

Supplemental Material to:

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**Proteolytic processing of Atg32 by the mitochondrial
i-AAA protease Yme1 regulates mitophagy**

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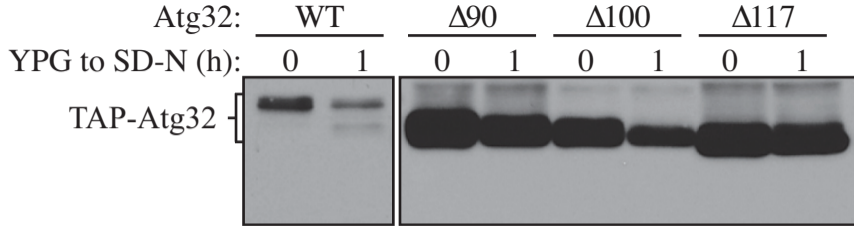


Figure S1. Atg32 processing occurs at its C terminus. Cells expressing *GAL1* promoter-driven TAP-Atg32 (KWY100), TAP-Atg32 $\Delta 90$ (KWY129), TAP-Atg32 $\Delta 100$ (KWY144) or TAP-Atg32 $\Delta 117$ (KWY145) were cultured in YPG to mid-log phase and shifted to SD-N for 1 h. TAP-Atg32 was monitored by immunoblotting with an antibody that binds to PA.

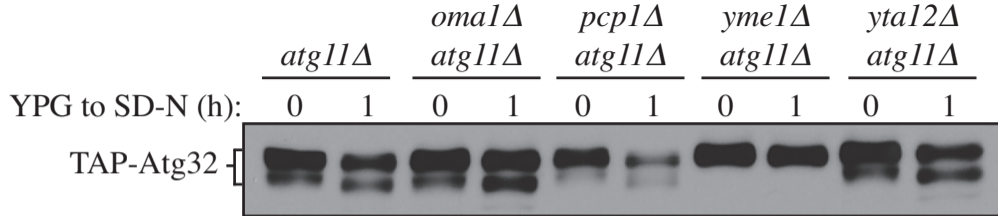


Figure S2. Screen for the protease that mediates Atg32 processing. Cells expressing *GAL1* promoter-driven TAP-Atg32 in *atg11Δ* (KWY104), *atg11Δ oma1Δ* (KWY113), *atg11Δ pcp1Δ* (KWY115), *atg11Δ yme1Δ* (KWY114) and *atg11Δ yta12Δ* (KWY117) backgrounds were cultured in YPG to mid-log phase and shifted to SD-N for 1 h. TAP-Atg32 was monitored by immunoblotting with an antibody that binds to PA.

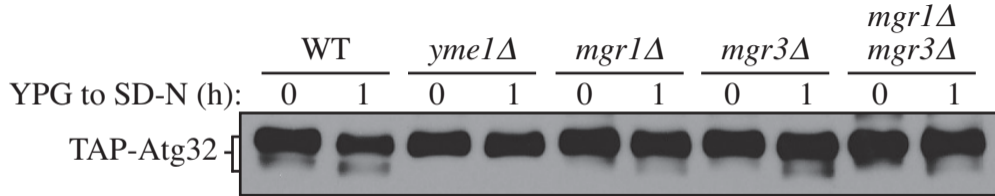
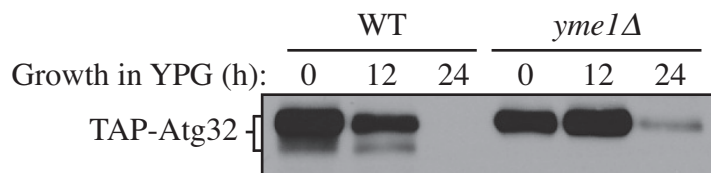


Figure S3. Mgr1 and Mgr3 are not critical for Atg32 processing. Cells expressing *GAL1* promoter-driven TAP-Atg32 in wild-type (WT; KWY100), *yme1*Δ (KWY118), *mgr1*Δ (KWY146), *mgr3*Δ (KWY147) and *mgr1*Δ *mgr3*Δ (KWY148) backgrounds were cultured in YPG to mid-log phase and shifted to SD-N for 1 h. TAP-Atg32 was monitored by immunoblotting with an antibody that binds to PA.

A



B

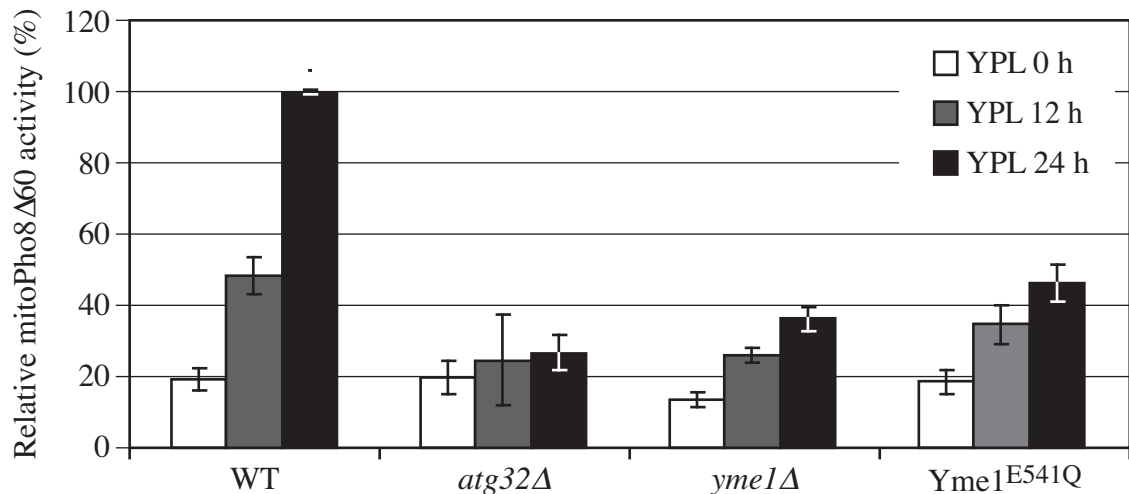


Figure S4. Yme1 is required for Atg32 processing and mitophagy during post-log phase growth. (A) Cells expressing *GALI* promoter-driven TAP-Atg32 in wild-type (WT; KWY100) and *yme1Δ* (KWY118) backgrounds were cultured in YPG to mid-log (0 h) and post-log (12, 24 h) phases. TAP-Atg32 was monitored by immunoblotting with an antibody that binds to PA. (B) Wild-type (KWY20), *yme1Δ* (KWY136) and *atg32Δ* (KWY22) strains expressing mitoPho8Δ60 were grown in YPL to mid-log (0 h) and post-log (12, 24 h) phases. Samples were collected and protein extracts were assayed for mitoPho8Δ60 activity. The results represent the mean and standard deviation (SD) of three independent experiments.

Table S1. Strains used in this study.

Strain	Genotype	Reference
KWY20	SEY6210 <i>pho8Δ::TRP1 pho13Δ::LEU2</i> pRS406- <i>ADHI-COX4-pho8Δ60</i>	1
KWY22	SEY6210 <i>pho8Δ::TRP1 pho13Δ::LEU2</i> pRS406- <i>ADHI-COX4-pho8Δ60 atg32Δ::KAN</i>	1
KWY90	SEY6210 <i>pho8Δ::KAN pho13Δ::Ble</i> pRS406- <i>ADHI-COX4-pho8Δ60</i>	This study
KWY100	SEY6210 <i>GAL1-TAP-ATG32::TRP1</i>	This study
KWY101	KWY100 <i>atg1Δ::HIS5 S.p.</i>	This study
KWY104	KWY100 <i>atg11Δ::LEU2</i>	This study
KWY110	KWY100 <i>ATG32-GFP::HIS3MX6</i>	This study
KWY111	SEY6210 <i>GAL1-GFP-ATG32::His3MX6</i>	This study
KWY113	KWY104 <i>oma1Δ::HIS5 S.p.</i>	This study
KWY114	KWY104 <i>yme1Δ::URA3</i>	This study
KWY115	KWY104 <i>pcp1Δ::HIS5 S.p.</i>	This study
KWY117	KWY104 <i>yta12Δ::URA3</i>	This study
KWY118	KWY100 <i>yme1Δ::HIS5 S.p.</i>	This study
KWY121	KWY90 <i>ATG32-GFP::HIS3MX6</i>	This study
KWY129	KWY100 <i>atg32Δ90::HIS5 S.p.</i>	This study
KWY134	KWY100 <i>YME1-GFP::HIS3MX6</i>	This study
KWY136	KWY20 <i>yme1Δ::HIS5 S.p.</i>	This study
KWY138	KWY20 <i>YME1-E541Q-GFP::HIS3MX6</i>	This study
KWY139	<i>pATG32-TAP-ATG32</i>	This study
KWY140	<i>pATG32-TAP-Atg32, yme1Δ::HIS5 S.p.</i>	This study

KWY141	KWY100 <i>YME1-E541Q-GFP::HIS3MX6</i>	This study
KWY142	SEY6210 <i>yme1Δ::HIS5 S.p.</i>	This study
KWY143	WLY176 <i>yme1Δ::HIS5 S.p.</i>	This study
KWY144	KWY100 <i>atg32Δ100::HIS5 S.p.</i>	This study
KWY145	KWY100 <i>atg32Δ117::HIS5 S.p.</i>	This study
KWY146	KWY100 <i>mgr1Δ::HIS5 S.p.</i>	This study
KWY147	KWY100 <i>mgr3Δ::HIS5 S.p.</i>	This study
KWY148	KWY146 <i>mgr3Δ::LEU2</i>	This study
SEY6210	MATα <i>his3-Δ200 leu2-3,112 lys2-801, trp1-Δ901 ura3-52 suc2-Δ9 GAL</i>	2
WLY176	SEY6210 <i>pho13Δ pho8Δ60</i>	1
WLY192	SEY6210 <i>pho13Δ::KAN pho8Δ60::URA3 atg1Δ::HIS5</i>	1

Supplemental references

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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.