

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-P70gp120} ^a
ddC	529 ± 198	430 ± 33 (0.8) ^b
<i>T-20-based peptides</i>		
T-20	51 ± 11	54 ± 15 (1.1)
T-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-20EK _{S138A}	0.60 ± 0.23	0.47 ± 0.20 (0.8)
T1249	0.32 ± 0.10	0.18 ± 0 (0.6)
<i>C34-based peptides</i>		
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)

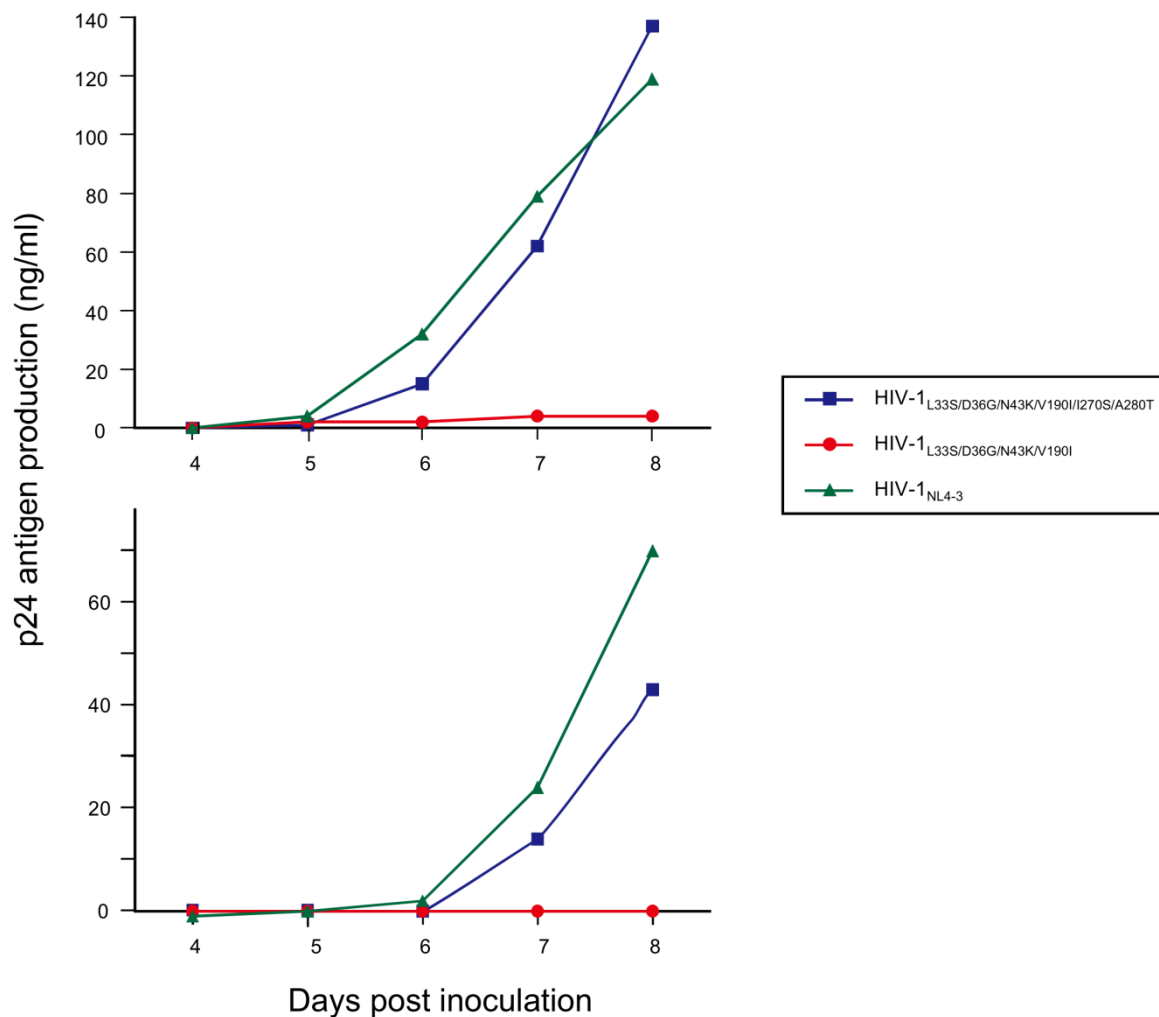
^a Molecular clone having S128N, S162N, N298S and A314T mutations in the gp120 of NL4.3.

^b Data represent the mean ± SD (fold of resistance) of at least three independent determinations.

		gp120										
	1										100	
NL4.3		MRVKEKYQHL	WRWGKWKGTM	LLGILMICS	TEKLWVTVY	GVPVWKEATT	TLFCASDAKA	YDTEVHNVWA	THACVPTDPN	PQEVVLVNVT	ENFNMWKNDM	
P.22		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
P.42		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
P.56		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
P.70		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
	101			128				162			200	
NL4.3		VEQMHEDIIS	LWDQSLKPCV	KLTPLCVSLK	CTDLKNDTNT	NSSSGRMIME	KGEIKNCSEFN	ISTTSIRDVKQ	KEYAFFYKLD	IVPIDNTSYR	LISCNTSVIT	
P.22		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
P.42		-----	-----	N	-----	-----	-----	N	-----	-----	-----	
P.56		-----	-----	N	-----	-----	-----	N	-----	-----	-----	
P.70		-----	-----	N	-----	-----	-----	N	-----	-----	-----	
	201										300	
NL4.3		QACPKVSFEP	IPIHYCAPAG	FAILKCNKKT	FNGTGPCTNV	STVQCTHGIR	PVVSTQLLLN	GSLAEEDVVI	RSANFTDNAK	TIIVQLNTSV	EINCTRPNNN	
P.22		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
P.42		-----	-----	-----	-----	-----	-----	-----	-----	-----	S	
P.56		-----	-----	-----	-----	-----	-----	-----	-----	-----	S	
P.70		-----	-----	-----	-----	-----	-----	-----	-----	-----	S	
	301		314								400	
NL4.3		TRKSIRIQRG	PGRAFVTIGK	IGNMRQAHCN	ISRAKWNATL	KQIASKLREQ	FGNKKTIIFK	QSSGGDPEIV	THSFNCGGEF	FYCNSTQLFN	STWFNSTWST	
P.22		-----	T	-----	-----	-----	-----	-----	-----	-----	-----	
P.42		-----	T	-----	-----	-----	-----	-----	-----	-----	-----	
P.56		-----	T	-----	-----	-----	-----	-----	-----	-----	-----	
P.70		-----	T	-----	-----	-----	-----	-----	-----	-----	-----	
	401										500	
NL4.3		EGSNNTEGSD	TITLPCRIRKQ	FINMWQEVGK	AMYAPPISGQ	IRCSSNITGL	LLTRDGGNNN	NGSEIFRPGG	GDMRDNRWSE	LYKYKVKVIE	PLGVAPTAKK	
P.22		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
P.42		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
P.56		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
P.70		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
			gp41									
	501	509	1			33	36	43		63	72	90
NL4.3		RRVVQREKR	AVGIGALFLG	FLGAAGSTMG	AASMTLTVQA	RQLLSDIVQQ	QNNLLRAIEA	QQHLLQLTVW	GIKQLQARIL	AVERYLKDQQ	LLGIWGCSSGK	
P.22		-----	-----	-----	-----	G	-----	-----	-----	-----	-----	
P.42		-----	-----	-----	S	G	K	-----	N	-----	-----	
P.56		-----	-----	-----	S	G	K	-----	-----	-----	-----	
P.70		-----	-----	-----	S	G	K	-----	-----	-----	-----	
	91							153				190
NL4.3		LICTTAVPWN	ASWSNKSLEQ	IWNMTWMEW	DREINNYTSL	IHSLIEESQN	QQEKNEQELL	ELDKWASLWN	WFNITNWLWY	IKLFIMIVGG	LVGLRIVFAV	
P.22		-----	-----	-----	-----	-----	-----	Y	-----	-----	-----	
P.42		-----	-----	-----	-----	-----	-----	-----	-----	-----	I	
P.56		-----	-----	-----	-----	-----	-----	-----	-----	-----	I	
P.70		-----	-----	-----	-----	-----	-----	-----	-----	-----	I	
	191	199	203						266	270	281	290
NL4.3		LSIVNRVRQG	YSPLSFQTHL	PIPRGPDRPE	GIEEEGGERD	RDRSIRLVNG	SLALIWDLLR	SLCLFSYHRL	RDLLLVTRI	VELLGRRGWE	ALKYWWNLLQ	
P.22		P	S	-----	-----	-----	-----	-----	-----	-----	-----	
P.42		-----	-----	-----	-----	-----	-----	-----	V	S	-----	
P.56		-----	-----	-----	-----	-----	-----	-----	-----	S	-----	
P.70		-----	-----	-----	-----	-----	-----	-----	-----	S	-----	
	291						345					
NL4.3		YWSQELKNSA	VNLLNATAIA	VAEGTDRVIE	VLQAAYRAIR	HIPRRIRQGL	ERILL	-----	-----	-----	-----	
P.22		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
P.42		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
P.56		T	-----	-----	-----	-----	-----	-----	-----	-----	-----	
P.70		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	

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 (ZeptoMetrix 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.