Supplemental Tables

Table S1. ***Related to Figure 2.*** Inhibitory activity against GT1a HCV NS3/4A protease and drug resistant variants with fold changes with respect to GT1a wild-type, and the Michaelis-Menten constants (K_m) of the protease variants.

Inhibitor	***K*i (nM) (Fold change)**			
	GT1a WT	**Y56H**	**D168A**	**Y56H/D168A**
Grazoprevir	0.21 ± 0.03	11.9 ± 1.1 (57)	49.1 ± 1.6 (234)	531.7 ± 29.7 (2532)
Paritaprevir	0.35 ± 0.04	61.8 ± 13.1 (177)	297 ± 23 (849)	2974 ± 230 (8497)
5172-mcP1P3	3.29 ± 0.52	101 ± 17 (31)	82.4 ± 4.4 (25)	1179 ± 73 (358)
JZ01-15	3.60 ± 0.44	109 ± 19 (30)	52.0 ± 2.4 (14)	784 ± 50 (218)
AM-07	1.0 ± 0.1	29.7 ± 4.2 (29)	1823 ± 347 (1770)	2375 ± 1200 (2306)
MG-28	7.18 ± 1.02	205 ± 33 (29)	190 ± 13 (26)	1773 ± 394 (247)
Danoprevir	1.0 ± 0.1	2.9 ± 0.3 (3)	199 ± 64 (199)	520.3 ± 58.0 (520)
	K_m (µM)			
	7.9 ± 1.2	5.3 ± 0.5	1.3 ± 0.1	3.2 ± 0.4

Table S2. ***Related to Figure 2.*** Antiviral activity against wild-type GT1a HCV and drug resistant variants, with fold changes with respect to wild-type.

Inhibitor	Replicon EC_{50} (nM) (Fold change)			
	GT1a	Y56H	D168A	Y56H/D168A
Grazoprevir	0.26	35.8 (138)	26.8 (103)	>500 (1923)
Paritaprevir	0.17	23.5 (138)	17.9 (105)	>500 (2941)
5172-mcP1P3	0.56	28.7 (51)	12.9 (23)	>500 (893)
JZ01-15	0.47	24.6 (52)	4.8 (10)	231 (491)
AM-07	1.43	90.6 (63)	177.7 (124)	>500 (350)
Danoprevir	0.57	6.2 (11)	50.6 (89)	136 (239)

Table S3. ***Related to Figure 3.*** Gibbs free energy of binding against HCV NS3/4A protease and drug resistant variants, calculated from the enzyme inhibition constants in Table S1.

Inhibitor	ΔG (kcal/mol)			
	GT1a	Y56H	D168A	Y56H/D168A
Grazoprevir	-12.97 ± 0.08	-10.62 ± 0.05	-9.79 ± 0.02	-8.41 ± 0.03
Paritaprevir	-12.67 ± 0.07	-9.66 ± 0.12	-8.75 ± 0.05	-7.40 ± 0.05
5172-mcP1P3	-11.37 ± 0.09	-9.37 ± 0.10	-9.49 ± 0.03	-7.94 ± 0.04
JZ01-15	-11.31 ± 0.07	-9.33 ± 0.10	-9.76 ± 0.03	-8.18 ± 0.04
AM-07	-12.04 ± 0.07	-10.08 ± 0.08	-7.69 ± 0.11	-7.54 ± 0.29
Danoprevir	-12.06 ± 0.06	-11.44 ± 0.06	-8.98 ± 0.19	-8.42 ± 0.06

Supplemental Figures

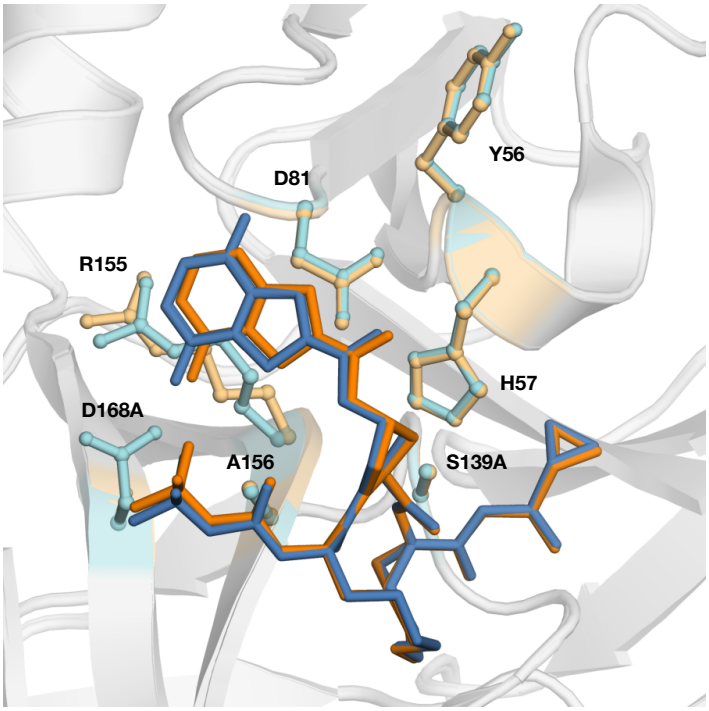

Figure S1. ***Related to Figure 4.*** **Crystal structure of danoprevir bound to WT and D168A HCV NS3/4A proteases.** Superposition of danoprevir bound to WT (blue) and D168A (orange) proteases. Danoprevir's P2 isoindoline moiety occupies two confirmations in the protease active site. Drug resistance residues and the catalytic triad residues (His57, Asp81 and S139A) are shown as sticks.

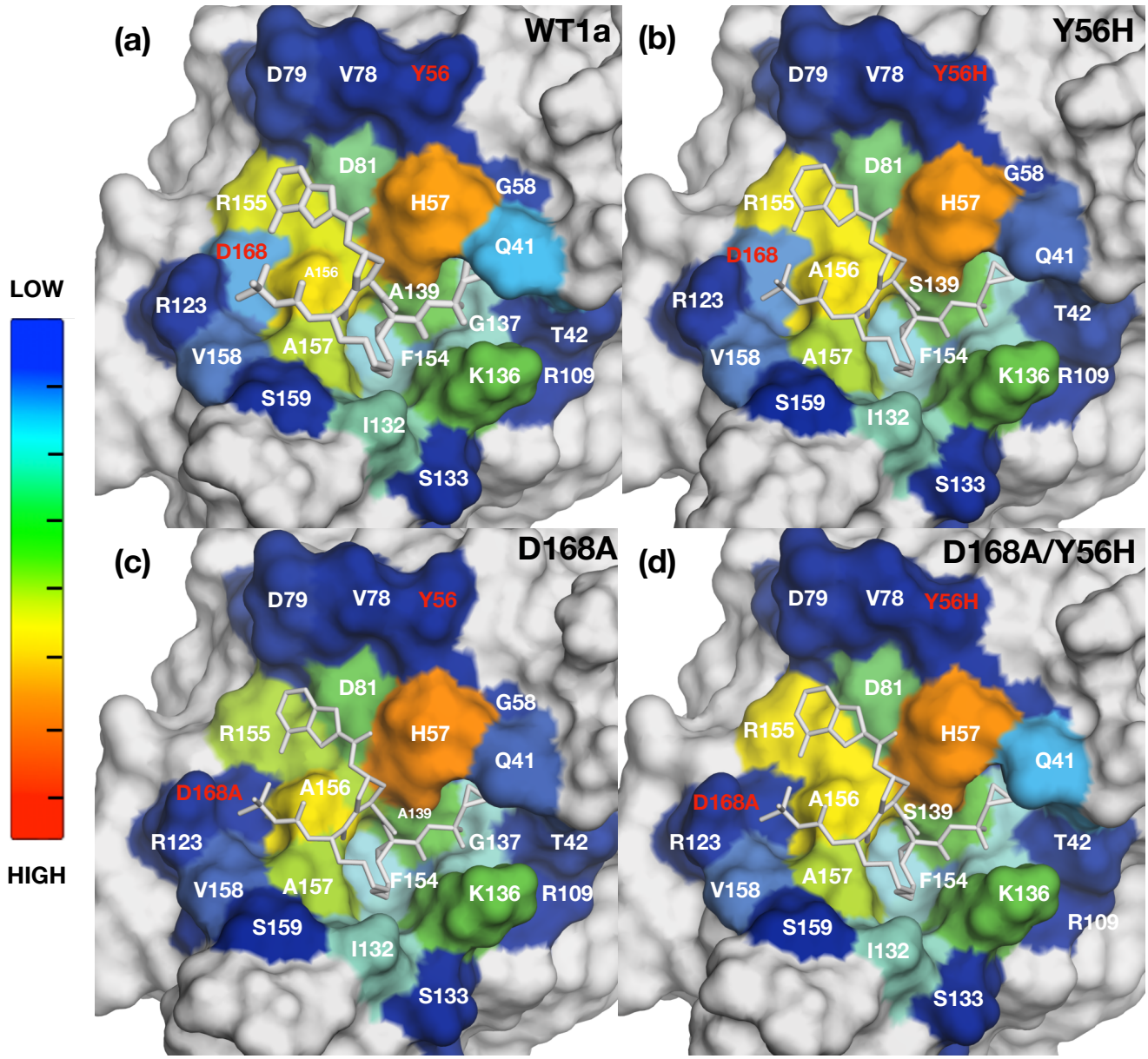

Figure S2. ***Related to Figure 5.*** **Packing of danoprevir in the active site of HCV NS3/4A protease variants.** The van der Waals (vdW) contact energies of danoprevir from crystal structures mapped onto the protease surface in (a) WT, (b) Y56H, (c) D168A and (d) Y56H/D168A proteases. The warmer (red) and cooler (blue) colors indicate more and less contacts with the inhibitor, respectively. Residues with substitutions among the variants are labeled in red.

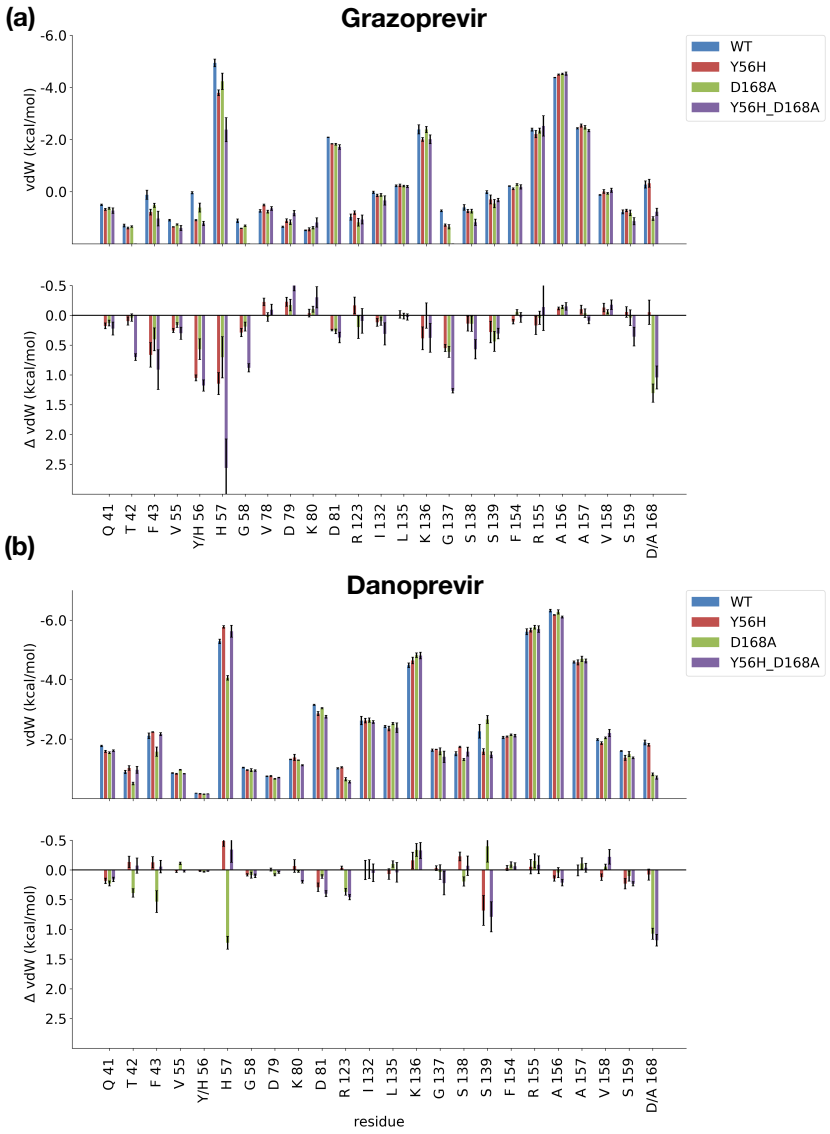

Figure S3. ***Related to Figure 5.*** **Inhibitor interactions with wildtype HCV NS3/4A protease and variants by residue.** The van der Waals (vdW) contact energies and change in vdW (ΔvdW) relative to wildtype for (a) grazoprevir, and (b) danoprevir bound to protease variants, calculated from MD trajectories. These vdW values are mapped onto the protease surface in Figure 5.