

Supplemental Figure 1: Identification of polarized memory CD4 T cells. (A): Flow cytometric gating strategy for identifying Th1, Th2, Th9 and Th17 memory CD4 T cells. (B): Pie charts reflecting relative contribution of Th1, Th2, Th9, Th17 and Thneg to memory CD4 T cell pool in different study cohorts. (C): Pie charts indicating relative contributions of indicated CD4 T cell populations to total CD4 T cell pool (left pie), and to total viral reservoir in CD4 T cells (right pie). (D): Proportions of cytokine-negative Th cells (Th-neg) in the indicated study cohorts. Significance was tested using a Kruskal-Wallis test with Dunn's multiple comparisons test.

Supplemental Figure 2



Supplemental Figure 2: Clonal expansion of cells harboring sequence-defective HIV-1. Bar diagram reflects number of identical, defective HIV-1 sequences in 41 different clusters. See supplemental Table 3 for details.

Supplemental Figure 3



Supplemental Figure 3: Analysis of HIV-1 DNA sequencing products from individuals with acute HIV-1 infection. Diagrams reflect genomic position of base pair variants between near full-length HIV-1 sequences collected from the two individuals with acute HIV-1 infection.

Supplemental Table 1a: Clinical and demographical characteristics of all study patients.

cohort	number of	age	Gender	CD4 T cell count	Viral load
	subjects		(% of male)	(cells/ul)	(HIV-1 RNA copies/ml)
Patients with acute	10	36	90%	768	18,150
HIV-1 infection		(21-67)		(549-905)	(178-22,300,000)
Chronic HIV-1	9	46	100%	387	44,500
progressors		(32-55)		(2-773)	(1,290-310,000)
ART-treated HIV-1	16	51	69%	876	<20
patients		(29-63)		(426-1475)	
HIV-1 controllers	22	52	89%	1006	75
		(26-66)		(700-1746)	(<20-5780)

Supplemental Table 1b: Clinical and demographical characteristics of five ART-treated patients selected for near full-length viral sequencing.

patient	age	Gender	time between HIV diagnosis	CD4 T cell count	Viral load	Time with continuous
			and treatment initiation	(cells/ul)	(HIV-1 RNA copies/ml)	suppressive HAART treatment
1	55	М	11 years	612	<20	13 years
2	59	М	12 years	599	<20	8 years
3	39	М	1 year	570	<20	4 years
4	nd	F	2 days	851	3800 copies/ml	0 days (started treatment on
						day of first PBMC sampling)
5	nd	F	3 days	731	<20	7 days (at the time of first
						PBMC sampling)

Supplemental Table 2. Frequency of intact HIV-1 sequences in Th1 vs non-Th1 cells in each study patient

Subject 1	Intact	Intact Defective			
Th1		8	125		
Non-Th1		4	353		

Fisher 2-sided p-value = 0.004385

Subject 2	Intact		Defective
Th1		7	17
Non-Th1		5	95

Fisher 2-sided p-value = 0.001831

Subject 3	Intact		Defective
Th1		2	0
Non-Th1		0	5

Fisher 2-sided p-value = 0.04762

Supplemental Table 3: Identification of identical HIV-1 sequences in ART-treated HIV-1 patients

							Number of ider	ntical sequenc	es per cell com	npartment							
Patient	Cluster	number of identical sequences	Length (bp)	position in HIV-1 genome (HXB2)	Sequence-Type	PBMC (2016)	CD4+ (2016)	Th1 (2016)	Th2 (2016)	Th9 (2016)	Th17 (2016)	Thneg (2016)	PBMC (2007)	PBMC (2008)	PBMC (2009)	PBMC (2011)	PBMC (2015)
Subject 1	1	2	9019	638→9632	5'DEFECT	0	1	0	0	0	0	1	NA	NA	0	0	NA
	2	3	9060	638 ightarrow 9632	Hypermut	1	1	0	1	0	0	0	NA	NA	0	0	NA
	3	2	8603	$1108 \rightarrow 9632$	Hypermut	0	1	1	0	0	0	0	NA	NA	0	0	NA
	4	3	9057	$638 \rightarrow 9632$	Hypermut	0	1	0	0	1	0	0	NA	NA	0	1	NA
	5	4	9073	638→9632	Hypermut	0	1	0	1	1	0	1	NA	NA	0	0	NA
	6	9	9105	638→9632	Hypermut	1	2	0	0	0	0	0	NA	NA	6	0	NA
	/	3	9104	638→9632	Hypermut	0	0	0	0	0	0	1	NA	NA	1	1	NA
	8	2	9104	638→9632	Hypermut	0	0	1	0	0	0	0	NA	NA	1	0	NA
	9	2	9091	000→9002 620 .0622	Hypermut	0	0	/	0	0	0	0	NA NA	NA NA	0	0	NA NA
	10	2	9085	030→9032 638→9632	Hypermut	0	0	3	2	0	0	0	NA	NA	0	0	NA
	12	3	9073	638 <u>→</u> 9632	Hypermut	1	0	0	1	0	0	0	NA	NΔ	1	0	NA
	13	2	9082	638→9632	Hypermut	0	1	1	0	0	0	0	NA	NA	0	ő	NA
	14	2	9079	638→9632	Hypermut	õ	0 0	ò	2	õ	õ	Ő	NA	NA	õ	õ	NA
	15	4	9106	638→9632	Hypermut	0	0	õ	ō	õ	õ	1	NA	NA	3	õ	NA
	16	8	9138	638→9632	Intact	0	2	4	0	0	0	1	NA	NA	0	1	NA
	17	2	1603	638→2228	Large deletion	0	0	1	0	1	0	0	NA	NA	0	0	NA
	18	4	3742	$638 \rightarrow 4286$	Large deletion	1	0	1	0	1	0	0	NA	NA	0	1	NA
	19	2	1365	638→1992	Large deletion	2	0	0	0	0	0	0	NA	NA	0	0	NA
	20	3	1683	638→2320	Large deletion	1	0	0	0	2	0	0	NA	NA	0	0	NA
	21	2	1173	1→601	Large deletion	0	0	2	0	0	0	0	NA	NA	0	0	NA
	22	15	1629	638→2274	Large deletion	0	0	0	0	15	0	0	NA	NA	0	0	NA
	23	2	3743	638→4286	Large deletion	0	0	0	0	0	0	1	NA	NA	0	1	NA
	24	2	1629	638→2262	Large deletion	0	0	0	0	2	0	0	NA	NA	0	0	NA
Subject 2	1	2	8573	1049→9632	5'DEFECT	1	0	0	0	0	0	0	0	0	1	NA	NA
	2	2	8982	638→9632	Hypermut	0	1	0	0	0	0	1	0	0	0	NA	NA
	3	2	8989	638→9633 638 0633	Hypermut	0	0	0	0	0	0	0	0	1	1	NA	NA
	4	9	9004	038→9032 638 .0633	Intact	1	1	5	1	0	0	1	0	0	0	NA	NA
	5	2	7402	030→9032 620 .0622	Indui	1	1	2	0	0	0	0	0	0	0	N/A	NA NA
	7	2	7493	030→9032 2555 \0632		0	5	1	4	0	0	6	1	0	1	NA	NA
	8	21	1047	2333→9032 638→1668	Large deletion	1	0	0	4	0	0	1	0	0	0	NA	NA
	9	3	1494	638→2113	Large deletion	1	1	õ	0	õ	Ő	1	0	õ	Ő	NA	NA
	10	3	8919	701→9632	Large deletion	3	0 0	õ	õ	õ	õ	0 0	õ	õ	õ	NA	NA
	11	2	4769	638→5424	Large deletion	1	0	0	0	0	0	1	0	0	0	NA	NA
	12	3	8757	848→9632	Large deletion	1	1	0	0	0	0	0	0	0	1	NA	NA
	13	2	8919	701→9632	Large deletion	2	0	0	0	0	0	0	0	0	0	NA	NA
	14	6	7949	1684→9632	Large deletion	2	0	3	0	0	0	1	0	0	0	NA	NA
	15	5	2905	6744→9686	Large deletion	0	1	0	0	0	0	4	0	0	0	NA	NA
	16	6	3448	638→4085	Large deletion	1	1	3	1	0	0	0	0	0	0	NA	NA
	17	2	1047	638→1668	Large deletion	0	1	0	0	0	0	1	0	0	0	NA	NA
	18	2	4050	638→4653	Large deletion	0	0	0	0	0	0	1	0	0	1	NA	NA
	19	3	4540	1909→6427	Large deletion	0	0	0	0	0	0	2	1	0	0	NA	NA
	20	5	2851	6744→9632	Large deletion	1	0	0	0	0	0	4	0	0	0	NA	NA
	21	2	7062	2555→9633	Large deletion	0	0	0	0	0	0	0	0	0	2	NA	NA
	22	0	2852	0/44→9033 2555 .0622	Large deletion	0	0	0	0	0	0	4	0	0	2	NA	NA
	23	2	/002	2000→9000		0	0	0	0	0	0	0	1	0	1	NA	NA
	24	2	4973	4090-9000	Large deletion	1	0	0	0	0	0	2	0	0	1	NA	NA
	26	7	7234	4090→9032 2382→9632	Large deletion	0	0	0	0	0	0	2	0	1	1	NA	NA
	27	4	420	638→1064	Large deletion	3	ő	ő	0	0	1	0	0	0	0	NA	NA
	28	2	939	638→1545	Large deletion	1	õ	1	õ	õ	0	õ	õ	õ	õ	NA	NA
	29	2	7541	2133←9632	Large deletion	O	õ	1	õ	ĩ	ŏ	õ	õ	ŏ	ŏ	NA	NA
	30	2	9075	522→9632	Scramble	Ō	ō	0	Ō	0	0	2	Ō	0	0	NA	NA
Subject 3	1	2	8955	638→9632	Intact	2	0	0	0	0	0	0	NA	NA	NA	NA	0
	2	2	8970	638→9632	Intact	2	0	0	0	0	0	0	NA	NA	NA	NA	0
	3	3	8976	638→9632	Intact	2	0	0	0	0	0	0	NA	NA	NA	NA	1
	4	2	8966	638→9632	Intact	0	0	2	0	0	0	0	NA	NA	NA	NA	0

Supplementary Table 4: Concordance between sequence-intact viruses determined by computational analysis of near full-length viral sequencing and functional viral outgrowth assays.

Count	Sequence length (bp)	qVOA results	In house Bioinformatics Pipeline Classification
1	9083	Positive [#]	Genome-intact
2	9138	Positive*	Genome-intact
3	9123	Positive*	Genome-intact
4	9086	Positive*	Genome-intact
5	9138	Positive*	Genome-intact
6	9138	Positive*	Genome-intact
7	9118	Positive*	Genome-intact
8	9007	Positive*	Genome-intact
9	9138	Positive*	Genome-intact
10	9138	Positive*	Genome-intact
11	9139	Positive*	Genome-intact
12	9007	Positive*	Genome-intact
13	9092	Positive*	Genome-intact
14	8991	Positive*	Genome-intact

Data from three different patients are shown

qVOA=quantitative viral outgrowth assay

*indicates viral outgrowth assays in which viral sequencing was performed from MOLT-4 cells co-cultured in a transwell system. #indicates viral outgrowth assays in which viral sequencing was performed MOLT-4 cells co-cultured without a transwell system.

	Intact	5'DEFECT	Premature Stop	Hypermutated	Large deletions
Subject 4. 2 days post-detection	9	0	1	0	1
Subject 4. 42 days post-detection	3	0	1	0	2
Subject 4. 164 days post-detection	1	0	0	0	1
Subject 5. 10 days post-detection	5	4	0	2	15
Subject 5. 46 days post-detection	0	0	1	0	1
Subject 5. 165 days post-detection	0	1	0	0	0

Supplemental Table 5. HIV-1 proviral DNA sequences in patients with acute HIV-1 infection (n=2).