New Phytologist Supporting Information

Article title: OsmiR530 acts downstream of OsPIL15 to regulate grain yield in rice

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The following Supporting Information is available for this article:

Fig. S1 Agronomic traits of OsmiR530-overexpressing (miR530-OE) and target mimic (MIM530) lines. (a) Results of the qRT-PCR analysis of the expression of OsmiR530 in rice miR530-OE lines. 5.8S rRNA was used as an internal control for qRT-PCR. Three biological replicates were analyzed. (b) Results of the qRT-PCR analysis of the expression of the OsmiR530 target mimic in MIM530 lines. OsEF-1 α was used as a reference gene for the qRT-PCR. Because the OsmiR530 target mimic was not expressed in rice wild-type (WT) plants, the expression level of the OsmiR530 target mimic in MIM530-#2 was set as the baseline. Three biological replicates were analyzed. (c) Rice transgenic and WT plants heights (n = 20 plants). (d) Phenotypes of the main panicle of rice transgenic and WT plants. Scale bars, 5 cm. (e) Primary panicle lengths of rice transgenic and WT plants (n = 20 panicles). (f) Seed-setting rates of rice transgenic and WT plants (n = 20 panicles). (g) Spikelet hull lengths and widths in rice transgenic and WT plants (n= 20 spikelets). For all histogram, the gray bar represents WT, blue bars represent miR530-OE transgenic lines #a1, #b5, and #c2 (from left to right), and red bars represent MIM530 transgenic lines #2, #3, and #6 (from left to right). The data in (a), (b), (c), (e), (f), and (g) are presented as the mean \pm standard deviation. Significant differences were determined with Student's *t*-test (* *P*< 0.05, ** *P*< 0.01).

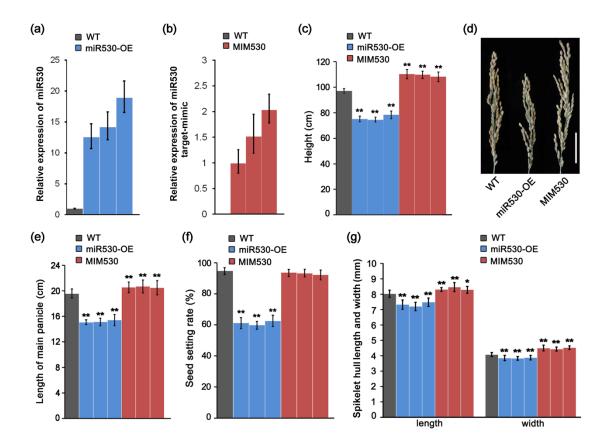


Fig. S2 Comparison of the peduncle in miR530-overexpressing (miR530-OE), target mimic (MIM530) and wild-type (WT) plants. (a) Comparison of the peduncle diameters of rice transgenic and WT plants (n = 10 plants). (b) Cross-sections of the peduncles of rice transgenic and WT plants. LV, large vascular bundles; SV, small vascular bundles. Scale bars, 200 µm. Comparison of the number of LV (c) and SV (d) in the peduncles of rice transgenic and WT plants (n = 10 plants). Data are presented as the mean ± standard deviation. Significant differences were determined with Student's *t*-test (* *P*< 0.05, ** *P* < 0.01).

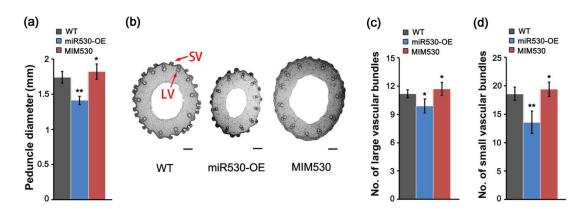


Fig. S3 Expression of OsmiR530 and *OsPL3* in various rice tissues. (a) Expression of OsmiR530 in various rice tissues. R, root; IN, internode; FL, flag leaf; SP1, spikelet (0.5-1 cm long); SP2, spikelet (3-5 cm long); E, embryo; S, immature seed. 5.8S rRNA was used as an internal control for the qRT-PCR. Data are presented as the mean \pm standard deviation of three biological replicates. (b) Expression of *OsPL3* in various rice tissues. R, root; IN, internode; FL, flag leaf; SP1, spikelet (0.5-1 cm long); SP2, spikelet (3-5 cm long); E, embryo; S, immature seed. *OsEF1a* was used as a reference gene for the qRT-PCR. Data are presented as the mean \pm standard deviation of three biological replicates.

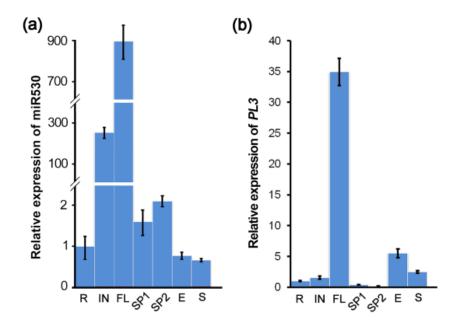


Fig. S4 Diagram of two single-guide RNAs (sgRNAs) targeting *OsPL3* in the rice genome.

Target sites are marked in red, whereas the protospacer-adjacent motif (PAM) is marked in green. Sequences in boxes are the target sequences in the wild-type (WT) rice. Black dashes represent deleted bases and inserted nucleotides are indicated with red lowercase letters.

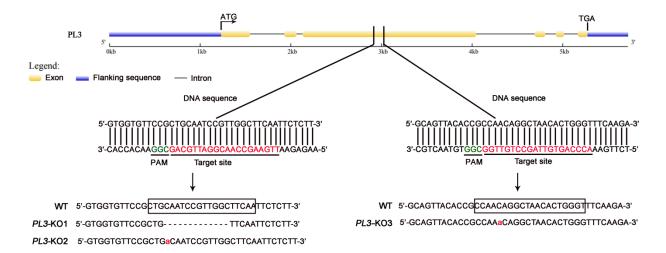


Fig. S5 Analysis of the OsPL3 protein sequence of in the wild-type (WT) and *OsPL3*-knockout (*PL3*-KO) lines. (**a**) Protein structure of OsPL3 in rice WT and *PL3*-KO lines. (**b**) Changes in the OsPL3 amino acid sequences in *PL3*-KO lines.

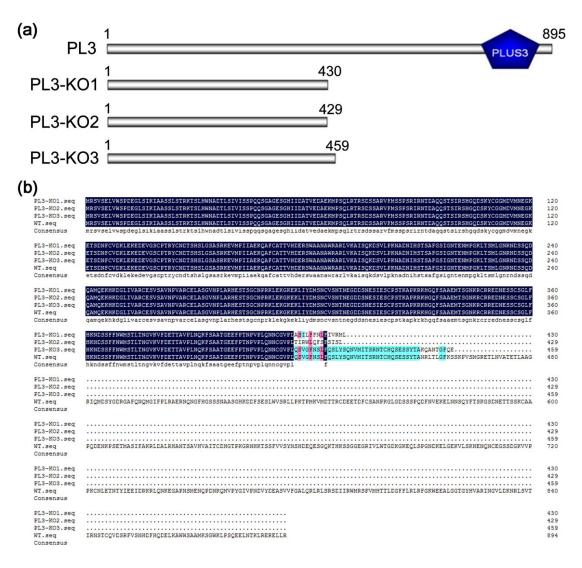


Fig. S6 Agronomic traits of *OsPL3*-knockout (*PL3*-KO) lines. (**a**) The rice *PL3*-KO and wildtype (WT) plant heights (n = 20 plants). (**b**) Phenotypes of the main panicle of rice *PL3*-KO and WT plants. (**c**) Primary panicle lengths of rice *PL3*-KO and WT plants (n = 20 panicles). (**d**) Number of primary branches per main panicle in rice *PL3*-KO and WT plants. (**e**) Seed-setting rates of rice *PL3*-KO and WT plants (n = 20 panicles). For all histograms, the gray bar represents WT and green bars represent *PL3*-KO transgenic lines #12, #13, and #16 (from left to right). The data in (**a**) and (**c**-**e**) are presented as the mean ± standard deviation. Significant differences were determined with Student's *t*-test (* *P*< 0.05, ** *P*< 0.01).

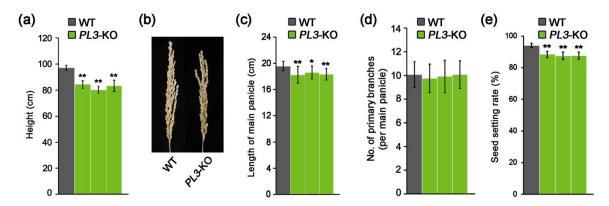


Fig. S7 Diagram of two single-guide RNAs (sgRNAs) targeting *OsPIL15* in the rice genome.

Target sites are marked in red, whereas the protospacer-adjacent motif (PAM) is marked in green. Sequences in boxes are the target sequences in the wild-type (WT) rice. Black dashes represent deleted bases and inserted nucleotides are indicated with red lowercase letters.

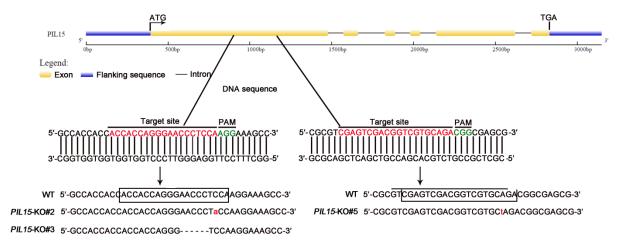


Fig. S8 Analysis of the OsPIL15 protein sequence in the rice wild-type (WT) and OsPIL15-

knockout (PIL15-KO) lines.

WT.sea	MSDGNDFAELLWENGCAVVHGRKKHPOPAFPPFGFFGGTGGGGGGSSSRACEROPGGIDAFAKVGGGFGALGMAPAVHDF	80
PL15-KO#2.seg	MSDGNDFAELLWENGQAVVNGRKKHPQFAFFFFGFFGGTGGGGGGGSSSGRQERQFGGIDAFAKVGGGFGALGMAFAVNDF	80
PIL15-KO#3.seg	MSDGNDFAELLWENGQAVVNGRKKHPQFAFFFFGFFGGTGGGGGGGSSSGRQERQFGGIDAFAKVGGGFGALGMAFAVNDF	80
PIL15-KO#5.seq	MSDGNDFAELLWENGCAVVHGRKKHPCFAFFFFGFFGGTGGGGGGGSSSGRQERCFGGIDAFAKVGGGFGALGMAFAVHDF	80
Consensus	msdgndfaellwengqavvhgrkkhpqpafppfgffggtggggggsssraqerqpggidafakvgggfgalgmapavhdf	00
WT.seq	ASGFGATTQDNGDDDTVPWIHYPIIDDEDAAAPAALAAADYGSDFFSELQAAAAAAAAAAPPTDLASLPASNHNGATNNR	160
PL15-KO#2.seg	ASGFGATTCDNGDDDTVPWIHYFIIDDEDAAAPAALAAADYGSDFFSELCAAAAAAAAAAAPPTDLASLPASNHNGATNNR	160
PIL15-KO#3.seg	ASGFGATTQDNGDDDTVPWIHYPIIDDEDAAAPAALAAADYGSDFFSELQAAAAAAAAAAPPTDLASLPASNHNGATNNR	160
PIL15-KO#5.seg	asgfgattcdngdddtvpwihyfiiddedaaaaaaaaadygsdffselcaaaaaaaaaapptdlaslpasnhngatnnr	160
Consensus	asgfgattqdngdddtvpwihypiiddedaaapaalaaadygsdffselqaaaaaaaaaapptdlaslpasnhngatnnr	
WT.seq	NAFVATTTTREPSKESHCELSVETTREEPOPOLAARKIFESSGSCEGEGVMNFSLFSREAVLARATLESACETOG	237
PL15-KO#2.seq	NAFVATTTTREFYQGKPRRFVGSHH <mark>FSRA</mark> AAATARRS <mark>QAA</mark> SVE <mark>RCRRRGRDELLALLFFGRFGEGDAGER</mark> AED	235
PIL15-KO#3.seq	NAPVATTTTRVCGKPRRPVCSHHESRAMAAATARRSQAASVERORRRGRDELLALLPPCREGEGDAGERAED	233
PIL15-KO#5.seq	NAPVATTTTR EPSKESHGELSVETTRZEPCFCFCPCLAAAKLERSSGSGEGEGVMNFSLFSREAVLARATLESACRTCG	237
Consensus	napvattttr g p a aa r g rp r	
WT.seq	T <mark>DNKASNVTASNRVESTVV</mark> QTASGPRSAPAFADQRAAAWPPQPKEMPFASTAAAPMA <mark>P</mark> AVNLHHEMGRDRAGRTMPVHKT	317
PL15-KO#2.seq	AGHRQZ	241
PIL15-KO#3.seq	AGHRQZ	239
PIL15-KO#5.seq	T <mark>DNKASNVTASNRVESTVV</mark> LDGERAKERTGVRRSEGGGVAAAAEGDAVRVHGSRSHG <mark>P</mark> GRZ	298
Consensus		
WT.seq	EARKAPEATVATSSVCSGNGAGSDELWRQQKRKCQAQAECSASQDDDLDDEPGVLRKSGTRSTKRSRTAEVHNLSERRRR	397
PL15-KO#2.seq		241
PIL15-KO#3.seq		239
PIL15-KO#5.seq		298
Consensus		
WT.seq	DRINEKMRALQELIPNCNKIDKASMLDEAIEYLKTLQLQVQMMSMGTGLCIPPMLLPTAMQHLQIPPMAHFPHLGMGLGY	477
PL15-KO#2.seq		241
PIL15-KO#3.seq		239
PIL15-KO#5.seq		298
Consensus		
WT.seq	GMGVFDMSNTGALQMPPMPGAHFPCPMIPGASPQGLGIPGTSTMPMFGVPGQTIPSSASSVPPFASLAGLPVRPSGVPQV	557
PL15-KO#2.seq		241
PIL15-KO#3.seq		239
PIL15-KO#5.seq	•••••••••••••••••••••••••••••••••••••••	298
Consensus		
WT.seq	SGAMANMVQDQQQGIANQQQQCLNKEAIQGANPGDSQMQIIMQGDNENFRIPSSAQTKSSQFSDGTGKGTNARERDGAET	637
PL15-KO#2.seq		241
PIL15-KO#3.seq		239
PIL15-KO#5.seq		298
Consensus		

Fig. S9 Expression of OsmiR530 in rice *OsPIL15* transgenic lines. The expression of mature OsmiR530 in *OsPIL15*-overexpressing (*PIL15*-OE) and knockout (*PIL15*-KO) lines. 5.8S rRNA was used as an internal control for the qRT-PCR. Data are presented as the mean \pm standard deviation of three biological replicates.

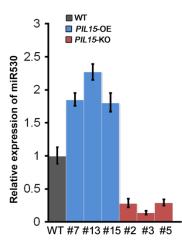


Fig. S10 Alignment of mature miR530 sequences identified in miRBase. tae, *Triticum aestivum*; mdm, *Malus domestica*; stu, *Solanum tuberosum*; ppe, *Prunus persica*; eun, *Eugenia uniflora*; mtr, *Medicago truncatula*; gma, *Glycine max*; cme, *Cucumis melo*; tcc, *Theobroma cacao*; fve, *Fragaria vesca*; aqc, *Aquilegia caerulea*; bdi, *Brachypdium distachyon*; ptc, *Populus trichocarpa*; csi, *Citrus sinensis*; lus, *Linum usitatissimum*; htu, *Helianthus tuberosus*; mes, *Manihot esculenta*; gra, *Gossypium raimondii*; sly, *Solanum lycopersicum*.

tae-miR530	UGCAGUGGCAUAUGCAACUCU	21
mdm-miR530a	UGCAUUUGCACCUGCACUUGU	21
mdm-miR530b	UGCAUUUGCACCUGCACUUGU	21
mdm-miR530c	UGCAUUUGCACCUGCACUUGU	21
stu-miR530	-UCUGCAUUUGCACCUGCACCU	21
ppe-miR530	-UCUGCAUUUGCACCUGCACCU	21
eun-miR530-5p	-UCUGCAUUUGCACCUGCACCU	21
mtr-miR530	UGCAUUUGCACCUGCACUUUC	21
gma-miR530a	UGCAUUUGCACCUGCACUUU-	20
gma-miR530b	UGCAUUUGCACCUGCACUUUA	21
gma-miR530c	UGCAUUUGCACCUGCACUUUA	21
gma-miR530d	UGCAUUUGCACCUGCACUUUA	21
gma-miR530e	UGCAUUUGCACCUGCACUUUA	21
cme-miR530b	UGCAUUUGCACCUACACCUU-	20
tcc-miR530a	UGCAUUUGCACCUGCACCUC-	20
fve-miR530	UGCAUUUGCACCUGCACCUCU	21
aqc-miR530	UGCAUUUGCACCUGCAUCUC	20
OsmiR530	UGCAUUUGCACCUGCACCUAC	21
bdi-miR530a	UGCAUUUGCACCUGCACCUAC	21
bdi-miR530b	UGCAUUUGCACCUGCACCUAC	21
ptc-miR530b	UGCAUUUGCACCUGCAUCUU-	20
csi-miR530b-5p	UGCAUUUGCACCUGCAUCUUG	21
mes-miR530b	UGCAUUUGCACCUGCACCUUA	21
ptc-miR530a	UGCAUUUGCACCUGCACCUU-	20
csi-miR530a-5p	UGCAUUUGCACCUGCACCUUG	21
tcc-miR530b	UGCAUUUGCACCUGCACCUU-	20
lus-miR530a	UGCAUUUGCACCUGCACCUU-	20
lus-miR530b	UGCAUUUGCACCUGCACCUU-	20
htu-miR530	UGCAUUUGCACCUGCACCUU-	20
cme-miR530a	UGCAUUUGCACCUGCACCUU-	20
mes-miR530a	UGCAUUUGCACCUGCACCUU-	20
gra-miR530a	AGGUGCAGAUGCAGUUGCAGG	21
gra-miR530b	AGGUGCAGGUGCAGGCGCAGC	21
sly-miR530	AGGUGUAGGUGUUCAUGCAGA	21
	** * * **	

Fig. S11 PHYB physically interacts with OsPIL15. (**a**) Yeast two-hybrid analysis of the interaction between PHYB and OsPIL15. The full-length *OsPIL15* open reading frame and the C-terminal *PHYB* cDNA sequences were inserted into GAL4-AD and GAL4-BD vectors to generate AD-*OsPIL15* and BD-*PHYB*-C constructs, respectively. Yeast AH109 cells were co-transformed with the two constructs. The AD/BD-*PHYB*-C combination was used as a negative control. Transformed yeast cells were selected on synthetic dextrose medium lacking Leu and Trp (SD/–L/–W) and then transferred to medium lacking Leu, Trp and His (SD/–L/–W/–H). (**b**) A firefly luciferase complementation imaging (LCI) assay revealed the interaction between PHYB and OsPIL15 in *Nicotiana benthamiana* leaves. The constructs are listed on the right. The signals were analyzed at 48 h after the infiltrations. At least 10 independent *N. benthamiana* leaves were infiltrated for each experiment. (**c**) Semi-*in vivo* pull-down assays confirming that PHYB specifically interacts with OsPIL15 under red light conditions.

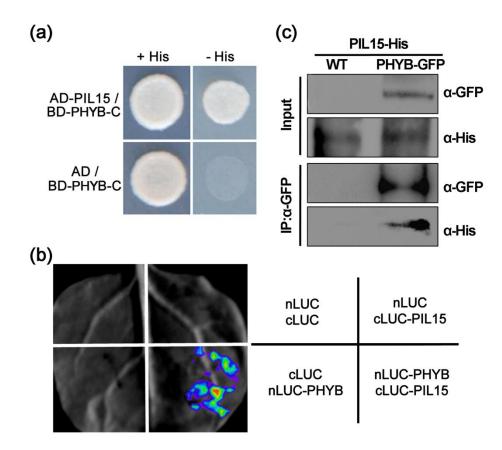
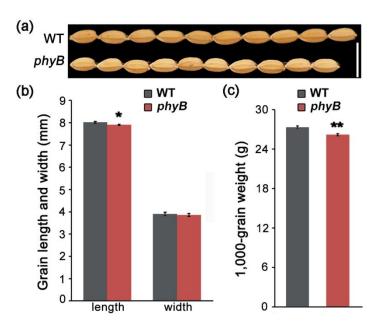


Fig. S12 Knockout of *PHYB* in rice results in smaller seed. (a) Grains with hulls of wild-type (WT) and *phyB* mutant. Bar, 1cm. (b) Grain length and width of WT and *phyB* mutant. (c) The 1,000-grain weight of WT and *phyB* mutant. The data in (b) and (c) are presented as the mean \pm standard deviation (b, *n* > 300 seeds; c, *n* = 3 replicates). Significant differences were determined with Student's *t*-test (* *P*< 0.05, ** *P*< 0.01).



miR530-OE-F	5'-gg actagtATGCTCATTGATTGAAACTTC-3'	For OsmiR530
miR530-OE-R	5'-cg ggatccCTGATCCACGTGTAAACCG-3'	overexpression construct
osa-5.8S	5'-GAACGACTCTCGGCGGCTA-3'	For detecting the expression of the 5.8S rRNA by qRT- PCR
osa-miR530	5'-cgccTGCATTTGCACCTGCACCTAC-3'	For detecting the expression of the mature OsmiR530 by qRT-PCR
MIM530-RF1	5'-TCTCTCTTCTCTATGCTTTG-3'	For detecting OsmiR530 target mimic by qRT-PCR
MIM530-RR1	5'-CACTGGTCTGACTATTCTC-3'	
miR530Pre-F	5'-AGGAAGATGAAGCAGCAA-3'	For detecting the precursor of
miR530Pre-R	5'-AAGAGACCAGGAACAACAT-3'	OsmiR530 by qRT-PCR
IPS1-F	5'-caccAAACACCACAAAAAACAAAAGA-3'	
IPS1-R	5'-ACTATAAAGAGAATCGGAAGCA-3'	
MIM530-F	5'-AATAGGTGCAGGTGTTGCAAATGCAAGCTTCGGTTCCCCTC- 3'	For OsmiR530 target mimic construct (MIM530)
MIM530-R	5'-CT TGCATTTGCAACACCTGCACCTA TTTCTAGAGGGAGA TAA-3'	
PHYB-F	5'- aatctaga ATGGCCTCGGGTAGCCG-3'	For PHYB-GFP construct
PHYB-R	5'- atggtacc GCTTGTCCCCCTACTT G-3'	Torring-orr construct
eGFP-F	5'- aaggtcaccATGGTGAGCAAGGGCGAG-3'	For eGFP amplification
eGFP-R	5'- atggtcaccTTACTTGTACAGCTCGTCCATG-3'	roi eorr ampinication
pMIR530-A	gageteCGGTCTTGGACGAGAAAGCAGCTTTGATACGCAAATCTTT AGCTGCCGTTGAACGGCCGGCCAAATCCGACAGAACGAAGCTT TTGGCTACATCTACATGTGACATGTACTGCAGCAGCTAGCAAT ATATCCAACATTGTTGCTAGCTAACTGATC <u>CACGTG</u> TAAACCGG <u>CACGTG</u> TCGCCGTGCCATTGGCCTGCCGTGCAAGTCCCATGTGA ACCGTGGCGATGTGCctcgag	For yeast one-hybrid (Fragment A, G-box were underlined)
pMIR530-Am	gageteCGGTCTTGGACGAGAAAGCAGCTTTGATACGCAAATCTTT AGCTGCCGTTGAACGGCCGGCCAAATCCGACAGAACGAAGCTT TTGGCTACATCTACATGTGACATGTACTGCAGCAGCTAGCAAT ATATCCAACATTGTTGCTAGCTAACTGATC <u>TGACTT</u> TAAACCGG <u>TGACTT</u> TCGCCGTGCCATTGGCCTGCCGTGCAAGTCCCATGTGA ACCGTGGCGATGTGCetegag	For yeast one-hybrid (mutant fragment A, mutant G-box were underlined)
pMIR530-B	gageteCGACAGGTAATATGGGACAGAGGTAGTAGATATTTTCC <u>C</u> <u>ACGTG</u> CACTACAGATGGTTTCTCTTAGAAGCTGCTAGCTAGCTct cgag	For yeast one-hybrid (Fragment B, G-box were underlined)
pMIR530-Bm	gageteCGACAGGTAATATGGGACAGAGGTAGTAGATATTTTCC <u>T</u> <u>GACTT</u> CACTACAGATGGTTTCTCTTAGAAGCTGCTAGCTAGCTct cgag	For yeast one-hybrid (mutant fragment B, mutant G-box were underlined)
Probe A-F	5'-TGATC <u>CACGTG</u> TAAACCGG <u>CACGTG</u> TCGCC-3'	For EMSA assay (Probe A,
Probe A-R	5'-GGCGA <u>CACGTG</u> CCGGTTTA <u>CACGTG</u> GATCA-3'	G-box were underlined)
Probe Am-F	5'-TGATC <u>TGACTT</u> TAAACCGG <u>TGACTT</u> TCGCC-3'	For EMSA assay (mutant probe A, mutant G-box were underlined)
Probe Am-R	5'-GGCGA <u>AAGTCA</u> CCGGTTTA <u>AAGTCA</u> GATCA-3'	
Probe B-F	5'- ATTTTCC <u>CACGTG</u> CACTACAGATGGTT -3'	For EMSA assay (Probe B, G-box were underlined)
Probe B-R	5'- AACCATCTGTAGTG <u>CACGTG</u> GGAAAAT -3'	

Table S1 Details regarding the primers and DNA fragments used in this study.

Probe Bm-F	5'- ATTTTCC <u>TGACTT</u> CACTATGACTTGTT -3'	For EMSA assay (mutant probe B, mutant G-box were
Probe Bm-R	5'- AACAAGTCATAGTG <u>AAGTCA</u> GGAAAAT-3'	underlined)
PHYB-1300- nLUC-Infusion-F PHYB-1300- nLUC-Infusion-R	5'-TCGGTACCCGGGATCCAATGGCCTCGGGTAGCCGC-3' 5'-ACGAGATCTGGTCGACTCAGCTTGTCCCCCTACTTGCTG-3'	For LCI assay
PIL15-1300- cLUC-Infusion-F PIL15-1300- cLUC-Infusion-R	5'-AGCTCTGCAGGTCGACTCAGCTTGTCCGACGGCAACGACTTC-3'	- For LCI assay
OsPIL15NdeF1 OsPIL15LxhoR1	5'-CAC ATA TGT CCG ACG GCA ACG ACT-3' 5'-ATC TCG AGT GTT TCA GCC CCA TCT CTC-3'	For yeast one-hybrid and two- hybrid assays
phyB-C-F phyB-C-R	5'-AACCATGGCAGAGGGCACAAGTAAC-3' 5'-ATCTGCAGCTTGTCCCCCTACTTG-3'	For yeast two-hybrid assay
PIL15-Infusion-F PIL15-Infusion-R	5'-CGAAATCGATGGATCCTATGTCCGACGGCAACGACTTC-3' 5'-ATTCGAGCTCACTAGTTTATGTTTCAGCCCCATCTCTCTC	For Luciferase activity assay
p530-LUC-F p530-LUC-R	5'-caccAGGAAGCGGATTACATAGAAC-3' 5'-GCTCTATCACACATCACTTGC-3'	Luciferase activity assay
p530Chip-AF p530Chip-AR	5'-GCTTTTGGCTACATCTACAT-3' 5'-CGGTTCACATGGGACTTG-3'	For ChIP-qPCR (Fragment A)
p530Chip-BF p530Chip-BR	5'-GGTAATATGGGACAGAGGTA-3' 5'-GAACTATTGTCGATGGGTT-3'	For ChIP-qPCR (Fragment B)
p530Chip-CF p530Chip-CR	5'-TTGACAACCGGCACCTAA-3' 5'-AAACATTTATGGGCTTATCGCTAT-3'	For ChIP-qPCR (Fragment C)
PL3-GSP1	5'-AACAGGATTCACAGCAGACACG-3'	For RLM5' -RACE
PL3-GSP2	5'-AGCCCATCCTTATGCTTTTCCTG-3'	For RLM5' -RACE
PL3-TS PL3-TAS	5'-cgggatccATGAGATCTGTCTCTGAGCTGGTG-3' 5'-gctctagaAAAACGAAGCAGCTCTCTT-3'	For subcellular localization of OsPL3
PL3-RS PL3-RAS	5'-AAGCTGTGAAATGAGATG-3' 5'-ATGCCGAATATTATGACTG-3'	For detection of <i>OsPL3</i> expression by qRT-PCR