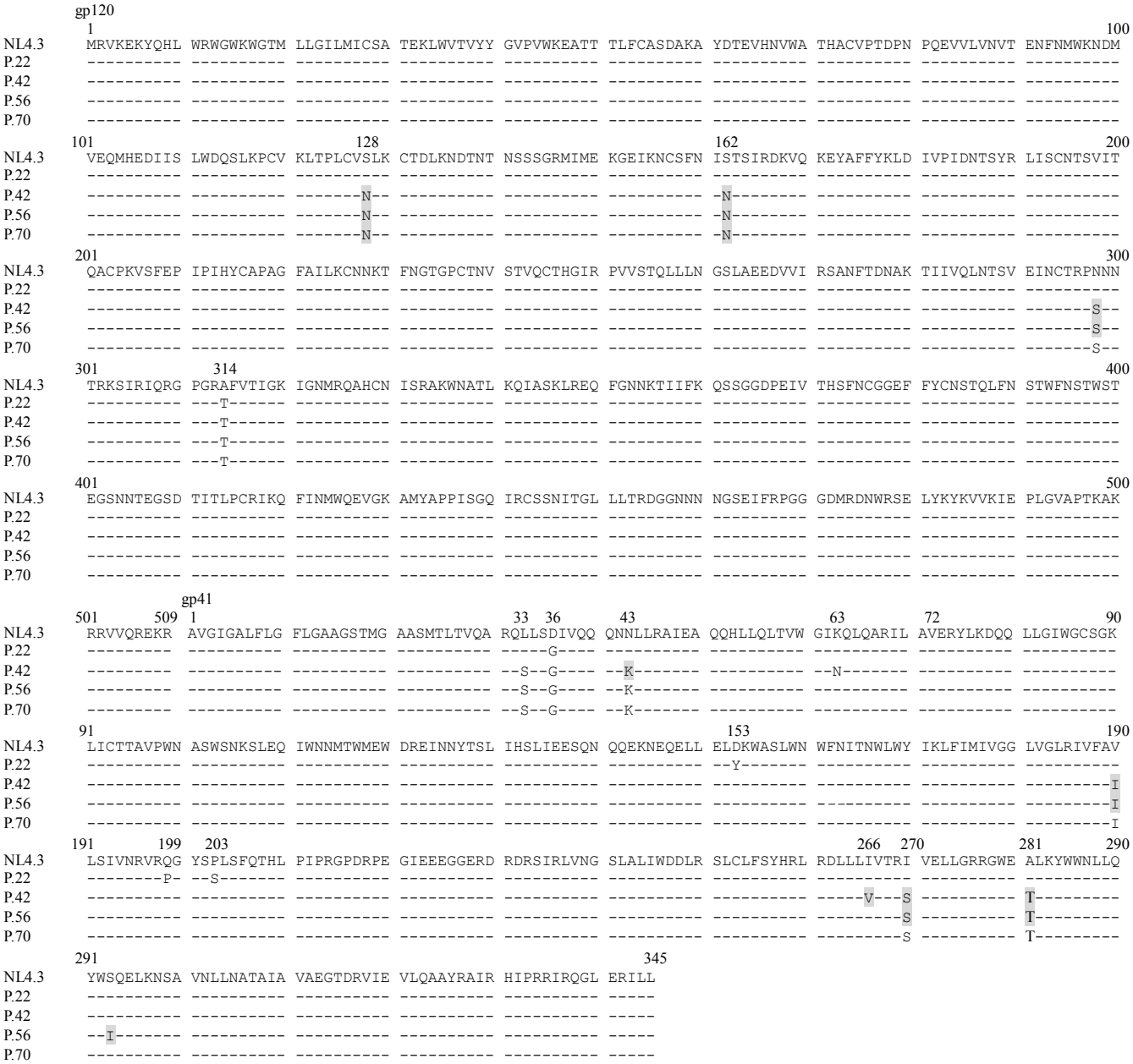
Supplemental Table 1. Effects of gp120 substitutions on resistance to fusion inhibitors[a]

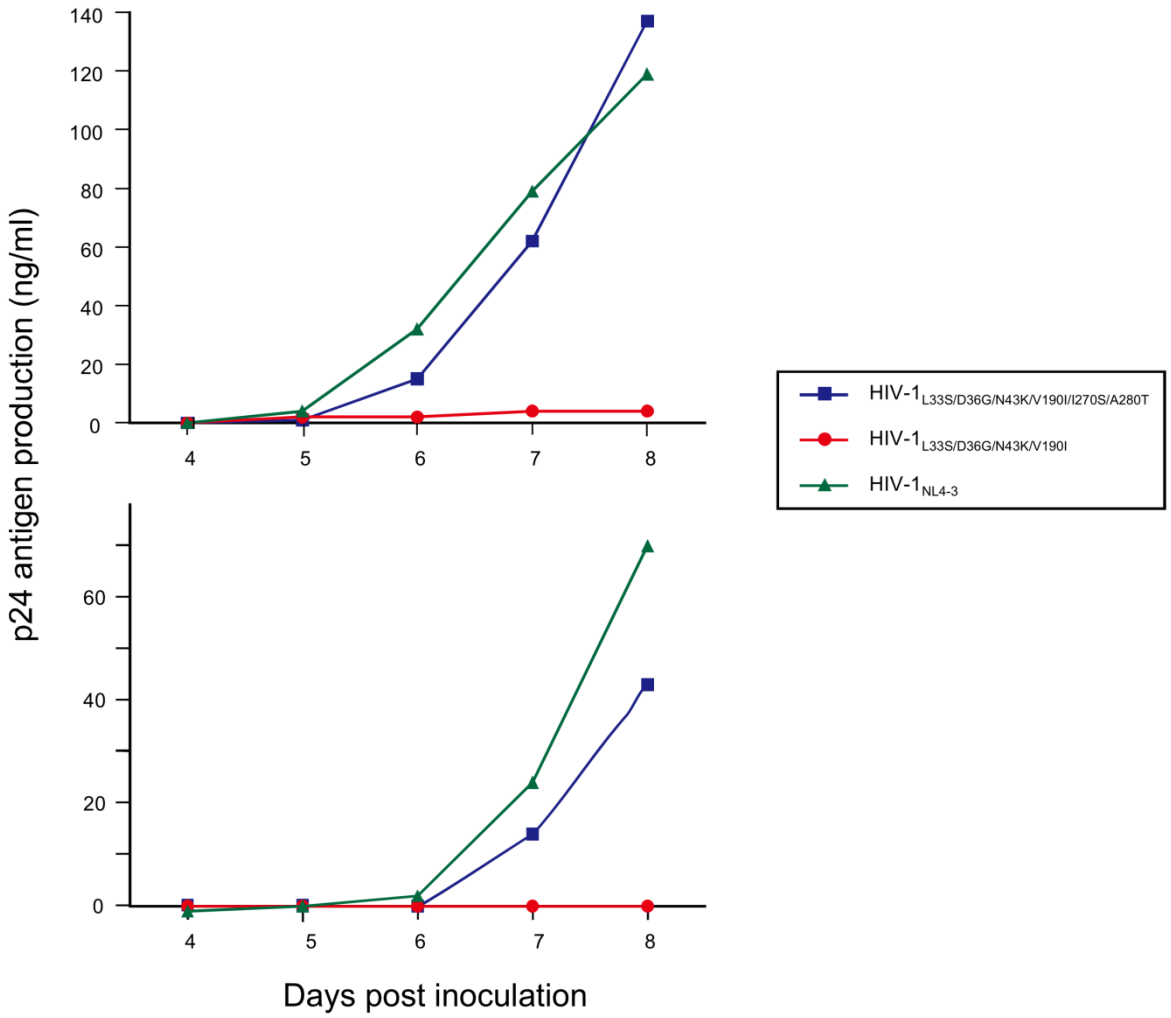
Peptide	EC_{50} (nM)	
	HIV-$1_{NL4.3}$	HIV-$1_{NL4.3\text{-}P70gp120}$ [a]
ddC	529 ± 198	430 ± 33 (0.8) [b]
T-20-based peptides		
T-20	51 ± 11	54 ± 15 (1.1)
$T\text{-}20_{S138A}$	2.6 ± 0.6	1.5 ± 0.6 (0.6)
T-20EK	2.9 ± 0.4	2.5 ± 1.0 (0.9)
$T\text{-}20EK_{S138A}$	0.60 ± 0.23	0.47 ± 0.20 (0.8)
T1249	0.32 ± 0.10	0.18 ± 0 (0.6)
C34-based peptides		
C34	2.8 ± 0.4	1.0 ± 0.4 (0.4)
SC35EK	0.51 ± 0.11	0.48 ± 0.01 (0.9)
Sifvirtide	0.99 ± 0.40	0.80 ± 0.31 (0.8)
T2410	1.5 ± 0.6	0.63 ± 0.19 (0.4)
T2429	0.91 ± 0.24	0.89 ± 0.26 (1.0)
T290676	0.27 ± 0.10	0.36 ± 0.08 (1.3)
T2635	0.38 ± 0.13	0.27 ± 0.06 (0.7)
T2544	0.49 ± 0.07	0.38 ± 0.06 (0.8)

[a] Molecular clone having S128N, S162N, N298S and A314T mutations in the gp120 of NL4.3.
[b] Data represent the mean ± SD (fold of resistance) of at least three independent determinations.



Supplemental Figure 1. Amino acid substitutions emerged in gp120 and gp41 during induction of T-20EK resistance.

Amino acid substitutions emerged in gp120 and gp41 at the indicated passage during T-20EK resistance induction are shown. Amino acid substitutions of R313R in gp120 at passage 22 and V72V in gp41 at passage 22, 42, 56 and 70 are silent mutation, AGA to AGG and GTG to GTA, respectively. Shaded letters mean mixture of substitutions from 30 to 70 %.



Supplemental Figure 2. Replication kinetics of resistant clones.

The effects of T-20EK-selected substitutions on the replication kinetics were analyzed. MT-2 cells were infected with each gp41-recombinant HIV-1 variant at 500 MAGI units. After 16 h incubation, the infected cells were washed and cultured for 8 days. The culture supernatants were harvested every 24 h and production of progeny viruses was monitored by a commercial available p24 antigen ELISA kit (ZeptroMetrix Co. NY). HIV-1_{NL4-3} was used as a wild-type control. Replication kinetics of HIV-$1_{L33S/D36G/N43K/V190I}$ and HIV-$1_{L33S/D36G/N43K/V190I/I270S/A280T}$ was compared. Substitutions in cytoplasmic domain of gp41 restore significantly reduced replication kinetics by those in ecto- and transmembrane-domains. Results shown are representative of two independent experiments each using two independent clones.