

Supplementary Material

pM to μ M: Elucidating the Role of Distal Mutations in HIV-1 Protease in Conferring Drug Resistance

Mina Henes^{+,1}, Gordon J. Lockbaum^{+,1}, Klajdi Kosovrasti¹, Florian Leidner¹, Gily S. Nachum¹, Ellen A. Nalivaika¹, Sook-Kyung Lee², Ean Spielvogel², Shuntai Zhou², Ronald Swanstrom², Daniel N.A. Bolon¹, Nese Kurt Yilmaz^{*,1}, and Celia A. Schiffer^{*,1}

¹Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01605, United States

²Department of Biochemistry and Biophysics and the UNC Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States

⁺Joint first authors

^{*}Corresponding Author

Celia A. Schiffer: Phone: +1 508 856 8008; Celia.Schiffer@umassmed.edu

Nese Kurt Yilmaz: Phone: +1 508 856 1867; Nese.KurtYilmaz@umassmed.edu

Table S1. Enzyme kinetics of HIV-1 protease variants measured using a natural substrate sequence: Michaelis-Menten constant (K_M), enzyme turnover number (k_{cat}), and catalytic efficiency (k_{cat}/K_M).

	K_M (μM)	k_{cat} (s^{-1})	k_{cat} / K_M ($\mu M^{-1} s^{-1}$)
WT	71.4 ± 6.8	1282.7 ± 0.1	17.1 ± 0.1
I84V	66.4 ± 4.3	782.3 ± 0.1	11.8 ± 0.1
2Mut	55.1 ± 12.4	11.8 ± 0.1	3.4 ± 0.2
4Mut	71.0 ± 4.6	690 ± 0.1	9.7 ± 0.1
8Mut	123.8 ± 30.1	430.7 ± 0.2	3.5 ± 0.2
9Mut-A71V	139.2 ± 35.3	16.9 ± 0.3	0.1 ± 0.2
9Mut-L76V	174.1 ± 44.8	17.7 ± 0.3	0.1 ± 0.3
10Mut	101.4 ± 14.8	532.8 ± 0.1	5.3 ± 0.1
11Mut	63.9 ± 22.6	81.9 ± 0.2	1.3 ± 0.4

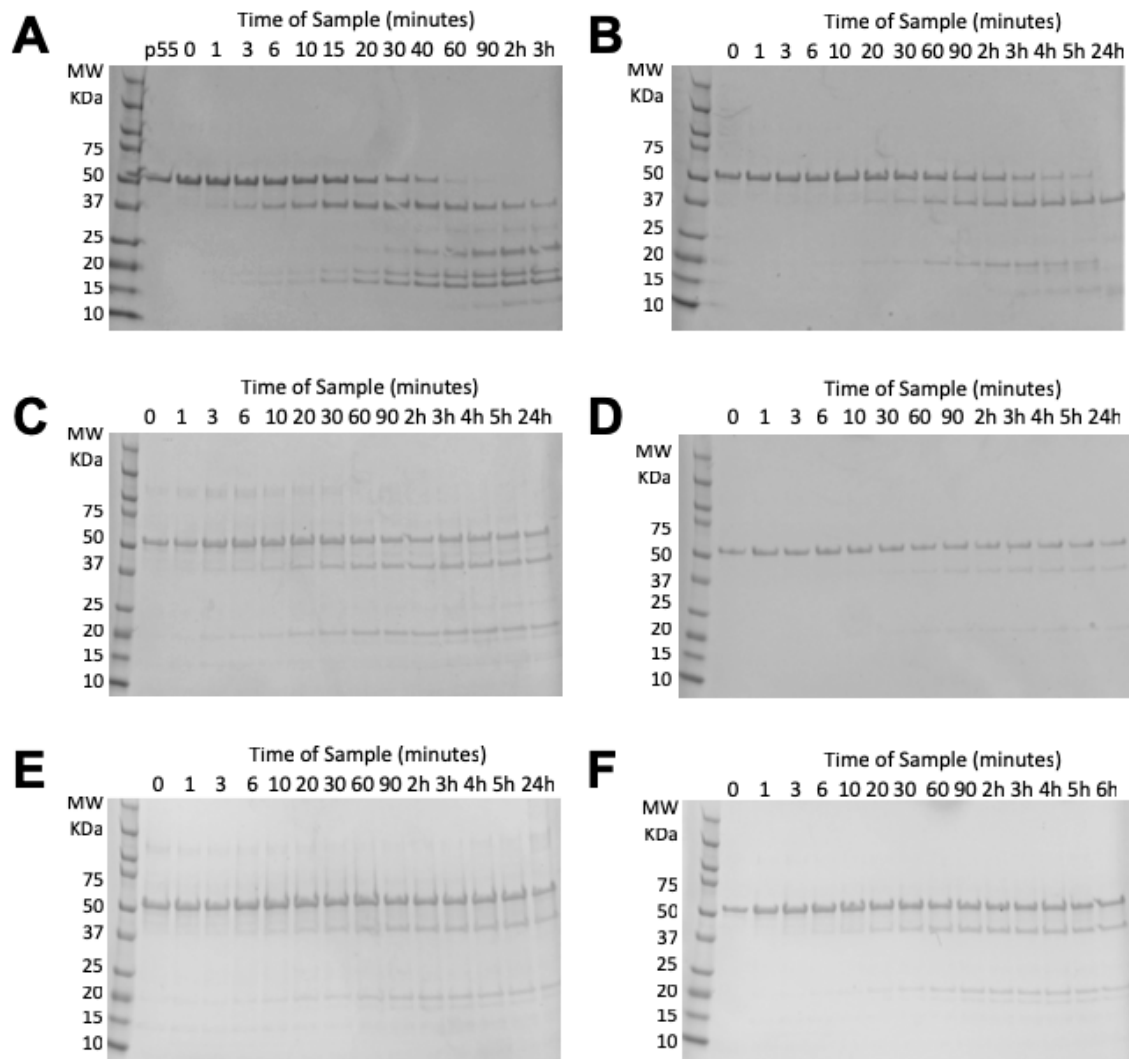


Figure S1. Time course cleavage of full-length gag polypeptide, p55, by HIV-1 protease variants. (A) WT, (B) 8Mut, (C) 9Mut-A71V, (D) 9Mut-L76V, (E) 10Mut, and (F) 11Mut.

Supplemental Method

Gag Polyprotein Cleavage Assay

The pET28a plasmid containing full length Pr55^{Gag}-TEV-His construct was a kind gift from Maria Bewley and John Flanagan. Protein expression and purification was done as described by Bewley et al. (*Protein Expr Purif*, 2017; 130:137-145) and consists of the removal of DNA and ammonium sulfate precipitation. On the day of the assay, ammonium sulfate pellets were dissolved in resuspension buffer (10 mM HEPES pH 7.5, 500 mM NaCl, 0.1 mM TCEP and 0.1 mM EDTA) and diluted 1:5 with the same buffer without NaCl. The final solution was centrifuged at 20k x g for 20 min. Cleavage of Pr55^{Gag} polyprotein by HIV-1 protease was monitored by SDS-PAGE of cleavage products visualized by Coomassie staining. Samples were taken from the reaction mixture at designated time points, and the cleavage reaction was quenched by adding gel running buffer containing SDS and boiling for 2 min.

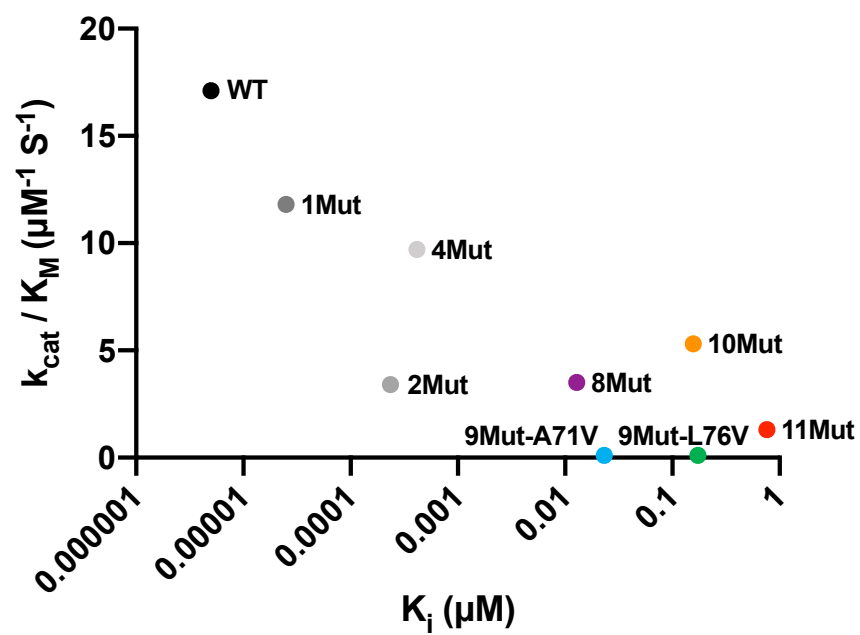


Figure S2. The relationship between DRV inhibition and enzyme catalytic efficiency of HIV-1 protease variants with increasing number of mutations.

Table S2. X-Ray Crystallography Statistics

	WT (NL4-3)	1MUT	2MUT	WT-HEX	4MUT	8MUT	9MUT-A71V	9MUT-L76V	10MUT	11MUT
INHIBITOR	DRV	DRV	DRV	DRV	DRV	DRV	DRV	DRV	DRV	DRV
PDB ID	6DGX	6DH0	6OPT	6OPS	6OPU	6OPV	6OPW	6OPX	6OPY	6OPZ
RESOLUTION (Å)	2.00	1.90	1.96	2.08	1.94	1.91	2.10	2.03	2.13	2.20
SPACE GROUP	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁	P6 ₁	P6 ₁	P6 ₁	P6 ₁	P6 ₁	P6 ₁	P6 ₁
TWIN LAW				h,-h-k,-l	h,-h-k,-l	h,-h-k,-l	h,-h-k,-l	h,-h-k,-l	h,-h-k,-l	h,-h-k,-l
A (Å)	51.0	51.1	50.8	62.3	61.5	61.7	62.1	61.9	62.1	61.8
B (Å)	58.3	58.1	57.9	62.3	61.5	61.7	62.1	61.9	62.1	61.8
C (Å)	61.9	61.8	61.5	82.3	81.3	81.2	82.3	81.5	82.6	82.4
COMPLETENESS	98.1	99.9	96.5	97.0	97.9	99.7	99.9	99.5	97.0	99.8
TOT. REFLECTIONS	84332	181306	252365	104283	126710	135511	110972	127376	53059	92665
UNIQ. REFLECT.	12758	15103	13103	10660	12722	13672	10571	11470	9912	9120
AVG. I/SIGMA	31.1	41.1	53.2	24.0	48.5	43.7	37.1	33.8	29.7	37.2
REDUNDANCY	6.6	12.0	19.3	9.8	10.0	9.9	10.5	11.1	5.4	10.2
R-MERGE (LINEAR)	0.057	0.057	0.056	0.086	0.045	0.052	0.064	0.077	0.048	0.059
RMSD BONDS*	0.003	0.003	0.003	0.003	0.002	0.006	0.004	0.003	0.006	0.004
RMSD ANGLES*	0.590	0.710	0.663	0.499	0.616	0.888	0.820	0.678	0.783	0.606
R-FREE*	22.8	21.2	22.8	25.1	23.2	22.3	28.5	25.6	22.6	26.8
R-WORK*	19.2	18.3	18.2	22.5	20.5	17.6	26.0	23.1	19.1	22.8

*Based on Phenix Program

Table S3. **A)** Protease–DRV per residue vdW contacts for all crystal structures. Darker red indicates more contacts. **B)** Difference in per residue protease–DRV vdW contacts relative to WT cocrystal structure in the same space group. Positive numbers and cooler colors indicate reduced vdW contacts and negative numbers and warmer colors indicate increased vdW contacts, relative to WT structure.

A											B							
	WT-P2			WT-P6			9Mut		9Mut		WT-P2							
	WT-P2	I84V	2Mut	WT-P6	4Mut	8Mut	A71V	L76V	10Mut	11Mut	I84V	2Mut	4Mut	8Mut	9Mut	9Mut	10Mut	11Mut
A23	-1.0	-1.0	-1.1	-1.1	-0.9	-1.0	-0.8	-0.9	-1.2	-1.2	0.1	0.0	0.2	0.2	0.3	0.2	-0.1	-0.1
A27	-3.8	-3.6	-3.8	-4.3	-3.9	-3.9	-4.0	-3.9	-4.0	-3.7	0.2	0.1	0.3	0.3	0.2	0.3	0.3	0.5
A28	-5.4	-5.6	-5.6	-5.4	-5.2	-5.2	-5.0	-5.1	-5.2	-5.5	-0.2	-0.2	0.2	0.2	0.4	0.3	0.2	-0.1
A29	-4.2	-4.2	-4.2	-4.1	-3.9	-3.9	-4.1	-3.6	-4.1	-4.0	0.0	0.0	0.2	0.1	-0.1	0.5	0.0	0.0
A30	-3.8	-3.8	-3.0	-3.0	-3.0	-3.1	-2.7	-3.0	-3.0	-3.5	0.0	0.8	-0.1	-0.1	0.2	0.0	-0.1	-0.6
A32	-1.2	-1.2	-1.1	-1.2	-1.1	-0.6	-0.7	-0.5	-0.7	-0.8	0.0	0.1	0.1	0.5	0.5	0.6	0.5	0.4
A47	-1.5	-2.7	-2.6	-2.0	-1.7	-2.1	-1.7	-1.7	-1.8	-1.9	-1.2	-1.1	0.3	-0.1	0.2	0.3	0.2	0.1
A48	-2.1	-2.7	-2.5	-2.6	-2.3	-2.3	-2.7	-2.1	-2.3	-2.3	-0.6	-0.4	0.3	0.2	-0.1	0.5	0.3	0.3
A49	-2.9	-3.3	-3.0	-3.2	-3.2	-2.9	-3.1	-2.7	-2.9	-2.4	-0.4	-0.1	0.0	0.4	0.1	0.5	0.3	0.9
A50	-6.1	-5.8	-5.7	-5.7	-6.3	-5.5	-5.6	-6.1	-5.3	-4.9	0.3	0.4	-0.6	0.2	0.1	-0.4	0.4	0.8
A81	-0.5	-0.5	-0.7	-0.6	-0.7	-0.9	-1.3	-0.9	-0.9	-0.9	0.0	-0.1	-0.2	-0.3	-0.8	-0.3	-0.3	-0.4
A82	-1.1	-1.2	-0.6	-1.4	-1.7	-1.6	-1.6	-1.7	-1.6	-1.6	-0.1	0.5	-0.3	-0.2	-0.2	-0.3	-0.2	-0.2
A84	-3.1	-2.0	-2.0	-2.8	-1.6	-1.4	-1.3	-1.3	-1.5	-1.9	1.2	1.1	1.2	1.4	1.5	1.5	1.4	0.9
B8	-1.4	-1.2	-0.7	-1.6	-0.9	-0.6	-1.0	-0.9	-0.7	-0.9	0.2	0.7	0.8	1.1	0.6	0.7	1.0	0.7
B23	-1.5	-0.8	-1.3	-1.5	-1.2	-1.3	-1.2	-1.3	-1.3	-1.3	0.7	0.2	0.2	0.2	0.3	0.1	0.1	0.2
B27	-2.8	-2.8	-2.8	-2.7	-2.7	-3.1	-3.3	-2.9	-2.7	-2.9	0.0	0.0	0.0	-0.3	-0.6	-0.2	0.0	-0.2
B28	-4.4	-3.5	-3.9	-4.8	-4.3	-4.3	-4.2	-4.2	-4.6	-4.6	0.9	0.4	0.4	0.5	0.6	0.5	0.1	0.2
B29	-1.6	-1.4	-1.6	-1.8	-1.5	-1.6	-2.4	-2.0	-2.1	-1.8	0.1	-0.1	0.3	0.2	-0.6	-0.2	-0.3	0.0
B30	-2.1	-2.1	-2.1	-2.5	-2.3	-2.5	-2.5	-2.7	-2.8	-2.7	0.1	0.1	0.2	0.0	0.0	-0.2	-0.3	-0.3
B31	-0.5	-0.4	-0.4	-0.5	-0.5	-0.4	-0.3	-0.3	-0.4	-0.3	0.0	0.0	-0.1	0.1	0.2	0.1	0.0	0.1
B32	-1.7	-2.1	-2.0	-1.7	-1.8	-1.0	-0.8	-0.8	-1.2	-1.0	-0.3	-0.3	-0.1	0.7	1.0	0.9	0.5	0.7
B47	-3.2	-2.9	-3.1	-2.2	-2.0	-1.9	-1.4	-1.6	-1.7	-1.7	0.3	0.1	0.2	0.3	0.8	0.6	0.5	0.5
B48	-1.8	-1.7	-1.8	-1.7	-1.5	-1.8	-1.7	-1.9	-1.7	-1.7	0.1	0.0	0.2	-0.1	0.0	-0.2	0.0	0.0
B49	-1.8	-1.8	-1.8	-2.1	-2.4	-2.5	-2.6	-2.6	-2.1	-2.0	0.0	0.0	-0.3	-0.4	-0.6	-0.5	-0.1	0.1
B50	-3.6	-3.5	-3.4	-4.3	-4.4	-4.5	-5.1	-4.7	-4.7	-4.4	0.2	0.2	-0.1	-0.2	-0.8	-0.4	-0.3	0.0
B76	-0.6	-0.5	-0.6	-0.5	-0.5	-0.4	-0.3	-0.1	-0.2	-0.1	0.1	0.0	0.0	0.1	0.2	0.4	0.3	0.4
B81	-1.6	-1.6	-1.6	-1.6	-1.8	-2.0	-1.9	-1.9	-2.0	-1.7	0.0	0.0	-0.2	-0.4	-0.3	-0.3	-0.4	-0.1
B82	-2.2	-3.2	-2.9	-2.4	-2.8	-2.4	-2.0	-2.7	-2.5	-2.3	-1.0	-0.7	-0.4	-0.1	0.4	-0.3	-0.1	0.0
B84	-4.0	-2.3	-2.4	-4.2	-2.7	-2.1	-1.8	-2.3	-2.1	-1.9	1.8	1.6	1.5	2.2	2.5	1.9	2.1	2.4
Total	-83.1	-80.5	-79.4	-84.9	-79.9	-77.8	-78.3	-77.4	-77.9	-76.7	2.6	3.7	5.1	7.2	6.7	7.5	7.0	8.2

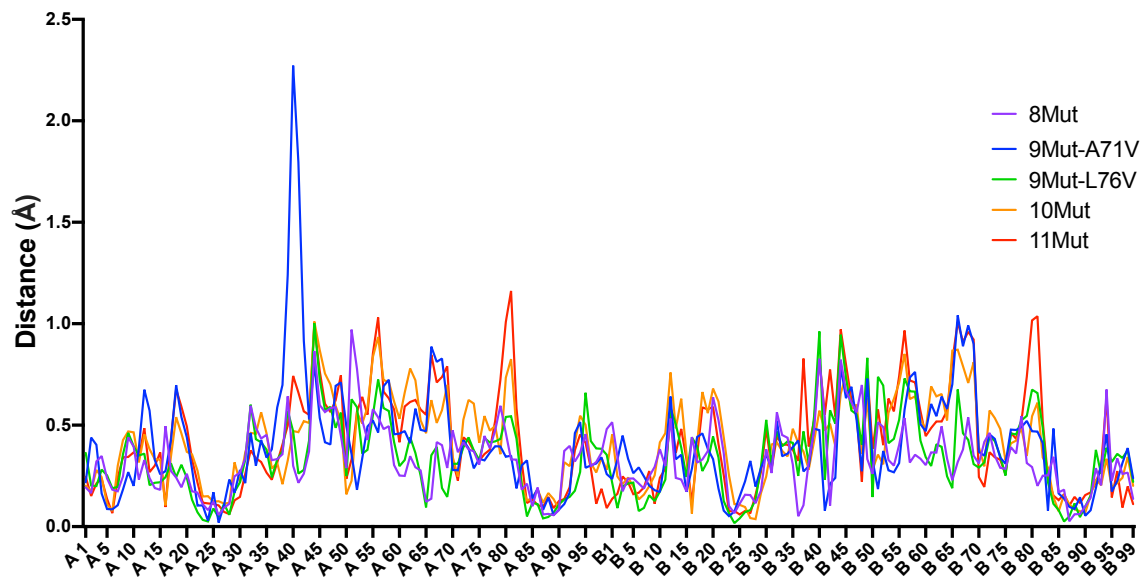


Figure S3. Deviation of internal C_{α} distances in protease variant crystal structures relative to WT-DRV complex.

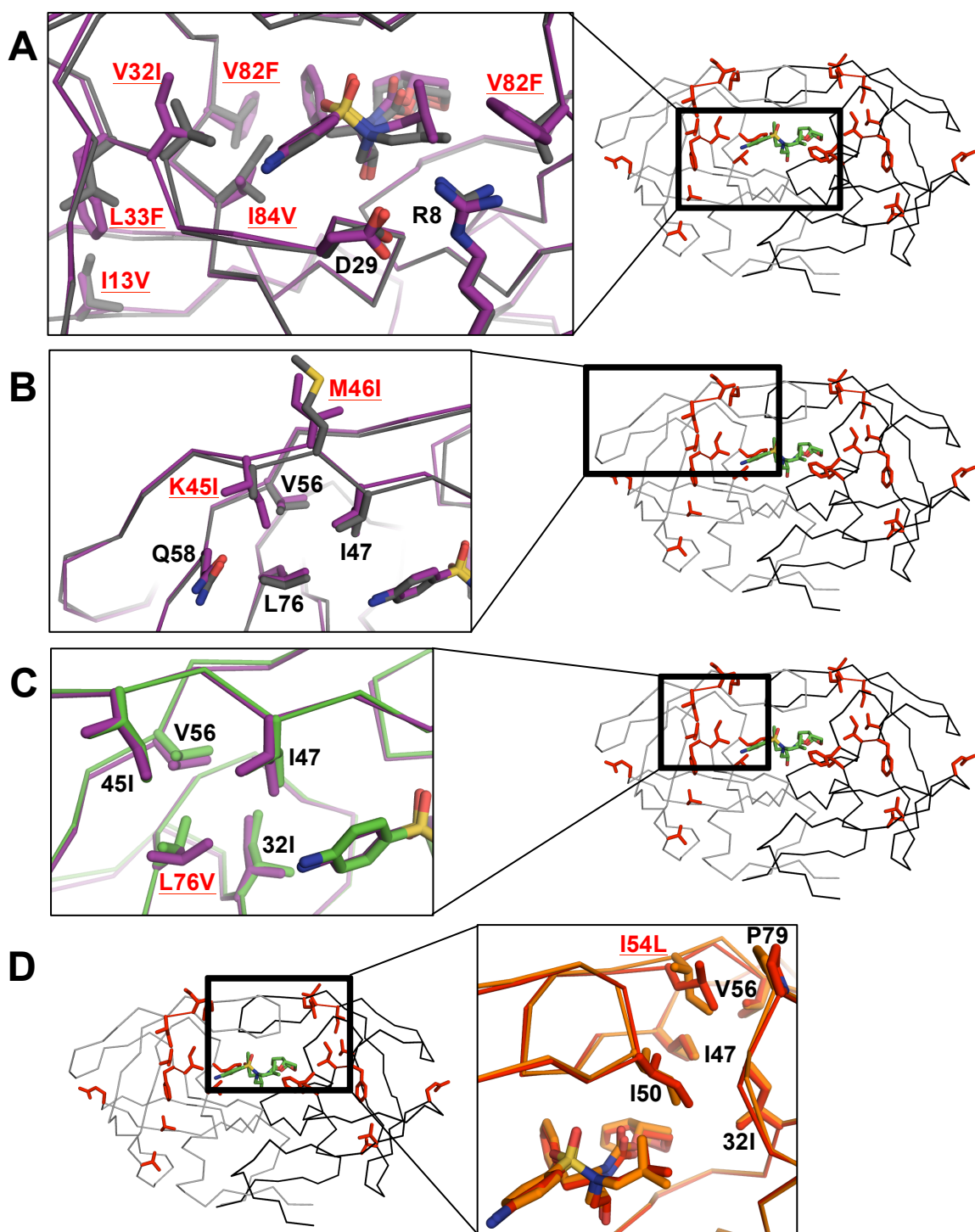


Figure S4. A,B) Comparison of WT (black) and 8Mut (purple) variants, focusing on the active site, core, and flaps. C) 8Mut (purple) and 9Mut-L76V (green) variants, focusing on the hydrophobic packing at base of the flaps. D) 10Mut (orange) and 11Mut (red) variants, which differ at I54L mutation, focusing on the hydrophobic packing upon P79.

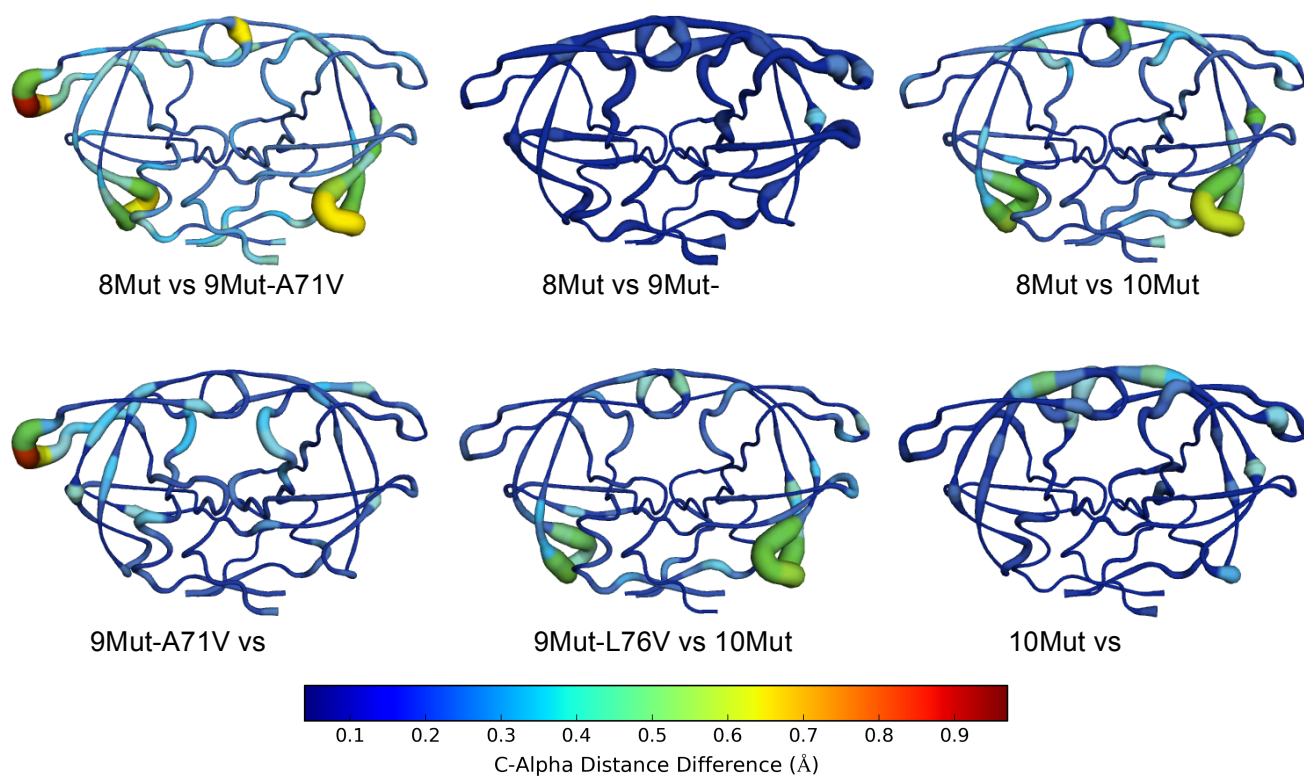


Figure S5. Internal C α distance differences in crystal structures mapped onto HIV-1 protease structure. Warmer colors and larger putty thickness indicate larger differences between the variants compared.

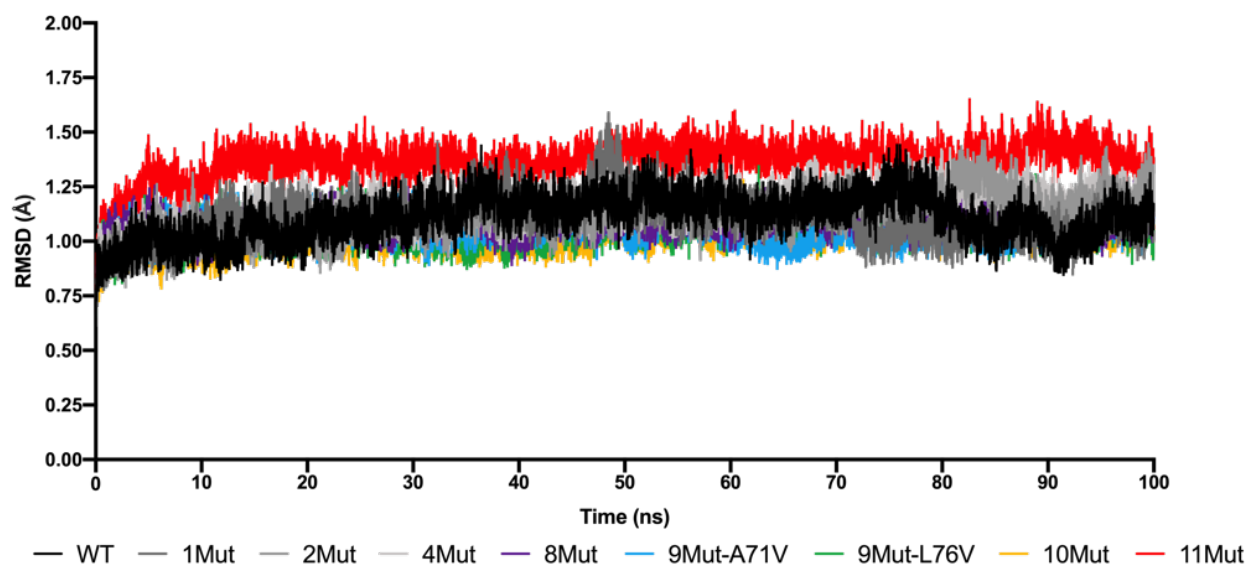


Figure S6. Root-Mean-Square Deviation (RMSD) of protein C α atoms during MD simulations.

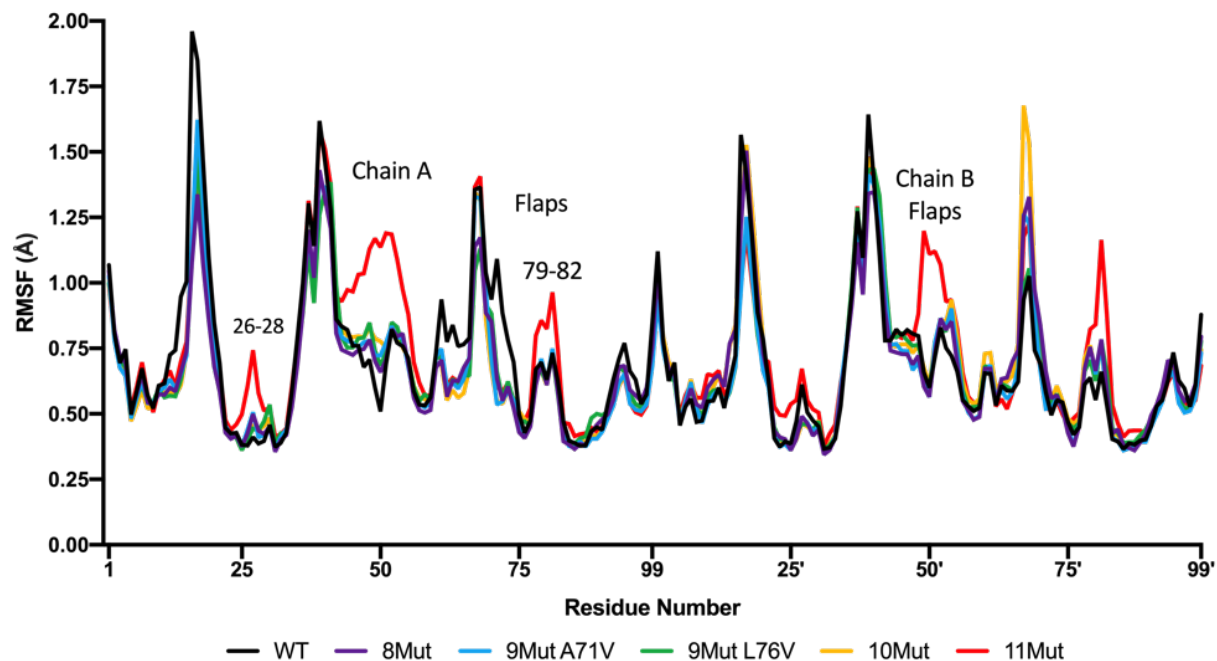


Figure S7. Root Mean Square Fluctuation (RMSF) of C_{α} atoms for WT, 8Mut, 9Mut-A71V, 9Mut-L76V, 10Mut, and 11Mut from MD simulations.

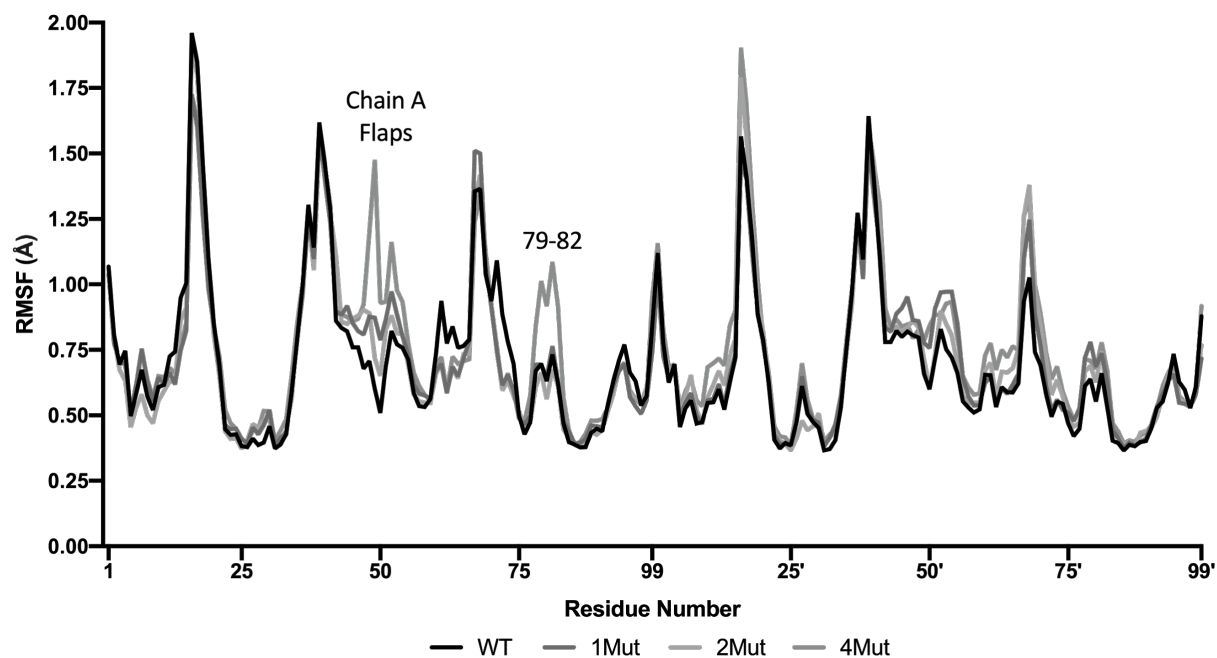


Figure S8. Root Mean Square Fluctuation (RMSF) of C_{α} atoms for WT, 1Mut, 2Mut, and 4Mut from MD simulations.

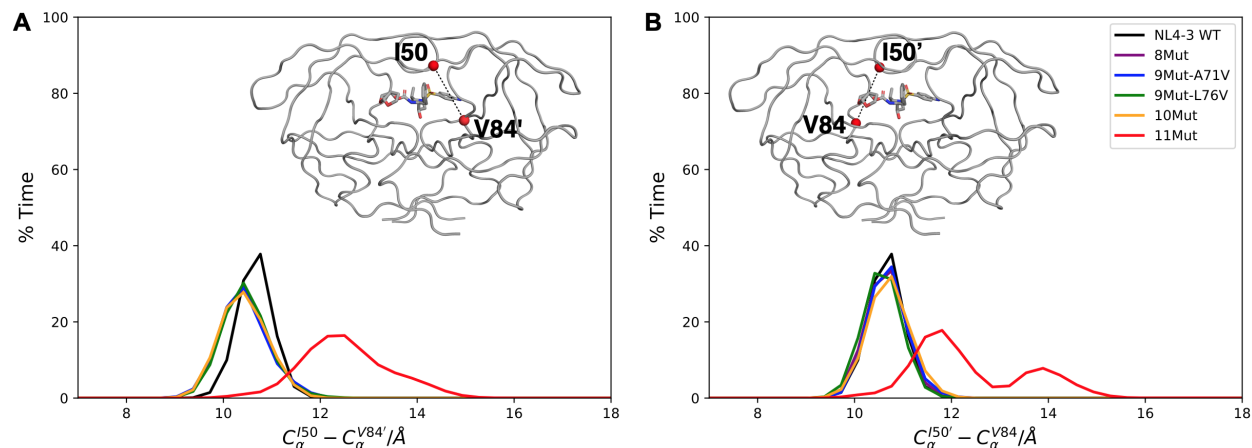


Figure S9. Distance between the C_{α} atoms of **A)** I50-V84' and **B)** I50'-V84 from MD simulations indicate that the flaps of the 11Mut are in a semi-open conformation.

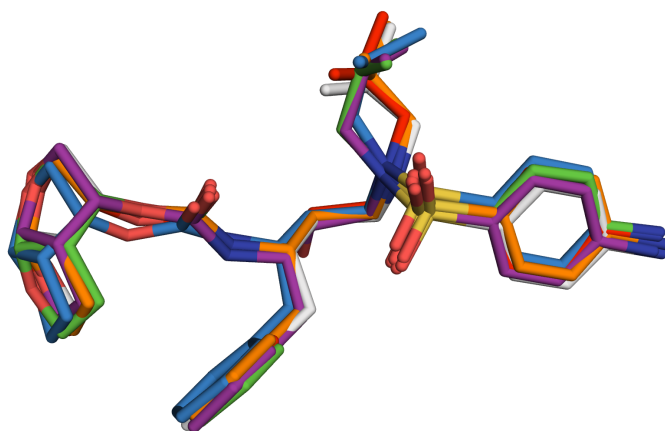


Figure S10. Alignment of DRV from crystal structures bound to WT (grey), 8Mut (purple), 9Mut-A71V (blue), 9Mut-L76V (green), 10Mut (orange) and 11Mut (red) variants.

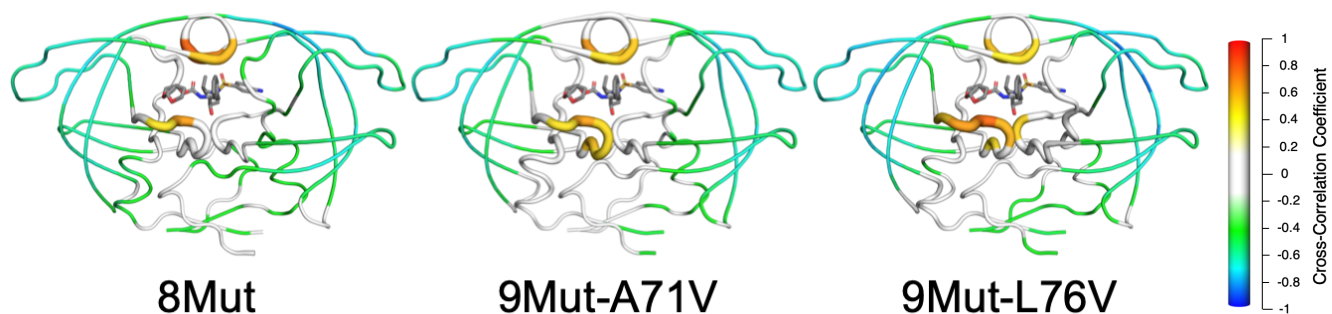


Figure S11. Cross-correlation of DRV fluctuations with 8Mut, 9Mut-A71V, or 9Mut-L76V from MD simulations. Mean cross-correlation coefficient for each residue mapped onto protease structure, shown as cartoon putty.