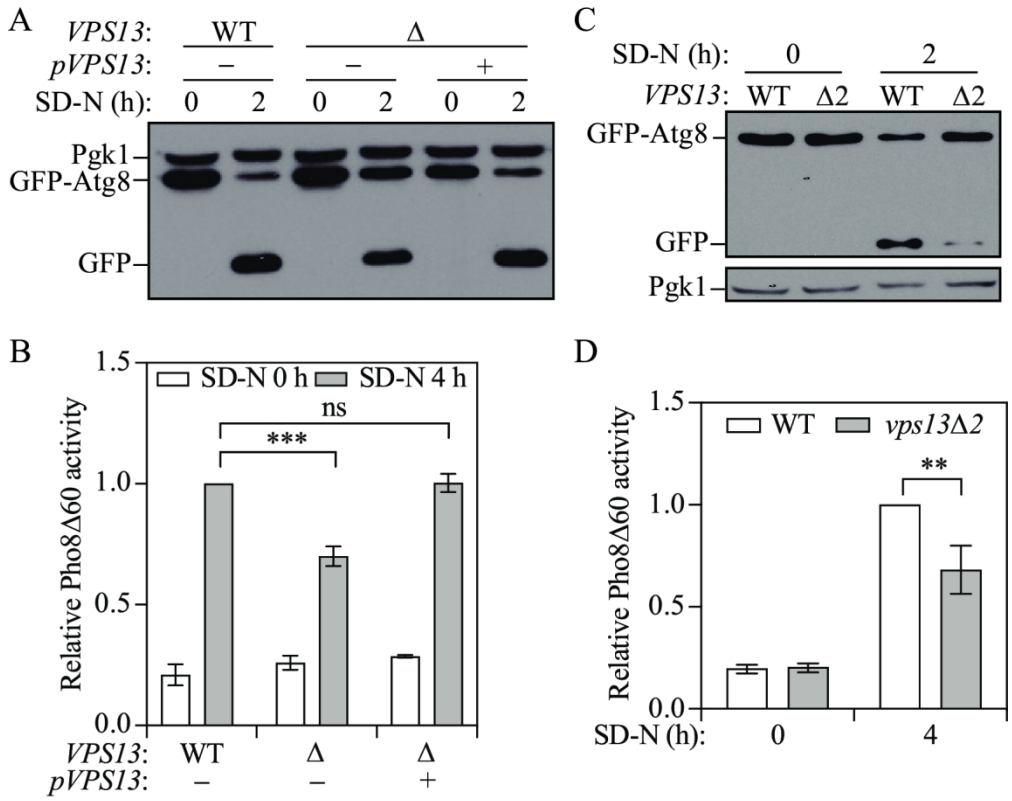

Figure S1. Vps13 is important for efficient autophagy. Protein samples were collected from (**A and B**) wild-type (YLY085) and *vps13*Δ cells (YLY086) with the indicated pRS313 plasmids or (**C and D**) from wild-type cells (YLY085) and cells with most of *VPS13* deleted (WXY222) after growth in YPD to mid-log phase (SD-N 0 h) and (**A and C**) 2 h or (**B and D**) 4 h after nitrogen starvation. Western blots were probed and Pho8Δ60 was quantified as indicated in Figure 1. **p<0.01, ***p<0.001, ns, not significant. Related to Fig. 1.

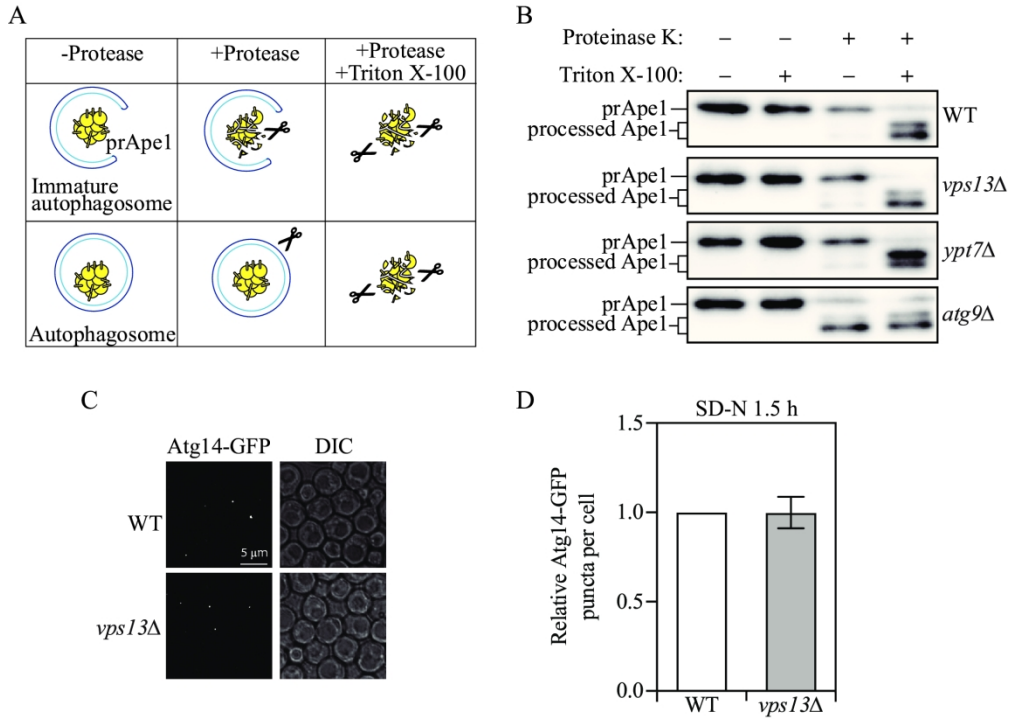

Figure S2. Autophagosome completion is not affected by *VPS13* deletion. (**A**) Schematic representation of the protease-protection assay. (**B**) Wild-type (YLY085), *vps13*Δ (YLY086), *ypt7*Δ (YLY162) and *atg9*Δ (YLY164) cells were cultured in YPD to mid-log phase and then transferred to nitrogen starvation medium (SD-N) for 3 h. A protease-protein assay was conducted as described in the Materials and Methods. (**C and D**) Wild-type (UNY176, Atg14-GFP) and *vps13*Δ (YLY145, Atg14-GFP) cell samples were collected 1.5 h after nitrogen starvation (SD-N) and imaged. The total puncta to total cell number ratio was quantified and normalized to wild-type cells. In the quantitative analysis, the error bar represents the SD of three independent experiments. DIC: differential interference contrast. Related to Fig. 2.

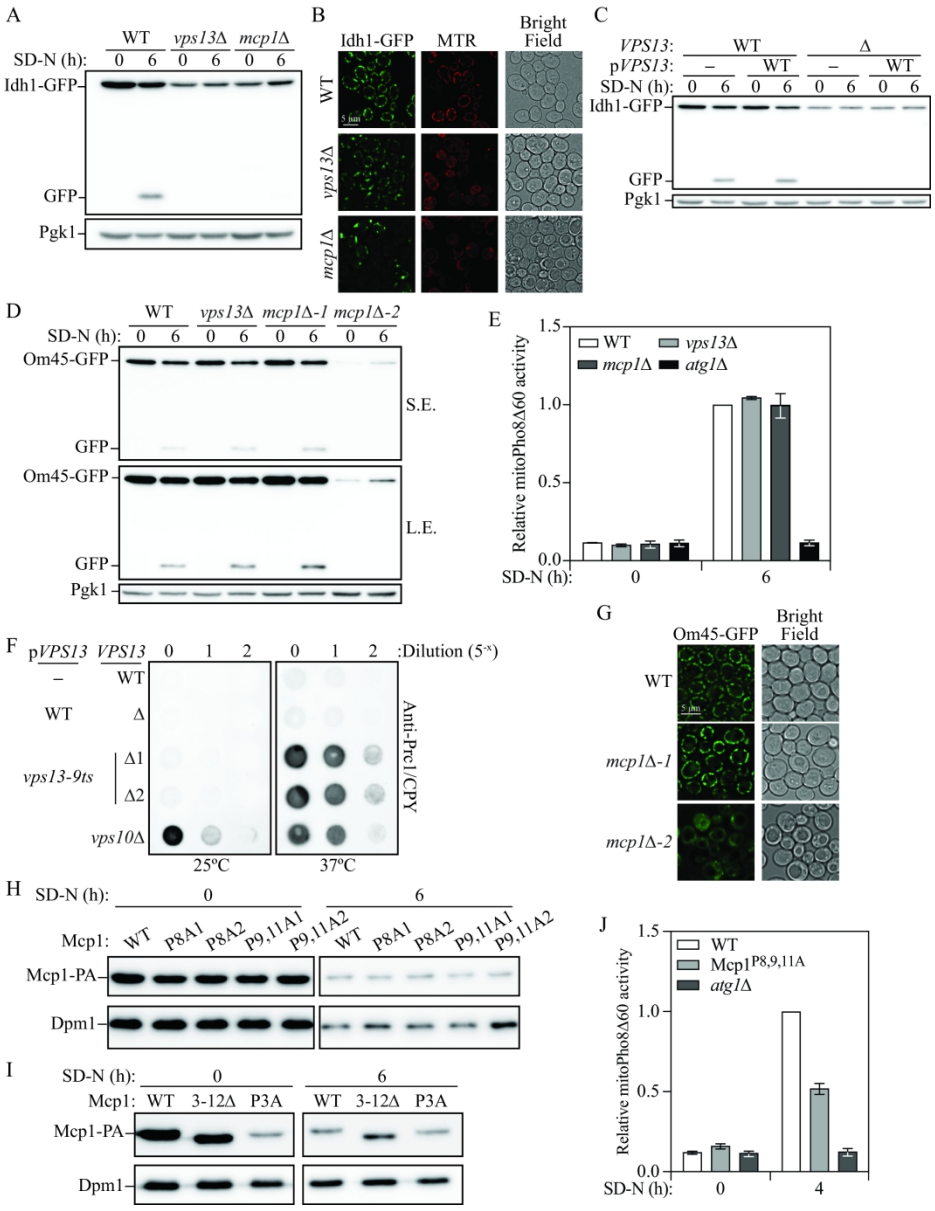

Figure S3. Neither Vps13 nor the interaction between Vps13 and Mcp1 is important for mitophagy. (**A**) Wild-type (YLY068, Idh1-GFP), *vps13*Δ (YLY123, Idh1-GFP) and *mcp1*Δ (YLY124, Idh1-GFP) cells were cultured as indicated in Materials and Methods to induce mitophagy. Protein samples were collected from the culture in YPL (SD-N 0 h) and 6 h after nitrogen starvation. Western blots were probed as indicated in Figure 3C. (**B**) The same cells as in (A) were cultured in YPD to mid-log phase, shifted to YPL for 18 h and stained with 50 nM MitoTracker Red (MTR) for 30 min before imaging. Single Z-sections of representative images were shown. (**C**) Wild-type (YLY068) or *vps13*Δ cells (YLY123) with an empty pRS313 vector or pRS313 vector bearing wild-type Vps13 were cultured as indicated in the Materials and Methods to induce mitophagy. Protein samples were collected from the culture in SML-His (SD-N 0 h) and 6 h after nitrogen

starvation (SD-N). Western blots were probed with anti-YFP and anti-Pgk1 antibodies or antisera. (**D**) Wild-type (TKYM23, Om45-GFP), *vps13*Δ (YLY083, Om45-GFP) and *mcp1*Δ (YLY084, Om45-GFP; two different colonies were included) cells were cultured as indicated in Materials and Methods to induce mitophagy. Two *mcp1*Δ colonies have the same apparent genotype, but *mcp1*Δ-2 cells grew slower, and the *mcp1*Δ-1 cells grew at the normal rate. Protein samples were collected from the culture in YPL (SD-N 0 h) and 6 h after nitrogen starvation. Western blots were probed as indicated in (C). (**E**) Wild-type (KDM1009), *vps13*Δ (YLY087), *mcp1*Δ (YLY088) and *atg1*Δ (KDM1024, as control) strains were cultured as described in Materials and Methods to induce mitophagy. Protein samples were collected from the culture in YPL (SD-N 0 h) and 6 h after nitrogen starvation. mitoPho8Δ60 was used to measured mitophagy activity. mitoPho8Δ60 activity was quantified as indicated in Figure 3. (**F**) The same cells as in Fig. 3D and *vps10*Δ (YLY279, as a control) cells were cultured in SMD-His or YPD at 25°C to mid-log phase and a Prc1/CPY secretion assay was performed as described in Materials and Methods. (**G**) The same cells in (D) were cultured in YPD to mid-log phase and shifted to YPL for 18 h before imaging. Single Z-sections of representative images are shown. (**H**) Wild-type (YLY110 Mcp1-PA), *MCP1* single mutant (YLY115, two colonies were included) and double mutant (YLY116, two colonies were included) cells or (**I**) *MCP1* truncated (YLY111) and triple mutant cells (YLY112; P3A) cells were cultured as indicated in Materials and Methods to induce mitophagy. Protein samples were collected from the culture in YPL (SD-N 0 h) and 6 h after nitrogen starvation. Western blots were probed with anti-PA and anti-Dpm1 antibodies. (**J**) Wild-type (YLY110, Mcp1-PA), *MCP1* triple mutant cells (YLY112) and *atg1*Δ (KDM1024, as control) cells were cultured as described in Materials and Methods to induce mitophagy. Protein samples were collected from the culture in YPL (SD-N 0 h) and 6 h after nitrogen starvation. mitoPho8Δ60 was used to measured mitophagy activity. mitoPho8Δ60 activity was quantified as indicated in Figure 3. Related to Fig. 3.