Supporting Information

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SI Materials and Methods

Strains, Plasmids, and Reagents. Strains used in this paper are summarized in Table S1.

Yeast strain construction. *ATG11* was tagged on the chromosome with triple hemagglutinin (3×HA) at the COOH terminus in NSY125 strain as described previously (1). Gene deletions were done as described previously (2). *TRS85* was tagged on the chromosome with yEGFP, and *ATG9* was tagged on the chromosome with mCherry at their COOH terminus in relevant strains as described previously (3).

Plasmid construction. Plasmids used in this study are summarized in Table S2. For recombinant protein interaction experiments, the Atg11 coiled-coil domain (CC)2-3 peptide (amino acids 626–859) was cloned into multiple cloning site 1 (MCS1) of pCDFDuet-1 (EMD Chemicals) in frame with the 6×His tag.

Fusion proteins: To create Atg11-Ypt1/Ypt1-1 fusion constructs, the ClaI–BamHI genomic DNA fragment containing the *YPT1* ORF was cloned into pRS315. The NdeI site was introduced just upstream of the *YPT1* ORF by site-directed mutagenesis. This plasmid was used to introduce T40K mutation. The *ATG11* ORF plus the AASS linker was cloned into the NdeI site, resulting in pRS315-Atg11-Ypt1/Ypt1-1 constructs. Later these constructs were subcloned into pRS317 using the NotI and SalI/PspXI restriction sites. To make *ATG11* expressed under the control of the *YPT1* promoter, we replaced the PstI fragment of pRS315-Atg11-Ypt1 with the PstI fragment from pGBDU-C2-*ATG11*, thus putting a stop codon after the *ATG11* ORF and removing the first 65 amino acids of Ypt1 from the construct as well.

Plasmids used in live-cell microscopy: To construct the yEGFP-tagged versions of Atg11, Atg8, and Atg1 expressed under the *ADH1* promoter, we first replaced the VF1 coding fragment in p416-VF1 (4) with yEGFP amplified from pKT127 [received from the European *Saccharomyces cerevisiae* Archive for Functional Analysis (EUROSCARF) (5)], using the BstXI and BspEI sites. We then used the construct obtained to clone the appropriate protein-coding sequence in frame with yEGFP. To make the mCherry-Atg8 chimera expressed under the *ADH1* promoter, we first replaced the VF1 coding fragment in p416-VF1 with mCherry amplified from pBS34 [obtained from the Yeast Resource Center (6)], using the XbaI and BspEI sites, and then cloned *ATG8* in frame with mCherry. Later the whole fragment, including the *ADH1* promoter, mCherry, *ATG8*, and *CYC1* terminator, was subcloned into pRS41H (7) using the SacI and KpnI sites.

Plasmids used in bimolecular fluorescence complementation (BiFC): The constructs used for multicolor BiFC include the NH2-terminus of yEVenus (172 amino acids)–Atg11, the Trs85-NH2 terminus of Cerulean (172 amino acids), and the COOH terminus of yECFP (amino acids 155–238)–Ypt1/ypt1-1/ypt1Q67L. All the constructs were expressed under the control of the *ADH1* promoter and *CYC1* terminator and included either a GGGG (Atg11 and Ypt1/ypt1-1/ypt1Q67L) or a GGGS (Trs85) linker. The plasmids carrying the constructs were pRS416, pRS415, and pRS413, respectively. The fragment length was selected in accordance with previously published methods (8). To make expression constructs for the YFP-*N*-Atg11, YFP-*N*-Atg1, YFP-*N*-Atg11ΔCC2, and YFP-*N*-Atg11ΔCC3 chimeras, the VF1 coding fragment in p416-VF1 was replaced by the fragment encoding the 172 NH2-terminal amino acids of yEVenus amplified from pKT103 [received from EUROSCARF (5)] using the SpeI/XbaI and BspEI sites. Then *ATG11* or *ATG1* was cloned downstream and in frame with this fragment, or *ATG11*ΔCC2 and *ATG11*ΔCC3 were subcloned from pNS1387 and 1388, respectively. For the Trs85-CFP-N chimera, we first cloned the fragment encoding 172 amino acids of Cerulean amplified from pBS10 [obtained from Yeast Resource Center (9)] into p415-VF2 (4) instead of VF2, using the BspEI and XhoI sites, followed by cloning *TRS85* without the upstream stop codon. To construct Y/CFP-C–tagged Ypt1, ypt1-1, ypt1Q67L, and Y/CFP-C-Atg1, the fragment encoding amino acids 155–238 of yECFP amplified from pKT102 [received from EUROSCARF (5)] was inserted into p416-VF1 using the SpeI/XbaI and BspEI restriction sites and replacing VF1. Then the piece containing the *ADH1* promoter, amino acids 155–238 of yECFP, and the *CYC1* terminator was subcloned into pRS413 using PvuII. Finally Ypt1, *ypt1-1*, ypt1Q67L, or Atg1 was cloned downstream of and in frame with amino acids 155–238 of yECFP. For the Atg19-Y/CFP-C construct, *ATG19* was cloned into p415-VF2.

All chemical reagents were purchased from Fisher Scientific, except for the following: Media components, other than amino acids, were purchased from US Biological; ProtoGel for Western blots from National Diagnostics; amino acids, GDP, and GTPγS, p-nitrophenyl phosphate, and protease inhibitors from Sigma-Aldrich; glutathione Sepharose 4B beads from Amersham Biosciences; EDTA-free protease inhibitor mixture from Roche Diagnostics; glass beads from BioSpec Products; restriction enzymes and buffers from New England BioLabs; isopropyl-β-D-thiogalactopyranoside (IPTG) from ACROS Organics; and DTT from Invitrogen.

Antibodies used in this study included rabbit anti–GAL4-AD, rabbit anti–GAL4-BD, mouse monoclonal anti-GFP (Santa Cruz Biotechnology); mouse monoclonal anti-HA (Covance); rabbit anti–glucose-6-phosphate dehydrogenase (G-6-PDH; Sigma-Aldrich), rabbit anti-GST (Molecular Probes), mouse monoclonal anti-HIS (R&D Systems; goat anti-rabbit HRP and goat anti-mouse HRP (GE Healthcare); anti-ApeI (10); and affinity purified rabbit anti-Ypt1 (11).

Yeast Culture Conditions and Viability Analysis. For yeast two-hybrid assays (Fig. 1 and Fig. S1), haploid cells were transformed with the relevant activation domain (AD) (pACT2) and binding domain (BD) (pGBDU-C2) plasmids and were mated. Diploid cultures were grown overnight at 26 °C in minimal synthetic dextrose medium, normalized to the same density by OD_{600}, and spotted onto agar plates in serial dilutions. Plates for the yeast two-hybrid assay, indicated in figure legends, were incubated at 26 °C. Medium preparation and yeast culture growth for nitrogen-starvation shift experiments were done as described (12). Growth of the diploids is shown on synthetic dextrose-Ura-Leu plates, whereas interaction is shown on synthetic dextrose-Ura-Leu-His plates; one or two 10-fold dilutions are shown from top to bottom. Empty AD and BD plasmids (Φ) served as negative controls for interaction.

Protein Level Analyses. To determine the expression level of yeast two-hybrid constructs, overnight cell cultures ($4.5 \times OD_{600}$) were spun down, resuspended in 100 μL of Laemmli buffer, boiled, vortexed with an equal volume of glass beads, and subjected to immunoblot analysis with anti–GAL4-AD, anti-HA, or anti–GAL4-BD. To check the level of GFP-tagged proteins or fusion constructs, exponentially growing cell cultures ($7 \times OD_{600}$) were spun down, resuspended in 100 μL of Laemmli buffer, boiled, vortexed with an equal volume of glass beads, and subjected to Western blot analysis with anti-GFP or anti-Ypt1. Preparation of yeast lysates for Ape1- and Atg8-GFP–processing analysis was

done as described (13). Quantification of bands was done using ImageJ software (National Institutes of Health).

Coprecipitation Analyses. Preparation of proteins for coprecipitation: Bacterially expressed GST-Ypt1, GST-Ypt1-1, or GST (as a negative control) proteins were expressed from pGEX-KT and purified as previously described (14). GST-tagged proteins were purified on glutathione agarose beads and loaded with GDP or GTP. The beads were incubated with lysates from yeast cells expressing full-length Atg11-3×HA or from bacterial cells expressing His6-tagged Atg11-CC2-3. To prepare lysates from yeast cells expressing Atg11-3×HA, cells (200 × OD_{600}) were spun down, washed twice with ice-cold water, resuspended in 10 mL of buffer containing 100 mM Tris·HCl (pH 9.4) and 20 mM DTT, and incubated for 15 min at 30 °C. Then cells were spun down, resuspended in 4 mL oxaliticase buffer [1 M sorbitol, 50 mM Na_2PO_4 (pH 7.4), 0.05 mg/mL oxaliticase], and incubated for 30 min at 30 °C with gentle mixing. Spheroplasts were pelleted by centrifugation at 4,000 × *g* for 5 min and were resuspended gently in 3 mL of ice-cold lysis buffer [20 mM Hepes (pH 6.8), 150 mM KOAc, 4 mM MgOAc, 250 mM sorbitol, 0.2% Triton X-100, 1 mM PMSF, and complete protease inhibitor mixture]. Protein lysate was aliquoted and stored at −80 °C. To prepare recombinant His-Atg11 CC2-3, BL21 cells were transformed with pCDF-Duet-1-Atg11 CC2-3, induced with 0.4 mM IPTG at OD_{600} 0.6–0.8 for 4 h, collected by centrifugation, resuspended in lysis buffer [50 mM PBS (pH 7.5), 1 mM PMSF, 0.5% Triton X-100], and lysed by sonication.

Coprecipitation of Atg11-3×HA or His-Atg11 CC2-3 with GST-Ypt1 or GST-Ypt1-1 was done as previously described (15), using GTP-γ-S instead of GTP. After precipitation, the levels of GST, GST-Ypt1, and GST-Ypt1-1 were determined by immunoblot analysis using anti-GST antibody, and the level of Atg11-HA or His6-tagged Atg11-CC2-3 that coprecipitated with the GST-tagged proteins was determined using anti-HA or anti-His6 antibodies, respectively.

Alkaline Phosphatase Activity Assay. The alkaline phosphatase activity assay of Pho8Δ60 was done as previously described (16).

Microscopy. Live-cell microscopy was done as follows: Wild-type (NSY125) and *ypt1-1* mutant (NSY2) cells carrying constructs for GFP- or mCherry-tagged protein expression were grown to midlog phase in appropriate selective media. Fluorescence microscopy was carried out using a deconvolution Axioscope microscope (Carl Zeiss) with FITC (GFP) and TexasRed (mCherry) filter sets. To visualize protein interactions in BiFC and multicolor BiFC assays, NSY128cells carrying the appropriate expression constructs were grown to midlog phase in appropriate selective media and visualized using a deconvolution Axioscope microscope with the filters optimized for the visualization of YFP and CFP (8). Immunofluorescence microscopy using affinity-purified anti-Ypt1 antibodies was done as previously described (11).

1. Longtine MS, et al. (1998) Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. *Yeast* 14:953–961.
2. Wach A, Brachat A, Pöhlmann R, Philippsen P (1994) New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast* 10:1793–1808.
3. Wach A, Brachat A, Alberti-Segui C, Rebischung C, Philippsen P (1997) Heterologous HIS3 marker and GFP reporter modules for PCR-targeting in *Saccharomyces cerevisiae*. *Yeast* 13:1065–1075.
4. Paquin N, et al. (2007) Local activation of yeast ASH1 mRNA translation through phosphorylation of Khd1p by the casein kinase Yck1p. *Mol Cell* 26:795–809.
5. Sheff MA, Thorn KS (2004) Optimized cassettes for fluorescent protein tagging in *Saccharomyces cerevisiae*. *Yeast* 21:661–670.
6. Shaner NC, et al. (2004) Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. *Nat Biotechnol* 22: 1567–1572.
7. Taxis C, Knop M (2006) System of centromeric, episomal, and integrative vectors based on drug resistance markers for *Saccharomyces cerevisiae*. *Biotechniques* 40: 73–78.
8. Hu CD, Kerppola TK (2003) Simultaneous visualization of multiple protein interactions in living cells using multicolor fluorescence complementation analysis. *Nat Biotechnol* 21:539–545.
9. Rizzo MA, Springer GH, Granada B, Piston DW (2004) An improved cyan fluorescent protein variant useful for FRET. *Nat Biotechnol* 22:445–449.
10. Klionsky DJ, Cueva R, Yaver DS (1992) Aminopeptidase I of Saccharomyces cerevisiae is localized to the vacuole independent of the secretory pathway. *J Cell Biol* 119(2): 287–299.
11. Segev N, Mulholland J, Botstein D (1998) The yeast GTP-binding YPT1 protein and a mammalian counterpart are associated with the secretion machinery. *Cell* 52(6): 915–924.
12. Segev N, Botstein D (1987) The ras-like yeast YPT1 gene is itself essential for growth, sporulation, and starvation response. *Mol Cell Biol* 7(7):2367–2377.
13. Cheong H, Klionsky DJ (2008) Biochemical methods to monitor autophagy-related processes in yeast. *Methods Enzymol* 451:1–26.
14. Jones S, Litt RJ, Richardson CJ, Segev N (1995) Requirement of nucleotide exchange factor for Ypt1 GTPase mediated protein transport. *J Cell Biol* 130(5):1051–1061.
15. Suvorova ES, Duden R, Lupashin VV (2002) *T*he Sec34/Sec35p complex, a Ypt1p effector required for retrograde intra-Golgi trafficking, interacts with Golgi SNAREs and COPI vesicle coat proteins. *J Cell Biol* 157(4):631–643.
16. Abeliovich H, Zhang C, Dunn WA, Jr., Shokat KM, Klionsky DJ (2003) Chemical genetic analysis of Apg1 reveals a non-kinase role in the induction of autophagy. *Mol Biol Cell* 14(2):477–490.

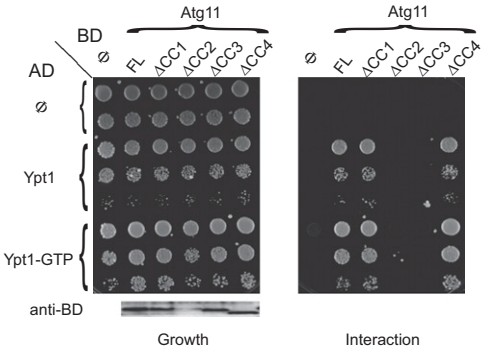


Fig. S1. Yeast-two hybrid interaction with Ypt1 requires CC2 and CC3 of Atg11. Ypt1 and Ypt1-GTP (Q67L) were cloned into the AD vector. Atg11 and mutants missing one of its four CC domains (CC1, CC2, CC3, or CC4) were cloned into the BD vector. Both the wild-type and the GTP-restricted form of Ypt1 interact with Atg11, Atg11ΔCC1, and ΔCC4 but not with Atg11ΔCC2 and ΔCC3 (growth is shown on the left and interaction on the right). (*Bottom Left*) Immunoblot analysis shows expression of the Atg11 proteins. Results are representative of at least two independent experiments.

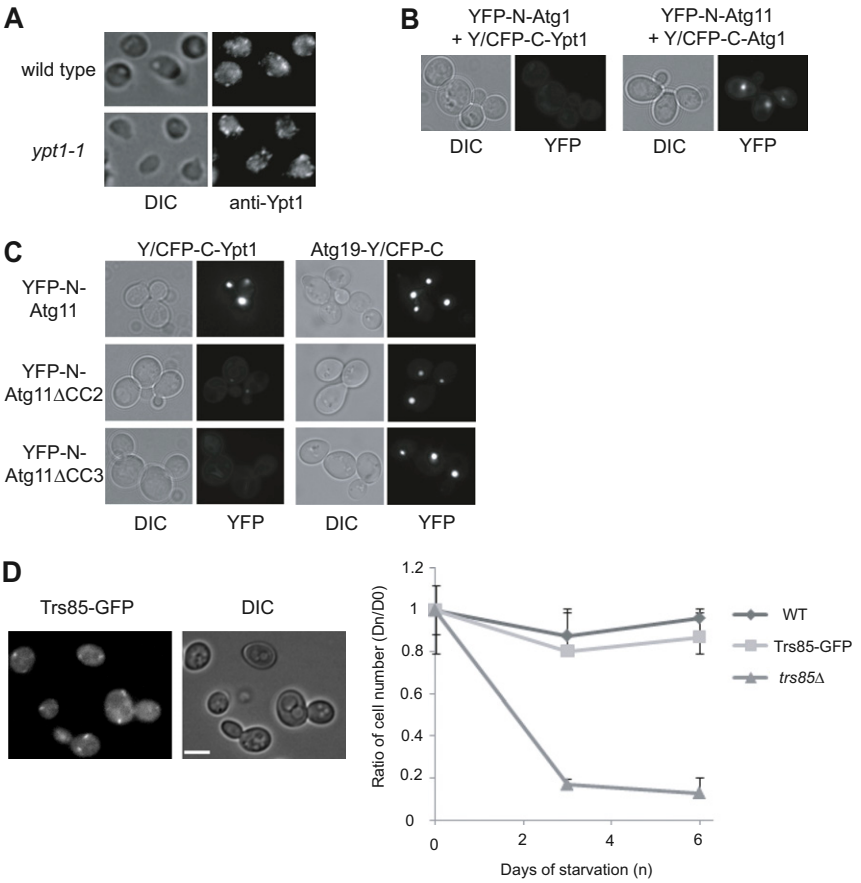
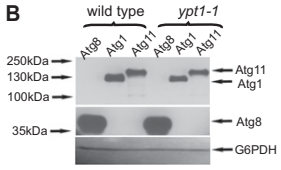


Fig. S2. Ypt1, Trs85, and Atg11 localization and multicolor BiFC controls. (*A*) The intracellular localization of the Ypt1-1 mutant protein is similar to that of the wild type. Ypt1 localization was determined by immunofluorescence microscopy using anti-Ypt1 antibody in wild-type (*Upper*) and *ypt1-1* mutant (*Lower*) cells. DIC, differential interference contrast. (*B*) Atg1 shows a positive protein-fragment complementation assay (PCA) with Atg11 but not with Ypt1. BiFC is seen only for Atg1 and Atg11 (*Right*) but not for Atg1 and Ypt1 (*Left*). (*C*) Atg11-CC2 and Atg11-CC3 are required for BiFC of Atg11 with Ypt1. BiFC with Ypt1 is seen only when YFP-N is tagged to wild-type Atg11 and not to Atg11ΔCC2 or Atg11ΔCC3 (*Left*). In contrast, BiFC with Atg19 is seen for wild-type Atg11, Atg11ΔCC2, and Atg11ΔCC3 tagged with YFP-N (*Right*). (*D*) Localization of Trs85 to multiple puncta. (*Left*) Endogenous Trs85 tagged at its C terminus with GFP localizes to multiple puncta. (Scale bar, 5 μm.) (*Right*) Trs85-GFP is functional. Yeast cells expressing Trs85-GFP from the chromosome are resistant to nitrogen starvation. Yeast cells carrying wild-type *TRS85* (as a positive control), *TRS85*-GFP, or the *trs85*Δ allele (as a negative control) were shifted to medium without nitrogen, and viability was tested at the indicated times. Viability is shown as percent of viability at day zero. Although *trs85*Δ cells are sensitive to nitrogen starvation, cells expressing Trs85-GFP are as resistant as wild-type cells. Error bars represent SD. Results are representative of at least two independent experiments.

A

	measured	strain	# cells	%
GFP-Atg11	> 1dot/cell	wild type	100	15
		ypt1-1	100	71
GFP-Atg8	> 1dot/cell	wild type	100	12
		ypt1-1	100	94
GFP-Atg1	no dot	wild type	100	18
		ypt1-1	100	84

B



C

Ypt/Atg11 plasmid	∅	Ypt1	Atg11-Ypt1	Ypt1-1	Atg11-Ypt1-1	Atg11
% GFP-Atg1 no dot	84	20	40	82	44	86
% GFP-Atg8 > 1dot/cell	94	12	44	94	46	86

50 cells scored for each strain

Fig. S3. Quantification of GFP-tagged Atg11, Atg8, and Atg1 proteins in wild-type and *ypt1-1*–mutant cells. (*A*) Quantification of micrographs used for Fig. 3*A*. (*B*) The level of proteins used for microscopy in Fig. 3*A* was determined by immunoblot analysis using anti-GFP antibody. G6PDH is shown as a loading control. (*C*) Quantification of micrographs used for Fig. 3*C*. Results are representative of at least two independent experiments.

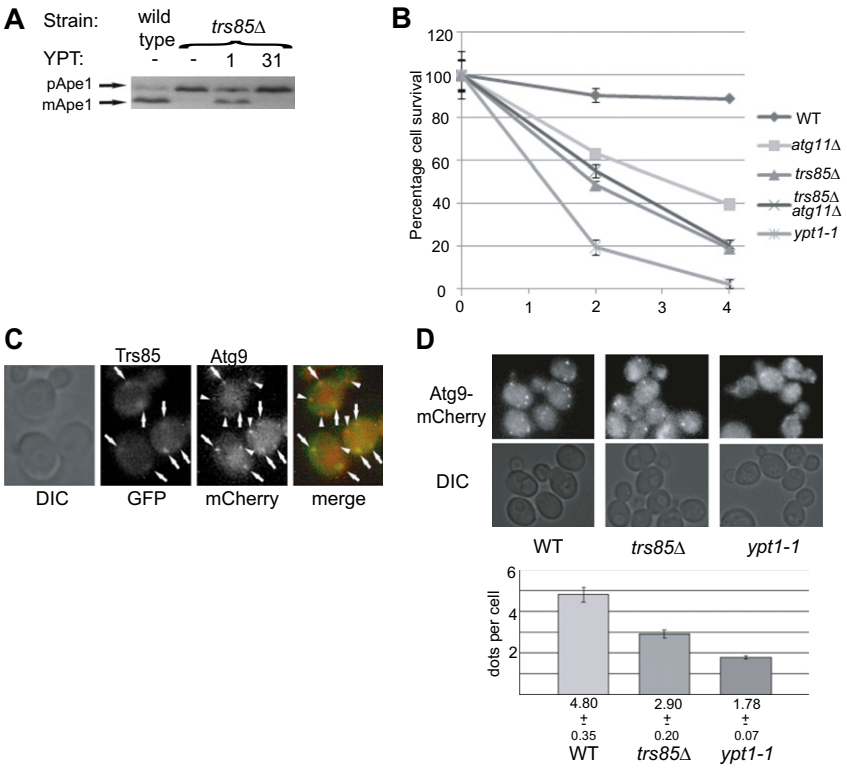


Fig. S4. The role of Ypt1, Trs85, and Atg11 in autophagy. (*A*) Overexpression of Ypt1, but not Ypt31, can suppress the Ape1-processing defect of *trs85*Δ mutant cells. The experiment was done as described in Fig. 4*A*, except that *trs85*Δ cells were transformed with 2μ plasmids: empty plasmid (–), a plasmid overexpressing Ypt1 (1), or Ypt31 (31). (*B*) The growth defect during nitrogen starvation of the *atg11*Δ*trs85*Δ double-deletion mutant cells is not more severe than that of the single-deletion mutants. The viability of wild-type and *atg11*Δ, *trs85*Δ, *atg11*Δ*trs85*Δ, and *ypt1-1* mutant cells was determined before and after the shift to medium without nitrogen. Shown is the percent of live cells 2 and 4 d after the shift compared with the number of cells before the shift. Both *atg11*Δ and *trs85*Δ single-deletion cells exhibit an intermediate nitrogen-starvation growth defect phenotype between that of the wild-type and *ypt1-1* mutant cells. The *trs85*Δ *atg11*Δ double-deletion mutant cells exhibit a growth defect similar to that of *trs85*Δ. (*C*) Trs85-GFP colocalizes with Atg9-mCherry. Endogenous Trs85 and Atg9 were tagged on the chromosome with GFP and mCherry, respectively. Shown from left to right are DIC, GFP, mCherry, and merged images. All the Trs85 puncta (green) puncta (arrowheads) overlap with the Atg9 puncta (red) (arrows). There are more red Atg9 puncta than the green Trs85 puncta. (*D*) The localization pattern of Atg9 is altered in *ypt1-1* and *trs85*Δ mutant cells. (*Upper*) Endogenous Atg9 was tagged on the chromosome with mCherry, and cells were visualized in the mCherry channel. At least 200 cells were visualized for each strain. (*Lower*) The number of Atg9 dots per cell is lower in *trs85*Δ and *ypt1-1* mutant cells than in wild-type cells. Error bars in *B* and *D* represent SD. Results are representative of at least two independent experiments.

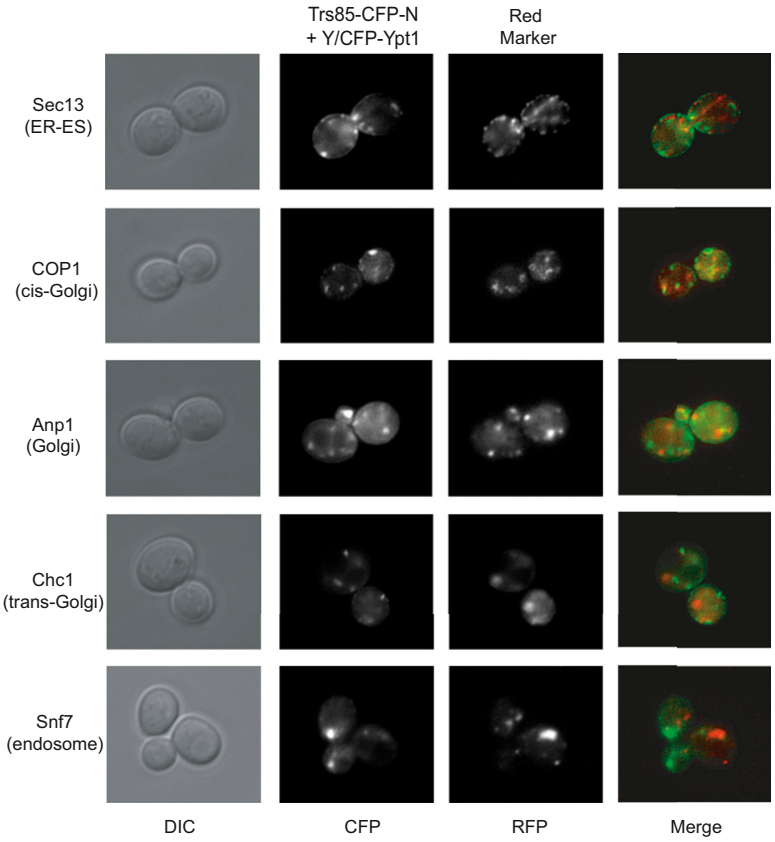


Fig. S5. Trs85 and Ypt1 PCA puncta do not overlap with secretory compartment markers. The experiment was done as described in Fig. 2C, except that the following compartmental markers were tagged on the chromosome with RFP: Sec13-ER-ES (endoplasmic reticulum exit sites), COP1 (*cis* Golgi), Anp1 (Golgi), Chc1 (*trans*-Golgi), and Snf7(endosomes) (1). Cells were visualized in the CFP and RFP channels. The blue (CFP) puncta do not overlap with red (RFP) fluorescence (Merge column), indicating that the Trs85-Ypt1 interaction does not occur in the ER, Golgi, or endosomes. DIC is shown in the far left column. Results are representative of at least two independent experiments.

1. Huh WK, et al. (2003) Global analysis of protein localization in budding yeast. *Nature* 425:686–691.

Table S1. Yeast strains used in this study

Strain	Alias	Genotype	Source
NSY468	PJ69-4A	*MATa trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ GAL2-ADE2 LYS2:GAL1-HIS3 met2::GAL7-lacZ*	(1)
NSY752	PJ69-4α	*MATα trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ gal2-ade2 lys2::gal1-his3 met2::gal7-lacZ*	(1)
NSY125	DBY1034	*MATa his4-539 lys2-801 ura3-52*	(2)
NSY2	*ypt1-1* (DBY1803)	*MATa his4-539 lys2-801 ura3-52 ypt1-T40K*	(3)
NSY128	DBY4975	*Matα ade2 his3Δ200 leu2-3,112 lys2-801 urs3-52*	D. Botstein
NSY825	BY4741	*MATa leu2Δ0 ura3Δ0 his3Δ1met15Δ0*	(4)
NSY1440	*trs85Δ*	NSY825 *TRS85Δ::HYGRO*	This study
NSY1499	*atg11Δ*	NSY825 *ATG11Δ::KAN*	This study
NSY1500	*trs85Δ atg11Δ*	NSY825 *ATG11Δ::KAN TRS85Δ::HYGRO*	This study
NSY1508	*ATG11-3xHA*	NSY125 *ATG11-3xHA::KAN*	This study
NSY1524	*TRS85-yEGFP*	NSY825 *TRS85-yEGFP::KAN*	This study
NSY1523	*ATG9-mCherry*	NSY825 *ATG9-mCherry::HYGRO*	This study
NSY1525	*TRS85-yEGFP,ATG9-mCherry*	NSY825 *TRS85-yEGFP::KAN ATG9-mCherry::HYGRO*	This study
NSY1526	*trs85Δ ATG9-mCherry*	NSY825 *TRS85Δ::KAN ATG9-mCherry::HYGRO*	This study
NSY1527	*ypt1-1 ATG9-mCherry*	NSY55 (*MAα his3-200 ura3-52 leu2-3,112 ypt1-T40K) ATG9-mCherry::KAN*	This study
NSY1528	TN124	*MATa leu2-3,112 trp1 ura3-52 pho8::pho8Δ60 pho13::LEU2*	(5)
NSY1529	*atg1Δ in* TN124	NSY1528 *ATG1Δ::KAN*	Y. Liang
NSY1530	TN124 *trs85Δ*	NSY1528 *TRS85Δ::HYGRO*	This study
NSY1531	TN124 *atg11Δ*	NSY1528 *ATG11Δ::KAN*	This study
NSY1532	TN124 *trs85Δ atg11Δ*	NSY1528 *ATG11Δ::KAN TRS85Δ::HYGRO*	This study

1. James P, Halladay J, Craig EA (1996) Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics* 144:1425–1436.
2. Jedd G, Mulholland J, Segev N (1997) Two new Ypt GTPases are required for exit from the yeast trans-Golgi compartment. *J Cell Biol* 137:563–580.
3. Baker D, Wuestehube L, Schekman R, Botstein D, Segev N (1990) GTP-binding Ypt1 protein and Ca2+ function independently in a cell-free protein transport reaction. *Proc Natl Acad Sci USA* 87:355–359.
4. Brachmann CB, et al. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14:115–132.
5. Noda T, Matsuura A, Wada Y, Ohsumi Y (1995) Novel system for monitoring autophagy in the yeast *Saccharomyces cerevisiae*. *Biochem Biophys Res Commun* 210:126–132.

Table S2. Plasmids used in this study

Plasmid	Alias	Genotype	Source
pNS196	pACT2	2μ, *LEU2*, Amp^r	Clontech
pNS1377		pACT2-*YPT1*	This study
pNS1378		pACT2-*YPT1-T40K*	This study
pNS1375		pACT2-*YPT1-S22N*	This study
pNS1374		pACT2-*YPT1-Q67L*	This study
pNS1376		pACT2-*YPT1-D124N*	This study
pNS206	pGBDU-C2	2μ, *URA3*, Amp^r	(1)
pNS1373		pGBDU-C2-*ATG11*	This study
pNS1385		pGBDU-C2-*ATG11-CC2-3*	This study
pNS1386		pGBDUC1-*ATG11ΔCC1*	This study
pNS1387		pGBDU-C1- *ATG11ΔCC2*	This study
pNS1388		pGBDU-C1- *ATG11ΔCC3*	This study
pNS1389		pGBDU-C1- *ATG11ΔCC4*	This study
pNS1390		pGBDU-C2-*SEC4-Q79L*	This study
pNS1391		pGBDU-C2-*YPT31-Q72L*	This study
pNS1392		pGBDU-C2-*YPT6-Q69L*	This study
pNS1348	pBS10	Cerulean-*HYGRO*, Amp^r	(2)
pNS1320	pBS34	mCherry-*KAN*, Amp^r	(3)
pNS719	pRS317	*CEN*, LYS2, Amp^r	(4)
pNS243	pRS313	*CEN*, HIS3, Amp^r	(5)
pNS245	pRS315	*CEN*, LEU2, Amp^r	(5)
pNS1359		p416-yEGFP-*ATG11*	This study
pNS1360		p416-yEGFP-*ATG8*	This study
pNS1361		p416-yEGFP-*ATG1*	This study
pNS1340		p416-VF1	(6)
pNS1341		p415-VF2	(6)
pNS1362		p416-mCherry-*ATG8*	This study
pNS1364		pRS315-*YPT1*	This study
pNS1365		pRS315-*YPT1-T40K*	This study
pNS1366		pRS315-*ATG11-YPT1*	This study
pNS1367		pRS315-*ATG11-YPT1-T40K*	This study
pNS1368		pRS317-*YPT1*	This study
pNS1369		pRS317-*YPT1-T40K*	This study
pNS1370		pRS317-*ATG11-YPT1*	This study
pNS1371		pRS317-*ATG11-YPT1-T40K*	This study
pNS1372		pRS317-*YPT1* promoter-*ATG11*- -*YPT1* terminator	This study
pNS1380		p413-Y/CFP C-*YPT1*	This study
pNS1381		p413-Y/CFP C-*YPT1-T40K*	This study
pNS1382		p413-Y/CFP C-*YPT1-Q67L*	This study
pNS1383		p416-YFP *N-ATG11*	This study
pNS1384		p415-*TRS85*-CFP N	This study
pNS1412		p416-YFP *N-ATG11ΔCC2*	This study
pNS1413		p416-YFP *N-ATG11ΔCC3*	This study
pNS1409		p415-*ATG19*-Y/CFP C	This study
pNS1410		p416-YFP *N-ATG1*	This study
pNS1411		p413-Y/CFP C-*ATG1*	This study
pNS274	YEp24	2μ, *URA3*, Amp^r	New England Biolabs, MA
pNS489		YEp24-*YPT1*	(7)
pNS229		YEp24-*YPT31*	(8)

1. James P, Halladay J, Craig EA (1996) Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics* 144:1425–1436.
2. Rizzo MA, Springer GH, Granada B, Piston DW (2004) An improved cyan fluorescent protein variant useful for FRET. *Nat Biotechnol* 22:445–449.
3. Shaner NC, et al. (2004) Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. *Nat Biotechnol* 22:1567–1572.
4. Eriksson P, Thomas LR, Thorburn A, Stillman DJ (2004) pRS yeast vectors with a LYS2 marker. *Biotechniques* 36:212–213.
5. Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* 122:19–27.
6. Paquin N, et al. (2007) Local activation of yeast ASH1 mRNA translation through phosphorylation of Khd1p by the casein kinase Yck1p. *Mol Cell* 26:795–809.
7. Morozova N, et al. (2006) TRAPPII subunits are required for the specificity switch of a Ypt-Rab GEF. *Nat Cell Biol* 8:1263–1269.
8. Jones S, et al. (1999) Genetic interactions in yeast between Ypt GTPases and Arf guanine nucleotide exchangers. *Genetics* 152:1543–1556.