Plant Physiology Preview. Published on February 19, 2019, as DOI:10.1104/pp.18.01503

Title: Proteolytic processing of SERK3/BAK1 regulates plant immunity, development and cell death

Authors: Jinggeng Zhou[1,2*], Ping Wang[3*], Lucas A. N. Claus[4,5], Daniel V. Savatin[4,5], Guangyuan Xu[1], Shujing Wu[3,6], Xiangzong Meng[2], Eugenia Russinova[4,5], Ping He[1], and Libo Shan[3#]

Author Affiliations:

[1]Department of Biochemistry and Biophysics, and Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, TX 77843;

[2]Shanghai Key Laboratory of Plant Molecular Sciences, College of Life Sciences, Shanghai Normal University, Shanghai, China;

[3]Department of Plant Pathology and Microbiology, and Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, TX 77843;

[4]Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Ghent, Belgium;

[5]Center for Plant Systems Biology, VIB, 9052 Ghent, Belgium;

[6]College of Horticulture, Shandong Agricultural University, Tai'an, China

Author contributions:

J.Z., P.W., E.R., P.H., and L.S. designed the research; J.Z., P.W., L.C., D.S., G.X., S.W., and X. M. performed the research; J.Z., P.W., L.C., D.S., G.X., and S.W. analyzed the data; and J.Z., E.R., P.H., and L.S. wrote the paper.*These authors contributed equally to the work.

[#]Corresponding author:

Libo Shan

Institute for Plant Genomics and Biotechnology

Texas A&M University

Phone: 979-845-8818

Fax: 979-862-4790

E-mail: lshan@tamu.edu

Running title: BAK1 cleavage regulates its multiple functions

One-sentence summary:

Arabidopsis BAK1, a co-receptor of multiple receptor-like kinases undergoes proteolytic cleavage process, which is essential for its functions in plant immunity, growth and cell death control.

Abstract

Plants have evolved many receptor-like kinases (RLKs) to sense extrinsic and intrinsic cues. The signaling pathways mediated by multiple leucine-rich repeat (LRR) RLK (LRR-RLK) receptors require ligand-induced receptor-coreceptor heterodimerization and transphosphorylation with BAK1/SERK family LRR-RLKs. Here we reveal an additional layer of regulation of BAK1 via a Ca^{2+}-dependent proteolytic cleavage process that is conserved in *Arabidopsis thaliana*, *Nicotiana benthamiana* and *Saccharomyces cerevisiae* . The proteolytic cleavage of BAK1 is intrinsically regulated in response to developmental cues and immune stimulation. The surface-exposed aspartic acid (D^{287}) residue of BAK1 is critical for its proteolytic cleavage and plays an essential role in BAK1-regulated plant immunity, growth hormone brassinosteroid-mediated responses and cell death containment. $BAK1^{D287A}$ mutation impairs BAK1 phosphorylation on its substrate BIK1, and its plasma membrane (PM) localization. Intriguingly, it aggravates BAK1 overexpression-triggered cell death independent of BIK1, suggesting that maintaining homeostasis of BAK1 through a proteolytic process is crucial to control plant growth and immunity. Our data reveal that in addition to layered transphosphorylation in the receptor complexes, the proteolytic cleavage is an important regulatory process for the proper functions of the shared co-receptor BAK1 in diverse cellular signaling pathways.

Introduction

Plants growing in their natural habitats are at constant risks for various potential attacks, meanwhile maintaining their active growth. To adapt, sessile plants have evolved a large number of cell surface-resident receptor-like kinases (RLKs) to sense extrinsic and intrinsic cues and elicit distinct biological responses (Shiu and Bleecker, 2003). A typical RLK usually contains a variable extracellular domain that perceives either self- or non-self signals on the cell surface, a single transmembrane domain, and an intracellular kinase domain that is important to relay signaling. A large portion of RLKs contains an extracellular leucine-rich repeat (LRR) domain with different number of LRR repeats. For example, the *Arabidopsis thaliana* genome encodes more than 200 LRR-RLKs that regulate a wide range of biological processes from plant growth, development, symbiosis to immunity (Belkhadir et al., 2014). The LRR-RLK BRASSINOSTEROID INSENSITIVE 1 (BRI1) is the receptor of plant hormone brassinosteroids (BRs), important in growth and development (Li and Chory, 1997; Belkhadir et al., 2006). Some LRR-RLKs function as pattern-recognition receptors (PRRs) that recognize microbe-associated molecular patterns (MAMPs) and induce the first line of plant immunity (Macho and Zipfel, 2014; Yu et al., 2017). Arabidopsis PRRs FLAGELLIN-SENSING 2 (FLS2) and ELONGATION FACTOR-TU (EF-Tu) RECEPTOR (EFR) recognize bacterial flagellin and EF-Tu, respectively, and initiate the convergent plant immune signaling (Gomez-Gomez and Boller, 2000; Zipfel et al., 2006; Boller and Felix, 2009).

A group of LRR-RLKs, named SOMATIC EMBRYOGENESIS RECEPTOR KINASES (SERKs) with five members in Arabidopsis, function as shared co-receptors of multiple LRR-RLKs (Liebrand et al., 2014; Ma et al., 2016; He et al., 2018). SERK3, also known as BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1), was originally identified as a BRI1 interacting protein that mediates BR signaling (Li et al., 2002; Nam and Li, 2002). SERK1 and SERK4 were also found to interact with BRI1 and mutation of SERK1, SERK3/BAK1 and SERK4 rendered plants insensitive to BR treatment (Karlova et al., 2006; He et al., 2007; Gou et al., 2012). SERK3/BAK1 was also shown to dimerize with FLS2 upon flagellin perception and plays a key role in flagellin-activated responses (Chinchilla et al., 2007; Heese et al., 2007). SERK3/BAK1 also heterodimerizes with several other PRRs, including EFR and PEP1 RECEPTOR 1 (PEPR1)

that perceives endogenous danger peptide PEPs (Postel et al., 2010; Roux et al., 2011). SERK4, but not SERK1 nor SERK2, performs a function redundant with that of SERK3/BAK1 in mediating PRR signaling (Roux et al., 2011). The crystal structure analysis indicates that BAK1 family RLKs are involved in ligand sensing through contacting the BRI1-BR- or FLS2-flagellin-binding interface respectively, and function as co-receptors (Santiago et al., 2013; Sun et al., 2013; Sun et al., 2013).

Recent studies have revealed additional functions of SERKs as co-receptors of different LRR-RLK receptors in regulating plant development and growth, including PHYTOSULFOKINE (PSK) RECEPTOR 1 (PSKR1) recognizing peptide hormone PSK for root growth (Wang et al., 2015), the ERECTA (ER) family sensing EPIDERMAL PATTERNING FACTORS (EPFs) for cell fate specification in stomatal patterning (Meng et al., 2015), the HAESA (HAE) family perceiving INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) for floral organ abscission (Meng et al., 2016; Santiago et al., 2016), ROOT MERISTEM GROWTH FACTOR (RGF) receptors perceiving peptide hormones RGF for regulating root meristem development (Ou et al., 2016; Song et al., 2016), EXCESS MICROSPOROCYTES1 (EMS1) perceiving TAPETUM DETERMINANT1(TPD1) in controlling anther cell fate determination (Li et al., 2017). In addition, SERK3/BAK1 and SERK4 (also named BAK1-LIKE 1, BKK1) negatively regulate cell death in a BR-independent manner, and the Arabidopsis *bak1serk4* mutant exhibits a seedling lethality and constitutive defense responses (He et al., 2007; Kemmerling et al., 2007). Recent studies suggest the involvement of protein *N*-glycosylation and nucleocytoplasmic trafficking in the cell death control regulated by BAK1 and SERK4 (de Oliveira et al., 2016; Du et al., 2016). Interestingly, excessive expression of BAK1 or its ectodomain could also trigger cell death in Arabidopsis with constitutive activation of defense (Dominguez-Ferreras et al., 2015). Thus, the homeostasis of BAK1 is important for its functions in plant growth and immunity.

A common theme for BAK1-associated RLK complex activation is rapid heterodimerization and transphosphorylation upon the cognate ligand perception (Wang et al., 2008; Perraki et al., 2018). Members of receptor-like cytoplasmic kinases (RLCKs), including BOTRYTIS-INDUCED KINASE 1 (BIK1), associate with multiple RLKs and can be phosphorylated by BAK1 (Lu et al., 2010; Zhang et al., 2010). It has been reported that members of mammalian receptor tyrosine kinases (RTKs) involved in growth regulation, and Toll-like

receptors (TLRs) involved in innate immunity, require proteolytic cleavage for activation (Park et al., 2008; Ancot et al., 2009; Chen and Hung, 2015). Recently, several plant RLKs, including rice LRR-RLK XANTHOMONAS RESISTANCE 21 (XA21) (Park and Ronald, 2012), Arabidopsis LYSM domain containing RLK CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) (Petutschnig et al., 2014), and legume RLK SYMBIOSIS RECEPTOR-LIKE KINASE (SYMRK) (Antolin-Llovera et al., 2014), are shown to undergo proteolytic cleavage. In this study, we report that BAK1 and other SERKs undergo Ca^{2+}-dependent proteolytic cleavage by a conserved protease(s) present in both plants and yeast. Bacteria or MAMP treatment promotes the accumulation of cleaved BAK1, which occurs between the transmembrane domain and ectodomain. Through an extensive mutagenesis screen, we identified the aspartate residue (D^{287}) of BAK1 as an important site for its proteolytic cleavage. The $BAK1^{D287A}$ mutation is impaired in FLS2-mediated immunity, BRI1-mediated BR signaling and cell death control. Our data suggest the proteolytic cleavage of plasma membrane-resident RLKs as a common mechanism in the regulation of intracellular signaling.

Results

BAK1 undergoes Ca^{2+}-dependent proteolytic cleavage

When the carboxyl (C)-terminal hemagglutinin (*HA*)-tagged *BAK1* under the control of the *35S* promoter was expressed in Arabidopsis Col-0 protoplasts, we observed a major protein band with a molecular weight (MW) of ~75 kilodalton (kD) corresponding to the full-length (FL) glycosylated BAK1-HA proteins, along with at least three polypeptide bands with MW ranging from 42 to 50 kD in an immunoblot (Fig. 1A). The truncated BAK1 fragments were also observed when BAK1 was tagged with FLAG (Supplemental Figure S1A) or GFP (Fig. S1B) at its C-terminus and expressed in protoplasts, and when *BAK1* was expressed in transgenic Arabidopsis plants under the control of either the *35S* promoter (*35S::BAK1-HA*) (Fig. 1B) or its native BAK1 promoter (*pBAK1::BAK1-GFP*) (Fig. 1C). To eliminate the potential complication from the C-terminal epitope tag (Ntoukakis et al., 2011), we expressed *BAK1* without any tag in Arabidopsis protoplasts and also observed the truncated fragments of BAK1 detected by anti (α)-BAK1 polyclonal antibodies directing against the C-terminal peptide (DSTSQIENEYPSGPR) in an immunoblot (Fig. 1D). Notably, among the truncated BAK1 polypeptide bands, the one with the MW of ~48 kD (~50 kD for HA- or FLAG-tagged BAK1 and ~75 kD for GFP-tagged BAK1) was the most abundant in different expression systems and named as C-terminal fragment (CTF) of BAK1.

To determine if $BAK1^{CTF}$ was derived from proteolytic cleavage or non-specific protein degradation, we applied different chemical inhibitors, including protease inhibitors, to explore the biochemical requirements for the formation of $BAK1^{CTF}$. The calcium (Ca^{2+}) chelating agents ethylenediaminetetraacetic acid (EDTA) and ethylene glycol tetra-acetic acid (EGTA) blocked the production of $BAK1^{CTF}$ (Fig. 1E). In addition, the Ca^{2+} channel blockers lanthanum chloride ($LaCl_3$) and gadolinium chloride ($GdCl_3$) markedly compromised the production of $BAK1^{CTF}$, suggesting the involvement of Ca^{2+} in the formation of $BAK1^{CTF}$ (Fig. 1E). MG132, a commonly used cell-permeable proteasome inhibitor, also blocked the production of $BAK1^{CTF}$ (Fig. 1F). However, lactacystin, a specific 26S proteasome inhibitor that binds and inhibits catalytic subunits of the proteasome (Fenteany et al., 1995), did not affect the production of $BAK1^{CTF}$ (Fig. 1F), suggesting the effect of MG132 on BAK1 cleavage may not be caused by its inhibition on the 26S proteasome. Notably, lactacystin has no reported effect on serine or

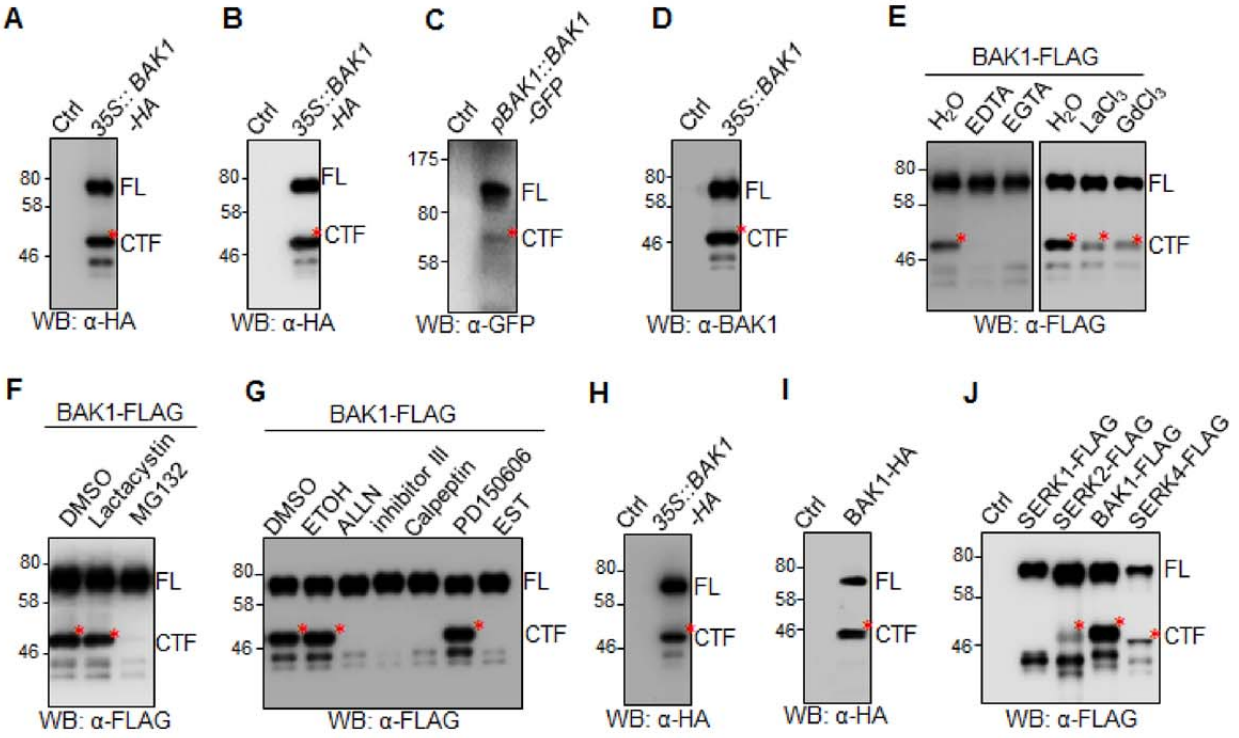

Figure 1. Proteolytic processing of BAK1 in plants by a conserved protease

(A) Expression of BAK1-HA in *Arabidopsis* protoplasts. Protein extracts from *Arabidopsis* protoplasts transfected with *35S::BAK1-HA* or a control vector (Ctrl) were analyzed by Western blot (WB) with an α-HA antibody. The upper band corresponding to the full-length BAK1 protein was indicated by FL, and the lower band corresponding to the C-terminal fragment of BAK1 was indicated by CTF, and labeled with a red star. **(B & C)** $BAK1^{CTF}$ is produced in *Arabidopsis* transgenic plants. Protein extracts from *35S::BAK1-HA* (B) and *pBAK1::BAK1-GFP* (C) transgenic plants were analyzed by WB with respective α-HA and α-GFP antibodies. Wild-type Col-0 *Arabidopsis* was used as Ctrl. **(D)** Non-tagged $BAK1^{CTF}$ is produced in *Arabidopsis* protoplasts. Protein extracts from *Arabidopsis* protoplasts transfected with *35S::BAK1* were analyzed by WB with an α-BAK1 antibody. **(E & F)** Effects of chemical inhibitors on the production of $BAK1^{CTF}$. 1 mM EDTA, 1 mM EGTA, 1 mM $LaCl_3$, 0.5 mM $GdCl_3$, 2 μM MG132 or 2 μM Lactacystin was added immediately after protoplast transfection with *35S::BAK1-FLAG*. Protein extracts were analyzed by WB with an α-FLAG antibody. Lactacystin and MG132 were stored in DMSO, and other chemicals were stored in H_2O. **(G)** Effects of Calpain inhibitors on the production of $BAK1^{CTF}$. 20 μM ALLN, Calpian inhibitor III, Calpeptin, PD150606 or EST (Calbiochem, cat.# 208733) was added immediately after protoplast transfection with *35S::BAK1-FLAG*. Protein extracts were analyzed by WB with an α-FLAG antibody. EST was stored in ethanol (ETOH), and other inhibitors were stored in DMSO. **(H & I)** $BAK1^{CTF}$ is produced in *Nicotiana benthamiana* and *Saccharomyces cerevisiae*. Protein extracts from *N. benthamiana* transiently expressing *35S::BAK1-HA* (H) and *S. cerevisiae* expressing *pGAL1::BAK1-HA* (I) were analyzed by WB with α-HA antibodies. **(J)** Proteolytic cleavage is conserved in SERK family members. Protein extracts from *Arabidopsis* protoplasts expressing FLAG-tagged SERK1, SERK2, SERK3 (BAK1) or SERK4 were analyzed by WB with α-FLAG antibodies.

The above experiments were repeated three times with similar results.

cysteine proteases whereas MG132 could also inhibit the activity of certain proteases, including calpains (Tsubuki et al., 1996), a family of cytosolic calcium-dependent cysteine proteases that regulate a wide variety of cellular processes (Zatz and Starling, 2005; Ono and Sorimachi, 2012). The Arabidopsis genome encodes one calpain gene *DEK1*, mutant of which is embryonic lethal

(Lid et al., 2005; Johnson et al., 2008), making it difficult to genetically assess the role of calpain in BAK1 cleavage. However, by using a wide collection of calpain inhibitors, we could show that pretreatment of certain calpain inhibitors, such as ALLN, calpain inhibitor III, calpeptin, or EST but not PD150606 nor the solvent DMSO and ethanol (ETOH) controls blocked the production of $BAK1^{CTF}$ (Fig. 1G). The data suggest that BAK1 undergoes Ca^{2+}-dependent cleavage in Arabidopsis, and calpain is a potential candidate that mediates BAK1 proteolytic cleavage. The protease that cleaves BAK1 is conserved in plants and yeast since $BAK1^{CTF}$ was observed when it was expressed in *Nicotiana benthamiana* (Fig. 1H) or *Saccharomyces cerevisiae* (Fig. 1I and S1C). Other SERK members, including SERK1, SERK2 and SERK4, also produced cleaved products when they were expressed in Arabidopsis protoplasts (Fig. 1J). BAK1 bears strong kinase activity. Pretreatment of the kinase inhibitor K252a did not affect the formation of $BAK1^{CTF}$ (Fig. S1D). Consistently, HA-tagged BAK1 kinase inactive mutant ($BAK1^{KM}$-HA) produced $BAK1^{CTF}$ similar as the wild-type (WT) BAK1-HA (Fig. S1E), suggesting that the kinase activity may not be required for the BAK1 cleavage.

BAK1 cleavage is MAMP-induced

As a co-receptor, BAK1 plays an important role in multiple cellular processes, including MAMP-mediated immunity and BR-mediated growth and development (Ma et al., 2016). We examined whether BAK1 cleavage is regulated upon the cognate ligand perception and signaling activation. When we treated the *pBAK1::BAK1-GFP* plants with the nonpathogenic bacterium *Pseudomonas syringae* pv. *Tomato* (*Pst*) DC3000 *hrcC*, the abundance of $BAK1^{CTF}$ was increased at 3, 6, and 12 hours post-inoculation (hpi) (Fig. 2A). *Pst hrcC* is a type III secretion mutant strain of *Pst* DC3000, which is defective in secretion of type III effectors, but possesses a variety of MAMPs. Furthermore, using the α-BAK1 antibody, we were able to detect both the endogenous $BAK1^{FL}$ proteins and $BAK1^{CTF}$ in WT Col-0 plants (Fig. 2B). Notably, *Pst hrcC* treatment enhanced the abundance of the endogenous $BAK1^{CTF}$ proteins (Fig. 2B). Treatment of purified MAMP flg22, the synthesized 22-amino-acid peptide derived from bacterial flagellin, also induced the production of $BAK1^{CTF}$ in Col-0 plants (Fig. 2B). Only a small portion of endogenous BAK1 is cleaved in the cells (Fig. 2B). The abundance of $BAK1^{CTF}$ was gradually reduced during the plant maturation process from the seedling to adult stages (Fig. 2C). $BAK1^{CTF}$ was mostly abundant at 8 to 13 days post-germination (dpg), indicating a potential

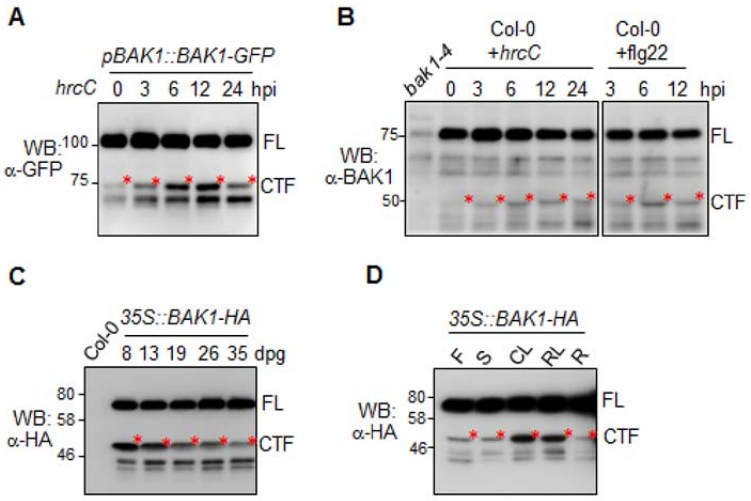

Figure 2. Regulation of BAK1^{CTF} production

(A) Production of BAK1^{CTF} in *pBAK1::BAK-GFP* transgenic plants upon *Pst hrcC* infection. Four-week-old soil-grown *Arabidopsis pBAK1::BAK-GFP* transgenic plants were hand-inoculated with *Pst hrcC* at 5×10^5 cfu. Total proteins from inoculated leaf extracts were analyzed by WB with an α-GFP antibody. hpi, hours post inoculation. **(B)** Production of BAK1^{CTF} in WT plants upon infections. Four-week-old soil-grown *Arabidopsis* Col-0 plants were hand-inoculated with *Pst hrcC* at 5×10^5 cfu or 1 μM flg22. Total proteins from inoculated leaf extracts were analyzed by WB with an α-BAK1 antibody. **(C)** Developmental regulation of BAK1^{CTF} production. Total proteins extracted from *Arabidopsis 35S::BAK1-HA* transgenic plants at different growth stages from 8 through 35 days post germination (dpg) on ½ MS plates were analyzed by WB with an α-HA antibody. **(D)** BAK1^{CTF} production in different tissues. Total proteins extracted from different tissues of two-month-old soil-grown *35S::BAK1-HA* transgenic plants were analyzed by WB with an α-HA antibody. F: flowers, S: stem; CL: cauline leaves; RL: rosette leaves; R: roots.

The above experiments were repeated three times with similar results.

1

endogenous regulatory mechanism during plant growth and development underlying the BAK1 cleavage (Fig. 2C). Additionally, we observed that BAK1^{CTF} was mostly observed in rosette and cauline leaves, but it was less abundant in stems, flowers and roots of four-week-old *35S::BAK1-*

HA transgenic plants (Fig. 2D). Taken together, the data indicate that BAK1 cleavage is a temporo-spatially regulated process and is also regulated upon pathogen recognition.

BAK1^{D287} is required for the production of BAK1^{CTF}.

BAK1 possesses an extracellular LRR domain €, a transmembrane domain (T), a juxtamembrane domain (J) and an intracellular kinase domain (K) (Fig. 3A). To map the BAK1 cleavage region, we first compared the expression pattern of BAK1^{FL} proteins with different BAK1 truncations, including the BAK1 kinase domain (BAK1^{K}), the juxtamembrane and kinase domains (BAK1^{JK}), and the juxtamembrane, transmembrane and kinase domains (BAK1^{TJK}) (Fig. S2A). BAK1^{TJK} exhibited a migration pattern similar to that of BAK1^{CTF} in an immunoblot detected by an α-BAK1 antibody (Fig. S2B), indicating that the BAK1 proteolytic cleavage may occur close to the transmembrane domain. We then performed an extensive alanine substitution mutagenesis screen to systemically mutate 35 residues in this region with each construct carrying three- or four-amino-acid substitution to alanine (A) (Fig. S2C). However, none of these mutations blocked the BAK1 cleavage (Fig. S2D). We extended our mutagenesis screen to the neighboring regions, in particular, the motifs showing certain homology to the cleavage sites of mammalian receptor tyrosine kinases (RTKs) by proteases (Fig. 3A). Notably, substitution of a four-amino-acid sequence at the residues 284-287 (BAK1^{VASD}) to alanine blocked the production of BAK1^{CTF} (Fig. 3A & 3B). Subsequent single-amino-acid substitution among these residues indicated that the aspartate-to-alanine mutation at the residue 287 (BAK1^{D287A}) was sufficient to block the production of BAK1^{CTF} (Fig. 3C). When transiently expressed in *N. benthamiana*, BAK1^{D287A} also blocked the production of BAK1^{CTF} (Fig. 3D). We further generated Arabidopsis transgenic plants stably expressing *HA*-tagged *BAK1^{D287A}* under the control of the *35S* promoter (*35S::BAK1^{D287A}-HA*) (Fig. 3E) and *GFP*-tagged *BAK1^{D287A}* under the control of its native *BAK1* promoter (*pBAK1::BAK1^{D287A}-GFP*) (Fig. 3F). BAK1^{CTF} was no longer detected in either *35S::BAK1^{D287A}-HA* or *pBAK1::BAK1^{D287A}-GFP* transgenic plants (Fig. 3E & 3F). Aspartate is a negatively charged amino acid. We also substituted aspartate to the negatively charged glutamate. Similarly, aspartate-to-glutamate (BAK1^{D287E}) substitution blocked the production of BAK1^{CTF} (Fig. 3G), suggesting that blocking of the cleavage in BAK1^{D287A} was not due to the loss of negative charge in residue 287. The aspartate 287 residue locates in the beginning of BAK1 kinase domain and is a surface-exposed residue according to the published BAK1 kinase domain

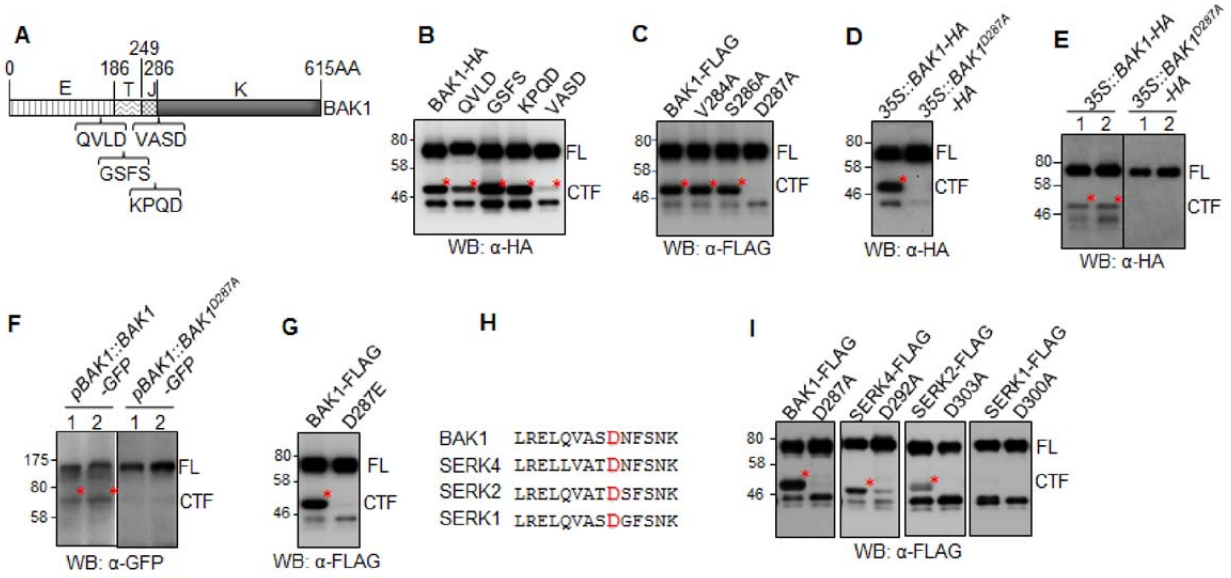

Figure 3. The D287A mutation of BAK1 blocks its cleavage

(A) Scheme of BAK1 protein domains and sites for mutagenesis. E: extracellular LRR domain; T: transmembrane domain; J: juxtamembrane domain; K: intracellular kinase domain. **(B)** VASD residues are required for BAK1 cleavage. *Arabidopsis* protoplasts expressing HA-tagged BAK1 mutants, in which the indicated four amino acids were mutagenized to alanine, were analyzed by WB with an α-HA antibody. **(C)** D287 residue is required for BAK1 cleavage in *Arabidopsis* protoplasts. **(D)** D287 residue is required for BAK1 cleavage in *N. benthamiana. N. benthamiana* leaves transiently expressing HA-tagged BAK1 or BAK1^{D287A} mutant were analyzed by WB with α-HA antibody. **(E)** D287 residue is required for BAK1 cleavage in *Arabidopsis 35S::BAK1-HA* transgenic plants. **(F)** D287 residue is required for BAK1 cleavage in *Arabidopsis pBAK1::BAK1-GFP* transgenic plants. **(G)** D287E mutation blocks BAK1 cleavage in *Arabidopsis* protoplasts. **(H)** D287 residue of BAK1 is conserved among *Arabidopsis* SERKs. **(I)** BAK1 D287 corresponding residue in other SERK members is required for cleavage in *Arabidopsis* protoplasts.

The above experiments were repeated three times with similar results.

1

235 structure (Yan et al., 2012). BAK1^{D287} is also conserved among SERKs (Fig. 3H). To determine
236 if this site is required for proteolytic cleavage of other SERKs, we substituted the cognate
237 aspartate residue to alanine in SERK1, SERK2 and SERK4, and found that the corresponding
238 cleaved fragment in SERK4 and SERK2 was reduced (Fig. 3I). The data indicate that the

conserved aspartate residue is required for the proteolytic cleavage of BAK1 and probably SERK4 and SERK2.

BAK1^{D287} is required for PTI signaling and responses.

BAK1 plays an important role in PTI signaling and the *bak1-4* mutant shows compromised immune responses (Chinchilla et al., 2007; Roux et al., 2011). To determine whether the cleavage is required for BAK1 function in PTI signaling, we generated the transgenic plants carrying the WT *BAK1* or *BAK1^{D287A}* under the control of the native *BAK1* promoter in the *bak1-4* mutant (*pBAK1::BAK1/bak1* or *pBAK1::BAK1^{D287A}/bak1*). Multiple transgenic lines were obtained and two representative lines for each construct with a comparable *BAK1* expression level between WT *BAK1* and *BAK1^{D287A}* were used to systematically characterize PTI responses (Fig. S3). As shown in Fig. 4, *pBAK1::BAK1/bak1*, but not *pBAK1::BAK1^{D287A}/bak1* transgenic plants, were able to complement the deficiency of the flg22-induced MAPK activation (Fig. 4A), ROS production (Fig. 4B) and callose deposition (Fig. 4C) in the *bak1-4* mutant. Pre-treatment of flg22 protected the WT Col-0 and *pBAK1::BAK1/bak1* transgenic plants to the infection of the virulent bacterium *Pst* DC3000 as indicated by the reduced *in planta* bacterial multiplication (Fig. 4D). However, similar to the *bak1-4* mutant, the *pBAK1::BAK1^{D287A}/bak1* transgenic plants lost the flg22-mediated resistance against *Pst* DC3000 infection. Taken together, the data indicate that BAK1^{D287}-mediated cleavage is critical for BAK1-dependent PTI responses.

BAK1 directly transphosphorylates BIK1 to relay PTI signaling (Lin et al., 2014). The *pBAK1::BAK1/bak1*, but not *pBAK1::BAK1^{D287A}/bak1*, transgenic plants restored the deficiency of flg22-induced BIK1 phosphorylation in the *bak1-4* mutant evidenced by phosphoprotein mobility shift in immunoblots (Fig. 4E). When BAK1 was expressed in Arabidopsis *bak1-4* protoplasts and immunoprecipitated for a kinase assay, the *in vivo* expressed BAK1^{D287A} showed reduced autophosphorylation compared to WT BAK1 (Fig. 4F). In addition, *in vivo* expressed BAK1^{D287A} showed reduced transphosphorylation activities towards BIK1^{KM} (Fig. 4F), consistent with the reduced BIK1 phosphorylation upon flg22 treatment (Fig. 4E). Notably, the cleaved BAK1^{CTF} also possessed autophosphorylation activity (Fig. 4F). Interestingly, an *in vitro* kinase assay showed that the cytosolic domain of BAK1 (BAK1^{CD}) and BAK1$^{CD\ D287A}$ fused with maltose-binding protein (MBP) purified from *Escherichia coli* possessed similar autophosphorylation and trans-phosphorylation activities towards BIK1 (Fig. 4G), suggesting

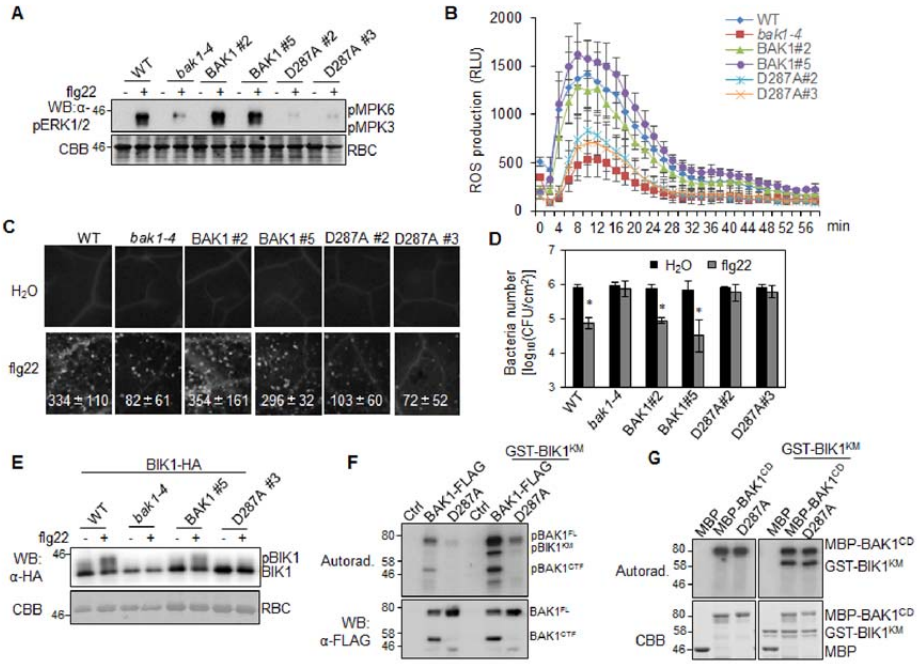

Figure 4. Compromised immune responses in *pBAK1::BAK1^{D287A}/bak1* transgenic plants

(A) flg22-induced MAPK activation. Two-week-old seedlings of WT Col-0, *bak1-4*, *pBAK1::BAK1/bak1* and *pBAK1::BAK1^{D287A}/bak1* were treated with 100 nM flg22 for 15 min. Phosphorylated MPK3 (pMPK3) and MPK6 (pMPK6) were detected by WB with an α-pERK antibody (top). Protein loading is shown by Coomassie Brilliant Blue (CBB) staining for RuBisCo (RBC) (bottom). **(B)** flg22-induced ROS production. Leaf discs from four leaves (technical repeats) of each of six five-week-old plants (biological repeats) of indicated genotypes were treated with 100 nM flg22, and ROS production was detected at the indicated time points. The data are shown as the mean ± SD from six biological repeats. **(C)** flg22-induced callose deposition. Leaves of four-week-old plants were collected for aniline blue staining 12 hr after inoculation with 500 nM flg22. Callose deposits were counted using ImageJ 1.43U software (http://rsb.info.nih.gov/ij/). The data are shown as the mean ± SD from six biological repeats. **(D)** flg22-mediated resistance to bacterial infection. Four-week-old plants were pretreated with 200 nM of flg22 or water and then infected with *Pst* DC3000 at 5×10^5 cfu/ml. The bacterial growth assays were performed 2 days after infection. The data are shown as the mean ± SD from three biological repeats. The asterisks indicate statistical significance compared to H_2O pretreatment by using student's *t*-test ($P<0.05$). **(E)** flg22-induced BIK1 mobility shift. *Arabidopsis* protoplasts isolated from Col-0, *bak1-4* mutant, *pBAK1::BAK1/bak1* and *pBAK1::BAK1^{D287A}/bak1* transgenic plants were used to express BIK1-HA and treated with 100 nM flg22 for 15 min. Mobility shift of BIK1 was detected by WB with an α-HA antibody (top). Equal loading of protein was indicated by CBB staining towards RBC (bottom). pBIK1, phosphorylated BIK1. **(F)** The kinase activity of BAK1 and BAK1^{D287A}. Full length BAK1-FLAG or BAK1^{D287A}-FLAG were expressed in *Arabidopsis* Col-0 protoplasts and precipitated with an α-FLAG antibody. Kinase activity of the precipitated proteins were detected using GST-BIK1^{KM} (kinase mutant) as a substrate. Phosphorylation was detected by autoradiography (top), and the protein loading is shown by WB with an α-FLAG antibody (bottom). **(G)** The *in vitro* kinase activity of the cytosolic domain of BAK1 and BAK1^{D287A}. GST-BIK1^{KM} protein was used as a substrate and MBP-BAK1^{CD} (cytosolic domain) or its D287A mutant was used as the kinase in an *in vitro* kinase assay. Phosphorylation was detected by autoradiography (top), and the protein loading is shown by CBB staining (bottom).

The above experiments were repeated three times with similar results.

that BAK1^{D287A} is a functionally competent kinase, and BAK1^{D287} mainly regulates its activity *in vivo*.

BAK1^{D287} is required for BAK1 function in BR signaling and cell death control.

BAK1 plays an important role in BR signaling and the *bak1-4* mutant resembles the weak *bri1* mutant with compacted rosette leaves and short petioles compared to WT Col-0 plants (Li et al., 2002; Nam and Li, 2002). The transgenic plants of *pBAK1::BAK1/bak1*, but not *pBAK1::BAK1*^{D287A}*/bak1*, restored the growth defect of *bak1-4* to WT Col-0 (Fig. S3). When grown in the dark, hypocotyls of *pBAK1::BAK1*^{D287A}*/bak1* transgenic plants and *bak1-4* were shorter than those of *pBAK1::BAK1/bak1* transgenic plants and WT Col-0 in the absence or presence of brassinazole (BRZ), an inhibitor of BR biosynthesis (Fig. 5A & 5B), suggesting an important role of BAK1^{D287} in mediating BR signaling. BAK1 positively regulates BR signaling by heterodimerization and transphosphorylation with BRI1 (Li et al., 2002; Nam and Li, 2002). Overexpression of functional *BAK1* partially alleviates the growth defects of the *bri1* mutants. We generated Arabidopsis transgenic plants expressing *BAK1* or *BAK1*^{D287A} under the control of the *35S* promoter in the *bri1-5* mutant (*35S::BAK1/bri1-5* and *35S::BAK1*^{D287A}*/bri1-5*). Multiple transgenic lines were obtained and two lines of each construct with comparable BAK1 protein levels were selected for further study (Fig. 5C & 5D). As previously reported, overexpression of *BAK1* in *bri1-5* partially rescued the dwarf phenotype of *bri1-5* with relatively big leaves, elongated stems, and long siliques compared to *bri1-5* (Nam and Li, 2002). In contrast, overexpression of BAK1^{D287A} in *bri1-5* did not recover the dwarf phenotype of *bri1-5*, instead enhanced the growth defects of *bri1-5*. The *35S::BAK1*^{D287A}*/bri1-5* transgenic plants were sterile (Fig. 5C). Taken together, the data suggest that BAK1^{D287} is required for BAK1 function in BR signaling.

BAK1 together with SERK4 (also called BAK1-LIKE 1 [BKK1]) play a redundant role in the regulation of cell death and the *bak1/serk4* mutant is seedling lethal associated with spontaneous cell death and constitutive H_2O_2 production (He et al., 2007). To assess the requirement of BAK1^{D287} in *BAK1/SERK4*-regulated cell death, we transformed *pBAK1::BAK1* and *pBAK1::BAK1*^{D287A} into the Arabidopsis *BAK1-4*$^{+/-}$*SERK4-1*$^{-/-}$ mutant and examined the growth phenotypes of T1 generation of transgenic plants. We further differentiated *BAK1* WT (*BAK1*$^{+/+}$), *BAK1* heterozygous (*BAK1*$^{+/-}$) and *BAK1* mutant (*BAK1*$^{-/-}$) genotypes by genotyping T-DNA insertions in the transgenic plants. Transgenic plants of *pBAK1::BAK1* and *pBAK1::BAK1*^{D287A} in *BAK1*$^{+/+}$*SERK4*$^{-/-}$ or *BAK1*$^{+/-}$*SERK4*$^{-/-}$ backgrounds displayed normal growth phenotype as the WT Col-0 plants (Fig. 5E). However, *pBAK1::BAK1*^{D287A} transgenic plants in the *BAK1*$^{-/-}$*SERK4*$^{-/-}$ background showed growth defects with much short petioles, and

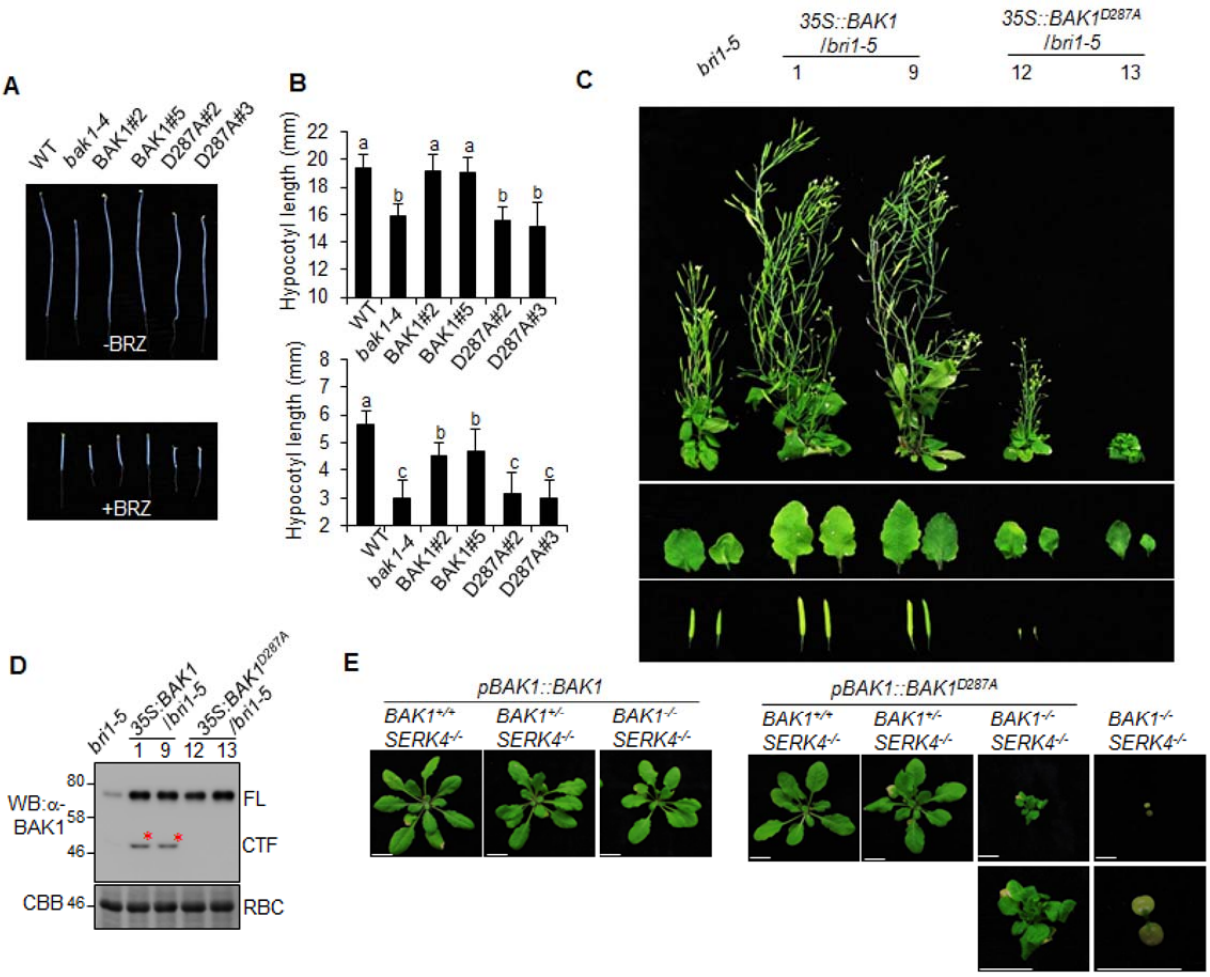

Figure 5. BAK1 D287 is critical for BR signaling and cell death control

(A) The reduced hypocotyl length of *pBAK1::BAK1*^{D287A}*/bak1* transgenic plants. The seedlings of WT, *bak1-4* and transgenic plants were grown in the dark for 5 days on ½ MS plats without or with 1 μM of BRZ. **(B)** Quantification of the hypocotyl length shown in (A). The data are shown as the mean ± SD from 20 biological repeats. The different letters indicate statistically significant difference analyzed with one-way ANOVA followed by Tukey's test (P<0.05). **(C)** BAK1^{D287A} mutant cannot restore *bri1-5* dwarf defect. Transgenic plants of *35S::BAK1* and *35S::BAK1*^{D287A} in *bri1-5* background were taken photos at two months old. The individual leaves and siliques are shown in the middle and bottom. **(D)** Expression of BAK1 protein in rosette leaves from four-week-old plants of (C) was detected by WB with an α-BAK1 antibody. **(E)** D287 is important for BAK1 function in cell death control. Arabidopsis *BAK1*$^{+/-}$*SERK4*$^{-/-}$ plants were transformed with *pBAK1::BAK1* or *pBAK1::BAK1*^{D287A}. Plants of T_1 generation after selection of BAK1 or BAK1^{D287A} transgene were genotyped for endogenous genomic BAK1. Pictures were taken four weeks after germination. *BAK1*$^{+/+}$*SERK4*$^{-/-}$ (BAK1: WT, SERK4: mutant); *BAK1*$^{+/-}$*SERK4*$^{-/-}$ (BAK1: heterozygous, SERK4: mutant); *BAK1*$^{-/-}$*SERK4*$^{-/-}$ (BAK1: mutant, SERK4: mutant). Scale bars, 1 cm.

The above experiments were repeated three times with similar results.

small leaves compared to the WT Col-0 plants (Fig. 5E). Notably, there are variations of growth defects of *pBAK1::BAK1*^{D287A} in the *BAK1*$^{-/-}$*SERK4*$^{-/-}$ background, with plants similar to or better than *BAK1*$^{-/-}$*SERK4*$^{-/-}$ (Fig. 5E). Thus, BAK1^{D287} is partially required for *BAK1/SERK4*-mediated cell death. In addition, silencing of *SERK4* in *bak1-4*, but not in WT Col-0 plants, using the

Agrobacterium-mediated virus-induced gene silencing (VIGS) triggers severe growth defects and cell death (de Oliveira et al., 2016) (Fig. S4A). Silencing of *SERK4* in *pBAK1::BAK1/bak1* transgenic plants via VIGS did not trigger growth defects, whereas silencing of *SERK4* in *pBAK1::BAK1^{D287A}/bak1* transgenic plants exhibited severe growth defects with chlorotic leaves and dwarfism (Fig. S4A). Trypan blue staining revealed spontaneous cell death in *bak1-4* and *pBAK1::BAK1^{D287A}/bak1* transgenic plants but not in Col-0 and *pBAK1::BAK1/bak1* transgenic plants upon silencing of *SERK4* (Fig. S4B). Additionally, RT-quantitative PCR (RT-qPCR) analysis indicated that transcripts of *pathogenesis-related protein 1* (*PR1*) and *PR2* were significantly increased in *bak1-4* and *pBAK1::BAK1^{D287A}/bak1* plants compared to Col-0 and *pBAK1::BAK1/bak1* plants upon silencing of *SERK4* (Fig. S4C). The data further elaborated that BAK1^{D287} is required for BAK1 and SERK4-regulated cell death.

BAK1^{D287} is required for BAK1 plasma membrane localization.

BAK1 is a plasma membrane-localized protein (Li et al., 2002; Nam and Li, 2002), that is also likely localized to endosomal compartments (Russinova et al., 2004). We tested whether D287A is important for BAK1 localization by generating *pBAK1::BAK1-GFP* and *pBAK1::BAK1^{D287A}-GFP* transgenic plants. As reported previously, BAK1-GFP was mainly localized in the plasma membrane, where it colocalized with FM4-64, a lipophilic dye that labels plasma membrane (Fig. 6A, top panels, &6B). However, the plasma membrane localization of BAK1^{D287A}-GFP was greatly reduced in the *pBAK1::BAK1^{D287A}-GFP* transgenic plants (Fig. 6A, bottom panels & 6B). The BAK1^{D287A}-GFP protein appeared retained in the endoplasmic reticulum (ER) as corroborated by colocalization analyses using the ER marker mCherry-HDEL (Nelson et al., 2007) (Fig. 6C) and the ER-Tracker Red (Fig. S5). The reduced plasma membrane localization of BAK1^{D287A} was also documented by protein fractionation assay. BAK1-HA and BAK1^{D287A}-HA were transiently expressed in *N. benthamiana*, and total proteins and plasma membrane-enriched fractions were analyzed with an α-HA immunoblot. The HA-tagged RLK PSKR1 was co-expressed with BAK1-HA or BAK1^{D287A}-HA as a control for the plasma membrane-localized proteins (Ladwig et al., 2015). Immunoblot for endogenous MPK6 with an α-MPK6 antibody was used as a cytoplasmic protein control (Collins et al., 2017). Significantly, compared to WT BAK1, the amount of BAK1^{D287A} in the plasma membrane-enriched fraction was greatly reduced, although the amount of PSKR1 in the plasma membrane-enriched fraction stayed the

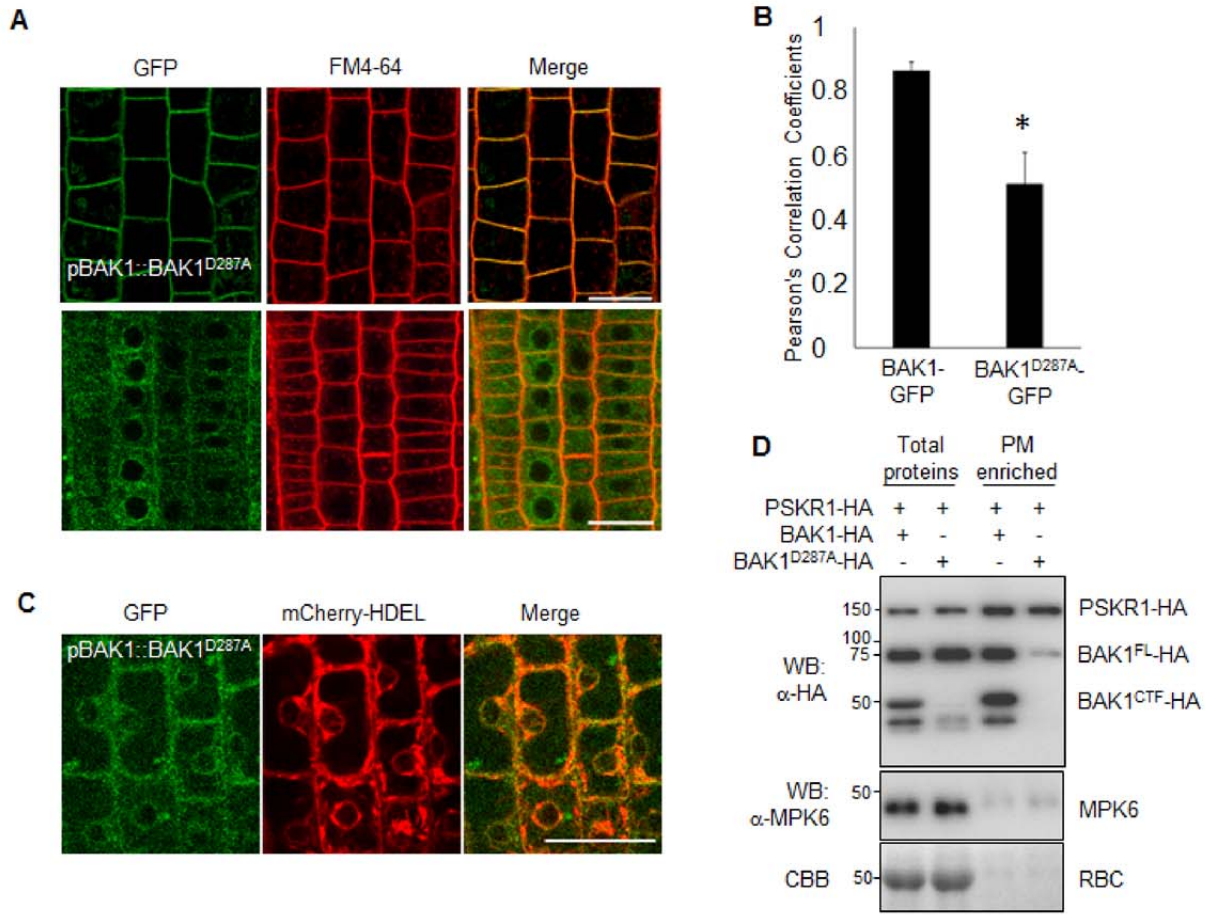

Figure 6. D287 is critical for the plasma membrane localization of BAK1

(A) Subcellular localization of BAK1-GFP (top panel) and BAK1^{D287A}-GFP (bottom panel) in root epidermis of five-day-old *Arabidopsis* seedlings. The plasma membrane was stained with FM4-64 (2 μM). **(B)** Pearson's correlation coefficient (*r* value) for the colocalization between GFP and FM4-64 fluorescence in the plasma membrane. These values were measured in 15 cells. Error bars indicate mean ± SD. P-value (*t*-test), * P<0.01. **(C)** Colocalization of BAK1^{D287A}-GFP with endoplasmic reticulum (ER) marker, mCherry-HDEL. Scale bars, 20 μm. **(D)** Leaves of four-week-old *N. benthamiana* were hand-inoculated with Agrobacteria carrying *35S::BAK1-HA* or *35S::BAK1^{D287A}-HA* together with *35S::PSKR1-HA*. BAK1 from total proteins and enriched plasma membrane (PM) proteins were analyzed by WB with an α-HA antibody. Cytosolic MPK6 was detected by α-MPK6 WB. PSKR1 is a plasma membrane-localized protein.
The above experiments were repeated three times with similar results.

same when it was co-expressed with BAK1 or BAK1^{D287A} (Fig. 6D). Thus, D287 is essential for BAK1 to localize in the plasma membrane, where it functions as a co-receptor to activate diverse signaling pathways.

Overexpression of *BAK1^{D287A}* triggers SOBIR1-depenent and BIK1-independent cell death. Overexpression of *BAK1* under a double *35S* (*2x35S*) promoter in Arabidopsis triggers plant dwarfism accompanied with cell death (Dominguez-Ferreras et al., 2015). Consistently, transient expression of *2x35S::BAK1* triggered cell death in *N. benthamiana*. Expression of *2x35S::BAK1^{D287A}* clearly aggravated the occurrence of cell death in *N. benthamiana* (Fig. 7A). The elevated cell death by *2x35S::BAK1^{D287A}* was also indicated by the autofluorescence signals under the UV light (Fig. 7B) and the electrolyte leakage (Fig. 7C). We did not detect cell death when *BAK1* was under the control of a single *35S* promoter (*35S::BAK1*). However, *35S::BAK1^{D287A}* triggered cell death when transiently expressed in *N. benthamiana* (Fig. 7A, 7B & 7C). No cell death was observed when *BAK1* or *BAK1^{D287A}* was expressed under the control of its native promoter (*pBAK1::BAK1 or pBAK1::BAK1^{D287A}*) (Fig. 7A, 7B & 7C). The occurrence of cell death by overexpression of *BAK1* appeared to be correlated with the level of BAK1 proteins as *2x35S::BAK1* produced more BAK1 proteins, including both BAK1^{FL} and BAK1^{CTF}, than *35S::BAK1* (Fig. 7A). We further generated Arabidopsis transgenic plants overexpressing *BAK1* and *BAK1^{D287A}* under the control of the *35S* promoter in the Col-0 (WT) background. Among 39 *35S::BAK1* plants with positive signals by α-BAK1 immunoblots, 33% (13 out of 39) of transgenic plants were small and showed dwarfism. However, 64% (9 out of 14) of *35S::BAK1^{D287A}* transgenic plants were small and dwarfed (Fig. 7D). The small plants were associated with cell death as stained by trypan blue and H_2O_2 accumulation as stained by DAB (Fig. 7E). RT-qPCR analysis revealed that the expression of *PR1* and *PR2* was elevated in the *35S::BAK1^{D287A}* transgenic plants than that in *35S::BAK1* plants (Fig. 7F). Taken together, our data indicate that cleavage of BAK1 might be a way to alleviate cell death when BAK protein level is high in plants.

BIK1 is a key component downstream of BAK1 in plant immunity (Lin et al., 2013; Liang and Zhou, 2018). We tested whether cell death caused by overexpression of *BAK1* or *BAK1^{D287A}* depends on BIK1 by transferring *35S::BAK1* or *35S*::*BAK1^{D287A}* into the *bik1* mutant. Compared to WT plants, the *bik1* mutant did not reduce the portion of small plants of *35S::BAK1* or *35S*::*BAK1^{D287A}* transgenic plants (Fig. 7G), indicating that cell death caused by over-expression of *BAK1* or *BAK1^{D287A}* is BIK1-independent. Thus, it is likely that cleavage of over-produced BAK1 negatively regulates cell death independently of flg22-triggered signaling. It has been reported that overexpression of *BAK1*-triggered cell death is dependent on *SOBIR1* (*Suppressor*

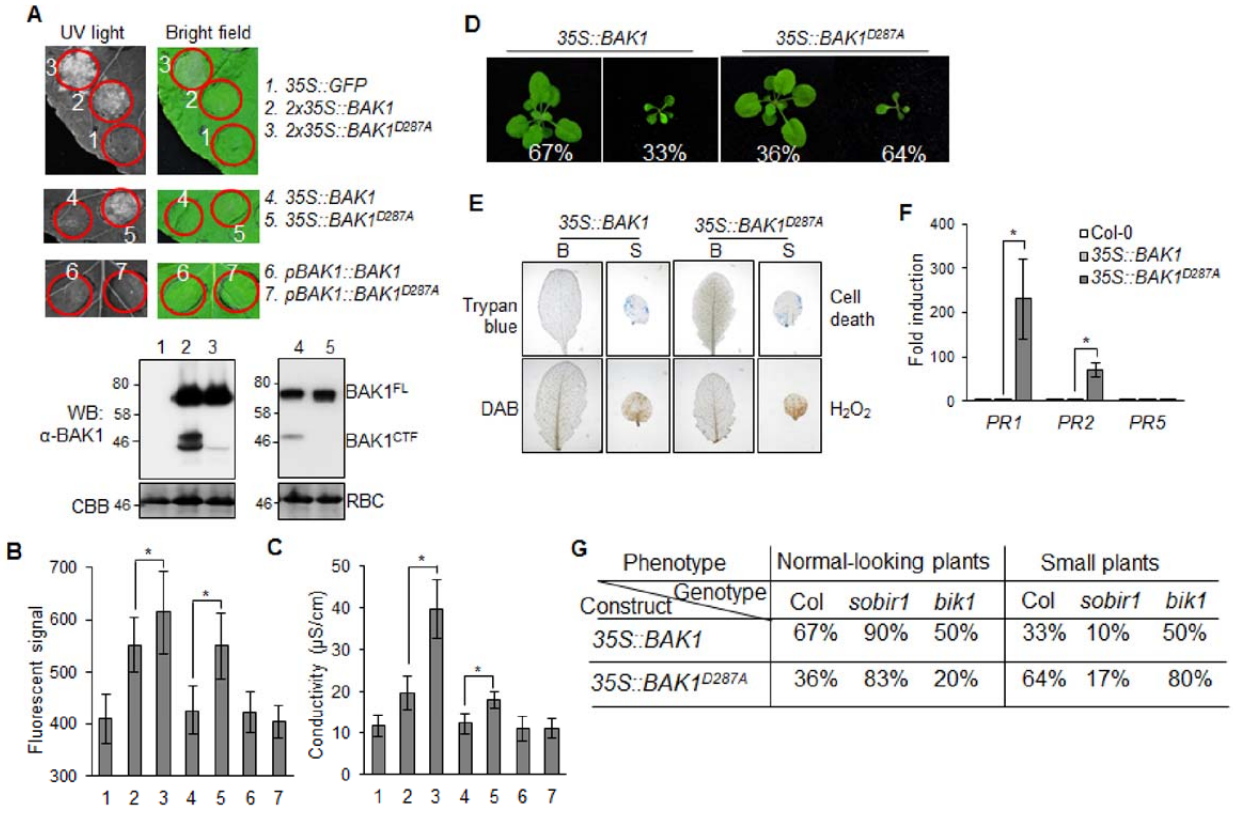

Figure 7. Over-production of BAK1 ^{D287A} enhances SoBIR1-dependent cell death

(A) Expression of BAK1 and BAK1^{D287A} under different promoters in *N. benthamiana*. Leaves of four-week-old *N. benthamiana* were hand-inoculated with *Agrobacteria* carrying *35S::GFP, 2x35S::BAK1, 2x35S::BAK1^{D287A}, 35S::BAK1, 35S::BAK1^{D287A}, pBAK1::BAK1 or pBAK1::BAK1^{D287A}*. Pictures were taken under UV light (left) or visible light (right) three days after inoculation. Two days after inoculation and before cell death progressed, protein levels of BAK1 were analyzed by WB with an α-BAK1 antibody (bottom). BAK1 proteins from *2x35S::BAK1* (2) and *2x35S::BAK1^{D287A}* (3) were detected by WB using SuperSignal™ West Pico Chemiluminescent Substrate, and BAK1 proteins from *35S::BAK1* (4) and *35S::BAK1^{D287A}* (5) were detected using Thermo Scientific™ SuperSignal™ West Femto Maximum Sensitivity Substrate. **(B)** The fluorescent signal from the circled areas in (A) was obtained under the UV light and was quantified as means ± SD from ten biological repeats. The asterisks indicate statistical significance by using student's *t*-test (P<0.05). **(C)** Measurement of electrolyte leakage of leaf discs from (A). Data are shown as means ± SD from three biological repeats. The asterisks indicate statistical significance by using student's *t*-test (P<0.05). **(D)** Overexpression of BAK1 and BAK1^{D287A} in *Arabidopsis*. *Arabidopsis* plants overexpressing *35S::BAK1* or *35S::BAK1^{D287A}* were taken photos at four-week-old stage. Each phenotype of T_1 generation plants was calculated as a percentage to the total transgenic plants of that genotype. **(E)** Cell death and H_2O_2 accumulation in the leaves of four-week-old plants were examined by trypan blue and DAB staining respectively. B: normal-looking plants; S: small plants. **(F)** Expression levels of *PR* genes in one-week-old transgenic plants were determined by qRT-PCR. The data are shown as means ± SD from three biological repeats. The asterisks indicate statistical significance by using student's *t*-test (P<0.05). **(G)** BAK1 and BAK1^{D287A} cell death is SoBIR1-dependent and BIK1-independent. *35S::BAK1* and *35S::BAK1^{D287A}* were transformed into *sobir1* and *bik1* mutant backgrounds. Each phenotype of T_1 generation at four-week-old stage was calculated as a percentage as in (D).
The above experiments were repeated three times with similar results.

Of BIR1-1), which encodes an LRR-RLK (Gao et al., 2009; Dominguez-Ferreras et al., 2015). We examined whether overexpression of *BAK1^{D287A}*-triggered cell death also requires SOBIR1 by transferring *35S::BAK1* or *35S::BAK1^{D287A}* into the *sobir1* mutant. Significantly, the ratio of dwarfed plants in *sobir1* was reduced for both *35S::BAK1* (33% to 10%) and *35S::BAK1^{D287A}*

379 (64% to 17%) transgenic plants compared to WT plants (Fig. 7G). Thus, over-expression of
380 $BAK1^{D287A}$ causes a SOBIR1-depenent, but BIK1-independent cell death.
381
382

Discussion

In animal studies, members of cell surface proteins have been observed to undergo multi-layered proteolytic cleavage processes to regulate transmembrane protein functions (Hayashida et al., 2010). For example, proteolytic cleavage of some RTKs upon ligand binding triggers their nuclear translocation to execute their functions in the nucleus (Carpenter and Liao, 2013). Immune receptors TLR7 and TLR9 are ectodomain-cleaved and form the functional receptors in the endolysosome (Ewald et al., 2008; Park et al., 2008; Petes et al., 2017). Similarly, rice LRR-RLK XA21, which confers broad-spectrum immunity to the Gram-negative bacterial pathogens, is cleaved and the intracellular domain is likely translocated to the nucleus, where it interacts with transcription factors for immune activation (Park and Ronald, 2012). The legume RLK SYMRK undergoes proteolytic cleavage and the cleaved ectodomain promotes the formation of a complex with NOD-FACTOR RECEPTOR 5 (NFR5), which is involved in root symbiosis (Antolin-Llovera et al., 2014). Arabidopsis chitin receptor RLK CERK1 is also likely cleaved, and a mutation that blocks cleavage generates a novel function of CERK1 in chitin-independent cell death control (Petutschnig et al., 2014). An ATP-binding proteomics analysis predicted that two uncharacterized LRR-RLKs (At3g02880 and At5g16590) exist in leaves only as cytoplasmic domains, suggesting that the extracellular domains are cleaved (Villamor et al., 2013). We show here that the shared coreceptor BAK1, and likely other SERK family members, are cleaved by a conserved protease in eukaryotes (Fig. 1). Importantly, this cleavage is enhanced by pathogen or MAMP treatments (Fig. 2), suggesting that the BAK1 cleavage is involved in plant responses to pathogen attacks. Together with previous findings, the proteolytic cleavage of plant RLKs is likely another layer of regulatory mechanism in addition to the well-studied phosphorylation, which controls RLK activation.

We have observed that BAK1 cleavage is Ca^{2+}-dependent (Fig. 1E), and multiple calpain inhibitors blocked BAK1 cleavage *in vivo* (Fig. 1G). Since the mutation of plant calpain DEK1 is embryonic lethal (Lid et al., 2005; Johnson et al., 2008), we attempted to silence Arabidopsis *DEK1* with VIGS. However, we did not observe the change of BAK1 cleavage in *DEK1*-silenced plants compared to control plants (Supplemental Figure S6). Notably, *DEK1*-silenced plants were phenotypically similar to control plants. It is possible that the reduced DEK1 level in *DEK1*-silenced plants is sufficient for its normal functions. Despite extensive studies on

414 mammalian calpains, the sequence and structural determinants of cleavage targets are little
415 understood (Johnson et al., 2005), and no substrates of calpain in plants have been reported yet.
416 Plant RLKs are functional counterparts of mammalian RTKs, cleavage of which is stimulated by
417 caspases, metalloproteases, γ-secretase complex or mRNA splicing (Ancot et al., 2009; Chen and
418 Hung, 2015). The most prevalent mechanism of RTK cleavages occurs within the membrane
419 through the action of a multisubunit γ-secretase complex (Carpenter and Liao, 2013). BAK1
420 cleavage likely occurs within or immediately after the transmembrane domain (Fig. S2B).
421 Arabidopsis also contains genes coding for γ-secretase homologous with the conserved amino
422 acid motifs essential for enzymatic activities (Smolarkiewicz et al., 2014). However, the
423 functions of γ-secretase subunits in plants are largely unknown. In a forward genetic screen for
424 suppressors of the growth phenotype of a weak brassinosteroid insensitive 1 allele *bri1-5*, BRS1
425 was identified and demonstrated as a secreted and active serine carboxypeptidase (Li et al., 2001;
426 Zhou and Li, 2005). Although BRS1's substrates remain unknown, this work reconciles the
427 importance of proteolytic cleavage processes in BR signaling. The proteolytic cleavage of BAK1
428 is required for its function in BR signaling and overexpression of $BAK1^{D287A}$ fails to alleviate the
429 growth defects of *bri1-5* (Fig. 5C & 5D). It remains possible that BRS1 proteolytically processes
430 BAK1 or plays a role in the BAK1 cleavage.
431 We have identified D287 as an important site for BAK1 cleavage (Fig. 3). This site is
432 conserved among other SERK family members and likely required for SERK2 and SERK4
433 cleavage (Fig. 3I). D287 locates at the beginning of BAK1 kinase domain (Fig. 3A). BAK1 is a
434 type I integral membrane protein with a single transmembrane domain, an extra-cytoplasmic
435 receptor domain and a cytoplasmic kinase domain. Based on the molecular weight, BAK1
436 cleaved products are bigger than the predicted products of cytoplasmic domain (Fig. S2A &
437 S2B). D287 may not be the cleavage site, but it is essential for the protease-mediated cleavage.
438 Importantly, D287 is essential for BAK1 functions in plant immunity (Fig. 4), BR responses
439 (Fig. 5A-C) and cell death control (Fig. 5D & 5E). Interestingly, the $BAK1^{D287A}$ mutant
440 exhibited an unaltered *in vitro* kinase activity (Fig. 4G), suggesting that $BAK1^{D287A}$ is still a
441 functional protein. There are several possibilities to explain why BAK1 cleavage is required for
442 its normal functions. Similar with XA21 (Park and Ronald, 2012), the cleaved BAK1 may be
443 released from the plasma membrane and translocated to the nucleus or other subcellular
444 compartments to execute the functions. However, we have not observed a claimable nuclear

localization of BAK1. It is also possible that the activated cytoplasmic domain of BAK1 is released to cytoplasm, where it phosphorylates different substrates for bifurcating its different functions. Additionally, the cleaved BAK1 ectodomain may have a higher affinity for the extracellular LRR domains of FLS2 and BRI1 than the full-length BAK1, and may promote the ligand-induced receptor complex formation. We have observed that the BAK1 ^{D287A} mutant has a reduced plasma membrane localization (Fig. 6). The cleaved BAK1 fragments may facilitate the translocation of BAK1 to the plasma membrane. The BAK1 ^{D287A} mutant is likely trapped in the endoplasmic reticulum (ER) during protein maturation process (Fig. 6C & 6D). ER-mediated protein quality control is essential for the abundance and signaling of some LRR-RLKs, such as EFR and BRI1 (Liu and Li, 2014; Tintor and Saijo, 2014).

Overexpression of BAK1 or its ectodomain in Arabidopsis triggers growth defects and constitutive activation of defense and cell death (Dominguez-Ferreras et al., 2015). It has been postulated that this cell death might be caused by unbalanced regulatory interactions, such as insufficient amount of the BAK1-INTERACTING RECEPTOR-LIKE KINASE 1 (BIR1), which can suppress BAK1 activity (Gao et al., 2009; Dominguez-Ferreras et al., 2015; Liu et al., 2016). We observed that overexpression of the BAK1^{D287A} mutant caused more extensive cell death than WT BAK1 in *N. benthamiana* and Arabidopsis when they were expressed at the same level (Fig. 7A-F). These data suggest that BAK1 cleavage might provide a means to avoid the detrimental effect of BAK1 overproduction. Both BAK1 and BAK1^{D287A}-induced cell death depends on SOBIR1 (Fig. 7G), which mediates *bir1*-induced cell death (Gao et al., 2009). SOBIR1 appears to be specifically required for immune responses triggered by certain LRR receptor-like proteins (LRR-RLPs), not LRR-RLKs (Liebrand et al., 2014). Thus, BAK1 overexpression-activated defense is likely mediated by a different mechanism with BAK1-mediated PTI in which BAK1 functions as a coreceptor of multiple LRR-RLKs. Indeed, BAK1 overexpression-activated defence is independent of a key PTI regulatory protein BIK1 (Fig. 7G). Thus, cleavage of BAK1 may have dual roles in plant immunity: mediating BIK1-dependent PTI signaling and attenuating SOBIR1-dependent autoimmunity. Notably, Dominguez-Ferreras et al. also observed the presence of multiple BAK1 polypeptides in BAK1 overexpression and WT plant extracts, and speculated that they were the products of proteolytic cleavage (Dominguez-Ferreras et al., 2015).

Pathogen effector-mediated proteolytic cleavage is a common mechanism in the activation or attenuation of plant immunity (Dean, 2011; Pogany et al., 2015; Hou et al., 2018). *P. syringae* effector AvrPphB, a cysteine protease, can cleave Arabidopsis RLCK AVRPPHB SUSCEPTIBLE 1 (PBS1) to initiate cytoplasmic immune receptor RESISTANCE TO P. SYRINGAE 5 (RPS5)-specified effector-triggered immunity (ETI) and cleave PBS1-like (PBL) kinases BIK1, PBL1, and PBL2 to inhibit PTI (Shao et al., 2003; Zhang et al., 2010). Interestingly, *P. syringae* effector HopB1 binds to FLS2 and cleaves immune-activated BAK1 to inhibit plant immunity (Li et al., 2016). In this study, we show that BAK1 proteolytic cleavage is dependent on a host-originated protease(s). In contrast to the HopB1-mediated BAK1 cleavage which occurs within the kinase domain between Arg297 and Gly298 (Li et al., 2016), the conserved eukaryotic protease-mediated BAK1 cleavage identified in this study occurs likely within the transmembrane domain, and the cleaved $BAK1^{CTF}$ has a normal kinase activity as the WT BAK1 (Fig. 4F). In the case of HopB1-mediated cleavage, the phosphorylation status of BAK1 is critical as two kinase-dead mutants ($BAK1^{K317E}$ and $BAK1^{D416N}$ in the ATP-binding site and catalytic site, respectively) and a phospho-site mutant of BAK1 ($BAK1^{T455A}$) are resistant to cleavage by HopB1 (Li et al., 2016). In contrast, our studies show that the host protease-mediated BAK1 cleavage does not require BAK1 kinase activity (Fig. S1D & S1E). Similarly, the proteolytic cleavage at the ectodomain of mammalian EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) does not require its kinase activity (Chen et al., 2008; Liao and Carpenter, 2012). These findings show that plants use a different type of protease with bacterial effector HopB1 to cleave BAK1 for a distinct function.

Materials and Methods

Plant materials, growth conditions and treatments. The following *Arabidopsis thaliana* mutant lines have been described previously: *bik1* (Salk_005291)*, sobir1-12, bak1-4, serk4-1* and *bak1/SERK4*$^{+/-}$ in Col-0 background (Gao et al., 2009; Lu et al., 2010; de Oliveira et al., 2016), *bri1-5* in Ws-0 background (Lin et al., 2013). Arabidopsis plants were grown in soil (Metro Mix 366) in a growth room with 23°C, 45% relative humidity, 85 μE m^{-2} s^{-1} light and a photoperiod of 12 hr light /12 hr dark for four weeks before protoplast isolation. To grow Arabidopsis seedlings on medium, the seeds were surface-sterilized with 50% bleach for 15 min,

washed with sterilized ddH_2O and then placed on the plates with half-strength Murashige and Skoog medium (½ MS) containing 0.5% sucrose, 0.8% agar and 2.5 mM MES at pH 5.7. The plates were first stored at 4°C for 3 days in the dark for seed stratification, and then moved to the growth room for different periods of time depending on the experiments.

Plasmid construction and generation of transgenic plants. Protoplasts expression vectors *pHBT-35S::BAK1-HA*, *pHBT-35S::BAK1-FLAG*, *pHBT-35S::BAK1-GFP*, *pHBT-35S::SERK1-FLAG*, *pHBT-35S::SERK2-FLAG*, *pHBT-35S::SERK4-FLAG*, *pHBT-35S::BIK1-HA*, *pHBT-35S::FLS2-HA*, protein expression vectors *pMAL-BAK1*^{CD}, *pGST-BIK1*^{KM}, and VIGS vector *pYL156-SERK4* were reported previously (Shan et al., 2008; Lu et al., 2010; Lu et al., 2011; Lin et al., 2013; Zhou et al., 2014; Meng et al., 2015; de Oliveira et al., 2016; Meng et al., 2016). The yeast expression vector *pESC-BAK1-MYC* and binary vector *pPZP212-pBAK1:BAK1-GFP* were obtained from Dr. Jianming Li (Nam and Li, 2002). The binary vector *pMDC32-2x35S::BAK1* was obtained from Dr. Chinchilla Delphine (Dominguez-Ferreras et al., 2015). The yeast expression vector *pYES2-BAK1-HA* was generated by sub-cloning *BAK1-HA* from *pHBT-35S::BAK1-HA* into a modified *pYES2* vector using primers listed in Table S1. Non-tagged full-length or truncated BAK1 in *pHBT* was cloned using *pHBT-35S::BAK1-FLAG* as the template and primers listed in Table S1. To construct *pCB302-35S::BAK1* and *pCB302-35S::BAK1-HA* binary vectors for *Agrobacterium*-mediated transformation in Arabidopsis, *BAK1* was sub-cloned into a modified plant transformation binary vector *pCB302* derivative under the control of the *35S* promoter with or without an HA-epitope tag at its C-terminus. To construct *pHBT-pBAK1::BAK1-GFP* and *pCB302-pBAK1::BAK1*, the *35S* promoter in *pHBT-35S::BAK1-GFP* or *pCB302-35S::BAK1* was substituted with the 1.5kb *BAK1* promoter that was amplified by PCR from Col-0 genomic DNA. BAK1/SERK point mutation variants were generated by site-directed mutagenesis with primers listed in Table S1 and using respective BAK1 or SERK4 *constructs* as the templates. Full-length *PSKR1* was amplified by PCR from Col-0 cDNA and cloned into a modified plant expression vector *pCAMBIA1300* with a HA tag.

The *BAK1* transgenic plants were generated by *Agrobacterium*-mediated transformation of Col-0, *bri1-5, sobir1-12, bik1* or *bak1/SRK4*$^{+/-}$, and screened with the herbicide BASTA (resistance conferred by the *pCB302* binary vector), antibiotic kanamycin (*pPZP212*) or hygromycin (*pMDC32*). BAK1 expression was detected by RT-PCR or Western blot with α-HA,

α-GFP or α-BAK1 antibodies. The homozygous lines were selected based on the survival ratio of T_2 and T_3 generation plants after BASTA spray or antibiotic selection.

pBAK1::BAK1^{D287A}-GFP plants expressing the ER marker were generated by *Agrobacterium*-mediated transformation with *p2x35S::mCherry-HDEL* (CD3-959, ER-rk) (Nelson et al., 2007). T1 plants were selected on kanamycin and transferred to ½ MS without antibiotic for 2 days before imaging.

Arabidopsis protoplast and *Nicotiana benthamiana* transient assays. For Arabidopsis protoplast transient expression, protoplasts were transfected with genes in the *pHBT* vector and incubated for 12 hr (He et al., 2007). The cell extracts were added with 2 × SDS loading buffer and subjected to immunoblot analysis.

For *N. benthamiana* transient expression, *Agrobacterium tumefaciens* strain GV3101 containing binary vector was cultured overnight in LB medium at 28 °C. Bacteria were harvested by centrifugation and resuspended with buffer (10 mM MES, pH 5.7, 10 mM $MgCl_2$, 200 μM acetosyringone) at A_{600} = 0.75. Leaves of four-week-old soil-grown *N. benthamiana* were hand-infiltrated using a needleless syringe with *Agrobacterium* cultures. Leaf samples were collected 36 hr after infiltration for protein isolation and immunoblot analysis. The cell death phenotype was observed and leaf pictures were taken three days after infiltration under UV light with a ChemiDoc system.

For electrolyte leakage assays, eight leaf discs (0.5 cm diameter) excised from *N. benthamiana* four days after infiltration was termed as one sample and was pre-floated in 10 ml of ddH_2O for 10-15 min to eliminate wounding effect. The ddH_2O was then exchanged and electrolyte leakage was measured using a conductivity meter (VWR; Traceable Conductivity Meter) as the average of three samples (n= 3).

***In vitro* phosphorylation, immunocomplex kinase and *in vivo* MAP kinase assays.** For *in vitro* kinase assay, reactions were performed in 30 μl of kinase buffer (20 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 5 mM EGTA, 100 mM NaCl, and 1 mM DTT) containing 10 μg of fusion proteins with 0.1 mM cold ATP and 5 μCi [^{32}P]-γ-ATP at room temperature for 3 hr with gentle shaking. The reactions were stopped by adding 4 x SDS loading buffer. The phosphorylation of fusion proteins was analyzed by autoradiography after separation with 12% SDS-PAGE.

For immunocomplex kinase assay, protoplasts were lysed with 0.5 mL of IP buffer (50 mM Tris·HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 1 mM DTT, 2 mM NaF, 2 mM Na_3VO_3, 1% Triton, and a protease inhibitor mixture from Roche). After centrifugation at 12,470 g for 10 min at 4°C, the supernatant was incubated with an α-FLAG antibody for 2 hr and then with protein-G–agarose beads for another 2 hr at 4°C with gentle shaking. The beads were collected and washed once with IP buffer and once with kinase buffer (20 mM Tris·HCl, pH 7.5, 20 mM $MgCl_2$, 5 mM EDTA, and 1 mM DTT). The kinase reactions were performed in 20 µl of kinase buffer with 2 µg of GST fusion proteins, 0.1 mM cold ATP, and 5 µCi of [^{32}P]-γ-ATP at room temperature for 1 hr with gentle shaking. The phosphorylation of GST fusion proteins was analyzed by 10% SDS-PAGE.

For detecting MAP kinase activity *in vivo*, two-week-old seedlings grown on ½ MS medium were transferred to water overnight and then treated with 100 nM flg22 or H_2O for the times indicated and frozen in liquid nitrogen. The seedlings were homogenized in IP buffer and equal amount of total protein was electrophoresed on 10% SDS–PAGE. An α-pERK antibody (1:2,000) (Cell Signaling) was used to detect phosphorylation status of MPK3 and MPK6 with an immunoblot.

Measurement of ROS production. Four leaves of each of six five-week-old Arabidopsis plants were excised into leaf discs of 0.25 cm^2, following an overnight incubation in 96-well plate with 100 µl of H_2O to eliminate the wounding effect. H_2O was replaced by 100 µl of reaction solution containing 50 µM luminol and 10 µg/ml horseradish peroxidase (Sigma) supplemented with 100 nM flg22. The luminescence was measured with a luminometer (Perkin Elmer, 2030 Multilabel Reader, Victor X3) immediately after adding the solution, with a 2 min interval reading time for a period of 60 min. The measurement value of ROS production from 24 leaf discs per treatment was indicated as the mean of RLU (Relative Light Units).

Callose deposition. The leaves of four-week-old plants were incubated with 500 nM flg22 for 12 hr at room temperature. Leaves were immediately cleared in alcoholic lactophenol [95% ethanol: lactophenol (phenol: glycerol: lactic acid: H_2O 1:1:1:1)=2:1] overnight. Samples were subsequently rinsed with 50% ethanol and H_2O. Cleared leaves were stained with 0.01% aniline blue in 0.15 M phosphate buffer (pH 9.5) and the callose deposits were visualized under a UV

599 filter using a fluorescence microscope. Callose deposits were counted using ImageJ 1.43U
600 software (http://rsb.info.nih.gov/ij/). The number of deposits was expressed as the mean of six
601 different leaf areas with standard error.
602
603 **Pathogen infection assays**. *Pseudomonas syringae* pv. *tomato* DC3000 strain was cultured
604 overnight at 28°C in KB medium with 50 μg/ml rifampicin. Bacteria were collected, washed and
605 diluted to the desired density with H_2O. Four-week-old Arabidopsis leaves were pre-inoculated
606 with 200 nM flg22 or H_2O control for 24 hr and then infiltrated with bacteria at a concentration
607 of 5 x 10^5 cfu/ml using a needleless syringe. To measure bacterial growth, two leaf discs were
608 ground in 100 μl of H_2O and serial dilutions were plated on KB medium with appropriate
609 antibiotics. Bacterial colony forming units (cfu) were counted two days after incubation at 28°C.
610 Each data point is shown as triplicates.
611
612 **Trypan blue and DAB staining.** For trypan blue staining, the leaves of four-week-old plants
613 were excised and subsequently immersed in boiled lactophenol (lactic acid: glycerol: liquid
614 phenol: distilled water 1:1:1:1) solution with 0.25 mg/ml trypan blue for 1 min. The stained
615 leaves were destained with 95% ethanol/lactophenol solution, and washed with 50% ethanol. For
616 DAB staining, the leaves of four-week-old plants were immersed in 1 mg/ml DAB (3,3'-
617 diaminobenzidine) (pH 3.8) solution with low vacuum pressure for 30 min, followed by an
618 overnight incubation at room temperature in the dark. The stained leaves were fixed and cleared
619 in alcoholic lactophenol (95% ethanol: lactic acid: phenol 2:1:1) at 65 °C, and rinsed once with
620 50% ethanol and twice with H_2O. The destained leaves were subjected to microscopic
621 observation.
622
623 ***Agrobacterium*-mediated virus-induced gene silencing assay.** Plasmids containing binary TRV
624 vectors *pTRV–RNA1* and *pTRV–RNA2* derivative *pYL156–SERK4* were introduced into
625 *Agrobacterium tumefaciens* strain GV3101 by electroporation. *Agrobacterium* cultures were first
626 grown overnight in LB medium containing 50 μg/ml kanamycin and 25 μg/ml gentamicin and
627 then sub-cultured in fresh LB medium containing 50 μg/ml kanamycin and 25 μg/ml gentamicin
628 supplemented with 10 mM MES and 20 μM acetosyringone overnight at 28 °C in a roller drum.
629 Cells were pelleted by 4,200 rpm centrifugation, resuspended in a solution containing 10 mM

$MgCl_2$, 10 mM MES and 200 μM acetosyringone, adjusted to A_{600}=1.5 and incubated at 25 °C for at least 3 hr. Agrobacterium cultures containing *pTRV–RNA1* and *pYL156–SERK4* were mixed in a 1:1 ratio and inoculated into the first pair of true leaves of two-week-old soil-grown plants using a needleless syringe.

Plasma membrane protein enrichment. Enrichment of plasma membrane proteins was performed as reported (Lee, 2017) with modifications. Briefly, *N. benthamiana* leaves weighing about 1g were ground in liquid N_2, and further lysed with 10 mL of ice-cold extraction buffer containing 100 mM Tris–HCl pH 8.8, 150 mM NaCl, 1 mM EDTA, 20% glycerol, 0.75% polyvinylpolypyrrolidone (PVPP), 20 mM NaF, 2 mM Na_3VO_4, 1 mM PMSF, and 1 complete protease inhibitor cocktail (Roche) per 50 mL. The lysate was centrifuged at 10,000 g at 4 °C for 10 min to pellet and remove intact cells, cell debris, and intact organelles. Microsomal membranes were then separated by ultracentrifugation at 100,000 g for 30 min at 4 °C and treated with detergent Brij58 (0.02%) to invert plasma membrane vesicles and release contaminating contents (Collins et al., 2017). Pellets were washed with extraction buffer and subjected to a final ultracentrifugation step to collect the enriched plasma membrane fraction.

Confocal microscopy and image analysis.

Five to seven-day-old Arabidopsis seedlings were imaged using Leica SP8X inverted confocal microscope equipped with a HC PL APO CS2 40×/1.10 water-corrected objective. The excitation wavelength was 488 nm for both GFP and FM4-64, and 561 nm for mCherry by using the white light laser. Emission was detected at 500–530 nm or 495-540 nm for GFP, 570–670 nm for FM4-64 and 600-700 nm for mCherry by using Leica hybrid detectors. Autofluorescence was removed by adjusting the time gate window between 1 and 4.5 ns or 0.3 and 6 ns. Intensities were manipulated with the Leica LAS-X standard software and FIJI software. The Pearson's correlation co-efficient (r value) was calculated using coloc-2 plugin included in Fiji software. ER-Tracker Red (BODIPY TR Glibenclamide; Life-Technologies) was used to stain ER in Arabidopsis roots following the manufacturer's protocol.

Accession Numbers

Sequence data from this article can be found in the Arabidopsis TAIR database under the following accession numbers: SERK1, AT1G71830; SERK2, AT1G34210; BAK1/SERK3, AT4G33430; SERK4, AT2G13790; FLS2, AT5G46330; BRI1, AT4G39400; BIK1, AT2G39660; SOBIR1, AT2G31880.

Supplemental Data

Supplemental Figure S1. Production of C-terminal BAK1 fragments

Supplemental Figure S2. Identification of BAK1 cleavage site by mutational analyses

Supplemental Figure S3. Phenotype of $BAK1^{D287A}$ transgenic plants

Supplemental Figure S4. The D287 site is critical for BAK1/SERK4-mediated cell death

Supplemental Figure S5. Colocalization of $BAK1^{D287A}$-GFP with endoplasmic reticulum (ER) marker

Supplemental Figure S6. The proteolytic cleavage of BAK1 in *DEK1*-silenced Arabidopsis

Supplemental Table S1. Primers for point mutations, gene cloning, genotyping and RT-qPCR

Figure Legends

Figure 1. Proteolytic processing of BAK1 in plants by a conserved protease

(A) Expression of BAK1-HA in Arabidopsis protoplasts. Protein extracts from Arabidopsis protoplasts transfected with *35S::BAK1-HA* or a control vector (Ctrl) were analyzed by Western blot (WB) with an α-HA antibody. The upper band corresponding to the full-length BAK1 protein was indicated by FL, and the lower band corresponding to the C-terminal fragment of BAK1 was indicated by CTF, and labeled with a red star. **(B & C)** $BAK1^{CTF}$ is produced in *Arabidopsis* transgenic plants. Protein extracts from *35S::BAK1-HA* (B) and *pBAK1::BAK1-GFP* (C) transgenic plants were analyzed by WB with respective α-HA and α-GFP antibodies. Wild-type Col-0 Arabidopsis was used as Ctrl. **(D)** Non-tagged $BAK1^{CTF}$ is produced in Arabidopsis protoplasts. Protein extracts from Arabidopsis protoplasts transfected with *35S::BAK1* were analyzed by WB with an α-BAK1 antibody. **(E & F)** Effects of chemical inhibitors on the production of $BAK1^{CTF}$. 1 mM EDTA, 1 mM EGTA, 1 mM $LaCl_3$, 0.5 mM $GdCl_3$, 2 μM MG132 or 2 μM Lactacystin was added immediately after protoplast transfection with *35S::BAK1-FLAG*. Protein extracts were analyzed by WB with an α-FLAG antibody. Lactacystin and MG132 were stored in DMSO, and other chemicals were stored in H_2O. **(G)** Effects of

Calpain inhibitors on the production of BAK1^{CTF}. 20 μM ALLN, Calpian inhibitor III, Calpeptin, PD150606 or EST (Calbiochem, cat.# 208733) was added immediately after protoplast transfection with *35S::BAK1-FLAG*. Protein extracts were analyzed by WB with an α-FLAG antibody. EST was stored in ethanol (ETOH), and other inhibitors were stored in DMSO. **(H & I)** BAK1^{CTF} is produced in *Nicotiana benthamiana* and *Saccharomyces cerevisiae.* Protein extracts from *N. benthamiana* transiently expressing *35S::BAK1-HA* (H) and *S. cerevisiae* expressing *pGAL1::BAK1-HA* (I) were analyzed by WB with α-HA antibodies. **(J)** Proteolytic cleavage is conserved in SERK family members. Protein extracts from Arabidopsis protoplasts expressing FLAG-tagged SERK1, SERK2, SERK3 (BAK1) or SERK4 were analyzed by WB with α-FLAG antibodies.

The above experiments were repeated three times with similar results.

Figure 2. Regulation of BAK1^{CTF} production

(A) Production of BAK1^{CTF} in *pBAK1::BAK-GFP* transgenic plants upon *Pst hrcC* infection. Four-week-old soil-grown Arabidopsis *pBAK1::BAK-GFP* transgenic plants were hand-inoculated with *Pst hrcC* at 5×10^5 cfu. Total proteins from inoculated leaf extracts were analyzed by WB with an α-GFP antibody. hpi, hours post inoculation. **(B)** Production of BAK1^{CTF} in WT plants upon infections. Four-week-old soil-grown Arabidopsis Col-0 plants were hand-inoculated with *Pst hrcC* at 5×10^5 cfu or 1 μM flg22. Total proteins from inoculated leaf extracts were analyzed by WB with an α-BAK1 antibody. **(C)** Developmental regulation of BAK1^{CTF} production. Total proteins extracted from Arabidopsis *35S::BAK1-HA* transgenic plants at different growth stages from 8 through 35 days post germination (dpg) on ½ MS plates were analyzed by WB with an α-HA antibody. **(D)** BAK1^{CTF} production in different tissues. Total proteins extracted from different tissues of two-month-old soil-grown *35S::BAK1-HA* transgenic plants were analyzed by WB with an α-HA antibody. F: flowers, S: stem; CL: cauline leaves; RL: rosette leaves; R: roots.

The above experiments were repeated three times with similar results.

Figure 3. The D287A mutation of BAK1 blocks its cleavage

(A) Scheme of BAK1 protein domains and sites for mutagenesis. E: extracellular LRR domain; T: transmembrane domain; J: juxtamembrane domain; K: intracellular kinase domain. **(B)** VASD

residues are required for BAK1 cleavage. Arabidopsis protoplasts expressing HA-tagged BAK1 mutants, in which the indicated four amino acids were mutagenized to alanine, were analyzed by WB with an α-HA antibody. **(C)** D287 residue is required for BAK1 cleavage in Arabidopsis protoplasts. **(D)** D287 residue is required for BAK1 cleavage in *N. benthamiana*. *N. benthamiana* leaves transiently expressing HA-tagged BAK1 or $BAK1^{D287A}$ mutant were analyzed by WB with α-HA antibody. **(E)** D287 residue is required for BAK1 cleavage in Arabidopsis *35S::BAK1-HA* transgenic plants. **(F)** D287 residue is required for BAK1 cleavage in Arabidopsis *pBAK1::BAK1-GFP* transgenic plants. **(G)** D287E mutation blocks BAK1 cleavage in Arabidopsis protoplasts. **(H)** D287 residue of BAK1 is conserved among Arabidopsis SERKs. **(I)** BAK1 D287 corresponding residue in other SERK members is required for cleavage in Arabidopsis protoplasts.

The above experiments were repeated three times with similar results.

Figure 4. Compromised immune responses in *pBAK1::BAK1^{D287A}/bak1* transgenic plants

(A) flg22-induced MAPK activation. Two-week-old seedlings of WT Col-0, *bak1-4*, *pBAK1::BAK1/bak1* and *pBAK1::BAK1^{D287A}/bak1* were treated with 100 nM flg22 for 15 min. Phosphorylated MPK3 (pMPK3) and MPK6 (pMPK6) were detected by WB with an α-pERK antibody (top). Protein loading is shown by Coomassie Brilliant Blue (CBB) staining for RuBisCo (RBC) (bottom). **(B)** flg22-induced ROS production. Leaf discs from four leaves (technical repeats) of each of six five-week-old plants (biological repeats) of indicated genotypes were treated with 100 nM flg22, and ROS production was detected at the indicated time points. The data are shown as the mean ± SD from six biological repeats. **(C)** flg22-induced callose deposition. Leaves of four-week-old plants were collected for aniline blue staining 12 hr after inoculation with 500 nM flg22. Callose deposits were counted using ImageJ 1.43U software (http://rsb.info.nih.gov/ij/). The data are shown as the mean ± SD from six biological repeats. **(D)** flg22-mediated resistance to bacterial infection. Four-week-old plants were pretreated with 200 nM of flg22 or water and then infected with *Pst* DC3000 at 5×10^5 cfu/ml. The bacterial growth assays were performed 2 days after infection. The data are shown as the mean ± SD from three biological repeats. The asterisks indicate statistical significance compared to H_2O pretreatment by using student's *t*-test ($P<0.05$). **(E)** flg22-induced BIK1 mobility shift. Arabidopsis protoplasts isolated from Col-0, *bak1-4* mutant, *pBAK1::BAK1/bak1* and

753 *pBAK1::BAK1^{D287A}/bak1* transgenic plants were used to express BIK1-HA and treated with 100
754 nM flg22 for 15 min. Mobility shift of BIK1 was detected by WB with an α-HA antibody (top).
755 Equal loading of protein was indicated by CBB staining towards RBC (bottom). pBIK1,
756 phosphorylated BIK1. **(F)** The kinase activity of BAK1 and BAK1^{D287A}. Full length BAK1-
757 FLAG or BAK1^{D287A}-FLAG were expressed in Arabidopsis Col-0 protoplasts and precipitated
758 with an α-FLAG antibody. Kinase activity of the precipitated proteins were detected using GST-
759 BIK1^{KM} (kinase mutant) as a substrate. Phosphorylation was detected by autoradiography (top),
760 and the protein loading is shown by WB with an α-FLAG antibody (bottom). **(G)** The *in vitro*
761 kinase activity of the cytosolic domain of BAK1 and BAK1^{D287A}. GST-BIK1^{KM} protein was used
762 as a substrate and MBP-BAK1^{CD} (cytosolic domain) or its D287A mutant was used as the kinase
763 in an *in vitro* kinase assay. Phosphorylation was detected by autoradiography (top), and the
764 protein loading is shown by CBB staining (bottom).
765 The above experiments were repeated three times with similar results.
766
767 **Figure 5. BAK1 D287 is critical for BR signaling and cell death control**

768 **(A)** The reduced hypocotyl length of *pBAK1::BAK1^{D287A}/bak1* transgenic plants. The seedlings
769 of WT, *bak1-4* and transgenic plants were grown in the dark for 5 days on ½ MS plats without or
770 with 1 μM of BRZ. **(B)** Quantification of the hypocotyl length shown in (A). The data are shown
771 as the mean ± SD from 20 biological repeats. The different letters indicate statistically significant
772 difference analyzed with one-way ANOVA followed by Tukey's test ($P<0.05$). **(C)** BAK1^{D287A}
773 mutant cannot restore *bri1-5* dwarf defect. Transgenic plants of *35S::BAK1* and *35S::BAK1^{D287A}*
774 in *bri1-5* background were taken photos at two months old. The individual leaves and siliques
775 are shown in the middle and bottom. **(D)** Expression of BAK1 protein in rosette leaves from
776 four-week-old plants of (C) was detected by WB with an α-BAK1 antibody. **(E)** D287 is
777 important for BAK1 function in cell death control. Arabidopsis *BAK1$^{+/-}$SERK4$^{-/-}$* plants were
778 transformed with *pBAK1::BAK1* or *pBAK1::BAK1^{D287A}*. Plants of T_1 generation after selection of
779 BAK1 or BAK1^{D287A} transgene were genotyped for endogenous genomic BAK1. Pictures were
780 taken four weeks after germination. *BAK1$^{+/+}$SERK4$^{-/-}$* (BAK1: WT, SERK4: mutant); *BAK1$^{+/-}$*
781 *SERK4$^{-/-}$* (BAK1: heterozygous, SERK4: mutant); *BAK1$^{-/-}$SERK4$^{-/-}$* (BAK1: mutant, SERK4:
782 mutant). Scale bars, 1 cm.
783 The above experiments were repeated three times with similar results.

Figure 6. D287 is critical for the plasma membrane localization of BAK1

(A) Subcellular localization of BAK1-GFP (top panel) and BAK1^{D287A}-GFP (bottom panel) in root epidermis of five-day-old Arabidopsis seedlings. The plasma membrane was stained with FM4-64 (2 μM). **(B)** Pearson's correlation coefficient (*r* value) for the colocalization between GFP and FM4-64 fluorescence in the plasma membrane. These values were measured in 15 cells. Error bars indicate mean ± SD. P-value (*t*-test), * P<0.01. **(C)** Colocalization of BAK1^{D287A}-GFP with endoplasmic reticulum (ER) marker, mCherry-HDEL. Scale bars, 20 μm. **(D)** Leaves of four-week-old *N. benthamiana* were hand-inoculated with Agrobacteria carrying *35S::BAK1-HA* or *35S::BAK1^{D287A}-HA* together with *35S::PSKR1-HA*. BAK1 from total proteins and enriched plasma membrane (PM) proteins were analyzed by WB with an α-HA antibody. Cytosolic MPK6 was detected by α-MPK6 WB. PSKR1 is a plasma membrane-localized protein.
The above experiments were repeated three times with similar results.

Figure 7. Over-production of BAK1 ^{D287A} enhances SoBIR1-dependent cell death

(A) Expression of BAK1 and BAK1^{D287A} under different promoters in *N. benthamiana*. Leaves of four-week-old *N. benthamiana* were hand-inoculated with *Agrobacteria* carrying *35S::GFP, 2x35S::BAK1, 2x35S::BAK1^{D287A}, 35S::BAK1, 35S::BAK1^{D287A}, pBAK1::BAK1 or pBAK1::BAK1^{D287A}*. Pictures were taken under UV light (left) or visible light (right) three days after inoculation. Two days after inoculation and before cell death progressed, protein levels of BAK1 were analyzed by WB with an α-BAK1 antibody (bottom). BAK1 proteins from *2x35S::BAK1* (2) and *2x35S::BAK1^{D287A}* (3) were detected by WB using SuperSignal™ West Pico Chemiluminescent Substrate, and BAK1 proteins from *35S::BAK1* (4) and *35S::BAK1^{D287A}* (5) were detected using Thermo Scientific™ SuperSignal™ West Femto Maximum Sensitivity Substrate. **(B)** The fluorescent signal from the circled areas in (A) was obtained under the UV light and was quantified as means ± SD from ten biological repeats. The asterisks indicate statistical significance by using student's *t*-test (P<0.05). **(C)** Measurement of electrolyte leakage of leaf discs from (A). Data are shown as means ± SD from three biological repeats. The asterisks indicate statistical significance by using student's *t*-test (P<0.05). **(D)** Overexpression of BAK1 and BAK1^{D287A} in Arabidopsis. Arabidopsis plants overexpressing *35S::BAK1* or *35S::BAK1^{D287A}* were taken photos at four-week-old stage. Each phenotype of T_1 generation

plants was calculated as a percentage to the total transgenic plants of that genotype. **(E)** Cell death and H_2O_2 accumulation in the leaves of four-week-old plants were examined by trypan blue and DAB staining respectively. B: normal-looking plants; S: small plants. **(F)** Expression levels of *PR* genes in one-week-old transgenic plants were determined by RT-qPCR. The data are shown as means ± SD from three biological repeats. The asterisks indicate statistical significance by using student's *t*-test ($P<0.05$). **(G)** BAK1 and $BAK1^{D287A}$ cell death is SoBIR1-dependent and BIK1-independent. *35S::BAK1* and *35S::BAK1*^{D287A} were transformed into *sobir1* and *bik1* mutant backgrounds. Each phenotype of T_1 generation at four-week-old stage was calculated as a percentage as in (D).

The above experiments were repeated three times with similar results.

Acknowledgements: We thank Jianming Li and Delphine Chinchilla for providing materials. The work was supported by National Institutes of Health (NIH) (R01GM092893) and the National Science Foundation (NSF) (IOS-1252539) to P.H., NIH (R01GM097247), the Robert A. Welch foundation (A-1795) to L.S., the Young Eastern Scholar (QD2016035) and Shanghai Sailing Program (17YF1406400) to J.Z., China Scholar Council to P. W. and G. X., and the Research Foundation-Flanders (G022516N and G0E5718N) to E.R. The authors have declared no conflict of interests.

833

Parsed Citations

Ancot F, Foveau B, Lefebvre J, Leroy C, Tulasne D (2009) Proteolytic cleavages give receptor tyrosine kinases the gift of ubiquity. Oncogene 28: 2185-2195
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Antolin-Llovera M, Ried MK, Parniske M (2014) Cleavage of the SYMBIOSIS RECEPTOR-LIKE KINASE ectodomain promotes complex formation with Nod factor receptor 5. Curr Biol 24: 422-427
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Belkhadir Y, Wang X, Chory J (2006) Arabidopsis brassinosteroid signaling pathway. Sci STKE 2006: cm5
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Belkhadir Y, Yang L, Hetzel J, Dangl JL, Chory J (2014) The growth-defense pivot: crisis management in plants mediated by LRR-RK surface receptors. Trends Biochem Sci 39: 447-456
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Boller T, Felix G (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu Rev Plant Biol 60: 379-406
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Carpenter G, Liao HJ (2013) Receptor tyrosine kinases in the nucleus. Cold Spring Harb Perspect Biol 5: a008979
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chen M, Chen LM, Lin CY, Chai KX (2008) The epidermal growth factor receptor (EGFR) is proteolytically modified by the Matriptase-Prostasin serine protease cascade in cultured epithelial cells. Biochim Biophys Acta 1783: 896-903
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chen MK, Hung MC (2015) Proteolytic cleavage, trafficking, and functions of nuclear receptor tyrosine kinases. FEBS J 282: 3693-3721
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nurnberger T, Jones JD, Felix G, Boller T (2007) A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature 448: 497-500
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Collins CA, Leslie ME, Peck SC, Heese A (2017) Simplified Enrichment of Plasma Membrane Proteins from Arabidopsis thaliana Seedlings Using Differential Centrifugation and Brij-58 Treatment. Methods Mol Biol 1564: 155-168
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

de Oliveira MV, Xu G, Li B, de Souza Vespoli L, Meng X, Chen X, Yu X, de Souza SA, Intorne AC, de AMAM, Musinsky AL, Koiwa H, de Souza Filho GA, Shan L, He P (2016) Specific control of Arabidopsis BAK1/SERK4-regulated cell death by protein glycosylation. Nat Plants 2: 15218
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Dean P (2011) Functional domains and motifs of bacterial type III effector proteins and their roles in infection. FEMS Microbiol Rev 35: 1100-1125
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Dominguez-Ferreras A, Kiss-Papp M, Jehle AK, Felix G, Chinchilla D (2015) An Overdose of the Arabidopsis Coreceptor BRASSINOSTEROID INSENSITIVE1-ASSOCIATED RECEPTOR KINASE1 or Its Ectodomain Causes Autoimmunity in a SUPPRESSOR OF BIR1-1-Dependent Manner. Plant Physiol 168: 1106-1121
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Du JB, Gao Y, Zhan YY, Zhang SS, Wu YJ, Xiao Y, Zou B, He K, Gou XP, Li GJ, Lin HH, Li J (2016) Nucleocytoplasmic trafficking is essential for BAK1-and BKK1-mediated cell-death control. Plant J 85: 520-531
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ewald SE, Lee BL, Lau L, Wickliffe KE, Shi GP, Chapman HA, Barton GM (2008) The ectodomain of Toll-like receptor 9 is cleaved to generate a functional receptor. Nature 456: 658-662
Pubmed: Author and Title

Google Scholar: Author Only Title Only Author and Title

Fenteany G, Standaert RF, Lane WS, Choi S, Corey EJ, Schreiber SL (1995) Inhibition of proteasome activities and subunit-specific amino-terminal threonine modification by lactacystin. Science 268: 726-731
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gao M, Wang X, Wang D, Xu F, Ding X, Zhang Z, Bi D, Cheng YT, Chen S, Li X, Zhang Y (2009) Regulation of cell death and innate immunity by two receptor-like kinases in Arabidopsis. Cell Host Microbe 6: 34-44
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gomez-Gomez L, Boller T (2000) FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. Mol Cell 5: 1003-1011
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gou X, Yin H, He K, Du J, Yi J, Xu S, Lin H, Clouse SD, Li J (2012) Genetic evidence for an indispensable role of somatic embryogenesis receptor kinases in brassinosteroid signaling. PLoS Genet 8: e1002452
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Hayashida K, Bartlett AH, Chen Y, Park PW (2010) Molecular and cellular mechanisms of ectodomain shedding. Anat Rec (Hoboken) 293: 925-937
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

He K, Gou X, Yuan T, Lin H, Asami T, Yoshida S, Russell SD, Li J (2007) BAK1 and BKK1 regulate brassinosteroid-dependent growth and brassinosteroid-independent cell-death pathways. Curr Biol 17: 1109-1115
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

He P, Shan L, Sheen J (2007) The use of protoplasts to study innate immune responses. Methods Mol Biol 354: 1-9
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

He Y, Zhou J, Shan L, Meng X (2018) Plant cell surface receptor-mediated signaling - a common theme amid diversity. J Cell Sci 131: jcs209353
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Heese A, Hann DR, Gimenez-Ibanez S, Jones AME, He K, Li J, Schroeder JI, Peck SC, Rathjen JP (2007) The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. Proc Natl Acad Sci USA 104: 12217-12222
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Hou S, Jamieson P, He P (2018) The cloak, dagger, and shield: proteases in plant-pathogen interactions. Biochem J 475: 2491-2509
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Johnson KL, Degnan KA, Ross Walker J, Ingram GC (2005) AtDEK1 is essential for specification of embryonic epidermal cell fate. Plant J 44: 114-127
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Johnson KL, Faulkner C, Jeffree CE, Ingram GC (2008) The phytocalpain defective kernel 1 is a novel Arabidopsis growth regulator whose activity is regulated by proteolytic processing. Plant Cell 20: 2619-2630
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Karlova R, Boeren S, Russinova E, Aker J, Vervoort J, de Vries S (2006) The Arabidopsis SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1 protein complex includes BRASSINOSTEROID-INSENSITIVE1. Plant Cell 18: 626-638
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kemmerling B, Schwedt A, Rodriguez P, Mazzotta S, Frank M, Qamar SA, Mengiste T, Betsuyaku S, Parker JE, Mussig C, Thomma BP, Albrecht C, de Vries SC, Hirt H, Nurnberger T (2007) The BRI1-associated kinase 1, BAK1, has a brassinolide-independent role in plant cell-death control. Curr Biol 17: 1116-1122
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ladwig F, Dahlke RI, Stuhrwohldt N, Hartmann J, Harter K, Sauter M (2015) Phytosulfokine Regulates Growth in Arabidopsis through a Response Module at the Plasma Membrane That Includes CYCLIC NUCLEOTIDE-GATED CHANNEL17, H+-ATPase, and BAK1. Plant Cell 27: 1718-1729
Pubmed: Author and Title

Google Scholar: Author Only Title Only Author and Title

Lee JS (2017) Purification of Plant Receptor Kinases from Plant Plasma Membranes. Methods Mol Biol 1621: 47-56
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Li J, Chory J (1997) A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell 90: 929-938
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Li J, Lease KA, Tax FE, Walker JC (2001) BRS1, a serine carboxypeptidase, regulates BRI1 signaling in Arabidopsis thaliana. Proc Natl Acad Sci USA 98: 5916-5921
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Li J, Wen J, Lease KA, Doke JT, Tax FE, Walker JC (2002) BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. Cell 110: 213-222
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Li L, Kim P, Yu L, Cai G, Chen S, Alfano JR, Zhou JM (2016) Activation-Dependent Destruction of a Co-receptor by a Pseudomonas syringae Effector Dampens Plant Immunity. Cell Host Microbe 20: 504-514
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Li Z, Wang Y, Huang J, Ahsan N, Biener G, Paprocki J, Thelen JJ, Raicu V, Zhao D (2017) Two SERK Receptor-Like Kinases Interact with EMS1 to Control Anther Cell Fate Determination. Plant Physiol 173: 326-337
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Liang X, Zhou JM (2018) Receptor-Like Cytoplasmic Kinases: Central Players in Plant Receptor Kinase-Mediated Signaling. Annu Rev Plant Biol 69: 267-299
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Liao HJ, Carpenter G (2012) Regulated intramembrane cleavage of the EGF receptor. Traffic 13: 1106-1112
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lid SE, Olsen L, Nestestog R, Aukerman M, Brown RC, Lemmon B, Mucha M, Opsahl-Sorteberg HG, Olsen OA (2005) Mutation in the Arabidopisis thaliana DEK1 calpain gene perturbs endosperm and embryo development while over-expression affects organ development globally. Planta 221: 339-351
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Liebrand TW, van den Burg HA, Joosten MH (2014) Two for all: receptor-associated kinases SOBIR1 and BAK1. Trends Plant Sci 19: 123-132
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lin W, Li B, Lu D, Chen S, Zhu N, He P, Shan L (2014) Tyrosine phosphorylation of protein kinase complex BAK1/BIK1 mediates Arabidopsis innate immunity. Proc Natl Acad Sci USA 111: 3632-3637
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lin W, Lu D, Gao X, Jiang S, Ma X, Wang Z, Mengiste T, He P, Shan L (2013) Inverse modulation of plant immune and brassinosteroid signaling pathways by the receptor-like cytoplasmic kinase BIK1. Proc Natl Acad Sci USA 110: 12114-12119
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lin W, Ma X, Shan L, He P (2013) Big roles of small kinases: the complex functions of receptor-like cytoplasmic kinases in plant immunity and development. J Integr Plant Biol 55: 1188-1197
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

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
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Liu Y, Li J (2014) Endoplasmic reticulum-mediated protein quality control in Arabidopsis. Front Plant Sci 5: 162
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lu D, Lin W, Gao X, Wu S, Cheng C, Avila J, Heese A, Devarenne TP, He P, Shan L (2011) Direct ubiquitination of pattern recognition

receptor FLS2 attenuates plant innate immunity. Science 332: 1439-1442
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lu D, Wu S, Gao X, Zhang Y, Shan L, He P (2010) A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. Proc Natl Acad Sci USA 107: 496-501
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ma X, Xu G, He P, Shan L (2016) SERKing Coreceptors for Receptors. Trends Plant Sci 21: 1017-1033
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Macho AP, Zipfel C (2014) Plant PRRs and the activation of innate immune signaling. Mol Cell 54: 263-272
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Meng X, Chen X, Mang H, Liu C, Yu X, Gao X, Torii KU, He P, Shan L (2015) Differential Function of Arabidopsis SERK Family Receptor-like Kinases in Stomatal Patterning. Curr Biol 25: 2361-2372
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Meng X, Zhou J, Tang J, Li B, de Oliveira MV, Chai J, He P, Shan L (2016) Ligand-Induced Receptor-like Kinase Complex Regulates Floral Organ Abscission in Arabidopsis. Cell Rep 14: 1330-1338
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Nam KH, Li J (2002) BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell 110: 203-212
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Nelson BK, Cai X, Nebenfuhr A (2007) A multicolored set of in vivo organelle markers for co-localization studies in Arabidopsis and other plants. Plant J 51: 1126-1136
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ntoukakis V, Schwessinger B, Segonzac C, Zipfel C (2011) Cautionary notes on the use of C-terminal BAK1 fusion proteins for functional studies. Plant Cell 23: 3871-3878
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ono Y, Sorimachi H (2012) Calpains: an elaborate proteolytic system. Biochim Biophys Acta 1824: 224-236
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ou Y, Lu X, Zi Q, Xun Q, Zhang J, Wu Y, Shi H, Wei Z, Zhao B, Zhang X, He K, Gou X, Li C, Li J (2016) RGF1 INSENSITIVE 1 to 5, a group of LRR receptor-like kinases, are essential for the perception of root meristem growth factor 1 in Arabidopsis thaliana. Cell Res 26: 686-698
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Park B, Brinkmann MM, Spooner E, Lee CC, Kim YM, Ploegh HL (2008) Proteolytic cleavage in an endolysosomal compartment is required for activation of Toll-like receptor 9. Nat Immunol 9: 1407-1414
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Park CJ, Ronald PC (2012) Cleavage and nuclear localization of the rice XA21 immune receptor. Nat Commun 3: 920
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Perraki A, DeFalco TA, Derbyshire P, Avila J, Sere D, Sklenar J, Qi X, Stransfeld L, Schwessinger B, Kadota Y, Macho AP, Jiang S, Couto D, Torii KU, Menke FLH, Zipfel C (2018) Phosphocode-dependent functional dichotomy of a common co-receptor in plant signalling. Nature 561: 248-252
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Petes C, Odoardi N, Gee K (2017) The Toll for Trafficking: Toll-Like Receptor 7 Delivery to the Endosome. Front Immunol 8: 1075
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Petutschnig EK, Stolze M, Lipka U, Kopischke M, Horlacher J, Valerius O, Rozhon W, Gust AA, Kemmerling B, Poppenberger B, Braus GH, Nurnberger T, Lipka V (2014) A novel Arabidopsis CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) mutant with enhanced pathogen-induced cell death and altered receptor processing. New Phytol 204: 955-967
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Pogany M, Danko T, Kaman-Toth E, Schwarczinger I, Bozso Z (2015) Regulatory Proteolysis in Arabidopsis-Pathogen Interactions. Int J Mol Sci 16: 23177-23194
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Postel S, Kufner I, Beuter C, Mazzotta S, Schwedt A, Borlotti A, Halter T, Kemmerling B, Nurnberger T (2010) The multifunctional leucine-rich repeat receptor kinase BAK1 is implicated in Arabidopsis development and immunity. Eur J Cell Biol. 89: 169-174
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Roux M, Schwessinger B, Albrecht C, Chinchilla D, Jones A, Holton N, Malinovsky FG, Tor M, de Vries S, Zipfel C (2011) The Arabidopsis leucine-rich repeat receptor-like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens. Plant Cell 23: 2440-2455
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Russinova E, Borst JW, Kwaaitaal M, Cano-Delgado A, Yin Y, Chory J, de Vries SC (2004) Heterodimerization and endocytosis of Arabidopsis brassinosteroid receptors BRI1 and AtSERK3 (BAK1). Plant Cell 16: 3216-3229
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Santiago J, Brandt B, Wildhagen M, Hohmann U, Hothorn LA, Butenko MA, Hothorn M (2016) Mechanistic insight into a peptide hormone signaling complex mediating floral organ abscission. Elife 5: e15075
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Santiago J, Henzler C, Hothorn M (2013) Molecular mechanism for plant steroid receptor activation by somatic embryogenesis co-receptor kinases. Science 341: 889-892
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Shan L, He P, Li J, Heese A, Peck SC, Nurnberger T, Martin GB, Sheen J (2008) Bacterial effectors target the common signaling partner BAK1 to disrupt multiple MAMP receptor-signaling complexes and impede plant immunity. Cell Host Microbe 4: 17-27
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Shao F, Golstein C, Ade J, Stoutemyer M, Dixon JE, Innes RW (2003) Cleavage of Arabidopsis PBS1 by a bacterial type III effector. Science 301: 1230-1233
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Shiu SH, Bleecker AB (2003) Expansion of the receptor-like kinase/Pelle gene family and receptor-like proteins in Arabidopsis. Plant Physiol 132: 530-543
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Smolarkiewicz M, Skrzypczak T, Michalak M, Lesniewicz K, Walker JR, Ingram G, Wojtaszek P (2014) Gamma-secretase subunits associate in intracellular membrane compartments in Arabidopsis thaliana. J Exp Bot 65: 3015-3027
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Song W, Liu L, Wang J, Wu Z, Zhang H, Tang J, Lin G, Wang Y, Wen X, Li W, Han Z, Guo H, Chai J (2016) Signature motif-guided identification of receptors for peptide hormones essential for root meristem growth. Cell Res 26: 674-685
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Sun Y, Han Z, Tang J, Hu Z, Chai C, Zhou B, Chai J (2013) Structure reveals that BAK1 as a co-receptor recognizes the BRI1-bound brassinolide. Cell Res 23: 1326-1329
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Sun Y, Li L, Macho AP, Han Z, Hu Z, Zipfel C, Zhou JM, Chai J (2013) Structural basis for flg22-induced activation of the Arabidopsis FLS2-BAK1 immune complex. Science 342: 624-628
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Tintor N, Saijo Y (2014) ER-mediated control for abundance, quality, and signaling of transmembrane immune receptors in plants. Front Plant Sci 5: 65
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Tsubuki S, Saito Y, Tomioka M, Ito H, Kawashima S (1996) Differential inhibition of calpain and proteasome activities by peptidyl aldehydes of di-leucine and tri-leucine. J Biochem 119: 572-576
Pubmed: Author and Title

Google Scholar: Author Only Title Only Author and Title

Villamor JG, Kaschani F, Colby T, Oeljeklaus J, Zhao D, Kaiser M, Patricelli MP, van der Hoorn RA (2013) Profiling protein kinases and other ATP binding proteins in Arabidopsis using Acyl-ATP probes. Mol Cell Proteomics 12: 2481-2496
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wang J, Li H, Han Z, Zhang H, Wang T, Lin G, Chang J, Yang W, Chai J (2015) Allosteric receptor activation by the plant peptide hormone phytosulfokine. Nature 525: 265-268
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wang X, Kota U, He K, Blackburn K, Li J, Goshe MB, Huber SC, Clouse SD (2008) Sequential transphosphorylation of the BRI1/BAK1 receptor kinase complex impacts early events in brassinosteroid signaling. Dev Cell 15: 220-235
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Yan L, Ma Y, Liu D, Wei X, Sun Y, Chen X, Zhao H, Zhou J, Wang Z, Shui W, Lou Z (2012) Structural basis for the impact of phosphorylation on the activation of plant receptor-like kinase BAK1. Cell Res 22: 1304-1308
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Yu X, Feng B, He P, Shan L (2017) From Chaos to Harmony: Responses and Signaling upon Microbial Pattern Recognition. Annu Rev Phytopathol 55: 109-137
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zatz M, Starling A (2005) Calpains and disease. N Engl J Med 352: 2413-2423
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zhang J, Li W, Xiang T, Liu Z, Laluk K, Ding X, Zou Y, Gao M, Zhang X, Chen S, Mengiste T, Zhang Y, Zhou JM (2010) Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted by a Pseudomonas syringae effector. Cell Host Microbe 7: 290-301
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zhou A, Li J (2005) Arabidopsis BRS1 is a secreted and active serine carboxypeptidase. J Biol Chem 280: 35554-35561
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zhou J, Wu S, Chen X, Liu C, Sheen J, Shan L, He P (2014) The Pseudomonas syringae effector HopF2 suppresses Arabidopsis immunity by targeting BAK1. Plant J 77: 235-245
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JD, Boller T, Felix G (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation. Cell 125: 749-760
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title