, Refsland EW *et al.* Human and rhesus APOBEC3D, APOBEC3F, APOBEC3G, and APOBEC3H demonstrate a conserved capacity to restrict Vif-deficient HIV-1. *J. Virol.* 85(21), 11220–11234 (2011).
109. Harris RS, Dudley JP. APOBECs and virus restriction. *Virology* 479–480, 131–145 (2015).
110. Russell RA, Moore MD, Hu WS, Pathak VK. APOBEC3G induces a hypermutation gradient: purifying selection at multiple steps during HIV-1 replication results in levels of G-to-A mutations that are high in DNA, intermediate in cellular viral RNA, and low in virion RNA. *Retrovirology* 6, 16 (2009).
111. Desimmie BA, Delviks-Frankenberrry KA, Burdick RC, Qi D, Izumi T, Pathak VK. Multiple APOBEC3 restriction factors for HIV-1 and one Vif to rule them all. *J. Mol. Biol.* 426(6), 1220–1245 (2014).
112. Mbisa JL, Barr R, Thomas JA *et al.* Human immunodeficiency virus type 1 cDNAs produced in the presence of APOBEC3G exhibit defects in plus-strand DNA transfer and integration. *J. Virol.* 81(13), 7099–7110 (2007).
113. Mbisa JL, Bu W, Pathak VK. APOBEC3F and APOBEC3G inhibit HIV-1 DNA integration by different mechanisms. *J. Virol.* 84(10), 5250–5259 (2010).
114. Holmes RK, Koning FA, Bishop KN, Malim MH. APOBEC3F can inhibit the accumulation of HIV-1 reverse transcription products in the absence of hypermutation. Comparisons with APOBEC3G. *J. Biol.Chem.* 282(4), 2587–2595 (2007).
115. Bishop KN, Holmes RK, Malim MH. Antiviral potency of APOBEC proteins does not correlate with cytidine deamination. *J. Virol.* 80(17), 8450–8458 (2006).
116. Newman EN, Holmes RK, Craig HM *et al.* Antiviral function of APOBEC3G can be dissociated from cytidine deaminase activity. *Curr. Biol.* 15(2), 166–170 (2005).
117. Pollpeter D, Parsons M, Sobala AE *et al.* Deep sequencing of HIV-1 reverse transcripts reveals the multifaceted antiviral functions of APOBEC3G. *Nat. Microbiol.* 3(2), 220–233 (2018).
118. Guo F, Cen S, Niu M, Saadatmand J, Kleiman L. Inhibition of formula-primed reverse transcription by human APOBEC3G during human immunodeficiency virus type 1 replication. *J. Virol.* 80(23), 11710–11722 (2006).
119. Guo F, Cen S, Niu M, Yang Y, Gorelick RJ, Kleiman L. The interaction of APOBEC3G with human immunodeficiency virus type 1 nucleocapsid inhibits tRNA3Lys annealing to viral RNA. *J. Virol.* 81(20), 11322–11331 (2007).
120. Yang Y, Guo F, Cen S, Kleiman L. Inhibition of initiation of reverse transcription in HIV-1 by human APOBEC3F. *Virology* 365(1), 92–100 (2007).
121. Luo K, Wang T, Liu B *et al.* Cytidine deaminases APOBEC3G and APOBEC3F interact with human immunodeficiency virus type 1 integrase and inhibit proviral DNA formation. *J. Virol.* 81(13), 7238–7248 (2007).
122. Li XY, Guo F, Zhang L, Kleiman L, Cen S. APOBEC3G inhibits DNA strand transfer during HIV-1 reverse transcription. *J. Biol. Chem.* 282(44), 32065–32074 (2007).
123. Iwatani Y, Chan DS, Wang F *et al.* Deaminase-independent inhibition of HIV-1 reverse transcription by APOBEC3G. *Nucleic Acids Res.* 35(21), 7096–7108 (2007).
124. Conticello SG, Harris RS, Neuberger MS. The Vif protein of HIV triggers degradation of the human antiretroviral DNA deaminase APOBEC3G. *Curr. Biol.* 13(22), 2009–2013 (2003).

125. Yu Y, Xiao Z, Ehrlich ES, Yu X, Yu XF. Selective assembly of HIV-1 Vif-Cul5-ElonginB-ElonginC E3 ubiquitin ligase complex through a novel SOCS box and upstream cysteines. *Genes Dev.* 18(23), 2867–2872 (2004).

126. Yu X, Yu Y, Liu B *et al.* Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. *Science* 302(5647), 1056–1060 (2003).

127. Marin M, Rose KM, Kozak SL, Kabat D. HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation. *Nat. Med.* 9(11), 1398–1403 (2003).

128. Sheehy AM, Gaddis NC, Malim MH. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. *Nat. Med.* 9(11), 1404–1407 (2003).

129. Mehle A, Strack B, Ancuta P, Zhang C, McPike M, Gabuzda D. Vif overcomes the innate antiviral activity of APOBEC3G by promoting its degradation in the ubiquitin-proteasome pathway. *J. Biol.Chem.* 279(9), 7792–7798 (2004).

130. Luo K, Xiao Z, Ehrlich E *et al.* Primate lentiviral virion infectivity factors are substrate receptors that assemble with cullin 5-E3 ligase through a HCCH motif to suppress APOBEC3G. *Proc. Natl Acad. Sci. USA* 102(32), 11444–11449 (2005).

131. Kao S, Miyagi E, Khan MA *et al.* Production of infectious human immunodeficiency virus type 1 does not require depletion of APOBEC3G from virus-producing cells. *Retrovirology* 1, 27 (2004).

132. Opi S, Kao S, Goila-Gaur R *et al.* Human immunodeficiency virus type 1 Vif inhibits packaging and antiviral activity of a degradation-resistant APOBEC3G variant. *J. Virol.* 81(15), 8236–8246 (2007).

133. Mehle A, Goncalves J, Santa-Marta M, McPike M, Gabuzda D. Phosphorylation of a novel SOCS-box regulates assembly of the HIV-1 Vif-Cul5 complex that promotes APOBEC3G degradation. *Genes Dev.* 18(23), 2861–2866 (2004).

134. Guo Y, Dong L, Qiu X *et al.* Structural basis for hijacking CBF-beta and CUL5 E3 ligase complex by HIV-1 Vif. *Nature* 505(7482), 229–233 (2014).

135. Jager S, Kim DY, Hultquist JF *et al.* Vif hijacks CBF-beta to degrade APOBEC3G and promote HIV-1 infection. *Nature* 481(7381), 371–375 (2012).

136. Hultquist JF, Binka M, LaRue RS, Simon V, Harris RS. Vif proteins of human and simian immunodeficiency viruses require cellular CBFbeta to degrade APOBEC3 restriction factors. *J. Virol.* 86(5), 2874–2877 (2012).

137. Kane JR, Stanley DJ, Hultquist JF *et al.* Lineage-specific viral hijacking of non-canonical E3 ubiquitin ligase cofactors in the evolution of Vif anti-APOBEC3 activity. *Cell Rep.* 11(8), 1236–1250 (2015).

138. Zhang W, Wang H, Li Z *et al.* Cellular requirements for bovine immunodeficiency virus Vif-mediated inactivation of bovine APOBEC3 proteins. *J. Virol.* 88(21), 12528–12540 (2014).

139. Berger A, Münk C, Schweizer M, Cichutek K, Schüle S, Flory E. Interaction of Vpx and apolipoprotein B mRNA-editing catalytic polypeptide 3 family member A (APOBEC3A) correlates with efficient lentivirus infection of monocytes. *J. Biol. Chem.* 285(16), 12248–12254 (2010).

140. Berger G, Durand S, Fargier G *et al.* APOBEC3A is a specific inhibitor of the early phases of HIV-1 infection in myeloid cells. *PLoS Pathog.* 7(9), e1002221 (2011).

141. Ikeda T, Symeonides M, Albin JS, Li M, Thali M, Harris RS. HIV-1 adaptation studies reveal a novel Env-mediated homeostasis mechanism for evading lethal hypermutation by APOBEC3G. *PLoS Pathog.* 14(4), e1007010 (2018).

142. Hache G, Abbink TE, Berkhout B, Harris RS. Optimal translation initiation enables Vif-deficient human immunodeficiency virus type 1 to escape restriction by APOBEC3G. *J. Virol.* 83(11), 5956–5960 (2009).

143. Hache G, Shindo K, Albin JS, Harris RS. Evolution of HIV-1 isolates that use a novel Vif-independent mechanism to resist restriction by human APOBEC3G. *Curr. Biol.* 18(11), 819–824 (2008).

144. Sawyer SL, Emerman M, Malik HS. Ancient adaptive evolution of the primate antiviral DNA-editing enzyme APOBEC3G. *PLoS Biol.* 2(9), E275 (2004).

145. OhAinle M, Kerns JA, Malik HS, Emerman M. Adaptive evolution and antiviral activity of the conserved mammalian cytidine deaminase APOBEC3H. *J. Virol.* 80(8), 3853–3862 (2006).

146. Nakano Y, Aso H, Soper A *et al.* A conflict of interest: the evolutionary arms race between mammalian APOBEC3 and lentiviral Vif. *Retrovirology* 14(1), 31 (2017).

147. Harris RS, Anderson BD. Evolutionary paradigms from ancient and ongoing conflicts between the lentiviral Vif protein and mammalian APOBEC3 enzymes. *PLoS Pathog.* 12(12), e1005958 (2016).

148. Compton AA, Hirsch VM, Emerman M. The host restriction factor APOBEC3G and retroviral Vif protein coevolve due to ongoing genetic conflict. *Cell Host Microbe* 11(1), 91–98 (2012).

149. Etienne L, Bibollet-Ruche F, Sudmant PH, Wu LI, Hahn BH, Emerman M. The role of the antiviral *APOBEC3* gene family in protecting chimpanzees against lentiviruses from monkeys. *PLoS Pathog.* 11(9), e1005149 (2015).

150. Bogerd HP, Doehle BP, Wiegand HL, Cullen BR. A single amino acid difference in the host APOBEC3G protein controls the primate species specificity of HIV type 1 virion infectivity factor. *Proc. Natl Acad. Sci. USA* 101(11), 3770–3774 (2004).

151. Schrofelbauer B, Chen D, Landau NR. A single amino acid of APOBEC3G controls its species-specific interaction with virion infectivity factor (Vif). *Proc. Natl Acad. Sci. USA* 101(11), 3927–3932 (2004).

152. Mangeat B, Turelli P, Liao S, Trono D. A single amino acid determinant governs the species-specific sensitivity of APOBEC3G to Vif action. *J. Biol. Chem.* 279(15), 14481–14483 (2004).

153. OhAinle M, Kerns JA, Li MM, Malik HS, Emerman M. Antiretroelement activity of APOBEC3H was lost twice in recent human evolution. *Cell Host Microbe* 4(3), 249–259 (2008).

154. Harari A, Ooms M, Mulder LC, Simon V. Polymorphisms and splice variants influence the antiretroviral activity of human APOBEC3H. *J. Virol.* 83(1), 295–303 (2009).

155. Cagliani R, Riva S, Fumagalli M *et al.* A positively selected APOBEC3H haplotype is associated with natural resistance to HIV-1 infection. *Evolution* 65(11), 3311–3322 (2011).

156. Wang X, Abudu A, Son S, Dang Y, Venta PJ, Zheng YH. Analysis of human APOBEC3H haplotypes and anti-human immunodeficiency virus type 1 activity. *J. Virol.* 85(7), 3142–3152 (2011).

157. Refsland EW, Hultquist JF, Luengas EM *et al.* Natural polymorphisms in human APOBEC3H and HIV-1 Vif combine in primary T lymphocytes to affect viral G-to-A mutation levels and infectivity. *PLoS Genet.* 10(11), e1004761 (2014).

158. Kim BH, Shenoy AR, Kumar P, Bradfield CJ, MacMicking JD. IFN-inducible GTPases in host cell defense. *Cell Host Microbe* 12(4), 432–444 (2012).

159. Carlow DA, Teh SJ, Teh HS. Specific antiviral activity demonstrated by TGTP, a member of a new family of interferon-induced GTPases. *J. Immunol.* 161(5), 2348–2355 (1998).

160. Gilly M, Wall R. The *IRG-47* gene is IFN-gamma induced in B cells and encodes a protein with GTP-binding motifs. *J. Immunol.* 148(10), 3275–3281 (1992).

161. Klamp T, Boehm U, Schenk D, Pfeffer K, Howard JC. A giant GTPase, very large inducible GTPase-1, is inducible by IFNs. *J. Immunol.* 171(3), 1255–1265 (2003).

162. Haller O, Staeheli P, Schwemmle M, Kochs G. Mx GTPases: dynamin-like antiviral machines of innate immunity. *Trends Microbiol.* 23(3), 154–163 (2015).

163. Anderson SL, Carton JM, Lou J, Xing L, Rubin BY. Interferon-induced guanylate binding protein-1 (GBP-1) mediates an antiviral effect against vesicular stomatitis virus and encephalomyocarditis virus. *Virology* 256(1), 8–14 (1999).

164. Olszewski MA, Gray J, Vestal DJ. *In silico* genomic analysis of the human and murine guanylate-binding protein (GBP) gene clusters. *J. Interferon Cytokine Res.* 26(5), 328–352 (2006).

165. Krapp C, Hotter D, Gawanbacht A *et al.* Guanylate binding protein (GBP) 5 is an interferon-inducible inhibitor of HIV-1 infectivity. *Cell Host Microbe* 19(4), 504–514 (2016).

166. Hotter D, Sauter D, Kirchhoff F. Guanylate binding protein 5: impairing virion infectivity by targeting retroviral envelope glycoproteins. *Small GTPases* 8(1), 31–37 (2017).

167. Fricke T, White TE, Schulte B *et al.* MxB binds to the HIV-1 core and prevents the uncoating process of HIV-1. *Retrovirology* 11, 68 (2014).

168. Matreyek KA, Wang W, Serrao E, Singh PK, Levin HL, Engelman A. Host and viral determinants for MxB restriction of HIV-1 infection. *Retrovirology* 11, 90 (2014).

169. Liu Z, Pan Q, Ding S *et al.* The interferon-inducible MxB protein inhibits HIV-1 infection. *Cell Host Microbe* 14(4), 398–410 (2013).

170. Goujon C, Moncorge O, Bauby H *et al.* Human MX2 is an interferon-induced post-entry inhibitor of HIV-1 infection. *Nature* 502(7472), 559–562 (2013).

171. Kane M, Yadav SS, Bitzegeio J *et al.* MX2 is an interferon-induced inhibitor of HIV-1 infection. *Nature* 502(7472), 563–566 (2013).

172. Meier K, Jaguva Vasudevan AA, Zhang Z, *et al.* Equine MX2 is a restriction factor of equine infectious anemia virus (EIAV). *Virology* 523, 52–63 (2018).

173. Li N, Zhang W, Cao X. Identification of human homologue of mouse IFN-gamma induced protein from human dendritic cells. *Immunol. Lett.* 74(3), 221–224 (2000).

174. Goldstone DC, Ennis-Adeniran V, Hedden JJ *et al.* HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. *Nature* 480(7377), 379–382 (2011).

175. Lahouassa H, Daddacha W, Hofmann H *et al.* SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates. *Nat. Immunol.* 13(3), 223–228 (2012).

176. Laguette N, Sobhian B, Casartelli N *et al.* SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. *Nature* 474(7353), 654–657 (2011).

177. Hrecka K, Hao C, Gierszewska M *et al.* Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. *Nature* 474(7353), 658–661 (2011).

178. Baldauf HM, Pan X, Erikson E *et al.* SAMHD1 restricts HIV-1 infection in resting CD4$^{(+)}$ T cells. *Nat. Med.* 18(11), 1682–1687 (2012).

179. Coquel F, Silva MJ, Techer H *et al.* SAMHD1 acts at stalled replication forks to prevent interferon induction. *Nature* 557(7703), 57–61 (2018).

180. Lim ES, Fregoso OI, McCoy CO, Matsen FA, Malik HS, Emerman M. The ability of primate lentiviruses to degrade the monocyte restriction factor SAMHD1 preceded the birth of the viral accessory protein Vpx. *Cell Host Microbe* 11(2), 194–204 (2012).

181. Lenzi GM, Domaoal RA, Kim DH, Schinazi RF, Kim B. Mechanistic and kinetic differences between reverse transcriptases of Vpx coding and non-coding lentiviruses. *J. Biol. Chem.* 290(50), 30078–30086 (2015).

182. Bibollet-Ruche F, Heigele A, Keele BF *et al.* Efficient SIVcpz replication in human lymphoid tissue requires viral matrix protein adaptation. *J. Clin. Invest.* 122(5), 1644–1652 (2012).

183. Moeller AH, Shilts M, Li Y *et al.* SIV-induced instability of the chimpanzee gut microbiome. *Cell Host Microbe* 14(3), 340–345 (2013).

184. Terio KA, Kinsel MJ, Raphael J *et al.* Pathologic lesions in chimpanzees (Pan trogylodytes schweinfurthii) from Gombe National Park, Tanzania, 2004-2010. *J. Zoo Wildl. Med.* 42(4), 597–607 (2011).

185. Barbian HJ, Jackson-Jewett R, Brown CS *et al.* Effective treatment of SIVcpz-induced immunodeficiency in a captive western chimpanzee. *Retrovirology* 14(1), 35 (2017).

186. Keele BF, Jones JH, Terio KA *et al.* Increased mortality and AIDS-like immunopathology in wild chimpanzees infected with SIVcpz. *Nature* 460(7254), 515–519 (2009).

187. Etienne L, Nerrienet E, LeBreton M *et al.* Characterization of a new simian immunodeficiency virus strain in a naturally infected *Pan troglodytes troglodytes* chimpanzee with AIDS related symptoms. *Retrovirology* 8, 4 (2011).

CHAPTER II

The cyclophilin A binding loop of capsid regulates the human TRIM5α sensitivity of non-pandemic HIV-1

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Twizerimana's contribution to this work:

1. Executed all the experiments in this work
2. Wrote the original draft of the manuscript

Manuscript ready for publication

The cyclophilin A binding loop of capsid regulates the human TRIM5α sensitivity of non-pandemic HIV-1

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Abstract

Despite their circulation, non-M HIV-1s have only appeared in a small number of cases in West Africa. The rather low cases of humans infected by HIV-1 N, O or P raise the question of their incomplete adaptation to humans. We hypothesized that early post-entry restrictions may be relevant for the impaired spread of these HIVs. One of the best-characterized species-specific restriction factors acting early in infection is TRIM5α. While HIV-1 M can escape human TRIM5α restriction by binding cyclophilin A (CYPA, also known as PPIA, peptidylprolyl isomerase A) to the so-called CYPA binding loop of its capsid protein. How non-M HIV-1s interact with huTRIM5α is ill-defined. By testing novel full-length reporter viruses (Δ *env*) of HIV-1 N, O, P, HIV-2AB and SIVgor, we found that in contrast to HIV-1 M, the non-pandemic HIVs and SIVgor showed restriction by human TRIM5α. To identify the viral determinant for the huTRIM5α inhibition, we transferred capsid coding regions of non-M HIV-1 and SIVgor to HIV-1 M and confirmed sensitivity to TRIM5α of such chimeric HIV-1s. Work to identify capsid residues that mediate susceptibility to huTRIM5α revealed that capsid residue 88 in the capsid CYPA binding loop, was important for such differences. HIV-1 M uses alanine to resist, while others have either valine or methionine, which avail them for huTRIM5α. Capsid residue 88 determines the sensitivity to TRIM5α in a novel way and likely influences the enzymatic cis-trans activity of the bound CYPA. These differential CYPA usages by pandemic and non-pandemic HIV-1 demonstrates that the enzymatic activity of CYPA on the viral core is important for its protective function against human TRIM5α.

Key words: TRIM5α, CYPA, pandemic, non-M HIV

Introduction

The species of human immunodeficiency virus 1(HIV-1) is subdivided into the four groups, M, N, O and P[1]. HIV-1s are the evolutionary results of rare successful transmission events of simian immunodeficiency viruses (SIV) to humans. Group M and group N HIV-1s are from SIV of chimpanzees (SIVcpz), while group O and group P are from SIV of gorillas (SIVgor)[2,3]. To halt retroviral replication, vertebrates use several cellular restriction factors[4–6]. It is ill-defined whether the low prevalence of non-M HIVs is associated to the activity of antiviral factors such as the capsid binding TRIM5α, which may limit their spread among humans[7].

TRIM proteins have a structure with a RING domain, a coiled-coil domain, one to two B box domains[7]. In addition, some TRIM proteins have a C-terminus domain which can be the PRYSPRY (B30.2) such in the alpha isoform of TRIM5, theTRIM5α and TRIM25 or CYPA such as in the TRIM-Cyclophilin A (TRIMCyp) fusion proteins[8–10].

The PRYSPRY or Cyclophilin A domains of TRIM5 proteins can bind retroviral capsids, oligomerize to form a higher-order self-association oligomers around the conical core to affect the core integrity and thereby impairs nuclear import but also integration of the reverse-transcribed viral genome[11,12]. The TRIM5α binding to capsid can also result in intracellular signaling events, for an extended viral restriction[13,14].

The anti-retroviral activity of TRIM5 proteins has been shown to mainly be PRYSPRY or CYPA domains dependent[15,16]. CYPA presence at the C-terminus of TRIM5 proteins is a result of evolutionary retrotransposition of *CYPA* in some primates, like the Old World rhesus monkeys (*Macaca mulatta*) or the New World *Aotus* night/owl monkeys[17]. The selective antiretroviral ability of TRIM5α is thought to have formed a selective protective shield to the retroviral spread in vertebrate hosts[18]. As an example, human TRIM5α restricts infections of humans by the horse lentivirus equine infectious anemia virus (EIAV)[19]. In contrast, the pandemic HIV-1 M escapes the antiviral activity of human TRIM5α by displaying a binding

site in the viral core for CYPA[18]. Once in interaction with the capsid CYPA binding loop, CYPA can influence many early steps of HIV infection[20,21].

CYPA is also encapsidated by HIV virions, with less defined role in the next round of infection[22]. TRIMCyp proteins on the other hand, differentially halt retroviral infection through the binding of their CYPA domain to capsid CYPA binding loop, early during infection, this inhibition can be blocked by cell treatment with cyclosporin A (CsA)[23]. For instance, owl monkey TRIMCyp is active against HIV-1 but not HIV-2 and rhTRIMCyp inhibits HIV-2 but not HIV-1 M[24].

It was recently documented that sequences in CYPA domain of rhTRIMCyp but also the capsid CYPA binding loop, have determined the ability of this protein to strongly restrict HIV-2, HIV-1 O but to the cost of loss of anti-HIV-1 M restriction[25,26]. However, such work did not cover other HIV-1 viruses from N and P groups. In addition, more is needed on CYPA usage by non-M HIVs. Understanding how sequences in the capsid affect viral infection is of paramount importance, particularly in the context of non-M HIVs.

Here we tested the antiviral property of human TRIM5α against non-M group HIVs but also SIVgor and identified viral determinants of sensitivity and assessed CYPA in TRIM5α mechanism to restrict HIVs.

Materials and methods.

Cells. CrFK and HEK293T cells were maintained in Dulbecco's high-glucose modified Eagle's medium (PAN Biotech, Aidenbach, Germany) with addition of 10% fetal bovine serum (FBS, PAN Biotech, Aidenbach, Germany), 2 mM L-glutamine (PAN Biotech, Aidenbach, Germany), 100 U/ml penicillin, and 100 µg/ml streptomycin (PAN Biotech, Aidenbach, Germany). TRIM5α knockout U87-MG and control cells were kindly donated by Michael Malim[27], CYPA knockdown and control U87-MG cells were cultured under 1 µg puromycin.

Plasmids. The replication competent HIV-1 N DJO0131, HIV-1 O RBF206, HIV-1 P RBF168, HIV-2 AB 7312A (=SNAG), SIVcpzPtt MB897 clones were kindly provided by Frank Kirchhoff. SIVgorCP2139, and SIVcpz*Pts* clone TAN1.910 clones were obtained from NIH AIDS repository[28,29]. The murine leukemia virus (MLV) packaging construct pHIT60, which encodes the *gag-pol* of Moloney MLV was provided by Jonathan Stoye[30].

Reporter viruses for HIV-1 N, HIV-1 O, HIV-1 P, HIV-2AB and SIVgor were constructed as follow: Using fusion PCR, *nanoluciferase* gene was cloned in replacement of *nef* of HIV-1 N, HIV-1 O, HIV-1 P, HIV-2 AB and SIVgor. Two stop codons were introduced in the envelope genes of these viruses, as VSV-G was to be used for envelope.

Capsid mutant reporter viruses of HIV-1 M, HIV-1 N, HIV-1 O, HIV-1 P and HIV-2AB were generated with fusion PCR using specific primers including those (forward and a reverse) with desired mutations.

HIV-1 M with HIV-1 N or HIV-1 O or HIV-1 P or SIVgor capsid were made by fusion PCR, their inserts were cloned in PmlI-MfeI digested pMDLg/pRRE vector to produce chimeric *gag-pol* of HIV-1 M with capsid genes of other viruses: pMDLg/pRRE.HIV-1 N CA, pMDLg/pRRE.HIV-1 O CA, pMDLg/pRRE.HIV-1 P CA, pMDLg/pRRE.SIVgor CA.

Complete *gag-pol* constructs for HIV-1 N, HIV-1 O and HIV-1 P were constructed using the HIV-1 M pMDLg/pRRE plasmid and replacing HIV-1 M *gag-pol* by digestion with PmlI and BspEI and insertion of *gag-pols* of interest, produced through a series of overlapping PCR reactions, to make pMDLg/pRRE.HIV-1 N, pMDLg/pRRE.HIV-1 O and pMDLg/pRRE.HIV-1 P.

CYPA binding loop capsid mutants were made through PCR with primers bearing specific CYPA binding loops sequences and their inserts were respectively cloned in specific vectors to make HIV-1 M. HIV-1 N loop, HIV-1 M.HIV-1 O loop and HIV-1 N.HIV-1 M loop. HIV-1 vector pSIN.PPT.CMV.Luc.IRES.GFP expresses firefly luciferase and GFP. psPAX2 was

obtained from the NIH AIDS Reagent Program (Cat# 11348), pRSV-Rev, pMDLg/pRRE and pMD.G (VSV-G) have been described.

Using fusion PCR on TRIMCyp cDNA in pLNCX2 plasmid[31], two primers from both rhTRIMCyp cDNA ends (a forward primer with XhoI site and a reverse primer with HA tag and NotI site), forward and reverse primers containing the desired mutations in the cyclophilin A domain; N66D, H69R or N66D-H69R rhTRIMCyp-HA mutants were generated. XhoI and NotI digested rhTRIMCyp mutant PCR products were ligated in pLNCX2 digested with same enzymes and plasmid propagation was done under ampicillin. DNA Sequencing confirmed desired mutations.

Transfection and viral particle production. The pMDLg/pRRE based viral particles production was done using a three (3) plasmid system: pMDLg/pRRE (800ng), pSIN.PPT.CMV.Luc.IRES.GFP (800ng), pRSV-Rev (400ng), and pMDG.VSV-G (200ng). For nanoluciferase-based reporter viruses (HIV-1 N, O, P, SIVgor, HIV-2, SIVcpz), 10^6 HEK293T cells were seeded, the following day, these cells were transfected using 200 ng VSV-G and 2000 ng of viral plasmids using polyjet (Tebubio GmbH, Offenbach, Germany). 48 hours post-transfection, viral particles were collected, short-centrifuged for 5 min at 5000 rpm at 4^{o}C, to remove any detached HEK293T cells, before being used for infection assay or transduction for stable cell lines. Where needed, the reverse transcriptase (RT) activity of viruses was quantified using the previously described approach[32].

Generation of stable cells expressing TRIM5 proteins. Viral particles produced in HEK293T cells using a pLNCX2.TRIMCyp construct, pHIT60 and pMD.G-VSV-G were used to transduce CrFK cells for three days, followed by a selection under 400 μg/ml G418 (Biochrom GmbH, Berlin, Germany), for the expression of HA-tagged wild type and mutant

rhTRIMCYP proteins. Protein expression was confirmed with immunoblots. Cells expressing empty pLNCX2 vector were used for control.

Single round infection assay. 10 x 10^4 CrFK cells or 5 x 10^3 (U-87 MG) cells were seeded into 96-well plates and infection was performed the following day. For experiments involving cyclosporin A (CsA, Sigma Aldrich, Germany), 1 to 10 μM of CsA or control DMSO were used to treat cells 2 hours before infection. Cells were then infected with different reporter viral particles and after 48 to 72 hours, luciferase activity was measured. For infection with nanoluciferase-containing reporter viruses, cells were washed with phosphate-buffered saline (PBS) three times before lysis and luciferase measurement, in addition to medium change 24 hours following infection, to eliminate the effect of background nanoluciferase.

Nanoluciferase activity was measured with Nano-Glo Luciferase system (Promega, Mannheim, Germany) and firefly luciferase activity was measured with the Steady-Glo Luciferase system (Promega, Mannheim, Germany), on a MicroLumat Plus luminometer (Berthold Detection Systems, Pforzheim, Germany). Each experiment was performed in triplicates for at least three times.

Pulldown assays and immunoblots. The GST-CYPA based pulldown experiments followed a protocol previously described[33]. To confirm expression of primate TRIM5 proteins, cells were lysed using RIPA lysis buffer (25 mM Tris-HCl [pH 8.0], 137 mM NaCl, 1% NP-40, 1% glycerol, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 2 mM EDTA, and protease inhibitor cocktail set III [Calbiochem, Darmstadt, Germany]).

The cell lysate was centrifuged at 14,800 rpm for 20 minutes at 4°C. The protein supernatant was denatured using Roti-load sample buffer (Roth, Karlsruhe, Germany) and was used for SDS PAGE. Mouse primary anti-HA (MMS-101P, Covance, Münster, Germany, 1:7.500

dilution) for HA-tagged proteins, anti-human TRIM5α (rabbit monoclonal, catalogue number 143265; Cell Signaling Technology Europe BV, Frankfurt, Germany), anti-CYPA (mouse monoclonal; Santa Cruz Biotechnology) (1:500 dilution), CYPA.GST was detected using mouse anti-GST (SAB4200237-200UL, Sigma-Aldrich, Germany,1:7500 dilution)) were used. For viral proteins, viral supernatant was centrifuged through 20% sucrose gradient for a minimum of 4 hours at 4°C, 14.800 rpm. Supernatant was discarded and viral pellet lysed using RIPA buffer for 5 min followed by denaturation at 95°C in Roti-load sample buffer for 5 minutes and SDS PAGE using anti-p24 antibody (NIH).

CYPA packaging by HIV-1. In each well of a six well plate, 10^6 HEK293T cells were seeded, one day later, they were transfected with plasmids for HIV-1 and HA-tagged CYPA, in the presence or absence of different amounts of CsA. Two days post-transfection, viral supernatant was centrifuged for 5 hours at 4°C on top of a 20% sucrose for viral concentration.
Cells and viral pellets were lysed and subjected to immunoblot for HA-tagged CYPA, p24, and tubulin.

Results

Construction of non-M HIV and SIVgor reporter viruses.

We constructed novel nanoluciferase-based reporter viruses for HIV-1 N, HIV-1 O, HIV-1 P, HIV-2AB CRF, and SIVgor. *Nanoluciferase* was cloned in replacement of *nef* gene. In addition, two stop codons were inserted in the *env*. Production of VSV-G-pseudotyped viral particles was done through transfection of HEK293T cells, and such virions were tested on human HeLa cells, for infectivity by nanoluciferase measurement, two days after infection (Suppl. Fig. S1A). We also constructed pMDLg/pRRE-based *gag-pol* constructs for HIV-1 N,

HIV-1 O, HIV-1 P and SIVgor. Such constructs were made by replacing HIV-1 M pMDLg/pRRE *gag-pol* sequences by non-M or SIVgor *gag-pol* sequences (data not shown).

Non-M HIVs are inhibited by human TRIM5α in human cells.

To test whether the replication of non-pandemic HIV is restricted by human TRIM5α, equal amounts of reporter viruses of non-M HIVs (HIV-1 N, HIV-1 O, HIV-1 P) HIV-2AB) and SIVs (SIVcpzPtt, SIVcpzPts, SIVgor) were used for infections of human wildtype (WT) U-87 MG and huTRIM5α knockout (KO) U-87 MG cells[27] (Fig. 1A). In addition, we included reporter viruses for HIV-1 M and for the equine infectious anemia infectious virus (EIAV) as a controls; EIAV is known to be sensitive to human TRIM5α[34].

While the infectivity of HIV-1 M was equal in WT and TRIM5 KO cells, infection by non-M HIVs but also SIVgor was enhanced when huTRIM5α was knocked down (Suppl. Figs. S1B, S1A). In fact, infection by HIV-1 N increased to up to three folds in the huTRIM5α KO cells as compared to control cells, infection by HIV-1 O, HIV-1 P, HIV-2 and SIVgor reached 2.5-, 3.5-, 2- and 2-folds, respectively in the absence of huTRIM5α, suggesting inhibition of these viruses by human TRIM5α. EIAV showed 6.5-fold higher infectivity in the absence of huTRIM5α, compared to WT cells. In contrast, SIVcpzPtt and SIVcpzPts did not significantly benefit from the absence of huTRIM5α (Fig. 1B), as described before[3]. We also compared infection results of HIV-1 M vs. non-M HIV-1s virions produced using their respective *gag-pol* plasmids. We used such virions (equal amount of virus) to infect WT U-87 MG cells, as shown in (Suppl. Fig. S1B), non-M HIV-1 infectivity was strongly reduced compared to infectivity of HIV-1 M.

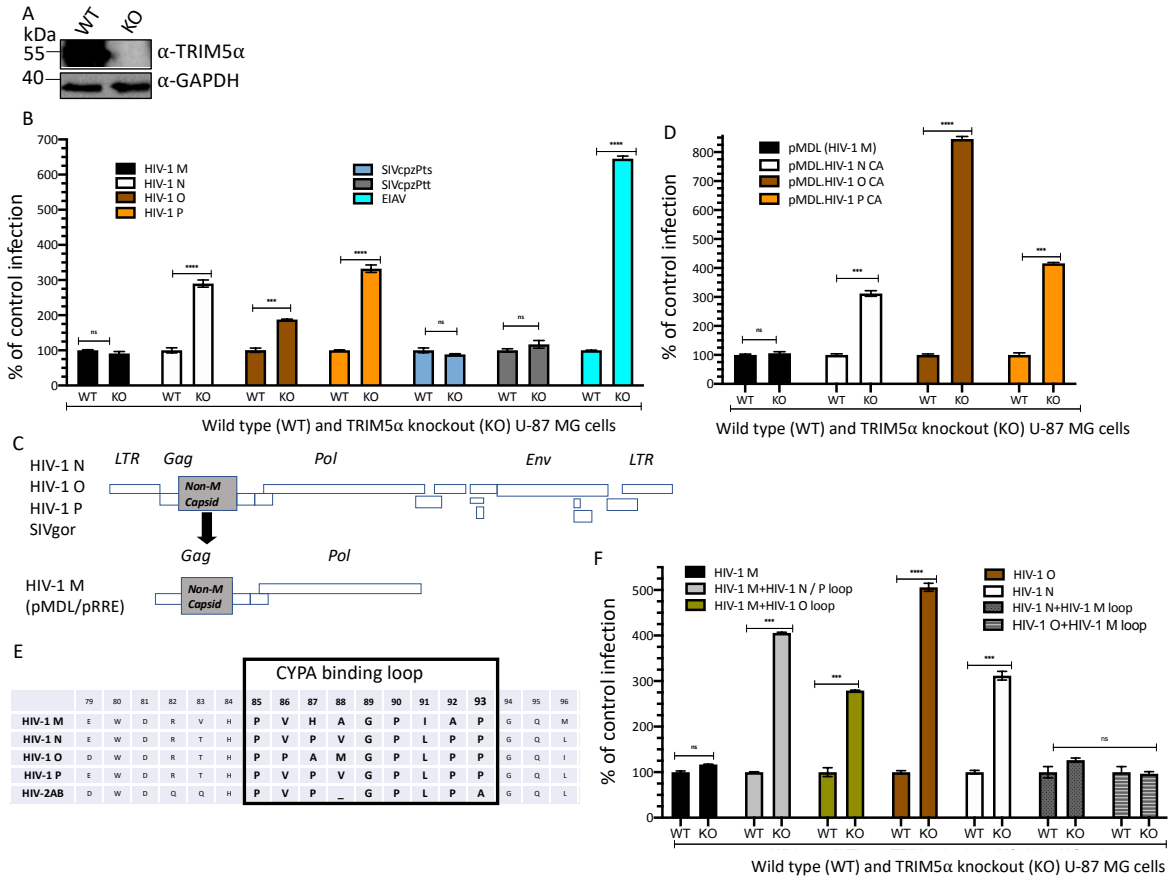

Fig. 1. **TRIM5α depletion in human U-87MG cells increases non-M HIVs/SIVgor infection in a capsid and CYPA binding loop dependent way.** (A) Immunoblot of WT and TRIM5α KO U-87 MG cells. Anti-TRIM5 antibody was used to detect TRIM5α; anti-GAPH antibody was used to ensure equal protein loading. (B) WT or TRIM5α KO U-87 MG cells were infected with equal amount of luciferase reporter viruses for HIV-1 M, HIV-1 N, HIV-1 O, HIV-1 P, SIVcpzPtt, SIVcpzPts and EIAV. Two to three days later, luciferase activity was measured and compared between WT and TRIM5α knockout cells. (C) HIV-1 N, HIV-1 O or HIV-1 P capsids were cloned in HIV-1 M *gag-pol* as replacements for its WT capsid. (D) HIV-1 M *gag-pol* with capsid of either HIV-1 N, O or P were tested in human WT and TRIM5α KO U-87MG cells. Two to three days after infection, luciferase activity was measured. (E) Protein sequence alignment of CYPA binding loop (box) regions of capsids of HIV-1 M, N, O and P and HIV-2. (F) Using their respective *GagPol* constructs, CYPA binding loop from HIV-1 N or HIV-1 O were cloned in HIV-1 M capsid and HIV-1 M CYPA binding loop was transferred to HIV-1 N and HIV-1 O capsids. These CYPA loop mutants were then used to infect WT or TRIM5α KO U-87 MG cells for two to three days, in triplicates. Luciferase activity was measured, compared. These experiments were repeated for a minimum of three times.

Capsid and its CYPA binding loop mediate differences in sensitivity to huTRIM5α.

Since TRIM5α is a capsid binding factor[35], we next opted to investigate on the mechanisms behind differences in sensitivity to huTRIM5α between HIV-1 M and non-pandemic HIVs. We transferred the capsid encoding sequence from HIV-1 N, HIV-1 O, HIV-1 P or SIVgor, to HIV-1 M in the *gag-pol* expression construct pMDLg/pRRE representing HIV-1 M sequence (Fig. 1C). In contrast to WT HIV-1 M, chimeric virus with capsid from non-M viruses were

inhibited 2.5 – 8-fold in WT cells compared to huTRIM5α KO cells (Fig. 1D), confirming the role of viral capsid for restriction by human TRIM5α.

Reports have described that in human cells the capsid interacting cellular protein cyclophilin A (CYPA) protects HIV-1 M against the antiviral activity of huTRIM5α [17,18].

In capsid, CYPA binds to a loop between helix 4 and helix 5, termed CYPA binding loop. Here CYPA directly interacts with residues G89 and P90[36], which are conserved in pandemic and non-pandemic HIVs. However, other residues between these viruses show variations and HIV-1 N and HIV-1 P share an identical CYPA binding loop (Fig. 1E).

To explore a possible differential role of CYPA in virus-specific infection patterns, we swapped in capsid the CYPA binding loops between HIV-1 M, HIV-1 N and HIV-O. Thus, we created HIV-1 M *gag-pol* constructs with a capsid CYPA binding loop of HIV-1 N (identical to HIV-1 P) or HIV-1 O. In reverse, we transferred the HIV-1 M capsid CYPA binding loop to HIV-1 N or HIV-1 O *Gag-Pol* constructs. Interestingly, viruses that had the CYPA binding loop of non-pandemic HIVs showed a restriction in WT cells compared to TRIM5α KO cells, and non-pandemic viruses with a CYPA binding loop of HIV-1 M escaped the restriction by TRIM5α (Fig. 1F). These findings pointed out that the capsid CYPA binding loops account for differences in infection levels of pandemic and non-pandemic HIVs in huTRIM5α KO cells.

Capsid residue at position 88 mediates HIV sensitivity to huTRIM5α.

In the capsid CYPA binding loop, the human CYPA protein binds strongly to residues G89 and P90[37] but a role of other loop residues has also been shown in the context of HIV-1 and HIV-2 interactions with rhTRIMCyp[26].

We hypothesized that variability in residue 88 in the CYPA binding loops could explain our observations regarding the differential sensitivity of HIV-1 M and the non-pandemic HIV-1s to TRIM5α. We infected both WT and TRIM5α KO cells with reporter viruses that had

mutations in the CYPA binding loop at position 88, HIV-1 M A88V, HIV-1 N V88A, HIV-1 O M88A or HIV-1 P V88A and compared their infectivity to the corresponding WT viruses. For HIV-1 M, we included the well characterized HIV-1 M G89V and A88V-G89V capsid mutants, which do not bind CYPA[38].

As expected, both mutations in HIV-1 M, A88V and G89V were strongly inhibited in WT U-87 MG cells compared to WT virus (Fig. 2A). However, in TRIM5α KO cells infections by both capsid mutants were not reduced compared to WT HIV-1 M, with infection by the A88V mutant even 4.3-fold higher than infection by WT virus (Fig. 2B, Suppl. Figs. S1C-E). Infections by capsid mutants of non-pandemic HIVs showed a contrasting pattern.

In WT cells, the infectivity of HIV-1 N V88A was 5-fold higher, of HIV-1 O M88A was 2.3 folds higher and of HIV-1 P V88A was 2.1 folds higher than infections by their WT viruses (Fig. 2C). However, these capsid mutants of non-pandemic viruses had a similar infectivity to WT virus in TRIM5α KO cells (Fig. 2D). Together, these findings show that the residue 88 in the CYPA binding loop of capsid is a determinant of TRIM5α restriction.

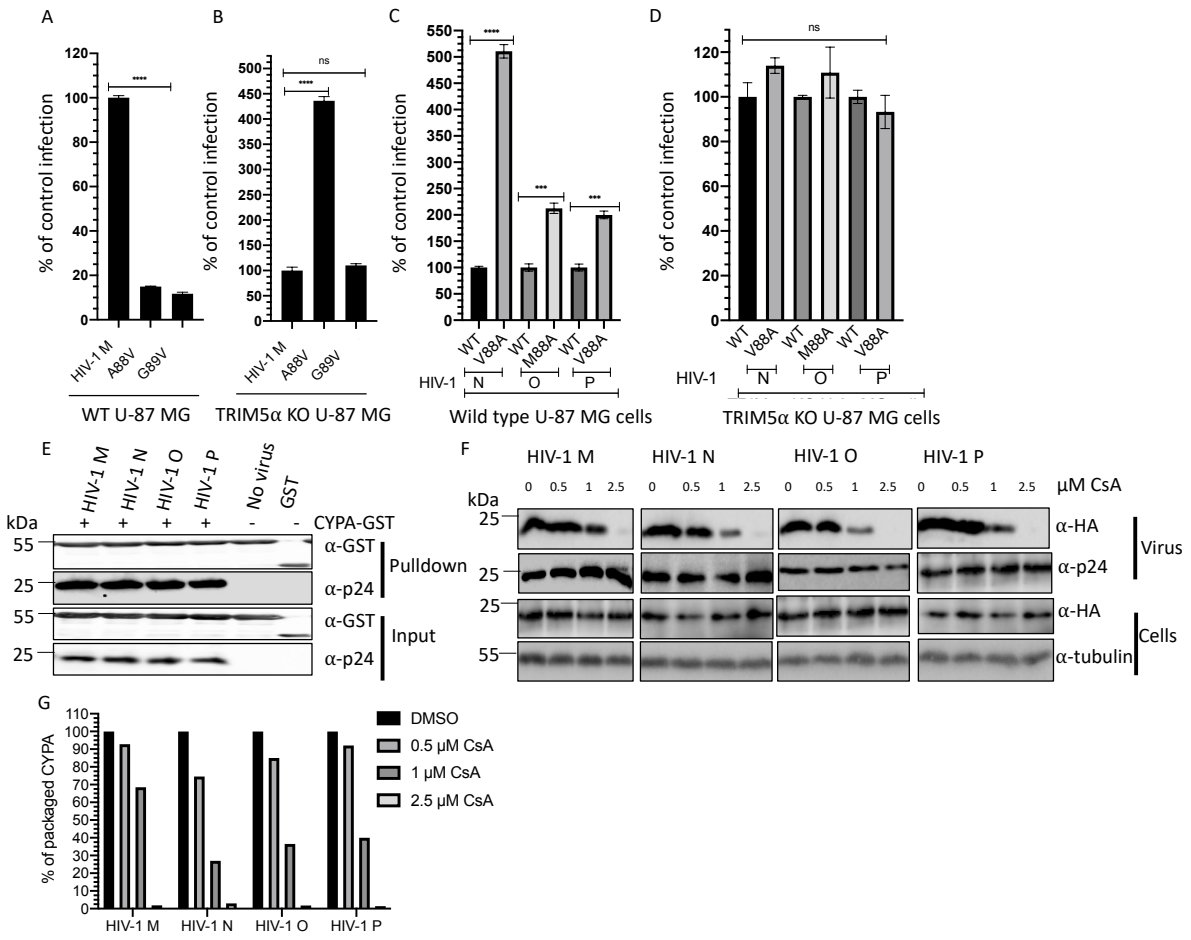

Fig 2. **Capsid residues 88 and 89 mediates susceptibility to huTRIM5α**. (A, B) A88V and G89V capsid mutations for HIV-1 M , (C, D) V88A for HIV-1 N, M88A for HIV-1 O and V88A mutation for HIV-1 P were introduced in their respective capsids. WT and capsid mutants were tested in infections of WT or TRIM5α KO U-87MG cells. Infection results of WT viruses were compared to their specific capsid mutants. (E) GST-pulldown of CYPA (CYPA-GST) with capsid proteins of HIV-1 M, N, O or P. GST: GST not fused to CYPA. Viral lysates and GST-tagged CYPA protein lysates were specifically incubated together with GST Sepharose beads. The elute was subjected to western blots to detect viral p24 (capsid) and GST-tagged CYPA (pulldown). Cell and viral lysates were also loaded as inputs for GST-tagged CYPA and p24, respectively. (F) Immunoblot of viral particles and corresponding virus producer cells. The level of CYPA packaged by virions was analyzed by anti-HA staining (for CYPA-HA), virus was confirmed by anti-p24 (capsid) antibody staining, anti-tubulin was used to confirm equal amounts of cell lysates loaded. Cells were treated with cyclosporine A (CsA) from 0 to 2.5 μM. (G) The amount of packaged CYPA in relation to the used CsA dose was quantified using ImageJ.

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Pandemic and non-pandemic HIV-1 capsids bind CYPA.

With the observed differences in capsid CYPA loop between M and non-M HIV-1s (Fig. 1F), we wanted to test whether all the four viruses interact with CYPA similarly. Using a GST-tagged CYPA, we assessed CYPA-capsid interaction in pulldown experiments. Immuno blots of these precipitations found similar levels of capsid protein for all four HIV-1s (Fig. 2E). In addition, we found that CYPA is packaged by virions of pandemic and non-pandemic HIV-1s. To understand on whether these viruses bind similarly to CYPA, we exposed them to CYPA

in the presence of increasing amounts of cyclosporine A (CsA) and then explored CYPA packaging patterns. Interestingly, non-M viruses lost CYPA packaging even with smaller doses of CsA as opposed to HIV-1 M. A CsA dose of 1 μM was enough to reduce packaged CYPA by around 70% in the context of non-M HIVs, as opposed to only 40% by HIV-1 M, suggesting differences in CYPA binding strength between capsids of these viruses (Figs. 2F-G).

Rhesus TRIMCyp inhibits non-M HIVs.

In TRIMCyp from rhesus macaques (rh), CYPA differs from human CYPA by two residues, N66D and H69R[39]. It was shown that HIV-1 M's resistance and HIV-1 O's inhibition by rhTRIMCyp was due to the presence of N66 and H69in this CYPA domain[40].

To further understand if rhTRIMCyp or a variant in which we reversed the two residues to D66 and R69 identical to human CYPA have differential antiviral activity to pandemic and non-pandemic HIV-1, we generated cell lines expressing WT rhTRIMCyp or its different mutants in CYPA domain, the N66D, H69R, N66D-H69H (Fig. 3A). We challenged such cells with reporter virions of HIV-1 M, HIV-1 N, HIV-1 O, HIV-1 P, HIV-2AB and SIVgor and analyzed against control cell infections. As expected, rhTRIMCyp did not reduce the infectivity of HIV-1 M, but strongly inhibited HIV-2AB and HIV-1 O.

In addition, all other viruses, HIV-1 N, HIV-1 P, SIVgor were also strongly inhibited in cells expressing rhTRIMCyp by or more than 90% (Figs. 3B-C and Suppl. Fig. S3). TRIMCyp with a human identical CYPA domain (N66D H69R, DR), recognized pandemic and non-pandemic viruses and displayed strong antiviral activity, further indicating that human CYPA interacts with all four viruses. Mutating only residue H69R (NR) in rhTRIMCyp caused a complete loss of antiviral activity against HIV-1 N and HIV-1 O, in addition to remaining inactive against HIV-1 M. However, HIV-1 P, HIV-2 and SIVgor were still inhibited by around 50%. In

contrast, mutating N66D (DH) in rhTRIMCyp, generated an antiviral protein that inhibited efficiently all tested viruses (Figs. 3B-C and Suppl. Fig. S3).

Previous data suggested that residue 88 in the CYPA binding loop mediates differential interaction of HIV-1 M and O with rhTRIMCyp[40].

To extend the understanding of the role of capsid residue 88 on the activity of rhTRIMCyp and its mutants on other HIVs, we tested viruses mutated in residue 88 in the capsid, and additionally the G89V mutant for HIV-1 M. For HIV-2, two mutants were analyzed, with alanine or methionine insertion at position equivalent to 88 in HIV-1, just before the glycine-proline (GP) motif.

The mutations in residue 88 reversed the resistance of HIV-1 M and the sensitivity of HIV-1 N, HIV-1 O and HIV-1 P to rhTRIMCyp (Figs. 3B-C). However, HIV-2 with insertion at position 87 were sensitive to rhTRIMCyp similar as WT HIV-2 (Suppl. Fig. S3). The non-pandemic HIVs with capsid mutations at position 88 were still sensitive to antiviral activity of TRIMCyp.DR, as was HIV-1 M A88V (Fig. 3B). The HIV-1 M G89V, was predictably neither inhibited by any TRIMCyp protein, likely because the G89V mutation prevented interaction of TRIMCyp (Fig. 3B).

These data suggest, that the human CYPA domain in TRIMCyp proteins and likely also free CYPA can recognize the incoming viral cores of non-pandemic HIV-1s.

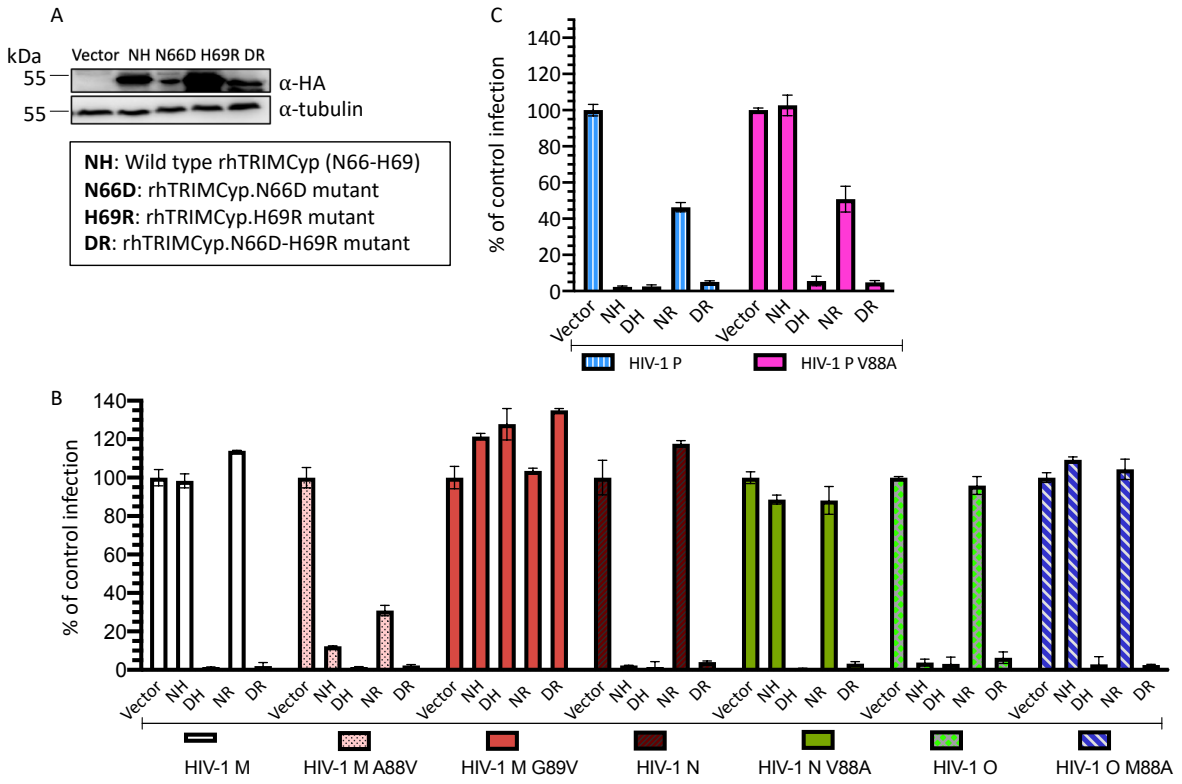

Fig. 3. **Wild type, N66D, H69R or N66D-H69R mutations in CYPA domain of rhTRIMCyp or capsid mutations at position 88 affect rhTRIMCyp antiviral activity in a virus-dependent way**. (A) Immunoblot of CrFK cells expressing WT rhTRIMCyp or its mutants with N66D (rhTRIMCyp.N66D) or H69R (rhTRIMCyp.H69R) or N66D-H69R (rhTRIMCyp.N66D.H69R). (B, C) CrFK cells expressing WT or mutated rhTRIMCyps were infection by WT luciferase reporter viruses from HIV-1 M, N, O or P or their capsid mutants at position 88. Two to three days after infection, cells were lysed, and luciferase activity was measured. Infectivity of each virus on control cells expressing the empty vector (vector) were used as reference. NH: wild type rhTRIMCyp with N66 and H69 in CYPA domain, DH: rhTRIMCyp with N66D mutation in CYPA, NR: rhTRIMCyp with H69R mutation in CYPA, DR: rhTRIMCyp with N66D and H69R mutations in CYPA. The experiment was done for at least three times and always in triplicates.

Pandemic and non-pandemic HIVs show different dependency on CYPA.

To test whether the depletion of CYPA affects pandemic and non-pandemic HIVs differently, a CYPA knockdown in U-87 MG cells and huTRIM5α KO U-87 MG using the CRISPR/Cas9 system was done (Fig. 4A).

CYPA knockdown in WT cells was associated with a strong and significant inhibition of HIV-1 M infectivity by up to 86%, and while the TRIM5α KO generated infections comparable to WT cells, the cells that lost TRIM5α and additionally CYPA expression were unexpectedly less infectable than WT or TRIM5α KO cells, suggesting that CYPA function for HIV-1 M is beyond protection against TRIM5α (Fig. 4B).

Non-M HIVs but also SIVgor were also tested in such conditions of CYPA knockdown or CYPA and huTRIM5α double depletions. The CYPA KD affected the non-pandemic HIVs less strongly than HIV-1 M and caused only 50% inhibition. In further contrast to the pandemic HIV-1 M, cells with no TRIM5α and no CYPA were much better infectable than the TRIM5α KO cells (Figs. 4C-E). Infections with HIV-2 and SIVgor differed to all HIV-1s and were not affected by CYPA KD in WT or TRIM5α KO cells, demonstrating that the CYPA KD did not impair cell vitality (Suppl. Figs. S2A-B).

In an additional approach, we tested CsA treatment of cells in infection experiments. Increasing levels of CsA (0.1 μM to 10 μM) inhibited up 91% of infection by HIV-1 M in WT U87 MG cells (Fig. 4F). In WT cells treated with 1 μM CsA, HIV-1 N was inhibited by 41%, HIV-1 O by 57%, and HIV-1 P by 66%. Interestingly, high CsA concentrations had no further effect on infections by HIV-1 N and HIV-1 O but inhibited 77% of infection of HIV-1 P (Fig. 4F).

As expected from experiments shown in Suppl. Fig. S2C, both, HIV-2AB and SIVgor did not react to CsA treatment of WT U-87 MG cells. In CYPA depleted cells, CsA treatment lost almost its antiviral activity and only HIV-1 M showed some mild inhibition using 10 μM CsA (Fig. 4G). In TRIM5α KO cells CsA pretreatment inhibited only against HIV-1 M but had no effect on non-M HIVs, mirroring data obtained with CYPA KD (Fig. 4H). In CYPA and TRIM5α double depleted cells, CsA had no significant inhibitory activity against any virus tested, suggesting that CsA itself does inhibit these viruses (Fig. 4I). Together, these data may suggest that the non-pandemic HIV-1s have a weak and partial protection against TRIM5α by its CYPA binding.

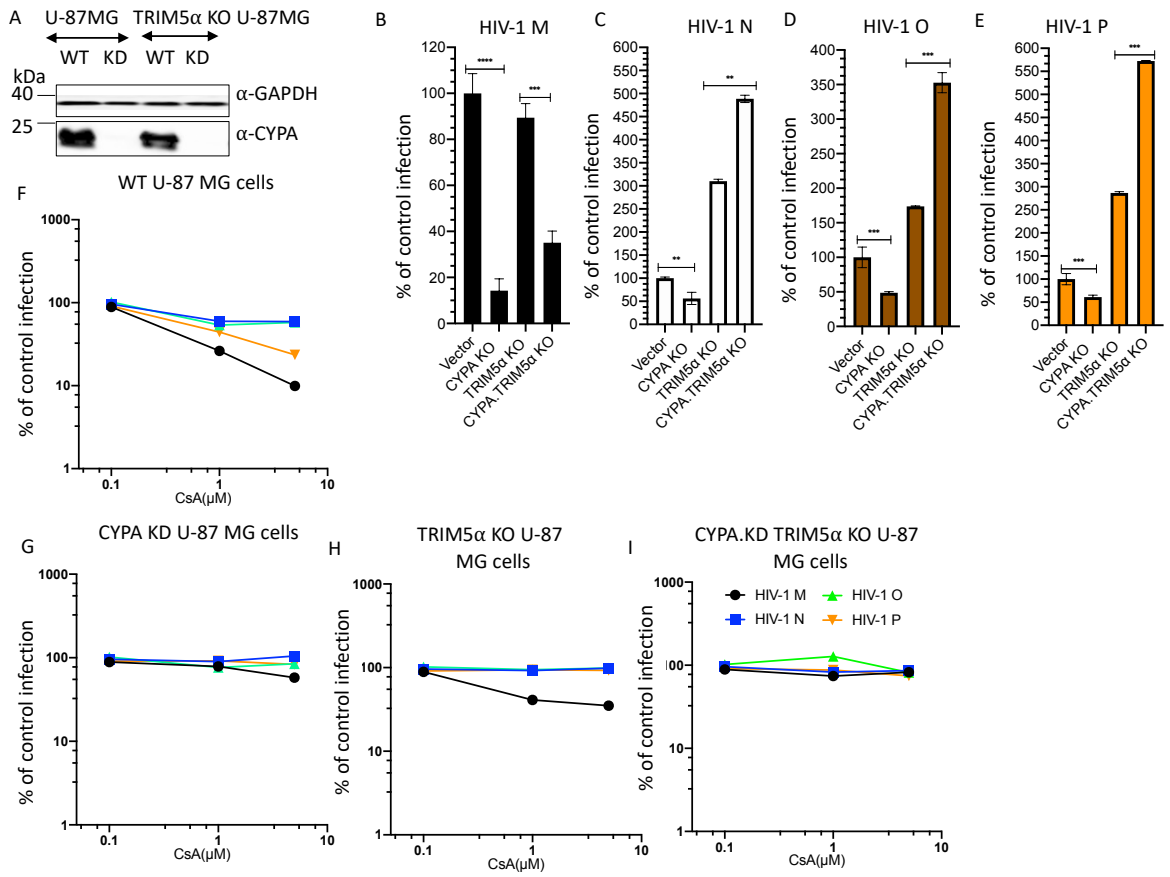

Fig. 4. **CYPA knockdown and CYPA, TRIM5α double depletion differentially affect infection by HIV-1 M, N, O and P.** (A) Immunoblot of CYPA KD in WT and in TRIM5α knockout U-87 MG cells. CYPA expression was detected by anti-CYPA antibodies and GAPDH detection served as control for equal protein loading. (B-E) WT and TRIM5α, CYPA, and double KO U-87 MG cells were infected by HIV luciferase reporter viruses from different groups M, N, O or P for 48 to 72 hours and luciferase activity was measured to assess infection.(F-I) HIV luciferase reporter viruses from groups M, N, O or P infection, in the presence of increasing amounts of cyclosporine A (CsA), of (F) WT U-87 MG cells, (G) CYPA KD U-87 MG cells, (H) TRIM5α KO U-87 MG cells, and (I) CYPA KD - TRIM5α double KO U-87 MG cells. Two to three days later, luciferase activity was assessed, and data analysis was done in comparison with control infection. Each experiment was repeated at least three times independently with similar findings.

Discussion

The reason(s) why non-M HIVs did not extensively spread in the human population remain elusive. Some reports have pointed out differences in GAG capsid sequences but also differences in functional activity of viral accessory proteins[41,424344,45]. Here, we found that non-pandemic HIV-1 are subject to restriction by human TRIM5α. While it was shown that CYPA forms a protective layer around the viral core of HIV-1 M to prevent destruction by human TRIM5α[18,43], the CYPA interaction with non-pandemic HIV-1 N, O and P does not give a strong protection against TRIM5α.

Our data demonstrate that the non-pandemic HIV-1s interact and bind CYPA to a level that appears similar to the level of binding of HIV-1 M. Surprisingly, our findings show that the viral capsid CYPA binding loop determines the different sensitivities to TRIM5α of HIV-1 M and the non-pandemic HIV-1s. A recent report by[44] has suggested that capsid residues such as residue at position 50 are important for differential sensitivity to TRIM5α by HIVs.

Our findings that capsid CYPA binding loop regulates HIV sensitivity to TRIM5α suggest that more than one region of the capsid are involved in regulation of TRIM5α activity.

Thus, we postulate that the nature of CYPA capsid interaction is a regulator of TRIM5α sensitivity. Despite identical GP motif of their capsid CYPA binding loops, these viruses engage CYPA differently, also suggesting important role from other residues in the loop, such as residue 88, described by our findings as a key residue for this differential susceptibility to TRIM5α.

Inhibition of non-M HIVs by rhTRIMCyp also confirms that these viruses engage with CYPA. A rescue of their infection when their capsids were specifically mutated at position 88 or when CYPA domain of rhTRIMCyp is mutated at position 66 or 69, show importance of the capsid CYPA binding loop for such restriction by rhTRIMCyp, as previously shown for HIV-1 M, HIV-1 O and HIV-2[25,26].

It is possible that the presence of valine (HIV-1 N and HIV-1 P) or methionine for HIV-1 O, at capsid position 88, as opposed to alanine in the HIV-1 M, changes capsid conformation and avails it to other host factors such as TRIM5α, in addition to CYPA binding. This would then explain a restriction of non-M HIVs by TRIM5α, but this hypothesis would need more clarification. We also postulate that the CYPA cis/trans activity on capsid G89-P90 peptide bond[45,46], differs between HIV-1 M and non-pandemic HIVs and that this peptide bond differential isomerization changes the binding strength of TRIM5α in an unknown way. The binding sites for TRIM5α are not well known[47].

CYPA has been highlighted to be involved in several steps of HIV-1 infection, including uncoating, nuclear import and beyond [48]. Our findings that HIV-1 M infection was strongly negatively affected, as compared to other viruses we tested in TRIM5α KO U-87 MG cells, when CYPA was blocked by CsA or knocked down also suggest another important protective role of CYPA for HIV-1 M. This constitutes an important difference between non-M group HIV-1s and the pandemic M group HIV-1.

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References

1. Villabona-Arenas CJ, Domyeum J, Mouacha F, et al. HIV-1 group O infection in Cameroon from 2006 to 2013: Prevalence, genetic diversity, evolution and public health challenges. Infect Genet Evol. 2015;36:210-216. doi:10.1016/j.meegid.2015.09.006
2. Bell SM, Bedford T. Modern-day SIV viral diversity generated by extensive recombination and cross-species transmission. Silvestri G, ed. PLoS Pathog. 2017;13(7):e1006466. doi:10.1371/journal.ppat.1006466
3. Twizerimana AP, Scheck R, Häussinger D, Münk C. Post-entry restriction factors of SIVcpz. Future Virol. 2018;13(10):727-745. doi:10.2217/fvl-2018-0093
4. Harris RS, Hultquist JF, Evans DT. The Restriction Factors of Human Immunodeficiency Virus. Journal of Biological Chemistry. 2012;287(49):40875-40883. doi:10.1074/JBC.R112.416925
5. Malim MH, Bieniasz PD. HIV Restriction Factors and Mechanisms of Evasion. Cold Spring Harb Perspect Med. 2012;2(5):a006940. doi:10.1101/cshperspect.a006940
6. Shi J, Friedman DB, Aiken C. Retrovirus Restriction by TRIM5 Proteins Requires Recognition of Only a Small Fraction of Viral Capsid Subunits. J Virol. 2013;87(16):9271-9278. doi:10.1128/JVI.00713-13
7. Reymond A, Meroni G, Fantozzi A, et al. The tripartite motif family identifies cell compartments. EMBO J. 2001;20(9):2140-2151. doi:10.1093/emboj/20.9.2140
8. D'Cruz AA, Babon JJ, Norton RS, Nicola NA, Nicholson SE. Structure and function of the SPRY/B30.2 domain proteins involved in innate immunity. Protein Science. 2013;22(1):1-10. doi:10.1002/pro.2185
9. Hatziioannou T, Cowan S, von Schwedler UK, Sundquist WI, Bieniasz PD. Species-specific tropism determinants in the human immunodeficiency virus type 1 capsid. J Virol. 2004;78(11):6005-6012. doi:10.1128/JVI.78.11.6005-6012.2004
10. Ganser-Pornillos BK, Pornillos O. Restriction of HIV-1 and other retroviruses by TRIM5. Nat Rev Microbiol. 2019;17(9):546-556. doi:10.1038/s41579-019-0225-2
11. Jolicoeur P, Baltimore D. Effect of Fv-1 Gene Product on Proviral DNA Formation and Integration in Cells Infected with Murine Leukemia Viruses (N-Tropic Murine Leukemia Virus/B-Tropic Murine Leukemia Virus/Provirus). Vol 73.; 1976. Accessed March 21, 2022. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC430510/pdf/pnas00037-0075.pdf

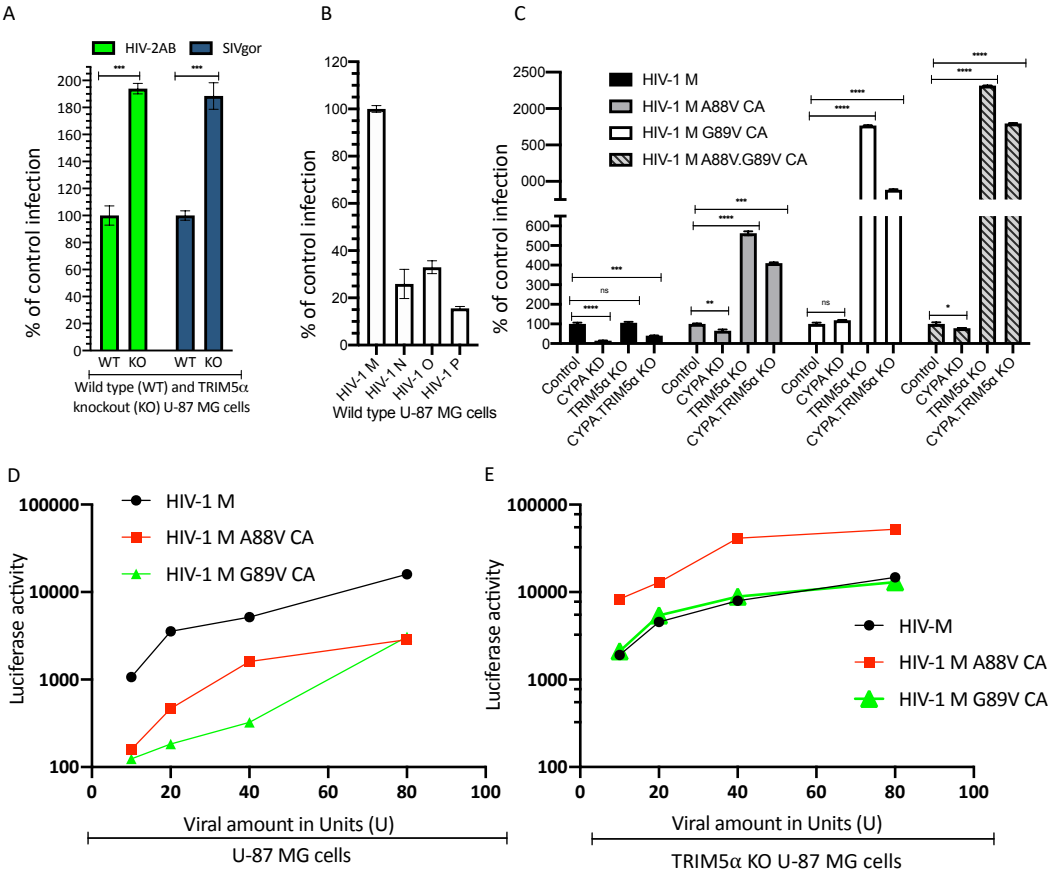
12. Black LR, Aiken C. TRIM5α Disrupts the Structure of Assembled HIV-1 Capsid Complexes In Vitro. J Virol. 2010;84(13):6564-6569. doi:10.1128/JVI.00210-10
13. Ganser-Pornillos BK, Pornillos O. Restriction of HIV-1 and other retroviruses by TRIM5. Nat Rev Microbiol. 2019;17(9):546-556. doi:10.1038/s41579-019-0225-2
14. Pertel T, Hausmann S, Morger D, et al. TRIM5 is an innate immune sensor for the retrovirus capsid lattice. doi:10.1038/nature09976
15. Brennan G, Kozyrev Y, Hu SL. TRIMCyp expression in Old World primates Macaca nemestrina and Macaca fascicularis. Proceedings of the National Academy of Sciences. 2008;105(9):3569-3574. doi:10.1073/pnas.0709511105
16. Nisole S, Lynch C, Stoye JP, Yap MW. A Trim5-cyclophilin A fusion protein found in owl monkey kidney cells can restrict HIV-1. Proceedings of the National Academy of Sciences. 2004;101(36):13324-13328. doi:10.1073/pnas.0404640101
17. Keckesova Z, Ylinen LMJ, Towers GJ. Cyclophilin A Renders Human Immunodeficiency Virus Type 1 Sensitive to Old World Monkey but Not Human TRIM5α Antiviral Activity. J Virol. 2006;80(10):4683-4690. doi:10.1128/JVI.80.10.4683-4690.2006
18. Selyutina A, Persaud M, Simons LM, et al. Cyclophilin A Prevents HIV-1 Restriction in Lymphocytes by Blocking Human TRIM5α Binding to the Viral Core. Cell Rep. 2020;30(11):3766-3777.e6. Accessed June 1, 2020. https://doi.org/10.1016/j.celrep.2020.02.100
19. Berthoux L, Sebastian S, Sokolskaja E, Luban J. Cyclophilin A Is Required for TRIM5-Mediated Resistance to HIV-1 in Old World Monkey Cells.; 2005. Accessed August 11, 2019. www.pnas.orgcgidoi10.1073pnas.0505659102
20. Braaten D, Luban J. Cyclophilin A regulates HIV-1 infectivity, as demonstrated by gene targeting in human T cells. EMBO J. 2001;20(6):1300-1309. doi:10.1093/emboj/20.6.1300
21. Braaten D, Ansari H, Luban J. The Hydrophobic Pocket of Cyclophilin Is the Binding Site for the Human Immunodeficiency Virus Type 1 Gag Polyprotein. Vol 71. J Virol; 1997. doi:10.1128/JVI.71.3.2107-2113.1997
22. Braaten D, Franke EK, Luban J. Cyclophilin A Is Required for the Replication of Group M Human Immunodeficiency Virus Type 1 (HIV-1) and Simian Immunodeficiency Virus SIV CPZ GAB but Not Group O HIV-1 or Other Primate Immunodeficiency Viruses. Vol 70.; 1996. Accessed December 5, 2021. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC190352/pdf/704220.pdf

23. Bastien Nisole S, Lynch C, Stoye JP, et al. A Trim5-Cyclophilin A Fusion Protein Found in Owl Monkey Kidney Cells Can Restrict HIV-1. Vol 101.; 2004:13324-13328. doi:10.1073/pnas.0404640101
24. Nakayama EE, Shioda T. TRIM5α and species tropism of HIV/SIV. Front Microbiol. 2012;3(JAN):1-12. doi:10.3389/fmicb.2012.00013
25. Nakayama EE, Shioda T. TRIM5α and Species Tropism of HIV/SIV. Front Microbiol. 2012;3:13. doi:10.3389/fmicb.2012.00013
26. Price AJ, Marzetta F, Lammers M, et al. Active site remodeling switches HIV specificity of antiretroviral TRIMCyp. Nat Struct Mol Biol. 2009;16(10):1036-1042. doi:10.1038/nsmb.1667
27. Jimenez-Guardeño JM, Apolonia L, Betancor G, Malim MH. Immunoproteasome activation enables human TRIM5α restriction of HIV-1. Nat Microbiol. 2019;4(6):933-940. doi:10.1038/s41564-019-0402-0
28. Takehisa J, Kraus MH, Ayouba A, et al. Origin and biology of simian immunodeficiency virus in wild-living western gorillas. J Virol. 2009;83(4):1635-1648. doi:10.1128/JVI.02311-08
29. Takehisa J, Kraus MH, Decker JM, et al. Generation of infectious molecular clones of simian immunodeficiency virus from fecal consensus sequences of wild chimpanzees. J Virol. 2007;81(14):7463-7475. doi:10.1128/JVI.00551-07
30. Bock M, Bishop KN, Towers G, Stoye JP. Use of a Transient Assay for Studying the Genetic Determinants of Fv1 Restriction. J Virol. 2000;74(16):7422-7430. doi:10.1128/JVI.74.16.7422-7430.2000
31. Wilson SJ, Webb BLJ, Ylinen LMJ, Verschoor E, Heeney JL, Towers GJ. Independent evolution of an antiviral TRIMCyp in rhesus macaques. Proc Natl Acad Sci U S A. 2008;105(9):3557-3562. doi:10.1073/pnas.0709003105
32. Vermeire J, Naessens E, Vanderstraeten H, et al. Quantification of reverse transcriptase activity by real-time PCR as a fast and accurate method for titration of HIV, lenti- and retroviral vectors. PLoS One. 2012;7(12):e50859. doi:10.1371/journal.pone.0050859
33. Twizerimana AP, Scheck R, Becker D, et al. Cell Type-Dependent Escape of Capsid Inhibitors by Simian Immunodeficiency Virus SIVcpz. J Virol. 2020;94(23). doi:10.1128/JVI.01338-20
34. Zhang F, Hatziioannou T, Perez-Caballero D, Derse D, Bieniasz PD. Antiretroviral potential of human tripartite motif-5 and related proteins. Virology. 2006;353(2):396-409. doi:10.1016/J.VIROL.2006.05.035

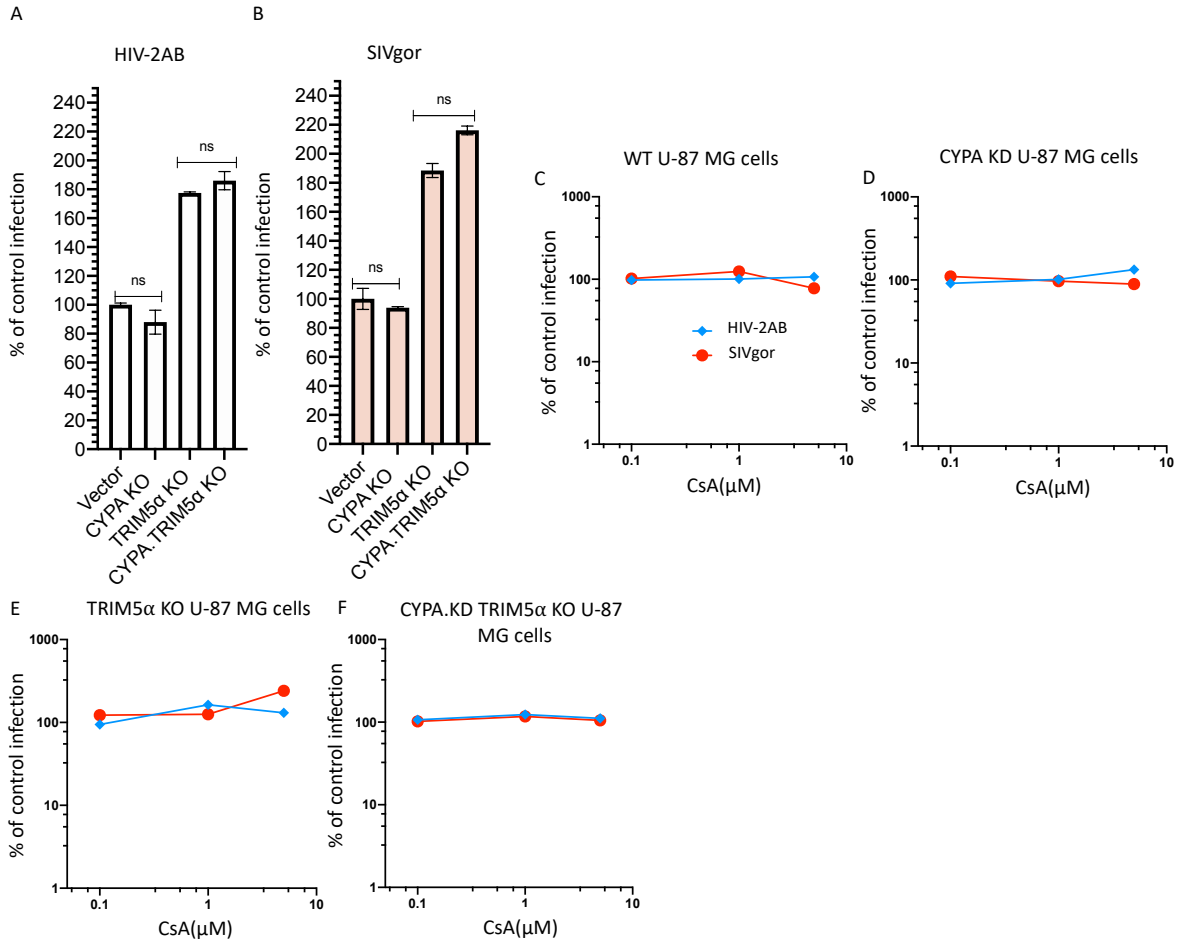
35. Grütter MG, Luban J. TRIM5 structure, HIV-1 capsid recognition, and innate immune signaling. Curr Opin Virol. 2012;2(2):142-150. doi:10.1016/j.coviro.2012.02.003
36. Tipper C, Sodroski JG. Contribution of glutamine residues in the helix 4-5 loop to capsid-capsid interactions in simian immunodeficiency virus of macaques. J Virol. 2014;88(18):10289-10302. doi:10.1128/JVI.01388-14
37. Sokolskaja E, Sayah DM, Luban J. Target Cell Cyclophilin A Modulates Human Immunodeficiency Virus Type 1 Infectivity. J Virol. 2004;78(23):12800-12808. doi:10.1128/JVI.78.23.12800-12808.2004
38. Saphire ACS, Bobardt MD, Gallay PA. Host cyclophilin A mediates HIV-1 attachment to target cells via heparans. EMBO J. 1999;18(23):6771-6785. doi:10.1093/emboj/18.23.6771
39. Virgen CA, Kratovac Z, Bieniasz PD, Hatziioannou T. Independent genesis of chimeric TRIM5-cyclophilin proteins in two primate species. Proc Natl Acad Sci U S A. 2008;105(9):3563-3568. doi:10.1073/pnas.0709258105
40. Price AJ, Marzetta F, Lammers M, et al. Active site remodeling switches HIV specificity of antiretroviral TRIMCyp. Nat Struct Mol Biol. 2009;16(10):1036-1042. doi:10.1038/nsmb.1667
41. Mack K, Starz K, Sauter D, et al. Efficient Vpu-Mediated Tetherin Antagonism by an HIV-1 Group O Strain. Silvestri G, ed. J Virol. 2017;91(6):e02177-16. doi:10.1128/JVI.02177-16
42. Cano-Ortiz L, Gu Q, de Sousa-Pereira P, et al. Feline Leukemia Virus-B Envelope Together With its GlycoGag and Human Immunodeficiency Virus-1 Nef Mediate Resistance to Feline SERINC5. J Mol Biol. 2022;434(6):167421. doi:10.1016/J.JMB.2021.167421
43. Kim K, Dauphin A, Komurlu S, et al. Cyclophilin A protects HIV-1 from restriction by human TRIM5α. Nat Microbiol. 2019;4(12):2044-2051. doi:10.1038/s41564-019-0592-5
44. Zuliani-Alvarez L, Govasli ML, Rasaiyaah J, et al. Evasion of cGAS and TRIM5 defines pandemic HIV. Nat Microbiol. 2022;7(11):1762-1776. doi:10.1038/s41564-022-01247-0
45. Howard BR, Vajdos FF, Li S, Sundquist WI, Hill CP. Structural insights into the catalytic mechanism of cyclophilin A. Nat Struct Mol Biol. 2003;10(6):475-481. doi:10.1038/nsb927

46. Bosco DA, Eisenmesser EZ, Pochapsky S, Sundquist WI, Kern D. Catalysis of cis/trans isomerization in native HIV-1 capsid by human cyclophilin A. Proceedings of the National Academy of Sciences. 2002;99(8):5247-5252. doi:10.1073/pnas.082100499
47. Yu A, Skorupka KA, Pak AJ, Ganser-Pornillos BK, Pornillos O, Voth GA. TRIM5α self-assembly and compartmentalization of the HIV-1 viral capsid. Nat Commun. 2020;11(1):1307. doi:10.1038/s41467-020-15106-1
48. Schaller T, Ocwieja KE, Rasaiyaah J, et al. HIV-1 Capsid-Cyclophilin Interactions Determine Nuclear Import Pathway, Integration Targeting and Replication Efficiency. Aiken C, ed. PLoS Pathog. 2011;7(12):e1002439. doi:10.1371/journal.ppat.1002439

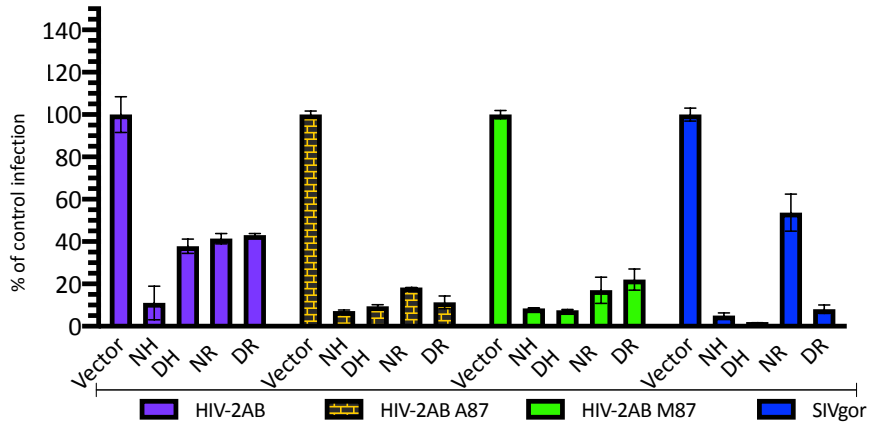
Supplementary figures



S1. Infection by HIV-2AB and SIVgor increases in the absence of TRIM5α, non-M HIV-1s show a reduced infection in wild type U-87 MG cells, HIV-1 M Capsid residues 88 and 89 mediate its susceptibility to huTRIM5α in U-87MG cells. Equal amounts of virions for HIV-2AB and SIVgor (A), HIV-1 M, HIV-1 N, HIV-1 O and HIV-1 P (B), HIV-1 M, its A88V, G89V and A88V.G89V capsid mutants (C-E) were used to infect wild type, TRIM5α KO, CYPA KD or CYPA and TRIM5α doubly depleted U-87 MG cells. Luciferase activity was measured two to three days after infection and infection patterns were compared. The experiment was repeated for a minimum of three times.



S2. CYPA knockdown does not affect HIV-2AB and SIVgor infection in U-87 MG cells. (A-B) WT and TRIM5α, CYPA, and double KO U-87 MG cells were infected by HIV-2AB and SIVgor nanoluciferase reporter viruses for 48 to 72 hours and nanoluciferase activity was measured to assess infection. (F-I) nanoluciferase reporter viruses for HIV-2AB and SIVgor were used for infection, in the presence of increasing amounts of cyclosporine A (CsA), of (C) WT U-87 MG cells, (D) CYPA KD U-87 MG cells, (E) TRIM5α KO U-87 MG cells, and (F) CYPA KD - TRIM5α double KO U-87 MG cells. Two to three days later, luciferase activity was assessed, and data analysis was done in comparison with control infection. Each experiment was repeated at least three times independently with similar findings.



S3. Wild type N66D, H69R or N66D-H69R mutations in CYPA domain of rhTRIMCYPA or capsid mutation at position 88 affect rhTRIMCYPA antiviral activity in a virus-dependent way. Wild type rhTRIMCYPA or rhTRIMCYP with N66D (rhTRIMCYPA.N66D) or H69R (rhTRIMCYPA.H69R) or N66D-H69R (rhTRIMCYPA.N66D.H69R) mutations in CYPA domain were stably expressed in CrFK and such stable cells were used to explore infection by HIV-2AB and SIVgor. Two to three days after infection, cells were lysed, and luciferase activity was measured. The experiment was done for at least three times and always in triplicates.

CHAPTER III

Anti-HIV-1 activity of Cyclophilin B is mediated by various residues of the capsid cyclophilin A binding loop.

Augustin Penda Twizerimana[1], Daniel Becker[2], Marianne Wammers[1], Tom Luedde[1], Holger Gohlke[2,3,4,5], Carsten Münk[1]. Anti-HIV-1 activity of Cyclophilin B is mediated by various residues of the capsid cyclophilin A binding loop.

Twizerimana's contribution to this work:

1. Executed all the experiments in this work.
2. Wrote the original draft of the manuscript.

Manuscript ready for publication.

Anti-HIV-1 activity of Cyclophilin B is mediated by various residues of the capsid cyclophilin A binding loop.

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Abstract

The cell entry of human immunodeficiency viruses (HIVs) or simian immunodeficiency viruses (SIVs), is associated with multiple events that culminate into activation or suppression of specific cellular pathways. Here, we have assessed the role of human cyclophilin B (CYPB, PPIB) during HIV-1 infection. CYPB core domain is a peptidylprolyl isomerase and shares 80% similarity with cyclophilin A (CYPA). CYPB depletion in HOS, HeLa or THP-1 cells enhanced infection by HIV-1 M but not of SIVgor. Non-M HIVs benefited from CYPB depletion in a more complex cell-line dependent way. Furthermore, CYPB overexpression or its fusion protein with human or rhesus TRIM5 (huTrimCYPB or rhTrimCYPB) inhibited all HIV-1 but SIVgor was inhibited only by TrimCYPB fusion proteins. In HIV-1 capsid, different residues are important for the CYPB activity and G89V, A92E-G94D mutations abolished CYPB or TrimCYPB inhibition of HIV-1. An uncoating assay, as well as the quantification of HIV-1 2-LTR circular DNA indicated that CYPB targets early steps of HIV-1 infection. Together, these findings show an antiviral signature of human cyclophilin B against viruses from the four groups of HIV-1.

Introduction

Following independent cross-species transmissions of simian immunodeficiency viruses (SIVs) to humans, transmitted viruses adapted to the human cellular environment as human immunodeficiency viruses of type 1 and type 2 (HIV-1 and HIV-2)[1,2] and successfully settled in a latent, chronic but also pathogenic profile that leads to acquired immunodeficiency syndrome or AIDS[2,3]. As of 2021, around 38.4 million people were living with HIV globally and approximately 40 million deaths related to HIV/AIDS have been recorded since the discovery of HIV, mainly due to the pandemic group M HIV-1 viruses[4–7]. Several cellular factors are known to play either a restrictive or enhancement role during HIV and SIV infection[8–14]. In addition, HIVs express accessory proteins to counteract restriction factors[15,16]. The HIV capsid core and its role during infection have been extensively studied and it is now clear that viral capsid is a binding site for diverse cellular factors[17]. Anti-retroviral drugs that target viral capsid are already in the clinics and new approaches are under study to develop additional capsid inhibitors[18–22]. Cyclophilin A (CYPA) is an example of a peptidylprolyl isomerase that binds the viral capsid to impact HIV infection[23,24]. In the HIV-1 capsid, CYPA binds in a loop from residues at position 85 to 93 that forms between helix 4 and helix 5, the so called capsid CYPA binding loop[25,26]. During HIV-1 infection, cyclophilin A affects multiple steps in replication spanning from viral uncoating to nuclear import[27,28]. For instance, CYPA is known to block human Tripartite Motif-containing protein 5 α (TRIM5α) from engaging with HIV-1 capsid and this facilitates infection[1]. CYPA is also packaged by HIV-1, but the biological relevance is unknown[29,30]. In some primates and through a retrotransposition process, CYPA has replaced the capsid binding domain of TRIM5α to form TrimCyp proteins which differentially restrict retroviruses[28,32]. In addition, CYPA affects antiviral activity of HIV capsid binding small molecules commonly referred to as capsid inhibitors such as PF74[33,34]. Cyclophilin B (CYPB, PPIB) is another peptidylprolyl isomerase of the immunophilin family

of proteins, which localizes across many cellular compartments and has endoplasmic reticulum and nuclear localization signals [35]. CYPB has a high similarity to CYPA and like CYPA, it configures peptidyl-prolyl bonds from cis- to the trans-state, a function important for protein folding, CYPB is also involved in many other cellular functions such as immune response[36,37]. CYPB binds HIV-1 GAG[38] and it was recently reported to support HIV-1 replication[37]. Here, we have assessed the role of human CYPB during infection by viruses from the four groups of HIV-1 and SIVgor.

Material and methods

Cells. Wild type HOS (ATCC CRL-1543), HeLa (ATCC CCL-2), HEK293T (ATCC CRL3216) and CRFK (ATCC CCL-94) cells were maintained in Dulbecco's Modified Eagle's Medium (PAN-Biotech, Aidenbach, Germany) containing 10% of fetal calf serum (FBS), 2 mM L-glutamine and 100 U/ml penicillin and 100 U/ml streptomycin at 37°C under a 5% CO2 milieu. One μg/ml of puromycin was used to select HOS and HeLa cells. Feline CrFK cells stably expressing specific proteins were selected under 400 μg/ml of G418 (Biochrom GmbH, Berlin, Germany), a concentration of 800 μg/ml of the same drug was used to select HeLa cells expressing CYPB.HA, huTRIM.HA or TRIMCYPB.HA proteins. RPMI 1640 medium (PAN-Biotech), supplemented with 10% FBS, 1% Glutamine, 100 U/ml of penicillin and 100 U/ml streptomycin was used to culture THP-1 suspension cells. For CYPB knockdown and control THP-1 cell selection, a 2 μg/ml concentration of puromycin was used in RPMI.

Plasmids. pHIT60 with MLV *Gag-Pol*, HIV-1 vector pSIN.PPT.CMV.Luc.IRES.GFP, pRSV-Rev, pMDLg/pRRE, pMDLg/pRRE.G89V and pMD.G (VSV-G) have been previously described[34]. The HIV-1 construct psPAX2 was obtained from the NIH AIDS Reagent Program (Cat# 11348). HIV-1 M capsid mutants were produced as follows: Using fusion PCR, the

region flanking Eco72I (PmlI) and MunI (MfeI) in pMDLg/pRRE was amplified at the same time introducing A88V, A88V-G89V, A92E, G94D and A92E-G94D mutations in the capsid p24. Mutants PCR products were cloned into pMDLg/pRRE plasmid digested with Eco72I (PmlI) and MunI (MfeI) (Thermo Fischer Scientific, Langenselbold, Germany) to generate the pMDLg/pRRE.A88V, pMDLg/pRRE.A88V-G89V, pMDLg/pRRE.A92E, pMDLg/pRRE.G94D and pMDLg/pRRE.A92E-G94D.

Gag-Pol constructs for HIV-1 N, HIV-1 O, HIV-1 P and SIVgor were made as follow: The *Gag-Pol* sequences for HIV-1 N molecular clone HIV-1 N DJO0131, HIV-1 O clone RBF206, HIV-1 P clone RBF168 (all kindly provided by Frank Kirchhoff) and SIVgor clone CP2139 (NIH AIDSREAGENTS cat# 11722), obtained from NIH[39], were cloned in the pMDLg/pRRE plasmid, by replacing HIV-1 M *Gag-Pol* with their respective sequences for *Gag-Pol.* This generated pMDLg/pRRE.HIV-1 N, pMDLg/pRRE.HIV-1 O, pMDRLg/pRRE.HIV-1 P and pMDLg/pRRE.SIVgor and they are referred to, in this manuscript as HIV-1 N, HIV-1 O, HIV-1 P and SIVgor. In addition, HIV-1 N Gag-Pol construct was used to introduce V88A, G89V, V88A-G89V, P92E, G94D, P92E-G94D mutations in the capsid, using fusion PCR.

PCR on human cDNA from HeLa cells-extracted mRNA was used to produce CYPB.HA. To produce HA-tagged human TRIM5α truncate lacking the SPRY domain (huTRIM.HA), a forward primer with XhoI site and then a reverse primer with HA tag and NotI site were used for PCR on a human TRIM5α cDNA. HuTrimCYPB.HA fusion protein was produced using fusion PCR between SPRY domain-lacking huTRIM5α and C-terminally HA-tagged human CYPB cDNA. For rhTRIMCyp truncate (rhTRIM.HA), the fragment coding for residues 1-309 was PCR amplified with introduction of a C-terminal HA-tag, using rhTRIMCyp cDNA in a pLNCX2[32], as template. As for rhTrimCYPB fusion protein, CYPA-lacking rhTRIMCyp cDNA was fused with C-terminally HA-tagged human CYPB cDNA. ΔCYPB was constructed by deleting the first 40 amino acids that contain endoplasmic reticulum and nuclear localization

signals, this mutant cDNA was also used to make ΔTrimCYPB. Such PCR-amplified cDNA were cloned in a pLNCX2 vector digested with XhoI and NotI enzymes (Thermo Fischer Scientific, Langenselbold, Germany).

To generate GST-tagged human CYPB or GST-tagged ΔCYPB, CYPB cDNA or ΔCYPB (CYPB lacking the first 40 residues) cDNAs with HindIII and BamHI restriction sites were cloned in HindIII and BamHI (Thermo Fisher Scientific) digested GST-containing pkMyc vector to generate pkMyc.CYPB.GST or pkMyc.ΔCYPB.GST.

Luciferase reporter viral production. Wild type or capsid mutants luciferase reporter virions were produced through transfection, using 10^6 HEK293T cells per well, seeded in a well of a 6-well plate, a day before transfection. Polyjet (Tebubio GmbH, Offenbach, Germany), was used to complex and deliver a total of 2200 ng of plasmid DNA into the cells (200 ng of pMD.G VSV-G, 800 ng of pSIN.PPT.CMV.Luc.IRES.GFP, 400 ng of pRSV-Rev and 800 ng of WT pMDLg/pRRE or capsid mutants), according to manufacturer's instructions.

Generation of human CYPB knockout HOS, HeLa and THP-1 cells using CRISPR/Cas9 system. Complementary oligonucleotides containing specific human CYPB sgRNA sequences (CACCGCAGGGCGGAGACTTCACCAG and AAACCTGGTGAAGTCTCCGCCCTGC) were ligated in a BsmBI digested pLentiCRISPRv2[40], to generate a specific functional vector for CYPB KO. LentiCRISPRv2 plasmid lacking sgRNA sequence was used as empty vector control. Transfection of HEK293T cells with empty or pLentiCRISPRv2 transfer vector containing CYPB knockout sgRNA, packaging plasmid psPAX2, pRSV-Rev, and VSV-G plasmid generated VSV-G pseudotyped viral particles which were used to transduce HeLa, HOS or THP-1 cells for three days and transduced cells were selected under 1 μg/ml (HeLa and HOS) or 2 μg/ml (THP-1) puromycin. For CYPB knockout in HOS cells, we were not able

to achieve a significant protein depletion in the cell pool and HOS single cell clones were established using flow cytometry (FACS ARIA III Sorter, BD, Heidelberg, Germany). CYPB knockout in HeLa and THP-1 cells was associated with a significant CYPB depletion and knockdown population cells were used for further experiments. CYPB depletion was confirmed by immuno blots.

Single round infection. 10,000 adherent cells were seeded into 96-well plates and infection was performed the following day. For THP-1 cells, a differentiation was done using 25 ng/ml of phorbol 12-myristate-13-acetate (PMA) for 48 hours before infection. 48 to 72 hours post-infection, firefly luciferase activity was measured with the Steady-Glo Luciferase system (Promega, Mannheim, Germany), following instructions from the manufacturer, on a MicroLumat Plus luminometer (Berthold Detection Systems, Pforzheim, Germany), each experiment was performed at least three times and always in triplicates.

GST based pulldown experiments. 10^6 HEK293T cells per well were seeded into a 6-well plate for one day before transfection. 2200 ng of plasmid DNA (200 ng of pMD.G VSV-G with 800 ng of pSIN.PPT.CMV.Luc.IRES.GFP, 400 ng of pRSV-Rev, 800 ng of pMDLg/pRRE wild type constructs or capsid mutants were used for virus production. In addition, 1000 ng of pk.GST or pkMyc.CYPB.GST, pkMyc.ΔCYPB.GST together with 1200 ng of pcDNA3.1 were used to produce GST-tagged CYPB or the control GST. 48 hours post-transfection, viral supernatant was collected and centrifuged through 20% sucrose for a minimum of 4 hours at 4°C at 14800 rpm, viral pellet was lysed using a light lysis buffer (50 mM Tris-HCl [pH 7.4], 130 mM NaCl, 0.8% NP-40, 10% glycerol, in water) and protease inhibitor cocktail set III (Calbiochem, Darmstadt, Germany). Cells were lysed using same buffer for 20 minutes on ice. The supernatant of the cell lysate was added to GST Sepharose beads together with viral lysates

and incubated according to the beads manufacturer's instructions (GE Healthcare, Solingen, Germany). Protein complexes were washed and eluted from the beads for immunoblotting.

Immunoblot. Denaturation of cell or viral lysate or pulldown elute protein was done at 95°C for 5 minutes in RotiLoad loading buffer (Roth, Karlsruhe, Germany), followed by western blot. GST-tagged proteins were detected using anti-GST SAB4200237-200UL (Sigma-Aldrich, 1:7500 dilution). Tubulin was detected with the anti-tubulin antibody, DM1A, mouse, monoclonal (Sigma-Aldrich, 1:20,000 dilution). An anti–CYPB antibody, rabbit (Abcam, ab238511) was used to detect CYPB. Viral p24/p27 MAb AG3.0 (1:250 dilution) was used to detect capsid p24. HA-tagged proteins were detected using mouse anti-HA (catalog number MMS-101P; Covance, Münster, Germany) at a dilution of 1:7500. Horseradish peroxidase conjugated anti-mouse (1:10,000 dilution, NA931V, GE Healthcare) and anti-rabbit (1:10,000 dilution, NA9340V, GE Healthcare) were used as secondary antibodies. ECL prime reagent (GE Healthcare, Solingen, Germany) was used for signals detection.

HIV-1 2-LTR circular DNA measurement. To quantify HIV-1 2-LTR, we produced VSV-G enveloped HIV-1 particles in 293T cells, and such reporter viral particles were treated with DNase I for one hour before being used to infect control cells. 12 hours post-infection, cells were collected, genomic DNA extracted. Using 500 ng of genomic DNA, the following primers were used for HIV-1 2-LTR measurement using qPCR: For HeLa cells GAPDH (forward: 5'CATCATCCCTGCCTCTACTGG, reverse: 5'GGTCCACCACTGACACGTT), CrFK GAPDH (forward: 5'GCG CCT GGT CAC CAG GGC TGC, reverse: 5'CCC ATT TGA TGT TGG CGG GAT C) and 2-LTR (forward: 5'AAC TAG GGA ACC CAC TGC TTA AG, reverse: 5'TCC ACA GAT CAA GGA TAT CTT GTC).

HIV-1 uncoating assay. The following primers (forward1: 5'ATGAATTCAGCGAAAGTAAAGCCAGAGGAGATC3', reverse1: 5'GTTTTTGGCGTCTTCCATCTCTCTCCTTCTAGCCTC, forward2: 5'GAGGCTAGAAGGAGAGAGATGGAAGACGCCAAAAAC and reverse2: 5'ATGCGGCCGCTTACACGGCGATCTTTCCGCCCTTC) were used to fuse HIV-1 pNL4-3 packaging signal (ψ) from long terminal repeats, with nanoluciferase reporter gene cDNA. The fused insert was then cloned in EcoRI-NotI digested pcDNA3.1. To produce viral-like particles (VLPs), 800 ng of HIV-1 ψ-nanoluciferase construct together with 350 ng of pRSV-Rev, 200ng of VSV-G, 800 ng of pMDLg/pRRE were used for co-transfection of 293T cells in wells of a six-well plate. Two days post-transfection, viral supernatant was collected, centrifuged through 20% sucrose for a minimum of 4 hours at 4^{o}C. Supernatant was discarded, VLPs were resuspended in DMEM medium and such particles were used to challenge CrFK cells expressing human CYPB, huTRIMCYPB or cells with control empty vector, for 8 hours and 12 hours. Transduced cells were washed for a minimum of three times to remove extra-cellular (background) nanoluciferase before being lysed using a nanoluciferase substrate-containing buffer (Promega, Mannheim/Germany), according to manufacturer's instructions. Luciferase was measured on a MicroLumat Plus luminometer (Berthold Detection Systems, Pforzheim, Germany).

Bioinformatics. Structural models of binding of human Cyclophilin B to HIV1 M CA protein was performed following a previously described approach[41].

Results

CYPB depletion increases HIV-1 but not SIVgor infection in a cell line dependent way

To understand the role of CYPB during infection of HIV-1 or SIVgor, we performed CYPB knockout in HOS, HeLa, THP-1 and CYPB knockout in CYPA-depleted HeLa cells (double knockdown), using the CRISPR/Cas9 system (Fig. 1A). This process significantly depleted CYPB expression in HeLa and THP-1 cells but CYPB knockout single clones had to be established in HOS cells, as the initial knockout approach did not significantly deplete CYPB levels. Such CYPB depleted cells were then used for infection with HIV-1 M, HIV-1 N, HIV-1 O, HIV-1 P and SIVgor reporter viruses; luciferase activity was measured 48 to 72 hours post infection. In the two CYPB depleted HOS single clones, number 1 and number 7, CYPB knockout increased infection by HIV-1 M up to 2.8-folds (Fig. 1B). Infection by HIV-1 N, HIV-1 O and HIV-1 P also increased to similar levels, by 2.2-folds, 2.4-folds, and 2.8-folds, respectively. On the other hands, infection by SIVgor was not affected by the absence of CYPB in HOS cells (Fig. 1B). CYPB depleted HeLa cells showed higher permissivity to HIV-1 M infection by up to 2.3- folds as compared to control cell infection (Fig. 1C). Similar to HIV-1M, HIV-1 N infectivity significantly increased following CYPB knockdown in HeLa cells. Interestingly, all other viruses were not affected, and showed equal infectivity in WT control and KD cells (Fig. 1C). HIV-1 infection was also tested in myeloid THP-1 cells with a CYPB knockdown. As seen in HOS and HeLa cells, CYPB depletion in THP-1 cells was associated with increased HIV-1 M infection by up to 2-folds, compared to vector control cells (Fig. 1D). In THP-1 cells, CYPB depletion had no effect on all non-M group HIV-1s and SIVgor, suggesting a cell type specificity of CYPB for the viruses we tested (Fig. 1E).

To exclude influence of CYPA, which shares a high similarity with CYPB, on our CYPB knockout cell infection results, we also tested infection in CYPB depleted HeLa cells knocked down of CYPA and CYPA KD HeLa cells were used as control cells. We also confirm the

integrity of CYPA in CYPB knockdown cells, by western blot (Fig. 1A). By testing HIV-1 in CYPB-CYPA double knockdown HeLa cells, we were able to confirm that an increase in HIV-1 infection in CYPB knockdown HeLa cells was not influenced by CYPA presence (Fig.1E). In such cells, HIV-1 M infection increased up to 2.5 folds compared to CYPA KD control cells, HIV-1 N infection was enhanced by to 1.6 folds, infection by other viruses confirmed the findings obtained with HeLa CYPB KD cells before (Figs. 1C, 1E). Together, these data suggest that CYPB depletion is beneficial for early infection events of HIV-1 M and may affect non-M HIVs in a more cell-type dependent manner.

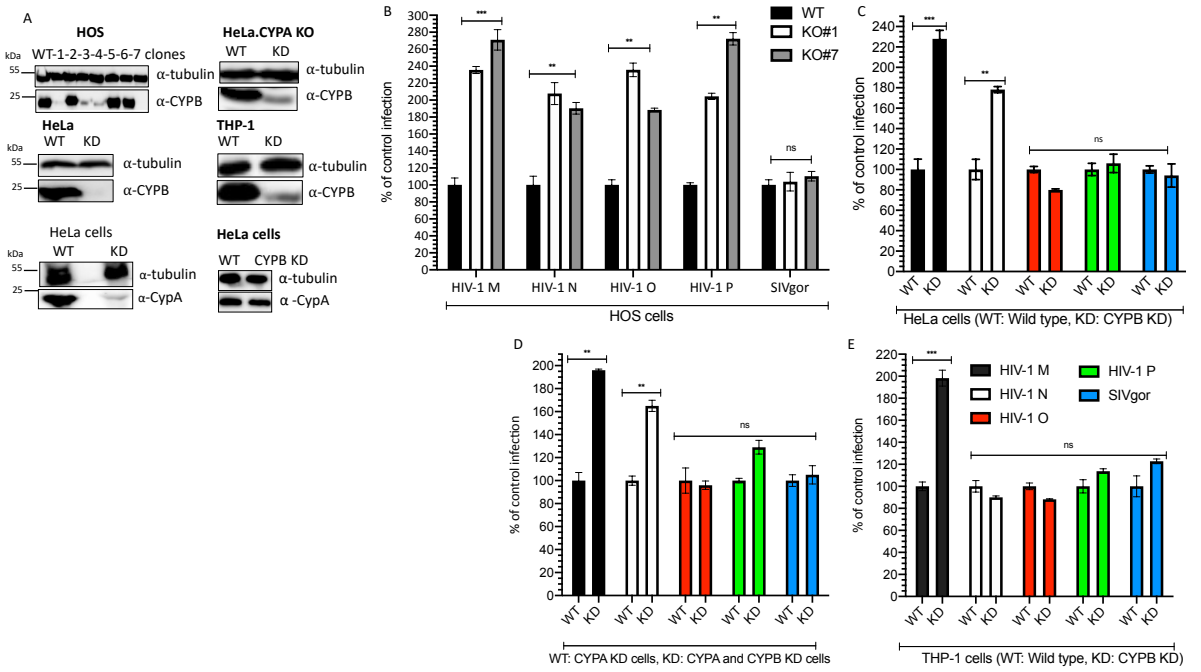
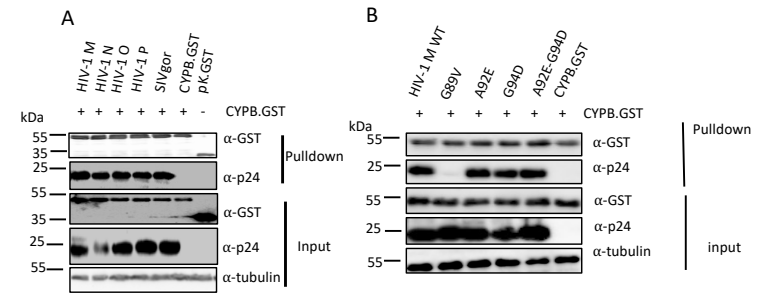


Fig. 1. Human CYPB inhibits HIV. (A) Immunoblots of CYPB depleted HOS, HeLa, CYPA KD HeLa and THP-1 cells. CYPA or CYPA were detected by anti-CYPA or anti-CYPB; anti tubulin served as a control for equal protein loading. (B, C, D, E) Luciferase reporter viruses of HIV-1 M, HIV-1 N, HIV-1 O, HIV-1 P and SIVgor were used to infect (B) WT HOS or HOS CYPB KD cells, or (C) WT HeLa or HeLa CYPB KD cells, or (D) HeLa CYPA KD or HeLa CYPB/CYPB KD cells, or (E) WT THP-1 or THP-1 CYPB KD cells. . Luciferase activity was measured two to three days post-infection and results were normalized to control cells. Means and SD (error bars) are shown. Each experiment was performed at least three times and in triplicates.

CYPB binds many residues of HIV-1 capsid CYPA binding loop

We next assessed CYPB binding to HIV-1 and SIVgor capsids using GST-tagged CYPB. For these experiments, GST-CYPB or viral Gag-Pol/Rev was expressed in 293T cells separately. Cell lysates were mixed and used for pulldown experiments. In the precipitated CYPB, we

detected capsid proteins from all four HIV-1s and SIVgor, demonstrating capsid binding to CYPB (Fig. 2A). GST expressed without a fused CYPB did not bind any viral capsid (data not shown). We then assessed CYPB binding of HIV-1 M bearing different mutations in CYPA binding loop, and only G89V but not G94D, A92E or A92E-G94D mutations in HIV-1 M capsid abolished GST-tagged CYPB binding to viral capsid (Fig. 2B).



D

	CYPA binding loop																				
	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
HIV-1 M	E	W	D	R	V	H	P	**V**	**H**	**A**	**G**	**P**	**I**	**A**	P	G	Q	M	R	E	P	R
HIV-1 N	E	W	D	R	T	H	P	**V**	**P**	**V**	**G**	**P**	**L**	**P**	P	G	Q	L	R	E	P	R
HIV-1 O	D	W	D	R	T	H	P	**P**	**A**	**M**	**G**	**P**	**L**	**P**	P	G	Q	I	R	E	P	T
HIV-1 P	E	W	D	R	T	H	P	**V**	**P**	**V**	**G**	**P**	**L**	**P**	P	G	Q	L	R	D	P	T
SIVgor	E	W	D	R	M	H	P	**A**	**V**	**V**	**G**	**P**	**L**	**P**	V	G	Q	L	R	E	P	S

Fig. 2. CYPB binds viral capsid protein. (A, B, C) Pulldown of CYPB-GST and viral capsids and capsid CYPA binding loop sequence alignment. (A) Test of CYPB binding to capsids from the four groups of HIV-1 and of SIVgor; (B) HIV-1 M WT capsid and mutations G89V, A92E, G94D or A92E-G94D were tested for binding to CYPB-GST. (C) Sequence of the capsid CYPA binding loop of the tested viruses binding to HIV-1 Capsids. Pk.GST: GST without fusion to CYPB was used as a control in all experiments. All pulldowns were repeated three times with identical findings.

We also performed a structural modelling of the binding of CYPB to HIV-1 M capsid protein, to explore on which residues are used by HIV capsid to interact with CYPB. Our binding modelling suggests that both CYPA and CYPB share the capsid interaction surface of the capsid binding loop (Fig. 3A-B).

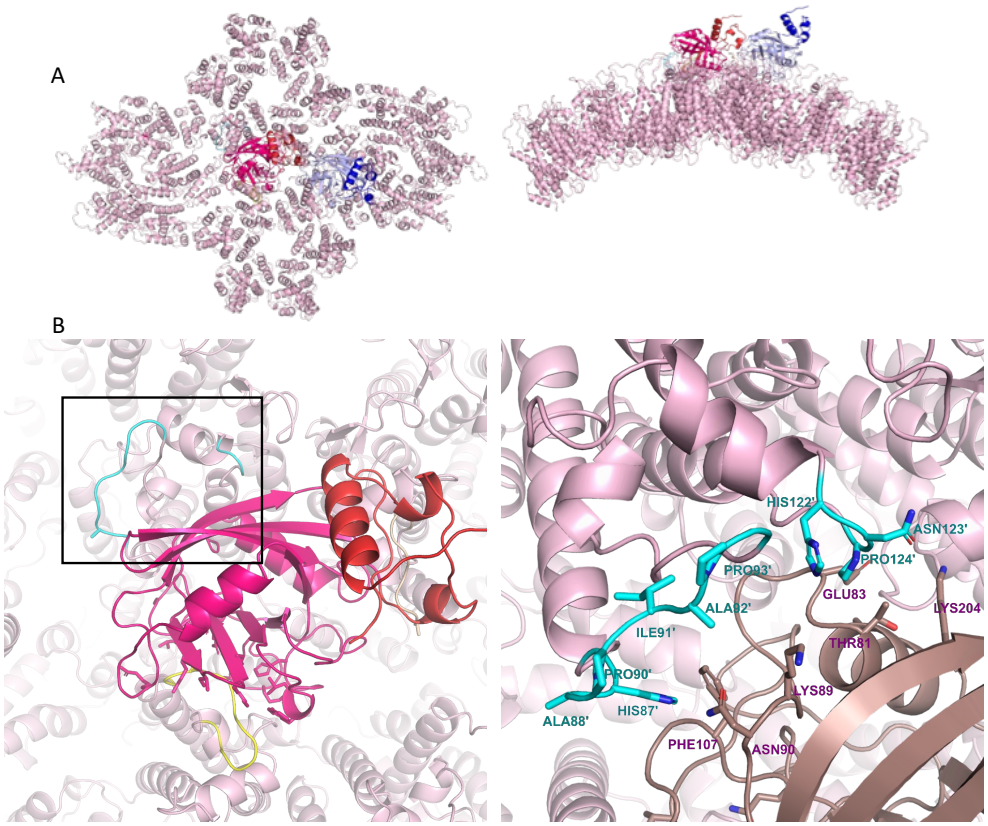


Fig. 3. Structural models of binding of human Cyclophilin B to HIV1 M CA protein. Model of two potential binding modes for CYPB (magenta/blue) on HIV1 M capsid surface (rose). Shown from top (left) and side perspective (right) binding poses were provided by[41]. Canonical binding loop bound in common binding pocket of CYPA is shown in yellow. Other interacting loops of CA monomers are shown in cyan and beige (left). Right: Blow up of interaction of canonical CYPA binding loop (residues 87-93) and small other loop (residues 122-124) with the surface of CYPB. these interactions are probably weak.

Next, we wanted to test the capsid mutants used in the GST pulldown assays in functional studies using HIV-1 M and HIV-1 N with capsid mutants G89V, A92E, G94D, double mutants or A92E-G94D for infection of CYPB knockout/knockdown cells. In both CYPB knockout HOS and CYPB knockdown HeLa cells, infection by HIV-1 M A88V capsid mutant followed same pattern as infections by HIV-1 M with wild type capsid. In both CYPB KO/KD/ HOS and HeLa cells, infection by this capsid mutant increased to up to 2.4- or 2.6-folds, suggesting that alanine 88 in HIV-1 capsid is not important for CYPB-capsid interaction. In contrast, HIV-1 with capsid mutations G89V, A92E, G94D or A92E-G94D had the same infectivity in WT or CYPB KO HOS cells (Fig. 4A). In HeLa cell infections, we found for some capsid mutants' infectivity phenotypes that differed from the findings obtained from HOS cells. The CYPB KD

in HeLa cells enhanced both the HIV-1 M WT and its A88V mutant by around 2.5-folds compared to WT cells. But different to results in HOS cells, HIV-1 bearing A92E and the double mutant A92E-G94 showed 2-fold higher infection in Hela CYPB KD cells and HIV-1 M with G94D was enhanced slightly by 0.5-fold. HIV-1 M G89V and A88V-G89V variants were similar as in HOS cells not enhanced by the CYPB depletion (Fig. 4B).

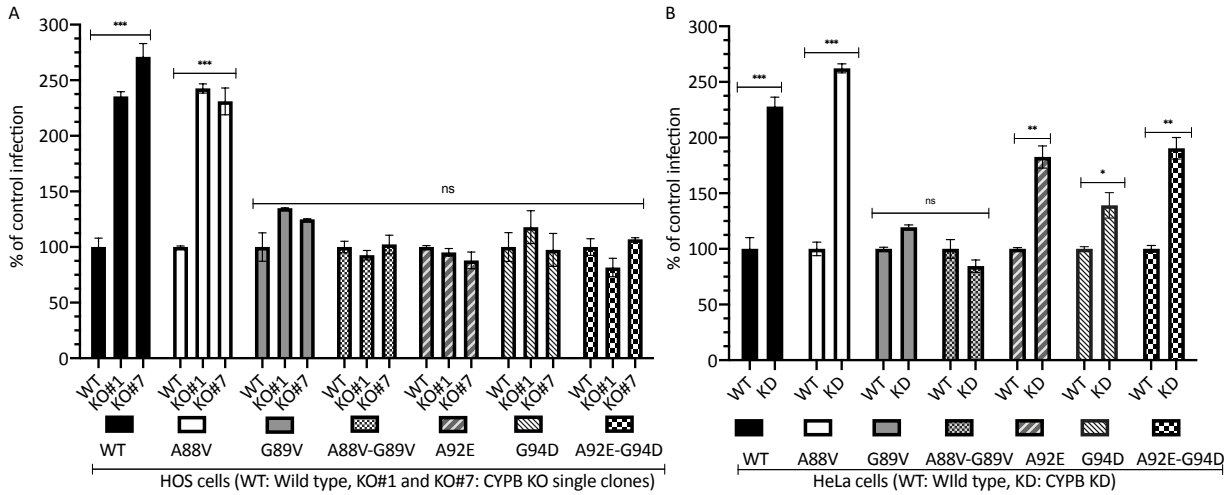


Fig. 4. Infection of CYPB knockout HOS and CYPB KD HeLa cells with HIV-1 capsid CYPA loop mutants. (A) CYPB knockout HOS or (B) CYPB KD HeLa cells were infected with luciferase reporter viruses of HIV-1 bearing wild type capsid or capsids with mutations in the CYPA loop (A88V, G89V, A88V-G89V, A92E, G94D, A92E-G94D). Luciferase activity was measured two to three days post-infection and results were normalized to vector control cells. Means and SD (error bars) are shown. Each experiment was performed at least three times and in triplicates.

Cyclophilin B and TrimCYPB proteins inhibit HIV-1 viruses

Artificial Trim5 fusion proteins in which the capsid-binding SPRY domain was replaced by a potential capsid binding domain have been used to discover and investigate mechanisms of cellular interactors of HIV[42,43]. Natural TrimCYPA proteins found in some monkeys can play a key role against viral cross species spread[44–46]. TrimCYPA proteins are known to selectively inhibit HIVs where the virus is targeted by the CYPA domain of the fusion protein. An example is rhesus TrimCYPA (rhTrimCYPA) that inhibits HIV-2 but not HIV-1 M or owl monkey TrimCYPA (omTrimCYPA), which inhibits HIV-1 but not HIV-2[32,47,48]. We hypothesized that based on similarity between CYPA and CYPB, fusing CYPB with TRIM5 proteins, would also

result in strong antiviral proteins. In this way, we constructed HA-tagged fusion proteins of human CYPB and human or rhesus TRIM5 proteins, human TrimCYPB (huTrimCYPB.HA) and rhesus TrimCYPB (rhTrimCYPB.HA). Human TrimCYPB.HA was constructed by replacing the SPRY domain of human TRIM5α by HA-tagged huCYPB, and rhTrimCYPB was made by replacing the CYPA domain of rhTrimCYPA[47] by HA-tagged human CYPB. In addition, HA-tagged huCYPB (CYPB.HA), HA-tagged huTRIM5α lacking the SPRY domain (huTRIM.HA), and HA-tagged rhTrimCYPA lacking the CYPA domain (rhTRIM.HA) were constructed (Fig. 5A). CrFK cells were generated to express these proteins (Fig. 5B). Such cells were subsequently used to test infection by HIV-1 viruses. In this cell system, HIV-1 M, N, O, and P were inhibited by expression of CYPB by up to 40 - 50%, indicating a mild antiviral activity. A much stronger antiviral activity against all four HIV-1s were detected in cells expressing human or rhesus TrimCYPB proteins that caused around 90% inhibition of infections. To conclude on whether this inhibition was from the fused CYPB, we tested huTRIM.HA and rhTRIM.HA (lacking the SPRY domains) and their expression did not affect infection by any HIV-1, as infection levels were statistically similar to infections of empty vector cells. (Fig. 5C). SIVgor was also inhibited by TrimCYPBs but the mild antiviral activity of CYPB overexpression was not detectable (Fig. 5C).

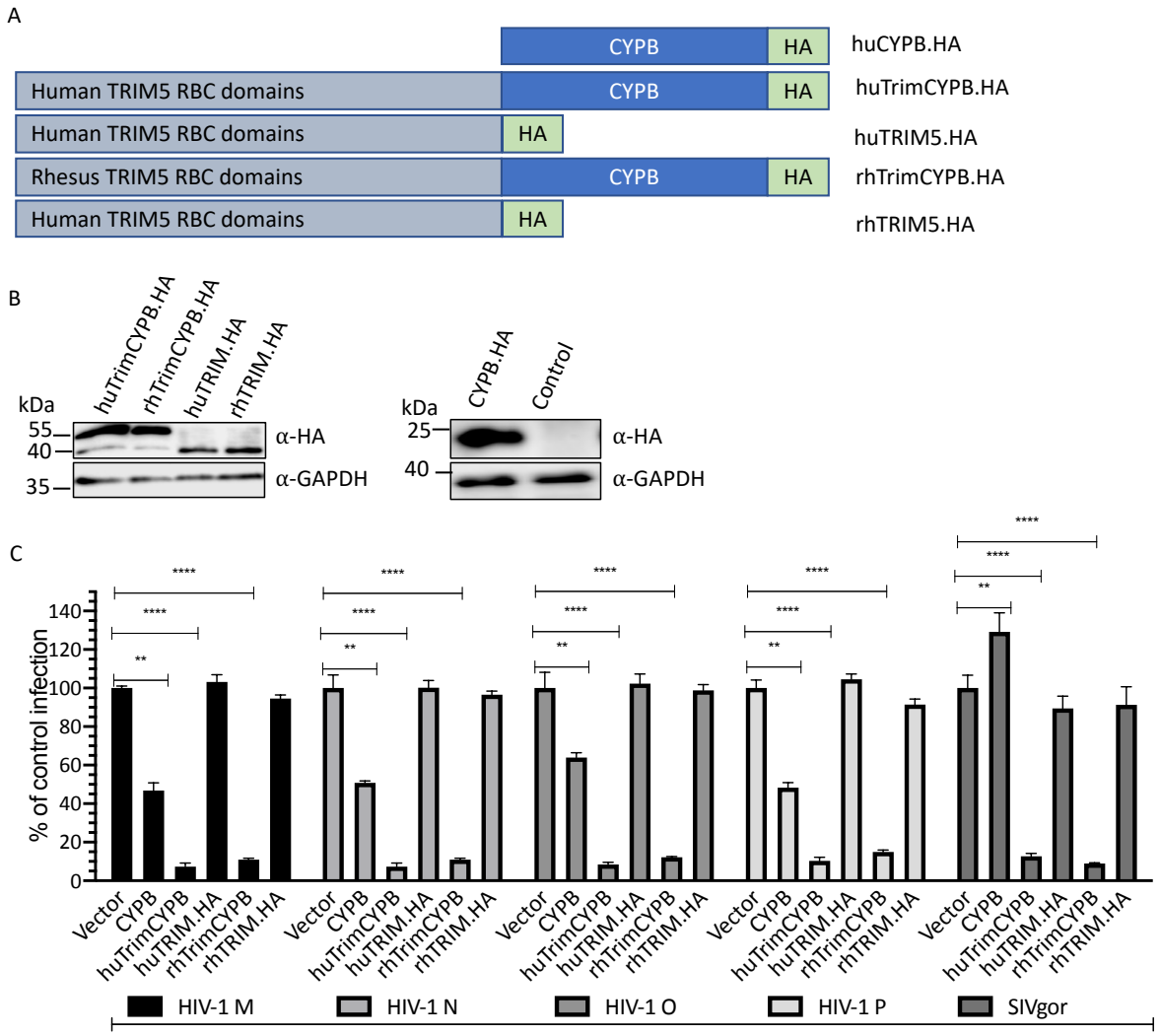


Fig. 5. Infection of CrFK cells overexpressing human CYPB and TRIMCYPB fusion proteins. (A) Scheme of HA-tagged human CYPB; huTrimCYPB and rhTrimCYPB in which the SPRY domains of TRIM5α were replaced by human CYPB; TRIM.HA isTRIM5α with an HA tag instead of the SPRY domain. (B) Immunoblots of stable CrFK cells overexpressing HA-tagged human CYPB, huTrimCYPB, rhTrimCYPB, huTRIM.HA and rhTRIM.HA. HA-taged proteins were detected by anti-HA antibody. Anti-GAPDH was used as a control. (C) CrFK cells expressing CYPB, hu or rhTrimCYPB, or hu or rhTRIM.HA were infected with luciferase reporter viruses of HIV-1 M, HIV-1 N, HIV-1 O, HIV-1 P and SIVgor. Luciferase activity was measured two to three days post-infection and results were normalized to control infection in cells expressing the empty (vector). Means and SD (error bars) are shown, Mann Whitney U test was performed. Each experiment was performed at least three times and in triplicates.

In the next step, we analyzed the HIV-1 M capsid mutants that we characterized in the CYPB depleted cells (Fig. 3) in the CYPB/TrimCYPB overexpression cell lines. HIV-1 M bearing the A88V mutation in the capsid was inhibited by CYPB expression similar as WT HIV-1 M and was also strongly blocked by the two TrimCYPB fusion proteins, huTrimCYPB and rhTrimCYPB. On the other hand, HIV-1 infection was restored when G89V or double A88V-G89V mutations were introduced in the capsid, as the two mutants fully resisted expressed CYPB and TrimCYPB inhibition, confirming again the role of capsid glycine 89 (G89) for the

activity of CYPB. The mild antiviral activity of CYPB was lost in HIV-1 M carrying A92E or G94D, but the strong antiviral activity of TrimCYPB protein did not change. The A92E-G94D capsid double mutant completely resisted CYPB as well as both TrimCYPB proteins (Fig. 6A). We tested corresponding capsid mutations also in HIV-1 N. Very similar to the findings with HIV-1 M, HIV-1 N A88V was sensitive to CYPB and huTrimCYPB overexpression, while G89V and P92E-G94D resisted CYPB and huTrimCYPB. HIV-1 N with either the mutation P92E or G94D were sensitive to huTrimCYPB but resistant to CYPB (Fig. 6B). These data suggest importance of CYPA binding loop residues at specific positions for CYPB-HIV capsid interaction, despite a diversity in such region among HIV-1 viruses.

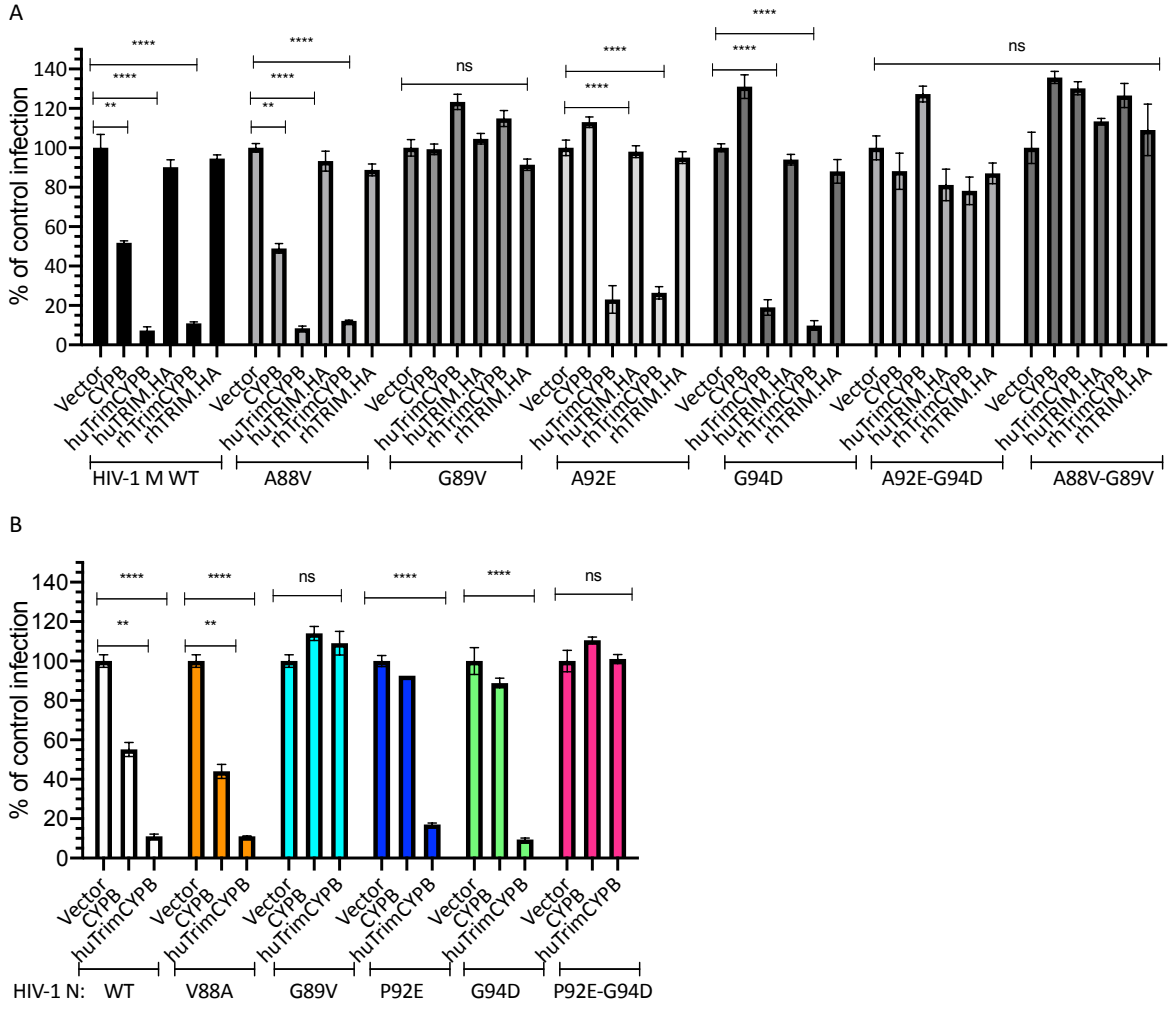


Fig. 6. Overexpressed human CYPB and TrimCYPB fusion proteins differentially inhibit (A) HIV-1 M and (B) HIV-1 N capsid CYPA binding loop mutants. Stable CrFK cells overexpressing HA-tagged human CYPB, huTrimCYPB, rhTrimCYPB, huTRIM.HA or

rhTRIM.HA were infected with luciferase reporter viruses of HIV-1 M, its capsid CYPA binding loop mutants, HIV-1 N or its capsid CYPA binding loop mutants. Luciferase activity was measured two to three days post-infection and results were normalized to control infection. Means and SD (error bars) are shown. Each experiment was performed at least three times and in triplicates.

CYPB lacking the ER/NLS localization domain retains HIV-1 GAG binding and anti-HIV-1 activity

CYPB has in its N-terminus both endoplasmic reticulum and nuclear localization signals (ER and NLS), which has been suggested to be important for the CYPB role during HIV-1 infection[37]. We generated a CYPB construct where the first 40 amino acids were deleted that carry the ER/NLS targeting signals, ΔCYPB. 293T cells were transiently transfected with increasing amounts (from 50 to 400 ng) of WT CYPB or ΔCYPB, infected with same amount of HIV-1 reporter viruses and luciferase was measured two days later. HIV-1 infection was gradually inhibited with increasing amounts of both proteins and inhibition was equally strong by CYPB or ΔCYPB with blocking infection to up to 80% (Fig. 7A-B). In our GST-pulldown assay, ΔCYPB.GST bound also to capsid, further supporting that the N-terminal part of CYPB is not involved in capsid binding (Fig. 7C). Using ΔCYPB, we also constructed a human Trim5 fusion protein designated TrimΔCYPB (Fig. 7D). Stable expression of TrimΔCYPB in CrFK cells strongly inhibited 97% of WT HIV-1 M but it also restricted in a similar way, infection by HIV-1 M with A88V, A92E and G94D mutations in the capsid. The weak antiviral activity of expressed CYPB was also seen with ΔCYPB expression. The G89V and the double mutant A92E-G94D resisted TrimΔCYPB inhibition (Fig. 7E).

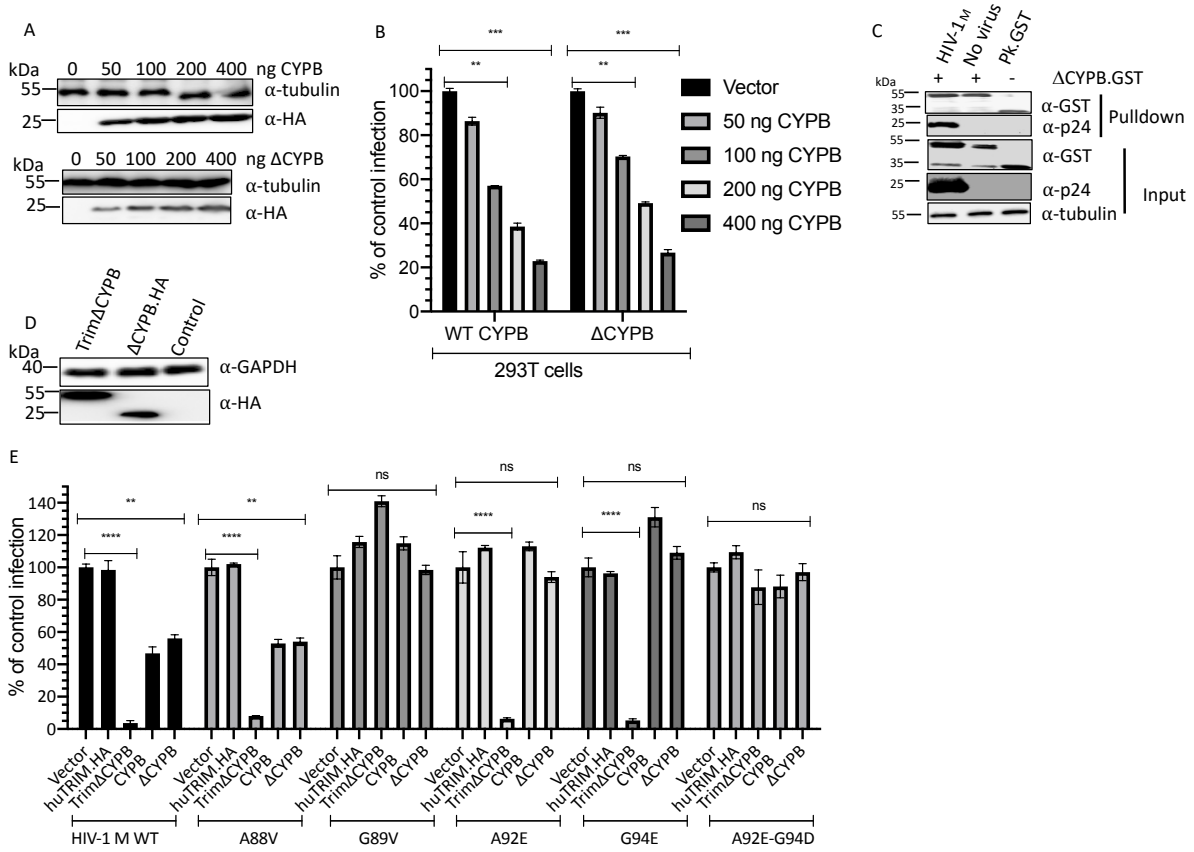


Fig. 7. **Infection of human 293T cells overexpressing human CYPB or ΔCYPB (lacking endoplasmic reticulum/nuclear localization signal sequences) and feline CrFK cells overexpressing human TRIM.HA, TrimΔCYPB, CYPB or ΔCYPB,** ΔCYPB binding to GAG**.** (A) Immunoblot of 293T cells expressing increasing amounts of CYPB or ΔCYPB. Anti-HA was used to detect the CYPB or ΔCYPB; anti tubulin was used to demonstrate equal protein loading. (B) 293T cells were transfected with increasing amounts of CYPB or ΔCYPB 24 hours later, these cells were infected by HIV-1 M reporter virions and luciferase was measured 48 hours following infection. (C) Test of CYPB lacking its endoplasmic reticulum/nuclear localization signal (ΔCYPB) for binding to HIV-1 M capsid; Pk.GST: GST without fusion to CYPB was used as a control in all experiments. (D) Immunoblots of CrFK cells expressing TrimΔCYPB or ΔCYPB, detection was done using anti-HA antibodies, anti-GAPDH served as control for equal protein loading; (E) CrFK cells overexpressing human CYPB.HA, ΔCYPB.HA, TrimΔCYPB or huTRIM.HA were infected with luciferase reporter viruses of HIV-1 M or its capsid mutants. Luciferase activity was measured two to three days post-infection and results were normalized to control infection. Means and SD (error bars) are shown, each experiment was performed at least three times and in triplicates.

CYPB inhibits early steps of HIV-1 infection

To understand more on CYPB's antiviral mechanisms, we assessed its target phase during infection by HIV-1 M. We tested a construct in which the HIV-1 packaging signal (ψ) was fused with a nanoluciferase reporter gene, for a nanoluciferase reporter cDNA based uncoating assay, similar as[49]. HIV-1 M viral-like particles (VLPs) were produced by cotransfection of the expression plasmids for *ψ.nanoluciferase* and HIV-1 gag-pol/rev and VSV-G. A negative control transfection lacking VSV-G plasmid was also added (data not shown). These VLPs were used to challenge WT CrFK cells, CrFK cells overexpressing human CYPB or huTrimCYPB. Nanoluciferase was measured 8 hours and 12 hours later. Cells expressing

empty vector or TRIM5α lacking the SPRY domain (TRIM5-HA) were used for controls. VLP infection of cells overexpressing CYPB or huTrimCYPB generated 2.1- and 3.5- folds more nanoluciferase after 8 hours, respectively, and similar results were obtained at 12 hours post-transduction, indicating CYPB involvement in HIV-1 core destabilizing (Fig. 8A). To show that the measured nanoluciferase was from *de novo* synthesis following entry of VLPs in target cells, we pre-treated CrFK target cells with 100 μM of Cycloheximide, a known protein synthesis inhibitor[50] and this concentration reduced more than 90% of nanoluciferase activity (results not shown), confirming that measured nanoluciferase was the result of post-entry translation.

We also assessed the amount of HIV-1 M 2-LTR circular DNA. We infected CrFK and HeLa cells expressing CYPB and huTrimCYPB or empty vector, with equal amount of WT HIV-1 but also A92E.G94D capsid mutant (shown to confer resistance to both CYPB and TrimCYPB), for 12 hours. This was followed by HIV-1 2-LTR circular DNA quantification on extracted genomic DNA using qPCR. In CYPB overexpressing HeLa and CrFK cells, the amount of WT HIV-1 2-LTR circles was significantly reduced as compared to control cells and this reduction was even higher in the presence of huTrimCYPB (Fig. 8B), indicating that CYPB inhibits HIV-1 before nuclear entry. 2-LTR circles were not reduced by CYPB or TrimCYPB in infections by HIV-1 M carrying a capsid with resistance mutations A92E-G94D mutations (Fig. 8C). Together, our results suggest that overexpressed CYPB inhibits HIV-1 early during infection before the viral core enters the nucleus.

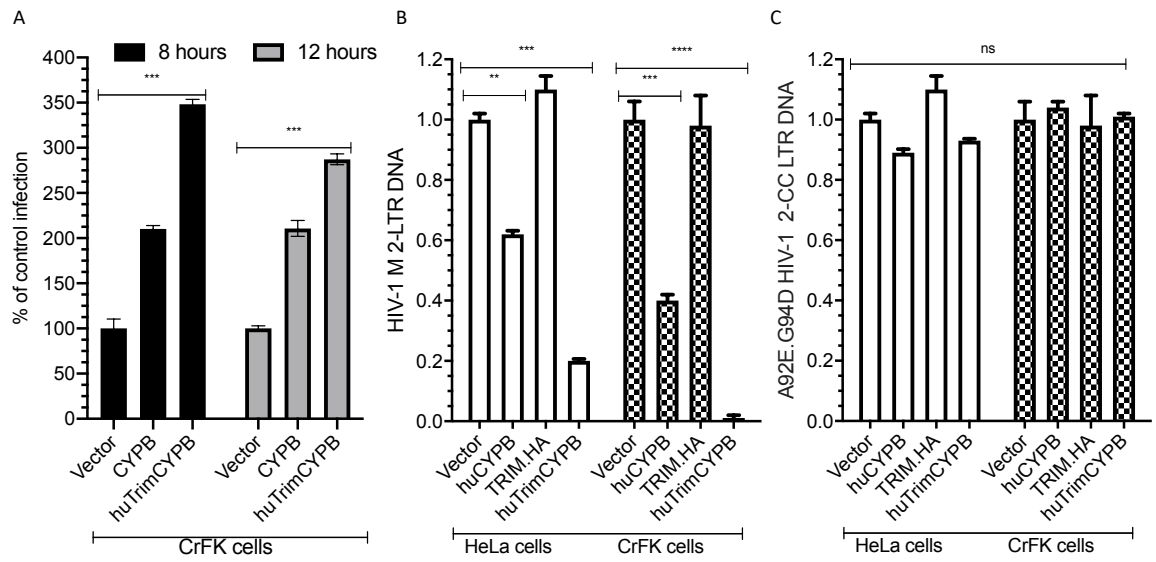


Fig. 8. CYPB and TRIMCYPB proteins enhance HIV core stability and reduce HIV 2-cicle LTRs. (A) CrFK cells expressing human CYPB or TrimCYPB were transduced with HIV-1 viral like particles produced using HIV-1 Ψ-nanoluciferase, *GagPol*, *Rev* and *VSV*-G plasmids. 8 and12 hours post-transduction, nanoluciferase activity was assessed and compared between control and CYPB, TrimCYPB expressing cells. (B, C) CrFK or HeLa cells expressing human CYPB, TRIM5-HA or TrimCYPB were transduced with (B) WT or (C) A92E.G94D HIV-1 viral particles. 24 hours post infection, cells were lysed and HIV-1 2-circle LTRs were quantified using qPCR. These experiments were done for a minimum of three times and always in triplicates.

Discussion

Most studies to understand cyclophilin activity on HIV focused on CYPA and particularly in the context of HIV-1 group M[29,30,51–56]. Any role and mechanisms of other cyclophilins during HIV/SIV infection is ill defined. As capsid-binding proteins, hugely determining the fate of HIV infection, both CYPB and CYPA are of major interest in the process to develop viral inhibitors targeting the viral capsid. In fact, CYPA binding has been shown to influence activity of other cellular interactors of capsid, like TRIM5α but also pharmacological inhibitors like PF74[34,57]. Using a CYPB gene knockout approach in different human cell lines, and CYPB overexpression, we identified a more antiviral activity of CYPB during infection by viruses from the four groups of HIV-1s and SIVgor. Furthermore, we consolidated our findings on the anti-HIV activity of CYPB and its dependency on capsid CYPA binding loop, using engineered human or rhesus TRIM5 and CYPB fusion proteins, the huTrimCYPB and rhTrimCYPB,

which strongly inhibited HIV-1 and SIVgor viruses but did not restrict HIV with specific mutations in the capsid, such as G89V or double mutant A92E-G94D.

CYPB depletion in HOS cells enhances infection by viruses from all groups of HIV-1 but not SIVgor. In HeLa cell, CYPB knockdown was beneficial for HIV-1 M and N but not for any of the other tested viruses and finally, in THP-1 cells lacking CYPB, only HIV-1 showed higher infectivity compared to WT cells. These complex cell type dependent effects of CYPB depletion on different HIV-1 groups may reflect a different interaction with cellular factors during the early phase of infections[8,31]. CYPA KO in HOS cells was found to inhibit HIV-1 M infection and CYPA KO in HeLa cells enhanced infection by HIV-1 M[34,58]. These data do not allow to describe a complete model. However, at least in some cells, CYPB could be a competitor of CYPA binding and CYPB depletion would allow more binding of CYPA. While both cyclophilins are highly related, we do not know if the cis-trans isomerase activity known of CYPA on HIV-1 M capsid is similarly available by CYPB.

In contrast to our observations, a recent report described that overexpression of CYPB enhanced HIV-1 infection by increasing nuclear import of viral DNA[37]. The reason for different results from our experiments to the previous study remains unknown.

Some monkeys encode TrimCYPA proteins which restrict specific retroviruses in a CYPA domain-dependent way soon after cell entry[59–61]. Our strong anti-HIV-1/SIVgor TrimCYPB fusion proteins, show that CYPB transposition to TRIM5 proteins would also make strong antiviral factors[43,62].

In CYPB depleted cells, differences in infection between capsid CYPA binding loop mutants and wild type HIV-1, highlights importance of such loop residues for CYPB activity[30,63]. A better infection by A92E capsid mutant of HIV-1 in the absence of CYPB, has also been reported in CYPA knockdown HeLa but not in Jurkat cells[58]. Our data showing an increase in infection by the HIV-1 M double mutant A92E-G94D but not the single mutant G94D in HeLa

cells depleted of CYPB, also confirms the infection results of the A92E during CYPB knockdown. HIV-1 intra-type sequence variability in capsid CYPA binding loop has been shown to affect the ability of host factors which need such loop, to bind and affect the course of infection; an example is the capsid residue 88 important for TrimCYPA proteins[46]. Capsid proline 92 is conserved between HIV-1 N, HIV-1 O, HIV-1 P, SIVgor (Fig. 2C) and even HIV-2AB (not shown). Our data shows that capsid P92 mediates HIV-1 sensitivity to CYPB and TrimCYPB proteins, as does A92. Why HIV-1 N and other viruses evolved with such proline instead of alanine, need further exploration. Investigations on whether binding of CYPB to capsid also has effects on other capsid binding host factors of HIV, is currently unknown.

Binding of CYPB to GAG from non-M group HIVs and SIVgor suggests that differences between these viruses in CYPA binding loop do not affect their binding to CYPB. The similarity between CYPA and CYPB[37], particularly in their capsid binding residues, can at least explain the sharing of a capsid binding loop between the cyclophilins.

G89V mutation in HIV-1 capsid renders its capsid unable to interact with CYPA[29]. Our pulldown experiment shows that CYPB also uses G89 for its binding to capsid. ΔCYPB lacking the first 40 residues retained HIV-1 capsid binding property, antiviral activity if overexpressed and showed in the fusion protein TrimΔCYPB, a strong antiviral activity. This retention of inhibitory activity can be explained by many residues in CYPB, outside the N-terminus, predicted by our protein interaction model (Fig. 4).

The loss of antiviral activity by TrimCYPB and CYPB when HIV capsid is specifically mutated, shows again how the CYPA binding loop is a crucial part of the viral capsid, for engaging with cyclophilins. It is possible that a weak binding of CYPB to HIV-1 with G94D or A92E or P92E mutations is unable to cause inhibition. In the context of TrimCYPB, we postulate that such weak binding of CYPB to capsid mutants, is compensated in TrimCYPB

by its oligomerization activity and may explain the restriction of HIVs with some capsid mutations, otherwise resistant to CYPB[23].

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Bibliography

1. Twizerimana AP, Scheck R, Häussinger D, Münk C. Post-entry restriction factors of SIVcpz. Future Virol. 2018;13(10):727-745. doi:10.2217/fvl-2018-0093
2. Gao F, Bailes E, Robertson DL, et al. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. Nature. 1999;397(6718):436-441. doi:10.1038/17130
3. Van Heuverswyn F, Li Y, Neel C, et al. SIV infection in wild gorillas. Nature. 2006;444(7116):164-164. doi:10.1038/444164a
4. Mahboubi-Rabbani M, Hajimahdi Z, Abbasi M, Zarghi A. HIV-1 Reverse Transcriptase/Integrase Dual Inhibitors: A Review of Recent Advances and Structure-activity Relationship Studies. Iranian Journal of Pharmaceutical Research. 2021;20(2):333-369. doi:10.22037/IJPR.2021.115446.15370
5. Merci NM, Emerence U, Augustin N, et al. No Title. 2017;26(14):14. doi:10.11604/pamj.2017.26.14.10488
6. Twizerimana AP, Mwatha J, Musabyimana JP, et al. IMMUNOLOGICAL PROFILES IN HIV POSITIVE PATIENTS FOLLOWING HAART INITIATION IN KIGALI, RWANDA. East Afr Med J. 2014;91(8):261-266. doi:10.4314/eamj.v91i8.
7. Unaids. F A C T S H E E T 2 0 2 2. Accessed February 17, 2023. https://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf
8. Braaten D, Aberham C, Franke EK, Yin L, Phares W, Luban J. Cyclosporine A-resistant human immunodeficiency virus type 1 mutants demonstrate that Gag encodes the functional target of cyclophilin A. J Virol. 1996;70(8):5170-5176. doi:10.1128/jvi.70.8.5170-5176.1996
9. Braaten D, Franke EK, Luban J. Cyclophilin A Is Required for the Replication of Group M Human Immunodeficiency Virus Type 1 (HIV-1) and Simian Immunodeficiency Virus SIV CPZ GAB but Not Group O HIV-1 or Other Primate Immunodeficiency Viruses. Vol 70.; 1996. Accessed December 5, 2021. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC190352/pdf/704220.pdf
10. Buffone C, Martinez-Lopez A, Fricke T, et al. Nup153 Unlocks the Nuclear Pore Complex for HIV-1 Nuclear Translocation in Nondividing Cells. J Virol. 2018;92(19). doi:10.1128/JVI.00648-18
11. Price AJ, Jacques DA, McEwan WA, et al. Host Cofactors and Pharmacologic Ligands Share an Essential Interface in HIV-1 Capsid That Is Lost upon Disassembly. Cullen BR, ed. PLoS Pathog. 2014;10(10):e1004459. doi:10.1371/journal.ppat.1004459

12. Saito A, Ferhadian D, Sowd GA, et al. Roles of Capsid-Interacting Host Factors in Multimodal Inhibition of HIV-1 by PF74. Kirchhoff F, ed. J Virol. 2016;90(12):5808-5823. doi:10.1128/JVI.03116-15
13. Zhang Z, Gu Q, de Manuel Montero M, et al. Stably expressed APOBEC3H forms a barrier for cross-species transmission of simian immunodeficiency virus of chimpanzee to humans. Ross SR, ed. PLoS Pathog. 2017;13(12):e1006746. doi:10.1371/journal.ppat.1006746
14. Zhong Z, Ning J, Boggs EA, et al. Cytoplasmic CPSF6 Regulates HIV-1 Capsid Trafficking and Infection in a Cyclophilin A-Dependent Manner. mBio. 2021;12(2). doi:10.1128/mBio.03142-20
15. Zhang Z, Gu Q, Jaguva Vasudevan AA, et al. Vif Proteins from Diverse Human Immunodeficiency Virus/Simian Immunodeficiency Virus Lineages Have Distinct Binding Sites in A3C. Ross SR, ed. J Virol. 2016;90(22):10193-10208. doi:10.1128/JVI.01497-16
16. Malim MH. Natural resistance to HIV infection: The Vif-APOBEC interaction. C R Biol. 2006;329(11):871-875. doi:10.1016/j.crvi.2006.01.012
17. Saito A, Yamashita M. HIV-1 capsid variability: viral exploitation and evasion of capsid-binding molecules. Retrovirology. 2021;18(1):32. doi:10.1186/s12977-021-00577-x
18. Balasubramaniam M, Zhou J, Addai A, et al. PF74 Inhibits HIV-1 Integration by Altering the Composition of the Preintegration Complex. J Virol. 2019;93(6). doi:10.1128/JVI.01741-18
19. Grütter MG, Luban J. TRIM5 structure, HIV-1 capsid recognition, and innate immune signaling. Curr Opin Virol. 2012;2(2):142-150. doi:10.1016/j.coviro.2012.02.003
20. Singh K, Gallazzi F, Hill KJ, et al. GS-CA Compounds: First-In-Class HIV-1 Capsid Inhibitors Covering Multiple Grounds. Front Microbiol. 2019;10:1227. doi:10.3389/fmicb.2019.01227
21. Li J, Tang S, Hewlett I, Yang M. HIV-1 capsid protein and cyclophilin a as new targets for anti-AIDS therapeutic agents. Infect Disord Drug Targets. 2007;7(3):238-244. doi:10.2174/187152607782110013
22. Yant SR, Mulato A, Hansen D, et al. A highly potent long-acting small-molecule HIV-1 capsid inhibitor with efficacy in a humanized mouse model. Nat Med. 2019;25(9):1377-1384. doi:10.1038/s41591-019-0560-x

23. Braaten D, Luban J. Cyclophilin A regulates HIV-1 infectivity, as demonstrated by gene targeting in human T cells. EMBO J. 2001;20(6):1300-1309. doi:10.1093/emboj/20.6.1300
24. Ganser-Pornillos BK, Pornillos O. Restriction of HIV-1 and other retroviruses by TRIM5. Nat Rev Microbiol. 2019;17(9):546-556. doi:10.1038/s41579-019-0225-2
25. Gamble TR, Vajdos FF, Yoo S, et al. Crystal Structure of Human Cyclophilin A Bound to the Amino-Terminal Domain of HIV-1 Capsid. Cell. 1996;87(7):1285-1294. doi:10.1016/S0092-8674(00)81823-1
26. Tipper C, Sodroski JG. Contribution of glutamine residues in the helix 4-5 loop to capsid-capsid interactions in simian immunodeficiency virus of macaques. J Virol. 2014;88(18):10289-10302. doi:10.1128/JVI.01388-14
27. Towers GJ. The control of viral infection by tripartite motif proteins and cyclophilin A. Retrovirology. 2007;4:40. doi:10.1186/1742-4690-4-40
28. Keckesova Z, Ylinen LMJ, Towers GJ. Cyclophilin A Renders Human Immunodeficiency Virus Type 1 Sensitive to Old World Monkey but Not Human TRIM5 Antiviral Activity. J Virol. 2006;80(10):4683-4690. doi:10.1128/jvi.80.10.4683-4690.2006
29. Franke EK, Yuan HEH, Luban J. Specific incorporation of cyclophilin a into HIV-1 virions. Nature. 1994;372(6504):359-362. doi:10.1038/372359a0
30. Sokolskaja E, Sayah DM, Luban J. Target Cell Cyclophilin A Modulates Human Immunodeficiency Virus Type 1 Infectivity CA-CypA interaction. J Virol. 2004;78(23):12800-12808. doi:10.1128/JVI.78.23.12800-12808.2004
31. Selyutina A, Persaud M, Simons LM, et al. Cyclophilin A Prevents HIV-1 Restriction in Lymphocytes by Blocking Human TRIM5α Binding to the Viral Core. Cell Rep. 2020;30(11):3766-3777.e6. doi:10.1016/j.celrep.2020.02.100
32. Wilson SJ, Webb BLJ, Ylinen LMJ, Verschoor E, Heeney JL, Towers GJ. Independent evolution of an antiviral TRIMCyp in rhesus macaques. Proc Natl Acad Sci U S A. 2008;105(9):3557-3562. doi:10.1073/pnas.0709003105
33. Shi J, Zhou J, Shah VB, Aiken C, Whitby K. Small-molecule inhibition of human immunodeficiency virus type 1 infection by virus capsid destabilization. J Virol. 2011;85(1):542-549. doi:10.1128/JVI.01406-10
34. Twizerimana AP, Scheck R, Becker D, et al. Cell Type-Dependent Escape of Capsid Inhibitors by Simian Immunodeficiency Virus SIVcpz. Kirchhoff F, ed. J Virol. 2020;94(23). doi:10.1128/JVI.01338-20

35. Rycyzyn MA, Clevenger C v. The intranuclear prolactin/cyclophilin B complex as a transcriptional inducer. Proc Natl Acad Sci U S A. 2002;99(10):6790-6795. doi:10.1073/pnas.092160699
36. Price ER, Jin M, Lim D, Pati S, Walsh CT, McKeon FD. Cyclophilin B trafficking through the secretory pathway is altered by binding of cyclosporin A. Proc Natl Acad Sci U S A. 1994;91(9):3931-3935. doi:10.1073/pnas.91.9.3931
37. DeBoer J, Madson CJ, Belshan M, J D, CJ M, M B. Cyclophilin B enhances HIV-1 infection. Virology. 2016;489:282-291. doi:10.1016/J.VIROL.2015.12.015
38. Luban J, Bossolt KL, Franke EK, Kalpana G v., Goff SP. Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B. Cell. 1993;73(6):1067-1078. doi:10.1016/0092-8674(93)90637-6
39. Takehisa J, Kraus MH, Ayouba A, et al. Origin and Biology of Simian Immunodeficiency Virus in Wild-Living Western Gorillas. J Virol. 2009;83(4):1635-1648. doi:10.1128/JVI.02311-08
40. Sanjana NE, Shalem O, Zhang F. Improved vectors and genome-wide libraries for CRISPR screening. Nat Methods. 2014;11(8):783-784. doi:10.1038/nmeth.3047
41. Ni T, Gerard S, Zhao G, et al. Intrinsic curvature of the HIV-1 CA hexamer underlies capsid topology and interaction with cyclophilin A. Nat Struct Mol Biol. 2020;27(9):855-862. doi:10.1038/s41594-020-0467-8
42. Rebensburg S v., Wei G, Larue RC, et al. Sec24C is an HIV-1 host dependency factor crucial for virus replication. Nat Microbiol. 2021;6(4):435-444. doi:10.1038/s41564-021-00868-1
43. Yap MW, Dodding MP, Stoye JP. Trim-cyclophilin A fusion proteins can restrict human immunodeficiency virus type 1 infection at two distinct phases in the viral life cycle. J Virol. 2006;80(8):4061-4067. doi:10.1128/JVI.80.8.4061-4067.2006
44. Brennan G, Kozyrev Y, Hu SL. TRIMCyp expression in Old World primates Macaca nemestrina and Macaca fascicularis. Proceedings of the National Academy of Sciences. 2008;105(9):3569-3574. doi:10.1073/pnas.0709511105
45. Nisole S, Stoye JP, Saïb A. TRIM family proteins: retroviral restriction and antiviral defence. Nat Rev Microbiol. 2005;3(10):799-808. doi:10.1038/nrmicro1248
46. Price AJ, Marzetta F, Lammers M, et al. Active site remodeling switches HIV specificity of antiretroviral TRIMCyp. Nat Struct Mol Biol. 2009;16(10):1036-1042. doi:10.1038/nsmb.1667

47. Newman RM, Hall L, Kirmaier A, et al. Evolution of a TRIM5-CypA splice isoform in old world monkeys. PLoS Pathog. 2008;4(2):e1000003. doi:10.1371/journal.ppat.1000003
48. Nisole S, Saïb A. Early steps of retrovirus replicative cycle. Retrovirology. 2004;1:9. doi:10.1186/1742-4690-1-9
49. da Silva Santos C, Tartour K, Cimarelli A, et al. A Novel Entry/Uncoating Assay Reveals the Presence of at Least Two Species of Viral Capsids During Synchronized HIV-1 Infection. Emerman M, ed. PLoS Pathog. 2016;12(9):e1005897. doi:10.1371/journal.ppat.1005897
50. SIEGEL MR, SISLER HD. Inhibition of Protein Synthesis in vitro by Cycloheximide. Nature. 1963;200(4907):675-676. doi:10.1038/200675a0
51. Experiments to confirm GAG CypA binding.
52. Lahaye X, Satoh T, Gentili M, King MC, Pawlotsky JM, Correspondence NM. Nuclear Envelope Protein SUN2 Promotes Cyclophilin-A-Dependent Steps of HIV Replication. Published online 2016. doi:10.1016/j.celrep.2016.03.074
53. Lin TY, Emerman M. Cyclophilin A interacts with diverse lentiviral capsids. Retrovirology. 2006;3:1-12. doi:10.1186/1742-4690-3-70
54. Thali M, Bukovsky A, Kondo E, et al. Functional association of cyclophilin A with HIV-1 virions. Nature. 1994;372(6504):363-365. doi:10.1038/372363a0
55. Kim K, Dauphin A, Komurlu S, et al. Cyclophilin A protects HIV-1 from restriction by human TRIM5α. Nat Microbiol. 2019;4(12):2044-2051. doi:10.1038/s41564-019-0592-5
56. Sayah DM, Sokolskaja E, Berthoux L, Luban J. Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1. Nature. 2004;430(6999):569-573. doi:10.1038/nature02777
57. Selyutina A, Persaud M, Bulnes-Ramos A, et al. Cyclophilin A Prevents HIV-1 Restriction in Lymphocytes by Blocking Human TRIM5α Binding to the viral Core. bioRxiv. 2019;(718):678037. doi:10.1101/678037
58. de Iaco A, Luban J. Cyclophilin A promotes HIV-1 reverse transcription but its effect Cyclophilin A promotes HIV-1 reverse transcription but its effect on transduction correlates best with its effect on nuclear entry of on transduction correlates best with its effect on nuclear entry of viral cDNA viral cDNA Repository Citation Repository Citation Cyclophilin A promotes HIV-1 reverse transcription but its effect on

transduction correlates best with its effect on nuclear entry of viral cDNA. Published online 2014. doi:10.1186/1742-4690-11-11

59. Coren L v, Trivett MT, Jain S, et al. Potent restriction of HIV-1 and SIVmac239 Replication by African Green Monkey TRIM5α. Retrovirology. 2015;12(1):11. doi:10.1186/s12977-015-0137-9

60. Schaller T, Ylinen LMJ, Webb BLJ, Singh S, Towers GJ. Fusion of cyclophilin A to Fv1 enables cyclosporine-sensitive restriction of human and feline immunodeficiency viruses. J Virol. 2007;81(18):10055-10063. doi:10.1128/JVI.00616-07

61. Perez-Caballero D, Hatziioannou T, Zhang F, Cowan S, Bieniasz PD. Restriction of Human Immunodeficiency Virus Type 1 by TRIM-CypA Occurs with Rapid Kinetics and Independently of Cytoplasmic Bodies, Ubiquitin, and Proteasome Activity. J Virol. 2005;79(24):15567-15572. doi:10.1128/JVI.79.24.15567-15572.2005

62. Ylinen LMJ, Keckesova Z, Wilson SJ, Ranasinghe S, Towers GJ. Differential restriction of human immunodeficiency virus type 2 and simian immunodeficiency virus SIVmac by TRIM5alpha alleles. J Virol. 2005;79(18):11580-11587. doi:10.1128/jvi.79.18.11580-11587.2005

63. Braaten D, Ansari H, Luban J. The Hydrophobic Pocket of Cyclophilin Is the Binding Site for the Human Immunodeficiency Virus Type 1 Gag Polyprotein. Vol 71. J Virol; 1997. doi:10.1128/JVI.71.3.2107-2113.1997

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I declare under oath that I have compiled my dissertation independently and without any undue assistance by third parties under consideration of the 'Principles for the Safeguarding of Good Scientific Practice at Heinrich Heine University Düsseldorf'.

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