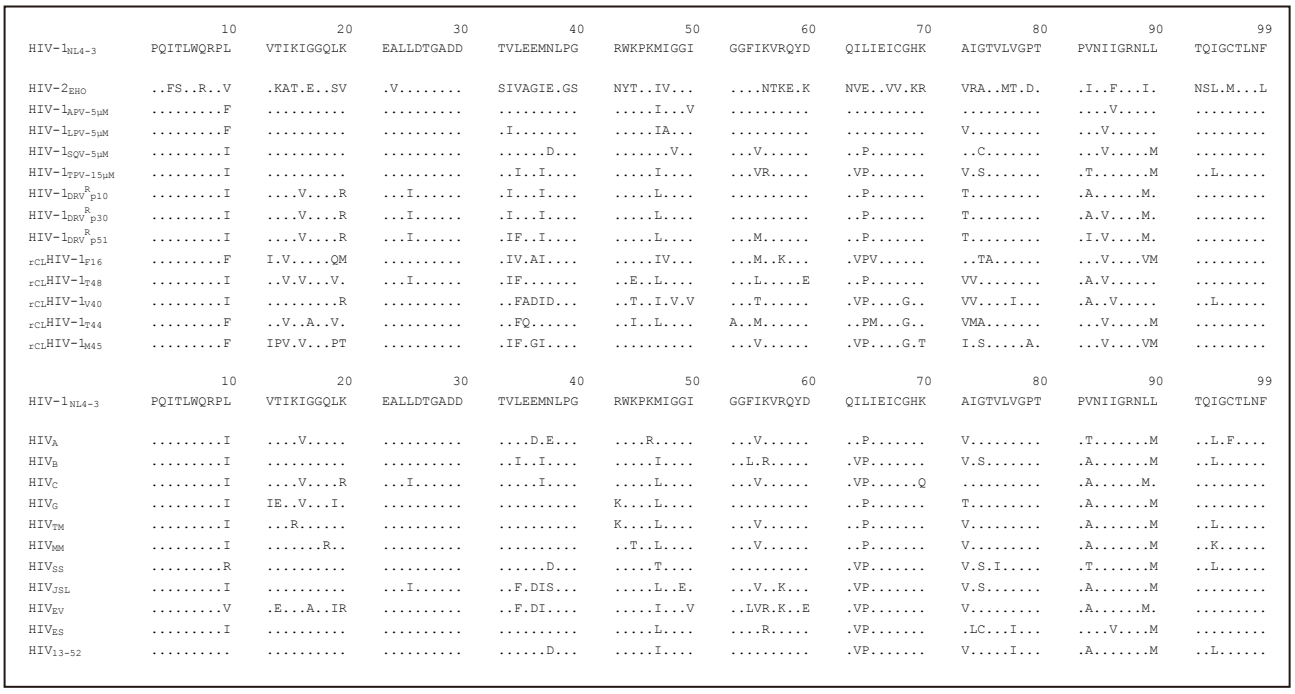

	10	20	30	40	50	60	70	80	90	99
HIV-1_{NL4-3}	PQITLWQRPL	VTIKIGGQLK	EALLDTGADD	TVLEEMNLPG	RWKPKMIGGI	GGFIKVRQYD	QILIEICGHK	AIGTVLVGPT	PVNIIGRNLL	TQIGCTLNF
HIV-2_{EHO}	..FS..R..V	.KAT.E..SV	.V.....	SIVAGIE.GS	NYT..IV...	....NTKE.K	NVE..VV.KR	VRA..MT.D.	.I..F...I.	NSL.M...L
HIV-$1_{APV-5\mu M}$	.....F	.....	.....	.....	.....I...V	.....	.....	.....	....V.....	.....
HIV-$1_{LPV-5\mu M}$	.....F	.....	.....	.I.....	.....IA...	.....	.....	V.....	...V.....	.....
HIV-$1_{SQV-5\mu M}$	I	.....	.....	.....D...	.....V..	...V.....	..P.....	..C.....	...V.....M	.....
HIV-$1_{TPV-15\mu M}$	I	.....	.....	..I..I....	.....I....	...VR.....	.VP.....	V.S.....	.T.....M	..L.....
HIV-$1_{DRV^{R}p10}$	I	....V....R	...I.....	.I...I....	.....L....	.....	..P.....	T.....	.A.....M.	.....
HIV-$1_{DRV^{R}p30}$	I	....V....R	...I.....	.I...I....	.....L....	.....	..P.....	T.....	.A.V....M.	.....
HIV-$1_{DRV^{R}p51}$	I	....V....R	...I.....	.IF..I....	.....L....	...M.....	..P.....	T.....	.I.V....M.	.....
$_{rCL}$HIV-1_{F16}	.....F	I.V.....QM	.....	.IV.AI....	.....IV...	...M..K...	.VPV.....	..TA.....	...V....VM	.....
$_{rCL}$HIV-1_{T48}	I	..V.V...V.	...I.....	.IF.....	..E..L....	...L.....E	..P.....	VV.....	.A.V.....	.....
$_{rCL}$HIV-1_{V40}	I	.....R	.....	..FADID...	..T..I.V.V	...T.....	.VP....G..	VV....I...	.A..V.....	..L.....
$_{rCL}$HIV-1_{T44}	.....F	..V..A..V.	.....	..FQ.....	..I..L....	A..M.....	..PM...G..	VMA.....	...V.....M	.....
$_{rCL}$HIV-1_{M45}	.....F	IPV.V...PT	.....	.IF.GI....	.....	...V.....	.VP....G.T	I.S.....A.	...V....VM	.....

	10	20	30	40	50	60	70	80	90	99
HIV-1_{NL4-3}	PQITLWQRPL	VTIKIGGQLK	EALLDTGADD	TVLEEMNLPG	RWKPKMIGGI	GGFIKVRQYD	QILIEICGHK	AIGTVLVGPT	PVNIIGRNLL	TQIGCTLNF
HIV_{A}	I	....V.....	.....	....D.E...	....R.....	...V.....	..P.....	V.....	.T.....M	..L.F....
HIV_{B}	I	.....	.....	..I..I....	.....I....	..L.R.....	.VP.....	V.S.....	.A.....M	..L.....
HIV_{C}	I	....V....R	...I.....	.....I....	.....L....	...V.....	.VP.....Q	.....	.A.....M.	.....
HIV_{G}	I	IE..V...I.	.....	.....	K....L....	.....	..P.....	T.....	.A.....M	.....
HIV_{TM}	I	...R.....	.....	.....	K....L....	...V.....	..P.....	V.....	.A.....M	..L.....
HIV_{MM}	I	.....R..	.....	.....	..T..L....	...V.....	..P.....	V.....	.A.....M	..K.....
HIV_{SS}	.....R	.....	.....	.....D...	.....T....	.....	.VP.....	V.S.I.....	.T.....M	..L.....
HIV_{JSL}	I	.....	...I.....	..F.DIS...	.....L..E.	...V..K...	.VP.....	V.S.....	.A.....M	.....
HIV_{EV}	V	.E...A..IR	.....	..F.DI....	.....I...V	..LVR.K..E	.VP.....	V.....	.A.....M.	.....
HIV_{ES}	I	.....	.....	.....	.....L....	....R.....	.VP.....	.LC...I...	....V....M	.....
HIV_{13-52}	.....	.....	.....	.....D...	.....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.

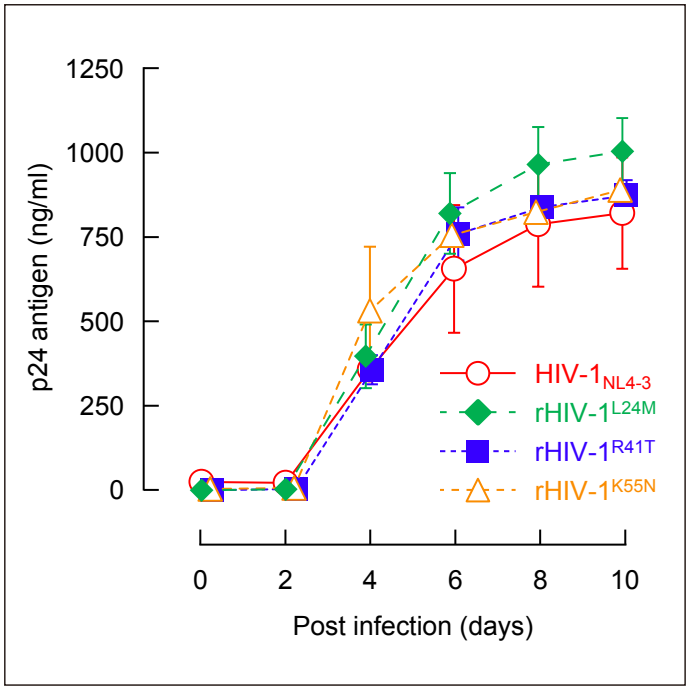

Figure S2. Viral replication kinetics of recombinant HIV-1 variants harboring single amino acid substitutions compared to wild-type HIV-1$_{NL4-3}$. All of the HIV-1 variants showed the compatible replication activity compared with HIV-1$_{NL4-3}$.

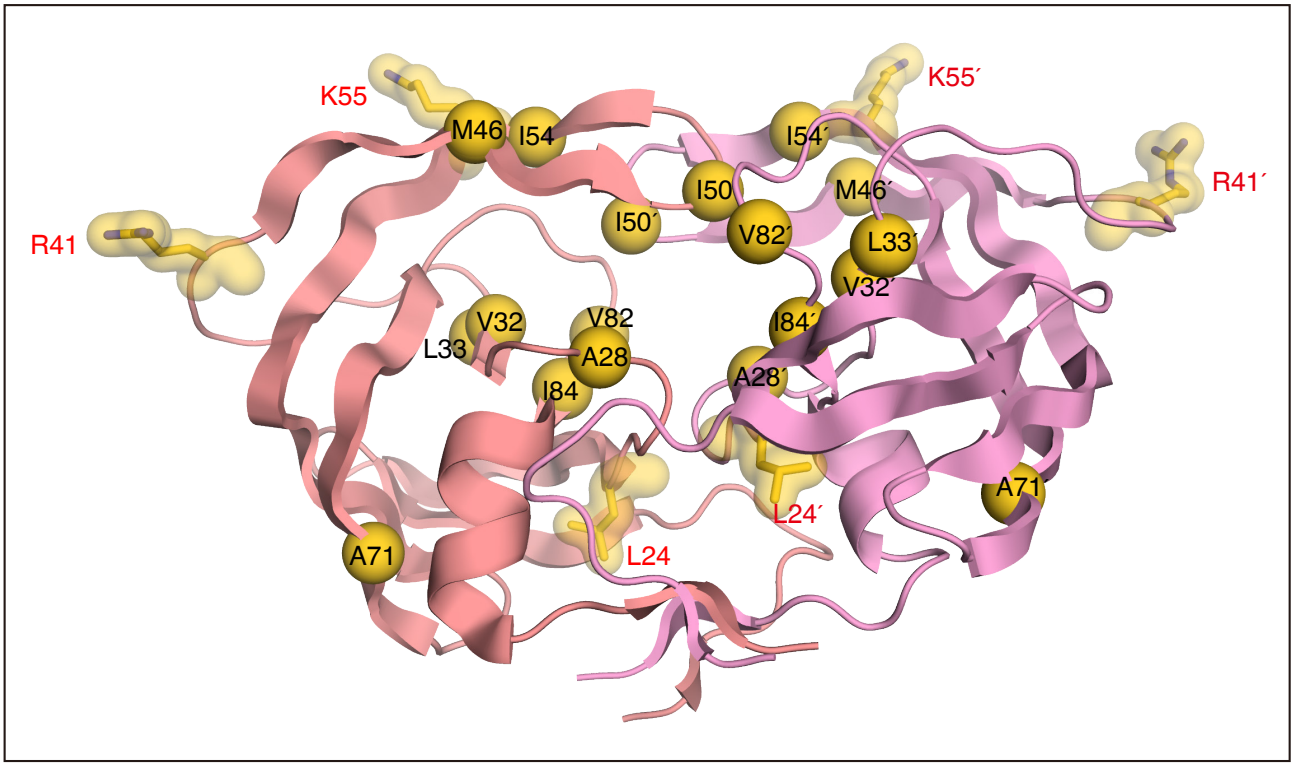

Figure S3. The locations of the amino acid substitutions often associated with HIV-1 resistance to PIs. Three relatively unique amino acid substitutions identified in the present study (L24M, R41T, and K55N) are also illustrated.

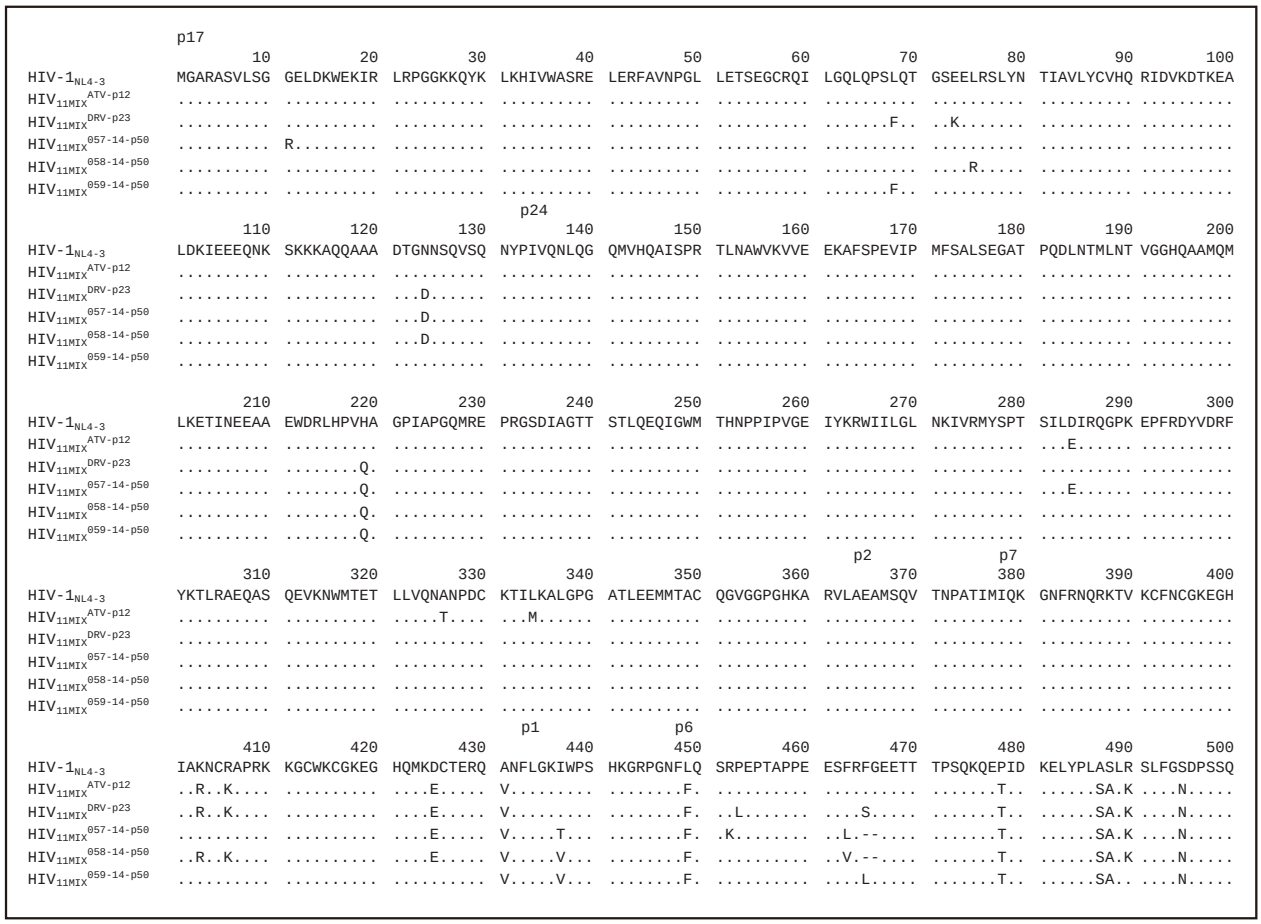

Figure S4. Amino acid sequences of the Gag-encoding region of the virus. The consensus HIV-1_{NL4-3} sequence is displayed at the top of the figures as a reference. Identity at individual amino acid positions is indicated by dots.

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

	EC_{50} (nM) against	
Compound	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 EC_{50} (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.