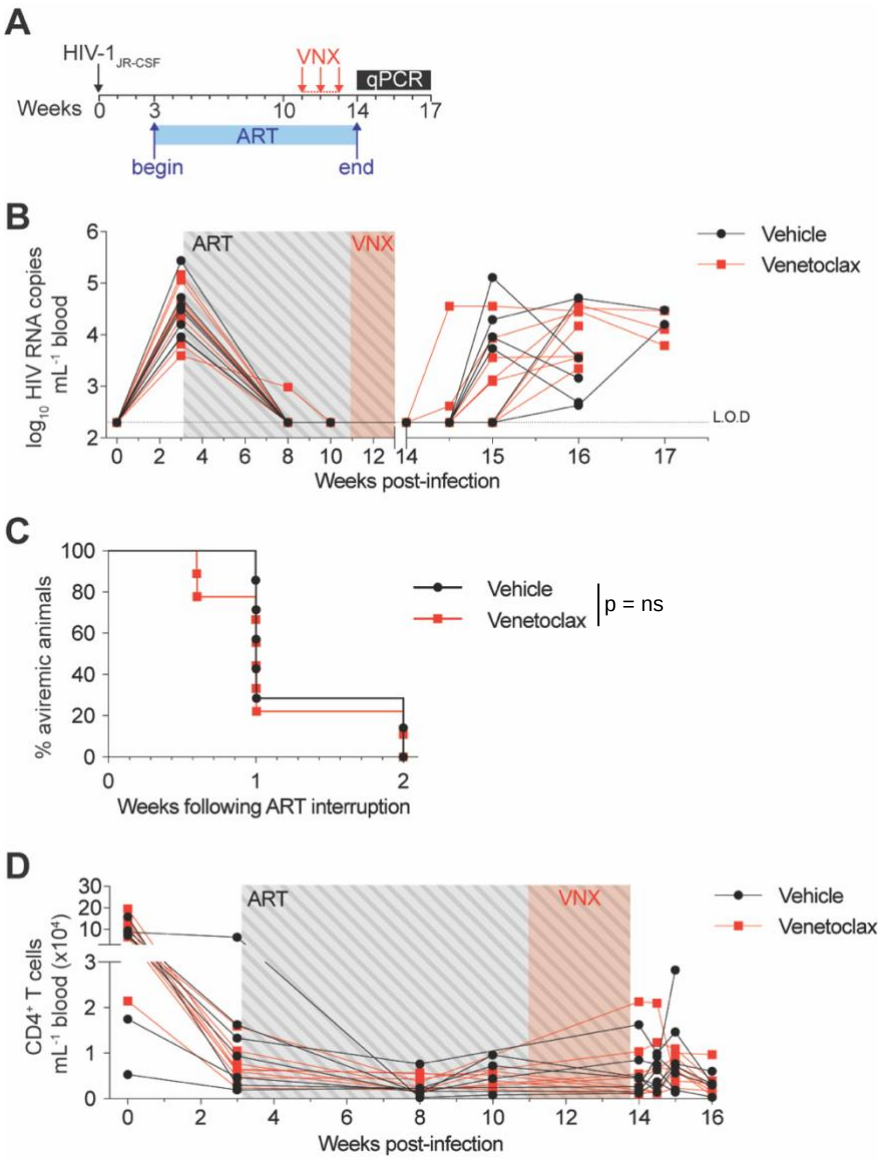
Cell Reports Medicine, Volume *4*

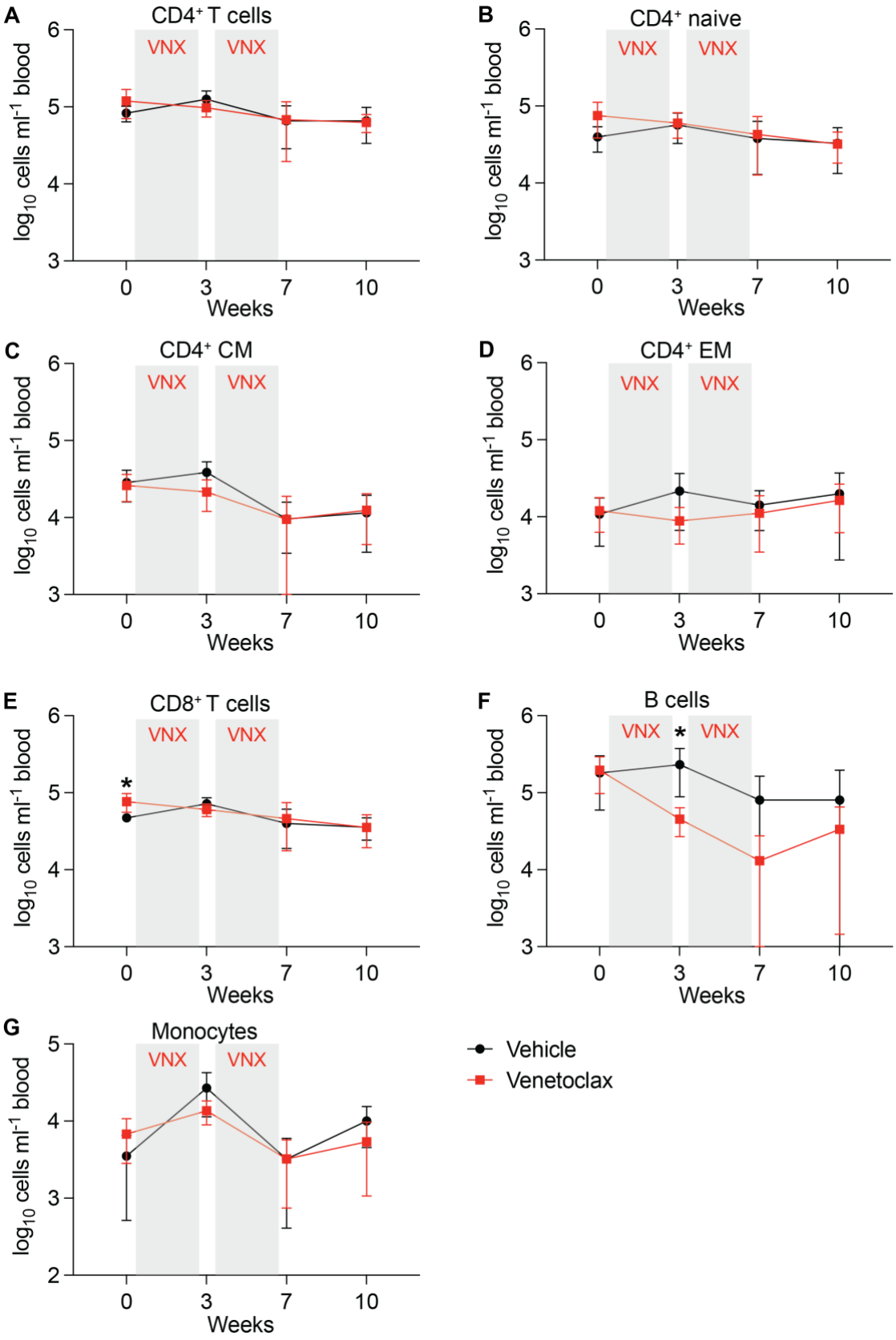
Supplemental information

Venetoclax, alone and in combination with the BH3 mimetic S63845, depletes HIV-1 latently infected cells and delays rebound in humanized mice

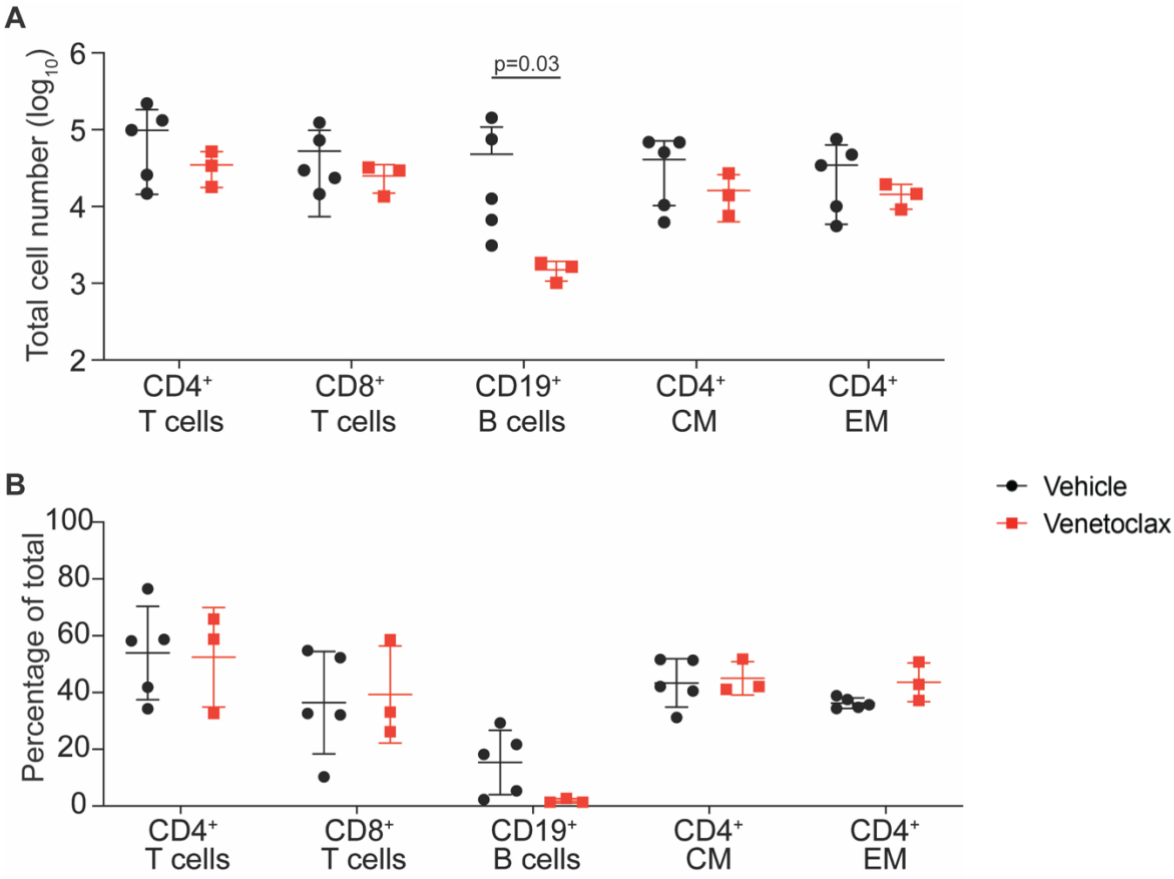
Philip Arandjelovic, Youry Kim, James P. Cooney, Simon P. Preston, Marcel Doerflinger, James H. McMahon, Sarah E. Garner, Jennifer M. Zerbato, Michael Roche, Carolin Tumpach, Jesslyn Ong, Dylan Sheerin, Gordon K. Smyth, Jenny L. Anderson, Cody C. Allison, Sharon R. Lewin, and Marc Pellegrini



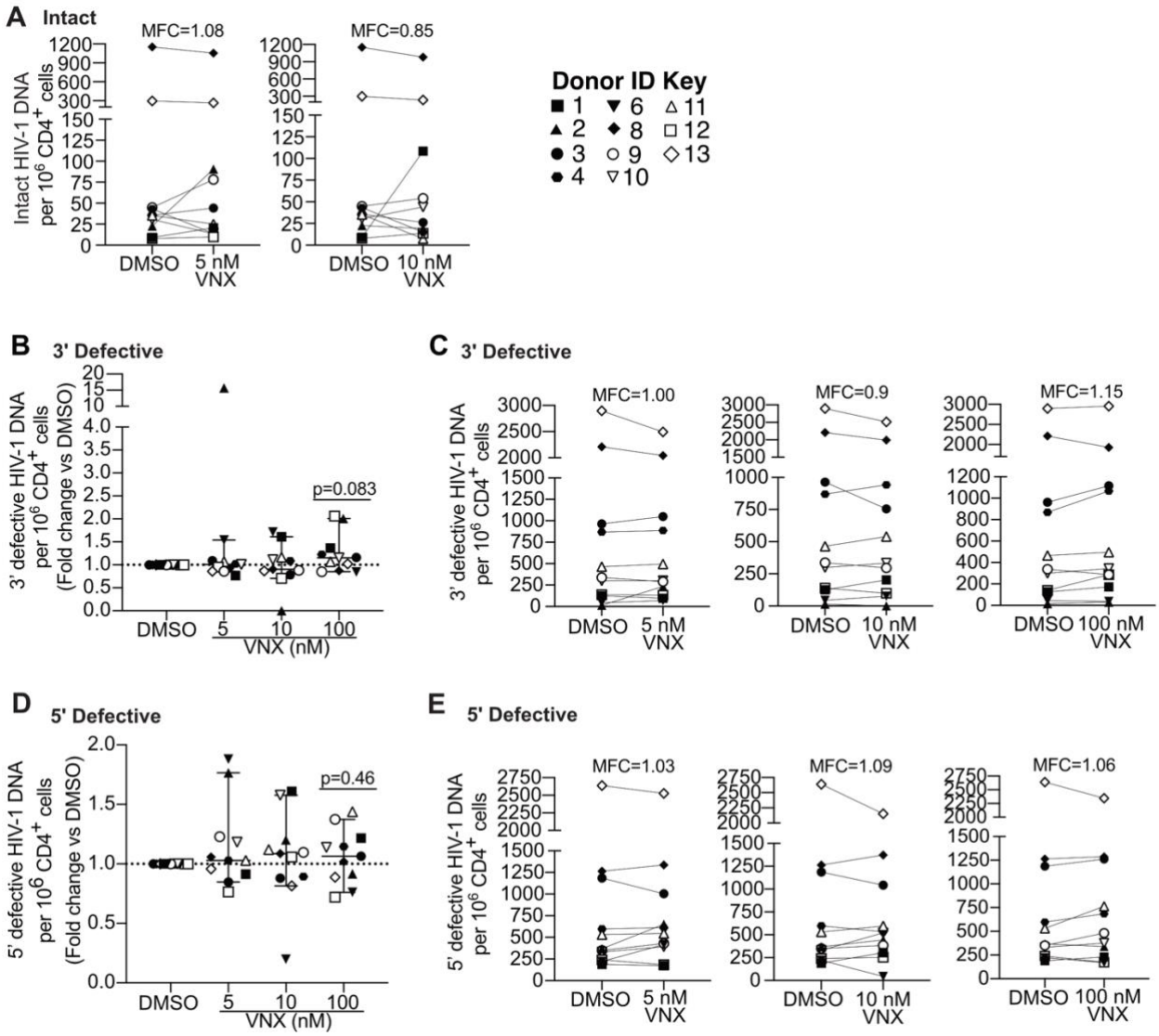
Supplementary Fig. S1. A delay in viral rebound was not detected after three weeks of venetoclax treatment. (A) Schematic timeline depicting HIV-1 infection, suppressive ART period and the beginning of each venetoclax treatment cycle (red arrows; administered every weekday). (**B)** Plasma viral loads of individual hu-mice over the course of the experiment (*n* = 7-8 mice per group). Limit of detection (L.O.D) is indicated with a dotted horizontal line (2.3 $\log_{10}$ RNA copies mL^{-1}). (**C)** Kaplan-Maier curve representing the time to viral rebound following ART interruption. (**D)** Peripheral $CD4^+$ T cell counts over the course of the experiment. *p* value for Kaplan-Maier curve (C) was calculated using a log-rank Mantel-Cox test. *p* value for each timepoint in (D) was analysed using an unpaired *t* test corrected for multiple comparisons with Holm-Šìdák method. Related to Figures 1, 2 and 3.


A
CD4+ T cells
B
CD4+ naive
C
CD4+ CM
D
CD4+ EM
E
CD8+ T cells
F
B cells
G
Monocytes
VNX
VNX
log10 cells ml-1 blood
Weeks
0
3
7
10
*
Vehicle
Venetoclax

Supplementary Fig. S2. Quantification of white blood cell subsets in uninfected hu-mice after six weeks of venetoclax treatment. Uninfected hu-mice were treated with 100 mg/kg venetoclax for 6 weeks by oral gavage, with a 1-week drug holiday. At the end of the final treatment week, mice were bled weekly and WBC subsets enumerated by flow cytometry. **(A)** Total $CD4^+$ T cells; (**B)** Naïve $CD4^+$ T cells; **(C)** Central memory $CD4^+$ T cells; **(D)** Effector memory $CD4^+$ T cells; **(E)** Total $CD8^+$ T cells; **(F)** Total $CD19^+$ B cells; and **(G)** Total monocytes. *$p \leq 0.05$. *p* value for each timepoint in (A)-(G) was analysed using an unpaired *t* test corrected for multiple comparisons with Holm-Šìdák method. Error bars show mean ± S.D. Related to Figure 1.



Supplementary Fig. S3 | Differences in CD4⁺ and CD8⁺ T cells were not detected in hu-mouse lymph nodes after six weeks of venetoclax treatment. Hu-mice were treated with venetoclax for 6 weeks while under cover of suppressive ART. At the end of the final treatment week, mice were sacrificed and lymph nodes (LN) pooled for flow cytometric analysis. **(A)** Total number of each cell type. (**B)** Percentage of each cell type. CM = central memory; EM = effector memory. *p* values in (A) and (B) were calculated using a Mann-Whitney two-tailed *t* test. Error bars show mean ± S.D. Related to Figure 1.



Supplementary Fig. S4 | Frequency of proviral HIV-1 DNA based on IPDA analysis of peripheral blood CD4$^+$ T cells treated with DMSO or venetoclax. Total CD4$^+$ T cells isolated from peripheral blood of PLWH on ART were co-cultured with venetoclax (VNX) or DMSO control for 24 hrs, before washing and harvesting 24 hrs later for quantification of HIV-1 DNA. **(A)** Absolute frequency of intact HIV-1 DNA per million CD4+ T cells for 5 nM and 10 nM doses. **(B)** Fold-change in 3' defective HIV-1 DNA per million CD4$^+$ T cells. **(C)** Absolute frequency of 3' defective HIV-1 DNA per million CD4$^+$ T cells for 5 nM, 10 nM and 100 nM doses. **(D)** Fold-change in 5' defective HIV-1 DNA per million CD4$^+$ T cells. **(E)** Absolute frequency of 5' defective HIV-1 DNA per million CD4$^+$ T cells for 5 nM, 10 nM and 100 nM doses. n = 11 donors. Each symbol represents a different donor. MFC = median fold-change. Error bars show median ± 95% CI. Related to Figure 4.

Supplementary Table 1 | Clinical information for HIV-1-infected individuals on ART. Related to Figure 4 and S4.

Donor ID	Age	Gender	Ethnicity	Years on ART	Current ART Regimen	Viral Load (copies/mL)	Nadir CD4 (cells/mm^3)	Current CD4 (cells/mm^3)
1	49	Male	Caucasian	20.3	Tenofovir disoproxil fumarate/emtricitabine/darunavir/ritonavir	<20	218	833
2	48	Male	Caucasian	6.8	elvitegravir/tenofovir alafenamide fumarate/emtricitabine/cobicistat	<20	538	864
3	56	Male	Caucasian	21.0	raltegravir, darunavir, ritonavir	<20	624	744
4	53	Male	Caucasian	14.2	abacavir/lamivudine/efavirenz	<20	300	735
5	68	Male	White/European American	23	abacavir/dolutegravir/lamivudine	<40	30	494
6	70	Male	Caucasian	32.7	abacavir/dolutegravir/lamivudine	<40	13	524
7	61	Male	Hispanic/Latino	25.3	abacavir/dolutegravir/lamivudine	<40	4	837
8	49	Male	Caucasian	> 3 years*	elvitegravir/cobicistat/emtricitabine/ tenofovir alafenamide fumarate	<20	42	474
9	67	Male	Caucasian	10.9	abacavir, dolutegravir, lamivudine	<20	315	534
10	69	Male	Caucasian	33.3	lamivudine, duranavir, ritonavir, dolutegravir	<40	98	800
11	66	Male	Caucasian	34.2	Emtricitabine, tenofovir disoproxil fumarate, darunavir, cabicistat	<40	54	466
12	46	Male	Hispanic/Latino	11.9	rilpivirine, tenofovir disoproxil fumarate, emtricitabine	<40	324	429
13	56	Male	Caucasian	31	darunavir, ritonavir, abacavir, dolutegravir, lamividune	<40	200	586

*Time on ART unknown. At study enrolment, ART information collected for previous 3 years only.