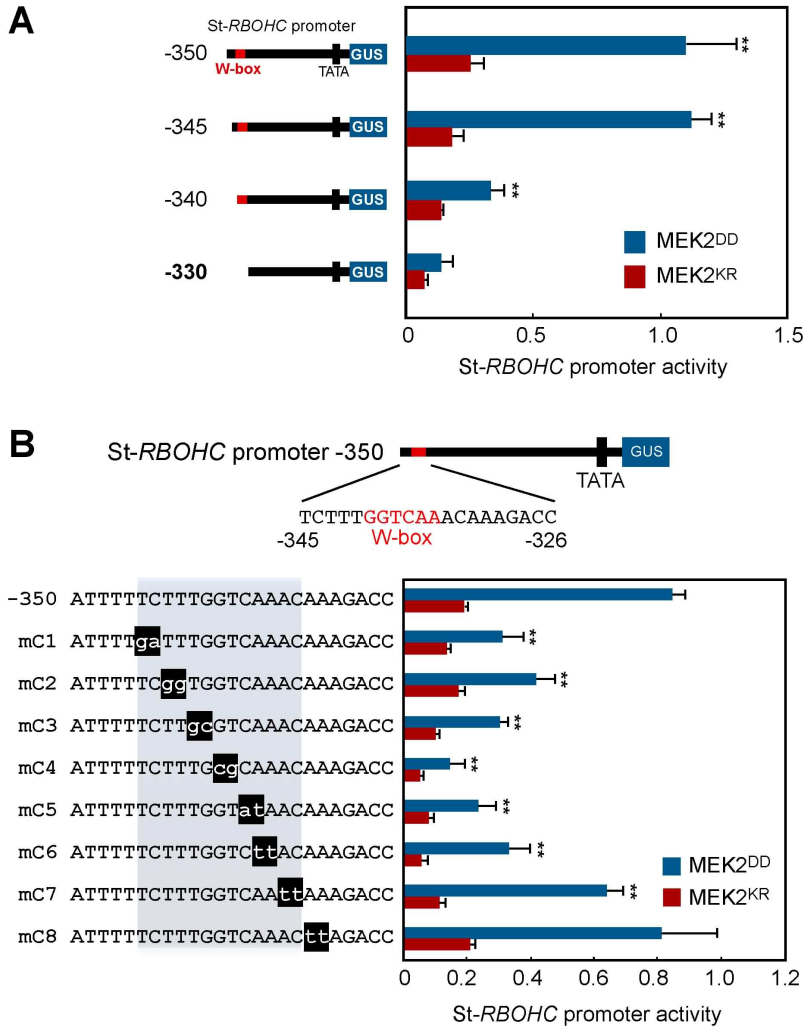
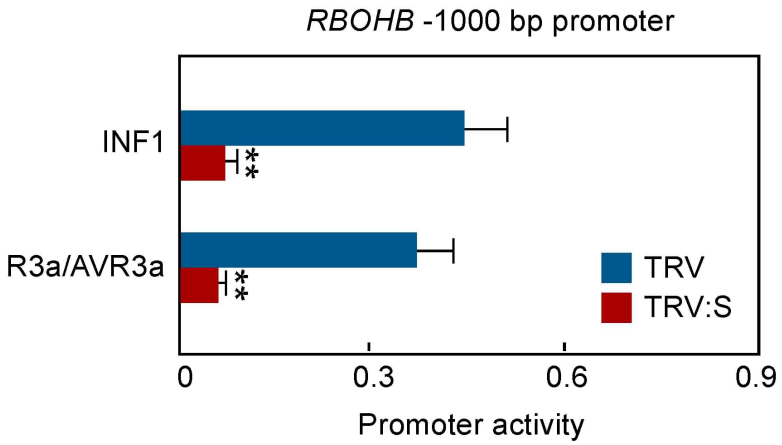


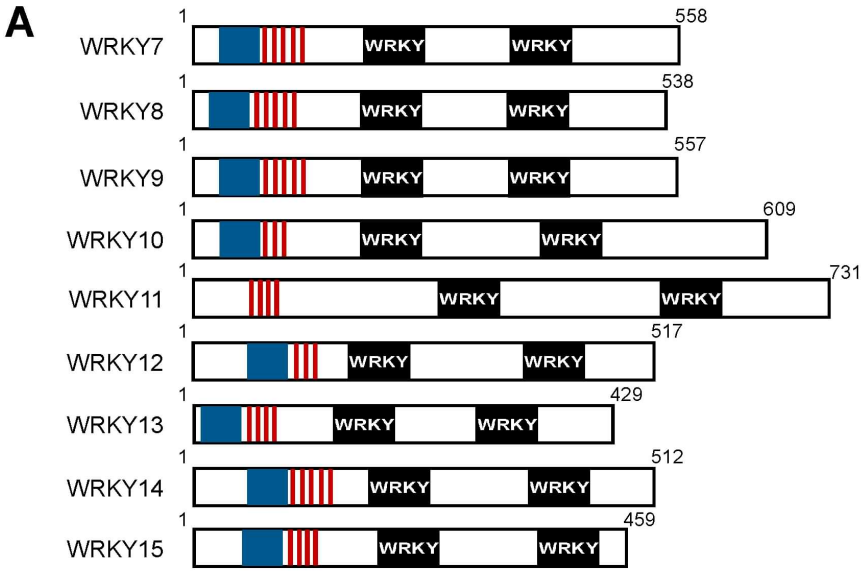
Supplemental Figure 1. Promoter Activity of St-*RBOHC* Induced by MEK2^{DD} via the *cis*-Element.

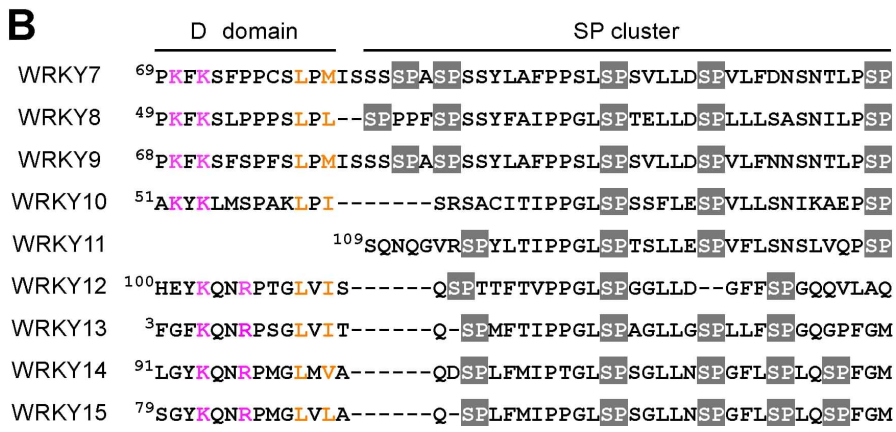
(A) Deletion analysis of the St-*RBOHC* promoter in response to MEK2^{DD} in *N. benthamiana* leaves. A series of 5'-deleted St-*RBOHC* promoter fragments was translationally fused to the *GUSint* reporter gene. The number indicates the distance from the St-RBOHC translation start site. A mixture of *Agrobacterium* cultures containing St-*RBOHC* promoter-*GUSint* (reporter) and *pER8:MEK2*^{DD} (effector), CaMV *35S* promoter-*LUCint* (reference) was coinfiltrated into leaves and 20 μM estradiol was injected 24 h later. Promoter activities were analyzed as described in Figure 2A.
(B) Analysis of 350 bp St-*RBOHC* promoter and mutated promoters containing two-base substitutions. Mutant bases are shown on a black background.
Asterisks indicate statistically significant differences compared with MEK2^{KR} **(A)** and -350 **(B)** (*t* test, **P < 0.01). Data are means ± SDs from at least three experiments.



Supplemental Figure 2. Analysis of INF1- or R3a/AVR3a-Induced *RBOHB* -1000 Promoter Activities in *SIPK*-Silenced Leaves.

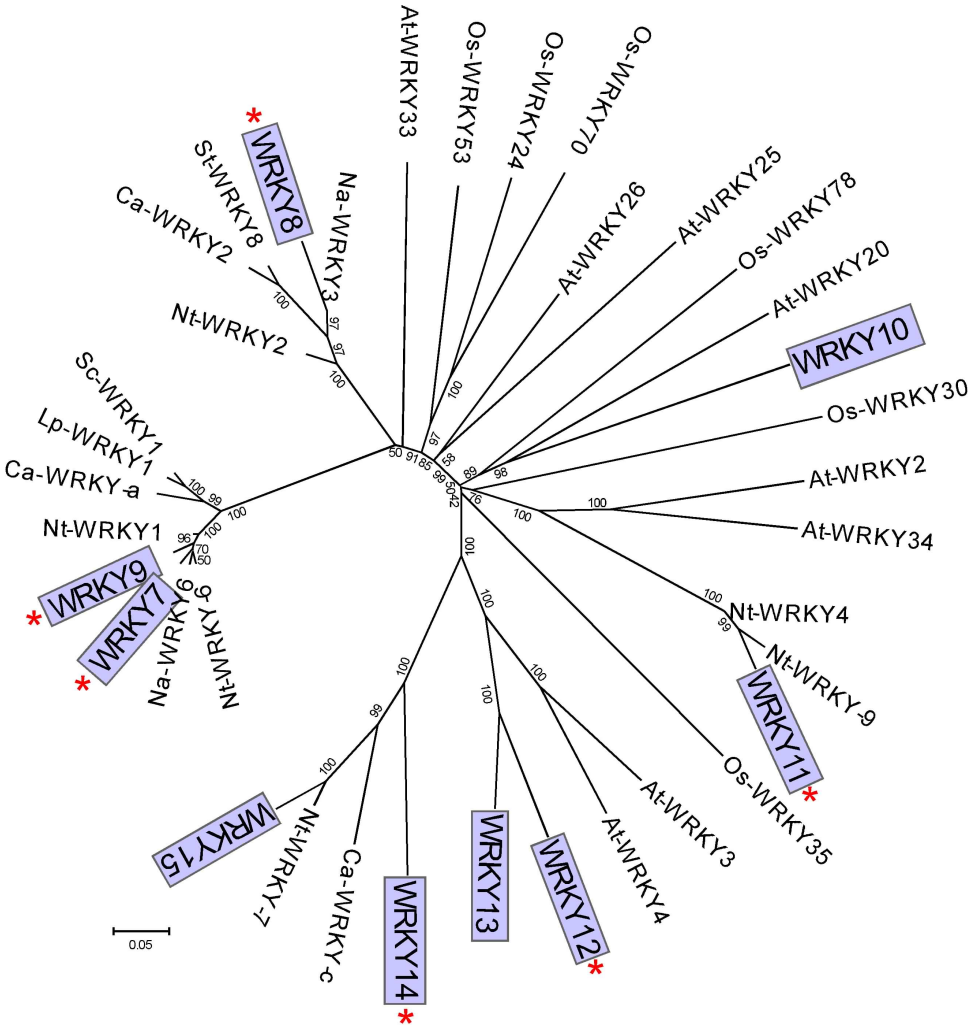
Leaves were inoculated with *Agrobacterium* carrying INF1 and R3a/AVR3a. Promoter activities were analyzed as described in Figure 2A.
Asterisks indicate statistically significant differences compared with TRV (*t* test, **P < 0.01). Data are means ± SDs from at least three experiments.





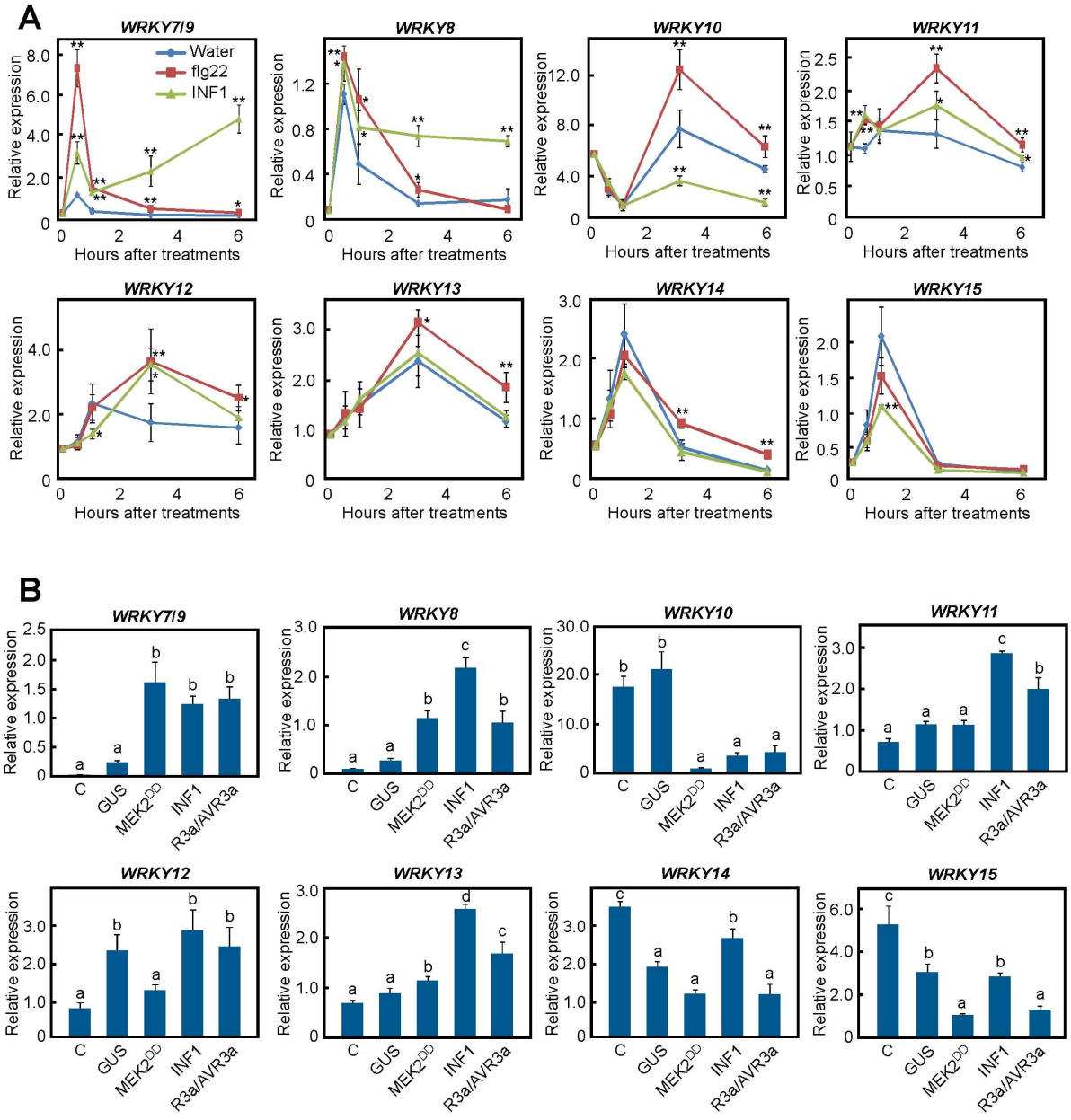
Supplemental Figure 3. Identification of WRKY Transcriptional Factors.

(A) Schematic representations of putative amino acid sequences of WRKY7, 8, 9, 10, 11, 12, 13, 14 and 15. WRKY domains are boxed in black, D domains and SP sequences are shown in blue and red, respectively.
(B) Amino acid sequences of the SP cluster and D domain adjacent to the N-terminal side of the SP cluster of each WRKY protein.



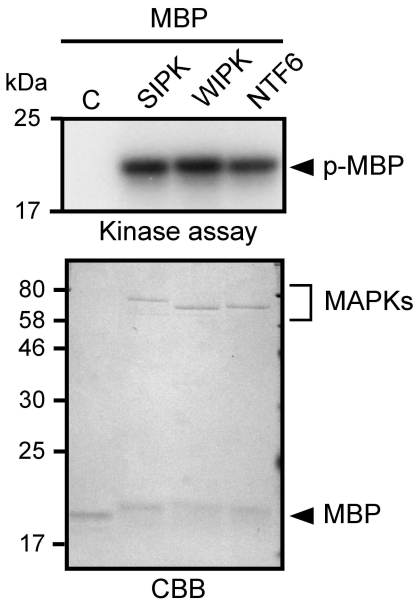
Supplemental Figure 4. Phylogenetic Tree of Group I WRKYs Containing the SP Cluster.

The phylogenetic tree comprised 37 members of the group I WRKY family considered to have the SP cluster in higher plants. Partial amino acid sequences containing the SP cluster and WRKY domain of 37 WRKYs were used to facilitate alignment. The unrooted tree of the group I WRKY family was constructed by using the neighbor-joining method. Species acronyms are included before the protein name: At, *Arabidopsis thaliana*; Ca, *Capsicum annuum*; Lp, *Lycopersicon peruvianum*; Na, *Nicotiana attenuata*; Nt, *Nicotiana tabacum*; Os, *Oryza sativa*; Sc, *Solanum chacoense*; St, *Solanum tuberosum*. *Nicotiana benthamiana* WRKYs used in this study are boxed, and asterisks indicate cell death-inducing WRKYs as shown in Figure 4 and Table 1. The percentage of replicate trees, in which the associated taxa clustered together in the bootstrap test (1000 replicates), are shown next to the branches. Phylogenetic analyses were done in MEGA5. Supplemental Table 7 lists the accession numbers. The scale bar represents 0.05 amino acid substitutions per site in the primary structure.



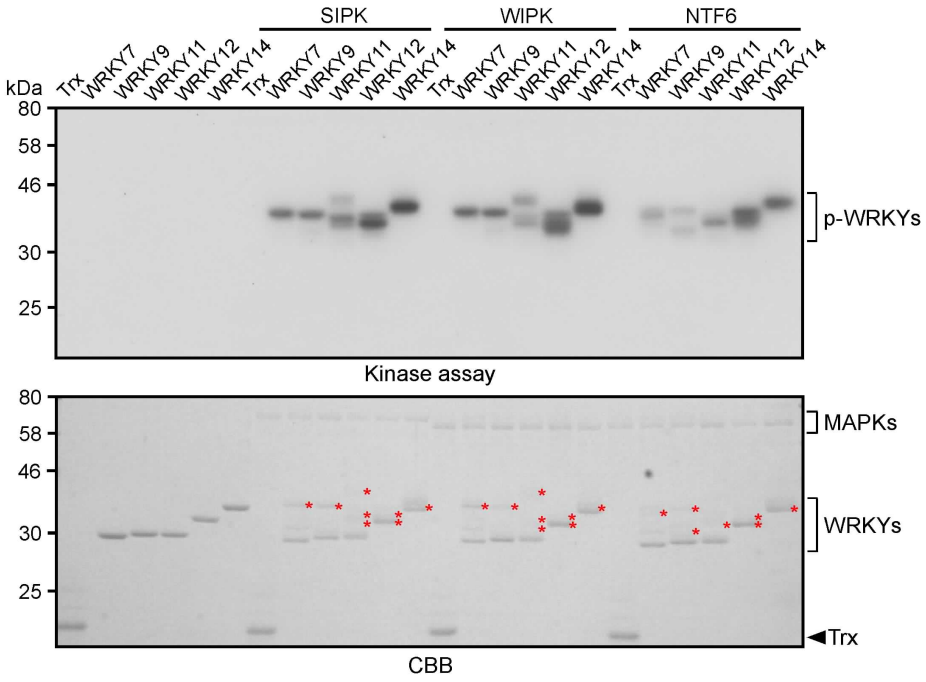
Supplemental Figure 5. Induction of *WRKY* Genes in Response to Flg22, INF1 and R3a/AVR3a.

(A) Expression levels of *WRKY* genes after flg22 and INF1 treatments. Total RNAs were extracted from *N. benthamiana* leaves at the indicated hours after treatments and were used for RT-qPCR. *EF-1α* was used as a normalization control.
(B) Expression levels of *WRKY* genes after MEK2^{DD}, INF1 and R3a/AVR3a expressions. Total RNAs were extracted from *N. benthamiana* leaves 36 h after agroinfiltrations and were used for RT-qPCR. *EF-1α* was used as a normalization control.
Asterisks indicate statistically significant differences compared with Water (*t* test, *P < 0.05, **P < 0.01). Letters represent each significance group, determined through Tukey's multiple range test. Data are means ± SD from at least three independent experiments.



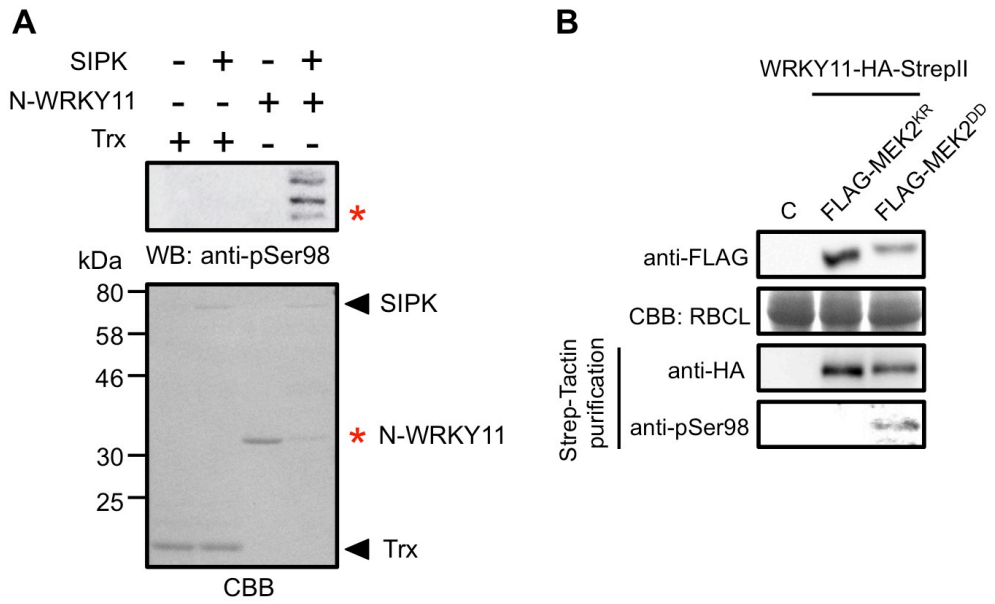
Supplemental Figure 6. In vitro Phosphorylation of MBP by Recombinant MAPKs.

Activated MAPKs (SIPK, WIPK and NTF6) by their corresponding constitutively active MAPKKs ($MEK2^{DD}$ and $MEK1^{DD}$), MBP and ^{32}P-labeled ATP were mixed. The reaction mixtures were separated using SDS-PAGE and were exposed to X-ray film. Phosphorylated MBPs (p-MBP), which reflect the activities of MAPKs, were visualized by autoradiography.



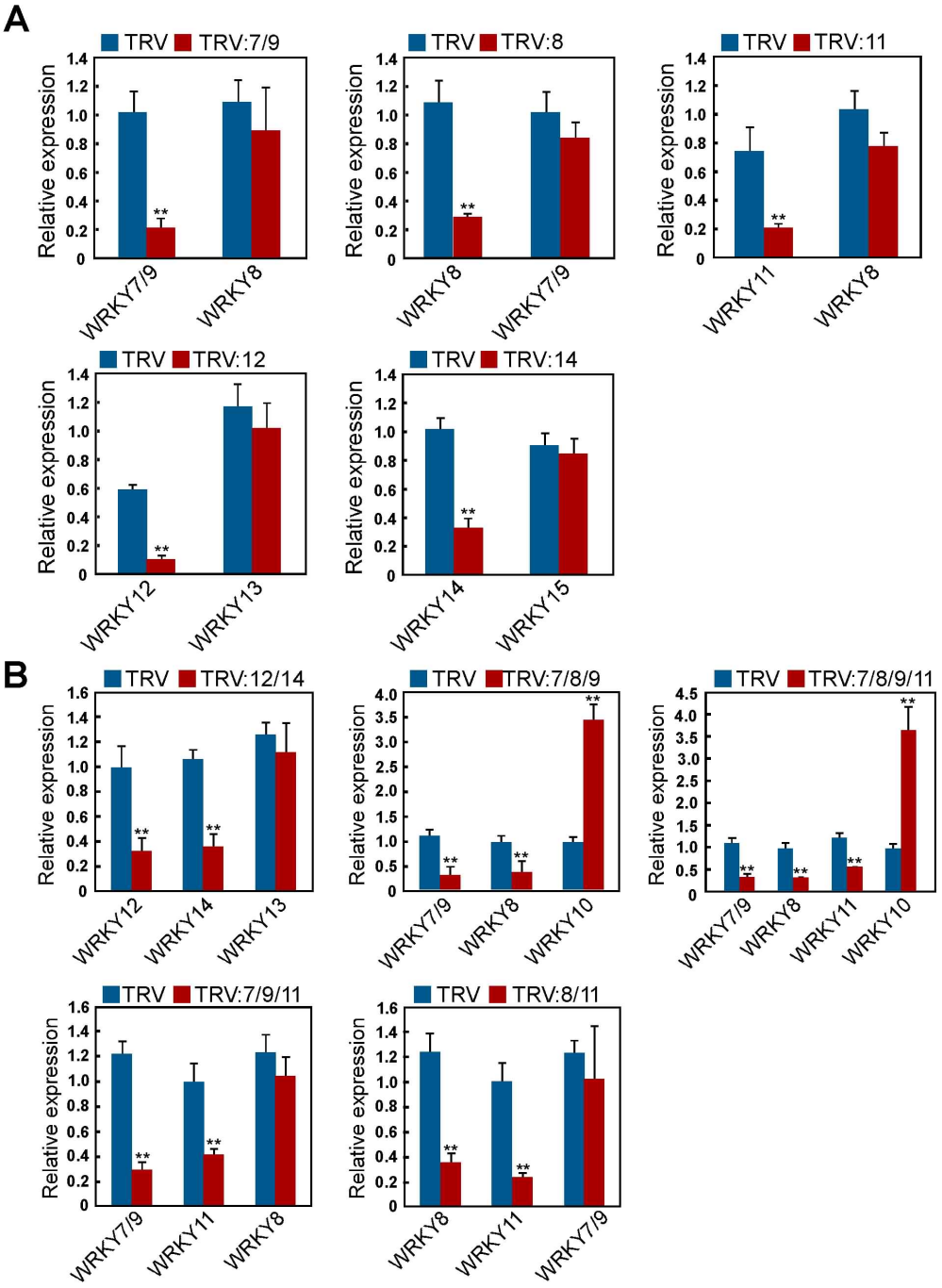
Supplemental Figure 7. Phosphorylation of WRKYs Inducing Cell Death by Pathogen-Responsive MAPKs in vitro.

Purified Trx and Trx-fused N-terminal regions of the WRKYs were used as substrates of active MAPKs. Proteins were separated by SDS-PAGE, were stained with CBB (bottom panel), and were exposed to X-ray film (kinase assay; top panel). Red asterisks indicate shifted bands by phosphorylation in SP cluster.



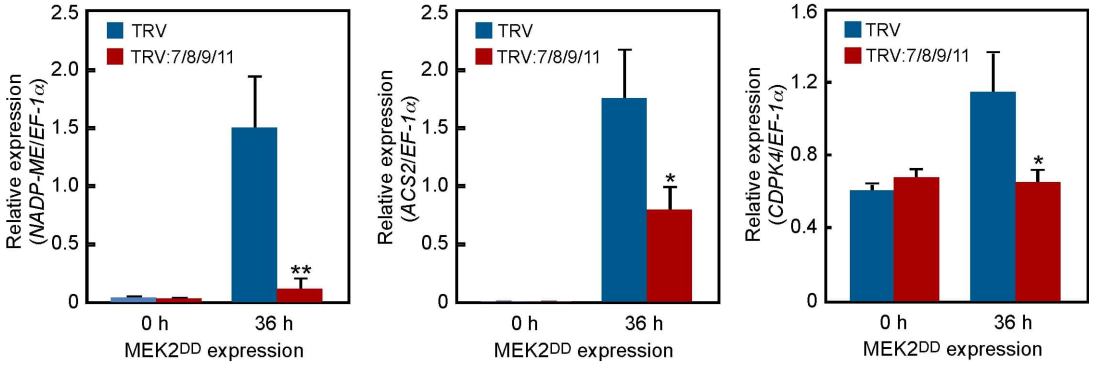
Supplemental Figure 8. MAPK-Dependent Phosphorylation of WRKY11 in vitro and in vivo.

(A) Cross-reaction of anti-WRKY8 pSer98 antibody to phosphorylated WRKY11 in vitro. Purified Trx and Trx-fused N-terminal region of WRKY11 were used as substrates of active SIPK. Proteins were separated by SDS-PAGE, were stained with CBB (bottom panel), and immunoblot analysis was done using anti-pSer98 antibody.
(B) Phosphorylation of WRKY11 by MEK2^{DD} expression. WRKY11-HA-StrepII was expressed with FLAG-MEK2^{KR} or FLAG-MEK2^{DD} in *N. benthamiana* leaves by agroinfiltration. Total proteins were prepared 36 h after agroinfiltration. Anti-FLAG antibody was used to detect accumulation of FLAG-MEK2^{KR} and FLAG-MEK2^{DD}. Protein loads were monitored by CBB staining of the bands corresponding to the RBCL. After Strep-Tactin purification, immunoblot analyses were done using anti-HA or anti-WRKY8 pSer98 antibody.



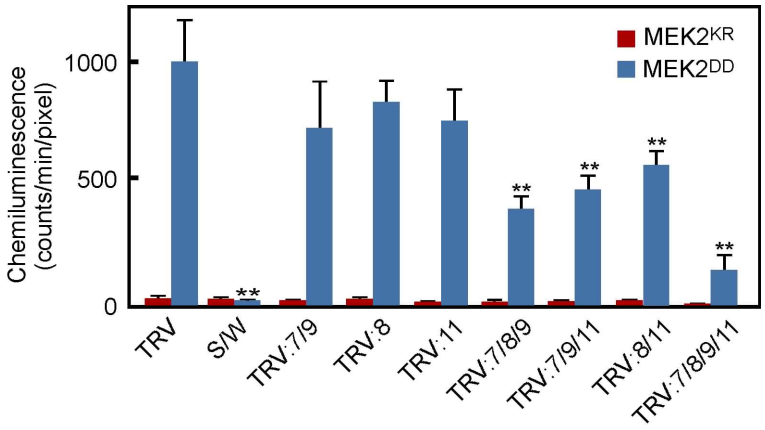
Supplemental Figure 9. Specific Gene Silencing of Single *WRKY* Gene or Multiple *WRKY* Genes in TRV:*WRKY*-Infected Plants.

(A) Specificity of single *WRKY* gene silencing. Single *WRKY* gene-silenced leaves were infiltrated with *Agrobacterium* carrying the $MEK2^{DD}$ gene 3 to 4 weeks after the TRV infection. Total RNAs were extracted from the leaves 36 h after agroinfiltration and were used for RT-qPCR. *EF-1*α was used as a normalization control.
(B) Specificity of multiple *WRKY* gene silencing. Multiple *WRKY* genes-silenced leaves were used for RT-qPCR as described in **(A)**.
Data are means ± SDs from at least three experiments. Asterisks indicate statistically significant differences compared with TRV (*t* test, **P < 0.01).



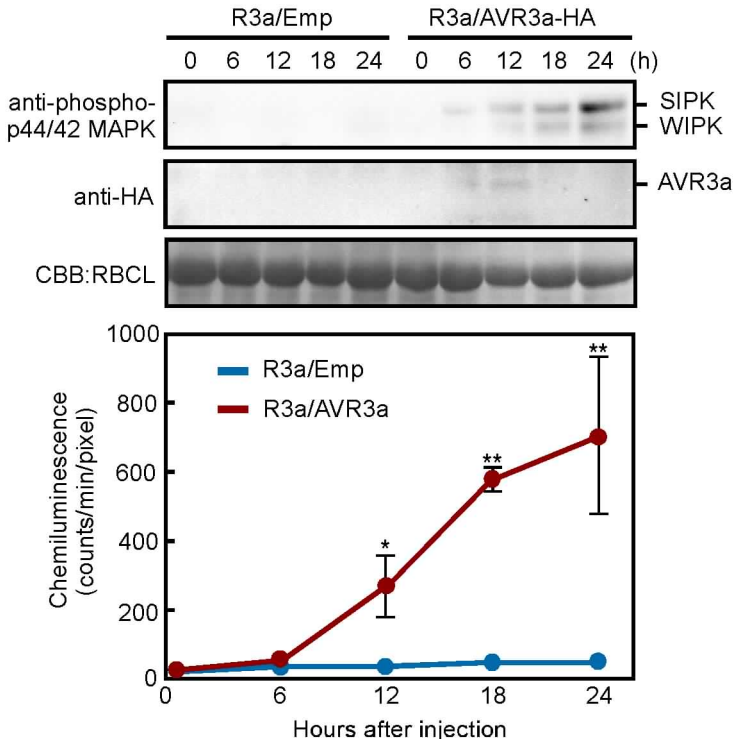
Supplemental Figure 10. Regulation of Defense-Related Gene Expression by Multiple WRKYs.

Total RNAs were extracted from multiple *WRKY* gene-silenced leaves 0 h or 36 h after agroinfiltration and were used for RT-qPCR. *EF-1α* was used as a normalization control. Data are means ± SD from three independent experiments. Asterisks indicate statistically significant differences compared with TRV (*t* test, *P < 0.1 and **P < 0.05).



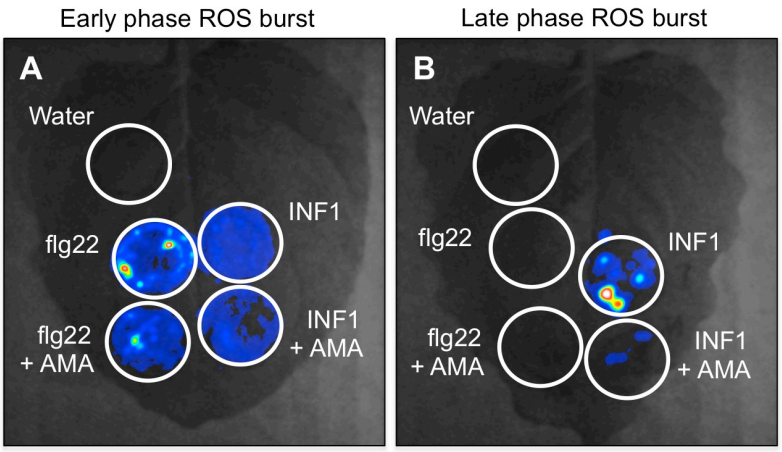
Supplemental Figure 11. Effects of Single or Multiple *WRKY* Gene Silencing on MEK2^{DD}-Dependent ROS Burst.

TRV control-, *SIPK*/*WIPK*- and single or multiple *WRKY* gene-silenced leaves were inoculated with *Agrobacterium* carrying *MEK2*^{DD} or *MEK2*^{KR} and were analyzed as described in Figure 6B. Data are means ± SE from six experiments. Asterisks indicate statistically significant differences compared with TRV (*t* test, **P < 0.05).



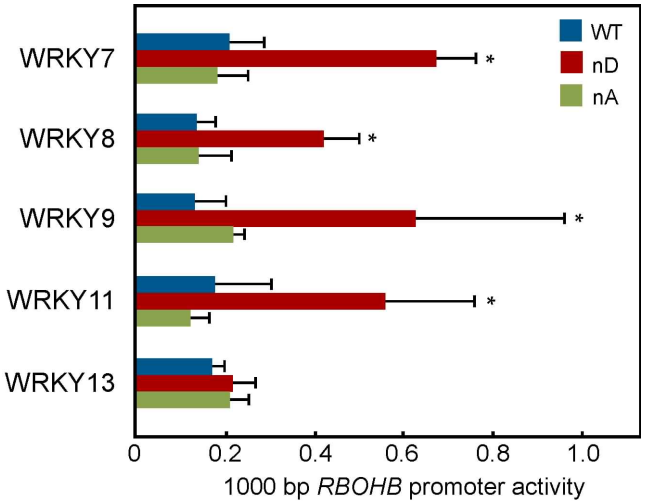
Supplemental Figure 12. Activation of SIPK and WIPK Induced by R3a/AVR3a.

Leaves were coinoculated with *Agrobacterium* carrying *pER8*:*AVR3a-HA* and *pBinPlus*:*R3a* and then were injected with 20 μM estradiol 24 h later. Total proteins were prepared at the indicated hours after estradiol injection, and phosphorylation of SIPK and WIPK was detected using anti-phospho-p44/42 MAPK antibody. To detect ROS generation, inoculation sites were infiltrated with 0.5 mM L-012 solution at the indicated hours after estradiol injection and were monitored by using a CCD camera. Chemiluminescence intensities were quantified by a program equipped with a photon image processor. Asterisks indicate statistically significant differences compared with R3a/Emp (*t* test, *P < 0.05, **P < 0.01). Data are means ± SE from three experiments.



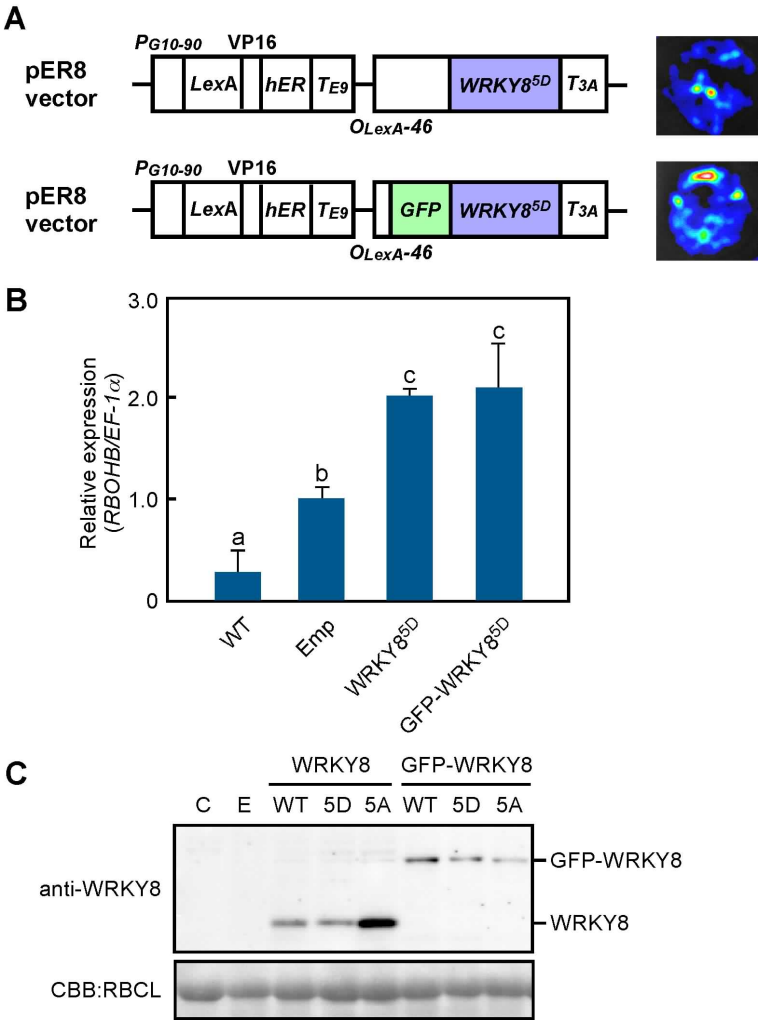
Supplemental Figure 13. Early and Late Phase ROS Bursts in Response to Flg22 and INF1.

(A) Early phase ROS bursts were monitored for 60 min after flg22 and INF1 treatments. 10 µg/mL α-amanitin (AMA) was coinfiltrated in the leaves with flg22 or INF1.
(B) Late phase ROS bursts were monitored for 5 min 12 h after the flg22 and INF1 treatments. 10 µg/mL AMA was additionally infiltrated into the leaves 6 h after initial treatment.



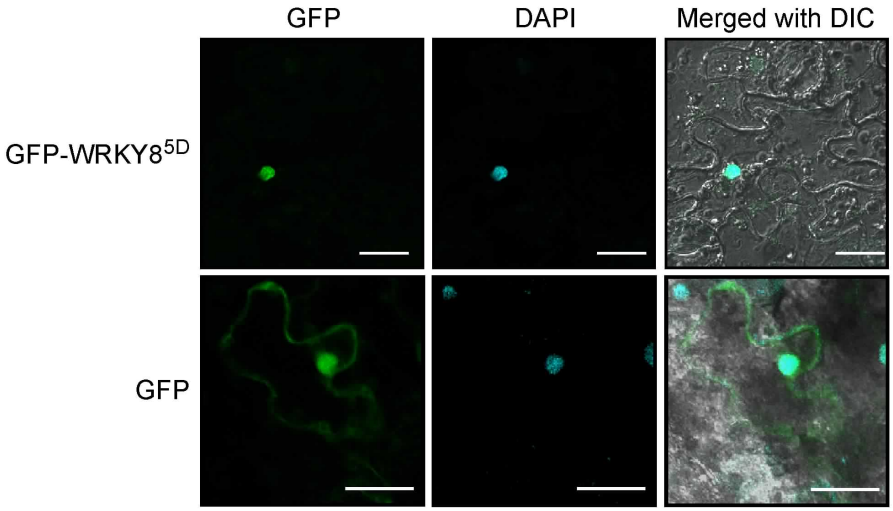
Supplemental Figure 14. Analysis of *RBOHB* -1000 Promoter in Response to WRKY Variants.

N. benthamiana leaves were inoculated with *Agrobacterium* carrying promoter assay constructs. *WRKY* variants were expressed as the effector. Promoter activities were analyzed as described in Figure 2A. Putative phosphorylated Ser residues in SP cluster were substituted with Asp (nD) or Ala (nA), respectively. Asterisks indicate statistically significant differences compared with WT (*t* test, *P < 0.05).



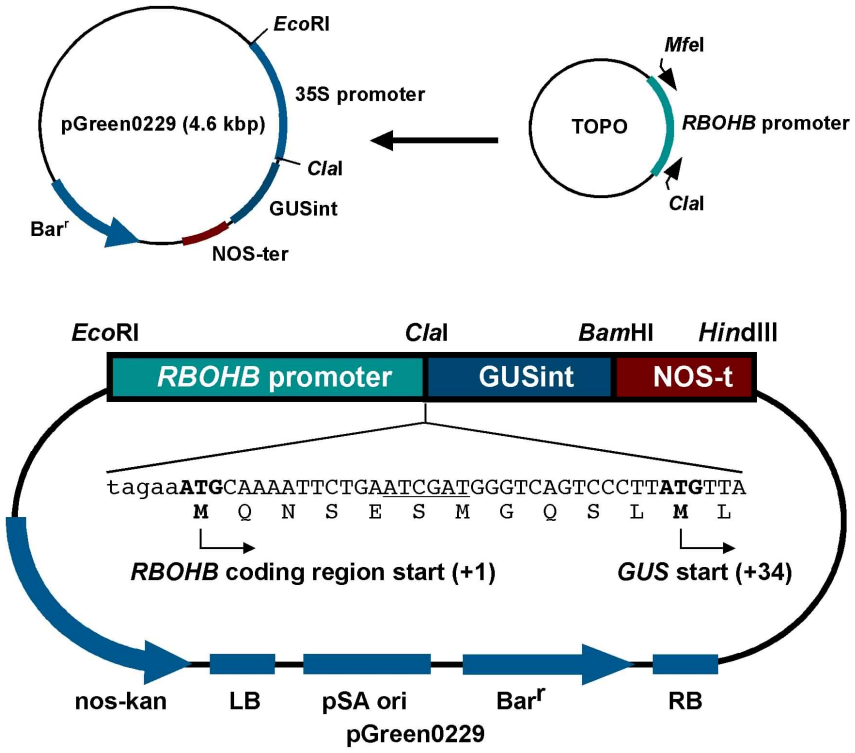
Supplemental Figure 15. Induction of ROS by GFP-Tagged WRKY8^{5D} or Non-Tagged WRKY8^{5D}.

(A) ROS generation by overexpression of GFP-WRKY8^{5D} or WRKY8^{5D}. *N. benthamiana* leaves were inoculated with *Agrobacterium* carrying *GFP-WRKY8^{5D}* or *WRKY8^{5D}* and were analyzed as described in Figure 6B.
(B) Expression of *RBOHB* in response to GFP-WRKY8^{5D} or WRKY8^{5D}. Leaves were inoculated with *Agrobacterium* carrying *pER8*:*GFP-WRKY8^{5D}* or *WRKY8^{5D}* and 20 μM estradiol was injected 24 h later. Total RNAs were extracted from the leaves 24 h after estradiol injection and were used for RT-qPCR. *EF-1α* was used as a normalization control. Data are means ± SD from three independent experiments. Letters represent each significance group, determined through Tukey's multiple range test.
(C) Detection of GFP-WRKY8^{5D} or WRKY8^{5D} proteins. Anti-WRKY8 antibody was used to detect accumulation of GFP-tagged or non-tagged WRKY8 variants. Protein loads were monitored by CBB staining of the bands corresponding to the RBCL.



Supplemental Figure 16. Subcellular Localization of GFP-WRKY8^{5D}.

N. benthamiana leaves were inoculated with *Agrobacterium* containing *pER8*:*GFP* or *GFP-WRKY8^{5D}* and 20 μM estradiol was injected 24 h later. The leaves were observed 24 h after estradiol injection. DAPI (4',6-diamidino-2-phenylindole) was used for nucleus staining. Images are single-plane confocal images. Scale bars, 30 μm.



Supplemental Figure 17. Schematic Representation of the Construct of *RBOHB* Promoter:*GUSint* Reporter Gene.

Supplemental Table 1. Phosphorylation Levels of WRKYs by Pathogen-Responsive MAPKs

Protein	SIPK	WIPK	NTF6
WRKY7	1.00	1.03	0.74
WRKY9	1.00	1.01	0.74
WRKY11	1.00	0.66	0.53
WRKY12	1.00	1.27	0.97
WRKY14	1.00	1.03	0.47

The pixel intensities of each band in Supplemental Figure 7 were quantified by zone densitometry assay. The signal intensity was calculated by subtraction of the background intensity and normalized by using each value phosphorylated by SIPK as a normalization control.

Supplemental Table 2. Primers Used for RT-qPCR

Name	Forward Primer (5'>3')	Reverse Primer (5'->3')
WRKY7/9	CACAAGGGTACAAACAACACAG	GGTTGCATTTGGTTCATGTAAG
WRKY8	AACAATGGTGCCAATAATGC	TGCATATCCTGAGAAACCATT
WRKY10	TCTGAGCAATTGTGAACGTC	ATTTGTCACGGGTCTAATTCC
WRKY11	ACCTACCCCAGGATTTAGCTA	CCCAGATATGAATGGACAGG
WRKY12	GCAACTGACCCTAAAGCAGTC	TTGCTCATTGTTTGGAAAGTC
WRKY13	AGTGAAGGTCAAAGTAATCAAAATC	ACAAATTTTCCACTGAAGCAC
WRKY14	CAGAGCTAACAATCCCACCT	GGTATTCAGATTCCGAGAAGAA
WRKY15	GTAAAAGATGGTGACTTCGAGG	TTGGTAATGGTATTTATGCTGC
RBOHB	TTTTCTCTGAGGTTTGCCAGCCACCA	GCCTTCATGTTGTTGACAATGTCTTT
NADP-ME	GTCTTCCTCTGTCACCTGACC	AAACATTTACGAAGGAAGTCGTG
ACS2	TGATTGTCAAGAGCCAGG	TGGTGAGTGAGGGATAGGAGATG
CDPK4	AGGACTACGCTCAGCCCGAGG	TAAGCCTGATTACTACCCGTACG
EF-1α	TGTGGAAGTTTGAGACCACCC	GCAAGCAATGCGTGCTCAC

Supplemental Table 3. Primers Used for PCR Targeting Silencing Inserts

Name	Forward Primer (5'>3')	Reverse Primer (5'->3')
WRKY7/9	GGACTAGTCCGATAAGCAAAGAAGAACC	CGGGATCCTGCCAACCAATCTATAGCTAAG
WRKY11	CCATCGATATGATGGGGGATTTATGTTTC	GGGGTACCTAGGGCTTATGCCCATTAAATA
WRKY12	GGACTAGTTGCAAGCGTGGACTTTCTA	CCATCGATATTCTCAGCCATTGATTTTG
WRKY14	GGACTAGTGGGTACATTCTCAGGCATATC	GGACTAGTGCTGAAGCTTTGAATTTTTC

Red characters indicate restriction sites.

Supplemental Table 4. Primers Used for PCR Targeting *WRKY* cDNA

Name	Forward Primer (5'>3')	Reverse Primer (5'->3')
WRKY9	GAATTCTTTCTTGTCCTGTTTTTATTTTC	GCTGCCAACCAATCTATAGCTA
WRKY10	AGATTCTCAGTCTCAGTCTCAGC	ATTTGTCACGGGTCTAATTCC
WRKY11	CTAAGCTATTTTTTCCACAGAAAGA	TAGGGCTTATGCCCATTAAATA
WRKY12	TGCAAGCGTGGACTTTCTA	ATTCGATTACTCCATGCTGAAA
WRKY13	TATGTAGAGCAGTGAAAGAAAGAAAC	CATAAAAAGCCAAAACATGTTTA
WRKY14	CTATAAACATCCAAAGAATCAACAAC	AGCAAGTAGCCATAAGATTTACAAA
WRKY15	TTCAGCGATGTCGTTTCC	AGAGATGCAAACGAAATATAACTTCTAA

Supplemental Table 5. Sequences of Linkers for Y1H Assay

Name	Forward Primer (5'>3')	Reverse Primer (5'->3')
3 × *cis*	AATTC-TATTCTTTGGTCAAACAAACAGCTC×3-GAGCT	C-GAGCTGTTTGTTTGACCAAAGAATA×3-G
3×mB4	AATTC-TATTCTTTGcgCAAACAAACAGCTC×3-GAGCT	C-GAGCTGTTTGTTTGcgCAAAGAATA×3-G

Red characters indicate restriction sites for linker ligation. Lowercases indicate mutation in W-box.

Supplemental Table 6. Primers Used for ChIP-qPCR

Name	Forward Primer (5'>3')	Reverse Primer (5'->3')
RBOHA	AAAATCTACGGCCAAAGAGC	AATTGACTGTTGCTATACAG
RBOHB	CGACACTTCATAAATTATGC	GAAGAAGCCGCATTCTATTG

Supplemental Table 7. Accession Numbers of the Amino Acid Sequences Included in the Phylogenic Tree

Name	Accession No.
At-WRKY2	NP_200438.1
A-WRKY3	NP_178433.1
At-WRKY4	NP_172849.1
At-WRKY20	NP_849450.1
At-WRKY25	NP_180584.1
At-WRKY26	NP_196327.1
At-WRKY33	NP_181381.2
At-WRKY34	NP_194374.1
Ca-WRKY2	ABD65255.1
Ca-WRKY-a	AAR26657.1
Ca-WRKY-c	AAW67002.1
Lp-WRKY1	ABI95141.1
Na-WRKY3	AAS13439.1
Na-WRKY6	AAS13440.1
Nb-WRKY7	BAI63295.1
Nb-WRKY8	BAI63296.1
Nb-WRKY9	
Nb-WRKY10	
Nb-WRKY11	
Nb-WRKY12	
Nb-WRKY13	
Nb-WRKY14	
Nb-WRKY15	
Nt-WRKY1	BAA82107.1
Nt-WRKY2	BAA77383.1
Nt-WRKY4	BAA86031.1
Nt-WRKY-6	BAB61053.1
Nt-WRKY-7	BAB61054.1
Nt-WRKY-9	BAB61056.1
Os-WRKY24	NP_001044675.1
Os-WRKY30	NP_001062148.1
Os-WRKY35	NP_001053057.1
Os-WRKY53	NP_001055252.1
Os-WRKY70	NP_001055828.1
Os-WRKY78	NP_001060116.1
Sc-WRKY1	AAQ72790.1
St-WRKY8	BAI63294.1.