Supplemental Information (Wu and Tu, "Selective Regulation of Autophagy by the Iml1p-Npr2p-Npr3p Complex in the Absence of Nitrogen Starvation")

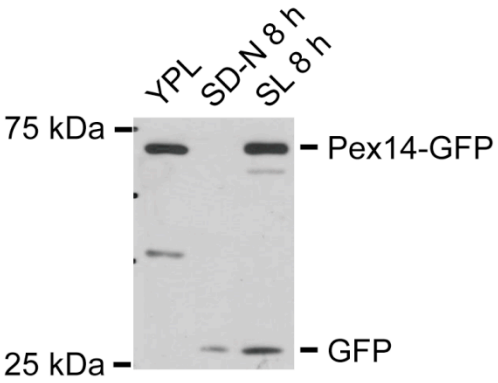

Figure S1. Pexophagy is Induced upon Switch to SL Medium.

Cells were grown in YPL to log phase before switch to either nitrogen starvation medium (SD-N) or non-starvation medium (SL). After 8 hours following medium switch, autophagic degradation of peroxisomes was assayed by Western blot using Pex14-GFP as the reporter. Pex14p is a peroxisomal membrane protein.

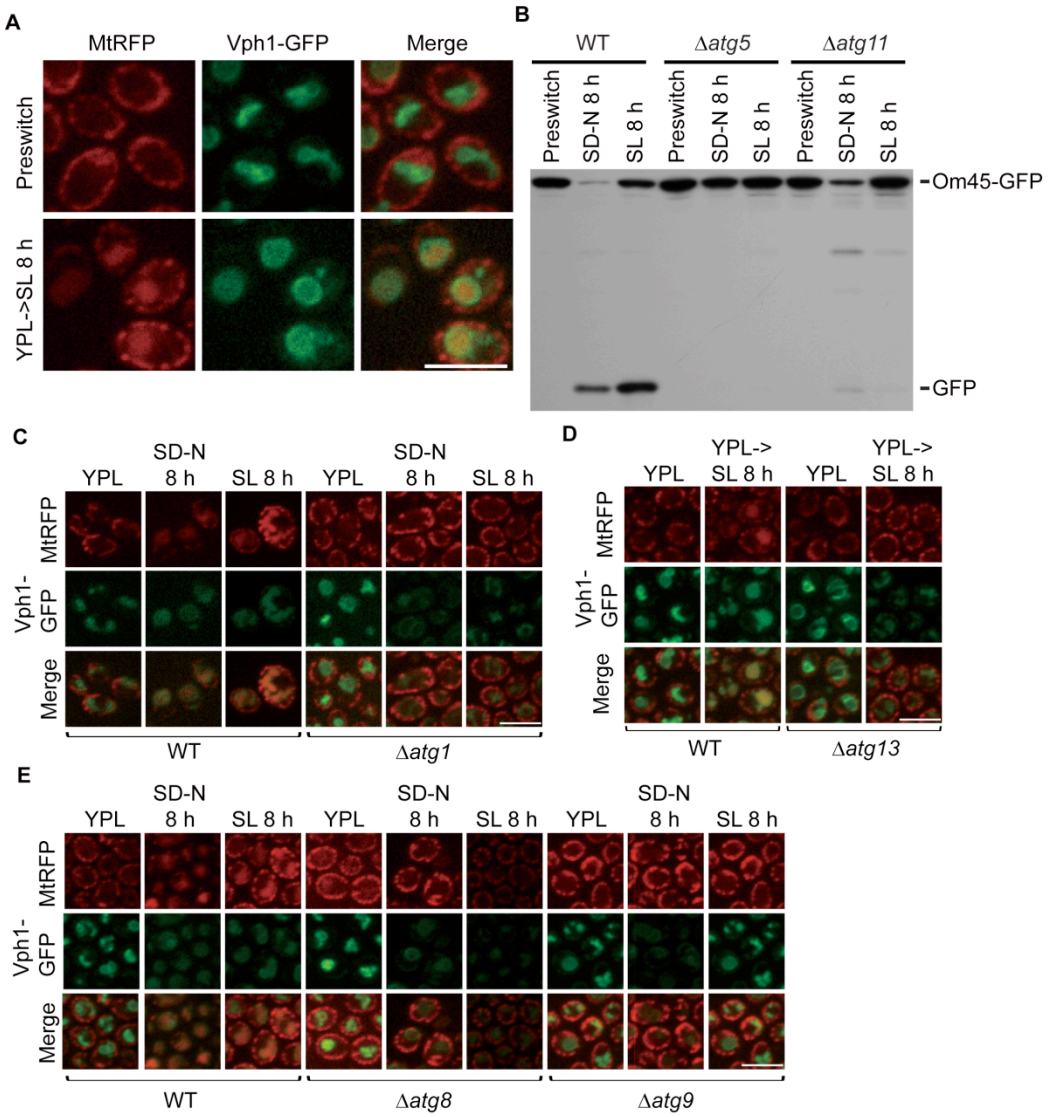

Figure S2. NNS-induced Mitophagy is Blocked upon Deletion of *ATG1, ATG5, ATG8, ATG9, ATG11* or *ATG13.*

Cells were cultured as described in Figure S1. After 8 hours following medium switch, mitophagy was assayed by imaging using MtRFP as the reporter or by Western blot using Om45-GFP as the reporter. MtRFP is a reporter targeted to the mitochondrial matrix. *OM45* encodes a mitochondrial outer-membrane protein. Vph1-GFP is a marker for the vacuole.

(A) Mitochondria reporter was detected in the vacuole after switch to SL medium for 8 h. Scale bar, 10 μm.

(B) The release of free GFP from Om45-GFP increased upon switch to autophagy-inducing media (SD-N and SL) and was blocked in Δ*atg5* and Δ*atg11* cells.

(C-E) In contrast to WT cells, the vacuolar mitochondrial signal was undetectable in Δ*atg1*, Δ*atg13*, Δ*atg8* and Δ*atg9* cells following switch to the autophagy-inducing media (SD-N and SL). Scale bar, 10 μm.

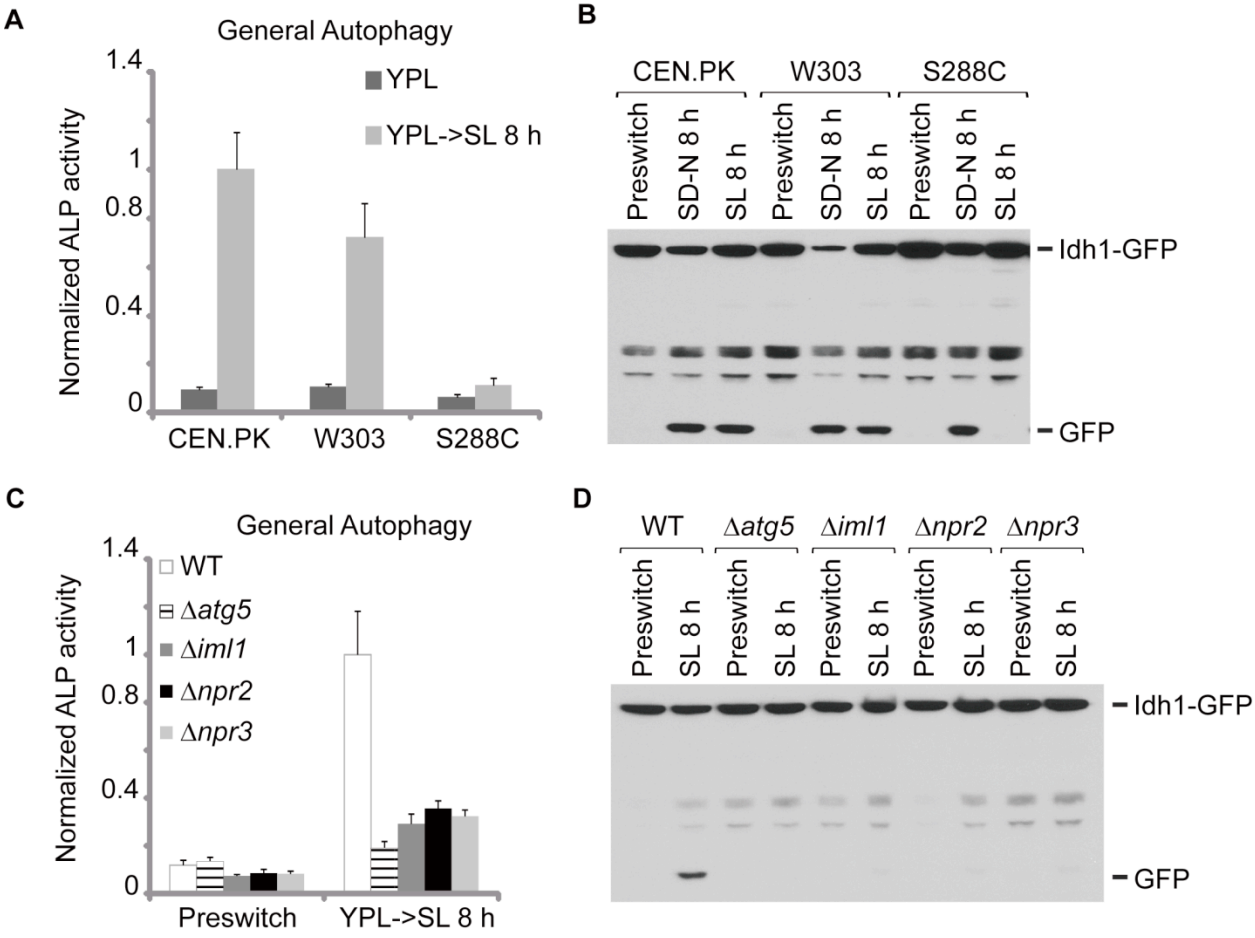

Figure S3. *IML1*, *NPR2*, and *NPR3* are Required for NNS-induced Autophagy in the W303 Strain Background.

The prototrophic versions of CEN.PK, W303 and S288C were cultured as described in Figure S1. For the ALP activity assays, data represent the averages of 3-5 samples with error bars for standard deviations.

(A and B) Medium switch from YPL to SL led to robust induction of autophagy in CEN.PK and W303 cells, but not in S288C cells. Another autophagy-inducing condition, nitrogen starvation, served as the positive control.

(C and D) Loss of *IML1*, *NPR2* or *NPR3* in W303 cells strongly inhibited NNS-induced autophagy. Δ*atg5* cells were used as the positive control.

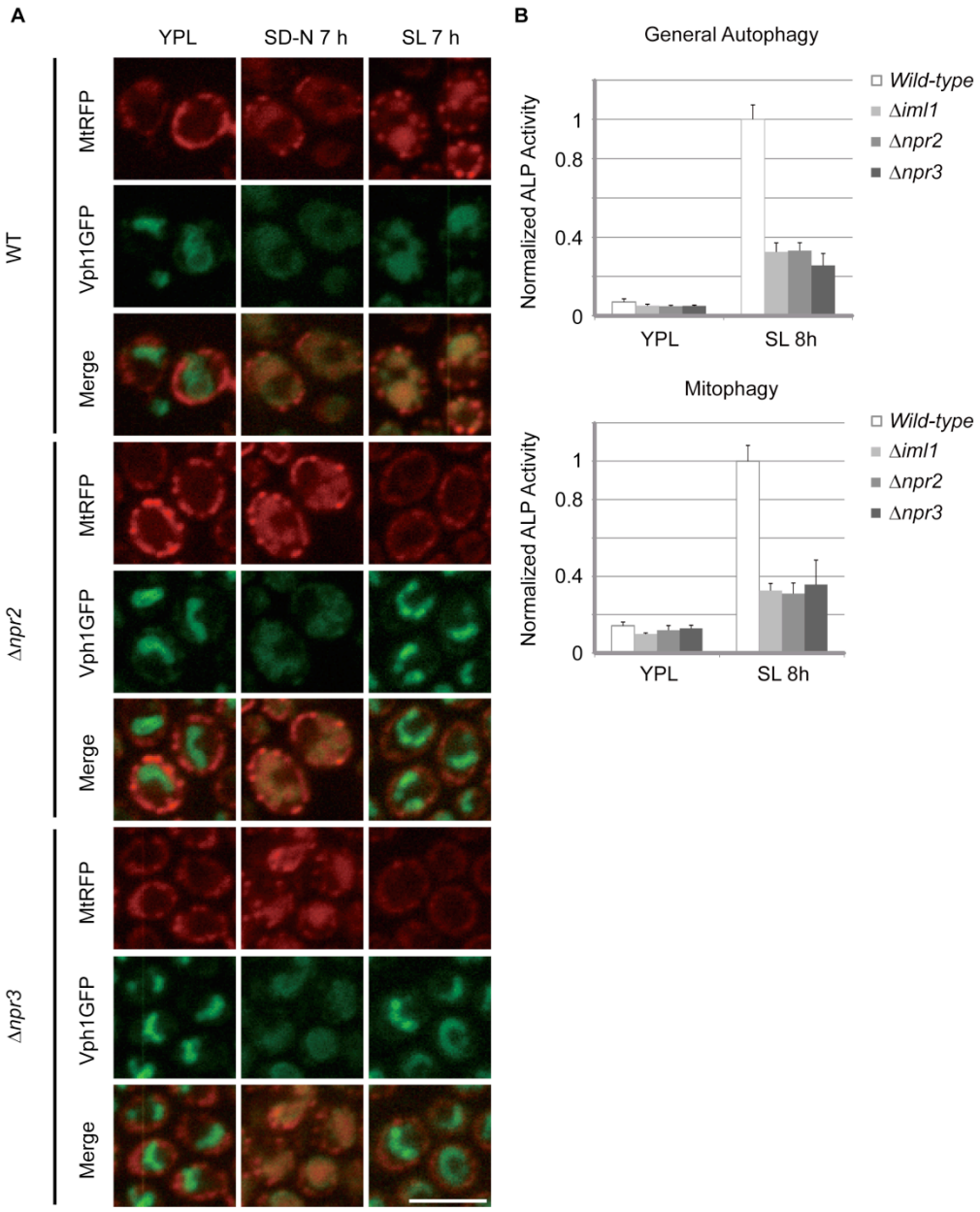

Figure S4. *IML1*, *NPR2* and *NPR3* are Selectively Required for NNS-Induced Autophagy

Cells were cultured as described in Figure S1. After 7 or 8 hours following medium switch, general autophagy or mitophagy was measured by ALP activity assay or by imaging using MtRFP as the reporter.

(A) Deletion of *NPR2* and *NPR3* inhibited NNS-induced mitophagy but did not affect nitrogen-starvation-induced mitophagy. Scale bar, 10 μm.

(B) Deletion of *IML1*, *NPR2* and *NPR3* led to about 50%-70% decrease in NNS-induced autophagy. Data represent averages of 3-5 samples with error bars for standard deviations.

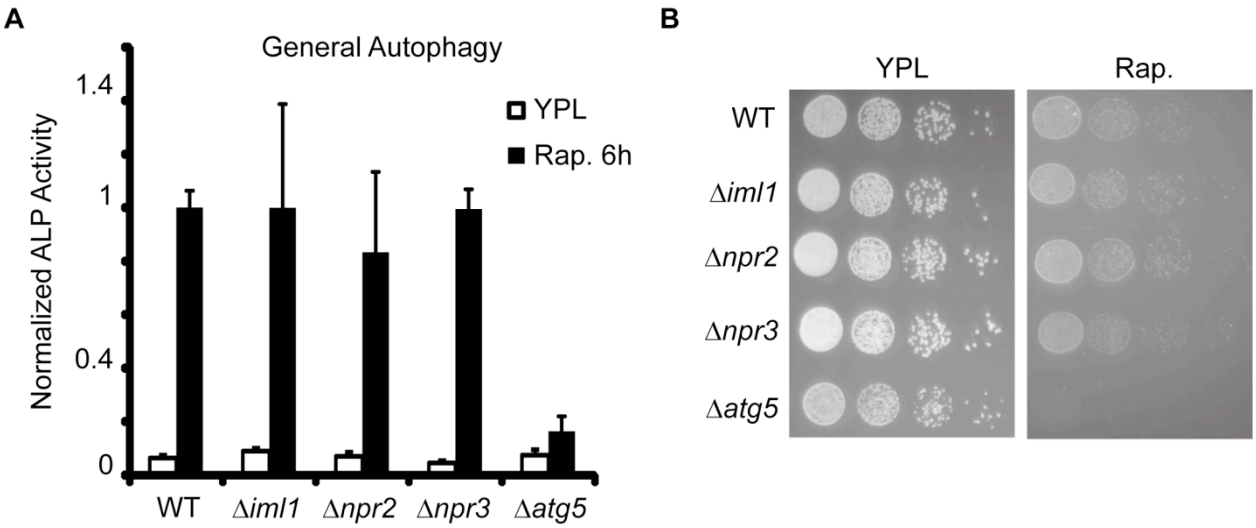

Figure S5. *IML1*, *NPR2* and *NPR3* are Not Required for Rapamycin-Induced Autophagy.

(A) Cells were grown in YPL to log phase (OD 0.5-0.8) and then continued to be grown in YPL in the presence or absence of rapamycin (0.2 μg/mL) for 6 hours. After rapamycin treatment, general autophagy was measured by ALP activity assay. Data represent the averages of 3-5 samples with error bars for standard deviations. Rap., rapamycin.

(B) Deletion of *IML1*, *NPR2* or *NPR3* did not affect rapamycin sensitivity. Cells of the indicated genotypes were grown in YPL to log phase and then serial 10-fold dilutions of cells were stamped onto YPL plates supplemented with or without rapamycin (0.2 μg/mL). Images were captured after cells had been incubated at 30˚C for 2-4 days.

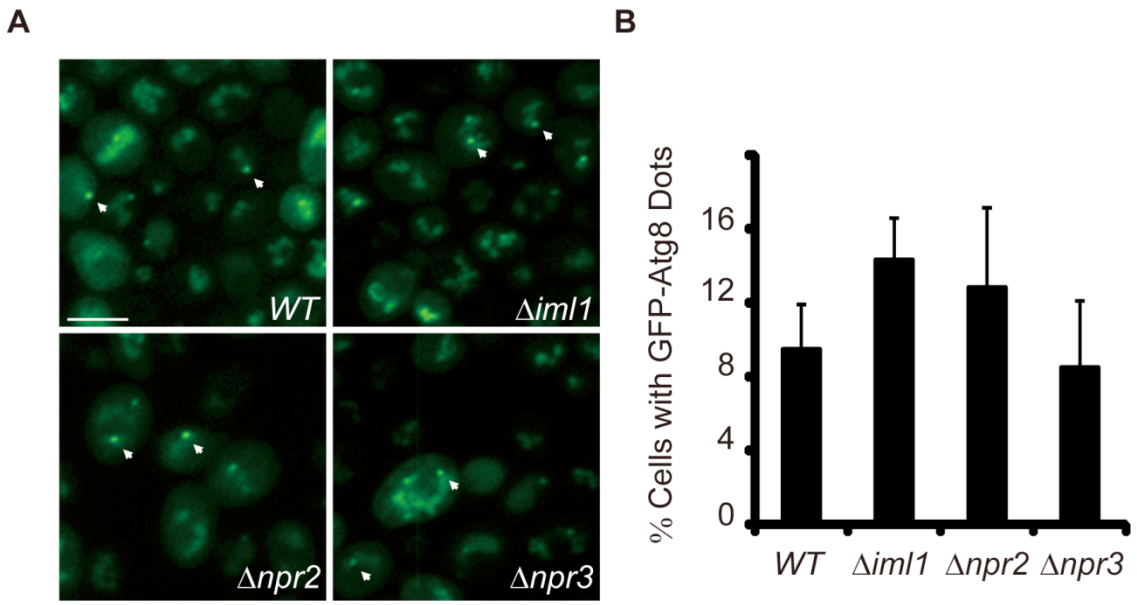

Figure S6. Deletion of *IML1*, *NPR2* or *NPR3* Does Not Affect Atg8p Dot Formation upon Switch to SL Medium.

WT or mutant cells that express GFP-Atg8 were grown in YPL to log phase and then switched to SL medium. Thirty minutes after medium switch, cells were collected for GFP imaging.

(A) Representative images showing the formation of Atg8p dots in SL as indicated by the arrows. Scale bar, 5 μm.

(B) Quantification of Atg8p dot formation in WT and mutant cells. At least 200 cells were counted for each genotype. Data represent averages from three independent experiments with error bars for standard deviations.

Figure S7. Multiple Sequence Alignment of Iml1p and its Orthologs.

Highly conserved residues are colored in black. Partially conserved residues are colored in gray. The DUF and DEP domains are highlighted by straight lines beneath the sequences. Mm, Mus musculus; Rn, Rattus norvegicus; Hs, Homo sapiens; Gg, Gallus gallus; Dm, Drosophila melanogaster; Sp, Schizosaccharomyces pombe; Sc, Saccharomyces cerevisiae.

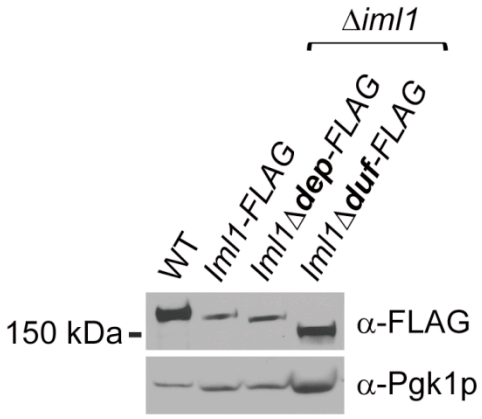

Figure S8. Ectopic Expression of Full-Length and the Domain-Deleted Versions of Iml1p.

The coding sequence for full length IML1-FLAG, IML1Δdep-FLAG or IML1Δduf-FLAG was cloned into a centromeric plasmid p417-CYC1. Plasmids expressing Iml1-FLAG, Iml1Δdep-FLAG or Iml1Δduf-FLAG were transformed into Δ*iml1* cells to determine the ectopic expression levels of full-length and the domain-deleted versions of Iml1p. Cells were collected and analyzed as described in Figure 5F. Pgk1p was used as the loading control. WT cells expressing Iml1-FLAG from the endogenous chromosomal locus are shown for comparison.

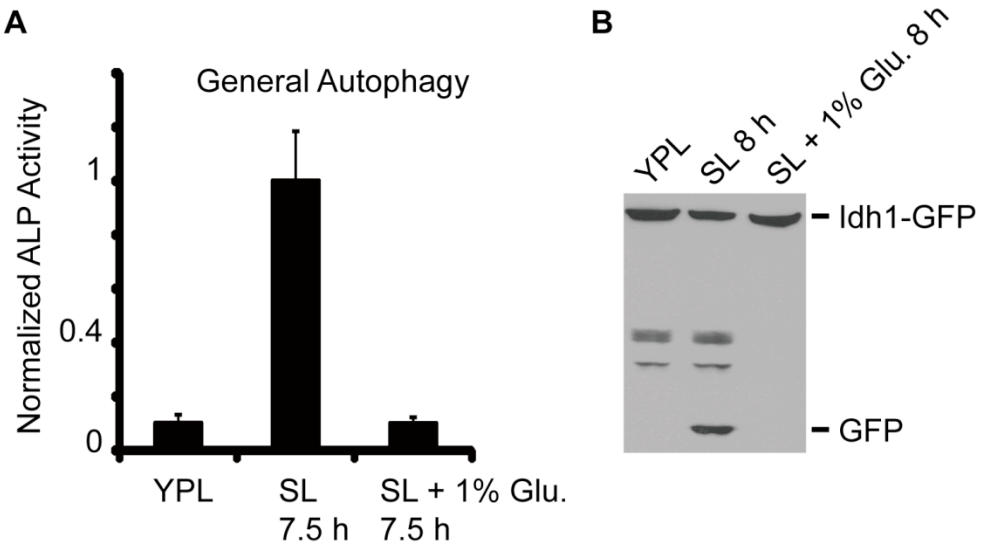

Figure S9. NNS-induced Autophagy Is Completely Blocked by Addition of Glucose.

Cells were cultured in YPL to log phase and then switched to SL medium in the presence or absence of 1% glucose for about 8 hours. Glu., glucose.

(A) NNS-induced general autophagy was totally blocked by addition of glucose. Data represent the averages of 3-5 samples with error bars for standard deviations.

(B) NNS-induced mitophagy was totally blocked by addition of glucose.

Table S1 Yeast Strains Used in This Study

Name	Genotype	Strain Background
YXW1	*MATa ho::ADH1^{P}-Mito-dsRed::kanMX4 vph1::VPH1-GFP::kanMX6*	protrotrophic CEN.PK
YXW2	*MATα om45::OM45-GFP::kanMX6 vph1::VPH1-mCherry::natNT2*	protrotrophic CEN.PK
YXW3	*MATα om45::OM45-GFP::kanMX6 vph1::VPH1-mCherry::natNT2 atg5::hphNT1*	protrotrophic CEN.PK
YXW4	*MATα om45::OM45-GFP::kanMX6 vph1::VPH1-mCherry::natNT2 atg11::hphNT1*	protrotrophic CEN.PK
YXW5	*MATa pho8::TEF1^{P}-OM-pho8Δ60::kanMX6 pho13::natNT2*	protrotrophic CEN.PK
YXW6	*MATa pho8::TEF1^{P}-OM-pho8Δ60::kanMX6 pho13::natNT2 atg5::hphNT1*	protrotrophic CEN.PK
YXW7	*MATa pho8::TEF1^{P}-OM-pho8Δ60::kanMX6 pho13::hphNT1 atg11::natNT2*	protrotrophic CEN.PK
YXW8	*MATa pho8::TEF1^{P}-OM-pho8Δ60::kanMX6 pho13::natNT2 atg32::hphNT1*	protrotrophic CEN.PK
YXW9	*MATa pho8::TEF1^{P}-OM-pho8Δ60::kanMX6 pho13::natNT2 iml1::hphNT1*	protrotrophic CEN.PK
YXW10	*MATa pho8::TEF1^{P}-OM-pho8Δ60::kanMX6 pho13::natNT2 npr2::hphNT1*	protrotrophic CEN.PK
YXW11	*MATa pho8::TEF1^{P}-OM-pho8Δ60::kanMX6 pho13::natNT2 npr3::hphNT1*	protrotrophic CEN.PK
YXW12	*MATa pho8::TEF1^{P}-OM-pho8Δ60::kanMX6 pho13::hphNT1 iml1::IML1-GFP::natNT2*	protrotrophic CEN.PK
YXW13	*MATa pho8::TEF1^{P}-OM-pho8Δ60::kanMX6 pho13::natNT2 npr2::NPR2-HA::hphNT1*	protrotrophic CEN.PK
YXW14	*MATa pho8::TEF1^{P}-OM-pho8Δ60::kanMX6 pho13::natNT2 npr3::NPR3-HA::hphNT1*	protrotrophic CEN.PK
YXW15	*MATa pho8::TEF1^{P}-pho8Δ60::kanMX6 pho13::natNT2*	protrotrophic CEN.PK
YXW16	*MATa pho8::TEF1^{P}-pho8Δ60::kanMX6 pho13::natNT2 atg5::hphNT1*	protrotrophic CEN.PK
YXW17	*MATa pho8::TEF1^{P}-pho8Δ60::kanMX6 pho13::hphNT1 atg11::natNT2*	protrotrophic CEN.PK
YXW18	*MATa pho8::TEF1^{P}-pho8Δ60::kanMX6 pho13::natNT2 iml1::hphNT1*	protrotrophic CEN.PK
YXW19	*MATa pho8::TEF1^{P}-pho8Δ60::kanMX6 pho13::natNT2 npr2::hphNT1*	protrotrophic CEN.PK
YXW20	*MATa pho8::TEF1^{P}-pho8Δ60::kanMX6 pho13::hphNT1 npr3::natNT2*	protrotrophic CEN.PK
YXW21	*MATa pho8::TEF1^{P}-pho8Δ60::kanMX6 pho13::hphNT1 iml1::IML1-GFP::natNT2*	protrotrophic CEN.PK
YXW22	*MATa pho8::TEF1^{P}-pho8Δ60::kanMX6 pho13::natNT2 npr2::NPR2-HA::hphNT1*	protrotrophic CEN.PK
YXW23	*MATa pho8::TEF1^{P}-pho8Δ60::kanMX6 pho13::natNT2 npr3::NPR3-HA::hphNT1*	protrotrophic CEN.PK
YXW24	*MATα idh1::IDH1-GFP::kanMX6*	protrotrophic CEN.PK
YXW25	*MATα iml1::IML1-FLAG::kanMX6 npr3::NPR3-HA::hphNT1*	protrotrophic CEN.PK
YXW26	*MATα iml1::IML1-FLAG::kanMX6 npr3::NPR3-HA::hphNT1 npr2::natNT2*	protrotrophic CEN.PK
YXW27	*MATα iml1::IML1-FLAG::natNT2 npr2::NPR2-HA::kanMX6*	protrotrophic CEN.PK

Table S1 Yeast Strains Used in This Study (Continued)

Name	Genotype	Strain Background
YXW28	*MATα iml1::IML1-FLAG::natNT2 npr2::NPR2-HA::kanMX6 npr3::hphNT1*	protrotrophic CEN.PK
YXW29	*MATα npr2::NPR2-FLAG::kanMX6 npr3::HA-NPR3::natNT2*	protrotrophic CEN.PK
YXW30	*MATα npr2::NPR2-FLAG::kanMX6 npr3::HA-NPR3::natNT2 iml1::hphNT1*	protrotrophic CEN.PK
YXW31	*MATα iml1::IML1-FLAG::kanMX6*	protrotrophic CEN.PK
YXW32	*MATα npr2::NPR2-FLAG::kanMX6*	protrotrophic CEN.PK
YXW33	*MATα npr3::NPR3-FLAG::kanMX6*	protrotrophic CEN.PK
YXW34	*MATα iml1::IML1-GFP::kanMX6*	protrotrophic CEN.PK
YXW35	*MATα iml1::IML1-GFP::kanMX6 vph1::VPH1-mCherry::natNT2*	protrotrophic CEN.PK
YXW36	*MATα iml1::IML1-GFP::kanMX6 ape1::APE1-mRFP1::hphNT1*	protrotrophic CEN.PK
YXW37	*MATα iml1::IML1-GFP::kanMX6 vph1::VPH1-mCherry::natNT2 npr2::hphNT1*	protrotrophic CEN.PK
YXW38	*MATα iml1::IML1-GFP::kanMX6 vph1::VPH1-mCherry::natNT2 npr3::hphNT1*	protrotrophic CEN.PK
YXW39	*MATα iml1::IML1-GFP::kanMX6 vph1::VPH1-mCherry::natNT2 atg8::hphNT1*	protrotrophic CEN.PK
YXW40	*MATα ho::ADH1^{P}-Mito-dsRed::kanMX4 leu2::hphNT1*	protrotrophic CEN.PK
YXW41	*MATα ho::ADH1^{P}-Mito-dsRed::kanMX4 leu2::hphNT1* [pRS41N-GFP-ATG8]	protrotrophic CEN.PK
YXW42	*MATa idh1::IDH1-GFP::kanMX6*	protrotrophic W303[1]
YXW43	*MATa idh1::IDH1-GFP::kanMX6 atg5::hphNT1*	protrotrophic W303
YXW44	*MATa idh1::IDH1-GFP::kanMX6 iml1::hphNT1*	protrotrophic W303
YXW45	*MATa idh1::IDH1-GFP::kanMX6 npr2::hphNT1*	protrotrophic W303
YXW46	*MATa idh1::IDH1-GFP::kanMX6 npr3::hphNT1*	protrotrophic W303
YXW47	*MATa pho8::TEF1^{P}-pho8Δ60::kanMX6 pho13::natNT2*	protrotrophic W303
YXW48	*MATα idh1::IDH1-GFP::kanMX6*	protrotrophic S288C[2]
YXW49	*MATα pho8::TEF1^{P}-pho8Δ60::kanMX6 pho13::natNT2*	protrotrophic S288C
YXW50	*MATa pho8::TEF1^{P}-pho8Δ60::kanMX6 pho13::natNT2 atg5::hphNT1*	protrotrophic W303
YXW51	*MATa pho8::TEF1^{P}-pho8Δ60::kanMX6 pho13::natNT2 iml1::hphNT1*	protrotrophic W303
YXW52	*MATa pho8::TEF1^{P}-pho8Δ60::kanMX6 pho13::natNT2 npr2::hphNT1*	protrotrophic W303
YXW53	*MATa pho8::TEF1^{P}-pho8Δ60::kanMX6 pho13::natNT2 npr3::hphNT1*	protrotrophic W303
YXW54	*MATα idh1::IDH1-GFP::natNT2 iml1::hphNT1 [p417-CYC1^{P}]*	protrotrophic CEN.PK

Table S1 Yeast Strains Used in This Study (Continued)

Name	Genotype	Strain Background
YXW55	*MATα idh1::IDH1-GFP::natNT2 iml1::hphNT1 [p417-CYC1^{P}-IML1-FLAG]*	protrotrophic CEN.PK
YXW56	*MATα idh1::IDH1-GFP::natNT2 iml1::hphNT1 [p417-CYC1^{P}-iml1Δdep-FLAG]*	protrotrophic CEN.PK
YXW57	*MATα idh1::IDH1-GFP::natNT2 iml1::hphNT1 [p417-CYC1^{P}-iml1Δduf-FLAG]*	protrotrophic CEN.PK
YXW58	*MATα pho8::TEF1^{P}-pho8Δ60::hphNT1 pho13::natNT2 iml1::hphNT1 [p417-CYC1^{P}]*	protrotrophic CEN.PK
YXW59	*MATα pho8::TEF1^{P}-pho8Δ60::hphNT1 pho13::natNT2 iml1::hphNT1 [p417-CYC1^{P}-IML1-FLAG]*	protrotrophic CEN.PK
YXW60	*MATα pho8::TEF1^{P}-pho8Δ60::hphNT1 pho13::natNT2 iml1::hphNT1 [p417-CYC1^{P}-iml1Δdep-FLAG]*	protrotrophic CEN.PK
YXW61	*MATα pho8::TEF1^{P}-pho8Δ60::hphNT1 pho13::natNT2 iml1::hphNT1 [p417-CYC1^{P}-iml1Δduf-FLAG]*	protrotrophic CEN.PK
YXW62	*MATα npr2::NPR2-HA::natNT2 iml1::hphNT1 [p417-CYC1^{P}-IML1-FLAG]*	protrotrophic CEN.PK
YXW63	*MATα npr2::NPR2-HA::natNT2 iml1::hphNT1 [p417-CYC1^{P}-iml1Δdep-FLAG]*	protrotrophic CEN.PK
YXW64	*MATα npr2::NPR2-HA::natNT2 iml1::hphNT1 [p417-CYC1^{P}-iml1Δduf-FLAG]*	protrotrophic CEN.PK
YXW65	*MATα npr3::NPR3-HA::natNT2 iml1::hphNT1 [p417-CYC1^{P}-IML1-FLAG]*	protrotrophic CEN.PK
YXW66	*MATα npr3::NPR3-HA::natNT2 iml1::hphNT1 [p417-CYC1^{P}-iml1Δdep-FLAG]*	protrotrophic CEN.PK
YXW67	*MATα npr3::NPR3-HA::natNT2 iml1::hphNT1 [p417-CYC1^{P}-iml1Δduf-FLAG]*	protrotrophic CEN.PK
YXW68	*MATa ho::ADH1^{P}-Mito-dsRed::kanMX4 vph1::VPH1-GFP::kanMX6 npr3::HA-NPR3::natNT2*	protrotrophic CEN.PK
YXW69	*MATa ho::ADH1^{P}-Mito-dsRed::kanMX4 vph1::VPH1-GFP::kanMX6 atg1::hphNT1*	protrotrophic CEN.PK
YXW70	*MATa ho::ADH1^{P}-Mito-dsRed::kanMX4 vph1::VPH1-GFP::kanMX6 atg13::hphNT1*	protrotrophic CEN.PK
YXW71	*MATa ho::ADH1^{P}-Mito-dsRed::kanMX4 vph1::VPH1-GFP::kanMX6 atg8::hphNT1*	protrotrophic CEN.PK
YXW72	*MATa ho::ADH1^{P}-Mito-dsRed::kanMX4 vph1::VPH1-GFP::kanMX6 atg9::hphNT1*	protrotrophic CEN.PK
YXW73	*MATα atg13::HA-ATG13::natNT2*	protrotrophic CEN.PK
YXW74	*MATα atg13::HA-ATG13::natNT2 iml1::hphNT1*	protrotrophic CEN.PK
YXW75	*MATα atg13::HA-ATG13::natNT2 npr2::hphNT1*	protrotrophic CEN.PK
YXW76	*MATα atg13::HA-ATG13::natNT2 npr3::hphNT1*	protrotrophic CEN.PK
YXW77	*MATα atg1::hphNT1*	protrotrophic CEN.PK

Table S1 Yeast Strains Used in This Study (Continued)

Name	Genotype	Strain Background
YXW78	*MATα atg6::hphNT1*	protrotrophic CEN.PK
YXW79	*MATα atg7::hphNT1*	protrotrophic CEN.PK
YXW80	*MATα atg8::hphNT1*	protrotrophic CEN.PK
YXW81	*MATα atg9::hphNT1*	protrotrophic CEN.PK
YXW82	*MATα atg10::hphNT1*	protrotrophic CEN.PK
YXW83	*MATα atg13::hphNT1*	protrotrophic CEN.PK
YXW84	*MATα atg5::kanMX6*	protrotrophic CEN.PK
YXW85	*MATα iml1::hphNT1*	protrotrophic CEN.PK
YXW86	*MATα npr2::kanMX6*	protrotrophic CEN.PK
YXW87	*MATα npr3::kanMX6*	protrotrophic CEN.PK
YXW88	*MATα pep4::kanMX6 prb1::hphNT1*	protrotrophic CEN.PK
YXW89	*MATα pep4::natNT2 prb1::hphNT1 npr2::kanMX6*	protrotrophic CEN.PK
YXW90	*MATα pep4::natNT2 prb1::hphNT1 atg5::kanMX6*	protrotrophic CEN.PK
YXW91	*MATα pep4::natNT2 prb1::kanMX6 iml1::hphNT1*	protrotrophic CEN.PK
YXW92	*MATα pep4::kanMX6 prb1::hphNT1 npr3::natNT2*	protrotrophic CEN.PK
YXW93	*MATα pex14::PEX14-GFP::kanMX6*	protrotrophic CEN.PK
YXW94	*MATα [p417-GFP-ATG8]*	protrotrophic CEN.PK
YXW95	*MATα iml1::hphNT1 [p417-GFP-ATG8]*	protrotrophic CEN.PK
YXW96	*MATα npr2::kanMX6 [pRS41H-GFP-ATG8]*	protrotrophic CEN.PK
YXW97	*MATα npr3::kanMX6 [pRS41H-GFP-ATG8]*	protrotrophic CEN.PK
YXW98	*MATα atg32::kanMX6*	protrotrophic CEN.PK

[1]The prototrophic version of W303 is a kind gift from Andrew Murray.

[2]The prototrophic version of S288C was purchased from ATCC (Catalog No. 204508).