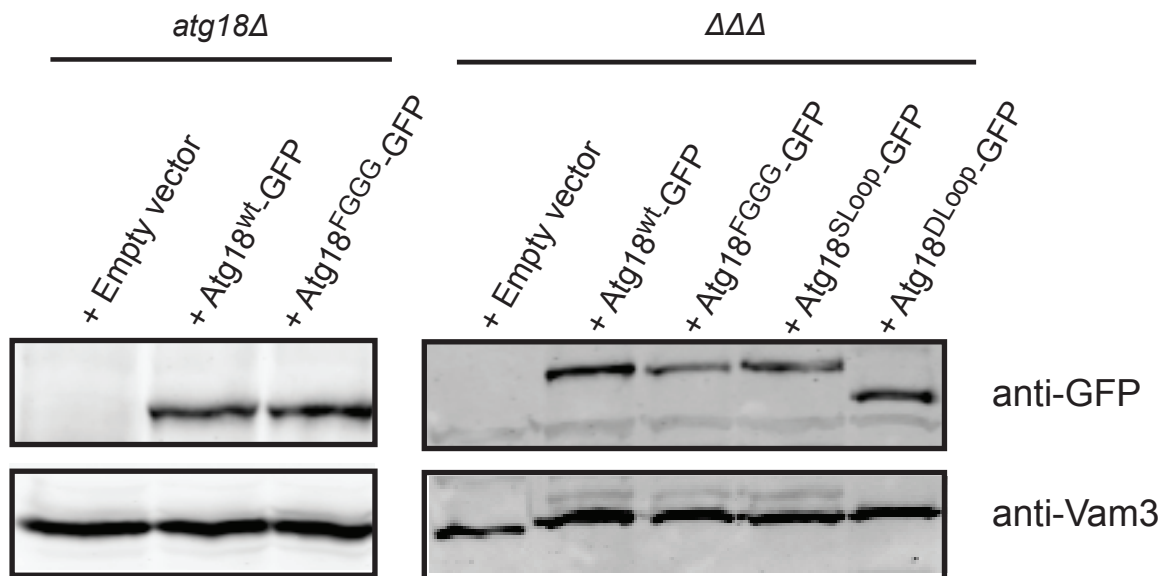


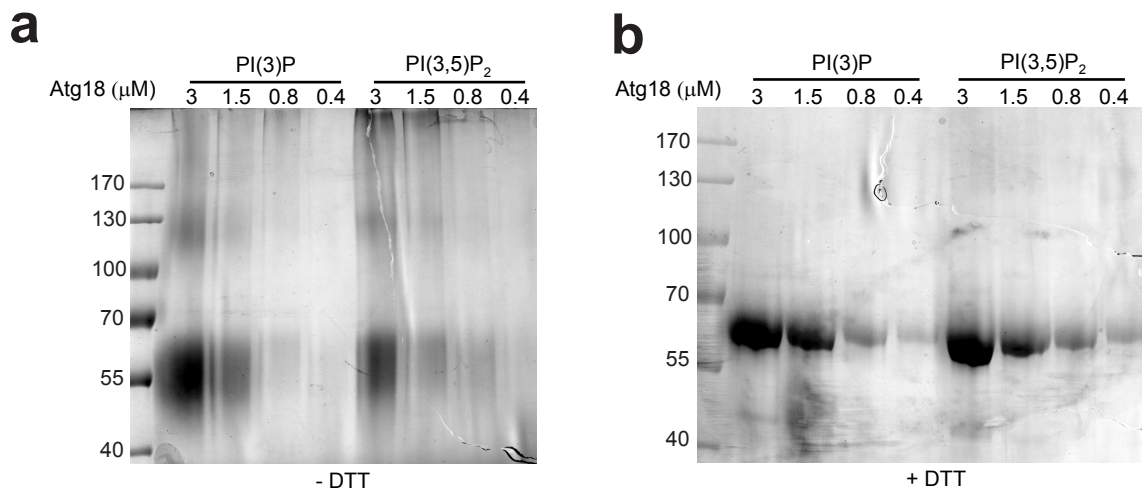
## Appendix file

### Table of content

	Page
Appendix Figure S1	2
Appendix Figure S2	3
Appendix Table O	4
Appendix Table O	6



**Appendix Figure G1.** Expression levels of Atg18wt-GFP and its mutant variants, resulting from the pRS316-based expression in *atg18Δ* and  $\Delta\Delta\Delta$  cells. Cells were grown as in Fig. 2, lysed with TCA and glass beads, and analysed by SDS-PAGE and Western blotting against GFP and the vacuolar integral membrane protein Vam3, which served a loading control.



**Appendix Figure S2.** Cross-linking of Atg18 at various concentrations of protein. 3  $\mu\text{M}$  to 0.4  $\mu\text{M}$  of Atg18 were incubated with 1 mg/mL of liposomes containing 5% PI3P or PI(3,5)P<sub>2</sub>. Cross-linking was performed as in Fig. 9.

**5 ddYbXJl Table G1: Yeast strains used**

Number	Strain	Genotype	Source
NG25	BJ3505	<i>MATa pep4::HIS3 prb1-Δ1.6R lys2-208 trp1-Δ101 ura3-52 gal2 can</i>	E. Jones
NG60	BJ3505 <i>atg18Δ</i>	BJ3505; <i>atg18::natNT2</i>	Zieger and Mayer (2012)
NG149	BJ3505 <i>atg18Δ atg21Δ hsv2Δ</i>	BJ3505; <i>atg18::natNT2; atg21::loxP-loxP; hsv2::loxP-KANMX-loxP</i>	This study
NG49	BJ3505 <i>atg18Δ</i> Atg18-GFP	NG60 + <i>pRS316-Atg18-3HA-GFP</i>	This study
	BJ3505 <i>atg18Δ</i> Atg18FGGG-GFP	NG60 + <i>pRS316-Atg18FGGG-3HA-GFP</i>	This study
NG140	BJ3505 <i>atg18Δ atg21Δ hsv2Δ</i> Atg18-GFP	NG149 + <i>pRS316-Atg18-3HA-GFP</i>	This study
NG172	BJ3505 <i>atg18Δ atg21Δ hsv2Δ</i> Atg18FGGG-GFP	NG149 + <i>pRS316-Atg18FGGG-3HA-GFP</i>	This study
NG176	BJ3505 <i>atg18Δ atg21Δ hsv2Δ</i> Atg18SLoop-GFP	NG149 + <i>pRS316-Atg18SLoop-3HA-GFP</i>	This study
TN124	Pho8 Δ60	Mat a, <i>ura3-52, leu2-3,-112, trp1-1, pho8::pho8Δ60, pho13::LEU2</i>	(Noda et al., 1995)
NG240	Pho8Δ60 <i>atg1Δ</i>	TN124 <i>atg1::KAN</i>	This study
NG241	Pho8Δ60 <i>atg18Δ</i>	TN124 <i>atg18::NAT + pRS316-Atg18</i>	This study
NG242	Pho8Δ60 <i>atg18Δ</i> Atg18	NG241 + <i>pRS316-Atg18</i>	This study
NG243	Pho8Δ60 <i>atg18Δ</i> Atg18FGGG	NG241 + <i>pRS316-Atg18FGGG</i>	This study
NG244	Pho8Δ60 <i>atg18Δ</i> Atg18SLoop	NG241 + <i>pRS316-Atg18SLoop</i>	This study
NG245	Pho8Δ60 <i>atg18Δ</i> Atg18DLoop	NG241 + <i>pRS316-Atg18DLoop</i>	This study
NG250	Pho8Δ60 <i>atg18Δ atg21Δ</i>	NG241 <i>atg21::KAN</i>	This study
NG251	Pho8Δ60 <i>atg18Δ atg21Δ</i> Atg18	NG250 + <i>pRS316-Atg18</i>	
NG252	Pho8Δ60 <i>atg18Δ atg21Δ</i> Atg18 Atg21	NG250 + <i>pRS316-Atg18 + pRS314-Atg21</i>	This study

NG253	Pho8Δ60 <i>atg18Δ</i> <i>atg21Δ</i> Atg18 Atg21SLoop	NG250 + <i>pRS316-Atg18</i> + <i>pRS314-Atg21SLoop</i>	This study
NG255	Pho8Δ60 <i>atg18Δ</i> <i>atg21Δ</i> Atg18SLoop Atg21	NG250 + <i>pRS316-Atg18SLoop</i> + <i>pRS314-Atg21</i>	This study
NG256	Pho8Δ60 <i>atg18Δ</i> <i>atg21Δ</i> Atg18SLoop Atg21SLoop	NG250 + <i>pRS316-Atg18SLoop</i> + <i>pRS314-Atg21SLoop</i>	This study
NG257	BJ <i>fab1Δ</i> Atg18-GFP	NG25 <i>fab1::NAT pRS316-Atg18-3HA-GFP</i>	This study
NG258	SEY <i>vps34Δ</i> (PHY102) Atg18-GFP	MATa <i>suc2-Δ9 ura3-52 leu2-3 his3-Δ200 trp1Δ911 lys 2-801 vps34Δ::TRP1 pRS316-Atg18-3HA-GFP</i>	(Herman and Emr 1990) This study

## 5 ddYbXJl Table G2: PCR primers used

KO Primers are for amplification of the deletion cassette from the pUG6 plasmid (loxP-KanMX-LoxP) (Güldener *et al*, 1996). Atg18 F and R primers are for amplification of full-length Atg18 for ligation into the pEXP5-NT/TOPO vector (Invitrogen)

Name	Sequence 5' – 3'
atg21-KO-F	GAC AAT TCC ACT CCT TTG GAT TTG AAA TAG ACA GAT AGA AAA GGA TCA GCT GAA GCT TCG TAC GC
atg21-KO-R	GAA TAC GTA CAA TAT CTA TTA AGA TTA TGA AAA CTG CAC ATA TGC AGC ATA GGC CAC TAG TGG ATC TG
atg21 KO ctrl F	AGG AGC GTG AGC TGC AGA AAG
atg21 KO ctrl R	CCC TCT TTA GCA ATT CAC ACG
hsv2-KO-F	CTG GAA AGG CAG CGA TTA TTA GAG GAC AAC TAT AAG CAT ACA TAA CTA GCA GAT GCG TAC GCT GCA GGT CGA C
hsv2-KO-R	TTG TAC GTA AAT GCA CAC TTT CTC TAT ACA TAT ATA TAT ATT TAT ATT CAT GTT AAT CGA TGA ATT CGA GCT CG
hsv2 KO ctrl F	CGG GGG CTA TTG TTA AAG GG
hsv2 KO ctrl R	CTC GCA TGA CGC CTA CCG
atg18-F	ATG TCT GAT TCA TCA CCT AC
atg18-R	TCA ATC CAT CAA GAT GGA ATA C
atg18_cterm-F	GCC AGT ACT TGG CGG TTA C
atg18 terminator R	GCA AAA GGA TAG ATT ATA CGC AGG AG
atg2 KO ctrl F	CCG AAA TGG AAC AGC AAG TGG
atg2 KO ctrl R	CCT CTA TAA TGG CAT CTT CCC CC
atg2 promotor F	CGA CTC ACT ATA GGG CGA ATT GGA GCT CCA CCG CGG TGA GCG GGT TGT ACT GTT TGC T
atg21 C term R	TTA CGC ATA GTC AGG AAC ATC GTA TGG GTA TGT AAA TTT ATT ATT TTT AGT CAG CAC ACA TTC
atg21 C term F	TAC CCA TAC GAT GTT CCT GAC TAT GCG TAA TGC ATA TGT GCA GTT TTC ATA ATC TT
atg21 terminator R	ACT AAA GGG AAC AAA AGC TGG GTA CCG GGC CCC CCA TCG TTT TCT TAA ACA TTG