Supplementary Table 2: *In vitro* selection for HIV-1 variants resistant to GS-CA1

GS-CA1		CA Amino Acid Substitutions in HIV-1 Outgrowth Assays	
		MT-2 cells[a,c]	**PBMC[b,c]**
Fixed Drug Concentration (nM)	**Fold EC_{95}**	**HIV-1_{IIIb}**	**HIV-1 isolates (n=5)**
1.58 – 12.6	2.5 – 20	L56I N57S M66I Q67H **N74D** Q67H/T107N	**Q67H** Q67Y N74D Q67H+M66I Q67H+K70R Q67H+N74D Q67H+T107N

[a] HIV-1_{IIIB}-infected MT-2 cell cultures were maintained in a fixed concentration of GS-CA1 (24 replicate cell cultures per test concentration) for 35 days and monitored daily for cytopathic effect (CPE). Cultures were passaged (1:5) every 3-4 days into fresh drug-containing media. Viral supernatants were harvested whenever the majority of the culture showed CPE, genotyped and their CA sequence compared to that of the input virus to identify amino acid substitutions.

[b] Human PBMCs were independently infected with each of 5 HIV-1 isolates (BaL, 92US657, 92HT593.1, 7467 and 7576) and cultured in a fixed concentration of GS-CA1 (6 replicate cell cultures per experimental condition) for 35 days. Half of the cell-free supernatants were harvested every 7 days and replenished with fresh PBMCs in drug-containing media over a 28 day selection period, at which point a small aliquot of supernatant (150 µL) was used to infect fresh PBMCs in drug-containing media. After 7 more days in culture (35 days post-infection), the final cell-free supernatants were collected and analyzed for HIV content by p24 ELISA and for infectivity in duplicate cell cultures using CEM-NK^R CCR5+Luc+ indicator cells. HIV+ samples with infectivity over mock-infected controls were genotyped and their CA sequence compared to that of the input virus to identify amino acid substitutions.

[C] Mutations in boldface indicate the single most predominant emergent HIV-1 variant observed under each assay format.