

Fig. S1. Comparisons of frequencies of intact proviruses and inducible proviruses.

Scatter plot showing the frequencies of intact proviruses and inducible proviruses measured by NFL sequencing and Q2VOA, respectively. Each dot represents a different participant. Horizontal bars indicate median values. Statistical significance was determined using two-tailed Mann-Whitney U test.

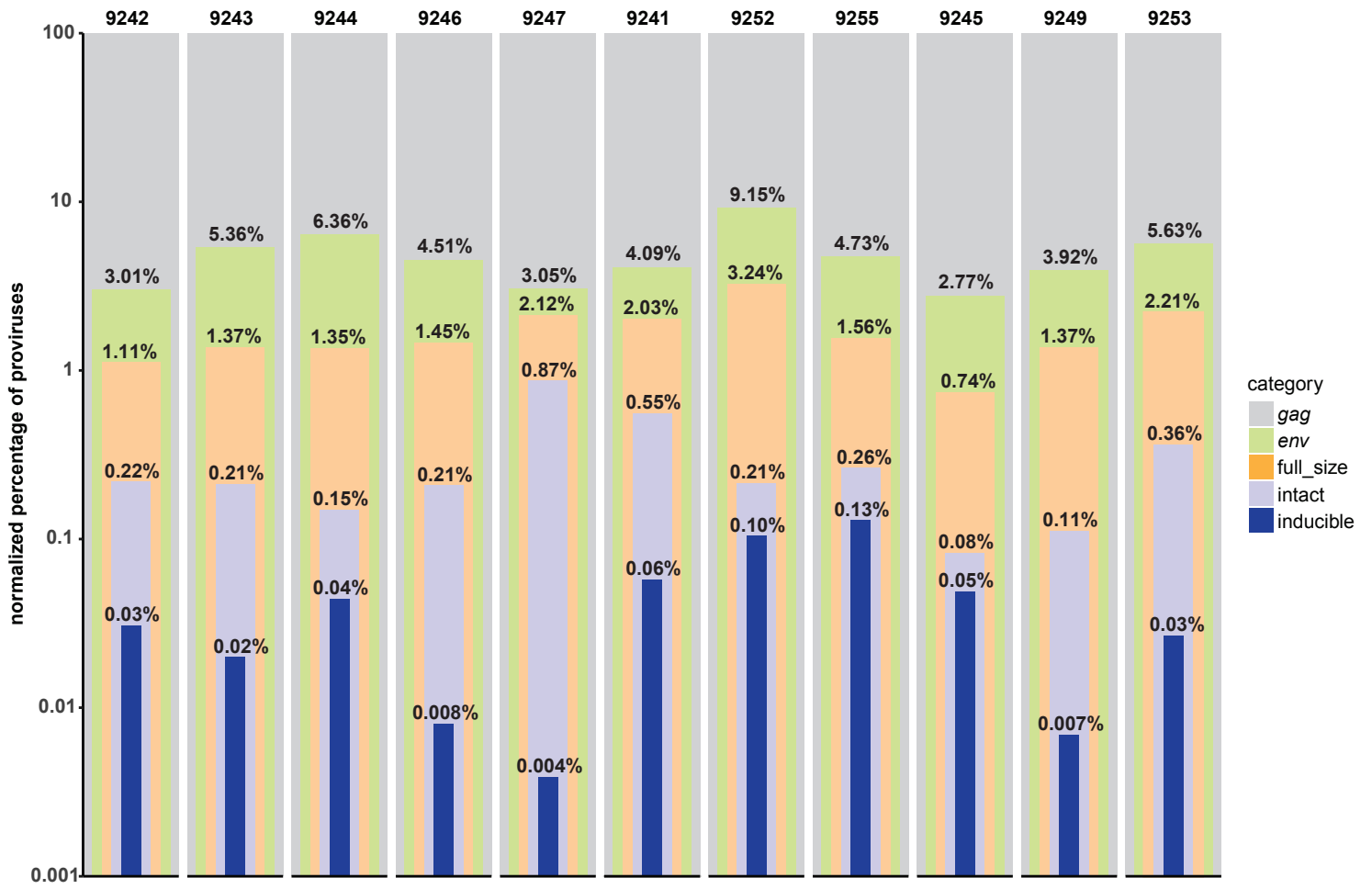


Fig. S2. Comparisons of different reservoir measurements.

Bar graph showing the proportion of env+ proviruses, near full size proviruses, intact proviruses, and inducible proviruses within gag+ proviruses population.

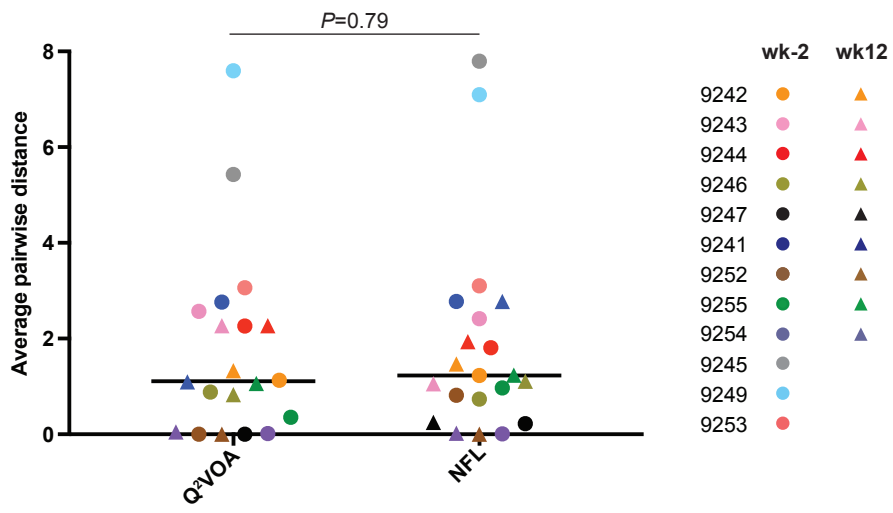


Fig. S3. HIV-1 env sequences diversity.

Scatter plot showing HIV-1 env sequence diversity from Q2VOA or NFL sequencing measured by average pairwise nucleotide distance. Each dot represents a different participant. Horizontal bars indicate median values. Statistical significance was determined using two-tailed Mann-Whitney U test.

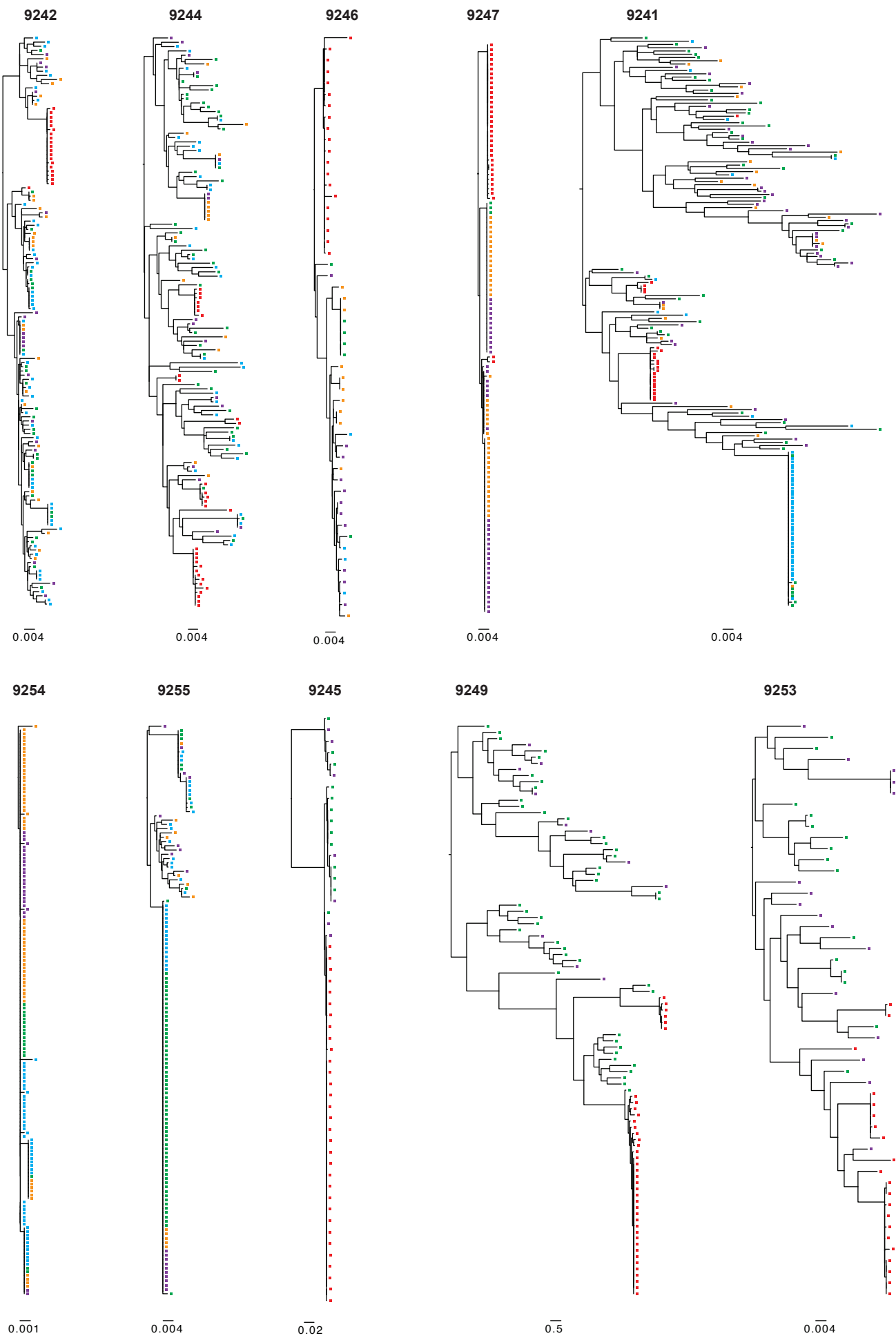


Fig. S4. Phylogenetic trees of env sequences.

Maximum-likelihood phylogenetic trees of env sequences obtained from Q2VOA at the pre-infusion (green) and week 12 (blue), NFL at the pre-infusion (purple) and week 12 (orange), and rebound viruses from SGA or outgrowth cultures (red).

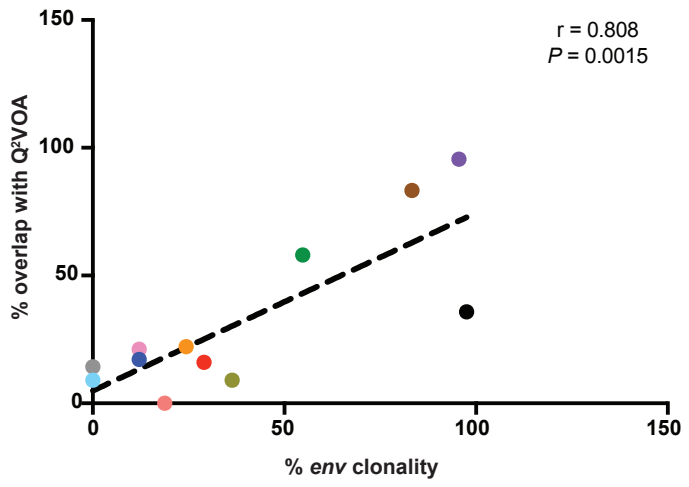


Fig. S5. Correlation between shared sequences from two methods and clonality. Scatter plot showing Pearson correlation between the percentage of clones and percentage of overlap between Q2VOA and NFL sequencing. Each dot represents a different participant.

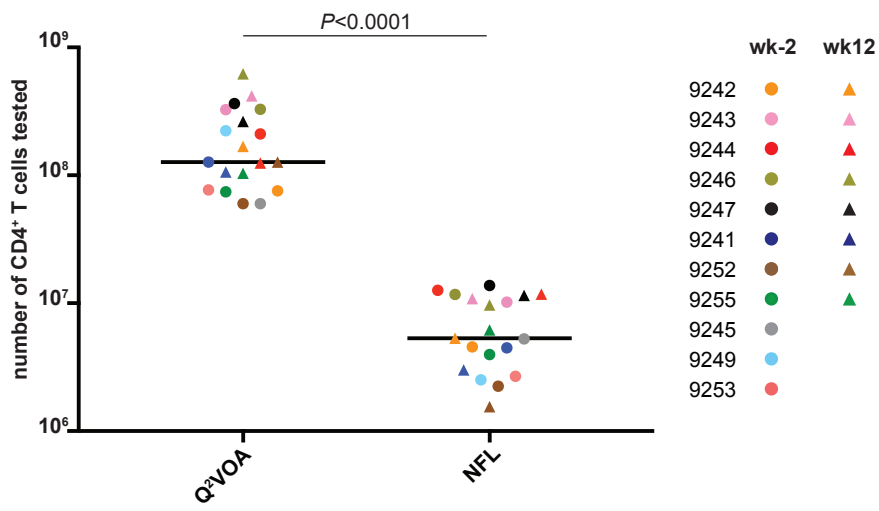


Fig. S6. Number of CD4+ T cells tested.

Scatter plot showing the number of CD4+ T cells tested in Q2VOA and NFL sequencing. Each dot represents a different participant. Horizontal bars indicate median values. Statistical significance was determined using two-tailed Mann-Whitney U test.

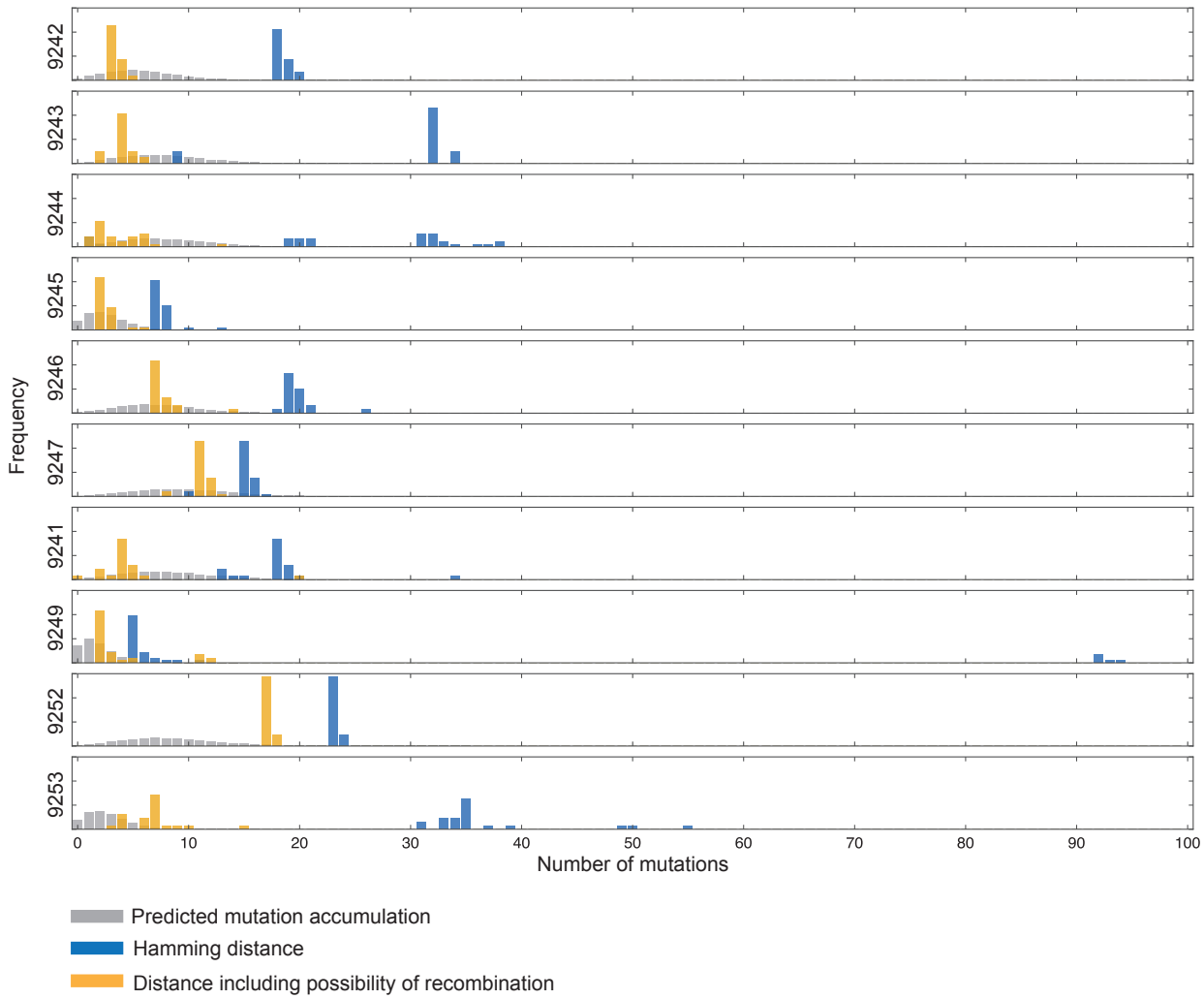


Fig. S7. Relationship between latent and rebound sequences.

Histograms show the proportion of env sequences (y-axis) and number of mutations (x-axis).

The blue bars represent the Hamming distance between rebound and NFL/Q2VOA sequences.

The grey bars represent the predicted distance between rebound and NFL/Q2VOA sequences based on a simulation of mutation accumulation during the ATI period for each participant.

Yellow bars represent the predicted distance between the rebound and NFL/ Q2VOA sequences when the possibility of recombination is included.

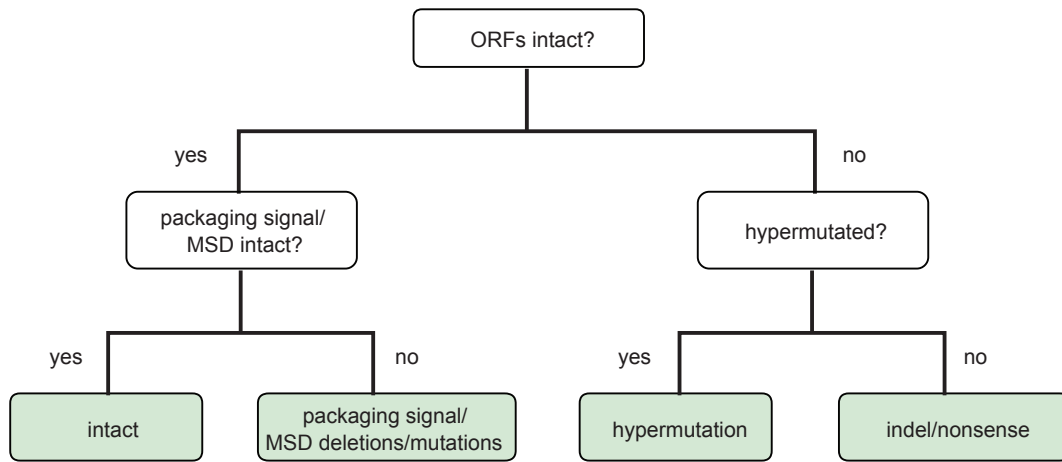


Fig. S8. Process used to identify intact and defective proviruses.

Assembled sequences were aligned to the HXB2 genome to identify premature stop codons, out-of-frame insertions or deletions (indel), or packaging signal (Ψ) and the major splice donor (MSD) site deletions and mutations.

Table 1. Quantitative analysis of the latent reservoir during treatment interruption.

	ID	Weeks to viral rebound	CD4+ T cells tested in Q ² VOA		CD4+ T cells tested in NFL		Gag+ proviruses per 10 ⁶ cells		Env+ proviruses per 10 ⁶ cells		full size proviruses per 10 ⁶ cells		intact proviruses per 10 ⁶ cells		Inducible proviruses per 10 ⁶ cells			
			week -2	week 12	week -2	week 12	week -2	week 12	week -2	week 12	week -2	week 12	week -2	week 12	week -2	week 12	week -2	week 12
late rebounder	9242	15	7.56E+07	1.68E+08	4.57E+06	5.32E+06	1938	2229	75.77	49.63	26.28	20.11	4.38	4.70	0.781	0.493		
	9243	20	3.26E+08	4.18E+08	1.02E+07	1.09E+07	816	674	42.31	37.58	11.98	8.45	2.06	1.10	0.170	0.126		
	9244	21	2.11E+08	1.25E+08	1.26E+07	1.18E+07	636	646	58.31	50.60	13.09	10.03	1.27	1.28	0.397	0.354		
	9246	19	3.28E+08	6.22E+08	1.17E+07	9.70E+06	526	475	19.87	25.26	5.57	8.97	0.94	1.13	0.066	0.014		
	9247	26	3.64E+08	2.64E+08	1.38E+07	1.15E+07	392	268	11.67	11.15	7.68	8.19	2.83	3.66	0.025	0.004		
	9241	21	1.27E+08	1.06E+08	4.48E+06	3.01E+06	1199	1531	51.52	60.15	28.10	27.25	8.48	6.65	0.743	0.828		
	9252	22	6.00E+07	1.27E+08	2.25E+06	1.55E+06	1068	1988	117.97	161.79	36.95	62.13	2.67	3.88	1.709	1.470		
9255	>30	7.44E+07	1.04E+08	3.97E+06	6.19E+06	1548	1007	85.92	34.91	28.22	11.80	4.79	1.94	1.890	1.400			
early rebounder	9245	5	6.00E+07		5.25E+06		1171		44.98		12.01		1.33		0.79			
	9249	3	2.23E+08		2.52E+06		3922		153.84		53.53		4.36		0.27			
	9253	5	7.68E+07		2.68E+06		1653		93.11		36.50		5.96		0.44			

Table 2. Analysis of near full size proviruses.

ID	intact proviruses				ψ /MSD mutation or deletion				hypermutation				indel/nonsense				total sequences	
	week -2		week 12		week -2		week 12		week -2		week 12		week -2		week 12		week -2	week 12
	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	no.
9242	20	18.7	25	30.1	4	3.7	2	2.4	75	70.1	50	60.2	8	7.5	6	7.2	107	83
9243	21	25.3	12	18.5	7	8.4	13	20	37	44.6	29	44.6	18	21.7	11	16.9	83	65
9244	16	13.9	15	17	33	28.7	36	40.9	47	40.9	29	33	19	16.5	8	9.1	115	88
9246	11	25	11	18.6	13	29.5	28	47.5	14	31.8	14	23.7	6	13.6	6	10.2	44	59
9247	39	7.2.2	42	72.4	5	9.3	11	19	5	9.3	5	8.6	5	9.3	0	0	54	58
9241	38	35.5	20	34.5	0	0	0	0	57	53.3	31	53.4	12	11.2	7	12.1	107	58
9252	6	6.8	6	6.8	50	56.8	55	62.5	1	1.1	2	2.3	31	35.2	25	28.4	88	88
9254	26	52	62	62.6	0	0	0	0	23	46	35	35.4	1	2	2	2	50	99
9255	19	26.4	12	20	2	2.8	1	1.7	44	61.1	35	58.3	7	9.7	12	20	72	60

Table 3. Qualitative analysis of the circulating latent reservoir.

	ID	Env sequences (seqs) by Q ² VOA						Env sequences (seqs) by NFL						total Env in rebound (no.)		
		week-2	week 12	total	clonal env seqs		overlap with NFL		week-2	week 12	total	clonal env seqs		overlap with Q ² VOA		Rebound
		no.	no.	no.	no.	(%)	no.	(%)	no.	no.	no.	no.	(%)	no.	(%)	no.
late rebounder	9242	28	45	73	27	37.0	10	13.7	20	25	45	11	24.4	10	22.2	19
	9243	25	34	59	22	37.3	7	11.9	21	12	33	4	12.1	7	21.2	13
	9244	38	32	70	12	17.1	6	8.6	16	15	31	9	29.0	5	16.1	31
	9246	6	4	10	4	40.0	4	40.0	11	11	22	8	36.4	2	9.1	20
	9247	3	0	3	3	100.0	3	100.0	39	42	81	79	97.5	29	35.8	35
	9241	45	50	95	46	48.4	44	46.3	38	20	58	7	12.1	1	17.2	22
	9252	41	57	98	98	100.0	98	100.0	6	6	12	10	83.3	10	83.3	14
	9254	18	50	68	65	95.6	65	95.6	26	62	88	84	95.5	84	95.5	NA
	9255	70	33	103	88	85.4	88	85.4	19	12	31	17	54.8	18	58.1	NA
early rebounder	9245	13			7		3	23.1	7			0	0	1	14.3	32
	9249	43			2		1	2.3	11			0	0	1	9.1	39
	9253	15			2		0	0.0	16			3	18.8	0	0.0	21
Total		650							435							246