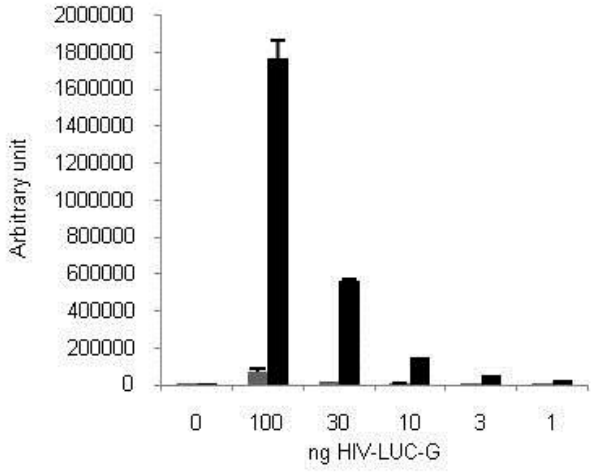
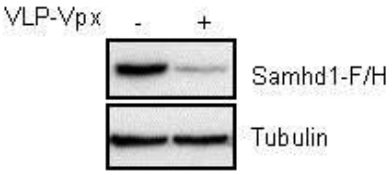
doi:10.1038/nature10117

Supplementary Figure 1 : Characterization of THP-1-Vpx cell line



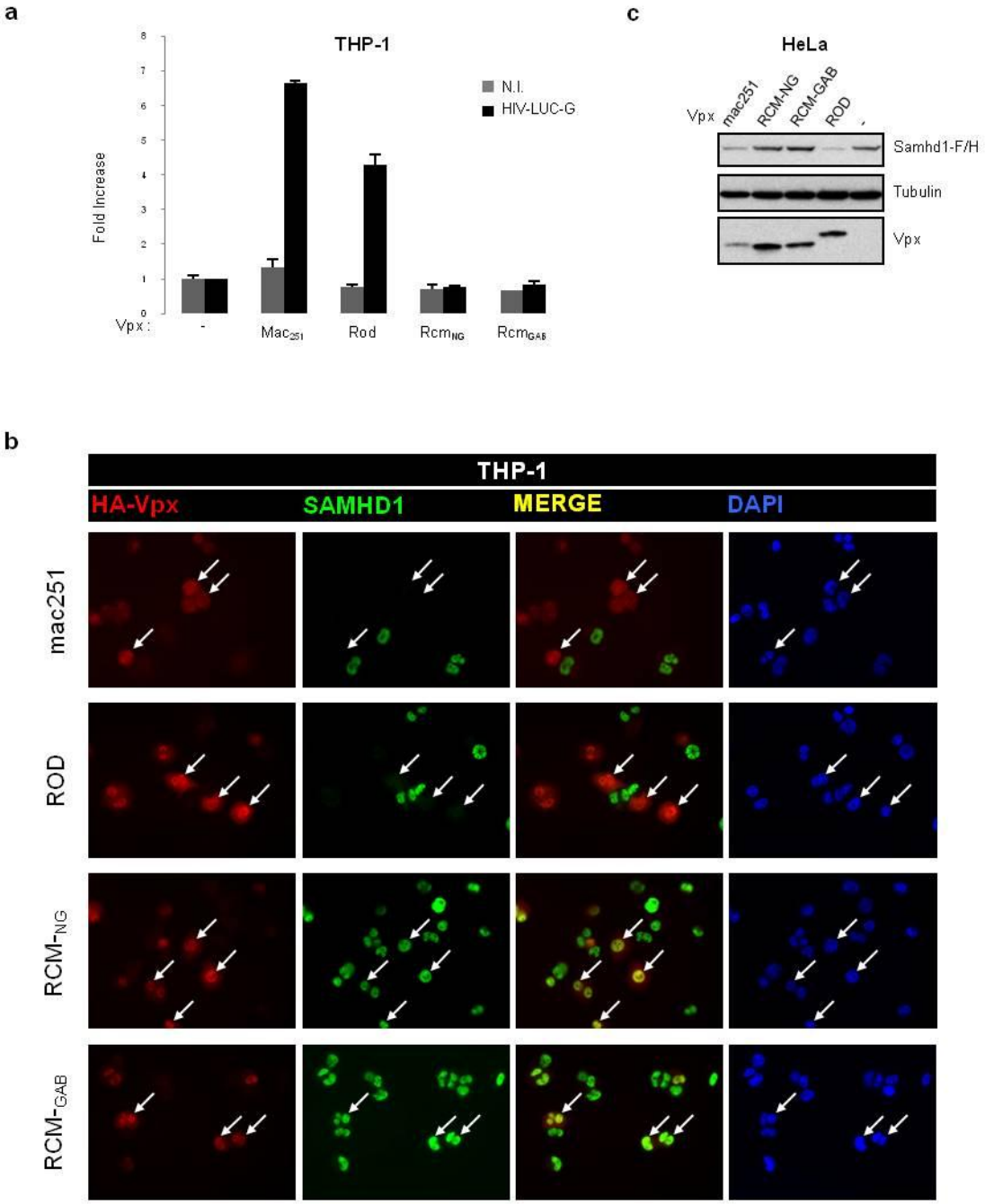
THP-1 and THP-1-Vpx cells were PMA-differentiated for 16 hrs and infected with serial dilutions of HIV-LUC-G. Luciferase activity was measured 24 hrs post infection, normalized for protein concentration of analyzed samples. Results are presented as arbitrary units. Graphs show mean ± Standard Deviation from a representative experiment, n=6)

Supplementary Figure 2: VLP-Vpx-treatment of HeLa cells decreases Samhd1 protein levels.

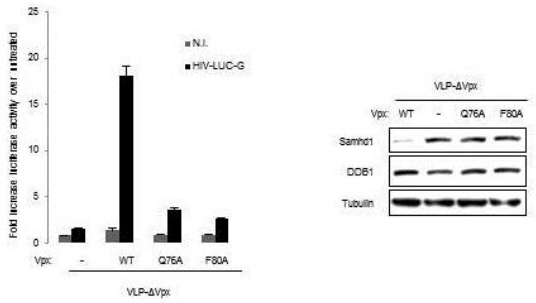


HeLa-Samhd1 cells were treated with VLP-Vpx for 2hrs prior to whole cell extraction and analysis by WB using antibodies allowing for detection of Samhd1-F/H and tubulin.

Supplementary Figure 3: Vpx variants ability to degrade Samhd1 correlates with their ability to facilitate infection of differentiated THP-1 and can be reconstituted in HeLa cells.



d

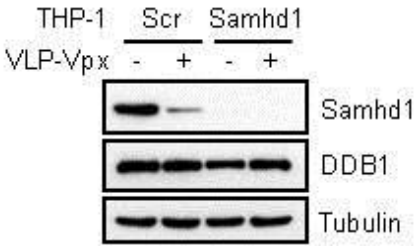


a, THP-1 cells were transduced with Vpx variants for 24 hrs, differentiated for a further 16 hrs prior to infection with HIV-LUC-G. Luciferase activity was measured and expressed as in Fig.S. Graphs show mean ± Standard Deviation from a representative experiment, n=4). **b,** Immunofluorescence against Flag- and HA-tagged Vpx variants and Samhd1 in THP-1 cells. Images are representative of 150 HA-positive analyzed cells. Vpx_{mac251}- and Vpx_{ROD}-expressing cells show dramatically reduced Samhd1 staining. $Vpx_{RCM\text{-}NG}$- and $Vpx_{RCM\text{-}GAB}$-expressing cells show no evident alteration of Samhd1 staining. **c**, HeLa cells were engineered to stably express Flag- and HA-tagged Samhd1 (HeLa- Samhd1). HeLa- Samhd1 were transduced with a retroviral vector expressing Vpx variants for 24 hrs. Whole cell extracts were prepared and analysis by WB for the expression of Samhd1-F/H, DDB1 and Tubulin using specific antibodies. **d**, Vpx-Q76A and Vpx-F80A fail to increase HIV infection and to decrease Samhd1 expression. Differentiated THP1 cells were treated with VLP-ΔVpx (-) or VLP-ΔVpx containing WT Vpx (WT) or VpxQ76A (Q76A) or VpxF80A (F80A) for 2 hrs prior to infection with 100 ng HIV-LUC-G. Luciferase activity was measured 24 hrs later, normalized for protein concentration. Results are expressed as fold increase luciferase activity over infected untreated cells (left panel) Graphs show mean ± Standard Deviation from a representative experiment, n=3). Cells from **d** were lysed and analyzed by WB using the indicated antibodies (right panel).

Supplementary Figure 4: WB analysis of Samhd1 levels in THP-1 stably expressing shRNA 1 to 5 targeting Samhd1 or srambled shRNA

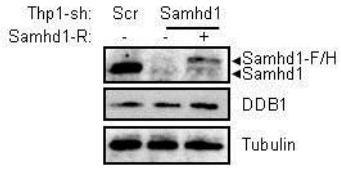
THP-1 cells were transduced with lentiviruses encoding scrambled shRNA or Samhd1 specific shRNAs. Stable cell lines were selected, whole cell extracts were prepared and analyzed by WB with the indicated antibodies.

Supplementary Figure 5: WB analysis of THP-1-shSamhd1 or THP-1shScr treated or not with VLP-Vpx



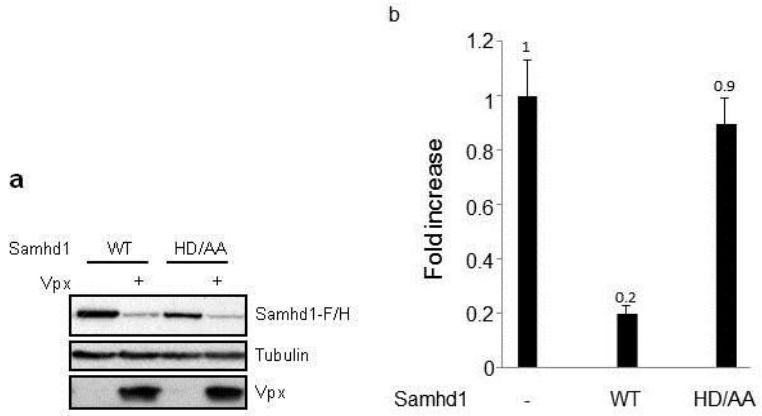
Cells from Fig 2 **b** were analyzed by WB with the indicated antibodies.

Supplementary Figure 6: Rescue of THP-1-shSamhd1 with ShRNA-resistant Samhd1 mutant (Samhd1-R).



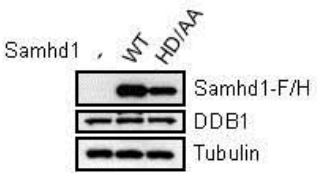
Cells from Fig 2 **d**, were analyzed by WB using Samhd1, DDB1 and Tubulin specific antibodies.

Supplementary Figure 7: Samhd1-mediated HIV-1 restriction can be reconstituted in permissive HeLa cells.



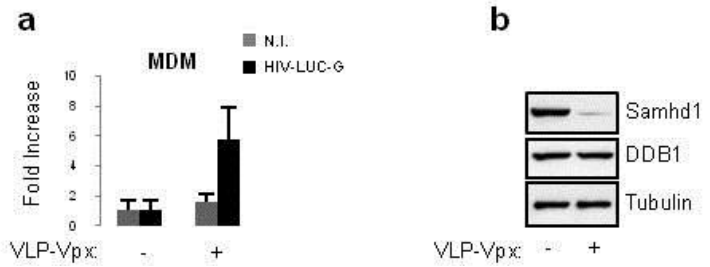
a, HeLa cells were transduced with Samhd1-F/H or Samdh1-HD/AA 24 hrs prior to transduction with Vpx_{mac251}. Cells were harvested 24 hrs later and whole cell extracts were analyzed by WB with antibodies allowing for detection of Samhd1-F/H, Tubulin and Vpx. **b**, HeLa cells were transduced with Samhd1-F/H or Samhd1-HD/AA 24 hrs prior to infection with HIV-LUC-G. Luciferase activity was measured. Results are expressed as fold increase luciferase activity in transduced over parental HeLa cells. Graphs show mean ± Standard Deviation from a representative experiment, n=4)

Supplementary Figure 8: Expression of WT-Samhd1 and Samhd1-HD/AA in U937.



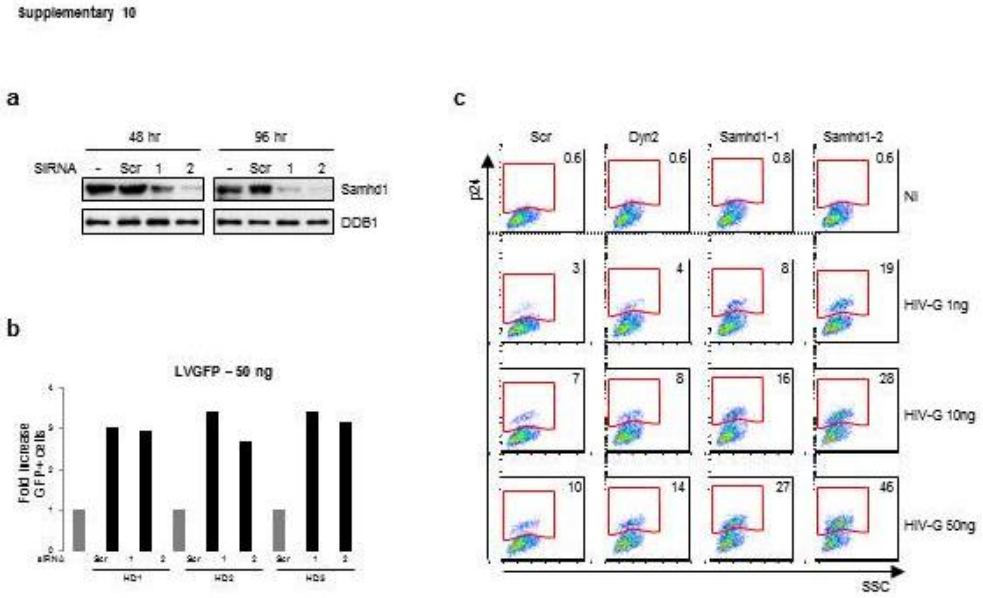
Cells from Fig 2 **e** were analyzed by WB with the indicated antibodies.

Supplementary Figure 9: VLP-Vpx treatment of monocyte-derived macrophages (MDM) causes increased HIV-1 infection and a decrease of Samhd1 levels



a, MDMs were VLP-treated for 2 hrs prior to infection with HIV-LUC-G. Luciferase was measured 48 hrs post infection. Results are expressed as fold increase luciferase activity in VLP-Vpx treated over untreated cells. Graphs show mean ± Standard Deviation from a representative experiment, n=3). **b**, Cells from **a**, were analyzed by WB with indicated antibodies.

Supplementary Figure 10: Samhd1 depletion in MDDCs enhanced their susceptibility to HIV-1.



a, MDDCs treated as in Fig. 3D were analyzed by WB with indicated antibodies.

b, HD1, HD2 and HD3 were treated and analyzed as in Fig. 2 **e**, except that infection was performed with 50 ng LV-GFP. Results are expressed as fold increase of GFP positive cells.

c, HIV-G-infected MDDCs from HD 5 were stained for p24 expression prior to flow cytometry analysis. All infections were performed at 48 hrs post siRNA treatment