

Supplemental Data
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Bacterial Effectors Target BAK1 to Disrupt MAMP Receptor-Signaling Complexes and Impede Plant Innate Immunity

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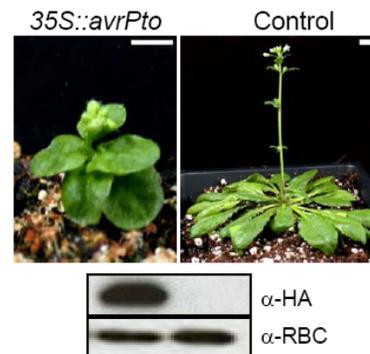


Figure S1. The *avrPto* transgenic plants phenotypically mimic brassinosteroid insensitive mutants. Transgenic *Arabidopsis* plants expressing *35S::avrPto* or a control vector at 6 weeks are shown. Scale bars: 1 cm. The expression of AvrPto (α -HA) and RuBisCO (α -RBC) was detected using specific antibodies and Western blot analysis.

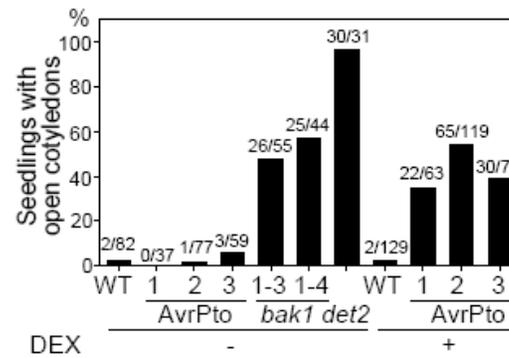


Figure S2. Quantitative analysis of the open-cotyledon phenotype in the DEX-inducible *avrPto* transgenic (1, 2 and 3), *bak1* (1-3 and 1-4), and *det2* mutant seedlings. Seedlings were grown in the dark for seven days with or without 10 μ M DEX. The experiments were repeated three times with similar results.

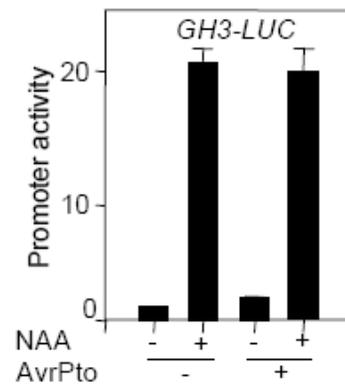


Figure S3. *AvrPto* does not affect auxin response. Protoplasts were transfected with an auxin-responsive reporter *GH3-LUC* with or without *AvrPto*, and incubated for 3 hrs before treatment with 1 μ M NAA for 3 hrs. The data are shown as means \pm standard errors from four independent biological replicates.

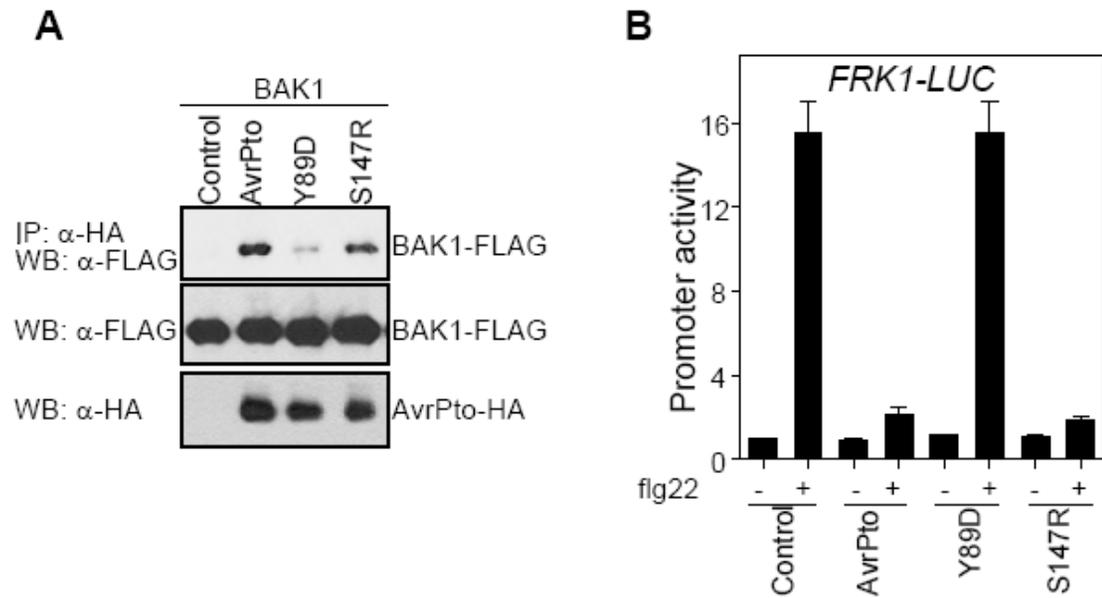


Figure S4. The AvrPtoY89D mutation significantly diminishes its association with BAK1 and disrupts its MAMP suppression activity. (A) AvrPtoY89D, but not S147R, significantly reduces its association with BAK1. The Co-immunoprecipitation (Co-IP) was performed with protoplasts co-expressing BAK1-FLAG and HA-tagged AvrPto or its mutants. The Co-IP was carried out with anti-HA-agarose (IP: α -HA), and the proteins were analyzed using Western blot with an anti-FLAG antibody (WB: α -FLAG). (B) The AvrPtoY89D mutation compromises its MAMP suppression activity. Protoplasts were co-transfected with *FRK1-LUC* and AvrPto or AvrPto mutants. Transfected protoplasts were incubated for 3 hrs to express AvrPto before treatment with 100 nM flg22 for 3 hrs. AvrPtoS147R did not affect its MAMP suppression activity (He et al., 2006). The experiments were repeated three times with similar results.

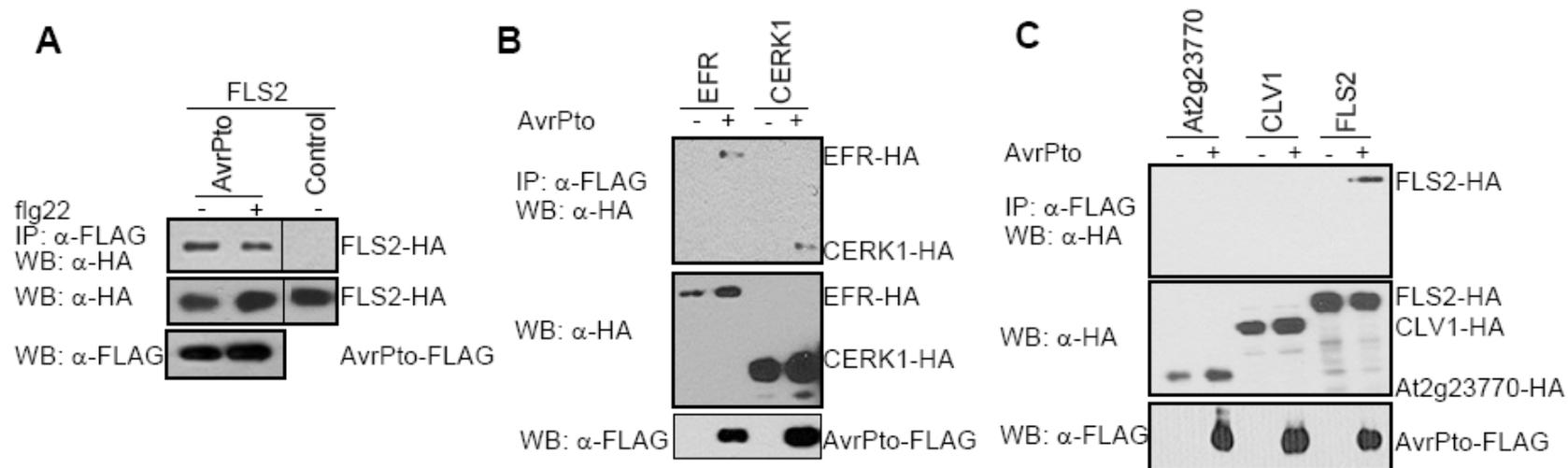


Figure S5. AvrPto associates with some receptor-like kinases. (A) AvrPto associates with FLS2 independent of flg22. Protoplasts were treated with 1 μ M flg22 for 5 min. The Co-immunoprecipitation (Co-IP) was performed with protoplasts co-expressing AvrPto-FLAG and FLS2-HA. The Co-IP was carried out with anti-FLAG-agarose (IP: α -FLAG), and the proteins were analyzed using Western blot with an anti-HA antibody (WB: α -HA). (B) AvrPto associates with EFR and CERK1. The Co-IP was performed with protoplasts co-expressing AvrPto-FLAG and EFR-HA, or CERK1-HA. (C) AvrPto does not associate with CLV1 or a putative LysM receptor-like kinase At2g23770, a close homolog of CERK1. The Co-IP was performed with protoplasts co-expressing AvrPto-FLAG and At2g23770-HA, CLV1-HA or FLS2-HA. The experiments were repeated three times with similar results.

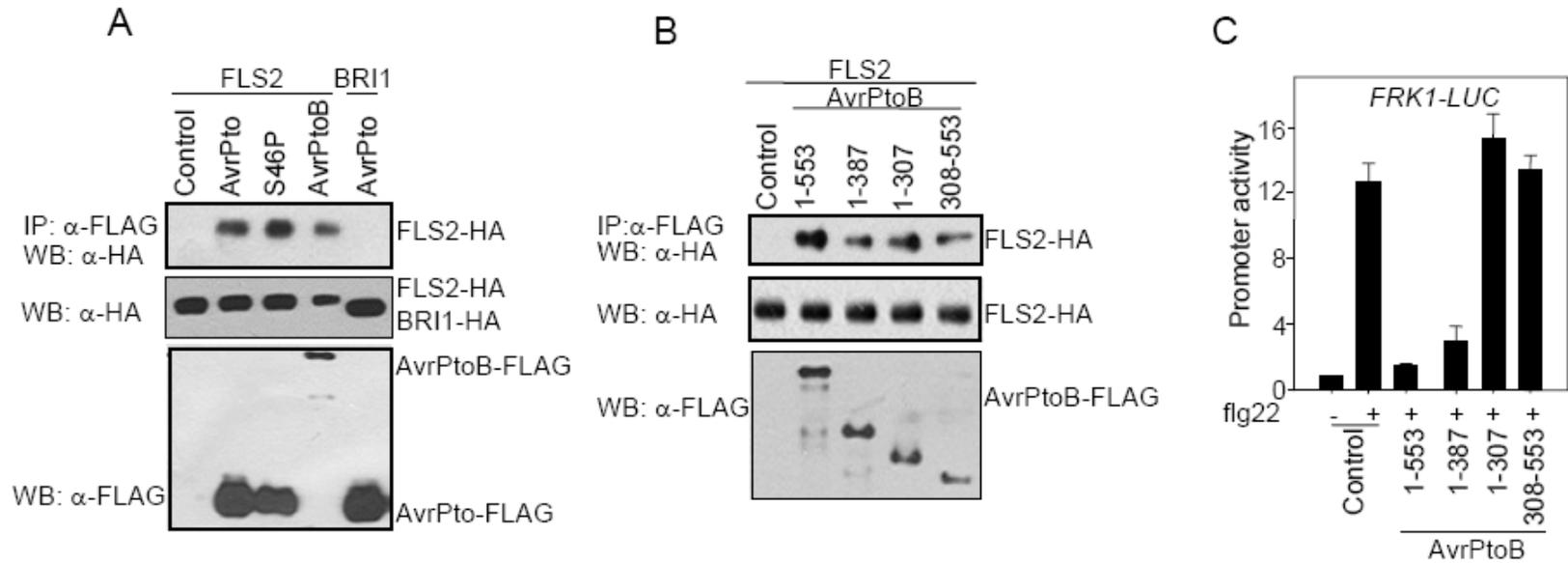


Figure S6. AvrPto and AvrPtoB loss-of-MAMP-suppression mutants still associate with FLS2. (A) AvrPtoS46P associates with FLS2. AvrPtoS46P does not have MAMP suppression activity (He, et al., 2006). The Co-immunoprecipitation (Co-IP) was performed with protoplasts co-expressing FLAG-tagged effectors and FLS2-HA or BRI1-HA. (B) AvrPtoB deletion mutants still associate with FLS2. The Co-IP was performed with protoplasts co-expressing FLS2-HA and FLAG-tagged AvrPtoB or AvrPtoB deletion mutants. (C) Some AvrPtoB deletions do not exhibit MAMP suppression activity. Protoplasts were co-transfected with *FRK1-LUC* and full-length AvrPtoB (1-553) or AvrPtoB deletion mutants. Transfected protoplasts were incubated for 3 hrs to express AvrPtoB before treated with 100 nM flg22 for 3 hrs. The experiments were repeated three times with similar results

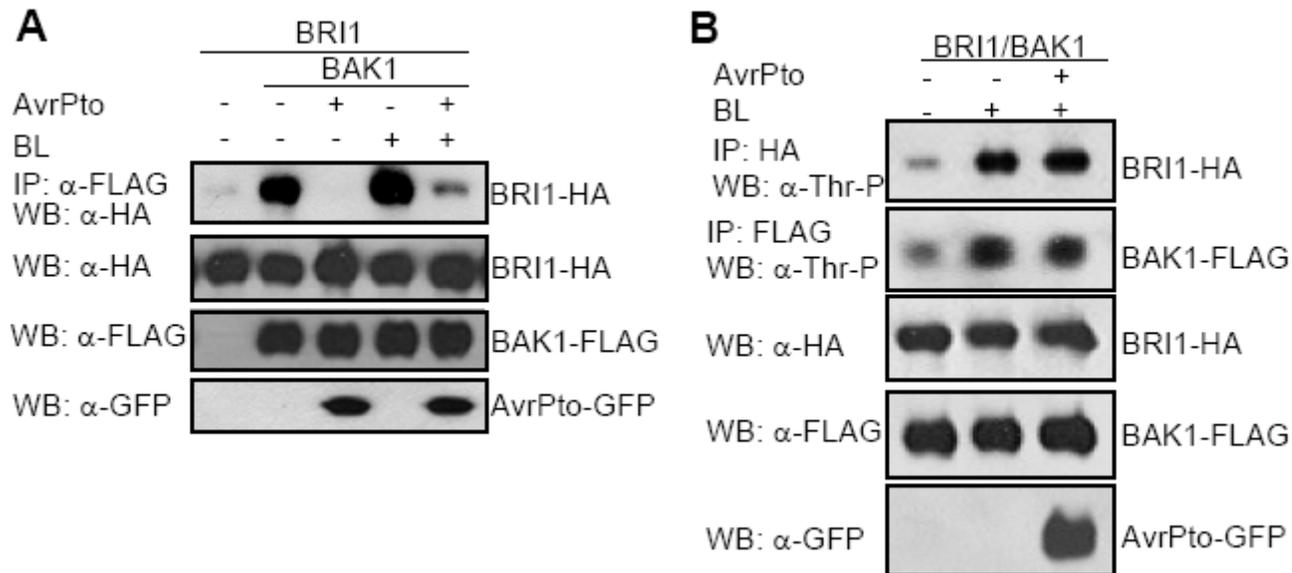


Figure S7. AvrPto suppresses BAK1 and BRI1 association but not BL-stimulated BAK1 and BRI1 phosphorylation. (A) AvrPto suppresses BAK1 and BRI1 association. The Co-immunoprecipitation (Co-IP) was performed with protoplasts co-expressing BAK1-FLAG and BRI1-HA with or without AvrPto-GFP. The Co-IP was carried out with an anti-FLAG antibody (IP: α -FLAG), and the proteins were analyzed using Western blot with an anti-HA antibody (WB: α -HA). (B) AvrPto does not affect BL-stimulated BAK1 and BRI1 phosphorylation. The immunoprecipitation (IP) was carried out with anti-FLAG-agarose (IP: α -FLAG) or anti-HA-agarose (IP: α -HA), and the proteins were analyzed using Western blot with an anti-phosphothreonine antibody (WB: α -Thr-P) (Wang et al., 2005) (Cell Signaling Biotechnology, Beverly, MA). Protoplasts were stimulated with 0.1 μ M BL for 90 min. The experiments were repeated twice with similar results.

A

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CLV1 -----ECLKEENIIGKGGAGIVYRGSMPNNDVAIKRLV--GRGTGRSDHGFTAEIQTILGRIRHRHIVRLLG-----YVANKDNTNLL
ERECTA -----ENLSEKYYIIGHGASSTVYKCVLKNCKPVAIKRLY--SHNP-QSMKQFETELEMSSIKHRNLVSLQA-----YSLSHLGSLL
CERK1 -----DNFNLSFKIQGGGFGAVVYAEELR-GEKAAIKKMD--MEASK----QFLAELKVLTRVHVHVNLRVRLIG-----YCVVEG-SLFL
2g23770 -----QSATSDFTSSSSSIGGSGYIGKIN-GDGAMIKKIE--GNAS-----EEVNLKSLNHLNLIIRLSG-----FCFHEGDWYL
Pto MGSKYSKATNSINDALSSSYLVPFESYRVPLVDLEAATNNFDHKFLIGHGVFGKVKYKGVLRDGAVALKRRT--PESSQG-IEEFETELETLSFCRHPHLVSLIG-----FCDERNEMIL
SERK4 -----DNFSNKNVLRGGGFGKVKYKGRADGNLVAVKRLK--EERTKGGELQFQTEVEMISMAVHRNLLRLRG-----FCMTPTERLL
SERK5 -----EKFSKRNVLGKGRFGILYKGRADDTLVAVKRLN--EERTKGGELQFQTEVEMISMAVHRNLLRLRG-----FCMTPTERLL
BAK1 -----DNFSNKNILGRGGFGKVKYKGRADGTLVAVKRLK--EERTQGGELQFQTEVEMISMAVHRNLLRLRG-----FCMTPTERLL
BRI1 -----NGFHNDSLIGSGGFGDVYKAILKDGSAVAIKKLI--HVSGQG-DREFMAEMETIGKIKHRNLVPLLG-----YCKVGDERLL
FLS2 -----DSFN SANIIGSSSLSTVYKQLEDC-TVIAVKVLNLRKFSAESDKWFYTEAKTLSQLKHRNLVKILG----FAWESGKTKAL
EFR -----SRFSSTNLIGSGNFGNFKGLLGPENKLVAVKVLNLLKHGATKS--FMACETFKGIRHRNLVKLITVCSLSDSEGNDFRAL

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ERECTA FYDYLENGSLWDLHGP-TKKKTLT-----WDTRLKIAYGAAQGLAYLHHDSCSPRIIHRDVKSNINLLDKLEARLTDGFIKSLVCSKS-HTSTYVM-----GTIGYIDPEYARTSRLT
CERK1 VYEVYENGNGLQHLHGSG--REPLP----WTKRVQIALDSARGLEYIHEHTVPVYVHRDIKSNANILIDQKFRAKVADFGTLK--LTEVGGSATRGAM-----GTFGYMAPETVYG-EVS
2g23770 VYEHASNGSLSEWIHTT--KSLLS----LTQKLQIALDIATGLNVLHNFADPPYVHRDLNNGNVLFDLLEFRAKIGSLGSAR--STEDFVLTKHVE-----GTRGYLAPEYLEHGLVS
Pto IYKYMENGNLKRHLYGSDLPMTSMS----WEQRLEICIGAARGLHYLHTR--AIHRDVKSNINLLDENFVPKITDFGISKKGTELDQTHLSTVVK----GTLGYIDPEYFIKGRLT
SERK4 VYPYMANGSVASCLRERPEGNPALD----WPKRKHIALGSSARGLAYLHDHCDQKIIHRDVKAANILLDEEFAVVGDFGLAK-LMNYNDSHVTTAVR-----GTIGHIAPEYLTGKSS
SERK5 VYPYMANGSVASCLRERPEGNPALD----WPKRKHIALGSSARGLAYLHDHCDQKIIHLDVKAANILLDEEFAVVGDFGLAK-LMNYNDSHVTTAVR-----GTIGHIAPEYLTGKSS
BAK1 VYPYMANGSVASCLRERPEQPPLD----WPKRQRIALGSSARGLAYLHDHCDPKIIHRDVKAANILLDEEFAVVGDFGLAK-LMDYKDHVTTAVR-----GTIGHIAPEYLTGKSS
BRI1 VYEFMKYGSLEDVLHDPKAGVKLN----WSTRRKIATGSARGLAFLHHCSPHIIHRDMKSNVLLDENLEARVSDFGMARLMSAMDTHLVSVTLA-----GTPGYVPEYQSFRC
FLS2 VLPFMENGNLEDTIHGSAAPIC-----SLEKIDLCVHIAGSIDYHSGYGFPIVHCDLKPANILLSDRVAHVSDFGTARILG--FREDGSTTASTSAFEGTIGYLAPEYFAYMRKVT
EFR VYEFMPKGSMDMLQLEDLERVNDHSRSLTPAEKLNIAIDVASALEYLHVHCHDPAVHADIKPSNILLDDDLTAHVSDFGLAQLLYKYDRESFLNQFSSAGVRGTIGYAAPEYGMGGQPS

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CLV1 EKSDVYSFGVVLLELIAGKKPVGFEFGEVDIVRWRNTEEEITQPSDAAIVVAIVDPRIT-G--YPLTSVIHVFKIAMMC
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CERK1 AKVDVYAFGVVLYELISAKGAVVKMTEAVGEFRGLVGVFEESFKETDKEEARLKIIDPRL-GDSYPPFDSVYKMAELGKAC
2g23770 TKLDVYAFGVVLEIVTGK-----EASELKEIDEKKAIDEILIH---GRLLPEGLTSFVERLVVDC
Pto EKSDVYSFGVVLFEVLCARSAIVQSLPR-----EMVNLAEWAVESHNNQLEQIVDPNL-ADKIRPESLRKFGDTAVKC
SERK4 EKTDFVGYGVMLLELITGQKAFDLARLAND----DDIMLLDWVKEVLKEKKLESIVDAEL-EGKYVETEVEQLIQMALLC
SERK5 EKTDFVGYGVMLLELITGQKAFDLARLAND----DDIMLLDWVKEVLKEKKLESIVDAEL-EGKYVETEVEQLIQMALLC
BAK1 EKTDFVGYGVMLLELITGQRAFDLARLAND----DDVMLLDWVKGLLKEKKLEALVDVDL-QGNYKDEEVEQLIQVALLC
BRI1 TKGDVYSYGVVLELLTGKRPDSDPFGDN-----NLVGVWVKQHAKLRI SDVFDPELMKEDPALEIELLQHLKVAVAC
FLS2 TKADVFSFGIIMMELMTKQRPTSLNDESDQDMTLRQLVEKSI GNGRKG MVVRVLD MELGDSIVSLKQEEAIEDFLKLCFLC
EFR IQGDVYSFGIILLEMFSGKKPT---DESFA GDY NLHSYTKSILSG-----CTSSGGSNAIDEGRLRLVLQVGIK

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CLV1 VEEEAARPTMREVVMHMLTNPPKSVANLIAF-----
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CERK1 TQENAQLRPSMRYIVVALS--TLFSTGNWVGNFQNEDELVSLMSGR-----
2g23770 LKKDHLNRPSMDENVMLS--KILAAATQNWEESY-----
Pto LALSSEDRPSMGDVLWKLE--YALRLQESVI-----
SERK4 TQSSAMERPDKMSEVVRMLEGDGLAERWEEWQKEEMPIHDFNYQAYPHAGTDWLIPIYSNSLIENDYPSGPR-----
SERK5 TQSSAMERPDKMSEVVRMLEGDGLAERWEEWQKEEMPIHDFNYQAYPHAGTDWLIPIYSNSLIENDYPSGPR-----
BAK1 TQSSAMERPDKMSEVVRMLEGDGLAERWEEWQKEEMFRQDFNYPTHHPAVSGWIIIGDSTSQIENEPYSGPR-----
BRI1 LDDRAWRRPTMVQVMAMFKEIQAGSGIDSQSTIRSIEDGGFSTIEMVDMSIKEVPEGKL-----
FLS2 TSSRPEDRPMNEILTHLMKLRGKANSFREDRNEEDREV-----
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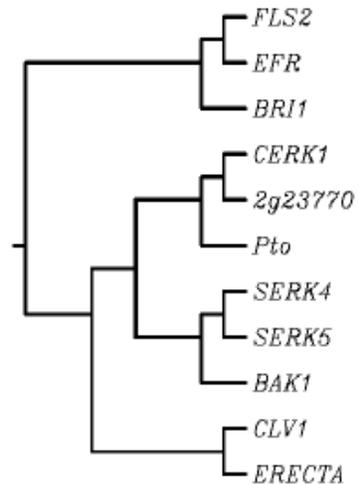
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Figure S8. Comparison of tomato Pto with different *Arabidopsis* receptor-like kinases. (A) Alignment of amino acid sequences of tomato Pto with different *Arabidopsis* receptor-like kinases. The kinase domains of BAK1, FLS2, EFR, CERK1, At2g23770, ERECTA, CLV1 and BRI1 are shown. Pto residues that form the two interfaces with AvrPto are in bold. Key Pto residues that determine the specific recognition of AvrPto are marked in red (Xing et al., 2007). (B) Phylogenetic tree of tomato Pto with different *Arabidopsis* receptor kinases. The alignment and phylogenetic tree were generated by CLUSTALW at <http://clustalw.genome.jp/sit-bin/clustalw>.

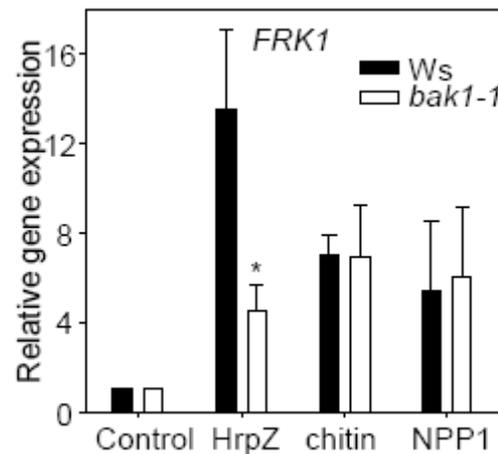


Figure S9. BAK1 is important in HrpZ- but not chitin- or NPP1-mediated activation of *FRK1*. Wild-type (Ws) and *bak1-1* mutant seedlings (12 days) were collected 1 hr after treatment with 1 μ M HrpZ, 50 μ g/ml chitin, or 20 nM NPP1. Endogenous *FRK1* expression was analyzed by real-time RT-PCR. The data are shown as means \pm standard errors from three independent biological replicates. * indicates a significant difference with $p < 0.05$ when compared with data from wild-type (Ws) based on the results of an unpaired Student's t-test.

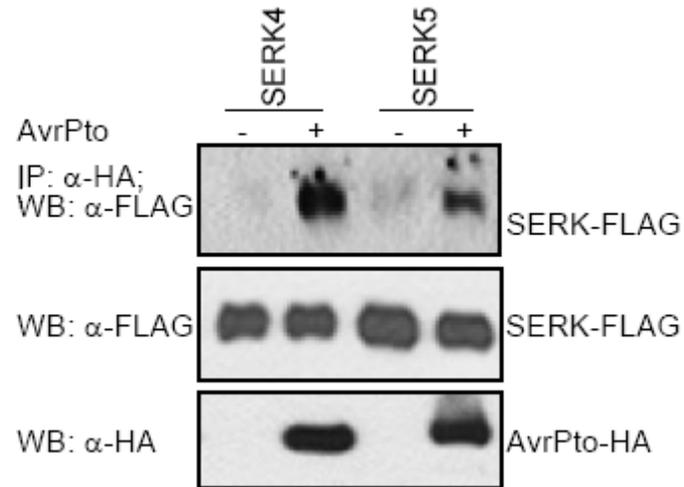


Figure S10. AvrPto associates with SERK4/BKK1 and SERK5 in *Arabidopsis* protoplasts. The Co-immunoprecipitation (Co-IP) was performed with protoplasts co-expressing AvrPto-HA and SERK4/BKK1-FLAG, or SERK5-FLAG. The Co-IP was carried out with anti-HA-agarose (IP: α -HA), and the proteins were analyzed using Western blot with an anti-FLAG antibody (WB: α -FLAG). The experiments were repeated twice with similar results.

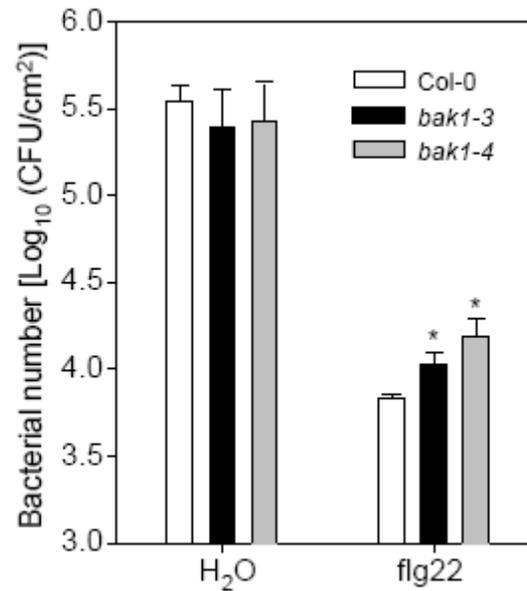


Figure S11. The *bak1* mutants reduce flg22-induced resistance to *P. s. tomato* DC3000 infection. *Arabidopsis* plants (4 weeks) were hand-inoculated with DC3000 at a concentration of 5×10^5 cfu/ml after infiltration with 200 nM flg22 for 24 hrs. The bacterial growth assay was performed two days after inoculation. Each data point is shown as triplicates. * indicates a significant difference with $p < 0.05$ when compared with data from wild-type (Col-0) based on the results of an unpaired Student's t-test. The experiments were repeated twice with similar results.

Supplemental Experimental Procedures

Primers for constructs in protoplast transient assays

BAK1 (*At4g33430*) was amplified with primers 5'-CATGCCATGGAACGAAGATTAATGATC-3' and 5'-GAAGGCCTTCTTGGACCCGAGGGGTATTC-3'. *FLS2* (*At5g46330*) was amplified with primers 5'-CGGGATCCATGAAGTTACTCTCAAAGAC-3' and 5'-TCCCCCGGGAAGTTCTCGATCCTCGTTACG-3'. *BR11* (*At4g39400*) was amplified with primers 5'-CGGGATCCATGAAGACTTTTTCAAGCTT-3' and 5'-GAAGGCCTTAATTTTCCTTCAGGAAC-3'. *RPS2* (*At4g26090*) was amplified with primers 5'-GGGGTACCATGGATTTTCATCTCATCTCTTATC-3' and 5'-TCCCCCGGATTTGGAACAAAGCGCGGTAA-3'. *BKK1/SERK4* (*At2g13790*) was amplified with primers 5'-CGGGATCCATGACAAGTTCAAAAATGGA-3' and 5'-GAAGGCCTTCTTGGACCCGAGGGGTAAT-3'. *SERK5* (*At2g13800*) was amplified with primers 5'-CATGCCATGGAACATG GATCATCCCG-3' and 5'-GAAGGCCTTCTTGGCCCCGAGGGGTAAT-3'. *CERK1* (*At3g21630*) was amplified with primers 5'-CGGGATCCATGAAGCTAAAGATTTCTCT-3' and 5'-GAAGGCCTCCGGCCGGACATAAGACTGAC-3'. *At2g23770* was amplified with primers 5'-CGGGATCCATGATCTCGTTTTTCATTTCA-3' and 5'-GAAGGCCTGTACGACGATTCTTCCCAGTT-3'. *CLV1* (*At1g75820*) was amplified with primers 5'-CGGGATCCATGGCGATGAGACTTTTGAA-3' and 5'-GAAGGCCTGAACGCGATCAAGTTCGCCA-3'. *EFR* (*At5g20480*) was amplified from Col-0 genomic DNA with primers 5'-CGGGATCCATGAAGCTGTCCTTTTCACTTG-3' and 5'-GAAGGCCTCATAGTATGCATGTCCGTATTTAAC-3'.

AvrPtoB deletion mutants were generated by following primers, AvrPtoB₁₋₃₈₇: 5'-CATGCCATGGCGGGTATCAATAGAGC-3' and 5'-GAAGGCCTCATCACCCGCAATCGTGTTG-3'; AvrPtoB₁₋₃₀₇: 5'-CATGCCATGGCGGGTATCAATAGAGC-3' and 5'-GAAGGCCTCATTACATGTCTTTCAAGGG-3'; AvrPtoB₃₀₈₋₅₅₃: 5'-CGGGATCCATGCAGCGCCTCCCTATCCC-3' and 5'-GAAGGCCTGGGGACTATTCTAAAAGCATAC-3'. BAK1 deletion mutants were generated by following primers, BAK1ETJ: 5'-CATGCCATGGAACGAAGATTAATGATC-3' and 5'-GAAGGCCT

CGAAGCAACTTGTAGTTCAC-3'; BAK1TJK: 5'-CATGCCATGGGTTCTTTTCACTTTTCACT-3' and 5'-GAAGGCCTTCTTGGACCCGAGGGGTATTC-3'; BAK1JK:5'-CGGGATCCATGCGA AGGAAAAAGC CGCAGGAC-3' and 5'-GAAGGCCTTCTTGGACCCGAGGGGTATTC-3'; BAK1K:5'-CATGCCATGGATAATTTTAGCAACAAG-3' and 5'-GAAGGCCTTCTTGGACCCGAGGGGTATTC-3'; BAK1 and AvrPto point mutations were generated by site-specific mutagenesis kit (Stratagene) using following primers, BAK1F300A: 5'-GGGTAGAGGTGGTGCTGGTAAAGTTTATAA-3' and 5'-TTATAAACTTTACCAGCACCACTCTACCC-3'; BAK1T455N: 5'-GCAGTGCCTGGGAACATTGGTCATATA-3' and 5'-TATATGACCAATGTTCCCACGCACTGC-3'; AvrPtoY89D: 5'-GACATGCAGCATAGGGACATGACGGGAG-3' and 5'-CTCCCGTCATGTCCCTATGCTGCATGTC-3'

Primers for yeast split-ubiquitin assay

The primers of *BAK1* are, 5'-ACAAGTTTGTACAAAAAAGCAGGCTCTCCAACCACCATGGAACGAA GATTAATGATC-3', and 5'-TCCGCCACCACCAACCACTTTGTACAAGAAAGCTGGGTATCTTGGACCCGAGGGGTA-3'. The primers of *BAK1ETJ* are, 5'-ACAAGTTTGTACAAAAAAGCAGGCTCTCCAACCACCATGGAACGAA GATTAATGATC-3', and 5'-TCCGCCACCACCAACCACTTTGTACAAGAAAGCTGGGTACGAAGCAACTTGTAGTTCAC-3'. The primers of *BAK1TJK* are, 5'-ACAAGTTTGTACAAAAAAGCAGGCTCTCCAACCACCATGGGTTCTTTTCACTTTTTCAC and 5'-TCCGCCACCACCAACCACTTTGTACAAGAAAGCTGGGTATCTTGGACCCGAGGGGTA-3'. The primers of *BR11* are, 5'-ACAAGTTTGTACAAAAAAGCAGGCTCTCCAACCACCATGAAGACTTTTTCAAGCTT-3' and 5'-TCCGCCACCACCAACCACTTTGTACAAGAAAGCTGGGTATAATTTTCCTTCAGGAAC-3'. The primers of *FLS2* are, 5'-ACAAGTTTGTACAAAAAAGCAGGCTCTCCAACCACCATGAAGTTACTCTCAAAGAC-3' and 5'-TCCGCCACCACCAACCACTTTGTACAAGAAAGCTGGGTAAACTTCTCGATCCTCGTTACG-3'.

The primers of *avrPto* are 5'-ACAAGTTTGTACAAAAAAGCAGGCTCTCCAACCACCATGGGAAATATATGTGTCGG-3', and 5'-TCCGCCACCACCAACCACTTTGTACAAGAAAGCTGGGTATTGCCAGTTACGGTACG-3'. The primers of *avrRpt2* are 5'-ACAAGTTTGTACAAAAAAGCAGGCTCTCCAACCACCATGAAAATTGCTCCAGTTGC-3', and 5'-TCCGCCACCACCAACCACTTTGTACAAGAAAGCTGGGTAGCGGTAGAGCATTGCGTGTG-3'

Primers for RT-PCR analysis

The RT-PCR primer sequences of *UBQ10* (*At4g05320*), *FRK1* (*At2g19190*), *At2g17740* and *CPD* (*At5g05690*) were described previously (He et al., 2005; He et al., 2006). The RT-PCR primer sequences of *SAUR-AC* (*At4g38850*) are 5'-CGTCGACACCAAGAGGATTC-3' and 5'-AAGTATGAAACCGGCACCAC-3'. The RT-PCR primer sequences of *IAA5* (*At1g15580*) are 5'-TCCTTGGGAAATGTTCCCTTG-3' and 5'-TCATCCTCTGTTACATGATCTCTTC-3'.

Supplemental References

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