

Extracellular leucine-rich repeats as a platform for receptor/coreceptor complex formation

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Receptor kinases with leucine-rich repeat (LRR) extracellular domains form the largest family of receptors in plants. In the few cases for which there is mechanistic information, ligand binding in the extracellular domain often triggers the recruitment of a LRR-coreceptor kinase. The current model proposes that this recruitment is mediated by their respective kinase domains. Here, we show that the extracellular LRR domain of BRI1-ASSOCIATED KINASE1 (BAK1), a coreceptor involved in the disparate processes of cell surface steroid signaling and immunity in plants, is critical for its association with specific ligand-binding LRR-containing receptors. The LRRs of BAK1 thus serve as a platform for the molecular assembly of signal-competent receptors. We propose that this mechanism represents a paradigm for LRR receptor activation in plants.

brassinosteroid signaling | flagellin signaling | plant innate immunity | Receptor-like kinase | signaling crosstalk

Leucine-rich repeat receptor kinases (LRR-RKs) form the largest family of receptors in plants (1). LRR-RKs bind a wide range of ligands, including small molecule hormones and peptides, and are involved in a variety of developmental and immune signaling processes (2, 3). In *Arabidopsis*, BAK1 (BRI1-ASSOCIATED KINASE1) is an LRR coreceptor kinase for several LRR-RKs, including the brassinosteroid (BR) receptor BRI1 (BRASSINOSTEROID-INSENSITIVE 1) and the flagellin receptor FLS2 (FLAGELLIN-SENSING 2) that are involved in growth and immune responses, respectively (3–5). Ligand perception at the cell surface by either BRI1 or FLS2 induces the subsequent recruitment of BAK1 to a ligand-bound receptor complex (6–10). This process triggers transphosphorylation at multiple serines and threonines of the respective kinase domains inside the cell (11–13). Perhaps because BRI1 is a long-lived protein that apparently cycles between the plasma membrane and endosomes (14), there are multiple mechanisms to maintain the kinase domain in a basal state. BRI1 kinase is auto-inhibited by its C-terminal tail (15), by auto-phosphorylation on threonine 872 (11), and by a protein, BRI1 KINASE INHIBITOR 1 (BKI1), which associates with BRI1's kinase domain (10, 16). BKI1 inhibits BR signaling by binding to the BRI1's kinase domain, thereby inhibiting the interaction between BRI1- and BAK1-kinase (10, 16). Upon ligand binding, BRI1 phosphorylates BKI1 on a tyrosine within its membrane-targeting region, which dissociates BKI1 from the cell membrane and targets it to the cytoplasm, where it is inactive (10). Dissociation of BKI1 from BRI1 allows formation of a stable BRI1-BAK1 complex that is competent to induce downstream signaling (17).

The interplay between BRI1 and BAK1 kinase domains is further regulated by BAK1 autophosphorylation on tyrosine 610 (tyr-610), which is required to stimulate BRI1 kinase activity in vitro and for proper BR signaling in vivo (18). Of note, BAK1 tyr-610 phosphorylation is not required for flagellin response and it is possible that tyr-610 phosphorylation might be involved in the proper interaction with its cognate receptors. However, tyr-610 mutations affect only BRI1 kinase activation but not its interaction with BRI1 intracellular domain (18). Therefore, a critical unanswered question is how ligand-bound LRR-RKs selectively recruit

BAK1. Here, we report that the LRR domain of BAK1 is required for its recruitment to a ligand-bound LRR-RK and allows the kinase domains to be in physical contact for subsequent reciprocal transphosphorylation. Furthermore, our data indicate that the extracellular domain (ECD) of BAK1 is critical for the high affinity formation of the correct receptor/coreceptor pair.

Results and Discussion

Gain-of-Function Phenotype of *bak1^{elg}* Allele in the Brassinosteroid Pathway. A previously described mutation in *BAK1*, *elg* (*elongated*), was originally identified as a suppressor of the gibberellin biosynthesis mutant, *ga4* (19). The *elg* mutation results in a substitution of an aspartic acid to an asparagine (D122N) in the third LRR of BAK1 (20) (Fig. 1A and Fig. S1). The *elg* mutant is also hypersensitive to exogenous BR treatment (20). We found that both *elg* and transgenic lines of a null *bak1* mutant (*bak1-3*) (9), expressing *bak1^{elg}* fused with mCITRINE, a monomeric yellow variant of GFP (*bak1^{elg}::CITRINE*), had slightly longer hypocotyls in the dark compared with control plants (Fig. 1B and Fig. S1). Cell elongation in etiolated seedlings is BRI1-dependent (4). Importantly, in the presence of brassinazole (BRZ), an inhibitor of BR biosynthesis, both *elg* and *bak1-3* transgenic plants expressing a *bak1^{elg}::CITRINE* fusion protein still displayed partially elongated hypocotyls compared with controls (Fig. 1B and Fig. S1). These phenotypes were not explained by differential protein accumulation (Fig. 1D). Moreover, when grown in the light, both *elg* and the *bak1^{elg}::CITRINE*-expressing *bak1-3* transgenic plants exhibited long twisted petioles and elongated leaf blades (Fig. 1C and Fig. S1), and a rosette phenotype reminiscent of plants either overexpressing BRI1 or treated exogenously with BR (21).

We asked whether *bak1^{elg}::CITRINE* growth promotion is BRI1-dependent. We introgressed both *bak1^{elg}::CITRINE* and a complementing *BAK1::CITRINE* transgene into a *bri1*-null mutant. Both *BAK1::CITRINE* and *bak1^{elg}::CITRINE* failed to induce hypocotyl and petiole elongation in *bri1* plants (Fig. 1B and C). Finally, we checked the phosphorylation status of the BRI1-EMS-SUPPRESSOR 1 (BES1) transcription factor in *BAK1::CITRINE* and *bak1^{elg}::CITRINE* expressing *bak1-3* transgenic plants (Fig. 1E). BES1 phosphorylation is a readout for BR activity, as phosphorylated BES1 (P-BES1) is a mark of low BR signaling and dephosphorylated BES1 is indicative of active BR signaling (22). We found that *bak1^{elg}::CITRINE* but not

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Fig. 1. Gain-of-function phenotype of *bak1^{elg}* allele for the brassinosteroid signaling pathway. (A) Schematic representation of BAK1 with its extracellular LRR domain in red and intracellular kinase domain in blue. TM: transmembrane segment. The position of the *elg* (D122N) mutation in BAK1 (LRR3) is indicated. (B) *BAK1prom::BAK1::CITRINE* expression complements the *bak1-3* hypocotyl growth defect. *BAK1prom::bak1^{elg}::CITRINE* expression in *bak1-3* leads to an elongated hypocotyl phenotype in the dark that is BRI1-dependent. Note that *BAK1prom::bak1^{elg}::CITRINE*-expressing hypocotyls still elongate when BR ligand is partially depleted by 1 μ M brassinazole, BRZ. Hypocotyl length is in mm \pm SD ($n = 25$), NT/T is the ratio of nontreated (NT) over BRZ-treated (T) hypocotyl length. (C) Pictures of rosette stage transgenic homozygous *Arabidopsis* (T3) expressing *BAK1prom::BAK1::CITRINE* or *BAK1prom::bak1^{elg}::CITRINE* under the control of *BAK1* promoter in the *bak1-3* background. The phenotypes associated with the overexpression of BRI1 (on the right, for comparison), narrow leaf blades, elongated and twisting petioles were recapitulated by driving the expression of the *bak1^{elg}::CITRINE* variant. Mean value of rosette radius is indicated in mm \pm SD ($n = 25$). (D) BAK1::CITRINE accumulates to a similar extent as *bak1^{elg}::CITRINE*. Microsomal protein extracts were prepared from wild-type Col-0, *BAK1prom::BAK1::CITRINE* in *bak1-3* and *BAK1prom::bak1^{elg}::CITRINE* in *bak1-3* plants. These extracts were subjected to an anti-GFP protein immunoblot analysis to detect the accumulation of the CITRINE-tagged proteins. Equal loading was ensured by protein quantification before loading and by Ponceau red staining of the membrane postprotein transfer. (E) BES1

BAK1::CITRINE plants accumulated dephosphorylated BES1 to a similar extent as plants overexpressing BRI1. We conclude that *elg* acts as a gain-of-function mutation that requires BRI1 to promote cell elongation.

Impaired Flagellin Signaling of *bak1^{elg}*. To address the phenotype of *elg* and *bak1^{elg}::CITRINE* plants with respect to innate immune-response signaling, we monitored various readouts that include both early and late responses to *g22* (an elicitor peptide from bacterial agellin) (3). Expression of BAK1::CITRINE, but not *bak1^{elg}::CITRINE*, in the *bak1-3* mutant almost completely rescued the induction of reactive oxygen species triggered by *g22*, one of the earliest readouts for agellin signaling (3) (Fig. 2A). Similarly, BAK1-CITRINE, but not *bak1^{elg}::CITRINE*, rescued the *bak1* phenotype with respect to loss of fresh weight and callose deposition triggered by *g22* late readouts of agellin signaling (Fig. 2 B and C). The *elg* mutant was also insensitive to *g22* treatments with respect to loss of fresh weight and callose deposition (Fig. S1 D and E). Additionally, *bak1^{elg}::CITRINE bak1-3* plants did not exhibit protection from *Pseudomonas syringae* pv. *tomato* (*Pto*) DC3000 infection, which is normally induced in wild-type by cotreatment with *g22* (23) (Fig. 2D). Together, these results suggest that both early and late responses to agellin are impaired by a single amino acid substitution in the ECD of BAK1. Importantly, *bak1^{elg}::CITRINE* selectively affected innate immune responses triggered by various MAMPs (microbe-associated molecular patterns) (Fig. S2). Together, our results indicate that the *bak1^{elg}* protein behaves differently with respect to BR signaling (gain-of-function) and agellin responsiveness (loss-of-function).

D122N Substitution in BAK1's ECD Modifies its Interaction with Both BRI1 and FLS2 LRR-RKs. Next, we addressed the mechanism by which the *bak1^{elg}* protein induces BR signaling and blocks agellin response. Control experiments showed that *bak1^{elg}::CITRINE* accumulates to similar levels as BAK1::CITRINE (Fig. 1D) and had a similar subcellular localization (Fig. S3A). In addition, the *elg* mutant had normal accumulation of BRI1 (Fig. S1), and expression of *bak1^{elg}::CITRINE* did not alter the accumulation of BRI1::CITRINE (Fig. 3A) or FLS2::GFP (Fig. 3B). Importantly, *bak1^{elg}::CITRINE* did not modify BRI1::mCITRINE or FLS2-GFP subcellular localization (Fig. S3 B and C). Therefore, we hypothesized that the phenotypes ascribed to *bak1^{elg}* in Fig. 1 are the result of alterations in the interaction between *bak1^{elg}* and either BRI1 or FLS2.

Both BAK1::CHERRY and *bak1^{elg}::CHERRY* coimmunoprecipitated with BRI1::CITRINE in the absence of the brassinosteroid biosynthesis inhibitor, BRZ (Fig. 3A). In contrast, only *bak1^{elg}::CHERRY* coimmunoprecipitated with BRI1::CITRINE in the presence of BRZ (Fig. 3A). As described previously, *g22* treatment induced the recruitment of wild-type BAK1 to FLS2 (8, 9) (Fig. 3B). However, *bak1^{elg}::6xHA* did not coimmunoprecipitate with FLS2::GFP under these conditions (Fig. 3B). We could immunoprecipitate only a fraction of BAK1 with FLS2 after *g22* treatment; therefore, we cannot exclude the possibility that BAK1^{elg} can still bind to FLS2, albeit more weakly than wild-type BAK1. Taken together, our results indicate that the *bak1^{elg}* variant interacts with BRI1, even when the BR concentration is very low, whereas its ligand-induced interaction with FLS2 is impaired. These differences in affinity likely explain the opposite gain- and loss-of-function phenotypes in BR and agellin signaling, respectively.

phosphorylation in *BAK1::CITRINE/bak1-3*, *BAK1-bak1^{elg}::CITRINE/bak1-3* and *OxBRI1* lines. P-BES is phosphorylated BES1. Equal loading was ensured by protein quantification before loading and by the signal intensity of a nonspecific band.

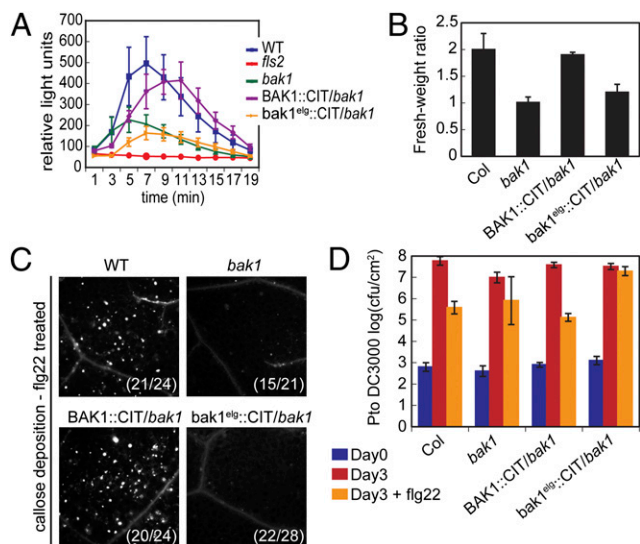


Fig. 2. *bak1^{elg}* has impaired flagellin response. (A) Oxidative burst triggered by 100 nM flg22 in wild-type Col-0 (blue), *fls2* (red), *bak1-3* (green), *BAK1prom:BAK1::CITRINE* in *bak1-3* (purple), and *BAK1prom:bak1^{elg}::CITRINE* in *bak1-3* (orange) leaf discs measured in relative light units (RLU). Results are mean \pm SD ($n = 24$). (B) Average fresh-weight ratio of 14-d-old seedlings grown for 7 d in either water or water plus 1 μ M flg22. The bar graph represents the average fresh-weight ratio from wild-type Col-0, *bak1-3* mutant, *BAK1prom:BAK1::CITRINE* in *bak1-3*, and *BAK1prom:bak1^{elg}::CITRINE* in *bak1-3*. Means and SDs were calculated from 48 seedlings (six random pools of eight seedlings). (C) Callose deposits stained with aniline blue from leaves of wild type Col-0, *bak1-3*, *BAK1prom:BAK1::CITRINE* in *bak1-3* and *BAK1prom:bak1^{elg}::CITRINE* in *bak1-3* treated with 1 μ M flg22. The number of leaves showing the displayed features over the total in a given genotype is indicated in parentheses. (D) Growth of *Pseudomonas syringae* pv. *tomato* (Pto DC3000) was measured on the genetic backgrounds indicated at bottom. Leaves from 4-wk-old plants were infiltrated with a bacterial inoculum of 10^5 cfu mL⁻¹ in the presence (orange) or absence (red) of 1 μ M flg22 peptide. The number of bacteria per square centimeter of leaf was plotted on a log₁₀ scale. Error bars represent two times the SE among four internal replicate samples from one of three experiments.

BAK1 Kinase Activity Is Not Required for *bak1^{elg}* Association with BRI1. Previous reports indicated that the isolated kinase domains of BRI1 and BAK1 interact directly *in vitro* and in yeast (6, 7, 16, 18). It was therefore unexpected that the *bak1^{elg}* ECD mutation modified its interaction with both BRI1 and FLS2. One simple explanation for this could be that the LRRs of BAK1 interact directly with LRRs of BRI1 and *bak1^{elg}* enhances that interaction. Alternatively, *bak1^{elg}* may indirectly activate BAK1 kinase activity, thus enhancing the binding affinity between the two kinase domains. To explore these possibilities, we took advantage of the fact that strong overexpression of kinase-dead BAK1 leads to a dwarf phenotype because of impaired BR signaling (7). This phenotype is likely caused by a dominant-negative effect of the kinase-dead BAK1 on BRI1 kinase activity. In contrast, expression of a BAK1 kinase-dead mutant (D434N) under the control of its own promoter in wild-type plants did not induce a dwarf phenotype, probably because at this lower expression level, *bak1^{D434N}* is unable to compete with endogenous BAK1 to inhibit BRI1 activity (Fig. 4A). We reasoned that if *bak1^{elg}* activates its own kinase activity, then a double-mutant *bak1^{elg} D434N* would suppress any effect of the *elg* mutation. Alternatively, if the enhanced *bak1^{elg}* interaction with BRI1 is mediated by their respective ECDs, then *bak1^{elg} D434N* would bring the catalytically dead BAK1 kinase domain into proximity with the BRI1 kinase domain potentially enhancing any intrinsic dominant-negative effect on BRI1 activity, even at native *bak1^{elg} D434N* expression levels. In fact, we found that at similar expression levels, *bak1^{elg} D434N::CITRINE* but not

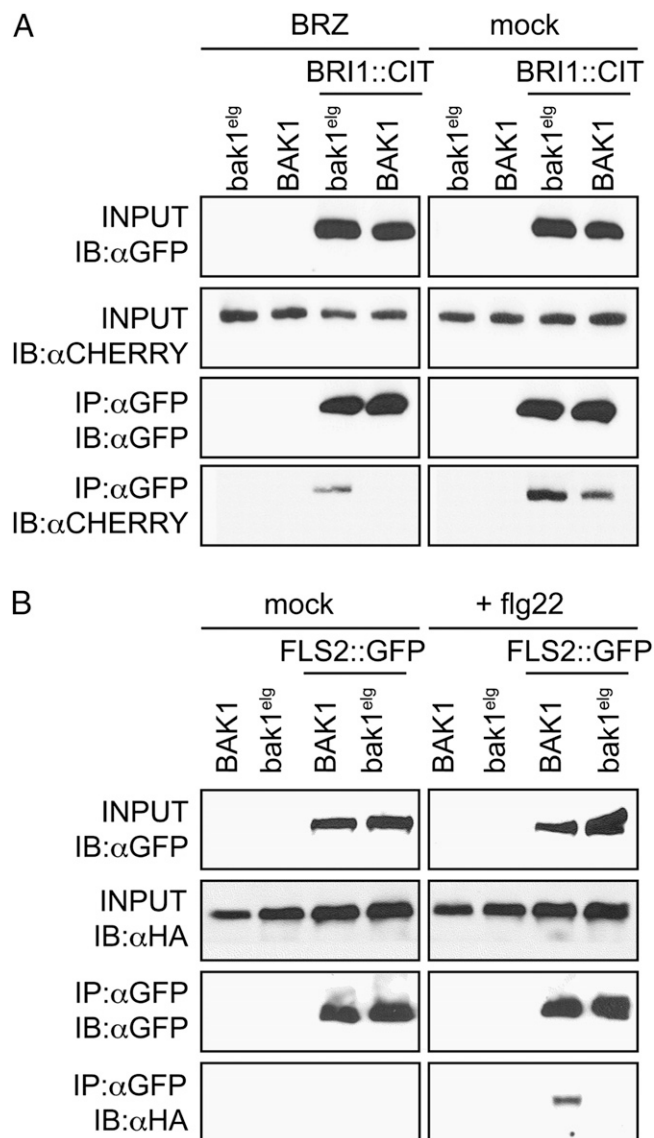


Fig. 3. A mutation in the extracellular LRR domain of BAK1 modifies its interaction with BRI1 and FLS2. (A) Transgenic *Arabidopsis* plants expressing either *BAK1prom:BAK1::CHERRY* or *BAK1prom:bak1^{elg}::CHERRY* alone or with *BRI1prom:BRI1::CITRINE* were grown with or without the BR biosynthesis inhibitor BRZ (5 μ M added from sowing of seeds). Total membrane protein was immunoprecipitated (IP) with anti-GFP antibodies and subjected to immunoblot (IB) analysis, as indicated. (B) Transgenic plants expressing either *BAK1prom:BAK1::6xHA* or *BAK1prom:bak1^{elg}::6xHA* alone or with *FLS2prom:FLS2::GFP* were grown on 1/2 LS media and treated 5 min before protein extraction with 10 μ M flg22. Total membrane protein was immunoprecipitated (IP) with anti-GFP antibodies and subjected to immunoblot (IB) analysis as indicated.

bak1^{D434N}::CITRINE resulted in a very strong dominant-negative phenotype; the plants were compact dwarfs that resembled mild to strong *bri1* mutants (Fig. 4A and B and Fig. S4). These results suggest that BAK1 is likely to interact with BRI1 through both its extracellular LRR domain, as well as its intracellular kinase domain, and that the *bak1^{elg}* mutation enhances this interaction.

BRI1 Receptor Complex Formation Involves a "Double-Lock" Mechanism. In conclusion, our study has identified a key role for the LRR ECD of the coreceptor BAK1 during recruitment to its receptors, BRI1 and FLS2. We propose a scenario in which LRR-containing coreceptors are recruited to their activated receptors

Confocal Microscopy, Hormone, and Inhibitor Treatments. Microscopy and drug treatments were performed as described previously (27). Confocal microscopy was performed with a Leica SP2 inverted microscope and image analysis was done as described previously (28). BRZ (Chemiclones; 10 mM stock in DMSO) was used at the indicated concentration and was supplemented into the agar medium from the onset of germination.

Protein Extraction from Plants and Immunoprecipitation. Monoclonal anti-GFP HRP-coupled (Miltenyi Biotec), anti-HA-HRP coupled (Miltenyi Biotec), anti-ACTIN (clone C4; MP Biomedicals), and polyclonal anti-CHERRY (DsRed polyclonal; Clontech) were used at 1:5,000. Polyclonal anti-BRI1 [raised against BRI1 C terminus in rabbit] (26)] was used at 1:1,000. Flg22 treatment before protein extraction was done in liquid medium (0.5× Linsmaier and Skoog medium) for 5 min under vacuum. The immunoprecipitation extraction buffer was supplemented with 10 μ M flg22; the mock condition corresponds to addition of the same volume of water. Similarly, BRZ was supplied in the immunoprecipitation extraction buffer at a concentration of 5 μ M in the BRZ-treated condition; the mock condition corresponds to the addition of the same volume of DMSO (BRZ solvent). All immunoprecipitations were performed as previously described (28). Approximately 100 mg of 14-d-old light-grown seedlings were harvested for Western blot experiments. Immunoprecipitation experiments required from 1 to 3 g of seedlings (14-d-old). Tissues were ground at 4 °C in a 15-mL tube containing 2-mL of ice-cold sucrose buffer [20 mM Tris, pH 8; 0.33M Sucrose; 1 mM EDTA, pH 8; protease inhibitor (Roche)] using a polytron (Brinkman). Samples were centrifuged for 10 min at 5,000 \times g at 4 °C or until the supernatants were clear. This total protein fractions were centrifuged at 4 °C for 45 min at 20,000 \times g to pellet microsomes. The pellet was resuspended in 1 mL of immunoprecipitation buffer (50 mM Tris pH 8, 150 mM NaCl, 1% Triton X-100) using a 2-mL potter-Elvehjem homogenizer (Wheaton) and left on a rotating wheel for 30 min at 4 °C. Samples were then pelleted for 10 min at 20,000 \times g and 4 °C. The supernatant corresponded to the fraction enriched in microsomal associated proteins. The proteins were quantified and immunoprecipitates were performed on 1 mg of microsomal proteins. Each experiment was repeated at least three times and showed consistent results.

MAMP Response Assays. Flg22 (QRLSTGSRINSKDDAAGLQIA) and elf18 (acetyl-MSKEKFERTKPHVNVGTI) peptides were synthesized at >95% purity by ezbiolab and dissolved to a 10-mM stock in water. A pectidoglycan (Sigma-Aldrich) stock solution was prepared at 10 mg/mL in water. A 10 mg/mL chitin from shrimp shell (Sigma-Aldrich) stock solution was prepared as follows. Chitin powder was suspended in sterile PBS and sonicated at 25% output

power three times for 5 min with a sonicator. The suspension was then filtered with 100-, 70-, and 40- μ m sterile cell strainers. Following centrifugation (2,800 \times g, 10 min), chitin fragments from the 40- to 70- μ m fraction were suspended in the desired volume of sterile PBS and autoclaved. Oxidative burst assays were performed as described previously (9, 23), except that luminescence was measured using a Tecan Sapphire plate reader. Loss of fresh-weight ratio was calculated on 14-d-old seedlings grown for 7 d in either water or 1 μ M flg22 ($n = 48$, six random pools of eight seedlings). For callose deposition assays, 14-d-old plants were completely submerged in individual 0.5-mL Eppendorf tube containing the elicitor at the indicated concentration. A vacuum was applied for 15 min and plants remained in the elicitor solution for another 16 h. Next, seedlings were fixed in a 3:1 ethanol:acetic acid solution for several hours. Seedlings were rehydrated in 70% ethanol for 2 h, 50% ethanol for an additional 2 h, and then with water overnight. Seedlings were then incubated in 150 mM K_2HPO_4 , pH 9.5, and 0.01% Aniline blue (Sigma-Aldrich) for several hours. Individual leaves were mounted on slides in 50% glycerol, and callose was observed immediately using a Leica DM5000B under UV (excitation, 390 nm; emission, 460 nm). Bacterial assays were performed as described earlier (23, 29) except that bacterial count were assayed at 3 d postinfection. Each experiment was repeated at least three times and showed consistent results.

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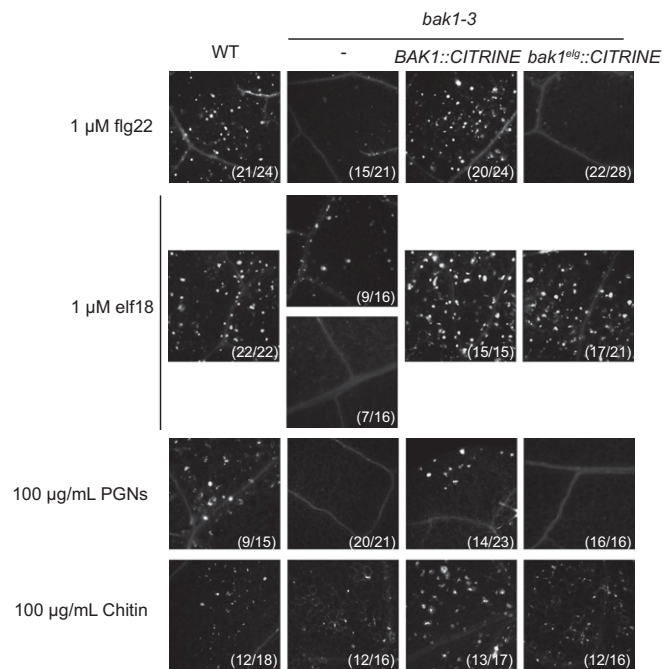


Fig. S2. *bak1^{elg}* selectively eliminates flg22- and peptidoglycan- (PGN) but not elf18-induced callose deposition. The first line of active defense relies on the recognition of microbe-associated molecular patterns (MAMPs) by pattern recognition receptors (PRRs) (1). Among these responses to MAMPs, some are BAK1-dependent and others are BAK1-independent. To test whether *bak1^{elg}* affects only flg22 response, all aspect of BAK1-dependent immunity or MAMP-triggered Immunity (MTI) in general, we tested several MAMPs known to be either BAK1-dependent or -independent. In *Brassicaceae*, a peptide corresponding to the *N*-acetylated N-terminal 18 amino acids of bacterial EF-Tu (elf18) is recognized by a receptor called EFR (for EF-Tu Receptor) and triggers MAMP-triggered Immunity (1). Like FLS2, EFR function is partially dependent on BAK1 (2). PGNs are a major cell-wall component of Gram-positive bacteria and are recognized as a MAMP in *Arabidopsis*. The receptor for PGNs is unknown but this response is BAK1-dependent (1). Finally, chitin, an important component of the cell wall of fungi, is also recognized as a MAMP in *Arabidopsis*. Interestingly, the plant chitin receptor RLK1/CERK1 is a LysM receptor-like Kinase and do not have LRR in its extracellular domain (3, 4). This finding is consistent with the observation that chitin response is BAK1-independent. Callose deposits were stained with aniline blue in the leaves of wild-type *Col-0*, *bak1-3*, *BAK1prom:BAK1::CITRINE* in *bak1-3*, *BAK1prom:bak1^{elg}::CITRINE* in *bak1-3* seedlings treated with 1 μM flg22, 1 μM elf18, 100 μg/mL of PGNs, or 100 μg/mL of chitin for 16 h. The fraction of leaf showing the displayed features is shown in parenthesis. Note that elf18-induced callose deposition was extremely robust in wild-type *Col-0* and was not completely abolished in about half of *bak1-3* plant analyzed. We saw this partial response when looking at well-emerged true leaves but not cotyledons. In contrast, PGNs and chitin-induced callose deposition was not observed in all of the wild-type *Col-0* leaves observed. Nevertheless, the PGN- but not chitin-induced callose deposition was clearly reduced in *bak1-3* and *BAK1prom:bak1^{elg}::CITRINE* in *bak1-3*. These results indicate that *bak1^{elg}* selectively affected innate immune responses triggered by various MAMPs, it behaves as a loss-of-function with respect to flg22/FLS2 and PNG responses, but it is neutral for elf18/EFR function.

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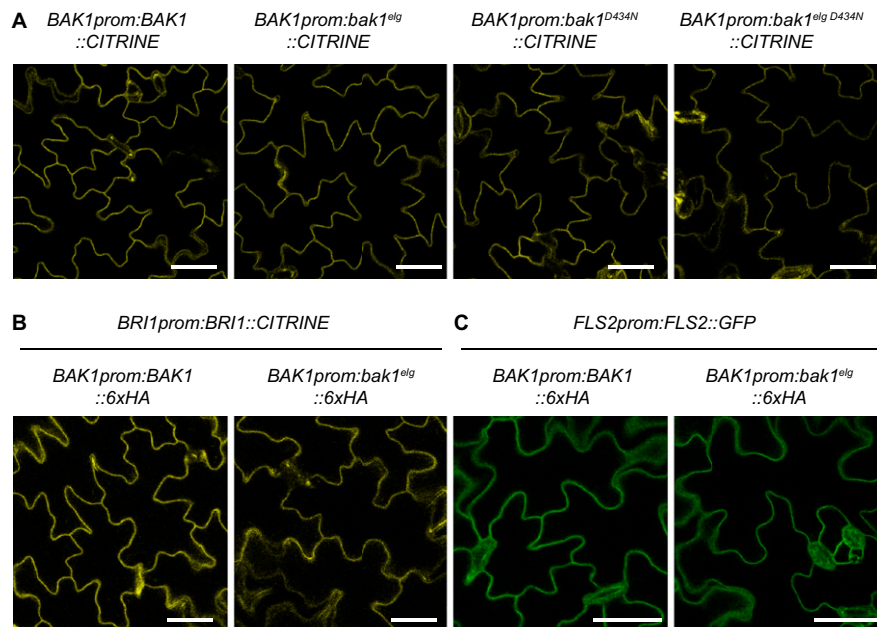


Fig. S3. Subcellular localization of the different BAK1 mutants and effect of $bak1^{elg}$ on BRI1/FLS2 subcellular localization. (A) Representative confocal pictures of the cotyledon of $BAK1prom:BAK1::CITRINE$, $BAK1prom:bak1^{elg}::CITRINE$, $BAK1prom:bak1^{D434N}::CITRINE$, and $BAK1prom:bak1^{elg D434N}::CITRINE$ T3 homozygous lines. Identical confocal settings were used for each of the picture shown. (B) Representative confocal pictures of cotyledon of $BRI1prom:BRI1::CITRINE$ in $BAK1prom:BAK1::6xHA$ and $BAK1prom:bak1^{elg}::6xHA$ expressing lines, respectively. The same confocal settings were used for both pictures. (C) Representative confocal pictures of cotyledon of $FLS2prom:FLS2::GFP$ in $BAK1prom:BAK1::6xHA$ and $BAK1prom:bak1^{elg}::6xHA$ expressing lines, respectively. The same confocal settings were used for both pictures. (Scale bars, 20 μm .)

T1			
	Wild type	Dwarf	Severe dwarf
$BAK1prom:BAK1^{D434N}::CITRINE$	189	16	0
$BAK1prom:bak1^{elg D434N}::CITRINE$	116	61	24

Fig. S4. Quantification of T1 phenotype of $BAK1prom:bak1^{D424N}::CITRINE$ and $BAK1prom:bak1^{elg D434N}::CITRINE$. Because some $bak1^{elg D434N}::CITRINE$ expressing T1 plants had a very strong *bri1*-like phenotype and could not set seeds, and some $bak1^{D434N}::CITRINE$ T1 lines showed a mild phenotype, we decided to score the phenotype of individual T1 plants. We divided these phenotypes into three different categories: no obvious phenotypes (wild-type), dwarf, and severe dwarves (plants in the severe dwarf category had a phenotype similar to a strong *bri1* and could not set seeds). We found no $BAK1prom:bak1^{D434N}::CITRINE$ plant in the severe dwarf category; 12% of $BAK1prom:bak1^{elg D434N}::CITRINE$ T1 plants (24 out of 201) were ranked in that category. Furthermore, only 8% of $BAK1prom:bak1^{D434N}::CITRINE$ T1 plants (16 of 205) showed a dwarf phenotype against 30% of $BAK1prom:bak1^{elg D434N}::CITRINE$ T1 plants (60 of 201). Next, we selected plants in T2 that harbored similar expression level. At similar expression, $BAK1prom:bak1^{elg D434N}::CITRINE$ plants showed already a strong phenotype but $BAK1prom:bak1^{D434N}::CITRINE$ were indistinguishable from wild-type (see Fig. 4A of the main text).

