Supplementary Information

SAMHD1 restricts the infection of myeloid cells with HIV-1 by depleting the intracellular pool of dNTPs

Hichem Lahouassa[1,2,3,10], Waaqo Daddacha[4,10], Henning Hofmann[5], Diana Ayinde[1,2,3], Eric C Logue[5], Loïc Dragin[1,2,3], Nicolin Bloch[5], Claire Maudet[1,2,3], Matthieu Bertrand[1,2,3], Thomas Gramberg[6], Gianfranco Pancino[7], Stéphane Priet[8], Bruno Canard[8], Nadine Laguette[9], Monsef Benkirane[9], Catherine Transy[1,2,3], Nathaniel R Landau[5], Baek Kim[4] & Florence Margottin-Goguet[1,2,3]

[1]Institut National de la Santé et de la Recherche Médicale U1016, Institut Cochin, Paris, France. [2]Centre National de la Recherche Scientifique UMR8104, Paris, France. [3]University of Paris Descartes, Paris, France. [4]Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, New York, USA. [5]Department of Microbiology, New York University School of Medicine, New York, New York, USA. [6]Virologisches Institut, Klinische und Molekulare Virologie, Universitat Erlangen-Nurnberg, Erlangen, Germany. [7]Institut Pasteur, Unité de Régulation des Infections Rétrovirales, Paris, France. [8]Laboratoire d'Architecture et Fonction des Macromolécules Biologiques, UMR6098, Centre National de la Recherche Scientifique–Université d'Aix-Marseille, Marseille, France. [9]Institut de Génétique Humaine, Laboratoire de Virologie Moléculaire, Centre National de la Recherche Scientifique UPR1142, Montpellier, France. [10]These authors contributed equally to this work. Correspondence should be addressed to N.R.L. (nathaniel.landau@med.nyu.edu), B.K. (baek_kim@urmc.rochester.edu) and F.M.-G. (florence.margottin-goguet@inserm.fr).

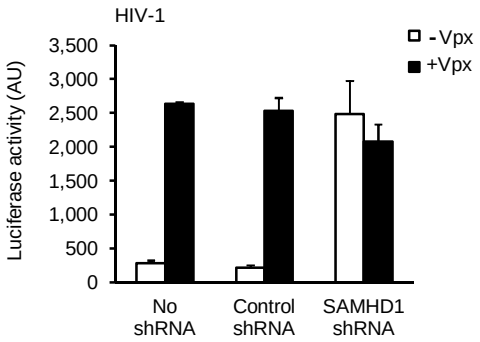
a

	Local - Global alignment of human SAMHD1 (Q9Y3Z3)
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%

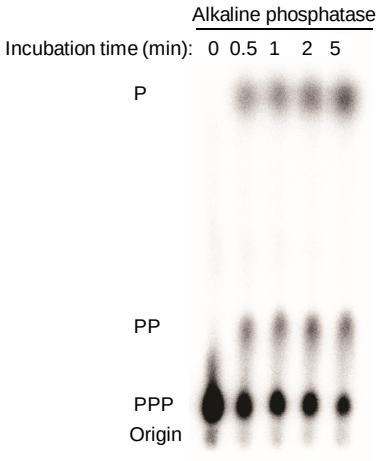
b

```
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
```

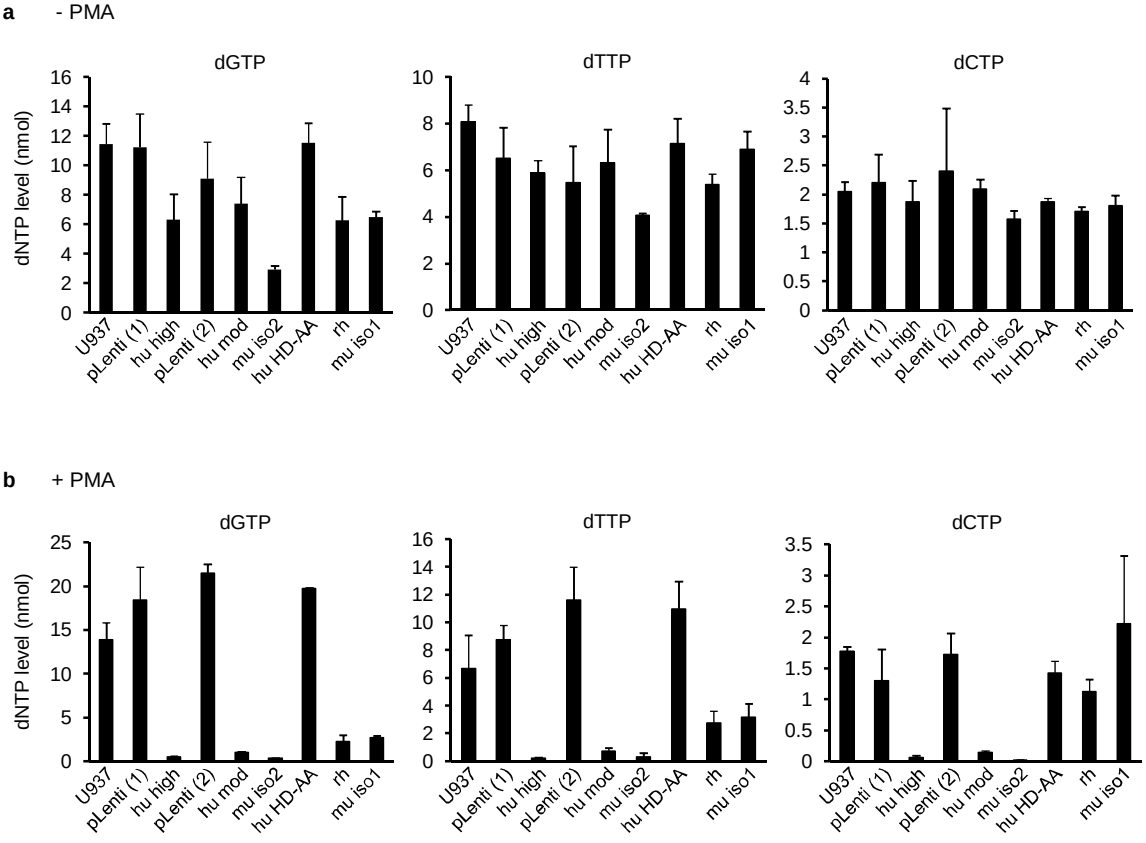
Supplementary Figure 1. SAMHD1 shares sequence similarity with a bacterial nucleotide metabolic enzyme. (**a)** Homology is indicated at the local level (91 residue region encompassing the HD motif) and at the global level. Accession numbers are indicated between brackets (Uniprot database). (**b**) Pairwise alignment of human SAMHD1 against EF1143, a bacterial nucleotide metabolic enzyme from *Enterococcus faecalis***.** The SAMHD1 sequence shares 17.8% identity and 31.6% similarity with 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.



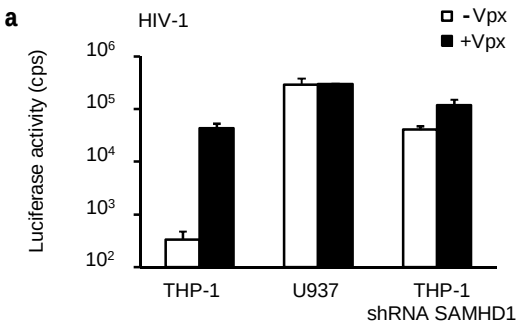
Supplementary Figure 2. Validation of the THP-1 cell system for SAMHD1-mediated restriction. THP-1, engineered to stably express scrambled shRNA (control) or shRNA specifically targeting SAMHD1 were differentiated overnight with PMA. The cells were pre-incubated for 2 h with Vpx-containing or control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



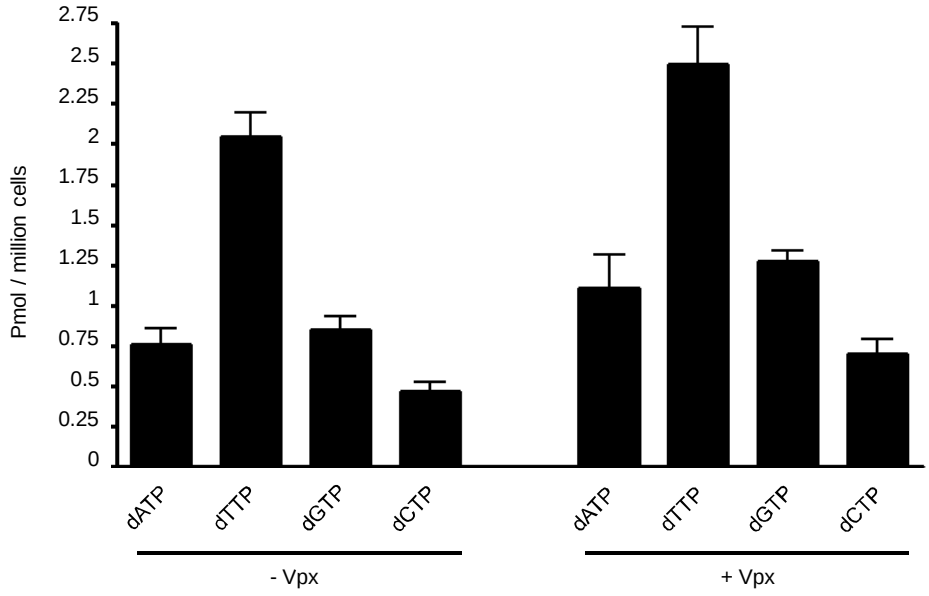
Supplementary Figure 3. SAMHD1 removes three phosphates from dNTP in a single step. The α-^{32}P-labeled reaction products from the recombinant SAMHD1 cleavage of dNTP were incubated with alkaline phosphatase for different times, then separated on thin layer chromatography. The position of mono-, di-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



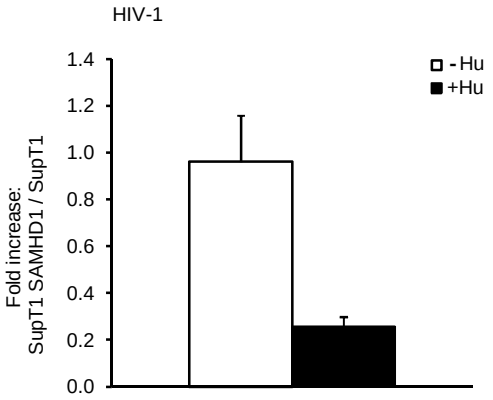
Supplementary Figure 4. SAMHD1 reduces the level of dCTP, dGTP and dTTP in HIV-1 permissive U937 cells. dCTP, dGTP and dTTP levels were determined for the SAMHD1-expressing U937 cells before **(a)** and after **(b)** differentiation with PMA. The data are the average of duplicate measurements ± the standard deviation. The experiment was done three times.



Supplementary Figure 5. U937 and THP-1 SAMHD1 knock-down cells support efficient HIV-1 infection but only THP-1 has a phenotype for Vpx. U937, THP-1 and THP-1 SAMHD1 knock-down cells were differentiated with PMA and then treated with Vpx-containing or control VLP and infected with HIV-1 luciferase reporter virus. The data are the average of triplicate measurements with error bars indicating the standard deviation.



Supplementary Figure 6. 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 5 μg/ml phytohemagglutinin and interleukin-2 (10 ng/ml). After three days, 1 X 10^6 $CD4^+$ cells were incubated for two hours with Vpx-containing and control VLP. On the following day, dNTPs were extracted and their concentration was determined. The data are the average of duplicates ± standard deviation from two donors.



Supplementary Figure 7. SAMHD1 activity is revealed in T cells by treatment with RNR inhibitor. SupT1 cells that stably expressed SAMHD1 were treated with or without 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. The data are the average of duplicates ± standard deviation and the experiment was repeated twice.

	dNTP content in fmol/10^6 cells (fold change compared to VLP -VPX -dN)					
	VLP -VPX -dN	VLP +VPX -dN	VLP -VPX +dN	VLP +VPX +dN	VLP -VPX -dN +HU	VLP +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 ± 1 318 (5X)	1011 ± 154 (17X)	20526 ± 9395 (>100X)	58 ± 8 (1X)	61 ± 2 (1X)

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 quantified. The data shown are the average of duplicate measurements derived from two donors ± the standard deviation.