SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURES

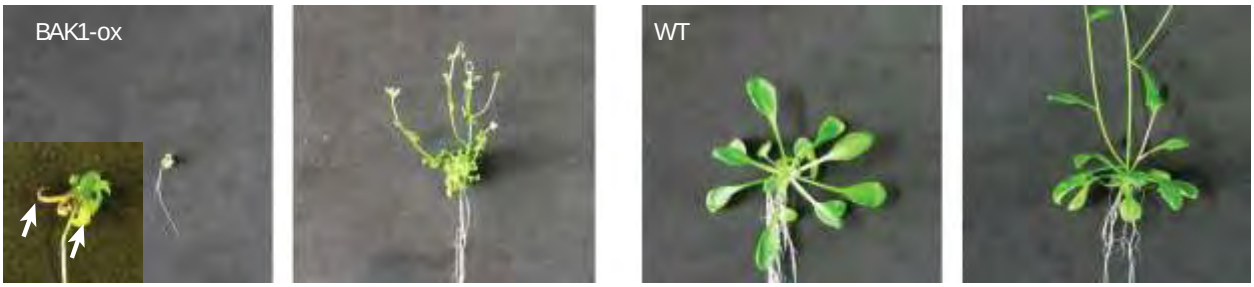


Figure S1. Phenotype of BAK1-ox Arabidopsis plants grown under axenic conditions. Necrosis (white arrows) and growth inhibition were observed on BAK1-ox plants but not on wild-type Col-0 plants (WT) after 6 weeks of growth under axenic conditions. This experiment was repeated twice with similar results.

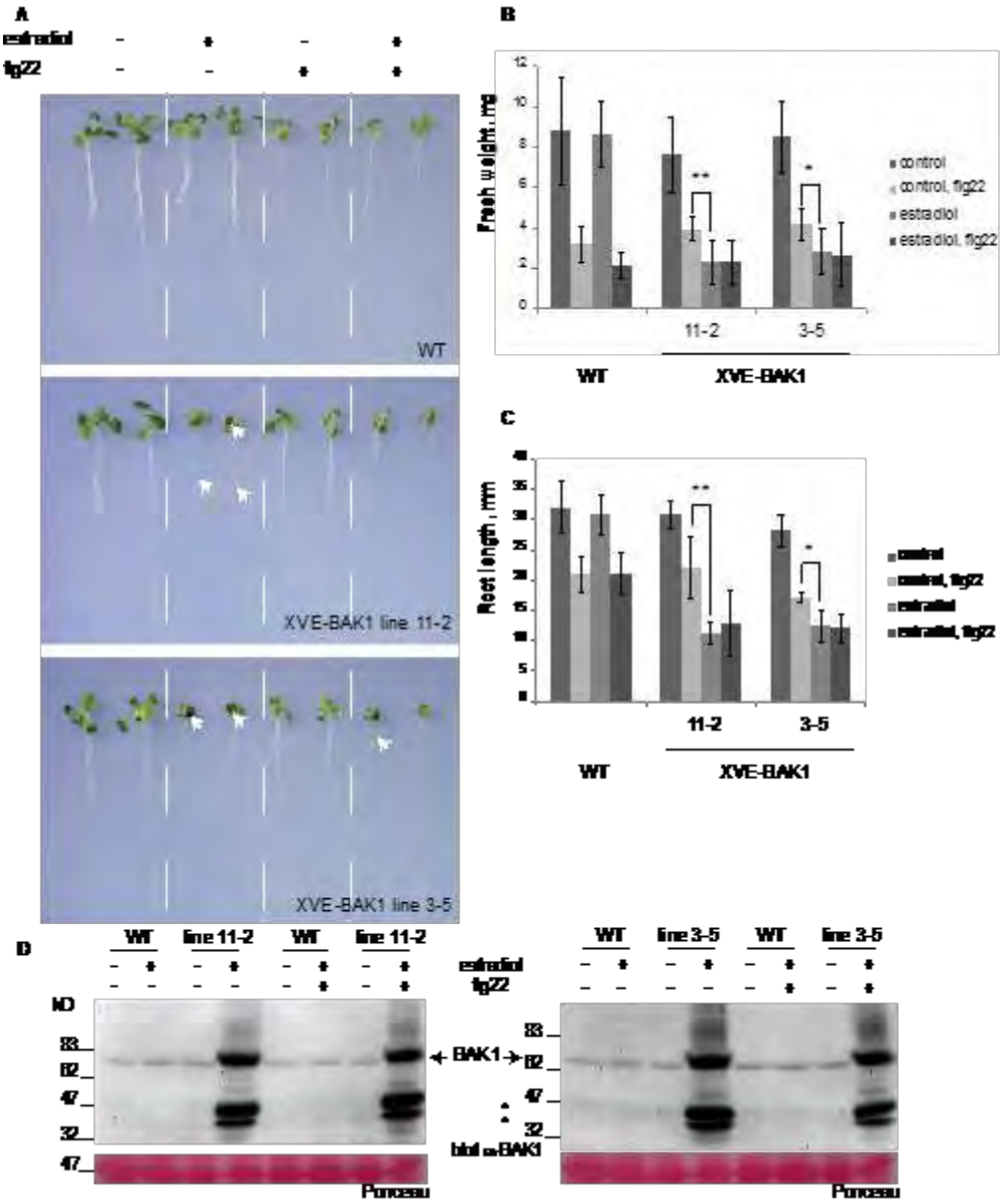


Figure S2. Seedling growth of XVE-BAK1 lines. A, Pictures of wild-type (WT) and transgenic seedlings (two independent XVE-BAK1 homozygous lines named 11-2 and 3-5) after 6 days of growth in presence or not of estradiol (1 µM) and/or flg22 (1 µM) used as MAMP control. White arrows indicate a brownish coloration on cotyledon leaves and roots. B and C, Quantification of fresh weight (mg) and root length (mm) of the corresponding seedlings. Bars show averages and standard deviations for n=6 technical replicates. Statistics were performed using ANOVA and significant differences between flg22 and estradiol treatments are marked by asterisks (*p< 0.05;

**p<0.01). D, BAK1 accumulation in the seedling samples analyzed by Western blot using anti-BAK1 antibodies. These experiments were reproduced three times with similar results.

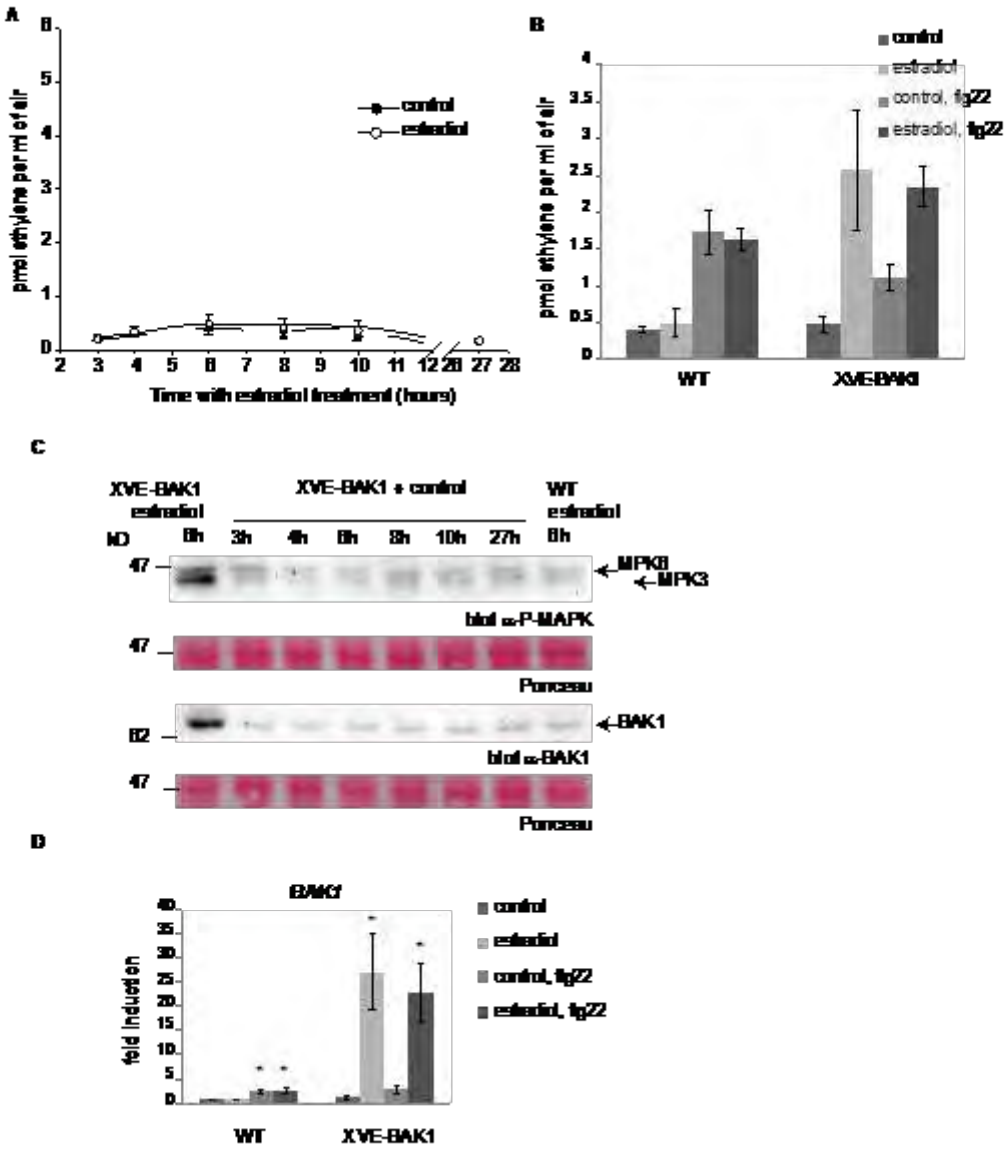


Figure S3. *BAK1* overexpression is required for constitutive defense activation in XVE-BAK1 seedlings. A, Ethylene production by wild-type seedlings treated or not with estradiol for various times. B, Ethylene production by wild-type and XVE-BAK1 seedlings treated or not with estradiol for 6h and/or flg22 for 3h. C, Western blot analysis of seedling extracts (XVE-BAK1) mock-treated for different times using anti-BAK1 antibodies (lower panel) or antibodies recognizing the phosphorylated form of MAPKs (upper panel). D, Accumulation of *BAK1* transcripts in WT and XVE-BAK1 seedlings after a 6-hour estradiol treatment. The bars show the average of three

biological replicates with error bars indicating standard errors. Statistics were performed using ANOVA and significant differences are marked by * ($p < 0.05$).

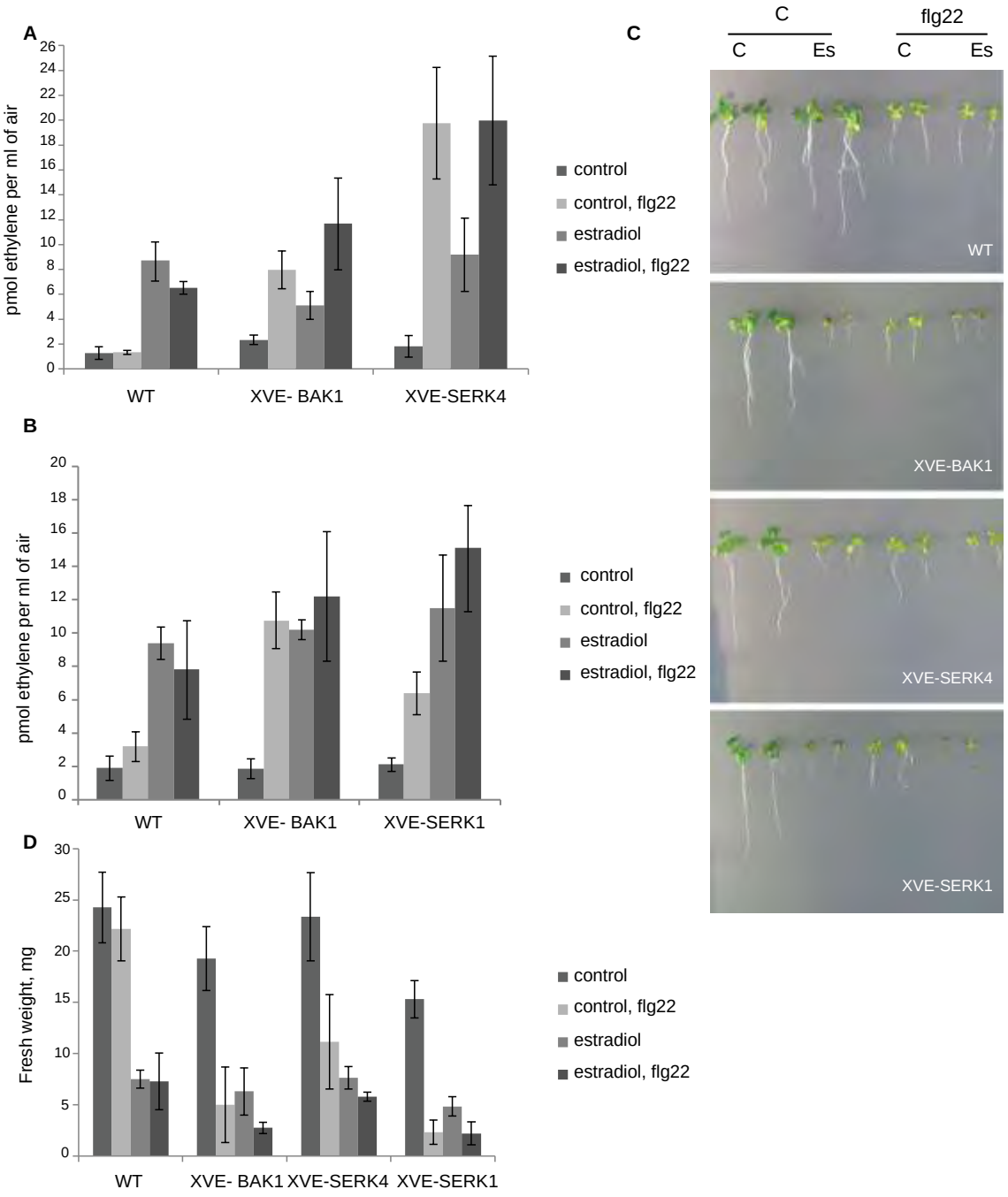
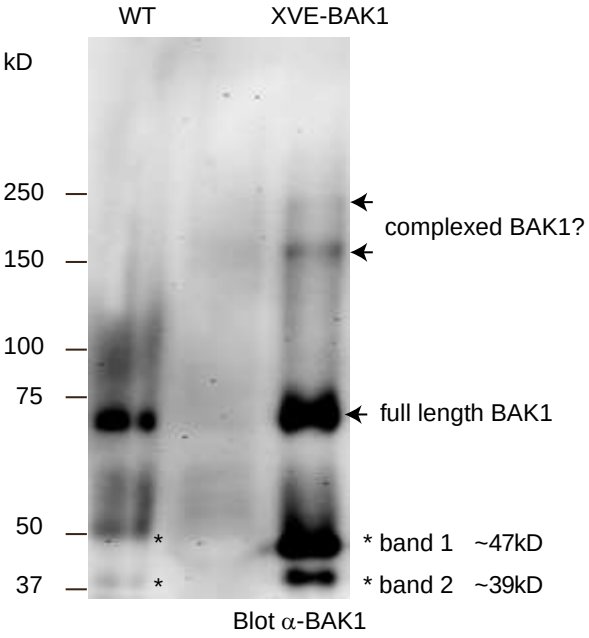


Figure S4. Overexpression of *SERK4* or *SERK1* induces defense responses in Arabidopsis. The genomic sequences of *SERK1* and *SERK4* were cloned downstream of the estradiol inducible promoter and transformed in Col-0 plants to generate transgenics (XVE-SERK1 and XVE-SERK4). A, The effect of *SERK4* overexpression on ethylene production was assessed in adult

leaves of XVE-SERK4 in presence (or not) of estradiol (10 μM) and/or flg22 (1 μM). Leaves from XVE-BAK1 adult plants are used as positive control. Graphs show means of n=6 technical replicates and bars represent standard deviations. B, Ethylene production by WT, XVE-BAK1 and XVE-SERK1 seedlings incubated in presence (or not) of estradiol (1 μM) and/or flg22 (1 μM). In this assay, a 24-hour-treatment was required to detect an effect in estradiol-treated samples of the XVE-SERK1 line. C, Growth of XVE-BAK1, XVE-SERK1 and XVE-SERK4 seedlings after flg22 (1 μM) and/or estradiol treatment (10 μM). D, Quantification of seedling fresh weight from C.



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LTELVSLDLYLNNLSGPIPSTLGR
LKKLRFLRLNNNSLSGEIPRSLTA
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AVRGTIGHIAPEYLSTGKSSEKTDVFGYGVMLLELITGQRAFDLARLAND
DDVMLLDWVKGLLKEKKLEALVDVDLQGNYKDEEVEQLIQVALLCTQSSP
MERPKMSEVVRMLEGDGLAERWEEWQKEEMFRQDFNYPTHHPAVSGWIIG
DSTSQIENEYPSGPR

~39 kD
~47 kD

Figure S5. BAK1 truncated forms can be immunoprecipitated from Arabidopsis seedling extracts and detected by mass spectrometry. BAK1 polypeptides were enriched by immunoprecipitation using BAK1 antibodies from solubilized seedling extracts (WT and XVE-BAK1) and analyzed by

LC-MS/MS. The sequence of the BAK1 protein is shown with the signal peptide highlighted in yellow and the trans-membrane domain highlighted in grey. Peptides identified by mass spectrometry are shown in green in the BAK1 sequence and were identical for the 75 kD and 47 kD BAK1 polypeptides, except for LNNNSLSGEIPR absent in the truncated form. Peptides identified for the 39 kD truncated version of BAK1, are highlighted with frames (see also Tables SI and SII for raw data). Asparagine and lysine residues are highlighted in red and N-glycosylation sites sub-lined and in bold.

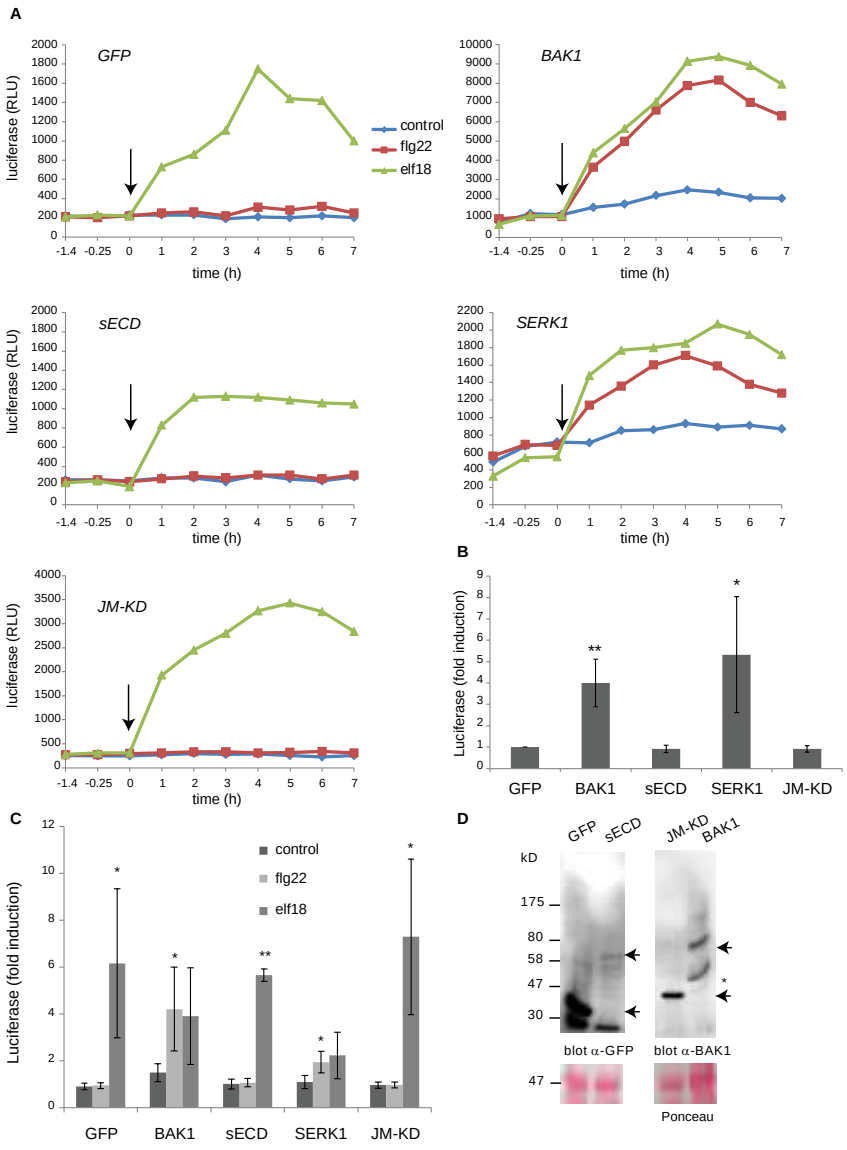

Figure S6. Effect of *BAK1*-derived constructs on the *FRK1* promoter activity in Arabidopsis cells. A, Protoplasts from the *bak1-4* mutants were co-transfected with the pFRK1-Luc construct and plasmids carrying the coding sequence for *BAK1*, *SERK1*, *sECD*, *JM-KD* and *GFP* (used as control). *FRK1* promoter activity was measured by recording luminescence after treating protoplasts at time zero with flg22 (100 nM), elf18 (100 nM) or buffer as control. B, Quantification of the basal luciferase activity obtained in A at time zero. C, Quantification of luciferase activity after mock, flg22 or elf18 treatment (4 hours) in the same protoplasts. Results shown in B and C represent three independent experiments. Asterisks indicate significant differences respect to control (**p<0.01; *p<0.05 according to an ANOVA-Test). D, Expression analysis of protoplast samples by Western blot using anti-GFP and anti-BAK1 antibodies.

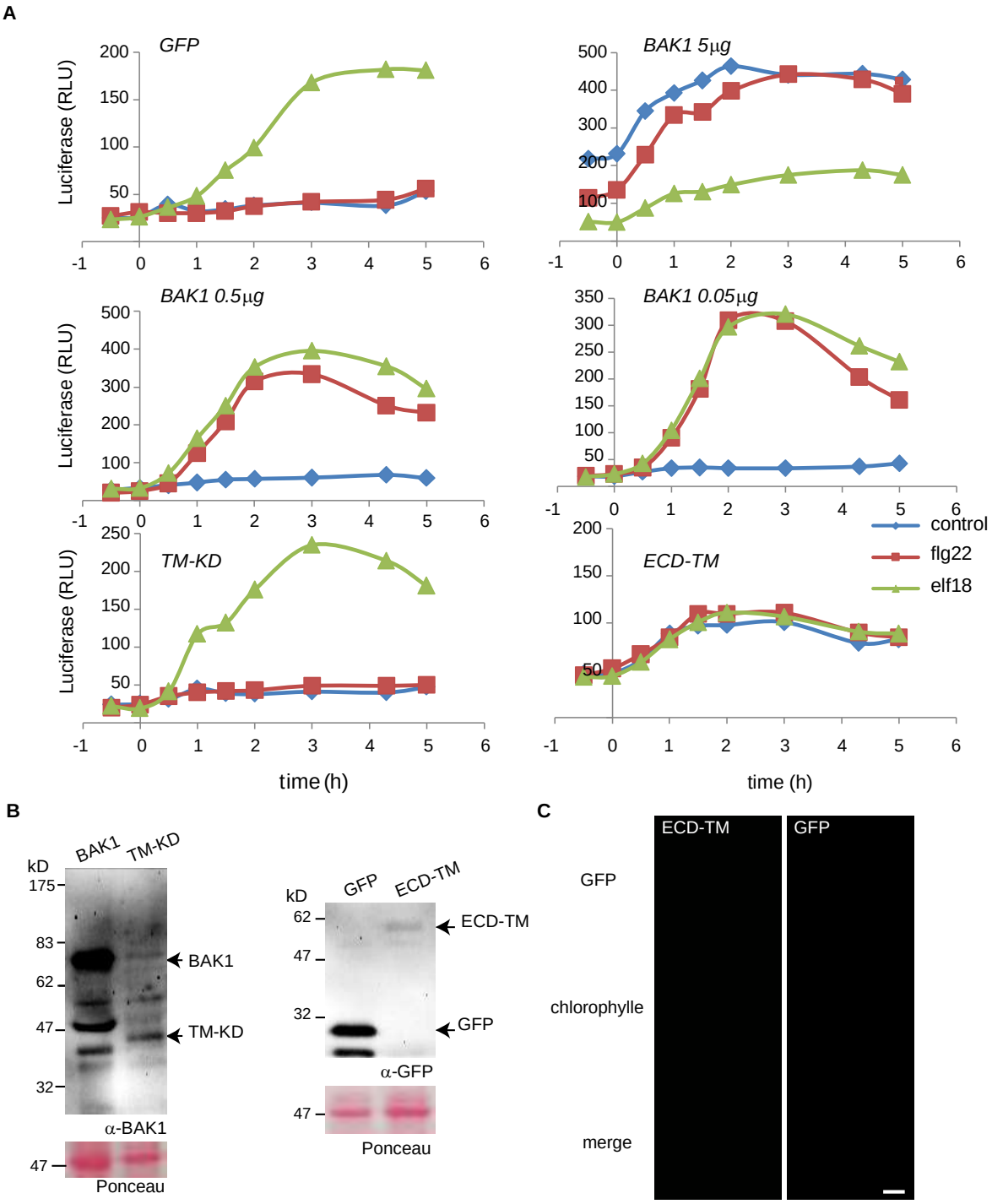

Figure S7. Effect of *BAK1*-derived constructs on the *FRK1* promoter activity in Arabidopsis cells. A, *bak1-4* protoplasts were co-transfected with the pFRK1-Luc construct and a plasmid carrying the genomic coding sequence for *BAK1*, used at various doses (5-0.5-0.05 µg). Other constructs,

including the cDNA forms of *ECD-TM* and *TM-KD*, were tested at 5 μg. Luciferase activity was measured by recording luminescence after treating protoplasts at time zero with MAMPs or buffer as negative control. B, Western blot analysis on protoplast extracts with anti-BAK1 or anti-GFP antibodies. C, Subcellular localization of ECD-TM in Arabidopsis protoplasts. Standard confocal micrographs of cells expressing ECD-TM or GFP show optical sections of cells. Bar=20mm.

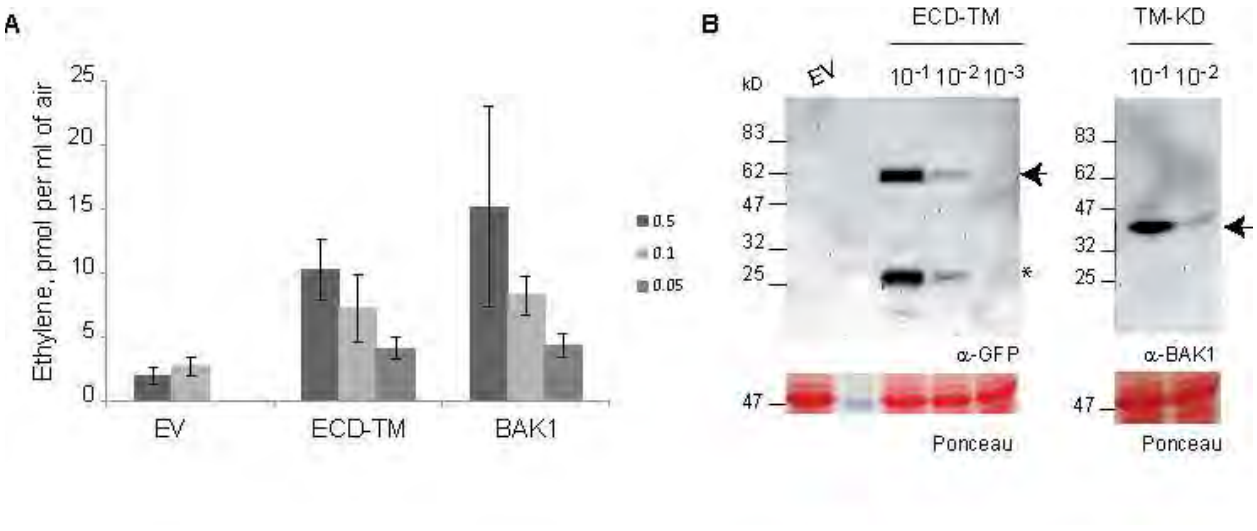

Figure S8. Defense phenotype in *N. benthamiana* leaves expressing *BAK1* variants. A, Ethylene production by leaves agro-transformed with various ODs of bacteria carrying *BAK1*, *ECD-TM* or *EV*. B, Western blot analysis of the *N. benthamiana* samples from Figure 6 using anti-GFP or anti-BAK1 antibodies.

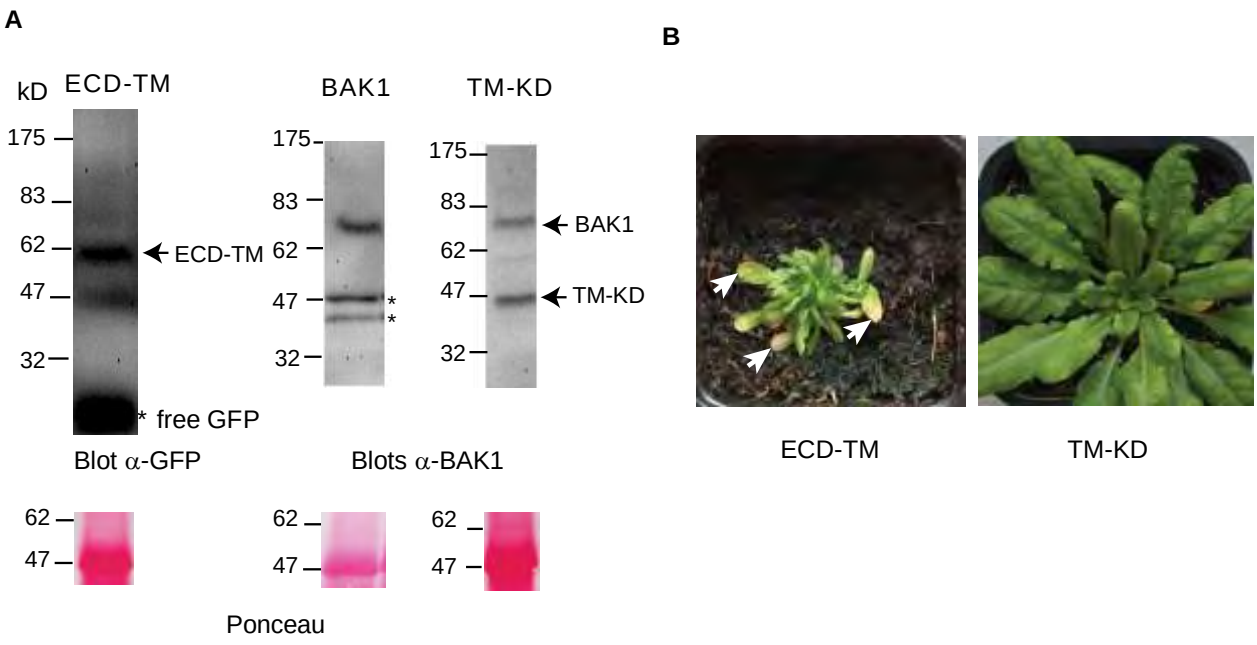

Figure S9. *ECD-TM* expression causes leaf necrosis at late stages of development. A, Protein accumulation in lines depicted in Figure 7 was checked by Western blot analysis using anti-GFP and anti-BAK1 antibodies. Ponceau S stainings are shown as protein loading controls. B, Phenotypes of plants shown in Figure 7, after 9 weeks of growth. White arrows indicate leaf necrosis.

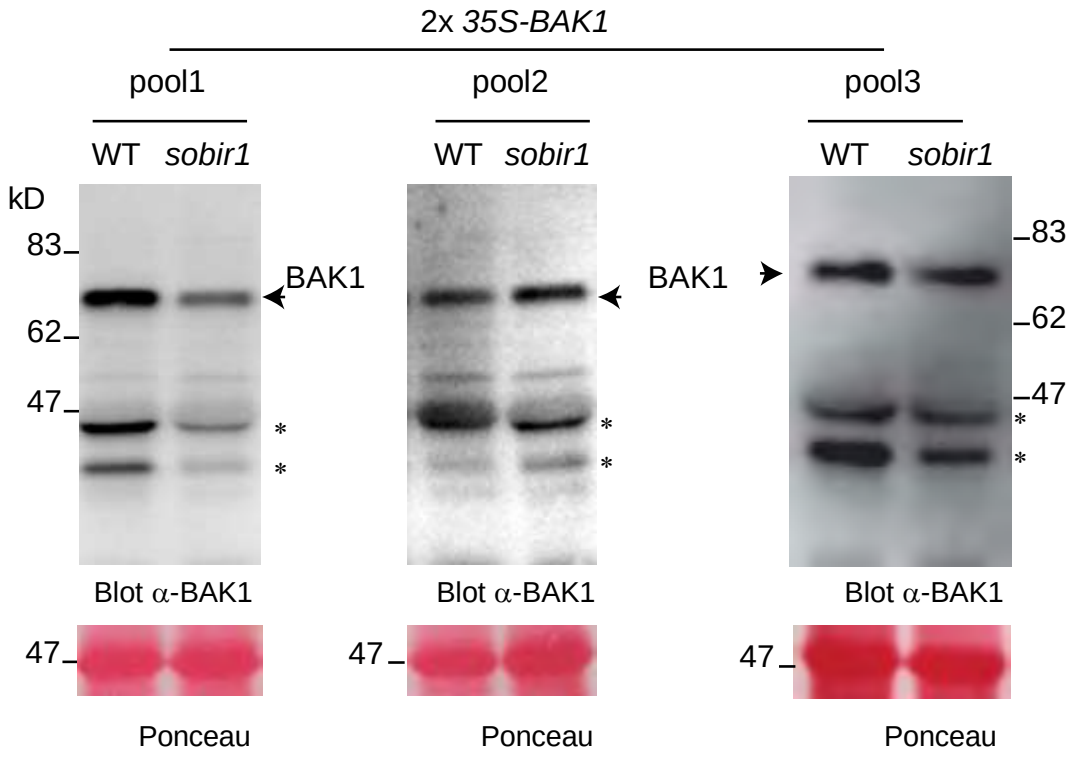

Figure S10. Western blot analysis of whole seedling extracts showing BAK1 accumulation. Six - 8 two-week old T1 seedlings from wild-type or *sobir1-13* plants transformed with 2x *35S-BAK1* were individually selected on hygromycin and pooled to assess BAK1 accumulation. Three independent repetitions are shown (pools 1 to 3).

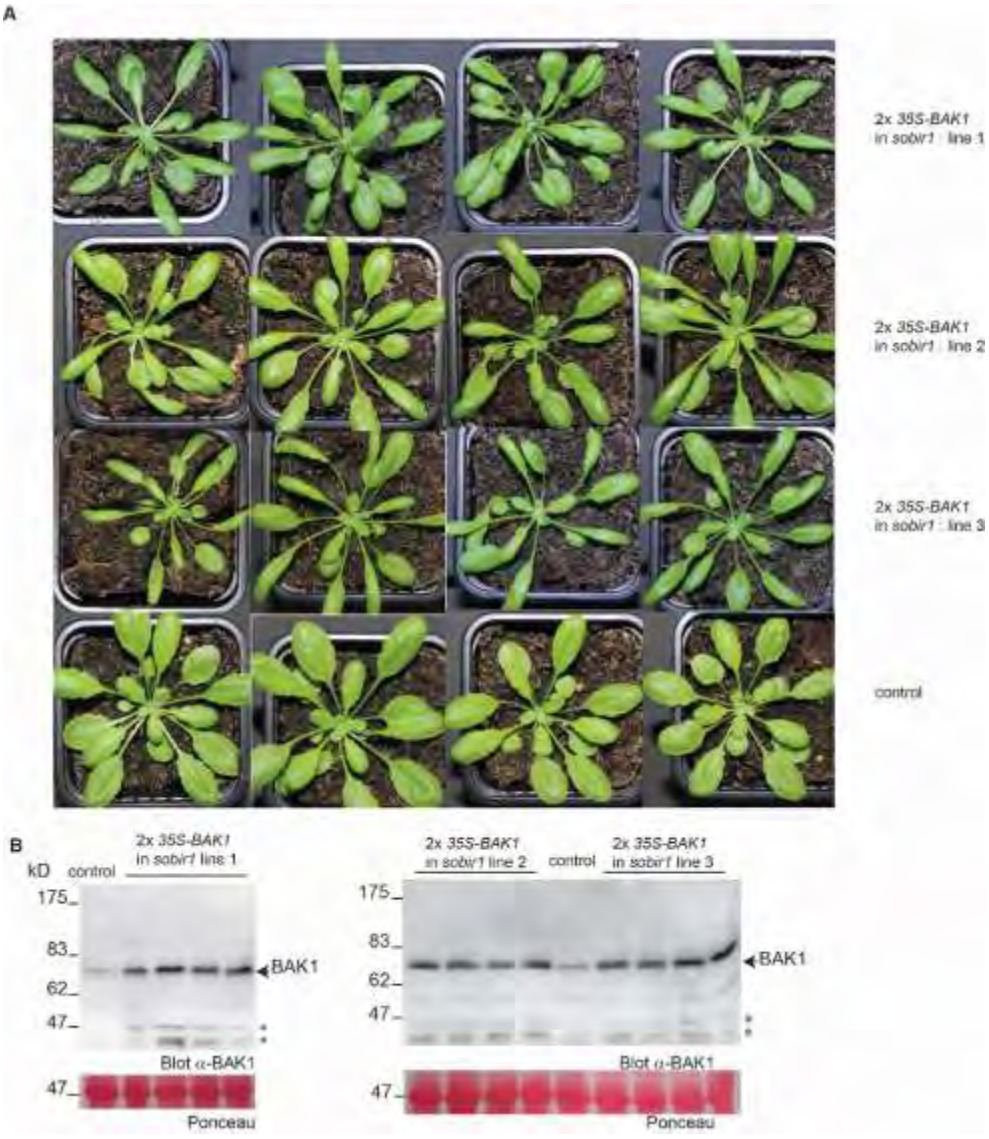

Figure S11. Analysis of the T2 lines from *sobir1-13* mutant plants overexpressing *BAK1* under the control of the 2x *35S* promoter. A, Pictures of four T2 plants from three independent lines transformed with 2x *35S-BAK1* or the empty vector (control). B, Western blot analysis of whole plant extracts from the presented lines with anti-BAK1 antibodies. Arrowhead indicates full length BAK1 and asterisks signal the presence of two additional bands. Protein loading controls by Ponceau S are shown. Because BAK1-ox plants in Col-0 died before setting seeds, the next generation could not be included as T2 positive control.

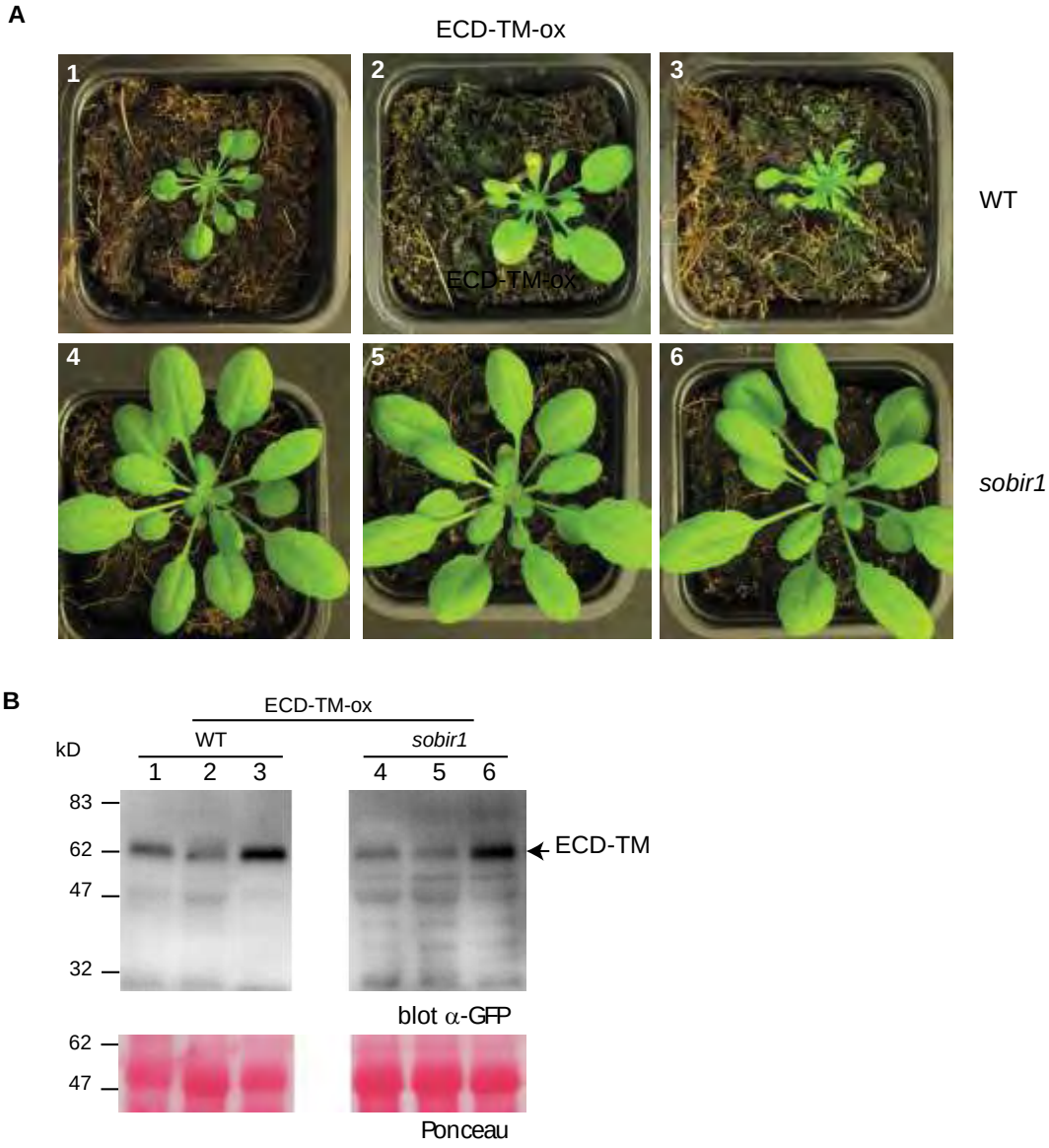

Figure S12. A mutation in the *SOBIR1* gene rescues the phenotype caused by *ECD-TM* overexpression. A, Pictures from 3 transgenic T1 plants overexpressing *ECD-TM* under the control of the 2x *35S* promoter in wild type Col-0 or in the *sobir1-13* mutant. B, Western blot analysis for ECD-TM in crude extracts of the respective lines. Protein samples extracted from the same amount of leaf material were separated on the same gel and analyzed in the presence of anti-GFP antibodies.

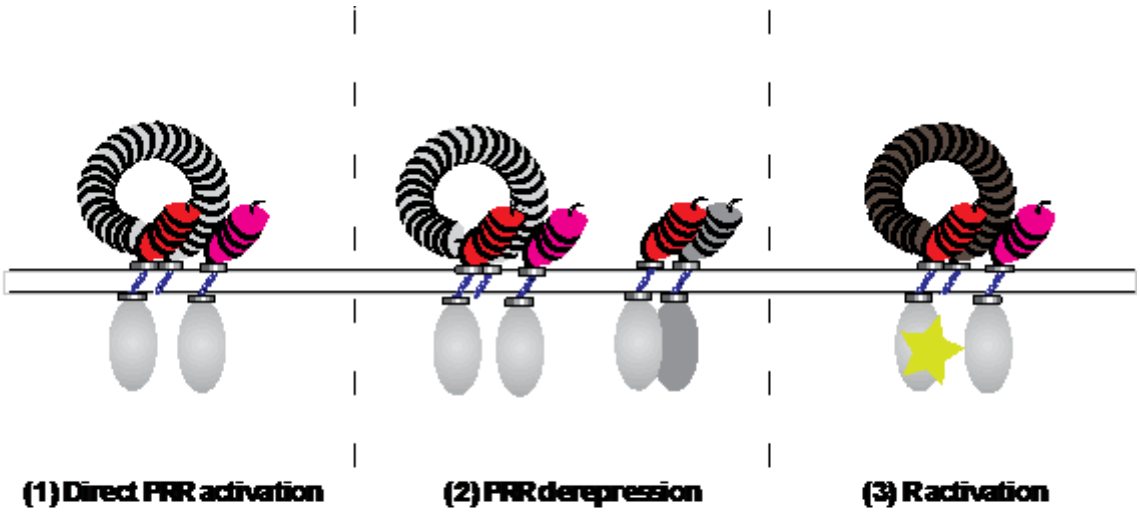

Figure S13. Model of activation of defense responses triggered by *BAK1* overexpression**.** We devised the following hypothetical models to illustrate possible defense pathways activated in response to BAK1 over-accumulation at the plasma membrane. (1) Extra BAK1 (in red) could interact and activate RLP PRRs (in grey) associated with SOBIR1 (in pink) in the absence of MAMPs. (2) Extra BAK1 (or ECD-TM) could sequester negative regulators (in dark grey), allowing derepression of PRR activity. (3) Extra BAK1 (or ECD-TM) could be detected by R proteins (possibly of the RLP class, as depicted in black), inducing immunity.

SUPPLEMENTAL MATERIAL AND METHODS

Tables SI and SII. *Protein and peptide identifications obtained by searching the MS data with Mascot and Sequest HT* (provided in separated Excel files)

Table SIII. *Oligonucleotides used for cloning*

name	**sequence 5' – 3'**
BAK1atgB1	AAAAAGCAGGCTCCATGGAACGAAGATTAATGATCCC
BAK1AttB2	AGAAAGCTGGGTGTCTTGGACCCGAGGGG
BAK1StopAttB2	AGAAAGCTGGGTGTTATCTTGGACCCGAGGGG
AttB1SERK1	AAAAAGCAGGCTAAATGGAGTCGAGTTATGTGGTGTTTATCTTACTTTC
AttB2SERK1stop	AGAAAGCTGGGTGTTACCTTGGACCAGATAACTCAACGGCGTGCAAATTG
AttR1SERK4	AAAAAGCAGGCTAAATGACAAGTTCAAAAATGGAAC
AttR2SERK4	AGAAAGCTGGGTGTTATCTTGGACCCGAGGGG
BAK1PROAttR2	AGAAAGCTGGGTGTCTATTACTCCCTGCAAGAATG
BAK1Ki2B	AAAAAGCAGGCTCCATGAGGAAAAAGCCGCAGG ACCAC

BAK1signpepURrev	ATCGCgcUGGCGTTGCCCGAGACTCTGAGAACCAAATC
BAK1tmURfor	AGCGCGaUgATTACTGGAGCGATTGCGGGAGG
BAK1tmURrev	ATCGCgcUtCGCCACCAAGCTAGTGCAATGGCCGG
GFP-URfor	AGCGCGaUgGTGAGCAAGGGCGAGGAGCTGTTCACCGGGG
GFPattB2rev	AGAAAGCTGGGTGTTACTTGTACAGCTCGTCCATGCCG

The destination vectors pMDC7 and pMDC32 were used to clone *BAK1*, *SERK1*, *SERK4*, *JM-KD*, *TM-KD* and *ECD-TM*. pMDC83 was used as destination vector for *ECDs*.

Table SIV. *Primers used for qRT-PCR analysis*

Beacon Designer 2.0 (PREMIER Biosoft International, USA) was used to design primers for qRT-PCR analysis with target T_m = 60°C +/- 1°C; primer length range from 18-25 bp; amplicon length from 100 to 250 bp. Primers for *FRK1*, *NHL10* and *EIF4a* are described in (Boudsocq et al., 2010).

name	**sequence 5' – 3'**	**gene or vector**
EIF4α qF	TCATAGATCTGGTCCTTGAAACC	*EIF4a*
EIF4α qR	GGCAGTCTCTTCGTGCTGAC	*EIF4a*
BAK1qF	TGTCCTGACGCTACAAGTTCTGG	*BAK1*

BAK1qR	AGCAACTCCTCCCGCAATCG	*BAK1*
FRK1qF	CGGTCAGATTTCAACAGTTGTC	*FRK1*
FRK1qR	AATAGCAGGTTGGCCTGTAATC	*FRK1*
NHL10qF	TTCCTGTCCGTAACCCAAAC	*NHL10*
NHL10qR	CCCTCGTAGTAGGCATGAGC	*NHL10*

Literature cited

Boudsocq M, Willmann MR, McCormack M, Lee H, Shan L, He P, Bush J, Cheng SH, Sheen J (2010) Differential innate immune signalling via Ca(2+) sensor protein kinases. Nature **464:** 418-422