

Type I interferons sensitise HIV-1-reactivating T-cells for NK cell-mediated elimination despite HDACi-imposed dysregulation of innate immunity

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SUPPLEMENTARY MATERIALS

Supplementary Table 1.

Supplementary Figure 1-11

Supplementary Movie 1.

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Donor ID	Seroconversion	Treated since	Viral Load (viral copies/ml)	CD4 counts (cells/ μ l)
1	2006	2008	< 50	1048
2	2008	2011	< 50	526
3	2017	2018	< 50	629

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24 **Supplementary Table 1. Clinical data of the three PLHIV.**

25 Shown are the dates of infection and ART initiation as well as viral loads and counts of CD4⁺
 26 T-cells in the peripheral blood. The detection limit for viral copies/ml is 50; samples with
 27 undetectable levels are indicated with "< 50".

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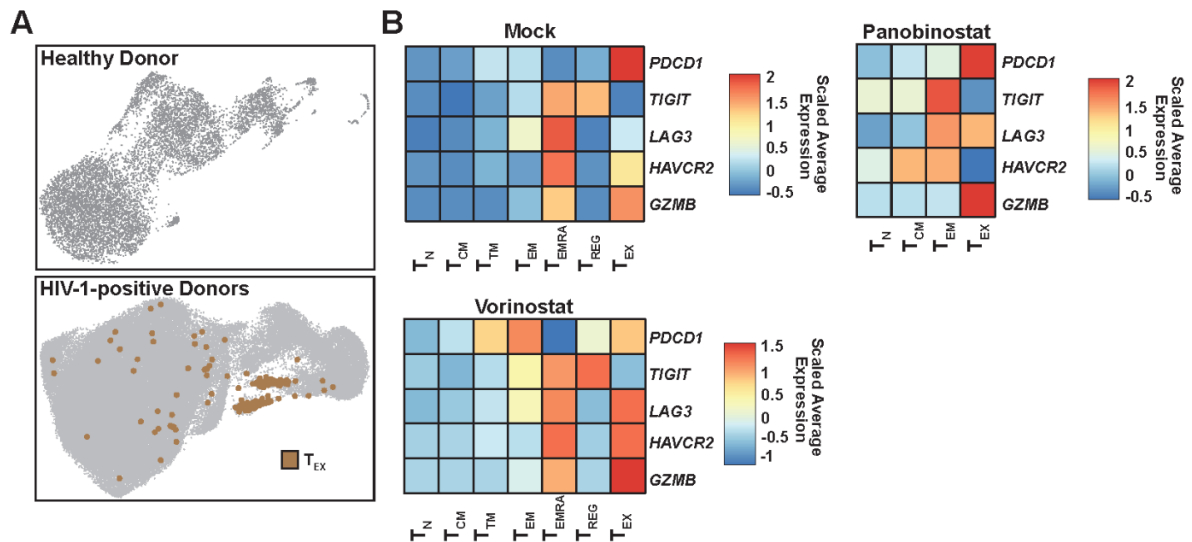
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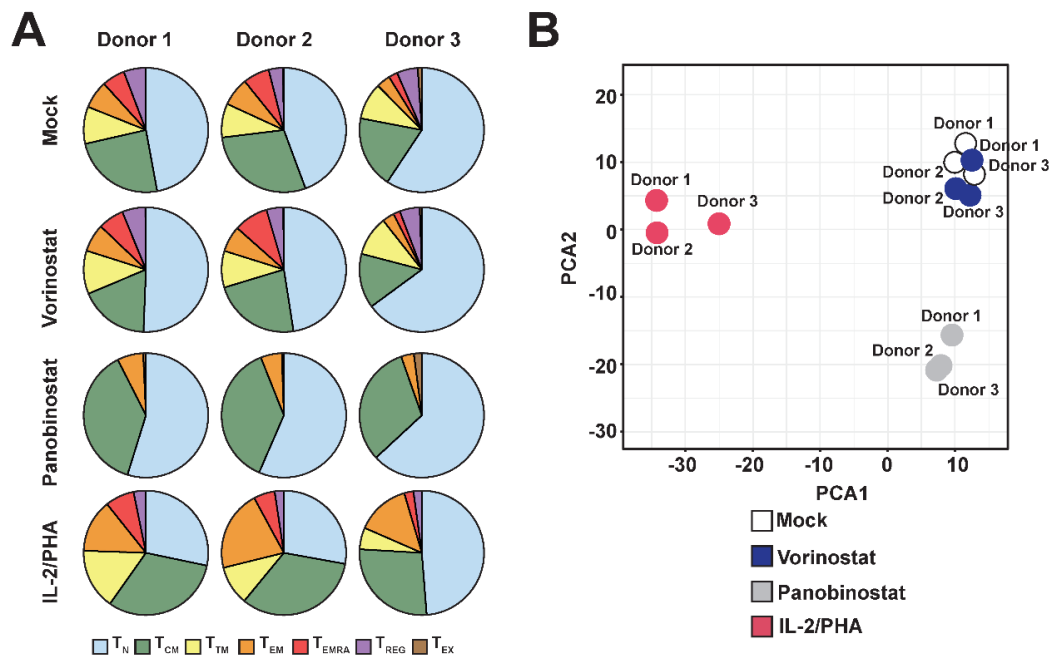
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Prigann *et al.*, Supplementary Figure 1

Supplementary Figure 1. A subset of CD4⁺ T-cells, identified specifically in cells isolated from PLHIV, resembles T_{EMRA} cells, with additional expression of the exhaustion marker *PDCD1*

(A) UMAP plots of CD4⁺ T-cells from an HIV-1-negative donor (n=1) and from aviremic PLHIV (n=3). Exhausted T-cells are coloured in brown.

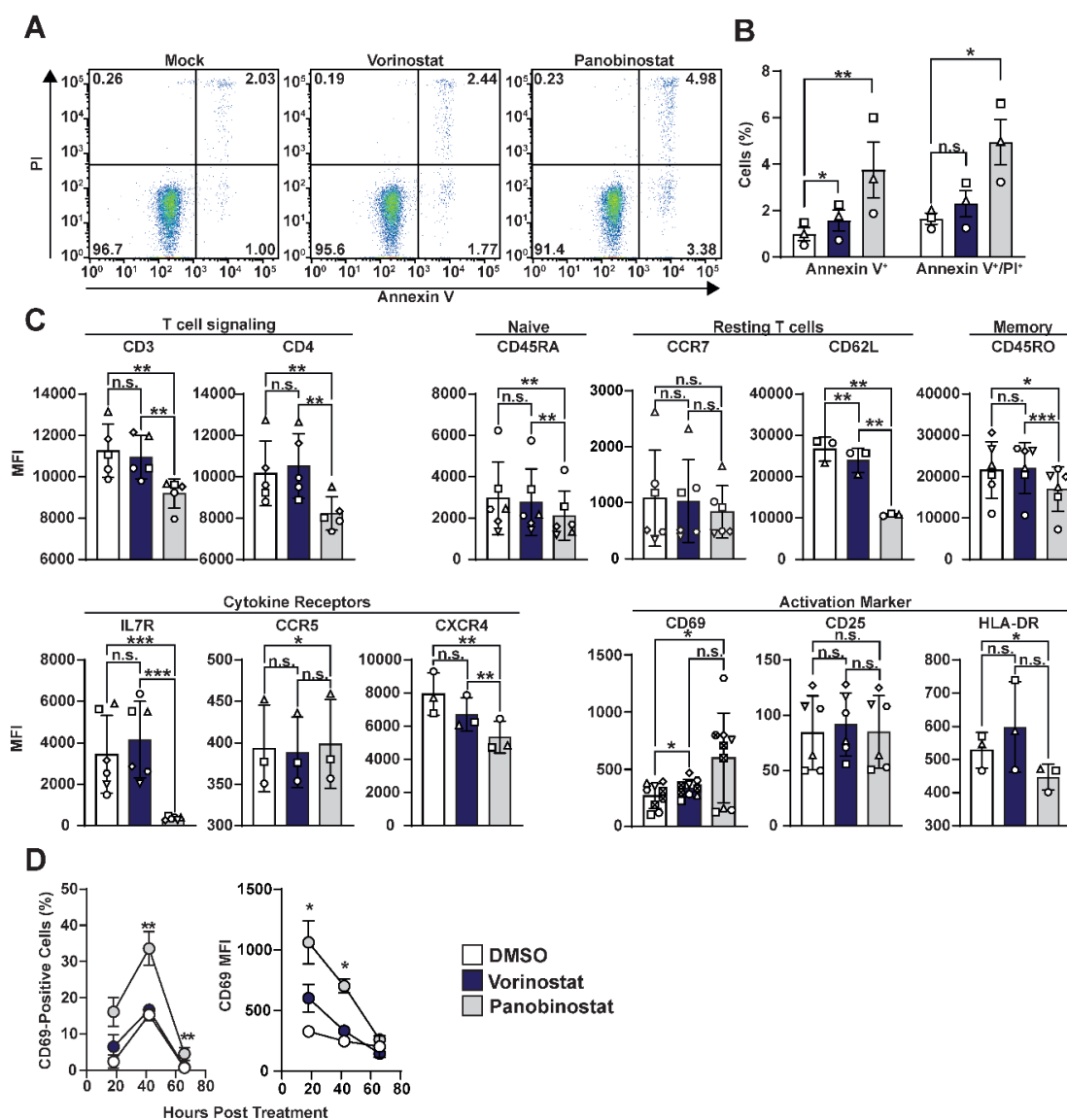
(B) Heatmaps showing the scaled average expression of T-cell exhaustion markers, *PDCD1*, *TIGIT*, *LAG3*, *HAVCR2*, *GZMB* in the different CD4⁺ T-cell subsets of PLHIV (n=3) upon mock-treatment or treatment with Vorinostat or Panobinostat.

Prigann *et al.*, Supplementary Figure 2

Supplementary Figure 2. CD4⁺ T-cell subset distribution and the transcriptomic landscape is determined by treatment condition in a donor-independent manner

(A) Pie charts displaying the CD4⁺ T-cell subset distribution of samples obtained from the individual PLHIV after indicated treatment.

(B) PCA based on the global gene expression profile of the individual samples after indicated treatment.

Prigann *et al.*, Supplementary Figure 3

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58 **Supplementary Figure 3. Panobinostat treatment modulates expression of CD4⁺ T-cell**
 59 **markers**

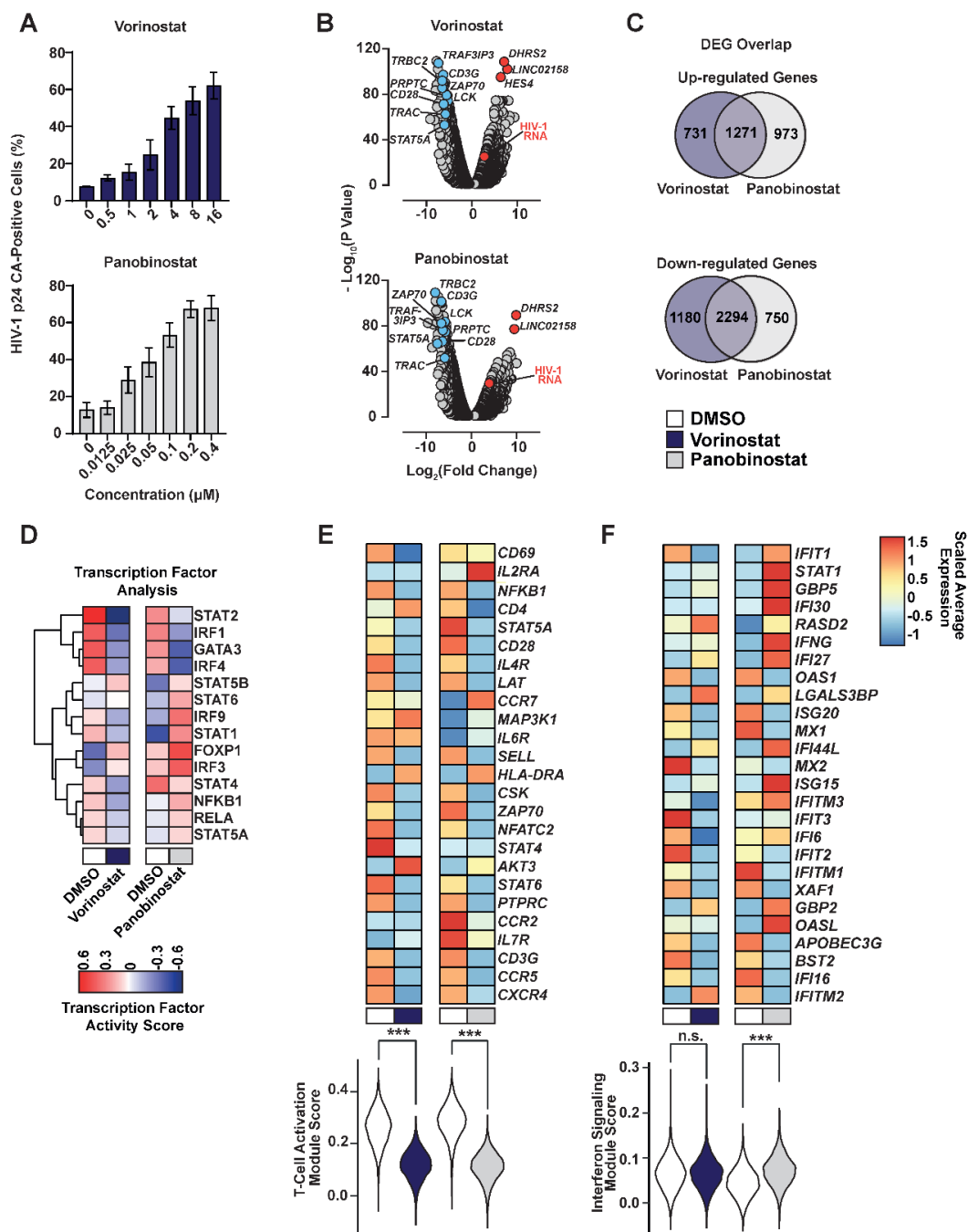
60 CD4⁺ T-cells from HIV-1-negative donors were treated with 0.5% DMSO (mock), Vorinostat
 61 (500 nM) or Panobinostat (50 nM).

62 **(A)** Representative Annexin V/PI cell apoptosis staining upon indicated treatment for 48 hours.

63 **(B)** Percentage of Annexin V⁺- and Annexin V⁺/PI⁺-positive CD4⁺ T-cells from three individual
 64 donors upon indicated treatment for 48 hours.

(C) Mean fluorescence intensity (MFI) of cell surface expression of indicated proteins upon indicated treatment for 48 hours.

(D) Percentage of CD69⁺ T-cells and MFI of CD69 expression upon indicated treatment of cells from three donors.

Prigann *et al.*, Supplementary Figure 4

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83 Supplementary Figure 4. HDACi-induced transcriptome in the J1.1 T-cell model of HIV- 84 1 latency

85 (A) J1.1 T-cells were treated with the indicated concentration of Vorinostat or Panobinostat for
86 40 hours before quantification of intracellular HIV-1 p24 CA expression by flow cytometry.

(B) - (F) J1.1 T-cells were treated with 16 μ M Vorinostat, 1.6% DMSO (DMSO I), 200 nM Panobinostat or 0.2% DMSO (DMSO II) for 40 hours prior to single cell RNA-sequencing analysis.

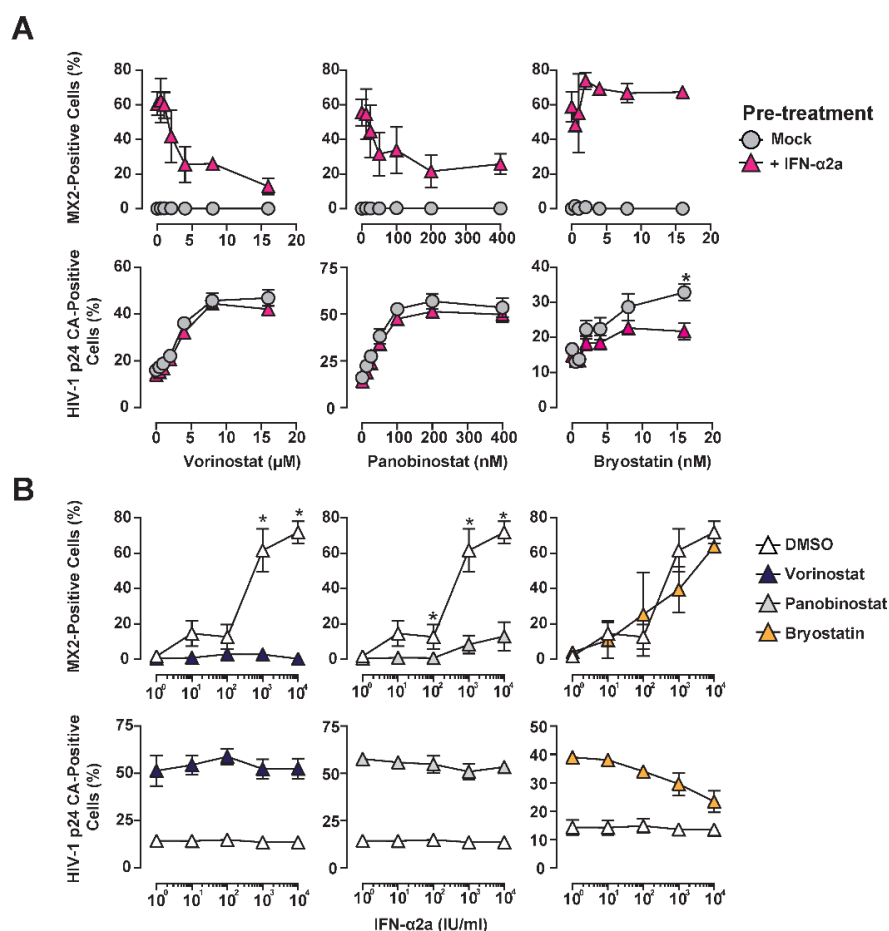
(B) Volcano plot showing significant DEGs of Vorinostat- or Panobinostat-treated samples compared to mock samples.

(C) Overlap of genes up- and down-regulated after Vorinostat- or Panobinostat-treatment compared to mock-treatment.

(D) Activity analysis of selected transcription factors.

(E) Heatmap showing expression of selected genes involved in T-cell activation and signalling and T-cell activation module scores. Statistical significance for the module score was tested using Wilcoxon signed-rank testing.

(F) Heatmap showing expression of selected ISGs and IFN signalling module scores. Statistical significance for the module score was tested using Wilcoxon signed-rank testing.

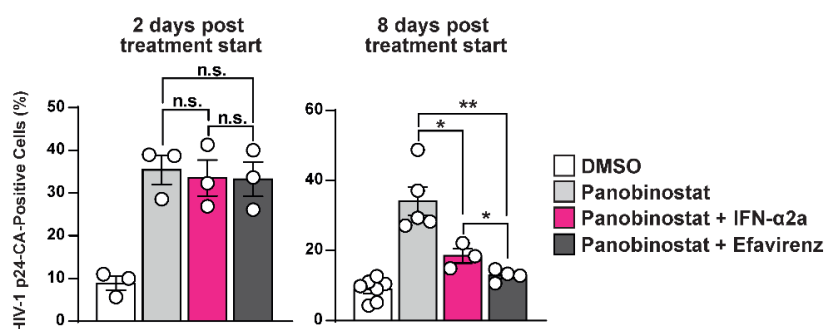
Prigann *et al.*, Supplementary Figure 5

Supplementary Figure 5. HDAC inhibition suppresses type I IFN-induced ISG expression

(A) J1.1 T-cells were pre-treated with IFN- α 2a (500 IU/ml) or mock-treated for 24 hours before incubation with indicated concentrations of Panobinostat, Vorinostat, Bryostatin or DMSO. Intracellular HIV-1 p24-CA and MX2 expression was quantified 40 hours post addition of LRAs by flow cytometry.

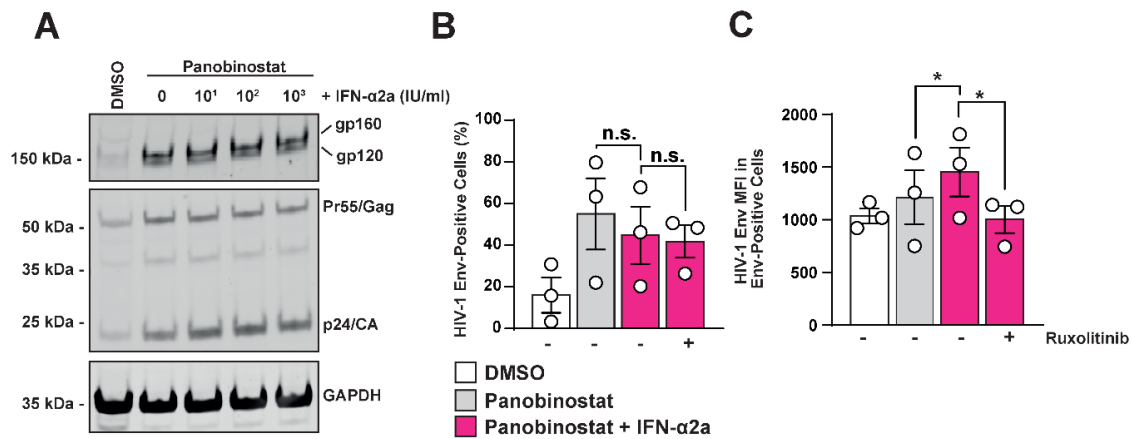
(B) J1.1 T-cells were cultured in the presence of Panobinostat (200 nM), Vorinostat (8 μ M), Bryostatin (10 nM) or DMSO in combination with indicated concentrations of IFN- α 2a. Intracellular HIV-1 p24 CA and MX2 expression was quantified after 40 hours.

Experiments were performed as 3-4 independent replicates.

Prigann *et al.*, Supplementary Figure 6

Supplementary Figure 6. HIV-1 reverse transcriptase inhibitor Efavirenz blocks spread of HIV-1 upon Panobinostat-triggered HIV-1 reactivation.

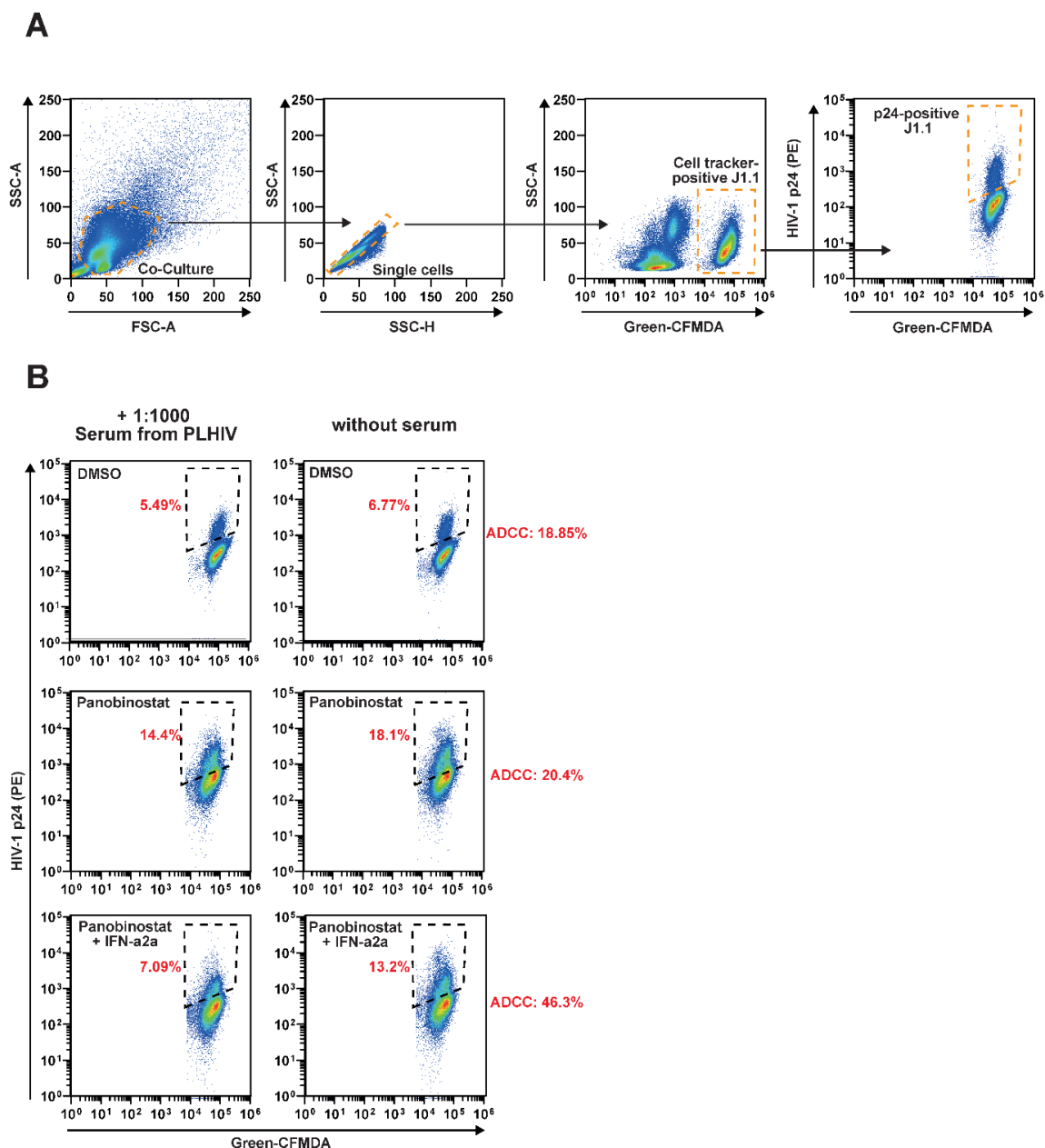
J1.1 cells were treated with 50 nM Panobinostat alone, in combination with either 100 IU/ml IFN-α2a or 100 nM Efavirenz or mock-treated with DMSO for 2 or 8 days before analysing HIV-1 p24 CA-positive cells by flow cytometry.

Prigann *et al.*, Supplementary Figure 7

Supplementary Figure 7. Influence of IFN-α2a signalling on HIV-1 gp160 processing and Env cell surface expression.

(A) J1.1 T-cells were treated with DMSO, 50 nM Panobinostat alone or in combination with indicated concentration of IFN-α2a for 48 hours and analysed for HIV-1 gp160, gp120, Pr55Gag and p24 CA protein expression and processing via immunoblotting.

(B-C) J1.1 T-cells were treated 50 nM Panobinostat in the absence or presence of 100 IU/ml IFN-α2a and 10 μM Ruxolitinib, immunostained for surface HIV-1 Env and analysed for (B) percentage of HIV-1 Env-positive cells or (C) Env MFI in Env-positive cells.

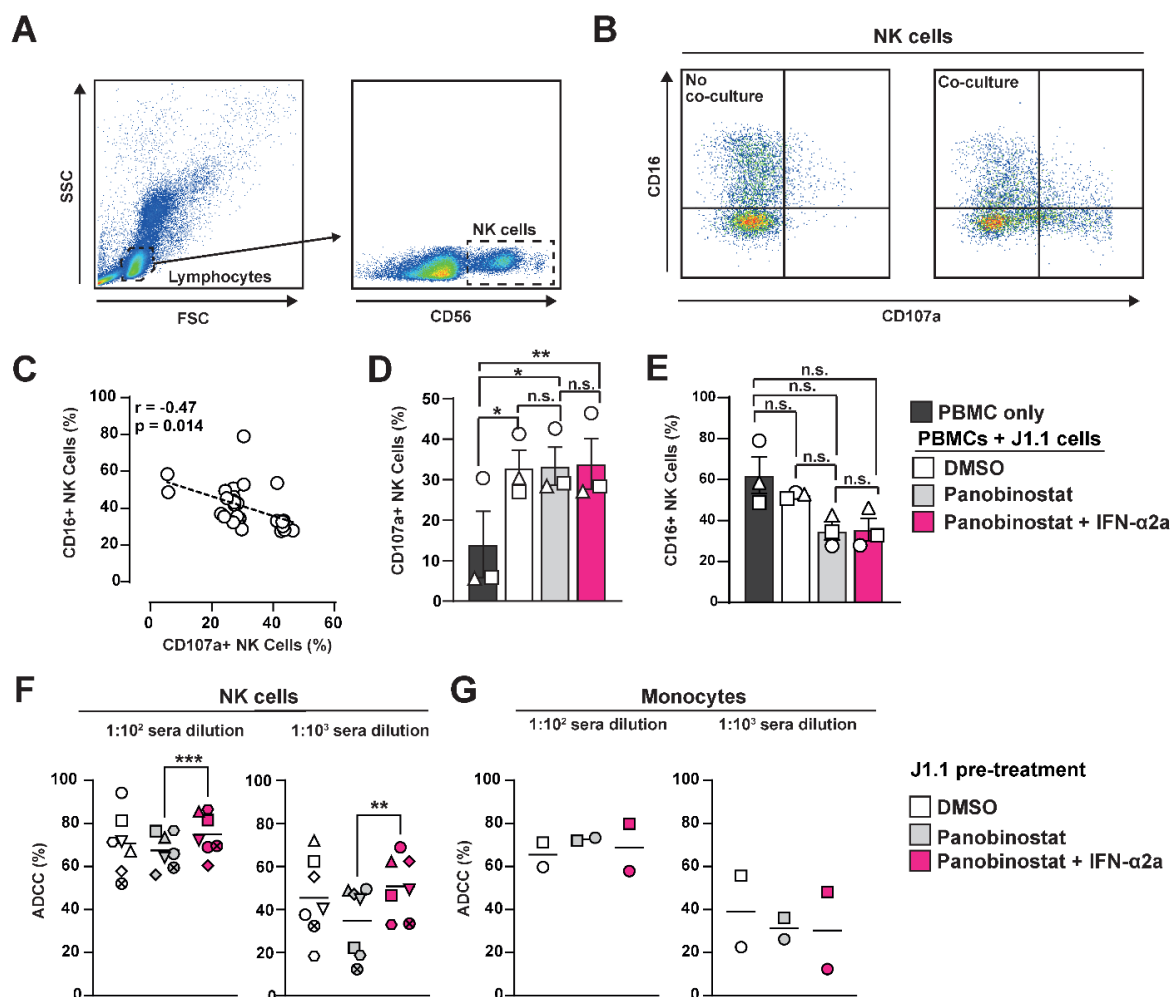
Prigann *et al.*, Supplementary Figure 8**Supplementary Figure 8. Gating strategy and analysis of the ADCC assay**

To characterise ADCC targeting reactivated J1.1 T-cells, freshly isolated PBMCs were co-cultured with cell-tracker Green-CFMDA stained J1.1 T-cells at a 1:1 ratio for four hours. The co-culture was then subjected to intracellular HIV-1 p24 CA-PE immunostaining.

(A) The gating strategy to determine the percentage of p24 CA-positive J1.1 T- cells in the co-culture is shown.

(B) A representative dataset is shown for which the ADCC percentage was calculated.

Prigann et al., Supplementary Figure 9



Supplementary Figure 9. NK cells display signs of degranulation and cellular activation upon co-culturing with J1.1 T-cells in an IFN-independent manner.

PBMC co-cultures with J1.1 T-cells were established as described before, but complemented with anti-CD107a-BV421 antibodies for the duration of the co-culture. After one hour of co-culture, Brefeldin A and Golgi Stop were added and after a total of four hours of co-culture, cells were immunostained for CD56 and CD16 cell surface expression.

(A) Representative plots showing the gating strategy to identify NK cells in the co-culture.

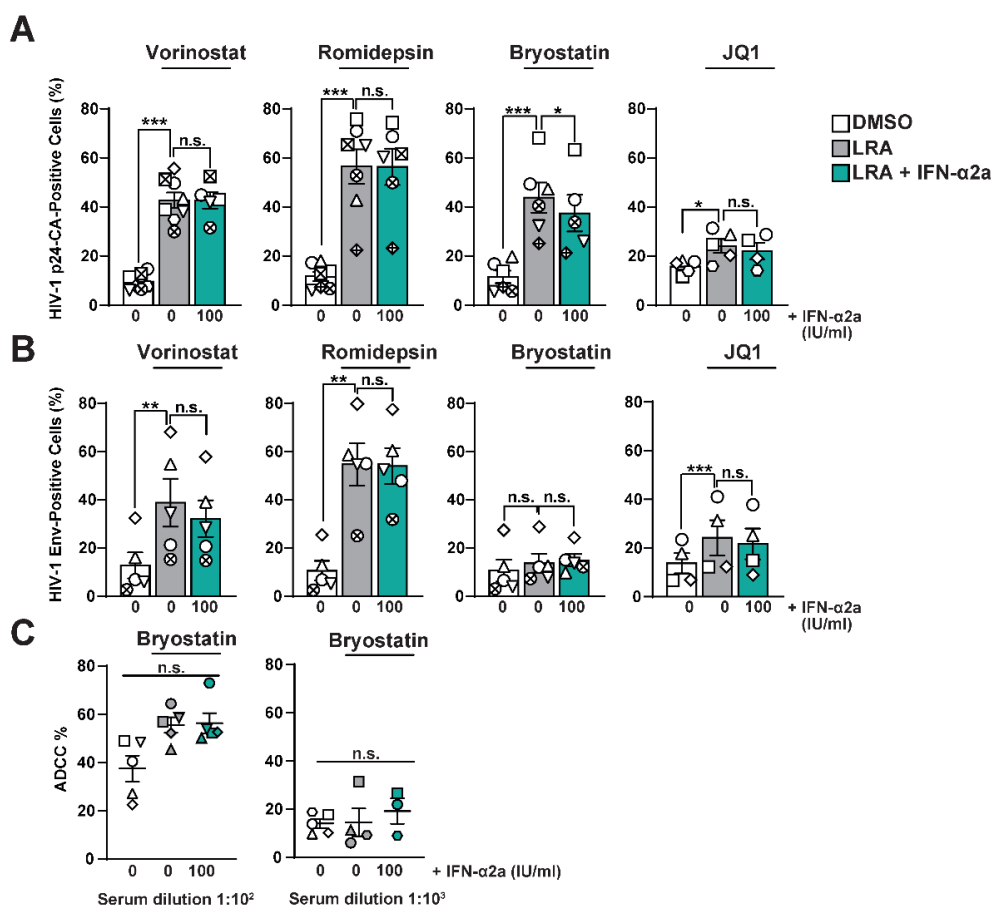
(B) Representative plots showing CD16- and CD107a-positive NK cells within PBMCs alone or in co-culture with J1.1 T-cells.

(C) Pearson's correlation analysis of the percentage of CD16- and CD107a-positive NK cells combining the data of all analysed co-cultures.

(D) Quantification of CD107a-positive cells within the NK cell populations following culturing PBMCs as indicated.

(E) Quantification of CD16-positive cells within the NK cell populations following culturing PBMCs as indicated.

(F) - (G) J1.1 T-cells were treated with DMSO, 50 nM Panobinostat alone or in combination with 100 IU/ml IFN- α 2a for 48 hours before co-culturing with isolated NK cells (F) or monocytes (G) for four hours in the presence of serum from PLHIV. The percentage of ADCC was calculated as described before. Symbols indicate individual experimental replicates.

Prigann *et al.*, Supplementary Figure 10

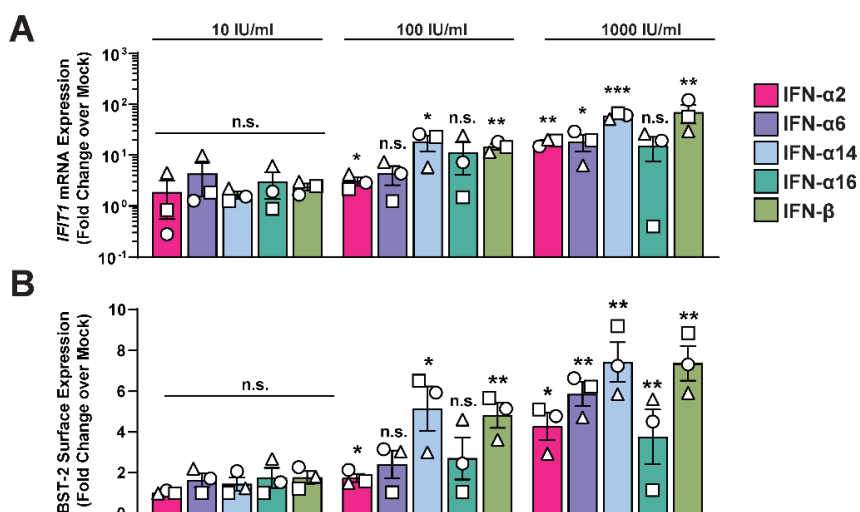
Supplementary Figure 10. IFN-α2a-enhanced HIV-1 Env cell surface accumulation and ADCC sensitization occur in the context of HDACi-, but not PKC agonist-mediated HIV-1 reactivation

J1.1 T-cells were treated with 4 μM Vorinostat, 25 nM Romidepsin, 20 nM Bryostatin or 10 μM JQ1 in the presence or absence of 100 IU/ml IFN-α2a or DMSO for 48 hours.

(A) Quantification of the percentage HIV-1 p24 CA-positive cells by flow cytometry.

(B) Quantification of HIV-1 Env cell surface-positive cells by flow cytometry.

(C) Bryostatin-treated cells were subjected to ADCC assays, as described before, using 1:100 and 1:1000 dilutions of serum from PLHIV

Prigann *et al.*, Supplementary Figure 11

Supplementary Figure 11. IFN-α14 and IFN-β exhibit most pronounced ability to induce ISG expression in latently HIV-1-infected J1.1 T-cells

J1.1 T-cells were treated with indicated concentrations of type I IFNs for 72 hours and analysed for **(A)** *IFIT1* mRNA expression via RT-Q-PCR and **(B)** BST-2/Tetherin cell surface expression via flow cytometry. Bars show the mean of three independent experiments.

Supplementary Movie 1. Addition of IFN-α2a to Panobinostat results in accelerated killing of HIV-1-reactivating T-cells.

J1.1 T-cells were treated with 50 nM Panobinostat alone or in combination with 100 IU/ml IFN-α2a for 48 hours and individually stained with CellTracker™ Green-CFMDA and Deep Red, respectively. Stained J1.1 T-cells were co-cultured with non-stained, freshly isolated NK cells at a 1:1:1 ratio in the presence of a cell death marker (blue) and a 1:100 dilution of sera from PLHIV containing anti-HIV-1 Env antibodies. The co-culture was monitored using live-cell imaging for four hours with images acquired every three minutes.