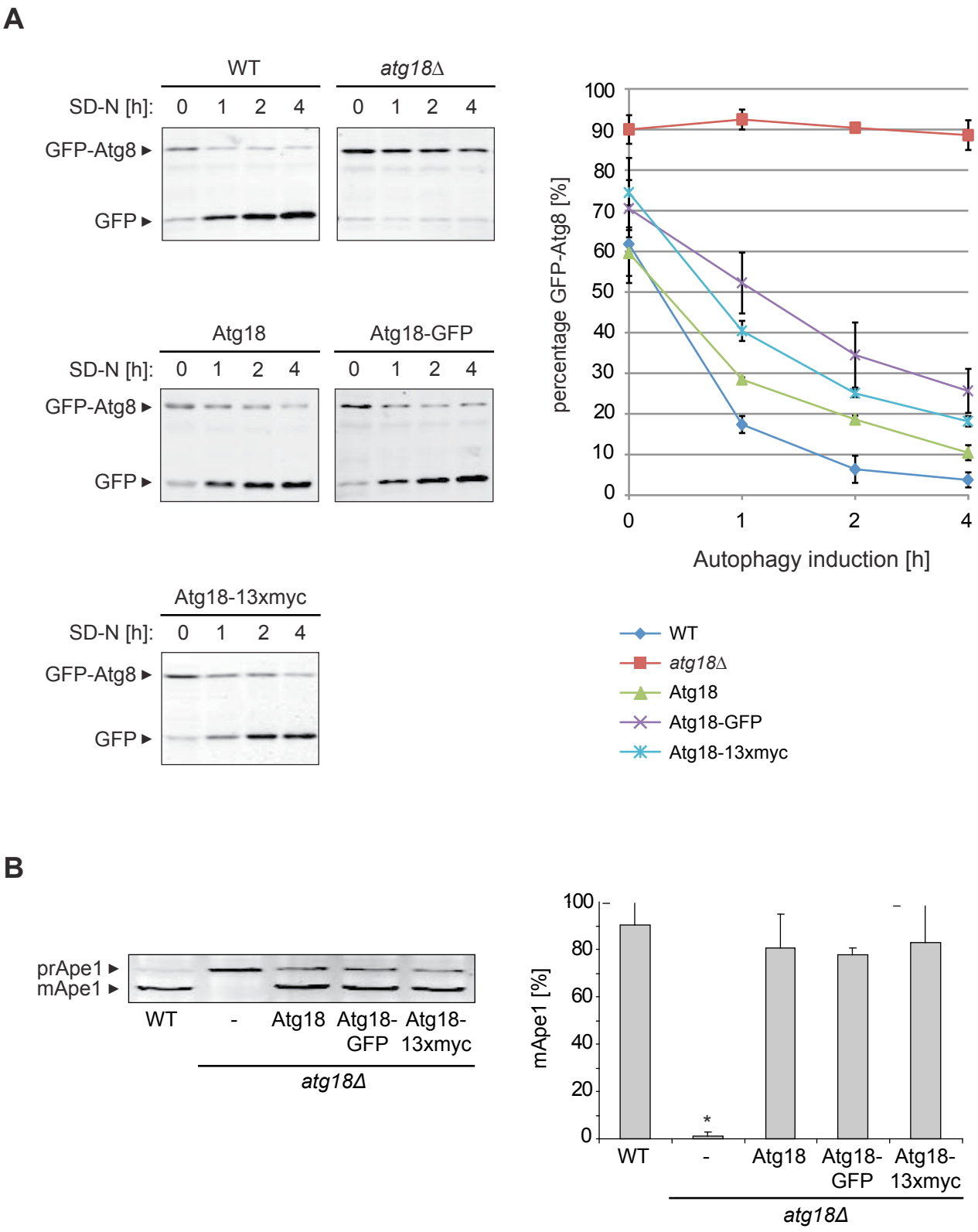

Fig. S1. GFP- and myc-tagged wild type Atg18 is functional. (A) Wild type (SEY6210) and *atg18*Δ (JGY3) cells carrying both the pCuGFPATG8414 construct and either the plasmid expressing untagged, myc-tagged or GFP-tagged wild type Atg18 under the control of the endogenous promoter were grown in rich medium to an early log phase and transferred to starvation SD-N medium to induce autophagy. Cell aliquots were taken at 0, 1, 2 and 4 h, before analyzing the cell extracts by western blot using an antibody against GFP. The detected bands were quantified using the Odyssey software and the percentages of GFP-Atg8 were plotted in a graph. Data represent the average of 3 experiments ± s.e.m. (B) Wild-type (SEY6210) and *atg18Δ* (JGY3) cells transformed with plasmids expressing the untagged, myc-tagged or GFP-tagged wild type Atg18 were grown in rich medium to an early log phase. Cell aliquots were collected and cell extracts analysed by western-blot using anti-Ape1 antibodies. The detected bands were quantified as in panel A and the percentages of mApe1 were plotted. The graphs represent the average of 2 experiments ± s.e.m. and asterisks indicate a significant difference with the WT (*$P < 0.05$).

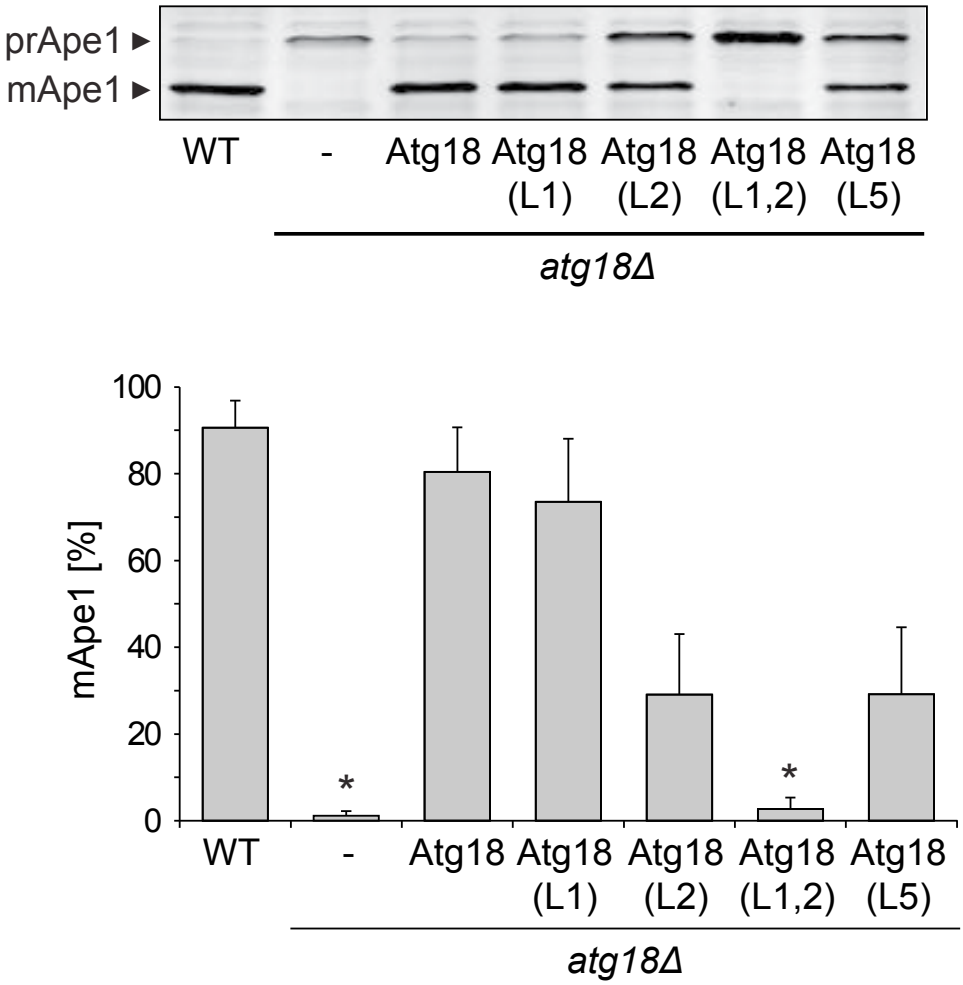

Fig. S2. Atg18-Atg2 interaction is essential for the Cvt pathway. Mutations in the Atg18 loops 1, 2 and 5 severely affect the Cvt pathway. Wild-type (SEY6210) and *atg18Δ* (JGY3) cells transformed with plasmids expressing the untagged Atg18 loop mutants were grown in rich medium and cell extracts analysed by western blot using anti-Ape1 antibodies. The detected bands were quantified using the Odyssey software and the percentages of mApe1 were plotted. The graphs represent the average of 3 experiments ± s.e.m. and asterisks indicate a significant difference with the WT (two-tailed *t*-test: $P < 0.05$).

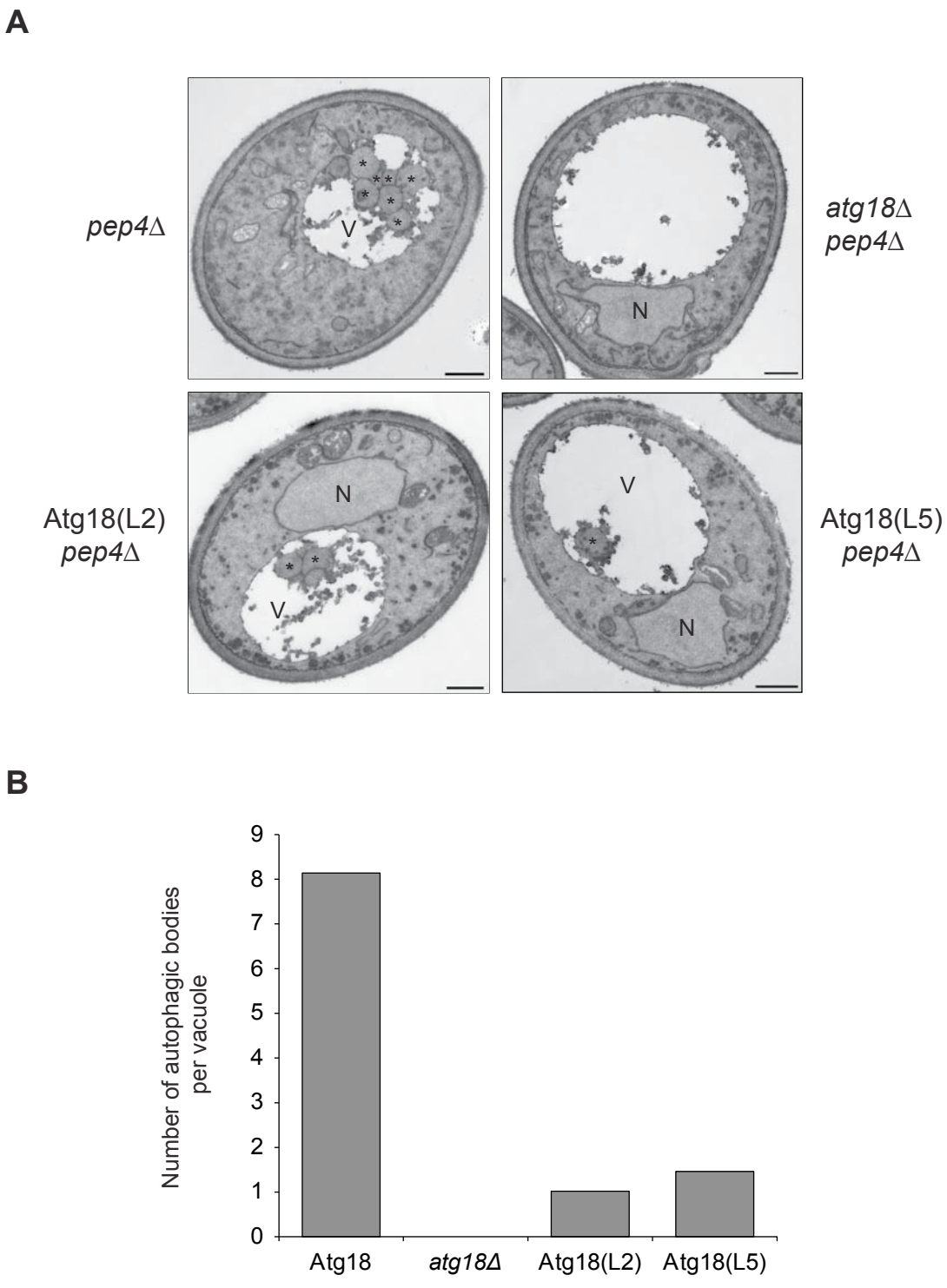

Fig. S3. Atg2-binding mutants of Atg18 have a defect in autophagosome biogenesis. (A) The Atg2-binding mutant of Atg18 blocks autophagosome biogenesis at an early stage. The *atg18Δ pep4Δ* (ERY060) cells carrying one of the plasmids expressing the untagged Atg18 loop mutants 2 or 5 were grown in rich medium and then transferred to SD-N medium for 3 h before processing them for EM. (B) Quantification of the autophagic bodies present in the vacuoles. The experiment shown in panel A was quantified as described in Materials and Methods, and the results are expressed as the average number of autophagic bodies per vacuole. N, nucleus; V, vacuole; autophagic body: *. Scale bar, 500 nm.

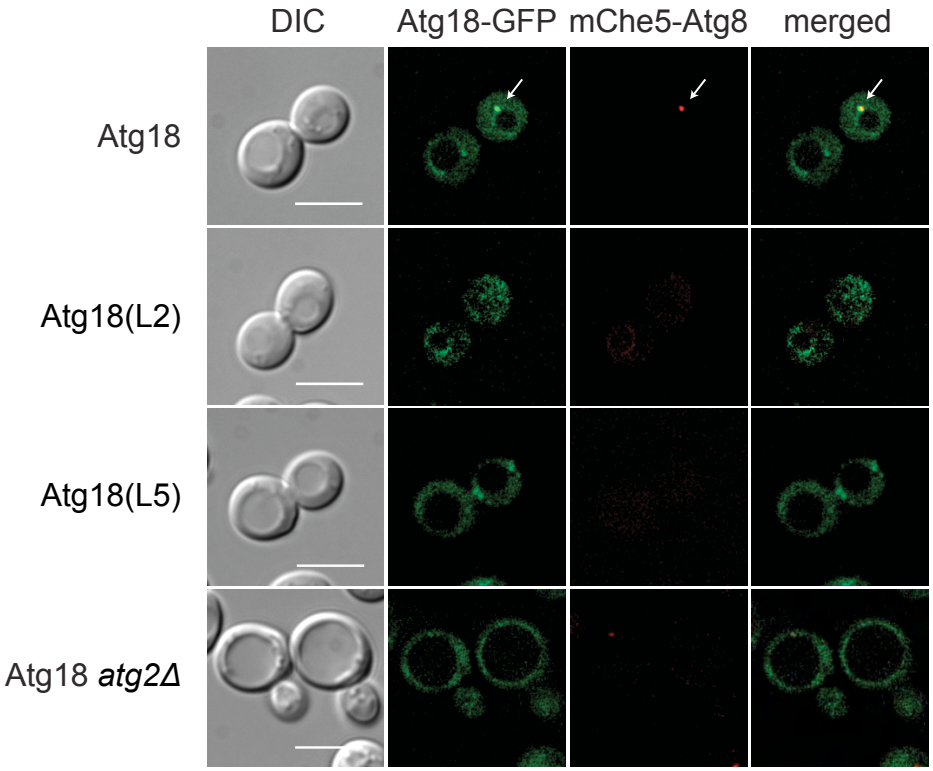

Fig. S4. Atg2-binding mutants of Atg18 do not localize to the PAS in growing conditions. The *atg18Δ* strain carrying the mCheV5-Atg8 fusion and genomically integrated GFP-tagged *ATG18* (ERY090) or the different GFP-tagged *ATG18* loop mutants (ERY091 and ERY093) and the *atg18Δ atg2Δ* strain carrying GFP-tagged *ATG18* (ERY102) were grown to an early log phase and imaged by fluorescence microscopy. White arrows highlight colocalization of the fluorescence signals. DIC, differential interference contrast. Scale bar, 5 μm. The quantification of the percentage of cells with colocalizing puncta is presented in Fig. 6B.

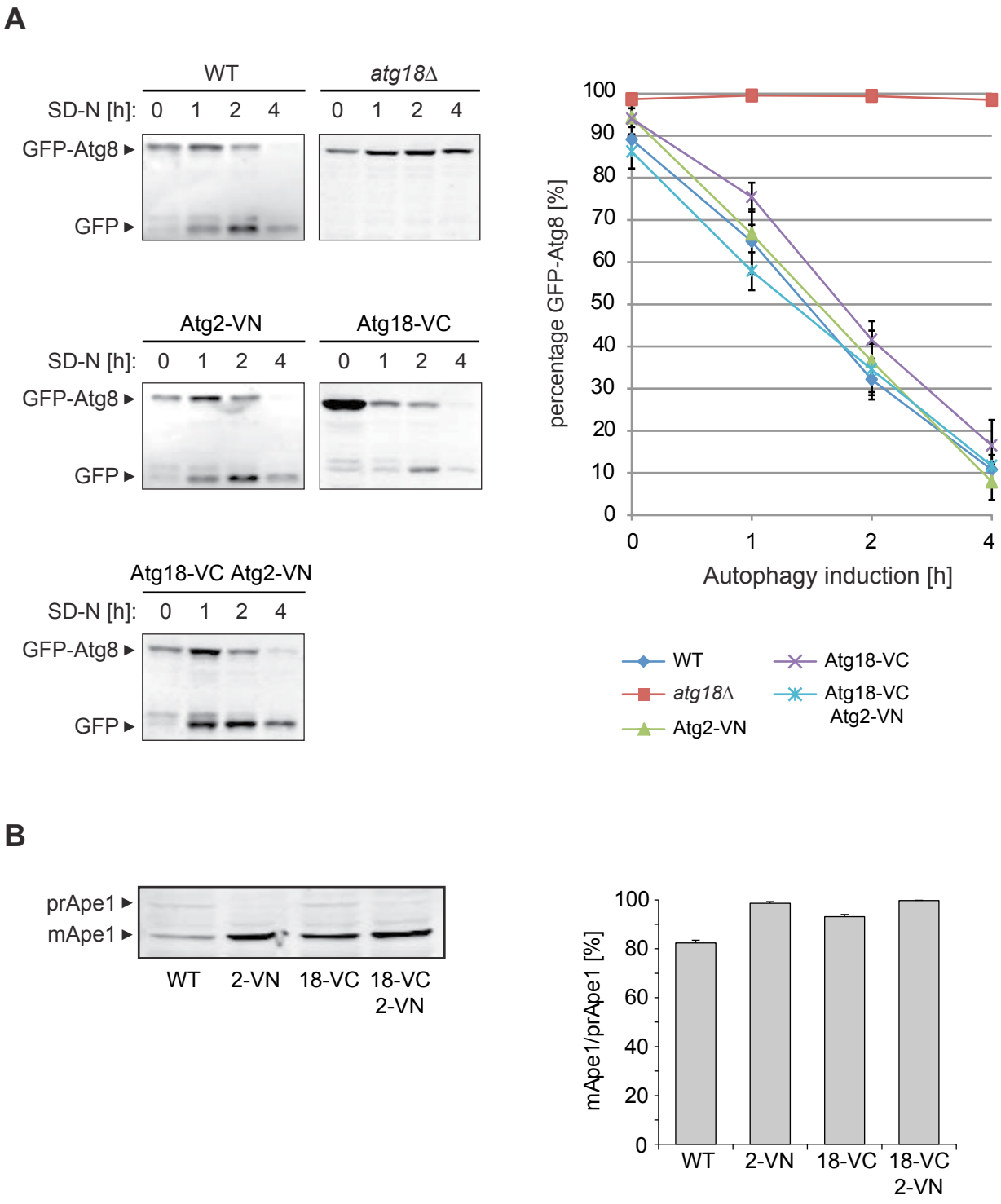

Fig. S5. VN-tagged Atg2 and VC-tagged Atg18 fusion proteins are functional for the Cvt pathway and autophagy. (A) Wild type cells and cells expressing endogenous Atg2-VN and/or Atg18-VC (ERY115, ERY116, and ERY117) carrying the pCuGFPATG8414 construct were grown in rich medium to an early log phase and transferred into SD-N medium to induce autophagy. Cell aliquots were taken at 0, 1, 2 and 4 h, before analyzing the cell extracts by western blot using an antibody against GFP. The detected bands were quantified using the Odyssey software and the percentages of GFP-Atg8 were plotted in a graph. Data represent the average of 2 experiments ± s.e.m. (B). Wild type cells expressing endogenous Atg2-VN and/or Atg18-VC (ERY115, ERY116 and ERY117) were grown in rich medium to an early log phase. Cell aliquots were collected and cell extracts analysed by western blot using anti-Ape1 antibodies. The detected bands were quantified as in panel A and the percentage of mApe1 was plotted. The graphs represent the average of 2 experiments ± s.e.m. and asterisks indicate a significant difference with the WT (*$P < 0.05$).

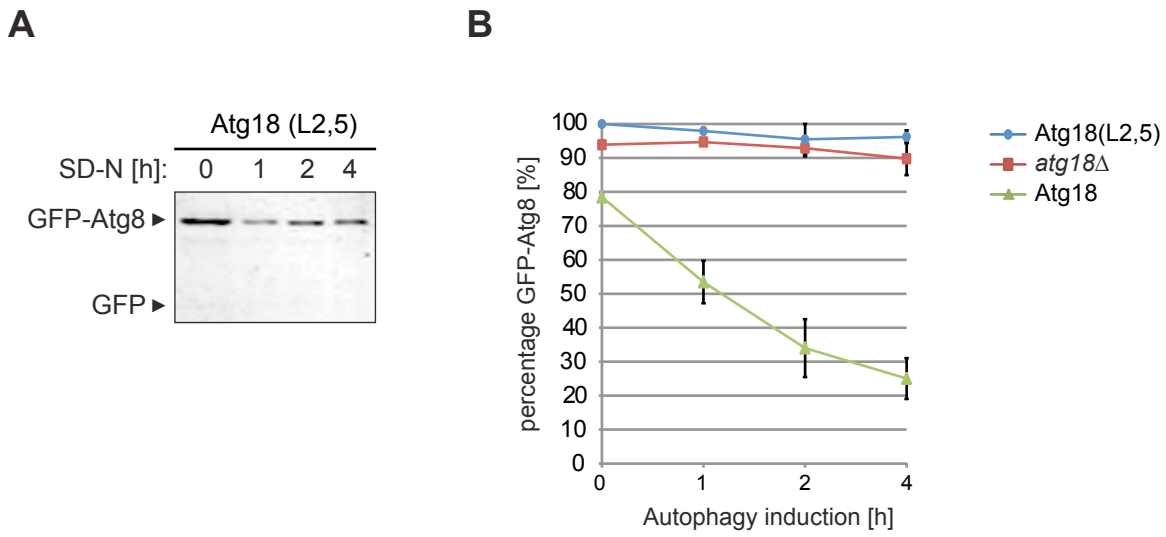

Fig. S6. The simultaneous defect of Atg18 in binding to Atg2 and PtdIns3P completely blocks autophagy. The *atg18*Δ (JGY3) strain transformed with the pCuGFPATG8414 construct and the plasmid expressing untagged Atg18(L2,5) was grown in rich medium and nitrogen starved to induce autophagy. Cell aliquots were taken at 0, 1, 2 and 4 h, before analyzing the cell extracts by western blot. The detected bands were quantified and presented as in Fig. 3 compared to cells expressing untagged wild type Atg18 or *atg18*Δ cells.

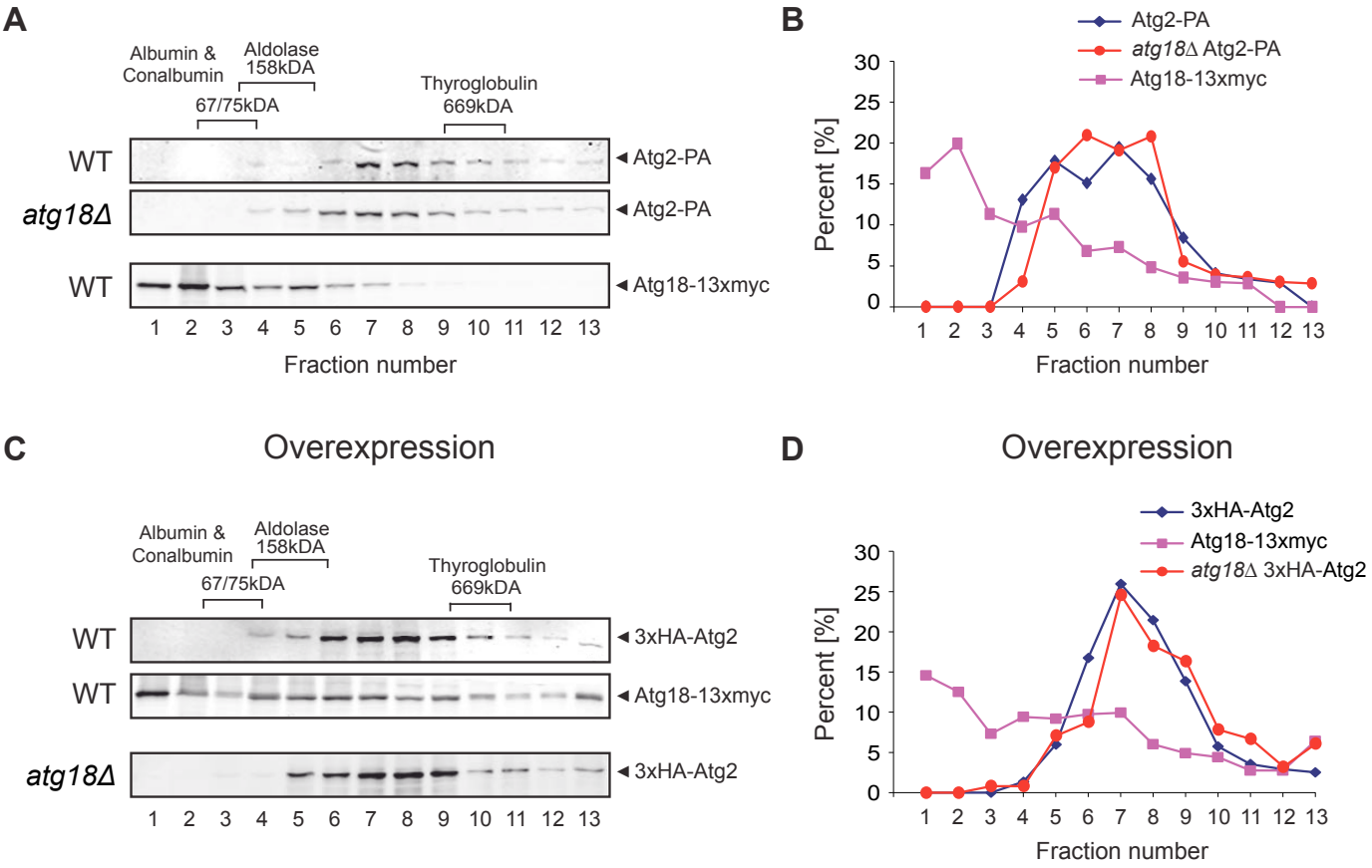

Fig. S7. Analysis of endogenous and overexpressed Atg2 and Atg18 on continuous glycerol gradients. (A) Analysis of Atg2 and Atg18 on continuous glycerol gradients. Cell lysates from Atg2-PA-expressing *atg18Δ* strain (ERY086) carrying endogenously 13xmyc-tagged Atg18 were fractionated on 10-15% glycerol gradients as described in *Materials and methods*. For each gradient 13 fractions were collected, resolved by SDS-PAGE and analyzed by western blot using antibodies against myc and PA. (B) Quantification of the immunoblots presented in panel A. The graphs represent the average of 2 experiments. **(**C) The *atg18Δ* strain overexpressing 3xHA-Atg2 (ERY052), and either untransformed or transformed with a plasmid expressing wild type *ATG18* tagged with 13xmyc under the control of the *GAL1* promoter, was grown on galactose for 3 h to induce protein overexpression. Cell lysates were fractionated on 10-15% glycerol gradients and analyzed using antibodies against HA and myc. (D) Quantification of the immunoblots presented in panel C. The graphs represent the average of two experiments.

Table S1. Strains used in this study

Name	Genotype	Reference/Origin
*atg18*Δ	BY4742 *atg18*Δ*::kanMX4*	Euroscarf
BY4742	*MATa his3*Δ*1 leu2*Δ*0 lys2*Δ*0 ura3*Δ*0*	Euroscarf
ERY052	BY4247 *atg18*Δ*::kanMX4 HIS3MX6::pGAL1-3HA-ATG2*	This study
ERY060	SEY6210 *atg18*Δ*::HIS5 S.p. pep4*Δ*::LEU2*	This study
ERY068	SEY6210 *atg18*Δ*::HIS5 S.p. CFP-ATG8::URA3*	This study
ERY070	SEY6210 *atg18*Δ*::HIS5 S.p. CFP-ATG8::URA3* *ATG18-13xmyc*::*LEU2*	This study
ERY072	SEY6210 *atg18*Δ*::HIS5 S.p CFP-ATG8::URA3* *ATG18(L2)-13xmyc::LEU2*	This study
ERY074	SEY6210 *atg18*Δ*::HIS5 S.p. CFP-ATG8::URA3* *ATG18(L5)-13xmyc::LEU2*	This study
ERY086	SEY6210 *atg2*Δ*::TRP1 atg18*Δ*::HIS5 S.p.*	This study
ERY087	SEY6210 *ATG2-GFP::HIS5 S.p. atg18Δ::TRP1*	This study
ERY090	SEY6210 *atg18*Δ*::HIS5 S.p. pCUP1-mCheV5-ATG8::URA3* *ATG18-GFP*::*LEU2*	This study
ERY091	SEY6210 *atg18*Δ*::HIS5 S.p. pCUP1- mCheV5-ATG8::URA3* *ATG18(L2)-GFP::LEU2*	This study
ERY093	SEY6210 *atg18*Δ*::HIS5 S.p. pCUP1- mCheV5-ATG8::URA3* *ATG18(L5)-GFP::LEU2*	This study
ERY094	SEY6210 *ATG2-GFP::HIS5 S.p. atg18Δ::TRP1* *ATG18-13xmyc*::*LEU2*	This study
ERY095	SEY6210 *ATG2-GFP::HIS5 S.p. atg18Δ::TRP1* *ATG18(L2)-13xmyc::LEU2*	This study
ERY097	SEY6210 *ATG2-GFP::HIS5 S.p. atg18Δ::TRP1* *ATG18(L5)-13xmyc::LEU2*	This study
ERY102	SEY6210 *atg18*Δ*::HIS5 S.p. pCUP1- mCheV5-ATG8::URA3* *ATG18-GFP::LEU2 atg2*Δ*::TRP1*	This study

ERY103	SEY6210 *ATG2-GFP::HIS5 S.p. atg18Δ::TRP1 atg21Δ::URA3 K.l.*	This study
ERY117	SEY6210 *ATG2-VN::HIS5 S.p. ATG18-VC::TRP1*	This study
ERY118	SEY6210 *ATG2-VN::HIS5 S.p. ATG18-VC::TRP1 atg3Δ::URA*	This study
ERY119	SEY6210 *ATG2-VN::HIS5 S.p. ATG18-VC::TRP1 atg13Δ::URA*	This study
ERY132	SEY6210 *ATG2-VN::HIS5 S.p. atg18Δ::TRP1 ATG18-VC::LEU2*	This study
ERY133	SEY6210 *ATG2-VN::HIS5 S.p. atg18Δ::TRP1 ATG18(L2)-VC::LEU2*	This study
ERY137	SEY6210 *ATG2-VN::HIS5 S.p. atg18Δ::TRP1 ATG18(L5)-VC::LEU2*	This study
ERY145	SEY6210 *ATG2-PA::TRP1 atg18Δ::HIS5 S.p. atg14Δ::NatMX6*	This study
ERY146	SEY6210 *ATG2-VN::HIS5 S.p. ATG18-VC::TRP1 atg14Δ::URA3*	This study
FRY387	SEY6210 *ATG2-PA::TRP1 atg18Δ::HIS5 S.p.*	This study
JGY3	SEY6210 *atg18Δ::HIS5 S.p.*	(Guan et al., 2001)
PJ69-4A	*MATa leu2-3,112 trp1-Δ901ura3-52 his3-Δ 200 gal Δ4 gal80Δ LYS::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ*	(James et al., 1996)
PSY102	SEY6210 *ATG2-GFP::HIS5 S.p.*	Reggiori lab collection
SEY6210	*MATa ura3-52 leu2-3,112 his3-Δ200 trp1-Δ901 lys2-801 suc2-Δ9 mel GAL*	(Robinson et al., 1988)