Supplementary data

Figure S1: Expression of HA-Atg32 protein. (A) *atg32Δ* mutant cells grown in lactate-containing medium and expressing HA-Atg32 recombinant protein were harvested in a mid-exponential phase of growth (T0) and after 8-, 24-, and 48 hours from T0. Total protein extracts were prepared afterwards and protein samples were analyzed by western blots. Anti-HA antibody was used to visualize HA-Atg32 recombinant protein and Pgk1, the cytosolic phosphoglycerate kinase, was used as loading control. **(B)** Atg32-V5 expression was quantified as the percentage of Atg32-V5 level of T0 (100%). Experiments were carried out three times independently.

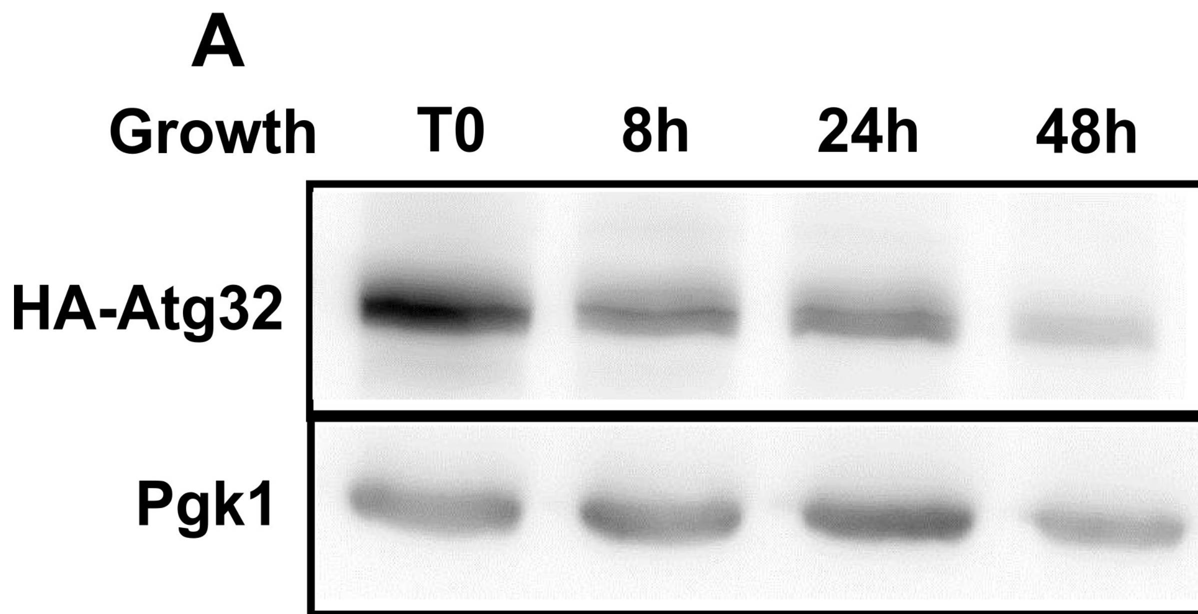
Figure S2: Study of Atg32-V5 expression in autophagy mutants. (A) *atg5Δ*, *atg8Δ,* and *atg11Δ* mutant cells transformed with a plasmid expressing Atg32-V5 were grown in a minimal synthetic medium containing lactate. Cells were harvested in early an exponential phase of growth (T0) and after 8-, 24-, and 48 hours of growth. **(B)** Atg32-V5/Pgk1 ratios were quantified at T0 and T48 for all tested strains. Three independent experiments were carried out for each condition. **(C)** *atg5Δ* cells expressing Atg5-V5 were treated with MG-132 (75 µM) and 0,003% SDS at time point 8h. Total protein extracts were prepared afterwards and protein samples were analyzed by western-blots. Anti-V5 antibody was used to visualize Atg32-V5 recombinant protein and Pgk1, the cytosolic phosphoglycerate kinase, was used as loading control.

Figure S3: The effect of MG-132 on rapamycin treatment and the Atg32 protein level in control strain (A) *atg32Δ* cells grown in a lactate-containing medium and expressing Atg32-V5 recombinant protein were harvested at T0 and treated with 1 µg/ml rapamycin was added to the cell culture for 3, 6 and 24h +/- 75 µM MG-132 and 0,003 % SDS. Total protein extracts were prepared afterwards and protein samples were analyzed by western blots. Anti-V5 antibody was used to visualize Atg32-V5 recombinant protein; Pgk1, the cytosolic phosphoglycerate kinase, was used as loading control. **(B)** BY4742 (wild-type) cells transformed with a plasmid expressing Atg32-V5 grown in a minimal synthetic medium supplemented with 2% lactate as a carbon source were harvested in an early exponential phase of growth (T0) and after 8-, 24-, and 48 hours of cell growth. To inhibit proteasome, 75 µM MG-132 and 0,003 % SDS were added to the cell culture at 8h time point. Total protein extracts were prepared afterwards and protein samples were analyzed by western blots. Anti-V5 antibody was used to visualize Atg32-V5 recombinant protein and Pgk1, the cytosolic phosphoglycerate kinase, was used as loading control. **(C)** Atg32-V5 expression was quantified as the percentage of Atg32-V5 level of T0 (100%). Experiments were carried out three times independently.

Figure S4: Effect of drugs treatment on growth and Atg32-V5 expression. (A) Addition of proteasome inhibitor MG-132 (75 µM MG-132 + 0,003 % SDS) and inhibitor of vacuolar proteolysis PMSF (2 mM) do not affect growth and growth yield in *atg32Δ* mutant expressing Atg32-V5 plasmid and grown in a minimal synthetic medium containing lactate. Y-axis is represented in logarithmic scale (n = 5 for control and MG-132 ; n = 3 for PMSF). **(B)** *atg32Δ* cells grown in a lactate-containing medium and expressing Atg32-V5 recombinant protein were harvested at different time points: T0 that represents a mid-exponential phase of growth and then after 24- and 48 hours of cell growth. To inhibit proteasome, 75 µM MG-132 + 0,003 % SDS were added to the cell culture at 8 h time point (T8). To inhibit vacuolar proteolysis, 2mM PMSF was added to the cell culture at T8.. Total protein extracts were prepared afterwards and protein samples were analyzed by western blots. Anti-V5 antibody was used to visualize Atg32-V5 recombinant protein; Pgk1, the cytosolic phosphoglycerate kinase, was used as loading control.

Figure S5: Mass spectrometry analysis. (A) SEQUEST spectra, (B) MASCOT spectra, and (C) Protein coverage.

Figure S1



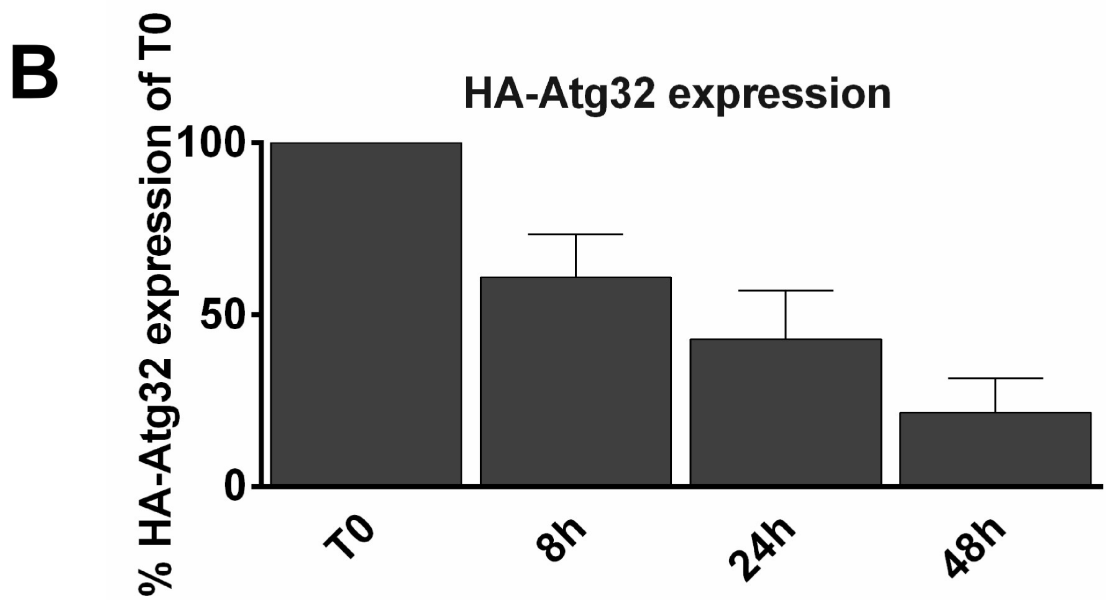
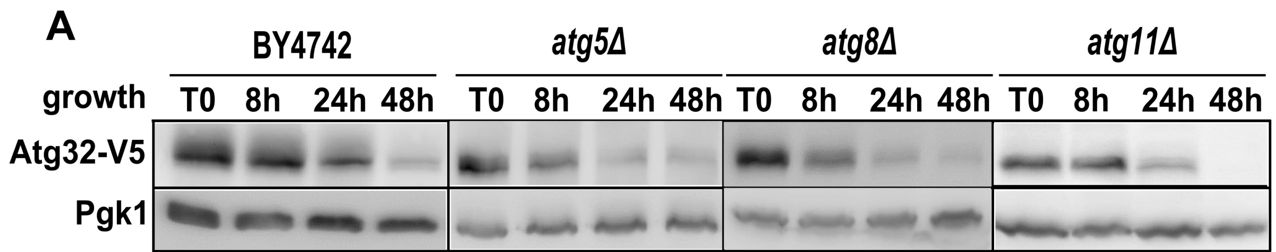
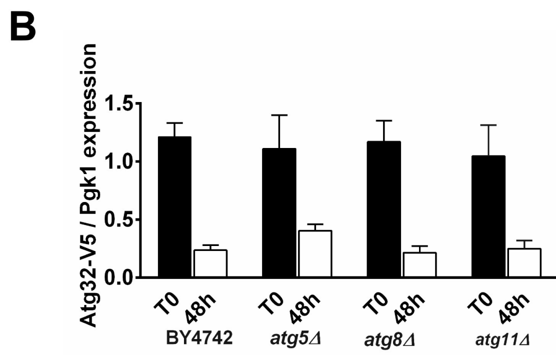


Figure S2





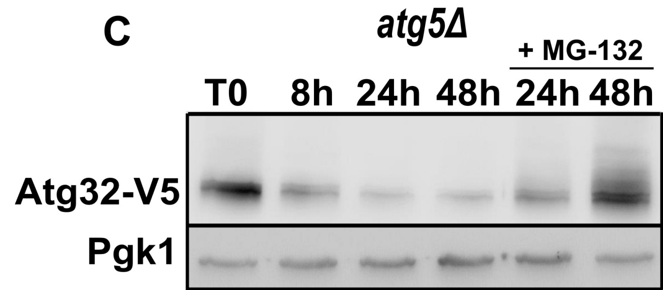
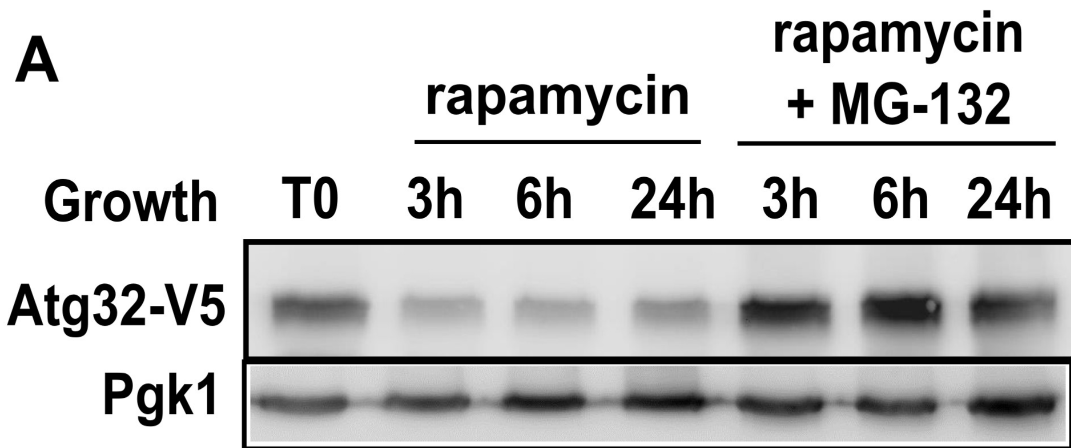
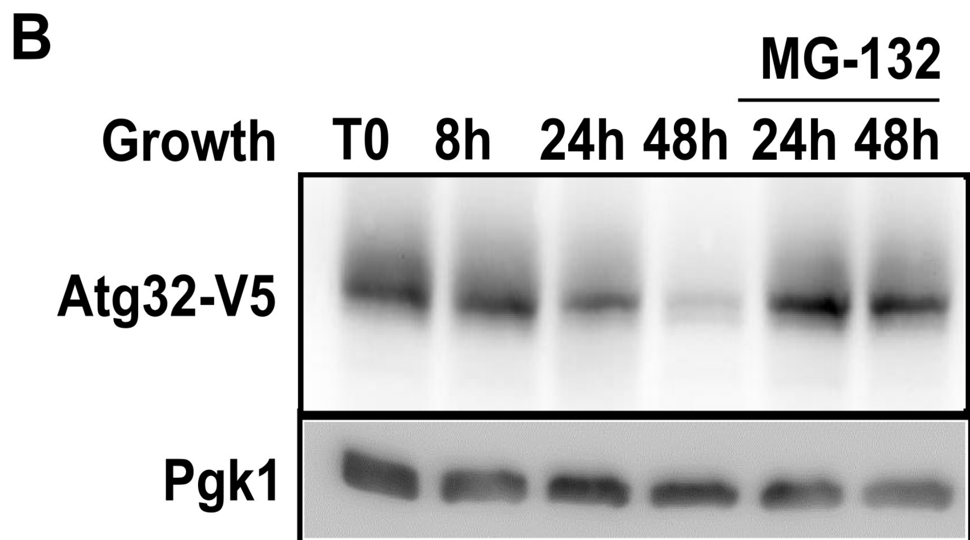


Figure S3





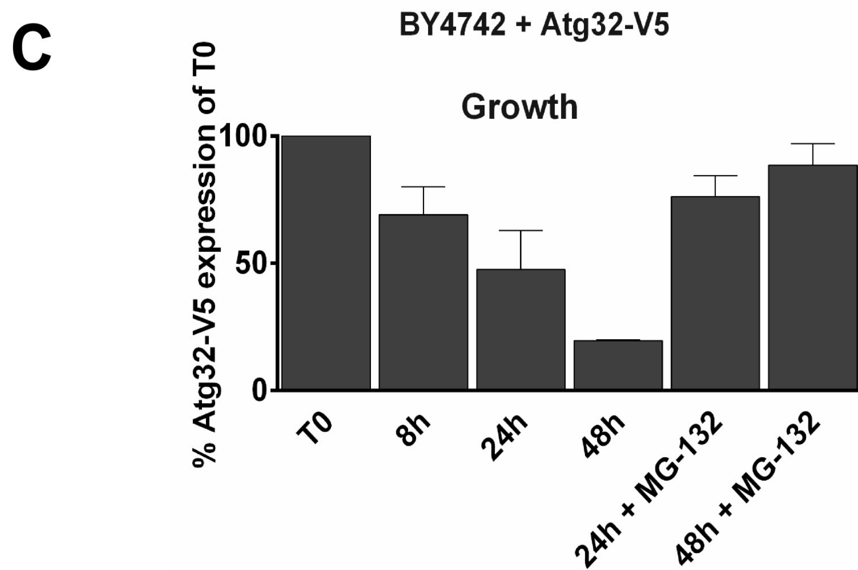
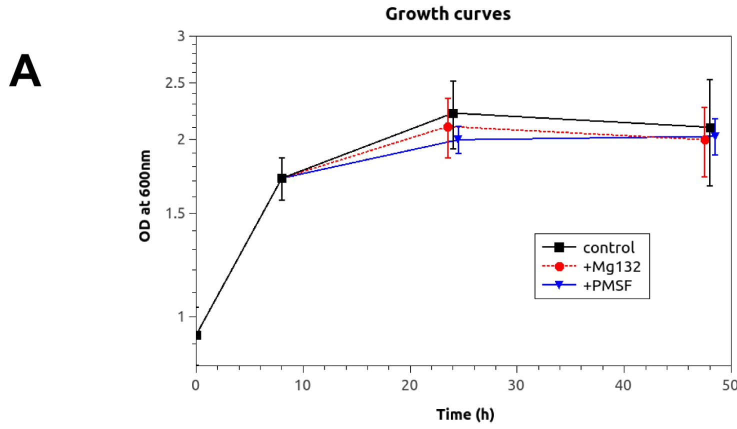


Figure S4

A



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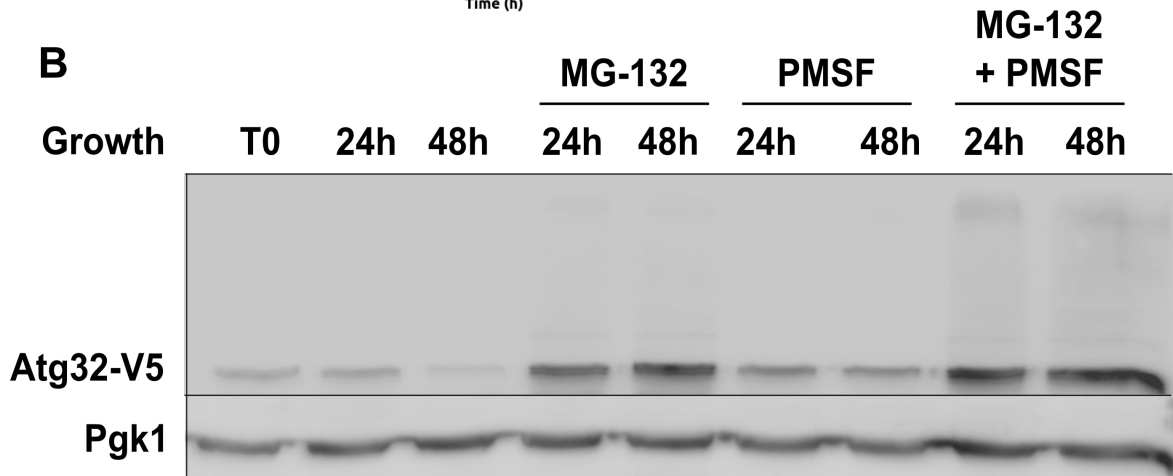
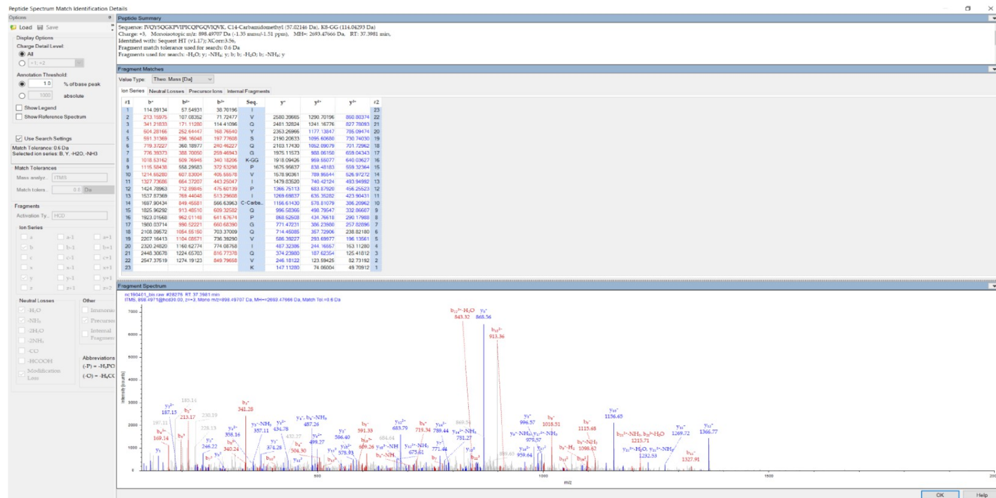
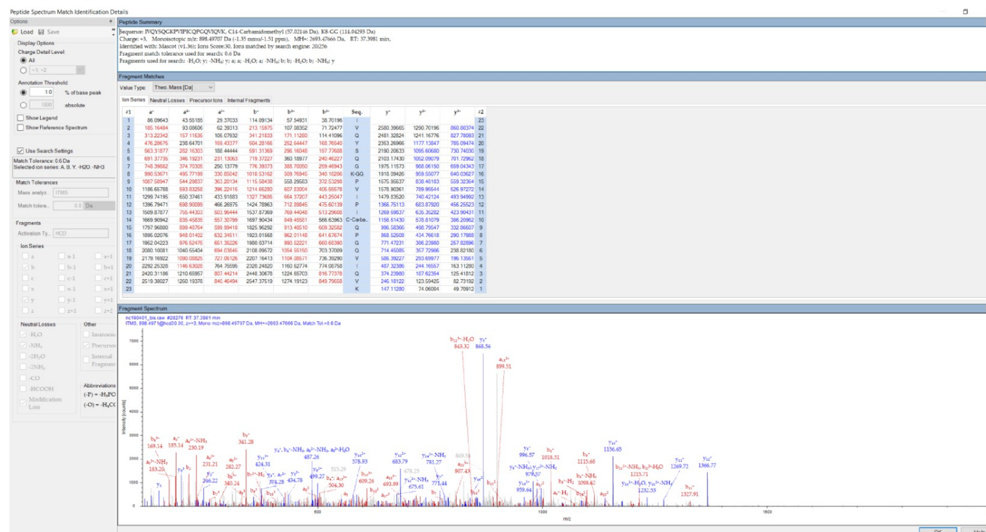


Figure S5

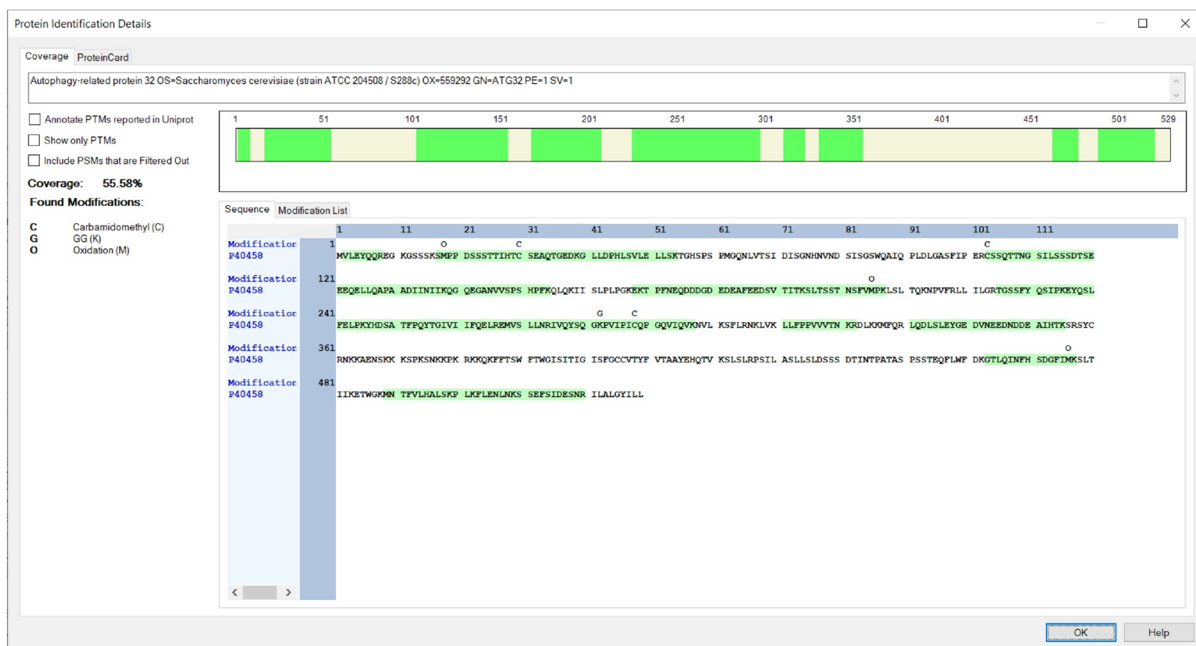
Peptide SEQUEST



Peptide MASCOT



Protein coverage



Supplementary Table 1

STRAINS

Souche	**Genotype**
BY4742 + Idp1-GFP+ Atg32-V5	Mat α ; *his3Δ1;leu2Δ0 ; lys2Δ0 ; ura3Δ0 ; pIDP1-GFP, pYES-Atg32-V5*
BY4742 + pPROM-*ATG32-β-galactosidase*,	Mat α ; *his3Δ1;leu2Δ0 ; lys2Δ0 ; ura3Δ0 ;* YEp357-prom*ATG32-lacZ*
Δ*atg32* + Idp1-GFP+ Atg32-V5	BY4742 *atg32*::*kanMX4* p*IDP1-GFP, pYES-Atg32-V5*
Δ*atg32* + HA- Atg32-V5	BY4742 *atg32*::*kanMX4* p*HA-Atg32*
Δ*bre5* + Idp1-GFP+ Atg32-V5	BY4742 *bre5*::*kanMX4* p*IDP1-GFP, pYES-Atg32-V5*
Δ*ubp3*+ Idp1-GFP+ Atg32-V5	BY4742 *ubp3*::*kanMX4* p*IDP1-GFP, pAtg32-V5*
Δ*yme1*+ Idp1-GFP+ Atg32-V5	BY4742 *yme1*::*kanMX4* p*IDP1-GFP, pAtg32-V5*
Δ*atg5* + Atg32-V5	BY4742 *atg5*::*kanMX4 pYES-Atg32-V5*
Δ*atg8* + Atg32-V5	BY4742 *atg8*::*kanMX4, ppYES-Atg32-V5*
Δ*atg11*+ Atg32-V5	BY4742 *atg11*::*kanMX4, pYES-Atg32-V5*
Δ*atg32* + Idp1-GFP	BY4742 *atg32*::*kanMX4, pIDP1-GFP*
Δ*atg32*+ HA-Atg32	BY4742 *atg11*::*kanMX4, pHA-Ag32*
pre2-2 + Atg32-V5	BY4742 *his3D1; leu2D0; met15D0; ura3D0 pre2-2::KanMX4 pYES-Atg32-V5*
Δ*atg32*Δ*pho8* + mtPho8	BY4742 *atg32*::*kanMX4 pho8::HIS3* pFL39-*COXIV-PHO8*Δ*60*