Supporting Information

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SI Materials and Methods

Map-Based Cloning. The *fin3* mutation was identified by crossing the mutant (in Col-0 background) to wild-type plants of the Landsberg *erecta* (L*er*-0) ecotype. Genomic DNA from recombinants displaying the mutant phenotype in the segregating F_2 population was extracted and used for subsequent genetic mapping analysis. The mutation was roughly mapped using a set of known simple sequence-length polymorphism markers (SSLP). Fine mapping was realized using PCR-based markers and sequencing SNP markers identified from The *Arabidopsis* Information Resource (TAIR) polymorphism/allele collection. Mutation was found associated to a 38.7-kb interval between the TAIR markers PERL0860777 (SNP position PCR-amplified using the primers 5′-GTCAATGAGTAGTAGTGAACATTCAAG-3′ and 5′-TCTTCGCTGGAAAACAGCTT-3′ and then sequenced) and PERL0861406 (SNP position PCR-amplified using the primers 5′-TTCTGGCTCCTTCTACGCTC-3′ and 5′-GGTGCAGCTGACAGAGTGTT-3′ and then sequenced). Direct sequencing of candidate genes was then used to identify mutations in *fin3* line.

cDNA Synthesis and Quantitative RT-PCR. First-strand cDNA was synthesized from 3 μg RNA using SuperScript RNA H- Reverse Transcriptase (Invitrogen) and an oligo (dT) primer, according to the manufacturer's instructions. cDNA was amplified in triplicate by quantitative PCR using SYBR Green JumpStart Taq ReadyMix (Sigma) and the PTC-200 Peltier Thermal Cycler (MJ Research). The relative expression values were determined using *U-box* as reference and the comparative Ct method ($2^{-\Delta\Delta Ct}$). Primers used for quantitative PCR are as follows: *FLS2* (*At5g46330*) forward 5′-ACTCTCCTCCAGGGGCTAAGGAT-3′ and reverse 5′-AGCTAACAGCTCTCCAGGGATGG-3′; *SIRK*/*FRK1* (*At2g19190*) forward 5′-ATCTTCGCTTGGAGCTTCTC-3′ and reverse 5′-TGCAGCGCAAGGACTAGAG-3′ (1); *PR1* (*At2g14610*) forward 5′-GGCACGAGGAGCGGTAGGCG-3′ and reverse 5′-CACGGCGGAGACGCCAGACA-3′; *U-box* (*At5g15400*) forward 5′-TGCGCTGCCAGATAATACACTATT-3′ and reverse 5′-TGCTGCCCAACATCAGGTT-3′ (2).

1. He P, et al. (2006) Specific bacterial suppressors of MAMP signaling upstream of MAPKKK in *Arabidopsis* innate immunity. *Cell* 125:563–575.

2. Nemhauser JL, Mockler TC, Chory J (2004) Interdependency of brassinosteroid and auxin signaling in *Arabidopsis*. *PLoS Biol* 2:e258.

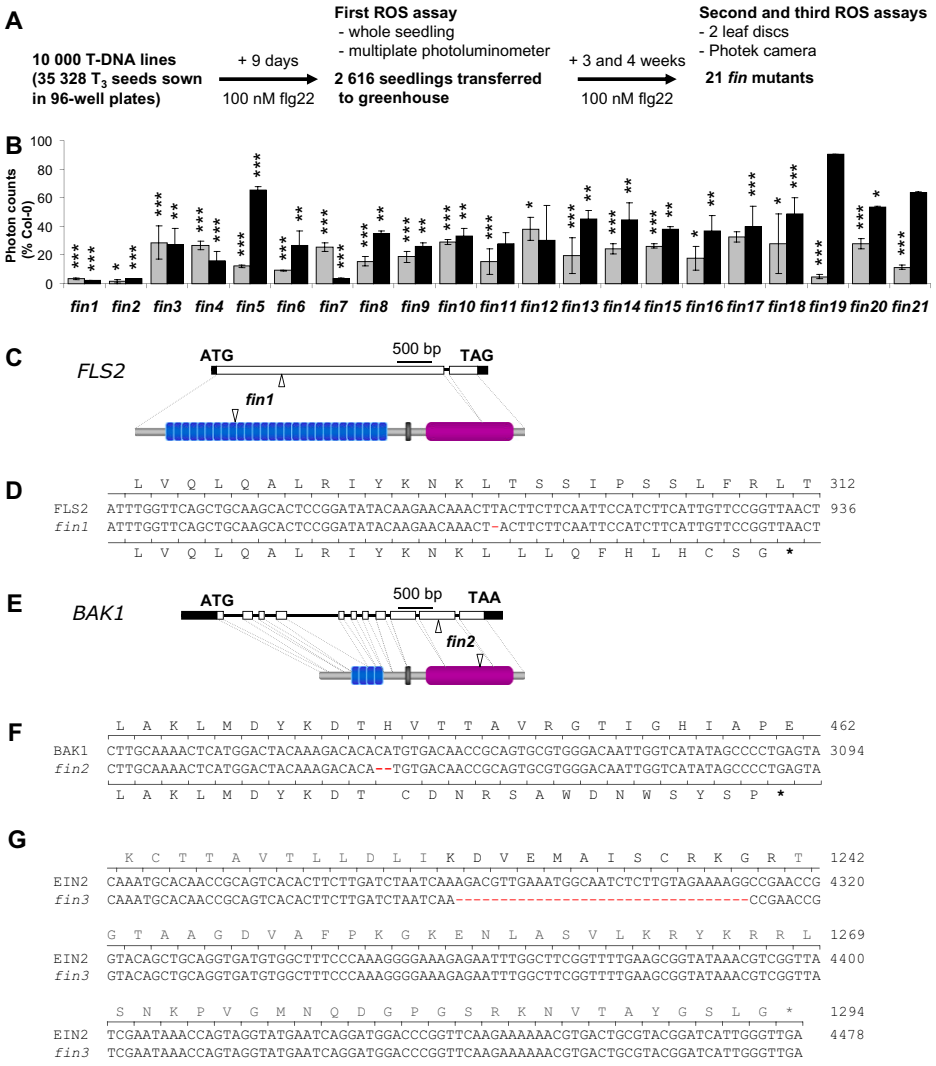

Fig. S1. *Fin* forward genetic screen. (*A*) Flowchart of the forward genetic screening developed to isolate *Arabidopsis flagellin*-insensitive (*fin*) mutants impaired in the oxidative burst induced by 100 nM flg22. (*B*) Total reactive oxygen species (ROS) production measured during the second (gray bar) and third (black bars) assays for 40 min after application of 100 nM flg22 in the 21 *fin* candidates. Values are expressed as percentage of photon counts measured in wild-type Col-0 with identical treatment and are mean ± SE ($n = 2$). (*C*) Schematic representations of *FLS2* gene (*At5g46330*) and deduced protein with position of the *fin1* mutation. Filled boxes and open boxes represent the 5′- and 3′-untranslated regions and exons of the gene, respectively. The *fin1* deletion (T) is at position +900 after start codon. The predicted leucine-rich domains are shown as blue rectangles, the transmembrane domain is gray, and the kinase domain is purple. (*D*) Alignment of the nucleotide sequences of *FLS2* gene and *fin1* mutant in the region of the detected mutation. The wild-type translated sequence is shown above. Numbers indicate amino acid and nucleotide position after the start codon. (*E*) Schematic representations of *BAK1/SERK3* gene (*At4g33430*) and deduced protein with position of the *fin2* mutation. Filled boxes and open boxes represent the 5′- and 3′-untranslated regions and exons of the gene, respectively. The *fin2* deletion (CA) is at position +3045/+3046 after the start codon. The predicted leucine-rich domains are shown as blue rectangles, the transmembrane domain is gray, and the kinase domain is purple. (*F*) Alignment of the nucleotide sequences of *BAK1/SERK3* and *fin2* in the region of the detected mutation. The wild-type translated sequence is shown above. Numbers indicate amino acid and nucleotide position after the start codon. (*G*) Alignment of the nucleotide sequences encoding the C-terminal regions of *EIN2* and *fin3*. The wild-type translated sequence is shown above. In *fin3*, an asparagine amino acid residue (N) replaces the wild-type KDVEMAISCRKG sequence. Numbers indicate amino acid and nucleotide position after the start codon.

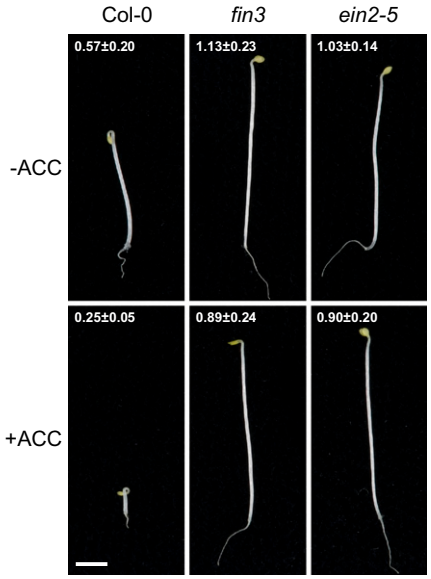

Fig. S2. The *fin3* mutant is ethylene-insensitive. Triple-response phenotype of 3-d-old *Arabidopsis* Col-0, *fin3*, and *ein2-5* seedlings grown in the dark and in presence or absence (±) of 100 μM of the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC). Mean hypocotyl lengths are given in millimeters ± SE (n = 8). (Scale bar, 2 mm.)

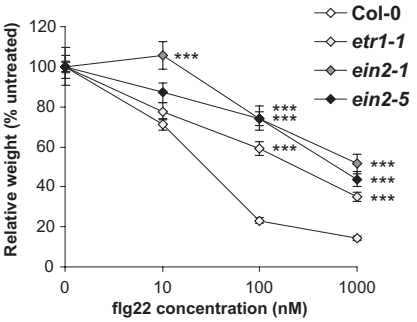

Fig. S3. Ethylene perception and signaling are required for flg22-induced seedling-growth inhibition (SGI). Growth inhibition of Col-0, *etr1-1*, *ein2-1*, and *ein2-5* seedlings treated with different concentrations of flg22 for 12 d. Values are mean ± SE (n = 8). Data are representative of one of three experiments. Statistical significance by comparison with Col-0 was assessed using one-way ANOVA followed by Dunnett's test. ***P < 0.001.

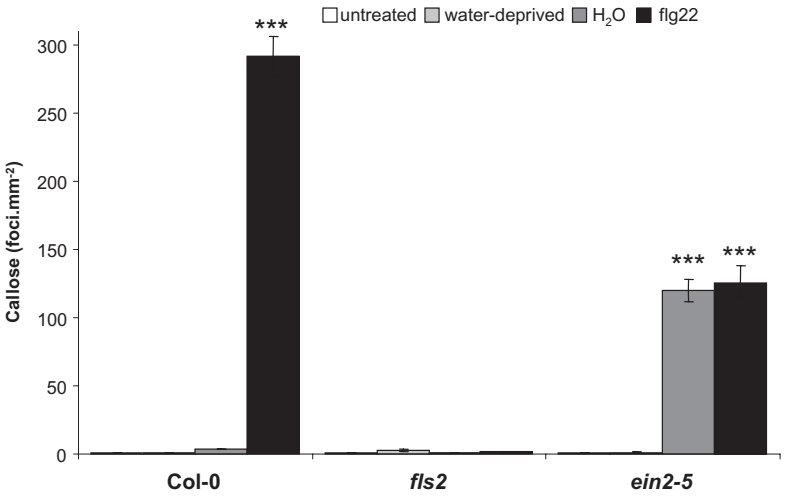

Fig. S4. Callose deposition in leaves of Col-0, *fls2*, and *ein2-5* plants. Callose was measured in watered plants (untreated), plants deprived of water for 24 h and then watered for the next 24 h (water-deprived), and plants deprived of water for 24 h, then infiltrated with water (H_2O) or 1 μM flg22 (flg22), and then watered for the next 24 h. Results showing quantification of callose deposits (foci/mm^{-2}) are mean ± SE (n = 4). Statistical significance by comparison with untreated Col-0 was assessed using one-way ANOVA followed by Dunnett's test. ***P < 0.001. Similar results were observed in at least two independent experiments.

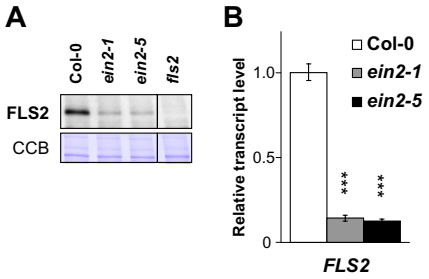

Fig. S5. FLS2 expression is impaired in *ein2-1* and *ein2-5* mutants. (*A*) Western blotting showing accumulation of FLS2 in Col-0, *ein2-1*, and *ein2-5* 13-d-old seedlings. The *fls2* mutant is used as a negative control for FLS2 accumulation. Thirty micrograms of total protein were loaded in each lane. Blot stained with CCB is presented to show equal loading. (*B*) Quantitative RT-PCR analysis of *FLS2* expression in Col-0, *ein2-1*, and *ein2-5* seedlings. Transcript levels are normalized to the *U-box* housekeeping gene and are presented as relative to Col-0. Statistical significance by comparison with Col-0 was assessed using one-way ANOVA followed by Dunnett's test. ***$P < 0.001$.

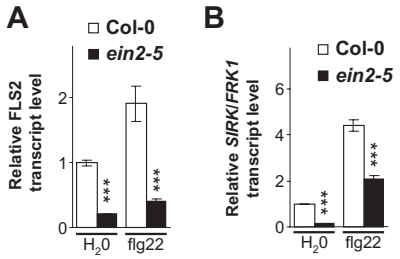

Fig. S6. Expression of *SIRK/FRK1* gene in *ein2-5*. (*A* and *B*) Quantitative RT-PCR analyses of (*A*) *FLS2* and (*B*) *SIRK/FRK1* expression in Col-0 and *ein2-5* seedlings treated for 1 h with water or 100 nM flg22. Transcript levels are normalized to the *U-box* housekeeping gene *At5g15400* and are presented as relative to expression in water-treated Col-0. Statistical significance by comparison with water-treated Col-0 was assessed using one-way ANOVA followed by Dunnett's test. ***$P < 0.001$. Similar results were observed in at least three independent experiments.

***FLS2* (At5g46330)**

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-1474 TAAGGCGCCAGATTTAGGCTCTGGTCCGAAAGGGCGTGGGTTCAAATCCCACAGCTGTCA
-1414 ATTTGTTTTTTTCTTTGACTCCTTTGTTCATCTTTATTTTAAAATCAATATAAACCCTTT
-1354 TCGGACATTCTAAATATTTTGACCCAACCTAATGATATTCAtTATTTGTCTAACGGGTCA
-1294 ACTTTCAAGTCTTCAAGTAAACATGATATGGAATTATATGGGTCAACTCCAAGTAAGAAC
-1234 AGTGCATGCATCTTGAAGACAAAATTGATTTTTTTTTTTGTTTTTAATTAATGGAAATTT
-1174 TGAAATACTAATGGGTCAAGATGAGGTTGTCCAAATCAACTGATATCATATATGACCAGA
-1114 TTTC-TCAATAGTCATTAGTCAATACAAAATCCATAATCTTTTTTAAAAATATAATCTTTT
-1054 TATTTTAAATCGCCTATTTTTTTCCATTCATTTGTCCAATGTGAAAAGCCATAAATCATA
 -994 TATTAAGAAGTTGTGAATTGTGATCATAGGAGTAATATTTCTATCTGAACAAAAATTGAA
 -934 TTTTGTTTTGCTTTTTGTTGAAAGATAaATAAAATGTTTGGCAGATTGTTTGTACAGAAC
 -874 ACAACTTGTTGATAAAACAAATTCCATCAAGACAGCTAATGAGCTTTTGATAGTCAGAGC
 -814 TTTGAAGACGTGTTAATGAATTAAACTGATTAGACCTAAAACCAAATATTTAGAAAGAAA
 -754 AAAATATGATAATTGTTATGTTATTTAATCAATAGTCAAATACTATGTTGAGTACCAATC
 -694 ATTTCAGTTTCAACGATTGCTACTCTTTTGACCAATAACAAAAAGGTTTTTATTTTTCAT
 -634 TTCTCAAACTCCATTTTTGTTTGTTTTTAGCATAAAAACAGCTAGAATGTGAGtTTCATA
 -574 TGTATTATATACTATAAGAAATCATATAAATTAACGACTAGAACATATTGTTTTCATTCT
 -514 AGTGGTATATCTGAGTTTGACTAGAACGTTTGGAGGCAGATTAAAGTGATTTCGCCTTGT
 -454 CAAAACTACTTTGTATATGTTATCAATCAATCGCTCAAAACTAAATCGGAAAAAAACTAC
 -394 ATAAATAAGATATGGAAAATGATACTAACACAAACTAGTAAGTAAGTAGTCACTACATAT
 -334 TCTGGTCCCATGAATaTGGCTTACATAGTAcATACATTGTGAGATTAAATGAATaTGATA
 -274 TATAATACAAGCATATAAGCATGTGCATGACTATGAAGTGGGAATACATTTAGTGCCTTT
 -214 ATATGCAACTTGACAGAATTACCAATTATGTGAATAGTGAAACTTATATTGCGTCTTGGC
 -154 CTTTTCACATCCGTGACATGATCCCTCAAATCTATATCTTCTTGCGGCATATGCTTATAT
  -94 ATAAACATATGACTTCGTCTATAGATTtATATCCAGATAATTAATTCTCTCATAATATCT
  -34 TCTCCACAATTTCAACTCTTCTAAAGTCTAAACC
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Fig. S7. In silico analysis of FLS2 promoter. Positions of potential EIN3 binding sites in the promoter of the *FLS2* gene (*At5g46330*). The relaxed primary ethylene response elements (PEREs) described in ref. 1. (AYGWAYCT) are highlighted in green. One mismatch (presented in lowercase) was allowed during the search for homology. The positions of the two regions (distal from −1417 to −1100; proximal from −379 to −149) identified by ChIP and followed by Illumina sequencing (ChIP-Seq) corresponding to the EIN3 binding sites to the *FLS2* promoter are underlined.

1. Kosugi S, Ohashi Y (2000) Cloning and DNA-binding properties of a tobacco Ethylene-Insensitive3 (EIN3) homolog. *Nucleic Acids Res* 28:960–967.

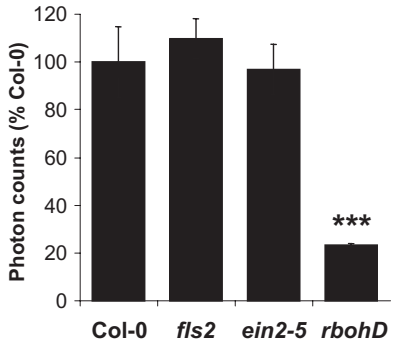

Fig. S8. The *ein2-5* mutation does not affect the oxidative burst triggered by calyculin A. Oxidative burst triggered by 500 nM calyculin A in wild-type Col-0, *fls2*, *ein2-5*, and *rbohD* mutant seedlings measured in relative light units (RLU). Total ROS production was measured for 14 h and is expressed as percentage of calyculin A-treated Col-0. Values are mean ± SE (n = 16). Similar results were observed in two independent experiments.

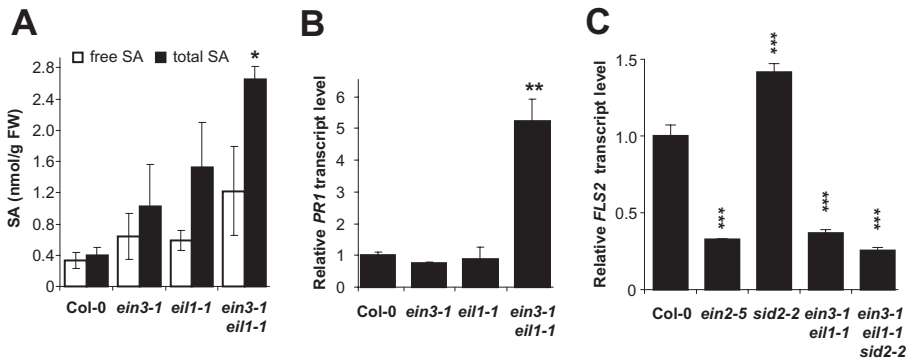

Fig. S9. The effect of the *ein3-1 eil1-1* mutation on *FLS2* expression is independent of salicylic acid (SA) levels. (*A*) Free (white bars) and total (black bars) SA content in 5-wk-old soil-grown Col-0, *ein3-1*, *eil1-1*, and *ein3-1 eil1-1* plants. Values are mean ± SE (n = 4). Data are representative from one of two independent experiments. (*B*) Quantitative RT-PCR analysis of *PR1* expression in old soil-grown Col-0, *ein3-1*, *eil1-1*, and *ein3-1 eil1-1* plants. Transcript levels are normalized to the *U-box* housekeeping gene and are presented as relative to Col-0. (*C*) Quantitative RT-PCR analysis of *FLS2* expression in Col-0, *ein2-5*, *sid2-2*, *ein3-1 eil1-1*, and *ein3-1 eil1-1 sid2-2* seedlings. Transcript levels are normalized to the *U-box* housekeeping gene and are presented as relative to Col-0. Statistical significance by comparison with Col-0 was assessed using one-way ANOVA followed by Dunnett's test. *P < 0.05; **P < 0.01; ***P < 0.001.

Table S1. Allelism test between *Arabidopsis ein2* null-mutants and *fin3* mutant.

	ROS (% of Col-0)	Fresh weight (% of untreated)
Col-0	100 ± 7.0	33.5 ± 4.3
ein2-1	58.3 ± 3.9	n.d.
ein2-5	n.d.	62.3 ± 7.4
fin3	70.3 ± 3.7	55.2 ± 7.0
♂ *ein2-1* x ♀ *fin3* F_1	62.4 ± 4.0	n.d.
♂ *ein2-5* x ♀ *fin3* F_1	n.d.	75.3 ± 5.2
♂ Col-0 x ♀ *fin3* F_1	111.1 ± 5.2	38.8 ± 2.7

The phenotype from the parents and the F_1 progeny have been assessed for ROS production following application of 100 nM flg22 (n=20), and for seedling growth inhibition after 14 days of treatment with 1 μM flg22. Same letter indicates statistically nonsignificant (one-way ANOVA; P > 0.05) differences between samples. n.d., not determined.