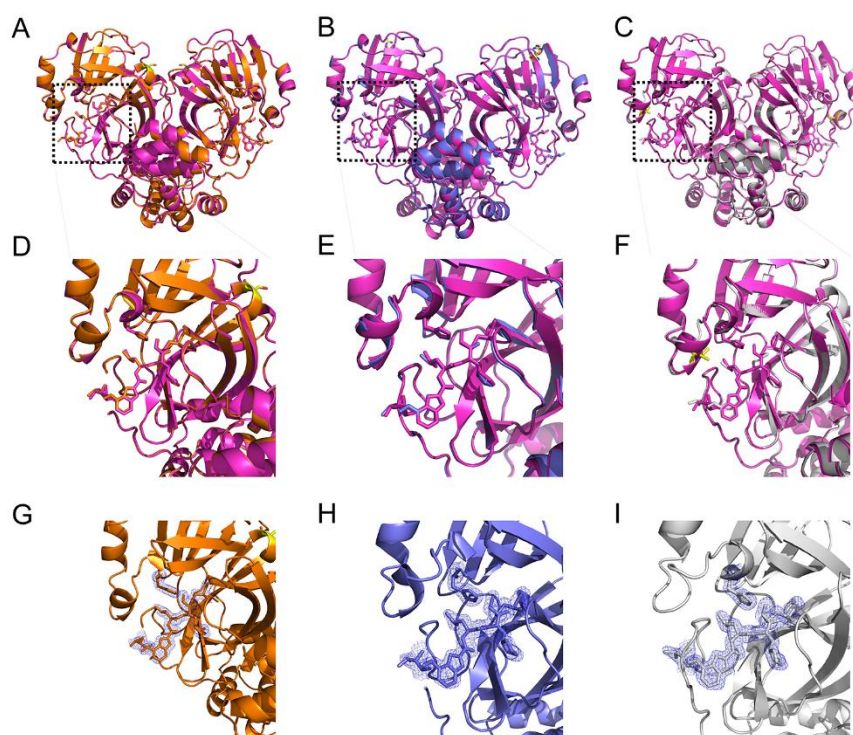
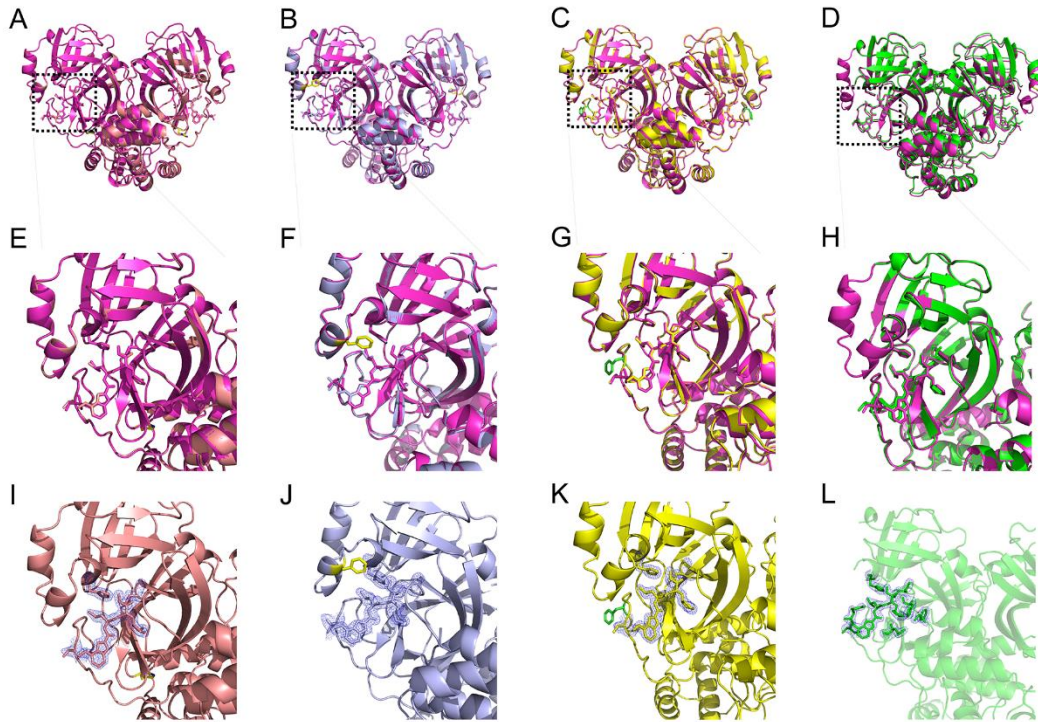


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2 **Supplementary Figure S1. Structural comparison between SARS-CoV-2**
3 **M^{pro}-PF-00835231 and available structures** (A) Structure of SARS-CoV-2
4 M^{pro}-PF-00835231. (B) Structural comparison between SARS-CoV-2
5 M^{pro}-PF-00835231 and SARS-CoV-2 M^{pro}-PF-00835231 (PDB ID 6XHM, Lime
6 green). (C) Structural comparison between SARS-CoV-2 M^{pro}-PF-00835231 and
7 SARS-CoV-2 M^{pro}-PF-00835231 (PDB ID 8DSU, cyan). SARS-CoV-2
8 M^{pro}-PF-00835231 is shown as a cartoon with light magenta (this research), lime green
9 (PDB ID 6XHM) and cyan (PDB ID 8DSU). (D–F) The zoomed-in view of the
10 substrate binding pocket of the main proteases. PF-00835231 and its catalytic dyad
11 residues are shown as sticks. (G) The *2Fo-Fc* electron density map (contoured at 1.0σ)
12 of the inhibitor and catalytic dyad residues His41, Cys145 and Thr190 in the
13 SARS-CoV-2 M^{pro}-PF-00835231 complex (this study) is shown as blue mesh. (H) The
14 *2Fo-Fc* electron density map (contoured at 1.0σ) of the inhibitor and catalytic dyad
15 residues His41, Cys145 and Thr190 in the SARS-CoV-2 M^{pro}-PF-00835231 complex
16 (PDB ID 6XHM) is shown as a blue mesh. (I) The *2Fo-Fc* electron density map
17 (contoured at 1.0σ) of the inhibitor and catalytic dyad residues His41, Cys145 and
18 Thr190 in the SARS-CoV-2 M^{pro}-PF-00835231 complex (PDB ID 8DSU) is shown as
19 a blue mesh.



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21 **Supplementary Figure S2. Structural comparison between SARS-CoV-2**
 22 **M^{pro}-PF-00835231 and the M^{pro} mutants PF-00835231 (G15S, K90R and M49I).**
 23 **S15, R90 and I49 are shown as yellow sticks** (A) Structural comparison between
 24 SARS-CoV-2 M^{pro}-PF-00835231 and G15S-PF-00835231. (B) Structural comparison
 25 between SARS-CoV-2 M^{pro}-PF-00835231 and M^{pro} K90R-PF-00835231. (C)
 26 Structural comparison between SARS-CoV-2 M^{pro}-PF-00835231 and M^{pro}
 27 M49I-PF-00835231. (D–F) The zoomed-in view of the substrate binding pocket of
 28 main proteases. PF-00835231 and catalytic dyad residues are shown as sticks. (G–H)
 29 *2Fo-Fc* electron density maps of PF-00835231 bound to different SARS-CoV-2 M^{pro}
 30 mutants. *2Fo-Fc* electron density maps (blue) were constructed at 1 σ . (G) The *2Fo-Fc*
 31 electron density map of PF-00835231 bound to the G15S mutant. (H) The *2Fo-Fc*
 32 electron density map of PF-00835231 bound to the K90R mutant. (I) The *2Fo-Fc*
 33 electron density map of PF-00835231 bound to the M49I mutant.



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35 **Supplementary Figure S3. Structural comparison between SARS-CoV-2**
 36 **M^{pro}-PF-00835231 and the M^{pro} mutant PF-00835231 (P132H, S46F, V186F and**
 37 **Y54C). H132, F46 and C54 are shown as yellow sticks, while F186 is shown as**
 38 **green sticks** (A) Structural comparison between SARS-CoV-2 M^{pro}-PF-00835231
 39 and P132H-PF-00835231. (B) Structural comparison between SARS-CoV-2
 40 M^{pro}-PF-00835231 and M^{pro} S46F-PF-00835231. (C) Structural comparison between
 41 SARS-CoV-2 M^{pro}-PF-00835231 and M^{pro} V186F-PF-00835231. (D) Structural
 42 comparison between SARS-CoV-2 M^{pro}-PF-00835231 and M^{pro} Y54C-PF-00835231.
 43 (E–H) The zoomed-in view of the substrate binding pocket of main proteases.
 44 PF-00835231 and catalytic dyad residues are shown as sticks. (I–L) *2Fo-Fc* electron
 45 density maps of PF-00835231 bound to different SARS-CoV-2 M^{pro} mutants. *2Fo-Fc*
 46 electron density maps (blue) were contoured at 1 σ . (I) The *2Fo-Fc* electron density map
 47 of PF-00835231 bound to the P132H mutant. (J) The *2Fo-Fc* electron density map of
 48 PF-00835231 bound to the S46F mutant. (K) The *2Fo-Fc* electron density map of
 49 PF-00835231 bound to the V186F mutant. (L) The *2Fo-Fc* electron density map of
 50 PF-00835231 bound to the Y54C mutant.

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