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Supplementary Materials for

The Origins of Sexually Transmitted HIV Among Men Who Have Sex with Men

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Table S1: Genetic differentiation between compartments

Transmission Pair		Intra-individual			Inter-individual		
		SBP-SSP	SBP-SSC	SSP-SSC	SBP-RBP	SSP-RBP	SSC-RBP
A	F_{st}	0.842	0.727	0.707	0.827	0.549	0.692
	sd	0.019	0.030	0.042	0.020	0.022	0.046
	P	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***
B	F_{st}	0.034	0.995	0.993	-0.010	0.045	0.996
	sd	0.046	0.002	0.002	0.037	0.046	0.002
	P	0.203	***<0.001***	***<0.001***	0.501	0.112	***<0.001***
C	F_{st}	0.010	0.992	0.991	-0.012	-0.008	0.990
	sd	0.086	0.004	0.003	0.060	0.117	0.004
	P	0.309	***<0.001***	***<0.001***	0.518	0.526	***<0.001***
D	F_{st}	0.004	0.579	0.563	0.156	0.132	0.460
	sd	0.027	0.057	0.060	0.072	0.077	0.078
	P	0.324	***<0.001***	***<0.001***	0.07	0.084	***<0.001***
E	F_{st}	-0.081	0.698	0.702	0.216	0.384	0.693
	sd	0.104	0.076	0.077	0.129	0.035	0.073
	P	0.441	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***
F	F_{st}	0.449	0.821	0.784	0.376	0.088	0.760
	sd	0.121	0.035	0.032	0.123	0.047	0.035
	P	***<0.001***	***<0.001***	***<0.001***	***<0.001***	**0.021**	***<0.001***

Legend: F_{st}: Pair-wise measures of genetic distance: SBP: Source Blood Plasma; SSP: Source Seminal Plasma; SSC: Source Seminal Cells; RBP: Recipient Blood Plasma, sd: bootstrapped estimates of the standard deviation of the mean, *P*: probability that a random assortment of sequences between compartments/individuals has equal or greater F_{st}, and values <0.05 are bolded. Significant *P* values with Bonferroni correction for multiple comparisons (P < 0.008) are in italics.

Table S2: Estimated divergence times of compartments

Pair	Compartments Investigated	T_{MRCA}	Standard error	ESS	P
A	RBP-SBP	1006.48 (161.78, 1463.68)[#]	63.54 (0.34, 1.05)[#]	358.70	
	RBP-SSP	242.14	13.79	562.86	
	RBP-SSC	1437.88	30.42	345.04	0.999
B	RBP-SBP	1155.96	7.05	397.25	
	RBP-SSP	1168.37	7.05	416.37	
	RBP-SSC	1453.66	29.8	262.91	0.972
C	RBP-SBP	1156.79	7.12	392.75	
	RBP-SSP	1168.86	7.09	412.24	
	RBP-SSC	1453.77	29.8	262.78	0.974
D	RBP-SBP	58.86	4.47	280.93	
	RBP-SSP	58.85	4.48	280.88	
	RBP-SSC	61.01	5.18	254.11	0.999
E	RBP-SBP	465.35	16.41	372.81	
	RBP-SSP	127.82	2.046	657.79	
	RBP-SSC	502.96	18.06	360.53	0.999

Legend: T_{MRCA}: Time to Most Recent Common Ancestor was estimated (S1) in days between source and recipient viral populations in the 5 transmission pairs for which time of infection could reliably be estimated; SBP: Source Blood Plasma HIV RNA; SSP: Source Seminal Plasma HIV RNA; SSC: Source Seminal Cell-associated HIV DNA; RBP: Recipient Blood Plasma HIV RNA; ESS: Effective Sample Size; P is the posterior probability that T_{MRCA} (RBP-SSP) <= T_{MRCA} (RBP-SSC). [#]Multiple runs failed to converge on the estimates of time to the most recent common ancestor. The means and standard errors of both modes are thus presented.

Table S3: Number of purifying (dN/dS < 1) and positive (dN/dS > 1) selection sites

Transmission Pair	FEL		iFEL		FEL & iFEL	
	dN/dS < 1	dN/dS > 1	dN/dS < 1	dN/dS > 1	dN/dS < 1	dN/dS > 1
A	6	2	8	2	5	2
B	3	0	3	0	2	0
C	2	0	1	0	1	0
D	10	0	4	0	4	0
E	8	0	7	0	3	0
F	14	4	11	6	11	4

Legend: FEL: Fixed Effects Likelihood inference of selection at individual sites across all lineages; iFEL: Fixed Effects Likelihood inference of selection at individual sites along internal branches; dN: non-synonymous rate, dS: synonymous rate. FEL results indicate sites experiencing significant ($P < 0.05$) positive/purifying selection along all lineages, whereas iFEL results are only at internal branches.

Table S4: Inferred substitutions along transmission branches for pairs A, E and F (panel A) and along pre-transmission branches for all pairs A-K (panel B).

Panel A		**Transmission Pairs (codon substitutions with aa)**			
hxb2 position	aa	A	E	F(1)*	F(2)*
285	I		CTA (L) > TTA(L)		
299	P	CTC(L) > CTT(L)			
301	N	AAC(N) > AAT(N)			
324	--				
329	Q	CAA(Q) > AAA(K)			CAA(Q) > CGA(R)
342	N			AAC(N) > GAC(D)	
345	K			AAA(K) > ACA(T)	
347	I				ATA(I) > GTA(V)

Panel B		**Transmission Pairs (codon substitutions with aa)**					
hxb2 position	aa	A	B	C	D	E	F
252	R						AGG(R) > AAG(K)
259	L				TTG(L) > CTG(L)		
260	L						CTG(L) > CTA(L)
261	L						TTA(L) > TTG(L)
269	E	GAG(E) > GGG(G)				GGG(G) > GAG(E)	
270	V						GTA(V) > GTC(V)
271	V						ATA(I) > ATG(M)
272	I					GTT(V) > ATT(I)	
275	V		GCC(A) > GTC(V)				GAG(E) > GAA(E)
276	N					AAC(N) > AAT(N)	
277	F						TTC(F) > CTC(L)
278	T	ACG(T) > TCG(S)	TCG(S) > ACG(T)	TCG(S) > ACG(T)		ACA(T) > ACG(T)	

279	D	GAC(D) > AAC(N)	AAC(N) > GAC(D)	AAC(N) > GAC(D)			
283	T				ACA(T) > ACC(T)		ACC(T) > AAC(N)
285	I					CTA(L) > ATA(I)	
287	Q						CAG(Q) > CAT(H)
288	L				CTA(L) > CTG(L)		
290	T					AAC(N) > AAA(K)	GAG(E) > CAG(Q)
293	E	GAA(E) > GTA(V)	GTA(V) > GAA(E)	GTA(V) > GAA(E)	CCC(P) > AAA(K)		AAA(K) > ACA(T)
295	N				CAT(H) > AAT(N)		
296	C				TGC(C) > TGT(C)		
297	T				ATA(I) > ACA(T)		
299	P	CCC(P) > CTC(L)	CTC(L) > CCC(P)	CTC(L) > CCC(P)			CCC(P) > CCT(P)
300	N					GGC(G) > AAC(N)	AAC(N) > AAT(N)
305	K					AAG(K) > AAA(K)	
306	R	AGT(S) > GGT(G)	GGT(G) > AGT(S)	GGT(G) > AGT(S)			
308	R		CAT(H) > CGT(R)		CCT(P) > CAT(H)		
318	V						TTT(F) > TAT(Y)
322	K	GAA(E) > GCC(A)			GAC(D) > GAA(E)	GAT(D) > GAA(E)	
323	I			GTA(V) > ATA(I)			
324	-		GTA(V) > ATA(I)				
329	Q		AAA(K) > CAA(Q)	AAA(K) > CAA(Q)			
331	H						CAT(H) > CAC(H)
332	C						TGT(C) > TGC(C)
334	I	ATT(I) > CTT(L)	CTT(L) > ATT(I)	CTT(L) > ATT(I)			
335	S				ACT(T) > AGT(S)		
336	R					AGT(S) > AGA(R)	AGA(R) > GGG(G)
337	A		ACA(T) > GCA(A)			ACA(T) > GCA(A)	GCA(A) > GAA(E)
338	K		GAA(E) > AAA(K)		AAT(N) > AAA(K)		
340	N				ACT(T) > AAT(N)		
341	-				ACT(T) > AAC(N)		
342	N				AGC(S) > ACT(T)		
345	K	AAA(K) > GAA(E)	GAA(E) > AAA(K)		CAA(Q) > AAA(K)	GAA(E) > AAA(K)	
346	Q				CTG(L) > CAG(Q)		CAG(Q) > AAG(K)
348	A					GTT(V) > GCT(A)	
349	S		AAA(K) > AGA(R)		GCA(A) > AGA(R)		AGA(R) > ACA(T)

351	L					TTG(L) > TTA(L)	
352	R				GGA(G) > AGA(R)		AAA(K) > CAA(Q)
353	E						GAA(E) > AAA(K)
354	Q						CAA(Q) > GAA(E)
355	F	TTT(F) > TAC(Y)	TAC(Y) > TTT(F)		TAT(Y) > TTT(F)		TTT(F) > TTC(F)
358	N				AAC(N) > AAT(N)		
359	K				GCA(A) > AAA(K)		
362	I	ATC(I) > GTC(V)					ATC(I) > GTC(V)
364	K					AAT(N) > AAG(K)	

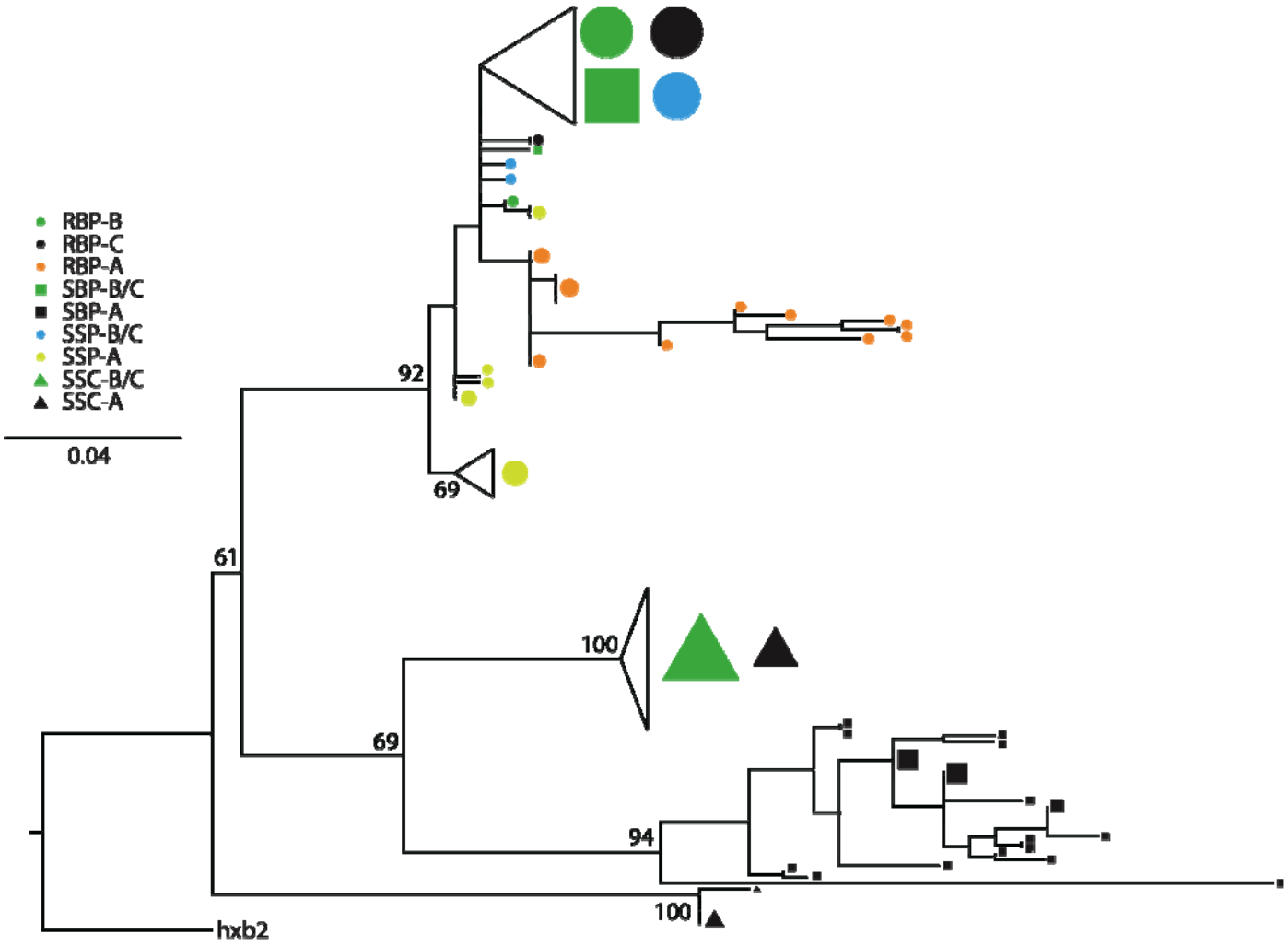
Legend: aa: one letter amino acid code; -: indel; >: substitution direction. *Transmission pair F had two potential transmission branches leading to each of two clades containing both source and recipient virus (see Figure 1).

Table S5: Marginal likelihoods, estimated as the harmonic mean of the sampled likelihoods in Bayesian MCMC analysis (S1).

Substitution model	Demographic model	Marginal likelihood
GTR+Γ	Constant coalescent	-1145.29 ± 0.16
GTR+Γ	Exponential coalescent	-1148.15 ± 0.18
GTR+Γ	Bayesian Skyline	-1145.27 ± 0.18
SRD06	Constant coalescent	-1146.27 ± 0.16
SRD06	Exponential coalescent	-1146.64 ± 0.16
SRD06	Bayesian Skyline	-1144.46 ± 0.18

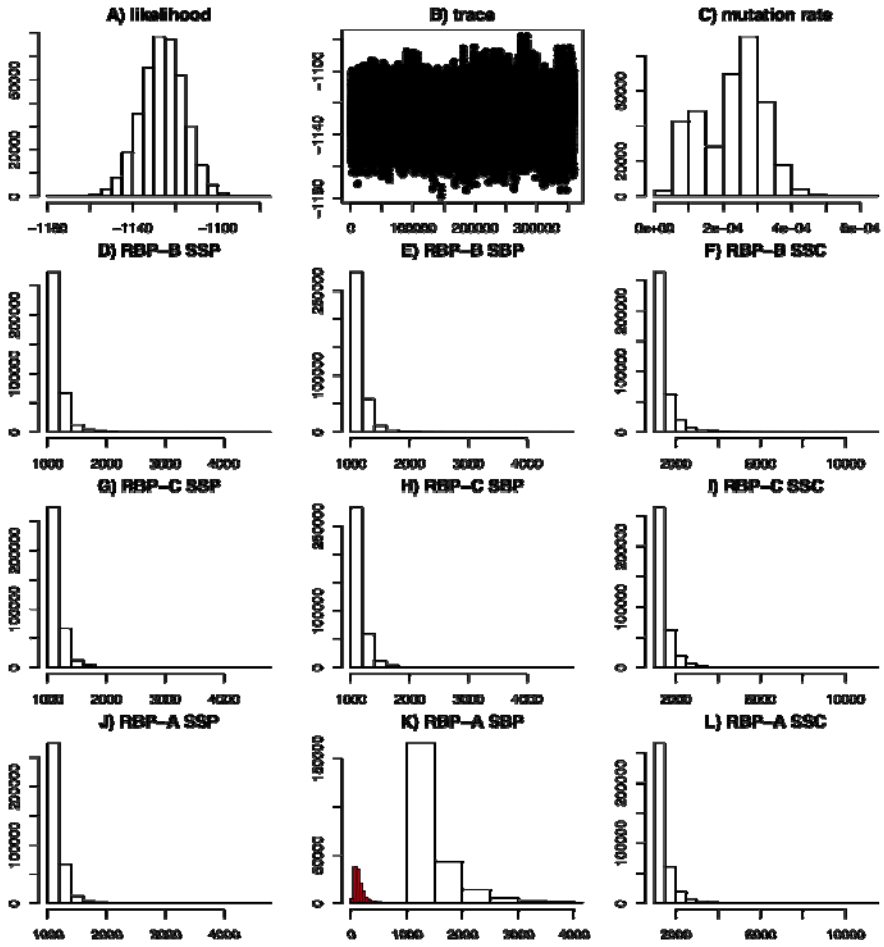
Figure Legends

Figure S1: Maximum likelihood phylogenetic tree of viral sequences from a source who infected multiple partners at two independent time points.



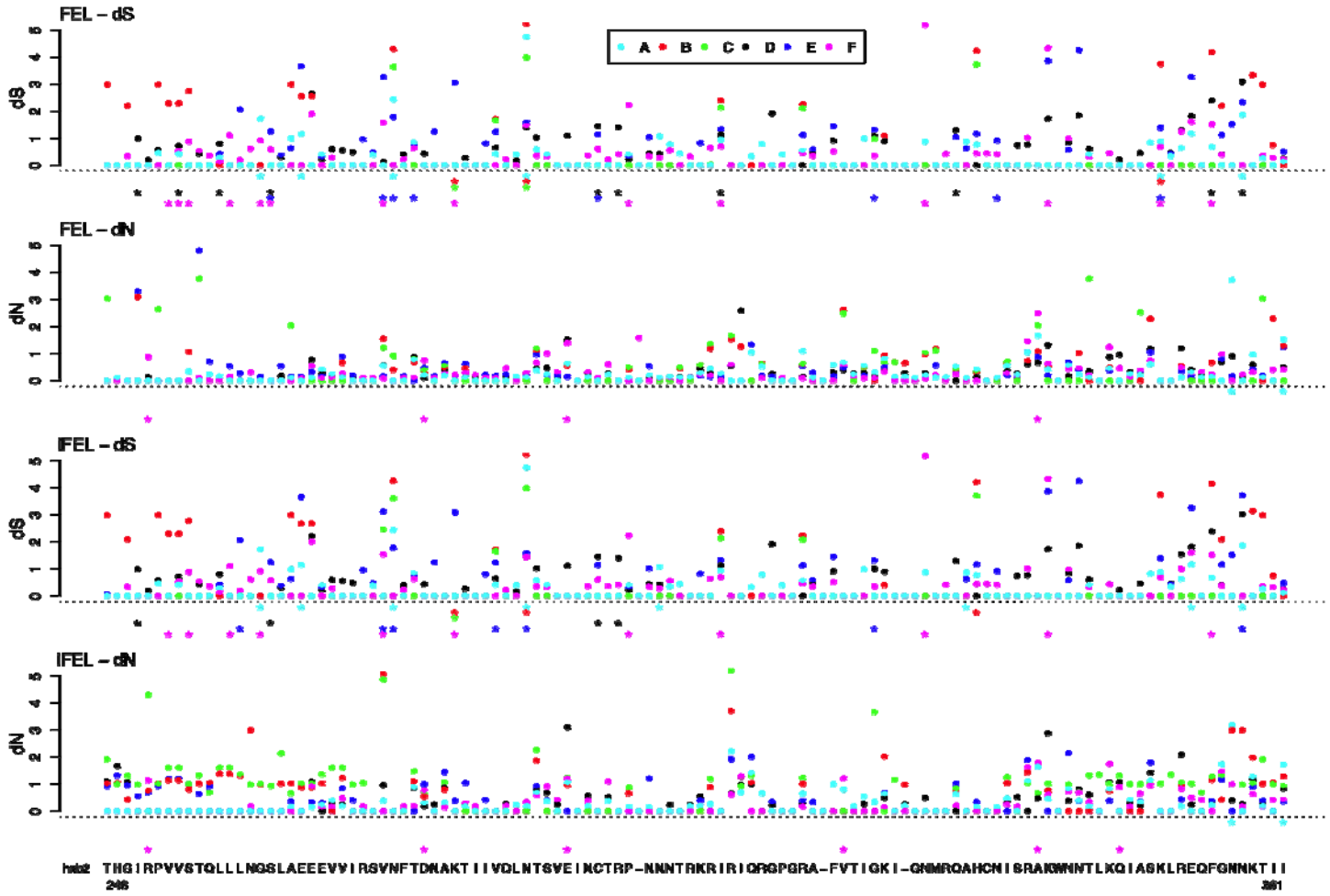
Legend: RBP: recipient HIV RNA in blood plasma, SBP: source HIV RNA in blood plasma, SSP: source HIV RNA in seminal plasma, SSC: source seminal cell-associated virus. B/C: first time point corresponding to transmission pairs B & C; A: second time point corresponding to transmission pair A.

Figure S2: Posterior distributions of parameters sampled during Bayesian MCMC analyses (S1).



Legend: As in Fig S1

Figure S3: Site-specific non-synonymous (dN) and synonymous (dS) rates of substitution.



Legend: FEL: Fixed Effects Likelihood; and iFEL: Internal Fixed Effects Likelihood. Estimated rates for each of the transmission pairs (A-F) are shown, with asterisks (*) below plots indicating sites with significant ($P < 0.05$) purifying (*dS* plots) or diversifying (*dN* plots) selection.

References

References

S1. A. J. Drummond, A. Rambaut, BEAST: Bayesian evolutionary analysis by sampling trees *BMC Evol Biol* **7**, 214 (2007).