

## Supplementary Figure Legends

**Supplementary Figure 1. Env variants with different co-receptor phenotype are interrelated among the individual subjects.** Full-length env sequences from different subjects were aligned with reference sequence HXB2 from the Los Alamos database using Clustal X and manually edited using BioEdit (version 7.1.3). DIVEIn (<http://indra.mullins.microbiol.washington.edu/DIVEIN>) was used to generate the maximum likelihood tree using GTR model. Subject IDs and HXB2 reference outgroup node are noted. Envs phenotyped as R5, dual, and X4 are noted as circles, triangles and boxes respectively. The tree was generated using a web tool at <http://www.hiv.lanl.gov/content/sequence/RAINBOWTREE/rainbowtree.html>.

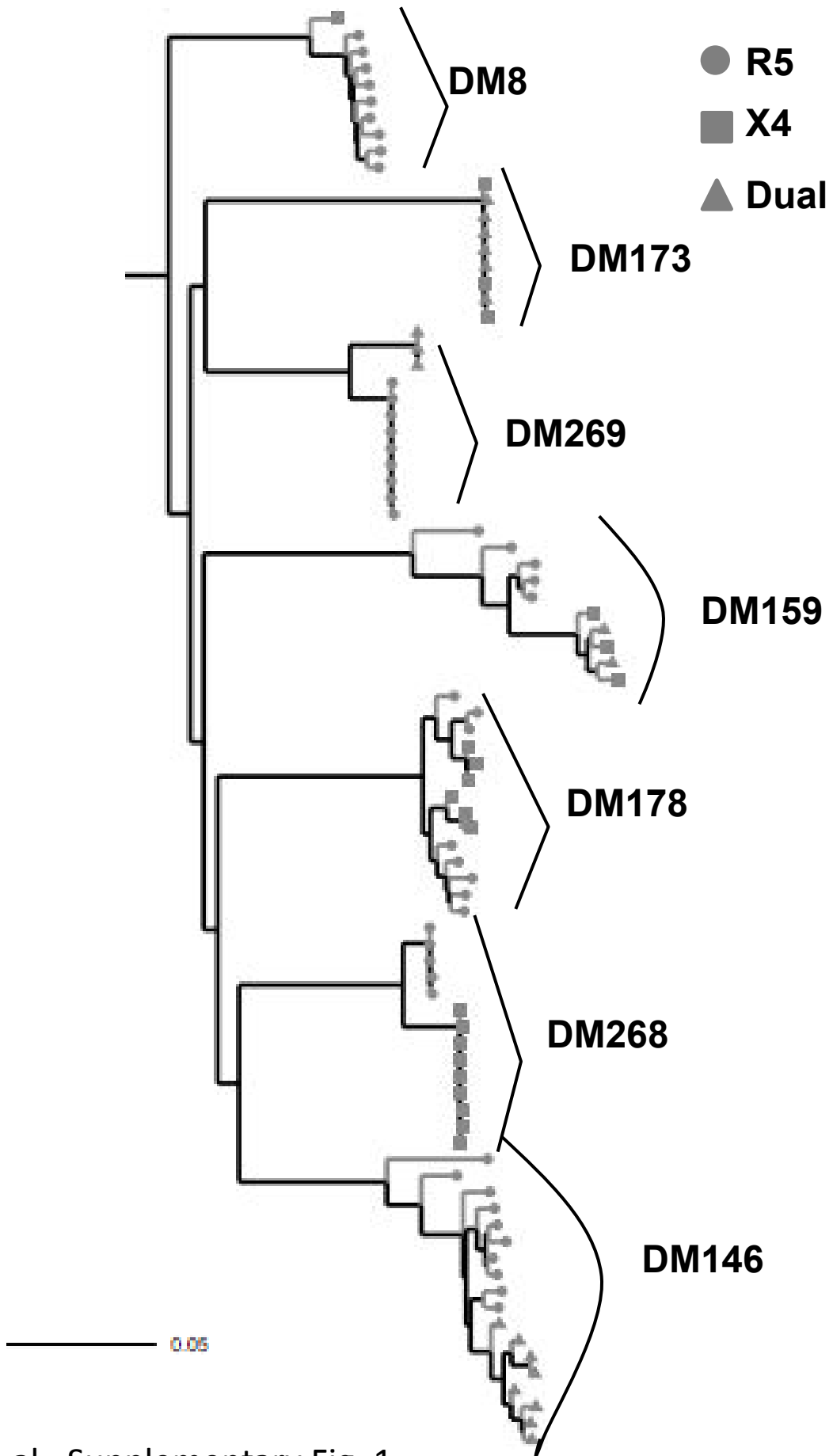
**Supplementary Figure 2. Single genome amplified HIV-1B X4 as compared to co-circulating R5 envs are more neutralization resistant to autologous contemporaneous plasma.** Neutralization  $IC_{50}$ s for R5 (circles), dual-tropic (triangles), and X4 (boxes) viruses against autologous contemporaneous plasma. Symbols represent one of the 8 independent single genome amplified envelopes from a subject infected with HIV-1B. Y-axis shows the  $IC_{50}$  against autologous contemporaneous plasma as reciprocal dilution. The long line represents the median of the dot plot, and the whiskers show the interquartile range. Star indicates statistically significant difference ( $p = 0.01$ ). The dashed line shows the highest tested plasma dilution (1:50). Symbols below the dotted lines are envs that were not neutralized by at least 50% at the highest tested plasma dilution (1:50). For statistical comparisons, these envs were assigned a midpoint value between 0 and 50.

**Supplementary Figure 3. DM8 X4 variant is equivalent susceptible to neutralization by a late plasma sample.** Graphs shows neutralization  $IC_{50}$ s against autologous plasma (reciprocal dilution) for DM8 R5 (circle) and X4 (box) variants. Graph a shows neutralization with a plasma sample collected around 6 months after the plasma from which the envs were isolated. The long line represents the median of the dot plot, and the whiskers show the interquartile range.

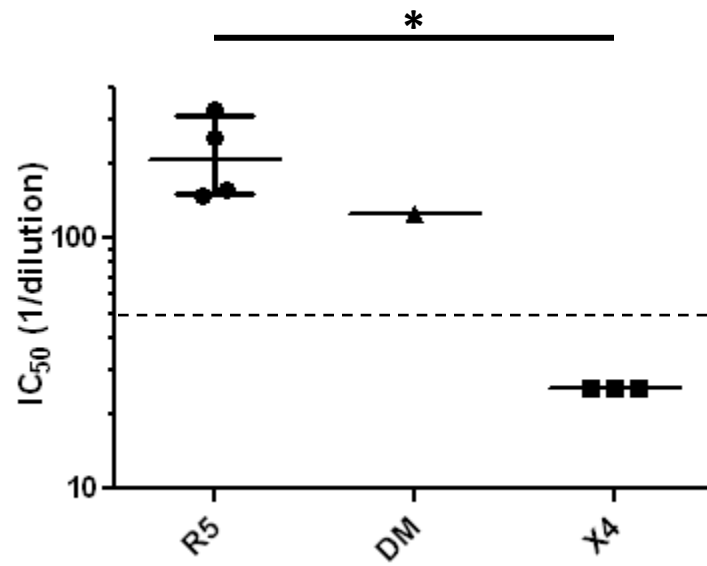
The dashed line shows the highest tested plasma dilution (1:50). Graph b shows the comparisons of the  $IC_{50}$ s between the contemporaneous versus late plasma sample. Star indicates statistically significant difference ( $p = 0.008$ ).

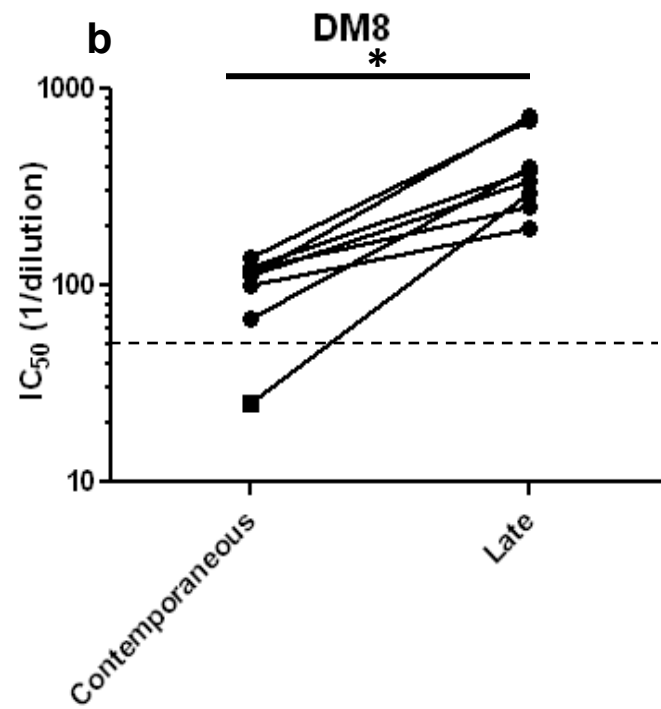
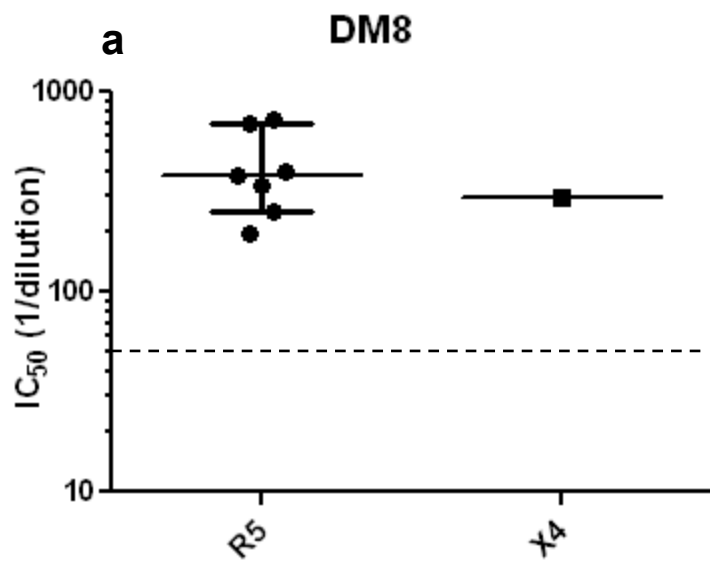
**Supplementary Figure 4. Passaged viruses show minimal env sequence changes**

**compared to the input stock.** The label at the left identifies the amino acid sequence of the env prior to passage (DM268-input), the env recovered from both U87 – CXCR4 and U87 – CCR5 culture supernatants after cells were exposed to day 75 plasma passaged virus stock (DM268+plasma), and env recovered from U87 – CCR5 culture supernatants after cells were exposed to day 75 virus passaged in the absence of plasma (DM268-plasma). Sequences from the beginning of env to the end of the ecto-domain are shown in single-letter amino acid code. The first box highlights the asparagine (N) change in DM268+plasma and the predicted N – linked glycosylation site. The second box shows the V3 loop sequence. Figure also shows the sites with mixture of amino acids in DM268-plasma. Nucleotide sequences were generated after RNA isolation from culture supernatants and direct sequencing of a bulk RT-PCR product. Amino acid sequence was generated using Bioedit (version 7.1.3).



Lin et. al., Supplementary Fig. 1





DM268-input	MRVMGIMRNC	QQWIIWVILG	FWMVMICNVV	GNLWVTVYYG	VPVWREAKAT	LFCASDAKAY	DREVHNVWAT	HACVPTDPNP	QEIVLGSVTE	NFNMWKNDMV	QMHEDIISL	WEESLKPCVK	
DM268+plasma	-----	-----	-----	-----	-----	-----	-----	-----	N	-----	-----	-----	
DM268-no-plasma	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	120
DM268-input	LTPLCVTLSC	KNVYKEGTP	DVDNKDEAKN	CSFNITTELK	DKKKQEYALF	YRVDIEPLNK	NNTENSTYSD	YRLINCNTSA	IAQACPKVSF	DPIPIHYCAP	AGFAILKCNN	KTFNGTGPCQ	
DM268+plasma	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
DM268-no-plasma	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	240
									<b>V3</b>				
DM268-input	NVSTVQCTHG	IKPVVSTQLL	LNGSLAEEGI	IIRSQNLTDN	AKTIIVHLNE	SIEIVCTRPS	NNRRSVRIG	PGQAFYTTGE	IIGDIRQAHK	NISEAKWNET	LQVGEKLRK	HFPNKTIEFK	
DM268+plasma	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
DM268-no-plasma	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	360
DM268-input	PSSGGDLEIT	THSPTCRGEF	FYCDTSKLPN	GTYNGTEGAS	TNSTNSTITL	PCRKIQFINM	WQEVGRAMYA	PPIAGNITCK	SNITGLLLTR	DGGNNNTGPE	TFRPGGGNMR	DNWRSELYKY	
DM268+plasma	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
DM268-no-plasma	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	480
DM268-input	KVVEVKPLGI	APTEAKRRVV	EREKRAVGIG	AVFLGFLGAA	GSTMGAASIT	LTVQARQLLS	GIVQQQSNLL	RAIEAQQHML	QLTVWGIKQL	QTRVLAIERY	LKDQQLLGIW	GCSGKLICTT	
DM268+plasma	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
DM268-no-plasma	-----	-----	-----	-----	-----	L	-----	-----	-----	E	-----	-----	600
						F				K			
DM268-input	AVPWNSSWSN	KSQKYIWENM	TWMQWEKEIS	NYTGIIYELL	EESQNQQEQN	EKDLLALDSW	KNLWTWPDIS	NWLWYIKIF					
DM268+plasma	-----	-----	-----	-----	-----	-----	-----	-----					
DM268-no-plasma	-----	-----	-----	-----	-----	-----	-----	-----					