Supporting Information

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SI Materials and Methods

Plant Growth. Expression of *BAK1::BAK1-Flag* (WT and site-directed mutants) in the *bak1-4 bkk1-1* double mutant background was achieved by transforming the viable heterozygous *BAK1-4_/bkk1-1 bkk1-1* (1, 2) with the pBIB-Hyg-PK1-BAK1-Flag construct and screening for homozygous *bak1-4 bkk1-1* double mutants expressing the transgene as described (2). PK1 refers to the native promoter of BAK1 located in the 1.7-kb sequence upstream of the gene. The site-directed mutants for K317E (kinase-inactive), Y610F and Y610E, were obtained using a full-length BAK1 cDNA with C-terminal Flag epitope tag as template.

Bacterial Inoculation. *A. thaliana* mutant plants were grown in soil in growth chambers with a day/night cycle of 12 h/12 h, a light intensity of 100 μE, and a constant temperature of 20 °C. Plants expressing WT BAK1-Flag or BAK1(Y610F)-Flag in the *bak1-4 bkk1-1* double mutant background were 3 to 4 wks old when used for experiments. Bacteria were grown with appropriate antibiotic in Luria–Bertani broth to the mid- to late logarithmic phase at 28 °C with shaking. Bacterial cultures were centrifuged to recover bacteria, which were resuspended in sterile water to a final OD_{600} of 0.2 and then diluted 100 times (equivalent to 1×10^6 cfu cm^{-2}). Fully expanded leaves were infiltrated with bacterial suspensions and sampled immediately after inoculation (day 0) or after 3 d, and bacteria were enumerated by dilution plating as described previously (3). For the day-0 sample, the average of bacterial numbers for both genotypes is reported.

Site-Directed Mutagenesis of Flag-BAK1-CD and Recombinant Protein Studies. The described Flag-BAK1-CD construct (2) was used as template for site-directed mutagenesis with the QuikChange XL Site-Directed Mutagenesis Kit (Stratagene) and 10 individual constructs were generated with the following substitutions: Y304F, Y363F, Y365F, Y403F, Y443F, Y463F, Y478F, Y530F, Y587F, and Y610F. All constructs were sequenced in both directions to verify specific mutations and lack of additional mutations. Recombinant proteins were analyzed by immunoblotting as described later. The ability of Flag-BAK1-CD (WT and Y610F-directed mutants) to transphosphorylate Flag-BRI1-CD and increase its kinase activity was monitored using the BR13 peptide (sequence GRJKKIASVEJJKK, where J is norleucine) as substrate as previously described (2).

Protein Interaction Studies. The real-time binding of Flag-BRI1 to GST-BAK1 or GST-BAK1(Y610F) was monitored using an Octet QK (ForteBio). The binding buffer contained 50 mM Mops (pH 7.5), 0.05% (vol/vol) Nonidet P-40 (Roche) and 10 mM $MgCl_2$ unless specified otherwise. Three micrograms of monoclonal anti-GST antibody (G1160; Sigma) was captured to each anti-Murine sensor (18-5024; ForteBio), followed by binding of the ligand, 250 nM GST-BAK, or GST-BAK1(Y610F) to the sensor. Then, replicate coated sensors were exposed to several concentrations of the Flag-BRI1 analyte for 15 min followed by a 15-min disassociation period. One-shot K_D was calculated based on the 1:1 model and the average K_D was extracted from binding results for six different concentrations of the analyte.

Preparation of Microsomal Membranes for Immunoblot Analysis. Microsomal membranes were isolated, solubilized with Triton X-100, and recombinant Flag-tagged protein affinity purified as described (4). Proteins were separated by SDS/PAGE, transferred to PVDF membranes, and analyzed by immunoblotting with anti-Flag antibodies, antiphosphothreonine (anti-pT), antiphosphotyrosine (anti-pY), or custom modification antibodies (anti-pY610 antibodies). The custom antibodies were produced by GenScript against the sequence IENEpYPSGPRC, where the terminal cysteine residue was added for coupling. The antibodies were sequentially affinity purified using the nonphosphorylated and phosphotyrosine-containing antigen peptides as immobilized ligands. Immunoblots were scanned with an Odyssey infrared imaging system (Li-Cor) for visualization. As indicated, blots were also stained with ProQ Diamond phosphoprotein stain (Invitrogen) and scanned using a Typhoon Molecular Dynamics imager.

Experimental Design: Plant Materials for the Microarray Experiments. Plants were grown under long-day conditions (16 h/8 h) for 25 d on soil. All tissues were collected except roots, frozen in liquid nitrogen, and stored at −80 °C. Total RNA was isolated and cleaned using RNeasy Plant Mini Kit (Qiagen). Total RNA was extracted from the above-soil portions of individual BAK1-Flag, WT, and BAK1(Y610F)-Flag, mutant plants in triplicate, with each array indicating the gene expression of a single plant.

Extract Labeling. RNA microarray analysis was performed by the W. M. Keck Center for Comparative and Functional Genomics in the Roy J. Carver Biotechnology Center at the University of Illinois at (Urbana, IL). RNA was prepared and hybridized to the GeneChip Arabidopsis ATH1 genome array (Affymetrix) in the Functional Genomics unit using the GeneChip 3′ Express Kit (Affymetrix) according to the manufacturer's instructions. Briefly, 200 ng of total RNA was spiked with four prokaryotic polyadenylated sense RNA controls, primed with a T7-oligo$(dT)_{24}$ primer containing a 5′ T7 promoter sequence, and reverse transcribed to produce single-stranded cDNA. RNase H was used to degrade remaining RNA, and DNA polymerase used to synthesize the second strand cDNA. The double-stranded cDNA template was in vitro transcribed and amplified using a biotinylated pseudouridine in addition to the four natural ribonucleotides to produce biotin-labeled aRNA. The aRNA was purified, quantified, and fragmented.

Hybridization Conditions and Chip Processing. Hybridization mixtures were prepared, adding biotinylated controls: four prokaryotic fragmented aRNAs and one synthetic oligonucleotide. Following a 16-h hybridization, the chip was washed, stained first with streptavidin-conjugated phycoerythrin dye (Invitrogen), and the stain enhanced with biotinylated goat anti-streptavidin antibody (Vector Laboratories), then counterstained with the conjugated phycoerythrin, using a GeneChip Fluidics Station 450 and the Affymetrix GeneChip Command Console software. The chips were scanned with a GeneChip Scanner (model 3000 7G Plus; Affymetrix). Files of the fluorescence signals were generated with Affymetrix Expression Console version 1.1, quality control values checked, and data files transferred to the Bioinformatics Unit for statistical analysis.

Microarray Analysis. Microarray data were preprocessed and analyzed using R (5) and Bioconductor (6) packages as indicated. After preprocessing the arrays with the GCRMA algorithm (7), we evaluated array quality by performing a PCA-based clustering from the affycoretools package (8). All three replicates of the BAK1 group clustered tightly, but only two of the three Y610F replicates clustered tightly. The third replicate of the Y610F group had had a lower cRNA yield during labeling, and the GCRMA algorithm was unable to normalize away this difference. Therefore, we excluded this sample from further analysis. To assess

differential expression between Y610F and BAK1, we used the limma package (9). After fitting the model, we filtered out probe sets that were called "absent" on all arrays using the Affymetrix Call Detection Algorithm (10); of the 22,810 probe sets on the array, 16,973 survived the filtering and had their *P* values corrected for multiple hypothesis testing using the false discovery rate method (11). Annotation of the probe sets was done by using the Bioconductor ath1121501.db package (version 2.2.13).

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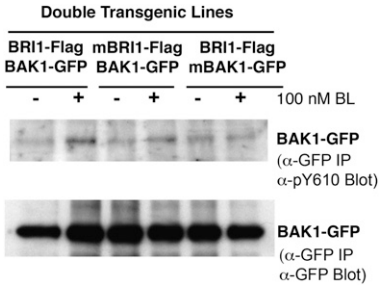

Fig. S1. BAK1 phosphorylation on Tyr-610 in vivo is BL dependent and requires an active BAK1 kinase domain. Homozygous transgenic Arabidopsis plants expressing both BRI1-Flag and BAK1-GFP or their kinase-inactive directed mutants (mBRI1 = K911E and mBAK1 = K317E). Plants were grown with the BR biosynthesis inhibitor Brz (1 μM added after 6 d of initial growth) and were then treated with BL or solvent for 90 min on day 11. Proteins immunoprecipitated from solubilized microsomal membranes with anti-GFP antibodies were analyzed by immunoblotting as indicated.

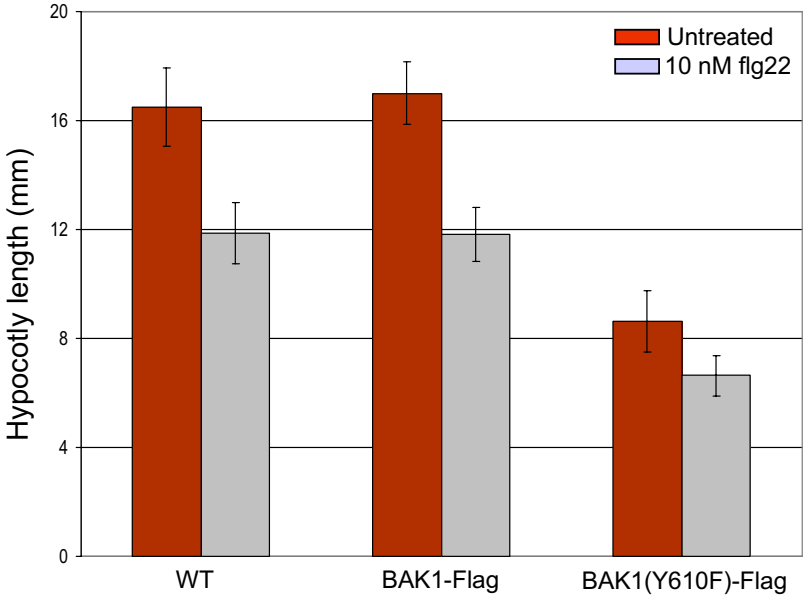

Fig. S2. Hypocotyl elongation of WT plants and transgenic plants expressing BAK1-Flag or BAK1(Y610F)-Flag in the *bak1-4 bkk1-1* double mutant background. Seedlings were grown on agar plates in the dark for 5 d, and hypocotyl length was measured. Values are means ± SEM; $n = 3$.

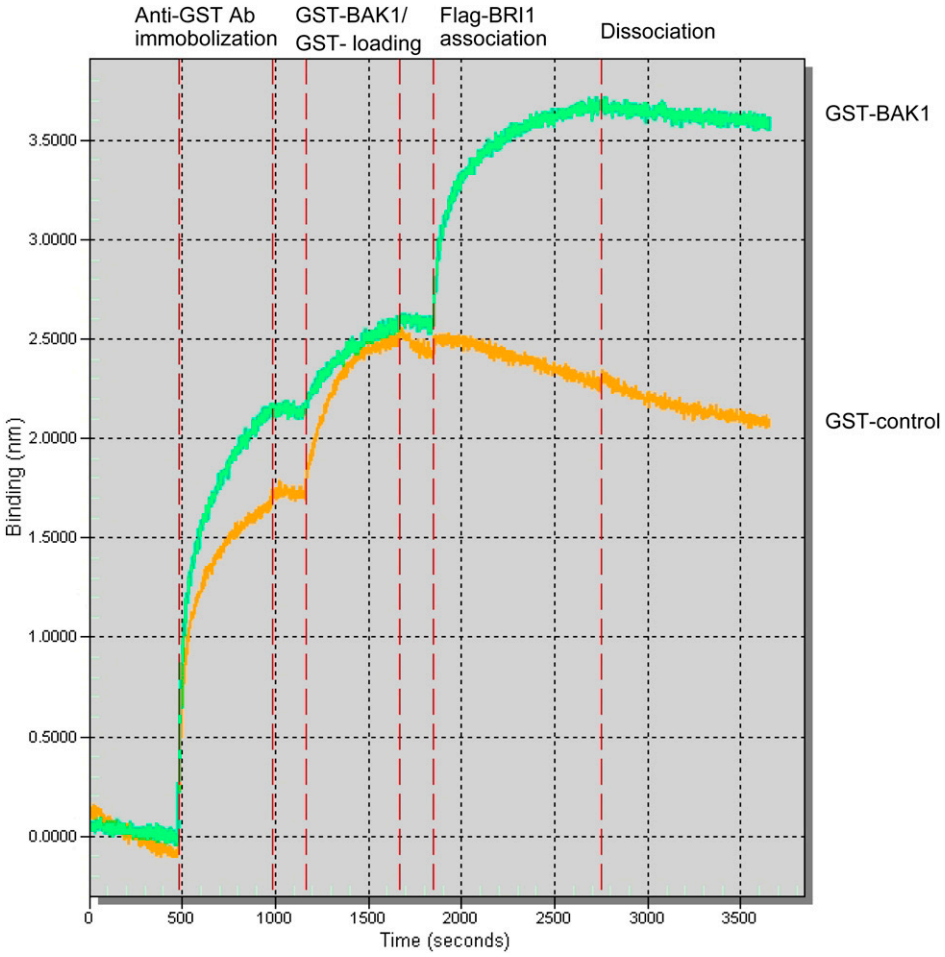

Fig. S3. Flag-BRI1-CD does not bind to immobilized GST, and thus the binding to immobilized GST-BAK1 reflects an interaction with sequences contained in the BAK1-CD protein.

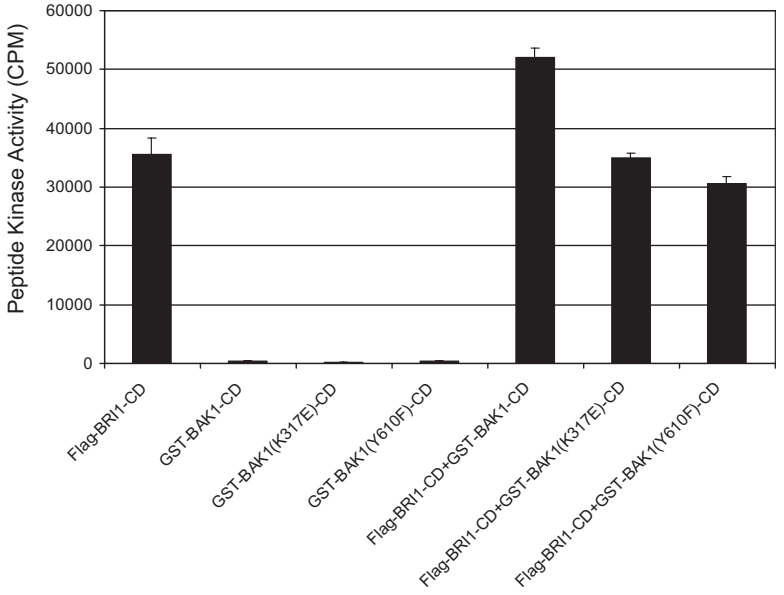

Fig. S4. Activation of Flag-BRI1-CD activity by WT BAK1-CD but not the Y610F or kinase-inactive K317E directed mutants monitored using the BR13 peptide substrate. The increase in BRI1 activity requires an active BAK1 kinase, which alone does not phosphorylate the BR13 peptide. Values are means ± SEM; $n = 3$.

Other Supporting Information Files

Table S1 (XLS)
Table S2 (XLS)
Table S3 (DOC)