SAMHD1 restricts HIV-1 replication in quiescent CD4\(^+\) T-cells.

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Supplementary figures and text.
Supplementary Figure 1. Loss of SAMHD1 expression in monocytes after Vpx treatment. Kinetic of SAMHD1 loss in CD14 monocytes after VLP-Vpx treatment was assessed by Facs using anti-SAMHD1 antibody (Representative experiment, n=2).
Supplementary Figure 2. Quiescent CD4+ T-cells and CD14+ monocytes display increased susceptibility to HIV-1 infection after Vpx treatment. a. Dot plots illustrating the susceptibility of quiescent CD4+ T-cells after VLP-Mock or VLP-Vpx treatment followed by HIV-CMV-EGFP infection as represented in Figure 2C, in healthy donors 1 to 4 and 6 (healthy donor #5 is shown in Figure 2a). b. Left panel: As in a., dot plots illustrating the susceptibility of CD14+ monocytes VLP-Mock or VLP-Vpx treatment followed by HIV-CMV-
EGFP infection in healthy donors 1 to 6. Right panel: Results are expressed as percentage of GFP positive cells. Graphic shows mean and standard deviation for these 6 healthy donors. c. Proportions of resting (CD69- HLA-DR-) CD4 T cells were assessed in 6 donors in uninfected, VLP-Mock and VLP-Vpx treated cells. Results are expressed as percentage of total CD4 T cells. d. Impact of Vpx treatment on TCR stimulated CD4 T cell susceptibility to HIV-CMV-EGFP infection. Left panel: after staining with the eFluor670 proliferation dye, PBMCs were stimulated for 3 days with anti-CD3 and anti-CD28 antibodies and IL-2, treated with VLP-Mock or VLP-Vpx for 12 hours and infected by HIV-CMV-EGFP for 2 days before FACS analysis (Representative dot plots from one experiment out of three is shown). Right panel: Results are expressed as percentage of GFP positive cells. Graphic shows mean and standard deviation for 3 healthy donors.
Supplementary Figure 3. Effect of Vpx on the permissiveness of quiescent CD4+ T-cells to HIV-EGFP infection. a. Left panel: Dot plots illustrating the susceptibility of quiescent CD4+ T-cells and CD14+ monocytes after VLP-Mock or VLP-Vpx treatment followed by HIV-EGFP infection as represented in Figure 2a, in healthy donors #5 and #6 (healthy donor #7 is shown in Figure 2e). Right panel: results are expressed as percentage of GFP positive cells. Graphic shows mean and standard deviation for 3 healthy donors. b. Flow cytometry histograms showing HIV-EGFP infection susceptibility of non-stimulated CD4 T cells treated with VLP-Mock or VLP-Vpx and of CD4 T cells stimulated through the TCR as describe in supplementary figure 2d.
Supplementary Figure 4. Enhanced permissiveness of purified CD4+ T-cells by VLP-Vpx correlates with loss of SAMHD1 expression. a. Experimental outline. CD4+ T cells were purified from PBMCs and activated with phytohemagglutinin (PHA) and IL-2. Cells were maintained in IL-2 for 14 to 20 days, until disappearance of the activation markers CD69 and Ki67. The resulting “resting post-activated cells” were then treated for 3h with VLP-Mock or VLP-Vpx.
VLP-Vpx and infected with HIV-CMV-EGFP lentiviral vector. Cells were then analyzed for GFP and SAMHD1 expression by flow cytometry at 96h post-infection. 

b. Left panel: Analysis of expression of CD69 and Ki67 activation markers by flow cytometry in resting post-activated cells compared to activated cells (at day 3 post PHA stimulation, right panel).

c. Vpx enhances lentiviral vector infection of post-activated resting CD4+ T cells. One representative donor out of seven is shown (right panel). The graph (right panel) represents mean ± SEM of the fold increase in GFP expression in SAMHD1 positive and negative populations following incubation with VLP-Vpx.