

SUPPORTING INFORMATION

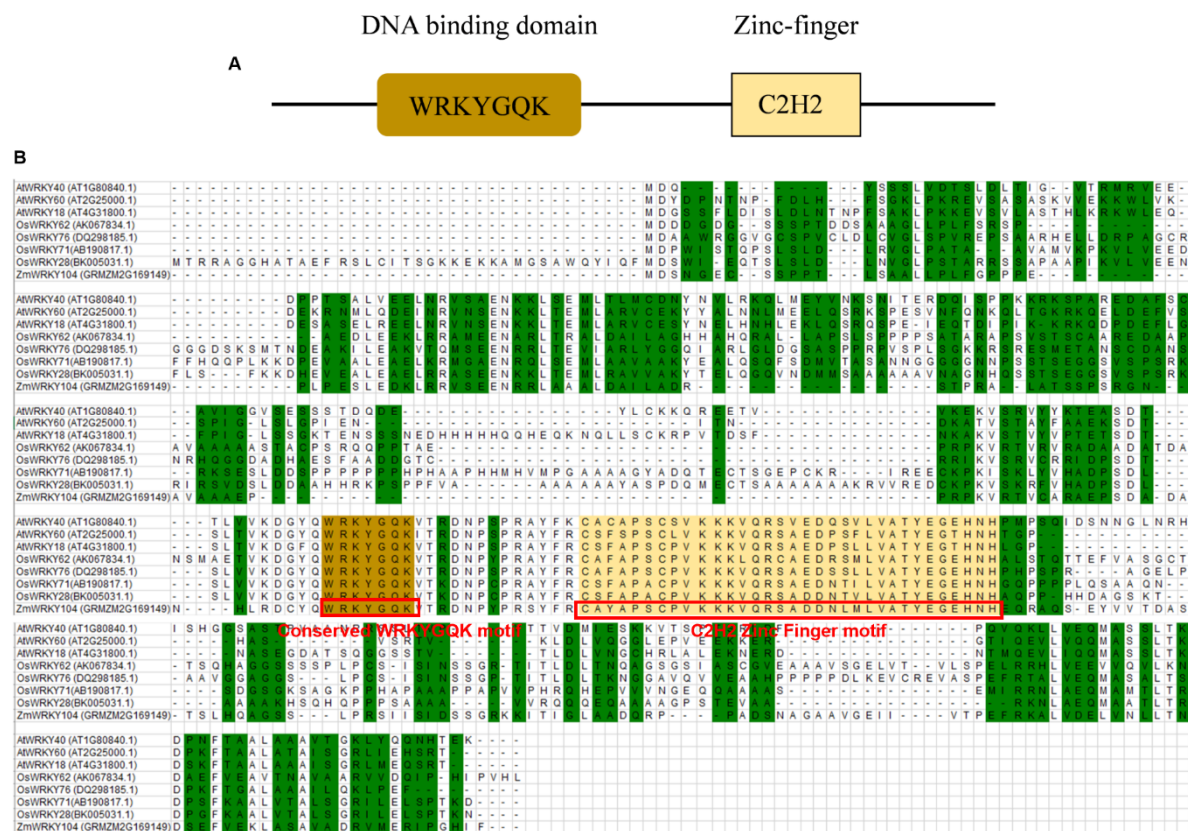


Figure S1. Multiple alignment of ZmWRKY104 with other orthologs in rice, *Arabidopsis*. (A) Conserved domains of ZmWRKY104. (B) Multiple alignment of ZmWRKY104 with other orthologs in rice, *Arabidopsis*. The first red box indicates the WRKYGQK motif, and the second indicates the conserved C2H2 zinc-finger motif. The accession numbers: AtWRKY40 (AT1G80840.1), AtWRKY60 (AT2G25000.1), AtWRKY18 (AT4G31800.1), OsWRKY62 (AK067834.1), OsWRKY76 (DQ298185.1), OsWRKY71(AB190817.1), OsWRKY28(BK005031.1), ZmWRKY104 (GRMZM2G169149). At, *Arabidopsis thaliana*; Zm, *Zea mays*; Os, *Oryza sativa*.

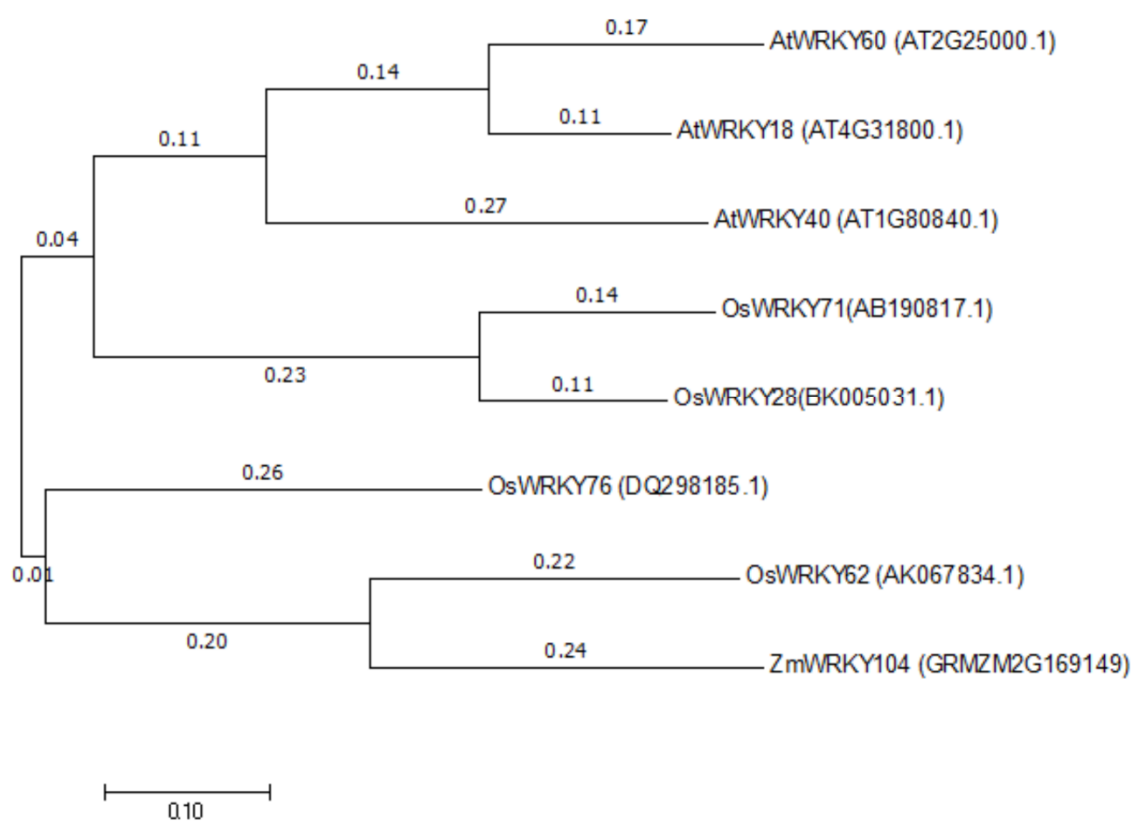
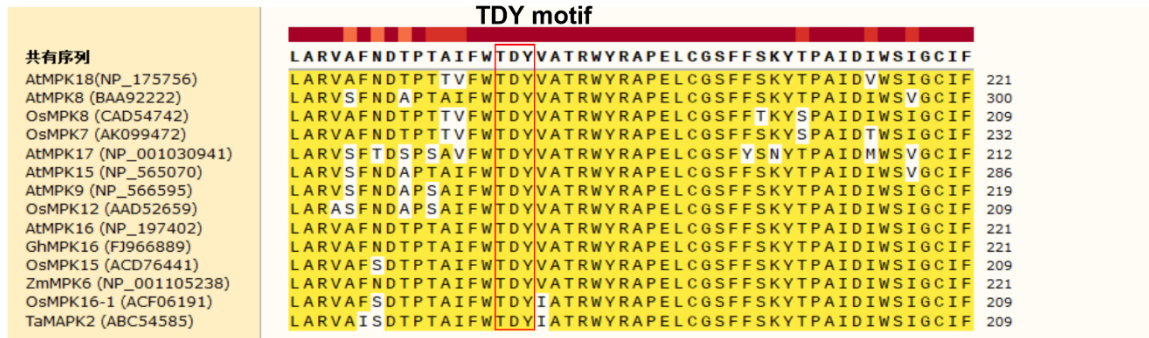


Figure S2. Phylogenetic relationships of ZmWRKY104 with other orthologs in rice, *Arabidopsis*. The amino acid sequences of WRKYs alignment were completed by ClustalW, and MEGA 7 was used to construct a phylogenetic tree using the neighbor-joining method.

A



B

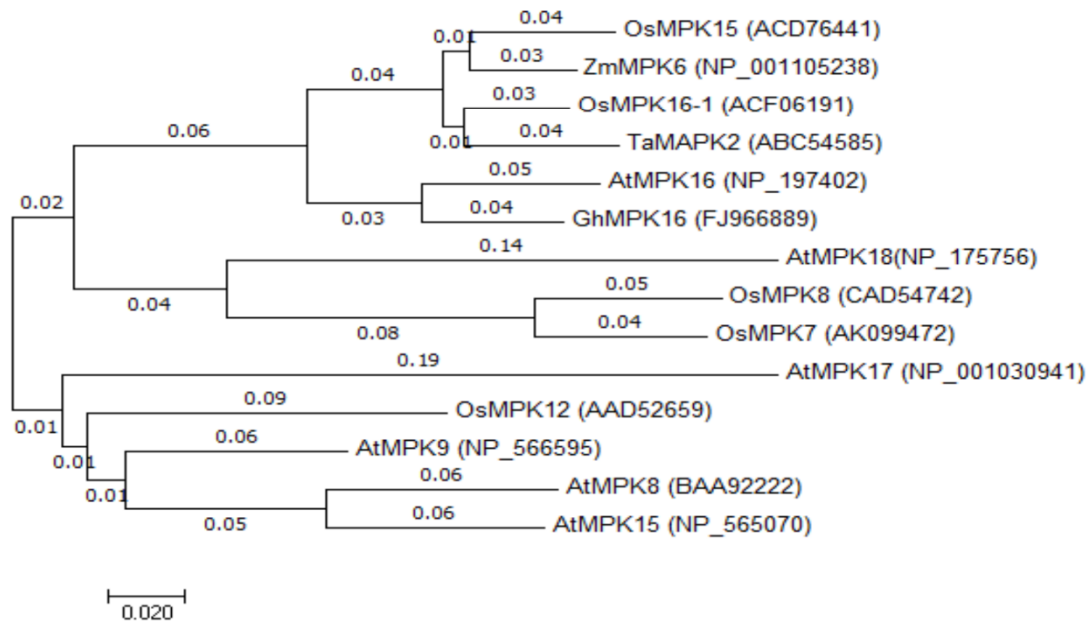


Figure S3. Multiple alignment and phylogenetic relationships of ZmMPK6 with other orthologs

in rice, *Arabidopsis*, wheat, cotton. (A) Multiple alignment of ZmMPK6 with other orthologs in rice,

Arabidopsis, wheat, cotton. Similar residues were shaded in yellow, respectively. The TDY activation

motif was boxed in red. (B) Phylogenetic relationships of ZmMPK6 with other orthologs in different

species. The amino acid sequences of MAPKs alignment were completed by ClustalW, and MEGA 7

was used to construct a phylogenetic tree using the neighbor-joining method. The accession numbers:

AtMPK18 (NP_175756), AtMPK8 (BAA92222), OsMPK8 (CAD54742), OsMPK7 (AK099472),

AtMPK17 (NP_001030941), AtMPK15 (NP_565070), AtMPK9 (NP_566595), OsMPK12

(AAD52659), AtMPK16 (NP_197402), GhMPK16 (FJ966889), OsMPK15 (ACD76441), ZmMPK6

(NP_001105238), OsMPK16-1 (ACF06191), TaMAPK2 (ABC54585). At, *Arabidopsis thaliana*; Zm,

Zea mays; Os, *Oryza sativa*; Ta, *Triticum aestivum*; *Gossypium hirsutum*.

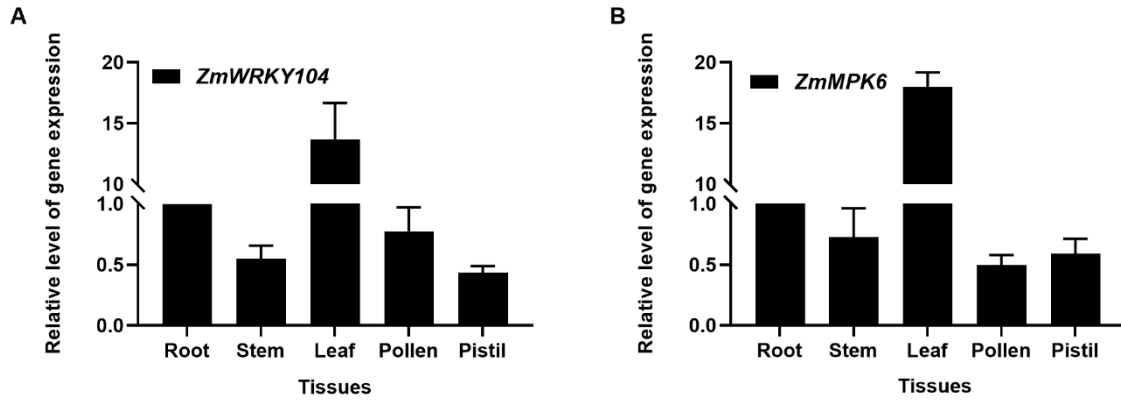


Figure S4. RT-qPCR analysis of *ZmWRKY104* and *ZmMPK6* expression in different tissues of maize. Total RNA was isolated from roots, stems, leaves, pollen, and pistil, the expression of (A) *ZmWRKY104* and (B) *ZmMPK6* relative to *ZmActin* was determined by RT-qPCR analysis. Data are the means \pm SE ($n = 3$).

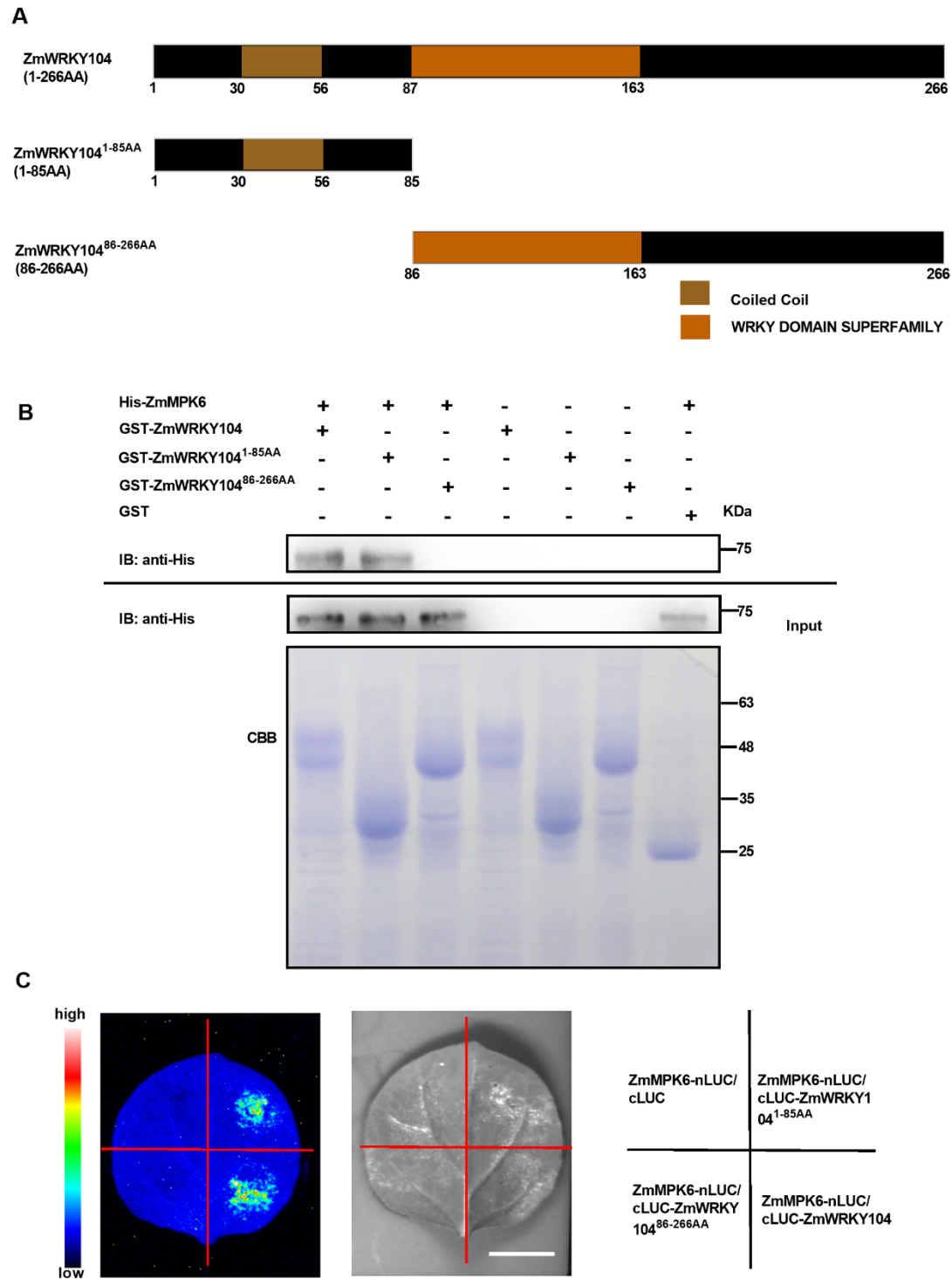


Figure S5. ZmMPK6 interacts with the truncations of ZmWRKY104. (A) The truncations of ZmWRKY104. ZmWRKY104^{1-85AA} consists of 1-85 amino acids of the N-terminal of ZmWRKY104 protein, ZmWRKY104^{86-266AA} consists of 86-266 amino acids of the C-terminal of ZmWRKY104 protein. (B) GST pull-down. GST-ZmWRKY104 fusion protein and its truncations (ZmWRKY104¹⁻

^{85AA}, ZmWRKY104^{86-266AA}) or GST alone was incubated with His-ZmMPK6 protein in GST beads. His-ZmMPK6 was detected by western blot using Anti-His antibody. GST-ZmWRKY104 fusion protein and its truncations or GST were then detected by SDS-PAGE. Molecular mass markers in kilodaltons are shown on the right. (C) LCI. *ZmMPK6* was fused to the nLUC, the full-length ZmWRKY104 protein and its truncations were fused to the cLUC, and then co-expressed in *Nicotiana benthamiana* leaves. ZmMPK6-nLUC/cLUC-ZmWRKY104 was used as a positive control, ZmMPK6-nLUC/cLUC was used as a negative control. Luciferase signals were captured using the Tanon-5200 image system. Scale bar, 1 cm. All the experiments were repeated at least three times with similar results.

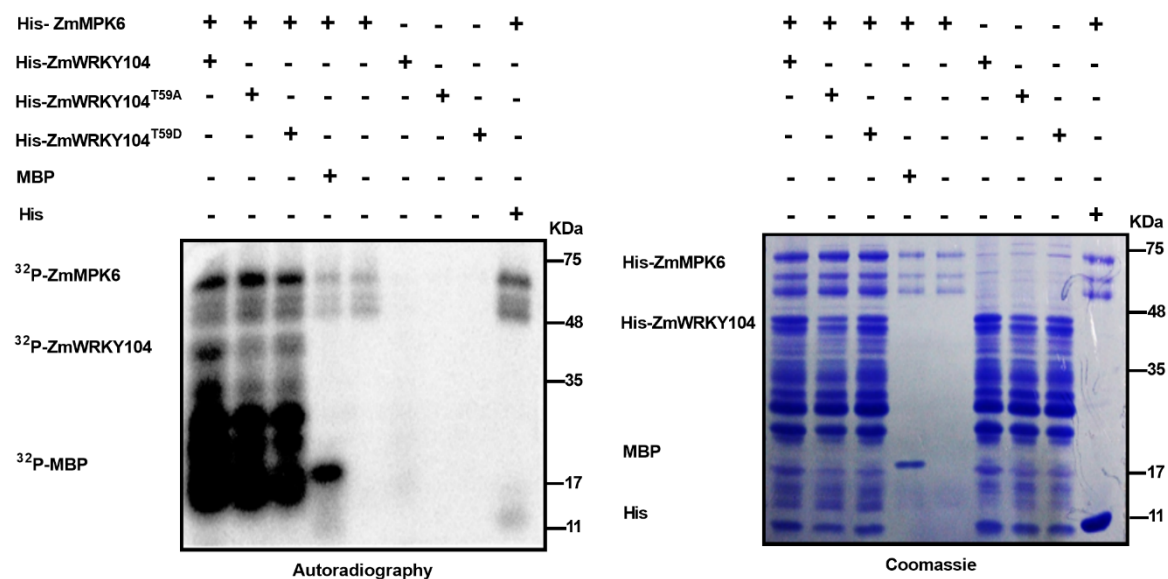


Figure S6. Phosphorylation of ZmWRKY104^{T59A} and ZmWRKY104^{T59D} by ZmMPK6 *in vitro*.

His-ZmMPK6, His-ZmWRKY104, His-ZmWRKY104^{T59A}, His-ZmWRKY104^{T59D} and His protein are expressed in *E. coli*. *In vitro* kinase assays are performed using the purified proteins. Recombinant His-ZmWRKY104, His-ZmWRKY104^{T59A}, His-ZmWRKY104^{T59D} or maltose binding protein (MBP) are used as substrate and subjected to in-gel kinase assay. Images show autoradiography (left panel), the corresponding coomassie staining (right panel). Molecular mass markers in kilodaltons are shown on the right. All the experiments were repeated at least three times with similar results.

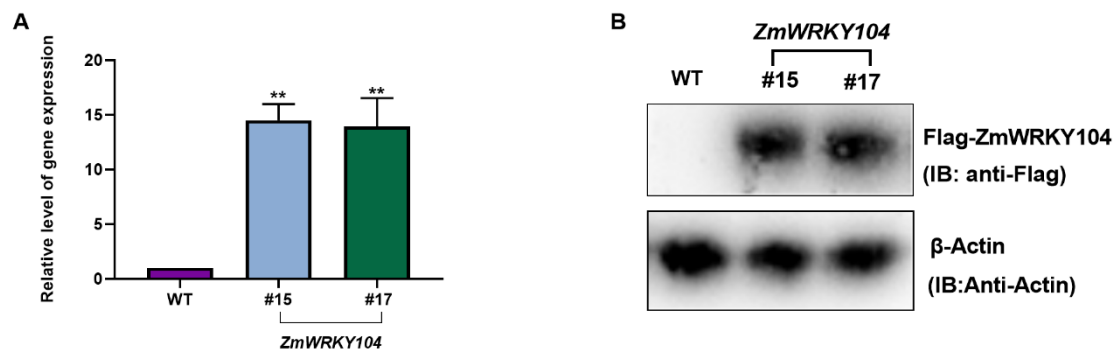


Figure S7. Analysis the gene expression and protein level of the *ZmWRKY104* transgenic lines. (A) The expression level of *ZmWRKY104* (#15, #17) in transgenic maize was analyzed by RT-qPCR. Values are the means \pm SE of three different experiments. P values were calculated by the Student's *t* tests (**P < 0.01). (B) Western blotting (WB) analysis of *ZmWRKY104* (#15, #17) protein, *ZmWRKY104* was analyzed by immunoblotting (IB) using an Anti-Flag antibody; β -actin was used as the total protein loading control.

Table S1. PCR Primers Used

Primer name	Vector	Primer sequence (5'–3')
Primers for gene clone		
<i>ZmWRKY104-F</i>		ATGGACAGTAACGGCGAGTGC
<i>ZmWRKY104-R</i>		CTAGAATATGTGCCCTGGTATTCTC
Primers for Y2H		
<i>AD-ZmWRKY104-F</i>	pGADT7	<u>GAATTC</u> ATGGACAGTAACGGCGAGTGC
<i>AD-ZmWRKY104-R</i>	pGADT7	<u>GGATCC</u> CTAGAATATGTGCCCTGGTATTCTC
<i>BD-ZmMPK6-F</i>	pGBKT7	<u>GGATCC</u> ATGCAGCACGACCAGAAGAAGAAGGCACC
<i>BD-ZmMPK6-R</i>	pGBKT7	<u>CTGCAGCT</u> ACCAGTGACCACCACGACTATCAACGGT
Primers for GST-pull down		
<i>GST-ZmWRKY104-F</i>	pGEX-4T-1	<u>GGATCC</u> ATGGACAGTAACGGCGAGTG
<i>GST-ZmWRKY104-R</i>	pGEX-4T-1	<u>GAATTC</u> GAATATGTGCCCTGGTATTCTC
<i>GST-ZmWRKY104^{1-85AA}-F</i>	pGEX-4T-1	<u>GGATCC</u> ATGGACAGTAACGGCGAGTG
<i>GST-ZmWRKY104^{1-85AA}-R</i>	pGEX-4T-1	<u>GAATTC</u> CCCGACCTTGGGCCG
<i>GST-ZmWRKY104^{86-266AA}-F</i>	pGEX-4T-1	<u>GGATCC</u> ATGACGGTGTGCGCGC
<i>GST-ZmWRKY104^{86-266AA}-R</i>	pGEX-4T-1	<u>GAATTC</u> GAATATGTGCCCTGGTATTCTC
<i>His-ZmWRKY104-F</i>	pET-30a	<u>GGATCC</u> ATGGACAGTAACGGCGAGTG
<i>His-ZmWRKY104-R</i>	pET-30a	<u>GAATTC</u> GAATATGTGCCCTGGTATTCTC
<i>His-ZmMPK6-F</i>	pET-30a	<u>GGATCC</u> ATGCAGCACGACCAGAAGAAGAAGGC
<i>His-ZmMPK6-R</i>	pET-30a	<u>GAGCTC</u> CCAGTGACCACCACGACTATCAACGGT
Primers for LCI		
<i>ZmMPK6-nLUC-F</i>	pCAMBIA1300	<u>GAGCTC</u> ATGCAGCACGACCAGAAGAAG

<i>ZmMPK6-nLUC-R</i>	pCAMBIA130 0	<u>GGATCCCCC</u> CAGTGACCACCACGACTATC
<i>ZmWRKY104-cLUC-F</i>	pCAMBIA130 0	<u>GGTACCAT</u> GGACAGTAACGGCGAGT
<i>ZmWRKY104-cLUC-R</i>	pCAMBIA130 0	<u>GGATCCGA</u> ATATGTGCCCTGGT
<i>ZmWRKY104¹⁻85AA-cLUC-F</i>	pCAMBIA130 0	<u>GGTACCAT</u> GGACAGTAACGGCGAG
<i>ZmWRKY104¹⁻85AA-cLUC-R</i>	pCAMBIA130 0	<u>GGATCCCCG</u> GACCTTGGGCCGCGG
<i>ZmWRKY104⁸⁶⁻266AA-cLUC-F</i>	pCAMBIA130 0	<u>GGTACCAT</u> GACGGTGTGCGCGCGC
<i>ZmWRKY104⁸⁶⁻266AA-cLUC-R</i>	pCAMBIA130 0	<u>GGATCCGA</u> ATATGTGCCCTGGTAT

Primers for Co-IP

<i>ZmWRKY104-3*flag-F</i>	1300-221-3*flag	<u>GGTACCAT</u> GGACAGTAACGGCGAGTG
<i>ZmWRKY104-3*flag-R</i>	1300-221-3*flag	<u>GGATCCTC</u> AGAATATGTGCCCTGGTATTCT C
<i>ZmMPK6-6*myc-F</i>	1300-221-6*myc	<u>TTCGAAAT</u> GCAGCACGACCAGAAGA
<i>ZmMPK6-6*myc-R</i>	1300-221-6*myc	<u>GGATCCCT</u> ACCAGTGACCACCACGA

Primers for qRT

<i>ZmMPK6-qRT-F</i>		CAGAAGAAGCCAGACCTGCA
<i>ZmMPK6-qRT-R</i>		CCACCACGACTATCAACGGT
<i>ZmWRKY104-qRT-F</i>		TCCGAGATCCTACTTCCGCT
<i>ZmWRKY104-qRT-R</i>		ATATTCGCTCTGGGCACGTT
<i>ZmActin2-qRT-F</i>		GCCATCCATGATCGGTATGG
<i>ZmActin2-qRT-R</i>		GTCGCACTTCATGATGGAGTTG

Primers used in subcellular localization

<i>ZmWRKY104-YFP-F</i>	1305-d35SYFP	<u>GGTACCAT</u> GGACAGTAACGGCGAGTG
<i>ZmWRKY104-YFP-R</i>	1305-d35SYFP	<u>GGATCCTC</u> AGAATATGTGCCCTGGTATTCT C
<i>ZmMPK6-YFP-F</i>	1305-d35SYFP	<u>TCTAGAAT</u> GCAGCACGACCAGAAGAAGA AGGCA

<i>ZmMPK6-YFP-R</i>	1305- d35SYFP	<u>GGATCCCCAGTGACCACCACGACTATCA</u> ACGGTAG
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Primers for dsRNA

<i>ds-ZmWRKY104-F</i>		<u>TAATACGACTCACTATAGGGGCCATACTC</u> GCCGACCGCT
<i>ds-ZmWRKY104-R</i>		<u>TAATACGACTCACTATAGGGCTGGCCGTA</u> CTTGCGCCAC

Primers used in maize protoplasts

<i>Ubi: ZmWRKY104-mcherry-F</i>	pXZP008	<u>GGATCCATGGACAGTAACGGCGAGTG</u>
<i>Ubi: ZmWRKY104-mcherry-R</i>	pXZP008	<u>GGTACCACGAATATGTGCCCTGGTATTCT</u> C
<i>Ubi: ZmWRKY104-SRDX-mcherry-F</i>	pXZP008	<u>GGATCCATGGACAGTAACGGCGAGTG</u>
<i>Ubi: ZmWRKY104-SRDX-mcherry-R</i>	pXZP008	<u>GGTACCGTCAAACGGAGTTCTAGATCCA</u> GGAATATGTGCCCTGGTATTCTC
<i>Ubi: ZmMPK6-mcherry-F</i>	pXZP008	<u>TCTAGAATGCAGCACGACCAGAAGAAGA</u> AGGCAC
<i>Ubi: ZmMPK6-mcherry-R</i>	pXZP008	<u>GGATCCCCAGTGACCACCACGACTATCA</u> ACGGT

Primers used in transgenic maize

<i>pCUN ZmWRKY104-F</i>	- pCUN-N-HF	<u>AAGCTTAATGGACAGTAACGGCGAG</u>
<i>pCUN ZmWRKY104-R</i>	- pCUN-N-HF	<u>GGTACCCTAGAATATGTGCCCTGGTATTCT</u>
<i>Primer 1-F</i>		ATCGAGACAAGCACGGTCAA
<i>Primer 1-R</i>		AAACCCACGTCATGCCAGTT
<i>Primer 2-F</i>		GGACGATGACGATAAGCTGGAAGT
<i>Primer 2-R</i>		CATCAGATTGTCGTCTGCGCTT

F, forward primer; R, reverse primer.

DNA oligos used for site-directed mutagenesis

Oligo Name (Gene-mutation)	Oligo for mutagenesis
<i>ZmWRKY104-T59A</i>	TCGCCGACCGCTCCGCCCCGCGAGCGCTCG
<i>ZmWRKY104-T59D</i>	TCGCCGACCGCTCCGACCCGCGAGCGCTCG