Supplemental Data

Specific Bacterial Suppressors of MAMP

Signaling Upstream of MAPKKK

in *Arabidopsis* Innate Immunity

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Supplemental Experimental Procedures

Primers for Type III Effector Constructs

avrRpm1: 5'- CGGGATCCATGGGCTGTGTATCGAGCAC -3' and 5'-GAAGGCCTAAAGTCATCTTCTGAGTCAG -3'; *avrB*: 5'- CGGGATCCATGGGCTGCGTCTCGTCA -3' and 5'-GAAGGCCTAAAGCAATCAGAATCTAG -3'; *avrRpt2*: 5'- CGGGATCCATGAAAATTGCTCCAGTTGC -3' and 5'- GAAGGCCTGCGGTAGAGCATTGCGTGTGG -3'; *avrPto*: 5'- CATGCCATGGGAAATATATGTGTCGG -3' and 5'- GAAGGCCTTTGCCAGTTACGGTACGG -3'; *avrPtoB*: 5'- CATGCCATGGCGGGTATCAATAGAGC -3' and 5'- GAAGGCCTGGGGACTATTCTAAAAGCATAC -3'; *hopPtoD2*: 5'-CGGGATCCATGAATCCCCTGCAACCTATTC -3' and 5'- GAAGGCCTTTCTAACGCTATTTTTGCGC -3'; *hopPtoE*: 5'- CGGGATCCATGAATAGAGTTTCCGGTAG -3' and 5'-GAAGGCCTGTCAATCACATGCGCTTGG -3'; *hopPtoK*: 5'- CGGGATCCATGAATCGCATTTCAACCAG -3' and 5'- GAAGGCCTGCAGTAGAGCGTGTCGC; *avrBsT*: 5'- CGGGATCCATGAAGAATTTTATGCGTTC -3' and 5'- GAAGGCCTTGATTCAATAGTTTTCCTAA -3'; *virPphA*: 5'-CGGGATCCATGCCGGGTATCAACGGAGC -3' and 5'- GAAGGCCTTGGAACAATTTTAAAAGCGTAC -3'; *hopAI1*: 5'- CGGGATCCATGCTCGCTTTGAAGCTGAAC -3' and 5'-GAAGGCCTGCGAGTCCAGGGCGGTGGC -3'.

Primers for AvrPto and AvrPtoB Mutations

Point mutations were generated by site-specific mutagenesis kit (Stratagene) using following primers, G2A: 5'- CCGTGGATCCATGGCAAATATATGTGTCG -3' and 5'-CGACACATATATTTGCCATGGATCCACGG -3'; S46P: 5'-CATCAACTTGCGGAGCCTGCTGGTCTACCAAG -3' and 5'-CTTGGTAGACCAGCAGGCTCCGCAAGTTGATG -3';
S94P: 5'-ACATGACGGGAGCGCCAGGAATCAATCCG -3' and 5'-CGGATTGATTCCTGGCGCTCCCGTCATGT -3'; I96T: 5'-GGGAGCGTCAGGAACCAATCCGGGAATGC -3' and 5'-GCATTCCCGGATTGGTTCCTGACGCTCCC -3'; P146L: 5'-GTTGCGACTATGAACCTGAGCGGATCAATTCG -3' and 5'-CGAATTGATCCGCTCAGGTTCATAGTCGCAAC -3'; S147R: 5'-CGACTATGAACCCGAGGGGATCAATTCGAATG -3' and 5'-CATTCGAATTGATCCCCTCGGGTTCATAGTCG -3'; F525A: 5'-GAAAAAACTTGCCCAAGCCCTCGCAGGCAAG -3' and 5'-CTTGCCTGCGAGGGCTTGGGCAAGTTTTTTC -3'

Primers for RT-PCR Analysis

FRK1 (*At2g19190*): 5'- ATCTTCGCTTGGAGCTTCTC -3' and 5'- TGCAGCGCAAGGACTAGAG -3'; *At1g51890*: 5'- CCAGTTTGTTCTGTAATACTCAGG -3' and 5'- CTAGCCGACTTTGGGCTATC -3'; *At2g17740*: 5'- TGCTCCATCTCTCTTTGTGC -3' and 5'- ATGCGTTGCTGAAGAAGAGG -3'; *At5g57220*: 5'- AATGGAGAGAGCAACACAATG -3' and 5'- ATACTGAGCATGAGCCCTTTG -3'; *WRKY46* (*At2g46400*): 5'- TCAACCAAGACAAGAACATC -3' and 5'- GTTCTCAATCTCATGGTTAG -3'; *UBQ10* (*At4g05320*): 5'- AGATCCAGGACAAGGAGGTATTC -3' and 5'-CGCAGGACCAAGTGAAGAGTAG -3'; *NHO1* (*At1g80460*): 5'- GCTGCTCCTAATGCTGTTGTC -3'

and 5'- GATTCAGGCTGATCTGATGG -3'.

Primers for *WRKY46* (*At2g46400*) Promoter

5'- CGGGATCCGCAGATGATAAGACATCATTTG -3' and 5'- CATGCCATGGTCACTTCAGAAAATTAAGAATC -3'.

Arabidopsis Protoplast Transient Expression

Typically, 0.1 ml protoplasts at a density of 2 x 10^5 /ml were transfected with 20 μg total DNA including different effector and reporter plasmids. The ratio of effector and reporter DNA was 1:1. *UBQ10-GUS* was always cotransfected with *FRK1-LUC* as an internal control, and the promoter activity was presented as LUC/GUS ratio. For protein expression and kinase assays, 0.2 ml protoplasts were transfected with 40 μg DNA including effector and kinase plasmids at a ratio of 1:2. The amount of different effector DNA was also adjusted to get equal level of effector protein expression. Protoplasts were collected 6 hr after transfection for protein expression, kinase activity and promoter activity assays. The detail protocol was described in He et al., 2006.

MAPK Assays

For in-gel kinase assay of endogenous MAPKs, protoplasts were lysed in 25 μl of MAPK extraction buffer (50 mM Hepes-KOH [pH7.6], 2 mM EDTA, 10 mM β-glycerophosphate, 20% glycerol, 1 mM Na_3VO_4, 1 mM NaF and 1% triton X-100). Protoplast exacts with equal amount of protein were fractioned in a 10% SDS-polyacrylamide gel with 0.25 mg/ml myelin basic protein (MBP). The gel was washed three times for 1 hr with washing buffer (25 mM Tris-HCl [pH7.5], 0.5 mM DTT, 5 mM NaF, 0.1 mM Na_3VO_4, 0.5 mg/ml BSA and 0.1% triton X-100), and then incubated for 18 hr with three changes of renaturation buffer (25 mM Tris-HCl [pH7.5], 0.5 mM DTT, 5 mM NaF, 0.1 mM Na_3VO_4). After equilibration of the gel for 30 min in the reaction buffer (25 mM Tris-HCl [pH7.5], 2 mM EDTA, 12 mM $MgCl_2$, 1 mM DTT and 0.1 mM Na_3VO_4), the kinase reaction was performed for 1 hr in the reaction buffer with 25 μM ATP and 50 μCi [γ-^{32}P] ATP. The reaction was stopped and washed 6 times by 5% TCA and 1% sodium pyrophosphate for 6 hr. The gel was dried and visualized by autoradiography.

For *in-vitro* kinase assay, protoplasts were lysed in 200 μl of immunoprecipitation (IP) buffer (150 mM NaCl, 50 mM Tris-HCl [pH7.5], 5 mM EDTA, 1 mM DTT, 2 mM NaF and 2 mM Na_3VO_4). To immunoprecipitate HA-tagged MPK3 or MPK6, protoplast extracts were incubated with an anti-HA antibody for 2 hr at 4°C with gently shaking, and additional 1 hr after adding 5 μl protein A agarose beads (Roche Applied Science). The HA-tagged kinase was precipitated by a brief centrifugation and washed once with IP buffer, once with kinase buffer (20 mM Tris-HCl [pH7.5], 20 mM $MgCl_2$, 5 mM EDTA and 1 mM DTT). Kinase reaction was performed for 30 min in 25 μl of kinase buffer with 0.25 mg/ml MBP, 100 μM ATP and 5 μCi [γ-^{32}P] ATP. The reaction was stopped by adding SDS-PAGE loading buffer. The ^{32}P-labeled MBP was separated by SDS/PAGE (15%) gel and visualized by autoradiography.

Supplemental References

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Joardar, V., Lindeberg, M., Jackson, R. W., Selengut, J., Dodson, R., Brinkac, L. M., Daugherty, S. C., Deboy, R., Durkin, A. S., Giglio, M. G., et al. (2005). Whole-genome sequence analysis of *Pseudomonas syringae* pv. *phaseolicola* 1448A reveals divergence among pathovars in genes involved in virulence and transposition. J. Bacteriol. *187*, 6488-6498.

Kang, L., Tang, X., and Mysore, K. S. (2004). *Pseudomonas* type III effector AvrPto suppresses the programmed cell death induced by two nonhost pathogens in *Nicotiana benthamiana* and tomato. Mol. Plant Microbe Interact. *17*, 1328-1336.

Kreps, J. A., Wu, Y., Chang, H. S., Zhu, T., Wang, X., and Harper, J. F. (2002). Transcriptome changes for *Arabidopsis* in response to salt, osmotic, and cold stress. Plant Physiol. *130*, 2129-2141.

Table S1. The Induction of *FRK1*, *At1g51890*, *At2g17740* and *At5g57220* by Flg22 in *Arabidopsis* Protoplasts, Leaves, and Seedlings (Fold Increase)

AGI	Annotation	Protoplasts 1 hr	Leaves 3 hr	Seedlings 3 hr
At2g19190 (FRK1)	Receptor-like protein kinase, PPC:1.8.1	21.1	21.4	43
At1g51890	Receptor-like protein kinase, PPC:1.8.1	9.2	9.1	30.5
At2g17740	CHP-rich zinc finger protein	12	17.4	23.8
At5g57220	CYP81F2 cytochrome P450	18	11.2	294

Table S2. Consistent Expression of Control Genes in *Arabidopsis* Leaves Inoculated with DC3000, DC3000*hrcC*, and *Psp* NPS3121 (Fold Change) (Kreps et al., 2002; AtGenExpress Database)

AGI	Annotation	DC3000 2 hr	*hrcC* 2 hr	*Psp* 2 hr	DC3000 6 hr	*hrcC* 6 hr	*Psp* 6 hr
At4g05320	UBQ10 polyubiquitin	0.93	0.93	1.07	0.93	1.07	1.07
At3g13920	AteIF4A-1protein synthesis factor	1.07	1.07	1.07	1.07	1.15	1.23
At2g09990	40S ribosomal protein S16	0.93	1	1	1.07	1.15	1.07
At3g18780	Actin 2	0.87	1.07	0.87	0.93	1.07	0.93
At2g18960	AHA1 pumps ATPases	1.07	1	1	0.93	1.07	0.93
At5g44340	Tubulin beta-4	0.93	1.15	0.87	1	1.07	1.07
At5g37780	CaM4 calmodulin	1.3	1.23	1.07	1	1.23	1.15
At4g23650	AtCPK3 calcium dependent protein kinase	0.87	1.07	0.93	1.23	1.23	1.07

Table S3. Summary of AvrPto Mutants on Virulence and Avirulence Functions in Different Hosts

AvrPto	Pto interaction (yeast two-hybrid)	Virulence in tomato	Avirulence in tomato	Avirulence in tobacco	Suppression of nonhost cell death in *Nicotiana benthamiana*	Suppression of MAMP-mediated immunity in *Arabidopsis*
WT	+	+	+	+	+	+
G2A	+	-	-	-	-	-
S46P	-	-	-	-	-	-
S94P	-	+	-	+	++	-
I96T	-	+	-	+	++	-
P146L	+	+	+	-	-	+
S147R	+	+ Slightly reduced	+	-	-	+
References	Shan et al., 2000a,b	Shan et al., 2000a	Shan et al., 2000b	Shan et al., 2000b	Kang et al., 2004	This work

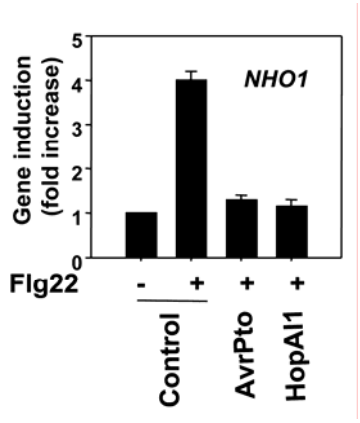

Figure S1. HopAI1 Suppresses Flg22-Mediated *NHO1* Induction

Protoplasts were transfected with empty vector (control), AvrPto or HopAI1. Transfected protoplasts were incubated for 3 hr before treated with 1 μM flg22 for 3 hr. The expression of *NHO1* was analyzed by real-time RT-PCR and normalized to the expression of *UBQ10*. The gene induction (fold change) was compared to the expression level without treatment.

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 A
      1            15 16           30 31           45 46           60 61
75 76          90
    1 TGGAACTCTTTCCTG CTCTTTTGCCACACA GCGCTGATCTTGCGG GTGATTCGGTCCGCA
GGCAGAAGATCGGAG AGGATCAGCATATGG
    2 TGGAACCTGTTCTTT CCCGATCGCCACACA TTGCTGATTTTGCGG GTGAGTCAGCCCGCA
GCCAGAAAACCTCGG AGGATCAGCATATGG
      91          105 106         120 121         135 136         150
151         165 166         180
    1 CGGGTATCAATAGAG CGGG-ACCATCGGGC GCTTATTTTGTTGGC CACACAGACCCCGAG
CCAGTATCGGGGCAA GCACACGGATCCGGC
    2 TGGGGATCAATAGAG CGGGGACCATCGGGC GCTTATATTGCCGGG CACTCAGACCACGAG
CCTGCATCAGGGCGC GCACGCGAATCCAGC
      181         195 196         210 211         225 226         240
241         255 256         270
    1 AGCGGCGCCAGCTCC TCGAACAGTCCGCAG GTTCAGCCGCGACCC TCGAATACTCCCCCG
TCGAACGCGCCCGCA CCGCCGCCAACCGGA
    2 AGCGGCGCCAACTCT TCGAACAGTCCGCAG GTGCCGCCGCCCCCG TCAGAGGCTTCCGCT
TCGCAGGCGCGAG-- ---------ATCGG-
      271         285 286         300 301         315 316         330
331         345 346         360
    1 CGTGAGAGGCTTTCA CGATCCACGGCGCTG TCGCGCCAAACCAGG GAGTGGCTGGAGCAG
GGTATGCCTACAGCG GAGGATGCCAGCGTG
    2 CGTGAGAGGCTTTTG CGATCCAGACCGCTG TCGCGCGAAACCAGG GAGTGGCTGGAGCAG
GGCATGCCGCCAACG GCGGAGGCTGAAGTG
      361         375 376         390 391         405 406         420
421         435 436         450
    1 CGTCGTAGGCCACAG GTGACTGCCGATGCC GCAACGCCGCGTGCA GAGGCAAGACGCACG
CCGGAGGCAACTGCC GATGCCAGCGCACCG
    2 CGTAGCAGACCGCAT GGGTCTGCCGATGCC GCAGCGCCGCATGCA CCTGCT-GAGACAAG
ACCCAGGC------- ----C----GCAGCA
      451         465 466         480 481         495 496         510
511         525 526         540
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TGATATTTCGCATAC TCGTAACATGCTCCG
    2 CGCAG--GGGTGATG GCACACGCTACATAG CATCGTTCAGCAATT GGTCAGCGCGGGCGC
TGATCTTGCCCATAC TCGTACCACGCTGCG
      541         555 556         570 571         585 586         600
601         615 616         630
    1 CAATGCAATGAATGG CGACGCAGTCGCTTT TTCTCGAGTAGAACA GAACATATTTCGCCA
GCATTTCCCGAACAT GCCCATGCATGGAAT
    2 CAATATCATAAGAGG CGAAGAGATGGCTCT TTCTAGAGCCGAACA GAGCATTTTGCGCGA
GCATTTCCCTAACAT GATTGCGACTGGAAT
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691         705 706         720
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ACAGGCGGCGTCAGC GCCAGTGAGGTCGCC
    2 CAACCCCCATTCAGA GCTCGCTATCGAGCT CCGTGATGCGCTCCG TAGAGCGGATCGCCA
ACAGGCAGCGTCAAC TCCAGCAAGGACACC
      721         735 736         750 751         765 766         780
781         795 796         810
    1 CACGCCAACACCGGC ------CAGCCCTGC GGCATCATCATCGGG CAGCAGTCAGCGTTC
TTTATTTGGACGGTT TGCCCGTTTGATGGC
    2 GACGCGGTCCCCGGC GGCACCCACCCCTAC GCAATCATCATCGAG TAGCAGCCAGCGTTC
ATTATTTGGTCGGTT CGCCCGGCTGATGAC
      811         825 826         840 841         855 856         870
871         885 886         900
    1 GCCAAACCAGGGACG GTCGTCGAACACTGC CGCCTCTCAGACGCC GGTCGACAGGAGCCC
GCCACGCGTCAACCA AAGACCCATACGCGT
    2 GCCTGATCAGAGACG TTCGTCAAACGCATC GACCTCTCAGACACC GGTAGACAGAAGCCC
GCCACGCGTCAATCA GGTACCCATACGCCC
      901         915 916         930 931         945 946         960
961         975 976         990
    1 CGACAGGGCTGCGAT GCGTAATCGTGGCAA TGACGAGGCGGACGC CGCGCTGCGGGGGTT
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AGTACAACAGGGGGT CAATTTAGAGCACCT

2 CGACAGGGCTGCGAT GCGTAATCGTGGCAA TAACCAGGCCGCCGC AGCCTTGCAGGGATT

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991 1005 1006 1020 1021 1035 1036 1050

1051 1065 1066 1080

1 GCGCACGGCCCTTGA AAGACATGTAATGCA GCGCCTCCCTATCCC CCTCGATATAGGCAG

CGCGTTGCAGAATGT GGGAATTAACCCAAG

2 GCGCACGGCGCTTGA AAGACATCTATTGCG GCACCAACCGATCCC CTTGGATATAGCGTA

CGCGTTGCAGAGCGT GGGCATTCCCTCAAG

1081 1095 1096 1110 1111 1125 1126 1140

1141 1155 1156 1170

1 TATCGACTTGGGGGA AAGCCTTGTGCAACA TCCCCTGCTGAATTT GAATGTAGCGTTGAA

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2 TGTTGACACGGCTGA GAGCCTTGTGGAAAG CCCGCTGATGGATTT GAGTGTTGCGCTGCA

CCGCGTGCTTGGGCC ACGTCCCGTTAGTGC

1171 1185 1186 1200 1201 1215 1216 1230

1231 1245 1246 1260

1 TGAAAGAGCGCCTCG TCCAGCCGTCCCCGT GGCTCCCGCGACCGC CTCCAGGCGACCGGA

TGGTACGCGTGCAAC ACGATTGCGGGTGAT

2 T------CCGCCTCG CCCAGCCGTTCCGAT GCATCCTCCAGCTGC CTCCAGGCGACCAGA

TGGCGCGCGTTCCAG CGCATTGCGGGTAAT

1261 1275 1276 1290 1291 1305 1306 1320

1321 1335 1336 1350

1 GCCGGAGCGGGAGGA TTACGAAAATAATGT GGCTTATGGAGTGCG CTTGCTTAACCTGAA

CCCGGGGGTGGGGGT AAGGCAGGCTGTTGC

2 TCCGGAGCGGGAGGA TTATGAAAATAATGT GGCTTACGGAATGCG CTTGCTTAACCTGAA

CCCGGGGGTTGGGGT AAGGAGGGTTGTTGC

1351 1365 1366 1380 1381 1395 1396 1410

1411 1425 1426 1440

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GGACCCTATAACCTC ACAATTCAATCAACT

1441 1455 1456 1470 1471 1485 1486 1500

1501 1515 1516 1530

1 GCGCACAATTTCGAA GGCCGATGCTGAATC TGAAGAGCTGGGTTT TAAGGATGCGGCAGA

TCATCACACGGATGA CGTGACGCACTGTCT

2 GCGTACGGTCTCCAA GGCCGTTGCTGAATC CCAAAATCCGCCCTT CATGGACGCGGCACA

CCATCACCCGGACGA TGCGACCCATTGCCT

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1591 1605 1606 1620

1 TTTTGGCGGAGAATT GTCGCTGAGTAATCC GGATCAGCAGGTGAT CGGTTTGGCGGGTAA

TCCGACGGACACGTC GCAGCCTTACAGCCA

2 TTTTGGTGAACCATT GTCGCTGGAAAATCC TGATCGGCAGGTGAT CGGCCTGGCGGGGAA

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1681 1695 1696 1710

1 AGAGGGAAATAAGGA CCTGGCGTTCATGGA TATGAAAAAACTTGC CCAATTCCTCGCAGG

CAAGCCTGAGCATCC GATGACCAGAGAAAC

2 GCAGGGAAATAAGGA GCTGGCGTTCATGGA TATGAAAAAACTTGC CCAATTCCTCGCAGG

CAAGCCTGAGCATCC GATGAACAGACAACC

1711 1725 1726 1740 1741 1755 1756

1 GCTTAACGCCGAAAA TATCGCCAAGTATGC TTTTAGAATAGTCCC CTGA

2 GCTTGACGCCAGAAC CATCGCTAACTACGC GTTTAGAATCGTGCC CTGA

B

>AvrPtoB-Psp, frame+1, 545 bases, E7F checksum.

MVGINRAGTIGRLYCRALRPRACIRARTRIQQRRQLFEQSAGAAAPVRGFRFAGARSA*EAFAIQTAVARNQ
GVAGAGHAANGGG*SA*QTAWVCRCRSAACTC*DKTQAAARRGDGTRYIASFSNWSARALILPILVPRCAIS
*EAKRWLFLEPNRAFCASISLT*LRLESTPIQSSLSSSVMRSVERIANRQRQLQQGHRRGPRRHPPLRNHHR
VAASVHYLVGSPG**RLIRDVRQTHRPLRHR*TEARHASIRYPYAPTGLRCVIVAITRPPQPCRDWFNKGSI
WKTCARRLKDIYCGTNRSPWI*RTRCRAWAFPQVLTRLRALWKAR*WI*VLRCTACLGHVPLVLRLAQPFRC
ILQLPPGDQMARVPAHCG*FRSGRIMKIMWLTECACLT*TRGLG*GGLLQPL*PIRRPGRQLWTISAQPGTL
*PHNSINCVRSPRPLLNPKIRPSWTRHTITRTMRPIAFLVNHCRWKILIGR*SAWRGIRRTRRSFTVSREIR
SWRSWI*KNLPNSSQASLSIR*TDNRLTPEPSLTTRLESCP

Figure S2. Sequence Analysis of AvrPtoB Homolog in *Psp* 1448A

(A) Alignment of the nucleotide sequence of *avrPtoB* from DC3000 (1) and its homolog in *Psp* 1448A (2) (Joardar et al., 2005) by ClustalW 1.8.
(B) Deduced amino acid sequence of AvrPtoB homolog in *Psp* 1448A (AvrPtoB-Psp). (* indicates stop codon).

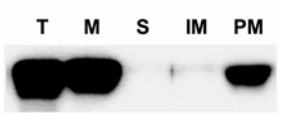

Figure S3. The Plasma Membrane Localization of AvrPto in Transgenic *Arabidopsis* Plants

Leaves from *avrPto* transgenic line 120 (L120) were collected 24 hr after spraying with 20 μM dexamethasone (DEX). The total protein (T) was extracted and separated by centrifugation into soluble (S) and membrane (M) fractions. Plasma membrane (PM) was separated from intracellular membrane (IM) by the two-phase partitioning system (Shan et al., 2000b). The expression of AvrPto was detected by anti-HA antibody.

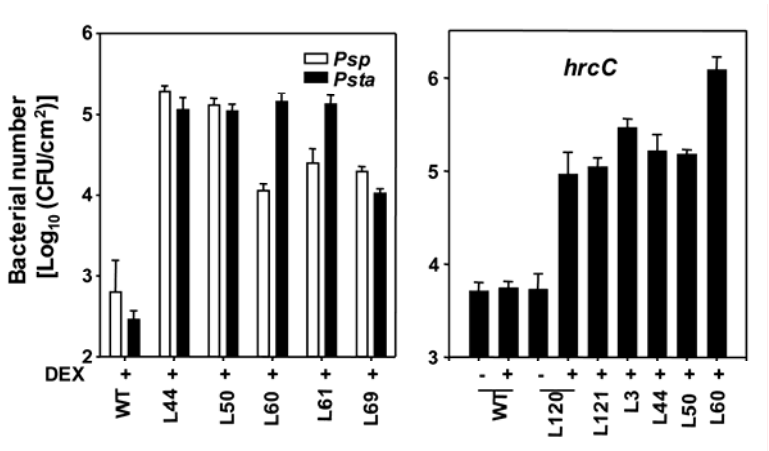

Figure S4. AvrPto Transgenic Plants Support Nonhost/Nonpathogenic Bacteria Growth

Independent *avrPto* transgenic lines were inoculated with *Psp* NPS3121 (*Psp*), *P. s. tabaci* (*Psta*) or DC3000*hrcC* (*hrcC*) at 5 x 10^5 cfu/ml 24 hr after spraying with 20 μM dexamethasone (DEX). Bacterial growth assay was carried out at 3 days after inoculation.

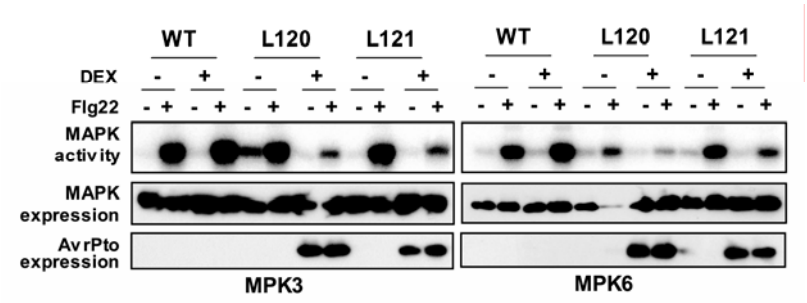

Figure S5. Expression of AvrPto Suppresses MPK3/6 Activation by Flg22 in Transgenic Plants

Protoplasts from *avrPto* transgenic plants were transfected with HA-tagged MPK3 or MPK6. Transfected protoplasts were treated with control or 20 μM dexamethasone (DEX) for 6 hr before 1 μM flg22 treatment for 10 min. An anti-HA antibody was used for immunoprecipitation of MPK3 and MPK6. Kinase activity was detected by an *in-vitro* kinase assay (top). Protein expression is shown for MAPKs (middle) and AvrPto (bottom).

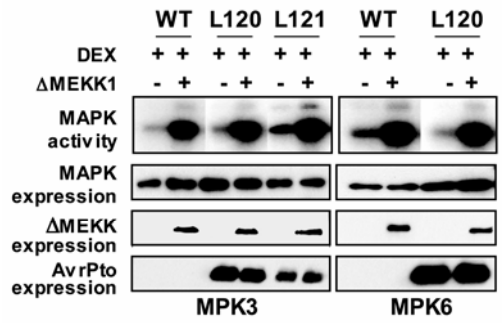

Figure S6. Expression of AvrPto Does Not Affect ΔMEKK1 Activity

Protoplasts from *avrPto* transgenic plants were treated with 20 μM dexamethasone (DEX) for 4 hr then cotransfected with HA-tagged MPK3 or MPK6 and Flag-tagged ΔMEKK1. Kinase activity was detected by an *in-vitro* kinase assay and protein expression is shown.

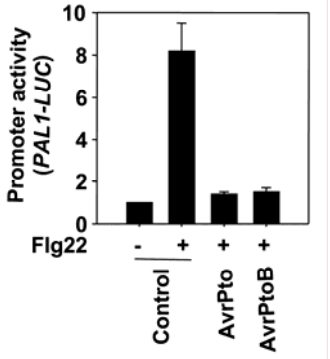

Figure S7. AvrPto and AvrPtoB Suppress Flg22-Mediated *PAL1-LUC* Induction

Protoplasts were cotransfected with an effector and *PAL1-LUC* reporter and incubated for 3 hr before treated with 100 nM flg22 for 3 hr.