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Rabbits as a model to study HIV-1 infection and the search for new innate immunity players in resting CD4 T cells

Habilitationsschrift

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(2018)

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1. Abstract

Once infected with human immunodeficiency virus (HIV), currently available pharmacotherapies can only partly control, but not cure infection. Thus, there remains an urgent need for more potent and conceptually novel antiviral therapeutics, including the development of prophylactic and therapeutic HIV vaccines. To develop such novel treatment strategies, the use of animal models is critical to study virus replication and disease progression *in vivo*. On the other side, understanding how innate immunity works in primary human HIV target cells is important to find novel measures to inhibit or even eradicate HIV. Furthermore, knowledge gained from innate immunity studies in human cells can be transferred to animal model development. Here, we focus on both establishing rabbits as a suitable candidate to study HIV pathogenesis and identifying SAM domain and HD domain-containing protein 1 (SAMHD1) and a currently unknown protein as important cellular factors inhibiting HIV replication in primary resting T cells.

Cells from New Zealand white rabbits display a remarkable HIV susceptibility *ex vivo* as they express only three blocks to full-length HIV replication. Deficits at the level of entry and reverse transcription could be overcome by transient expression of human CD4/CCR5 on primary rabbit macrophages and by using a HIV/ simian immunodeficiency virus (SIV) capsid chimera to avoid recognition by rabbit tripartite motif-containing protein 5 (TRIM5). The nature of the third barrier, causing a HIV infectivity defect in primary rabbit macrophages, remains elusive. As the phenotype resembles the antiviral activity of serine incorporator proteins 3/5 (SERINC3/5), we analyzed SERINC3/5 orthologs from mouse, rat and rabbit, and compared them to the human counterparts. We found that all orthologs are highly conserved at amino acid level. In the absence of viral antagonists, all rodent and lagomorph SERINC3 and SERINC5 orthologs displayed anti-HIV activity comparable to the human orthologs, generally with lower restriction activities for SERINC3 than for SERINC5. Interestingly, HIV Nef, murine leukemia virus (MLV) GlycoGag and equine infectious anemia (EIAV) S2 proteins counteracted the antiviral activity of all SERINC3/5 orthologs with comparable efficiencies. Thus, our results demonstrate that the antiviral activity of SERINC proteins is conserved also in rodents and rabbits, and can be overcome by all three thus far identified viral antagonists. These findings indicate that SERINC3/5 restrictions do not pose a significant barrier for the development of immunocompetent animal models for HIV-1 infection.

Resting CD4 T cells are one of the major target cells for HIV. Since two decades it was known that resting CD4 T cells are highly resistant to productive infection by inhibiting early reverse transcription of incoming viral genomes, but its underlying nature remained elusive. Here, we identified the deoxynucleoside triphosphate triphosphohydrolase SAMHD1 as a major

restriction factor acting also in resting CD4 T cells. SAMHD1 reduces intracellular dNTP pools, which are a major substrate for the reverse transcription of HIV-1 RNA to cDNA. This restriction is overcome by HIV-1 or HIV-2 virions into which viral protein X (Vpx) is artificially or naturally packaged, respectively, or by addition of exogenous deoxynucleosides. Vpx from the SIVmac (rhesus macaque)/HIV-2 lineage mediates proteasomal degradation of SAMHD1, which leads to the elevation of intracellular deoxynucleotide pools and successful infection of Vpx-carrying HIV.

Subsequently, we found that virion-packaged Vpx proteins from a second SIV lineage, SIV of red-capped mangabeys or mandrills (SIVrcm/mnd-2), increased HIV infection in resting CD4 T cells, but not in macrophages. Surprisingly, these Vpx proteins did not induce SAMHD1 degradation, dNTP pool elevation, or change SAMHD1 phosphorylation. We mimicked enhancement of early post entry steps in a Vpx rcm/mnd-2-like fashion by generating single amino acid changes in the SAMHD1-degrading Vpx mac239 protein. In addition, SIVmac239 Vpx enhanced HIV-1 infection of SAMHD1-deficient resting CD4 T cells of a patient with Aicardi-Goutières syndrome. Thus, our results indicate that Vpx can also counteract an additional block at the level of reverse transcription that acts independently of the SAMHD1-mediated restriction and is specific to resting CD4 T cells.

Summarizing, identification, characterization and surmounting of barriers to HIV replication will increase our knowledge on HIV innate immunity and help to build an immunocompetent small animal model to HIV infection.

2. Abbreviations

2-LTR	two-long terminal repeats
AGS	Aicardi-Goutières syndrome
AIDS	acquired immunodeficiency syndrome
ALLN	N-acetyl-Leu-Leu-Norleu-al; Calpain Inhibitor I
AMD3100	Plerixafor, CXCR4-antagonist
APOBEC	apolipoprotein B mRNA editing catalytic polypeptide-like
BD	Below detection
BLT	humanized bone marrow-liver-thymus mouse
CCR5	C-C chemokine receptor type 5
CD	cluster of differentiation
CD25	α-chain of the IL-2 receptor, expressed on activated T cells
CD3	T cell coreceptor
CD317	Tetherin, BST-2
CD4	Surface receptor on T helper cells, monocytes, macrophages, and dendritic cells
CD69	transmembrane C-Type lectin protein, early activation antigen
cDNA	complementary DNA

CRISPR/Cas	Clustered Regularly Interspaced Short Palindromic Repeats / CRISPR-associated protein, genome editing tool
CRM1	Chromosomal Maintenance 1, Exportin 1
CXCR4	C-X-C chemokine receptor type 4, fusin
CycT1	Cyclin T1
dN	deoxynucleosides
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide
EFV	Efavirenz, Sustiva
EIAV	equine infectious anemia virus
env / Env	envelope (gene/protein)
F1	first filial generation
FACS	fluorescence-activated cell sorting
FITC	fluorescein isothiocyanate
gag / Gag	group of specific antigen (gene/protein)
GBP	guanylate binding protein
GFP	green fluorescent protein
h	human
H/SCA	HIV capsid switch mutant with 1-149aa of SIV capsid
HAART	highly active antiretroviral therapy
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
IFITM	Interferon-induced transmembrane protein
KO	knockout
LTR	long terminal repeats
mABs	monoclonal antibodies
mac	macacca
MARCH	Membrane Associated Ring-CH-Type Finger
MG132	carbobenzoxy-Leu-Leu-leucinal, proteasomal inhibitor
µM	micromolar
mM	millimolar
MLV	murine leukemia virus
mnd	mandrill
Nef	Negative Regulatory Factor
NHP	non-human primates
p24	HIV capsid
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PE	phycoerythrin
PERT	qPCR-based product-enhanced RT
pol / Pol	polymerase (gene/protein)
qPCR	quantitative PCR
R	redundant, region of LTR
R5	CCR5-tropic
rcm	red-capped mangabeys
Rev	regulator of virion expression
RNA	ribonucleic acid
RNAseq	RNA sequencing
RT	reverse transcriptase
s.d.	standard deviation
s.e.m.	standard error of the mean
S2	EIAV regulatory protein
SAMHD1	SAM domain and HD domain-containing protein 1
SCID	severe combined immunodeficiency
SERINC	serine incorporator

SG-PERT	SYBR Green I-based PERT assay
SHIV	SIV/HIV chimera
SIV	Simian immunodeficiency virus
T20	Enfuvirtide, fusion inhibitor
Tat	trans-activator of transcription
TRIM	tripartite motif-containing protein
U	unique, region of LTR
Vif	viral infectivity factor
Vpr	viral protein R
Vpu	viral protein U
Vpx	viral protein X
wt	wildtype
X4	CXCR4-tropic
YFP	yellow fluorescent protein

3. Introduction

HIV has become one of the most devastating pandemic in recorded history. Currently over 36.7 million people are living with HIV-1 and about 1.1 million died in the same year (UNAIDS /WHO, 2016). The introduction of antiretroviral therapy in 1995 (HAART: highly active antiretroviral therapy) resulted in a marked reduction of mortality and morbidity caused by HIV-1/ acquired immunodeficiency syndrome (AIDS), as determined by a decreased incidence of opportunistic infections, and deaths in the developed world. Despite the therapeutic advances made during the last decade, once an individual has become infected, eradication of the virus still remains impossible and no protective vaccine against HIV is in sight.

All retroviruses possess three major coding domains between the two long terminal repeats (LTR). *gag* (group specific antigen) encodes internal structural proteins, *pol* (polymerase) the viral enzymes, and *env* (envelope) components of the envelope protein (Fig. 1). Late in the timing of infection, three primary HIV translation products, all encoding structural proteins, are synthesized as polyprotein precursors that are further processed by viral or cellular proteases, yielding mature particle-associated proteins. In addition to that, being a complex retrovirus, HIV possesses accessory genes which are *vif*, *vpr*, *nef*, *tat*, *rev* and *vpu*.

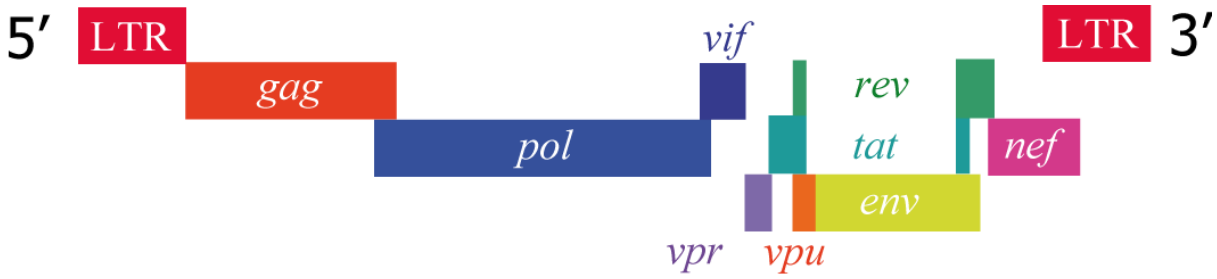

Figure 1: Organization of the HIV genome. The relative locations of the HIV open reading frames *gag*, *pol*, *env*, *vif*, *vpr*, *vpu*, *nef*, *tat*, and *rev* are indicated as well as the 5′ and 3′ LTRs (U3, R and U5 regions noted).

3.1 HIV animal models

An immunocompetent, permissive small animal model would be valuable for the study of HIV-1 pathogenesis and for the testing of drug and vaccine candidates.

Non-human primate (NHP) models are used for the study of human diseases because they exhibit remarkable similarities to humans in virtually every aspect of their anatomy, physiology and endocrinology. Most pathogenesis and vaccine studies of retrovirus infections have been conducted in either the SIV- or SHIV chimera-macaque model including testing of inhibitors of the HIV reverse transcriptase and protease[1-3]. While acute viremia and persistent low level infection were found *in vivo* in pigtailed macaques using a minimally modified simian-tropic HIV-1 strain[4], signs for AIDS-like disease were only reported after serial *in vivo* passage of the virus[5]. Disadvantages of NHPs are their limited accessibility due to their popularity as an HIV animal model, experiments result in high costs and the origin of the animals raises ethical concerns. Furthermore, rhesus macaques have to be infected with the related lentivirus SIV or SHIV chimeras.

Since HIV does not replicate in mice and rats[6] the majority of preclinical testing of anti-HIV drugs has been performed in various xenotransplant models, in which human hematopoietic cells or tissues are transplanted into SCID (severe combined immunodeficiency) mice. Selective aspects of HIV pathogenesis could be investigated using modified versions of the SCID mice. Recently, successful HIV-1 infection and sustained viremia was achieved via rectal, vaginal and mucosal exposure of BLT (bone marrow-liver-thymus) mice[7-9], which was efficiently prevented by pre-exposure prophylaxis treatment[8-10]. Humanized NOD/SCID/IL2Rγnull (hNOG) mice, which were constructed by transplanting human fetal- or cord blood-derived hematopoietic stem cells in lymphoid tissue[11-16], were used for long-term evaluation of anti-HIV drugs and broadly neutralizing antibodies (bNAbs)[8,17-19] and selected aspects of HIV pathogenesis[20,21]. However, a disadvantage of all these systems is the lack of proper humoral and cellular immune responses. This is mainly due to the absence of HLA class I and II expression in the mouse thymus, which is required to support the selection of T cells following human stem cell engraftment[21,22]. A further disadvantage is the variation in human donors but also the reconstitutions. For the generation of BLT mice embryonic material has to be used, which raises ethical concerns. Such a model may therefore not replace but should help to limit the number of studies that are required in NHPs and humans and efforts to reduce the current limitations of this model are in progress[22,23].

An alternative approach to HIV animal model development has been the identification and surmounting of species-specific barriers that HIV encounters along its replication cycle in cells from small animals. These species-specific barriers are either due to missing cellular co-factors, which are hijacked by HIV-1 at different steps of its life cycle for efficient replication, or due to the presence of restriction factors, which block HIV-1 replication at different steps. We

and others have characterized several barriers that limit HIV-1 replication in rodent cells[24-29] (Fig. 2). The ultimate goal is to use this knowledge to generate immunocompetent transgenic animals that are HIV-1 susceptible. In mice, barriers at the level of HIV-1 entry and transcription have been successfully overcome by transgenic expression of appropriate human cofactors, CD4, CCR5, and Cyclin T1[29,30]. Parallel efforts in the rat species in the laboratory of Prof. Keppler have accomplished marked HIV susceptibility following systemic challenge of CD4/CCR5-transgenic animals[31]. This has allowed limited drug testing and contributed to selected aspects of studies into HIV pathogenesis and vaccine development[31-36]. However, several significant limitations exist in the current form of the transgenic rat model, including low and transient viremia and lack of HIV disease. Analogous to the mouse species this is due to ill-defined limitations in the later steps of the replication cycle that limit HIV production (Fig. 2). As a consequence, the search for an alternative small animal model, that either encounters less species-specific restrictions to HIV-1 replication or in which surmounting of those barriers is efficient, is required to develop a more permissive immunocompetent small animal model for HIV-1.

Rabbits have been investigated as potential alternative animal models by either infecting rabbits *in vivo* with cell-free HIV-1[37,38] or with HIV-1-infected human cells[39-43].

However, these initial promising results could not be reproduced and further limitations at the level of virus entry and reverse transcription were identified[44-50].

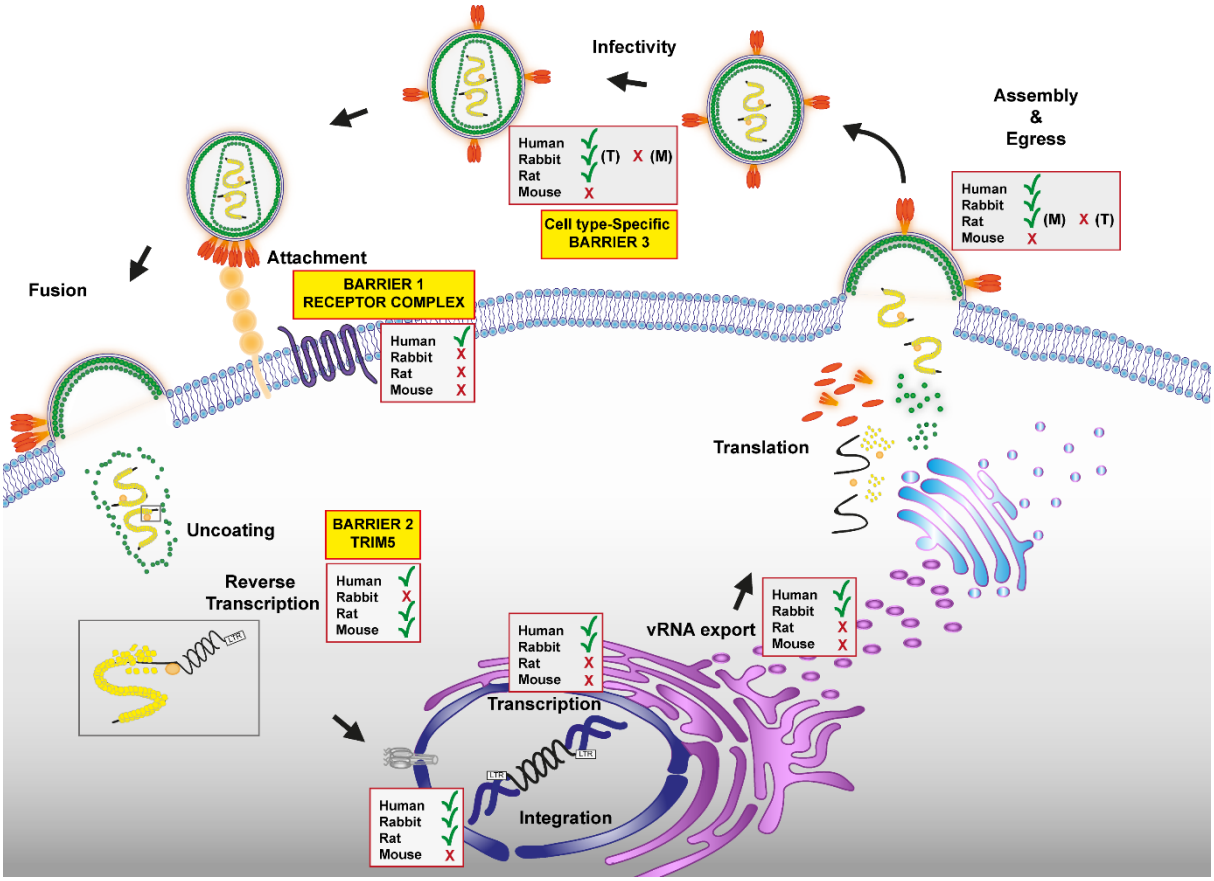

Figure 2: Summary of the efficiency of steps in the HIV-1 replication in primary cells of human, rat, mouse and rabbit origin. Schematic representation of consecutive steps in the HIV-1 replication cycle and the ability of primary cells (T = T cells; M = macrophages) from the respective species to support these steps (√ = efficient; **x** = inefficient or completely blocked). Yellow boxes indicate blocks in the rabbit species[51].

3.2. Resting CD4 T cells

The main targets of HIV-1 are CD4 T cells, macrophages and microglia, yet lymphoid tissues are the major viral reservoir of HIV[52-54], compromising mainly latently infected resting CD4 T cells that carry integrated replication-competent HIV, even in patients under antiretroviral therapy[55-61]. Once latently infected resting CD4 T cells get activated, virus production is triggered, leading to the generation of fully-infectious HIV[55]. A SIV *Macaca nemestrina* (pig-tailed macaques) model could mimic these findings by detecting SIV DNA in tissues containing residing resting CD4 T cells as well as in resting CD4 T cells from the peripheral blood[62]. In addition, we could recently show a lack of significant changes of integrated HIV-1 cDNA in patients treated with the integrase inhibitor Raltegravir, suggesting that most integrated DNA is archival[63]. *In vitro* infection studies with resting CD4 T cells, however, demonstrated that despite binding to and entering resting CD4 T cells, HIV is not able to replicate within this large cell population[64,65]. This is mainly due to inefficient reverse transcription[65] as well as a block to integration of proviral DNA[64], yet the cellular factor responsible for this block as well as its nature remained unidentified. These findings indicate that the block in resting CD4 T cells might be a driving force for HIV latency in infected patients and boosting of this factor might activate those latently infected reservoirs.

3.3. SAMHD1 acts as innate immunity factor against HIV

When mammals are attacked by pathogens, innate immunity represents the first line of defense before adaptive immunity is turned on. Specific pathogen-recognition receptors (PRRs) are expressed by cells of the innate immune system that recognize pathogen-associated molecular patterns (PAMPs) of bacterial and viral pathogens. One important category of innate factors are so-called restriction factors. In the case of retroviruses, host cell proteins have been identified to interfere at different steps of the retroviral life cycle. Some factors have been shown to recognize virus-specific proteins or structural motifs to trigger an immune response and block virus infection of the cell. Among the proteins encoded by these antiviral genes are e.g. APOBEC3, TRIM5α, and CD317[66]. One of the more recent restriction factors is SAMHD1[67,68], which limits HIV at the level of reverse transcription in myeloid cells (Fig. 3) and acts as a dGTP-regulated deoxynucleotide triphosphohydrolase[69,70].

Phosphorylation at position T592 was shown to be a key regulator of SAMHD1's HIV restrictive capacity[71-74].

According to the current dogma, this potent restriction can be overcome by overriding SAMHD1's triphosphohydrolase activity by providing an excess of nucleic acid substrates or by lentiviral incorporated Vpx proteins, which trigger subsequent proteasomal degradation of SAMHD1. Vpx proteins are naturally encoded by the less-pathogenic HIV-2 or SIV, yet HIV-1 has lost an antagonism for SAMHD1, which may contribute to innate sensing-mediated T cell immunopathology and immune evasion in the development of AIDS[75].

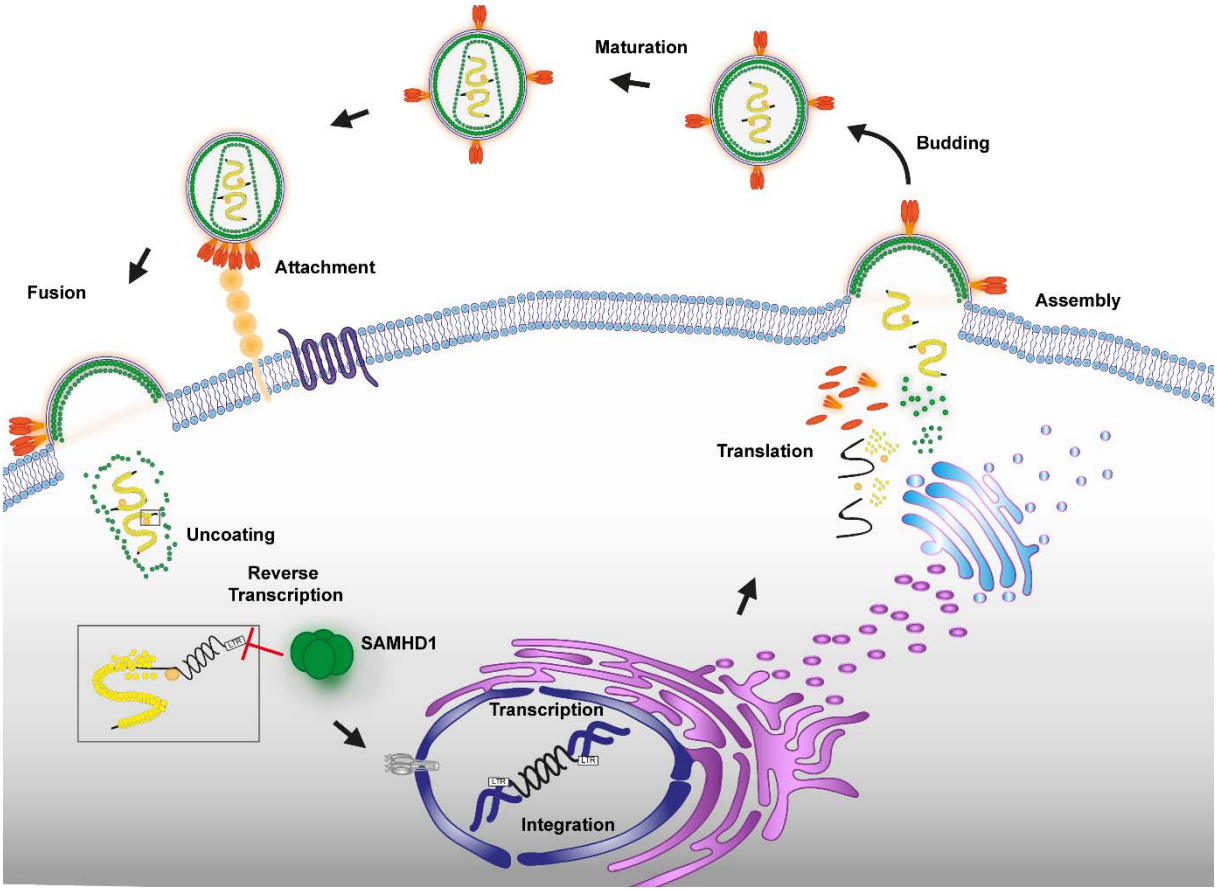

Figure 3: Schematic representation of the replication cycle of HIV-1 in infected cells. Interactions between gp120, CD4 and chemokine receptors (CCR5 or CXCR4) lead to gp41-mediated fusion followed by virion uncoating, reverse transcription of the RNA genome, nuclear import of the viral pre-integration complex, integration of the double-stranded viral cDNA into the host chromosome and transcription of HIV-1 genes. Late steps in the replication cycle include translation, assembly, budding, and maturation of HIV-1 particles. SAMHD1 (green) is active as a tetramer and inhibits HIV-1 at the level of reverse transcription.

4. Results and Discussion

4.1. Rabbits are a better model to study HIV-1 infection

4.1.1. High natural permissivity of primary rabbit cells with a virion infectivity defect in primary rabbit macrophages (Tervo and Keppler, J. Virol. 2010)

Currently no animal model exists which recapitulates major aspect of HIV-1 disease in humans and which could be used to study HIV-1 pathogenesis and to test both new drugs and vaccine treatments. Within the past decades, many efforts were made to identify species-specific limitations to HIV-1 replication in mouse, rat and rabbit cells[24-29,47,49,50]. These limitations can be either due to the lack of cellular factors needed by HIV-1, so-called co-factors, as for example the HIV-1 receptor complex or CyclinT1[76-79]. On the other hand, limitations can occur due to cellular proteins which block HIV-1 replication at distinct stages of its replication cycle and cannot be overcome by HIV-1's accessory proteins, e.g. mouse / rat CD317 by HIV-1 Vpu[80] or rabbit APOBEC1 by HIV-1 Vif [81].

Within this study, we confirmed that rabbits possess a barrier to HIV-1 replication at the level of entry and reverse transcription[47,50]. Entry could be readily overcome by transient overexpression of human CD4 and CCR5 on rabbit macrophages and the block imposed by rabbit TRIM5 was surmounted by introducing the first 149 amino acids of SIV capsid in the context of HIV (H/SCA) [51] (Fig. 4).

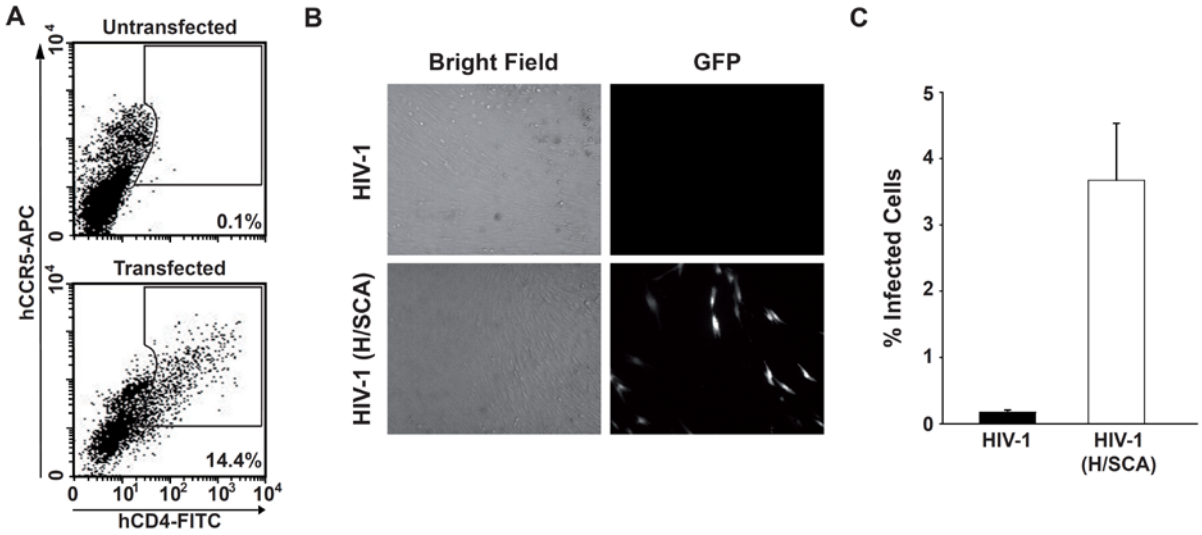

Figure 4: Primary rabbit macrophages are rendered permissive to R5 HIV-1 viruses by coexpression of human CD4 and CCR5. (A) FACS dot plots of hCD4 and hCCR5 expression on rabbit macrophages, which had been transfected with corresponding expression constructs. The FACS gate indicates the receptor-positive cell population. **(B)** Microscopic images of transfected macrophages from panel A, which were subsequently challenged with JR-FL Env-pseudotyped HIV-1 or HIV-1 (H/SCA) GFP vectors. **(C)** Percentages of infected (GFP-positive) rabbit macrophages from panel B as determined by flow cytometry 3 days postinfection.

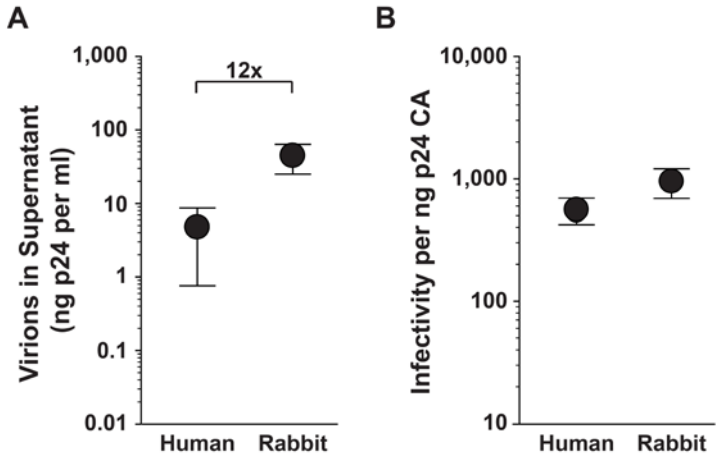

Figure 5: HIV-1 released by primary human and rabbit T cells is equally infectious. Primary human and rabbit T cells were transfected with full-length HIV-1_{NL4-3} proviral DNA. 36 hours post-transfection, supernatants were concentrated and harvested for p24 capsid ELISA **(A)** and TZM-bl cells were inoculated. **B)** Infectious titer was calculated as infectivity per ng p24 capsid. The arithmetic means ± s.e.m. of individual experiments in human (n=4) and rabbit (n=8) T cells are depicted.

Yet, a third cell-type specific defect at the level of infectivity of released virions was identified. As shown in Figure 5, primary T cells of rabbit and human origin were transfected with full-length HIV-1 proviral DNA and physical particles released as well as their infectivity was measured by p24 capsid-ELISA and blue cell assay on TZM-bl reporter cells, respectively. The amount of released viral particles was higher in rabbit T cells than in human T cells, yet the relative infectivity of released particles was comparable. This was in clear contrast to the scenario in primary macrophages. Primary human macrophages were infected with infectious 49.5, a CCR5-tropic variant of HIV_{NL4-3}, and primary rabbit macrophages transfected with proviral HIV-1_{NL4-3} DNA (Fig. 6). The amount of released particles was calculated as percent release of particles measured in the supernatant to the total amount of p24 capsid.

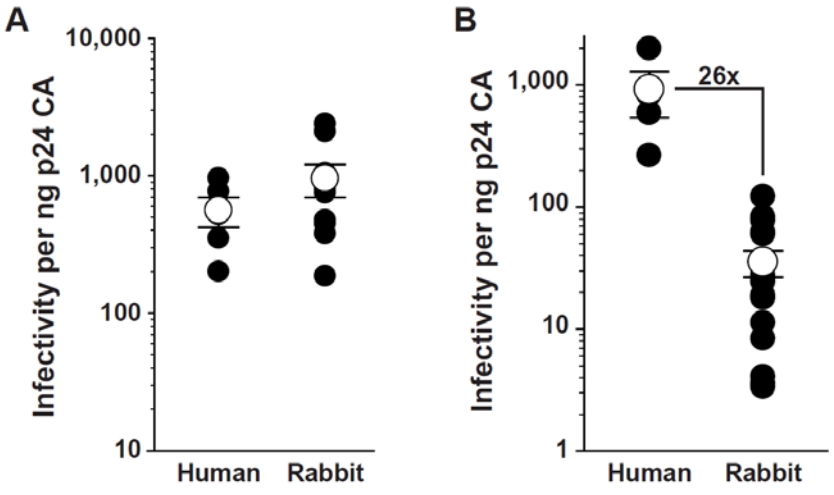

Figure 6: Primary rabbit macrophages display an infectivity defect of released HIV particles. Primary rabbit macrophages were transfected with full-length HIV-1_{NL4-3} proviral DNA. Primary human

macrophages infected with 49.5, a R5-tropic variant of HIV-1_{NL4-3}, served as control. **A)** HIV-1 release was quantified 5 days post transfection or infection as the percentage of total p24 capsid (in cells and supernatant) that was secreted as virion-associated p24 capsid. **B)** The relative infectivity was determined as described for Figure 3. The arithmetic means ± s.e.m. of individual experiments of human macrophages (n=4) and rabbit macrophages (n=16) are depicted.

As depicted in Figure 6, the percent release of HIV-1 was comparable between both species. Yet, relative infectivity of released virions was reduced by 26-fold in primary rabbit macrophages. This could be due to the lack of a co-factor needed for HIV-1's infectivity or the presence of a restriction factor that is incorporated into the budding particle. However, the latter scenario is more likely as different restriction factors have nowadays been described to inhibit HIV-1 infectivity by different mechanisms. Rabbit APOBEC1 was described to reduce HIV-1 infectivity by hypermutating its *de novo* transcribed cDNA in the next round of infection[81]. We also analyzed the mutation pattern in TZM-bl reporter cells that were inoculated with filtered supernatants of HIV-1_{NL4-3} transfected primary rabbit macrophages. Yet, we could not identify APOBEC-specific hypermutation patterns within the amplified fragments and they were quite similar to those found in TZM-bl cells inoculated with filtered supernatants of infected primary human macrophages (data not shown).

More recently, IFITMs (interferon inducible transmembrane proteins), MARCH8 (membrane associated ring-CH-type finger 8), GBP-5 (guanylate binding protein 5), 90K and SERINC3/5 (serine incorporators 3/5) were described to influence HIV-1 infectivity[82-87]. IFITMs reduce virion infectivity by disturbing incorporation and processing of the envelope protein[88]. The mechanism by which MARCH8 blocks envelope incorporation into budding virions is currently not fully understood[83], but it is highly expressed in terminally differentiated myeloid cells. GBP-5 expression levels in primary macrophages inversely correlate with infectious HIV-1 yield. Furthermore, GBP-5 interferes with Env processing and incorporation[87]. 90K was also recently described to reduce particle infectivity by affecting proteolytic cleavage of Env precursor molecules[84]. SERINC proteins do not affect Env maturation but rather inhibit a step prior to small pore formation and thus impair fusion of the virus with the next target cell[89].

Analyses of the individual putative restriction factor orthologs in an ectopic overexpression context in otherwise permissive cells would shed light whether they have an impact on HIV-1 infectivity. In addition, RNAseq and mass spectrometric analyses of primary rabbit T cells and macrophages could directly identify several candidates that might be more abundantly expressed in primary rabbit macrophages. Knockdown or knockout approaches in primary rabbit macrophages could then directly show whether one of the currently known candidates is the cause for the infectivity defect of HIV-1. Once, the candidate is known, strategies to overcome this specific factor would have to be set-up.

Summarizing our findings, rabbit cells pose only three replication barriers to HIV-1, which is far less than in rodents[25,26]. Currently the nature of only one limitation remains elusive. In combination with recent knock-in and knock-out strategies also in this species [90-92], rabbits are an attractive candidate to become a fully permissive small animal model to study HIV-1 and test antiviral strategies.

4.1.2. SERINC3 and SERINC5 do not pose a barrier for HIV animal model development (de Sousa-Pereira *et al*., *submitted*)

Generation of immunocompetent human immunodeficiency virus (HIV)-permissive animal models have been hampered by the fact that HIV encounters replication barriers in rodents and lagomorphs and most of them are still not characterized or overcome[25,29,31,36]. These barriers are in some instances due to missing or incompatible cellular co-factors. This is especially the case for CD4, CCR5 and CXCR4. Here, HIV entry is supported only by the human orthologs[31,93,94]. In addition, a single species-specific amino acid change or variant (C261Y) in rodent CyclinT1 abrogates Tat-mediated transcription elongation[95,96]. Recently, Sherer *et al*. identified surface-exposed elements in CRM1, which are unique to higher primates and important for Rev-regulated nuclear export of unspliced and singly spliced viral mRNA[97]. Expression of human CyclinT1 and/or human CRM1 enhance HIV gene expression in T cells and macrophages from transgenic rats[36,98], yet further undefined limitations exist in the late phase of HIV replication in T cells from hCD4/hCCR5/hCyclin T1 transgenic rats[36].

On the other side, HIV encounters barriers that are due to the presence of intrinsic innate immunity factors, so-called restriction factors. HIV has evolved accessory proteins that counteract these factors for successful replication in human cells. HIV reverse transcription is affected by lagomorph TRIM5 proteins whose restriction can be evaded by exchanging the first 149 amino acids of HIV capsid by simian immunodeficiency virus (SIV) capsid[50,51,99]. SAMHD1 restricts HIV replication at the level of reverse transcription by reducing intracellular dNTP pools and is degraded by lentiviral Vpx proteins[67,68], yet the antiretroviral activity of murine SAMHD1 cannot be antagonized by Vpx[100]. CD317/BST-2/Tetherin tethers mature virions at the plasma membrane and its antiviral activity is counteracted by HIV-1 Vpu/HIV-2 Env[101,102]. However, rodent CD317 proteins are resistant to currently known antagonists[80,103], and murine leukemia virus (MLV) seems devoid of a CD317 antagonist[104]. Members of the APOBEC cytidine deaminase family get incorporated into budding virions and trigger G to A hypermutations during HIV reverse transcription in the next round of infection. Virion inclusion is prevented by lentiviral Vif proteins. However, rat APOBEC1, mouse APOBEC3 and rabbit APOBEC1 are still incorporated in the presence of Vif[81,105,106]. Thus, species-specific

comparisons may not only add valuable information to the mode of action and interacting motifs, but also identify factors that are critical for building HIV-permissive small animal models. Here, we are interested in a species-specific comparison with rodent and lagomorph orthologs of SERINC3/5, two newly described antiviral factors, as the antiviral phenotype of SERINC3/5 resembles in part the infectivity defect recently reported in primary rabbit macrophages, which manifests itself by twentyfold decreased HIV infectivity compared to human macrophages[51].
SERINC3 and SERINC5 were recently discovered to reduce virion infectivity[85,86]. They have been proposed to belong to a family of serine incorporators[107], yet they are not involved in lipid biosynthesis[108]. SERINC3/5 are highly conserved in terms of amino acid sequences among eukaryotes and primate SERINC3/5 do not show any signatures of positive selection or difference in their antiviral activity[109,110]. Their exact mode of action is still under debate, yet fusion to the next target cell is impaired. Recently, Sood and colleagues demonstrated that virion-associated SERINC3/5 interfere with HIV entry prior to small pore formation with the new target cell[89]. This step is critical within the HIV entry process. First, HIV envelope molecules attach to the cell membrane and bind to its major receptor CD4 and subsequently to its co-receptors CXCR4 or CCR5. Second, co-receptor binding induces exposure and insertion of the gp41 fusion peptide into the host cell membrane. Small pore formation is finally induced by gp41 pre-bundles, which culminate in the formation of six-helix bundles (reviewed in [111]). Those six-helix bundles most likely stabilize and expand the opening pores (reviewed in [111]).
Importantly, viruses have developed strategies to evade the inhibitory effect of restriction factors. HIV Nef, MLV GlycoGag and equine infectious anemia virus (EIAV) S2 proteins counteract human, primate, murine and equine SERINC3/5 and increase virus particle infectivity[85,86,110,112]. How this antagonism works is not clear yet, but it was suggested that both virion exclusion of cellular SERINC5 and inactivation of virion-associated SERINCs is driven by HIV-1 Nef[113]. An interplay with GlycoGag and Envelope seems to be important to overcome SERINC5 antiviral activity against MLV[114]. In addition, equine SERINC5 is also inhibiting EIAV in an Env-dependent manner and EIAV S2 uses similar motifs as HIV Nef for its counteraction[112].
As access to primary rabbit T cells and macrophages is currently limited, we assessed whether ectopically expressed rodent and rabbit SERINCs possess antiviral activities and can be counteracted by HIV-1 Nef, MLV GlycoGag and EIAV S2 proteins.

First, we aligned rodent and rabbit orthologs of SERINC3 and SERINC5 with human counterparts and found that their amino acid sequences are quite conserved. They share 78-93 % amino acid identity for SERINC3 and 81-91 % for SERINC5 (Fig. 7A-B). Further, we calculated the overall mean diversity, which defines the number of base differences per site from averaging over all sequence pairs. The overall mean diversity for SERINC3 is 0.155+/-0.007 and for SERINC5 0.177+/-0.007. In addition, putative transmembrane domains,

highlighted in grey, are well-preserved (Fig. 7A-B). Thus, SERINC3/5 orthologs are quite conserved also among rodents, lagomorphs and humans and none of them seems to be under positive selection. As we considered only sequences from four different species, we could not make any statement whether the orthologs experienced evolutionary pressure due to the arms race between the host and the virus.

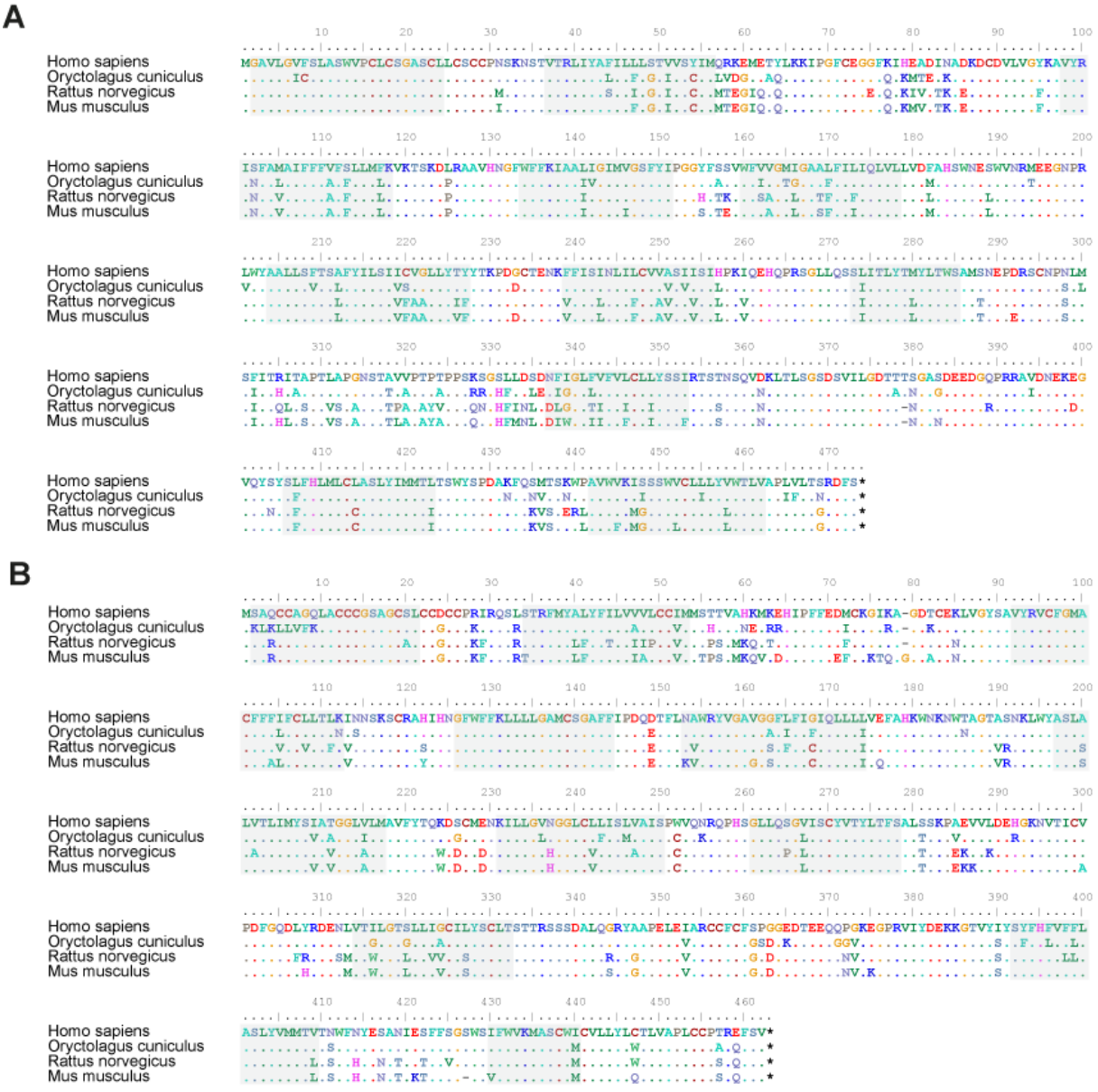

Figure 7: Rodent and rabbit SERINC3/5 are highly conserved on amino acid level. A-B) Translation of the nucleotide sequences for *Homo sapiens* (NM_006811 and NM_001174072), *Oryctolagus cuniculus* (XM_002721072 and XM_008261873), *Rattus norvegicus* (NM_001008312 and NM_133395) and *Mus musculus* (NM_012032 and NM_172588) SERINC3 **(A)** and SERINC5 **(B)**. Highlighted in grey are the transmembrane domains predicted using the web tool PredictProtein [115] and in accordance with [107].

We then isolated rodent and rabbit orthologs of SERINC3 and SERINC5 from mouse, rat and rabbit splenocytes and cloned them into pcDNA- (strong expression) and pBJ6-based (weak expression) vectors.

HEK293T cells, expressing low endogenous SERINC levels, were co-transfected with a proviral HIV-1_{NL4-3} lacking Nef (HIV-1ΔNef) and increasing concentrations of plasmids encoding for the different SERINC3 and SERINC5 orthologs. Supernatants were collected 48 hours post transfection and TZM-bl reporter cells inoculated to further assess virion infectivity. In addition, physical HIV particles were quantified via an in-house p24 capsid ELISA of supernatants and cells, and supernatants were collected for a quantitative RT activity assay[116] (Fig. 8A). Figure 8B shows the relative infectivity, calculated as the infectivity measured on TZM-bl reporter cells per Unit RT activity. Nef-defective HIV-1 was inhibited by SERINC3/5 orthologs in a dose-dependent manner. The reduction imposed by SERINC5 ranged between 1.4 and 151-fold whereas SERINC3 was less potent (2.1- to 29.2-fold). SERINC5 reduced virion infectivity to maximal 0.43-0.85% and SERINC3 only to 3.43-5.49%. The protein abundance of SERINC3/5 was determined in parallel by Western Blotting (Fig. 8B) and flow cytometry (data not shown). For both analyses, increasing levels of HA-tagged SERINC3/5 could be detected for all orthologs with increasing amount of plasmid DNA used for transfection. Yet, as seen in Figure 8B, HA-tagged SERINC3 orthologs show only one prominent band at the expected size of ~ 53 kDa. The expression pattern for SERINC5, in contrast, is more complex and is most likely due to different levels of glycosylation and phosphorylation.

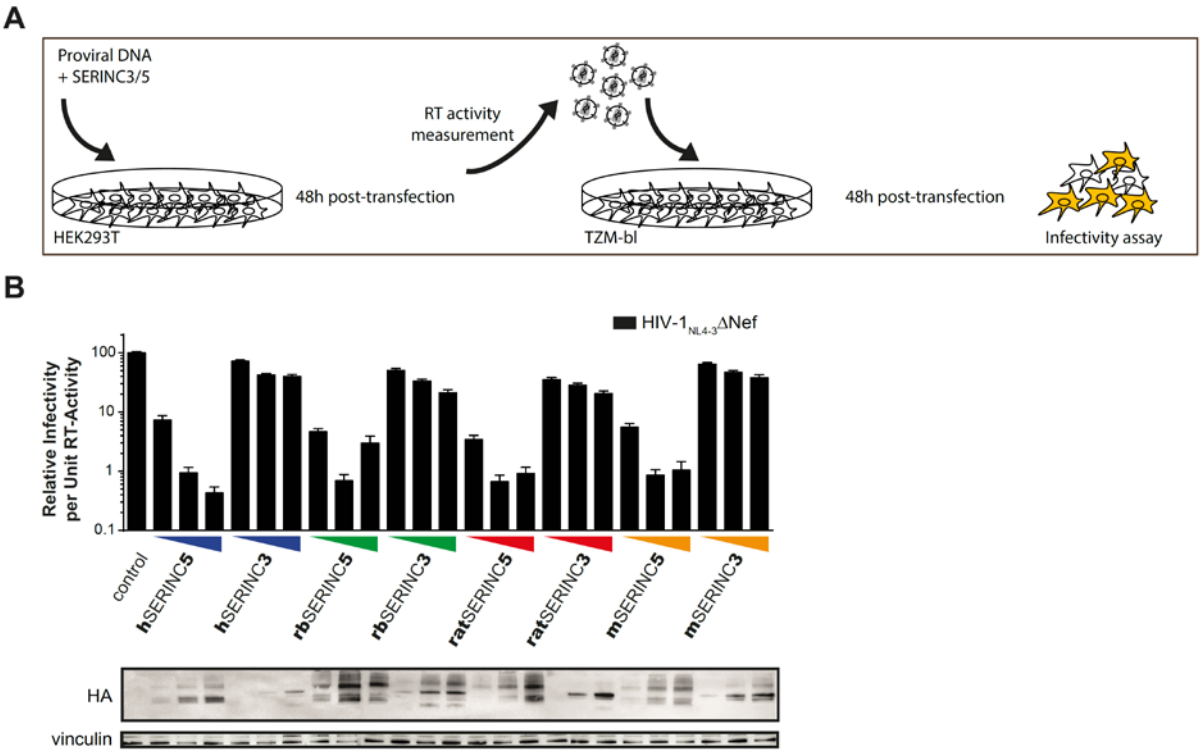

Figure 8: SERINCs from rodents and rabbits are restricting HIV in a dose-dependent manner. HEK293T cells were transfected with proviral HIV-1_{NL4-3} plasmid DNA lacking Nef (HIV-1_{NL4-3}ΔNef) and increasing amounts of expression plasmids encoding for the different SERINC3/5 orthologs. Supernatants were collected 48 h post transfection and analyzed for reverse transcriptase (RT) activity of released viral particles using SG-PERT. In parallel, TZM-bl cells were inoculated with harvested supernatants and firefly luciferase activity measured 48 hours post inoculation. In addition, HEK293T cells were harvested and SERINC3/5 expression levels monitored by Western Blotting. Relative HIV-1 infectivity was calculated as a ratio of firefly luciferase counts to RT units and normalized to control

(empty expression plasmid). Shown are arithmetic means +/- s.e.m. of three independent experiments. * < 0.05; ** < 0.01; *** < 0.001; ns = not significant.

To assess at which step of the HIV replication cycle SERINC3/5 orthologs act as restriction factors, we assessed the fusion capacity of HIV-1ΔNef virions produced in the presence or absence of individual SERINC proteins by performing BlaM-Vpr fusion assays[117]. Identical RT Units were applied onto TZM-bl cells and virion fusion was measured by flow cytometry[118]. Figure 9A depicts representative dot plots of fusion events in TZM-bl cells. Uninfected and fusion inhibitor T20 control-treated, infected TZM-bl cells did not display infection levels above background. Fusion of particles produced in the presence of SERINC5 and SERINC3 dropped from 78% in control viruses to 0.2- to 3.6% and to 6.4- to 27%, respectively (Fig. 9A).

Over a range of multiple experiments, virus entry was significantly reduced from 3 to 257-fold when HIV-1ΔNef virions were produced in the presence of SERINC3/5 orthologs (Fig. 9B). In order to judge whether these virion fusion analyses correlate with the virion infectivity read-outs performed in parallel, we plotted the log10 of relative virion fusion and relative virion infectivity. Both parameters correlate highly with each other with R^2 of 0.9113 and $p < 0.0001$ (Fig. 9C). Thus, our assay approach shows that the extent of inhibition of fusion is similar to the extent of infection inhibition. In contrast to Rosa *et al*., our data does not indicate a further limitation after virus entry[85].

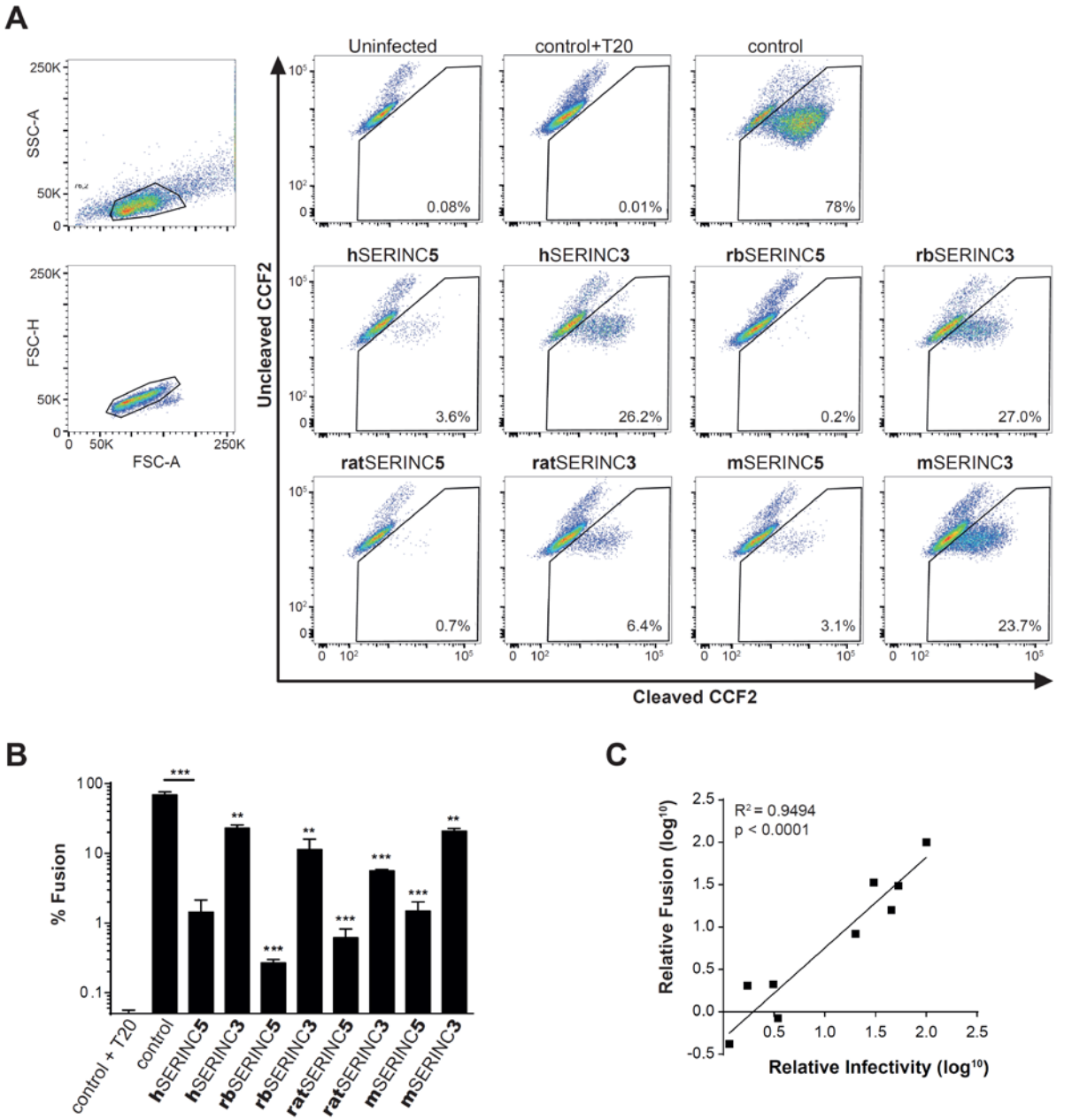

Figure 9: Rodent and rabbit SERINC3/5 orthologs interfere with HIV prior to virus entry. A) HEK293T cells were transfected with proviral HIV-1_{NL4-3}ΔNef plasmid DNA, a BlaM-Vpr expression plasmid together with expression plasmids encoding for the SERINC3/5 orthologs. Viral particles were harvested 48h post transfection and pelleted via sucrose cushion. Equal RT units (determined by SG-PERT) were used to infect TZM-bl cells. Fusion was analyzed 4 hours post infection via flow cytometry, measuring the shift in fluorescence caused by the cleavage of CCF2 upon cellular entry. T20 was used as fusion inhibitor. Shown are representative dot plots. **B)** Graphical representation summarizing the raw data presented in **(A)**. Shown are arithmetic means +/- s.e.m. of two independent experiments. **C)** Correlation between fusion events and relative infectivity measured in parallel. * < 0.05; ** < 0.01; *** < 0.001; ns = not significant.

In order to assess whether SERINC3/5 orthologs can be antagonized by known viral counteractors of SERINC proteins, HIV-1ΔNef and SF2Nef virions were produced in the presence or absence of SERINC expressed from pBJ6-based plasmids. SF2Nef was chosen as one of the strongest SERINC3/5 antagonist of HIV-1[85]. The expression of pBJ6-driven plasmids is much weaker than from CMV-driven ones[85,113], which further allows Nef

antagonism studies. Due to this, SERINC3/5 protein levels could not be detected by Western Blotting, yet expression could be verified by measuring HA-tag levels by flow cytometry (data not shown). First, we titrated the amount of pBJ6-driven SERINC3/5 to get the optimal dose that shows maximal inhibition of HIV-1ΔNef and best rescues by SF2Nef virions (data not shown).

As expected, the impact of pBJ6-driven SERINC on virion infectivity was less pronounced as with CMV-driven expression plasmids. Nevertheless, individual SERINC5 proteins reduced virion infectivity of HIV-1ΔNef virions by 2.8 to 9.2 fold, whereas SERINC3 had only a marginal 1.3 to 2.9-fold effect on virion infectivity of HIV-1ΔNef particles (Fig. 10A). In the presence of SF2Nef, SERINC3/5's antiviral activity was antagonized. Specifically, SF2Nef significantly increased virion infectivity of SERINC5-containing particles from 2.6- to 9.2-fold. This effect was less pronounced for SERINC3-containing particles with 1.1- to 1.9-fold and no significant effect was detected for murine SERINC3.

In addition to HIV-1 Nef, MLV GlycoGag was shown to antagonize SERINC3/5[85]. Thus, we produced HIV-1ΔNef virions in the presence or absence of pBJ6-based SERINC3/5 and MLV GlycoGag. Regardless which SERINC3/5 orthologs were used during virus production, the antiviral activity was antagonized by GlycoGag (Fig. 10B). Here, GlycoGag significantly increased virion infectivity of SERINC5-containing particles from 2.8- to 7-fold. As already seen for SF2Nef, infectivity enhancement for SERINC3-containing particles was less pronounced with 1.2- to 2.9-fold, without being significant for rabbit SERINC3.

In a last approach, we produced HIV-1ΔNef virions in the presence or absence of pBJ6-based SERINC3/5 and EIAV S2. Here, we could also detect no antiviral activity of SERINC3/5 when S2 was present during virion production (Fig. 10C). More important, EIAV S2 significantly increased virion infectivity for SERINC5-containing particles with 2.8- to 5.4-fold. Infectivity of SERINC3-containing particles were only enhanced 1.7- to 2.4-fold in the presence of EIAV S2, without being significant for murine SERINC3.

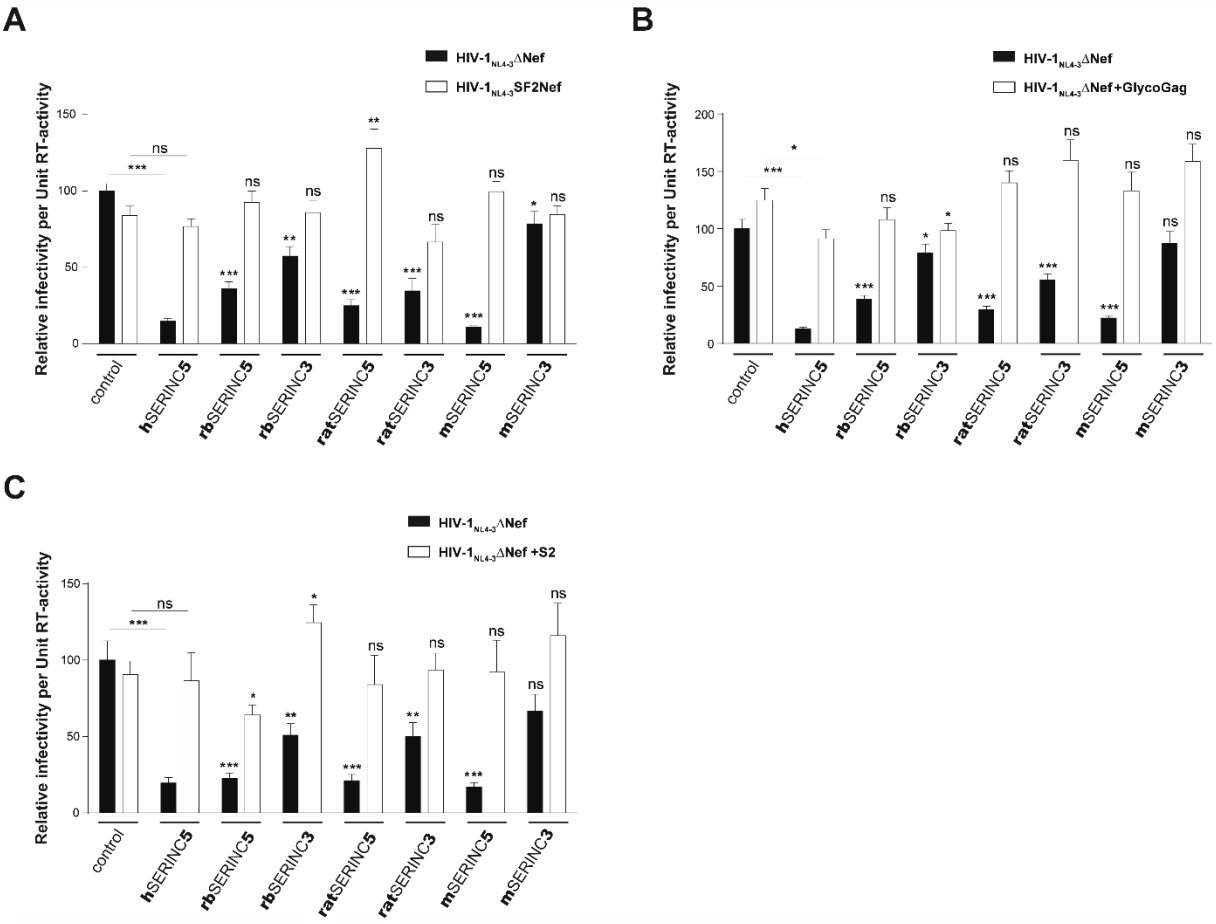

Figure 10: Rodent and rabbit SERINC3/5 orthologs are counteracted by three different viral proteins. A-C) HEK293T cells were transfected with plasmids encoding for SERINCs and proviral HIV-1_{NL4-3}ΔNef or HIV-1_{NL4-3}SF2 Nef plasmid DNA **(A)**; proviral HIV-1_{NL4-3}ΔNef plasmid DNA in the presence or absence of a MLV (Murine leukemia virus) GlycoGag expression plasmid **(B)** or EIAV (Equine Infectious Anemia Virus) S2 expression plasmid **(C)**. Relative infectivity values were calculated as described before. Shown are arithmetic means +/- s.e.m. of three independent experiments. * < 0.05; ** < 0.01; *** < 0.001; ns = not significant.

Summarizing our results, we show that human, rodent and lagomorph SERINC3 and SERINC5 orthologs are conserved in regards of their amino acid sequences. Analyses with simian orthologs showed that these genes do not exert typical signatures of an arms race with pathogens[109]. Normally, it is an arm race between the host and the virus and through continuous evolution, mutations manifest themselves within the host and/or the virus as a result of evolutionary pressure. This was recently shown for human restriction factors like APOBEC3G or BST-2 in contrast to human SERINC3 and SERINC5[109]. Of course, rodent and rabbit SERINC3 and SERINC5 orthologs have not encountered HIV, yet other retro- or lentiviruses, like MLV or RELIK [119], may have posed pressure on those genes.

We observed that rodent and lagomorph SERINC3 and SERINC5 are as antivirally active as human and simian orthologs in the absence of viral antagonists[85,86,110]. Both are acting in a dose-dependent manner, whereas the magnitude of inhibition is less pronounced for SERINC3 orthologs.

In the absence of a viral antagonist, SERINC3/5's antiviral activity is displayed prior to virus entry. This is highly correlated with their infectivity defect in the same experimental set-up (R^2 = 0.9494, p <0.0001; Fig. 3c), which is consistent with other reports[85,113]. Our data does not support the additional post-entry defect that was suggested by Rosa *et al*.[85], indicating that it might dependend on the experimental system whether or not SERINC3/5's antiviral activity is displayed only prior virus entry or additionally post-entry.

To our surprise, the three known antiviral factors, HIV-1 Nef, MLV GlycoGag and EIAV S2[85,86,112], were able to counteract all SERINC3 and SERINC5 orthologs. Normally, HIV restriction factors orthologs are antivirally active, yet the block induced by these factors cannot be surmounted with the viral antagonists. Rodent CD317/BST-2/Tetherin inhibits release of HIV-1, HIV-2, SIV and MLV and known viral antagonists, like HIV-2_{ROD-10} Env, Ebola GP, KSHV K5, or HIV-1 Vpu, are unable to rescue infection[80,103]. HIV-1 Vif cannot exclude incorporation of murine APOBEC3G into budding virions[106]. Furthermore, rodent and rabbit APOBEC1 have also antiviral activity against HIV, SIV and MLV without being counteracted by Vif proteins[81]. Thus, our results show that the domains responsible for the antiviral activity are highly conserved among the SERINC3/5 orthologs. In addition, interacting domains necessary for counteraction have to be preserved to a very high degree that viral antagonists expressed by complex lentiviruses (HIV, EIAV) and a simple gamma retroviruses (MLV) are able to counteract. However, this does not mean that all three viral proteins target similar motifs or antagonize via direct interaction. HIV-1 Nef, MLV GlygoGag and EIAV S2 proteins most likely have evolved independently to counteract SERINC3/5 proteins. HIV-1 Nef and EIAV S2 proteins share similar interacting motifs[85,112] and together with MLV Glycogag they localize to cellular membranes. Recently, Dai *et al*. identified the intracellular loop 4 of SERINC5, specifically amino acids 9-26, to confer sensitivity to Nef[120]. Within this stretch, amino acid sequences between human, rodent and rabbit SERINC5 present 1-3 amino acid differences (Fig. 7), in contrast to 6-7 amino acid differences for frog and zebrafish SERINC5[120]. This might explain the high conservation of Nef antagonism. Yet, little is known about important domains in SERINC3/5 orthologs and domains essential for counteraction by MLV GlycoGag or EIAV S2 proteins. Thus, the underlying interactions need to be determined.

In summary, rodent and lagomorph SERINC3/5 orthologs are restricting HIV infectivity and can be counteracted by HIV-1 Nef, MLV GlycoGag and EIAV S2 proteins. Our main conclusion is that rodent and lagomorph SERINC3/5 orthologs do not pose any barrier for HIV animal model development.

As SERINC5's antiviral function is highly conserved among mammals and virus-encoded countermeasures have evolved in a diverse set of pathogens (HIV Nef, SIV Nef, MLV GlycoGag, EIAV S2), we will now use murine leukemia virus (MLV) and its natural host as a

model system to study SERINC5's impact in an endogenous setting *(funded by Else-Kröner Fresenius Stiftung starting 04/2018)*. In particular, we will elucidate **(i)** the mode of action for SERINC5's antiviral activity and antagonism by studying the effect of exogenous SERINC5 orthologs on MLV infectivity, **(ii)** we will measure endogenous SERINC5's impact *in vitro* and **(iii)** investigate its role in viral dissemination and immune response *in vivo*. Furthermore, **(iv)** we will study how HIV Nef affects SERINC5 *in vivo*.

We anticipate that our results will lead to a gain-of-knowledge for virus host interactions in general and in particular *in vivo* and furthermore open new options for drug design approaches.

4.1.3. Generation of hCD4/hCCR5 transgenic rabbits *(unpublished)*

Based on our findings, the generation of transgenic rabbits carrying the HIV-1 entry receptor complex on target cells would be a prerequisite for the generation of a susceptible immunocompetent animal model for HIV-1. hCD4/hCCR5 transgenic rats and mice are susceptible to HIV-1 infection and this was dependent on both receptors[31,94,121]. We hypothesized that the hCD4 and hCCR5 transgenic constructs used in rodents[29,31] may support cell-type specific transgene expression also in rabbits. Analogous to their strategy, we generated transgenic rabbits expressing hCD4/hCCR5 in collaboration with a commercial vendor (AGROBIOGEN GmbH., Hilgertshausen, Germany) by co-microinjection of both constructs. Until now, we have generated five founder lines, three of which transmit both transgenes to their offsprings. We sacrificed one pair of littermates (non-transgenic and transgenic) and performed immunohistochemical (Fig. 11) and flow cytometric (Fig. 12) analyses. Both analyses showed that hCD4 and hCCR5 are expressed in relevant HIV target organs, i.e. thymus, spleen and bone marrow.

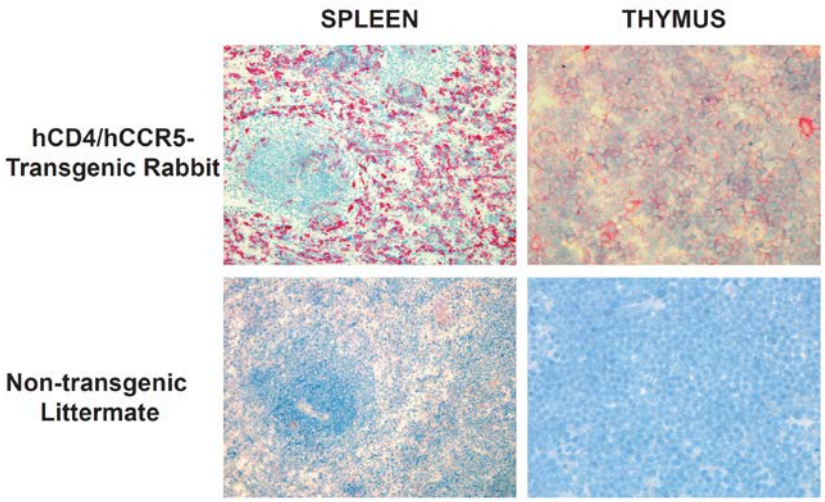

Figure 11: hCD4 expression in rabbit spleen and thymus. Transgenic and non-transgenic F1 animals were sacrificed. Tissue sections from spleen and thymus were stained for hCD4 expression by immunohistochemistry using New Fuchsin and hematoxylin as nuclear counter stain.

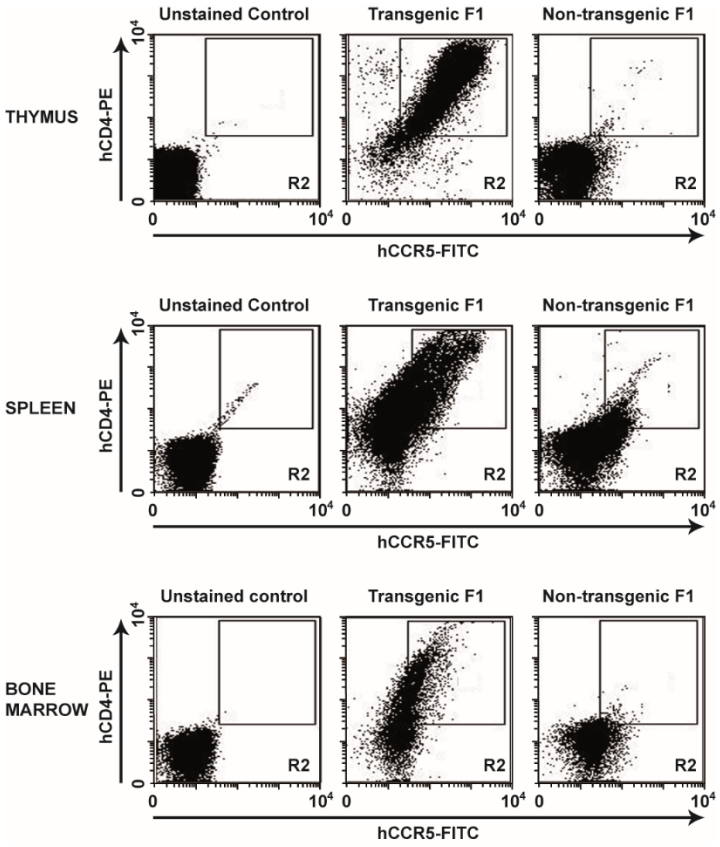

Figure 12: CD4/hCCR5 are expressed in lymphatic organs. Thymocytes (upper panel), splenocytes (middle panel) and bone marrow cells (lower panel) of transgenic and non-transgenic animals were isolated and stained with mABs for hCD4 and hCCR5 coupled to PE and FITC, respectively, and expression levels analyzed by flow cytometry. Unstained control with cells of transgenic F1 animal.

Summarizing, we succeeded to generate hCD4/hCCR5 transgenic rabbits that express the HIV receptor complex on relevant HIV target cells. As CRISPR/Cas manipulation strategies are now available for rabbits as well[90-92], we will express hCD4 and hCCR5 in rabbit TRIM5 KO rabbits. Yet, HIV may not be able to readily infect these genetically modified rabbits. The adaption of the virus to the rabbit background has to be considered on hCD4 and hCCR5-positive TRIM5 KO rabbit cell lines or primary *ex vivo* cultures from genetically modified animals, in which HIV can replicate and evolve. Virus replication will be monitored over time by SG-PERT[116] and once replication has been established, we will isolate the virus and sequence for adaptation-induced mutations. Alternatively, we will perform a similar evolution approach *in vivo* as recently published for NHPs[5].

4.2. Resting CD4 T cells express a potent restriction factor other than SAMHD1 limiting the early phase of infection

4.2.1. SAMHD1 restricts HIV-1 infection in resting CD4 T cells (Baldauf, Pan *et al*., Nature Medicine, 2012)

SAMHD1 is a dGTP-regulated deoxynucleotide triphosphohydrolase and was identified as a restriction factor in myeloid cells[67-70], which restricts HIV by depleting intracellular dNTP pools.

This potent restriction can be overcome by overriding SAMHD1's triphosphohydrolase activity by an excess of nucleic acid substrates or by lentiviral incorporated Vpx proteins, which trigger proteasomal degradation of SAMHD1. Vpx proteins are naturally encoded by the less-pathogenic HIV-2 or SIV[67,68,122].

As HIV-1 does not encode for Vpx proteins, we generated a HIV-1_{NL4-3} proviral plasmid, which encodes a Vpx packaging signal within the p6 domain (HIV-1*GFP). Viruses were produced in HEK293T cells in the presence or absence of Vpx expression plasmids and co-packaging into virions confirmed by Western blotting (Fig. 13D). We then challenged resting CD4 T cells with equivalent infectious units of HIV-1*GFP virions with (+Vpx) or without (Control) incorporated Vpx from SIVmac239 and measured GFP expression, as a surrogate marker for early viral gene expression, by flow cytometry three days post infection. We determined in parallel also levels of 2-LTR circles by quantitative PCR. As 2-LTR circles are a by-product of integrated proviral DNA and only present in the nucleus, it can be used as a surrogate marker for nuclear entry. Figure 13A shows primary dot plots for our gating strategy of infected resting CD4 T cells. Both the percentage of GFP positive cells and the amounts of 2-LTR circles were elevated, in the case of GFP expression by more than 30-fold (mean: 31.3 ± 8.4; range: 2.2–199.4-fold), when cells were infected with Vpx-carrying virions (Fig. 13B-C).

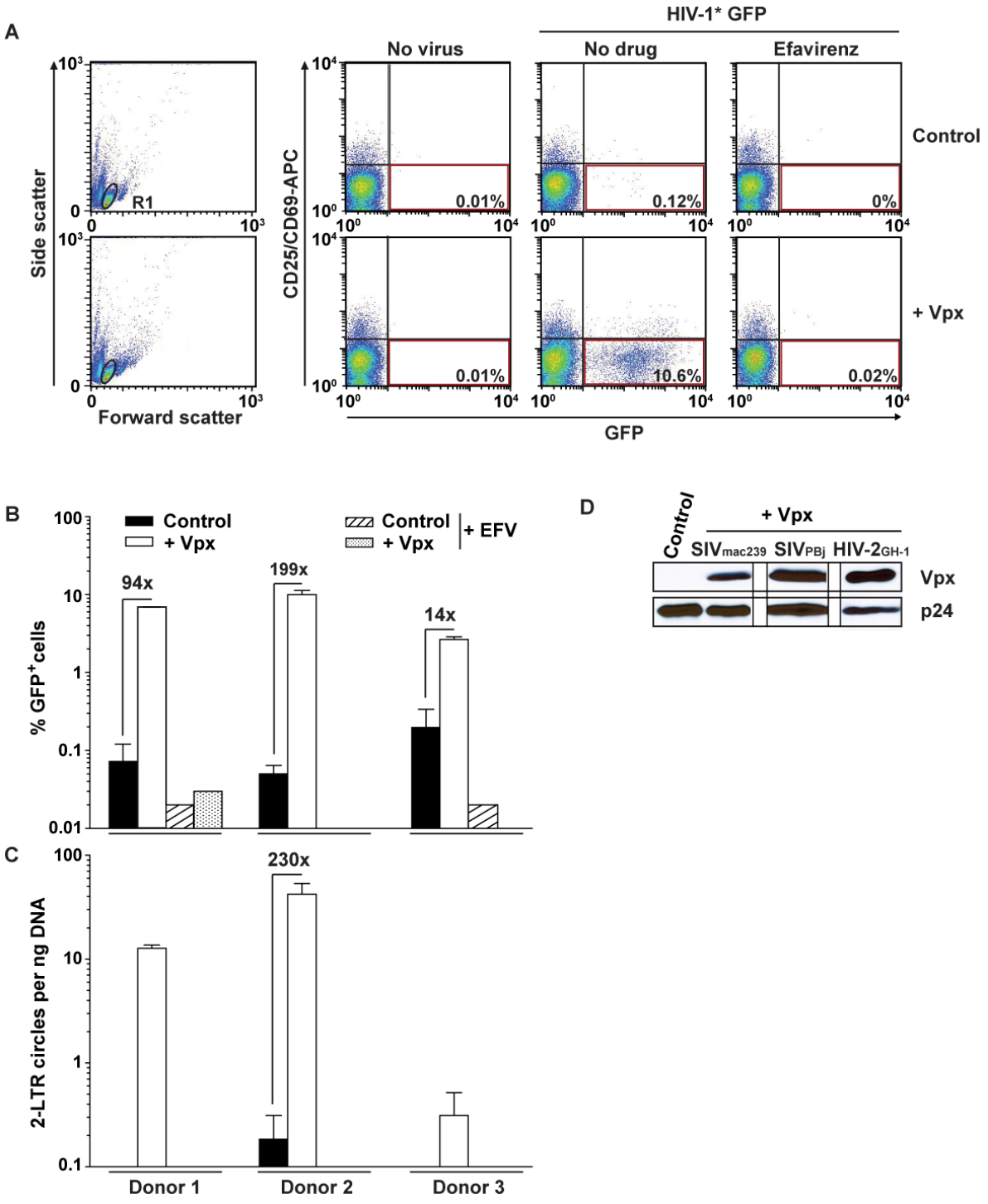

Figure 13: Vpx overcomes a restriction to HIV-1 infection in resting human CD4 T cells. A-C) Resting CD4 T cells were challenged with equivalent infectious units of HIV-1* GFP virions with (+Vpx) or without (Control) incorporated Vpx from SIV_{mac239} and analyzed on day 3 after infection. The reverse transcriptase inhibitor efavirenz (EFV) served as specificity control. **A)** Dot plots of flow cytometric analysis for 1 out of 23 donors. The percentages of viable (gate R1, left graphs) resting ($CD25^{-}CD69^{-}$) and infected (GFP^{+}) CD4 T cells are shown in the bottom right quadrants. **B-C)** Percentage of GFP^{+} cells **(B)** and relative copy numbers of HIV-1 2-LTR circles **(C)**. Bars represent means of triplicates + s.d., with the factors of increase by Vpx indicated. **D)** Immunoblotting of HIV-1 virions for incorporated epitope-tagged Vpx proteins from SIV_{mac239}, SIV_{PBj} or $HIV\text{-}2_{GH\text{-}1}$. HIV-1 p24 served as a loading control.

We next were interested to see whether the phenotype we observe in resting CD4 T cells is linked to SAMHD1. We established a staining approach of SAMHD1 to monitor its expression levels in parallel to HIV infection in resting CD4 T cells by flow cytometry. We observed that SAMHD1 was degraded in resting CD4 T cells when infected with HIV-1* GFP virions containing Vpx. This degradation was very fast as we observed it as early as 6 hours after virion challenge (Fig. 14A-B). We next determined whether this SAMHD1 degradation depends on virus entry and the release of Vpx from disassembled viral capsid. Thus, we added the peptidic virion fusion inhibitor T20 or the CXCR4 antagonist AMD3100 and determined that SAMHD1 degradation is dependent on efficient virus entry (Fig. 14C). We further showed that SAMHD1 depletion and infection enhancement in resting T cells was abrogated, when the cells were treated with the proteasome inhibitors MG132 (Fig. 14C) or ALLN (data not shown).

SAMHD1's function is to hydrolyze intracellular dNTPs, which are required for efficient HIV-1 reverse transcription. Thus, SAMHD1 might lower the dNTP concentrations below a certain threshold[69,70,122]. Exogenous addition of pyrimidine and purine deoxynucleosides (dNs) as dNTP precursors to the culture medium should thus provide sufficient levels of dNTPs. Addition of dNTP precursors enhanced the permissivity of resting CD4 T cells for HIV-1 GFP ninefold (n = 8) over solvent-treated control cells in the absence of Vpx proteins (Fig. 14D). Collectively, these results showed that intracellular dNTPs pools are rate limiting for HIV reverse transcription[123] and that SAMHD1 may be a key regulator of this cellular antiviral state.

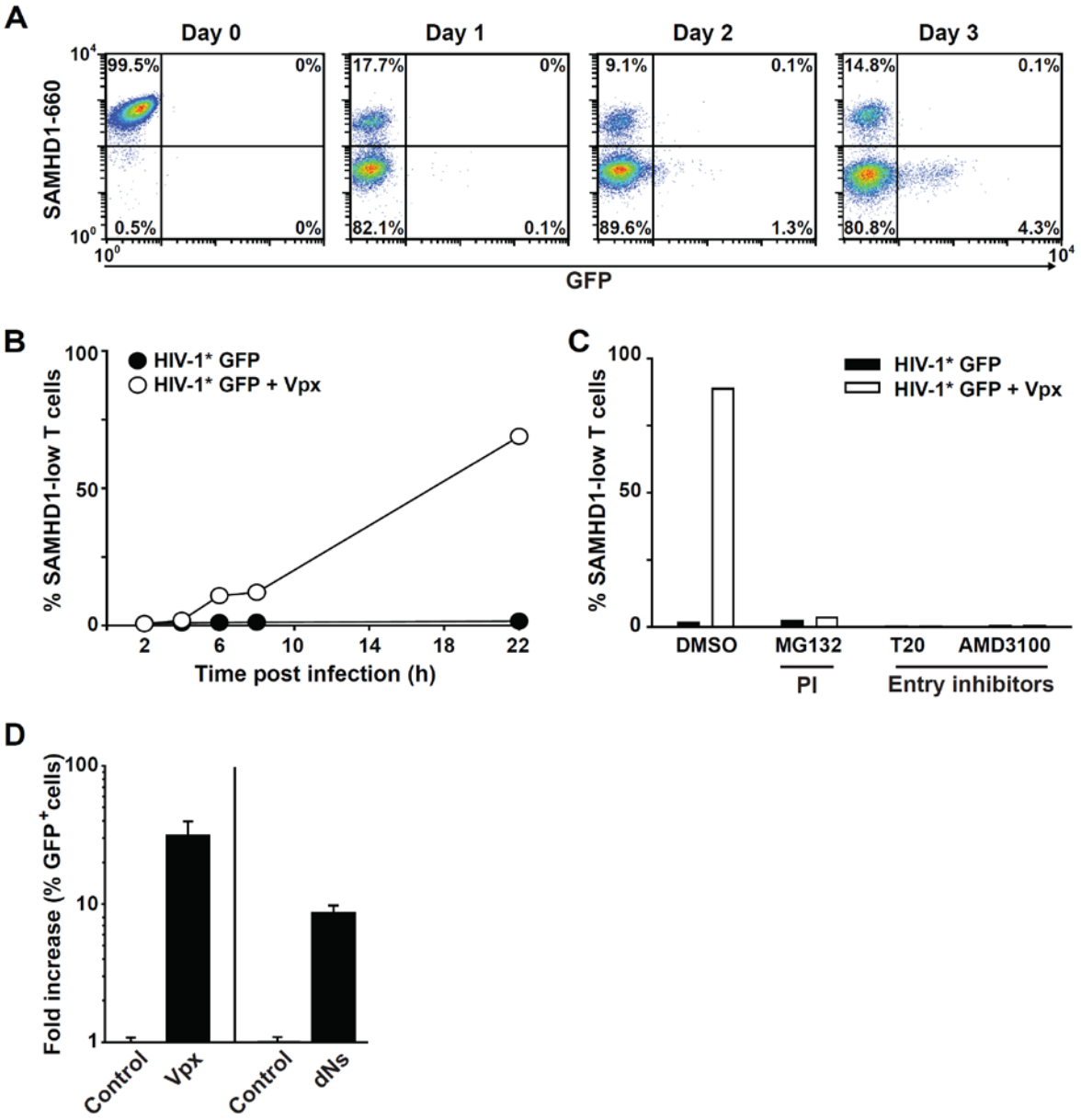

Figure 14: Susceptibility of resting CD4+ T cells to Vpx-carrying HIV-1 is paralleled by proteasomal degradation of SAMHD1. A) Time course of SAMHD1 and GFP expression in resting CD4 T cells after challenge with HIV-1* GFP + Vpx (SIV_{mac239}). The percentages of cells in the respective quadrants are shown. **B)** Quantification of SAMHD1 expression in resting CD4 T cells within the first 22 hours after exposure to HIV-1* GFP with (+ Vpx) or without (Control) incorporated Vpx. Data points mark the percentages for cells with low SAMHD1 levels (corresponding to the bottom left quadrants in FACS panels shown in **(A)**. **C)** Effect of proteasome or HIV-1 entry inhibitors on SAMHD1 levels in resting CD4 T cells after exposure to HIV-1* GFP ± Vpx. Resting CD4 T cells were pretreated for 1 hour with either DMSO, the proteasome inhibitor (PI) MG132 (10 µM), the fusion inhibitor T20 (50 µM) or the CXCR4 antagonist AMD3100 (5 µM) before infection with HIV-1*GFP with or without virion-packaged Vpx. Drugs were removed 20 h later. Shown are the percentages of resting CD4 T cells with low levels of SAMHD1 24 h after infection for one of three donors. **D)** Relative factor of increase of infection of resting CD4 T cells with HIV-1* GFP by virion-packaged Vpx (n = 15) or by incubation with deoxynucleosides (dNs) (n = 8). Means + s.e.m. *P < 0.05; ***P < 0.005.

In addition to RNAi experiments, we had the chance to infect resting peripheral blood mononuclear cells (PMBCs) with a nonsense mutation in *SAMHD1* obtained from a patient

with Aicardi-Goutières syndrome (AGS)[124,125]. We were unable to detect SAMHD1 in $CD25^{-}CD69^{-}$ $CD3^{+}CD4^{+}$ T cells from this patient. Unlike cells from healthy donors, resting CD4 T cells from this patient with AGS were intrinsically permissive for HIV-1 GFP infection (Fig. 15A-C).

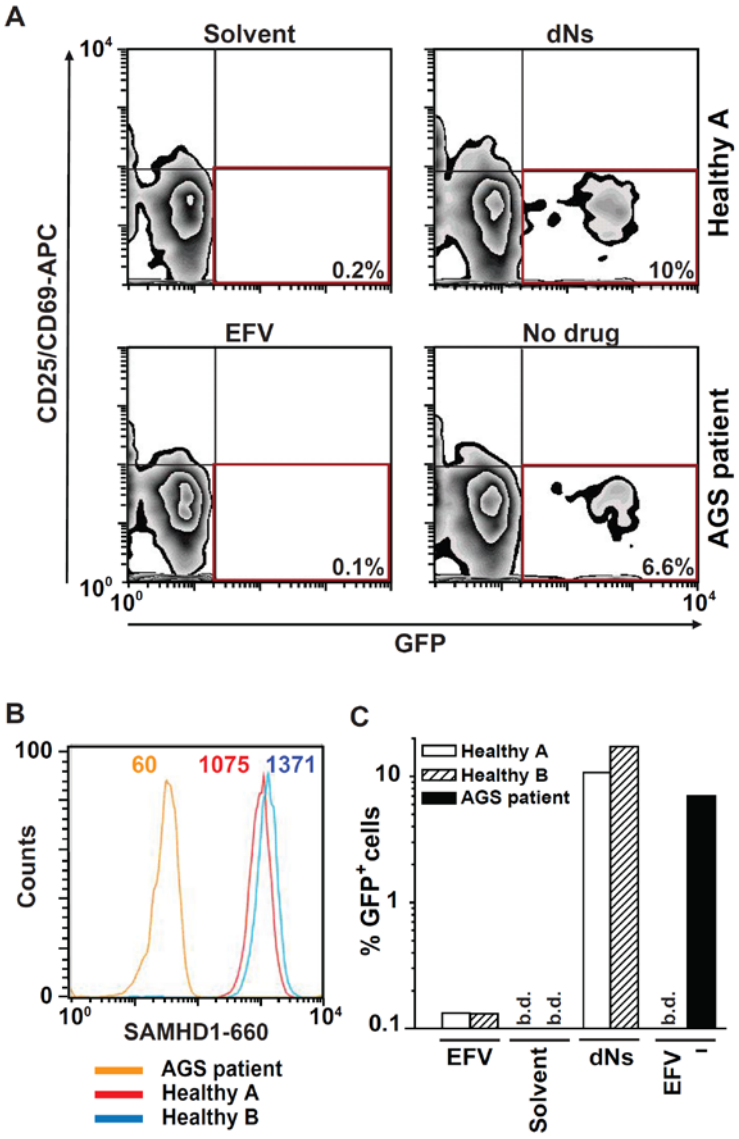

Figure 15: A homozygous nonsense mutation renders resting CD4 T cells permissive for HIV-1 infection. A) Flow cytometric analyses of PBMCs from healthy donor A (solvent- or dN-treated, top graphs) or PBMCs from a patient with AGS with a homozygous nonsense mutation in the *SAMHD1* gene (EFV-treated or untreated, bottom graphs) 3 days after challenge with HIV-1 GFP (multiplicity of infection = 1). The percentages of resting ($CD25^{-}CD69^{-}$), infected (GFP^{+}) $CD3^{+}CD4^{+}$ PBMCs are shown in the bottom right quadrants boxed in red, with the percentage of infected cells indicated. **B)** Intracellular SAMHD1 expression in resting CD4 T cells of PBMCs from the patient with AGS and from healthy donors A and B, as determined by flow cytometry. The mean fluorescence intensity of SAMHD1 levels is indicated. **C)** Quantification of the percentage of infected (GFP^{+}) resting CD4 T cells from the patient with AGS and from healthy donors. Shown are arithmetic means of duplicates. BD, below detection.

Summarizing our findings, HIV-1 reverse transcription is actively suppressed in resting human CD4 T cells, and we identify SAMHD1 as a long-sought cellular factor that is responsible for

this restriction. SAMHD1 thus emerges as a ubiquitous and potent barrier to productive HIV-1 infection in dendritic and myeloid cells[67,68,122,125] and also in the large pool of noncycling CD4 T cells *in vivo*.

4.2.2. Vpx overcomes a SAMHD1-independent block to HIV-1 reverse transcription in resting CD4 T cells (Baldauf *et al*., PNAS 2017)

During our initial studies[126], we however realized that two Vpx proteins behave different to the others we have tested. As seen in Figure 16, Vpx proteins from the second Vpx^+ lentiviral lineage, represented by SIVrcm and SIVmnd-2, enhanced HIV-1*GFP infection in resting T cells 3 days post infection by 8- to 54-fold for SIVmnd-2 Vpx and SIVrcm Vpx, respectively. In our previous study, we showed that infection with X4 HIV-1*GFP with packaged Vpx from SIVmac239 resulted in a massive depletion of SAMHD1 and GFP expression was detected almost exclusively within the $SAMHD1^{low}$ population (Fig. 16C, *Lower Right* quadrant). We were surprised to see that infection with SIVrcm or SIVmnd-2 Vpx incorporated virions did not influence SAMHD1 levels and GFP^+ cells were observed in the $SAMHD1^{high}$ population.

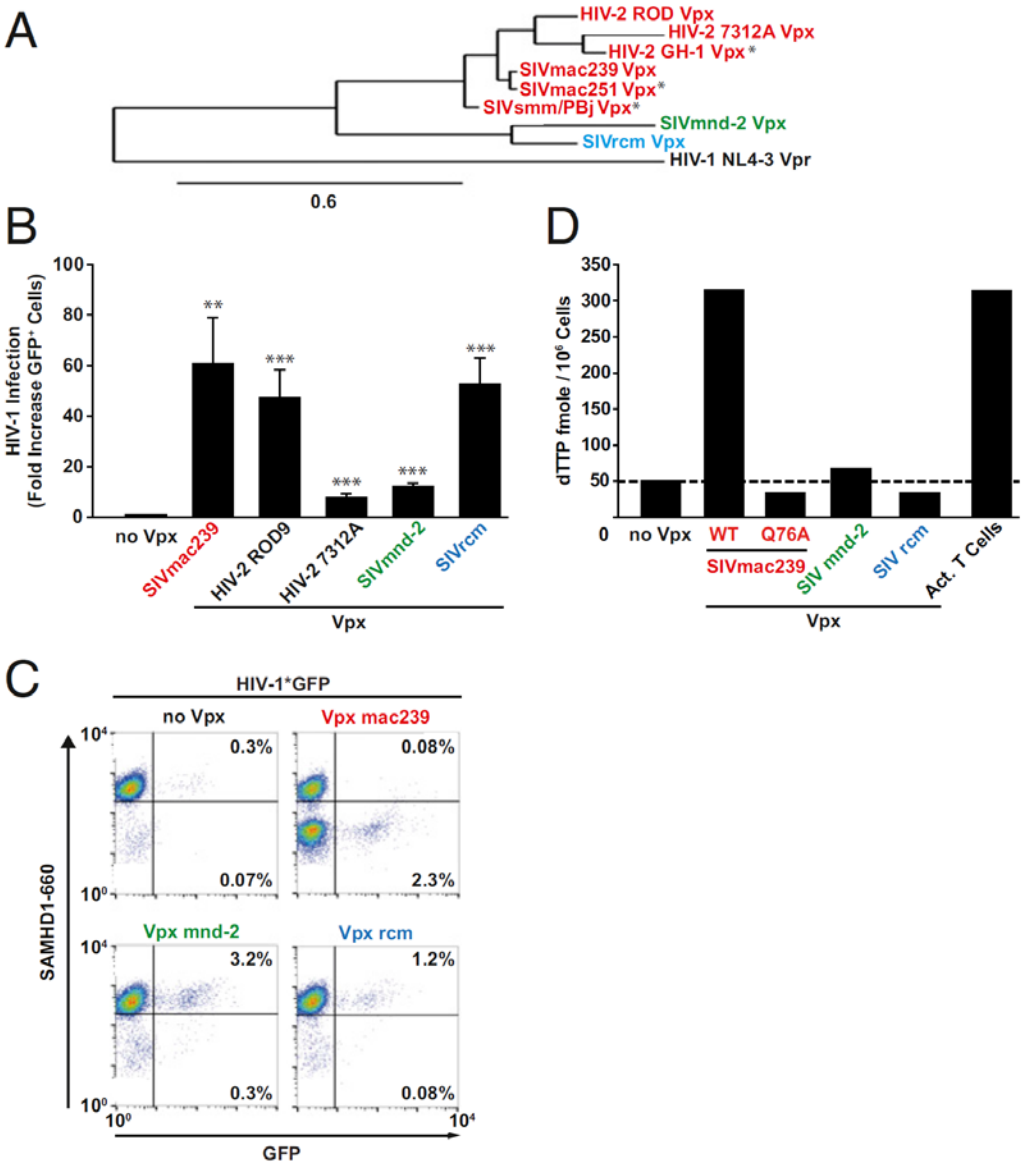

Figure 16: Vpx of SIVmnd-2 and SIVrcm enhance HIV-1 infection in resting CD4 T cells in the absence of degradation of SAMHD1. A) Phylogenetic tree analysis of Vpx proteins from HIV-2 and the two Vpx-carrying SIV lineages used in the current and previous studies. The figure was generated using the online tool on www.phylogeny.fr with MUSCLE for alignment and PhyML for the generation of the phylogenetic tree. Depicted are SAMHD1-degrading (red) and nondegrading (SIVmnd-2 in green; SIVrcm in blue) Vpx proteins of HIV-2 and SIV. HIV-1 NL4-3 Vpr was used as a reference. Stars denote previously analyzed Vpx alleles[126]. **B-C)** Resting CD4 T cells were challenged with equivalent infectious units of X4 HIV-1*GFP virions without (no Vpx; *n* = 15) or with incorporated Vpx from SIVmac239 (*n* = 15), HIV-2 ROD9 (*n* = 10), HIV-2 7312A (*n* = 11), SIVmnd-2 (*n* = 10), or SIVrcm (*n* = 12) and analyzed 3 d later for expression of GFP and SAMHD1, in principle as reported [126]. **B)** Factor of increase of Vpx-mediated HIV-1 infection enhancement. Shown are arithmetic means + s.e.m. **C)** Dot plots of flow cytometric analysis of intracellular SAMHD1 and GFP expression for one representative donor.

We further investigated whether exogenous addition of dNTP precursors would be beneficial for HIV infection in resting CD4 T cells and primary macrophages that were challenged with virions that had different Vpx proteins incorporated. Addition of dNs did not have any effect in resting CD4 T cells, when the cells were challenged with Vpx containing particles (Fig. 17A).

To our surprise, primary macrophages were refractory to infection with SIVrcm or SIVmnd-2 containing particles and infection was elevated when the cells were treated with dNs (Fig. 17B). Thus, Vpx from SIVrcm and SIVmnd-2 can only enhance infection of resting CD4 T cells, but not of primary macrophages. In addition, intracellular dNTP concentrations are rate limiting for HIV-1 in primary macrophages than in resting CD4 T cells.

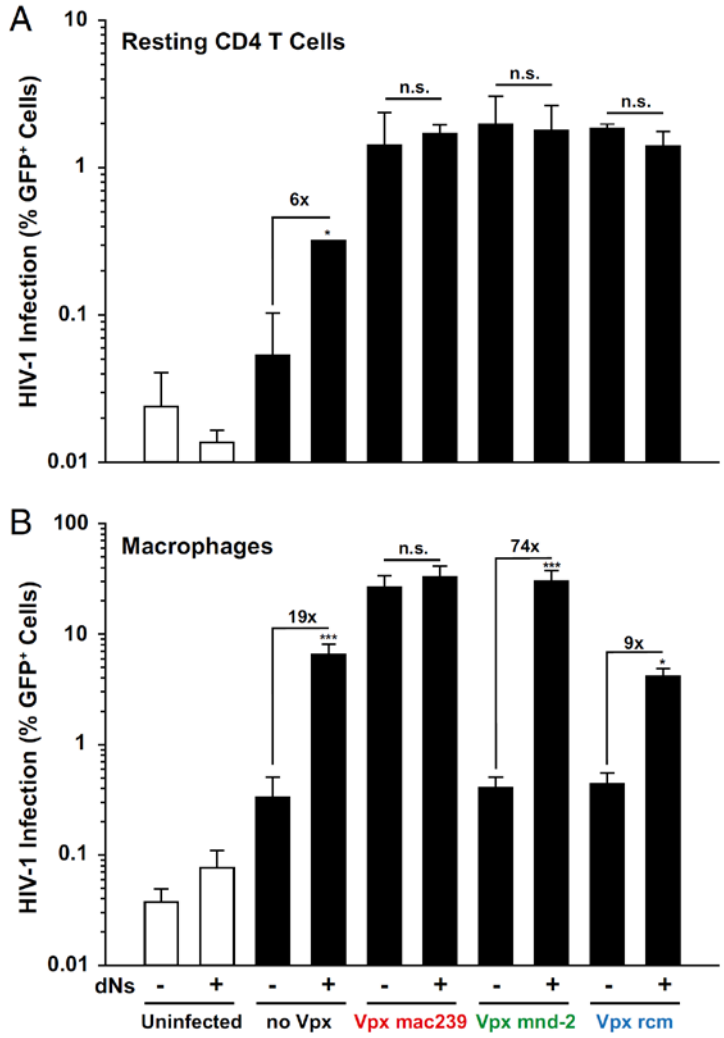

Figure 17: Exogenous addition of dNs has a different impact on HIV-1 infection in resting CD4 T cells and macrophages. A) Resting CD4 T cells were challenged with equal infectious units of X4 HIV-1*GFP virions with or without (no Vpx) incorporated Vpx from SIVmac239, SIVmnd-2, or SIVrcm in the presence or absence of 2 mM dNTP precursors (dNs) and analyzed on day 3 postinfection for GFP expression and SAMHD1 levels. The percentages of GFP^+ cells are shown for one representative donor out of three. **B)** MDMs were challenged with equal infectious units of VSV-G pseudotyped HIV-1*GFP virions with or without (no Vpx) incorporated Vpx from SIVmac239, SIVmnd-2, or SIVrcm in the presence or absence of 4 mM dNs and analyzed on day 3 post infection for GFP expression and SAMHD1 levels. The arithmetic means + s.e.m. of four donors are depicted for the percentage of GFP^+ cells.

We next investigated whether SIVmac239 Vpx is only able to enhance HIV-1 infection of resting CD4 T cells by degrading SAMHD1 and thereby elevating dNTP levels or whether it carries a SIVrcm/mnd-2-like activity in the absence of SAMHD1 degradation. Thus, we generated a panel of SIVmac239 Vpx mutants with a focus on residues that are conserved

among Vpx proteins of the two lentiviral lineages and are thought to be involved in binding to SAMHD1 (L25) or zinc (H39 and H82) or fail to target SAMHD1 for degradation for unknown reasons (W56)[125,127,128]. Interestingly, alanine mutations at positions 25, 39, 56 and 86 enhanced HIV infection of resting CD4 T cells in the absence of SAMHD1 degradation (Fig. 18A-B). Furthermore, these SIVmac239 mutants did not enhance dNTP levels (Fig. 18C), but strongly enhanced levels of reverse transcribed HIV-1 cDNAs and 2-LTR circles (Fig. 18D). Thus, single amino acid replacements in Vpx mac239 lost their interactions with SAMHD1, which resulted in accessory proteins that phenocopied the infection-enhancing ability of SIVmnd-2 and SIVrcm Vpx.

Finally, we analyzed resting CD4 T cells with a nonsense mutation in *SAMHD1* obtained from one patient with AGS[125,126]. As already shown in our previous report [126], $CD25^{-}CD69^{-}CD3^{+}CD4^{+}$ T cells from this donor were intrinsically more permissive for X4 HIV-1*GFP infection compared with resting CD4 T cells from healthy donors (Fig. 18 E-F), underscoring the relevance of SAMHD1 in this process. We increased HIV-1 infection of noncycling CD4 T cells from healthy donors with SIVmac239 Vpx by 159-fold, but, importantly, Vpx also boosted infection of SAMHD1-deficient AGS cells by 11-fold (Fig. 18 E-F). Due to highly limited fresh cell samples available and the low survival rate of previously cryopreserved resting CD4 T cells from patients with AGS, SIVmnd-2/rcm could, unfortunately, not be analyzed in this experiment and cells from additional donors were not accessible. These results provide direct evidence that Vpx can target a SAMHD1-independent restriction in resting CD4 T cells.

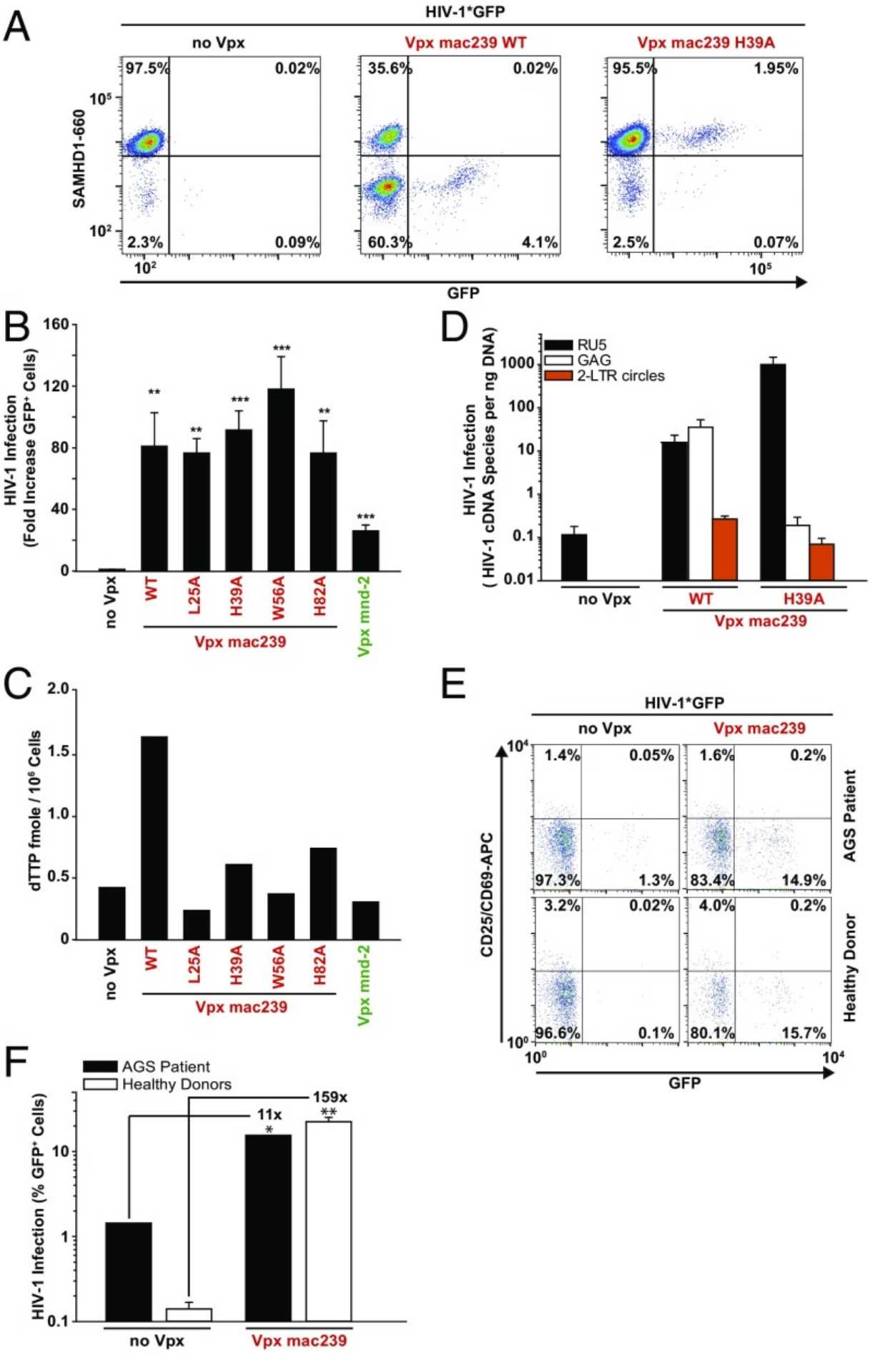
A
HIV-1*GFP
no Vpx
Vpx mac239 WT
Vpx mac239 H39A
97.5%
0.02%
2.3%
0.09%
35.6%
0.02%
60.3%
4.1%
95.5%
1.95%
2.5%
0.07%
SAMHD1-660
GFP
B
HIV-1 Infection
(Fold Increase GFP+ Cells)
no Vpx
WT
L25A
H39A
W56A
H82A
Vpx mnd-2
Vpx mac239
C
dTTP fmole / 10^6 Cells
D
HIV-1 Infection
(HIV-1 cDNA Species per ng DNA)
RU5
GAG
2-LTR circles
E
HIV-1*GFP
CD25/CD69-APC
AGS Patient
Healthy Donor
1.4%
0.05%
1.6%
0.2%
97.3%
1.3%
83.4%
14.9%
3.2%
0.02%
4.0%
0.2%
96.6%
0.1%
80.1%
15.7%
F
AGS Patient
Healthy Donors
HIV-1 Infection (% GFP+ Cells)
11x
159x

Figure 18: Antagonism of a SAMHD1-independent early postentry restriction for HIV-1 in resting CD4 T cells is a conserved feature of Vpx proteins. A-B) Resting CD4 T cells from healthy donors were challenged with equivalent infectious units of X4 HIV-1*GFP virions without (no Vpx) or with incorporation of the indicated Vpx alleles and point mutants and analyzed 3 days later for GFP expression and SAMHD1 levels. **A)** Dot plots of flow cytometric analysis of intracellular SAMHD1 and GFP levels for one representative donor. **B)** Factor of increase of Vpx-mediated HIV-1 infection enhancement 3 d post challenge. Shown are arithmetic means + s.e.m. of data from at least three donors. **C)** Resting CD4 T cells were cotransfected with pDisplay-YFP and expression constructs for the indicated Vpx constructs, sorted for YFP surface expression, and analyzed for dTTP levels. Shown are the arithmetic means from two independent experiments. **D)** Resting CD4 T cells were challenged with equivalent infectious units of the indicated DNase-treated virus stocks and harvested 3 d later for qPCR analyses. Shown are levels of early (RU5) and late (GAG) RT products as well as 2-LTR circles presented as arithmetic means + SEM of five donors. **E - F)** Resting CD4 T cells from a patient with AGS with SAMHD1 deficiency and from two healthy donors were challenged with equivalent infectious units of X4 HIV-1*GFP virions without (no Vpx) or with incorporation of Vpx from SIVmac239 and analyzed 3 d later for expression of GFP and activation markers CD25/CD69. **E)** Representative FACS dot plots and **F)** arithmetic means of the percentages of GFP^+ cells of duplicate infections.

Summarizing our findings, degradation of SAMHD1 and subsequent elevation of cellular dNTPs is critically required for efficient infection of primary macrophages, both events are not necessary in resting CD4 T cells (Fig. 19). Our data suggests the presence of a restriction factor other than SAMHD1 that acts at the level of reverse transcription (RT) and is specific for resting CD4 T cells. This factor limits HIV RT, which would be consistent with an RNase activity, and might be the driving force for HIV latency. SIVmac239 Vpx wt degrades SAMHD1 and presumably also targets this factor inefficiently. In contrast, SAMHD1 non-degrading SIVmnd-2 and SIVrcm Vpx as well as non-degrading SAMHD1 SIVmac239 Vpx mutants primarily or exclusively target this restriction factor, resulting in an up to 1000-fold accumulation of early RT products compared to Vpx-negative particles. Interestingly, these enhanced levels of RT products do apparently not efficiently enter into the nucleus. Thus, another yet unidentified block at the level of or prior to nuclear import seems to be active in resting CD4 T cells.

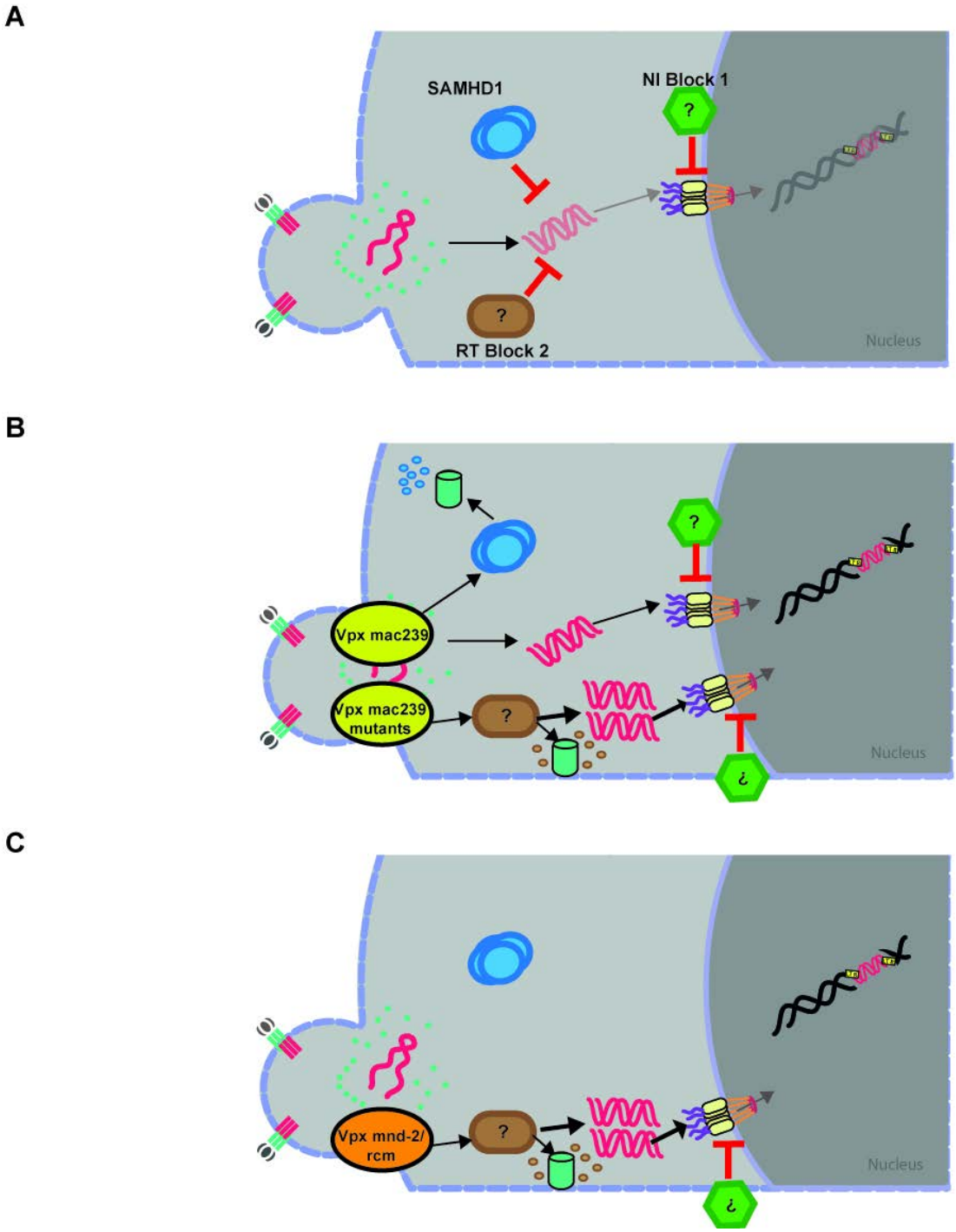

Figure 19: Proposed model for HIV restrictions in primary resting CD4 T cells and counteraction by SIV Vpx variants. A) Both SAMHD1 (RT block 1) and an unknown factor (RT block 2) are able to restrict HIV at the level of reverse transcription. Downstream, an unknown factor limits nuclear import of the preintegration complex (NI block 1). **B)** SIVmac239 Vpx WT targets SAMHD1 for degradation to overcome RT block 1. In the presence of SAMHD1 and RT block 2, SAMHD1 is the preferred target of SIVmac239 Vpx WT, but in the absence of SAMHD1, RT block 2 is targeted. SAMHD1 degradation-deficient mutants of SIVmac239 Vpx target RT block 2 similarly to SIVmnd-2 and SIVrcm Vpx through a mechanism that likely involves proteasomal degradation. **C)** SIVmnd-2 and SIVrcm Vpx are unable to target SAMHD1 for degradation but apparently overcome the major restriction at the level of reverse transcription by targeting RT block 2. Despite highly efficient reverse transcription, levels of 2-LTR circles are similar to those observed with SIVmac239 Vpx, indicating that also under these conditions, nuclear import is restricted by NI block 1.

5. Perspectives

5.1. Immunocompetent, genetically modified rabbit model of HIV infection

Current lentiviral animal models face profound shortcomings to recapitulate HIV disease in humans. Our goal is to generate genetically modified rabbits with an intact immune system that are susceptible to HIV infection and develop key aspects of HIV pathogenesis in humans such as loss of CD4 T cells and AIDS. Building on my exciting recent characterizations of HIV susceptibility in the rabbit species, I aim to **I)** generate and characterize TRIM5-knockout rabbits expressing hCD4 and hCCR5 in a lineage-specific manner, **II)** identify remaining barrier(s) to high HIV infectivity in primary rabbit macrophages to further improve the model, and **III)** establish genetically modified rabbits as a unique *in vivo* screening platform for pre-clinical testing of HIV vaccine candidates. The broad application range of the rabbit animal model will tremendously stimulate studies on viral pathogenesis and on virus-host interactions occurring during HIV infection *in vivo*. In addition, studies on HIV's impact on the immune system and even co-infection studies will be feasible in this rabbit model of HIV infection.

5.2. Characterization of HIV entry in rabbit cells

HIV-1 entry is dependent on the expression of human CD4 and the human co-receptors CXCR4 or CCR5. In collaboration with Dr. Olga Kalinina (Max-Planck-Institute for Computational Biology; Structural Bioinformatics of Protein Interactions), we modelled interaction of gp120 with human and rabbit CD4 orthologs using the 3D structure of the complex of CD4 with the core domain of HIV-1 gp120 (PDB in 2QAD[129]). As seen in Figure 20A, the overall interaction is generally very well conserved. Yet, an insertion between the residues 41G and 42S (human), corresponding to 66G-71S in rabbit, and 44L → 73W + 45T → 74L substitution were identified. Based on this finding, four models were built: (1) wt rabbit CD4; (2) rabbit CD4 without polyS insertion; (3) rabbit CD4 without polyS insertion and with W73L+L74T mutations; (4) wt human CD4 (for comparison purposes) (Fig. 20B). In these models, the non-conserved rabbit-specific residues form a substantial additional volume, which leads to the formation of non-favorable "too tight" contacts and potential clashes. The complex energy in each model of the rabbit CD4 was estimated with FoldX (http://foldxsuite.crg.eu/) and compared to the complex energy of the model of the human CD4 (Fig. 20C). It must be noted that in all cases the energy is positive, which is generally non-favorable.

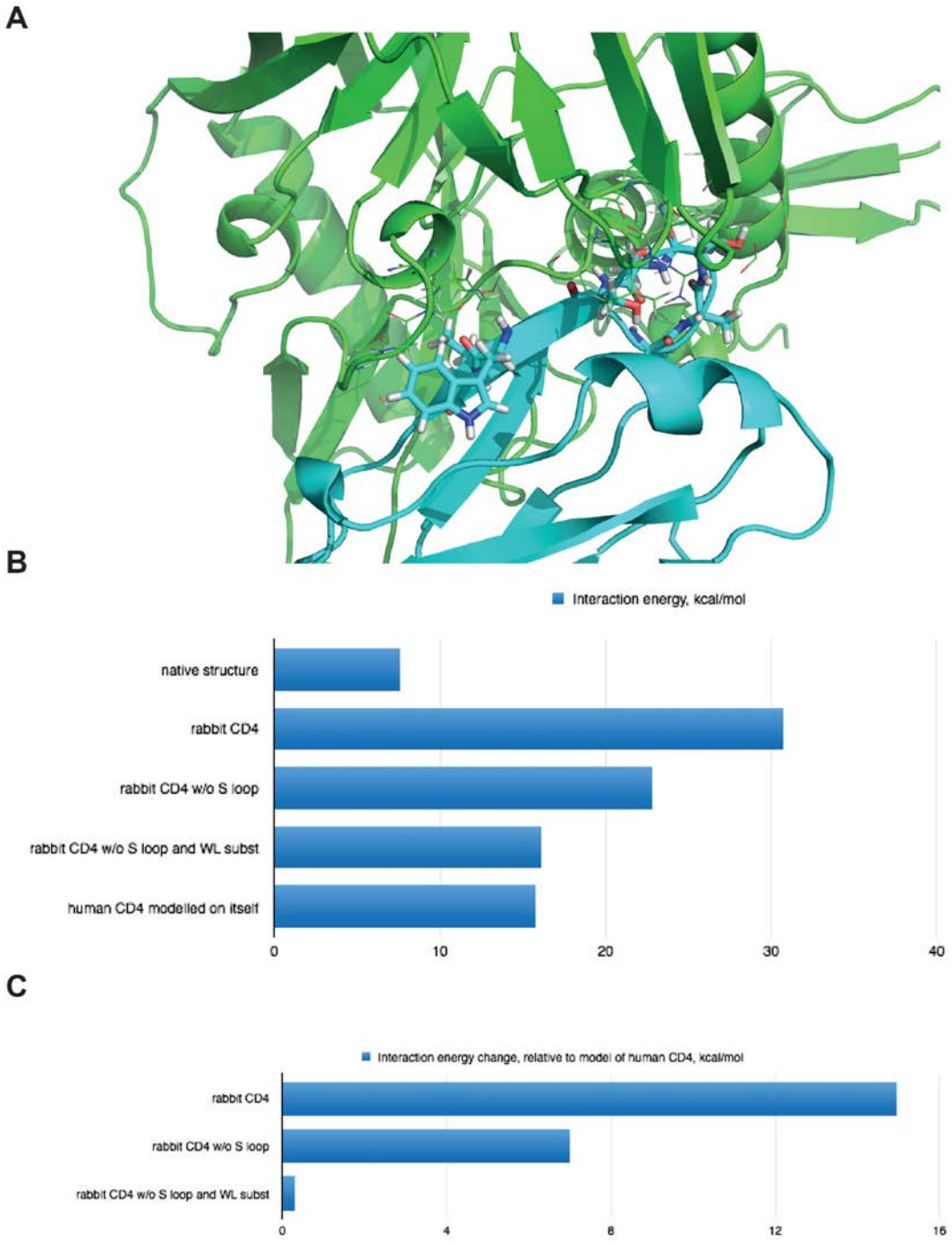

Figure 20: Modelling of CD4 with gp120. A) The 3D structure of the complex of CD4 with the core domain of HIV-1 gp120 (PDB id 2QAD[129]) was taken as basis for modelling. The complex of HIV-1 gp120 core domain (green) with wt rabbit CD4 (blue) is modelled in which all non-conserved elements in the wt rabbit CD4 are presented in the stick model. **B)** Shown is the interaction energy that was calculated for four models: (1) wt rabbit CD4; (2) rabbit CD4 without polyS insertion; (3) rabbit CD4 without polyS insertion and with W73L+L74T mutations and compared to wt human CD4. **C)** Depicted is the interaction energy change relative to human CD4 with wt rabbit CD4, rabbit CD4 without polyS insertion and rabbit CD4 without polyS insertion and WL substitution.

We performed similar analyses for human, rat and rabbit CCR5. Most of the interacting residues are conserved in all three species, with one exception (Fig. 21). Position I198 in

human is mutated to N in rabbit (conserved in rat). It has been shown that the substitution I198A strongly reduces the HIV-1 coreceptor and maraviroc binding [130]. Taken together, N-terminal residues 1-24 and I198 might be the key specificity determinants.

Based on these initial observations, we are currently addressing the impact of rabbit wildtype and mutated CD4 and CCR5 on HIV entry efficiency.

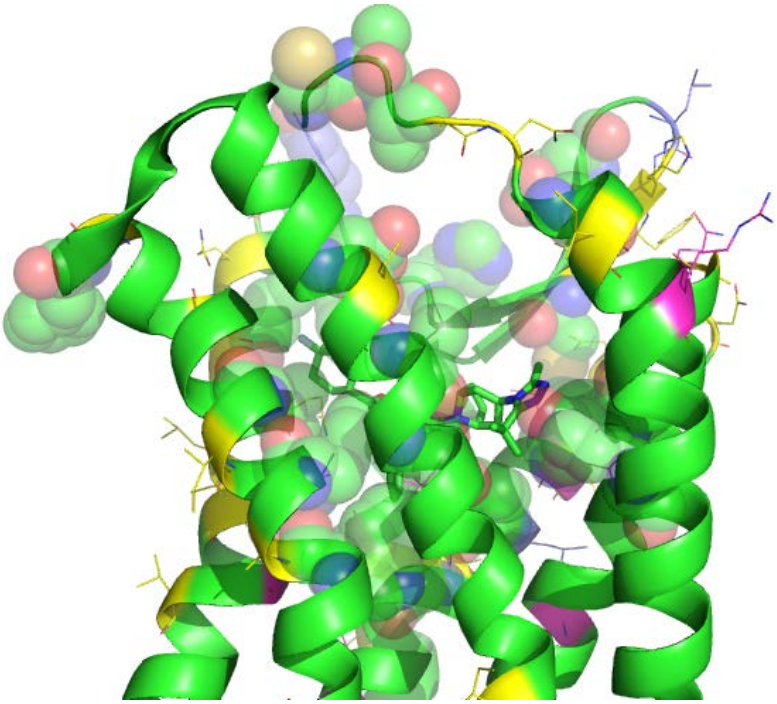

Figure 19: Comparison of non-conserved residues in human, rabbit and rat CCR5. The residues conserved in rat and rabbit, but not in human, are depicted in red, the residues conserved in human and either in rat or rabbit are in yellow, and the residues different in all species are in blue. The residues involved in HIV-1 coreceptor activity are shown as spheres. The non-conserved residues and residues implicated in HIV-1 binding in the structure of human CCR5. Only the part of the protein close to the extracellular side of the membrane is shown. The view is along the membrane plane.

5.3. Finding factor X in resting CD4 T cells

We recently screened a set of Vpx alleles for their ability to overcome the SAMHD1 barrier in resting CD4 T cells. For the majority of functional Vpx alleles, successful infection correlated with proteasomal degradation of SAMHD1 and an elevation of dNTP pools. However, we identified two Vpx alleles from SIV strains, SIVmnd-2 and SIVrcm, the virion packaging of which enhanced the HIV susceptibility of resting CD4 T cells in the absence of SAMHD1 degradation. In collaboration with Prof. Oliver Fackler (University Hospital Heidelberg, Department of Infectious Diseases, Section Integrative Virology) and Prof. Oliver Keppler (LMU Munich, Max von Pettenkofer-Institute, Virology), we are currently identifying this novel factor in resting CD4 T cells. One hypothesis is that the putative new restriction factor in resting CD4 T cells is degraded by SIVmnd-2 and SIVrcm Vpx via the highly conserved interaction with the E3 ubiquitin ligase complex. In this scenario, Vpx and this new restriction factor are

physically interacting. Strategies to identify the interaction partner of SIVmnd-2 and SIVrcm Vpx include mass spectrometry after enrichment of Vpx-transfected resting CD4 T cells or immunoprecipitations of flag-tagged Vpx proteins. Another hypothesis is that interaction occurs indirectly in a bigger complex or via an adaptor protein. Whole cell analysis as well RNAseq of resting CD4 T cells in comparison to primary macrophages, which most likely do not express the novel factor, might help to identify the novel factor in resting CD4 T cells.

6. References

1 Giuffre, A. C., Higgins, J., Buckheit, R. W., Jr. & North, T. W. Susceptibilities of simian immunodeficiency virus to protease inhibitors. *Antimicrobial agents and chemotherapy* **47**, 1756-1759 (2003).

2 Hu, S. L. Non-human primate models for AIDS vaccine research. *Current drug targets. Infectious disorders* **5**, 193-201 (2005).

3 North, T. W. *et al.* Suppression of virus load by highly active antiretroviral therapy in rhesus macaques infected with a recombinant simian immunodeficiency virus containing reverse transcriptase from human immunodeficiency virus type 1. *Journal of virology* **79**, 7349-7354, doi:10.1128/jvi.79.12.7349-7354.2005 (2005).

4 Hatziioannou, T. *et al.* A macaque model of HIV-1 infection. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 4425-4429, doi:10.1073/pnas.0812587106 (2009).

5 Hatziioannou, T. *et al.* HIV-1-induced AIDS in monkeys. *Science (New York, N.Y.)* **344**, 1401-1405, doi:10.1126/science.1250761 (2014).

6 van Maanen, M. & Sutton, R. E. Rodent models for HIV-1 infection and disease. *Current HIV research* **1**, 121-130 (2003).

7 Denton, P. W. & Garcia, J. V. Mucosal HIV-1 transmission and prevention strategies in BLT humanized mice. *Trends in microbiology* **20**, 268-274, doi:10.1016/j.tim.2012.03.007 (2012).

8 Kovarova, M. *et al.* A long-acting formulation of the integrase inhibitor raltegravir protects humanized BLT mice from repeated high-dose vaginal HIV challenges. *The Journal of antimicrobial chemotherapy* **71**, 1586-1596, doi:10.1093/jac/dkw042 (2016).

9 Sun, M. *et al.* VRC01 antibody protects against vaginal and rectal transmission of human immunodeficiency virus 1 in hu-BLT mice. *Archives of virology* **161**, 2449-2455, doi:10.1007/s00705-016-2942-4 (2016).

10 Denton, P. W. *et al.* Antiretroviral pre-exposure prophylaxis prevents vaginal transmission of HIV-1 in humanized BLT mice. *PLoS medicine* **5**, e16, doi:10.1371/journal.pmed.0050016 (2008).

11 Ito, M. *et al.* NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood* **100**, 3175-3182, doi:10.1182/blood-2001-12-0207 (2002).

12 Gimeno, R. *et al.* Monitoring the effect of gene silencing by RNA interference in human CD34+ cells injected into newborn RAG2-/- gammac-/- mice: functional inactivation of p53 in developing T cells. *Blood* **104**, 3886-3893, doi:10.1182/blood-2004-02-0656 (2004).

13 Traggiai, E. *et al.* Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science (New York, N.Y.)* **304**, 104-107, doi:10.1126/science.1093933 (2004).

14 Chicha, L. *et al.* Human adaptive immune system Rag2-/-gamma(c)-/- mice. *Annals of the New York Academy of Sciences* **1044**, 236-243, doi:10.1196/annals.1349.029 (2005).

15 Miyake, A. *et al.* Rapid dissemination of a pathogenic simian/human immunodeficiency virus to systemic organs and active replication in lymphoid tissues following intrarectal infection. *The Journal of general virology* **87**, 1311-1320, doi:10.1099/vir.0.81307-0 (2006).

16 Brehm, M. A. *et al.* Parameters for establishing humanized mouse models to study human immunity: analysis of human hematopoietic stem cell engraftment in three immunodeficient strains of mice bearing the IL2rgamma(null) mutation. *Clinical immunology (Orlando, Fla.)* **135**, 84-98, doi:10.1016/j.clim.2009.12.008 (2010).

17 Nakata, H. *et al.* Potent anti-R5 human immunodeficiency virus type 1 effects of a CCR5 antagonist, AK602/ONO4128/GW873140, in a novel human peripheral blood mononuclear cell nonobese diabetic-SCID, interleukin-2 receptor gamma-chain-knocked-out AIDS mouse model. *Journal of virology* **79**, 2087-2096, doi:10.1128/jvi.79.4.2087-2096.2005 (2005).

18 Watanabe, S. *et al.* Humanized NOD/SCID/IL2Rgamma(null) mice transplanted with hematopoietic stem cells under nonmyeloablative conditions show prolonged life spans and allow detailed analysis of human immunodeficiency virus type 1 pathogenesis. *Journal of virology* **81**, 13259-13264, doi:10.1128/jvi.01353-07 (2007).

19 Klein, F. *et al.* HIV therapy by a combination of broadly neutralizing antibodies in humanized mice. *Nature* **492**, 118-122, doi:10.1038/nature11604 (2012).

20 Nie, C. *et al.* Selective infection of CD4+ effector memory T lymphocytes leads to preferential depletion of memory T lymphocytes in R5 HIV-1-infected humanized NOD/SCID/IL-2Rgammanull mice. *Virology* **394**, 64-72, doi:10.1016/j.virol.2009.08.011 (2009).

21 Berges, B. K., Akkina, S. R., Remling, L. & Akkina, R. Humanized Rag2(-/-)gammac(-/-) (RAG-hu) mice can sustain long-term chronic HIV-1 infection lasting more than a year. *Virology* **397**, 100-103, doi:10.1016/j.virol.2009.10.034 (2010).

22 Brehm, M. A., Shultz, L. D., Luban, J. & Greiner, D. L. Overcoming current limitations in humanized mouse research. *The Journal of infectious diseases* **208 Suppl 2**, S125-130, doi:10.1093/infdis/jit319 (2013).

23 Akkina, R. *et al.* Improvements and Limitations of Humanized Mouse Models for HIV Research: NIH/NIAID "Meet the Experts" 2015 Workshop Summary. *AIDS research and human retroviruses* **32**, 109-119, doi:10.1089/aid.2015.0258 (2016).

24 Wei, P., Garber, M. E., Fang, S. M., Fischer, W. H. & Jones, K. A. A novel CDK9-associated C-type cyclin interacts directly with HIV-1 Tat and mediates its high-affinity, loop-specific binding to TAR RNA. *Cell* **92**, 451-462 (1998).

25 Bieniasz, P. D. & Cullen, B. R. Multiple blocks to human immunodeficiency virus type 1 replication in rodent cells. *Journal of virology* **74**, 9868-9877 (2000).

26 Keppler, O. T. *et al.* Susceptibility of rat-derived cells to replication by human immunodeficiency virus type 1. *Journal of virology* **75**, 8063-8073 (2001).

27 Goffinet, C. *et al.* Primary T-cells from human CD4/CCR5-transgenic rats support all early steps of HIV-1 replication including integration, but display impaired viral gene expression. *Retrovirology* **4**, 53, doi:10.1186/1742-4690-4-53 (2007).

28 Tervo, H. M., Goffinet, C. & Keppler, O. T. Mouse T-cells restrict replication of human immunodeficiency virus at the level of integration. *Retrovirology* **5**, 58, doi:10.1186/1742-4690-5-58 (2008).

29 Zhang, J. X., Diehl, G. E. & Littman, D. R. Relief of preintegration inhibition and characterization of additional blocks for HIV replication in primary mouse T cells. *PloS one* **3**, e2035, doi:10.1371/journal.pone.0002035 (2008).

30 Sun, J. *et al.* CD4-specific transgenic expression of human cyclin T1 markedly increases human immunodeficiency virus type 1 (HIV-1) production by CD4+ T lymphocytes and myeloid cells in mice transgenic for a provirus encoding a monocyte-tropic HIV-1 isolate. *Journal of virology* **80**, 1850-1862, doi:10.1128/jvi.80.4.1850-1862.2006 (2006).

31 Keppler, O. T. *et al.* Progress toward a human CD4/CCR5 transgenic rat model for de novo infection by human immunodeficiency virus type 1. *The Journal of experimental medicine* **195**, 719-736 (2002).

32 Goffinet, C., Allespach, I. & Keppler, O. T. HIV-susceptible transgenic rats allow rapid preclinical testing of antiviral compounds targeting virus entry or reverse transcription. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 1015-1020, doi:10.1073/pnas.0607414104 (2007).

33 Munch, J. *et al.* Semen-derived amyloid fibrils drastically enhance HIV infection. *Cell* **131**, 1059-1071, doi:10.1016/j.cell.2007.10.014 (2007).
34 Bosch, V. *et al.* HIV pseudovirion vaccine exposing Env "fusion intermediates"-response to immunisation in human CD4/CCR5-transgenic rats. *Vaccine* **27**, 2202-2212, doi:10.1016/j.vaccine.2009.02.014 (2009).
35 Goffinet, C. *et al.* Pharmacovirological impact of an integrase inhibitor on human immunodeficiency virus type 1 cDNA species in vivo. *Journal of virology* **83**, 7706-7717, doi:10.1128/jvi.00683-09 (2009).
36 Michel, N. *et al.* Human cyclin T1 expression ameliorates a T-cell-specific transcriptional limitation for HIV in transgenic rats, but is not sufficient for a spreading infection of prototypic R5 HIV-1 strains ex vivo. *Retrovirology* **6**, 2, doi:10.1186/1742-4690-6-2 (2009).
37 Filice, G., Cereda, P. M. & Varnier, O. E. Infection of rabbits with human immunodeficiency virus. *Nature* **335**, 366-369, doi:10.1038/335366a0 (1988).
38 Reina, S. *et al.* Serological, biological, and molecular characterization of New Zealand white rabbits infected by intraperitoneal inoculation with cell-free human immunodeficiency virus. *Journal of virology* **67**, 5367-5374 (1993).
39 Kulaga, H., Folks, T. M., Rutledge, R. & Kindt, T. J. Infection of rabbit T-cell and macrophage lines with human immunodeficiency virus. *Proceedings of the National Academy of Sciences of the United States of America* **85**, 4455-4459 (1988).
40 Kulaga, H. *et al.* Infection of rabbits with human immunodeficiency virus 1. A small animal model for acquired immunodeficiency syndrome. *The Journal of experimental medicine* **169**, 321-326 (1989).
41 Gordon, M. R. *et al.* Evidence for HIV-1 infection in rabbits. A possible model for AIDS. *Annals of the New York Academy of Sciences* **616**, 270-280 (1990).
42 Tseng, C. K., Leibowitz, J. & Sell, S. Defective infection of rabbit peripheral blood monocyte cultures with human immunodeficiency virus type 1. *AIDS research and human retroviruses* **10**, 285-293, doi:10.1089/aid.1994.10.285 (1994).
43 Yamamura, Y. *et al.* Infection of human CD4+ rabbit cells with HIV-1: the possibility of the rabbit as a model for HIV-1 infection. *International immunology* **3**, 1183-1187 (1991).
44 Dunn, C. S. *et al.* Human immunodeficiency virus type 1 infection of human CD4-transgenic rabbits. *The Journal of general virology* **76 (Pt 6)**, 1327-1336, doi:10.1099/0022-1317-76-6-1327 (1995).
45 Leno, M., Hague, B. F., Teller, R. & Kindt, T. J. HIV-1 mediates rapid apoptosis of lymphocytes from human CD4 transgenic but not normal rabbits. *Virology* **213**, 450-454, doi:10.1006/viro.1995.0017 (1995).
46 Snyder, B. W. *et al.* Developmental and tissue-specific expression of human CD4 in transgenic rabbits. *Molecular reproduction and development* **40**, 419-428, doi:10.1002/mrd.1080400405 (1995).
47 Speck, R. F. *et al.* Rabbit cells expressing human CD4 and human CCR5 are highly permissive for human immunodeficiency virus type 1 infection. *Journal of virology* **72**, 5728-5734 (1998).
48 Besnier, C., Takeuchi, Y. & Towers, G. Restriction of lentivirus in monkeys. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 11920-11925, doi:10.1073/pnas.172384599 (2002).
49 Cutino-Moguel, T. & Fassati, A. A phenotypic recessive, post-entry block in rabbit cells that results in aberrant trafficking of HIV-1. *Traffic (Copenhagen, Denmark)* **7**, 978-992, doi:10.1111/j.1600-0854.2006.00449.x (2006).
50 Schaller, T., Hue, S. & Towers, G. J. An active TRIM5 protein in rabbits indicates a common antiviral ancestor for mammalian TRIM5 proteins. *Journal of virology* **81**, 11713-11721, doi:10.1128/jvi.01468-07 (2007).
51 Tervo, H. M. & Keppler, O. T. High natural permissivity of primary rabbit cells for HIV-1, with a virion infectivity defect in macrophages as the final replication barrier. *Journal of virology* **84**, 12300-12314, doi:10.1128/JVI.01607-10 (2010).

52 Pantaleo, G. *et al.* Lymphoid organs function as major reservoirs for human immunodeficiency virus. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 9838-9842 (1991).
53 Embretson, J. *et al.* Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature* **362**, 359-362, doi:10.1038/362359a0 (1993).
54 Pantaleo, G. *et al.* HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature* **362**, 355-358, doi:10.1038/362355a0 (1993).
55 Chun, T. W. *et al.* In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. *Nature medicine* **1**, 1284-1290 (1995).
56 Chun, T. W. *et al.* Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* **387**, 183-188, doi:10.1038/387183a0 (1997).
57 Finzi, D. *et al.* Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science (New York, N.Y.)* **278**, 1295-1300 (1997).
58 Perelson, A. S. *et al.* Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature* **387**, 188-191, doi:10.1038/387188a0 (1997).
59 Wong, J. K. *et al.* Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science (New York, N.Y.)* **278**, 1291-1295 (1997).
60 Finzi, D. *et al.* Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nature medicine* **5**, 512-517, doi:10.1038/8394 (1999).
61 Persaud, D. *et al.* A stable latent reservoir for HIV-1 in resting CD4(+) T lymphocytes in infected children. *The Journal of clinical investigation* **105**, 995-1003, doi:10.1172/jci9006 (2000).
62 Shen, A. *et al.* Resting CD4+ T lymphocytes but not thymocytes provide a latent viral reservoir in a simian immunodeficiency virus-Macaca nemestrina model of human immunodeficiency virus type 1-infected patients on highly active antiretroviral therapy. *Journal of virology* **77**, 4938-4949 (2003).
63 Stephan, C. *et al.* Impact of raltegravir on HIV-1 RNA and DNA forms following initiation of antiretroviral therapy in treatment-naive patients. *The Journal of antimicrobial chemotherapy* **69**, 2809-2818, doi:10.1093/jac/dku213 (2014).
64 Stevenson, M., Stanwick, T. L., Dempsey, M. P. & Lamonica, C. A. HIV-1 replication is controlled at the level of T cell activation and proviral integration. *The EMBO journal* **9**, 1551-1560 (1990).
65 Zack, J. A. *et al.* HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell* **61**, 213-222 (1990).
66 Altfeld, M. & Gale, M., Jr. Innate immunity against HIV-1 infection. *Nature immunology* **16**, 554-562, doi:10.1038/ni.3157 (2015).
67 Hrecka, K. *et al.* Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. *Nature* **474**, 658-661, doi:10.1038/nature10195 (2011).
68 Laguette, N. *et al.* SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. *Nature* **474**, 654-657, doi:10.1038/nature10117 (2011).
69 Goldstone, D. C. *et al.* HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. *Nature* **480**, 379-382, doi:10.1038/nature10623 (2011).
70 Powell, R. D., Holland, P. J., Hollis, T. & Perrino, F. W. Aicardi-Goutieres syndrome gene and HIV-1 restriction factor SAMHD1 is a dGTP-regulated deoxynucleotide triphosphohydrolase. *The Journal of biological chemistry* **286**, 43596-43600, doi:10.1074/jbc.C111.317628 (2011).
71 White, T. E. *et al.* The retroviral restriction ability of SAMHD1, but not its deoxynucleotide triphosphohydrolase activity, is regulated by phosphorylation. *Cell host & microbe* **13**, 441-451, doi:10.1016/j.chom.2013.03.005 (2013).
72 Pauls, E. *et al.* Cell cycle control and HIV-1 susceptibility are linked by CDK6-dependent CDK2 phosphorylation of SAMHD1 in myeloid and lymphoid cells. *Journal of immunology (Baltimore, Md. : 1950)* **193**, 1988-1997, doi:10.4049/jimmunol.1400873 (2014).
73 Arnold, L. H. *et al.* Phospho-dependent Regulation of SAMHD1 Oligomerisation Couples Catalysis and Restriction. *PLoS pathogens* **11**, e1005194, doi:10.1371/journal.ppat.1005194 (2015).

74 Yan, J. *et al.* CyclinA2-Cyclin-dependent Kinase Regulates SAMHD1 Protein Phosphohydrolase Domain. *The Journal of biological chemistry* **290**, 13279-13292, doi:10.1074/jbc.M115.646588 (2015).
75 Yan, N. & Lieberman, J. SAMHD1 does it again, now in resting T cells. *Nature medicine* **18**, 1611-1612, doi:10.1038/nm.2980 (2012).
76 Deng, H. *et al.* Identification of a major co-receptor for primary isolates of HIV-1. *Nature* **381**, 661-666, doi:10.1038/381661a0 (1996).
77 Dragic, T. *et al.* HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* **381**, 667-673, doi:10.1038/381667a0 (1996).
78 Lores, P. *et al.* Expression of human CD4 in transgenic mice does not confer sensitivity to human immunodeficiency virus infection. *AIDS research and human retroviruses* **8**, 2063-2071 (1992).
79 Fraldi, A., Licciardo, P., Majello, B., Giordano, A. & Lania, L. Distinct regions of cyclinT1 are required for binding to CDK9 and for recruitment to the HIV-1 Tat/TAR complex. *Journal of cellular biochemistry. Supplement* **Suppl 36**, 247-253 (2001).
80 Goffinet, C. *et al.* HIV-1 antagonism of CD317 is species specific and involves Vpu-mediated proteasomal degradation of the restriction factor. *Cell host & microbe* **5**, 285-297, doi:10.1016/j.chom.2009.01.009 (2009).
81 Ikeda, T. *et al.* The antiretroviral potency of APOBEC1 deaminase from small animal species. *Nucleic acids research* **36**, 6859-6871, doi:10.1093/nar/gkn802 (2008).
82 Compton, A. A. *et al.* IFITM proteins incorporated into HIV-1 virions impair viral fusion and spread. *Cell host & microbe* **16**, 736-747, doi:10.1016/j.chom.2014.11.001 (2014).
83 Tada, T. *et al.* MARCH8 inhibits HIV-1 infection by reducing virion incorporation of envelope glycoproteins. *Nature medicine* **21**, 1502-1507, doi:10.1038/nm.3956 (2015).
84 Lodermeyer, V. *et al.* 90K, an interferon-stimulated gene product, reduces the infectivity of HIV-1. *Retrovirology* **10**, 111, doi:10.1186/1742-4690-10-111 (2013).
85 Rosa, A. *et al.* HIV-1 Nef promotes infection by excluding SERINC5 from virion incorporation. *Nature* **526**, 212-217, doi:10.1038/nature15399 (2015).
86 Usami, Y., Wu, Y. & Gottlinger, H. G. SERINC3 and SERINC5 restrict HIV-1 infectivity and are counteracted by Nef. *Nature* **526**, 218-223, doi:10.1038/nature15400 (2015).
87 Krapp, C. *et al.* Guanylate Binding Protein (GBP) 5 Is an Interferon-Inducible Inhibitor of HIV-1 Infectivity. *Cell host & microbe* **19**, 504-514, doi:10.1016/j.chom.2016.02.019 (2016).
88 Yu, J. *et al.* IFITM Proteins Restrict HIV-1 Infection by Antagonizing the Envelope Glycoprotein. *Cell reports* **13**, 145-156, doi:10.1016/j.celrep.2015.08.055 (2015).
89 Sood, C., Marin, M., Chande, A., Pizzato, M. & Melikyan, G. B. SERINC5 protein inhibits HIV-1 fusion pore formation by promoting functional inactivation of envelope glycoproteins. *The Journal of biological chemistry* **292**, 6014-6026, doi:10.1074/jbc.M117.777714 (2017).
90 Guo, R. *et al.* Generation and evaluation of Myostatin knock-out rabbits and goats using CRISPR/Cas9 system. *Scientific reports* **6**, 29855, doi:10.1038/srep29855 (2016).
91 Yang, D. *et al.* Identification and characterization of rabbit ROSA26 for gene knock-in and stable reporter gene expression. *Scientific reports* **6**, 25161, doi:10.1038/srep25161 (2016).
92 Liu, H. *et al.* Multiple homologous genes knockout (KO) by CRISPR/Cas9 system in rabbit. *Gene*, doi:10.1016/j.gene.2018.01.044 (2018).
93 Maddon, P. J. *et al.* The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* **47**, 333-348 (1986).
94 Browning, J. *et al.* Mice transgenic for human CD4 and CCR5 are susceptible to HIV infection. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 14637-14641 (1997).
95 Chen, D., Fong, Y. & Zhou, Q. Specific interaction of Tat with the human but not rodent P-TEFb complex mediates the species-specific Tat activation of HIV-1 transcription. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 2728-2733 (1999).

96 Bieniasz, P. D., Grdina, T. A., Bogerd, H. P. & Cullen, B. R. Recruitment of a protein complex containing Tat and cyclin T1 to TAR governs the species specificity of HIV-1 Tat. *The EMBO journal* **17**, 7056-7065, doi:10.1093/emboj/17.23.7056 (1998).
97 Sherer, N. M. *et al.* Evolution of a species-specific determinant within human CRM1 that regulates the post-transcriptional phases of HIV-1 replication. *PLoS pathogens* **7**, e1002395, doi:10.1371/journal.ppat.1002395 (2011).
98 Okada, H. *et al.* Synergistic effect of human CycT1 and CRM1 on HIV-1 propagation in rat T cells and macrophages. *Retrovirology* **6**, 43, doi:10.1186/1742-4690-6-43 (2009).
99 Fletcher, A. J., Hue, S., Schaller, T., Pillay, D. & Towers, G. J. Hare TRIM5alpha restricts divergent retroviruses and exhibits significant sequence variation from closely related lagomorpha TRIM5 genes. *Journal of virology* **84**, 12463-12468, doi:10.1128/jvi.01514-10 (2010).
100 Rehwinkel, J. *et al.* SAMHD1-dependent retroviral control and escape in mice. *The EMBO journal* **32**, 2454-2462, doi:10.1038/emboj.2013.163 (2013).
101 Van Damme, N. *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**, 245-252, doi:10.1016/j.chom.2008.03.001 (2008).
102 Neil, S. J., Zang, T. & Bieniasz, P. D. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature* **451**, 425-430, doi:10.1038/nature06553 (2008).
103 Goffinet, C., Schmidt, S., Kern, C., Oberbremer, L. & Keppler, O. T. Endogenous CD317/Tetherin Limits Replication of HIV-1 and Murine Leukemia Virus in Rodent Cells and Is Resistant to Antagonists from Primate Viruses. *Journal of virology* **84**, 11374-11384, doi:10.1128/JVI.01067-10 (2010).
104 Liberatore, R. A. & Bieniasz, P. D. Tetherin is a key effector of the antiretroviral activity of type I interferon in vitro and in vivo. *Proc Natl Acad Sci U S A* **108**, 18097-18101, doi:10.1073/pnas.1113694108 (2011).
105 Bishop, K. N. *et al.* Cytidine deamination of retroviral DNA by diverse APOBEC proteins. *Current biology : CB* **14**, 1392-1396, doi:10.1016/j.cub.2004.06.057 (2004).
106 Mariani, R. *et al.* Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. *Cell* **114**, 21-31 (2003).
107 Inuzuka, M., Hayakawa, M. & Ingi, T. Serinc, an activity-regulated protein family, incorporates serine into membrane lipid synthesis. *The Journal of biological chemistry* **280**, 35776-35783, doi:10.1074/jbc.M505712200 (2005).
108 Trautz, B. *et al.* The host-cell restriction factor SERINC5 restricts HIV-1 infectivity without altering the lipid composition and organization of viral particles. *The Journal of biological chemistry* **292**, 13702-13713, doi:10.1074/jbc.M117.797332 (2017).
109 Murrell, B., Vollbrecht, T., Guatelli, J. & Wertheim, J. O. The Evolutionary Histories of Antiretroviral Proteins SERINC3 and SERINC5 Do Not Support an Evolutionary Arms Race in Primates. *Journal of virology* **90**, 8085-8089, doi:10.1128/jvi.00972-16 (2016).
110 Heigele, A. *et al.* The Potency of Nef-Mediated SERINC5 Antagonism Correlates with the Prevalence of Primate Lentiviruses in the Wild. *Cell host & microbe* **20**, 381-391, doi:10.1016/j.chom.2016.08.004 (2016).
111 Melikyan, G. B. Common principles and intermediates of viral protein-mediated fusion: the HIV-1 paradigm. *Retrovirology* **5**, 111, doi:10.1186/1742-4690-5-111 (2008).
112 Chande, A. *et al.* S2 from equine infectious anemia virus is an infectivity factor which counteracts the retroviral inhibitors SERINC5 and SERINC3. *Proceedings of the National Academy of Sciences of the United States of America* **113**, 13197-13202, doi:10.1073/pnas.1612044113 (2016).
113 Trautz, B. *et al.* The Antagonism of HIV-1 Nef to SERINC5 Particle Infectivity Restriction Involves the Counteraction of Virion-Associated Pools of the Restriction Factor. *Journal of virology* **90**, 10915-10927, doi:10.1128/jvi.01246-16 (2016).
114 Ahi, Y. S. *et al.* Functional Interplay Between Murine Leukemia Virus Glycogag, Serinc5, and Surface Glycoprotein Governs Virus Entry, with Opposite Effects on Gammaretroviral and Ebolavirus Glycoproteins. *mBio* **7**, doi:10.1128/mBio.01985-16 (2016).

115 Yachdav, G. *et al.* PredictProtein--an open resource for online prediction of protein structural and functional features. *Nucleic Acids Res* **42**, W337-343, doi:10.1093/nar/gku366 (2014).
116 Vermeire, J. *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* **7**, e50859, doi:10.1371/journal.pone.0050859 (2012).
117 Cavrois, M., De Noronha, C. & Greene, W. C. A sensitive and specific enzyme-based assay detecting HIV-1 virion fusion in primary T lymphocytes. *Nature biotechnology* **20**, 1151-1154, doi:10.1038/nbt745 (2002).
118 Geuenich, S. *et al.* Aqueous extracts from peppermint, sage and lemon balm leaves display potent anti-HIV-1 activity by increasing the virion density. *Retrovirology* **5**, 27, doi:10.1186/1742-4690-5-27 (2008).
119 Katzourakis, A., Tristem, M., Pybus, O. G. & Gifford, R. J. Discovery and analysis of the first endogenous lentivirus. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 6261-6265, doi:10.1073/pnas.0700471104 (2007).
120 Dai, W., Usami, Y., Wu, Y. & Gottlinger, H. A Long Cytoplasmic Loop Governs the Sensitivity of the Anti-viral Host Protein SERINC5 to HIV-1 Nef. *Cell reports* **22**, 869-875, doi:10.1016/j.celrep.2017.12.082 (2018).
121 Sawada, S. *et al.* Disturbed CD4+ T cell homeostasis and in vitro HIV-1 susceptibility in transgenic mice expressing T cell line-tropic HIV-1 receptors. *J Exp Med* **187**, 1439-1449 (1998).
122 Lahouassa, H. *et al.* SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates. *Nature immunology* **13**, 223-228, doi:10.1038/ni.2236 (2012).
123 Plesa, G. *et al.* Addition of deoxynucleosides enhances human immunodeficiency virus type 1 integration and 2LTR formation in resting CD4+ T cells. *Journal of virology* **81**, 13938-13942, doi:10.1128/jvi.01745-07 (2007).
124 Thiele, H. *et al.* Cerebral arterial stenoses and stroke: novel features of Aicardi-Goutieres syndrome caused by the Arg164X mutation in SAMHD1 are associated with altered cytokine expression. *Human mutation* **31**, E1836-1850, doi:10.1002/humu.21357 (2010).
125 Berger, A. *et al.* SAMHD1-deficient CD14+ cells from individuals with Aicardi-Goutieres syndrome are highly susceptible to HIV-1 infection. *PLoS pathogens* **7**, e1002425, doi:10.1371/journal.ppat.1002425 (2011).
126 Baldauf, H. M. *et al.* SAMHD1 restricts HIV-1 infection in resting CD4(+) T cells. *Nature medicine* **18**, 1682-1687, doi:10.1038/nm.2964 (2012).
127 Schaller, T., Bauby, H., Hue, S., Malim, M. H. & Goujon, C. New insights into an X-traordinary viral protein. *Frontiers in microbiology* **5**, 126, doi:10.3389/fmicb.2014.00126 (2014).
128 Schwefel, D. *et al.* Molecular determinants for recognition of divergent SAMHD1 proteins by the lentiviral accessory protein Vpx. *Cell host & microbe* **17**, 489-499, doi:10.1016/j.chom.2015.03.004 (2015).
129 Huang, C. C. *et al.* Structures of the CCR5 N terminus and of a tyrosine-sulfated antibody with HIV-1 gp120 and CD4. *Science (New York, N.Y.)* **317**, 1930-1934, doi:10.1126/science.1145373 (2007).
130 Garcia-Perez, J. *et al.* Allosteric model of maraviroc binding to CC chemokine receptor 5 (CCR5). *The Journal of biological chemistry* **286**, 33409-33421, doi:10.1074/jbc.M111.279596 (2011).

7. Publication index

7.1. Peer-reviewed paper as first author

1. **Baldauf HM**, Stegmann L, Schwarz SM, Ambiel I, Trotard M, Martin M, Burggraf M, Lenzi GM, Lejk H, Pan X, Fregoso OI, Lim ES, Abraham L, Nguyen LA, Rutsch F, König R, Kim B, Emerman M, Fackler OT, Keppler OT. Vpx overcomes a SAMHD1-independent block to HIV reverse transcription that is specific to resting CD4 T cells. Proc Natl Acad Sci U S A. 2017;114(10):2729-2734. **(IF 2017: 9.661)**

2. Schneider C*, Oellerich T*, **Baldauf HM***, Schwarz SM*, Thomas D, Flick R, Bohnenberger H, Kaderali L, Stegmann L, Cremer A, Martin M, Lohmeyer J, Michaelis M, Hornung V, Schliemann C, Berdel WE, Hartmann W, Wardelmann E, Comoglio F, Hansmann ML, Yakunin AF, Geisslinger G, Ströbel P, Ferreirós N, Serve H, Keppler OT, Cinatl J Jr. SAMHD1 is a biomarker for cytarabine response and a therapeutic target in acute myeloid leukemia. Nat Med. 2017;23(2):250-255.* shared first co-author **(IF 2017: 29.886)**

3. Stephan C*, **Baldauf HM***, Barry J*, Giordano F, Bartholomä C, Haberl A, Bickel M, Schmidt M, Laufs S, Kaderali L, Keppler OT. Impact of raltegravir on HIV-1 RNA and DNA species following initiation of antiretroviral therapy in treatment-naïve patients. J Antimicrob Chemother. 2014;69(10):2809-18. * shared first co-author **(IF 2014: 5.313)**

4. **Baldauf HM***, Pan X*, Erikson E, Schmidt S, Daddacha W, Burggraf M, Schenkova K, Ambiel I, Wabnitz G, Gramberg T, Panitz S, Flory E, Landau NR, Sertel S, Rutsch F, Lasitschka F, Kim B, König R, Fackler OT, Keppler OT. SAMHD1 restricts HIV-1 infection in resting CD4(+) T cells. Nature Medicine 2012, 18(11):1682-7. * shared first co-author **(IF 2012: 22.864)**

5. **Tervo HM**, Homann S, Ambiel I, Fritz JV, Fackler OT, Keppler OT. ß-TrCP is dispensable for Vpu's ability to overcome the CD317/Tetherin-imposed restriction to HIV-1 release. Retrovirology 2011 ; 10;8:9. **(IF 2011: 6.47)**

6. **Tervo HM**, Keppler OT. High natural permissivity of primary rabbit cells for HIV-1, with a virion infectivity defect in macrophages as the final replication barrier. J Virol. 2010 84(23):12300-14. **(IF 2010: 4.606)**

7. **Tervo HM**, Goffinet C, Keppler OT. Mouse T-cells restrict replication of human immunodeficiency virus at the level of integration. Retrovirology 2008; 5:58.**(IF 2008: 4.042)**

8. **Tervo HM**, Allespach I, Keppler OT. High-level transfection of primary rabbit T lymphocytes. J Immunol. Methods 2008; 336(1):85-9. **(IF 2008: 1.858)**

7.2. Peer-reviewed paper as co-author

1. de Sousa-Pereira P, Abrantes J, **Baldauf HM**, Esteves PJ. Evolutionary studies on the betaretrovirus RERV-H in the Leporidae family reveal an endogenization in the ancestor of Oryctolagus, Bunolagus and Pentalagus at 9 million years ago. Virus Res. 2017. pii: S0168-1702(17)30174-0. **(IF 2017: 2.628)**

2. de Sousa-Pereira P, Abrantes J, **Baldauf HM**, Keppler OT, Esteves PJ. Evolutionary study of leporid CD4 reveals a hotspot of genetic variability within the D2 domain. Immunogenetics. 2016;68(6-7):477-82. **(IF 2016: 2.303)**

3. Anni Tuulasvaara A, Vanhanen R, **Baldauf HM**, Puntila J, Arstila TP. Interleukin-7 promotes human regulatory T cell development at the CD4+CD8+ double positive thymocyte stage. J Leukoc Biol. 2016;100(3):491-8. **(IF 2016: 4.289)**

4. Hetemäki I, Jarva H, Kluger N, **Baldauf HM**, Laakso S, Bratland E, Husebye E, Kisand K, Ranki A, Peterson P, Arstila TP. Anticommensal Responses Are Associated with Regulatory T Cell Defect in Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy Patients. J Immunol. 2016;196(7):2955-64. **(IF 2016: 4.922)**

5. Posch W, Steger M, Knackmuss U, Blatzer M, **Baldauf HM**, Doppler W, White TE, Hörtnagl P, Diaz-Griffero F, Lass-Flörl C, Hackl H, Moris A, Keppler OT, Wilflingseder D. Complement-Opsonized HIV-1 Overcomes Restriction in Dendritic Cells. PLoS Pathog. 2015;11(6):e1005005.**(IF 2015: 7.003)**

6. Mueller B, Anders M, Akiyama H, Welsch S, Glass B, Nikovics K, Clavel F, **Tervo HM**, Keppler OT, Kraeusslich HG. Human immunodeficiency virus (HIV1) Gag processing intermediates trans-dominantly interfere with HIV-1 infectivity. J Biol Chem. 2009; 284(43):29692-703. **(IF 2009: 5.328)**

7. Seckert CK, Renzaho A, **Tervo HM**, Krause C, Deegen P, Kühnapfel B, Reddehase MJ, Grzimek NK. Liver sinusoidal endothelial cells are a site of murine cytomegalovirus latency and reactivation. J Virol. 2009; 83(17):886984. **(IF 2009: 5.15)**

8. Goffinet C, Allespach I, Homann S, **Tervo HM**, Habermann A, Rupp D, Oberbremer L, Kern C, Tibroni N, Welsch S, Krijnse-Locker J, Banting G, Kräusslich HG, Fackler OT, Keppler OT. HIV-1 antagonism of CD317 is species specific and involves Vpu-mediated proteasomal degradation of the restriction factor. Cell Host Microbe 2009; 5(3):285-97. **(IF 2009: 13.021)**

9. Goffinet C, Michel N, Allespach I, **Tervo HM**, Hermann V, Kräusslich HG, Greene WC, Keppler OT. Primary T-cells from human CD4/CCR5-transgenic rats support all early steps of HIV-1 replication including integration, but display impaired viral gene expression. Retrovirology 2007; 4:53. **(IF 2007: 4.42)**

10. Simon CO, Holtappels R, **Tervo HM**, Bohm V, Daubner T, Oehrlein-Karpi SA, Kuhnapfel B, Renzaho A, Strand D, Podlech J, Reddehase MJ, Grzimek NK. CD8 T cells control cytomegalovirus latency by epitope-specific sensing of transcriptional reactivation. J Virol. 2006; 80 (21):10436-56. **(IF 2006: 5.341)**

7.3. Reviews

1. Esteves PJ, Abrantes J, **Baldauf HM**, BenMohamed L, Chen Y, Christensen N, González-Gallego J, Giacani L, Hu J, Kaplan G, Keppler OT, Knight KL, Kong XP, Lanning DK, Le Pendu J, Lemos de Matos A, Liu J, Liu S, Lopes AM, Lu S, Lukehart S, Manabe YC, Neves F, McFadden G, Pan R, Peng X, de Sousa-Pereira P, Pinheiro A, Rahman M, Ruvoën-Clouet N, Subbian S, Tuñón MJ, van der Loo W, Vaine M, Via LE, Wang S, Mage R. The wide utility of rabbits as models of human diseases. Experimental & Molecular Medicine 2018 *accepted* **(IF 2018: 5.063)**

2. Pan X, **Baldauf HM**, Keppler OT, Fackler OT. Restrictions to HIV-1 replication in resting CD4+ T lymphocytes. Cell Res. 2013;23(7):876-85. **(IF 2013: 11.981)**

Printouts of relevant publications

1. **Tervo HM**, Keppler OT. High natural permissivity of primary rabbit cells for HIV-1, with a virion infectivity defect in macrophages as the final replication barrier. J Virol. 2010 84(23):12300-14. doi: 10.1128/JVI.01607-10.

2. de Sousa-Pereira P, Abrantes J, Bauernfried S, Pierini V, Esteves PJ, Keppler OT, Pizzato M, Hornung V, Fackler OT, **Baldauf HM**. The antiviral activity of rodent and lagomorph SERINC3 and SERINC5 is counteracted by known viral antagonists. J Gen Virol. 2019 Feb;100(2):278-288. doi: 10.1099/jgv.0.001201.

3. **Baldauf HM***, Pan X*, Erikson E, Schmidt S, Daddacha W, Burggraf M, Schenkova K, Ambiel I, Wabnitz G, Gramberg T, Panitz S, Flory E, Landau NR, Sertel S, Rutsch F, Lasitschka F, Kim B, König R, Fackler OT, Keppler OT. SAMHD1 restricts HIV-1 infection in resting CD4(+) T cells. Nature Medicine 2012, 18(11):1682-7. doi: 10.1038/nm.2964.

4. **Baldauf HM**, Stegmann L, Schwarz SM, Ambiel I, Trotard M, Martin M, Burggraf M, Lenzi GM, Lejk H, Pan X, Fregoso OI, Lim ES, Abraham L, Nguyen LA, Rutsch F, König R, Kim B, Emerman M, Fackler OT, Keppler OT. Vpx overcomes a SAMHD1-independent block to HIV reverse transcription that is specific to resting CD4 T cells. Proc Natl Acad Sci U S A. 2017;114(10):2729-2734. doi: 10.1073/pnas.1613635114.