

Additional file 2: Figures

Title:

Functional conservation of HIV-1 gag: implications for rational drug design

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Figure S1. The distribution of natural polymorphisms at 500 gag positions on 8 HIV-1 subtypes. HXB2 indices at each individual protein are shown in the first column, followed by HXB2 indices in the full-length gag. Drug binding positions are marked with red stars. The colors in the second column distinguish the gag domains: light green for matrix (position: 1-132), light blue for capsid (133-363), dark green for p2 (364-377) and p1 (433-448), dark blue for nucleocapsid (378-432) and gray for p6 (449-500). The remaining columns list the consensus amino acid for each subtype followed its natural variation(s) and the corresponding proportion(s) in blue (prevalence above 5%) and orange (prevalence at or below 5%).

Figure S2: Surface representation of conservation index of full-length gag in 8 HIV-1 subtypes. 4 gag proteins (monomers) and 2 spacer peptides are annotated and displayed in schematic view at the top. Drug binding sites (red) are mapped onto HIV-1 gag protein structures. For each subtype figure, surface spectrum colors indicate each position's CI, from the most conserved (blue CI = 0) to the least conserved positions (pink CI ≥ 0.1). Crystallized inhibitors are shown in sticks view inside their binding pockets. Visualization software: PyMOL V1.5 (<http://www.pymol.org/>).

Figure S3: Surface representation of five drug binding pockets in 8 HIV-1 subtypes. For each subtype figure, surface spectrum colors indicate each position's CI, from the most conserved (blue CI = 0) to the least conserved positions (pink CI $\geq$ 0.1). Crystallized inhibitors are shown in sticks view inside their binding pockets. Visualization software: PyMOL V1.5 (<http://www.pymol.org/>).

Figure S4: The structure of capsid hexamer superimposed with 8 crystallized inhibitors. The capsid hexamer structure is shown in the top, side and bottom views from Fig S1(A) to S1(C). The drug binding pockets are annotated in each figure. Three figures on the left side show the N-terminal domains (NTD, position: 1-146) and C-terminal domains (CTD, position: 151-231), colored blue and pink, respectively. Red circles indicate drug binding pockets, whose targets are within the interfaces of NTD-NTD, NTD-CTD or CTD-CTD interactions. Three figures on right side map the CIs to the structure and visualize the conservation of the drug binding pockets. It shows that drug binding pocket 1 in NTD is situated on the NTD-CTD interaction interface, drug binding pocket 2 in NTD is situated on the NTD-NTD interaction interface, drug binding pocket 3 in CTD is situated on the NTD-CTD interaction interface, and drug binding pocket 4 is inside NTD. Note that drug binding pockets 1 and 3 are situated on the opposite sides of the same NTD-CTD interface. The superimposed crystallized inhibitors were mapped onto the capsid hexamer using PyMOL V1.5 (PDB: 3H4E). Visualization software: PyMOL V1.5 (<http://www.pymol.org/>). Inhibitor references are available in Additional file 1.

Figure S5: Conserved NTD and CTD domains in HIV-1 capsid. The capsid hexamer structure is shown in top (A), side (B) and bottom (C) views. Conserved NTD-NTD interaction domains are colored yellow (capsid positions: 30-70, gag positions: 162-202). Conserved NTD-CTD interaction domains are colored red (capsid positions: 155-176, gag positions: 287-308). PDB: 3H4E. Visualization software: PyMOL V1.5 (<http://www.pymol.org/>).

Figure S6: Conserved zinc-finger domains in HIV-1 nucleocapsid. The structures of nucleocapsid–RNA and nucleocapsid–inhibitor complexes are shown in top (A), side (B) and bottom (C) views. The first zinc-finger domain is colored red (nucleocapsid positions: 14-29, gag positions: 389-404) and the second zinc-finger domain (nucleocapsid positions: 35-50, gag positions: 410-425) are colored orange. PDB: 1A1T, 2M3Z. Visualization software: PyMOL V1.5 (<http://www.pymol.org/>).

Figure S5 and S6 visualizes the conserved regions identified as three minimum conserved regions using our conservation analysis (Figure 2).

Figure S1. Natural variations of gag positions in 8 major HIV-1 subtypes and CRFs

B		A1	C	D	F1	G	01_AE	02_AG
gag								
1	M	M1.9	M	M	M	M	M	M
2	G	G	G	G	G	G	G	G
3	A	A	A	A	A	A	A	A
4	R	R S2.1	R S5.7	R S5.7	R	R	R	R
5	A	A	A	A	A	A	A	A
6	S	S V1.66	S V27.5	S V1.38	S V	S V	S V	S V
7	V	V I16.2	V V27.5	V I3.8	V	V	V	V
8	L	L I4.0	L R K11.6	L S R5.2	L	L	L	L
9	S	S T1.5, R1.3	S R K11.6	S R5.2	S	S	S	S
10	G	G	G	G	G	G	G	G
11	G	G A2.0	G E42.6 K1.4	G E5.7	G	G	G	G
12	E	E K25.4 Q15.5 D2.5, G1.5	E K N3.5	E K R11.3 S4.8, Q4.1, E3.4	E K	E K	E K	E K
13	L	L	L	L	L	L	L	L
14	D	D E4.5, N9	D	D	D	D	D	D
15	R	R K25.7 Q3.3, S1.3, A.9	R T7.4, S4.1, K4.1, M1.8, Q1.3, E1.3	R D9.5, A6.3	R D17.6	R K A39.6	R T3.7, S2.3, R1.2	R A S23.0
16	W	W	W	W	W	W	W	W
17	E	E	E	E	E	E	E	E
18	K	K R7.7, G2.1	K R28.1	K R19.9	K	K	K	K
19	I	I T1.3	I R Q3.0, E1.6	I R Q24.0	I	I	I	I
20	R	R	R	R	R	R	R	R
21	L	L	L	L	L	L	L	L
22	R	R	R	R	R	R	R	R
23	P	P	P	P	P	P	P	P
24	G	G	G	G	G	G	G	G
25	G	G	G	G	G	G	G	G
26	K	K R8.1, N2.3, S2.1	K R6.6, N1.8, S1.1	K R3.7	K	K	K	K
27	K	K	K	K	K	K	K	K
28	K	K Q21.7 R10.8 T2.2	K Q18.5 R3.4, T1.7	K K16.4 Q11.9 C11.3 R10.4 S2.6, T1.7	K Q26.5	K K Q39.6 R10.4	K R3.7, Q1.2	K Q18.0
29	Y	Y	Y	Y	Y	Y	Y	Y
30	K	K R32.7 Q9.8	K R8.6, Q1.2	K R13.7 Q6.5, K5.6	K R20.6	K R45.8	K R33.6 Q5.8	K R13.1
31	L	L	L I4.9, I3.8	L I15.4 V9.5	L L23.5	L L33.3	L L30.4 I9.5	L L
32	K	K	K	K	K	K	K	K
33	H	H	H	H	H	H	H	H
34	I	I L22.8 V2.5	I L13.7	I L31.7	I L	I L	I L	I L
35	V	V I1.5	V I4.9	V V I4.2	V	V	V	V
36	W	W	W	W	W	W	W	W
37	A	A	A	A	A	A	A	A
38	S	S G2.1	S	S G2.0	S	S	S	S
39	R	R	R	R K2.7	R	R	R	R
40	E	E	E	E Q4.5	E	E	E	E
41	L	L M1.9	L	L	L	L	L	L
42	E	E D8.1	E D2.4, G1.9	E	E	E	E	E
43	R	R K2.5	R K14.1	R	R	R	R	R
44	F	F Y1.5	F	F Y3.4	F	F	F	F
45	A	A	A S1.2	A	A	A	A	A
46	V	V L9.0, I3.2	V L24.2 V1.4	V L24.2 V1.4	V L	V L	V L	V L
47	N	N	N D4.7	N D4.8	N	N	N	N
48	P	P S2.1	P S2.9	P	P	P	P	P
49	G	G S6.0	G D10.9 S4.8	G S12.0	G	G	G	G
50	L	L	L	L P1.6	L	L	L	L
51	L	L	L	L	L	L	L	L
52	E	E	E	E	E	E	E	E
53	T	T S2.3	T S1.3	T S6.6	T	T	T	T
54	S	S A18.5 T1.7, P1.3	S A14.2 P3.7, K3.5, S2.8, R1.3	S T8.6, A7.2, P5.4, E4.1	S P26.5	S A T16.7	S S6.3, T5.0, G3.3	S G11.5
55	E	E D8.7, G6.7, A1.5, Q1.2	E Q2.1, G1.2, A.9	E D5.1, A2.1, G1.7	E	E	E	E
56	G	G	G	G	G	G	G	G
57	C	C	C	C	C	C	C	C
58	R	R K12.0	R K17.1 N1.0	R K11.3	R K26.5	R	R	R
59	Q	Q K4.6	Q R2.2, K1.3	Q	Q	Q	Q	Q
60	I	I S2.3, M3.7	I A1.8	I L4.5	I	I	I	I
61	L	L I5.2, M3.7	L I38.6 L13.0	L L3.9	L	L	L	L
62	G	G E17.7 A4.1, R3.0, V1.9, Q1.5, I1.0	G K7.7, G5.5, N2.4, I1.8, D1.8, A1.5	G E30.3 A19.2	G A17.6	G V16.7 T12.5	G	G
63	Q	Q	Q K1.6	Q A4.1, K1.4	Q	Q	Q	Q
64	L	L	L I3.6	L I4.3	L	L	L	L
65	H	H	H	H	H	H	H	H
66	P	P S2.3	P S28.2 A1.2	P S2.0	P	P	P	P
67	S	S A31.2 T2.0	S A4.6, T1.7	S S4.0	S	S	S	S
68	L	L I4.1	L I13.5	L I14.7 V7.7	L	L	L	L
69	Q	Q K4.8, R3.2, P2.7	Q K10.8 N7.3, Q3.9, G1.5	Q K24.7 N4.1, P2.0	Q	Q	Q	Q
70	T	T	T	T	T	T	T	T
71	G	G	G	G	G	G	G	G
72	S	S T2.0	S S6.1	S	S	S	S	S

Figure S1 (continue)

gag	B	A1	C	D	F1	G	01 AE	02 AG
73	E	E K51	E K48	E K48	E	E	E	E
74	E	E	E G10.4	E G10.4	E	E	E	E
75	L F7.5, I4.8	L I12.8F4.5, V1.8	L F8.5, I2.8	L I24.3M2.0, V2.0	L	L I41.7	L I6.1, V4.2, F1.6, Y1.2	L
76	K R42.4	K R36.6	K K41.2 I3.7, V1.3	K R7.2	K R38.2	K R39.6	K R17.4	K R18.0
77	S	S	S	S	S	S	S	S
78	L V2.7	L	L S1.1	L S1.1	L	L	L	L
79	F F34.2H1.1	F Y34.7H2.1	F Y40.6H5.0	F Y26.9	F Y35.3	F Y37.5	F Y32.5H2.9	F Y31.1
80	N	N	N	N	N	N	N	N
81	T A9.0, L1.0	T A4.5	T	T	T	T A12.5	T A9.2, I4.1	T
82	V I28.5	V I2.8	V I4.0	V I4.1	V I29.4	V I33.3	V I32.1L5.4, T1.3	V V32.8L8.2
83	A V1.7	A	A V1.2	A	A V29.4	A	A V42.2T1.3	A
84	T V46.4	T V1.3	T V7.1	T V5.0, A1.1	T	T V43.8	T V19.4	T
85	L	L I1.5	L	L	L	L	L	L
86	F F34.W1.2	F C1.9	F	F W4.5	F Y35.3C14.7	F	F Y3.0, L1.5, R1.1	F Y26.2
87	C	C	C	C	C	C	C	C
88	V	V I3.5	V	V	V	V	V I1.5	V
89	H	H	H	H	H	H	H	H
90	Q R31.1, K9	Q R4.3, L2.0	Q K25.5A15.8S6.3, R4.3, T3.9, Q2.4	Q A19.2K7.5, Q6.3	Q	Q	Q R3.5, K1.6	Q R8.2
91	K R27.0Q9.3, N6.6 Q3.8	K R14.3Q4.8	K G21.4R19.1N15.4D8.2, E4.5, Q1.2	K R15.2G2.0	K R50.0	K R29.2G10.4	K R8.7, G5.8	K
92	I V1.2	I M2.6	I V3.9	I K4.8	I V38.2E17.6	I	I V3.0, M2.5	I L16.4
93	E D44.1G2.8	E D28.6G8.8, A3.0, N2.6	E D25.3K8.0, P3.7, N1.4	E K6.8, D1.4	E	E	E D21.1G3.5, Q2.0	E D45.9
94	V I27.0	V I5.3	V I9.7	V I11.5	V I26.5	V I18.8	V I18.7	V I19.7
95	K R11.9	K R3.3	K R8.0, H7.0 Q4.6, L2.8	K A37.3K16.7	K	K R10.4	K R7.1, Q1.3	K R9.8
96	D	D	D	D N6.6	D	D	D	D
97	T	T	T	T	T	T	T	T
98	K R1.0	K	K	K Q3.4	K	K	K Q1.6	K
99	E	E	E	E	E	E	E	E
100	A	A	A	A	A	A	A	A
101	L V1.7	L V3.8	L	L	L	L	L	L
102	D E30.3	D E1.4	D	D E7.2	D D35.3	D	D E2.3	D
103	K R8.4	K	K R15.1	K R2.9	K	K	K	K
104	I V7.8	I V4.1	I V10.6L2.9	I I6.6, M1.6	I	I I4.6	I I30.4M15.9V4.7	I V18.0L13.1
105	E	E G1.9	E K5.5	E	E	E	E K2.5	E
106	E	E	E	E	E	E	E	E
107	E	E M17.7L9.0, V1.5	E	E I7.0, G1.4	E	E L20.8A10.4	E A18.6I7.5, E3.1, M1.4	E V23.0V9.8, A8.2
108	Q	Q K7.8	Q	Q R4.8	Q	Q	Q L.9	Q K13.1
109	N S1.8, E1.6	N K10.8S3.2	N K6.3, R4.8, S1.6	N A34.8N16.7	N	N	N K35.0S2.6, R1.8, T1.6	N K8.2
110	K R1.0	K R10	K R1.4	K	K	K N16.7	K R5.2	K
111	S C9.0, I1.4	S N10.6G1.3	S C33.2G4.2, I2.5	S	S	S	S N8.3, R2.3, G2.3, H1.5	S N16.4
112	K E1.6, Q1.4	K R2.3, T1.8, Q1.6	K K1.1	K Q2.3	Q	Q	Q K4.6, R2.5	Q
113	K E1.6, Q1.4	K R2.3, T1.8, Q1.6	K Q2.3	K Q2.3	Q	Q K16.7	Q R2.0, P1.5	Q
114	K R4.7, Q1.6, N1.4	K R4.5, N3.2, Q2.9	K R4.5, N3.2, Q2.9	K R2.9	K	K E14.6	K Q3.8, R2.2, N1.5	K R14.8
115	A V8.2, T4.3, E1.3	T A7.8, P3.9, S2.9	T A7.6, I5.8, V2.9, M2.0	A V5.7, T2.7	T	T	T K4.1, A1.1	T I14.8
116	Q	Q	Q K5.5, P1.3	Q	Q	Q	Q	Q
117	Q P3.0	Q	Q K2.3	Q	Q	Q K16.7	Q	Q
118	A T7.9, E3.0, V3.0	A T5.2, E3.0, V1.0	A V7.5, E4.8, T3.0	A T4.5	A	A	A T2.9	A T23.0
119	A T2.5, V1.6, E1.2	A T3.6	E K38.4A9.6, Q2.2, T1.3, G1.2	E A14.9	A	A	A T1.8, V1.8	A E16.4
120	A D1.2, T1.0	A T3.8	A E14.2V4.7, M3.7, K2.7, G1.4	A	A	A	A T4.3	A
121	D G12.5N4.7, A4.3	D A14.3G7.0, E3.0, N2.6, V1.4	D A13.4, D3.3	D G1.1	D	D	D D22.5S3.7, A2.9, N1.3	D
122	T A7.9, P2.4	T A2.8, P2.8	T A2.8, P2.8	T P4.8, A2.0	T	T E29.2K20.8	T A5.3, P3.0, S1.1	T A16.4
123	G E3.9, R1.2	G E3.5	G D20.5G17.6E10.7A5.0, R1.2	R K31.7G21.3	G	G E18.8	G E.9	G
124	N S10.2K4.7, T2.3, D1.0	N S25.9D1.6	N S25.9D1.6	N T5.7	N	N	N S13.3	N
125	S N24.6K5.8, R1.7, G1.6, T.9	S N21.1, R2.0	S N10.6G2.5	S N15.2G5.4, K2.0	S	S N25.0	S N13.5	S
126	S N9.1, G3.6, K1.9	S R4.0, G2.7, K1.5, N1.4	S R4.0, G2.7, K1.5, N1.4	S N15.2G5.4, K2.0	S	S N16.7	S N2.6, G1.3	S
127	Q K6.5, P4.6	Q Q24.2T2.5, N2.2, R2.1, S1.9	Q Q24.2T2.5, N2.2, R2.1, S1.9	Q	Q	Q	Q N3.8, T3.4, Q2.6	Q
128	V A4.2, G1.5	V T2.0, G1.8, A1.7, I1.5	V T2.0, G1.8, A1.7, I1.5	V V	V	V A10.4	V I1.6	V
129	S	S	S	S	S	S	S	S
130	Q H2.5, R.9	Q	Q R5.9	Q	Q	Q	Q H8.6, Y1.5, L1.3	Q
131	N	N	N	N	N	N	N	N
132	Y F4.6	Y	Y F9.4	Y F10.2	Y	Y	Y F1.8	Y F24.6
133	P	P	P	P	P	P	P	P
134	I	I V2.1	I	I V6.3	I	I	I	I
135	V	V	V	V	V	V	V I1.0	V
136	Q R1.0	Q	Q	Q	Q	Q	Q	Q
137	N	N S1.8	N	N	N	N	N	N
138	L I22.5M8.8	L	L A9.8, I4.5, H2.2, P1.6, V1.2	L	L	L	L A2.9	L
139	Q	Q	Q	Q	Q	Q	Q	Q
140	G	G	G	G	G	G	G	G
141	Q	Q	Q	Q	Q	Q	Q	Q
142	M	M	M	M	M	M	M	M
143	V	V I17.4T4.7, M2.9	V	V Y4.1	V	V	V T3.9, I2.9, A1.7, M1.0	V
144	H	H Y4.0, L3.4	H	H	H	H	H	H

Figure S1 (continue)

gag		B	A1	C	D	F1	G	01_AE	02_AG
15	145	Q	Q	Q	Q	Q	Q	Q	Q
16	146	A	S	A	A	S	A	P	S
17	147	I	I	I	I	I	I	L	N
18	148	S	T	T	T	T	T	V	I
19	149	P	P	P	P	P	P	P	M
20	150	R	R	R	R	R	R	R	P
21	151	T	T	T	T	T	T	T	R
22	152	L	L	L	L	L	L	L	T
23	153	N	N	N	N	N	N	N	L
24	154	A	A	A	A	A	A	A	N
25	155	W	W	W	W	W	W	W	W
26	156	V	V	V	V	V	V	V	V
27	157	K	K	K	K	K	K	K	K
28	158	V	V	V	V	V	V	V	V
29	159	V	V	V	V	V	V	V	V
30	160	E	E	E	E	E	E	E	E
31	161	E	E	E	E	E	E	E	E
32	162	K	K	K	K	K	K	K	K
33	163	N	N	N	N	N	N	N	N
34	164	F	F	F	F	F	F	F	F
35	165	S	S	S	S	S	S	S	S
36	166	P	P	P	P	P	P	P	P
37	167	E	E	E	E	E	E	E	E
38	168	V	V	V	V	V	V	V	V
39	169	I	I	I	I	I	I	I	I
40	170	P	P	P	P	P	P	P	P
41	171	M	M	M	M	M	M	M	M
42	172	F	F	F	F	F	F	F	F
43	173	S	S	S	S	S	S	S	S
44	174	A	A	A	A	A	A	A	A
45	175	L	L	L	L	L	L	L	L
46	176	S	S	S	S	S	S	S	S
47	177	E	E	E	E	E	E	E	E
48	178	G	G	G	G	G	G	G	G
49	179	A	A	A	A	A	A	A	A
50	180	T	T	T	T	T	T	T	T
51	181	P	P	P	P	P	P	P	P
52	182	Q	Q	Q	Q	Q	Q	Q	Q
53	183	D	D	D	D	D	D	D	D
54	184	L	L	L	L	L	L	L	L
55	185	N	N	N	N	N	N	N	N
56	186	T	T	T	T	T	T	T	T
57	187	M	M	M	M	M	M	M	M
58	188	L	L	L	L	L	L	L	L
59	189	N	N	N	N	N	N	N	N
60	190	I	I	I	I	I	I	I	I
61	191	V	V	V	V	V	V	V	V
62	192	G	G	G	G	G	G	G	G
63	193	G	G	G	G	G	G	G	G
64	194	H	H	H	H	H	H	H	H
65	195	Q	Q	Q	Q	Q	Q	Q	Q
66	196	A	A	A	A	A	A	A	A
67	197	A	A	A	A	A	A	A	A
68	198	M	M	M	M	M	M	M	M
69	199	Q	Q	Q	Q	Q	Q	Q	Q
70	200	M	M	M	M	M	M	M	M
71	201	L	L	L	L	L	L	L	L
72	202	K	K	K	K	K	K	K	K
73	203	E	E	E	E	E	E	E	E
74	204	T	T	T	T	T	T	T	T
75	205	I	I	I	I	I	I	I	I
76	206	N	N	N	N	N	N	N	N
77	207	E	E	E	E	E	E	E	E
78	208	E	E	E	E	E	E	E	E
79	209	A	A	A	A	A	A	A	A
80	210	A	A	A	A	A	A	A	A
81	211	E	E	E	E	E	E	E	E
82	212	W	W	W	W	W	W	W	W
83	213	D	D	D	D	D	D	D	D
84	214	R	R	R	R	R	R	R	R
85	215	L	L	L	L	L	L	L	L
86	216	H	H	H	H	H	H	H	H

Figure S1 (continue)

gag	B	A1	C	D	F1	G	01_AE	02_AG
85	217 P	P	P	P	P	P	P	P
218	V A5.6, T.9	V I1.6, A1.1	V I1.6, A1.1	V A2.3	V A17.6	P Q43.8	V A2.9	V Q13.1
219	H Q25.3 P1.7	H Q18.3 P3.7	H Q18.3 P3.7	H Q13.1	H Q35.3	Q	H Q22.2 P1.1	H Q13.1
220	A	A	A	A	A	A	A	A
221	G	G	G	G	G	G	G	G
222	P	P	P	P	P	P	P	P
223	I V7.7, A2.7, F2.4, L.9	I V33.3 A11.9 N2.8	I V33.3 A11.9 N2.8	I V14.9 P11.3 A6.3, S5.4, N3.6	I I	I L10.4 F10.4	I F3.4, N1.6, V1.3	I A9.8
224	A P3.2	A P3.6	A P3.6	A P3.2	A P	A A33.3	A A4.3	A A18.0
225	P	P	P	P	P	P	P	P
226	G	G	G	G	G	G	G	G
227	Q	Q	Q	Q	Q	Q	Q	Q
228	M L12.1 I8.9	M I20.1 L5.0, S1.1	M I20.1 L5.0, S1.1	M I15.2	M I32.4	I	M I13.5 L8.5	M
229	R	R	R	R K2.0	R	R	R	R
230	E D6.0	E D6.0	E D6.0	E D7.0	E	E D20.8	E D2.1	E
231	P	P	P	P	P	P	P	P
232	R	R	R	R	R	R	R	R
233	G	G	G	G	G	G	G	G
234	S	S	S	S	S	S	S	S
235	D	D	D	D	D	D	D	D
236	I	I	I	I	I	I	I	I
237	A	A	A	A	A	A	A	A
238	G	G	G	G	G	G	G	G
239	T S2.8	T	T	T	T	T	T S1.2	T
240	T	T	T	T	T	T	T	T
241	S	S	S	S	S	S	S	S
242	T N9.5	T	T N25.3 S1.9	T N23.1 S12.7	T	T N22.9	T N19.6	T N9.8
243	L	L P1.7, A7.4, T7.2, L3.2, V.9	L P3.2, R1.7	L P4.1	L P17.6	L	L	L
244	Q	Q	Q	Q A2.5	Q	Q	Q	Q
245	E	E	E	E	E	E	E	E
246	Q	Q	Q	Q	Q	Q	Q	Q
247	I V2.5	I L14.1 V4.1	I V15.7	I V15.7	I	I V10.4	I	I
248	G A23.8 T3.8	G Q12.9 A6.4, T2.1, R1.8	G T14.2 G3.6, Q1.8	G A29.9 T11.8	G	R T33.3 A16.7	G A11.2 T2.2	G
249	W	W	W	W	W	W	W	W
250	M	M	M	M	M	M	M	M
251	T	T	T	T	T	T	T	T
252	N S23.0 H17.7 G.10	N S G19.2 N16.4 A3.0, H1.2	N S G19.2 N16.4 A3.0, H1.2	N S38.0 A2.5, H2.3, G1.1	N S G14.7	N N10.4	N S35.8 G10.7 H.9	N S
253	N	N	N	N	N	N	N	N
254	P	P	P	P	P	P	P	P
255	P A1.8	P A2.2	P A2.2	P V6.3	P	P	P A8.3 S4.1	P
256	I V3.6	I V2.4, F1.8	I V35.6	I V6.3	I	I V10.4	I V1.8	I V14.8
257	P	P	P	P	P	P	P	P
258	V	V	V	V	V	V	V	V
259	G	G	G	G	G	G	G	G
260	E D6.0	E E6.4 N1.9	E E29.0	E D13.1	E D14.7	E D12.5	E E8.7	E D31.1
261	I	I	I	I	I	I	I	I
262	Y	Y	Y	Y	Y	Y	Y	Y
263	K	K R1.0	K	K R7.2	K	K	K	K
264	R K5.8	R K5.5, G2.0	R	R	R	R	R	R
265	W	W	W	W	W	W	W	W
266	I	I	I	I	I	I	I	I
267	I V1.1	I V3.6	I V3.6	I V1.4	I	I	I	I
268	L M10.7 I3.4	L M2.4	L M2.4	L M7.0	L	L M29.2	L	L
269	G	G	G	G K2.0	G	G	G	G
270	L	L	L	L	L	L	L	L
271	N	N	N	N	N	N	N	N
272	K	K	K	K	K	K	K	K
273	I	I	I	I	I	I	I	I
274	V	V	V	V	V	V	V	V
275	R	R	R	R	R	R	R	R
276	M	M	M	M	M	M	M	M
277	Y	Y	Y	Y	Y	Y	Y	Y
278	S	S	S	S	S	S	S	S
279	P	P	P	P	P	P	P	P
280	V22.1 S3.5, I2.5, A2.1	V T1.8	V T1.8	V	V	V	V T10.2 I2.5	V T18.0
281	S	S	S	S	S	S	S	S
282	I	I	I	I	I	I	I	I
283	L	L	L	L	L	L	L	L
284	D	D	D	D	D	D	D	D
285	I	I	I	I	I	I	I	I
286	R K29.0	R	R	R K9.0	R K32.4	R K45.8	R K12.7	R K27.9
287	Q	Q	Q	Q	Q	Q	Q	Q
288	G	G	G	G	G	G	G	G

Figure S1 (continue)

gag	B	A1	C	D	F1	G	01_AE	02_AG
289	P	P	P	P	P	P	P	P
290	K	K	K	K	K	K	K	K
291	E	E	E	E	E	E	E	E
292	P	P	P	P	P	P	P	P
293	F	F	F	F	F	F	F	F
294	R	R	R	R	R	R	R	R
295	D	D	D	D	D	D	D	D
296	Y	Y	Y	Y	Y	Y	Y	Y
297	V	V	V	V	V	V	V	V
298	D	D	D	D	D	D	D	D
299	R	R	R	R	R	R	R	R
300	F	F	F	F	F	F	F	F
301	Y	Y	Y	Y	Y	Y	Y	Y
302	K	K	K	K	K	K	K	K
303	T	T	T	T	T	T	T	T
304	L	L	L	L	L	L	L	L
305	R	R	R	R	R	R	R	R
306	A	A	A	A	A	A	A	A
307	E	E	E	E	E	E	E	E
308	Q	Q	Q	Q	Q	Q	Q	Q
309	A	A	A	A	A	A	A	A
310	S	S	S	S	S	S	S	S
311	Q	Q	Q	Q	Q	Q	Q	Q
312	D	D	D	D	D	D	D	D
313	V	V	V	V	V	V	V	V
314	K	K	K	K	K	K	K	K
315	N	N	N	N	N	N	N	N
316	W	W	W	W	W	W	W	W
317	M	M	M	M	M	M	M	M
318	T	T	T	T	T	T	T	T
319	E	E	E	E	E	E	E	E
320	T	T	T	T	T	T	T	T
321	L	L	L	L	L	L	L	L
322	L	L	L	L	L	L	L	L
323	V	V	V	V	V	V	V	V
324	Q	Q	Q	Q	Q	Q	Q	Q
325	N	N	N	N	N	N	N	N
326	A	A	A	A	A	A	A	A
327	N	N	N	N	N	N	N	N
328	P	P	P	P	P	P	P	P
329	D	D	D	D	D	D	D	D
330	C	C	C	C	C	C	C	C
331	K	K	K	K	K	K	K	K
332	I	I	I	I	I	I	I	I
333	I	I	I	I	I	I	I	I
334	L	L	L	L	L	L	L	L
335	K	K	K	K	K	K	K	K
336	A	A	A	A	A	A	A	A
337	L	L	L	L	L	L	L	L
338	G	G	G	G	G	G	G	G
339	P	P	P	P	P	P	P	P
340	A	A	A	A	A	A	A	A
341	A	A	A	A	A	A	A	A
342	T	T	T	T	T	T	T	T
343	L	L	L	L	L	L	L	L
344	E	E	E	E	E	E	E	E
345	E	E	E	E	E	E	E	E
346	M	M	M	M	M	M	M	M
347	M	M	M	M	M	M	M	M
348	T	T	T	T	T	T	T	T
349	A	A	A	A	A	A	A	A
350	C	C	C	C	C	C	C	C
351	Q	Q	Q	Q	Q	Q	Q	Q
352	G	G	G	G	G	G	G	G
353	V	V	V	V	V	V	V	V
354	G	G	G	G	G	G	G	G
355	G	G	G	G	G	G	G	G
356	P	P	P	P	P	P	P	P
357	G	G	G	G	G	G	G	G
358	H	H	H	H	H	H	H	H
359	K	K	K	K	K	K	K	K
360	A	A	A	A	A	A	A	A

Figure S1 (continue)

gag	B	A1	C	D	F1	G	01_AE	02_AG
230	361 R	R I4.3	R V I1.3	R V I5.0	R V	R	R	R
362	V I0.5	L	L	L	V	V	L	V
363	L	A	A	A	L	L	L	L
364	A	E	E	E	A	A	A	A
365	E	A	A	A	E	E	E	E
366	A	A	A	A	A	A	A	A
367	M	M	M	M	M	M	M	M
368	S	S	S	S	S	S	S	S
369	Q H1.9	Q H1.8	Q L1.7 R1.0	Q V16.1	Q V41.2	Q H10.4	S N2.5 G1.5	Q
370	V A20.M6.1 I 1.7 T1.1	V A16.5 I1.9 L1.4	V22.5 T15.4	T N15.4 S8.8	T Q14.7	A V10.4	Q H30.2 N7.5 S 2.7 K1.3	V A31.1
371	T S1.1	H31.1 N1.3	N G13.0 S6.5 T1.3 Q1.0	T N14.5 G13.6 K12.7 V4.8 Q3.4 T2.3	S T16.7	S T16.7	Q V28.1 T4.0	Q H16.4
372	N G8.3 S6.6 T2.5 Q1.5	H Q29.4 N1.5 G1.1	N S20.4 H16.0 Q4.9	N S14.5 G13.6 K12.7 V4.8 Q3.4 T2.3	G A29.4	G A29.4	Q H30.2 N7.5 S 2.7 K1.3	Q
373	S P17.7 S4.4 A1.1 Q3.5	T P18.0 A9.7	T A25.2 N8.4 I6.6 K6.2 V5.4 G4.6 P2.2 S1.8 M1.3	G A22.4 L11.5 N8.6 S7.2 V7.2 E6.6 T1.8	T A29.4	T A29.4	Q H30.2 N7.5 S 2.7 K1.3	Q
374	A T12.6 N9.1 P6.2 S1.8 V1.5 G1.5	N S2.4	N S14.9 H13.3 A1.7 V1.1	A V9.7 T7.7	A T12.5	A T12.5	Q H30.2 N7.5 S 2.7 K1.3	Q
375	T A18.5 N12.3 S5.3	I V7.8	I V8.0 T1.3	I V18.8 L6.3 M3.6 A1.6	S A26.5 T23.5	S A26.5 T23.5	A T25.9 V18.3 P1.3 Q1.2	S A29.5 T16.4 P13.1
376	I V13.0 M2.2	M L12.2	M L12.2	M L12.2	V I44.1	V I44.1	N T7.4 S3.6 K3.2 H2.3	N S9.8
377	M L1.2	M L1.3	M L1.2	M L1.2	M	M	I V15.4 M1.3	I V34.4
378	M L2.1 V1.1	M V2.0	M I2.4 V1.3	M L5.7 I1.4	M	M	M L6.9	M
379	Q	Q	Q	Q	Q	Q	M I3.0	M
380	R K35.8 G1.3	R K9.6	R K25.2	R K8.8 G4.1	R K14.6	R K14.6	Q	Q
381	S A41.1 N3.8	G S2.1	S G16.5 N16.4	S G20.1	S G29.2	S G29.2	R K11.3 G2.6	R
382	N K1.2	F L1.3 P.9	F P6.0 V2.1	F K R8.1	F	F	G S1.9 D.9	G
383	F Y2.1 I1.5 L1.2	R K26.7 S1.2	R K7.6 S4.0	R K R8.1	R K	R K	N I2.7 K1.0	N
384	R K16.3 G1.3	G A7.9 N6.7	G S1.3	G	G	G	Q P12.3 R16.4 H1.3	Q
385	N G6.6 S4.6 D2.3 T1.3	P S29.6 T7.6 A2.4 H1.9 L1.3	P S29.6 T7.6 A2.4 H1.9 L1.3	P Q15.6 T6.3 S5.0	P	P	R K14.4 N1.0	R
386	Q P3.3	Q P9.0 G1.0	Q R38.5 N2.2	Q R7.5	Q	Q	G W1.4 N1.2 D.9	G
387	K K7.4 G1.5	R K8.1 G1.4 Q1.3	R K11.2	R K7.5	R K38.2	R K38.2	Q P12.3 R16.4 H1.3	Q
388	K R9.2	R K10.3 T1.7 N1.3 M1.1	R K11.2	R K7.5	R	R	R K21.5 N2.6 Q1.2	R
389	T T58.8 P7.5 S6.6 A4.6 N2.3 V1.6 M1.5 K1.4	I	I T14.8 P5.1 N4.7 V2.2 R1.5 S1.3 M1.0	I T17.6 N10.9 S5.2	I	I	R K10.9 S4.0 G2.2 N1.2 I1.2 T.9	I
390	V I5.2	I	I L13.3 V9.0	I L13.3 V9.0	I	I	I F1.1	I
391	K	K	K	K	K	K	K	K
392	C	C	C	C	C	C	C	C
393	F	F	F Y1.7	F	F	F	F	F
394	N	N	N	N	N	N	N	N
395	C	C	C	C	C	C	C	C
396	G	G	G	G	G	G	G	G
397	K R11.0	K	K R6.7	K R6.7	K	K	K R25.3	K
398	E D5.5 V3.9 Q3.0 I.10	E V2.6 D2.4	E	E	E	E	E D.9	E
399	G	G	G	G	G	G	G	G
400	H	H	H	H	H	H	H	H
401	L11.5 V18.1 T1.5	L	L L2.7	L L14.5 T13.3	L	L	L	L
402	A	A	A	A	A	A	A	A
403	K R48.1	R K6.0	R K24.9	R K31.7	R	R	R K3.6	R
404	N H1.8	N	N	N	N	N	N	N
405	C	C	C	C	C	C	C	C
406	R K7.3	R	R K7.0	R K9.7	R	R	R K9.9	R
407	A	A	A	A	A	A	A	A
408	P	P	P	P	P	P	P	P
409	R	R	R	R	R	R	R	R
410	K R1.8	K	K	K	K	K	K	K
411	K R17.8	K	K R11.9	K R39.8	K	K	K R2.9	K
412	G	G	G	G	G	G	G	G
413	C	C	C	C	C	C	C	C
414	W	W	W	W	W	W	W	W
415	K	K	K	K	K	K	K	K
416	C	C	C	C	C	C	C	C
417	G	G	G	G	G	G	G	G
418	K R14.4 Q4.6	K R1.9	K R7.5 Q4.4	K R35.3 G2.5	K R35.3	K R10.4	K R14.4 Q1.3	K
419	E	E D1.1	E	E	E	E	E D1.8	E
420	G	G	G	G	G	G	G	G
421	H	H	H	H	H	H	H	H
422	Q	Q	Q	Q	Q	Q	Q	Q
423	M	M	M	M	M	M	M	M
424	K	K	K	K	K	K	K	K
425	D E5.7	D E3.7	D E2.0	D E1.6	D	D	D E1.9	D
426	C	C	C	C	C	C	C	C
427	T N4.6 S3.9 I2.1	T E1.4 D1.3	T	T	T	T	T N4.6	T
428	E G1.6	E D1.2 G.9	E	E	E	E	E	E
429	R K1.5 G10	R K2.2	R	R	R	R	R	R
430	Q	Q	Q	Q	Q	Q	Q	Q
431	A	A	A	A	A	A	A V1.0	A
432	N	N	N	N	N	N	N	N

Figure S1 (continue)

gag	B	A1	C	D	F1	G	01 AE	02 AG
1	433 F	F	F	F	F	F	F	F
	434 L	L	L	L	L	L	L	L
	435 G	G	G	G	G	G	G	G
5	436 K R33	K R257	K R50	K R81	K R235	K R146	K R223	K R213
	437 I L2.4. V1.7	I	I L2.6. F1.0. V1.0	I F14.3L13.3	I	I	I L17.4 F4.9	I
	438 W	W	W	W	W	W	W	W
	439 P S1.4	P	P	P S7.0	P	P	P	P
	440 S	S	S	S	S	S	S P3.6	S
10	441 H Y7.1 N6.0. S5.9. C2.3. Q1.5	S N16.0H5.2 R1.5	H Q9.3 N7.8 R3.2 L2.2 Y1.8	H N9.0 R5.7 C5.2 Q4.3 Y1.4 L1.1	N H32.4	N S33.3	N H18.4 S4.8	N S29.5
	442 K R2.3	K	K Q2.4. R2.0	K Q5.4 R1.4	K	K	K	K
	443 G E1.5	G	G	G	G	G	G	G
	444 R	R	R	R	R	R	R	R
	445 P	P	P	P	P	P	P	P
	446 G	G	G	G R1.8	G	G	G	G
15	447 N	N	N	N	N	N	N	N
	448 F	F	F	F	F	F	F	F
	449 L P7.7	L L4.4	L I5.2. P2.7	L P1.4	L	L	L	L
	450 Q	Q	Q	Q	Q	Q	Q	Q
	451 S N16.3	N S35.0K3.3	N S42.1R1.2	N S14.5R2.0	N S41.2	N S10.4	N S3.2	N
5	452 R	R S1.6. K1.0	R	R	R	R	R	R
	453 P L7.3. T1.4	L P31.5M1.0. V1.0	P L11.3T3.4. A2.8. I1.6	P T3.6	P	P T43.8	P T13.3L5.0	P
	454 E T3.2	E	E	E	E	E	E	E
	455 P	P	P	P	P	P	P	P
	456 T S18.1	T S4.5	T S4.4	T	T	T	T S18.5	T S11.5
	457 A	A	A	A	A	A	A	A
10	458 P	P	P	P	P	P	P	P
	459 P T3.6. S1.3	P	P L1.3	P	P	P	P	P
	460 E V2.3	E	E A L10.0E4.2. V1.2	E A T2.7	E	E	E	E
	461 E V2.3	E	E	E	E	E	E	E
15	462 S N2.7. G2.2. I2.1	I L20.7M4.0. S2.9. T2.6. N2.4	S Q3.9	S	S	S	S N19.5H2.2. S1.3	S
	463 F V2.8. L2.0	F C26.8L10.4S3.2. W1.7	F S3.9. A1.6. Q1.2	F L6.6	F	F L10.4	F	F
	464 R G5.2. S1.5. K1.3	R	R K13.2P3.9. E3.5. G1.6. S1.4	R S7.7	R	R	R	R
	465 F R1.3. V1.0	M F2.6. I2.4. V1.2	M	M	M	M	M	M
	466 E G1.6	G R8.1. E4.2. A2.1	G R8.1. E4.2. A2.1	G	G	G	G	G
	467 E G1.6	E	E Q4.6. G3.1. R3.0	E	E	E	E	E
20	468 E G5.7	E	E G1.2	E	E	E	E	E
	469 T K2.5. A2.5. I2.4	I T7.3. M4.9. V4.7	I	I	I	I	I	I
	470 T A17.0 T1.8	T	T	T	T	T	T	T
	471 T A8.3. P1.9. N1.3	T	T	T	T	T	T	T
25	472 P	P	P	P	P	P	P	P
	473 S P33.7A1.7. V1.5	A T35.3V3.5. S1.5	A	A	A	A	A	A
	474 Q	Q	Q	Q	Q	Q	Q	Q
	475 K R5.2	S P9.8. F3.8	S	S	S	S	S	S
	476 Q P4.6. K2.4	P S4.6. H1.1	P	P	P	P	P	P
	477 E G7.7 D2.9	P L17.8Q10.7	P	P	P	P	P	P
30	478 P T14.1Q12.9S5.8. L2.7. K1.1	K Q2.0	K	K	K	K	K	K
	479 I T11.5K5.4. R4.8. V3.2. M1.3	R R18.5T2.1. E1.3	R	R	R	R	R	R
	480 D N2.7. E1.4	D E8.4. N2.9. G1.3	D	D	D	D	D	D
	481 K E6.1. R4.6. Q2.3	R K15.2G1.4	R	R	R	R	R	R
	482 E D10.2G4.2	E D11.1. N1.0	E	E	E	E	E	E
35	483 L M13.5R3.8. K2.2. Q1.9. T1.7. V1.5	Q P5.1. L4.6. R1.2	Q	Q	Q	Q	Q	Q
	484 Y P2.1	A G1.0. V1.0	A	A	A	A	A	A
	485 P	P	P	P	P	P	P	P
	486 L S6.3. M2.6. V1.5	L S20.0A1.8	L	L	L	L	L	L
	487 A T34.5V1.2. S.9	V I42.6T5.2. A1.7	V	V	V	V	V	V
40	488 S A1.2	S	S	S	S	S	S	S
	489 L	L	L	L	L	L	L	L
	490 R K44.2	R	R	R	R	R	R	R
	491 S	S	S	S	S	S	S	S
45	492 L	L	L	L	L	L	L	L
	493 F	F	F	F	F	F	F	F
	494 G	G	G	G	G	G	G	G
	495 N S10.0	N S2.4	N	N	N	N	N	N
	496 D	D	D	D	D	D	D	D
50	497 P H.9	P L24.5	P	P	P	P	P	P
	498 S L14.9	S L9.7	S	S	S	S	S	S
	499 S L6.5	S L3.1	S	S	S	S	S	S
	500 Q	Q	Q	Q	Q	Q	Q	Q

Most conserved Least conserved Drug binding positions

Figure S2



Drug binding positions

Subtype A1

Subtype B

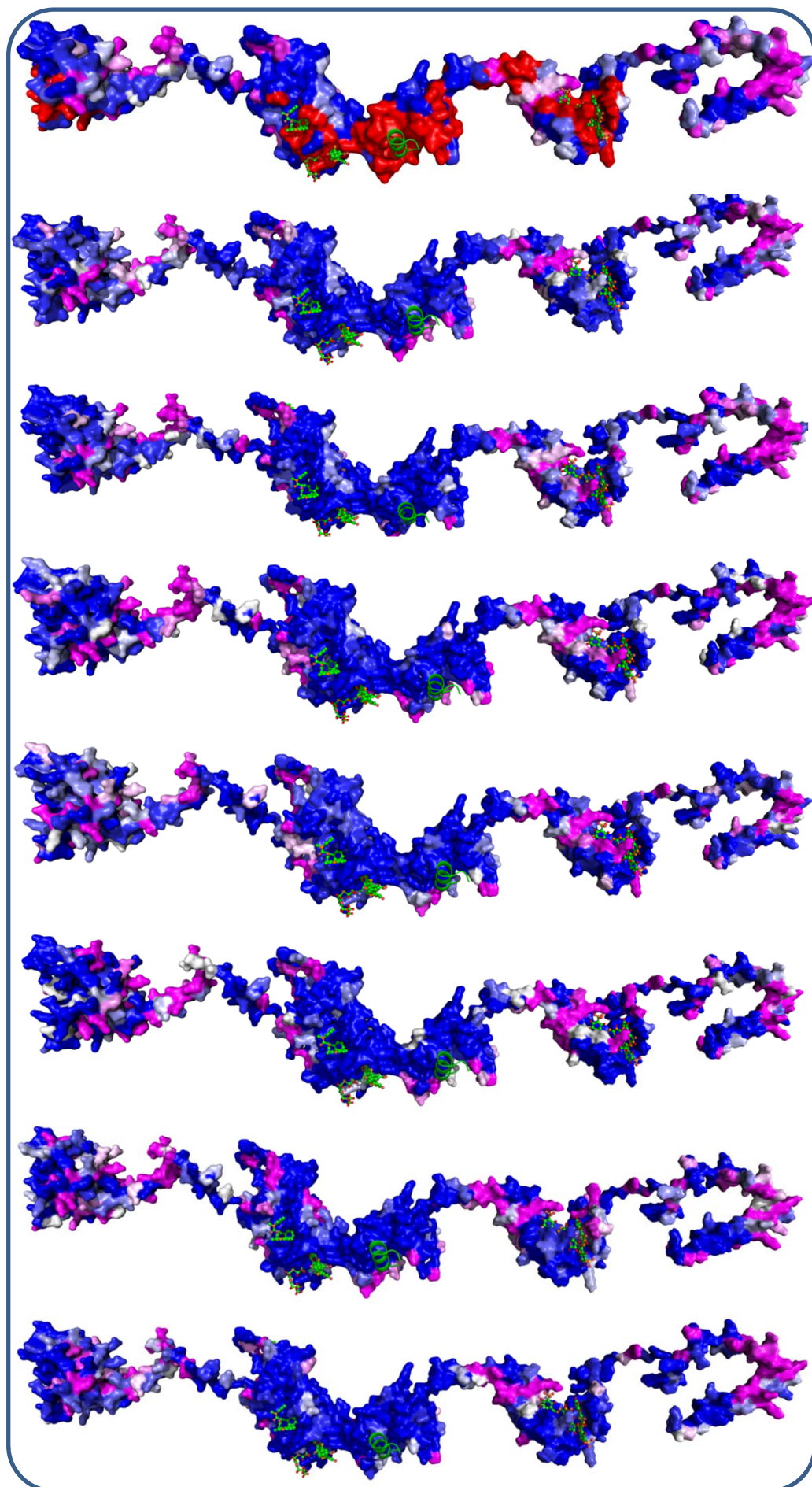
Subtype C

Subtype D

Subtype F1

Subtype G

CRF 01_AE



**Figure S2
(continue)**

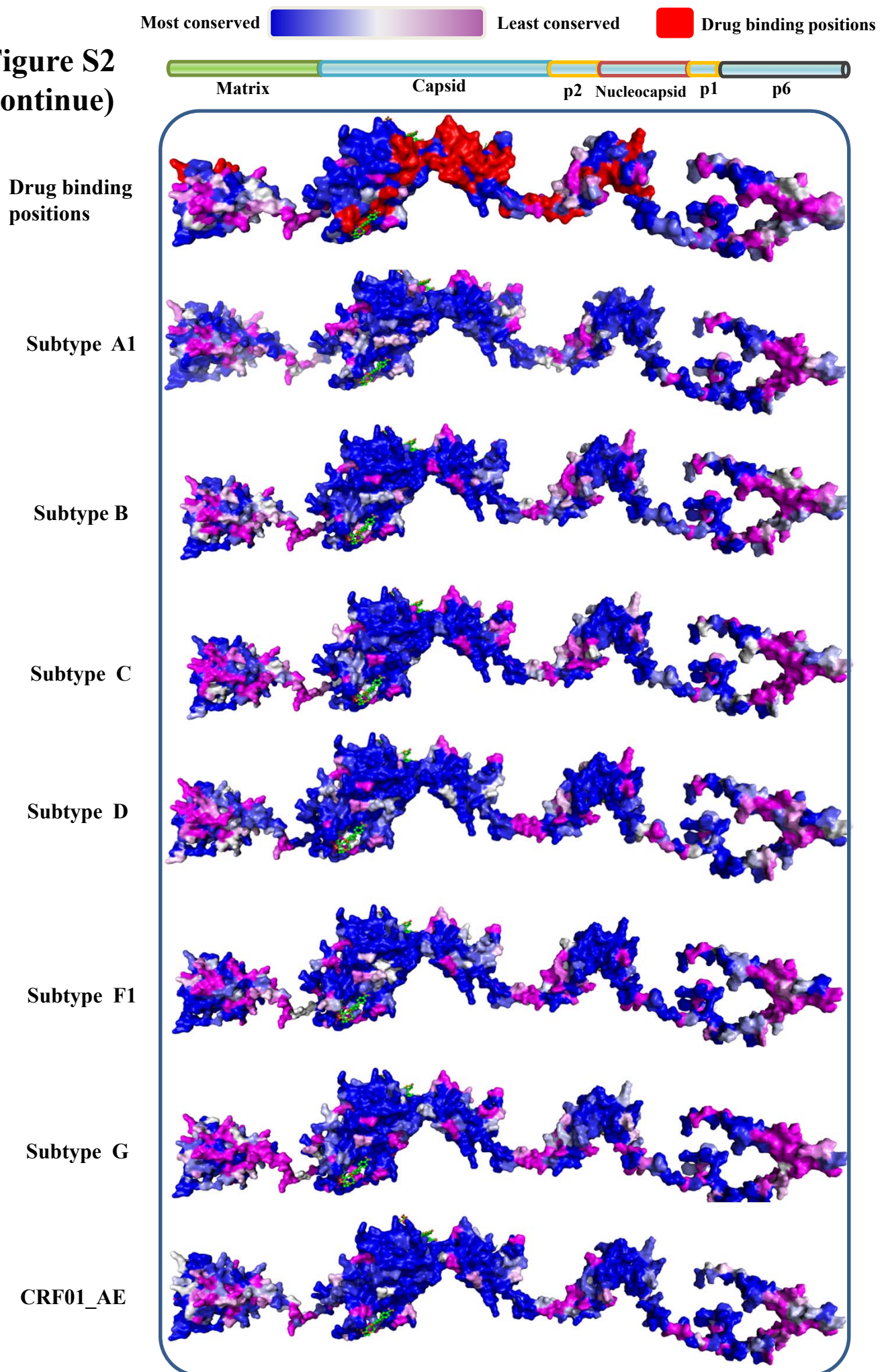


Fig S3 Binding pockets 1 Binding pockets 2 Binding pockets 3 Binding pockets 4 Binding pockets 5

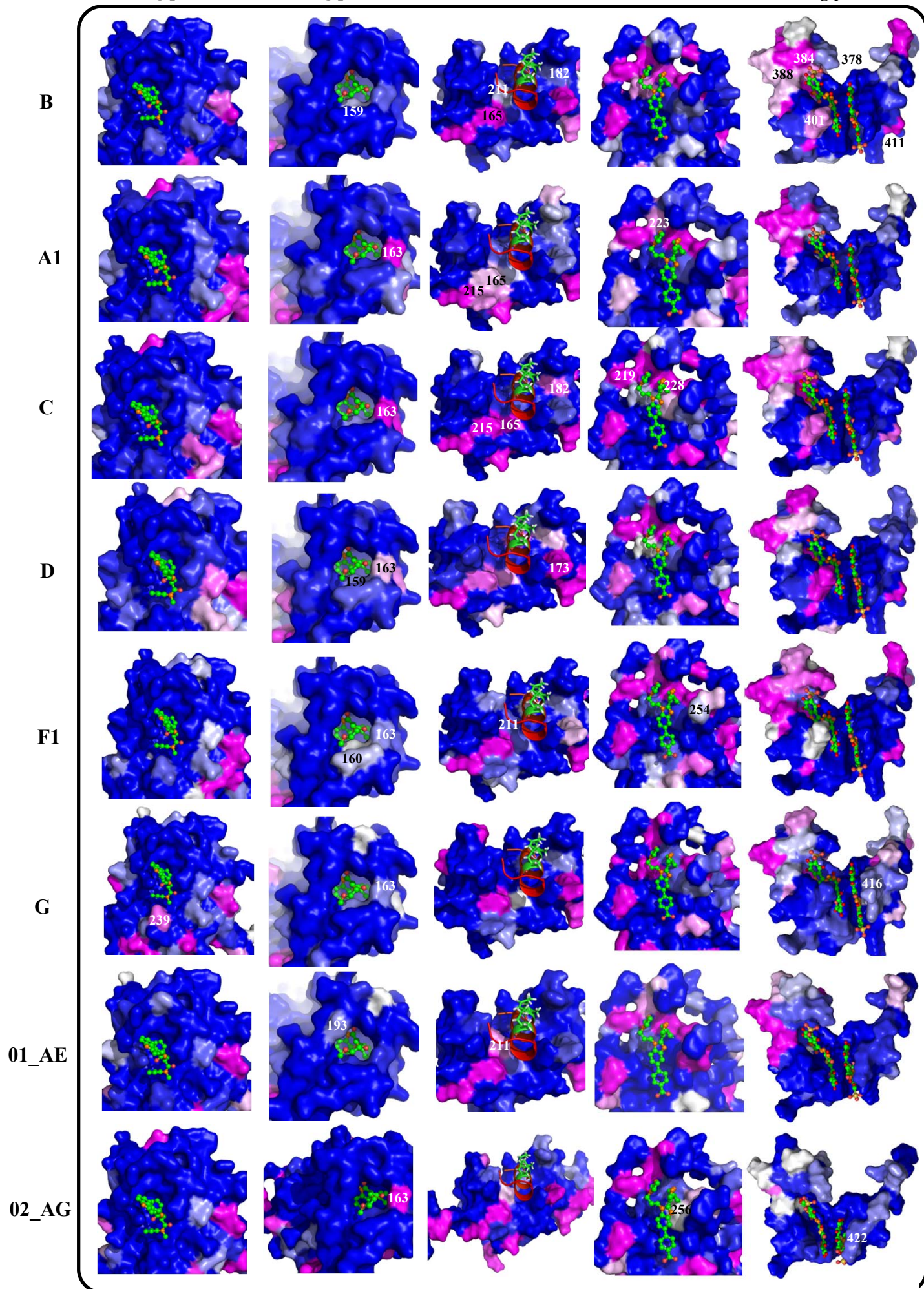


Fig S4(A)

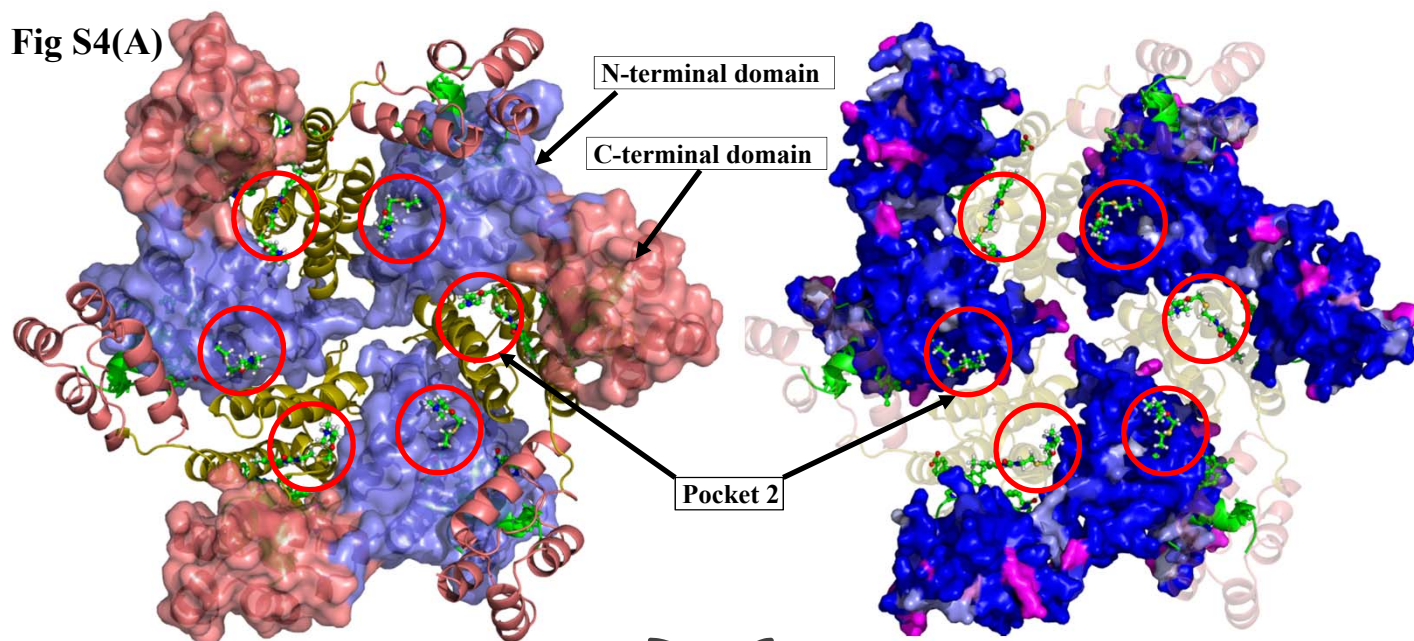
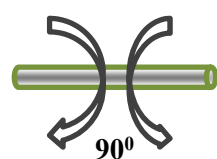
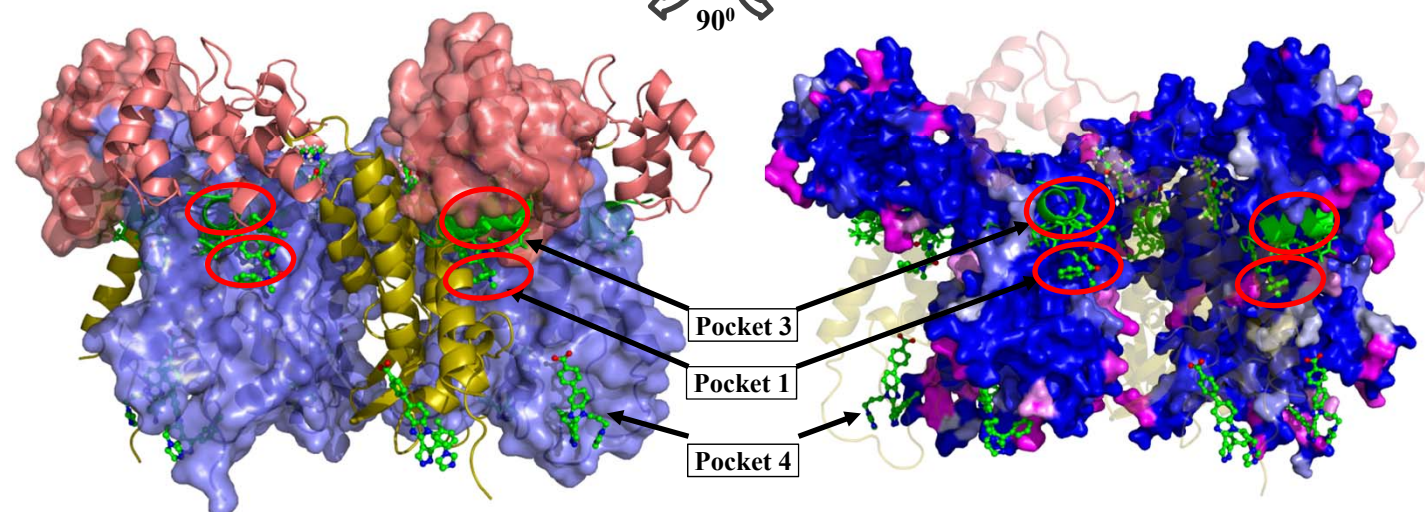


Fig S4(B)



Pocket 2: NTD-NTD interaction



Pocket 1: NTD-CTD interaction

Pocket 3: NTD-CTD interaction

Fig S4(C)

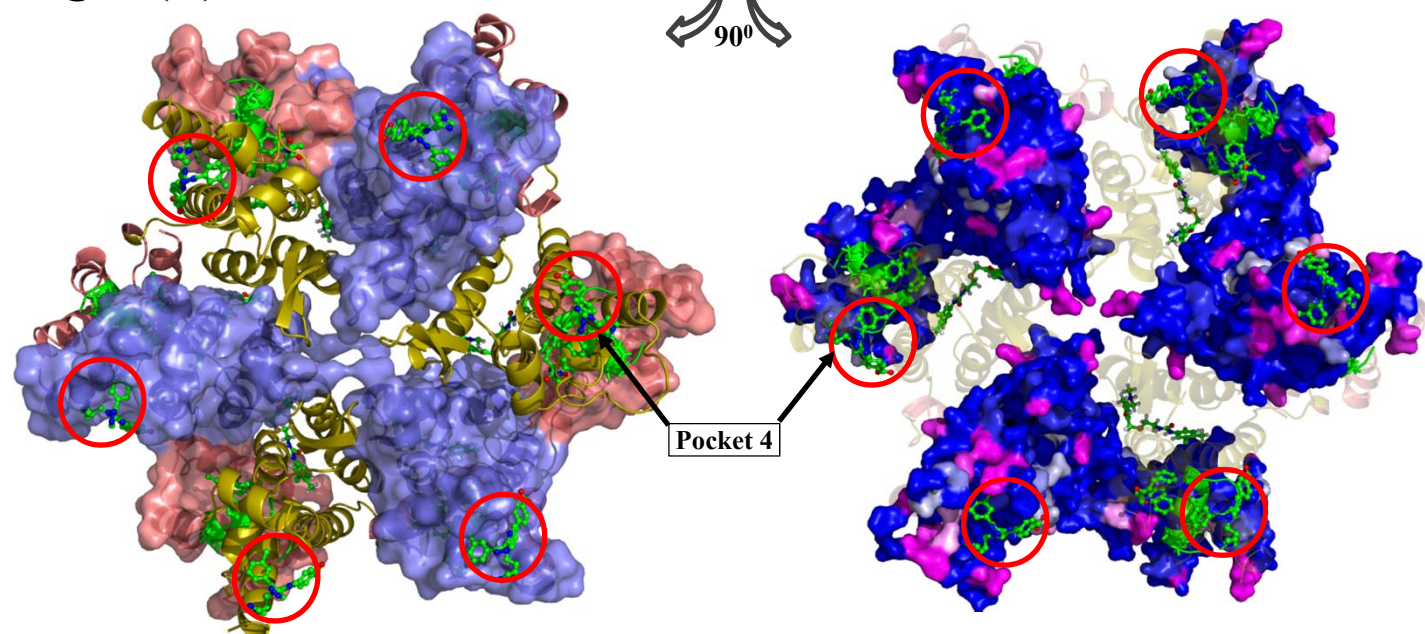


Fig S5(A)

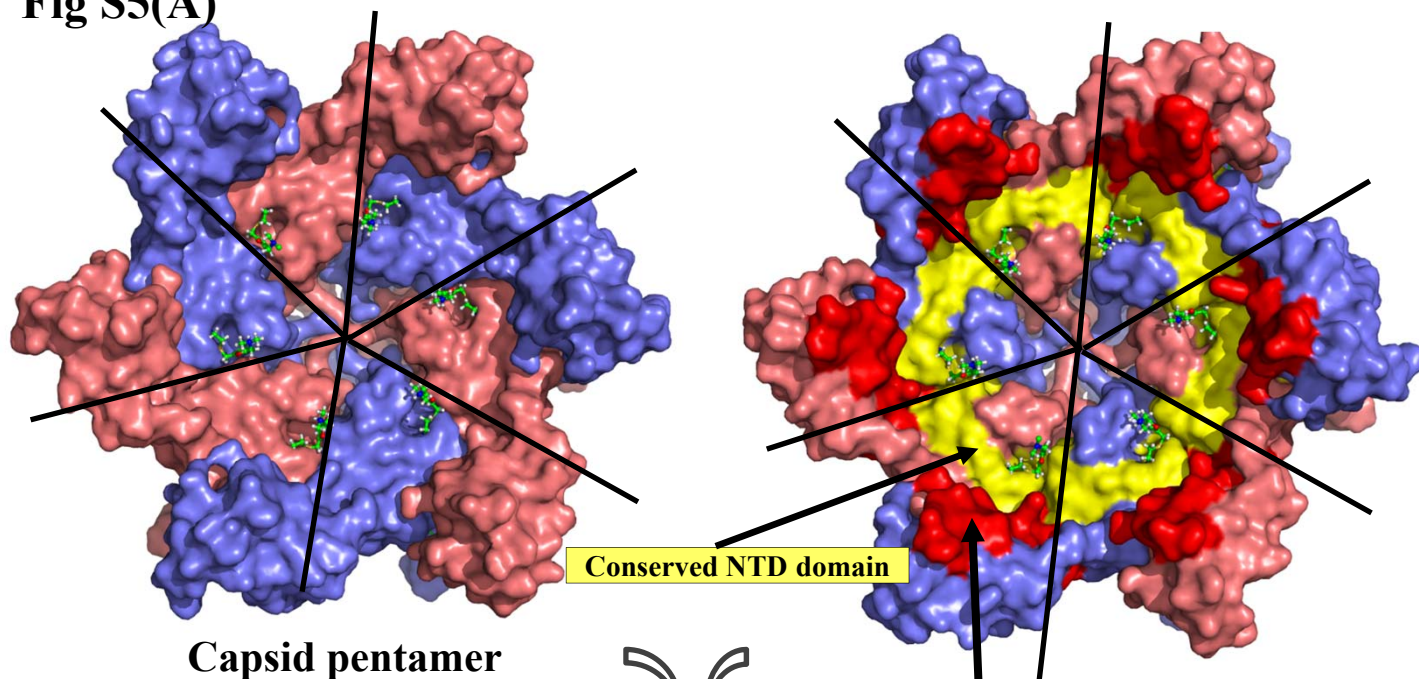


Fig S5(B)

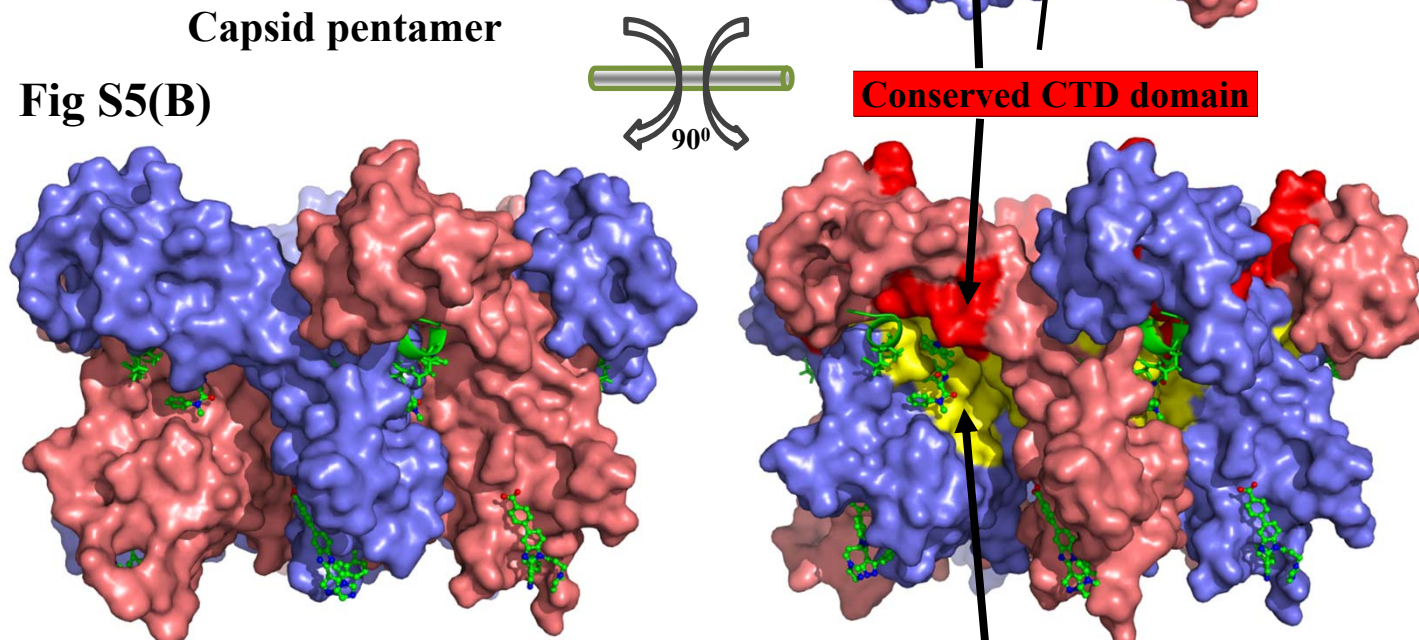


Fig S5(C)

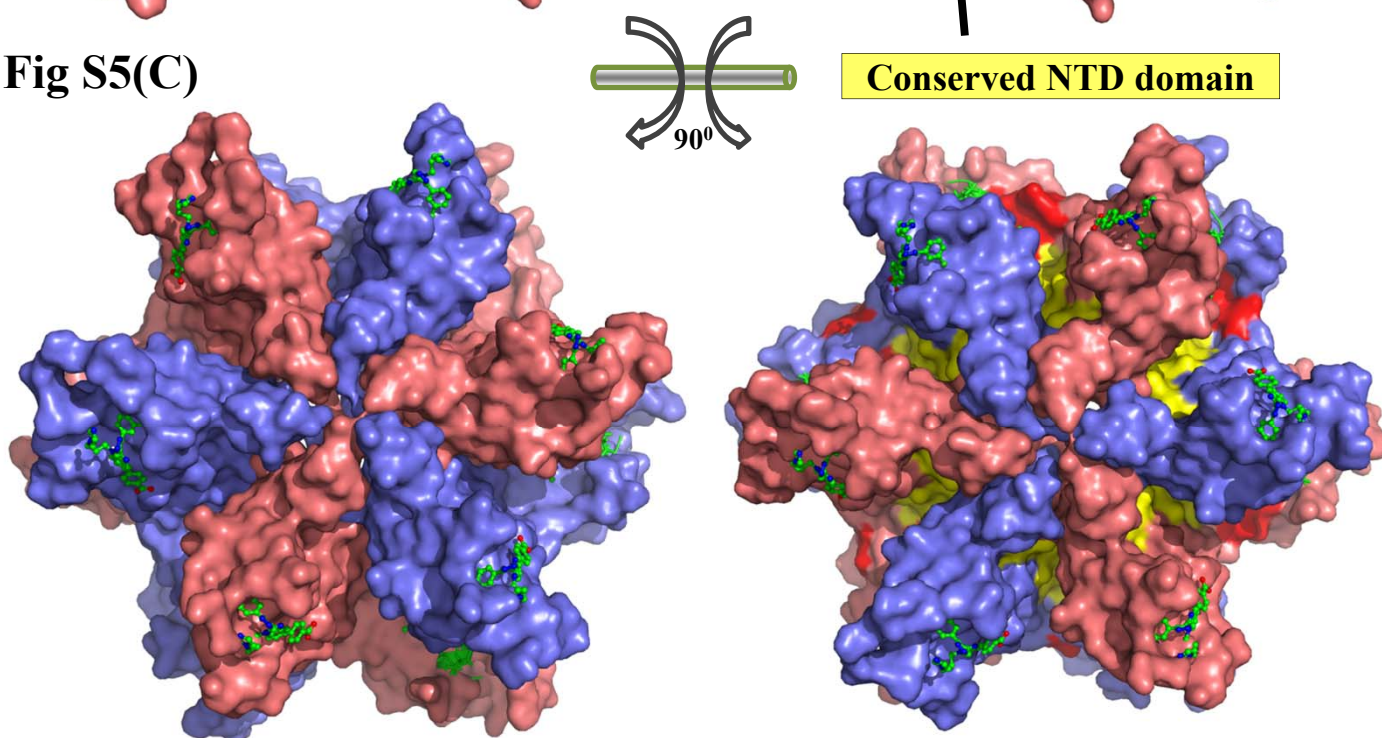


Fig S6(A)

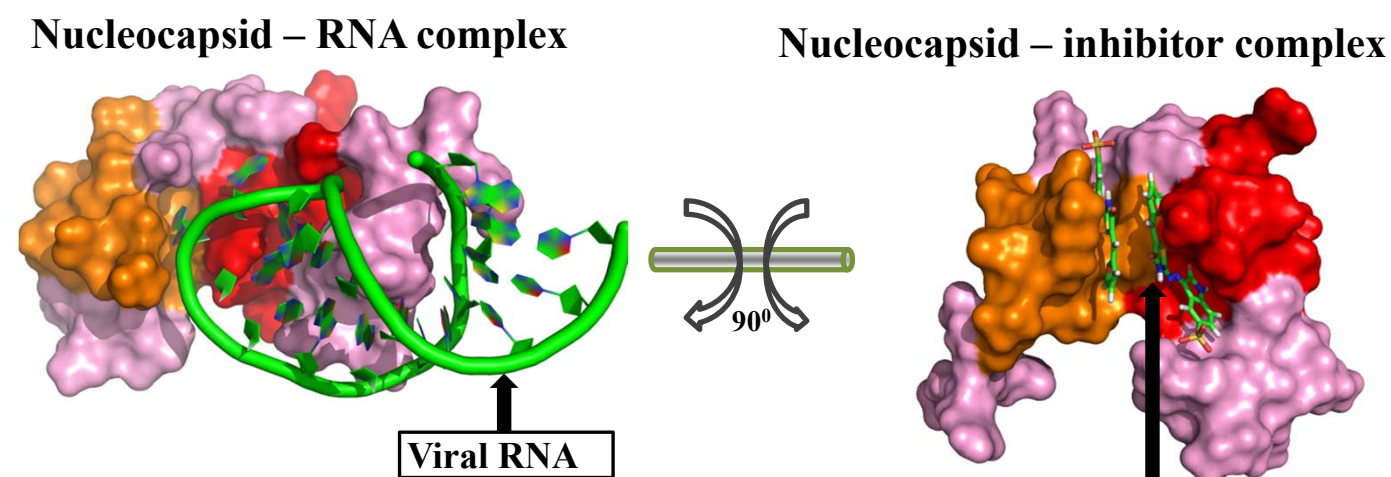


Fig S6(B)

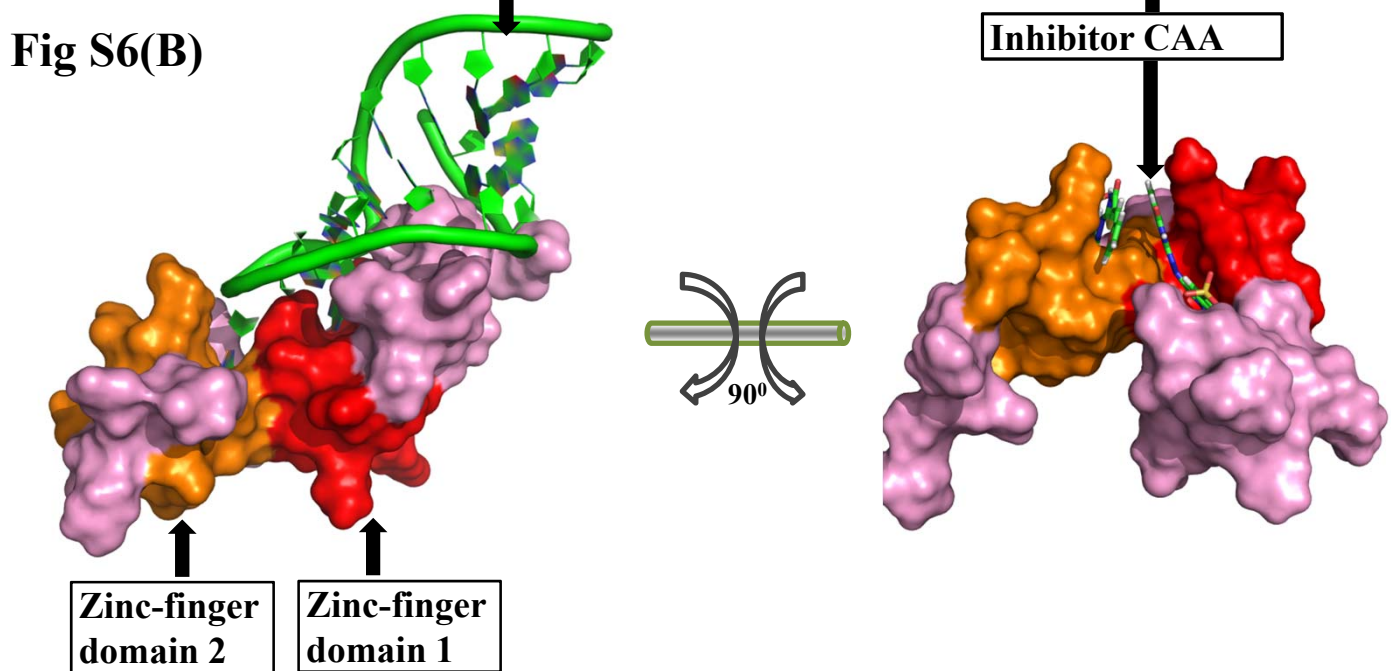


Fig S6(C)

