

Supplemental Figure 1. Morphology of *ien1*, SALK_048091 (*sfr6-2*) and their F₁ progeny

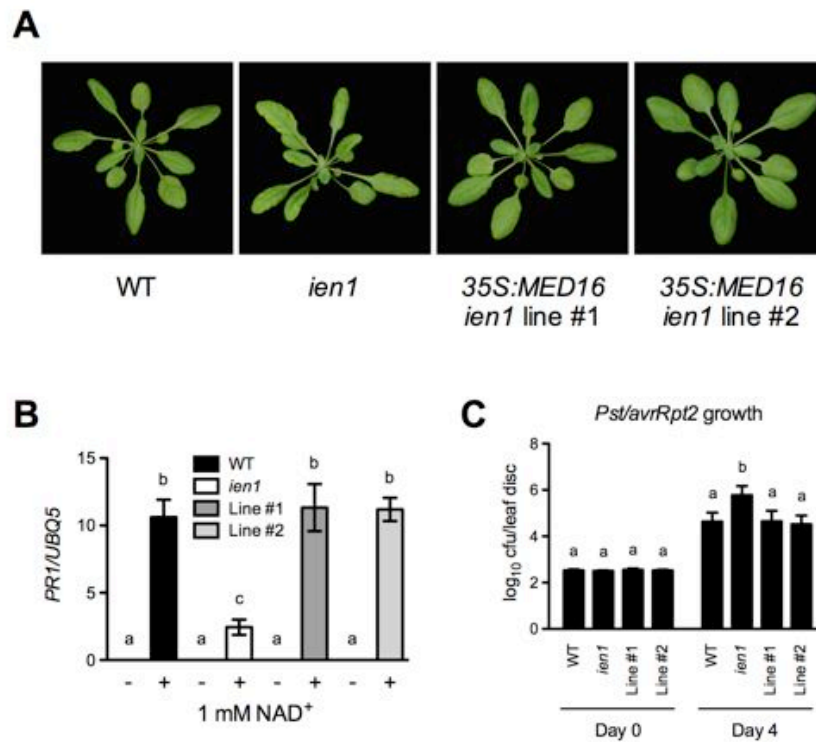
(A) A wild-type(WT) plant.

(B) An *ien1* plant.

(C) A SALK_048091 (*sfr6-2*) plant.

(D) An F₁ plant from a cross between *ien1* and SALK_048091.

Representative photos were taken 4 weeks after germination.

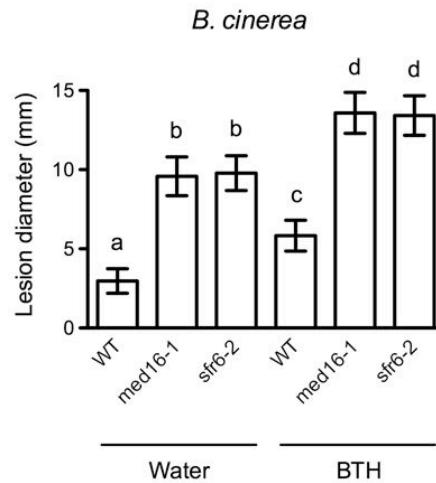


Supplemental Figure 2. Genetic complementation of the *ien1* mutant

(A) Morphology of a wild-type (WT) plant, an *ien1* plant, and plants of two independent *35S:MED16 ien1* transgenic lines. Representative photos were taken 4 weeks after germination.

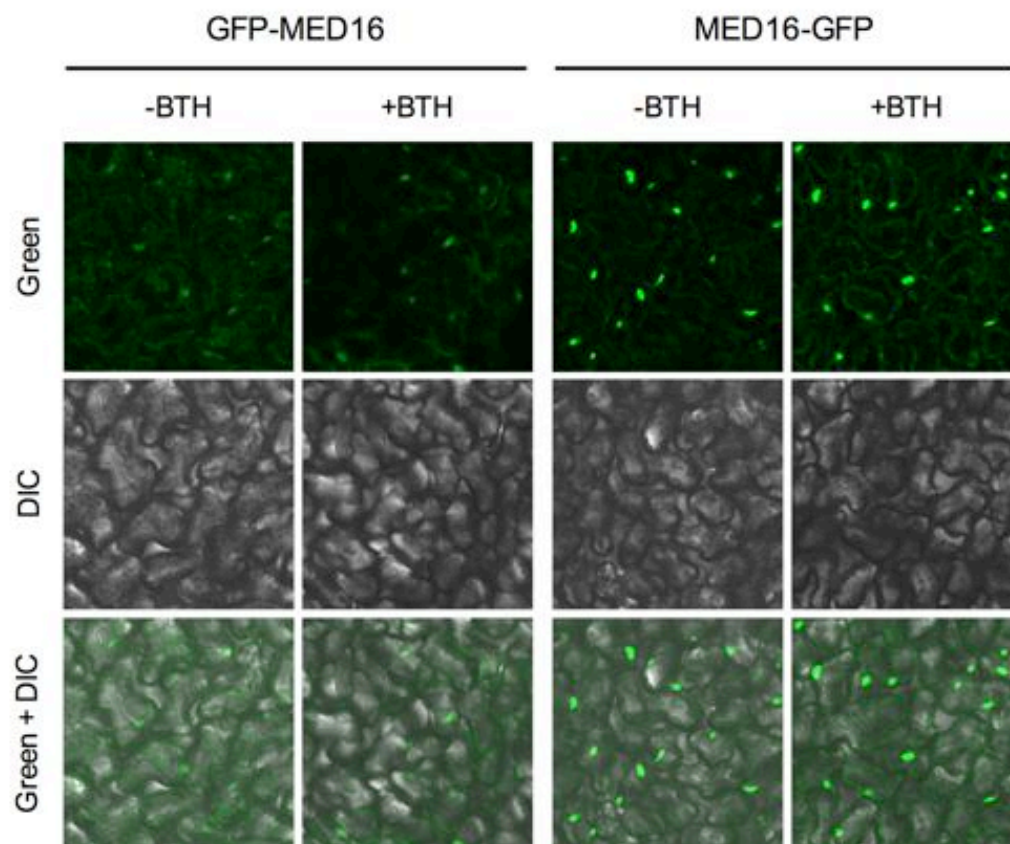
(B) Exogenous NAD⁺-induced *PR1* gene expression in WT, *ien1*, and *35S:MED16 ien1* transgenic plants. Data represent the mean of 3 independent samples with standard deviation. Different letters above the bars indicate significant differences ($p < 0.05$, Student's *t*-test).

(C) Growth of *Pst* DC3000/*avrRpt2* in WT, *ien1*, and *35S:MED16 ien1* transgenic plants. cfu, colony-forming units. Data represent the mean of 8 independent samples with standard deviation. Different letters above the bars indicate significant differences ($p < 0.05$, Student's *t*-test). The comparison was made separately among genotypes for each time point. Experiments in (B) and (C) were repeated with similar results.



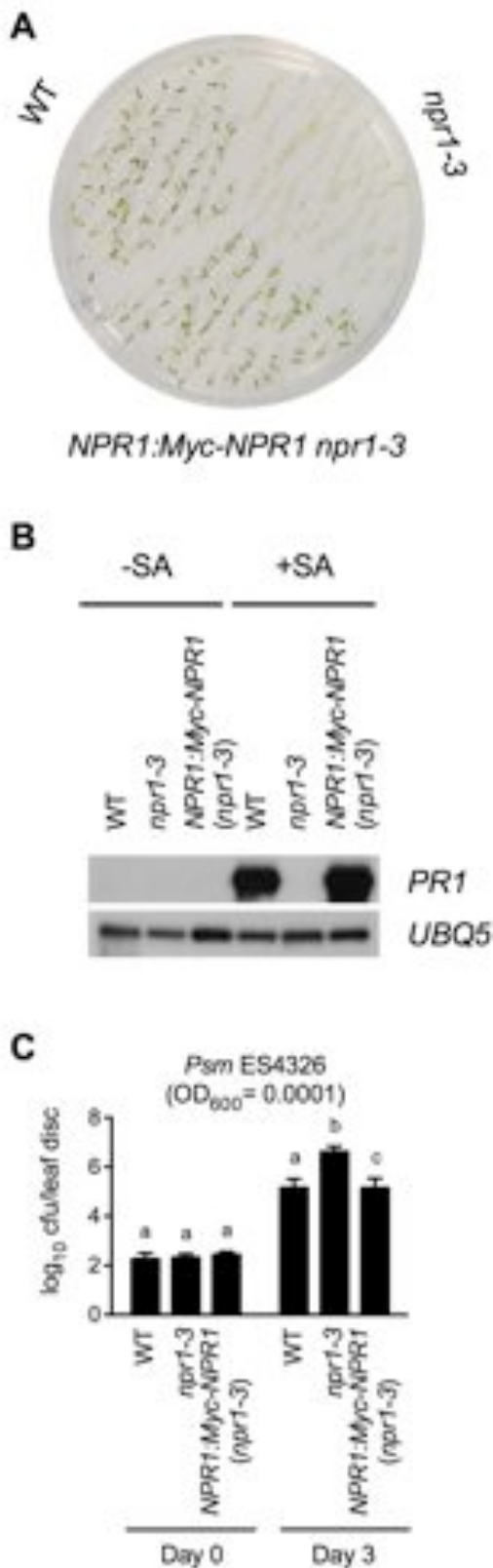
Supplemental Figure 3. Effect of BTH treatment on resistance to *B. cinerea* in *med16/sfr6* mutants

Size of the necrotic lesions formed on BTH-treated wild-type (WT) and *med16/sfr6* plants after *B. cinerea* infection. Four-week-old soil-grown WT and *med16/sfr6* plants were treated with soil drenches plus foliar sprays of 0.3 mM BTH solution. After 24 hours, the plants were inoculated with *B. cinerea* spores and the inoculated leaves were scored 4 days later. Data represent the mean of lesion sizes on 36 leaves with standard deviation. Different letters above the bars indicate significant differences ($P < 0.05$, Student's *t*-test).



Supplemental Figure 4. Effect of BTH treatment on the subcellular localization of the MED16 protein.

Two-week-old soil-grown *35S::GFP-MED16* and *35S::MED16-GFP* plants were treated with or without soil drenches plus foliar sprays of 0.3 mM BTH solution. After 24 hours, leaf pieces were mounted in water and viewed with a confocal microscope.

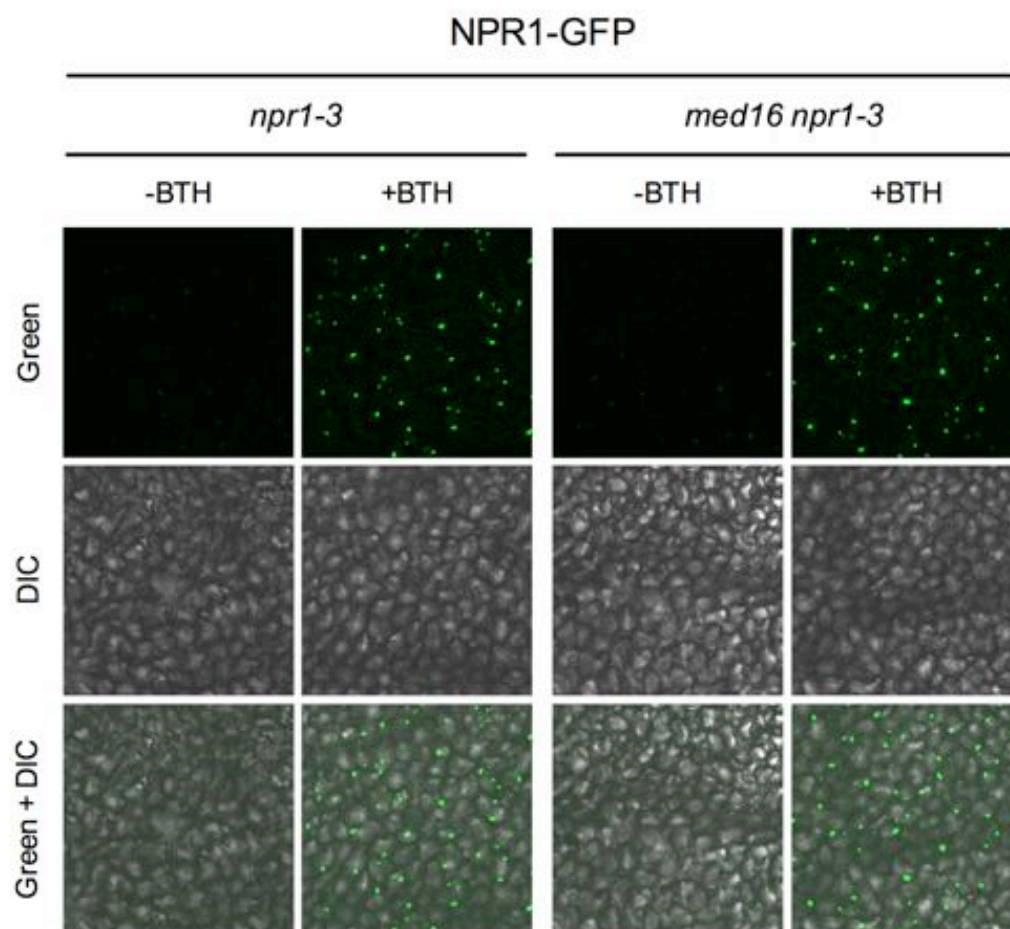


Supplemental Figure 5. Characterization of the *NPR1:Myc-NPR1* transgene.

(A) Seeds of wild type (WT), *npr1-3*, and *NPR1:Myc-NPR1 npr1-3* were placed on ½ MS agar medium containing 0.5 mM SA. After 3 days of stratification, the plate was transferred to a growth chamber and photographed 10 days later.

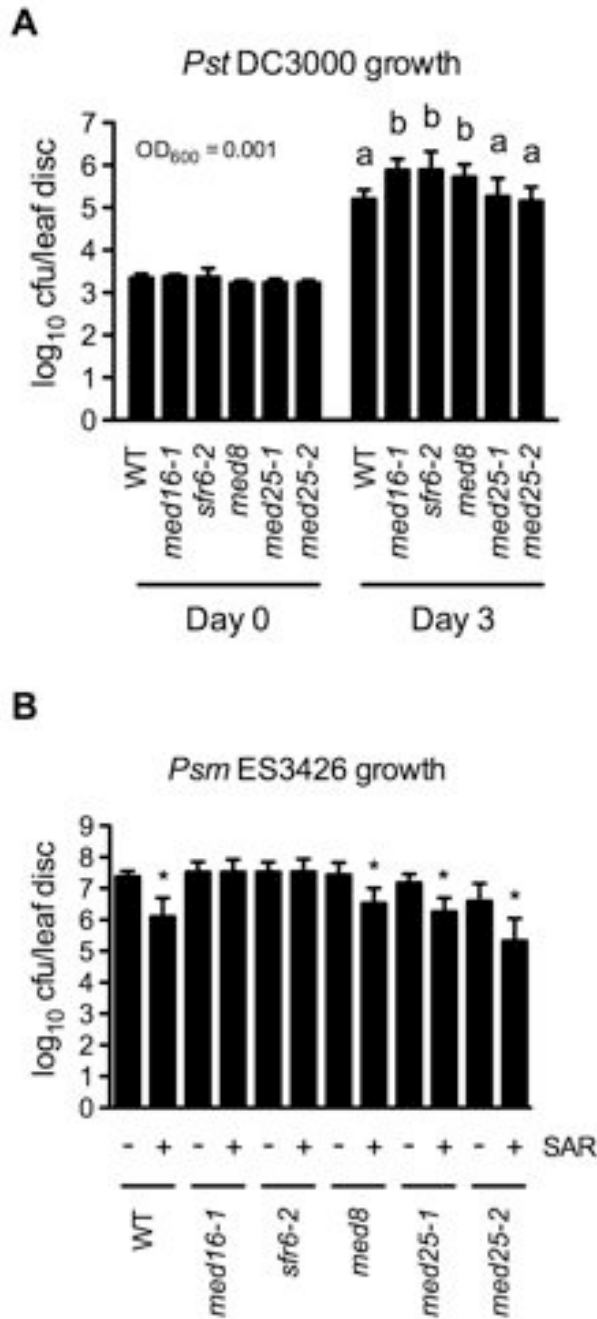
(B) SA-induced *PR1* gene expression in WT, *npr1-3*, and *NPR1:Myc-NPR1 npr1-3* plants.

(C) Growth of *Psm* ES4326 in WT, *npr1-3*, and *NPR1:Myc-NPR1 npr1-3* plants. Leaves of 4-week-old plants were inoculated with *Psm* ES4326 (OD₆₀₀ = 0.0001). The in planta bacterial titers were determined immediately and 3 days postinoculation. cfu, colony-forming units. Data represent the mean of 8 independent samples with standard deviation. Different letters above the bars indicate significant differences ($P < 0.05$, Student's *t*-test). The comparison was made separately for each time point.



Supplemental Figure 6. Subcellular localization of NPR1-GFP in *med16* plants.

Two-week-old soil-grown *35S:NPR1-GFP npr1* and *35S:NPR1-GFP med16-1 npr1* plants were treated with or without soil drenches plus foliar sprays of 0.3 mM BTH solution. After 24 hours, leaf pieces were mounted in water and viewed with a confocal microscope.



Supplemental Figure 7. Basal resistance and SAR induction in *med8* and *med25* mutants

(A) Growth of *Pst* DC3000 in *med16/sfr6*, *med8*, *med25*, and wild-type (WT) plants. Leaves of 4-week-old plants were inoculated with *Pst* DC3000 ($OD_{600} = 0.001$). The in planta bacterial titers were determined immediately and 3 days postinoculation. cfu, colony-forming units. Data represent the mean of 8 independent samples with standard deviation. *Pst* DC3000 grew significantly more in the *med16/sfr6* and *med8* plants than in *med25* and the wild-type plants. Different letters above the bars indicate significant differences ($P < 0.05$, Student's *t*-test).

(B) SAR-mediated resistance in *med16/sfr6*, *med8*, *med25*, and wild-type plants. Three lower leaves on each plant were inoculated with *Psm* ES4326 ($OD_{600} = 0.002$) (+SAR) or mock-treated with 10 mM $MgCl_2$ (-SAR). Three days later, two upper uninfected/untreated leaves were challenge-inoculated with *Psm* ES4326 ($OD_{600} = 0.001$). The in planta bacterial titers were determined 3 days after challenge inoculation. cfu, colony-forming units. Data represent the mean of 8 independent samples with standard deviation. An asterisk (*) indicates that *Psm* ES4326 grew significantly less in SAR-treated systemic leaves than in mock-treated

systemic leaves ($p < 0.05$, Student's *t*-test). The comparison was made between SAR-treated and mock-treated plants of the same genotype.

All experiments were repeated 3 times with similar results.

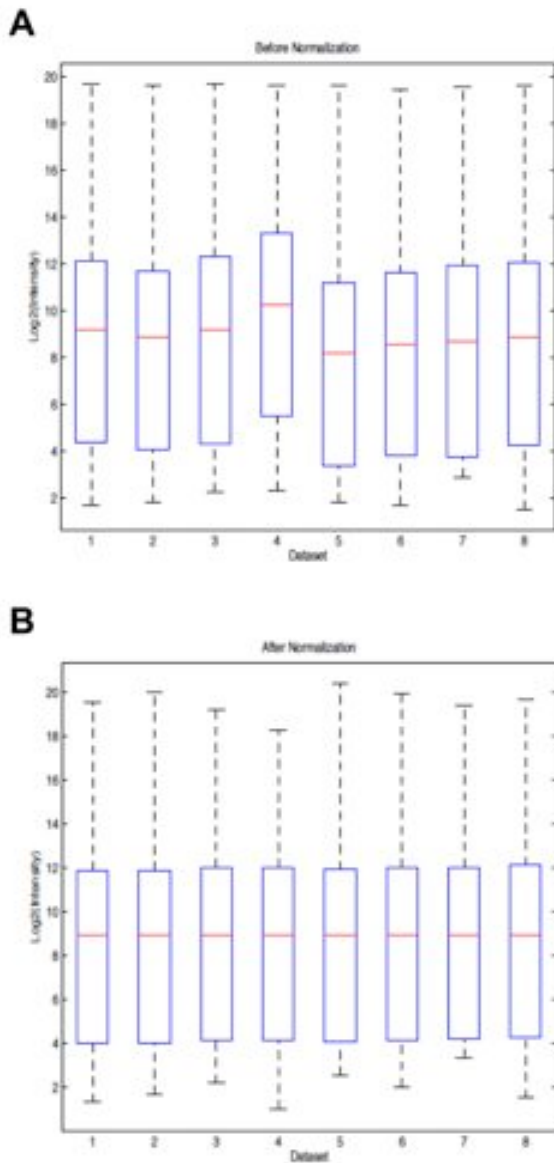
Supplemental Figure 8. Normalization of the microarray datasets obtained from the eight microarray probes

Individual signal intensity values obtained from the 8 microarray probes were log transformed (using 2 as the base) and normalized to ensure that meaningful biological comparisons can be performed.

(A) Box plots of the 8 datasets before normalization. The pre-normalized signal intensity datasets had different median intensities and dynamic ranges (i.e., height of the box).

(B) Box plots of the 8 datasets after normalization. The individual log-transformed signal intensity datasets were scaled so that all datasets had comparable lower quartile, median and upper quartile values.

The x-axis represents the 8 datasets, and the y-axis represents the log transformed signal intensity values. The boxes have lines at the lower quartile, median and upper quartile values. The whiskers are lines extending from each end of the boxes to show the extent of the rest of the data. Outliers are intensity values beyond the ends of the whiskers.



Supplemental Table 1. Defense genes differentially expressed between *med16-1* and wild type during *Pst* DC3000/*avrRpt2* infection

AGI Locus	Gene Name	<i>med16-1</i> /Wild type				AGI Description
		0 hr Log ₂ (FC)	4 hr Log ₂ (FC)	8 hr Log ₂ (FC)	24 hr Log ₂ (FC)	
At4g18470	<i>SNII</i>		1.093			SUPPRESSOR OF NPR1-1, INDUCIBLE 1
At1g02450	<i>NIMIN1</i>	2.031	1.477			NIM-INTERACTING 1
At3g25882	<i>NIMIN2</i>	1.514	1.843			NIM-INTERACTING 2
At1g09415	<i>NIMIN3</i>		1.254			NIM-INTERACTING 3
At5g22570	<i>WRKY38</i>	2.95	3.077			WRKY DNA-binding protein 38
At3g01080	<i>WRKY58</i>		1.413			WRKY DNA-binding protein 58
At5g01900	<i>WRKY62</i>	4.449	4.208			WRKY DNA-binding protein 62
At4g39030	<i>EDS5</i>		1.354			ENHANCED DISEASE SUSCEPTIBILITY 5
At5g13320	<i>PBS3</i>		1.366			AVRPPHB SUSCEPTIBLE 3
At2g13810	<i>ALDI</i>	2.99	2.203		1.088	AGD2-LIKE DEFENSE RESPONSE PROTEIN 1
At1g19250	<i>FMO1</i>		1.47			FLAVIN_DEPENDENT MOMOOXYGENASE 1
At4g14400	<i>ACD6</i>		-1.425			ACCELERATED CELL DEATH 6
At4g12470	<i>AZII</i>	-1.415	-2.347	-1.253	-2.236	AZELAIC ACID INDUCED 1
At2g40690	<i>SFD1</i>		-1.242			SUPPRESSOR OF FATTY ACID DESATURASE DEFICIENCY 1
At2g14610	<i>PR1</i>	1.265	3.165		-1.635	PATHOGENESIS-RELATED GENE 1
At3g57260	<i>PR2</i>	1.49	1.37			PATHOGENESIS-RELATED GENE 2
At1g75040	<i>PR5</i>			-1.704	-1.529	PATHOGENESIS-RELATED GENE 5
At2g43570		1.186	1.748			Chitinase, putative
At5g64120		1.701			-1.04	Peroxidase, putative
At1g30900	<i>VSR6</i>		1.207			Vacuolar sorting receptor 6
At5g24110	<i>WRKY30</i>	-2.066	-2.65	-2.502		WRKY DNA-binding protein 30
At4g23810	<i>WRKY53</i>	-1.167				WRKY DNA-binding protein 53
At2g40750	<i>WRKY54</i>	1.891	1.233			WRKY DNA-binding protein 54
At2g21900	<i>WRKY59</i>		2.022			WRKY DNA-binding protein 59
At1g80590	<i>WRKY66</i>		2.632			WRKY DNA-binding protein 66
At3g56400	<i>WRKY70</i>	1.357	1.14			WRKY DNA-binding protein 70
At5g44420	<i>PDF1.2</i>	-7.958	-6.710	-4.129	-5.339	PLANT DEFENSIN 1.2

Supplemental Table 2. Primers for identification of homozygous T-DNA insertion lines

Line	Forward primer (5'-3')	Reverse primer (5'-3')
SALK_092406	GAGTCAGATACCAGCACTTC	GTCACCCAACATTTGAGACG
SALK_048091	GAGTGTTCCTGAATTGTGCC	AACTTGGCTTGGAGGACAAC
SALK_059316	GCTGACTTCCTTGTGTTTCG	GATTCAGAGGCGAAGTCTG
SALK_129555	TTGCTTGGTACAACGGAGTG	TGACTTAAGGCAGCACATGC

Supplemental Table 3. The CAPS marker for the *med16-1* mutation

Marker	Forward primer (5'-3')	Reverse primer (5'-3')	Restriction enzyme	WT (bp)	<i>med16-1</i> (bp)
<i>med16-1</i>	ATGATGCTGCTAAGGACTGC	AACACTGCATGAACCGTTCC	NcoI	1193, 181, 96	1193, 277

Supplemental Table 4. Primers used for qPCR in this study

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>NPR1</i>	AGCATTCTCTCAAAGGCCGAC	TGAGACGGTCAGGCTCGAGG
<i>SNII</i>	TTGTTTCAGATCCTCATCGGC	CTTGAGTACCATTTCCTGCAG
<i>NIMIN1</i>	CAAGAAGCACGGAAACGTTAG	CATCAATGGCGGCTTCAAAC
<i>NIMIN2</i>	AAGTTGTTTCGGACGGTAACG	TCCAACGAGTTTCTCAACCC
<i>NIMIN3</i>	AGAGCCATCATACTCGTTGG	TCAACAGCTCCATGGAGAAG
<i>WRKY38</i>	CGTCGTAGTAAATCGGATCC	CCAGAAACCGAAGATGATCAG
<i>WRKY58</i>	AGTCGATCTTCTCGAGATG	AATCACAGCCTTAGCATCCG
<i>WRKY62</i>	GTATTTCTCCAGAGGAAGC	ACCACCAAGACGATCAATCC
<i>DIR1</i>	GAGCCAGGATGAGTTGAATG	GAAGCGAGTTCAGGATCAAC
<i>AZII</i>	TGTCTATGCACTGCTCTGAG	CGATATTGTGCACTGGCATT
<i>PDF1.2</i>	GCATTAACCTTGAAGGAGCC	GTTACTCATAGAGTGACAGAG
<i>CHIB</i>	GGTTCTGGATGACTGCTCAG	CTATACGATCGGCGACTCTC
<i>HEL1</i>	GTGAGTGCTTATTGCTCCAC	ACATCCAAATCCAAGCCTCC

Supplemental Methods

Plant Materials

For generation of the *PR1:luciferase* transgenic line, a pair of primers *XbaI-PR1PF* (5'-GCTCTAGAAATCTCATTATCCGTTTCGC-3') and *SmaI-PR1PR* (5'-TCCCCGGGATTTTGGGGTTCGTAAACATCG-3') was used to amplify the *PR1* gene promoter (1968bp), and a pair of primers *SmaI-LucF* (5'-TCCCCGGGTACTGTTGGTAAAGCCAC-3') and *SacI-LucR* (5'-ATCGAGCTCGTATCATGTCTGCTCGAAGCG-3') was used to amplify the coding region of a luciferase gene. The PCR products were digested with *XbaI/SmaI* and *SmaI/SacI*, respectively, and then ligated into the corresponding sites of the T-DNA binary vector pBI101 (Clontech, Mountain View, CA). For the *NPR1:Myc-NPR1* transgenic line, a pair of primers *SalI-NPR1PF* (5'-GCGTCGACTCTGAACGGAAGAAGCAACG-3') and *BamHI-NPR1PR* (5'-CGGGATCCAACAGGTTCCGATGAATTG-3') was used to amplify the *NPR1* gene promoter (2020bp), and a pair of primers *EcoRI-NPR1F* (5'-GGAATTCCATGGACACCACCATTGATGG-3') and *SacI-NPR1R* (5'-CGAGCTCTCACCGACGACGATGAGAGAG-3') was used to amplify the coding region of *NPR1* cDNA. The PCR products were digested with *SalI/BamHI* and *EcoRI/SacI*, respectively, and then ligated into the corresponding sites of the T-DNA binary vector pBI101 and a pBS-6×Myc plasmid (gift from Dr. Hongquan Yang, Shanghai Jiaotong University, China), respectively. The pBS-6×Myc-NPR1 plasmid was digested with *ClaI*, blunted with the Klenow fragment of DNA polymerase I, and then digested with *SacI*. The 6×Myc-NPR1 fragment was recovered and ligated into *SmaI/SacI*-digested pBI101-NPR1 promoter plasmid. For 35S:*MED16* transgenic lines, a pair of primer *SalI-MED16F* (5'-GCGTCGACATGAATCAGCAAACCCAGAAG-3') and *EcoRI-StrepII-MED16R* (5'-GGAATTCTATTTTCAAATTGAGGATGAGACCATAACAACACGGACCCACGTTC-3') was used to amplify the coding region of *Med16* genomic DNA. The PCR products were digested with *SalI/EcoRI*, and then ligated into the corresponding sites of the T-DNA vector pBI1.4T (Mindrinos et al., 1994). The plasmids, pBI101-PR1:luciferase, pBI101-NPR1:6×Myc-NPR1, and pBI1.4T-MED16, were introduced into the *Agrobacterium* strain GV3101(pMP90) by electroporation and transformed into Col-0, *npr1-3*, and *ien1*, respectively, following the floral dip method (Clough and Bent, 1998).

The T-DNA insertion mutants *med8*, *sfr6-2*, *med25-1*, and *med25-2* are SALK_092406, SALK_048091, SALK_059316, and SALK_129555, respectively. All T-DNA insertion lines were obtained from ABRC at The Ohio State University (Columbus, OH). Homozygous plants of the T-DNA insertion lines were confirmed with primers flanking the T-DNA insertions (Supplemental Table 2) and the left border primer LBa1 (Alonso et al., 2003).

Microscopy

Confocal microscopy was performed as described previously (Xiong et al., 2009).

Supplemental References

- Alonso, J.M., Stepanova, A.N., Leisse, T.J., Kim, C.J., Chen, H., Shinn, P., Stevenson, D.K., Zimmerman, J., Barajas, P., Cheuk, R., Gadrinab, C., Heller, C., Jeske, A., Koesema, E., Meyers, C.C., Parker, H., Prednis, L., Ansari, Y., Choy, N., Deen, H., Geralt, M., Hazari, N., Hom, E., Karnes, M., Mulholland, C., Ndubaku, R., Schmidt, I., Guzman, P., Aguilar-Henonin, L., Schmid, M., Weigel, D., Carter, D.E., Marchand, T., Risseuw, E., Brogden, D., Zeko, A., Crosby, W.L., Berry, C.C., and Ecker, J.R.** (2003). Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* **301**: 653-657.
- Clough, S.J. and Bent, A.F.** (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.* **16**: 735–743.
- Mindrin M., Katagiri F., Yu G.L., Ausubel F.M.** (1994). The *A. thaliana* disease resistance gene *RPS2* encodes a protein containing a nucleotide-binding site and leucine-rich repeats. *Cell* **78**: 1089-1099.
- Xiong, Y., DeFraia, C., Williams, D., Zhang, X., and Mou, Z.** (2009). Characterization of *Arabidopsis* 6-phosphogluconolactonase T-DNA insertion mutants reveals an essential role for the oxidative section of the plastidic pentose phosphate pathway in plant growth and development. *Plant Cell Physiol.* **50**: 1277-1291.