Fig. S1

Figure S1. *LSM1* deletion lowers autophagy activity after nitrogen starvation by decreasing *ATG* mRNA and protein levels. Related to Figure 1.

(**A**) Autophagy activity was measured by the Pho8Δ60 assay in WT, *lsm1Δ* and *lsm1Δ pat1Δ* strains under growing conditions (+N) and following 3 h of nitrogen starvation (−N). Error bars indicate the standard deviation of 4 independent experiments. ANOVA, ***P <0.001. ns, no statistical significance.

(**B**) Autophagy was measured by GFP-Atg8 processing in WT and *lsm1Δ* strains under growing conditions and after 1 and 2 h of nitrogen starvation. Atg1 and Atg9 protein levels were also measured; representative images are shown.

(**C**) *ATG1*, *ATG2*, *ATG7* and *ATG9* mRNA levels were determined by RT-qPCR in WT and *lsm1Δ* strains under growing conditions and following 1 h of nitrogen starvation.

(**D**) Pat1 levels were measured by western blot in a Pat1 auxin-inducible degron (AID) strain in nutrient-rich conditions in the presence of DMSO (vehicle) or 300 µM auxin; the upper left panel shows the loss of Pat1-AID-MYC at the 1-h time point. *ATG1*, *ATG2* and *ATG7* mRNA levels were quantified by RT-qPCR in Pat1-AID-MYC strains incubated with auxin and/or the synthetic transcriptional inhibitor 1,10 phenanthroline [200 µg/ml], following 1 h of nitrogen starvation. Error bars indicate the standard deviation of 5 independent experiments. Student's t-test, ANOVA, * P <0.05, ** P <0.01, *** P <0.001.

A

B

C
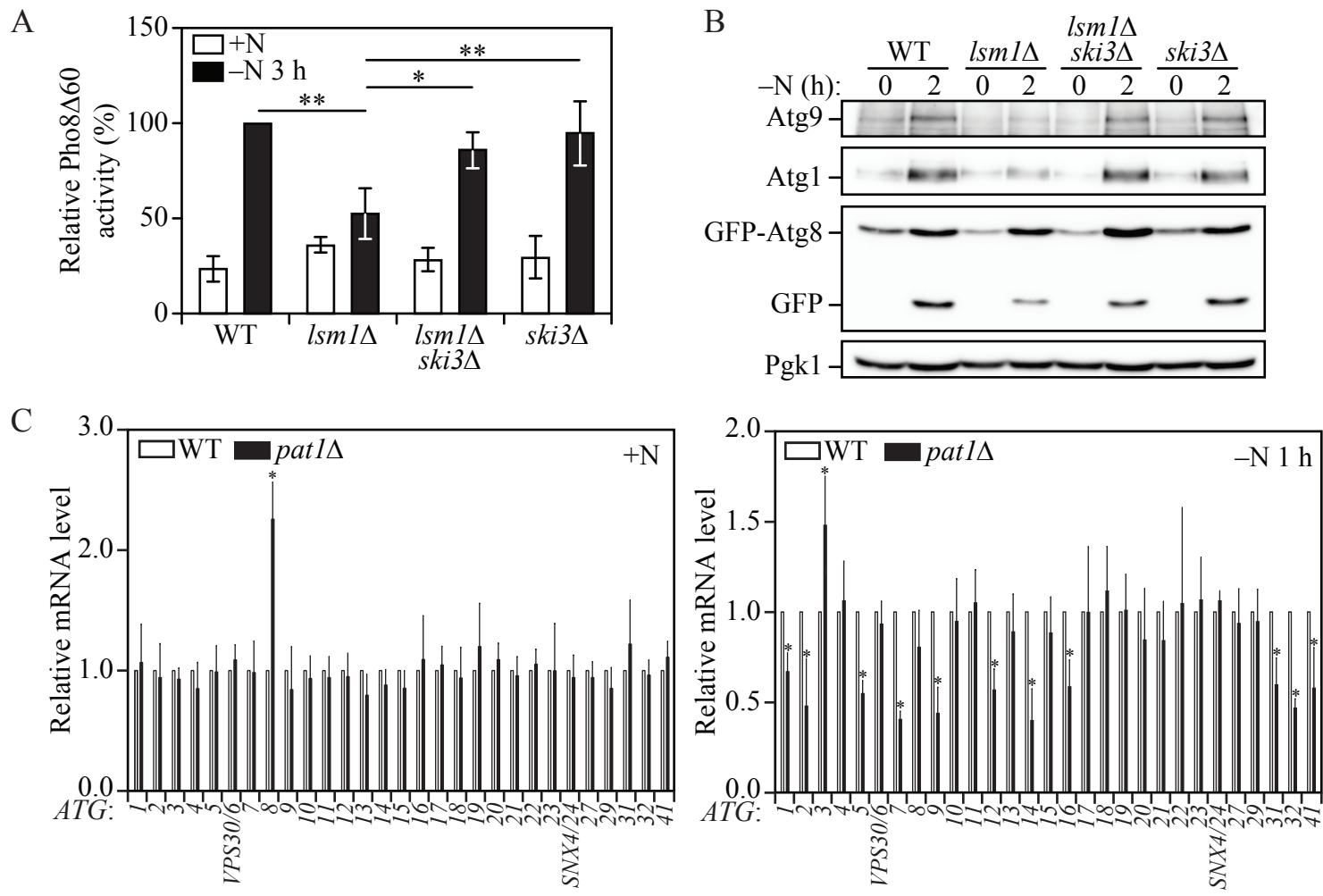
Figure S2. *SKI3* deletion prevents the *lsm1Δ*-mediated decrease in autophagy. Related to Figure 2.

(**A**) Autophagy activity was measured by the Pho8Δ60 assay in WT, *lsm1Δ*, *ski3Δ* and *lsm1Δ ski3Δ* strains under growing conditions (+N) and following 3 h of nitrogen starvation (−N). Error bars indicate the standard deviation of 3 independent experiments. ANOVA, *P <0.05 and ** P <0.01.

(**B**) Autophagy was measured by GFP-Atg8 processing in WT, *lsm1Δ*, *ski3Δ* and *lsm1Δ ski3Δ* strains under growing conditions and following 2 h of nitrogen starvation. Atg1 and Atg9 protein levels were also measured; representative images are shown.

(**C**) mRNA levels were determined by RT-qPCR in WT and *pat1Δ* cells for the indicated *ATG* genes under growing conditions and after 1 h of nitrogen starvation. Error bars indicate the standard deviation of 3 independent experiments. Student's t-test, * P<0.05.

A

Pat1-PA: WT   AA

p-Pat1-PA—

Pat1-PA—

-N (min):   0   30   60   0   30   60

B

Pat1-PA:p-Pat1-PA

**

+N   −N
60 min
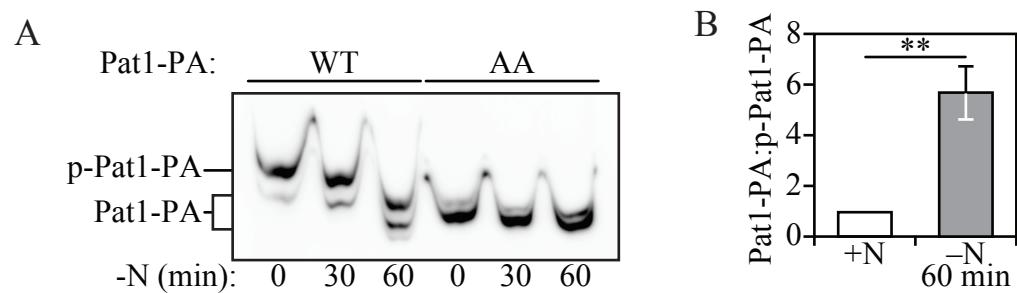
**Figure S3. The Pat1 protein is dephosphorylated under nitrogen-starvation conditions. Related to Figure 3.**

(**A**) Pat1 phosphorylation (p-) levels were determined in Pat1-PA strains by migration shift in a Phos-tag SDS-PAGE gel under growing conditions (0 min) and following , 30 and 60 min of nitrogen starvation. The Pat1-PA AA strain was used as a positive control. Representative images are shown.

(**B**) Quantification of the ratio between dephosphorylated and phosphorylated Pat1-PA under growing conditions and after 60 min of nitrogen starvation. Error bars indicate the standard deviation of 3 independent experiments. Student's t-test, **P<0.01.

A

+N
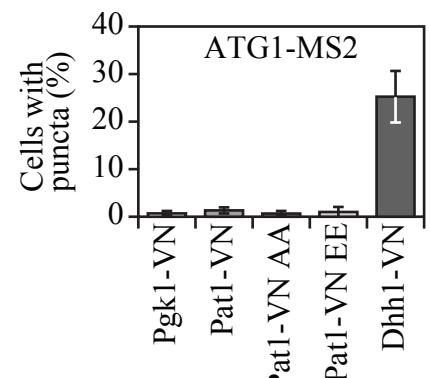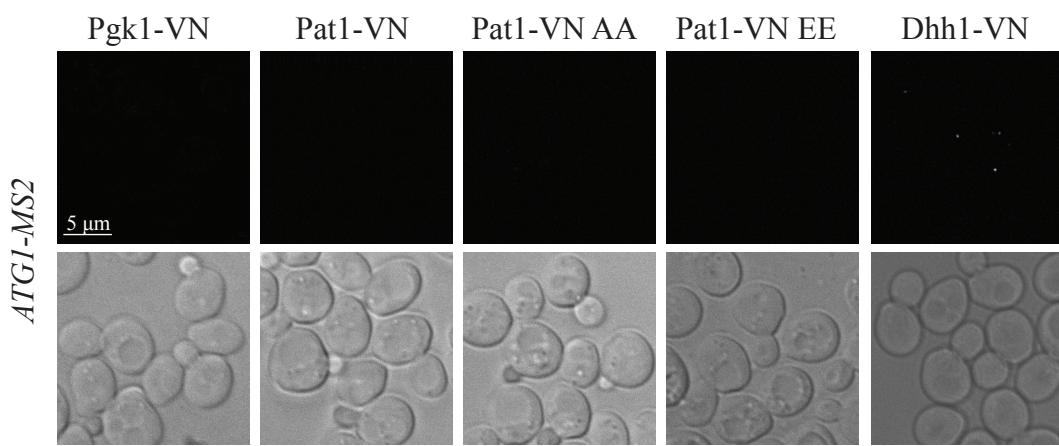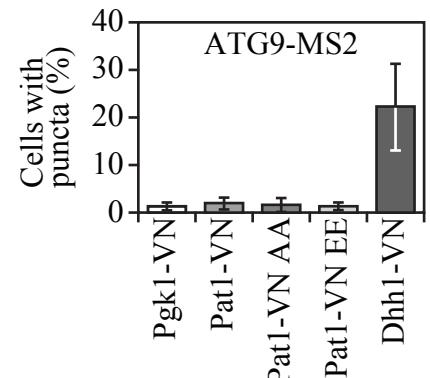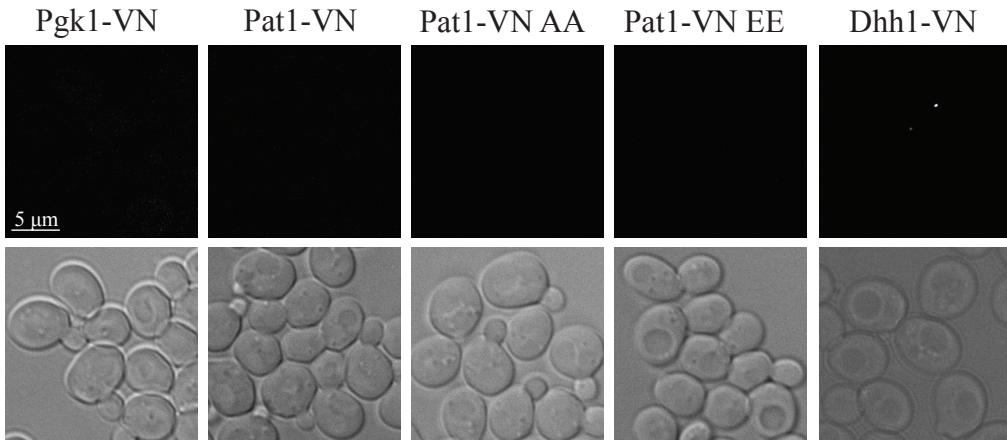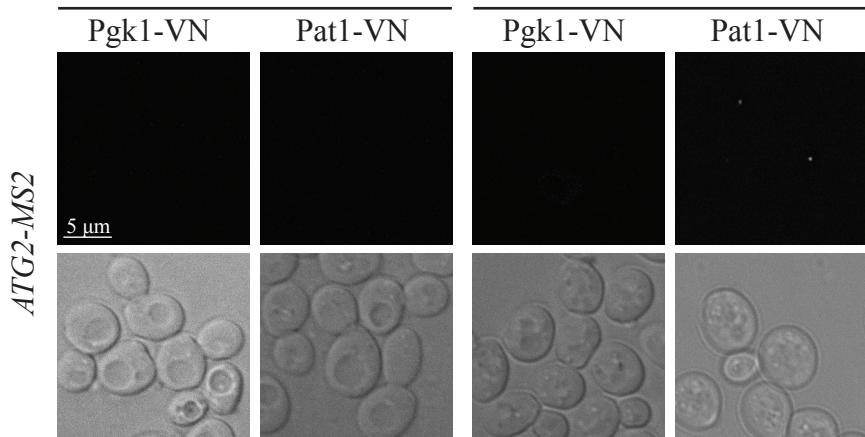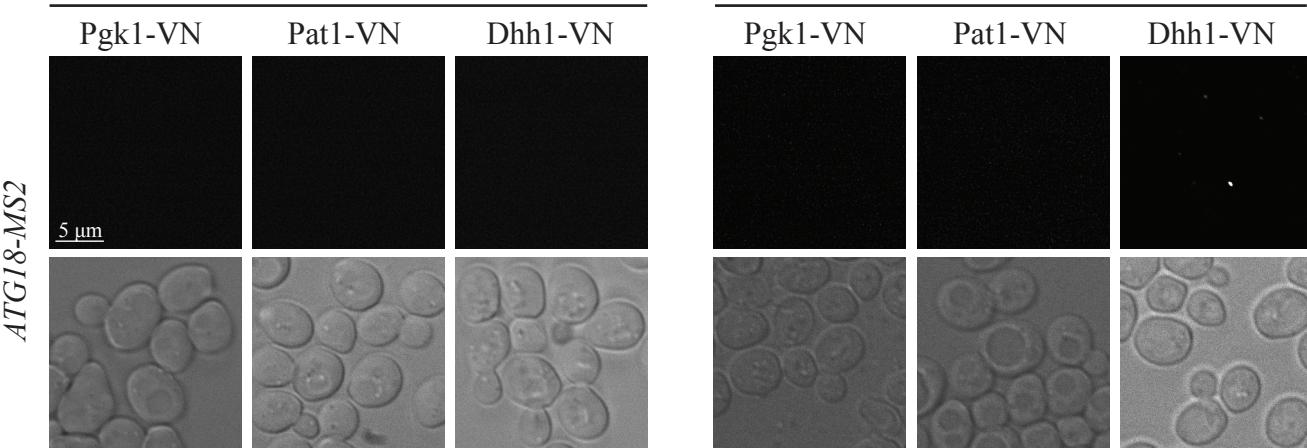
Figure S4. Protein-RNA BiFC of *ATG2*-MS2, *ATG17*-MS2 and *ATG18*-MS2. Related to Figure 4.

(**A**) Protein-RNA BiFC was used to determine the interaction of Pgk1-VN, Pat1-VN, Pat1-VN AA, Pat1-VN EE and Dhh1-VN with *ATG1-MS2*- and *ATG9-MS2*-tagged mRNA during nutrient-replete conditions (+N). Quantification of the percent of cells with puncta is shown on the right.

(**B**) Protein-RNA BiFC was used to determine the interaction of Pgk1-VN and Pat1-VN with *ATG2-MS2*-tagged mRNA during nutrient-replete conditions and following 2 h of nitrogen starvation (−N).

(**C**) Protein-RNA BiFC was used to determine the interaction of Pgk1-VN, Pat1-VN and Dhh1-VN with *ATG18-MS2*-tagged mRNA during nutrient-replete conditions and following 2 h of nitrogen starvation.

Nutrients

Decapping

Dcp1 Dcp2

P

p

m7Gpp

Xrn1

5'→3' degradation

Pat1-Lsm

P

P

ATG mRNA
decapping and degradation

AAAAAAA

Autophagy
inhibited
(basal)

Starvation

m7Gppp

Pat1-Lsm

AAAAAAA

ATG mRNA
accumulation and translation

Autophagy
induced

Xrn1

5'→3' degradation

Exosome
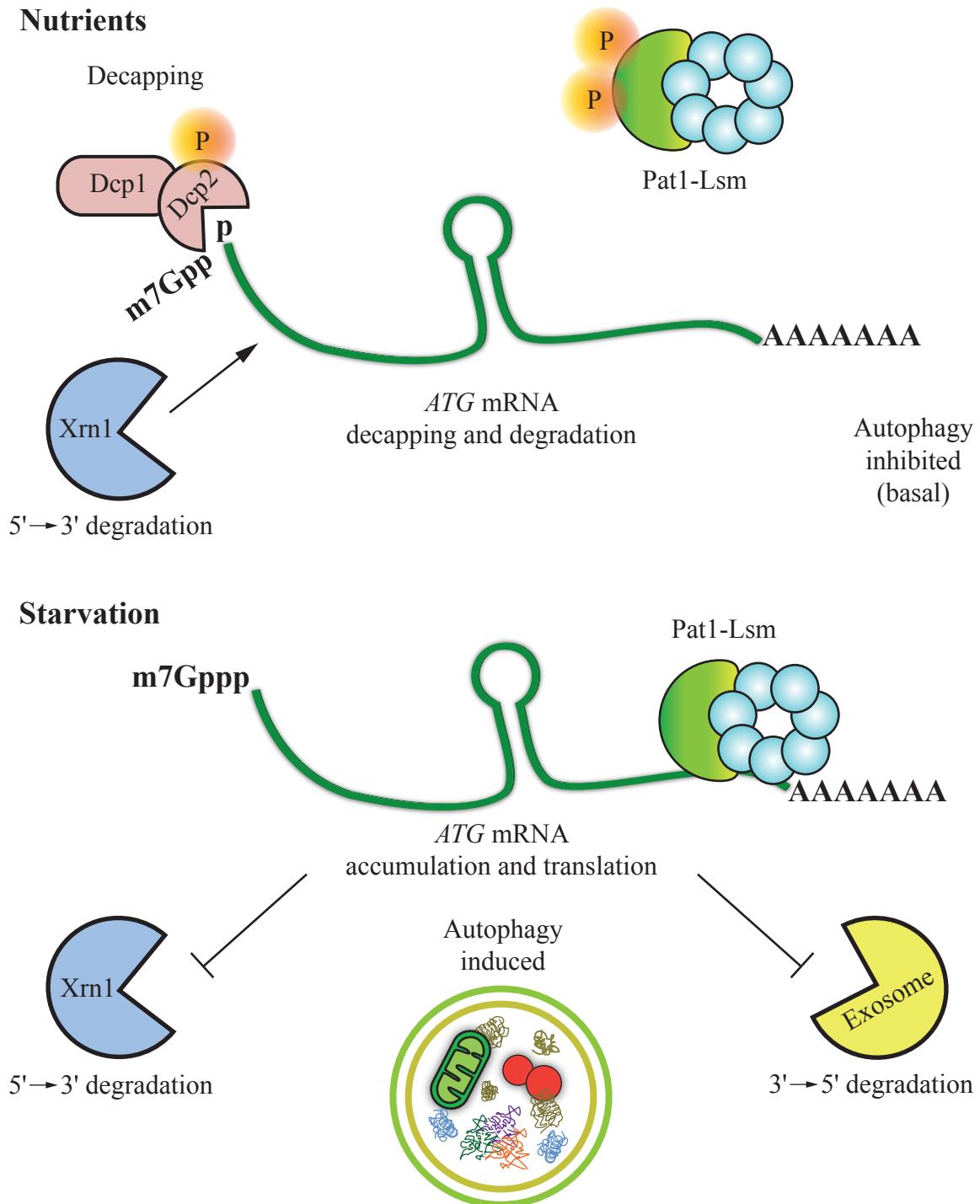
3'→5' degradation

Figire S5. Pat1 stabilizes a subset of *ATG* mRNAs during nitrogen starvation-induced autophagy by preventing 3'-5' mRNA degradation. Related to Figures 1-7.

Under normal growth conditions Dcp2 is phosphorylated, leading to *ATG* transcript decapping and subsequent 5' to 3' degradation by Xrn1 (Hu et al., 2015; Delorme-Axford et al., 2018). This process occurs in parallel with *ATG* gene transcription inhibition by inactivation of several transcription factors (not shown). Pat1 is phosphorylated on residues Ser456 and Ser457. Nutrient deficiency leads to Dcp2 and Pat1 dephosphorylation. The former prevents decapping and Xrn1-mediated 5' to 3' degradation of *ATG* mRNAs, whereas the latter leads to Pat1-Lsm binding to *ATG* transcripts and prevents their exosome-mediated 3' to 5' degradation. This process, in concert with the transcriptional activation of *ATG* mRNA synthesis, facilitates *ATG* transcript accumulation and autophagy induction.

Table S1. *S. cerevisiae* strains used in this study. Related to STAR methods.

Name	Genotype
WLY176	SEY6210 *pho13Δ pho8*::*pho8Δ*60
DGY001	WLY176 *pat1Δ*::*LEU2*
DGY002	DGY001 *ski2Δ*::*HIS3*
DGY003	DGY001 *ski3Δ*::*HIS3*
DGY004	WLY176 *ski2Δ*::*HIS3*
DGY005	WLY176 *ski3Δ*::*HIS3*
JMY347	WLY176 p*ZEO1-pho8Δ*60 pCu-GFP-*ATG8*::*LEU2*
DGY006	WLY176 p*ZEO1-pho8Δ*60 pCu-GFP-*ATG8*::*LEU2 pat1*::*URA3*
DGY007	DGY006 *ski3Δ*::*HIS3*
DGY008	JMY347 *ski3Δ*::*HIS3*
JMY316	WLY176 *ATG2-PA ATG7*-PA
DGY009	JMY316 *pat1Δ*::*URA3*
DGY010	DGY009 *ski3Δ*::*HIS3*
DGY011	JMY316 *ski3Δ*::*HIS3*
DGY012	JMY316 *PAT1-HA*::*HIS3*
DGY013	JMY316 *PAT1-HA* S456E S457E::*HIS3*
DGY014	JMY316 *PAT1-HA* S456A S457A::*HIS3*
DGY015	DGY012 *ski3Δ*::*URA3*
DGY016	DGY013 *ski3Δ*::*URA3*
DGY017	WLY176 *PAT1-PA*::*TRP1*
DGY018	WLY176 *PAT1-PA* S456E S457E::*TRP1*
DGY019	WLY176 *PAT1-PA* S456A S457A::*TRP1*
DGY020	DGY018 *ski2Δ*::*HIS3*
DGY021	DGY018 *ski3Δ*::*HIS3*
DGY022	JMY347 *PAT1-PA*::*TRP1*
DGY023	JMY347 *PAT1-PA* S456E S457E::*TRP1*
DGY024	JMY347 *PAT1-PA* S456A S457A::*TRP1*
DGY025	DGY022 *ski3Δ*::*LEU2*
DGY026	DGY023 *ski3Δ*::*LEU2*
DGY027	WLY176 *PAT1-HA*::*HIS3*
DGY028	WLY176 *PAT1-HA* S456E S457E::*HIS3*
DGY029	WLY176 *PAT1-HA* S456A S457A::*HIS3*
DGY030	DGY027 *ski3Δ*::*URA3*
DGY031	DGY028 *ski3Δ*::*URA3*
DGY032	SEY6210 *ATG1-MS2 pCu-MCP-VC*::*HIS3 PGK1-VN*::*KAN*
DGY033	SEY6210 *ATG1-MS2 pCu-MCP-VC*::*HIS3 PAT1-VN*::*KAN*
DGY034	SEY6210 *ATG1-MS2 pCu-MCP-VC*::*HIS3 PAT1-VN* S456E S457E::*KAN*
DGY035	SEY6210 *ATG1-MS2 pCu-MCP-VC*::*HIS3 PAT1-VN* S456A S457A::*KAN*
DGY036	SEY6210 *ATG1-MS2 pCu-MCP-VC*::*HIS3 DHH1-VN*::*KAN*
DGY037	SEY6210 *ATG9-MS2 pCu-MCP-VC*::*HIS3 PGK1-VN*::*KAN*
DGY038	SEY6210 *ATG9-MS2 pCu-MCP-VC*::*HIS3 PAT1-VN*::*KAN*
DGY039	SEY6210 *ATG9-MS2 pCu-MCP-VC*::*HIS3 PAT1-VN* S456E S457E::*KAN*

DGY040   SEY6210 *ATG9-MS2 pCu-MCP-VC::HIS3 PAT1-VN* S456A S457A*::KAN*
DGY041   SEY6210 *ATG9-MS2 pCu-MCP-VC::HIS3 DHH1-VN::KAN*
DGY042   SEY6210 *ATG2-MS2 pCu-MCP-VC::HIS3 PGK1-VN::KAN*
DGY043   SEY6210 *ATG2-MS2 pCu-MCP-VC::HIS3 PAT1-VN::KAN*
DGY044   SEY6210 *ATG18-MS2 pCu-MCP-VC::HIS3 DHH1-VN::KAN*
DGY045   SEY6210 *ATG18-MS2 pCu-MCP-VC::HIS3 PGK1-VN::KAN*
DGY046   SEY6210 *ATG18-MS2 pCu-MCP-VC::HIS3 PAT1-VN::KAN*
DGY047   SEY6210 pNHK53::URA3 *PAT1*-AID-MYC::HIS3

Table S2. Primers used in this study. Related to STAR methods.

Primer Name	Sequence
PAT1 deletion For	AGCAAAGGTTTTAACCGGAAGTAAGAGCAGCAAGAAGCACTAGCACAGCTGAAGCTTCGTACGC
PAT1 deletion Rev	AAAAAAAAATACATGCGTAAGTACATTAAAATTACAGGAAAAATCGCATAGGCCACTAGTGGATCTG
LSM1 deletion For	TAAAAGAAAGCAGCCCTCGAATCGAATTAATTCACCAAAACAGCTGAAGCTTCGTACGC
LSM1 deletion Rev	TACTCCAGGATATATGTTGGTAGTATTGTGTTTTTCTTTCGCATAGGCCACTAGTGGATCTG
PAT1 C terminal TAG For	GGGTTGGTGTATCGCGATGGTGAAATATCAGAACTAAAGCGGATCCCCGGGTTAATTAA
PAT1 C terminal TAG Rev	AAAATACATGCGTAAGTACATTAAAATTACAGGAAAAATCGAATTCGAGCTCGTTTAAAC
PAT1-S456E S457E For	GCCGCTGCTGTTGCTTCTAAGCAAAGAAGAAGAGAAGAGTACGCGTTCAACAACGGTAAT
PAT1-S456A S457A For	GCCGCTGCTGTTGCTTCTAAGCAAAGAAGAAGAGCTGCATACGCGTTCAACAACGGTAAT
PAT1-AID-Myc TAG For	GGGGTTGGTGTATCGCGATGGTGAAATATCAGAACTAAAGCTTCGTACGCTGCAGGTCGA
PAT1-AID-Myc TAG Rev	AAAATACATGCGTAAGTACATTAAAATTACAGGAAAAATCCATCGATGAATTCGAGCTCG
SKI2 deletion For	AACCTAACTCACAAAATTTACTGTACTAATACTAATTTATCAGCTGAAGCTTCGTACGC
SKI2 deletion Rev	TTTATAAACATGACTCACATTGAGAATAAATGAGCTCTGCATAGGCCACTAGTGGATCTG
SKI3 deletion For	ACTAAGAACACAGAAAAGAAACACGAAGAGCAGAGGAAATCAGCTGAAGCTTCGTACGC
SKI3 deletion Rev	TACATTAAGGTTTGATTGACTATCTCGAATCCAAATTTGCATAGGCCACTAGTGGATCTG
ATG17 Template-FastCloning For	CCTTTTTATTGGGGTTCTTGTGTGATCTGAGATGCAAAAGCG
ATG17 Template-FastCloning Rev	ACCGTATCCTTTTTTTCCTTTTTTTTAATTTTGGTGGTTCATCTTCTG
ATG18 Template-FastCloning For	GGCAGCTCTCTTAGCAAAATAATGATCTGAGATGCAAAAGCG
ATG18 Template-FastCloning Rev	GCGAGACACTTCCGTGATTAATTTTGGTGGTTCATCTTCTG
ATG7 Template-FastCloning For	ACATTAATTTGGCATTCATATCTAAATGATCTGAGATGCAAAAGCG
ATG7 Template-FastCloning Rev	TGTACCAATGCTATTATATGCAAAATATTAATTTTGGTGGTTCATCTTCTG
ATG17 Insert-FastCloning For	CAGAAGATGAACCACCAAAATTAAAAAAAAGGAAAAAAAGGATACGGT
ATG17 Insert-FastCloning Rev	CGCTTTTGCATCTCAGATCACACAAGAACCCCAATAAAAAGG
ATG18 Insert-FastCloning For	CAGAAGATGAACCACCAAAATTAATCACGGAAGTGTCTCGC
ATG18 Insert-FastCloning Rev	CGCTTTTGCATCTCAGATCATTATTTTGCTAAGAGAGCTGCC
ATG7 Insert-FastCloning For	CAGAAGATGAACCACCAAAATTAATATTTTGCATATAATAGCATTGGTACA
ATG7 Insert-FastCloning Rev	CGCTTTTGCATCTCAGATCATTTAGATATGAATGCCAAATTAATGT
ATG1 deletion For	CAGGTTGAAAATATTGAGGCAGAAGATGAACCACCAAAATCAGCTGAAGCTTCGTACGC
ATG1 deletion Rev	GGTCATTTGTACTTAATAAGAAAACCATATTATGCATCACGCATAGGCCACTAGTGGATCTG
ATG1 MS2-TAG For	GTTGAAAATATTGAGGCAGAAGATGAACCACCAAAATTAACCGCTCTAGAACTAGTGGAT
ATG1 MS2-TAG Rev	GGTCATTTGTACTTAATAAGAAAACCATATTATGCATCACGCATAGGCCACTAGTGGATC
ATG2 MS2-TAG For	AATCAATGATAAGTACAAGTCCAATCGGACTGATTCGTAACCGCTCTAGAACTAGTGGAT
ATG2 MS2-TAG Rev	ATATGAATTGAATATATATCAAAAATGTCTGCAAAAATTTGCATAGGCCACTAGTGGATC
ATG9 MS2-TAG For	TGTTAAAGAGTATTACAAGAAGTCTGACGTCGGAAGATAACCGCTCTAGAACTAGTGGAT
ATG9 MS2-TAG Rev	TATATAGTTATATTGGATGATGTACACGACACAGTCTGCCGCATAGGCCACTAGTGGATC
ATG18 MS2-TAG For	TTGCTTAATATTGTCACAGTATTCCATCTTGATGGATTGACCGCTCTAGAACTAGTGGAT
ATG18 MS2-TAG Rev	CGTTGTGACGTACGGAAGGCAGCGCGAGACACTTCCGTGAGCATAGGCCACTAGTGGATC
MCP vYFP-C TAG For	GGTCTCCTAAAAGATGGAAACCCGATTCCCTCAGCAATCGCAGCAAACTCCGGCATCTACCCAGCTGAAGCTTCGTACGCT
MCP vYFP-C TAG Rev	ACCATGATTACGCCAAGCGCGCAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCCGAATTCGAGCTCGTTTAAAC
DHH1 vYFP-N TAG For	TATCCTCCACAGCAGGAACATTTCATGGCGATGCCACCTGGTCAGTCACAACCCCAGTATCGGATCCCCGGGTTAATTAA
DHH1 vYFP-N TAG Rev	ATTCTTGTTCAAAATCAATAGTAAAAGTATGGTTACAAAGTAATGTAATTCACAATGGAGATTTGGAATTCGAGCTCGTTTAAAC

PGK1 vYFP-N TAG For	GGAATTATTGGAAGGTAAGGAATTGCCAGGTGTTGCTTTCTTATCCGAAAAGAAACGGATCCCCGGGTTAATTAA
PGK1 vYFP-N TAG Rev	GGATGGGGAAAGAGAAAAGAAAAAAATTGATCTATCGATTTCAATTCAATTCAATGAATTCGAGCTCGTTTAAAC
PAT1 vYFP-N TAG For	CAGGTTGAAAATATTGAGGCAGAAGATGAACCACCAAAATCGGATCCCCGGGTTAATTAA
PAT1 vYFP-N TAG Rev	GGTCATTTGTACTTAATAAGAAAACCATATTATGCATCACGAATTCGAGCTCGTTTAAAC
PATL1-S-KPN1	CGGCGGGGTACCATGTTCCGCTACGAGTCTTTG
PATL1-A-NOT1	CGGCGGGCGGCCGCTTATCGTATCCCCTGAACTAGC
PATL1-EE1	TCTAACAGGAGGACTGCCAATGATAGGATCAGTATCCCGCCTTGGTAATGCTCGTTCAGAAAG
PATL1-EE2	CTTTCTGAACGAGCATTACCAAGGCGGGATACTGATCCTATCATTGGCAGTCCTCCTGTTAGA
PATL1-AA1	CTGCCAATGATAGGTGCAGTTGCCCGCCTTGGTAATGCT
PATL1-AA2	AGCATTACCAAGGCGGGCAACTGCACCTATCATTGGCAG