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Anti-SAMHD1 Strategies of Feline Immunodeficiency Virus

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Anti-SAMHD1 Strategies of Feline Immunodeficiency Virus

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B.S., The College of New Jersey, 2014

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Abstract

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By Sarah A. Mereby

Human immunodeficiency virus (HIV) is classified as a lentivirus. There are many different lentiviruses that infect various species of primates and non-primates. Feline immunodeficiency virus is a non-primate lentiviruses and it is a more ancestral lentivirus as compared to primate lentiviruses. The goal of this project was to characterize host and virus coevolution for the mechanism that lentiviruses counteract host sterile α motif domain and HD domain-containing protein 1 (SAMHD1) during nondividing myeloid cell infection by employing feline immunodeficiency virus (FIV) and feline SAMHD1 as a model system. SAMHD1 restricts HIV-1 in myeloid cells by depleting cellular dNTPs and suppressing viral reverse transcription. First, I show that FIV does not degrade feline SAMHD1 and SIV viral protein x (Vpx), which proteosomally degrades primate SAMHD1, does not target feline SAMHD1. Next, I showed that human SAMHD1 inhibits FIV infection in human primary monocyte derived macrophages. To show that feline SAMHD1 is a dNTPase in cells, I isolated dNTPs in feline SAMHD1 expressing monocytic THP-1 cells. Indeed, feline SAMHD1 expression lowered dATP and dGTP levels. Then I showed that feline SAMHD1 expression in THP-1 cells inhibits infection of HIV by flow cytometry. Finally, I purified FIV reverse transcriptase (RT). In the future, we will determine the enzymatic efficiency of FIV RT as it compares to HIV-1 RT and simian immunodeficiency virus (SIV) RT. These data demonstrate that, unlike SIV targeting its host SAMHD1 proteosomally by Vpx, FIV does not degrade feline SAMHD1 proteosomally, supporting the possibility that FIV may counteract feline SAMHD1 by its enzymatically efficient RT in myeloid cells as HIV-1 does.

Anti-SAMHD1 Strategies of Feline Immunodeficiency Virus

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Table of Contents

Introduction

HIV and AIDS

Approximately 36 million people worldwide have human immunodeficiency virus (HIV)[1]. The virus infects T cells which are dividing cells and macrophages which are non-dividing cells[2,3]. As the viral infection progresses, the infected person's T cells die. Once a patient has a T cell count less than 200 cells/mm^3 he or she is diagnosed with Acquired Immunodeficiency Syndrome (AIDS)[4]. Patients with AIDS are severely immunocompromised and can die of a secondary infection. While there are drugs that decrease an infected person's viral load, there currently is no treatment that rids the body entirely of HIV[5].

Virus and host coevolution: an evolutionary arm's race

Virus and host coevolution is a phenomenon that is an ongoing arms race that has been occurring for millions of years[6]. The goal of a virus is to thrive within the host. What this usually means for most viruses is making as many infectious virus particles as possible to potentially infect more hosts and start the process over again. The goal of a host is to restrict the viral infection at the smallest possible cost. Hosts have evolved to better evade viral infections and in turn viruses evolve to find better ways to infect their hosts[7]. This same pattern is present in lentiviruses and their hosts[8]. Understanding how lentiviruses have evolved from a non-primate host to a primate host can help us better understand lentiviral evolutionary history. Non primate lentiviruses such as equine infectious anemia virus (EIAV), bovine immunodeficiency virus (BIV) and feline immunodeficiency virus (FIV) are more ancestral lentiviruses as compared to HIV[9,10]. EIAV is the most ancestral, followed by BIV and FIV[10,11]. In this study I focus upon FIV.

Feline Immunodeficiency Virus (FIV)

FIV infects many species of cats from domestic house cats to large wild cats[12]. FIV infection is similar to that seen in humans infected with HIV-1. T cells are depleted and felines can develop what is referred to as "feline AIDS". At this stage felines are immunocompromised and are subject to secondary issues such as gingivitis, tumors, stomatitis and neurological diseases. However not all cats will develop "feline AIDS". In a study, 50 percent of cats infected with FIV did not show any systems two years after infection[13]. FIV infects both T cells and macrophages like HIV. It also contains the core proteins gag, pol and env like HIV. However, the accessory proteins differ in that FIV contains orfA, dUTPase and lacks vpr[11].

Cell tropism of lentiviruses: T cells vs. macrophages

HIV infects both CD4 T cells and macrophages[14,15]. Activated T cells are dividing cells, whereas macrophages are non-dividing cells. Cellular dNTPs levels are 20 to 40nM in macrophages, which is 100 times less than the cellular dNTPs found in CD4 T cells[17,18]. Key enzymes that are responsible for the difference in cellular dNTP levels in macrophages versus T cells are ribonucleotide reductase (RNR) and sterile α motif domain and HD domain-containing protein 1 (SAMHD1)[19]. RNR is an enzyme involved in dNTP biogenesis whereas SAMHD1 is an enzyme that depletes cellular dNTP pools[20]. In T cells, RNR is highly expressed whereas SAMHD1 protein levels are low[14]. The opposite is found in macrophages where SAMHD1 is more highly expressed and RNR protein levels are low. This thesis focuses upon SAMHD1 and lentiviral co-evolution in macrophages.

SAMHD1 host restriction factor and viral protein x coevolution

SAMHD1 is an evolutionarily conserved host restriction factor[21,22,23]. In macrophages SAMHD1 acts as a dNTP triphosphohydrolase meaning that it degrades dNTPs and lowers

cellular pools[24,25]. Limiting dNTP pools in macrophages is advantageous for a host because lentiviruses require host dNTPs for reverse transcription[26]. HIV-2 and some SIVs have evolved to counteract SAMHD1 by using their viral protein x (Vpx) accessory protein to target SAMHD1 to proteasomal degradation[27]. Vpx does this by binding to SAMHD1 and bringing it to association with E3 ubiquitin ligase complex, which then increases cellular dNTP levels[28]. This leads to a more permissive infection in macrophages in HIV-2 and SIV while HIV-1 infection is seen as restrictive since HIV-1 does not have a Vpx to degrade SAMHD1.

HIV-1 RT and SAMHD1

Since HIV-1 does not target SAMHD1 for proteasomal degradation, HIV-1 has evolved a way to overcomes SAMHD1 restriction by having a more efficient reverse transcriptase (RT)[29,30]. HIV-1 RT can incorporate dNTPs at a low concentration more efficiently and thus have a lower K_m than that of HIV-2 and SIVs that encode Vpx[29]. Pre-steady-state enzymatic assays have shown that HIV-1 has a faster k_{pol} step as compared to HIV-2 and SIVs that encode Vpx[30]. Additionally, Vpx encoding lentiviruses pause more during incoroporation[30]. This evidence shows that HIV-1 has evolved a mechanism to overcome SAMHD1 mediated lowered dNTP pools by having a more efficient RT.

Whether FIV contains a Vpx-like protein that degrades host SAMHD1 and FIV's RT efficiency is unknown. Because Vpx has appeared more recently in evolutionary history we hypothesize that FIV does not have a Vpx like proteins that degrade SAMHD1 and therefore has a more efficient RT like that of HIV-1 to overcome SAMHD1 mediated lowered dNTP pools in macrophages[11].

Summary

Studying FIV, a non-primate lentivirus, can give more insight into how lentiviruses have evolved to counteract host SAMHD1. By understanding this history, we can see how lentiviruses have adapted in many different hosts over time. In this thesis, I aim to discover conserved mechanistic crosstalk among host SAMHD1, RTs of lentiviruses, and cellular dNTPs in myeloid target cells.

Results:

FIV does not degrade host SAMHD1

To determine if FIV targets feline SAMHD1 I transfected HEK 293T cells and feline kidney cells (CRFK cells) with feline SAMHD1 HA tag and FIV packaging plasmid. As a negative control I transfected feline SAMHD1 HA tag and FIV transfer plasmid. After probing for feline SAMHD1, there was no difference in feline SAMHD1 protein levels across the different conditions (Figure 1A-B). I then repeated the same experiment to validate the assay in 293T cells by transfecting human SAMHD1 with SIV wildtype packaging plasmid and SIV packaging plasmid ΔVpx. Human SAMHD1 protein degraded when co-transfected with SIV WT as compared to human SAMHD1 and SIV ΔVpx (Figure 1C). I tested if the pattern seen in FIV and feline SAMHD1 would be similar in equine infectious anemia virus (EIAV), also a non-primate lentivirus. I transfected 293T cells with equine SAMHD1 and EIAV packaging and transfer plasmid. I saw the same result I saw for feline SAMHD1: there was no change in equine SAMHD1 protein levels across the different conditions (Figure 1D). The results suggest that non-primate lentiviruses do not target their host SAMHD1 proteins.

Vpx does not target feline SAMHD1

To better understand why feline SAMHD1 is not degraded by FIV, I transfected 293T cells with feline SAMHD1 and SIV wildtype packaging plasmid and SIV packaging plasmid ΔVpx. There were no changes in feline SAMHD1 levels suggesting that Vpx does not target feline SAMHD1 (Figure 2).

Human SAMHD1 inhibits FIV infection

Because we know that human SAMHD1 inhibits HIV infection, I wanted to determine if human SAMHD1 could inhibit FIV infection. This would inform us if the relationship between

FIV and SAMHD1 would be similar to that of HIV and SAMHD1. To determine if human SAMHD1 inhibits FIV infection I transduced human primary monocyte derived macrophages first with FIV GFP vector and then I transduced with either virus like particle (VLP) with or without viral protein x (Vpx). Because Vpx targets SAMHD1, the VLP Vpx+ would show the effects of FIV infection without SAMHD1 (Figure 3A). After flow cytometry analysis for percent GFP positive cells, VLP Vpx+ significantly increased FIV infection as compared to VLP Vpx- condition (Figure 3B). This experiment strongly suggests that human SAMHD1 inhibits FIV infection. This could mean that feline SAMHD1 could restrict FIV in a similar manner.

Generation of feline SAMHD1 expressing THP-1s

To generate a feline SAMHD1 expressing cell line I first transduced human SAMHD1 knockout THP-1 cells (Figure 4A) with feline SAMHD1 mCherry vector. As a control I transduced SAMHD1 knockout THP-1 cells with an empty mCherry vector. I then FACS sorted for mCherry positive cells. Then I differentiated the cells with PMA and verified that the feline SAMHD1 expressing cells expressed feline SAMHD1 protein and that the empty mCherry and initial knockout that I transduced did not express feline SAMHD1 (Figure 4B).

Feline SAMHD1 is a dNTPase in cells

Then to answer the question if feline SAMHD1 lowers dNTPs in cells, I isolated dNTPs from human SAMHD1, human SAMHD1 KO +mCherry, and human SAMHD1 KO feline SAMHD1 expressing THP-1s and performed a radiolabeled primer extension assay. dATP and dGTP levels in feline SAMHD1 expressing cells was lower than that of SAMHD1 KO mCherry cells, whereas dCTP and dTTP levels did not differ between feline SAMHD1 and the SAMHD1 KO mCherry cells (Figure 5). This data suggests that feline SAMHD1 readily targets purines as compared to pyrimidine bases.

Feline SAMHD1 inhibits infection of HIV

To then determine if feline SAMHD1 inhibits infection of HIV, I differentiated feline SAMHD1, SAMHD1 KO mCherry, and SAMHD1 expressing THP-1 cells with PMA. After differentiation, I infected cells with HIVD3-GFP vector. After flow cytometry analysis for percent GFP positive cells at 24 hours post infection, feline SAMHD1 expressing cells showed comparable infection to human SAMHD1 control and lower HIV infection when compared to SAMHD1 knockout mCherry cells (Figure 6).

FIV RT purification

To do further studies on FIV RT, I cloned FIV RT from FIV FP93 packaging plasmid and FIV PPR plasmid into a pET28a backbone. I then purified the proteins on His tag gravity filtration column (Figure 7). We hope to use these purified proteins to do enzyme kinetic assays in the future.

Discussion & Conclusion:

This study sought to understand how FIV counteracts host SAMHD1 in myeloid cells. FIV is a more ancestral lentivirus as compared to primate lentiviruses such as HIV-1, HIV-2 and SIVs. We chose feline SAMHD1 and FIV to study them in the context of non-primate evolution and compared them to primate lentiviruses. I show that unlike SIV targeting its host SAMHD1 proteosomally, FIV does not target feline SAMHD1 protein levels. SIV Vpx also does not target feline SAMHD1 protein levels, indicting the limited host specificity of Vpx. However, human SAMHD1 in human primary monocyte derived macrophages does inhibit FIV infection. I showed that feline SAMHD1 is a dNTPase in cells, and that the expression of feline SAMHD1 inhibits HIV infection in PMA-differentiated THP-1 cells. Collectively, these data support the likelihood that FIV may counteract feline SAMHD1-mediated dNTP depletion by using its enzymatically efficient RT as HIV-1 lacking Vpx does. For testing this, I purified FIV RT to compare the enzymatic efficiency of FIV RT with that of HIV-1 and SIV.

Further validation for the anti-SAMHD1 strategy of ancestral non-primate lentiviruses, which employ their enzymatically efficient RTs instead of the proteosomal degradation of SAMHD1, requires similar experimental investigations with other non-primate lentiviruses including equine infectious anemia virus (EIAV) and bovine immunodeficiency virus (BIV). Future studies include comparing the enzymatic efficiency of FIV, EIAV and BIV RT with that of HIV-1 and SIV RT. Also we will test if BIV and EIAV target their host SAMHD1 proteins. Indeed, preliminary data I performed showed that EIAV also does not target equine SAMHD1 which is the same pattern observed with FIV and feline SAMHD1. Overall, this study provides valuable clues about how ancestral non-primate lentiviruses mechanistically overcome the

dNTPase/SAMHD1 mediated antiviral restriction during their replication in nondividing myeloid cells.

Figures:

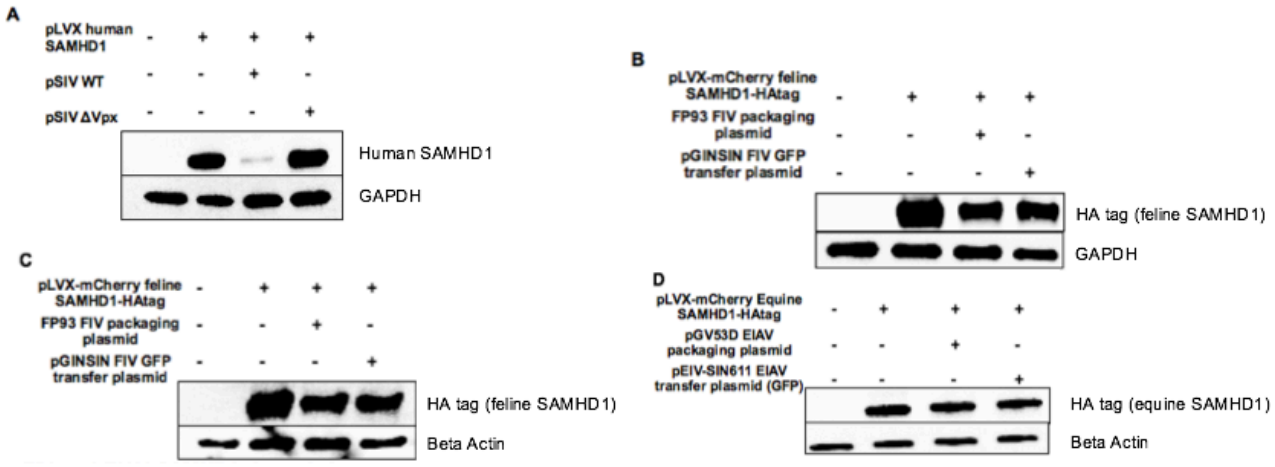


Figure 1: FIV and EIAV SAMHD1 degradation assays by western blot. (A) Co-transfection of pLVX human SAMHD1 and pSIV WT or pSIV ΔVpx packaging plasmids in 293T cells. **(B)** Co-transfection of pLVX-mCherry feline SAMHD1 HA tag and FP93 FIV packaging plasmid or pGINSIN FIV GFP transfer plasmid in 293T cells. **(C)** Same as B, but in CRFK cells. **(D)** Co-transfection of pLVX-mCherry equine SAMHD1 HA tag and pGV53D EIAV packaging plasmid or pEIV-SIN611 EIAV transfer plasmid in 293T cells. Molecular weights of proteins: human SAMHD1 72 kDa, GAPDH: 35.8 kDa, HA tagged feline SAMHD1: ~70kDa, Beta actin: 42kDa.

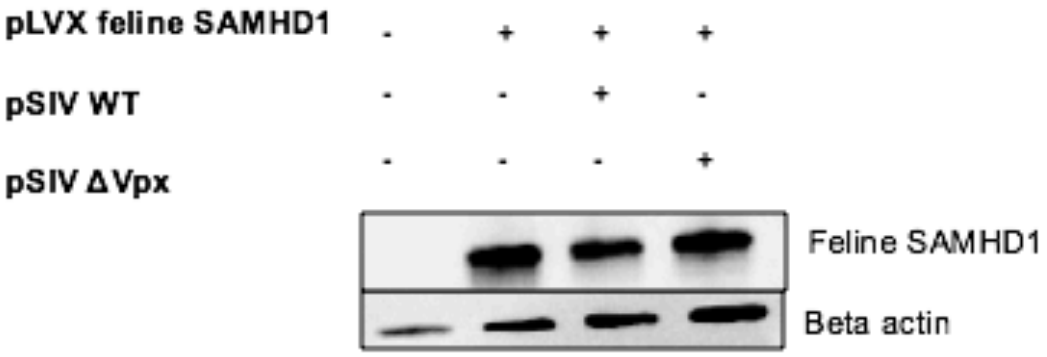
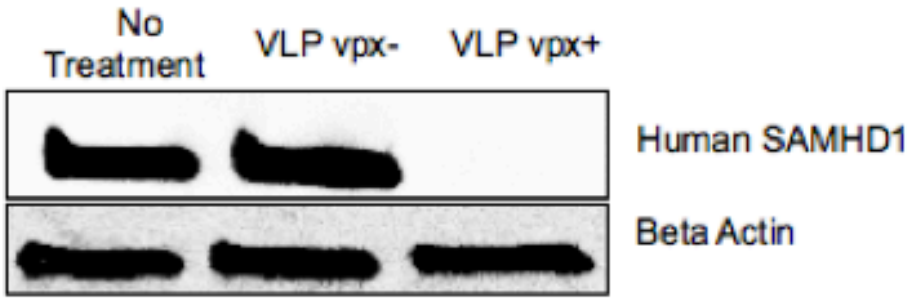


Figure 2: Vpx does not target feline SAMHD1 protein levels. Co-transfection of pLVX human SAMHD1 and pSIV WT or pSIV ΔVpx packaging plasmids in 293T cells. Cells were lysed 48 hours post transfection and assayed via western blot with HA tag (feline SAMHD1) and beta actin antibodies. Molecular weights of proteins: HA tagged feline SAMHD1: ~70kDa, Beta actin: 42kDa.

A



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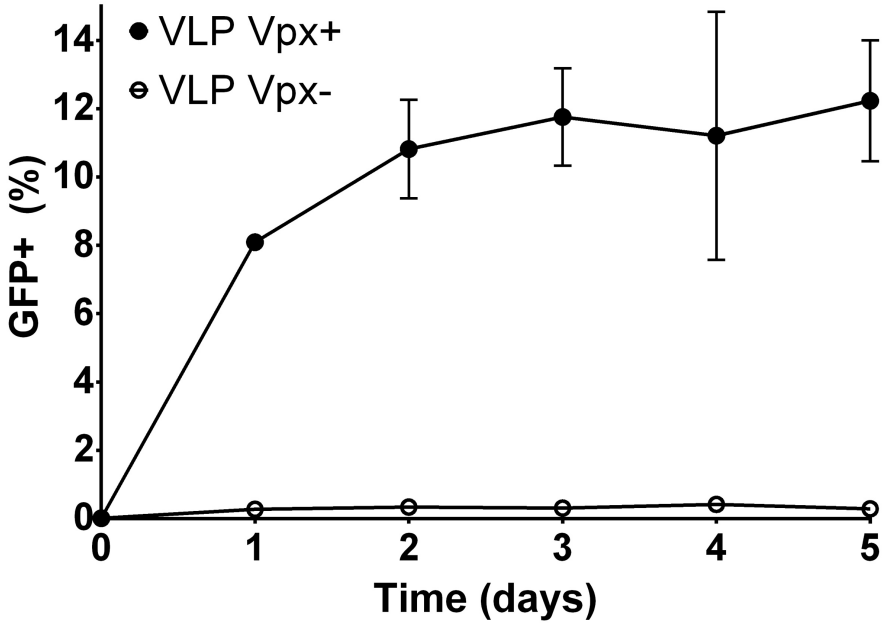

Figure 3: FIV infectivity assays in human primary monocyte derived macrophages. A) Human primary monocyte derived macrophages were treated with Vpx- and and Vpx+ VLPs, confirming Vpx degrades human SAMHD1 in the primary macrophages. B) Human macrophages pre-treated with Vpx+ and Vpx- VLPs were transduced with FIV-GFP vector, and the percent GFP positive cells were determined by FACS in triplicate. Error bars indicate standard deviation. Molecular weights of proteins: human SAMHD1 72 kDa, Beta actin: 42kDa.

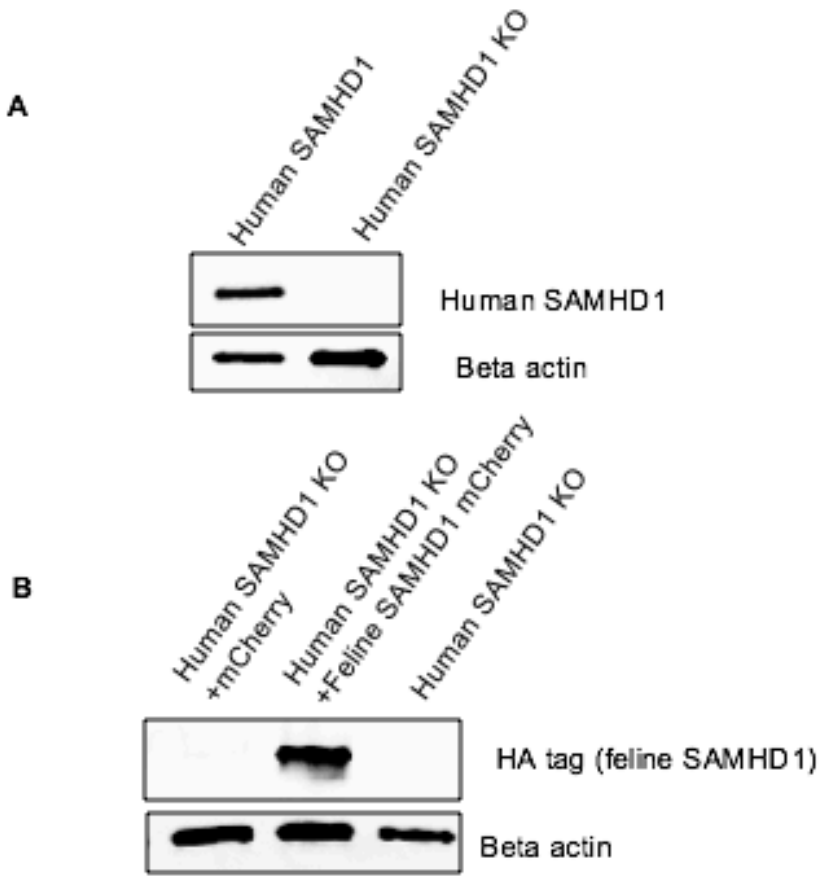

Figure 4: Generation of feline SAMHD1 expressing THP-1 cells. A) human SAMHD1 and knockout THP-1 cells. B) Human SAMHD1 KO THP-1 cells were transduced to generate 1) human SAMHD1 KO THP-1 cells expressing mCherry. 2) human SAMHD1 KO THP-1 cells expressing feline SAMHD1 mCherry. THP-1 cells were differentiated with PMA and then lysed and assayed via western blot for human SAMHD1, HA tag (feline SAMHD1) and beta actin control protein levels. Molecular weights of proteins: GAPDH: 35.8 kDa, HA tagged feline SAMHD1: ~70kDa, Beta actin: 42kDa.

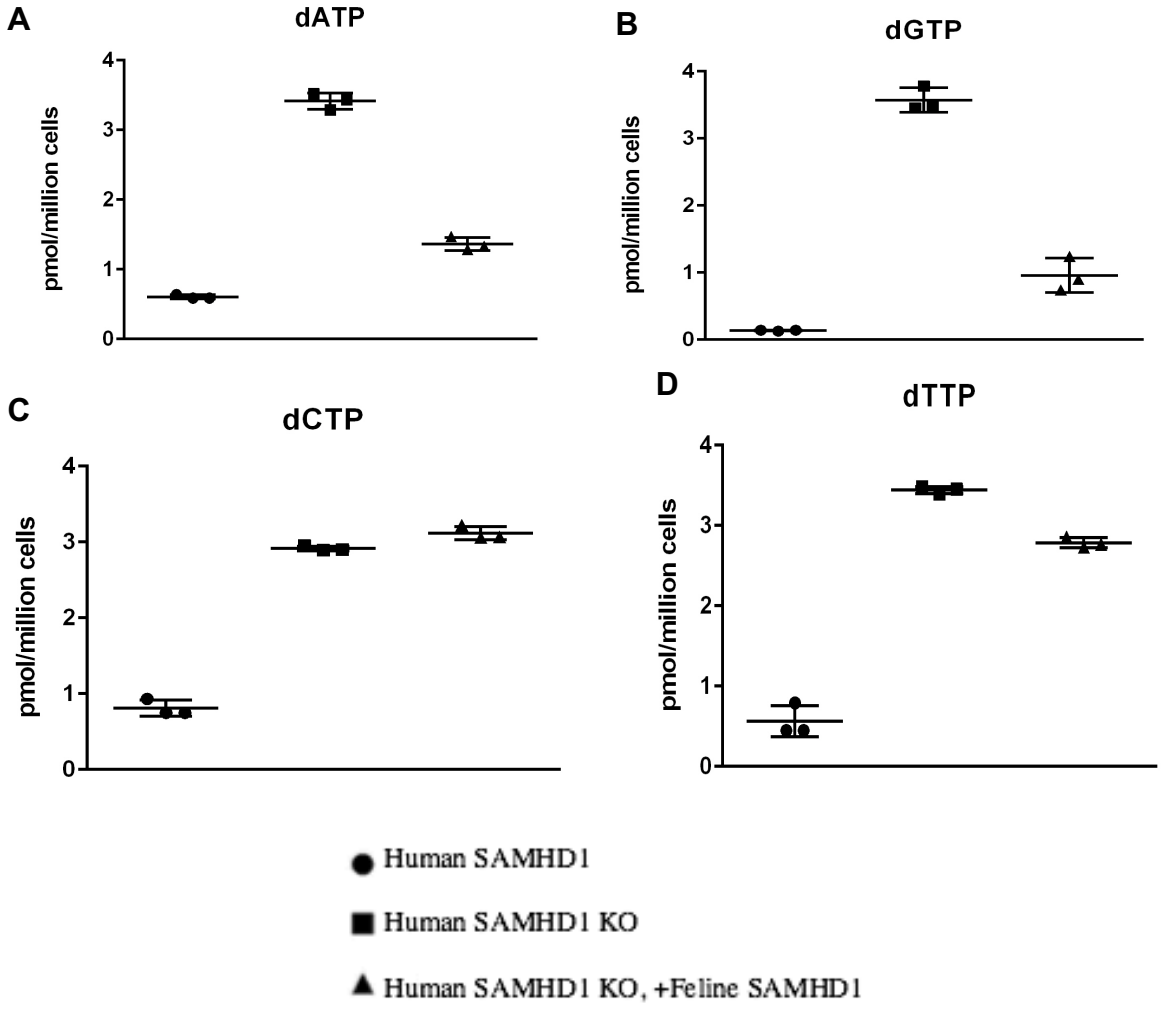

Figure 5: Feline SAMHD1 lowers dNTPs in in PMA-differentiated THP-1 cells. After differentiation, THP-1 cells were collected for dNTP isolation. Radiolabeled primer extension assay was performed to determine dNTP levels pmol/million cells. A) dATP B) dGTP C) dCTP D) dTTP. Assays were conducted with human SAMHD1, human SAMHD1 KO and human SAMHD1 KO feline SAMHD1 expressing THP-1 cells in triplicate.

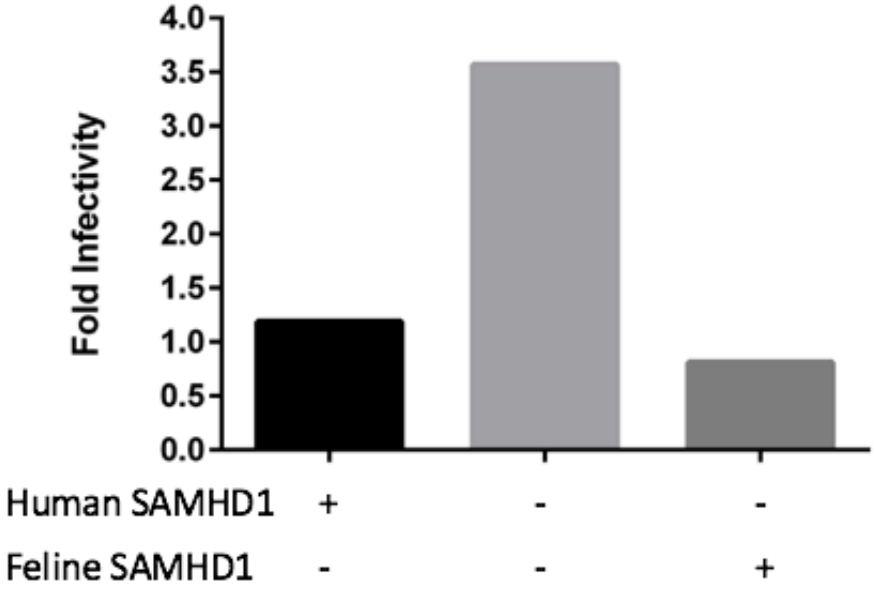

Figure 6: Feline SAMHD1 inhibits HIV infection in PMA-differentiated THP-1 cells. Relative infectivity of human SAMHD1, human SAMHD1 KO and human SAMHD1 KO expressing feline SAMHD1 PMA differentiated-THP-1 cells transduced with HIVD3-GFP. Cells were collected at 24 hours post transduction and the percent GFP positive cells were determined by FACS in triplicate.

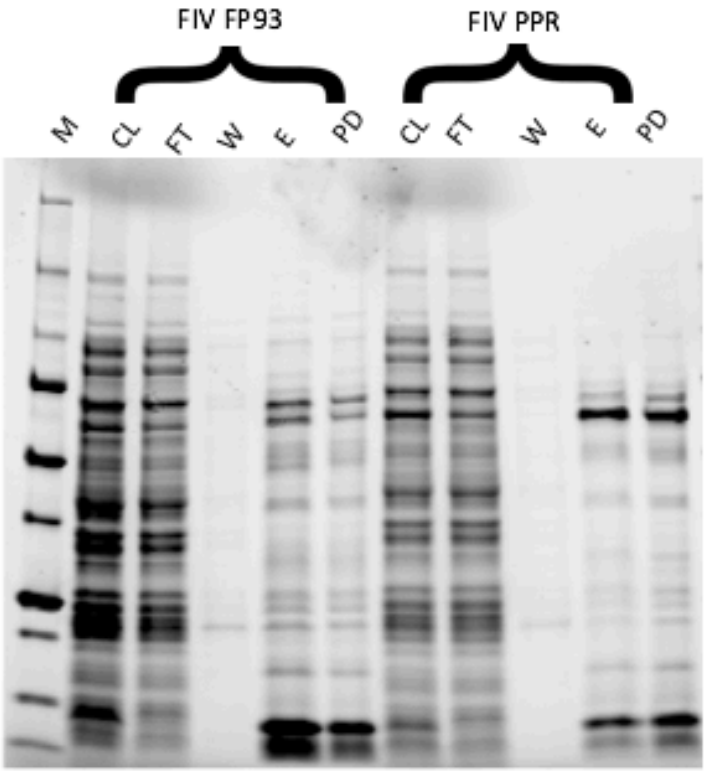

Figure 7: Purification of FIV RT. Lanes 2-6 FIV FP93, lanes 7-11 FIV PPR, M=marker, CL= clear lysate, FT= flow through, W= wash, E= elute, PD= post dialysis

Experimental Procedures:

Cell Culture and Lentiviral Vectors

HEK 293FT cells (Invitrogen) were cultured in DMEM (Gibco) supplemented with 10% FBS and 1% penicillin/streptomycin. CRFK *Felis catus* kidney cells (ATCC CCL-94) were cultured in EMEM (ATCC) supplemented with 10% FBS and 1% penicillin/streptomycin. THP-1 cells (ATCC TIB-202) were cultured in RPMI 1640 medium (Corning) supplemented with 10% FBS and 1% penicillin/streptomycin. All cells were grown at 37 °C, 5% CO2. THP-1 cells were differentiated with 150 nM PMA (Sigma) for 7 days. Media was replaced with media containing PMA every 2 days.

293FT cells were transfected to make lentiviral vector. 293FT cells were grown until they were 70% confluent. Media was then replaced before transfection to serum free DMEM. Then, they were transfected using polyethylenimine (Sigma). Media was removed after 8 hours and replaced with serum containing DMEM. Media was collected at 24 and 48 h post-transfection. Media was filtered through a 0.45um filter. Then the viral vector was concentrated using a Beckman Coulter Optima XE90 ultracentrifuge. After centrifugation the pellets were resuspended in Hanks' balanced salt solution (Gibco) and stored at -80 °C.

Western Blotting

Cells were lysed in 1X Ripa solution. 2x Laemmli sample buffer (Biorad) was added to the lysates. Then samples were run on SDS-PAGE gel 4–15% (Bio-Rad) and transferred to nitrocellulose membrane (Biorad). Primary antibodies and dilutions in 5% milk-TBST used include: HA tag 1:5000 (Cell Signaling #3724), anti-human SAMHD1 1:3000 (Abcam ab119751), anti-GAPDH 1:3000 (Cell Signaling #14C10), anti-beta actin 1:10,000 (Abcam ab6276). Secondary antibodies were diluted at 1:1000 in 5% milk-TBST. Blots were visualized

by using chemiluminescence (SuperSignal West Femto maximum sensitivity substrate, Thermo Scientific) and imaged using a ChemiDoc Touch imaging system (Bio-Rad).

Macrophage isolation and differentiation

Macrophages were isolated from buffy coats of human donors with CD14 microbeads. Then they were differentiated with 5ng of GM-CSF for 7 days.

Generation of feline SAMHD1 expressing THP-1 cells

THP-1 human SAMHD1 knockout cells were transduced with pLVX mCherry feline SAMHD1 vector and empty mCherry vector. After 48 hours, cells were FACS sorted for mCherry positive cells. Cells were pooled and grown to generate a large enough cell population to perform experiments.

dNTP isolation

dNTPs were isolated from differentiated THP-1 cells by washing with PBS and then adding trypsin for 5 minutes at 37C. Cells were spun down and then resuspended in cold 65% methanol. Cells were then vortexed and lysed at 95C. Cells were then centrifuged and the supernatant was dried in CentriVap Complete Vacuum Concentrator (Labconco). Samples were resuspended in water before doing assay.

dNTPase assay

Assay was performed as described[18]. Pharos FX Plus Molecular Imager, Biorad was used to image the gel. Image Lab 5.2.1 (Biorad) software was used to determine dNTP concentrations.

FIV RT purification

pET28a FIV RT clones were transformed into Rosetta cells. RT expression was induced by adding 1mM IPTG for 3 hours at 37C. Cells were harvested and resuspended in 1x Binding

buffer (Novagene). Sample was sonicated and centrifuged. Lysate was loaded onto nickel column with resin and eluted by gravity filtration.

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