

## *Supporting information*

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

**Dongwon Baek<sup>1,†</sup>, Won Tae Yang<sup>2,†</sup>, Soyeon Hong<sup>3,†</sup>, Hye Jeong Kim<sup>2</sup>, Sunok Moon<sup>4</sup>, Ki Hong Jung<sup>4</sup>, and Doh Hoon Kim<sup>2,\*</sup>**

<sup>1</sup> Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju 52828, Republic of Korea; [dw100@hanmail.net](mailto:dw100@hanmail.net) (D.B.).

<sup>2</sup> College of Life Science and Natural Resources, Dong-A University, Busan 49315, Republic of Korea; [wtyang@dau.ac.kr](mailto:wtyang@dau.ac.kr) (W.T.Y.), [hjkim83@dau.ac.kr](mailto:hjkim83@dau.ac.kr) (H.J.K.), [dhkim@dau.ac.kr](mailto:dhkim@dau.ac.kr) (D.H.K.).

<sup>3</sup> National Agrobiodiversity Center, National Institute of Agricultural Sciences, Rural Development Administration, Jeonju 54874, Republic of Korea; [hsy1203@korea.kr](mailto:hsy1203@korea.kr) (S.H.).

<sup>4</sup> Graduate School of Biotechnology and Crop Biotech Institute, Kyung Hee University, Yongin 17104, Republic of Korea; [moonsun@khu.ac.kr](mailto:moonsun@khu.ac.kr) (S.M.), [kjhjung2010@khu.ac.kr](mailto:kjhjung2010@khu.ac.kr) (K.H.J.).

<sup>†</sup> These authors contributed equally to this work

\* Correspondence: [dhkim@dau.ac.kr](mailto:dhkim@dau.ac.kr); Tel.: +82-51-200-7507 (D.H.K.)

## **Supplementary Materials:**

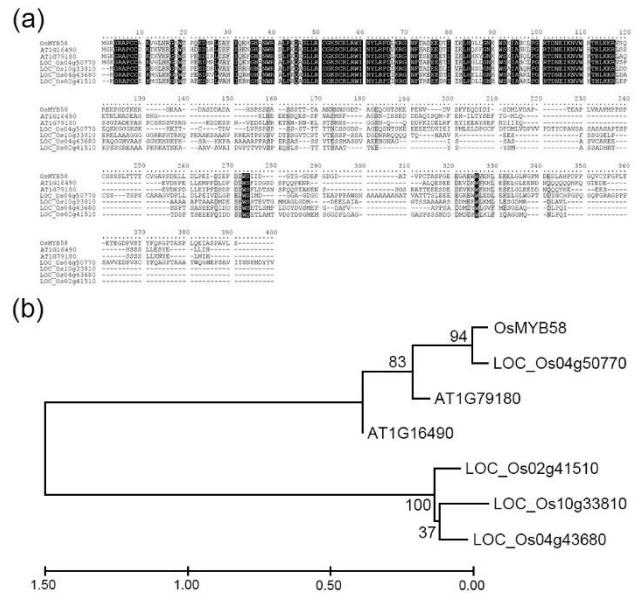
**Supplementary Figure S1.** Sequence alignment and phylogenetic tree analysis of R2R3-type MYB transcription factors in Arabidopsis and rice.

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

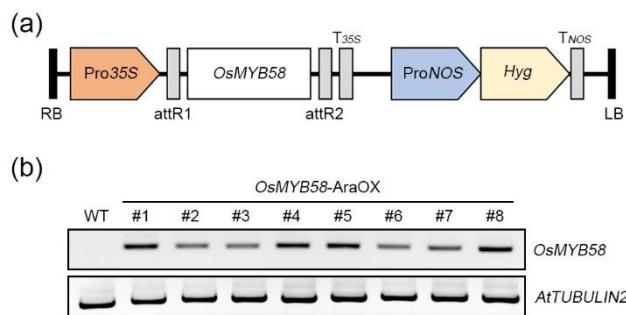
**Supplementary Figure S3.** Generation of *OsMYB58* overexpressing rice plants.

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

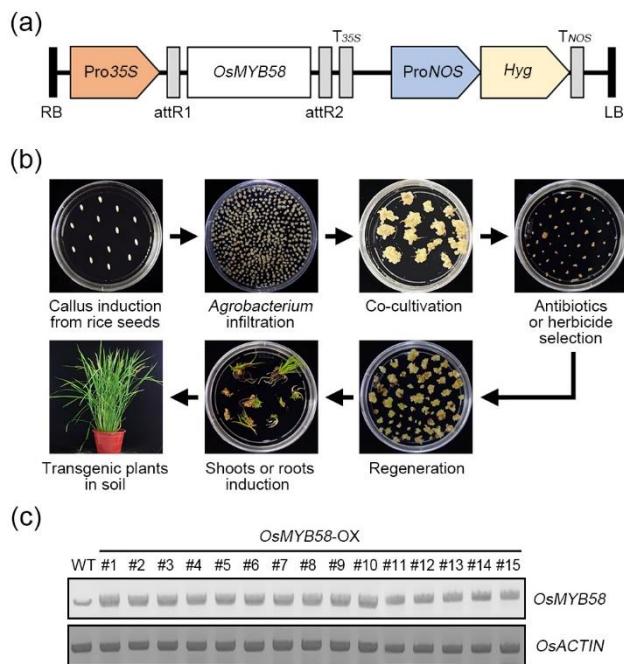
**Supplementary Table S1.** Lists of primers in this study.



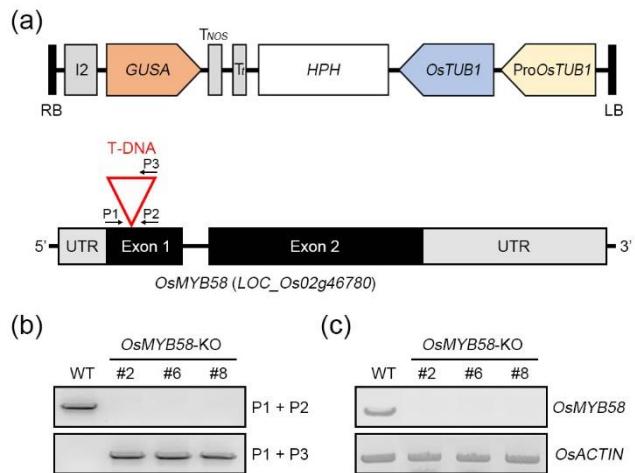
**Supplementary Figure S1. Sequence alignment and phylogenetic tree analysis of R2R3-type MYB transcription factors in *Arabidopsis* and rice.** (A) Multiple protein sequence alignment of R2R3-type MYB protein in *Arabidopsis* and rice was generated by the Clustal Omega program (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). 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. *GUSA*; native *E. coli*  $\beta$ -glucuronidase reporter, *ProOsTUB1*; promoter of coding sequence (*OsTUB1*), I2 and Tt; second (I2) intron and terminator sequence (Tt) of the rice  $\alpha$ -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	ATGCTCTCCCCATGCTATC	Analysis of qRT-PCR or RT-PCR
	Reverse	TCTTCCTTGCTCATCCTGTC	
<i>AtTUBULIN2</i> -RT	Forward	TGGCATCAACTTCATTGGA	Analysis of qRT-PCR or RT-PCR
	Reverse	ATGTTGCTCTCGCTTCTGT	
<i>OsmiR399a</i> -qRT	Forward	GCTGGAAATGATGCTGGTAGC	Analysis of qRT-PCR or RT-PCR
	Reverse	CTCCTTGGCACGAGATCTGT	
<i>OsmiR399j</i> -qRT	Forward	GGAGCATGTAAGTCTTTGTAGC	Analysis of qRT-PCR or RT-PCR
	Reverse	GGCAACTCTCCTTGGCAGA	
<i>OsIPSI</i> -qRT	Forward	CTAAGGTAGGGCAACTTGTATC	Analysis of qRT-PCR or RT-PCR
	Reverse	TTATTAGAGCAAGGACCGAAC	
<i>OsPHO2</i> -qRT	Forward	GGTGCAGCTGGAACACCTTA	Analysis of qRT-PCR or RT-PCR
	Reverse	GCACCGGAATGGTAGTGAA	
<i>OsPT2</i> -qRT	Forward	GACGAGACCGCCCAAGAAC	Analysis of qRT-PCR or RT-PCR
	Reverse	TTTCAGTCACTCACGTCGAGAC	
<i>OsPT4</i> -qRT	Forward	TTCTGCTAGTGTACCAAACAAAATTACA	Analysis of qRT-PCR or RT-PCR
	Reverse	CTAAGTGGCATTATAATATCAACAGTAACC	
<i>OsACTIN</i> -qRT	Forward	GAACCTGGTATGGTCAAGGCTG	Genotyping from RNAi-plants
	Reverse	ACACGGAGCTCGTTGTAGAAG	
<i>OsMYB58</i> -KO	P1	CGAACACGCAAGAATTAAC	Genotyping from RNAi-plants
	P2	GCTAAGCACACGTGTAGGAT	
	P3	GGTGAATGGCATCGTTGAA	