Developmental Cell *14*

Supplemental Data

PpAtg30 Tags Peroxisomes for Turnover by Selective Autophagy

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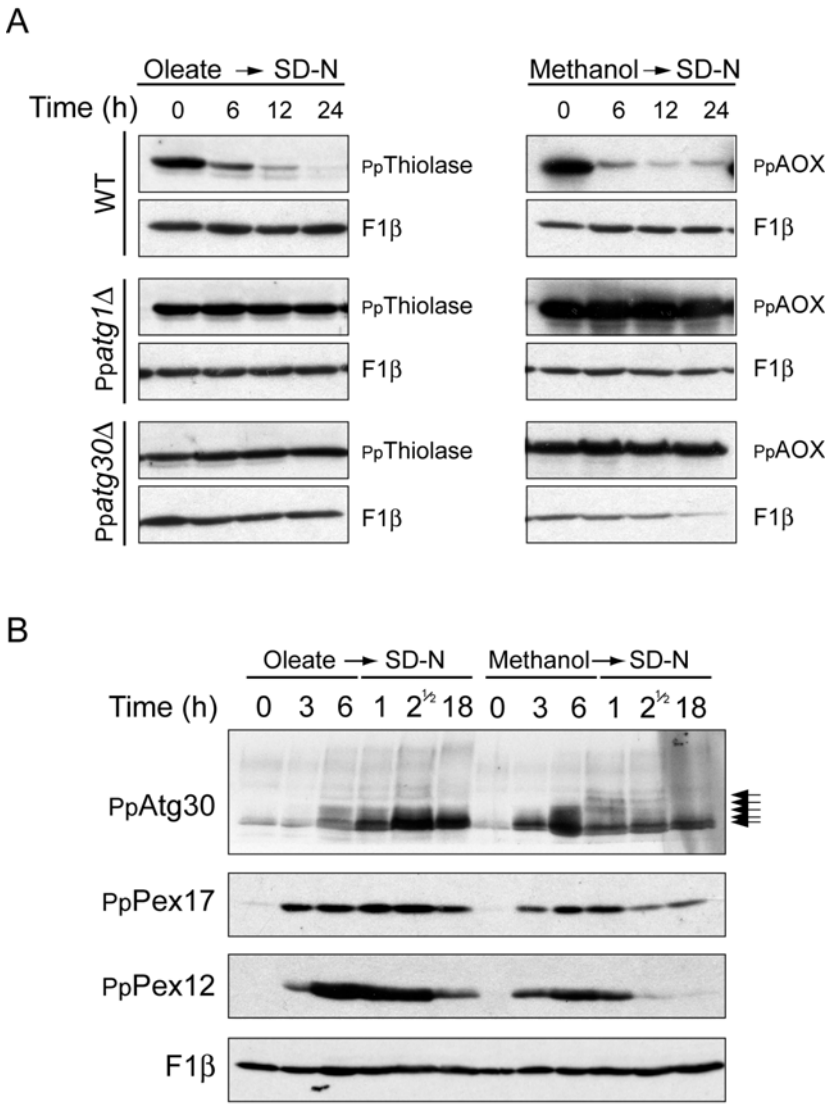

Figure S1. PpAtg30 Is Required for Pexophagy of Both Oleate- and Methanol-Grown Cells

(A) PpAtg30 is essential for pexophagy of peroxisomes induced by methanol or oleate medium. Wild-type (PPY12)**,** Pp*atg1*Δ (R12) and Pp*atg30*Δ (SJCF44) were grown in methanol or oleate medium overnight and shifted to nitrogen starvation medium for the indicated times. One ml of cells was TCA precipitated and analyzed by immunoblotting as described in Experimental Procedures. AOX and thiolase levels were used to follow peroxisome degradation whereas mitochondrial F1β served as loading control. **(B)** PpAtg30 behaves like a peroxin with respect to its induction in oleate and methanol medium in *P. pastoris* and it is phosphorylated when shifted to pexophagy conditions. Pp*atg30*Δ cells complemented with PpAtg30-GFP driven by its own promoter (SJCF632) were grown in oleate (0.67% yeast nitrogen base without amino acids, 0.5% [v/v] oleate acid and 0.025% Tween-80, supplemented with the appropriate Complete Supplement Mixture (CSM) of amino acids) or in methanol medium for up to 6 h and shifted to nitrogen starvation medium.

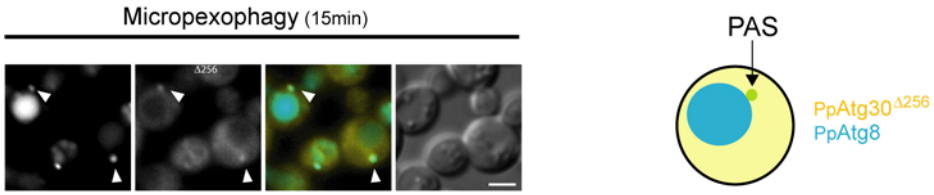

Figure S2. The Deletion of the First 256 Amino Acids of PpAtg30 (PpAtg30$^{\Delta 256}$) Abolished Its Peroxisomal Localization but Preserved Its Localization to a Dot-like Structure, Where It Colocalized with PpAtg8

Pp*atg30*Δ cells co-expressing PpAtg30$^{\Delta 256}$-YFP and CFP-PpAtg8 (SJCF752) were grown overnight in methanol medium and then adapted to glucose medium for 15 min and examined by fluorescence microscopy. White arrow: colocalization of PpAtg30$^{\Delta 256}$-YFP with CFP-PpAtg8, probably at the PAS. PpAtg30 was expressed from the endogenous Pp*ATG30* promoter. Bars, 2 µm.

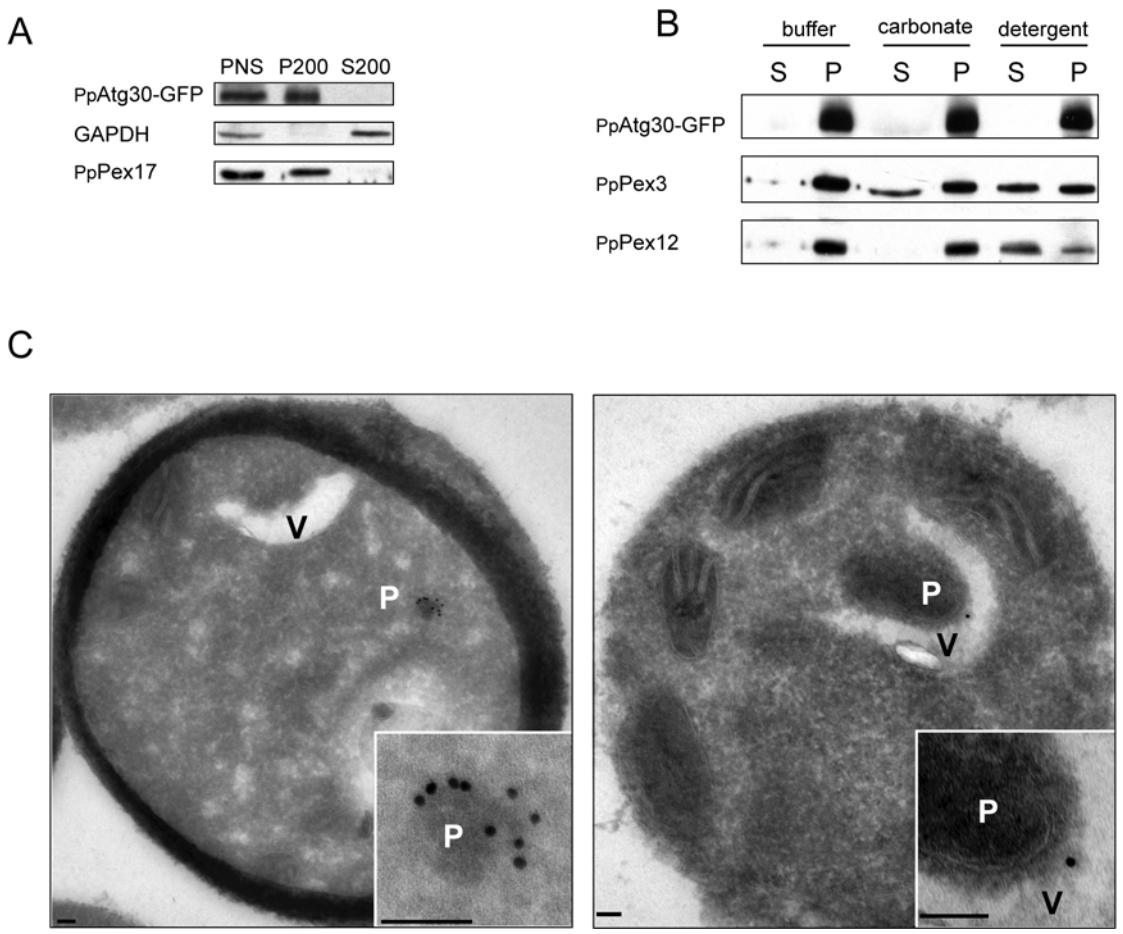

Figure S3. PpAtg30 Is Membrane-Associated

(A) PpAtg30 localizes to the membrane fraction. Yeast cells expressing PpAtg30-GFP (SJCF600) were grown to mid-logarithmic phase in YPD medium, washed with sterile de-ionised water and incubated in methanol medium for 6 h at a density of 0.5 OD/ml (150-200 total ODs). The cells were centrifuged at 2,000 *g* for 10 min at room temperature to harvest the cells. The cells were resuspended in reducing buffer (100 mM Tris-HCl, pH 7.5, 50 mM EDTA, 10 mM NaN_3, 10 mM DTT) at 10-20 OD/ml. After incubation at 30˚C for 20 min, the cells were washed once with 20 mM potassium phosphate buffer (pH 7.4) and spheroplasted by incubating them at 30˚C for 30-60 min in spheroplasting buffer (20 mM potassium phosphate, pH 7.4, 1.2M sorbitol, 10 mM NaN_3) with Zymolyase 20T (Seikagaku Corp., Japan) at the concentration of 12 mg/1000 OD units. The spheroplasts were collected by centrifugation at 1,000 *g* for 10 min at 4˚C. Spheroplasts were washed with 10 ml of cold spheroplasting buffer without NaN_3 and subsequently homogenized using a Dounce homogenizer. Ten firm strokes were applied

to break the spheroplasts in the presence of ice-cold Dounce buffer (1M sorbitol, 5 mM MES, pH 6.0, 0.5 mM EDTA, 1 mM KCl, 0.1% ethanol) containing protease and phosphatase inhibitors (protease inhibitor cocktail, Sigma), 1 mM PMSF, 50 mM NaF. The unbroken spheroplasts, cell debris, and nuclei were removed by centrifuging the homogenate at 2,000 *g* for 10 min at 4˚C twice and the supernatant was considered as the post nuclear supernatant (PNS). Ultracentrifugation of the PNS (200,000 *g*, Optima Max-E, Beckman) generated the cytosol fraction (S200) and the membrane fraction (P200). Fractions were resolved on a 10% SDS-PAGE and immunoblotted with anti-GFP (PpAtg30-GFP), anti-ScGAPDH and anti-PpPex17 antibodies. **(B)** PpAtg30 is resistant to carbonate and detergent extraction. The PNS was centrifuged at 27,000 *g* to generate the membrane fraction (P27), which was gently resuspended in either buffer (10 mM Tris-HCl, pH 8), carbonate (100 mM Na_2CO_3 in 10 mM Tris-HCl, pH 11.5) or detergent (1% Triton X-100, 1% CHAPS in 10 mM Tris-HCl, pH 8) and incubated on ice for 30 min with intermittent mixing. Samples were subjected to ultracentrifugation (200,000 *g*, Optima Max-E, Beckman) to generate supernatant (S) and pellet (P) and analyzed as above. **(C)** PpAtg30 localized at the peroxisomal membrane. Pp*atg26*Δ cells overexpressing PpAtg30-GFP (SJCF858) were induced in methanol medium for 6 h, shifted for 30 min to glucose medium and processed for immunoelectron microscopy by standard procedures. Briefly, cells were fixed overnight with 4% paraformaldehyde and PpAtg30 was labeled using mouse monoclonal anti-GFP (dilution 1/50) and goat anti-mouse 10-nm gold conjugate antibodies. P, peroxisome; V, vacuole. Bar, 100 nm.

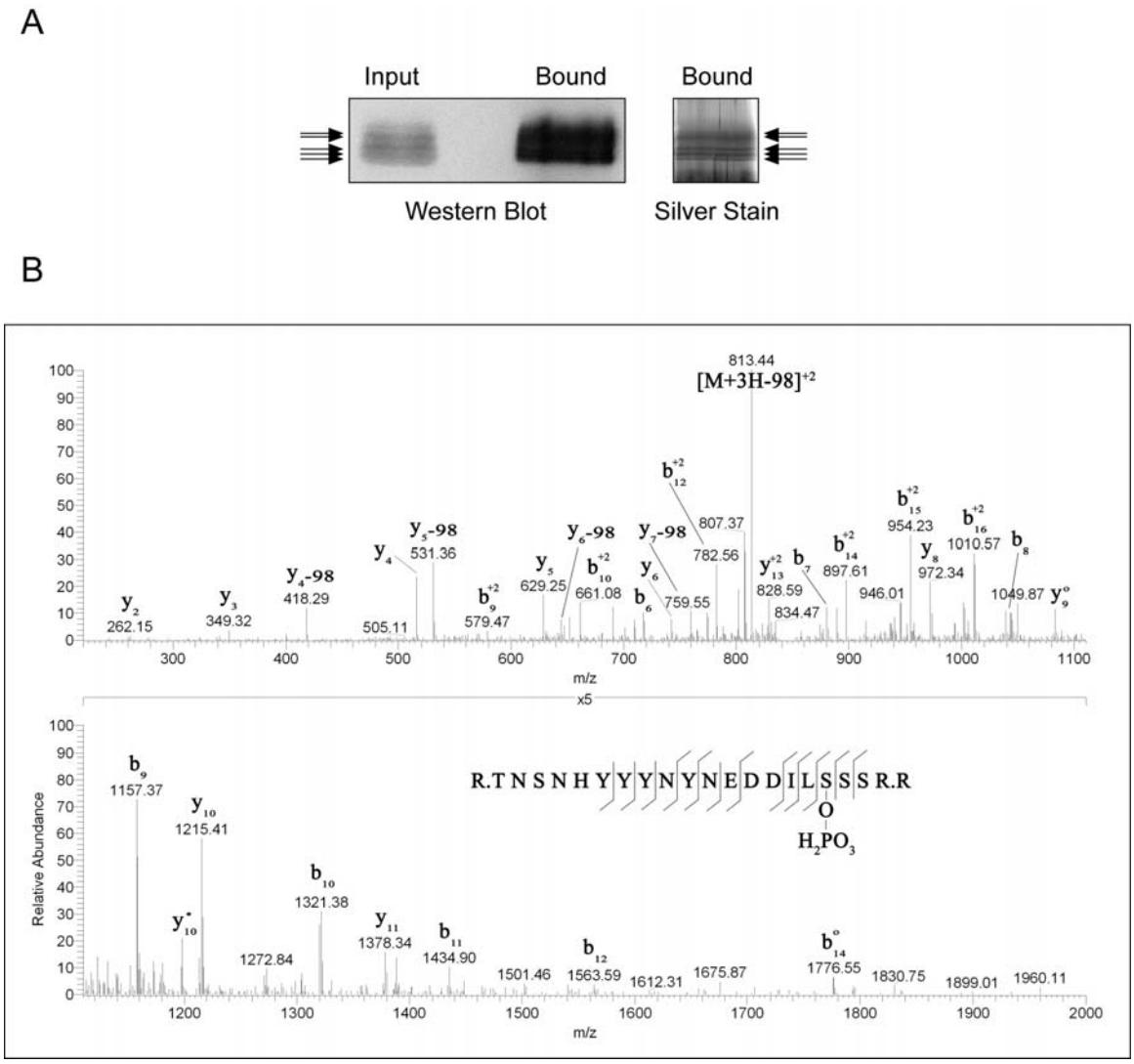

Figure S4. MS-MS Chromatograms of Affinity-Purified PpAtg30 Showing Phosphorylation at S112

(A) Affinity purification of PpAtg30. PpAtg30-Prot.A (SJCF767) was purified essentially as described in "Experimental Procedures". The entire process was scaled up for 2500 ODs. A fraction (1/50th of total purified protein) of the total lysate (Input, I) and the human IgG bound PpAtg30-Prot.A (Bound, B) was analyzed by silver staining and western blotting using anti-Calmodulin binding protein (CBP) antibody. The rest of the sample was then resolved on a 10% Bis-Tris gel with MOPS running buffer (NuPAGE, Invitrogen Corp.), Coomassie stained with (BioSafe Coomasie stain, BioRad) and a broad band corresponding to PpAtg30-Prot.A was excised. **(B)** Identification of S112 phosphorylation in PpAtg30-Prot.A. The excised band (corresponding to 1/4th of total

purified protein) was treated with iodoacetamide and subjected to trypsin digestion. Half of the peptide mixture was subjected to phosphopeptide enrichment. Peptide identification and phosphorylation site assignment were carried out with SEQUEST software and verified manually. The fragmentation pattern of the peptide 95 to 116 is indicated along with phosphoserine S112 (Serine-O-H_2PO_3). The ions labeled with (-98) were generated from the peptide in which the phosphoserine had been converted to dehydroalanine by β–elimination (loss of phosphoric acid, 98 Da).

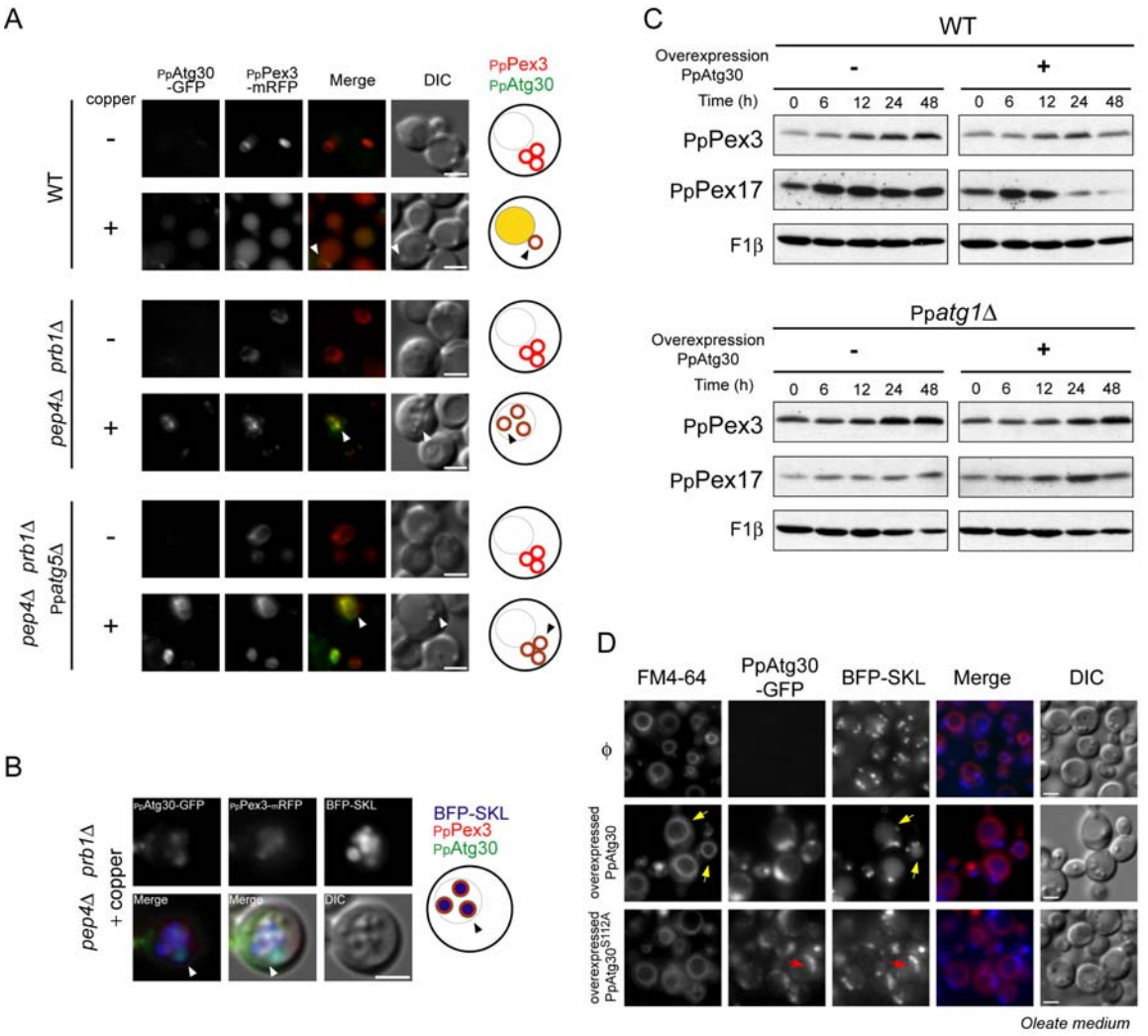

Figure S5. **PpAtg30 Overexpression in Methanol and Oleate Growing Cells Induces Pexophagy**

(A) PpAtg30 overexpression in methanol induced pexophagy, rather than inactivating peroxisomal matrix protein import. PpPex3 (like most peroxins) is mainly degraded in

the vacuole, in a PpAtg5-dependent manner. Fluorescence and DIC microscopy pictures of methanol-grown cells (wild-type [SJCF543], *pep4*Δ *prb1*Δ [SJCF529], *pep4*Δ *prb1*Δ Pp*atg5*Δ [SJCF562]) expressing PpPex3-mRFP as a peroxisomal marker and PpAtg30-GFP under the control of the copper-inducible *CUP1* promoter (Koller et al., 2000). Cells were grown for 6 h in methanol medium and for an additional 2 h in presence or absence of 100 μM $CuSO_4$. Arrowhead shows colocalization between PpPex3-mRFP and PpAtg30-GFP. Bars, 2 μm. **(B)** Fluorescence and DIC microscopy pictures of *pep4*Δ *prb1*Δ cells expressing PpPex3-mRFP and BFP-SKL as peroxisomal markers and PpAtg30-GFP under the control of the *CUP1* promoter (SJCF539). Cells were grown for 6 h in methanol medium and for an additional 2 h in the presence of 100 μM $CuSO_4$. Arrowhead shows colocalization between PpPex3-mRFP, PpAtg30-GFP and BFP-SKL inside the vacuole. Bars, 2 μm. **(C)** Overexpression of PpAtg30 in oleate medium induces pexophagy. In oleate medium, overexpression of PpAtg30, unlike the situation for cells grown in methanol medium did not show a strong biogenesis-defect phenotype, but reduced levels of peroxins were detected. After 24 h, PpPex17 and PpPex3 levels decreased in wild-type cells expressing PpAtg30 from the GAPDH promoter (SJCF600, +) when compared to wild-type cells without overexpression (PPY12, -) and Pp*atg1*Δ cells with or without PpAtg30 overexpression (R12, -; SJCF471, +). Cell lysates prepared at different time intervals from strains described above were analyzed by immunoblotting. F1β served as a loading control. (D) Fluorescence microscopy of cells overexpressing PpAtg30 in oleate medium show pexophagy. The cells were grown in oleate medium for 16 h. Wild-type cells expressing BFP-SKL from GAPDH promoter grown (SJCF883, Φ) in oleate medium show several small peroxisomes. When PpAtg30-GFP was overexpressed (SJCF884) it colocalized with BFP-SKL. Furthermore, considerable amount of BFP-SKL was detected in the vacuole consistent with pexophagy. However, mutation of S112 in PpAtg30 (SJCF885) abolished the pexophagy phenotype induced by overexpression. Yellow arrow indicates BFP-SKL inside the vacuole. Red arrow indicates colocalization of PpAtg30 and BFP-SKL. Bars, 2 μm.

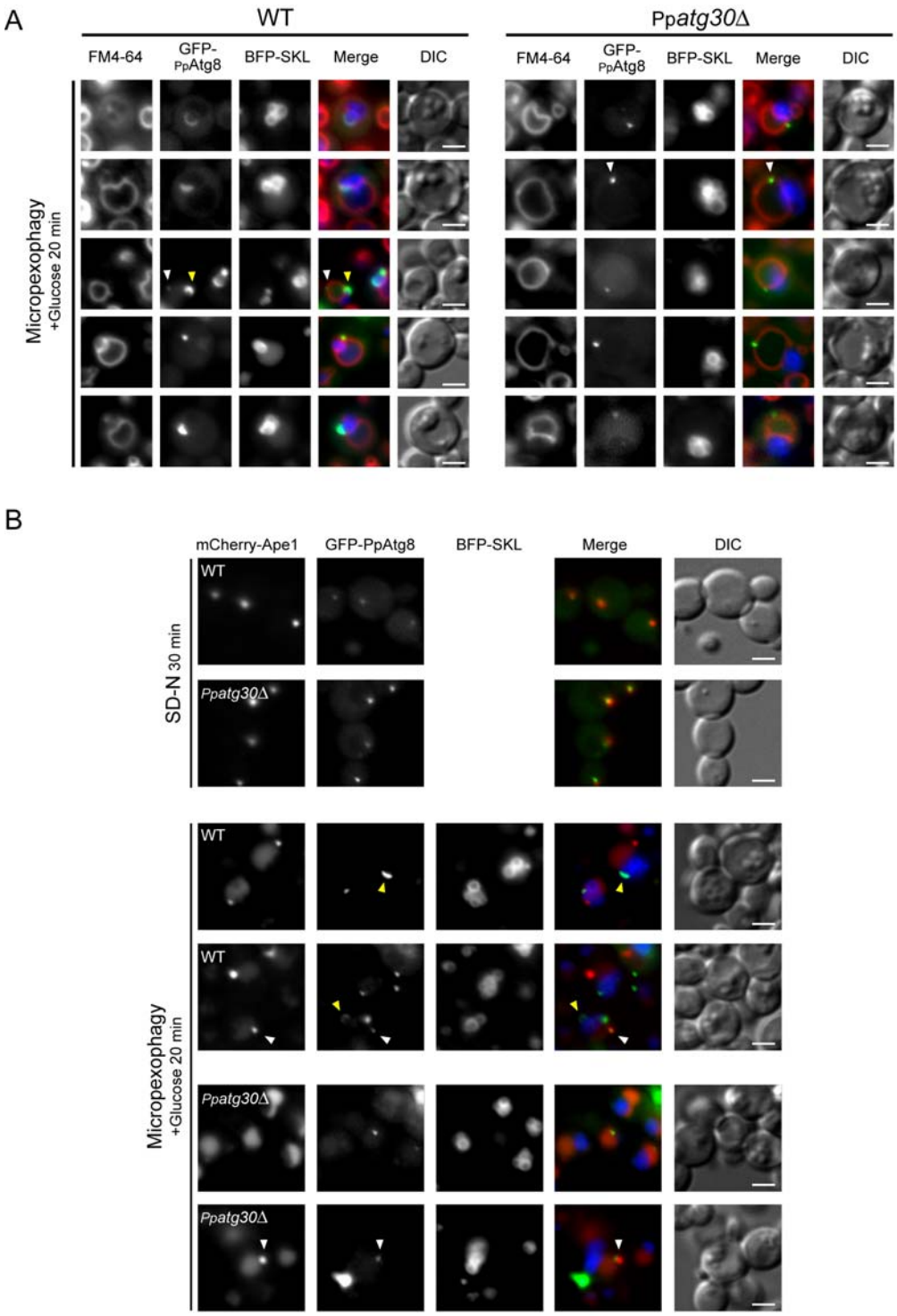

Figure S6. **PpAtg30 Is Required for PpAtg8 Localization near the Peroxisome Cluster or at the MIPA during Micropexophagy Conditions**

During micropexophagy, PpAtg8 is found in different locations. It mainly localizes as a dot in close proximity to the peroxisome cluster or at the MIPA (yellow arrowhead), and it could be also found as a dot arbitrarily on the vacuolar membrane (white arrowhead).

In the absence of PpAtg30, PpAtg8 localized as a dot randomly on the vacuolar membrane (white arrowhead). The MIPA was not formed in the absence of PpAtg30.

We further investigated the relevance of PpAtg8 localized on the vacuolar membrane. Under starvation condition PpAtg8 entirely colocalized with PpApe1 (mCherry fusion) at the PAS. During micropexophagy, some of the arbitrary PpAtg8-dot on the vacuolar membrane colocalized with PpApe1 (white arrowhead). In the absence of PpAtg30, most of PpApe1 was in the vacuolar lumen. However in the extremely rare event when it appeared as a dot (PAS), it always colocalized with PpAtg8 (white arrowhead). These data suggests that the PpAtg8 localized as a dot in Pp*atg30*Δ cells are probably involved in the Cvt or autophagy pathway rather than in pexophagy. **(A)** Fluorescence and DIC microscopy of wild-type (SJCF320) and Pp*atg30*Δ (SJCF376) cells expressing BFP-SKL and GFP-PpAtg8 under the control of the Pp*ATG8* promoter. **(B)** Fluorescence and DIC microscopy of wild-type (SSJ04) and Pp*atg30*Δ (SSJ05) cells expressing mCherry-Ape1 and GFP-PpAtg8 under the control of the Pp*ATG8* promoter. In (A) and (B), the cells were grown overnight in methanol medium and shifted to glucose medium for 20 min (micropexophagy). For induction of starvation conditions, cells were initially grown to mid-log phase in SD medium and then shifted to starvation medium (SD-N) for 30 min. Bars, 2 μm.

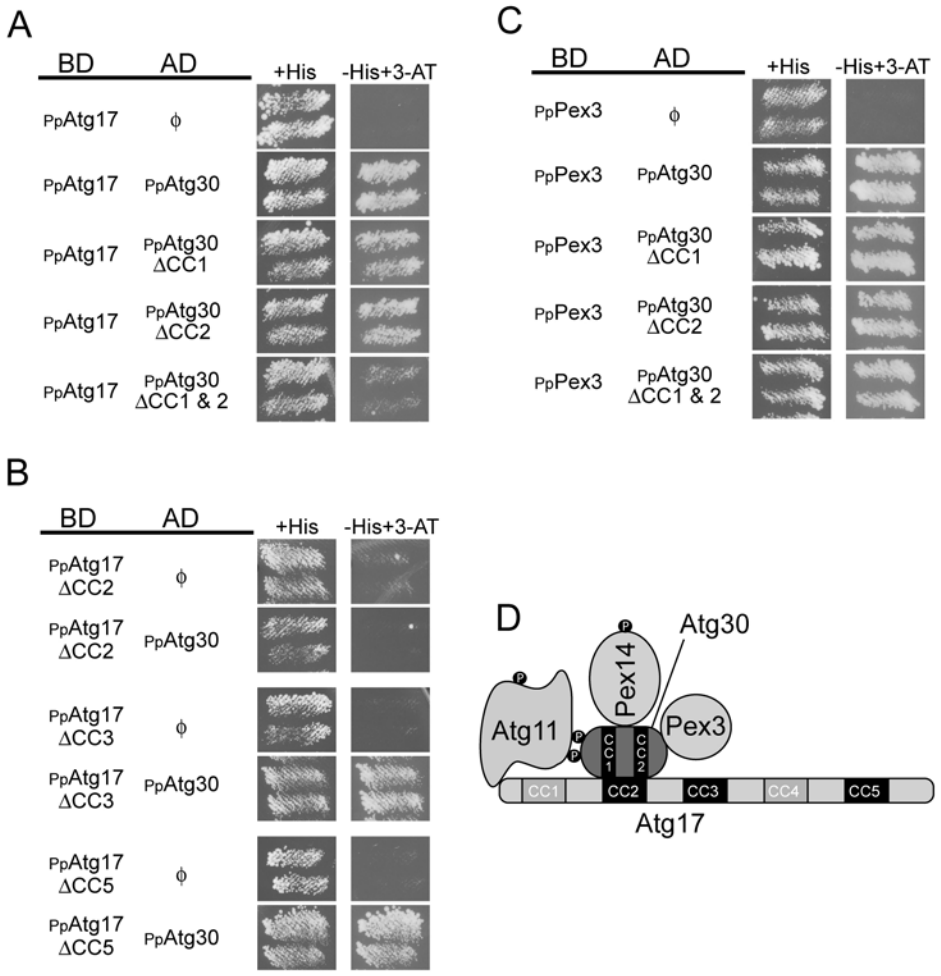

Figure S7. **Mapping of the PpAtg17 and PpAtg30 Binding Sites by a Yeast Two-Hybrid Assay**

The interaction by yeast two-hybrid assays was determined by growth on medium lacking histidine supplemented with 15 to 100 mM 3-aminotriazole (3-AT). The two-hybrid strain (AH109) was transformed with plasmids containing the binding domain (BD)-fused to wild-type or mutant proteins and, the activation domain (AD)-fused to wild-type or mutant PpAtg30 as indicated: **(A)** Wild-type PpAtg17-BD, and wild-type and coiled-coil domain deletion mutants (ΔCC1 and ΔCC2) of PpAtg30-AD. **(B)** Wild-type PpAtg30-AD and coiled-coil domain deletion mutants (ΔCC2, ΔCC3 and ΔCC5) of PpAtg17-BD. **(C)** Wild-type PpPex3 and coiled-coil domain deletion mutants (ΔCC1 and ΔCC2) of PpAtg30-AD. **(D)** Summary of the interaction domains of PpAtg30, PpPex3, PpPex14, PpAtg11 and PpAtg17. •: indicates phosphorylation

Supplemental Experimental Procedures

Yeast strains, plasmids and media

The *P. pastoris* strains and plasmids used are listed in Table S1. Growth media components were as follows: YPD medium (2% glucose, 2% Bactopeptone, and 1% yeast extract), glucose medium (0.67% yeast nitrogen base without amino acids, 2.0% glucose), nitrogen starvation medium (0.67% yeast nitrogen base without ammonium sulfate and amino acids, 2.0% glucose), methanol medium (0.67% yeast nitrogen base without amino acids, 0.5% [v/v] methanol), or ethanol medium (containing 0.67% yeast nitrogen base without amino acids, 0.5% [v/v] ethanol), supplemented with the appropriate Complete Supplement Mixture (CSM) of amino acids.

Strains Name	Description	Genotype	Source
WDK011	*atg2*Δ	GS115 *atg1*Δ::Zeocin^r his4	Strømhaug et al., 2001
Δatg28	*atg28*Δ	GS200 *atg28*Δ::ARG4 his4 arg4	Stasyk et al., 2006
JC121	*pex8*Δ	GS200 *pex8*Δ::ARG4 his4 arg4	Liu et al., 1995
JC404	*pex14*Δ	GS200 *pex14*Δ::ARG4 his4 arg4	Johnson et al., 2001
GS115	WT	his4	Cregg et al., 1985
GS200	WT	his4 arg4	Waterham et al., 1996
OP5	*vps15*Δ	GS200 *vps15*Δ::ARG4 his4 arg4	Stasyk et al., 1999
Pdg3D	*atg26*Δ	GS200 *atg26*Δ::ARG4 his4 arg4	Stasyk et al., 2003
PPF1	WT	his4 arg4	Yuan et al., 1997
PPY12	WT	his4 arg4	Gould et al., 1992
R8	*atg11*Δ	GS115 *atg11*Δ::Zeocin^r his4	Kim et al., 2001
R12	*atg1*Δ	GS115 *atg1*Δ::Zeocin^r his4	Strømhaug et al., 2001
R19	*atg9*Δ	GS115 *atg9*Δ::Zeocinr his4	Strømhaug et al., 2001
SEW1	*pex3*Δ	PPY12 *pex3*Δ::ARG4 his4 arg4	Wiemer et al., 1996
SMD1163	*pep4*Δ / *prB1*Δ	pep4 prB1 his4	Tuttle and Dunn, 1995
STK108	*pex4*Δ	PPY12 *pex4*Δ::ARG4 his4 arg4	Subramani laboratory
WDKO7	*atg7*Δ	PPF1 *atg7*Δ::ARG4 his4 arg4	Yuan et al., 1999
SSJ04	WT / mCherry-Ape1 / GFP-Atg8	PPY12 GFP-Atg8::HIS4 mCherry-Ape1::ARG4 his4 arg4	This study
SSJ05	*atg30*Δ / mCherry-Ape1 / GFP-Atg8	SJCF44 GFP-Atg8::HIS4 mCherry-Ape1::ARG4 his4 arg4	This study
SJCF20	WT / pr*GAPDH*-Atg30-GFP	PPY12 pr*GAPDH*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF44	*atg30*Δ	PPY12 *atg30*Δ::Zeocin^r his4 arg4	This study
SJCF137	*atg30*Δ / pr*ATG30*-Atg30-CFP	PPY12 *atg30*Δ::pr*ATG30*-Atg30-CFP(Geneticin^r) his4 arg4	This study
SJCF247	WT / BFP-SKL	PPY12 BFP-SKL::pr*AOX*(Blasticidin^r) his4 arg4	This study
SJCF257	*atg8*Δ	PPY12 *atg8*Δ::Geneticin^r his4 arg4	This study
SJCF279	*atg8*Δ / pr*GAPDH*-Atg30-GFP	SJCF257 pr*GAPDH*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF314	WT / BFP-SKL / pr*GAPDH*-Atg30-GFP	PPY12 BFP-SKL::pr*AOX*(Blasticidin^r) pr*GAPDH*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF320	WT / BFP-SKL / GFP-Atg8	PPY12 BFP-SKL::pr*AOX*(Blasticidin^r) GFP-Atg8::HIS4 his4 arg4	This study
SJCF330	*pex3*Δ / pr*ATG30*-Atg30-GFP	SEW1 pr*ATG30*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF332	*atg30*Δ / BFP-SKL	SJCF44 BFP-SKL::pr*AOX*(Blasticidin^r) his4 arg4	This study
SJCF366	*pex14*Δ / pr*ATG30*-Atg30-GFP	JC404 pr*ATG30*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF376	*atg30*Δ / BFP-SKL / GFP-Atg8	SJCF44 BFP-SKL::pr*AOX*(Blasticidin^r) GFP-Atg8::HIS4 his4 arg4	This study
SJCF385	*atg30*Δ / pr*ATG30*-Atg30-GFP / BFP-SKL	SJCF44 pr*ATG30*-Atg30-GFP::HIS4 BFP-SKL::pr*AOX*(Blasticidin^r) his4 arg4	This study
SJCF387	*atg7*Δ / pr*ATG30*-Atg30-GFP / BFP-SKL	WDKO7 pr*ATG30*-Atg30-GFP::HIS4 BFP-SKL::pr*AOX*(Zoecin^r) his4 arg4	This study
SJCF389	*atg8*Δ / pr*ATG30*-Atg30-GFP / BFP-SKL	SJCF257 pr*ATG30*-Atg30-GFP::HIS4 BFP-SKL::pr*AOX*(Zoecin^r) his4 arg4	This study
SJCF390	*atg17*Δ / pr*ATG30*-Atg30-GFP / BFP-SKL	PPY12 *atg17*Δ::Geneticin^r pr*ATG30*-Atg30-GFP::HIS4 BFP-SKL::pr*AOX*(Zoecin^r) his4 arg4	This study
SJCF391	*vps15*Δ / pr*ATG30*-Atg30-GFP / BFP-SKL	OP5 pr*ATG30*-Atg30-GFP::HIS4 BFP-SKL::pr*AOX*(Zoecin^r) his4	This study
SJCF393	WT / BFP-SKL / pr*ATG30*-Atg30-GFP	SJCF247 pr*ATG30*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF409	WT / BFP-Atg17 / pr*ATG30*-Atg30-GFP	PPY12 BFP-Atg17::ARG4 pr*ATG30*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF420	*atg11*Δ / pr*ATG30*-Atg30-GFP	R8 pr*ATG30*-Atg30-GFP::HIS4 his4	This study
SJCF432	*atg30*Δ / BFP-SKL / GFP-Atg11	SJCF44 BFP-SKL::pr*AOX*(Blasticidin^r) GFP-Atg11::HIS4 his4 arg4	This study
SJCF471	*atg1*Δ / pr*GAPDH*-Atg30-GFP	R12 pr*GAPDH*-Atg30-GFP::HIS4 his4	This study
SJCF473	*atg1*Δ / pr*ATG30*-Atg30-GFP	R12 pr*ATG30*-Atg30-GFP::HIS4 his4	This study
SJCF483	WT / Ape1-CFP	PPY12 Ape1-CFP::HIS4 his4 arg4	This study
SJCF529	*pep4*Δ / *prB1*Δ / pr*COPPER*-Atg30-GFP / Pex3-mRFP	SMD1163 pr*COPPER*-Atg30-GFP::HIS4(Geneticin^r) Pex3-mRFP::ARG4(Zeocin^r) his4	This study
SJCF539	*pep4*Δ / *prB1*Δ / pr*COPPER*-Atg30-GFP / BFP-SKL / Pex3-mRFP	SMD1163 pr*COPPER*-Atg30-GFP::HIS4(Geneticin^r) BFP-SKL::pr*AOX*(Blasticidin^r) Pex3-mRFP::ARG4(Zeocin^r) his4	This study
SJCF543	WT / pr*COPPER*-Atg30-GFP / Pex3-mRFP	PPY12 pr*COPPER*-Atg30-GFP::HIS4(Geneticin^r) Pex3-mRFP::ARG4 his4 arg4	This study
SJCF547	WT / GFP-Atg8	PPY12 GFP-Atg8::HIS4 his4 arg4	This study
SJCF557	*pep4*Δ / *prB1*Δ / GFP-Atg8	SMD1163 GFP-Atg8::HIS4 his4	This study
SJCF562	*pep4*Δ / *prB1*Δ / *atg5*Δ / pr*COPPER*-Atg30-GFP / Pex3-mRFP	SMD1163 atg5Δ::Zeocinr pr*COPPER*-Atg30-GFP::HIS4(Geneticin^r) Pex3-mRFP::HIS4 his4	This study
SJCF578	WT / pr*COPPER*-Atg30-GFP / BFP-SKL	PPY12 pr*COPPER*-Atg30-GFP::HIS4(Geneticin^r) BFP-SKL::pr*AOX*(Zoecin^r) his4 arg4	This study
SJCF587	*pex14*Δ / Pex3-RFP / pr*ATG30*-Atg30-GFP	JC404 Pex3-mRFP::ARG4(Zoecin^r) pr*ATG30*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF590	*atg30*Δ / pr*ATG30*-Atg30-HA	PPY12 *atg30*Δ::pr*ATG30*-Atg30-HA(Geneticin^r) his4 arg4	This study
SJCF594	WT / BFP-SKL / GFP-Atg11	PPY12 BFP-SKL::pr*AOX*(Blasticidin^r) GFP-Atg11::HIS4 his4 arg4	This study
SJCF600	WT / pr*ATG30*-Atg30-GFP	PPY12 pr*ATG30*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF623	*atg11*Δ / pr*ATG30*-Atg30-GFP / BFP-SKL	R8 pr*ATG30*-Atg30-GFP::HIS4 BFP-SKL::pr*AOX*(Blasticidin^r) his4	This study
SJCF624	*atg1*Δ / pr*ATG30*-Atg30-GFP / BFP-SKL	R12 pr*ATG30*-Atg30-GFP::HIS4 BFP-SKL::pr*AOX*(Blasticidin^r) his4	This study
SJCF632	*atg30*Δ / pr*ATG30*-Atg30-GFP	SJCF44 pr*ATG30*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF651	*atg30*Δ / Ape1-CFP	SJCF44 Ape1-CFP::HIS4 his4 arg4	This study
SJCF736	*atg30*Δ / pr*ATG30*-Atg30^{S112A}-GFP	SJCF44 pr*ATG30*-Atg30^{S112A}-GFP::HIS4 his4 arg4	This study
SJCF752	*atg30*Δ / pr*ATG30*-Atg30^{256}-YFP / CFP-Atg8	SJCF44 pr*ATG30*-Atg30^{256}-YFP::HIS4 CFP-Atg8::ARG4 his4 arg4	This study
SJCF757	*atg30*Δ / pr*ATG30*-Atg30^{S112A}-GFP / BFP-SKL	SJCF44 pr*ATG30*-Atg30^{S112A}-GFP::HIS4 BFP-SKL::pr*AOX*(Blasticidin^r) his4 arg4	This study
SJCF758	WT / pr*GAPDH*-Atg30^{S112A}-GFP	PPY12 pr*GAPDH*-Atg30^{S112A}-GFP::HIS4 his4 arg4	This study
SJCF764	*atg8*Δ / BFP-Atg8 / pr*ATG30*-Atg30-GFP	SJCF257 BFP-Atg8::ARG4 pr*ATG30*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF765	*atg26*Δ / GFP-Atg17 / Flag-Atg11	Pdg3D GFP-Atg17::HIS4 Flag-Atg11::ARG4(Zeocin^r) his4 arg4	This study
SJCF766	*atg26*Δ / GFP-Atg17 / Flag-Atg11 / pr*GAPDH*-Atg30^{S112A}-Prot.A	Pdg3D GFP-Atg17::HIS4 Flag-Atg11::ARG4(Zeocin^r) pr*GAPDH*-Atg30^{S112A}-Prot.A::HIS4(Geneticin^r) his4 arg4	This study
SJCF767	*atg26*Δ / GFP-Atg17 / Flag-Atg11 / pr*GAPDH*-Atg30-Prot.A	Pdg3D GFP-Atg17::HIS4 Flag-Atg11::ARG4(Zeocin^r) pr*GAPDH*-Atg30-Prot.A::HIS4(Geneticin^r) his4 arg4	This study
SJCF768	WT / Pex3-mRFP / pr*ATG30*-Atg30-GFP	PPY12 Pex3-mRFP::ARG4 pr*ATG30*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF769	*pex4*Δ / Pex3-RFP / pr*ATG30*-Atg30-GFP	STK108 Pex3-mRFP::ARG4(Zoecin^r) pr*ATG30*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF770	*pex8*Δ / Pex3-RFP / pr*ATG30*-Atg30-GFP	JC121 Pex3-mRFP::ARG4(Zoecin^r) pr*ATG30*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF777	*atg11*Δ / GFP-Atg8	R8 GFP-Atg8::HIS4 his4	This study
SJCF823	*atg8*Δ / pr*ATG30*-Atg30-GFP	SJCF257 pr*ATG30*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF826	*atg30*Δ / GFP-Atg8	SJCF44 GFP-Atg8::HIS4 his4 arg4	This study
SJCF827	*atg2*Δ / pr*GAPDH*-Atg30-GFP	WDK011 pr*GAPDH*-Atg30-GFP::HIS4 his4	This study
SJCF829	*atg11*Δ / pr*GAPDH*-Atg30-GFP	R8 pr*GAPDH*-Atg30-GFP::HIS4 his4	This study
SJCF837	*atg2*Δ / pr*ATG30*-Atg30-GFP	WDK011 pr*ATG30*-Atg30-GFP::HIS4 his4	This study
SJCF839	*atg28*Δ / pr*GAPDH*-Atg30-GFP	Δatg28 pr*GAPDH*-Atg30-GFP::HIS4 his4	This study
SJCF843	*atg9*Δ / GFP-Atg8	R19 GFP-Atg8::HIS4 his4	This study
SJCF848	*atg28*Δ / pr*ATG30*-Atg30-GFP	Δatg28 pr*ATG30*-Atg30-GFP::HIS4 his4	This study
SJCF858	*atg26*Δ / pr*AOX*-Atg30-GFP	Pdg3D pr*AOX*-Atg30-GFP::HIS4his4 arg4	This study
SJCF883	WT / pr*GAPDH*-BFP-SKL	PPY12 pr*GAPDH*-BFP-SKL::ARG4 his4 arg4	This study
SJCF884	WT / pr*GAPDH*-BFP-SKL / pr*GAPDH*-Atg30-GFP	PPY12 pr*GAPDH*-BFP-SKL::ARG4 pr*GAPDH*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF885	WT / pr*GAPDH*-BFP-SKL / pr*GAPDH*-Atg30^{S122A}-GFP	PPY12 pr*GAPDH*-BFP-SKL::ARG4 pr*GAPDH*-Atg30-GFP^{S112A}::HIS4 his4 arg4	This study
SJCF892	*pex14*Δ / pr*GAPDH*-Atg30-GFP	JC404 pr*GAPDH*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF893	*pex3*Δ / pr*GAPDH*-Atg30-GFP	SEW1 pr*GAPDH*-Atg30-GFP::HIS4 his4 arg4	This study
SJCF907	*atg1*Δ / pr*COPPER*-Atg30-GFP / BFP-SKL	R12 pr*COPPER*-Atg30-GFP::HIS4(Geneticin^r) BFP-SKL::pr*AOX*(Higromycin^r) his4	This study
SJCF908	*atg11*Δ / pr*COPPER*-Atg30-GFP / BFP-SKL	R8 pr*COPPER*-Atg30-GFP::HIS4(Geneticin^r) BFP-SKL::pr*AOX*(Higromycin^r) his4	This study
SJCF909	*atg30*Δ / pr*COPPER*-Atg30-GFP / BFP-SKL	SJCF44 pr*COPPER*-Atg30-GFP::HIS4(Geneticin^r) BFP-SKL::pr*AOX*(Blasticidin^r) his4 arg4	This study
SJCF910	WT / pr*ATG30*-Atg30^{S112A}-GFP	PPY12 pr*ATG30*-Atg30^{S112A}-GFP::HIS4 his4 arg4	This study
SJCF911	WT / HA-Atg8	PPY12 HA-Atg8::HIS4 his4 arg4	This study
SJCF912	*atg7*Δ / HA-Atg8	WDKO7 HA-Atg8::HIS4 his4 arg4	This study
SJCF913	*atg30*Δ / HA-Atg8	SJCF44 HA-Atg8::HIS4 his4 arg4	This study

Table S1. All Strains Indicated in the Table Are the Yeast *Pichia pastoris* Plasmids are indicated in red.

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