Supporting Information

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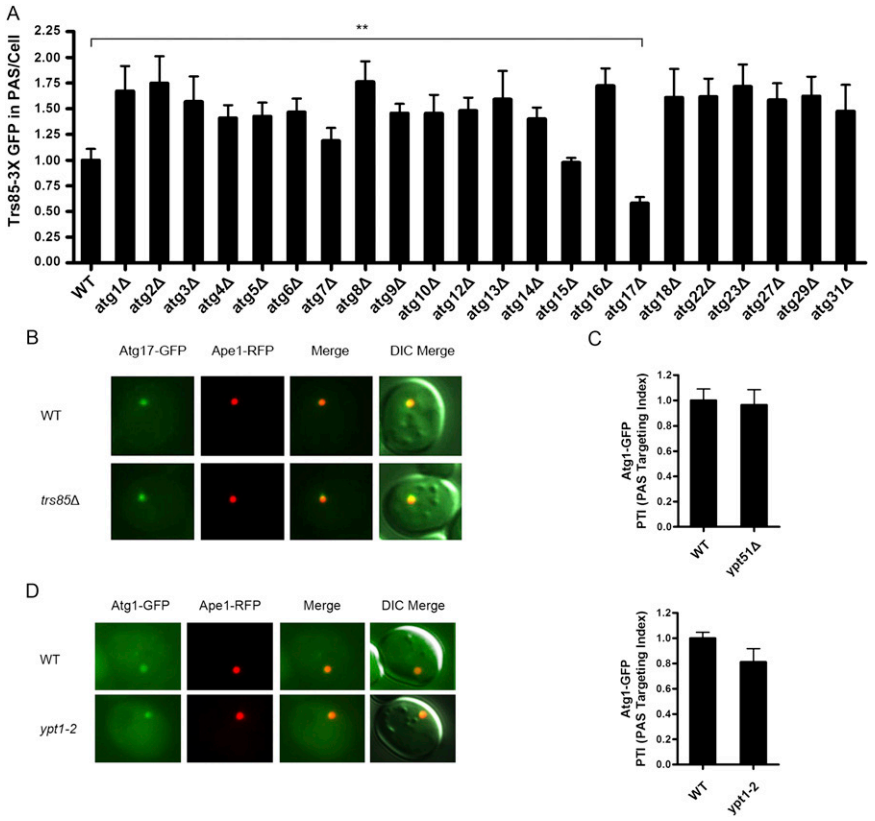

Fig. S1. Screen of the *atg* mutants for defects in the localization of Trs85–3XGFP to the preautophagosomal structure (PAS). (*A*) Known *atg* mutants defective in macroautophagy were screened for defects in the recruitment of Trs85–3XGFP to the PAS. Wild-type and *atg* mutants expressing Trs85-3× GFP and amino peptidase I fused to Red fluorescent protein (ApeI-RFP) were grown to log phase in synthetic complete (SC)-Leu medium. The cells were pelleted, resuspended in synthetic minimal medium lacking nitrogen (SD-N), and incubated for 4 h before they were examined by fluorescence microscopy. The ratio of the Trs85–3XGFP signal that resides at the PAS divided by the Trs85–3XGFP signal of the whole cell was calculated in 15 cells. The ratio in wild-type was set at 1.00. Error bars represent SEM, $n = 45$ cells from three separate experiments. (*B*) Wild-type and *trs85*Δ cells expressing Atg17–GFP and Ape1–RFP were grown to log phase in SC-Leu medium and then shifted to SD-N medium as in *A*. (*C*) Wild-type and *ypt51*Δ cells expressing Atg1–GFP and Ape1–RFP were grown in SC-Leu medium and then shifted to SD-N medium as in *A*. The PAS targeting index (PTI) in wild-type was set at 1.00. Error bars represent SEM, $n = 150$ cells from three separate experiments. (*D*) The localization of Atg1–GFP to the PAS is not significantly disrupted when the *ypt1–2* mutant is grown in nutrient-rich conditions. Wild-type and *ypt1–2* cells expressing Atg1–GFP and Ape1–RFP were grown to log phase in nutrient-rich conditions. The PTI in wild-type was set at 1.00. Error bars represent SEM, $n = 150$ cells from three separate experiments. The slight decrease observed in the *ypt1–2* mutant is not statistically significant.

A

Gene name	Subgroup	Macroautophagy	Cvt
ATG1	Atg1 kinase and its regulators	+	+
ATG13	Atg1 kinase and its regulators	+	+
ATG17	Atg1 kinase and its regulators	+	-
ATG29	Atg1 kinase and its regulators	+	-
ATG31	Atg1 kinase and its regulators	+	-
ATG2	Atg2-Atg18 complex	+	+
ATG18	Atg2-Atg18 complex	+	+
ATG3	Atg8 conjugation system	+	+
ATG4	Atg8 conjugation system	+	+
ATG8	Atg8 conjugation system	+	+
ATG7	Atg8 conjugation system and Atg12 conjugation system	+	+
ATG5	Atg12 conjugation system	+	+
ATG10	Atg12 conjugation system	+	+
ATG12	Atg12 conjugation system	+	+
ATG16	Atg12 conjugation system	+	+
ATG14	PI3K complex	+	+
VPS15	PI3K complex	+	+
VPS30	PI3K complex	+	+
VPS34	PI3K complex	+	+
ATG9	Atg9	+	+

B

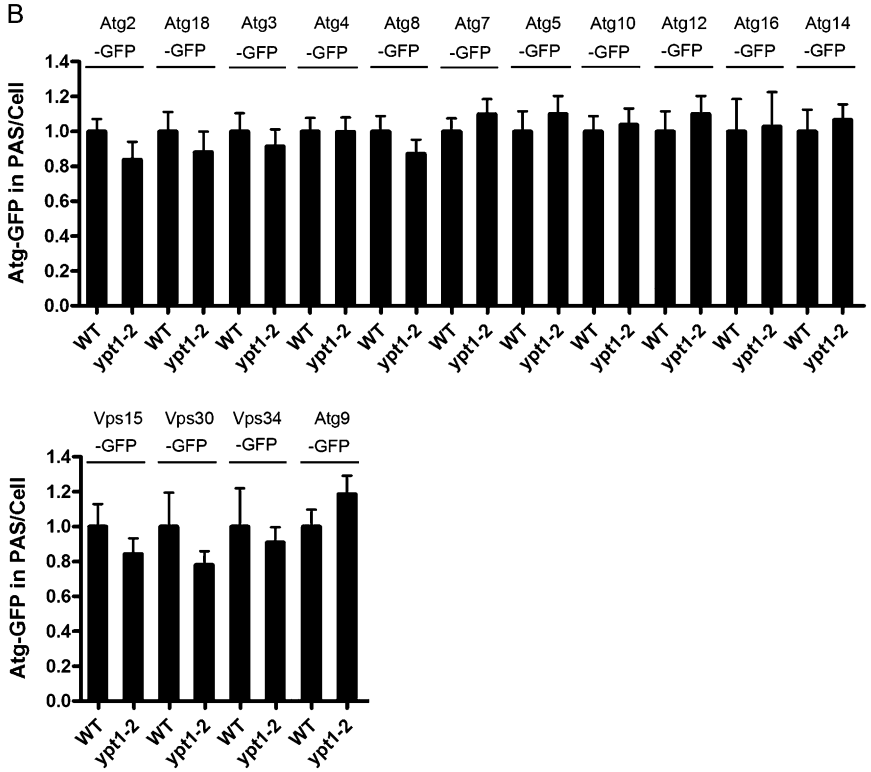

Fig. S2. Screen of Atgs for defects in PAS recruitment in the *ypt1–2* mutant. (*A*) The table lists all known Atg proteins and three Vps proteins that associate with Atg14. Also listed is the subgroup in which the Atg functions, and whether it acts in macroautophagy and/or the cytoplasm to vacuole targeting (Cvt) pathway (*4*). (*B*) Wild-type and *ypt1–2* cells expressing GFP-tagged Atg proteins and Ape1–RFP were grown to log phase and shifted to SD-N medium for 4 h before they were examined by fluorescence microscopy. Fifteen cells were used to calculate the ratio of the GFP signal that resides at the PAS by the GFP signal of the whole cell. The ratio in wild-type was set at 1.00. Error bars represent SEM, $n = 15$ cells.

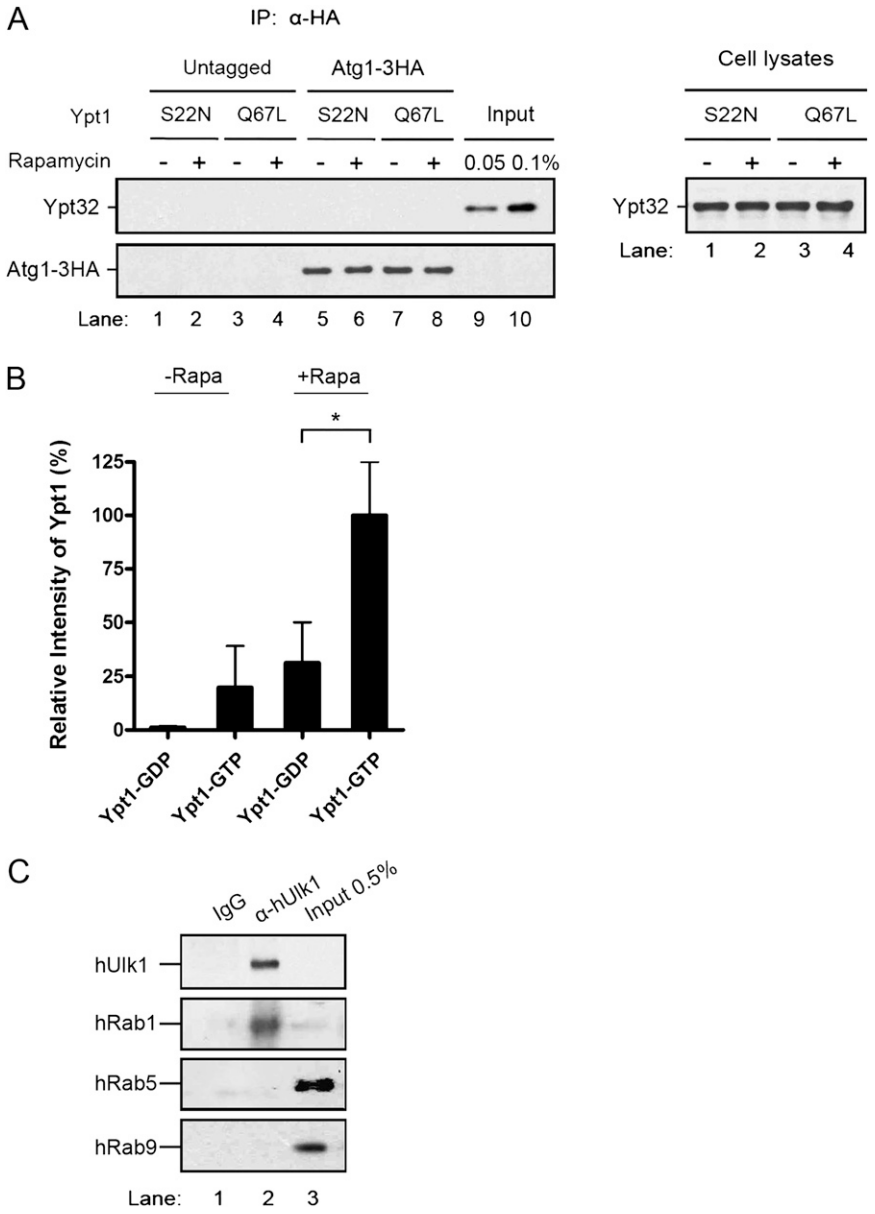

Fig. S3. Atg1–HA does not coprecipitate with Ypt32. (*A*, *Left*) Cells expressing the GTP (Q67L) or GDP (S22N) form of Ypt1 were converted to spheroplasts and treated with or without rapamycin for 30 min. The lysates were immunoprecipitated with HA affinity matrix and immunoblotted with anti-Ypt32 and anti-HA antibodies. (*Right*) Lysates were immunoblotted with anti-Ypt32 antibody. (*B*) Atg1–HA preferentially coprecipitates with the GTP form of Ypt1. Cells expressing the GTP or GDP form of Ypt1 were converted to spheroplasts, treated with or without rapamycin, and processed as in Fig. 5*B*. The data from three separate experiments are graphed. Error bars represent SD, $n = 3$. *$P < 0.05$ Student *t* test. A statistically significant difference was only seen in samples treated with rapamycin. (*C*) Rab1, but not Rab5 (Santa Cruz Biotechnology) or Rab 9, coprecipitate with Ulk1 from HeLa cell lysates.