

Supplementary Information

Mechanism and functions of membrane binding by the Atg5-Atg12/Atg16 complex during autophagosome formation

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Supplementary Methods

Protein expression and purification

Expression and purification of *Saccharomyces cerevisiae* Atg3 (NP_014404). Full length Atg3 was expressed as an N-terminal GST fusion protein from pGEX4T1 in *E. coli*. Rosetta pLySS cells were grown at 37°C to an OD₆₀₀ of 0.8, induced with 50µM IPTG and grown for a further 16h at 18°C. Cells were pelleted and resuspended in a buffer containing 50mM HEPES pH 7.5, 300mM NaCl, 2mM MgCl₂, 1mM DTT, complete protease inhibitors (Roche) and DNase I (Sigma). Cells were lysed by freeze thawing and the lysate was centrifuged at 40000rpm (Beckman Ti45 rotor) for 40 minutes at 4°C. The supernatant was incubated with glutathione-beads (GE Healthcare) for 2h at 4°C. Beads were washed 5x with 50mM HEPES pH 7.5, 300mM NaCl, 1mM DTT followed by 2 washes with 50mM HEPES pH 7.5, 1000mM NaCl, 1mM DTT and two washes with 50mM HEPES pH 7.5, 300mM NaCl, 1mM DTT. The protein was cleaved off from the GST tag by incubation with thrombin protease (Serva) overnight at 4°C. The supernatant containing the cleaved off Atg3 protein was diluted to reach a final salt concentration of 150mM NaCl and further purified using a 16/60 Q-Sepharose column. The protein was eluted on a gradient ranging from 150mM-1M NaCl. Fractions containing Atg3 were pooled, concentrated and run on a 16/60 S75 size exclusion column in 50mM HEPES pH 7.5, 150mM NaCl, 1mM DTT. Fractions containing pure Atg3 were pooled, concentrated, diluted 1:1 with glycerol, and stored at -20°C.

Expression and purification of *Saccharomyces cerevisiae* Atg7 (NP_012041.1). Full length Atg7 was expressed as an N-terminal His-tagged protein from pOPThrsTEV in *E. coli*. Rosetta pLySS cells were grown at 37°C to an OD₆₀₀ of 0.8, induced with 50µM IPTG and grown for a further 16h at 18°C. Cells were pelleted and resuspended in a buffer containing 50mM HEPES pH 7.5, 300mM NaCl, 10mM Imidazole, 1mM MgCl₂, 2mM β-mercaptoethanol, complete protease inhibitors (Roche) and DNase I (Sigma). Cells were lysed by freeze thawing and the lysate was centrifuged at

40000rpm (Beckman Ti45 rotor) for 40 minutes at 4°C. The supernatant was incubated with nickel beads (5 Prime) for 2h at 4°C. Beads were washed with 50mM HEPES pH 7.5, 300mM NaCl, 10mM Imidazole, 2mM β-mercaptoethanol and the His-tag was cleaved off with TEV protease at room temperature. The Atg7 protein was diluted to reach a final salt concentration of 150mM and the protein was further purified on 16/60 Q-Sepharose column. The protein was eluted using a gradient reaching from 150mM-1M NaCl. Fractions containing Atg7 were pooled, concentrated and run on a 16/60 S200 size exclusion column in 50mM HEPES pH 7.5, 150mM NaCl, 1mM DTT. Fractions containing pure Atg7 were pooled, concentrated, diluted 1:1 with glycerol, and stored at -20°C.

Expression and purification of *Saccharomyces cerevisiae* Atg8 (NP_009475.1). Atg8 lacking the C-terminal arginine was expressed as N-terminal His-tagged protein from pOPC-His-TEV-Atg8 in *E. coli*. Rosetta pLySS cells were grown at 37°C to an OD₆₀₀ of 0.8, induced with 500μM IPTG and grown for a further 3h at 37°C. Cells were pelleted and resuspended in a buffer containing 50mM HEPES pH 7.5, 300mM NaCl, 10mM Imidazole, 1mM MgCl₂, 2mM β-mercaptoethanol, complete protease inhibitors (Roche) and DNase I (Sigma). Cells were lysed by freeze thawing and the lysate was centrifuged at 40000rpm (Beckman Ti45 rotor) for 40 minutes at 4°C. The supernatant was incubated with nickel beads (5 Prime) for 2h at 4°C. Beads were washed with 50mM HEPES pH 7.5, 300mM NaCl, 10mM Imidazole, 2mM β-mercaptoethanol and the His-tag was cleaved off for several hours with TEV protease at room temperature. The Atg8 protein was diluted to reach final salt concentration of 150mM and the protein was further purified on 16/60 SP-Sepharose column. The protein was eluted using a gradient reaching from 150-1000mM NaCl. Fractions containing Atg8 were pooled, concentrated and run on a 16/60 Superdex S75 size exclusion column in 50mM HEPES pH 7.5, 150mM NaCl, 1mM DTT. Fractions containing pure Atg8 were pooled, concentrated, diluted 1:1 with glycerol, and stored at -20°C.

Expression of N-terminal eGFP, monomeric eGFP and mCherry fusion of *Saccharomyces cerevisiae* Atg8 (NP_009475.1). All fusion genes coded for an N-terminal His-tag followed by a TEV protease recognition site and were expressed from pETDuet-1 in *E. coli*. Rosetta pLySS cells were grown at 37°C until an OD₆₀₀ of 0.8, induced with 50µM IPTG and grown for a further 16h at 18°C. Cells were pelleted and resuspended in a buffer containing 50mM HEPES pH 7.5, 300mM NaCl, 10mM Imidazole, 1mM MgCl₂, 2mM β-mercaptoethanol, complete protease inhibitors and DNase I. Cells were lysed by freeze thawing and the lysate was centrifuged at 40000rpm (Beckman Ti45 rotor) for 40 minutes at 4°C. The supernatant was passed over a nickel column and the protein was eluted in step gradient ranging from 50-300mM Imidazole. The His-tag was cleaved off with TEV protease at room temperature. The Atg8 protein was diluted to reach final imidazole concentration of 40mM and the TEV protease containing a N-terminal His-tag was removed by incubation with nickel beads. The supernatant containing Atg8 was concentrated and run on a 16/60 Superdex S75 size exclusion column in 50mM HEPES pH7.5, 150mM NaCl, 1mM DTT. Fractions containing pure Atg8 fusion proteins were pooled, concentrated and stored at -80°C.

Expression and purification of the *Saccharomyces cerevisiae* Atg5-16 complex and Atg5 bound to aa 1-46 of Atg16 (Atg5: NP_015176.1, Atg16: NP_013882.1) from *E. coli*.

Rosetta pLySS cells were co-transformed with pETDuet-1-His-TEV-Atg5 and pCDFDuet-1-Atg16 or pCOLADuet-1-Atg16(1-46), grown at 37°C to an OD₆₀₀ of 0.8, induced with 500mM IPTG and further grown for 4h at 37°C. Cells were pelleted and resuspended in a buffer containing 50mM HEPES pH 7.5, 300mM NaCl, 10mM Imidazole, 1mM MgCl₂, 2mM β-mercaptoethanol, complete protease inhibitors (Roche) and DNase I (Sigma). Cells were lysed by freeze thawing and the lysate was centrifuged at 40000rpm (Beckman Ti45 rotor) for 40 minutes at 4°C. The supernatant was passed over a nickel column (GE Healthcare) and the protein was eluted in step gradient ranging

from 50-300mM Imidazole. Fractions containing the Atg5-16 complex were concentrated and run on a 16/60 Superdex S200 size exclusion column in 50mM HEPES pH7.5, 150mM NaCl, 1mM DTT. Fractions containing Atg5-16 complex pooled, concentrated and stored at -80°C.

Expression and purification of the Atg5-12 conjugate and the Atg5-12-16 complex (Atg5: NP_015176.1, Atg12: NP_009776.1, Atg16: NP_013882.1)

The Atg5-12 conjugate and the Atg5-12-16 complex were produced in *E. coli* Rosetta pLySS by coexpression of Atg12, hexahistidine-tagged Atg5, Atg7, Atg10 and, if applicable, Atg16.

Cells were grown at 37°C to an OD₆₀₀=0.8, induced with 1mM IPTG and grown for another 4h at 37°C. Harvested cells were resuspended in the resuspension buffer (300mM NaCl, 50mM HEPES pH 7.5, 10mM Imidazole, 2.5mM Pefablock (Roth), 1mM MgCl₂, 2mM β-mercaptoethanol, 10μg/ml DNase), and disrupted by freeze-thaw method and sonication. The cleared lysate was applied to a HisTrap column (GE Healthcare) and the proteins were eluted by a step-wise imidazole gradient at 150mM imidazole. For Atg5-12, the eluate was concentrated using Amicon Ultra centrifugal filter (MW cut-off 30kDa) and further purified using a 16/60 S200 gel exclusion column (GE Healthcare). The protein was eluted from the column with 150mM NaCl, 50mM HEPES pH 7.5, 1mM DTT. For Atg5-12-16, the HisTrap fractions containing the protein were concentrated and run on a Q-sepharose column (GE Healthcare). The protein was eluted in continuous NaCl gradient at 530mM NaCl. The eluate was concentrated and applied to a 16/60 S200 gel exclusion column (GE Healthcare). The protein was eluted with 150mM NaCl, 50mM HEPES pH 7.5, 1mM DTT.

The Atg5-12-16 complex as well as Atg5(K160E, R171E)-12-16 and Atg5-12-16-eGFP (or 16-monomeric-eGFP) was alternatively produced by co-expression of the Atg5-12 conjugate (with Atg5 mutations, where applicable) with Atg10 and 7 and purification as described above followed

by incubation with purified Atg16 at a molar ratio of 1:1 at 4°C for 6h and purification of the resulting complex by size exclusion chromatography on Superdex S200.

Expression of Atg16, Atg16-eGFP, Atg16-monomeric-eGFP and Atg5

GST-tagged Atg16 was expressed in *E. coli* Rosetta pLySS from pOPTG-Atg16. Cells were grown at 37°C to an OD₆₀₀=0.8, induced with 1 mM IPTG and grown for another 4 h. Cells were disrupted by freeze thawing and the cleared lysate was incubated with glutathione beads (Glutathione Sepharose 4B, GE Healthcare). The protein was cleaved off from the beads with TEV protease, concentrated and applied to a 16/60 S75 gel exclusion column (GE Healthcare). The protein was eluted with 150mM NaCl, 50mM HEPES pH 7.5, 1mM DTT.

Atg16 with N-terminal hexahistidine-tag followed by a TEV cleavage site and C-terminal eGFP or monomeric-eGFP was expressed in *E. Coli* Rosetta pLysS. Cells were grown at 37 °C to an OD₆₀₀ of 0.4 and induced with 50µM IPTG for 18h at 18°C. Purification procedure was as described for Atg8 fusion proteins but with size exclusion chromatography on Superdex S200.

Atg5 was expressed as a hexahistidine-tagged protein in *E. coli* Rosetta pLysS from pOPTH-Atg5. Cells were grown at 37 °C to an OD₆₀₀ of 0.3 and induced with 50µM IPTG for 16h at 18°C. The purification procedure was the same as for Atg5-Atg12.

Liposome co-sedimentation assays

Lipids were mixed, dried under argon stream and desiccated for 1h. Buffer (150mM NaCl, 50mM HEPES pH 7.5, 1mM DTT) was added to give a final concentration of 1mg/ml lipids. After 30 min rehydration, the lipids were sonicated for 5 min in a water-bath sonicator and extruded using 400nm and 100nm filters. 5µg protein was incubated with 0.5mg/ml liposomes for 30 min at room temperature. For the Atg3 recruitment experiments, proteins were added to the liposomes at the final concentration 1.6 µM each. The reactions were centrifuged for 10 minutes at 180,000 g at

22°C, supernatants and pellets were separated and equal amounts were run on 12 % SDS/polyacrylamide gels. Quantification was done using ImageJ.

The following lipids were used: Folch lipids type 1 (Sigma #B1502), POPC (Avanti #850457), POPE (Avanti #850757), POPS (Avanti #840034), PI3P (Avanti #850150), liver PI (Avanti #840042), egg PC (Avanti #840051), brain PE (Avanti #840022), brain PS (Avanti #840032), DAG (Avanti #845875).

For quantifications of the gels ImageJ was used.

Preparation of GUVs

GUVs were prepared by electroformation. 3µl of a lipid mixture (10mg/ml in chloroform/methanol (3:1)) were applied onto the surface of indium-tin-oxide coated glass slides in a drop wise manner and desiccated for at least 3h under vacuum. Subsequently, electroformation chambers were assembled using silicon gaskets and the chambers were filled with a solution containing 300mM sucrose. The electroformation protocol contained 3 phases. Phase 1: 30 minutes. Sine wave with frequency $f = 10\text{Hz}$. Peak-to-peak amplitude ramps up linearly from 0.05V to 1.41V. Phase 2: 120 minutes. Sine wave with frequency $f=10\text{Hz}$. Peak-to-peak amplitude held constant at 1.41V. Phase 3: 30 minutes. Square wave with frequency $f=4.5\text{Hz}$. Peak-to-peak amplitude held constant at 2.12V. The electroformation was conducted either at 24°C or at 60°C dependent on the phase transition temperature of the lipids. The GUVs were diluted in 15mM HEPES pH 7.5, 135mM NaCl.

The GUVs contained the following lipid compositions: POPC: 39%, POPS: 35%, POPE: 20%, PI3P: 5%, PE-Rh: 1% (Figure 4A, C, E, Figure 5A, D9 and Figure 6), DOPC: 39.5%, DOPS: 35%, DOPE: 20%, PI3P: 5%, PE-Rh: 0.5% (Figure 4D, F and Supplementary Movie 1), Egg-PC: 39%, Brain-PS: 35%, Brain-PE: 20%, PI3P: 5%, PE-Rh: 1% (Figure 4B, Figure 5B), Egg-PC: 59%,

Brain-PS: 35%, Brain-PE: -%, PI3P: 5%, PE-Rh: 1% (Figure 5C). All lipids were from Avanti Polar Lipids Inc..

Liposome aggregation assay

The measurements were taken with Hitachi U-2000 spectrophotometer. Liposomes were prepared as described for the liposome co-sedimentation assays, using 400 nm filter membrane for extrusion. The absorbance at 450 nm of 0.5 mg/ml liposomes was measured for 24-30 s before the protein was added to a final concentration of 0.04 mg/ml. The absorbance at 450 nm was further measured for a total of 20 min, in 6 s intervals. For the sample without protein, buffer (150 mM NaCl, 50 mM HEPES pH 7.5, 1 mM DTT) was added to the liposomes after 24-30 s to reach the same final liposome concentration as for the reactions with the proteins. As a control, the absorbance at 450 nm of 0.04mg/ml protein solution was measured where no increase in absorbance was detected (not shown).

Yeast strains

Yeast strains were BY4741 wt (Mat a; *his3Δ1*; *leu2Δ0*; *met15Δ0*; *ura3Δ0*, Euroscarf), and BY4741 *atg5Δ::kanMX4* generated as described in (Kraft et al, 2008).

The following yeast strains were used in this study

Supplemental Table 1

Strain Name	ID	Background	Genotype	Reference
wt	yCK566	S288C	Mat a	Euroscarf
<i>atg5Δ</i>	yCK408	S288C	Mat a; <i>atg5Δ::kanMX6</i>	this study
<i>atg12Δ</i>	yCK748	S288C	Mat a; <i>atg12Δ::kanMX6</i>	this study
<i>atg8Δ</i>	yCK412	S288C	Mat a; <i>atg8Δ::kanMX6</i>	this study
<i>atg3Δ</i>	yCK750	S288C	Mat a; <i>atg3Δ::kanMX6</i>	this study
<i>atg16Δ</i>	yCK753	S288C	Mat a; <i>atg16Δ::kanMX6</i>	this study
<i>ypt7Δ</i>	yCK814	S288C	Mat a; <i>ypt7Δ::natMX6</i>	this study
<i>atg5Δypt7Δ</i>	ySM16	S288C	Mat a; <i>atg5Δ::kanMX6</i> , <i>ypt7Δ::natMX6</i>	this study
<i>atg16Δatg12Δ</i>	ySM18	S288C	Mat a; <i>atg16Δ::kanMX6</i> , <i>atg12Δ::natMX6</i>	this study
<i>atg5Δatg16Δ</i>	ySM20	S288C	Mat alpha; <i>atg5Δ::natMX6</i> , <i>atg16Δ::kanMX6</i> ; <i>MET15</i>	this study
<i>atg5Δatg12Δ</i>	ySM22	S288C	Mat a; <i>atg5Δ::natMX6</i> , <i>atg12Δ::kanMX6</i>	this study
<i>atg8Δatg5Δ</i>	ySM30	S288C	Mat a; <i>atg8Δ::kanMX6</i> , <i>atg5Δ::natMX6</i>	this study

The genotype of *S. cerevisiae* S288C genetic background used in this study is *his3Δ1*; *leu2Δ0*; *met15Δ0* (or *MET15* where indicated); *ura3Δ0*: Knockout strains were purchased as diploids from Euroscarf and haploid spores were selected.

Yeast culture and assay conditions

Plasmid DNA was transformed to yeast in stationary phase grown on YPD-agar according to a LiOAc/ssDNA/PEG transformation protocol.

Transformed yeast strains were grown in synthetic defined minimal medium (SD; 1.7g/l yeast nitrogen base without amino acids and ammonium sulphate (Formedium, UK), 5g/l ammonium sulphate, 2g/l glucose) supplemented with the appropriate amino acid drop-out mix (CSM; Formedium) to log phase and subjected to nitrogen starvation for 5 hours. Lysates were prepared from cultures in log phase and after starvation by adding 1 volume of cold 50% Trichloroacetic acid to 6 volumes of culture followed by incubation on ice for 10 minutes and centrifugation at

13000rpm at 4°C. Pellets were washed with cold acetone and resuspended in urea/SDS loading buffer (120 mM TrisHCl pH 6.8, 5% glycerol, 8 M urea, 143 mM β-mercaptoethanol, 8% SDS) at a concentration of 1 unit OD₆₀₀ per 25μl buffer.

For immunoprecipitation of Myc-tagged Atg5 transformed yeast cells were grown in SD-Ura until log phase. Whole-cell lysates of yeast cells were prepared as described previously (Fellner et al, 2003). Briefly, 200 OD₆₀₀ units of cells were washed once in cold ddH₂O and then resuspended in cold yeast lysis buffer (50 mM Tris-Cl at pH 7.6, 150 mM NaCl, 1% (v/v) Triton X-100, and 1 mM EDTA) supplemented with 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.03 units/ml aprotinin (Sigma), and Complete inhibitor (Roche). Cells were lysed by FastPrep for 2x40 sec at 6m/s. Myc-tagged Atg5 was immunoprecipitated with anti-Myc antibody (clone 4A6) covalently cross-linked to protein A sepharose beads (GE Healthcare). Lysate containing 10mg total protein was incubated with 30μl solid beads for 2h at 4°C under agitation. Samples were washed once with 1 ml ice cold lysis buffer and three times with 1 ml Tris-buffered saline (TBS; 25 mM Tris-HCl at pH 7.4, 137 mM NaCl, 2.7 mM KCl). Immunoprecipitates were boiled in Lämmli-buffer, separated by 11% SDS-PAGE, transferred to 0.2 μm nitrocellulose membrane and proteins were detected with the monoclonal anti-Atg5 (8B12-3B11) or Atg12 (7B11-1F9) antibody.

Yeast constructs.

Atg5-3xmCherry and Atg5dbE-3xmCherry were expressed from pRS316, under the control of the endogenous promoter and the *cyc1* terminator. The *cyc1* terminator was cloned via the XhoI and KpnI sites into pRS316 to give rise to pRS316-*cyc1*. The 3xmCherry was amplified from pFA6a-linker-3mCherry-dcu-hphNT1 (a gift from Michael Knop, Heidelberg University) and inserted into pRS316-*cyc1* via the HindIII and Sall sites. A genomic fragment containing the Atg5 ORF including 800 upstream nucleotides was cloned upstream of the 3xmCherry via the NotI and XmaI sites. Mutations were introduced using the QuickChange protocol (Stratagene).

Atg16-3mCherry was expressed from pRS315. A genomic fragment from 800 nucleotides upstream to 300 nucleotides downstream of the Atg16 ORF was cloned into pRS315 via the SalI and NotI sites. A codon optimized 3xmCherry tag (see above) was inserted into a PstI site introduced just before the stop codon of Atg16.

Yeast microscopy

Yeast were grown to the log phase in the appropriate selective medium and subjected to a 5h treatment with 220 nM rapamycin. Cells were immobilized with concavalinA (Sigma). Stacks of images were taken using Deltavision Epifluorescence Deconvolution Microscope. Image processing and data analysis was done using ImageJ.

For the quantification of Atg5 localization to the PAS, approximately 150 cells were counted per experiment per construct (3 experiments each). Only cells emitting fluorescence above the background levels (i.e. above the fluorescence of wt strain transformed with the appropriate empty plasmids) were counted.

For the quantification of PAS localization of Atg16-3xmCherry, in average 250 cells per experiment (3 in total) were counted. Due to the low Atg16-3xmCherry signal we were unable to unambiguously distinguish between cells displaying a purely cytoplasmic signal and cells displaying no signal. We therefore calculated the percentage of cells displaying Atg16 puncta relative to all cells.

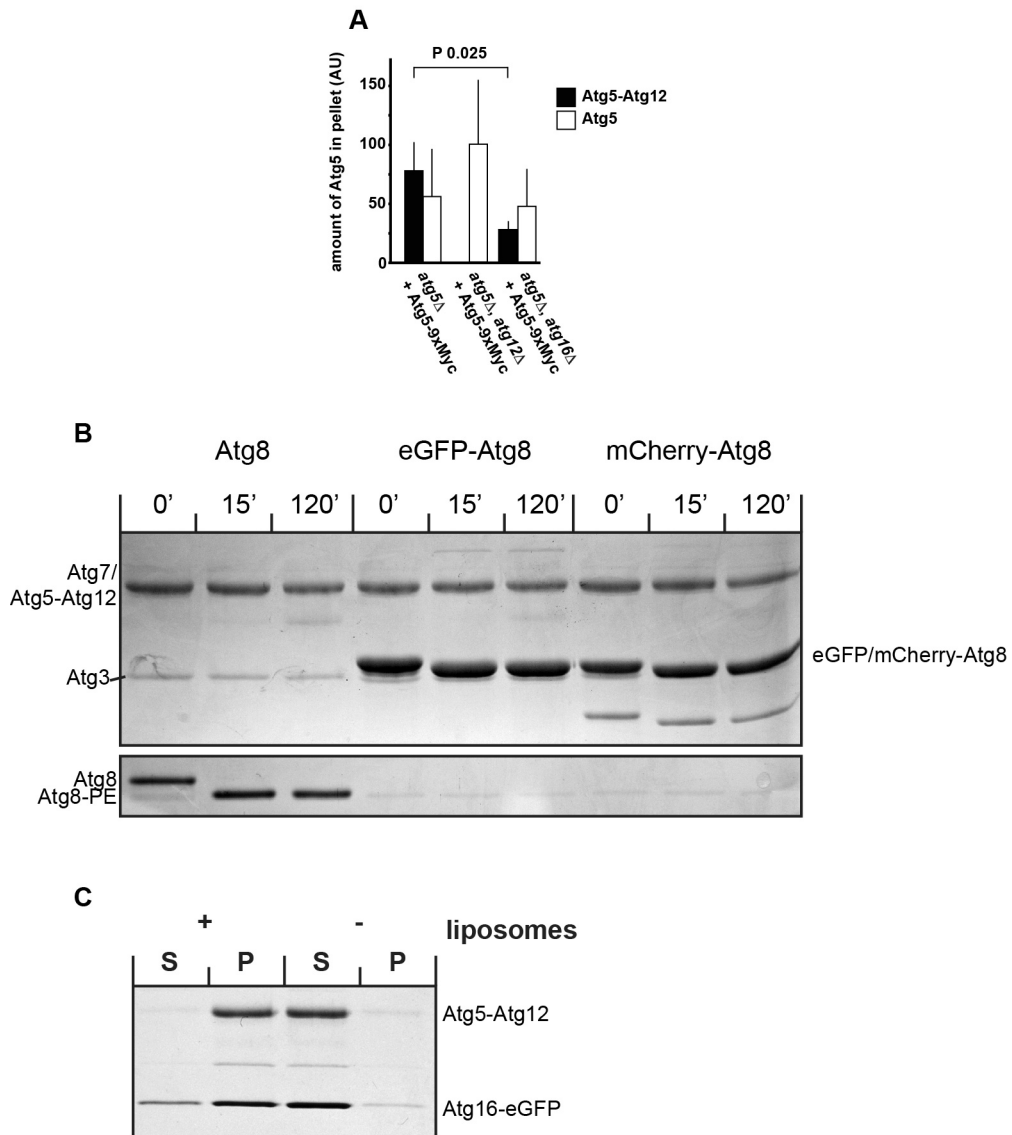
Supplementary References

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Supplementary Figure 1



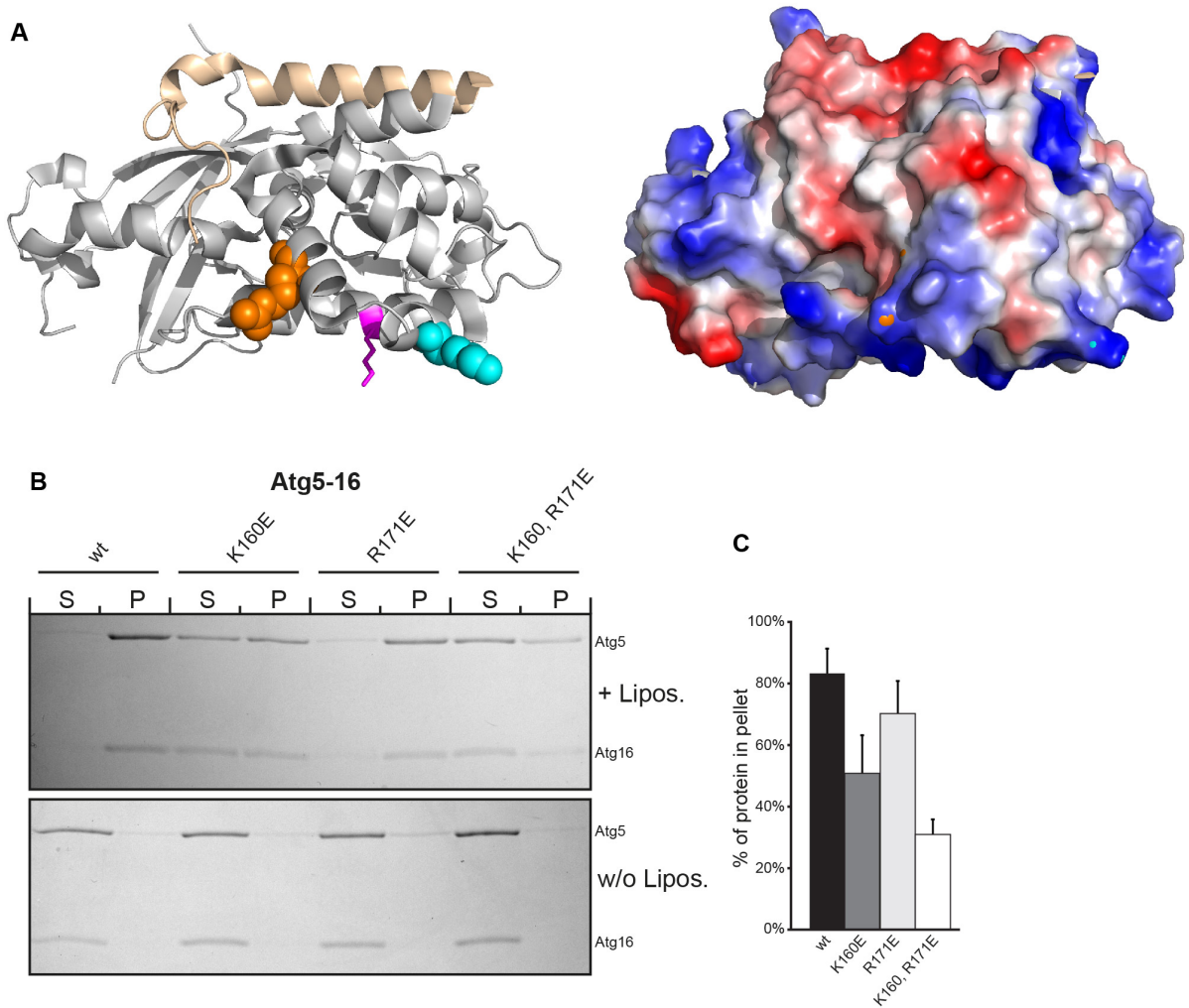
Amount of Atg5 in the pellet fractions of different deletion strains and functionality of fluorescently labeled Atg8 and Atg16 *in vitro*

(A) Graph based on 3 anti-Myc western blots showing the amount of Atg5 and Atg5-Atg12 in the pellet fraction of the indicated yeast strains. The graph is based on the same data as the graph shown in Figure 1C but has not been normed to the amount of Atg5 and Atg5-Atg12 in the pellet fractions of Atg5D cells expressing Atg5-9xMyc. The quantification is based on 3 independent experiments and shown are the averages and the standard deviations.

(B) eGFP- and mCherry-Atg8 fusion proteins are efficiently conjugated to PE. Atg8, eGFP-Atg8 and mCherry-Atg8 were incubated with Atg3, Atg7, the Atg5-12 conjugate, liposomes and ATP. The reactions were stopped after the indicated time points and run on a 16% urea-SDS gel. The PE conjugated form of eGFP- and mCherry-Atg8 shows the characteristic band shift.

(C) The Atg5-12-16-eGFP complex binds to liposomes. The Atg5-12-16-eGFP complex was incubated with Folch liposomes, centrifuged and equal amounts of the resulting supernatants and pellets were run on a 12% gel.

Supplementary Figure 2



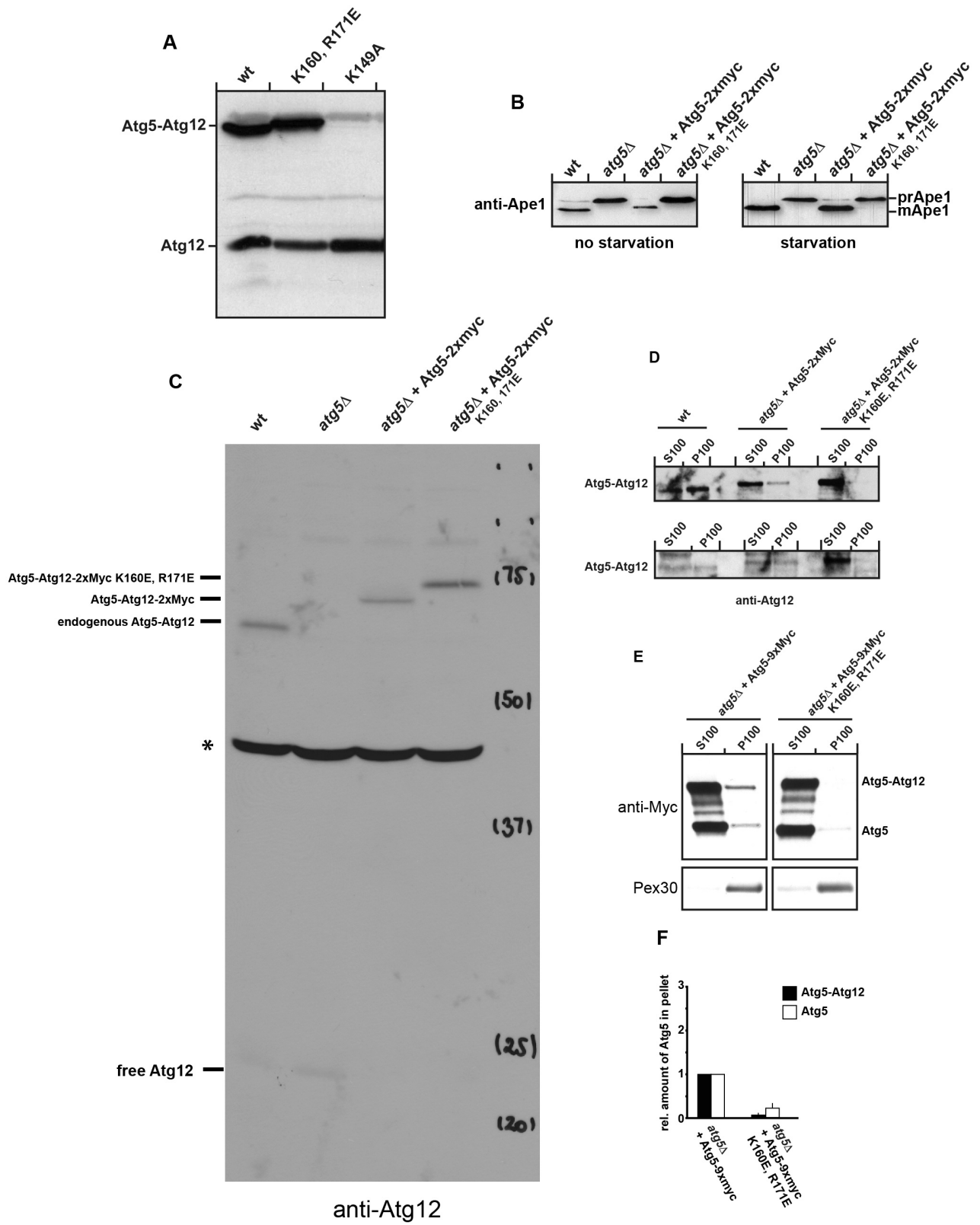
The Atg5 K160E, R171E double mutant shows a decrease in membrane binding *in vitro*

(A) Structure of yeast Atg5 (grey colored) in complex with the N-terminus of yeast Atg16 (wheat colored). Left: Lysine 149 (pink) as well as lysine 160 (cyan) and arginine 171 (orange) are highlighted. Right: surface charge representation of the same structure. The figure was created using Pymol from structure 2DYO (www.pdb.org) (Matsushita et al, 2007).

(B) Coomassie stained gel of a liposome co-sedimentation assay with wild type Atg5 and the indicated mutants in complex with full-length Atg16 using Folch liposomes. (S: Supernatant; P: Pellet)

(C) Quantification of 3 liposome co-sedimentation assays as shown in (b). The total amount of protein in the supernatant and pellet was set to 100%. Shown are the averages and the standard deviations.

Supplementary Figure 3



The Atg5 K160E, R171E double mutant is efficiently conjugated to Atg12 *in vitro* and *in vivo*.

(A) Western blot of *E. coli* cell lysates co-expressing Atg3, Atg7, Atg12 and the indicated versions of Atg5. The cell lysates were subjected to anti-Atg12 western blotting. Note that the K160, R171E mutant Atg5 is still efficiently conjugated. In contrast, the K149A mutant Atg5 lacking the Atg12 acceptor site is not.

(B) Starvation assay with the indicated yeast strains and the indicated constructs using an anti-Ape1 antiserum for western blotting.

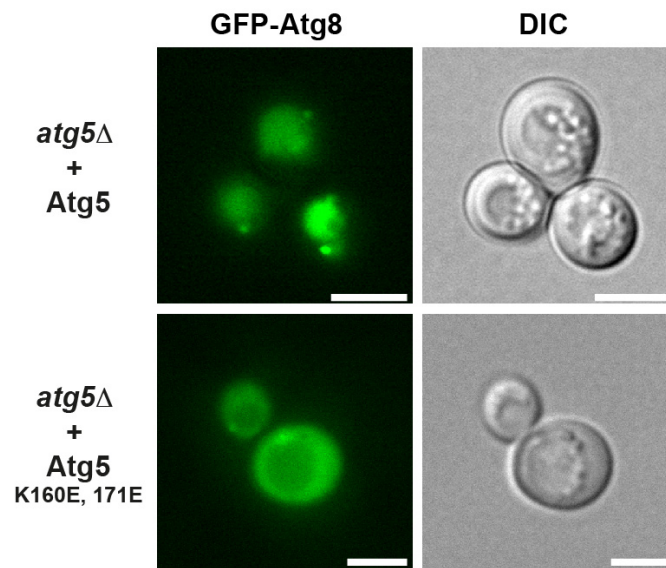
(C) Yeast cell lysates of the indicated strains were subjected to Western blotting using the monoclonal anti-Atg12 antibody. The asterisk indicated a strong background band running between 40-50kDa. The numbers in brackets indicate the molecular weight in kDa. The upper two brackets indicate the position of the 150kDa and 100kDa marker bands, respectively.

(D) Anti-Atg12 western blot of cell fractions prepared from rapamycin treated yeast cells.

(E) Anti-Myc and anti-Pex30 western blots of cell fractions of Atg5 deficient yeast cells expressing wild type or the K160E, R171E mutant Atg5.

(F) Quantification based on three independent experiments as shown in (E). The amount of wild type Atg5-9xmyc and Atg5-9xmyc-Atg12 in the pellet fraction relative to the Pex30 signal was set to 1. Shown are the averages and standard deviations.

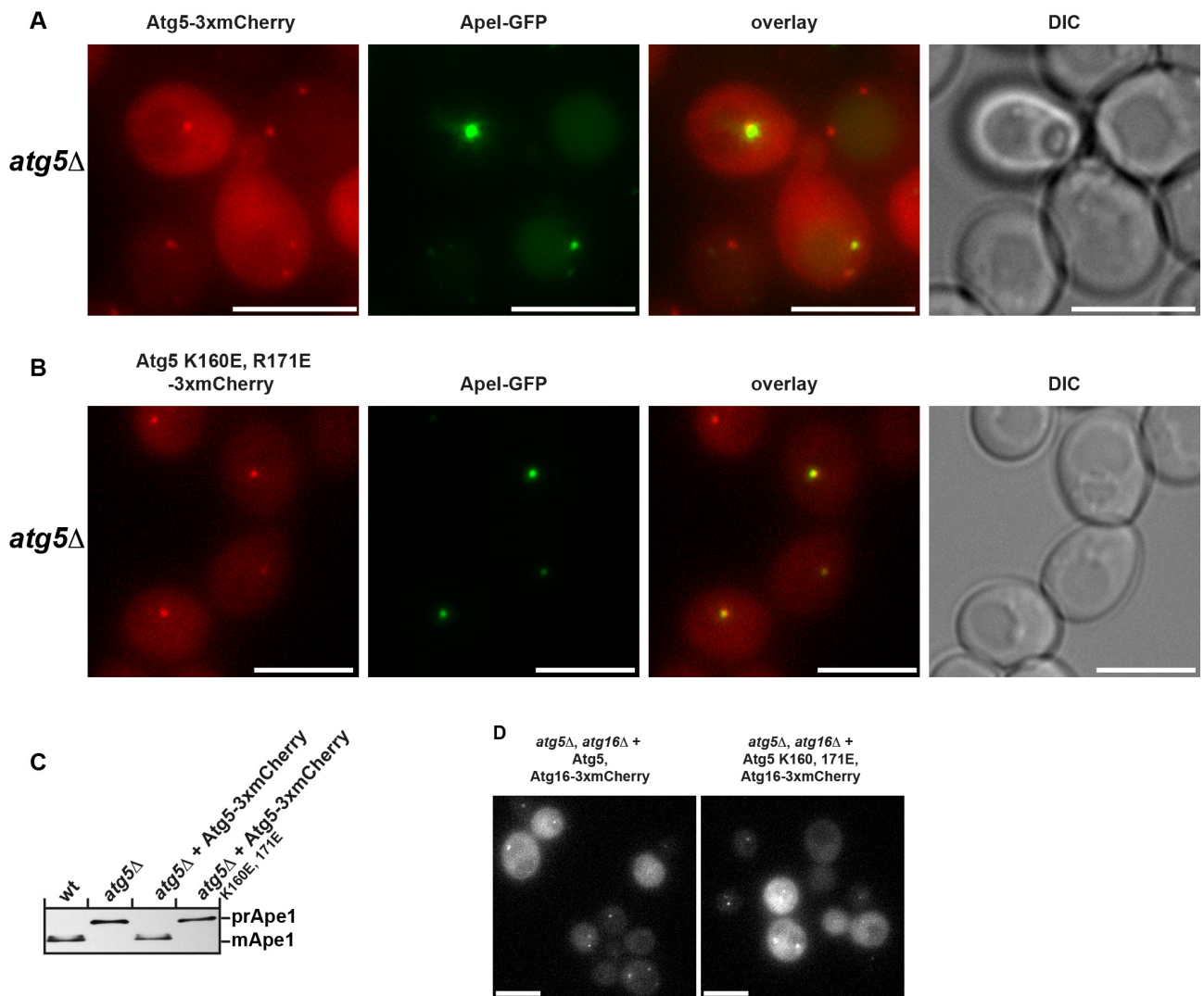
Supplementary Figure 4



The Atg5 K160E, R171E mutant shows a severe defect in the delivery of GFP-Atg8 to vacuolar lumen

Atg5 Δ yeast cells were co-transformed with wild type Atg5 or the Atg5 K160, R171E mutant and GFP-Atg8. GFP-Atg8 localization was analyzed after overnight starvation using an Olympus CellR fluorescence microscope. Note that GFP-Atg8 is delivered to the vacuolar lumen in cells expressing wild type Atg5 but not in cells expressing the Atg5 K160E, R171E mutant. DIC: differential interference contrast. Scale bars: 5 μ m.

Supplementary Figure 5



The Atg5 K160E, R171E mutant still localizes to the PAS as defined by Apel

(A, B) Maximum projection of Atg5 deficient yeast cells expressing the indicated fusion proteins that were treated with rapamycin for 5h and imaged using a Deltavision microscope.

(C) Anti-ApelI western blot of Atg5 deficient yeast cells treated with rapamycin for 5h expressing the indicated fusion proteins.

(D) Maximum projections of rapamycin treated (5h) yeast cells of the indicated genotype expressing the indicated proteins and that were imaged using a Deltavision microscope.

Scale bars: 5 μ M.

Supplementary Movie 1

The Atg5-12-16 complex forms mobile clusters on the surface of a GUV.

The movie shows a time lapse of the Atg5-12-16-monomericGFP complex on the top surface of a GUV. Images were taken every 500 milliseconds. The movie is accelerated 8 times. The diameter of the GUV is 4 μ m.