

Viral and host mediators of non-suppressible HIV-1 viremia

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Supplementary Tables

Supplementary Table 1. Characteristics of the non-suppressible viremia (NSV) participants.

ID	Age Group	Sex	Race	NSV Duration (years)	CD4 cell Count (cells/mm ³)	ART Regimen ^a	GSS ^b
LV1	70-79	M	Hispanic	8	390	TAF/FTC/DTG/DRVr	3.25
LV2	50-59	M	Native American	2	998	TAF/FTC/DTG/MVC/DRVc	5
LV3	60-69	M	White	3	531	TAF/FTC/DTG	2.25
LV4	50-59	M	White	1	1472	ABC/3TC/DTG	3
LV5	60-69	M	Black	3	598	TAF/FTC/BIC/DOR/DRVr	2.25
LV7	60-69	M	White	5	672	TAF/FTC/DTG	3
LV8	40-49	F	Black	10	1039	TAF/FTC/BIC	3
LV9	50-59	M	White	11	923	TAF/FTC/BIC	3

^aART regimen at the time of initial sample collection

^bGSS, genotypic susceptibility score of plasma virus against the last ART regimen (number of active antiretroviral medications, see Methods section)

ART, antiretroviral therapy; TAF, tenofovir alafenamide; FTC, emtricitabine; DTG, dolutegravir; MVC, maraviroc; DRVr, darunavir-ritonavir; DRVc, darunavir-cobicistat; DOR, doravirine; BIC, bictegravir; ABC, abacavir; 3TC, lamivudine

Supplementary Table 2. Characteristics of the ART-suppressed participants for sequencing analysis.

Age Group	Sex	Race	ART Duration (years)	CD4 cell Count (cells/mm ³)	NFL-seq ^a	RNA-seq ^b
30-39	F	Hispanic	7	1097	Y	N
40-49	M	White	7.7	736	Y	Y
50-59	M	White	7.7	1652	Y	N
50-59	M	Black	6.3	806	Y	N
30-39	M	White	5.4	691	Y	Y
50-59	F	White	5.7	941	Y	N
50-59	F	White	7	781	Y	Y
40-49	F	Black	6.6	685	Y	Y
40-49	M	Black	5.4	1462	Y	N
40-49	M	White	3.2	469	Y	N
30-39	F	White	6.5	1319	N	Y

^aNFL-seq, near-full length sequencing performed

^bRNA-seq, bulk CD4+ T cell RNA transcript sequencing performed

Supplementary Table 3. Characteristics of the ART-suppressed comparator participants for T cell activation and phenotyping.

Age Group	Sex	Race	ART Duration (years)	CD4 cell Count (cells/mm ³)
40-49	M	White	9.25	508
70-79	M	White	9.5	961
60-69	F	Black	14	953
50-59	F	Black	12.5	945
60-69	M	White	13	602
70-79	M	White	13	633
50-59	M	White	11	793

Supplementary Table 4. Characteristics of the viremic controller (VC) comparator participants for T cell activation and phenotyping.

Age Group	Sex	Race	Viral load (copies/ml)	CD4 cell Count (cells/mm ³)
40-49	M	White	48	496
60-69	M	NA	103	NA
60-69	M	Black	<48	1004
80-89	M	White	160	650
50-59	M	White	396	940
60-69	F	Black	95	838
60-69	F	White	146	1015

Supplementary Table 5. Drug concentrations for NSV participants were quantified using two different methodologies.

a. For two participants LV1 and LV2, antiretroviral concentrations were quantified in plasma b. For other five participants, antiretroviral concentrations were quantified in dried blood spots.

		Plasma Concentration (mcg/mL)				Dried Blood Spots (fmol or pmol/punches)			
PID	Current ART	TP1 ^a		TP2					
		DTG	DRV	DTG	DRV				
LV1	TAF/FTC/DTG/DRVr	0.29	1.52	2.88	7.21				
LV2	TAF/FTC/DTG/DRVr	1.52	6.27	ND ^b	ND				
		TP1	TP2	TP3	LLOQ ^c				
		TFV-DP	FTC-TP	TFV-DP	FTC-TP	TFV-DP	FTC-TP	TFV LLOQ	FTC LLOQ
LV3	DTG/FTC/TAF	2771	5.1	ND	ND	ND	ND	25	0.1
LV5	TAF/BIC/FTC/DOR/DRV	6684	6	ND	ND	ND	ND	25	0.1
LV7	FTC/TAF/BIC	3632	6.7	4306	5.2	4941	5.7	25	0.1
LV8	FTC/TAF/BIC	3844	4.5	3041	5	3322	4.3	25	0.1
LV9	FTC/TAF/BIC	3773	4.5	3480	3.2	ND	ND	25	0.1

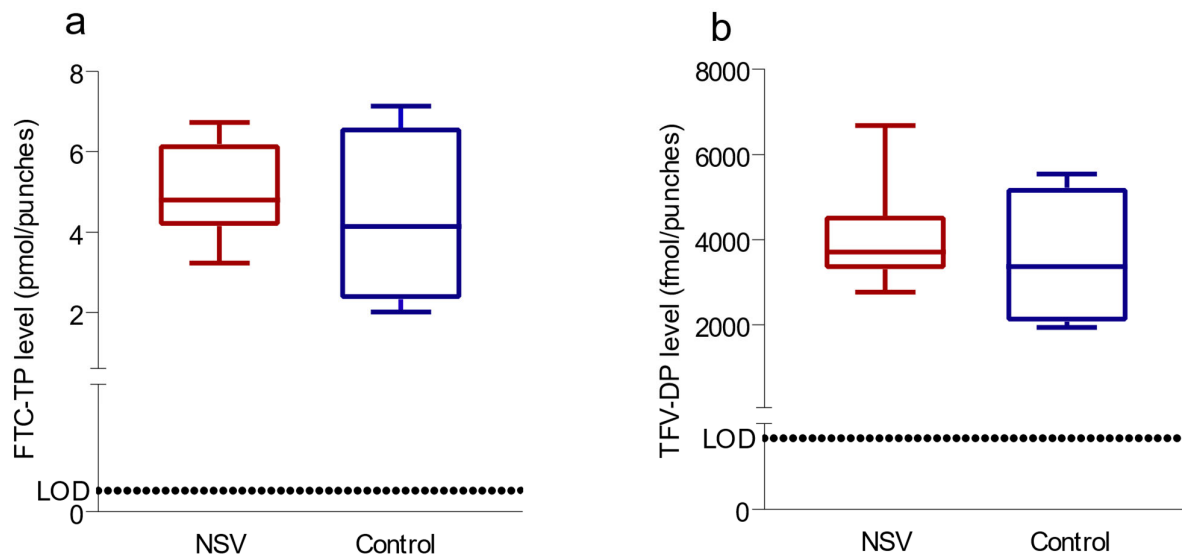
^aTP, time points

^bND, Not done

^cLLOQ, lower limit of detection

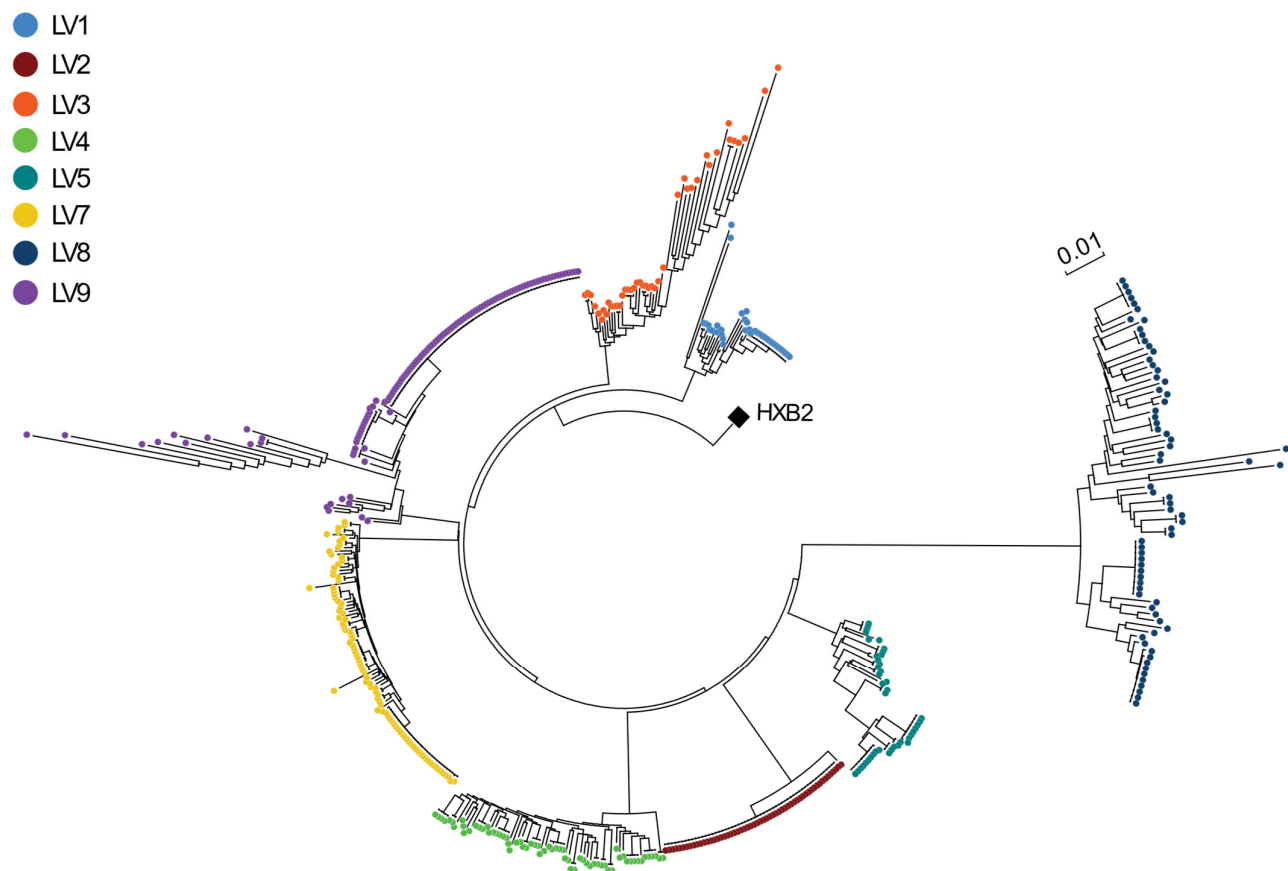
“-DP”, diphosphate; “-TP”, triphosphate.

Supplementary Figures



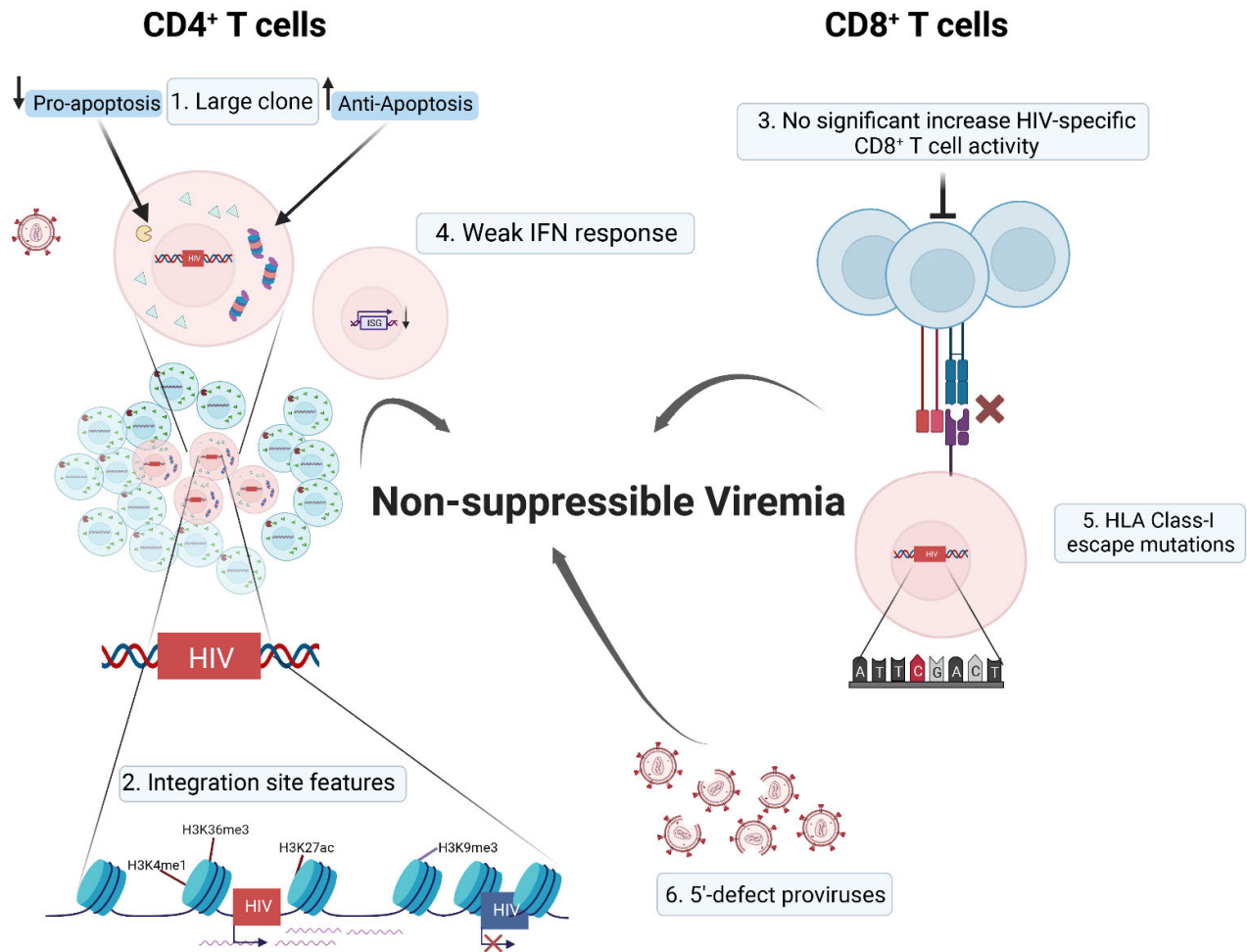
Supplementary Fig. 1. Levels of intracellular emtricitabine-triphosphate (FTC-TP) and tenofovir-diphosphate (TFV-DP) levels in dried blood spots.

(a) Levels of FTC-TP in NSV participants compared to a previously published group of HIV-uninfected individuals receiving daily FTC by directly observed therapy. (b) Levels of TFV-DP in NSV participants compared to a previously published group of ART-suppressed individuals. N=10 biologically independent NSV samples from different time points were compared to n=5 HIV-uninfected samples. Bars show median values and error bars indicate 95% confidence interval. Mann Whitney test was used. Box plot shows median, Q1, Q3, and whiskers show range.



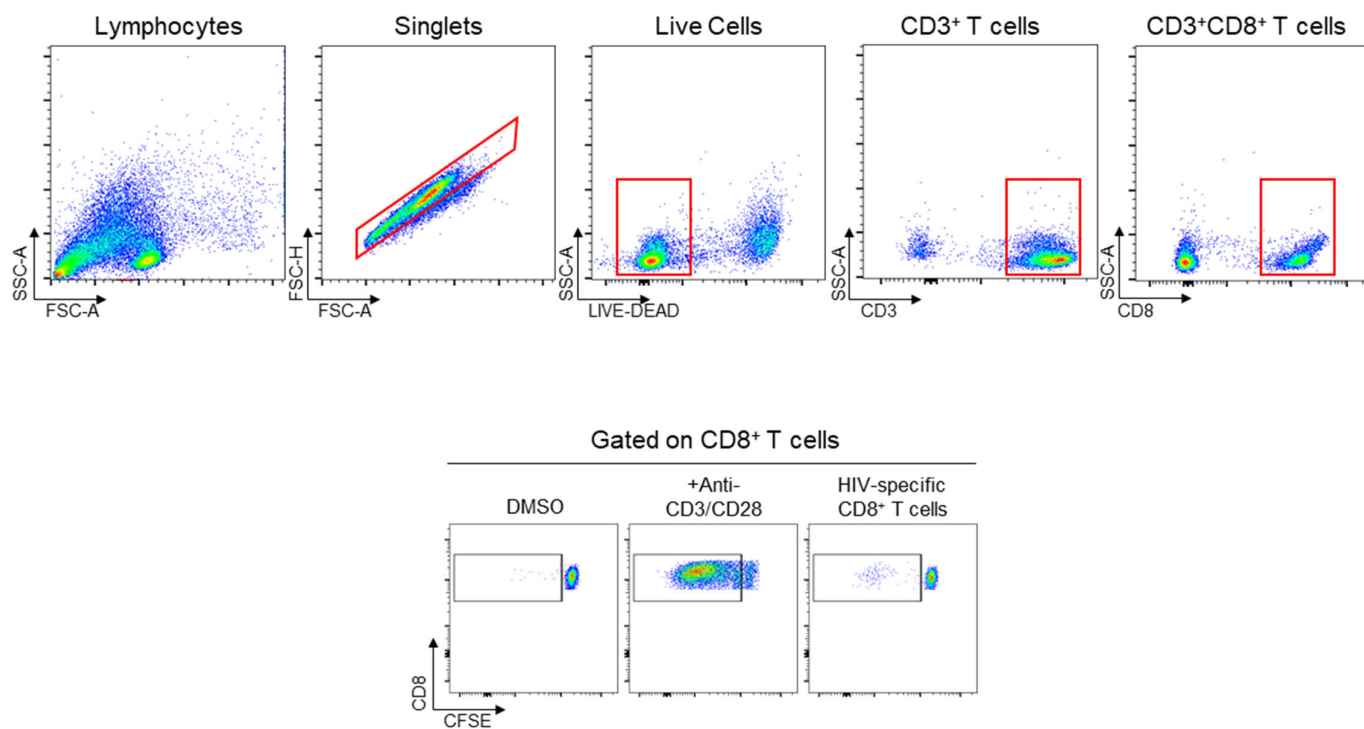
Supplementary Fig. 2. Neighbor joining tree of all near-full length proviral sequences from NSV participants.

The predominant outlier LV8 (dark blue) harbored HIV-1 subtype C virus, while others harbored HIV-1 subtype B virus.



Supplementary Fig. 3. Non-suppressible viremia is driven by critical intersection of factors at viral genetic, epigenetic, and cellular level.

The NSV phenotype was highlighted by the presence of large, clonally-expanded reservoirs of proviruses (1) integrated in transcriptionally-permissive chromosomal regions (2), within a CD4⁺ T cell environment primed for survival, with a muted HIV-specific immune responses (3, 4), and harboring certain viral mutations (5, 6). Graphics in in this figure were created with Biorender and license was obtained.



Supplementary Fig. 4. Gating strategy for CFSE proliferation assay.

Representative gating strategy for identification of proliferating CD3⁺ CD8⁺ CFSE low T cells in response to HIV peptides. The gate establishing the frequency of CFSE low CD8⁺ T cells was chosen based on minimizing responses in two negative-control (DMSO) wells and verified using positive control (CD3/CD28) wells.