Mao et al., http://www.jcb.org/cgi/content/full/jcb.201102092/DC1

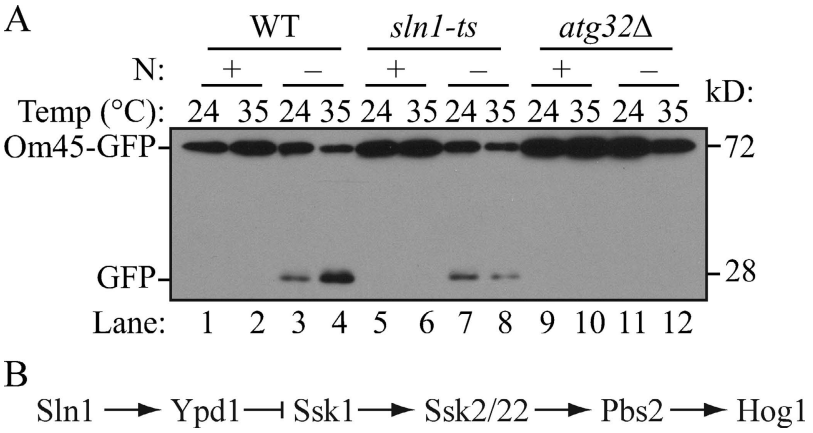

Figure S1. **Sln1 is required for efficient mitophagy.** (A) *OM45* was chromosomally tagged with GFP in the wild-type (TKYM22), *sln1-ts* (KDM2036), and *atg32Δ* (TKYM130) strains. Cells were cultured in YPL to mid-log phase, then shifted to SD-N and incubated for 6 h. Samples were taken before (+) and after (−) starvation. Immunoblotting was done with anti-YFP antibody and the positions of full-length Om45-GFP and free GFP are indicated. (B) The Sln1 branch of the Hog1 pathway.

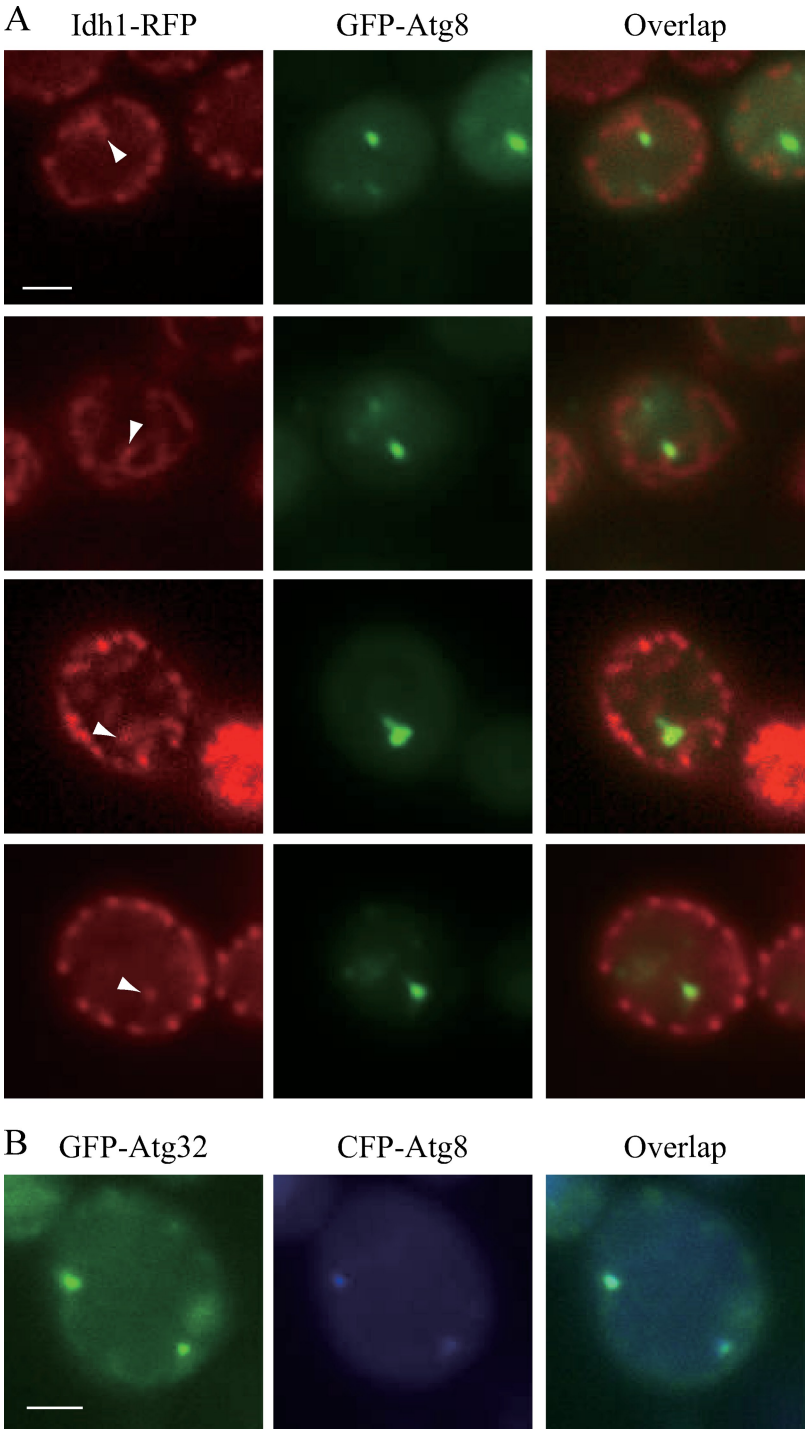

Figure S2. **A portion of the total mitochondria population accumulates at the PAS under mitophagy-inducing conditions.** (A) Idh1-RFP colocalized with GFP-Atg8 during mitophagy. *IDH1-RFP atg1Δ* (TKYM203) cells transformed with a plasmid expressing GFP-Atg8 were cultured in SML to mid-log phase and shifted to SD-N. Samples were taken after 2 h culturing in SD-N and observed by fluorescence microscopy. White arrowheads mark mitochondria that overlap with GFP-Atg8. Representative pictures from single Z-section images are shown. (B) The *atg1Δ* (WHY001) cells transformed with plasmids encoding CFP-Atg8 and GFP-Atg32 were cultured in SML to mid-log phase and shifted to SD-N. Samples were taken after 2 h culturing in SD-N and observed by fluorescence microscopy. Representative pictures from single Z-section images are shown. Bars, 2.5 μm.

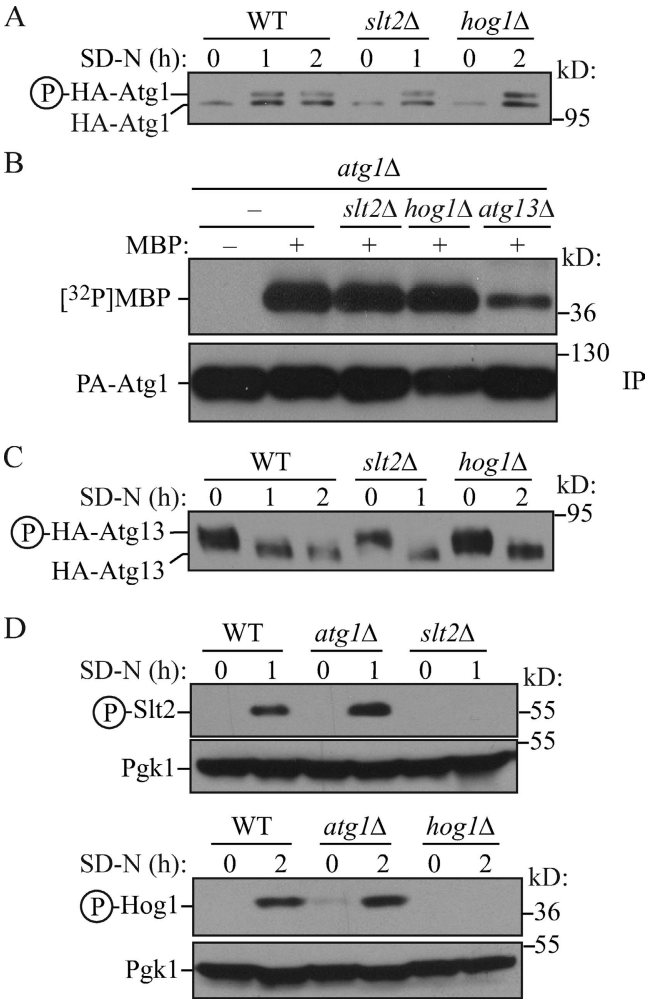

Figure S3. **Slt2 and Hog1 regulate mitophagy independent of the Atg1-Atg13 complex.** (A) Slt2 and Hog1 have no effect on Atg1 phosphorylation. Wild-type (SEY6210), *slt2Δ* (KDM1213), and *hog1Δ* (KDM1214) cells transformed with plasmid encoding HA-Atg1 were cultured in SML to mid-log phase and shifted to SD-N. Samples were collected before or at 1 or 2 h after nitrogen starvation. Immunoblotting was done with anti-HA antibody. (B) Slt2 and Hog1 have no effect on Atg1 kinase activity. The *atg1Δ* (WHY001), *atg1Δ slt2Δ* (KDM 1203), *atg1Δ hog1Δ* (KDM 1211), and *atg1Δ atg13Δ* (UNY29) cells transformed with protein A–tagged Atg1 (PA-Atg1) were cultured in SML medium to mid-log phase and shifted to SD-N for 2 h. PA-Atg1 from the indicated strains was immunoprecipitated with IgG sepharose. MBP was used as substrate in an in vitro kinase assay with immunoprecipitated PA-Atg1 from different mutant cells. Phosphorylated MBP was detected by autoradiography and the protein input was shown by immunoblot; the molecular mass indicated is approximate. (C) Wild-type (SEY6210), *slt2Δ* (KDM1213), and *hog1Δ* (KDM1214) cells transformed with a plasmid encoding HA-Atg13 were treated as in A. Immunoblotting was performed with anti-HA antibody. (D) Atg1 has no effect on the phosphorylation of Slt2 and Hog1. Wild-type (SEY6210), *atg1Δ* (WHY001), *slt2Δ* (KDM1213), and *hog1Δ* (KDM1214) cells were treated as in A. Immunoblotting was done with anti–phospho-Slt2 or anti–phospho-Hog1 antibodies.