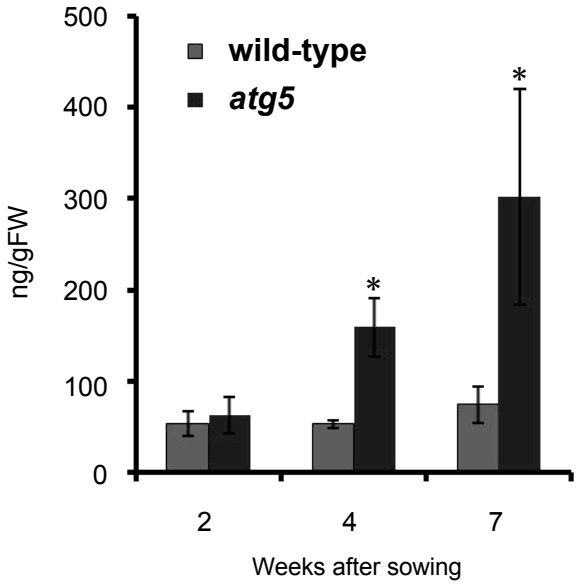
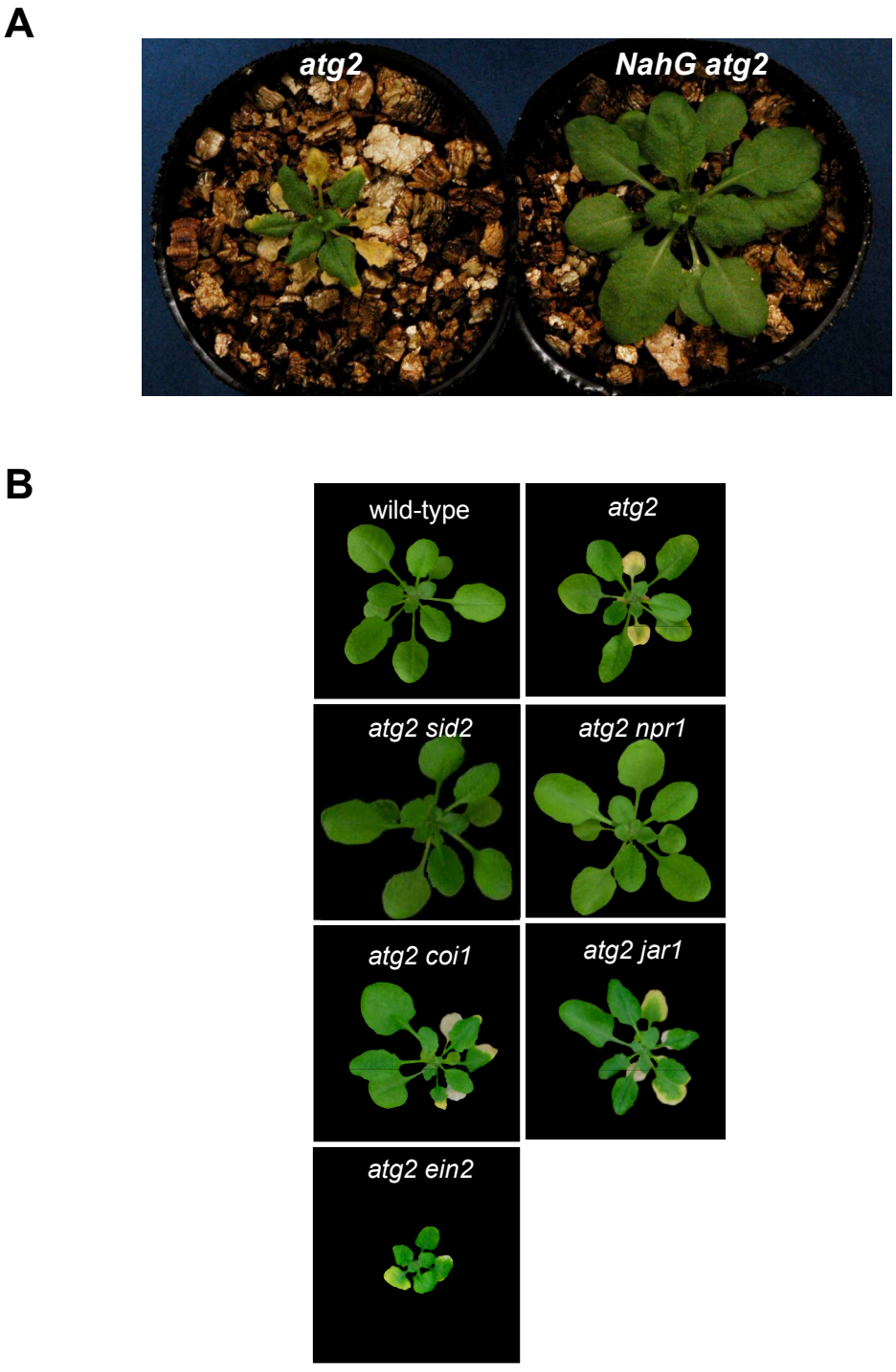
Supplemental Data. Yoshimoto et al. (2009). Plant autophagy negatively regulates cell death by controlling NPR1-dependent salicylic acid signaling during senescence and the innate immune response in *Arabidopsis*.

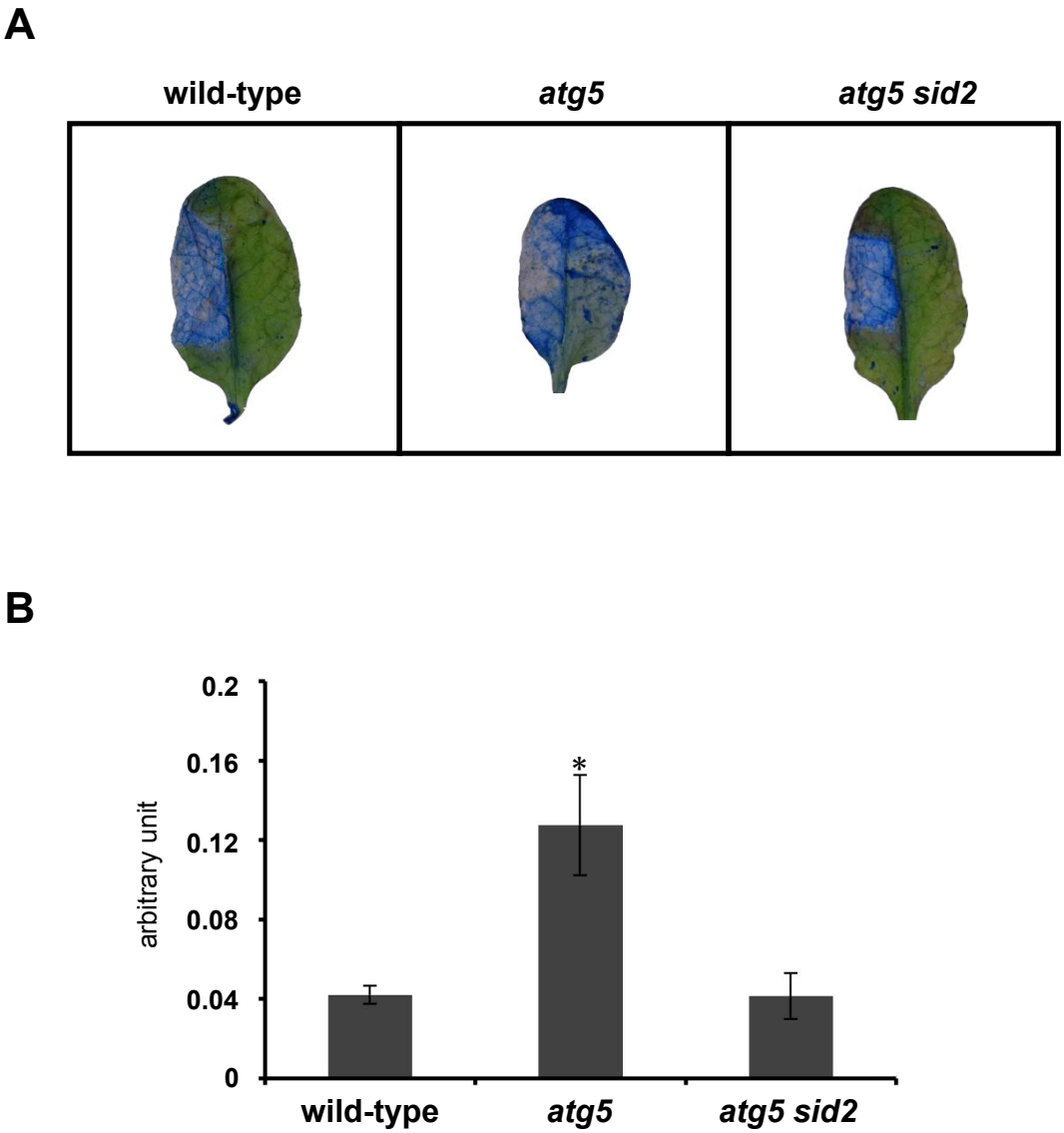


Supplemental Figure 1. *atg5* mutant shows a developmentally controlled increase in SA levels.

Time-course analysis of endogenous SA levels in rosette leaves of wild-type and *atg5* mutant plants grown under short-day conditions. Error bars indicate S.D. (n = 3). Asterisks indicate a significant difference from wild-type ($P < 0.01$; Student's *t*-test).



Supplemental Figure 2. Early senescence phenotype of autophagy-defective mutants was suppressed by inactivation of the SA-signaling pathway.
(A) *atg2 NahG* plants were created by crossing. Photographs of six-wk-old plants grown on vermiculites supplied with a rich nutrient solution.
(B) *atg2* double mutants with *sid2*, *npr1*, *jar1*, *coi1*, and *ein2*. Photographs of five-wk-old plants grown on rockwool supplied with a rich nutrient solution. Results were reproduced in at least two independent experiments using four plants in each experiment.

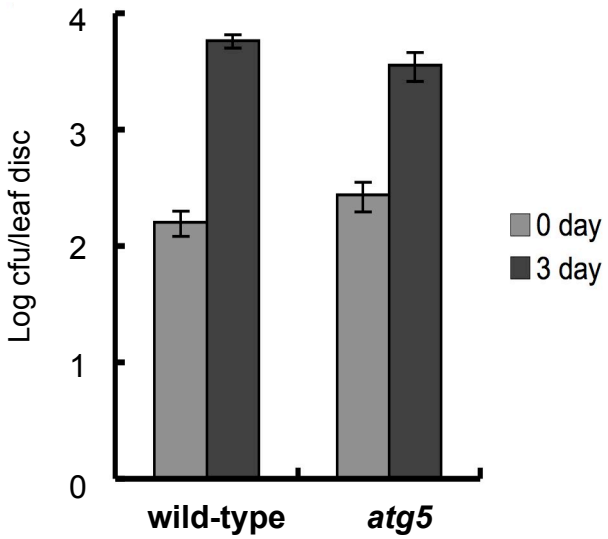


Supplemental Figure 3. Pathogen-induced chlorotic cell death suppressed by SA signaling pathway.

Leaves were infected with *Pst-avrRpm1* as described in Figure 3 legend.

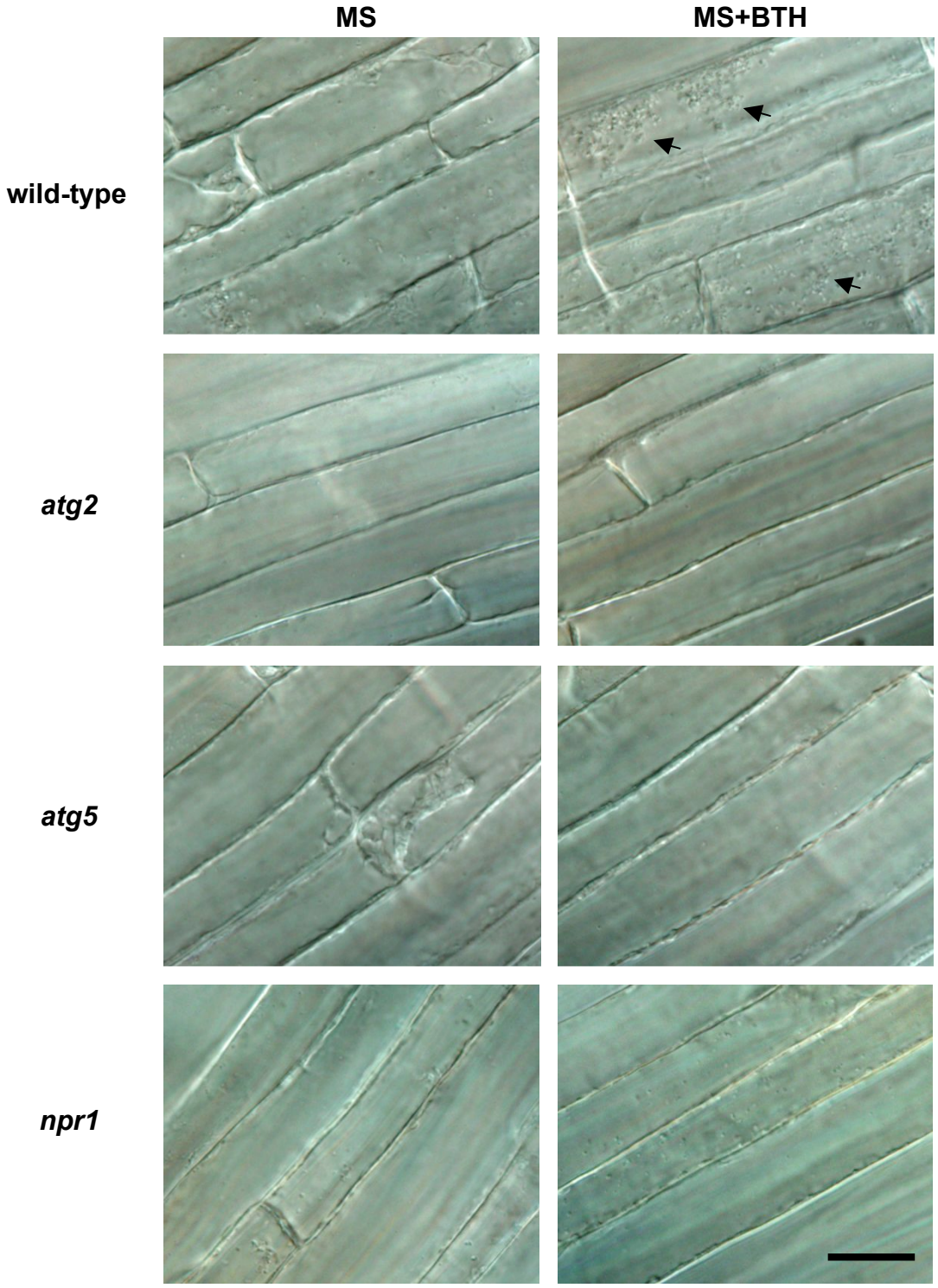
(A) Images of leaves of wild-type, *atg5*, and *atg5 sid2* plants stained with Evans blue, an indicator of cell death, were taken at 6-9 d after infection.

(B) Cell death was quantified spectrophotometrically as Evans blue staining in wild-type, *atg5*, and *atg5 sid2* detached leaves as described in Supplemental Methods. Error bars indicate S.D. (n = 5). Asterisks indicate a significant difference from wild-type ($P < 0.01$; Student's *t*-test).

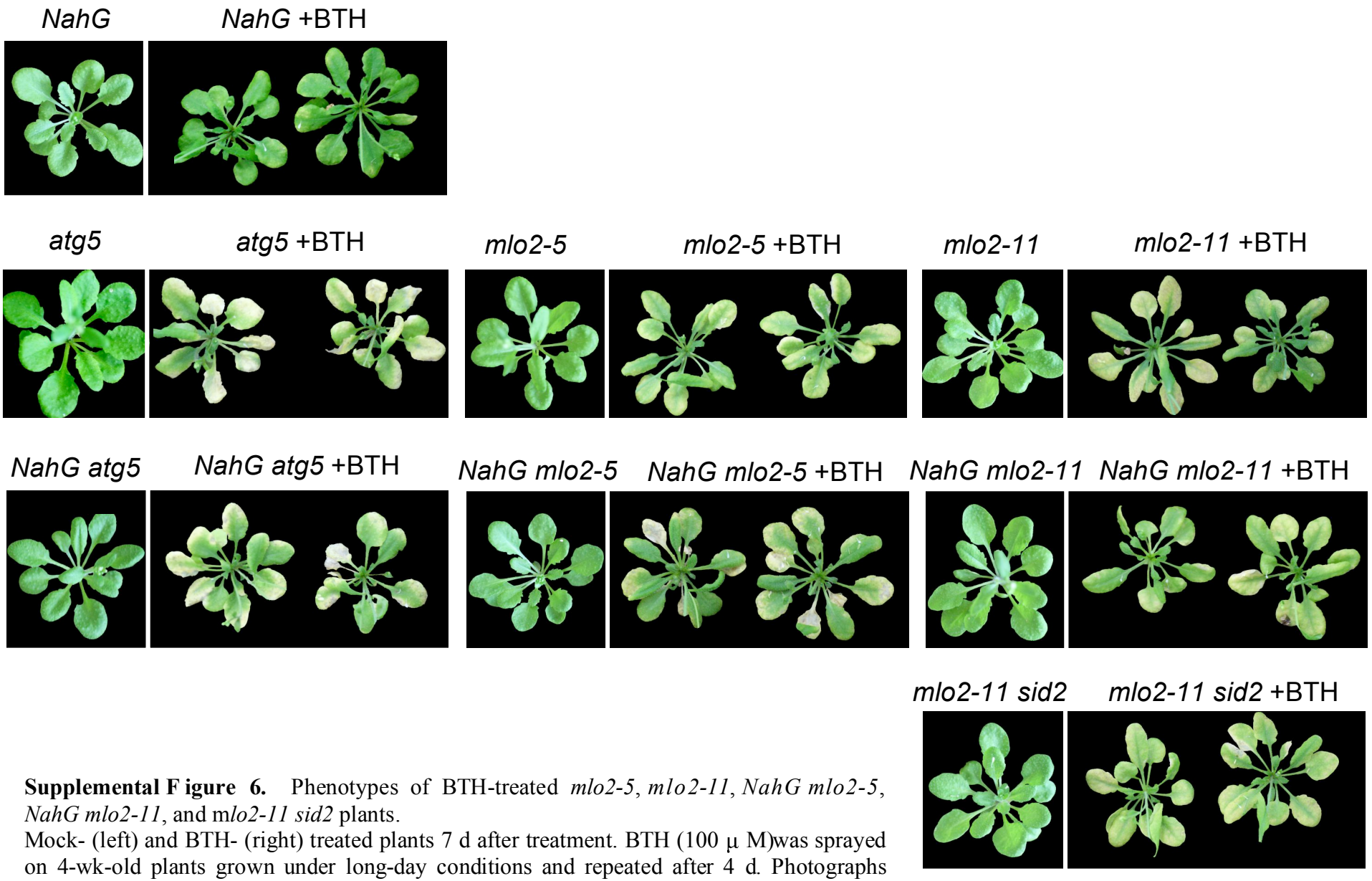


Supplemental Figure 4. Bacterial growth analysis of *Pst-avrRpm1* in wild-type and *atg5* plants.

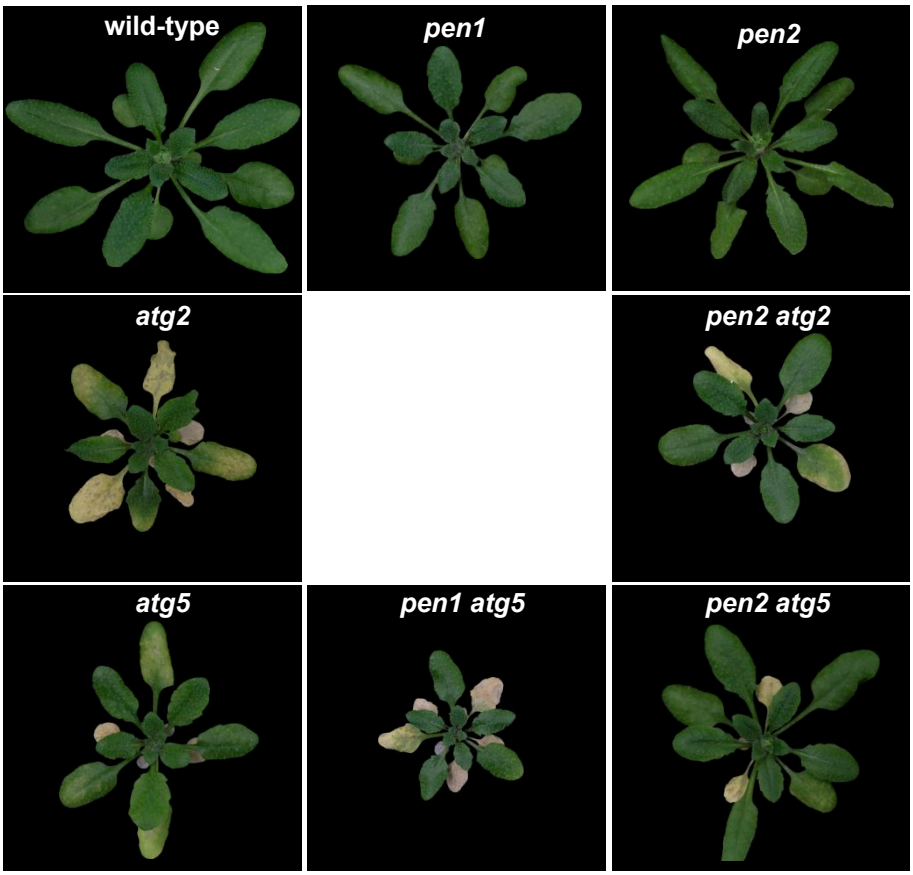
Pst-avrRpm1 (1 x 10^5 cfu / ml) was hand-infiltrated with the needless syringe into the leaves of 8-wk-old plants. Leaves were harvested immediately after infiltration (light gray column) or at 3days post inoculation (dark gray column). Error bars represent S.D. of three samples.



Supplemental Figure 5. Autophagy is induced by BTH treatment.
Roots of seven-day-old seedlings were excised and transferred to MS liquid medium containing E-64d (10 μM) with (*right*) or without (*left*) BTH (100 μM) for 3-5 h, and then observed by conventional transmission light microscopy. Arrows indicate highly accumulated autophagic bodies. Results were r eproduced in at least three independent experiments using five seedlings in each experiment. Bar = 20 μm.



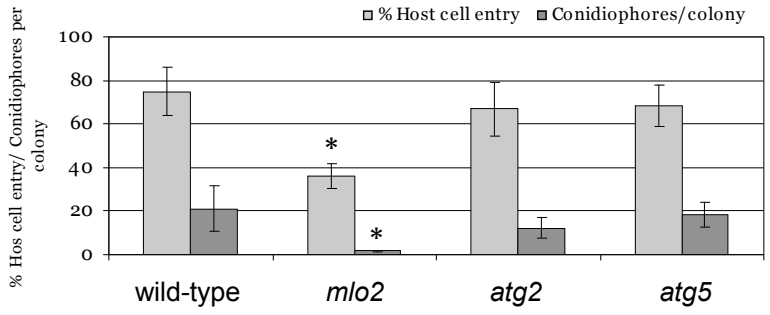
Supplemental F igure 6. Phenotypes of BTH-treated *mlo2-5*, *mlo2-11*, *NahG mlo2-5*, *NahG mlo2-11*, and m*lo2-11 sid2* plants.
Mock- (left) and BTH- (right) treated plants 7 d after treatment. BTH (100 μ M)was sprayed on 4-wk-old plants grown under long-day conditions and repeated after 4 d. Photographs were taken 3 d a fter the last BTH treatment. Results were reproduced in at least two independent experiments using four plants in each experiment.



Supplemental Figure 7. Early senescence phenotype of autophagy-defective mutants was accelerated by *pen1* and partially suppressed by *pen2*.
The *atg2* and *atg5* double mutants with *pen1* and *pen2* were made by crossing. Photographs of five-wk-old plants grown on rockwool supplied with a rich nutrient solution. Results were reproduced in at least two independent experiments using four plants in each experiment.

A

B



Supplemental F igure 8. The *atg* mutants do not show obvious resistance against a powdery mildew, *G. orontii,* unlike *mlo2*.
(A) Phenotypes of *G. orontii* growth on wild-type, *mlo2-5*, *atg2*, *atg5* mutant plants. Photographs were taken 10 d post inoculation.
(B) Quantitative assessment of host cell entry (48 h post inoculation; pale gray bars) and conidiation (7 d post inoculation; gray bars). Data represent the mean ± S.D. of three independent experiments. Asterisks indicate a significant difference from wild-type ($P < 0.01$; Student's *t*-test).

Supplemental Table 1. Relative levels of free amino acids in wild-type and *atg5* mutant plants

Amino acids	wild-type	*atg5*
Aspartate	1.00 ± 0.09	1.36 ± 0.09
Threonine	1.00 ± 0.08	1.05 ± 0.05
Serine	1.00 ± 0.08	1.21 ± 0.10
Asparagine	1.00 ± 0.15	1.73 ± 0.15
Glutamate	1.00 ± 0.08	1.19 ± 0.04
Glutamine	1.00 ± 0.11	1.23 ± 0.10
Proline	1.00 ± 0.07	1.15 ± 0.25
Glycine	1.00 ± 0.23	1.39 ± 0.32
Alanine	1.00 ± 0.20	0.95 ± 0.18
Valine	1.00 ± 0.02	0.96 ± 0.03
Cysteine	1.00 ± 0.66	1.03 ± 0.66
Methionine	1.00 ± 0.32	1.63 ± 0.41
Isoleucine	1.00 ± 0.06	0.99 ± 0.07
Leucine	1.00 ± 0.12	1.03 ± 0.15
Tyrosine	1.00 ± 0.12	1.01 ± 0.11
Phenylalanine	1.00 ± 0.05	0.98 ± 0.16
Lysine	1.00 ± 0.16	1.07 ± 0.09
Histidine	1.00 ± 0.08	1.05 ± 0.22
Tryptophan	nd	nd
Arginine	1.00 ± 0.28	0.99 ± 0.09

Data represent the mean ± S.D. of at least six experiments. Each value was determined relative to the average value of the sample in wild-type plants.

Supplemental Table 2. LC conditions

Method No.	Solvent A	Solvent B	Gradient (composition of solvent B)
1	Water containing 0.01% acetic acid	MeCN, 0.05 % acetic acid	3 to 50 % over 20 min
2	Water containing 0.01% acetic acid	MeCN, 0.05 % acetic acid	3 % to 25 % over 27 min
3	Water containing 0.1 % formic acid	MeCN, 0.1 % formic acid	3 to 98 % over 10 min

Supplemental Table 3. Parameters for LC-ESI-MS/MS analysis (Agilent 1200-6410)

	LC method	Retention time on LC (min)	ESI	MS/MS transitions for quantifications (*m*/*z*)	Collision energy (V)	Fragmentor (V)
ABA D_6-ABA	1	10.8	-	263/153 269/159	8	140
GA_1 D_2-GA_1	1	8.0	-	347/273 349/275	24	150
GA_4 D_2-GA_4	1	14.1	-	331/257 333/259	26	150
IAA D_2-IAA	1	9.9	+	176/130 178/132	18	110
JA D_2-JA	1	12.6	-	209/59 211/59	10	150
JA-Ile $^{13}C_6$-JA-Ile	1	14.7	-	328/136 322/130	14	160
SA D_6-SA	3	6.1	-	137/93 143/99	16	100
tZ D_5-tZ	2	7.8	+	220/136 226/136, 137	16	110
DHZ D_3-DHZ	2	8.1	+	222/136 225/136	20	110
iP D_6-iP	2	17.4	+	204/136 210/137	14	100

SUPPLEMENTAL METHODS

Plant materials

Arabidopsis mutant alleles *mlo2-5*, *mlo2-11*, *NahG mlo2-5*, *NahG mlo2-11*, *mlo2-11 sid2* (Consonni et al., 2006) were used for the BTH treatment experiment, and *pen1-1* (Collins et al., 2003) and *pen2-2* (Lipka et al., 2005) were used for intermutant crosses with either *atg2* or *atg5*.

Cytology

Pathogen-induced cell death was quantified spectrophotometrically by Evans blue staining of detached leaves infected with *Pst-avrRpm1* as described by Guo et al. (2005) with minor modifications. Briefly, detached leaves were completely submerged in a 0.1 % (w/v) aqueous solution of Evans blue dye and vacuumed for 5min. The leaves were washed three times with distilled water (15 min each) and then leaf discs were made from topside of the uninfected site. Dye bound to dead cells was solubilized in 50% (v/v) methanol and 1% (w/v) SDS at 60°C for 30 min and then quantified by absorbance at 590 nm. For observation of induction of autophagy by BTH, excised roots (approximately 4 cm) were treated with E-64d (10 μM), a membrane-permeable cysteine protease inhibitor, in the presence or absence of BTH (100 μM) for 3-5h. The roots were mounted in water and observed by conventional transmission light microscopy (Olympus BX51).

Bacterial growth analysis

Pst-avrRpm1 were grown overnight with kanamycin (25 μg / ml) and rifampicin (100 μg / ml), washed twice in 10 mM $MgCl_2$, and resuspended to a density of 1 x 10^5 colony-forming units (cfu) / ml for *in planta* growth assay. Bacterial suspensions were infiltrated into abaxial leaf surfaces by using needless syringe. *Pst-avrRpm1* bacteria were counted according to the protocol by Katagiri et al. (2002).

Amino acid analyses

Aerial parts of 3-wk-old plants were harvested and then analyzed by using a gas chromatography time-of-flight mass spectrometry (GC-TOF/MS). The levels of amino acids were analyzed as described previously (Kusano et al., 2007).

Genetic analyses

For isolation of *atg pen1* and *atg pen2*, homozygous *atg* mutants were selected from F2 seedlings by PCR as described by Inoue et al. (2006). *atg pen1* double mutants were identified by CAPS using *Mlu* I polymorphism as described by Lipka et al. (2005). *atg pen2* double mutants were selected by PCR using gene specific primers. Gene-specific primers used are as follows: for verifying *pen2-2* mutant (GABI-KAT 134C04), 5′-CCCATTTGGACGTGAATGTAGACAC-3′ designated as T-DNA-GABI-Kat, and 5′-CTCTTTGGAACTGCTTCATCTTCT-3′ and 5′-CATAGCCCGCGACACTGTAGACCC-3′.

Powdery mildew inoculations

Four-wk-old plants were inoculated with the powdery mildew fungus, *Golovinomyces orontii*, and samples were collected at 48hpi for quantification of fungal entry rates. In addition, samples were collected at 7dpi for quantification of conidiophore formation. Finally, pictures of inoculated plants were taken at 10 dpi. Fungal entry rate and conidiophore formation were quantified as described by Consonni et al. (2006).

SUPPLEMENTAL REFERENCES

Collins, N.C., Thordal-Christensen, H., Lipka, V., Bau, S., Kombrink, E., Qiu, J.L., Huckelhoven, R., Stein, M., Freialdenhoven, A., Somerville, S.C., and Schulze-Lefert, P. (2003). SNARE-protein-mediated disease resistance at the plant cell wall. Nature **425:** 973-977.

Consonni, C., Humphry, M.E., Hartmann, H.A., Livaja, M., Durner, J., Westphal, L., Vogel, J., Lipka, V., Kemmerling, B., Schulze-Lefert, P., Somerville, S.C., and Panstruga, R. (2006). Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. Nat. Genet. **38:** 716-720.

Guo, F.-Q., and Crawford, N.M. (2005). Arabidopsis nitric oxide synthase 1 is targeted to mitochondria and protects against oxidative damage and dark-induced senescence. Plant Cell **17:** 3436-3450.

Inoue, Y., Suzuki, T., Hattori, M., Yoshimoto, K., Ohsumi, Y., and Moriyasu, Y. (2006). *AtATG* genes, homologs of yeast autophagy genes, are involved in constitutive autophagy in *Arabidopsis* root tip cells. Plant Cell Physiol. **47:** 1641-1652.

Katagiri, F., Thilmony, R., and He, S.Y. (2002). The Arabidopsis thaliana-Pseudomonas syringae interaction. The Arabidopsis Book. Rockville, MD: American Society of Plant Biologists. doi: 10.1199/tab.0039, http://www.aspb.org/publications/arabidopsis

Kusano, M., Fukushima, A., Arita, M., Jonsson, P., Moritz, T., Kobayashi, M., Hayashi, N., Tohge, T., and Saito, K. (2007). Unbiased characterization of genotype-dependent metabolic regulations by metabolomic approach in *Arabidopsis thaliana*. BMC Syst. Biol. **1:** 53-69.

Lipka, V., Dittgen, J., Bednarek, P., Bhat, R., Wiermer, M., Stein, M., Landtag, J., Brandt, W., Rosahl, S., Scheel, D., Llorente, F., Molina, A., Parker, J.,

Somerville, S., and Schulze-Lefert, P. (2005). Pre- and postinvasion defenses both contribute to nonhost resistance in *Arabidopsis*. Science **310:** 1180-1183.