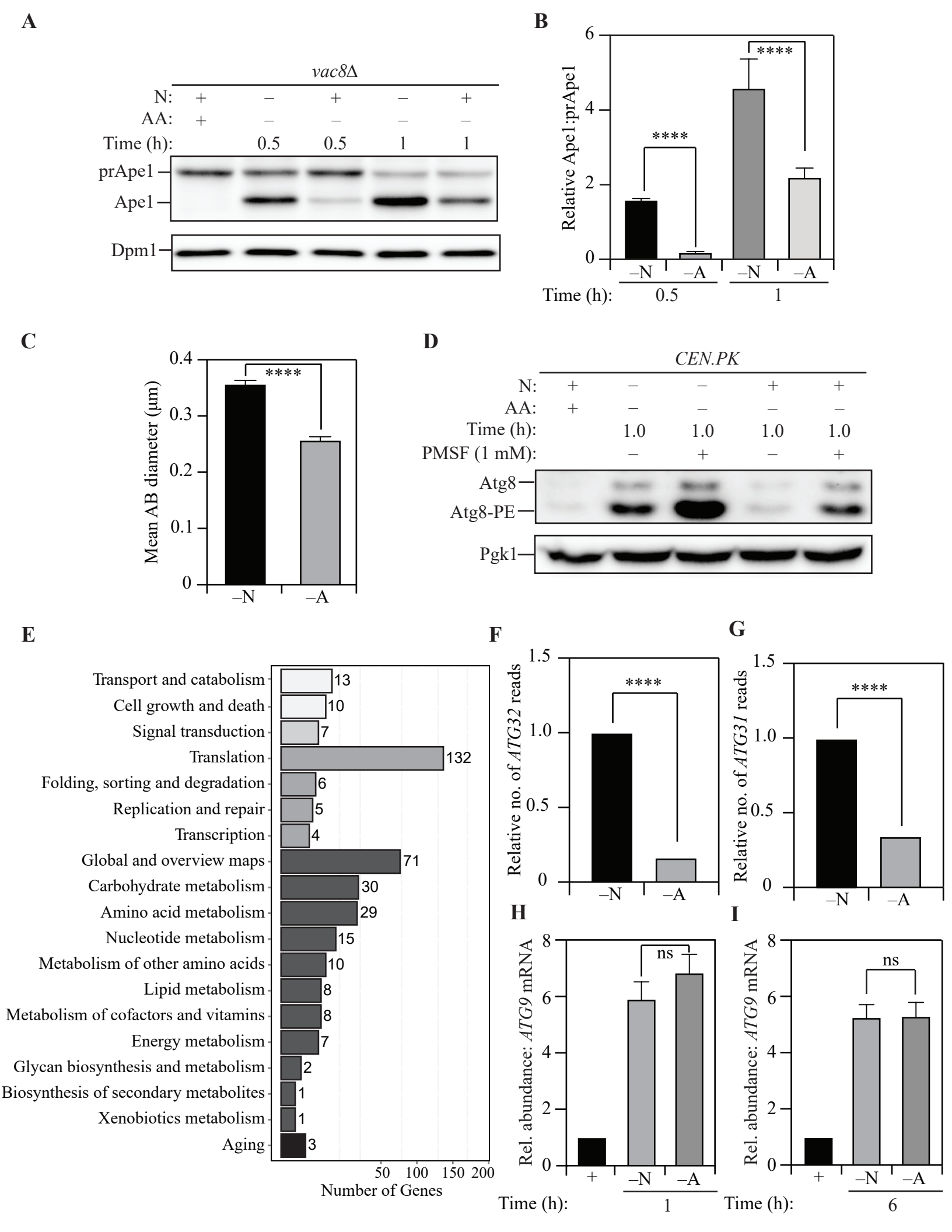
A
vac8Δ
N:
AA:
Time (h):
prApe1
Ape1
Dpm1
B
Relative Ape1:prApe1
****
****
–N
–A
Time (h):
C
Mean AB diameter (μm)
****
–N
–A
D
CEN.PK
N:
AA:
Time (h):
PMSF (1 mM):
Atg8
Atg8-PE
Pgk1
E
Transport and catabolism
Cell growth and death
Signal transduction
Translation
Folding, sorting and degradation
Replication and repair
Transcription
Global and overview maps
Carbohydrate metabolism
Amino acid metabolism
Nucleotide metabolism
Metabolism of other amino acids
Lipid metabolism
Metabolism of cofactors and vitamins
Energy metabolism
Glycan biosynthesis and metabolism
Biosynthesis of secondary metabolites
Xenobiotics metabolism
Aging
Number of Genes
F
Relative no. of ATG32 reads
****
G
Relative no. of ATG31 reads
****
H
Rel. abundance: ATG9 mRNA
ns
Time (h):
I
Rel. abundance: ATG9 mRNA
ns
Time (h):

Figure S1. Differential autophagy flux during distinct nutrient stresses is not determined by *ATG* transcription. (**A**) Higher autophagy flux during nitrogen starvation compared to amino acid starvation, demonstrated by the precursor Ape1 (prApe1) maturation assay: SEY6210 *vac8*Δ cells were harvested in nutrient-replete conditions or after starvation for the indicated times. Conversion of prApe1 to Ape1 indicates autophagy flux. Dpm1 was used as a loading control (**B**) Densitometric analysis of (A) from three independent biological replicates. (**C**) The size of autophagosomes is larger during nitrogen starvation relative to amino acid starvation: Quantification of the diameter of autophagic bodies from SEY6210 *pep4*Δ *vps4*Δ cells starved for nitrogen or amino acids. Data from 100 cell profiles per condition across two independent biological replicates. (**D**) Elevated Atg8-lipidation in nitrogen starvation relative to amino acid starvation in the absence and presence of the serine protease inhibitor PMSF: CEN.PK cells were harvested in nutrient-replete conditions or after starvation for the indicated times. Pgk1 was used as a loading control. (**E**) Identified DEGs were grouped according to cellular function. (**F and G**) Abundance of *ATG32* and *ATG31* transcripts is significantly lower in amino acid starvation relative to nitrogen starvation as determined by RNA-Sequencing. Data represent the mean of three independent biological replicates. (**H and I**) Transcriptional upregulation of *ATG9* is similar during nitrogen and amino acid starvation: qRT-PCR-based detection of *ATG9* mRNA in WT (SEY6210) cells after 1 h (H) or 6 h (I) of starvation. *ALG9* was used as a reference gene for normalization. Data are from three independent biological replicates. Data in (B), (C), (H) and (I) represent mean ± SEM from indicated number of replicates. Statistical analysis for (B) was carried out using one-way analysis of variance (ANOVA) while (C), (H) and (I) were analyzed using an unpaired Student's t-test. Data in (F) and (G) represent mean from indicated number of replicates. Statistical analysis for (F) and (G) was carried out using one-way analysis of variance (ANOVA). Multiple comparisons were carried out using Tukey's multiple comparisons test. *p < 0.05, **p < 0.005, ***p < 0.001, ****p < 0.0001 ns: not significant. Related to Figure 1.

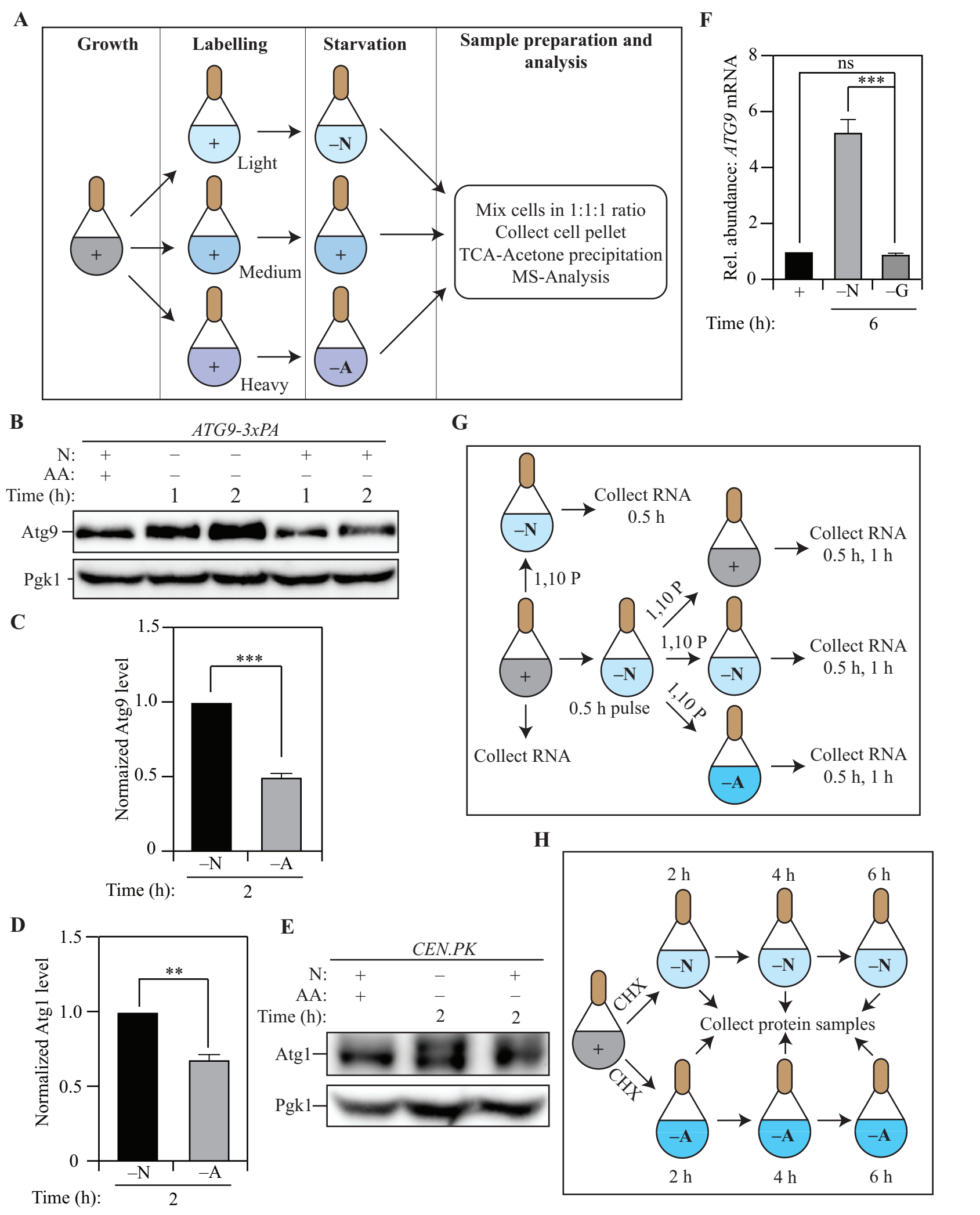
A
Growth
Labelling
Starvation
Sample preparation and analysis
Light
Medium
Heavy
–N
–A
Mix cells in 1:1:1 ratio
Collect cell pellet
TCA-Acetone precipitation
MS-Analysis
F
Rel. abundance: ATG9 mRNA
ns
***
Time (h):
6
B
ATG9-3xPA
N:
AA:
Time (h):
Atg9
Pgk1
C
Normaized Atg9 level
***
Time (h):
2
D
Normalized Atg1 level
**
Time (h):
2
E
CEN.PK
Atg1
Pgk1
G
Collect RNA
0.5 h
1,10 P
0.5 h pulse
Collect RNA
0.5 h, 1 h
H
2 h
4 h
6 h
CHX
Collect protein samples

Figure S2. Post-transcriptional activation of *ATG* gene expression is a critical node determining autophagy during nitrogen starvation. (**A**) Scheme of the triplex SILAC experiment. "Light", "Medium" and "Heavy" refer to the nature of arginine and lysine isotopes present in the media. Across different biological replicates, the nature of the medium used for growing cells for each treatment (nutrient-replete, nitrogen starvation or amino acid starvation) was alternated. (**B**) Atg9 levels are higher during nitrogen starvation compared to amino acid starvation: WT (SEY6210) cells were harvested in nutrient-replete conditions or after starvation for the indicated times and Atg9 protein levels were assessed by western blot. Pgk1 was used as a loading control. (**C**) Densitometric analysis of (B) from three independent biological replicates. (**D**) Densitometric analysis of Atg1 levels in WT cells after 2 h of nitrogen or amino acid starvation (from Figure 2B) from three independent biological replicates. (**E**) Atg1 levels increase substantially more during nitrogen versus amino acid starvation: CEN.PK cells were harvested in nutrient-replete conditions or after starvation for the indicated times and protein levels analyzed by western blot. Pgk1 was used as a loading control. (**F**) Transcriptional upregulation of *ATG9* mRNA occurs during nitrogen, but not glucose, starvation: qRT-PCR-based detection of *ATG9* mRNA in WT cells after 1 h of starvation. *ALG9* was used as a reference gene for normalization. Data are from three independent biological replicates. (**G**) Schematic for *ATG1* mRNA stability assay by 1,10-phenanthroline (1,10 P) chase (Figure 2G). (**H**) Schematic for Atg1 stability assay by cycloheximide chase (Figures 2H and 2I). Data in (C), (D) and (F) represent mean ± SEM from indicated number of replicates. Statistical analysis for (C) and (D) was carried out using an unpaired Student's t-test while (F) was analyzed using one-way analysis of variance (ANOVA). Multiple comparisons were carried out using Tukey's multiple comparisons test. $p < 0.05$, $^{**}p < 0.005$, $^{***}p < 0.001$, $^{****}p < 0.0001$ ns: not significant. Related to Figure 2.

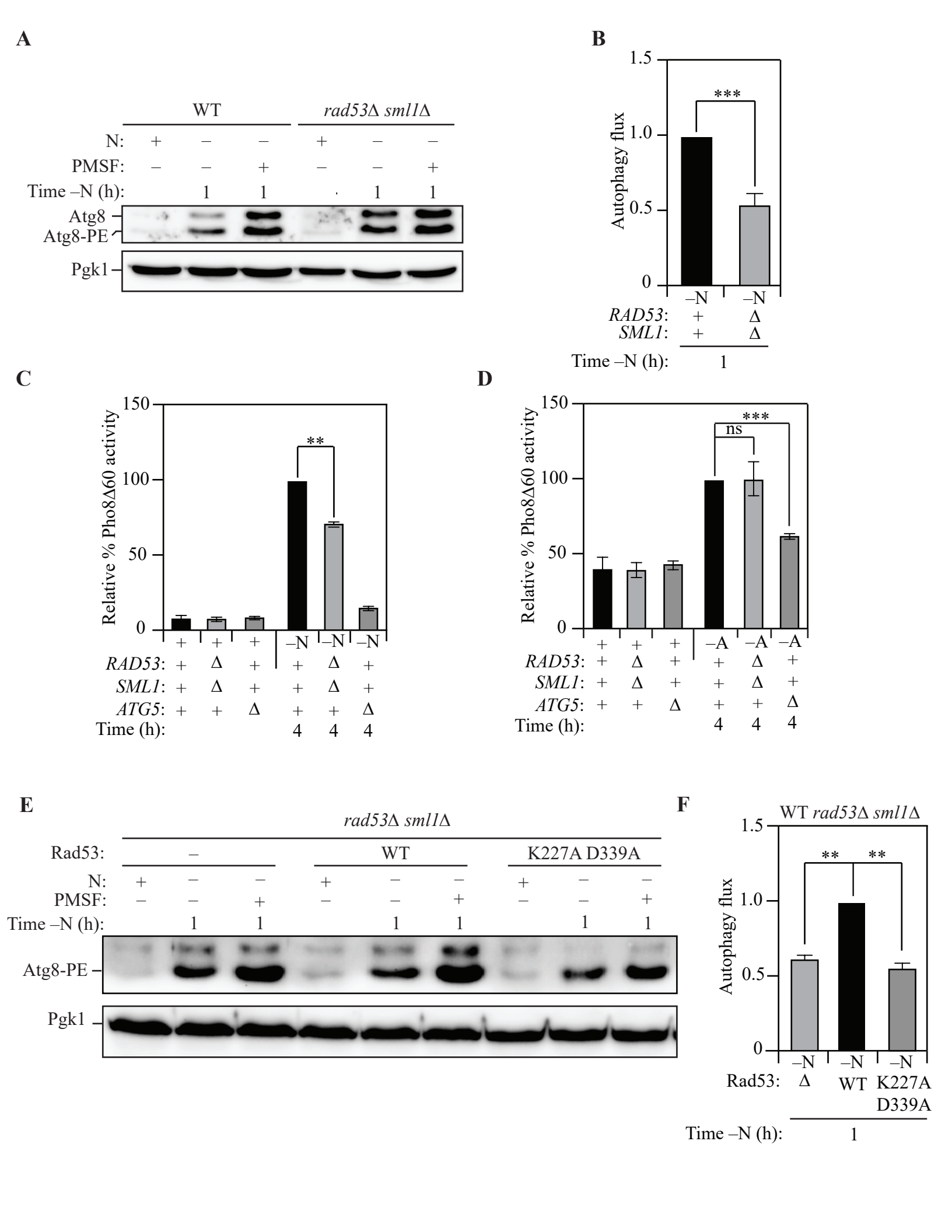
A
WT
rad53Δ sml1Δ
N:
PMSF:
Time –N (h):
Atg8
Atg8-PE
Pgk1
B
Autophagy flux
***
–N
RAD53:
SML1:
Time –N (h):
C
Relative % Pho8Δ60 activity
**
RAD53:
SML1:
ATG5:
Time (h):
D
Relative % Pho8Δ60 activity
***
ns
–A
E
rad53Δ sml1Δ
Rad53:
WT
K227A D339A
F
WT rad53Δ sml1Δ
Autophagy flux
**
Rad53:
K227A
D339A

Figure S3. Post-transcriptional regulation of *ATG1* expression by Rad53 facilitates nitrogen starvation-induced autophagy. (**A and B**) The loss of Rad53 function reduces autophagy flux during nitrogen starvation but not amino acid starvation, as demonstrated by the Pho8Δ60 assay: WT (WLY176) and *rad53*Δ *sml1*Δ cells were harvested during nutrient-replete conditions or after nitrogen starvation (A) or amino acid starvation (B) for the indicated times. Pho8Δ60 enzyme activity was measured by colorimetry. An increase in Pho8Δ60 activity indicates increased autophagic flux. Negative control: SEY6210 *atg5*Δ cells. Data from three independent biological replicates. (**C**) The loss of Rad53 function impairs autophagy during nitrogen starvation as demonstrated by the Atg8-lipidation assay: WT (SEY6210) and *rad53*Δ *sml1*Δ cells were harvested during nutrient-replete conditions or after starvation with or without PMSF treatment. Increased Atg8–PE accumulation upon PMSF treatment (relative to no treatment control) indicates autophagy flux. (**D**) Densitometric analysis of (C) from three independent biological replicates. (**E**) Abolishing Rad53 kinase activity reduces autophagy flux during nitrogen starvation: WLY176 *rad53*Δ *sml1*Δ cells expressing either Rad53 or Rad53^{K227A,D339A} (kinase dead Rad53) from a centromeric plasmid were harvested during nutrient-replete conditions or after starvation with or without PMSF treatment. Increased Atg8–PE accumulation upon PMSF treatment (relative to the no-treatment control) indicates autophagy flux. (**F**) Densitometric analysis of (E) from three independent biological replicates. Data in (A), (B), (D) and (F) represent mean ± SEM from the indicated number of replicates. Statistical analysis for (A) and (B) was carried out using two-way analysis of variance (ANOVA) while (D) was analyzed using an unpaired Student's t-test. Statistical analysis for (F) was carried out using one-way analysis of variance (ANOVA). Multiple comparisons were carried out using Tukey's multiple comparisons test. *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$, ****$p < 0.0001$ ns: not significant. Related to Figure 3.

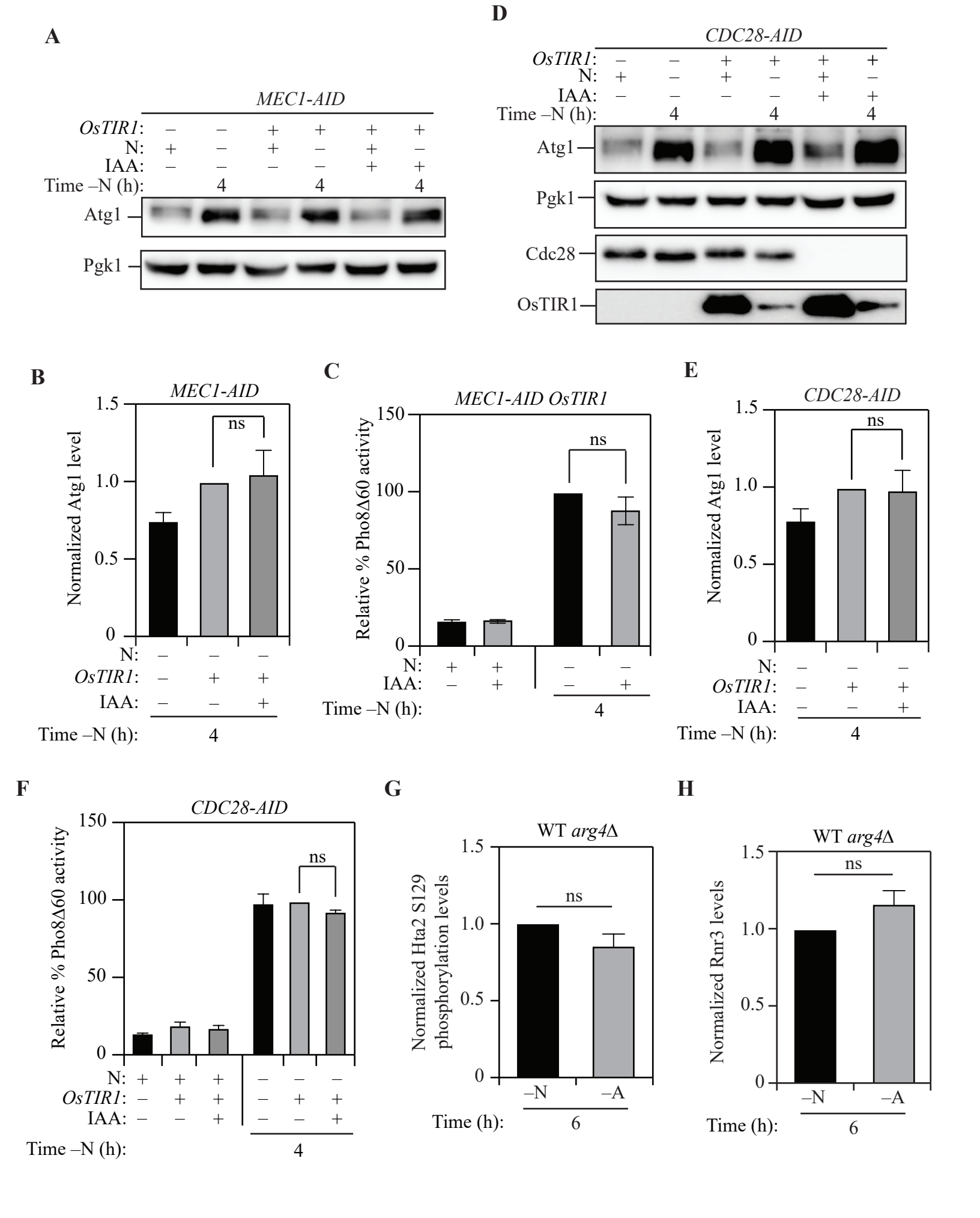
A
MEC1-AID
OsTIR1: – – + + + +
N: + – + – + –
IAA: – – – – + +
Time –N (h): 4 4 4
Atg1
Pgk1
D
CDC28-AID
OsTIR1: – – + + + +
N: + – + – + –
IAA: – – – – + +
Time –N (h): 4 4 4
Atg1
Pgk1
Cdc28
OsTIR1
B
MEC1-AID
Normalized Atg1 level
1.5
1.0
0.5
0
ns
N: – – –
OsTIR1: – + +
IAA: – – +
Time –N (h): 4
C
MEC1-AID OsTIR1
Relative % Pho8Δ60 activity
150
100
50
0
ns
N: + + – –
IAA: – + – +
Time –N (h): 4
E
CDC28-AID
Normalized Atg1 level
1.5
1.0
0.5
0
ns
N: – – –
OsTIR1: – + +
IAA: – – +
Time –N (h): 4
F
CDC28-AID
Relative % Pho8Δ60 activity
150
100
50
0
ns
N: + + + – – –
OsTIR1: – + + – + +
IAA: – – + – – +
Time –N (h): 4
G
WT arg4Δ
Normalized Hta2 S129 phosphorylation levels
1.5
1.0
0.5
0
ns
–N –A
Time (h): 6
H
WT arg4Δ
Normalized Rnr3 levels
1.5
1.0
0.5
0
ns
–N –A
Time (h): 6

Figure S4. Mec1 and Cdc28 are not involved in Rad53 activation during nitrogen starvation-induced autophagy. Mec1 and Cdc28 are not involved in Rad53 activation during nitrogen starvation-induced autophagy. (**A**) The acute loss of Mec1 has no effect on Atg1 expression during nitrogen starvation: WT (WLY176) *CUP1p-GFP-ATG8 MEC1-AID* cells without OsTIR1 expression and WT *CUP1p-GFP-ATG8 MEC1-AID OsTIR1* cells were harvested during nutrient-replete conditions or after nitrogen starvation with or without IAA treatment. Atg1 proteins levels were examined by western blot. Pgk1 was used as a loading control. (**B**) Densitometric analysis of three independent biological replicates from (A). (**C**) The acute loss of Mec1 does not affect autophagy flux during nitrogen starvation: WLY176 *CUP1p-GFP-ATG8 MEC1-AID OsTIR1* cells were harvested as in (A) and Pho8Δ60 enzyme activity was measured by colorimetry. An increase in Pho8Δ60 activity indicates increased autophagic flux. Data from three independent biological replicates. (**D**) The acute loss of Cdc28 has no effect on Atg1 expression: WT *CUP1p-GFP-ATG8 CDC28-AID* cells without OsTIR1 expression and WT *CUP1p-GFP-ATG8 CDC28-AID OsTIR1* cells were harvested and examined as in (A). Pgk1 was used as a loading control. (**E**) Densitometric analysis of three independent biological replicates from (D). (**F**) The acute loss of Cdc28 does not affect autophagy flux during nitrogen starvation: WLY176 *CUP1p-GFP-ATG8 CDC28-AID OsTIR1* cells were harvested and measured as in (C). Data from three independent biological replicates. (**G and H**) No differential DNA damage in nitrogen starvation relative to amino acid starvation. Hta2 S129 phosphorylation levels (G) and Rnr3 levels (H) are similar during nitrogen and amino acid starvation (see text for details). Data in (B), (C), and (E-H) represent the mean ± SEM from the indicated number of replicates. Statistical analysis for (B) and (E) was carried out using one-way analysis of variance (ANOVA). Statistical analysis for (C) and (F) was carried out using two-way analysis of variance (ANOVA). Statistical analysis for (G) and (H) was carried out using Unpaired t-test. Multiple comparisons were carried out using Tukey's multiple comparisons test. *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$, ****$p < 0.0001$, ns: not significant. Related to Figure 3.

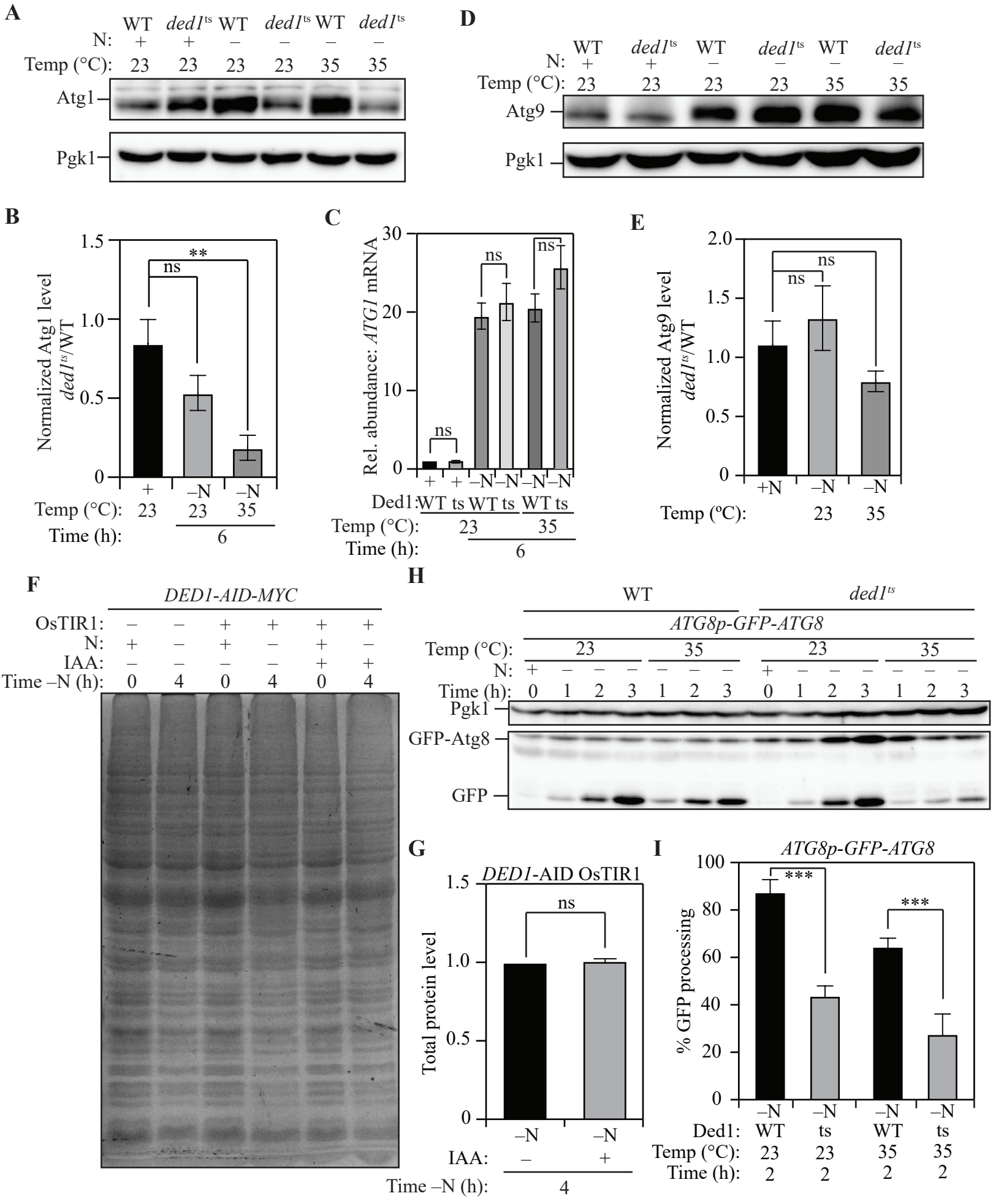
A
WT ded1ts WT ded1ts WT ded1ts
N: + + – – – –
Temp (°C): 23 23 23 23 35 35
Atg1
Pgk1
D
WT ded1ts WT ded1ts WT ded1ts
N: + + – – – –
Temp (°C): 23 23 23 23 35 35
Atg9
Pgk1
B
Normalized Atg1 level ded1ts/WT
1.5
1.0
0.5
0
**
ns
+ –N –N
Temp (°C): 23 23 35
Time (h): 6
C
Rel. abundance: ATG1 mRNA
30
20
10
0
ns
ns
ns
+ + –N –N –N –N
Ded1: WT ts WT ts WT ts
Temp (°C): 23 35
Time (h): 6
E
Normalized Atg9 level ded1ts/WT
2.0
1.5
1.0
0.5
0
ns
ns
+N –N –N
Temp (ºC): 23 35
F
DED1-AID-MYC
OsTIR1: – – + + + +
N: + – + – + –
IAA: – – – – + +
Time –N (h): 0 4 0 4 0 4
H
WT ded1ts
ATG8p-GFP-ATG8
Temp (°C): 23 35 23 35
N: + – – – – – – + – – – – – –
Time (h): 0 1 2 3 1 2 3 0 1 2 3 1 2 3
Pgk1
GFP-Atg8
GFP
G
DED1-AID OsTIR1
Total protein level
1.5
1.0
0.5
0
ns
–N –N
IAA: – +
Time –N (h): 4
I
ATG8p-GFP-ATG8
% GFP processing
100
80
60
40
20
0
***
***
–N –N –N –N
Ded1: WT ts WT ts
Temp (°C): 23 23 35 35
Time (h): 2 2 2 2

Figure S5. Ded1 regulates Atg1 expression and autophagy in yeast. (**A**) The loss of Ded1 activity impairs Atg1 expression during nitrogen starvation: WT (SEY6210) or *ded1*^{ts} (non-permissive temperature: 35°C) strains were harvested during nutrient-replete conditions or after nitrogen starvation at 23°C or 35°C. Atg1 levels were examined using western blot. Pgk1 was used as a loading control. (**B**) Densitometric analyses of Atg1 expression in *ded1*^{ts} relative to SEY6210 from three independent biological replicates. (**C**) The loss of Ded1 activity has no effect on *ATG1* transcription during nitrogen starvation: Strains and conditions as in (A) were used to measure the levels of *ATG1* mRNA by qRT-PCR. *ALG9* was used as a reference gene for normalization. Data from three independent biological replicates. (**D**) The loss of Ded1 activity has no effect on Atg9 expression: WT (SEY6210) or *ded1*^{ts} strains were harvested as in (A). Atg9 levels were examined using western blot. Pgk1 was used as a loading control. (**E**) Densitometric analyses of Atg9 expression in *ded1*^{ts} relative to the WT (SEY6210) from three independent biological replicates. (**F**) Total protein profile reveals that the loss of Ded1 activity does not promote changes in general translation during nitrogen starvation: WT (WLY176) *CUP1p-GFP-ATG8 DED1-AID* cells without OsTIR1 expression and WT (WLY176) *CUP1p-GFP-ATG8 DED1-AID OsTIR1* cells harvested during nutrient-replete conditions or after nitrogen starvation with or without IAA treatment. Proteins were stained using Coomassie Brilliant Blue. (**G**) Total protein quantified by densitometric analysis of five prominent bands from the total profile across three independent biological replicates. (**H**) The loss of Ded1 activity impairs autophagy flux during nitrogen starvation, assessed by GFP-Atg8 processing: WT (SEY6210) or *ded1*^{ts} (non-permissive temperature: 35°C) cells were transformed with an *ATG8p-GFP-ATG8* plasmid (expressing *ATG8* under the endogenous promoter) and harvested in nutrient-replete conditions or after starvation for the indicated times at either 23°C or 35°C. Proteins were examined by western blot. The appearance of free GFP indicates autophagy flux. Pgk1 was used as a loading control. (**I**) Densitometric analysis from three independent biological replicates. Data in (B), (C), (E), (G) and (I) represent the mean ± SEM from the indicated number of replicates. Statistical analysis for (B), (C), (E) and (I) was carried out using one-way analysis of variance (ANOVA). Statistical analysis for (G) was carried out using an unpaired Student's t-test. Multiple comparisons were carried out using Tukey's multiple comparisons test. *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$, ****$p < 0.0001$ ns: not significant. Related to Figure 5.

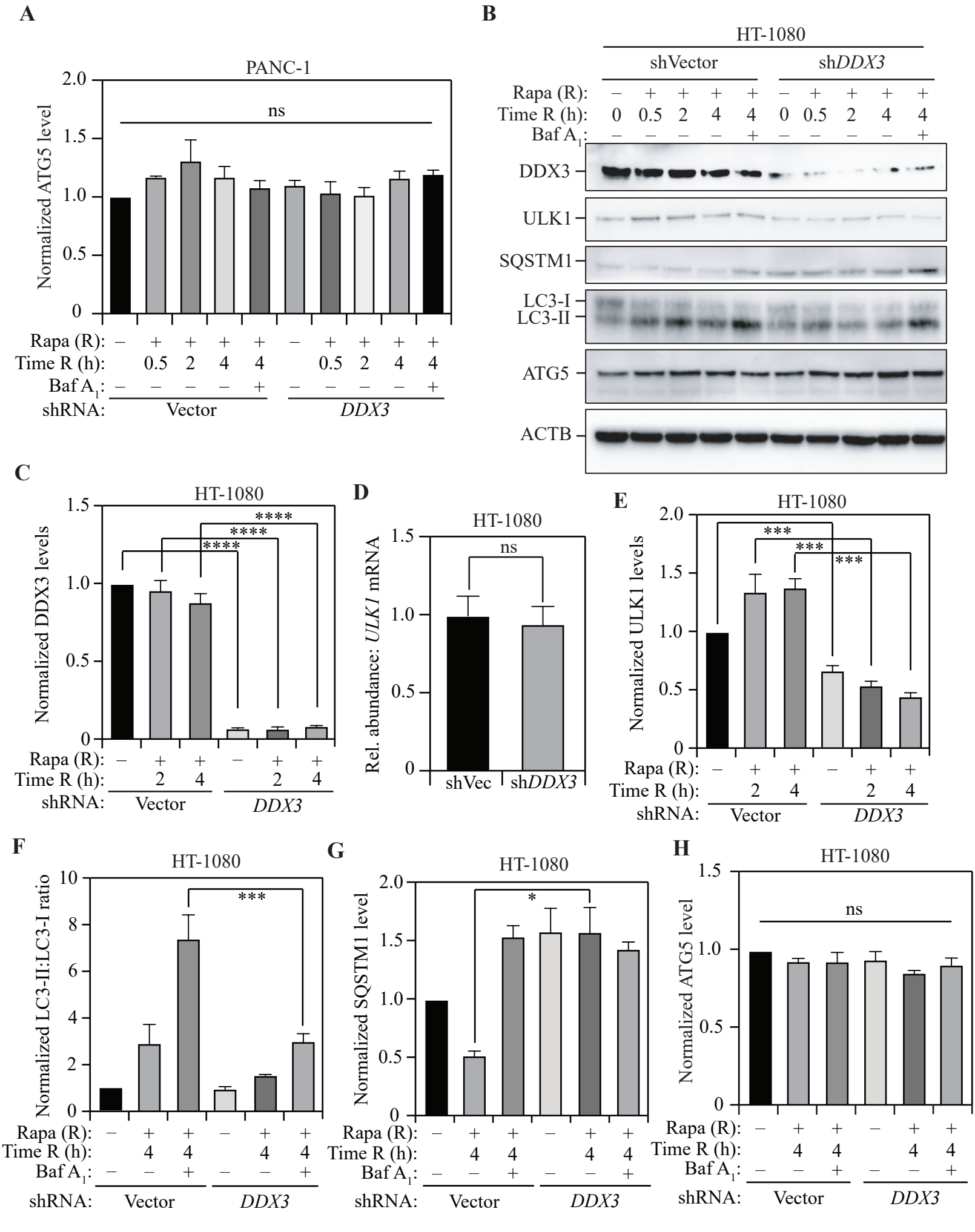
A
PANC-1
Normalized ATG5 level
ns
Rapa (R):
Time R (h):
Baf A1:
shRNA:
Vector
DDX3
B
HT-1080
shVector
shDDX3
Rapa (R):
Time R (h):
Baf A1:
DDX3
ULK1
SQSTM1
LC3-I
LC3-II
ATG5
ACTB
C
HT-1080
Normalized DDX3 levels
****
Rapa (R):
Time R (h):
shRNA:
Vector
DDX3
D
HT-1080
Rel. abundance: ULK1 mRNA
ns
shVec
shDDX3
E
HT-1080
Normalized ULK1 levels
***
Rapa (R):
Time R (h):
shRNA:
Vector
DDX3
F
HT-1080
Normalized LC3-II:LC3-I ratio
***
Rapa (R):
Time R (h):
Baf A1:
shRNA:
Vector
DDX3
G
HT-1080
Normalized SQSTM1 level
*
Rapa (R):
Time R (h):
Baf A1:
shRNA:
Vector
DDX3
H
HT-1080
Normalized ATG5 level
ns
Rapa (R):
Time R (h):
Baf A1:
shRNA:
Vector
DDX3

Figure S6. DDX3 regulates autophagy in mammalian cells. (**A**) The loss of DDX3 has no effect on ATG5 expression: PANC-1 cells, stably transfected with either vector shRNA (control) or shRNA targeting *DDX3*, were treated with rapamycin (Rapa) for the indicated times with or without co-treatment with bafilomycin A_1 (Baf A_1). Normalized ATG5 level at the indicated time points with the indicated treatments. (**B**) The loss of DDX3 impairs ULK1 expression and autophagy in mammalian cells: HT-1080 cells, stably transfected with either vector shRNA (control) or shRNA targeting *DDX3*, were treated with rapamycin for the indicated times with or without co-treatment with bafilomycin A_1. A representative blot shows the levels of DDX3, ULK1, SQSTM1, LC3-I and LC3-II, and ATG5 with ACTB as a loading control, upon harvesting cells at the indicated time points after rapamycin treatment. (**C**) Normalized DDX3 levels at the indicated time points with the indicated treatments. Data represent three independent biological replicates. (**D**) Relative abundance of *ULK1* mRNA in sh*DDX3* cells compared to control cells. *GAPDH* was used as a reference gene. Data represent three independent biological replicates. (**E**) Normalized DDX3 levels at the indicated time points with the indicated treatments. Data represent three independent biological replicates. (**F, G and H**) Normalized LC3-II:LC3-I ratio (F), normalized SQSTM1 level (G) and normalized ATG5 level (H) at the indicated time points with the indicated treatments. Decreased LC3-II:LC3-I (F) ratio in the presence of bafilomycin A_1 indicates reduced autophagy flux. An increased SQSTM1 accumulation (G) indicates reduced autophagy flux. Data represent three independent biological replicates. Data in (A), and (C-H) represent mean ± SEM from the indicated number of replicates. Statistical analysis for (A), (C), and (E-H) was carried out using one-way analysis of variance (ANOVA) while (D) was analyzed using an unpaired Student's t-test. Multiple comparisons were carried out using Tukey's multiple comparisons test. *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$, ****$p < 0.0001$ ns: not significant. Related to Figure 6.

Table 1. Yeast strains used in this study.

Strain	Genotype	Reference
SEY6210	*MATα leu2-3,112 ura3-52 his3-Δ200 trp1-Δ901 suc2-Δ9 lys2-801; GAL*	[61]
VLY001	SEY6210 *GCN4-3xPA::TRP1*	This study
VLY002	WLY176 *CUP1p-GFP-ATG8::LEU2*	This study
VLY003	SEY6210 pep4Δ*::KAN*	This study
VLY004	SEY6210 pep4Δ*::KAN vps4Δ::LEU2*	This study
VLY005	SEY6210 vac8Δ*::KAN*	This study
VLY006	SEY6210 arg4Δ*::HIS*	This study
VLY007	SEY6210 ATG9-*3xPA::TRP1*	This study
VLY008	SEY6210 *atg1*Δ*::HIS3*	This study
VLY009	SEY6210 *sml1*Δ*::HIS3*	This study
VLY010	SEY6210 *sml1*Δ*::HIS3 rad53*Δ*::URA3*	This study
VLY011	SEY6210 *PGI1-GFP::TRP1*	[58]
VLY012	SEY6210 *sml1*Δ*::HIS3 PGI1-GFP::TRP1*	This study
VLY013	SEY6210 *sml1*Δ*::HIS3 rad53*Δ*::URA3 PGI1-GFP::TRP1*	This study
VLY014	WLY176 *RAD53-AID-MYC::HIS3*	This study
VLY015	VLY014 *pNHK53::URA3*	This study
VLY016	VLY002 *MEC1-AID-MYC::HIS3*	This study
VLY017	VLY016 *pNHK53::URA3*	This study
VLY018	VLY002 *CDC28-AID-MYC::HIS3*	This study
VLY019	VLY018 *pNHK53::URA3*	This study
VLY020	SEY6210 *DED1-3xPA::TRP1*	This study
VLY021	SEY6210 *DED1-13xMYC::HIS3*	This study
VLY022	VLY010 *DED1-13xMYC::HIS3*	This study
	ded1-95	[113]
VLY022	VLY002 *DED1-AID-MYC::HIS3*	This study
VLY023	VLY022 *pNHK53::URA3*	This study

VLY024	VLY004 *DED1-AID-MYC::HIS3*	This study
VLY025	VLY024 *pNHK53::URA3*	This study
WLY176	SEY6210 *pho13*Δ *pho8*::*pho8*Δ60	[30]

Primers used in this study:

Table 2. Primers for yeast genetics.

Name	Sequence (5'-3')
GCN4 T F	AAATGAGGTTGCCAGATTAAAGAAATTAGTTGGCGAACGCCGGATCCCCGGGTTAATTAA
GCN4 T R	GAGAATGAAATAAAAAATATAAAATAAAAGGTAAATGAAAGAATTCGAGCTCGTTTAAAC
GCN4 T C	TCCACTGAAGAAGTTTCTCT
PEP4 D F	AAAGAAAAAAAAAAAAGCCTAGTGACCTAGTATTTAATCCAAATAAAATTCAAACAAAAACCAAAA CTAACCGGATCCCCGGGTTAATTAA
PEP4 D R	TTGTTATCTACTTATAAAAGCTCTCTAGATGGCAGAAAAGGATAGGGCGGAGAAGTAAGAAAAGT TTAGCGAATTCGAGCTCGTTTAAAC
PEP4 D C	CGTTTTCAATATCTTGAGCTCCTCAATTGTATTTG
VPS4 D F	TTGAGGACATGGAAGACAAAAATAAAGCAGCATAGAGTGCCTATAGTAGATGGGGTACAACAGCT GAAGCTTCGTACGC
VPS4 D R	TTTTTTTATTTTTTATTTTCATGTACACAAGAAATCTACATTAGCACGTTAATCAATTGAGCATAGG CCACTAGTGGATCTG
VPS4 D C	GTGTCATCTGTTGCAGTCG
VAC8 D F	CAGGAACTGAGCAAACTATAAGGGTGTTCTTTCTTCTGTACTATATATACATTTGCAACTCAGCTGA AGCTTCGTACGC
VAC8 D R	AACTTCTGAGAAGAAAATTTTGATAAAAATTATAATGCCTAGTCCCGCTTTTGAAGAAAAGCATAG GCCACTAGTGGAT
VAC8 D C	GAGCCCTTAAGGAGGACTC
ARG4 D F	GCTCAAAAGCAGGTAACTATATAACAAGACTAAGGCAAACCAGCTGAAGCTTCGTACGC
ARG4 D R	CCAGACCTGATGAAATTCTTGCGCATAACGTCGCCATCTGGCATAGGCCACTAGTGGATCTG
ARG4 D C	CAGCGGTAGATGTAAGCC

ATG9 T F	CTTGTTAAAGAGTATTACAAGAAGTCTGACGTCGGAAGACGGATCCCCGGGTTAATTAA
ATG9 T R	ATATAGTTATATTGGATGATGTACACGACACAGTCTGCCGAATTCGAGCTCGTTTAAAC
ATG9 T C	CCGAAGACCATAGCGATAAAG
ATG1 D F	ATTTGAAGCTACCCCATATTTTCAAATCTCTTTTACAACACCAGACGAGAAATTAAGAAACAGCTG AAGCTTCGTACGC
ATG1 D R	AGATACTTGAAAATATAGCAGGTCATTTGTACTTAATAAGAAAACCATATTATGCATCACGCATAG GCCACTAGTGGATCTG
ATG1 D C	TCCCCCATCAGCATCAGTTTGTG
SML1 D F	CCTTTGTGATCTTACGGTCTCACTAACCTCTCTTCAACTGCTCAATAATTTCCCGCTCAGCTGAAGC TTCGTACGC
SML1 D R	GAAAAGAACAGAACTAGTGGGAAATGGAAAGAGAAAAGAAAAGAGTATGAAAGGAACTGCATAG GCCACTAGTGGATCTG
SML1 D C	CATTGCCGTCGAACGTC
RAD53 D F	TCTTAAGCTTTAAAAGAGAGAATAGTGAGAAAAGATAGTGTTACACAACATCAACTAAAACAGCT GAAGCTTCGTACGC
RAD53 D R	GGTATCTACCATCTTCTCTCTTAAAAAGGGGCAGCATTTTCTATGGGTATTTGTCCTTGGGCATAGG CCACTAGTGGATCTG
RAD53 D C	GCTCAGCACCTACCTAAATG
RAD53 A F	GGTTAAAAGGGCAAAATTGGACCAAACCTCAAAAGGCCCCGAGAATTTGCAATTTTCGCTTCGTAC GCTGCAGGTCGA
RAD53 A R	TATCTACCATCTTCTCTCTTAAAAAGGGGCAGCATTTTCTATGGGTATTTGTCCTTGGCATCGATGA ATTCGAGCTCG
RAD53 A C	GCATGTATGAATCTCCGGC
MEC1 A F	AGAAGCAACATCAGAAGACAATCTAAGCAAGATGTATATTGGTTGGCTTCCATTTTGGCTTCGTAC GCTGCAGGTCGA
MEC1 A R	TGCAGTGATGGTTAGATCAAGAGGAAGTTCGTCTGTTGCCGAAAATGGTGGAAAGTCGCATCGATG AATTCGAGCTCG
MEC1 A C	CATGGAACAGGTAGATAAATTTCC

CDC28 A F	CCCTATTAACCGGATTAGCGCCAGAAGAGCAGCCATCCACCCCTACTTCCAAGAATCACTTCGTAC GCTGCAGGTCGA
CDC28 A R	AGGCTATAATGACAGTGCAGTAGCATTTGTAATATAATAGCGAAATAGATTATAATGCCATCGATG AATTCGAGCTCG
CDC28 A C	GGACCAACCGTTAGGAGC
DED1 A F	TGGGGTAACAGCGGTGGTTCAAACAACTCTTCTTGGTGGCTTCGTACGCTGCAGGTCGA
DED1 A R	GCAGAAAACGAAGAATCCTCACCCTAGTTTGTCTGAAACATCGATGAATTCGAGCTCG
DED1 A C	GAGCTACCGCCATTCATG
RA	TCGACCTGCAGCGTACGAAG
RD	GCGTACGAAGCTTCAGCTG
RT	TTAATTAACCCGGGGATCCG

A, AID tagging; C, PCR-based forward detection primer; D, deletion; F, forward primer; R, reverse primer; RA, PCR-based reverse detection primer for AID tagging; RD, PCR-based reverse detection primer for deletion; RT, PCR-based reverse detection primer for tagging; T, tagging.

Table 3. Primers for qRT-PCR.

Name	Sequence (5'-3')
ATG1 F	ATCTAAGATGGCCGCACATATG
ATG1 R	AGGGTAGTCACCATAGGCATTC
ATG9 F	CGTACTAACAGAGTCTTTCCTTG
ATG9 R	CTAAGACACCACCCTTATTGAG
ALG9 F	CACGGATAGTGGCTTTGGTGAACAATTAC
ALG9 R	TATGATTATCTGGCAGCAGGAAAGAACTTGGG
ATG1 5'-UTR F	TAGGCCGAGGTTAATTCTAGAACG
ATG1 5'-UTR R	ATAGTACTGTTCTCTGTTTCCCCAGA
ATG1 CDS F	GAGCTTCCAATCATTTGGAGTTATTC
ATG1 CDS R	CTATTCTTTGGGCTGGATCAAATGTC
ATG1 3'-UTR F	GAGGCAGAAGATGAACCACCAAA
ATG1 3'-UTR R	GTAAAGCATTTCGAGAGTAGCATAAC
PGK1 CDS	GAAGGACAAGCGTGTCTTCATCAG
PGK1 CDS	CGTACTTGATGGTTGGCAAAGCAG
hGAPDH F	GTCTCCTCTGACTTCAACAGCG
hGAPDH R	ACCACCCTGTTGCTGTAGCCAA
hULK1 F	GCAAGGACTCTTCCTGTGACAC
hULK1 R	CCACTGCACATCAGGCTGTCTG

Table 4. Primers for shRNA-mediated knockdown.

Name	Sequence (5'-3')
DDX3F	CCGGCGGAGTGATTACGATGGCATTCTCGAGAATGCCATCGTAATCACTCCGTTTTT
DDX3R	CCGGCGTAGAATAGTCGAACAAGATCTCGAGATCTTGTTCGACTATTCTACGTTTTT

References

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