Persistence of an intact HIV reservoir in phenotypically naive T cells

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Supplementary Data

Table S1. Clinical characteristics of the subjects enrolled in the study

Subject	Age*	Sex	Year of infection	CD4 T-cell nadir (cells/µl (year))	Viral load zenith (copies/ml (year))
1	47	M	1984	295 (1998)	114,422 (1998)
2	36	M	1993	0 (1999)	225,000 (2001)

*Age at the time of first apheresis collection

Abbreviations: M, man

Table S2. Virological and immunological parameters at two time points after ART initiation in the subjects enrolled in the study

Subject	Year	Time on ART (years)	ART	Viral load (copies/ml)	CD4 T-cells (cells/µl)
1	2008	1.9	ATV/r TDF/FTC	<50	617
	2015	8.2	ATV/r TDF/FTC	<20	718
2	2007	2.1	ATV/r 3TC D4T	<50	629
	2015	9.9	ATV/r 3TC RAL	<40	768

Abbreviations: ABC, abacavir; ART, antiretroviral therapy; ATV, atazanavir; D4T, stavudine; r, ritonavir; RAL, raltegravir; TDF/FTC, tenofovir/emtricitabine; 3TC, lamivudine.

	Subject 1 (Half-lives with 95% C.I., years)			
	Intact	Intact (repeated sequences removed)	Defective	Defective (repeated sequences removed)
T_N	2.8 (1.8-5.5)	2.5 (1.6-4.8)	(9.4-Inf)	(9.7-Inf)
T_{CM}	(2.5-Inf)	(1.7-Inf)	25 (13-273)	17 (9.7-62)
T_{TM}	(0.9-Inf)	(0.9-Inf)	(11-Inf)	7.3 (4.8-16)
T_{EM}	(1.5-Inf)	(0.9-Inf)	(14-Inf)	5.2 (3.4-10)
CD45RA$_{dim}$	1.8 (0.8-7.8)	1.8 (0.8-7.7)	(30-Inf)*	(6.4-Inf)*
	Subject 2 (Half-lives with 95% C.I., years)			
	Intact	Intact (repeated sequences removed)	Defective	Defective (repeated sequences removed)
T_N	2.8 (1.6-6.6)	2.8 (1.6-6.6)	(68-Inf)*	(47-Inf)*
T_{CM}	2.6 (1.0-89)	2.6 (1.0-88)	(34-Inf)*	(13-Inf)
T_{TM}	(2.3-Inf)	(2.6-Inf)	(7.4-Inf)*	(10-Inf)*
T_{EM}	(3.8-Inf)	2.1 (0.9-10)	6.4 (4.4-12)	3.3 (2.3-5.4)
CD45RA$_{dim}$	(3.7-Inf)	(1.8-Inf)	(27-Inf)*	(14-Inf)

*Median fit is actually growth, not decay

Table S3. Half-life of intact and defective proviruses in cellular subsets before and after removing repeated sequences.
The median decay rate is reported only for those subsets with a statistically significant decay rate.
Data are reported in years (95% confidence interval (C.I.)). Confidence intervals were computed as exact posteriors, using log-normal variance computed from repeated measures for qPCR measurements and Binomial statistics for intact vs. defective ratios as in (1)

(1) Luo R, Piovoso MJ, Zurakowski R. Modeling uncertainty in single-copy assays for

HIV. *J Clin Microbiol.* 2012;50(10):3381-3382. doi:10.1128/JCM.01254-12

	Subject 1: 2 years						Subject 2: 2 years						
	T_N	T_{CM}	T_{TM}	T_{EM}	CD45RA$_{dim}$		T_N	T_{CM}	T_{TM}	T_{EM}	CD45RA$_{dim}$		
Proviral sequences (N)	76	100	66	91	32		48	52	45	70	48		
Unique sequences (N)	75	89	46	51	29		41	39	17	29	37		
% repeated sequences	1.3	11	30	44	13		15	25	62	59	23		
Intact proviral sequences (N)	23	2	1	3	3		10	3	7	10	4		
Repeated sequences among intact proviruses (N)	0	0	0	0	0		0	0	4	6	2		

	Subject 1: 9 years						Subject 2: 9 years						
	T_N	T_{CM}	T_{TM}	T_{EM}	CD45RA$_{dim}$	CD95-T_N	T_N	T_{CM}	T_{TM}	T_{EM}	CD45RA$_{dim}$	CD95-T_N	T_{SCM}
Proviral sequences (N)	83	100	128	118	95	53	89	82	72	80	83	215	91
Unique sequences (N)	82	80	57	27	45	53	74	51	38	12	56	203	71
% clones	1.2	20	55	79	53	0	17	38	47	85	33	5.6	22
Intact proviral sequences (N)	6	2	0	1	0	3	2	0	4	14	5	8	3
Repeated sequences among intact proviruses (N)	1	0	0	1	0		0	0	1	14	4	1	1

Table S4. Number of proviruses sequenced in cellular subsets at two time points and percentage of repeated sequences among total and intact proviruses
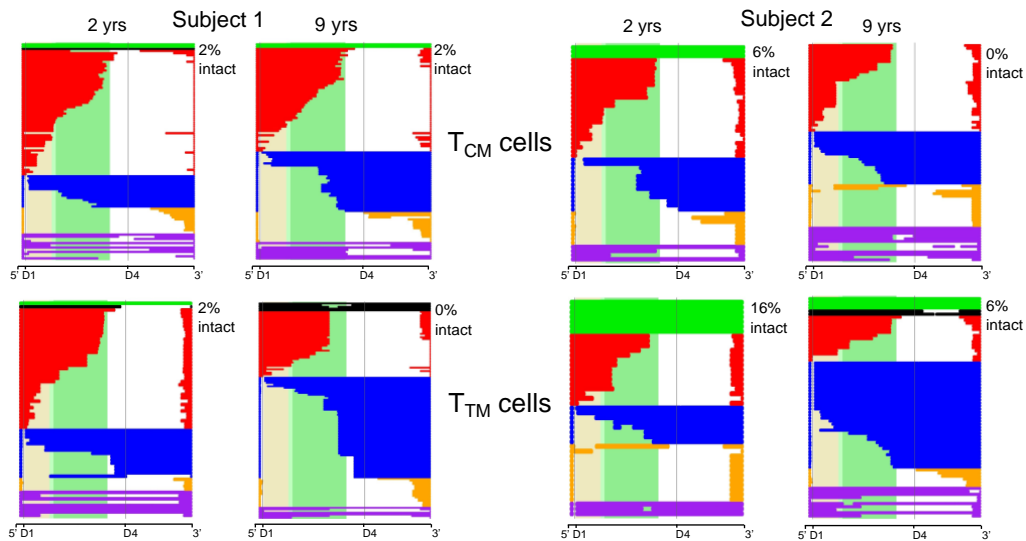
Abbreviations: T_N: Naive CD4 T cells; T_{CM}: Central memory CD4 T cells; T_{TM}: Transitional memory CD4 T cells; T_{EM}: Effector memory CD4 T cells; T_{SCM}: Stem-cell like memory CD4 T cells; yrs: years after ART initiation.

	Subject 1					
		Deleted				
	Intact	D1+D4+	D1+D4-	D1-D4+	D1-D4-	Hypermutated
T$_N$ 2 yrs	23 (30%)	9 (12%)	21 (28%)	10 (13%)	7 (9%)	6 (8%)
T$_{CM}$ 2 yrs	2 (2%)	1 (1%)	58 (58%)	15 (15%)	12 (12%)	12 (12%)
T$_{TM}$ 2 yrs	1 (2%)	1 (2%)	37 (56%)	15 (23%)	4 (6%)	8 (12%)
T$_{EM}$ 2 yrs	3 (3%)	12 (13%)	26 (29%)	39 (43%)	5 (5%)	6 (7%)
CD45RA$_{dim}$ 2 yrs	3 (9%)	1 (3%)	15 (47%)	9 (28%)	3 (9%)	1 (3%)
T$_N$ 9 yrs	6 (7%)	1 (2%)	44 (53%)	17 (20%)	5 (6%)	10 (12%)
T$_{CM}$ 9 yrs	2 (2%)	0 (0%)	48 (48%)	28 (28%)	14 (14%)	8 (8%)
T$_{TM}$ 9 yrs	0 (0%)	5 (4%)	39 (30%)	60 (47%)	17 (13%)	7 (5%)
T$_{EM}$ 9 yrs	1 (1%)	2 (2%)	23 (19%)	83 (70%)	2 (2%)	7 (6%)
CD45RA$_{dim}$ 9 yrs	0 (0%)	0 (0%)	29 (31%)	58 (61%)	4 (4%)	4 (4%)
CD95- T$_N$ 9 yrs	3 (6%)	0 (0%)	27 (51%)	13 (25%)	6 (11%)	4 (8%)

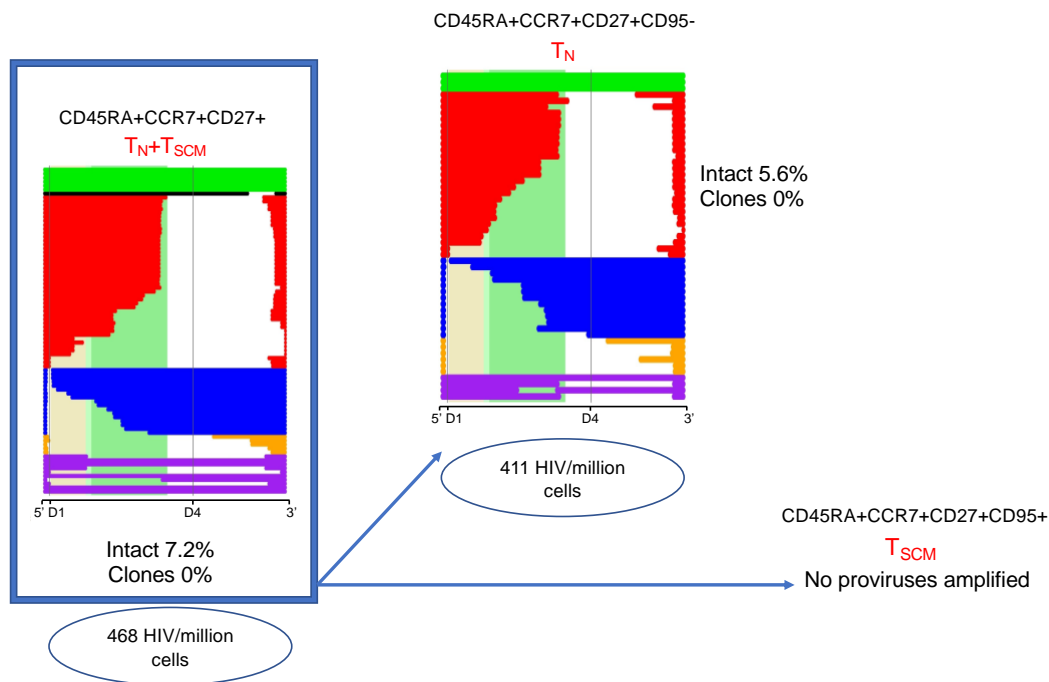|	**Subject 2**					
		Deleted				
	Intact	**D1+D4+**	**D1+D4-**	**D1-D4+**	**D1-D4-**	**Hypermutated**
T$_N$ 2 yrs	10 (21%)	11 (23%)	9 (19%)	14 (29%)	3 (6%)	1 (2%)
T$_{CM}$ 2 yrs	3 (6%)	0 (0%)	24 (46%)	13 (25%)	8 (15%)	4 (8%)
T$_{TM}$ 2 yrs	7 (16%)	0 (0%)	15 (33%)	8 (18%)	12 (27%)	3 (7%)
T$_{EM}$ 2 yrs	10 (14%)	1 (1%)	32 (46%)	16 (23%)	6 (9%)	5 (7%)
CD45RA$_{dim}$ 2 yrs	4 (8%)	1 (2%)	23 (48%)	16 (34%)	3 (6%)	1 (2%)
T$_N$ 9 yrs	2 (2%)	0 (0%)	45 (51%)	34 (38%)	6 (7%)	2 (2%)
T$_{CM}$ 9 yrs	0 (0%)	0 (0%)	33 (40%)	20 (24%)	16 (20%)	13 (16%)
T$_{TM}$ 9 yrs	4 (6%)	2 (3%)	15 (21%)	35 (49%)	6 (8%)	10 (14%)
T$_{EM}$ 9 yrs	14 (18%)	0 (0%)	1 (1%)	48 (60%)	1 (1%)	16 (20%)
CD45RA$_{dim}$ 9 yrs	5 (6%)	1 (1%)	18 (22%)	41 (49%)	12 (14%)	6 (7%)
CD95- T$_N$ 9 yrs	8 (4%)	6 (3%)	83 (38%)	71 (33%)	28 (13%)	19 (9%)
T$_{SCM}$ 9 yrs	3 (3%)	2 (2%)	41 (45%)	34 (37%)	3 (3%)	8 (9%)

Table S5. Number of proviruses sequenced at two time points based on proviral categories

Abbreviations: T$_N$: Naive CD4 T cells; T$_{CM}$: Central memory CD4 T cells; T$_{TM}$: Transitional memory CD4 T cells; T$_{EM}$: Effector memory CD4 T cells; T$_{SCM}$: Stem-cell like memory CD4 T cells; yrs: years after ART initiation.

Figure S1. Deletion map analysis of T_CM and T_TM cells reveals a genetic make-up in between T_N and T_EM cells. DNA was purified and sequenced from sorted T_CM and T_TM cells. The sequenced amplicons were then analyzed using our bioinformatic pipeline. Intact and defective proviruses from T_CM and T_TM cells were aligned to HXB2 at an early and late time point. Green proviruses are intact (D1+D4+), black proviruses are nearly intact (D1+D4+), red proviruses are 3' deleted (D1+D4−), blue proviruses are 5' deleted (D1−D4+), and yellow proviruses are massively deleted (D1−D4-). Hypermutated proviruses are represented in purple. The shaded beige, light green, and dark green regions correspond to the gag, gag-pol, and pol regions of HXB2, respectively. The percentage of intact proviruses is reported in the upper right corner of each deletion map.
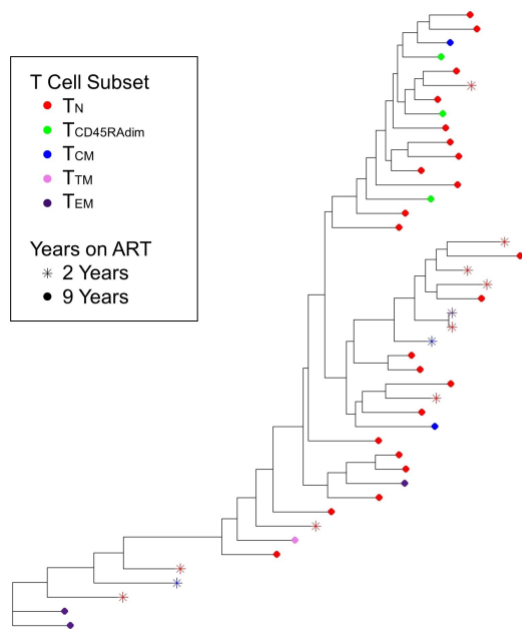
Abbreviations: T_CM: central memory CD4 T cells; T_TM: transitional memory CD4 T cells; yrs: years after ART initiation.
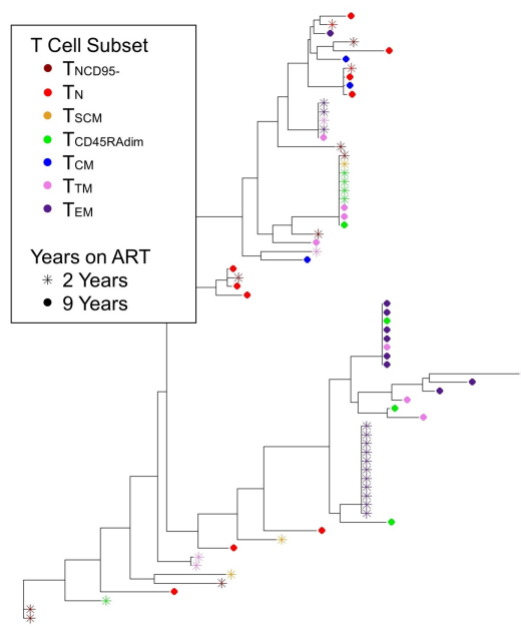
Figure S2. Deletion map of T~N~ *vs.* T~SCM~ cells for Subject 1. The sort experiment described in Figure 1 was repeated separating T~SCM~ from CD95-T~N~ cells. CD95-T~N~ cells were defined as CD45RA+CCR7+CD27+CD95- while T~SCM~ cells were CD45RA+CCR7+CD27+CD95+. Proviral DNA could not be sequenced from T~SCM~ cells due to the extremely low levels of sorted cells. Proviral sequences retrieved from CD95-T~N~ cells showed a similar percentage of intact HIV compared to CD45RA+CCR7+CD27+ T~N~ cells.

Abbreviations: T~N~, Naive CD4 T cells; T~CM~, Central memory CD4 cells; T~TM~, Transitional memory CD4 T

cells; T~EM~, Effector memory CD4 T cells; T~SCM~, Stem-cell like memory T cells.

Figure S3. Phylogenetic tree of intact proviruses for Subject 1 (panel A) and Subject 2 (panel B). The phylogenetic tree included 44 intact proviral sequences for Subject 1 and 70 sequences for Subject 2. For Subject 1, we identified a proviral clone in T_N and T_{EM} cells at the second time point. For Subject 2, 4 clones were identified. For both subjects, identical intact proviral sequences were found among T_N cells and more differentiated subsets, suggesting the potential for naive cells to repopulate the memory reservoir.

Abbreviations: T_N, Naive CD4 T cells; T_{CM}, Central memory CD4 cells; T_{TM}, Transitional memory CD4 T cells; T_{EM}, Effector memory CD4 T cells; T_{SCM}, Stem-cell like memory T cells.