

Supporting information

OsMYB58 negatively regulates phosphate acquisition via *OsmiR399*-dependent phosphate starvation signaling in rice.

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Supplementary Materials:

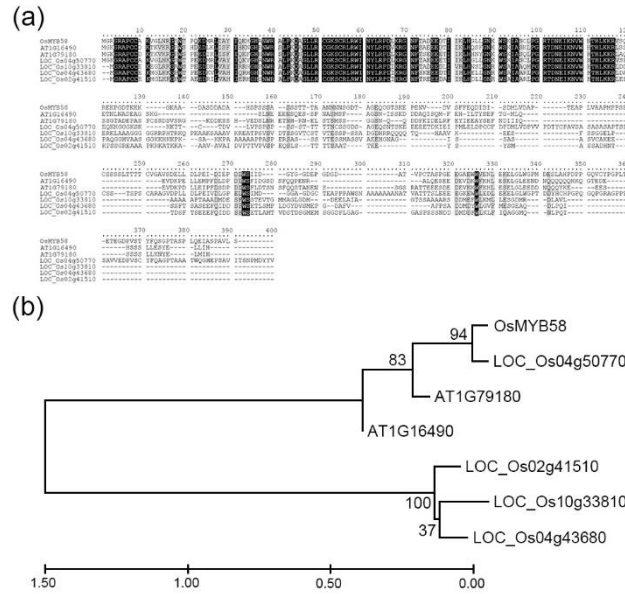
Supplementary Figure S1. Sequence alignment and phylogenetic tree analysis of R2R3-type MYB transcription factors in Arabidopsis and rice.

Supplementary Figure S2. Generation of *OsMYB58* overexpressing Arabidopsis plants.

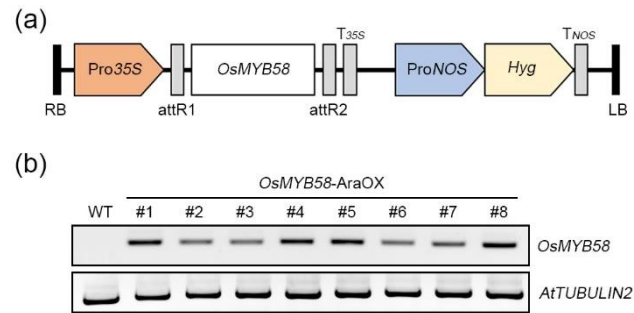
Supplementary Figure S3. Generation of *OsMYB58* overexpressing rice plants.

Supplementary Figure S4. Generation of *OsMYB58* T-DNA tagging knock-out mutant rice plants.

Supplementary Table S1. Lists of primers in this study.

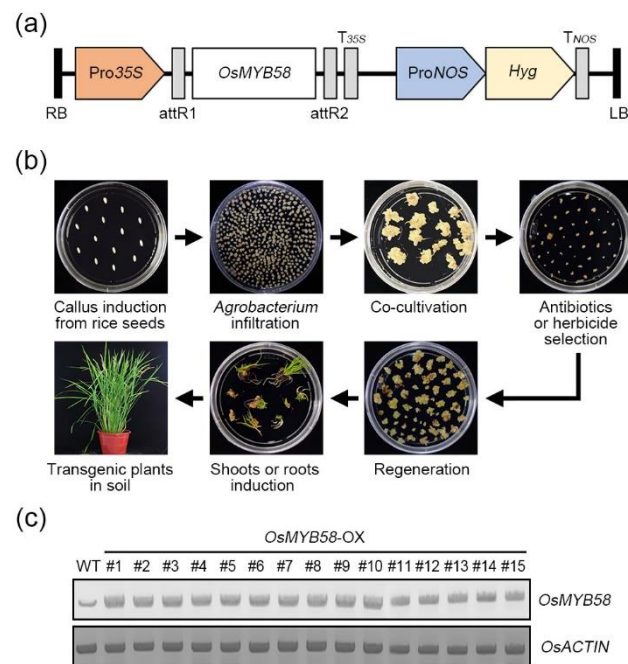


Supplementary Figure S1. Sequence alignment and phylogenetic tree analysis of R2R3-type MYB transcription factors in Arabidopsis and rice. (A) Multiple protein sequence alignment of R2R3-type MYB protein in Arabidopsis and rice was generated by the Clustal Omega program (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). Identical protein sequences are shaded in black and similar protein sequences are shaded in gray. (B) The phylogenetic tree of Arabidopsis and rice MYB proteins was constructed with the Neighbor-Joining method in MEGA X (<https://www.megasoftware.net/>) using R2R3 domain sequences.

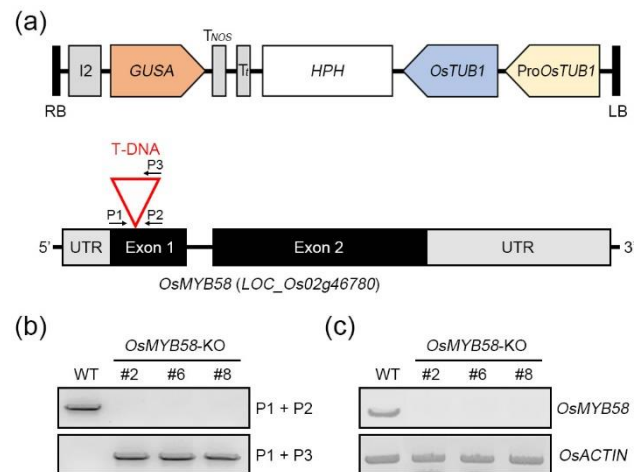


Supplementary Figure S2. Generation of *OsMYB58* overexpressing Arabidopsis plants. (a)

For overexpressing *OsMYB58* into Arabidopsis Col-0 plants, the diagram showed the plasmid DNA construct including the hygromycin (Hyg) selection marker. (b) *OsMYB58* expression in *OsMYB58*-AraOX plants by RT-PCR analysis. Total RNA was extracted from selected *OsMYB58*-AraOX plants by hygromycin. *AtTUBULIN2* is an internal control.



Supplementary Figure S3. Generation of *OsMYB58* overexpressing rice plants. (a) For overexpressing *OsMYB58* into rice plants, the diagram showed the plasmid DNA construct including the hygromycin (Hyg) selection marker. (b) The process diagram indicated the rice transformation by *Agrobacterium*-mediated methods. (c) *OsMYB58* expression in *OsMYB58*-OX plants by RT-PCR analysis. Total RNA was extracted from selected *OsMYB58*-OX rice plants by hygromycin. *OsACTIN* is an internal control.



Supplementary Figure S4. Generation of *OsMYB58* T-DNA tagging knock-out mutant rice plants. (a) For mutation of *OsMYB58* into rice plants using T-DNA tagging vector (*pGA2707*) systems, diagram showed the plasmid DNA construct including the hygromycin phosphotransferase (*HPH*) selectable gene. *GUS*A; native *E.coli* β -glucuronidase reporter, *ProOsTUB1*; promoter of coding sequence (*OsTUB1*), I2 and Tt; second (I2) intron and terminator sequence (Tt) of the rice α -TUBULIN A1 gene. (b) Genotyping PCR analysis in *OsMYB58*-KO plants. For selecting T-DNA-inserted transgenic plants, diagnostic PCR was performed in wild-type (WT) and *OsMYB58*-KO plants using a combination of gene-specific (P1 and P2) or T-DNA-specific (P3) primers. (c) *OsMYB58* expression in *OsMYB58*-KO plants by RT-PCR analysis. Total RNA was extracted from selected *OsMYB58*-KO rice plants by hygromycin. *OsACTIN* is an internal control.

Supplementary Table S1. Lists of primers in this study

Gene	Direction	Sequence (5'→3')	Purpose
<i>OsMYB58</i> -RT	Forward	CATCGCCTACATCCAGAAG	Analysis of qRT-PCR or RT-PCR
	Reverse	GAGATGTCGATGTCTTGCTC	
<i>OsACTIN</i> -RT	Forward	ATGCTCTCCCCCATGCTATC	
	Reverse	TCTTCCTTGCTCATCCTGTC	
<i>AtTUBULIN2</i> -RT	Forward	TGGCATCAACTTTCATTGGA	
	Reverse	ATGTTGCTCTCCGCTTCTGT	
<i>OsmiR399a</i> -qRT	Forward	GCTGGAAATGATGCTGGTAGC	
	Reverse	CTCCTTTGGCACGAGATCTGT	
<i>OsmiR399j</i> -qRT	Forward	GGAGCATGTAAGTCTTTGTAGC	
	Reverse	GGCAACTCTCCTTTGGCAGA	
<i>OsIPS1</i> -qRT	Forward	CTAAGGTAGGGCAACTTGTATC	
	Reverse	TTATTAGAGCAAGGACCGAAAC	
<i>OsPHO2</i> -qRT	Forward	GGTGCAGCTGGAACACCTTA	
	Reverse	GCACCGGAATGGTAGTGAA	
<i>OsPT2</i> -qRT	Forward	GACGAGACCGCCCAAGAAC	
	Reverse	TTTTCAGTCACTCACGTCGAGAC	
<i>OsPT4</i> -qRT	Forward	TTCTGCTAGTGTACCAAACAAAATTACA	
	Reverse	CTAAGTGGCATTATAATATCAACAGTAACC	
<i>OsACTIN</i> -qRT	Forward	GAACTGGTATGGTCAAGGCTG	
	Reverse	ACACGGAGCTCGTTGTAGAAG	
<i>OsMYB58</i> -KO	P1	CGAACACGCAAGAATTAAC	Genotyping from RNAi- plants
	P2	GCTAAGCACACGTGTAGGAT	
	P3	GGTGAATGGCATCGTTTGAA	