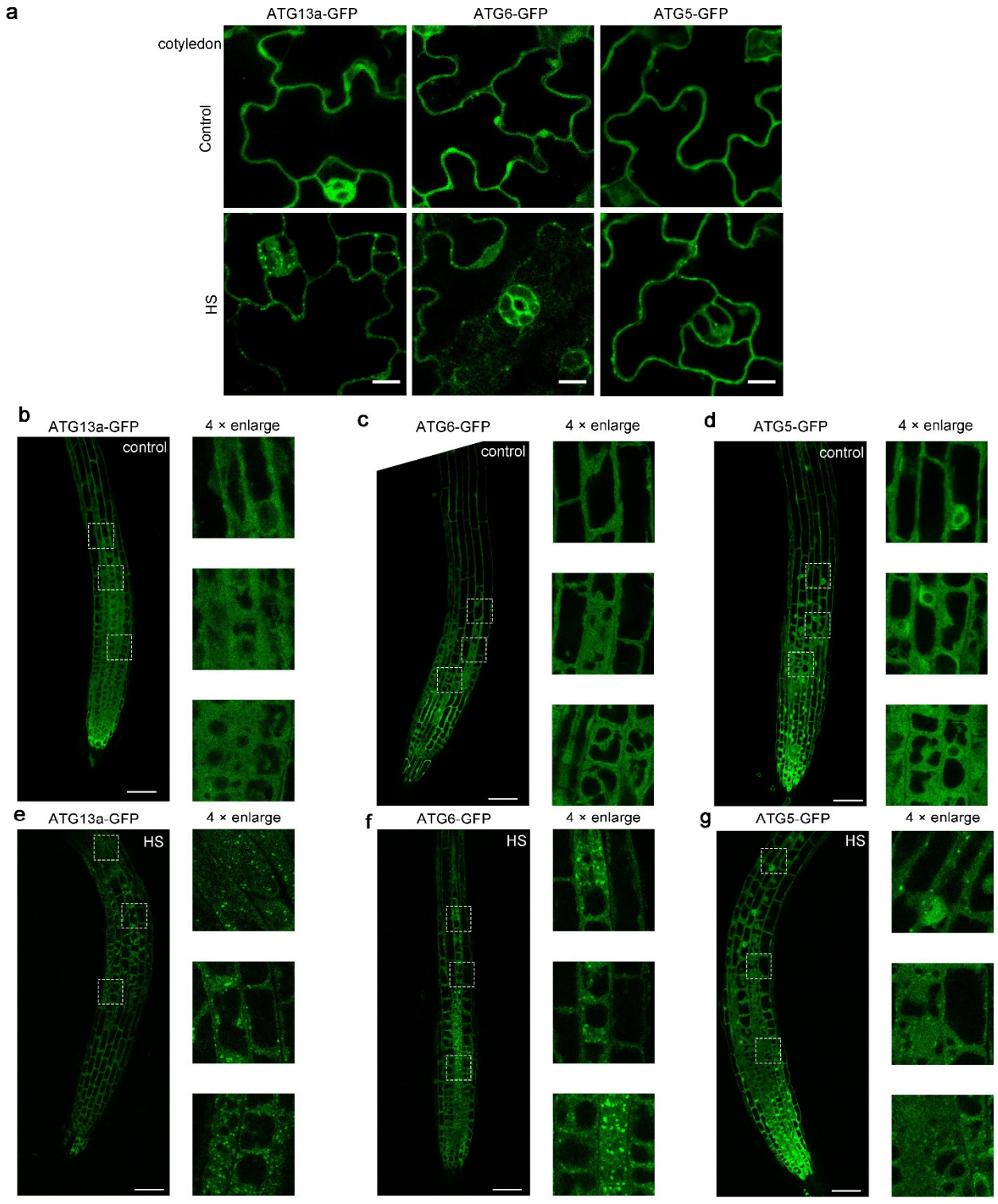
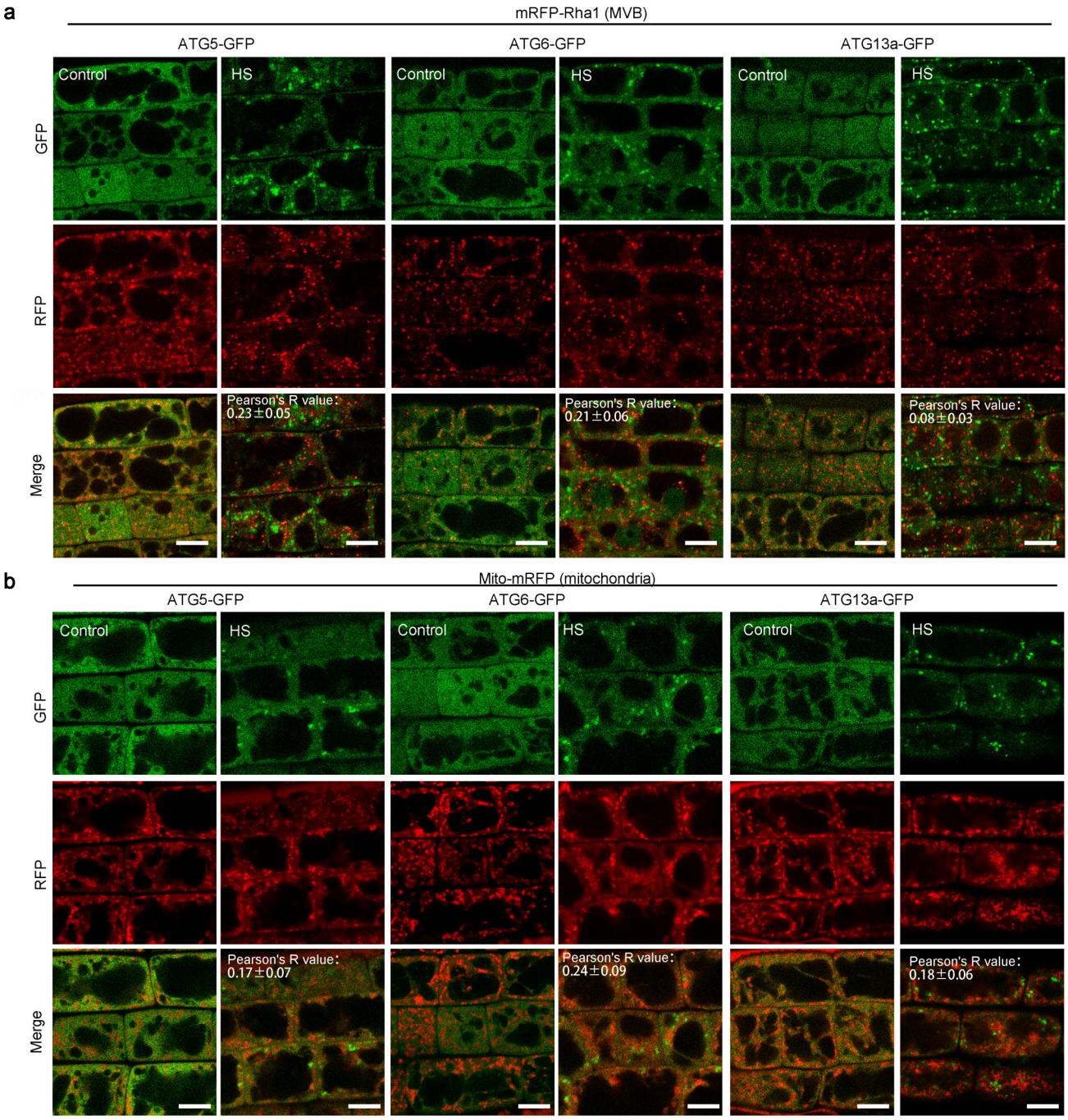


Supplementary Fig. 1 Subcellular localization analyses of autophagy-related proteins in response to HS.

(a) The 5-day-old ATG13a-GFP, ATG6-GFP, and ATG5-GFP plants were subjected to treatment

22°C (control) or 38°C (HS) for 1 h. Cotyledons of indicated samples were observed using a confocal microscope. Scale bar=10 μm.

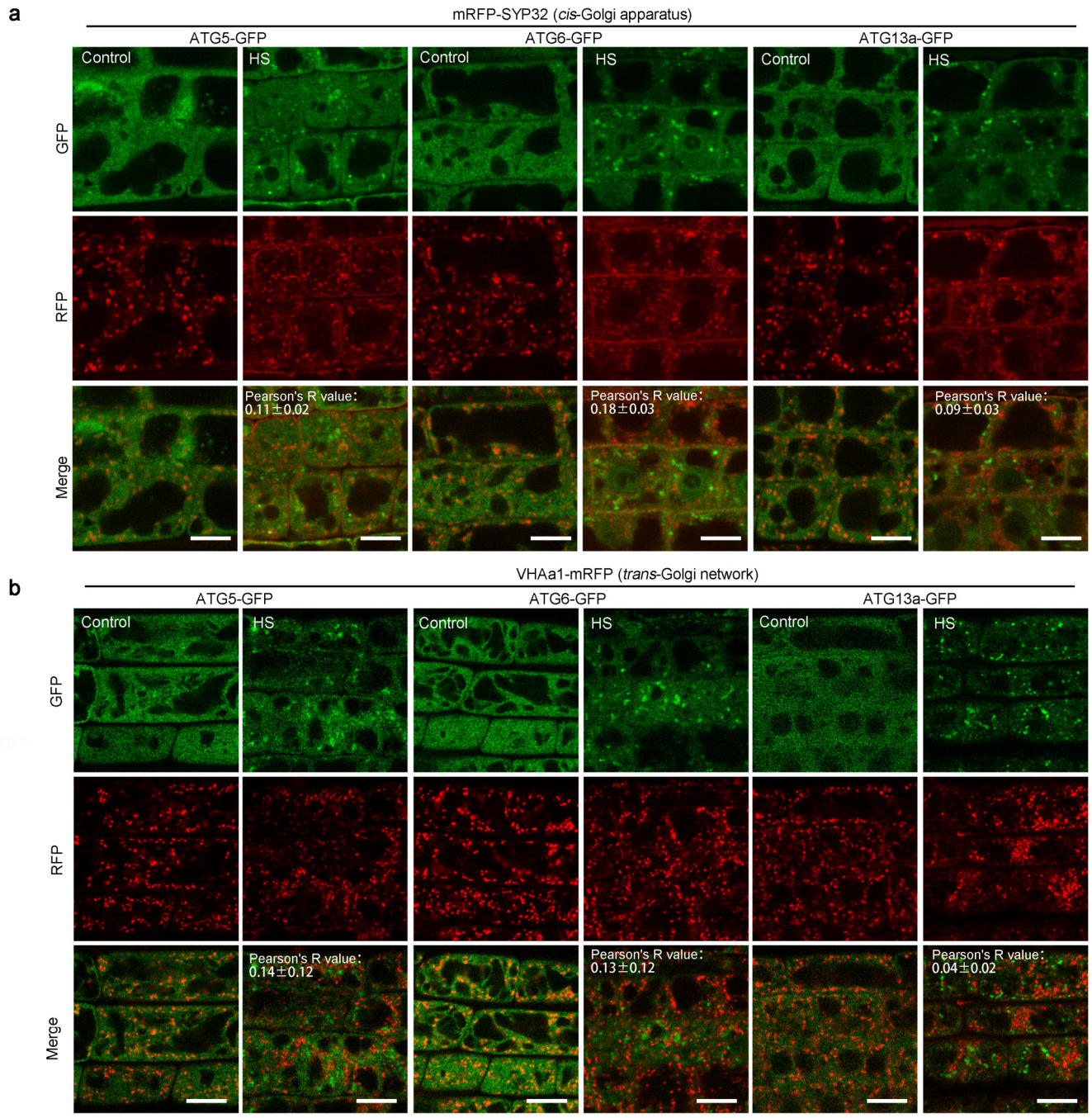
(b-g) The 5-day-old ATG13a-GFP, ATG6-GFP, and ATG5-GFP plants were subjected to treatment 22°C (control) or 38°C (HS) for 1 h. Root of indicated samples were observed using a confocal microscope. The tile scan module of Carl Zeiss LSM880 confocal imaging was used to assemble large image fields. Tiles=25. Scale bar=50 μm.



Supplementary Fig. 2 ATG proteins do not colocalize with MVB and mitochondria during HS phase.

(a) The 5-day-old hybrid plant, including the ATG13a-GFP × mRFP-Rha1 (F1), ATG6-GFP ×mRFP-Rha1 (F1), ATG5-GFP ×mRFP-Rha1 (F1) plants, and (b) ATG13a-GFP × Mtio-mRFP (F1), ATG6-GFP × Mtio-mRFP (F1), ATG5-GFP × Mtio-mRFP (F1) plants were subjected to treatment at 22°C (control) or 38°C (HS) for 1 h. Meristem zone of indicated samples were observed using a confocal microscope. mRFP-Rha1 serves as Multivesicular Body (MVB) marker. Mtio-mRFP serves as mitochondria marker. Scale bar=10 μm. The value represents the Pearson's correlation coefficient. Data represent mean ± SEM, *n*=3. Three different merged

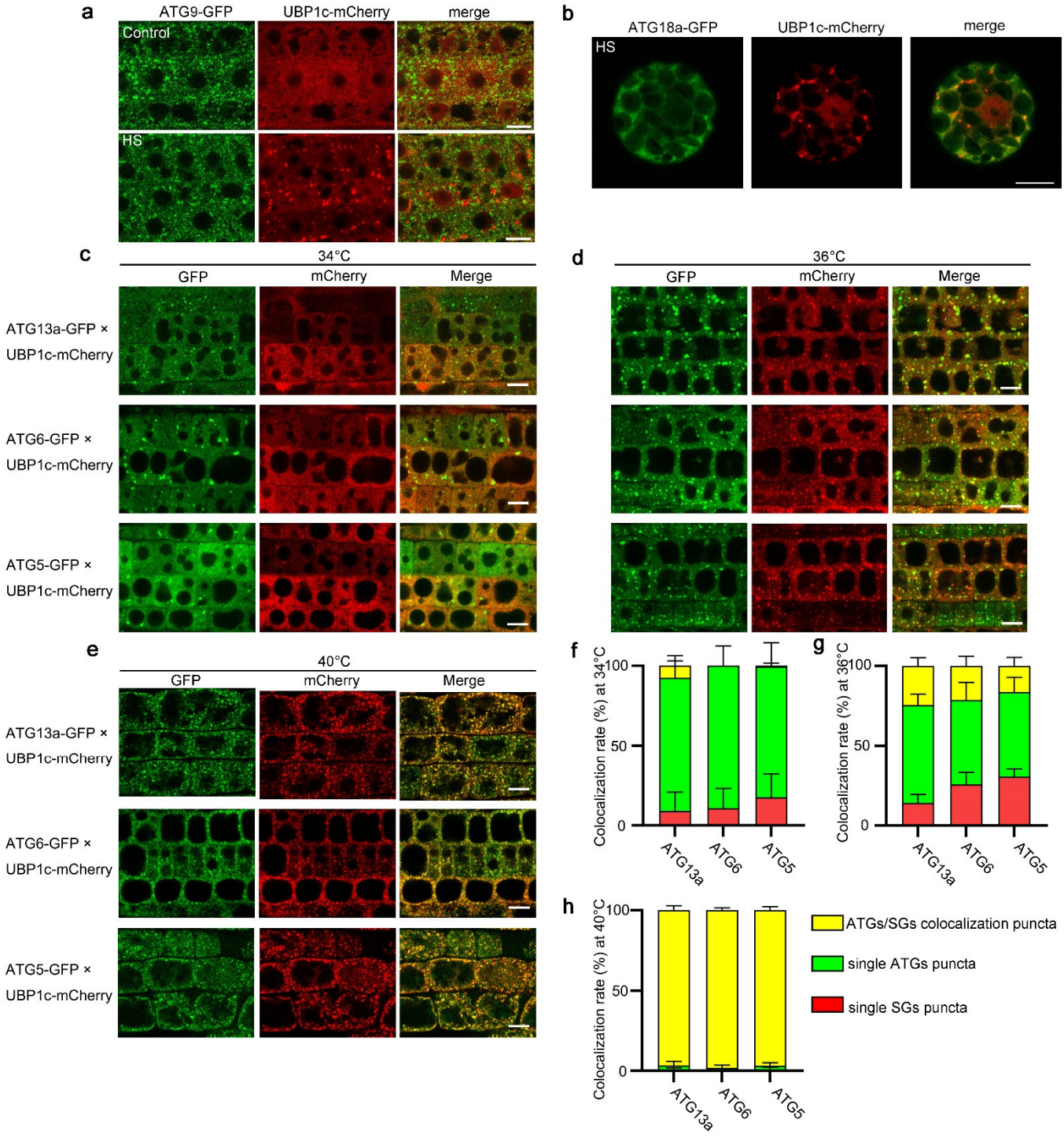
images were used for calculations. Source data are provided as a Source Data file.



Supplementary Fig. 3 ATG proteins do not colocalize with the *cis*-Golgi apparatus the *trans*-Golgi apparatus during HS phase.

(a) The 5-day-old hybrid plants, including the ATG13a-GFP × mRFP-SYP32 (F1), ATG6-GFP × mRFP-SYP32 (F1), ATG5-GFP × mRFP-SYP32 (F1) plants, and (b) ATG13a-GFP × VHAa1-mRFP (F1), ATG6-GFP × VHAa1-mRFP (F1), ATG5-GFP × VHAa1-mRFP (F1) were subjected to treatment at 22°C (control) or 38°C (HS) for 1 h. Meristem zone of indicated samples were observed using a confocal microscope. mRFP-SYP32 serves as *cis*-Golgi apparatus marker. VHAa1-mRFP serves as *trans*-Golgi network marker. Scale bar=10 μm. The values represent the

Pearson’s correlation coefficient. Data represent the mean ± SEM, *n*=3. Three different merged images were used for calculations. Source data are provided as a Source Data file.



Supplementary Fig. 4 ATG9 and ATG18a do not colocalize with SGs during HS phase.

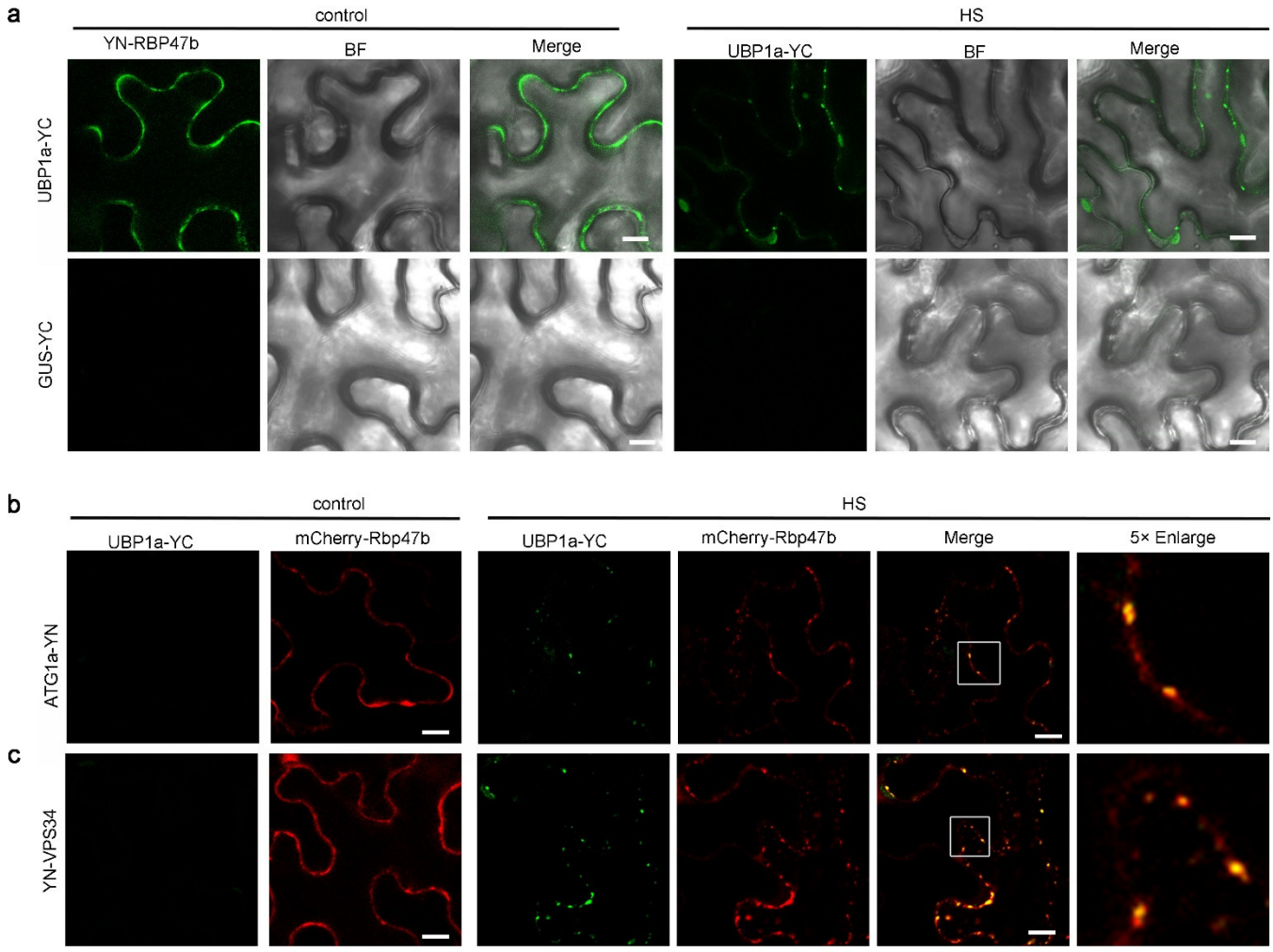
(a) The 5-day-old Arabidopsis ATG9-GFP × UBP1c-mCherry seedlings were subjected to treatment 22°C (control) or 38°C (HS) for 1 h. Meristem zone of indicated samples were observed by confocal microscope. UBP1c-mCherry serves as SGs marker. Scale bar=10 μm.

(b) The Arabidopsis protoplast co-expressing ATG18a-GFP and UBP1c-mCherry was observed

by confocal microscopy after HS treatment at 38°C for 1 h. UBP1c-mCherry serves as SGs marker. Scale bar=10 μm.

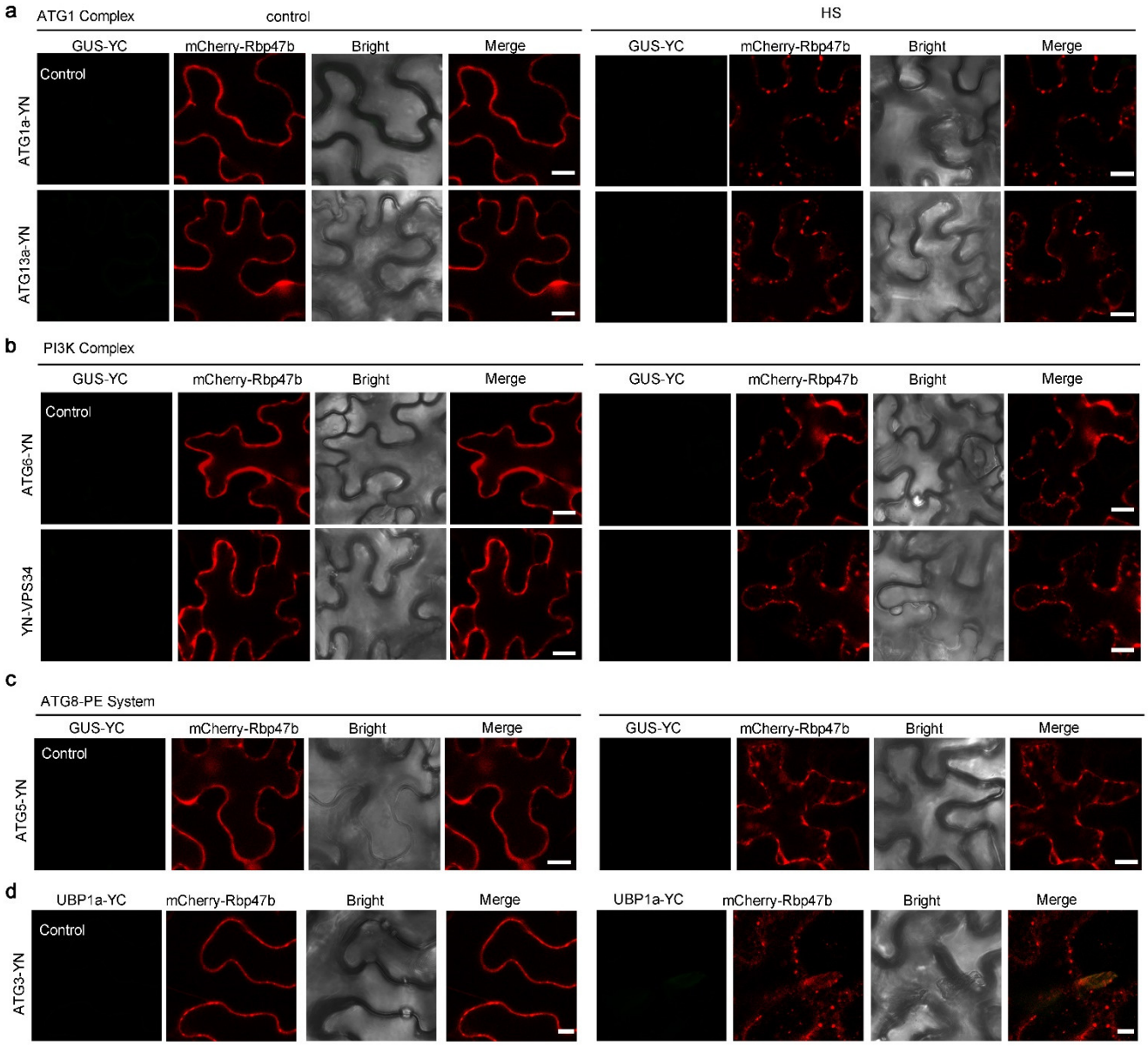
(c-e) The 5-day-old ATG13a-GFP, ATG6-GFP, and ATG5-GFP plants were subjected to HS treatment at 34°C, 36°C, or 40°C for 1 h. Meristem zone of indicated samples were observed using a confocal microscope. Scale bar=10 μm.

(f-h) Colocalization analysis of ATG protein puncta with SGs after 38°C HS in Supplementary Fig.4 c-e. Data represent the mean ± SD; *n*=8. Eight different 50×50 μm^2 areas in the meristem zone were used for the colocalization analysis. Source data are provided as a Source Data file.



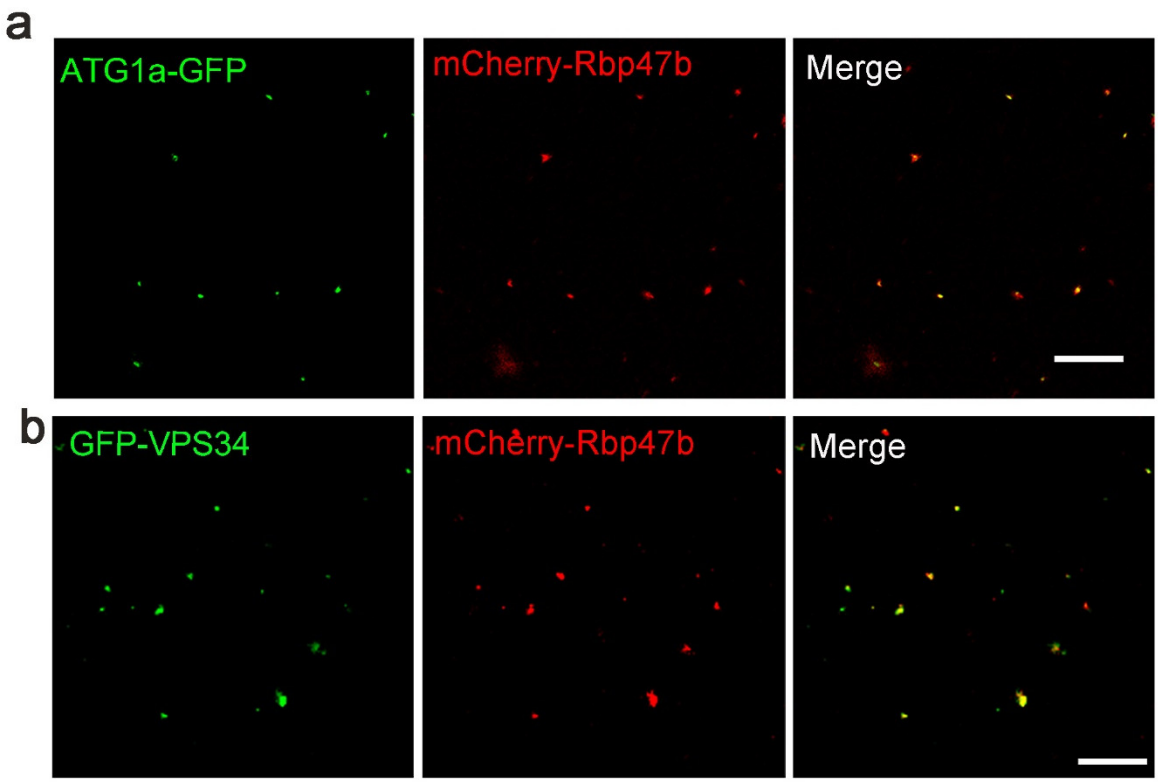
Supplementary Fig. 5 BiFC assays demonstrates the interaction of UBP1a with ATG1a, VPS34 *in vivo* under HS.

(a) UBP1a-YC and YN-RBP47b, (b-c) ATG1a-YN, or YN-VPS34 and UBP1a-YC were co-expressed in tobacco leaves. mCherry-RBP47b was used as an SGs marker. For HS treatment, the isolated tobacco leaves were placed in a 38°C incubator for 1h. Scale bar=10 μm.



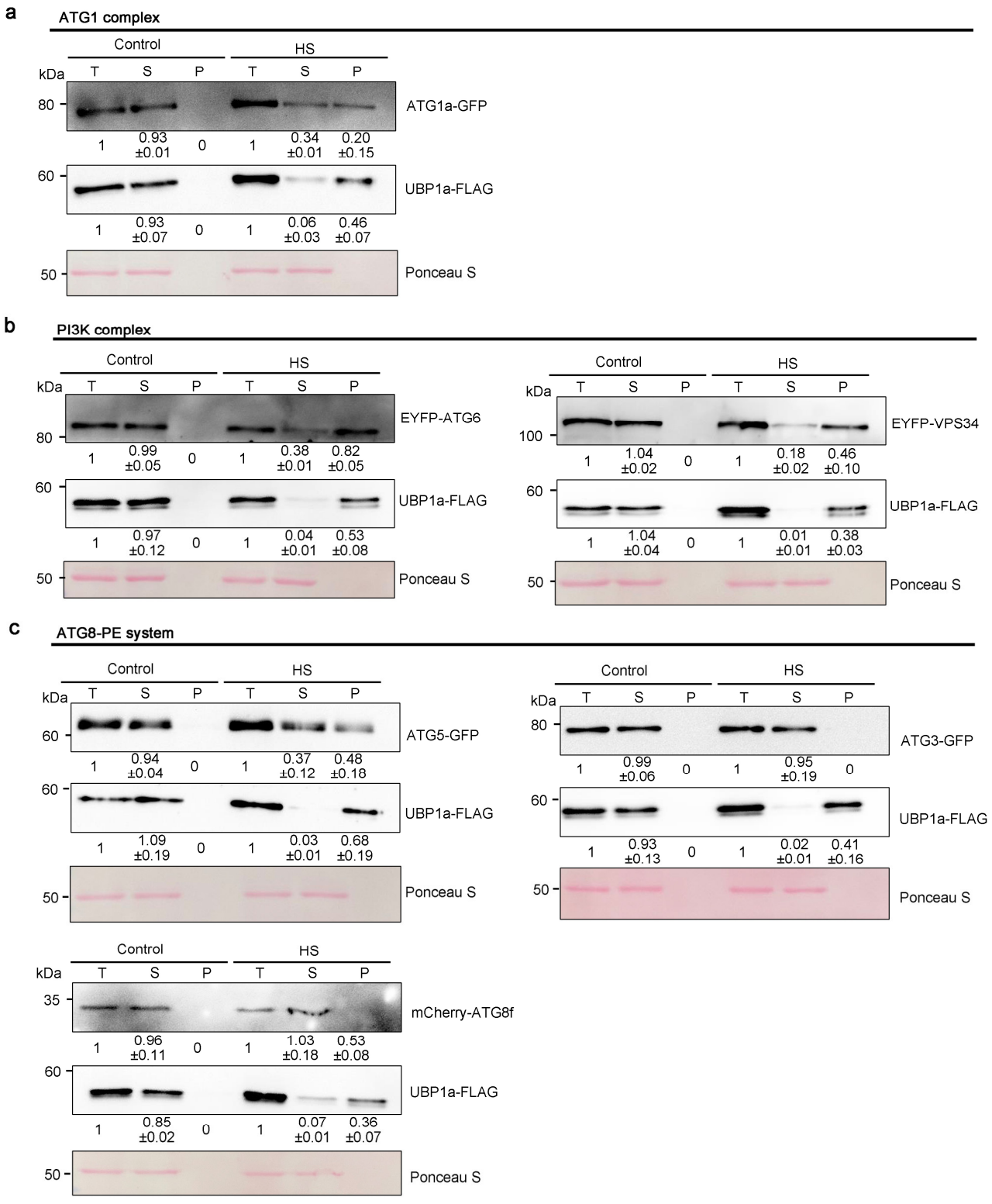
Supplementary Fig. 6 Negative control of BiFC between UBP1a with ATG protein.

(a-c) ATG13a-YN, ATG1a-YN, YN-ATG6, YN-VPS34, or ATG5-YN with GUS-YC, and (d) UBP1a-YC with ATG3-YN were co-expressed in tobacco leaves. mCherry-RBP47b was used as an SGs marker. For HS treatment, the isolated tobacco leaves were placed in a 38°C incubator for 1h. Scale bar=10 μm.



Supplementary Fig. 7 Confocal detection of SGs separated by differential centrifugal method.

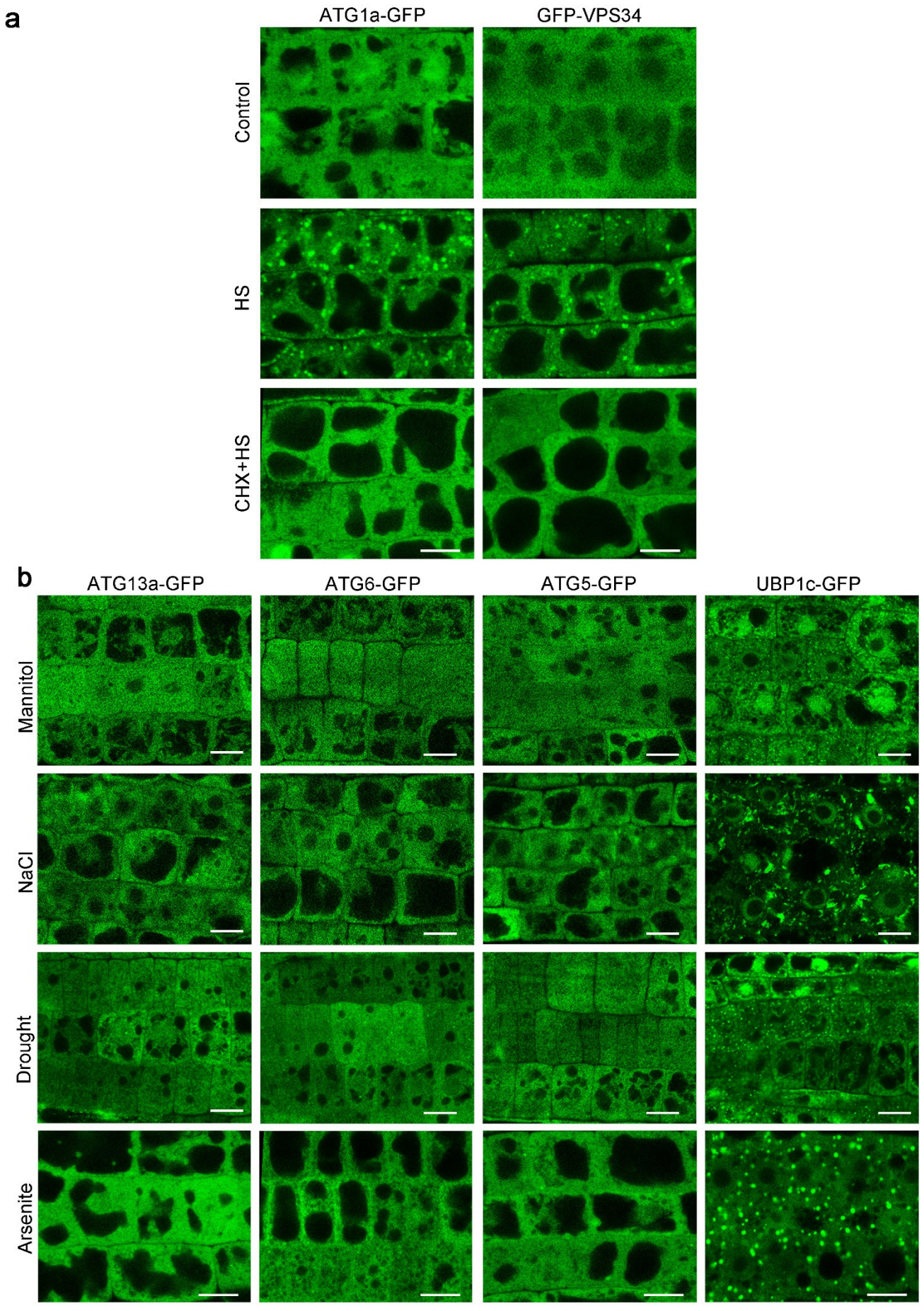
(a, b) *Arabidopsis* cells co-expressing mCherry-RBP47b and ATG1a-GFP, GFP-VPS34 were subjected to HS treatment at 38°C for 1 h, followed by differential centrifugation to separate SGs. Precipitate was re-suspended in a few of wash buffer, followed by observation using confocal microscopy. Scale bar =10 μm.



Supplementary Fig. 8 The ATG proteins were co-precipitated with SGs.

(a-c) *Arabidopsis* cells co-expressing UBP1a-FLAG/ATG1a-GFP, UBP1a-FLAG/EYFP-ATG6, UBP1a-FLAG/EYFP-VPS34, UBP1a-FLAG/ATG5-GFP, UBP1a-FLAG/ATG3-GFP, or UBP1a-FLAG/mCherry-ATG8f were subjected to treatment at 22°C (control) or 38°C (HS) for 1 h, followed

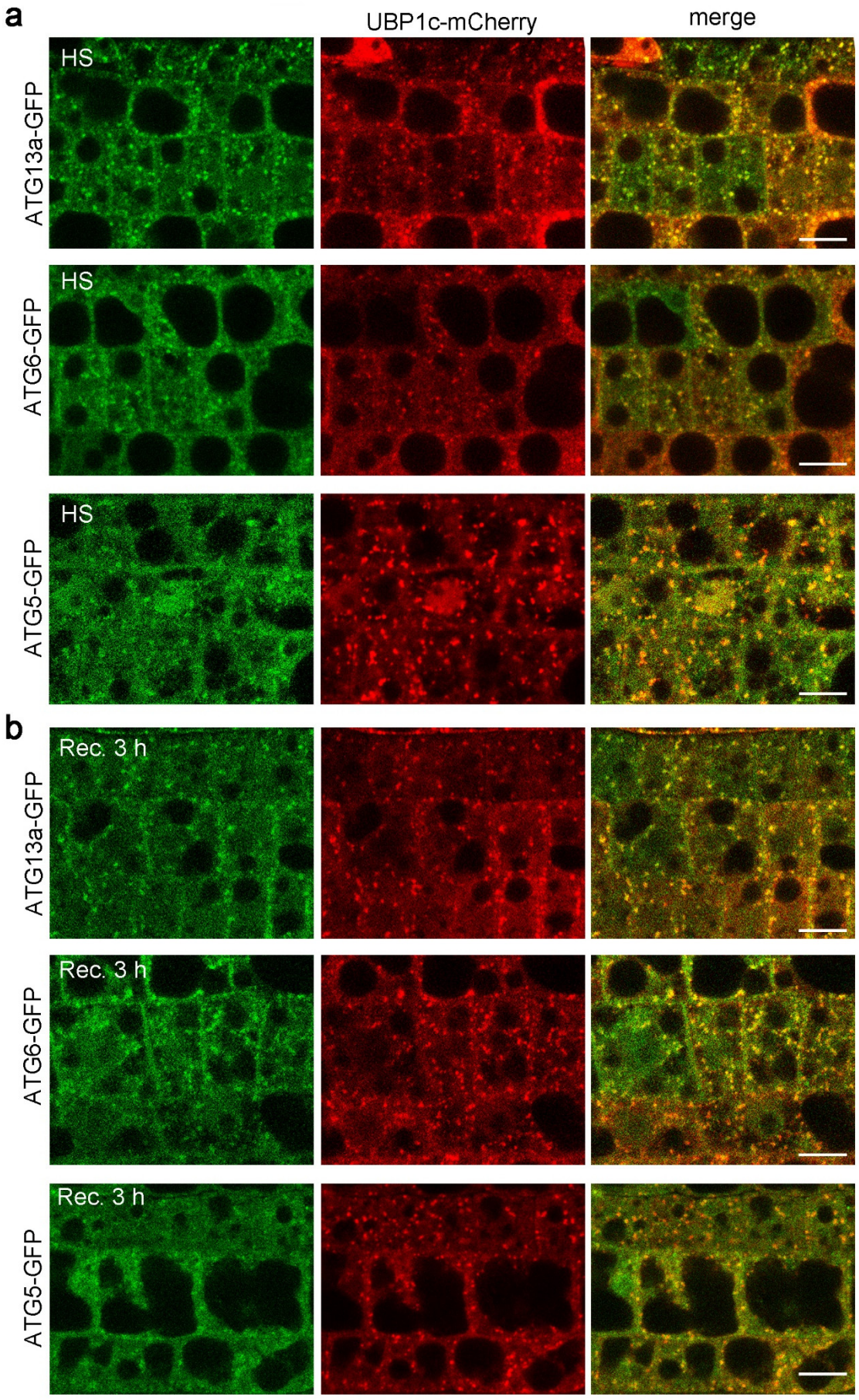
by differential centrifugation to separate SGs. Equal proportion of denatured samples (T: total protein. S: supernatant. P: precipitate) were separated by 10% SDS-PAGE, followed by immunoblotting with anti-GFP, and anti-FLAG antibody. Ponceau staining represents the Rubisco large subunit were used as the reference of loading control. The value represents the S or P vs. T. Data represent mean ± SEM; *n*=2. Source data are provided as a Source Data file.


a
ATG1a-GFP
GFP-VPS34
Control
HS
CHX+HS
b
ATG13a-GFP
ATG6-GFP
ATG5-GFP
UBP1c-GFP
Mannitol
NaCl
Drought
Arsenite

Supplementary Fig. 9 ATGs proteins do not form aggregates under other abiotic stresses

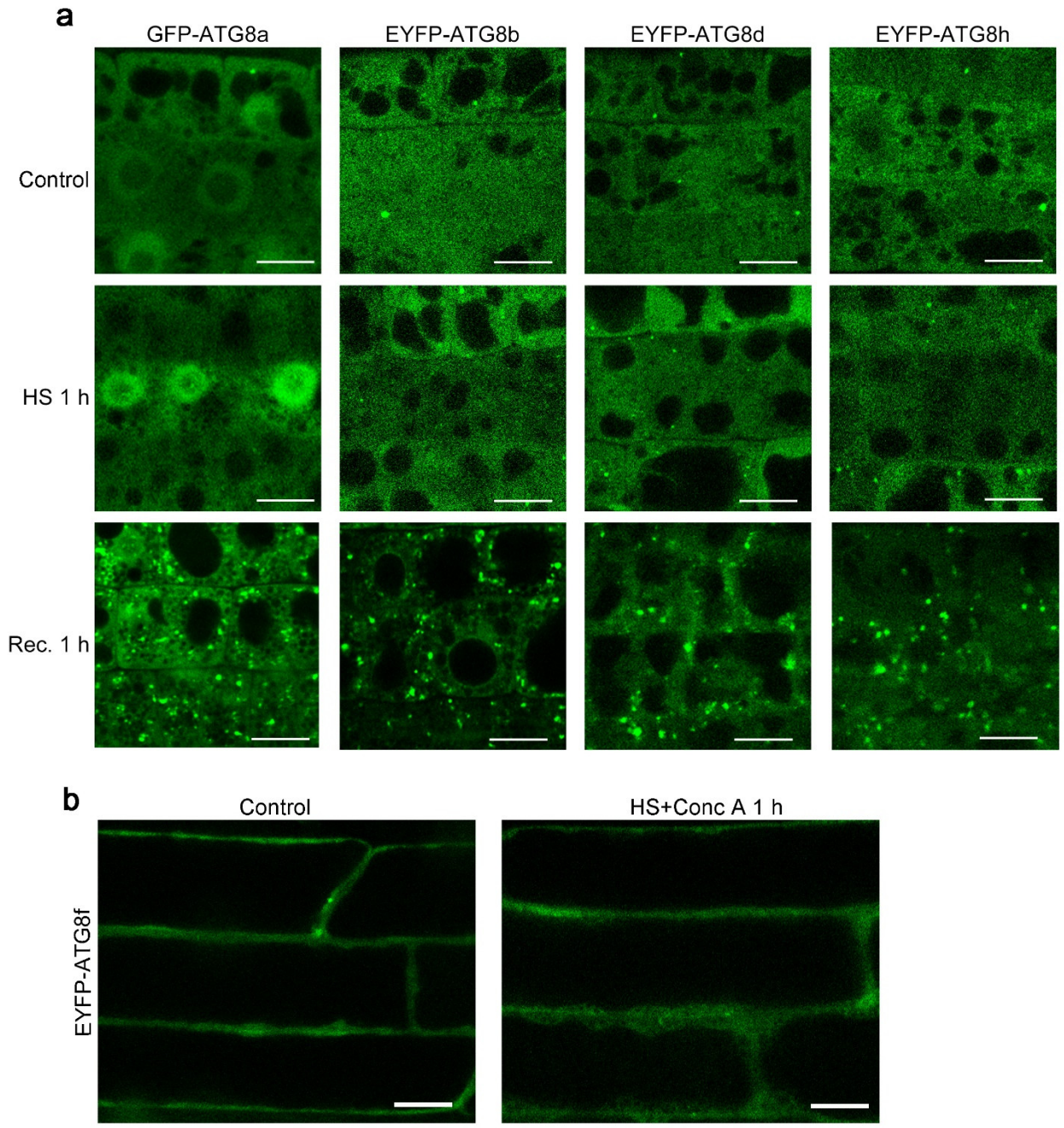
(a) The 5-days old ATG1a-GFP and GFP-VPS34 plant were subjected to 22°C (control), 38°C (HS) or 50 μM CHX + 38°C HS treatment for 1 h. Meristem zone of indicated samples were observed using a confocal microscope. Scale bar =10 μm.

(b) The 5-days old ATG13a-GFP, ATG6-GFP, ATG5-GFP, and UBP1c-GFP plants were subjected to treatment in 1/2 MS liquid medium containing 300 mM mannitol for 6 h (osmotic stress), 300 mM NaCl (salt stress) for 6 h, 20% (w/v) PEG4000 for 6 h (drought stress) and 0.5 mM Arsenite (oxidative stress) for 1 h at 22°C, respectively. Meristem zone of indicated samples were observed using a confocal microscope. Scale bar =10 μm.


a
UBP1c-mCherry
merge
HS
ATG13a-GFP
HS
ATG6-GFP
HS
ATG5-GFP
b
Rec. 3 h
ATG13a-GFP
Rec. 3 h
ATG6-GFP
Rec. 3 h
ATG5-GFP

Supplementary Fig. 10 With the HS was relieved, autophagy-related proteins was released from SGs.

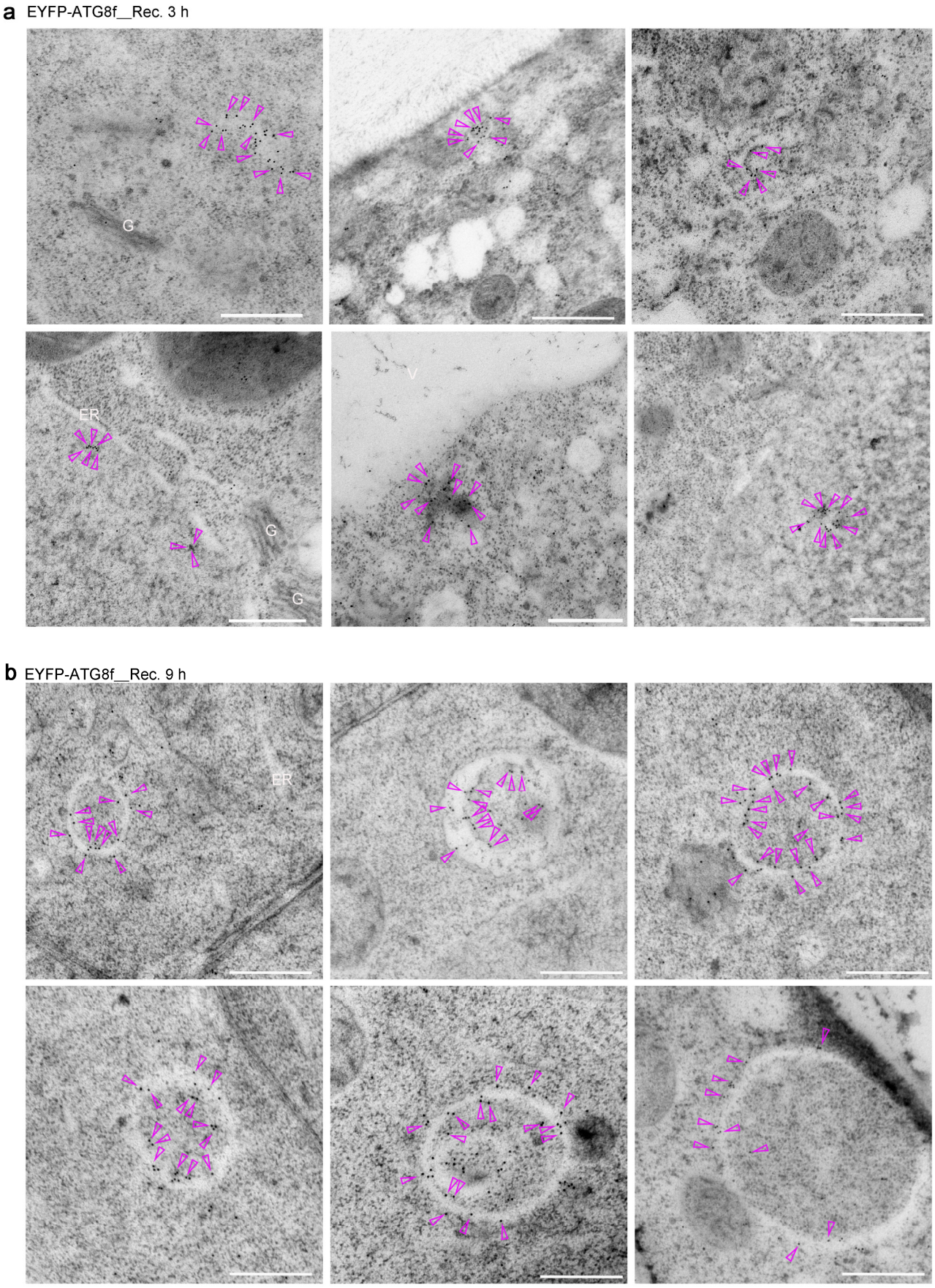
(a, b) The 5-day-old *ATG-GFP* × *UBP1c-mCherry* plants were subjected to 38°C HS 1 h, followed by recovery at 22 °C for 3 h. Root tips of indicated samples were observed using a confocal microscope. Scale bar =10 μm.



Supplementary Fig. 11 The localization pattern of the homologous protein of ATG8 in Arabidopsis is consistent in response to heat stress.

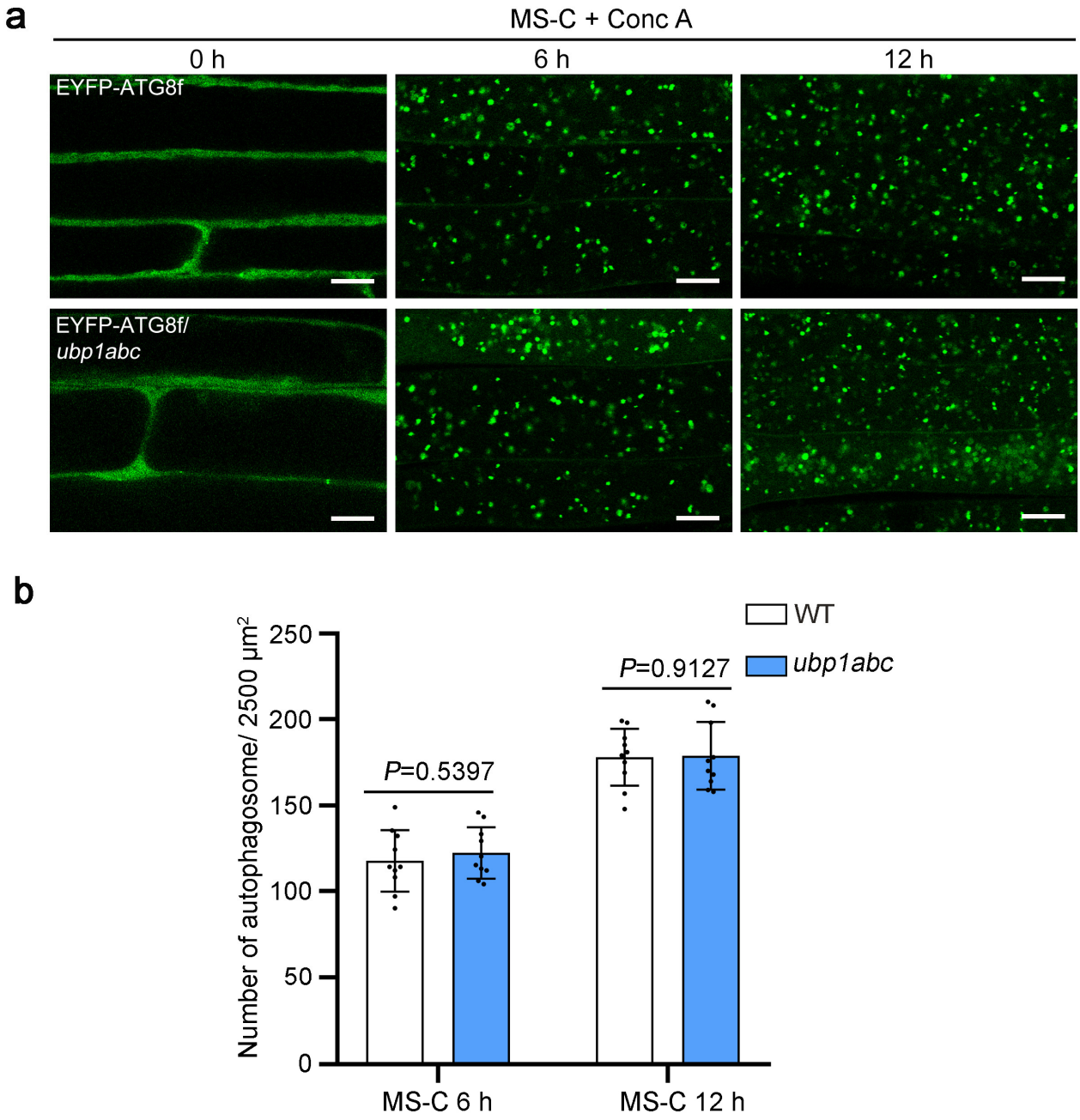
(a) The 5-day-old GFP-ATG8a, EYFP-ATG8b, EYFP-ATG8d, and EYFP-ATG8h plants were subjected to treatment at 22°C (control) or 38°C (HS) for 1 h, followed by heat stressed plants recovery at 22°C for 1 h. Meristem zone of indicated samples were observed using a confocal microscope. Scale bar =10 μm.

(b) The 5-day-old EYFP-ATG8f plants were subjected to treatment in 1/2 MS liquid medium at 22°C or supplemented with 1 μM Conc A at 38°C for 1 h. Maturation zone of the roots from the indicated samples were observed using a confocal microscope. Scale bar =10 μm.



Supplementary Fig. 12 TEM images of the HS recovery phase.

TEM image of EYFP-ATG8f plant during recovery phase. EYFP-ATG8f plants were subjected to HS treatment at 38°C for 1 h, followed by recovery at 22°C for 3 h (a) or 9 h (b). Immuno-gold labelling with GFP antibody showing the location of EYFP-ATG8f. The magenta arrowheads indicate the GFP-antibody coated gold particles. Scale bar =500 nm.

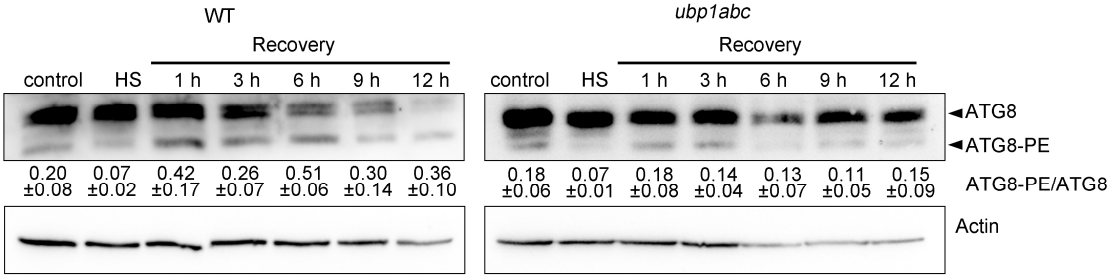


Supplementary Fig. 13 Carbon starvation-induced macroautophagy was not affected in the *ubp1abc* mutant.

(a) The 5-day-old EYFP-ATG8f and EYFP-ATG8f/*ubp1abc* plants were subjected to MS-C treatment in 1/2 MS liquid medium without sucrose supplemented with 1 μM Conc A for 6 h, 12 h in darkness. Maturation zone of the roots from the indicated samples were observed using a confocal microscope. Scale bar =10 μm.

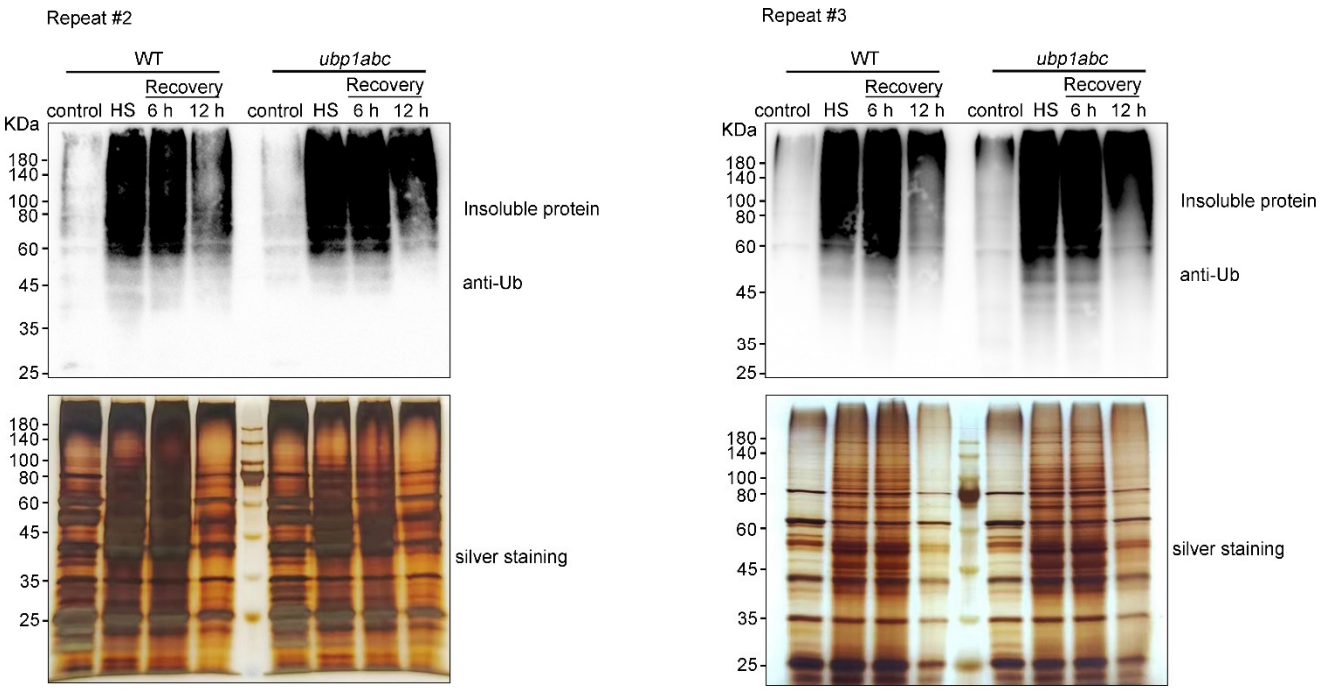
(b) Statistical analysis of number of autophagosome in Supplementary Fig. 13a. Data represent mean ± SD; *n*=10. Ten different 50×50 μm^2 area in maturation zone were used for colocalization analysis. Statistical analysis was performed using a two-tailed unpaired Student's *t* test. 'ns'

represents no significant difference. Source data are provided as a Source Data file.



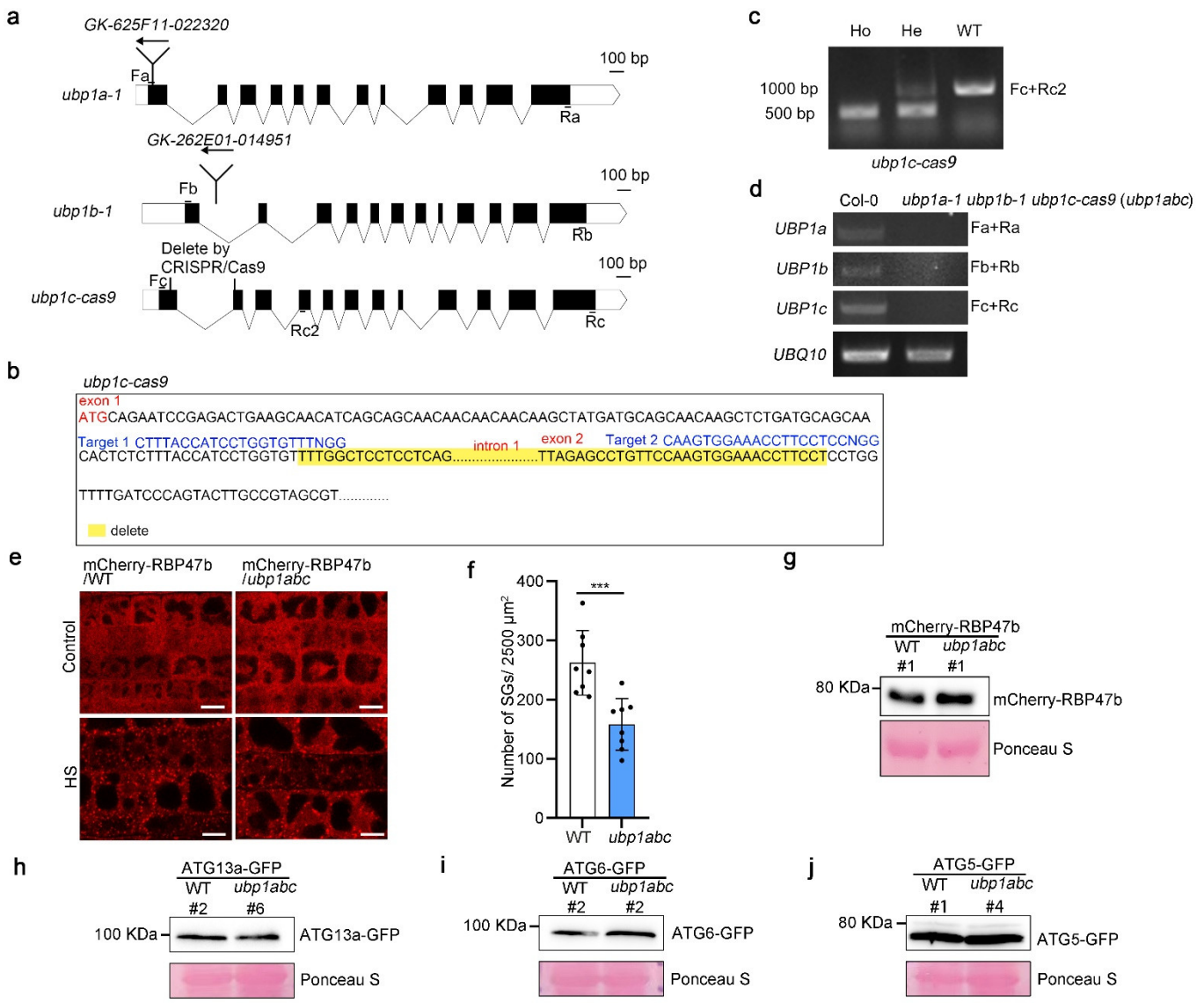
Supplementary Fig. 14 The changes of ATG8-PE modification in WT and *ubp1abc* mutants before and after heat stress.

The 5-day-old WT and *ubp1abc* plants were subjected to treatment at 22°C (control) or 38°C (HS) for 1 h and then heat stressed plants recovered at 22°C for 1h, 3h, 6 h, 9 h, and 12 h. Total proteins were extracted and then loaded onto 12% SDS-PAGE gels for detection of ATG8 and ATG8-PE, with actin as the loading control. The value represents the ATG8-PE vs. ATG8. Data represent mean ± SEM; *n*=2. Source data are provided as a Source Data file.



Supplementary Fig. 15 Detection of ubiquitinated insoluble proteins during HS and recovery phases

The 5-day-old WT and *ubp1abc* plants were subjected to treatment at 22°C (control) or 38°C (HS) for 1 h and then heat stressed plants recovered at 22°C for 6 h and 12 h. The Insoluble protein was extracted and then loaded onto 12% SDS-PAGE gels for ubiquitination detection. The same amount of insoluble protein was separated by SDS-PAGE and then stained with silver stain, which was used as a loading control.



Supplementary Fig. 16 Acquisition and identification of the *ubp1abc* triple mutant.

(a) Schematic of *ubp1a-1* locus (At1g54080), *ubp1b-1* locus (At1g17370) and *ubp1c-cas9* locus (At3g14100) gene indicate the T-DNA insertions (arrows), exons (black boxes) introns (thin lines).

(b) Schematic of *ubp1c-cas9* mutant indicate the delete region (Yellow highlight), and target 1 and target 2 (CRISPR-Cas9 target sequence).

(c) The *ubp1c-cas9* homozygous genome was identified using PCR. The Fc+Rc2 primer, labeled in Fig. S16a, was used with total DNA as the template.

(d) Semi-quantitative analysis of *ubp1abc* triple mutant (*ubp1a-1 ubp1b-1 ubp1c-cas9*) by PCR. The Fa+Ra (UBP1a), Fb+Rb (UBP1b), Fc+Rc (UBP1c) primer labeled in Fig. S16a, was used with total cDNA of *ubp1abc* as the template. cDNA of WT (Col-0) was used as a control, and *UBQ10* was used as the internal control.

(e) The 5-old-day mCherry-RBP47b and mCherry-RBP47b*/ubp1abc* were subjected to treatment at 22°C (control) or 38°C (HS) for 1 h. Meristem zone of indicated samples were observed using a confocal microscope. Scale bar=10 μm.

(f) Statistical analysis of number of punctate signals in Fig. S16e. Data represent mean ± SD; n=8. Statistical analysis was performed using a two-tailed unpaired Student's t test. ***represents P < 0.001.

(g) Transgenic lines with mCherry-RBP47b and mCherry-RBP47b*/ubp1abc* in Fig. S16e were analyzed using WB to detect protein expression. Ponceau S staining was used as a loading control.

(h-j) Transgenic lines with autophagy proteins fused to GFP tags in Fig. 2b were analyzed using WB to detect protein expression. Ponceau S staining was used as a loading control. Source data are provided as a Source Data file.

Supplementary Table 1. Primers used in This study.	
Usage	Sequece(5' to 3')
Transgenic assay	
pUBQ10::UBP1c-mCherry/GFP	
Forward	CGCGGATCCATGCAGAATCCGAGACTGAAGCAAC
Reverse	CGCGGTACCCTGATAGTACATGAGTTGCTGTGCG
pUBQ10::UBP1a-FLAG/YC	
Forward	CCCGGATCCATGCAGAATCAAAGGCTTAT
Reverse	CCCGGTACCCTGATAGTACATGAGCTGCT
pUBQ10::mCherry/YN-RBP47b	
Forward	GGGACTAGTATGCAGACAACCAACGGCTC
Reverse	TATCGATGCCCACCCATTCTCCCCATGATAGTTGTTG
pUBQ10::GUS-YC	
Forward	CGCGGATCCATGGTAGATCTGAGGGTAAATTT
Reverse	CGCGGTACCTTGTTTGCCTCCCTGCTG
pUBQ10::ATG6-GFP/FLAG/YN	
Forward	CGCGGATCCATGAGGAAAGAGGAGATTCCA
Reverse	GCTATGACAGAATTCTTTCACACAGGAAACAGCTAT
pUBQ10::ATG1a-GFP/YN	
Forward	CGCGGATCCATGGAGTCGGCACGACTT
Reverse	CGCGGTACCAAGATGAGACCGACGATGCTG
pUBQ10::ATG13a-GFP/FLAG/YN	
Forward	CGCGGATCCATGGATTTTCCAGAGAATTTGCCTTCA
Reverse	GCCGGTACCGTGGACGCGAGTTGGTCCAGA
pUBQ10::ATG5-GFP/FLAG/YN	
Forward	GCCGGATCCATGGCGAAGGAAGCGGTCAAG
Reverse	ACTAGTCCTTTGAGGAGCTTTCACAAG
pUBQ10::EYFP/YN-VPS34	
Forward	CGCACTAGTATGGGTGCGAACGAGTTTCGT
Reverse	CGCGGTACCTCAACGCCAGTATTGAGCCCA
pUBQ10::ATG3-GFP/YN	
Forward	CGCGGATCCATGGTGCTTTCGCAAAAGCT
Reverse	GCCGGTACCGGTGCTTGAGCTACCGAGAT
pUBQ10::ATG7-GFP	
Forward	GGGACTAGTATGGCTGAGAAAGAAACTCCA
Reverse	GCCGGTACCAAGATCTACAGCTACATCGTC
Genotyping	
ubp1a-LP/ *ubp1c*-Rc2	ATCTTCGATCAAAGTAGTGAACAAA
ubp1a-RP	CTTGGGCTTGATCCGTCAAC
ubp1b-LP	ACACTGCGGCAAGTACTTGG
ubp1b-RP	CGTAAGAGCCACTCGATTGC
ubp1c-Fc	CGCGGATCCATGCAGAATCCGAGACTGAAGCAAC
semi-Qutitative PCR	
ubp1a-Fa	CCCGGATCCATGCAGAATCAAAGGCTTAT
ubp1a- Ra	CCCGGTACCCTGATAGTACATGAGCTGCT
ubp1b-Fb	GGGACTAGTATGCAGAGGTTGAAGCAGCA
ubp1b-Rb	CCCGGTACCCTGGTAGTACATGAGCTGCT
ubp1c-Fc	CGCGGATCCATGCAGAATCCGAGACTGAAGCAAC
ubp1c-Rc	CGCGGTACCCTGATAGTACATGAGTTGCTGTGCG
CRISPR/Cas9 for UBP1c	
Target 1	ATATATGGTCTCGATTGCTTTACCATCCTGGTGTTTGTTTTAGAGC TAGAAATAGC
Target 2	ATTATTGGTCTCGAAACGGAGGAAGGTTTCCACTTGCAATCTCTT AGTCGACTCTAC