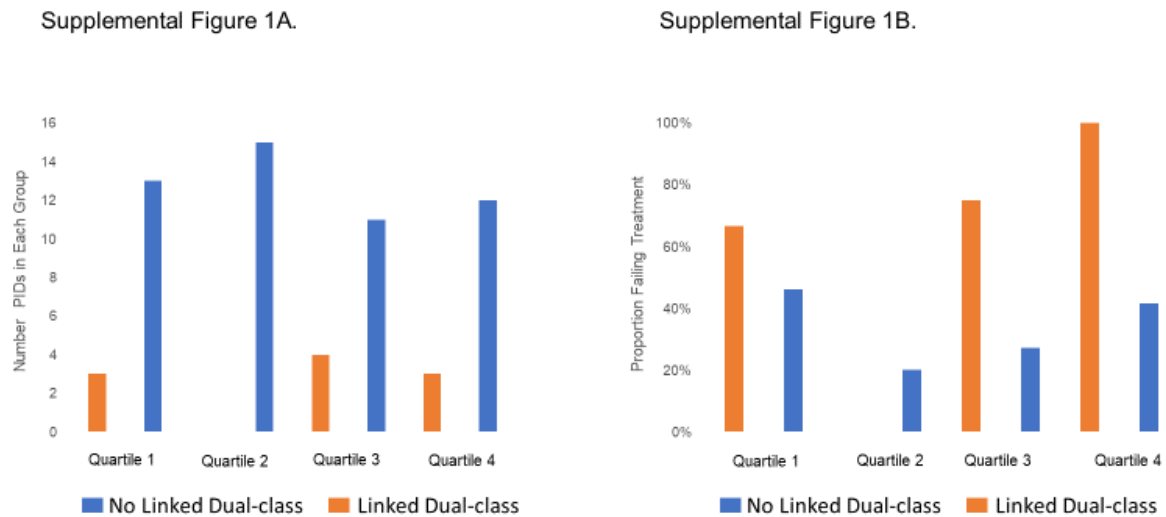


Supplemental Material:

Linked Dual-Class HIV Resistance Mutations Are Associated with Treatment Failure



Supplemental Figure 1. **Distribution of NVP DRM frequencies with and without linked dual-class linkage. (A)** Plotted on the Y axis are the number of samples in each of 2 categories: containing linked dual-class DRMs (orange bars) or not (blue bars). The X axis stratifies NVP resistant variant frequencies by quartiles. As shown, the distribution of linked dual-class resistance is independent of the frequency of NVP resistant variants. **(B)** Using the same classification (with and without dual-class resistance) and the same quartile distribution of NVP resistance frequency, the Y axis now shows the proportion failing treatment for each quartile. The proportions of treatment failure are higher for all quartiles having linked dual-class resistance.

Supplemental Table 1. Donor samples according to outcome, numbers of uSGS obtained and dual-class linkage of DRMs detected.

PID/Failure Outcome	#uSGS	Dual-class linkage	PID/non-Failure Outcome	#uSGS	Dual-class linkage
T1F01	12427		T1S11	341	
T1F02	696		T1S12	6080	
T1F03	4687	Yes	T1S13	25408	
T1F04	13747	Yes	T1S14	368	
T1F05	20327		T1S15	3951	
T1F06	3789		T1S16	8443	
T1F07	31531	Yes	T1S17	13865	
T1F08	27132	Yes	T1S25	157	
T1F09	16373		T1S26	13861	Yes
T1F10	3017		T1S27	3064	
T1F18	12692	Yes	T1S28	1958	
T1F19	46		T1S29	662	
T1F20	4190		T1S30	43321	
T1F21	3836		T1S31	2571	
T1F22	262		T1S32	1340	
T1F23	12155	Yes	T1S33	8063	
T1F24	3010		T1S34	2709	
T1F42	6165		T1S35	3450	
T1F43	11898		T1S36	2959	

T1F44	44124	Yes	T1S37	4023	
T1F45	273		T1S38	210	
T1F46	627		T1S39	2796	
T1F47	57278	Yes	T1S40	4998	Yes
T1F48	55327		T1S41	476	
T1F49	4531		T1S52	14016	
T1F50	23754		T1S53	8551	
T1F51	1191		T1S54	34653	
			T1S55	358	
			T1S56	28271	
			T1S57	45053	
			T1S58	20085	
			T1S59	4119	
			T1S60	46163	
			T1S61	21010	

Supplemental Table 2. Expected versus observed numbers of linked dual-class linked DRMs on NVP resistant genomes.

<u>Current Study</u>				
		Number of Dual-class linked DRMs (NVP/r+184I)		
Outcome	Sequences with NVP resistance	Expected*	Observed	p (Binomial)
ART Failure	12486	1.7	14	<0.000001
ART Non-Failure	3556	0.44	2	0.082
<u>Reference Study*</u>				
	Average number of sequences	M184I	Error rate	SD
	23022**	3**	1.3×10^{-4}	3.8×10^{-5}

* Calculated from the experiment shown in Table 3 of (37) for the ATG to ATA (M184I) mutation. This rate was used to estimate the number of linked resistance mutations expected as the result of assay background error on a template already containing an NVP resistance mutation in the study presented here.

** Average of 4 replicate analyses.

Supplemental Table 3. Ultrasensitive Single Genome Sequencing results of Donor T1F03

Linked drug resistance mutations detected by uSGS pre-therapy	Linked drug resistance mutations detected by uSGS at failure (# of genomes)
65R/ 181C ^A (2)	181C/184V (2271)
184I /103N (1)	108I/181C/184V (144)
101E/103N (1)	74I/181C/184V (142)
103N/190E (1)	181C/184I (125)
	74I/108I/181C/184V (26)
	74V/181C/184V (9)
	70E/181C/184V (4)
	67N/181C/184I (4)
	101E/181C/184I (1)
	67N/181C/184V (1)
	181C/184V/190E (1)
	74I/184V (1)

^ABoldface type indicates possible recombination between two different variants with dual-class linked DRMs detected pre-ART (65R/**181C** and **184I**/103N) and a variant with dual-class linked DRMs (**181C/184I**) at treatment failure