SUPPLEMENTAL INFORMATION TABLES AND FIGURES

SUPPLEMENTARY TABLE S1. Overview of Last Gift Participants.

SUPPLEMENTARY TABLE S2. Viral Characteristics of the Sampled Compartments.

The total number (n=676) and the number of intact (n=605) full length (FL) envelope (*env*) sequences is reported. See method section for the identification of defective or hypermutated FL *env* sequences. CI: Confidence Interval; †The tropism of each variant was inferred from the V3 amino acid sequence using geno2pheno(1) with conservative 10% false positive rate threshold for co-receptor CXCR4 usage * Digital droplet PCR using skGag primer; results reported in DNA copy per 10^6 cells.

SUPPLEMENTARY TABLE S3. Statistical analysis of population structure of all full length *env* HIV sequences used in the present study. The level of spatial structure was quantified using the Simmonds association index (AI) implemented in BaTS v1.0(98) on all sequences (A) and after exclusion of identical FL *env* sequences within compartments (B). AI, association index. PS, parsimony score. MC, monophyletic clade statistic. 95% CI, 95% credibility interval. *Statistically significant (P<0.05). N/A, not available because of the observed 95% CI contains the null 95% CI.

SUPPLEMENTARY FIGURE S1. **Clinical Courses of the 6 Last Gift Participants.**

SUPPLEMENTARY FIGURE S2. **Distribution of Pairwise Distance between Intact full length *env* HIV DNA within Compartments.** The pairwise genetic distance between sequences from a compartment was measured using the Tamura Nei 93 (TN93) algorithm (1). Horizontal bars represent the median, the 25th and 75th percentiles and 95% Confidence Intervals.

SUPPLEMENTARY FIGURE S3. **Approximate Maximum Likelihood (ML) Phylogeny Including All Data**. Maximum-likelihood phylogeny reconstruction was performed by using IQtree(2). No sample cross-contamination was observed.

SUPPLEMENTARY FIGURE S4. **Maximum Likelihood (ML) Phylogenies and Clonal Populations (Full Length Envelope).** Phylogenies were estimated using IQtree(4) from the full length (FL) HIV *env* sequences

obtained from pre-mortem blood plasma and from tissues and PBMC collected during rapid autopsy. **A.** ML phylogeny for Individual LG04 who stopped therapy. **B.** ML phylogeny for Individual LG05, LG06 and LG08 who remained virally suppressed. Tips are colored by compartment as in the legend. Size and distribution of nearly identical FL *env* populations (99% identical, populations of at least 3 identical proviruses) for each participant are presented in the middle of each tree. Colors represent tissues described in Left Panel legend. For LG01, nearly identical FL *env* populations including HIV RNA viruses sampled in blood plasma during viral rebound are marked with *. See also **Figure 4**.

SUPPLEMENTARY FIGURE S5. **Approximate maximum likelihood phylogenies and Genotropism.** Phylogenies were estimated using IQtree(4) from the full length HIV *env* sequences obtained from pre-mortem blood plasma and from tissues and PBMC collected during rapid autopsy. Tips colored by tropism according to the tropism of each variant inferred from the V3 amino acid sequence using geno2pheno(1) with conservative 10% false positive rate threshold for co-receptor CXCR4 usage.

SUPPLEMENTARY FIGURE S6. Proportion of Supported Transition Events between Compartments for Participants Who Stopped ART (A) or Remained Virally Suppressed on ART (B) . Sankey plot showing the proportion of transition events between locations for which the adjusted BF ≥ 3 (at least positive evidence). The adjusted BF support for each transition type is given next to the corresponding color. The source locations are depicted on the left side of the plots while the destination locations are given at the right side. See also **Figure 6**.

SUPPLEMENTARY FIGURE S7. Generalized Linear Model Analyses. Box plots of the GLM coefficients for all tested predictors. Bayes Factors (BFs) of each predictor in the model is indicated in red on the right. The original BF support are presented along with the adjusted BF (in dark grey) (see Methods for details). Adjusted BF≥3 are in bold. The GLM coefficients are conditional on inclusion of predictor in the model.

SUPPLEMENTARY TABLE S1. Overview of Last Gift Participants. ALS: amyotrophic lateral sclerosis; AML: Acute Myeloid Leukemia. ABC: abacavir, 3TC: lamivudine, DTG: dolutegravir, FTC: emtricitabine.*estimated based on medical records (the exact date of infection is unknown.)

ID	Age	Diagnosis	Years since diagnosis*	Stopped ART	Last ART Regimen	Last CD4 count (cells/mm^3)	Last HIV RNA level	Deceased
LG01	58	ALS	21	Yes	ABC+3TC+DTG	864	280	July 2017
LG03	72	Pancreatic Tumor	23	No	FTC/TAF+DTG	330	undetectable	May 2018
LG04	69	AML	35	Yes	FTC/TAF+DTG	497	48,000	March 2018
LG05	57	ALS	23	No	RPV/TAF/FTC+DTG	347	undetectable	March 2019
LG06	57	Oral Cancer	28	No	FTC/TAF+DTG	174	undetectable	May 2018
LG08	52	Rectal cancer	13	No	DRV/c+DTG	224	undetectable	Dec 2018

SUPPLEMENTARY TABLE S2. Viral Characteristics of the Sampled Compartments.

The total number (n=676) and the number of intact (n=605) full length (FL) envelope (*env*) sequences is reported. See method section for the identification of defective or hypermutated FL *env* sequences. CI: Confidence Interval; †The tropism of each variant was inferred from the V3 amino acid sequence using geno2pheno(1) with conservative 10% false positive rate threshold for co-receptor CXCR4 usage * Digital droplet PCR using skGag primer; results reported in DNA copy per 10^6 cells.

IDs	Compartment	Number of FL env (pro)viruses (% total)	Number of FL intact env (pro)viruses (% total)	Mean Pairwise Distance [25-75% CI]	% X4 tropic†	*gag* copies /10^6 cells* [Min-Max]	HIV RNA Levels (cp/mL)
LG01	PBMC	19 (24%)	16 (22%)	0.034 [0.017-0.058]	81.0%	94.3 [68.3-125.7]	
(n=75)	Blood Plasma	17 (21%)	17 (23%)	0.018 [0.001-0.029]	100.0%		13,500
	Gut	11 (14%)	11 (15%)	0.03 [0.019-0.055]	81.8%	138 [94.9-181.2]	
	Ileum	7 (9%)	5 (7%)	0.045 [0.041-0.051]	50.0%	66.1 [35.1-97]	
	Lymph Nodes (Peritracheal)	6 (8%)	5 (7%)	0.04 [0.03-0.058]	100.0%	58.6 [38.5-79.3]	
	Prostate	7 (9%)	7 (10%)	0.034 [0.007-0.058]	71.4%	10.7 [4-26.7]	
	Spleen	6 (8%)	5 (7%)	0.038 [0.022-0.059]	66.7%	53.5 [31.1-83.3]	
	Liver	7 (9%)	7 (10%)	0.043 [0.017-0.066]	86.0%	19.9 [2.1-37.7]	
	Frontal Lobe Motor	2 (3%)	2 (3%)	0.054 [0.054-0.054]	100.0%	0 [0-10.7]	
LG03	PBMC	11 (9%)	10 (9%)	0.032 [0.016-0.057]	80.0%	24.9 [5.8-45.3]	
(n=113)	Duodenum	7 (6%)	7 (6%)	0.028 [0.009-0.055]	85.7%	101.3 [56.9-147.6]	
	Ileum	5 (4%)	3 (3%)	0 [0-0]	100.0%	176.7 [127.2-226.1]	
	Right Colon	10 (8%)	9 (8%)	0.04 [0.017-0.062]	44.4%	95.7 [65.3-134.9]	
	Rectum	10 (8%)	10 (9%)	0.021 [0-0.059]	80.0%	34.5 [14.8-66.6]	
	Lymph Nodes (Aortic)	12 (10%)	11 (10%)	0.029 [0.012-0.058]	81.8%	73.9 [52-102.1]	
	Lymph Nodes (Axillary)	12 (10%)	12 (11%)	0.031 [0.013-0.059]	83.3%	44.8 [33.4-57.9]	
	Prostate	9 (7%)	9 (8%)	0.022 [0.012-0.025]	100.0%	56 [34.7-85.3]	
	Testis	6 (5%)	5 (4%)	0.041 [0.022-0.058]	60.0%	22.7 [8.5-42.6]	
	Spleen	5 (4%)	5 (4%)	0.047 [0.034-0.064]	60.0%	39.5 [6.2-73.8]	
	Pancreas	8 (7%)	7 (6%)	0.045 [0.029-0.06]	57.1%	391.5 [329.2-453.7]	
	Liver	11 (9%)	11 (10%)	0.034 [0.014-0.061]	72.7%	20.1 [5.7-40.1]	
	Occipital Lobe	2 (2%)	2 (2%)	0.051 [0.051-0.051]	0.0%	1.7 [0-9.1]	
	Frontal Lobe Motor	1 (1%)	1 (1%)	0 [0-0]	0.0%	2.9 [0-15.8]	
	Pericardial Adipose	12 (10%)	11 (10%)	0.03 [0.015-0.059]	82.0%	31.1 [15.6-53.7]	
LG04	PBMC	24 (14%)	23 (15%)	0.001 [0-0.001]	0.0%	95.5 [56.9-137.7]	
(n=152)	Blood Plasma	10 (6%)	10 (7%)	0.001 [0-0.001]	10.0%		48,000
	Cardiac Serum	6 (3%)	6 (4%)	0.007 [0.005-0.009]	0.0%	101 [76.3-132.1]	
	Esophagus	10 (6%)	7 (5%)	0.026 [0.007-0.046]	0.0%	304.8 [262-342.2]	
	Duodenum	9 (5%)	8 (5%)	0.029 [0-0.044]	62.5%	76.1 [52.2-106.8]	
	Jejunum	7 (4%)	7 (5%)	0 [0-0]	100.0%	264.8 [210.9-329.5]	
	Ileum	6 (3%)	5 (3%)	0.023 [0.004-0.053]	40.0%	351.8 [268-452.3]	
	Right Colon	7 (4%)	6 (4%)	0.047 [0.032-0.064]	16.7%	37.6 [22.3-61]	
	Left Colon	10 (6%)	10 (7%)	0.032 [0.024-0.041]	0.0%	77.4 [52-110.7]	
	Rectum	6 (3%)	5 (3%)	0.043 [0.041-0.046]	20.0%	37.1 [17.4-67.6]	
	Lymph Nodes (Axillary)	7 (4%)	7 (5%)	0.015 [0.001-0.048]	14.3%	658.8 [580.4-752.9]	
	Lymph Nodes (Inguinal)	6 (3%)	3 (2%)	0.027 [0.021-0.04]	0.0%	513.4 [424.7-600.2]	
	Lymph Nodes (Mesentary)	10 (6%)	9 (6%)	0.031 [0.007-0.048]	11.1%	369.1 [303.8-435.8]	
	Seminal Vesicle	8 (5%)	8 (5%)	0.022 [0.001-0.043]	0.0%	7.5 [0-21.1]	
	Testis	9 (5%)	5 (3%)	0.035 [0.039-0.042]	20.0%	18.7 [7.2-37.4]	
	Prostate	5 (3%)	4 (3%)	0.042 [0.037-0.047]	50.0%	33.1 [14.2-52]	
	Kidney	8 (5%)	7 (5%)	0.018 [0.004-0.043]	0.0%	66.5 [41.4-99]	
	Liver	7 (4%)	4 (3%)	0.003 [0.001-0.004]	0.0%	15.8 [-2.4-32.7]	
	Pancreas	8 (5%)	8 (5%)	0.042 [0.031-0.053]	25.0%	23 [10.9-41.2]	
	Spleen	7 (4%)	7 (5%)	0.025 [0.004-0.041]	0.0%	48.5 [17.6-101.3]	
	Occipital Lobe	3 (2%)	2 (1%)	0.051 [0.051-0.051]	50.0%	0 [0-4.6]	
	Frontal Lobe Motor	1 (1%)	1 (1%)	0 [0-0]	0.0%	1.9 [0-10.4]	
LG05	PBMC	5 (9%)	5 (9%)	0.042 [0.038-0.051]	40.0%	172.6 [123.5-232.5]	
(n=55)	Duodenum	11 (19%)	9 (16%)	0.001 [0-0.001]	100.0%	40.1 [23.9-58.3]	
	Jejunum	8 (14%)	8 (15%)	0.001 [0-0.001]	100.0%	169.7 [131.5-216.4]	
	Lymph Nodes (Aortic)	11 (19%)	11 (20%)	0.021 [0.01-0.029]	82.0%	153.8 [109.1-209.3]	
	Lymph Nodes (Axillary)	10 (18%)	10 (18%)	0.027 [0.01-0.056]	80.0%	28.2 [18.9-38.4]	
	Spleen	7 (12%)	7 (13%)	0.041 [0.021-0.062]	100.0%	120.8 [83.4-161]	
	Frontal Lobe Motor	5 (9%)	5 (9%)	0.007 [0.005-0.011]	0.0%	10.6 [1.8-33.6]	
LG06	PBMC	20 (16%)	16 (15%)	0.054 [0.014-0.089]	81.0%	172.7 [132.8-219.8]	

(n=104)	Duodenum	7 (6%)	6 (6%)	0.025 [0.001-0.072]	0.0%	216.2 [170.2-269.2]
	Ileum	11 (9%)	8 (8%)	0.045 [0.006-0.069]	25.0%	143.2 [107.7-187.1]
	Jejunum	7 (6%)	6 (6%)	0.026 [0.002-0.066]	16.7%	206.9 [164-255]
	Right Colon	8 (6%)	5 (5%)	0.051 [0.039-0.065]	0.0%	276.3 [206.6-348.6]
	Rectum	7 (6%)	7 (7%)	0.055 [0.041-0.081]	28.6%	134.8 [81.7-192]
	Lymph Nodes (Aortic)	7 (6%)	7 (7%)	0.034 [0.011-0.083]	85.7%	123.4 [90.8-159.9]
	Lymph Nodes (Axillary)	13 (10%)	13 (13%)	0.03 [0.01-0.021]	92.3%	23.8 [17.9-30.8]
	Lymph Nodes (Mediastinal)	7 (6%)	5 (5%)	0.044 [0.017-0.083]	80.0%	99.8 [31.5-168.2]
	Seminal Vesicle	7 (6%)	6 (6%)	0.005 [0-0.016]	0.0%	14.4 [4.8-40.9]
	Testis	7 (6%)	6 (6%)	0 [0-0]	100.0%	2.6 [0-13.1]
	Prostate	5 (4%)	3 (3%)	0.068 [0.053-0.096]	66.7%	12.6 [3.1-28.3]
	Liver	11 (9%)	10 (10%)	0.066 [0.048-0.083]	20.0%	9.9 [3.3-23]
	Spleen	8 (6%)	6 (6%)	0.063 [0.039-0.086]	33.0%	74.7 [49.3-108]
LG08	PBMC	15 (13%)	14 (13%)	0.044 [0.042-0.05]	14.3%	41.9 [21.8-72.8]
(n=106)	Duodenum	6 (5%)	4 (4%)	0.037 [0.033-0.046]	0.0%	176.6 [137.7-224.7]
	Ileum	9 (8%)	8 (8%)	0.043 [0.035-0.052]	12.5%	107.1 [76.6-143.6]
	Jejunum	9 (8%)	7 (7%)	0.047 [0.044-0.052]	0.0%	194.4 [90.6-292]
	Right Colon	7 (6%)	7 (7%)	0.048 [0.042-0.051]	14.3%	72.3 [49.1-99.1]
	Rectum	9 (8%)	9 (8%)	0.036 [0.026-0.048]	0.0%	14.6 [5.6-30.2]
	Prostate	6 (5%)	6 (6%)	0.027 [0.001-0.046]	12.5%	55.8 [34.9-81.4]
	Seminal Vesicle	11 (9%)	9 (8%)	0.033 [0.001-0.055]	0.0%	9.9 [0-23.7]
	Spleen	9 (8%)	8 (8%)	0.048 [0.043-0.059]	0.0%	39.9 [27.8-54.5]
	Liver	7 (6%)	7 (7%)	0.024 [0-0.046]	0.0%	29.7 [12.2-48.1]
	Lymph Nodes (Aortic)	7 (6%)	6 (6%)	0.023 [0-0.04]	0.0%	79.5 [48.9-112.5]
	Lymph Nodes (Hilar)	7 (6%)	6 (6%)	0.048 [0.047-0.052]	33.0%	28 [14-44.5]
	Frontal Lobe Motor	2 (2%)	2 (2%)	0.05 [0.05-0.05]	0.0%	4.2 [0-21.2]
	Esophagus	6 (5%)	6 (6%)	0.015 [0-0.045]	0.0%	53.7 [20.7-85.4]
	Pancreas	7 (6%)	7 (7%)	0.014 [0-0.048]	0.0%	95 [65.2-131.8]

SUPPLEMENTARY TABLE S3. Statistical analysis of population structure of all full length *env* HIV sequences used in the present study. The level of spatial structure was quantified using the Simmonds association index (AI) implemented in BaTS v1.0(98) on all sequences (A) and after exclusion of identical env sequences within compartments (B). AI, association index. PS, parsimony score. MC, monophyletic clade statistic. 95% CI, 95% credibility interval. *Statistically significant (P<0.05). N/A, not available because of the observed 95% CI contains the null 95% CI.

A. All sequences included.

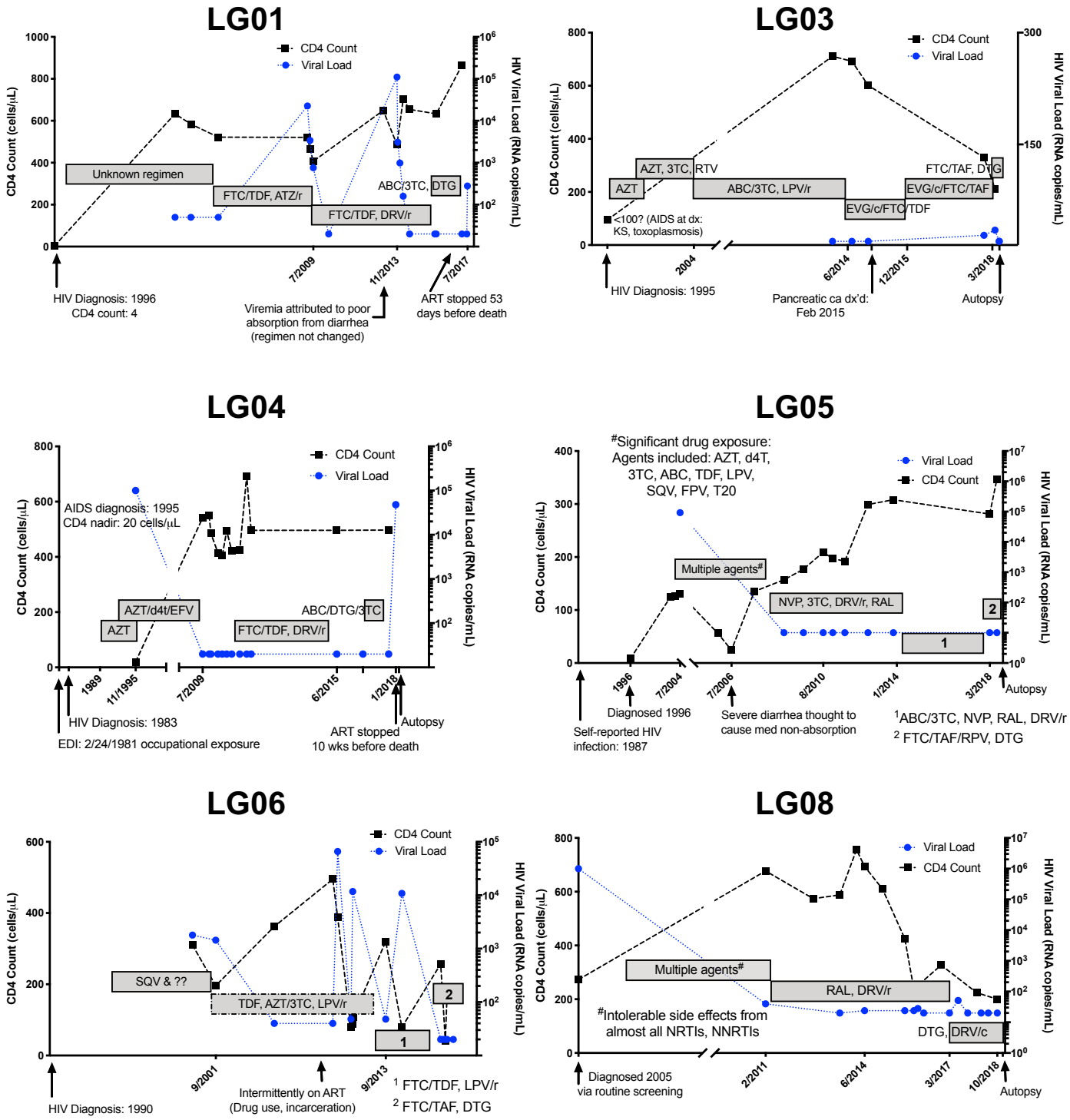
Participant	Statistic	Observed mean (95% CI)	Null mean (95% CI)	*Adjusted AI*	*P*-value
LG01	AI	3.546 [3.094-3.912]	5.334 [4.714-5.877]	0.665 [0.656-0.666]	<0.001
LG03	AI	8.558 [7.958-9.399]	10.166 [9.481-10.727]	0.842 [0.839-0.876]	<0.001
LG04	AI	11.062 [10.16-12.269]	14.059 [13.401-14.6]	0.787 [0.758-0.84]	<0.001
LG05	AI	2.737 [2.138-3.073]	4.22 [3.635-4.702]	0.649 [0.588-0.653]	<0.001
LG06	AI	5.148 [4.636-5.613]	8.476 [7.853-8.992]	0.607 [0.59-0.624]	<0.001
LG08	AI	8.228 [6.982-9.495]	9.668 [9.054-10.16]	0.851 [0.771-0.935]	<0.001

B. After exclusion of identical env sequences within compartments

Participant	Statistic	Observed mean (95% CI)	Null mean (95% CI)	*Adjusted AI*	*P*-value
LG01	AI	3.625 [3.123-4.002]	5.196 [4.572-5.738]	0.698 [0.683-0.697]	<0.001
LG03	AI	7.579 [7.088-7.909]	7.971 [7.317-8.49]	0.951 [0.969-0.931]	<0.001
LG04	AI	10.107 [9.399-11.001]	12.105 [11.5-12.59]	0.835 [0.817-0.874]	<0.001
LG05	AI	2.087 [1.555-2.437]	3.668 [3.096-4.133]	0.569 [0.502-0.59]	<0.001
LG06	AI	5.173 [4.67-5.798]	7.63 [6.969-8.18]	0.678 [0.67-0.709]	<0.001
LG08	AI	7.688 [7.3-7.962]	8.845 [8.228-9.336]	0.869 [0.887-0.853]	<0.001

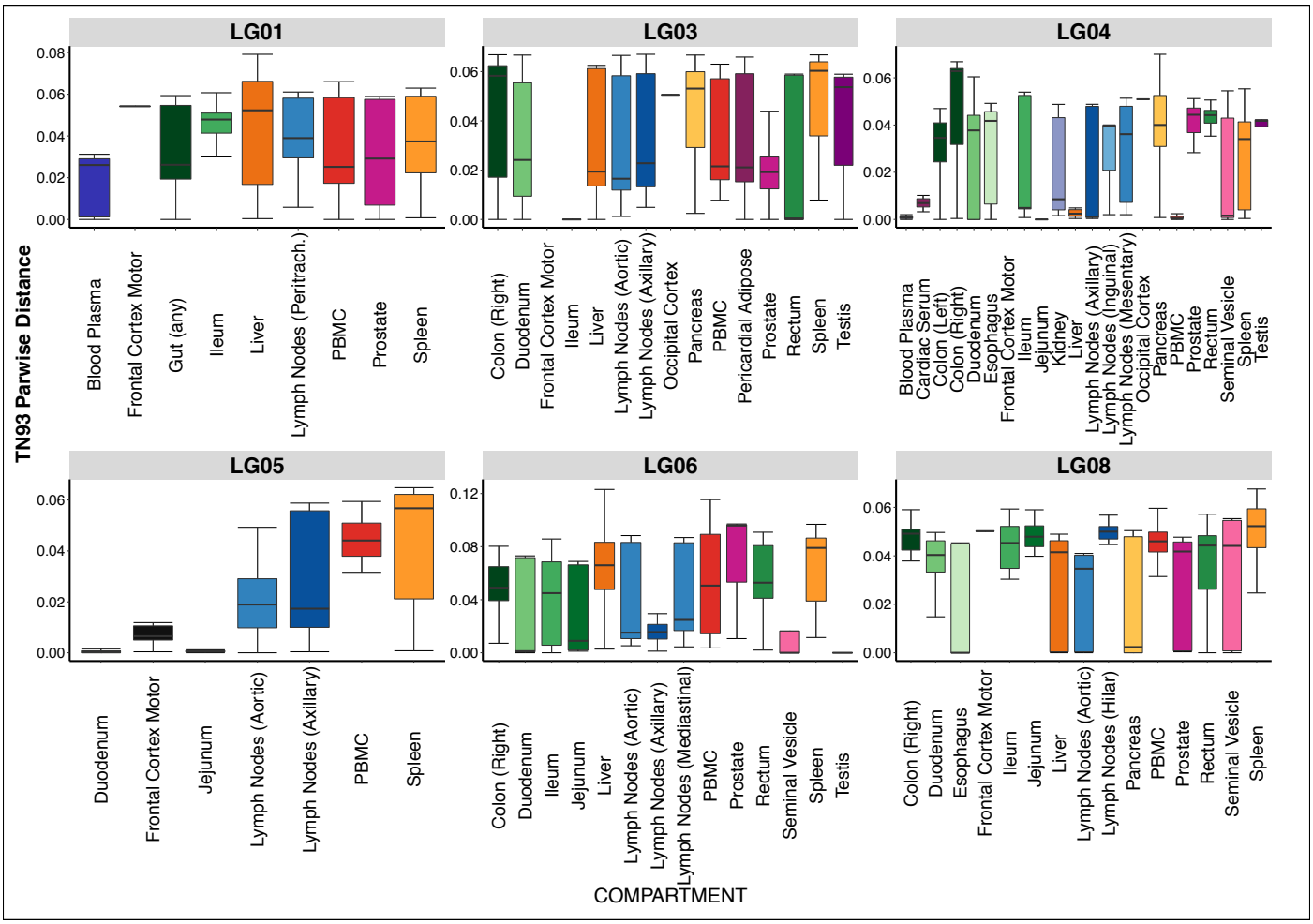
Participant	Statistic	Observed mean (95% CI)	Null mean (95% CI)	*Adjusted AI*	*P*-value
LG01	AI	3.625 [3.123-4.002]	5.196 [4.572-5.738]	0.698 [0.683-0.697]	<0.001
LG03	AI	7.579 [7.088-7.909]	7.971 [7.317-8.49]	0.951 [0.969-0.931]	<0.001
LG04	AI	10.107 [9.399-11.001]	12.105 [11.5-12.59]	0.835 [0.817-0.874]	<0.001
LG05	AI	2.087 [1.555-2.437]	3.668 [3.096-4.133]	0.569 [0.502-0.59]	<0.001
LG06	AI	5.173 [4.67-5.798]	7.63 [6.969-8.18]	0.678 [0.67-0.709]	<0.001
LG08	AI	7.688 [7.3-7.962]	8.845 [8.228-9.336]	0.869 [0.887-0.853]	<0.001

SUPPLEMENTARY FIGURE S1. Clinical Courses of the 6 Last Gift Participants.

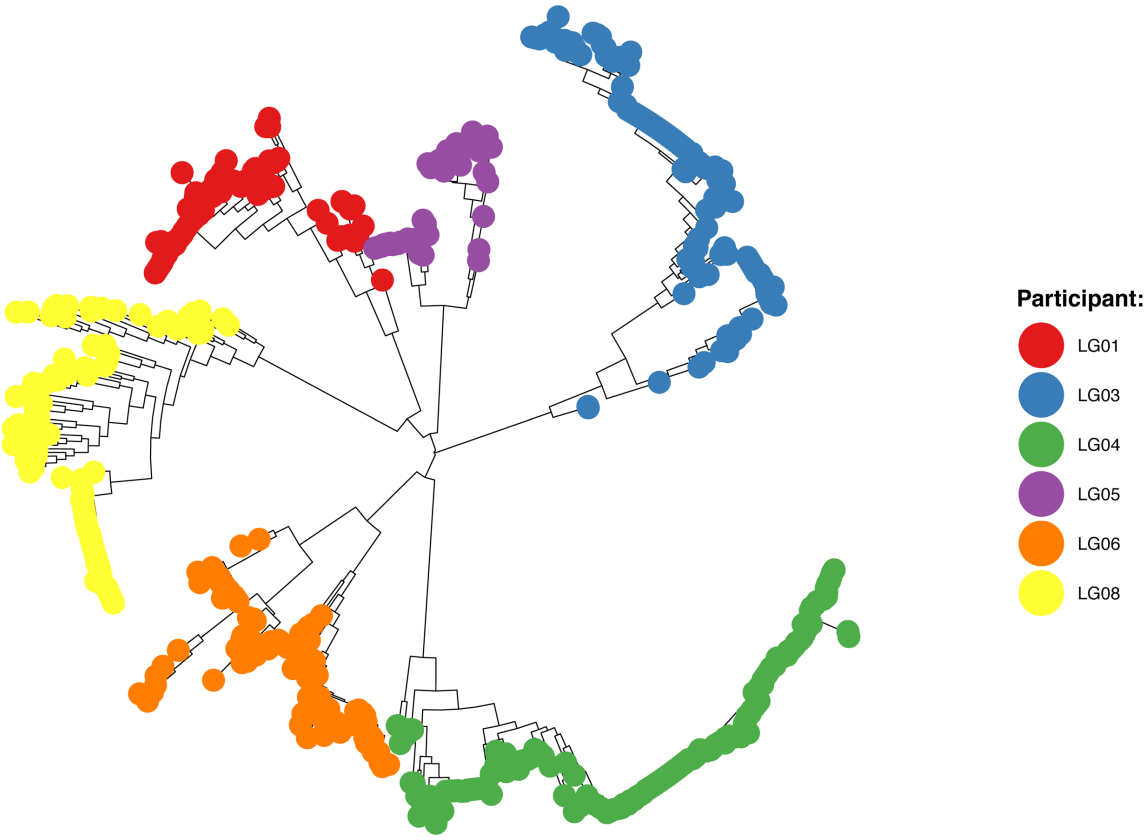


EDI: estimated date of infection; ABC: abacavir, 3TC: lamivudine, DTG: dolutegravir, FTC: emtricitabine; d4t: stavudine; AZT: Atazanavir; DRV/r: , EFV: Efavirenz ; LPV: Lopinavir; SQV: Saquinavir; EVG: Elvitegravir; TDF: Tenofovir ; TAF: tenofovir alafenamide.

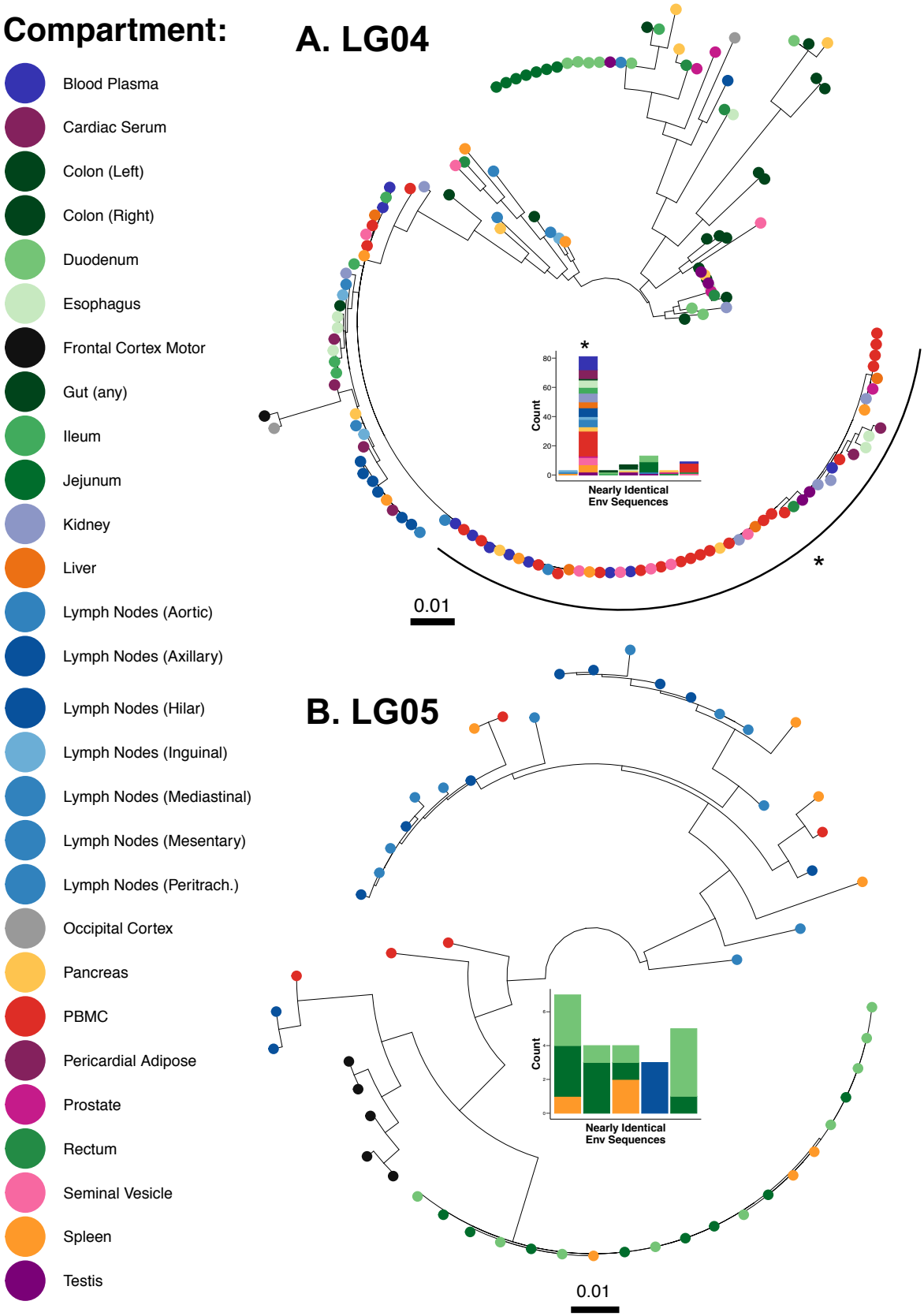
SUPPLEMENTARY FIGURE S2. **Distribution of Pairwise Distance between Intact full length *env* HIV DNA within Compartments. .** The pairwise genetic distance between sequences from a compartment was measured using the Tamura Nei 93 (TN93) algorithm (1). Horizontal bars represent the median, the 25th and 75th percentiles and 95% Confidence Intervals.

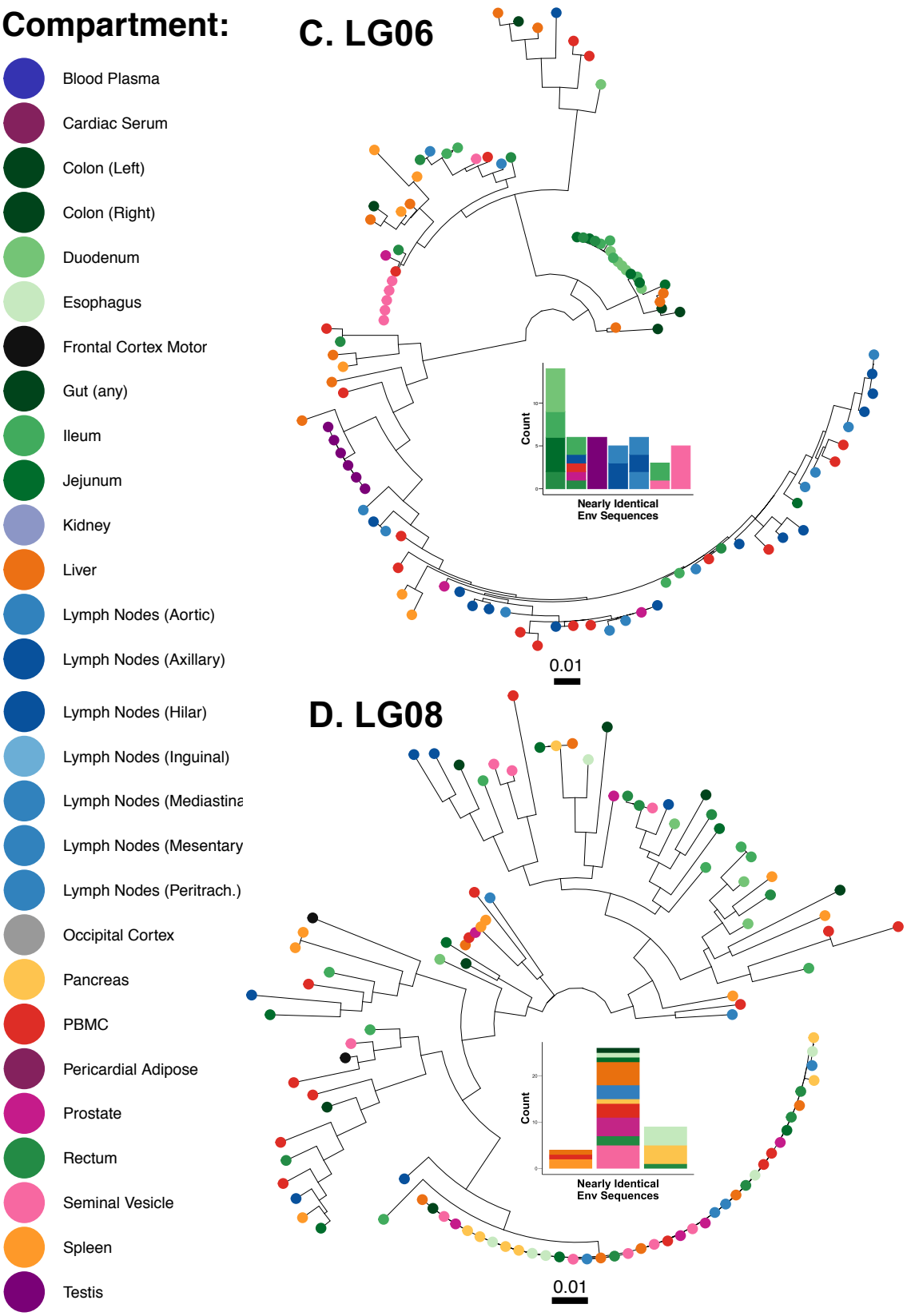


SUPPLEMENTARY FIGURE S3. Approximate Maximum Likelihood (ML) Phylogeny Including All Data. Maximum-likelihood phylogeny reconstruction was performed by using IQtree(2). No sample cross-contamination was observed.

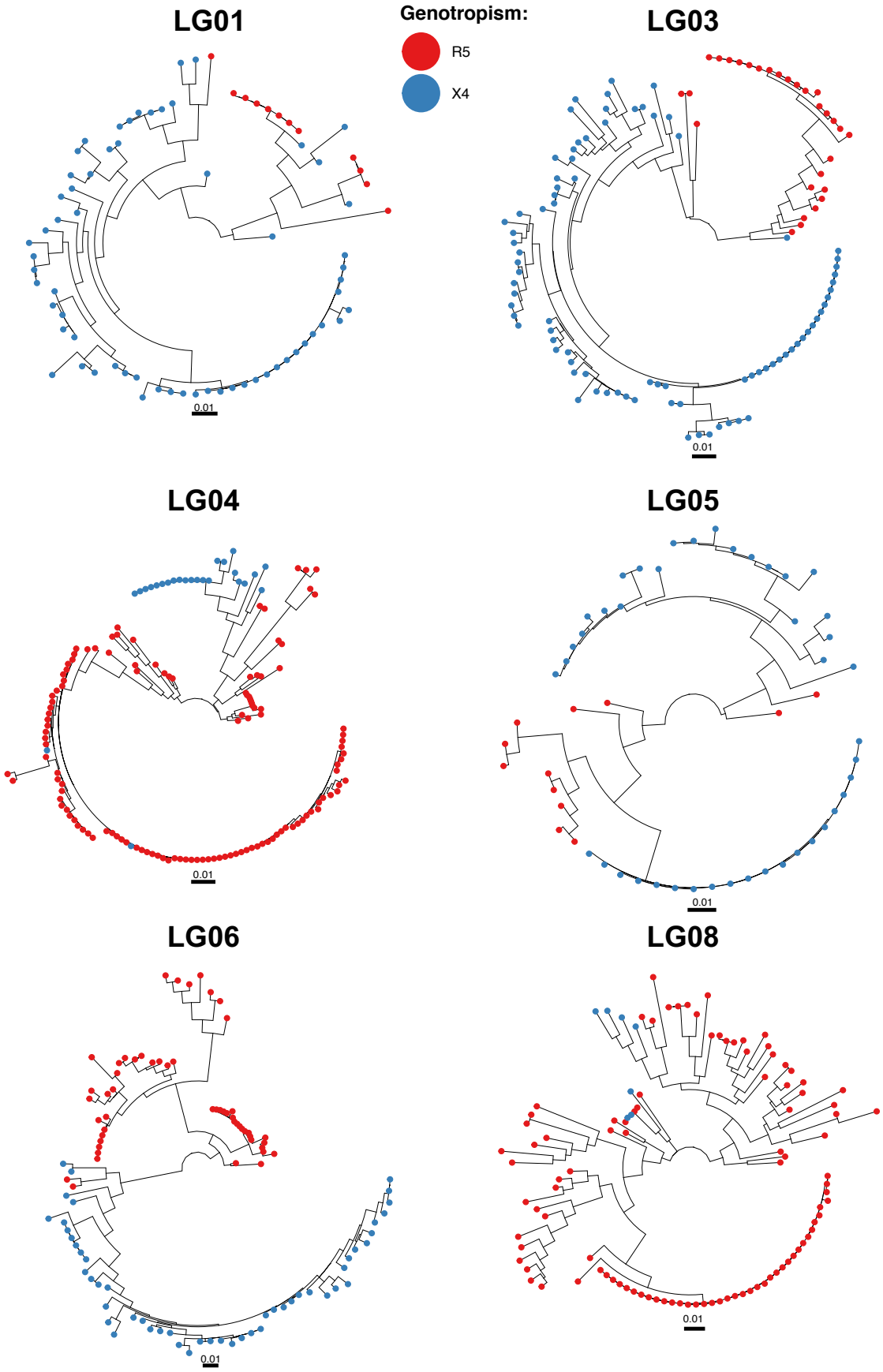


SUPPLEMENTARY FIGURE S4. **Maximum Likelihood (ML) Phylogenies and Clonal Populations (Full Length Envelope).** Phylogenies were estimated using IQtree(4) from the full length (FL) HIV *env* sequences obtained from pre-mortem blood plasma and from tissues and PBMC collected during rapid autopsy. **A.** ML phylogeny for Individual LG04 who stopped therapy. **B.** ML phylogeny for Individual LG05, LG06 and LG08 who remained virally suppressed. Tips are colored by compartment as in the legend. Size and distribution of nearly identical FL *env* populations (99% identical, populations of at least 3 identical proviruses) for each participant are presented in the middle of each tree. Colors represent tissues described in Left Panel legend. For LG01, nearly identical FL *env* populations including HIV RNA viruses sampled in blood plasma during viral rebound are marked with *. See also **Figure 4**.



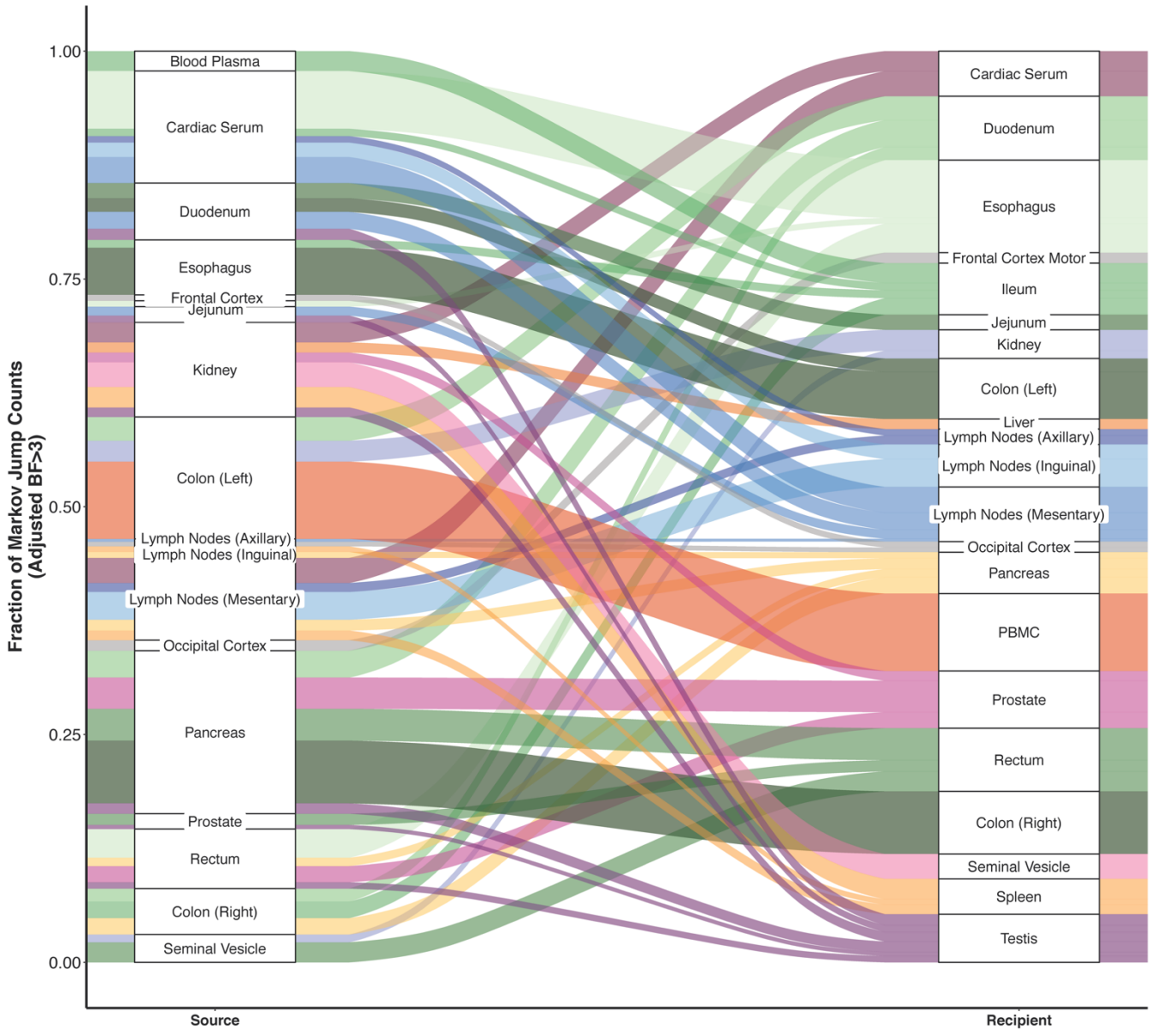

Compartment:
Blood Plasma
Cardiac Serum
Colon (Left)
Colon (Right)
Duodenum
Esophagus
Frontal Cortex Motor
Gut (any)
Ileum
Jejunum
Kidney
Liver
Lymph Nodes (Aortic)
Lymph Nodes (Axillary)
Lymph Nodes (Hilar)
Lymph Nodes (Inguinal)
Lymph Nodes (Mediastina
Lymph Nodes (Mesentary
Lymph Nodes (Peritrach.)
Occipital Cortex
Pancreas
PBMC
Pericardial Adipose
Prostate
Rectum
Seminal Vesicle
Spleen
Testis
C. LG06
Count
Nearly Identical
Env Sequences
0.01
D. LG08
Count
Nearly Identical
Env Sequences
0.01

SUPPLEMENTARY FIGURE S5. **Approximate maximum likelihood phylogenies and Genotropism.** Phylogenies were estimated using IQtree(4) from the full length HIV *env* sequences obtained from pre-mortem blood plasma and from tissues and PBMC collected during rapid autopsy. Tips colored by tropism according to the tropism of each variant inferred from the V3 amino acid sequence using geno2pheno(1) with conservative 10% false positive rate threshold for co-receptor CXCR4 usage.

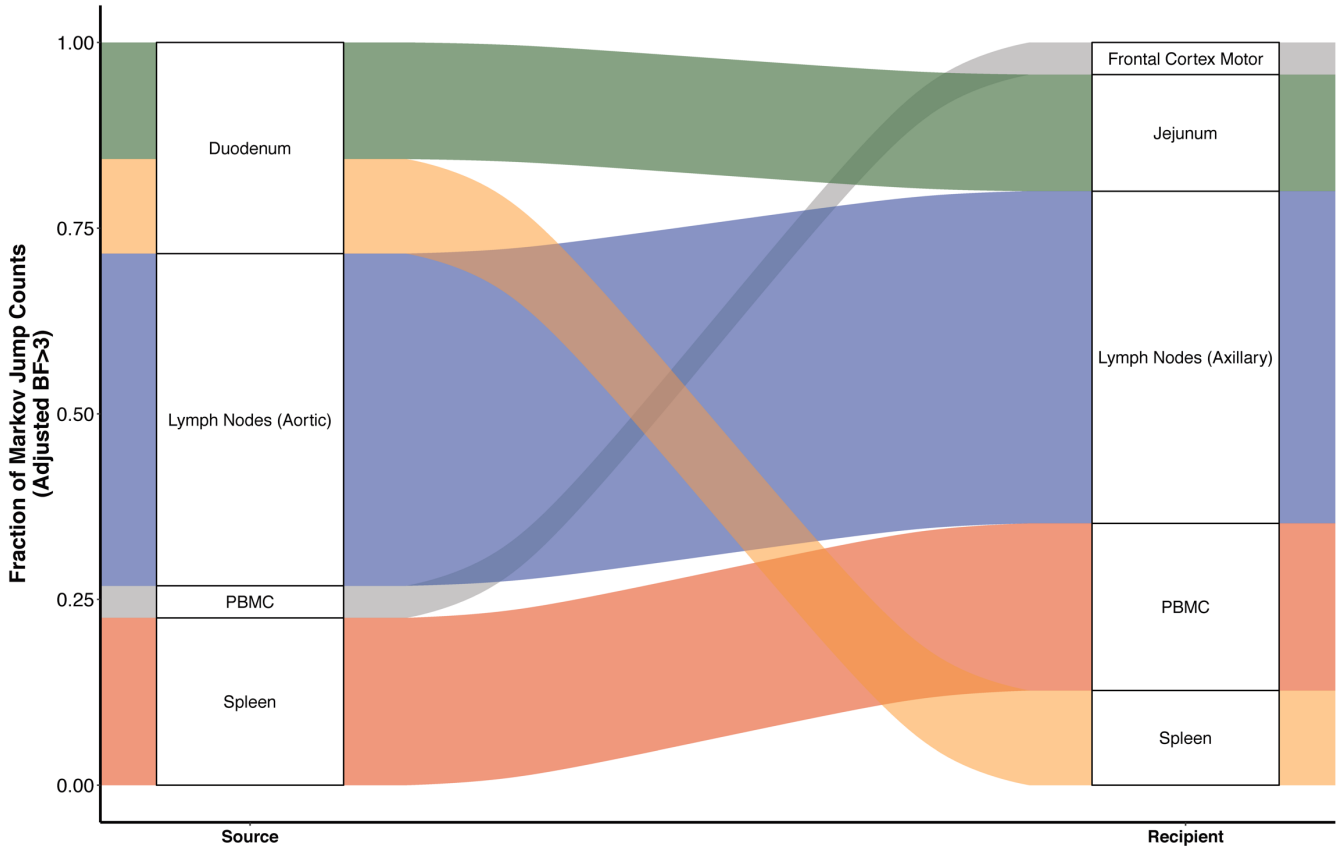


SUPPLEMENTARY FIGURE S6. Proportion of Supported Transition Events between Compartments for Participants Who Stopped ART (A) or Remained Virally Suppressed on ART (B) . Sankey plot showing the proportion of transition events between locations for which the adjusted BF ≥ 3 (at least positive evidence). The adjusted BF support for each transition type is given next to the corresponding color. The source locations are depicted on the left side of the plots while the destination locations are given at the right side. See also **Figure 6**.

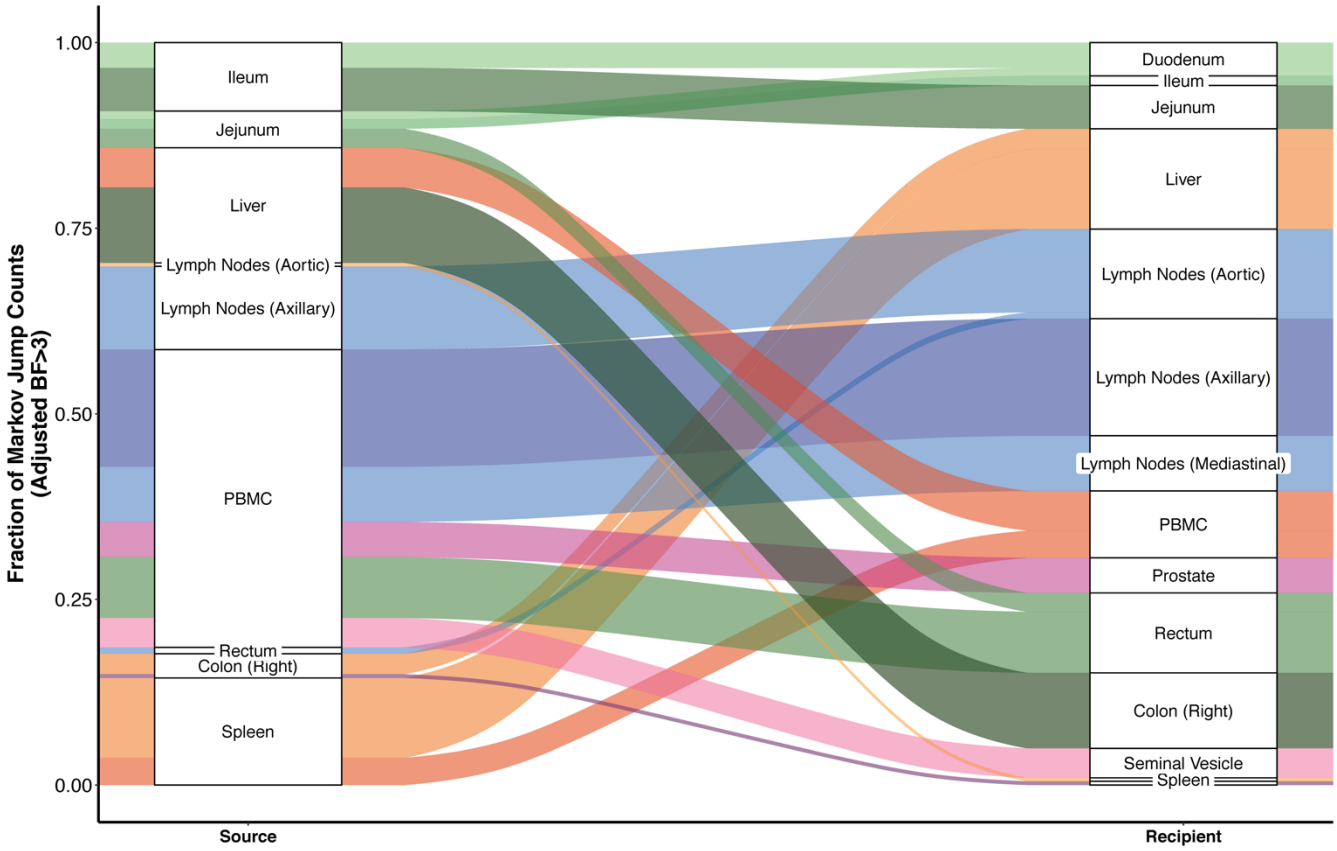
A. LG04



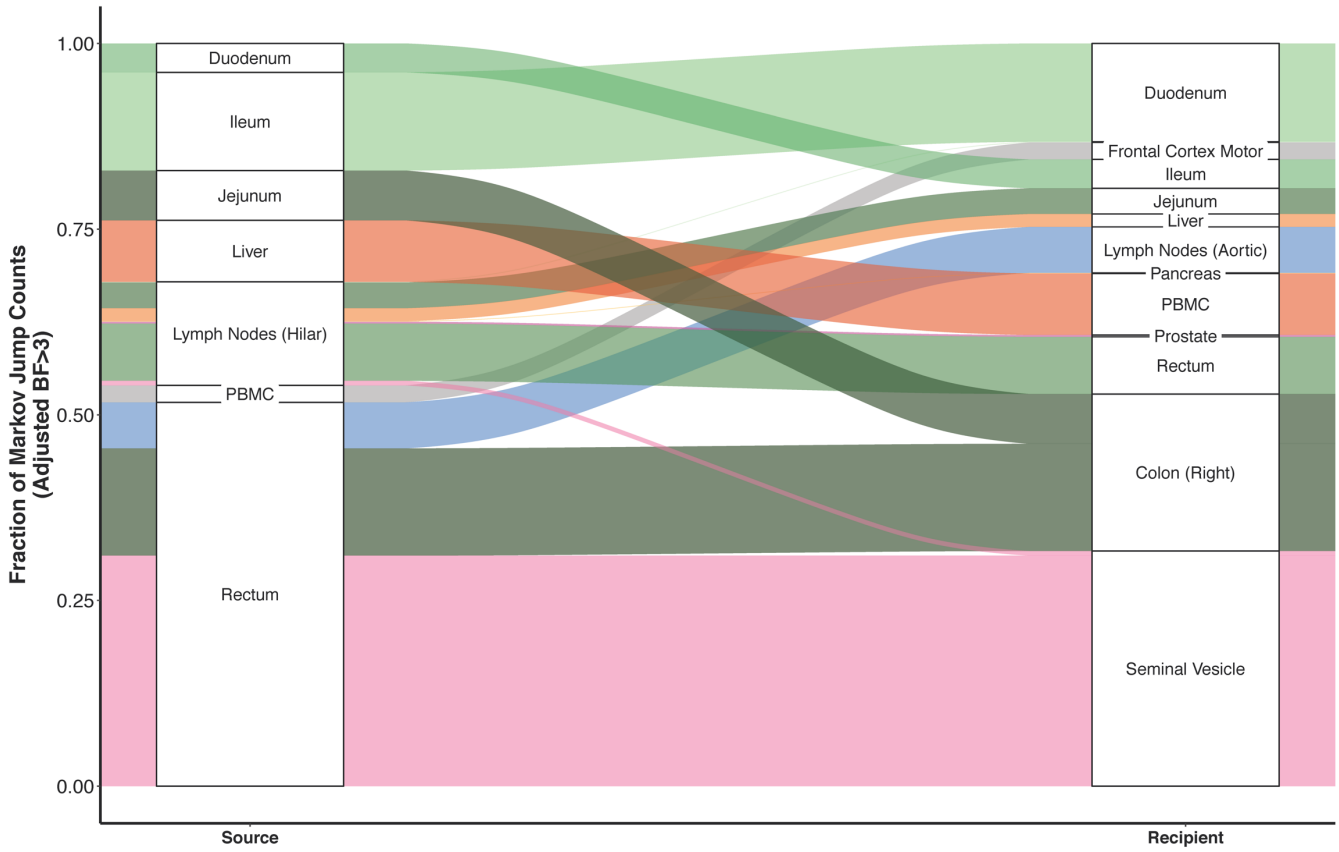
B. LG05


Fraction of Markov Jump Counts (Adjusted BF>3)
1.00
0.75
0.50
0.25
0.00
Duodenum
Lymph Nodes (Aortic)
PBMC
Spleen
Frontal Cortex Motor
Jejunum
Lymph Nodes (Axillary)
PBMC
Spleen
Source
Recipient

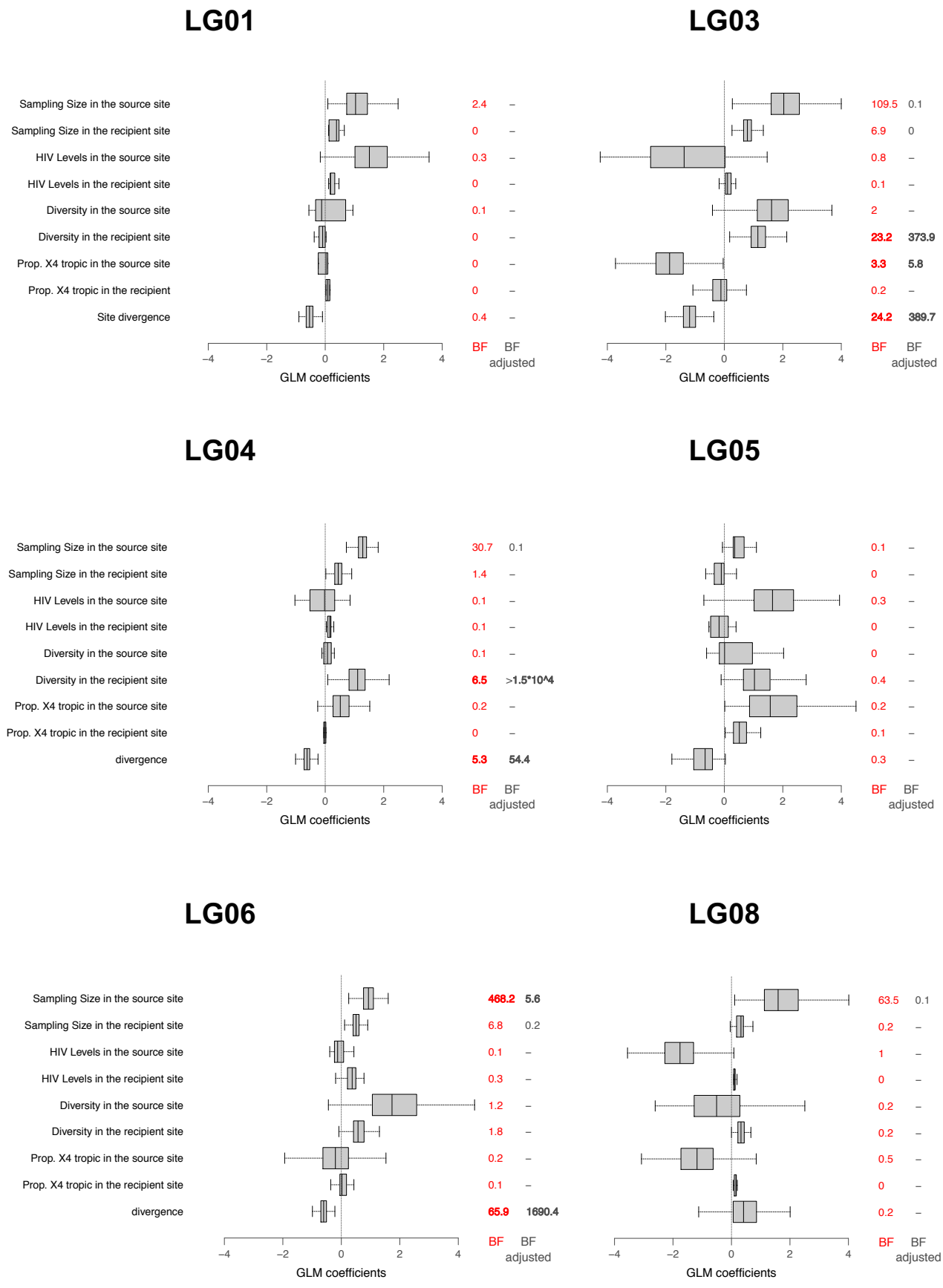
C. LG06


Fraction of Markov Jump Counts
(Adjusted BF>3)
1.00
0.75
0.50
0.25
0.00
Ileum
Jejunum
Liver
Lymph Nodes (Aortic)
Lymph Nodes (Axillary)
PBMC
Rectum
Colon (Right)
Spleen
Source
Duodenum
Ileum
Jejunum
Liver
Lymph Nodes (Aortic)
Lymph Nodes (Axillary)
Lymph Nodes (Mediastinal)
PBMC
Prostate
Rectum
Colon (Right)
Seminal Vesicle
Spleen
Recipient

D. LG08


Fraction of Markov Jump Counts (Adjusted BF>3)
1.00
0.75
0.50
0.25
0.00
Duodenum
Ileum
Jejunum
Liver
Lymph Nodes (Hilar)
PBMC
Rectum
Source
Duodenum
Frontal Cortex Motor
Ileum
Jejunum
Liver
Lymph Nodes (Aortic)
Pancreas
PBMC
Prostate
Rectum
Colon (Right)
Seminal Vesicle
Recipient

SUPPLEMENTARY FIGURE S7. Generalized Linear Model Analyses. Box plots of the GLM coefficients for all tested predictors. Bayes Factors (BFs) of each predictor in the model is indicated in red on the right. The original BF support are presented along with the adjusted BF (in dark grey) (see Methods for details). Adjusted BF≥3 are in bold. The GLM coefficients are conditional on inclusion of predictor in the model.



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STROBE Statement

TITLE AND ABSTRACT

1. Title and abstract

The Last Gift Study is an observational study. The study design is identified in the abstract.
The proposed study is entitled:
"HIV persists throughout deep tissues and blood is the main source of viral migration"

The study abstract is below and in the main manuscript :

Understanding HIV persistence and dynamics across the human body is important for HIV cure efforts. This goal has been hampered by technical difficulties and the challenge to obtain fresh tissues. This observational study evaluated 6 persons with HIV (4 virally suppressed with antiretroviral therapy and 2 with rebound viremia after stopping therapy) who provided blood serially before death and their bodies for rapid autopsy. Virologic data were generated from samples, and analyses showed: 1) emergence of large, clonal, intact HIV RNA populations (full length envelope) in blood after stopping therapy, which repopulated tissues throughout the body, 2) multiple sites can act as hubs for HIV dissemination within the body but blood and lymphoid tissues were the main source, and 3) viral exchanges occur within brain areas and across the blood brain barrier, and 4) within-body migration associated with low HIV divergence between sites and higher HIV diversity at the recipient site. These results suggest that HIV reservoirs persist in all deep tissues, and blood is the main source of most viral dispersal between tissues. This may explain why eliminating HIV susceptibility in circulating T cells via bone marrow transplants allowed some people with HIV to have therapy free remission, even though deeper tissue reservoirs were not targeted.

INTRODUCTION

2. Background

As discussed in the papers' Introduction:

"Despite extensive investigations in humans (1-13) much remains unclear about HIV reservoirs that persist during ART(14-18). In part, this is because of technical limitations and limited access to appropriately collected tissues for such studies. To address tissue availability, a peri-mortem observational research cohort, 'The Last Gift', was developed and enrolled PWH diagnosed with a terminal illness from a non-HIV condition. Participants consented to blood sampling before death and donated their bodies for a rapid autopsy after death(19-21). This allows to collect ante-mortem blood and post-mortem tissues. Post-mortem tissues are collected within 6 hours of death to maximally preserve tissue and viral integrity(21). In the Last Gift cohort, some participants decide that they no longer wanted to continue their ART in the days and weeks

before death. This provides the opportunity to observe and characterize rebounding viral populations in blood and compare them to viral populations found in tissues."

3. Objectives

The objective are stated in the introduction of the manuscript and below:

"In our observational Last Gift cohort, we hypothesized to see differential patterns of HIV population and clonality within tissues. Among participants who decided to stop their HIV therapy, we expected to see repopulation of tissues consistent with reservoirs that persisted ion tissues among participants who chose to remain on their HIV therapy until death. To characterize the HIV reservoirs in collected specimens, we combined new technologies that could deep sequence near full-length (FL) env HIV genomes and sensitively quantify HIV DNA(22, 23) with established molecular epidemiology inference methods to assess the viral diversity, divergence, predicted cellular tropism, replication competence, compartmentalization, and migration across the human body."

METHODS

4. Study design

This study is an observational study, as described below and in the Methods

Rationale for involvement of terminally ill, HIV infected persons. The choice to involve a terminally ill patient population is motivated by i) the absence of any reasonable expectation of clinical benefit by the volunteers, ii) the desire that many terminally ill persons voice to "give back" and contribute in some way prior to their death, iii) the expectation that both the volunteers and the research scientists may accept a greater risk-to-benefit ratio for these HIV cure studies. Local foundational work in this area demonstrates that the concept of this research is widely accepted among local HIV communities.

Persons who are making end-of-life decisions may be considered to be vulnerable; however, we work closely with individuals, family, caregivers, and physicians to provide as much information as possible about the Last Gift program and the process involved with possible organ donation upon death. We expect a significant portion of our participants to have some degree of cognitive impairment. If a participant lacks the capacity to consent to study entry, we will attempt to obtain surrogate consent.

Protocol development. A Last Gift observational study protocol has already been developed and approved by the Human Research Protection Program (HRPP).

5. **Setting**

Recruitment sites: Participants were recruited primarily from the UCSD Owen Clinic (largest HIV primary care provider in San Diego County), the HIV Neurobehavioral Research Center (HNRC), the AntiViral Research Center (AVRC, home of the UCSD AIDS Clinical Trials Group (ACTG). Additional sources of referral are listed in **Table 1**.

Table 1. Referral Sources in San Diego County	
Primary Medical Care	
Organization	Gender & Minority Population Served
UCSD Owen Clinic and Inpatient Service	Men, Women, Hispanic, African American, older adults
San Diego Hospice and Institute for Palliative Medicine	Men and Women with Advanced HIV Disease
San Diego V.A. Healthcare System	Veteran men and women, older adults
Naval Medical Center	Active Duty men, women, Dependents
Family Health Centers of San Diego	Men, Women, Gay Identified, Hispanic, African American
San Ysidro Health Center	Hispanic men, women
San Diego American Indian Health Center	Native American Men and Women
Vista Community Clinic	Hispanic men, women
Residential & Case Management	
Organization	Service Provided
Josue Homes	Transitional Living for people with HIV/AIDS
North County Health Services	Case Management, County Wide
Townspeople	Residential Shelter for Advanced HIV
Neighborhood House Association HIV/AIDS Service	Case Management
Services for Minorities, Women, & Youth	
Organization	Service Provided
Being Alive San Diego	Peer Advocacy, Support Groups, Education for people living with HIV
County of San Diego HIV, STD and Hepatitis Branch	HIV Prevention, Counseling and Testing, and AIDS Case Management
Center for Social Support (CSSE)	Housing, Education, and Social Support for African Americans
Christie's Place	Social Support and Services for Women & Children with HIV
Lesbian & Gay Men's Community Center	Lesbians, Gay Men and transgender persons
Substance Abuse Treatment & Recovery	
Organization	Service Provided
CRASH Recovery Services	Substance Use Recovery
Community Connections	Outpatient Residential Treatment for formerly incarcerated
MAAC Project Casa de Milagros	Residential alcohol/drug treatment for Latinas
Stepping Stone Recovery Services	Substance Use Recovery
Community Networking Groups	
Group Name	Attendees

Table 1. Referral Sources in San Diego County	
Coalition of Latino AIDS Service Providers (CLASP)	Outreach Workers from Various HIV Service Organizations working with Latino clients
San Diego POZabilities	Social Organization for older HIV positive men, drug and alcohol free events

Recruitment procedures. See table 2 below.

Table 2. Recruitment Procedures
Community Education: Increase individual awareness and understanding of the value of research and promote volunteerism. Conducted through: formal group presentations; brochures and flyers; and mass media advertising (internet and radio).
Referral Networks: Provide information and awareness to providers who treat the health conditions being investigated. Network nodes include health care providers; case managers; recovery programs; and community opinion leaders.
Event Outreach: Increase community level awareness regarding research and volunteerism.
Community Outreach: Make personal contact with potential participants over the phone and in community venues to answer questions and facilitate study enrollment.

CAB. We will also hold presentations and open discussions with UCSD HIV CABs, (AVRC, CFAR Disparities Core and HNRC). Similar to the Town Halls, we will collect a survey on the attitudes of HIV research at the end of life before and after each CAB discussions. We will establish our own CAB with broad membership from the community and bi-annual meetings, including interested community members on the study team.

Enrollment procedures. To eliminate any possibility of economic coercion (or appearance thereof), minimal monetary reimbursement will be provided directly to the participants ($10 for each visit with or without blood draw respectively), but we will cover costs for body transport and crematory fees (up to $1,000 per participant). This will ensure that only highly motivated, well-informed, altruistic individuals participate in the study. Use of monetary reimbursement will be a main discussion point in the community forums, the HIV bioethics groups and our local Human Research Protections Program.

6. Participants

This cohort consists of altruistic person with HIV (PWH) on long-term ART who are terminally-ill with a non-HIV related disease.

Relevant inclusion and exclusion criteria include:

Inclusion criteria: age ≥18 (although the project will allow inclusion of any eligible adult, given the nature of the project, we expect to enroll mostly older participants), capacity to provide informed consent in English, HIV infected (confirmed by serologic and virologic tests at screening). To participate in the Last Gift study, participants must agree to an autopsy and organ donation upon death. Participant Informed Concent for autopsy will be reviewed and discussed with next of kin in order to minimize concerns or conflicts at the time of death.

Exclusion criteria: unwilling or unable to comply with study-related procedures or pregnant, non-English speaking.

7. Variables

Ante-mortem clinical data: demographics, HIV risk factors, blood viral load, CD4 T-cell counts, estimated duration of HIV infection, current and past ART use, lifetime history of drug use. Recent history of stimulant use, as indicated on PRISM/CIDI (24-26) or urine toxicology within 2 years of entering palliative care.
Post-mortem histological data: Similar to published procedures (27), the rapid pathology protocol was developed with a focus on nervous system evaluation and expanded to also exploit other specimen resources that can be obtained from the autopsy. The protocol was designed to: 1) comprehensively sample tissues that are susceptible to injury from HIV and represent an important HIV DNA reservoir; 2) preserve tissues for a wide range of research methodologies; and 3) whenever possible collect tissue within 6-8 hours death to maximize assay precision.

8. Data Sources /Measurement

All cohort participants receive the Comprehensive Assessment including:

Blood sampling. Approximately 65 ml of blood will be collected by a licensed phlebotomist for the following laboratory tests: routine hematologic and biochemical tests, targeted biomarkers, lymphocyte subset enumeration, viral serologies for HIV and Hepatitis C, HIV viral quantitation, and other blood tests (e.g. for syphilis).

Tissue sample collection. All tissue samples will be collected during the rapid autopsy procedure.

Measurements: We detailed the following cohort measurements in the manuscript.

"*Six participants enrolled in the Last Gift cohort (n=2 who stopped ART and n=4 who remained virally suppressed on ART until death) were included in this study. (1) Pre-mortem blood plasma (n=2 participants) and peripheral blood mononuclear cell (PBMC) samples (n=6 participants) were collected before death and tissues were collected during the rapid autopsy procedure. (2) HIV RNA and DNA were extracted from blood plasma and PBMC/tissues for quantification of HIV DNA/RNA (digital droplet PCR); (3) and (4) HIV full length envelopes were sequenced via single genome amplification and sequencing. Intact full length env sequences from all samples were used to (5) characterize the HIV populations within each compartments and in blood, (6) assess viral dispersal across tissues using Bayesian phylodynamic models and (7) evaluate factors associated with viral dispersal*."

9. Bias

Our study only evaluated men with HIV. Recent estimates show that approximately 5% of current persons living with HIV/AIDS in San Diego are women. If eligible, women with HIV are encouraged to participate. The

outreach team work hard to ensure that eligible women in this research study are informed about the research study and are invited to participate.

10. Study size

Given that this was an observational cohort, we evaluated participants as they enrolled in the study with completion of the rapid autopsy. No selection occurred before evaluation. After evaluation of six participants, enough measures were sufficient to see clear patterns of HIV population and repopulation.

11. Quantitative variables

Quantitative variables derived from our validated assays are described above in the Methods in the manuscript.

12. Statistical methods

Also, our statistical analyses are detailed below and in the manuscript.

"Statistical analyses. Multivariable logistic regression carried out in R version 3.6.1 using function glm and binomial link function, was used to compare the proportion of sequences that were intact and identical (clonal). The independent variables in these analyses were participant and anatomical location. For the continuous average pairwise distance (diversity) outcome, a multiple linear regression was used with assumptions of constant variance and normality of residuals checked and met. Mixed models for both binary and continuous outcomes were analyzed with glmer and lmer from the lme4 R library. These mixed models used participant as the grouping factor and included a random term for either intercept alone, or intercept and compartment. Due to the sparse nature of the data, all mixed models had difficulty converging with poor model fits. As such the results and P-values of these models are not reliable and so the results were not presented. Given the large number of tissue compartments, the sparse sampling across participants, and the expected effect modification by participant and compartment, comparisons across specific compartments is not informative."

RESULTS

13. Participants

It was expected that the demographics of adults recruited to the Last Gift study cohort will reflect the demographics of HIV in San Diego, with men who have sex with men (MSM) representing the majority of enrolled participants, which was consistent with the six male participants who were enrolled in this study. See

Supplementary Table 1 and Supplementary Figure S1 for details. All eligible participants who were evaluated for the study were enrolled and completed all study evaluations, including rapid autopsy, except for one man who decided to disenroll before death. His decision to not finish the study was his personal concern over logistics of the autopsy.

14. Descriptive data

Descriptive study data detailing the characteristics of the study participants, including demographics, behavioral, clinical and social factors), time on study, and potential confounders are detailed in the manuscript, and summarized in Supplementary Table S1 and Supplementary Figure S1. Missing clinical and behavioral data were rare, and discussed in the Discussion.

15. Outcome data

Outcomes of the study include HIV DNA measures, viral migration and population of cellular reservoirs. Counts of these measures are detailed in Figures 2-6, and are reported in the Results of the manuscript and detailed for each participant.

16. Main results

Main results of the study are provided in the manuscript, and in the Supplementary Material. These results include all the applicable confounded-adjusted estimates and precision measures.

17. Other analyses

All analyses performed are reported in the manuscript.

DISCUSSION

18. Key results

Knowledge gathered addresses the pathogenesis and impact of HIV-disease and how the HIV persists and distribute across the human body. Therefore, these findings may have important implications for developing interventions and informing public health policy. The limited risks of this protocol are reasonable in view of the importance of this knowledge to be gained for treatment and planning policy.

19. Limitations

The limitations of the study are detailed in the Discussion and below.

“Our study has a number of limitations. The main limitation is the small number of participants, especially having only two participants who stopped their ART. Nonetheless, it allowed us to unprecedentedly observ the population of tissue reservoirs from the blood during rebound viremia. Another limitation is that the participants were all in the process of dying, which may limit the generalizability to healthy PWH. Also, this study, as others(28, 29), focused on the HIV env gene, which has the greatest amount of molecular diversity and evolution of all coding regions(30-35), but we acknowledge that by sequencing only the env region, we may have incorrectly inferred that some viruses intact in the env coding region were replication competent, when they may have had defects in other genome regions. Further, the study found many identical HIV DNA env single genomes, consistent with previous reports,(36-39) but we did not directly assess cellular clonal expansion, which is thought to be an important mechanism for HIV persistence(40-42). However, others have shown that multiple identical env sequences in proviruses provide a strong indication for clonal expansion(43, 44). Sequencing near FL proviral genome (45-47) would increase the sensitivity of the analyses of intactness and clonality but these approaches require a high cellular input which may limit the ability to explore reservoirs such as the central nervous system. Therefore, we are confident that our approach provided a good surrogate for the extensive analysis of HIV reservoirs. Further, our study did not perform phenotypic testing of CCR5 tropism, and every participant in our study had X4-tropic virus by genotypic analysis. This is likely because participants were infected for a long time before cohort enrollment(48), and emphasizes that people with longstanding HIV infection are unlikely to benefit from measures focusing only on CCR5 tropic viruses, as was the case for the two PWH who received CCR5 mutated bone marrow transplants and have been cured of HIV so far (49, 50). Finally, we cannot exclude the possibility of blood T cell contamination in tissues obtained during autopsy. This is likely to be a small impact on our analysis given the small size of capillaries compared to overall tissue mass and settling of blood in tissues, i.e. livor mortis. While we cannot completely rule out such contamination, our sequence analyses showed viral compartmentalization for all participants, which suggested that possible blood contamination did not significantly impact our analyses.”

20. Interpretation

As discussed in our limitations in the manuscript, we caution the readers that the number of participants were small with a large number of detailed assays. This is an issue with most other autopsy studies. Further, the analyses were placed in the context of the current literature.

21. Generalizability

As with the limitations discussed above and in the Discussion, we cite the issue of generalizability of our cohort, especially concerning people who are at the end of their life. In particular people who are at the end of their life and with a terminal illness may not represent the same situation as with people with HIV who are otherwise healthy. This was discussed in the manuscript.

OTHER INFORMATION

22. Funding

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