Supplemental Information

EXTENDED EXPERIMENTAL PROCEDURES

Anthocyanin Measurement

Anthocyanin quantification was performed as described by Deikman and Hammer (1995). Ten day old Arabidopsis seedlings were pre-weighed, placed into 1 mL extraction buffer (18% 1-propanol, 1% HCl, and 81% H_2O), boiled for 3 min, and then incubated in darkness overnight at room temperature. A_{535} and A_{650} of the extraction solution were measured. The amount of anthocyanins was reported as (A535–A650) g^{-1} fresh weight (FW). Three replicates were measured for each treatment.

JA Content Measurement

For JA measurement, 200 mg plant tissue was homogenized and extracted for 24 h in methanol containing Jasmonic-d5 Acid (CDN Isotopes Inc.) as internal standard. The sample was centrifuged and purified by using Oasis Max cartridge (150mg/6cc, Waters). The entire sample was then injected into a LC/MS/MS system consisting of an Acquity Ultra Performance Liquid Chromatograph (Acquity UPLC; Waters) and a triple quadruple tandem mass spectrometer (Quattro Premier XE; Waters). Two biological replicates were analyzed for each treatment.

SUPPLEMENTAL REFERENCE

Deikman, J., and Hammer, P.E. (1995). Induction of anthocyanin accumulation by cytokinins in *Arabidopsis thaliana*. Plant Physiol. *108*, 47-57.

Figure S1. Relates to Figure 1. AvrB-induced JA responses and effects of GDA treatment on AvrB protein accumulation and bacterial growth in medium. (A) GDA does not affect AvrB stability. *rpm1* and *rpm1/AvrB-3xFLAG* plants pre-treated with 20 μM estradiol were infiltrated with 2.5μM GDA or DMSO for the indicated times prior to protein isolation. The AvrB-3xFLAG protein was detected by immunoblot using anti-FLAG antibodies. (B) GDA does not affect *P. syringae* bacterial growth. *P. syringae hrcC* bacteria was grown in liquid KB medium with 2.5 μM GDA or equal volume of DMSO, and optical density was measured at the indicated times. The experiment was repeated twice with similar results. (C) GDA inhibits AvrB-induced Thi2.1 expression. (D) RAR1 is required for AvrB-induced *THI2.1* expression. Quantitative RT-PCR measurement of *THI2.1* expression in plants of the indicated genotypes. Plants were treated with Estradiol, GDA, and/or DMSO for 24 hours prior to RNA isolation (C and D). (E) Accumulation of anthocyanin in plants. 10 day old seedlings of the indicated genotypes were grown in the presence of estradiol or DMSO, and anthocyanin was measured. The amount of anthocyanin is expressed as (A535-A650)/g fresh weight (FW). Error bars indicate standard deviation. The experiment was repeated twice with similar results. (F) Expression of AvrB does not alter JA content. Plants of the indicated genotypes were treated with estradiol or DMSO for 24 hrs, and JA content was measured. Error bars indicate standard deviation. The experiment was repeated twice with similar results.

Figure S2. Relates to Figure 2. AvrB-RAR1 interaction occurs in the absence of EDTA**.** Plants of the indicated genotypes were treated with estradiol for 24 hrs prior to protein isolation and co-IP experiments. EDTA was omitted from all buffer used.

Figure S3. Relates to Figure 3. Control experiments for AvrB-induced MPK4 phsphorylation. (A) Transgenic AvrB induces MPK4 phosphorylation. Plants of the indicated genotypes were treated with estradiol to induce AvrB expression, and MPK4 was isolated by immunoprecipitation using anti-MPK4 antibodies. The level of phosphorylated and total MPK4 was detected by immnuoblot using anti-phospho-ERK and anti-MPK4 antibodies, respectively. *mpk4* was included as a negative control. The anti-MPK4 antibodies did not detect any signal in *mpk4* plants, indicating that the antibodies were specific. (B) Transgenic AvrB induces MPK4 activity. Anti-MPK4 antibodies were used to immunoprecipitate MPK4 proteins from estradiol-treated *rpm1* plants with (+) or without (-) the *AvrB-3xFLAG* transgene. Immunoprecipitates isolated with Protein-A agrose alone (P-A IP) were included as a negative control. Kinase activity of the immunoprecipitated MPK4 protein was determined using an *in vitro* kinase assay employing myelin basic protein (MBP) as a substrate. Equal loading of immunoprecipitated proteins was confirmed by immunoblot using anti-MPK4 antibodies. (C) Bacterially-delivered AvrB induces MPK4 phosphorylation. *rpm1* plants were either untreated or infiltrated with the indicated bacterial strains for 6 hrs, immunoprecipitated with anti-MPK4 antibodies, and the level of phosphorylated and total MPK4 was determined as in (B).

Figure S4. Relates to Figure 5. Control experiment for AvrB-MPK4 interaction *in vivo*. Co-IP experiment was done as in Figure 6B. *mpk4* plants were included as a control for the specificity of anti-MPK4 antibodies.

Figure S5. Relates to Figure 7. Role of RIN4 in AvrB-induced plant susceptibility and the relationship between RIN4 and MPK4. (A) An *AvrB* transgene does not induce plant susceptibility to *P. syringae* in an *rps2*/*rin4* mutant. Plants of the indicated genotypes were pre-treated with estradiol, inoculated with *hrcC* mutant bacteria, and bacterial populations within leaves determined at the indicated times. Two independent transgenic lines expressing AvrB at a level comparable to the *AvrB-3xFLAG/rpm1* transgenic line (Shang *et al.,* 2006) were tested with similar results. Data from one transgenic line are shown. (B) MPK4 phosphorylates RIN4 *in vitro*. Uninduced and flg22-induced MPK4-3xFLAG was expressed in protoplasts were induced by flg22, enriched by immunoprecipitation, and incubated with RIN4-His in an *in vitro* kinase assay. RIN4 phosphorylation was detected by autoradiography. CBB stain indicate the amount of RIN4-His. A p-RIN4 signal was detected only when both MPK4-3xFLAG and RIN4-His were present. (C) AvrB induces MPK4 phosphorylation normally in *rps2/rin4* mutant plants. Arabidopsis *rps2/rin4* plants were inoculated with DC3682 with (+) or without (-) *avrB* 3 hrs prior to immunoprecipitation of MPK4. The phosphorylation state of the immunoprecipitated MPK4 was determined by immunoblot using anti-phospho-ERK1 antibodies.

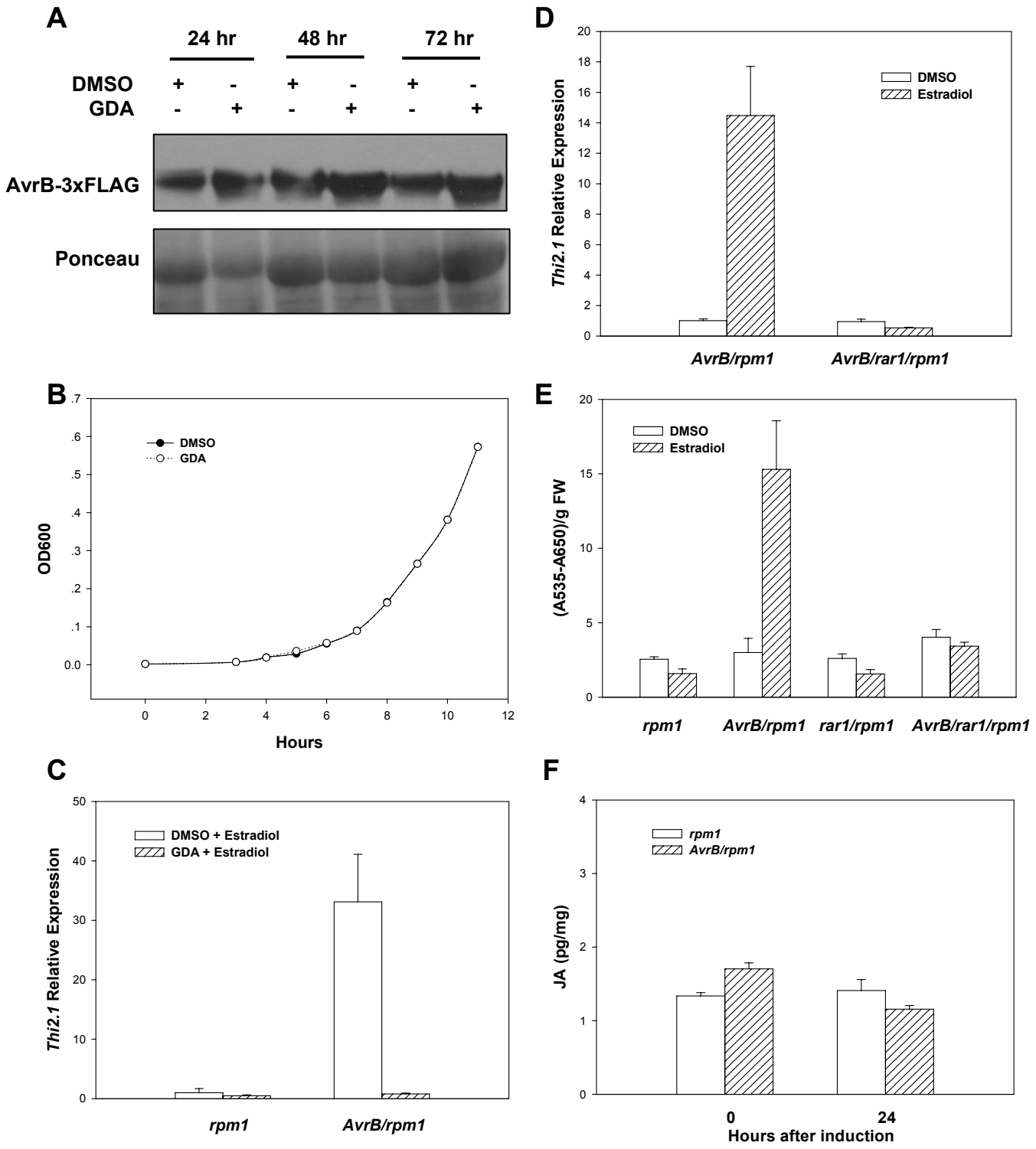


Fig. S1. Relates to Figure 1

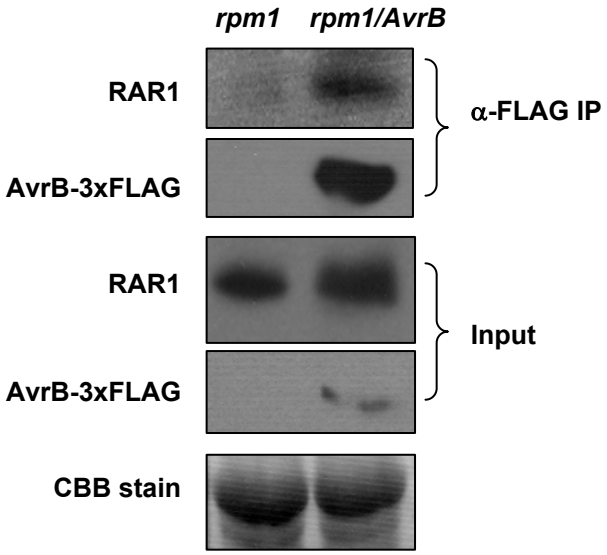


Fig. S2. Relates to Figure 2

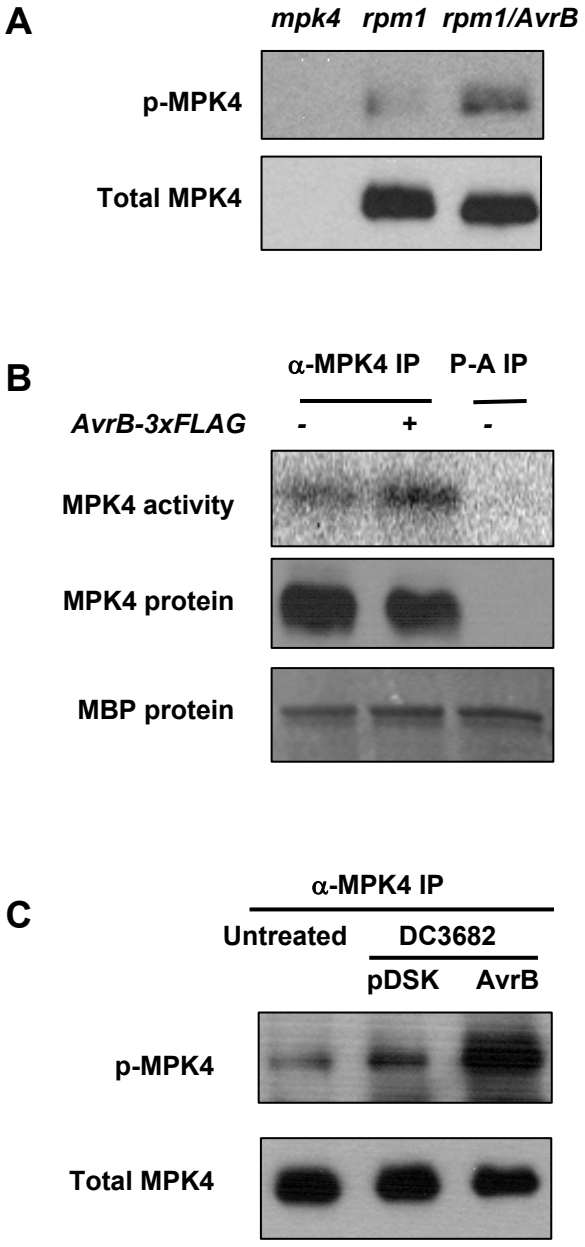


Fig. S3. Relates to Figure 3

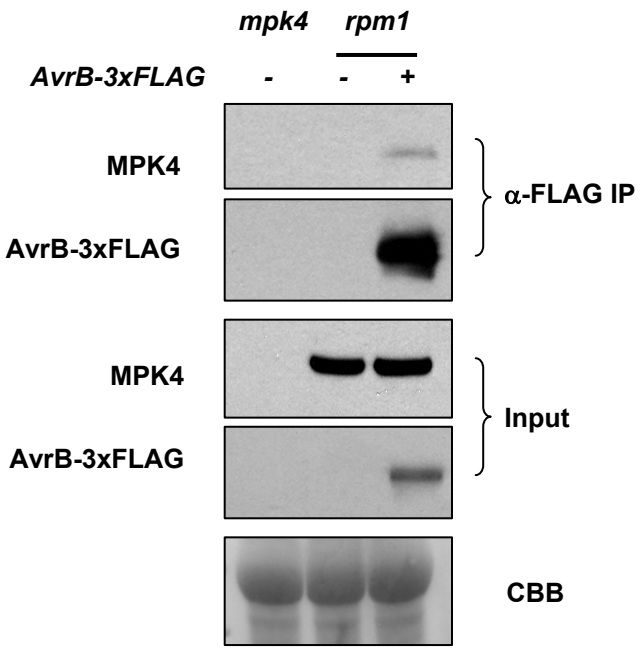
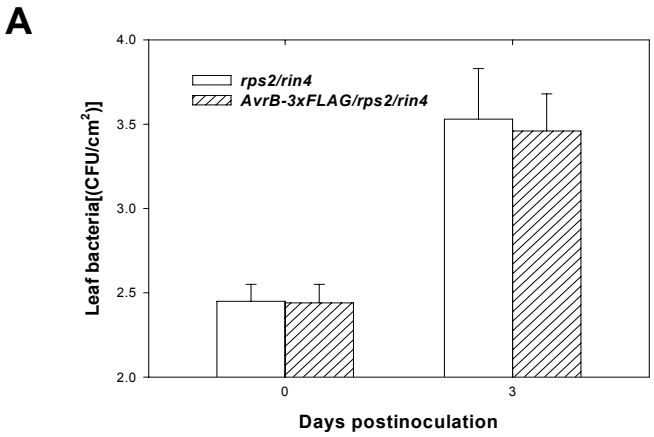
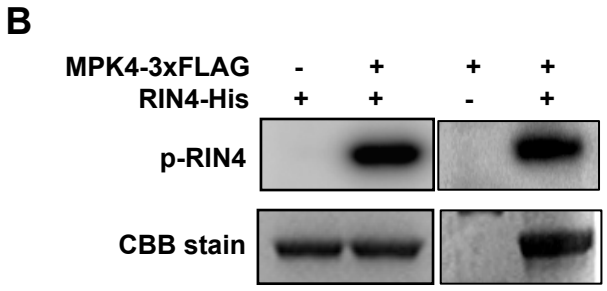


Fig. S4. Relates to Figure 5





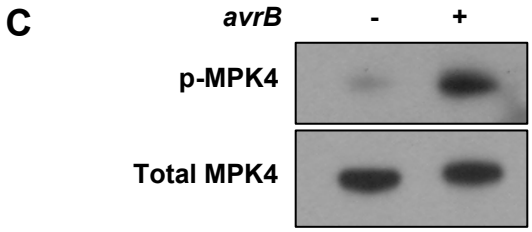


Fig. S5. Relates to Figure 7