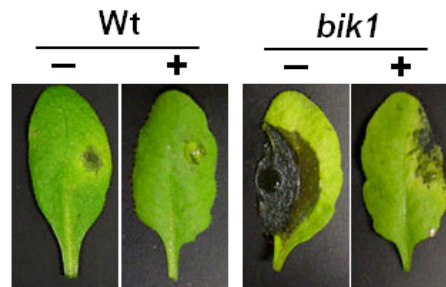
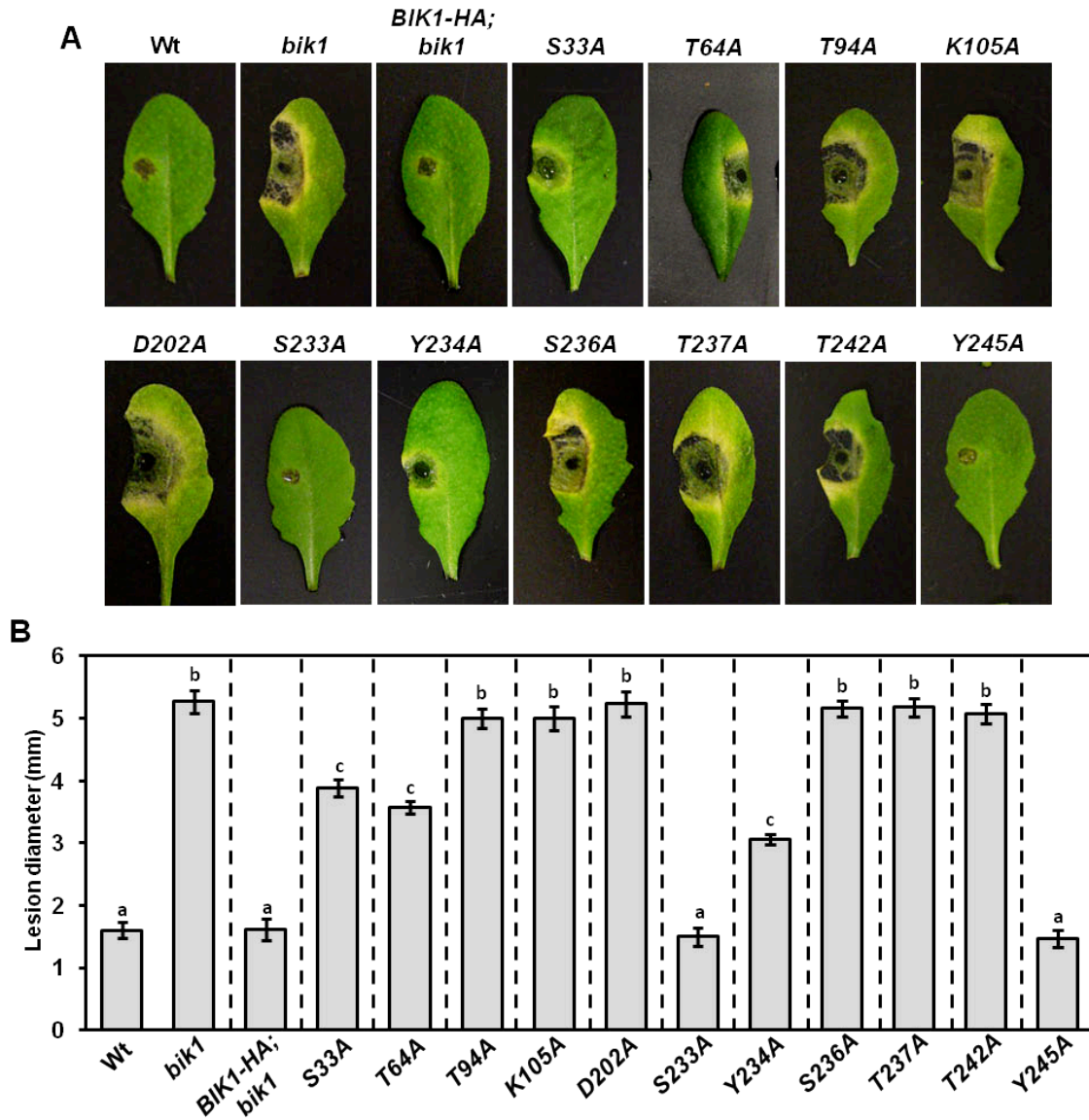


Supplemental Figures and Tables



Supplemental Figure 1. Wound-induced immunity to *B. cinerea* requires BIK1

Disease symptoms of non-wounded (-) and forceps-wounded (+) Arabidopsis leaves 3 days post-drop-inoculation with *B. cinerea* (2.5×10^5 spores/mL). Wt, wild-type.



Supplemental Figure 2. Response of the *BIK1* substitution mutants to *A. brassicicola* infection (A) Disease symptoms and (B) lesion diameters of *bik1* and *BIK1* substitution mutants 4 days post-inoculation with *A. brassicicola* (5×10^5 spores/mL). Data in (B) represent mean \pm SE from a minimum of 30 disease lesions. Experiments were repeated at least three times with similar results. Statistical analysis was performed as described in the legend of Figure 2. All substitutions are in the *bik1* background. Wt, wild-type.

BIK1 (AT2G39660) promoter region

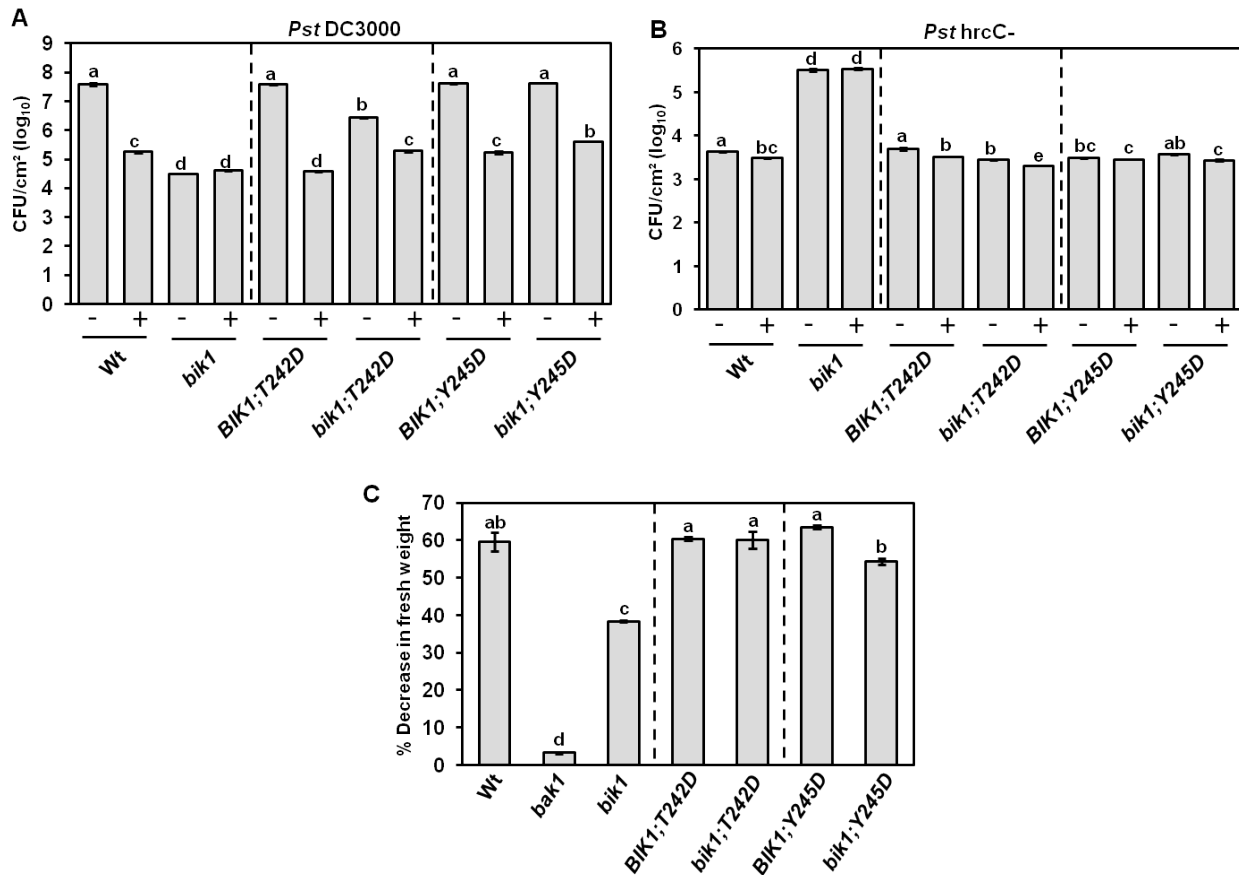
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-1287  atttaaacctgtgctcataatcttacttttacaattattacttttccataaattgtggaaa
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-1167  gagtttttagatacttgggaagatatatgcattttatgaatacagattacagacacatacta
-1107  gtactactgtatgtctgtatgatggatacaaaaaaaaaatcatgtatgaataactaaaatttta
-1047  tttagaaatctattttcaattggttgcaacaatcaagttgtcaaattttatttttgtaaccg
-987   ttaaacaaacaaatcatcgatttaggtttctaactgaattgacatctcaacaaaaaagg
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-867   gaagaaaattcaggtgacttttagaaccattataaccggaagaaaagggtgaattttaatt
-807   ttttagctgtgtggaagacacggcaagtccaagtagtaccttcgtacgtcaatattgtcca
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-387   gtaaaaattcatgggtacaaacatcgaatataattttctgcttacacacaccaattaac
-327   gtggatagaccaattgaaatattttgttacgacaaaagcaaaaacaaaacacgtcatgtt
-267   tcgctgtttgtttgtcgtcccgttaatggtaatctttcagacacatacagtacccaaac
-207   agtaatttgactaaaatttctctctgtctaaatttcagaagaaaaaaaaacttttaggat
-147   atattgccaaaagatcttaaaaaatgggtcatatcattttgatcatatagaatccaacgac
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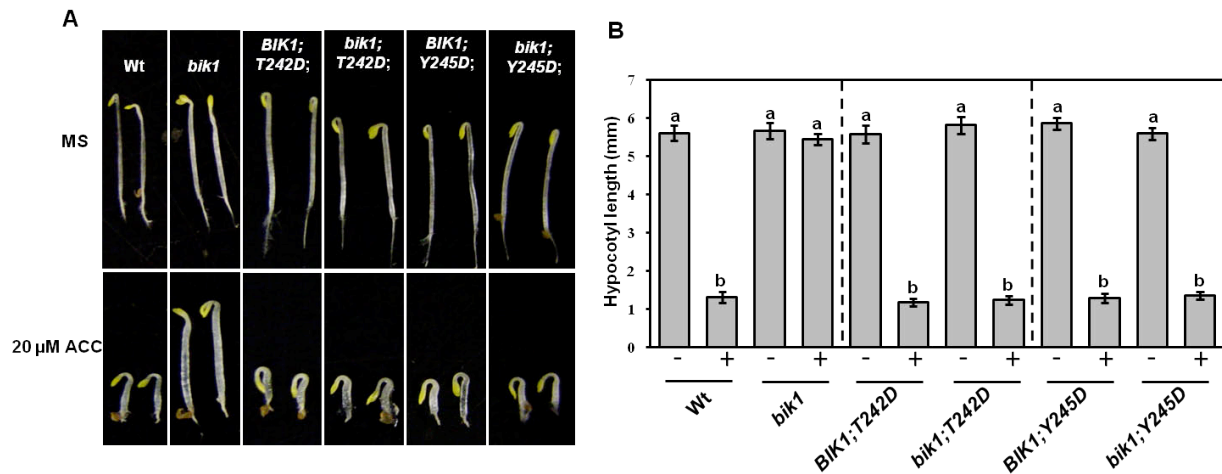
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Supplemental Figure 3. The BIK1 promoter sequence and location of putative EIN3-binding sequences in the *BIK1* promoter.

The primary ethylene response elements (AYGWAYCT) as described (Kosugi and Ohashi 2000) are highlighted in yellow. Two predicted EIN3 binding regions (Bik1 promoter region A from -1221 to -774 and region B from -494 to -166) are highlighted in red and primers designed for CHIP-PCR are highlighted in blue.

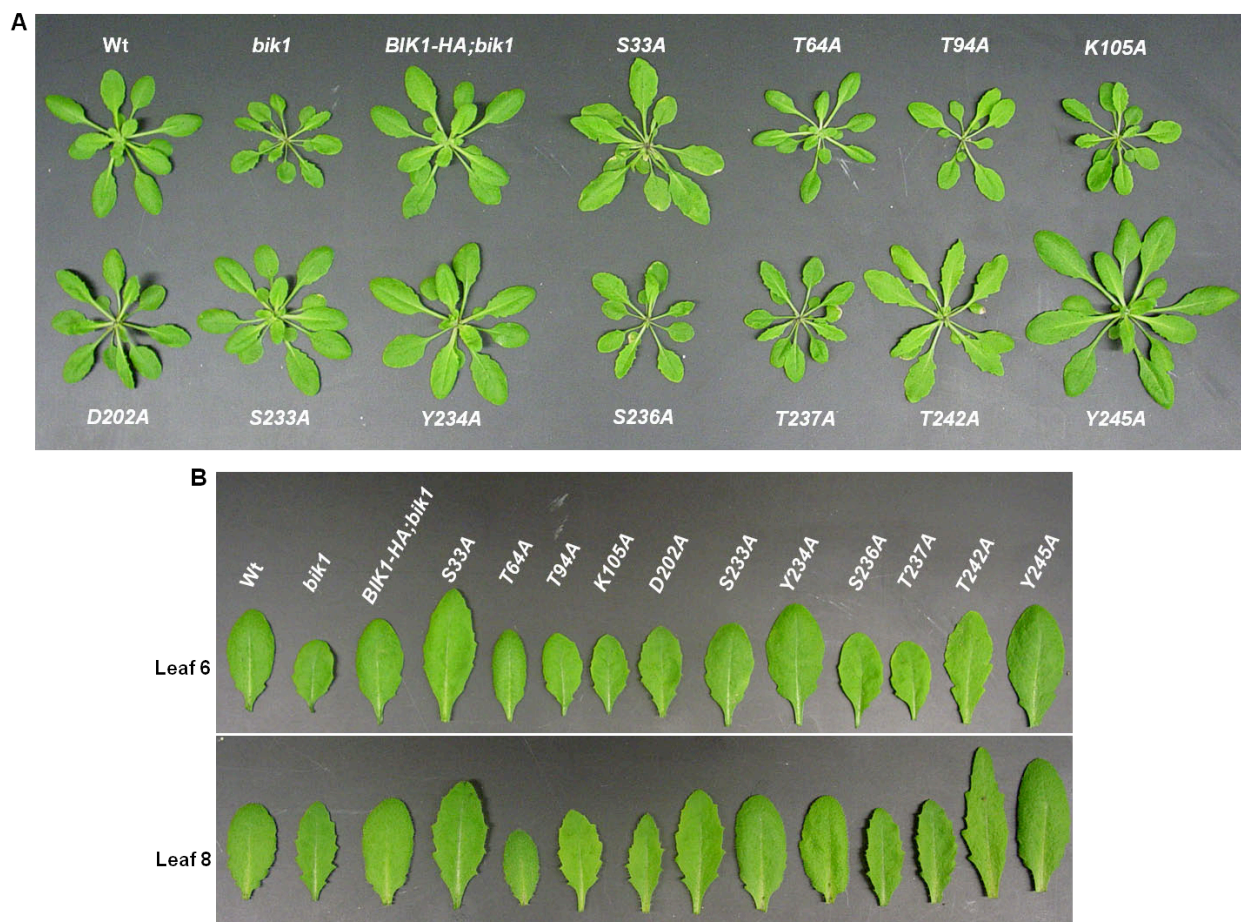


Supplemental Figure 4. Bacterial and flagellin responses of the BIK1 phospho-mimic mutants
 Bacterial growth (colony forming units [CFU]/cm² leaf area) in water (-) and flg22 (+) pre-treated *bik1* and *BIK1* substitution mutants 3 days post-inoculation with (A) *Pst*DC3000 or (B) the non-pathogenic *Pst hrp* mutant *hrcC-*. (C) Percent decrease in fresh weight of *bik1* and *BIK1* substitution seedlings after growth in 10 nM flg22. Data represent mean values \pm SE from three experiments and a minimum of 120 seedlings for bacterial growth and fresh weights, respectively. Experiments were repeated at least three times with similar results. Statistical analysis was performed as described in the legend of Figure 2. Wt, wild-type; CFU, colony-forming units.



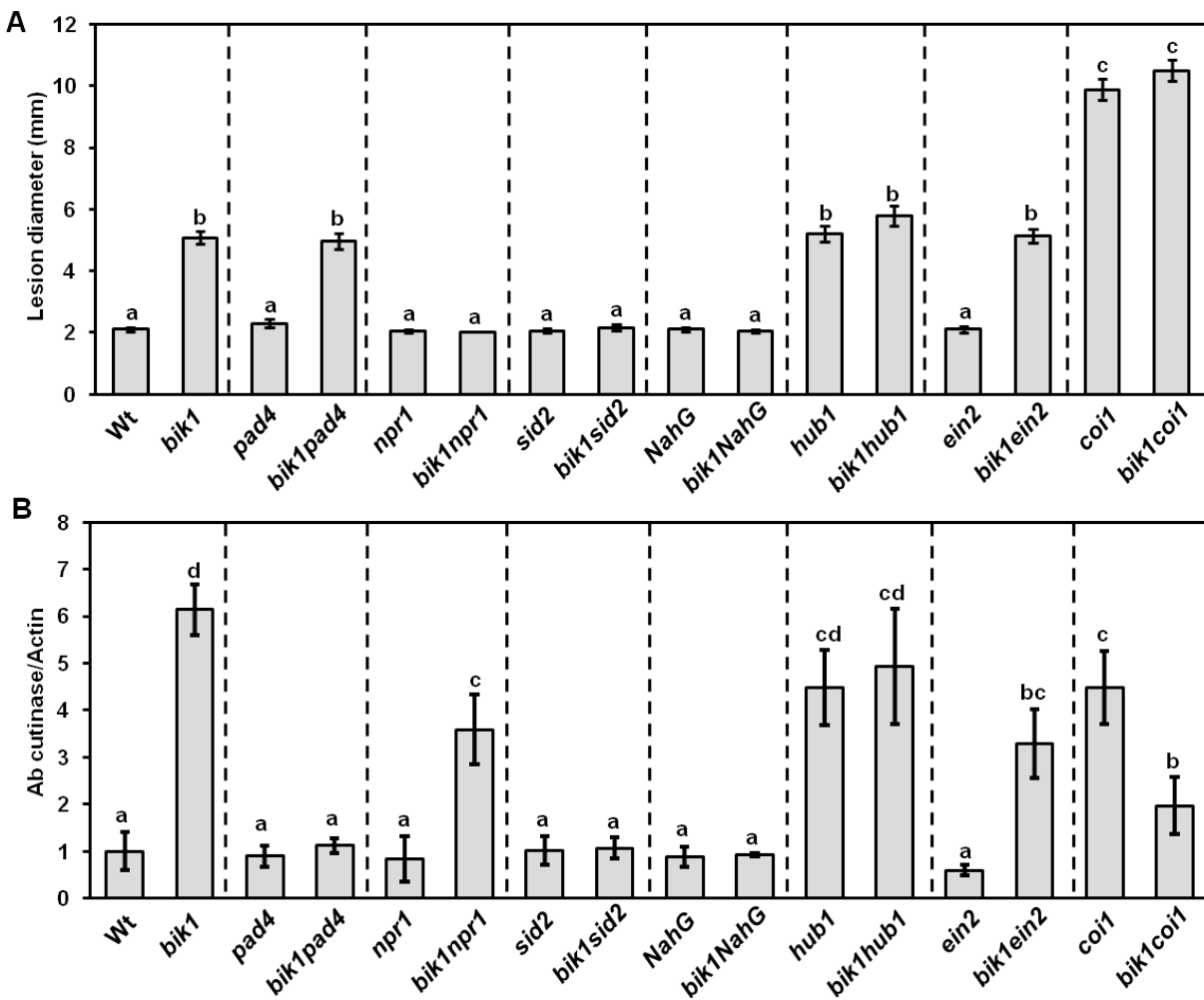
Supplemental Figure 5. The triple response of BIK1 T242 and Y245 phospho-mimic mutations at 20 μ M ACC

(A) Triple response and (B) average hypocotyl length of the *BIK1^{T242D}* and *BIK1^{Y245D}* substitution mutants on 20 μ M ACC. Data in (B) represents mean \pm SE from a minimum of 60 seedlings. Experiments were repeated at least three times with similar results. Statistical analysis was performed as described in the legend of Figure 2. Wt, wild-type.



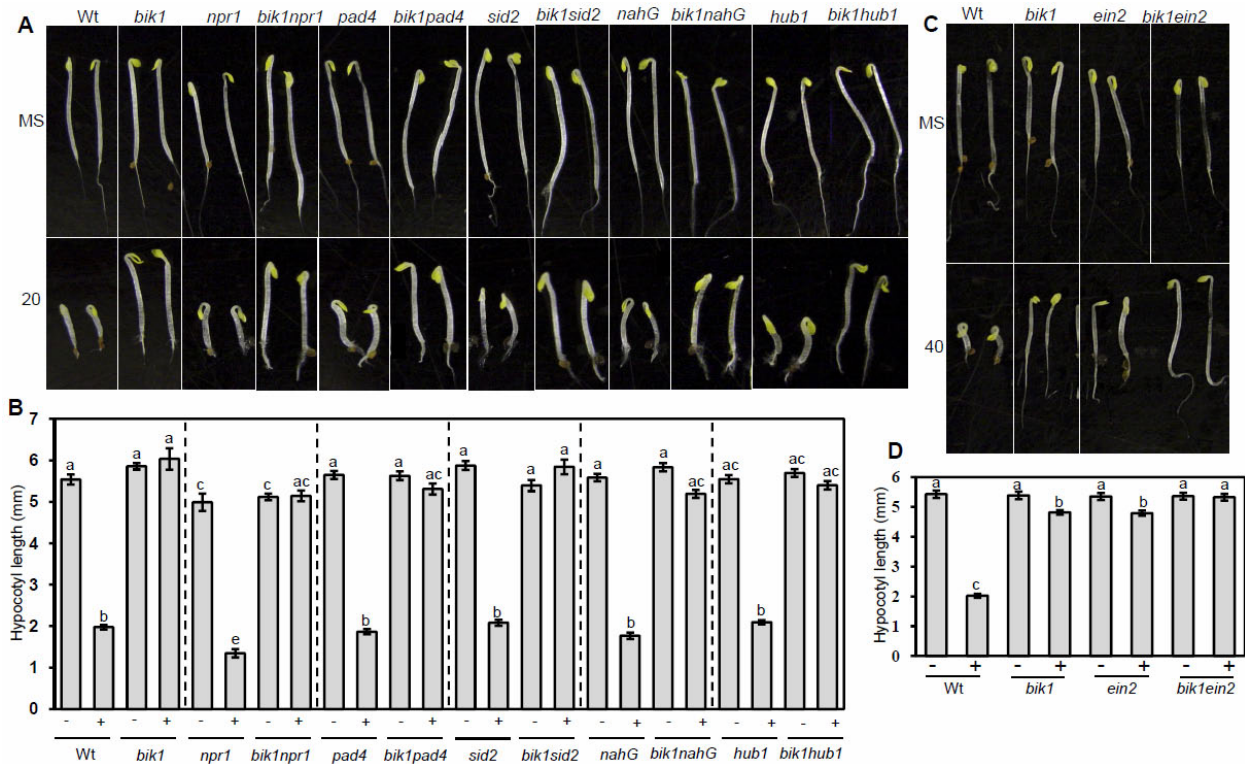
Supplemental Figure 6. Growth morphology of the *BIK1* substitution mutants

(A) Comparison of rosettes from 4-week old wild-type (Wt) and *bik1* plants with those of the substitution mutants. **(B)** Close-up images of rosette leaves 6 (upper panel) and 8 (lower panel).



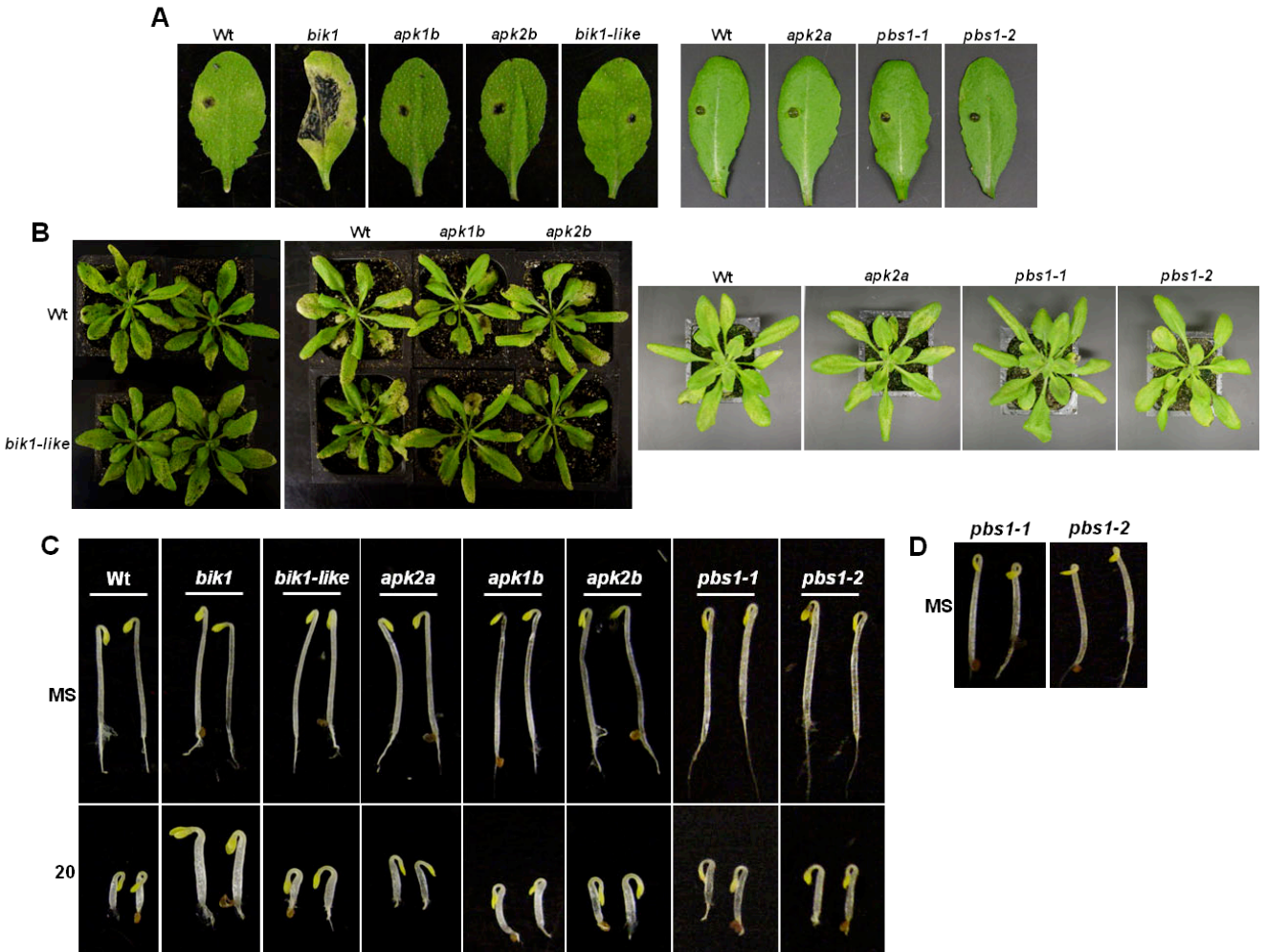
Supplemental Figure 7. Disease responses of the *bik1* double mutants to *A. brassicicola*

(A) Lesion diameter and (B) fungal growth in single and double mutants 4 days post-inoculation with *A. brassicicola* (5×10^5 spores/mL). Fungal accumulation based on qPCR amplification of *A. brassicicola* *CutinaseA* relative to Arabidopsis *Actin2*. Data in (A) represents the mean \pm SE from a minimum of 30 disease lesions. Experiments were repeated at least three times with similar results. Statistical analysis was performed as described in the legend of Figure 2. Wt, wild-type.



Supplemental Figure 8. The BIK1-regulated ET growth response is independent of SA

(A, C) Triple response and (B, D) average hypocotyl lengths of single and double mutants on 20 or 40 μ M ACC (+) and un-supplemented MS media (MS; -). Data in (B) and (D) represent mean \pm SE from a minimum of 60 seedlings. Experiments were repeated at least three times with similar results. Statistical analysis was performed as described in the legend of Figure 2. Wt, wild-type.



Supplemental Figure 9. BIK1 function in necrotrophic defense and ET-responses is unique among related protein kinases

Disease response phenotypes of T-DNA insertion alleles of *APK1B*, *APK2B*, *BIK1-LIKE*, and *PBS1* in response to (A) *A. brassicicola* and (B) *B. cinerea* 4 and 3 days post-inoculation, respectively. (C) Triple response of *bik1* and the related kinase mutant alleles on 20 μ M ACC. (D) Constitutive triple response phenotype of *pbs1-1* and *pbs1-2* seedlings when grown in the dark on un-supplemented MS media (MS).

Supplemental Table 1. Primers used in the *BIK1* study. Sequences read 5' to 3'.

(For pGEX-4T)

BIK1 full length-F: CTAGGATCCATGGGTTCTTGCTTCAGTTCTCGAGT

BIK1 full length-R: AAGGAATTCCTACACAAGGTGCCTGCCAAAAGGT

(For pCAMBIA 99-1-3xHA)

BIK1 full length-F: AGACGGTGGATGGGTTCTTGCTTCAGTTCT

BIK1 full length-R: GCGCCGGGCACAAGGTGCCTGCCAAAAGG

BIK1mS33A-F: CGGAAATCGGCGTCGACTGTA

BIK1mS33A-R: TACAGTCGACGCCGATTTC

BIK1mT35A-F: TCGTCTTCGGCGGATGCGGCG

BIK1mT35A-R: CGCCGCATCCGCCGAAGACGA

BIK1mT64A-F: AAATCGCCGCGAGAACTTC

BIK1mT64A-R: GAAGTTTCTCGCGGCGAGTTT

BIK1mS71A-F: AGACCAGATGCGGTGATCGGA

BIK1mS71A-R: TCCGATCACCGCATCTGGTCT

BIK1mT94A-F: CTCACTCCGGCGAAACCTGGA

BIK1mT94A-R: TCCAGGTTTCGCCGAGTGAG

BIK1mK105A-F: ATCGCCGTTGCGAAGCTTAAC

BIK1mK105A-R: GTTAAGCTTCGCAACGGCGAT

BIK1mS206A-F: ATTAAAGCCGCGAACATCTTA

BIK1mS206A-R: TAAGATGTTTCGCGGCTTTAAT

BIK1mS233A-F: GGTGATTTGGCGTATGTTAGT

BIK1mS233A-R: ACTAACATACGCCAAATCTCC

BIK1mY234A-F: GATTTGAGTGCGGTTAGTACA

BIK1mY234A-R: TGTACTAACCGCACTCAAATC

BIK1mS236A-F: AGTTATGTTGCGACAAGGGTC

BIK1mS236A-R: GACCCTTGTCGCAACATAACT

BIK1mT237A-F: TATGTTAGTGCGAGGGTCATG

BIK1mT237A-R: CATGACCCTCGCACTAACATA

BIK1mY243A-F: ATGGGTACTGCGGGGTACGCC

BIK1mY243A-R: GGCGTACCCCGCAGTACCCAT

BIK1mY245A-F: ACTTATGGGGCGGCCGCGCCT
 BIK1mY245A-R: AGGCGCGGCCGCCCCATAAGT

BIK1mS253A-F: TACATGTCAGCGGGTCACTTG
 BIK1mS253A-R: CAAGTGACCCGCTGACATGTA

BIK1mS274A-F: GAGATTTTAGCGGGTAAGCGA
 BIK1mS274A-R: TCGCTTACCCGCTAAAATCTC

BIK1mS333A-F: CAGTGTCTCGCGTTTGAACCC
 BIK1mS333A-R: GGGTTCAAACGCGAGACTG

BIK1mY245D-F: ACTTATGGGGACGCCGCGCCT
 BIK1mY245D-R: AGGCGCGGCGTCCCCATAAGT

BIK1mT242D-F: GTCATGGGTGACTATGGGTAC
 BIK1mT242D-R: GTACCCATAGTCACCCATGAC

qA. brassicicola CutinaseA-F: CACTGCGCCCAATGATGAAC
qA. brassicicola CutinaseA-R: GTAGCCGAACAACACGACACC

qPRI-F: GTGGGTTAGCGAGAAGGCTA
qPRI-R: ACTTTGGCACATCCGAGTCT

qERF4-F: ATGGGGATCGGTAACGTAGG
qERF4-R: CGATCTAAACGCCGATGTC

qORA59-F: TCGCGGCCGAGATAAGAGACTC
qORA59-R: TCCGGAGAGATTCTTCAACGACATCC

qERF104-F: CACGGCAGTGTCGTAAGTCCCA
qERF104-R: GAGTGAGACGAAGACCGTGGG

qMKK9-F: CAGTTGATGCGAGAGATGGA
qMKK9-R: GCGAGTTTTTGCTCCGTTAC

qMPK6-F: CGTCCCGGGATGGACGGTG
qMPK6-R: ATAGCGGCCGCCTATTCGTGATAT

qPDF1.2-F: TTCTCTTTGCTGCTTTTCGACGCA
qPDF1.2-R: TTGCATGATCCATGTTTGGCTCCTT

qA. thaliana Actin2-F: GTCGTACAACCGGTATTGTGCTG
qA. thaliana Actin2-R: CTCTCTCTGTAAGGATCTTCATGAGGT

qFRK1-F: ATCTTCGCTTGGAGCTTCTC

qFRK1-R: TGCAGCGCAAGGACTAGA

qBIK1-F: ACTTATGGGTACGCCGCGCCTGAGT

qBIK1-R: GGCACGGACCACTTGGTCCA

qPDF1.2-F: TTCTCTTTGCTGCTTTCGACGCA

qPDF1.2-R: TTGCATGATCCATGTTTGGCTCCTT

qA. thaliana Actin2-F: GTCGTACAACCGGTATTGTGCTG

qA. thaliana Actin2-R: CTCTCTCTGTAAGGATCTTCATGAGGT

qFRK1-F: ATCTTCGCTTGGAGCTTCTC

qFRK1-R: TGCAGCGCAAGGACTAGA

qBIK1-F: ACTTATGGGTACGCCGCGCCTGAGT

qBIK1-R: GGCACGGACCACTTGGTCCA

ChIP primers

Actin F: 5'-CGTTTCGCTTTCCTTAGTGTTAGCT-3'

Actin R: 5'-AGCGAACGGATCTAGAGACTCACCTTG-3'

BIK1 promoter region A-F: 5'- CTGTAACATCCGATTCAACTGGAG-3'

BIK1 promoter region A-R: 5'- TACTTGGACTTGCCGTGTCTTCCA-3'

BIK promoter region B-F: 5'- CGAGTTCCTACGACAAGATAC-3'

BIK promoter region B-R: 5'- CTTCTGAAATTTAGACAGAGAGAA-3'

References:

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