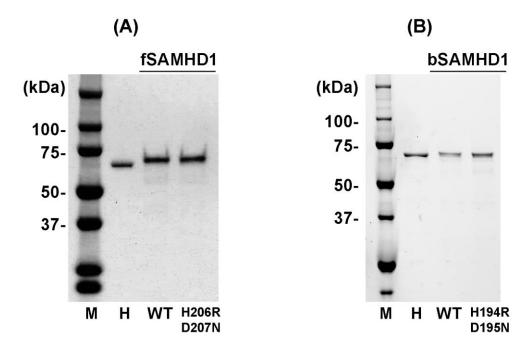
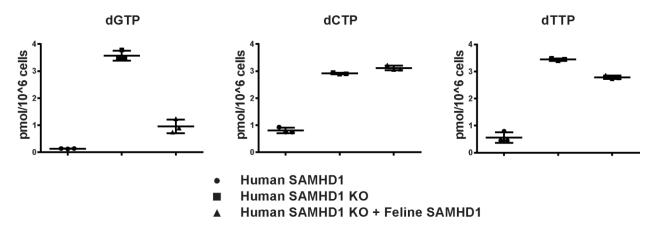
Figure S1



**Figure S1: Purified SAMHD1 proteins in** *E. coli***.** The purified GST tag free full-length fSAMHD1 (**A**) and bSAMHD1 (**B**) proteins were analyzed in SDS-PAGE. M: Protein molecular weight markers, H: purified hSAMHD1 (tag free, full-length), WT: wild type SAMHD1 proteins, and HD: catalytically inactive active site mutant SAMHD1 proteins.

Figure S2



**Figure S2: dNTP level in THP-1 cell expressing fSAMHD1.** The levels of dGTP, dCTP and dTTP in the differentiated parental THP-1 cells with human SAMHD1 (circle), differentiated human SAMHD1 KO THP-1 cells (square), and differentiated human SAMHD1 KO THP-1 cells expressing feline SAMHD1 expression (triangle) were determined by the RT-based dNTP assay in triplicates as described for dATP measurement (Figure 2B). The error bars; SDs.

Human	SAMHD1	111	HVDTMKVINDPIHGHIELHPLLVRIIDTPQ	140
Feline	SAMHD1	111	HVDAM <mark>KV</mark> INDPIHGHIELHPLLIRII <mark>D</mark> TPQ	140
Equine	SAMHD1	111	DLDTM <mark>KV</mark> INDPIHGHIELHPLLIRIIDTPQ	140
Bovine	SAMHD1	101	DTM <mark>KV</mark> INDPIHGHIEFHPLLMRII <mark>D</mark> TPQ	128
Human	SAMHD1	141	FQRLRYIKQLGGGY <mark>YVF</mark> PGASHNRFE <mark>H</mark> SLG	170
Feline	SAMHD1	141	F <mark>Q</mark> RL <mark>R</mark> YIKQLGGSY <mark>YIF</mark> PGASHNRFE <mark>H</mark> SLG	170
Equine	SAMHD1	141	F <mark>Q</mark> RL <mark>R</mark> YIKQLGGGY <mark>YVF</mark> PGASHNRFE <mark>H</mark> SLG	170
Bovine	SAMHD1	129	F <mark>Q</mark> RL <mark>R</mark> YIKQLGGGY <mark>YVF</mark> PGASHNRFE <mark>H</mark> SLG	158
			_	
Human	SAMHD1	196	VLCVQIAGLCHDLGHGPFSHMFDGRFIPLA	225
Feline	SAMHD1	196	VLCVQIAGLC <mark>HD</mark> LG <mark>H</mark> GPFS <mark>H</mark> MF <mark>D</mark> GRFIPLA	225
Equine	SAMHD1	196	MLCVQIAGLC <mark>HD</mark> LG <mark>H</mark> GPFS <mark>H</mark> MF <mark>D</mark> GRFIPLA	225
Bovine	SAMHD1	184	ILCVQIAGLC <mark>HD</mark> LG <mark>H</mark> GPFS <mark>H</mark> MF <mark>D</mark> GRFIPLA	213
Human	SAMHD1	351	ARDKEVGNLYDMFHTRNSLHRRAYQHKVGN	380
Feline	SAMHD1	351	TRDKEVGNLYDMFHT <mark>R</mark> TSL <mark>H</mark> RRA <mark>Y</mark> QHKVGN	380
Equine	SAMHD1	283	FSEVGNLYDMFHTRNCLHRRAYQHKVGN	310
Bovine	SAMHD1	340	TREKEVGNLYDMFHT <mark>R</mark> NCL <mark>H</mark> RRA <mark>Y</mark> QHKVGN	369
Human	SAMHD1	572	WCADRNFTKPQDGDVIAPLITPQKKEWNDS	601
Feline	SAMHD1	573	WCLIRNFTKPQDSDVVAPLITPRKEEWKNP	602
Equine	SAMHD1	503	WCLVRNFTKPQDGDVVAPLITPLKKEWNCT	532
Bovine	SAMHD1	561	WCLINDFTKPQIKKLPLRKLKKELTTA	587
			Allosteric Site 1 Allosteric Site 2	
			Catalytic Site Phophorylation site	
			- Table one	

**Figure S3: Sequence comparison of human, feline, bovine and equine SAMHD1 proteins.** The amino acid sequences of the HD active site (red), A1 (blue) and A2 (green) allosteric sites, and C-terminal phosphorylation sites (dark grey) of the four SAMHD1 proteins were compared. The sequence sources are: NCBI reference sequences: NM\_015474.3 (hSAMHD1), XM\_003983547.2 (fSAMHD1), NM\_001075861.1 (bSAMHD1), and XM\_008541885.1 (eSAMHD1).

## SIV/hSAMHD1/293T cells pHuman SAMHD1 : + + + pSIV WT : - + + MG132 : - - + Human SAMHD1 GAPDH

Figure S4: Reversal of the Vpx induced degradation of hSAMHD1 by MG132. Human 293T cells were co-transfected with pLVX-IRES-mCherry co-expressing HA-tagged hSAMHD1 and mCherry protein ("pHuman SAMHD1",  $0.1\mu g$ ) and a plasmid expressing SIVmac251 proteins with ("pSIV WT",  $2\mu g$ ). In order to verify functional Vpx expression, MG132 was added ( $1\mu M$ , final) 2hrs prior to the transfection and kept at the same concentration throughout the experiment. Cells were harvested 48 hours post transfection, and the hSAMHD1 levels were determined by western blots with anti-hSAMHD1 antibody. GAPDH was used as a loading control. Transfection efficiency for the cells expressing hSAMHD1 were monitored by the mCherry protein expression.