Aquaporin PIP1;3 of rice and harpin Hpa1 of bacterial blight pathogen cooperate in a type III effector translocation

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Supplementary Data

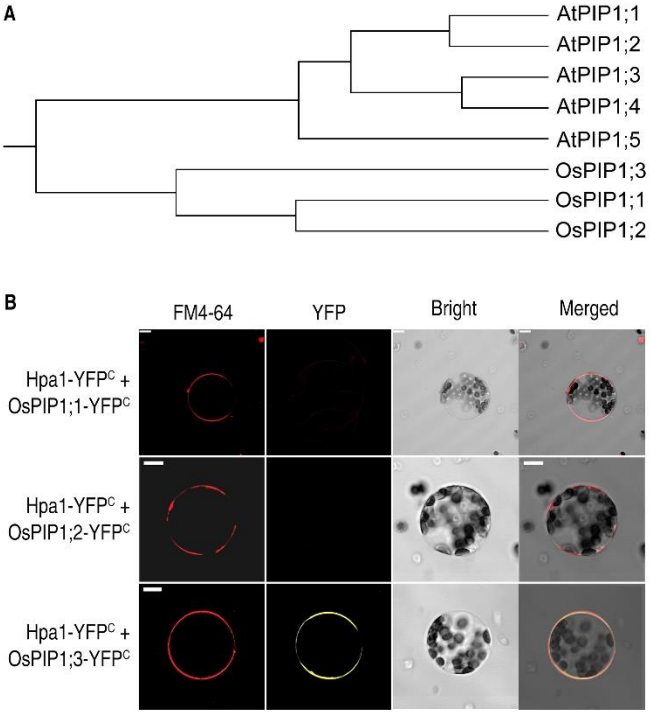
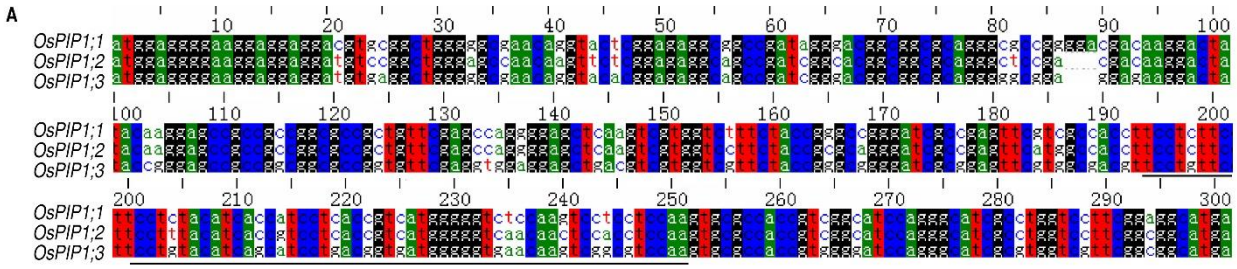
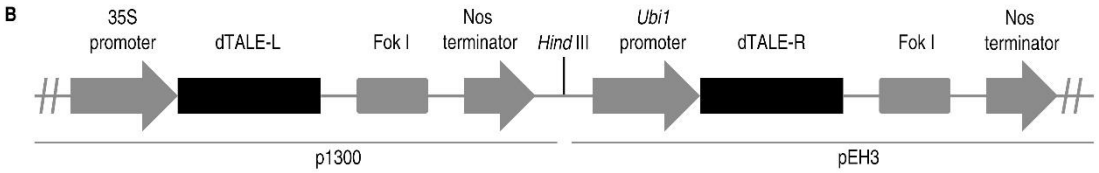
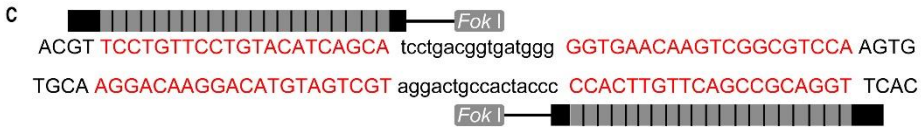


Fig. S1. A phylogenetic tree of Arabidopsis and rice PIP1s established by the MEGA4 program (A) and BiFC tests of OsPIP1s and Hpa1 (B).







D

OsPIP1;3⁻	#6			#9			#15	#25			#36		
T1	-5 (-13/+3)			-7* -4			-11 -11	-23 +4			-8* wt		
T2	-5 -5	-5 (-13/+3)	(-13/+3) (-13/+3)	-7 -7	-7 -4	-4 -4	-11 -11	-23 -23	-23 +4	+4 +4	-8 -8	-8 wt	wt wt
Ratio	3/15	8/15	4/15	6/25	10/25	9/25	14/14	5/22	14/22	3/22	3/19	10/19	6/19

E

TCCTGTTCCTGTACATCAGCAtcctgacggtgatgggGGTGAACAAGTCGGCGTCCA	WT
TCCTGTTCCTGTACATCAGCAtcctg-----gatgggGGTGAACAAGTCGGCGTCCA	-5
TCCTGTTCCTGTACATCAGCAtcc----GAA------GGTGAACAAGTCGGCGTCCA	-13/+3
TCCTGTTCCTGTACATCAGCAtcctgacg-------gGGTGAACAAGTCGGCGTCCA	-7
TCCTGTTCCTGTACATCAGCA----gacggtgatgggGGTGAACAAGTCGGCGTCCA	-4
TCCTGTTCCTGTACATCAGCAtc----------gggGGTGAACAAGTCGGCGTCCA	-11
TCCTGTTCCTGTACATCAGCA----------AAGTCGGCGTCCA	-23
TCCTGTTCCTGTACATCAGCAtcctgacggtAATAgatgggGGTGAACAAGTCGGCGTCCA	+4
TCCTGTTCCTGTACATCAGCAt-------tgatgggGGTGAACAAGTCGGCGTCCA	-8

Fig. S2. Analyses of *OsPIP1;3* knockout by TALENs. (A) Selection of designer TALEN target sites according to *OsPIP1;3* sequence analysis. *OsPIP1;1–1;3* nucleotide sequences were aligned by BioEdit. The underlined sequence region was selected as target of designer TALENs. (B) Schematic diagram of a two-gene expression cassette in a single binary vector designed for *Agrobacterium*-mediated rice transformation. The expression cassette includes *35S* promoter to drive expression of the TALEN-L gene and *Ubi1* promoter to direct transcription of the TALEN-R gene. (C) Designer TALEN constructs and their DNA targets in 191–247 sites of chromosomal *OsPIP1;3* gene. Lowercase letters represent regions wherein two *Fok*I domains dimerize to cause a double stranded DNA break. (D) Genetic segregation of T1 and T2 plants derived from self-pollination of 5 independent T0 primary transgenic plants. Totally 40 independent T0 primary transgenic plants were defined as #1–40 transgenic lines. Each of the two alleles of an individual plant are designated as being wild type (wt) or as having a nucleotide insertion (+) or a deletion

(−) and are separated top and bottom by a dividing line. The designation "−5/(−13/+3)" indicates that one allele contains a deletion of 5 bp and that the other allele has both a deletion of 13 bp and an insertion of 3 bp. Asterisks denote that T1 progenies from lines #9 or #36 lack any selection marker and TALEN gene. The hygromycin resistance gene and TALEN genes were not detected by PCR assays in these TALEN-modified T1 rice plants or their self-pollinated progenies. (E) Sequences of *OsPIP1;3* mutations induced by the designer TALENs with deletions (dashes) and insertions (red letters). TALEN-binding sequences are underlined in WT.

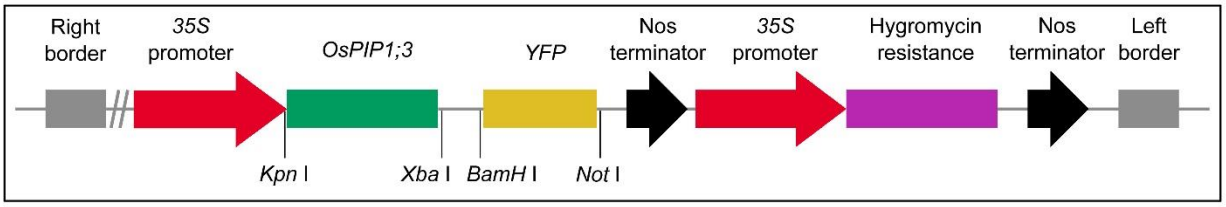


Fig. S3. Schematic diagram of *OsPIP1;3* overexpression construction. Recombinant gene cassette was designed for rice embryo transformation under mediation by *Agrobacterium tumefaciens*. Elements shown are important for gene expression modulations, such as *35S* promoter from cauliflower mosaic virus and maize, respectively. Other elements are required for screening of transgenic plants, such as antibiotic resistance.

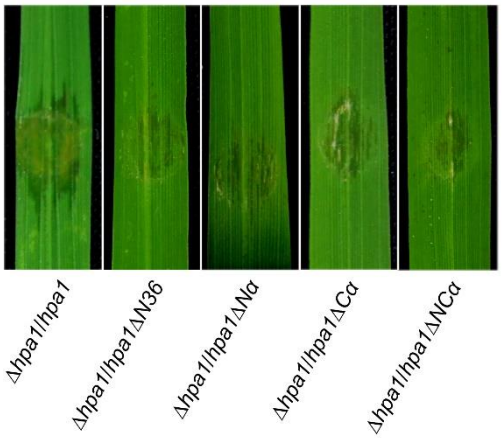


Fig. S4. Deletions of *hpa1* sequence regions affect the virulent function of PthXo1. Here, Rice seedlings were inoculated by leaf infiltration with every bacterial suspension of the indicated strains; leaves were photographed at 5 dpi.

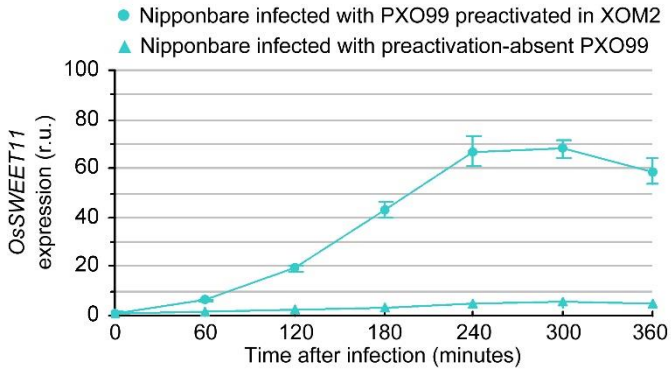


Fig. S5. *Xoo* preactivation is required for infection of rice protoplasts. Preactivation of POX99 cells by induction in the XOM2 medium are capable of infecting rice protoplasts but non-induced bacteria are not. Approximately 2×10^5 protoplasts were infected by co-incubation with a bacterial suspension of the bacterial strains preactivated or not preactivated. Relative units (r.u.) of *SWEET11* gene expression in protoplasts were analyzed by RT-qPCR at the indicated time points. The average expression levels of gene expression at 0 min were defined as 1 to assess gene expression extents at the other time points. Data shown are means ± SEMs; $n = 9$ repetitions from 3 independent experiments.

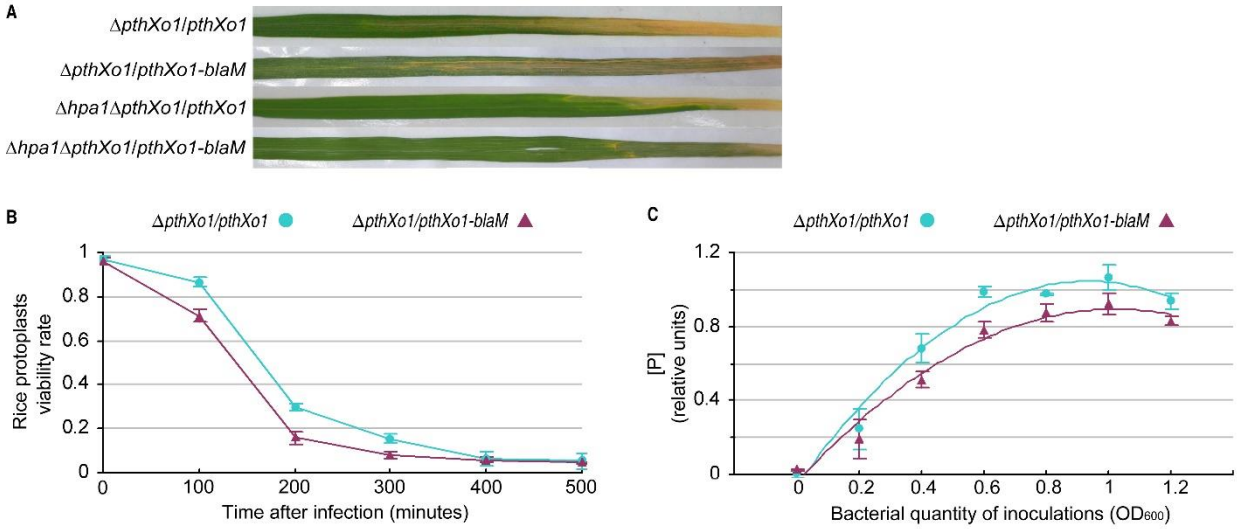


Fig. S6. Optimization of BlaM reporter assays. (A) Nipponbare plants were inoculated by leaf top clipping with a bacterial suspension of the indicated *Xoo* strain. Leaves were photographed at 12 dpi. (B) Nipponbare protoplasts were infected by co-incubated with the indicated bacterial strain. Bacteria had been preactivated by incubation in XOM2 medium. The viability of protoplasts was determined by the FDA staining method. (C) Correlation between bacterial density of inoculation and relative concentrations of [P], the product from enzymatic activity of TALE-BlaM. In all curves, data shown are means ± SEMs; $n = 9$ repetitions from 3 independent experiments.

Fig. S7. Compositions in OsPIP1s.

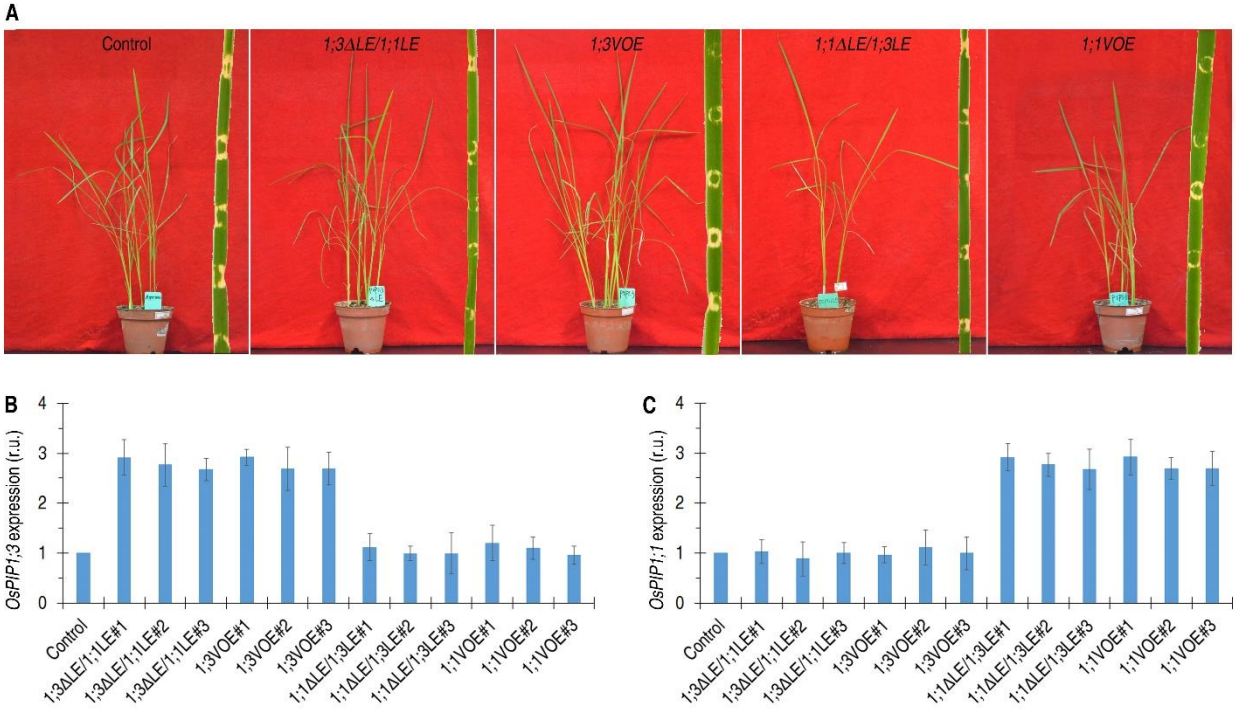


Fig. S8. The rice VMGOE efficiency. (A) Photos of 30-day-old Nipponbare seedlings and leaves at 15 days after transformation with the empty VMGOE vector (Control) or the recombinant vector carrying an insert of the indicated gene. The vector was constructed by using rice tungro bacilliform virus. (B, C) Gene expression was analyzed by RT-qPCR and quantified as relative unit (r.u.) in contrast to the expression defined as 1 in control. Data show are mean values ± SEM estimates; different letters indicate significant differences by Duncan's multiple range tests; $P < 0.01$; $n = 15$ plants tested in 3 independent experiments.

Table S1 Strains and plasmids used and created in this study

Strains or Plasmids	Relevant characteristics	Source/Reference
Strains		
Escherichia coli		
DH5α	F^- 80d*lacZ* M15(*lacZYA-argF*) U169 *endA1 deo*R *rec*A1 *hsd*R17(r_K^- m_K^+) *pho*A *sup*E44 λ^- *thi-l gyr*A96 *rel*A1	This lab
BL21(DE3)	F^- *omp*T *hsd*SB (r_B^- m_B^-) *gal dcm* (DE3)	Novagen
Yeast		
NMY51	MATa his3D200 *trp1-901 leu2-3,112 ade2 LYS2::(lexAop) 4-HIS3 ura3::(lexAop) 8-lacZ ade2::(lexAop)8-ADE2 GAL4*	Obrdlik et al. 2004
Agrobacterium tumefaciens		
GV3101	$Gent^R$	This lab
X. oryzae pv. *Oryzae*		
$PXO99^A$	Philippine race 6; azacytidine resistant clone of $PXO99^A$, virulent to rice cultivars Nipponbare and IRBB10	This lab
P*Δhpa1*	$PXO99^A$ *hpa1* unmarked mutant	This study
P*ΔpthXo1*	$PXO99^A$ *pthXo1* unmarked mutant	This study
P*Δhpa1ΔpthXo1*	$PXO99^A$ *hpa1pthXo1* double unmarked mutant	This study
P*ΔhrcU*	$PXO99^A$ *hrcU* knock-out mutant	This lab
P*Δhpa1/hpa1*	$PXO99^A$ *hpa1* mutant complemented with pHM*hpa1*	This study
P*ΔpthXo1/pthXo1*	$PXO99^A$ *pthXo1* mutant complemented with pHM*pthXo1*	This study
P*Δhpa1ΔpthXo1/hpa1/pthXo1*	$PXO99^A$ *hpa1pthXo1* mutant complemented with pHM*hpa1pthXo1*	This study
$PXO99^A$/*avrXa10*	$PXO99^A$ transformed with pHM*avrXa10*	This study
P*Δhpa1/avrXa10*	$PXO99^A$ *hpa1* mutant transformed with pHM*avrXa10*	This study
P*ΔpthXo1/avrXa10*	$PXO99^A$ *pthXo1* mutant transformed with pHM*avrXa10*	This study
P*Δhpa1ΔpthXo1/avrXa10*	$PXO99^A$ *hpa1pthXo1* mutant transformed with pHM*avrXa10*	This study
P*ΔhrcU/avrXa10*	$PXO99^A$ *hrcU* mutant transformed with pHM*avrXa10*	This study
P*ΔpthXo1/pthXo1-cya*	$PXO99^A$ *pthXo1* mutant complemented with pHM*pthXo1-cya*	This study
P*Δhpa1ΔpthXo1/pthXo1-cya*	$PXO99^A$ *hpa1pthXo1* mutant complemented with pHM*pthXo1-cya*	This study
P*Δhpa1ΔpthXo1/hpa1/pthXo1-cya*	$PXO99^A$ *hpa1pthXo1* mutant complemented with pHM*hpa1pthXo1-cya*	This study
P*ΔhrcU/pthXo1-cya*	$PXO99^A$ *hrcU* mutant transformed with pHM*pthXo1-cya*	This study
$PXO99^A$/*avrXa10-cya*	$PXO99^A$ transformed with pHM*avrXa10-cya*	This study
P*Δhpa1/avrXa10-cya*	$PXO99^A$ *hpa1* mutant transformed with pHM*avrXa10-cya*	This study
P*ΔpthXo1/avrXa10-cya*	$PXO99^A$ *pthXo1* mutant transformed with pHM*avrXa10-cya*	This study
P*ΔhrcU/avrXa10-cya*	$PXO99^A$ *hrcU* mutant transformed with pHM*avrXa10-cya*	This study
P*ΔpthXo1/pthXo1-blaM*	$PXO99^A$ *pthXo1* mutant complemented with pHM*pthXo1-blaM*	This study
P*Δhpa1ΔpthXo1/pthXo1-blaM*	$PXO99^A$ *hpa1 pthXo1* mutant complemented with pHM*pthXo1-blaM*	This study
P*ΔhrcU/pthXo1-blaM*	$PXO99^A$ *ΔhrcU* mutant transformed with pHM*pthXo1-blaM*	This study
$PXO99^A$/*avrXa10-blaM*	$PXO99^A$ transformed with pHM*avrXa10-blaM*	This study
P*Δhpa1/avrXa10-blaM*	$PXO99^A$ *hpa1* mutant transformed with pHM*avrXa10-blaM*	This study
P*ΔhrcU/avrXa10-blaM*	$PXO99^A$ *hrcU* mutant transformed with pHM*avrXa10-blaM*	This study
P*Δhpa1/hpa1ΔN36*	$PXO99^A$ *hpa1* mutant complemented with pHM*hpa1ΔN36* (*hpa1* sequence with a deletion of 1-36 sites in N-terminal)	This study
P*Δhpa1/hpa1ΔNα*	$PXO99^A$ *hpa1* mutant complemented with pHM*hpa1ΔNα* (*hpa1* sequence with a deletion of α-helix in N-terminal)	This study
P*Δhpa1/hpa1ΔCα*	$PXO99^A$ *hpa1* mutant complemented with pHM*hpa1ΔCα* (*hpa1* sequence with a deletion of α-helix in C-terminal)	This study
P*Δhpa1/hpa1ΔNCα*	$PXO99^A$ *hpa1* mutant complemented with pHM*hpa1ΔNCα* (*hpa1* sequence with two deletions of α-helices in NC-terminals)	This study
P*Δhpa1ΔpthXo1/pthXo1-cya/hpa1ΔN36*	$PXO99^A$ *hpa1 pthXo1* mutant complemented with pHM*hpa1ΔN36-pthXo1-cya*	This study

P*Δhpa1ΔpthXo1/pthXo1-cya/hpa1ΔNα*	PXO99^{A} *hpa1 pthXo1* mutant complemented with pHM*hpa1ΔNα-pthXo1-cya*	This study
P*Δhpa1ΔpthXo1/pthXo1-cya/hpa1ΔCα*	PXO99^{A} *hpa1 pthXo1* mutant complemented with pHM*hpa1ΔCα-pthXo1-cya*	This study
P*Δhpa1ΔpthXo1/pthXo1-cya/hpa1ΔNCα*	PXO99^{A} *hpa1 pthXo1* mutant complemented with pHM*hpa1ΔNCα-pthXo1-cya*	This study
P*Δhpa1ΔpthXo1/pthXo1-blaM /hpa1*	PXO99^{A} *hpa1 pthXo1* mutant complemented with pHM*hpa1-pthXo1-blaM*	This study
P*Δhpa1ΔpthXo1/pthXo1-blaM /hpa1ΔN36*	PXO99^{A} *hpa1 pthXo1* mutant complemented with pHM*hpa1ΔN36-pthXo1-blaM*	This study
P*Δhpa1ΔpthXo1/pthXo1-blaM /hpa1ΔNα*	PXO99^{A} *hpa1 pthXo1* mutant complemented with pHM*hpa1ΔNα-pthXo1-blaM*	This study
P*Δhpa1ΔpthXo1/pthXo1-blaM /hpa1ΔCα*	PXO99^{A} *hpa1 pthXo1* mutant complemented with pHM*hpa1ΔCα-pthXo1-blaM*	This study
P*Δhpa1ΔpthXo1/pthXo1-blaM /hpa1ΔNCα*	PXO99^{A} *hpa1 pthXo1* mutant complemented with pHM*hpa1ΔNCα-pthXo1-blaM*	This study
Plasmids		
pK18*sacB*	Suicide vector derivative from pK18mobGII, *sacB*$^{+}$, Km^{R}	This lab
pHM1	Broad-host range vector with pUC19 polylinker, Sp^{R}	This lab
pZW*pthXo1*	PthXo1 fused to *lacZ* promoter of pBluescript II KS(+)	Yang et al. 2006
pZW*avrXa10*	AvrXa10 fused to *lacZ* promoter of pBluescript II KS(+)	Makino et al. 2006
pZW*pthXo1-cya*	Cya tag inserted in the *Sac* I site of pZW*pthXo1*	This study
pZW*avrXa10-cya*	Cya tag inserted in the *Sac* I site of pZW*avrXa10*	This study
pZW*pthXo1-blaM*	BlaM tag inserted in the *Sal* I site of pZW*pthXo1*	This study
pZW*avrXa10-blaM*	BlaM tag inserted in the *Sal* I site of pZW*avrXa10*	This study
pXNubG-gate21(SUS1)	Prey vector (8194 bp), *TRP1*, Amp^{R}, 2μ	Obrdlik et al. 2004
pNubGX-gate32(SUS2)	Prey vector (8177 bp), *TRP1*, Amp^{R}, 2μ	Obrdlik et al. 2004
pMetYCub-gate(SUS5)	Bait vector (10033 bp), *LEU2*, Amp^{R}, CEN/ARS	Obrdlik et al. 2004
pKAT1-Cub(SUS6)	Control vector, *LEU2*, Amp^{R}, CEN/ARS	Obrdlik et al. 2004
pNubG-KAT1(SUS8)	Postive control vector, *TRP1*, Amp^{R}, CEN	Obrdlik et al. 2004
pNubG-SUT2(SUS9)	Negetive control vector, *TRP1*, Amp^{R}, CEN	Obrdlik et al. 2004
pMD18-T simple	pUC *ori*, cloning vector, Amp^{R}	Takara
pMS107	pLC20H containing the PCR fragment of *cyaA* from nucleotide 4 to nucleotide 1221, Amp^{R}	This lab
pET30a (+)	pBR322 origin, *T7* promoter His-tag, Km^{R}	Novagen
pET41a (+)	pBR322 origin, *T7* promoter His-tag, GST-tag, Km^{R}	Novagen
pCAMBIA1300	Binary vector, *35S* promoter, Km^{R}, Hyg^{R}	This lab
pCAMBIA1301	Binary vector, *35S* promoter, Km^{R}, Hyg^{R}	This lab
pCAMBIA1301-YFP^{N}	pCAMBIA1301 inserted with N-terminal (1-155) of YFP	This lab
pCAMBIA1301-YFP^{C}	pCAMBIA1301 inserted with N-terminal (156-239) of YFP	This lab
pCAMBIA1300-YFP	pCAMBIA1300 inserted with YFP tag	This lab
pTCK303	Binary vector, *Ubi1* promoter, Km^{R}, Hyg^{R}	This lab
pTL-n	TALEN construction vector, Tet^{R}	Li et al. 2012
psk/TALEN	TALEN construction vector, Amp^{R}	Li et al. 2012
p1300-*35S*	TALEN construction vector, *35S* promoter, Km^{R}	Li et al. 2012
pEH3-*Ubi1*	TALEN construction vector, *Ubi1* promoter, Amp^{R}	Li et al. 2012
pRTBV-VMGOE	Gene overexpression vector adopted from the tungro bacilliform virus-mediated gene silencing vector by insertion of full-length, instead of partial short sequence, of a gene to be tested, *Km*^{R}	Purkayastha *et al.*, 2010

Table S2 Information on genes tested and primers used in this study

	Primers / product size (bp) / subjects
hpa1 (ACD56757)	5’-ATGAATTCTTTGAACACACAAT-3’, 5’-TTACTGCATCGATGCGCTGTCG-3’ / 420 / coding sequence cloning by PCR
	5’-TTCTCAACAACGCCCCGCGGATTTG -3’, 5’-TTACTGCATCGATGCGCTGTCG-3’ / 639 / promoter + coding sequence
	Upstream homologous arm: 5’-CGGGATCCCGCTGCTGCAAATAGAATACG-3’ (*Bam*H I), 5’-GTAGGGGCGACCAACAGTTCTCGTGACGATTCCTCTCTGATT-3’ / 537, Downstream homologous arm: 5’-AATCAGAGAGGAATCGTCACGAGAACTGTTGGTCGCCCCTAC-3’, 5’-GCTCTAGATCACTCGCACATGCTGGTTCT-3’ (*Xba* I) / 536 / deletion
	5’-AACTGCAGTTCTCAACAACGCCCCGCGGATTTG-3’ (*Pst* I), 5’-GGGGTACCTTActtatcgtcgtcatccttgtaatc(flag)CTGCATCGATGCGCTGTCGCT-3’ (*Kpn* I) / 639 / complemention
	5’-acaagtttgtacaaaaaagcaggctctccaaccacc(homologous arm)ATGAATTCTTTGAACACACAAT-3’, 5’-tccgccaccaccaaccactttgtacaagaaagctgggta(homologous arm)CTGCATCGATGCGCTGTCGC-3’ / 417 / SUB Y2H
	5’-CGGGGTACCATGAATTCTTTGAACACACAAT-3’ (*Kpn* I), 5’-CGCGGATCCCTGCATCGATGCGCTGTCG-3’ (*Bam*H I) / 417 / BiFC
	5’-CGGGGTACCATGAATTCTTTGAACACACAAT-3’ (*Kpn* I), 5’-CGCGGATCCTTActtatcgtcgtcatccttgtaatc(flag)CTGCATCGATGCGCTGTCG-3’ (*Bam*H I) / 420 / Co-IP
	5’-CGCGGATCCATGAATTCTTTGAACACACAAT-3’ (*Bam*H I), 5’-CCCAAGCTTTTACTGCATCGATGCGCTGTCG-3’ (*Hind* III) / 420 / GST-Pulldown.
hpa1ΔN(1-36)	5’-TTCTCAACAACGCCCCGCGGATTTG -3’, 5’-ACTGGTCCAGTTGCTTTTCCATCGTGACGATTCCTCTCTGATTA-3’ / 219 / promoter 5’-TAATCAGAGAGGAATCGTCACGATGGAAAAGCAACTGGACCAGT-3’, 5’-CTGCATCGATGCGCTGTCGCT-3’ / 309 / *ΔN(1-36)* coding sequence / recombination
	5’-AACTGCAGTTCTCAACAACGCCCCGCGGATTTG-3’ (*Pst* I), 5’-GGGGTACCTTActtatcgtcgtcatccttgtaatc(flag)CTGCATCGATGCGCTGTCGCT-3’ (*Kpn* I) / 531 / complemention
	5’-acaagtttgtacaaaaaagcaggctctccaaccacc(homologous arm)ATGGAAAAGCAACTGGACCAGT-3’, 5’-tccgccaccaccaaccactttgtacaagaaagctgggta(homologous arm)CTGCATCGATGCGCTGTCGC-3’ / 309 / SUB Y2H
	5’-CGGGGTACCATGGAAAAGCAACTGGACCAGTTGC-3’ (*Kpn* I), 5’-CGCGGATCCCTGCATCGATGCGCTGTCG-3’ (*Bam*H I) / 309 / BiFC
	5’-CGGGGTACCATGGAAAAGCAACTGGACCAGTTGC-3’ (*Kpn* I), 5’CGCGGATCCTTACTTATCGTCGTCATCCTTGTAATCCTGCATCGATGCGCTGTCG-3’ (*Bam*H I) / 312 / Co-IP
	5’-CGCGGATCCATGGAAAAGCAACTGGACCAGTTGC-3’ (*Bam*H I), 5’-CCCAAGCTTTTACTGCATCGATGCGCTGTCG-3’ (*Hind* III) / 312 / GST-Pulldown
hpa1ΔNa-helix	5’-TTCTCAACAACGCCCCGCGGATTTG -3’, 5’-CCTCAGCATTTTTGCTCGACTGCGAGATGCCCTGGTTGCCGC-3’ / 327 / promoter + coding sequence (1-108) 5’-GCGGCAACCAGGGCATCTCGCAGTCGAGCAAAAATGCTGAGG-3’,

	5’-CTGCATCGATGCGCTGTCGCT-3’ / 261 / coding sequence (157-417) / recombination
hpa1ΔCa-helix	5’-TTCTCAACAACGCCCCGCGGATTTG-3’, 5’-CACCAGCACCACCACCATTCTGCGAGGGGCCATTCTGCTGCCCA-3’ / 477 / promoter + coding sequence (1-258) 5’-TGGGCAGCAGAATGGCCCCTCGCAGAATGGTGGTGGTGCTGGTG-3’, 5’-CTGCATCGATGCGCTGTCGCT-3’ / 111 / coding sequence (307-417) / recombination
hpa1ΔNCa-helices	5’-TTCTCAACAACGCCCCGCGGATTTG -3’, 5’-CCTCAGCATTTTTGCTCGACTGCGAGATGCCCTGGTTGCCGC-3’ / 327 promoter + coding sequence (1-108) 5’-GCGGCAACCAGGGCATCTCGCAGTCGAGCAAAAATGCTGAGG-3’, 5’-CACCAGCACCACCACCATTCTGCGAGGGGCCATTCTGCTGCCCA-3’ / 102 / coding sequence (157-258) 5’-TGGGCAGCAGAATGGCCCCTCGCAGAATGGTGGTGGTGCTGGTG-3’, 5’-CTGCATCGATGCGCTGTCGCT-3’ / 111 / coding sequence (307-417) / recombination
pthXo1 (WP_041182630)	Upstream homologous arm: 5’-CG**GGATCC**CTATTCGGATTTCGTGCTGTAC-3’ (*Bam*H I), 5’-CAAACTGCTATGCCCAGGCTTATCGTTGTGCTCGTCCCTTTTG-3’ / 513 Downstream homologous arm: 5’-CAAAAGGGACGAGCACAACGATAAGCCTGGGCATAGCAGTTTG-3’, 5’-GC**TCTAGA**ATCGTGGAATTCGCCAAGGAAT-3’ (*Xba* I) / 605 / deletion
OsPIP1;1 (AK061769)	5’-ATGGAGGGGAAGGAGGAGGACGTGC-3’, 5’-TTAAGACCTGCTCTTGAATGGGATCGC-3’ / 870 / cloning
	5’-acaagtttgtacaaaaaagcaggctctccaaccacc(homologous arm)ATGGAGGGGAAGGAGGAGGACGT-3’, 5’-tccgccaccaccaaccactttgtacaagaaagctgggta(homologous arm)AGACCTGCTCTTGAATGGGATCG-3’ / 867 / SUB Y2H
	5’-CGG**GGTACC**ATGGAGGGGAAGGAGGAGGACGT-3’ (*Kpn* I), 5’-GC**TCTAGA**AGACCTGCTCTTGAATGGGATCG-3’ (*Xba* I) / 867 / BiFC
	5’-CGG**GGTACC**ATGGAGGGGAAGGAGGAGGACGT-3’ (*Kpn* I), 5’-GC**TCTAGA**TTAagcgtagtctgggacgtcgtatgggta(HA) AGACCTGCTCTTGAATGGGATCG-3’ (*Xba* I) / 870 / Co-IP and expression
	5’-G**ACGCGT**ATGGAGGGGAAGGAGGAGGACGTGC-3’ (*Mlu I*), 5’-CCGGCC**TTAATTAA**AGACCTGCTCTTGAATGGGATCGC-3’ (*Pac I*)/867/Vigs mediated overexpression
OsPIP1;1(ΔLE→C)-*1;3*LE→C	5’-ATGGAGGGGAAGGAGGAGGACGT-3’, 5’-CGGGGTTGATGCCGGTGCCGGTGATGGGGATGGTGGCCAGGTG-3’ / 696 / *OsPIP1;1* up 5’-CACCTGGCCACCATCCCCATCACCGGCACCGGCATCAACCCCG-3’, 5’-GTCCCGGCTCTTGAAGGGGATT-3’ / 171 / recombination / *OsPIP1;3* LE + TM6 + COOH
*OsPIP1;1*ΔLE-*1;3*LE	5’-ATGGAGGGGAAGGAGGAGGACGT-3’, 5’-GACCAACCCAGAAGATCCAATGGTCGTGCCATGCGTGGGCGCG-3’ / 771 / *OsPIP1;1* ΔLOOPE-*1;3* LE UP 5’-CGCGCCCACGCATGGCACGACCATTGGATCTTCTGGGTTGGTC-3’, 5’-AGACCTGCTCTTGAATGGGATCG-3’ / 99 / recombination / *OsPIP1;1* TM6 + COOH
OsPIP1;2 (AK098849)	5’-ATGGAGGGGAAGGAGGAGGATGTCC-3’, 5’-TTACGACCTGCTCTTGAATGGAATTGC-3’ / 867 / cloning
	5’-ACAAGTTTGTACAAAAAAGCAGGCTCTCCAACCACCATGGAGGG

	GAAGGAGGAGGAT-3', 5'-TCCGCCACCACCAACCACTTTGTACAAGAAAGCTGGGTACGACCTG CTCTTGAATGGA-3' / 864 / SUB Y2H
OsPIP1;3 (AK102174)	5'-ATGGAGGGGAAGGAGGAGGATGTGAGG-3', 5'-TTAGTCCCGGCTCTTGAAGGGGATTGC-3' / 867 / cloning
	5'-CGG**GGTACC**ATGGAGGGGAAGGAGGAGGAT-3' (*Kpn* I), 5'-GC**TCTAGA**GTCCCGGCTCTTGAAGGGGAT-3' (*Xba* I) / 864 / overexpression, BiFC and Co-IP
	5'-GG**GGTACC**GGCATGATCTTCGCGCTCGTCTA-3' (*Kpn* I), 5'-CG**GGATCC**GTGGCGGAGAAGACGGTGTAGAC-3' (*Bam*H I) / 302 5'-GG**ACTAGT**GGCATGATCTTCGCGCTCGTCTA-3' (*Spe* I), 5'-C**GAGCTC**GTGGCGGAGAAGACGGTGTAGAC-3' (*Sac* I) / 302 / hairpin construction
	5'-GCCGTCTTCCTCGTCCACCTC-3', 5'-CGATGAACGGACCAACCCAGA-3' / 142 / real-time RT-PCR
	5'-acaagtttgtacaaaaaagcaggctctccaaccacc(homologous arm)ATGGAGGGGAAGGAGGAGGAT-3', 5'-tccgccaccaccaaccactttgtacaagaaagctgggta(homologous arm)GTCCCGGCTCTTGAAGGGGAT-3' / 864 / SUB Y2H
	5'-CGG**GGTACC**ATGGAGGGGAAGGAGGAGGAT-3' (*Kpn* I), 5'-GC**TCTAGA**TTAagcgtagtctgggacgtcgtatgggta(HA)GTCCCGGCTCTTGAAGGG GAT-3' (*Xba* I) / 867 / Co-IP and expression
	5'-G**ACGCGT**ATGGAGGGGAAGGAGGAGGAT-3' (*Mlu I*), 5'-CCGG**TTAATTAA**GTCCCGGCTCTTGAAGGGGAT-3' (*Pac I*)/864/Vigs mediated overexpression
TALEN (*OsPIP1;3*)	5'-AAAGAAGCAGGAAGCCACTC-3', 5'-CTGCATCGCCATGTAGAACACC-3' / 554 / cloning
	5'-AGTAGAAAGAAGAAGAGGCAAATT-3' / sequencing
*OsPIP1;3*Δ(N+TM1)-*1;1*(N+TM1)	5'-ATGGAGGGGAAGGAGGAGGACGT-3', 5'-CGCACTTGGACGCCGACTTGTTGACCCCCATGACGGTGAGGATG-3' / 234 / *OsPIP1;1* NH3 + TM1 5'-CATCCTCACCGTCATGGGGGTCAACAAGTCGGCGTCCAAGTGCG-3', 5'-GTCCCGGCTCTTGAAGGGGATT-3' / 633 / *OsPIP1;3* DOWN
*OsPIP1;3*Δ(LA+TM2)-*1;1*(LA+TM2)	5'-ATGGAGGGGAAGGAGGAGGATGT-3', 5'-CGCACTTGGAGGAGGACTTGGACACCCCCATCACCGTCAGGATG-3' / 231 / *OsPIP1;3* up 5'-CATCCTGACGGTGATGGGGGTGTCCAAGTCCTCCTCCAAGTGCG-3', 5'-CGAACGTCACCGCCGGGTTGATGTGTCCTCCGGAGATGCCGGC-3' / 111 / *OsPIP1;1* LA + TM2 5'-GCCGGCATCTCCGGAGGACACATCAACCCGGCGGTGACGTTCG-3', 5'-GTCCCGGCTCTTGAAGGGGATT-3' / 522 / *OsPIP1;3* down
*OsPIP1;3*Δ(LB+TM3)-*1;1*(LB+TM3)	5'-ATGGAGGGGAAGGAGGAGGATGT-3', 5'-CAAAAGTAACTGCTGGGTTGATGTGCCCGCCGGAGATGCCGGC-3' / 342 / *OsPIP1;3* up 5'-GCCGGCATCTCCGGCGGGCACATCAACCCAGCAGTTACTTTTG-3', 5'-ACAACCCCCGCTGGAACCCCTTCACAACTCCAGCTCCGCAGATG-3' / 114 / *OsPIP1;1* LB + TM3 5'-CATCTGCGGAGCTGGAGTTGTGAAGGGGTTCCAGCGGGGGTTGT-3', 5'-GTCCCGGCTCTTGAAGGGGATT-3' / 408 / *OsPIP1;3* down
*OsPIP1;3*Δ(LC+TM4)-*1;1*(LC+TM4)	5'-ATGGAGGGGAAGGAGGAGGATGT-3', 5'-TACAGACCCTGCTGGAAGCCCTTCACCACGCCGGCGCCGCAGAT-3' / 456 / *OsPIP1;3* up 5'-ATCTGCGGCGCCGGCGTGGTGAAGGGCTTCCAGCAGGGTCTGTA-3',

	5’-TTGCGCTTGGCGTCGGTGGCTGAGAAGACGGTGTAGACCAGG-3’ / 132 / *OsPIP1;1* LC + TM4 5’-CCTGGTCTACACCGTCTTCTCAGCCACCGACGCCAAGCGCAA-3’, 5’-GTCCCGGCTCTTGAAGGGGATT-3’ / 276 / *OsPIP1;3* down
*OsPIP1;3*Δ(LD+TM5)-*1;1*(LD+TM5)	5’-ATGGAGGGGAAGGAGGAGGATGT-3’, 5’-CATTCCTCTTGGCATCAGTGGCGGAGAAGACGGTGTAGACGAG-3’ / 588 / *OsPIP1;3* up 5’-CTCGTCTACACCGTCTTCTCCGCCACTGATGCCAAGAGGAATG-3’, 5’-CGGGGTTGATGCCGGTGCCGGTGATGGGGATGGTGGCCAGGTG-3’ / 105 / *OsPIP1;1* LD + TM5 5’-CACCTGGCCACCATCCCCATCACCGGCACCGGCATCAACCCCG-3’, 5’-GTCCCGGCTCTTGAAGGGGATT-3’ / 171 / *OsPIP1;3* down
OsPIP1;3(ΔLE→C)-*1;1*(LE→C)	5’-ATGGAGGGGAAGGAGGAGGATGT-3’, 5’-CTGGGTTGATGCCAGTACCGGTGATGGGGATGGTGGCGAGGTG-3’ / 693 / *OsPIP1;3* up 5’-CACCTCGCCACCATCCCCATCACCGGTACTGGCATCAACCCAG-3’, 5’-AGACCTGCTCTTGAATGGGATCG-3’ / 171 / *OsPIP1;1* LE + TM6 + COOH
*OsPIP1;3*ΔLE-*1;1*LE	5’-ATGGAGGGGAAGGAGGAGGATGT-3’, 5’-GACCAACCCAGAAAATCCAGTGGTCATTCCAGGCATGGTCCTT-3’ / 768 / *OsPIP1;3* ΔLE-1;1 LE up 5’-AAGGACCATGCCTGGAATGACCACTGGATTTTCTGGGTTGGTC-3’, 5’-GTCCCGGCTCTTGAAGGGGA-3’ / 96 / *OsPIP1;3* TM6 + COOH
SWEET11 (AK070510)	5’-TGGTTCTGCTACGGCCTCTT-3’, 5’-GGTACCAGAAGTAGAGCCCCATCT-3’ / 103 / real-time RT-PCR
OsEF1α (AF030517)	5’-CGTGCCTGTGGGTCGTGTTG-3’, 5’-TCCTGGAGAGCCTCGTGGTG-3’ / 120 / real-time RT-PCR

Table S3 Open reading frame sequences of two designer TALENs

The start (ATG) and stop (TAA) codons are shown in red. Restriction sites used for cloning and constructing are in blue. The repeat variable di-residues (RVD) of each repeat are in light blue. Pink sequence of nucleotides corresponds to the FokI nuclease domain.

***OsPIP1,3*$_{(191\text{-}247)}$-F**

GGTACCATGGCTCCAAAGAAGAAGCGTAAGGTATACCCATACGATGTTCCTGACTATGCGGGCTATCCCTATGACGTGCCGGACTATGCAGGATCGTATCCA
TATGACGTTCCAGATTACGCTGCTCATGGCACCGTGGATCTACGCACGCTCGGCTACAGCCAGCAGCAACAGGAGAAGATCAAACCGAAGGTTCGTTCGACA
GTGGCGCAGCACCACGAGGCACTGGTCGGCCACGGGTTTACACACGCGCACATCGTTGCGCTCAGCCAACACCCGGCAGCGTTAGGGACCGTCGCTGTCAAG
TATCAGGACATGATCGCAGCGTTGCCAGAGGCGACACACGAAGCGATCGTTGGCGTCGGCAAACAGTGGTCCGGCGCACGCGCTCTGGAGGCCTTGCTCACG
GTGGCGGGAGAGTTGAGAGGTCCACCGTTACAGTTGGACACAGGCCAACTTCTCAAGATTGCAAAACGTGGCGGCGTGACCGCAGTGGAGGCAGTGCATGCA
TGGCGCAATGCACTGACGGGTGCCCCCCTGAAC
CTGACCCCGGACCAAGTGGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCACTAGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCTCTTGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAAGTCGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGTTGGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCATGGC
CTGACCCCGGCCCAAGTGGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAAGTGGTGGCCATCGCCAACAATAACGGCGGCAAGCAGGCAGTTGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCATGGC
CTGACCCCGGCCCAAGTGGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCTGTAGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCATGGC
CTGACCCCGGCCCAAGTGGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCCGTGGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCACTGGAAACACTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCACTAGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCATGGC
CTGACCCCGGCCCAAGTGGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCTCTTGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAAGTGGTGGCCATCGCCAACAATAACGGCGGCAAGCAGGCGTTGGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCATGGC
CTGACCCCGGCCCAAGTGGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAGGTCGTGGCCATCGCCAGCAATATTGGCGGCAAGCAGGCAGTTGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAAGTCGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCTGTAGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAGGTCGTGGCCATCGCCAGCAATATTGGCGGCAAGCAGGCCGTGGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCATGGC
CTGACCCCGGCCCAAGTGGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCGCTGGAAACTGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAAGTCGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCACTAGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
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ACCAATCGCCGTATTCCCGAACGCACATCCCATCGCGTTGCCGGATCCCAACTAGTCAAAAGTGAACTGGAGGAGAAGAAATCTGAACTTCGTCATAAATTG
AAATATGTGCCTCATGAATATATTGAATTAATTGAAATTGCCAGAAATCCCACTCAGGATAGAATTCTTGAAATGAAGGTAATGGAATTTTTTATGAAAGTT
TATGGATATAGAGGTGAGCATTTGGGTGGATCAAGGAAACCGGACGGAGCAATTTATACTGTCGGATCTCCTATTGATTACGGTGTGATCGTGGATACTAAG
GCTTATAGCGGAGGTTATAATCTGCCAATTGGCCAAGCACGAGAAATGCAACGATATGTCGAAGAAAATCAAACACGAAACAAACATATCAACCCTAATGAA
TGGTGGAAAGTCTATCCATCTTCTGTAACGGAATTTAAGTTTTTATTTGTGAGTGGTCACTTTAAAGGAAACTACAAAGCTCAGCTTACACGATTAAATCAT
ATCACTAATTGTAATGGAGCTGTTCTTAGTGTAGAAGAGCTTTTAATTGGTGGAGAAATGATTAAAGCCGGCACATTAACCTTAGAGGAAGTGAGACGGAAA
TTTAATAACGGCGAGATAAACTTTTAATGAGAGCTC

***OsPIP1,3*$_{(191\text{-}247)}$-R**

GGTACCATGGCTCCAAAGAAGAAGCGTAAGGTATACCCATACGATGTTCCTGACTATGCGGGCTATCCCTATGACGTGCCGGACTATGCAGGATCGTATCCA
TATGACGTTCCAGATTACGCTGCTCATGGCACCGTGGATCTACGCACGCTCGGCTACAGCCAGCAGCAACAGGAGAAGATCAAACCGAAGGTTCGTTCGACA
GTGGCGCAGCACCACGAGGCACTGGTCGGCCACGGGTTTACACACGCGCACATCGTTGCGCTCAGCCAACACCCGGCAGCGTTAGGGACCGTCGCTGTCAAG
TATCAGGACATGATCGCAGCGTTGCCAGAGGCGACACACGAAGCGATCGTTGGCGTCGGCAAACAGTGGTCCGGCGCACGCGCTCTGGAGGCCTTGCTCACG
GTGGCGGGAGAGTTGAGAGGTCCACCGTTACAGTTGGACACAGGCCAACTTCTCAAGATTGCAAAACGTGGCGGCGTGACCGCAGTGGAGGCAGTGCATGCA
TGGCGCAATGCACTGACGGGTGCCCCCCTGAAC
CTGACCCCGGACCAAGTGGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCACTAGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAAGTGGTGGCCATCGCCAACAATAACGGCGGCAAGCAGGCTCTTGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAAGTGGTGGCCATCGCCAACAATAACGGCGGCAAGCAGGCGTTGGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAGGTCGTGGCCATCGCCAGCAATATTGGCGGCAAGCAGGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAAGTCGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCAGTTGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
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CTGACCCCGGACCAAGTCGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCCGTGGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCACTGGAAACACTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
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CTGACCCCGGACCAGGTGGTGGCCATCGCCAGCAATATTGGCGGCAAGCAGGCTCTTGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAAGTCGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGTTGGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCATGGC
CTGACCCCGGCCCAAGTGGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCGCTCGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCATGGC
CTGACCCCGGACCAAGTGGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCAGTTGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
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CTGACCCCGGACCAAGTCGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCACTAGAAACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
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ACCAATCGCCGTATTCCCGAACGCACATCCCATCGCGTTGCCGGATCCCAACTAGTCAAAAGTGAACTGGAGGAGAAGAAATCTGAACTTCGTCATAAATTG
AAATATGTGCCTCATGAATATATTGAATTAATTGAAATTGCCAGAAATCCCACTCAGGATAGAATTCTTGAAATGAAGGTAATGGAATTTTTTATGAAAGTT
TATGGATATAGAGGTGAGCATTTGGGTGGATCAAGGAAACCGGACGGAGCAATTTATACTGTCGGATCTCCTATTGATTACGGTGTGATCGTGGATACTAAG
GCTTATAGCGGAGGTTATAATCTGCCAATTGGCCAAGCACGAGAAATGCAACGATATGTCGAAGAAAATCAAACACGAAACAAACATATCAACCCTAATGAA
TGGTGGAAAGTCTATCCATCTTCTGTAACGGAATTTAAGTTTTTATTTGTGAGTGGTCACTTTAAAGGAAACTACAAAGCTCAGCTTACACGATTAAATCAT
ATCACTAATTGTAATGGAGCTGTTCTTAGTGTAGAAGAGCTTTTAATTGGTGGAGAAATGATTAAAGCCGGCACATTAACCTTAGAGGAAGTGAGACGGAAA
TTTAATAACGGCGAGATAAACTTTTAATGAGAGCTC