Table S1: Reagents and strains REAGENT

Antibodies	Source	Identifier
Mouse monoclonal Pgk1	Thermofisher	**Cat #** 459250
Mouse monoclonal Porin	Thermofisher	**Cat# 459500, RRID:AB_2532239**
Goat polyclonal Atg8	Santa Cruz	**Cat# sc-15639, RRID:AB_633983**
Mouse monoclonal V5	Thermofisher	**Cat#14-6796-80, RRID:AB_10717814)**
Mouse monoclonal GFP	ROCHE	**Cat# 11814460001, RRID:AB_390913**

STRAINS

Souche	**Génotype**
BY4742 (WT)	MATα; *his3Δ1*; *leu2Δ0*; *lys2Δ0*; *ura3Δ0*
BY + GFP-Atg8	BY4742 *pRS416-GFP-ATG8*
BY + Idp1-GFP	BY4742 p*IDP1-GFP*
Δ*psd1* + GFP-Atg8	BY4742 *psd1*::*kanMX4* pRS416-*GFP-ATG8*
Δ*psd1* + Idp1-GFP	BY4742 *psd1*::*kanMX4* p*IDP1-GFP*
Δ*psd1* + Idp1-GFP + p*PSD1*	BY4742 *psd1*::*kanMX4* p*IDP1-GFP* pRS416-*PSD1*
Δ*psd2* + GFP-Atg8	BY4742 *psd2*::*kanMX4* pRS416-*GFP-ATG8*
Δ*psd2* + Idp1-GFP	BY4742 *psd2*::*kanMX4* p*IDP1-GFP*
Δ*ept1* + GFP-Atg8	BY4742 *ept1*::*kanMX4* pRS416-*GFP-ATG8*
Δ*ept1* + Idp1-GFP	BY4742 *ept1*::*kanMX4* p*IDP1-GFP*
Δ*ale1* + GFP-Atg8	BY4742 *ale1*::*kanMX4* pRS416-*GFP-ATG8*
Δ*ale1* + Idp1-GFP	BY4742 *ale1*::*kanMX4* p*IDP1-GFP*
Δ*tgl3* + GFP-Atg8	BY4742 *tgl3*::*kanMX4* pRS416-*GFP-ATG8*
Δ*tgl3* + Idp1-GFP	BY4742 *tgl3*::*kanMX4* p*IDP1-GFP*
Δ*dpl1* + GFP-Atg8	BY4742 *dpl1*::*HIS3* pRS416-*GFP-ATG8*
Δ*dpl1* + Idp1-GFP	BY4742 *dpl1*::*HIS3* p*IDP1-GFP*
Δ*pho8* +mtPho8	BY4742 *pho8*::*kanMX4* + pFL39-*COXIV-PHO8Δ60*
Δ*psd1*Δ*pho8* +mtPho8	BY4742 *psd1*::*kanMX4 pho8::HIS3* pFL39-*COXIV-PHO8Δ60*
Δ*atg32*Δ*pho8* +mtPho8	BY4742 *atg32*::*kanMX4 pho8::HIS3* pFL39-*COXIV-PHO8Δ60*
Δ*psd1* + mtPsd2-V5 + Idp1-GFP	BY4742 *psd1*::*kanMX4 pho8::HIS3* pESC-*HIS3-COXIVmts-PSD2-V5* p*IDP1-GFP*
BY4742 + Atg3-GFP	BY4742 *atg3*::*ATG3-GFP*

BY4742 + Atg4-GFP	BY4742 *atg4*::*ATG4-GFP*
BY4742 + Atg32-V5	BY4742 pYES-*ATG32-V5*
Δ*psd1* + Atg32-V5	BY4742 *psd1*::*kanMX4* pYES-*ATG32-V5*
BY4742 + Ilv3-RFP + GFP-Atg8	*BY4742 pRS416-GFP-ATG8 pIlv3-GFP*

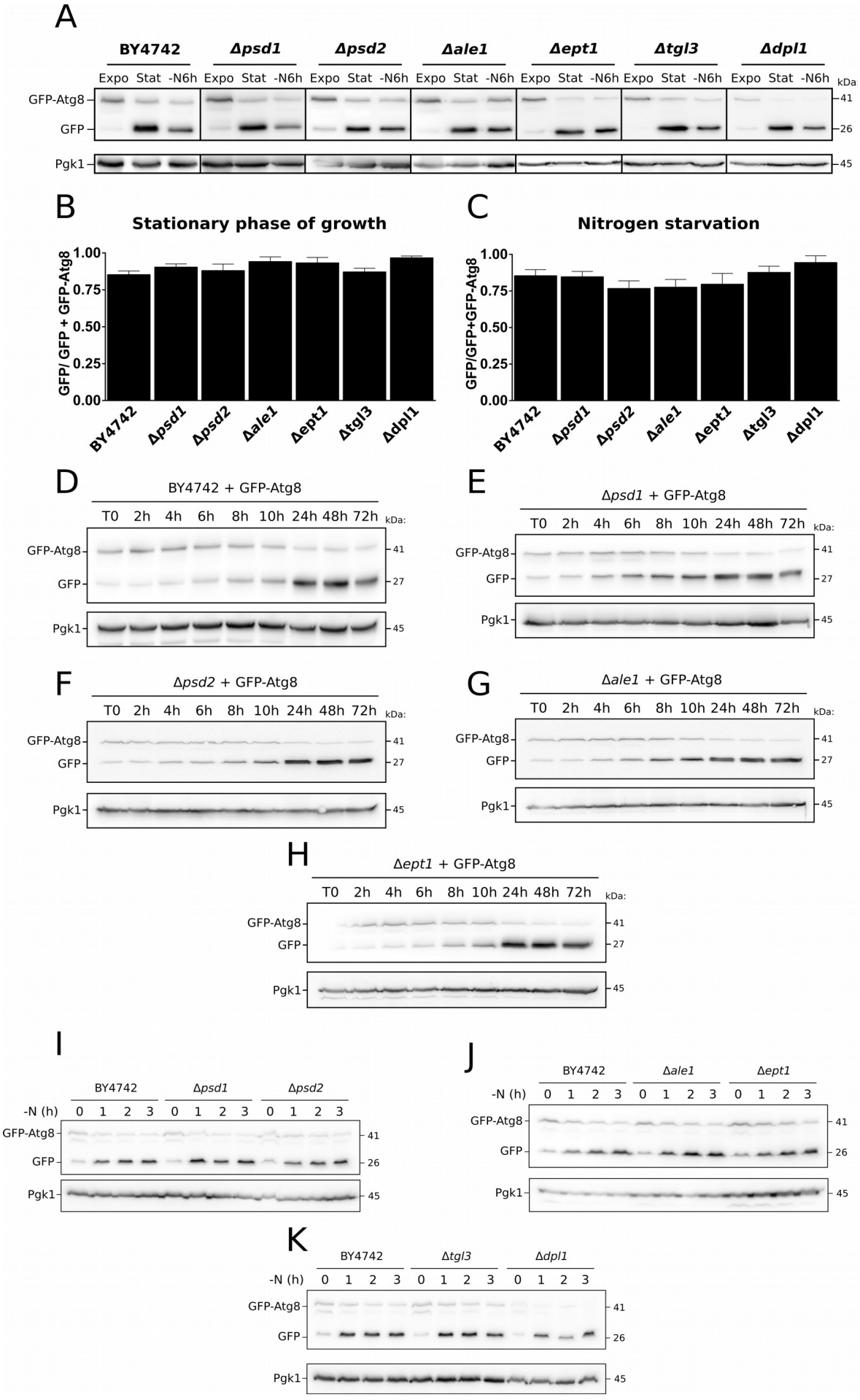

Figure S1: Autophagy induction is not altered in strains in which a single phosphatidylethanolamine pathway is impaired. (**A**) BY4742, *Δpsd1*, *Δpsd2*, *Δale1*, *Δept1*, *Δtgl3*, and *Δdpl1* strains grown in a respiratory carbon source medium and expressing GFP-Atg8, were harvested in a mid-exponential phase of growth (Expo) and submitted either for 6 hours of nitrogen starvation (-N6h) or 1 day of stationary phase of growth (Stat). Total protein extracts were prepared, separated in SDS PAGE and analyzed by western blots. For immunodetection, anti-GFP antibody was used to detect GFP-Atg8 and residual GFP. The cytosolic phosphoglycerate kinase, Pgk1, was used as loading control. Autophagy induction was quantified by calculating GFP/(GFP+GFP-Atg8) ratio in stationary phase of growth (**B**) or nitrogen starvation (**C**). Data were obtained from five independent experiments. BY4742 WT (**D**), *Δpsd1* (**E**), *Δpsd2* (**F**), *Δale1* (**G**) and *Δept1* (**H**) strains expressing GFP-Atg8, were harvested in a mid-exponential phase of growth (T0) and at the different time points until 72h after T0. Then, total protein extracts were prepared, proteins were separated by SDS-PAGE and GFP protein was visualized by western blots using Roche anti-GFP antibody. Pgk1, the cytosolic phosphoglycerate kinase was used as loading control. Experiments were performed twice with the same outcome. The same experiment was performed twice on cells used in (D-H) submitted for 1h, 2h or 3h of nitrogen starvation (**I**, **J**, **K**).

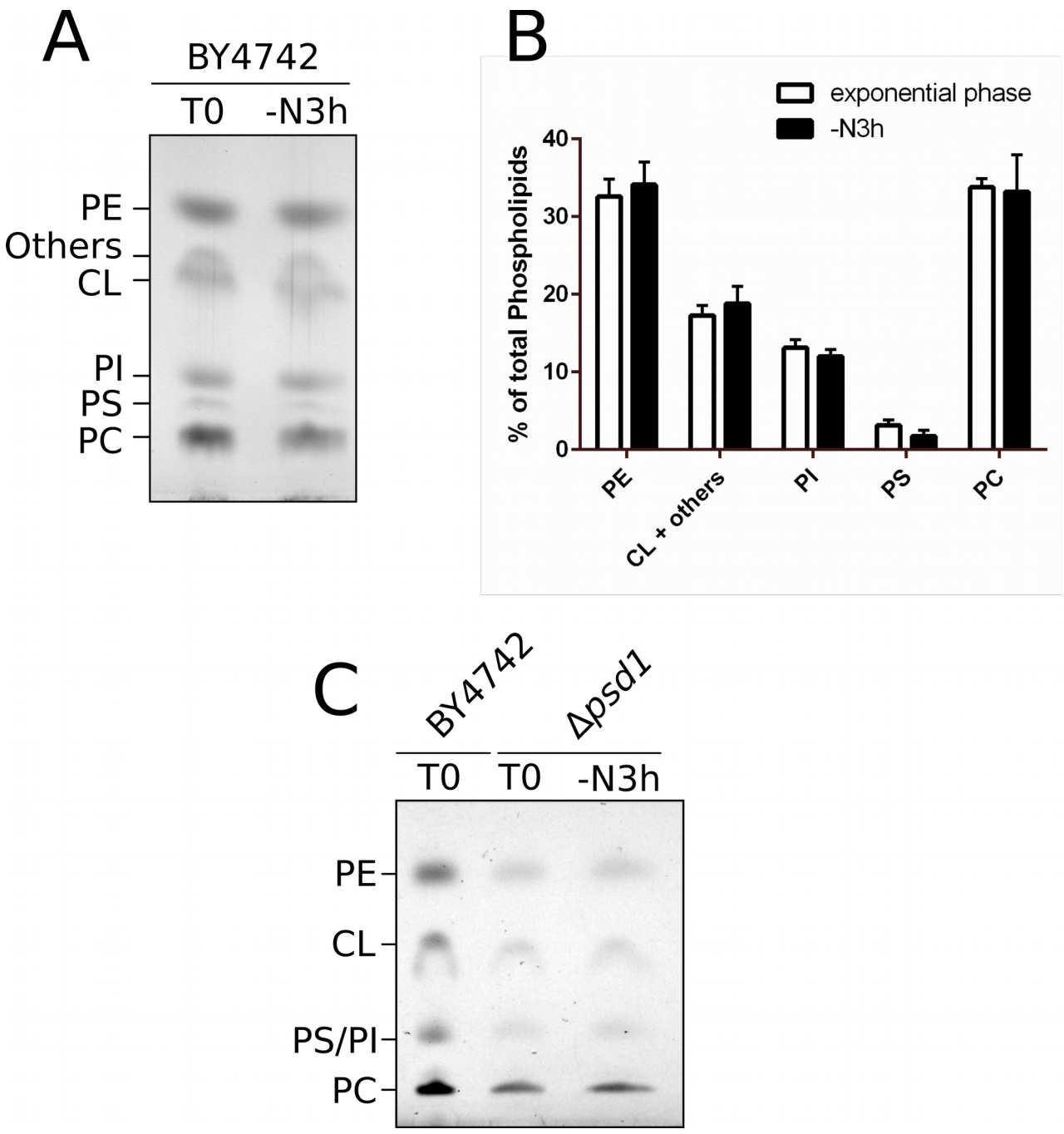

Figure S2: Nitrogen starvation does not affect lipid composition of mitochondria. (**A**) Mitochondria lipids were isolated from BY4742 cells harvested either in a mid-exponential phase of growth or after 3 hours of nitrogen starvation, separated by TLC afterward as described in the Material and Methods. (**B**) Lipids were quantified from TLC plates using ImageJ software (NIH). (**C**) The same experiment as described in (A) was carried out with mitochondria purified from Δ*psd1* cells, harvested in a mid-exponential phase of growth or after 3 hours of nitrogen starvation.

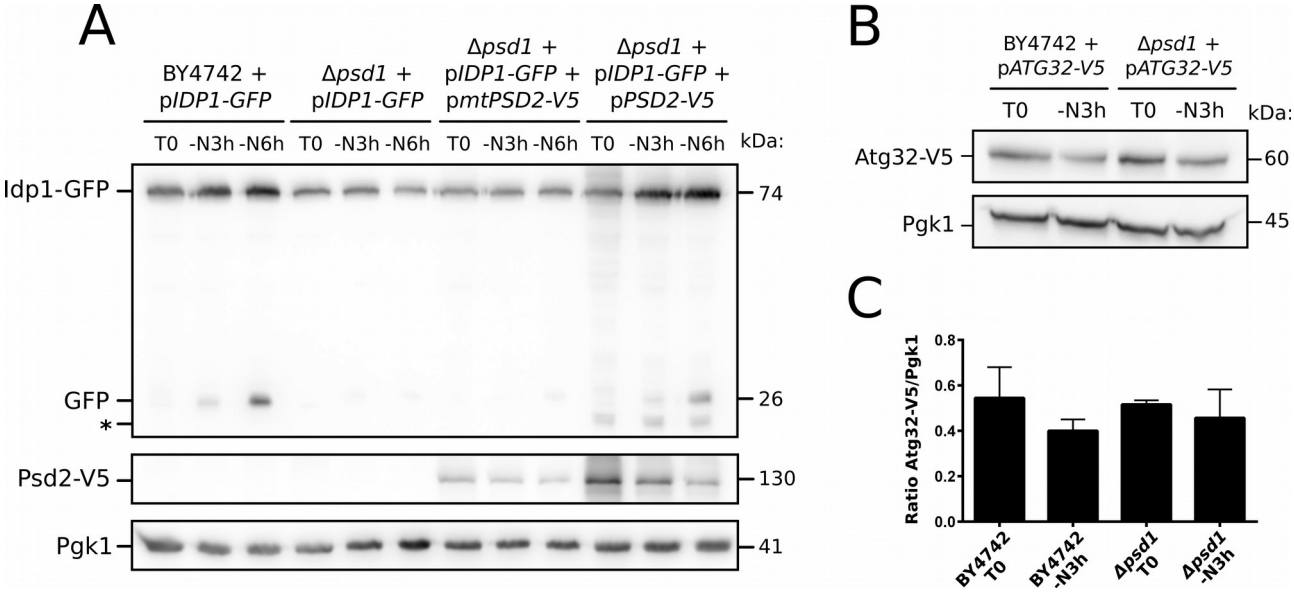

Figure S3: Overexpression of Psd2 rescues mitophagy defect in *Δpsd1* cells under nitrogen starvation. (**A**) Mitophagy was assessed using Idp1-GFP tool in *Δpsd1* cells overexpressing Psd2-V5 or a mitochondria-targeted version of Psd2-V5 (mtPSD2-V5). Cells were harvested in an exponential phase of growth (T0) or after 3 hours (-N3h) or 6 hours (-N6h) of nitrogen starvation, respectively. The corresponding total protein extracts were separated by SDS PAGE and analyzed by western-blots using anti-GFP antibody and anti-V5 antibody. Pgk1, the cytosolic phosphoglycerate kinase, was used as loading control. Results were obtained from 3 independent experiments. (**B**) Lactate-grown cells expressing Atg32-V5 were harvested either in an exponential phase of growth (T0) or after 3 hours of nitrogen starvation (-N3h). Then, total protein extracts were prepared, separated in SDS-PAGE and analyzed by western-blots. Anti-V5 antibody was used to detect Atg32-V5 protein. Pgk1, the cytosolic phosphoglycerate kinase, was used as loading control. (**C**) The ratio Atg32-V5/Pgk1 was calculated to appreciate Atg32-V5 expression in the corresponding strains and conditions. Results were obtained from three independent experiments.

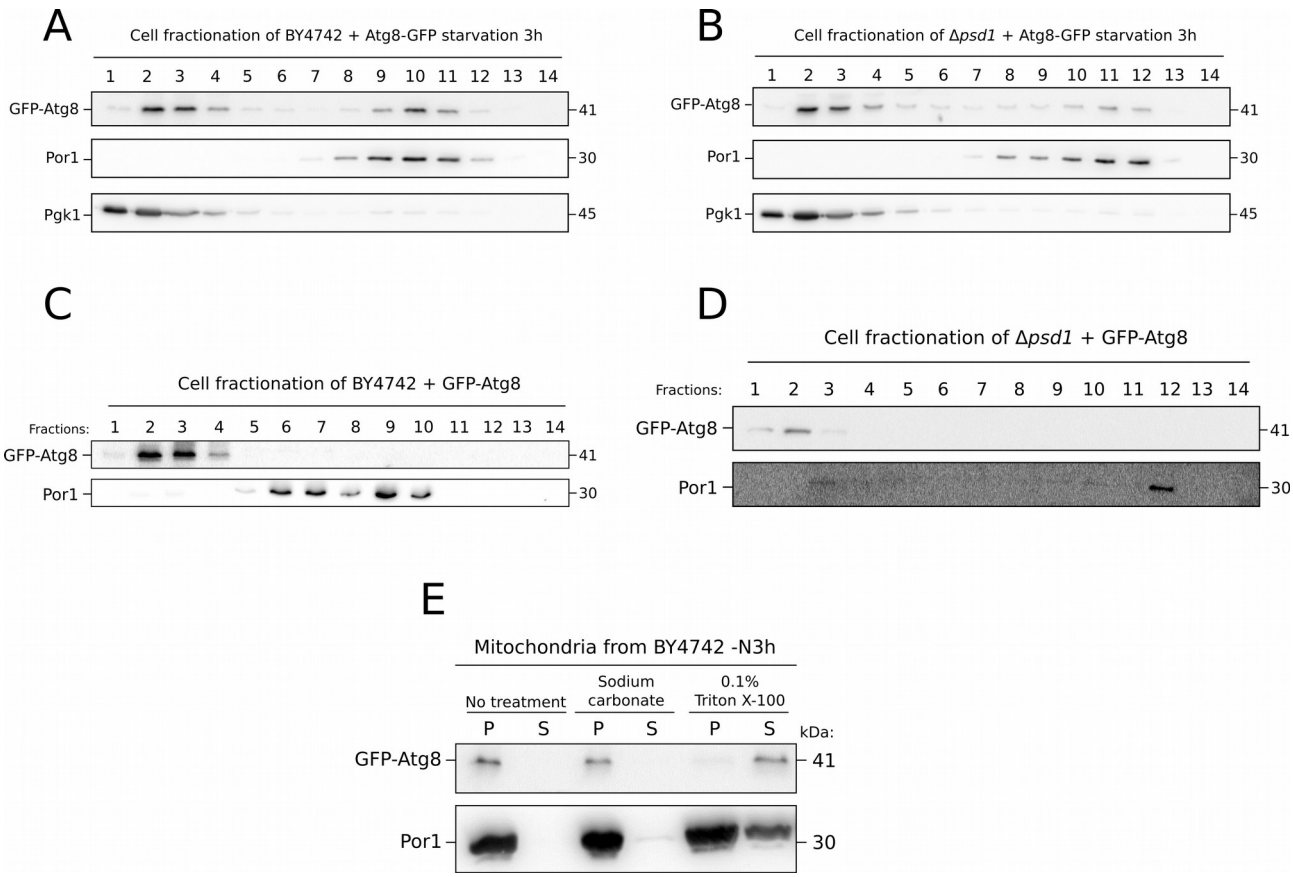

Figure S4: Study of GFP-Atg8 localization by cell fractionation. BY4742 and *Δpsd1* cells expressing GFP-Atg8 were grown in lactate-containing medium (**A**, **B**) or a glucose-containing medium (**C**, **D**) until a mid-exponential phase of growth and submitted to 3 hours of nitrogen starvation. Then, cells were harvested, lysed and cell lysates were separated in a 10-60% OptiprepTM density gradient. Fractions were collected after centrifugation and analyzed by western-blots. Anti-GFP antibody was used to visualize GFP-Atg8 and anti-Por1 to detect mitochondria-containing fractions. Pgk1 was used as a cytosolic marker. Each experiment was performed twice. (**E**) Mitochondria, purified from BY4742 cells submitted to 3 hours of nitrogen starvation, were treated with 100 mM sodium carbonate or 0.1% Triton X-100. After centrifugation, pellets (P) and supernatants (S) were analyzed by western-blots.

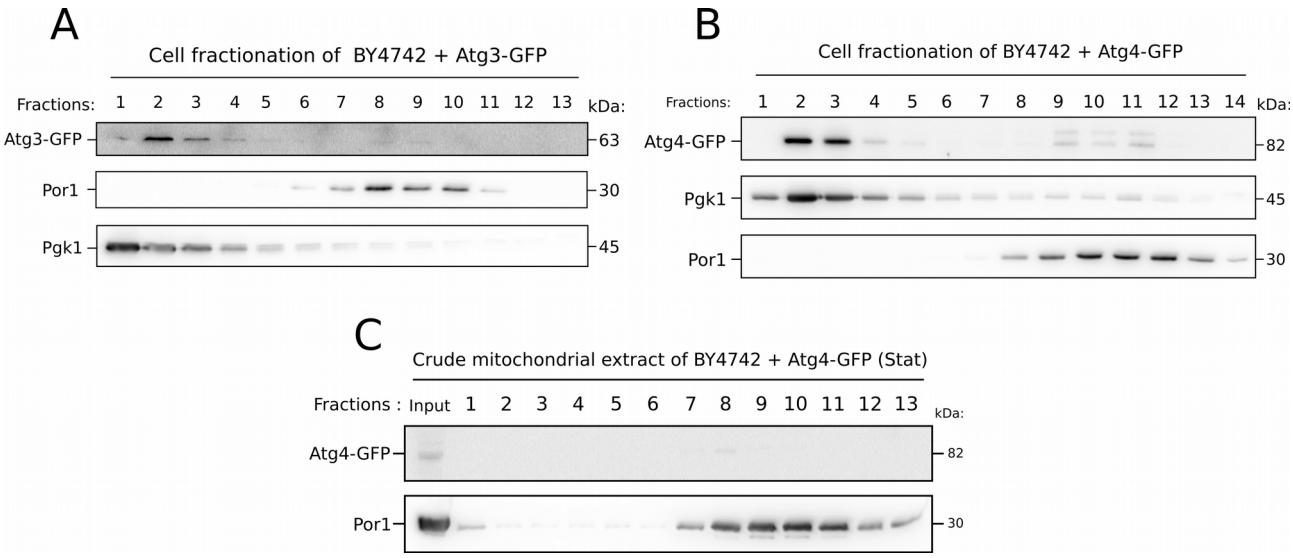

Figure S5: Study of Atg3-GFP and Atg4-GFP localization by cell fractionation. BY4742 cells expressing Atg3-GFP or Atg4-GFP were grown in lactate-containing medium until a mid-exponential phase of growth and submitted to 3 hours of nitrogen starvation (**A**, **B**) or one day of stationary phase of growth (**C**). Then, cells were harvested, lysed and cell lysates were separated in a 10-60% OptiprepTM density gradient. Fractions were collected after centrifugation and analyzed by western-blots. Anti-GFP antibody was used to visualize Atg3-GFP and Atg4-GFP, anti-Por1 to detect mitochondria-containing fractions. Pgk1 was used as a cytosolic marker. Experiments were performed twice.