

Table S1. Detailed analysis of TEM data.

Strain	Autophagic body size				
	Measured cross-sections		Estimated original		
	Mean rad ^a	SD rad	Mean rad	SD rad	Volume ^b
<i>ATG41-GFP</i>	187.065	54.430	219.400	37.065	4.81E+07
<i>COF1p-ATG41-GFP</i>	173.260	47.824	202.738	30.962	3.74E+07
<i>atg41Δ</i>	187.273	52.571	221.008	32.479	4.82E+07
Strain	Vacuole size				
	Measured cross-sections		Estimated original		
	Mean rad	SD rad	Mean rad	SD rad	
<i>ATG41-GFP</i>	882.267	251.897	952.992	238.480	
<i>COF1p-ATG41-GFP</i>	859.125	268.753	895.624	258.352	
<i>atg41Δ</i>	865.867	266.114	904.460	260.716	
Strain	Autophagic body number				
	Measured cross-sections/cell		Estimated bodies/cell		
<i>ATG41-GFP</i>	7.330		25.640		
<i>COF1p-ATG41-GFP</i>	4.539		15.862		
<i>atg41Δ</i>	4.331		14.185		
Strain	Total flux (%) ^c		Number of cells analyzed		
<i>ATG41-GFP</i>	100.00		183		
<i>COF1p-ATG41-GFP</i>	48.07		170		
<i>atg41Δ</i>	55.41		136		

^aRad, radius in nm.^bExpressed in nm³.^cTotal flux was calculated as the estimated average autophagic body volume × the estimated autophagic body number per cell. The values were normalized to the *ATG41-GFP* sample, which was set to 100%.

Table S2. Strains used in this study.

Name	Genotype	Ref
FRY143	SEY6210 <i>vps4Δ::TRP1 pep4Δ::LEU2</i>	1
PJ69-4A	MATa <i>trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ LYS2::GAL1-HIS GAL2-ADE2 met2::GAL7-lacZ</i>	2
SEY6210	MATa <i>leu2-3, 112 ura3-52 his3-Δ200 trp1-Δ901 suc2-Δ9 lys2-801 GAL</i>	3
SKB171	<i>atg1Δ::ble atg9::KAN ATG9-3GFP::LEU2 RFP-APE1::URA3 SEY6210</i>	This study
WLY176	SEY6210 <i>pho13Δ pho8Δ60</i>	4
ZYY101	WLY176 <i>pRS405-GFP-ATG8::LEU2</i>	This study
ZYY102	WLY176 <i>pRS405-GFP-ATG8::LEU2 atg41Δ::HIS3</i>	This study
ZYY103	WLY176 <i>pRS405-GFP-ATG8::LEU2 atg3Δ::HIS3</i>	This study
ZYY104	WLY176 <i>atg9Δ::LEU2</i>	This study
ZYY105	WLY176 <i>atg41Δ::HIS3</i>	This study
ZYY106	WLY176 <i>icy1Δ::LEU2</i>	This study
ZYY107	WLY176 <i>ATG41-GFP::HIS3</i>	This study
ZYY108	WLY176 <i>ATG41-PA::HIS3</i>	This study
ZYY109	WLY176 <i>COF1p::KAN-ATG41-GFP::HIS3</i>	This study
ZYY110	FRY143 <i>atg41Δ::HIS3</i>	This study
ZYY111	FRY143 <i>COF1p::KAN-ATG41-GFP::HIS3</i>	This study
ZYY112	FRY143 <i>ATG41-GFP::HIS3</i>	This study
ZYY113	WLY176 <i>ATG41-VC::HIS3 ATG9-VN::TRP1</i>	This study
ZYY114	WLY176 <i>ATG41-VC::HIS3 ATG2-VN::TRP1</i>	This study
ZYY115	WLY176 <i>ATG41-VC::HIS3 ATG27-VN::TRP1</i>	This study
ZYY116	WLY176 <i>ATG41-VC::HIS3</i>	This study
ZYY117	WLY176 <i>atg41Δ(91-110)-VC::HIS3</i>	This study
ZYY118	WLY176 <i>atg41Δ(127-136)-VC::HIS3</i>	This study
ZYY119	WLY176 <i>atg41Δ(91-110)-VC::HIS3 ATG9-VN::TRP1</i>	This study
ZYY120	WLY176 <i>atg41Δ(127-136)-VC::HIS3 ATG9-VN::TRP1</i>	This study
ZYY121	WLY176 <i>atg41Δ(127-136)-GFP::HIS3</i>	This study
ZYY122	WLY176 <i>gcn4Δ::LEU2</i>	This study
ZYY123	WLY176 <i>gcn4Δ::LEU2 ATG41-PA::HIS3</i>	This study
ZYY124	WLY176 <i>GCN4-PA::LEU2</i>	This study
ZYY125	WLY176 <i>ATG41-GFP::HIS3; ATG9-mCherry::TRP1</i>	This study
ZYY126	SKB171 <i>atg41Δ::HIS3</i>	This study
ZYY127	WLY176 <i>ZEO1p::HIS3-ATG41 gcn4Δ::LEU2</i>	This study
ZYY128	WLY176 <i>ZEO1p::HIS3-ATG41</i>	This study
ZYY129	WLY176 <i>ATG41-GFP::HIS3 atg1Δ::LEU2</i>	This study

1. Cheong H, Yorimitsu T, Reggiori F, Legakis JE, Wang C-W, Klionsky DJ. Atg17 regulates the magnitude of the autophagic response. *Mol Biol Cell* 2005; 16:3438-53.
2. James P, Halladay J, Craig EA. Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics* 1996; 144:1425-36.
3. 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.
4. Kanki T, Wang K, Baba M, Bartholomew CR, Lynch-Day MA, Du Z, Geng J, Mao K, Yang Z, Yen W-L. A genomic screen for yeast mutants defective in selective mitochondria autophagy. *Mol Biol Cell* 2009; 20:4730-8.

Table S3. Amino acid, nucleoside and vitamin stock solutions.

20× amino acid and nucleic acid base complete stock
(1 L in distilled H₂O); store at 4°C.

Amino acid or base	Concentration (%)
Adenine	0.06
Histidine	0.04
Leucine	0.10
Lysine	0.06
Tryptophan	0.10
Uracil	0.04

200x vitamins (1 L in distilled H₂O); filter sterilize and store
at store at 4°C or -20°C.

Vitamin or precursor	Concentration
4-Aminobenzoic acid (p-Aminobenzoic acid)	0.3 mM
Biotin (vitamin H)	2.0 μM
Ca pantothenate (vitamin B5)	330 μM
Folic acid (vitamin B9)	1.0 μM
Inositol (<i>myo</i> -inositol; vitamin B8)	2.22 mM
Niacin (nicotmic acid, vitamin B3)	0.65 mM
Pyridoxine HCl (vitamin B6)	0.4 mM
Riboflavin (vitamin B2)	0.1 mM
Thiamine HCl (vitamin B1)	0.24 mM

Table S4. Primers used in qPCR, ChIP and promoter replacement.

Name	Sequence	Use
ATG41-CHIP1-F	GCGGCTCTGCGTAAAAAGGT	ChIP-qPCR
ATG41-CHIP2-F	CGGAAAGGGTCTGGAGCAAC	ChIP -qPCR
ATG41-CHIP3-F	GCGCGAAGATGACACATTGC	ChIP -qPCR
ATG41-CHIP4-F	CCGTCCGGAAATCTACAGAA	ChIP -qPCR
ATG41-CHIP5-F	CCAAGAAGTCCCTTGGGGTT	ChIP -qPCR
ATG41-CHIP6-F	ACACCATACCCACTGTCCTG	ChIP -qPCR
ATG41-CHIP7-F	TCTCCCTTCCGGGTAAGCAC	ChIP -qPCR
ATG41-CHIP8-F	GCTCTCCCCCAAAGCTAGTT	ChIP -qPCR
ATG41-CHIP9-F	GTTCTTTCACCAGAGCCAAG	ChIP -qPCR
ATG41-CHIP1-R	TTGGTTGGTTTGGTGTAGCC	ChIP -qPCR
ATG41-CHIP2-R	GGGGGAAGATTCCACAGAGG	ChIP -qPCR
ATG41-CHIP3-R	ACCTTTTTACGCAGAGCCGC	ChIP -qPCR
ATG41-CHIP4-R	GCAATGTGTTCATCTTCGCGC	ChIP -qPCR
ATG41-CHIP5-R	TTCTGTAGATTTCCGGACGG	ChIP -qPCR
ATG41-CHIP6-R	AACCCCAAGGGACTTCTTGG	ChIP -qPCR
ATG41-CHIP7-R	CAGGACAGTGGGTATGGTGT	ChIP -qPCR
ATG41-CHIP8-R	GTGCTTACCCGGAAGGGAGA	ChIP -qPCR
ATG41-CHIP9-R	AACTAGCTTTGGGGGAGAGC	ChIP -qPCR
HIS5-F	TGGTATAGTGACGTAGTTAGT GC	ChIP -qPCR
HIS5-R	GAACAGAACTGTGTGCATCC	ChIP -qPCR
TFC1-F	CCCCATCTTTAAGCTCTGCT	ChIP -qPCR, <i>ATG41</i> mRNA level check
TFC1-R	AAGACCAAAAGCGAAGAAGC	ChIP -qPCR, <i>ATG41</i> mRNA level check
ATG41_F1	CGAGTACTGAAGACGATTGCA T	<i>ATG41</i> mRNA level check
ATG41_R1	TGCGACATTGGCAAAGGCAT	<i>ATG41</i> mRNA level check
ATG41_F2	GCACGCACACTAAAGATAAGA CG	<i>ATG41</i> mRNA level check
ATG41_R2	ACTTGCAAAGGCAAACCCAG	<i>ATG41</i> mRNA level check
ATG41_COF1-P-F	CTTGTTTTATTCTTGAGAAAAC CAAATAAATTGGCTACAGAA TTCGAGCTCGTTTAAAC	<i>COF1</i> promoter change
ATG41_COF1-P-R	CTTCATAGCGCGAGATGGGGG AAGATTCCACAGAGGACATCT TCTTTTGTAGTTTTTTG	<i>COF1</i> promoter change
ATG41-ZEO1-P-F	CTTGTTTTATTCTTGAGAAAAC CAAATAAATTGGCTACAGAA TTCGAGCTCGTTTAAAC	<i>ZEO1</i> promoter change
ATG41-ZEO1-P-R	CTTCATAGCGCGAGATGGGGG	<i>ZEO1</i> promoter

	AAGATTCCACAGAGGACATAA TTAATTGATATAAACGTA	change
ATG7-F	ATGAGCATTGTCCAGCATGTA G	<i>ATG7</i> mRNA level check
ATG7-R	GACCTCCTGCTTTATGACTGA C	<i>ATG7</i> mRNA level check
ATG9-F	CGTACTAACAGAGTCTTTCCTT G	<i>ATG9</i> mRNA level check
ATG9-R	CTAAGACACCACCCTTATTGA G	<i>ATG9</i> mRNA level check





