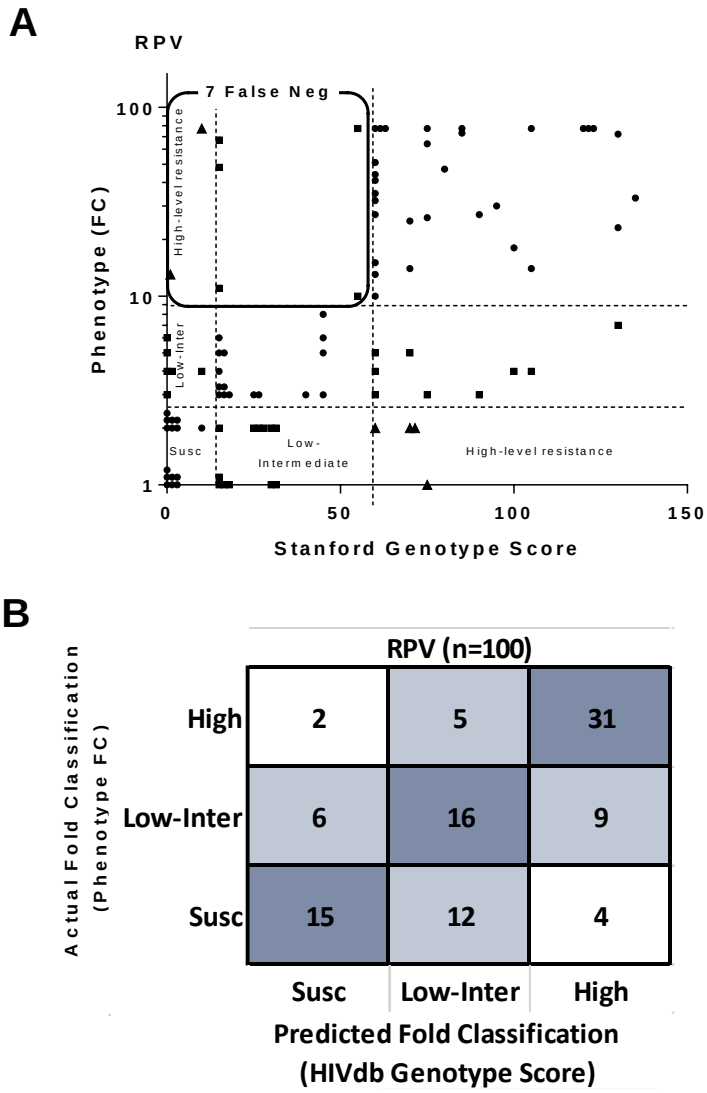
Supplemental Methods

The RPV and DPV genotyping and phenotyping data used in this analysis has been described previously[1,2]. HIVdb genotypic interpretation algorithms scores in the supplemental material were determined using the Stanford HIVdb resistance interpretation algorithm version 8.4 (HIVdb v8.4)[3] by uploading HIV-1 population genotyping FASTA files into the HIVdb website (https://hivdb.stanford.edu/hivdb/by-sequences/). In Supplemental Figure 1, we used the RPV conventional biological cutoff (BCO) of 2.5-fold as previously described[24] for 'low-intermediate resistance' and >10 fold as 'high level resistance' for comparison to HIVdb v8.4. These cutoff values are not based on clinical data, as with etravirine, because these data are unavailable. In Supplemental Figure 2A and B we reanalyzed our genotyping ETR weight using the Tibotec system[4] and compared it to the HIVdb v8.4 (Supplemental Figure 3) using Pearson's correlation coefficient calculated with Graph pad Prism version 7. To validate our findings on the association between ETR resistance and the K65R mutation, we accessed a genotypic and phenotypic dataset previously published by Melikian et al. 2012[5] accessed through the Stanford RT Phenotype Query (Supplemental Table 4A). In addition, there was no significant association between these NRTI mutations and RPV or DPV resistance (Supplemental Figure 3B)[1,2].

Supplemental References

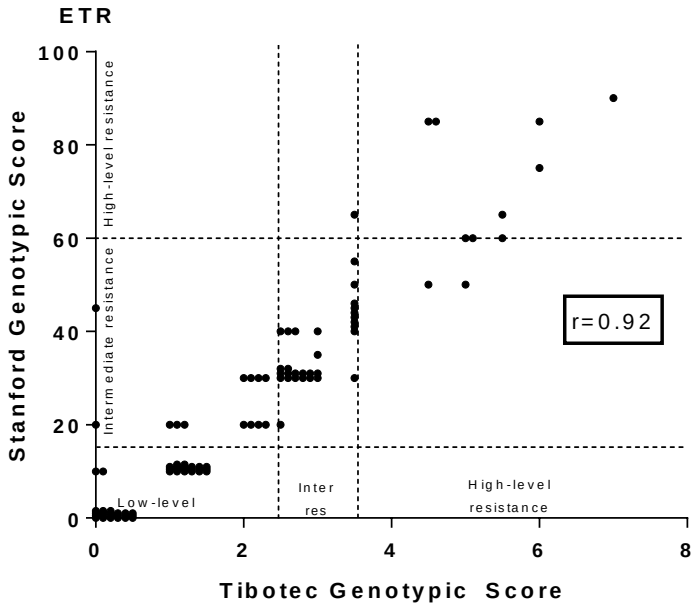
1. Penrose, K. J. *et al.* Frequent Cross-Resistance to Dapivirine in HIV-1 Subtype C-Infected Individuals on Failing First-Line Antiretroviral Therapy in South Africa. *Antimicrob. Agents Chemother.* AAC.01805-16 (2016). doi:10.1128/AAC.01805-16
2. Penrose, K. J. *et al.* Frequent cross-resistance to rilpivirine among subtype C HIV-1 from first-line antiretroviral therapy failures in South Africa. *Antivir. Chem. Chemother.* **26**, 204020661876298 (2018).
3. Liu, T. F. & Shafer, R. W. Web Resources for HIV Type 1 Genotypic-Resistance Test Interpretation. *Clin. Infect. Dis.* **42**, 1608–1618 (2006).
4. Vingerhoets, J. *et al.* Tibotec Etravirine Weighted Genotype Score to Predict Responsetle. in *XVII HIV Drug Resistance Workshop* (2008).
5. Melikian, G. L. *et al.* Standardized Comparison of the Relative Impacts of HIV-1 Reverse Transcriptase (RT) Mutations on Nucleoside RT Inhibitor Susceptibility. *Antimicrob. Agents Chemother.* **56**, 2305–2313 (2012).

Supplemental Figures and Tables

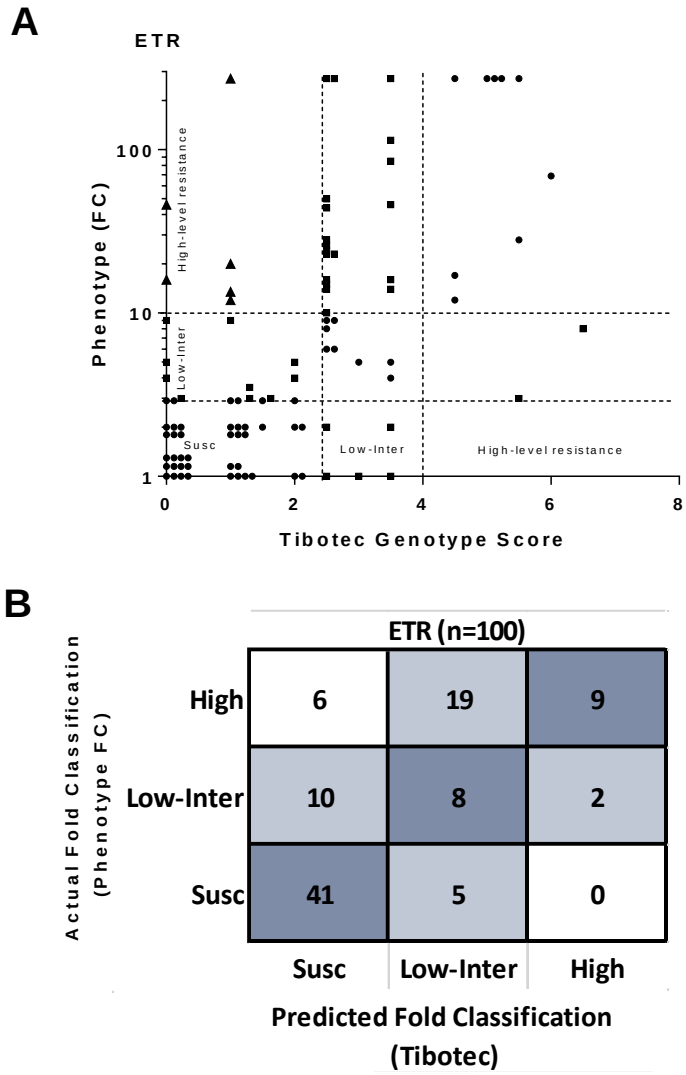


Supplemental Figure 1. RPV genotypic interpretation algorithm scores accurately predicted the RPV phenotype resistance in HIV-1 subtype C isolates. (A) RPV genotypic interpretation algorithm scores more accurately predicted the phenotype as 62% of samples were completely concordant (●) and only 32 were partially concordant (■). (B) Error matrixes of actual fold phenotypic resistance vs predicted resistance for RPV. Few samples with high phenotypic RPV resistance (FC >10) were misclassified as having low or intermediate resistance misclassified

samples (7/100). Genotypic interpretation algorithm scores were determined using the Stanford HIVdb v8.4.

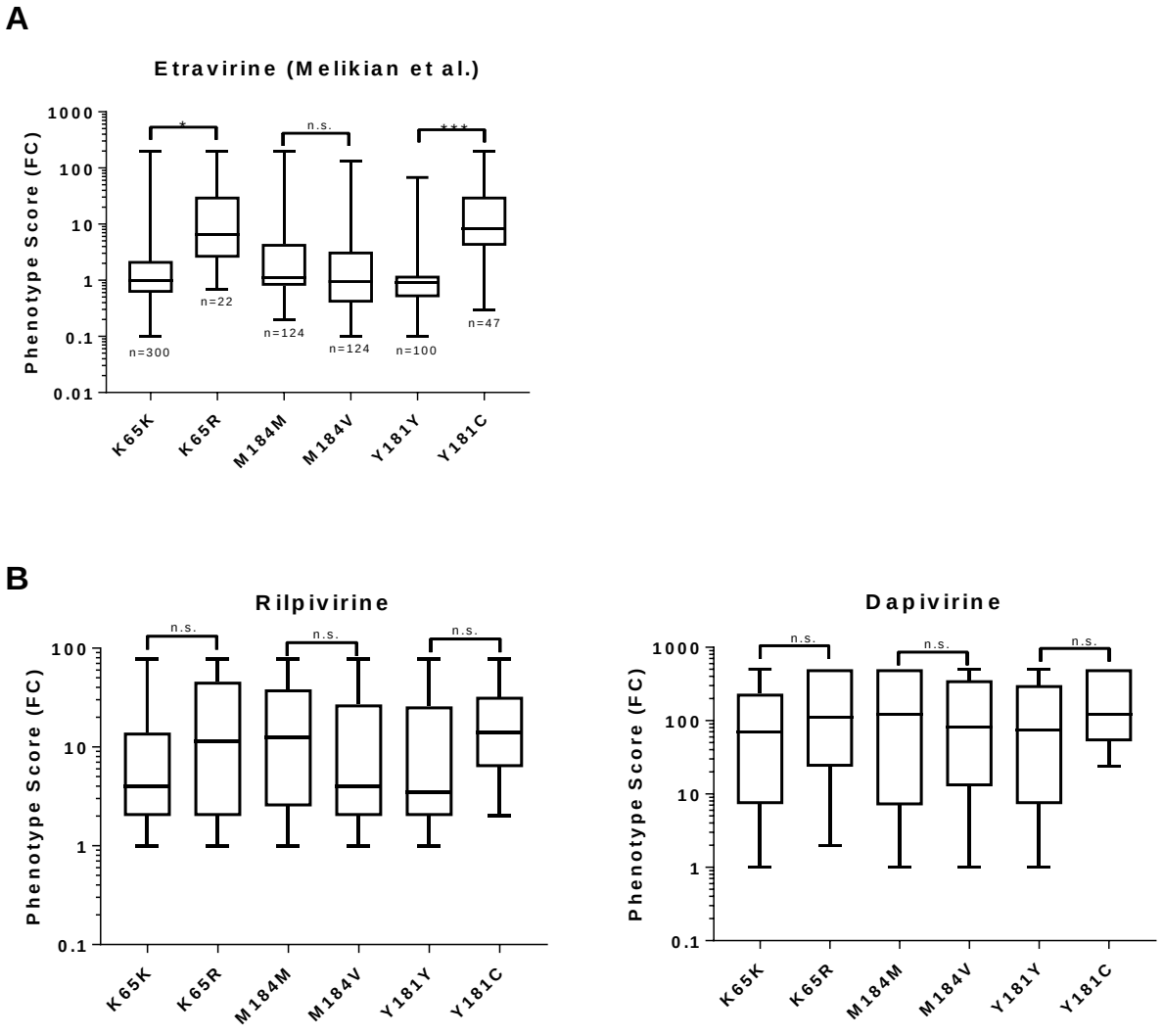


Supplemental Figure 2 Tibotec weighted scores significantly correlated with the HIVdb v8.4 (r=0.92) in HIV-1 subtype C isolates.



Supplemental Figure 3. The ETR phenotype and Tibotec weighted genotype scores are discordant in HIV-1 subtype C isolates (A) ETR Phenotype (Fold-Change in IC50) does not strongly correlate with Tibotec score (r=0.45) for HIV-1 subtype C isolates. 58% of genotype scores were concordant (●; classifications matched), 36% partially discordant (■; Tibotec predicted 1 classification different) and 6% completely discordant (▲; Tibotec predicted 2 classifications different) relative to the phenotype clinical cut-offs. (B) Error matrixes of actual

fold phenotypic resistance vs TIbotec predicted resistance for ETR. Tibotec failed to predict the ETR phenotype resistance in 42%.



Supplemental Figure 4. K65R is associated with ETR resistance, but not ETR NNRTI analogs such as RPV and DPV (A) K65R was associated with higher ETR phenotypic resistance in a previously published dataset (Melikian et al. 2012) obtained from the Stanford RT Phenotype Query. *p<0.05, ***p<0.005. (B) Fold-change in Rilpivirine (RPV) or Dapivirine (DPV) phenotype resistance was evaluated based on the presence or absence of K65R, M184V or Y181C.

*n.s.= "no signifigant difference using Fisher's Exact".*There was no significant association between viruses in our dataset containing K65R and phenotypic resistance to RPV or DPV.

Supplemental Table 1. ETR susceptibility in recombinant HIV-1 virus clones containing K65R or reversion to 65K.

Sample	Sample Type	ETR IC50 (nM)	Mutations		
			NRTI	NNRTI	Other
Wild-type	Batch	1.2	None	None	none
Sample 1	Batch	17.5	M41LM, K65R, M184V	V106M, E138A, V179D	T39DE, K103KR, I135IL
	Single 65R clone 1	2.0	M41L, K65R, M184V	V106M, E138A, V179D	T39D, K102R
	65R reverted clone 1	1.9	M41L, M184V	V106M, E138A, V179D	T39D, K102R
	Single K65R clone 2	74.8	M41M, K65R, M184V	V106M, E138A, V179D	T39E, K103R, I135L
	65R reverted clone 2	76.0	M41M, M184V	V106M, E138A, V179D	T39E, K103R, I135L
Sample 2	Batch	29.8	A62V, K65R, M184I	V106M, V179D, M230L	none
	Single clone 1	41.7	A62V, K65R, M184I	V106M, V179D, M230L	none
	65R reverted clone 1	58.0	A62V, M184I	V106M, V179D, M230L	none

Supplemental Table 2. K65R is associated with the NNRTI mutations V179DFT, Y181CIV and M230L

NNRTI RAMs	K65R n=35 (%)	K65K n=77 (%)	P Value (Fisher's Exact)	P value summary
V90I	3 (9)	2 (3)	0.1752	*n.s.
A98G	2 (6)	12 (16)	0.2188	n.s.
L100I	7 (20)	6 (8)	0.1071	n.s.
K101EHP	5 (14)	8 (10)	0.5401	n.s.
V106I	1 (3)	1 (1)	0.5293	n.s.
E138AGKQ	5 (14)	9 (12)	0.7609	n.s.
V179DFT	9 (26)	5 (6)	0.0105	p<0.05
Y181CIV	10 (29)	6 (8)	0.0070	p<0.01
G190SA	9 (26)	18 (23)	0.8143	n.s.
M230L	8 (23)	3 (4)	0.0037	p<0.01

**n.s.= "no significant difference" using Fisher's Exact*