Supporting Information

Structural Analysis of Asunaprevir Resistance in HCV NS3/4A Protease

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Table S1. Crystallography statistics for HCV NS3/4A protease-inhibitor structures.

Complexes/crystal	WT-ASV	R155K-ASV	WT-ASVmc
PDB ID	4WF8	4WH6	4WH8
Resolution (Å)	1.6	1.9	2.7
Space Group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Molecules in AU[a]	1	1	2
Cell Dimensions			
a	39.35	54.92	58.43
b	60.89	58.41	54.96
c	80.87	60.09	59.88
β (°)	90	90	90
Completeness (%)	92.7	91.7	99.8
Measured Reflections	170394	61851	26199
Unique Reflections	22158	12673	6134
Average I/σ[c]	11.6 (7)	9.2 (4.3)	10.5
Redundency	7.7	4.9	4.3
R_{sym} (%)[b, c]	6.8 (31.5)	10 (32)	6.8 (21.2)
RMSD[d] in			
Bonds (Å)	0.013	0.006	0.015
Angles (°)	1.67	1.5	1.6
R_{factor}[e]	19.2	16.8	17.6
R_{free}[f]	21.8	21.9	24.8

a, AU, asymmetric unit.
b, $R_{sym} = \Sigma|I - \langle I \rangle|/\Sigma I$, where I = observed intensity, <I> = average intensity over symmetry equivalent.
c, values in parentheses are for the highest resolution shell.
d, RMSD, root mean square deviation.
e, $R_{factor} = \Sigma||F_o| - |F_c||/\Sigma|F_o|$.
f, R_{free} was calculated from 5% of reflections, chosen randomly, which were omitted from the refinement process.

Table S2. Intra and intermolecular hydrogen bond analysis of protease-inhibitor complexes

	Complex Structure								
	WT-ASV			R155K-ASV			WT-ASVmc		
	Donnor Atom	Acceptor Atom	Distance (Å)	Donnor Atom	Acceptor Atom	Distance (Å)	Donnor Atom	Acceptor Atom	Distance (Å)
S2 Subsite Residues	His57 Hδ	Asp81 COδ1	1.8	His57 NH	Asp81 COδ1	1.9	His57 NH	Asp81 COδ1	1.7
	His57 NH	Asp81 COδ2	1.6	His57 Hδ1	Asp81 COδ2	1.7	His57 NHδ1	Asp81 COδ2	1.6
	Arg155 NH	Asp168 CO	1.9	Lys155 NH	Asp168 CO	2.0	Arg155 NH	Asp168 CO	1.9
	Arg155 NεH	Asp168 Oδ1	1.6	Arg123 NεH	Asp168 COδ1	1.6	Arg155 NH12	Asp168 COδ1	2.0
	Arg123 NεH	Asp168 Oδ2	2.7	Arg123 NH21	Asp81 COδ2	2.1	Arg155 NεH	Asp168 COδ1	1.7
							Arg123 NH22	Asp168 COδ2	2.9
							Arg123 NHε	Asp168 COδ2	1.6
	Protease Atom	Inhibitor Atom	Distance (Å)	Protease Atom	Inhibitor Atom	Distance (Å)	Protease Atom	Inhibitor Atom	Distance (Å)
Protease Backbone	Ala157 CO	HN17	1.9	Ala157 CO	NHBA	1.9	His57 Nε2	NH39	2.1
	Ala157 NH	O15	1.9	Ala157 NH	OAL	2.0	Ala157 NH	O34	2.1
	Arg155 CO	NH06	1.9	Lys155 CO	NHBB	2.0	Arg155 CO	H35	4.0
	His57 Nε2	NH45	2.2	His57 Nε2	NHBC	2.4	Arg155 CO	H13	2.3
	Ser138 NH	O44	2.9	Ser138 NH	OAK	2.6	Ser138 NH	O38	3.0
	Gly137 NH	O44	2.0	Gly137 NH	OAK	2.1	Gly137 NH	O38	2.3
	Gly137 NH	O48	2.3	Gly137 NH	OAM	2.2	Ala139 NH	O38	2.8
	Ser139 NH	O44	2.6	Ser139 NH	OAK	2.5	Gly137 NH	O41	2.4
	Ser139 NH	O47	3.1	Ser139 NH	OAN	2.7	Gly137 NH	O42	2.5
	Ser139 Hγ	O47	2.1	Ser139 Hγ	OAN	1.8	Ala139 NH	O42	2.7
	Water Molecule	Inhibitor Atom	Distance (Å)	Water Molecule	Inhibitor Atom	Distance (Å)			
Water Molecules	HW1 HOH 26	O20	2.1	HW1 HOH 29	OBF	1.9			
	HW1 HOH 122	O08	1.8	HW1 HOH 142	NAZ	2.0			
	HW2 HOH 135	O48	2.6	HW1 HOH 145	O	1.9			
				HW1 HOH 139	O	1.9			
				HW2 HOH 139	OAM	2.0			
				HW2 HOH 60	OAM	2.1			

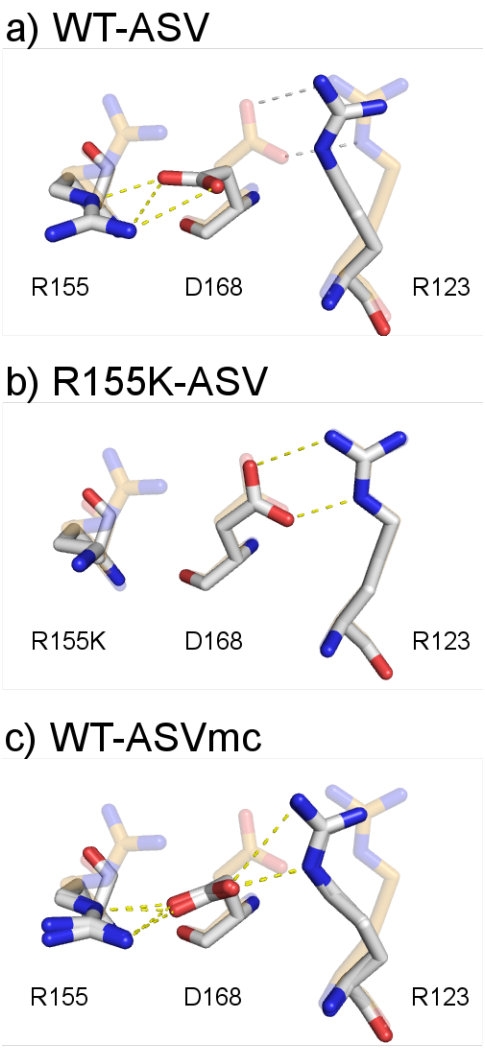

Figure S1. Electrostatic network spanning Arg155-Asp168-Arg123 is not conserved in the mutant structure: (a) in the apo NS3/4A (orange transparent sticks, PDB: 3RC6), Asp168 is oriented toward Arg123 for interactions (grey dashed lines). The WT-ASV complex (white sticks) has Asp168's carbonyl oxygens oriented towards Arg155's nitrogens forming a salt-bridge (yellow dashed lines). (b) In the R155K complex (white sticks), Asp168 rotates away from Lys155 and towards Arg123 for interaction, as is observed in the apo structure. (c) In the WT-ASVmc (white sticks), Asp168's position enables an extended salt-bridge formation spanning Arg155-Asp168-Arg123. Both conformers of the crystal structure are represented.

Figure S2

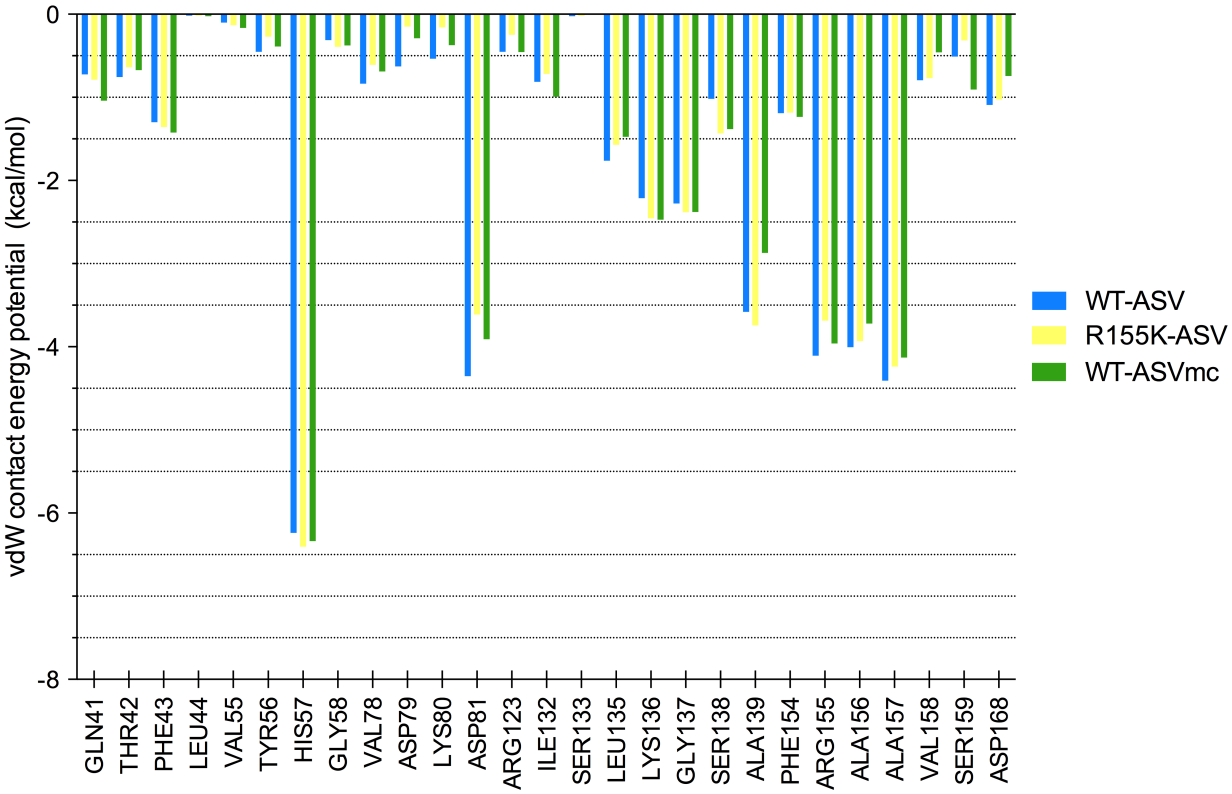

Figure S2. The van der Waals contact energy of protease residues in WT-ASV (blue), R155K-ASV (yellow) and WT-ASVmc (green) structures, which are mapped onto the structure in main manuscript Figure 2. ASV's binding mode is characterized by high vdW contact energy with catalytic residues His57, Asp81 as well as S_2 residues Arg155, Ala156 and Ala157.

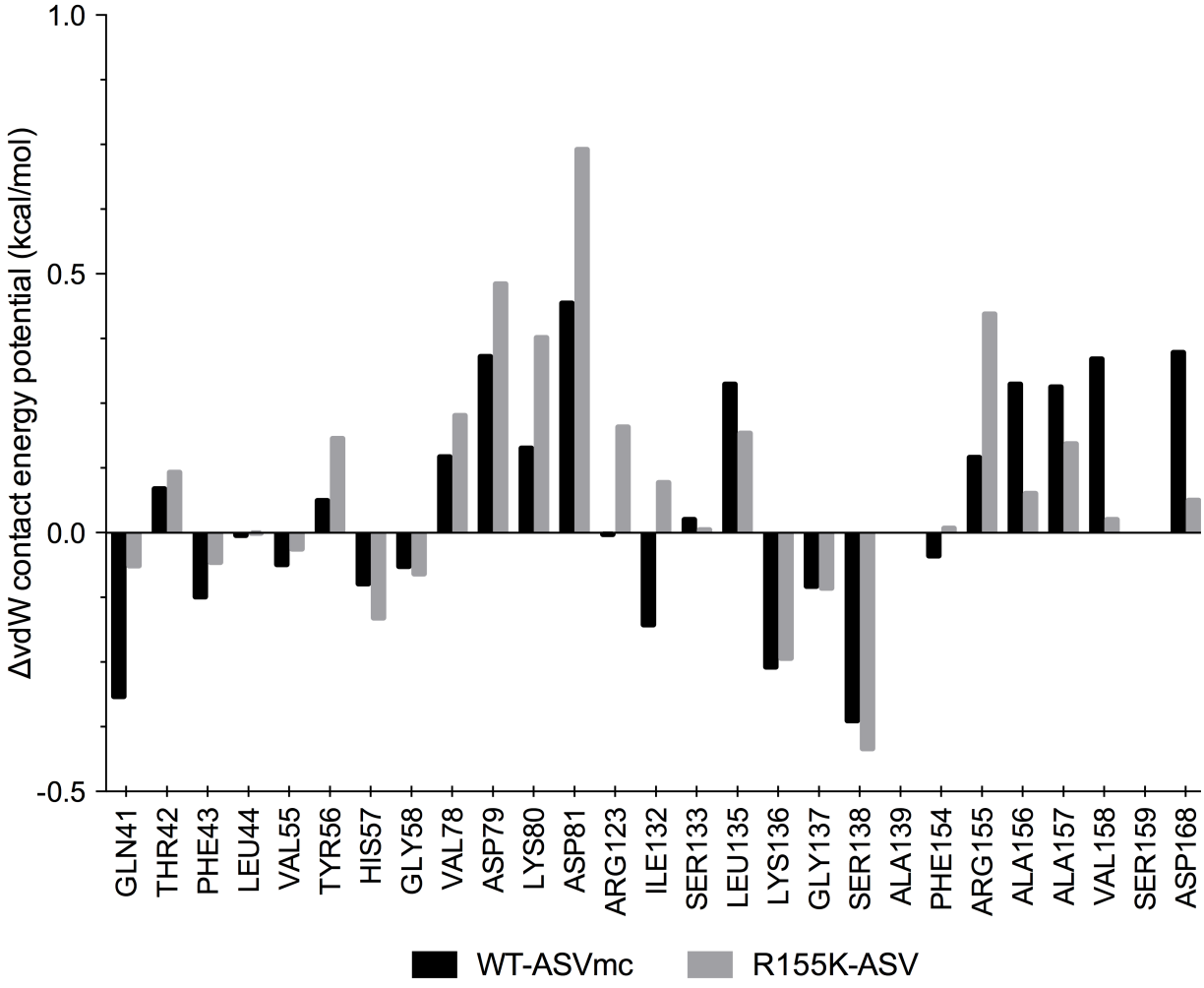

Figure S3. Change in van der Waals contact energy of protease residues with the inhibitor in WT-ASVmc (black) and R155K-ASV (grey) relative to the WT-ASV structure.