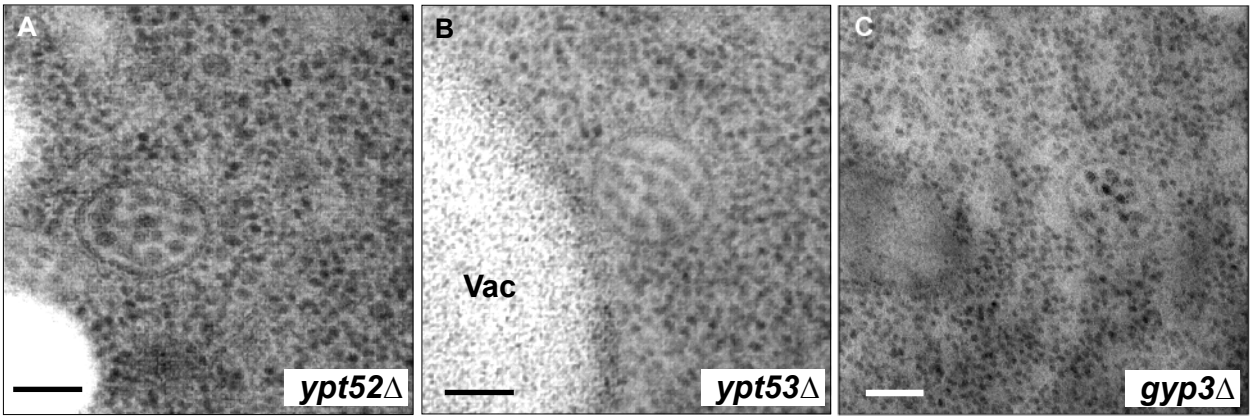
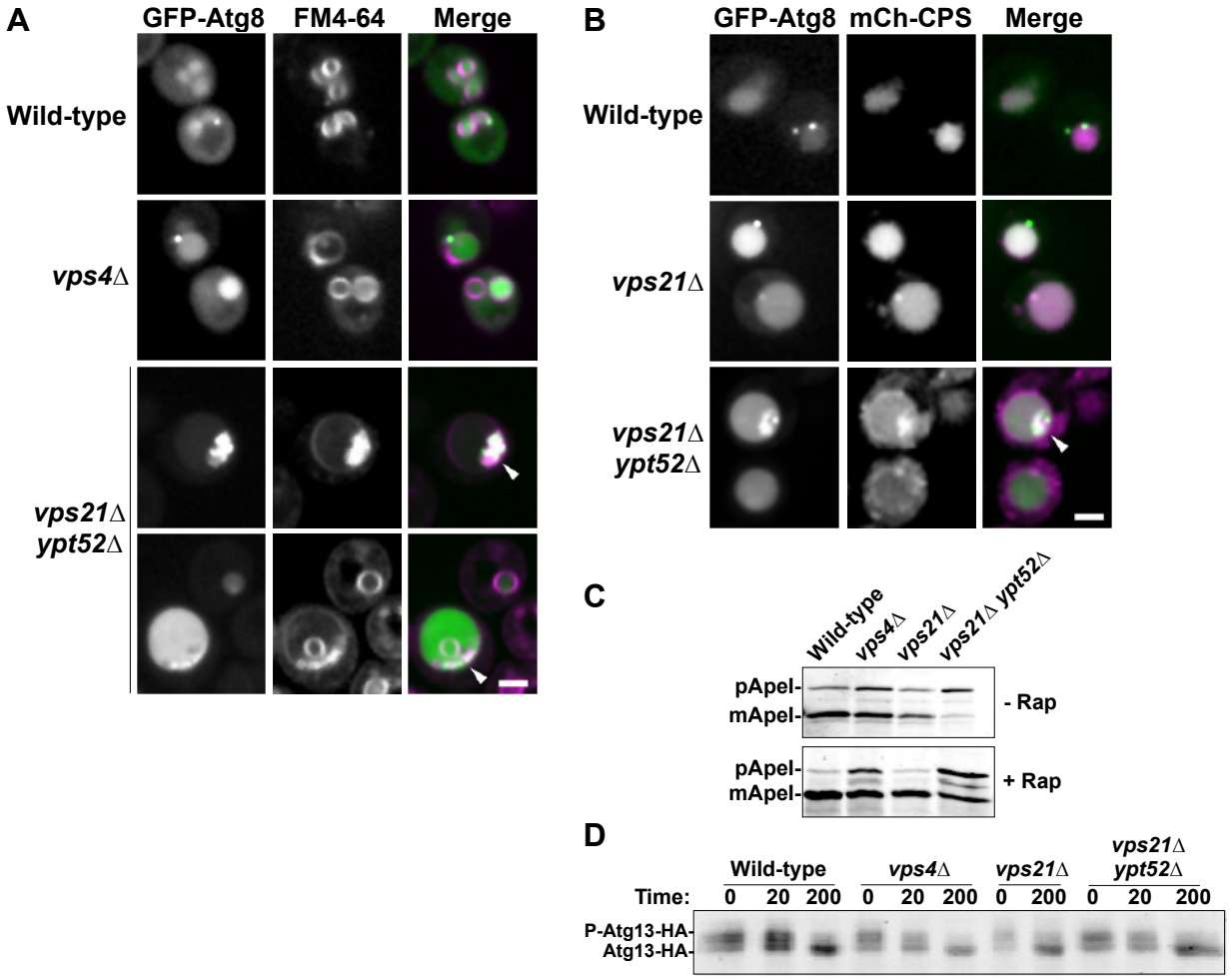
Supplemental Figure 1



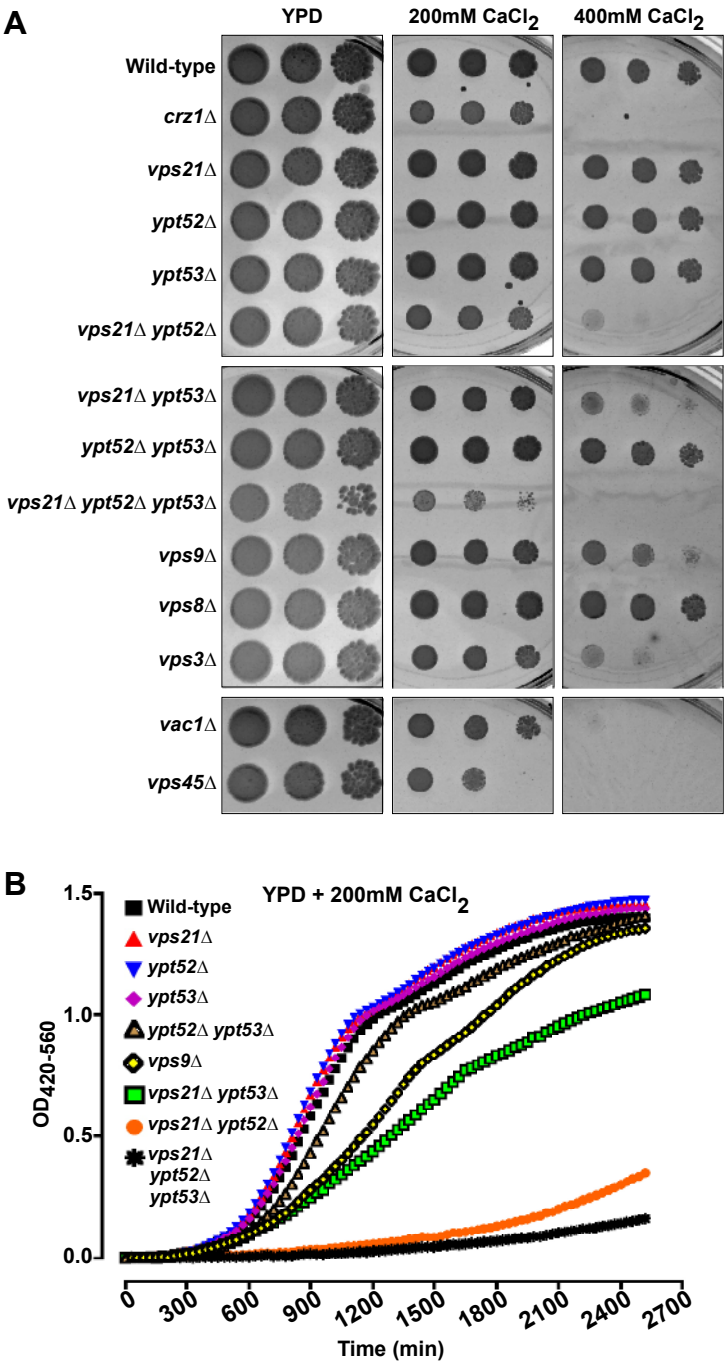
Supplementary Figure S1. Endosome morphology. (A-C) Thin-section electron micrographs show endosomes in *ypt52*Δ (A), *ypt53*Δ (B) or *gyp3*Δ (C) cells, all possessing characteristic MVB morphology. Bar, 100 nm. Vac, vacuole.

Supplemental Figure 2



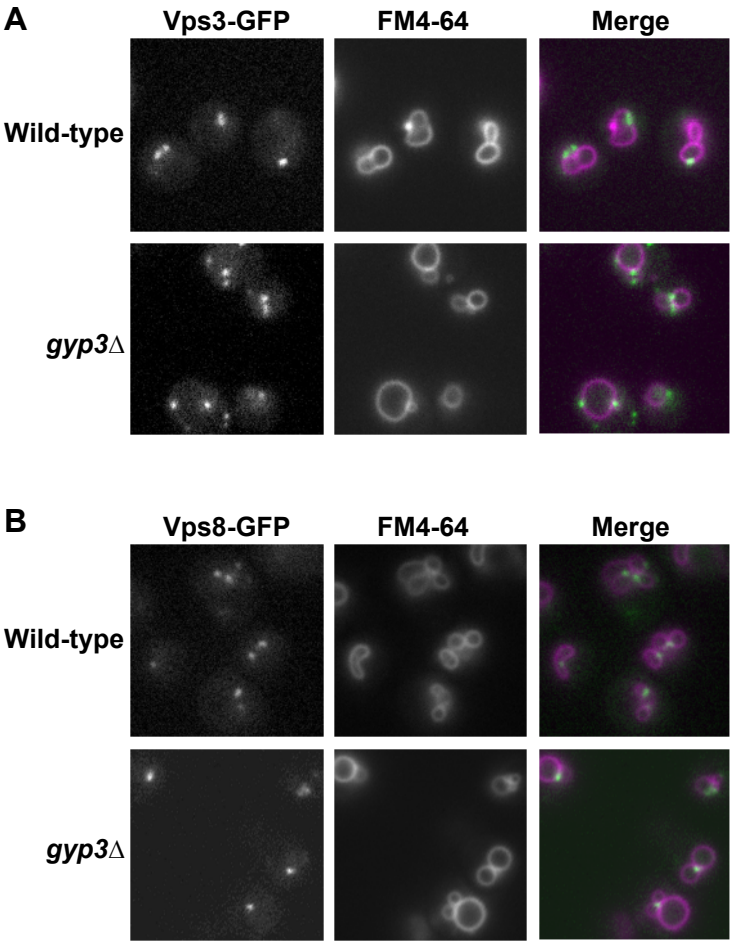
Supplementary Figure S2. Autophagy in Rab5-deficient cells. (A-B) Confocal fluorescence microscopy of GFP-Atg8 expressed in cells stained with FM 4-64 (A) or co-expressing MVB cargo mCherry-CPS (B). Scale bars, 2 μm. (C) Western blot analysis of ApeI maturation in cell lysates with or without rapamycin treatment. (D) Western blot analysis of a timecourse following rapamycin treatment, indicating dephosphorylation of the TOR kinase substrate Atg13. pApeI, premature ApeI. mApeI, mature ApeI. P-Atg13-HA, phospho-Atg13-HA.

Supplemental Figure 3



Supplementary Figure S3. Rab5 signaling and effectors are required to resist Ca^{2+} stress. (A) Limiting dilution plate growth assay using YPD agar with the indicated concentrations of added Ca^{2+}. (B) Growth curves in YPD broth supplemented with 200 mM Ca^{2+}. For all experiments, cells were grown at 30° C.

Supplemental Figure 4



Supplementary Figure S4. Peri-vacuolar localization of Rab5 effector molecules in wild-type and *gyp3*Δ cells. (A-B) Fluorescence microscopy of FM 4-64-stained cells expressing either Vps3-GFP (A) or Vps8-GFP (B).