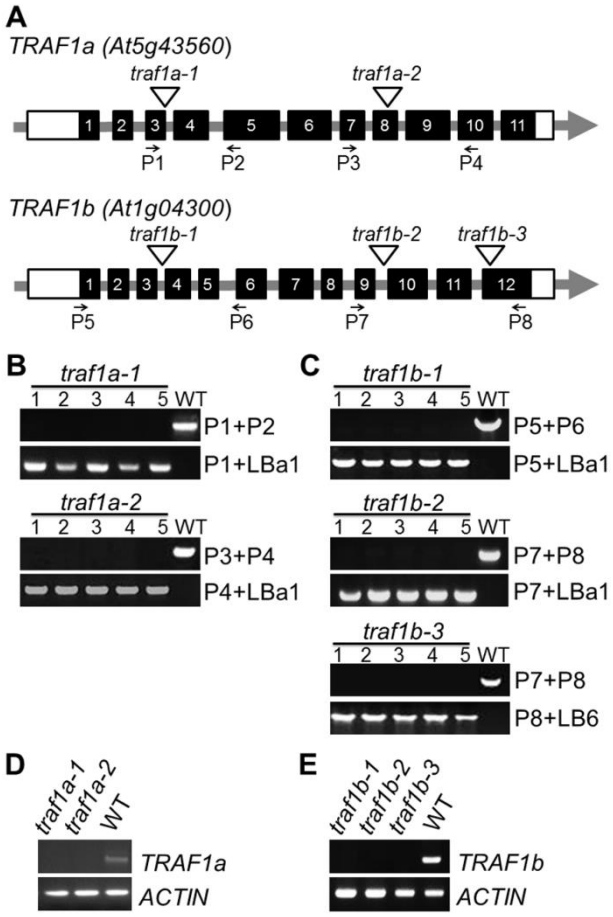
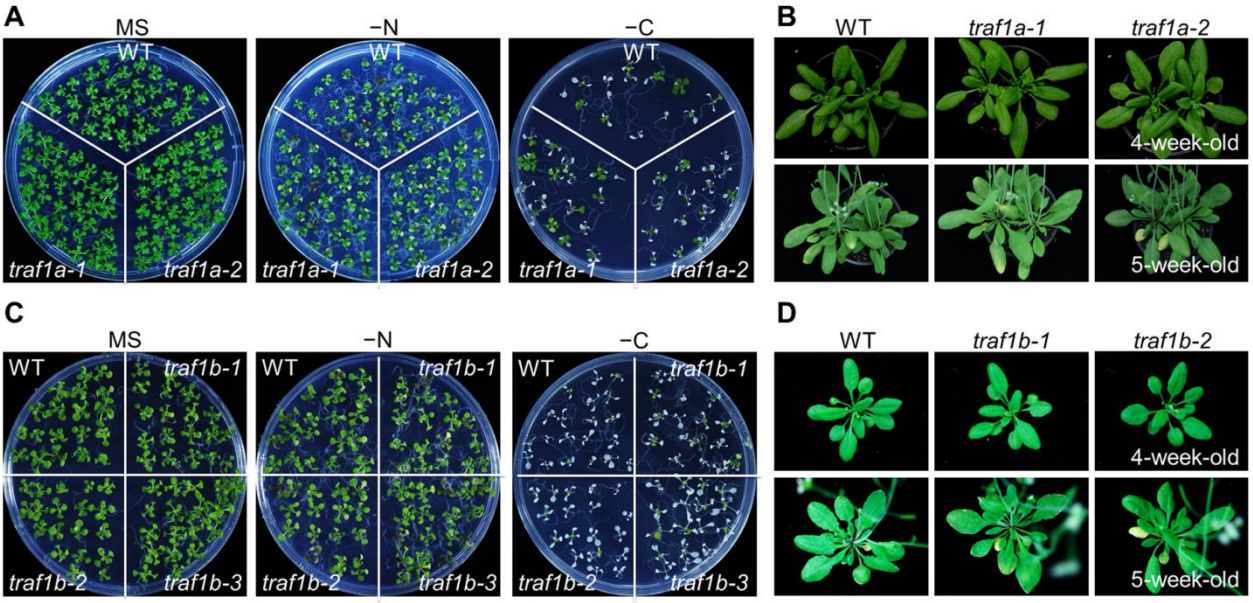


Supplemental Figure 1. Molecular Identification of *TRAF1a* and *TRAF1b* Knockout Mutants.

(A) T-DNA insertion sites in *traf1a-1* (SALK_059309C)*, traf1a-2* (SALK_120387)*, traf1b-1* (SALK_146938C), *traf1b-2* (SALK_035658), and *traf1b-3* (CS821400). Primers used for genotyping are indicated by arrows. White and black boxes indicate UTRs and exons, respectively. Lines between black boxes indicate introns.

(B) and (C) Genotyping of the *traf1a* (*traf1a-1* and *traf1a-2*) (B) and *traf1b* (*traf1b-1*, *traf1b-2*, and *traf1b-3*) mutants (C) by PCR analysis. Genomic DNA isolated from wild type (WT), *traf1a-1*, *traf1a-2, traf1b-1*, *traf1b-2*, and *traf1b-3* was amplified using the primer pairs indicated on the right. The primers are listed in Supplemental Table 2.

(D) and (E) RT-PCR analysis showing the absence of *TRAF1a* and *TRAF1b* expression in *traf1a* (D) and *traf1b* (E). Total RNA isolated from WT, *traf1a*, and *traf1b* was used for RT-PCR using the primer pairs shown in Supplemental Table 2. The expression of *ACTIN2* was used as a control.



Supplemental Figure 2. Phenotypic Characterization of *traf1a* and *traf1b* Single Mutants.

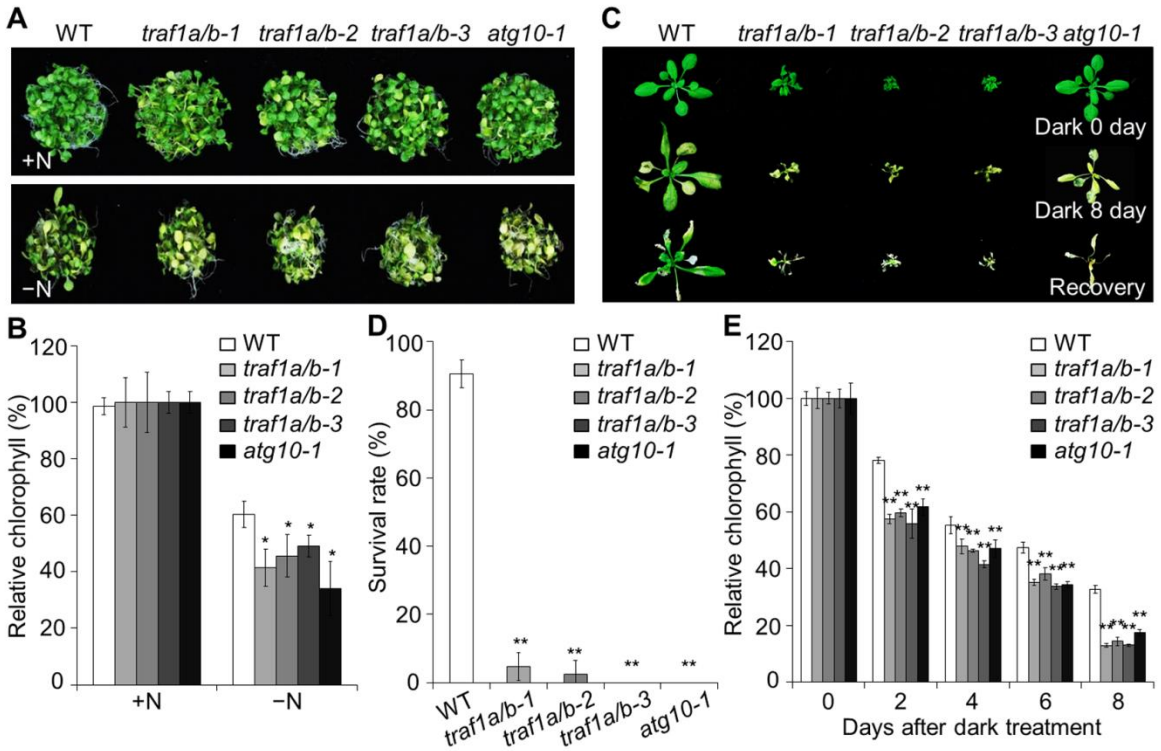
(A) Images of wild-type (WT) and *traf1a* mutant (*traf1a-1* and *traf1a-2*) seedlings grown on MS medium, MS medium without nitrogen (–N), and MS medium without sugar followed by darkness (–C) treatment.

(B) Images showing the onset of leaf senescence in WT and *traf1a* mutant (*traf1a-1* and *traf1a-2*) grown under normal light/dark growth conditions. Photographs were taken at 4 and 5 weeks after germination.

(C) Images of WT and *traf1b* mutant (*traf1b-1*, *traf1b-2*, and *traf1b-3*) seedlings grown on MS medium, MS medium without nitrogen (–N), and MS medium without sugars followed by darkness (–C) treatments.

(D) Images showing the onset of leaf senescence in WT and *traf1b* mutant (*traf1b-1* and *traf1b-2*) grown under normal light/dark growth conditions. Photographs were taken at 4 and 5 weeks after germination.

Seeds of WT, *traf1a* mutants (*traf1a-1* and *traf1a-2*), and *traf1b* mutants (*traf1b-1*, *traf1b-2*, and *traf1b-3*) were germinated on solid MS medium with 2% sucrose for 7 d. For nitrogen starvation treatment, 7-d-old seedlings grown on MS were transferred to MS or N-deficient medium for 5 d under a normal light/dark cycle. For carbon starvation treatment, 7-d-old seedlings were transferred to MS or MS without sucrose (–C) and grown in constant darkness for 10 d, followed by exposure to normal light/dark conditions for a 7-d recovery.



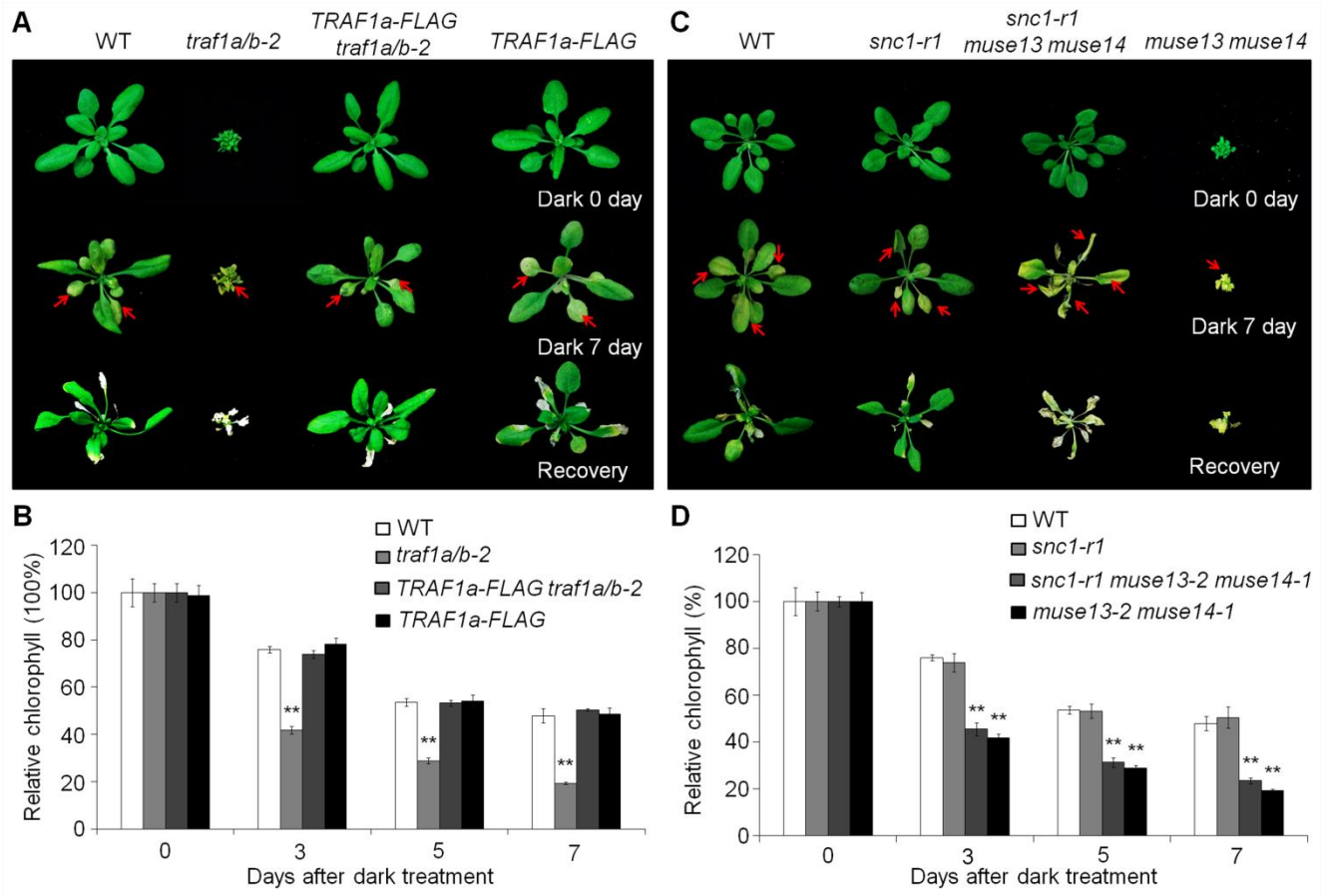
Supplemental Figure 3. Phenotypic Analyses of *traf1a/b* Double Mutants.

(A) Sensitivity of wild-type (WT), *traf1a/b* plants (*traf1a/b-1*, *traf1a/b-2*, and *traf1a/b-3*), and *atg10-1* to nitrogen starvation. Seedlings were grown for 1 week on MS liquid medium, followed by transfer to nitrogen-rich (+N) or nitrogen-deficient (–N) medium for an additional 4 d.

(B) Relative chlorophyll contents of seedlings with or without nitrogen starvation shown in (A). Bars represent the average ± SD (n = 3) of three biological replicates. For each technical replicate, at least 15 seedlings were pooled for chlorophyll extraction.

(C) Sensitivity of soil-grown WT, *traf1a/b* (*traf1a/b-1*, *traf1a/b-2*, and *traf1a/b-3),* and *atg10-1* plants to carbon starvation. Three-week-old plants were grown in normal light/dark conditions (+C), followed by growth in constant darkness for 8 d (–C). The plants were recovered for 7 d (Recovery) before photographing.

(D) and (E) Survival rates (D) and relative chlorophyll contents (E) of plants after 8-d carbon starvation treatment. Data are average values ± SD (n = 3) calculated from three independent experiments. For each experiment, 5 whole plants (technical replicates) were used per genotype. Asterisks indicate significant differences from WT (*P < 0.05 **P < 0.01 by Student's t-test).



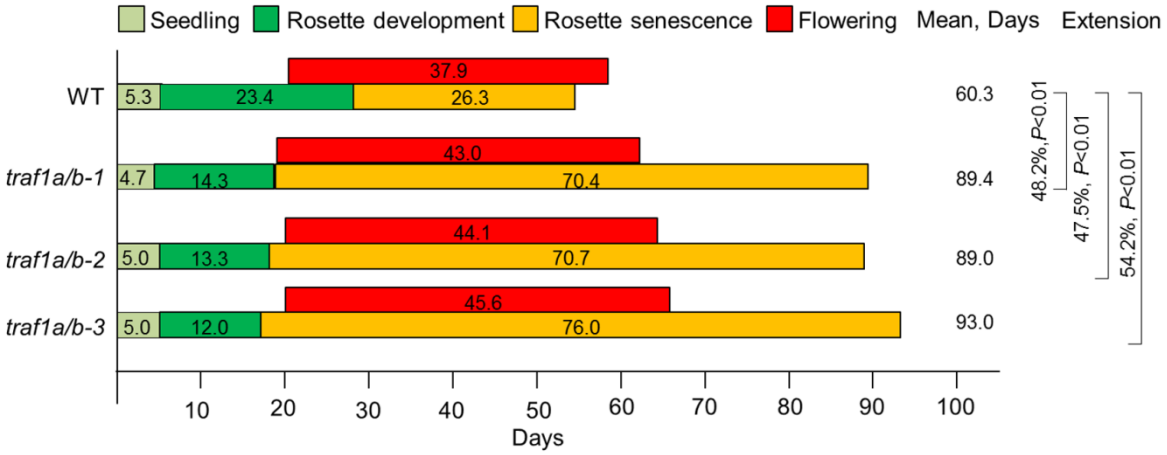
Supplemental Figure 4. Phenotypic Analyses of *TRAF1a traf1a/b-2* and *snc1-r1 muse13-2 muse14-1* Mutants.

(A) Sensitivities of soil-grown wild-type (WT), *TRAF1a-FLAG traf1a/b-2* and *traf1a/b-2* mutants to carbon starvation. Three-week-old plants were grown in normal light/dark conditions (0 day), followed by growth in constant darkness for 7 d (7 day). The plants were recovered for 7 d (Recovery) before photographing.

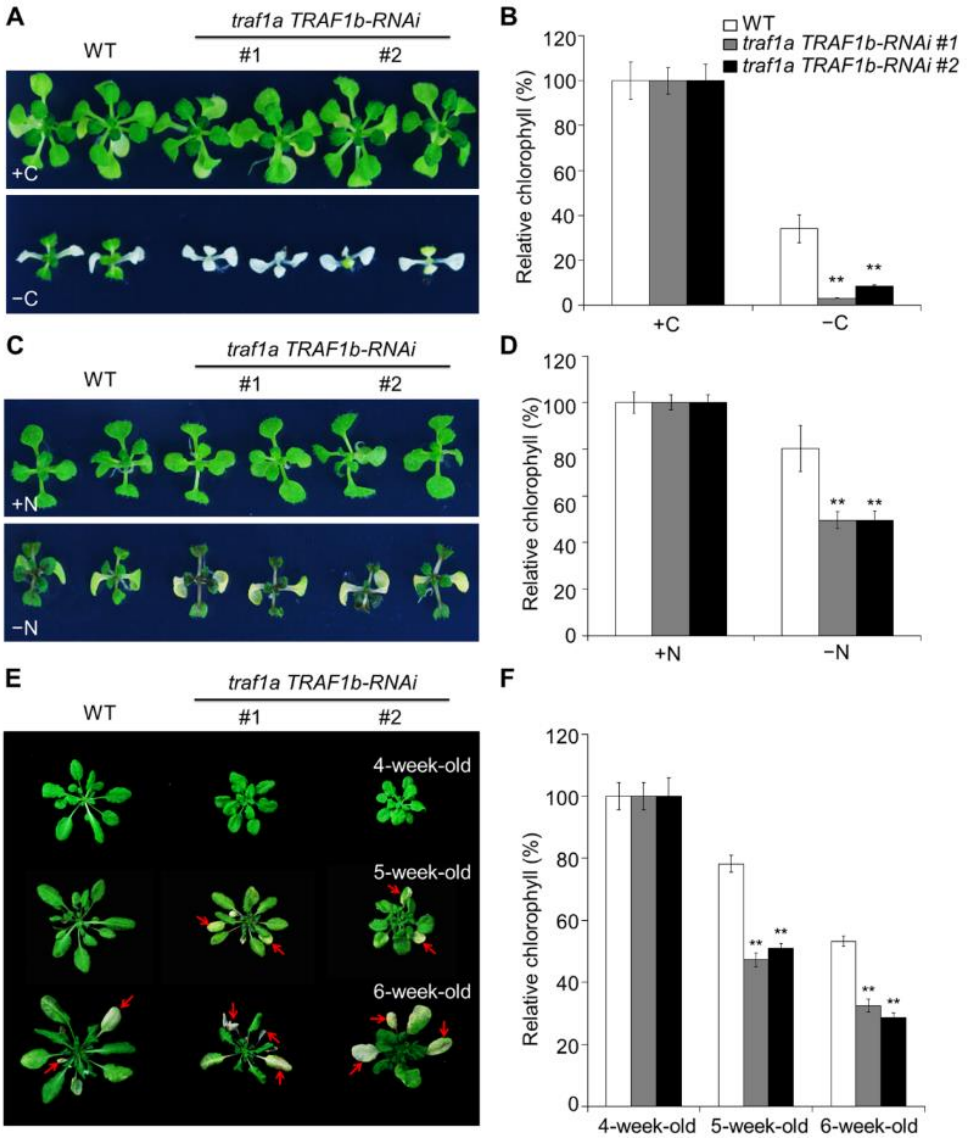
(B) Relative chlorophyll contents of plants in (A) after carbon starvation treatment for 0, 3, 5, and 7 d. Data are average values ± SD (n = 3) calculated from three independent experiments. For each experiment, 5 whole plants (technical replicates) were used per genotype. Asterisks indicate significant differences from WT (**P < 0.01 by Student's t-test).

(C) Sensitivities of soil-grown WT, *snc1-r1 muse13-2 muse14-1, muse13-2 muse14-1* and *snc1-r1* plants to carbon starvation. Three-week-old plants were grown in normal light/dark conditions (0 day), followed by growth in constant darkness for 7 d (7 day). The plants were recovered for 7 d (Recovery) before photographing.

(D) Relative chlorophyll contents of plants in (D) after carbon starvation treatment for 0, 3, 5, and 7 d. Data are average values ± SD (n = 3) calculated from three independent experiments. For each experiment, 5 whole plants (technical replicates) were used per genotype. Asterisks indicate significant differences from WT (**P < 0.01 by Student's t-test).



Supplemental Figure 5. Lifespan of the Wild Type (WT) and *traf1a/b* Mutants.
Life cycles of WT and *traf1a/b* mutants were measured under LD conditions. The x-axis indicates days after radicle emergence. Numbers on the horizontal bars indicate mean length (days) of the developmental stages. *P* values indicate the probability of lifespan extension by LD. The seedling stage indicates the plants from radical emergence to 2 rosette leaves > 1 mm; the rosette development stage indicates the plants from 2 rosette leaves > 1 mm to the beginning of senescence. The rosette senescence stage indicates the plants from the beginning of senescence to the completion of senescence, and the flowering stage indicates the plants from inflorescence emergence to the end of flowering.



Supplemental Figure 6. Phenotypic Analyses of *traf1a TRAF1b-RNAi* Transgenic Plants.

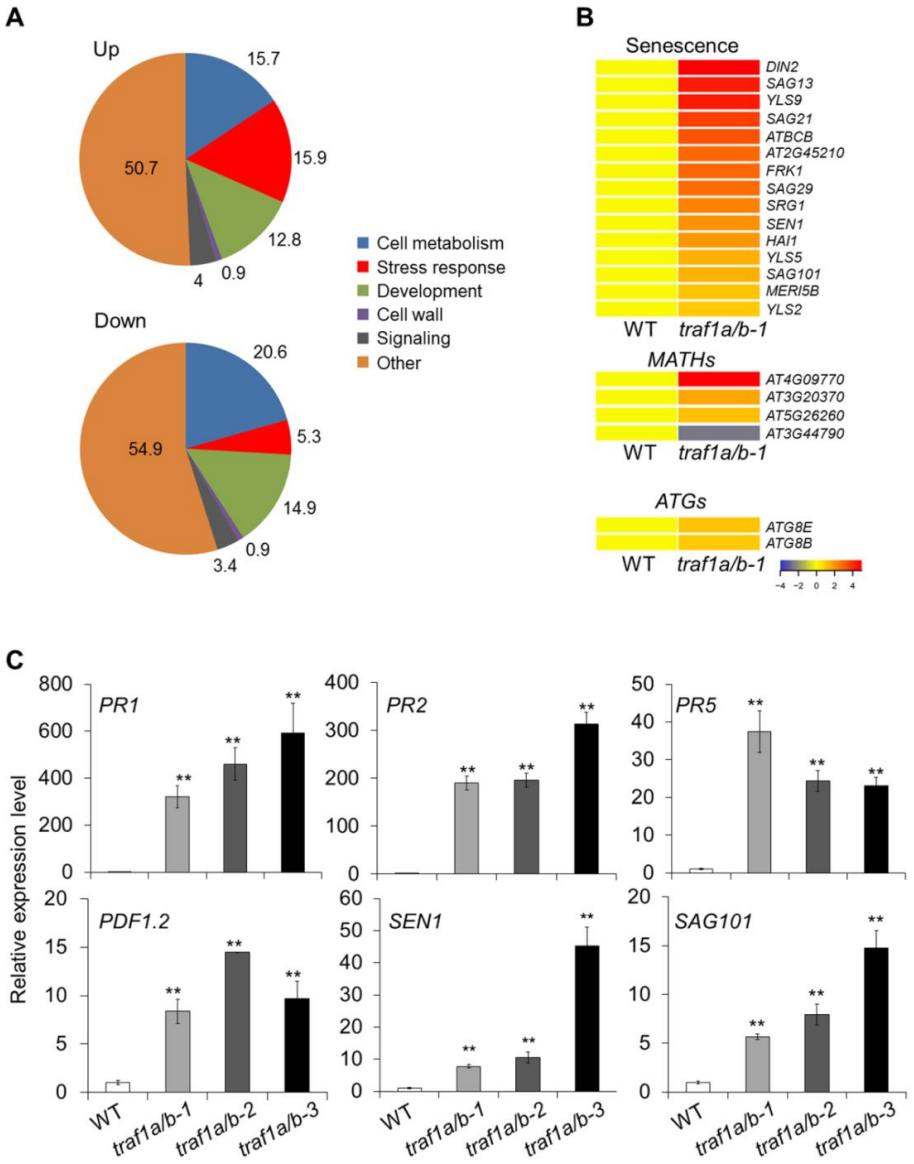
(A) Phenotypes of *traf1a TRAF1b-RNAi* in response to carbon starvation. One-week-old wild-type (WT) and *traf1a TRAF1b-RNAi* seedlings grown on MS solid medium for 1 week. The seedlings were transferred to MS agar with sucrose (+C) or MS agar plates without sucrose followed by constant dark treatment (-C) for 7 d. The images were recorded after a 7-d recovery.

(B) Relative chlorophyll contents of WT and *traf1a TRAF1b-RNAi* seedlings upon 7-d carbon starvation following recovery. The relative chlorophyll contents were calculated by comparing the values in –C seedlings versus +C seedlings. Data are average values ± SD (n = 3) calculated from three independent experiments. For each experiment, 5 technical replicates pooled with 20 seedlings were used per genotype.

(C) Phenotypes of *traf1a TRAF1b-RNAi* in response to nitrogen starvation. One-week-old wild-type (WT) and *traf1a TRAF1b-RNAi* seedlings grown on MS solid medium for 1 week. The seedlings were transferred to N-rich (+N) or N-deficient (–N) medium and photographed at 5 d after treatment.

(D) Relative chlorophyll contents of WT and *traf1a TRAF1b-RNAi* seedlings upon 5-d nitrogen starvation. The relative chlorophyll contents were calculated by comparing the values in –N seedlings versus +N seedlings. For each experiment, 5 technical replicates pooled with 20 seedlings were used per genotype.

(E) Images showing the onset of leaf senescence in wild type (WT) and *traf1a TRAF1b-RNAi* lines grown under short day (SD, 8h light/16h dark) growth conditions. Photographs were taken at 4, 5, and 6 weeks after germination. Arrows indicate senescent leaves.
(F) Relative chlorophyll content of plants grown under SD growth conditions for the indicated times. The values of 4-week-old WT and *traf1a TRAF1b-RNAi* plants were set at 100%, and the relative chlorophyll contents of WT and *traf1a TRAF1b-RNAi* leaves at the other stages were calculated accordingly. Data are average values ± SD ($n = 3$) calculated from three independent experiments. For each experiment, 5 leaves (technical replicates) harvested from independent plants were used per genotype. Asterisks indicate significant differences from WT (**$P < 0.01$ by Student's *t*-test).

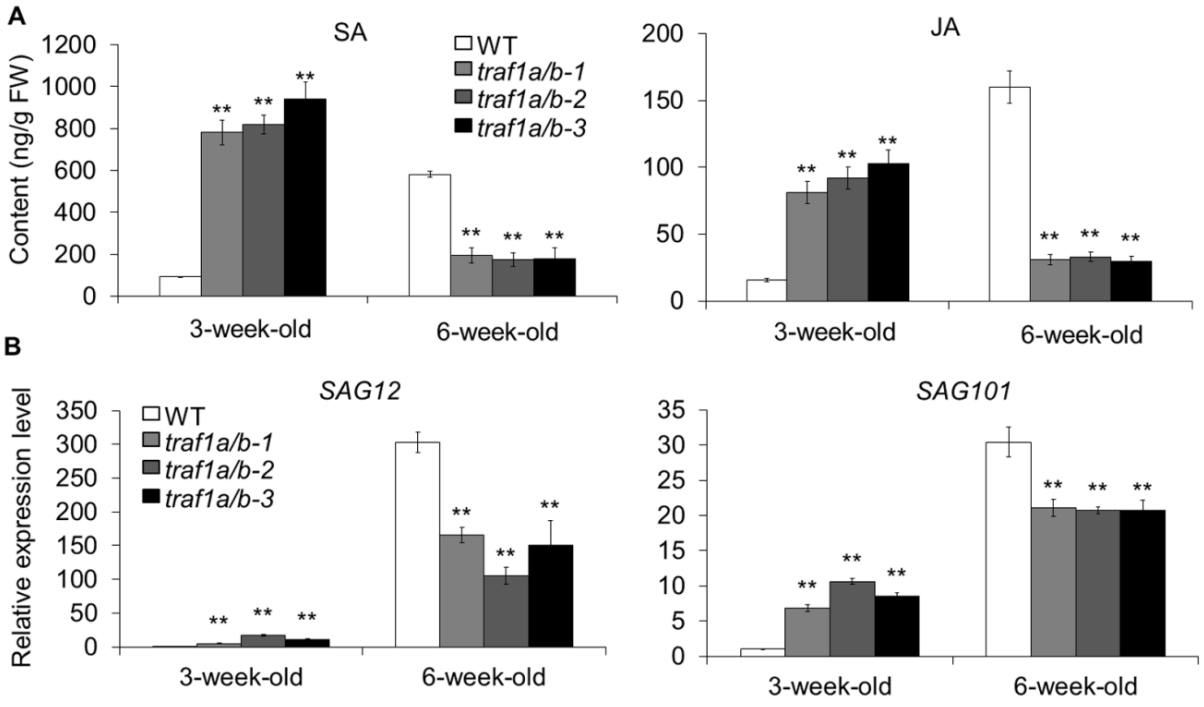


Supplemental Figure 7. Gene Expression Profiling of *traf1a/b* by RNA-seq Analysis.

(A) Functional classification of 2523 upregulated and 346 downregulated genes in the *traf1a/b-1* double mutant compared to wild type (WT). Differentially expressed genes (DEGs) were identified using the DEseq package in R with a two-fold change and *FDR* < 0.05 cutoff.

(B) Hierarchical cluster analysis of the 15 DEGs in the senescence-related category, four DEGs in the MATH family gene category, and two DEGs in the ATG-related gene category in the *traf1a/b-1* double mutant versus wild type (WT). The transcriptional profiles of relative gene expression values were analyzed using the heatmap 2.0 command of the R language. Red and blue represent upregulated and downregulated genes, respectively.

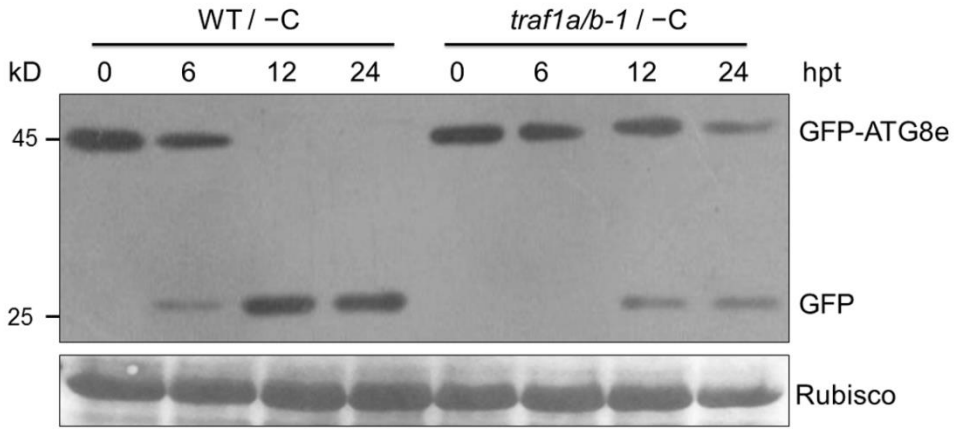
(C) qRT-PCR analysis showing the relative expression of *PR1*, *PR2*, *PR5*, *PDF1.2*, *SEN1*, and *SAG101* in WT and the *traf1a/b* mutants (*traf1a/b-1*, *traf1a/b-2*, and *traf1a/b-3*). Total RNA was isolated from rosettes of 3-week-old soil-grown plants under normal light/dark conditions. Transcript levels relative to WT were normalized to the levels of *ACTIN2*. The experiments were repeated three times (biological replicates) with similar results, and representative data from one replicate are shown. Data are average values ± SD (n = 3) of three technical replicates. For each replicate, 3 plants were pooled for RNA extraction. Asterisks indicate significant differences from WT (**P < 0.01 by Student's t-test).



Supplemental Figure 8. Endogenous SA and JA contents and Senescence-Associated Gene Expression in the 6-week-old Wild Type (WT) and *traf1a/b* Double Mutants.

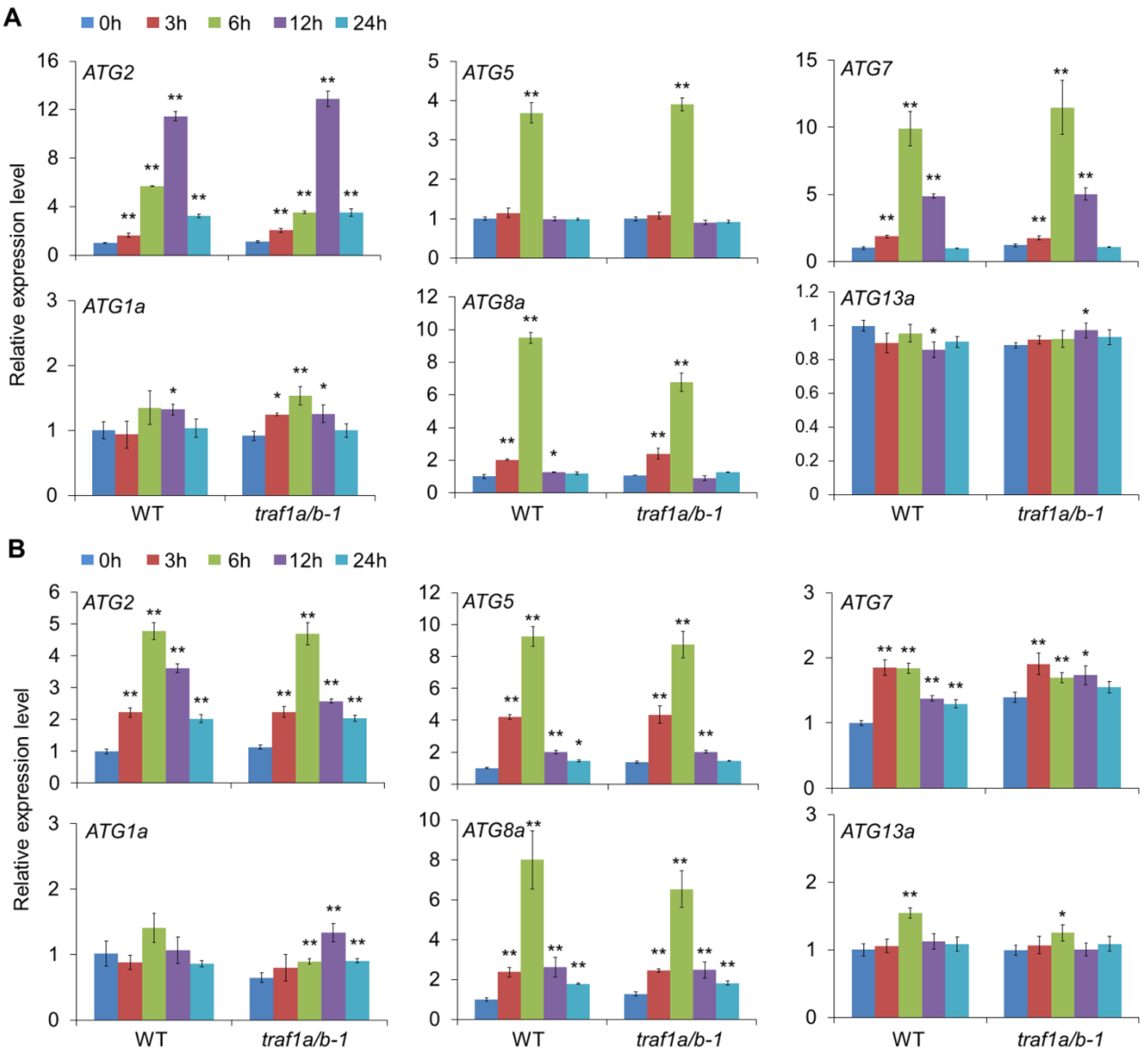
(A) Endogenous SA and JA contents in the WT and *traf1a/b* double mutants. Rosettes of 3- and 6-week-old WT and *traf1a/b* mutants grown under a normal light/dark cycle were harvested for plant hormone extraction following LC/MS analysis. D_4-SA and D_5-JA were added as internal quantitative standards. The experiment was repeated twice (biological replicates) with similar results. Data are average values ± SD (n = 5) calculated from five independent technical replicates (for each replicate, 2-3 plants were pooled) per experiment. Asterisks indicate significant differences from WT (**P < 0.01 by Student's t-test).

(B) qRT-PCR analysis showing the relative expression of senescence marker genes *SAG12* and *SAG101* in WT and *traf1a/b* double mutants at 3- and 6-week-old under LD conditions. Total RNA was isolated from 3- and 6-week-old plants and subjected to qRT-PCR analysis. Transcript levels relative to WT were normalized to the levels of *ACTIN2*. The experiments were repeated three times (biological replicates) with similar results, and representative data from one replicate are shown. Data are average values ± SD (n = 3) of three technical replicates. Asterisks indicate significant differences from WT (**P < 0.01 by Student's t-test).



Supplemental Figure 9. Immunoblot Analysis Showing the Processing of GFP-ATG8e in Wild-Type and *traf1a/b-1* Plants in Response to Carbon Starvation.

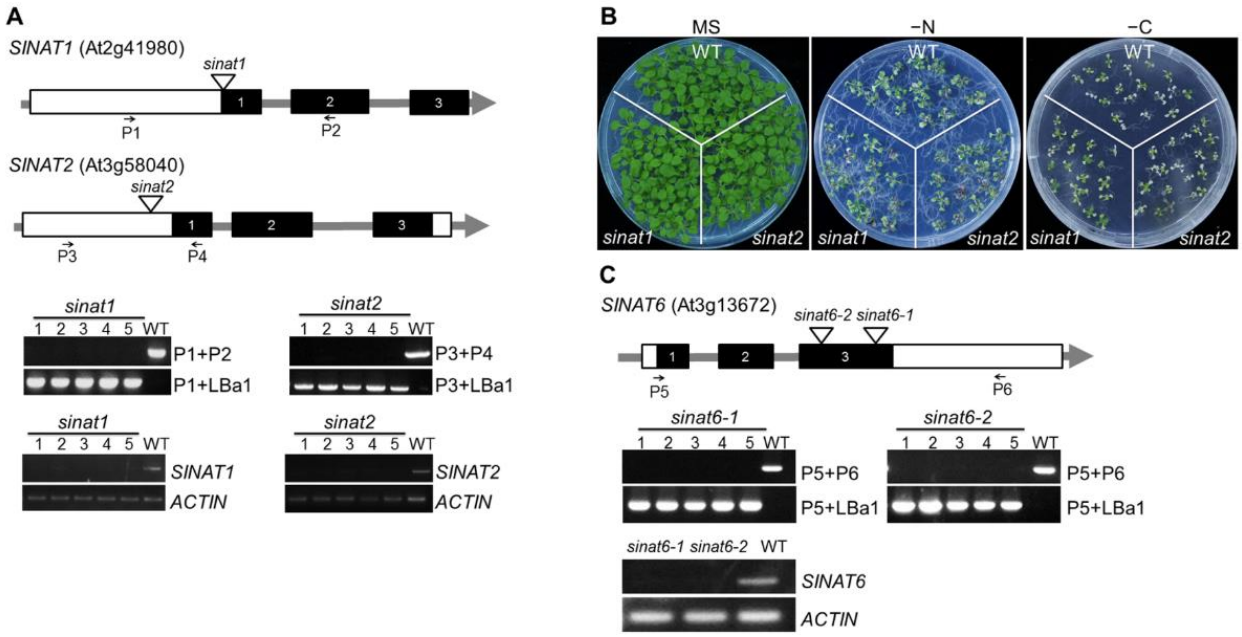
Two-week-old seedlings were collected at 0, 6, 12, and 24 h after treatment (hpt) and anti-GFP antibodies were used for immunoblotting. The GFP-ATG8e fusion and free GFP form are indicated on the right. The numbers on the left indicate the molecular weight (kD) of each size marker. Ponceau S-stained membranes are shown below the blot to indicate the amount of protein loaded per lane.



Supplemental Figure 10. *ATG* Gene Expression in the Wild Type and *traf1a/b* by qRT-PCR Analysis.

(A) qRT-PCR analysis showing the relative expression of *ATG2*, *ATG5*, *ATG7*, *ATG1a*, *ATG8a*, and *ATG13a* in WT and the *traf1a/b-1* after fixed-C starvation treatment. Total RNA was isolated from 1-week-old seedlings after fixed-C starvation treatment for the indicated times.

(B) qRT-PCR analysis showing the relative expression of *ATG2*, *ATG5*, *ATG7*, *ATG1a*, *ATG8a*, and *ATG13a* in WT and the *traf1a/b-1* after nitrogen starvation treatment. Total RNA was isolated from 1-week-old seedlings after nitrogen starvation treatment for the indicated times. Transcript levels relative to WT were normalized to the levels of *ACTIN2*. The experiments were repeated three times (biological replicates) with similar results, and representative data from one replicate are shown. Data are average values ± SD (n = 3) of three technical replicates. For each replicate, 20 seedlings were pooled for total RNA extraction. Asterisks indicate significant differences from WT (* $P < 0.05$, ** $P < 0.01$ by Student's t-test).



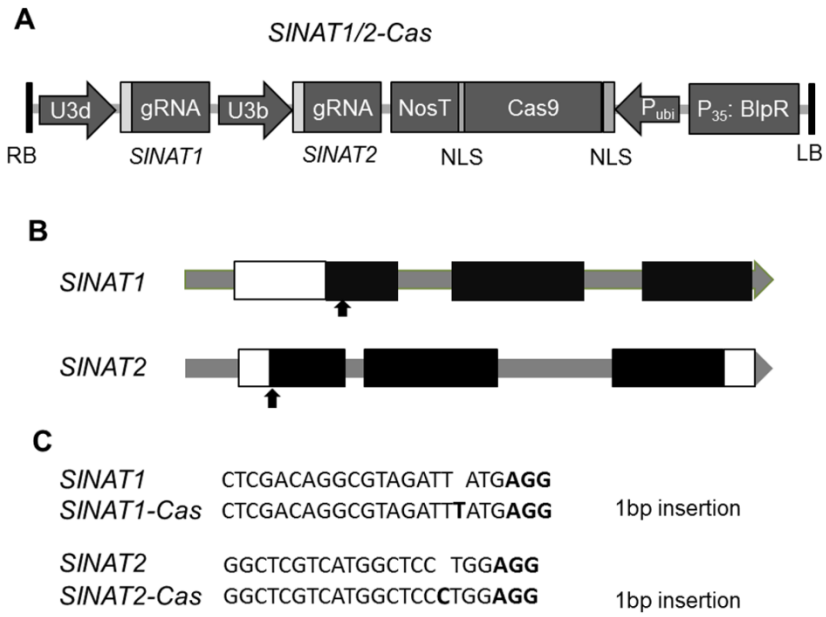
Supplemental Figure 11. Analysis of *sinat1*, *sinat2*, and *sinat6* Single Mutants.

(A) Molecular identification of *sinat1* and *sinat2*. White and black boxes represent UTRs and exons, respectively. Lines between black boxes indicate introns. T-DNA insertion sites in *sinat1* (SALK_010417C) and *sinat2* (SALK_002174C) are marked with triangles. Primers used for genotyping are indicated by arrows. Genomic DNA isolated from wild type (WT), *sinat1*, and *sinat2* was amplified using the primer pairs indicated on the right. The primers are listed in Supplemental Table 2. RT-PCR analysis showing that *SINAT1* and *SINAT2* are not expressed in *sinat1* and *sinat2*. Total RNA isolated from WT, *sinat1*, and *sinat2* was subjected to RT-PCR using the primer pairs listed in Supplemental Table 2. The expression of *ACTIN2* was used as a control.

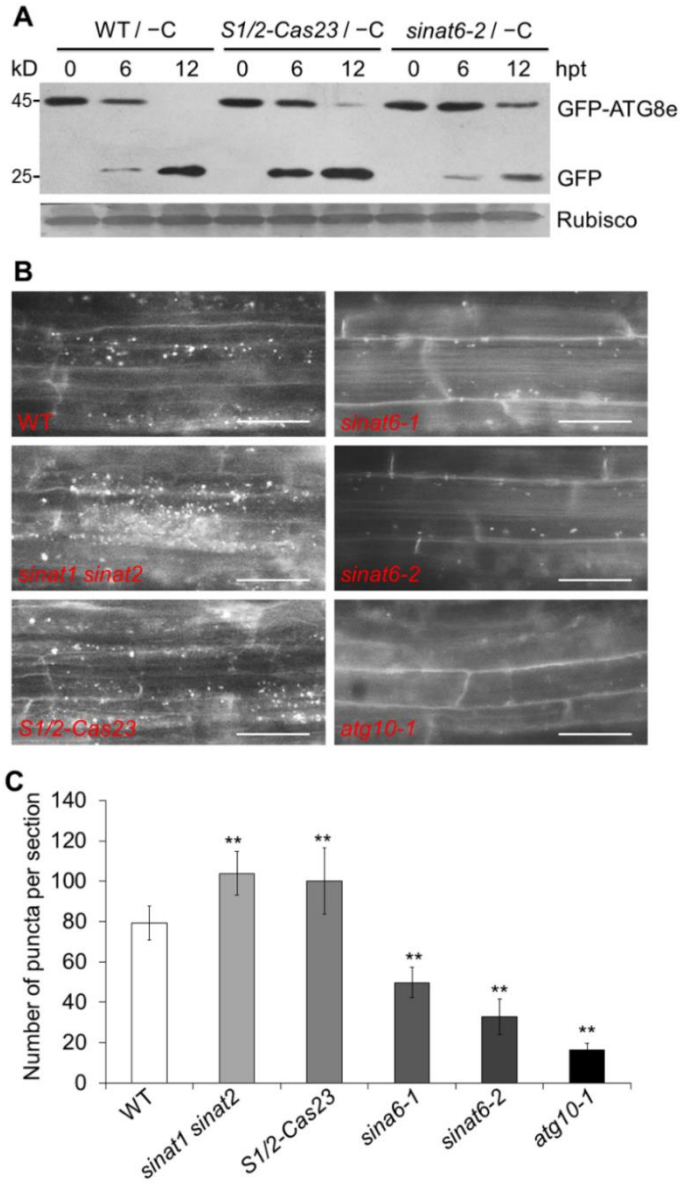
(B) Sensitivity of WT, *sinat1*, and *sinat2* to carbon and nitrogen starvation. Images of WT, *sinat1*, and *sinat2* seedlings grown on MS medium, MS medium without nitrogen (–N), or MS medium without sugar followed by darkness (–C).

Seeds of WT, *sinat1*, and *sinat2* were germinated on solid MS medium with 2% sucrose for 7 d. For nitrogen starvation treatment, 7-d-old seedlings grown on MS were transferred to MS or N-deficient medium for 5 d under a normal light/dark cycle. For carbon starvation treatment, 7-d-old seedlings were transferred to MS or MS without sucrose (–C) in constant darkness for 8 d, followed by exposure to normal light/dark conditions for a 7-d recovery.

(C) Molecular identification of *sinat6*. White and black boxes represent UTRs and exons, respectively. Lines between black boxes indicate introns. T-DNA insertion sites in *sinat6-1* (SALK_077413) and *sinat6-2* (SALK_129594) are marked with triangles. Primers used for genotyping are indicated by arrows. Genomic DNA isolated from WT, *sinat6-1*, and *sinat6-2* was amplified using the primer pairs in Supplemental Table 2. The primers are listed in Supplemental Table 2. RT-PCR analysis showing that *SINAT6* are not expressed in *sinat6-1* and *sinat6-2*. Total RNA isolated from WT, *sinat6-1*, and *sinat6-2* was subjected to RT-PCR. The expression of *ACTIN2* was used as a control.



Supplemental Figure 12. Generation of *SINAT1/2-Cas* Transgenic Lines.
(A) and (B) Diagram of construction structure (A) and target sites (B) of *SINAT1* and *SINAT2* genes. Black boxes indicate exons and white boxes represent UTR. Lines between black boxes indicate introns. Arrows mark the target sites of CRISPR-Cas9 editing.
(C) The genome editing efficiency of transgenic lines was tested by PCR products sequencing. 1-bp insertion was observed at the expected cleavage site in both *SINAT1* and *SINAT2* genes.



Supplemental Figure 13. Autophagosome Formation in the *sinat* Mutants.

(A) Immunoblot analysis showing the processing of GFP-ATG8e in wild type (WT) and *sinat* mutants (*S1/S2-Cas23* and *sinat6-2*) in response to carbon starvation. Two-week-old seedlings were collected at 0, 6, and 12 h after treatment (hpt) and anti-GFP antibodies were used for immunoblotting. The GFP-ATG8e fusion and free GFP form are indicated on the right. Ponceau S-stained membranes are shown below the blot to indicate the amount of protein loaded per lane. The numbers on the left indicate the molecular weight (kD) of each size marker.

(B) MDC staining showing the autophagosome numbers in the root cells of WT and *sinat* mutants (*sinat1 sinat2*, *S1/S2-Cas23*, *sinat6-1*, and *sinat6-2*) after nitrogen and carbon starvation. The *atg10-1* mutant was used as a control. One-week-old seedlings were starvation-treated (–C, –N) for 16 h with 1 μM CA, followed by staining with MDC. The labeled autophagosomes were visualized under an epifluorescence microscope. Bars = 50 μm.

(C) The number of puncta per root section in the root mature cells of WT and *sinat* mutants (*sinat1 sinat2*, *S1/S2-Cas23*, *sinat6-1*, and *sinat6-2*). One-week-old seedlings were subjected to starvation treatment (–C, –N) and the puncta number was quantified. Data are average values ± SD (n = 3) of three independent biological replicates. For each experiment, 15 sections were calculated per genotype. Asterisks indicate significant differences from WT (**P < 0.01 by Student's t-test).