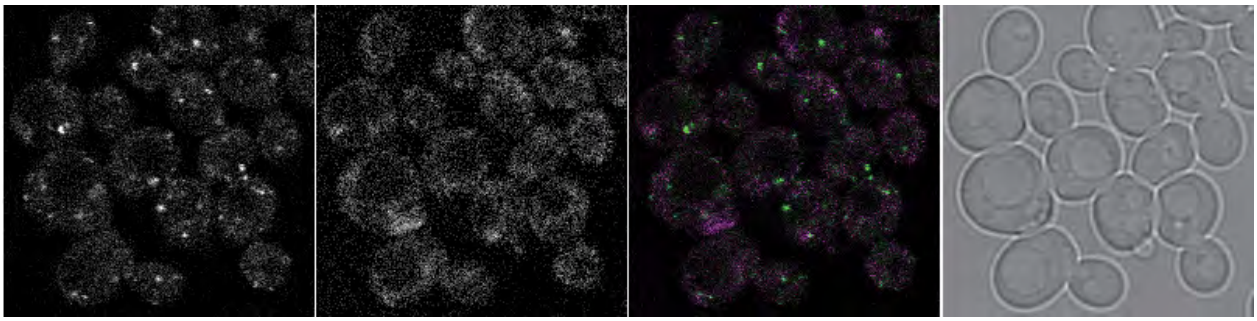
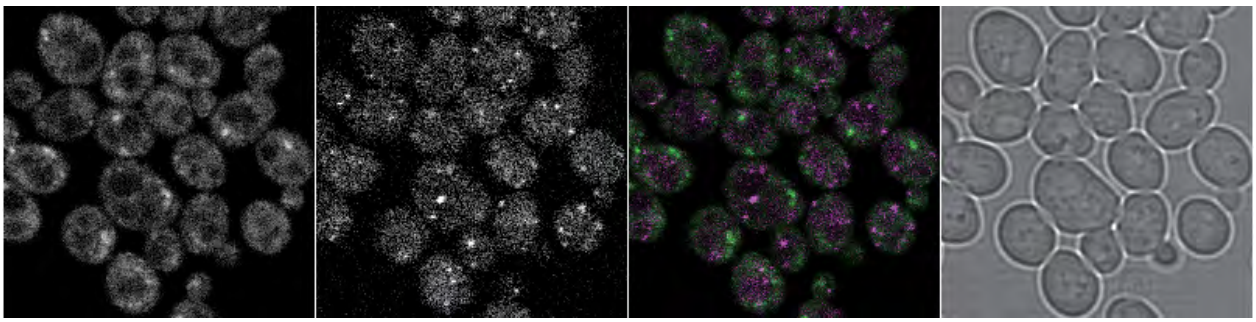
A



B



Supplemental figure 1. A part of Atg9 resides with TRAPPIII and Ypt1.

A. Wild-type cells harboring chromosomally tagged Atg9-3×GFP and Trs85-3×mCherry were incubated in YPD at 30˚C. Logarithmic growing cells were observed using Leica TCS SP8 microscope. B. Wild-type cells harboring pKN38 (GFP-Ypt1) and chromosomally tagged Atg9-3×mCherry were incubated in YPD at 30˚C. Logarithmic growing cells were observed using Leica TCS SP8 microscope.

Supplemental figure 1

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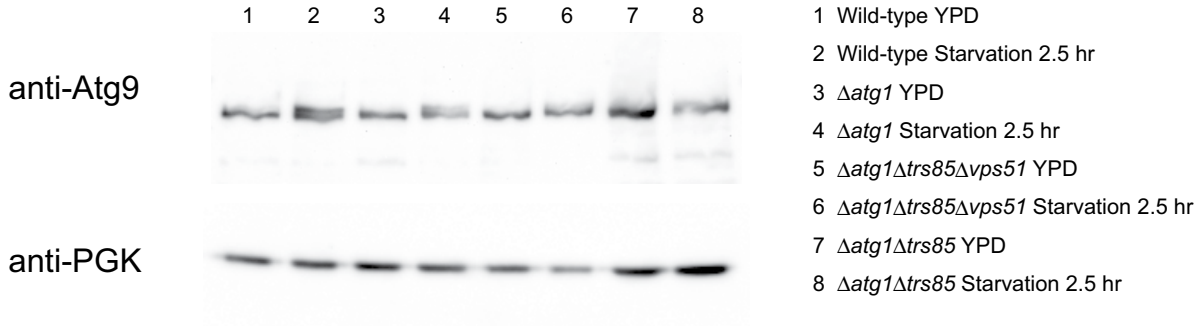

Supplemental figure 2. Atg9 protein levels are unaffected by the endosomal mutations. Wild-type, atg1Δ, atg1Δtrs85Δvps51Δ, and atg1Δtrs85Δ cells were incubated at 25˚C in YPD, shifted to the nitrogen starvation, and harvested at the indicated time points. Lysates were prepared by the glass beads lysis method, and subjected to SDS-PAGE, followed by immunoblotting for Atg9 and PGK.

Supplemental figure 2
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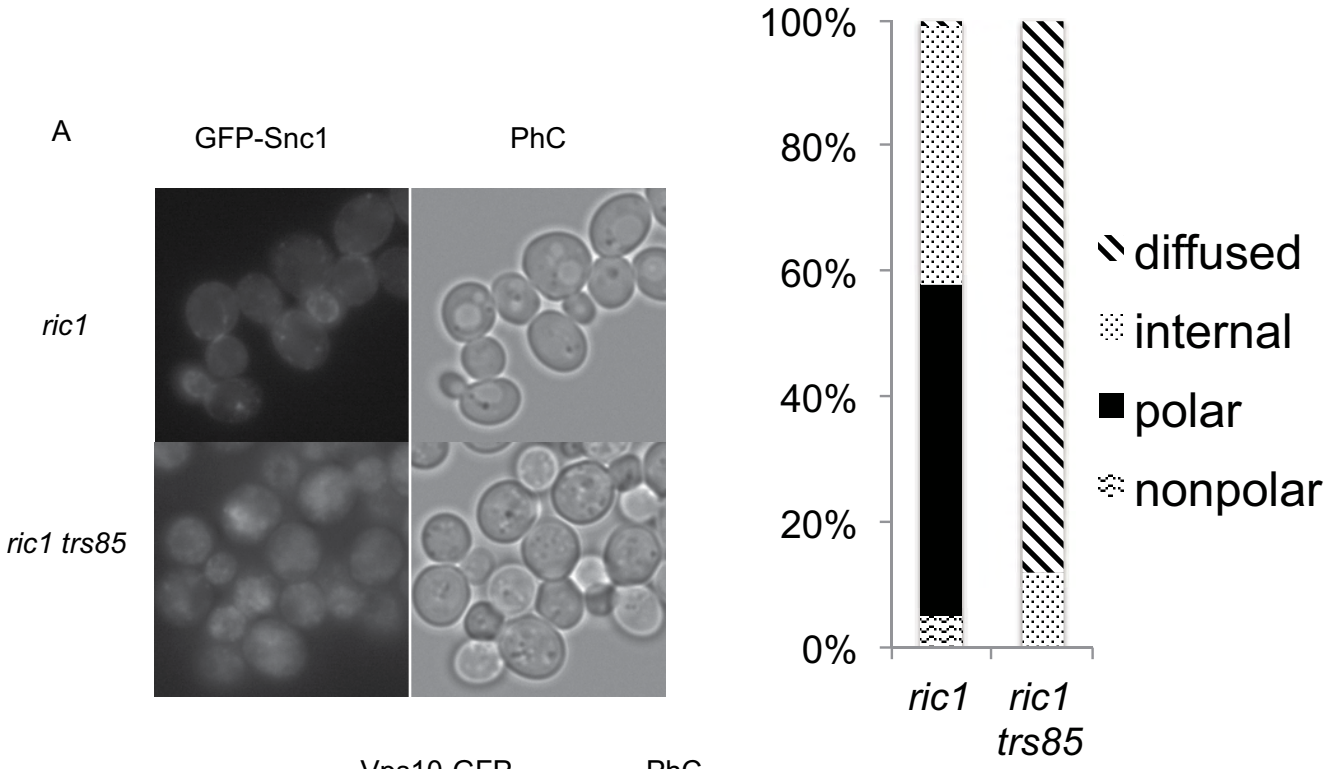

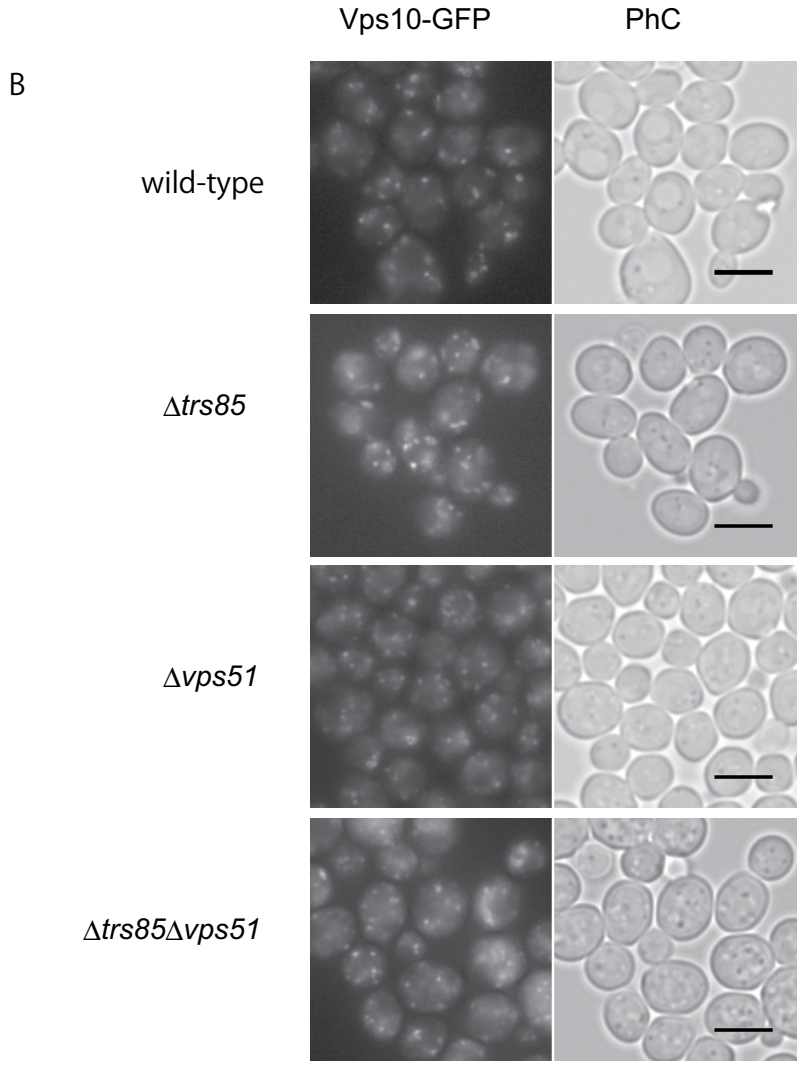

Supplementa figure 3. Endosome-to-Golgi trafficking is disrupted by a combination of TRAPPIII and GARP complex mutations. A. Strains (KNY351 and 352) expressing GFP-Snc1 were cultured at 26˚C in YPD and GFP-derived signals were observed using Leica DIM6000B microscope. The cells were categorized into four groups as described in Figure 6 and relative size of each population was determined (n > 400 cells). Diffused, GFP signals were diffusely located throughout the cell. Internal, GFP signals were observed as internal puncta. Polar, in addition to internal puncta, GFP signals were observed on the plasma membrane of a bud. Nonpolar, in addition to a bud, GFP signals were observed on the plasma membrane of the mother cell. Representative results from two independent experiments are shown. B. Strains (KNY353, 354, 356 and 357) harboring chromosomally tagged Vps10 were cultured at 26˚C in YPD and Vps10-GFP signals were observed using Leica DIM6000B microscope. Bars indicate 5 μm.

Supplemental figure 3
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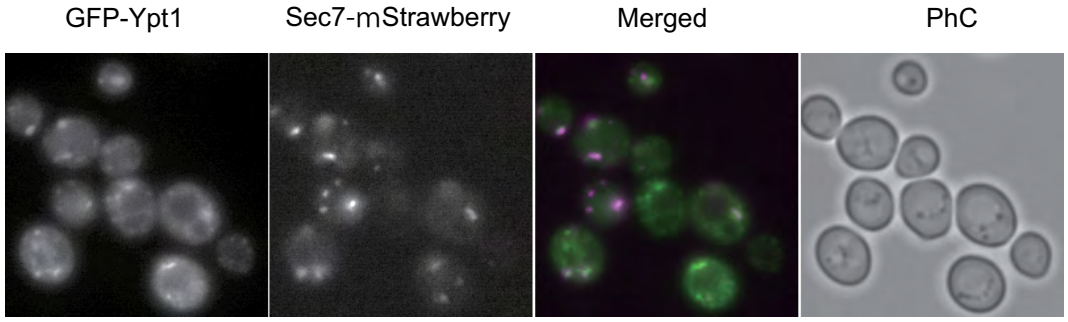
A



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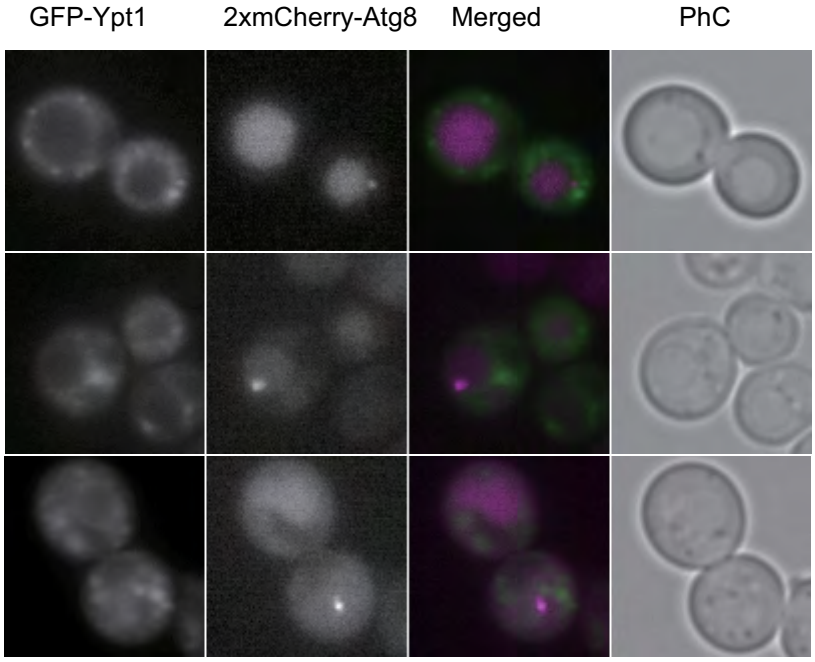

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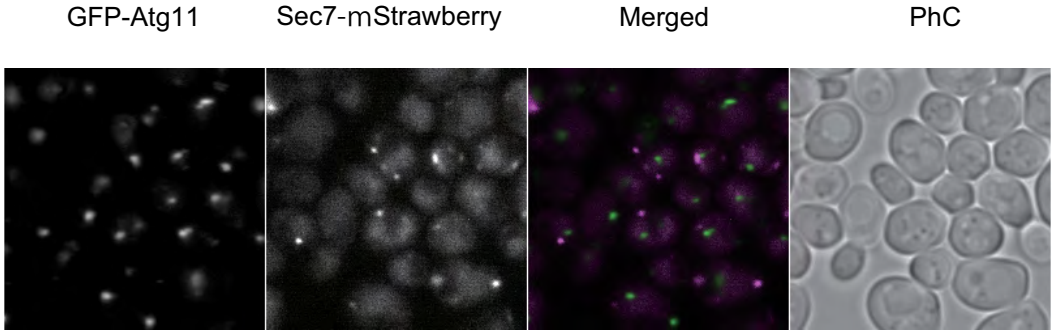

Supplemental figure 4. Localization of Ypt1 and Atg11 with organelle markers

A. Wild-type strain harboring pKN38 (GFP-Ypt1) and chromosomally tagged Sec7-mStrawberry was incubated in YPD and observed using Leica DIM 6000B microscope. Note that approximately 40-50% of signals were colocalized. B. Wild-type strain harboring pKN38 (GFP-Ypt1) and pRS314-2×mCherry-Atg8 was incubated in SCD and observed using Leica DIM6000B microscope. C. Wild-type strain harboring chromosomally tagged GFP-Atg11 and Sec7-mStrawberry was incubated in YPD and observed using Leica DIM 6000B microscope.

Supplemental figure 4

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