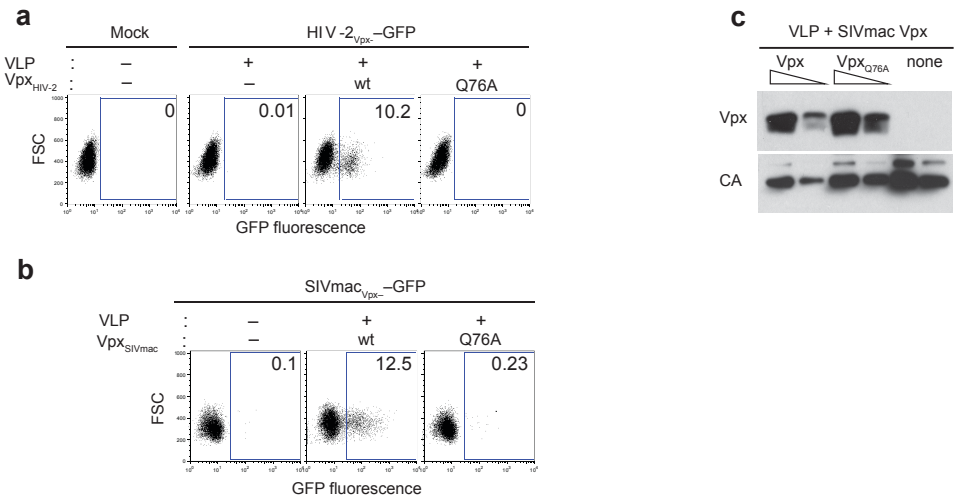
Tagged Subunit(s): / NCBI gene:	HA-Vpx; ↓ FLAG-Cul4	HA-DCAF1 ↓ FLAG-Cul4	HA-FLAG-Vpx	Mock
Vpx (SIVmac)	**100**[(a)]; 0.165369[(b)]	–	**33**; 0.633056	–
DCAF1	**166**; 0.024591	**500**; 0.074839	**36**; 0.006131	–
DDB1	**179**; 0.035054	**403**; 0.079739	**12**; 0.002702	–
DDA1	**12**; 0.026265	**26**; 0.057497	**4**; 0.010064	–
Cul4A	**313**; 0.092064	**405**; 0.120361	–	–
SAMHD1	**37**; 0.013195	_	**6**; 0.002460	–
TUBB2C	**13**; 0.006489	_	**17**; 0.009915	–
EEF1A1	**4**; 0.001933	_	**7**; 0.003889	**1**; 0.00004
PRKDC	**16**; 0.000872	_	**2**; 0.000125	–
SLC25A13	**6**; 0.001929	_	**7**; 0.002661	–
RPN1	**3**; 0.001103	_	**4**; 0.001691	–
AIFM1	**3**; 0.001100	_	**2**; 0.000843	–
KPNB1	**3**; 0.000765	_	**2**; 0.000586	–
TCP1	**3**; 0.001670	_	**1**; 0.000640	–
MIOS	**2**; 0.000510	_	**4**; 0.001173	–
ATP2A2	**2**; 0.000429	_	**2**; 0.000493	–
ATP5C1	**2**; 0.001503	_	**1**; 0.000864	–
CAD	**2**; 0.000201	_	**1**; 0.000115	–
SLC25A12	**1**; 0.000384	_	**0**;	–
ATP5A1	**1**; 0.000404	_	**3**; 0.001392	–
ATP1A1	**1**; 0.000218	_	**3**; 0.000753	–
WDR59	**1**; 0.000229	_	**1**; 0.000264	–

Supplementary Table I. MudPIT identification of cellular proteins that specifically co-purify with Cul4^{DCAF1} E3 complex in the presence of Vpx.

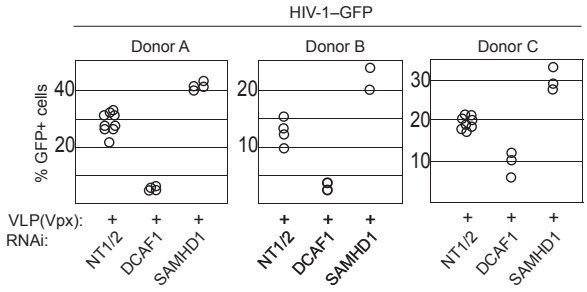
Protein complexes were purified by two sequential immunoprecipitation via HA-tag and then via FLAG-tag, each followed by competitive elution with peptide epitope and analyzed by MudPIT. Upper rows: [(a)]spectral count and [(b)d]NSAF values for subunits of CRL4^{DCAF1} and Vpx are shown; Lower rows: proteins that co-purified with HA-FLAG-Vpx and with Vpx-CRL^{DCAF1} complex, but not with CRL^{DCAF1} complex in the absence of Vpx, are shown.

[(a)]spectral count, number of mass spectrometry spectra matching peptides from the indicated protein

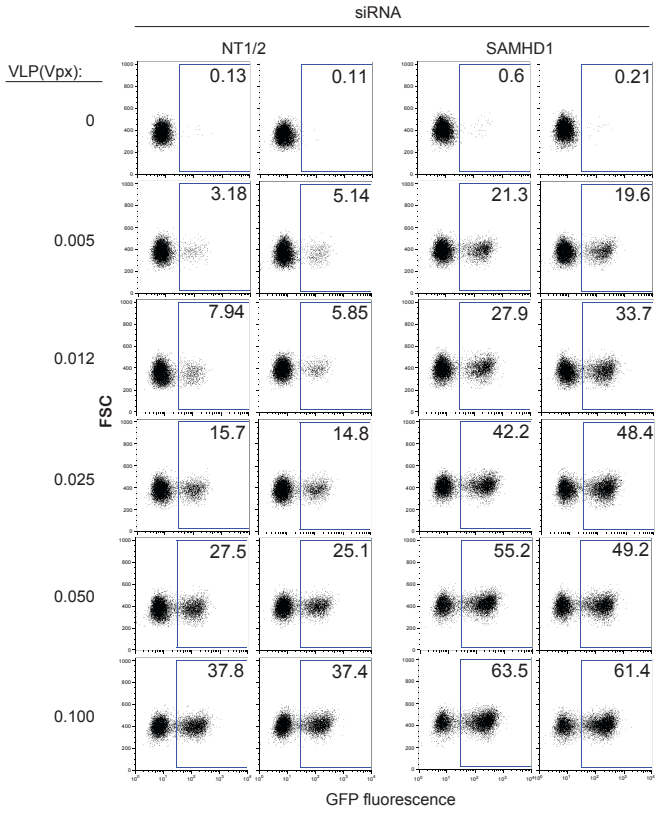
[(b)d]NSAF, distributed normalized spectral abundance factor



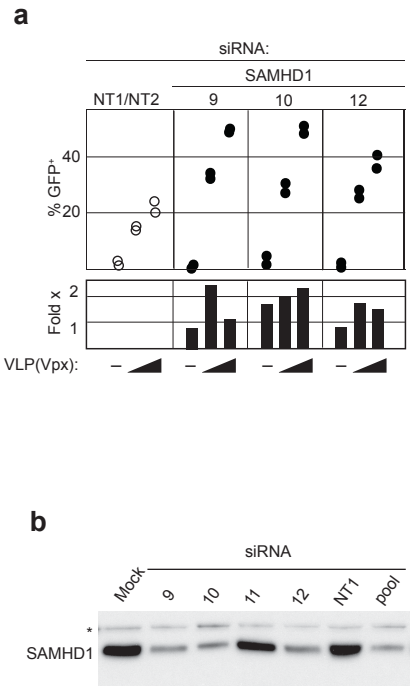
Supplementary Figure 1. Vpx-mediated relief of the inhibition of HIV-2 & SIVmac infection in MDM requires Vpx glutamine Q76. MDM were infected with *vpx*-defective HIV-2 (HIV-$2_{vpx–}$–GFP) **(a)**, or SIVmac 239 ($SIVmac_{vpx–}$–GFP) **(b)**, single cycle reporter viruses alone, or in combination with SIV VLP loaded with wild type (wt) or Q76A -substituted (Q76A) HIV-2 Rod (a), or SIVmac 239 (b) Vpx proteins. **(c)** All SIV VLP were normalized for their p24 CA and Vpx contents, by Western blotting.



Supplementary Figure 2. HIV-1–GFP transduction efficiency of MDM following RNAi to SAMHD1 or DCAF1. MDM subjected to the siRNA targeting DCAF1, SAMHD1, or non-targeting siRNA (NT1/2), in a protocol described in Figure 3c, were infected with SIV VLP loaded with SIVmac Vpx. Two days later cells were challenged with HIV-1–GFP reporter virus. GFP-positive cells were quantified 3 days later by flow cytometry. Results of three typical experiments with MDM from three donors are shown.



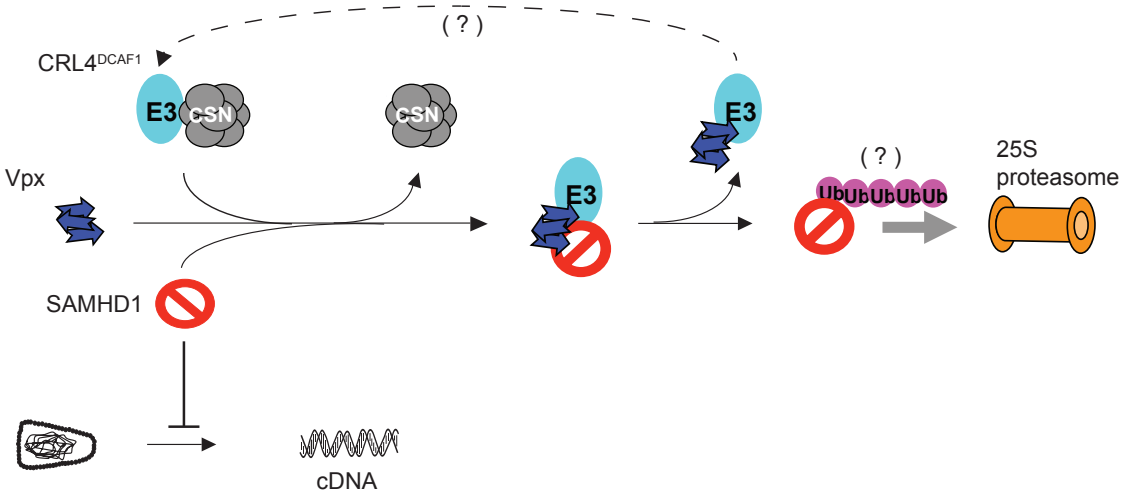
Supplementary Figure 3. Flow cytometry analysis of the effects of SAMHD1 depletion on HIV-1–GFP transduction of MDM. Primary data for the experiment with MDM from Donor 6, in Figure 4a, are shown.



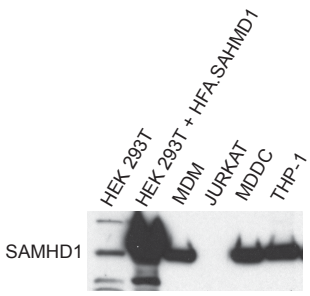
Supplementary Figure 4. Increased permissiveness to HIV-1–GFP infection of SAMHD1-depleted MDM is not due to off target effects of siRNA pool targeting SAMHD1.

a. To exclude the possibility that the observed enhancement of macrophage transduction following RNAi to SAMHD1 is caused by off target effects of the siRNA pool targeting SAMHD1, individual siRNAs from the ON-TARGETplus SMARTpool SAMHD1 (J-013950-09, J-013950-10 and J-013950-12) were tested using the assay described in Figure 4. The enhanced MDM transduction by HIV-1–GFP was observed with all three out of three siRNAs to SAMHD1 that we tested (panel a). Thus, it is unlikely that the enhanced MDM transduction by HIV-1–GFP following RNAi to SAMHD1 is due to an off target effect of the siRNA pool targeting SAMHD1.

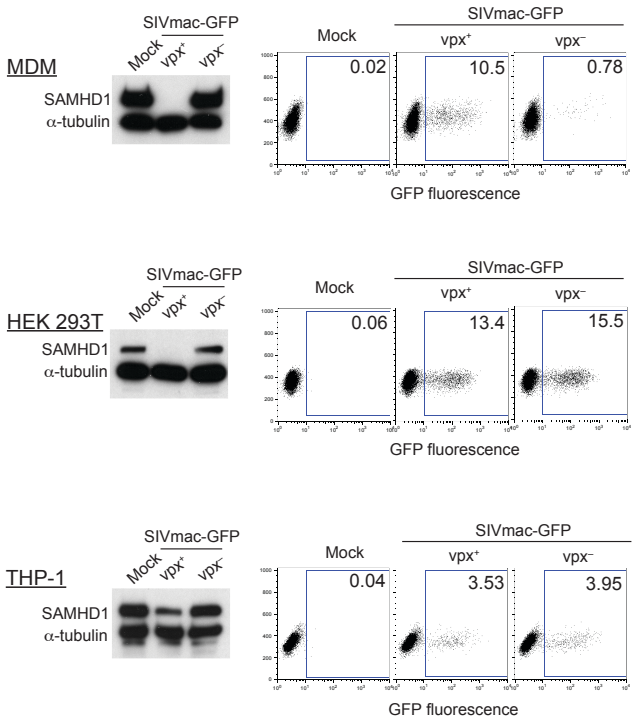
b. SAMHD1 protein levels in cells transfected with individual siRNA duplexes targeting SAMHD1. U2OS cells stably expressing human FLAG- tagged SAMHD1 were transfected with the indicated siRNAs targeting SAMHD1 (J-013950-09 (9), J-013950-10 (10), J-013950-11 (11) and J-013950-12 (12)), the SMARTpool SAMHD1 (pool), or non-targeting siRNA pool (NT1), as described previously[12]. Detergent extracts were prepared from the cells 3 days after initiation of RNAi and SAMHD1 was visualized by immunoblotting for the FLAG epitope. Mock – not transfected U2OS cells.



Supplementary Figure 5. Model for Vpx-mediated relief of the inhibition of HIV-1 infection in myeloid cells by SAMHD1. SAMHD1 interferes with HIV-1 infection at an early post-entry step preventing efficient viral cDNA synthesis, probably by interfering with the reverse transcription process itself. Vpx relieves SAMHD1-imposed inhibition by binding to SAMHD1 and loading the protein onto $CRL4^{DCAF1}$ E3 ubiquitin ligase complex, *via* its DCAF1 substrate receptor subunit. Vpx targets specifically DCAF1-DDB1 complex that is not associated with the inhibitory COP9 Signalosome (CSN), or displaces CSN upon binding to DCAF1[20]. $CRL4^{DCAF1}$ E3 poly-ubiquitylates SAMHD1 and directs it for degradation by the proteasome.



Supplementary Figure 6. SAMHD1 protein levels in myeloid cells and established cell lines. Detergent extracts prepared from monocyte-derived macrophages and dendritic cells (MDM & MDDC), as well as HEK 293T cells, Jurkat T cells, and monocytic THP-1 cells were normalized for their protein concentrations, resolved by SDS-PAGE and immunoblotted for endogenous SAMHD1. Extract from HEK 293T cells transiently over-expressing FLAG- epitope tagged SAMHD1 (HEK 293T + HFA.SAMHD1) provided positive control.



Supplementary Figure 7. SAMHD1 does not restrict SIVmac–GFP infection in HEK 293T and THP-1 cells. Monocyte-derived macrophages (MDM), monocytic THP-1 cells and epithelial HEK 293T cells were infected with SIVmac-GFP reporter viruses containing wild type (vpx^+) or inactivated (vpx^-) *vpx* open reading frames. Three days after infection the cells were harvested for flow cytometry quantification of GFP-positive productively infected cells, and for immunoblot analysis of SAMHD1 levels in the infected cells. Mock – non-infected control cells. It is evident that SIVmac-GFP transduction rates are not affected by endogenous SAMHD1 levels in HEK 293T and THP-1 cells. Differences between transduction efficiencies of HEK 293T and THP-1 cells reflect differences in infection multiplicities.