TABLE S1 Summary of the oligonucleotide primers used in this study*

A. Reverse Transcription-PCR Primers

Position$_{HXB2}$	**Name**	**Sequence**
2703→2722	RT_Sense	CCTGAAAATCCATACAATAC
3786←3804	RT_Antisense	TATTGACAAACTCCCACTC

B. AS-PCR Primers

Position$_{HXB2}$	**Name**	**Sequence**
2942→2960	138_Total	CATACCTAGTATAAACAAT
2942→2962	138A_GC	CATACCTAGTATAAAC**TT**T**GC**
	138G_GG	CATACCTAGTATAAAC**TC**T**GG**
	138K_AA	CATACCTAGTATAAAC**TG**T**AA**
	138Q_CA	CATACCTAGTATAAAC**TC**T**CA**
	138R_CG	CATACCTAGTATAAAC**TC**T**AG**
	138V_GT	CATACCTAGTATAAAC**TC**T**GT**
3212←3230	138_Antisense	GAATGGAGGTTCTTTCTGA

*Sequences are provided 5′ to 3′. Bolded nucleotides were intentionally mismatched; bolded and underlined nucleotides base-pair with the specified mutant codon.

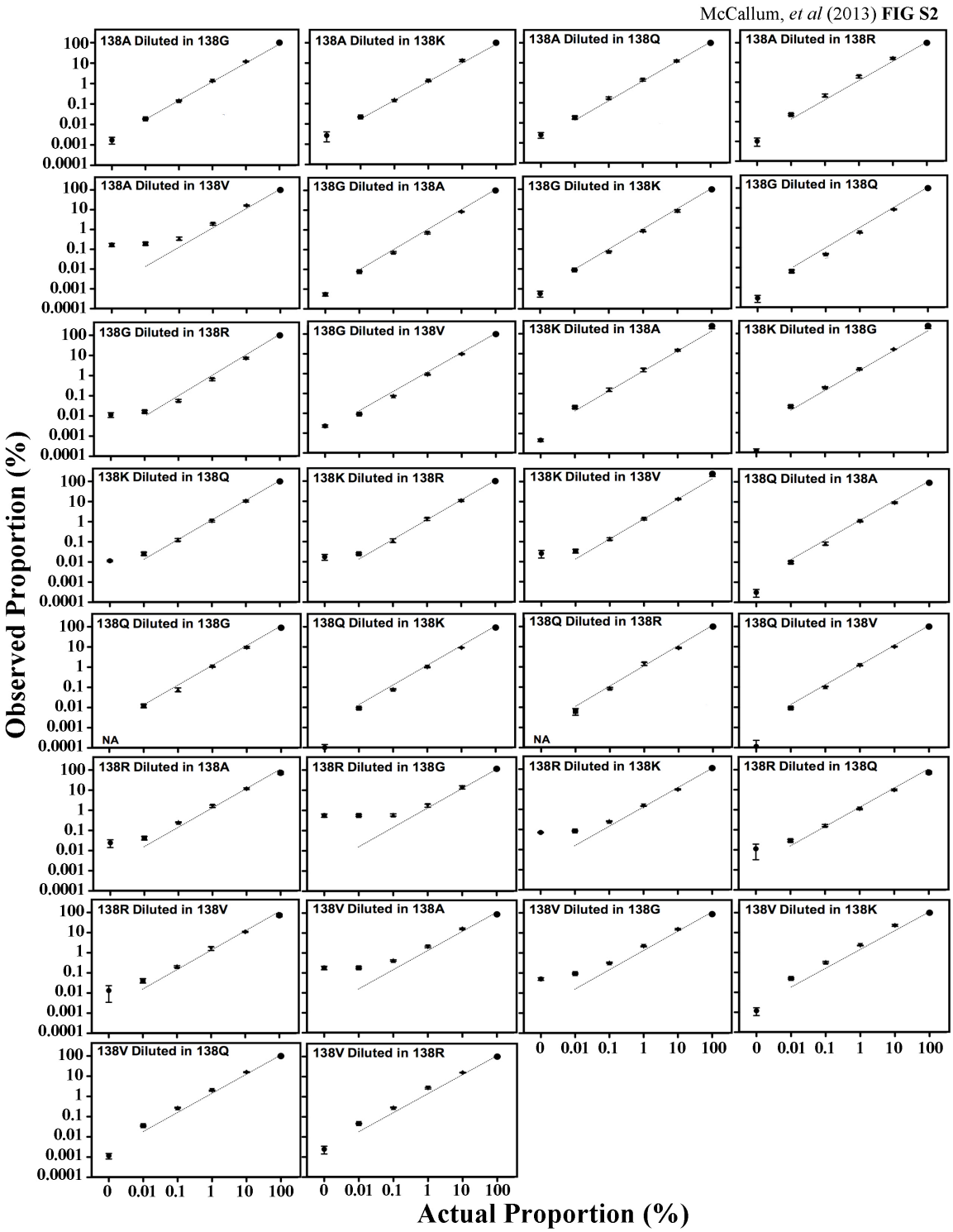

FIG S2 E138 drug resistance substitutions serially diluted in other E138 drug resistance substitutions and measured by AS-PCR. DNA used for dilutions are PCR amplicons from pNL4-3 vector containing specified mutations. Data are representative of the mean±SEM of two independent trials performed in triplicate. (Dotted line) equivalence between actual and observed proportion. (NA) the PCR reaction with the allele-specific primer failed to amplify.

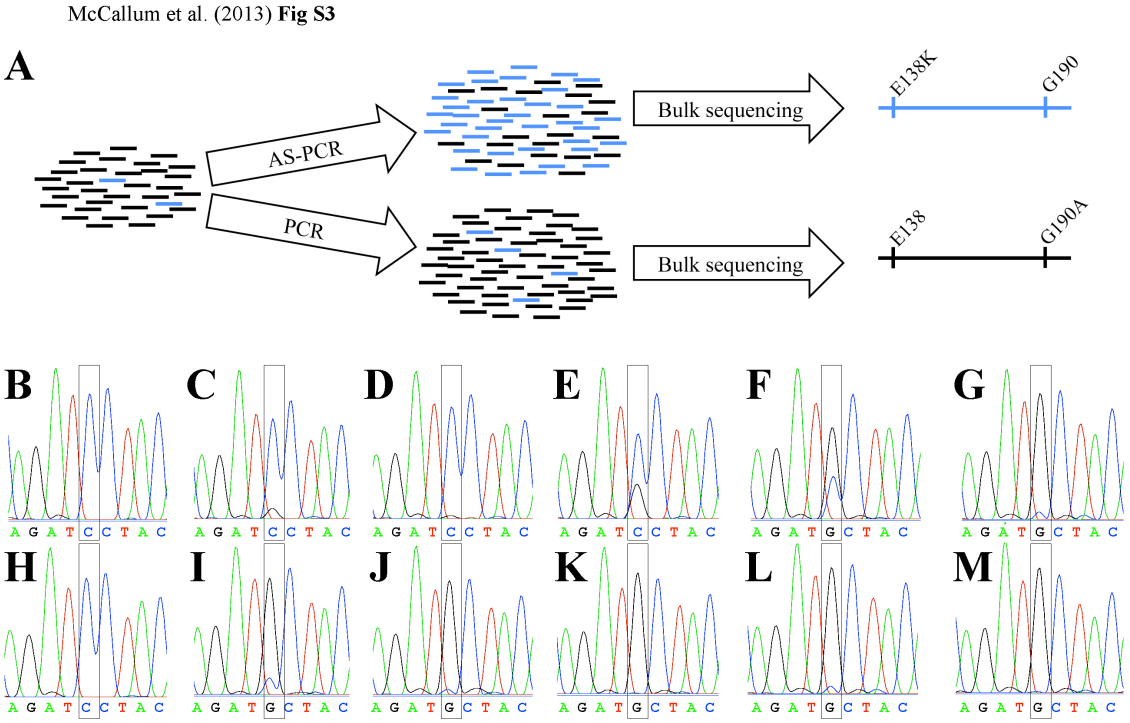

FIG S3 Sequencing the amplicon of AS-PCR reactions with the antisense primer demonstrating that this method is not susceptible to RT-PCR recombination events. (A) Schematic representation of the method; horizontal bars represent HIV-1 DNA and genetically linked mutations. (B-M) Viruses containing G190 and the E138K substitution were serially diluted in viruses with E138 and the G190A substitution; after RT-PCR and AS-PCR, the PCR amplicon was sequenced with the 138_Antisense primer. (B-G) AS-PCR was performed with the 138K_AA AS-primer preceding sequencing. (H-M) AS-PCR was performed with the 138_Total non-allele-specific primer preceding sequencing. The viral dilution series with E138K virus diluted in G190A virus was as follows: (B & H) 100% E138K; (C & I) 10% E138K; (D and J) 1% E138K; (E and K) 0.1% E138K; (F and L) 0.01% E138K, (G and M) 0% E138K. Note that the G190 anticodon is TCC and the G190A anticodon is TGC.

TABLE S4 Sequencing of the E138K AS-PCR from etravirine selection experiments

Isolate	Passage	E138K Proportion (%)*	Not Allele-Specific	E138K-Linked
5326	8	1	None	E138K
	12	30	E138E/K, Y181Y/C, V189V/I	E138K, V189I
	16	20	E138E/K, Y181C	E138K, Y181C
	25	100	E138K, V179D, Y181C	E138K, V179D, Y181C
5331	5	0.1	None	E138K
	8	0.9	None	E138K
	12	20	E138E/K, V189V/I	E138K, V189V/I
	16	40	E138K/E, V189I/V	E138K, V189I/V
	25	100	E138K, V189I	E138K, V189I
8116	12	1	G190A	E138K, G190A
	16	14	G190A	E138K, G190A
	25	100	E138Q/K, G190A	E138K, G190A
8336	5	3	G190A	G190A
	8	30	E138E/K, G190A	E138K, G190A
	12	100	E138K, G190A	E138K, G190A
	16	80	E138K, G190A	E138K, G190A
	25	100	E138K, G190A	E138K, G190A
BG-05	5	0.2	None	E138K
	8	8	None	E138K
	12	80	E138K/E, Y181Y/C	E138K
	16	30	E138E/K, Y181C	E138K, Y181C
	24	100	E138K, Y181C	E138K, Y181C

*Proportion corrected based on fold decrease in observed proportion due to primer mismatches

TABLE S5 Mutations specifically enriched with E138K by sequencing of allele-specific PCR amplicon from six drug-naïve plasma samples testing positive for E138K

Subtype (Patient)	Measured Proportion of E138K (%)	Nucleotide Mutations*				Corresponding E138K-Linked Substitutions
		$Position_{HxB2}$	Total		E138K-Linked	
B(4)	0.4	2972	G/A		G	Silent
		2993	G		G/A	Silent
		3050	C		C/T	Silent
		3125	C		C/T	Silent
B(15)	0.1	2972	G/A		A	Silent
B(18)	0.08	3026	A/C		A	Silent
		3046	A/G		A	K/R166K
		3118	G/C		G	G/A190G
		3161	G/A		A	Silent
B(21)	0.09	3002	G/A		A	Silent
B(22)	0.1	3035	C/T		C	Silent
C(1)	0.2	3041	G	→	A	M164I
		3065	G	→	A	Silent
		3098	T/C		C	Silent
		3102	G	→	A	D185N
		3105	G	→	A	D186N
		3110	G	→	A	Silent
		3118	G	→	A	G190E
		3123	G	→	A	D192N
		3129	G	→	A	E194K
		3135	G	→	A	G196K
		3136	G	→	A	G196K
		3159	G	→	A	E204K
		3166	G	→	A	R206K
		3168	G	→	A	E207K

*Only mutations between position 2963 to ~3160—the region amplified during AS-PCR—were screened. All mutations linked to E138K are not reported; simply the mutations that were different between amplicons produced with non-allele specific primer 138_Total and the AS-primer E138K_AA have been reported. A slash indicates that the mutation is in a mixture with the species on the left of the slash in greater proportion that that on the right. Also, arrows highlight G→A mutations.