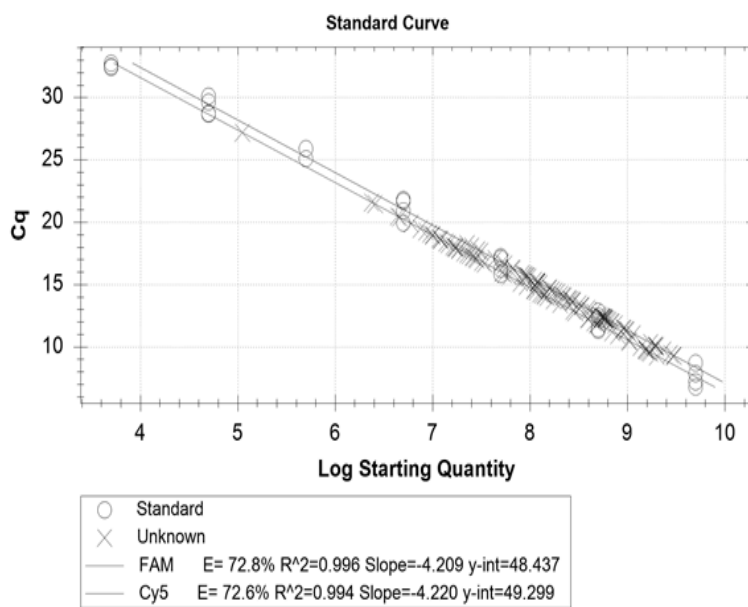
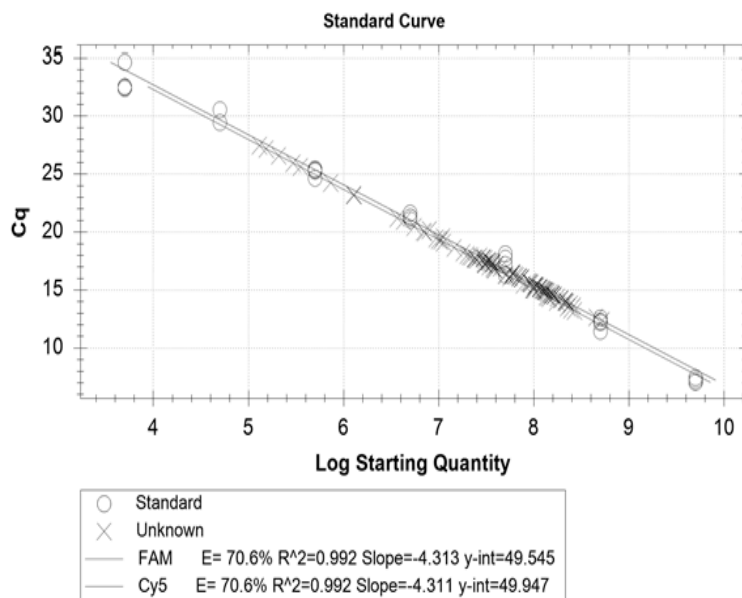


Supplemental Figure 1.
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Supplemental Table 2.

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Average relative mutant frequency							
RT variant	Number of replicates	Mean	Std. Deviation	Std. Error	Lower 95% CI	Upper 95% CI	P-value
wild type	8	1.000	N/A	N/A	N/A	N/A	N/A
A62V	8	1.334	0.0711	0.0214	1.195	1.489	*
K65R	8	0.658	0.0863	0.0273	0.571	0.759	***
L74V	8	0.928	0.0416	0.0132	0.866	0.994	n/s
Y115F	8	1.205	0.0592	0.0171	1.105	1.313	*
Y115A	4	4.203	0.1775	0.0888	2.193	8.056	***
V148I	4	0.510	0.2782	0.1391	0.184	0.707	***
M184I	8	0.605	0.0862	0.0305	0.513	0.714	***
M184V	8	0.749	0.0790	0.0279	0.644	0.872	*
K65R/M184V	5	0.685	0.0805	0.0360	0.544	0.863	**
L74V/Y115F/M184V	8	0.780	0.0941	0.0284	0.682	0.893	*

N/A is not applicable; n/s is not significant. * P value < 0.05; ** P value < 0.01; *** P value < 0.001

Supplemental Table 3.
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Fitness difference (W_D) relative to reference strain

	Replicate number						Mean	STD	Dunnett's Multiple Comparison Test		
	1	2	3	4	5	6			Mean diff.	95% CI of diff.	P-value
wild type	0.888	0.918	1.220	1.070	0.956	0.904	0.993	0.129	N/A	N/A	N/A
A62V	0.202	0.442	1.052				0.565	0.438	0.427	-0.077 to 0.504	n/s
K65R	0.036	0.614	0.058				0.236	0.327	0.757	0.088 to 0.669	**
L74V	0.874	0.502	1.628				1.001	0.574	0.009	-0.295 to 0.286	n/s
Y115F	0.448	1.142	0.860				0.817	0.349	0.176	-0.203 to 0.369	n/s
Y115A	0.0045	0.0013	0.0023				0.003	0.002	0.990	0.204 to 0.786	***
V148I	0.051	0.068	0.015				0.045	0.027	0.948	0.183 to 0.765	***
M184I	0.242	0.033	0.033				0.103	0.121	0.890	0.154 to 0.736	**
M184V	0.692	0.504	0.904				0.700	0.200	0.293	-0.144 to 0.437	n/s
K65R/M184V	0.085	0.614	0.342				0.347	0.265	0.646	0.032 to 0.614	*
L74V/Y115F/M184V	1.132	0.758	1.262				1.051	0.262	0.058	-0.320 to 0.262	n/s

N/A is not applicable; n/s is not significant. * P value < 0.05; ** P value < 0.01; *** P value < 0.001

Table S1. Summary of fitness, processivity, and fidelity measures for HIV-1 RT variants used in this study.

RT variant	Location	Resistance Phenotype	Effect on Fitness	Assay	Effect on Processivity	Effect on Fidelity	Assay	Citation	
A62V	Finger subdomain	Q151M complex	No Change	Parallel growth				1	
K65R	Finger subdomain	ABC, ddI, ddC, 3TC, FTC, TDF	↓ (29-fold)	Dual competition				1	
			↓ (40%)	Single-cycle RC			2		
			No Change	Parallel growth			3		
			↓ (47%)	Single-cycle RC	↓ (31%)			4	
								5	
						↑ (8.1-fold)	<i>In vitro</i> FMA		6
			↓ (80%)	Single-cycle RC: patient samples					7
			↓ (15%)	Dual competition					8
						↑ (3.3-fold)	<i>In vivo</i> single-cycle FMA		9
L74V	Finger subdomain	ABC, ddI	↓ (11%)	Parallel growth				10	
			↓	Parallel growth	↓			10	
			↓ (2-fold)	Dual competition				11	
			No Change	Parallel growth	↓			3	
								5	
						↑ (1.7-fold)	<i>In vitro</i> FMA		6
						↑ (2-6-fold)	Gel-based assay		12
						↑ (3.5-fold)	<i>In vitro</i> FMA		13
						No Change	<i>In vivo</i> single-cycle FMA		9
					No Change	Parallel growth			1
Y115F	dNTP binding site	ABC			No change			14	
					No Change	<i>In vitro</i> FMA	15		
					No Change	<i>In vivo</i> single-cycle FSA	16		
Y115A	dNTP binding site	N/A			↓			14	
								15	
			↓ (100%)	Parallel growth				17	
					↓ (4.0-fold)	<i>In vitro</i> FMA		13	
					↓ (2.3-fold)	<i>In vivo</i> single-cycle FMA		9	
V148I	Finger subdomain	N/A				↑ (8.1-fold)	<i>In vitro</i> FMA	18	
						↑ (8.7-fold)	<i>In vitro</i> FMA	19	
						↑ (3.33-fold)	<i>In vivo</i> single-cycle FMA	19	
M184I	dNTP binding site	ABC, 3TC, FTC	↓ (vs M184V)	Parallel growth	↓ (83%-49%)			20	
			↓ (11.6-21.9%)	<i>In vivo</i> reversion				21	
			↓ (10-fold)	Dual competition				22	
			↓ (5-fold)	Single-cycle RC				23	
			No Change	Single-cycle RC				24	
			↓ (30%)	Single-cycle RC	↓			25	
								26	
			↓ (0-99.5%)	Single-cycle RC		↑ (4-fold)	<i>In vitro</i> FMA		27
								28	
M184V	dNTP binding site	ABC, 3TC, FTC	↓	Parallel growth	↓ (75%-33%)			20	
			↓ (11.6-21.9%)	<i>In vivo</i> reversion				21	
			↓ (14-fold)	Dual competition				11	
			↓ (4-fold)	Single-cycle RC				23	
			↓ (35%)	Single-cycle RC	↓			2	
								5	
			No Change	Single-cycle RC				24	
			↓ (4-8%)	<i>In vivo</i> reversion	↓			29	
							↑ (1.2 fold)	<i>In vitro</i> FMA	13
						↓			26
							↑ (1.6-fold)	<i>In vitro</i> FMA	27
							↑ (1.6-fold)	<i>In vitro</i> FMA	30
↓ (3-6.2 fold)	Parallel growth					31			
				↑ (1.3-fold)	<i>In vivo</i> single-cycle FMA	32			
K65R/M184V		ABC, 3TC, FTC	↓ (65%)	Single-cycle RC				2	
			↓ (76%)	Single-cycle RC	↓ (69%)			4	
L74V/Y115F/M184V		ABC				↓ (1.91-fold)	<i>In vivo</i> single-cycle FSA	16	

Q151M multidrug resistance complex consists of A62V/V75I/F77L/F116Y/Q151M. ABC = Abacavir; ddI = Didanosine; ddC = Zalcitabine; FTC = Emtricitabine; 3TC = Lamivudine; TDF = Tenofovir. N/A means not applicable. Parallel growth describes fitness assay that replicated wt and mutant HIV-1 clones in separate cultures. Dual competition describes fitness assay that replicated wt and mutant HIV-1 clones together in co-culturing conditions. Single-cycle RC, replicative capacity, describes assay that compares transduction efficiency of wt and mutant HIV-1 vectors after one round of replication. *In vivo* reversion describes a fitness measure that

takes into account the reversion time of a particular mutant back to wt after therapy is withdrawn. *In vitro* FMA describes a cell-free forward mutation assay. Gel-based assay describes a cell-free assay that compares the ability of wt and mutant RTs to insert and extend mispairs on a specified template. *In vivo* single-cycle FMA is a cell-based forward mutation assay. *In vivo* single-cycle FSA is a cell-based frame shift detection assay.

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