Supplementary data

Rice Inositol Polyphosphate Kinase (OsIPK2) Directly Interacts with OsIAA11 to Regulate Lateral Root Formation

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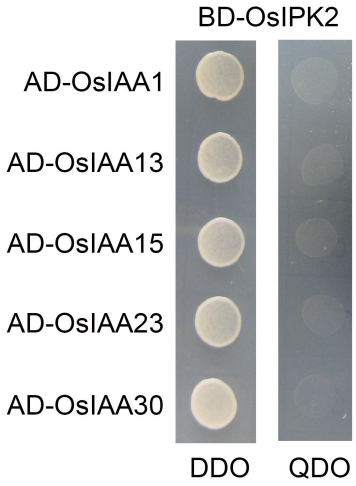
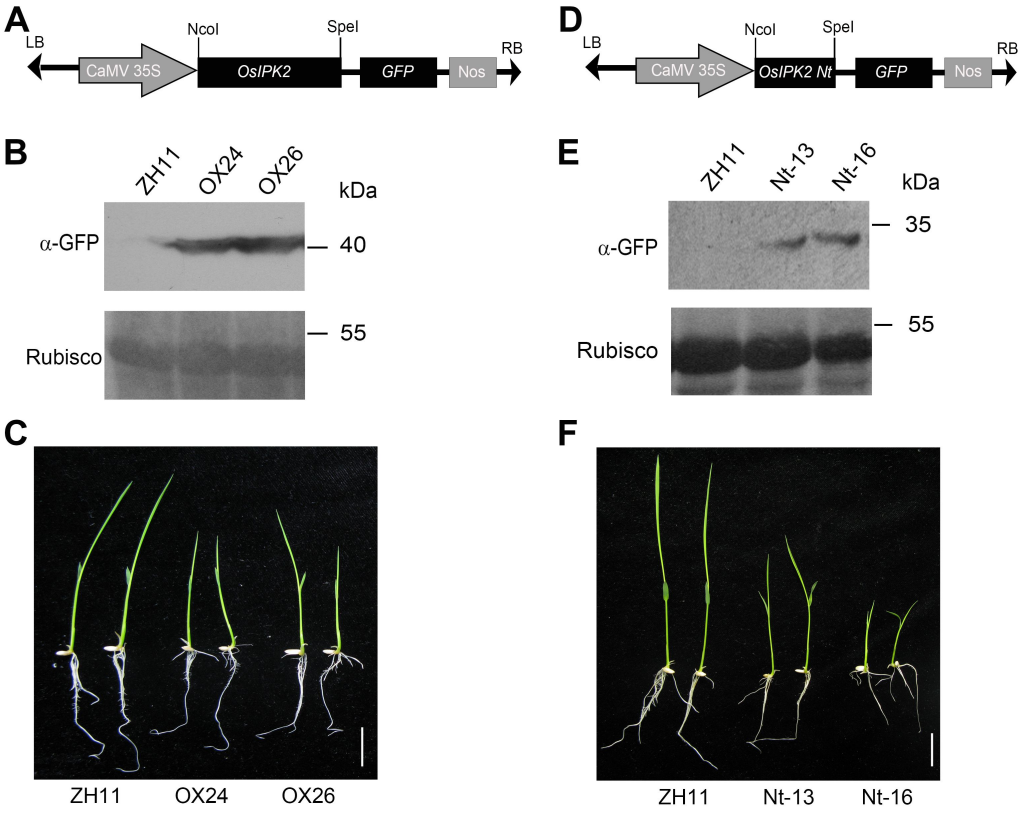
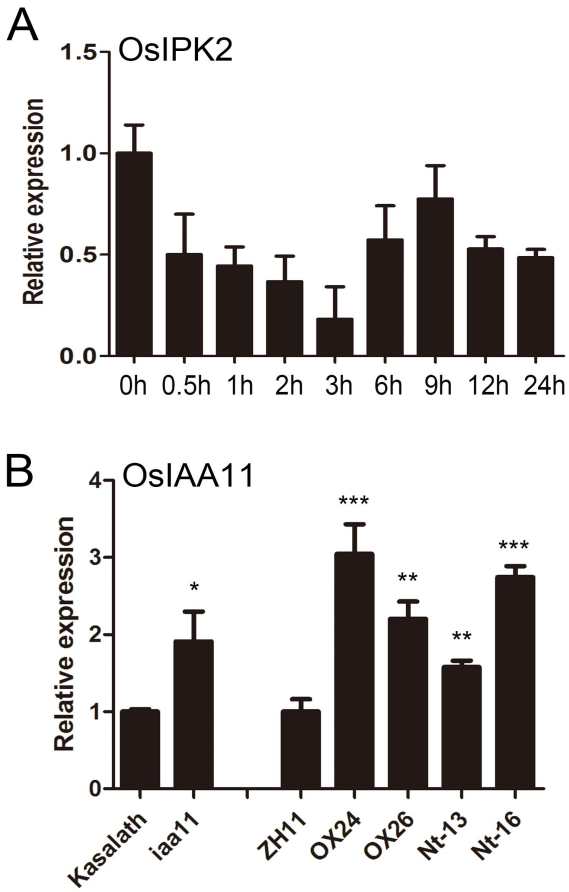


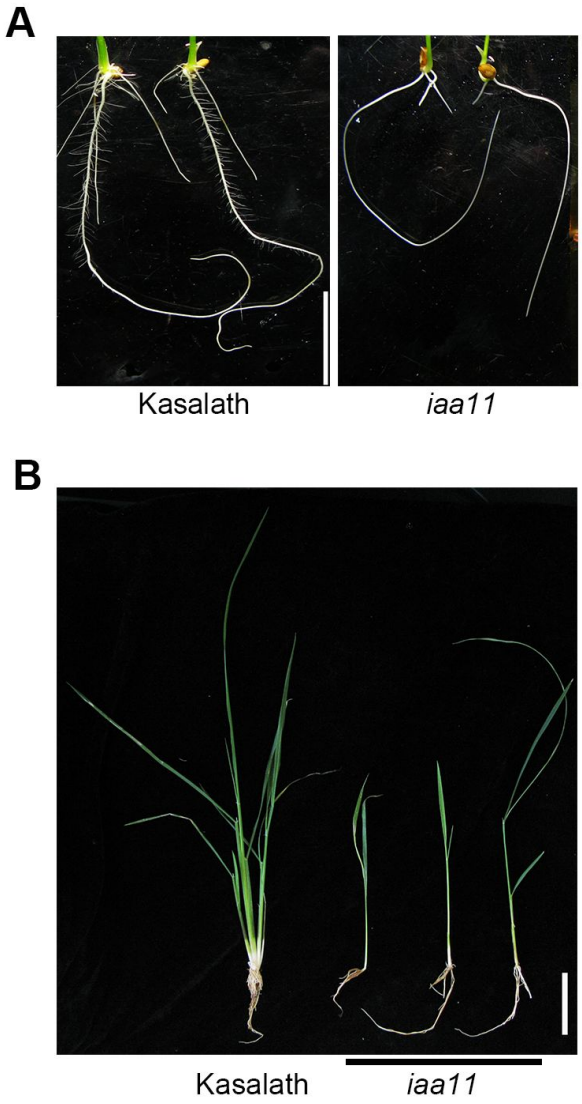
Supplementary Fig. S1. Yeast two-hybrid assays to detect interaction between OsIPK2 and Aux/IAA in the OsIAA11 clade. Aux/IAAs were fused to GAL4 activation domain (AD) as prey and OsIPK2 was fused to GAL4 DNA-binding domain (BD) as bait. The interactions were examined on the double dropout (DDO) medium (SD/-Leu/-Trp) and quaternary dropout (QDO) medium (SD/-Ade/-His/-Leu/-Trp).



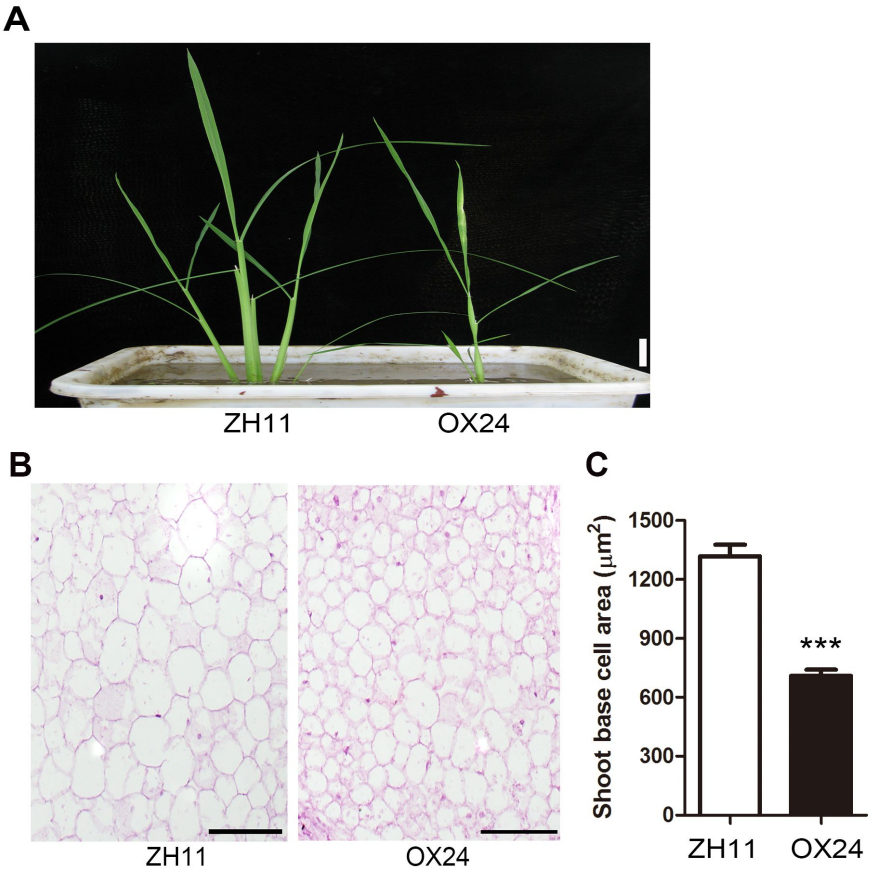
Supplementary Fig. S2. Construction of *35S:OsIPK2-GFP* and *35S:OsIPK2 Nt-GFP* transgenic rice. (**A, D**) Schematic representation of rice transformations. The black boxes indicate coding sequences. (**B, E**) Identification of *35S:OsIPK2-GFP*, *35S:OsIPK2 Nt-GFP* transgenic plants. OsIPK2 and OsIPK2-1-101 peptide were visualized by immunoblotting using anti-GFP antibody. (**C, F**) Phenotype of 7-day-old ZH11 (wild type), *35S:OsIPK2-GFP* and *35S:OsIPK2 Nt-GFP* transgenic plant seedlings. Scale bars=2 cm.



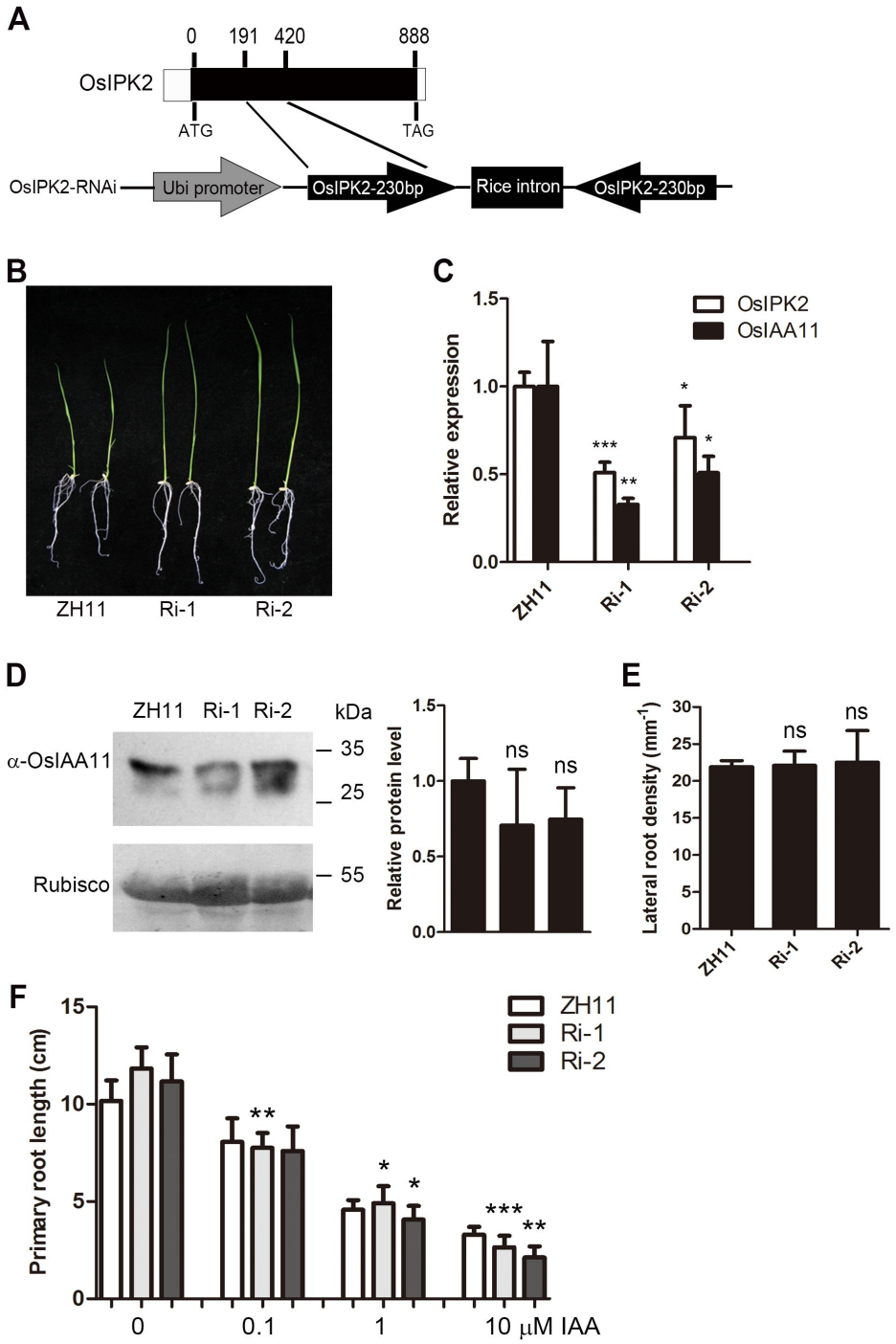
Supplementary Fig. S3. Analysis of the mRNA level of *OsIAA11*. (**A**) Accumulation of *OsIPK2* transcript of wild-type (WT) seedlings in response to 1 μM IAA. Data shown are means ± SD of three biological replicates. (**B**) The qRT-PCR analysis of the transcript level of *OsIAA11* gene in WT (Kasalath, ZH11), *iaa11* mutant, *35S:OsIPK2-GFP* and *35S:OsIPK2 Nt-GFP* transgenic plants. Data shown are means ± SD from three biological replicates. Asterisks indicate significant differences by Student's *t*-test (*P < 0.05, **P < 0.01, ***P < 0.001). These experiment were repeated twice with similar results.



Supplementary Fig. S4. Deficient phenotypes of *iaa11* mutant. (**A**) Roots of 7-day-old Kasalath (wild type) and *iaa11* mutant plant seedlings. *iaa11* mutant showed extreme defects in lateral root formation. Scale bar=2 cm. (**B**) Phenotype of 4-week-old Kasalath and *iaa11* mutant plants. *iaa11* mutant showed a dwarf phenotype. Scale bar=6 cm.

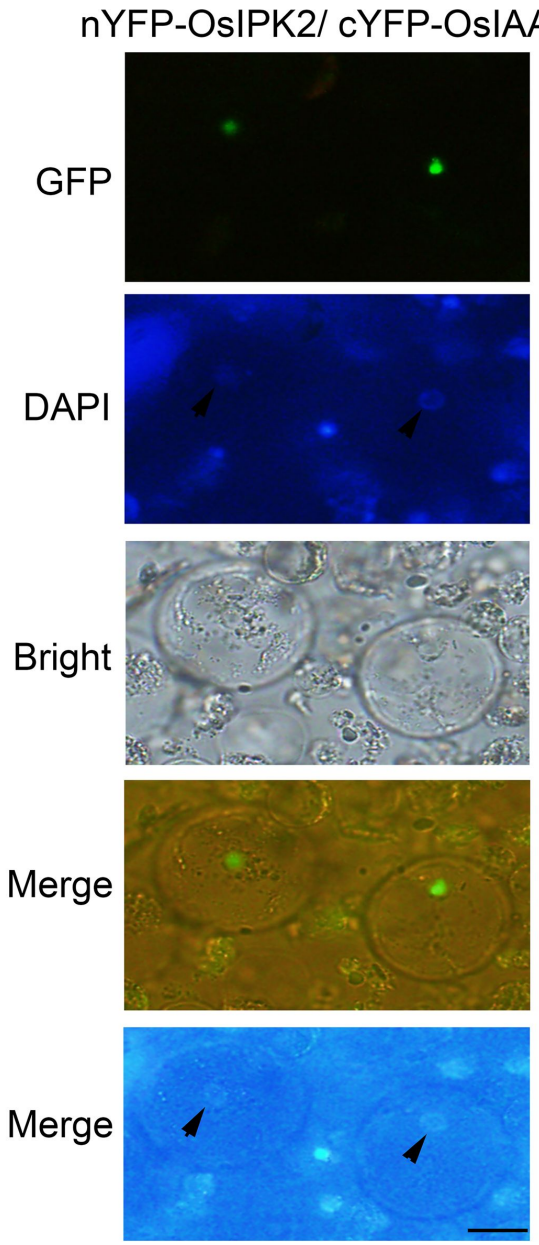


Supplementary Fig. S5. *OsIPK2* overexpression transgenic rice shows a dwarf phenotype. (**A**) The phenotype of ZH11 (left, wild type) and OX24 (right) plants grown on soil for 4 weeks. Scale bar= 3 cm. (**B**) Cross-section of the shoot base of ZH11 and OX24 plants. Scale bars= 100 μm. (**C**) Quantification analysis of the cell size in the shoot base of ZH11 and OX24 plants. Mean is shown with SD (n>20). Asterisks indicate significant differences between OX24 and ZH11 by Student's *t*-test (***P < 0.001).



Supplementary Fig. S6. Auxin signaling is altered in *OsIPK2* RNAi transgenic plants. (**A**) Schematic representation of constructs. The black box indicates an ORF, and open boxes indicate UTRs. The arrows indicate the fragment selected from the corresponding sequence in *OsIPK2*. (**B**) The phenotype of ZH11 (left, wild type) and *OsIPK2*-RNAi (right, Ri-1 and Ri-2) seedlings grown on 1/2 MS medium for 7 days. (**C**)

Quantitative RT-PCR analysis of the expression of *OsIPK2* and *OsIAA11* in ZH11 and RNAi transgenic plants. Data shown are means ± SD from three biological replicates. Asterisks indicate significant differences between WT and RNAi lines by Student's *t*-test (*P < 0.05, **P < 0.01, ***P < 0.001). The experiment was repeated twice with similar results. (**D**) Immunodetection of the accumulation of OsIAA11 protein in the ZH11 and *OsIPK2* RNAi transgenic lines with anti-OsIAA11 antibody. The PVDF membrane was stained with Ponceau to visualize the large subunit of Rubisco as loading control. On the right was the quantification of the relative OsIAA11 levels of the western blots. The average values were obtained from three independent experiments. ns, not significant by Student's *t*-test. (**E**) The lateral root densities of 10-day-old ZH11 and *OsIPK2* RNAi transgenic plant seedlings. All means are shown with SD (n>15). ns, not significant by Student's *t*-test. The experiment was repeated twice with similar results. (**F**) The response of root growth of ZH11 and RNAi lines to exogenous IAA (0-10 μM). Root lengths of 10-day-old ZH11 and RNAi transgenic lines grown on 1/2 MS medium were measured. The treatment × genotype interaction effect (two-way ANOVA) is indicated (*P < 0.05, **P <0.01, ***P <0.001). All means are shown with SD (n>15). The experiment was repeated twice with similar results.



Supplementary Fig. S7. Interaction between OsIPK2 and OsIAA11 in rice protoplasts. The bimolecular fluorescence complementation (BiFC) assays indicate OsIPK2 interacts with OsIAA11 in the nucleus of rice cell. nYFP-OsIPK2 and cYFP-OsIAA11 were co-expressed in rice protoplasts prepared from 12-day-old etiolated rice leaf. Nuclei are shown with DAPI staining. From top to bottom: GFP; DAPI, nucleus stained with 4', 6-Diamidino-2-phenylindole; Bright field; Merged, combined fluorescence from GFP or DAPI with bright field. Scale bar=5 μm.

Supplementary Table S1. Sequences of primers used in this work.

Yeast two-hybrid	Primer Sequence (5' to 3')
IAA11-yeast-F	AACCCGGGTATGGCTGGCCTCGGGTTC
IAA11-yeast-R	AAGGATCCGCTCTTGCATTTCTCCAC
IAA11-DI-R	AAGGATCCGTCGGCATCTTCCTTGTC
IAA11-DII-F	AACCCGGGTGACAAGGAAGATGCCGAC
IAA11-DII-R	AAGGATCCCTTGCATTTCTCGGCGAG
IAA11-DIII-F	AACCCGGGTCTCGCCGAGAAATGCAAG
IAA11-DIII-R	AAAGGATCCATGGGACCCACACTTGCCGAT
IAA11-DIV	AACCCGGGTCAGCAGCTGAAGGAGAGCA
IAA11-mI-F	GCCGAGACCATCGACGCCAAGGCCAAGGCGCAGCCGG CGGCG
IAA11-mI-R	CGCCGCCGGCTGCGCCTTGGCCTTGGCGTCGATGGTCT CGGC
IAA11-mIIab-F	CGCAGGTGGTGGGGTGGTCGTCGGTCCGGTCGTTCAGG AA
IAA11-mIIab-R	TTCCTGAACGACCGGACCGACGACCACCCCACCACCT GCG
IAA11-BM2-F	GCGCTGGTGGCGGTGAGCATGGACGGCGCGCCGTACC TGGCGAAGATCGA
IAA11-BM2-R	TCGATCTTCGCCAGGTACGGCGCGCCGTCCATGCTCAC CGCCACCAGCGC
IAA11-AM2-F	GTGCCGACGTACGAGGCGAAGGCGGGCGACTGGATGC TCG
IAA11-AM2-R	CGAGCATCCAGTCGCCCGCCTTCGCCTCGTACGTCGGC AC
IAA1-F	AACCCGGGTATGTCGGTGGAGACGGAG
IAA1-R	AAGGATCCTCATTGAGCGGCTCTTGG
IAA13-F	AACCCGGGTATGGCCGGCGCCGACGTG
IAA13-R	AAGGATCCTCAGCTCTTGTTCTTGTA
IAA15-F	AACCCGGGTATGTCGGTGGAGACGGAG

IAA15-R	GCGGGATCCATCAAGCAGATCTTGGTG
IAA23-F	AACCCGGGTATGTCGACGAGCTCCGGC
IAA23-R	AAGGATCCTTATCTGGATGATCGTCT
IAA30-F	AACCCGGGTATGGCGGCGGACCTGGCCTTCG
IAA30-R	AACTCGAGTCAGCTTCTGTTCTTGCA
OsIPK2-yeast-F	AACCCGGGTATGGCCTCCGACCTGCGC
OsIPK2-yeast-R	AAGGATCCTCAAGAATGATCTGAAGA
OsIPK2-Nt-R	AAGGATCCGGACGGGAGGCCCGCGAGGAGGTC
OsIPK2-ΔN-F	AAGAATTCATGCCCTGCGTCGCCGACGTC
Rice transformation	
OsIPK2p-GUS-F	AACTGCAGGTGACACGAGACCCAGAGCAGC
OsIPK2p-GUS-R	AAAGATCTAAAGAATGATCTGAAGACGCCT
OsIPK2-OE-F	AACCATGGCTATGGCCTCCGACCTGCGCCCG
OsIPK2-OE-R	CGGGTACCCGTCAAGAATGATCTGAAGACGCCT
OsIPK2 Nt-OE-F	AACCATGGCTATGGCCTCCGACCTGCGCCCG
OsIPK2 Nt-OE-R	AAACTAGTGGACGGGAGGCCCGCGAGGAGGTC
OsIPK2-RNAi 1-F	GGACTAGTCCGCGCCTTCTTCCCGCGCTTC
OsIPK2-RNAi 1-R	TCCGAATTCGGAGAAGCCGAGGAGCGCGCTGG
OsIPK2-RNAi 2-F	CGGGATCCCGGCGCCTTCTTCCCGCGCTTC
OsIPK2-RNAi 2-R	GCCATGGCGAAGCCGAGGAGCGCGCTGG
BiFC	
OsIAA11-F	AAGGTACCAATGGCTGGCCTCGGGTTCGA
OsIPK2-F	AAGGTACCAATGGCCTCCGACCTGCGC
Protein expression	
Myc-tag-F	GGGTCTAGAATGACTAGTGGTGAACAAAA
Myc-tag-R	AAAGGTACCAATCTAGGCTGCAGCCCGGG
GFP-tag-F	AAGGTACCATGGTGAGCAAGGGCGAG
GFP-tag-R	AAGGATCCTCACGAGCTGAGTCCGGACTT
OsIPK2-MBP-F	AAGAATTCATGGCCTCCGACCTGCGC
OsIPK2-MBP-R	AAGTCGACTCAAGAATGATCTGAAGA
OsIAA11-His-F	AACCATGGAATGGCTGGCCTCGGGTTCGA
qRT-PCR assays	

OsIPK2-qRT-F	AGGAGCAAACCCTGTACCACTTCT
OsIPK2-qRT-R	AAGTCCACCAGCTTCACCCTTACA
OsIAA11-qRT-F	GCGCTGGTGAAGGTGAGCAT
OsIAA11-qRT-R	ACGTACTCCAGGTCATCTCT
UBQ5-qRT-F	ACCACTTCGACCGCCACTAC
UBQ5-qRT-R	ACGCCTAAGCCTGCTGGTT