

## Supplementary File 2

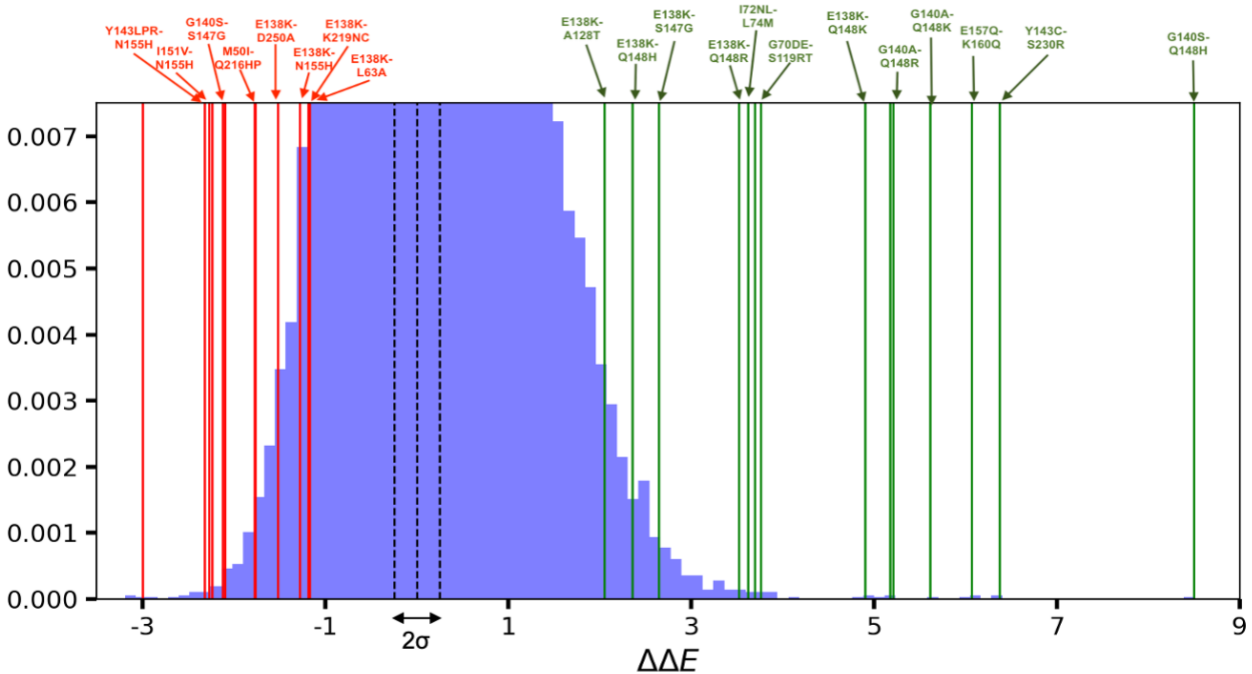
### Limits to detecting epistasis in the fitness landscape of HIV

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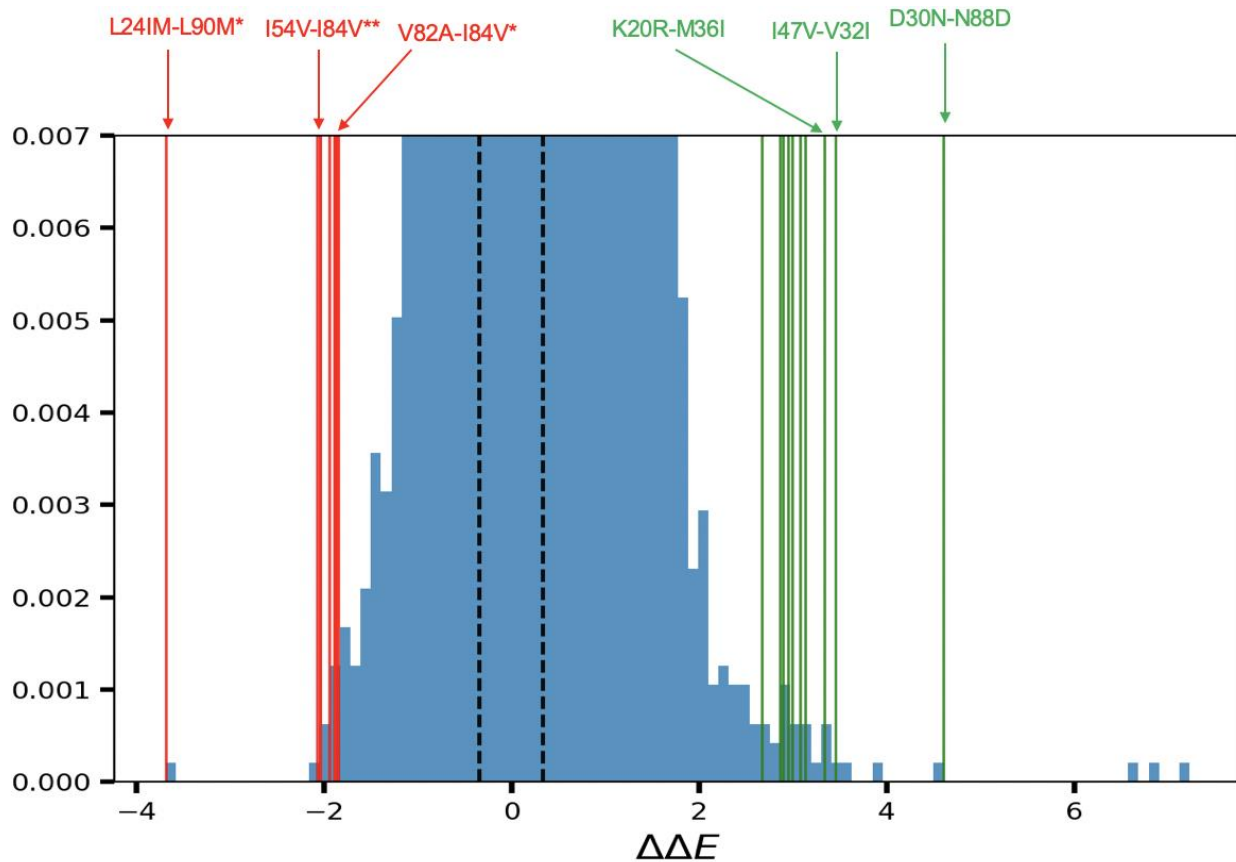


**Figure S2A:** Figure shows the distribution of Potts model predicted double mutant cycles ( $\Delta\Delta E$ ) in HIV-1 Integrase (blue) with respect to the NL4-3 background.  $\Delta\Delta E$  values above zero (0) indicate positive epistasis while  $\Delta\Delta E$  values below zero (0) indicate negative epistasis. The location of zero (0) and the standard deviations of  $\Delta\Delta E$  are shown as black 'dashed' lines. Some of the strongly interacting double mutations which involve drug-resistance mutations are indicated and labelled by colored lines; and are studied in the literature. Many of the strongest double mutant cycles captured by the Potts model are for drug-resistance mutations.

**Supplementary Table S2A: Potts model predicted strongest, positive double mutant cycle effects involving mutations (at least one amongst the pair) at drug-resistance-associated sites with corresponding literature references in HIV-1 Integrase.**

Mutation pair	Potts $\Delta\Delta E$	Literature reference*
G140S-Q148H	8.50879	1, 2, 3, 4, 5
Y143C-S230R	6.37754	6
E157Q-K160Q	6.0882	---
G140A-Q148K	5.61299	1, 2, 3, 4
G140A-Q148R	5.2042	1, 2, 3, 4
G140A-Q148K	4.91495	1, 2, 3, 4
E138K-Q148K	4.90052	5, 6, 7, 8
G70DE-S119RT	3.76054	----
Y143ASGH - S230R	3.7007	6
I72NL-L74M	3.62676	9
E138K-Q148R	3.5258	4, 6, 7, 8
E138K-Q148H	2.3615	4,6,7,8

\* Literature references are mentioned only for pairs where the coupled effect of the mutations is studied in literature.



**Figure S2B:** Figure shows the distribution of Potts model predicted double mutant cycles ( $\Delta\Delta E$ ) in HIV-1 Protease (blue).  $\Delta\Delta E$  values above zero (0) indicate positive epistasis while  $\Delta\Delta E$  values below zero (0) indicate negative epistasis. The location of zero (0) and the standard deviations of  $\Delta\Delta E$  are shown as black 'dashed' lines. Some of the strongly interacting double mutations which involve drug-resistance mutations are indicated and labelled by colored lines; and are studied in the literature. Many of the strongest double mutant cycles captured by the Potts model are for drug-resistance.

**Supplementary Table S2B: Potts model predicted strongest, positive double mutant cycle effects involving mutations (at least one amongst the pair) at drug-resistance-associated sites with corresponding literature references in HIV-1 Protease.**

<b>Mutation pair</b>	<b><i>Potts</i> <math>\Delta\Delta E</math></b>	<b>Literature reference*</b>
D30N-N88D	4.61	10, 11, 13, 14
I47V—V32I	3.45	15, 16
K20R-M36I	3.33	17
G48VALMQ-I54ATS	3.14	---
D30N-K45Q	3.08	----
G16E-P39S	2.99	---
I54ATS-V82A	2.95	12, 13, 18
P39S-I62K	2.89	----
I54V-V82A	2.86	21
M46I-L76V	2.67	14, 19, 20

\* Literature references are mentioned only for pairs where the coupled effect of the mutations is studied in literature.

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