Supplementary Information

Direct-acting antiviral resistance of Hepatitis C virus is promoted by epistasis

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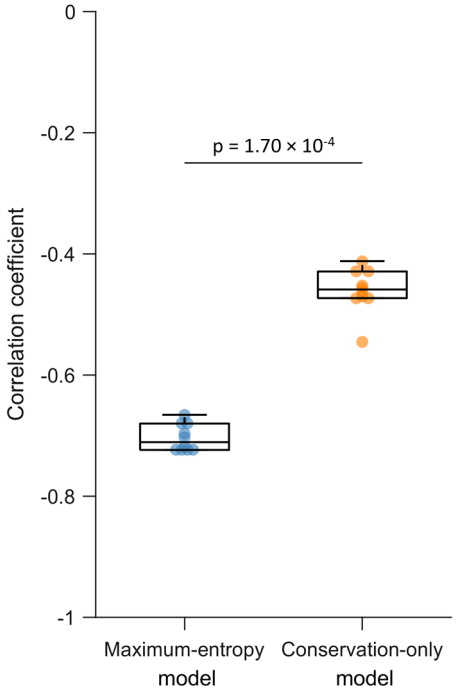
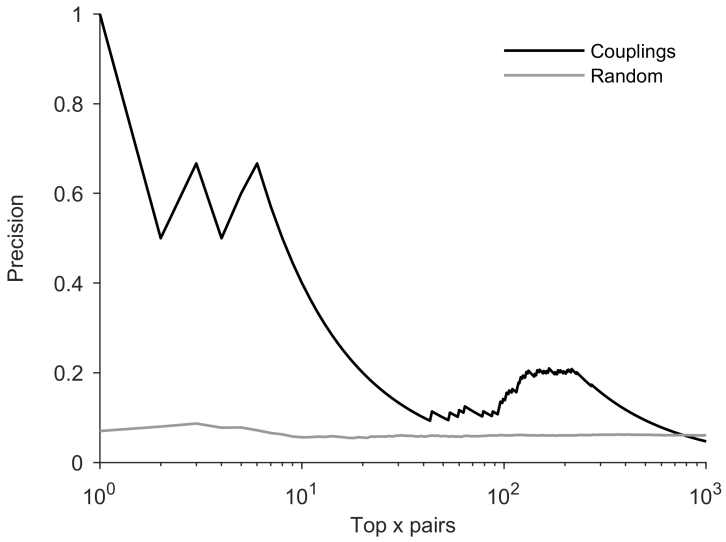
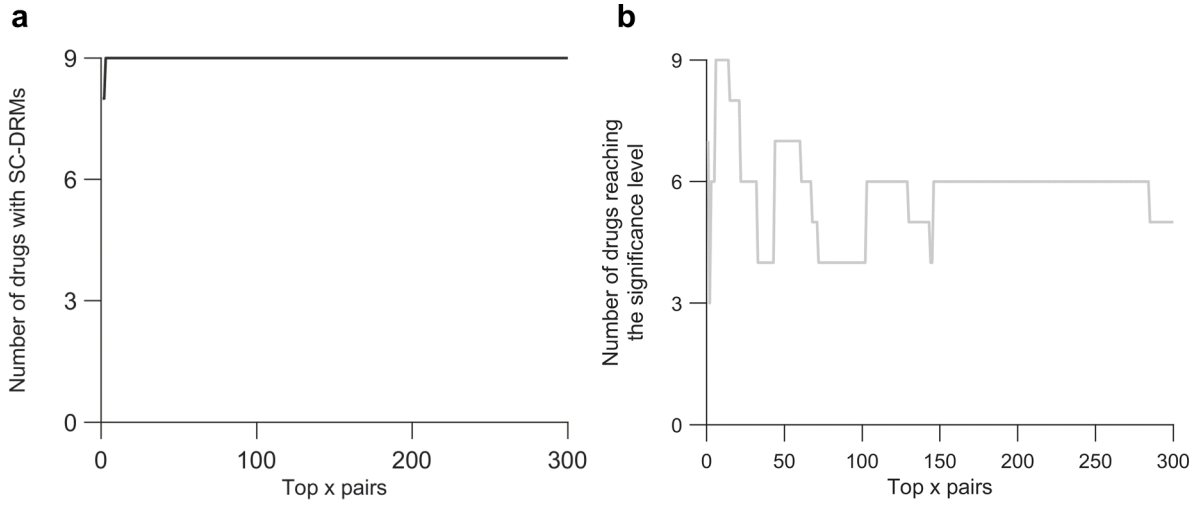
Supplementary Figures



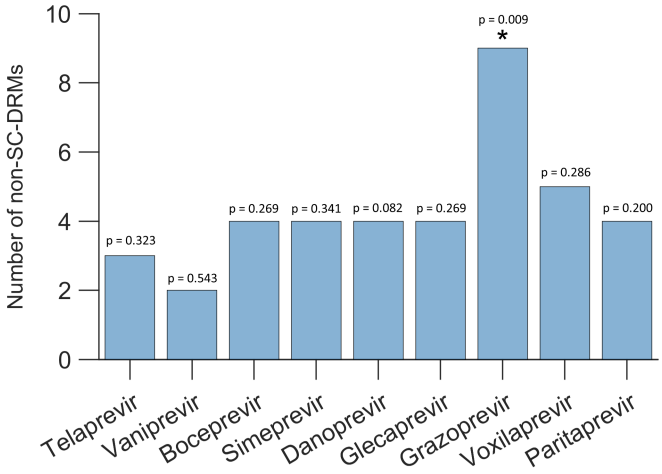
Supplementary Figure 1: **Robustness of the correlation observed between model energies and experimental fitness values. Results are shown for the maximum-entropy model that considers epistatic interaction and for the conservation-only model that ignores epistasis.** The sequence data used for inferring each model was generated by a standard bootstrap procedure using ten samples. The reported p-value was calculated using the two-sided Mann-Whitney test ($n_1 = 9$ SC-DRMs and $n_2 = 11$ remaining DRMs). Source data are provided as a Source Data file.



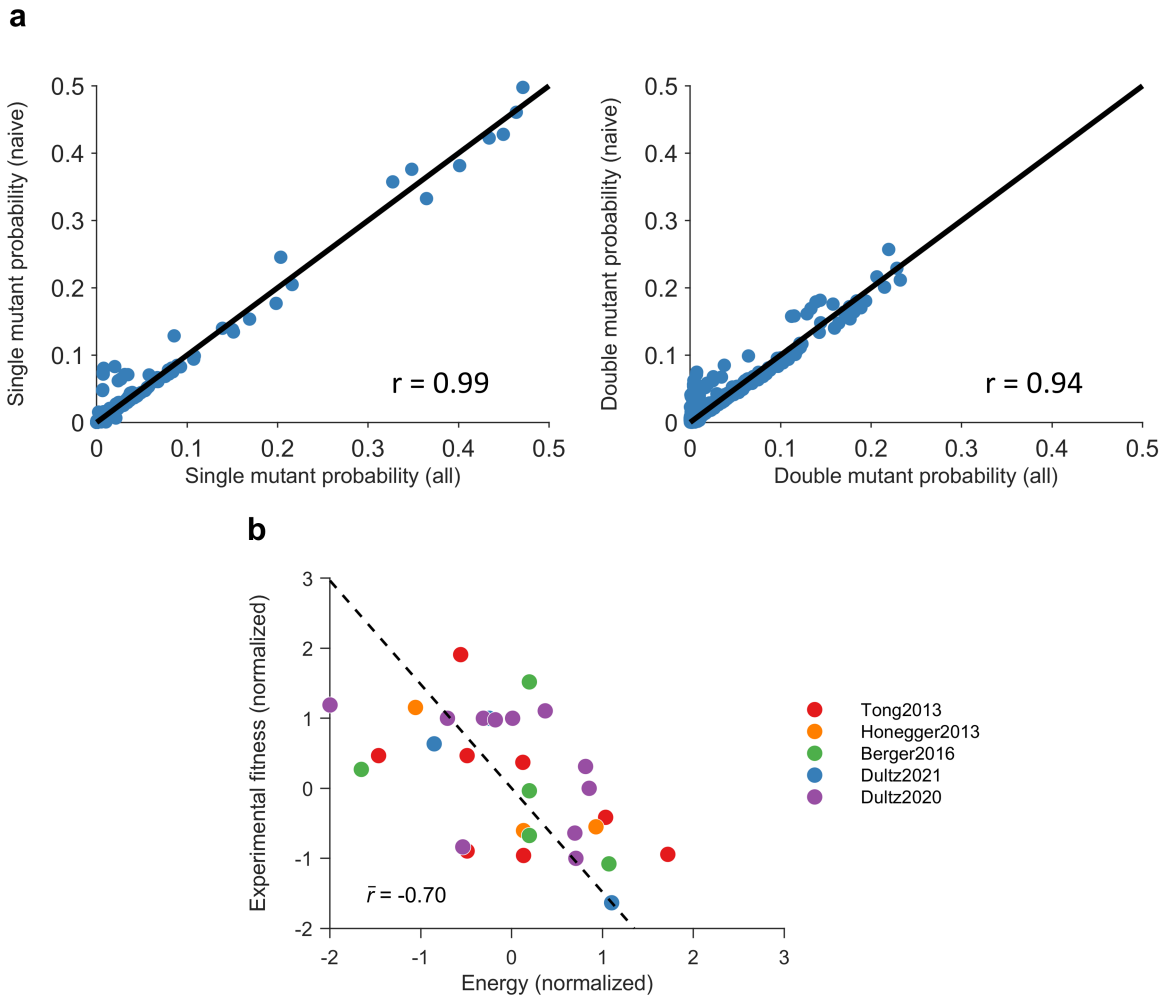
Supplementary Figure 2: **Top ranked pairs of mutations based on the strength of their couplings (ranked by the values of -J from Eq. 1) are more likely to involve DRMs as compared to pairs picked randomly.** Precision is the proportion of top x pairs that involve at least one DRM based on the model couplings (shown in black) or picking pairs randomly (shown in gray; results averaged over 10 random realizations). Source data are provided as a Source Data file.



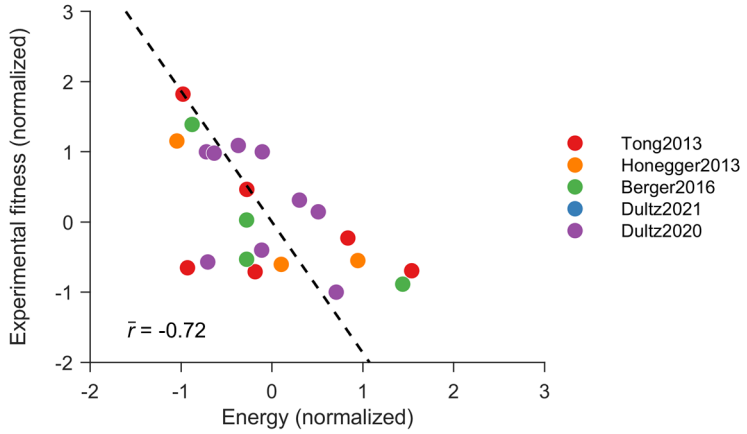
Supplementary Figure 3: **Robustness of the enrichment of SC-DRMs in each drug (Figure 4) to the number of top-coupled pairs of mutations used to define SC-DRMs.** (**a**) Number of drugs with at least one SC-DRM vs. top x (x $\in$ [0, 300]) pairs of mutations used to define SC-DRMs. Pairs of mutations were ranked based on the couplings of the inferred model (ranked by the values of -J from Eq. 1), and DRMs appearing among the top x pairs of mutations were considered to be SC-DRMs. (**b**) Number of drugs for which the p-value associated with the number of SC-DRMs reached the significance level (p-value < 0.05) vs. top x (x $\in$ [0, 300]) pairs of mutations used to define SC-DRMs. Statistically significant enrichment of SC-DRMs was observed for the majority of drugs ($\geq$5) for almost all values of x. Here, the p-value measures the probability of observing by a random chance at least the observed number of SC-DRMs among all DRMs associated with a drug (see Methods for details). DRMs against each drug are listed in Table 1.[1–4]Source data are provided as a Source Data file.



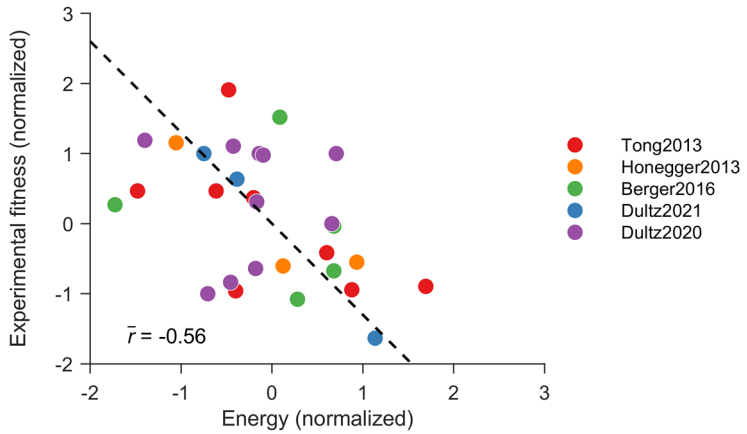
Supplementary Figure 4: **Statistical significance of the number of non-SC-DRMs associated with each drug.** Non-SC-DRMs are DRMs not appearing in the top 300 pairs of mutations based on the couplings of the inferred model (ranked by the values of -J from Eq. 1). The p-value measures the probability of observing by a random chance at least the observed number of non-SC-DRMs among all DRMs associated with a drug (see Methods for details). Statistical significant results (p-value < 0.05) are marked with a star on the top of each bar. Source data are provided as a Source Data file.



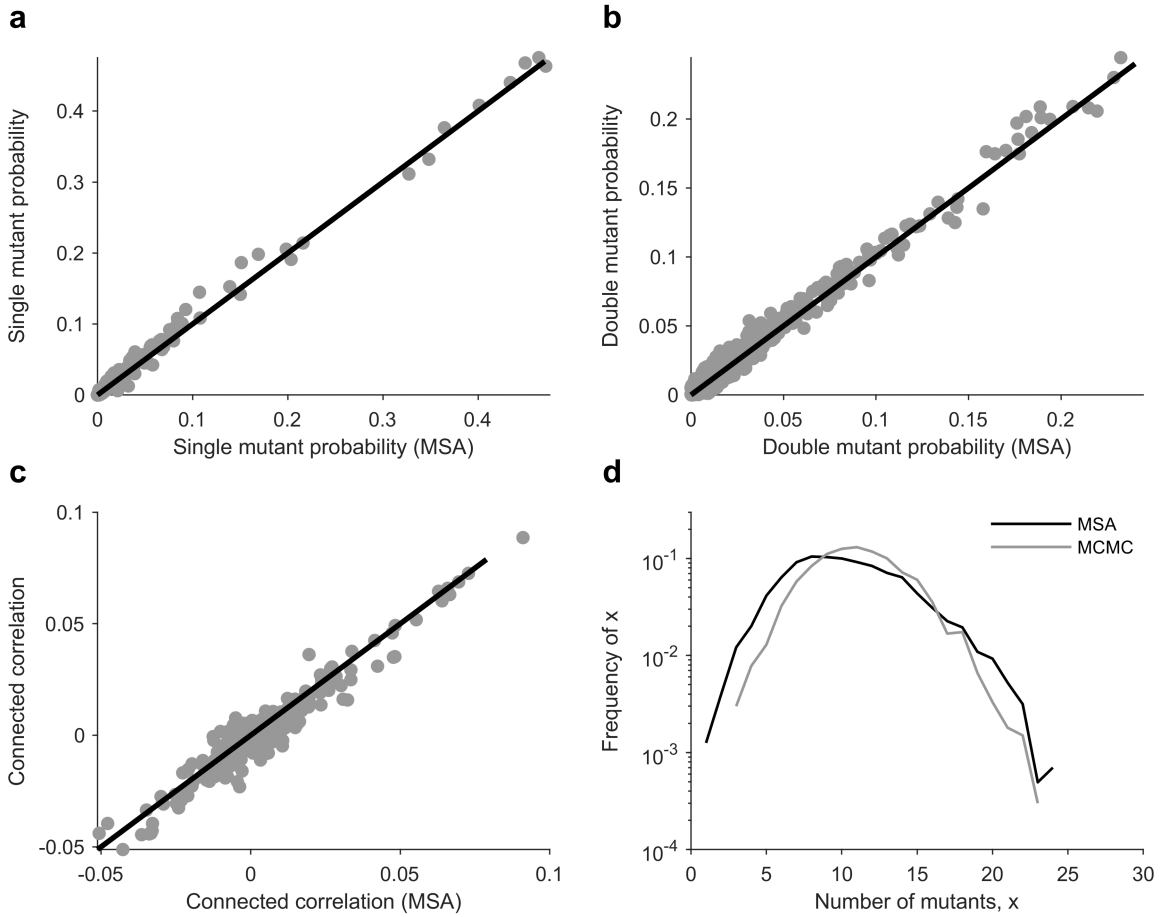
Supplementary Figure 5: **Comparison of statistical properties and model predictions based on complete data with those based on a subset of drug-naïve patients.** (**a**) Correlation of single mutant probabilities (left panel) and double mutant probabilities (right panel) between sequences from all patients (7370 sequences) and the subset of drug-naïve patients (5877 sequences). (**b**) Correlation between model predicted energies and experimental fitness measurements compiled from different studies (mentioned in the legend). Normalization of both fitness measurements and predicted model energies was performed by subtracting the mean from each data set and dividing by its standard deviation. Source data are provided as a Source Data file.



Supplementary Figure 6: **Correlation between the model predicted energies and 36 experimental fitness measurements that are associated with DRMs.** These fitness measurements were compiled from different studies that are mentioned in the legend. Normalization of both fitness measurements and predicted model energies was performed by subtracting the mean from each data set and dividing by its standard deviation. Source data are provided as a Source Data file.



Supplementary Figure 7: **Correlation between the sequence energy obtained from newly inferred model and in-vitro infectivity measurements.** This model was inferred by including all sequences (9683 sequences) and weighted each sequence without patient information (2167 sequences) as 1. These fitness measurements were compiled from different studies that are mentioned in the legend. Normalization of both fitness measurements and predicted model energies was performed by subtracting the mean from each data set and dividing by its standard deviation. Source data are provided as a Source Data file.



Supplementary Figure 8: **Statistical validation of the inferred model for the HCV NS3 protein.** Comparison of the (**a**) single mutant probabilities, (**b**) double mutant probabilities, (**c**) connected correlations, and (**d**) distribution of the number of mutants per sequence obtained from the MSA and those predicted by the inferred model. Connected correlations represent correlations which cannot be explained by lower order mutant probabilities and is given by $f_{ij}(a,b) - f_i(a)f_j(b)$, where $f_i(a)$ is the probability of observing mutant a at residue i while f_{ij} is the probability of simultaneously observing mutants a and b at residues i and j respectively. The number of mutants per sequence is the number of amino acids that are different in a sequence from those of the consensus sequence (sequence constructed with the most-frequent amino acid at each residue). Samples were generated from the inferred model using the Markov Chain Monte Carlo method.[5] Source data are provided as a Source Data file.

Supplementary Tables

Supplementary Table 1: **List of NS3 residues in each co-evolutionary sector inferred using RocaSec[6,7]**

Sectors	Residues in sector
1	29, 40, 51, 53, 54, 55, 64, 66, 67, 80, 86, 87, 94, 121, 122, 124, 150, 151, 174, 178, 185, 186, 187, 192, 197, 200, 247, 248, 264, 295, 315, 318, 329, 333, 334, 337, 338, 358, 370, 371, 372, 379, 383, 394, 395, 417, 418, 484, 485, 490, 517, 518, 586, 593, 594, 610, 614, 615
2	36, 40, 42, 51, 52, 53, 54, 55, 64, 69, 70, 71, 80, 87, 94, 124, 151, 155, 156, 157, 170, 171, 174, 185, 186, 187, 192, 197, 224, 225, 247, 248, 264, 295, 315, 318, 337, 338, 340, 341, 358, 370, 371, 372, 379, 383, 384, 386, 394, 395, 401, 402, 417, 418, 484, 485, 517, 518, 557, 558, 593, 594, 609, 610, 614, 615
3	452, 453, 459, 460, 461, 462, 463, 468, 471, 511, 512
4	29, 36, 40, 42, 51, 52, 53, 54, 55, 64, 66, 80, 86, 87, 91, 124, 150, 151, 155, 156, 157, 170, 171, 178, 192, 197, 200, 224, 225, 247, 248, 295, 318, 329, 333, 334, 337, 338, 340, 341, 370, 371, 372, 379, 383, 384, 386, 394, 395, 401, 402, 417, 418, 484, 485, 490, 505, 517, 518, 541, 576, 586, 593, 594, 609, 610
5	1, 2, 3, 4, 5, 6, 11, 12, 15, 19, 24, 29, 36, 42, 51, 52, 53, 54, 55, 64, 67, 80, 87, 91, 124, 150, 151, 155, 156, 157, 170, 171, 192, 196, 200, 213, 329, 337, 338, 340, 341, 357, 370, 371, 401, 402, 403, 404, 469, 470, 472, 484, 485, 517, 518, 535, 570, 614, 615
6	2, 3, 4, 5, 6, 11, 12, 15, 19, 24, 29, 36, 42, 51, 52, 53, 54, 55, 64, 67, 80, 86, 87, 91, 121, 122, 124, 150, 151, 155, 156, 157, 170, 171, 192, 196, 197, 200, 318, 329, 333, 334, 337, 338, 340, 341, 357, 370, 371, 383, 386, 401, 402, 403, 404, 417, 418, 469, 470, 472, 484, 485, 490, 517, 518, 586, 614, 615
7	29, 40, 51, 53, 54, 55, 66, 67, 86, 87, 91, 94, 121, 122, 124, 150, 151, 178, 185, 186, 187, 192, 196, 197, 200, 224, 225, 247, 248, 252, 295, 309, 310, 315, 318, 333, 334, 337, 338, 357, 358, 370, 371, 379, 384, 394, 395, 399, 401, 402, 403, 404, 469, 470, 472, 484, 485, 517, 518, 557, 558, 576, 586, 593, 594

Supplementary Table 2: **List of NS3 residues in experimentally-known biochemical domains**

Biochemical Domains	**Acronyms**	**Residues involved in each domain**	**References**
NS4A interface for protease activation	NS3-NS4A-Pro-Act	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22	8
NS3-NS4A membrane association	NS3-NS4A-Mem-Asso	10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24	9
NS5A hyper-phosphorylation	NS5A-Hyper-Phos	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 139	10,11
Motif important for enzymatic and helicase activities in NS3	NS3-Motif-Enz-Heli	460, 461, 462, 463, 464, 465, 466, 467	12
Intra-dimer interface in NS3 helicase	NS3-Intra-Dimer-Int	435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 545, 546, 547, 548, 549, 550, 551, 552, 553, 584, 585, 586, 587, 588, 589, 590, 591	13

Supplementary Table 3: **List of top-coupled mutations that are predicted to be compensatory for SC-DRMs**

SC-DRM	**Total number of selected sequence backgrounds**	**Associated compensatory mutation**	**Percentage of sequence backgrounds where mutation has a compensatory effect**
41R	7362	168E	100%
168E	7318	41R	100%
170V	7011	174S	73.2%
80K	4553	615V	61.1%
122G	6953	174S	60.2%
122C	7207	174S	51.8%
55A	7250	40T	29.6%
80K	4553	91S	22.7%
36L	7292	197Y	11.5%
122C	7207	197Y	10.3%
122C	6953	318T	10%

Each row shows the SC-DRM, the number of MSA sequences lacking the SC-DRM, the associated compensatory mutation (among top 300 pairs of mutations with large values of $-J_{ij}$), and the percentage of sequences where the associated mutation was found to compensate for the SC-DRM.

Supplementary Table 4: **List of DRM-associated residues in the binding residues of drugs with known structure**

Drug	Residues with DRMs in binding residues
Danoprevir	**41**, 43, **55**, **80**, 132, 138, 155, 156, 158, **168**
Vaniprevir	**41**, 43, **55**, **80**, 132, 138, 155, 156, 158, **168**
Telaprevir	**41**, 43, **55**, 132, 138, 155, 156, 158, **168**
Grazoprevir	**41**, 43, **55**, 56, 132, 138, 155, 156, 158, **168**

SC-DRMs are shown in **bold** and DRMs from other drugs exclusively are underlined.

Supplementary Table 5: **List of binding residues of drugs with known structures.**

Drug	Binding residues
Danoprevir	41, **42**, 43, 55, **57**, **58**, **78**, **79**, 80, **81**, **123**, 132, **135**, **136**, **137**, 138, **139**, **154**, 155, 156, **157**, 158, **159**, 168
Vaniprevir	41, **42**, 43, 55, **57**, **58**, **78**, **79**, 80, **81**, **123**, 132, **135**, **136**, **137**, 138, **139**, **154**, 155, 156, **157**, 158, **159**, 168
Telaprevir	41, **42**, 43, 55, **57**, **81**, **123**, 132, **135**, **136**, **137**, 138, **139**, **154**, 155, 156, **157**, 158, **159**, 168
Grazoprevir	41, **42**, 43, 55, 56, **57**, **58**, **78**, **81**, **123**, 132, **135**, **136**, **137**, 138, **139**, **154**, 155, 156, **157**, 158, **159**, 168

Residues that are not associated with any DRMs are shown in **bold**. Of these, residues that are associated with strong compensatory interactions based on our model (top 300 pairs of mutations with large values of $-J_{ij}$) are also underlined.

Supplementary Table 6: **Efficacy of each NS3-targeting drug and the number of SC-DRMs associated with them**

Drug	DAA regimen	Number of SC-DRMs	Weighted average of SVR rates (%)	Total number of patients	References
Telaprevir	Telaprevir	3	68.5	992	[14–16]
Vaniprevir	Vaniprevir	2	73.6	110	[17,18]
Boceprevir	Boceprevir	5	63.7	947	[19,20]
Simeprevir	Simeprevir	6	71.8	1132	[21–24]
	Simeprevir-sofosbuvir		91.8	425	[25,26]
Danoprevir	Danoprevir	2	72.0	1264	[27–29]
	Danoprevir-sofosbuvir		100	58	[30]
Glecaprevir	Glecaprevir-pibrentasvir	5	99.4	703	[31]
Grazoprevir	Elbasvir-grazoprevir	7	96.6	1297	[32]
Voxilaprevir	Sofosbuvir-velpatasvir-voxilaprevir	8	95.2	461	[33,34]
Paritaprevir	Ombitasvir-paritaprevir-ritonavir / Ombitasvir-paritaprevir-ritonavir-dasabuvir	4	96.8	5046	[35]

Supplementary Table 7: **Details of infectivity measurements obtained from each study (listed in Supplementary Data 1).**

Reference	Figure/Table	Type of experiment	Number of measurements
[36]	Table 1 and Figure 2	replicative capacity	22
[37]	Figure 6	replicative capacity	5
[38]	Table 4	replicative capacity	8
[39]	Figure 3f	replicative capacity	3
[40]	Supplementary Table H	replicative capacity	5

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