

	10	20	30	40	50	60	70	80	90	99
HIV-1 _{NL4-3}	PQITLWQRPL	VTIKIGGQLK	EALLDTGADD	TVLEEMNLPG	RWKPKMIGGI	GGFIKVRQYD	QLLIEICGHK	AIGTVLVGPT	PVNIIGRNL	TQIGCTLNF
HIV-2 _{EHO}	..FS..R..V	.KAT.E..SV	.V.....	SIVAGIE.GS	NYT..IV...	...NTKE.K	NVE..VV.KR	VRA..MT.D.	.I..F...I.	NSL.M...L
HIV-1 _{1PV-5μM}FI..VV....
HIV-1 _{1PV-5μM}FI.....IA...	V.....	...V....
HIV-1 _{1QV-5μM}ID..V..	..V.....	..P.....	..C.....	...V....M
HIV-1 _{1PV-15μM}II..I..I...	..VR.....	..VP.....	V.S.....	.T.....M	..L.....
HIV-1 _{1PRV⁸p10}I	...V...R	..I.....	.I...I..L...P.....	T.....	.A.....M.
HIV-1 _{1PRV⁸p30}I	...V...R	..I.....	.I...I..L...P.....	T.....	.A.V....M.
HIV-1 _{1PRV⁸p51}I	...V...R	..I.....	.IF..I..L...	..M.....	..P.....	T.....	.I.V....M.
r _{CL} HIV-1 _{p16}F	I.V...QMIV.AI...IV...	..M..K...	..VPV....	..TA.....	...V....VM
r _{CL} HIV-1 _{p48}I	.V.V...V.	..I.....	.IF.....	..E..L...	..L...E	..P.....	VV.....	.A.V....
r _{CL} HIV-1 _{p40}IRFADID..	..T..I.V.V	..T.....	..VP...G..	VV...I..	.A..V....	..L.....
r _{CL} HIV-1 _{p44}F	.V..A..V.FQ...	..I..L...	A..M.....	..PM...G..	VMA.....	...V....M
r _{CL} HIV-1 _{M45}F	IPV.V...PTIF.GI...V.....	..VP...G.T	I.S...A.	...V....VM
HIV-1 _{NL4-3}	PQITLWQRPL	VTIKIGGQLK	EALLDTGADD	TVLEEMNLPG	RWKPKMIGGI	GGFIKVRQYD	QLLIEICGHK	AIGTVLVGPT	PVNIIGRNL	TQIGCTLNF
HIV _AI	...V....D.E...	...R....	..V.....	..P.....	V.....	.T.....M	..L.F...
HIV _BII..I..I...	..L.R.....	..VP.....	V.S.....	.A.....M	..L.....
HIV _CI	...V...R	..I.....I...L...	..V.....	..VP...QA.....M.
HIV _GI	IE..V...I.	K...L...P.....	T.....	.A.....M
HIV _{HM}I	..R.....	K...L...	..V.....	..P.....	V.....	.A.....M	..L.....
HIV _{HM}I	...R..T..L...	..V.....	..P.....	V.....	.A.....M	..K.....
HIV _{SS}RD..T...VP.....	V.S.I....	.T.....M	..L.....
HIV _{SL}II.....	..F.DIS..L..E.	..V..K...	..VP.....	V.S.....	.A.....M
HIV _{EV}V	.E...A..IRF.DI...I..V	..LVR.K..E	..VP.....	V.....	.A.....M.
HIV _{ES}IL...R.....VP.....	.LC...I..	...V....M
HIV ₁₃₋₅₂D..I...VP.....	V...I..	.A.....M	..L.....

Figure S1. Amino acid sequences of the protease-encoding region of the virus. (A) HIV-2_{EHO} and PI-resistant HIV-1 variants used in drug susceptibility assay. **(B)** Each of the eleven HIV-1 clinical strains. The consensus HIV-1_{NL4-3} sequence is displayed at the top of the figures as a reference. Identity at individual amino acid positions is indicated by dots.

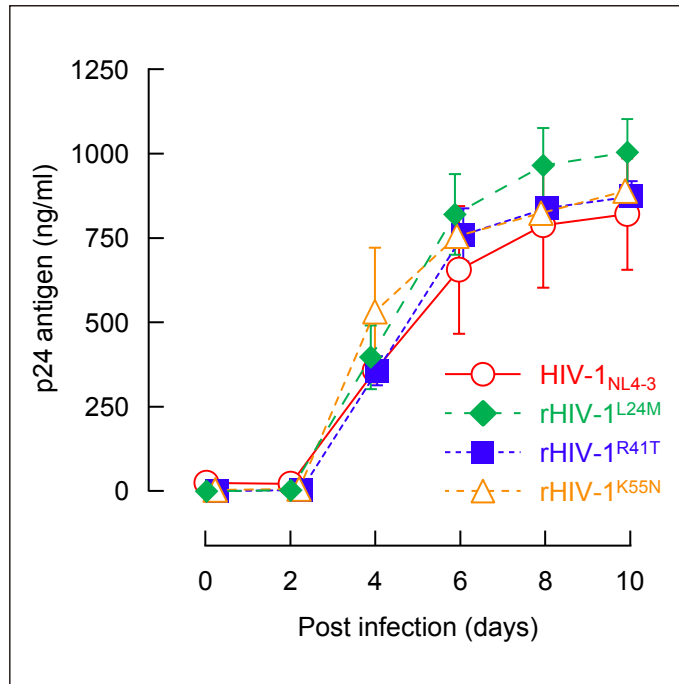


Figure S2. Viral replication kinetics of recombinant HIV-1 variants harboring single amino acid substitutions compared to wild-type HIV-1_{NL4-3}. All of the HIV-1 variants showed the compatible replication activity compared with HIV-1_{NL4-3}.

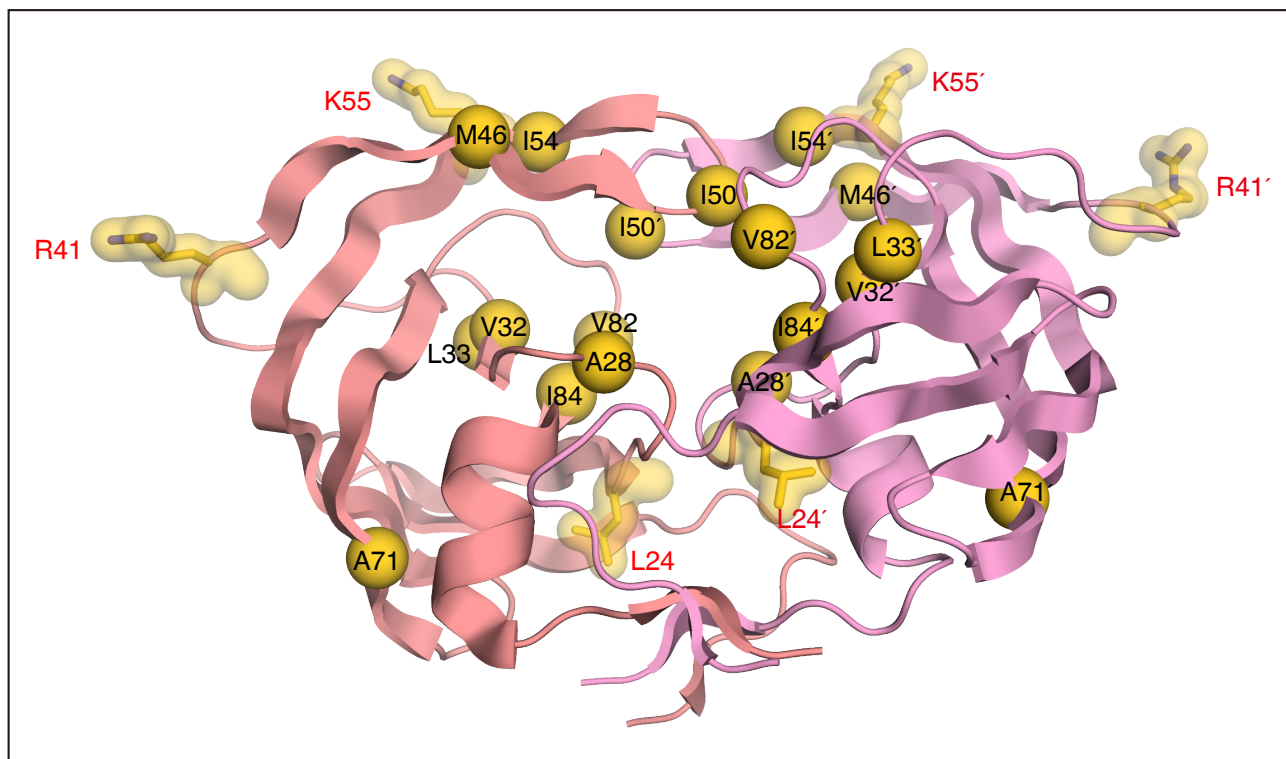


Figure S3. The locations of the amino acid substitutions often associated with HIV-1 resistance to PIs. Three relatively unique amino acid substitutions identified in the present study (L24M, R41T, and K55N) are also illustrated.

	p17									
	10	20	30	40	50	60	70	80	90	100
HIV-1 _{NL4-3}	MGARASVLSG	GELDKWEKIR	LRPGGKKQYK	LKHIVWASRE	LERFAVNPGL	LETSEGCROI	LGQLQPSLQT	GSEELRSLYN	TIAVLYCVHQ	RIDVKDTKEA
HIV _{11MIX} ^{ATV-p12}
HIV _{11MIX} ^{DRV-p23}F.....K.....
HIV _{11MIX} ^{057-14-p50}	R.....
HIV _{11MIX} ^{058-14-p50}R.....
HIV _{11MIX} ^{059-14-p50}F.....
				p24						
	110	120	130	140	150	160	170	180	190	200
HIV-1 _{NL4-3}	LDKIEEEQNK	SKKKAQAAAA	DTGNNSQVSQ	NYPIVQNLOG	QMVHQAI SPR	TLNAMVKVVE	EKA FSPEVIP	MFSALSEGAT	PQDLNTMLNT	VGGHQAAMQM
HIV _{11MIX} ^{ATV-p12}
HIV _{11MIX} ^{DRV-p23}D.....
HIV _{11MIX} ^{057-14-p50}D.....
HIV _{11MIX} ^{058-14-p50}D.....
HIV _{11MIX} ^{059-14-p50}
	210	220	230	240	250	260	270	280	290	300
HIV-1 _{NL4-3}	LKETINEEAA	EWDRLHPVHA	GPIAPGQMR E	PRGSDIAGTT	STLQEQIGWM	THNPPIPVGE	IYKRWIILGL	NKIVRMYSP T	SILDIRQGPK	EPFRDYVDRF
HIV _{11MIX} ^{ATV-p12}E.....
HIV _{11MIX} ^{DRV-p23}Q.....
HIV _{11MIX} ^{057-14-p50}Q.....E.....
HIV _{11MIX} ^{058-14-p50}Q.....
HIV _{11MIX} ^{059-14-p50}Q.....
	310	320	330	340	350	360	p2	p7	390	400
							370	380		
HIV-1 _{NL4-3}	YKTLRAEQAS	QEVKNWMTET	LLVQNaNPDC	KTILKALGPG	ATLEEMMTAC	QGVGGPGHKA	RVLAEAMSQV	TNPATIMI QK	GNFRNQRKT V	KCFNCGKEGH
HIV _{11MIX} ^{ATV-p12}T.....M.....
HIV _{11MIX} ^{DRV-p23}
HIV _{11MIX} ^{057-14-p50}
HIV _{11MIX} ^{058-14-p50}
HIV _{11MIX} ^{059-14-p50}
				p1		p6				
	410	420	430	440	450	460	470	480	490	500
HIV-1 _{NL4-3}	IAKNCRAPRK	KGCKWCKGKEG	HQMKDCTERQ	ANFLGKIWPS	HKGRPGNFLQ	SRPEPTAPPE	ESFRFG EETT	TPSQKQEPID	KELYPLASLR	SLFGSDPSSQ
HIV _{11MIX} ^{ATV-p12}	..R..K.....E.....	V.....F.....T.....SA.K.....N.....
HIV _{11MIX} ^{DRV-p23}	..R..K.....E.....	V.....F.....	..L.....S.....T.....SA.K.....N.....
HIV _{11MIX} ^{057-14-p50}	..R..K.....E.....	V.....T.....F.....	..K.....	..L.....T.....SA.K.....N.....
HIV _{11MIX} ^{058-14-p50}	..R..K.....E.....	V.....V.....F.....V.....T.....SA.K.....N.....
HIV _{11MIX} ^{059-14-p50}	..R..K.....E.....	V.....V.....F.....L.....T.....SA.....N.....

Figure S4. Amino acid sequences of the Gag-encoding region of the virus. The consensus HIV-1_{NL4-3} sequence is displayed at the top of the figures as a reference. Identity at individual amino acid positions is indicated by dots.

Table S1. Antiviral activity of PIs against X4-tropic subtype-A and R5-tropic subtype-C clinical isolates in PHA-stimulated PBMCs.

Compound	EC ₅₀ (nM) against	
	HIV-1 _{92/UG/029} (subtype-A, X4)*	HIV-1 _{97/ZA/003} (subtype-C, R5)*
GRL-057-14	3.9 ± 0.3	4.0 ± 1.0
GRL-058-14	11.0 ± 1.0	13.0 ± 3.0
GRL-059-14	37.0 ± 5.0	21.0 ± 7.0
DRV	4.0 ± 0.8	4.0 ± 1.0

The EC₅₀ (50% effective concentration) values were determined by using PHA-stimulated PBMC as target cells and the inhibition of p24 Gag protein production by each drug was used as an endpoint. All assays were conducted in duplicate, and the data shown represent mean values (± 1 S.D.) derived from the results of two independent experiments. PHA-stimulated PBMCs were derived from a single donor in each independent experiment. *‘‘X4’’ and ‘‘R5’’ denote X4-tropic HIV-1 strain and R5-tropic HIV-1 strain, respectively.