Supplemental Methods

CRISPR/Cas Construct Assembly

All constructs were assembled using the Golden Gate cloning method (Weber et al. 2011). Level 1 constructs carrying sgRNAs placed under the control of the Arabidopsis (*Arabidopsis thaliana*) U6 promoter were assembled as described (Belhaj et al. 2013). For knocking out of the *NRC4* genes in tomato, forward primers JC_sgrna2_F, JC_sgrna3_F, JC_sgrna4_F, and JC_sgrna5_F were used in combinations with the JC_sgrna_R reverse primer (Supplemental Table S1) to clone sgRNAs 2, 3, 4 and 5, respectively. Level 1 constructs pICH47732::NOSp::NPTII (Addgene no. 51144; www.addgene.org), pICH47742::35S::Cas9 (Addgene no. 49771), pICH47751::AtU6p::sgRNA2, pICH47761::AtU6p::sgRNA3, pICH47772::AtU6p::sgRNA4, pICH47781::AtU6p::sgRNA5 and the linker pICH41822 (Addgene no. 48021) were assembled into the level 2 vector pAGM4723 (Addgene no. 48015) as described (Weber et al. 2011) resulting in the level 2 construct pAGM4723::NPTII::Cas9::sgRNA2::sgRNA3::sgRNA4::sgRNA5. For knocking out of *NRC4s* in *N. benthamiana*, forward primers CHW_sgNbNRCs (Supplemental Table S1) were used in combinations with the JC_sgrna_R reverse primer to clone sgRNA4.1, sgRNA4.2, sgRNA2.1-4, sgRNA3.1-4 into the level 1 vectors for different positions. Level 1 constructs pICSL11017 (pICH47732::NOSp::BAR, Addgene no. 51145), pICH47742::35S::Cas9, and combinations of sgRNAs targeting *N. benthamiana NRCs* were assembled into level 2 vector pICSL4723 (Castel et al., 2019).

Plant transformation

Tomato cultivar GCR758 (Balint-Kurti et al. 1995) was transformed with the pAGM4723::NPTII::Cas9::sgRNA2::sgRNA3::sgRNA4::sgRNA5 construct as previously described (Fillatti et al. 1987). T0 transgenic plants were selected in the medium with kanamycin (100mg/L) and then transferred into the soil. *N. benthamiana* were transformed with the binary vector pICSL4723 containing BAR selection marker gene, Cas9 expression cassette, and sgRNAs targeting *NRC4* or multiple *NRCs*. T0 transgenic plants were selected in the medium with phosphinothricin (2mg/L) and then transferred into the soil.

Plant genotyping

Genomic DNA was extracted from tomato leaves as described (Nekrasov et al. 2017). Tomato plants were genotyped using PCR with respective primers (Supplemental Table S1) followed by Sanger sequencing. DNeasy Plant DNA Extraction Kit (Qiagen) was used to extract the genomic DNA of *NRCs* knockout *N. benthamiana* lines. Primers aligning to each *NRC* gene (Supplemental Table S1) were used to amplify the region targeted by the sgRNAs in multiplex PCR reactions. The amplified DNA fragments were then sequenced with the amplicon sequencing services provided by Floodlight Genomics (USA) or Genewiz (Germany). Illumina sequencing reads were aligned to the reference *N. benthamiana* draft genome Niben.genome.v0.4.4 [Sol Genomics Network (SGN), https://solgenomics.net/]. Reads aligning to *NRC4a* (NbS00016103g0004.1) and *NRC4b* (NbS00002971g0007.1), *NRC2a* (NbS00018282g0019.1), *NRC2b* (NbS00026706g0016.1), and *NRC3* (NbS00011087g0003.1) were further analysed. Raw Illumina sequencing data were submitted to the Sequence Read Archive (SRA) database of NCBI under the BioProject ID PRJNA577826. T3 populations from the selected T2 plants were used for further experiments.

Measurement of ROS

ROS production after flg22 peptide treatment was monitored by a luminol-based assay. 6.25 mm diameter tomato or *N. benthamiana* leaf discs were incubated for 16 hours in 150 µL of water in a 96-

well plate. Before the measurement of ROS, the water was removed and 100 µL of ROS detection solution [17 mM luminol (Sigma); 1 µM horseradish peroxidase (Sigma); 100 nM flg22 (EzBiolab)] was added to each well. Chemiluminescence was measured by using an ICCD photon-counting camera (Photek, East Sussex, UK).

Detection of MAPK phosphorylation

Six leaf discs (6.25 mm diameter) of tomato or *N. benthamiana* leaves and were homogenised in 200 µL of extraction buffer [10% glycerol; 25 mM Tris, pH 7.5; 1 mM EDTA; 150 mM NaCl; 2% w/v PVPP; 10 mM DTT; 1% v/v protease inhibitor cocktail 2 (Sigma, P5726); 1% v/v protease inhibitor cocktail 3 (Sigma, P0044); 0.2% v/v IGEPAL (Sigma). After centrifugation at 12,000 *xg* for 10 min, 50 µL of supernatant was mixed with equal amount of 2x SDS-PAGE sample buffer and used for SDS-PAGE. Immunoblotting was done with an antibody against phosphorylated MAPK (Phospho-p44/42 MAPK Erk 1/2 Thr202/Tyr204 XP Rabbit mAb #4370; Cell Signaling Technology).

Cell death assay on tomato and *N. benthamiana* leaves

Transient expression of GFP, Rpi-blb2, AVRblb2, and Rpi-vnt1 and AVRvnt1 in tomato leaves were performed according to the method described previously in Bos et al. (2006) with modifications. Suspensions of *Agrobacterium tumefaciens* AGL1 strains harboring the expression vector of different proteins were prepared in infiltration buffer (10mM MES, 10mM MgCl2, and 150µM acetosyringone, pH5.6) and adjusted to the final OD_{600} of 0.3 for each expression construct. Leaflets of the 4th leaves from four-week-old tomato plants were syringe-infiltrated with *A. tumefaciens* strains harboring the expression vector of different proteins as indicated. Cell death assays in *N. benthamiana* leaves were performed according to the method described previously in Wu et al. (2017).

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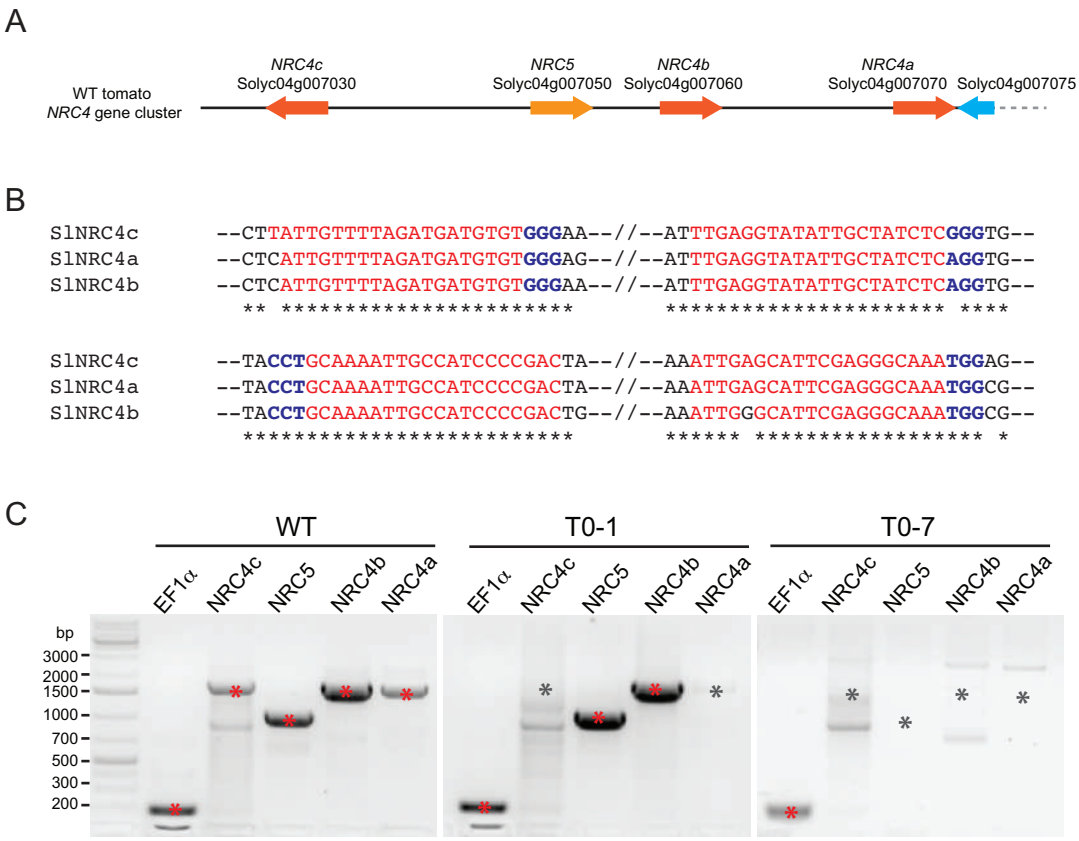
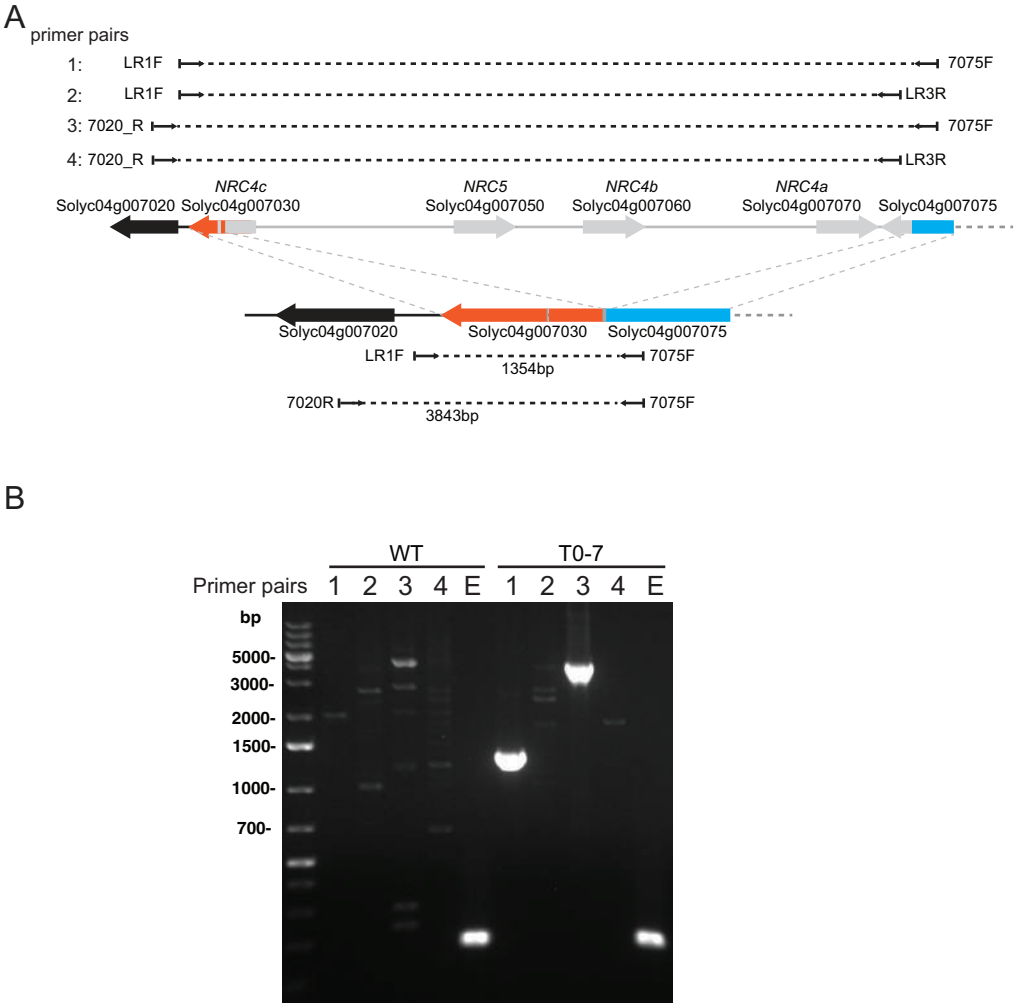
Supplemental Data

Supplemental Table S1. Primers used in this study.

Primer name	Sequence	Note
JC_sgrna2_F	tgtggtctcaATTGATTGTTTTAGATGATGTGTgttttagagctagaaatagcaag	sgRNA
JC_sgrna3_F	tgtggtctcaATTGTTGAGGTATATTGCTATCTCgttttagagctagaaatagcaag	sgRNA
JC_sgrna4_F	tgtggtctcaATTGATTGAGCATTCGAGGGCAAAgttttagagctagaaatagcaag	sgRNA
JC_sgrna5_F	tgtggtctcaATTGGTCGGGGATGGCAATTTTGCgttttagagctagaaatagcaag	sgRNA
JC_sgrna_R	tgtggtctcaAGCGTAATGCCAACTTTGTAC	sgRNA
JC_7050F	GAATTCTTAATGGAGTGAGT	NRC5 specific
JC_7050R	GTAATCTCTCTCGACACAGT	NRC5 specific
JC_1.3R	GTTGATACCACCCTTGTAAGCACC	NRC4a/c specific
JC_2R	GCA GATTCCACCTCTGGATTCAAG	NRC4b specific
JC_1F	CCAGGGTGAGGATGATGA	NRC4c specific
JC_2.3F	TCAGGGTCCTTCTTTGGA	NRC4a/b specific
RCG_7075F	AATCCTGTACTGCAAGTCCT	Primer pair 1/3
RCG_LR1F	TTGGAAATCTTAACGAAGATACG	Primer pair 1/2
RCG_7020R	AAAAGGTTAGCAAGCAGTTG	Fig 1, Fig S2
RCG_LR3R	AGCTGTACATAATGTCAGGCC	Fig 1, Fig S2
CHW_7070F1	AAATCTATAATGATCCTAAGCT	Fig S4A
Cas9_6F	gagGAAGACAAaTCT CCCGAAGATAATGAGCAGAAG	Fig S4A
Nos_R2	GGATCTAGTAACATAGATGACAC	Fig S4A
JC_EF-1α_qpcr_F	TGAGGCAAACTGTTGCTGTC	Fig 1B, Fig S1C, Fig S2B
JC_EF-1α_qpcr_R	TGGAAACACCAGCATCACAC	Fig 1B, Fig S1C, Fig S2B
EF1α_F	AAGGTCCAGTATGCCTGGGTGCTTGAC	Fig S4A
EF1α_R	AAGAATTCACAGGGACAGTTCCAATACCAC	Fig S4A
CHW_sgNbNRC4.1	tgtggtctcaATTGAAAAACGGTACATACCGCAGgttttagagctagaaatagcaag	sgRNA4.1
CHW_sgNbNRC4.2	tgtggtctcaATTGAGTCAGGAATCTTGCAGCTGgttttagagctagaaatagcaa	sgRNA4.2
CHW_sgNbNRC2.1	tgtggtctcaATTGTAAGCTTCACAAGGACAAAGgttttagagctagaaatagcaag	sgRNA2.1
CHW_sgNbNRC2.2	tgtggtctcaATTGGAACGATGTCCACAAAGAATgttttagagctagaaatagcaag	sgRNA2.2
CHW_sgNbNRC2.3	tgtggtctcaATTGTGGTGAAGAAAATTAAGACTGgttttagagctagaaatagcaag	sgRNA2.3
CHW_sgNbNRC2.4	tgtggtctcaATTGCTTCACAAGGACAAAGGGGTgttttagagctagaaatagcaag	sgRNA2.4
CHW_sgNbNRC3.1	tgtggtctcaATTGTCAAACAAGCAGCTAAATCAgttttagagctagaaatagcaag	sgRNA3.1
CHW_sgNbNRC3.2	tgtggtctcaATTGTGATGAGATTAAAACTATAAgttttagagctagaaatagcaag	sgRNA3.2
CHW_sgNbNRC3.3	tgtggtctcaATTGTGACTTGATTATTGGTATAAgttttagagctagaaatagcaag	sgRNA3.3
CHW_sgNbNRC3.4	tgtggtctcaATTGAATCACTAGTAAAGAAGATAgttttagagctagaaatagcaag	sgRNA3.4
CHW_NRC4_1_F	GGAAGTGCAAAGGGAGAGTT	Fig S5
CHW_NRC4_1_R	TCGCCTGAACCACAAACTTA	Fig S5
CHW_NRC4_2_F	GGCAAGAATTTTGGATGTGG	Fig S5
CHW_NRC4_2_R	CGAGGAACCCTTTTTAGGCAG	Fig S5
CHW_NRC4_ApS_F	acactctttccctacacgacgctcttccgatctatggcagatgcagtagtgaattttc	Fig S6
CHW_NRC4_ApS_R	gactggagttcagacgtgtgctcttccgatctgtttccgaggaacccttttaggcag	Fig S6
CHW_NRC2_ApS_F	acactctttccctacacgacgctcttccgatctatggcgaacgttgcggtggagtttc	Fig S6
CHW_NRC2_ApS_R	gactggagttcagacgtgtgctcttccgatctttcttggacacctttggcagattga	Fig S6
CHW_NRC3_ApS_F	acactctttccctacacgacgctcttccgatctatggcagatgtagcagcagatgtag	Fig S6
CHW_NRC3_ApS_R	gactggagttcagacgtgtgctcttccgatctcttggttgaaattatcataagaagt	Fig S6



Supplemental Figure S1. Deleting the *NRC4* gene cluster in tomato with CRISPR/Cas9. A. Cartoon showing the *NRC4* gene cluster (Solyc04g007030, Solyc04g007060 and Solyc04g007070) targeted by CRISPR/Cas9 in this study. Orange, NRC4 paralogs; yellow, NRC5; blue, Solyc04g007075, which contains incomplete CNL sequence information due to a sequencing gap in the reference genome. Another putative gene, Solyc04g007040, also exists in this gene cluster. This gene encodes a very short peptide of 45aa that does not show significant homology to any known proteins. Furthermore, it is not expressed according to the published tomato RNAseq data and thus is not included for further analyses. B. Sequence alignment of the region targeted by sgRNA in the NRC4 paralogs. The PAM motifs are marked in blue, and the sgRNA sequences are marked in red. C. PCR-genotyping for the presence of *NRC4* and *NRC5* genes in T0 transgenic lines. Amplification bands with expected size are labelled with red stars, whereas missing bands at the corresponding size are labelled with grey stars.



Supplemental Figure S2. Primer design and characterisation of the large deletion in mutant T0-7. A. Cartoon showing the *NRC4* gene cluster and positions of primers for characterising the mutant T0-7. B. Agarose gel electrophoresis of the PCR amplification results obtained with primer pairs indicated in A. None of the primer pairs amplified fragments with expected size when the genomic DNA from the WT plant was used. Primer pairs 1:LR1F x 7075F and 3:7020R x 7075F amplified fragments of 1354bp and 3848bp with the genomic DNA from mutant T0-7. Primer pairs 2: LR1R x LR3R and 4: 7020R x LR3R failed to amplify any fragments because of the reverse primer LR3R locates on the region that is deleted in the mutant T0-7. Primer pairs for EF1α (E) was used as amplification control.

DNA sequence:

GTAGTCTTGTTGGAAATCTTAACGAAGATACGTGTAGAGAATTCTTTATAGCAGAGGAGGCATGTCAATAACA
CTGCATATTTCACGATTCAAACTAGTATTGCAAAAGAAACTTACAATAGATTATTTACACAAATGAAAACAAAG
CAAATACAACCATAACAAAATAGGATAAGATGCAAAACAGTTATTTGTCTGTGGCTTCCACACAAAGGGGGAA
CTCGAAATGCAACAGAGTTAGCCTTATTTGAAATACTCTCCGCTAGCTATTTGTGTGATGATCCCTGGCTTGC
TTTGAGAAAGCTCAATCTCAAAGTTCTCTCCTGCAGAAGAAGAGGAAAATTAAGTTTTAGTAACTGGAACAAG
GAGAGGAAGATTGTGGCGGATAAACAAATGAATTCGACTGACCTTTCAACTTCACTGTGATCAGCATCACGA
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TTTGATTGTGTTCTCCAACTTCATCTCGCGAAGATTAGGTATATCAGCCAGCTCAACTGGTACAGCGTTGAGC
TTATCACAAGAGACAAGAACAAGGTTCCTAAGGATGGGGTAGTTAAGATTAGACGCCTCCCAAGTTTCTAATT
CTGCCCTCTCGATCCACAATACCCGGAGTTTGCAAAAACCTCCCAACTCCGGCTTCCAAGTATCACCCACGA
ATGCATTTTCTTTCAACTTTAGAACCTCAAGGGATTCCAATTGCCCTAATTTTTCTGCCTCACTCCATGAAAAC
CTTGTGTTTCTCAAAGTTAACTTCTTCACAGTACGTACAAGTCTGAAGAATGTTGCAGGAAGTTGAACTGTTT
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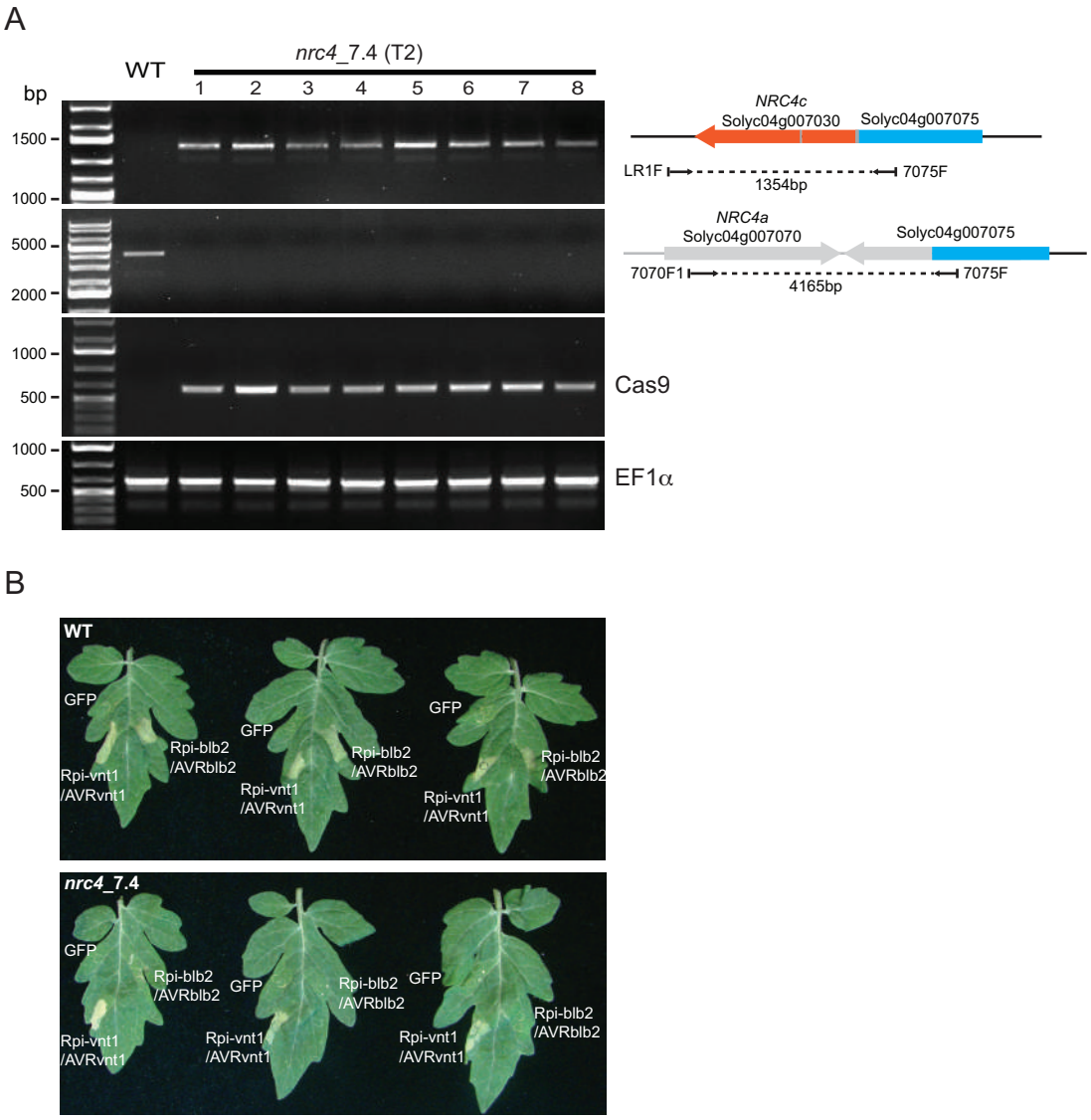
Translation (from direction of Solyc04g007075):

DPLSLLFESCTASPCELPFIDYDPIILGFLPCFNLLRVLVIIHDEMLRT*MLAVDCLFNHLISLNFSPQNLLQNM*DLSI
VFPQNKSQSTCLLWKPNSFTMHFHLLESWMLNLSILNSPHTSTNYFI*GILL*MEAFLGAYKGGINNLEELKRLEHL
KLLNDVPHYMNKTVQLPATFFRLVRTVKKLTLRNTRFSWSEAEKLGQLESLEVLKLKENAFVGDTWKPELGGFCK
LRVLWIERAELETWEASNLNYPILRNLVLVSCDKLNAVPVELADIPNLREMKLENTIKAVKSAKDILERKKSKDEKF
KCSIFPRDADHSEVERSVEFICLSATIFLSLFQLLKLNFPLLLQERTLRLSFLKASQGSSHK*LAESISNKANSVAFRV
PPLCGSHRQITVLHLILFCYGCICFVFICVNNLL*VSFAILV*IVKYAVLLTCLLCYKEFSTRIFVKIS

Supplemental Figure S3. Sanger sequencing results of the *NRC4* deletion allele from T0-7. In the DNA sequence, positions of the primers LR1F and 7075F used to amplify across the deleted region are highlighted in green. Regions of the NRC4 gene cluster present in the PCR amplicon are highlighted in yellow. Positions of the sgRNAs targets are underlined. Deleted regions are shown in blue. In the translation, amino acid residues belonging to Solyc04g007075 are marked in grey, and amino acid residues belonging to NRC4c (Solyc04g007030) are marked in orange. "*" represents stop codons.



Supplemental Figure S4. The *NRC4* knockout homozygous T2 line (*nrc4*_7.4) is impaired in Rpi-blb2 mediated cell death. A. Genotyping of the T2 line *nrc4*_7.4. Primer pair LR1F x 7075F and primer pair 7070F1 x 7075F were used to characterise the NRC4 gene cluster in the WT and *nrc4*_7.4 plants. Eight T2 plants from the line *nrc4*_7.4 were used. Primers for amplification of Cas9 and EF1α were used as controls. B. Cell death assay on leaves from WT and *nrc4*_7.4 plants. Transient expression of Rpi-blb2 and AVRblb2 induced cell death in WT, but not in *nrc4*_7.4 leaves. Constructs expressing GFP, Rpi-vnt1 and AVRvnt1 were used as controls.

A

*nrc4*_9.1.3

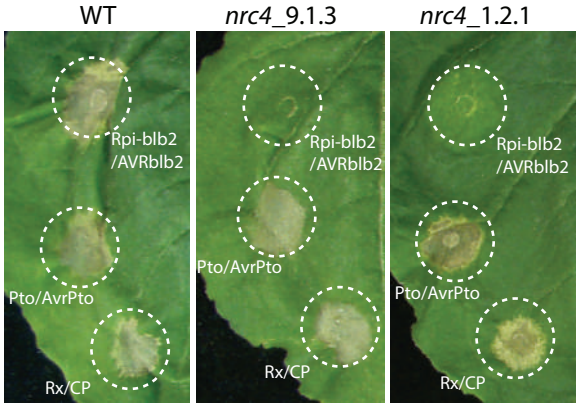
	sgRNA4.1	sgRNA4.2	
WT	ATACAAAAAACGGTACATACCG--CAG**AGG**ATGC—(80bp)—CTACAGTCAGGAATCTTGCAGCTG**AGG**TTCAA		
NRC4a	ATACAAAAAACGGTACATACCGACAG**AGG**ATGC—(80bp)—CTACAGTCAGGAATCTTGCAGCTG**AGG**TTCAA	premature stop 77aa , 44%	
NRC4a	ATACAAAAAA----------CG--CAG**AGG**ATGC—(80bp)—CTACAGTCAGGAATCTTGCAGCTG**AGG**TTCAA	premature stop 119aa , 54%	
NRC4b	ATACAAAAAACGGTACATACC----CAG**AGG**ATGC—(80bp)—CTACAGTCAGGAATCTTGCAGCTG**AGG**TTCAA	premature stop 123aa , 99%	

B

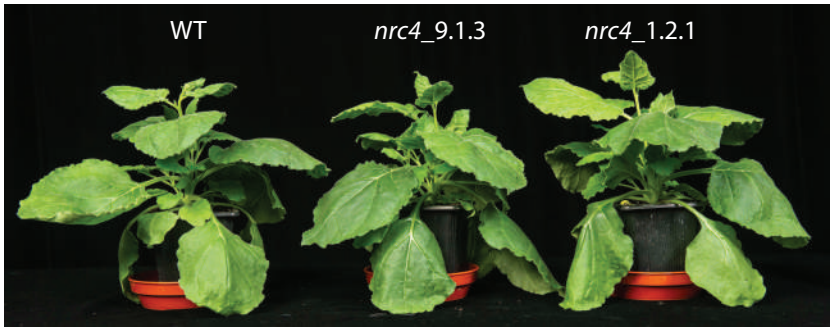
*nrc4*_1.2.1

	sgRNA4.1	sgRNA4.2	
WT	ATACAAAAAACGGTACATACCG--CAG**AGG**ATGC—(80bp)—CTACAGTCAGGAATCTTGCAG--CTG**AGG**TTCAA		
NRC4a	ATACAAAAAACGGTACATACCGACAG**AGG**ATGC—(80bp)—CTACAGTCAGGAATCTTGCAG--CTG**AGG**TTCAA	premature stop 77aa, 56%	
NRC4a	ATACAAAAAACGGTACATACCGTCAG**AGG**ATGC—(80bp)—CTACAGTCAGGAATCTT----------CTG**AGG**TTCAA	premature stop 77aa, 43%	
NRC4b	ATACAAAAAACG----------CG--CAG**AGG**ATGC—(80bp)—CTACAGTCAGGAATCTTGCAGACTG**AGG**TTCAA	premature stop 107aa, 63%	
NRC4b	ATACAAAAAACG----------CG--CAG**AGG**ATGC—(80bp)—CTACAGTCAGGAATCTTGCAG--CTG**AGG**TTCAA	full length -3aa, 20%	
NRC4b	ATACAAAAAACG----------CG--CAG**AGG**ATGC—(80bp)—CTACAGTCAGGAATCTT----------CTG**AGG**TTCAA	premature stop 118aa, 16%	

C



D



Supplemental Figure S5. Genotypes and phenotypes of *NRC4* knockout *N. benthamiana*. A and B. Amplicon sequencing results of the *NRC4* loci of the *NRC4a/b* knockout *N. benthamiana* lines *nrc4*_9.1.3 and *nrc4*_1.2.1. Sequences of the two sgRNAs are marked in red. C. Rpi-blb2-mediated HR cell death was compromised in the *NRC4* knockout lines. Rpi-blb2/AVRblb2, Pto/AvrPto and Rx/CP were transiently expressed in leaves of wild type and *NRC4* knockout *N. benthamiana* lines according to the method described previously in Wu et al. (2017). The pictures were taken at 7 days after agroinfiltration. D. *NRC4* knockout lines did not exhibit any growth defects when compared to the wild type plants. Six-week-old wild type and *NRC4* knockout *N. benthamiana* lines were used in the photograph.

A

*nrc234*_4.3.1

	sgRNA2.2	sgRNA2.3	sgRNA2.1	sgRNA2.4
WT	CTGAGAACGATGTCCACAAAGAATTGGTGAAGAAAATTAAGACTGTGGTCAACTCTGCTGAAGATGCCATTGATAAGTTTGTGATTGAGGCTAAGCTTCACAAGGACAAAGG--GGTTGGCA			
NRC2a	CTGAGAACGATGTCCACAAAGAATTGGTGAAGAAAATTAAGACTGTGGTCAACTCTGCTGAAGATGCCATTGATAAGTTTGTGATTGAGGCTAAGCTTCACAAGGACAAAGGTGGTTGGCA			premature stop 97aa,100%
NRC2b	CTGAGAACGATGTCCACAAAGAATTGGTGAAGAAAATTAAGACTGTGGTCAACTCTGCTGAAGATGCCATTGATAAGTTTGTGATTGAGGCTAAGCTTCACAAGGACAAAGGTGGTTGGCA			premature stop 97aa, 53%
NRC2b	CTGAGAACGATGTCCACAAAGAATTGGTGAAGAAAATTAAGACTGTGGTCAACTCTGCTGAAGATGCCATTGATAAGTTTGTGATTGAGGCTAAGCTTCAC----------AAAGG--GGTTGGCA			premature stop 95aa, 47%

	sgRNA3.3	sgRNA3.1	sgRNA3.4	sgRNA3.2
WT	ACGCTGACTTGATTATTGGTATAAAGGG-(46bp)-CTCAAACAAGCAGCTAAATCAAGGA-(17bp)-AAATCACTAGTAAAGAAGATAAGGA-(127bp)-CTGATGAGATTAAAACTATAAGGGAAAG			
NRC3	ACGCTGACTTGATTATTGGTATAAAGGG-(46bp)-CTCAAACAAGCAGCTA----------GA-(17bp)-AAATCACTAGTAAAGAAGATAAGGA-(127bp)-CTGATGAGATTAAAACTATAAGGGAAAG			premature stop 60aa, 100%

	sgRNA4.1	sgRNA4.2	
WT	ATACAAAAAACGGTACATACCG--CAGAGGATGC—(80bp)—CTACAGTCAGGAATCTTGCAGCTGAGGTTCAA		
NRC4a	ATACAAAAAACGGTACATACC-----CAGAGGATGC—(80bp)—CTACAGTCAGGAATCTTGCAGCTGAGGTTCAA		premature stop 122 aa, 12%
NRC4a	ATACAAAAAACGGTACATACC-----CAGAGGATGC—(80bp)—CTACAGTCAGGAATCTTGCA--CTGAGGTTCAA		premature stop 108aa, 12%
NRC4a	ATACAAAAAACGGTACATACCGTCAGAGGATGC—(80bp)—CTACAGTCAGGAATCTTGCA--CTGAGGTTCAA		premature stop 76aa, 76%
NRC4b	ATACAAAAAACGGTACATACC-----CAGAGGATGC—(80bp)—CTACAGTCAGGAATCTTGCAGCTGAGGTTCAA		premature stop 117aa, 39%
NRC4b	ATACAAAAAACGGTACATACC-----CAGAGGATGC—(80bp)—CTACAGTCAGGAATCTTGCA--CTGAGGTTCAA		premature stop 108aa, 39%
NRC4b	ATACAAAAAACGGTACATACCGTCAGAGGATGC—(80bp)—CTACAGTCAGGAATCTTGCA--CTGAGGTTCAA		premature stop 76aa, 22%

B

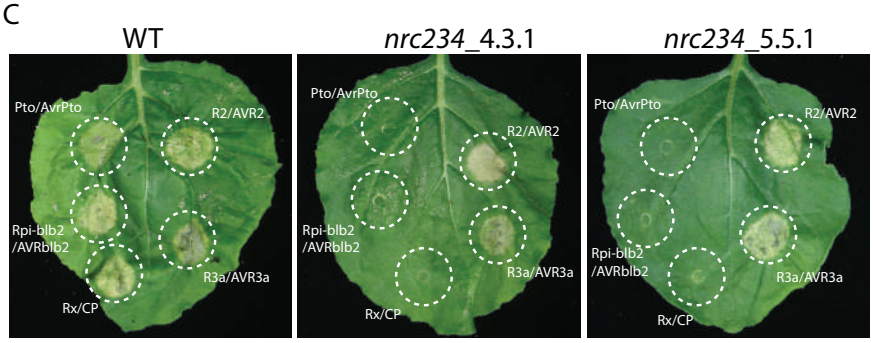
*nrc234*_5.5.1

	sgRNA2.2	sgRNA2.3	sgRNA2.1	sgRNA2.4
WT	CTGAGAACGATGTCCACAAAG--AATTGGTGAAGAAAATTAAG--ACTGTGGTCAACTCTGCTGAAGATGCCATTGATAAGTTTGTGATTGAGGCTAAGCTTCACAAGGACAAAGGGGTTGGCA			
NRC2a	CTGAGAACGATGTCCACAAAGTAATTGGTGAAGAAAATTAAGAACTGTGGTCAACTCTGCTGAAGATGCCATTGATAAGTTTGTGATTGAGGCTAAGCTTCACAA-----(Δ9bp)----GGTTGGCA			premature stop 65aa,100%
NRC2b	CTGAGAACGATGTCCACAAAGTAATTGGTGAAGAAAATTAAGAACTGTGGTCAACTCTGCTGAAGATGCCATTGATAAGTTTGTGATTGAGGCTAAGCTTC-----(Δ9bp)----AAGGGGTTGGCA			premature stop 65aa, 20%
NRC2b	CTGAGAACGATGTCCACAAAGTAATTGGTGAAGAAAATTAAG--ACTGTGGTCAACTCTGCTGAAGATGCCATTGATAAGTTTGTGATTGAGGCTAAGCTTC-----(Δ9bp)----AAGGGGTTGGCA			premature stop 65aa, 12%
NRC2b	CTGAGAACGATGTCCACAAAG--AATTGGTGAAGAAAATTAAG--ACTGTGGTCAACTCTGCTGAAGATGCCATTGATAAGTTTGTGATTGAGGCTAAGCTTC-----(Δ9bp)---AAGGGGTTGGCA			fullength -3aa, 68%

	sgRNA3.3	sgRNA3.1	sgRNA3.4	sgRNA3.2
WT	ACGCTGACTTGATTATTGGT--ATAAAGGG-(46bp)-CTCAAACAAGCAGCTAAATCAAGGA-(17bp)-AAATCACTAGTAAAGAAGATAAGGA-(127bp)-CTGATGAGATTAAAACTATAAGGGAAAG			
NRC3	ACGCTGACTTGATTATTGGTGATAAAGGG-(46bp)-CTCAAACAAGCAGC----------(Δ43bp)----------AGATAAGGA-(127bp)-CTGATGAGATTAAAACT----------AAAG			premature stop 33aa, 100%

	sgRNA4.1	sgRNA4.2	
WT	ATACAAAAAACGGTACATACCG--CAGAGGATGC—(80bp)—CTACAGTCAGGAATCTTGCAGCTGAGGTTCAA		
NRC4a	ATACAAAAAACG----------GCAGAGGATGC—(80bp)—CTACAGTCAGGAATCTTGCAGCTGAGGTTCAA		full length -3aa, 15%
NRC4a	ATACAAAAAACGGTACATACCGCCAGAGGATGC—(80bp)—CTACAGTCAGGAATCTTGCAGCTGAGGTTCAA		premature stop 76aa, 85%
NRC4b	ATACAAAAAACGGTACATACCGCCAGAGGATGC—(80bp)—CTACAGTCAGGAATCTTGCAGCTGAGGTTCAA		premature stop 76aa, 14%
NRC4b	ATACAAAAAACG----------GCAGAGGATGC—(80bp)—CTACAGTCAGGAATCTTGCAGCTGAGGTTCAA		full length -3aa, 86%

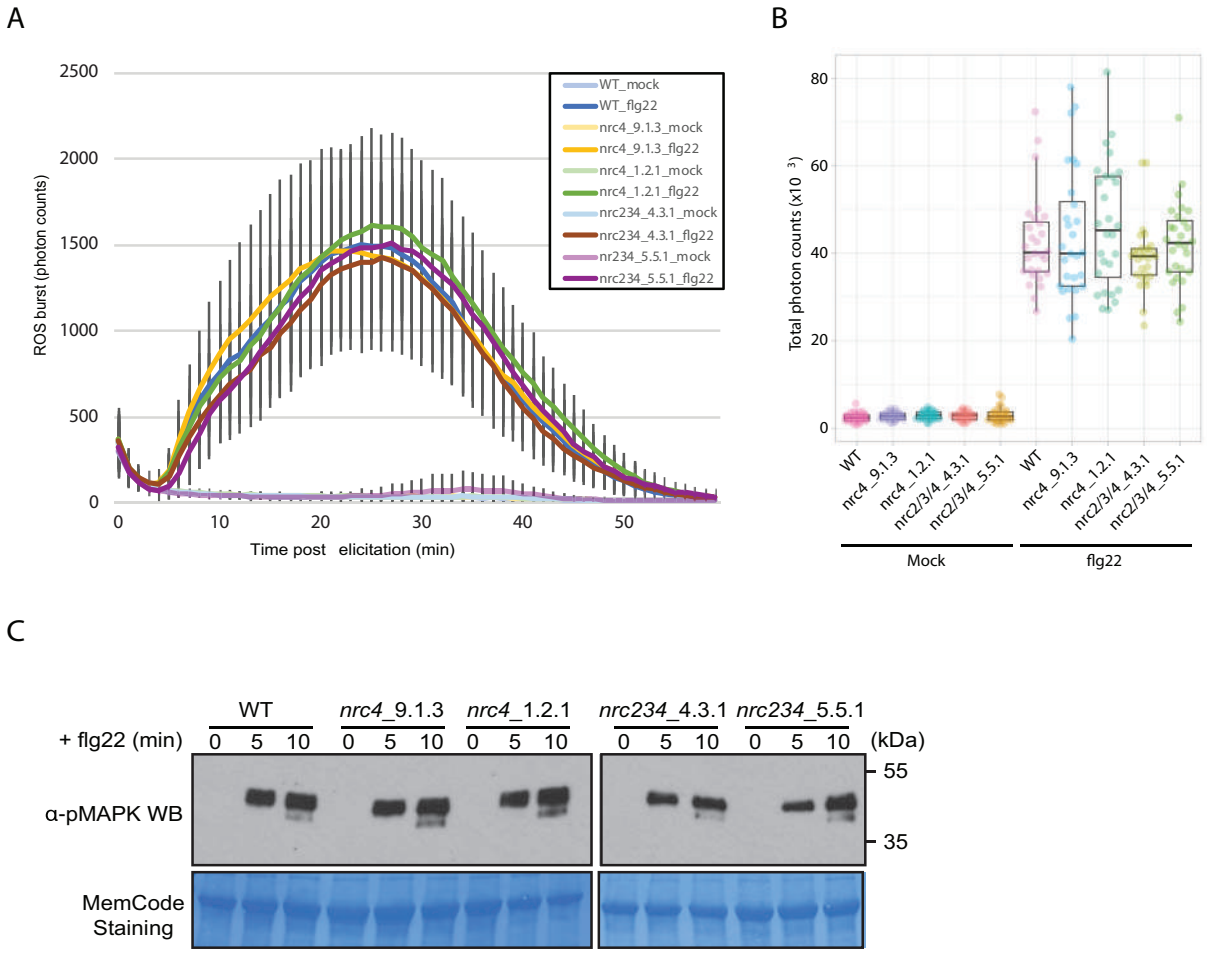
C



D

Supplemental Figure S6. Genotypes and phenotypes of *NRC2/3/4* knockout *N. benthamiana*. A and B. Amplicon sequencing results of the *NRC* loci of the *NRC2/3/4* knockout *N. benthamiana* lines *nrc234*_4.3.1 and *nrc234*_5.5.1. Sequences of the sgRNAs are marked in red. C. Rpi-blb2-, Pto- and Rx-mediated HR cell death were compromised in the *NRC2/3/4* knockout plants. Combinations of R/AVR were transiently expressed in leaves of wild type and *NRC2/3/4* knockout *N. benthamiana* lines according to the method described previously in Wu et al. (2017). R2/AVR2 and R3a/AVR3a were used as *NRC2/3/4*-indpendent controls. The pictures were taken at 5 days after agroinfiltration. D.

NRC2/3/4 knockout lines did not exhibit any growth defects when compared to the wild type plants. Six-week-old wild type and *NRC2/3/4* knockout *N. benthamiana* lines were used in the photograph.



Supplemental Figure S7. Knocking out of *NRC2/3/4* in *N. benthamiana* did not affect flg22-induced defence responses. A. Flg22-triggered ROS bursts were measured for 60 min using leaf discs of the WT and *NRCs* knockout lines. Data are presented as means ± SD B. Scatter plot and box plot of total photon counts of each treatment in A. C. Flg22-triggered MAPK activation was analysed by immunoblots with α-pMAPK. Proteins were extracted from leaf discs of the WT and *NRCs* knockout *N. benthamiana* lines at 0, 5 or 10 min after treatment with flg22 peptides.