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Supplementary Materials for

Epistatic pathways can drive HIV-1 escape from integrase strand transfer inhibitors

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This PDF file includes:

Figs. S1 to S7

Fig. S1

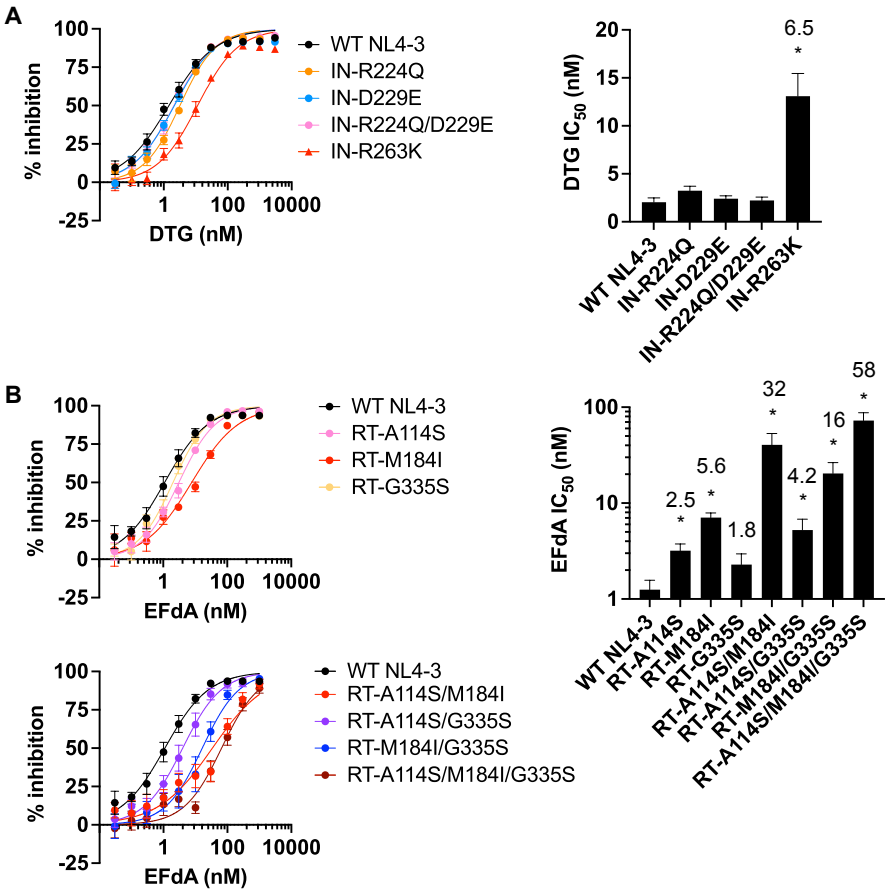
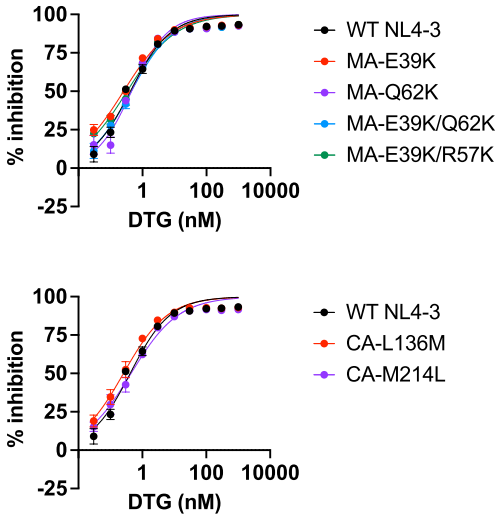


Fig. S1. Sensitivity of selected mutants in the drug- target gene to DTG or EFdA. TZM-bl cells were incubated with 100 $TCID_{50}$ of the indicated NL4-3-derived mutants in the presence of various concentrations of DTG and incubated for 48 h. (A) DTG sensitivity of the selected IN mutants shown in Fig. 1A. (B) EFdA sensitivity of the selected RT mutants shown in Fig. 1D. Data from at least three independent experiments are shown as means ± SEMs.

Fig. S2

A. Gag

Drugs	Viruses	Passage (weeks)	Conc. (nM)	Gag: MA E39K	MA L40M	MA R57K	MA Q62K	CA A116T	CA L136M	CA M214L	NC N8S	NC N17S	NC G19S	NC V25A	NC N27K/I	NC R29G/M	NC R32G	NC G35D	NC G40E	NC G43E	NC M46I
DTG (INSTI)	WT NL4-3 cul 1	46	2,000																		
	WT NL4-3 cul 2	39	1,000																		
	WT NL4-3 cul 3	37	1,000												K						
	Env-A541V cul1	41	1,000																		
	Env-A541V cul2	32	4,000													G					
	Env-A541V cul3	36	4,000																		
	IN-G118R	39	2,000																		
	WT NL(AD8) cul 1	31	1,000												I						
	WT NL(AD8) cul 2	35	500													M					
	WT NL(AD8) cul 3	31	500																		
	WT CH185 cul 1	36	6,400																		
	WT CH185 cul 1	36	500																		
	Env-T541I cul 1	36	20,000																		
	Env-T541I cul 2	36	20,000																		
EFdA (NRTTI)	WT NL4-3 cul 1	38	250																		
	WT NL4-3 cul 2	17	16																		



B. Pol

Drugs	Viruses	Passage (weeks)	Conc. (nM)	Pol: RT V21I	RT K46R	RT V75I	RT A114S	RT T132I	RT A138T	RT T165A	RT R172K	RT **M184I**	RT V276I	RT V314I	RT G335S	RT E399K	RT V381L	RT I382L	RT T409I	RT V467I	RT D471I	IN A23T	IN V54L	IN **G118R**	IN A128T	IN V150I	IN R224Q	IN D229E	IN **R263K**
DTG (INSTI)	WT NL4-3 cul 1	46	2,000																										
	WT NL4-3 cul 2	39	1,000																										
	WT NL4-3 cul 3	37	1,000																										
	Env-A541V cul1	41	1,000																										
	Env-A541V cul2	32	4,000																										
	Env-A541V cul3	36	4,000																										
	IN-G118R	39	2,000																										
	WT NL(AD8) cul 1	31	1,000																										
	WT NL(AD8) cul 2	35	500																										
	WT NL(AD8) cul 3	31	500																										
	WT CH185 cul 1	36	6,400																										
	WT CH185 cul 2	36	500																										
	Env-T541I cul 1	36	20,000																										
	Env-T541I cul 2	36	20,000																										
EFdA (NRTTI)	WT NL4-3 cul 1	38	250																										
	WT NL4-3 cul 2	17	16																										

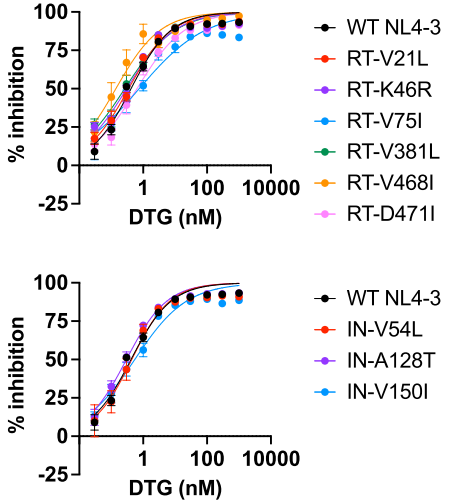


Fig. S2

C. gp120

Drugs	Viruses	Passage (weeks)	Conc. (nM)	V3M	K6N	V75I	T77I	V84I	E87D	E91K	N94D	T138I	A142T	N145D	N160K	S162K/R	I165R	D167N/G	V181I	S190R	N197D	Q203R	A204T	P206T	T236I	R298K	S306N	R308K	I309M	D322N	A329T	A336T	Q363R	S364P	G404E	I414V	V424I	E429K	N448K	V488A	L494I	I496V
				gp120																																						
				SP		C1						V1			V2						C2					V3						C3			V4		C4			C5		
gp120-gp41 interface																																										
PNGS																																										
DTG (INSTI)	WT NL4-3 cul 1	46	2,000													K																										
DTG (INSTI)	WT NL4-3 cul 2	39	1,000																																							
DTG (INSTI)	WT NL4-3 cul 3	37	1,000															N																								
DTG (INSTI)	Env-A541V cul1	41	1,000													K																										
DTG (INSTI)	Env-A541V cul2	32	4,000															G																								
DTG (INSTI)	Env-A541V cul3	36	4,000															N																								
DTG (INSTI)	IN-G118R	39	2,000																																							
DTG (INSTI)	WT NL(AD8) cul 1	31	1,000																																							
DTG (INSTI)	WT NL(AD8) cul 2	35	500																																							
DTG (INSTI)	WT NL(AD8) cul 3	31	500																																							
DTG (INSTI)	WT CH185 cul 1	36	6,400																																							
DTG (INSTI)	WT CH185 cul 2	36	500																																							
DTG (INSTI)	Env-T541I cul 1	36	20,000																																							
DTG (INSTI)	Env-T541I cul 2	36	20,000																																							
EFdA (NRTTI)	WT NL4-3 cul 1	38	250																																							
EFdA (NRTTI)	WT NL4-3 cul 2	17	16													R																										

D. gp41

Drugs	Viruses	Passage (weeks)	Conc. (nM)	A525T	A541V/T541I	A558T	A561T	T578I	S620N	N636S	T639K	T651I	N677D	M687I	Y712C	G737D	A818T	A819T	V822A	A823V	A832T/I832M	L833S	A835V
				gp41																			
				FP	HR1				DSL	HR2			MPER	MSD	CT								
gp120-gp41 interface																							
PNGS																							
DTG (INSTI)	WT NL4-3 cul 1	46	2,000		A/V																		
DTG (INSTI)	WT NL4-3 cul 2	39	1,000		A/V																		
DTG (INSTI)	WT NL4-3 cul 3	37	1,000		A/V																		
DTG (INSTI)	Env-A541V cul1	41	1,000		A/V																		
DTG (INSTI)	Env-A541V cul2	32	4,000		A/V																		
DTG (INSTI)	Env-A541V cul3	36	4,000		A/V																A/T		
DTG (INSTI)	IN-G118R	39	2,000		A/V																		
DTG (INSTI)	WT NL(AD8) cul 1	31	1,000																				
DTG (INSTI)	WT NL(AD8) cul 2	35	500																				
DTG (INSTI)	WT NL(AD8) cul 3	31	500																				
DTG (INSTI)	WT CH185 cul 1	36	6,400		T/I																		
DTG (INSTI)	WT CH185 cul 2	36	500																		I/M		
DTG (INSTI)	Env-T541I cul 1	36	20,000		T/I																		
DTG (INSTI)	Env-T541I cul 2	36	20,000		T/I																		
EFdA (NRTTI)	WT NL4-3 cul 1	38	250																				
EFdA (NRTTI)	WT NL4-3 cul 2	17	16																				

E

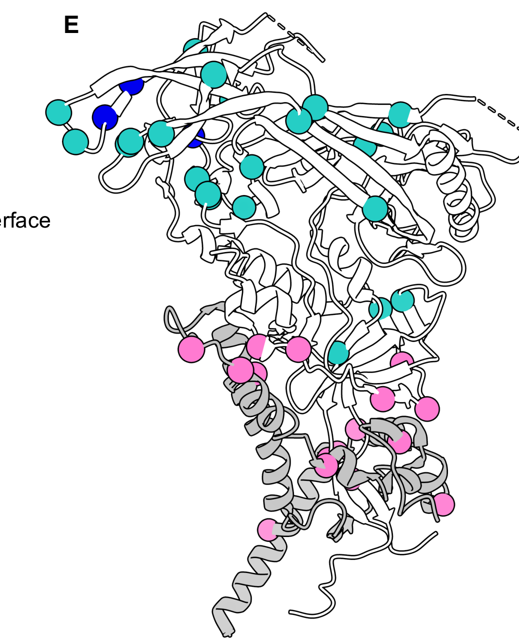


Fig. S2. Summary of mutations in Gag, Pol, and Env-coding regions obtained with NL4-3, NL(AD8), and CH185 selections in the presence of DTG and EFdA. The selected mutations in (A) Gag, (B) Pol, (C) gp120, and (D) gp41 in the presence of DTG or EFdA. Viruses used to initiate the selection are shown in the second column. The third column indicates the time of sampling. Repeated selection experiments are indicated as culture 1 (cul 1), cul 2, etc. Pol mutations highlighted in bold are established resistance mutations to DTG or EFdA. Dominant or mixed mutations at the indicated time points are highlighted in red or orange, respectively. TZM-bl cells were incubated with 100 $TCID_{50}$ of the indicated NL4-3-derived mutants in the presence of various concentrations of DTG and incubated for 48 h; luciferase activity was measured at 48 h postinfection. (E) Env structure of subtype B JR-FL SOSIP.664 (PDB accession number 5FYK) with the position of the Env mutations highlighted. Mutations in the gp120-gp41 interface are highlighted in pink. Several mutations in gp120 V1/V2 region disrupt N-linked glycosylation (highlighted in blue).

Fig. S3

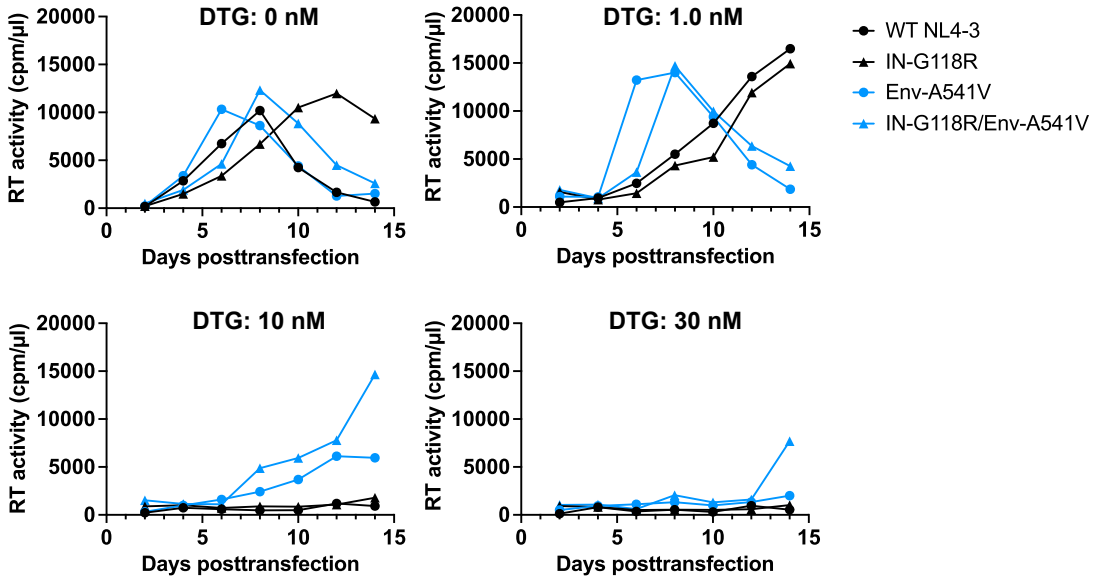


Fig. S3. Replication kinetics of IN-G118R/Env-A541V in the presence of DTG. The SupT1 T-cell line was transfected with WT NL4-3 or the indicated IN- or Env-mutant proviral clones in the absence or presence of serial dilutions of DTG (0.01 – 3,000 nM); data are shown for 0, 1.0, 10, and 30 nM DTG. Virus replication kinetics were monitored by measuring RT activity at the indicated time points. Data are representative of three independent experiments.

Fig. S4

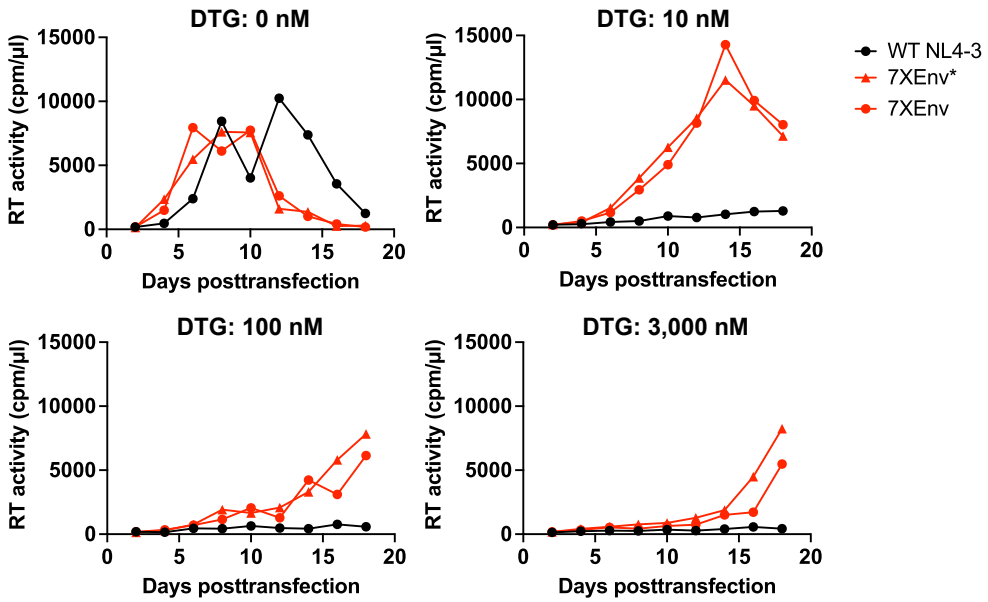


Fig. S4. Replication kinetics of 7XEnv* and 7XEnv in the presence of DTG. The SupT1 T-cell line was transfected with WT NL4-3 or the indicated Env-mutant proviral clones in the absence or presence of serial dilutions of DTG (0.01 – 3,000 nM). Data for 0, 10, 100, and 3,000 nM DTG are shown. Virus replication kinetics were monitored by measuring RT activity at the indicated time points. 7XEnv does not contain the mutation in Vpu/Env-SP region (Vpu-V60L/SP-K6N). The comparison of WT vs 7XEnv and WT vs 7XEnv* was performed at least three times with similar results.

Fig. S5

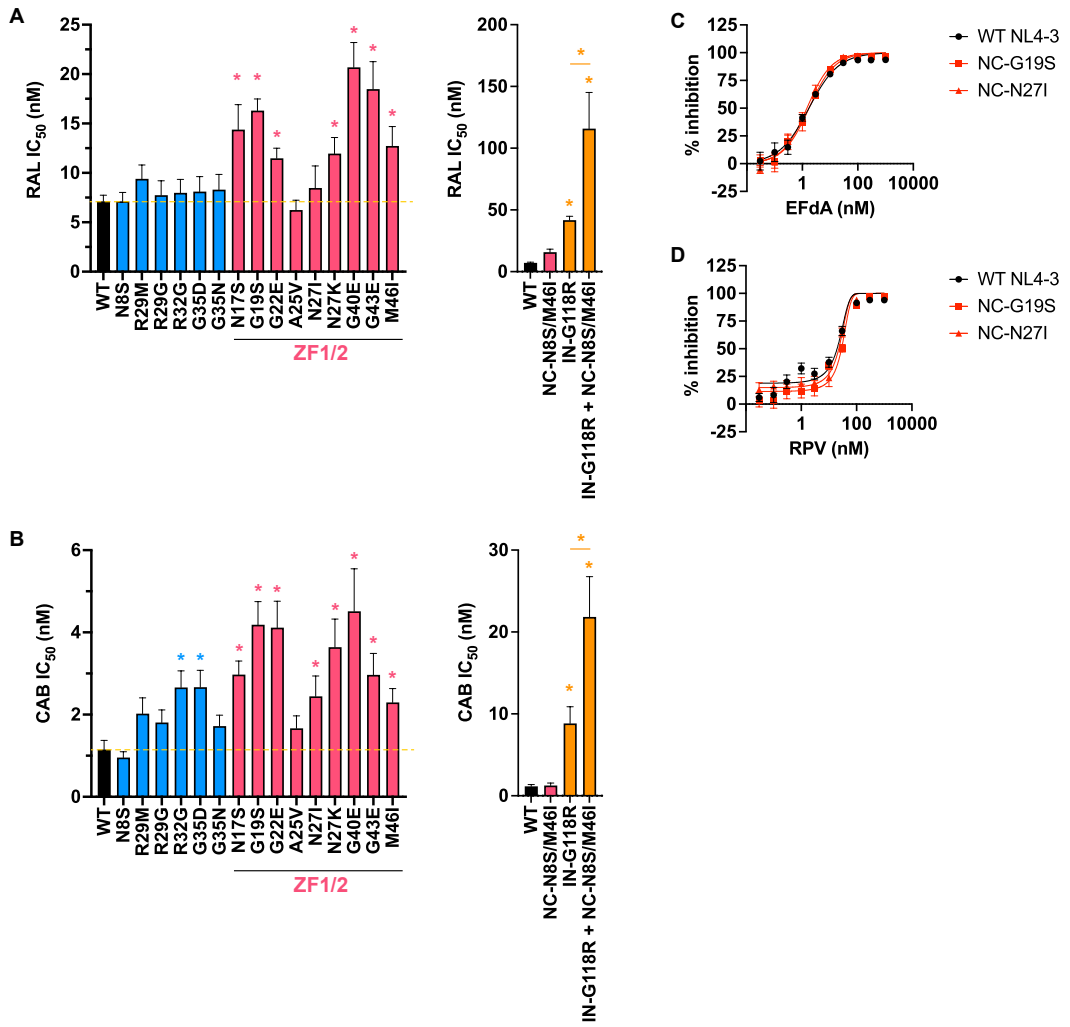


Fig. S5. Sensitivity of the Gag-NC mutants to INSTIs and RT inhibitors.
Sensitivity of the NC mutants to (A) RAL and (B) CAB. Mutations within or outside the zinc-finger domains (ZF1/2) are shown in blue and red, respectively. TZM-bl cells were exposed to 100 $TCID_{50}$ of WT or the NC mutants in the presence of various concentrations of DTG (from 0.03 to 1,000 nM INSTIs). Luciferase activity was measured at 48 h postinfection. Sensitivity of NC mutants to (C) NRTTI EFdA and (D) NNRTI RPV. Data from at least three independent experiments are shown as means ± SEMs. *, $P < 0.05$ by one-way ANOVA and Tukey's multiple-comparison test or unpaired t-test.

Fig. S6

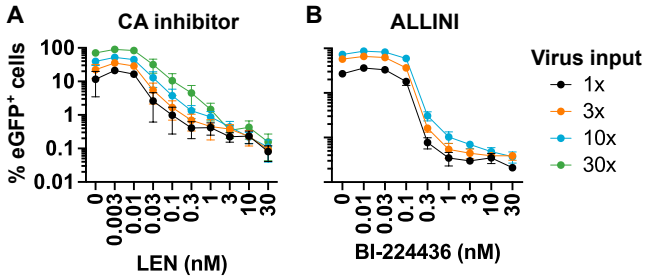


Fig. S6. Sensitivity of VSV-G-pseudotyped eGFP reporter virus to a CA inhibitor and an ALLINI over a range of viral input. A) CA inhibitor LEN, and B) ALLINI BI-224436. The SupT1 T-cell line was exposed to a 30-fold range of VSV-G pseudotyped eGFP reporter virus at the indicated inhibitor concentrations. The number of eGFP-positive cells was enumerated by flow cytometry.

Fig. S7

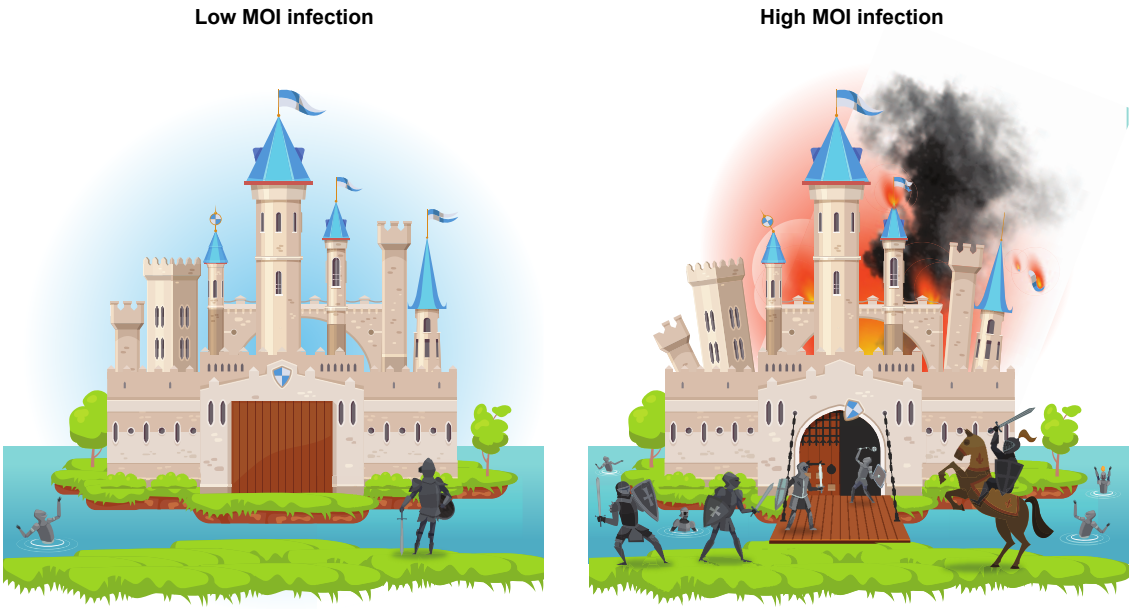


Fig S7. Model for Env-mediated drug resistance by increasing the MOI via enhanced cell-cell transfer. Left, low-MOI conditions; right, high-MOI conditions. In this cartoon, the soldiers represent viruses, the moat represents the ARV, and the castle represents the cell. Under low-MOI conditions, the ARVs completely block productive infection. Under high-MOI conditions, productive infection can still occur.