The transcription factor Spt4-Spt5 complex regulates the expression of *ATG8* and *ATG41*

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Supplementary data

Table S1. Strains used in this study.

Name	Genotype	Ref
BY4741	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	[1]
BY4742	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	[1]
DGY047	WLY176 *SPT5-MYC::TRP1*	This study
DGY048	WLY176 *SPT5-MYC* S1009A S1015A S1025A S1032A S1043A S1052A S1058A*::TRP1*	This study
DGY049	WLY176 *SPT5-MYC* S1009E S1015E S1025E S1032E S1043E S1052E S1058E*::TRP1*	This study
DGY050	WLY176 *GFP-ATG8(405)::LEU2 SPT5-MYC::TRP1*	This study
DGY051	WLY176 *GFP-ATG8(405)::LEU2 SPT5-MYC* S1009A S1015A S1025A S1032A S1043A S1052A S1058A*::TRP1*	This study
DGY052	WLY176 *GFP-ATG8(405)::LEU2 SPT5-MYC* S1009E S1015E S1025E S1032E S1043E S1052E S1058E*::TRP1*	This study
JMY97	WLY176, *ume6Δ::HIS3*	[2]
JMY347	SEY6210, *ZEO1p-pho13Δ pho8Δ60, CUP1p-GFP-ATG8(405)::LEU2*	This study
SEY6210	MATα *leu2-3,112 ura3-52 his3-Δ200 trp1-Δ901 suc2-Δ9 lys2-801; GAL*	[1]
TVY1	MAT*α leu2–3,112 ura3–52 his3-Δ200 trp1-Δ901 lys2–801 suc2-Δ9 GAL pep4Δ::LEU2*	[3]
WLY176	SEY6210 *pho13Δ pho8Δ60*	[4]
WXY100	TVY1 *spt4Δ::HIS3*	This study
WXY101	TVY1 *bur2Δ::URA3*	This study
WXY102	WLY176 *SPT5-PA::TRP1*	This study
WXY103	WLY176 *spt4Δ::KAN SPT5-PA::TRP1*	This study
WXY104	WLY176 *bur2Δ::URA3 SPT5-PA::TRP1*	This study
WXY105	WLY176 *spt4Δ::HIS3*	This study
WXY106	WLY176 *SPT4-PA::TRP1*	This study
WXY107	WLY176 *SPT5-GFP::KAN*	This study
WXY108	WLY176 *ATG5-PA::TRP1*	This study
WXY109	WLY176 *spt4Δ::KAN ATG5-PA::TRP1*	This study
WXY110	WLY176 *ATG20-PA::TRP1*	This study
WXY111	WLY176 *spt4Δ::URA3 ATG20-PA::TRP1*	This study
WXY112	WLY176 *CUP1p-GFP-ATG8(405)::LEU2*	This study
WXY113	WLY176 *spt4Δ::URA3 CUP1p-GFP-ATG8(405)::LEU2*	This study
WXY114	ZYY108 *spt4Δ::URA3*	This study
WXY115	WLY176 *bur2Δ::KAN*	This study
WXY116	WLY176 *bur2Δ::KAN ATG41-PA::TRP1*	This study
WXY117	WLY176 *SPT5-PA::TRP1 SGV1-MYC::KAN*	This study
WXY120	WLY176 *rim15Δ::URA3 SPT5-PA::TRP1 SGV1-MYC::KAN*	This study
WXY121	BY4741 *vac8Δ::HIS3*	This study
WXY122	BY4741 *spt4Δ::URA3 vac8Δ::HIS3*	This study
WXY123	WLY176 *rim15Δ::URA3*	This study

WXY124	ZYY108 *rim15Δ::URA3*	This study
WXY125	WLY176, *pNHK53::URA3 SPT5-AID-MYC::KAN*	This study
WXY126	SEY6210, *atg1Δ::HIS3*	This study
WXY127	ZYY108 *SPT5-MYC::TRP1*	This study
WXY128	ZYY108 *SPT5-MYC* S1009A S1015A S1025A S1032A S1043A S1052A S1058A*::TRP1*	This study
WXY129	ZYY108 *ume6Δ::URA3*	This study
WXY130	ZYY124 *spt4Δ::URA3*	This study
WXY131	JMY347, *spt4Δ::URA3*	This study
WXY132	ZYY108 *SPT5-MYC* S1009E S1015E S1025E S1032E S1043E S1052E S1058E*::TRP1*	This study
WXY133	WXY125, *CUP1p-GFP-ATG8(405)::LEU2*	This study
WXY134	WXY125, *ATG41-PA:: TRP1*	This study
YZY007	BY4742 *arg4Δ::URA3*	This study
YZY076	BY4742 *arg4Δ::URA3 rim15Δ::KAN*	This study
ZYY108	WLY176 *ATG41-PA::HIS3*	[5]
ZYY124	WLY176 *GCN4-PA::LEU2*	[5]

Supplemental references

[1] Winston F, Dollard C, Ricupero-Hovasse SL. Construction of a set of convenient *Saccharomyces cerevisiae* strains that are isogenic to S288C. Yeast. 1995;11:53-5.

[2] Bartholomew CR, Suzuki T, Du Z, Backues SK, Jin M, Lynch-Day MA, et al. Ume6 transcription factor is part of a signaling cascade that regulates autophagy. Proc Natl Acad Sci U S A. 2012;109:11206-10.

[3] Gerhardt B, Kordas TJ, Thompson CM, Patel P, Vida T. The vesicle transport protein Vps33p is an ATP-binding protein that localizes to the cytosol in an energy-dependent manner. J Biol Chem. 1998;273:15818-29.

[4] Kanki T, Wang K, Baba M, Bartholomew CR, Lynch-Day MA, Du Z, et al. A genomic screen for yeast mutants defective in selective mitochondria autophagy. Mol Biol Cell. 2009;20:4730-8.

[5] Yao Z, Delorme-Axford E, Backues SK, Klionsky DJ. Atg41/Icy2 regulates autophagosome formation. Autophagy. 2015;11:2288-99.

Table S2. Oligonucleotide primers used in this study.

Name	Sequence	Use
ATG1_F	ATCTAAGATGGCCGCACATATG	*ATG1* mRNA level check
ATG1_R	AGGGTAGTCACCATAGGCATTC	*ATG1* mRNA level check
ATG5_F	TCGGTCAACGAAGCTCGAAA	*ATG5* mRNA level check
ATG5_R	GATGAGCGGTATATGTCGCG	*ATG5* mRNA level check
ATG7_F	ATGAGCATTGTCCAGCATGTAG	*ATG7* mRNA level check
ATG7_R	GACCTCCTGCTTTATGACTGAC	*ATG7* mRNA level check
ATG8_F	GAAGGCCATCTTCATTTTTGTC	*ATG8* mRNA level check
ATG8_R	TTCTCCTGAGTAAGTGACATAC	*ATG8* mRNA level check
ATG9_F	CGTACTAACAGAGTCTTTCCTTG	*ATG9* mRNA level check
ATG9_R	CTAAGACACCACCCTTATTGAG	*ATG9* mRNA level check
ATG20_F	CAGGTAGCGGTGGGAAATCA	*ATG20* mRNA level check
ATG20_R	TGCGGTATGCGGATCTGTTT	*ATG20* mRNA level check
ATG41_F	CGAGTACTGAAGACGATTGCAT	*ATG41* mRNA level check *ATG41* ChIP-qPCR check
ATG41_R	TGCGACATTGGCAAAGGCAT	*ATG41* mRNA level check *ATG41* ChIP-qPCR check
YAT1_F	CTCGGTTTGCGTCGCTCATG	*YAT1* mRNA level check
YAT1_R	GTAGCCGGACAACAGGTATT	*YAT1* mRNA level check
ALG9_F	CACGGATAGTGGCTTTGGTGAACAATTAC	RT-qPCR check
ALG9_R	TATGATTATCTGGCAGCAGGAAAGAACTTGGG	RT-qPCR check
TFC1_F	GCTGGCACTCATATCTTATCGTTTCACAATGG	ChIP-qPCR check
TFC1_R	GAACCTGCTGTCAATACCGCCTGGAG	ChIP-qPCR check
ATG1-500_F	TCCTTGTTCGTTTCGTGTATCTG	*ATG1* ChIP-qPCR check

ATG1-500_R	GGTTTAAGAAATCAGAGCCGAGC	*ATG1* ChIP-qPCR check
ATG1+80_F	TAAAGATCACACAACCTCTGTGAACC	*ATG1* ChIP-qPCR check
ATG1+80_R	GATACTTCCTTTATGGCTACATGCTG	*ATG1* ChIP-qPCR check
ATG1+330_R	ATTGACTGTGAACGAACATCAACAG	*ATG1* ChIP-qPCR check
ATG1+330_F	GGTTCTCACTCGGTGGAGGGTATTT	*ATG1* ChIP-qPCR check
ATG41-100_F	GCGGCTCTGCGTAAAAAGGT	*ATG41* ChIP-qPCR check
ATG41-100_R	TTGGTTGGTTTGGTGTAGCC	*ATG41* ChIP-qPCR check
ATG41+20_F	GGCTACACCAAACCAACCAA	*ATG41* ChIP-qPCR check
ATG41+20_R	GGCTCGAAAGCAACGTTTGA	*ATG41* ChIP-qPCR check
ATG41+110_F	TCAAACGTTGCTTTCGAGCC	*ATG41* ChIP-qPCR check
ATG41+110_R	GTGCATTCTGACTCATCGAC	*ATG41* ChIP-qPCR check
ATG41+200_F	TTTTCCGCTATCGTACCCGA	*ATG41* ChIP-qPCR check
ATG41+200_R	TCCTTACTAGCAGCAGCAAC	*ATG41* ChIP-qPCR check
ATG8-20_F	GGGAACCATTAAAGGTTGAGGAG	*ATG8* ChIP-qPCR check
ATG8-20_R	AATCCTCTCCGACTCCGCCTTC	*ATG8* ChIP-qPCR check
ATG8+50_F	ACTAGAGACATGAAGTCTACATTTAAGTCT	*ATG8* ChIP-qPCR check
ATG8+50_R	TCAGCAGGAACTAGATATTTACGCTTATCA	*ATG8* ChIP-qPCR check
ATG8+150_F	GGAGAGGATTGCTGACAGGTTCAAG	*ATG8* ChIP-qPCR check
ATG8+150_R	AAATGAAGATGGCCTTCTCAGGGGG	*ATG8* ChIP-qPCR check

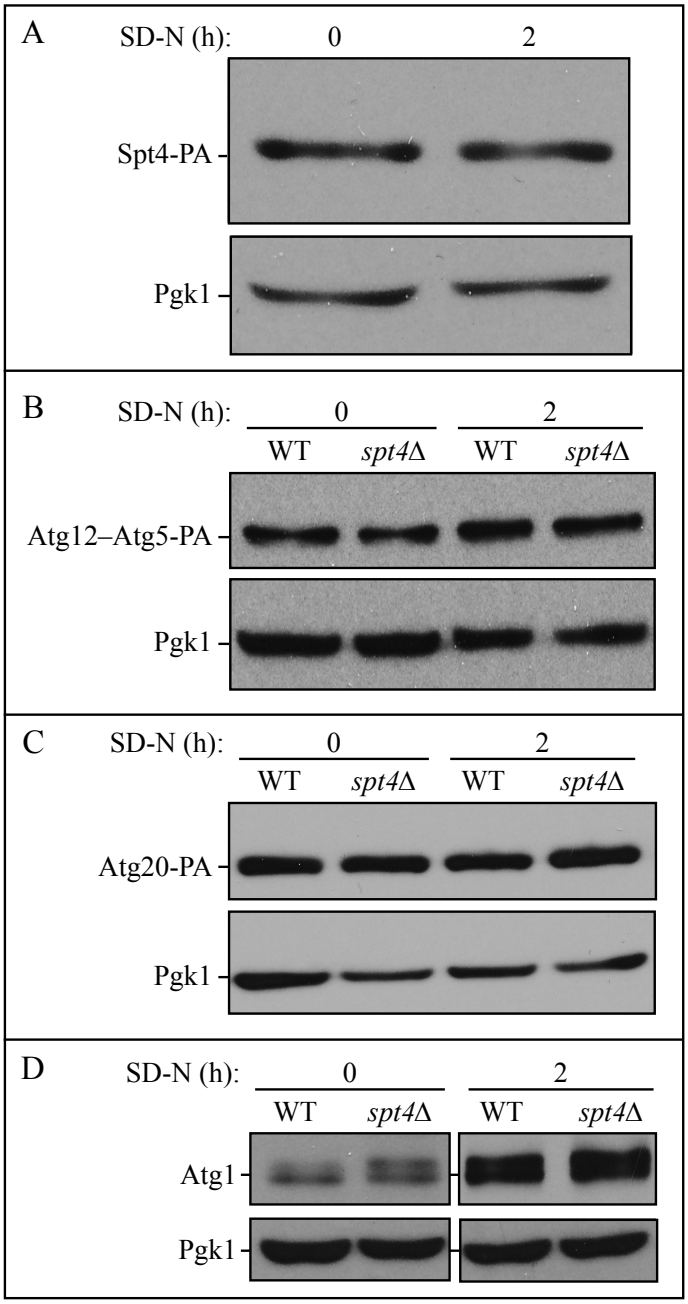


Figure S1. Spt4 protein level does not change after starvation, and protein levels of Atg5, Atg20 and Atg1 remain similar in *spt4*Δ cells. (**A**) Samples were collected from a strain expressing PA-tagged Spt4 (WXY106) under both growing and starvation conditions, and protein extracts were analyzed by western blot using antibodies to PA; Pgk1 was used as the loading control. (**B-C**) The anti-PA antibody was used to detect Atg5 (as the Atg12–Atg5 conjugate) and Atg20 protein levels, by probing for the PA tag in wild-type (WXY108, WXY110) and *spt4*Δ (WXY109, WXY111) cells in growing (YPD) and starvation (SD-N, t=2 h) conditions. Pgk1 was used as a loading control. (D) Samples from the wild-type (WLY176) and *spt4*Δ (WXY105) strains were collected in growing (YPD) and starvation (SD-N, t=2 h) conditions. The samples were analyzed by western blot with anti-Atg1 antibodies; Pgk1 was used as a loading control.

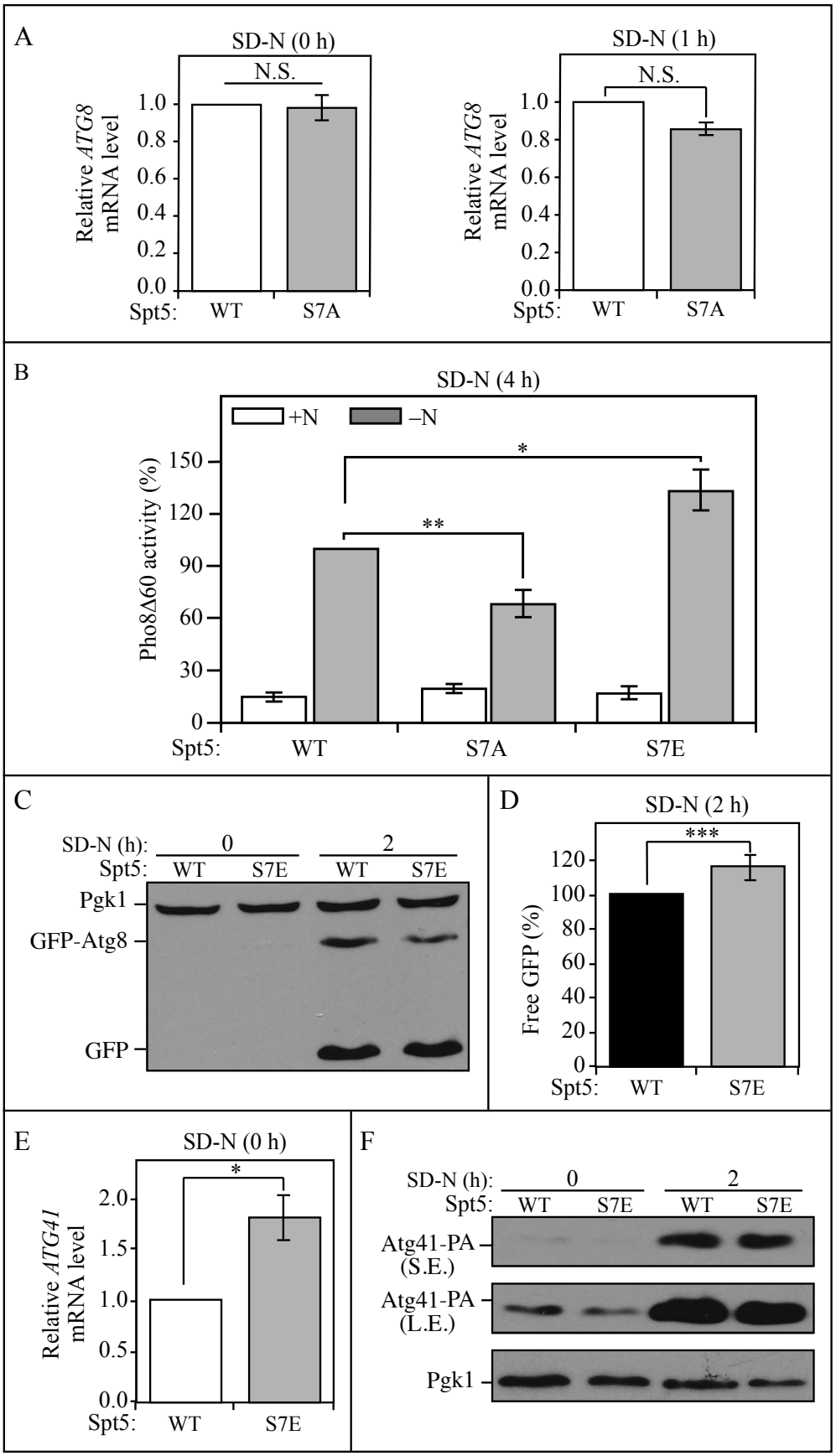

A
SD-N (0 h)
N.S.
Relative ATG8 mRNA level
1.0
0.8
0.6
0.4
0.2
0.0
Spt5: WT S7A
SD-N (1 h)
N.S.
Relative ATG8 mRNA level
1.0
0.8
0.6
0.4
0.2
0.0
Spt5: WT S7A
B
SD-N (4 h)
+N
–N
*
**
Pho8Δ60 activity (%)
150
120
90
60
30
0
Spt5: WT S7A S7E
C
SD-N (h): 0 2
Spt5: WT S7E WT S7E
Pgk1
GFP-Atg8
GFP
D
SD-N (2 h)
***
Free GFP (%)
120
100
80
60
40
20
0
Spt5: WT S7E
E
SD-N (0 h)
*
Relative ATG41 mRNA level
2.0
1.5
1.0
0.5
0.0
Spt5: WT S7E
F
SD-N (h): 0 2
Spt5: WT S7E WT S7E
Atg41-PA (S.E.)
Atg41-PA (L.E.)
Pgk1

Figure S2. Autophagy activity and *ATG41*/Atg41 expression are upregulated in cells expressing phosphomimetic Spt5[S7E]. (**A**) The RT-qPCR analysis of the *ATG8* mRNA level in WT (DGY047) and Spt5[S7A] (DGY048) cells in growing and starvation conditions. Error bars represent the SEM of at least 3 independent experiments, and WT was set as 1 in both conditions; other values were normalized. N.S., not significant. (**B**) Autophagy was measured with the quantitative Pho8Δ60 assay in WT (DGY047), Spt5[S7A] (DGY048) and Spt5[S7E] (DGY049) cells under growing conditions (+N) and after 4 h of nitrogen starvation (-N). Error bars represent the SEM of at least 3 independent experiments. *, $p<0.05$; **, $p<0.01$. (**C-D**) Autophagy activity was measured with the GFP-Atg8 processing assay in WT (DGY050) and Spt5[S7E] (DGY052) cells. Proteins were analyzed by western blot with anti-YFP antibody and anti-Pgk1 (loading control) antiserum. The quantitative analysis of processed GFP after starvation is shown in (D), and the error bar represents the SEM of 3 independent experiments. The processed GFP ratio after starvation has been set as 1, and other values were normalized. ***, $p<0.005$. (**E**) The mRNA level of *ATG41* was measured by RT-qPCR in WT (DGY047) and Spt5[S7E] (DGY049) cells in growing conditions. Error bars represent the SEM of at least 3 independent experiments, and WT was set as 1; other values were normalized. *, $p<0.05$. (**F**) The Atg41-PA protein level was tested by western blot and analyzed using anti-PA antibody in WT (WXY127) and Spt5[S7E] (WXY132) cells.

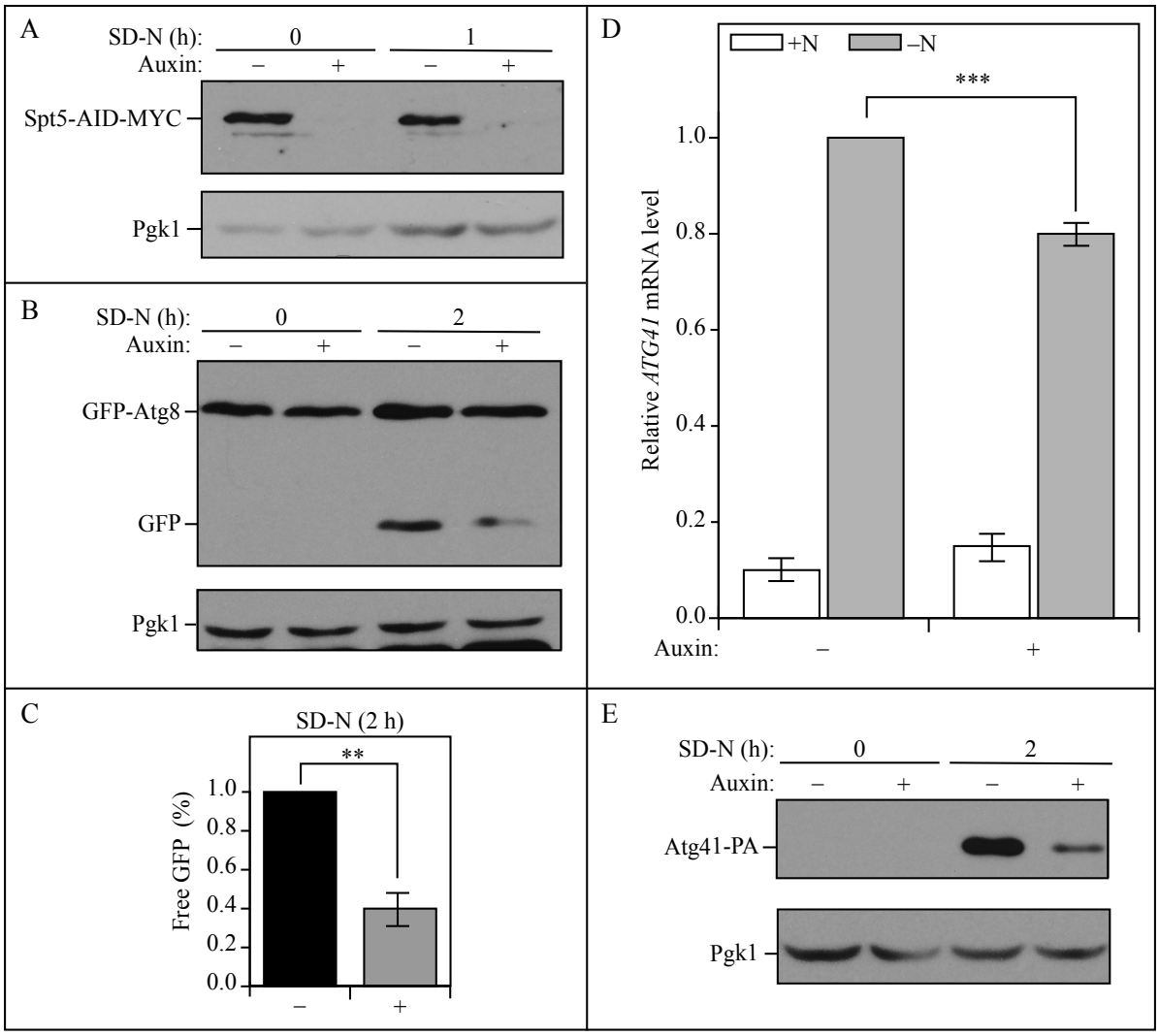
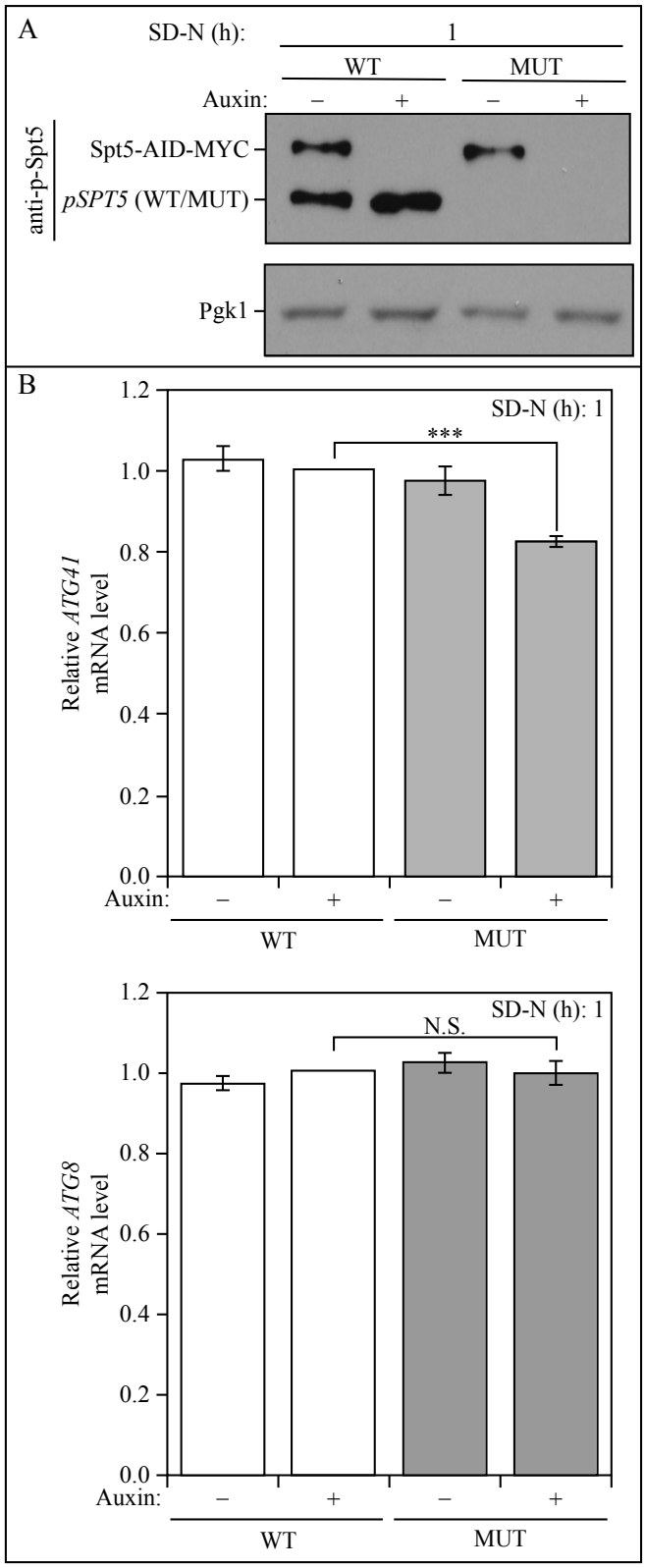


Figure S3. The temporal depletion of Spt5 using an Spt5-inducible degradation strain leads to decreased autophagy activity. (**A**) Spt5 levels were measured by western blot in an Spt5 auxin-inducible degron (AID) strain (WXY125) in both nutrient-rich and starvation conditions in the presence of DMSO (vehicle) or 300 μM auxin; the loss of Spt5-AID-MYC was detected with anti-MYC antibody after auxin treatment. (**B-C**) Autophagy activity was measured by the GFP-Atg8 processing assay. The Spt5-AID-MYC strain (WXY133) was incubated with DMSO or auxin in both growing (YPD, mid-log phase) and starvation (SD-N, t=2 h) conditions. Anti-YFP antibody and anti-Pgk1 (loading control) antiserum were used to detect the corresponding proteins. The quantitative analysis of processed GFP is shown in (C), and the error bar represents the SEM of 3 independent experiments. The processed GFP after 2 h of starvation was set as 1, and other values were normalized. (**D**) The mRNA level of *ATG41* was quantified by RT-qPCR in both growing (+N) and starvation (-N) conditions. The value of the strain treated with DMSO in -N was set as 1, and other values were normalized. The error bars indicate the SEM of at least 3 independent experiments. ***, $p<0.005$. (**E**) Anti-PA antibody was used to detect the protein level of Atg41-PA in the Spt5-AID-MYC strain (WXY134) when the strain was incubated with DMSO or auxin in either growing (SD-N, t=0 h) or starvation (SD-N, t=2 h) conditions.

Figure S4. The nonphosphorylable mutant of Spt5 in the Spt5-inducible degradation strain displays a decreased *ATG41* mRNA level. (**A**) The centromeric *HIS3*-marked Spt5 WT (WT) and Spt5[S15A] (MUT) plasmids were transformed into the Spt5-inducible degradation strain (WXY125). The anti-p-Spt5 antibody was used to detect Spt5 phosphorylation after starvation. (**B**) The mRNA level of *ATG41* and *ATG8* were quantified by RT-qPCR in Spt5-AID-MYC strains with WT and MUT plasmids incubated with DMSO and auxin in the nutrient-depleted condition. The value of WT with auxin was set as 1, and other values were normalized. The error bars indicate the SEM of at least 3 independent experiments. N.S., not significant; ***, p<0.005.



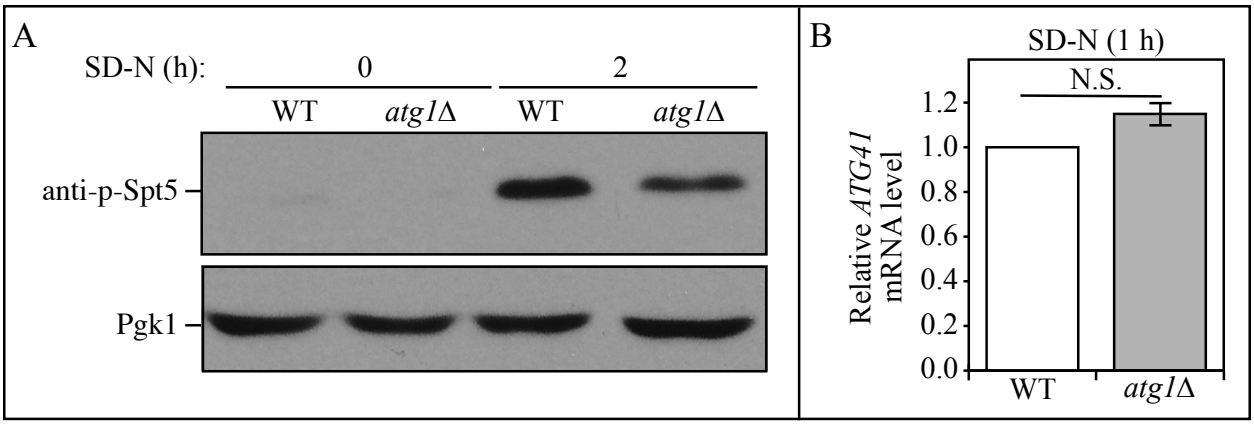


Figure S5. The deletion of *ATG1* results in a partial block of Spt5 phosphorylation but has no effect on *ATG41*. (**A**) The phosphorylation of Spt5 was detected with anti-p-Spt5 antibody in western blot samples of WT (SEY6210) and *atg1*Δ (WXY126) cells collected from growing and starvation (SD-N=2 h) conditions. (**B**) The RT-qPCR analysis of *ATG41* mRNA level in WT and *atg1*Δ cells after starvation. Error bars represent the SEM of at least 3 independent experiments, and WT was set as 1; the other value was normalized. N.S, not significant.

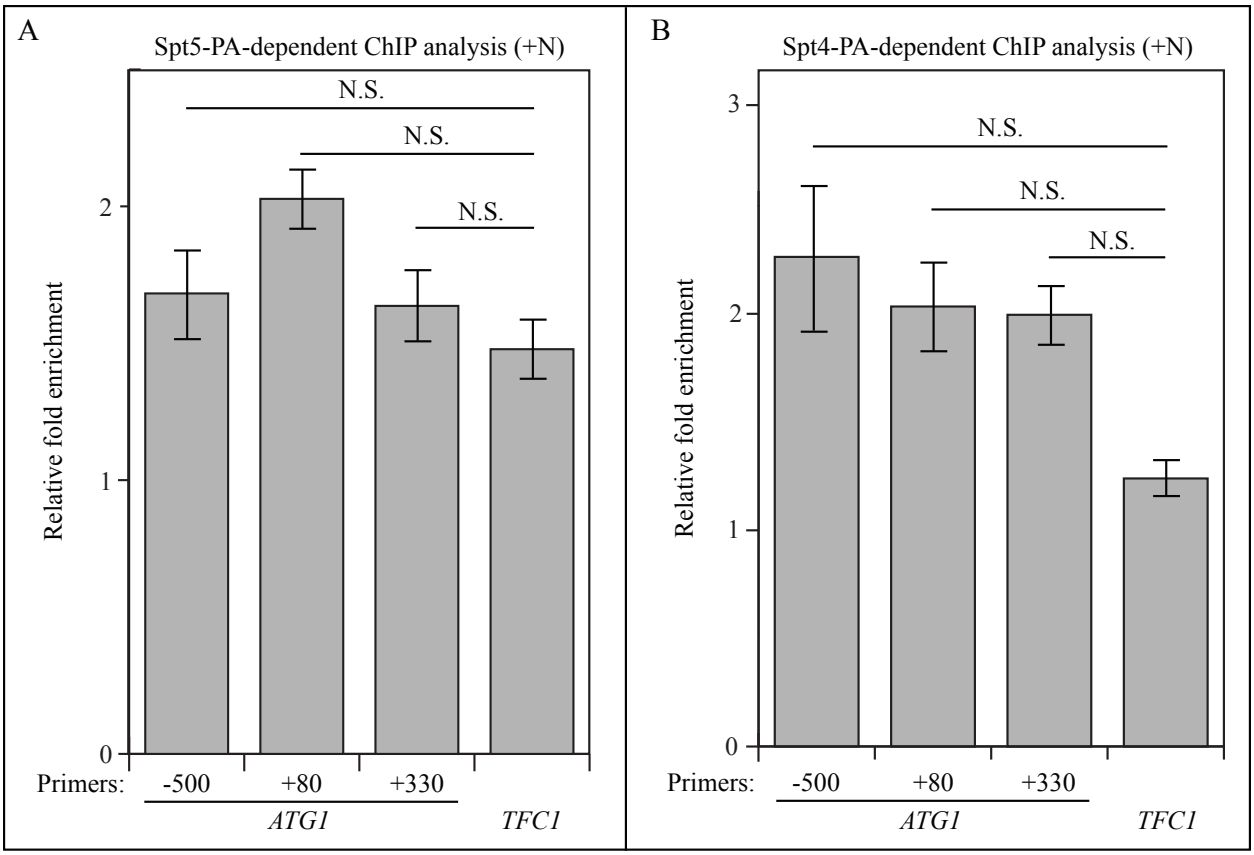


Figure S6. ChIP analysis shows that Spt5 and Spt4 may not bind to *ATG1* DNA. (**A-B**) ChIP analysis was conducted using the protein A-tagged (**A**) Spt5 (WXY102) and (**B**) Spt4 (WXY106) strains on 3 regions of DNA at the *ATG1* locus: -500 base pairs (bps), +80 bps, and +330 bps. The ChIP results were normalized to the input DNA, and *TFC1* was used as a negative control for both strains. N.S., not significant.

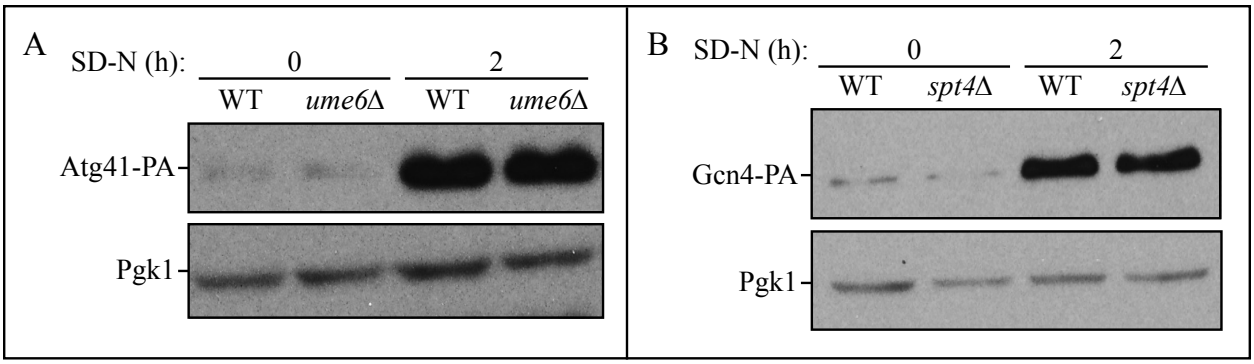


Figure S7. The transcription factors Ume6 and Gcn4 are not involved in Spt4-Spt5 complex regulation on autophagy. (**A**) The Atg41-PA protein level was detected with anti-PA antibody in WT (ZYY108) and *ume6*Δ (WXY129) cells in growing (SD-N, t=0 h) and starvation (SD-N, t=2 h) conditions. Anti-Pgk1 antiserum was used to detect the loading control. (**B**) The Gcn4-PA protein level was analyzed by western blot with the anti-PA antibody in WT (ZYY124) and *spt4*Δ (WXY130) strains.

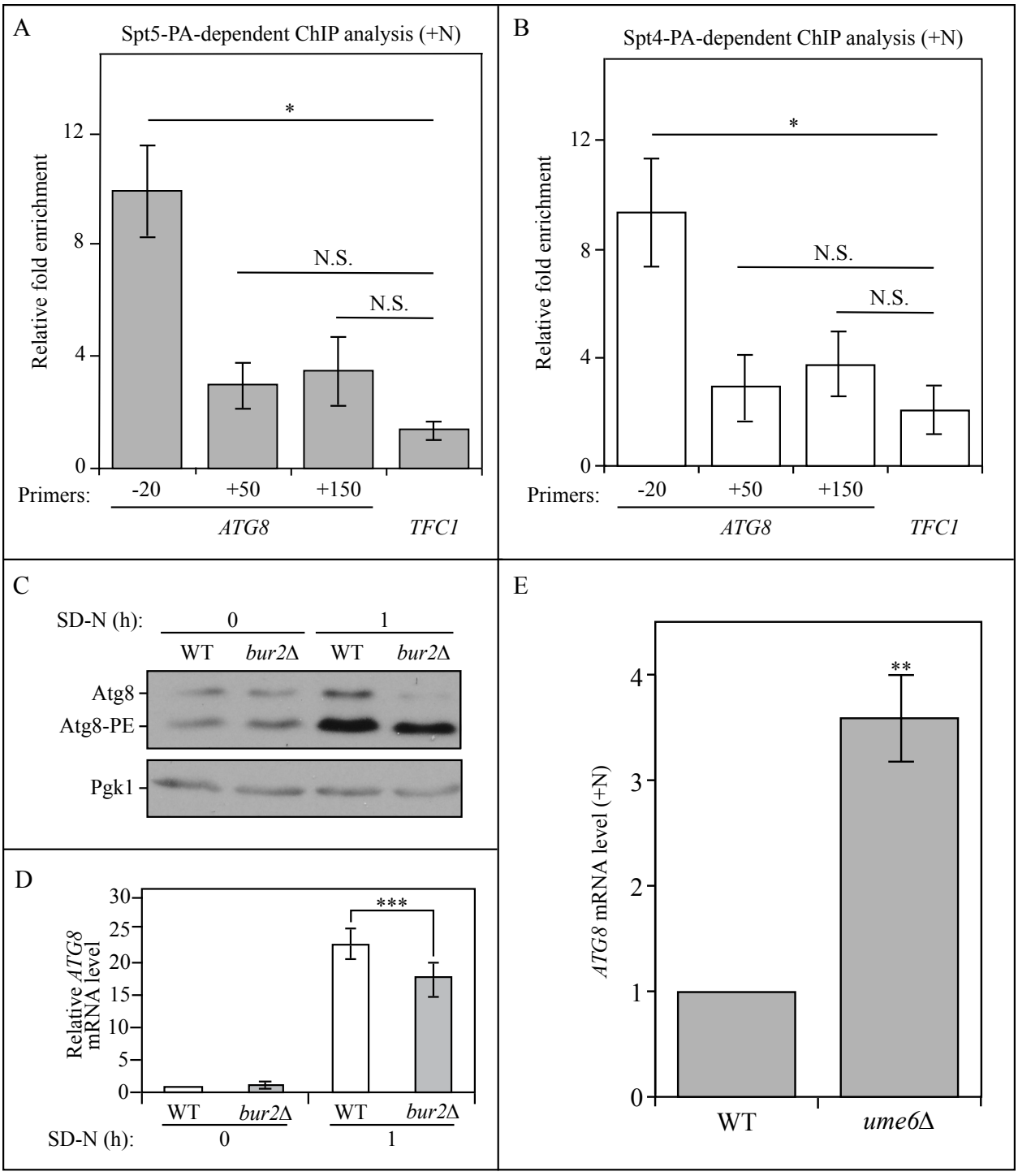


Figure S8. The direct binding of the Spt4-Spt5 complex with *ATG8* and the effect of the Sgv1/Bur1-Bur2 complex on *ATG8*/Atg8. (**A-B**) Spt5- and Spt4-dependent ChIP analysis (WXY102, WXY106) on different regions (-20, +50, +150) of *ATG8*. Results were normalized to the input DNA, and *TFC1* was used as a negative control for both strains. The error bars show the SEM of at least 3 independent experiments: *, $p<0.05$; N.S., not significant. (**C**) The Atg8 protein level was detected by western blot with anti-Atg8 antiserum in wild-type (TVY1) and *bur2*Δ

(WXY101) samples collected from both growing (YPD) and starvation (SD-N, 1 h) conditions. (**D**) The wild-type (WLY176) and *bur2*Δ (WXY115) cells were cultured until mid-log phase in growing conditions (YPD) and then shifted to starvation (SD-N) for 1 h. The mRNA level of *ATG8* was measured by RT-qPCR. The error bar represents the SEM of 3 independent experiments, and p values are reported for the comparison between the wild-type and *bur2*Δ strains during starvation. ***, $p<0.005$. (**E**) The *ATG8* mRNA level was measured by RT-qPCR in both WT and *ume6*Δ (JMY97) cells in growing conditions (YPD). Error bars represent the SEM of at least 3 independent experiments, and WT was set as 1. **, $p<0.01$.