Plant Communications, Volume *5*

Supplemental information

WRKY33 negatively regulates anthocyanin biosynthesis and coopera-tes with PHR1 to mediate acclimation to phosphate starvation

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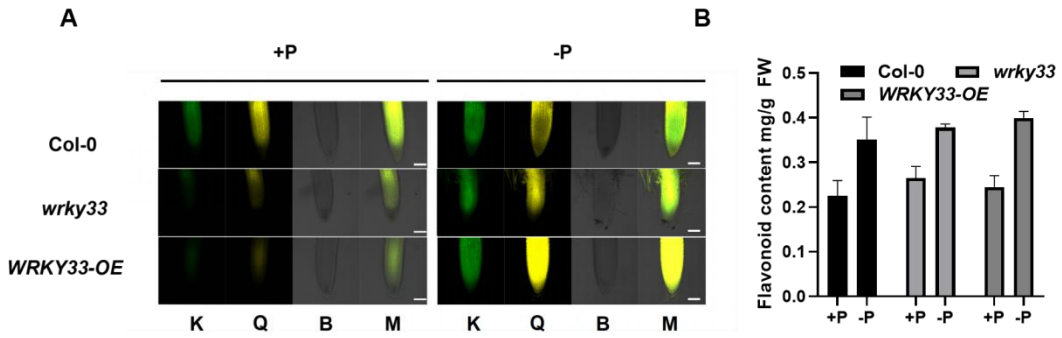
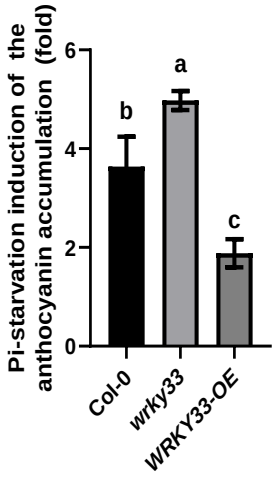


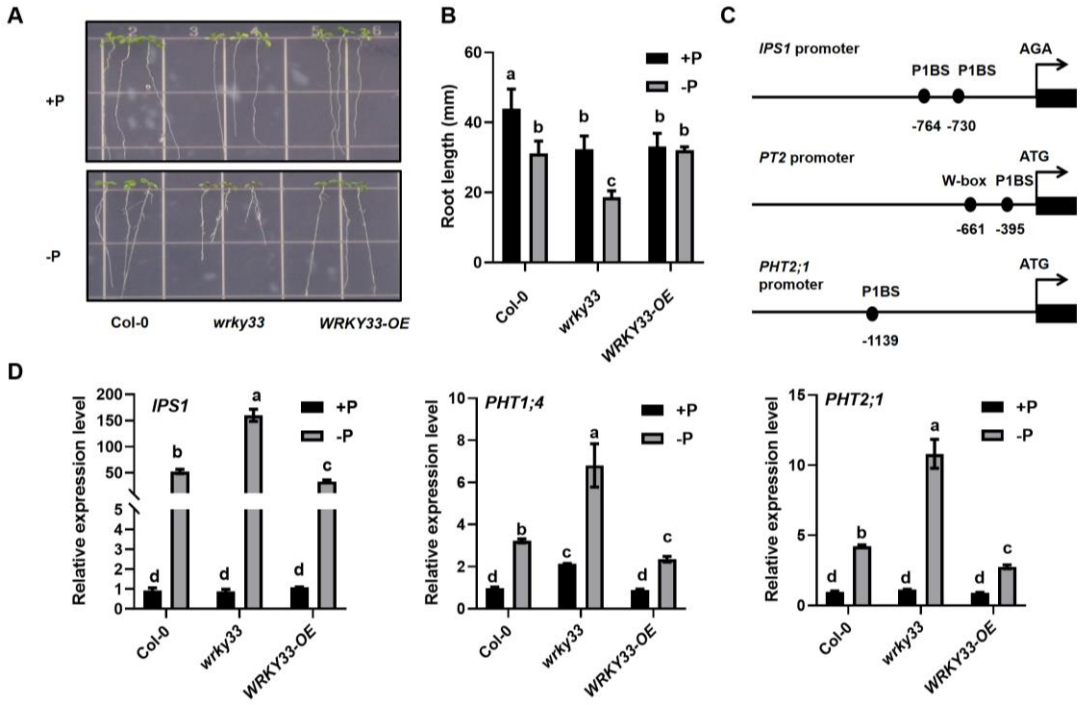
Supplemental Figure 1. The effects of WRKY33 on flavonoids in *Arabidopsis thaliana*

(A) In situ flavonol staining of 9-day-old Col-0, *wrky33* and *WRKY33-OE* seedlings grown on ½ MS +P (1.25 mM Pi) and -P (0 mM Pi) media. Flavonols in ethanol-bleached inflorescences were stained with diphenylboric acid 2-aminoethylester (DPBA) to saturation and imaged with a Leica TCS SP5 confocal laser-scanning microscope. K: kaempferol; Q: quercetin; B: Bright; M: merged. **(B)** Flavonoids contents of 9-day-old Col-0, *wrky33* and *WRKY33-OE* plants grown under Pi-sufficient and Pi-starvation conditions. Error bars indicate the SD of four biological replicates.



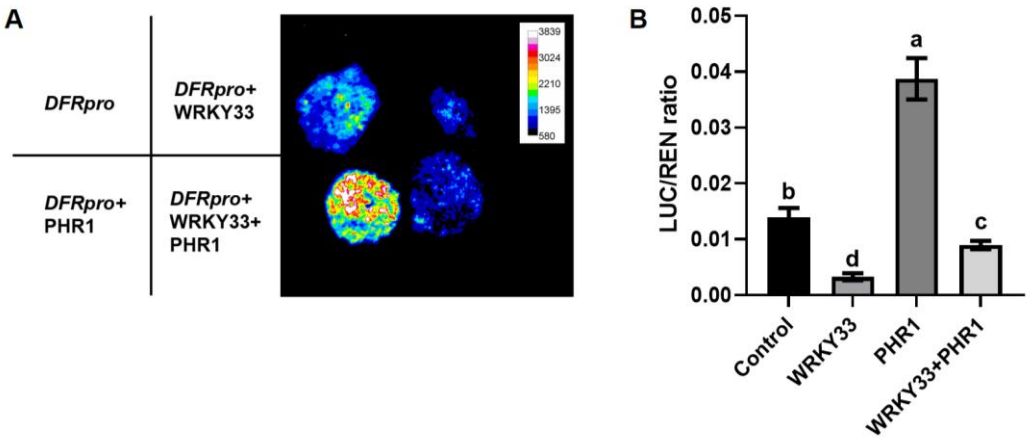
Supplemental Figure 2. Ratios of the anthocyanin accumulation in -Pi/+Pi conditions

9-day-old Col-0, *wrky33* and *WRKY33-OE* seedlings grown on ½MS +P (1.25 mM Pi) and -P (0 mM Pi) media. Different letters indicate significant differences (ANOVA, Fisher's LSD tests; $P < 0.05$). Error bars indicate the SD of four biological replicates.



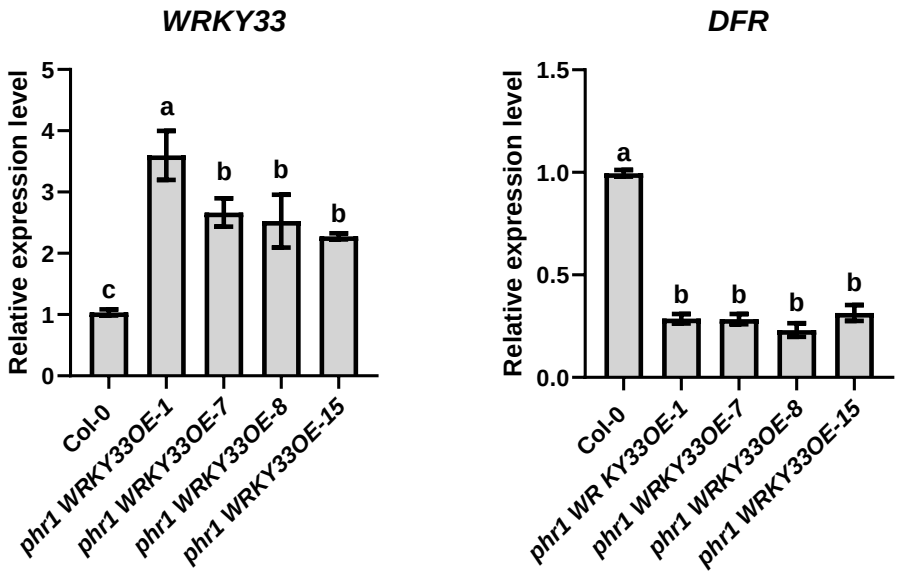
Supplemental Figure 3. The root growth and relative expression levels of PSI genes in WRKY33-related mutants

(A) Phenotype of 9-day-old Col-0, *wrky33* and *WRKY33-OE* seedlings grown on +Pi and -Pi media. **(B)** Root length of Col-0, *wrky33* and *WRKY33-OE* seedlings grown on +Pi and -Pi media for 9 days. Different letters indicate significant differences. **(C)** The diagram of the presence / absence of W-boxes and / or P1BS elements in the promoters of *IPS1, PHT1;4* and *PHT2;1* **(D)** qRT-PCR analysis of Pi starvation-responsive (PSI) genes expression in *Col-0*, *wrky33* and *WRKY33-OE* grown on +Pi/-Pi media s for 9 days. Error bars indicate the SD of four biological replicates. Different letters above the bars indicate significant differences between groups ($P < 0.05$; ANOVA with Fisher's LSD test).



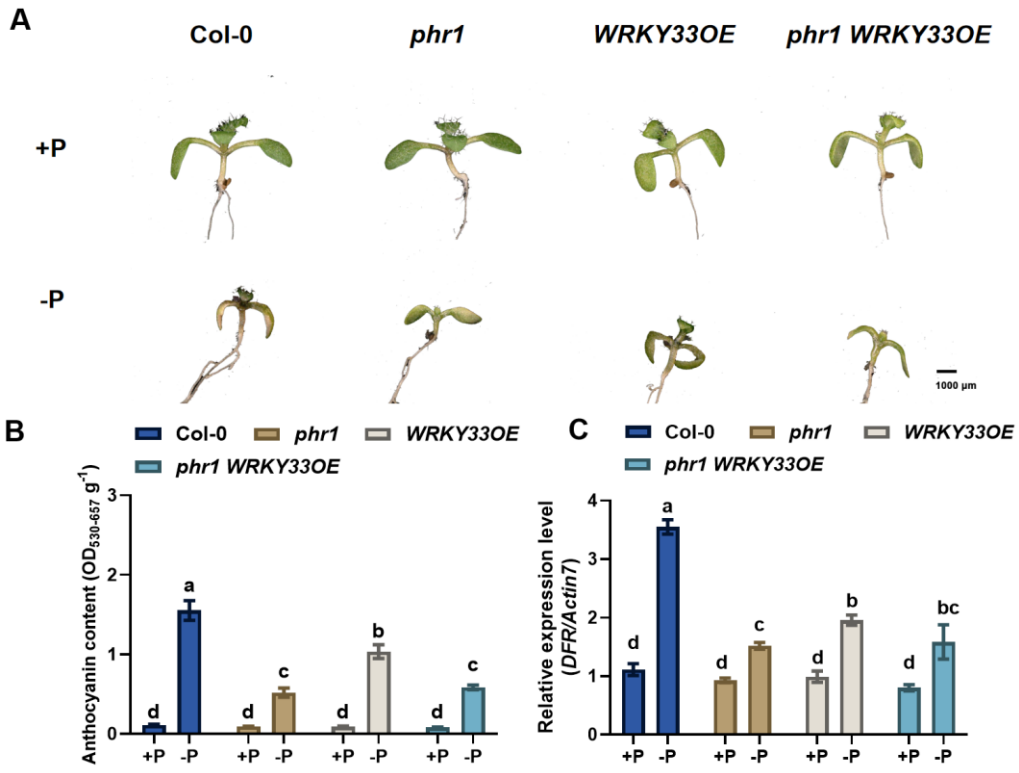
Supplemental Figure 4. PHR1 suppresses the inhibition of *DFR* promoter by WRKY33

(A) The transient expression assay showed the inhibition of WRKY33 to *DFR* promoter was compromised when PHR1 coexpressed with WRKY33 in *N. benthamiana* leaves. **(B)** Relative firefly LUC to REN ratios from transient expression assays. These represent the activity of the *DFR* promoter in the absence/presence of WRKY33 and PHR1. Error bars indicate the SD of four biological replicates. Different letters above the bars indicate significant differences between groups ($P < 0.05$; ANOVA with Fisher's LSD test).



Supplemental Figure 5. The expression levels of *WRKY33* and *DFR* in *phr1/WRKY33OE* homozygous plants

Homozygous T3 lines were screened using hygromycin. *phr1WRKY33OE-1*, *phr1WRKY33OE-7*, *phr1WRKY33OE-8*, *phr1WRKY33OE-15* were different homozygous lines. Error bars indicate the SD of three biological replicates. Different letters above the bars indicate significant differences between groups ($P < 0.05$; ANOVA with Fisher's LSD test).



Supplemental Figure 6. The phenotype of *Col-0, phr1, WRKY33OE* and *phr1WRKY33OE* under +Pi or -Pi conditions.

(A) The anthocyanin accumulation and phenotype of Col-0, *phr1,WRKY33OE* and *phr1WRKY33OE* under +Pi or -Pi conditions. (B-C) The anthocyanin content (B) and relative expression levels of anthocyanin biosynthesis genes *DFR* (C) in Col-0, *phr1,WRKY33OE* and *phr1WRKY33OE* under +Pi or -Pi conditions. Error bars indicate the SD of three biological replicates. Different letters indicate statistically significant differences ($P < 0.05$)

Supplemental Table 1. Primer sequences in this study

Primer names	Primer sequence (5'--3')	Purpose
CHS-qRT-F	GGAGAAGTTCAAGCGCATGTG	qRT-PCR
CHS-qRT-R	ATGTGACGTTTCCGAATTGTCG	qRT-PCR
CHI-qRT-F	CTCTCTTACGGTTGCGTTTTCG	qRT-PCR
CHI-qRT-R	CACCGTTCTTCCCGATGATAGA	qRT-PCR
DFR-qRT-F	AGCCGCCAAGGGACGTTATATTTG	qRT-PCR
DFR-qRT-R	CCGGGAGAAAACCCTTTTGACGA	qRT-PCR
DFR-W1-F	GTGGTGGTTACCTCGTCCAC	qRT-PCR-ChIP
DFR-W1-R	CTACACCAAAGACGCTTGGC	qRT-PCR-ChIP
DFR-W2-F	AGTACCAACCGGAGAAGCAC	qRT-PCR-ChIP
DFR-W2-R	AAGTCACCCACACGTCTCAC	qRT-PCR-ChIP
Lic-WRKY33-F	CgACgACAAgACCgTCACCatgATGGCTGCTTCTTTTCTTACAATG	BiFC
Lic-WRKY33-R	gAggAgAagAgCCgTCgGGGCATAAACGAATCGAAAAAT	BiFC
pABAi-DFR-HindIII-F	AAAATGATGAATTGAAAAGCTTCTCTGACGTCTTACGATACAACAAATTG	Y1H
pABAi-DFR-SalI-R	GAGCACATGCCTCGAGGTCGACTTTTGTGGTTATATGATAGATTGTGC	Y1H
DFR-Prob-1-F	GTACCGGTGGGTGAAATACGTTGACTTCGATTTGTTTGGTGAGAC	EMSA
DFR-Prob-1-R	GTCTCACCAAACAAATCGAAGTCAACGTATTTCACCCACCGGTAC	EMSA
DFR-Prob-2-F	GAGAAGAGGTCAGCTTAATTTTGACTCTCCTCCAAACAGAGAGAC	EMSA
DFR-Prob-2-R	GTCTCTCTGTTTGGAGGAGAGTCAAAATTAAGCTGACCTCTTCTC	EMSA