

Supplementary Tables and Figures

Table S1: HIV-1 subtype B and C lab-adapted strains.

HIV-1 Subtype	Strain	GeneBank accession number	Publication
B	DS9	MH234640	Obasa <i>et al.</i> , 2019 [64]
	LTNP5	AY835779	Mikhail <i>et al.</i> , 2005 [65]
	SM1	KY060873	Ledwaba <i>et al.</i> , 2019 [66]
	SM2	KF469956	Maldarelli <i>et al.</i> , 2013 [67]
C	CM9	AF411967	Papathanasopoulos <i>et al.</i> , 2002 [68]
	DU151	AF544009	Williamson <i>et al.</i> , 2003 [69]
	DU179	AY043174	van Harmelen <i>et al.</i> , 2001 [70]
	DU422	AF544005	Williamson <i>et al.</i> , 2003 [69]

Table S2: SDM primers used to generate the single and combination NNRTI profiles.

DRM Profile	Primer Name*	Mutation	Sequence (5' → 3')**	optimised T _m (°C)
A98G, K103N, P225H	A98G, K103N forward	A98G	AGGGTTAAAAAAGAATAAATCAGTAACAGTATTAG	63
A98G, K103N	A98G, K103N reverse		CCTGGGTGCGGTATTCCTAATTGAAC	63
L100I, K103N	L100I, K103N forward	L100I	AAAAAAGAATAAATCAGTAACAGTATTAGATGTG	56.9
L100I, K103N, P225H	L100I, K103N reverse		ATCCCTGCTGGGTGCGGTATTCCTAA	55.1
K101P, K103N	K101P, K103N forward	K101P	AAAGAATAAATCAGTAACAGTATTAGATGTGGGG	61.3
	K101P, K103N reverse		GGTAACCCTGCTGGGTGCGGTATTCC	
K103N, V106M, F227L	K103N, V106M forward	K103N	TAAATCAATGACAGTATTAGATGTGGGGGATG	63
K103N, V106M	K103N, V106M reverse	V106M	TTCTTTTTTAACCCTGCTGGGTGCGG	62
K101E, V106M, G190A	V106M forward	V106M	GACAGTATTAGATGTGGGGGATGCA	64.4
	K101E, V106M reverse		ATTGATTTTTTCTTCTCTAACCCTGC	
K103S, V106M	K103S, V106M reverse		ATTGATTTGCTTTTTTTAACCCTGC	58.3
K103N, V108I	K103N, V108I forward	V108I	ATTAGATGTGGGGGATGCATATTTTTTC	63.2
K103N, V108I, P225H	K103N, V108I reverse		ATTGTTACTGATTTATTCTTTTTTTAACCCTG	63.2
K103N, E138A	E138A forward	E138A	AATAGTCATCTATCAATATATGGATGATTTGTATG	59.6
	E138A reverse		GCTGGATTTTGTGTCCTAAAGGGCTC	
K103N, Y188L	Y188L forward	Y188L	AGTAGGATCTGATTTAGAAATAGGGC	54
V106M, Y188L	Y188L reverse		AACAAATCATCCATATATTGATAGATGACTATTTTC	55
V179D, Y188L	V179D, Y188L reverse		AACAAATCATCCATATATTGATAGATGTCTATTTTC	57
V106M, G190A	G190A forward	G190A	ATCTGATTTAGAAATAGGGCAACATAG	54
V106M, G190A, F227L	G190A reverse		GCTACATACAAATCATCCATATATTGATAGATG	54
V106M, V179D, F227L	F227L forward	F227L	ACTTTGGATGGGGTATGAACTCCATC	62.7
	F227L reverse		AATGGGGGTTCTTTCTGATGTTTCTTGTC	

* Some sets of primers were occasionally used to introduce more than one nucleotide change

** The nucleic bases that were changed to introduce mutations (■)

Table S3: SDM primers used to generate single mutants in lab-adapted strains.

Mutation	Primer direction	Sequence (5' → 3')***	optimised T _m (°C)
V106M*	forward	CCACATCCAGTACTGT C ATTGATTTTTTC TTTTTTAA	58.2
	reverse	TTAAAAAAGAAAAAATCA A T G ACAGTACT GGATGTGG	
V106M** in NSX	forward	G ACAGTACTGGATGTGGGCG	59
	reverse	A TTGATTTTTTCTGTTTTAACCTGCAGG	
F227L**	forward	A CTTTGGATGGGTTATGAACTCCATC	58
	reverse	AATGGAGGTTCTTTCTGATGTTTTTTGTC	

*SDM with overlapping primers was performed

**SDM with non-overlapping primers was performed

***The nucleic bases that were changed to introduce mutations are highlighted (■)

Table S4: IC₅₀ values and FC for the single NNRTI mutations.

Mutation	Average IC ₅₀ (standard deviation) (µM)		Average Fold Change (standard deviation)		p-value**
A98G	0.017	(0.000)	2.99	(0.02)	<0.0001
L100I	0.008	(0.003)	1.36	(0.50)	0.188
K101E	0.010	(0.003)	1.79	(0.51)	0.114
K101P	0.005	(0.001)	0.85	(0.20)	0.465
K103N	0.005	(0.000)	0.96	(0.02)	0.274
K103S	0.007	(0.002)	1.23	(0.29)	0.300
V106M	0.097	(0.030)	17.33	(5.31)	0.001
V108I	0.016	(0.001)	2.84	(0.21)	0.046
E138A	0.009	(0.001)	1.56	(0.16)	0.119
V179D	0.004	(0.000)	0.64	(0.03)	<0.0001
Y181C	0.002	(0.000)	0.35	(0.07)	0.014
Y188L	0.500*	(0.000)	89.16	(0.00)	<0.0001
G190A	0.014	(0.003)	2.52	(0.55)	0.041
P225H	0.022	(0.009)	3.86	(1.60)	0.090
F227L	0.008	(0.001)	1.34	(0.24)	0.031
MJ4	0.006	(0.002)	1.00	(0.33)	-

*IC₅₀ values exceed the maximum concentration tested which was 0.5 µM

**an unpaired Student's t-test test compared the FC of each mutant to MJ4 and the significant p-values are bolded

*** each mutant was tested in at least two independent assays in duplicate

Table S5: IC₅₀ values and FC for V106M in subtype B and C lab-adapted strains.

Mutation	Lab Adapted Strain	Average IC ₅₀ (standard deviation) (μ M)		Average Fold Change (standard deviation)		p-value*
V106M	DS9	0.044	(0.005)	13.40	(1.66)	0.006
	LTNP5	0.101	(0.009)	30.69	(2.75)	0.003
	SM1	0.068	(0.014)	20.55	(4.31)	0.016
	SM2	0.089	(0.019)	26.93	(5.63)	0.015
	NSX	0.042	(0.006)	12.69	(1.86)	0.008
	NSX WT	0.003	(0.003)	1.00	(0.33)	-
V106M	CM9	0.132	(0.013)	23.59	(2.36)	0.004
	DU151	0.043	(0.004)	7.73	(0.64)	0.003
	DU179	0.211	(0.051)	37.67	(9.02)	0.020
	DU422	0.109	(0.023)	19.46	(4.03)	0.016
	MJ4	0.097	(0.035)	17.33	(5.31)	0.0007
	MJ4 WT	0.006	(0.002)	1.00	(0.33)	-

* an unpaired Student's t-test test compared the FC of each lab-adapted strain to the subtype wild-type reference and the significant p-values are bolded

** each lab strain was tested in at least two independent assays in duplicate

Table S6: IC₅₀ values and FC for F227L in subtype B and C lab-adapted strains.

Mutation	Lab Adapted Strain	Average IC ₅₀ (standard deviation) (μ M)		Average Fold Change (standard deviation)		p-value*
F227L	DS9	0.004	(0.0003)	1.30	(0.11)	0.013
	LTNP5	0.012	(0.003)	3.75	(1.01)	0.040
	SM1	0.008	(0.001)	2.45	(0.17)	<0.0001
	SM2	0.013	(0.001)	3.90	(0.35)	0.002
	NSX	0.003	(0.001)	0.97	(0.17)	0.824
	NSX WT	0.003	(0.003)	1.00	(0.33)	-
F227L	CM9	0.008	(0.001)	1.35	(0.11)	0.023
	DU151	0.005	(0.001)	0.91	(0.11)	0.290
	DU179	0.001	(0.000)	0.26	(0.07)	<0.0001
	DU422	0.004	(0.001)	0.69	(0.14)	0.053
	MJ4	0.008	(0.001)	1.34	(0.24)	0.0307
	MJ4 WT	0.006	(0.002)	1.00	(0.33)	-

* an unpaired Student's t-test test compared the FC of each lab-adapted strain to the subtype wild-type reference and the significant p-values are bolded

** each lab strain was tested in at least two independent assays in duplicate

Table S7: IC₅₀ values and FC for combination NNRTI mutations.

Mutation	Average IC ₅₀ * (standard deviation) (μ M)		Average Fold Change (standard deviation)		p-value**
A98G,K103N	0.045	(0.013)	8.1	(2.3)	0.032
A98G,K103N,P225H	0.451	(0.083)	80.5	(14.8)	0.011
L100I,K103N	0.035	(0.008)	6.3	(1.5)	0.026
L100I,K103N,P225H	0.164	(0.057)	29.3	(10.2)	0.012
K101P,K103N	0.005*	(0.001)	0.8	(0.1)	0.104
K103N,V106M	0.459	(0.070)	81.9	(12.5)	0.008
K103N,V106M,F227L	0.500*	(0.000)	89.2	(0.0)	<0.0001
K103N,V108I	0.016	(0.002)	2.8	(0.3)	0.067
K103N,V108I,P225H	0.098	(0.001)	17.5	(0.3)	0.006
K103N,E138A	0.007	(0.001)	1.2	(0.2)	0.334
K103N,Y188L	0.500*	(0.000)	89.2	(0.0)	<0.0001
K103N,P225H	0.054	(0.012)	9.7	(2.2)	0.020
K101E,V106M,G190A	0.500*	(0.000)	89.2	(0.0)	<0.0001
K103S,V106M	0.500*	(0.000)	89.2	(0.0)	<0.0001
V106M,V179D	0.037	(0.002)	6.7	(0.3)	0.025
V106M,V179D,F227L	0.500*	(0.000)	89.2	(0.0)	<0.0001
V106M,Y188L	0.500*	(0.000)	89.2	(0.0)	<0.0001
V106M,G190A	0.478	(0.038)	85.3	(6.7)	0.002
V106M,G190A,F227L	0.500*	(0.000)	89.2	(0.0)	<0.0001
V179D,Y188L	0.500*	(0.000)	89.2	(0.0)	<0.0001
A98G,K103N	0.006	(0.002)	89.2	(0.0)	0.032
MJ4	0.045	(0.013)	1.00	(0.3)	0.011

* IC₅₀ values exceed the maximum concentration tested which was 0.5 μ M

** an unpaired Student's t-test test compared the FC of each mutation profile to wild type subtype C (MJ4) and the significant p-values are bolded

*** each combination mutation was tested in at least two independent assays in duplicate