Aquaporin OsPIP2;2 Links the H_2O_2 Signal and a Membrane-anchored Transcription Factor to Promote Plant Defense

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Supplemental Materials

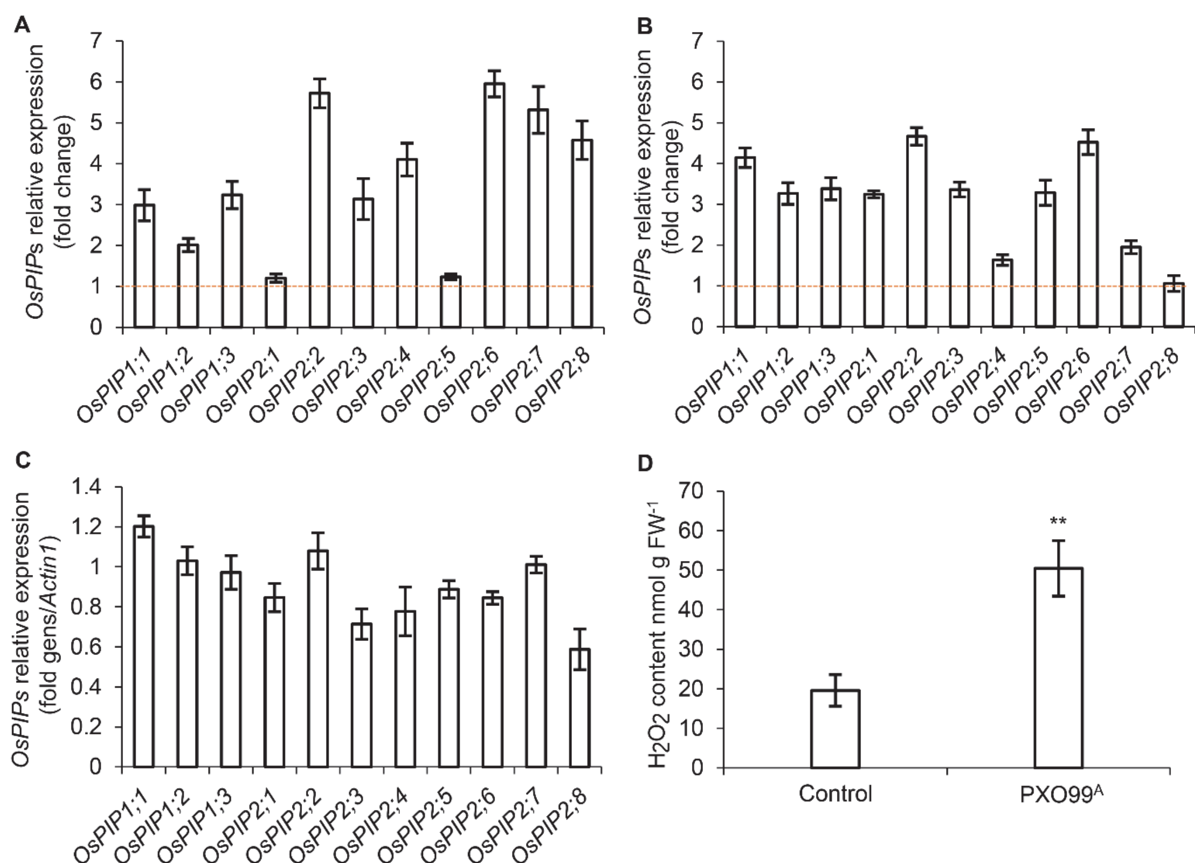


Figure S1. The expression profiles of *OsPIP*s in response to PXO99^A. A, Relative expression of *OsPIPs* after PXO99^A inoculation. The rice leaves were inoculated with PXO99^A (OD_{600} = 0.6). Eight hours later, total RNA was extracted for RT-qPCR analysis. B, Relative expression of *OsPIPs* upon H_2O_2 treatment. The leaves were treated with 2 mM H_2O_2. Two hours later, gene expression was analyzed by RT-qPCR and expression level of each gene was shown as the ratio of transcript quantities between treatment and control plants. C, Relative expression of *OsPIP*s to *OsActin1* in normal growth condition. D, H_2O_2 content in 2-week-old rice leaves 8 h after inoculation with PXO99^A. FW, fresh weight. Data are shown as means ± SEM (n = 4). Asterisks indicate significant differences compare with control by Student's t test (** $P \leq 0.01$). These experiments were performed at least twice with similar results.

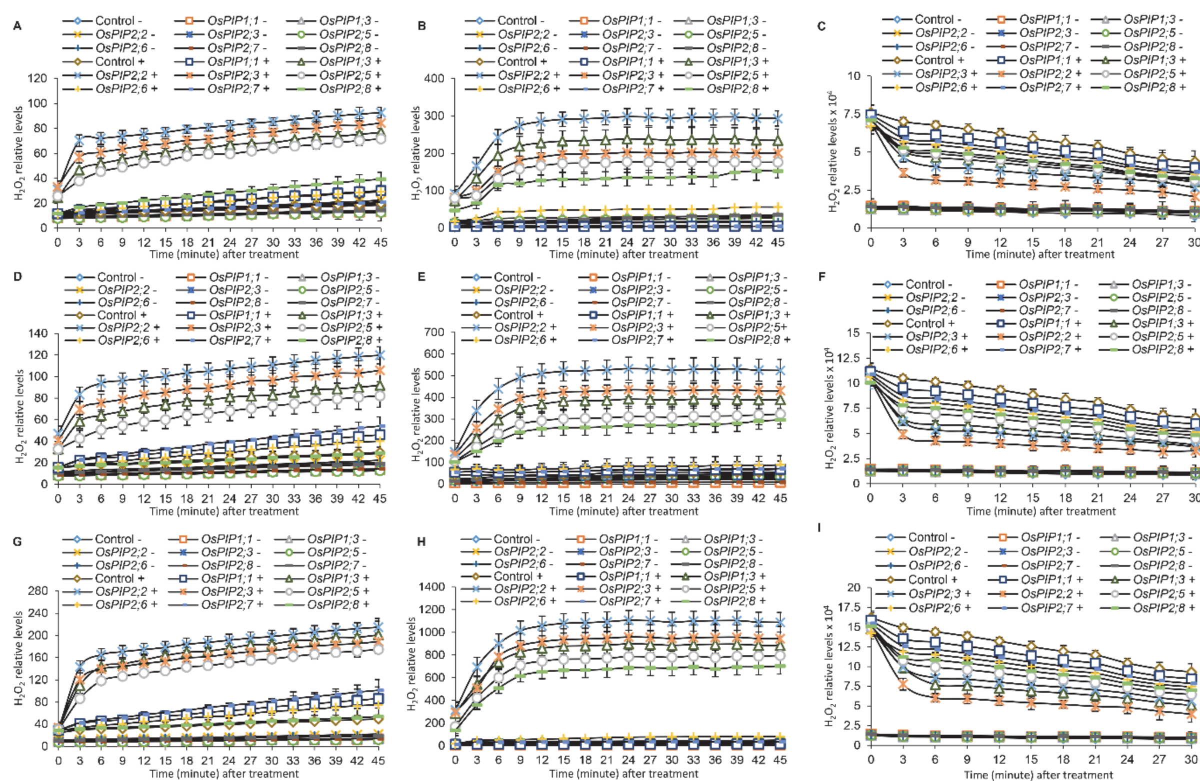


Figure S2. H_2O_2 transport mediated by OsPIPs in yeast. A-C, Chronological changes of H_2O_2 content in yeast cells after treatment with 0 or 300 μM H_2O_2 detected by 2',7'-dichlorofluorescin diacetate (H_2DCF-DA), Amplex Red (AR), and Amplex Ultra Red (AUR) probes respectively. D-F, Chronological changes of H_2O_2 content in yeast cells after treatment with 0 or 500 μM H_2O_2 detected by H_2DCF-DA, AR, and AUR probes respectively. G-I, Chronological changes of H_2O_2 content in yeast cells after treatment with 0 or 1000 μM H_2O_2 detected by H_2DCF-DA, AR, and AUR probes respectively. + indicates supplied with H_2O_2, - indicates supplied with H_2O as control. Data are shown as means ± SEM (n = 8). These experiments were performed at least twice with similar results.

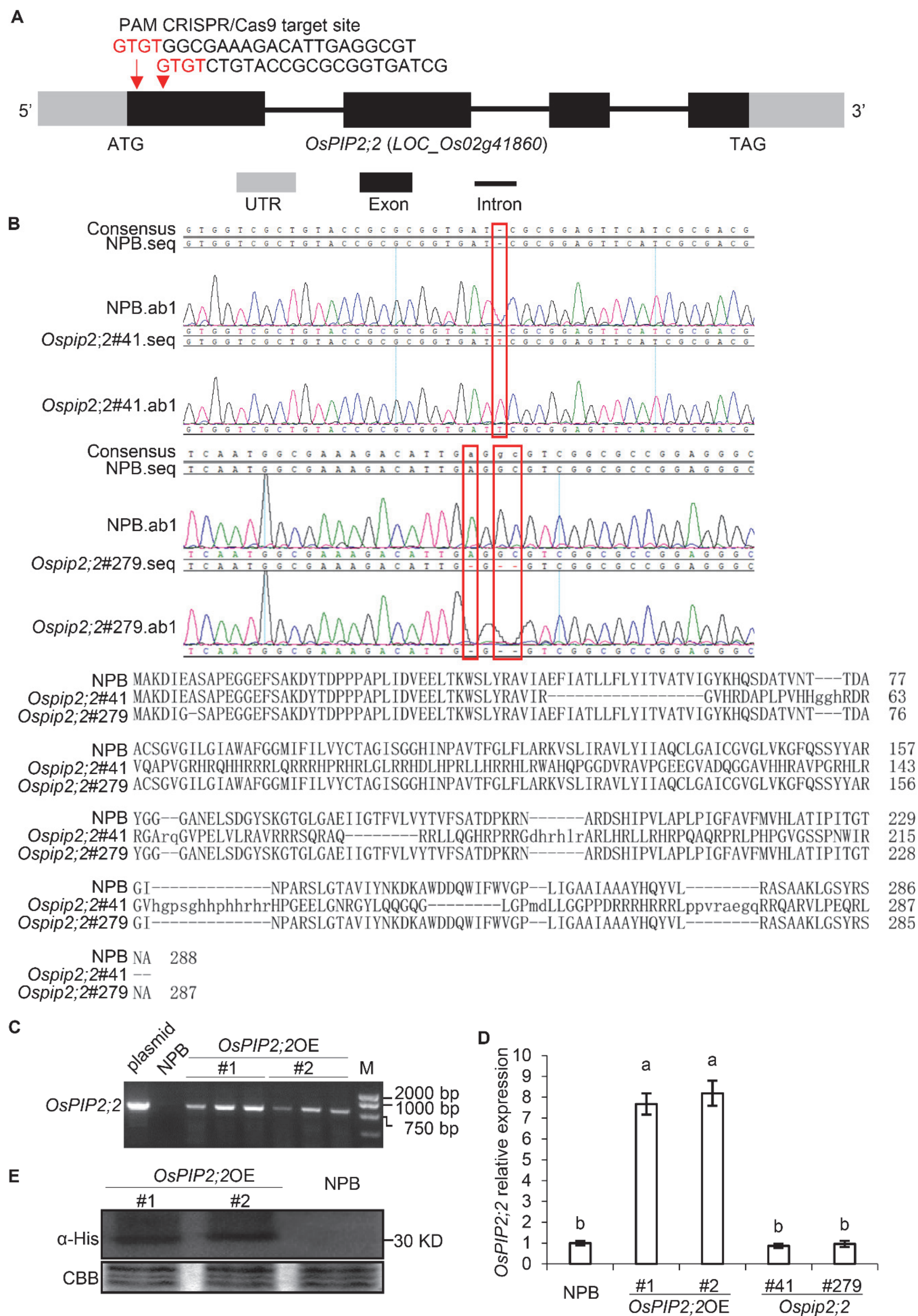
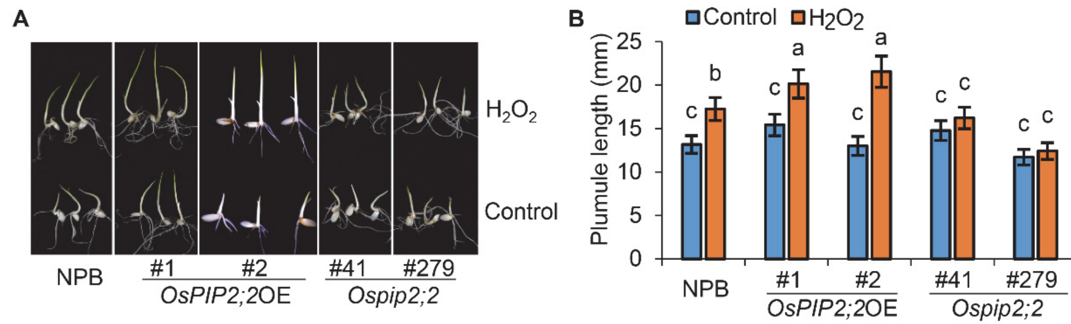


Figure S3. Confirmation of the *OsPIP2;2* mutation and overexpression lines. A, Schematic presentation of the OsPIP2;2 structure and gene editing sites. PAM, Protospacer

adjacent motif. Red arrows were used to show editing sites in *OsPIP2;2*. B, Sanger sequencing chromatograph of the target sites in *OsPIP2;2* and amino acid sequence alignment between OsPIP2;2 and the mutant lines. The red boxes were used to show mutation sites in *OsPIP2;2*. C, Confirmation of the *OsPIP2;2* overexpression lines by genomic PCR. M, DL2000 DNA marker. Plasmid, the pCAMBIA1301-*OsPIP2;2*-*His* plasmid as a positive control. D, Relative expression of *OsPIP2;2* in NPB, *OsPIP2;2*OE and *Ospip2;2* lines. Expression level of *OsPIP2;2* was shown as the ratio of transcript quantities between transgenic and NPB plants. Data are shown as means ± SEM (n = 4). NPB, Wild-type Nipponbare. Lowercase letters indicate significant differences at $P \leq 0.01$ followed Duncan's multiple range tests and one-way ANOVA. The experiment was performed twice with simliar results. E, Detection of OsPIP2;2 protein expression by western blotting. The OsPIP2;2 protein was detected by His antibody, and coomassie brilliant blue (CBB) staining was used to show protein loading (bottom panel).



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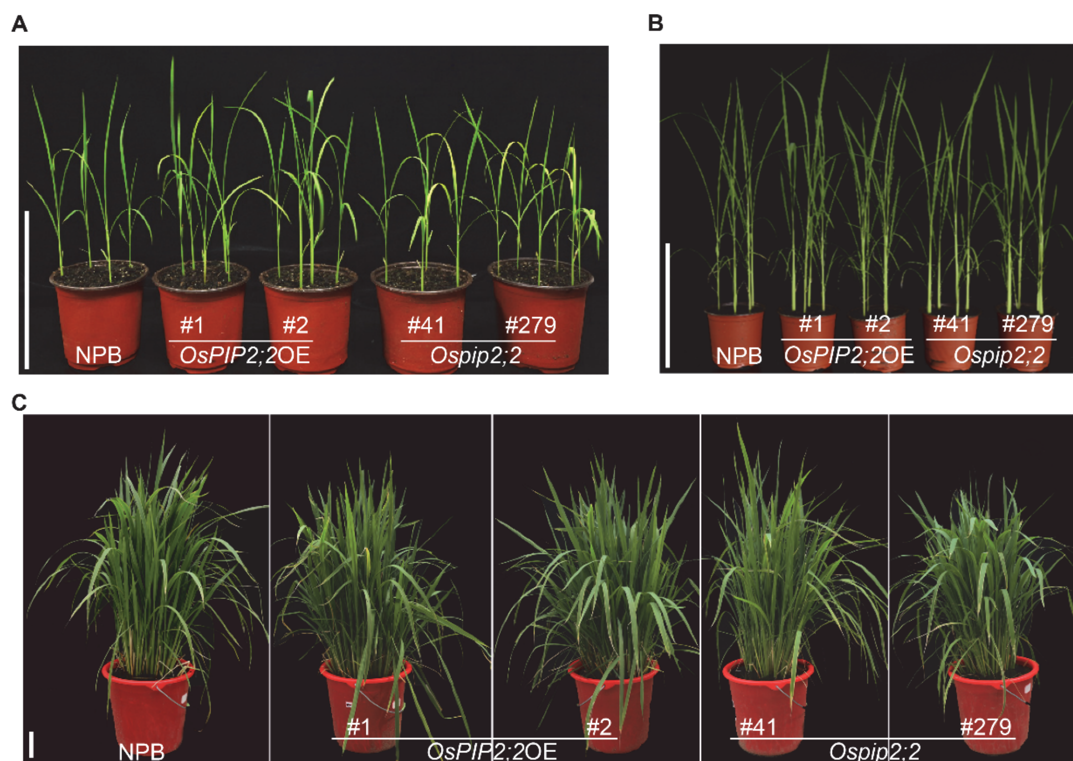
2 **Figure S4. Contribution of OsPIP2;2 in H_2O_2-mediated cell elongation.** A, Wild-type
3 Nipponbare (NPB), *OsPIP2;2*OE, and *Ospip2;2* seedlings were grown on 1/2 MS medium
4 containing 0.5 mM H_2O_2 at 28ºC for 7 days. Data are shown as means ± SEM (n = 10). B,
5 Quantification of plumule lengths after H_2O_2 treatment. Lowercase letters indicate significant
6 differences at $P \leq 0.01$ followed Duncan's multiple range tests and one-way ANOVA. The
7 experiment was performed at least twice with similar results.



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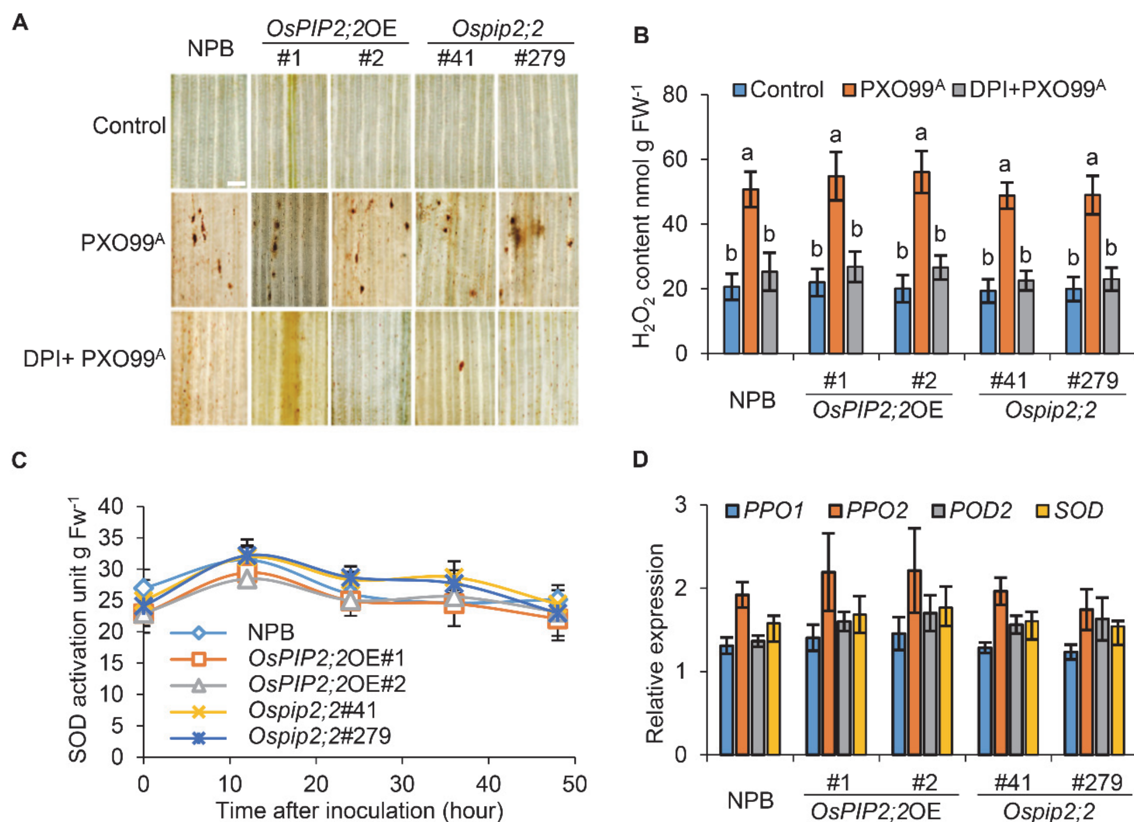
9 **Figure S5. Growth and development of transgenic lines in the greenhouse.** A, Fifiteen-
10 day-old rice seedlings. B, One-month-old seedlings. C, Three-month-old rice plants. Scale
11 bar, 10 cm.



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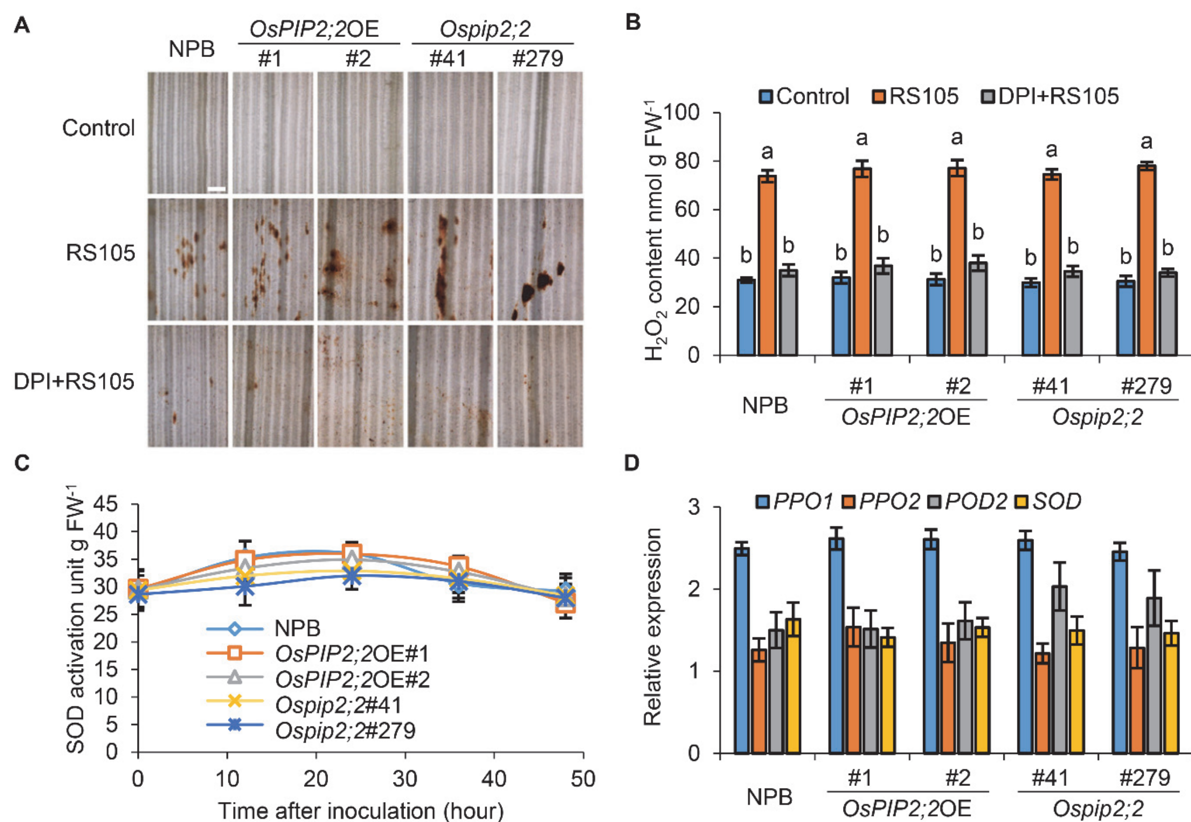
13 **Figure S6. Analysis of the H_2O_2 content in rice after infection with PXO99^A.** A, 3,3-
14 diaminobenzidine (DAB) staining of H_2O_2 production in NPB, *OsPIP2;2*OE, and *Ospip2;2*
15 plants at 24 h after inoculation with PXO99^A. Scale bar = 50 µm, applying to all the images.
16 B, Total H_2O_2 content in rice 8 h post inoculation with PXO99^A. Leaf tissues were ground
17 into powder, and mixed with 20 mM phosphate buffer (pH 6.5). The Amplex Red probe was
18 used to detecte total H_2O_2 content. Data are shown as means ± SEM (n = 4). C, Chronological
19 changes of superoxide dismutase (SOD) activity in leaves of rice in response to PXO99^A.
20 Data are shown as means ± SEM (n = 4). D, Relative expression of H_2O_2-related genes after
21 PXO99^A inoculation. Data are shown as means ± SEM (n = 3). DPI, diphenylene iodonium.
22 Lowercase letters indicate significant differences at $P \leq 0.01$ followed Duncan's multiple
23 range tests and one-way ANOVA. These experiments were performed at least twice with
24 similar results.



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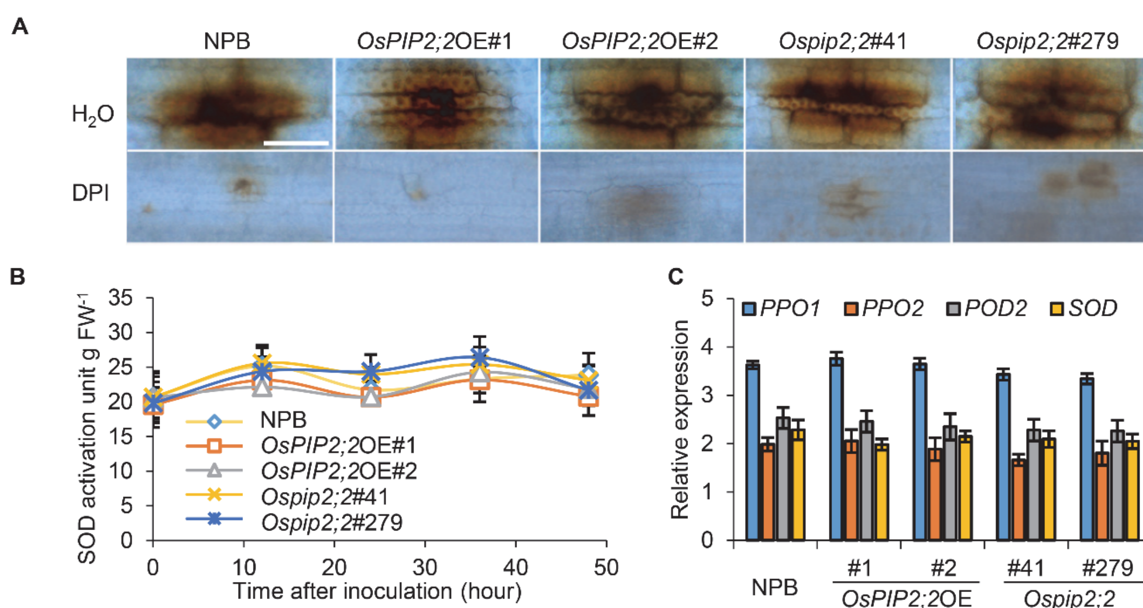
26 **Figure S7. Analysis of the H_2O_2 content in rice after infection with RS105.** A, 3,3-
27 diaminobenzidine (DAB) staining of H_2O_2 production in rice at 24 h after inoculation with
28 RS105. Scale bar = 50 µm, applying to all the images. B, H_2O_2 content in rice 8 h post
29 inoculation with RS105, Amplex Red was used to detecte total H_2O_2 in leaves of rice. Data
30 are shown as means ± SEM (*n* = 4). C, Chronological changes of superoxide dismutase
31 (SOD) activity in leaves of rice in response to RS105. Data are shown as means ± SEM (*n* =
32 4). D, Relative expression of H_2O_2-related genes after inoculation with RS105. Data are
33 shown as means ± SEM (*n* = 3). DPI, diphenylene iodonium. Lowercase letters indicate
34 significant differences at $P \leq 0.01$ followed Duncan's multiple range tests and one-way
35 ANOVA. These experiments were performed at least twice with similar results.



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37 **Figure S8. Analysis of the H_2O_2 content in rice after infection with HB3.** A, 3,3-

38 diaminobenzidine (DAB) staining of H_2O_2 production in rice at 24 h after infection with

39 HB3. Scale bar = 50 µm, applying to all the images. B, Chronological changes of superoxide

40 dismutase (SOD) activity in leaves of rice in response to HB3. Data are shown as means ±

41 SEM (*n* = 4). C, Relative expression of H_2O_2-related genes after inoculation with HB3. Data

42 are shown as means ± SEM (*n* = 3). DPI, diphenylene iodonium. These experiments were

43 performed at least twice with similar results.

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                                                            TMD1
OsPIP2;2    MAKDIEASAPEGGEFSAKDYTDPPPAPLIDVEELTKWSLYRAVIAEFIATLLFLYITVAT 60
ZmPIP2;5    MAKDIEAAAAHEG----KDYSDPPPAPLVDAEELTKWSLYRAVIAEFVATLLFLYITVAT 56
AtPIP2;1    MAKDVEAVPGEGFQT—RDYQDPPPAPFIDGAELKKWSFYRAVIAEFVATLLFLYITVLT 58
SoPIP2;1    MGKDIEVG-GDHRRELAKDYQDPPPSPLFDGEELGKWSFYRALIAEFIATMLFLYITVLT 59
            *.**:*.    .       :** ****:*:.*  ** ***:***:****:**:******* *
                                         TMD2
OsPIP2;2    VIGYKHQSDATVNTTDAACSGVGILGIAWAFGGMIFILVYCTAGISGGHINPAVTFGLFL 120
ZmPIP2;5    VIGYKHQTDAAASGPDAACGGVGVLGIAWAFGGMIFILVYCTAGVSGGHINPAVTFGLFL 116
AtPIP2;1    VIGYKIQSDTDAGG—VDCGGVGILGIAWAFGGMIFILVYCTAGISGGHINPAVTFGLFL 116
SoPIP2;1    VIGHKSQ------NATDQCGGVGILGIAWAFGGMIFVLVYCTAGISGGHINPAVTFGLLL 113
            ***:* *           *.***:************:*******:*************:*
                          TMD3
OsPIP2;2    ARKVSLIRAVLYIIAQCLGAICGVGLVKGFQSSYYARYGGGANELSDGYSKGTGLGAEII 180
ZmPIP2;5    ARKVSLVRALLYIVAQCLGAICGVGLVKGFQSAFYVRYGGGANELSAGYSKGTGLAAEII 176
AtPIP2;1    ARKVSLPRALLYIIAQCLGAICGVGFVKAFQSSYYTRYGGGANSLADGYSTGTGLAAEII 176
SoPIP2;1    ARKLSLVRAILYMVAQCLGAICGVGLVKAFQSAYYHEYGGGANTLSQGYSKGTGLAAEII 173
            ***:** **:**::***********:**.***::* .****** *: ***.****.****
              TMD4                          TMD5
OsPIP2;2    GTFVLVYTVFSATDPKRNARDSHIPVLAPLPIGFAVFMVHLATIPITGTGINPARSLGTA 240
ZmPIP2;5    GTFVLVYTVFSATDPKRNARDSHVPVLAPLPIGFAVFMVHLATIPITGTGINPARSLGAA 236
AtPIP2;1    GTFVLVYTVFSATDPKRSARDSHVPVLAPLPIGFAVFMVHLATIPITGTGINPARSFGAA 236
SoPIP2;1    GTFVLVYTVFSATDPKRSARDSHVPVLAPLPIGFAVFMVHLATIPVTGTGINPARSFGAA 233
            *****************.*****:*********************:**********:*:*
                            TMD6
OsPIP2;2    VIYNKDKAWDDQWIFWVGPLIGAAIAAAYHQYVLRASAAKLGSYRSNA--- 288
ZmPIP2;5    VIYNNDKAWDDHWIFWVGPFIGAAIAAAYHQYVLRASAAKLGSSASFSR-- 287
AtPIP2;1    VIYNKSKPWDDHWIFWVGPFIGAAIAAFYHQFVLRASGSKSLGSFRSAANV 285
SoPIP2;1    VIFNSKQAWADQWIFWVGPMIGAAIAAIYHQYILRAGFVKALGSFRSSSNM 284
            **:*..:.* *:*******:******* ***::***.   *  .     :
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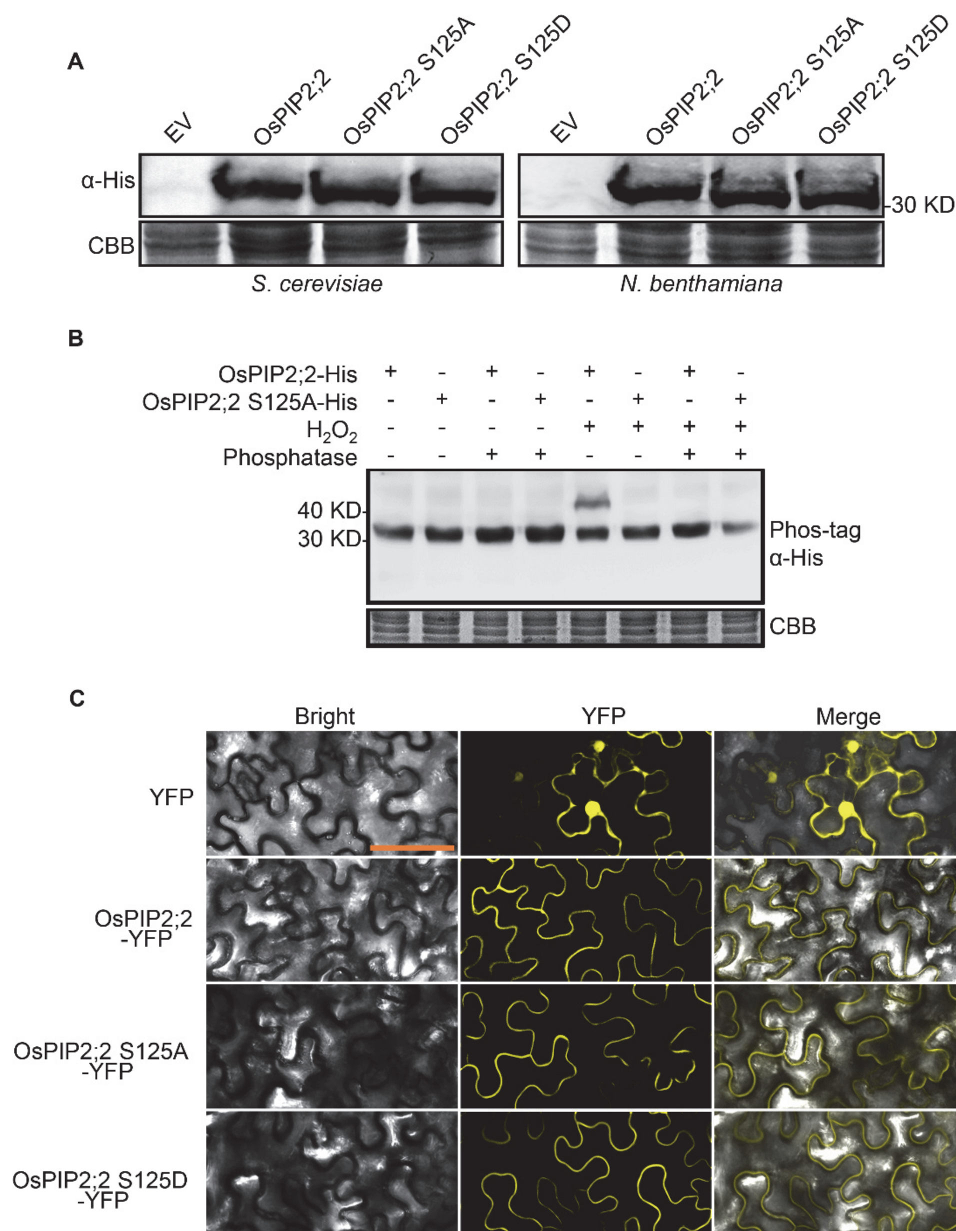
Figure S9. Protein alignment of several PIPs proteins showing conserved S125 residue in OsPIP2;2. TMD1-6, transmembrane domain 1-6. Green boxes, conserved asparagine-proline-alanine (NPA) domain. Red boxes, conserved Ser residue in several PIPs.



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49 **Figure S10. Phosphorylation of OsPIP2;2 at S125 does not change its expression level.**

50 A, Western blot analyzed OsPIP2;2 and its mutant expression in yeast (left) and tobacco

51 (right). Empty vector (EV) was used as negative control. α-His, anti-His antibody. coomassie

52 brilliant blue (CBB) was used to show loading protein (bottom panel). B, Immunoblotting

53 detection of phosphorylation of OsPIP2;2 at S125 induced by H_2O_2 after Phos-tag gel

54 separation. Phospho-proteins can be dephosphorylated by phosphatase. Protein samples

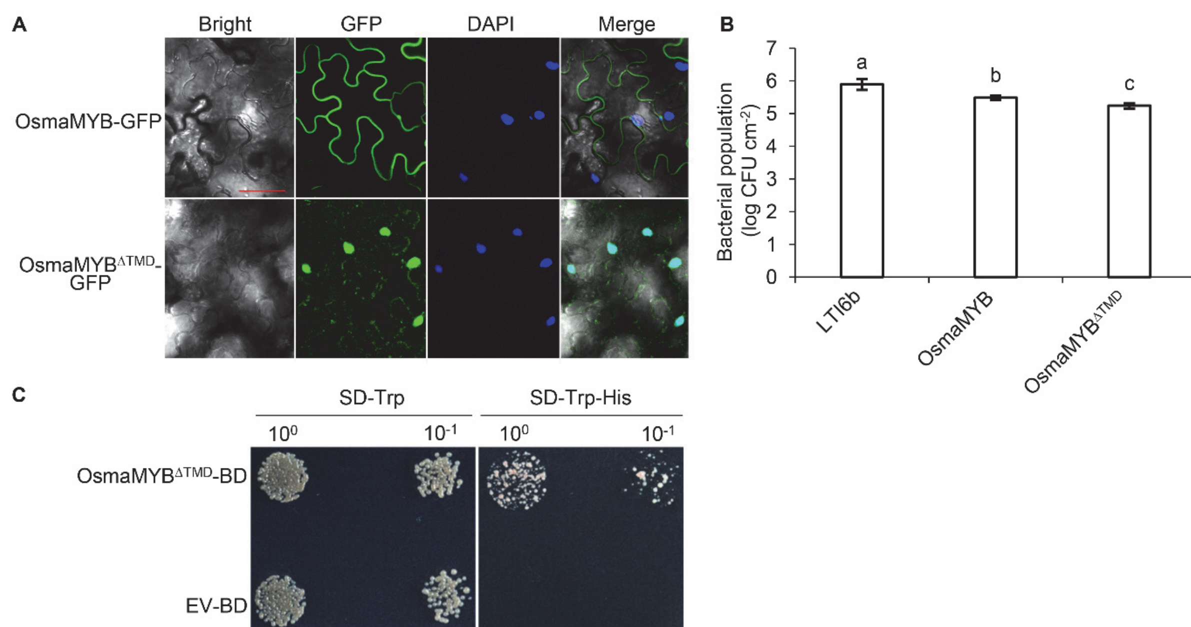
55 treated with (+) or without (–) calf intestinal alkaline phosphatase were separated by Phos-tag

56 gels. The yeast cells were treated with 300 μM H_2O_2 for 30 min. S125A, phosphodeficient

57 mutant of OsPIP2;2; S125D, phosphomimetic mutant of OsPIP2;2, coomassie brilliant blue
58 (CBB) was used to show loading protein (bottom panel). These experiments were performed
59 at least twice with similar results. C, Subcellular localization of OsPIP2;2 and its mutants in
60 *N. benthamiana* (Figure 4F duplicate placed here to show coorelations of the responses with
61 each other). Scale bar = 50 µm, applying to all the images. S125A, phosphodeficient mutant
62 of OsPIP2;2; S125D, phosphomimetic mutant of OsPIP2;2.

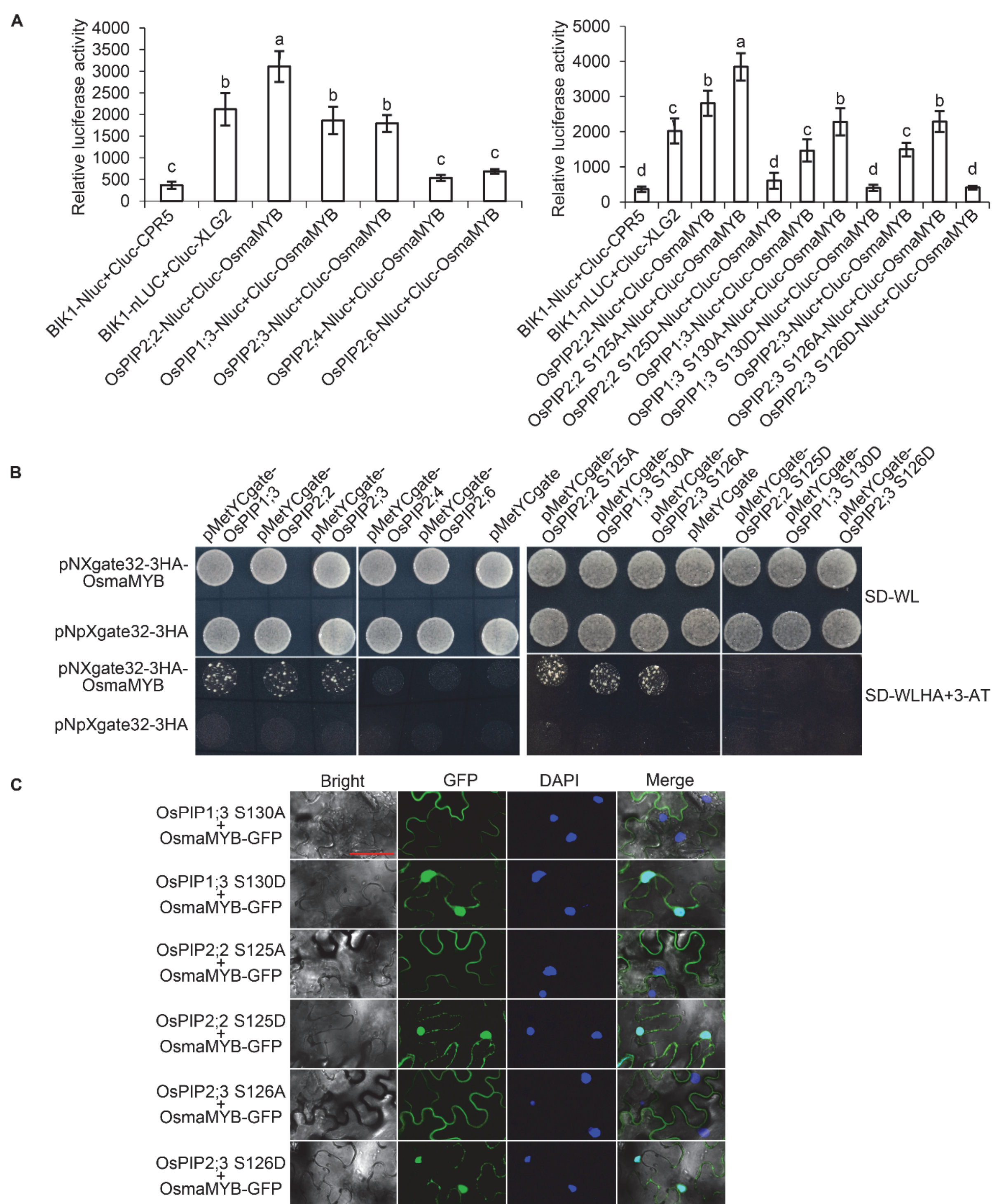
63

64 **Figure S11. Phylogenetic analysis of maMYBs.** A, Protein sequence alignment of
65 maMYBs. TMD, transmembrane domain. R2, a conserved MYB domain. NLS, nuclear
66 localization signal. B, Cluster analysis of maMYBs. Bootstrap values indicate the confidence
67 of each branch, and the scale indicates branch length (an average of 0.2 substitutions per site).
68 Os, *Oryza sativa*. Td, *Triticum dicoccoides*. Zm, *Zea mays*. At, *Arabidopsis thaliana*. Rh,
69 *Rosa hybrida*. Sly, *Solanum lycopersicum*.



70

71 **Figure S12. Promotion of plant resistance by OsmaMYB C terminus.** A, Subcellular
72 localization of OsmaMYB and OsmaMYB$^{\Delta TMD}$ in *N. benthamiana*. GFP, green-fluorescent
73 protein. DAPI, 4,6-diamidino-2-phenylindole, a nuclear staining dye. To provide
74 fluorescence-undetectable background, GFP and DAPI fluorescence signals were captured
75 under lasers intensity setings as 2% and 6%, respectively. Scale bar = 50 µm, applying to all
76 the images. B, Titers of DC3000 ΔhopQ1-1 3 days post-inoculation. *A. tumefaciens* was used
77 to transiently express OsmaMYB and OsmaMYB$^{\Delta TMD}$ on one-half of an *N. benthamiana* leaf
78 and LTI6b control on the other. Data are shown as means ± SEM (n = 6). ΔTMD, deletion of
79 the transmembrane domain (TMD). Lowercase letters indicate significant differences at $P \leq$
80 0.01 followed Duncan's multiple range tests and one-way ANOVA. C, Transactivation
81 activity assay of OsmaMYB in yeast. BD, GAL4 DNA-binding domain; ΔTMD, deletion of
82 the transmembrane domain. Empty vector (EV) served as a negative control. These
83 experiments were performed at least twice with similar results.



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85 **Figure S13. Association between OsmaMYB and OsPIPs.** A, Association of OsPIP1;3,
86 OsPIP2;2, OsPIP2;3 or their mutants with OsmaMYB indicated by the split-luciferase
87 complementation assay. The indicated constructs were co-expressed in *N. benthamiana*
88 leaves and then luciferase activities were examined. Cluc-CPR5 and BIK1-Nluc were used as
89 negative control, and Cluc-XLG2 and BIK1-Nluc were used as positive control. Data are
90 shown as means ± SEM (n = 8). Lowercase letters indicate significant differences at $P \leq 0.01$

91 followed Duncan's multiple range tests and one-way ANOVA. B, Association of OsPIP1;3,
92 OsPIP2;2, OsPIP2;3 or their mutants with OsmaMYB in the split-ubiquitin yeast two-hybrid
93 system. Yeast cells co-transformed with bait and prey vectors were grown on SD-Trp-Leu-
94 His-Ade (SD-WLHA) medium supplemented with 10 mM of 3-amino-1,2,4-triazole (3-AT).
95 C, Subcellular localization of OsmaMYB-GFP after co-expression with phosphorylation
96 mutants of OsPIP1;3, OsPIP2;2, or OsPIP2;3 in *N. benthamiana*. To provide fluorescence-
97 undetectable background, GFP and DAPI fluorescence signals were captured under lasers
98 intensity setings as 2% and 6%, respectively. To provide fluorescence-undetectable
99 background, GFP and DAPI fluorescence signals were captured under lasers intensity setings
100 as 2% and 6%, respectively. S130A, phosphonull mutant of OsPIP1;3. S130D,
101 phosphomimetic mutant of OsPIP1;3. S126A, phosphonull mutant of OsPIP2;3. S126D,
102 phosphomimetic mutant of OsPIP2;3. GFP, green-fluorescent protein. DAPI, 4,6-diamidino-
103 2-phenylindole, a nuclear staining dye. Scale bar = 50 μm, applying to all the images.

104 **Table S1.** List of primers used in this study.

Name	Gen ID	Primer sequence (5' to 3')
Primers for qRT-PCR		
q*PIP1;1*-F	LOC_Os02 g44630	CGCAATCGTGATGTCCTGTT
q*PIP1;1*-R		CACGATTGAGTTGTTCAGGGTT
q*PIP1;2*-F	LOC_Os04 g47220	GCTCCGACGACAAGGACTAC
q*PIP1;2*-R		AGGAAGGTGGCCATGAACTC
q*PIP1;3*-F	LOC_Os02 g57720	ACCGTCTGGTGATCGATGAAG
q*PIP1;3*-R		TCCGCACACACAAGTACCAT
q*PIP2;1*-F	LOC_Os07 g26690	GCAGCCATTGTTGGGGGATA
q*PIP2;1*-R		GCTGCTGAAACAAAACGACCA
q*PIP2;2*-F	LOC_Os02 g41860	TGCATTTCGCCTCGTGGATA
q*PIP2;2*-R		CAAACTTGGAAGCACCAGCG
q*PIP2;3*-F	LOC_Os04 g44060	AGCTGACCAAGTGGTCCCTG
q*PIP2;3*-R		GACTGGTGCTTGTACCCGAT
q*PIP2;4*-F	LOC_Os07 g26630	ACAGAGCACCTGTTCGTCAG
q*PIP2;4*-R		AGACAACAGAGGGACAGAGTTT
q*PIP2;5*-F	LOC_Os07 g26660	CCGTGTTCATGGTGCACCT
q*PIP2;5*-R		CCGGGTTGATGCCGGT
q*PIP2;6*-F	LOC_Os04 g16450	GCGGCGTATCACCAGTACA
q*PIP2;6*-R		ATGACCAAGTCCAACCAGGC
q*PIP2;7*-F	LOC_Os09 g36930	GTGTGCGTATGTTGTCGTGG
q*PIP2;7*-R		GAATGGCGATCGCATGCATTA
q*PIP2;8*-F	LOC_Os03 g64330	CTGTTGGTGTGCATCAGTGT
q*PIP2;8*-R		GCAGTACACCAGCACGAAGAT
q*Actin1*-F	Os05g0438 800	TGAAGATCAAGGTGGTCGCC
q*Actin1*-R		CACAATGGATGGGCCAGACT
q*PPO1*-F	LOC433705 5	TATGCGACTTGCTGGACGAC
q*PPO1*-R		ACTTAGCGTAGCCGATGCTG
q*PPO2*-F	LOC432412	CTCAAGTGCCAGTTAGGCCA

q*PPO2*-R		ATTCGCATCACACACGCAAC
q*POD1*-F	LOC4326969	TTCCACGACTGCTTCGTCAG
q*POD1*-R		GGCGTCGATCACCTCGAAA
q*SOD*-F	LOC4340091	GATAGATAGCGCGGCAGAGG
q*SOD*-R		GCGGAGTAGTGAGAGCGTAG
q*PR1a*-F	LOC4342317	CGTGTCGGCGTGGGTGT
q*PR1a*-R		GGCGAGTAGTTGCAGGTGATG
q*PR10*-F	LOC_Os03g18850	CCATGAAGCTTAACCCCGATG
q*PR10*-R		AGCTTGCCCACCTTGCTTT
q*Pot2*-F	MGG_05850	ACGACCCGTCTTTACTTATTTGG
q*Pot2*-R		AAGTAGCGTTGGTTTTGTTGGAT
Primers for over-expression constructs to H_2O_2 translocation assay		
*PIP1;1-Bam*H I -F		GGGATCCATGGAGGGGAAGGAGGAGGAC
PIP1;1-Xba I-R		GCTCTAGAAGACCTGCTCTTGAATGGGATC
*PIP1;2-Bam*H I-F		GGGATCCCATGGAGGGGAAGGAGGAGG
PIP1;2-Xba I-R		GCTCTAGACGACCTGCTCTTGAATGGAATT
*PIP1;3-Bam*H I-F		GGGATCCATGGAGGGGAAGGAGGAGGATG
PIP1;3-Xba I-R		GCTCTAGAGTCCCGGCTCTTGAAGGGGA
*PIP2;1-Bam*H I-F		GGGATCCATGGGGAAGGACGAGGTGATG
PIP2;1-Xba I-R		GCTCTAGACGCGTTGCTCCTGAAGGAGC
*PIP2;2-Bam*H I-F		GGGATCCATGGCGAAAGACATTGGGTCG
PIP2;2-Xba I-R		GCTCTAGAGGCGTTGCTCCGGTAGGAC
*PIP2;3-Bam*H I-F		GGGATCCATGGCGAAGGACATTGAGGC
PIP2;3-Xba I-R		GCTCTAGAGCCGCGGAAGGAGGAGGAAGA
*PIP2;4-Bam*H I-F		GGGATCCATGGGCAAAGAGGTGGACGT
PIP2;4-Xba I-R		GCTCTAGACGCGTTGCTCCGGAAGGAG
*PIP2;5-Bam*H I-F		GGGATCCATGGGCAAAGAGGCCGACGTC
PIP2;5-Xba I-R		GCTCTAGAAGCATTGCTCCGGAAGGAG
*PIP2;6-Bam*H I-F		GGGATCCATGTCGAAGGAGGTGAGCGAG
PIP2;6-Xba I-R		GCTCTAGAGTTGCTGGGGTTGCTCCGGAA
*PIP2;7-Bam*H I-F		GGGATCCATGGCGTCGAAGGAGGAGGT
PIP2;7-Xba I-R		GCTCTAGACGCCGTCACGCTGGTGC
PIP2;8-BamH I-F		GGGATCCATGGCTGCAGGCAGCGGCA
PIP2;8-Xba I-R		GCTCTAGAAAAATGGGGTGATCGGTAGGAGG
Primers for PIPs mutation (overlap-PCR)		
PIP1;3 S130A-mutation-F		ATTAAGCTTGGTACCGAGCTCATGGAGGGGAAGGAGGAGGATGTG
PIP1;3 S130A-mutation-		AACACCGCCCGCGTCAGCGCCAGCTTCCGCGCCA

R		
PIP1;3 S130A-mutation-F		TGGCGCGGAAGCTGGCGCTGACGCGGGCGGTGTT
PIP1;3 S130A-mutation-R		TGATGGATATCTGCAGAATTC GATTAGTCCCGGCTCTTGAAGGGGAT
PIP1;3 S130D-mutation-F		ATTAAGCTTGGTACCGAGCTCATGGAGGGGAAGGAGG AGGATGTG
PIP1;3 S130D-mutation-R		AACACCGCCCGCGTCAGGTCCAGCTTCCGCGCCA
PIP1;3 S130D-mutation-F		TGGCGCGGAAGCTGGACCTGACGCGGGCGGTGTT
PIP1;3 S130D-mutation-R		TGATGGATATCTGCAGAATTC GATTAGTCCCGGCTCTTGAAGGGGAT
PIP2;2 S125A-mutation-F		ATTAAGCTTGGTACCGAGCTCATGGCGAAAGACATTGAG GCGTCGGCGCC
PIP2;2 S125A-mutation-R		CCGCCCTGATCAGCGCCACCTTCCTCGCC
PIP2;2 S125A-mutation-F		GGCGAGGAAGGTGGCGCTGATCAGGGCGG
PIP2;2 S125A-mutation-R		TGATGGATATCTGCAGAATTCGGCGTTGCTCCGGTAGGA CCCGAGCTTGG
PIP2;2 S125D-mutation-F		ATTAAGCTTGGTACCGAGCTCATGGCGAAAGACATTGAG GCGTCGGCGCC
PIP2;2 S125D-mutation-R		CCGCCCTGATCAGGTCCACCTTCCTCGCC
PIP2;2 S125D-mutation-F		GGCGAGGAAGGTGGACCTGATCAGGGCGG
PIP2;2 S125D-mutation-R		TGATGGATATCTGCAGAATTC GGCGTTGCTCCGGTAGGACCCGAGCTTGG
PIP2;3 S126A-mutation-F		ATTAAGCTTGGTACCGAGCTCATGGCGAAGGACATTGAG GCGGCGGCGG
PIP2;3 S126A-mutation-R		GCGCGCACCAGCGCCACCTTGCGCGC
PIP2;3 S126A-mutation-F		GCGCGCAAGGTGGCGCTGGTGCGCGC
PIP2;3 S126A-mutation-R		TGATGGATATCTGCAGAATTCGCCGCGGAAGGAGGAGG AAGAGCCGAGC
PIP2;3 S126D-mutation-F		ATTAAGCTTGGTACCGAGCTCATGGCGAAGGACATTGAG GCGGCGGCGG
PIP2;3 S126D-mutation-R		GCGCGCACCAGCGTCACCTTGCGCGC
PIP2;3 S126D-mutation-F		GCGCGCAAGGTGACGCTGGTGCGCGC
PIP2;3 S126D-mutation-R		TGATGGATATCTGCAGAATTCGCCGCGGAAGGAGGAGG AAGAGCCGAGC
Primers for yeast two-hybrid (homologous recombination)		

PIP1;3/*PIP1;3 S130A*/*PIP1;3 S130D*-F		ACAAGTTTGTACAAAAAAGCAGGCTCTCCAACCACCATGGAGGGGAAGGAGGAGGATGTG
PIP1;3/*PIP1;3 S130A*/*PIP1;3 S130D*-R		TCCGCCACCACCAACCACTTTGTACAAGAAAGCTGGGTAGTCCCGGCTCTTGAAGGGGAT
PIP2;2/*PIP2;2 S125A*/*PIP2;2 S125D*-F		ACAAGTTTGTACAAAAAAGCAGGCTCTCCAACCACCATGGCGAAAGACATTGAGGCGTC
PIP2;2/*PIP2;2 S125A*/*PIP2;2 S125D*-R		TCCGCCACCACCAACCACTTTGTACAAGAAAGCTGGGTAGGCGTTGCTCCGGTAGGACC
maMYB-F	LOC4344711	ACAAGTTTGTACAAAAAAGCAGGCTCTCCAACCACCATGGAGTTCATCGACGACGACTG
maMYB-R		TCCGCCACCACCAACCACTTTGTACAAGAAAGCTGGGTATGGAGCTGCCTCCGATGCTG
PIP2;3/*PIP2;3 S126A*/*PIP2;3 S126D*-F		ACAAGTTTGTACAAAAAAGCAGGCTCTCCAACCACCATGGCGAAGGACATTGAGGCG
PIP2;3/*PIP2;3 S126A*/*PIP2;3 S126D*-R		TCCGCCACCACCAACCACTTTGTACAAGAAAGCTGGGTAGCCGCGGAAGGAGGAGGAAGA
PIP2;4-F		ACAAGTTTGTACAAAAAAGCAGGCTCTCCAACCACCATGGGCAAAGAGGTGGACGT
PIP2;4-R		TCCGCCACCACCAACCACTTTGTACAAGAAAGCTGGGTACTACGCGTTGCTCCGGA
PIP2;6-F		ACAAGTTTGTACAAAAAAGCAGGCTCTCCAACCACCATGTCGAAGGAGGTGAGCGA
PIP2;6-R		TCCGCCACCACCAACCACTTTGTACAAGAAAGCTGGGTAGTTGCTGGGGTTGCTCC
Primers for co-IP		
PIP2;2-Kpn I-F		GGTACCCATCATCACCATCACCATATGGCGAAAGACATTGAGGCGTC
PIP2;2-Xba I-R		TCTAGAGGCGTTGCTCCGGTAGGACC
maMYB-Kpn I-F		GGTACCATGGAGTTCATCGACGACGACTG
maMYB-Xba I-R		CTTGTCATCGTCATCCTTGTAGTCGATGTCATGATCTTTATAATCACCGTCATGGTCTTTGTAGTCTGGAGCTGCCTCCGATGCTG
Primers for Split-luciferase complementation assays		
PIP1;3/*PIP1;3 S130A*/*PIP1;3 S130D-Kpn* I-F		GGTACCATGGAGGGGAAGGAGGAGGATGTG
PIP1;3/*PIP1;3 S130A*/*PIP1;3 S130D-Sac* I-R		GTCGACTAGTCCCGGCTCTTGAAGGGGAT
PIP2;2/*PIP2;2 S125A*/*PIP2;2 S125D-Kpn* I-F		GGTACCATGGCGAAAGACATTGAGGCGTC
PIP2;2/*PIP2;2 S125A*/*PIP2;2 S125D-Sac* I-R		GTCGACGGCGTTGCTCCGGTAGGACC

PIP2;3/*PIP2;3 S126A*/*PIP2;3 S126D-Kpn* I-F		GGTACCATGGCGAAGGACATTGAGGCG
PIP2;3/*PIP2;3 S126A*/*PIP2;3 S126D-Sac* I-R		GTCGACGCCGCGGAAGGAGGAGGAAGA
maMYB-Kpn I-F		GGTACCATGGAGTTCATCGACGACGACTG
maMYB-Sac I-R		GTCGACTGGAGCTGCCTCCGATGCTG
PIP2;4-Kpn I-F		GGTACCATGGGCAAAGAGGTGGACGT
PIP2;4-Sac I-R		GTCGACCTACGCGTTGCTCCGGA
PIP2;6-Kpn I-F		GGTACCATGTCGAAGGAGGTGAGCGA
PIP2;6-Sac I-R		GTCGACGTTGCTGGGGTTGCTCC
Primers for subcellular localization construct		
PIP2;2/*PIP2;2 S125A*/ *PIP2;2 S125D-Kpn* I-F		GGTACCATGGCGAAAGACATTGAGGCGTC
PIP2;2/*PIP2;2 S125A*/ *PIP2;2 S125D-Xba* I-R		TCTAGATCTAGAGGCGTTGCTCCGGTAGGACC
maMYB-Kpn I-F		GGTACCATGGAGTTCATCGACGACGACTG
maMYB-Xba I-R		TCTAGATGGAGCTGCCTCCGATGCTG
homologous recombination		
maMYB$^{\Delta TMD}$-F		GCTTTCGCGAGCTCGGTACCATGACCGGCGGCGACGTCTC
maMYB$^{\Delta TMD}$-R		CTAGAGGATCCCCGGGTACCTGGAGCTGCCTCCGATGC
Transactivation assay (homologous recombination)		
maMYB$^{\Delta TMD}$-F		TGGCCATGGAGGCCGAATTCACCGGCGGCGACGTCTC
maMYB$^{\Delta TMD}$-F		CGACGGATCCCCGGGAATTCTGGAGCTGCCTCCGATGC
Primers for transgenic plants		
*pip*2;2-F (mutation)		GTGTGGCGAAAGACATTGAGGCGT/GTGTGCTGTACCGCGCGGTGATCG
*pip*2;2-R (mutation)		AAACACGCCTCAATGTCTTTCGCC/AAACCGATCACCGCGCGGTACAGC
35S:*PIP2;2-Bam*H I-F		GGATCCATGGCGAAAGACATTGAGGCGTC
35S:*PIP2;2-Hin*d III-R		ATGGTGATGGTGATGATGAAGCTTGGCGTTGCTCCGGTAGGACC
Primers for detection transgenic plants		
pip2;2-F		TGAGGCTAAGTCGGTTGTGG
pip2;2-R		ATTTGGGACCTGAGATGCCG
PIP2;2-F		TTCATTTGGAGAGAACACGGGGGAC
PIP2;2-R		CTCTGGAACCCCTTGACGAGCC

105