Supplemental Material to:

A Pseudo-Receiver Domain in Atg32 is Required for Mitophagy

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***Equal contribution**

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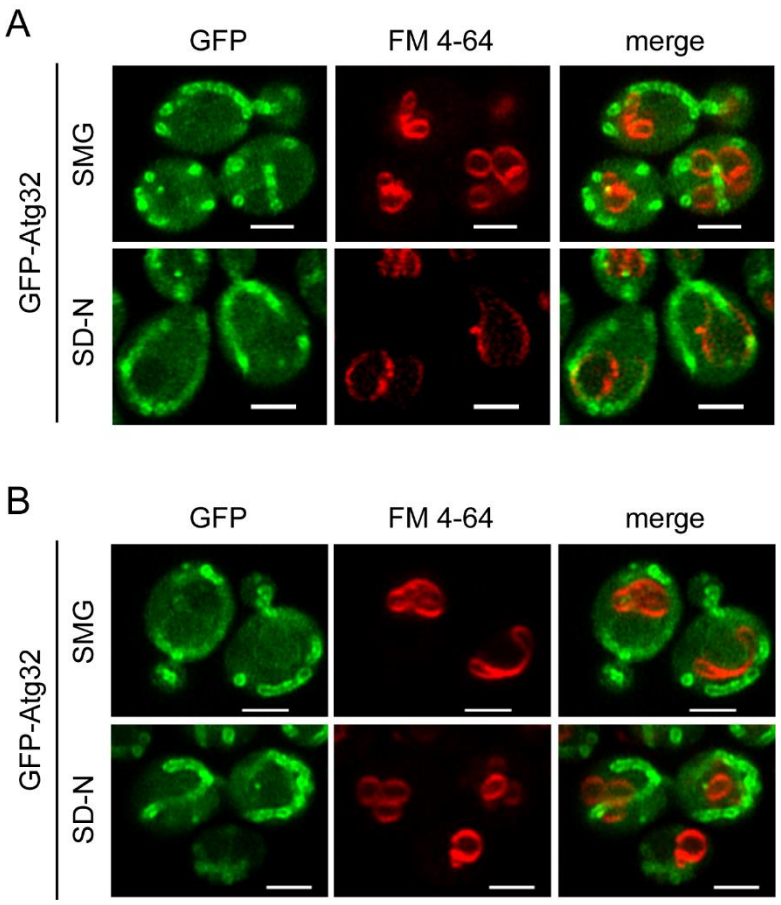

Figure S1. GFP-Atg32 vacuole localization requires mitophagy. The localization of GFP-Atg32 was monitored in (**A**) *atg1Δ* and (**B**) *atg11Δ S. cerevisiae* in nutrient rich (SMG) and starvation (SD-N) conditions. The vacuole was stained using FM 4-64. Over 500 cells were imaged per condition, and of these 2% or less of the cells contained GFP in the vacuole. Scale bar: 2 µm.

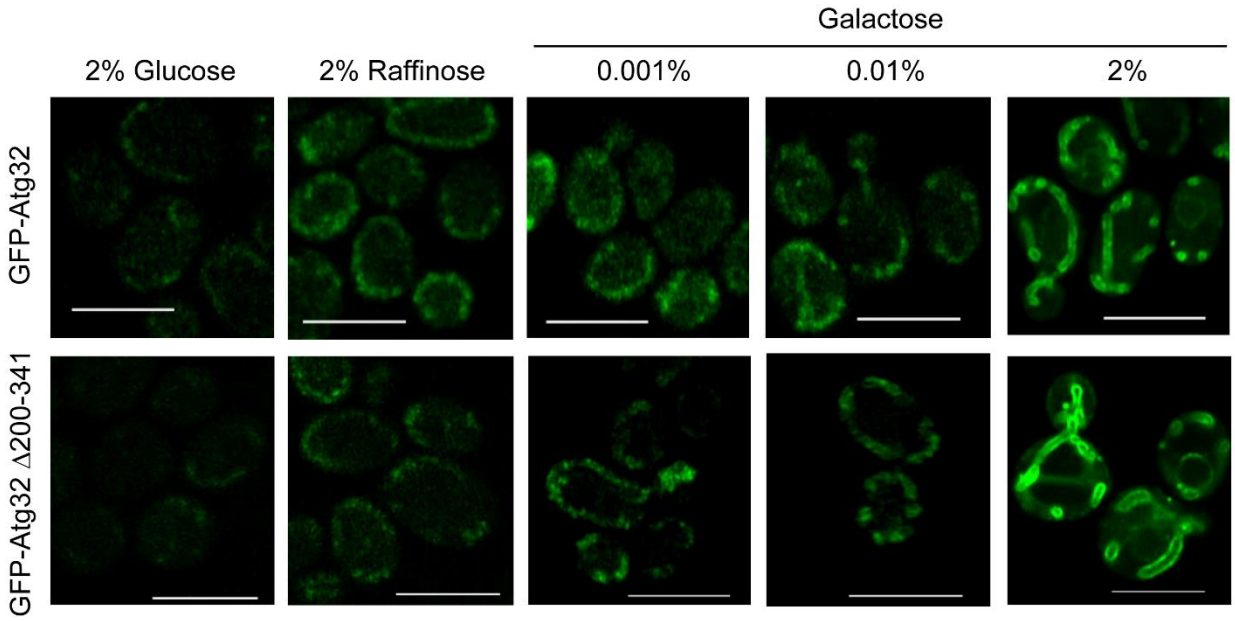

Figure S2. Expression of GFP-Atg32 with varying levels of induction. GFP-Atg32 expression was driven by the GAL1 promoter which is activated by galactose. Different concentrations of galactose were added to the media and the localization of GFP-Atg32 and GFP-Atg32Δ200-341 was monitored. In the samples cultured with 0.001% or 0.01% galactose, raffinose was supplemented to achieve a total sugar concentration of 2%. All samples were imaged with the same settings. The 2% galactose samples were processed using a wider linear range to reveal the location of GFP-Atg32. Scale bar: 5 µm.

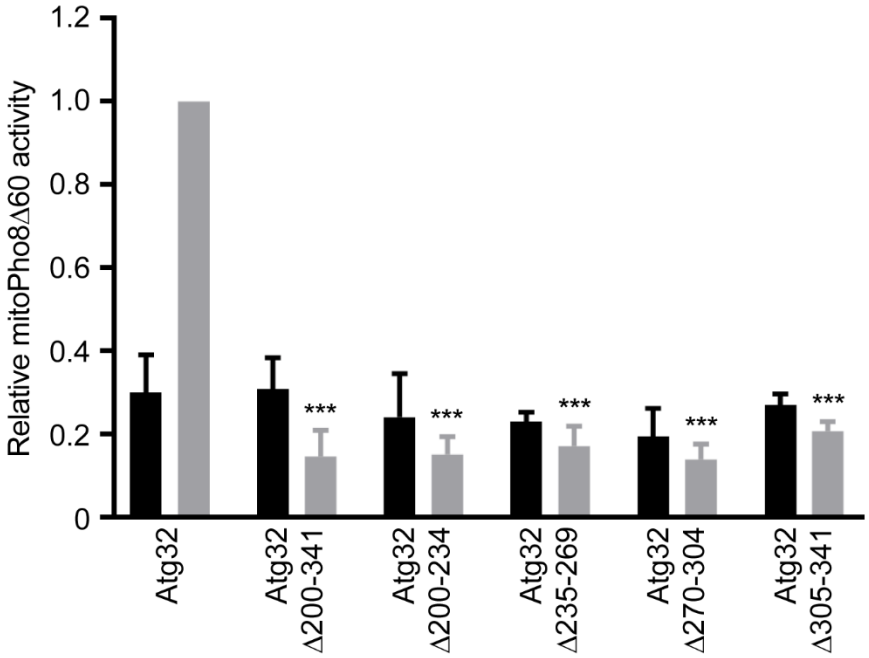

Figure S3. Mitophagy activity with different Atg32 deletions. XX1 cells were transformed with Atg32 or Atg32 deletions and expression was driven by their endogenous promoters. Phosphatase activity for cells grown in SML is shown as black bars and SD-N is shown as grey bars. Results were normalized to phosphatase activity in cells expressing Atg32 that were starved. Error bars represent the average of 3 independent measurements. Significance was determined by 2-way ANOVA where $p<0.001$ was considered to be significant (***).

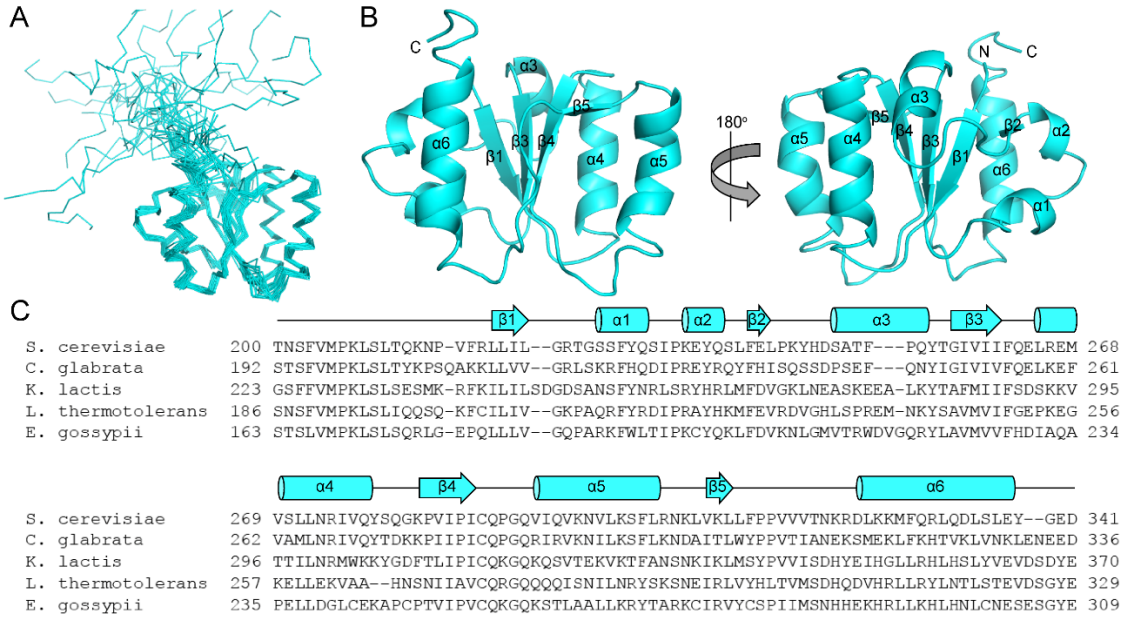

Figure S4. The solution structure of Atg32[200-341]. (**A**) The bundle of the 20 energy lowest energy structures superimposed over residues 216-336. (**B**) Cartoon representation of Atg32 residues 216-341. Two orientations of the structure are shown rotated by 180º. All secondary structure is labeled. (**C**) Sequence alignment of Atg32[200-341] from five different yeast species. Residue numbers for the first and last amino acid in each line are labeled. Secondary structure from the *S. cerevisiae* Atg32[200-341] solution structure is shown above the alignment.

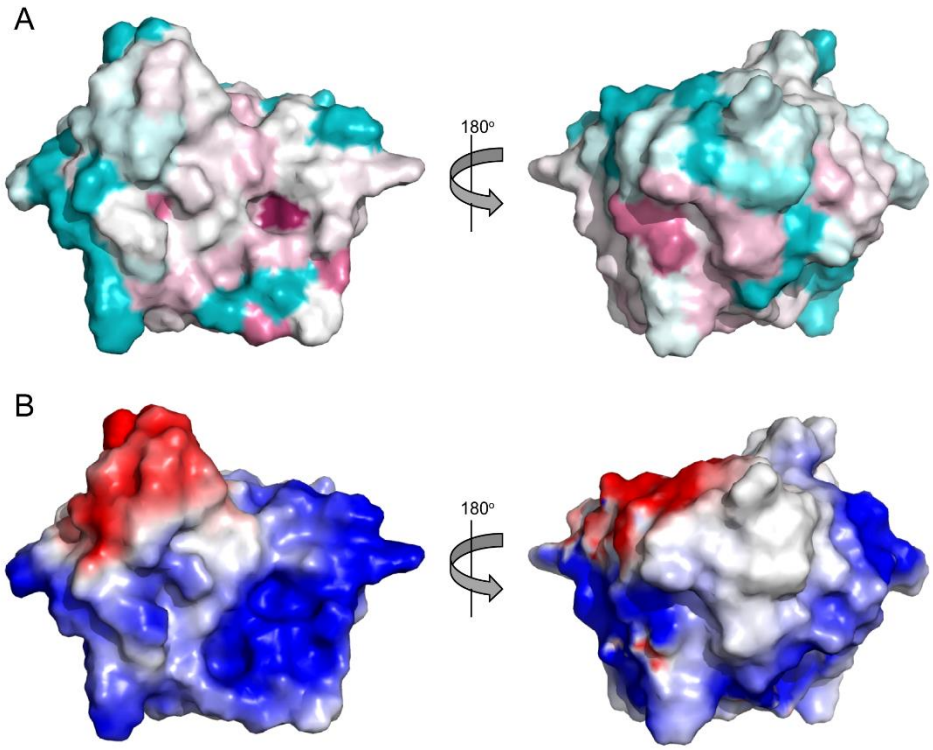

Figure S5. Surface properties of Atg32[200-341]. (**A**) Surface conservation generated using the ConSurf Server. A sequence alignment was generated using 13 additional Atg32 sequences from different yeast species. Turquoise represents low conservation, white represents average conservation and maroon represents highly conserved residues. (**B**) Electrostatic surface representation of the Atg32[200-341] generated using PyMOL. Two views of the surface are shown in both (**A**) and (**B**) and are rotated by 180°.

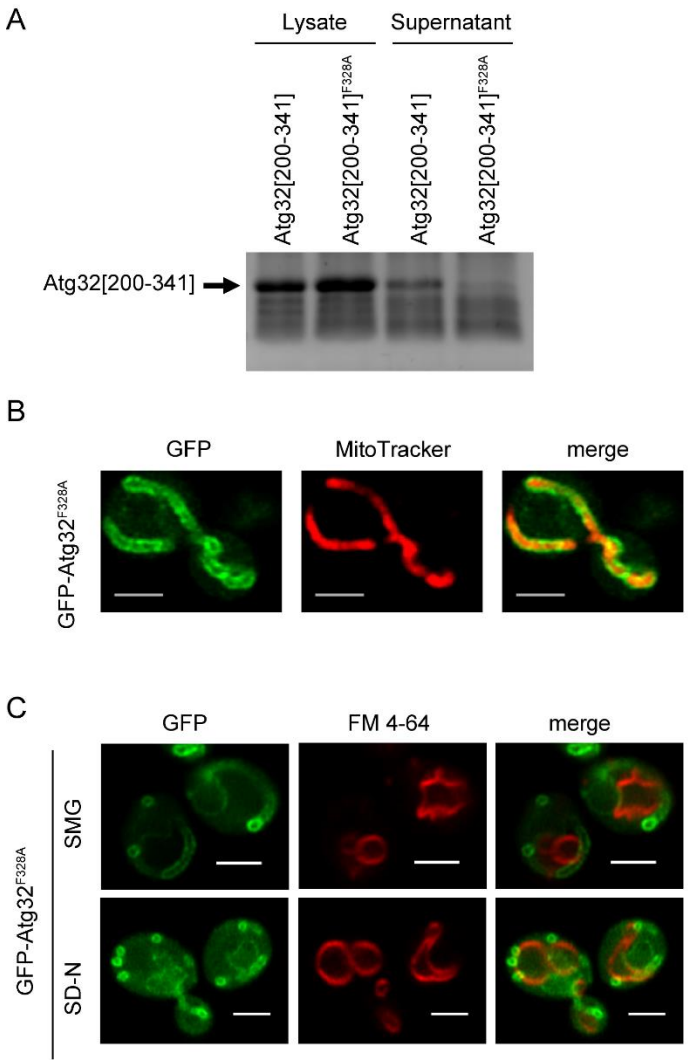

Figure S6. Characterization of Atg32^{F328A}. (**A**) Atg32[200-341]^{F328A} was expressed in *E. coli*. Total lysate and supernatant after clearing the lysate are shown. The band corresponding to the recombinant protein is labeled. (**B**) GFP-Atg32^{F328A} localization to mitochondria stained with MitoTracker. (**C**) GFP-Atg32^{F328A} localization in nutrient rich conditions (SMG) and after 1 h of starvation (SD-N). The vacuole was stained with FM 4-64. Over 500 cells were imaged per treatment group, and of these 2% or less contained GFP in the vacuole. Scale bar: 2 µm.

Table S1. Yeast strains used in this study.

Strain	Genotype	Source or reference
atg1Δ	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 atg1Δ::KAN*	Invitrogen
atg11Δ	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 atg11Δ::KAN*	Invitrogen
atg32Δ	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 atg32Δ::KAN*	Invitrogen
KMB010	SEY6210 *atg32Δ::NAT*	This study
KWY90	SEY6210 *pho8Δ::KAN pho13Δ::Ble pRS406-ADH1-COX4-pho8Δ60*	1
SEY6210	MATα *his3-Δ200 leu2-3,112 lys2-801, trp1-Δ901* 2*ura3-52 suc2-Δ9 GAL*	2
TKYM130	SEY6210 *OM45-GFP::TRP1 atg32Δ::URA3*	3
XX1	KWY90 *atg32Δ::HIS*	This study

References

1. Wang K, Jin M, Liu X, Klionsky DJ. Proteolytic processing of Atg32 by the mitochondrial i-AAA protease Yme1 regulates mitophagy. Autophagy 2013; 9:1828-36. doi:10.4161/auto.26281.

2. Robinson JS, Klionsky DJ, Banta LM, Emr SD. Protein sorting in Saccharomyces cerevisiae: isolation of mutants defective in the delivery and processing of multiple vacuolar hydrolases. Mol Cell Biol 1988; 8:4936-48.

3. Mao K, Wang K, Zhao M, Xu T, Klionsky DJ. Two MAPK-signaling pathways are required for mitophagy in Saccharomyces cerevisiae. J Cell Biol 2011; 193:755-67. doi:10.1083/jcb.201102092.