Table S1: Donor characteristics

Participant identifier (PID)	Gender	Maximum documented viral load[e] (c/ml)	CD4+ T cell nadir (cells/µl)	Approximate duration of infection before initial ART (years)	Current ART regimen	Total years of ART experience	Duration of continuous viral suppression on ART at sampling (years)	Long-term plasma viral load on ART (c/ml)	CD4+ on long-term ART (cells/µl)
3720[a]	Male	20,794	452	<1.0	DTG/ABC/3TC	1.8	1.8	<40	636
2661[b]	Male	77,839	613	0.3	ABC/3TC, RGV	17.8	12.9	<40	739
1683[c]	Male	134,406	452	2.0	FTC/TDF, RTV, DRV	5.7	5.4	<40	1348
1079[c]	Male	183,855	207	4.3	FTC/TDF, ETV	13.0	11.4 at first sample, 12.8 at second sample	<40	799
2669[d]	Male	unknown	205	unknown	ABC/3TC/DTG	<15.8	4.3 at first sample, 5.5 at second sample	<40	681

[a] Subtype C infection

[b] Had a brief treatment interruption about 4.7 years after ART initiation followed by full viral suppression for additional 12.9 years; samples were obtained from treatment interruption and after subsequent 12.9 years of full suppression of viremia on ART

[c] Continuous suppression on ART since initiation; samples obtained from pre-ART and after 5.7 years or 11.4 and 12.8 years on ART

[d] Previously failed ART due to drug resistance. Had sustained viral suppression at <40 copies/ml throughout sampling period of 5.5 years on new regimen containing dolutegravir.

[e] Abbott Diagnostics RealTime HIV-1 PCR Assay

Table S2. Samples Analyzed

Patient Identifier	Sample Date	Time since viral suppression (years)	Sample Type
3720	3/14/2016	viremic: pre-ART	PBMC, LNMC
	1/9/2018	1.8	PBMC, LNMC
2661	3/13/2003	viremic: brief interruption after 4.8 years on ART	Plasma
	4/25/2016	12.9	PBMC, LNMC
1683	8/26/2010	viremic: pre-ART	PBMC
	5/10/2016	5.4	PBMC, LNMC
1079	6/8/2004	viremic: pre-ART	PBMC
	4/25/2016	11.4	PBMC, LNMC
	8/8/2017	12.8	PBMC, LNMC (right and left)
	9/26/2017	viremic: rebound	Plasma
2669	3/14/2016	4.3	PBMC, LNMC
	6/9/2017	5.5	PBMC, LNMC (left and right)

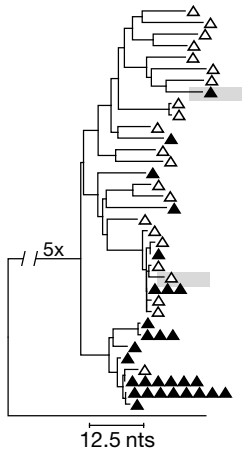
Table S3. Percent of infected cells with HIV-1 RNA calculated by determining the number of total infected cells with various primer/probe sets

PID	Number infected cells/million MC						Percent infected cells with unspliced HIV RNA[c]								
	R-U5 primers[a]		Gag primers[a]		Pol primers[b]		R-U5 primers		p value[d]	Gag primers		p value[d]	Pol primers		p value[d]
	PB	LN	PB	LN	PB	LN	PB	LN		PB	LN		PB	LN	
3720[e]	109	466	<18	<18	233	994	21	100		N/A	N/A		10	100	
3270[f]	<7	---	<7	---	6	---	25	---		25	---		31	---	
2661	<3	<3	<6	24	6	<4	<17	<17	0.4	<9	2	0.7	9	<13	0.1
1683	151	200	53	147	192	154	7	5		19	7		5	7	
1079	163	297	116	183	137	194	7	13		9	21		8	20	
2669	460	733	292	558	429	1673	6	29		9	39		6	13	

[a]ddPCR protocol in methods; corrected, assuming 2 LTRs per infected cell. Gag primers not designed for detection of HIV subtype C.
[b]Integrase cell-associated DNA (iCAD) protocol (31)
[c]All p-values are significant when p<0.017 to account for multiple comparisons
[d]Wilcoxon signed rank test
[e]pre-ART—excluded from statistical tests
[f]1.8 year suppressed on ART

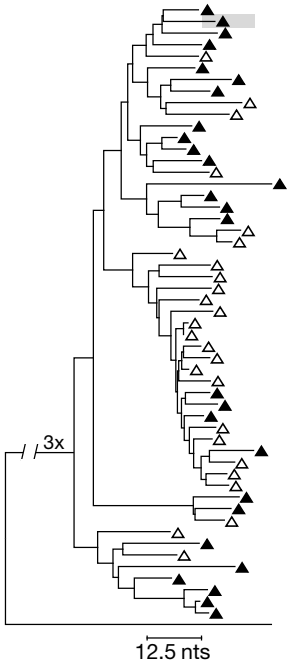
Supplemental Figure 1

A. 1683 *env*
(pre-ART PBMC & 5.4 years suppressed PBMC)
△ 0 days before ART PBMC DNA
▲ 5.4 years on ART PBMC DNA
Contains STOP codon(s)



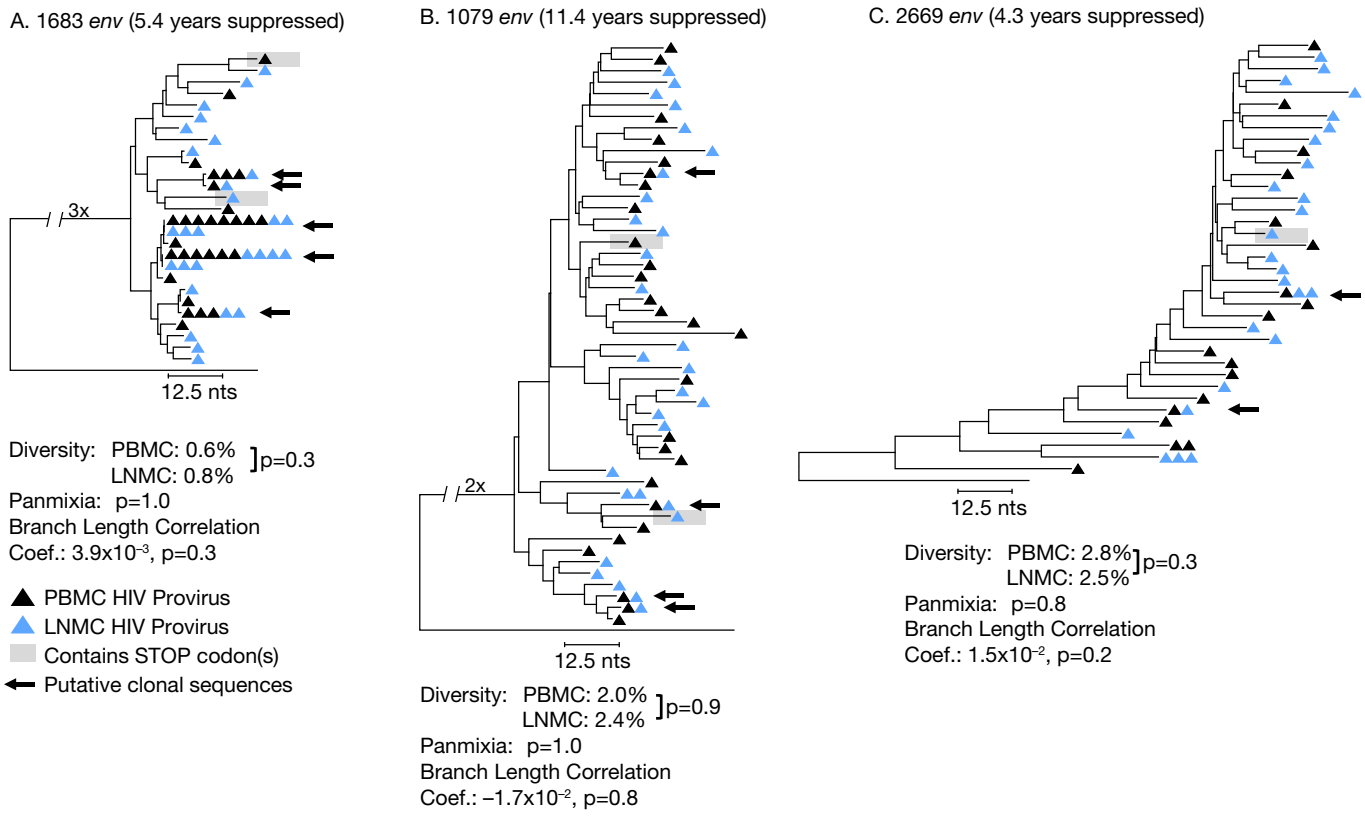
Diversity: Pre ART: 1.3%
Long-term ART: 0.6%] p<0.0001
Panmixia: p=0.003
Root to Tip Slope: -2.1×10^{-3}

B. 1079 *env*
(pre-ART PBMC & 11.4 years suppressed PBMC)
△ 2.5 months before ART PBMC DNA
▲ 11.4 years on ART PBMC DNA
Contains STOP codon(s)



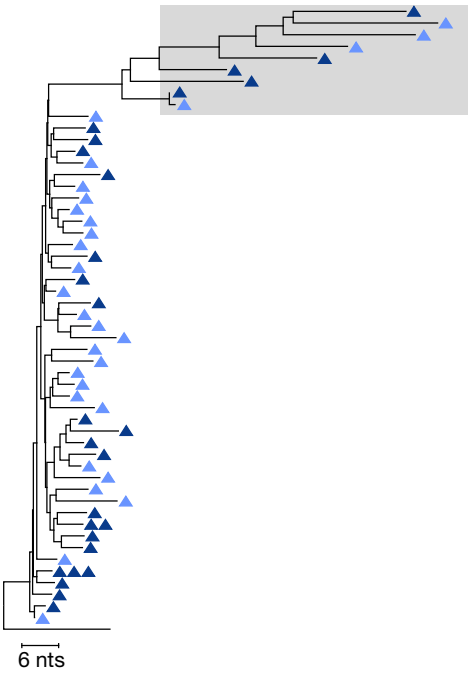
Diversity: Pre ART: 1.6%
Long-term ART: 2.0%] p=0.06
Panmixia: p=0.0002
Root to Tip Slope: -7.3×10^{-4}

Supplemental Figure 2



Supplemental Figure 3

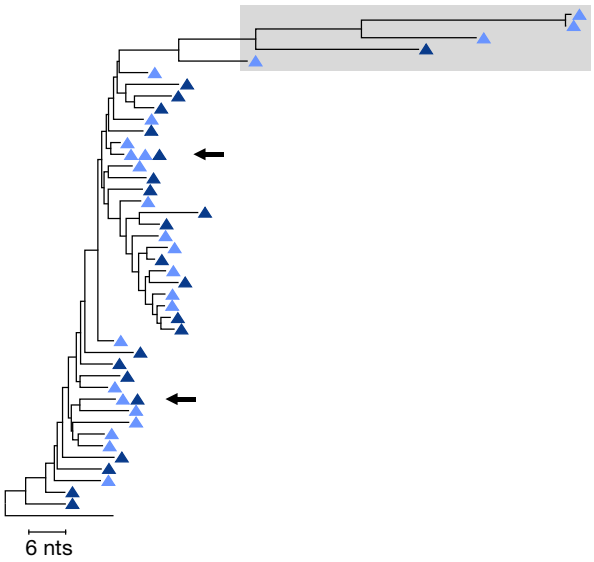
A. 1079 p6-PR-RT (12.8 years suppressed)



Diversity: Right LN: 1.0%
Left LN: 1.0%
p=0.8
Panmixia: p=0.3
Branch Length Correlation
Coef.: 3.6×10^{-2}, p=0.05

▲ Right inguinal LN LNMC DNA
▲ Left inguinal LN LNMC DNA
← Putative clonal sequences
Contains STOP codon(s)

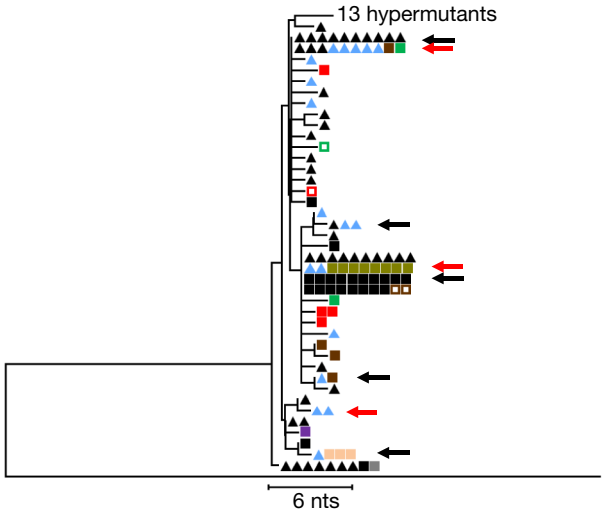
B. 2669 p6-PR-RT (5.5 years suppressed)



Diversity: Right LN: 1.6%
Left LN: 1.3%
p=0.3
Panmixia: p=0.7
Branch Length Correlation
Coef.: -1.5×10^{-3}, p=0.7

Supplemental Figure 4

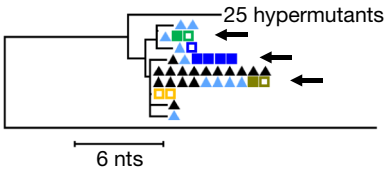
A.3720 p6-PR-RT(1.8 years suppressed)



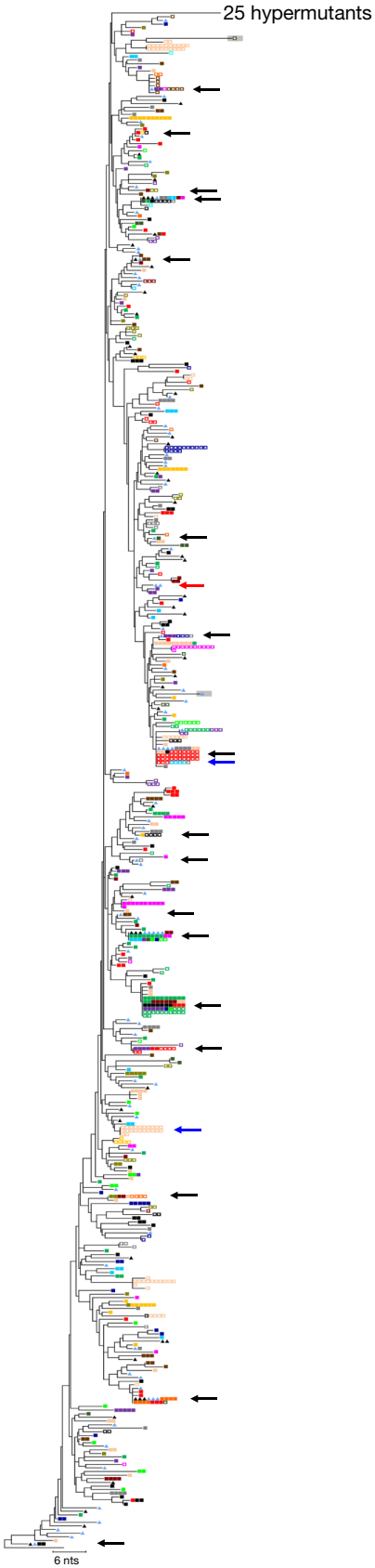
PBMC HIV provirus
LNMC HIV provirus
PBMC HIV RNA
LNMC HIV RNA
Putative clonal sequences
Replication competent
High expressing
Contain STOP codon(s)

Different colored squares indicate different aliquots with few expressing cells

B.2661 p6-PR-RT(12.9 years suppressed)



C. 2669 p6-PR-RT (4.3 years suppressed)



Supplemental Figure Legend

Figure S1. HIV-1 full-length *env* DNA sequences prior to and during long-term ART. Neighbor joining trees were constructed from single-genome full-length *env* proviral sequences obtained from PBMCs prior to continuous viral suppression on ART (hollow black triangles) and after 5.4-11.4 years of viral suppression on ART (solid black triangles). Diversity, divergence, and root-to-tip distances were measured as described in the legend to Figure 1. Both trees are rooted on the subtype B consensus sequence. Sequences containing G to A hypermutation and/or stop codons within open reading frames (indicated by shaded boxes) were excluded from all analyses. The limited number of infected cells on ART in donors 3720 and 2661 (<10 copies HIV DNA/million PBMC) prevented *env* SGS on these samples. Pre-ART sample was not available from donor 2669. Results from a total of 4 samples – two samples each from 2 patients – are represented in this figure.

Figure S2. HIV-1 full length *env* proviral sequences in lymph nodes and peripheral blood during ART. Neighbor joining trees were constructed from single-genome full-length *env* proviral sequences obtained from PBMC (black triangles) and LNMCs (blue triangles) after 4.3-18.0 years of continuous viral suppression on ART. Diversity and divergence, were measured as described in the legend to Figure 1; branch length correlation coefficients were calculated as described in the legend to Figure 2. Black arrows indicate identical sequences that were found in both locations, likely due to clonal expansion of infected cells. Sequences containing G to A hypermutation and/or stop codons within open reading frames (indicated by shaded boxes) were excluded from all analyses. Results from a total of 6 samples – two samples each from 3 patients – are represented in this figure.

Figure S3. HIV-1 p6-PR-RT proviral DNA sequences obtained from paired lymph node samples during ART. Neighbor joining trees were constructed from single-genome p6-PR-RT proviral sequences obtained from LNMC collected from one right (dark blue triangles) and one left (light blue triangles) inguinal lymph node at a single time point for each donor (after 12.8 or 5.5 years of continuous viral suppression on ART). Diversity and divergence, were measured as described in the legend to Figure 1; branch length correlation coefficients were calculated as described in the legend to Figure 2. Black arrows indicate identical sequences that were found in both lymph nodes, likely due to clonal expansion of infected cells. Both trees are rooted on the HIV-1 subtype B consensus sequence. Sequences containing G to A hypermutation and/or stop codons within open reading frames (indicated by shaded boxes) were excluded from all analyses. Results from a total of 4 samples – two samples each from 2 patients – are represented in this figure.

Figure S4. HIV-1 p6-PR-RT cell-associated RNA in peripheral blood and lymph nodes during ART. Neighbor joining trees were constructed from single-genome *p6-PR-RT* proviral and cell-associated HIV-1 RNA sequences obtained from paired PBMC and LNMC samples during continuous viral suppression on ART. Black and blue triangles indicate HIV-1 DNA sequences from PBMC and LNMC, respectively; solid squares and hollow squares indicate HIV-1 RNA sequences from PBMC and LNMC, respectively. Squares of the same color with no genetic distance indicate sequences obtained from the same aliquot of cells, and thus represent the level of viral RNA within a single infected cell. Black arrows indicate identical sequences detected in both PBMC and LNMC, which likely derive from clonally expanded cells. Blue arrows indicate cells that have high levels of viral RNA (<20 copies). The red arrows indicate sequences matching virus that grew in the viral outgrowth assay. **A** is rooted on the HIV-1 subtype C consensus sequence, and **B** and **C** are rooted on the subtype B consensus sequence. Results from a total of 12 samples – four samples each from 3 patients – are represented in this figure.