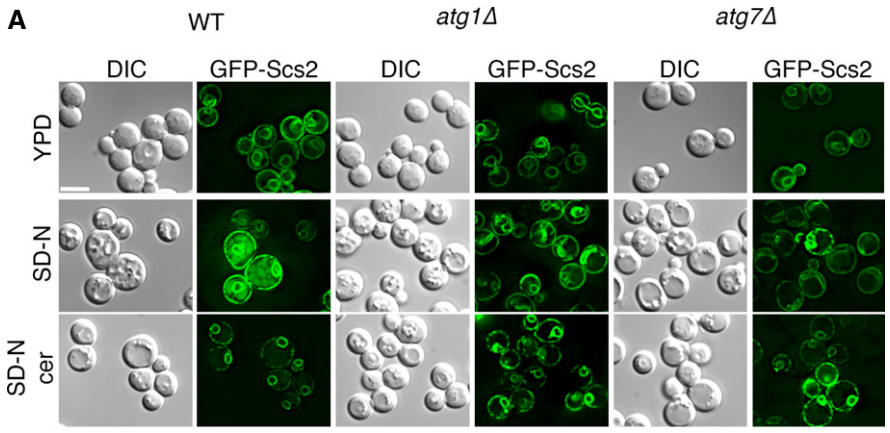
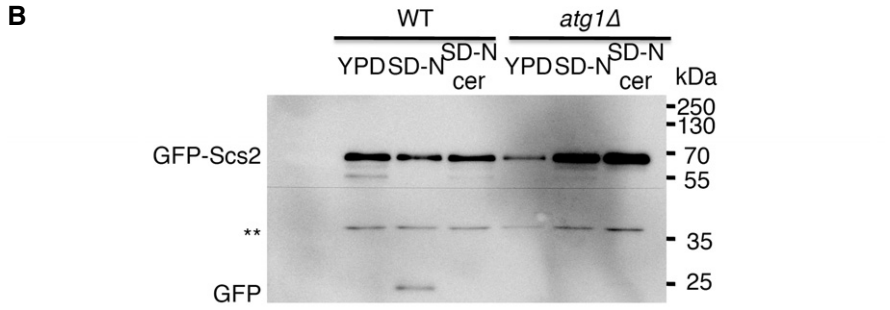
Expanded View Figures





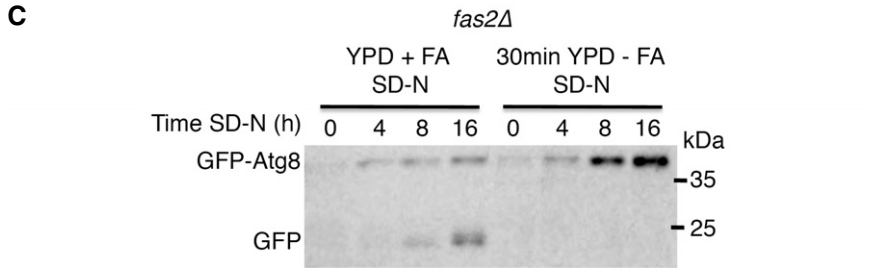


Figure EV1. Autophagy is inhibited upon depletion of free fatty acids.

A WT (BY4741), *atg1*Δ (TOS001), and *atg7*Δ (TOS005) cells expressing GFP-Scs2 were grown to mid-log phase in YPD and shifted to SD-N for 12 h in the presence (SD-N cer) or absence (SD-N) of 50 μM cerulenin and visualized by fluorescence microscopy. Scale bar, 5 μm.

B WT (BY4741) and *atg1*Δ (TOS001) cells were grown as in (A). Lysates were subjected to SDS–PAGE, followed by Western blot analysis using anti-GFP antibodies. **, non-specific band.

C *fas2*Δ (TOS030) cells expressing GFP-Atg8 were grown to mid-log phase in YPD + 0.1 mM palmitic/stearic/myristic acids and shifted either to the same medium or to YPD without fatty acids for 30 min. The cells were then shifted to SD-N for the indicated time periods. Cell lysates were subjected to SDS–PAGE, followed by Western blot analysis using anti-GFP antibodies.

Data information: cer, cerulenin; SD-N, nitrogen starvation medium; WT, wild type; YPD, complete medium.

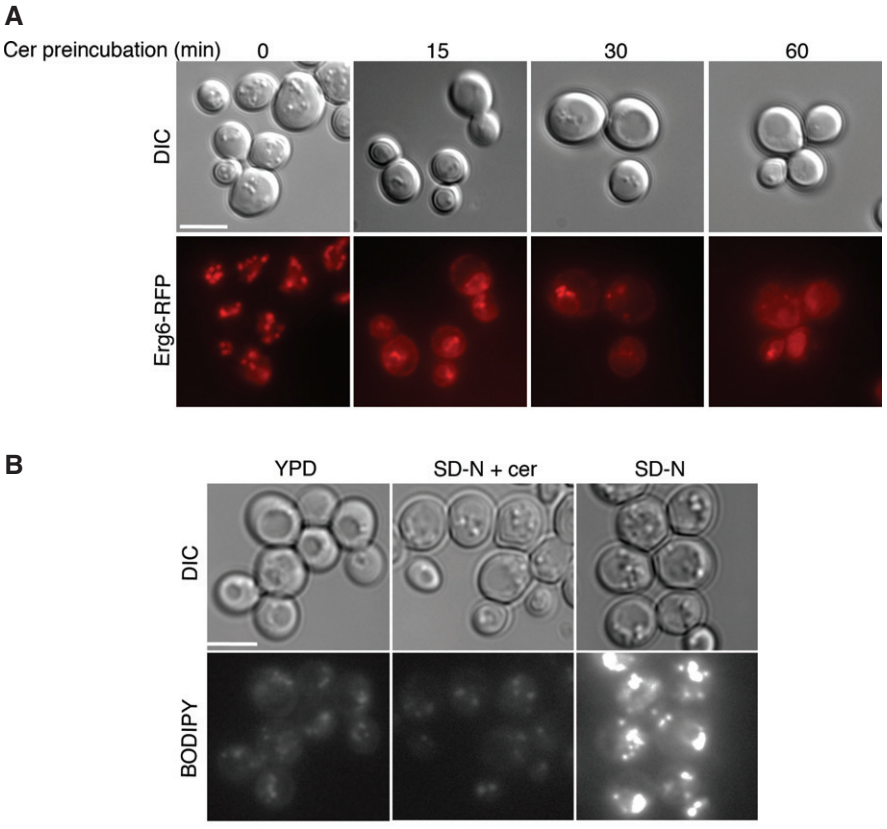


Figure EV2. Lipid droplets are important for autophagy.

A RFP-Erg6 (TOS039)-expressing cells were grown to mid-log phase in YPD and preincubated with 50 μM cerulenin for the indicated time periods (Cer preincubation) or with DMSO (0). Cells were shifted to SD-N with 50 μM cerulenin for 3 h and then visualized by fluorescence microscopy. Scale bar, 5 μm.

B WT cells were grown to mid-log phase in YPD and shifted to SD-N in the presence or absence of 50 μM cerulenin for 16 h. Cells were stained with BODIPY and visualized by fluorescence microscopy. Scale bar, 5 μm.

C WT (SCY62) and *tagΔsteΔ* (H1246) cells were grown to mid-log phase in YPD and shifted to SD-N for 2 h. Cells were lysed and the lysate was subjected to subcellular fractionation as described in Materials and Methods. An equal volume of each fraction was subjected to immunoblotting with anti-Atg8, anti-Kar2, anti-Ape1, and anti-Atg3 antibodies.

Data information: cer, cerulenin; SD-N, nitrogen starvation medium; WT, wild type; YPD, complete medium.
Source data are available online for this figure.

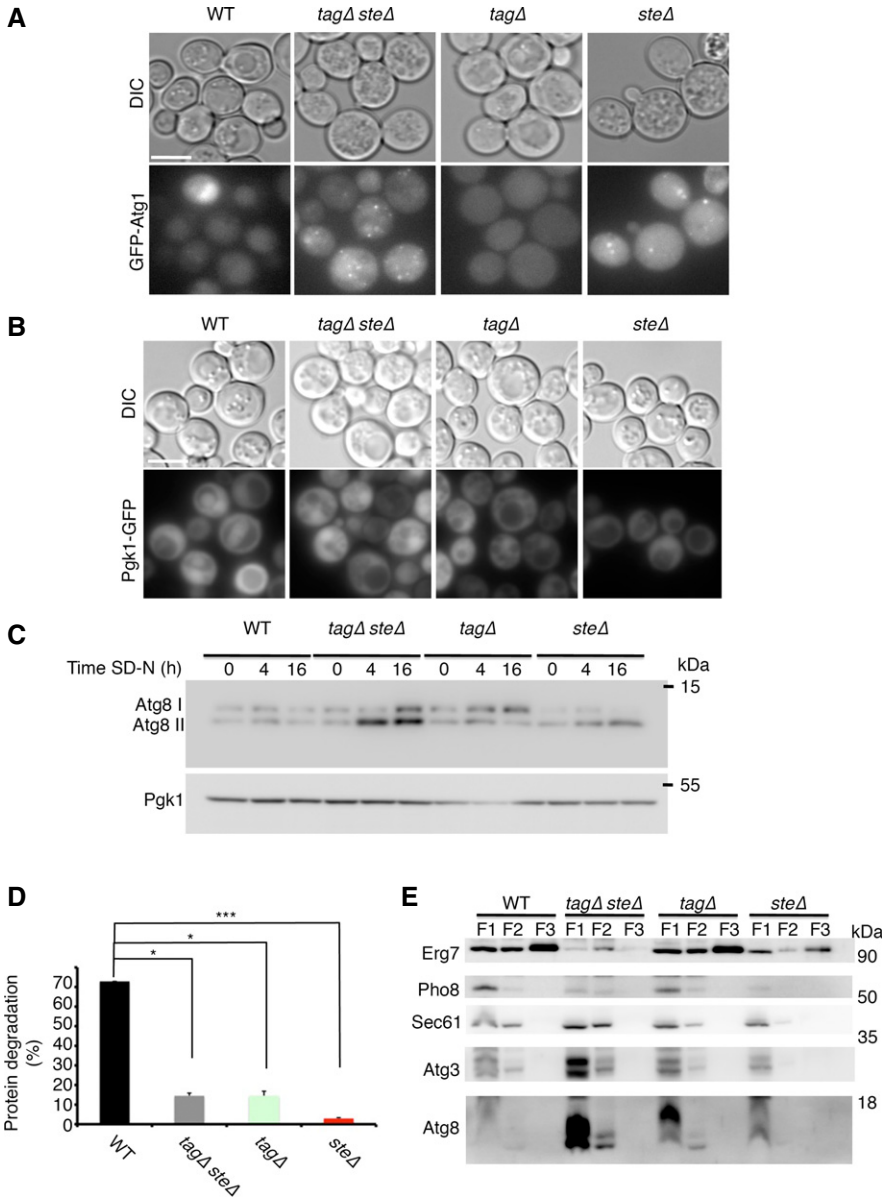


Figure EV3. TAG and STE are both essential for efficient autophagy.

A, B WT (SCY62), *tag*Δ*ste*Δ (H1246), *tag*Δ (H1226), and *ste*Δ (H1112) cells expressing Atg1-GFP (A) or Pgk1-GFP (B) were grown to mid-log phase in YPD and shifted to SD-N for 2 h. GFP was visualized by fluorescence microscopy. Scale bar, 5 μm.

C WT (SCY62), *tag*Δ*ste*Δ (H1246), *tag*Δ (H1226), and *ste*Δ (H1112) cells were grown to mid-log phase in YPD and shifted to SD-N for the indicated time periods. Lysates were subjected to SDS–PAGE in urea gel, followed by Western blot analysis using anti-Atg8 and anti-Pgk1 antibodies.

D WT (SCY62), *tag*Δ*ste*Δ (H1246), *tag*Δ (H1226), and *ste*Δ (H1112) cells were grown to mid-log phase in YPD and pulse-labeled for 16 h with [^{35}S] methionine and cysteine. Cells were chased on non-radioactive starvation medium. Acid-soluble small peptides generated by proteolysis were determined after 8 h in SD-N, as described in Materials and Methods. Error bars represent the s.e.m. of three independent experiments. *$P < 0.05$, ***$P < 0.001$ (Student's *t*-test).

E WT (SCY62), *tag*Δ*ste*Δ (H1246), *tag*Δ (H1226), and *ste*Δ (H1112) cells were grown to mid-log phase in YPD and shifted to SD-N for 4 h. Cells were lysed and lipid droplets were isolated by three successive flotations (F1, F2, F3) as described in Materials and Methods. The flotation fractions were subjected to SDS–PAGE, followed by Western blot analysis using anti-Atg8, anti-Sec61, anti-Erg7, anti-Atg3, and anti-Pho8 antibodies. WT sucrose %: 1–8.4%, 2–13%, 3–19.5%, 4–23.8%, 5–26%, 6–28.5%, 7–28.8%, 8–29.5%, 9–32%, 10–34.2%, 11–39.5%, 12–48.5%, 13–48.5%. *tag*Δ*ste*Δ sucrose %: 1–8.4%, 2–12%, 3–13%, 4–18.5%, 5–23.8%, 6–26.5%, 7–28.2%, 8–31%, 9–31.5%, 10–33.8%, 11–38%, 12–45%, 13–48%.

Data information: SD-N, nitrogen starvation medium; WT, wild type; YPD, complete medium. Source data are available online for this figure.

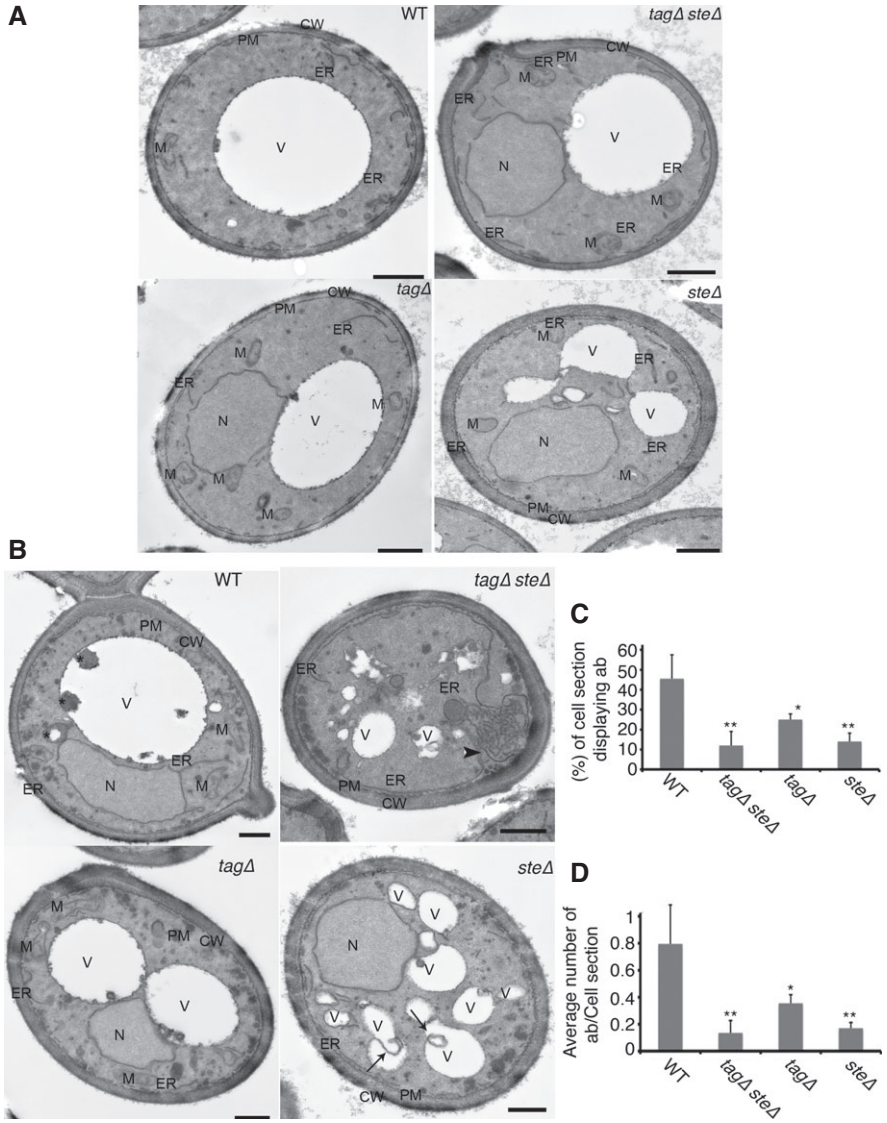


Figure EV4. Lack of lipid droplets inhibits starvation-induced formation of autophagosomes.

A, B WT (SCY62), *tag*Δ (H1226), *ste*Δ (H1112), and *tag*Δ*ste*Δ (H1246) cells were grown to an exponential phase in rich medium and were then processed for electron microscopy (A) or starved in SD-N for 2 h, prior to electron microscopy analysis (B). Scale bars, 500 nm. CW, cell wall; ER, endoplasmic reticulum; M, mitochondrion; V, vacuole; SD-N, nitrogen starvation medium; WT, wild type. Arrows highlight homotypic membrane fusion of a vacuolar remnant. Arrowheads point to proliferating ER.

C, D Average number of autophagic bodies per cell section (C) and percentages of cell sections displaying autophagic bodies in the vacuolar lumen (D) were determined by counting 100 randomly selected cell profiles. Error bars represent standard deviations from counting of the three grids. $*P < 0.05$, $**P < 0.01$ (Student's *t*-test). WT, wild type.

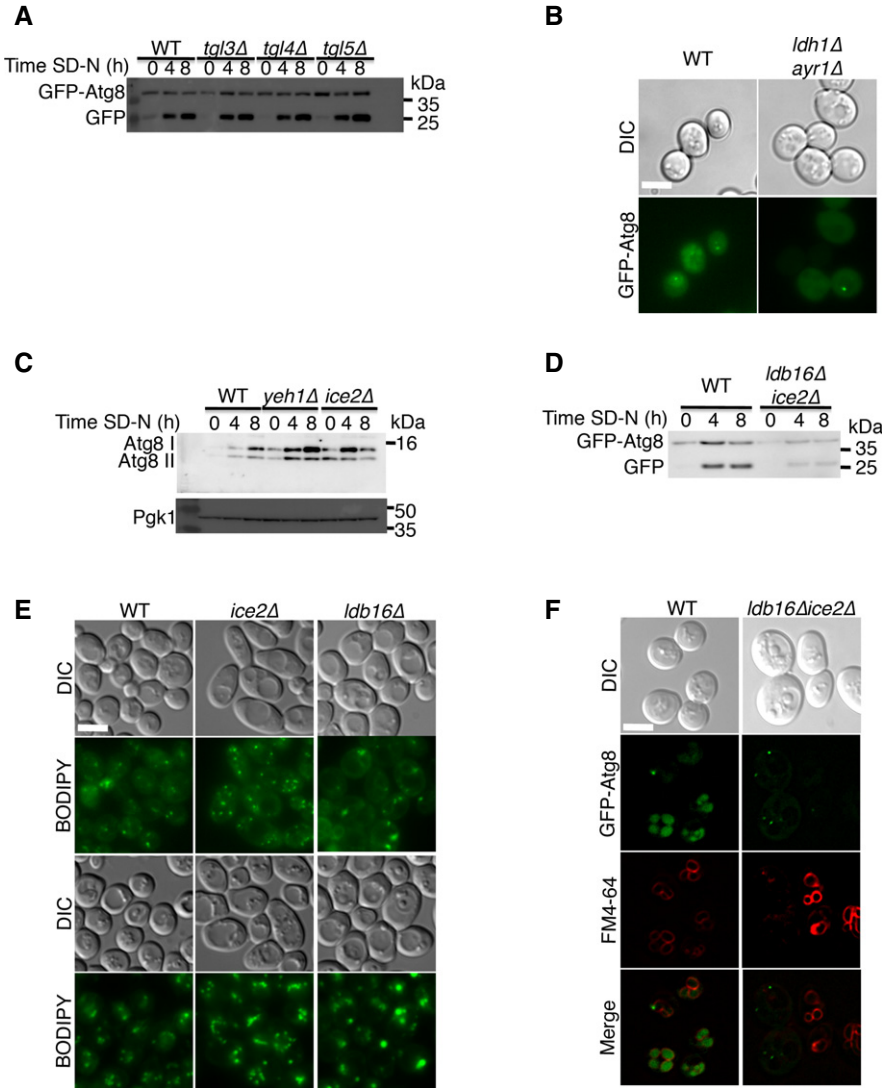


Figure EV5. Lipolysis of TAG and STE is essential for autophagy.

A WT (BY4741), *tgl3*Δ (TOS042), *tgl4*Δ (TOS043), and *tgl5*Δ (TOS044) cells expressing GFP-Atg8 were grown to mid-log phase in YPD and shifted to SD-N for the indicated time periods. Cells were lysed and subjected to SDS–PAGE, followed by Western blot analysis using anti-GFP antibodies.

B WT (BY4741) and *ldh1*Δ*ayr1*Δ (TOS056) cells were grown to mid-log phase in YPD, shifted to SD-N for 4 h, and then visualized by fluorescence microscopy. Scale bar, 5 μm.

C WT (BY4741), *tgl1*Δ (TOS041), *yeh1*Δ (TOS045), and *yeh2*Δ (TOS046) cells were grown to mid-log phase in YPD and shifted to SD-N for the indicated time periods. Lysates were subjected to SDS–PAGE in urea gel, followed by Western blot analysis using anti-Atg8 and anti-Pgk1 antibodies.

D WT (BY4741) and *ldb16*Δ*ice2*Δ (TOS057) cells expressing GFP-Atg8 were grown to mid-log phase in YPD and shifted to SD-N for the indicated time periods. Cells were lysed and subjected to SDS–PAGE, followed by Western blot analysis using anti-GFP antibodies.

E WT (BY4741), *ice2*Δ (TOS047), and *ldb16*Δ (TOS048) cells were grown to mid-log phase in YPD and shifted to SD-N for 2 h. Cells were stained with BODIPY and visualized by fluorescence microscopy. Scale bar, 5 μm.

F WT (BY4741) and *ldb16*Δ*ice2*Δ (TOS057) cells expressing GFP-Atg8 were grown to mid-log phase in YPD, shifted to SD-N for 4 h, and visualized by fluorescence microscopy. Scale bar, 5 μm.

Data information: SD-N, nitrogen starvation medium; WT, wild type; YPD, complete medium. Source data are available online for this figure.