Supplementary information

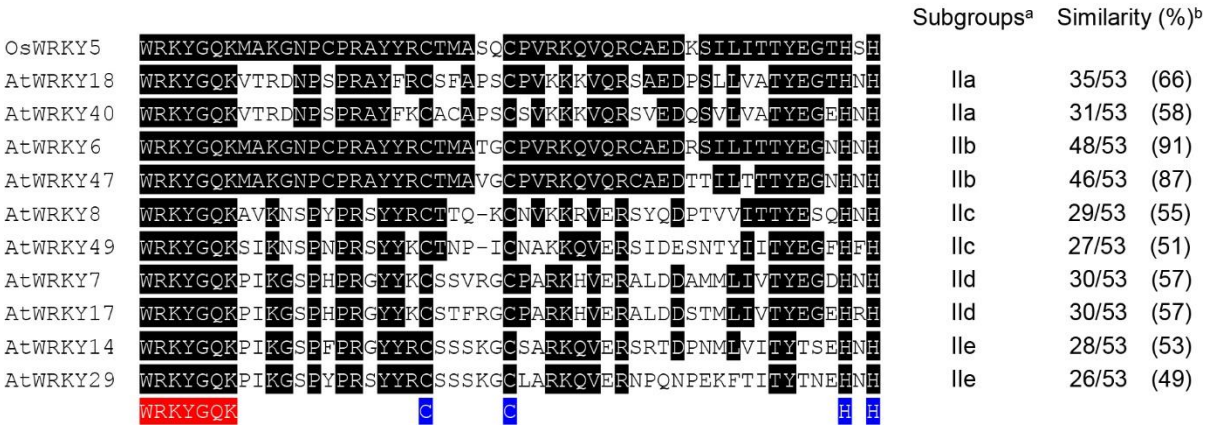

Fig. S1. Amino acid sequence alignments of WRKY domains between OsWRKY5 and group II AtWRKY proteins.

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. [a]Eulgem et al. (2000) classified group II AtWRKY proteins into five subgroups [37]. [b]Amino acid similarity of AtWRKY proteins compared with OsWRKY5. Os, *Oryza sativa*; At, *Arabidopsis thaliana*.

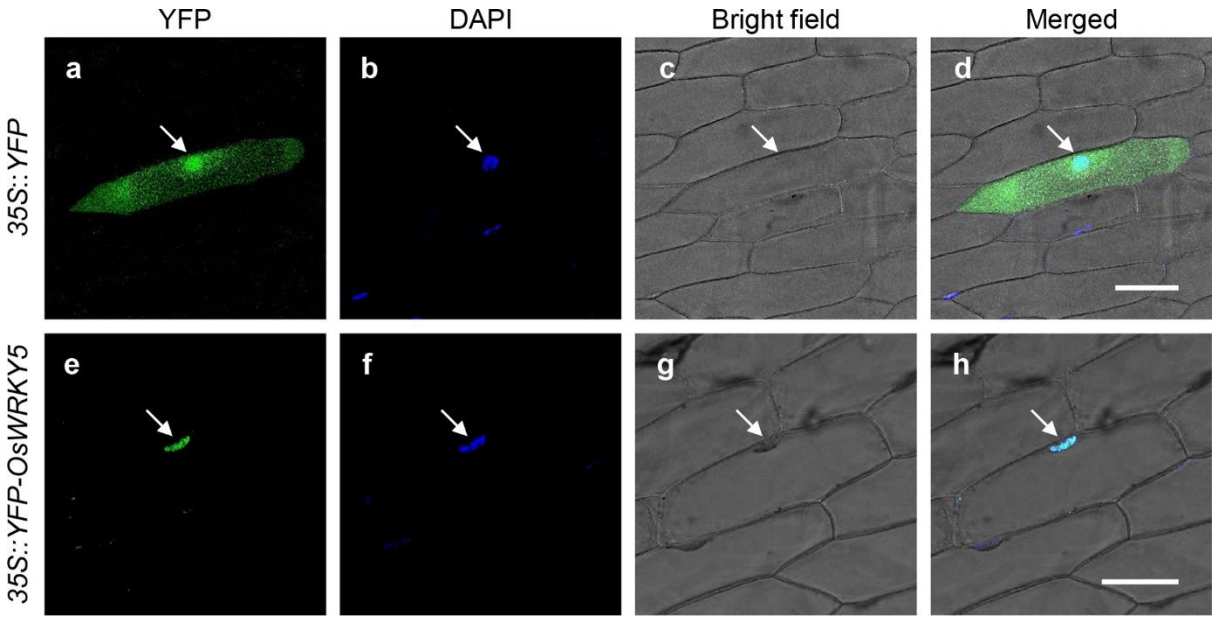

Fig. S2. Subcellular localization of OsWRKY5.

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 μm.

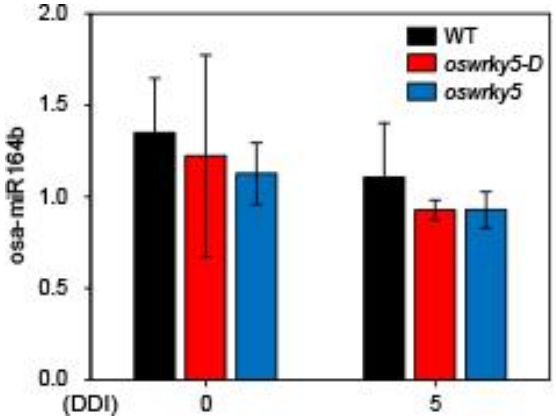

Fig. S3. Endogenous osa-miR164b levels in *oswrky5-D* and *oswrky5*.

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.

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	CATTAAAGCTGGACCAGATGG	AACCACTTGCGGATTAATGC
PFG_3A-06060	TTGGATGCCTGATTAAGGTTG	CCGTTCTTGCACATTCACAC
pGA2715	CTAGAGTCGAGAATTCAGTACA	TTGGGGTTTCTACAGGACGTAAC
B. Primers for subcellular localization		
Gene	Forward primers (5’ → 3’)	Reverse primers (5’ → 3’)
OsWRKY5	ATGGAGATGATGGTGCAGAAGCA	TCAGGTGGGAGACGTGCCGCAA
C. Primers for RT-qPCR		
Genes	Forward primers (5’ → 3’)	Reverse primers (5’ → 3’)
OsWRKY5	GGCTCCAATGATCAGTGATGGA	AGCCATTGTGCATCGGTAGT
SGR	AGGGGTGGTACAACAAGCTG	GCTCCTTGCGGAAGATGTAG
NYC3	TGTCGTTGCCATGTGAAGAT	TTGGTCACGCCACAAATCTA
OsPAO	GGAAATCCTAGCCAAGAAGTGTTG	CGCAGGAATCCCAGCAGTT
Osh69	CCACAACACGGATAACTT	GGTGAACACTATGGAACA
Osh36	GCACGGAGGCGAACGA	TTGAGCGGTAGCACCCATT
Osl85	GAGCAACGGCGTGGAGA	GCGGCGGTAGAGGAGATG
OsNAP	CAAGAAGCCGAACGGTTC	GTTAGAGTGGAGCAGCAT
OsNAC2	CAACTCCTGGAGAGCTGCAA	GATCTCCGGGTTCACGTCG
OsNCED3	GTGGTGCTCGACAAGGAGAA	CAGAGGTGGAAGCAGAAGCA
OsNCED4	GAGGTACGACTTCCATGGGC	TTGAGGTACGGCTTGGACAC
OsNCED5	CCCAGCTTGAAGCTTTTGCT	ACAACACTGCAACTATCCCTATCACT
OsUBQ5	ACCACTTCGACCGCCACTACT	ACGCCTAAGCCTGCTGGTT
D. Primers for pulsed RT-qPCR of miR164b		
Primer names	Forward primers (5’ → 3’)	Reverse primers (5’ → 3’)
miR164b_pulsed RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGCACG	
miR164b_qPCR	GCTCTGGAGAAGCAGGGC	GTGCAGGGTCCGAGGT
U6 snRNA_qPCR	CAACGGATATCTCGGCTCT	CAACTTGCGTTCAAAGACTC
E. Primers for yeast one-hybrid assay		
Primer names	Forward primers (5’ → 3’) [a]	Reverse primers (5’ → 3’) [a]
OsWRKY5_GAD424 _*Eco*RI and *Pst*I	GAATTCATGGAGATGATGGTGCAG AAGCAACGA	CTGCAGTCAGGTGGGAGACGTGCCG CAA
OsNAP-1_*Eco*RI and *Xba*I	GAATTCAGGTGTGAAAACAAATAA GA	CTCGAGAATAGTACCGCTGTGGTGA A
OsNAP-2_*Eco*RI and *Xba*I	GAATTCATCACGTCGTTTTTCAACT A	CTCGAGGTACCAAGGTGCTAAGATA C

OsNAC2-1_*Sal*I and *Xho*I	GTCGACTGTGTTCAGTTTGTCTCTTC	CTCGAGGTTAACAAGCCAGAACAAAC
OsNAC2-2_*Sal*I and *Xho*I	GTCGACCGGGGACATTTCAGACGTTT	CTCGAGTGGTTTTGTGGGGCTTAGAA
OsNAC2-3_*Sal*I and *Xho*I	GTCGACCCACTGCTATTACACAATAG	CTCGAGATCTCCCAGGAGATAAGCCA
OsNAC2-4_*Sal*I and *Xho*I	GTCGACTTCTCGGTTGCTGCTCGGCT-	CTCGAGGGCTAGTGATCCATCAGATC
OsNAC2-5_*Sal*I and *Xho*I	GTCGACCTAGCTAGTACTCCATCCGT	CTCGAGCAGGTTACTACTCCCTCCAT

[a] The underlined nucleotides represent the restriction site for restriction enzymes.