

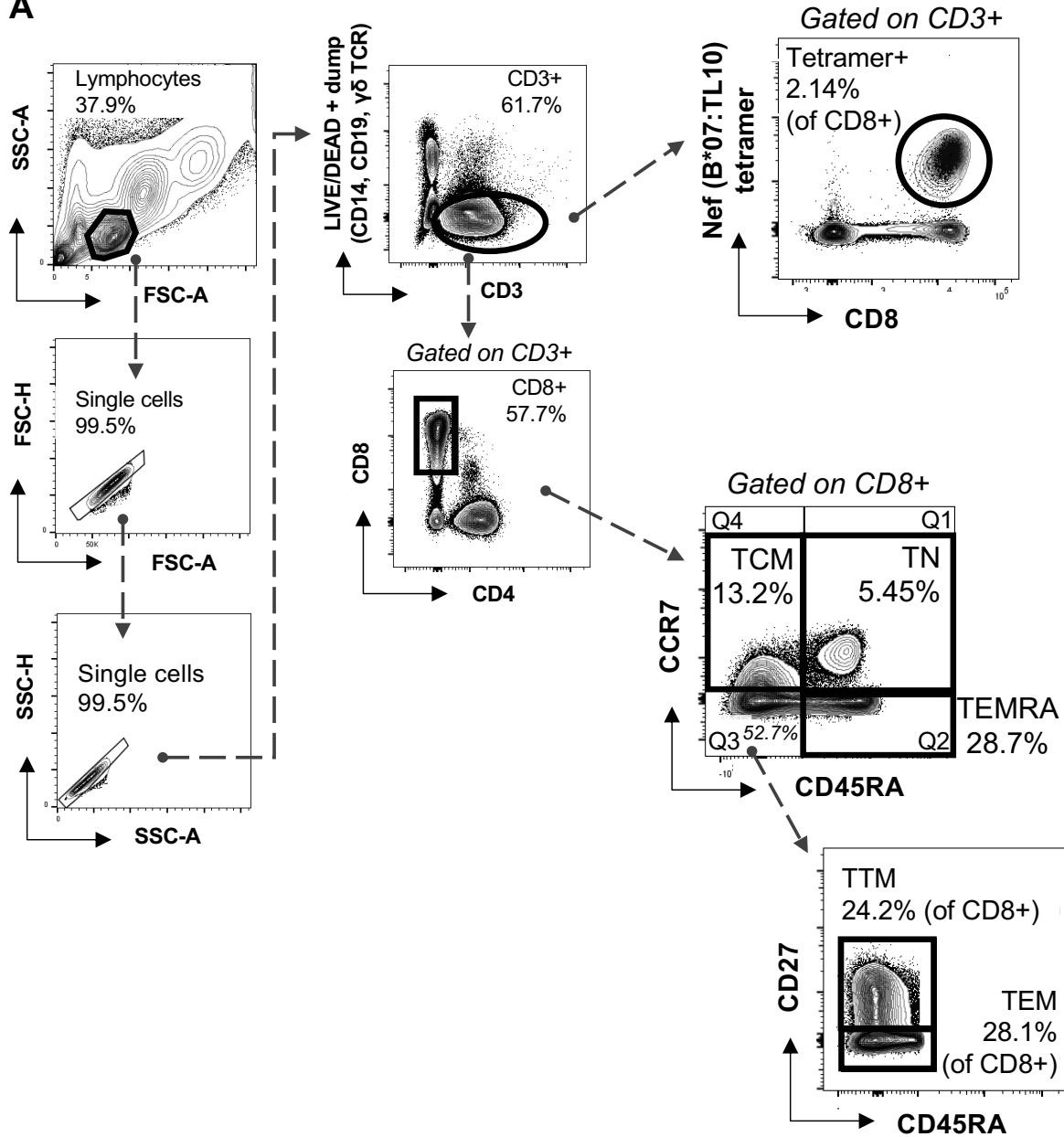
Clinical characteristics of study participants

Clinical Group	Age (years)	Sex	Ethnicity	CD4 count (cells/mL)	HIV Viral Load (copies/mL)	Tetramer Target	Tetramer specificity (HLA:peptide)	ART regimen (years on ART)
Viremic (n=8)	35	M	Caucasian	301	986,418	Gag	A*02:FLGKIQWPSYK	-
	41	F	Caucasian	480	28,122	Gag	A*02:FLGKIQWPSYK	-
	38	M	Caucasian	771	10,787	Gag	A*02:SLYNTVATL	-
	40	M	Caucasian	376	13,600	Gag	A*02:SLYNTVATL	-
	44	M	African American	623	3,375	Gag	A*02:SLYNTVATL	-
	46	M	Caucasian	484	119,163	Gag	A*02:SLYNTVATL	-
	39	M	Caucasian	588	8,123	-	-	-
	54	F	Caucasian	382	41,206	-	-	-
<i>Median</i>	<i>40.5</i>	-	-	<i>482</i>	<i>20,861</i>			
ART-suppressed (n=11)	61	M	Caucasian	612	<40	Gag	A*02:FLGKIQWPSYK	ABC/3TC, RGV (14)
	63	M	Caucasian	276	<40	Gag	A*02:FLGKIQWPSYK	TDF/FTC, ATV/r (8)
	59	M	Caucasian	894	<40	Gag	A*02:SLYNTVATL	ABC/3TC/DTG (19)
	44	F	Caucasian	512	<40	Gag	A*02:SLYNTVATL	TDF/FTC, ATV/r (12)
	54	M	Caucasian	400	<75	Pol	B*07:SPAIFQSSM	DDI, TDF/FTC, LPV/r, ENF (3)
	53	M	Caucasian	563	<20	Nef	B*07:TPGPGVRYPL	TDF/FTC, ATV/r (6)
	62	M	Caucasian	399	<40	Nef	B*07:TPGPGVRYPL	TDF/FTC, DTG (15)
	67	M	Caucasian	954	<40	Gag	B*27:KRWIIMGLNK	DRV/r, RGV (10)
	54	M	Caucasian	416	<40	-	-	TDF/FTC, NVP (12)
	56	M	Caucasian	756	<48	-	-	TDF, ABC/3TC, LPV/r (15)
	57	M	African American	513	<40	-	-	TDF, ABC/3TC, ETV, DRV/r (18)
<i>Median</i>	<i>57</i>	-	-	<i>513</i>	<i>-</i>			
Controller (n=9)	57	M	Caucasian	733	<40	Gag	A*02:FLGKIQWPSYK	-
	57	M	Caucasian	742	<40	Gag	A*02:SLYNTVATL	-
	33	M	Caucasian	657	<40	Gag	A*03:RLRPGGRKR	-
	40	M	Caucasian	693	<40	Nef	A*24:RYPLTFGW	-
	47	M	Caucasian	1098	<40	Pol	B*07:SPAIFQSSM	-
	53	M to F	Mixed Race	-	<40	Nef	B*07:TPGPGVRYPL	-
	52	F	African American	582	<40	-	-	-
	53	M	Caucasian	1003	<40	-	-	-
	75	M	African American	809	<20	-	-	-
<i>Median</i>	<i>53</i>	-	-	<i>733</i>	<i>-</i>			
Uninfected (n=10)	37	M	African American	-	-	-	-	-
	38	M	Caucasian	-	-	-	-	-
	50	M	Asian	-	-	-	-	-
	50	M	African American	-	-	-	-	-
	53	M	Caucasian	-	-	-	-	-
	56	M	Caucasian	-	-	-	-	-
	57	M	African American	-	-	-	-	-
	58	M	Caucasian	-	-	-	-	-
	62	M	African American	-	-	-	-	-
	67	M	African American	-	-	-	-	-
<i>Median</i>	<i>54.5</i>	-	-	<i>-</i>	<i>-</i>			

Supplemental Digital Content 1. Clinical characteristics of study participants. 3TC=lamivudine, ABC=abacavir, ATV/r=atazanavir/ritonavir, DDI=didanosine, DRV/r=darunavir/ritonavir, DTG=dolutegravir, ENF=enfuvirtide, ETV=etravirine, FTC=emtricitabine, LPV/r=lopinavir/ritonavir, NVP=nevirapine, RGV=raltegravir, TAF=tenofovir alafenamide, TDF=tenofovir disoproxil fumarate.

Target	Fluorochrome	Clone	Supplier	Panel
CD14 (dump)	BV510	M5E2	Biolegend	1,2,3
CD19 (dump)	BV510	H1B19	Biolegend	1,2,3
TCR- $\gamma\delta$ (dump)	BV510	B1	Biolegend	1,2,3
CD3	BV650, PE-Cy5.5	SK7	Thermo Fisher Scientific	1,2,3
CD4	BV650, BV711	OKT4	Biolegend	1,2,3
CD8 α	APC-R700	RPA-T8	BD Biosciences	1,2,3
CD45RA	APC-Cy7	HI100	Biolegend	1,2,3
CCR7	BV785	G043H7	Biolegend	1,2,3
CD27	BV570	O323	Biolegend	1,2,3
CD127	BV605	A019D5	Biolegend	1,2,3
PD-1	BV650/PE/PE-Cy7	EH12.2H7	Biolegend	1,2,3
Granzyme B	FITC	GB11	Biolegend	3
Perforin	PE-Cy7	B-D48	Biolegend	3

Supplemental Digital Content 2. List of antibodies used for flow cytometry. Cells in each panel were also stained for Mitochondrial Mass and ROS content (Panel 1), Mitochondrial Membrane Potential (Panel 2), or stained with CellTrace Violet with and without peptide stimulation (Panel 3).

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Supplemental Digital Content 3. General gating strategy used to identify bulk CD8⁺ T cell subsets and MHC Class I tetramer⁺ HIV-specific CD8⁺ T cells.

HIV-uninfected participants

MitoGREEN MFI	TN	TCM	TTM	TEM
TN	2,033	-	-	-
TCM	4,185	0.455	-	-
TTM	5,688	0.007	>0.999	-
TEM	5,347	0.027	>0.999	>0.999
TEMRA	3,597	>0.999	>0.999	0.719
				>0.999

%JC-1+	TN	TCM	TTM	TEM
TN	13%	-	-	-
TCM	62%	0.001	-	-
TTM	52%	0.278	>0.999	-
TEM	48%	0.051	>0.999	>0.999
TEMRA	25%	>0.999	0.093	>0.999
				>0.999

CellROX MFI	TN	TCM	TTM	TEM
TN	1,754	-	-	-
TCM	2,271	>0.999	-	-
TTM	2,368	>0.999	>0.999	-
TEM	1,462	>0.999	0.061	>0.999
TEMRA	1,691	>0.999	>0.999	>0.999

Supplemental Digital Content 4. MM, MMP, and ROS content of bulk CD8+ T cell subsets in HIV-uninfected participants. Statistically significant differences in these characteristics between different bulk CD8+ T cell subsets (TN=naïve, TCM=central memory, TTM-transitional memory, TEM=effector memory, TEMRA=effector memory RA) from HIV-uninfected participants. Median values are shown in second column. P-values based on result of Friedman test with post-hoc testing by Dunn's multiple comparison test. Grey shading, $p < 0.05$.

All HIV-infected participants

MitoGREEN MFI	TN	TCM	TTM	TEM	TEMRA
TN	4,761	-	-	-	-
TCM	8,600	0.011	-	-	-
TTM	12,496	<0.001	<0.001	-	-
TEM	12,640	<0.001	<0.001	>0.999	-
TEMRA	9,470	<0.001	>0.999	0.002	<0.001
HIV-specific	11,045	<0.001	0.005	>0.999	0.034

%JC-1%	TN	TCM	TTM	TEM	TEMRA
TN	20%	-	-	-	-
TCM	66%	<0.001	-	-	-
TTM	67%	<0.001	>0.999	-	-
TEM	69%	<0.001	>0.999	>0.999	-
TEMRA	49%	0.029	0.017	>0.999	0.041
HIV-specific	80%	<0.001	0.215	0.079	0.461
					<0.001

CellROX MFI	TN	TCM	TTM	TEM	TEMRA
TN	2,750	-	-	-	-
TCM	3,615	0.015	-	-	-
TTM	5,123	<0.001	0.314	-	-
TEM	4,082	0.059	>0.999	0.164	-
TEMRA	2,902	>0.999	>0.999	0.002	>0.999
HIV-specific	7,818	<0.001	0.324	>0.999	0.259
					0.005

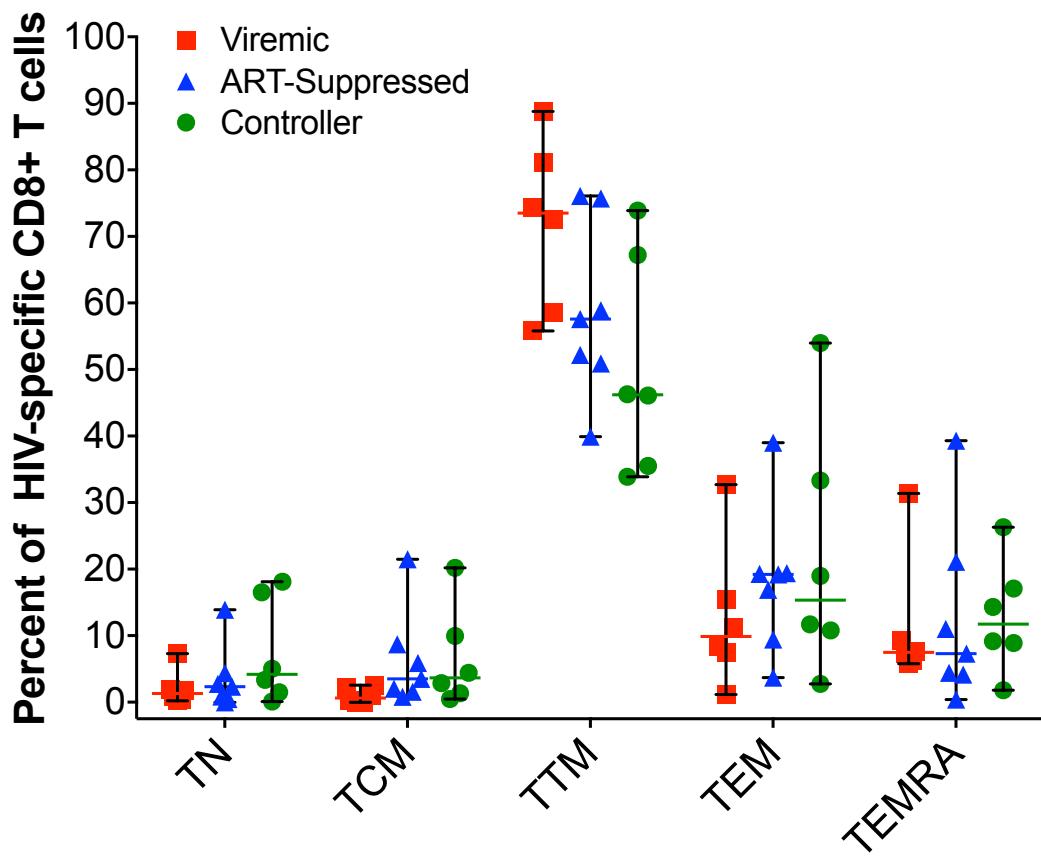
Supplemental Digital Content 5. MM, MMP, and ROS content characteristics of bulk CD8+ T cell subsets and HIV-specific CD8+ T cells in HIV-infected individuals. Statistically significant differences in these characteristics between different bulk CD8+ T cell subsets (TN=naïve, TCM=central memory, TTM-transitional memory, TEM=effector memory, TEMRA=effector memory RA) and tetramer+ HIV-specific CD8+ T cells from HIV-infected individuals. Median values are shown in second column. P-values calculated by Friedman test with post-hoc testing by Dunn's multiple comparison test. Grey shading, p<0.05.

Mann-Whitney Test of median MitoGREEN MFI							
			TN	TCM	TTM	TEM	TEMRA
Uninfected		MFI	2,033	4,185	5,688	5,347	3,597
Uninfected vs:	ART-Suppressed	MFI	5,395	9,250	13,569	14,225	8,217
		p value	0.028	0.075	0.005	0.008	0.055
	Controller	MFI	3,967	7,512	10,364	9,620	9,470
		p value	0.147	0.381	0.060	0.060	0.112
	Viremic	MFI	5,715	9,298	12,835	12,983	11,535
		p value	0.045	0.127	0.011	0.011	0.019
	All HIV-infected	MFI	4,761	8,600	12,496	12,640	9,470
		p value	0.022	0.088	0.003	0.004	0.019

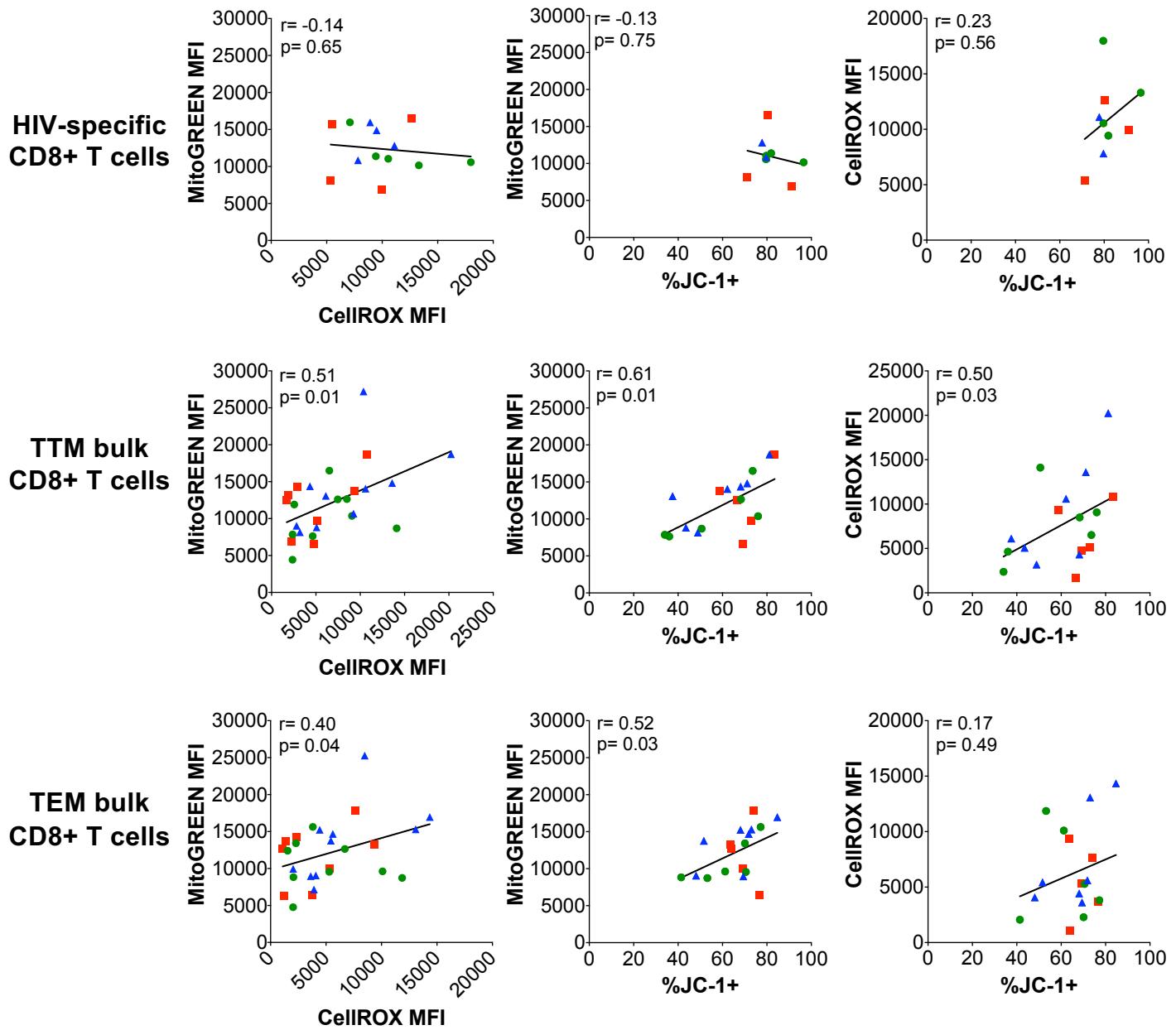
Mann-Whitney Test of median %JC-1+							
			TN	TCM	TTM	TEM	TEMRA
Uninfected		%	13	62	52	48	25
Uninfected vs:	ART-Suppressed	%	25	62	56	69	46
		p value	0.065	0.833	0.350	0.032	0.031
	Controller	%	11	63	59	66	51
		p value	0.662	0.792	0.507	0.178	0.052
	Viremic	%	20	66	69	69	58
		p value	0.095	0.548	0.008	0.008	0.008
	All HIV-infected	%	20	66	67	69	49
		p value	0.088	0.629	0.101	0.010	0.001

Mann-Whitney Test of median CellROX MFI							
			TN	TCM	TTM	TEM	TEMRA
Uninfected		MFI	1,754	2,271	2,368	1,462	1,691
Uninfected vs:	ART-Suppressed	MFI	2,923	5,229	7,685	4,934	3,813
		p value	0.007	0.099	<0.001	0.001	0.019
	Controller	MFI	2,424	3,265	6,519	3,809	2,528
		p value	0.313	0.298	0.042	0.019	0.240
	Viremic	MFI	2,877	3,620	3,809	3,012	2,608
		p value	0.045	0.093	0.019	0.284	0.354
	All HIV-infected	MFI	2,750	3,615	5,123	4,082	2,902
		p value	0.023	0.076	0.008	0.008	0.067

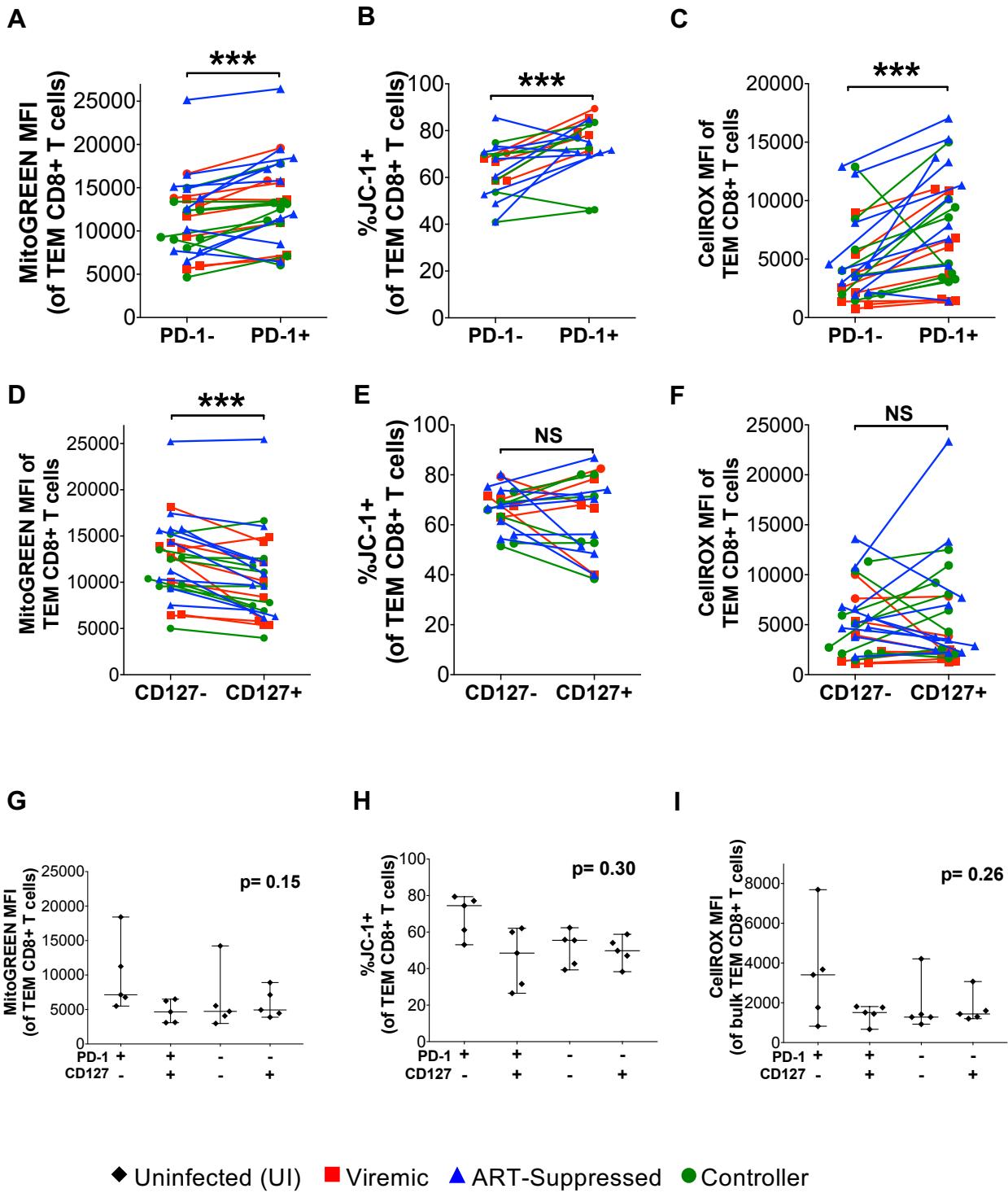
Supplemental Digital Content 6. Differences in MM, MMP, and ROS content of bulk CD8+ T cell subsets in different clinical groups of HIV-infected individuals. Statistically significant differences in these characteristics in bulk CD8+ T cell subsets between HIV-uninfected compared to HIV-infected individuals in the different clinical groups versus uninfected individuals. Median values for each T cell subpopulation within each clinical group (or across all HIV-infected participants) are shown. P-values calculated using Mann-Whitney U test. Grey shading, p<0.05.



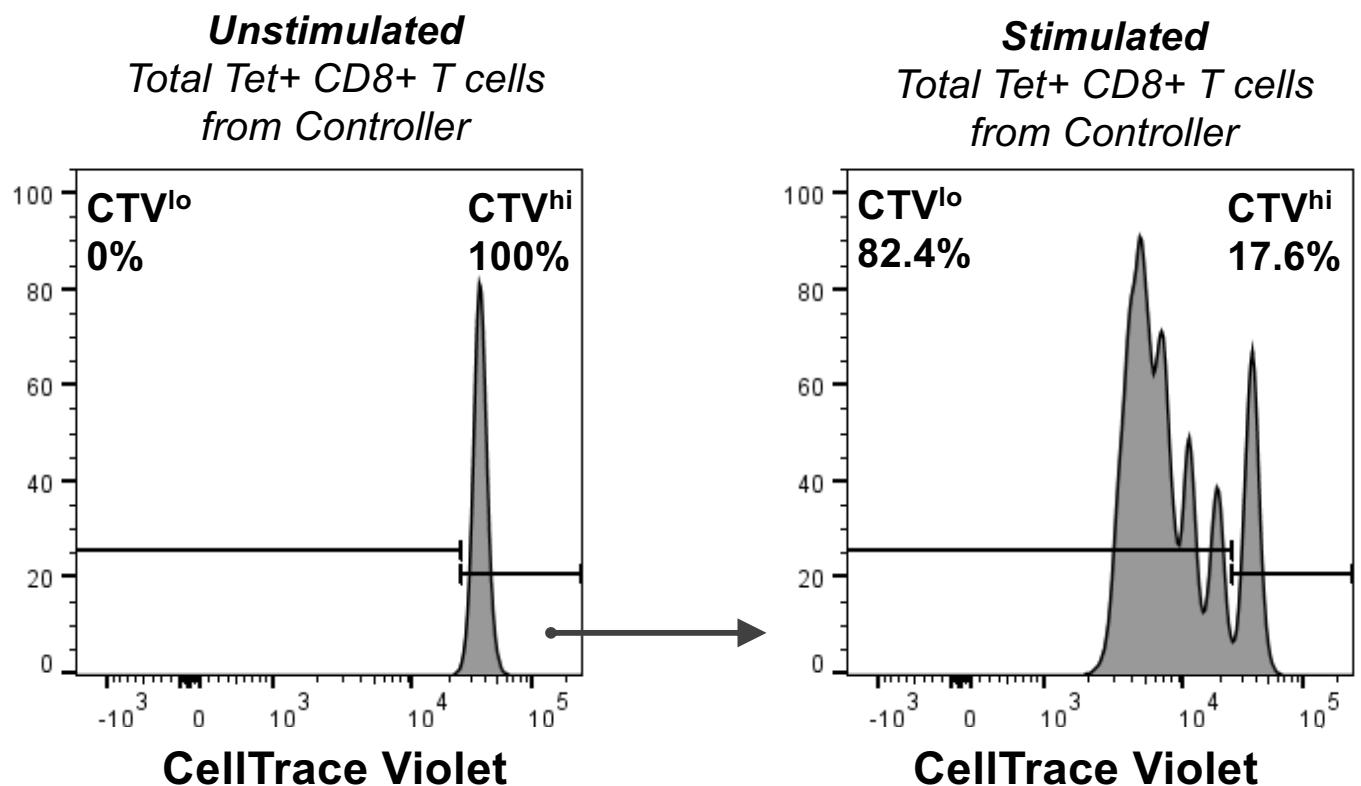
Supplemental Digital Content 7. Effector-memory subset distribution of tetramer+ HIV-specific CD8+ T cells. Tetramer+ CD8+ T cells were gated according to markers outlined in Figure 1.



Supplemental Digital Content 8. Correlations between MM, MMP, and ROS content of CD8+ T cell populations. MitoGREEN MFI, %JC-1+, and %CellROX+ of HIV-specific CD8+ T cells, TTM and TEM bulk CD8+ T cells. r and p -values calculated using Spearman's rank correlation test.



Supplemental Digital Content 9. MM, MMP, and ROS content of bulk TEM CD8+ T cell subpopulations. Bulk TEM CD8+ T cells from HIV-infected individuals gated according to the expression of PD-1 (A-C) and CD127 (D-F). P-values calculated using Wilcoxon signed-rank test. NS, no significant difference, *** $p < 0.001$. Bulk TEM CD8+ T cells from HIV-uninfected individuals gated according to co-expression of PD-1 and CD127 (G-I). P-values calculated by Friedman test.



Supplemental Digital Content 10. Gating strategy to identify proliferating cells. Proliferating tetramer⁺ HIV-specific CD8⁺ T cells were identified as cells that had reduced fluorescence of CellTrace Violet (CTV) dye. Gates to identify proliferating cells were individually set for each stimulated sample according to the first peak of CTV fluorescence in the corresponding unstimulated sample.