**SUPPLEMENTARY MATERIALS AND METHODS** (*Kaminska et al., 2016*)

## Plasmids and plasmid construction

The plasmids used in this study: YEp351 (Hill *et al*., 1986); YCp33lac and YEp181lac (Gietz and Sugino, 1988); pGBT9, pGAD424 (TaKaRa Clontech Kusatsu, Shiga, Japan); pYES-NEDD4 and pYES-NEDD4w4 (Gajewska *et al*., 2003); pGFP-ATG8 (Suzuki *et al*., 2001); pGEM-ATG2-XS, p415-$P_{GPD1}$-GFP-ATG2 and pGBT9-ATG2 (Romanyuk *et al*., 2011); pTS102 bearing *ATG2* (Shintani *et al*., 2001); PS-159 bearing *AD-ATG18* fusion (gift from D. Klionsky, University of Michigan, USA); pFL46S-HSP104 and pFL46S-SSB1 (Chacinska *et al*., 2001).

To test for suppression of the Nedd4w4-dependent growth defect, the plasmid YEp351-ATG2 was constructed by transfer of fragments SphI-SacI and SacI-SacI from pTS102 to YEp351. Then YEp351-ATG2-XS containing XmaI and SalI sites flanking *ATG2* ORF was obtained by replacing the wild type fragments with *in vitro*-mutated fragments of pGEM-ATG2-XS. To construct plasmid expressing *ATG2-N* fragment (*ATG2* promoter and fragment encoding aa 1-320 of Atg2) the PCR product obtained by using specific primers introducing flanking XmaI and SalI sites and YEp351-ATG2 as a template was digested with XmaI and SalI and ligated with YEp351. Plasmid expressing *ATG2-C* (encoding aa 1105-1592) was constructed similarly, the PCR product containing *ATG2-C* was obtained by using specific primers introducing flanking XmaI and SalI and was transferred to YEp351, and then the fragment containing promoter amplified using YEp-ATG2-XS as a template was digested with SphI and XmaI and inserted between the SphI and XmaI sites in front of *ATG-C*. To construct plasmid YEp-ATG2-ΔN, XmaI site and a new ATG codon before codon 320 of *ATG2* in the NcoI-BgtZI DNA fragment was introduced by *in vitro* mutagenesis and the wild type fragment was replaced in YEp-ATG-XS. Then XmaI-XmaI fragment was deleted. YEp-ATG2-ΔC and YEp-ATG2-ΔNΔC were constructed by digestion of YEp351-ATG2 or YEp-ATG2-ΔN with EagI and religation.

Plasmids used for localization and suppression studies, p415-GFP-ATG2-C1, p415-GFP-ATG2-C2, p415-GFP-ATG2-C3 and p415-GFP-ATG2-C4, were constructed by PCR amplification of respective fragments based on plasmid bearing *ATG2*, digestion with HindIII and SalI and ligation into HindIII/SalI-digested p415-$P_{GPD}$-GFP.

Plasmids used in two-hybrid experiments, derivatives of pGBT9 and pGAD424, containing *ATG2* fragments were constructed similarly as described above. Also plasmids encoding fragment of *ATG2* bearing three domains from the Atg2 N terminus, pGAD424-Atg2-N3 and pGBT9-Atg2-N3, were constructed by digestion of pGAD424-Atg2-XS and pGBT9-Atg2-XS with ClaI and SalI, filling ends with Klenow polymerase and religation. The pGBT9-Atg2-ΔC plasmid was constructed by in-frame deletion of 1032-bp EagI-EagI fragment from pGBT9-Atg2-XS.

Plasmids used for protein expression in *E. coli* are based on the pGEX-4T-1 vector, which is a part of the glutathione S-transferase (GST) gene fusion system from GE Healthcare. The Atg2-C-encoding sequence was subcloned into EcoRI/SalI-linearized pGEX-4T-1 as a 1497-bp EcoRI-SalI fragment of pGBT9-ATG2-C giving pGEX-ATG2-C. The pKF300TU vector was constructed by replacement of the multicloning site (30-bp

BamHI-NotI part) in pGEX-4T-1 with a 105-bp synthetic linker encoding a TEV protease cleavage site followed in-frame by several restriction sites and three STOP codons (BamHI-TEV_c.s.-NdeI-NcoI-XbaI-SpeI-BglII-SmaI-EcoRI-HindIII-ClaI-SalI-XhoI-3×STOP-NotI). A control plasmid, pKF463, containing STOP codon directly downstream of the TEV cleavage site, was created by ligation of a 4978-bp BamHI-EcoRI fragment of pKF300TU and 33-bp BamHI/EcoRI-digested dsDNA specific linker containing the AarI recognition site positioned downstream of the STOP codon. The AarI enzyme is a type IIS endonuclease and allows, in this particular case, the creation of a seamless fusion of a sequence encoding a protein of interest with the TEV cleavage site. The sequence encoding Atg2-S1074-P1348 (isolated APT1 domain) was PCR-amplified from strain S288C genomic DNA with specific primers and cloned as an 843-bp Esp3I-Esp3I fragment into AarI/SalI-linearized pKF463 yielding pKF481.

To observe cellular localization of Nedd4w4, the plasmid pYES-$P_{GAL}$-mCherry-Nedd4w4 was constructed with the use of fusion PCR. Based on $P_{GAL1}$-*NEDD4w4*, two PCR products were obtained: *GAL1* promoter and fragment of *NEDD4w4* gene. The mCherry encoding sequence was amplified from pFA-mCherry-NatMX plasmid (gift from M. Kaksonen lab,University of Geneva, Switzerland). Three PCR products were mixed and final PCR was performed to get $P_{GAL1}$-*mCherry-NEDD4w4*. Obtained DNA was AgeI/BlnI digested and used to replace the AgeI-BlnI fragment of pYES-NEDD4w4.

**Purification of proteins from bacteria**

For liposome assay and high speed pelleting assay with actin, the Atg2 fragments were purified as follows. Plasmid bearing *GST-ATG2-C* was transformed into *E.coli* strain OverExpress™ C43(DE3) (Lucigen Corporation, Middleton, WI, USA). Bacteria were grown to $OD_{600}$ ~0.6 in 2×YT medium at 37°C. Protein expression was induced by addition of IPTG to 1 mM, then cells were pelleted, resuspended in phosphate-buffered saline (PBS) pH 7.0 and lysed by sonication. Then Triton X-100 was added to 1% and samples were incubated for 30 min at 4°C. After centrifugation 20 min 20 000×g 10 ml supernatant was filtered through Millex filter unit (Millipore) and incubated with 250 µl of glutathione transferase (GST)-beads (GE Healthcare). GST-Atg2-C was eluted using reduced glutathione. Buffer was exchanged using PD Minitrap G-25 columns (GE Healthcare Little Chalfont, UK) into buffer G (2 mM Tris-HCl pH 8.0, 0.2 mM $CaCl_2$, 0.2 mM ATP, 0.5 mM dithiothreitol) or buffer B (20 mM HEPES pH 7.2, 150 mM NaCl).

**Liposome assay**

For preparation of liposomes, 25 µl of a 25 mg/ml solution of Folch fraction I from bovine brain (Sigma-Aldrich) was dried under nitrogen and resuspended in 250 µl of buffer B (20 mM HEPES pH 7.2, 150 mM NaCl) at 60°C for 1 h. GST-Atg2-C was mixed with 20 µl of liposomes and incubated at room temperature for 30 minutes. Liposomes and bound proteins were pelleted by centrifugation at 250 000 × g for 15 minutes, and samples were analyzed by SDS–PAGE.

**Monitoring of authophagy by Pho8Δ60 alkaline phosphatase test**

For measurements of autophagic activity, the alkaline phosphatase test was performed as described previously (Noda *et al*., 1995). Strains KJK146 *pho13*Δ *pho8*Δ*60* (a *pho13*Δ derivative of TN125; Shintani *et al*., 2001) and YPJ2 *pho8*Δ*60* strain (Romanyuk *et al*., 2011) were used. Four independent experiments were performed.

**SUPPLEMENTARY FIGURES**

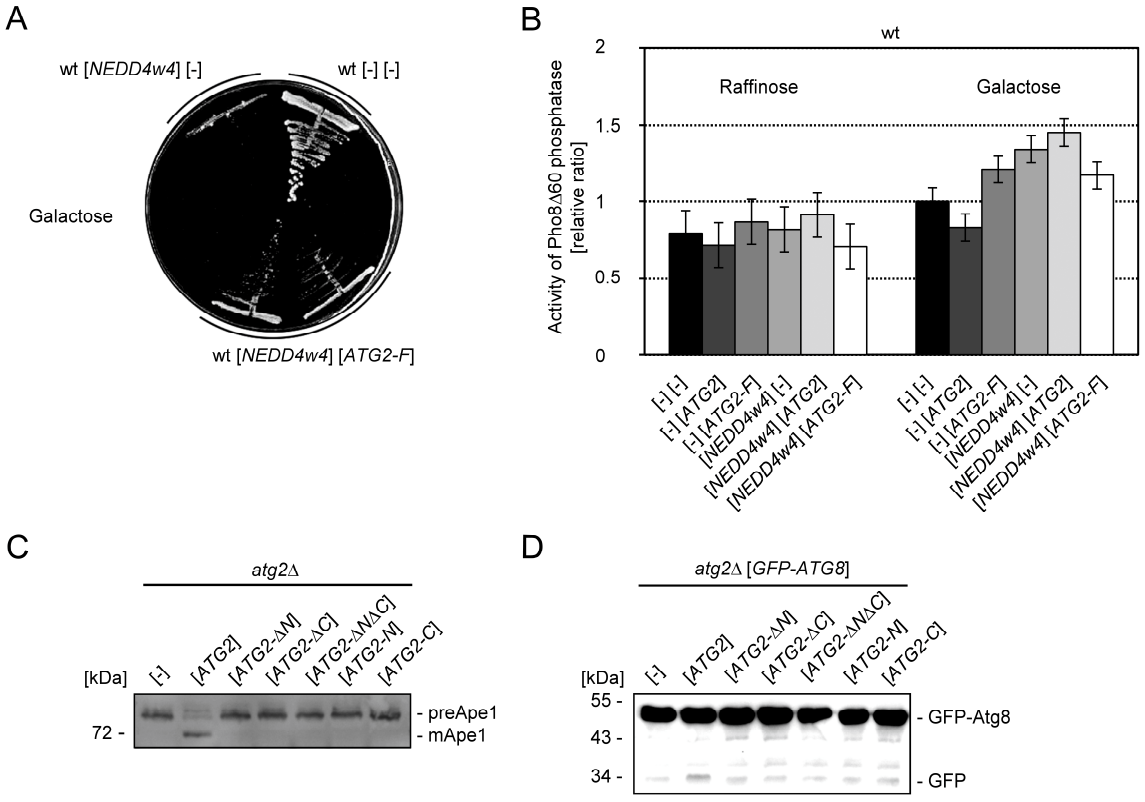


**Figure S1. *NEDD4w4* and *ATG2-F* affect cell growth but do not affect nonselective autophagy and fragments of *ATG2* do not complement defects of *atg2*Δ strain in Cvt pathway or nonselective autophagy.**

**A.** Growth of wild type strain (INV) transformed with empty vectors, and wild type strain expressing *NEDD4w4* and transformed with empty vector or plasmid bearing fragment of *ATG2* (*ATG2-F*) on SCgal-ura-leu (galactose) plate at 28°C for 5 days.

**B**. Activity of alkaline phosphatase of *pho8Δ60* strain (YPJ2; Romanyuk *et al*., 2011) transformed with plasmid carrying *NEDD4w4* and empty vector or *ATG2* or *ATG2f* fragment. Transformants bearing two empty vectors or expressing *ATG2* or *ATG2f* alone were used as a control. Cells were grown on raffinose-containing medium and shifted to galactose medium for 4 hours. Four independent experiments were performed and means ±SD (standard deviation) are shown.

**C**. Western blot analysis of preApe1 maturation in *atg2*Δ strain (BY4741 genetic background) transformed with empty vector or plasmids carrying *ATG2*, *ATG2*-Δ*N*, *ATG2*-Δ*C*, *ATG2*-Δ*N*Δ*C*, *ATG2-N*, *ATG2-C* grown on SC-ura-leu, shifted to nitrogen starvation and analyzed by Western blotting using anti-Ape1 antibody. Wild type strain transformed with empty vector was used as a control.

**D.** Analysis of GFP-Atg8 degradation. The *atg2*Δ strain (BY4741) was transformed with plasmid carrying *GFP-ATG8* and empty vector or *ATG2*, *ATG2*-Δ*N*, *ATG2*-Δ*C* or *ATG2*-Δ*N*Δ*C* fragments. Protein extracts were prepared and Western blotting was performed using anti-GFP antibody.

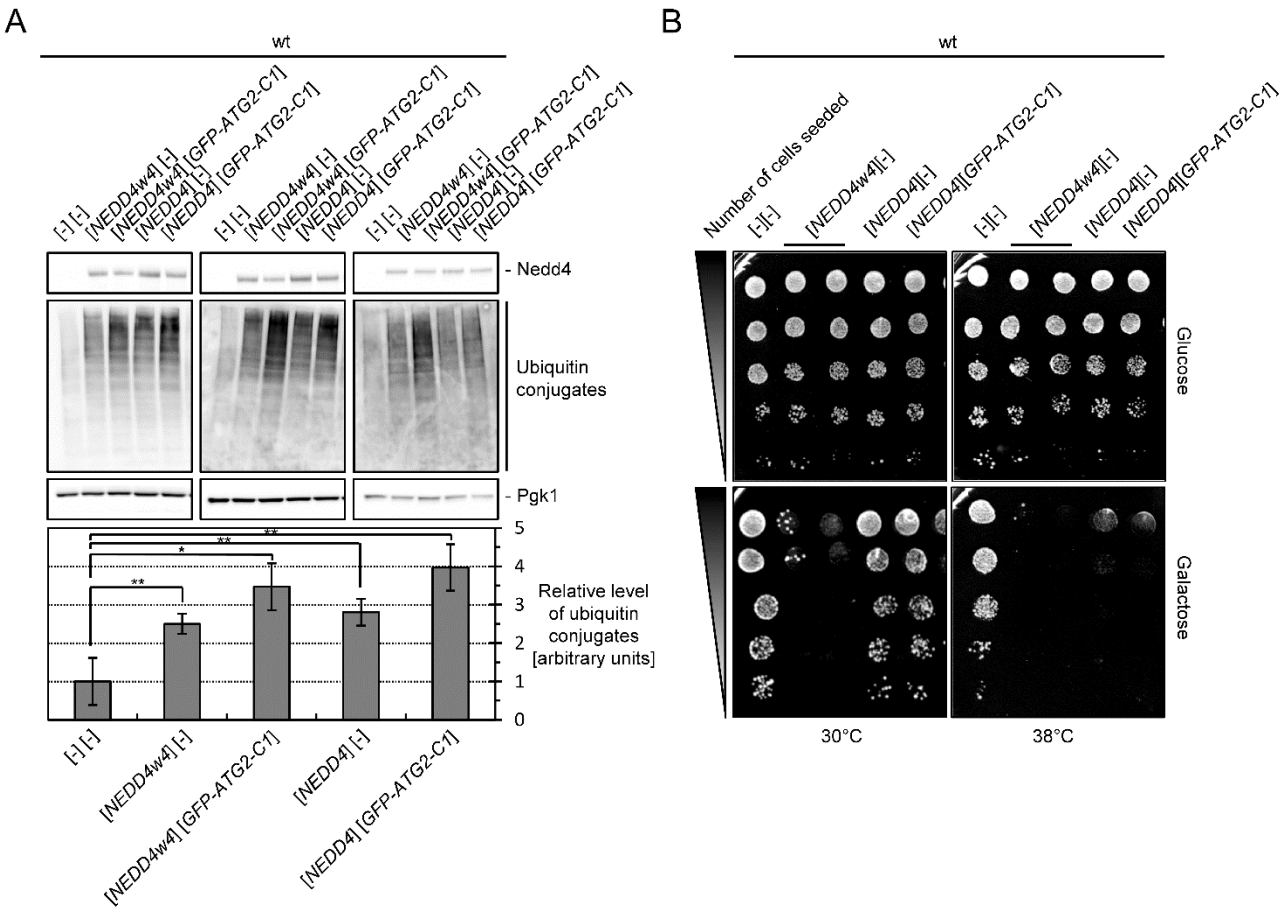


**Figure S2. Nedd4 and Nedd4w4 similarly affect the level of ubiquitinated proteins but, in contrast to Nedd4w4, the wild type Nedd4 is not toxic in yeast at 30ºC.**

**A**. Wild type **(**BY4741) cells transformed with empty plasmids or plasmids carrying *NEDD4*, *NEDD4w4* or *GFP-ATG2-C1* were grown at 30ºC in SCraf (raffinose) and transferred to SCgal (galactose) for 3.5 h to induce expression of *NEDD4* and *NEDD4w4*. Protein extracts were prepared by glass beads method. Western blots were developed with anti-ubiquitin, anti-Nedd4 and anti-Pgk1 antibodies. Pgk1 level was used as a loading control. Quantification of results obtained in three independent experiments is shown in the lower panel. The value obtained for wild type strain bearing empty vector was set to 1. Statistical significance level: *, $p<0.02$; **, $p<0.01$.

**B**. Growth of transformants as in (**A)** on SC (glucose) and SCgal (galactose) at temperatures indicated. Plates were incubated for 3-5 days.

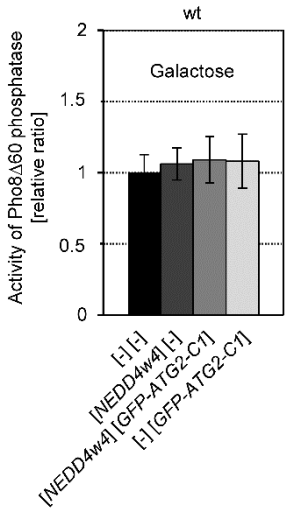


**Figure S3. GFP-Atg2-C1 does not induce nonselective autophagy in *NEDD4w4*-expressing cells.**

The *pho13*Δ *pho8*Δ*60* strain (KJK146, a *pho13*Δ derivative of TN125; Shintani *et al*., 2001) was transformed with empty vector or with plasmid bearing *NEDD4w4* and with empty vector or *GFP-ATG2-C1*. Transformants were grown in SCraf-ura-leu at 28ºC to $OD_{600}$ 0.4-0.5, then galactose was added to 2% final concentration and cells were grown for additional 4 h. The activity of Pho8Δ60 phosphatase was measured in four independent biological samples of each transformant type and means ±SD (standard deviation) are shown.

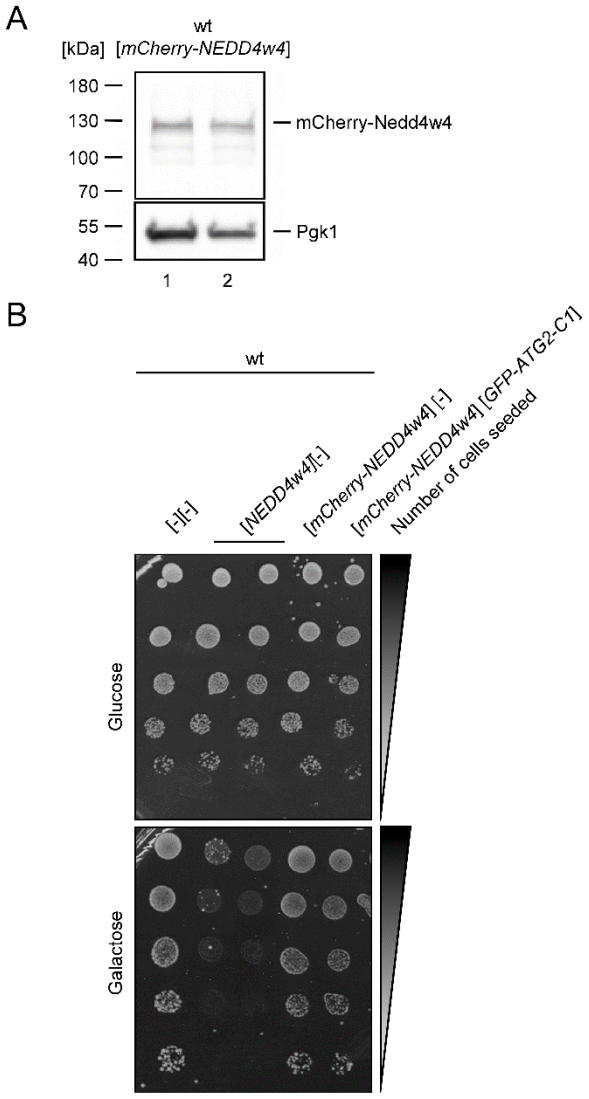


**Figure S4. The mCherry-Nedd4w4 is not toxic to yeast.**

**A**. Wild type strain (BY4741) was transformed with plasmid bearing *mCherry-NEDD4w4*. The level of fusion protein was then analyzed in two independent clones by Western blot using anti-Nedd4 antibody. Pgk1 was used as a loading control.
**B**. Growth of wild type strain bearing empty vector or vector containing *NEDD4w4* or *mCherry-NEDD4w4* and the second empty vector or vector containing *GFP-ATG2-C1* was checked by drop test on SC (glucose; repression) and SCgal (galactose; induction of *NEDD4w4* or *mCherry-NEDD4w4* expression) media and the results obtained after incubation at 30°C for 3-7 days are shown.

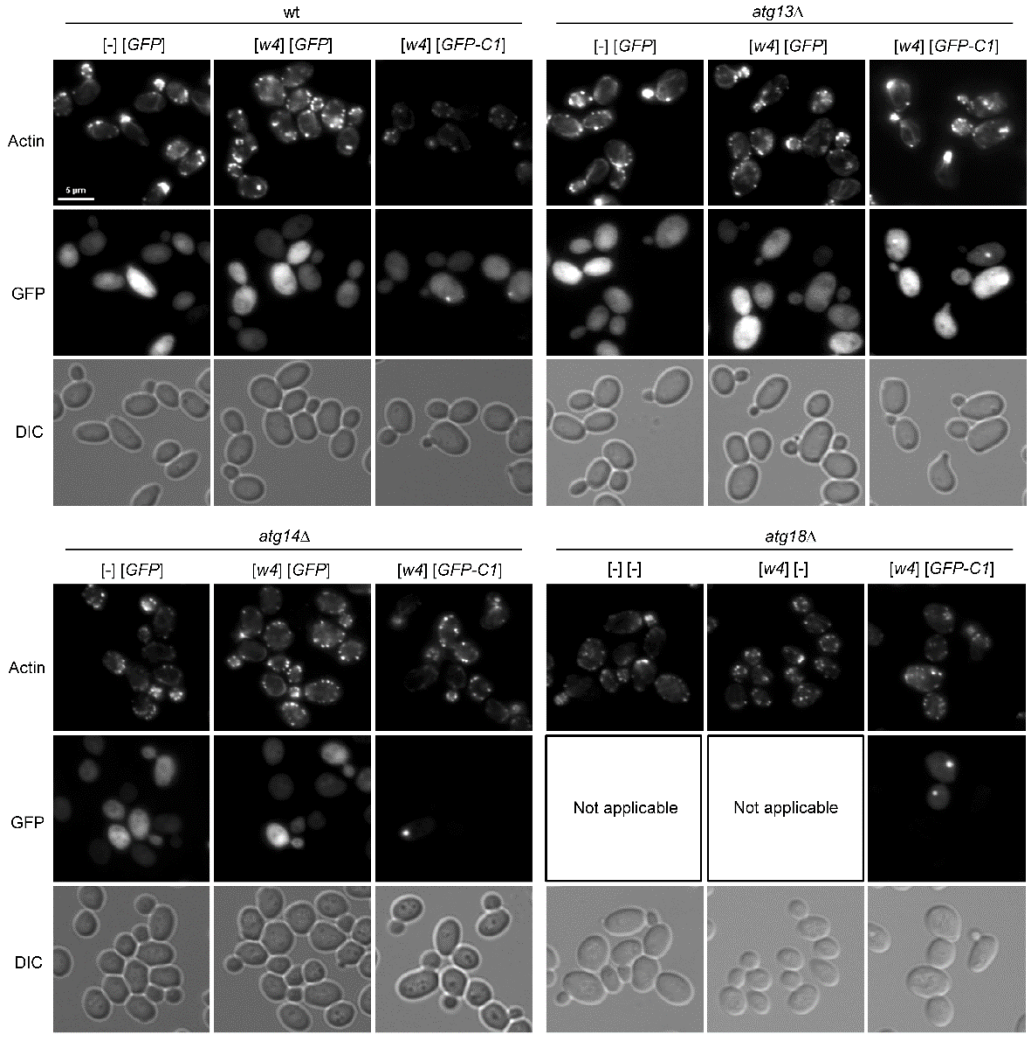


**Figure S5. The *atg13*Δ, *atg14*Δ and *atg18*Δ mutations variously affect organization of actin cytoskeleton in cells expressing *NEDD4w4* and *GFP-ATG2-C1*.**

Wild type, *atg13*Δ, *atg14*Δ and *atg18*Δ strains (BY4741 genetic background) bearing plasmids carrying *NEDD4w4* and *GFP-ATG2-C1* were growth to log-phase in SCraf-ura-leu. Expression of *NEDD4w4* was induced with galactose for 3.5 h at 28°C. Then cells were fixed using formaldehyde, pelleted, stained for actin filaments using Alexa fluor-conjugated phalloidin and observed using fluorescence microscopy.

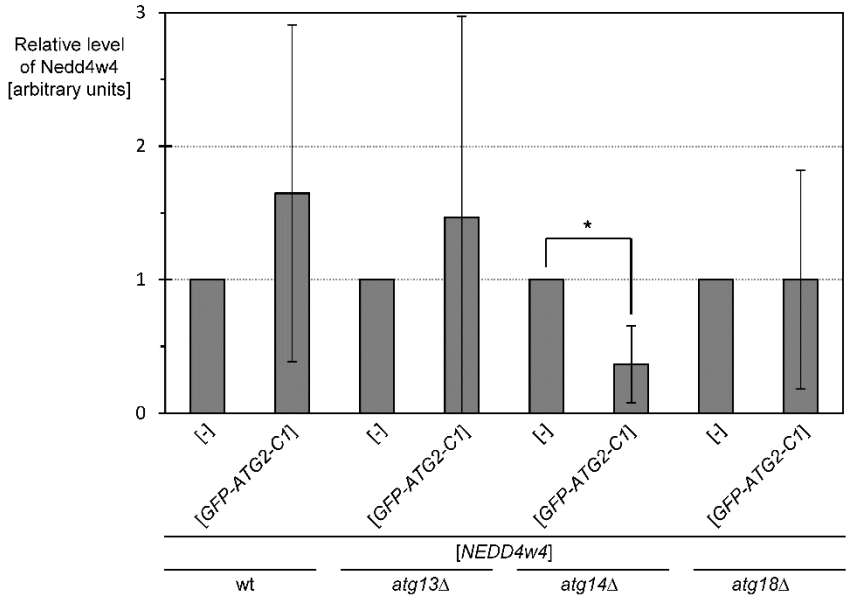


**Figure S6. The level of Nedd4 in *atg14*Δ is affected when GFP-Atg2-C1 is also present.**

Wild type, *atg13*Δ, *atg14*Δ and *atg18*Δ strains (in BY4741 background) transformed with empty plasmid or plasmids carrying *NEDD4w4* or *GFP-ATG2-C1* were grown as in Figure 7. Protein extracts were prepared by glass beads method and analyzed by SDS-PAGE and Western blot. Three independent experiments were performed. Blots were quantified and analyzed statistically, and relative levels of Nedd4w4 normalized to Pgk1 are shown. Statistical significance at $p < 0.02$ level is indicated by an asterisk (*).

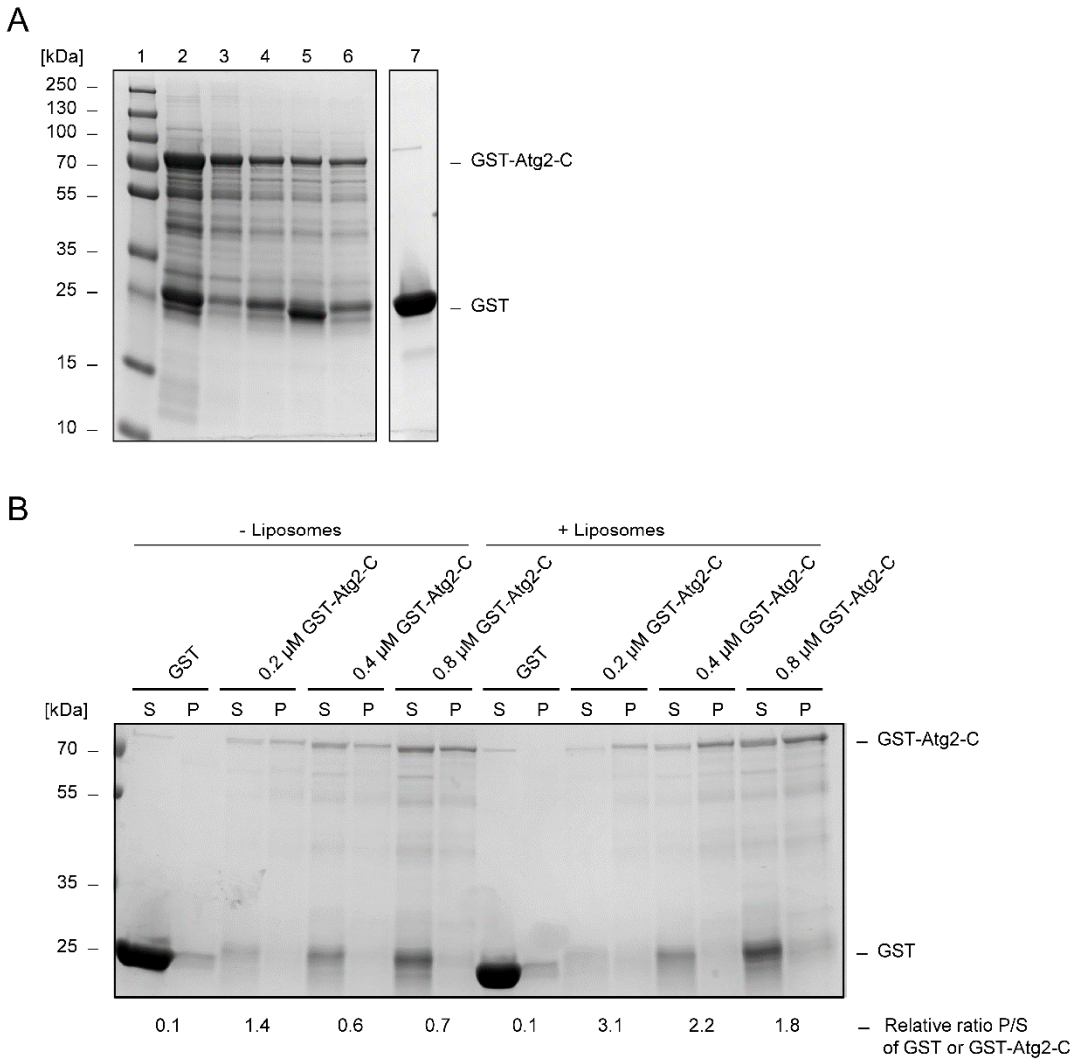


**Figure S7. GST-Atg2-C binds to liposomes.**

**A.** *E. coli* cells bearing pGEX-4T-GST-ATG2-C plasmid were grown at 37°C and induced with IPTG at 30°C for 7 h. GST-Atg2-C was purified using glutathione-sepharose beads and eluted with reduced glutathione. Buffer was exchanged using PD Minitrap G-25 columns. Known amounts of actin were used as a marker to estimate the amount of purified protein. 1. Molecular weight marker; 2. Beads after binding of Atg2-C; 3. Beads after elution of GST-Atg2-C; 4. Elution; 5, 6. Elution after buffer exchange; 7. GST purified in the same way.
**B**. GST-Atg2-C protein at concentration indicated or GST were incubated with liposomes or with lipid buffer alone and sedimented at 250 000 × g for 15 min. Pellets (P) and supernatants (S) were analyzed by SDS-PAGE and stained with Coomassie Brilliant Blue. Intensity of GST-Atg2-C bands in each fraction was measured densitometrically using Image Lab software and P/S ratio is shown.

**SUPPLEMENTARY REFERENCES**


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