Supplemental Table 1. Additional strains used in this study

Name	Genotype	Reference/Origin
AVY066	BY4742 *spo14Δ*::kanMX *pep4Δ::LEU2*	This study
AVY067	BY4742 *rud3Δ*::kanMX4 *pep4Δ::LEU2*	This study
AVY068	SEY6210 *gga1Δ::HIS5 gga2Δ::TRP1 pep4Δ::LEU2*	This study
chc1Δ	BY4742 *chc1Δ*::kanMX4	Euroscarf
MBY004	SEY6210 *gga1Δ::HIS5 gga2Δ::TRP1*	Black *et al.*, 2000
pep4Δ	BY4742 *pep4Δ*::kanMX4	Euroscarf
rud3Δ	BY4742 *rud3Δ*::kanMX4	Euroscarf
spo14Δ	BY4742 *spo14Δ*::kanMX	Euroscarf

Supplemental reference

Black. M. W. and Pelham, H. R. (2000). A selective transport route from Golgi to late endosomes that requires the yeast GGA proteins. J. Cell Biol. *151*, 587-600.

Supplemental Figure 1. Rapamycin treatment and nitrogen starvation induce similar autophagy responses. The experiment described in Figure 1 was repeated in conditions where autophagy was induced by transferring cells to nitrogen starvation (SD-N) medium. Quantification of the GFP-Atg8 processing was done using an Odyssey system.

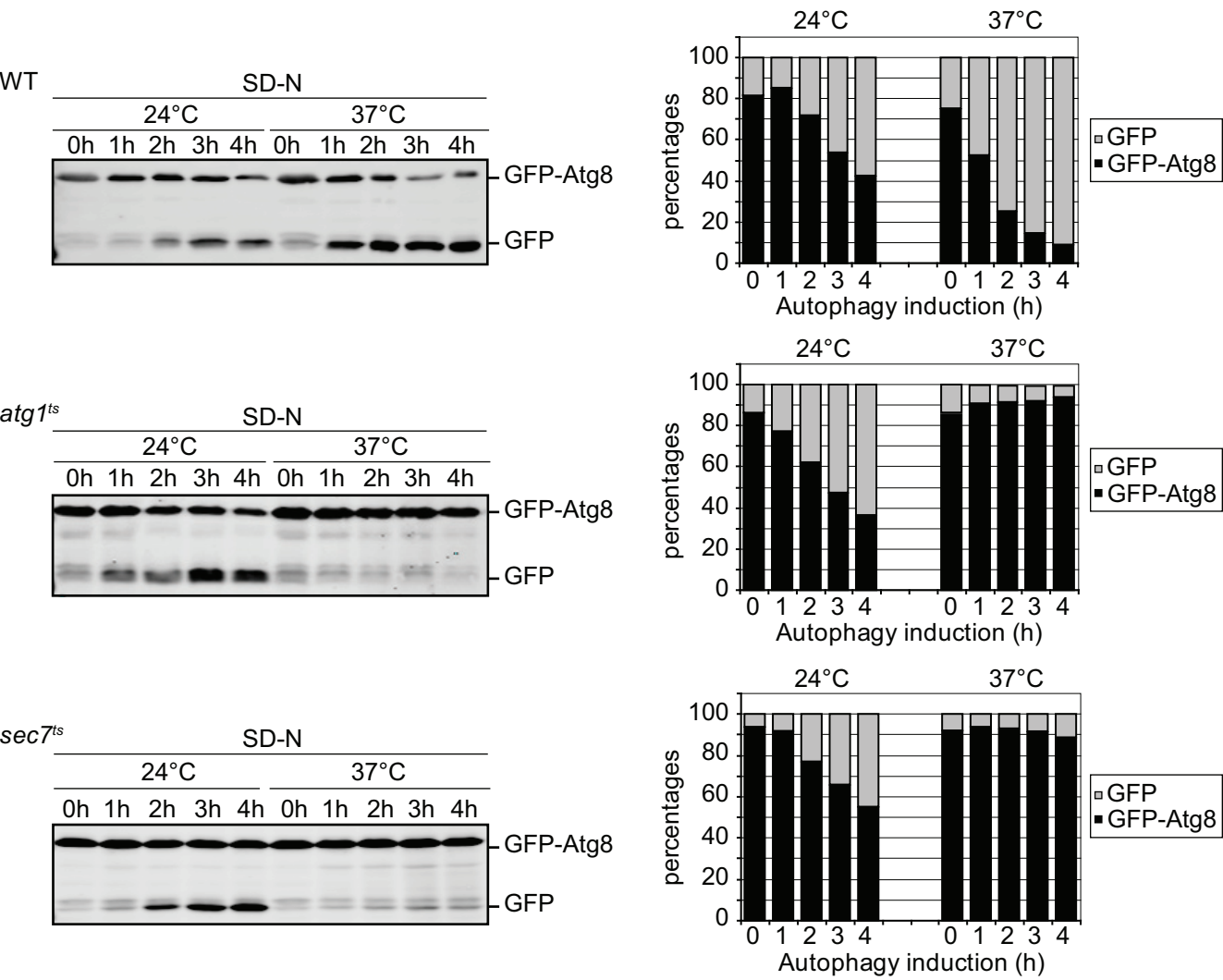
Supplemental Figure 2. The cleavage of GFP-Atg8 in the *ret3^{ts}* strain occurs in the vacuole. The *ret3^{ts} pep4Δ* (AVY037) cells carrying the pCuGFPAtg8416 plasmid were grown at 24˚C to an early log phase. Autophagy was induced by addition of rapamycin and cells were placed either at 24˚C or 37˚C. Culture aliquots were collected at intervals of 1h during a period of 4h, and GFP-Atg8 cleavage was determined by Western-blot analysis of the cell extracts.

Supplemental Figure 3. Rapamycin treatment and nitrogen starvation induce similar autophagy responses in *ret3^{ts}* cells. The experiment described in Figure 4C was repeated in conditions where autophagy was induced by rapamycin treatment. Error bars represent the standard deviation of 3 experiments.

Supplemental Figure 4. The Golgi-Arf effectors Spo14, Rud3, Gga1/Gga2 and clathrin (Chc1) are not essential for autophagy. (A) The *spo14Δ*, *rud3Δ*, *gga1Δ gga2Δ* (MBY004) and *chc1Δ* strains carrying the pCuGFPAtg8416 plasmid were grown at 30˚C to an early log phase. Autophagy was then induced by addition of rapamycin and culture aliquots were collected at intervals of 1h during a period of 4h. GFP-Atg8 cleavage was determined by Western-blot analysis of the cell extracts. Bands were quantified using the Odyssey software and the percentages of GFP-Atg8 (black) and GFP (grey) were plotted. (B) The *pep4Δ*, *spo14Δ pep4Δ* (AVY066), *rud3Δ pep4Δ* (AVY067) and *gga1Δ gga2Δ pep4Δ* (AVY068)

strains were grown in rich medium at 30˚C to an early log phase and then transferred to the SD-N medium for 2h at the same temperature. Culture aliquots were collected at the beginning and at the end of each incubation before being processed for EM and determining the average number of autophagic bodies per vacuole as indicated in Figure 2. Error bars represent the standard deviation in the counting of two different grids. N: nucleus, V: vacuole; *: autophagic body. Scale bar: 500 nm.

Supplemental Figure 1



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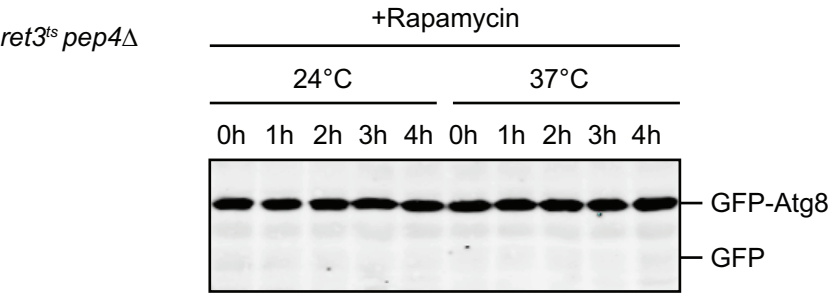
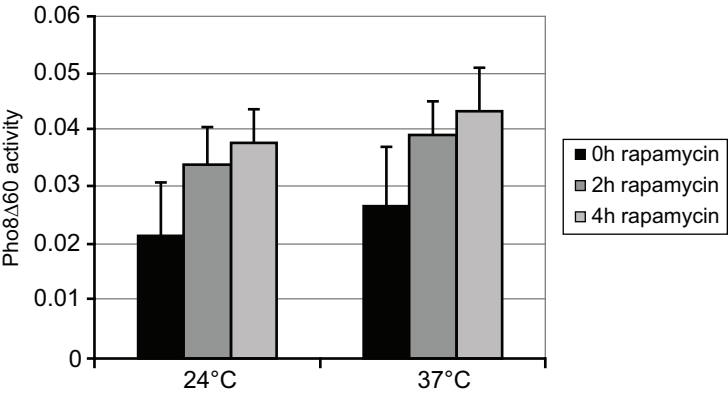
Supplemental Figure 2



Supplemental Figure 3



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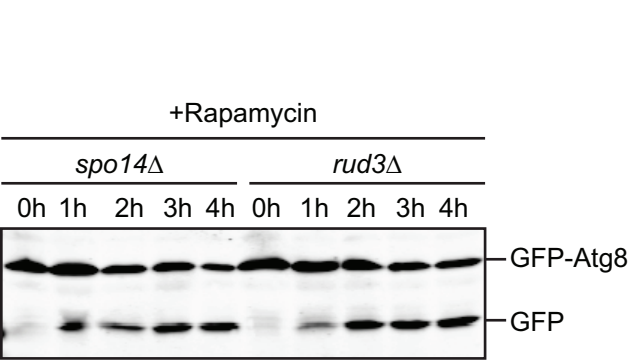
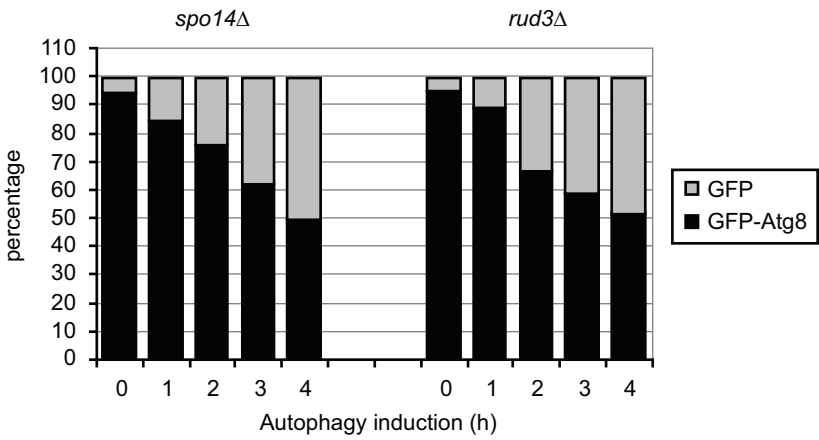
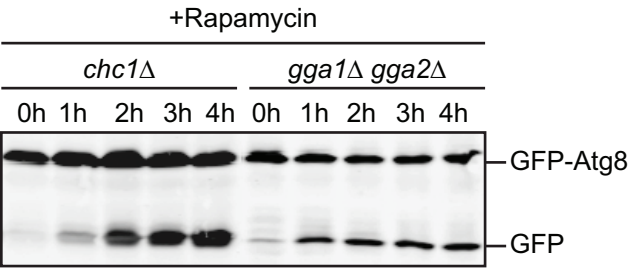
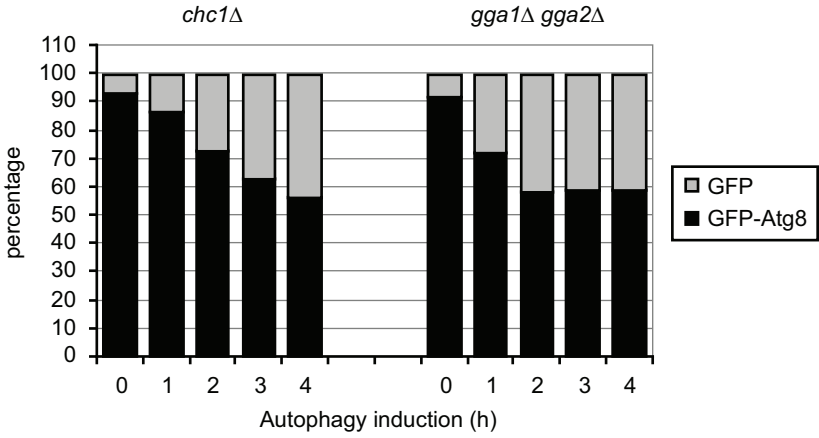
Supplemental Figure 4

A









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