# Supplemental Information

**Structural Basis for Assembly and Activation of the Hetero-tetrameric SAGA Histone H2B Deubiquitinase Module**

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**Figure S1 related to Figure 2.** Sequence alignment of Ubp8 and USP22 orthologues. Secondary structure elements of *S. cerevisiae* Ubp8 are indicated by cylinders (helices), arrows (β-strands), solid lines (loops), and dashed lines (disordered loops). Secondary structure elements are colored in blue for the Zn-UBP domain and Ubp8 linker, grey for the Thumb domain, cyan for the Fingers domain, and blue for the Palm domain. Amino acids that are strictly conserved are highlighted in light blue. Residues involved in forming the six zinc-coordinating sites are highlighted in yellow with the site numbers indicated below the sequences. Critical catalytic residues are highlighted in salmon. The amino acids of *S. cerevisiae* Ubp8 are numbered.

**Figure S2 related to Figure 4.** Analyses of the three zinc-binding sites in the Ubp8 catalytic domain.
A. A close-up view of the 4$^{th}$ and 5$^{th}$ zinc-coordinating sites in the Ubp8 catalytic domain. The Thumb domain (grey) is shown in ribbons with secondary structure elements labeled. The Fingers (cyan) and Palm (blue) domains are shown in surface representation. Residues involved in coordinating the zinc ions are shown in sticks. Site 4 is formed by one histidine and three cysteine residues from the H10-loop-H11 region, whereas site 5 is coordinated by two histidine and two cysteine residues of the H13-loop-H14 region. Both sites are strictly conserved in Ubp8 and USP22 orthologs from yeast to humans (see Figure S1). The location of these two zinc-binding sites suggests that they are minimally involved in maintaining the structural integrity of the Ubp8 catalytic domain. Based on the USP2 structure (Renatus et al. 2006), the Ubp8 catalytic domain is predicted to possess yet a third zinc-binding site (site 6) at the tip of the Fingers domain (see Figure S1). This zinc-coordinating site is disordered in the DUB module crystal, presumably due to structural distortion induced by crystal packing.
B. Ub-AMC hydrolysis assay with the indicated GST-purified recombinant DUB tetramers or Ub-AMC alone. Consistent with a role in maintaining local structural integrity, disruption of either site leads to reduced enzymatic activity of the DUB module and a greater susceptibility to proteolysis during purification.
C. Purification of the recombinant yeast DUB module containing the Ubp8 zinc-binding site mutants. Coomassie staining shows composition of the complexes. Diamond marks an Sgf11 fragment, open circles Ubp8 degradation products as determined by mass spectrometry.

**Figure S3 related to Figure 5.** Protein expression level of C-terminally 6His-tagged alleles of *SGF11*. The indicated plasmids were transformed into *sgf11*Δ cells. After normalizing cell densities, whole cell extracts (WCE) were prepared by trichloroacetic acid precipitation and bead lysis, followed by SDS-PAGE and western blotting using anti-His antibodies. Arc1 (a yeast cytosolic marker) was detected as a loading control.

**Figure S4 related to Figure 6.** Sequence and mutational analyses of the Sgf73 N-terminus.
A. Sequence alignment of the N-terminal regions of the yeast Sgf73 proteins. Secondary structure elements of *S. cerevisiae* Sgf73 N-terminal fragment are indicated by cylinders (helices), arrows (β-strands), and solid lines (loops). Amino acids that are conserved are highlighted in orange. Residues involved in forming the zinc-coordinating sites are highlighted in yellow. The amino acids of *S. cerevisiae* Sgf73 are numbered.

B. Mutations in the N-terminus of full-length Sgf73 abolish the recruitment of Ubp8 to SAGA. The yeast SAGA complex was purified by tandem affinity-tagged Ada2 in the background of wild type Sgf73, Sgf73 W32A/K33A/H93A, and Sgf73 deletion mutant. Calmodulin eluates were analyzed by SDS-PAGE (4–12% gradient gel, MOPS buffer) and Coomassie staining. Indicated bands are *bona fide* SAGA subunits, which were assigned according to their molecular mass; filled circles designate Ada2, arrow marks Ubp8, which was detected by mass spectrometry.

**Figure S5 related to Figure 7.** Conformational changes of the "blocking loops" of the Ubp8 catalytic domain is not a part of the activation mechanism. The catalytic domains of Ubp8 (blue) is superimposed with the catalytic domain of USP7 (orange) in complex with ubiquitin (grey) (Hu et al. 2002). The BL1 and BL2 loops of Ubp8 in the free DUB module appear to impose steric hindrance for ubiquitin binding. However, since the DUB module is already in an activated state, these two loops must be free to undergo conformational changes to accommodate the C-terminal tail of ubiquitin when the substrate is present.

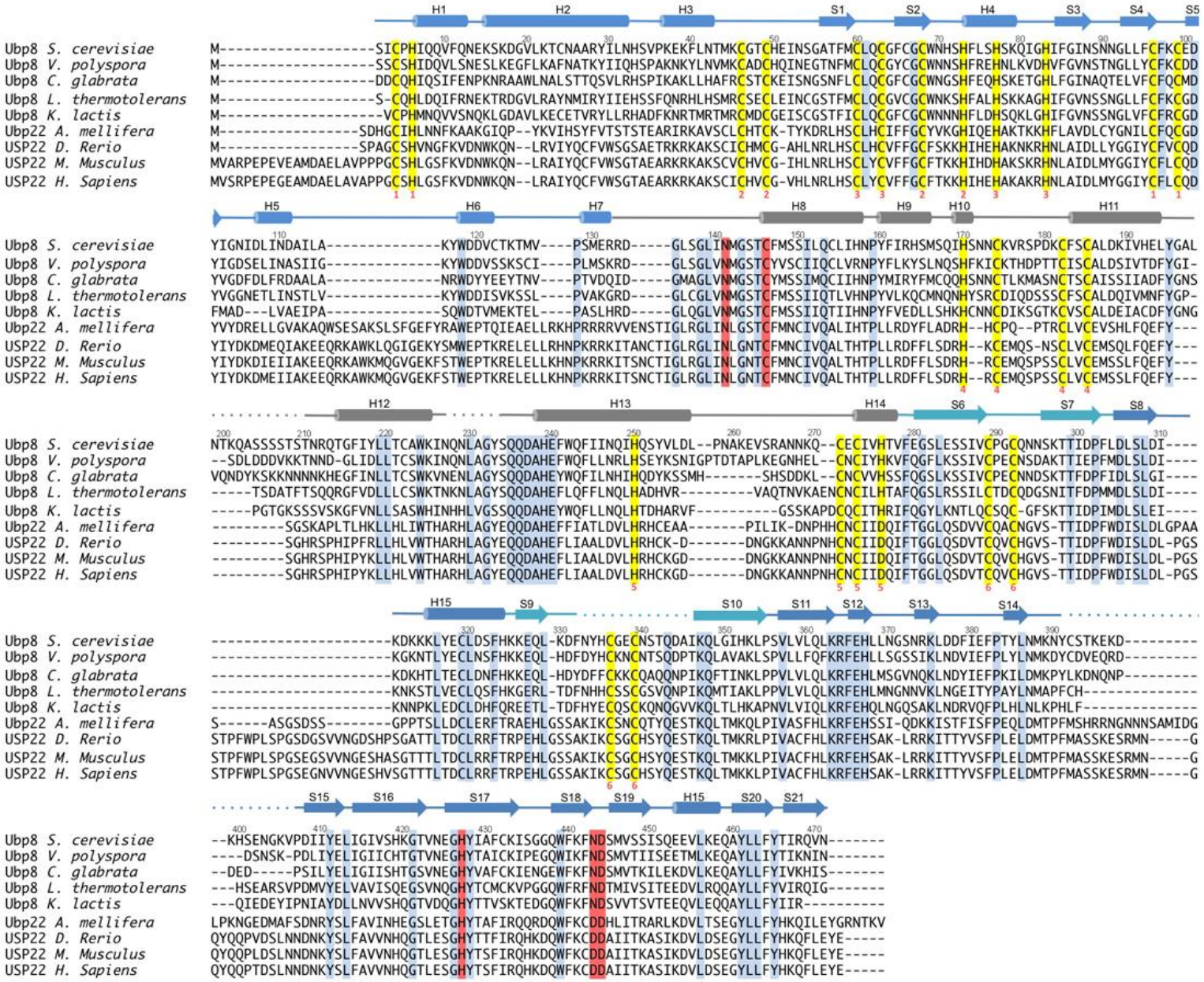
**SUPPLEMENTAL REFERENCES**

Hu, M., Li, P., Li, M., Li, W., Yao, T., Wu, J.W., Gu, W., Cohen, R.E., and Shi, Y. (2002). Crystal structure of a UBP-family deubiquitinating enzyme in isolation and in complex with ubiquitin aldehyde. Cell 111, 1041-1054.

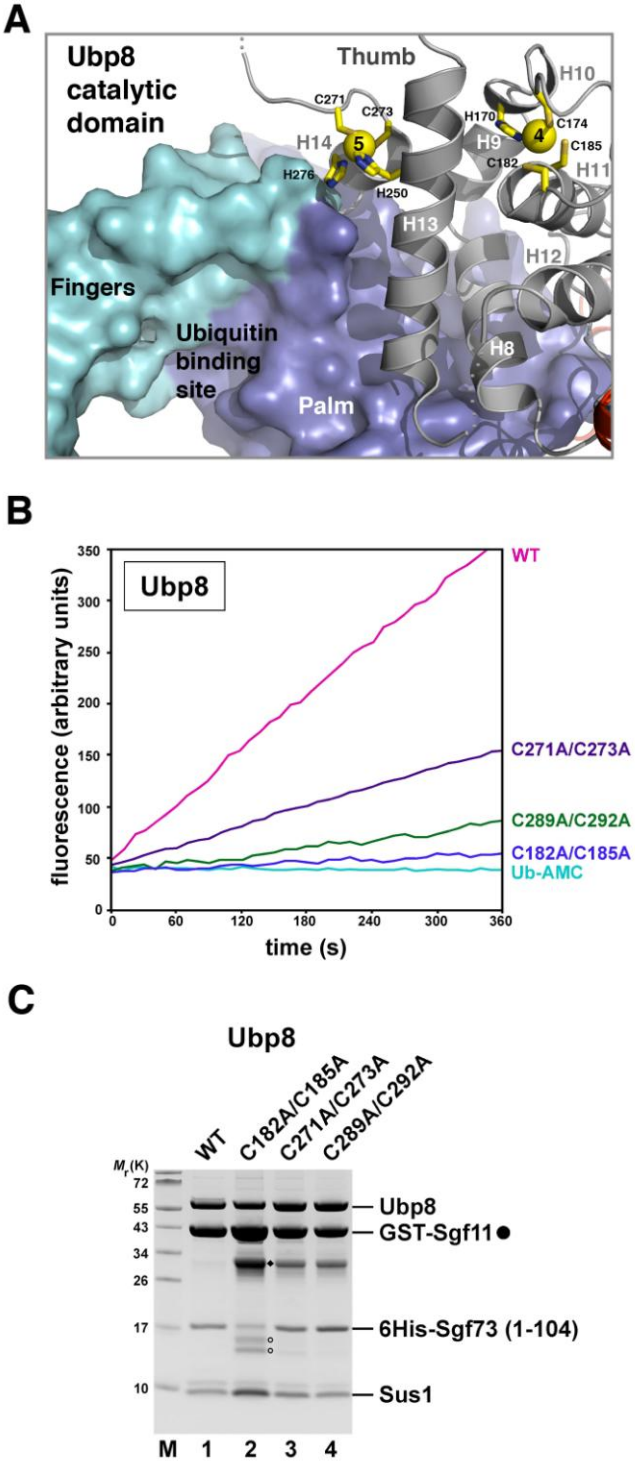
Renatus, M., Parrado, S.G., D'Arcy, A., Eidhoff, U., Gerhartz, B., Hassiepen, U., Pierrat, B., Riedl, R., Vinzenz, D., Worpenberg, S., et al. (2006). Structural basis of ubiquitin recognition by the deubiquitinating protease USP2. Structure 14, 1293-1302.

Robzyk, K., Recht, J. & Osley, M. A. Rad6-dependent ubiquitination of histone H2B in yeast. Science 287, 501-4 (2000).

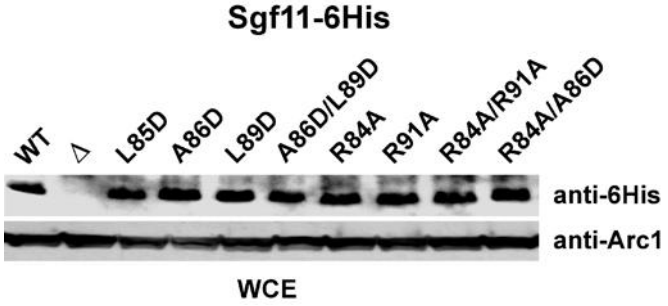
**SUPPLEMENTAL FIGURE 1**



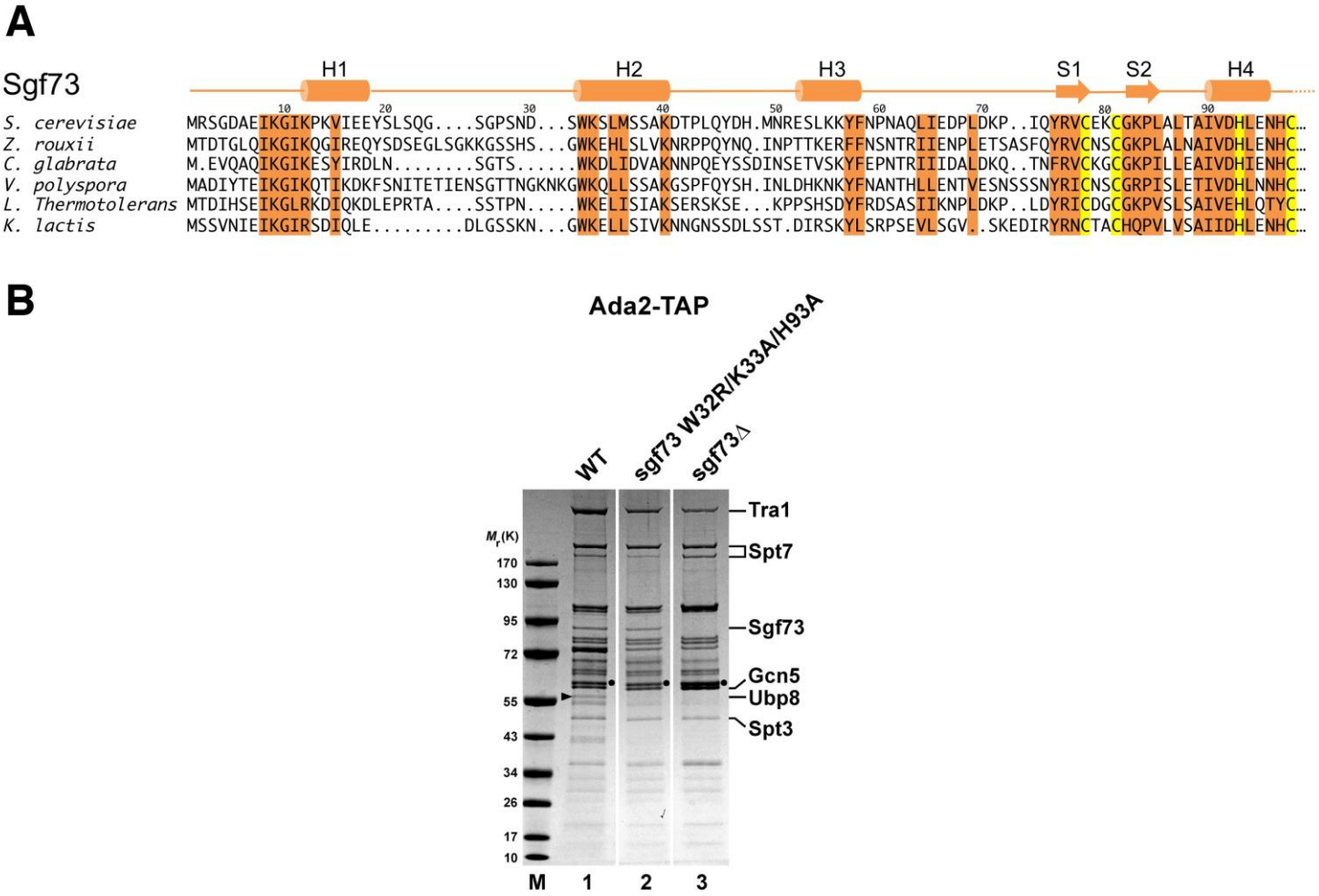
## SUPPLEMENTAL FIGURE 2



**SUPPLEMENTAL FIGURE 3**



**SUPPLEMENTAL FIGURE 4**



**SUPPLEMENTAL FIGURE 5**