Supporting Information for

Discovery of COVID-19 Inhibitors Targeting the SARS-CoV2 Nsp13 Helicase

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Table S1. Comparison of Available Nsp13 Models/Templates.

	SARS	MERS	Sc Upf1
PDB id	6JYT	5WWP	2XZL
Resolution (Å)	2.8	3.0	2.4
Model(A) CC	0.85	0.89	0.90
RC outliers (%)	5.0	0.9	0.4
Rotomer Outliers (%)	0.8	4.7	1.4
Walker-A Site	Empty?	(2) SO4	ADP-Al_4
Cis-Peptides (%)[1]	0.80	0.48	0
Clash-score	29	15	8
#RSRZ>2 Percentile	1st	82nd	84th
By-Chain Cα-RMSD	**A / B**	**A / B**	**Sc Upf1**
S1A (Å)	0 / 1.18	1.83 / 1.73	24
S1A[2] **(Å)**	0 / 1.28	1.77 / 1.70	21
S1A-CH (Å)	0 / 0.34	0.88 / 0.38	5
S1A-Stalk (Å)	0 / 0.19	0.38 / 0.38	5
S1A-1B (Å)	0 / 0.65	na /	21
S1A-Rec1A[3] **(Å)**	0 / 0.63	0.63 / 0.64	11
S1A-Rec2A (Å)	0 / 1.81	1.61 / 1.43	11
M1B (Å)	1.73 / 1.66	1.38 / 0	22
M1B[2] **(Å)**	1.70 / 1.55	0.98 / 0	18
M1B-CH (Å)	0.74 / 0.81	0.58 / 0	8
M1B-Stalk (Å)	0.38 / 0.39	0.50 / 0	10
M1B-1B (Å)	1.24 / 1.43	na / 0	8
M1B-Rec1A[3] **(Å)**	0.64 / 0.77	0.43 / 0	14
M1B-Rec2A (Å)	1.43 / 1.39	1.65 / 0	4

[1] Number of non-proline Cis-peptides. [2] Excluding CH Zn-binding domain. [3] Excluding the 333-353 loop which interacts with domain 1B.

Table S2. The Cα-RMSD (Å) values for the four apo-SARS-CoV2 Nsp13 models.

Nom\Template	SARS_2A	SARS_2B	MERS_2A	MERS_2B
S2A (Å)	0	0.74	1.88	1.67
S2B (Å)	0.74	0	1.90	1.71
M2A (Å)	1.88	1.90	0	1.61
M2B (Å)	1.67	1.71	1.61	0

The SARS_CoV Nsp13 (6JYT) chains A and B templates were used to create models S2A and S2B respectively. The MERS_CoV Nsp13 (5WWP) chains A and B templates were used to create models M2A and M2B respectively.

Table S3. The Molprobity Validation of Models.

	S2A	S2B	M2A	M2B	S2C
Resolution (Å)	2.8	2.8	3.0/MD	3.0/MD	MD
MP Score (%ile)	2.38 (54%)	2.11 (69%)	1.92 (80%)	2.04 (73%)	1.96 (78%)
Clash Score (%ile)	58.2 (2%)	43.3 (6%)	22.07 (18%)	36.53 (9%)	1.51 (99%)
RMS Bonds (Å)	0.016	0.020	0.015	0.017	0.035
Ramachandran					
Favoured	97.3%	98.8%	99.2%	99.8%	90.1%
Allowed	2.4%	1.2%	0.8%	0.2%	7.6%
Outliers	0.3%	0.0%	0.0%	0.0%	2.3%
Rotomer Outliers	0.2%	0.4%	0.0%	0.0%	5.0%
Cβ-Deviations	1.6%	1.6%	0.6%	0.9%	11.6%

Table S5. The Top-Twenty Drug Hits for each SARS-CoV2 Nsp13 model.*

NN	(apo)	DRUG_Name	(S2A)	DRUG_Name	(S2B)	DRUG_Name	(M2A)	DRUG_Name	(M2B)	DRUG_Name	(ATP)	DRUG_Name
1	**-10.3**	**Cepharanthine**	**-10.3**	**Cepharanthine**	**-10.1**	**Dihydroergotamine**	**-10.2**	**Cefoperazone**	-9.2	Ledipasvir	**-11.1**	**Lumacaftor**
2	**-10.2**	**Cefoperazone**	**-10**	**Ergoloid**	**-10**	**Ergotamine**	**-10**	**Cefpiramide**	-9.1	Idarubicin	**-10.6**	**Emend**
3	**-10.1**	**Dihydroergotamine**	**-10**	**Ergotamine**	**-10**	**Netupitant**	-9.9	Dpnh	-9	Daunorubicin	**-10.5**	**Nilotinib**
4	**-10**	**Cefpiramide**	-9.9	Dihydroergotamine	-9.9	Ergoloid	-9.9	Lifitegrast	-9	Dpnh	**-10.4**	**Irinotecan**
5	**-10**	**Ergoloid**	-9.7	Irinotecan	-9.9	Nilotinib	-9.8	Raltegravir	*-8.9*	*Carindacillin*	**-10.3**	**Enjuvia**
6	**-10**	**Ergotamine**	-9.7	Nilotinib	-9.9	Tubocurarin	-9.7	Daunorubicin	*-8.8*	*Cefonicid*	**-10.1**	**Zelboraf**
7	**-10**	**Netupitant**	-9.7	Vumon	-9.8	Idarubicin	-9.6	Avodart	*-8.8*	*Cefpiramide*	**-10**	**Cromolyn**
8	-9.9	Dpnh	-9.6	Conivaptan	-9.8	Oxytetracycline	-9.6	Idarubicin	*-8.8*	*Diosmin*	**-10**	**Diosmin**
9	-9.9	Lifitegrast	-9.6	Fosaprepitant	-9.7	Dihydroergotoxine	-9.6	Nilotinib	*-8.8*	*Rutin*	-9.9	Differin
10	-9.9	Nilotinib	-9.4	Biosone	-9.7	Dpnh	-9.5	Carindacillin	*-8.7*	*Azelnidipine*	-9.9	Risperdal
11	-9.9	Tubocurarin	-9.4	Ceftobiprole	-9.7	Metocurine	-9.5	Valrubicin	*-8.7*	*Carindacillin*	-9.9	Rutin
12	-9.8	Idarubicin	-9.4	Venetoclax	-9.7	Otc	-9.4	Delamanid	*-8.7*	*Demeclocycline*	-9.9	Valrubicin
13	-9.8	Oxytetracycline	-9.3	Ponatinib	-9.7	Venetoclax	-9.4	Ellence	*-8.7*	*Lumacaftor*	-9.8	Pemetrexed
14	-9.8	Raltegravir	-9.2	Avodart	-9.6	Daunorubicin	-9.4	Exjade	*-8.7*	*Meclocycline*	-9.7	Dihydroergotamine
15	-9.7	Daunorubicin	-9.2	Biosone	-9.6	Doxycycline	-9.4	Fosaprepitant	*-8.6*	*Cefoperazone*	-9.7	Ergotamine
16	-9.7	Dihydroergotoxine	-9.2	Bromocriptine	-9.6	Imatinib	-9.3	Carindacillin	*-8.6*	*Doxycycline*	-9.7	Idarubicin
17	-9.7	Irinotecan	-9.2	Cialis	-9.6	Lumacaftor	-9.3	Ceftobiprole	*-8.6*	*Methacycline*	-9.7	Lifitegrast
18	-9.7	Metocurine	-9.2	Rutin	-9.6	Meclocycline	-9.3	Dolutegravir	*-8.6*	*Raltegravir*	-9.6	Amaryl
19	-9.7	Otc	-9.2	Saquinavir	-9.6	Methacycline	-9.3	Doxorubicin	*-8.6*	*Sultamicillin*	-9.6	Avodart
20	-9.7	Venetoclax	-9.2	Tasosartan	-9.5	Biosone	-9.3	Erismodegib	*-8.6*	*Tasosartan*	-9.6	Ellence

*The (apo) column is the highest scoring hit for any of the apo-ATP sites. The SARS_CoV Nsp13 (6JYT) chains A and B templates were used to create models S2A and S2B respectively. The MERS_CoV Nsp13 (5WWP) chains A and B templates were used to create models M2A and M2B respectively. The apo-Nsp13 ATP sites were selected as targets. The Upf1 guided Nsp13:ATP:ssRNA (S2C) complex ATP site was targeted. Dpnh: Dihydronicotinamide Adenine Dinucleotide Disodium Salt Hydrate (NADH). Cardinacillin is in the database as different stereoisomers (+/-). Otc: an isomer of oxytetracycline. The Vina docking scores are given in the column preceding the drug name. The column header specifies the target.

Table S6. The Ligand-Protein interacting Pairs of Figures 3 & 4

Residue			ATP	Cepharanthine	Idarubicin	Nilotinib	Lumacaftor	Emend	Zelborafor	Cromolyn	Risperdal
Ser	264	Og	.	.	.	.	.	.	.	.	-3.0
Asn	265	Nd	.	.	.	.	-3.3	.	.	-3.2	.
Gly	285	N	-2.8	.	.	.	-3.1	-3.3	.	-3.2	.
Gly	285	O	.	.	.	.	.	.	.	.	-3.3
Thr	286	N	-2.9	.	.	3.1	.	.	.	-3.0	.
Thr	286	O	.	.	.	.	-3.4	.	.	-3.5	.
Gly	287	N	-3.2	-3.2	.	3.0	.	.	.	-3.0	.
Gly	287	N	-3.3	-3.1	.	.	.	.	.	.	.
Lys	288	N	-3.1	.	.	.	.	.	.	-3.1	.
Ser	289	N	-3.1	.	.	.	-3.1	.	-2.9	-2.8	.
Ser	289	Og	.	.	-3.0	.	.	.	.	.	.
Ser	289	Og	.	.	-3.2	.	.	.	.	.	.
His	290	N	-2.7	.	.	.	.	.	-3.1	.	.
His	290	Nd	-3.4	.	.	.	-3.2	-3.1	.	.	.
Lys	320	Nz	-2.6	.	-2.7	.	.	.	-3.0	.	.
Glu	375	Oe	.	.	-2.8	.	.	.	.	.	.
Asp	374	Od	-3.2	.	3.3	.	.	3.0	.	-3.2	.
Asp	374	Od	-3.1	.	-3.0	.	.	-3.5	.	.	.
Lys	465	Nz	-3.3	.	-2.7	.	.	.	.	.	.
Arg	442	Ne	.	.	.	.	.	.	.	-3.1	.
Arg	442	Nz	.	.	.	.	-3.2	.	.	.	-3.2
Arg	443	Nz	-2.6	.	.	.	-3.0	.	-2.9	.	.
Arg	443	Nz	-3.1	.	.	.	.	.	.	.	.
Gly	439	N	.	.	.	.	-3.5	.	.	.	.
Gln	537	Oe	-3.0	.	.	.	.	.	.	.	.
Gln	537	Ne	-2.9	-3.2	-3.0	.	.	.	.	.	.
Gly	538	N	-3.1	.	-3.0	.	.	.	.	.	.
Glu	540	Oe	-2.9	.	.	3.2	.	.	.	.	.
Glu	540	Oe	-3.2	.	.	3.3	.	.	.	.	.
Arg	567	Nz	-3.1	.	-3.3	.	.	.	.	.	.
Arg	567	Nz	-3.1	.	.	.	.	.	.	.	.

Hydrogen bond Distances and ligand atom type - Oxygen, + Nitrogen. The Ligands are ATP, Cepharanthine, Idarubicin, Nilotinib, Lumacaftor, Emend, Zelboraf, Cromolyn, and Risperdal.

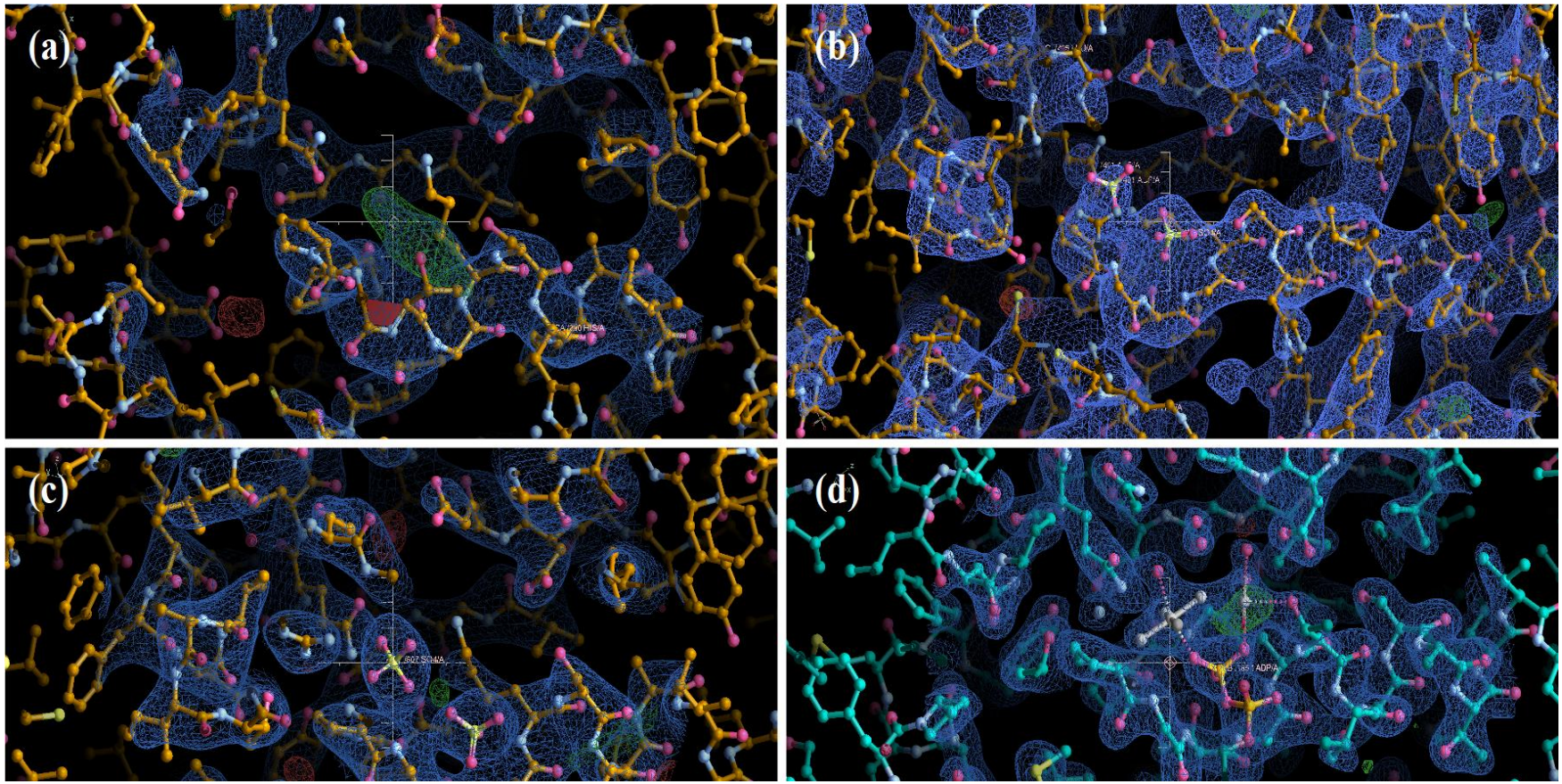

Figure S1. The electron density of the Walker-A site in each structure. (a) The SARS Nsp13 (6JYT) structure before and (b) after rebuilding the NTP site. (c) The MERS Nsp13 (5WWP) NTP binding site with two SO4 ions. (d) The yeast Upf1 (2XZL) ADP-AlF4 bound NTP site used as a template for the Nsp13 complex model.

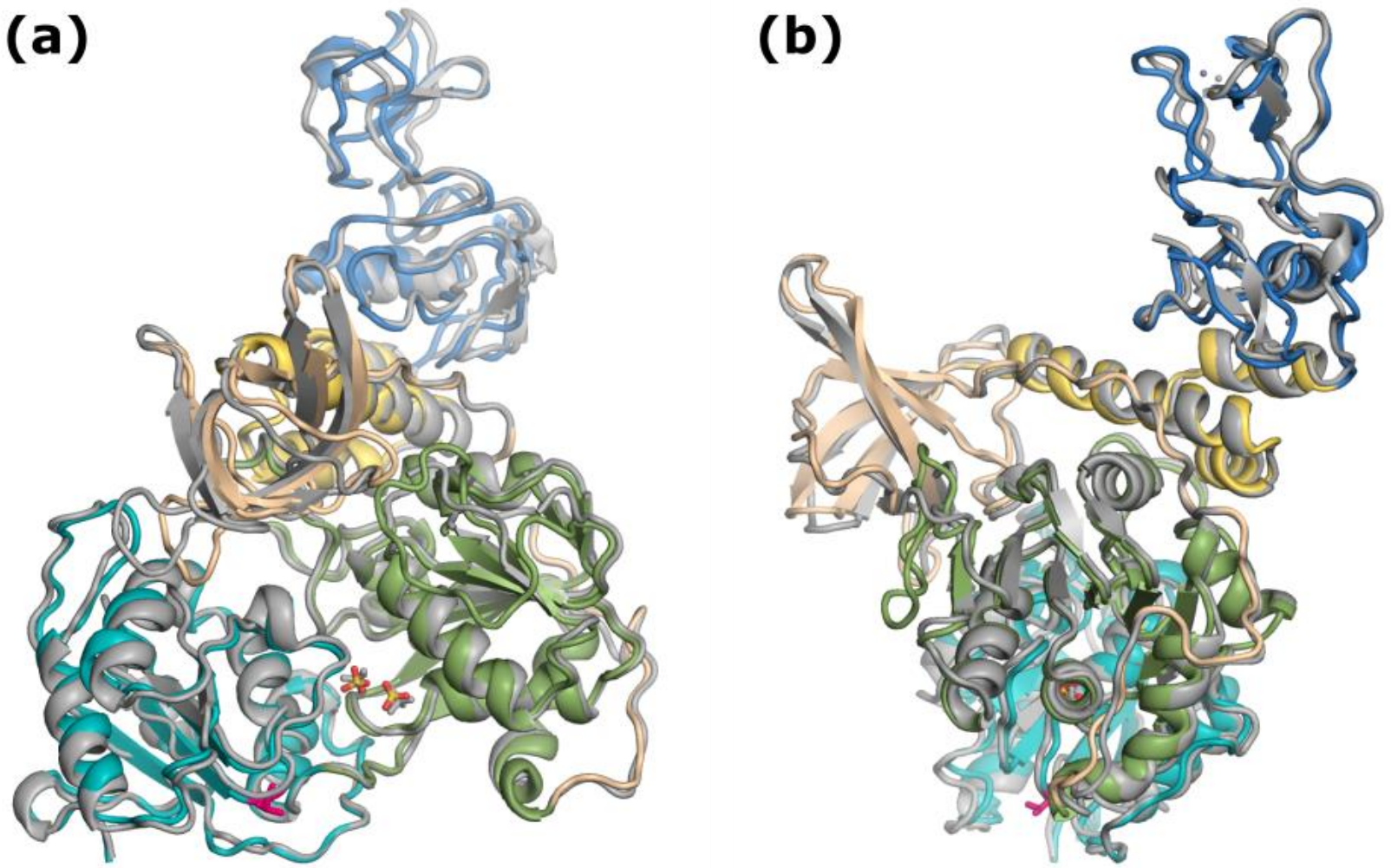

Figure S2. Comparison of the apo-Nsp13 Models. Superposition of the (M2B) apo-SARS-CoV2 Nsp13 homology model based on MERS Nsp13 helicase (5WWP), coloured-by-domain as in Figure 1, and the (S2A) apo-SARS-CoV2 Nsp13 structural model (grey) based on the SARS Nsp13 (6JYT) with the I570V mutation, show as red sticks. The two views (a and b) are rotated by 90° about the vertical. The Walker-A site is occupied by two SO_4 ions, lower center, in the apo crystal structures. See Figure S5 for superposition of other apo-Nsp13 models.

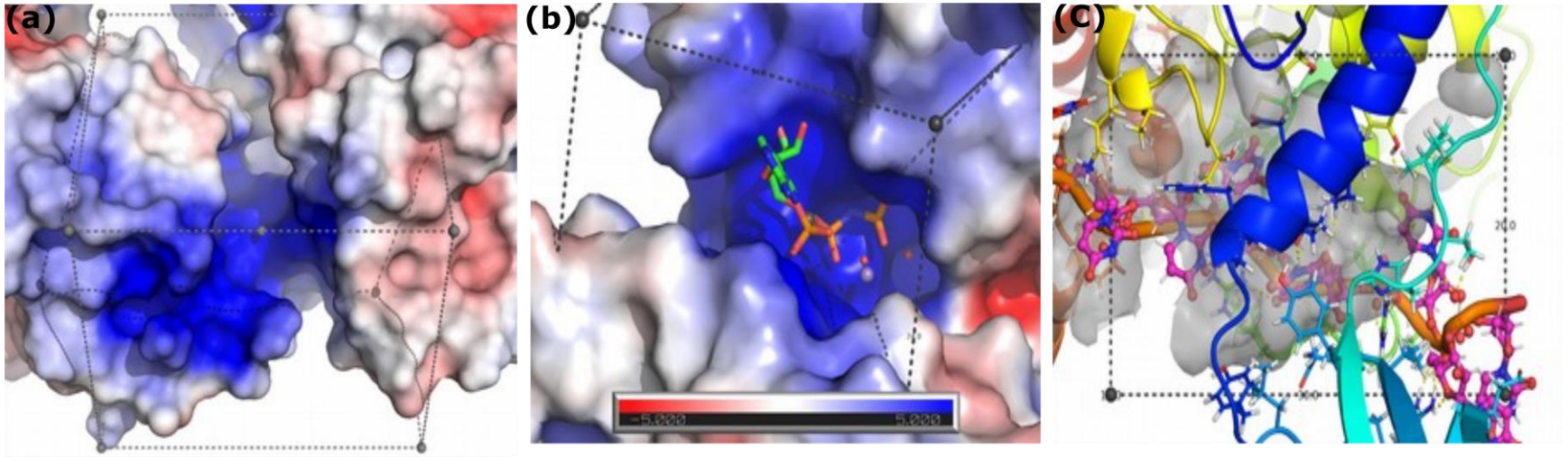

Figure S3. SARS-CoV2 models' ATP-binding Site for HTvS. Views of the (a) SARS-CoV (S2A), (b) MERS (M2B), and (c) Upf1 (S2C-ATP) based models of the SARS-CoV2 NTP binding site. ATP was modeled based on the Upf1 structure. The bounding box for AutoDockVina is shown as a dashed-line with gray spheres at each corner and the center.

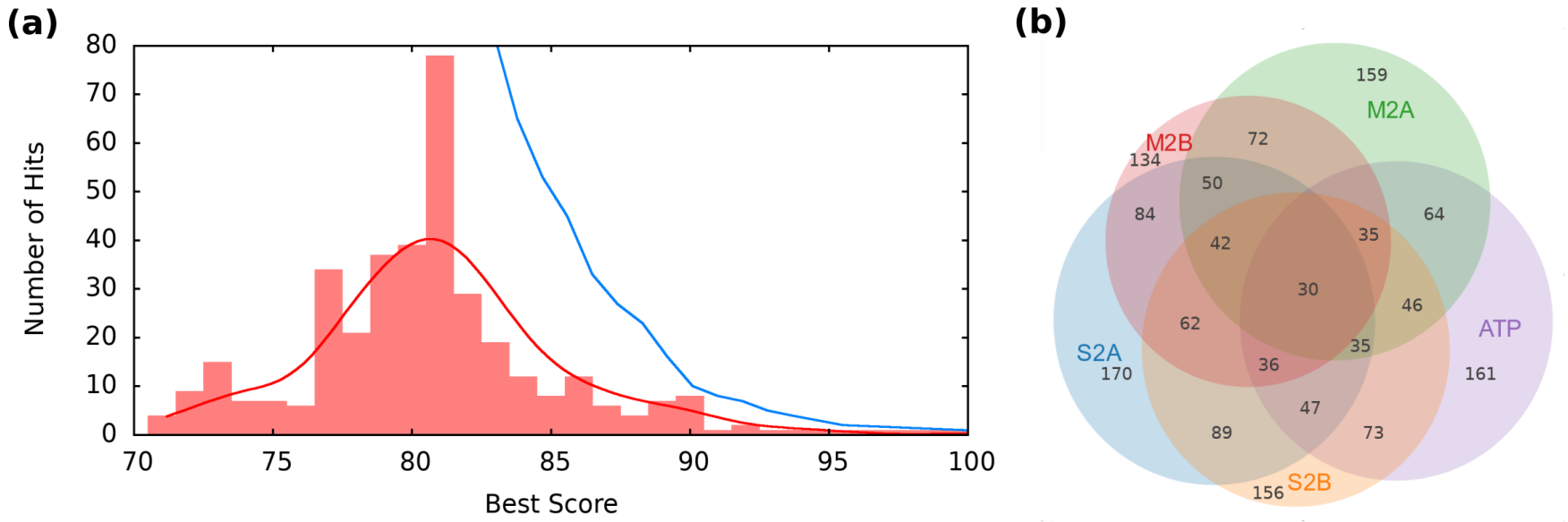

Figure S4. Selected top hits. (a) Histogram of the 369 drugs discovered using in-silico screening of the SARS-CoV2 Nsp13 helicase models (Table S4). The best score (x-axis) is the best normalized Vina docking score for any model, higher is better. The red line is the k-density smoothed distribution. The cut-off filtered results (blue line) is the cumulative number of drugs as the cut-off is lowered. (b) The Venn diagram of the ATP binding site hits for the five ATP templates. Each intersection has the hit count number.

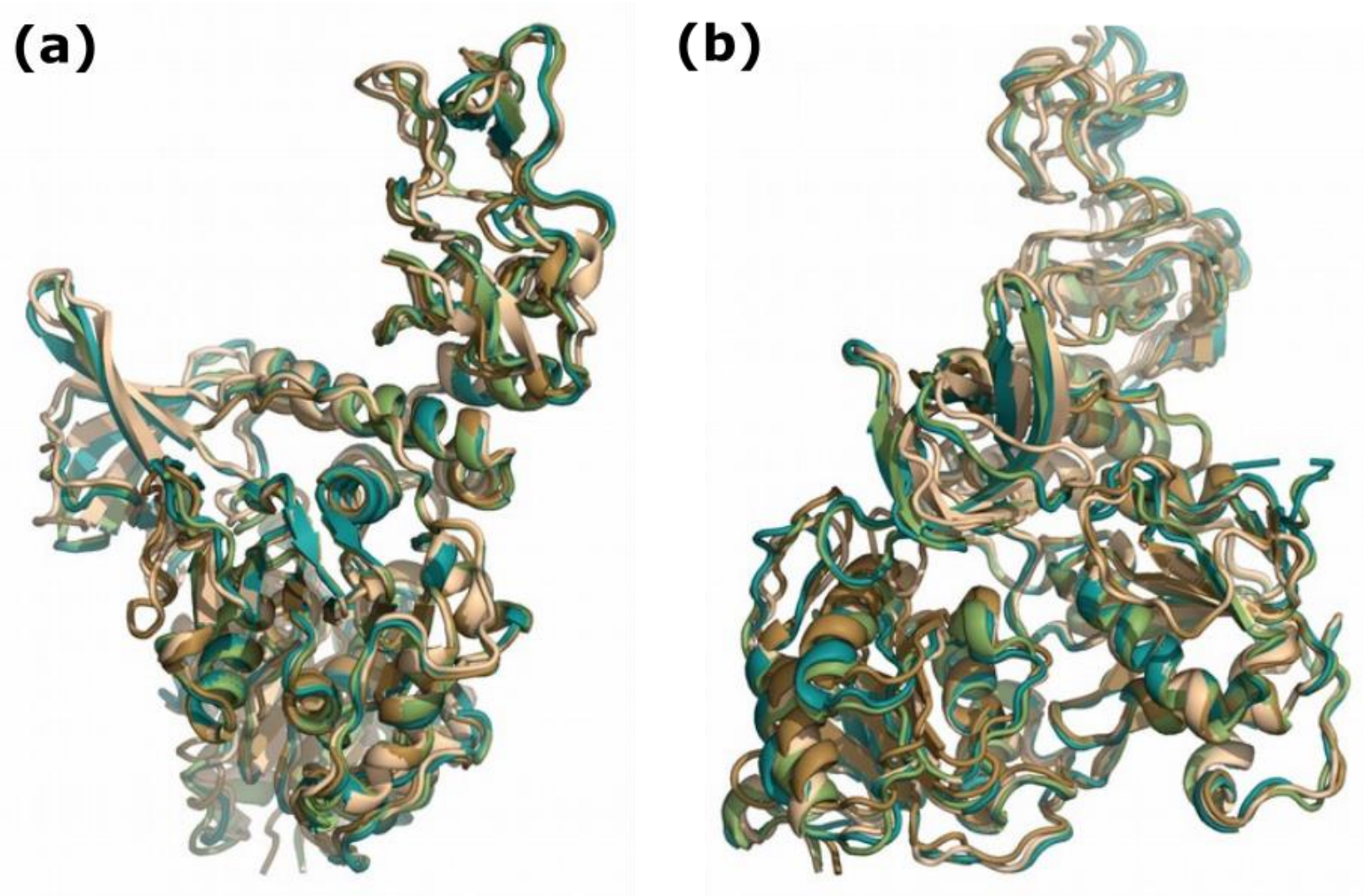

Figure S5. Superposition of the four (apo) SARS-CoV2 Nsp13 homology models: S2A (cyan), S2B (green), M2A (brown), M2B (tan). Inter-chain Cα-RMSDs are 1.6 Å for MERS, and 0.74 Å for SARS. The inter-species model Cα-RMSDs range from 1.7 to 1.9 Å (Table S-2). The two views (a and b) are rotated by 90° about the vertical.