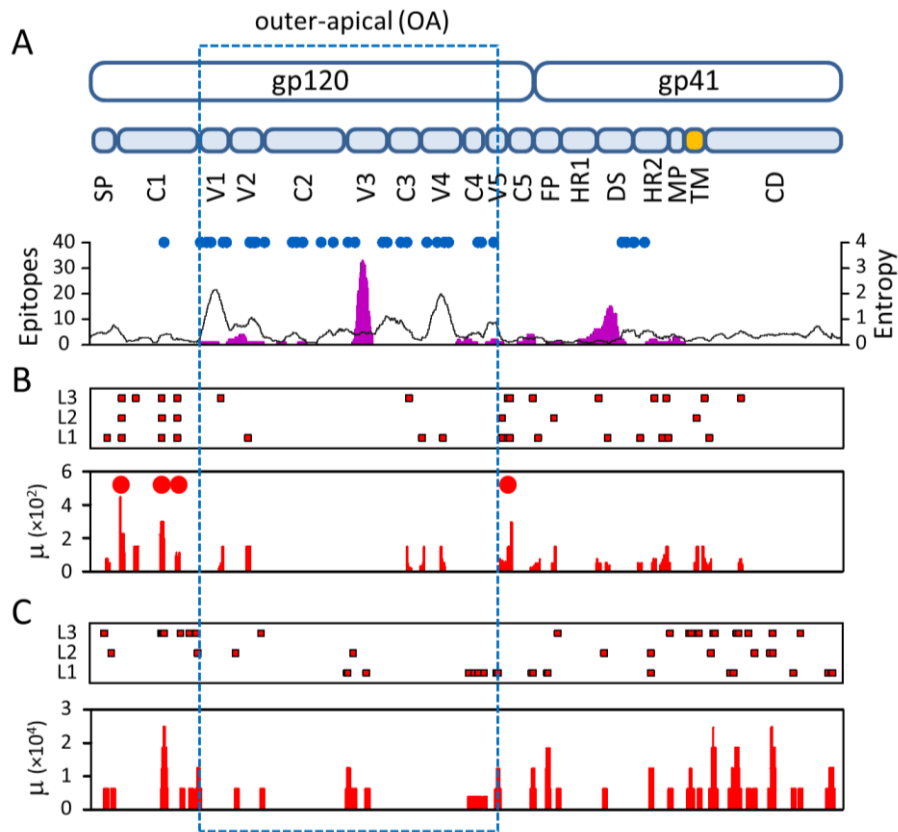**Supplementary Figure 1. Location of spontaneous mutations across the *int-vif-vpr* region.** On top is shown the genetic map of the region. Nucleotide substitutions found for each of the lines L1-L3 after four infection cycles are show as red squares (middle). Bottom: mutation rate (μ) averaged over 15-base sliding window (red bars). Significant mutation clusters are indicated with red circles.

Supplementary Figure 2. Location of A3-like and RT mutations across *env*. The *env* genetic map is shown on top (**A**). Abbreviations are defined in Figure 2 legend. The 1 kb region encoding the extensively glycosylated outer-apical domains of gp120 is boxed, and glycosylation sites (blue dots), number of B-cell epitopes (pink histogram), and protein sequence variability calculated as the Shannon entropy averaged over a 15-residue sliding window (black skyline) are indicated. **B.** A3-like (G→A substitutions in GG or GA motifs) found in each of the lines L1-L3 are shown with red squares. Red bars indicate the mutation rate (μ) averaged over 15-base sliding window. Notice that the per-target mutation rate is considerably higher than for non-A3 mutations. A3 targets that were mutated more than once in different lines are shown with red circles. **C.** Substitutions attributed to the HIV-1 RT mutations (all except A3-like mutations) in each of the lines L1-L3 are shown with red squares. Red bars indicate the mutation rate (μ) averaged over 15-base sliding window.

Supplementary Table 1. Shuttle vector primers used for cloning and PCR.

Name	Orientation	Sequence (5'-3')
Env_F_Mlu	Forward	ACCCGACACGCGTTGGCAATGAGAGTGATGGG
Env_R_Xho	Reverse	GACTAGCTCGAGTTTTGACCACTTGCCACCC
IVV_F_Mlu	Forward	ACCCGACACGCGTTTTTTAGATGGGATAGATAAG
IVV_R_Xho	Reverse	GACTAGCTCGAGGCTATGTTGGCACCCAATTC
Env_F	Forward	GGCAATGAGAGTGATGGGGA
Env_R	Reverse	TTTTTGACCACTTGCCACCCA
IVV_F	Forward	TTTTTAGATGGGATAGATAAGGCTC
IVV_R	Reverse	GCTATGTTGGCACCCAATTC

Supplementary Table 2. Primers used for nested limiting-dilution PCR of patient samples.

Patients	Name	Orientation	Sequence (5'-3')	HXB2 location
All except	294TU	Forward	ATGGCTTAGGGCAACATATCTATG	5677-5700
R14-R115	163U5D	Reverse	CTGAGGGATCTCTAGTTACCAGAG	9664-9687
	551VU	Forward	GGAAGCCATAATAAGAATTCTGCAACAACTGC	5729-5760
	555U5	Reverse	CCAGAGTCACACAACCGCGGGGCACACACTACT	9637-9669
R14	3'HIV-O-S	Forward	CAAAATTTTCGGGTTTATTACAGGGACA	4890-4917
	163U5D	Reverse	CTGAGGGATCTCTAGTTACCAGAG	9664-9687
	294TU	Forward	ATGGCTTAGGGCAACATATCTATG	5677-5700
	555	Reverse	CCAGAGTCACACAACCGCGGGGCACACACTACT	9637-9669
R15	3'HIV-O-S 3	Forward	TACAGTGCAGGGGAAAGAATAATAGACATAATA	4809-4834
	555U5	Reverse	CCAGAGTCACACAACCGCGGGGCACACACTACT	9637-9669
	551	Forward	GGAAGCCATAATAAGAATTCTGCAACAACTGC	5729-5760
	2R3.B6R	Reverse	TGAAGCACTCAAGGCAAGCTTTATTGAGGC	9607-9636