

Supplemental Data Chen et al., (2009) ETHYLENE INSENSITIVE 3 and ETHYLENE INSENSITIVE 3-LIKE 1 repress *SID2* expression to negatively regulate plant innate immunity in Arabidopsis

WT

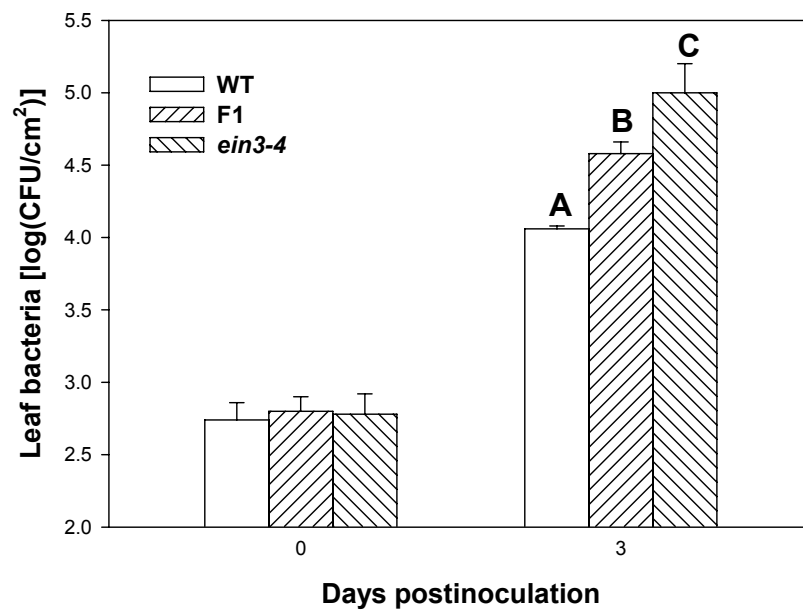


ein3-4



Supplemental Figure 1. Enhanced Disease Symptoms in *ein3-4* Plants.

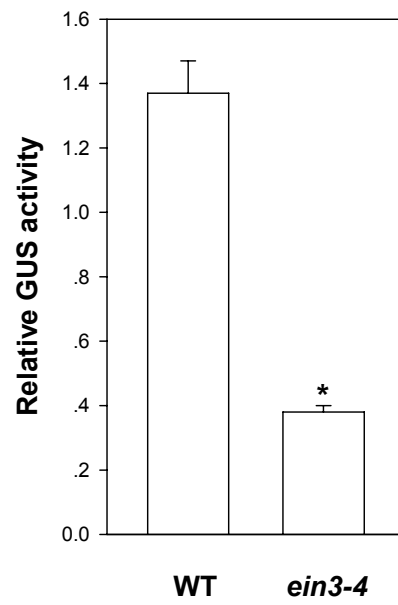
WT *RAP2.6-LUC* transgenic and *ein3-4* plants were dip-inoculated with 10^8 CFU/mL DC3000 bacteria, and disease symptoms were photographed 4 days post inoculation.



Supplemental Figure 2. *ein3-4* Is A Semidominant Mutation that Enhances Disease Susceptibility.

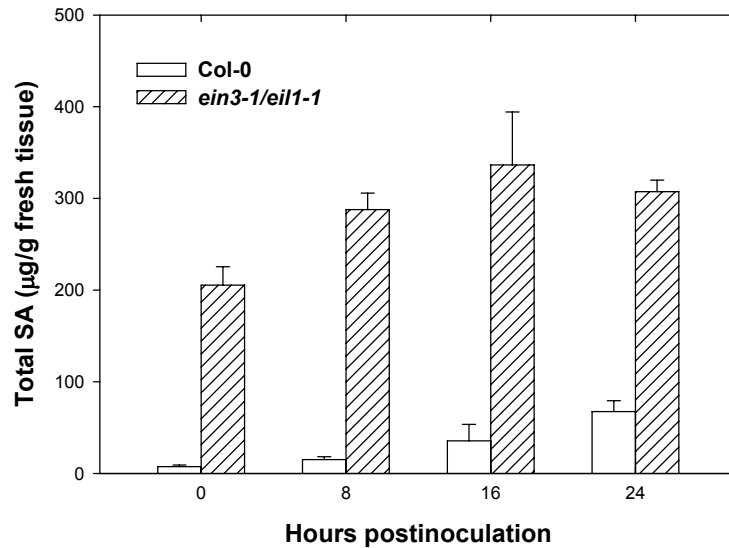
WT *RAP2.6-LUC* transgenic plants, *ein3-4*, and *ein3-4* x WT F1 were infiltrated with 10^5 CFU/mL DC3000 (*avrB*) bacteria, and leaf bacterial population was determined at the indicated times. Each data point consisted of three replicates. Error bars indicate standard deviation. Different letters above bars denote significant difference at 0.01 (Student *t* test). The experiment was performed twice with similar results.

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Supplemental Figure 3. *ein3-4* Shows Greater Repression of *SID2* Promoter. The *SID2* promoter-*GUS* reporter plasmid was transfected into protoplasts from WT *RAP2.6-LUC* transgenic or *ein3-4* plants, and GUS activity was determined. Each data point consisted of 3 replicates. Error bars indicate standard deviation. * indicates significant difference at 0.05 between *ein3-4* and WT. The experiment was performed twice with similar results.

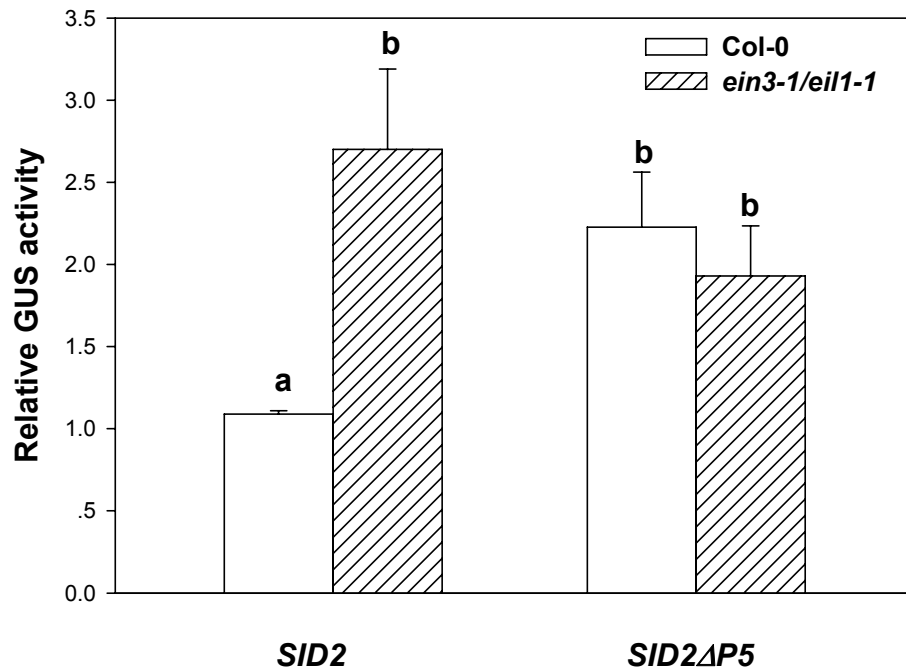
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Supplemental Figure 4. Total SA Level in *ein3-1/eil1-1* Double Mutant Plants After Bacterial Infection.

Plants were inoculated with DC3000 bacteria (10^6 CFU/mL) for the indicated hrs, and tissues were collected for SA extraction. Each data point consisted of three replicates. Error bars indicate standard deviation. The experiment was repeated twice with similar results.

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Supplemental Figure 5. *SID2* P5 Sequence Contributes to Transcriptional Repression by EIN3 and EIL1. The *SID2-GUS* reporter plasmid and *SID2ΔP5-GUS* construct carrying a 73 bp deletion of the P5 sequence (Fig. 7) were introduced into Col-0 or *ein3-1/eil1-1* protoplasts, and GUS activity was determined. Each data point consisted of 3 replicates. Error bars indicate standard deviation. Different letters denote significant difference at 0.05 (Student *t* test). The experiment was done twice with similar results.

SUPPLEMENTAL DATA

Supplemental Table 1. Complementation Test of *rrb3* and *ein3-1*.

Genotype	Seedlings scored	ACC insensitive	ACC sensitive
<i>rrb3</i> x <i>ein3-1</i> F1	23	23	0
<i>ein3-1</i> x <i>rrb3</i> F1	19	19	0
WT	28	0	28
<i>rrb3</i>	15	15	0

Supplemental Table 2. Quantitative Real-Time RT-PCR Analysis of Genes

Up-Regulated in *ein3-1/eil1-1*.

Gene	Protein encoded	*Fold increase in <i>ein3-1/eil1-1</i>
At4g30530	Putative defense-related protein	2.49 ± 0.04
At4g39940	Adenosine-5'-phosphosulfate-kinase (AKN2)	2.89 ± 0.11
At3g48090	Enhanced Disease Susceptibility 1 (EDS1)	11.89 ± 0.09
At2g31880	Leucine rich repeat transmembrane protein	14.01 ± 1.19
At3g01830	calmodulin-related protein	41.92 ± 0.53
At2g42360	C3HC4-type RING finger family protein	90.92 ± 3.77
At5g13320	PBS3 (<i>avrPphB</i> susceptible)	155.13 ± 2.14
At1g02450	NIM1-Interacting 1 (NIMIN-1)	286.69 ± 4.86
At1g65970	Thioredoxin-dependent peroxidase 2	575.12 ± 4.6

*Fold change was normalized to the value of WT plants.

Supplemental Table 3. Primers Used for Quantitative PCR Analyses..

Primer name	Sequence	Used for
<i>Actin</i> -forward	5'-GGTGCATGGTTGGTATGGGTC-3'	Quantitative RT PCR
<i>Actin</i> -reverse	5'-CCTCTGTGAGTAGAACTGGGTGC-3'	Quantitative RT PCR
<i>PR1</i> -forward	5'-TACGCAGAACAATAAGAGG-3'	Quantitative RT PCR
<i>PR1</i> -reverse	5'-TCGTTACATAATTCCCACG-3'	Quantitative RT PCR
<i>PR2</i> -forward	5'-TCGATGAGAATAAGAAGGAACCAAC-3'	Quantitative RT PCR
<i>PR2</i> -reverse	5'-ATAACAACATACTACACGCTGAAAG-3'	Quantitative RT PCR
<i>SID2</i> -forward	5'-GAGACTTACGAAGGAAGATGATGAG-3'	Quantitative RT PCR
<i>SID2</i> -reverse	5'-TGATCCCGACTGCAAATTCCTCTC-3'	Quantitative RT PCR
At4g30530-forward	5'-GAAAACCTTACGGAGGATACCACAAC-3'	Quantitative RT PCR
At4g30530-reverse	5'-AAGGATCCAATCATCTCTCAAAG-3'	Quantitative RT PCR
At4g39940-forward	5'-GGTAGTGCTGAAACACACAGGAGAC-3'	Quantitative RT PCR
At4g39940-reverse	5'-ATGATAGTGTGACGAAGCGAGAC-3'	Quantitative RT PCR

At3g48090-forward	5'-AGGAAAATCGGTAAAGGTGATGTAG-3'	Quantitative RT PCR
At3g48090-reverse	5'-CAAGTGCGTGAAGATAGAG-3'	Quantitative RT PCR
At2g31880-forward	5'-TGCCAGATGCAGTCACACATATTAC-3'	Quantitative RT PCR
At2g31880-reverse	5'-GTTCTCATCCACTTTATCAGACTC-3'	Quantitative RT PCR
At3g01830-forward	5'-TTTTCTCTATCTCTCGCTCAAGTC-3'	Quantitative RT PCR
At3g01830-reverse	5'-AGCTCCAACGATTTATCATCAGTC-3'	Quantitative RT PCR
At2g42360-forward	5'-AGTGGCTTCTTTATCTGGAGTTATC-3'	Quantitative RT PCR
At2g42360-reverse	5'-GCCATGACTGTGGGATTGAG-3'	Quantitative RT PCR
At5g13320-forward	5'-ACAACCTGTTAGAGGAGATCATCAC-3'	Quantitative RT PCR
At5g13320-reverse	5'-TGTGCTCCTCCAGAAGTTCCAG-3'	Quantitative RT PCR
At1g02450-forward	5'-TCAATCACTTTCTCTAAATGTATCC-3'	Quantitative RT PCR
At1g02450-reverse	5'-TTTCAGCTAACTCTTCTCGTCTAC-3'	Quantitative RT PCR
At1g65970-forward	5'-AACTCTTCTATTTTCTCTGTCTTC-3'	Quantitative RT PCR
At1g65970-reverse	5'-AAGCACCAGGAACACCAAAG-3'	Quantitative RT PCR
P2-forward	5'-ACTTCTAAAGTCTAGTCC-3'	Chromatin IP
P2-reverse	5'-TCTTCATTAGATCATTGC-3'	Chromatin IP
P3-forward	5'-GGTCTCAAAGAGCCTAAG-3'	Chromatin IP
P3-reverse	5'-CTCTTCTCGTTTGATACG-3'	Chromatin IP
P4-forward	5'-GAGCATGCGTGTAATGCC-3'	Chromatin IP
P4-reverse	5'-ACATACAGACCGGATTCG-3'	Chromatin IP
P5-forward	5'-ATTTCATGAAATATTAATGCACGAC-3'	Chromatin IP
P5-reverse	5'-GAGATATAAATAGGGACTGATGTAGC-3'	Chromatin IP
<i>SID2</i> intron 2-forward	5'-TTTCGTTCTCTAGCTTGCTGTCTTG-3'	Chromatin IP
<i>SID2</i> intron 2-reverse	5'-CCGAGTTCGAGTGTGTGTTTTATTTC-3'	Chromatin IP
<i>Actin</i> promoter-forward	5'-GATCCTAGTCTTTTAGTGTGCATTC-3'	Chromatin IP
<i>Actin</i> promoter-reverse	5'-ATTAAATGATTGATCGGTTTTTCGTG-3'	Chromatin IP
MQC12-forward	5'-AAATCCTCCACCAGCACCACCAC-3'	Mapping
MQC12-reverse	5'-GTCTCGAGGACCAAAGACATTCTTC-3'	Mapping
MOE17-forward	5'-ACGATATACTACGGGTGAAATCCTAC-3'	Mapping
MOE17-reverse	5'-GAATGTATTCGTGATTCACAAATGTG-3'	Mapping
