Supplemental Materials

Molecular basis for PHF7-mediated ubiquitination of histone H3

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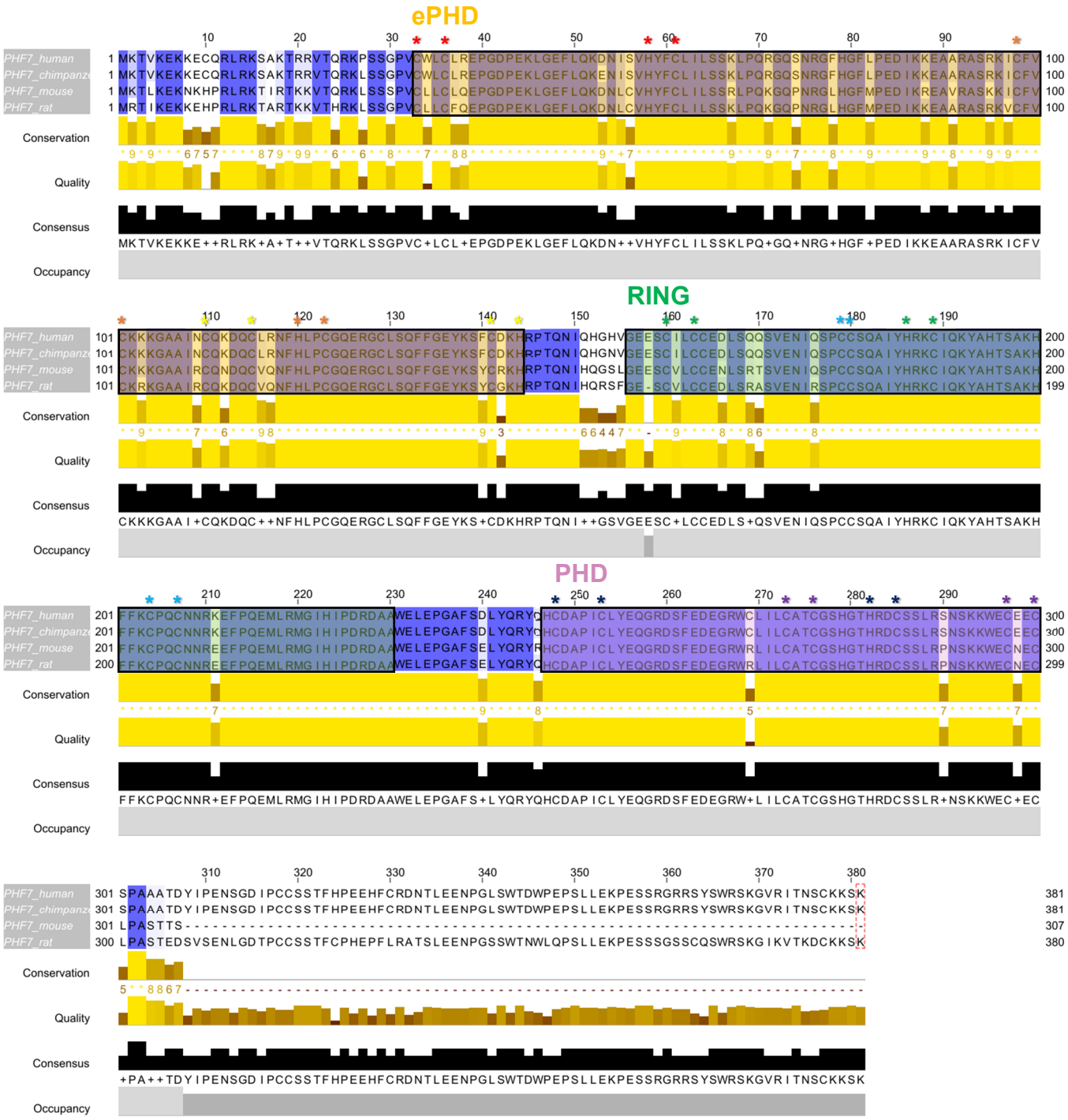
Table S1
Supplemental Figures S1 – S14

Table S1. Data collection and refinement statistics.

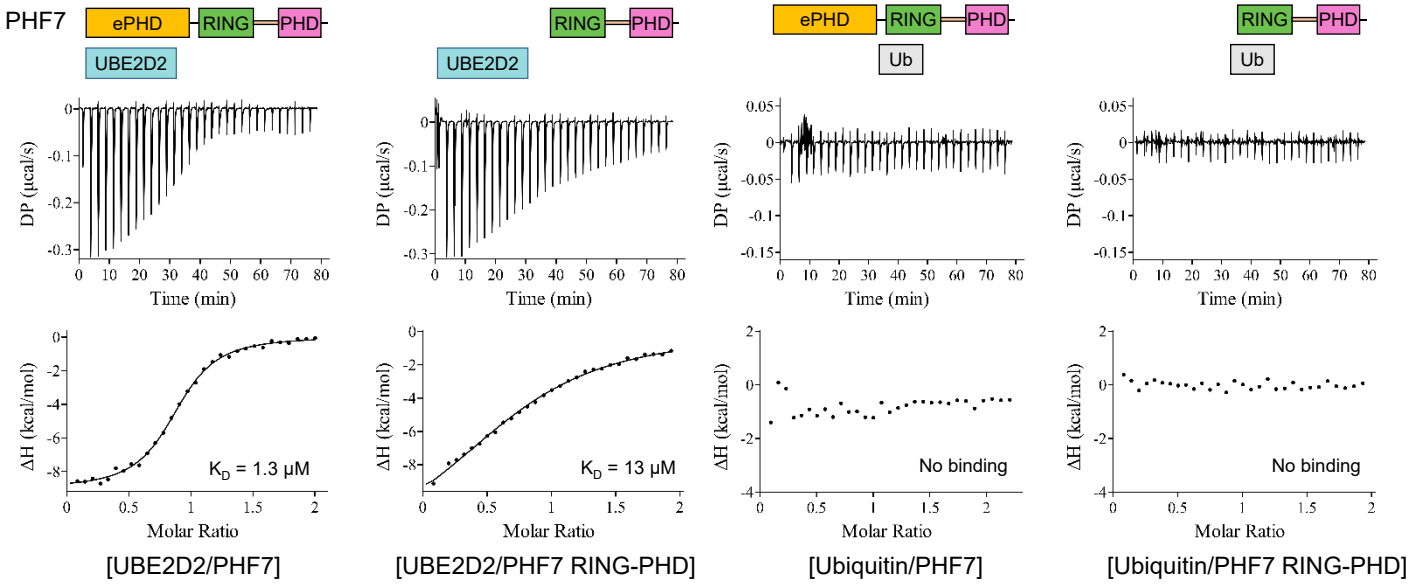
Data Sets	PHF7-sortase-UBE2D2	PHF7-GSGG3-UBE2D2	PHF7 ePHD
PDB ID	8JWJ	8JWU	8JWS
Data Collection			
Space group	P 21 21 21	C 1 2 1	P 21 21 21
Cell dimensions			
a, b, c (Å)	102.304 106.825 145.759 90 90 90	210.54 102.05 78.64 90 95.922 90	37.014 59.145 170.396 90 90 90
Resolution (Å)	47.35 – 2.961 (3.067 – 2.961)	29.89 - 3.58 (3.708 - 3.58)	30.86 - 2.0 (2.072 - 2.0)
R_{merge}	0.06656 (0.7055)	0.1378 (1.179)	0.09503 (0.5245)
Mean I/σ(I)	14.50 (1.51)	11.16 (2.02)	8.33 (2.80)
Completeness (%)	96.49 (94.39)	99.32 (99.69)	97.00 (98.01)
Multiplicity	4.5 (4.6)	6.9 (7.3)	3.2 (3.2)
Refinement			
Rwork/Rfree	0.192/0.216	0.2160/0.2656	0.1964/0.2227
R.m.s. deviations			
Bond lengths (Å)	0.007	0.006	0.012
Bond angles (°)	0.95	0.95	1.23
No. reflections	146142 (14527)	135962 (13930)	81808 (8090)
No. atoms	6695	9349	3147
Protein	6631	9326	2802
Ligand	64	23	29
Solvent			316
Average B factors (Å^2)	100.78	149.07	26.48
Protein	100.73	149.04	26.07
Ligand	105.97	158.84	33.54
Solvent			29.52
Ramachandran plot (%)			
Favored	95.66	93.21	98.55
Allowed	4.34	6.79	1.45
Outliers	0.00	0.00	0.00

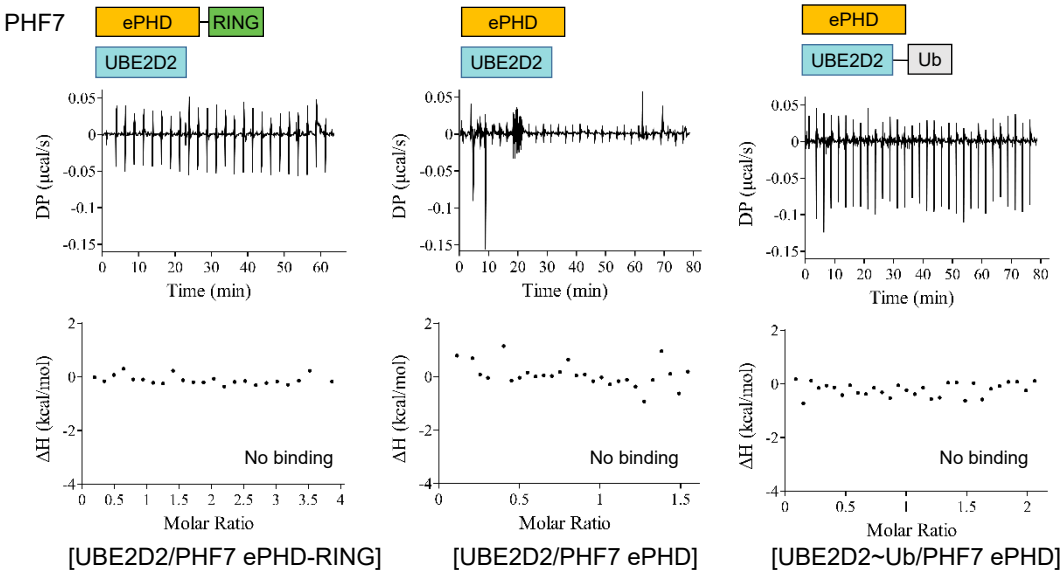
*Values in parentheses refer to that of the highest resolution shell.

Supplemental Figure S1. Sequence alignment of PHF7 in various species. PHF7 protein sequences were aligned by Clustal Omega and visualized by Jalview. Conserved domains are highlighted. Zinc coordinating residues are marked with colored asterisks.

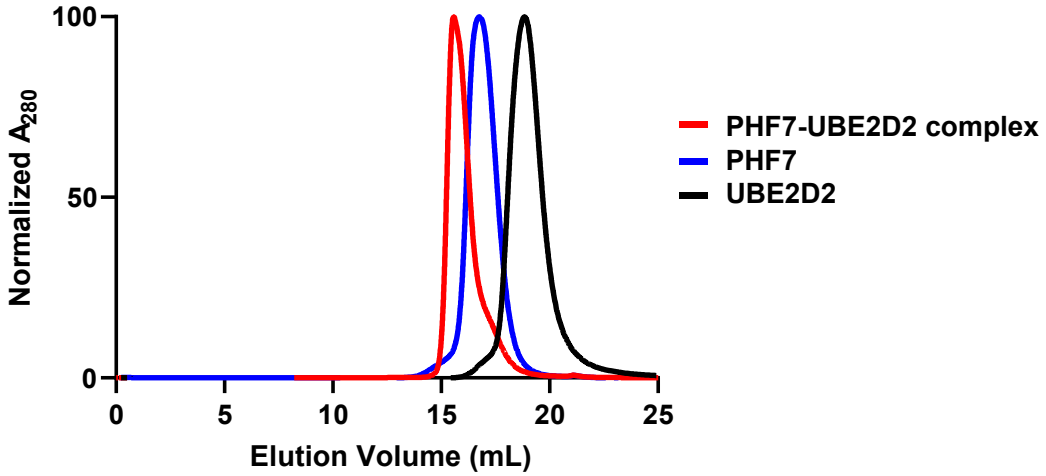


Supplemental Figure S2. Isothermal titration calorimetry binding curves of PHF7 domains bound to UBE2D2 or ubiquitin. UBE2D2 or ubiquitin was titrated against indicated PHF7 domains. The K_D values are shown and also summarized in Table 2.

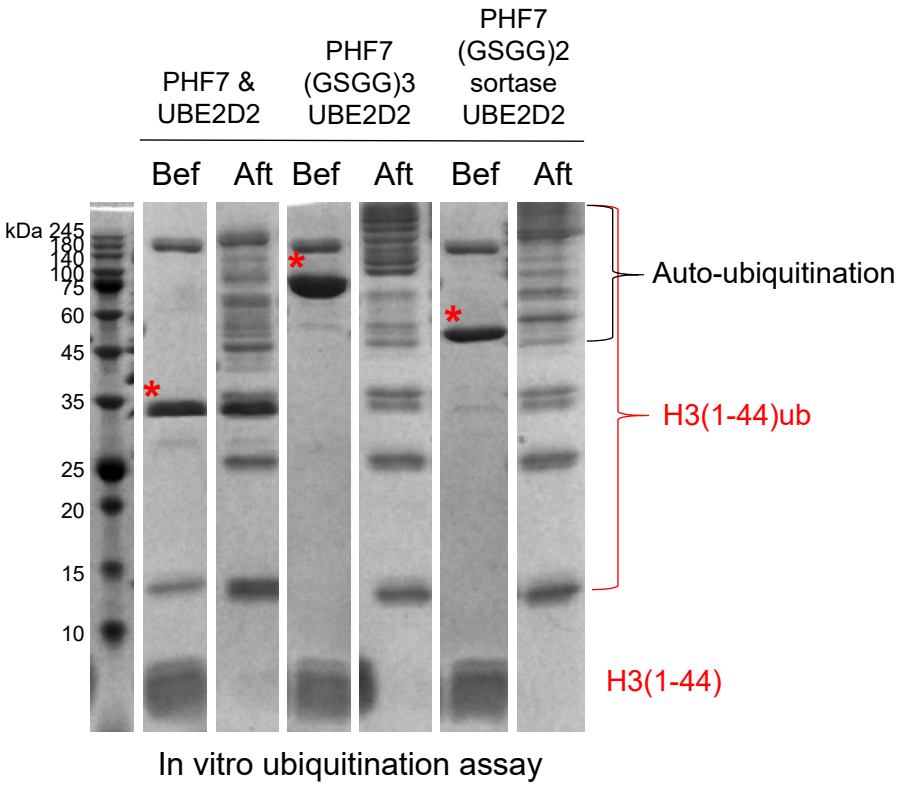




Supplemental Figure S3. Size exclusion chromatography (SEC) profile of PHF7, UBE2D2, and their complex. Proteins were injected into a Superdex 200 Increase (10/300), pre-equilibrated in 20 mM HEPES at pH 7.5 and 150 mM NaCl. The PHF7-UBE2D2 complex was formed by mixing equimolar amounts of the two proteins and incubating at 4°C for 30 min prior to injection. Each profile was normalized by its peak absorbance at 280 nm. Complex formation was confirmed by a noticeable peak shift.

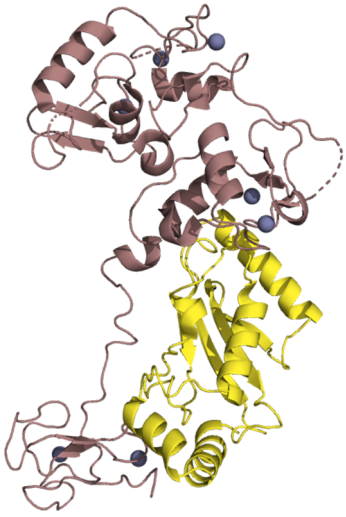


Supplemental Figure S4. Confirmation of ubiquitination activity in PHF7-UBE2D2 fusion constructs used for crystallization. (GSGG)3- and sortase A-fusion complexes were cloned and purified with wildtype versions of UBE2D2. In vitro ubiquitination was performed with H3(1-44) as substrate. Wildtype PHF7 & UBE2D2 were used as control. Fused and wildtype PHF7 proteins before ubiquitination assay are marked by red asterisks. After incubation, the original H3(1-44) and PHF7 bands disappear while multiple bands of higher molecular weights are observed, indicating H3 ubiquitination and PHF7 auto-ubiquitination activity.

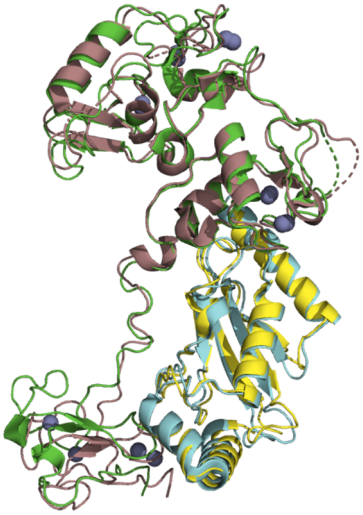


In vitro ubiquitination assay

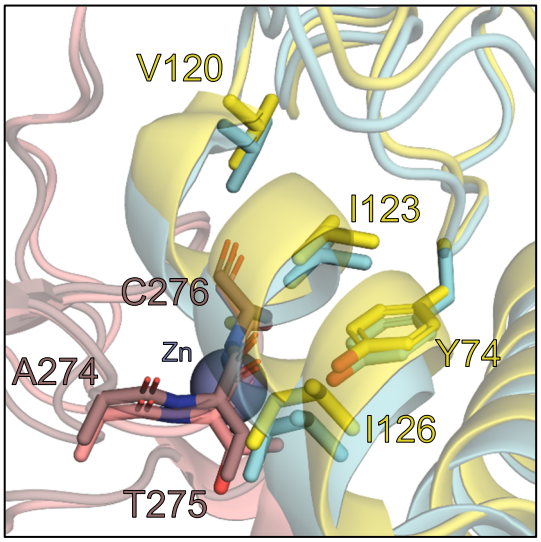
Supplemental Figure S5. Structure alignment of PHF7-UBE2D2 fusion structures. (GSGG)3-linker fusion structure was superimposed with the two chains in the crystal asymmetric unit of sortase A-fused structure. Chain C of sortase A-fused structure aligns well with (GSGG)3-linker fusion structure, and was therefore used as the primary reference for structural descriptions in the manuscript. PHD-E2 interactions, conserved in both fusion structures, is boxed.

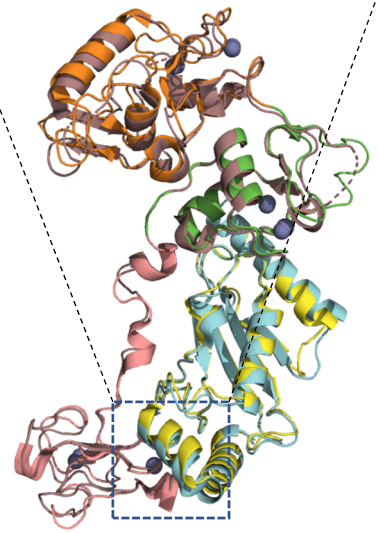


PHF7-(GSGG)3-UBE2D2
Chain B



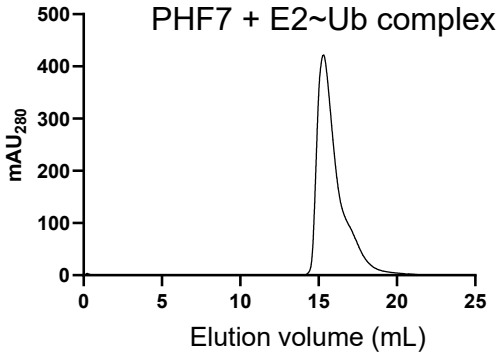
Aligned with Chain A of sortase-linked structure





Aligned with Chain C of sortase-linked structure
(Used for structural description)

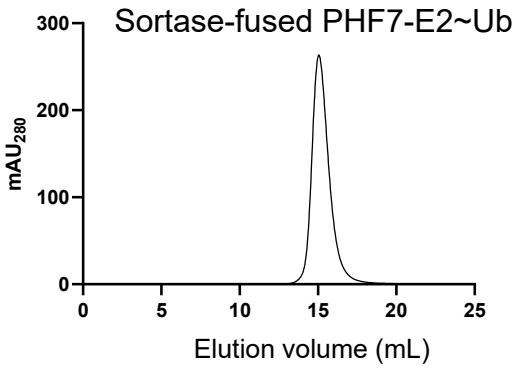
Supplemental Figure S6. Size exclusion chromatography (SEC) analysis and domain movements in sortase-fused PHF7-E2~Ub complex. SEC analysis was performed to compare the elution volumes and estimated molecular weights (MW) of PHF7 and E2~Ub complex in solution with those of the sortase-fused PHF7-E2~Ub complex, with calculated MW based on the amino acid sequence of the monomeric complex. The structures of the two PHF7-E2 complexes in the asymmetric unit of sortase-fused PHF7-E2~Ub complex were superimposed with respect to RING domain and E2. Labeled amino acids were used to measure the distances for reference.

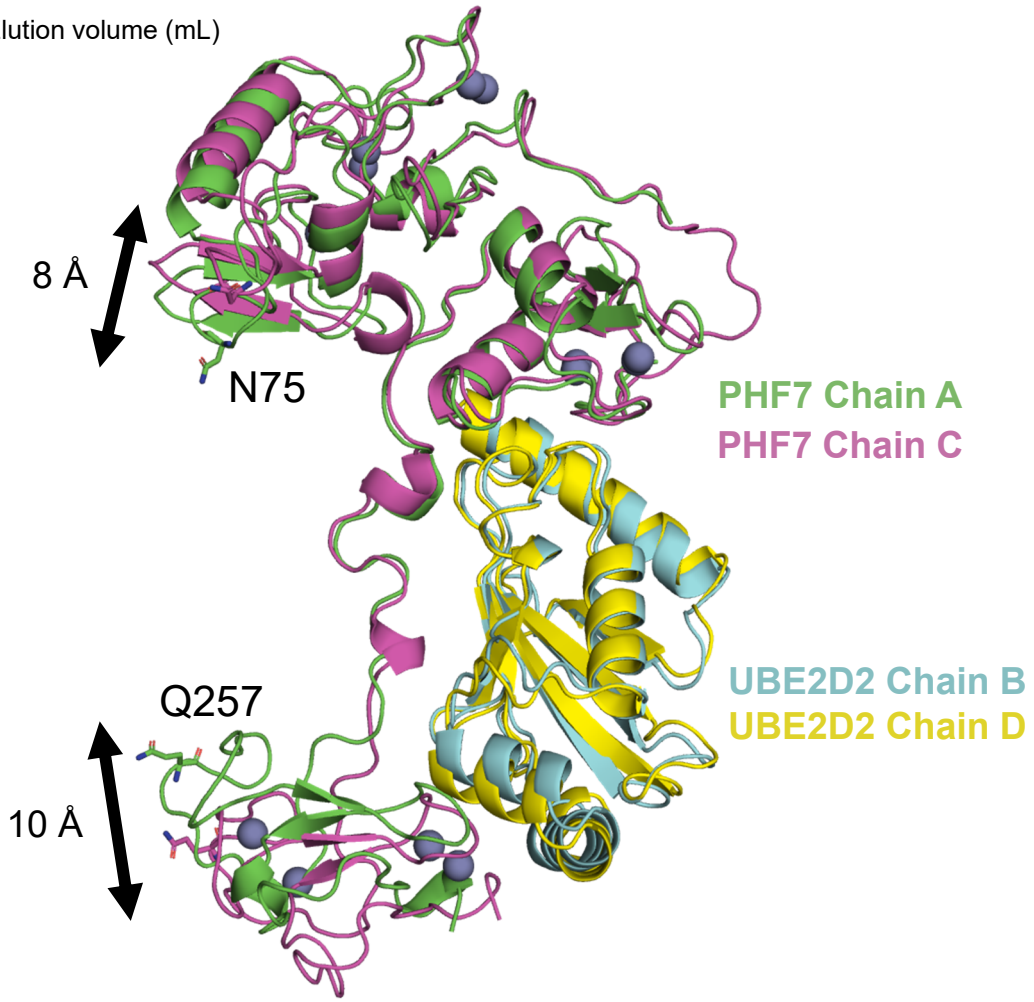


SEC analysis

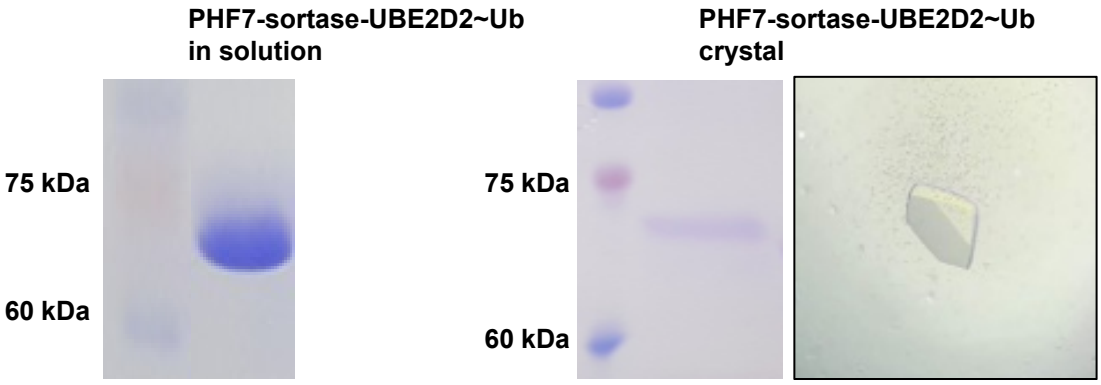
Sample	Elution volume (mL)	Estimated MW (kDa)
PHF7 + E2~Ub	15.1	55
PHF7-E2~Ub sortase	15.1	55

*Calculated MW of the complex = 59 kDa

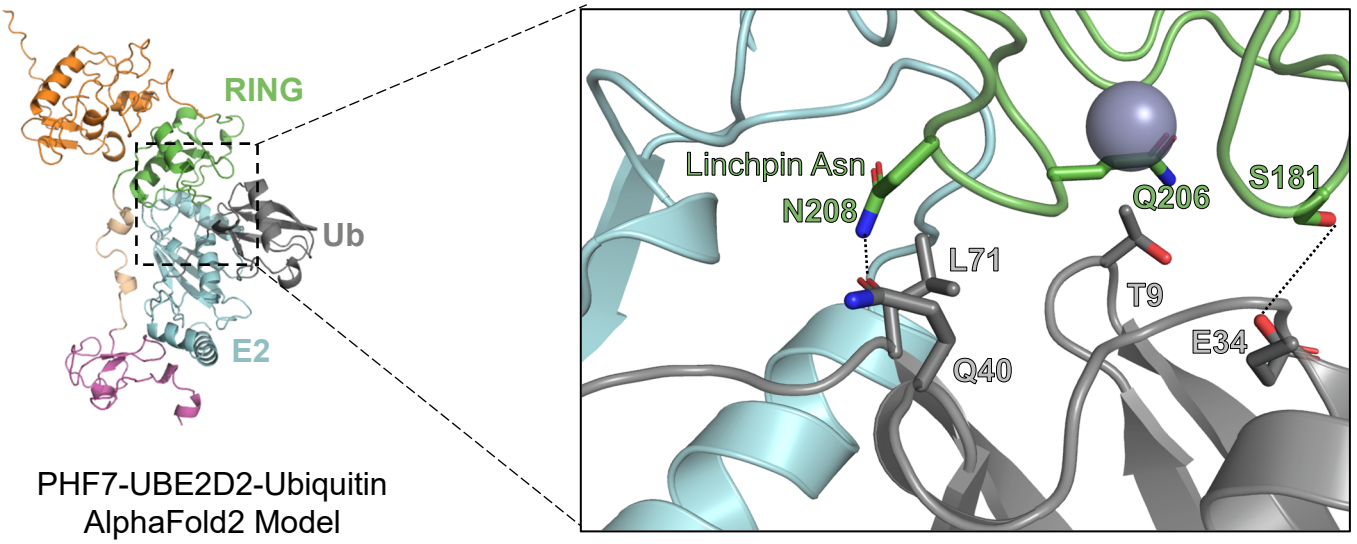




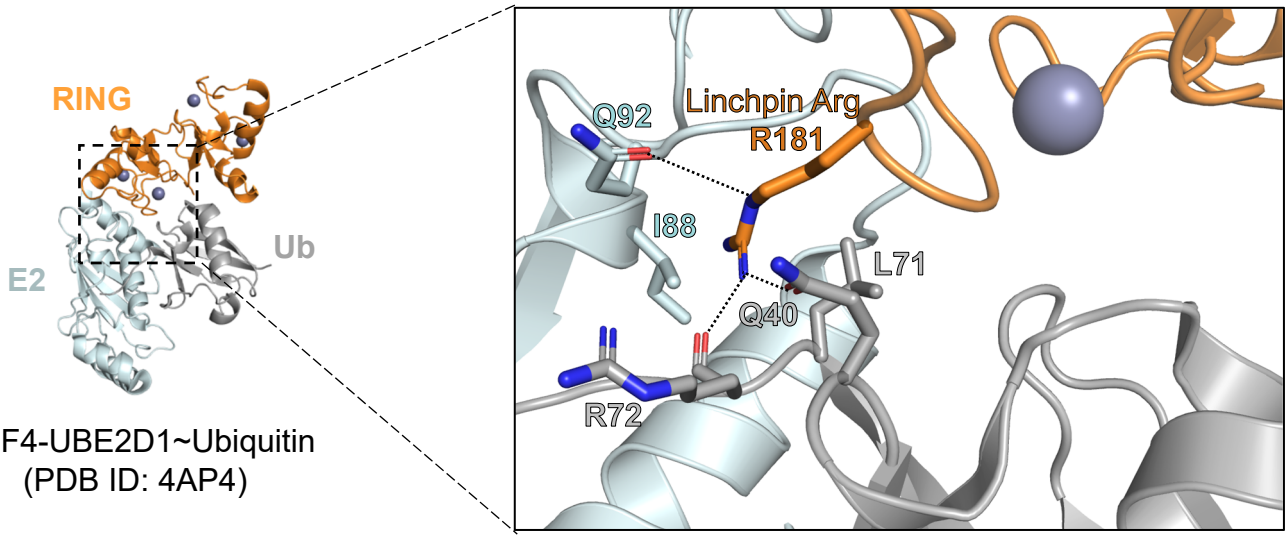
Supplemental Figure S7. SDS-PAGE analysis of sortase A-fused PHF7-UBE2D2~Ub protein crystal. Several sortase A-fused PHF7-UBE2D2~Ub crystals (shown in picture) were picked up and washed, then dissolved for SDS-PAGE analysis in comparison with purified protein in solution. The molecular weight of sortase A-fused PHF7-UBE2D2~Ub is approximately 59 kDa, but the sample migrates slowly in SDS-PAGE gel.



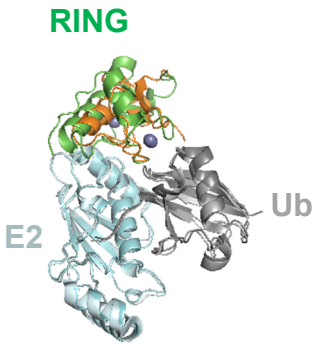
Supplemental Figure S8. Ubiquitin binding model of PHF7-UBE2D2 complex. PHF7 and UBE2D2 were modeled in complex with ubiquitin (Ub) using AlphaFold2. For comparison, the crystal structure of RNF4 in complex with ubiquitin-UBE2D1, showing the linchpin Arg of RING domain inserting between E2 and Ub, was aligned. Interactions within 4 Å are shown. Western blot using antibody against histone H3 was used to show in vitro ubiquitination assay result of NCP using PHF7 mutants targeting Ub interaction sites.



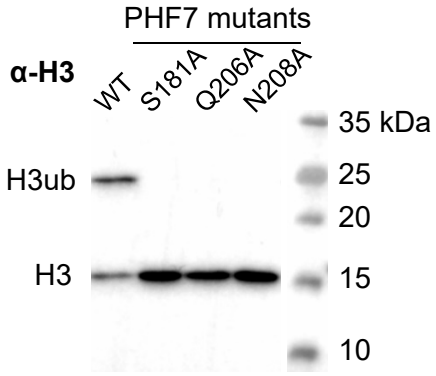
PHF7-UBE2D2-Ubiquitin
AlphaFold2 Model



RNF4-UBE2D1~Ubiquitin
(PDB ID: 4AP4)

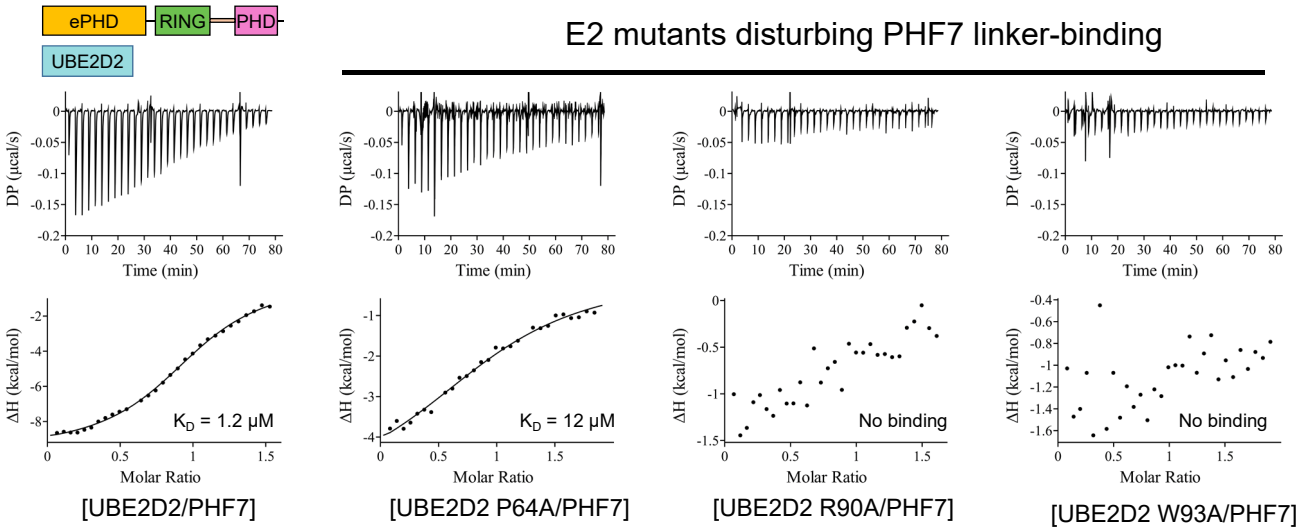


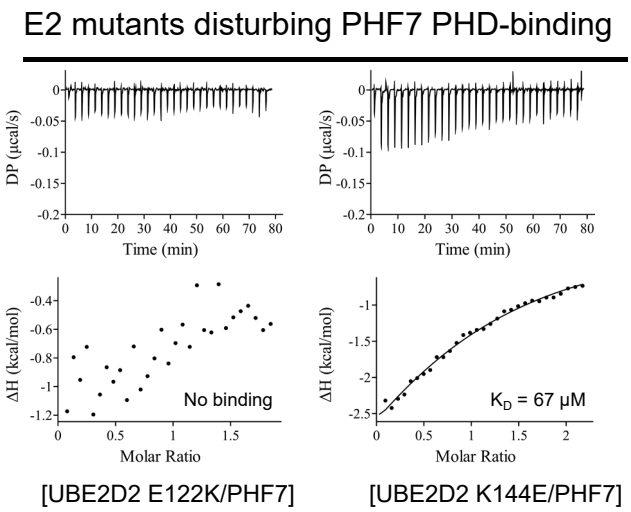
Aligned

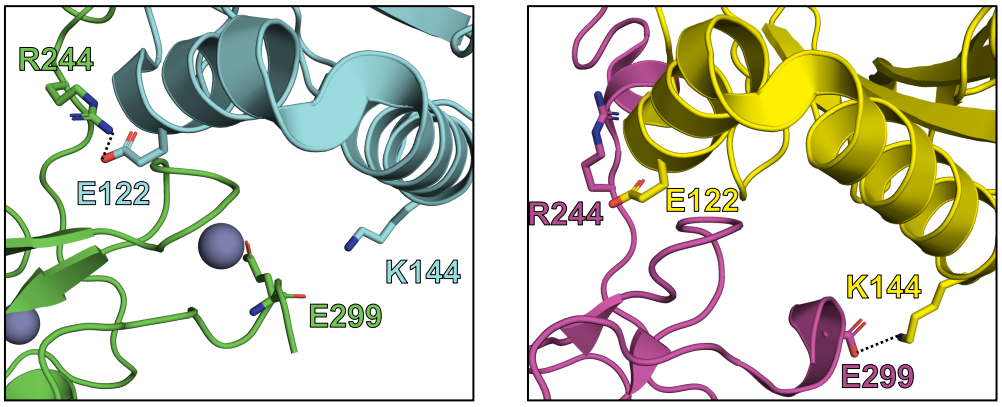


NCP in vitro ubiquitination assay

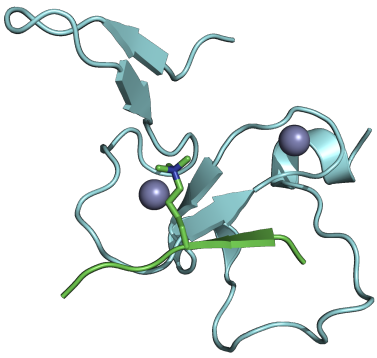
Supplemental Figure S9. Isothermal titration calorimetry binding curves of UBE2D2 mutants with PHF7. E2 residues that interact with PHF7 were mutated, then titrated against wile-type PHF7. Refer to Figure 3A. UBE2D2 E122 and K144 interact with PHF7 differently in the two copies of the complex in the asymmetric unit (bottom figures). The K_D values are indicated and also summarized in Table 2.



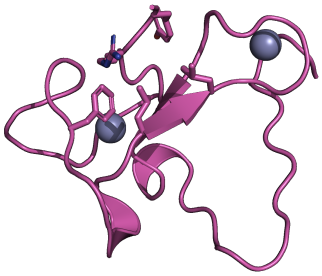




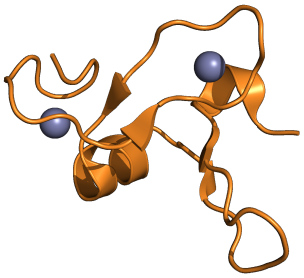
Supplemental Figure S10. Structural alignment of the PHD fingers. Alignment of the PHD and ePHD zinc fingers of PHF7 was performed using the PHD finger of TAF3 bound to H3K4me3 (PDB ID 5WXH) as the reference. For ePHD, only the zinc finger containing Zn2 and Zn3 was used. Amino acids of PHF7 PHD that occupy analogous positions and potentially constitute the histone H3 binding site were labeled for reference.



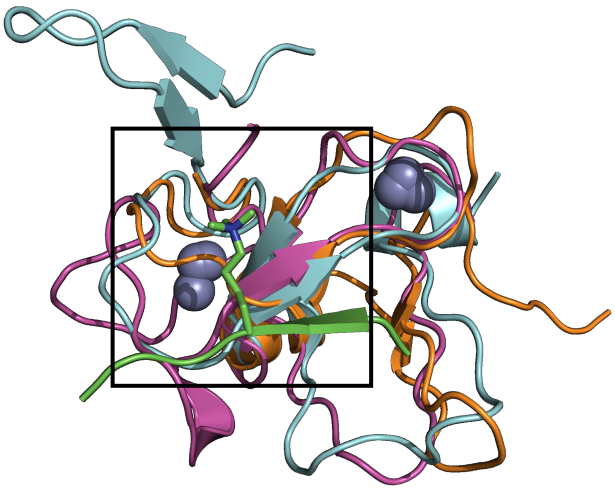
TAF3 PHD bound to H3K4me3
(PDB ID 5WXH)



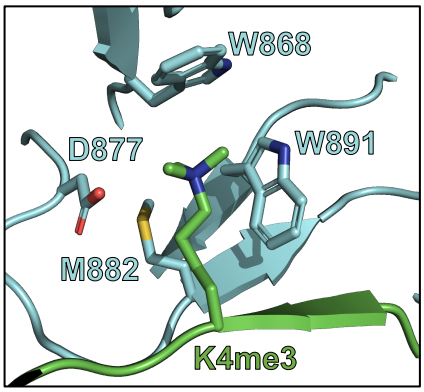
PHF7 PHD
(244-301)



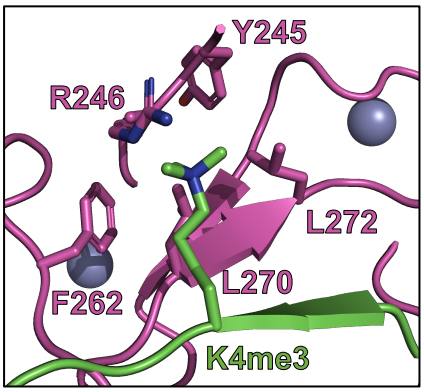
PHF7 ePHD without Zn1
(97-146)



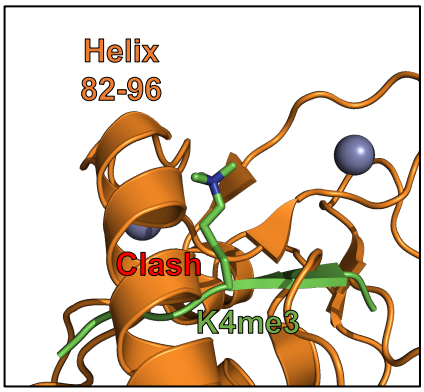
Aligned PHD fingers with H3K4me3
(RMSD 2.4 Å)



TAF3 PHD bound to H3K4me3
(PDB ID 5WXH)

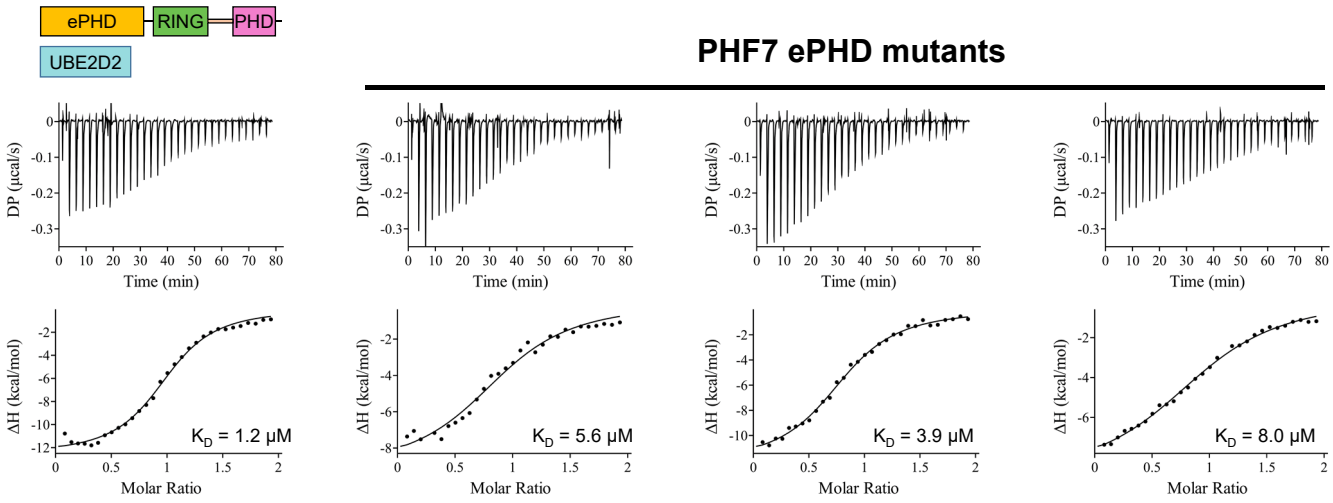


PHF7 PHD
(244-301)



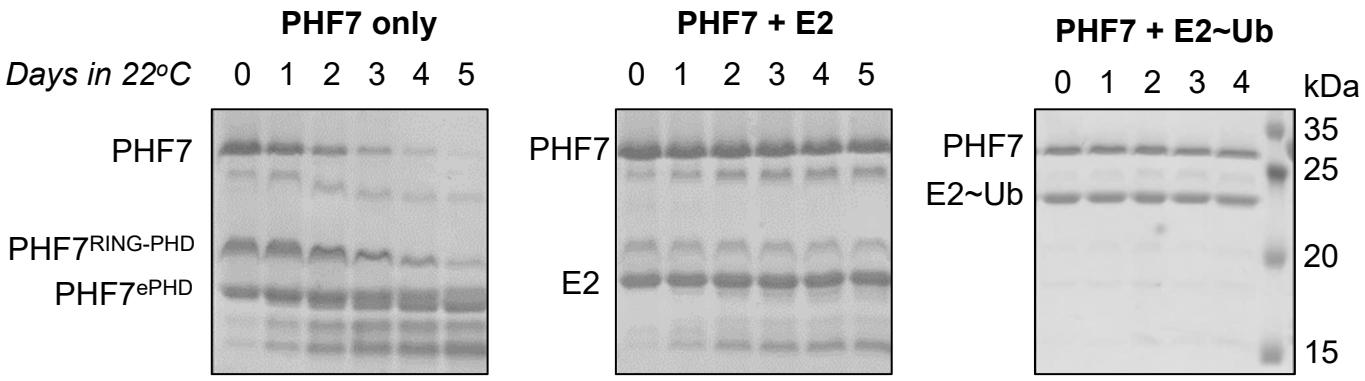
PHF7 ePHD
(30-146)

Supplemental Figure S11. Contribution of ePHD to E2 binding. PHF7 ePHD residues that indirectly affect E2 binding were mutated, then titrated against UBE2D2. Refer to Figure 4B. The K_D values are indicated and also summarized in Table 2.

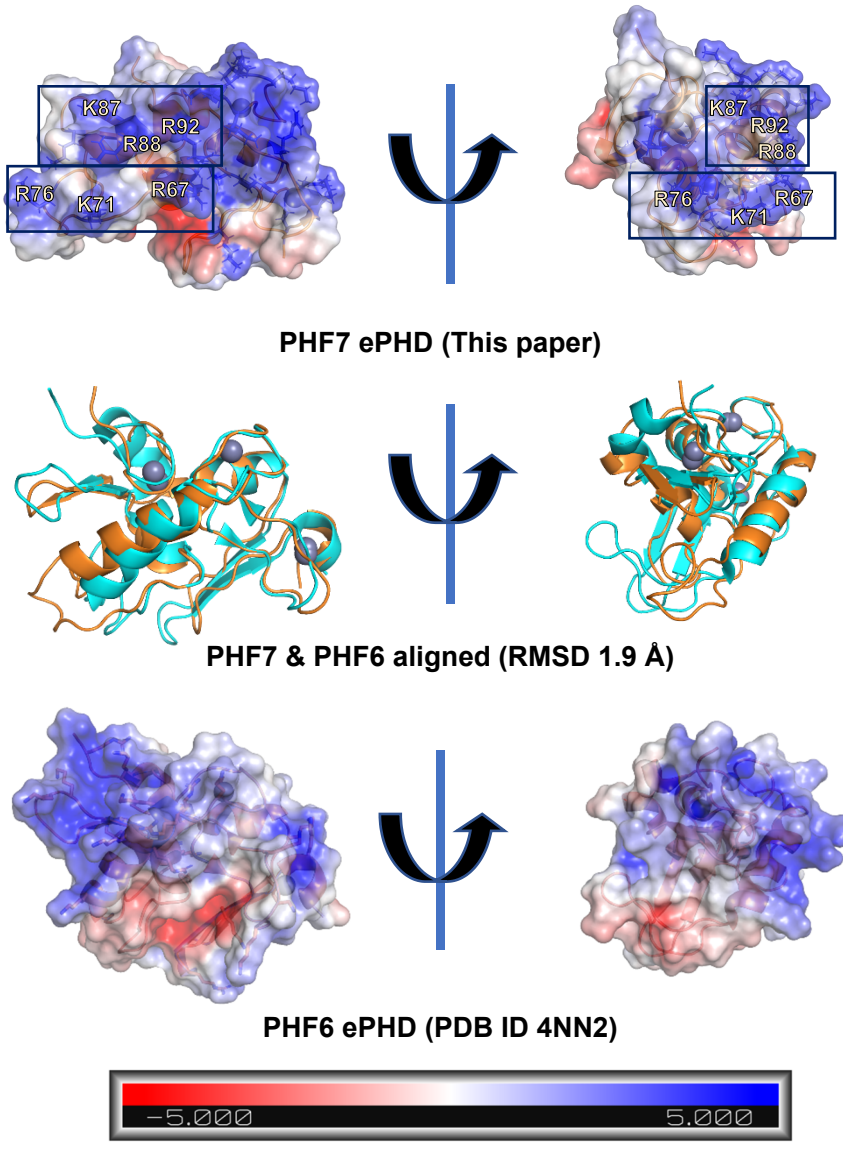


[UBE2D2/PHF7] [UBE2D2 /PHF7 E44K] [UBE2D2/PHF7 Q148A] [UBE2D2/PHF7 E44K/Q148A]

Supplemental Figure S12. Spontaneous cleavage of PHF7 is prevented by E2 binding. Purified PHF7 spontaneously cleaves into two parts, identified as 28-149 and 150-307 by mass spectrometry. Addition of E2 or E2~Ub shields PHF7 from degradation.



Supplemental Figure S13. APBS charge distribution of ePHD domains of PHF7 and PHF6. PDB2PQR, followed by APBS charge calculation, was performed on ePHD of PHF7 (orange) and PHF6 (cyan). The solvent accessible surface was colored based on electrostatic potential, with blue surface indicating a positive charge. In subsequent experiments (Figure 5B), in vitro ubiquitination assays and electrophoretic mobility shift assays were conducted using mutations targeting the labeled positive patches on PHF7.



Jurrus E, Engel D, Star K, Monson K, Brandi J, Felberg LE, Brookes DH, Wilson L, Chen J, Liles K, Chun M, Li P, Gohara DW, Dolinsky T, Konecny R, Koes DR, Nielsen JE, Head-Gordon T, Geng W, Krasny R, Wei GW, Holst MJ, McCammon JA, Baker NA. Improvements to the APBS biomolecular solvation software suite. Protein Sci. 2018 Jan;27(1):112-128. doi: 10.1002/pro.3280. Epub 2017 Oct 24. PMID: 28836357; PMCID: PMC5734301.

Supplemental Figure S14. Model of PHF7-UBE2D2~Ub bound to NCP. (A) Representative RING-E2 complex structures bound to NCP. PHF7-UBE2D2 structure from this paper was aligned to UBE2D3. The areas of PHF7 that clash with NCP are boxed. (B) A schematic diagram of PHF7 and UBE2D2~Ub binding cooperatively to NCP. The PHF7 ePHD and PHD are linked to the RING domain by flexible linkers, shown as blue lines and domain movement in PHF7 alone is demonstrated by red arrows.