

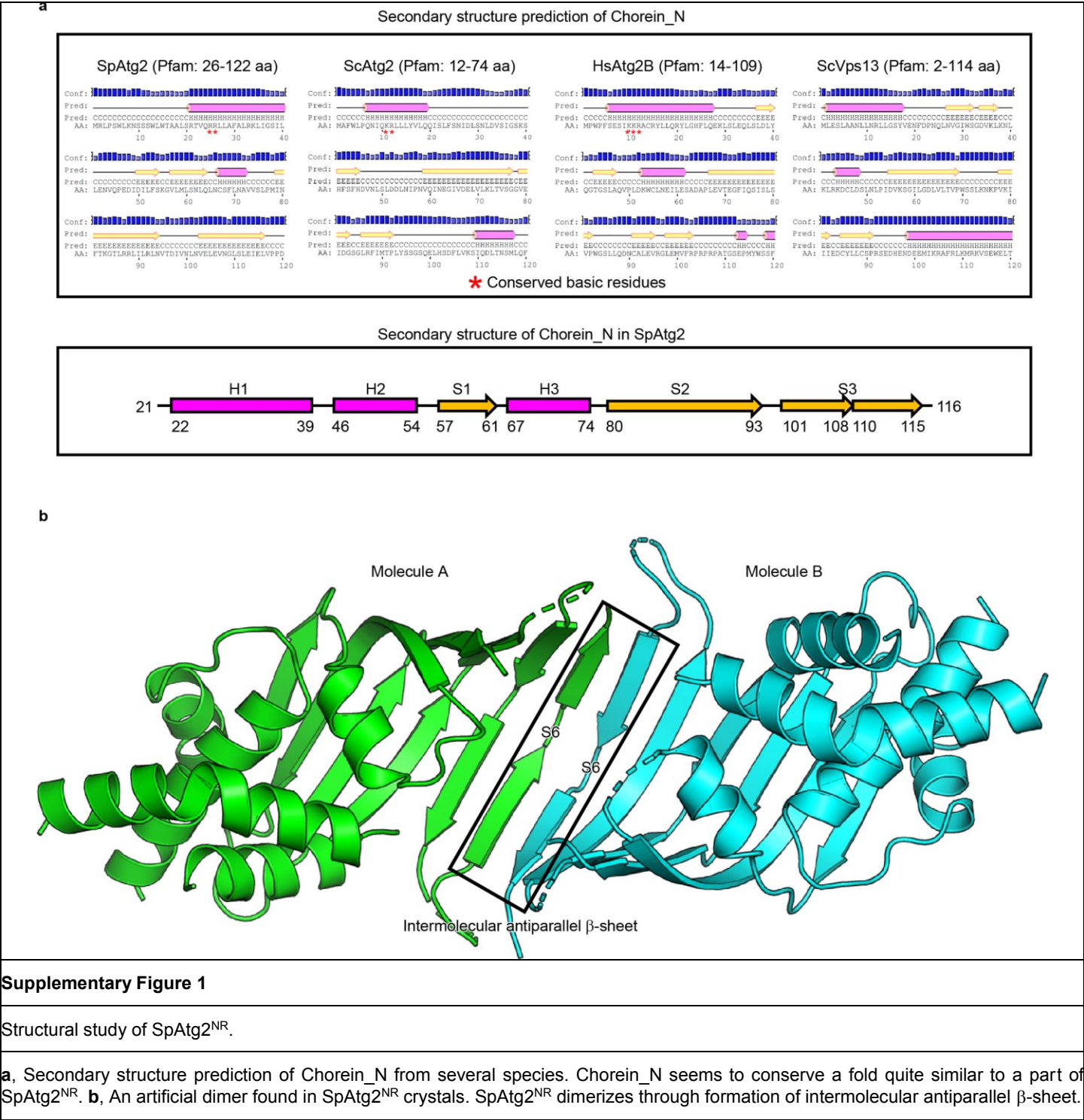
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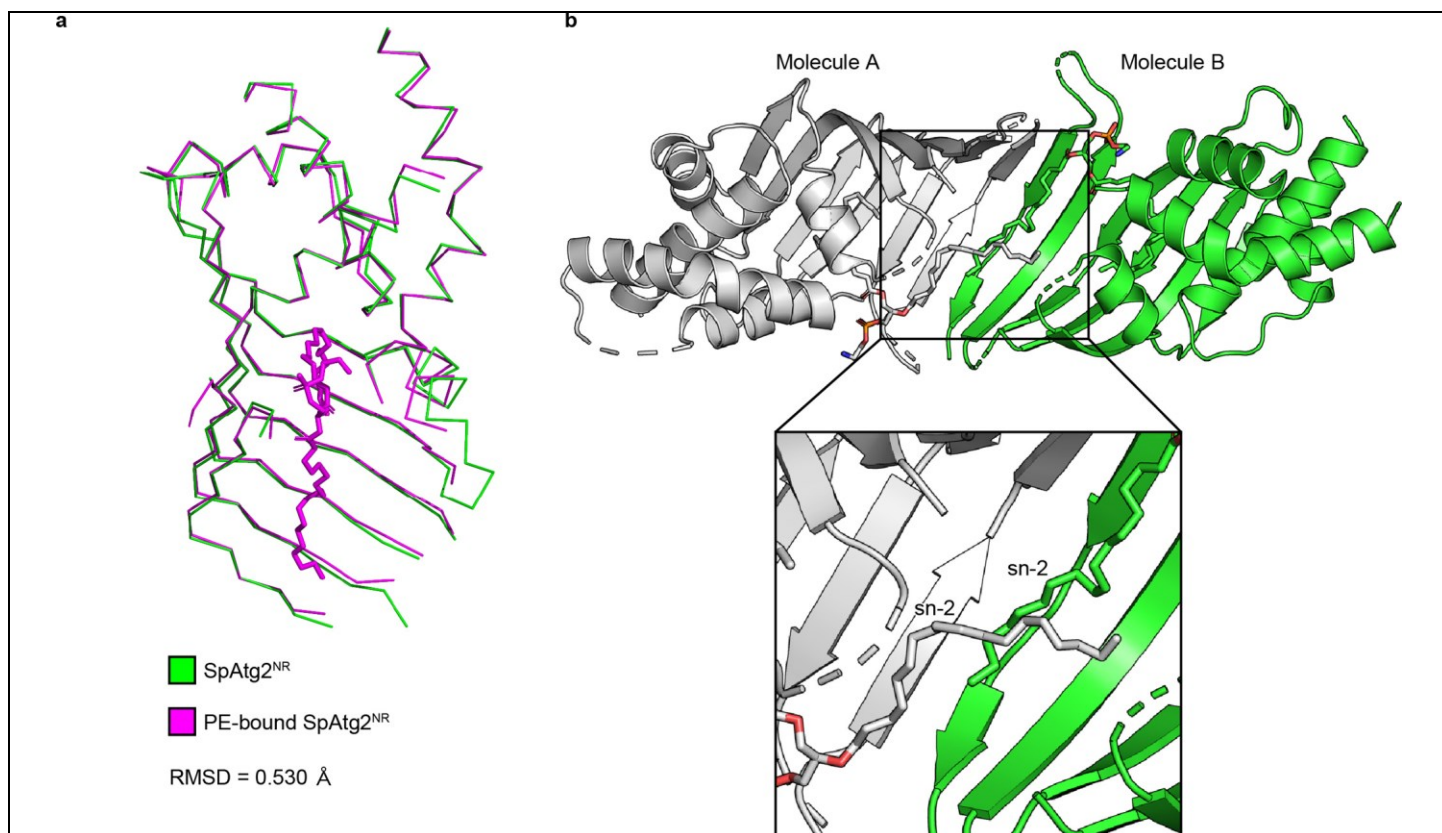
Atg2 mediates direct lipid transfer between membranes for autophagosome formation

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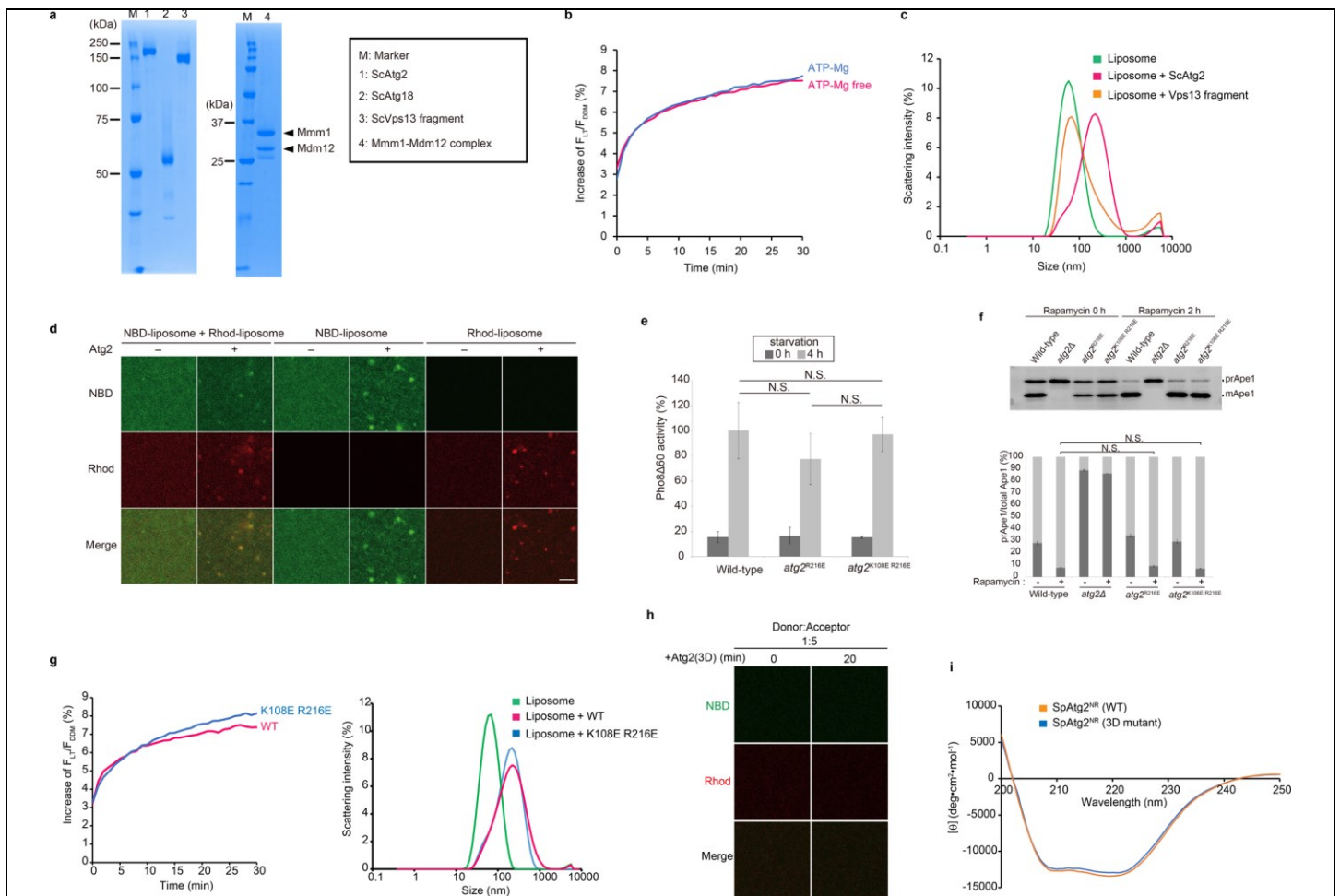




Supplementary Figure 2

Structural study of PE-bound SpAtg2^{NR}.

a, Structural superposition of SpAtg2^{NR} and its PE-bound form. Both structures are represented by C α -trace. **b**, Interaction between sn-2 acyl chains within the dimer found in crystal packing.



Supplementary Figure 3

Mutational analyses of ScAtg2.

a, SDS-PAGE of purified recombinant proteins used for in vitro analyses. Protein bands were stained with Coomassie Brilliant Blue. Uncropped gel images are shown in Supplementary Data Set 1. **b**, The effect of ATP on phospholipid transfer mediated by Atg2. **c**, Comparison of the MT activity between Atg2 and the Vps13 fragment analyzed by dynamic light scattering. **d**, MT activities of Atg2 as assessed by fluorescence microscopy of NBD-PE and Rhod-PE liposomes. Scale bar indicates 5 μ m. **e**, Bulk autophagic activity measured by the ALP assay. In **e** and **f**, data are presented as mean $\pm$ s.d. from $n = 3$ independent experiments. N.S. means not statistically significant (two-tailed Student's test). **f**, Maturation of a selective autophagy cargo aminopeptidase I (Ape1) via autophagy monitored by western blotting. Uncropped blot image is shown in Supplementary Data Set 1. **g**, LT and MT activities of the K108E R216E mutant. **h**, Confocal microscopic analysis of liposomes mixed with the 3D mutant of ScAtg2. **i**, Circular dichroism spectra of wild-type and 3D mutant of MBP-fused SpAtg2^{NR}.

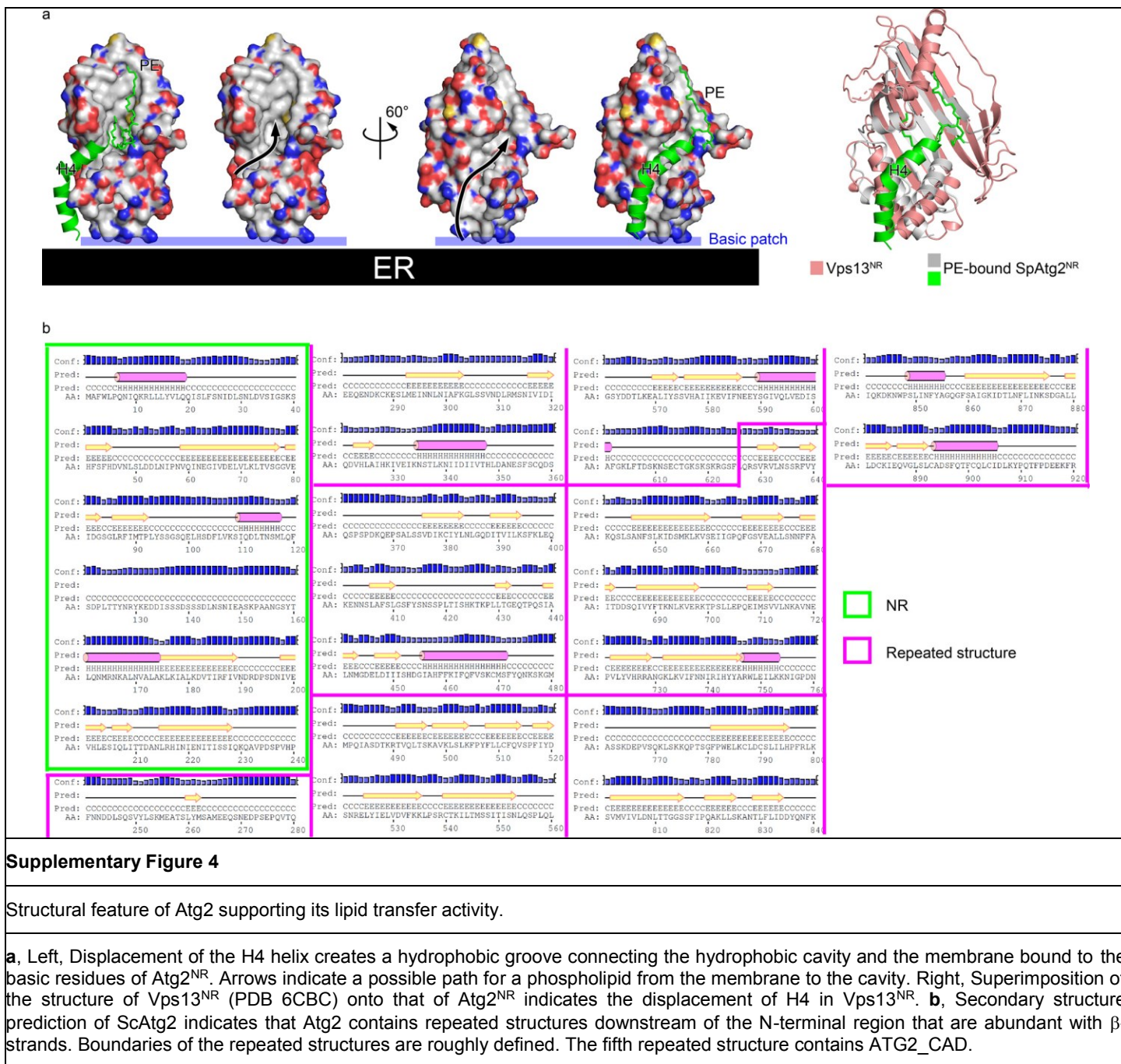


Figure 1c

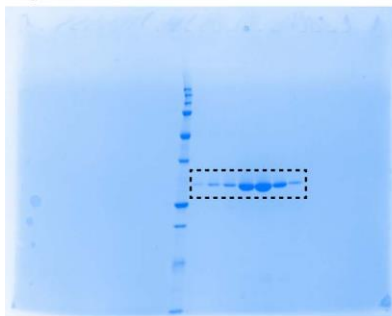


Figure 4e, anti-HA



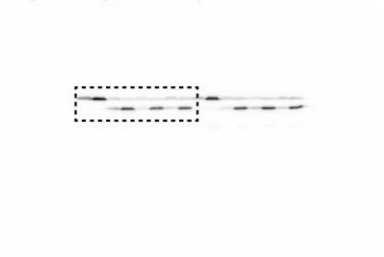
Figure 4g, anti-HA



Figure 4e, anti-GFP



Figure 4g, anti-Ape1



Supplementary Fig. 3a

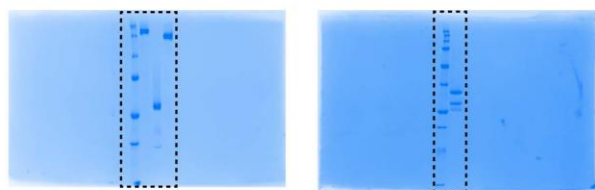
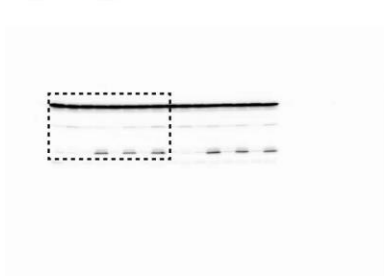
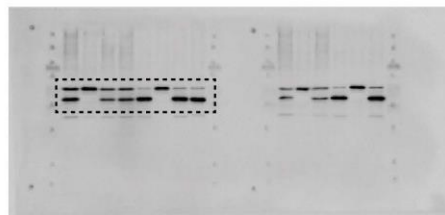


Figure 4g, anti-GFP



Supplementary Fig. 3f



Supplementary Data Set 1 Uncropped images.

Supplementary Table 1 Yeast strains used in this study.

Strain	Genotype	Source
SEY6210	<i>MATα lys2 suc2 his3 leu2 trp1 ura3</i>	<i>Mol. Cell. Biol.</i> 8 , 4936-4948 (1988)
GYS104	SEY6210; <i>atg2Δ::HIS3</i>	<i>J. Biol. Chem.</i> 276 , 30452-30460 (2001)
GYS1304	SEY6210; <i>atg2Δ::Atg2-2×YEGFP-kanMX6</i>	This study
GYS1308	SEY6210; <i>atg2Δ::Atg2(R216E)-2×YEGFP-kanMX6</i>	This study
GYS1312	SEY6210; <i>atg2Δ::Atg2(K108E R216E)-2×YEGFP-kanMX6</i>	This study
BY4741	<i>MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	<i>Yeast</i> 14 , 115-132 (1998)
YNH393	BY4741; <i>EGFP-ATG8-ATG8ter-hphNT1 atg2Δ::kanMX</i>	This study
ScTK298	BY4741; <i>his3::pRS303-PGK1-EGFP atg2Δ::natNT2</i>	Ref 15

Supplementary Table 2 Plasmids used for functional analysis in this study.

Name	Alias	Properties	Marker	Source
pFA6a-2×YEGFP-kanMX6		PCR template for generation of C-terminally 2×YEGFP-integrated strains	<i>Kan^r</i>	Ref 7
pRS316[mRuby3-Atg8]		Centromeric plasmid for expression of mRuby-Atg8 from the <i>ATG8</i> promoter	<i>URA3</i>	This study
pRS424[<i>P_{CUP1}</i> -prApe1]		2μ plasmid for expression of prApe1 from the <i>CUP1</i> promoter	<i>TRP1</i>	This study
pRS426[Pho8Δ60]	pCK6	2μ plasmid for expression of Pho8Δ60	<i>URA3</i>	This study
pRS316- <i>ATG2-3×HA-EGFP</i>		Centromeric plasmid for expression of Atg2-3×HA-EGFP from the <i>ATG2</i> promoter	<i>URA3</i>	Lab stock
pRS316- <i>atg2^{3D}(atg2^{10-12D})-3×HA-EGFP</i>	pTKO 78	Centromeric plasmid for expression of Atg2 ^{3D} (<i>atg2^{10-12D}</i>)-3×HA-EGFP from the <i>ATG2</i> promoter	<i>URA3</i>	Kotani et al., unpublished