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Supplemental Information

Evolutionary and Functional Analyses of the Interaction between the Myeloid Restriction Factor SAMHD1 and the Lentiviral Vpx Protein

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Supplemental Experimental Procedures

Primate material and SAMHD1 sequencing

Predicted SAMHD1 coding sequences were retrieved from genome assembly projects on Ensembl (http://www.ensembl.org/info/about/species.html) for Human (GRCh37 of *Homo sapiens*), Chimpanzee (CHIMP2.1 of *Pan troglodytes*), Sumatran orangutan (PPYG2 of *Pongo abelii*), Gorilla (gorGor3.1 of *Gorilla gorilla*), Northern white-cheeked gibbon (Nleu1.0 of *Nomascus leucogenys*), Rhesus macaque (MMUL_1 of *Macaca mulatta*), Common marmoset (C_jacchus3.2.1 of *Callithrix jacchus*), Tarsier (tarSyr1 of Tarsius syrichta), Bushbaby (BUSHBABY1 of *Otolemur garnettii*), Gray mouse lemur (micMur1 of *Microcebus murinus*) and for Tree shrew (TREESHREW of *Tupaia belangeri*). SAMHD1 sequences were aligned and used to design specific primers in conserved regions of the 5‘ and 3’ untranslated regions (UTRs) for PCR amplification.

Samples from NHPs were collected during their annual health surveys or at necropsy in accordance with each institution’s animal care and use committee guidelines. Total RNA was extracted from tissue and cells from a panel of 25 primate species (Supplementary table 1) using Qiagen RNeasy Plus minikit. cDNA was prepared by reverse transcription using Transcriptor High Fidelity cDNA Synthesis Kit (Roche). Multiple independent PCRs were performed on cDNA with Pfx Supermix using primers in UTRs (Life Technologies) and subsequently sequenced. The PCR products were cloned into the pCR-BluntII-TOPO vector (Life Technologies) and several individual clones (n=5) were sequenced to identify allelic variants.

Cell lines, transfections, transductions and infections

Adherent (HeLa and 293T) and suspension (THP-1 and U937) cells were cultured in DMEM or RPMI supplemented with 10% fetal calf serum (FCS), ultraglutamine and antibiotics. THP-1 cells silenced for endogenous SAMHD1 expression through shRNA were maintained in media supplemented with 1µg/mL puromycin. All cell culture reagents were purchase from Lonza. U937 and THP-1 cells were differentiated overnight with 30ng/mL PMA (Sigma). Transfection of 293T cells was achieved using the standard phosphate calcium transfection protocol. HeLa cells were transfected using the jetPEI kit (Polyplus). Expression of SAMHD1 and Vpx in U937 and THP-1 cells was achieved through transduction with MLV particles. THP-1 cells were infected with 100ng equivalent of p24 of HIV-LUC-G.

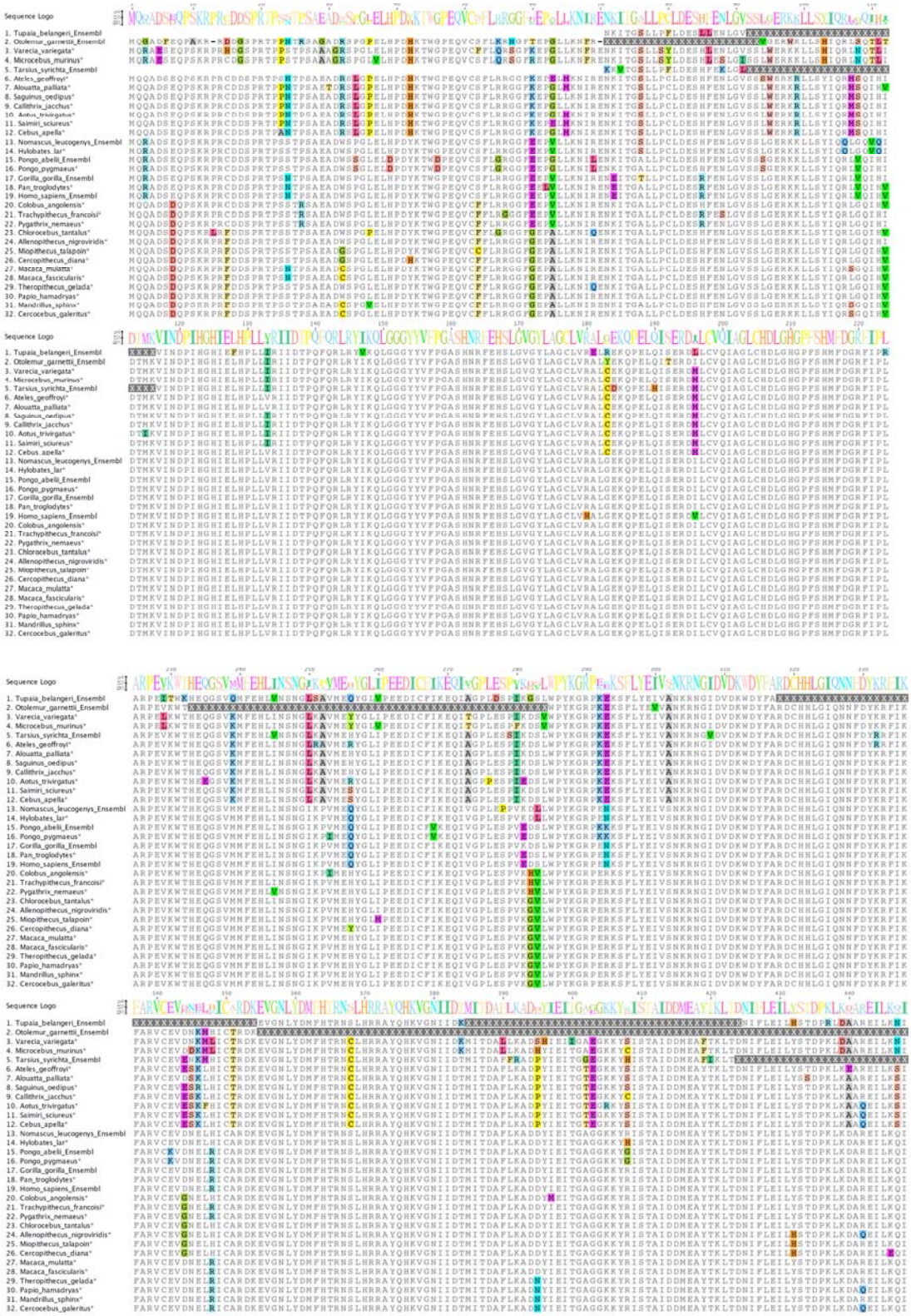
Virus production

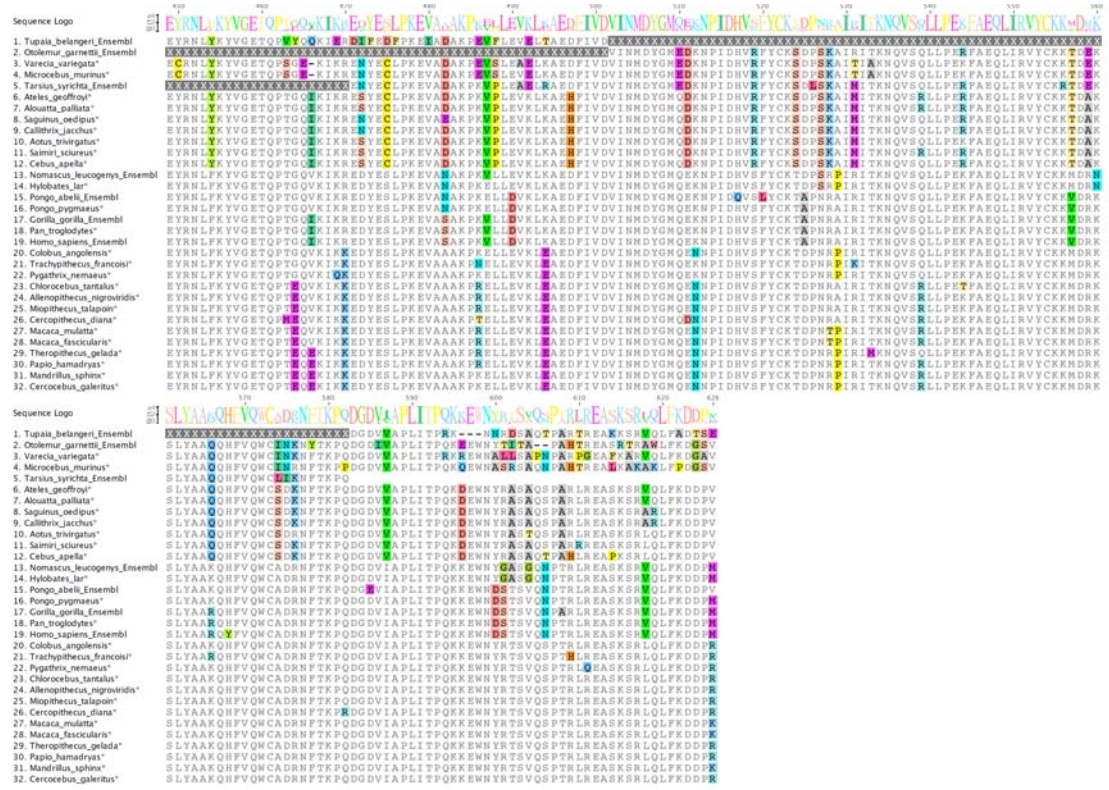
Viral particles were produced from 293T cells using the standard phosphate calcium transfection protocol. Briefly, for HIV-LUC-G production, 293T cells were transfected with 8µg HIV-LUC and 2µg VSV-G encoding plasmid, for shRNA production 4µg shRNA construct, 4µg packaging plasmid, 2µg VSV-G encoding plasmid, for MLV transduction particules 5µg pOZ construct, 2.5µg packaging plasmid and 2.5µg A-MLV envelope encoding plasmid and for VLP-Vpx production, 8µg SIV3+ was co-transfected or not with 2µg VSV-G encoding plasmid. Media was replaced 16 hrs post transfection and viruses were harvested 24 hrs later, filtered at 0.45µm. When required, p24 concentration was measured by ELISA (Innogenetics).

Cell extracts preparation and Western blot analysis

Whole cell extracts were prepared with 0.5% triton, 150 mM NaCl, 10 mM KCL, 1.5 mM MgCl2, 0.5 mM EDTA 10 mM β-mercaptoethanol, 0.5 mM PMSF. Mouse anti-SAMHD1, and anti-DDB1 were purchased from Abcam. HA (11 clone 16B12) and Tubulin antibodies were from Covance/Eurogentec and Sigma, respectively.

A





B

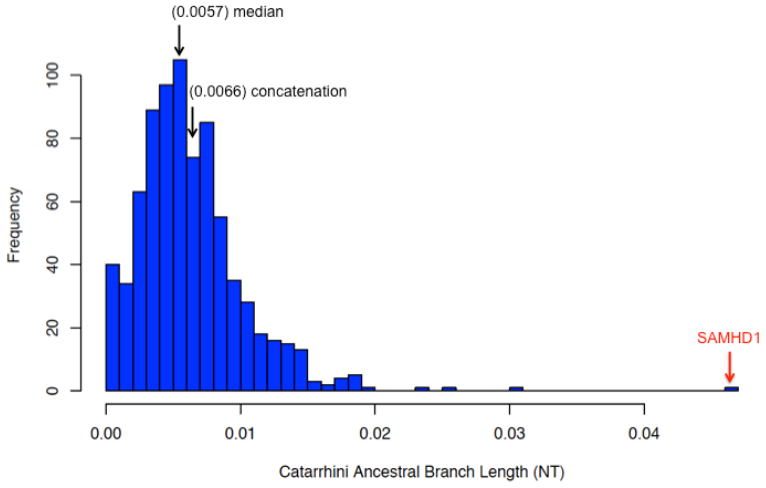


Figure S1. Related to Figure 1

(A) Multiple sequences alignment of amino acids from 31 primates with *Tupaia belangeri* as an outgroup. (B) Distribution of the ancestral Catarrhini nucleotide branch length inferred across the 786 1:1 orthologous CDS from OthroMaM v6 (36 taxa) gene trees showing that SAMHD1 is an extreme outlier with a branch length longer than that inferred from the concatenation

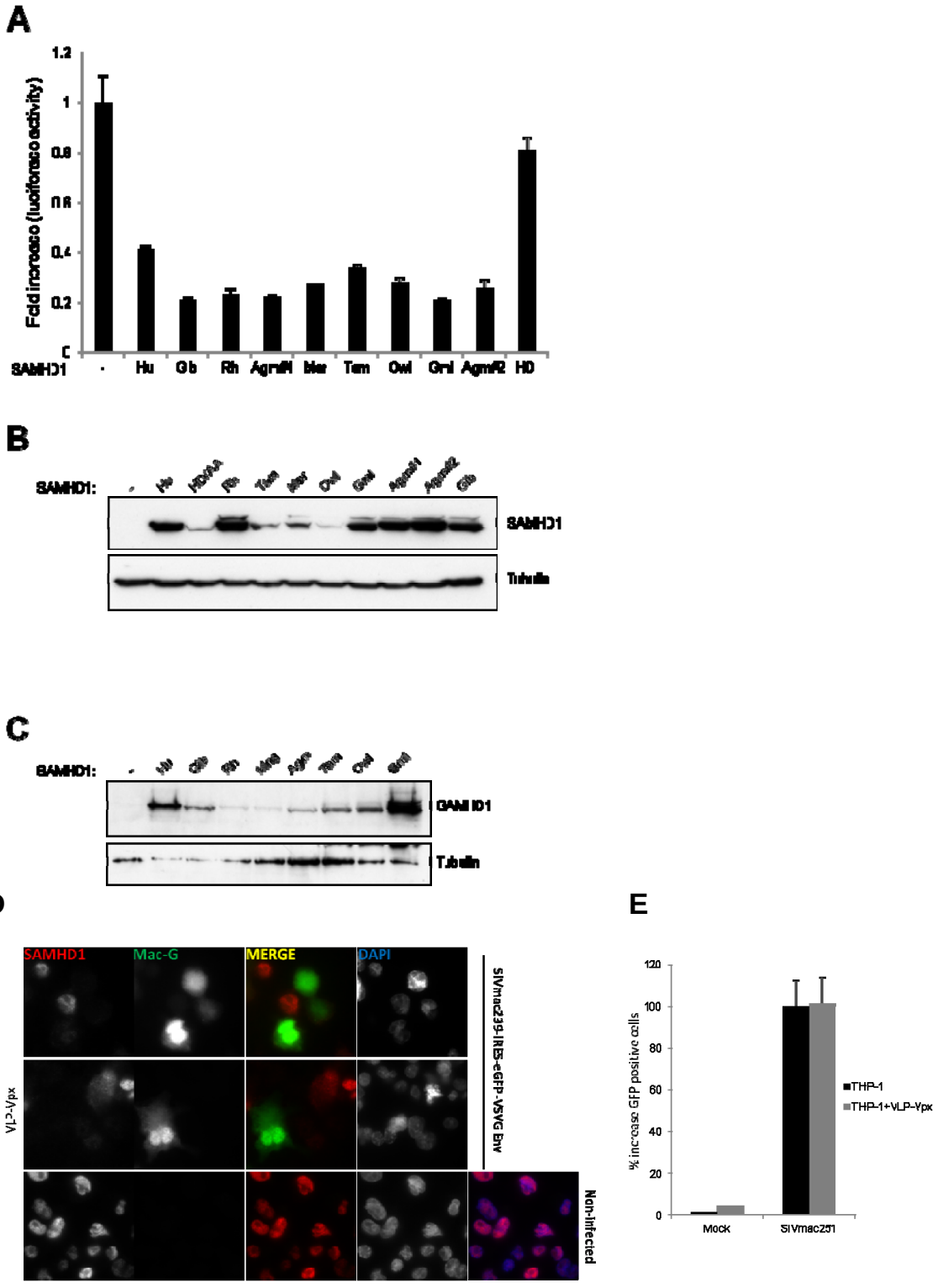
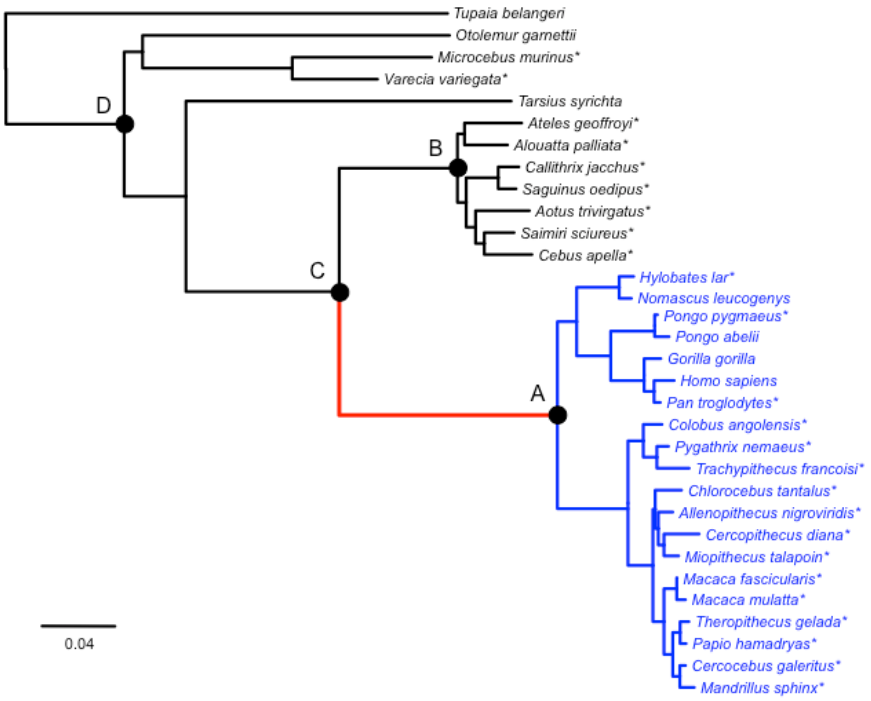


Figure S2. Related to Figure 2

(A) U937 cells were transduced with a retroviral vector allowing for expression of FLAG and HA tagged SAMHD1. 48hrs post transduction, cells were differentiated. Sixteen hours later transduced cells were infected with HIV-Luc-G. Luciferase activity was measured 24 hrs post

infection, normalized for protein concentration of analyzed samples. Results are presented as fold increase luciferase activity over parental U937 cells. (B) Cells from A were harvested and whole cell extracts were analyzed by WB with antibodies allowing for detection of SAMHD1, Tubulin and Vpx. (C) Cells from Figure 2 C were analyzed as in B. SIVmac251 infection causes huSAMHD1 degradation. (D) THP-1 cells were differentiated for 16hrs on polylysine-treated coverslips prior to infection with a VSV-G pseudotyped molecular clones of SIVmac251 harbouring an IRES-eGFP cassette as a reporter (mac-G). Cells were fixed 48hrs post infection and immunostained with anti-SAMHD1 antibody. Nuclei were DAPI-stained. (E) Flow cytometry analysis of cells from A. Results are presented as % of GFP positive cells in THP-1 cells. Error bars represent the standard deviation from the mean.

A



B

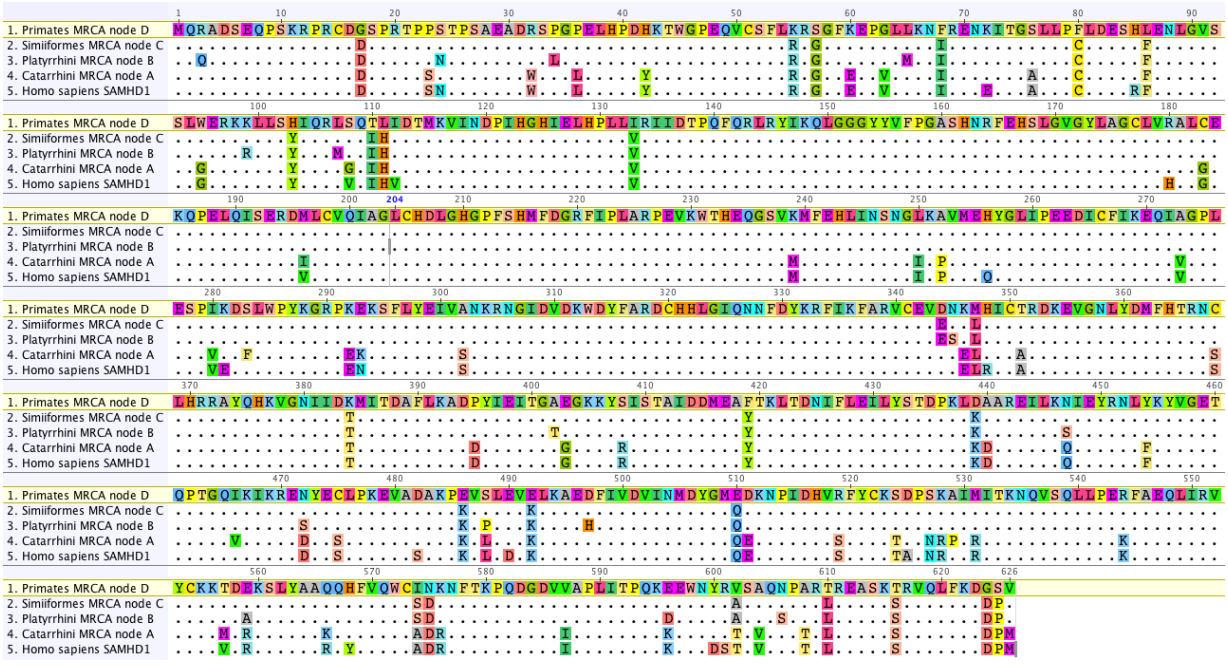


Figure S3. Related to Figure 3

Maximum Likelihood reconstruction of SAMHD1 ancestral sequences. The ancestral sequences were inferred using joint reconstruction under the best-fitting branch specific codon model M3ω with three separated ω values (see Figure 2A). (A) gene tree. (B) Sequence alignment.

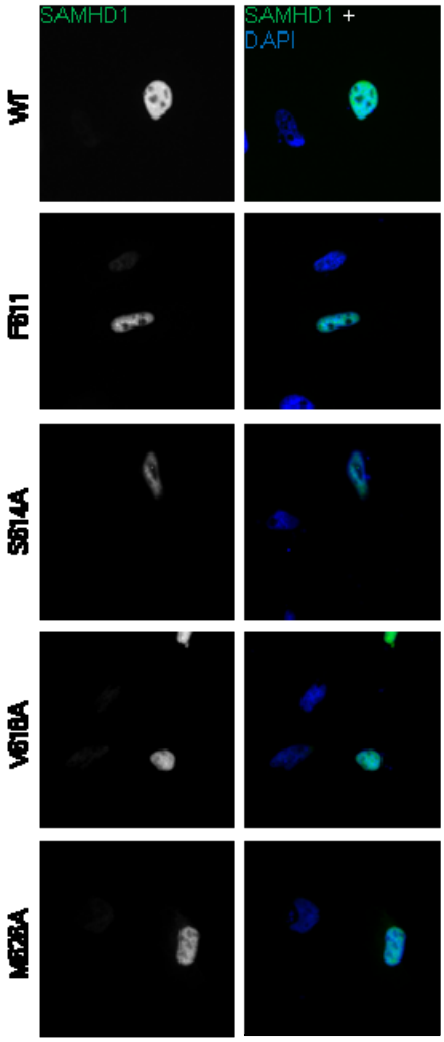


Figure S4. Related to Figure 4
Subcellular localization of huSAMHD1 mutants. HeLa cells were transfected with 250 ng of FLAG-tagged WT-huSAMHD1, SAMHD1-F611, SAMHD1-S614A, SAMHD1-V618A and SAMHD1M626A. Cells were fixed 48hrs post transfection and immunostained with anti-SAMHD1 antibody. Nuclei were stained with DAPI.

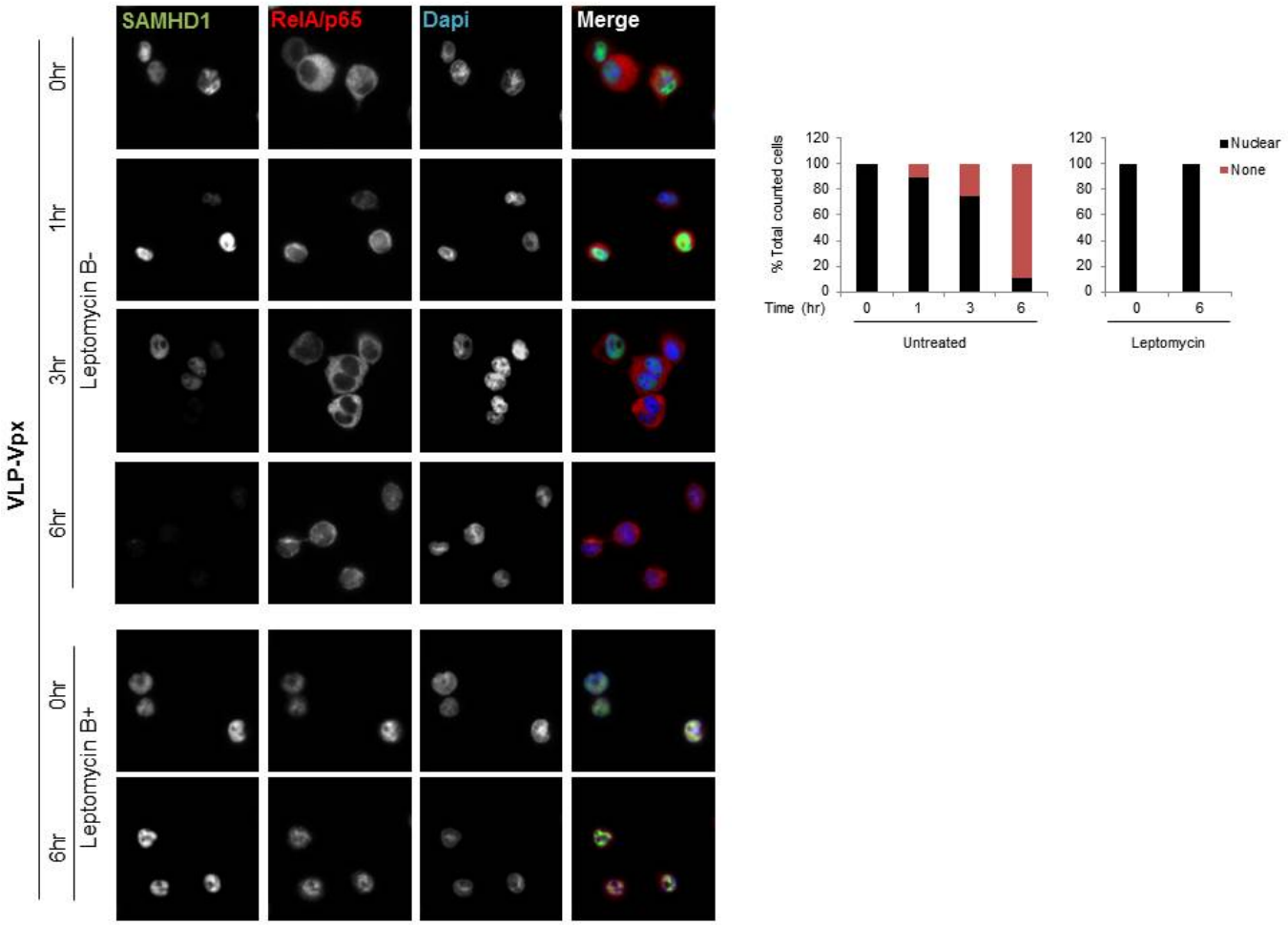


Figure S5. Related to Figure 5

Leptomycin B prevents SAMHD1 from degradation by Vpx. (Left panel) Differentiated THP1 cells were mock-treated or treated with Leptomycin B for 4 hrs. Mock-treated and Leptomycin B treated cells were incubated with VLP-Vpx for the indicated time. Cells were fixed and the expression of SAMHD1 and RelA/p65 was analyzed by immunofluorescence using specific antibodies. Nuclei were stained in mounting media with DAPI. (right panel) SAMHD1-positive cells from the left panel were counted. Results are expressed as %SAMHD1positive cells.

Table S1. Accession Numbers of Primate *SAMHD1* Alleles, Related to Figure 1

Taxonomy	Species	Common name	Cell type	Accession number
Hominoid	*Pan troglodytes*	*Chimpanzee*	PBMC	JN936887
	Pongo pygmaeus	*Bornean orangutan*	EB185(JC) lymphoblast line	JN936888
	Hylobates lar	*White-handed gibbon*	MLA-144 lymphoblast cell line	JN936889
Old World Monkeys	*Papio Hamadryas*	*Hamadryas baboon*	26-CB1 lymphoblast line	JN936890
	Chlorocebus tantalus	*African green monkey*	COS-7 kidney cell line	JN936891 JN936892
	Macaca fascicularis	*Crab-eating macaque*	CYNOMK-1 skin fibroblast	JN936893
	Macaca mulatta	*Rhesus macaque*	LLCMK-2 kidney cell line	JN936894
	Macaca mulatta	*Rhesus macaque*	FRhK-4 kidney cell line	JN936895
	Theropithecus gelada	*Gelada baboon*	PBL	JN936896
	Mandrillus sphinx	*Mandrill*	PBL	JN936897
	Cercocebus galeritus chrysogaster	*Tana River mangabey*	PBL	JN936898 JN936899
	Allenopithecus nigroviridis	*Allen's swamp monkey*	PBL	JN936900
	Miopithecus talapoin	*Angolan talapoin*	PBL	JN936901
	Cercopithecus diana	*Diana monkey*	PBL	JN936902
	Pygathrix nemaeus nemaeus	*Red shanked douc langur*	PBL	JN936903
	Trachypithecus francoisi	*Francois's langur*	PBL	JN936904
	Colobus angolensis palliatus	*Angola colobus*	PBL	JN936905
New World Monkeys	*Callithrix jacchus*	*Common marmoset*	liver	JN936906
	Aotus trivirgatus	*Northern owl monkey*	OMK kidney cell line	JN936907
	Saguinus oedipus	*Cotton-top tamarin*	B95-8 kidney cell line	JN936908
	Saimiri sciureus	*Squirrel monkey*	PBL	JN936909
	Cebus apella	*Tufted capuchin*	PBL	JN936910
	Ateles geoffroyi	*Black-handed spider monkey*	PBL	JN936911
	Alouatta villosa palliata	*Mantled howler*	PBL	JN936912
Lemurs	*Varecia variegata variegata*	Black-and-white ruffed lemur	PBL	JN936913
	Microcebus murinus	*Gray mouse lemur*	liver	JN936914

Table S2. *vpx* Alleles from SIV and HIV-2 Strains, Related to the Experimental Procedures

	Description	Strains	Species	Sequence
SIV	SIVmac251 Vpx	mac251	Rhesus macaque *Macaca mulatta*	ATGTCAGATCCCAGGGAGAGAATCCCACCTGGAAACAGTGGAGAAGAGACAATAGGAGAGGCCTTCGA ATGGCTAAACAGAACAGTAGAGGAGATAAACAGAGAGGCAGTAAACCACCTACCAAGGGAGCTGATTT TCCAGGTTTGGCAAAGGTCTTGGGAATACTGGCATGATGAACAAGGGATGTCACAAAGCTATGTAAAAT ACAGATACTTGTGTTTAATGCAAAAGGCTTTATTTATGCATTGCAAGAAAGGCTGTAGATGTCTAGGGGA AGGACACGGGGCAGGAGGATGGAGACCAGGACCTCCTCCTCCTCCCCCTCCAGGACTAGCATAA
	SIVmac239 Vpx	mac239	Rhesus macaque *Macaca mulatta*	ATGTCAGATCCCAGGGAGAGAATCCCACCTGGAAACAGTGGAGAAGAGACAATAGGAGAGGCCTTCGA ATGGCTAAACAGAACAGTAGAGGAGATAAACAGAGAGGCGGTAAACCACCTACCAAGGGAGCTAATTT TCCAGGTTTGGCAAAGGTCTTGGGAATACTGGCATGATGAACAAGGGATGTCACCAAGCTATGTAAAAT ACAGATACTTGTGTTTAATACAAAAGGCTTTATTTATGCATTGCAAGAAAGGCTGTAGATGTCTAGGGGA AGGACATGGGGCAGGGGGATGGAGACCAGGACCTCCTCCTCCTCCCCCTCCAGGACTAGCATAA
	SIVsmm Vpx	PGm53	Sooty mangabey *Cercocebus atys*	ATGTCAGATCCCAGAGAGAGGATCCCACCTGGAAACAGTGGAGAAGAAACAATAGGAGAGGCATTCGA ATGGCTAAACAGAACAGTAGAAGAAATAAACAGGGCAGCAGTGAATCACTTGCCGAGGGAGCTAATTTT CCAGGTTTGGCGAAGGTCTTGGGAATACTGGCGTGATGAAATGGGGATGTCAGAGAGCTACACAAAAT ACAGATACTTGTGCTTAATACAGAAAGCTCTGTTTGTGCATTGCAAGAGAGGGTGTAGGTGCTTAGGAG AAGAGCATGGGGCAGGGGGATGGAGATCAGGGCCTCCTCCTCCTCCCCCTCCAGGACTAGCATAA
	SIVrcm-ng Vpx	RCM.NG	Red-capped mangabey *Cercocebus torquatus*	ATGGCAGAGGGCAGAGAAAGAGTGCCAGAGGCCCCCACTGGGGCTGGAGATGTAGAGTTTGCCCCCT GGCTTCACAGAATGCTAACAGAAGTCAACTTAGAAGCCAGGTTGCACTTTCATCCAGAGTTCATTTTCC GTCTGTGGCGTACTTGTGTGGAACACTGGCATGATAGGCTTGGAAGAAGCCTTGAGTATGCAGGCTAT AGATATCTGCTTCTGATGCAAAAAGCTCTGTTTATTCATTGCCAATCAGGGTGTTCTCAGAGACATGGAC AGGGACAAGCAAGGGAAGCAGGAGAAAGAATCCAGATTCTTCCGGGAATGTAA
	SIVmnd2 Vpx	5440	Mandrill *Mandrillus*	ATGGCAGAGAGGGCACCAGAGGCGCCAGAAGGAGCAGGAGAGGTAGGACTGGAGCAATGGCTGGAA ACGTCACTGGAGAGAATCAACCGGGAGGCCCGGTTACACTTCCACCCAGAGTTCCTTTTCCGTCTCTG GAACACATGTGTAGAACACTGGCATGATAGACATCAGAGATCTCTTGATTATGCCAAGTATAGATACCT GCTGTTGATGCATAAGGCCATGTATACTCACATGCAACAGGGATGCCCATGTAGAAATGGGCGCCCAA GGGGACCTCCTCCTCCAGGGATGGCTTAA
	SIVdrl1 Vpx	FAO	Drill *Mandrillus leucophaeus*	ATGGCAGAAAGACAGTCAGTGGAGAGAGCTCCAGCGGAGCCAATGGGAGCAGGAGAGGTAGAGTTAG AAGAATGGCTACAGAGGAGTCTCTTAAGAATCAACCAGGAGGCTCGATTACACTTCCACCCAGAGTTCC TCTTCCGTCTTTGGAACACCTGCATGGAGCACTACCATGATGCTCTTCAGTTATCTTTTACTTACAGCAA GTATAGATACCTACTTTTGTTACAGAAGGCCATGTTCATGCACTTTCAGCAAGGATGCTCATGTCTGCAG GGAAGGCATCCACCTCCCCTCAGACCAGCAGGAGATAGACTTCCTCCTCCTCCTCCTCCTCCATGA
HIV-2	HIV-2 ROD Vpx	Rod	Human *H. sapiens*	ATGACAGACCCCAGAGAGACAGTACCACCAGGAAACAGCGGCGAAGAGACTATCGGAGAGGCCTTCG CCTGGCTAAACAGGACAGTAGAAGCCATAAACAGAGAAGCAGTGAATCACCTACCCCGAGAACTTATTT TCCAGGTGTGGCAGAGGTCCTGGAGATACTGGCATGATGAACAAGGGATGTCAGAAAGTTACACAAAG TATAGATATTTGTGCATAATACAGAAAGCAGTGTACATGCATGTTAGGAAAGGGTGTACTTGCCTGGGG AGGGGACATGGGCCAGGAGGGTGGAGACCAGGGCCTCCTCCTCCTCCCCCTCCAGGTCTGGTCTAA
	HIV-2A ROD Vpx	Rod	Human *H. sapiens*	ATGACAGACCCCAGAGAGACAGTACCACCAGGAAACAGCGGCGAAGAGACTATCGGAGAGGCCTTCG CCTGGCTAAACAGGACAGTAGAAGCCATAAACAGAGAAGCAGTGAATCACCTACCCCGAGAACTTATTT TCCAGGTGTGGCAGAGGTCCTGGAGATACTGGCATGATGAACAAGGGATGTCAGAAAGTTACACAAAG TATAGATATTTGTGCATAATACAGAAAGCAGTGTACATGCATGTTAGGAAAGGGTGTACTTGCCTGGGG AGGGGACATGGGCCAGGAGGGTGGAGACCAGGGCCTCCTCCTCCTCCCCCTCCAGGTCTGGTCTAA
	HIV-2B Vpx	7312	Human *H. sapiens*	ATGGATCCCAGGGAGAGAGTGCCACCAGGAAACAGCGACGAAGAGACAGTAGGAGAGGCATTCGCAT GGCTAGAAAGAACAATAGTAGAACTCAACAGGGAAGCAGTCAACCATTTGCCCCGAGAACTTATTTTCC AGGTCTGGCAAAGGTCTTGGGCATATTGGCGTGATGATCAGGGCATGTCAATTAGCTACACCAAGTATA GGTACTTGCTTCTGATACAAAAAGCAATGTTTGTACATTTTGCAAAGGGCTGTACATGCCTGCGGGGAG GCCATGGGCCAGGGGGATGGAGACGAGGACCTCCTCCTCCTCCTCCCCCAGGCCTAGCCTAA