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Supplemental information

Progressive transformation of the HIV-1 reservoir cell profile over two decades of antiviral therapy

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Supplemental Materials for

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Figure S1

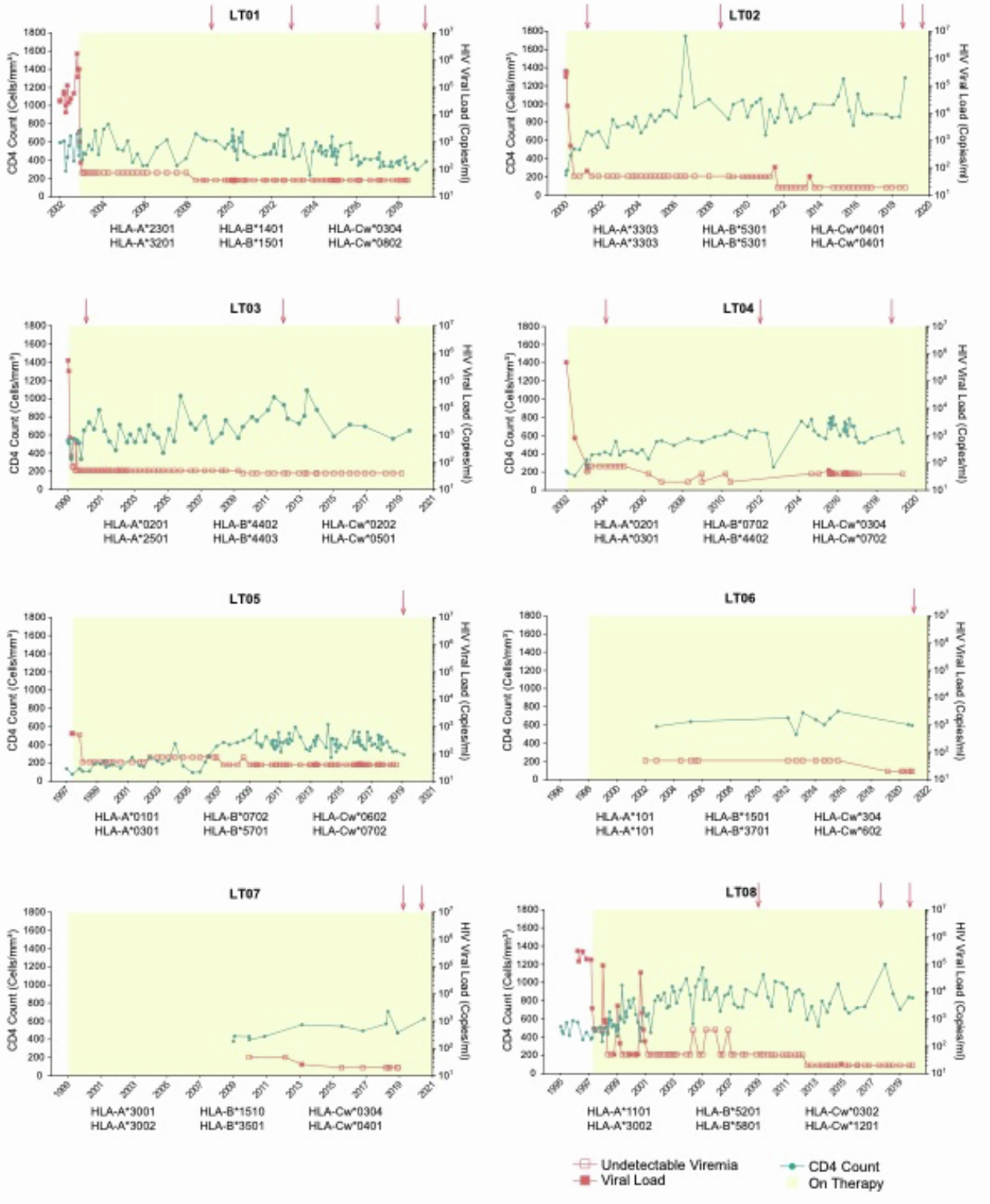

LT01
HLA-A*2301
HLA-A*3201
HLA-B*1401
HLA-B*1501
HLA-Cw*0304
HLA-Cw*0802
LT02
HLA-A*3303
HLA-A*3303
HLA-B*5301
HLA-B*5301
HLA-Cw*0401
HLA-Cw*0401
LT03
HLA-A*0201
HLA-A*2501
HLA-B*4402
HLA-B*4403
HLA-Cw*0202
HLA-Cw*0501
LT04
HLA-A*0201
HLA-A*0301
HLA-B*0702
HLA-B*4402
HLA-Cw*0304
HLA-Cw*0702
LT05
HLA-A*0101
HLA-A*0301
HLA-B*0702
HLA-B*5701
HLA-Cw*0602
HLA-Cw*0702
LT06
HLA-A*101
HLA-A*101
HLA-B*1501
HLA-B*3701
HLA-Cw*304
HLA-Cw*602
LT07
HLA-A*3001
HLA-A*3002
HLA-B*1510
HLA-B*3501
HLA-Cw*0304
HLA-Cw*0401
LT08
HLA-A*1101
HLA-A*3002
HLA-B*5201
HLA-B*5801
HLA-Cw*0302
HLA-Cw*1201
CD4 Count (Cells/mm³)
HIV Viral Load (Copies/ml)
Undetectable Viremia
Viral Load
CD4 Count
On Therapy

Figure S1 (Related to Figure 1): Longitudinal viral loads and CD4 T cell counts in the eight study participants. Timepoints of PBMC sampling are indicated by red arrows. HLA class I alleles are also listed. Duration of ART is indicated by yellow shading. For study participants LT06 and LT07, original viral load and CD4 T cell count data during early stages of ART could not be independently reviewed by study team; however, suppression of viremia during ART was confirmed through provider reports.

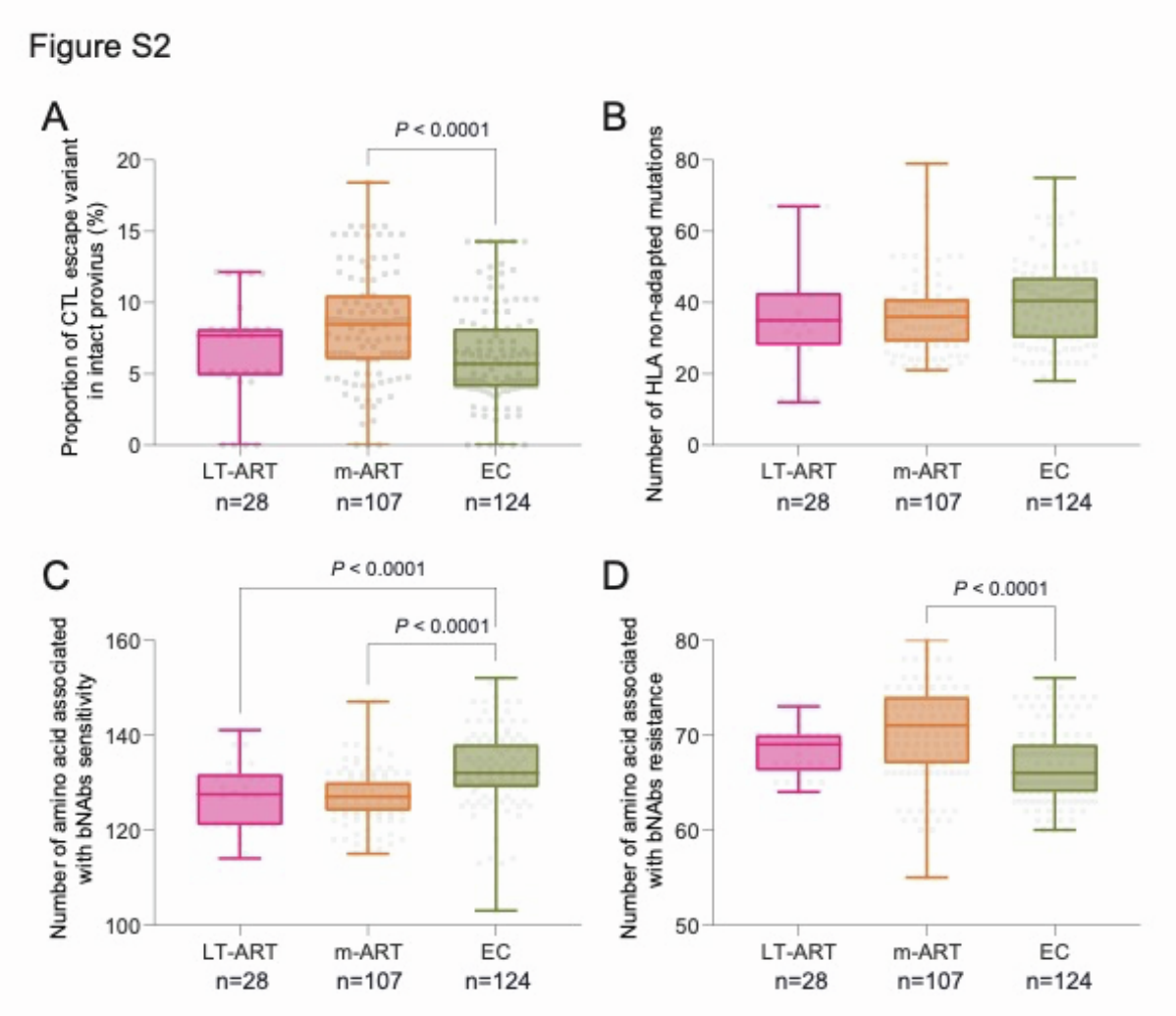
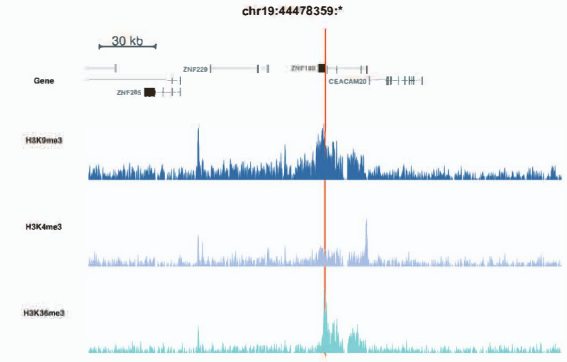


Figure S2 (Related to Figure 1): Immune footprints in intact HIV-1 proviruses. (A): Proportions of clade B CTL epitopes (restricted by autologous HLA class I alleles) within intact proviruses that harbor known escape variants. Defined escape mutations listed in the LANL HIV Immunology Database (www.hiv.lanl.gov) were counted. (B): Numbers of sequence variations without statistically significant associations with autologous HLA class I alleles are shown, determined as described by Carlson et al [S1]. Each dot represents one intact provirus. (C-D): Numbers of amino acid residues associated with sensitivity (C) or resistance (D) to broadly-neutralizing antibodies, calculated as described by Bricault et al [S2]. (A-D): Each symbol represents one intact proviral sequence from indicated study cohort; all intact clade B sequences were included. Clonal sequences are shown once. P-values were calculated using FDR-adjusted two-sided Kruskal-Wallis nonparametric test.

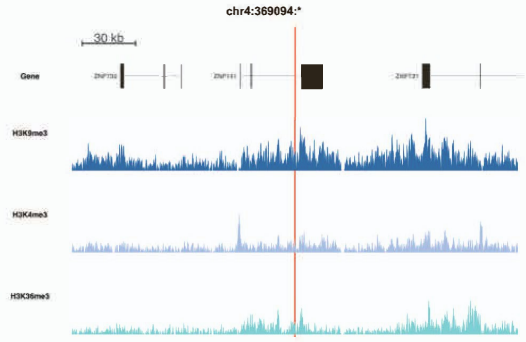
Figure S3 (Related to Figure 2): Sequences of viral-host junctions of proviruses integrated in satellite/microsatellite DNA or ZNF genes: Sequences of viral-host junctions are shown for selected proviruses from study participants LT01, LT04, LT07, LT08. Sequences of the 1.U5 primer from the ISLA protocol [S3] are underlined.

Figure S4

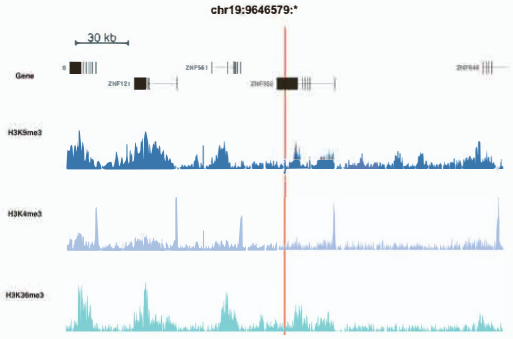
LT02



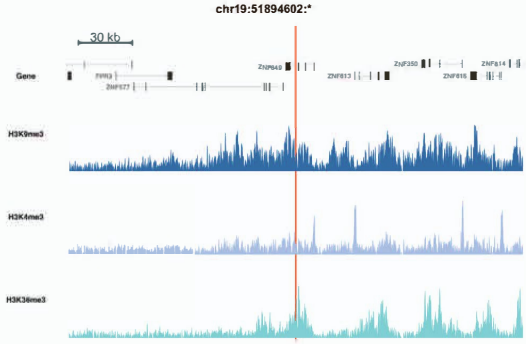
LT05



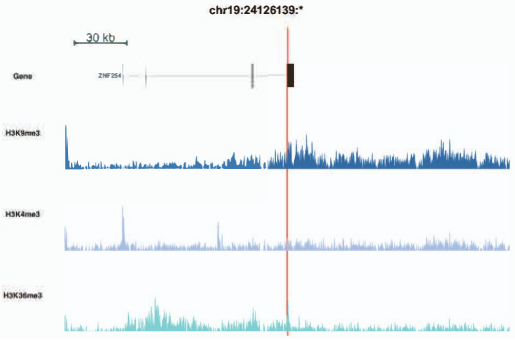
LT06



LT06



LT07



LT08

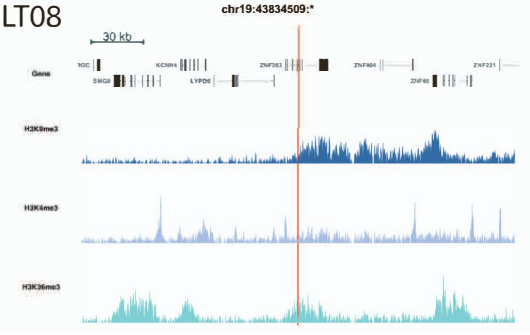


Figure S4 (Related to Figure 2): Chromatin environment of intact proviruses integrated in KRAB-ZNF genes, inferred from reference data. Genome browser shapshots of chromomal integration sites of intact proviruses located in ZNF genes are shown; ChIP-Seq tracks of the inhibitory histone markers H3K9me3, and the activating chromatin mark H3K4me3 from primary CD4 T cells evaulated in the ROADMAP consortium [S4] are included. ChIP-Seq tracks of H3K36me3 (associated with context-depedent transcriptional activation or repression [S5]), are also shown.

Figure S5

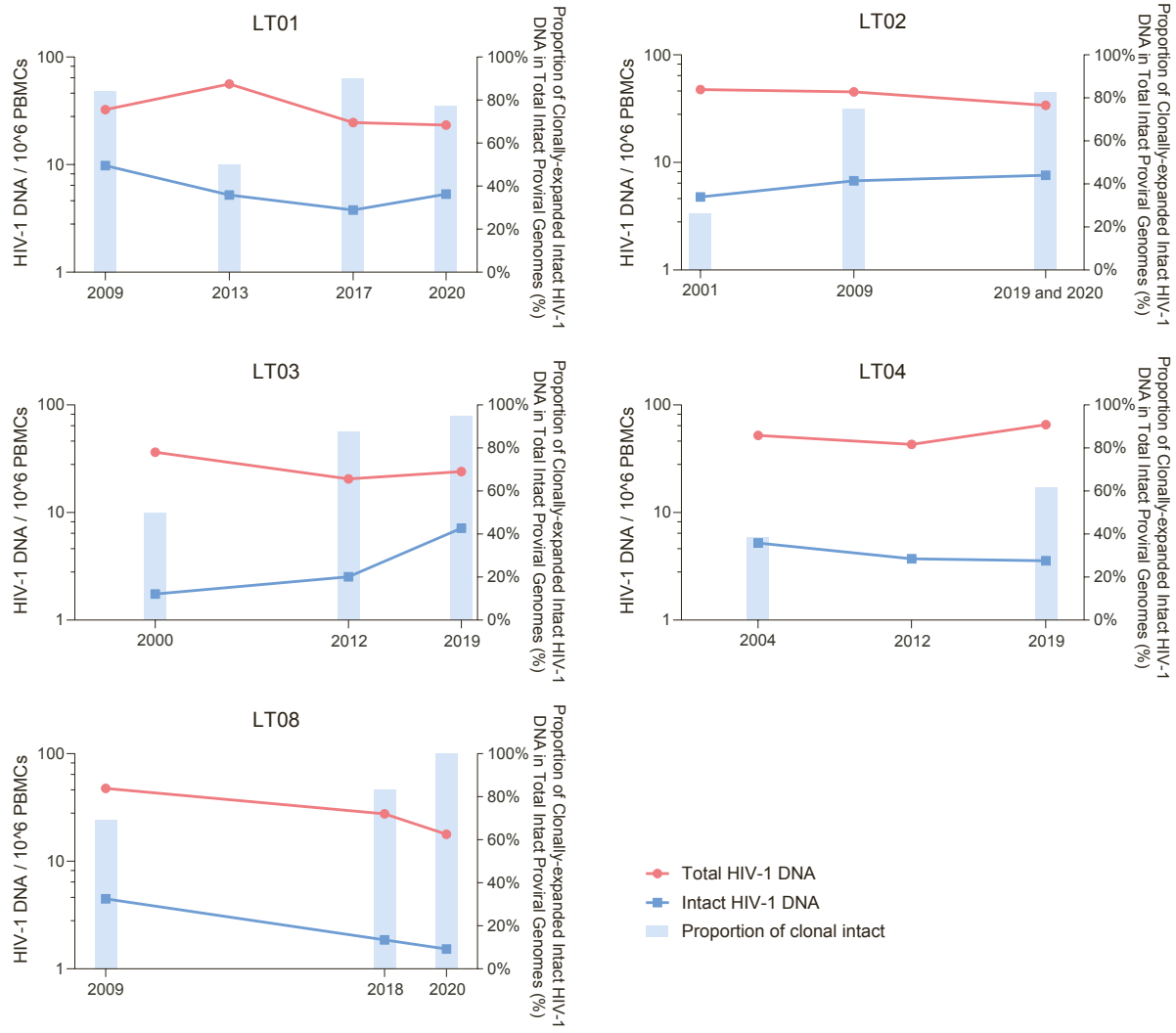


Figure S5 (Related to Figure 4): Frequencies of total and intact HIV-1 proviruses over time in the five longitudinally-followed study participants undergoing long-term ART. Proportions of clonal intact proviruses among all intact proviruses are also indicated.

Figure S6

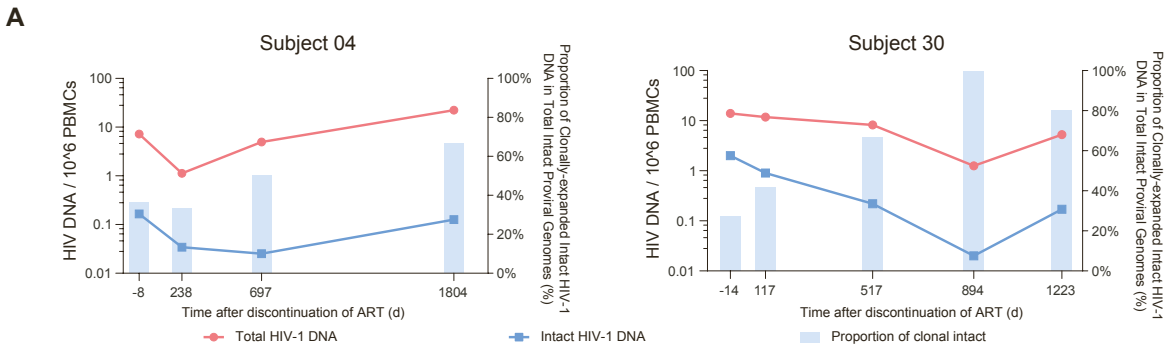


Figure S6 (Related to Figure 6): Longitudinal frequencies of HIV-1 proviruses in study persons 04 and 30. Longitudinal frequencies of total and intact HIV-1 proviruses in the 2 individuals with post-treatment control. Proportion of clonal intact proviruses among all intact proviruses is also indicated.

Table S2 (Related to Figures 2 and 4): Numbers of cells analyzed at each timepoint in indicated study participants.

Study Participant No.	Time point	Cells assayed
LT01	2009	1.94E+06
	2013	1.53E+06
	2017	2.64E+06
	2020	6.57E+06
LT02	2001	3.98E+06
	2009	1.78E+06
	2019	2.57E+06
	2020	1.15E+06
LT03	2000	2.82E+06
	2012	3.50E+06
	2019	2.66E+06
LT04	2004	1.15E+06
	2012	1.65E+06
	2019	3.64E+06
LT05	2019	8.06E+06
LT06	2021	1.27E+07
LT07	2019	8.01E+06
LT08	2009	4.85E+05
	2018	5.37E+06
	2020	2.62E+06
subject 04	on ART	1.66E+07
	d238 off ART	2.55E+07
	d697 off ART	2.43E+07
	d1804	3.61E+07
subject 30	on ART	6.60E+06
	d117 off ART	3.21E+06
	d517 off ART	5.75E+06
	d894 off ART	1.03E+07
	d1223 off ART	6.09E+06
subject 01	on ART	2.66E+07
subject 22	on ART	1.43E+06
subject 25	on ART	5.98E+06

Table S3 (Related to STAR Methods): Numbers of cells analyzed in quantitative viral outgrowth assays.

Study Participant	CD4 cells assayed	Estimated minimum number of intact proviruses based on FLIP-Seq results	Luciferase-positive wells after 21 days of culture
LT02	2.51E+06	42	1
LT03	1.93E+06	119	1
LT06	4.08E+06	20	0
LT07	1.62E+06	62	0
LT08	5.55E+06	64	0

Table S4 (Related to STAR Methods): List of Primers/Probes

Workflow	Amplicon/ Target (HXB2 Coordinates)	Round/ Orientation	Oligo Name	Oligo Sequence (5'->3')
gDNA ddPCR	LTRgag (HXB2 684-810)		LTRgag F	TCTCGACGCAGGACTCG
			LTRgag R	TACTGACGCTCTCGCACC
			LTRgag P	/56-FAM/CTCTCTCCT/ZEN/TCTAGCCTC/31ABkFQ/
	RPP30		RPP30 F	GATTTGGACCTGCGAGCG
			RPP30 R	GCGGCTGTCTCCACAAGT
			RPP30 P	/56-FAM/CTGACCTGA/ZEN/AGGCTCT/31ABkFQ/
Proviral DNA Sequencing	Near full-length (638-9632)	1F	U5-623F	AAATCTCTAGCAGTGGCGCCCGAACAG
		1R	U5-601R	TGAGGGATCTCTAGTTACCAGAGTC
		2F	U5-638F	GCGCCCGAACAGGGACYTGAAARCGAAAG
		2R	U5-547R	GCACTCAAGGCAAGCTTTATTGAGGCTTA
	Promoter (76-818)	1F	24F	CGAAGACAAGATATCCTTGATCTGTGG
		1R	962R	CTACAGCCTTCTGATGTTTCTAACAGG
		2F	76F	CTGATTAGCAGAACTACACACCAGG
		2R	818R	CCGCTTAATACTGACGCTCTCG
	Promoter (367-643)	1F	350F	GGGACTTTCCACTGGGGACTTTC
		1R	661R	GCTTTCAGGTCCCTGTTCGG
		2F	367F	ACTTTCCAGGGAGGCGTGG
		2R	Kumar R	GGGCGCCACTGCTAGAGA
	A1mod2 (638-2724)	1F	U5-623F	AAATCTCTAGCAGTGGCGCCCGAACAG
		1R	NE1	CCACTAACTTCTGTATGTCATTGACAGTCCAGCT
		2F	U5-638F	GCGCCCGAACAGGGACYTGAAARCGAAAG
		2R	ProC-	GAGTATTGTATGGATTTTCAGGCCCAAT
	Pol (2011-3798)	1F	5CP1	GAAGGGCACACAGCCAGAAATTGCAGGG
		1R	RT3.1	GCTCCTACTATGGGTTCTTTCTCTAACTGG
		2F	2.5	CCTAGGAAAAAGGGCTGTTGGAAATGTGG
		2R	RT3798R	CAAACTCCCACTCAGGAATCCA
	C (3626-5980)	1F	RT3597mixF	AAAACAGGAAARTATGCAA
		1R	SC05R	AGCTCTTCGTCGCTGTCTCCGCTT
		2F	RT3626F	TGCCCACACTAATGATGTAA
		2R	SC02R	CTTCCTGCCATAGGAGATGCCTA
	A2 (5550-7760)	1F	VP5450F	CAGGACATAACAAGGTAGGATC
		1R	CO602	GCCCATAGTGCTTCCTGCTGCTCCCAAGAACC
		2F	VP5549F	AGAGGATAGATGGAACAAGCCCCAG
		2R	V3CR	TGCTCTTTTTTCTCTCTSCACCACT
	B2 (7652-9610)	1F	GP41Fo	TTCAGACCTGGAGGAGGAGATAT
		1R	3LTRi	TCAAGGCAAGCTTTATTGAGGCTTAA
		2F	GP41Fi	GGACAATTGGAGAAGTGAATTAT
		2R	3UTRi	AGGCTTAAGCAGTGGGTTCCCTAG
Reverse Transcription	Long LTR (643)	RT	Bio Long LTR	/5BiotinTEG/AAGCAGTGGTATCAACGCAGAGTACGGGC GCCACTGCTAGAGA
	Pol (2662)	RT	Bio Pol	/5BiotinTEG/AAGCAGTGGTATCAACGCAGAGTACCAAAT TTCTACTAATGCTTTTATTTTTTC
	Nef (9040)	RT	Bio Nef	/5BiotinTEG/AAGCAGTGGTATCAACGCAGAGTACTGTAA GTCATTGGTCTTAAAGGTACCTGAGG
	PolyA (9635+25)	RT	Bio PolyA	/5BiotinTEG/AAGCAGTGGTATCAACGCAGAGTACTTTTT TTTTTTTTTTTTTTTTTTTTTGAAG
	Tat-Rev (8459)	RT	Bio Tat-Rev	/5BiotinTEG/AAGCAGTGGTATCAACGCAGAGTACGGATC TGTCTCTGTCTCTCTCTCCACC
	Read-through (582; 9667)	RT	Bio Readth	/5BiotinTEG/AAGCAGTGGTATCAACGCAGAGTACAGAGT CACACAACAGACGG
cDNA Amplification	NA	NA	Template-switching oligo (TSO)	AAGCAGTGGTATCAACGCAGAGTACATrGrG+G-3

	NA	NA	ISPCR	AAGCAGTGGTATCAACGCAGAGT
cDNA ddPCR	Long LTR (522-643)	F	Kumar F	GCCTCAATAAAGCTTGCCTTGA
		R	Kumar R	GGGCGCCACTGCTAGAGA
		R (alternate)	625R	TTTTCCACACTGACTAAAAKGGTC
		Probe	Kumar P	/56-FAM/CCAGAGTCA/ZEN/CACAACAGACGGGCACA/3IABkFQ/
	Pol (2536-2662)	F	Pol mf299	GCACTTTAAATTTTCCCATTAGTCCTA
		R	Pol mf1	CAAATTTCTACTAATGCTTTTATTTTTTC
		Probe	Pol P	/56-FAM/AAGCCAGGA/ZEN/ATGGATGGCC/3IABkFQ/
	Nef (8883-9040)	F	F8883-03	GGTGGGAGCAGYATCTCGAGA
		R	R9040-10	TGTAAGTCATTGGTCTTAAAGGTACCTGAGG
		Probe	P8967-50	/56-FAM/CCAGGCACA/ZEN/AKCAGCATT/3IABkFQ/
	PolyA (9496-9635+25)	F	Freadth-2	GCCCTCAGATGCTRCATATAA
		R	5T25	TTTTTTTTTTTTTTTTTTTTTTTTTTGAAG
		Probe	Preadth-1	/56-FAM/TGCCTGTAC/ZEN/TGGGTCTCTCTGGTTAG/3IABkFQ/
	Tat-Rev (5956-8459)	F	mf1	CTTAGGCATCTCCTATGGCAGGAA

Supplemental Reference List

[S1] Carlson, J.M., Brumme, C.J., Martin, E., Listgarten, J., Brockman, M.A., Le, A.Q., Chui, C.K., Cotton, L.A., Knapp, D.J., Riddler, S.A., et al. (2012). Correlates of protective cellular immunity revealed by analysis of population-level immune escape pathways in HIV-1. J Virol *86*, 13202-13216. 10.1128/JVI.01998-12.

[S2] Bricault, C.A., Yusim, K., Seaman, M.S., Yoon, H., Theiler, J., Giorgi, E.E., Wagh, K., Theiler, M., Hraber, P., Macke, J.P., et al. (2019). HIV-1 Neutralizing Antibody Signatures and Application to Epitope-Targeted Vaccine Design. Cell Host Microbe *26*, 296. 10.1016/j.chom.2019.07.016.

[S3] Wagner, T.A., McLaughlin, S., Garg, K., Cheung, C.Y., Larsen, B.B., Styrchak, S., Huang, H.C., Edlefsen, P.T., Mullins, J.I., and Frenkel, L.M. (2014). HIV latency. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. Science *345*, 570-573. 10.1126/science.1256304.

[S4] Roadmap Epigenomics, C., Kundaje, A., Meuleman, W., Ernst, J., Bilenky, M., Yen, A., Heravi-Moussavi, A., Kheradpour, P., Zhang, Z., Wang, J., et al. (2015). Integrative analysis of 111 reference human epigenomes. Nature *518*, 317-330. 10.1038/nature14248.

[S5] Wagner, E.J., and Carpenter, P.B. (2012). Understanding the language of Lys36 methylation at histone H3. Nat Rev Mol Cell Biol *13*, 115-126. 10.1038/nrm3274.