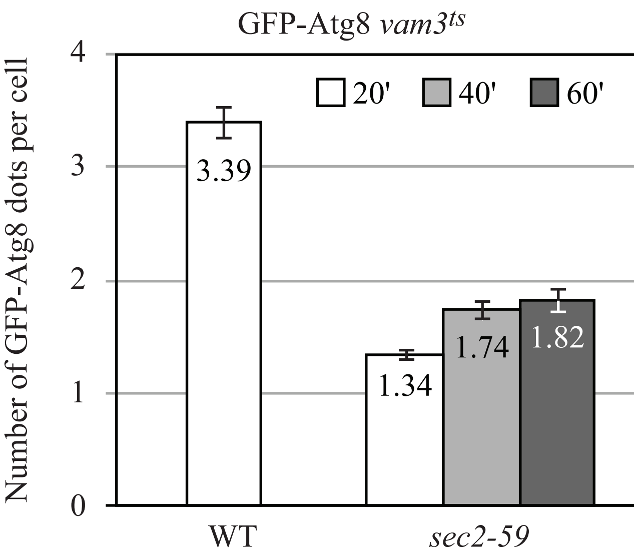


Figure S1. In the *vam3^{ts}* background *sec2-59* cells accumulated fewer GFP-Atg8 dots in the cytosol even after longer starvation. *vam3^{ts}* (JGY169) and *vam3^{ts} sec2-59* (JGY172) cells were starved at 37°C and fixed samples at the indicated time points were checked by microscopy. In the control cells, only a 20 min sample was quantified because after longer starvation there were too many overlapping GFP-Atg8 dots to allow accurate counting. Error bar, S.E.M., n = 100.

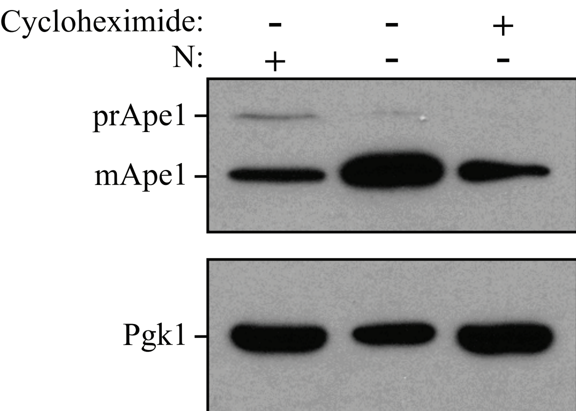


Figure S2. Cycloheximide blocked protein synthesis. During starvation, the protein level of Ape1 is upregulated, but in the presence of cyclohexmide the level of Ape1 remained unchanged. Wild-type (SEY6210) cells were cultured in YPD to mid-log phase and shifted to SD-N with or without 10 ng/ml cycloheximide. Samples were collected before and after 2 h starvation and examined by western blot. Pgk1 was used as a loading control.

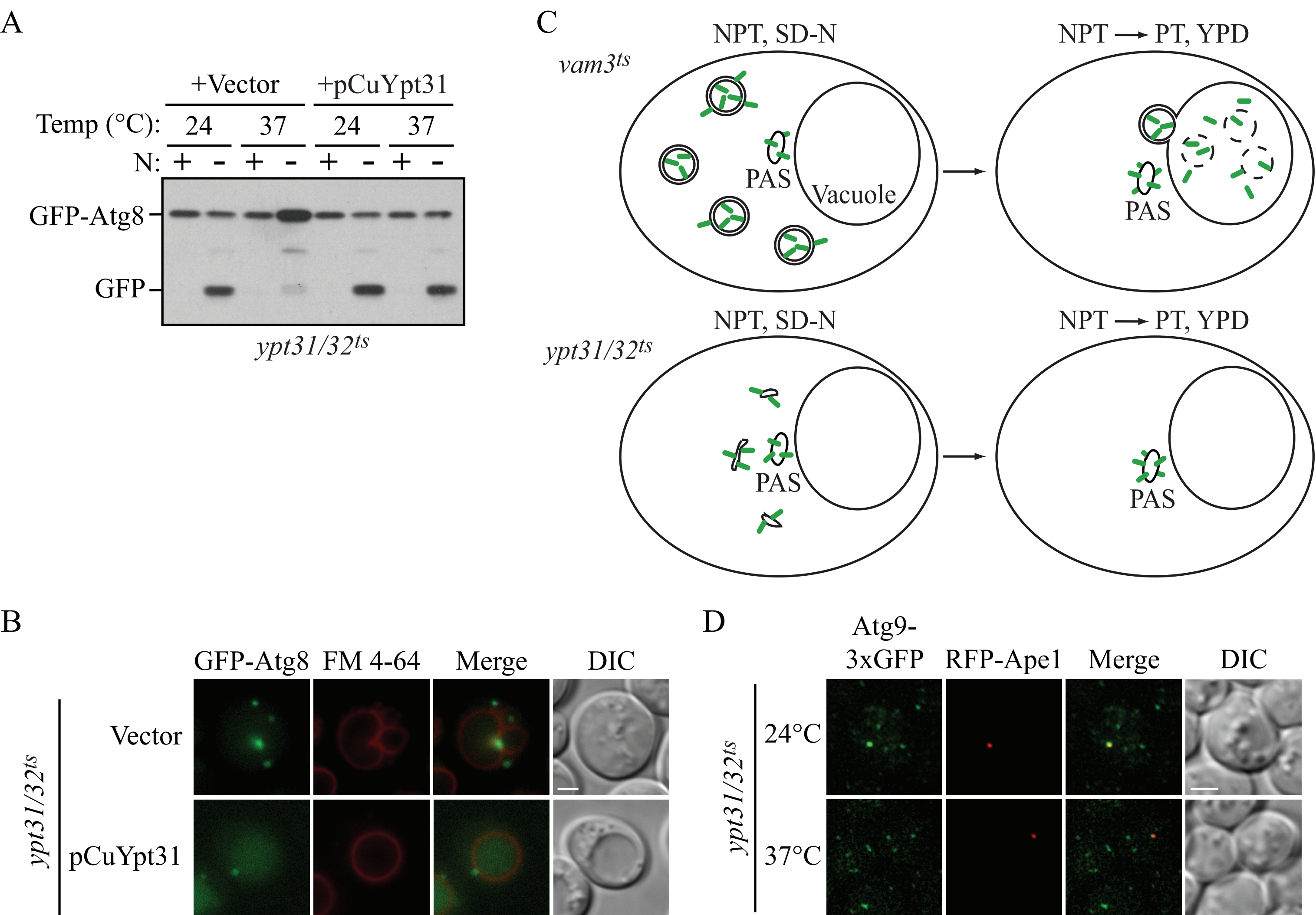
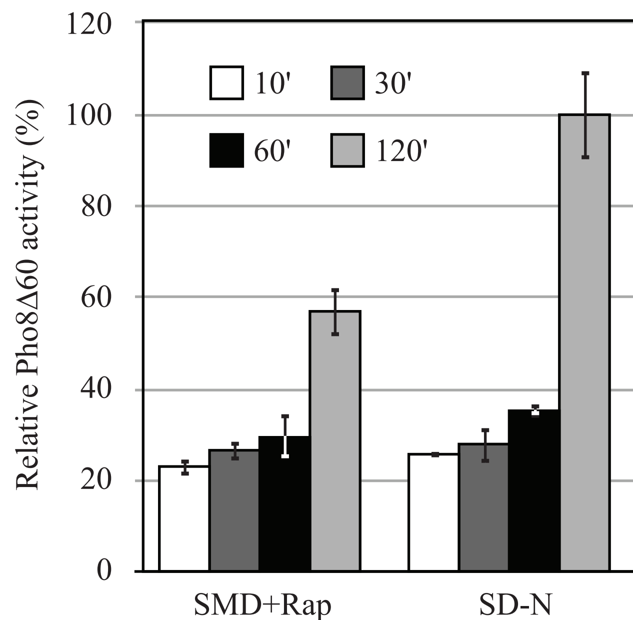


Figure S3. (A) Expression of Ypt31 rescues the autophagic defect in the *ypt31/32^{ts}* mutant. *ypt31/32^{ts}* GFP-Atg8 (JGY210) cells expressing empty vector or pCuYpt31(416) were examined as in Figure 1A. (B) Exogenous expression of Ypt31 restored Atg8 localization in *ypt31/32^{ts}* cells at the NPT. *ypt31/32^{ts}* GFP-Atg8 cells expressing empty vector or pCuYpt31(416) were inactived at 37°C for 30 min and then starved at 37°C for 2 h. Cell samples were then checked by microscopy. Vacuolar membrane was stained with FM 4-64. For each strain, only one Z-section with a clear vacuole was shown. Scale bar, 2µm. (C) Schematic diagram illustrating the interpreted phenotypes for the *vam3^{ts}* and *ypt31/ypt32^{ts}* strains after shifting from NPT in SD-N to PT in YPD. (D) Ypt31/32 are not required for the localization of Atg9 to its peripheral sites. *ypt31/32^{ts}* Atg9-3xGFP RFP-Ape1 (JGY207) cells were grown at 24°C. Half of the culture was incubated at 37°C for 30 min and the other half remained at 24°C. Cells were shifted to SD-N for 2 h and samples were collected after starvation. After fixation, cells were examined by microscopy as described in Materials and Methods. Scale bar, 2 µm.



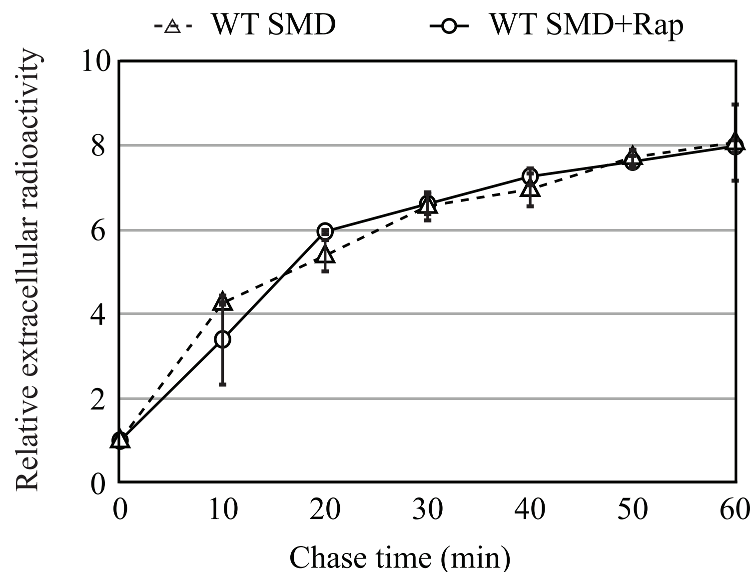


Figure S4. Protein secretion is not regulated by rapamycin treatment. (A) Induction of autophagy by rapamycin treatment. A Pho8Δ60 strain (TN124) was treated with 0.8 µg/ml rapamycin or incubated in SD-N medium. Pho8Δ60-dependent alkaline phosphatase activity was measured as described in Materials and Methods. Error bar, S.D. from three independent experiments. (B) Rapamycin treatment has no effect on the kinetics of protein secretion. After [^{35}S] labelling, cells were chased in SMD medium with or without 0.8 µg/ml rapamycin. Protein secretion was quantified as described in Figure 7B. Error bar, S.D. from three independent experiments.