

Supplementary information

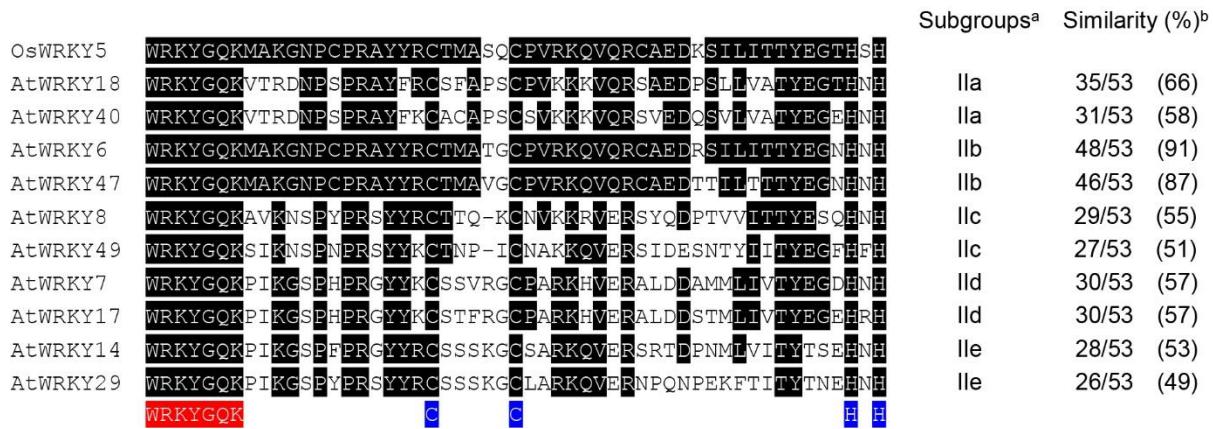


Fig. S1. Amino acid sequence alignments of WRKY domains between OsWRKY5 and group II AtWRKY proteins.

Amino acid sequences of OsWRKY5 and group II AtWRKY proteins were obtained from the Rice Genome Annotation Project (<http://rice.plantbiology.msu.edu/>) and The Arabidopsis Information Resource (TAIR, <https://www.arabidopsis.org/>), respectively. Sequence alignment was performed using ClustalW with default parameters. Sequences are as follows: OsWRKY5, Os05g04640; AtWRKY18, At4g31800; AtWRKY40, At1g80840; AtWRKY6, At1g62300; AtWRKY47, At4g01720; AtWRKY8, At5g46350; AtWRKY49, At5g43290; AtWRKY7, At4g24240; AtWRKY17, At2g24570; AtWRKY14, At1g30650; AtWRKY29, At4g23550. Black boxes represent amino acids of AtWRKY proteins that are identical to those of OsWRKY5. Red and blue boxes represent the conserved WRKYGQK sequence and zinc finger motif of the WRKY domain, respectively. ^aEulgem et al. (2000) classified group II AtWRKY proteins into five subgroups [37]. ^bAmino acid similarity of AtWRKY proteins compared with OsWRKY5. Os, *Oryza sativa*; At, *Arabidopsis thaliana*.

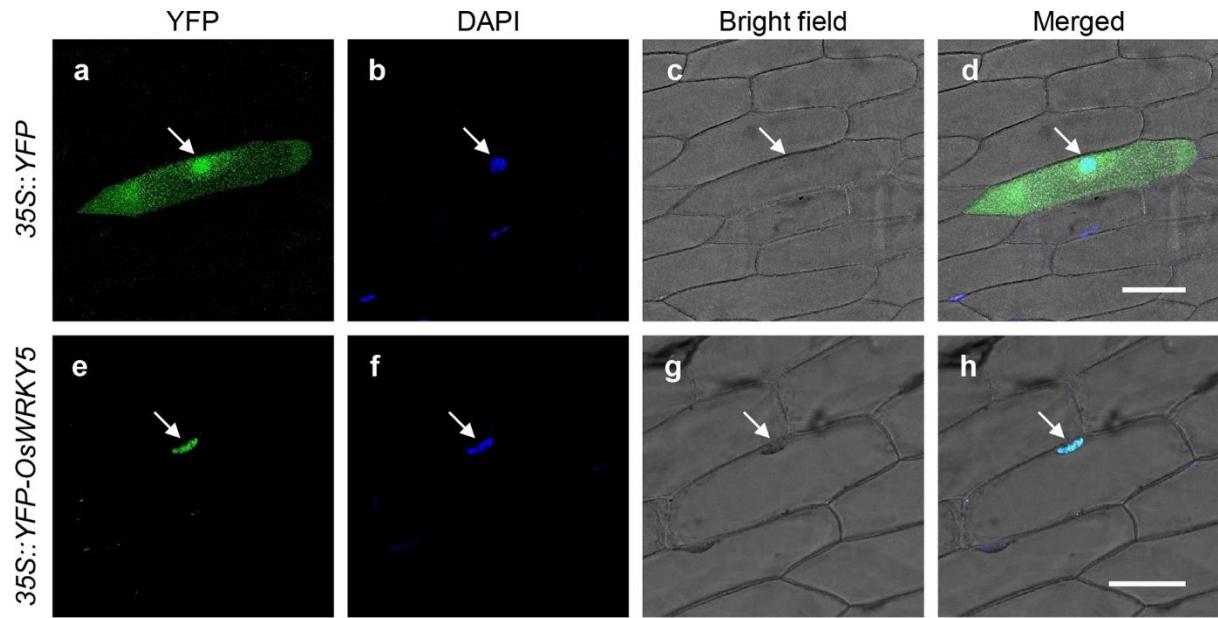


Fig. S2. Subcellular localization of OsWRKY5.

YFP-fused OsWRKY5 proteins transiently expressed in onion epidermal cells. Upper panels show fluorescence from the YFP control (**a-d**), which was distributed throughout the cell. Lower panels show the fluorescent signal of YFP-OsWRKY5 exclusively localized to the nucleus (**e-h**). Nuclei were stained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI). Experiments were repeated twice with similar results. White arrows indicate the position of the nucleus. Bars = 100 μ m.

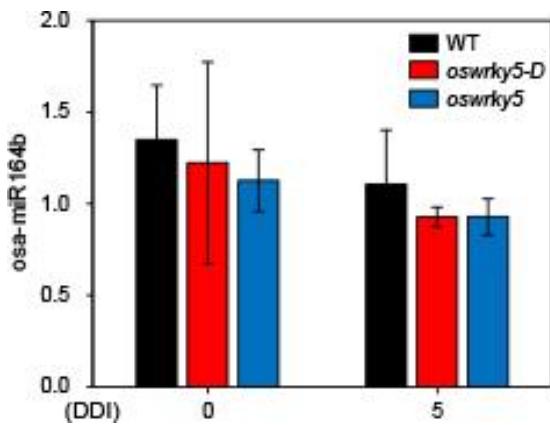


Fig. S3. Endogenous osa-miR164b levels in *oswrky5-D* and *oswrky5*.

Total RNA was extracted from detached leaves of WT and mutant lines (*oswrky5-D* and *oswrky5*) at 0 and 5 DDI under DIS as shown in Figure 2B. First-strand cDNA was synthesized by stem-loop pulsed RT-PCR. Transcript levels of osa-miR164b were determined by RT-qPCR and normalized using that of U6 snRNA. Mean and SD values were obtained from more than three biological replicates. No significant difference was found between WT and mutant lines by Student's *t*-test. DDI, day(s) of dark incubation.

AGAAACAA CCTACCCTAGCTGGTTTGCACTCTGAAGTCTAAACTGAAAAGGTCTCAGTTCTGATATATATG
 CAGATATTGTCAACTACTAGCTGATGAAGGCC **TTGACT** CTAGCTAAATACTAAGCCTCATGTGTCAATTCTCA
 TGATTCTCCACCCATATTGCGGCTGGATAATGTACACTTAGGCATCAGTCATGGTGTGAATGTGCAAGA
 ACGGATCCAACACGCTAGGTAGAGTCGTAGAGATATATAGATCCGGCGTGAAAATGCCATCAAACCTTCT
 CATGAAACTACGTTATGAAGAAAGAGAGTTACACATGTTATCTTTGAATTTCATGCATATTGATTTCGAT
 TGGCTACAAGTGCATGCGCATTAATCCGCAAGTGGTGACACTGGTTATTACTTCTTACATAGCAAAAAAA
 AAAATAATGAAACTATGGTTGTGTTAGTCCCTTAAGCTCCAAAAATTCCGTACATCAAATGTTGGATAC
 ATGCATAGAGCATTAAATGTGGACGAAAAAAACCAATTACACAGTTGCATGTAATTACGAGACGAATCTT
 GAGCCTAATTACGCCGTGATTGACAATGTGGTGTACAGTAAACATTGCTAATGGCAAATTAAATTAGACTTA
 ATAAATTCTGTCACAGTTACAGGCGGAATCTGTAATTGTTTATTATTAGTATATTTAATACCAAATGTG
 TGTCCGTATTCTCAAAAAAAAATTGGAGGGAGGAACAAACACAGCCTATATTAAAAAAATGTATTTGCAAG
 ATAAATCTAACATGATGTTCTGTAATTGATATCA **AGTCAA** GTAAAAGAGAGATGATTAGAAGA
 AGCGAACATATTAGGAAGAACCTAACATCAGATAATTAGAAGAACGAGACTTAAACTAGATCATCTAGCAC
 ACAACTTATAGATGCTAGCCAAAAAATCATGGACGTTGAGAGAGAGAGAGATCGTACTGAATGGAGTAA
 TTGTTGCTGCAGAGCGTGGCATATCCTCATGCACGCACCATCACTCCTAGGCTAGCTATCTCGATCGAT
 CAACTGGTGATCGAAAGGGAGTCTACATCGCG **TTGACT** CCG **TTGACC** CGGCCATGCAGTA **CACGTG** GAC
 GCGAGTGCCTCATCCACCTGTACGCTACGTCAGGCCGCTGACATGGCTGGCCCACCATCCCCATCGATT
 CCGATCCCCATTCTTGCATAATTGGTCCAAGTCCAAGTCTTCGTTACGTTACATATGCTAGCTTCTCGT
 CGTTAGCTAGCTAGGTTAAACTACTTACTAATTCTCACTCTCTCTCTCTCTGGAACCTAGCTAACT
 AGCTAGGAGTAGTAGTAGGAGCAAGAGCCATATAAGCTAGCTAGCTACGACCTAGCTAGCTCTCCCTACTT
 TAATTGATTCTCTCCTCTCACTCTACTGATCGATCGAGCTATCC **ATGGAGATG**
+1

Fig. S4. The *Cis*-elements in *OsWRKY5* promoter region.

The *cis*-elements were identified in the 1,500-bp upstream of the transcription initiation site (+1) represented by bent arrow. Red, blue, and black shaded sequences represent the W-boxes, G-box, and start codon, respectively.

Table S1. Primers used in this study.

A. Primers for verification of T-DNA insertion		
Primer names	Left primers (5' → 3')	Right primers (5' → 3')
PFG_3A-15928	CATTAAGCTGGACCAGATGG	AACCACTGCGGATTAATGC
PFG_3A-06060	TTGGATGCCTGATTAAGGTTG	CCGTTCTGCACATTACAC
pGA2715	CTAGAGTCGAGAATTCACTACA	TTGGGGTTCTACAGGACGTAAC
B. Primers for subcellular localization		
Gene	Forward primers (5' → 3')	Reverse primers (5' → 3')
OsWRKY5	ATGGAGATGATGGTGCAGAAC A	TCAGGTGGAGACGTGCCGCAA
C. Primers for RT-qPCR		
Genes	Forward primers (5' → 3')	Reverse primers (5' → 3')
OsWRKY5	GGCTCCAATGATCAGTGATGGA	AGCCATTGTGCATCGGTAGT
SGR	AGGGGTGGTACAACAAGCTG	GCTCCTGCGGAAGATGTAG
NYC3	TGTCGTTGCCATGTGAAGAT	TTGGTCACGCCACAAATCTA
OsPAO	GGAAATCCTAGCCAAGAAGTGT G	CGCAGGAATCCCAGCAGTT
Osh69	CCACAACACGGATAACTT	GGTGAACACTATGGAACA
Osh36	GCACGGAGGCGAACGA	TTGAGCGGTAGCACCCATT
Osl85	GAGCAACGGCGTGGAGA	GCGGCGGTAGAGGAGATG
OsNAP	CAAGAAGCCAACGGTTC	GTTAGAGTGGAGCAGCAT
OsNAC2	CAACTCCTGGAGAGCTGCAA	GATCTCCGGGTTACGTCG
OsNCED3	GTGGTGCTCGACAAGGAGAA	CAGAGGTGGAAGCAGAAGCA
OsNCED4	GAGGTACGACTTCCATGGC	TTGAGGTACGGCTTGGACAC
OsNCED5	CCCAGCTTGAAGCTTTGCT	ACAACACTGCAACTATCCCTATCAC T
OsUBQ5	ACCACTCGACCGCCACTACT	ACGCCTAAGCCTGCTGGTT
D. Primers for pulsed RT-qPCR of miR164b		
Primer names	Forward primers (5' → 3')	Reverse primers (5' → 3')
miR164b_pulsed RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACTGGATACGACTGCAC G	
miR164b_qPCR	GCTCTGGAGAACGAGGGC	GTGCAGGGTCCGAGGT

U6 snRNA_qPCR CAACGGATATCTCGGCTCT CAACTTGC GTTCAAAGACTC

E. Primers for yeast one-hybrid assay

Primer names	Forward primers (5' → 3') ^a	Reverse primers (5' → 3') ^a
OsWRKY5_GAD42	<u>GAATT</u> CATGGAGATGATGGTGCA	<u>CTGCAG</u> TCAAGGTGGGAGACGTGCC
4_EcoRI and <i>Pst</i> I	GAAGCAACGA	GCAA
OsNAP-1_EcoRI and <i>Xba</i> I	<u>GAATT</u> CAGGTGTGAAAACAAAT AAGA	<u>CTCGAG</u> AATAGTACCGCTGTGGTG AA
OsNAP-2_EcoRI and <i>Xba</i> I	<u>GAATT</u> CATCACGTCGTTTCAAC TA	<u>CTCGAG</u> GTACCAAGGTGCTAACAT AC
OsNAC2-1_SalI and <i>Xho</i> I	<u>GTCGACT</u> GTGTTCAGTTGTCTCT TC	<u>CTCGAG</u> GTAAACAAGCCAGAAC AAC
OsNAC2-2_SalI and <i>Xho</i> I	<u>GTCGACC</u> CGGGGACATTCAGACG TTT	<u>CTCGAG</u> TGGTTTGTGGGGCTTAG AA
OsNAC2-3_SalI and <i>Xho</i> I	<u>GTCGACCC</u> ACTGCTATTACACAA TAG	<u>CTCGAG</u> ATCTCCCAGGAGATAAGC CA
OsNAC2-4_SalI and <i>Xho</i> I	<u>GTCGACT</u> TCGGTTGCTGCTCG GCT-	<u>CTCGAG</u> GGCTAGTGATCCATCAGA TC
OsNAC2-5_SalI and <i>Xho</i> I	<u>GTCGAC</u> CTAGCTAGTACTCCATC CGT	<u>CTCGAG</u> CAGGTTACTACTCCCTCC AT

^a The underlined nucleotides represent the restriction site for restriction enzymes.