Supporting Information

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SI Materials and Methods

Reagents, Antibodies, and Plasmids. Yeast nitrogen base was purchased from Difco and cerulenin from Fermentek. Rabbit anti-FAS antibodies were a kind gift from Michael Thumm (Stuttgart, Germany). Mouse anti-Pgk1 was from Molecular Probes, mouse anti-GFP was from Covance, and rabbit anti-GFP was from Invitrogen. Anti-ApeI polyclonal antibodies were prepared by using two synthetic peptides that were conjugated to keyhole limpet hemocyanin and then injected into rabbits to produce anti-ApeI antiserum that recognizes both precursors. The pRS316-GFP-Atg8 plasmid under the promoter of Atg8 and the Pyes-GFP-Atg8 plasmid under the galactose promoter were previously described (1). All mutations in the Atg8 ORF were created with the QuikChange Site-Directed Mutagenesis Kit (Stratagene). pEW410 Atg24-GFP under the promoter of Atg24 was a kind gift from Hugh Pelham (Cambridge, UK).

Microscopy. Fluorescence microscopy was performed using DeltaVision microscope (Applied Precision). DeltaVision images were deconvoluted using the SoftWoRx software (Applied Precision). For FM4-64 staining, 40 μM FM4-64 (Invitrogen) was added to the cells at room temperature for 15–30 min. The cells were then collected (1,500 × *g*, 5 min) and resuspended in fresh medium (YPD or SD-N) for 15–30 min, and the chase was repeated once. Cells were washed (1,500 × *g*, 5 min) and visualized in a fluorescence microscope.

Coimmunoprecipitation. For immunoprecipitation experiments, cells (50 OD_{600} units) were harvested during log-phase or under nitrogen starvation, washed with double distilled water (DDW), and resuspended in breaking buffer (PBS + 0.5% Nonidet P-40) with glass beads and a mixture of protease inhibitors. Cells were lysed by vortexing for 1 min and then incubated for 1 min on ice. This process was repeated seven times. Extracts were centrifuged at 1,000 × *g* for 5 min at 4 °C to remove intact cells and cell-wall debris. The total yeast lysate was then centrifuged at 245,000 × *g* for 30 min and supernatant was taken for immunoprecipitation experiments using rabbit polyclonal anti-GFP antibody. Proteins (2 mg) were immunoprecipitated from total cellular material and subjected to SDS/PAGE followed by Western blot analysis.

Silver Staining. After samples were separated on SDS gel, proteins were fixed for 30 min in 50:5:45 (vol/vol/vol) methanol:acetone:water. Gels were washed twice with DDW and incubated for 1 h on a shaker with DDW. Gel sensitization was performed for 1–2 min in sensitizing solution (0.02% sodium thiosulfate) and washed twice with water. Gels were then incubated for 30 min at 4 °C with chilled 0.1% $AgNO_3$. The silver nitrate was discarded and the gel was washed twice with DDW and developed in 0.04% formaldehyde, 2% sodium carbonate (wt/vol). Staining was quenched with 1% acetic acid.

Mass Spectrometry. Mass spectrometry (MS) analysis was performed at the Biological Mass Spectrometry Unit in the Department of Biological Services at the Weizmann Institute of Science. Samples were analyzed in LTQ-Orbitrap (Thermo Fisher Scientific) operated in the positive-ion mode nano-LC-ESI-MS/MS. Peptide mixtures were separated by online reversed-phase nanoscale capillary LC and analyzed by ESI-MS/MS. For LC-MS/MS, samples were injected into an in-house-made 15-cm reversed phase spraying fused-silica capillary column (ID 75 μm) packed with 3 μm ReproSil-Pur C18 A18 media (Dr. Maisch), using an UltiMate 3000 Capillary/Nano LC System, consisting of FamosTMMicro Autosampler, SwitchosTM Micro Column Switching Module (LC Dionex/Packings). The LC setup was connected to the LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific) equipped with a nanoelectrospray ion source (Thermo Fisher Scientific). The flow rate through the column was 250 nL/min. An acetonitrile gradient was used with a mobile phase containing 0.1% and 0.2% formic acid in Milli-Q water in buffers A and B, respectively. The injection volume was 5 μL. Peptides were separated with 50 min gradients from 5 to 65% CH3CN (vol/vol). In the nano-electrospray ionization source, the end of the capillary from the nano-LC column was connected to the emitter with pico-tip silica tubing, ID 20 μm (New Objective) by stainless steel union, with a PEEK sleeve for coupling the nanospray with the on-line nano-LC. The voltage applied to the union to produce an electrospray was 2.4 kV. Helium was introduced as a collision gas at a pressure of 3 psi. The mass spectrometer was operated in the data-dependent mode. Survey MS scans were acquired in the Orbitrap with the resolution set to 60,000. Up to the seven most intense ions per scan were fragmented and analyzed in the linear trap. For analysis of tryptic peptides, survey scans were recorded in the FT-mode followed by data-dependent collision-induced dissociation of the seven most intense ions in the linear ion trap (LTQ). Raw spectra were processed using open-source software DTA SuperCharge (msquant.sourceforge.net). The data were searched with MASCOT (Matrix Science) against a Swissprot or National Center for Biotechnology Information database. Search parameters included variable modifications of 57.02146 Da (carboxyamidomethylation) on Cys, 15.99491 Da (oxidation) on Met, and 0.984016 Da (deamidation) on Asn and Gln. The search parameters were as follows: maximum two missed cleavages, initial precursor ion mass tolerance 6 ppm, fragment ion mass tolerance 0.6 Da. The peptides were identified from the detected collision-induced dissociation products by Mascot, Protein Discoverer (Thermo Fisher) and were confirmed by Scaffold Software (Proteome Software) and by manual inspection of the fragmentation series.

1. Amar N, Lustig G, Ichimura Y, Ohsumi Y, Elazar Z (2006) Two newly identified sites in the ubiquitin-like protein Atg8 are essential for autophagy. *EMBO Rep* 7(6):635–642.

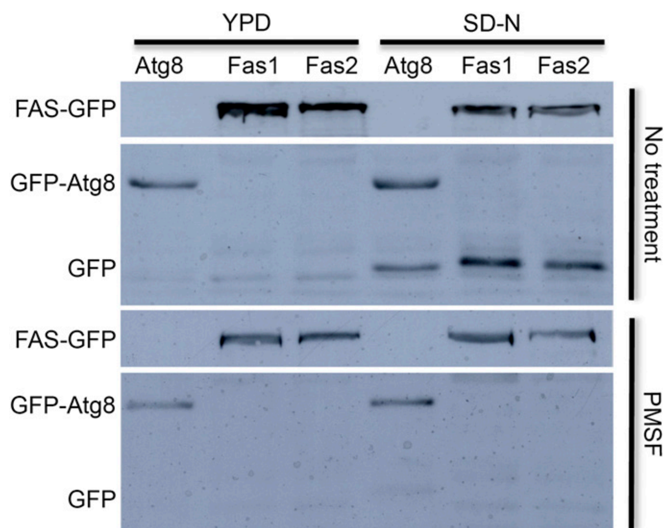

Fig. S1. FAS is rapidly degraded under nitrogen starvation. *atg8*Δ (TOS006) *Saccharomyces cerevisiae* strain-expressing GFP-Atg8 from the promoter of Atg8, *FAS1-GFP* (TOS016) and *FAS2-GFP* (TOS017) strains were grown to midlog phase and shifted to SD-N medium for 4 h in the presence of ethanol or 1 mM PMSF. Cell lysates were subjected to SDS/PAGE, followed by Western blot analysis using anti-GFP antibodies.

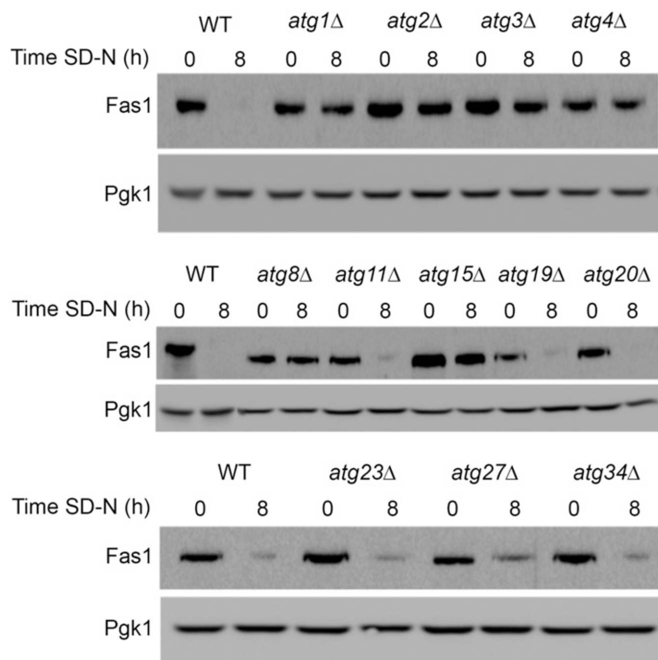

Fig. S2. FAS degradation depends on the core autophagy genes. WT (BY4741) and the indicated autophagy mutants (TOS001–TOS004 and TOOS6–TOS014) were grown to midlog phase and shifted to SD-N medium for 8 h. Cell lysates were subjected to SDS/PAGE, followed by Western blot analysis using anti-Fas1 and anti-Pgk1 antibodies.

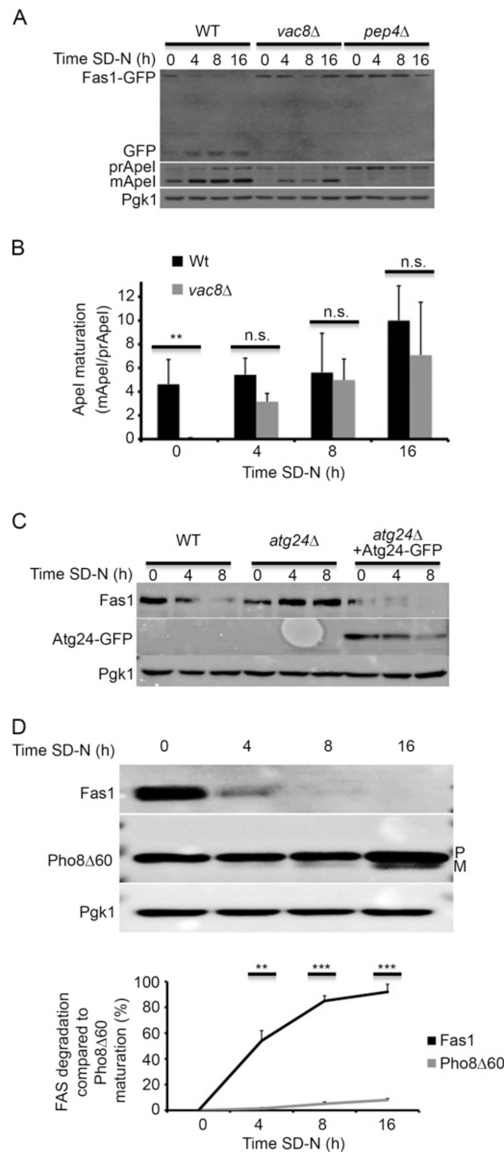

Fig. S3. Vac8 and Atg24 are essential for efficient degradation of FAS. (*A*) *FAS1-GFP* (TOS016) and *FAS1-GFP vac8*Δ (TOS019) *S. cerevisiae* cells were grown to midlog phase and shifted to SD-N medium for the indicated time periods. Cell lysates were subjected to SDS/PAGE, followed by Western blot analysis using anti-GFP, anti-Pgk1, and anti-Ape1 antibodies. (*B*) Quantification of ApeI maturation in WT (BY4741) and *vac8*Δ (TOS028). Error bars represent the SDs of three independent experiments (*Right*) n.s., not significant; $^{**}P < 0.01$ (Student's *t* test). (*C*) WT (BY4741), *atg24*Δ (TOS012), and *atg24*Δ (TOS012) cells expressing Atg24-GFP from the promoter of Atg24 were grown as in *A*. Cell lysates were subjected to SDS−PAGE electrophoresis, followed by Western blot analysis using anti-Fas1 and anti-GFP antibodies. (*D*) *pho8*Δ*:: pho8*Δ*60 pho13*Δ (TN124) *S. cerevisiae* cells were grown to midlog phase and shifted to SD-N medium for the indicated time periods. Cell lysates were subjected to SDS−PAGE electrophoresis, followed by Western blot analysis using anti-Pho8 and anti-Pgk1 antibodies. Error bars represent the SDs of three independent experiments (down), $^{**}P < 0.01$, $^{***}P < 0.001$ (Student's *t* test).

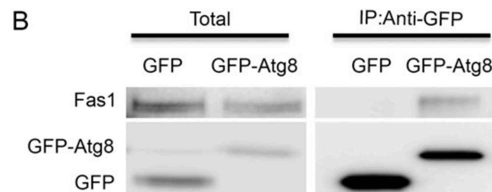

Fig. S4. FAS coimmunoprecipitates with Atg8 in an N-terminal-dependent manner. (*A*) Mass spectrometric analysis of the high molecular-weight band that specifically coimmunoprecipitated with the full-length GFP-Atg8. Peptides identified by mass spectrometry (bold red) are presented on the sequence of Fas1 and Fas2. (*B*) *atg8*Δ (TOS006) cells expressing pYES-GFP-Atg8 or pYES-GFP were grown to midlog. Cell lysates were immunoprecipitated using anti-GFP antibodies. Immunoprecipitates were subjected to SDS/PAGE, followed by Western blot analysis using anti-Fas1 and anti-GFP antibodies. Total is 5% of the lysate.

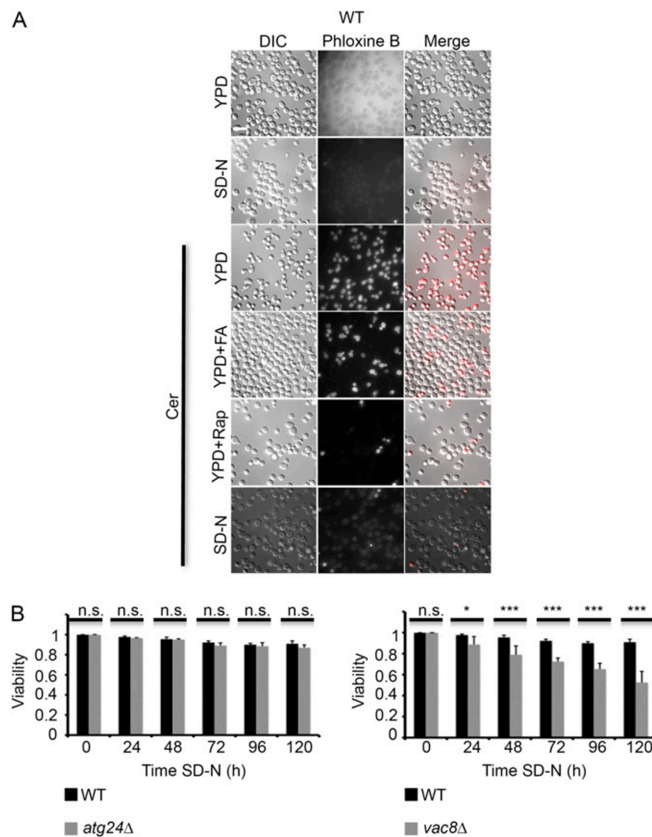

Fig. S5. FAS degradation is important for cell survival under nitrogen starvation. (*A*) WT (BY4741) *S. cerevisiae* cells were grown to midlog phase and incubated with DMSO, 50 μM cerulenin (cer), 50 μM cerulenin + 0.1 mM palmitic/stearic/myristic acids (cer + FA), or 50 μM cerulenin + 200 nM rapamycin. Cell viability was determined after 16 h using phloxine B. (Scale bar, 10 μm.) (*B*) WT (BY4741), *atg24*Δ (TOS012), and *vac8*Δ (TOS028) *S. cerevisiae* cells were grown to midlog phase and shifted to SD-N medium for the indicated time periods. Cell viability was determined using phloxine B at the indicated time periods. Error bars represent the SDs of three independent experiments. n.s., not significant; $^{*}P < 0.05$, $^{***}P < 0.001$ (Student's *t* test).

Table S1. List of *S. cerevisiae* strains used in this study

Strain	Genotype	Source
BY4741 (WT)	MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0	(1)
TOS001	BY4741 MATa *atg1Δ*::KAN	(2)
TOS002	BY4741 MATa *atg2Δ*::KAN	(2)
TOS003	BY4741 MATa *atg3Δ*::KAN	(2)
TOS004	BY4741 MATa *atg4Δ*::KAN	(2)
TOS005	BY4741 MATa *atg7Δ*::KAN	(2)
TOS006	BY4741 MATa *atg8Δ*::KAN	(2)
TOS007	BY4741 MATa *atg11Δ*::KAN	(2)
TOS008	BY4741 MATa *atg15Δ*::KAN	(2)
TOS09	BY4741 MATa *atg19Δ*::KAN	(2)
TOS010	BY4741 MATa *atg20Δ*::KAN	(2)
TOS011	BY4741 MATa *atg23Δ*::KAN	(2)
TOS012	BY4741 MATa *atg24Δ*::KAN	(2)
TOS013	BY4741 MATa *atg27Δ*::KAN	(2)
TOS014	BY4741 MATa *atg34Δ*::KAN	(2)
TOS015	BY4741 MATa *pep4Δ*::KAN	(2)
TOS016	BY4741 MATa *FAS1-GFP*::HIS	(2)
TOS017	BY4741 MATa *FAS2-GFP*::HIS	(2)
TOS018	BY4741 MATa *PGK1-GFP*::HIS	(2)
TOS019	BY4741 MATa *FAS1-GFP*::HIS *vac8*Δ::HYGRO	Present study
TOS020	BY4741 MATa *FAS1-GFP*::HIS *pep4*Δ::HYGRO	Present study
TOS021	BY4741 MATa *FAS2-GFP*::HIS *vac8*Δ::HYGRO	Present study
TOS022	BY4741 MATa *FAS2-GFP*::HIS *pep4*Δ::Hygro	Present study
TOS023	BY4741 MATa *FAS2-GFP*::HIS *atg8*Δ::HYGRO	Present study
TOS024	S288C MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 *FAS1-proteinA*::HIS3	TAP fusion library (Open Biosystems) (3)
TOS025	S288C MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 *FAS2-proteinA*::HIS3	TAP fusion library (Open Biosystems) (3)
TN124	*MATa leu2–3,112 trp1 ura3-52 pho8::pho8Δ60 pho13Δ::LEU2*	(4)
TOS026	*MATa leu2–3,112 trp1 ura3-52 pho8::pho8Δ60 pho13Δ::LEU2 atg24*Δ::HYGRO	Present study
TOS027	*MATa leu2–3,112 trp1 ura3-52 pho8::pho8Δ60 pho13Δ::LEU2 vac8*Δ::HYGRO	Present study
TOS028	BY4741 MATa *vac8Δ*::KAN	(2)
TOS029	BY4741 MATa *fas1*Δ::TRP1	Present study
TOS030	BY4741 MATa *fas2*Δ::TRP1	Present study
TOS031	BY4741 *DaMP-fas1*::KAN	(5)
TOS032	BY4741 *DaMP-fas1*::KAN *atg7*Δ::NAT	Present study

1. Brachmann CB, et al. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14(2):115–132.
2. Giaever, G, et al. (2002) Functional profiling of the Saccharomyces cerevisiae genome. *Nature* 418(6896):387–391.
3. Ghaemmaghami S, et al. (2003) Global analysis of protein expression in yeast. *Nature* 425(6959):737–741.
4. Noda T, Matsuura A, Wada Y, Ohsumi Y (1995) Novel system for monitoring autophagy in the yeast *Saccharomyces cerevisiae*. *Biochem Biophys Res Commun* 210(1):126–132.
5. Breslow DK, et al. (2008) A comprehensive strategy enabling high-resolution functional analysis of the yeast genome. *Nat Methods* 5(8):711–718.