Expanded View Figures

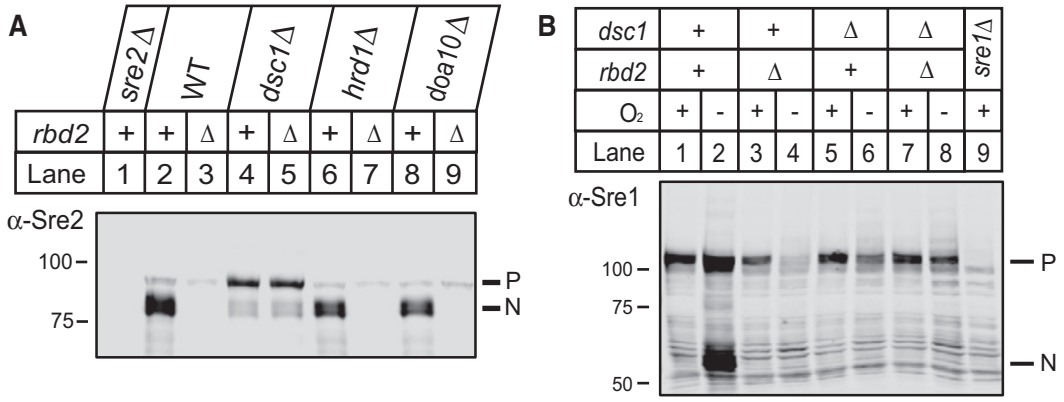

Figure EV1. Sre1 and Sre2 precursor degradation requires *dsc1*.

A Western blot of phosphatase-treated, whole-cell lysates from indicated strains was probed with anti-Sre2 IgG. P and N denote Sre2 precursor and cleaved forms, respectively.

B Western blot was probed with anti-Sre1 IgG of phosphatase-treated, whole-cell lysates from wild-type cells and the indicated mutants grown for 3 h in the presence or absence of oxygen.

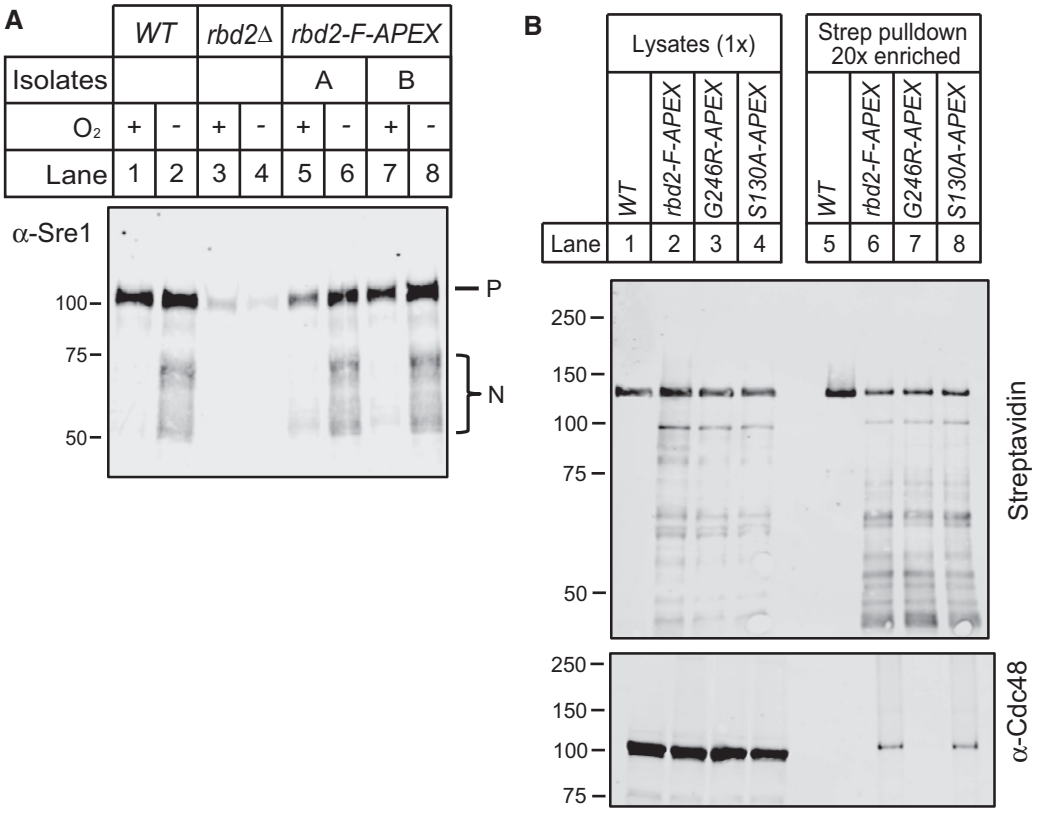

Figure EV2. Rbd2 catalytic dead mutant binds Cdc48.

A WT, *rbd2*Δ, or two isolates (A and B) of yeast expressing *rbd2-Flag-APEX2* under control of the constitutive *adh1*$^{+}$ promoter in *rbd2*Δ background were assayed for Sre1 cleavage. Western blot was probed with anti-Sre1 IgG of whole-cell lysates from the indicated strains grown for 3 h in the presence or absence of oxygen. P and N denote Sre1 precursor and cleaved forms, respectively. Note that whole-cell lysates were not treated with phosphatase, and multiple bands of Sre1N represent phosphorylated forms.

B *rbd2*Δ cells expressing *rbd2-Flag-APEX2*, *rbd2-G246R-Flag-APEX2*, or *rbd2-S130A-Flag-APEX2* from a plasmid were lysed after biotin-labeling reaction, and proteins were denatured by heating the cells in a lysis buffer containing 1% SDS. Biotinylated proteins were then enriched using streptavidin magnetic beads. Lysates and 20×-enriched eluates were analyzed by Western blot with IRDye 800CW Streptavidin, anti-Cdc48 serum, and anti-Flag IgG.

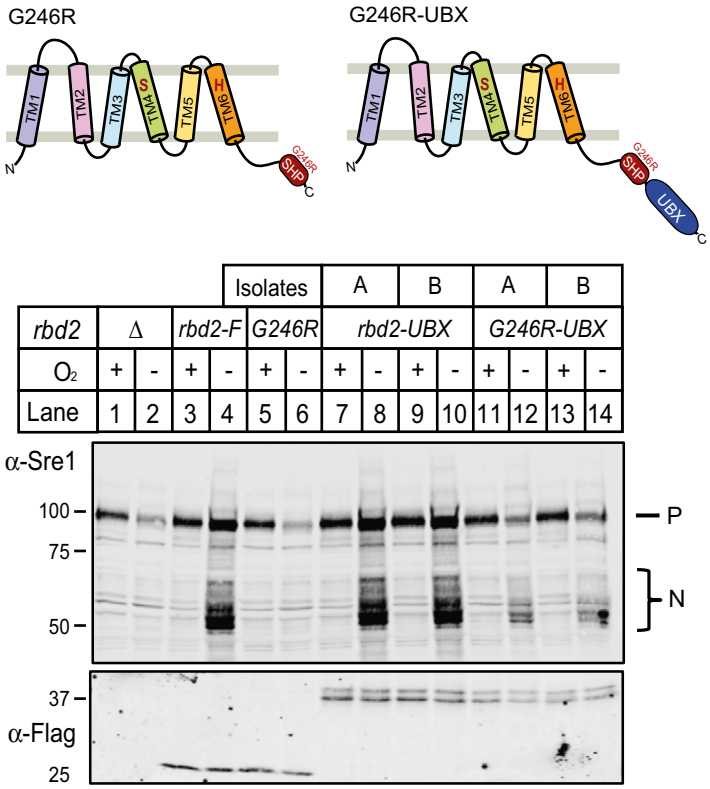

Figure EV3. Fusion of UBX domain to Rbd2-G246R partially rescues Sre1 cleavage.
Yeast strains containing Flag-tagged *rbd2-UBX* and *rbd2-G246R-UBX* (diagrammed) were generated in *rbd2*Δ background by chromosomal integration. Whole-cell lysates from indicated strains expressing Flag-tagged Rbd2 variants grown for 3 h in the presence or absence of oxygen were analyzed by Western blotting with anti-Sre1 or anti-Flag IgG.

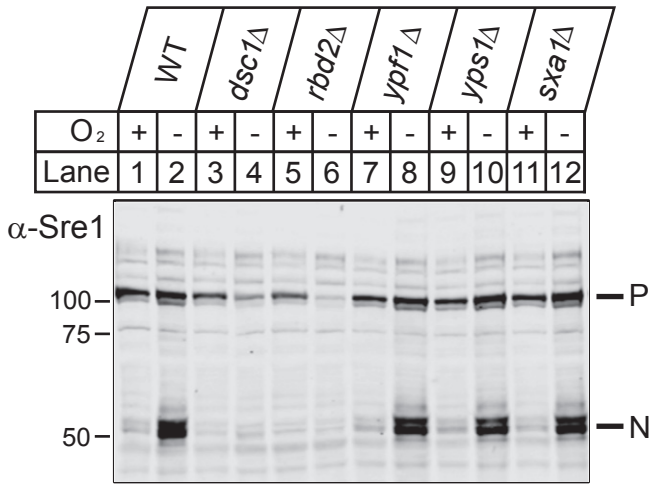

Figure EV4. Sre1 cleavage does not require signal peptide peptidase *ypf1*.
Western blot of phosphatase-treated, whole-cell lysates from wild-type cells and the indicated mutants grown for 3 h in the presence or absence of oxygen was probed with anti-Sre1 IgG. P and N denote Sre1 precursor and cleaved forms, respectively.

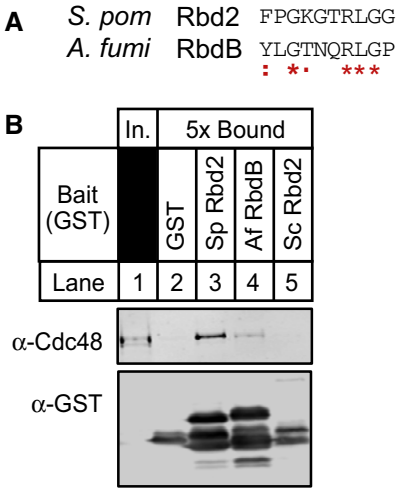

Figure EV5. *Aspergillus fumigatus* RbdB contains a conserved SHP box.

A Alignment of SHP box sequences from *Schizosaccharomyces pombe* Rbd2 C-terminal SHP box (aa 242–251) and *A. fumigatus* RbdB C-terminal SHP box (aa 263–272). Asterisks denote identical residues, colon marks conservative substitution, and dots mark semi-conservative substitutions

B Recombinant proteins GST-fused *S. pombe* Rbd2 C-terminus (aa 200–251), *A. fumigatus* RbdB C-terminus (aa 211–272), *S. cerevisiae* Rbd2 C-terminus (aa 192–262), and GST were bound to GST magnetic beads and incubated with *S. pombe* cytosol fraction from wild-type cells. 5× bound fractions were probed for anti-Cdc48 and anti-GST IgG. The *S. pombe* cytosol fraction was used for 1× input (In.) loading.