Wang et al., http://www.jcb.org/cgi/content/full/jcb.201404115/DC1

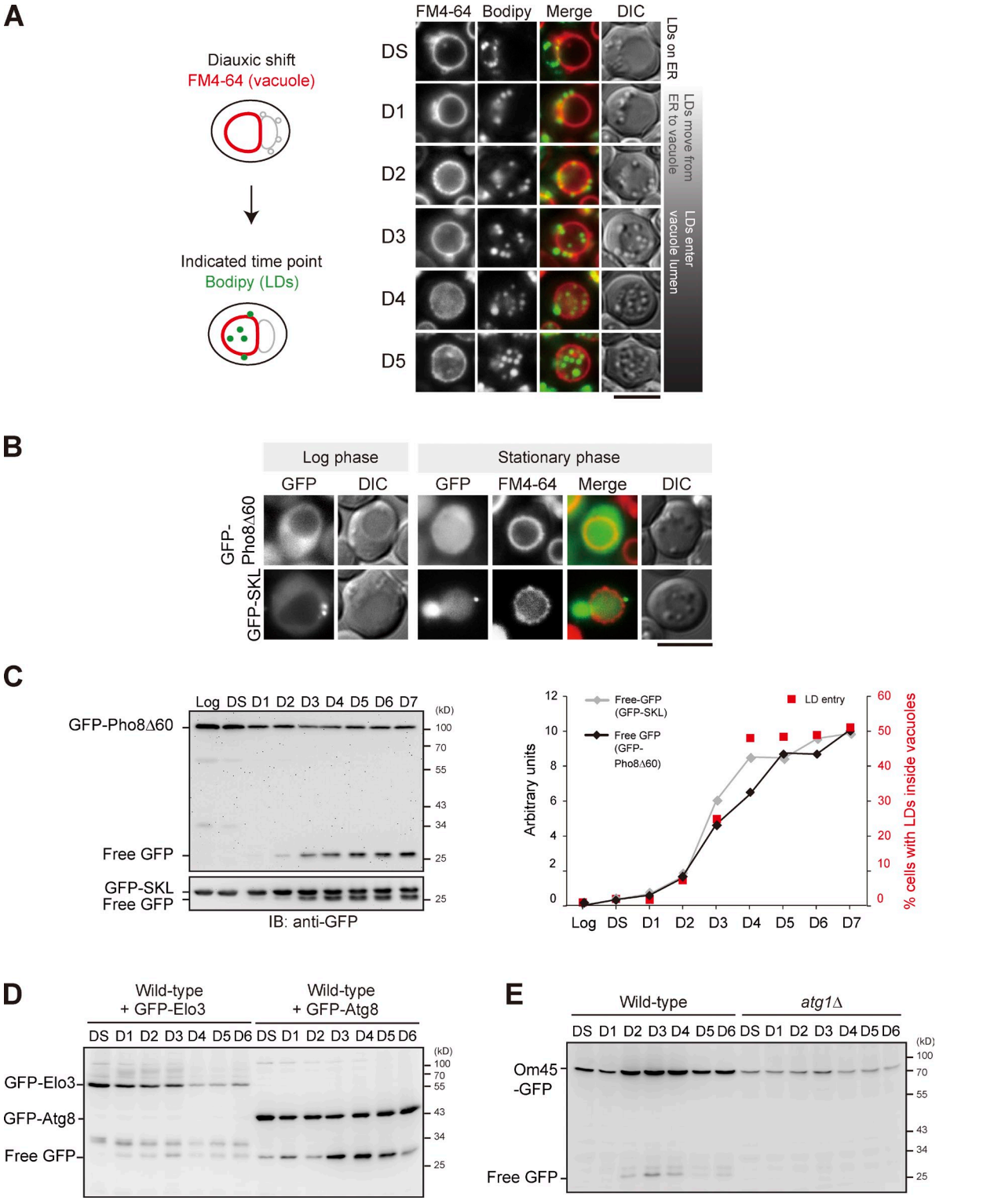


Figure S1. **Various forms of autophagy are activated during stat-phase.** (A, left) The scheme of vacuole and LD staining. (right) Representative images of LD association with vacuoles in wild-type cells grown from DS to D5. (B) Cells expressing GFP-Pho8Δ60 and GFP-SKL were imaged by fluorescence microscopy under growth conditions as indicated. (C, left) Cells expressing GFP-Pho8Δ60 and GFP-SKL under growth conditions as indicated were lysed, and the lysates were analyzed by immunoblotting (IB) with the anti-GFP antibody. (right) The free GFP signals were quantified and plotted. The percentage of cells containing LDs inside vacuole lumen in A on the same days was compared. The data shown are from a single representative experiment out of three repeats. (D) Wild-type cells expressing GFP-Elo3 or GFP-Atg8 under growth conditions as indicated were lysed, and the lysates were analyzed by immunoblotting with anti-GFP antibody. (E) Wild-type and *atg1Δ* cells harboring endogenous Om45-GFP under growth conditions as indicated were lysed, and the lysates were analyzed by immunoblotting with the anti-GFP antibody. DIC, differential interference contrast. Bars, 5 µm.

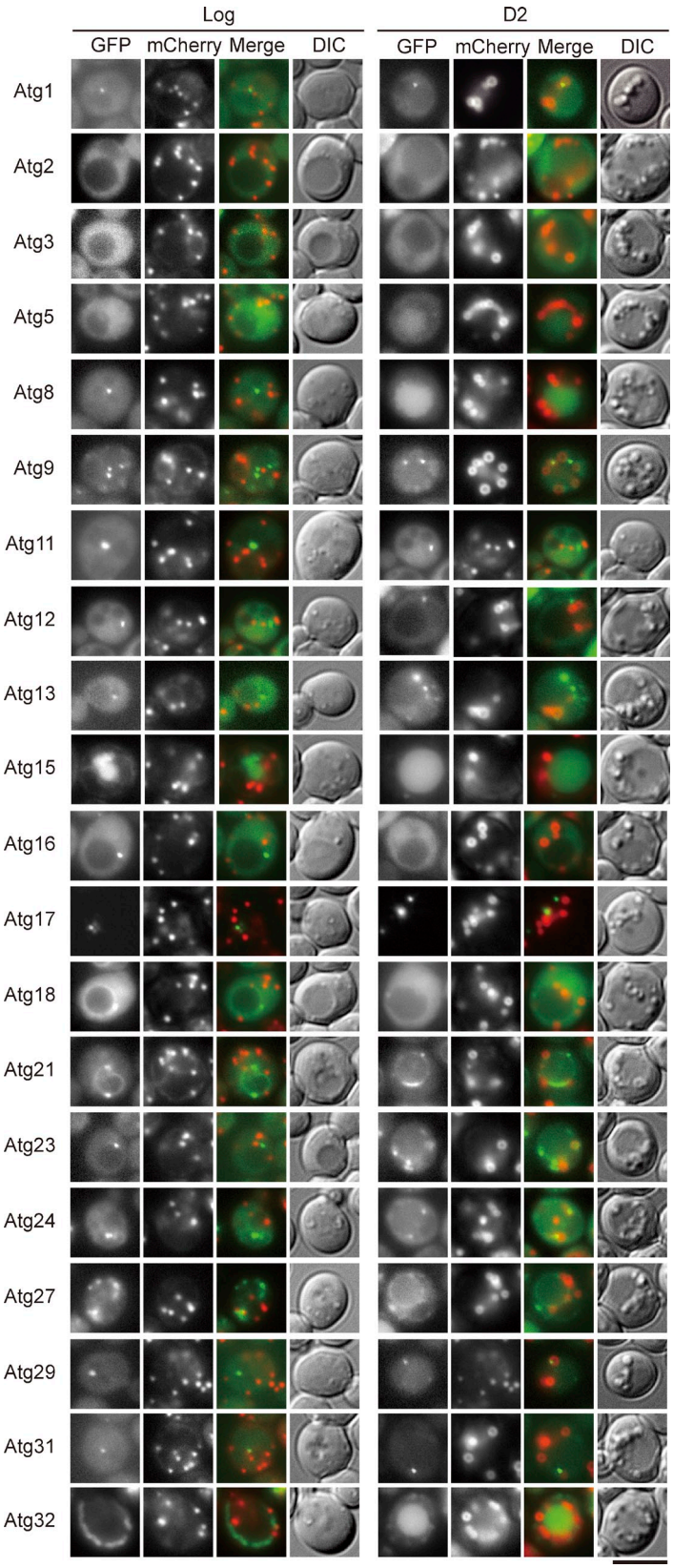


Figure S2. **Localization of various GFP-tagged Atg proteins during log and stat-phase.** Cells expressing Erg6-mCherry and various GFP-tagged Atg proteins as indicated grown in SC medium to log phase or D2 were imaged by fluorescence microscopy. DIC, differential interference contrast. Bar, 5 µm.

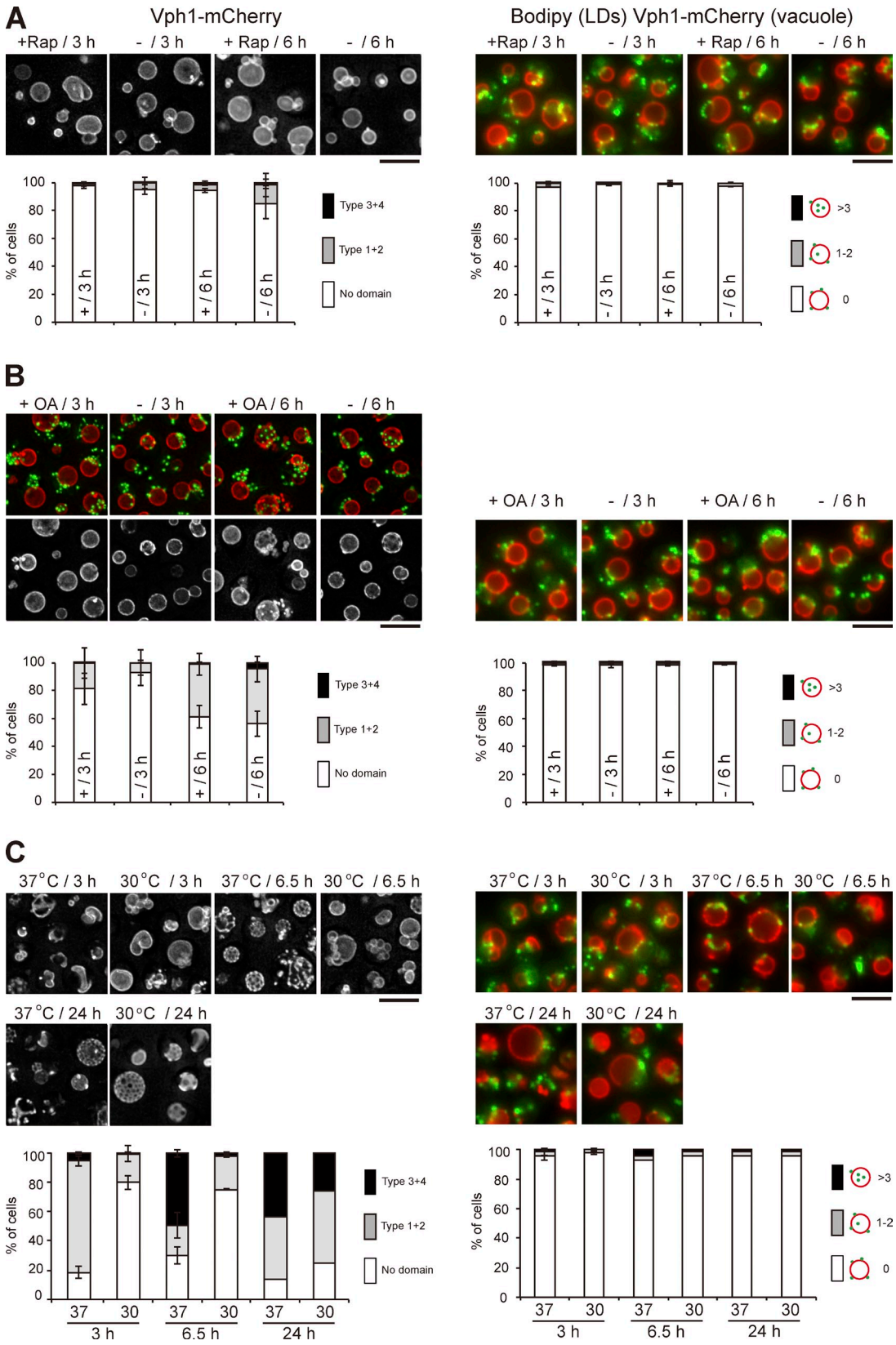


Figure S3. **Rapamycin, oleate, and higher temperature shift did not induce LD translocation into vacuoles.** (A) Cells expressing Vph1-mCherry grown in SC medium at 30°C to log phase were treated with (+) or without (−) 0.2 µg/ml rapamycin (Rap) for 3 or 6 h. (left) The representative images of Vph1-mCherry processed by deconvolution and maximal projection. The data were quantified based on the three indicated patterns. (right) Cells were stained with BODIPY (LDs) and imaged by fluorescence microscopy. The data were quantified based on the three indicated patterns. (B) Same as A, except that cells were grown in SC medium with 1% Brij to log phase and treated with (+) or without (−) 1% oleic acids (OA). (left) The merged Vph1-GFP and LDs (BODIPY) images were also shown for the comparison of LD numbers. (C) Same as A, except that cells were grown in SC medium to DS followed by 1:1 diluted into SC medium prewarmed to 30 or 37°C and incubated for 3, 6.5, or 24 h. Data are means ± SEM. Bars, 5 µm.

Table S1. **Yeast strains and plasmids used in this study**

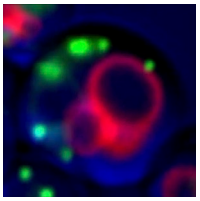
Strain	Description	Source
BY4742	*MATα leu2Δ his3Δ ura3Δ lys2Δ*	Laboratory collection
CWY6290	*ELO3-VENUS::LEU HIS::GPD-CFP-PHO8 ERG6-mCherry::KAN* BY4742	This study
CWY6302	*atg1Δ::HYG* BY4742	This study
CWY6304	*atg2Δ::HYG* BY4742	This study
Laboratory collection	*atg3Δ::KAN* BY4742	Invitrogen
CWY6469	*atg4Δ:::KAN* BY4742	This study
Laboratory collection	*atg5Δ::KAN* BY4742	Invitrogen
CWY6306	*atg6Δ::HYG* BY4742	This study
CWY6308	*atg7Δ::HYG* BY4742	This study
CWY6471	*atg8Δ::KAN* BY4742	This study
Laboratory collection	*atg9Δ::KAN* BY4742	Invitrogen
CWY6473	*atg10Δ::KAN* BY4742	This study
Laboratory collection	*atg11Δ::KAN* BY4742	Invitrogen
CWY6475	*atg12Δ::KAN* BY4742	This study
Laboratory collection	*atg13Δ::KAN* BY4742	Invitrogen
CWY6497	*atg14Δ::LEU* BY4742	This study
Laboratory collection	*atg15Δ::LEU* BY4742	Invitrogen
Laboratory collection	*atg16Δ::KAN* BY4742	Invitrogen.
CWY6499	*atg17Δ::LEU* BY4742	This study
Laboratory collection	*atg18Δ::KAN* BY4742	Invitrogen
Laboratory collection	*atg19Δ::KAN* BY4742	Invitrogen
Laboratory collection	*atg20Δ::KAN* BY4742	Invitrogen
CWY6501	*atg21Δ::LEU* BY4742	This study
Laboratory collection	*atg22Δ::KAN* BY4742	Invitrogen
Laboratory collection	*atg23Δ::KAN* BY4742	Invitrogen
Laboratory collection	*atg24Δ::KAN* BY4742	Invitrogen
Laboratory collection	*atg26Δ::KAN* BY4742	Invitrogen
Laboratory collection	*atg27Δ::KAN* BY4742	Invitrogen
Laboratory collection	*atg29Δ::KAN* BY4742	Invitrogen
Laboratory collection	*atg31Δ::KAN* BY4742	Invitrogen
Laboratory collection	*atg32Δ::KAN* BY4742	Invitrogen
Laboratory collection	*atg33Δ::KAN* BY4742	Invitrogen
CWY6503	*atg34Δ::LEU* BY4742	This study
Laboratory collection	*atg36Δ::KAN* BY4742	Invitrogen
CWY7336	*FAA4-GFP::HIS* BY4742	This study
CWY7464	*atg1Δ::LEU FAA4-GFP::HIS* BY4742	This study
CWY7466	*atg6Δ::LEU FAA4-GFP::HIS* BY4742	This study
CWY6294	*atg1Δ::HYG ELO3-VENUS::LEU HIS::GPD-CFP-PHO8 ERG6-mCherry::KAN* BY4742	This study
CWY6312	*atg2Δ::HYG ELO3-VENUS::LEU HIS::GPD-CFP-PHO8 ERG6-mCherry::KAN* BY4742	This study
CWY6298	*atg7Δ::HYG ELO3-VENUS::LEU HIS::GPD-CFP-PHO8 ERG6-mCherry::KAN* BY4742	This study
CWY6758	*VPH1-GFP::LEU ERG6-mCherry::KAN* BY4742	This study
CWY7085	*GTR2-3×GFP::HIS ERG6-mCherry::LEU* BY4742	This study
CWY6760	*IVY1-GFP::LEU ERG6-mCherry::KAN* BY4742	This study
CWY7129	*VPH1-mCherry::LEU* BY4742	This study
CWY7183	*ATG6-3×GFP::HIS VPH1-mCherry::LEU* BY4742	This study
CWY7325	*ATG6-3×GFP::HIS GTR2-mCherry::LEU* BY4742	This study
CWY7226	*ATG14-3×GFP::HIS VPH1-mCherry::LEU* BY4742	This study
CWY7327	*ATG14-3×GFP::HIS IVY1-mCherry::LEU* BY4742	This study
CWY7169	*VPH1-mCherry::LEU fab1Δ::KAN* BY4742	This study
CWY7161	*VPH1-mCherry::LEU vps4Δ::KAN* BY4742	This study
CWY7159	*VPH1-mCherry::LEU nem1Δ::KAN* BY4742	This study
Laboratory collection	*fab1Δ::KAN* BY4742	Invitrogen
Laboratory collection	*vps4Δ::KAN* BY4742	Invitrogen
Laboratory collection	*nem1Δ::KAN* BY4742	Invitrogen
Laboratory collection	*pep4Δ::KAN* BY4742	Invitrogen
CWY7266	*VPH1-mCherry::LEU pep4Δ::KAN* BY4742	This study
CWY7204	*VPH1-mCherry::LEU atg1Δ::HYG* BY4742	This study
CWY7212	*VPH1-mCherry::LEU atg6Δ::HYG* BY4742	This study

Table S1. **Yeast strains and plasmids used in this study** *(Continued)*

Strain	Description	Source
CWY7216	*VPH1-mCherry::LEU atg7Δ::HYG* BY4742	This study
CWY7220	*VPH1-mCherry::LEU atg8Δ::KAN* BY4742	This study
CWY7234	*VPH1-mCherry::LEU atg11Δ::KAN* BY4742	This study
CWY7250	*VPH1-mCherry::HIS atg14Δ::LEU* BY4742	This study
CWY7238	*VPH1-mCherry::LEU atg19Δ::KAN* BY4742	This study
CWY7254	*VPH1-mCherry::HIS atg21Δ::LEU* BY4742	This study
CWY7242	*VPH1-mCherry::LEU atg32Δ::KAN* BY4742	This study
CWY7246	*VPH1-mCherry::LEU atg33Δ::KAN* BY4742	This study
CWY4836	*are1Δ::KAN are2Δ::HIS* BY4742	This study
CWY3626	*dga1Δ::KAN lro1Δ::HIS* BY4742	Wang and Lee, 2012
CWY7167	*VPH1-mCherry::LEU are1Δ::KAN are2Δ::HIS* BY4742	This study
CWY7442	*OM45-GFP::LEU* BY4742	This study
CWY7444	*atg1Δ::HYG OM45-GFP::LEU* BY4742	This study
CWY6555	*ATG1-GFP::LEU ERG6-mCherry::KAN* BY4742	This study
CWY6558	*ATG3-GFP::LEU ERG6-mCherry::KAN* BY4742	This study
CWY6592	*ATG9-GFP::LEU ERG6-mCherry::KAN* BY4742	This study
CWY6572	*ATG13-GFP::LEU ERG6-mCherry::KAN* BY4742	This study
CWY6580	*ATG21-GFP::LEU ERG6-mCherry::KAN* BY4742	This study
CWY6582	*ATG23-GFP::LEU ERG6-mCherry::KAN* BY4742	This study
CWY6584	*ATG24-GFP::LEU ERG6-mCherry::KAN* BY4742	This study
CWY6586	*ATG27-GFP::LEU ERG6-mCherry::KAN* BY4742	This study
CWY6679	*ATG29-GFP::HIS ERG6-mCherry::KAN* BY4742	This study

Table S2. **Plasmids used in this study**

Plasmid	Description/reference
pRS416-P_{GPD}-GFP-Pho8Δ60-T_{CYC1}	*GPD* promoter–*GFP-PHO8Δ60-CYC1* terminator cloned into pRS416
pRS416-P_{GPD}-GFP-SKL-T_{CYC1}	*GPD* promoter–*GFP-SKL-CYC1* terminator cloned into pRS416
pBS-GTR2-3×GFP-His3	*GTR2* ORF residues 778–1,025 and the residues 50–411 after stop codon were cloned into HindIII–BamHI and SacI sites, respectively, of pBS-3×GFP-His3 (Toshima et al., 2006)
pBS-ATG6-3×GFP-His3	*ATG6* ORF residues 1,248–1,672 and the residues 57–572 after stop codon were cloned into HindIII–BamHI and SacI sites, respectively, of pBS-3×GFP-His3 (Toshima et al., 2006)
pBS-ATG14-3×GFP-His3	*ATG14* ORF residues 763–1,033 and the residues 44–455 after stop codon were cloned into HindIII–BamHI and SacI sites, respectively, of pBS-3×GFP-His3 (Toshima et al., 2006)
pFA6a-His3-GPD-CFP	*GPD* promoter–N-terminal *CFP* integration plasmid with a *His3* marker
pFA6a-VENUS-LEU2	C-terminal *VENUS* integration plasmid with a *LEU2* marker
pFA6a-mCherry-KanMX6	C-terminal *mCherry* integration plasmid with a *KanMX6* marker
pFA6a-mCherry-LEU2	C-terminal *mCherry* integration plasmid with a *LEU2* marker
pFA6a-mCherry-His3	C-terminal *mCherry* integration plasmid with a *His3* marker
pFA6a-GFP-LEU2	C-terminal *GFP* integration plasmid with a *LEU2* marker
pFA6a-GFP-His3MX6	Longtine et al., 1998
pFA6a-His3MX6	Longtine et al., 1998
pFA6a-HYG	Deletion plasmid with a *Hygromycin* resistance gene marker
pFA6a-LEU2	Deletion plasmid with a *LEU2* marker
pFA6a-KanMX6	Longtine et al., 1998
pRS416-P_{GPD}-GFP-Elo3-T_{CYC1}	*GPD* promoter–*GFP-ELO3-CYC1* terminator cloned into pRS416
pRS416-P_{Cu}-GFP-Atg8 (Aut7)	Kim et al., 2002
pRS416-P_{ADH1}-ATG2-GFP-T_{CYC1}	*ADH1* promoter–*ATG2-GFP-CYC1* terminator cloned into pRS416
pRS416-P_{ADH1}-ATG5-GFP-T_{CYC1}	*ADH1* promoter–*ATG5-GFP-CYC1* terminator cloned into pRS416
pRS416-P_{Cu}-GFP-Atg11 (Cvt9)	Kim et al., 2001
pRS416-P_{ADH1}-GFP-ATG12-T_{CYC1}	*ADH1* promoter–*GFP-ATG12-CYC1* terminator cloned into pRS416
pRS416-P_{ADH1}-GFP-ATG15-T_{CYC1}	*ADH1* promoter–*GFP-ATG15-CYC1* terminator cloned into pRS416
pRS416-P_{ADH1}-ATG16-GFP-T_{CYC1}	*ADH1* promoter–*ATG16-GFP-CYC1* terminator cloned into pRS416
pRS426-P_{Cu}-GFP-ATG17	Cheong et al., 2005
pRS426-ATG18-GFP	*ATG18-GFP* cloned into pRS426
pRS416-P_{Cyc1}-GFP-Atg32-T_{CYC1}	*CYC1* promoter–*GFP-ATG32-CYC1* terminator cloned into pRS416



Video 1. **Localization of LDs in wild-type cells during stat-phase.** Wild-type cells stained with FM4-64 (vacuole) at DS and with BODIPY (LDs) at the indicated time points. Images were analyzed by time-lapse microscopy using the Delta Vision system (Applied Precision). Frames were taken every 30 s for 30 min. Cells in D4 were also imaged for z sections. Frames from top to bottom were taken every 0.2 µm for 5 µm. The dotted circles outline the boundaries of yeast cells. N, nucleus; V, vacuole.

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