SUPPLEMENTARY MATERIAL

Molecular and Dynamic Mechanism Underlying Drug Resistance in Genotype 3 Hepatitis C NS3/4A Protease

Djadé I. Soumana[†], Nese Kurt Yilmaz, Akbar Ali, Kristina L. Prachanronarong, and Celia A. Schiffer*

Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA 01605, USA

*__Correspondence__: Celia.Schiffer@umassmed.edu

Table S1. The enzyme inhibition constants (K_i values) and associated standard deviations for seven PIs were determined for four enzyme variants: GT-1a, GT-3a, GT-1a with D168Q, and GT-1a with all three active site polymorphisms (R123T/I132L/D168Q) in GT-3a.

Inhibitor	GT-1a*	GT-1a–D168Q	GT-1a–R123T I132L-D168Q	GT-3a
Telaprevir	33.3 ± 3.9	38.5 ± 4.1	123 ± 26	266 ± 11
Boceprevir	33.0 ± 3.9	21.6 ± 2.2	21.0 ± 2.1	71.5 ± 9.1
Asunaprevir	2.7 ± 0.5	193 ± 45	2816 ± 1407	2633 ± 259
Danoprevir	1.00 ± 0.13	29.4 ± 3.4	1056 ± 376	879 ± 39
Vaniprevir	0.70 ± 0.09	31.9 ± 3.9	773 ± 293	373 ± 32
Grazoprevir	0.14 ± 0.02	14.10 ± 0.94	14.1 ± 4.3	33.7 ± 0.1
MK-6325	0.10 ± 0.01	0.40 ± 0.04	33.4 ± 2.5	31.1 ± 0.8

*Previously measured (Ali, A.; Aydin, C.; Gildemeister, R.; Romano, K. P.; Cao, H.; Ozen, A.; Soumana, D.; Newton, A.; Petropoulos, C. J.; Huang, W.; Schiffer, C. A. *ACS Chem Biol* **2013**, *8*, 1469).

Table S2. Crystallographic and refinement statistics for structures of chimeric 1a3a HCV NS3/4A protease bound to inhibitors asunaprevir, danoprevir and vaniprevir (PDB IDs: 5EQS, 5EGR, 5ESB). Statistics for the highest resolution shell are given in square brackets.

	Asunaprevir (ASV)	Danoprevir (DAN)	Vaniprevir (VAN)
Complexes	1a/3a	1a/3a	1a/3a
PDB Code	5EQS	5EGR	5ESB
Resolution range (Å)	25.3 – 1.8 [1.9–1.8]	50.0 – 1.9 [2.0–1.9]	33.3 – 2.2 [2.3–2.2]
Space Group	P212121	P212121	P212121
Molecules in AS	1	1	1
Cell Dimensions			
a	39.1	54.9	55.1
b	60.3	58.4	58.3
c	79.6	59.9	59.9
β (°)	90	90	90
Completeness (%)	92 [60]	97 [85]	94 [92]
Measured Reflections	69558 [2160]	60292 [2353]	39105 [1656]
Unique Reflections	15609 [995]	14041 [1202]	9488 [536]
Mean I/sigma(I)	10.2 [2.1]	7.7 [3.8]	17.8 [8.2]
Redundancy	4.4 [2.2]	4.3 [3.7]	4.5 [3.1]
R-merge (%)	9	9	6
RMSD in			
Bonds (Å)	0.015	0.012	0.042
Angles (°)	1.6	1.3	1.8
Rfactor	19.3	16.5	18.2
Rfree	24.5	20	23.2
Wilson B-factor	13.4	15.6	12.8
Ramachandran Favored (%)	98	98	99
Ramachandran Allowed (%)	1.6	1.6	0
Ramachandran Outliers (%)	0	0	0
Rotamer Outlier (%)	0.6	0	0
Clashscore	1.4	3.7	2.1
Average B-factor	16.3	25.0	21.4
Macromolecules	16.5	25.4	21.1
Ligands	10.6	24.0	35.5
Solvent	18.4	28.2	25.5

Crystallographic statistics were calculated using the Molprobity and the Phenix Software suite.

Table S3. Intramolecular hydrogen bonding network. Hydrogen bonds at the protease active site in crystal structures (distance in Å) and their averaged persistence during 100 ns simulations (percent time) for HCV GT-1 and 1a3a chimeric construct.

		Asunaprevir		Danoprevir		Vaniprevir	
		WT	1a3a	WT	1a3a	WT	1a3a
TYR56-Main-N	ASP81-Main-O	2.1 (80.4%)	2.0 (18.0%)	2.2 (81.6%)	>3.5 (50.1%)	2.2 (80.4%)	>3.5 (23.0%)
TYR56-Main-N	ASP81-Side-Oδ2	>3.5 (<20%)	>3.5 (<20%)	>3.5 (<20%)	>3.5 (<20%)	>3.5 (<20%)	>3.5 (<20%)
HIS57-Main-N	ASP81-Side-Oδ	1.8 (72.5%)	1.9 (93.8%)	1.9 (72.1%)	1.8 (82.6%)	1.9 (72.5%)	1.8 (85.1%)
HIS57-Side-Nδ1	ASP81-Side-Oδ	1.6 (96.8%)	1.6 (89.2%)	1.6 (98.4%)	1.6 (96.4%)	1.6 (96.8%)	1.8 (97.0%)
ARG123-Side-Nε	ASP168-Side-Oδ1	2.7 (<20%)	>3.5 (<20%)	2.4 (<20%)	>3.5 (<20%)	1.9 (<20%)	>3.5 (<20%)
ARG123-Side-NH2	ASP168-Side-Oδ1	>3.5 (31.3%)	>3.5 (<20%)	>3.5 (<20%)	>3.5 (<20%)	3.4 (31.3%)	>3.5 (<20%)
LYS136-Main-N	ILE132-Main-O	2.3 (28.1%)	2.0 (25.8%)	2.2 (<20%)	2.3 (<20%)	2.1 (28.1%)	2.5 (<20%)
SER138-Side-Oγ	LYS136-Main-O	>3.5 (10.0%)	>3.5 (<20%)	>3.5 (33.5%)	>3.5 (21.2%)	>3.5 (<20%)	>3.5 (<20%)
SER138-Side-Oγ	GLY137-Main-O	>3.5 (<20%)	>3.5 (<20%)	>3.5 (<20%)	>3.5 (<20%)	>3.5 (<20%)	>3.5 (<20%)
GLY140-Main-N	PHE154-Main-O	1.8 (90.0%)	1.9 (92.2%)	1.9 (85.4%)	1.8 (80.4%)	1.9 (90.0%)	1.9 (88.4%)
GLY141-Main-N	SER138-Main-O	>3.5 (<20%)	2.2 (<20%)	2.1 (<20%)	2.2 (19.2%)	2.1 (<20%)	2.2 (<20%)
PHE154-Main-N	GLY141-Main-O	1.8 (96.0%)	1.8 (96.2%)	1.8 (96.0%)	1.8 (94.2%)	1.8 (96.0%)	1.7 (94.8%)
ARG155-Main-N	ASP168-Main-O	1.9 (81.8%)	1.9 (67.7%)	2.0 (76.1%)	1.9 (60.1%)	1.9 (81.8%)	1.9 (69.9%)
ARG155-Side-Nε	ASP168-Side-Oδ2	1.6 (99.2%)	>3.5 (25.0%)	1.7 (99.0%)	>3.5 (<20%)	1.9 (99.2%)	>3.5 (<20%)
ARG155-Side-Nε	GLN168-Side-Oε1	>3.5 (<20%)	1.8 (47.7%)	>3.5 (<20%)	2.1 (78.2%)	>3.5 (<20%)	1.7 (63.7%)
ARG155-Side-NH1	GLN168-Side-Oε1	>3.5 (<20%)	>3.5 (<20%)	>3.5 (<20%)	2.6 (<20%)	>3.5 (<20%)	>3.5 (<20%)
ARG155-Side-NH1	ASP168-Side-Oδ1	2.3 (94.2%)	>3.5 (<20%)	2.4 (54.3%)	>3.5 (<20%)	>3.5 (94.2%)	>3.5 (<20%)
ARG155-Side-NH1	ASP168-Side-Oδ2	>3.5 (<20%)	>3.5 (<20%)	>3.5 (39.3%)	>3.5 (<20%)	1.8 (<20%)	>3.5 (<20%)
ARG155-Side-NH2	LYS80-Main-O	1.9 (76.1%)	1.7 (46.1%)	2.0 (81.6%)	2.2 (78.0%)	2.0 (76.1%)	2.1 (67.7%)
ALA156-Main-N	ASP168-Main-O	2.1 (50.1%)	2.1 (86.6%)	2.1 (71.5%)	2.2 (90.2%)	2.1 (50.1%)	1.9 (88.4%)
VAL158-Main-N	ALA166-Main-O	1.9 (63.3%)	1.9 (60.1%)	1.9 (82.8%)	1.8 (75.3%)	1.9 (63.3%)	2.2 (69.9%)
LYS165-Main-N	VAL158-Main-O	2.6 (<20%)	2.2 (19.0%)	2.3 (46.9%)	2.0 (<20%)	2.3 (<20%)	2.4 (<20%)
ASP168-Main-N	ALA156-Main-O	1.8 (71.3%)	1.8 (77.1%)	1.8 (85.2%)	1.6 (70.1%)	1.8 (71.3%)	1.6 (66.1%)

Table S4. Intermolecular hydrogen bonds between the protease and inhibitor. Hydrogen bonds between protease active site residues and the inhibitor in crystal structures (distance in Å) and their averaged persistence during 100 ns simulations (percent time) for HCV GT-1 and 1a3a chimeric construct.

		Asunaprevir		Danoprevir		Vaniprevir	
		WT	1a3a	WT	1a3a	WT	1a3a
SER1139-Main-N	P1' SO1	3.1 (48.1%)	2.9 (89.4%)	2.7 (<20%)	2.7 (<20%)	2.7 (86.6%)	2.8 (<20%)
SER139 H	P1' SO1	1.9 (<20%)	>3.5 (<20%)	>3.5 (<20%)	>3.5 (<20%)	>3.5 (<20%)	>3.5 (<20%)
SER1139-Side-Oγ	P1' SO1	2.1 (17.4%)	>3.5 (38.7%)	>3.5 (<20%)	2.0 (19.8%)	>3.5 (92.6%)	2.1 (20.2%)
GLY1137-Main-N	P1' SO2	2.2 (70.1%)	2.2 (81.6%)	2.3 (83.2%)	2.3 (85.2%)	2.1 (84.6%)	2.2 (88.2%)
HIP1057-Side-Nε2	P1' NH	2.1 (<20%)	2.0 (62.1%)	2.1 (34.9%)	2.2 (59.3%)	2.0 (61.3%)	2.1 (58.1%)
GLY1137-Main-N	P1 CO	2.0 (<20%)	2.1 (<20%)	2.0 (<20%)	2.1 (<20%)	2.3 (<20%)	2.2 (<20%)
SER1138-Main-N	P1 CO	2.9 (<20%)	3.0 (<20%)	2.7 (<20%)	2.5 (<20%)	2.8 (<20%)	2.6 (<20%)
SER1139-Side-Oγ	P1 CO	2.6 (<20%)	2.8 (<20%)	2.6 (<20%)	2.5 (<20%)	2.7 (<20%)	2.5 (<20%)
ARG1155-Main-O	P1 NH	1.9 (57.5%)	2.0 (84.4%)	1.9 (66.7%)	2.0 (84.4%)	2.0 (92.6%)	1.9 (83.6%)
ALA1157-Main-O	P3 NH	1.9 (43.3%)	1.9 (41.9%)	1.9 (82.0%)	1.9 (79.2%)	2.0 (88.2%)	2.0 (82.0%)
ALA1157-Main-N	P3 CO	1.9 (75.1%)	1.9 (60.7%)	1.9 (85.4%)	2.0 (80.4%)	1.9 (83.0%)	1.9 (83.0%)

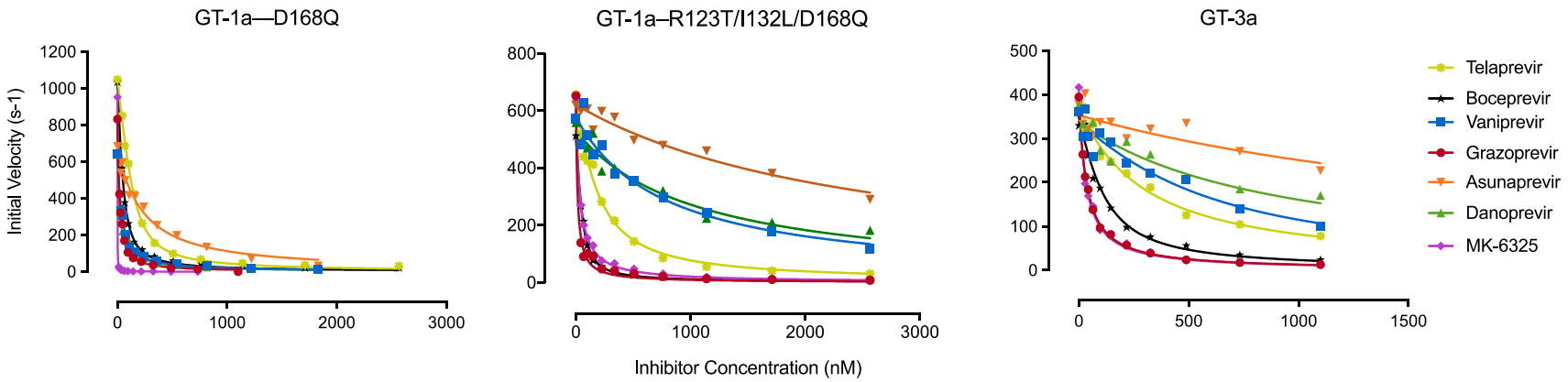


Figure S1. Inhibitor titration curves for representative assays used to determine the enzyme inhibition constants in Table S4 and Figure 2 of the manuscript. The K_i values for GT-1a were previously published (Ali, A.; Aydin, C.; Gildemeister, R.; Romano, K. P.; Cao, H.; Ozen, A.; Soumana, D.; Newton, A.; Petropoulos, C. J.; Huang, W.; Schiffer, C. A. *ACS Chem Biol* **2013**, *8*, 1469).

PERCENT IDENTITY MATRIX

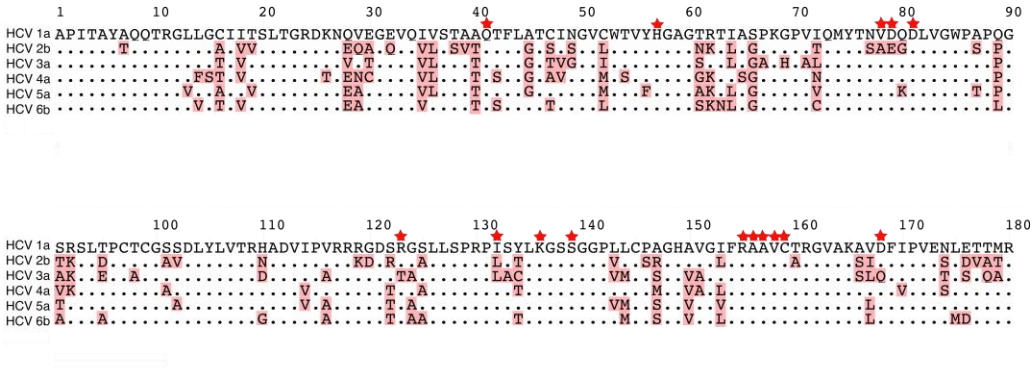
HCV.1a.M62321	100.0%					
HCV.2b.D10988	71.1%	100.0%				
HCV.3a.D17763	76.1%	70.6%	100.0%			
HCV.4a.DQ418788	80.0%	73.3%	75.0%	100.0%		
HCV.5a.AF064490	82.8%	74.4%	77.8%	80.0%	100.0%	
HCV.6b.D84262	82.2%	73.3%	79.4%	81.7%	84.4%	100.0%
	HCV.1a.M62321	HCV.2b.D10988	HCV.3a.D17763	HCV.4a.DQ418788	HCV.5a.AF064490	HCV.6b.D84262

PERCENT SIMILARITY MATRIX

HCV.1a.M62321	100.0%					
HCV.2b.D10988	83.3%	100.0%				
HCV.3a.D17763	87.8%	82.2%	100.0%			
HCV.4a.DQ418788	90.0%	86.1%	86.7%	100.0%		
HCV.5a.AF064490	93.3%	87.8%	87.8%	90.6%	100.0%	
HCV.6b.D84262	92.2%	86.7%	90.6%	92.8%	92.2%	100.0%
	HCV.1a.M62321	HCV.2b.D10988	HCV.3a.D17763	HCV.4a.DQ418788	HCV.5a.AF064490	HCV.6b.D84262

Figure S2. NS3/4A protease amino acid sequence similarity across genotypes. The percent identity and similarity matrix for the protease sequences of the 6 HCV genotypes.

A)



B)

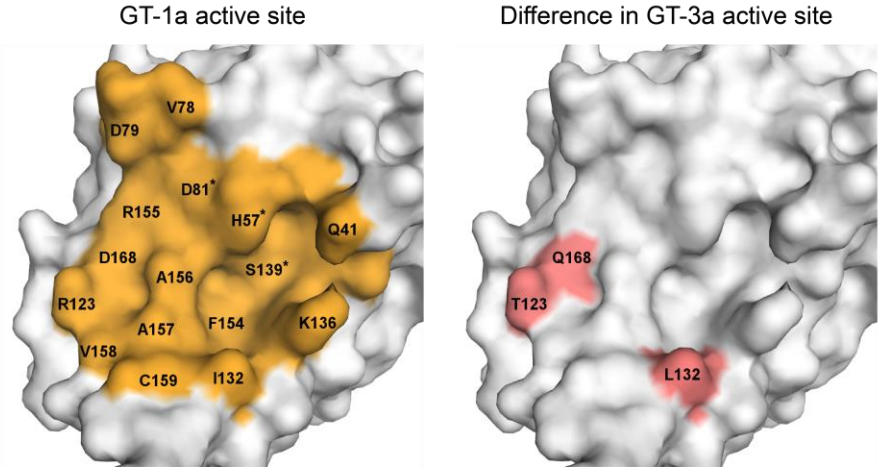


Figure S3. HCV NS3/4A protease polymorphisms. (A) Amino acid sequence alignment of protease from the 6 genotypes showing changes with respect to genotype 1a. The active site residues are indicated by red stars. (B) Active site residues (red stars in panel A) mapped onto the GT-1a protease structure (orange) with catalytic triad indicated by asterisks, and the polymorphisms in GT-3a active site relative to GT-1a (red).

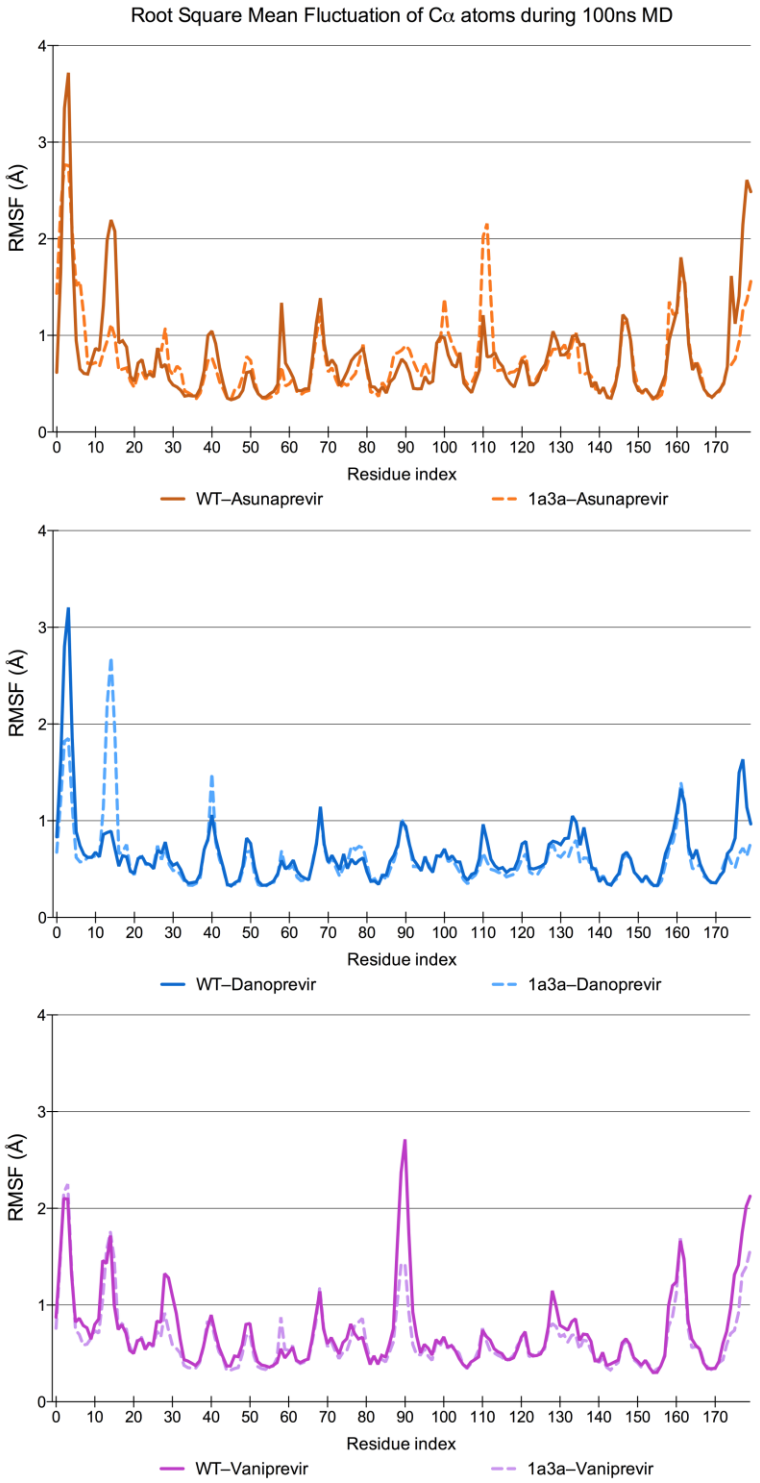


Figure S4. Root square mean fluctuations of protease Cα atoms during MD simulations.

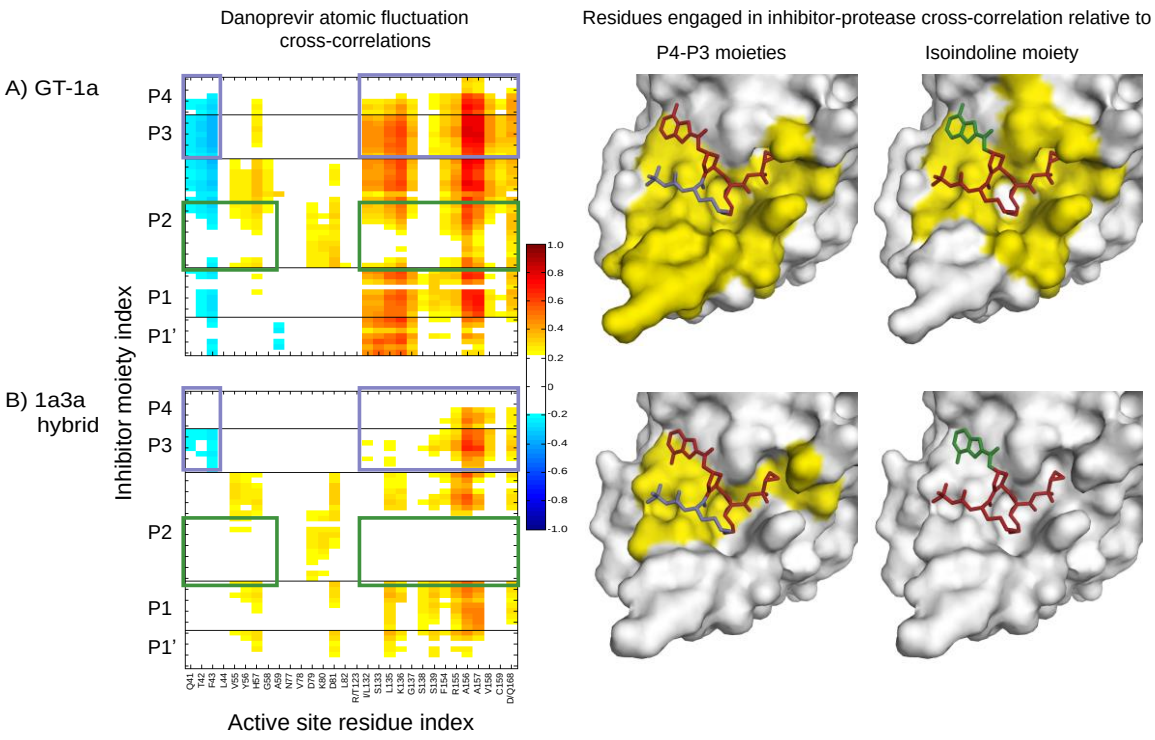


Figure S5. Effect of GT-3 polymorphisms on protease-danoprevir dynamic coupling. Cross-correlations between atomic fluctuations of protease active site residues and danoprevir atoms displayed on the crystal structure of (**A**) GT-1 and (**B**) chimeric 1a3a construct.

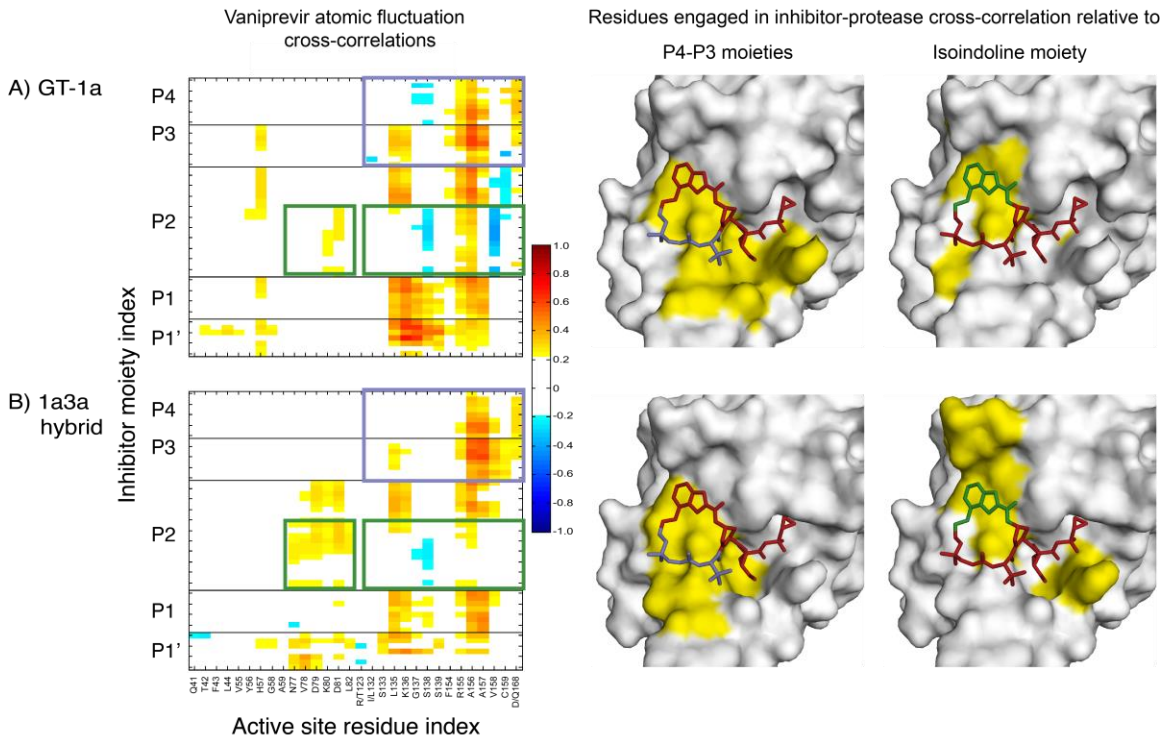


Figure S6. Effect of GT-3 polymorphisms on protease-vaniprevir dynamic coupling. Cross-correlations between atomic fluctuations of protease active site residues and vaniprevir atoms displayed on the crystal structure of (**A**) GT-1 and (**B**) chimeric 1a3a constructs.