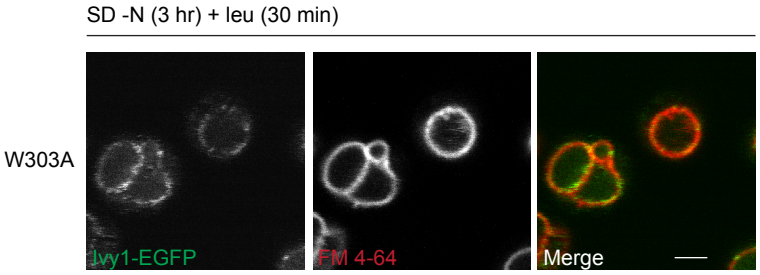
Table S1: Strains used in this work

Strain	Genotype	Reference
W303A	*MAT***a**; *ade2-1*; *leu2-3,112*; *his3-11,15*; *trp1-1*; *ura3-1*; *can1-100*	
PY_104	*MAT***a**; *ade2-1*; *leu2-3,112*; *his3-11,15*; *trp1-1*; *ura3-1*; *can1-100*; Δ*gtr1*::*HIS3*; Δ*gtr2*::KAN	This work
PY_116	*MAT***a**; *ade2-1*; *leu2-3,112*; *his3-11,15*; *trp1-1*; *ura3-1*; *can1-100*; Δ*ivy1*::KAN	This work
mgjf_33	*MAT***a**; *ade2-1*; *leu2-3,112*; *his3-11,15*; *trp1-1*; *ura3-1*; *can1-100*; Δ*vps1*::NAT	This work
PY_124	*MAT***a**; *ade2-1*; *leu2-3,112*; *his3-11,15*; *trp1-1*; *ura3-1*; *can1-100*; Δ*vps1*::NAT; Δ*ivy1*::KAN	This work
PY_136	*MAT***a**; *ade2-1*; *leu2-3,112*; *his3-11,15*; *trp1-1*; *ura3-1*; *can1-100*; *VPH1*-EGFP::KAN	This work
PY_142	*MAT***a**; *ade2-1*; *leu2-3,112*; *his3-11,15*; *trp1-1*; *ura3-1*; *can1-100*; Δ*vps1*::NAT; *VPH1*-EGFP::KAN	This work
PY_144	*MAT***a**; *ade2-1*; *leu2-3,112*; *his3-11,15*; *trp1-1*; *ura3-1*; *can1-100*; Δ*gtr1*::*HIS3*; Δ*gtr2*::KAN; Δ*ivy1*::NAT	This work
PY_187	*MAT*α; *ade2-1*; *leu2-3,112*; *his3-11,15*; *trp1-1*; *ura3-1*; *can1-100*; *GTR1*-VN::*HIS3*	This work
PY_191	*MAT*α; *ade2-1*; *leu2-3,112*; *his3-11,15*; *trp1-1*; *ura3-1*; *can1-100*; *GTR2*-VN::*HIS3*	This work
PY_196	*MAT***a**; *ade2-1*; *leu2-3,112*; *his3-11,15*; *trp1-1*; *ura3-1*; *can1-100*; *GTR1*-VC::KAN	This work
PY_200	*MAT***a**; *ade2-1*; *leu2-3,112*; *his3-11,15*; *trp1-1*; *ura3-1*; *can1-100*; Δ*tor1*::*HIS3*	This work
PY_202	*MAT***a**; *ade2-1*; *leu2-3,112*; *his3-11,15*; *trp1-1*; *ura3-1*; *can1-100*; KAN::p*GAL1*-VC-*IVY1*	This work
PY_206	*MAT***a**; *ade2-1*; *leu2-3,112*; *his3-11,15*; *trp1-1*; *ura3-1*; *can1-100*; *IVY1*-VC::KAN	This work

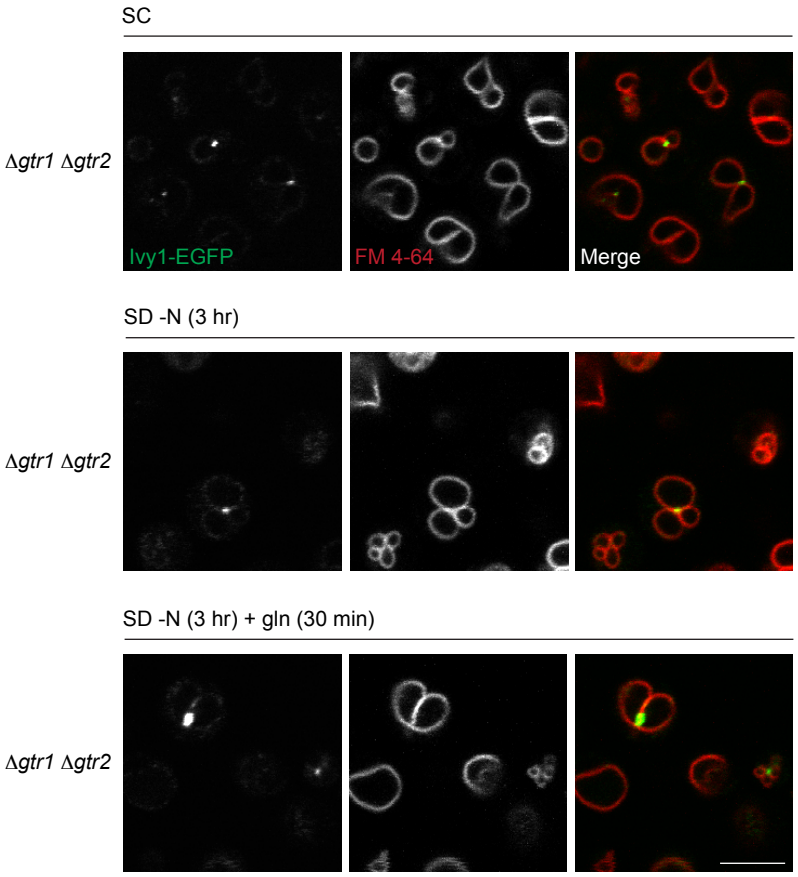
Table S2: Plasmids used in this work

Plasmid	Details	Reference
IVY1-EGFP	pRS316 *S cer. IVY1*-EGFP	This work
IVY1-mCherry	pRS315 *S cer. IVY1*-mCherry	This work
pCM190 *IVY1*	pCM190 *S cer. IVY1*	This work
pCM190 *IVY1*-EGFP	pCM190 *S cer. IVY1*-EGFP	This work
GTR1-EGFP	pRS316 *S cer. GTR1*-EGFP	(Varlakhanova et al., 2017)
GTR2-EGFP	pRS316 *S cer. GTR2*-EGFP	(Varlakhanova et al., 2017)
GTR1 Q65L	pRS314 *S cer. GTR1* Q65L	This work
GTR2 S23L	pRS315 *S cer. GTR2* S23L	(Varlakhanova et al., 2017)
EGFP-*TOR1*	pRS316 EGFP-*S cer*. *TOR1*	This work
TOR1 L2134M	pRS426 *S cer*. *TOR1* L2134M	(Varlakhanova et al., 2017)
Gtr1-Gtr2 complex expression	pRSFDuet-1 S cer. *GTR1*, S cer. *GTR2*-Myc	This work
Ivy1 expression	pET-15b S cer. *IVY1*	This work

A



B



C

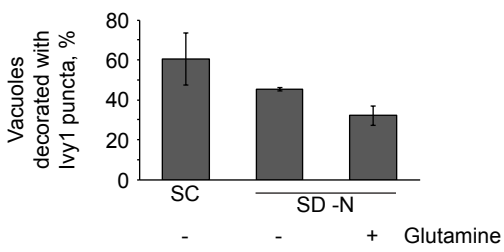
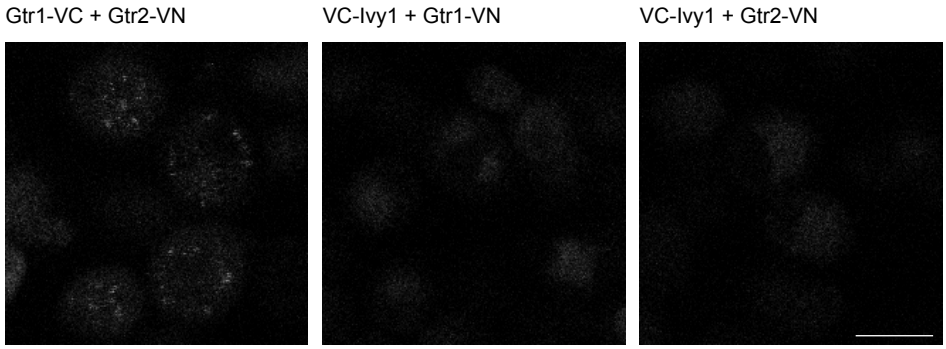


Figure S1. Changes in Ivy1 vacuolar distribution in response to leucine. A) W303A cells expressing Ivy1-EGFP were grown in SC. For nitrogen starvation, cells were transferred to SD –N for 3 hr. Leucine (leu, 3 mM) was added for 30 min, where indicated. Vacuoles were labeled with FM 4-64 (10 μM in the appropriate medium) for 1 hr prior to imaging. B) Ivy1 vacuolar distribution in Δ*gtr1* Δ*gtr2* cells. Δ*gtr1* Δ*gtr2* cells expressing Ivy1-EGFP were grown in SC. For nitrogen starvation, cells were transferred to SD –N for 3 hr. Glutamine (gln, 3 mM) was added for 30 min, where indicated. Vacuoles were labeled with FM 4-64 (10 μM in the appropriate medium) for 1 hr prior to imaging. C) Quantification of the data presented in A). For each condition and each field of cells, Z-stacks were obtained and the number of puncta associated with the vacuole in each stack was determined. Shown is mean ± s.d. Scales 5 μm.

A



B

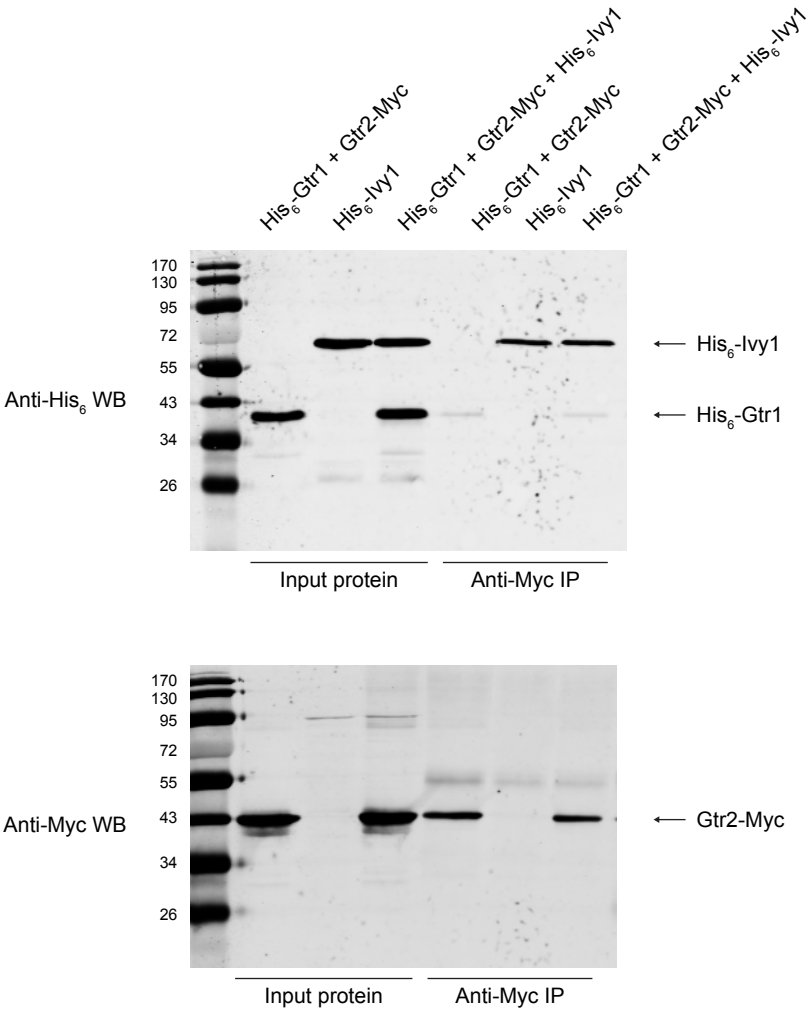


Figure S2. Controls for the split YFP assay. W303A/α cells expressing genomically integrated Ivy1, Gtr1 or Gtr2 N- or C-terminally tagged with either the N-terminal (VN) or C-terminal (VC) fragments of YFP, as indicated, were assessed for YFP fluorescence. Expression of N-terminally tagged VC-Ivy1 is regulated by the *GAL1* promoter. Hence, cells were grown in YPGal medium overnight. Note that tagging Ivy1 at the N-terminus does not produce functional complementation. Scale 5 μm. B) *In vitro* co-immunoprecipitation assays. Purified His_6-Gtr1/Gtr2-Myc was incubated in the absence or presence of His_6-Ivy1. Gtr2-Myc was immune-precipitated using magnetic beads conjugated with an anti-Myc antibody. The Western blot was performed using antibodies against Myc or His_6 as indicated.

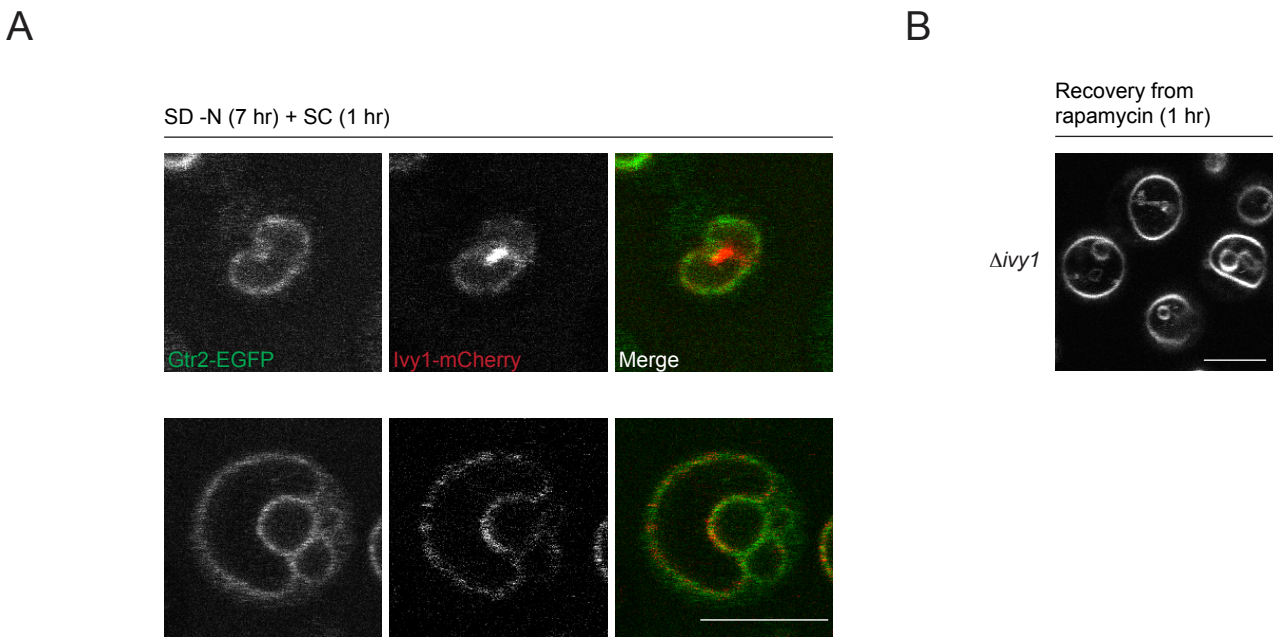
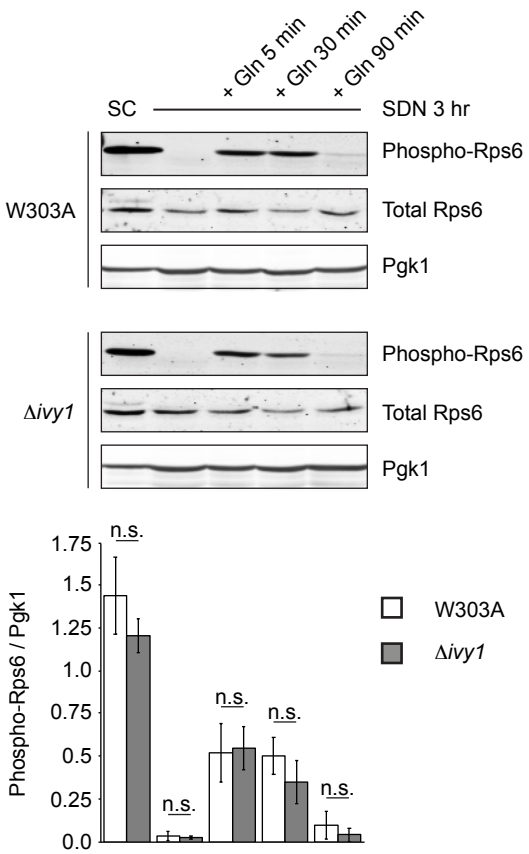
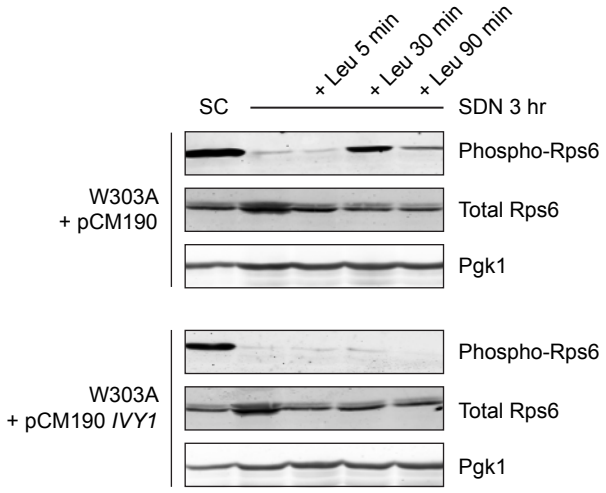


Figure S3. Cells lacking Ivy1 still exhibit microautophagic membrane invaginations. A) W303A cells expressing Gtr2-EGFP and Ivy1-mCherry were grown to saturation in SC, after which they were transferred to SD –N for 7 hr. Cells were then re-fed with SC for 1 hr prior to imaging. Some cells exhibited vacuolar invaginations, labeled by Ivy1-mCherry (top). Some cells lacked vacuolar membrane invaginations and in these Gtr2-EGFP and Ivy1-mCherry were distributed throughout the vacuolar membrane (bottom). B) Δ*ivy1* cells have microautophagic invaginations. Δ*ivy1* cells were grown in YPD, treated with rapamycin (200 ng/ml) for 3 hr, washed and recovered in YPD for 1 hr prior imaging. Vacuolar membrane was stained with FM 4-64. Scale 5 μm.

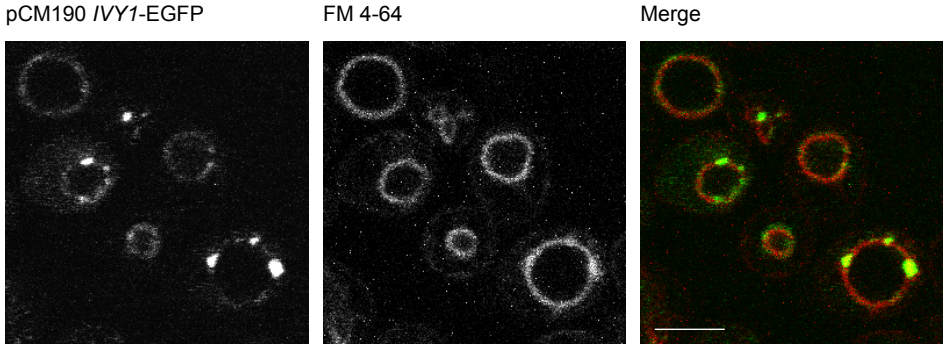
A



B



C



D

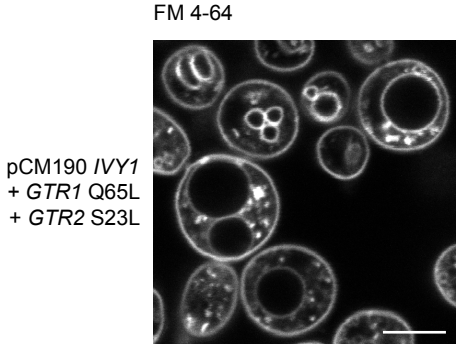
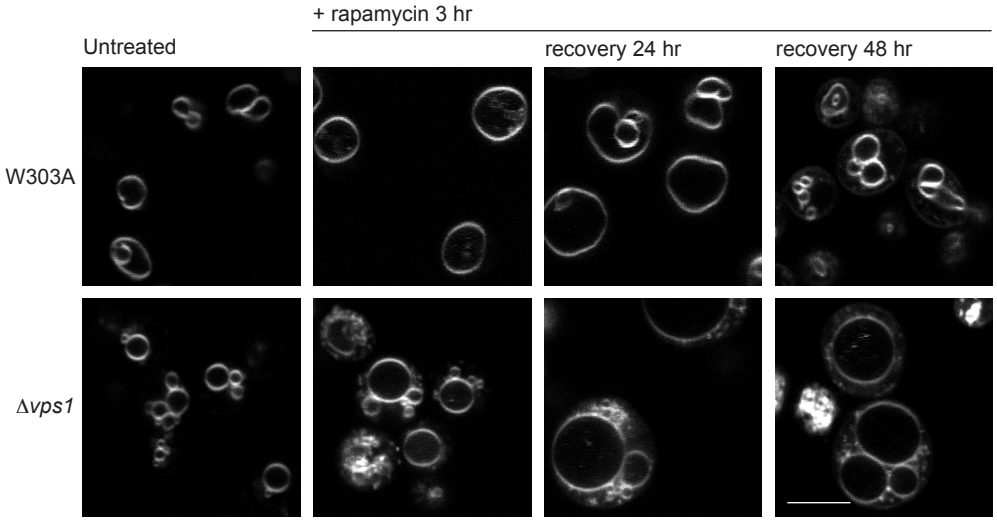
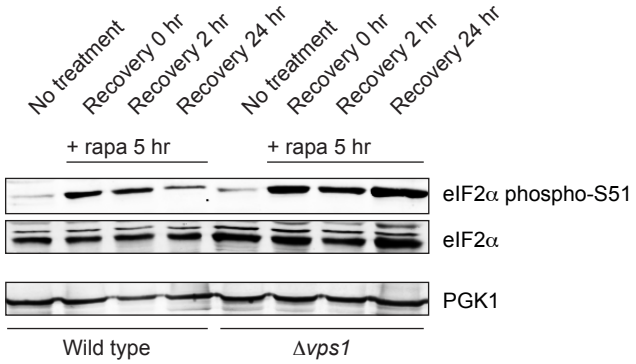


Figure S4. TORC1 activation in Δ*ivy1* cells in response to glutamine. A) Phosphorylation levels of Rps6 in W303A or Δ*ivy1* cells were evaluated under the indicated conditions. Untreated cells were grown in SC. Cells were nitrogen-starved by incubating in SD –N for 3 hr. For stimulation, cells were treated with SD –N supplemented with glutamine (gln, 3 mM) for the indicated times prior to lysis and processing. Pgk1 and total Rps6 were used as loading control. Representative blots are shown. Quantification of the 3 replicates of the blots is shown below. Shown are the means of the ratios of phosphorylated Rps6 (phospho-Rps6) to Pgk1 (mean ± s.d.) for each condition, Differences between means for untreated and treated W303A and Δ*ivy1* cells were assessed using a two-sample *t*-test. No significant differences were observed between W303A and Δ*ivy1* cells were observed under any of the conditions tested. n.s. – not significant. B) Phosphorylation levels of Rps6 were evaluated under the indicated conditions. Untreated cells were grown in SC. Cells were nitrogen-starved by incubating in SD –N for 3 hr. For stimulation, cells were treated with SD –N supplemented with either leucine (leu, 3 mM) and were incubated for the indicated times prior to lysis and processing. Pgk1 and total Rps6 were used as loading control. Representative blots are shown. C) Vacuolar morphology in cells overexpressing Ivy1-EGFP. Ivy1-EGFP was overexpressed in W303A cells using the pCM190 *IVY1*-EGFP plasmid. To overexpress Ivy1-EGFP, cells were cultured overnight in SD –uracil, lacking doxycycline. Cells were then diluted into SC lacking doxycycline and were allowed to grow for several generations. Vacuolar membranes were stained with FM 4-64. D) Vacuolar morphology of W303A cells overexpressing Ivy1 and expressing constitutively active forms of Gtr1 (Gtr1 Q65L) and Gtr2 (Gtr2 S23L). Ivy1 was overexpressed using pCM190 *IVY1*. Cells were stained with FM 4-64. Scales 5 μm.

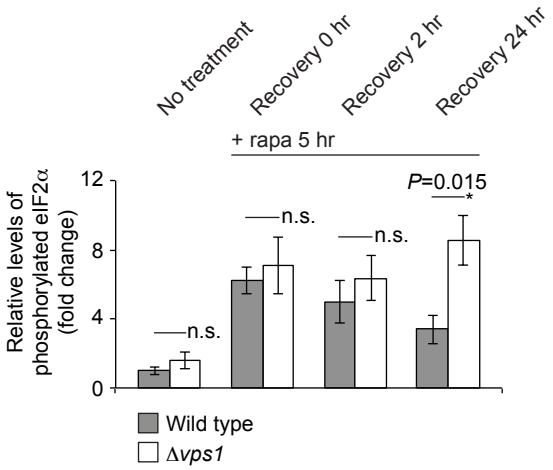
A



B



C



D

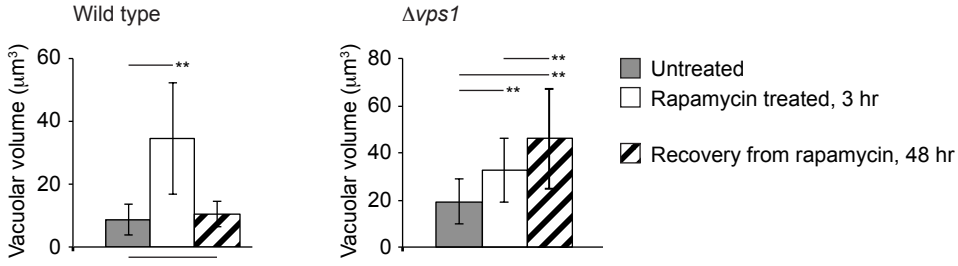
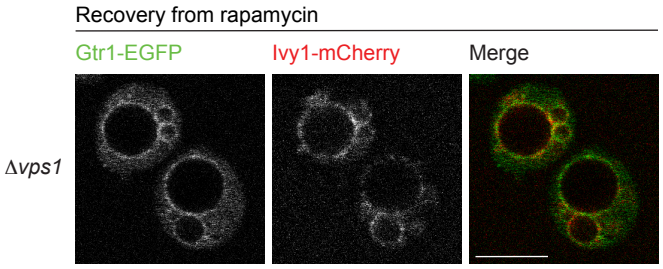
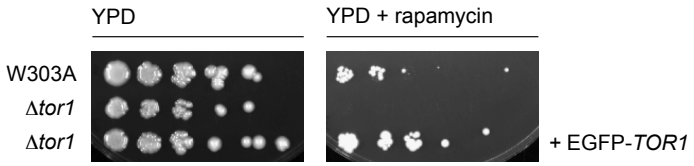


Figure S5. Δ*vps1* cells are defective in recovery from rapamycin exposure. A) W303A or Δ*vps1* cells were stained with FM 4-64 prior to visualization by confocal microscopy. Where indicated, cells were treated with rapamycin (200 ng/ml) for 3 hr, followed by washing and recovery in YPD. Representative images are shown. Scale 5 μm. B) Western blot analysis of levels of eIF2α and its phosphorylated form eIF2α S51 in W303A and Δ*vps1* cells after treatment with rapamycin (200 ng/ml) for 5 hr at 30 °C. Cells were washed and recovered for the indicated periods of time. A representative blot is shown. C) Quantification of the data presented in B). Ratios of phosphorylated to total eIF2α for each measurement were normalized to the mean ratio of phosphorylated to total eIF2α for untreated W303A cells. Two-sample *t*-tests were performed to assess the significance of differences between means for the W303A and Δ*vps1* cells for each condition (mean ± s.e.m. for 5 independent experiments). D) W303A or Δ*vps1* cells expressing genomically-integrated Vph1-EGFP as an established vacuolar membrane marker were treated as indicated prior to imaging by confocal microscopy. Vacuolar volumes were determined as described in Methods. Mean vacuolar volumes were analyzed by one-way ANOVA (W303A: $F_{2,130}$ = 83.2, P = 1.1E-16; Δ*vps1*: $F_{2,98}$ = 26.0, P = 9.E-10). Pairs of means not significantly different from one another (Tukey-Kramer *post-hoc* test) are indicated with horizontal lines below the graph (P > 0.05). Pairs of means that are significantly different from one another are indicated above the graph (**, P < 0.01). n.s. – not significant.

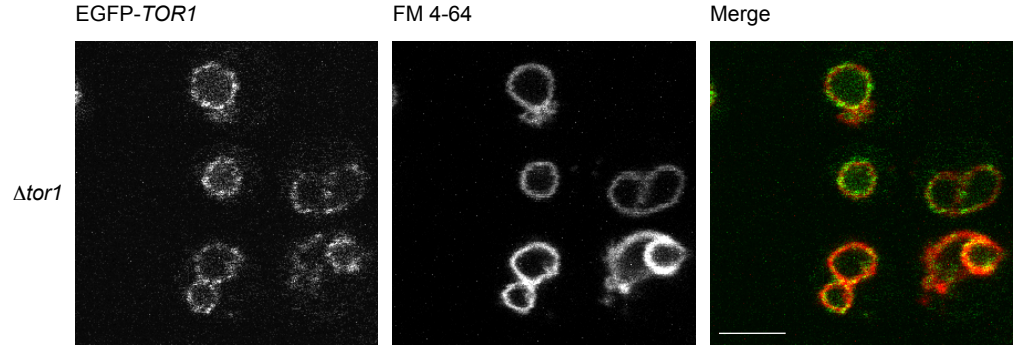
A



B



C



D

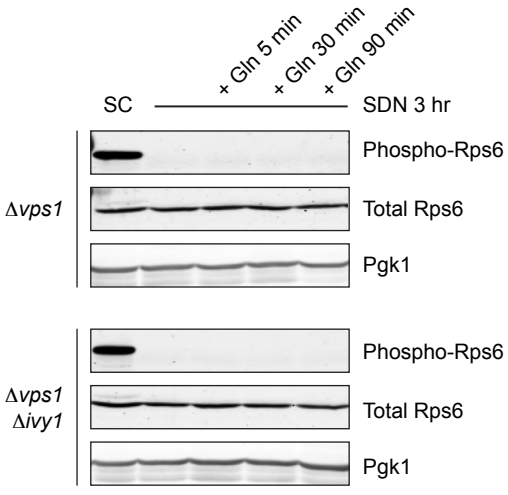


Figure S6. A) Ivy1 does not separate from Gtr1 in Δ*vps1* cells recovering from exposure to rapamycin. Δ*vps1* cells expressing Ivy1-mCherry and Gtr1-EGFP were treated with rapamycin (200 ng/ml) for 3 hr, washed and recovered in YPD for 1 hr prior to visualization. Representative cells are shown. B) W303A, Δ*tor1* or Δ*tor1* cells expressing EGFP-Tor1 were plated in serial dilution on YPD (left) or YPD supplemented with 2.5 ng/ml rapamycin (right) and were incubated at 30 °C for 3 days. The left-most spot in each case corresponds to 2 μl of a culture with OD_{600} 0.5. Spots to the right of this correspond to 2 μl of sequential 5-fold dilutions. C) EGFP-Tor1 localizes to vacuolar when expressed in Δ*tor1* cells. Cells were grown in SC and vacuolar membranes were labelled with FM 4-64. D) TORC1 is not activated by glutamine in Δ*vps1* or Δ*vps1* Δ*ivy1* cells. Phosphorylation levels of Rps6 in W303A or Δ*ivy1* cells were evaluated under the indicated conditions. Untreated cells were grown in SC. Cells were nitrogen-starved by incubating in SD –N for 3 hr. For stimulation, cells were treated with SD –N supplemented with glutamine (gln, 3 mM) for the indicated times prior to lysis and processing. Pgk1 and total Rps6 were used as loading control. Representative blots are shown. Scales 5 μm.