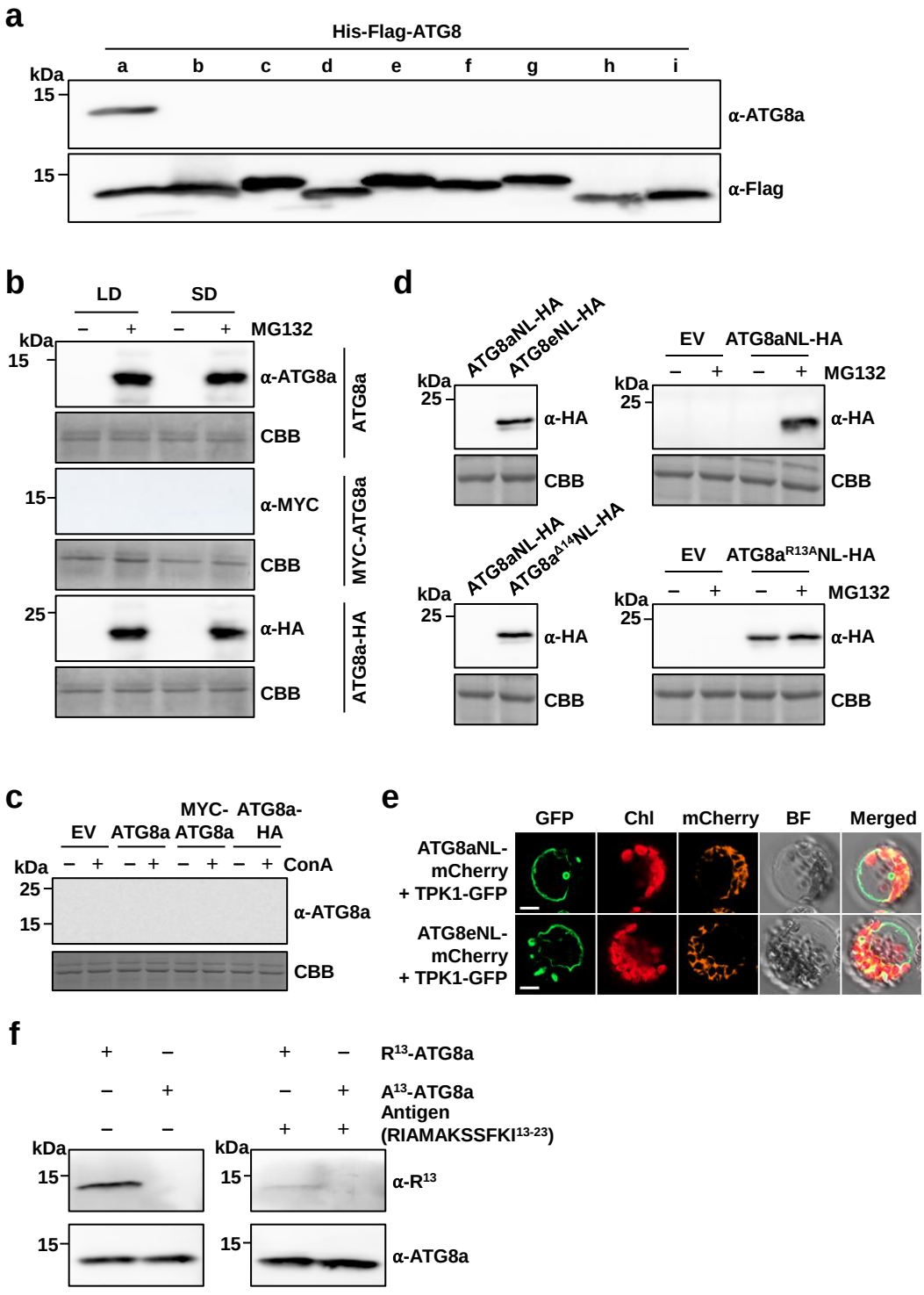

a
His-Flag-ATG8
a b c d e f g h i
kDa
15
α-ATG8a
15
α-Flag
b
LD SD
– + – + MG132
kDa
15
α-ATG8a
CBB
ATG8a
15
α-MYC
CBB
MYC-ATG8a
25
α-HA
CBB
ATG8a-HA
c
EV ATG8a MYC-ATG8a ATG8a-HA
kDa – + – + – + – + ConA
25
15
α-ATG8a
CBB
d
ATG8aNL-HA
ATG8eNL-HA
kDa
25
α-HA
CBB
EV ATG8aNL-HA
– + – + MG132
kDa
25
α-HA
CBB
ATG8aNL-HA
ATG8a^A14NL-HA
kDa
25
α-HA
CBB
EV ATG8a^R13ANL-HA
– + – + MG132
kDa
25
α-HA
CBB
e
GFP Chl mCherry BF Merged
ATG8aNL-mCherry + TPK1-GFP
ATG8eNL-mCherry + TPK1-GFP
f
+ – + – R13-ATG8a
– + – + A13-ATG8a
– – + + Antigen (RIAMAKSSFKI13-23)
kDa
15
α-R13
15
α-ATG8a

Supplementary Fig. 1. ATG8a undergoes 26S proteasome-dependent degradation independent of photoperiods and C-terminal cleavage.

a Specificity test of the anti-ATG8a antibody. Recombinant proteins of His-Flag-ATG8 isoforms (ATG8a-i) were immunoblotted with the anti-ATG8a and anti-Flag antibodies.

b ATG8a expression in different photoperiods. Untagged ATG8a, MYC-ATG8a, and ATG8a-HA were expressed in protoplasts prepared from Col-0 plants grown under long-day or short-day conditions, followed by treatments with cycloheximide (100 μM) and MG132 (10 μM) for 3 h.

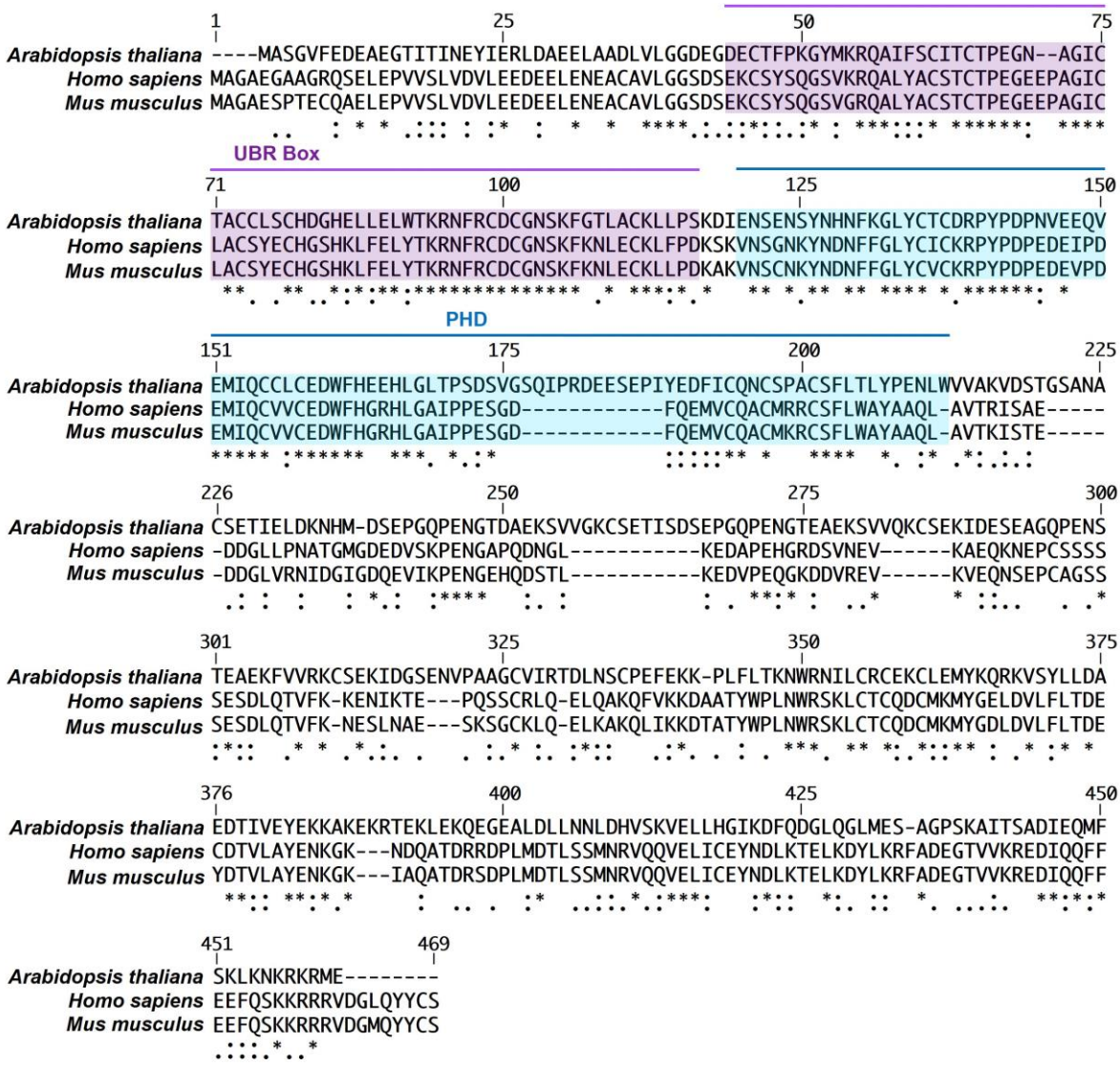
c Stability test of ATG8a. ATG8a, MYC-ATG8a, and ATG8a-HA were expressed in Col-0 protoplasts, followed by treatment with Concanamycin A (1 μM) for 16 h. ConA, Concanamycin A.

d Expression of non-lipidated (NL) ATG8a. ATG8aNL-HA, ATG8eNL-HA, ATG8a$^{\Delta 14}$NL-HA, and ATG8a^{R13A}NL-HA were expressed in Col-0 protoplasts, followed by treatments with cycloheximide (100 μM) and MG132 (10 μM) for 3 h.

e Subcellular localization of ATG8aNL and ATG8eNL. ATG8aNL-mCherry and ATG8eNL-mCherry were co-expressed with the tonoplast marker TPK1-GFP in Col-0 protoplasts. Fluorescence signal was visualized under a confocal microscope. Chl, chlorophyll; BF, bright field. Bars, 10 μm.

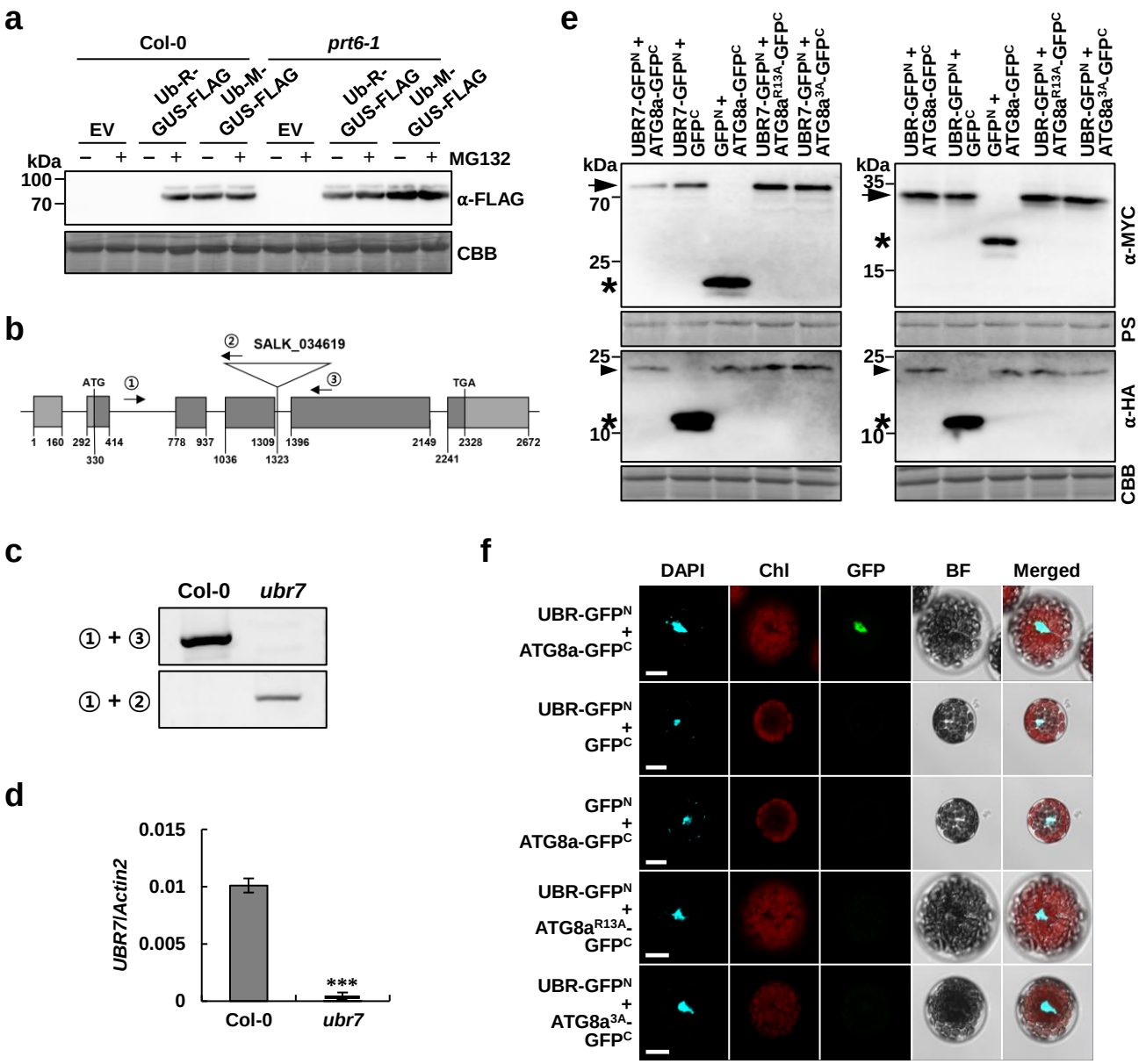
f Specificity test of the anti-R^{13}-ATG8a antibody. Recombinant proteins of R^{13}-ATG8a and A^{13}-ATG8a prepared using the LC3B-fusion technique were immunoblotted with the anti-R^{13}-ATG8a and anti-ATG8a antibodies in the presence or absence of the antigen peptide RIAMAKSSFKI at a 1:12 molar ratio of ATG8a to peptide. α-R^{13}, anti-R^{13}-ATG8a antibody.

ATG8a proteins were analyzed by immunoblotting with respective antibodies, and Coomassie brilliant blue (CBB) staining served as a loading control.



Supplementary Fig. 2. Multiple sequence alignment of UBR7 homologs in *Arabidopsis*, human, and mouse.

Sequences were aligned using Clustal Omega online (https://www.ebi.ac.uk/Tools/msa/clustalo/). Conserved UBR and PHD domains are shaded in purple and blue, respectively. Consensus symbols are as follows: asterisks indicate identical residues; colons and periods indicate conserved and semiconserved substitutions, respectively.


a
Col-0
prt6-1
EV
Ub-R-GUS-FLAG
Ub-M-GUS-FLAG
kDa
MG132
100
70
α-FLAG
CBB
b
SALK_034619
ATG
TGA
1
160
292
330
414
778
937
1036
1309
1323
1396
2149
2241
2328
2672
c
Col-0
ubr7
① + ③
① + ②
d
UBR7/Actin2
0.015
0.01
0.005
0
***
Col-0
ubr7
e
UBR7-GFP^N + ATG8a-GFP^C
UBR7-GFP^N + GFP^C
GFP^N + ATG8a-GFP^C
UBR7-GFP^N + ATG8a^R13A-GFP^C
UBR7-GFP^N + ATG8a^3A-GFP^C
UBR-GFP^N + ATG8a-GFP^C
UBR-GFP^N + GFP^C
GFP^N + ATG8a-GFP^C
UBR-GFP^N + ATG8a^R13A-GFP^C
UBR-GFP^N + ATG8a^3A-GFP^C
kDa
70
25
10
35
15
α-MYC
PS
α-HA
CBB
f
DAPI
Chl
GFP
BF
Merged
UBR-GFP^N + ATG8a-GFP^C
UBR-GFP^N + GFP^C
GFP^N + ATG8a-GFP^C
UBR-GFP^N + ATG8a^R13A-GFP^C
UBR-GFP^N + ATG8a^3A-GFP^C

Supplementary Fig. 3. UBR7 interacts with ATG8a through the UBR box domain *in vivo*.

a R-GUS-FLAG generated through the Ub fusion technique is degraded in wild-type but stabilized in *prt6-1* mutant. Ub-R/M-GUS-FLAG were expressed in Col-0 and *prt6-1* protoplasts, followed by treatments with cycloheximide (100 μM) and MG132 (10 μM) for 3 h. GUS proteins were analyzed by immunoblotting with the anti-FLAG antibody, and Coomassie brilliant blue (CBB) staining served as a loading control. EV, empty vector.

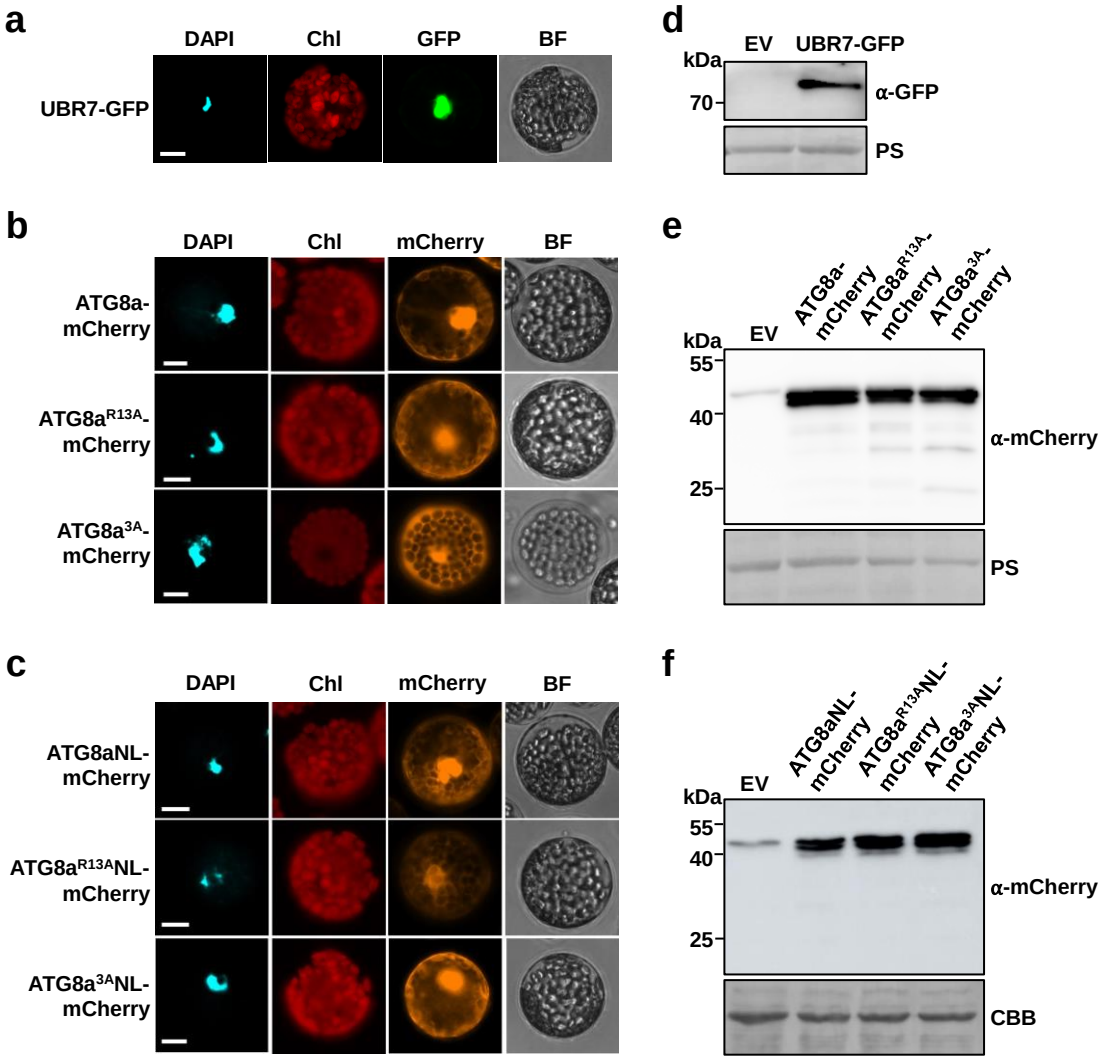
b Genomic structure of *UBR7* gene. Triangle and arrows indicate the positions of the T-DNA insertion and primers used for PCR, respectively. Genomic DNA sequences are represented by exons (dark gray boxes), introns (lines), and UTRs (light gray boxes). Numbers refer to nucleotides of *UBR7* gene.

c Genotyping of *ubr7* mutant. PCR using primers indicated in (**b**) verified T-DNA insertion.

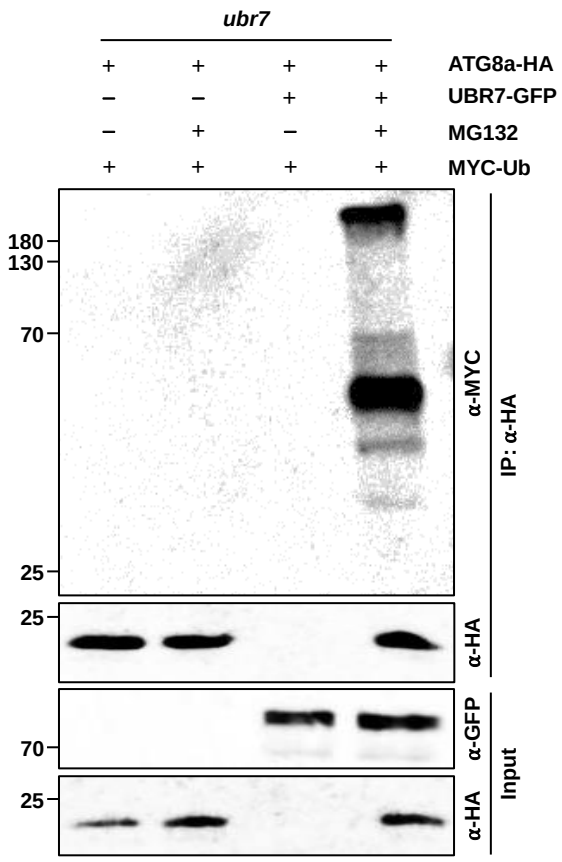
d RT-qPCR analysis of *UBR7* expression in Col-0 and *ubr7* plants. *Actin2* was used as a control. Data represent means ± SD (n = 4 biological replicates). Asterisks indicate significant differences between Col-0 and *ubr7* plants (t test; ***P < 0.001).

e Validation of expression of GFP^N-fused UBR7 (left) and UBR box (right) and GFP^C-fused ATG8a, $ATG8a^{R13A}$, and $ATG8a^{3A}$ in transfected protoplasts. Protein lysates were prepared from Col-0 protoplasts transfected with the indicated constructs, followed by treatment with MG132 (10 μM) for 3 h. UBR7/UBR box fused with N-terminal MYC and ATG8a/$ATG8a^{R13A}$/$ATG8a^{3A}$ fused with C-terminal HA were used and therefore detected by immunoblotting with the anti-MYC and anti-HA antibodies, respectively. Ponceau S (PS) and Coomassie brilliant blue (CBB) staining served as loading controls.

f BiFC assay for *in vivo* interaction between ATG8a and UBR box. GFP^N, GFP^C, and their fusions with UBR box, ATG8a, $ATG8a^{R13A}$, and $ATG8a^{3A}$ were co-expressed in Col-0 protoplasts, followed by treatment with MG132 (10 μM) for 3 h. Reconstituted GFP fluorescence was visualized under a confocal microscope. DAPI staining indicates the location of nuclei. UBR, UBR box; Chl, chlorophyll; BF, bright field. Bars, 10 μm.

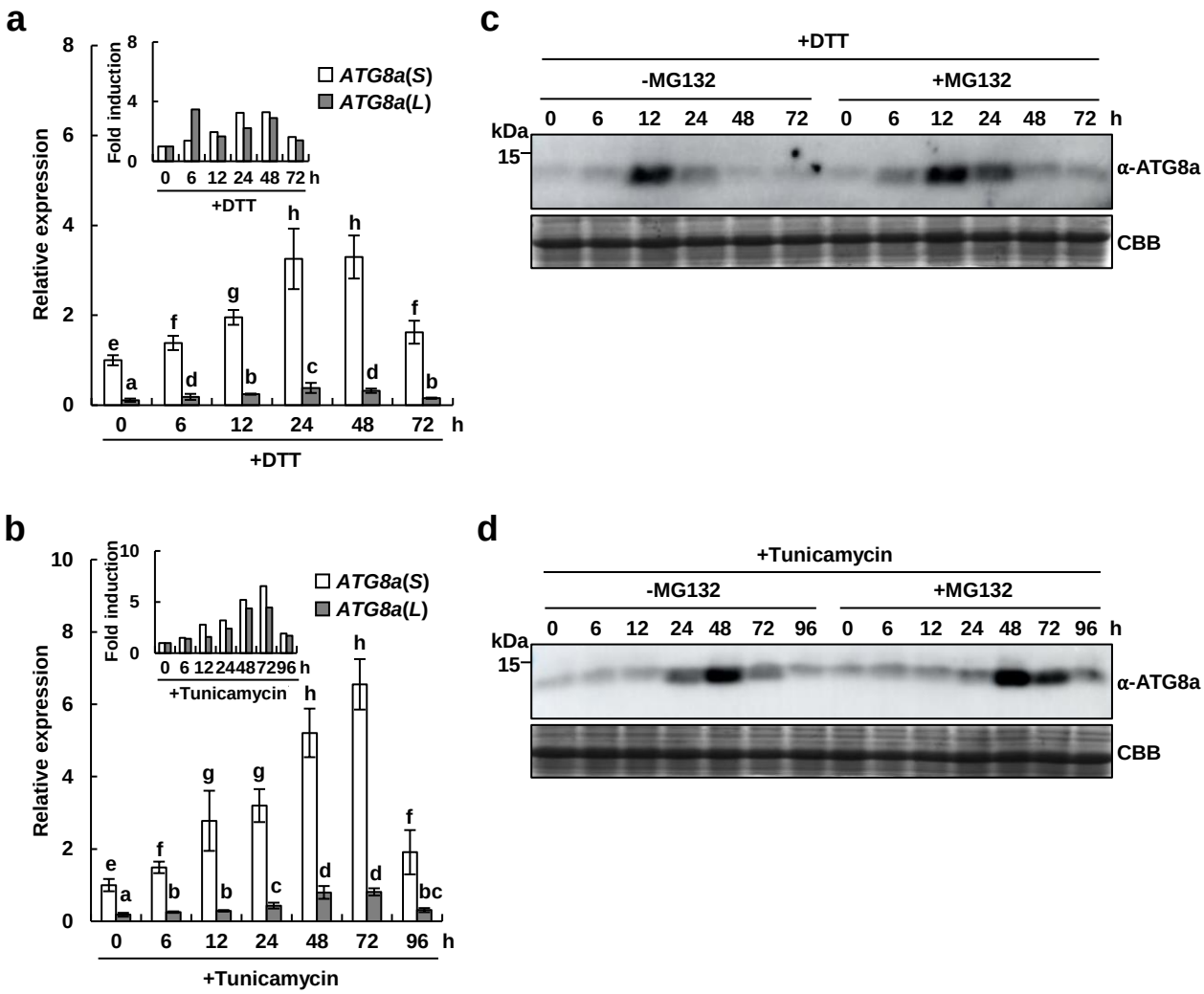


Supplementary Fig. 4. UBR7 and ATG8a are co-localized in the nucleus.
a-c Subcellular localization of UBR7 (**a**), ATG8a (**b**), and ATG8aNL (**c**). UBR7-GFP, ATG8a/ATG8a^{R13A}/ATG8a^{3A}-mCherry, and ATG8aNL/ATG8a^{R13A}NL/ATG8a^{3A}NL-mCherry were expressed in Col-0 protoplasts as indicated. Fluorescence signal was visualized under a confocal microscope. DAPI staining indicates the location of nuclei. Chl, chlorophyll; BF, bright field. Bars, 10 μm.
d-f Expression of UBR7 (**d**), ATG8a (**e**), and ATG8aNL (**f**) in transfected protoplasts. Protein lysates were prepared from Col-0 protoplasts transfected with the indicated constructs, followed by treatment with MG132 (10 μM) for 3 h. UBR7 and ATG8a proteins were detected by immunoblotting with the anti-GFP and anti-mCherry antibodies, respectively. Ponceau S (PS) and Coomassie brilliant blue (CBB) staining served as loading controls. EV, empty vector.



Supplementary Fig. 5. *In vivo* ubiquitination assay for UBR7-dependent ATG8a polyubiquitination.

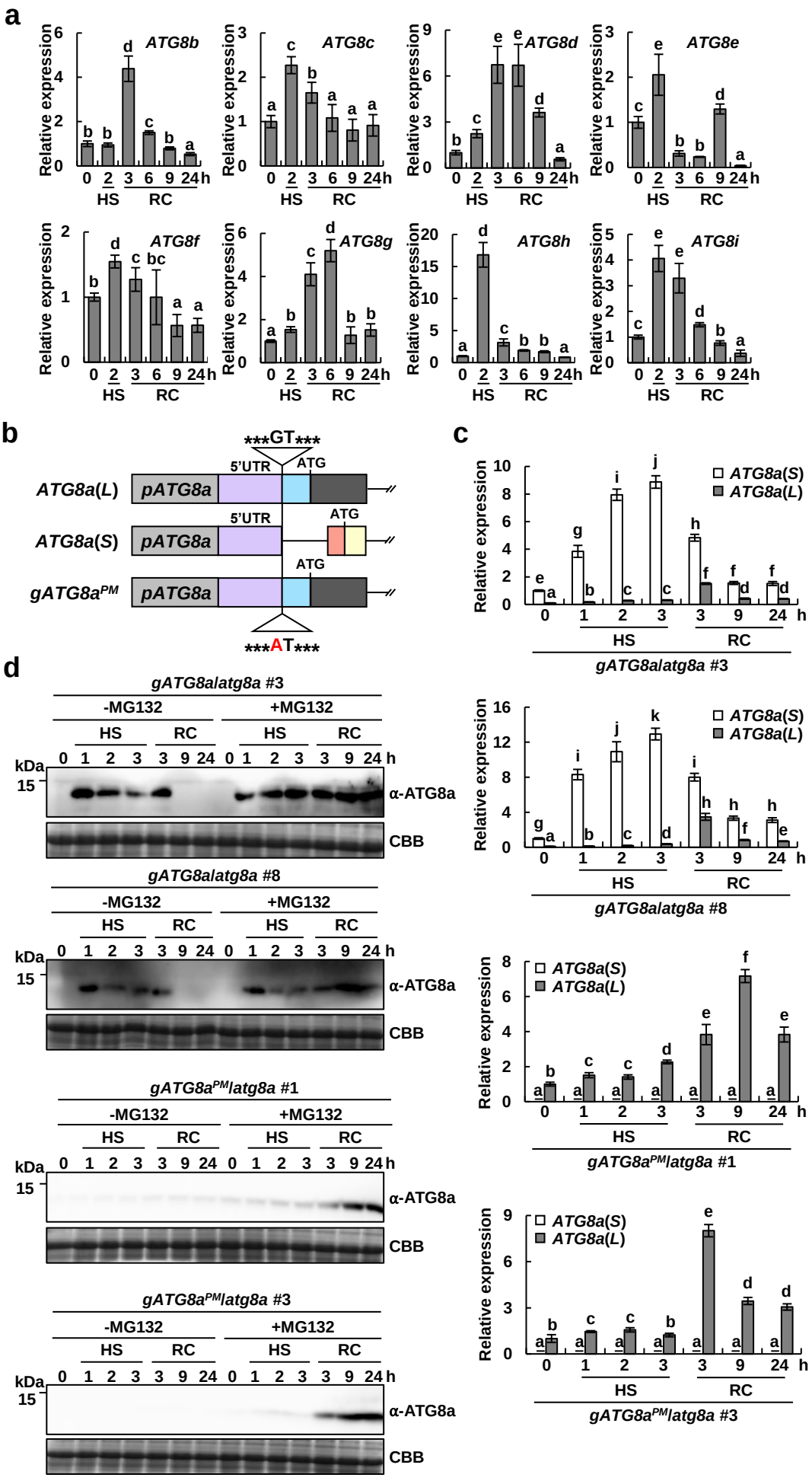
ATG8a-HA was expressed together with MYC-Ub and UBR7-GFP in *ubr7* protoplasts, followed by treatment with cycloheximide (100 μM) and MG132 (10 μM) for 3 h. Protein lysates were subjected to immunoprecipitation with the anti-HA antibody. Input shows 2% of the amount used in reactions. IP, immunoprecipitation.



Supplementary Fig. 6. N-degron-mediated ATG8a degradation is not related to ER stress responses.

a, b *ATG8*(*S*) expression is gradually increased in response to ER stressors, DTT (**a**) and tunicamycin (**b**). Transcript levels of *ATG8*(*S*) and *ATG8a*(*L*) were analyzed by RT-qPCR in Col-0 plants treated with DTT (8 mM) and tunicamycin (250 ng/ml) for the indicated times. Data represent means ± SD (*n* = 4 biological replicates). Different letters indicate significant differences (Tukey's HSD test; $P < 0.05$).

c, d ATG8a protein levels are not altered by MG132 treatment under ER stressors, DTT (**c**) and tunicamycin (**d**). ATG8a expression was monitored in the presence or absence of MG132 (10 μM) in Col-0 plants treated with DTT (8 mM) and tunicamycin (250 ng/ml) for the indicated times. Total proteins were extracted from plants and analyzed by immunoblotting with the anti-ATG8a antibody. Coomassie brilliant blue (CBB) staining served as a loading control.


a
ATG8b
ATG8c
ATG8d
ATG8e
ATG8f
ATG8g
ATG8h
ATG8i
Relative expression
0 2 3 6 9 24h
HS RC
b
GT
5'UTR
ATG
ATG8a(L)
pATG8a
ATG8a(S)
gATG8a^PM
AT
c
ATG8a(S)
ATG8a(L)
gATG8alatg8a #3
gATG8alatg8a #8
gATG8a^PMlatg8a #1
gATG8a^PMlatg8a #3
d
gATG8alatg8a #3
-MG132
+MG132
kDa
15
α-ATG8a
CBB
gATG8alatg8a #8
gATG8a^PMlatg8a #1
gATG8a^PMlatg8a #3

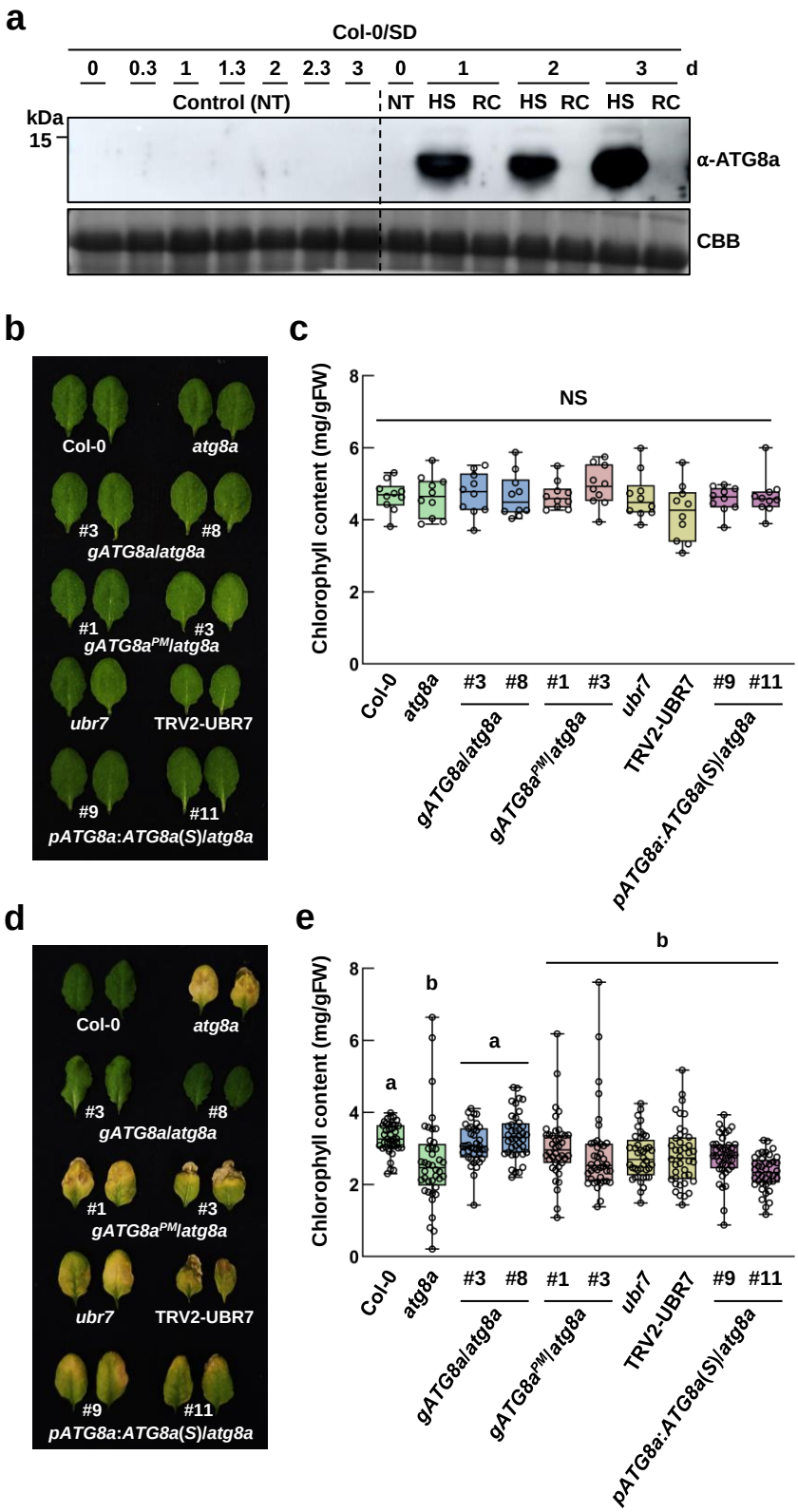
Supplementary Fig. 7. Expression analysis of ATG8 isoforms in wild-type and *atg8a* complemented with *ATG8a* genomic DNAs with either the wild-type sequence (*gATG8a*/*atg8a*) or a splice site mutation (*gATG8a*^{PM}/*atg8a*).

a Expression of several *ATG8* genes is induced during the HS recovery phase. Transcript levels of *ATG8* genes were analyzed by RT-qPCR in Col-0 plants exposed to HS (42°C) for 2 h and recovery (23°C) for the indicated times. Data represent means ± SD (n = 4 biological replicates). Different letters indicate significant differences (Tukey's HSD test; $P < 0.05$).

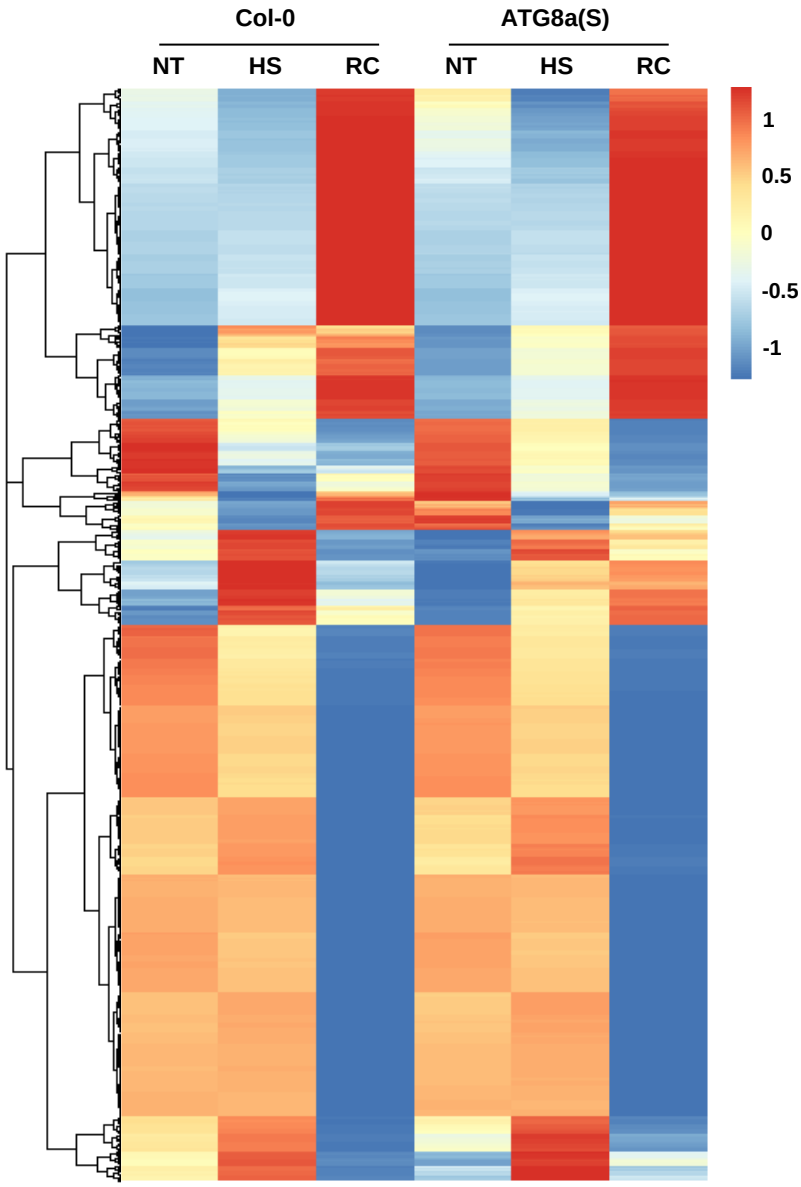
b Genomic structures of *ATG8*(*L*), *ATG8a*(*S*), and *gATG8a*^{PM} with a G to A splice site mutation in the retained intron. Exons are indicated by yellow and dark gray (coding) and purple, red, and blue (UTRs) boxes. Introns are shown in lines. Triangles indicate the position of a G to A splice site mutation.

c *ATG8*(*S*) is barely expressed in *gATG8a*^{PM}/*atg8a* lines. *ATG8*(*S*) and *ATG8a*(*L*) expression were analyzed by RT-qPCR in *gATG8a*/*atg8a* and *gATG8a*^{PM}/*atg8a* lines exposed to HS (42°C) and recovery (23°C) for the indicated times. Data represent means ± SD (n = 4 biological replicates). Different letters indicate significant differences (Tukey's HSD test; $P < 0.05$).

d ATG8a expression is decreased in *gATG8a*^{PM}/*atg8a* lines under HS. ATG8a expression was monitored in the presence or absence of MG132 during HS and recovery in *gATG8a*/*atg8a* and *gATG8a*^{PM}/*atg8a* lines. For MG132 treatment, plants were sprayed with MG132 (10 μM) prior to exposure to HS and recovery conditions. Total proteins were extracted from plants exposed to HS (42°C) and recovery (23°C) for the indicated times and analyzed by immunoblotting with the anti-ATG8a antibody. Coomassie brilliant blue (CBB) staining served as loading controls.


a
Col-0/SD
0 0.3 1 1.3 2 2.3 3 0 1 2 3 d
Control (NT)
NT HS RC HS RC HS RC
kDa
15
α-ATG8a
CBB
b
Col-0
atg8a
#3
#8
gATG8a/atg8a
#1
#3
gATG8a^PM/atg8a
ubr7
TRV2-UBR7
#9
#11
pATG8a:ATG8a(S)/atg8a
c
Chlorophyll content (mg/gFW)
NS
Col-0
atg8a
#3 #8
gATG8a/atg8a
#1 #3
gATG8a^PM/atg8a
ubr7
TRV2-UBR7
#9 #11
pATG8a:ATG8a(S)/atg8a
d
Col-0
atg8a
#3
#8
gATG8a/atg8a
#1
#3
gATG8a^PM/atg8a
ubr7
TRV2-UBR7
#9
#11
pATG8a:ATG8a(S)/atg8a
e
Chlorophyll content (mg/gFW)
a
b
a
b
Col-0
atg8a
#3 #8
gATG8a/atg8a
#1 #3
gATG8a^PM/atg8a
ubr7
TRV2-UBR7
#9 #11
pATG8a:ATG8a(S)/atg8a

Supplementary Fig. 8. ATG8a turnover is important for plant thermotolerance.
a Fluctuations in ATG8a abundance coincide with daily HS and recovery cycles. Col-0 plants grown in short days were exposed to 42°C (HS) for 8 h during the day and maintained at 23°C (recovery) for the remaining 16 h. Total proteins were extracted from Col-0 plants exposed to HS and recovery repeatedly for 3 days and analyzed by immunoblotting with the anti-ATG8a antibody. Coomassie brilliant blue (CBB) staining served as a loading control. SD, short day; NT, no treatment; HS, heat stress; RC, recovery.
b Leaf phenotypes of Col-0, *atg8a*, *ubr7*, TRV2-UBR7, and complementation lines grown in short days. Plants were maintained at 23°C under short-day conditions during HS experiments.
c Quantification of chlorophyll content in control plants in (**b**). Data represent means ± SD (n = 10 leaves). NS, not significant.
d Thermotolerance phenotypes of Col-0, *atg8a*, *ubr7*, TRV2-UBR7, and complementation lines under recurring HS and recovery conditions. Plants grown in short days were exposed to 42°C (HS) for 8 h during the day and maintained at 23°C (recovery) for the remaining 16 h, which was repeated for 5 days. Plants were allowed to recover for an additional 3 days and observed for leaf phenotypes.
e Quantification of chlorophyll content in plants exposed to HS and recovery in (**d**). Data represent means ± SD (n = 40 leaves). Different letters indicate significant differences (Tukey's HSD test; $P < 0.05$).



Supplementary Fig. 9. Heatmap of proteomics profiles in Col-0 and *pATG8:ATG8a*(*S*)*/atg8a* plants under NT, HS, and RC conditions.
Heatmap was generated using the pheatmap package in R Studio. NT, no treatment; HS, heat stress; RC, recovery.