**Supporting Information for**

# Structural basis for catalysis and ubiquitin recognition by the severe acute respiratory syndrome coronavirus papain-like protease

Chi-Yuan Chou[1,*], Hsing-Yi Lai[1,#], Hung-Yi Chen[1,#], Shu-Chun Cheng[1,#], Kai-Wen Cheng[1], and Ya-Wen Chou[1]

[1]Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei 112, Taiwan

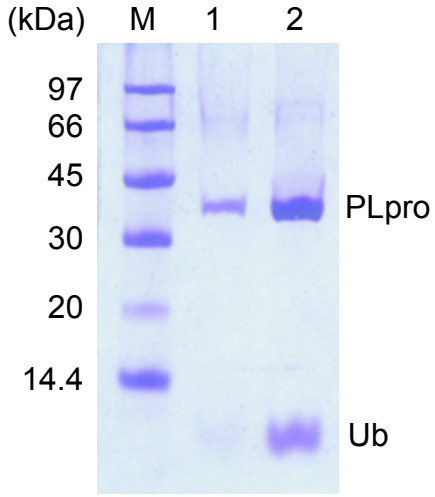
[#]Equal contributions

[*]Correspondence information for Chi-Yuan Chou

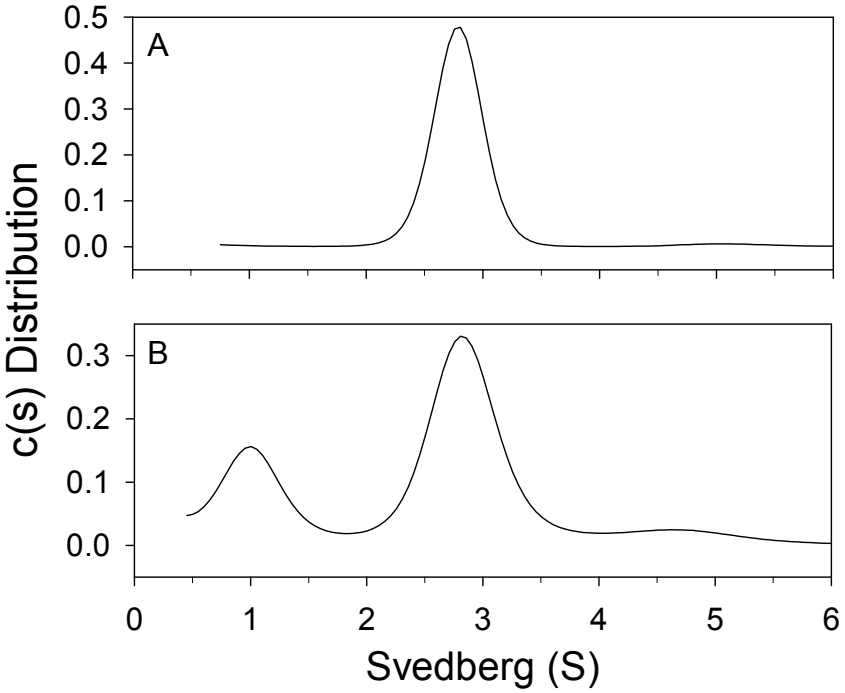
Phone: +886-2-28267168; FAX: +886-2-28202449

E-mail: cychou@ym.edu.tw

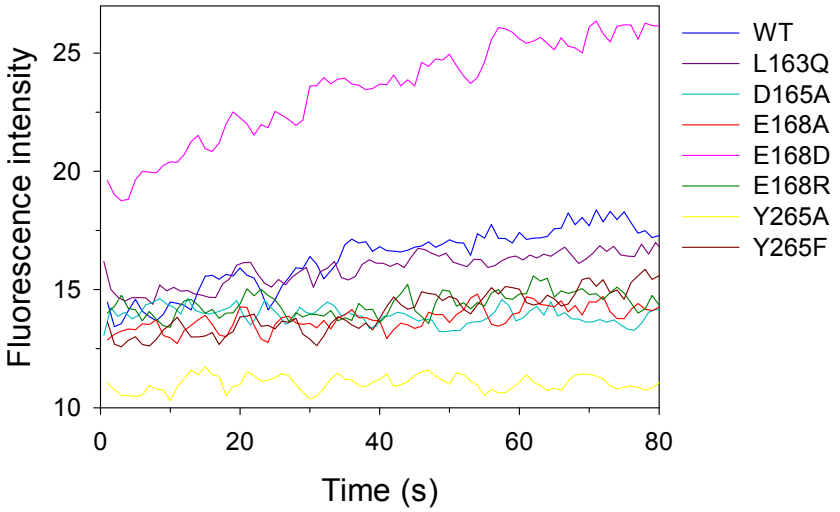
5 supplement figures included.



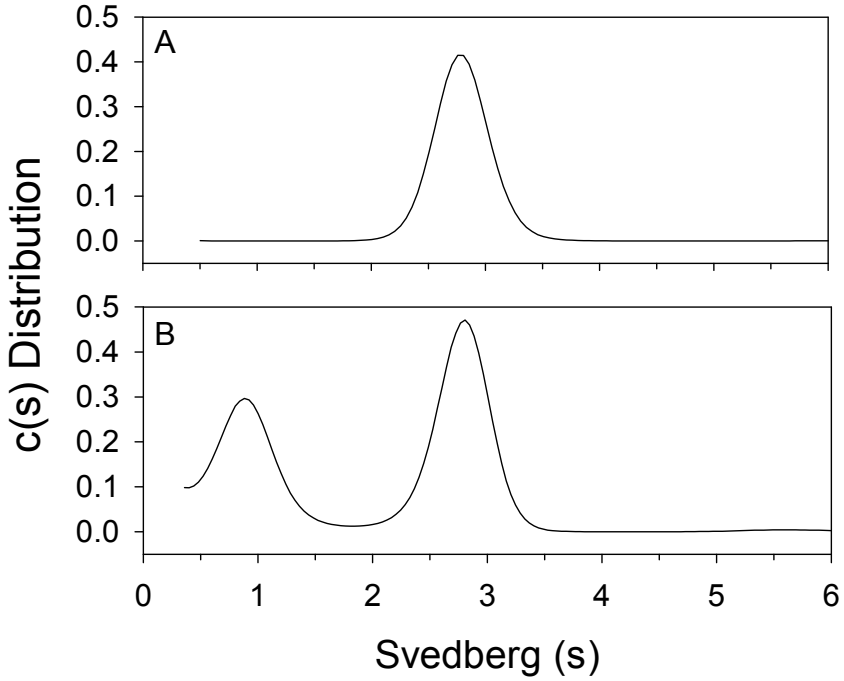

**Supplement Fig. 1. Protein content analysis of PL$^{pro}$ C112S-Ub co-crystals by SDS-PAGE.** Lane 1 and 2 show the results of two crystals with size of 0.2 and 0.8 mm, respectively.



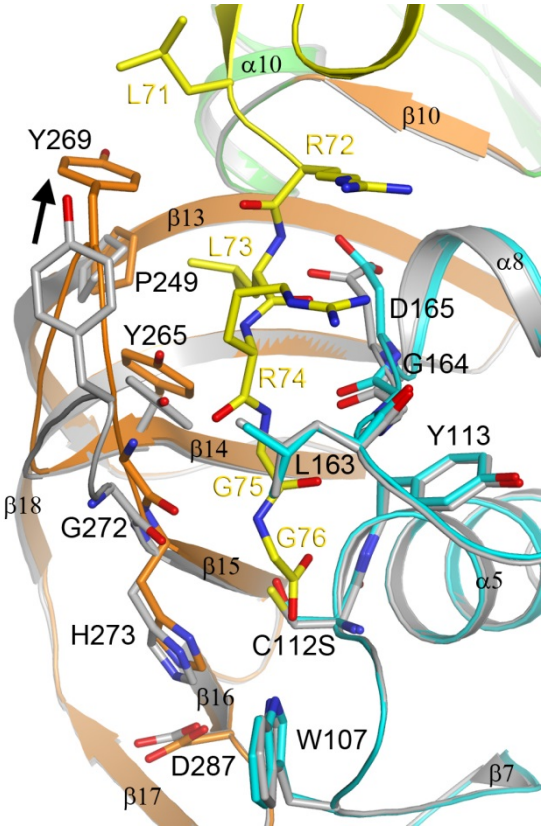

**Supplement Fig. 2. Continuous c(s) distributions of SARS-CoV PL$^{pro}$ without (A) and with Ub (B) by AUC analysis.** The protein concentration of wild-type PL$^{pro}$ in panel A was 0.2 mg/ml. In panel B, wild-type PL$^{pro}$ (0.2 mg/ml) and Ub (1 mg/ml) were overnight incubated at 4 $^{o}$C and then analyzed by AUC. The detailed AUC and analyzing methods are described in experimental procedures.



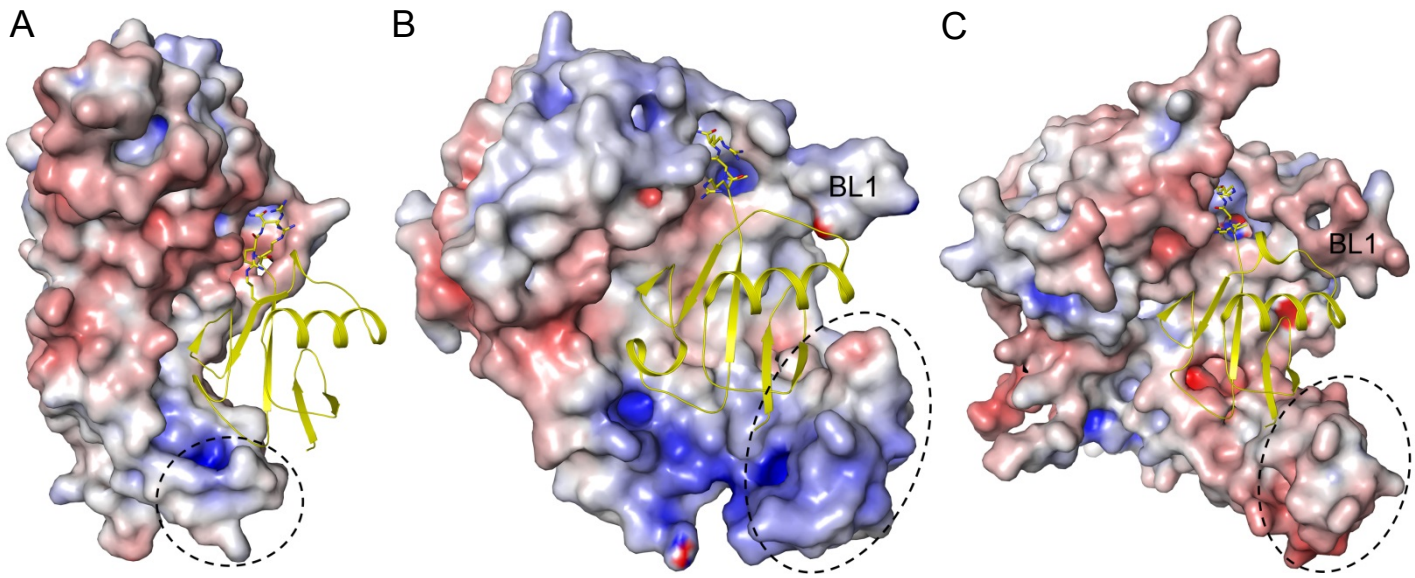

**Supplement Fig. 3. Deubiquitinating activity assay of SARS-CoV PL$^{pro}$ and its mutants.** Fluorescence versus time progress curves for hydrolysis of Ub-AFC (0.5 μM) at 30 $^{o}$C. The results of wild-type, L163Q, D165A, E168A, E168D, E168R, Y265A, and Y265F mutants are colored by blue, purple, cyan, red, magenta, green, yellow, and brown, respectively. The protein concentration of wild-type, L163Q, E168D, and Y265F mutant used for the assay was 0.17 μM, while that of D165A, E168A, E168R, and Y265A was 0.51 μM. The excitation and emission wavelength was 350 and 485 nm, respectively.




**Supplement Fig. 4. Continuous c(s) distributions of SARS-CoV PL$^{pro}$ C112S/E168R double mutant without (A) and with Ub (B) by AUC analysis.** The protein concentration of PL$^{pro}$ C112S/E168R double mutant in panel A was 0.2 mg/ml. In panel B, PL$^{pro}$ C112S/E168R double mutant (0.2 mg/ml) and Ub (1 mg/ml) were overnight incubated at 4 $^{o}$C and then analyzed by AUC. The detailed AUC and analyzing methods are described in experimental procedures.




**Supplement Fig. 5. Comparison with the active site of free wild-type SARS-CoV PL$^{pro}$.** Overlay of the active site region of the PL$^{pro}$ C112S mutant (color) in complex with Ub (yellow) and that of free PL$^{pro}$ (grey). The arrow indicates the movement of the residue Tyr269 on the BL2 loop.




**Supplement Fig. 6. Comparison of the ubiquitin-binding surfaces of SARS-CoV $PL^{pro}$, USP2, and USP14.** The ubiquitin molecule is shown as a ribbon diagram (in yellow) and the molecular surface of $PL^{pro}$ (A), human USP2 (B, PDB code: 2HD5), and USP14 (C, PDB code: 2AYO) are colored by potential (red for negative and blue for positive charge). The tip of the fingers domain is indicated by oval circles and location of the binding loop 1 (BL1) of USP2 and USP14 is labeled, respectively.