Supplementary Information

Supplementary Figure 1. Homology between SAMHD1 and bacterial nucleotide metabolic enzymes. **(a)** Homology between human SAMHD1 and bacterial nucleotide enzyme sequences is indicated at the local level (91 residue region encompassing the HD motif) and at the global level. Accession numbers are indicated between brackets (Uniprot database). (**b**) Pairwise alignment of human SAMHD1 against EF1143, a bacterial nucleotide metabolic enzyme from *Enterococcus faecalis***.** The sequence of SAMHD1 shares 17.8% identity and 31.6% similarity to EF1143. EF1143 has been biochemically and structurally characterized regarding its triphosphohydrolase activity. Most of the residues implicated in the enzymatic activity of EF1143 are conserved in SAMHD1 (highlighted in grey). The SAM domain of SAMHD1 (underlined with a solid line) is not conserved in EF1143 but the HD domain (boxed residues) is well-represented by the metal binding motif (H…HD…D) in both sequences (residues marked in bold). The solid vertical lines (|) between the sequences indicate the identities. Double dots (:) and single dots (.) indicate high and low similarities between the corresponding amino acids, respectively. EMBOSS Stretcher of EMBL-EBI (http://www.ebi.ac.uk) was used for the alignments.

Supplementary Figure 2. Validation of the THP1 cell system for SAMHD1-mediated restriction. THP-1, engineered to stably express shRNA scrambled (control) or shRNA specifically targeting SAMHD1, were differentiated overnight with PMA. The cells were pre-incubated for 2 h with Vpx-containing or control VLP before infection with wild-type HIV-1-luc virus (MOI=1). Luciferase activity was quantified after three days.

Supplementary Figure 3. SAMHD1 removes three phosphates from dNTP in a single step. The γ-^{32}P-labeled reaction products from the recombinant SAMHD1 cleavage of dNTP were incubated with alkaline phosphatase for different times, then separated on thin layer chromatography. The position of mono-, di- and triphosphate are indicated.

Supplementary Figure 4. Vpx has no effect on dNTP levels in activated $CD4^+$ T cells. CD4+ T cells were purified from buffy coats by positive selection with antibody-coated immunomagnetic beads (Miltenyi). Activated CD4 T cells were obtained after stimulation for three days with phytohemagglutinin (PHA, 5 μg/ml) and interleukin-2 (10ng/ml) (NIH AIDS Research and Reference Reagent Program). On day 3, CD4 cells (one million per point) were incubated for two hours with Vpx-containing and control VLP. dNTP extraction was carried out on day 4.

Supplementary Figure 5. SAMHD1 activity is revealed in T cells by treatment with RNR inhibitor. SupT1 cells that stably expressed SAMHD1 were treated or not with HU and then infected with HIV-1 luciferase reporter virus. The data are expressed as the ratio of luciferase activity in SAMHD1 cells over control cells. A ratio of 1 indicates that SAMHD1-SupT1 cells and control cells were infected with the same efficiency.

Supplementary Fig. 6. (a) The infectability of U937, THP1 and THP1 SAMHD1 knock-down cells by HIV-1 is compared. U937 cells are highly infectable as they lack SAMHD1 expression. **(b)** and **(c)** SAMHD1 reduces the level of each dNTP in the

stably transduced U937 cells. Individual dNTP levels were determined were for each of the SAMHD1 expressing U937 cells.

Supplementary Table 1 Results of dNTP quantification in MDM. The dNTP concentration of MDM pre-incubated control or Vpx-containing VLP, and treated or not with dN or with HU, were determined by enzyme-based dNTP assay. SD are based on independent experimental replicates from two donors.

Supplementary Figure 1

a

	Human SAMHD1 (Q9Y3Z3) Local - Global alignment
EF1143 (Q836G9)	55.2% - 31.6%
PA1124 (Q9I4L1)	43.1% - 29.6%
PA3043 (Q9HZG5)	41.4% - 27.4%
TT1383 (Q76DY8)	39.6% - 26.5%
Ec-dGTPase (P15723)	32.1% - 38.9%

b

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Human-SAMHD1       1 MQRADSEQPSKRPRCDDSPRTPSNTPSAEADWSPGLELHPDYKTWGPEQV     50
                     |.....||           |.|..
EF1143             1 MTIPYKEQ-----------RLPIE-------------------------     13

Human-SAMHD1      51 CSFLRRGGFEEPVLLKNIRENEITGALLPCLDESRFENLGVSSLGERKKL    100

EF1143            14 -------------------------------------------------     13

Human-SAMHD1     101 LSYIQRLVQIHVDTMKVINDPIHGHIEL-HPLLVRIIDTPQFQRLRYIKQ    149
                                 ||..||:|.:|.: |.::::.:|::.:.||||.|||
EF1143            14 ---------------KVFRDPVHNYIHVQHQVILDLINSAEVQRLRRIKQ     48

Human-SAMHD1     150 LGGGYYVFPGASHNRFEHSLGVGYLAGCLVHALGEKQPELQISER-----    194
                     ||...:.|.||.|:||.|||||..:...:...........::.|.
EF1143            49 LGTSSFTFHGAEHSRFSHSLGVYEITRRICEIFQRNYSVERLGENGWNDD     98

Human-SAMHD1     195 DVLCVQIAGLCHDLGHGPFSHMFDGRFIPLARPEVKWTHEQGSVMMFEHL    244
                     :.|....|.|.||:||||:||.|                      ||:
EF1143            99 ERLITLCAALLHDVGHGPYSHTF----------------------EHI    124

Human-SAMHD1     245 INSNGIKPVMEQYGLIPEEDICFIKEQIVGPLESPVEDSLWPYKGRPENK    294
                     .::|. :.:..|....||.::..|..::.......|...:.....|:
EF1143           125 FDTNH-EAITVQIITSPETEVYQILNRVSADFPEKVASVITKQYPNPQ--    171

Human-SAMHD1     295 SFLYEIVSNKRNGIDVDKWDYFARDCHHLGIQ-NNFDYKRFIKFARVCEV    343
                       :.:::|::   ||.|:.||..||.:..|.: ..||..|.::..|..:.
EF1143           172 --VVQMISSQ---IDADRMDYLLRDAYFTGTEYGTFDLTRILRVIRPYKG    216

Human-SAMHD1     344 DNELRICARDKEVGNLYDMFHTRNSLHRRAYQHKVGNIIDTMITDAFLKA    393
                     .    |.....:.:.|...:|..::.:.|.|.|...::.::.....:|
EF1143           217 G----IAFAMNGMHAVEDYIVSRYQMYVQVYFHPVSRGMEVILDHLLHRA    262

Human-SAMHD1     394 DDYIEITGAGGKKYRISTAID------DMEAYTKLTDNIFLEILYSTDPK    437
                     .:..| ........:.|..:.      .::.|.||.|.: |...::....
EF1143           263 KELFE-NPEFDYDLQASLLVPFFKGDFTLQEYLKLDDGV-LSTYFTQWMD    310

Human-SAMHD1     438 LKDAREILKQIEYRNLFKYVGETQPTGQIKIKREDYESLPKEVASAKPKV    487
                     :.|:  ||..:..|.|.:     :|     :|...:.:..:..|:.....
EF1143           311 VPDS--ILGDLAKRFLMR-----KP-----LKSATFTNEKESAATIAYLR    348

Human-SAMHD1     488 LLDVKLKAEDFIVDVINMDYGMQEKNPIDHVSFYCKTAPNRAIRITKNQV    537
                     .|..|:.........||..|.:    |.|   ||   .||:....|:.::
EF1143           349 ELIEKVGFNPKYYTAINSSYDL----PYD---FY---RPNKDRHRTQIEL    388

Human-SAMHD1     538 SQLLPEKFAEQLIRVYCKKVDRKSLYAARQYFVQWCADRNFTKPQDGDVI    587
                     .|.........:.....:..:|....|.||.:...|:...|..|
EF1143           389 MQKDGSLVELATVSPLVAALAGQSQGDERFYFPKEMLDQGNKKHYD----    434

Human-SAMHD1     588 APLITPQKKEWNDSTSVQNPTRLREASKSRVQLFKDDPM    626
                       |.....:|:  |:.:.|...:.:.
EF1143           435 --LFDETYREF--SSYIHNGALVLKK------------    456
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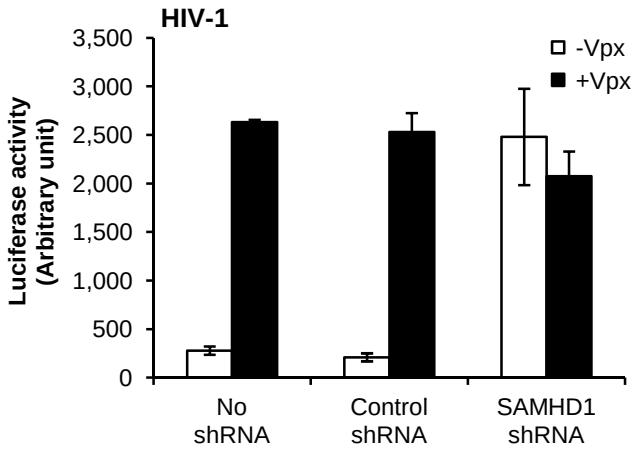
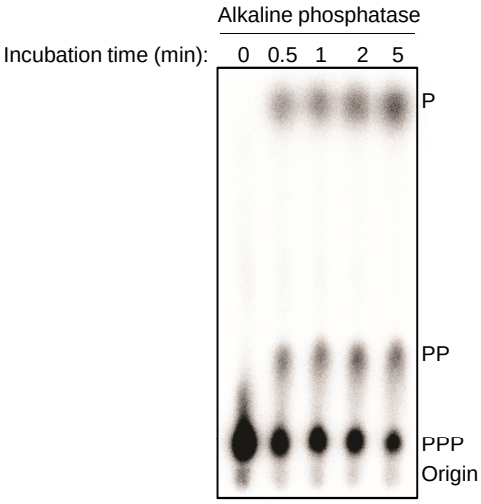
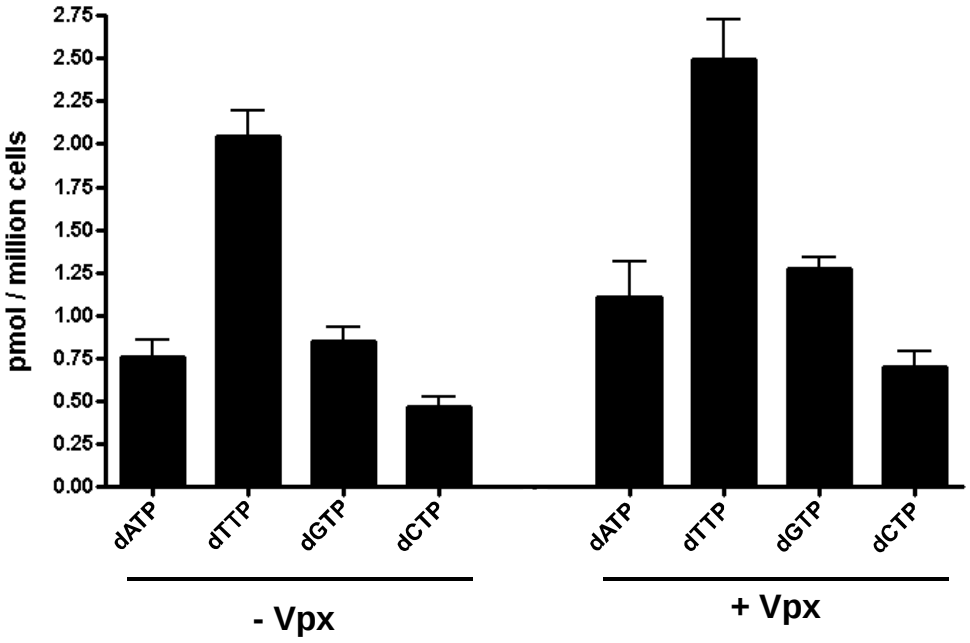
Supplementary Figure 2



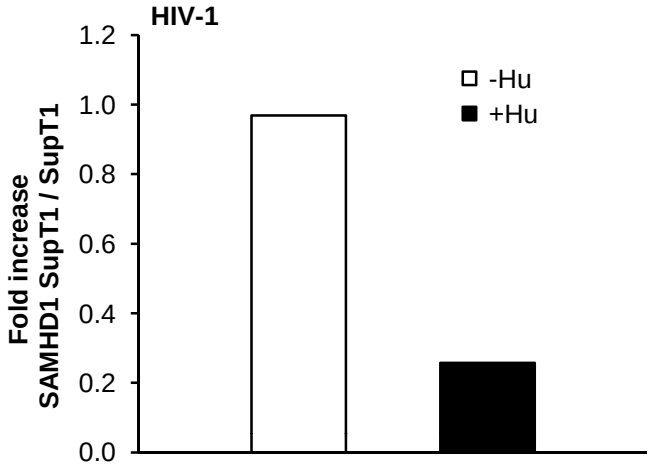
Supplementary Figure 3



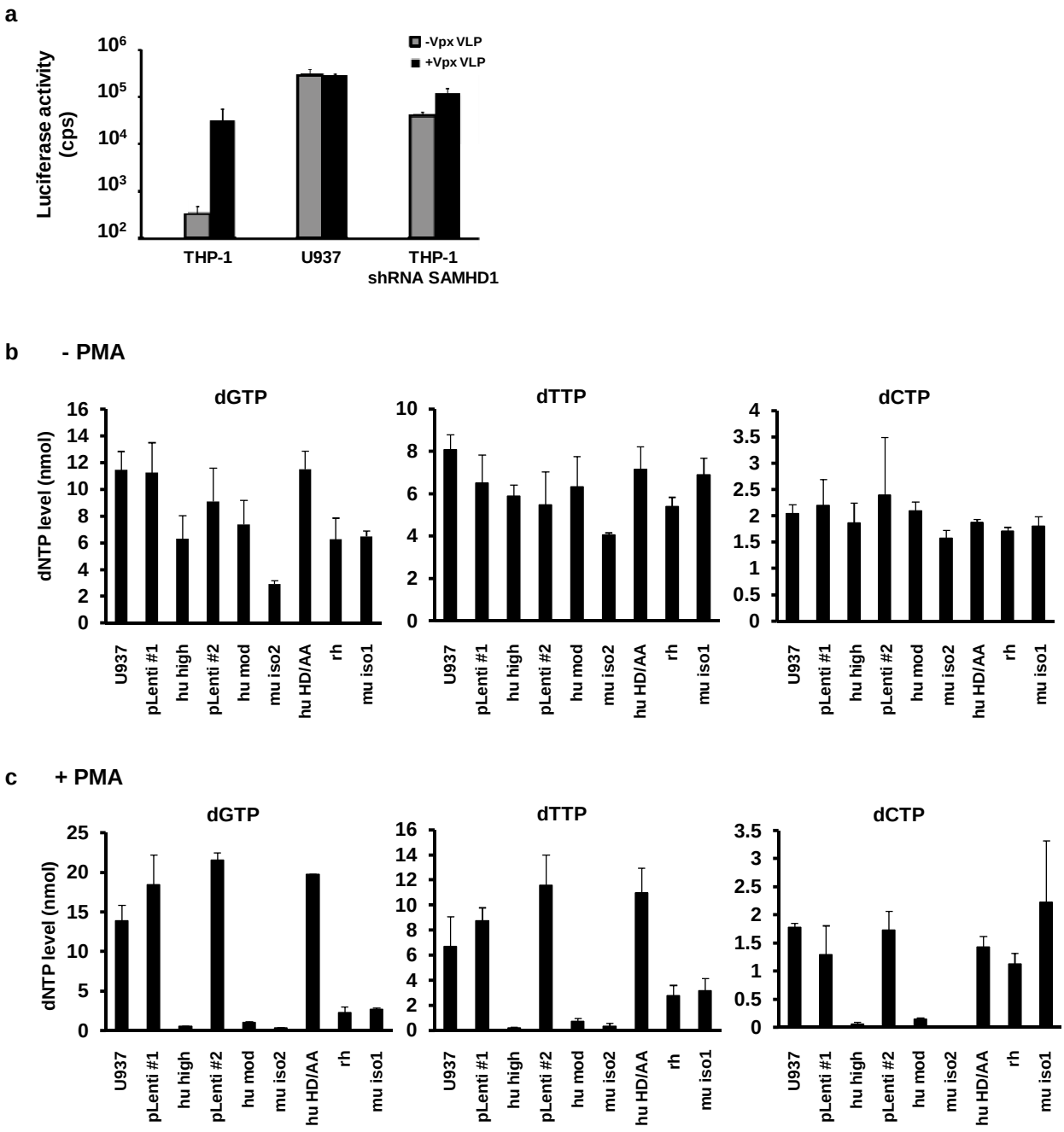
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Supplementary Table 1

	dNTP content in fmol/10^6 macrophages (fold change compared to -Vpx -dN)					
	-Vpx -dN	**+Vpx -dN**	**-Vpx +dN**	**+Vpx +dN**	**-Vpx -dN +HU**	**+Vpx -dN +HU**
dATP	**35 ± 4 (1X)**	**640 ± 12 (18X)**	**434 ± 112 (12X)**	**750200 ± 133039 (>100X)**	**44 ± 14 (1X)**	**50 ± 28 (1X)**
dTTP	**36 ± 3 (1X)**	**1175 ± 84 (33X)**	**1181 ± 31 (33X)**	**445360 ± 79 (>100X)**	**37 ± 3 (1X)**	**44 ± 2 (1X)**
dGTP	**56 ± 5 (1X)**	**640 ± 239 (11X)**	**1452 ± 425 (26X)**	**790938 ± 10 (>100X)**	**40 ± 1 (1X)**	**54 ± 16 (1X)**
dCTP	**61 ± 1 (1X)**	**317 ± 1 (5X)**	**1011 ± 154 (17X)**	**20526 ± 93395 (>100X)**	**58 ± 8 (1X)**	**61 ± 2 (1X)**