Supporting Information

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SI Experimental Procedures

Primers for Kinase Constructs and BIK1, BAK1, and FLS2 Point Mutations. The following primers were used in our study:

BIK1-F CGGGATCCATGGGTTCTT GCTTCAG,
BIK1-R GAAGGCCTCACAAGGTGCCTGCCA,
BSK1-F CG GGATCCATGGGTTGTT GTCAATCC,
BSK1-R TCCCCCGGGAGATCCTCTGCCGCCTCG,
OXI1-F CG GGATCCATGCTAGAGG GAGATGAGAA,
OXI1-R GAAGGCCTAAATACCAAAAAATTGTTATCAC,
BIK1S233A-F ATGGGTGATTTGGCTTATGTTAGTAC,
BIK1S233A-R GTACTAACATAAGCCAAATCACCCAT,
BIK1S236A-F TTGAGTTATGTTGCTACAAGGGTCAT,
BIK1S236A-R ATGACCCTTGTAGCAACATAACTCAA,
BIK1T237A-F AGTTATGTTAGTGCAAGGGTCATGG,
BIK1T237A-R CCATGACCCTTGCACTAACATAACT,
BIK1T242A-F GGGTCATGGGTGCTTATGGGTACG,
BIK1T242A-R CGTACCCATAAGCACCCATGACCC,
BIK1KM-F GTCATCGCCGTTGCAGCGCTTAACCAAGAA,
BIK1KM-R TTCTTGGTTAAGCGCTGCAACGGCGATGAC,
FLS2-KM-F GATTGCAGTAATGGTATTGAATCTAAAG,
FLS2-KM-R CTTTAGATTCAATACCATTACTGCAATC,
BAK1KM-F CTTTAGTGGCCGTTATGAGGCTAAAAGAG,
BAK1KM-R CTCTTTTAGCCTCATAACGGCCACTAAAG.

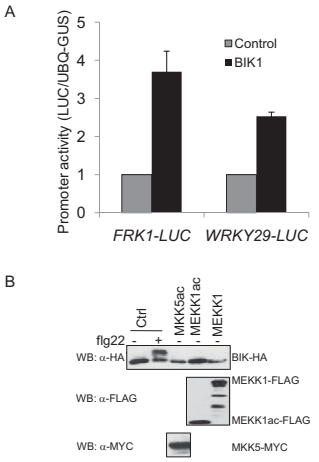

Fig. S1. BIK1 functions upstream of the MAPK cascade. (*A*) Expression of BIK1 activates *FRK1-LUC* and *WRKY29-LUC* in protoplasts. Protoplasts were co-transfected with *BIK1* and *FRK1-LUC* or *WRKY29-LUC* reporter and incubated for 6 h. *UBQ10-GUS* was included as a transfection control, and the promoter activity was presented as a LUC:GUS ratio. (*B*) BIK1 phosphorylation is upstream or independent of the MAPK cascade. Protoplasts were cotransfected with BIK1 and an active form of MKK5ac and MEKK1ac or full-length MEKK1 and incubated for 6 h. The phosphorylation of BIK1 protein was analyzed by Western blot with 12% SDS/PAGE. The expression of MEKK1, MEKK1ac, and MKK5ac is shown.

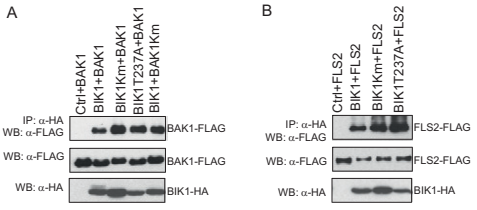

Fig. S2. The kinase activity of BIK1, BAK1, and FLS2 is not required for their association. (*A*) The association of BIK1, BIK1Km, and BIK1T237A with BAK1 and BAK1Km. (*B*) The association of BIK1, BIK1Km, and BIK1T237A with FLS2. The protoplasts were coexpressed with HA-tagged BIK1 or BIK1 mutants and FLAG-tagged FLS2 or BAK1. The control (Ctrl) was an empty vector control. Co-IP was carried out with an anti-HA antibody (IP: α-HA), and the proteins were analyzed using Western blot with an anti-FLAG antibody (WB: α-FLAG). (*Top*) BIK1 coimmunoprecipitates with BAK1 or FLS2. (*Middle* and *bottom*) The expression of BAK1 or FLS2 and BIK1 proteins.

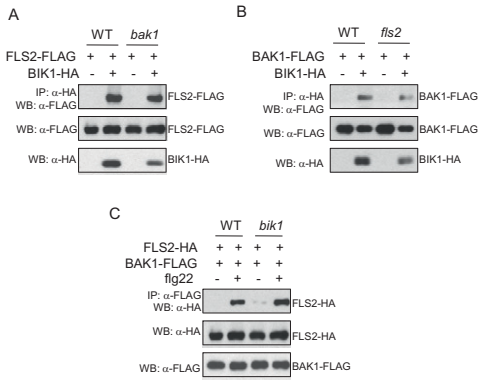

Fig. S3. The association among BIK1, FLS2, and BAK1 in *bak1*, *fls2*, and *bik1* mutants. (*A*) BIK1–FLS2 association is independent of BAK1. (*B*) BIK1–BAK1 association is independent of FLS2. (*C*) flg22-induced FLS2 and BAK1 association is BIK1-independent. The protoplasts were isolated from Col-0 [wild type (WT)], *bik1*, *bak1-4*, and *fls2* mutants and coexpressed with HA- or FLAG-tagged BIK1, FLS2, or BAK1. Co-IP was carried out with an anti-HA or anti-FLAG antibody (IP: α-HA or α-FLAG), and the proteins were analyzed using Western blot with an anti-FLAG or anti-HA antibody (WB: α-FLAG or α-HA). (*Top*) Coimmunoprecipitation results. (*Middle* and *bottom*) The expression of FLS2, BAK1, and BIK1 proteins. Protoplasts were stimulated with 1 μM flg22 for 10 min in *C*.

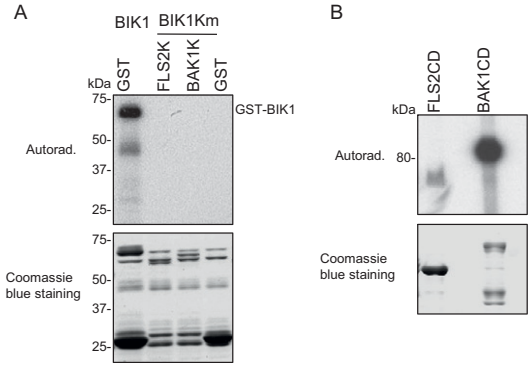

Fig. S4. Autophosphorylation activity of BIK1, BAK1CD, and FLS2CD. (*A*) GST-BIK1 exhibits strong autophosphorylation activity. An in vitro kinase assay was performed with incubating GST-BIK1 with GST and GST-BIK1Km with GST, GST-BAK1K, and GST-FLS2K in the presence of [^{32}P]-γ-ATP. (*B*) Autophosphorylation activity of BAK1CD and FLS2CD. An in vitro kinase assay was performed with incubating MBP-BAK1CD or GST-FLS2CD in the presence of [^{32}P]-γ-ATP. Proteins were separated with 7.5% SDS/PAGE and analyzed by autoradiography (*Upper*), and the protein loading control was shown by Coomassie Blue staining (*Lower*).

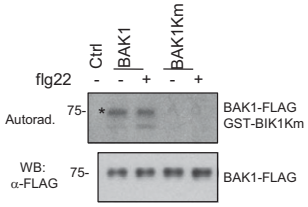

Fig. S5. BAK1 phosphorylates BIK1. An immunocomplex kinase assay was performed with protoplasts expressing FLAG-tagged BAK1 or BAK1Km. Immunoprecipitation was carried out with an anti-FLAG antibody, and the immunoprecipitated proteins were subjected to an in vitro kinase assay with GST-BIK1Km as a substrate in the presence of [^{32}P]-γ-ATP. Proteins were separated with SDS/PAGE and analyzed by autoradiography (*Upper*), and the protein expression was detected by Western blot (*Lower*). The BAK1 autophosphorylation band (marked with an asterisk) is also shown.

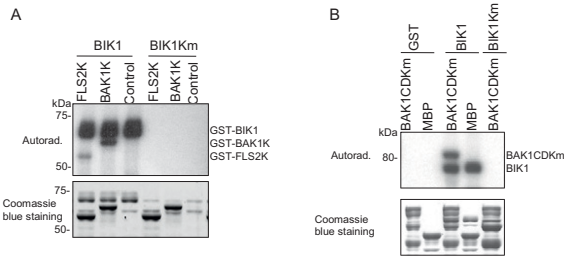

Fig. S6. BIK1 phosphorylates BAK1 and FLS2 in vitro. (*A*) BIK1 phosphorylates GST-BAK1K and GST-FLS2K. (*B*) BIK1 phosphorylates MBP-BAK1CDKm. An in vitro kinase assay was performed by incubating GST-FLS2K, GST-BAK1K, or MBP-BAK1CDKm with GST-BIK1 or its kinase mutant. Proteins were separated with SDS/PAGE and analyzed by autoradiography (*Upper*). The protein loading control was shown by Coomassie blue staining (*Lower*).

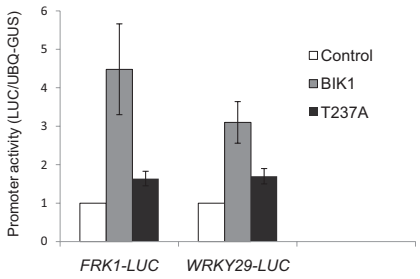

Fig. S7. T237 is required for BIK1 activation of *FRK1-LUC* and *WRKY29-LUC* in protoplasts. Protoplasts were cotransfected with *BIK1* or *BIK1T237A* and *FRK1-LUC* or *WRKY29-LUC* reporter and incubated for 6 h. *UBQ10-GUS* was included as a transfection control, and the promoter activity was presented as a LUC:GUS ratio.

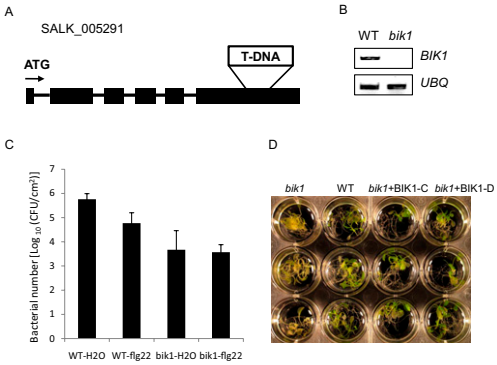

Fig. S8. BIK1 is involved in MAMP signaling. (*A*) T-DNA insertion site in *bik1* with exons (solid boxes). (*B*) RT–PCR analysis of *BIK1* and *UBQ10* (control) in wild-type (WT) and *bik1* mutant plants. (*C*) *bik1* mutant lost flg22-mediated immunity. Four-week-old plants were pretreated with 200 nM flg22 or H_2O for 24 h before being inoculated with *Pst* DC3000 at the concentration of 5×10^5 cfu/mL. Bacterial counting was performed 3 days after infection. (*D*) *bik1* mutants are compromised in plant immunity to *Pst* DC3000 *hrcC*. Twelve-day-old WT, *bik1*, and BIK1 complementation (*bik1*+BIK1-C and *bik1*+BIK1-D) seedlings were infected with *Pst* DC3000 *hrcC*. The photo was taken 6 days after infection.