Nazarko et al., http://www.jcb.org/cgi/content/full/jcb.201307050/DC1

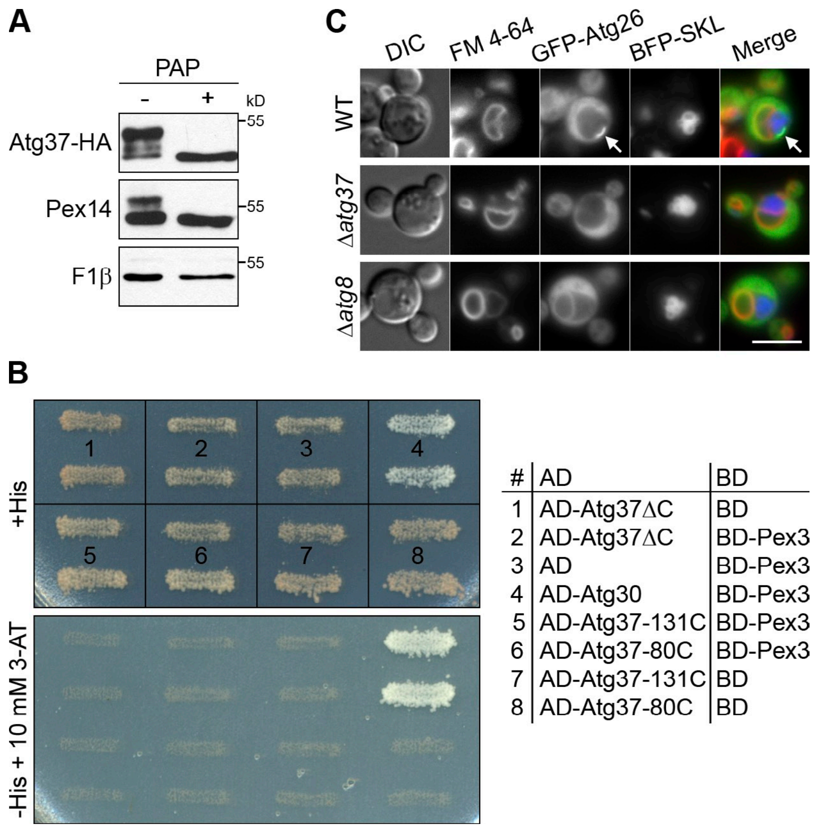

Figure S1. **Supplemental data on Atg37 modification, interaction, and the role in phagophore formation.** (A) Atg37 is a phosphoprotein. Cell lysates were incubated with (+) or without (−) PAP. (B) Atg37 does not interact with Pex3 in Y2H. Interaction was measured by the growth of cells on −His plates with 10 mM 3-AT. 3-AT, 3-amino-1,2,4-triazole; AD, activation domain; BD, binding domain. (C) Atg37 is essential for MIPA formation. Methanol-grown cells were adapted to glucose medium. Arrows point to the GFP-Atg26 labeled MIPA in the WT cell. DIC, differential interference contrast. Bar, 5 µm.

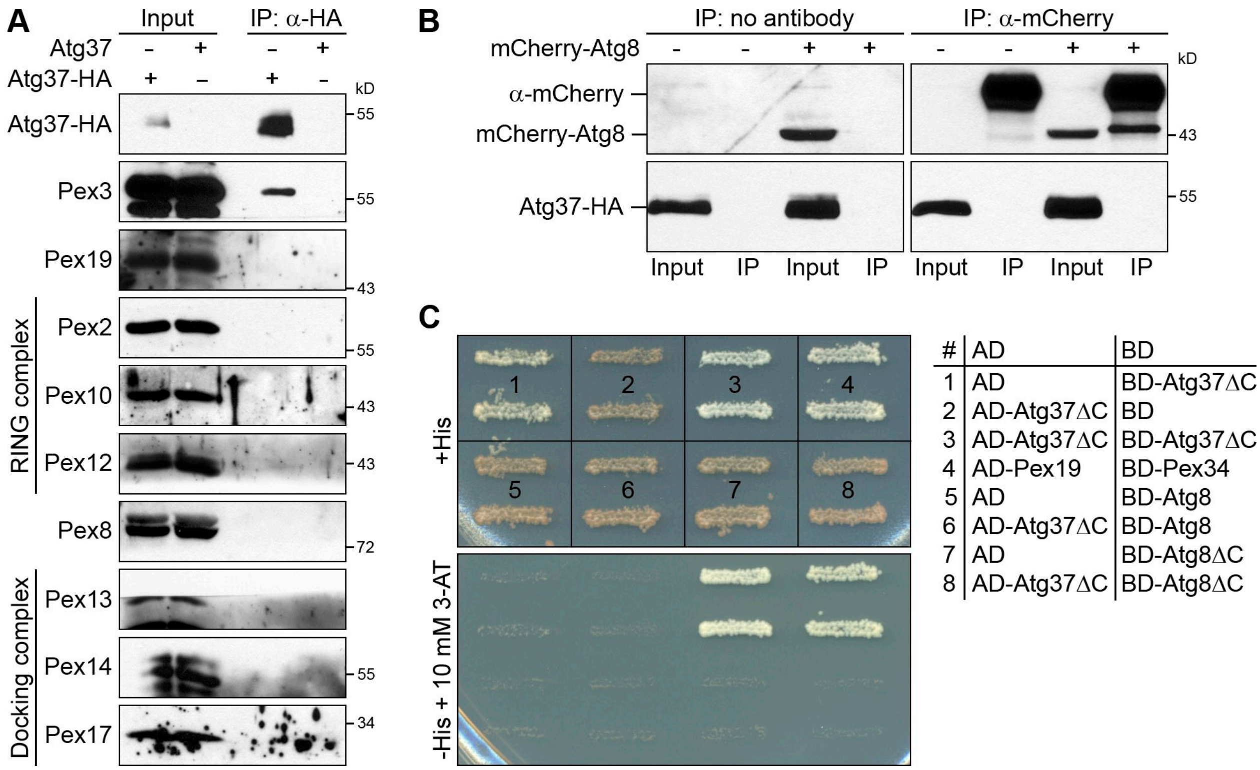

Figure S2. **Atg37 binds Pex3, but not Atg8, under pexophagy conditions.** (A) Pex3 coimmunoprecipitates with Atg37 under pexophagy conditions. Methanol-grown cells were adapted to glucose medium for 0.5 h. (B) Atg37 does not coimmunoprecipitate with Atg8. Methanol-grown cells were adapted to glucose medium for 0.5 h. The α-mCherry band represents the heavy chain of the antibody. (C) Atg37 does not interact with Atg8 in Y2H. Atg37ΔC self-interacted, but did not interact with either Atg8 or Atg8ΔC (aa 1–115).

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HsATG37    1 MADTRSVHETRFEAAVKVIQSLPK....NGSFQPTNEMMLKFYSFYKQATEGPCKLSRPGFWD.PIG........RYKWD
RnAtg37    1 MADTRSVYETRFEAAVKVIQSLPK....NGSFQPTNEMMLRFYSFYKQATEGPCKLSRPGFWD.PIG........RYKWD
MmAtg37    1 MADTPSVYETRFEAAVKVIQSLPK....NGSFQPTNEMMLKFYSFYKQATEGPCKLSRPGFWD.PIG........RYKWD
PpAtg37    1 ...MSESIDRVFVKAIGTIRTLSSRTGYGGLPRPPIENRVKLYGLYKQATEGDVA....GVMERPLGDSPEAEAAKRKWD
PaAtg37    1 .MSSADVIDALFVKTVDMVQSLPK....SGPIQTSYEEKLALYSLYKQATEGDVSSKRPGMLD.MLG........RAKWD
UhAtg37    1 .MSSADVIDALFVKTVDMVQSLPK....SGPIQTSYEEKLALYSLYKQATEGDVQSKRPGMLD.MLG........RAKWD
SrAtg37    1 .MSSADVIDALFVKTVDMVQSLPK....SGPIQTSYEEKLALYSLYKQATEGDVSSKRPGMLD.MLG........RAKCD
                                        YxxL

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SrAtg37   67 AWSKVSGLPPRDAKQMYVESMLRILRRF.SDRPQAASLIQELENFSGQVAQCVMDGSLAQVDSDEESITDTESDDQQRLD
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PaAtg37  146 PTGLHQPPSGPSRQQPPPPQLPAHLAP.....HPPSVTGTSTA..ATRGGG...AASAISTQR......TPLASESEQDS
UhAtg37  146 PTSLHQPPSGPSR..AAQPHLPRHLAPTAPSITAPSITATSTA..ATRGAG...HPSAVSPGR......TPMASESEEET
SrAtg37  146 PTALHQ...QPSR..AAQSNLPRHLAP.....NAPSVAGTSTA..ATRGGVGDAASAISPGR......TPIASESEQDS

HsATG37  213 SLNGRSTEEVKPIDENLGQ.TGKSAVCIHQDINDDHVEDVTGIQHLTS..........DSDSEVYCDSMEQFGQEESLDS
RnAtg37  209 SRSARRSEDKKPTDQSSQQ.TGNTVLCVHQDTNEDPGEDASGVHHLTS..........DSDSEVYCDSMEQFGQEE....
MmAtg37  211 SRSTRSSEDEKPGDESSQQ.TGHTIVCAHQDRNEDPSEDASGIHHLTS..........DSDSEVYCDSMEQFGQEE....
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PaAtg37  210 SDDDHLGSQPGPFRSVARHPTPHAPPSIHSYRPQPRPGSVSATEGYAR.GPPSRSNVPTSSVHEASTTLRPRGPPSLQGG
UhAtg37  213 SEDDRLGSQPGPFRGASRHPAPHRPSSVQSYRPQPRAGSISATEGYNR.RPPSRSQVSALAQYESSQGLRPRGPPSLQGG
SrAtg37  208 SNDDRLGSQPGPFRSAPRHSSSHAPPSVQSYRSQPRAGSISATEGYNRGGPPSRSQVSTSAQYDNPQALRSRGPPSLQGG
                         YxxL

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MmAtg37  276 .........YYLGGDPTQHLESSGFCEDAQQSP......GNGSIGKMWMVAVKGKGEVKHGGEDGRSSSGAPHRETRGGE
PpAtg37  253 ASD.....PLYSKRGS..DLTHDDFVND...........ISSSSSNRRFRARRNQPLVS...................KV
PaAtg37  289 YTR.....HTYAPQSTAASVAGSNYPRQPEPGHYQRSTYAASSVGGRSVGTRTGPAPVYPPRPPSNAGSSHQPPRPEMEL
UhAtg37  292 YSR.....PQYAPKSTAASVAGSNYPRQGEPGDYQRSTYAASSVGGRSVGRRTGPPPIYPPRPPSNAGTARPQPRPEMEM
SrAtg37  288 YSR.....PQYAPQSTAASVAGSNYPRQ...PEYQRSTYAASSVGGRSIGTRTGPPSIYPPRPPSNAGTAQHQPRAEMES

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UhAtg37  367 ALESIQASLAALHERLNRV....EGRSSGASPDDPRRA...GSGQSSSARLYTAIMNVVEDIKHLIGLNVVARGARSEAL
SrAtg37  360 ALQSIQASLAALHERLNRV....ESRSPAGGADDPRRSRP.GSGQSSAAYFYNAIMNVVDDFKQLLGLNGVARGQRSEAL

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MmAtg37  416 TASQAKLS...LQTSNQPSSQRPAWWPFEMS......PGALAF...AIIWPFIAQWLAHLYYQ...........RRRRKL
PpAtg37  348 HHSELESNSSIKGDSNGG..........RLTIVLPFINGKDFFQENSLLGKLLKVFHDYV........DHVSRIRLIKRN
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HsATG37  490 N....................
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MmAtg37  473 N....................
PpAtg37  410 .....................
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UhAtg37  516 QQAILRRSPR.AKQFVDQATA
SrAtg37  513 QQAVLRRSPA.AKQLVAEASV
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Figure S3. **Multiple sequence alignment of Atg37 orthologues.** Identical residues are indicated with black boxes and similar residues with gray boxes. HsATG37, *Homo sapiens* ACBD5 isoform 2 (NP_001035938.1); RnAtg37, *Rattus norvegicus* Acbd5 (NP_001071103.1); MmAtg37, *Mus musculus* Acbd5 (AAH61484.2); PpAtg37, *Komagataella* (formerly *Pichia*) *pastoris* GS115 Atg37 (CAY71862.1); PaAtg37, *Pseudozyma antarctica* T-34 Atg37(GAC77071.1); UhAtg37, *Ustilago hordei* Atg37 (CCF51006.1); SrAtg37, *Sporisorium reilianum* SRZ2 Atg37 (CBQ71469.1). The potential AIMs of PpAtg37 are underlined below the aligned sequences.

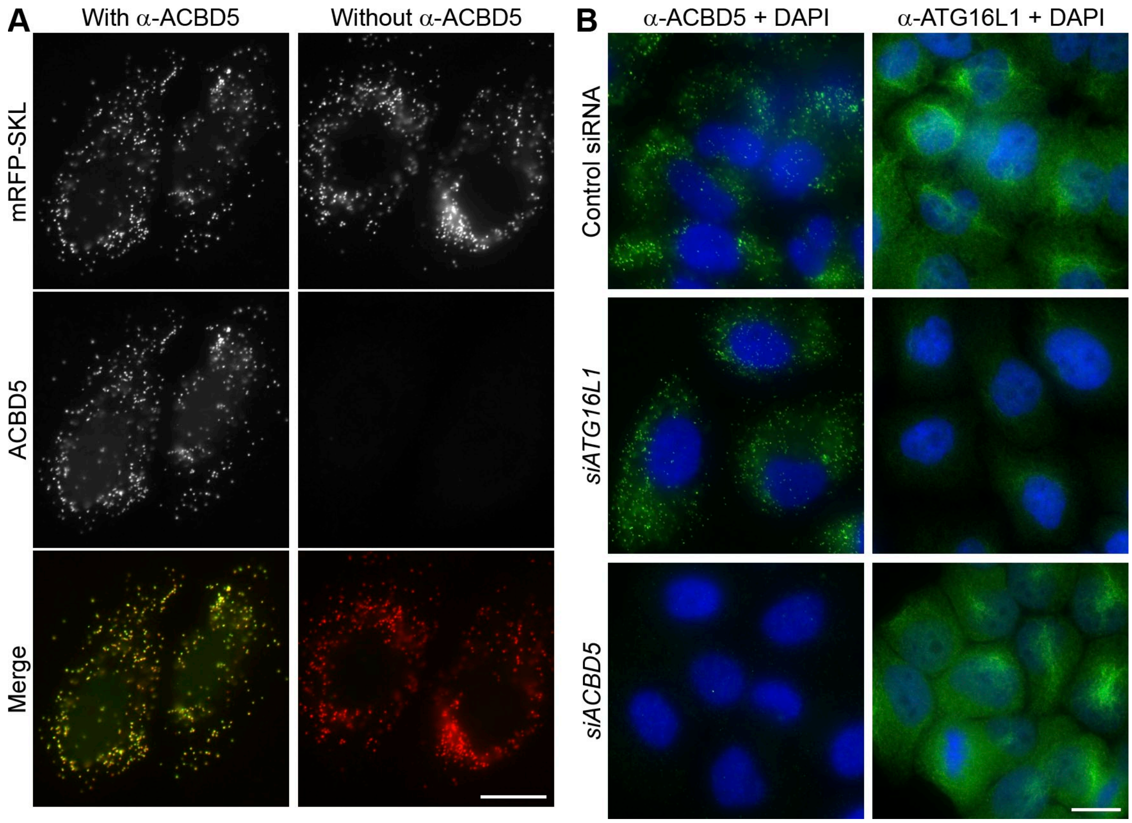

Figure S4. **ATG37/ACBD5 localizes to peroxisomes in human cells.** (A) ACBD5 is localized to peroxisomes in HeLa cells. Cells expressed peroxisomally targeted mRFP-SKL and were immunostained with antibody to ACBD5. Secondary antibody alone did not show any signal in the respective channel (right). Bar, 10 µm. (B) siRNAs efficiently down-regulate ATG16L1 and ACBD5 in HeLa cells. Cells were transfected with nontargeting control siRNA or siRNA targeting ACBD5 or ATG16L1 for 3 d and immunostained with antibodies to ACBD5 (left) or ATG16L1 (right). DAPI stained nuclei. Bar, 10 µm.

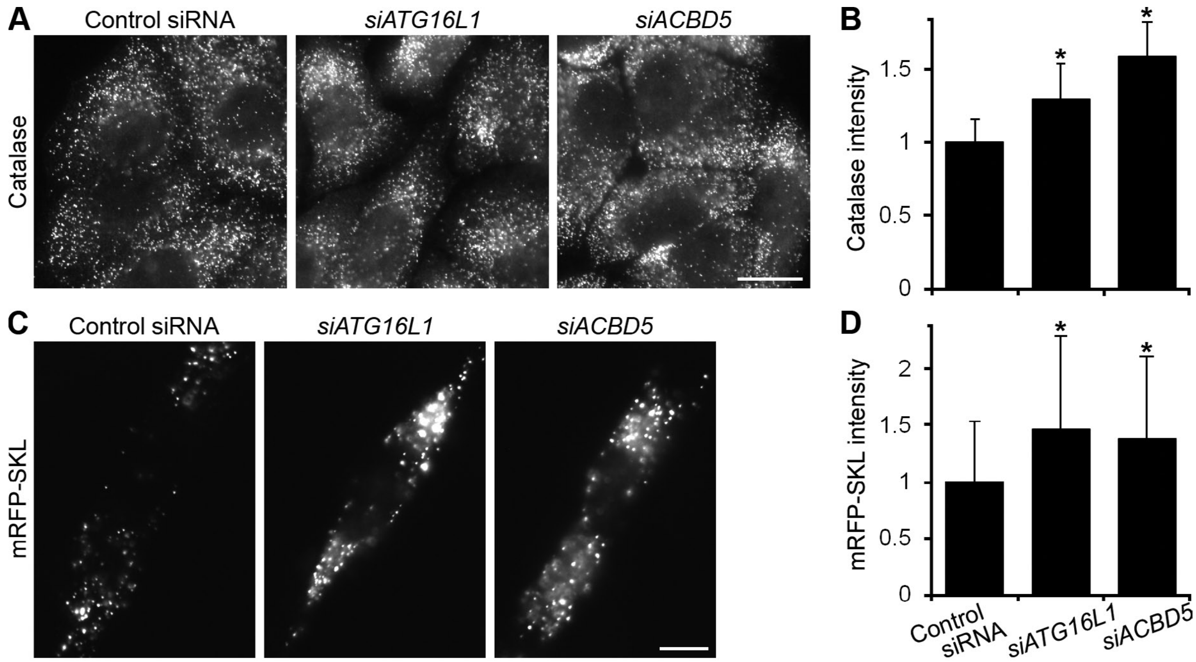

Figure S5. **Role of ATG37/ACBD5 in pexophagy.** (A) Knockdown of ACBD5 increases the peroxisome abundance in HeLa cells. Immunostaining with antibodies to peroxisomal catalase is shown. Bar, 10 µm. (B) Quantitation of data in A. Catalase intensity of cells transfected with control siRNA was set as 1. Relative catalase intensity is shown as the mean + SD (error bars; $n \geq 250$; *, $P < 0.05$ vs. control siRNA). (C) Knockdown of ACBD5 increases the peroxisome abundance in primary fibroblasts of a patient with a PBD caused by a missense mutation in the *PEX16* gene. Bar, 10 µm. (D) Quantitation of data in C. mRFP-SKL intensity of cells transfected with control siRNA was set as 1. Relative mRFP-SKL intensity is shown as the mean + SD (error bars; $n \geq 20$; *, $P < 0.05$ vs. control siRNA).

Table S1. ***P. pastoris* strains and plasmids used in this study**

Name	Description	Genotype and plasmid	Source
PDG2d	Δ*atg28*	GS200 Δ*atg28*::*ScARG4*	Stasyk et al., 2006
PPY12h	WT	*arg4 his4*	Gould et al., 1992
PPY12m	WT	*arg4 his4*	Gould et al., 1992
R8	Δ*atg11*	GS115 *atg11-2*::$Zeocin^R$	Kim et al., 2001
SEW1	Δ*pex3*	PPY12h Δ*pex3*::*ARG4*	Wiemer et al., 1996
SKF13	Δ*pex19*	PPY12h Δ*pex19*::$Zeocin^R$	Snyder et al., 1999
SJCF51	WT	PPY12m *his4*::pTW51 (P_{AOX1}-*GFP-SKL, HIS4)*	Nazarko et al., 2009
SJCF320	WT	SJCF247 *his4*::pJCF208 (P_{ATG8}-*GFP-ATG8, HIS4)*	Farré et al., 2008
SJCF376	Δ*atg30*	SJCF332 *his4*::pJCF208 (P_{ATG8}-*GFP-ATG8, HIS4)*	Farré et al., 2008
SJCF547	WT	PPY12m *his4*::pJCF208 (P_{ATG8}-*GFP-ATG8, HIS4)*	Farré et al., 2008
SJCF925	Δ*atg8*	PPY12h Δ*atg8*::$Geneticin^R$ (pJCF182)	Nazarko et al., 2009
SJCF936	Δ*atg30*	PPY12h Δ*atg30*::$Zeocin^R$ (pJCF56)	Nazarko et al., 2009
SJCF1821	Δ*atg30*	SJCF959 P_{AOX1}::pPICz-BFP-SKL (P_{AOX1}-*BFP-SKL*, $Zeocin^R$)	Farré et al., 2013
SMD1163	*pep4 prb1*	*pep4 prb1 his4*	Tuttle and Dunn, 1995
SMY154	WT	PPY12h *PEX3*::pMY126 (P_{PEX3}-*PEX3-TAP*, $Zeocin^R$)	This paper
SMY431	Δ*pmp47*	PPY12m Δ*pmp47*::$Zeocin^R$ (pMY243)	This paper
SMY432	Δ*atg37*	PPY12m Δ*atg37*::$Zeocin^R$ (pMY92)	This paper
SMY433	WT	SMY432 *his4*::pMY244 (P_{GAPDH}-*GFP-ATG37, HIS4*)	This paper
SMY437	Δ*atg37*	SMY432 *his4*::pTW51 (P_{AOX1}-*GFP-SKL, HIS4)*	This paper
SMY448	WT	PPY12m *his4*::pMY244 (P_{GAPDH}-*GFP-ATG37, HIS4*)	This paper
SRRM197	Δ*ypt7*	PPY12h Δ*ypt7*::$Geneticin^R$	Manjithaya et al., 2010
STN29	Δ*atg1*	R12 *his4*::pTW51 (P_{AOX1}-*GFP-SKL, HIS4)*	Nazarko et al., 2007
STN48	Δ*atg37*	SMY432 P_{AOX1}::pRDM054 (P_{AOX1}-*BFP-SKL*, $Hygromycin^R$)	This paper
STN50	Δ*atg37*	STN48 *his4*::pJCF208 (P_{ATG8}-*GFP-ATG8, HIS4)*	This paper
STN52	Δ*atg37*	SMY432 *his4*::pJCF208 (P_{ATG8}-*GFP-ATG8, HIS4)*	This paper
STN66	Δ*atg1*	R12 *his4*::pJCF208 (P_{ATG8}-*GFP-ATG8, HIS4)*	Nazarko et al., 2009
STN95	Δ*atg30* Δ*atg37*	SJCF936 Δ*atg37*::$Geneticin^R$ (pMY92Kan)	This paper
STN96	Δ*atg37*	PPY12h Δ*atg37*::$Geneticin^R$ (pMY92Kan)	This paper
STN117	WT	PPY12h *his4*::pJCF392 (P_{GAPDH}-*GFP-ATG26, HIS4*)	This paper
STN119	Δ*atg8*	SJCF925 *his4*::pJCF392 (P_{GAPDH}-*GFP-ATG26, HIS4*)	This paper
STN126	WT	SJCF936 *his4*::pJCF213 (P_{ATG30}-*ATG30-GFP, HIS4*)	This paper
STN128	WT	STN96 *his4*::pMY323 (P_{ATG37}-*ATG37-GFP, HIS4*)	This paper
STN130	WT	SJCF936 *his4*::pJCF45 (P_{GAPDH}-*ATG30-GFP, HIS4*)	This paper
STN132	WT	STN96 *his4*::pTYN4 (P_{GAPDH}-*ATG37-GFP, HIS4*)	This paper
STN139	WT	STN96 *his4*::pMY244 (P_{GAPDH}-*GFP-ATG37, HIS4*)	This paper
STN148	Δ*atg30*	STN95 *his4*::pTYN4 (P_{GAPDH}-*ATG37-GFP, HIS4*)	This paper
STN149	Δ*atg37*	STN95 *his4*::pJCF45 (P_{GAPDH}-*ATG30-GFP, HIS4*)	This paper
STN155	Δ*atg30*	STN95 *his4*::pMY323 (P_{ATG37}-*ATG37-GFP, HIS4*)	This paper
STN161	WT	STN128 P_{AOX1}::pRDM054 (P_{AOX1}-*BFP-SKL*, $Hygromycin^R$)	This paper
STN163	Δ*atg30*	STN155 P_{AOX1}::pRDM054 (P_{AOX1}-*BFP-SKL*, $Hygromycin^R$)	This paper
STN199	WT	STN126 P_{AOX1}::pRDM054 (P_{AOX1}-*BFP-SKL*, $Hygromycin^R$)	This paper
STN201	Δ*atg37*	STN95 *his4*::pJCF213 (P_{ATG30}-*ATG30-GFP, HIS4*)	This paper
STN203	Δ*atg37*	STN201 P_{AOX1}::pRDM054 (P_{AOX1}-*BFP-SKL*, $Hygromycin^R$)	This paper
STN215	WT	STN96 *his4*::pMY320 (P_{ATG37}-*ATG37-2xHA, HIS4*)	This paper
STN245	WT	PPY12h *ATG37*::pMY328 (P_{ATG37}-*ATG37-GFP, ARG4*)	This paper
STN249	Δ*pex19*	SKF13 *ATG37*::pMY328 (P_{ATG37}-*ATG37-GFP, ARG4*)	This paper
STN255	WT	STN245 *his4*::pKSN183 (P_{PEX3}-*PEX3-mRFP1, HIS4*)	This paper
STN260	Δ*pex19*	STN249 *his4*::pKSN183 (P_{PEX3}-*PEX3-mRFP1, HIS4*)	This paper
STN286	WT	STN288 P_{AOX1}::pRDM054 (P_{AOX1}-*BFP-SKL*, $Hygromycin^R$)	This paper
STN288	WT	STN128 *arg4*::pJCF477 (P_{ATG8}-*mCHERRY-ATG8, ARG4*)	This paper
STN292	*acbs*	STN96 *his4*::pTYN21 (P_{ATG37}-$ATG37^{Y40F, K44A}$-*2xHA, HIS4*)	This paper
STN294	$Atg37^{\Delta TMD}$	STN96 *his4*::pTYN24 (P_{ATG37}-$ATG37^{\Delta 290\text{-}339}$-*GFP, HIS4*)	This paper
STN300	*acbs*	STN96 *his4*::pTYN22 (P_{ATG37}-$ATG37^{Y40F, K44A}$-*GFP, HIS4*)	This paper
STN352	*acbs*	STN300 P_{AOX1}::pRDM054 (P_{AOX1}-*BFP-SKL*, $Hygromycin^R$)	This paper
STN356	$Atg37^{\Delta TMD}$	STN294 P_{AOX1}::pRDM054 (P_{AOX1}-*BFP-SKL*, $Hygromycin^R$)	This paper
STN363	WT	STN215 *arg4*::pJCF477 (P_{ATG8}-*mCHERRY-ATG8, ARG4*)	This paper
STN556	WT	STN96 *his4*::pKO8 (P_{ATG37}-*GFP-ATG37, HIS4*)	This paper
STN563	Δ*atg30*	STN95 *his4*::pMY320 (P_{ATG37}-*ATG37-2xHA, HIS4*)	This paper

Table S1. ***P. pastoris* strains and plasmids used in this study** *(Continued)*

Name	Description	Genotype and plasmid	Source
STN566	Δ*atg30* Δ*ypt7*	SJCF1736 *ATG37*::pMY328 (P_{ATG37}-*ATG37-GFP*, *ARG4*)	This paper
STN567	Δ*ypt7*	SJCF1809 *ATG37*::pMY328 (P_{ATG37}-*ATG37-GFP*, *ARG4*)	This paper
STN605	Δ*atg37*	STN96 *his4*::pJCF392 (P_{GAPDH}-*GFP-ATG26*, *HIS4*)	This paper
STN612	WT	STN117 P_{AOX1}::pRDM054 (P_{AOX1}-*BFP-SKL*, *Hygromycin*^{R})	This paper
STN614	Δ*atg8*	STN119 P_{AOX1}::pRDM054 (P_{AOX1}-*BFP-SKL*, *Hygromycin*^{R})	This paper
STN616	Δ*atg37*	STN605 P_{AOX1}::pRDM054 (P_{AOX1}-*BFP-SKL*, *Hygromycin*^{R})	This paper
STN618	WT	PPY12h P_{AOX1}::pPICz-BFP-SKL (P_{AOX1}-*BFP-SKL*, *Zeocin*^{R})	This paper
STN620	Δ*atg37*	STN96 P_{AOX1}::pPICz-BFP-SKL (P_{AOX1}-*BFP-SKL*, *Zeocin*^{R})	This paper
STN622	Δ*ypt7*	SRRM197 *arg4*::pJCF340 P_{ATG11}-*2xFLAG-ATG11*, *ARG4*)	This paper
STN630	Δ*ypt7*	STN622 *his4*::pJCF343 (P_{GAPDH}-*ATG30-TAP*, *HIS4*)	This paper
STN632	Δ*ypt7*	STN630 *his4*::pTYN57 (P_{GAPDH}-*GFP-ATG17*, *Hygromycin*^{R})	This paper
STN634	Δ*ypt7*	STN622 *his4*::pTYN57 (P_{GAPDH}-*GFP-ATG17*, *Hygromycin*^{R})	This paper
STN636	WT	STN618 *arg4*::pJCF291 P_{ATG11}-*GFP-ATG11*, *ARG4*)	This paper
STN639	Δ*atg37*	STN620 *arg4*::pJCF291 P_{ATG11}-*GFP-ATG11*, *ARG4*)	This paper
STN640	Δ*atg30*	SJCF1821 *arg4*::pJCF291 P_{ATG11}-*GFP-ATG11*, *ARG4*)	This paper
STN641	Δ*ypt7* Δ*atg37*	STN632 Δ*atg37*::*Zeocin*^{R} (pMY92) colony #2	This paper
STN642	Δ*ypt7* Δ*atg37*	STN632 Δ*atg37*::*Zeocin*^{R} (pMY92) colony #3	This paper

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