New Phytologist

***New Phytologist* Supporting Information**

Article title: **OsWRKY21 and OsWRKY108 Function Redundantly to Promote Phosphate Accumulation Through Maintaining the Constitutive Expression of *OsPHT1;1* under Phosphate-Replete Conditions**

Authors: **Jun Zhang, Mian Gu, Ruisuhua Liang, Xinyu Shi, Lingling Chen, Xu Hu, Shichao Wang, Xiaoli Dai, Hongye Qu, Huanhuan Li, and Guohua Xu**

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The following Supporting Information is available for this article:

Fig. S17 Pi concentration of *pht1;8*, *pht1;1 pht1;8* mutants and wild-type plants.

Fig. S18 Pi concentration of *pht1;2* mutants and wild-type plants.

Fig. S19 Phylogenetic analysis of the Group II and Group III type WRKY transcription factors from rice and *A. thaliana* according to Ross *et al*. (2007).

Fig. S20 Effect of *WRKY21* and *WRKY108* overexpression or mutation on transcript level of *PHO1;2* in rice.

Fig. S21 The total P concentration of wild-type plants, *pht1;1*, *wrky21*/*wrky108* single mutants, *wrky21 wrky108* double mutants and *WRKY21-SRDX* transgenic lines, at different Pi levels in a soil-based experiment.

Table S1 Primers used for RT-qPCR analysis.

Table S2 Primers used for constructs for generating transgenic plants.

Table S3 Primers used for constructs for subcellular location, Y1H, EMSA, Y2H, BiFC and pull-down.

Table S4 Positive interactions from Yeast One-Hybrid screening.

Table S5 Candidates from Yeast Two-Hybrid screening.

Fig. S1

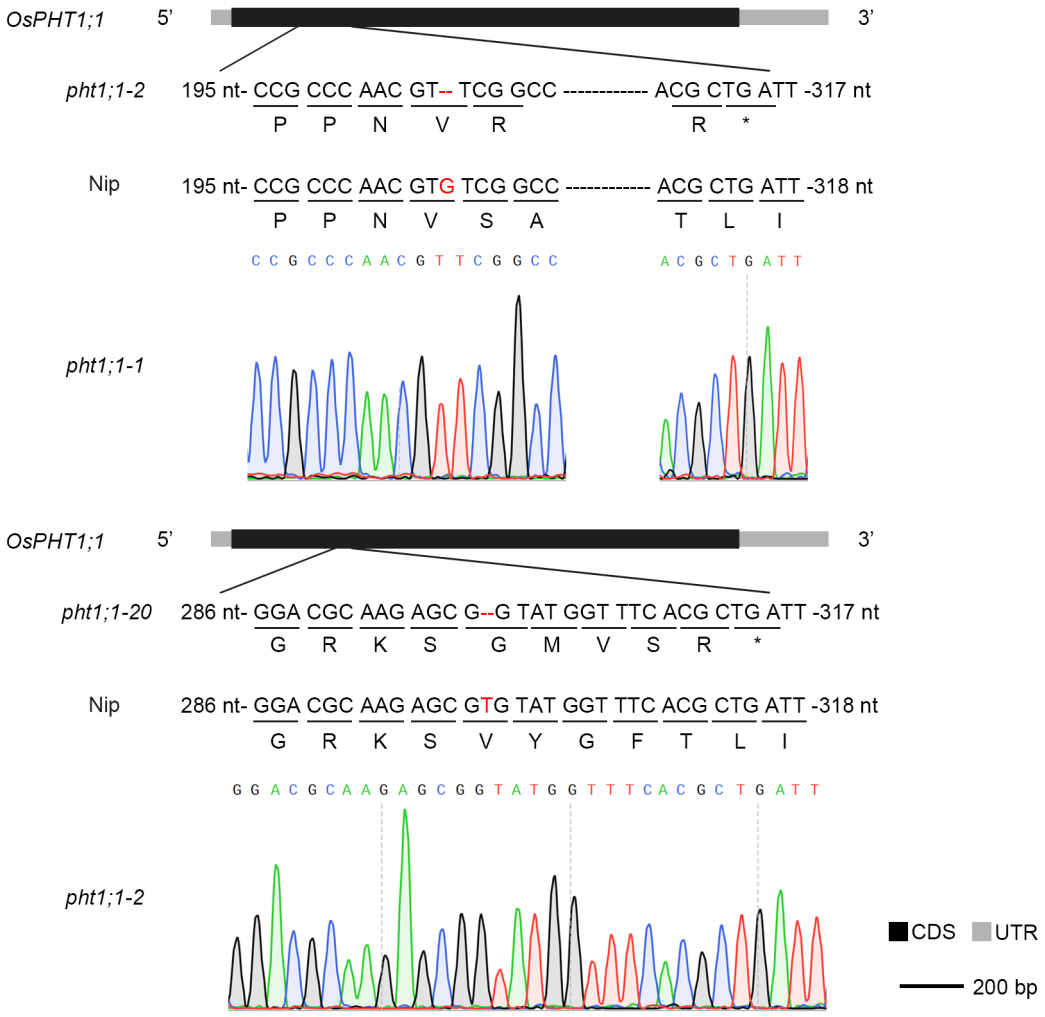

Fig. S1 Identification of *pht1;1* mutant lines. The gene structure of *OsPHT1;1* along with the mutation sites of two independent lines are present in each panel (upper and lower). The coding sequences (CDS) and the untranslated regions (UTR) are indicated by black rectangles and grey rectangles, respectively. The red letters indicate newly generated sequences by the CRISPR-Cas9 system. The letters underlying the nucleotide sequences are amino acid sequences.

Fig. S2

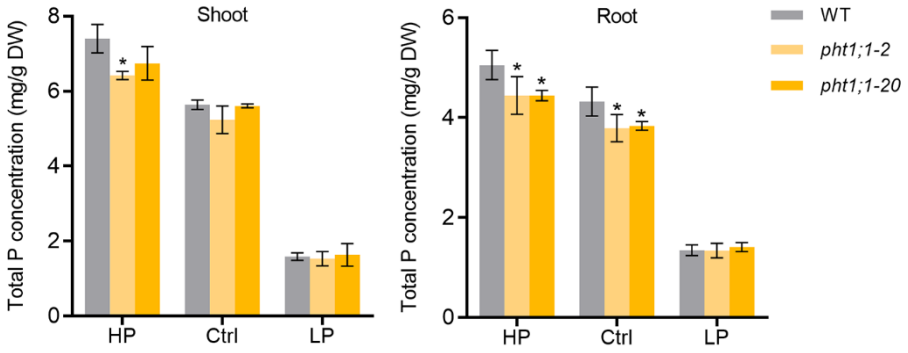

Fig. S2 Total P concentration of *pht1;1* mutants and wild-type plants. Plants were grown under the same conditions as that in Fig. 1. Total P concentration was measured in shoot (left) and Root (right). Error bars indicate SD (n=4). Data significantly different from the corresponding controls are indicated (*$P < 0.05$, Student's *t* test). DW, dry weight.

Fig. S3

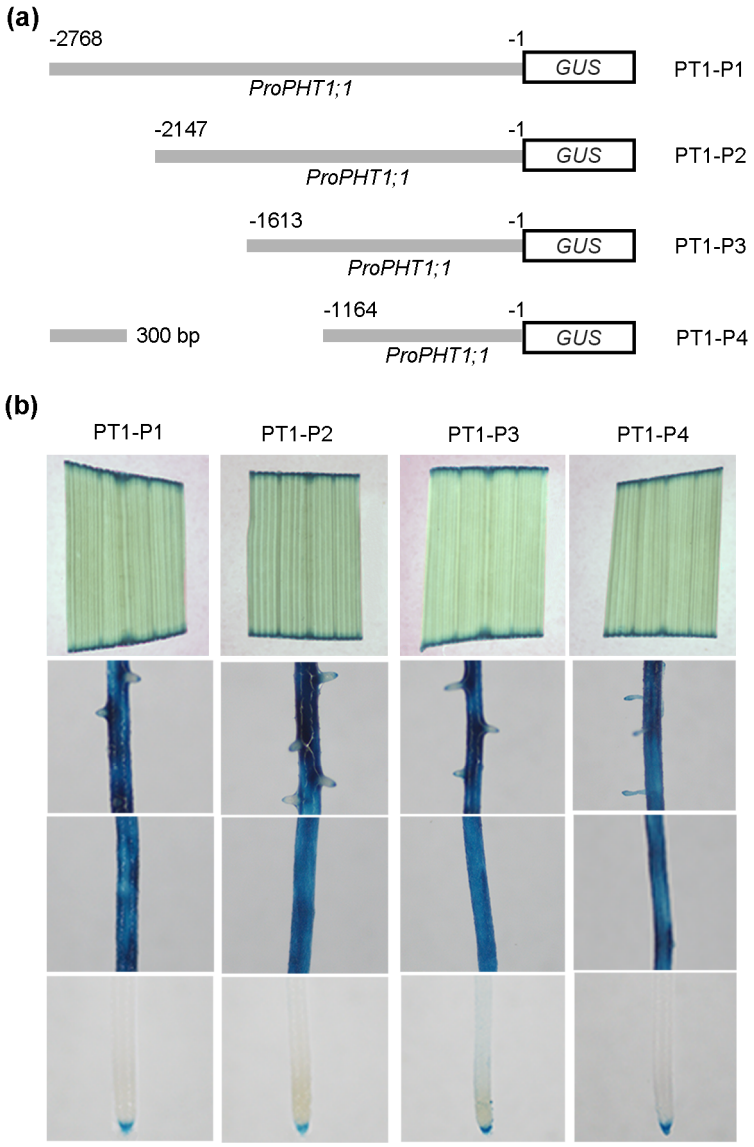

Fig. S3 Histochemical localization of different truncations of *PHT1;1* promoter fused with *GUS* reporter gene in transgenic plants. (a) Schematic diagram of truncated promoters of *PHT1;1.* The grey rectangle indicates the promoter region of *PHT1;1*. (b) Histochemical GUS staining analysis of different truncations of *PHT1;1* promoters fused with *GUS* reporter gene in transgenic plants.

Fig. S4

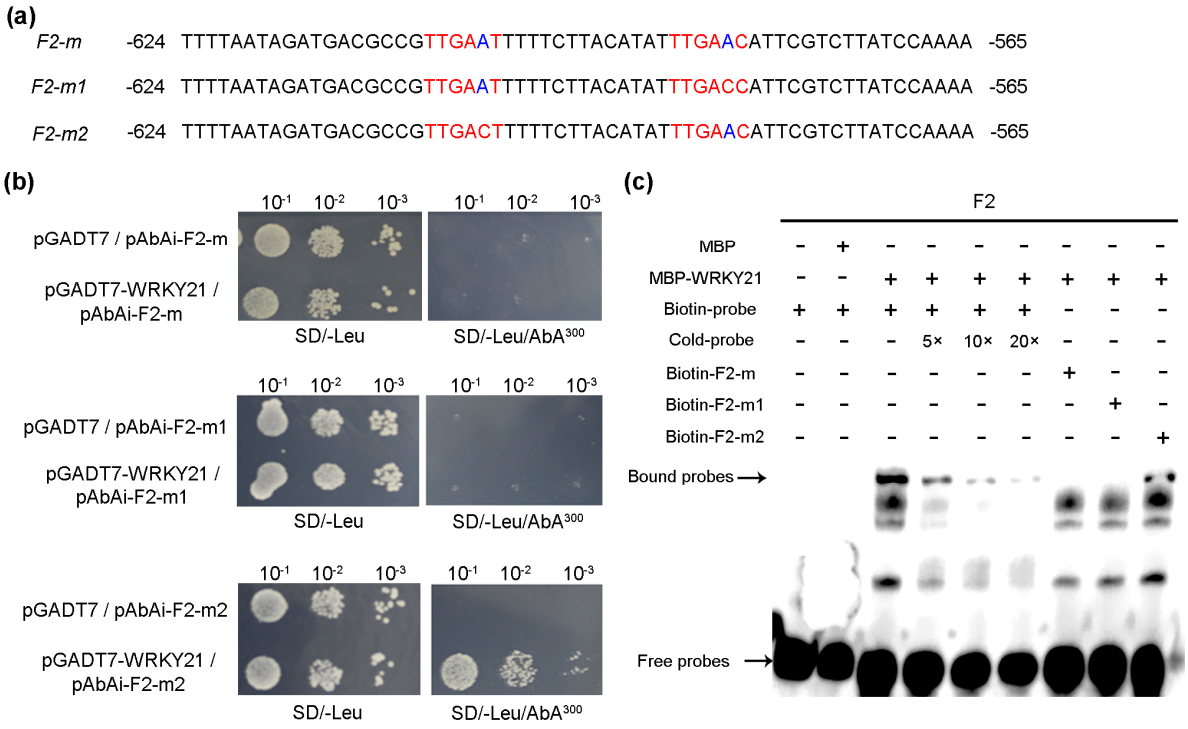

Fig. S4 WRKY21 binds to F2 fragment of *PHT1;1* promoter through the specific W-box. (a) The sequences of synthesized DNA probes used in Y1H and EMSA analysis. F2-m indicates the mutated F2 shown in Fig. 2b, with 1 bp mutation in both Wy and Wz (corresponding to the sequence in blue). F2-m1 and F2-m2 indicate the mutated F2 with 1 bp mutation in either Wz or Wy. (b) WRKY21 binds to F2 fragment of *PHT1;1* promoter through Wz in Y1H assay. Yeast cells were transformed with a bait vector, containing a promoter fragment F2-m, F2-m1 or F2-m2 fused to *AUR1-C* reporter gene, and a prey vector, containing WRKY21 fused to a *GAL4* activation domain. Yeast cells were grown in liquid media to an OD_{600} of 1.0 and diluted in a 10× dilution series (10^{-1} to 10^{-3}). From each dilution, 5 µL was spotted on SD/-Leu media selecting for plasmids, and SD/-Leu supplemented with 300ng/mL aureobasidin A (AbA) selecting for interaction. (c) EMSA assay to test the binding of WRKY21 to F2 fragment of *PHT1;1* promoter through Wz. Each biotin-labeled probe was incubated with MBP-WRKY21 protein. An excess amount of unlabeled probes (cold probe) were only added to compete with labeled F2 DNA probes. Biotin-labeled probes alone or biotin-labeled probes incubated with MBP protein served as negative controls. The WRKY21-DNA complex (bound probes) and free DNA probes are indicated by black arrows, respectively.

Fig. S5

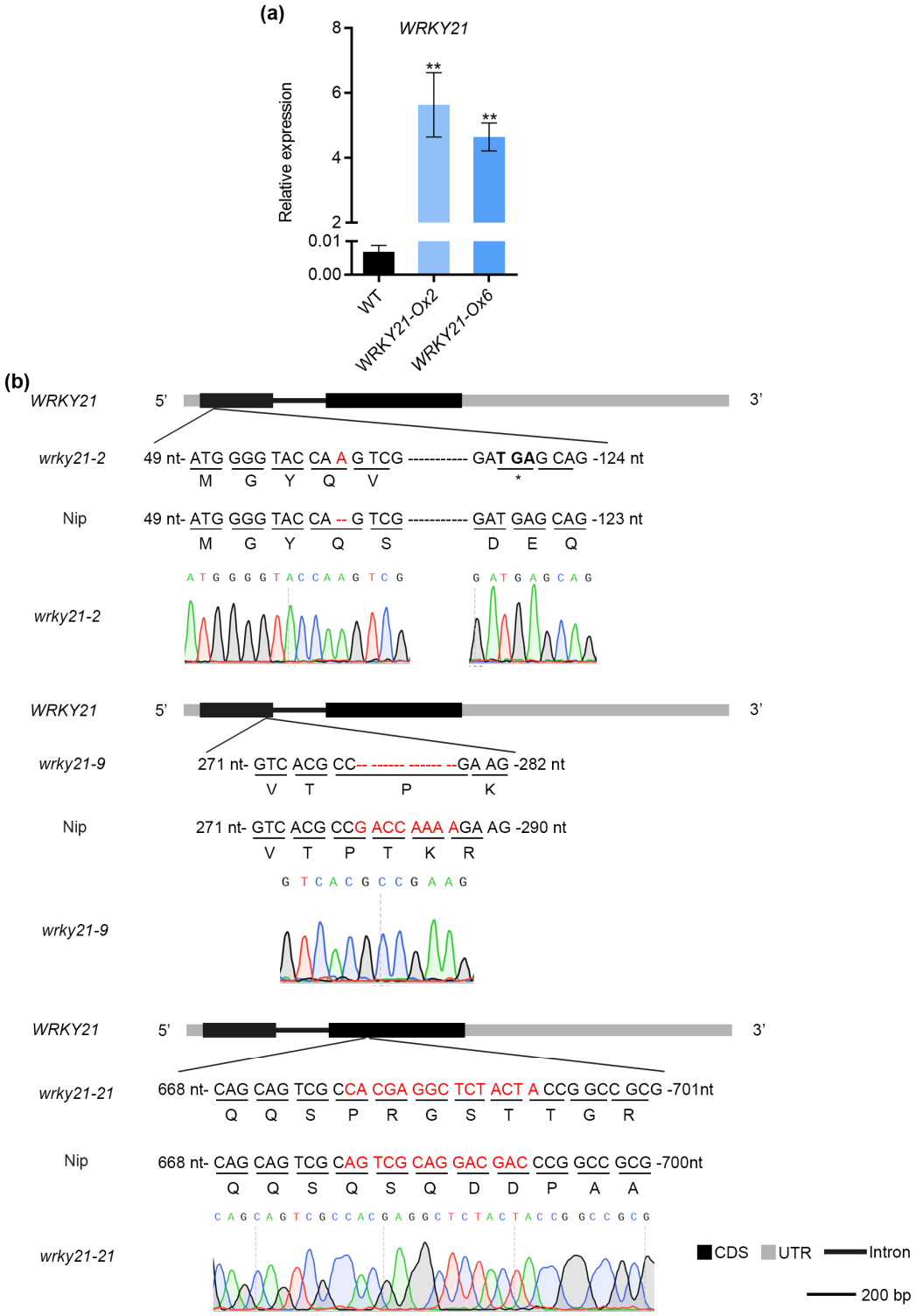

Fig. S5 Identification of *WRKY21* overexpression and mutant lines. (a) RT-qPCR analysis of the expression of *WRKY21* in *WRKY21* overexpression and wild-type plants. Rice seeds of *WRKY21* overexpression plants and wild-type plants were germinated in deionized H_2O and cultured

hydroponically in Pi-sufficient (200 μM Pi) condition. Plant roots were collected for RNA extraction and RT-qPCR analysis. The rice housekeeping gene *OsActin1* (LOC_Os03g50885) was used as an internal control. Error bars indicate SD (*n*=4). Data significantly different from the corresponding controls are indicated (**$P < 0.01$, Student's *t* test). (b) Identification of *wrky21* mutant lines. The gene structure of *OsWRKY21* along with the mutation sites of three independent lines are present in each panel (upper, middle and lower). The coding sequences (CDS), the untranslated regions (UTR) and the introns are indicated by black rectangles, grey rectangles and black bars, respectively. The red letters indicate mutations newly generated by the CRISPR-Cas9 system. The letters underlying the nucleotide sequences are amino acid sequences.

Fig. S6

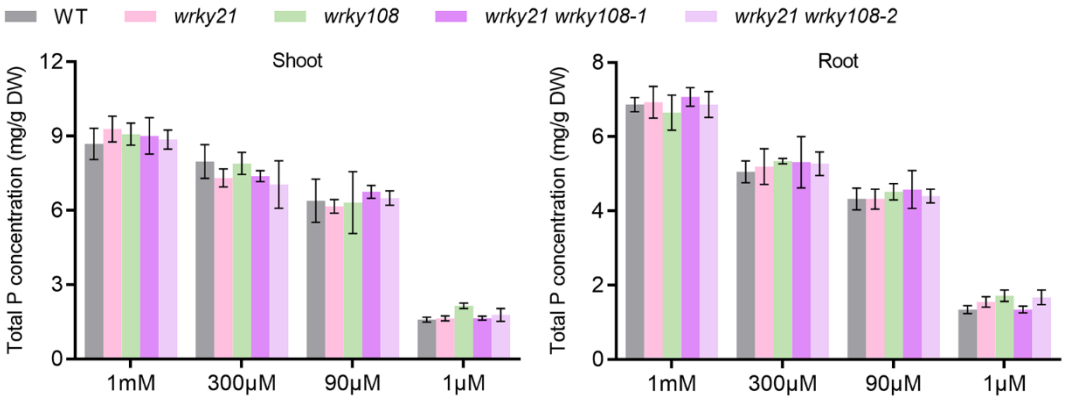

Fig. S6 Total P concentration of *wrky21*, *wrky108*, *wrky21 wrky108* mutants and wild-type plants under different Pi supplied hydroponic conditions. Plants were grown under the same conditions as that in Fig. 7. Total P concentration was measured in shoot (left) and Root (right). Error bars indicate SD (n = 4). DW, dry weight.

Fig. S7

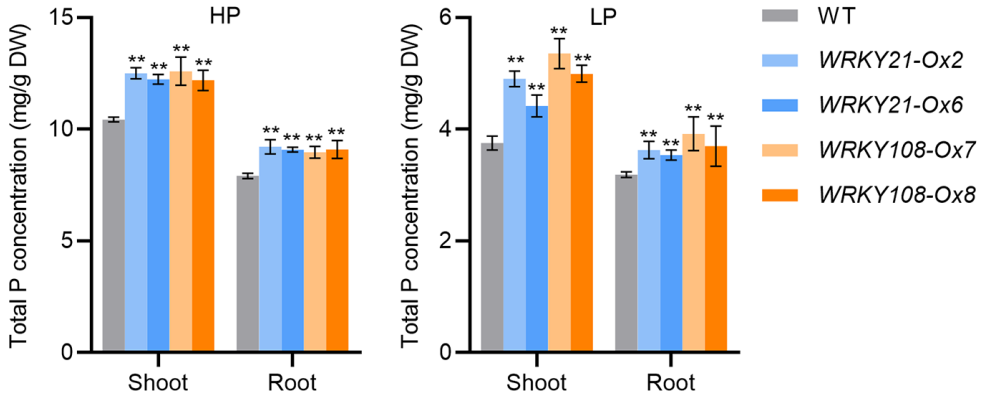

Fig. S7 Total P concentration of *WRKY21* and *WRKY108* overexpression lines and wild-type plants. Four-leaf-old seedlings were grown under half-strength Kimura B nutrient solution supplied with HP (200 μM Pi) or LP (10 μM Pi) until the seventh leaf blades were fully expanded. Total P concentration was measured in plants grown under HP (left) and LP (right) conditions. Plant shoot and root were collected for measurement. Error bars indicate SD (*n*=4). Data significantly different from the corresponding controls are indicated (**$P < 0.01$, Student's *t* test). DW, dry weight.

Fig. S8

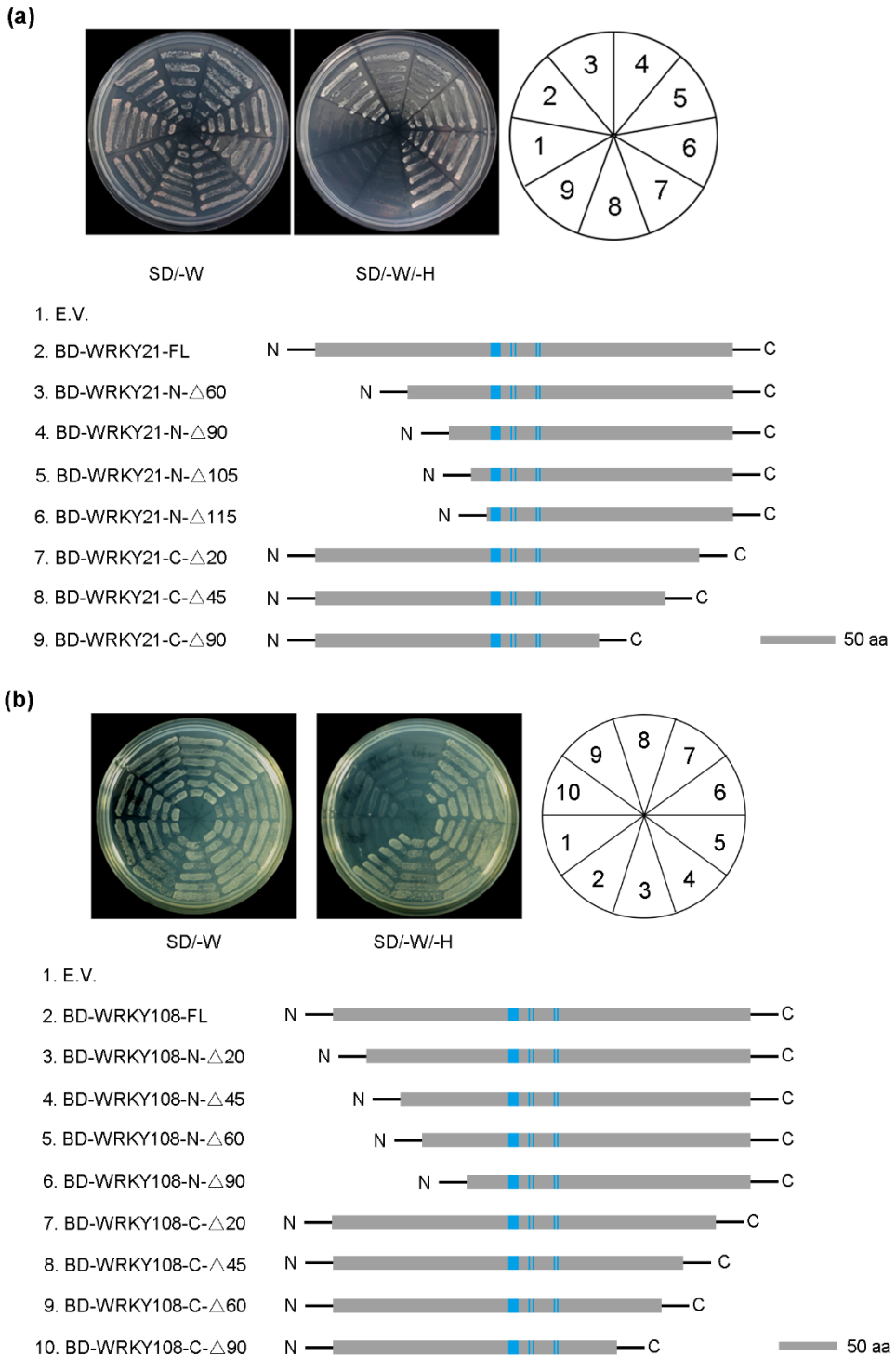

Fig. S8 Transactivation activity of the WRKY21 and WRKY108 proteins in yeast. (a) Deletion of 45 amino acids (236-280 aa) in C-terminal of WRKY21 protein abolished the transactivation activity of WRKY21. Schematic diagrams of the full-length and truncated WRKY21 constructs

was shown (lower panel), the grey rectangle indicates the amino acid peptide, the blue rectangles indicate WRKYGQK and zinc-finger motif. Empty vector (E.V.) was used as a negative control. Yeast strain Y2HGold was used in the transactivation activity analysis. The transformants were streaked on to the SD/-W or SD/-W/-H media, respectively (upper panel). SD/-W, -Trp; SD/-W/-H, -Trp-His. (b) Deletion of 45 amino acids (314-358 aa) in C-terminal of WRKY108 protein abolished the transactivation activity of WRKY108. Schematic diagrams of the full-length and truncated WRKY108 constructs was shown (lower panel), the grey rectangle indicates the amino acid peptide, the blue rectangles indicate WRKYGQK and zinc-finger motif. Empty vector (E.V.) was used as a negative control. Yeast strain Y2HGold was used in the transactivation activity analysis. The transformants were streaked on to the SD/-W or SD/-W/-H medium, respectively (upper panel). SD/-W, -Trp; SD/-W/-H, -Trp-His.

Fig. S9

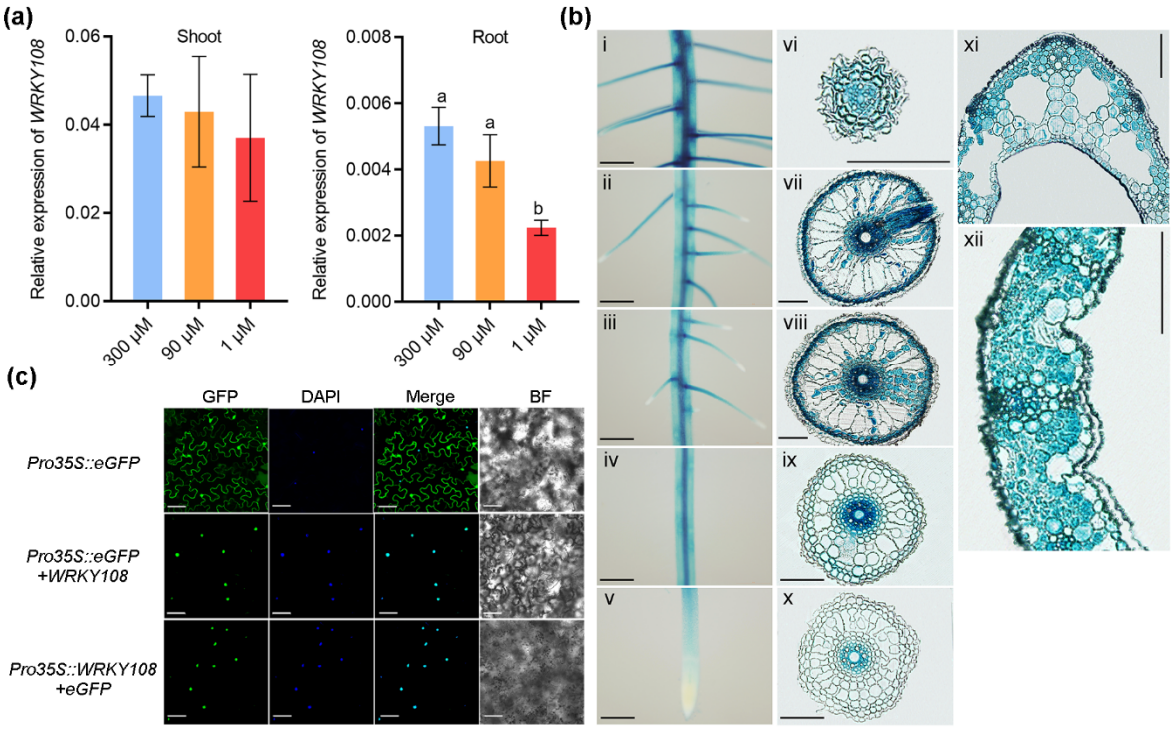

Fig. S9 Expression patterns and subcellular localization of WRKY108. (a) Expression of *WRKY108* in response to different Pi supplies. Rice seeds were germinated in deionized H_2O and supplied with 300 μM, 90 μM or 1 μM Pi. Plant shoots and roots were collected for from seedlings. RT-qPCR analysis was performed using the rice housekeeping gene *OsActin1* (LOC_Os03g50885) as an internal control. Values presented are the means±SD of biological replicates (n=4). Different letters indicate significant differences (P<0.05, Duncan's test). (b) Histochemical staining for GUS activity in transgenic plants expression a *ProWRKY108:GUS* fusion. Plants were grown hydroponically and supplied with sufficient Pi. (i-v) Different zones of primary root. (vi) Cross-sections of the basal part of lateral root. (vii-x) Cross-sections of root segment shown in ii(vii), iii(viii), iv(ix) and v(x). (xi) Cross-section of leaf sheath. (xii) Cross-section of leaf blade. Scale bars in i-v and xi-xii indicate 100 μm; bars in vi-x indicate 500 μm. (c) Subcellular localization of WRKY108. Fusion proteins of WRKY108:eGFP and eGFP:WRKY108 and eGFP along were expressed in the *N. benthamian* leaf epidermal cells by *A. tumefaciens*-mediated infiltration. The green signals indicate GFP, and the blue signals indicate cell nucleus that were specially stained with DAPI. Scale bars = 10 μM. BF, bright field.

Fig. S10

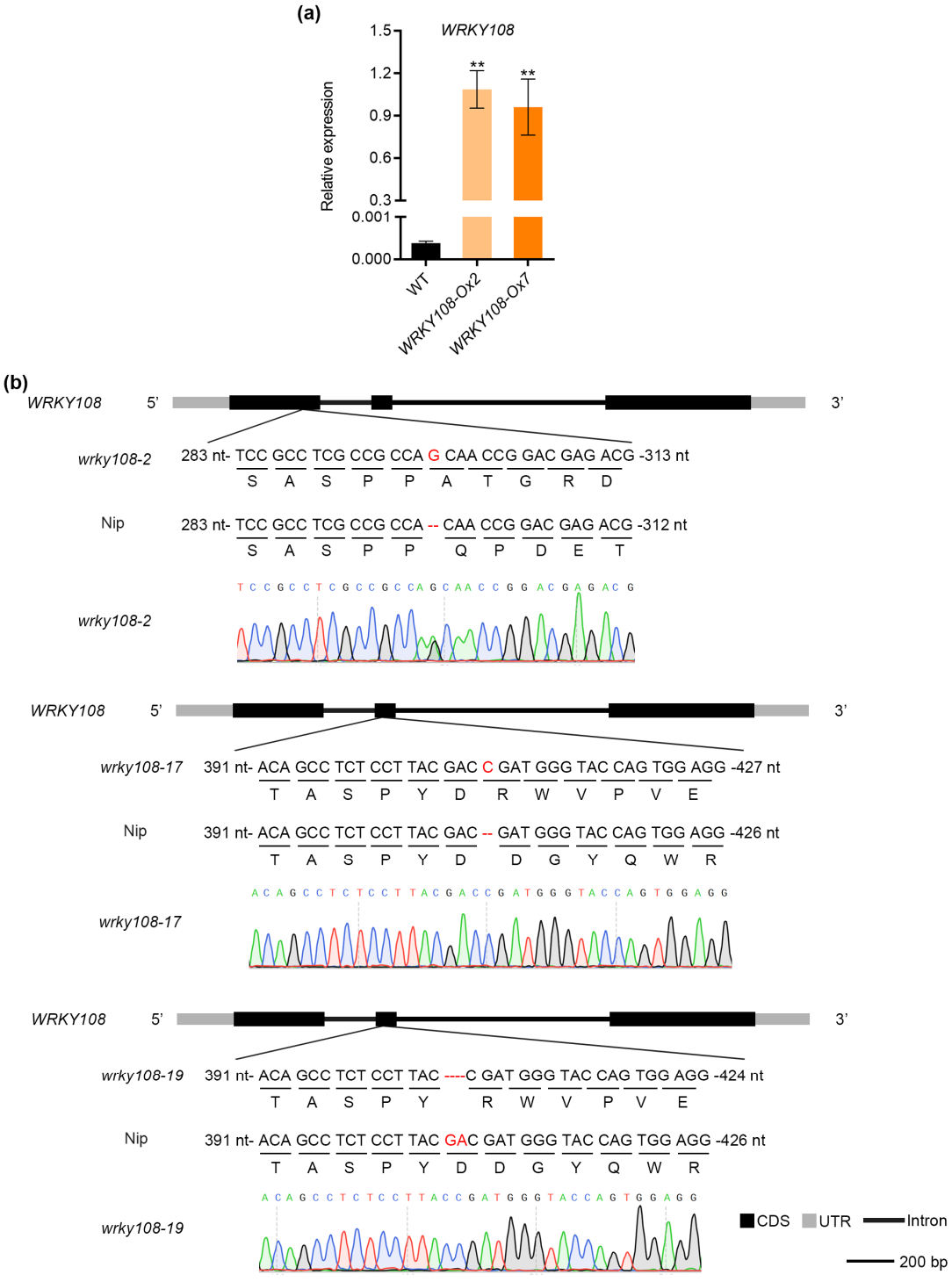

Fig. S10 Identification of *WRKY108* overexpression and mutant lines. (a) RT-qPCR analysis of the expression of *WRKY108* in *WRKY108* overexpression and wild-type plants. Rice seeds of *WRKY108* overexpression plants and wild-type plants were germinated in deionized H_2O and cultured hydroponically in Pi-sufficient (200 μM Pi) condition. Plant roots were collected for

RNA extraction and RT-qPCR analysis. The rice housekeeping gene *OsActin1* (LOC_Os03g50885) was used as an internal control. Error bars indicate SD (n=4). Data significantly different from the corresponding controls are indicated (**P < 0.01, Student's t test). (b) Identification of *wrky108* mutant lines. The gene structure of *WRKY108* along with the mutation sites of three independent lines are present in each panel (upper, middle and lower). The coding sequences (CDS), the untranslated regions (UTR) and the introns are indicated by black rectangles, grey rectangles and black bars, respectively. The red letters indicate mutations newly generated by the CRISPR-Cas9 system. The letters underlying the nucleotide sequences are amino acid sequences.

Fig. S11

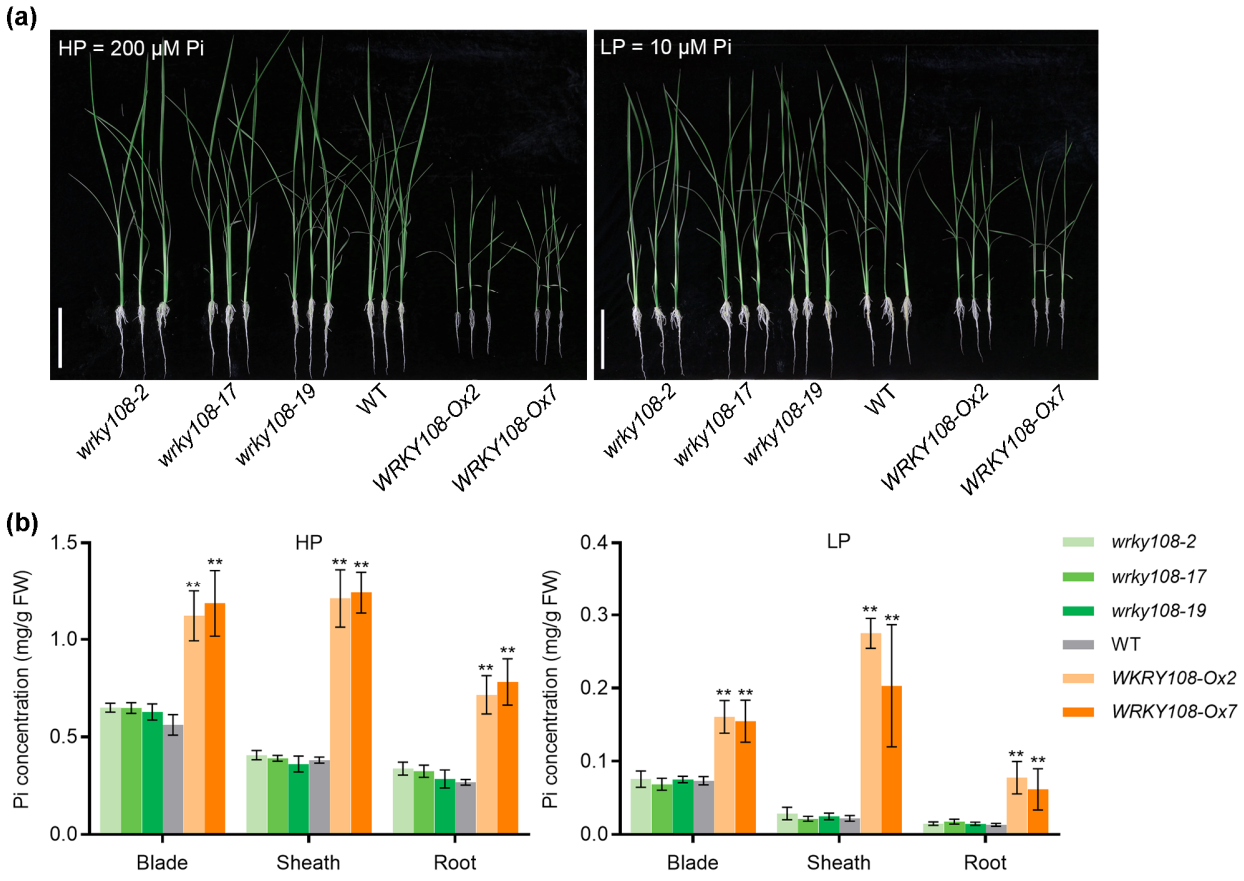

Fig. S11 WRKY108 positively affects phosphate (Pi) accumulation in rice. (a) The phenotype of *WRKY108* transgenic plants and wild-type plants grown under HP (200 μM Pi) and LP (10 μM Pi) hydroponic conditions. Scale bars = 10 cm. (b) Pi concentration analysis in leaf tissues and roots of lines (as above). Four-leaf-old seedlings were grown under full-strength Yoshida nutrient solution supplied with HP (200 μM Pi) or LP (10 μM Pi) until the seventh leaf blades were fully expanded. The Pi concentration was measured in plants grown under HP (left) and LP (right) conditions. Leaf blades, leaf sheaths and roots were collected for measurement. Error bars indicate SD (n=4). Data significantly different from the corresponding controls are indicated (**$P < 0.01$, Student's t test). FW, fresh weight.

Fig. S12

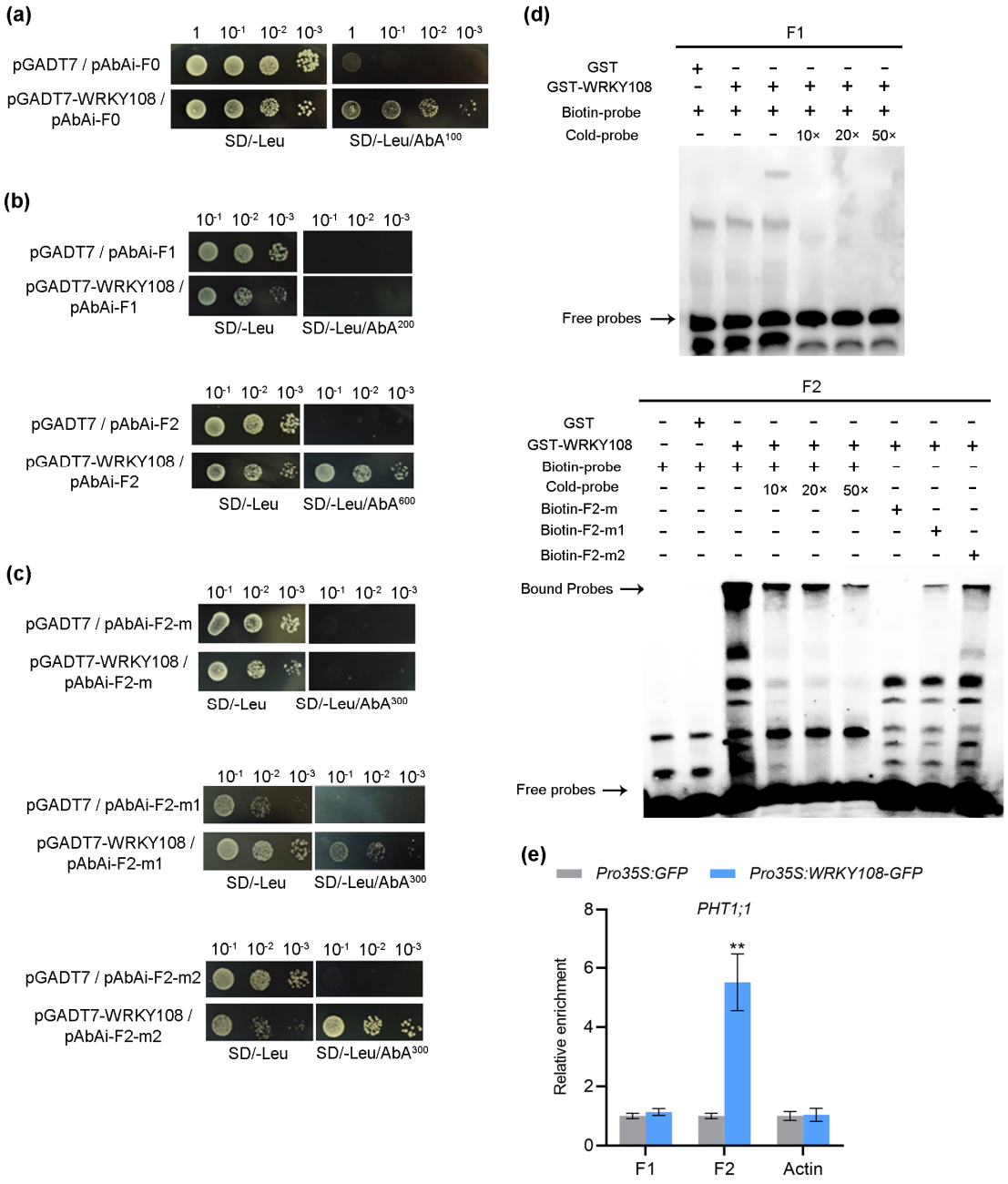

Fig. S12 WRKY108 binds to *PHT1;1* promoter region in *vivo* and in *vitro.* (a-c) WRKY108 binds to *PHT1;1* promoter region in the Y1H assay. Yeast cells were transformed with a bait vector, containing a promoter fragment F0, F1, F2, F2-m, F2-m1 or F2-m2 (the same as Fig. 2a, 2b, S3a) fused to *AUR1-C* reporter gene, and a prey vector, containing WRKY108 fused to a *GAL4* activation domain. Yeast cells were grown in liquid media to an OD_{600} of 1.0 and diluted in a 10× dilution series (10^{-1} to 10^{-3}). From each dilution, 5 µL was spotted on SD/-Leu media selecting

for plasmids, and SD/-Leu supplemented with aureobasidin A (AbA) selecting for interaction. (d) EMSA assay to test the binding of WRKY108 to *PHT1;1* promoter fragments. Each biotin-labeled probe was incubated with GST-WRKY108 protein. An excess amount of unlabeled probes (cold probe) were only to compete with biotin-labeled probes. Biotin-labeled probes alone or biotin-labeled probes incubated with GST protein served as negative controls. The WRKY108-DNA complex (bound probes) and free DNA probes are indicated by black arrows, respectively. (e) ChIP-qPCR analysis of WRKY21 binding to the *PHT1;1* promoter region. Rice seeds of *Pro35S:GFP* and *Pro35S:WRKY108-GFP* transgenic plants were germinated in deionized H_2O and supplied with sufficient Pi. The whole plants were harvested for ChIP analysis. Enriched DNA fragments (F1 and F2) in the *PHT1;1* promoter were quantified using RT-qPCR. Values represent means±SD (*n*=3). The enrichment was calculated as the ratio of immunoprecipitation to Input. Data significant different from the control are indicated. P values were determined by Student's *t* test. *Pro35S:WRKY108:GFP* versus *Pro35S:GFP*; $^{**}P < 0.01$.

Fig. S13

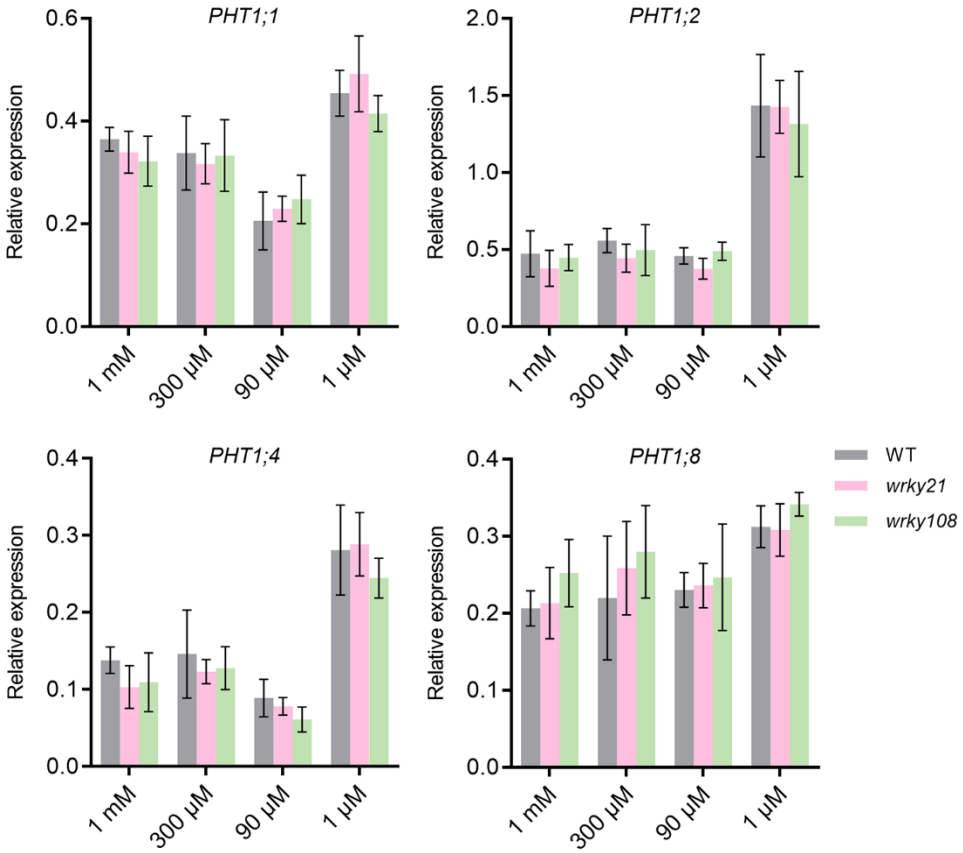

Fig. S13 RT-qPCR analysis of effect of *WRKY21/WRKY108* mutation on expression of four PHT1 genes. Root samples were collected from the same plants as that in Fig. 7 for RNA extraction and RT-qPCR analysis. The rice housekeeping gene *OsActin1* (LOC_Os03g50885) was used as an internal control. Values represent means ± SD of biological replicates (*n*=4).

Fig. S14

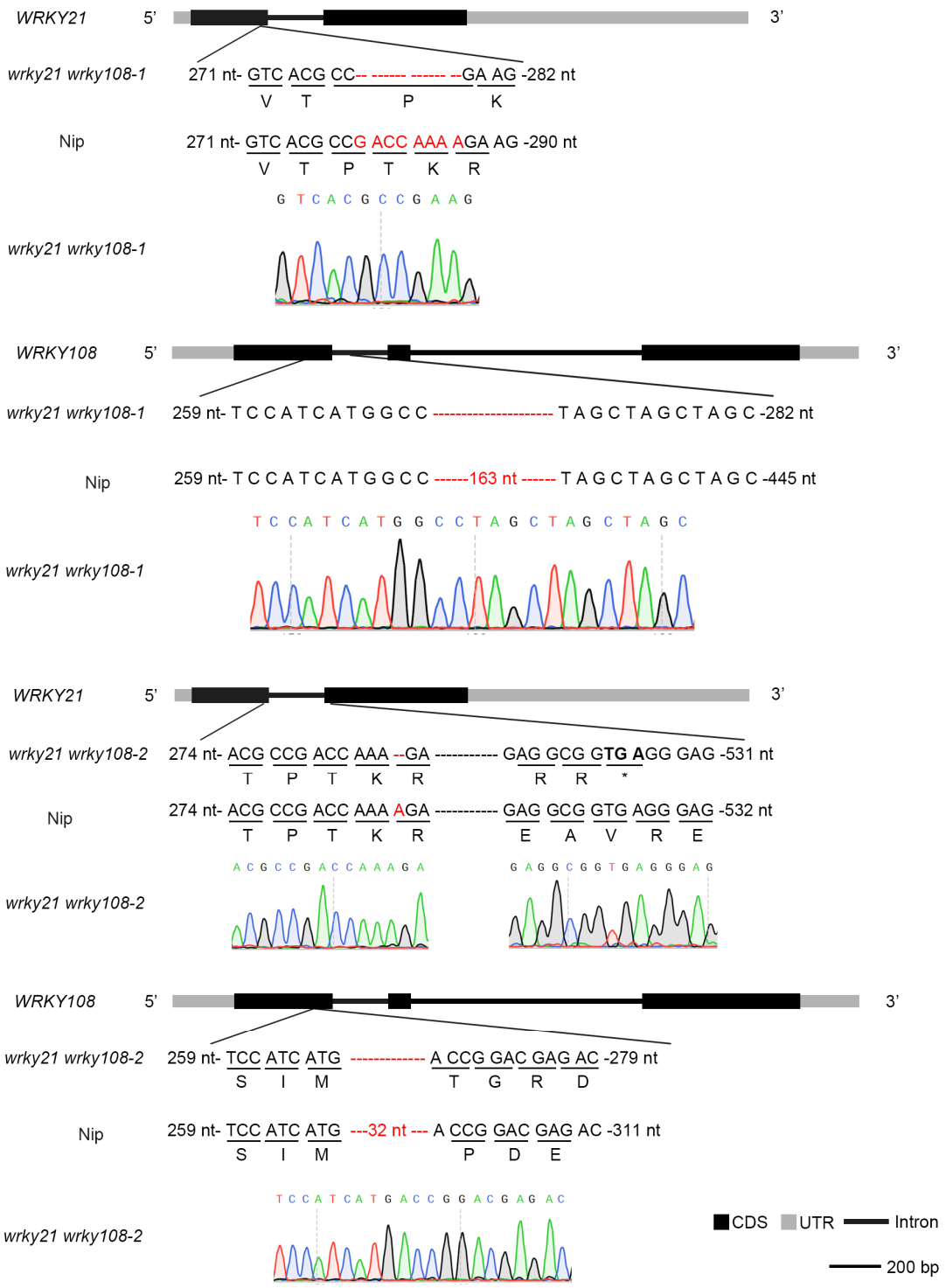

Fig. S14 Identification of *wrky21 wrky108* double mutants. The gene structures of *WRKY21* and *WRKY108* along with the mutation sites of two independent lines are present in each panel (upper and lower). The coding sequences (CDS), the untranslated regions (UTR) and the introns

are indicated by black rectangles, grey rectangles and black bars, respectively. The red letters indicate mutations newly generated by the CRISPR-Cas9 system. The letters underlying the nucleotide sequences are amino acid sequences.

Fig. S15

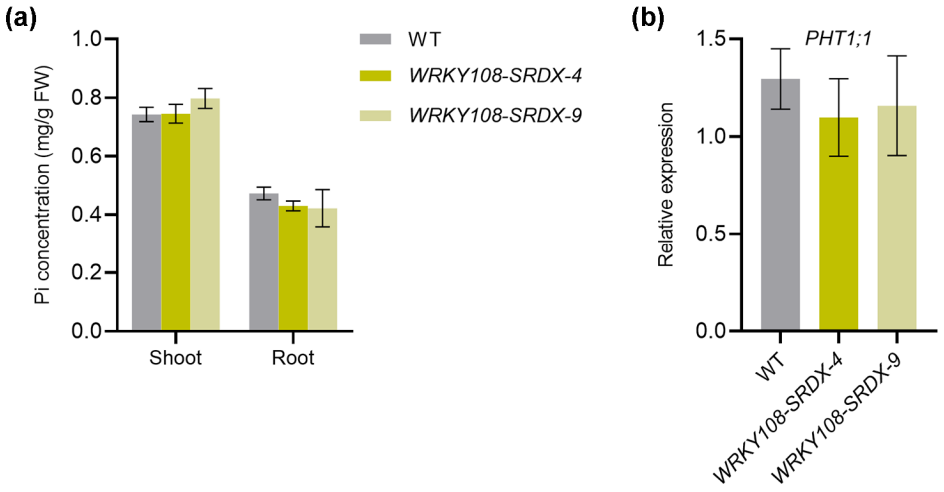

Fig. S15 Pi concentration and RT-qPCR analysis of *PHT1;1* in *Pro35S:WRKY108-SRDX* transgenic plants and wild-type plants. (a) The cellular Pi concentration analysis of *Pro35S:WRKY108-SRDX* transgenic plants and wild-type plants. Four-leaf-old seedlings were grown under half-strength Kimura B nutrient solution supplied 90 μM Pi until the seventh leaf blades were fully expanded. The Pi concentration was measured in shoot and Root. Error bars indicate SD (*n*=4). FW, fresh weight. (b) RT-qPCR analysis of *PHT1;1* in *Pro35S:WRKY108-SRDX* transgenic plants and wild-type plants. Plant roots were collected for RNA extraction and RT-qPCR analysis. The rice housekeeping gene *OsActin1* (LOC_Os03g50885) was used as an internal control. Error bars indicate SD (*n*=4).

Fig. S16

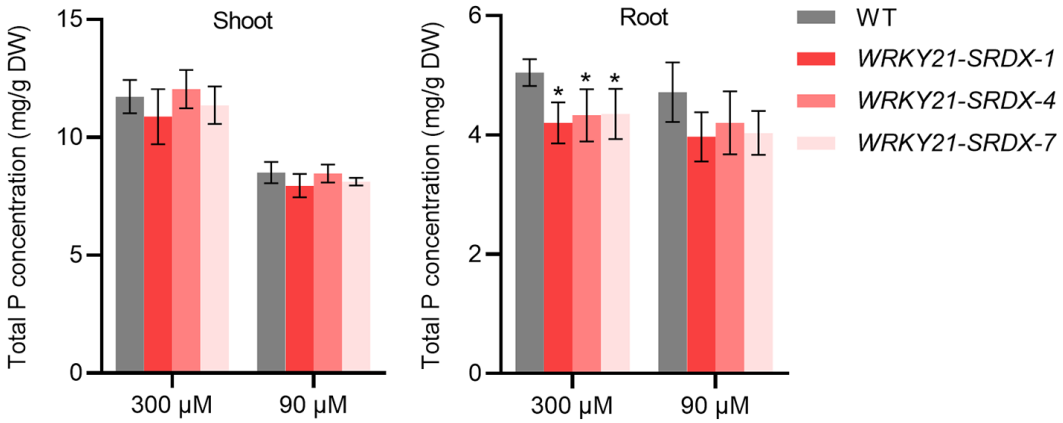

Fig. S16 Total P concentration of *Pro35S:WRKY21-SRDX* transgenic lines and wild-type plants. Plants were grown under the same conditions as that in Fig. 8. Total P concentration was measured in shoot (left) and Root (right). Error bars indicate SD (n=4). Data significantly different from the corresponding controls are indicated (*P < 0.05, Student's t test). DW, dry weight.

Fig. S17

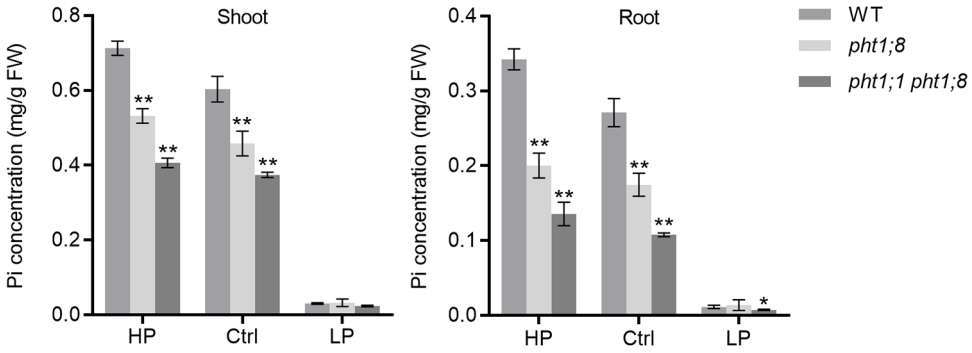

Fig. S17 Pi concentration of *pht1;8*, *pht1;1 pht1;8* mutants and wild-type plants. *pht1;8* and *pht1;1 pht1;8* mutant plants were grown along with *pht1;1* mutants and wild-type plants under the same conditions as that in Fig. 1. Pi concentration was measured in shoot (left) and Root (right). Error bars indicate SD (n=4). Data significantly different from the corresponding controls are indicated (**P* < 0.05, ***P* < 0.01, Student's *t* test). FW, fresh weight.

Fig. S18

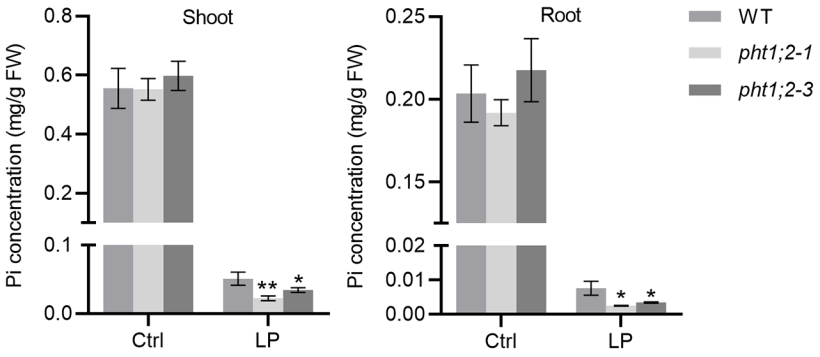

Fig. S18 Pi concentration of *pht1;2* mutants and wild-type plants. Four-leaf-old seedlings were grown under half-strength Kimura B nutrient solution supplied with Ctrl (90 µM Pi) or LP (1 µM Pi) until the seventh leaf blades were fully expanded. The Pi concentration was measured in shoot (left) and Root (right). Error bars indicate SD (n=4). Data significantly different from the corresponding controls are indicated (*$P < 0.05$, **$P < 0.01$, Student's *t* test). FW, fresh weight.

Fig. S19

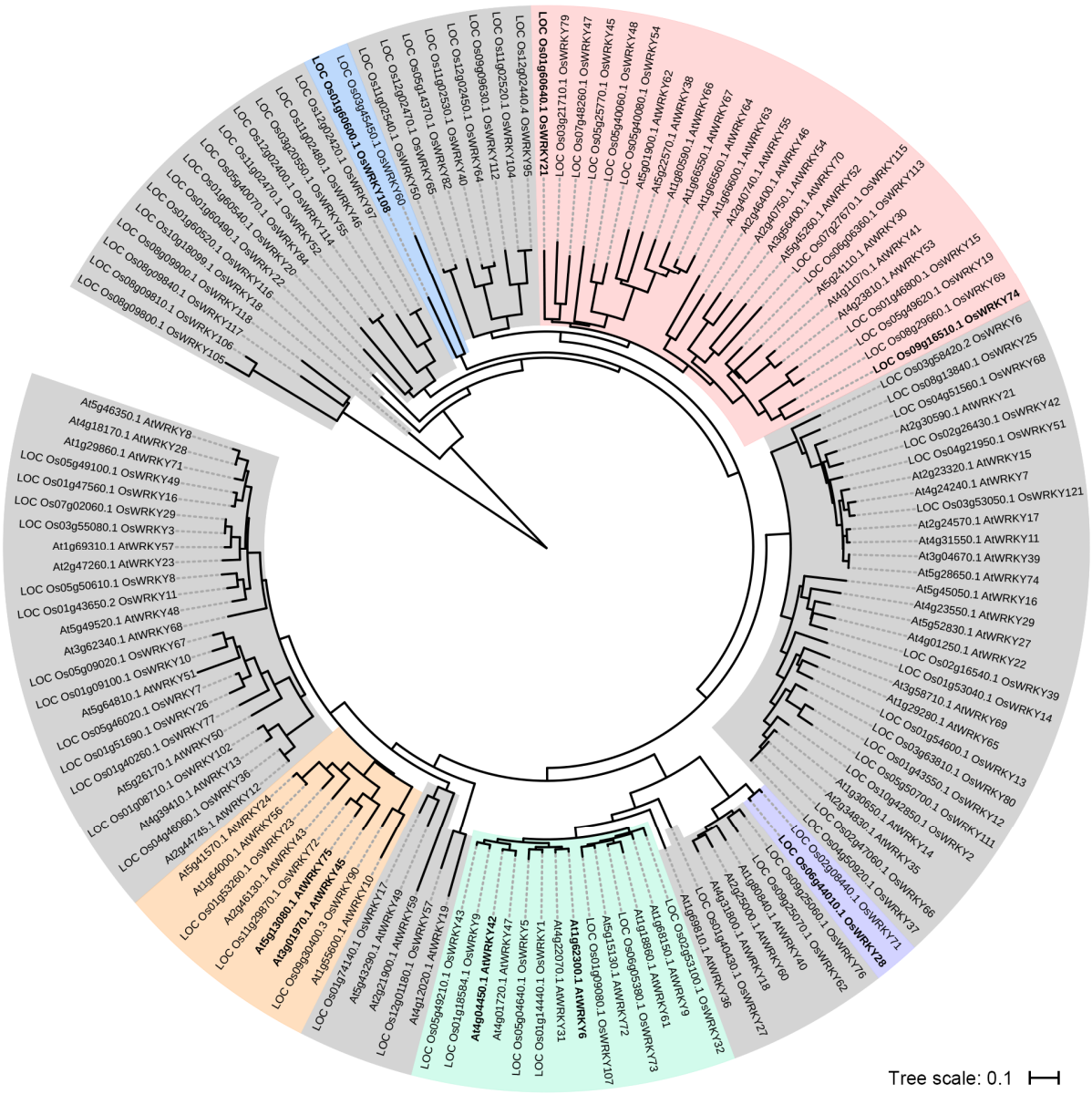

Fig. S19 Phylogenetic analysis of the Group II and Group III type WRKY transcription factors from rice and *A. thaliana* according to Ross *et al*. (2007). The phylogenetic tree was generated using the WRKY zinc finger domain of the candidates. The full-length amino acid sequences were download from Center for Bioinformatics, Peking University (http://atrm.cbi.pku.edu.cn). The gene names of six reported WRKY transcription factors involved in Pi signaling and *OsWKRY21* and *OsWRKY108* are highlighted in bold, and the clades to which these eight members belong are highlighted in blue, red, purple, green and orange.

Fig. S20

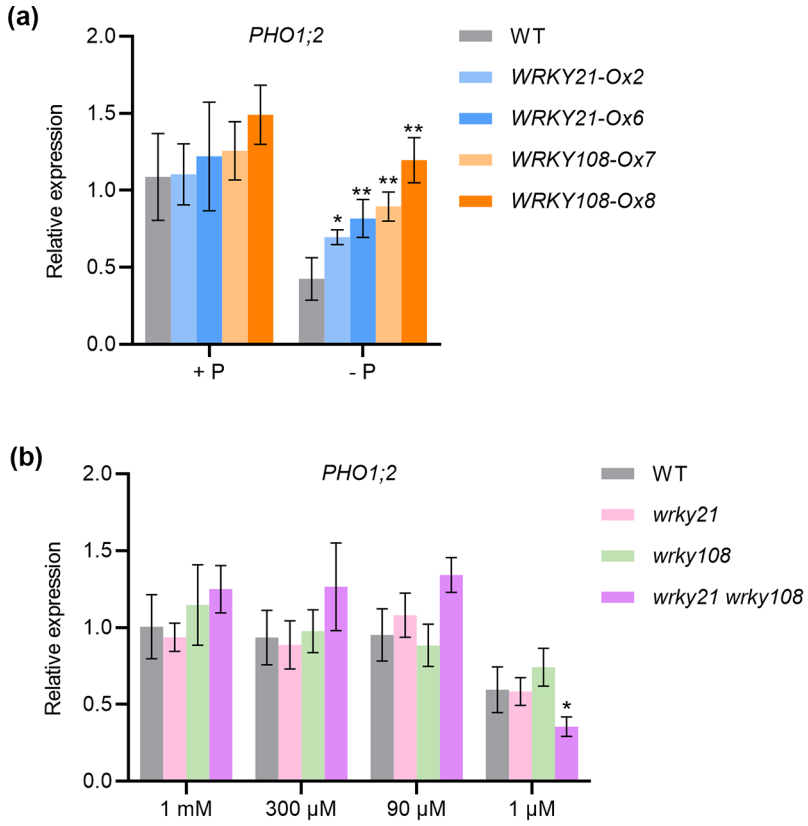

Fig. S20 Effect of *WRKY21* and *WRKY108* overexpression or mutation on the transcript level of *PHO1;2* in rice. (a) RT-qPCR analysis of *PHO1;2* in *WRKY21* and *WRKY108* overexpression lines and wild-type plants. The same templates as that in Fig. 6 were used for RT-qPCR analysis. (b) RT-qPCR analysis of *PHO1;2* in *wrky21*, *wrky108* single mutant, *wrky21 wrky108* double mutant and wild-type plants. The same templates as that in Fig. 8c were used for RT-qPCR analysis. Error bars indicate SD (n=4). Data significantly different from the corresponding controls are indicated (*P < 0.05, **P < 0.01, Student's t test).

Fig. S21

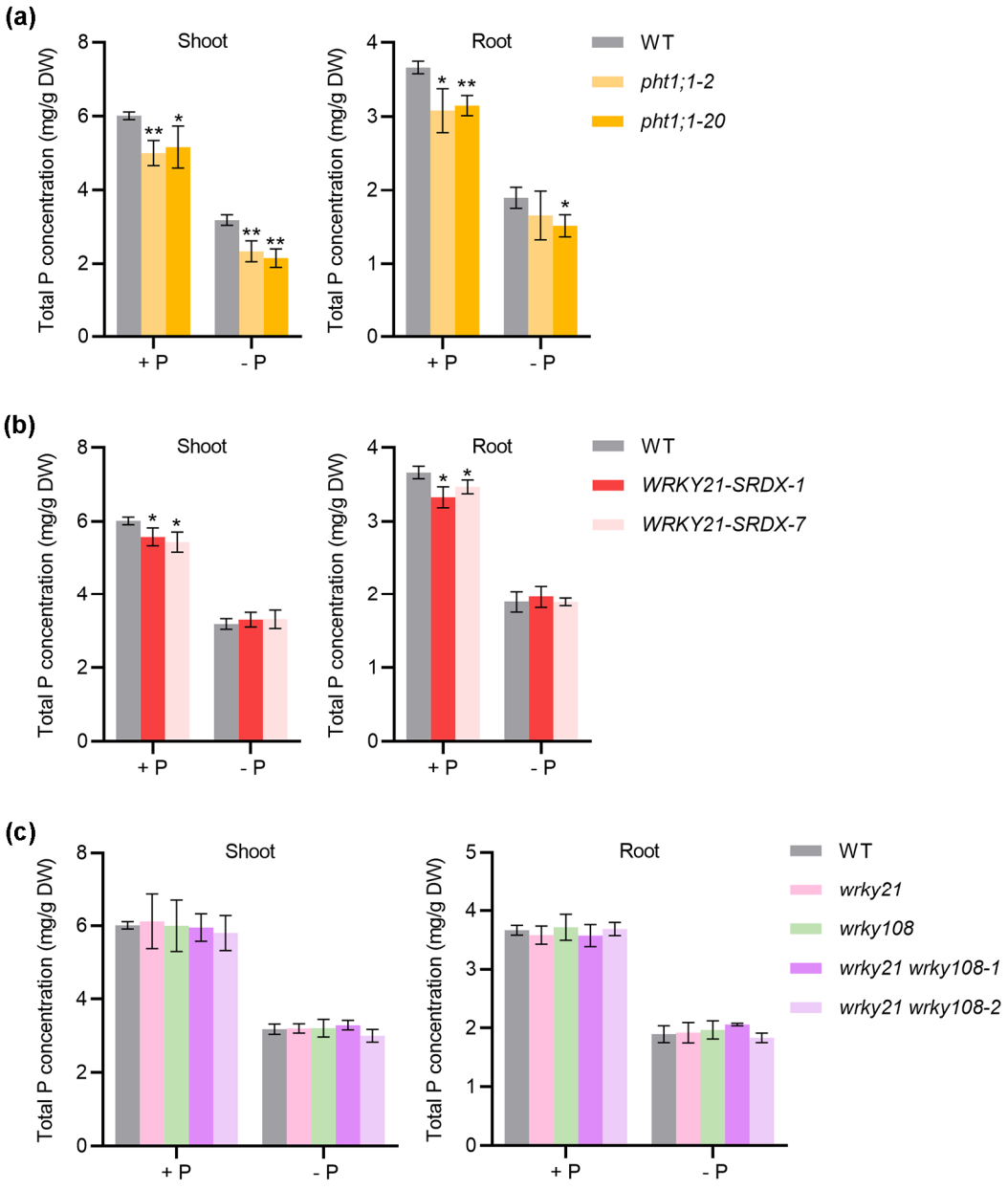

Fig. S21 Total P concentration of wild-type plants, *pht1;1*, *wrky21*/*wrky108* single mutants, *wrky21 wrky108* double mutants and *WRKY21-SRDX* transgenic lines, at different Pi levels in a soil-based experiment. (a) Total P concentration of *pht1;1* mutants and wild-type plants. (b) Total P concentration of *WRKY21-SRDX* transgenic lines and wild-type plants. (c) Total P concentration of *wrky21* and *wrky108* mutants, *wrky21 wrky108* double mutants and wild-type plants. Three-leaf-old seedlings were grown in a pot filled with soil at +P (200 mg fertilizer Pi kg^{-1} soil) or –P (0 mg fertilizer Pi kg^{-1} soil) condition until eighth leaf blades were fully expanded.

Plant shoots and roots were collected for total P concentration measurement. Wild-type plants under +P or –P condition in (a-c) were the same. Error bars indicate SD (n=4). Data significantly different from the corresponding controls are indicated (*$P < 0.05$, **$P < 0.01$, Student's *t* test). DW, dry weight.

Table S1 Primers used for RT-qPCR analysis.

Primers	Sequence(5’ to 3’)	Amplicon/bp
Actin-qRT-F	GAGTCTGGCCCATCCATTGT	60
Actin- qRT-R	AGCATTCTTGGGTCCGAAGA	
WRKY21-3’-qRT-F	AGTCTTTGCAAAACGCACAAAA	56
WRKY21-3’-qRT-R	CGCAAACGCGGGAAATT	
WRKY108-3’-qRT-F	TCAGGCGCGCATCGAT	57
WRKY108-3’-qRT-R	CACCGCGACTCGTCATATGT	
WRKY21-ORF-qRT-F	GGTCTCCCTCCTTCTCGTC	131
WRKY21-ORF-qRT-R	GGAGTTGATGACGAATTCCC	
WRKY108-ORF-qRT-F	CGTCCCGCGAAAGAGATTATT	69
WRKY108-ORF-qRT-R	TGACCGTACTTCCTCCACTGGTA	
PHT1;1-qRT-F	CGCTTCCGTACGAGTGGTAGT	146
PHT1;1-qRT-R	GGTTCTTTCAAATCCAGGGAAA	
PHT1;2-qRT-F	GACGAGACCGCCCAAGAAG	74
PHT1;2-qRT-R	TTTTCAGTCACTCACGTCGAGAC	
PHT1;4-qRT-F	TATTGCGGCTTAGATTGCATTAG	72
PHT1;4-qRT-R	TCCAAATCAAATGGGCACTAAG	
PHT1;8-qRT-F	AGAAGGCAAAAGAAATGTGTGTTAAAT	114
PHT1;8-qRT-R	AAAATGTATTCGTGCCAAATTGCT	
ChIP-Actin-F	TTCCCGTGCTTTGTTGTCC	100
ChIP-Actin-R	TATCAACCGCAAGCGTCCA	
ChIP-F1-F	ACGGCAACCATTAGAGTTGA	100
ChIP-F1-R	CCGCTGAACTGACTGTTCTC	
ChIP-F2-F	AACAAACACGGCATTCAGCT	149
ChIP-F2-R	GTGTGATTTATATCTTATAC	

Table S2 Primers used for constructs for generating transgenic plants.

Primer	Sequence(5' to 3')	Note	Construct/ Usage
For Overexpression constructs of WRKY21 and WRKY108			
1305-WRKY21-F	TTggtaccATGGCGATGCTGGGGAGCT	*Bam*HI	pCAMBIA1305-GUSPlus
1305-WRKY21-R	TTctgcagTCAGAGGGAGTTGATGACGAAT	*Pst*I	
1305-WRKY108-F	TTgagctcATGCAGGCGCAATCCCGCCTC	*Sac*I	pCAMBIA1305-GUSPlus
1305-WRKY108-R	TTtctagaTTAATTAATTAGATCAAAACAG	*Xba*I	
For promoter-*GUS* expression constructs of WRKY21 and WRKY108			
1300-WRKY21-F	TTaagcttGTCATCTTGGGATTTATGTTTG	*Hind*III	pCAMBIA1300-GN
1300-WRKY21-R	TTggtaccGCTCCTCACTCTCGCACGACA	*Kpn*I	
1300-WRKY108-F	TTaagcttGCCTAGCTCGACCACCTC	*Hind*III	pCAMBIA1300-GN
1300-WRKY108-R	TTggatccGGTTCGTGTTGTTCGCTTCG	*Kpn*I	
1300-PT1-P1-F	TTtctagaGAAAATAGCAGCGAATTTGTT	*Xba*I	pCAMBIA1300-GN
1300-PT1-P1-R	TTggtaccGGCTTCCCAACTCTTTGAGCT	*Kpn*I	
1300-PT1-P2-F	TTtctagaTTCGTACGCCGTCATCGTCC	*Xba*I	pCAMBIA1300-GN
1300-PT1-P2-R	TTggtaccGGCTTCCCAACTCTTTGAGCT	*Kpn*I	
1300-PT1-P3-F	TTtctagaACGGCAACGGAGTTCTGGCG	*Xba*I	pCAMBIA1300-GN
1300-PT1-P3-R	TTggtaccGGCTTCCCAACTCTTTGAGCT	*Kpn*I	
1300-PT1-P4-F	TTtctagaCGTCATTCGCGGGGAGTAGTT	*Xba*I	pCAMBIA1300-GN
1300-PT1-P4-R	TTggtaccGGCTTCCCAACTCTTTGAGCT	*Kpn*I	
For CRISPR/Cas9 mutation of *PHT1;1*, *WRKY21* and *WRKY108*, and identification of mutants			
PT1-SP1-F	GGCAGTGACGGCGGCCGACACGTT		pOs-sgRNA pH-Ubi-Cas9-7
PT1-SP1-R	AAACAACGTGTCGGCCGCCGTCAC		
PT1-SP2-F	GGCACTCGGACGCAAGAGCGTGTA		
PT1-SP2-R	AAACTACACGCTCTTGCGTCCGAG		

WRKY21-SP1-F	GGCAGATGACGATGGGGTACCAGT		
WRKY21-SP1-R	AAACACTGGTACCCCATCGTCATC		
WRKY21-SP2-F	GGCACCGTCACGCCGACCAAAAGA		pOs-sgRNA pH-Ubi-Cas9-7
WRKY21-SP2-R	AAACTCTTTTGGTCGGCGTGACGG		
WRKY21-SP3-F	GGCAGTCGCAGTCGCAGGACGACC		
WRKY21-SP3-R	AAACGGTCGTCCTGCGACTGCGAC		
WRKY108-SP1-F	GGCAGGCGTCTCGTCCGGTTGTGG		
WRKY108-SP1-R	AAACCCACAACCGGACGAGACGCC		pOs-sgRNA pH-Ubi-Cas9-7
WRKY108-SP2-F	GGCAACAGCCTCTCCTTACGACGA		
WRKY108-SP2-R	AAACTCGTCGTAAGGCGAGGCTGT		
Cas9-PCR-F	ACAAGGGCAGGGATTTCG		Identification of transgenic plants
Cas9-PCR-R	ACTGGTGGATGAGGGTGGC		
PT1-PCR-F	CTGTGCCCCTGCTCTGCTTT		Identification of *pht1;1* mutants
PT1-PCR-R	GTTGTCGGAGTAGGACGGCG		
WRKY21-PCR-F	GGGGCGGGGTTCTATAAATACG		Identification of *wrky21* mutants
WRKY21-PCR-R	GGAGTTGATGACGGCAGGTGGC		
WRKY108-PCR-F	GAGCGGTGGCATTAGCAGGT		Identification of *wrky108* mutants
WRKY108-PCR-R	TTGTCTCTTCCGTCCCCGGT		
For constructs of transgenic plants for ChIP assay			
1305-PA-F	cTTAATTAAGGTACCCGGGGATCCTCTAGAGT CGACGGCGCGCCctgca	*Sac*I	pCAMBIA1305-GUSPlus
1305-PA-R	gGGCGCGCCGTCGACTCTAGAGGATCCCCG GGTACCTTAATTAAgagct	*Pst*I	
1305-GFP-F	TTttaattaaATGGTGAGCAAGGGCGAGGAG	*Pac*I	pCAMBIA1305-GUSPlus
1305-GFP-R	TTggcgcgccTCACTTGTACAGCTCGTCCAT	*Asc*I	
1305-WRKY21+GFP-F	TTttaattaaATGGCGATGCTGGGGAGCT	*Pac*I	pCAMBIA1305-GUSPlus
1305-WRKY21+GFP-R	TTggcgcgccTCACTTGTACAGCTCGTCCAT	*Asc*I	

1305-WRKY108+GFP-F	TTttaattaaATGCAGGCGCAATCCCGCCTC	*Pac*I	pCAMBIA1305-GUSPlus
1305-WRKY108+GFP-R	TTggcgcgccTCACTTGTACAGCTCGTCCAT	*Asc*I	
For constructs of Chimeric repressors of WRKY21 and WRKY108			
WRKY21-SRDX-F	GTCATCAACTCCCTCctgcagCTCGACCTGGACCTCGAGCTGCGGCTCGGCTTCGCGTGActgcagGTCCGCAAAAATCAC	One step cloning	pCAMBIA1305-WRKY21
WRKY21-SRDX-R	GTGATTTTTGCGGACctgcagTCACGCGAAGCCGAGCCGCAGCTCGAGGTCCAGGTCGAGctgcagGAGGGAGTTGATGAC		
WRKY108-SRDX-F	TTTGATCTAATTAATtctagaCTCGACCTGGACCTCGAGCTGCGGCTCGGCTTCGCGTAAtctagaGTCGACCTGCAGGTC	One step cloning	pCAMBIA1305-WRKY108
WRKY108-SRDX-R	GACCTGCAGGTCGACtctagaTTACGCGAAGCCGAGCCGCAGCTCGAGGTCCAGGTCGAGtctagaATTAATTAGATCAAA		

Table S3 Primers used for constructs for subcellular location, Y1H, EMSA, Y2H, BiFC and pull-down.

Primer	Sequence(5' to 3')	Note	Construct/ Usage
For subcellular location of WRKY21 and WRKY108			
WRKY21+GFP-F	TTagatctATGGCGATGCTGGGGAGCT	*Bgl*II	pSAT6A-EGFP-N1
WRKY21+GFP-R	TTcccgggGAGGGAGTTGATGACGAAT	*Xma*I	
GFP+WRKY21-F	TTagatctATGGCGATGCTGGGGAGCT	*Bgl*II	pSAT6-EGFP-C1
GFP+WRKY21-R	TTggatccTCAGAGGGAGTTGATGACGAAT	*Bam*HI	
WRKY108+GFP-F	TTagatctATGCAGGCGCAATCCCGCCTC	*Bgl*II	pSAT6A-EGFP-N1
WRKY108+GFP-R	TTcccgggATTAATTAGATCAAAACAG	*Xma*I	
GFP+WRKY108-F	TTagatctATGCAGGCGCAATCCCGCCTC	*Bgl*II	pSAT6-EGFP-C1
GFP+WRKY108-R	TTggatccTTAATTAATTAGATCAAAACAG	*Bam*HI	
For yeast one-hybrid assay			
pAbA-F0-F	TTgagctcCGTCATTCGCGGGGAGTAGTT	*Sac*I	pAbA Vector
pAbA-F0-R	TTctcgagCAGGTCCAGTGTTGAATGCTC	*Xho*I	
pAbA-F1-F	cTAAGAACGGCAACCATTAGAGTTGACT AGTTTGAGCATTCAACACTGGAc	*Sac*I *Xho*I	pAbA Vector
pAbA-F1-R	tcgagTCCAGTGTTGAATGCTCAAACTAGTCAA CTCTAATGGTTGCCGTTCTTAgagct		
pAbA-F2-F	cTTTTAATAGATGACGCCGTTGACTTTTTCT TACATATTTGACCATTCGTCTTATCCAAAAc	*Sac*I *Xho*I	pAbA Vector
pAbA-F2-R	tcgagTTTTGGATAAGACGAATGGTCAAATATGTA AGAAAAAGTCAACGGCGTCATCTATTAAAAgagct		
pAbA-F2-m-F	cTTTTAATAGATGACGCCGTTGAATTTTTCT TACATATTTGAACATTCGTCTTATCCAAAAc	*Sac*I *Xho*I	pAbA Vector
pAbA-F2-m-R	tcgagTTTTGGATAAGACGAATGTTCAAATATGTA AGAAAAATTCAACGGCGTCATCTATTAAAAgagct		

pAbA-F2-m1-F	cTTTTAATAGATGACGCCGTTGAATTTTTCT TACATATTTGACCATTCGTCTTATCCAAAAc	*Sac*I *Xho*I	pAbA Vector
pAbA-F2-m1-R	tcgagTTTTGGATAAGACGAATGGTCAAATATGTA AGAAAAATTCAACGGCGTCATCTATTAAAAgagct		
pAbA-F2-m2-F	cTTTTAATAGATGACGCCGTTGACTTTTTCT TACATATTTGAACATTCGTCTTATCCAAAAc	*Sac*I *Xho*I	pAbA Vector
pAbA-F2-m2-R	tcgagTTTTGGATAAGACGAATGTTCAAATATGTA AGAAAAAGTCAACGGCGTCATCTATTAAAAgagct		
pGADT7-W21-F	TTcatatgATGGCGATGCTGGGGAGCT	*Nde*I	pGADT7 AD
pGADT7-W21-R	TTctcgagTCAGAGGGAGTTGATGACGAAT	*Xho*I	
pGADT7-W108-F	TTcatatgATGCAGGCGCAATCCCGCCTC	*Nde*I	pGADT7 AD
pGADT7-W108-R	TTctcgagTTAATTAATTAGATCAAAACAG	*Xho*I	
For yeast two-hybrid assay			
BD-W21-FL-F	TTcccgggAAATGGCGATGCTGGGGAGCT	*Xma*I	pBD-GAL4 Cam
BD-W21-FL-R	TTctgcagTCAGAGGGAGTTGATGACGAAT	*Pst*I	
BD-W21-N△60-F	TTcccgggAACGGGGAGGAGCCACTGGTGCT	*Xma*I	pBD-GAL4 Cam
BD-W21-N△60-R	TTctgcagTCAGAGGGAGTTGATGACGAAT	*Pst*I	
BD-W21-N△90-F	TTcccgggAAGTCACGCCGACCAAAAGAAG	*Xma*I	pBD-GAL4 Cam
BD-W21-N△90-R	TTctgcagTCAGAGGGAGTTGATGACGAAT	*Pst*I	
BD-W21-N△105-F	TTcccgggAAGAGGTGAGGAGCGGCACGAC	*Xma*I	pBD-GAL4 Cam
BD-W21-N△105-R	TTctgcagTCAGAGGGAGTTGATGACGAAT	*Pst*I	
BD-W21-N△115-F	TTcccgggAATTCATCTGGAGGAAGTACGG	*Xma*I	pBD-GAL4 Cam
BD-W21-N△115-R	TTctgcagTCAGAGGGAGTTGATGACGAAT	*Pst*I	
BD-W21-C△20-F	TTcccgggAAATGGCGATGCTGGGGAGCT	*Xma*I	pBD-GAL4 Cam
BD-W21-C△20-R	TTctgcagTCACGCCGACGAGTCGGCTCCGGC	*Pst*I	
BD-W21-C△45-F	TTcccgggAAATGGCGATGCTGGGGAGCT	*Xma*I	pBD-GAL4 Cam
BD-W21-C△45-R	TTctgcagTCACCCCTGCGACGTCTCGCTGGG	*Pst*I	
BD-W21-C△90-F	TTcccgggAAATGGCGATGCTGGGGAGCT	*Xma*I	pBD-GAL4

BD-W21-C△90-R	TTctgcagTCACATTAGTTCCCCGTCCCGGCA	*Pst*I	Cam
BD-W108-FL-F	TTgaattcATGCAGGCGCAATCCCGCC	*Eco*RI	pBD-GAL4 Cam
BD-W108-FL-R	TTcccgggTTAATTAATTAGATCAAAAC	*Xma*I	
BD-W108-N△20-F	TTgaattcGGGAGCGGTGGCATTAGCAG	*Eco*RI	pBD-GAL4 Cam
BD-W108-N△20-R	TTcccgggTTAATTAATTAGATCAAAAC	*Xma*I	
BD-W108-N△45-F	TTgaattcGGGCACGAGCTGACGGCGC	*Eco*RI	pBD-GAL4 Cam
BD-W108-N△45-R	TTcccgggTTAATTAATTAGATCAAAAC	*Xma*I	
BD-W108-N△60-F	TTgaattcCTGCGCGGGCAGGGGCAGG	*Eco*RI	pBD-GAL4 Cam
BD-W108-N△60-R	TTcccgggTTAATTAATTAGATCAAAAC	*Xma*I	
BD-W108-N△90-F	TTgaattcAGCGCCTCCCCGTCCGCCTC	*Eco*RI	pBD-GAL4 Cam
BD-W108-N△90-R	TTcccgggTTAATTAATTAGATCAAAAC	*Xma*I	
BD-W108-C△20-F	TTgaattcATGCAGGCGCAATCCCGCC	*Eco*RI	pBD-GAL4 Cam
BD-W108-C△20-R	TTcccgggTTAGCCGAAAGGCCCCCAGAAC	*Xma*I	
BD-W108-C△45-F	TTgaattcATGCAGGCGCAATCCCGCC	*Eco*RI	pBD-GAL4 Cam
BD-W108-C△45-R	TTcccgggTTAGGGCCCGGACGACGACGAC	*Xma*I	
BD-W108-C△60-F	TTgaattcATGCAGGCGCAATCCCGCC	*Eco*RI	pBD-GAL4 Cam
BD-W108-C△60-R	TTcccgggTTAAGGCGCGCGCGCGTGCACG	*Xma*I	
BD-W108-C△90-F	TTgaattcATGCAGGCGCAATCCCGCC	*Eco*RI	pBD-GAL4 Cam
BD-W108-C△90-R	TTcccgggTTAGGCGAGGGACGAGACGAGC	*Xma*I	
AD-W21-F	TTggtaccATGGCGATGCTGGGGAGCT	*Bam*HI	pAD-GAL4-2.1
AD-W21-R	TTctgcagTCAGAGGGAGTTGATGACGAAT	*Pst*I	
AD-W108-F	TTgaattcATGCAGGCGCAATCCCGCC	*Eco*RI	pAD-GAL4-2.1
AD-W108-R	TTagatctTTAATTAATTAGATCAAAAC	*Bgl*II	
For BiFC assay			
pENTR-WRKY21-F	CACCATGGCGATGCTGGGGAGCT	Gateway system	pENTR/D-TOPO pGTQL-YC pGTQL-YN
pENTR-WRKY21-R	GAGGGAGTTGATGACGAAT		
pENTR-WRKY108-F	CACCATGCAGGCGCAATCCCGCC		
pENTR-WRKY108-R	ATTAATTAGATCAAAACAGC		

For EMSA and pull-down assay			
GST-WRKY108-F	TTggatccATGCAGGCGCAATCCCGCC	*Bam*HI	pGS-21a
GST-WRKY108-R	TTgaattcTTAATTAATTAGATCAAAAC	*Eco*RI	
MBP-WRKY21-F	TTcatatgATGGCGATGCTGGGGAGCT	*Nde*I	pMal-c5x
MBP-WRKY21-R	TTgtcgacTCAGAGGGAGTTGATGACGAAT	*Sal*I	
F1-F	TAAGAACGGCAACCATTAGAGTTGACT AGTTTGAGCATTCAACACTGGA	Biotin-labeled/-unlabled,	
F1-R	TCCAGTGTTGAATGCTCAAACTAGTCAA CTCTAATGGTTGCCGTTCTT	Biotin-labeled/-unlabled	
F2-F	TTTTAATAGATGACGCCGTTGACTTTTTCT TACATATTTGACCATTCGTCTTATCCAAAA	Biotin-labeled/-unlabled	
F2-R	TTTTGGATAAGACGAATGGTCAAATATGTA AGAAAAAGTCAACGGCGTCATCTATTAAAA	Biotin-labeled/-unlabled	
F2-m-F	TTTTAATAGATGACGCCGTTGAATTTTTCT TACATATTTGAACATTCGTCTTATCCAAAA	Biotin-labeled	
F2-m-R	TTTTGGATAAGACGAATGTTCAAATATGTA AGAAAAATTCAACGGCGTCATCTATTAAAA	Biotin-labeled	
F2-m1-F	TTTTAATAGATGACGCCGTTGAATTTTTCT TACATATTTGACCATTCGTCTTATCCAAAA	Biotin-labeled	
F2-m1-R	TTTTGGATAAGACGAATGGTCAAATATGTA AGAAAAATTCAACGGCGTCATCTATTAAAA	Biotin-labeled	
F2-m2-F	TTTTAATAGATGACGCCGTTGACTTTTTCT TACATATTTGAACATTCGTCTTATCCAAAA	Biotin-labeled	
F2-m2-R	TTTTGGATAAGACGAATGTTCAAATATGTA AGAAAAAGTCAACGGCGTCATCTATTAAAA	Biotin-labeled	

Table S4 Positive interactions from Yeast One-Hybrid screening.

Locus identification no.	Description	The number of clones identified
LOC_Os01g06510	Similar to arginyl-tRNA synthetase	1
LOC_Os01g49770	RING finger protein OsRFPH2-23	1
LOC_Os01g60640	Rice WRKY gene21	3
LOC_Os01g66379	Similar to predicted protein	1
LOC_Os02g05480	MAP kinase MAPK2	1
LOC_Os02g47180	Similar to WD-repeat protein	1
LOC_Os03g04050	RECEPTOR-LIKE CYTOPLASMIC KINASE 98	1
LOC_Os03g05290	TONOPLAST INTRINSIC PROTEIN 1;1	1
LOC_Os03g19510	CHLOROPLAST PROTEASE 5	1
LOC_Os03g48471	Conserved hypothetical protein	1
LOC_Os03g64250	ASCH domain domain containing protein	1
LOC_Os04g55520	ETHYLENE RESPONSE FACTOR 8	1
LOC_Os05g15510	Glycoside hydrolase, family 5 protein;Similar to cellulase containing protein	1
LOC_Os05g33680	Conserved hypothetical protein	1
LOC_Os05g41550	Conserved hypothetical protein	1
LOC_Os06g44970	PIN 2	1
LOC_Os07g35500	Conserved hypothetical protein	1
LOC_Os09g14880	Homeodomain-like containing protein	1
LOC_Os09g14960	Homeodomain-like containing protein	1
LOC_Os09g26390	Conserved hypothetical protein	1
LOC_Os11g08120	BETA-GLUCOSIDASE 35	1
LOC_Os11g08569	2,3-oxidosqualene cyclase, Triterpene synthase, Parkeol synthase (Os11t0189600-01);Similar to cDNA clone:J013062J12, full insert sequence. (Os11t0189600-02)	1
LOC_Os11g14220	TWISTED DWARF 1	1
LOC_Os12g07110	Long-chain acyl-CoA synthetase 1	1
LOC_Os12g19350	Similar to Dual specificity protein phosphatase 8	1
LOC_Os12g24170	Similar to Relative to SR12 protein	1

Table S5 Candidates from Yeast Two-Hybrid screening.

Locus identification no.	Description	The number of clones identified
LOC_Os01g14950	IMPORTIN Subunit Alpha-1a	11
LOC_Os02g50010	APO protein 2, chloroplastic	19
Unknown	Unknown protein	19
LOC_Os01g08700	Protein GIGANTEA	8
LOC_Os01g04010	Conserved hypothetical protein	9
LOC_Os03g60740	NAD(P)-binding domain containing protein	5
LOC_Os03g63910	Pentatricopeptide repeat domain containing protein	4
LOC_Os05g33100	Similar to Endo-1,3;1,4-beta-D-glucanase	5
LOC_Os10g35370	Similar to Protochlorophyllide reductase B, chloroplastic	4
LOC_Os02g08380	Uncharacterized protein family UPF0133 domain containing protein	5
LOC_Os02g36350	Protein kinase PKN/PRK1, effector domain containing protein	3
LOC_Os01g60600	WRKY108, probable WRKY transcription factor	4
LOC_Os09g39070	Stem bromelain, hypothetical conserved gene	4

Reference

Ross CA, Liu Y, Shen QJ. 2007. The WRKY gene family in Rice (*Oryza sativa*). *Journal of Integrative Plant Biology* **6:** 827-842.