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Supplementary Materials for

Ubiquitination of OsCSN5 by OsPUB45 activates immunity by modulating the OsCUL3a-OsNPR1 module

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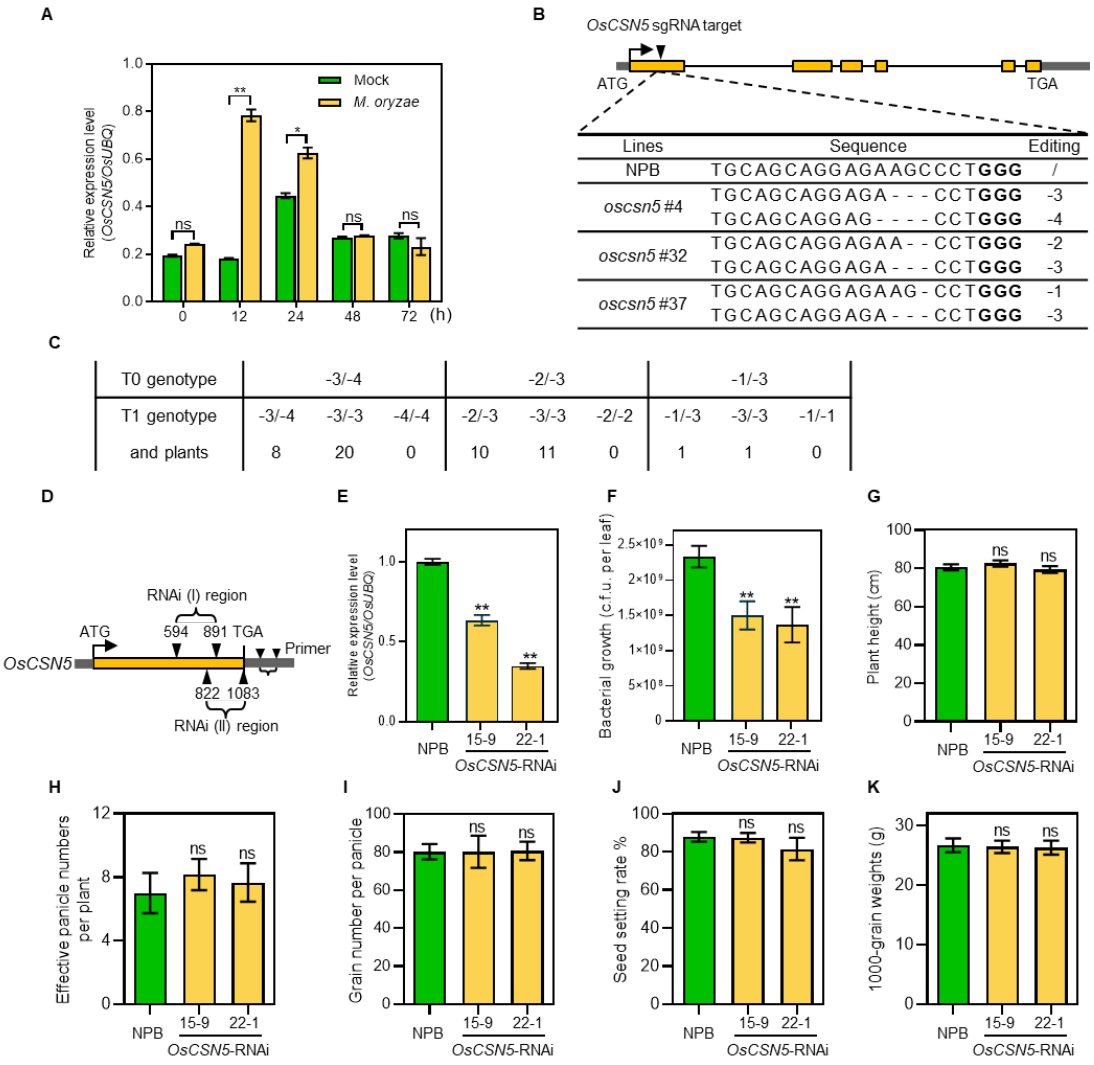

Fig. S1. Expression patterns of *OsCSN5*, genotype of *oscsn5* mutant and major agronomic traits of *OsCSN5*-RNAi plants.

(A) The expression of *OsCSN5* in NPB plants inoculated with the compatible *M. oryzae* isolate RB22, as determined by qRT-PCR. A mock-inoculation control was prepared using ddH_2O containing 0.1% (v/v) Tween 20. The rice *UBIQUITIN* (*OsUBQ*) gene was used as the reference gene to normalize gene expression levels. Values are means ± SD (n = 3, technical repeats).

(B) Diagram of mutations in *oscsn5* mutants generated by CRISPR/Cas9 technology. The yellow boxes represent exons and grey boxes represent untranslated regions (UTRs).

(C) Genotypes of *oscsn5* mutant plants in T1 generation.

(D) Diagram of the *OsCSN5*-silencing regions in *OsCSN5*-RNAi plants. The yellow boxes represent coding sequence (CDS) and grey boxes represent untranslated regions (UTRs).

(E) The expression of *OsCSN5* in *OsCSN5*-RNAi transgenic plants by qRT-PCR. *UBIQUITIN* (*OsUBQ*) was used as an internal control. Values are means ± SD (n = 3, technical repeats).

(F) Bacterial growth as indicated by the numbers of colony-forming units (c.f.u.) per leaf for *OsCSN5*-RNAi and NPB plants (n = 3).

(G-K) Major agronomic traits measured in *OsCSN5*-RNAi plants: plant height (G), effective panicle numbers per plant (H), grain number per panicle (I), seed setting rate % (J), thousand-grain weights (K). For (G)-(K), values are means ± SD (n = 6). 'ns' indicates no statistical significance at $p > 0.05$ according to Student's t test.

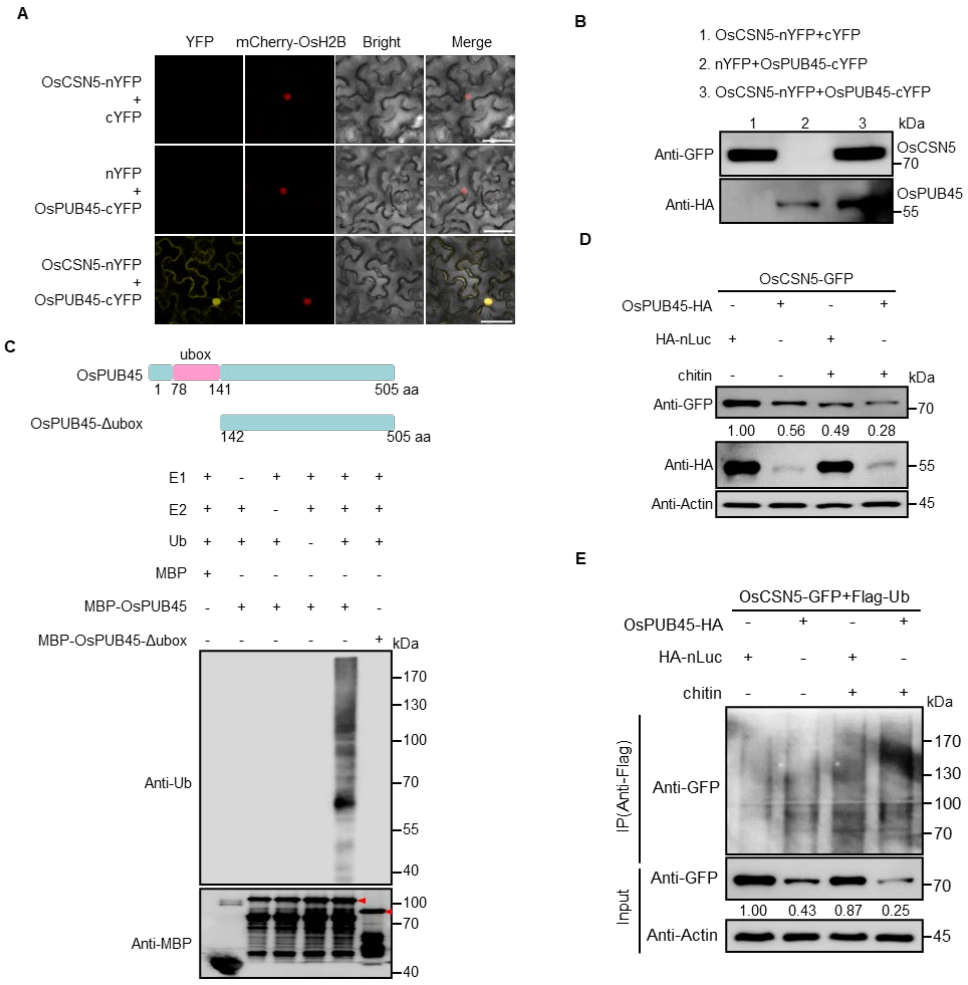

Fig. S2. OsPUB45 displays E3 ligase activity and degrades OsCSN5 in rice protoplasts.

(A) BiFC assay to confirm the interaction between OsCSN5 and OsPUB45 in *N. benthamiana*. Scale bars, 50 μm. mCherry-OsH2B was co-expressed to indicate the nucleus.

(B) Detection of protein expression in BiFC assay shown in (A). Anti-GFP and anti-HA antibodies were used to detect OsCSN5-nYFP and OsPUB45-cYFP, respectively.

(C) E3 ubiquitin ligase activity of OsPUB45. E1, E2, Ub, and rice total extract preincubated MBP-OsPUB45 or MBP-OsPUB45-Δubox were incubated in the reactions. Ubiquitin-bound proteins were detected by immunoblot with anti-Ub and anti-MBP antibodies. Arrowheads indicate the target bands of MBP-OsPUB45 and MBP-OsPUB45-Δubox.

(D) Degradation of OsCSN5 by OsPUB45 in rice protoplasts with chitin treatment. Co-expressed OsCSN5-GFP with HA-nLuc or OsPUB45-HA in rice protoplasts with or without 80 nM chitin treatment for 1 h. Anti-GFP and anti-HA antibodies were used to check OsCSN5-GFP and OsPUB45-HA, respectively.

(E) Ubiquitination levels of OsCSN5 by OsPUB45 with chitin treatment. Flag-Ub was transiently co-expressed in rice protoplasts with OsCSN5-GFP and OsPUB45-HA or HA-nLuc with or without 80 nM chitin for 1 h. Total proteins were extracted and were then subject to immunoprecipitation with anti-Flag. The polyubiquitination of OsCSN5-GFP was detected by immunoblot with an anti-GFP antibody. The input proteins were probed with anti-GFP. Actin was served as an internal control.

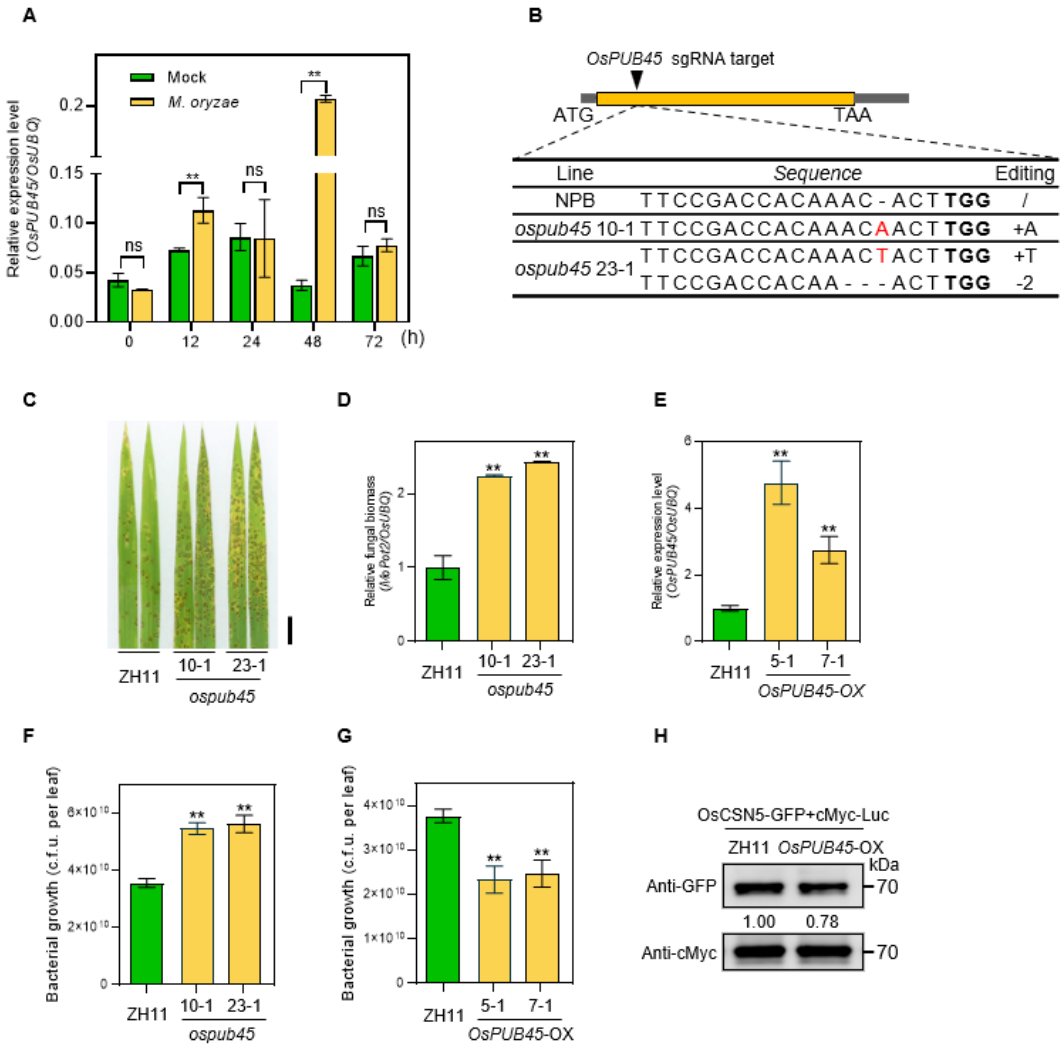

Fig. S3. Expression patterns of *OsPUB45*, genotype and phenotype of *OsPUB45* transgenic plants.

(A) The expression of *OsPUB45* in NPB plants inoculated with the compatible *M. oryzae* isolate RB22, as determined by qRT-PCR. A mock-inoculation control was prepared using ddH_2O containing 0.1% (v/v) Tween 20. Values are means ± SD (n = 3, technical repeats).

(B) Diagram of the mutation types in *ospub45* mutant lines generated by CRISPR/Cas9 technology. The yellow boxes represent exons and grey boxes represent untranslated regions (UTRs).

(C and D) Disease symptoms (C) and relative fungal biomass (D) of representative leaves from *ospub45* mutants and ZH11 plants after spray inoculation with *M. oryzae* isolate RB22. Scale bar, 2 cm.

(E) Transcript levels of *OsPUB45* in *OsPUB45*-OX transgenic plants was measured by qRT-PCR.
(F) Bacterial growth as indicated by the numbers of colony-forming units (c.f.u.) per leaf for *ospub45* mutants and ZH11 plants (n = 3).
(G) Bacterial growth as indicated by the numbers of colony-forming units (c.f.u.) per leaf for *OsPUB45*-OX and ZH11 plants (n = 3).
(H) Detection of OsCSN5 protein levels in ZH11 and *OsPUB45*-OX protoplasts. OsCSN5-GFP and cMyc-Luc were transiently expressed in ZH11 and *OsPUB45*-OX rice protoplasts and OsCSN5-GFP was detected by immunoblotting using an anti-GFP antibody. cMyc-Luc served as loading control. The relative OsCSN5-GFP band intensities were quantified with ImageJ. Similar results were obtained from three independent biological experiments.

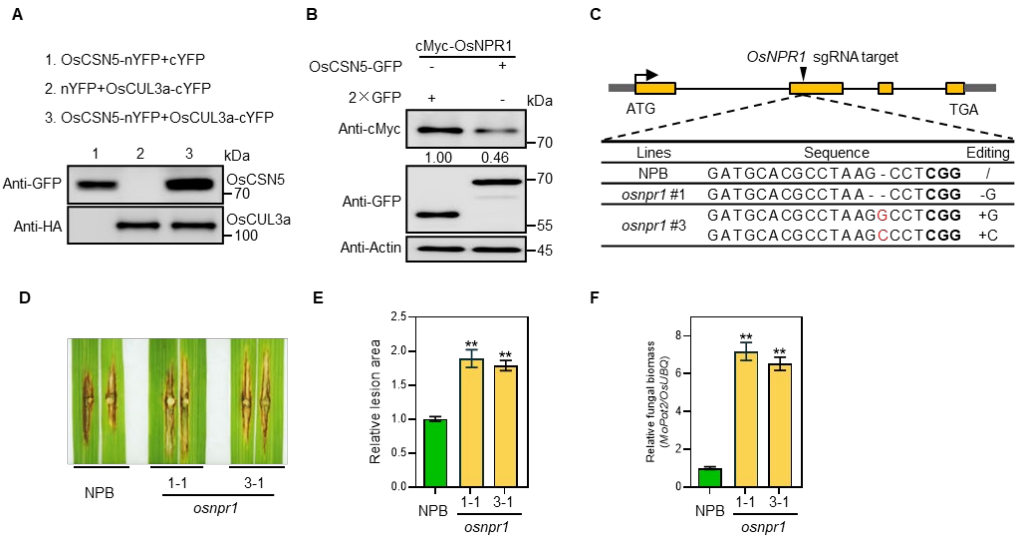

Fig. S4. Mutation type and blast resistance analysis in *osnpr1* mutant plants.

(A) Detection of protein expression in Fig. 4B. Anti-GFP and anti-HA antibodies were used to detect OsCSN5-nYFP and OsCUL3a-cYFP, respectively.

(B) OsCSN5 promotes the degradation of OsNPR1. *2×GFP* or *OsCSN5-GFP* and *cMyc-OsNPR1* plasmids were co-expressed in rice protoplasts. The relative cMyc-OsNPR1 band intensities were quantified with ImageJ. Actin serves as an internal control. Similar results were obtained from three independent biological experiments.

(C) Diagram of the mutation types in *osnpr1* mutants generated by CRISPR/Cas9 technology. The yellow boxes represent exons and grey boxes represent untranslated regions (UTRs).

(D-F) Disease symptoms (D), the relative leaf area with lesions, as measured by ImageJ (E), and relative fungal biomass (F) of representative leaves of *osnpr1* and NPB plants after punch inoculation with *M. oryzae* isolate RB22. Values are means ± SD (n = 3, technical repeats).

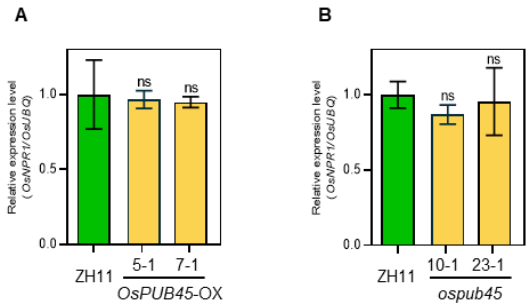

Fig. S5. Transcript levels of *OsNPR1* in *OsPUB45*-OX and *ospub45* plants.

(A) Relative transcript levels of *OsNPR1* in *OsPUB45*-OX and ZH11 plants as determined by qRT-PCR.

(B) Relative transcript levels of *OsNPR1* in *ospub45* mutants and ZH11 plants as determined by qRT-PCR.

Values are means ± SD (n = 3, technical repeats). 'ns' indicates no statistical significance at $p > 0.05$ according to Student's t test.

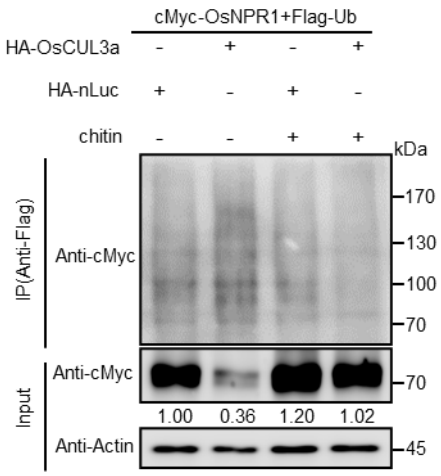

Fig. S6. The ubiquitination and degradation analysis of OsNPR1 by OsCUL3a with chitin treatment.

In vivo ubiquitination assay to show the ubiquitination levels and degradation of OsNPR1 after co-expression with OsCUL3a in rice protoplasts with chitin treatment. Flag-Ub was transiently co-expressed with cMyc-OsNPR1 and HA-OsCUL3a. Transfected protoplasts were treated with 80 nM chitin for 1 h. Total proteins were extracted and were then subject to immunoprecipitation with anti-Flag. The polyubiquitination of cMyc-OsNPR1 was detected by immunoblotting with an anti-cMyc antibody. Actin was served as an internal control.

Supplemental Table 1. List of primers used in this study

Primer Name	Primer Sequence （5'-3'）
Primers for vectors used for plant transformation	
CRISPR-*OsPUB45*-F	TGTTGCGTTCCGACCACAAACACT
CRISPR-*OsPUB45*-R	AAACAGTGTTTGTGGTCGGAACGC
CRISPR-*OsNPR1*-F	TGTTGTTGATGCACGCCTAAGCCT
CRISPR-*OsNPR1*-R	AAACAGGCTTAGGCGTGCATCAAC
CRISPR-*OsCSN5*-F	TGTTGTCGTCCACGCCCGCGCCGG
CRISPR-*OsCSN5*-R	AAACCCGGCGCGGGCGTGGACGAC
OsPUB45-OX-F	TCCCCGGGTGAGCTCGGTACCATGGACCCCTCATCCTCCT
OsPUB45-OX-R	CACCATAGCGGCCGCACTAGTGAAAGGCATGATGTGGGTG
Primers for co-IP and degradation assay	
OsCSN5-GFP-F	TAGAACTAGTGGATCCATGGAGCCCACCTCGTCG
OsCSN5-GFP-R	CCCCCTCGAGGTCGACTGCTTCAACCATAGGCTCAG
OsPUB45-HA-F	TAGAACTAGTGGATCCATGGACCCCTCATCCTCCTC
OsPUB45-HA-R	CCCCCTCGAGGTCGACGAAAGGCATGATGTGGGTG
*OsPUB45-ubox*HA-F	GCTCTAGAACTAGTGGATCCATGGCCTCCACGGCCGCGC
*OsPUB45-ubox*HA-R	GGGCCCCCCCTCGAGGTCGACGAAAGGCATGATGTGGGTG
pRTVnHA-*OsCLU3a*-F	GTTCCAGATTACGCTGGATCCATGAGCGGGGGCGGGC
pRTVnHA-*OsCLU3a*-R	TCACTTAGCGGCCGCACTAGTCTATGCAAGATAGCGATATAAC
pRTVcMyc-*OsNPR1*-F	GAGGACTTGAATTCGGGATCCATGGAGCCGCCGACCAG
pRTVcMyc-*OsNPR1*-R	TCACTTAGCGGCCGCACTAGTTCATCTCCTTGGTCGAATGG
Primers for yeast two hybrid assay	
pGBKT7-*OsCSN5*-F	ATGGCCATGGAGGCCGAATTCATGGAGCCCACCTCGTCG
pGBKT7-*OsCSN5*-R	TGCGGCCGCTGCAGGTCGACTCATGCTTCAACCATAGGC
Primers for BiFC assay	
p2YN-*OsCSN5*-F	CATTTACGAACGATAGTTAATTAAATGGAGCCCACCTCGTCG
p2YN-*OsCSN5*-R	CACTGCCACCTCCTCCACTAGTTGCTTCAACCATAGGCTCAG
p2YC-*OsPUB45*-F	CATTTACGAACGATAGTTAATTAAATGGACCCCTCATCCTCCT
p2YC-*OsPUB45*-R	CACTGCCACCTCCTCCACTAGTGAAAGGCATGATGTGGGTG
p2YC-*OsCLU3a*-F	CATTTACGAACGATAGTTAATTAAATGAGCGGGGGCGGGC
p2YC-*OsCLU3a*-R	CACTGCCACCTCCTCCACTAGTTGCAAGATAGCGATATAACTTC
Primers for qRT-PCR and Fungal biomass determination	
OsPAL1-qF	TGAATAACAGTGGAGTGTGGAG
OsPAL1-qR	AACCTGCCACTCGTACCAAG
OsPR10-qF	CCCTGCCGAATACGCCTAA
OsPR10-qR	CTCAAACGCCACGAGAATTTG
OsPUB45-qF	ATGGACCCCTCATCCTCCTC
OsPUB45-qR	GGTGTTGCAACAATGGCGAT
OsCSN5-qF	CCTTTTGTTGATGCTGGATTCA

OsCSN5-qR	TTGACGATAAAACGCAGCTAAC
OsUBQ-qF	CGCAAGAAGAAGTGTGGTCA
OsUBQ-qR	GGGAGATAACAACGGAAGCA
MoPot2-qF	ACGACCCGTCTTTACTTATTTGG
MoPot2-qR	AAGTAGCGTTGGTTTTGTTGGAT
OsgUBQ-qF	TTCTGGTCCTTCCACTTTCAG
OsgUBQ-qR	ACGATTGATTTAACCAGTCCATGA
Primers for RT-PCR	
OsCSN5-F	GGTCTCATGTCACAGGTCATTA
OsCSN5-R	TGAATCCAGCATCAACAAAAGG
OsNPR1-F	GAGCCCTTGACTCTGACGAT
OsNPR1-R	CCTCGCAGCAATGTGAAGAA
Primers for ubiquitination assay	
pGEX-6P-1-*OsCSN5*-F	TTCCAGGGGCCCCTGGGATCCATGGAGCCCACCTCGTCG
pGEX-6P-1-*OsCSN5*-R	ATGCGGCCGCTCGAGTCGACTCATGCTTCAACCATAGGC
pMAL-c2X-*OsPUB45*-F	ATTTCAGAATTCGGATCCATGGACCCCTCATCCTCCTC
pMAL-c2X-*OsPUB45Δubox*-F	ATTTCAGAATTCGGATCCATGGCCTCCACGGCCGCGC
pMAL-c2X-*OsPUB45Δubox*-R	GCTTGCCTGCAGGTCGAC TTAGAAAGGCATGATGTGGGT
Primers for Genotyping knockout plants	
Genotype-CRISPR-*OsPUB45*-F	GAAAAGGCCACCACTGCCTA
Genotype-CRISPR-*OsPUB45*-R	CGGAAGAACGTGGGTACCTT
Genotype-CRISPR-*OsNPR1*-F	ATTGCTAGCCCAATGCATGTC
Genotype-CRISPR-*OsNPR1*-R	CCTCGTAGACTCTCACCTGC
Genotype-CRISPR-*OsCSN5*-F	TGGGAGCTGGAGAACAACATC
Genotype-CRISPR-*OsCSN5*-R	GGCAGGGAAAGCAGATAACG