Araki et al., http://www.jcb.org/cgi/content/full/jcb.201304123/DC1

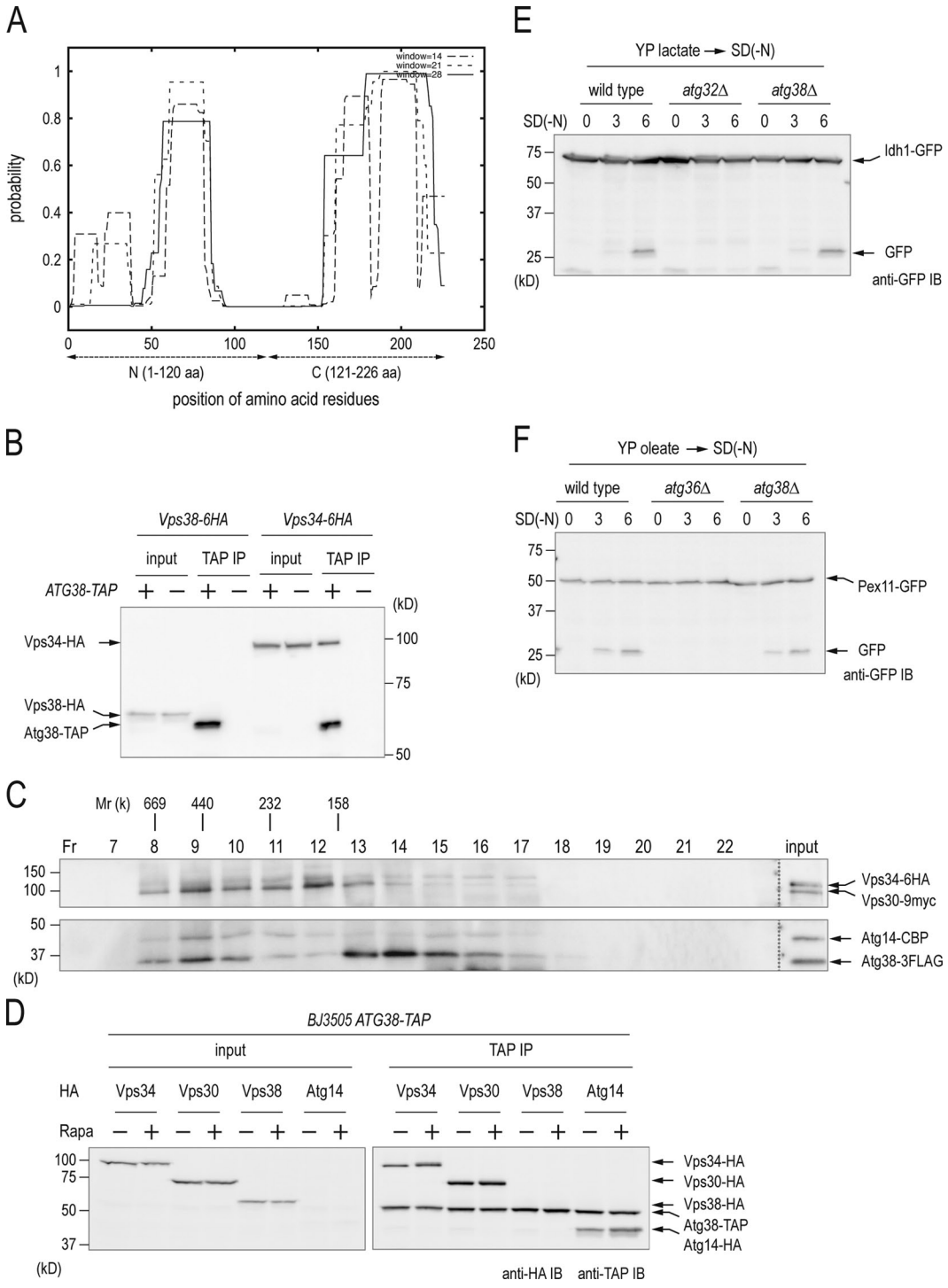

Figure S1. **Atg38 is a component of complex I.** (A) The presence of two coiled-coil domains is predicted within Atg38. (B) Cell extracts were prepared from yeast strains expressing TAP-tagged Atg38 and the indicated HA-tagged Vps38 and Vps34 proteins. The extracts were immunoprecipitated by IgG-Dynabeads. The whole-cell extract (input) and the precipitated proteins (TAP IP) were analyzed by immunoblotting with the indicated antibodies. (C) Complex I comprising Atg14-TAP was purified with IgG-Dynabeads and then eluted with TEV protease. The eluate was fractionated over a gel filtration column. Each fraction was analyzed by immunoblotting with the indicated antibodies. (D) *ATG38-TAP* cells with the indicated genotypes were grown in YPD and treated with rapamycin for 3 h at 30°C. Lysates from each group of cells were analyzed as in Fig. 1 B. (E) Cells expressing Idh1-GFP were grown in YPL medium, then shifted to SD(-N) medium. The whole-cell extracts were analyzed by immunoblotting with anti-GFP antibody. (F) Cells expressing Pex11-GFP were grown in YM2 oleate medium, then shifted to SD(-N) medium. The whole-cell extracts were analyzed by immunoblotting with anti-GFP antibody.

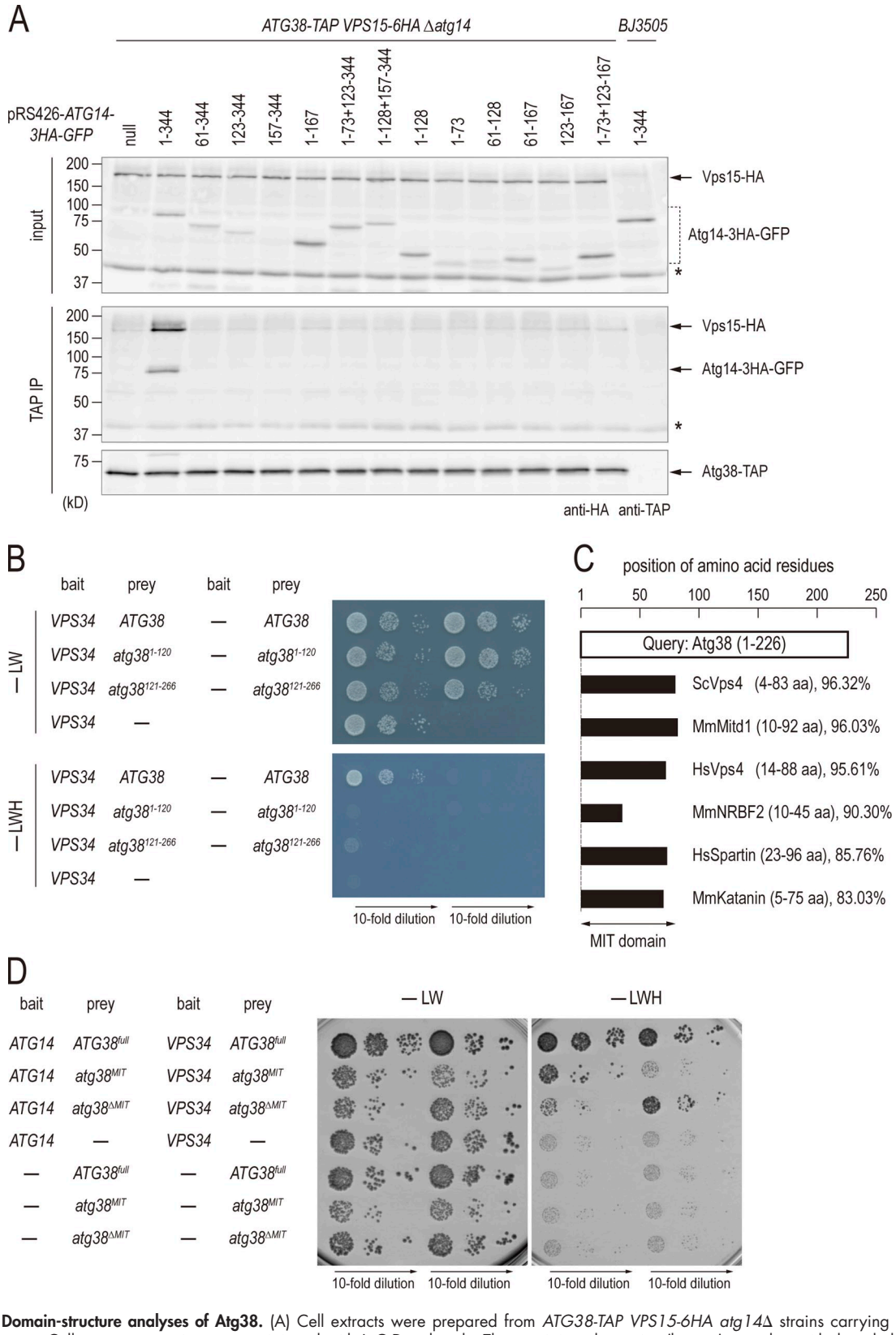

Figure S2. **Domain-structure analyses of Atg38.** (A) Cell extracts were prepared from *ATG38-TAP VPS15-6HA atg14*Δ strains carrying the indicated *ATG14* constructs. Cell extracts were immunoprecipitated with IgG-Dynabeads. The precipitated proteins (bottom), together with the whole-cell extracts (top), were immunoblotted with the indicated antibodies. (B) Interactions between the indicated fusion proteins were analyzed as in Fig. 5 A. (C) HHpred results for a search of Atg38 against genome database. Black bars indicate homologous regions between query and target proteins. The names of proteins that share homologous regions with Atg38 are indicated on the left. Sc: *Saccharomyces cerevisiae*; Mm: *Mus musculus*; Hs: *Homo sapiens*. (D) Interactions between the indicated fusion proteins were analyzed as in Fig. 5 A. Constructs are represented as $ATG38^{full}$ (1–226 aa), $ATG38^{MIT}$ (1–80 aa), and $ATG38^{\Delta MIT}$ (81–226 aa).

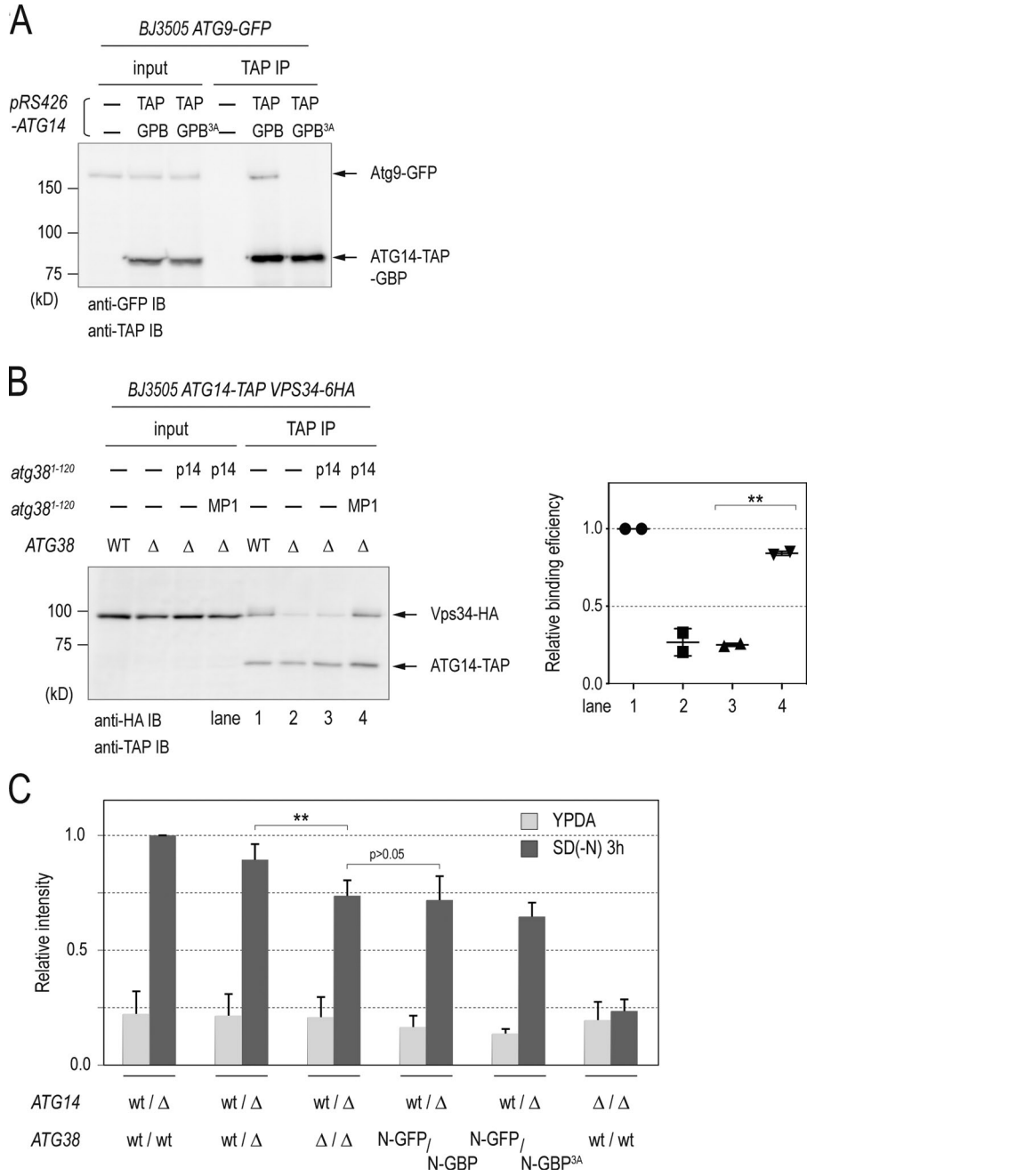

Figure S3. **An artificial tether facilitates the formation of PI3K complex I, but does not restore the defect in autophagy in *atg38Δ* cells.** (A) Introduction of three mutations (S33A R35A Y37A) to GBP compromises its interaction with GFP. Cell extracts were prepared from *ATG9-GFP atg14Δ* strains carrying the indicated *ATG14* constructs. The cell extracts were immunoprecipitated with IgG-Dynabeads. The precipitated proteins (TAP IP), together with the whole-cell extracts (input), were immunoblotted with the indicated antibodies. (B) Cell extracts were prepared from *ATG14-TAP* and either *VPS34-6HA* or *VPS15-6HA* strains expressing Atg38$^{1-120}$ fused with p14 and MP1. Interactions between the indicated proteins were analyzed as in A (left). Relative binding efficiency was determined as described in Fig. 3 A. Relative binding efficiency is shown as mean ± SD of two independent experiments (right). (C) Cells expressing Pho8Δ60 were grown in YPD and shifted to SD(-N) medium for 3 h at 30°C. Lysates from each group of cells were tested for ALP activity. ALP activity is shown as mean ± SD of three independent experiments with the activity of wild-type cells normalized to 1.

Table S1. **Yeast strains**

Name	Genotype	Figure used
BJ3505	*MATa pep4::HIS3 prb1-D1.6R HIS3 lys2-208 trp1-D101 ura3-52 gal2 can1*	S2 A; Jones et al., 1982
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Brachmann et al., 1998
BY4743	*MATa/alpha his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0*	Brachmann et al., 1998
YAY1187	BJ3505 *VPS34-TAP-hphNT1*	1 A
YAY1298	BJ3505 *VPS34-6HA-natNT2*	1 B, 1 D, S1 B
YAY1299	BJ3505 *VPS15-6HA-natNT2*	1 B, 1 D
YAY1300	BJ3505 *VPS30-6HA-natNT2*	1 B, 1 D
YAY1301	BJ3505 *VPS38-6HA-natNT2*	1 B, S1 B
YAY1302	BJ3505 *ATG14-6HA-natNT2*	1 B
YAY1318	BJ3505 *ATG38-TAP-hphNT1 VPS34-6HA-natNT2*	1 B, 1 D, S1 B, S1 D
YAY1319	BJ3505 *ATG38-TAP-hphNT1 VPS15-6HA-natNT2*	1 B, 1 D
YAY1320	BJ3505 *ATG38-TAP-hphNT1 VPS30-6HA-natNT2*	1 B, 1 D, S1 D
YAY1321	BJ3505 *ATG38-TAP-hphNT1 VPS38-6HA-natNT21*	1 B, S1 B, S1 D
YAY1322	BJ3505 *ATG38-TAP-hphNT1 ATG14-6HA-natNT2*	1 B, S1 D
YAY1303	BJ3505 *ATG38-TAP-hphNT1*	1 C
YAY1358	BJ3505 *VPS34-6HA-natNT2 ATG14-TAP-hphNT1*	1 C, 4 A, 4 B, 7 A, S3 B
YAY1355	BJ3505 *ATG38-TAP-hphNT1 VPS34-6HA-natNT2 atg14Δ::kanMX4*	1 D
YAY1356	BJ3505 *ATG38-TAP-hphNT1 VPS15-6HA-natNT2 atg14Δ::kanMX4*	1 D, S2 A
YAY1357	BJ3505 *ATG38-TAP-hphNT1 VPS30-6HA-natNT2 atg14Δ::kanMX4*	1 D
YAY1394	BY4741 *ATG17-2mcherry-hphNT1 ATG38-2GFP-kanMX6*	1 E
YAY1427	BY4741 *ATG17-2mcherry-hphNT1 ATG38-2GFP-kanMX6 atg14Δ::natNT2*	1 E
TK1050	BY4741 *kanMX4-GPD promoter-pho8Δ60*	2 A, 2 B, 2 C
YAY1332	BY4741 *kanMX4-GPD promoter-pho8Δ60 atg38Δ::natNT2*	2 A, 2 B, 2 C, 4 C
YAY1334	BY4741 *kanMX4-GPD promoter-pho8Δ60 atg14Δ::natNT2*	2 A, 2 B, 2 C
YAY1640	BY4741 *kanMX4-GPD promoter-pho8Δ60 ATG14-yeGFP-hphNT1*	2 A, 2 B
YAY1641	BY4741 *kanMX4-GPD promoter-pho8Δ60 atg38Δ::CgURA3 ATG14-yeGFP-hphNT1*	2 A, 2 B
YAY1414	BY4741 *kanMX4-GPD promoter-pho8Δ60 vps30Δ::natNT2*	2 C
YAY1778	BY4741 *ATG17-2mcherry-hphNT1 mRFP-APE1-URA3 VPS34-3myeGFP-KanMX6*	3A, 3B
YAY1781	BY4741 *ATG17-2mcherry-hphNT1 mRFP-APE1-URA3 VPS34-3myeGFP-KanMX6 atg38Δ::natNT2*	3A, 3B
YAY1780	BY4741 *ATG17-2mcherry-hphNT1 mRFP-APE1-URA3 VPS30-3myeGFP-KanMX6*	3A
YAY1783	BY4741 *ATG17-2mcherry-hphNT1 mRFP-APE1-URA3 VPS30-3myeGFP-KanMX6 atg38Δ::natNT2*	3A
YAY1776	BY4741 *ATG17-2mcherry-hphNT1 mRFP-APE1-URA3 ATG14-3myeGFP-KanMX6*	3A, 3B
YAY1777	BY4741 *ATG17-2mcherry-hphNT1 mRFP-APE1-URA3 ATG14-3myeGFP-KanMX6 atg38Δ::natNT2*	3A, 3B
YAY1774	BY4741 *ATG17-2mcherry-hphNT1 mRFP-APE1-URA3 ATG2-3myeGFP-KanMX6*	3A
YAY1786	BY4741 *ATG17-2mcherry-hphNT1 mRFP-APE1-URA3 ATG2-3myeGFP-KanMX6 atg38Δ::natNT2*	3A
YAY1359	BJ3505 *VPS15-6HA-natNT2 ATG14-TAP-hphNT1*	4 A, 7 A, S3 B
YAY1360	BJ3505 *VPS30-6HA-natNT2 ATG14-TAP-hphNT1*	4 A
YAY1368	BJ3505 *VPS34-6HA-natNT2 ATG14-TAP-hphNT1 atg38Δ::kanMX4*	4 A, 4 B, 7 A, S3 B
YAY1369	BJ3505 *VPS30-6HA-natNT2 ATG14-TAP-hphNT1 atg38Δ::kanMX4*	4 A, 7 A, S3 B
YAY1370	BJ3505 *VPS15-6HA-natNT2 ATG14-TAP-hphNT1 atg38Δ::kanMX4*	4 A
YAY1390	BJ3505 *VPS34-6HA-natNT2 ATG14-TAP-hphNT1 atg38Δ::kanMX4 $ATG38^{1\text{-}120}$-yEGFP-URA3 $ATG38^{1\text{-}120}$-GBP6His-TRP*	4 B,7 A
YAY1814	BY4741 *kanMX4-GPD promoter-pho8Δ60 ATG14-TAP-hphNT1*	4 C
YAY1815	BY4741 *kanMX4-GPD promoter-pho8Δ60 atg38Δ::CgURA3 ATG14-TAP-hphNT1*	4 C
YAY1536	BJ3505 *VPS34-6HA-natNT2 ATG14-TAP-hphNT1 VPS30-9myc-klTRP1 ATG38-3FLAG-10His-kanMX4*	4 D,S1 C
AH109	*MATa, trp1-901, leu2-3, 112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::$GAL1_{UAS}$-$GAL1_{TATA}$-HIS3, MEL1, $GAL2_{UAS}$-$GAL2_{TATA}$-ADE2, URA3::$MEL1_{UAS}$-$MEL1_{TATA}$-lacZ*	5 A, 5 B, 6 A, S2 B, S2 D (Takara Bio Inc.)
YAY1531	BJ3505 *VPS34-TAP-hphNT1 atg14Δ::natNT2 vps38Δ::kanMX4 vps30Δ::CgTRP1*	5 C
YAY1391	BJ3505 *VPS34-6HA-natNT2 ATG14-TAP-hphNT1 atg38Δ::kanMX4 $ATG38^{1\text{-}120}$-yEGFP-URA3 $ATG38^{1\text{-}120}$-GBP^{3A}6His-TRP*	7 A
YAY1392	BJ3505 *VPS15-6HA-natNT2 ATG14-TAP-hphNT1 atg38Δ::kanMX4 $ATG38^{1\text{-}120}$-yEGFP-URA3 $ATG38^{1\text{-}120}$-GBP6His-TRP*	7 A

Table S1. **Yeast strains** *(Continued)*

Name	Genotype	Figure used
YAY1393	BJ3505 *VPS15-6HA-natNT2 ATG14-TAP-hphNT1 atg38Δ::kanMX4 ATG38$^{1-120}$-yEGFP-URA3 ATG38$^{1-120}$-GBP^{3A}6His-TRP*	7 A
YKM65	BY4741 *ADHp-OsTIR1-9myc-URA3 IDH1-GFPhy-kanMX6*	S1 E
YKM90	BY4741 *ADHp-OsTIR1-9myc-URA3 IDH1-GFPhy-kanMX6 atg32Δ::hphNT1*	S1 E
YAY1733	BY4741 *ADHp-OsTIR1-9myc-URA3 IDH1-GFPhy-kanMX6 atg38Δ::hphNT1*	S1 E
YNH798	BY4741 *ADHp-OsTIR1-9myc-URA3 PEX11-eGFP-kanMX4*	S1 F
YNH799	BY4741 *ADHp-OsTIR1-9myc-URA3 PEX11-eGFP-kanMX4 atg36Δ:natNT2*	S1 F
YAY1747	BY4741 *ADHp-OsTIR1-9myc-URA3 PEX11-eGFP-kanMX4 atg38Δ:natNT2*	S1 F
YAY1541	BJ3505 *ATG9-yEGFP-kanMX4*	S3 A
YAY1436	BJ3505 *VPS34-6HA-natNT2 ATG14-TAP-hphNT1 atg38Δ::kanMX4 ATG38$^{1-120}$-p14-TRP1*	S3 B
YAY1459	BJ3505 *VPS34-6HA-natNT2 ATG14-TAP-hphNT1 atg38Δ::kanMX4 ATG38$^{1-120}$-p14-TRP1 ATG38$^{1-120}$-MP1-URA3.*	S3 B
YAY1583	BY4743 *kanMX4-GPD promoter-pho8Δ60/pho8Δ ATG14/atg14ΔATG38/atg38Δ*	S3 C
YAY1584	BY4743 *kanMX4-GPD promoter-pho8Δ60/pho8Δ ATG14/atg14Δ atg38Δ/atg38Δ*	S3 C
YAY1585	BY4743 *kanMX4-GPD promoter-pho8Δ60/pho8Δ ATG14/atg14ΔATG38/ATG38*	S3 C
YAY1586	BY4743 *kanMX4-GPD promoter-pho8Δ60/pho8Δ atg14Δ/atg14ΔATG38/ATG38*	S3 C
YAY1587	BY4743 *kanMX4-GPD promoter-pho8Δ60/pho8Δ ATG14/atg14Δ atg38Δ/atg38Δ ATG38$^{1-120}$-yEGFP-URA3 ATG38$^{1-120}$-GBP6His-HIS3*	S3 C
YAY1588	BY4743 *kanMX4-GPD promoter-pho8Δ60/pho8Δ ATG14/atg14Δatg38Δ/atg38Δ ATG38$^{1-120}$-yEGFP-URA3 ATG38$^{1-120}$-GBP^{3A}6His-HIS3*	S3 C

Table S2. **Plasmids**

Name	Description	Figure used
pGBKT7-p53	pGBKT7-p53	5 A (Takara Bio Inc.)
pGADT7-large T	pGADT7-large T	5 A (Takara Bio Inc.)
pYA259	pGADT7-*VPS30*	5 A
pYA260	pGADT7-*ATG14*	5 A, 5 B, S2 D
pYA261	pGADT7-*VPS34*	5 A, S2 D
pYA633	pGADT7-*ATG38*	5 A, 5 B, 6 A, S2 B
pYA643	pGBKT7-*ATG38*	5 A, 5 B, 6 A, S2 B
pYA656	pGADT7-*ATG38*$^{1-120}$	5 B, 6 A
pYA657	pGADT7-*ATG38*$^{121-226}$	5 B, 6 A
pYA638	pGEX6P-*ATG38*	5 C, 6 B, 6 C
pYA658	pGEX6P-*ATG38*$^{1-120}$	5 C
pYA659	pGEX6P-*ATG38*$^{121-226}$	5 C
pYA662	pGBKT7-*ATG38*$^{1-120}$	5 A
pYA664	pGBKT7-*ATG38c*$^{121-226}$	5 A
pOK15	pRS426-*ATG14*-3HA-yEGFP	S2 A
pOK16	pRS426-*ATG14*$^{61-344}$-3HA-yEGFP	S2 A
pOK17	pRS426-*ATG14*$^{123-344}$-3HA-yEGFP	S2 A
pOK18	pRS426-*ATG14*$^{157-344}$-3HA-yEGFP	S2 A
pOK19	pRS426-*ATG14*$^{1-167}$-3HA-yEGFP	S2 A
pOK20	pRS426-*ATG14*$^{1-73+123-344}$-3HA-yEGFP	S2 A
pOK21	pRS426-*ATG14*$^{1-128+157-344}$-3HA-yEGFP	S2 A
pOK22	pRS426-*ATG14*$^{1-128}$-3HA-yEGFP	S2 A
pOK26	pRS426-*ATG146*$^{1-73}$-3HA-yEGFP	S2 A
pOK27	pRS426-*ATG146*$^{61-128}$-3HA-yEGFP	S2 A
pOK31	pRS426-*ATG146*$^{61-167}$-3HA-yEGFP	S2 A
pOK32	pRS426-*ATG146*$^{123-167}$-3HA-yEGFP	S2 A
pOK33	pRS426-*ATG146*$^{1-73+123-167}$-3HA-yEGFP	S2 A
pYA874	pGADT7-*ATG38*$^{1-80}$	S2 D
pYA875	pGADT7-*ATG38*$^{81-226}$	S2 D
pYA775	pPS306-*ATG14*-TAP-GBP ^{3A}	S3 A
pYA777	pPS306-*ATG14*-TAP-GBP	S3 A

Table S3 is available as a Microsoft Excel file.

References

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