SUPPLEMENTARY MATERIAL

HIV-1 evolution in breakthrough infections in a human trial of oral pre-exposure prophylaxis with emtricitabine and tenofovir disoproxil fumarate

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Supplementary Table 1. Characteristics of infected individuals from the TDF2 PrEP trial

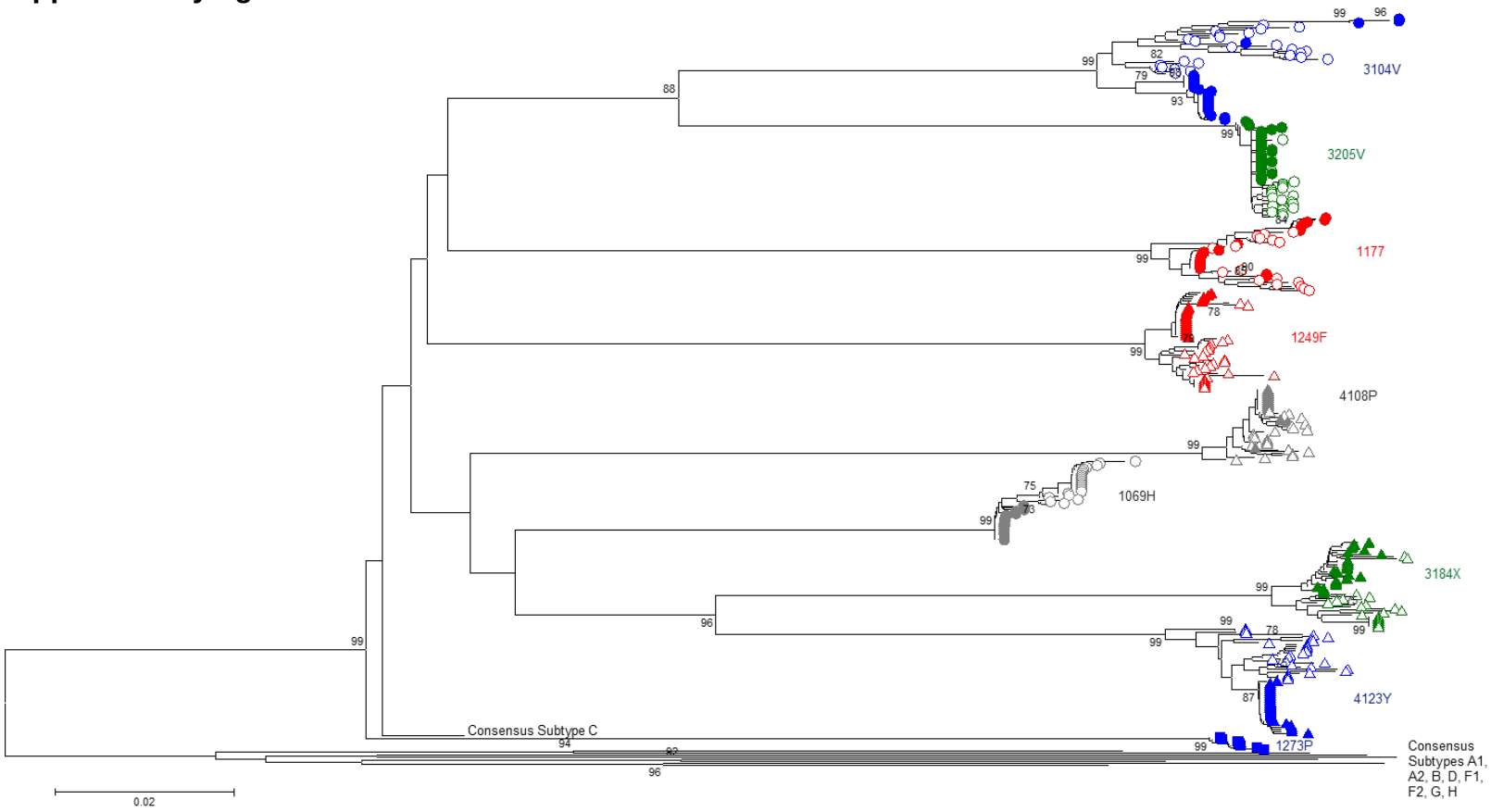
	Participant Gender	**Plasma TFV (ng/mL) at the seroconversion visit**	**Plasma FTC (ng/mL) at the seroconversion visit**	**Log_{10} RNA copies/mL at the seroconversion visit**	**Total follow up (months)**
Participants taking FTC/TDF					
1069H	F	<5*	1.73	4.4	12
4108P	M	1.7	17.2	4.0	9
3104V	F	BLQ	BLQ	3.4	12
1273P	F	BLQ	BLQ	3.6	n.a.
Participants not taking FTC/TDF*					
1249F	F	BLQ	BLQ	3.8	9
3184X	F	BLQ	BLQ	4.1	8
1177E	F	BLQ	BLQ	4.4	10
3205B	F	BLQ	BLQ	5.1	5
4123Y	M	BLQ	BLQ	5.0	11

n.a.; follow up samples were not available; BLQ, below limit of quantification

**detected as <5 ng/mL (lower limit of detection of the assay = 0.3 ng/ml); sample volume was inadequate to repeat with a more sensitive assay (4).*

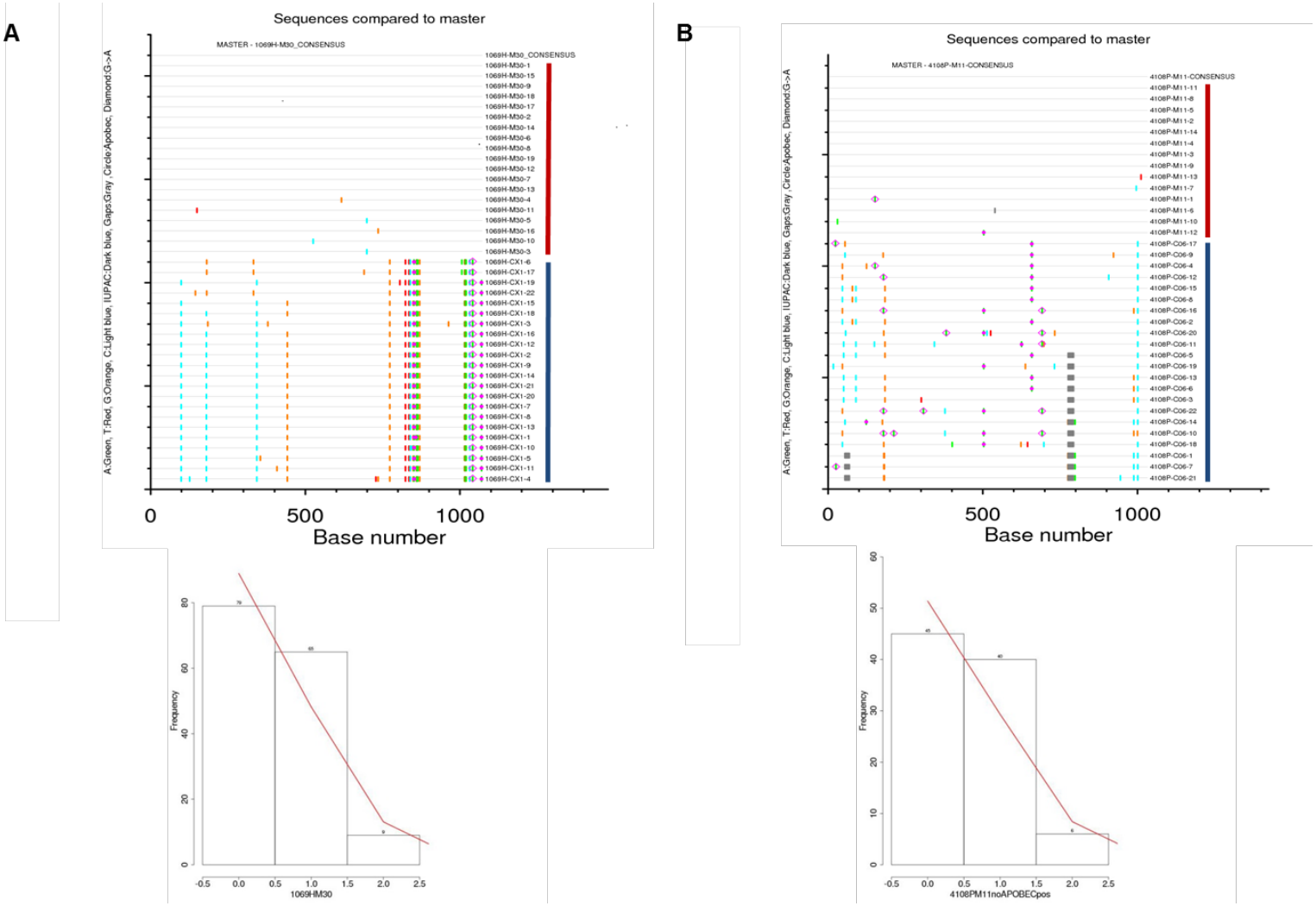
** Of the 5 seroconverters that were not on FTC/TDF, 4 were in the placebo arm and 1 (1249F) was in the FTC/TDF arm but was infected 30 days after the last reported dose of study medication*

Supplementary figure 1

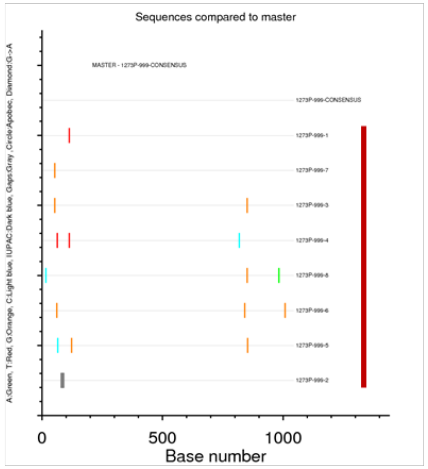


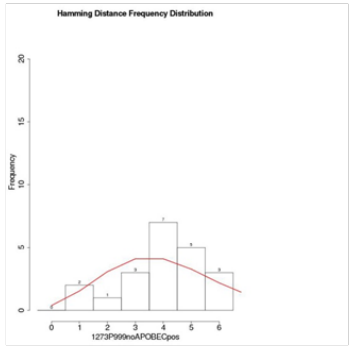
Supplementary Figure 1. Neighbor-joining tree of HIV env sequences from breakthrough infections in the TDF2 PrEP trial. Participants 3104V, 4108P, 1069H, and 1273P were in the active drug arm. The remaining participants were not on study drug. Closed symbols denote sequences obtained at the seroconversion visit. Open symbols denote sequences obtained after median of 10 (range, 5-12) months, with the exception of 1273P who was lost to follow up. Consensus subtypes A1, A2, B, C, D, F1, F2, G, and H reference sequences were retrieved from http://www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html. Only bootstrap values higher than 75 are shown.

Supplementary figure 2

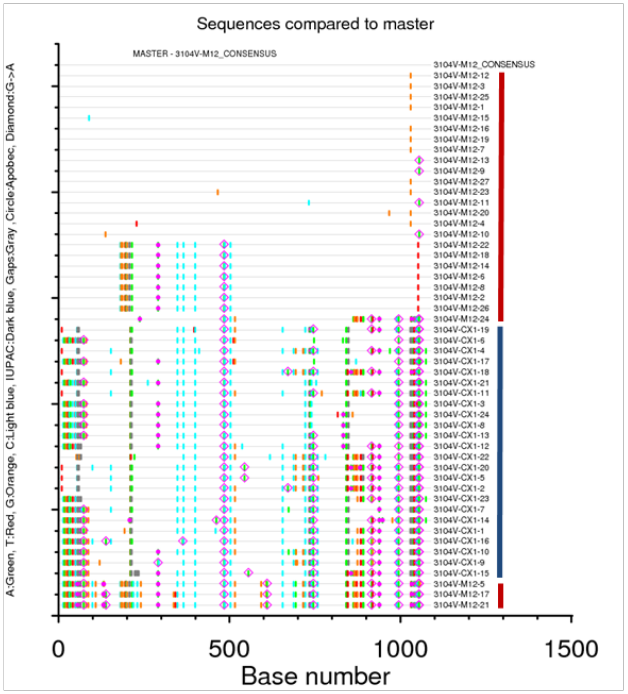


C



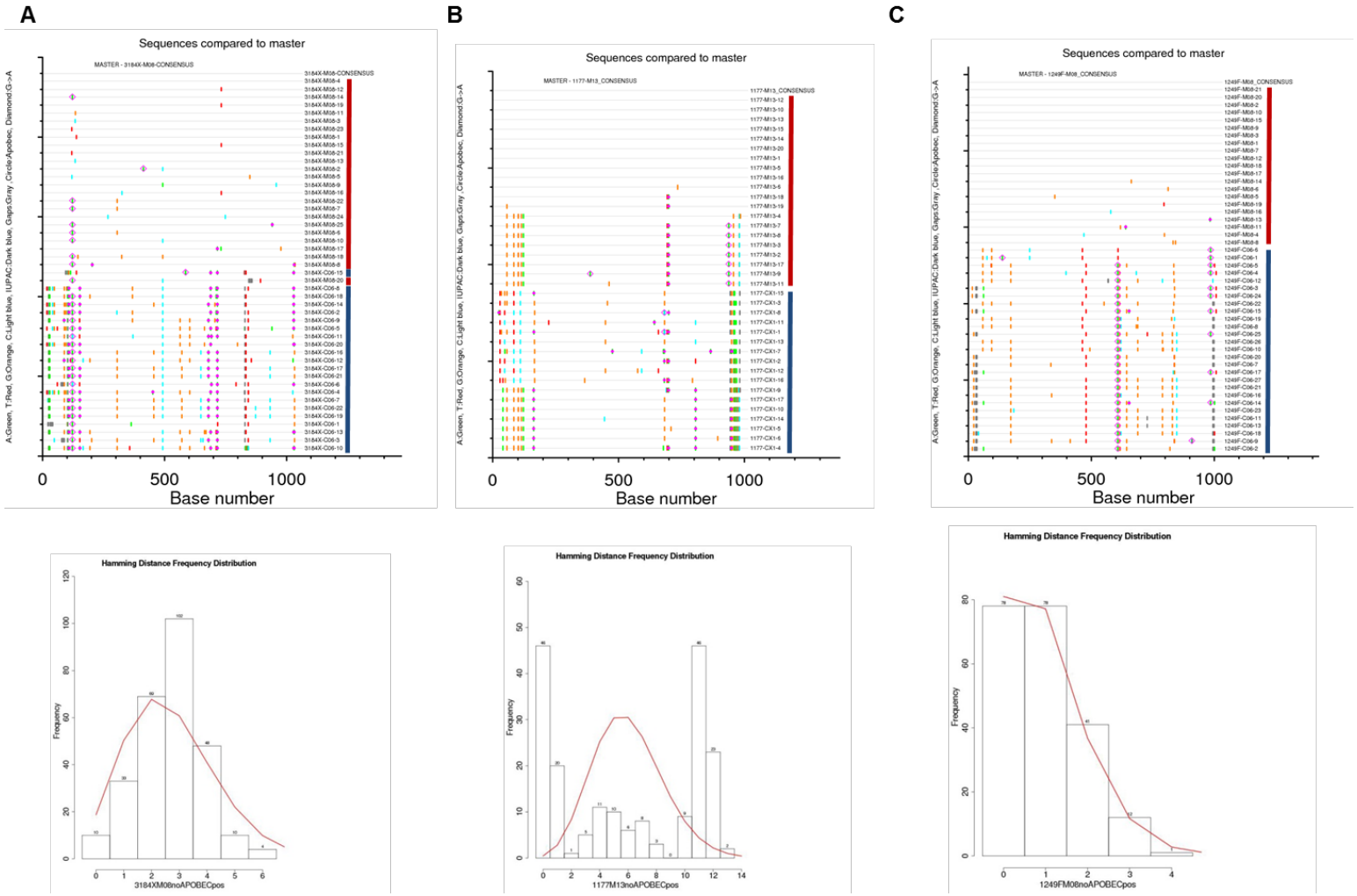


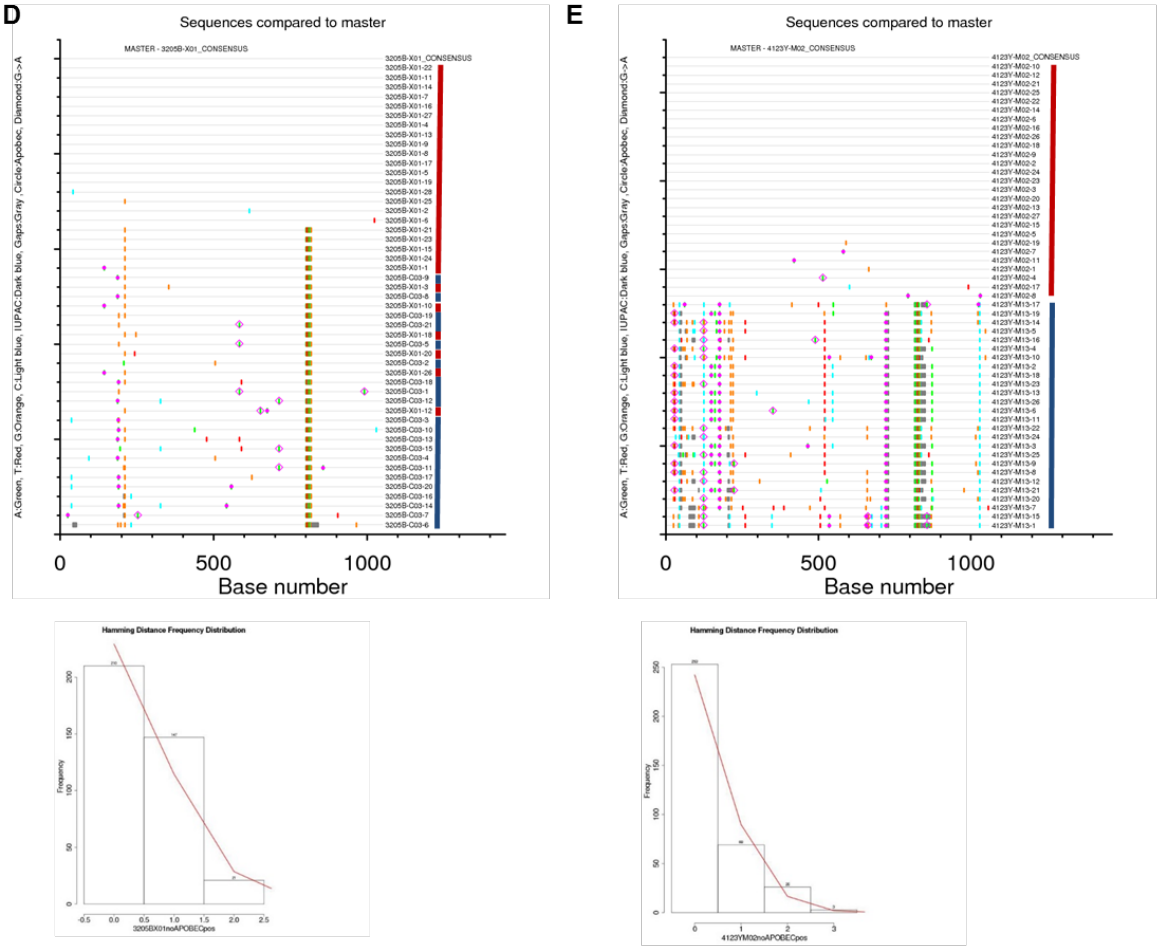
D



Supplementary figure 2. Highlighter plot and Poisson-Fitter analysis of env sequences from TDF2 cases assigned to FTC/TDF with (1069H, 4108P) or without (1273P, 3104V) detectable FTC or TFV in plasma. (A) Highlighter (top) and Poisson-Fitter plots (bottom) for 1069H. (B) Highlighter and Poisson-Fitter plots for 4108P. (C) Highlighter and Poisson-Fitter plots for 1273P. (D) Highlighter plot for 3104V. Nucleotide changes in the highlighter plots are colored as follows: A, green; T, red; G, orange; C, light blue. Gaps are indicated in gray. Circles denote APOBEC-mediated G-to-A hyper mutation. Diamonds represent G-to-A conversions. Poisson-Fitter plots exclude APOBEC mutations. Vertical red line denotes sequences obtained at seroconversion. Vertical blue line denotes sequences obtained at follow up (12 months for 1069H, 9 months for 4108P, and 12 months for 3104V). Due to limited sample availability and low plasma viremia we were only able to obtain 8 sequences from participant 1273P, all of which were obtained from the seroconversion visit specimen.

Supplementary figure 3





Supplementary figure 3. Highlighter plot and Poisson-Fitter analysis of env sequences from seroconverters from TDF2 that were not on study drug. (A) Highlighter plots (top) and Poisson-Fitter plots (bottom) for (A) 3184X; (B) 1177E; (C) 1249F; (D) 3205B; (E) 4123Y; Nucleotide changes in the highlighter plots are colored as follows: A, green; T, red; G, orange; C, light blue. Gaps are indicated in gray. Circles denote APOBEC-mediated G-to-A hyper mutation. Diamonds represent G-to-A conversions. Poisson-Fitter plots exclude APOBEC mutations. Vertical red line denotes sequences obtained at seroconversion. Vertical blue line denotes sequences obtained at follow up (8 months for 3184X, 10 months for 1177E, 9 months for 1249F, 5 months for 3205B, and 11 months for 4123Y)