

Immune mobilising T cell receptors redirect polyclonal CD8⁺ T cells in chronic HIV infection to form immunological synapses

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Supplementary Information

Supplementary Table S1. Percentage reduction in Gag⁺ cells by ImmTAV-redirected CD8⁺ T cells*

	[ImmTAV]	m121	m121	m134	m134
Effector source		HIV+	HIV-	HIV+	HIV-
E:T = 1:1	10 nM	60% (20%)	87% (6%)	72% (14%)	82% (11%)
E:T = 1:10	10 pM	55% (17%)	63% (23%)	55% (15%)	71% (18%)

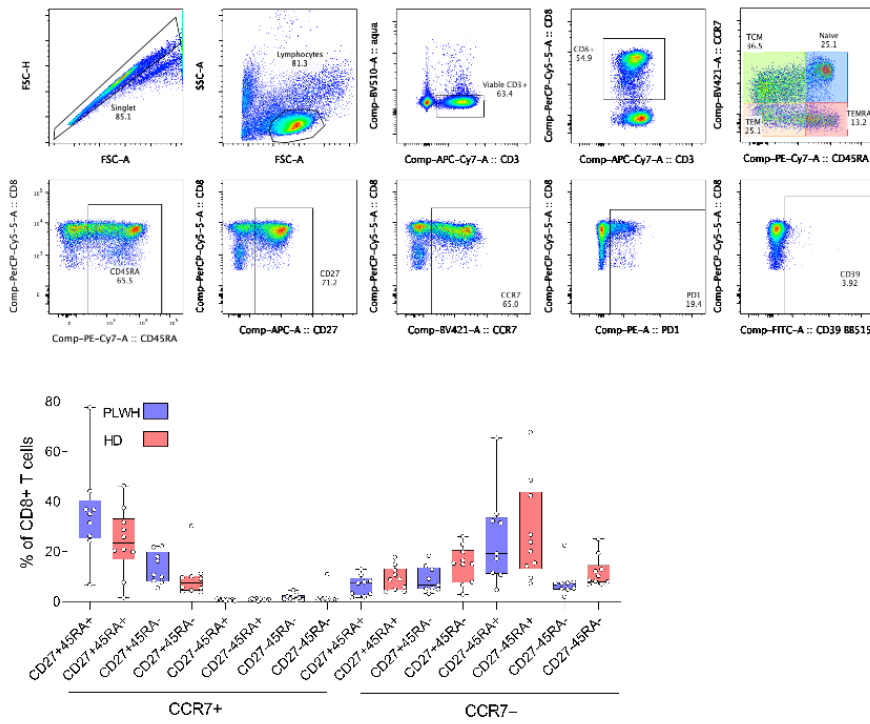
*Previously published in (Yang et al., 2016), indicate mean (SD).

Supplementary Table S2. PLWH cohort characteristics

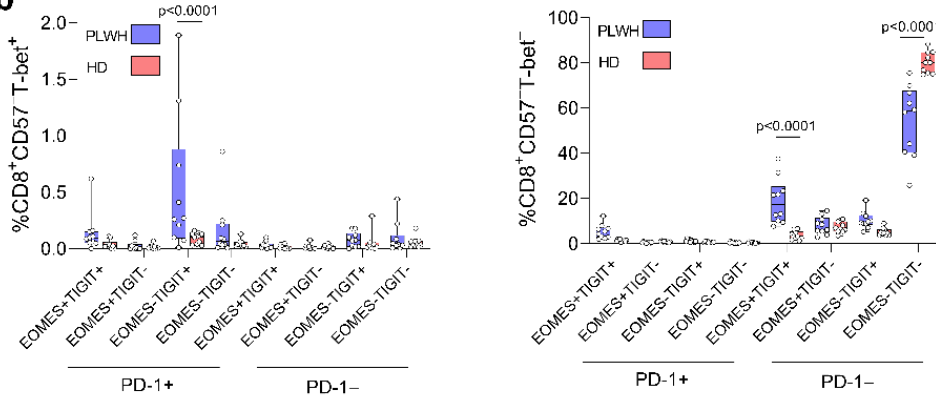
Donor ID	Gender	Age	ART duration (years)	CD4⁺ T cell count (cells/μl)
OX006	F	43	4.5	680
OX007	M	55	1	640
OX004	M	29	2	400
OX003	M	46	5	610
OX002	M	44	15	720
OX001	M	52	12	760
OX005	M	33	2	390
OX009	M	28	>1*	N/A
OX010	M	45	2	370
OX011	F	41	6	870
OX012	M	46	4	690
OX013	F	33	3	400
OX014	M	44	4.5	490
OX015	F	44	9	990
OX016	M	46	>1*	360
OX017	F	46	2	430
OX018	M	40	12	1060
OX019	M	46	2	730
OX020	M	46	2.5	390
OX021	M	55	12	2010
OX022	M	50	5.5	560
OX023	F	37	4	440
OX024	M	39	3	450
OX025	M	48	2	630
OX026	M	43	1.5	930
OX027	M	60	1	360
OX028	F	27	1.5	1270
OX029	M	N/A	7.5	N/A
OX008	F	33	4	720

* On ART for at least 1 year but exact duration not known

a

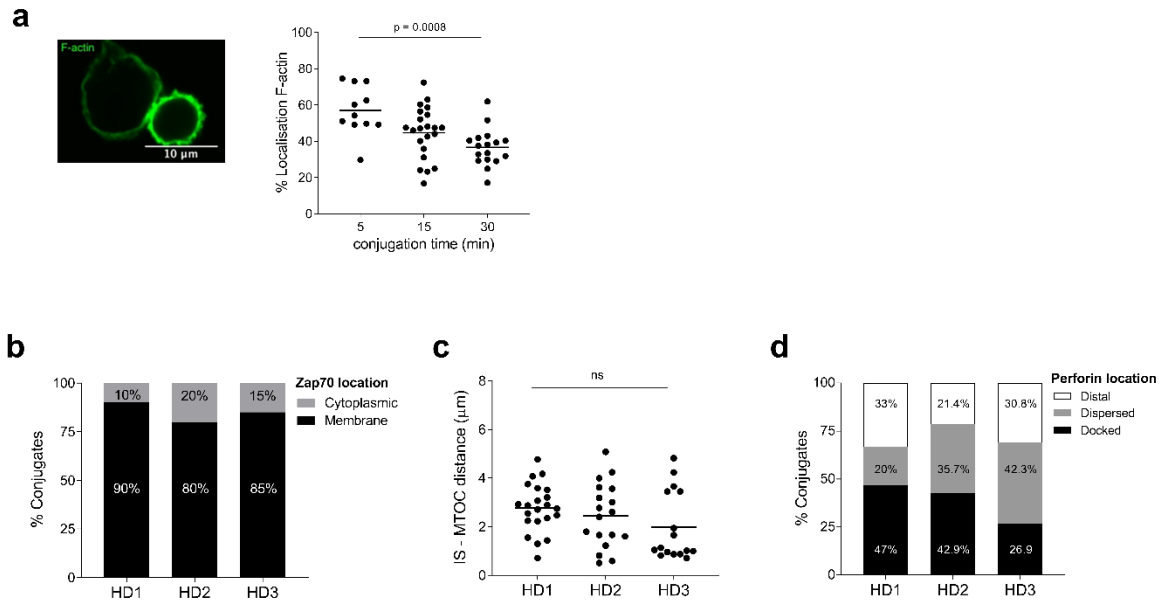


b



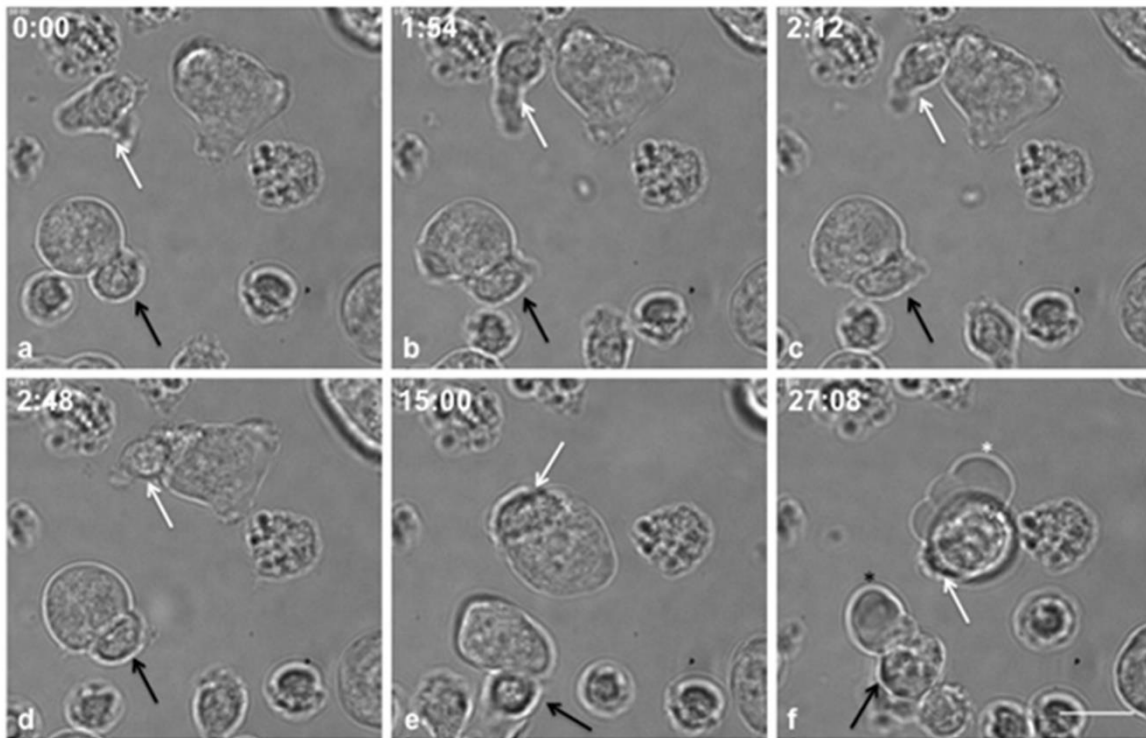
Supplementary Figure S1. Phenotype of PLWH and HD T cells.

CD8⁺ T cells from nine PLWH (blue) and ten HIV-naïve donors (red) were analysed for expression of (A) CD45RA, CCR7 and CD27 (effector memory or terminally differentiated populations - gating shown: top row shows gating for single, lymphocyte, alive CD3⁺CD8⁺ T cells and memory populations (CD45RA/CCR7); bottom row shows gating for CD45RA, CD27, CCR7, PD-1 and CD39 on CD8⁺ T cells) with no significant difference between PLWH and HD. A second set of nine PLWH donors (blue) and ten HIV-naïve donors (red) were analysed for expression of (B) CD57, Eomes, PD-1, T-bet and TIGIT (CD57⁻ population shown). Groups were analysed by 2-way ANOVA with Sidak's multiple comparisons. See Supplementary Table 2 for PLWH details.



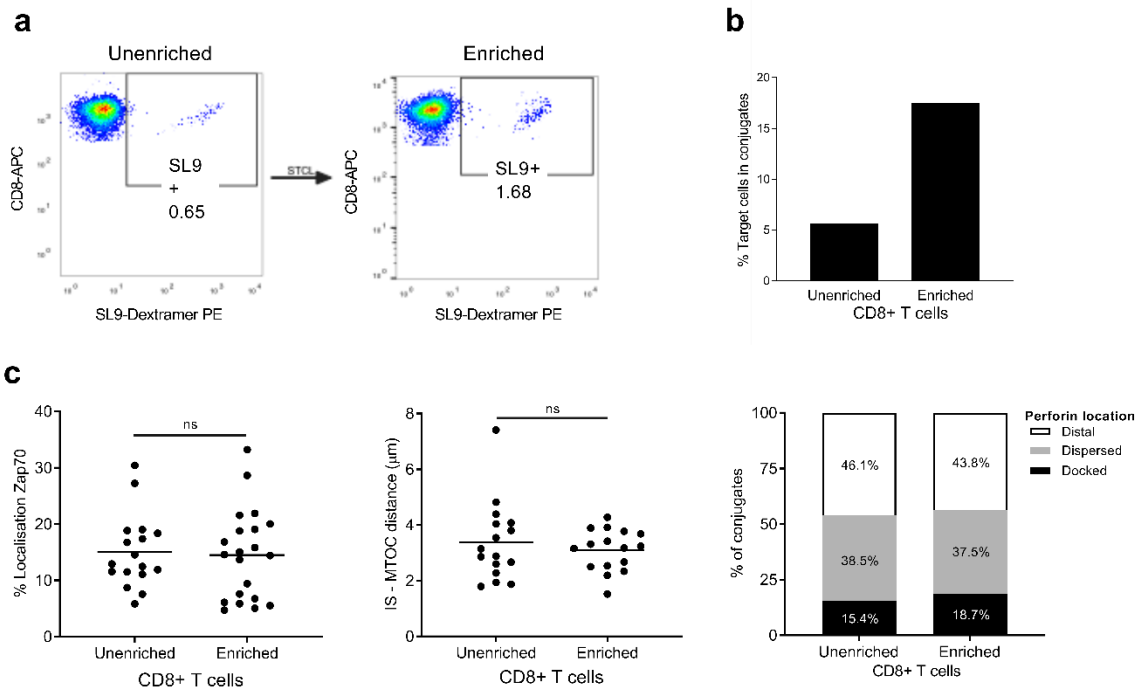
Supplementary Figure S2. Reproducibility of immune synapse formation with CD8⁺ T cells from three HIV-naïve donors (HD).

(A) Confocal microscopy image of bright F-actin expression in a conjugate formed between a HD CD8⁺ T cell/SL9-pulsed T2 cell and % localisation of F-actin at the IS over time (mean shown). Similar distribution of (B) Zap70, (C) MTOC and (D) perforin in CD8⁺ T cells from three HD when cultured with SL9-pulsed T2 cells at a ratio of 1:1 in the presence of ImmTAV (0.5 nM).



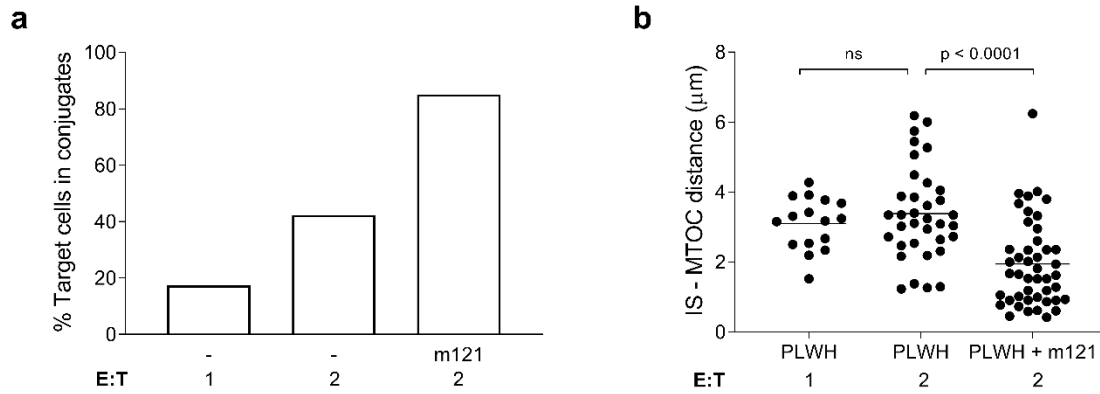
Supplementary Figure S3 and Video S1. Time-lapse microscopy of ImmTAV-redirection CD8⁺ T cell killing of virus-infected CD4⁺ T cells.

HIV-infected, HLA-A2 transduced Jurkat T cells were co-cultured with HD CD8⁺ T cells (E:T of 1:1 and m121 ImmTAV at 5 nM). Timestamp on each image is in relation to the start of imaging at 0:00, 15 minutes after co-culture began. Images show stages of synapse formation for top conjugate (white arrow marking CD8⁺ T cell) including (a) the migration of the CD8⁺ T cell, (b) the CD8⁺ T cell scanning the target, (c) the CD8⁺ T cell making contact with the target, (d) the formation of a tight synapse, (e) the CD8⁺ T cell roaming around the target and (f) the blebbing of the cell membrane of the target (*). Second conjugate already formed at 0:00 marked in black. Scale bar represents 10 μ m. See Video S1.



Supplementary Figure S4. Comparison of synapses formed with unenriched and enriched SLY-specific CD8⁺ T cells.

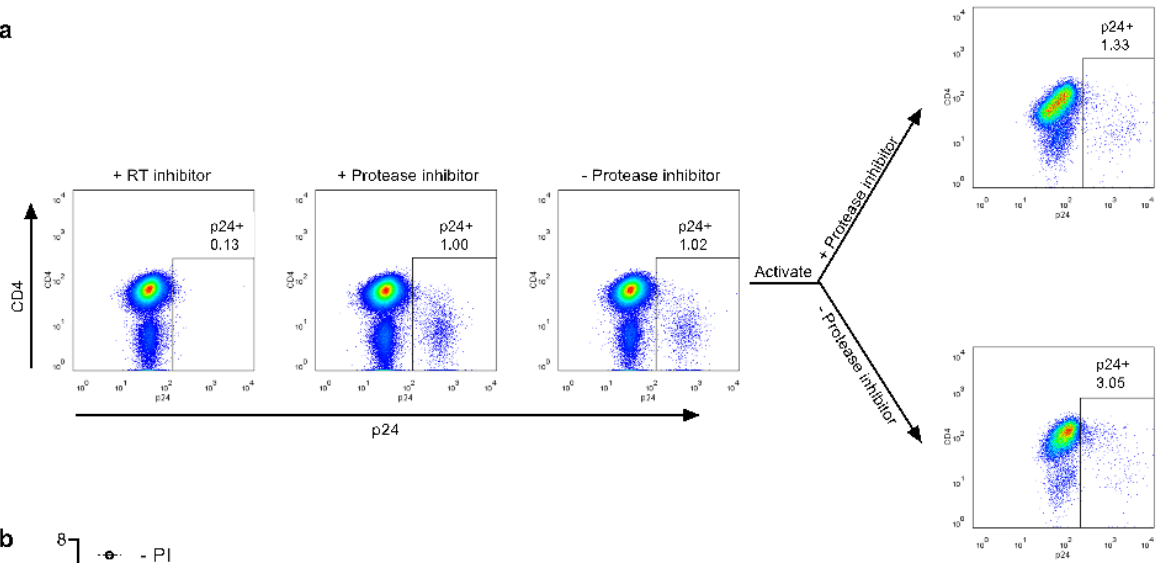
Enrichment of SLY-specific CD8⁺ T cells from PLWH donors as seen (A) by flow cytometry staining with an SLY-dextramer (B) increases the percentage of target cells in conjugates with SLY-pulsed T2s but does not affect (C) percentage localisation of Zap70 to the IS, localisation of the MTOC (μm from synapse) or perforin distribution (% of conjugates with perforin docked vs. dispersed vs. distal to the synapse). One donor shown (each dot represents one T2/CD8⁺ T cell conjugate); data were analysed by unpaired t test.



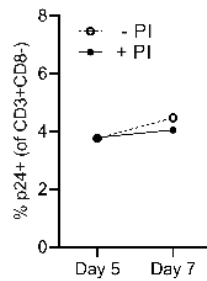
Supplementary Figure S5. Effect of E:T ratio and ImmTAV on conjugate formation.

Increasing the E:T or addition of ImmTAV to co-cultures of PLWH donor CD8⁺ T cells and SL9-pulsed T2s (A) increases the percentage of target cells in conjugates but (B) only addition of ImmTAV impacts the distance of the MTOC from the IS (µm). One donor shown (each dot represents one T2-CD8⁺ T cell conjugate); data were analysed by one-way ANOVA with multiple comparisons.

a



b



Supplementary Figure S6. Downregulation of CD4 in resting infected cells.

(A) Resting infected CD4⁺ T cells show downregulation of CD4 (y-axis) and expression of Gag p24 (x-axis) but do not show spreading infection (no difference in p24 expression when cultured +/- protease inhibitor (PI) until activated with PHA) (B) through the 7-day assay duration.