TEMPLATE FOR JIPB SUPPORTING INFORMATION

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Differential requirement of BAK1 C-terminal tail in development and immunity: The function of BAK1 C-terminal tail

SUPPLEMENTARY METHODS

Growth conditions and mutant generation

Arabidopsis plants were grown in soil at 23℃ under long-day conditions (16-h light/8-h dark), unless otherwise stated. When growing *Arabidopsis* under sterile conditions, stratified seeds were plated on half strength Murashige and Skoog medium (1/2 MS), after which plates were incubated in a growth chamber under long-day conditions (16-h light, 22 ℃/8-h dark 19℃).

The *sobir7-1 bir1-1 pad4-1* triple mutant was as previously described (Liu et al. 2016). To generate a *sobir7-1* single mutant, *sobir7-1 bir1-1 pad4-1* was crossed with wild-type Col-0 plants. To generate the *sobir7-1 bkk1-1* double mutant, *sobir7-1 bir1-1 pad4-1* was crossed with *bak1-4*$^{+/-}$ *bkk1-1*. The desired mutants were subsequently isolated from the respective F_2 population through genotyping all related loci.

Plasmid construction

Full-length *BAK1* DNA sequence was amplified with Phusion DNA Polymerase (Fisher Scientific International, Inc.) using primer pairs detailed in Table S1. The resulting PCR fragments were digested and then ligated into different vectors, according to experimental design. Specifically, for transient expression in *Nicotiana benthamiana* (*N.B.*), full-length *BAK1* DNA sequence was ligated into a modified pCambia1300 vector, containing the *35S* CaMV promoter and a C-terminal 3xHA tag. For biomolecular fluorescence complementation assays, full-length *BAK1* cDNA was incorporated into the pUC19-YCE vector. For heterologous expression of the BAK1 kinase domain (KD) in *E. coli*, the BAK1 kinase domain cDNA sequence was ligated into the pET24c vector to allow for

production of 6×His-tagged BAK1 KD protein in bacteria. Subsequently, the same set of constructs was generated for the mutant *BAK1* carrying *sobir7-1* mutation with the corresponding wild-type *BAK1* plasmids as templates, using primers listed in Table S1.

Measurement of PAMP-triggered reactive oxygen species (ROS)

Measurement of PAMP-triggered ROS production was performed, as previously described (Liu et al. 2013). Essentially, leaf strips of 4-week-old short-day grown *Arabidopsis* plants were treated with elicitor solution, consisting of 20 ìM luminol and 10 mg/ml horseradish peroxidase, and 1 μM elicitor peptide (flg22 or elf18). Chemiluminescence was then monitored using a microplate reader (Tecan Group Ltd., Tecan M200).

PAMP-triggered activation of MAP kinases

To measure flg22-triggered MAP kinase activation, two-week-old *Col-0*, *bak1-4* and *sobir7-1* seedlings grown on ½ MS medium were thoroughly sprayed with 100 nM flg22 solution supplemented with 0.01% silwet. Seedling samples were pooled at both 0 and 15 min post treatment. Subsequently, phosphorylated MAPKs were detected by anti-phospho-p44/42-ERK antibody (Cell Signaling Technology, Inc., #9102).

RNA extraction and quantitative reverse transcription PCR (qRT-PCR)

For gene expression analysis, 12-day-old seedlings grown on 1/2 MS plates were pooled for RNA extraction with EZ-10 Spin Column Plant RNA Mini-Preps Kit (Bio Basic Inc., Amherst, NY, USA), following the manufacturer's instructions. The first strand of cDNA was synthesized by reverse transcriptase from M-MuLV reverse transcriptase (New England Biolabs Inc., Ipswich, MA, USA), after which real-time PCR was performed with the SYBR Premix Ex TaqII kit (Takara, Kusatsu, Japan) to quantify the amount of target transcripts.

Measurement of *Arabidopsis* hypocotyl growth

To measure hypocotyl elongation under Epibrassinolide (Sigma-Aldrich, E1641) treatment, *Arabidopsis* seeds were plated on 1/2 MS medium solidified with 12g/L agar and supplemented with 100 nM Epibrassinolide, or mock solution. Plates were relocated into a light chamber for 2 d to allow for efficient germination. The plates were subsequently kept vertical, in the dark, for another six days before measuring hypocotyl

length. At least 30 seedlings from each experimental group were randomly chosen for hypocotyl measurement.

Biomolecular fluorescence complementation (BiFC) assay

All plasmids used in this assay were purified through the PureYield™ Plasmid Midiprep System (Promega Corporation). Preparation of *Arabidopsis* mesophyll cell protoplast was asprevious described (Wu et al. 2009). Protoplasts were transfected, via polyethylene glycol (PEG)-mediated transformation, and then incubated under weak light for approximately 16 h. Following incubation, protoplasts were harvested by slow-speed centrifugation and then examined with a Nikon ECLIPSE 80i confocal microscope to detect fluorescence.

Trypan blue staining

Trypan Blue staining was carried out as previously described (Thordal-Christensen et al. 1997). Essentially, seedlings were immersed into lactophenol Trypan Blue work solution (10 mg Trypan Blue, 10 g phenol, 10 mL lactic acid, 10 mL glycerol and 10 ml water, diluted 1:1 in ethanol to make work solution). After boiling for 3 min, staining solution was removed and samples were then de-stained using 2 mL chloralhydrate solution (2.5 g/mL chloralhydrate) with gentle shaking for 2 h. Samples were further de-stained, overnight, with new chloralhydrate solution before examination with a light microscope (Olympus Stereo Microscope SZX 10).

Purification of *E. coli*-expressed His-tagged BAK1 kinase domain

Constructs expressing the BAK1 kinase domain (BAK1-KD) and SOBIR7-1 kinase domain (SOBIR7-1-KD) were indivisually transformed into E. coli strain BL21 (New England BioLabs). The transformed *E. coli* strains were grown in 500 mL liquid LB to reach an approximate density of OD600 = 0.4, after which expression of the transgene was induced by 0.4 mM IPTG in 17℃ for 16 h. Following induction, bacteria were harvested by centrifugation and then re-suspended in lysis buffer, consisting of 25 mM Tris-HCl (pH 8.0) and 150 mM NaCl and 1 mM PMSF. Bacterial cells were then lysed by sonication, after which the resulting cell lysate was filtered through Ni-NTA affinity resin (QIAGEN N.V.). The Ni-NTA matrix was then thoroughly rinsed with wash buffer (25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 15 mM imidazole) before His-tagged protein was eluted with 2 mL

elution buffer (25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 250 mM imidazole). Eluted protein was subjected to dialysis prior to storage in a -80 ℃ freezer.

In subsequent immunoblotting assays to determine the autophosphorylation status of BAK1-KD and SOBIR7-1-KD, approximately 1 μg protein was loaded during gel electrophoresis. Anti-phospho-Ser/Thr antibody (BD Transduction Laboratories™) was used to probe the Western blot membrane to determine the autophosphorylation level of BAK1-KD and SOBIR7-1-KD.

REFERENCES:

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Liu Y, Huang X, Li M, He P, Zhang Y (2016) Loss-of-function of *Arabidopsis* receptor-like kinase BIR1 activates cell death and defense responses mediated by BAK1 and SOBIR1. **New Phytol** 212: 637–645

ThordalChristensen H, Zhang ZG, Wei YD, Collinge DB (1997) Subcellular localization of H2O2 in plants. H2O2 accumulation in papillae and hypersensitive response during the barley-powdery mildew interaction. **Plant J** 11: 1187–1194

Wu FH, Shen SC, Lee LY, Lee SH, Chan MT, Lin CS (2009) Tape-*Arabidopsis* Sandwich - a simpler *Arabidopsis* protoplast isolation method. **Plant Methods** 5: 16

Figure S1. Morphology of a F1 plant generated by crossing *bir1-1 pad4-1* with *sobir7-1 bir1-1 pad4-1*

Image of five-week-old soil-grown *Arabidopsis* plants. A representative of twelve F1 plants is shown.

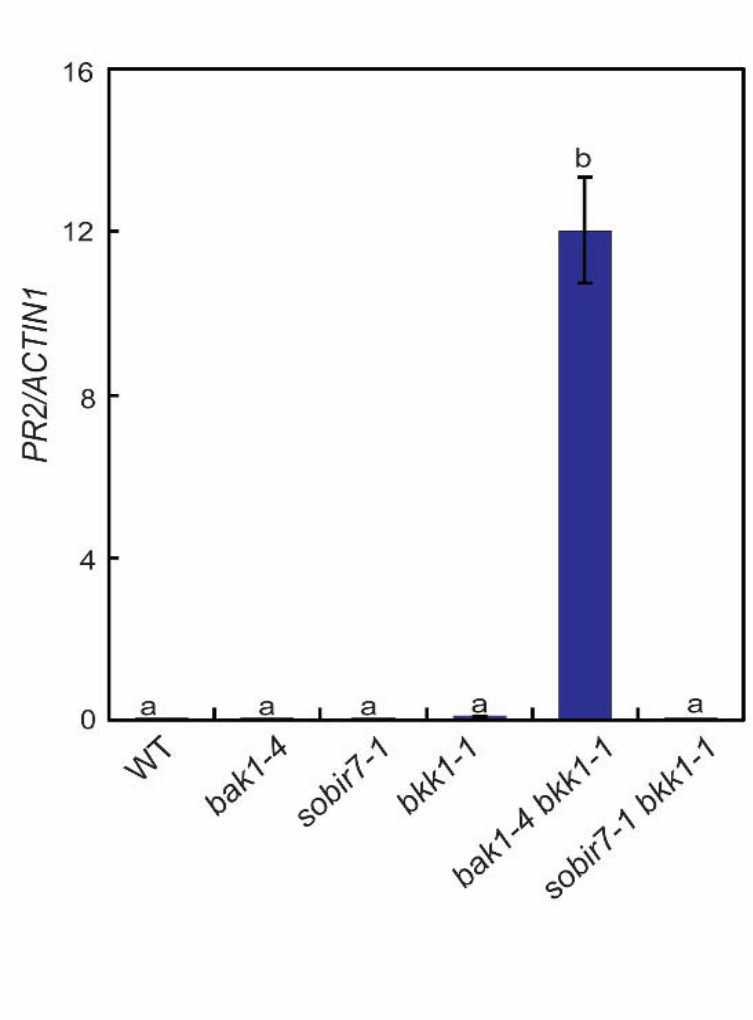

Figure S2. *PR2* expression in wild-type (WT), *bak1-4, sobir7-1, bkk1-1, bak1-4 bkk1-1* and *sobir7-1 bkk1-1* plants

Different letters indicate statistically significant difference ($p < 0.05$).

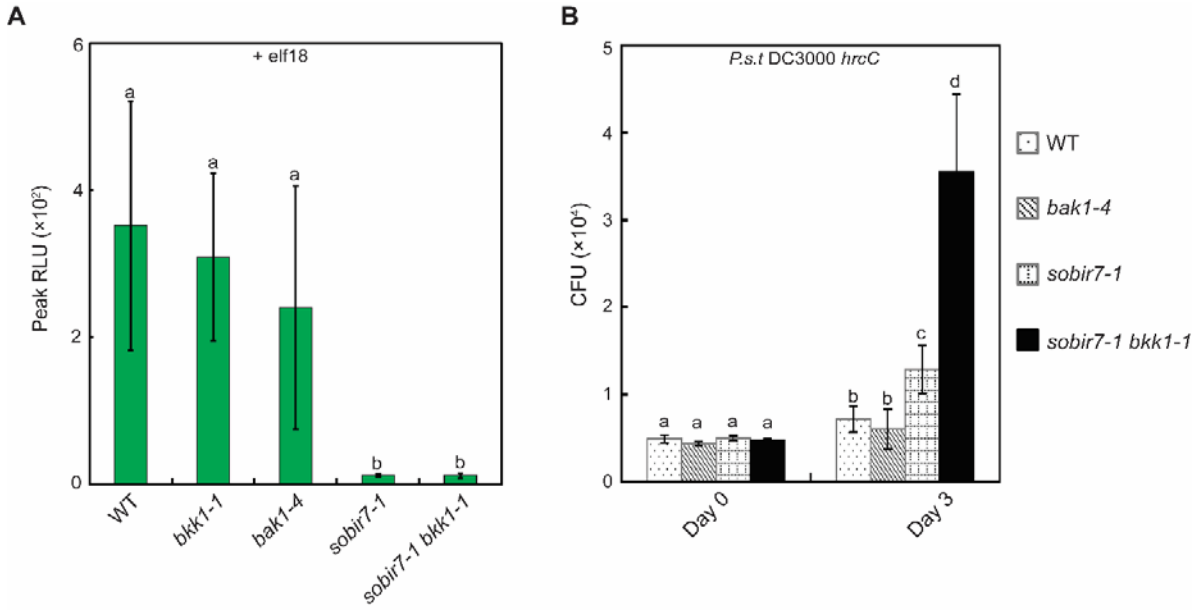

Figure S3. The *sobir7-1* single and *sobir7-1 bkk1-1* double mutant are defective in PTI responses

(**A**) elf18-triggered ROS production in *sobir7-1* and *sobir7-1 bkk1-1* mutants.

(**B**) *P.s.t.* DC3000 *hrcC* growth on *sobir7-1* and *sobir7-1 bkk1-1* mutants.

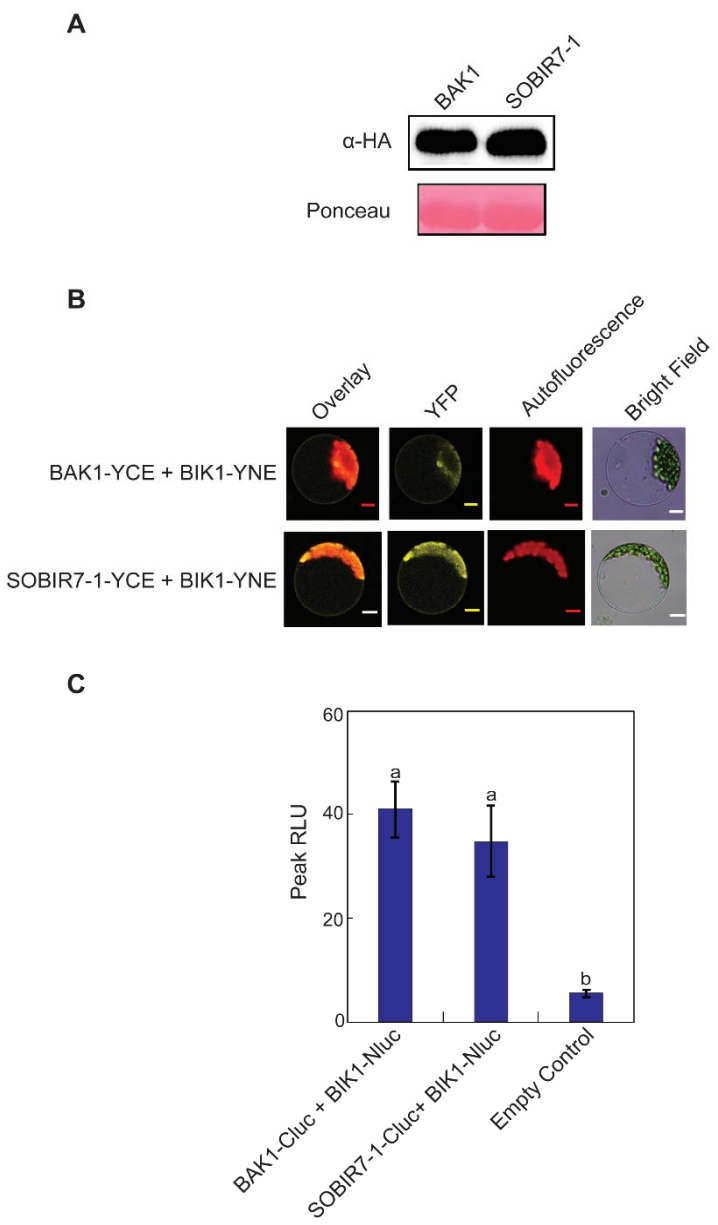

Figure S4. Analysis of the accumulation of BAK1 and SOBIR1-7 and their interactions with BIK1

(**A**) Immunoblotting with anti-HA antibody to examine the protein accumulation of HA-tagged BAK1 and SOBIR7-1 transiently expressed in *N. benthamiana*. Expression of both *BAK1-HA* and *sobir7-1-HA* was under the control of a *35S* promoter.

(**B**) Biomolecular fluorescence complementation assay showing the interaction between BAK1 or SOBIR7-1 with BIK1. Scale bars = 10 µm.

(**C**) Split luciferase assay showing the quantitative results of the interaction between BAK1 or SOBIR7-1 with BIK1. Different letters indicate statistically significant difference ($p < 0.05$).

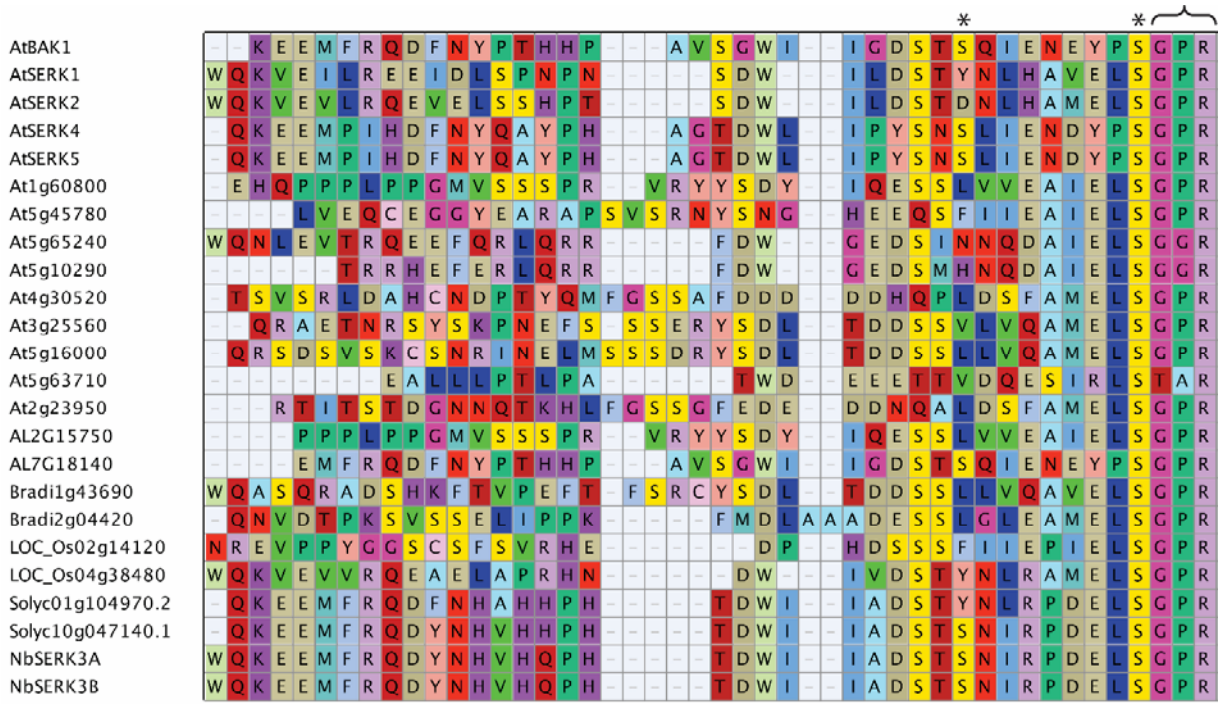

Figure S5. Multiple alignment of the CT sequence from BAK1 and its homologs

Bracket represents the conserved "GPR" motif. Asterisks indicate the two previously identified Serine auto-phosphorylation sites of the BAK1 intracellular domain. Left column lists gene name of BAK1 homologs in *Arabidopsis* and other plant species. Specifically, "At" represents *Arabidopsis thaliana*; "AL" is *Arabidopsis lyrata*; "Bradi" is *Brachypodium distachyon*; "Os" is *Oryza sativa* (rice); "Soly" is *Solanum lycopersicum* (tomato); "Nb" is *Nicotiana benthamiana* (tobacco).

Table S1. Primers used in this study

Primer name	Sequence (5' to 3')	Function
BAK1-pG229HAN-Kpn1-F	CGGGGTACCATGGAACGAAGATTAATGATCCCTTG	Cloning pCambia1300-35S-BAK1-3×HA and pCambia1300-35S-SOBIR7-1-3×HA, pairing with BAK1-StuI-R and tBAK1-StuI-R, respectively
BAK1-StuI-R	CGCGGATCCTTATCTTGGACCCGAGGGGTATTC	Cloning pCambia1300-35S-BAK1-3×HA
tBAK1-StuI-R	GAAGGCCTGCCAGACACGGCTGGATG	Cloning pCambia1300-35S-SOBIR7-1-3×HA
BAK1-BiFC-BamHI-XhoI-F	CGGGGATCCCTCGAGATGGAACGAAGATTAATGATCC	Cloning BAK1-pUC19-YCE and SOBIR7-1-pUC19-YCE, pairing with BAK1-BiFC-XhoI-R and Bak1W644KpniR, respectively
BAK1-BiFC-XhoI-R	CGGCGGCTCGAGTCTTGGACCCGAGGGGTATTCG	Cloning BAK1-pUC19-YCE
Bak1W644KpniR	CGGGGTACCGCCAGACACGGCTGGATG	Cloning SOBIR7-1-pUC19-YCE
Bak1-NdeI-F	GGAATTCCATATGGGACAACTGAAGAGGTTTTCATTG	Cloning BAK1-KD-pET24c and SOBIR7-1-KD-pET24c, pairing with BAK1-BiFC-XhoI-R and tBAK1-XhoI-R, respectively
BAK1-BiFC-XhoI-R	CGGCGGCTCGAGTCTTGGACCCGAGGGGTATTCG	Cloning for BAK1-KD-pET24c
tBAK1-XhoI-R	CGGCGGCTCGAGGCCAGACACGGCTGGATG	Cloning for SOBIR7-1-KD-pET24c
WtSobir7_1F2	TCCAGCCGTGTCTGGCAGG	Genotyping for *sobir7-1* SNP, pairing with BAK1-s1_F
BAK1_s1_F	CATGAATCTTCTAGGCTACTATG	Genotyping for *sobir7-1* SNP, pairing with WtSobir7_F2
Bak1-4-F	GGCCACTAAAGTACCATCAG	Genotyping for *bak1-4* T-DNA homozygote, pairing with Bak1-4-R
Bak1-4-R	CCTCTCACCGGAGATATTCCT	Genotyping for *bak1-4* T-DNA homozygote, pairing with Bak1-4-F
P745	AACGTCCGCAATGTGTTATTAAGTTGTC	Genotyping for *bir1-1* T-DNA presence, paring with BIR1-179-F
BIR1-179-F	AGAACGCAGTTGCATGCTAC	Genotyping for *bir1-1* T-DNA presence, pairing with P745

BKK1-1-NF	CCAGCCATTGCGTTTGCTTG	Genotyping for *bkk1-1* T-DNA homozygote, pairing with BKK1-1-NR
BKK1-1-NR	GCGTACAGCAGTTGTCACA	Genotyping for *bkk1-1* T-DNA homozygote, pairing with BKK1-1-NF
pad4-1-MT-F	GCATAAGACTAGCTAAGTTTCA	Genotyping for *pad4-1* presence, paring with pad4-1-R
pad4-1-R	AAGTCTCCATTGCGTCACTC	Genotyping for *pad4-1* presence, paring with pad4-1-MT-F
Lba1	TGGTTCACGTAGTGGGCCATCG	T-DNA Genotyping