Supplementary Figure S1. A whole-genome screen for components of the Doa10 pathway.

A. Growth of deletion mutants expressing *Deg1*-Ura3-HA3 after 7 d at 30˚C on medium lacking uracil (SD-ura). Mutants with evidence for enhanced growth in the primary screen were spotted onto a single plate and retested by pinning onto SD-ura. The *hrd1Δ* mutant (boxed) was used as a control. Deletion of the genes marked in bold gave reproducibly enhanced growth on SD-ura. Underlined genes were implicated previously in *Deg1*-mediated proteolysis.

B. Assays of *Deg1*-βgal and Ubc6-HA stability in selected mutants. The chase was initiated by addition of cycloheximide, and proteins were followed by anti-βgal and anti-HA immunoblotting. The *hrd1Δ* strain served as a negative control. Asterisk, a 90 kD in vivo cleavage product of βgal.

C. The *Deg1*-Ura3-stabilizing mutation in the library *nup120Δ* strain is in *DOA10*. Indicated diploids were grown at 30˚C for 4 d. Anti-Doa10 blot on right shows the loss of full-length Doa10 in this strain and accumulation of a truncated protein (arrowhead).

Supplementary Figure S2. Cue1 is important for Matα2 degradation. Kinetics of Matα2 degradation were measured by pulse-chase analysis in the indicated mutants.

Supplementary Figure S3. Analysis of different *Deg1*-derived fusion proteins by pulse-chase and immunoblot/cycloheximide chase analyses.

A. Immunoblot/cycloheximide chase analysis at 37˚C of *Deg1*-βgal and *Deg1*-GFP_2 expressed in the same cells. The plasmid encoding the fusion of *Deg1* to a tandem GFP cassette is from Lenk et al. (2000). To avoid *Deg1*-GFP_2 overexpression from the *CUP1* promoter in this high-copy plasmid, no copper was added to the medium. Immunoblots were probed with a mixture of antibodies to βgal, GFP, and PGK.

B. Pulse-chase analysis at 37˚C of the same cells as in panel A. Proteins were immunoprecipitated with an anti-α2 antiserum. The data were quantitated on a phosphorimager, and protein degradation rates were estimated by linear regression (lower panels). The half-life of *Deg1*-βgal was calculated to be 12 min, 14 min, and >100 min in wild-type (WT), *cdc48-6*, and *doa10Δ* cells, respectively. The half-life of *Deg1*-GFP_2 was calculated to be 18 min, 19 min, and 32 min in the same three strains.

C. Immunoblot/cycloheximide chase analysis at 37˚C of *Deg1*-GFP expressed the indicated strains. The plasmid encoding the fusion of *Deg1* to a single GFP moiety is from Bays et al. (2001). Immunoblotting was done with a mixture of antibodies to GFP and PGK. Cntl, control strain that did contain the *Deg1*-GFP reporter.