Supplemental Information

The SIK1 kinase ensures a robust extracellular ROS burst and anti-bacterial immunity by regulating BIK1 stability and RBOHD activation

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Supplemental Figures

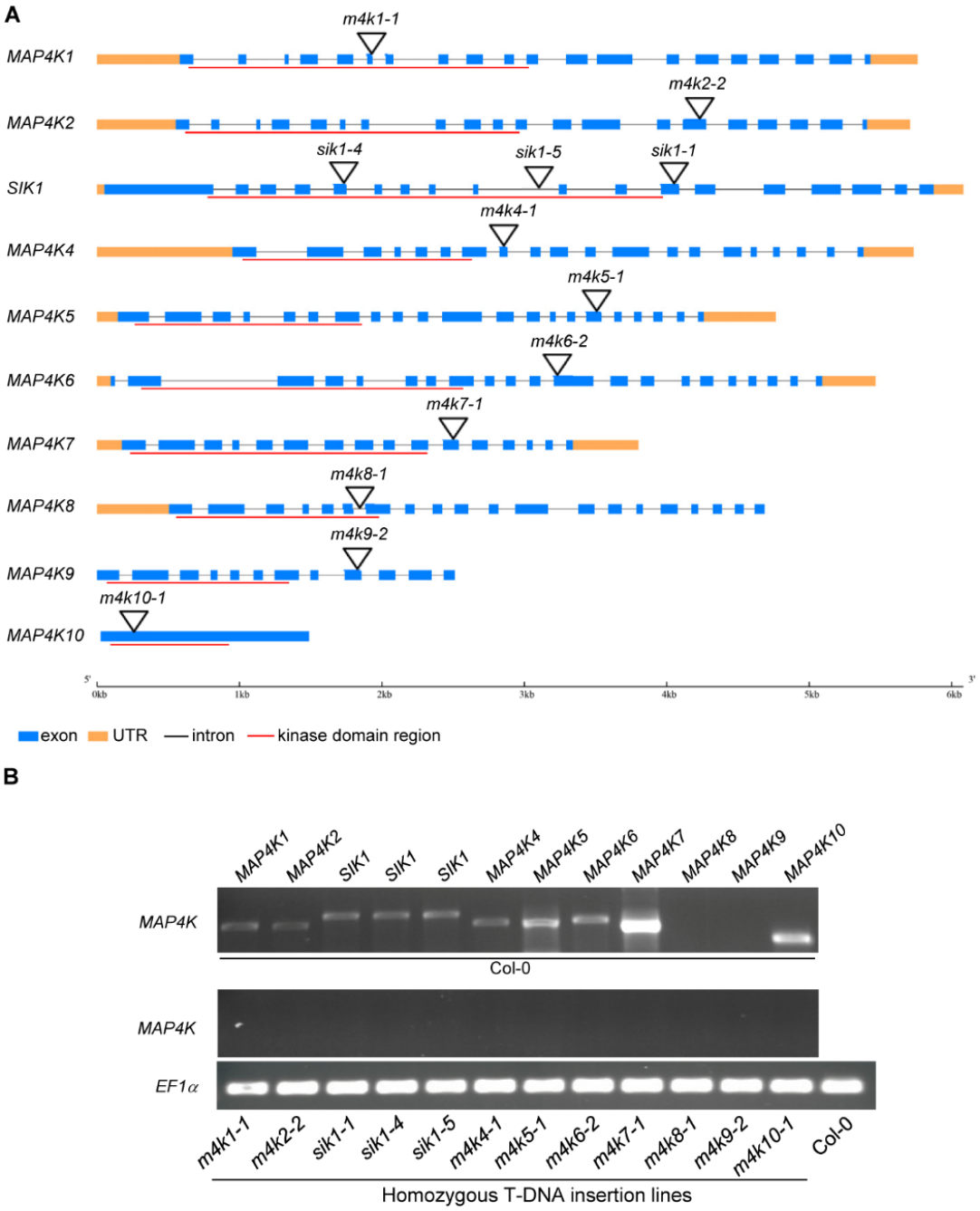

Figure S1. Related to Figure 1. RT-PCR verified the absence of full-length CDS transcripts in each *map4k* T-DNA line. (A) Gene structure of the *Arabidopsis MAP4K* family members and T-DNA insertion sites. (B) The absence of full-length transcripts in each *map4k* T-DNA line by RT-PCR. Top: transcript accumulation in wild-type Col-0. Bottom: transcript accumulation in individual T-DNA insertion lines.

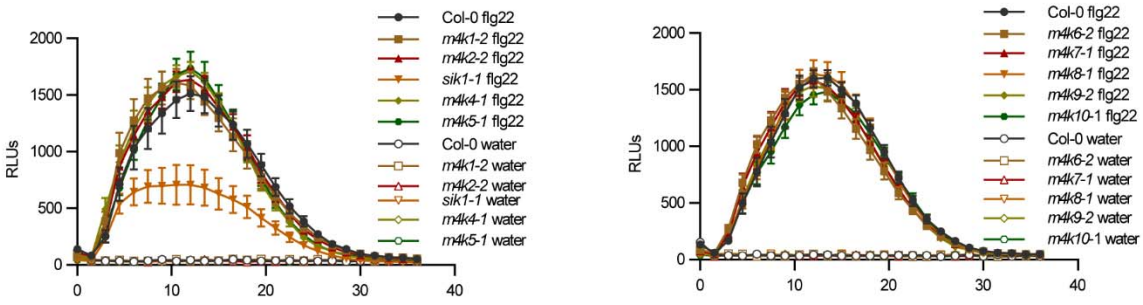

Figure S2. Related to Figure 1. ROS burst in the *MAP4K* T-DNA mutants.
The ROS burst in the indicated lines after treatment with 100 nM flg22 or water. Values are means ± SEM of RLU (n=12). Asterisks indicate significant differences (Fisher's LSD, $p < 0.01$). Similar results were obtained in three independent experiments.

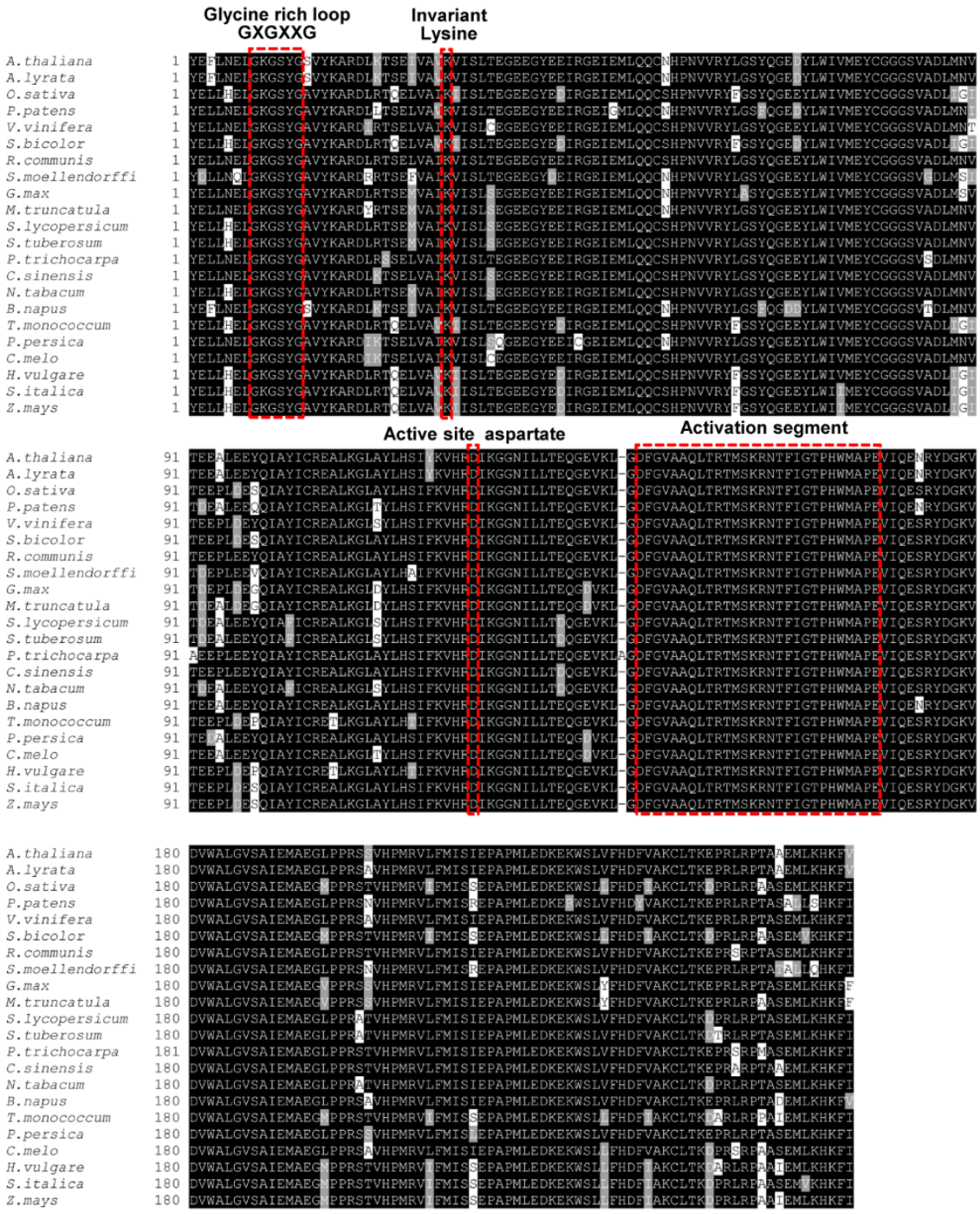

Figure S3. Related to Figure 2. Conservation of the SIK1 kinase domain. Amino acid alignment of the kinase domain of SIK1 homologs from different land plants. Important motifs required for kinase activity are highlighted.

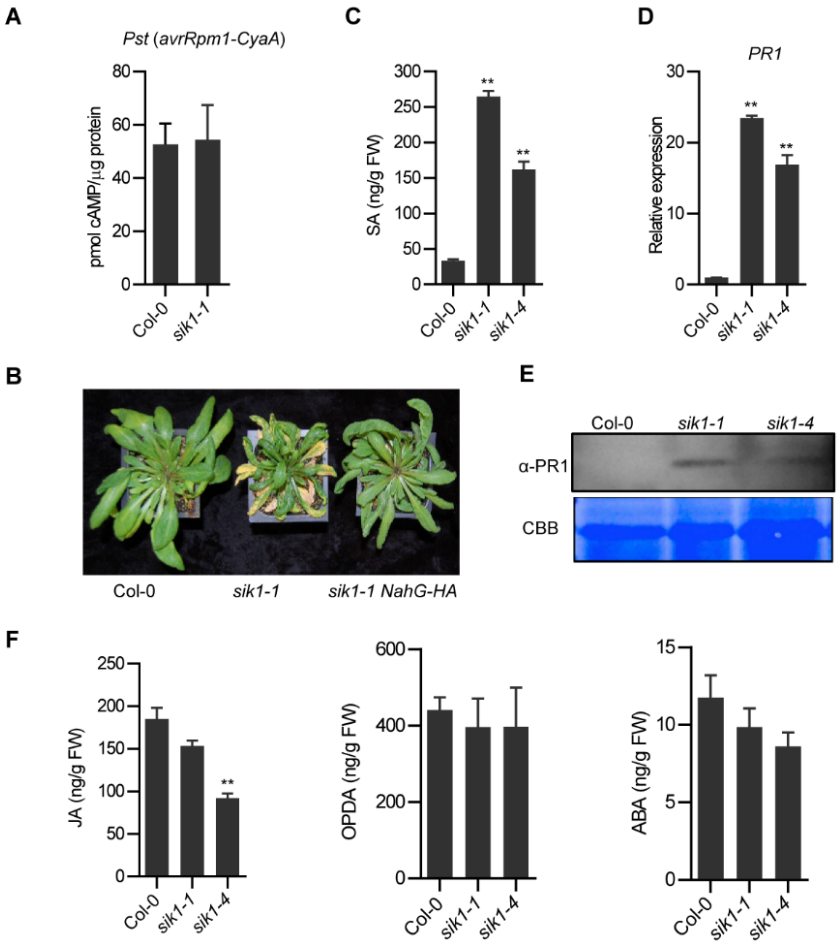

Figure S4. Related to Figure 3. Autoimmune responses in *sik1* mutants.
(A) The translocation of the AvrRpm1 effector is not compromised in *sik1-1*. The adenylate cyclase (CyaA) delivery assay was performed by infiltrating Col-0 and *sik1-1* with *P. syringae* carrying *avrRpm1-CyaA* at a concentration of 3×10^7 CFU ml^{-1}. CyaA activity depends on calmodulin in eukaryotic cells. Only fusion proteins injected into eukaryotic cells produce cAMP. Effector translocation levels were determined by quantifying cAMP after 8 h. The data are shown as means ± SD. Similar results were obtained in three independent experiments. (B) The lesion mimic phenotype of six-week-old *sik1-1*. Flowers were removed for a clearer image. The transgenic *sik1-1* line expressing *NahG-HA* was able to rescue the lesion mimic phenotype. (C) Total SA levels in Col-0 and *sik1* mutants. (D) Relative expression of the *PR1* gene in Col-0 and *sik1* mutants using qPCR. In panels (C and D) the data are shown as means ± SD (n=3). (E) Anti-PR1 immunoblot demonstrating PR1 protein accumulation in wild-type Col-0 and *sik1* mutant lines at a resting state. CBB = Coomassie brilliant blue. (F) Other phytohormone levels in *sik1* mutant lines. Total jasmonic acid (JA), cis-(+)-12-oxo-phytodienoic acid (OPDA), and abscisic acid (ABA) levels in Col-0 and *sik1* mutant lines. Hormones were extracted from five-week-old plants. The data are shown as means ± SD (n=3). Asterisks indicate significant differences (Dunnett's test, $p < 0.01$). Similar results were obtained in three independent experiments.

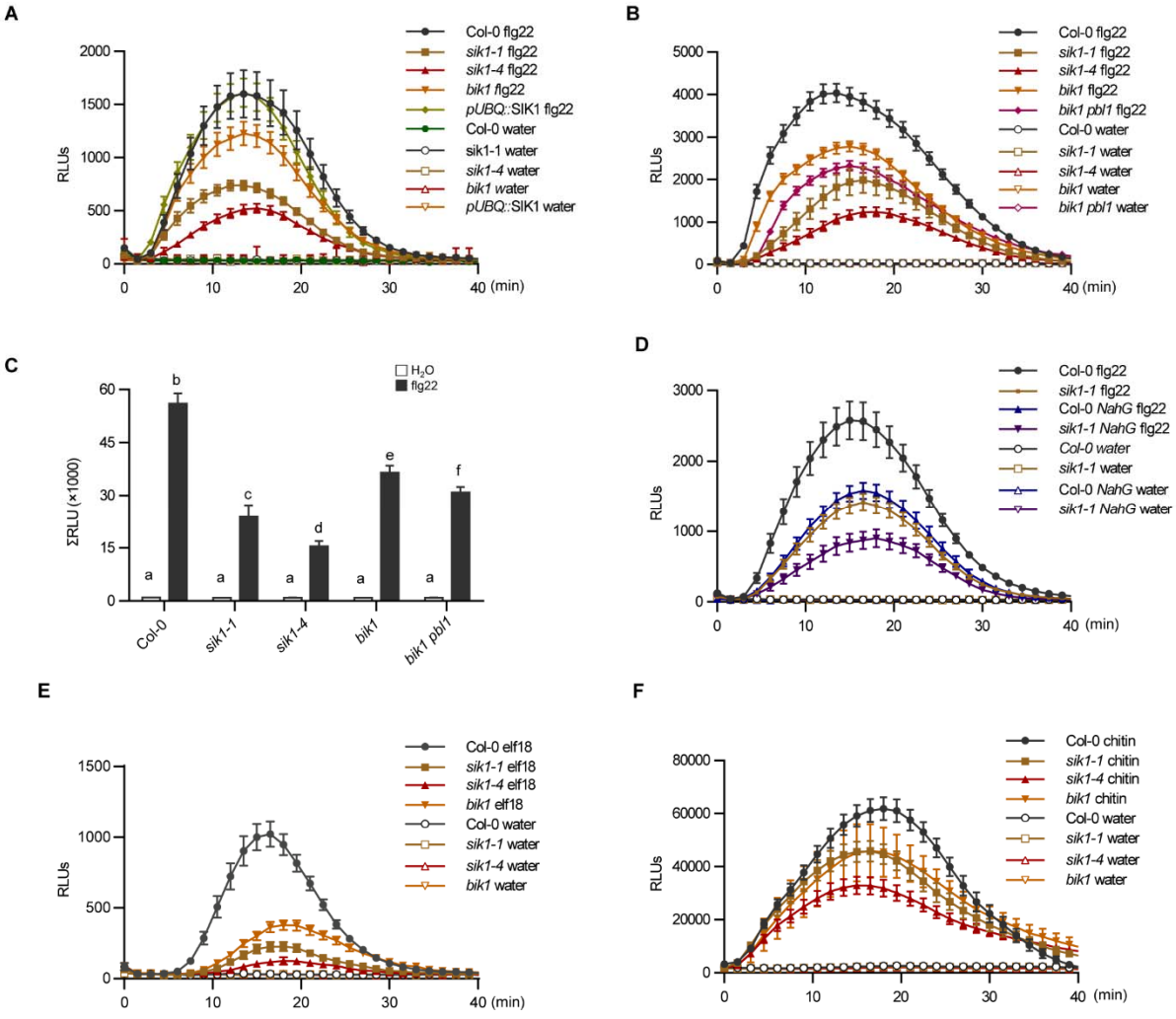

Figure S5. Related to Figure 4. Compromised PAMP-induced ROS burst in *sik1* mutants.

(A and B) The ROS burst in the indicated lines after treatment with 100 nM flg22 or water. Total relative luminescent units (RLU) were detected over a 40-min period. Values are means ± SEM of RLU (A, n=12; B, n=14). (C) The ROS in the indicated lines after treatment with 100 nM flg22 or water. Total relative luminescent units (RLU) were detected over a 40-min period. Values are means ± SEM of RLU (n=14). Asterisks indicate significant differences (Dunnett's test, $p < 0.01$). (D) The ROS burst in the indicated lines after treatment with 100 nM flg22 or water. Values are means ± SEM of RLU (n=18). (E) The ROS burst in the indicated lines after treatment with 100 nM elf18 or water. Values are means ± SEM of RLU (n=12). (F) The ROS burst in the indicated lines after treatment with 10 μM chitin or water. Values are means ± SEM of RLU (n=18). These experiments were repeated at least twice with similar results.

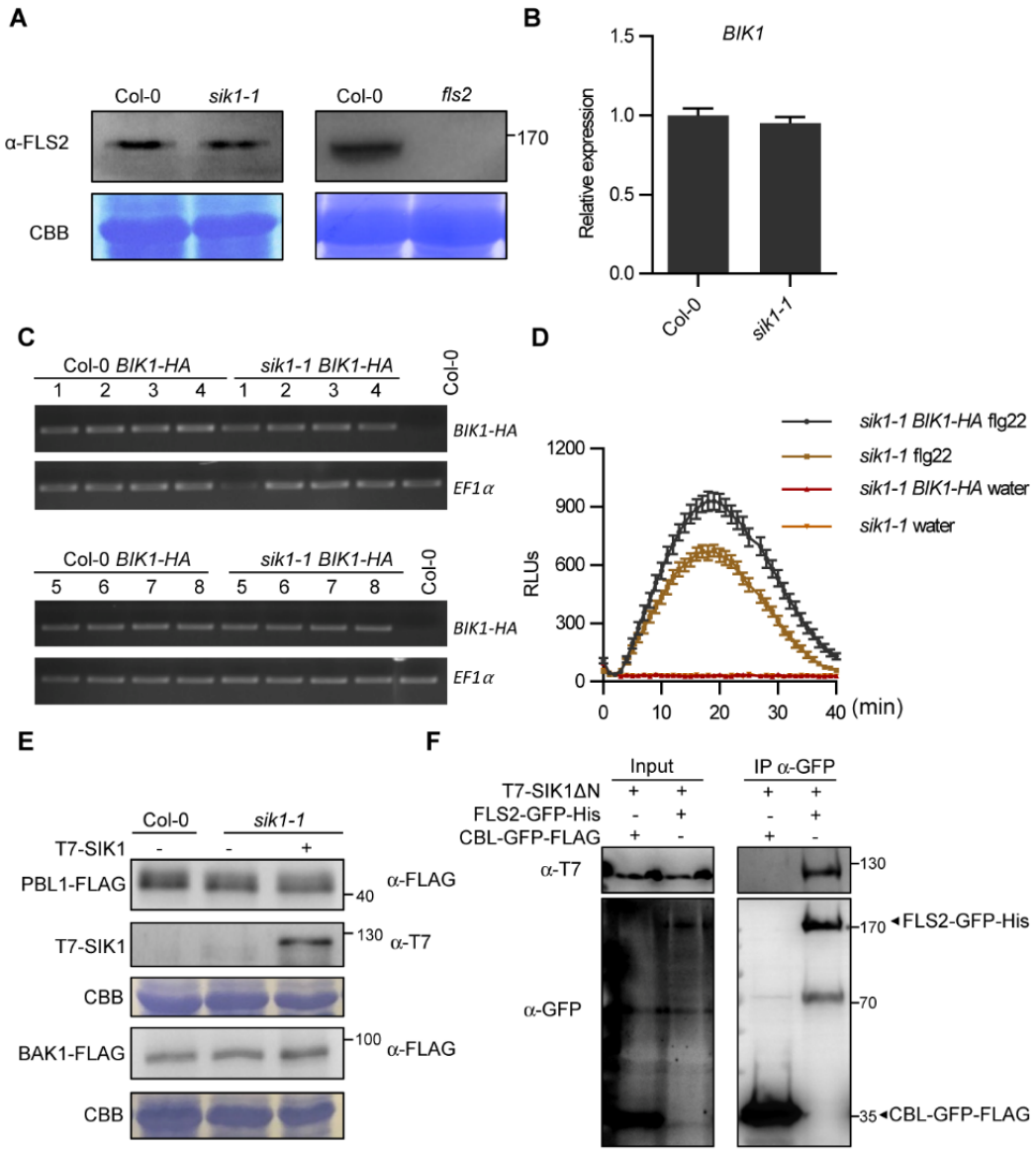

Figure S6. Related to Figure 6. SIK1 specifically affects BIK1 protein accumulation and can associate with the FLS2 immune receptor.(A) FLS2 abundance is not altered in *sik1-1*. Total proteins from Col-0, *fls2*, and *sik1-1* plants were extracted and FLS2 protein was detected by anti-FLS2 immunoblot analyses. CBB = Coomassie brilliant blue. (B) Relative expression of *BIK1* in indicated lines using qPCR. Data are shown as means ± SD (n=3). (C) Relative transcript expression of *BIK1-HA* in independent transgenic lines using RT-PCR. *EF1*α was used as a reference. (D) The ROS burst in the indicated lines after treatment with 100 nM flg22 or water. Values are means ± SEM of RLU (n=14). (E) PBL1 accumulation is not affected in *sik1-1*. *PBL1-FLAG*, *BAK1-FLAG*, and *T7-SIK1* plasmids were expressed in Col-0 and *sik1-1* protoplasts. Protein levels were determined by immunoblot analyses. (F) Co-immunoprecipitation of T7-SIK1ΔN and FLS2-GFP-his after co-expression in *Nicotiana benthamiana.*

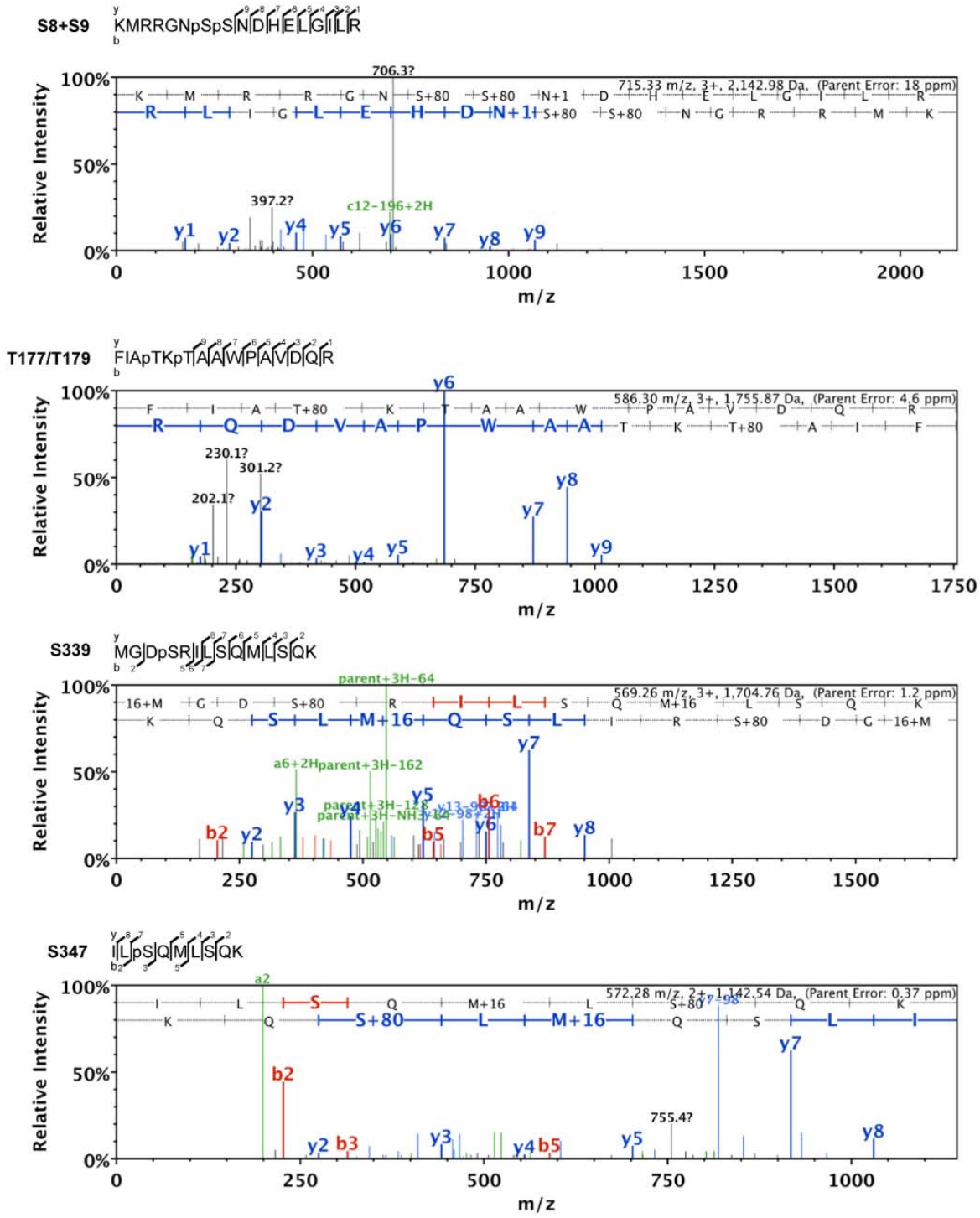

Figure S7. Related to Figure 7. MS/MS spectra of phosphopeptides in RBOHD's N-terminus. MS2 mass spectra after *in vitro* kinase assay with MBP-SIK1ΔN but not the kinase dead variant MBP-SIK1ΔN^{KD}.

Supplemental Tables

Table S1. Related to Figure 5. Phosphorylated BIK1 residues identified by LC/MS/MS.

Phosphorylation sites	Peptide modified sequence	Probability	Site ambiguity	Previously identified	Description	Reference
S26	SSDLYGLS[+80]LSSR	98%	No	Yes	by autocatalysis and BAK1.	Lin et al., 2014
S32	KS[+80]SSTVAAAQKTEGEILSSTPVK	99%	Yes	Yes	by autocatalysis.	Lin et al., 2014
S33	KSS[+80]STVAAAQKTEGEILSSTPVK	99%	Yes	Yes	by autocatalysis.	Laluk et al., 2011; Lin et al., 2014; Xu et al., 2013
S34	KSSS[+80]TVAAAQKTEGEILSSTPVK	99%	Yes	No		
T35	KSSST[+80]VAAAQKTEGEILSSTPVK	99%	No	Yes	by autocatalysis.	Laluk et al., 2011; Lin et al., 2014
T42	KSSSTVAAAQKT[+80]EGEILSSTPVK	99%	No	Yes	by autocatalysis.	Laluk et al., 2011; Lin et al., 2014
T50	KSSSTVAAAQKTEGEILSST[+80]PVK	99%	No	No		
S54	TEGEILSSTPVKS[+80]FTFNELK	91%	No	Yes	by autocatalysis.	Lin et al., 2014; Xu et al., 2013
T56	TEGEILSSTPVKSFT[+80]FNELK	91%	Yes	Yes	by autocatalysis.	Lin et al., 2014; Xu et al., 2013
T64	LAT[+80]RNFRPDSVIGEGGFGCVFK	99%	Yes	Yes		Laluk et al., 2011
S71	NFRPDS[+80]VIGEGGFGCVFK	99%	No	Yes	by autocatalysis and BAK1.	Xu et al., 2013
T120	EWLT[+80]EINYLGQLSHPNLVK	99%	No	No		
Y168	GAY[+80]FKPLPWFLR	98%	No	Yes	by autocatalysis and BAK1.	Xu et al., 2013
S193	GLAFLHS[+80]DPVKVIYR	98%	No	No		
S206	DIKAS[+80]NILLDADYNAK	99%	No	Yes	by autocatalysis and BAK1.	Laluk et al., 2011; Lin et al., 2014; Xu et al., 2013
S219	ASNILLDADYNAKLS[+80]DFGLAR	95%	No	No		
S233	DGPMGDLS[+80]YVSTR	99%	No	Yes	by autocatalysis and BAK1.	Laluk et al., 2011; Lin et al., 2014; Xu et al., 2013
S236	DGPMGDLSYVS[+80]TR	99%	No	Yes	by autocatalysis, BAK1 and PEPR1.	Laluk et al., 2011; Lin et al., 2014; Xu et al., 2013; Zhang et al., 2010
T314	VLLIVDNRLDT[+80]QYLPEEAVR	99%	No	Yes	by autocatalysis.	Xu et al., 2013
T341	SRPT[+341]MDQVVR	97%	No	No		
S360	ALQQLQDNLGKPS[+80]QTNPVKDTKK	98%	Yes	Yes	by autocatalysis.	Xu et al., 2013
T362	ALQQLQDNLGKPSQT[+80]NPVKDTKK	98%	Yes	Yes	by autocatalysis and BAK1.	Lin et al., 2014; Xu et al., 2013
T368	ALQQLQDNLGKPSQTNPVKDT[+80]KK	99%	No	Yes	by autocatalysis and BAK1.	Lin et al., 2014; Xu et al., 2013
T375	LGFKT[+80]GTTKSSEKR	93%	No	Yes	by autocatalysis.	Lin et al., 2014
T377	LGFKTGT[+80]TKSSEKR	93%	No	No		

Table S2. Related to STAR Methods. T-DNA insertion lines and accession numbers.

Gene	Locus	Allele	Stock number
MAP4K1	AT1G53165	*m4k1-1*	SALK_060372
MAP4K2	AT3G15220	*m4k2-2*	CS925354
MAP4K3 (*SIK1*)	AT1G69220	*sik1-1*	SALK_046158
		sik1-4	SALK_051369
MAP4K4	AT5G14720	*m4k4-1*	SALK_065417
MAP4K5	AT4G24100	*m4k5-1*	SALK_208908
MAP4K6	AT4G10730	*m4k6-2*	SALK_127267
MAP4K7	AT1G70430	*m4k7-1*	SALK_108286
MAP4K8	AT1G79640	*m4k8-1*	SALK_067866
MAP4K9	AT1G23700	*m4k9-2*	SALK_152867
MAP4K10	AT4G14480	*m4k10-1*	SALK_202941
FLS2	AT5G46330	*fls2*	SALK_062054
BIK1	AT2G39660	*bik1*	SALK_005291

Table S3. Related to STAR Methods. Primers used in this study.

Name	Sequence 5' to 3'	Purpose
LBb1.3	ATTTTGCCGATTTCGGAAC	T-DNA left border primer
M4K1-LP	AGGTTCTCCTTTTGCCATCTC	*m4k1-1* T-DNA line genotyping
M4K1-RP	CATGGAATATATGGCTGGTGG	
M4K2-LP	TCCATCATCACAAGGGATCTC	*m4k2-2* T-DNA line genotyping
M4K2-RP	GGGAACTTGGTTAAGTTTCACG	
M4K3-LP1	TGTCCAAATCATCCACATGTG	*sik1-1* T-DNA line genotyping
M4K3-RP1	ATGGTTCGAAAATGCAGATTG	
M4K3-LP2	ATTTGGTATCTGGATTGGCAG	*sik1-4* T-DNA line genotyping
M4K3-RP2	TGTGGAGTCCCAATGAACTTC	
M4K3-LP3	CAGCTATTGAGATGGCAGAGG	*sik1-5* T-DNA line genotyping
M4K3-RP3	TCAGAGCGCTTAAAGGTCAAC	
M4K4-LP	TTGCATTATCCTCGTCCTCAC	*m4k4-1* T-DNA line genotyping
M4K4-RP	CAAAAGATTCTCGAAAGTGCG	
M4K5-LP	TCAAACAGTTTGTCTGCTCCC	*m4k5-1* T-DNA line genotyping
M4K5-RP	CTCTGAAGCCACAGGTTCAAC	
M4K6-LP	ACTAGAAGATGTCCAGCTGCG	*m4k6-2* T-DNA line genotyping
M4K6-RP	ATGATTTCAGTTGCCGTTCAC	
M4K7-LP	GGTCACAGACTATGCCTTTGG	*m4k7-1* T-DNA line genotyping
M4K7-RP	TAACCTGATTACTTGCGCCTG	
M4K8-LP	ACACCTGAATCTCCGATTGTG	*m4k8-1* T-DNA line genotyping
M4K8-RP	TTCTTCTTGTCACAGGATGGC	
M4K9-LP	TTTCATGATTGTTTTCAGGTGC	*m4k9-2* T-DNA line genotyping
M4K9-RP	ACCAGCAGTTGGATCACAGAG	
M4K10-LP	CGTGGACTGGACTCAGAAGAG	*m4k10-1* T-DNA line genotyping
M4K10-RP	CTTGATGCTGAAGCCTACGAG	
M4K1-RT-F	ATGGATGATGTTGCTGGTTTAC	Full length *MAP4K1* CDS
M4K1-RT-R	TCAACTTTGGTTAAGATCACGC	
M4K2-RT-F	ATGGACGATGTTGCGGGTTTAC	Full length *MAP4K2* CDS
M4K2-RT-R	TTAAAGATCACGTGATGATTGGC	
SIK1-RT-F	ATGGATCATAACTCGCCGAAATC	Full length *SIK1* CDS
SIK1-RT-R	TTAGAGGCGGAGAATAGTGCG	
M4K4-RT-F	ATGGAATCGGGTTCAGAGAAAAAG	Full length *MAP4K4* CDS
M4K4-RT-R	TCAATCATTTCTGTGTGTTAATGC	
M4K5-RT-F	ATGGTGGGAGGAGGAGGAGG	Full length *MAP4K5* CDS
M4K5-RT-R	TTAGTGTTCGCGACCCGTGAATG	
M4K6-RT-F	ATGGTGTCTCGGTTTCGTCTTG	Full length *MAP4K6* CDS
M4K6-RT-R	TTACAATTGCTCGCGACCGGT	
M4K7-RT-F	ATGGCTGGTTCATCAACGAAAC	Full length *MAP4K7* CDS
M4K7-RT-R	TTAAGAGATGTTGTTGCTGCTAATC	
M4K8-RT-F	ATGGGTACAATGGAGAAGAAGAAG	Full length *MAP4K8* CDS
M4K8-RT-R	TTAGTTACTCATACTTACTTGGGC	

M4K9-RT-F	ATGACGAGTTCACCGGAAACG	Full length *MAP4K9* CDS
M4K9-RT-R	TTAAAGCTTGTGGAATTTATCATTACC	
M4K10-RT-F	ATGGCTCGGAACAAGCTCGAG	Full length *MAP4K10* CDS
M4K10-RT-R	TTAACCCAAAACACTATCTTTATCAGC	
PR1-F	CGGAGCTACGCAGAACAACT	qPCR
PR1-R	CTCGCTAACCCACATGTTCA	
EF-1α-F	CTGGATTCGAGGGAGACAACA	qPCR
EF-1α-R	GCACCGTTCCAATACCACCAA	
BIK1-RT-F	TGGGCTCGACCGTACCTCACA	qPCR
BIK1-RT-R	CGGGCGCGACTTGGGTTCAA	
BIK1-HA-RT-F	CAGGACAACTTGGGAAAACCG	T-PCR
BIK1-HA-RT-R	TAGGATCCTGCATAGTCCGGG	
NahG-F	CACCATGAAAAACAATAAACTTGGCTTGCGC	Clone *NahG* gene
NahG-R	CCCTTGACGTAGCGCACCC	
SIK1-F	CACCATGGATCATAACTCGCCGAAATC	Clone *SIK1* gene
SIK1-R	TTAGAGGCGGAGAATAGTGCGAAG	
SIK1ΔN -F	CACCATGTACGAATTCCTCAATGAACTTG	Clone *SIK1ΔN*
SIK1-K278E-F	CAGAGATTGTTGCTGTGGAAGTCATATCACTTAC	Generate *SIK1* mutant (K278E)
SIK1-K278E-R	GTAAGTGATATGACTTCCACAGCAACAATCTCTG	
BAK1-F	CCGCTCGAGATGGAACGAAGATTAATGAT	Clone *BAK1* gene into pUC19-35S-FLAG
BAK1-R	TTTATATTCGAATCTTGGACCCGAGGGGTATT	
PBL1-KpnI-F	CGGGTACCATGGGTTCTTGTCTCAGTTC	Clone *PBL1* into pUC19-35S-FLAG
PBL1-BstBI-R	AGGTTCGAACAATCCAACGGTTTTTTTGTTTAAACC	
RBOHD-F	CACCATGAAAATGAGACGAGGCAA	Clone *RBOHD-N* into MBP tag vector
RBOHD-N-R	CTATCTCTGCCAATTGTCAAGTAT	
HA/His-MBP-BSK1-F	AAAACCTGTACTTCCAATCCAATATGGGTTGTTGTCAATCCTTGTTTTCCGGC	Clone *BSK1* gene into MBP tag vector
HA/His-MBP-BSK1-R	GGATCCGTTATCCACTTCCAATTCAAGATCCTCTGCCGCCTCGTTGTCTCTT	
GST-RbohD-N-F	GAAAACCTGTACTTCCAATCCAATATGAAAATGAGACGAGGCAATTCA	Clone *RBOHD-N* into GST tag vector

GST-RbohD-N-R	CGGATCCGTTATCCACTTCCAATTCATCTCTGCCAATTGTCAAGTA	Clone *RBOHD-N* into GST tag vector
GST/MBP-SIK1ΔN-F	CCTGTACTTCCAATCCAATGAATTCCTCAATGAACTTGGG	Clone *SIK1ΔN* into GST or MBP tag vector
GST/MBP-SIK1ΔN-R	CCGTTATCCACTTCCAATTTAGAGGCGGAGAATAGTGCGA	
His-MBP-BIK1-F	CTGTACTTCCAATCCAATATGGGTTCTTGCTTCAGT	Clone *BIK1* into MBP tag vector
His-MBP-BIK1-R	CCGTTATCCACTTCCAATTTACACAAGGTGCCTGCCAAA	

Table S4. Related to Figure 7. Isolation list for PRM. Modified and unmodified peptide sequences used to quantify phosphorylation by PRM are noted. CID = collision induced dissociation, m/z = mass to charge, z = charge state.

Peptide sequence	Peptide modified sequence	m/z	z	CID Collision Energy (%)
GNSSNDHELGILR	GNSSNDHELGILR	706.35	2	27
	GNS[+80]SNDHELGILR	746.333	2	27
	GNSS[+80]NDHELGILR	746.333	2	27
	GNS[+80]S[+80]NDHELGILR	786.316	2	27
ILSQMLSQK	ILSQMLSQK	524.297	2	27
	ILS[+80]QMLSQK	564.28	2	27
	ILSQM[+16]LSQK	532.294	2	27
	ILSQMLS[+80]QK	564.28	2	27
	ILS[+80]QM[+16]LSQK	572.278	2	27
	ILS[+80]QMLS[+80]QK	604.263	2	27
	ILSQM[+16]LS[+80]QK	572.278	2	27
	ILS[+80]QM[+16]LS[+80]QK	612.261	2	27