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Supplementary Materials for

Different human resting memory CD4$^+$ T cell subsets show similar low inducibility of latent HIV-1 proviruses

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at stm.sciencemag.org/cgi/content/full/12/528/eaax6795/DC1)

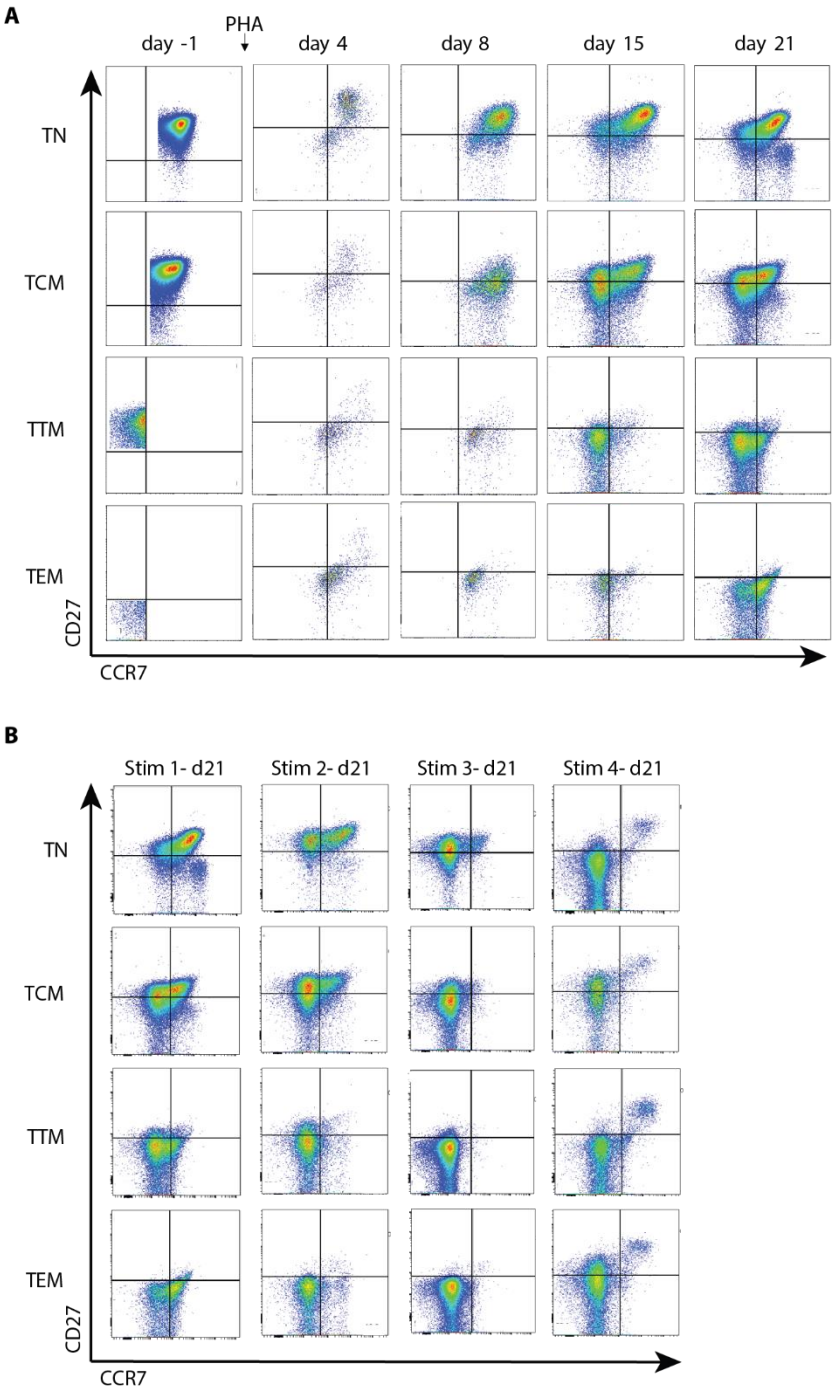

Fig. S1. Representative flow cytometry plots of T cell subset phenotypes after stimulation. (A) CCR7 and CD27 expression levels at time of sort and at 4, 8, 15, and 21 days after one stimulation from representative participant 2006. **(B)** CCR7 and CD27 expression levels 21 days after each respective stimulation from participant 2006.

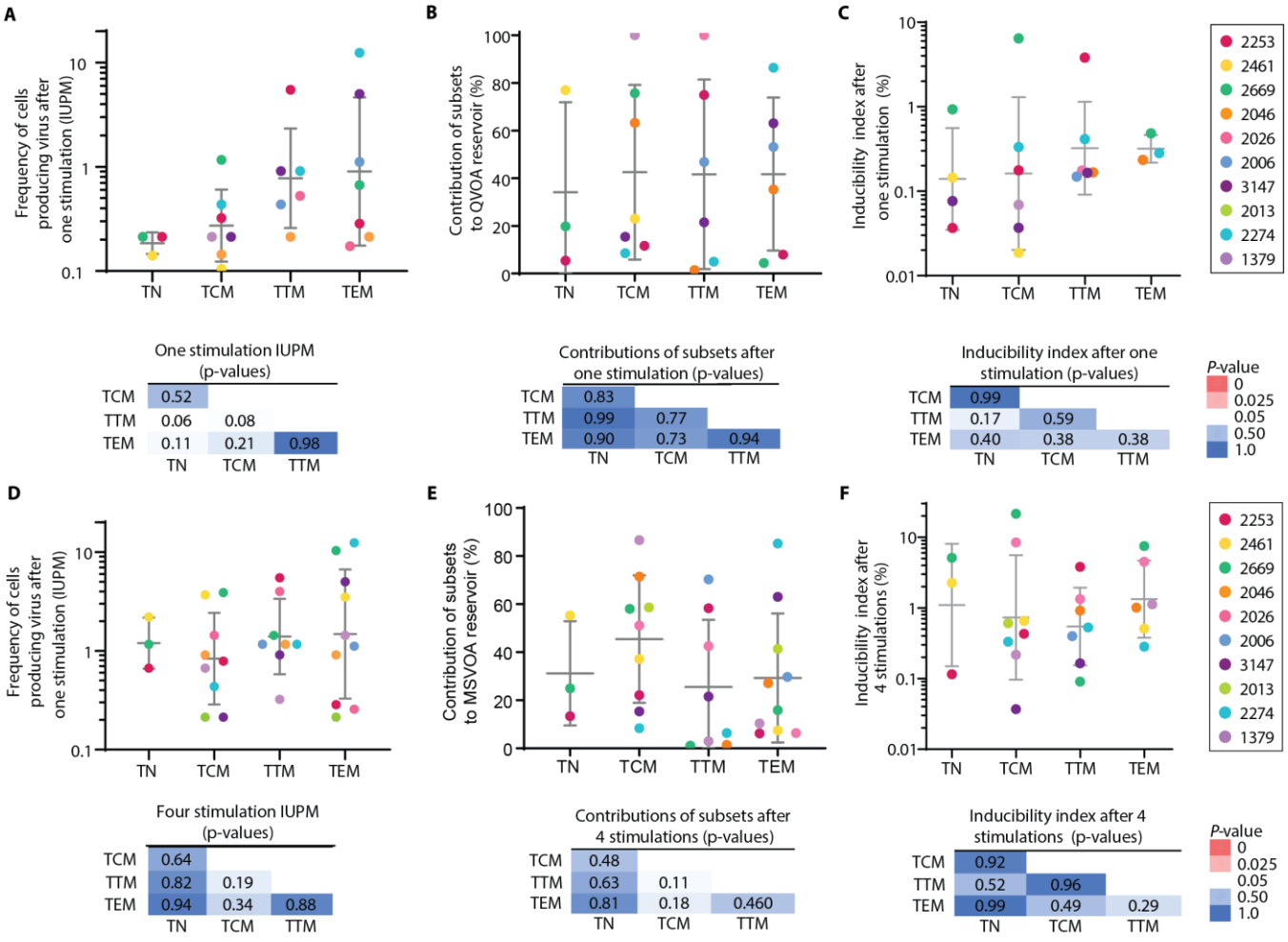

Fig. S2. Results of the multiple stimulation viral outgrowth assay and intact proviral DNA assay (excluding data points below the limit of detection). Data reanalyzed excluding samples where no intact proviruses were found (below limit of detection) or no viral outgrowth was observed. See Table S2 for raw data. Two-tailed *P*-values were calculated using Mann Whitney U-tests.

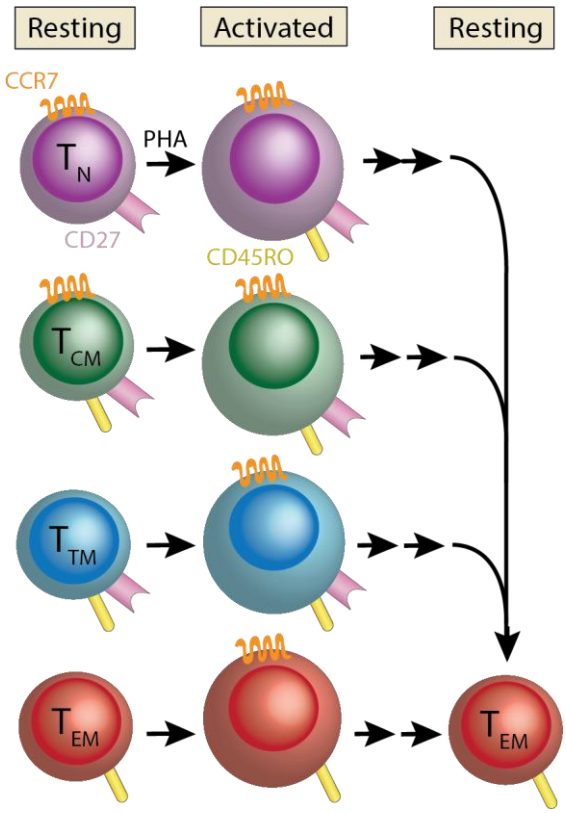

Fig. S3. Illustration of surface marker differences on resting versus activated CD4$^+$ T cell subsets. After activation with PHA, expression of the canonical subset-distinguishing surface markers changes. Naïve cells begin expressing CD45RO as they differentiate into memory cells. The initially resting subset cells differentiate toward the effector memory phenotype (CCR7$^-$CD27$^-$). TTM and TEM express CCR7 after activation. This highlights the importance of distinguishing resting vs total CD4$^+$ T cells which include activated cells. For example, the activated TTM cell exhibits the same surface markers as the activated TCM cell; even if they are functionally different, they would be sorted into the same population based on the canonical usage of CCR7 and CD27 to sort memory subsets.

Table S1. Characteristics of study participants.

SCOPE ID	Age	Sex	Ethnicity	Estimated minimum length of HIV infection at sample date (years)	Estimated minimum length of viral suppression at sample date (years)	CD4 Nadir (cells/µl)	CD4 count at sample date (cells/µl)	Viral Load at sample date (copies/ml)	ARV Regimen[a]
2253	67	M	White	18	3.4[b]	376	500	<40	ABC/TCV/3TC
2461	64	M	White	34	18.7	408	531	<40	RPV, TCV
2669	59	M	White	28	7.9[c]	180	516	<40	ABC/TCV/3TC
2046	54	M	Pacific Islander	26	19.9	10	456	<40	EGV/TAF/FTC/COBI
2026	64	M	White	30	17.6	132	377	<40	ABC/TCV/3TC
2006	68	M	White	23	21.0	11	428	<40	ABC/TCV/3TC
3147	62	M	Latino	25	10.8[d]	4	1089	<40	ABC/TCV/3TC
2013	70	M	White	32	21.9	13	588	<40	ABC/TCV/3TC
2274	57	M	White	16	14.9	234	383	<40	NVP, FTC/TAF
1379	50	M	African American	13	8.3	208	612	<40	DRV, RTV, FTC/TDF

[a] ABC= abacavir, COBI= cobicistat, DRV= darunavir, EGV= elvitegravir, FTC=emtricitabine, NVP= nevirapine, RPV= rilpivirine, RTV= ritonavir, TAF= tenofovir alafenamide, TCV= dolutegravir, TDF= tenofovir disoproxil fumarate, 3TC= lamivudine

[b] Minimum length of viral suppression calculated after two non-consecutive blips of 68 c/mL (7/17/2014) and 217 c/mL (8/07/2014) were documented during an experimental study of PRO-140 monotherapy. Otherwise, fully suppressed on standard regimen since 8/16/2006.

[c] Per participant report, viral suppression maintained since 2000, but earliest available documented viral loads start in 2010. Estimated length of minimal viral suppression is based on documented viral loads.

[d] Minimum length of viral suppression calculated after isolated blip of 620 c/mL on 6/27/2007, otherwise suppressed since 8/20/2001.

Table S2. Frequencies of proviral copies, infection frequencies, and assay input cell numbers for samples below the limit of detection. Raw data for samples with no detected intact proviral copies by IPDA or no viral outgrowth seen after 4 stimulations (highlighted cells). Cell inputs for IPDA and MSVOA are shown.

Participant ID	Subset	3' del/hyp copies per million cells	5' del copies per million cells	ICPM	QVOA IUPM	MSVOA IUPM	IPDA Total Cell Input	MSVOA Total Cell Input
2253	EM	1470	5603	0	0.29	0.29	$1.8x10^4$	$7.2x10^6$
2461	TM	1250	0	0	N/A	N/A	$1.5x10^3$	N/A
2046	N	14	0	0	0.14	0.14	$1.1x10^5$	$4.8x10^6$
2046	CM	492	2503	0	0.14	0.91	$1.5x10^4$	$4.8x10^6$
2026	N	181	399	0	0.17	0.17	$3.5x10^4$	$4.8x10^6$
2006	N	209	503	0	0.14	0.14	$5.2x10^4$	$4.8x10^6$
2006	CM	397	592	111	0.14	0.14	$3.1x10^4$	$4.8x10^6$
2006	EM	6469	328	0	1.12	1.12	$1.8x10^4$	$2.0x10^6$
3147	N	218	322	279	0.14	0.14	$3.2x10^4$	$4.8x10^6$
3147	EM	1251	1469	0	5.00	5.00	$7.5x10^4$	$8.0x10^6$
2013	N	88	436	0	0.14	0.14	$1.4x10^5$	$4.8x10^6$
2013	TM	513	82	0	0.29	0.29	$1.8x10^4$	$4.8x10^6$
2013	EM	363	187	0	0.14	0.21	$2.1x10^5$	$2.4x10^6$
2274	N	88	0	0	0.14	0.14	$4.3x10^4$	$4.8x10^6$
1379	N	272	272	200	0.14	0.14	$3.3x10^4$	$4.8x10^6$
1379	TM	4985	7315	0	0.22	0.32	$2.8x10^4$	$3.2x10^6$

Table S3. Frequencies of proviral copies, infection frequencies, and inducibility indices for all T cell subsets from all 10 participants. For samples where no proviral copies were detected, the LOD (4 copies/10^6 cells) was used in calculations.

Patient	Subset	Subset Frequency in Peripheral Blood (%)	3' del/hyp copies per million cells	5' del copies per million cells	ICPM	QVOA IUPM	MSVOA IUPM
2253	N	24.9	4699	7068	852	0.21	0.67
	CM	34.9	5452	7139	269	0.32	0.79
	TM	13.2	2796	2817	212	5.49	5.49
	EM	27.0	1470	5603	0	0.29	0.29
2461	N	67.2	280	424	143	0.14	2.20
	CM	26.9	1575	901	824	0.11	3.70
	TM	0.2	1250	0	0	N/A	N/A
	EM	5.7	62	138	1008	0.29	3.50
2669	N	56.0	635	412	34	0.21	1.17
	CM	39.0	26	50	27	1.17	3.90
	TM	2.0	19235	6602	2342	0.87	1.44
	EM	4.0	3848	18419	203	0.67	10.40
2046	N	75.4	14	0	0	0.14	0.14
	CM	17.6	492	2503	0	0.14	0.91
	TM	0.3	884	610	188	0.21	1.17
	EM	6.7	821	3538	133	0.21	0.91
2026	N	40.0	181	399	0	0.17	0.17
	CM	30.0	65	94	25	0.14	1.44
	TM	9.0	2539	2513	441	0.53	3.99
	EM	21.0	10	123	8	0.17	0.26
2006	N	41.3	209	503	0	0.14	0.14
	CM	48.2	397	592	111	0.14	0.14
	TM	7.3	1957	1243	430	0.44	1.17
	EM	3.2	6469	328	0	1.12	1.12
3147	N	43.5	218	322	279	0.14	0.14
	CM	37.7	4851	7804	848	0.21	0.21
	TM	12.3	7651	20722	814	0.91	0.91
	EM	6.6	1251	1469	0	5.00	5.00
2013	N	60.4	88	436	0	0.14	0.14
	CM	21.0	255	2110	52	0.14	0.21
	TM	3.9	513	82	0	0.29	0.29
	EM	14.8	363	187	0	0.14	0.21
2274	N	24.4	88	0	0	0.14	0.14
	CM	46.2	1552	1901	192	0.44	0.44
	TM	13.0	3803	3486	324	0.91	1.17
	EM	16.4	2621	2156	6461	12.42	12.42
1379	N	45.4	272	272	200	0.14	0.14
	CM	48.5	4113	5229	453	0.21	0.67
	TM	3.5	4985	7315	0	0.22	0.32
	EM	2.7	4013	10897	188	0.29	1.44
HD1	N	38.5	N/A				
	CM	40.7					
	TM	7.2					
	EM	13.6					