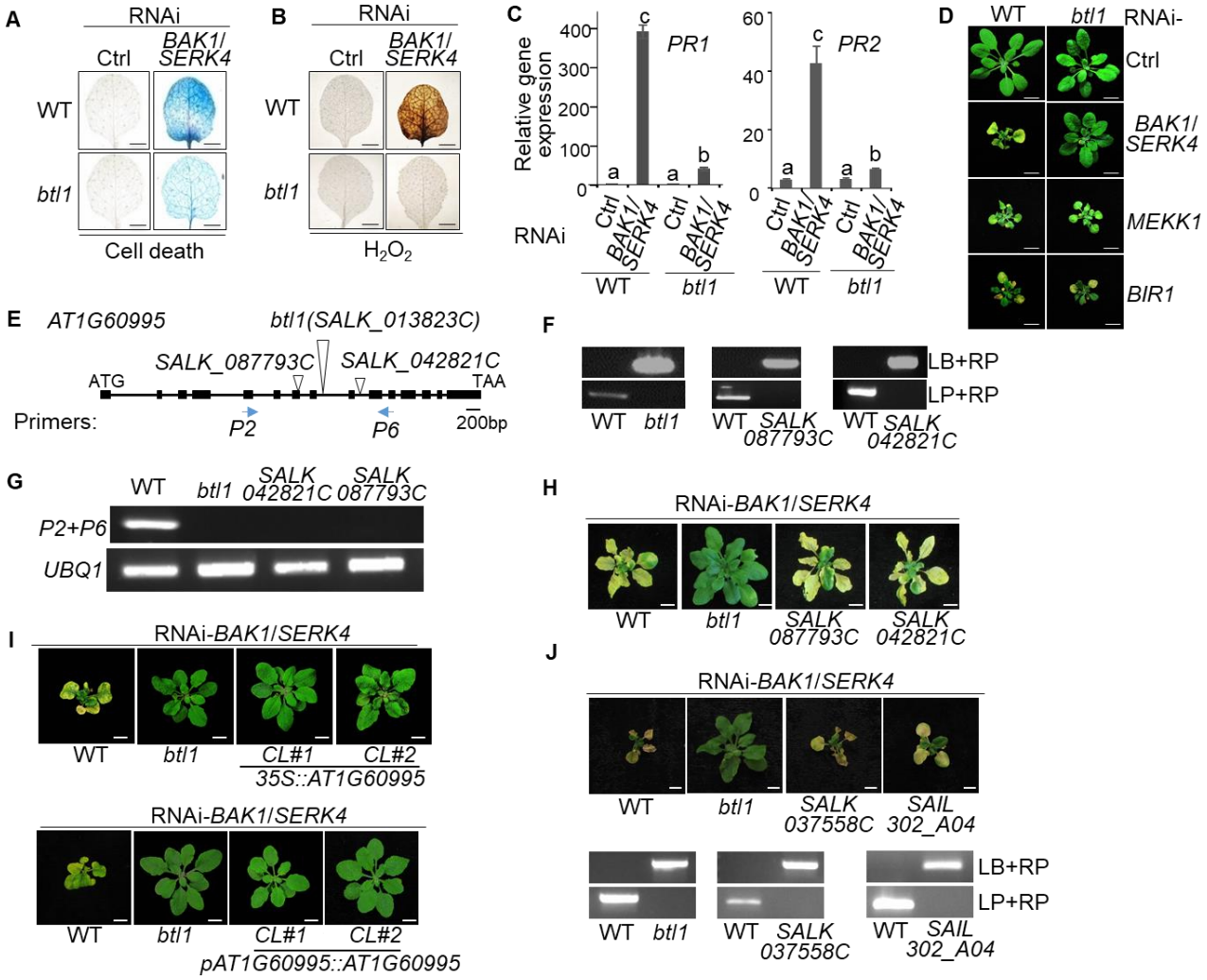


Figure S1. RNAi-based genetic screen identified *btl1* specifically suppressing *BAK1*/*SERK4*-mediated cell death. Related to Figure 1.

(A) The *btl1* mutant suppresses cell death triggered by RNAi-*BAK1/SERK4*. True leaves of WT (Col-0) and *btl1* after VIGS of *BAK1/SERK4* or a vector control were stained with trypan blue for cell death. Bar=2 mm.

(B) The *btl1* mutant suppresses H_2O_2 production triggered by RNAi-*BAK1/SERK4*. True leaves of WT and *btl1* after VIGS of *BAK1/SERK4* or a vector control were stained with DAB for H_2O_2 accumulation. Bar=2 mm.

(C) The *btl1* mutant suppresses *PR1* and *PR2* expression triggered by RNAi-*BAK1/SERK4*. The expression of *PR1* and *PR2* was normalized to the expression of *UBQ10*. The data are shown as

mean ± SE from three independent repeats. The different letters denote statistically significant difference according to one-way ANOVA followed by Tukey test ($p<0.05$).

(D) The *btl1* mutant does not suppress cell death triggered by RNAi-*MEKK1* or RNAi-*BIR1*. Plant phenotypes are shown two weeks after VIGS of *BAK1/SERK4*, *MEKK1*, *BIR1* or a vector control (Ctrl). Bar=5mm.

(E) Scheme of *AT1G60995* with annotated T-DNA insertion sites in different SALK line mutants. Solid bars indicate exons and lines indicate introns. Arrows indicate the primers used for RT-PCR analysis in G. Bar=200 bp.

(F) PCR confirmation of three T-DNA insertion mutants of *AT1G60995*. The genomic DNAs from WT, *btl1*, *SALK_087793C* and *SALK_042821C* were PCR-amplified to confirm all three lines are homozygous mutants for the annotated T-DNA insertions. The primer pair of LP and RP amplified the genomic DNA fragment of *AT1G60995* and the primer pair of LB and RP amplified the T-DNA insertions.

(G) RT-PCR confirmation of the T-DNA insertion mutants of *AT1G60995*. The cDNA fragments from WT, *btl1*, *SALK_087793C* and *SALK_042821*C were PCR-amplified with the indicated primers. *UBQ1* was used as an internal control.

(H) Unlike *btl1*, two independent alleles of T-DNA insertion mutants of *AT1G60995* do not suppress growth defects by RNAi-*BAK1/SERK4*. Plant phenotypes are shown from WT, *btl1*, *SALK_087793C* and *SALK_042821C* two weeks after VIGS of *BAK1/SERK4*. Bar=5 mm.

(I) Complementation of *btl1* with *AT1G60995* driven by the *35S* promoter (top panel) or the native promoter (bottom panel) does not restore growth defects by RNAi-*BAK1/SERK4*. Bar=5 mm.

(J) Unlike *btl1*, two independent T-DNA insertion alleles of *AT1G11020* do not suppress growth defects by RNAi-*BAK1/SERK4*. Plant phenotypes are shown from WT, *btl1*, *SALK_037558C* and *SAIL_302_A04* two weeks after VIGS of *BAK1/SERK4*. Bar=5 mm. Genotyping PCR confirmation of T-DNA insertion in *SALK_037558C* and *SAIL_302_A04* is shown on the bottom. The above experiments were repeated three times with similar results.

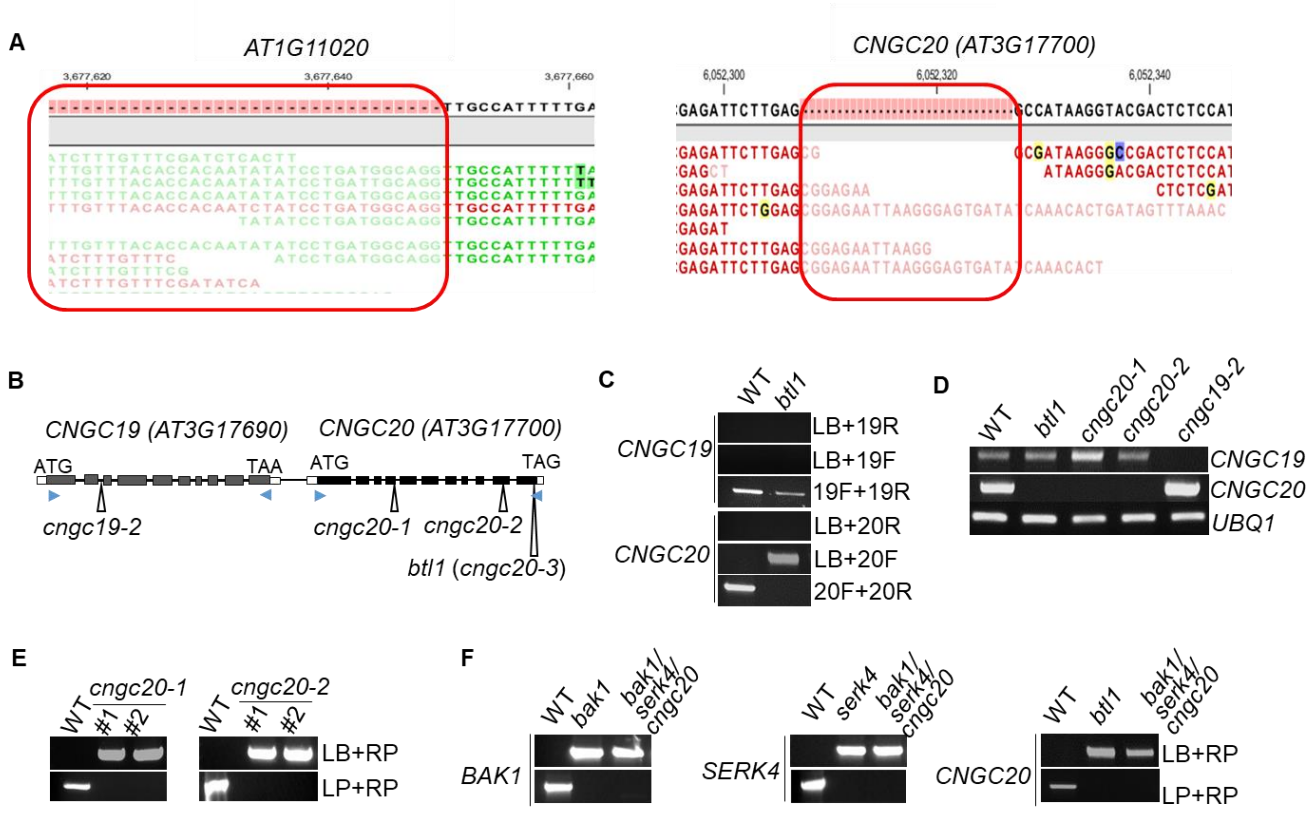


Figure S2. Characterization of the T-DNA insertions in the *cngc20* mutants. Related to Figure 1.

(A) Identification of T-DNA insertions in *AT1G11020* and *CNGC20* by whole genome sequencing analysis. Next-generation sequence reads of *btl1* are mapped and aligned to the genomic DNA sequence of *AT1G11020* and *CNGC20*, respectively. The red boxed regions indicate T-DNA fragments.

(B) Scheme of the *CNGC19* and *CNGC20* genes with annotated T-DNA insertion sites in *cngc19-2* (*SALK_007105*), *cngc20-1* (*SALK_129133C*), *cngc20-2* (*SALK_074919C*) and *btl1* (*cngc20-3*). Solid bars indicate exons and lines indicate introns. Triangles indicate the T-DNA insertion sites. Arrows indicate the primers used for genotyping analysis.

(C) Genotyping PCR analysis to confirm that *btl1* carries a T-DNA insertion in *CNGC20* but not *CNGC19*. DNAs from WT and *btl1* were PCR-amplified with the indicated primers to confirm the T-DNA insertion.

(D) RT-PCR analysis of *CNGC20* and *CNGC19* expression in *btl1*, *cngc20-1*, *cngc20-2* and *cngc19-2*. The cDNAs of *CNGC19* or *CNGC20* from different plants were PCR-amplified. *UBQ1* was used as an internal control.

(E) Genotyping PCR analysis of the T-DNA insertions in *cngc20-1* and *cngc20-2*. DNAs from WT, *cngc20-1*, and *cngc20-2* were PCR-amplified with the indicated primers to confirm that the two lines used are homozygous for the T-DNA insertions.

(F) Genotyping PCR analysis of the *bak1-4/serk4-1/cngc20-3* triple mutant. Genomic DNAs from WT, *bak1-4*, *serk4-1*, *btl1*(*cngc20-3*), and *bak1-4/serk4-1/cngc20-3* were PCR-amplified with specific primers to confirm that *bak1/serk4/cngc20* is a triple homozygous mutant.

The above experiments were repeated at least two times with similar results.

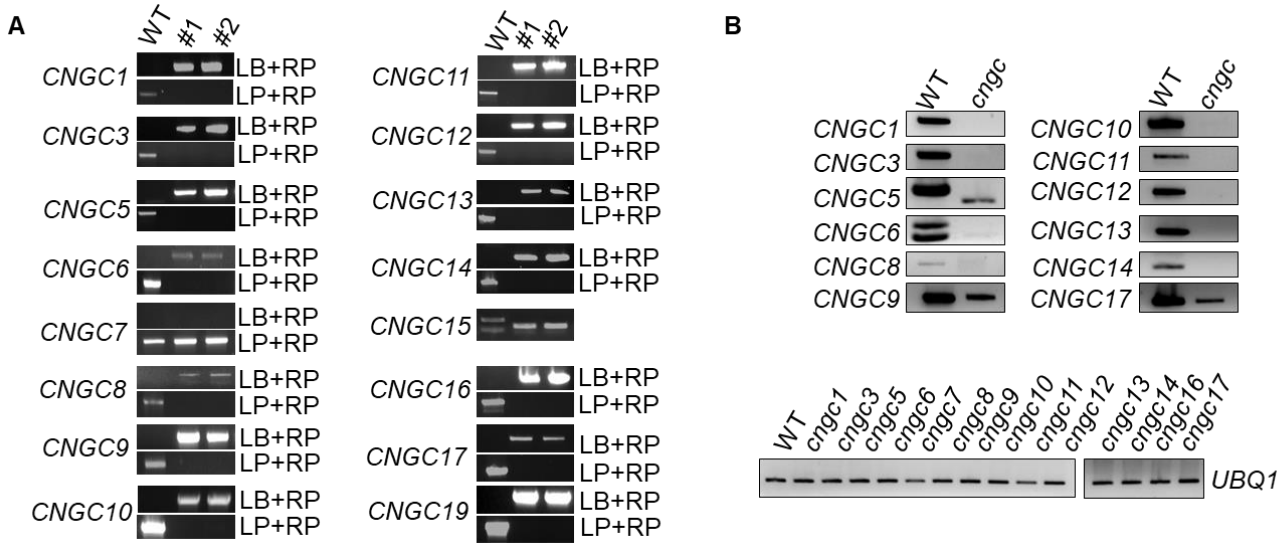


Figure S3. Characterization of *cngc* mutants. Related to Figure 2.

(A) Genotyping PCR analysis of *cngc* mutants. Genomic DNAs from WT and *cngc* mutants were PCR-amplified with the indicated primers to confirm that the lines used are homozygous except *cngc7*. #1 and #2 are two independent plant samples from each mutant. The genotyping for *cngc15* which bears a point mutation was performed by NlaIII digestion upon PCR-amplification.

(B) RT-PCR analysis of *CNGC* expression in *cngc* mutants. The cDNAs of *CNGCs* from WT and *cngc* mutants were PCR-amplified. *CNGC16* cannot be amplified from WT cDNA. *UBQ1* was used as an internal control.

The above experiments were repeated two times with similar results.

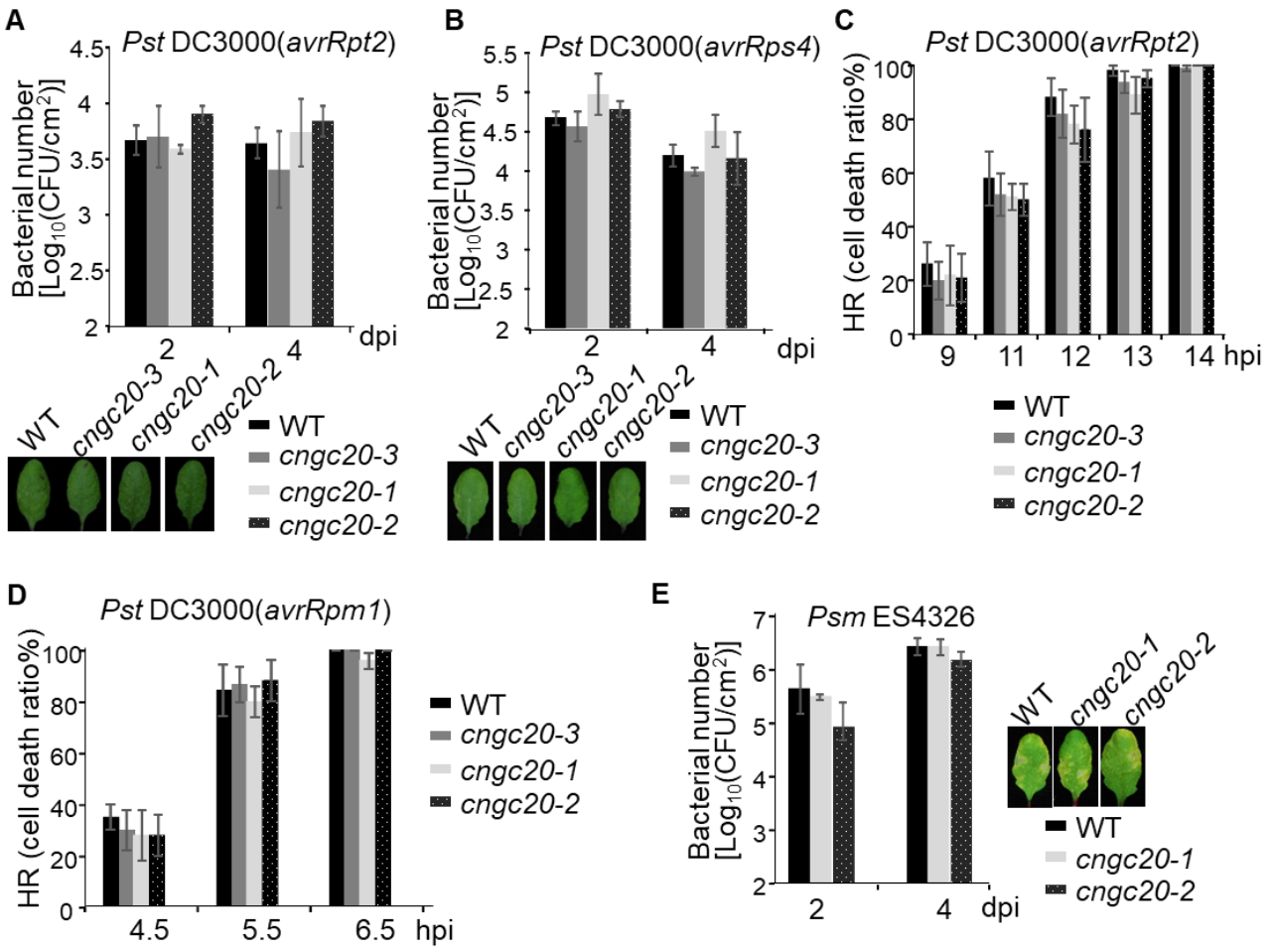


Figure S4. Unaltered plant immune responses in the *cngc20* mutants. Related to Figure 2.

(A and B) The *cngc20* mutants exhibit similar resistance to *Pst* DC3000 carrying *avrRpt2* (A) and *Pst* DC3000 carrying *avrRps4* (B) as WT plants. Four-week-old WT, *cngc20-3*, *cngc20-1* and *cngc20-2* plants were hand-inoculated with bacteria at OD_{600} =5×10^{-4}, and the bacterial counting was performed 2 and 4 days post-inoculation (dpi). The data are shown as mean ± SE from three independent repeats. The disease symptom is shown at 4 dpi (bottom panel).

(C and D) Comparable HR in *cngc20* mutants and WT triggered by *Pst* DC3000 *avrRpt2* (C) and *Pst* DC3000 *avrRpm1* (D). Four-week-old plants were hand-inoculated with bacteria at OD_{600}=0.1. HR was examined by counting the percentage of wilting leaves of total inoculated leaves (n=18) at different time points after inoculation.

(E) The *cngc20* mutants exhibit similar resistance to *Psm* infection as WT plants. The assays were performed as (A) with *Psm*.

The above experiments were repeated three times with similar results.

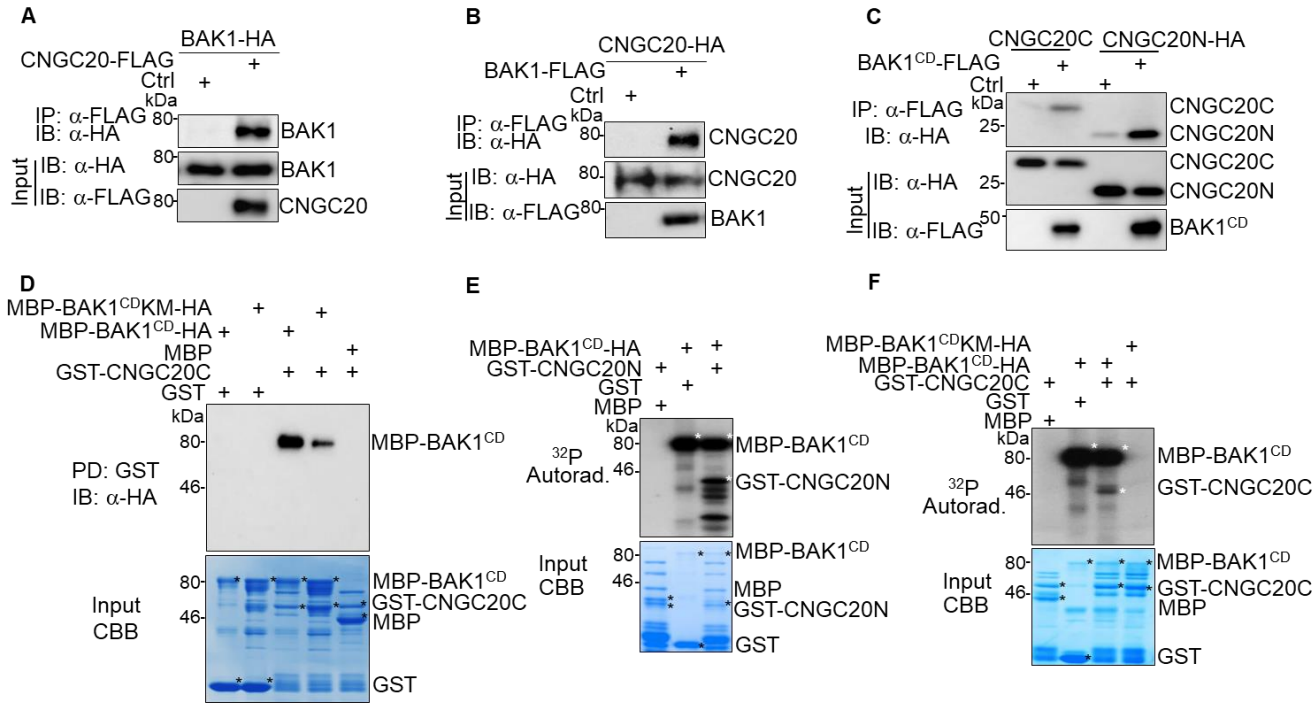


Figure S5. BAK1 interacts with and phosphorylates CNGC20. Related to Figure 3 & 4.

(A) CNGC20 associates with BAK1 in *Arabidopsis* protoplasts. CNGC20-FLAG and BAK1-HA were transiently co-expressed in *Arabidopsis* protoplasts. Protein extracts were immunoprecipitated with α-FLAG agarose beads (IP: α-FLAG) and immunoblotted with α-HA antibody (IB: α-HA) (top panel). The protein inputs are shown with immunoblotting before immunoprecipitation (bottom two panels).

(B) CNGC20 associates with BAK1 in *N. benthamiana*. CNGC20-HA and BAK1-FLAG were transiently co-expressed in *N. benthamiana* for Co-IP assay.

(C) Both CNGC20C and CNGC20N interact with BAK1^{CD} in *Arabidopsis* protoplasts. BAK1^{CD}-FLAG and CNGC20N-HA or CNGC20C-HA were transiently co-expressed in *Arabidopsis* protoplasts for Co-IP assay.

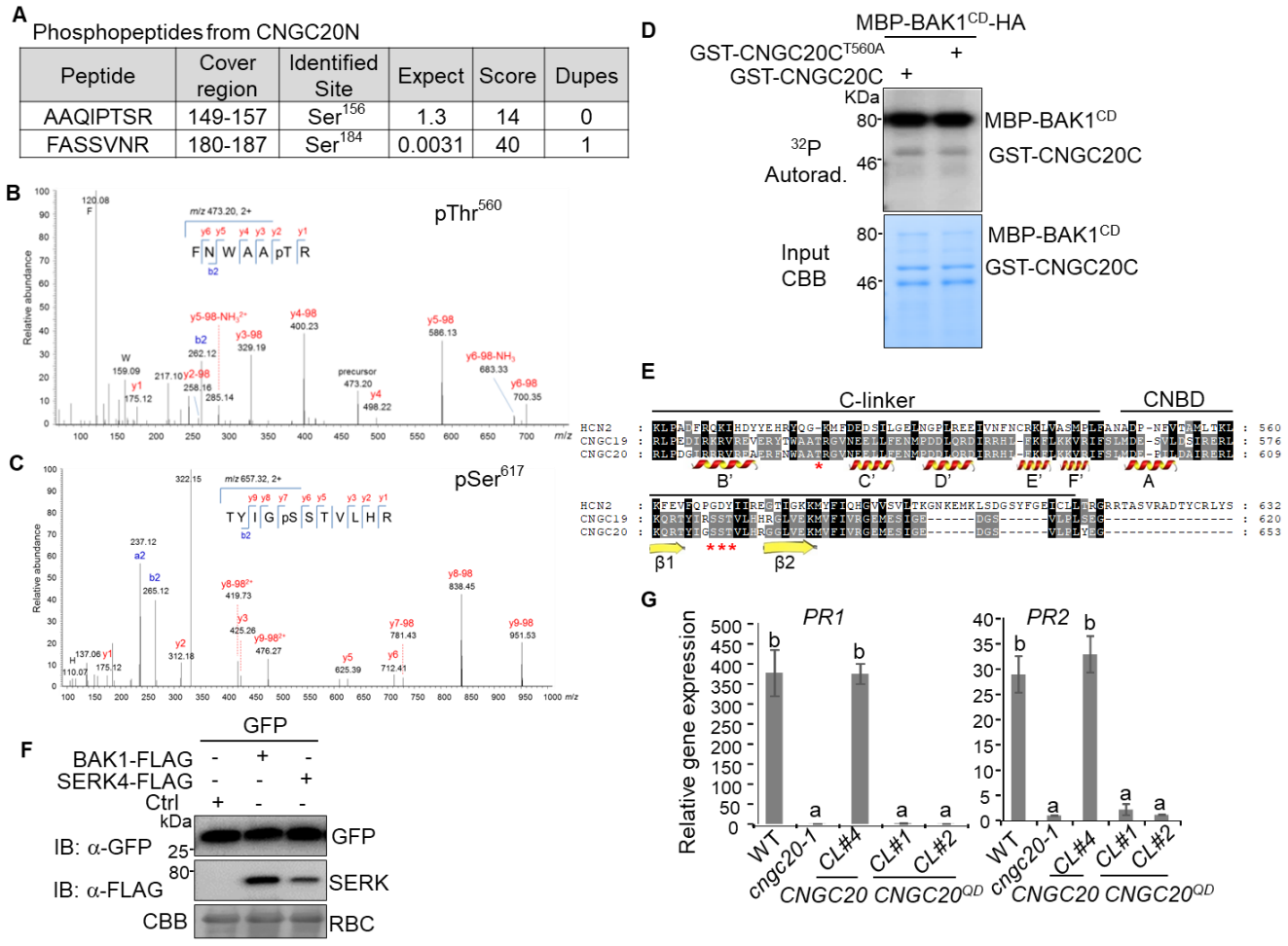
(D) The BAK1 kinase activity contributes to its interaction with CNGC20C. GST or GST-CNGC20C immobilized on glutathione sepharose beads was incubated with MBP, MBP-BAK1^{CD}-HA or MBP-BAK1^{CD}KM-HA proteins. The beads were washed and pelleted for immunoblotting analysis with α-HA antibody (PD: GST; IB: α-HA) (top panel). CBB staining of input proteins is shown on the bottom.

(E) BAK1 phosphorylates CNGC20N *in vitro*. The kinase assay was performed by incubating MBP or MBP-BAK1^{CD}-HA with GST or GST-CNGC20N as a substrate. The phosphorylation of

CNGC20N by BAK1^{CD} is shown with autoradiography (top panel). The protein loading control is shown by CBB (bottom panel).

(F) BAK1 phosphorylates CNGC20C *in vitro*. Similar assay using CNGC20C as a substrate was performed as in (E).

The above experiments were repeated three times with similar results.



Peptide	Cover region	Identified Site	Expect	Score	Dupes
AAQIPTSR	149-157	Ser^{156}	1.3	14	0
FASSVNR	180-187	Ser^{184}	0.0031	40	1

Figure S6. LC-MS/MS analysis of *in vitro* phosphorylation of CNGC20N and CNGC20C by BAK1^{CD}. Related to Figure 4.

(A) Phosphorylation sites and the corresponding phosphopeptides from CNGC20N region identified from LC-MS/MS analysis.

(B and C) LC-MS/MS analysis reveals that Thr^{560} (B) and Ser^{617} (C) of CNGC20C were phosphorylated by BAK1^{CD}.

(D) The phosphorylation of CNGC20C by BAK1^{CD} is not affected by the T560A mutation of CNGC20C. The kinase assays were performed using CNGC20C, CNGC20C^{T560A} proteins as substrates of BAK1^{CD}. The CBB staining of input proteins is shown (bottom panel).

(E) Amino acid sequence alignment of the C-linker and cyclic nucleotide-binding domain (CNBD) among human CNGC protein HCN2, *Arabidopsis* CNGC19 and CNGC20. The secondary

structures for the α-helix and β-sheet are labeled with letters. Red stars under the sequence indicate CNGC20 phosphorylation residues by BAK1.

(F) GFP protein accumulation is not affected by co-expression with BAK1 or SERK4. GFP was co-expressed with vector control (Ctrl), BAK1-FLAG or SERK4-FLAG in *Arabidopsis* protoplasts for 12 hr. Protein expression was analyzed with α-GFP or α-FLAG immunoblot.

(G) Complementation of *cngc20-1* with $CNGC20^{QD}$ does not induce *PR1* and *PR2* expression triggered by RNAi-*BAK1/SERK4*. CL#1 and CL#2 are two representative lines. CL#4 is WT *CNGC20* complementation control. The expression of *PR1* and *PR2* was normalized to the expression of *UBQ10*. The data are shown as mean ± SD from two independent repeats. The different letters denote statistically significant difference according to one-way ANOVA followed by Tukey test ($p<0.05$).

The above experiments were repeated three times with similar results.

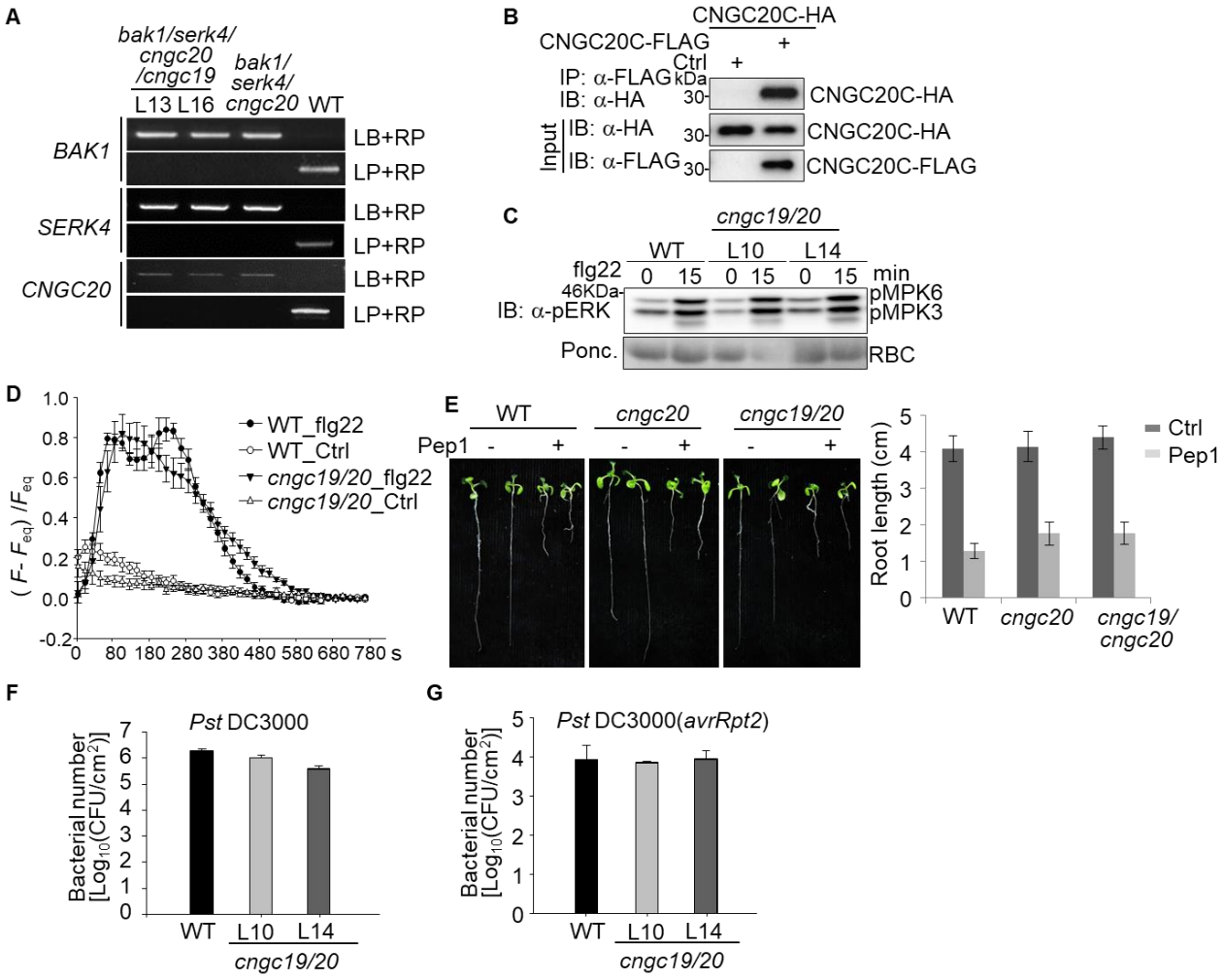


Figure S7. Unaltered immune responses in the *cngc19/cngc20* mutants. Related to Figure 7.

(A) Genotyping PCR analysis of the T-DNA insertions in *bak1/serk4/cngc20/cngc19* quadruple mutant. DNAs from WT, *bak1-4/serk4-1/cngc20-1*, and CRISPR/Cas9 *cngc19/bak1-4/serk4-1/cngc20-1* (line13 and line16) were PCR-amplified with specific primers.

(B) CNGC20C self-associates. CNGC20C-FLAG and CNGC20C-HA were transiently co-expressed in *Arabidopsis* protoplasts. Protein extracts were immunoprecipitated with α-FLAG agarose beads (IP: α-FLAG) and immunoblotted with α-HA antibody (IB: α-HA) (top panel). The protein inputs are shown with immunoblot before immunoprecipitation (bottom two panels).

(C) Comparable flg22-induced MAPK activation in the *cngc19/cngc20* mutant and WT. Ten-day-old seedlings were treated without or with 100 nM flg22 for 15 min. The MAPK activation was

analyzed by immunoblot with α-pERK antibody (top panel), and the protein loading is shown by Ponceau S staining for RBC (bottom panel).

(D) The *cngc19/cngc20* mutants exhibit similar flg22-induced Ca^{2+} influx as WT. GCaMP3 was expressed in protoplasts from WT and *cngc19/cngc20-1* plants for 12 hr. The signal was measured upon 100 nM flg22 or ddH_2O (Ctrl) treatment. The difference of absolute fluorescence values with the control value for each experiment was normalized to the control value as $(F-F_{eq})/F_{eq}$ (where F was the measured fluorescence at a given time point and F_{eq} was the averaged measurement for the samples at the final resting time point measured). Averages of five replicates ± SE are shown.

(E) The *cngc19/cngc20* mutants exhibit similar Pep1-induced seedling growth inhibition as WT. Four-day-old seedlings were transferred from ½MS plate to liquid ½MS medium supplemented without (black bar) or with (gray bar) 1 μM Pep1 and grew for another 7 days. Averages of root length from eight seedlings are shown as mean ± SD (left panel).

(F and G) The *cngc19/cngc20* mutants exhibit similar susceptibility as WT plants to *Pst* DC3000 (F) and *Pst* DC3000 *avrRpt2* (G). The bacterial counting was performed at 3 dpi.

The above experiments were repeated twice with similar results.

Gene Locus	Gene Identity	T-DNA Insertion Position in *btl1*	Additional T-DNA lines analyzed
AT1G60995	Unknown protein, containing membralin domain	*8th* intron	SALK_087793C (T-DNA in *7th* exon)
			SALK_042821C (T-DNA in *9th* intron)
AT1G11020	RING/FYVE/PHD zinc finger superfamily protein	*1st* intron	SALK_037558C (T-DNA in *1st* intron)
			SAIL_302_A04 (T-DNA in *1st* intron)
AT3G17700	*CNGC20*	*11th* exon	SALK_129133C (T-DNA in *4th* exon)
			SALK_074919C (T-DNA in *10th* exon)

Table S1. T-DNA insertions identified from the whole genome-sequence analysis of the *btl1* (*SALK _013823C*) mutant. Related to Figure 1.

ATGGCTTCCCACAACGAAAACGATGATATTCCCATGCTTCCGATTTCAGACCCATCA
TCTCGTACTAGAGCCAGAGCTTTCACTTCCAGGAGTCGTAGCGTTTCTCTCTCAAACC
CTACTTCCTCCATTGAAGGATTTGACACTTCCACTGTGGTTTTAGGCTACACGGGTCC
TCTTCGAACTCAGAGACGTCCTCCTTTAGTTCAAATGAGTGGTCCTCTTACCTCTACT
CGCAAGCATGAACCTCTCTTTCTTCCTCATCCTTCTTCTGATTCCGTTGGTGTCTCTTC
TCAGCCTGAGAGGTATCCTTCTTTTGCTGCTCTTGAACATAAAAACTCCTCAGAGGA
TGAGTTCGTTTTGAAACACGCAAATCTCTTGAGGTCTGGACAATTGGGAATGTGTAA
TGATCCTTACTGTACTACTTGCCCTTCTTACTACAACCGTAAGGCTGCTCAAATCCCT
ACTTCTAGAGTTTCTGCCCTTTTTGATTCCACGgtaaagttttgatttttgttactttacattcacaaatatagcttca
ggatcaagtaaatgtcgttgaaacagagtcttttgttttcttgctagTTCCATAACGCTCTGTATGATGATGCTAAA
GGTTGGGCAAGGAGATTTGCTTCCTCTGTTAATAGATACTTACCTGGAATCATGAAT
CCTCATGCCAAAGAGGTTCAAACCTGGACTAAATTCTTCGCCCTTTCATGCTTGTTAG
CTATTTTTATAGATCCCCTCTTCTTCTTCCTCATAAAAGTCCAAGAGgtatgtttttccttcagaga
tatcttttgcttttccaagtgtttttatcttgcctttacaccacctttttgaccatctttatttttctcactgtagCAAAACAAATGTATTA
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TATTCACTATGAACATTCTACTTCAGgtgcttttcttttgcttacatttttccatctctttataaacaaaatatgttggcttta
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AGCTGGCCAGTTAGTTAGTCATCCCAAAAAAATTGCCCTTCATTACCTCAAAGGAAA
GTTTTTCCTTGACTTGTTCATAGTGATGCCACTTCCACAGgtattgttacgttagagctcctactggtttta
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TTGGGTGCATCCGGGGCAAACTATGCGAAAAACCTTCTACGAGCTGCAGTTCTTTTC
CAATACATTCCAAAGTTATATAGACTTCTACCGTTTCTTGCTGGACAAACACCTACC
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TTGCTGGACATGTTGTTGGTTCTTGCTGGTATCTATTTGGTCTGCAGgtatgacaaactctcaaa
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CAGTGCCTTCGAAATGCTTGCGGTAATTTTGGGCGTGAATGTCAAGATCTTATAGAT
TGTGGTAATGGAAATAGCAGTGTATTAGTACGAGCTACCTGGAAAGATAATGCGAG
TGCCAATGCTTGTTTCCAAGAAGATGGTTTTCCTTATGGAATCTATTTAAAAGCAGTC
AATCTTACCAATCATTCTAATCTCTTCACAAGATACAGTTACTCTCTCTTCTGGGGCT
TCCAGgtaactgtttttttttttctctctttcagtttttagtaggaaactaagaacaactactcaactactttgcattgattggcatacttggaact
cttcattaagatcattaattttataattctcatagCAAATCAGCACTCTTGCTGGCAACCAAGTCCCAAGCTA
CTTTCTAGGGGAGGTCTTCTTTACTATGGGTATCATTGGACTAGGGCTTTTACTTTTT
GCGCTTCTTATTGGTAATATGCAAAATTTCCTTCAAGCTCTTGGTAAAAGgtaaacaattcca
tcattgtactaaaatagatatttcaagacataatggttggttctataaacactaactactcaggtgattgtgttttgtccatattagGAATTTG
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GAGAGCATTGGAGAAGATGGTTCTGTTCTTCCATTATATGAAGGCGATGTTTGTGGT
GAAGAACTCCTCACTTGGTGCCTCGAACGCTCTTCTGTAAACCCCGgtaccatccctttgactct
atctctatgtgttcataaatcccaaaaagtttggttccttagatgacacttcgttaagaaaaagcttggaactgagtatgttctgaaaccttcag

ATGGGACGAGGATAAGGATGCCATCAAAGGGATTGCTTAGTAGCAGAAATGTAAGG
TGTGTGACAAATGTGGAGGCGTTTTCGCTGAGTGTAGCCGATCTGGAAGACGTAACG
AGCTTGTTTTCGAGATTCTTGAG**GAGT**CATAGAGTCCAAGGAGCCATAAGGTACGA
CTCTCCATATTGGAGGCTACGAGCGGCTAGGCAGATTCAAGTGGCGTGGAGATACC
GTAGGAGACGGCTTCATAGATTATGCACTCCTCAGTCTAGTTATAGCCTT**TAG**

Table S2. Sequence of *CNGC20* with the T-DNA insertion site underlined in the *btl1 (cngc20-3)* mutant. Related to Figure 1.

Note: The start codon (ATG) and stop codon (TAG) are in bold. The capital letters denote exons while the small letters indicate introns. GAGT, which are in bold and underlined, bears a T-DNA insertion based on the whole genome-sequence analysis of *btl1*. This was further confirmed by targeted Sanger-sequence analysis of this region.

CNGCs	Gene Locus	Salk Line Number	T-DNA Insertion Position
CNGC1	*AT5G53130*	SAIL_443_B11	*4th* exon
CNGC2	*AT5G15410*	*dnd1-1*	Trp_{290}→stop codon
CNGC3	*AT2G46430*	SALK_056832C	*3rd* exon
CNGC4	*AT5G54250*	*dnd2-1*	Trp_{89}→stop codon
CNGC5	*AT5G57940*	SALK_149893C	*5th* exon
CNGC6	*AT2G23980*	SALK_042207	*2nd* intron
CNGC7	*AT1G15990*	SALK_060871C	*1st* exon
CNGC8	*AT1G19780*	GABI_101C03	*4th* intron
CNGC9	*AT4G30560*	SALK_026086	*5th* intron
CNGC10	*AT1G01340*	SALK_015952C	*7th* exon
CNGC11	*AT2G46440*	SALK_026568C	*7th* intron
CNGC12	*AT2G46450*	SALK_092657	*5th* exon
CNGC13	*AT4G01010*	SALK_060826	*3rd* exon
CNGC14	*AT2G24610*	WiscDsLox437E09	2nd exon
CNGC15	*AT2G28260*	CS93507	point mutation
CNGC16	*AT3G48010*	SAIL_726_B04	*3rd* exon
CNGC17	*AT4G30360*	SALK_041923	*5th* exon
CNGC19	*AT3G17690*	SALK_007105	*2nd* intron
CNGC20	*AT3G17700*	SALK_129133C	*4th* exon
CNGC20	*AT3G17700*	SALK_074919C	*10th* exon

Table S3. T-DNA insertion mutants of *CNGC* family members. Related to Figure 2.