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Supplemental Information

The Monocot-Specific Receptor-like Kinase SDS2 Controls Cell Death and Immunity in Rice

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Supplemental Figures

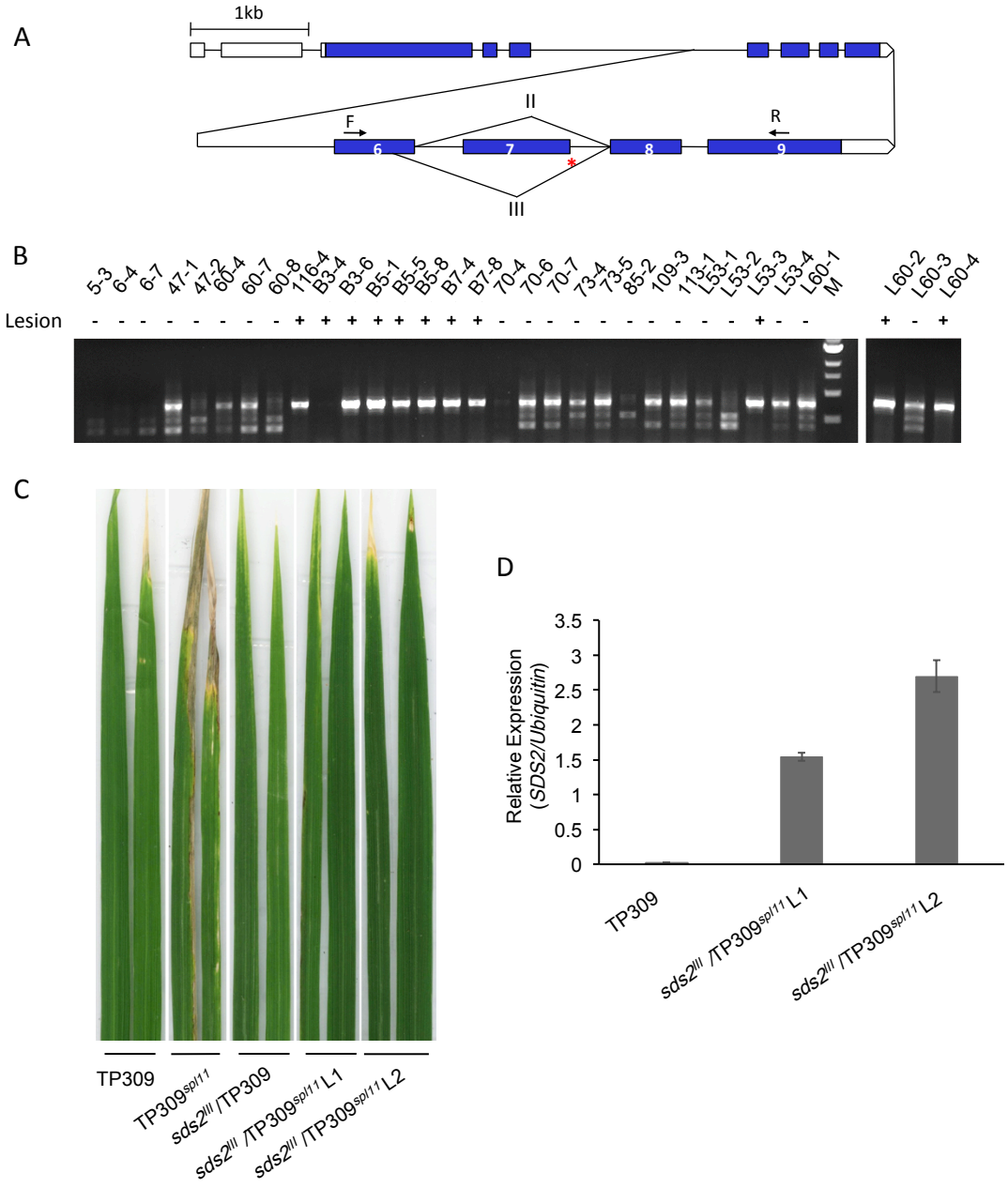

Figure S1. Related to Figure 1: Characterization of the *sds2* Mutant. (A) The *sds2* mutation leads to alternative splicing. Schema of the *SDS2* gene structure and alternative splicing. Boxes and lines indicate exons and introns, respectively. Blue boxes indicate coding regions. Red star shows the G/A transition. F and R indicate primers used for qRT-PCR amplification. II and III show the alternative splicing of Band II and Band III in Figure 1C. (B) Co-segregation assay of lesion suppression and *sds2* mutation. + and – indicate lesion positive and lesion negative plants, respectively. (C) Suppression of *spl11* lesion mimics by over-expression of $sds2^{III}$ in transgenic plants. (D) qRT-PCR analysis of the $sds2^{III}$ transgene transcript levels of $sds2^{III}$ transgenic plants compared to the wild-type TP309. Values are means ± SD calculated from 3 technical repeats.

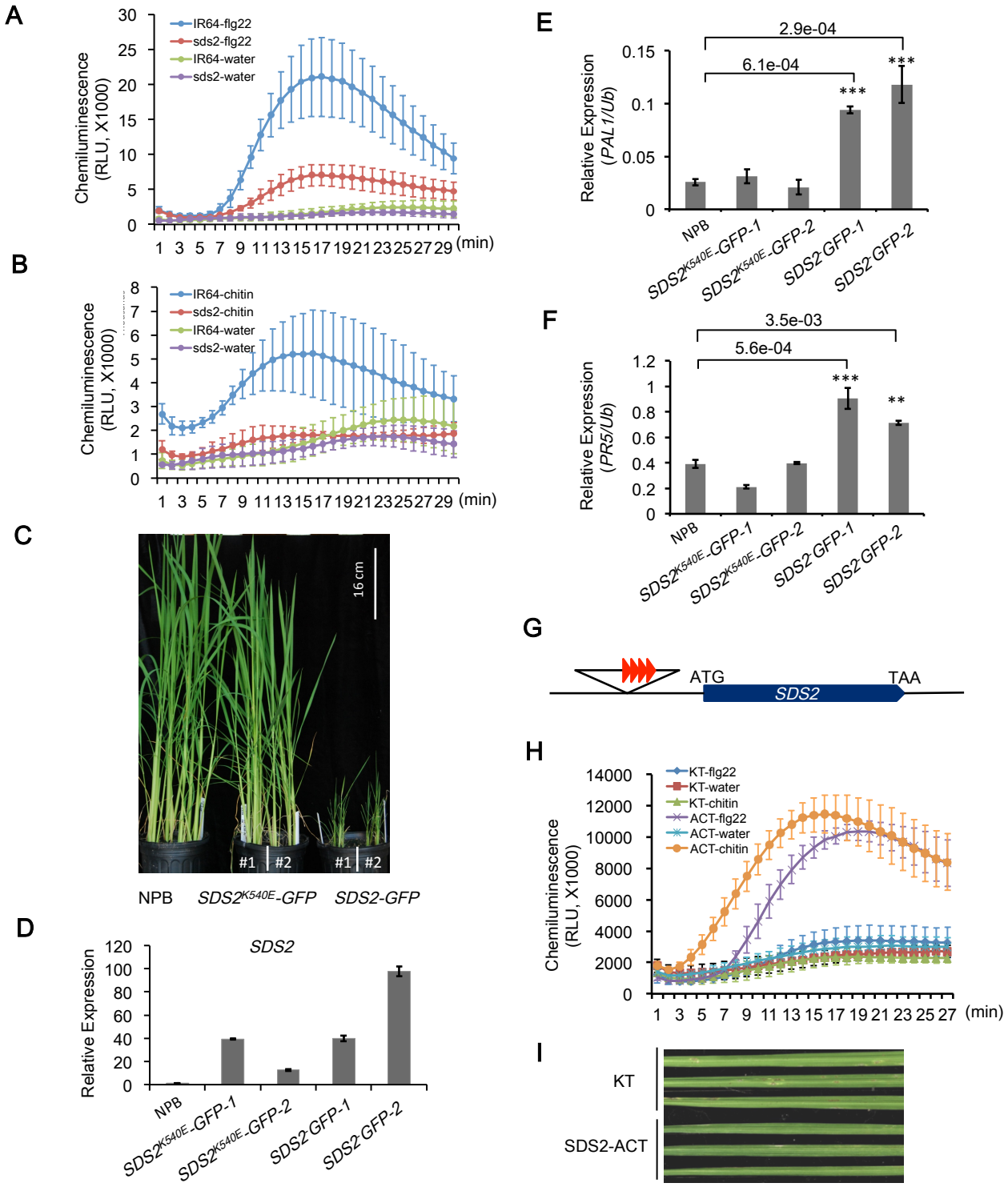

Figure S2. Related to Figure 2: SDS2 Positively Regulates Rice Immunity. (A) and (B) ROS induction in *sds2* and IR64 plants. Leaf discs were treated with 100μM flg22 (A) and 10μg/ml chitin (B), respectively. ROS generation is monitored for 30 min. Bars indicate means of SD calculated from three technical repeats. (C) *SDS2-GFP* overexpression suppresses rice growth (2 months old). (D) qRT-PCR analysis of *SDS2-GFP* transgene expression levels. (E) and (F) PR genes *PAL1* (E) and *PR5* (F) expression in *SDS2-GFP* over-expression plants. Values are means ± SD, n = 3 (technical repeats). Asterisks represent significant difference determined by Student's *t* test (** $p < 0.01$, *** $p < 0.001$). *p* values are labeled. (G) Schematic representation of SDS2-ACT line. Start codon and stop codon are indicated. Red triangles indicate quadrupleCaMV 35S enhancers. . (H) ROS induction in *SDS2-ACT* plants. KT and ACT indicate wild-type Kitaake and *SDS2-ACT* plants, respectively. ROS generation was monitored for 30 min. Bars indicate means of SD calculated from three technical repeats. (I) Spray-inoculated leaves of *SDS2-ACT* plants with blast isolate RO1-1 at 6 dpi. This inoculation was replicated twice with similar results.

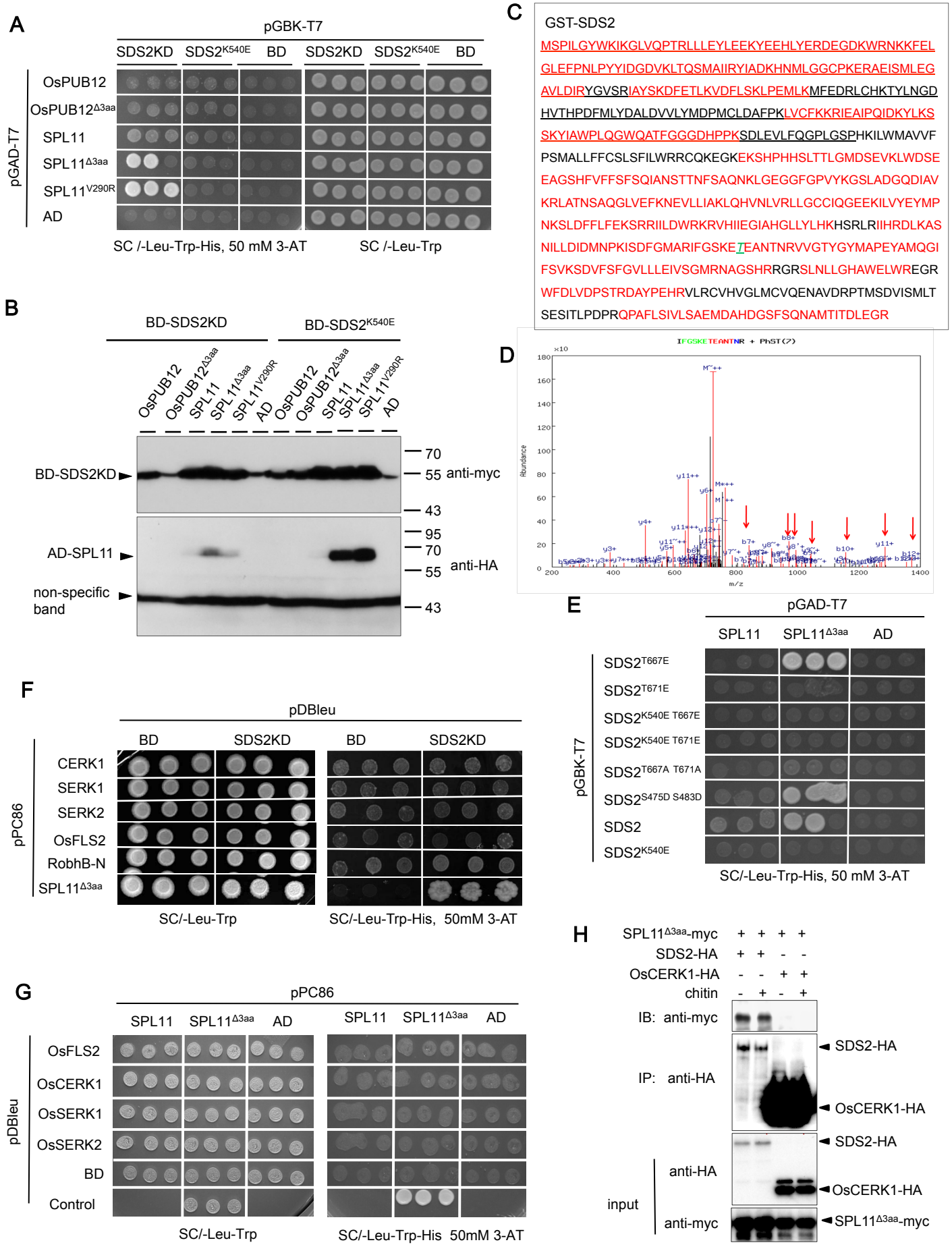
A
pGBK-T7
SDS2KD
SDS2K540E
BD
SDS2KD
SDS2K540E
BD
pGAD-T7
OsPUB12
OsPUB12Δ3aa
SPL11
SPL11Δ3aa
SPL11V290R
AD
SC /-Leu-Trp-His, 50 mM 3-AT
SC /-Leu-Trp
B
BD-SDS2KD
BD-SDS2K540E
OsPUB12
OsPUB12Δ3aa
SPL11
SPL11Δ3aa
SPL11V290R
AD
BD-SDS2KD
AD-SPL11
non-specific band
70
55
43
95
70
55
43
anti-myc
anti-HA
C
GST-SDS2
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEG
AVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGD
HVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKS
SKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPHKILWMAVVF
PSMALLFFCSLSFILWRRCQKEGKEKSHPHHSLTTLGMDSEVKLWDSE
EAGSHFVFFSFSQIANSTTNFSAQNKLGEGGFGPVYKGSLADGQDIAV
KRLATNSAQGLVEFKNEVLLIAKLQHVNLVRLLGCCIQGEEKILVYEYMP
NKSLDFFLFEKSRRIILDWRKRVHIIEGIAHGLLYLHKHSRLRIIHRDLKAS
NILLDIDMNPKISDFGMARIFGSKETEANTNRVVGTYGYMAPEYAMQGI
FSVKSDVFSFGVLLLEIVSGMRNAGSHRRGRSLNLLGHAWELWREGR
WFDLVDPSTRDAYPEHRVLRCVHVGLMCVQENAVDRPTMSDVISMLT
SESITLPDPRQPAFLSIVLSAEMDAHDGSFSQNAMTITDLEGR
D
IFGSKETEANTNR + PhST(7)
Abundance
m/z
E
pGAD-T7
SPL11
SPL11Δ3aa
AD
pGBK-T7
SDS2T667E
SDS2T671E
SDS2K540E T667E
SDS2K540E T671E
SDS2T667A T671A
SDS2S475D S483D
SDS2
SDS2K540E
SC/-Leu-Trp-His, 50 mM 3-AT
F
pDBleu
BD
SDS2KD
BD
SDS2KD
pPC86
CERK1
SERK1
SERK2
OsFLS2
RobhB-N
SPL11Δ3aa
SC/-Leu-Trp
SC/-Leu-Trp-His, 50mM 3-AT
G
pPC86
SPL11
SPL11Δ3aa
AD
SPL11
SPL11Δ3aa
AD
pDBleu
OsFLS2
OsCERK1
OsSERK1
OsSERK2
BD
Control
SC/-Leu-Trp
SC/-Leu-Trp-His 50mM 3-AT
H
SPL11Δ3aa-myc + + + +
SDS2-HA + + - -
OsCERK1-HA - - + +
chitin - + - +
IB: anti-myc
IP: anti-HA
SDS2-HA
OsCERK1-HA
input
anti-HA
SDS2-HA
OsCERK1-HA
anti-myc
SPL11Δ3aa-myc

Figure S3. Related to Figure 3: SDS2 Interacts with SPL11 but not with OsCERK1, OsSERK1 (OsBAK1), OsSERK2, OsFLS2 and OsRbohB. (A**)** Detection of the SDS2-SPL11 interaction with the TaKaRa yeast two-hybrid system (pGBK-T7 and pGAD-T7). OsPUB12 and its mutant OsPUB12$^{\Delta 3aa}$ (C269 P270 K271 three amino acids deletion in the U-box domain) were used as the controls. (B) Detection of protein levels for Y2H assay. Anti-myc and anti-HA antibodies are applied to detect BD fusions and AD fusions in this Y2H system. Arrows indicate specified proteins. Molecular weights in kD are labeled on right. (C) Mass spectrum (MS) analysis of GST-SDS2 auto-phosphorylation sites. Red and black sequences indicate covered and uncovered peptides in the MS data. Green italic letter highlights the detected auto-phosphorylation sites. Underlined sequence is GST tag. (D) A representative plot of MS peptide peaks. Peptide is labeled on the top of the plot. Red arrows indicate phosphorylated peptides. (E) Removal of the auto-phosphorylation site (T667) and its potential alternative site (T671) abolishes the SDS2-SPL11 interaction. (F) SDS2 does not interact with rice OsCERK1, OsSERK1 (OsBAK1), OsSERK2, OsFLS2 and OsRbohB. These genes are constructed in pPC86 and SDS2 (intracellular domain) is in pDBleu. Co-transformants are grown on selection media to detect interaction. (G) No interaction between SPL11 and OsFLS2, OsCERK1 and OsSERK1/2. SPL11 and SPL11$^{\Delta 3aa}$ were fused with AD while candidate kinase genes were fused with BD. Co-transformants were grown on selection media to detect interactions. Positive control was intact GAL4 transformants. (H) Co-IP assay to show SPL11 interacts with SDS2 but not with OsCERK1 and the interaction is not affected by PAMP treatment. SDS2-HA and OsCERK1-HA are used as baits and SPL11$^{\Delta 3aa}$-myc is used as prey. The assay is conducted with rice protoplasts. Protoplasts are treated with (+) or without (-) 5μg/ml chitin for 15 min.

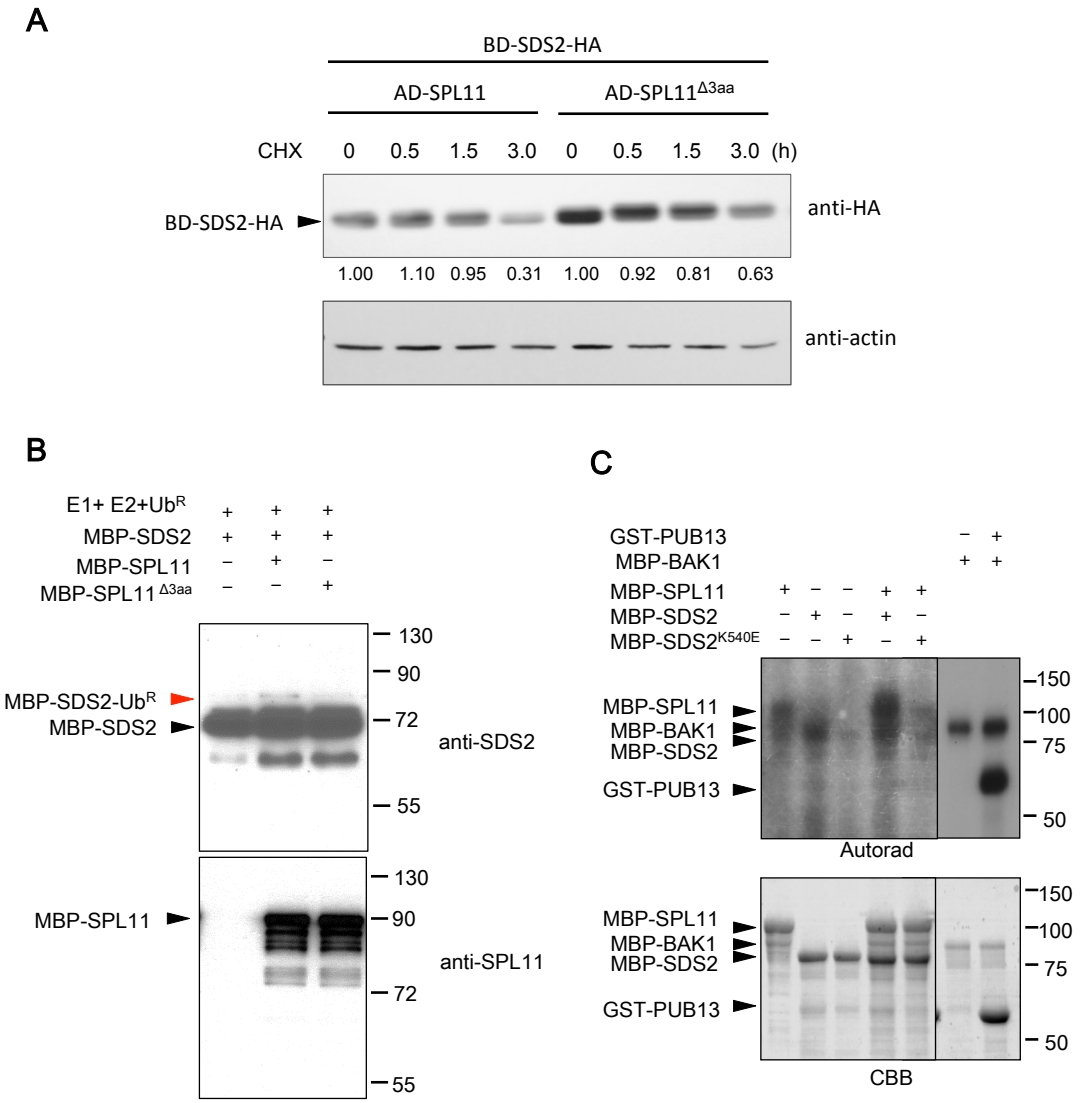

Figure S4. Related to Figure 4: SDS2 is ubiquitinated and degraded by SPL11 and phosphorylate the later. (A) Degradation of SDS2 by SPL11 in Yeast. SDS2 tagged with HA was constructed in pDBleu which is co-transformed into yeast with pPC86-SPL11/SPL11 $^{\Delta 3aa}$. SDS2-HA was detected by western blot. Actin was detected as loading control. Signal intensity is determined by ImageJ software. (B) *In vitro* ubiquitination assay of SDS2 by SPL11. Mutant ubiquitin (Ub^R) (all the Lys were substituted to Arg) was used in this assay to block formation of poly-ubiquitin chain. – and + indicate absent or present of proteins labeled on the left. Black triangles indicate corresponding protein bands labeled on the left. Red arrow indicates the ubiquitinated SDS2 band. (C) Trans-phosphorylation of SPL11 by SDS2. The assay was performed using MBP-SDS2 and MBP-BAK1 (intracellular domain) as kinases and MBP-SPL11 and GST-PUB13-ARM (ARM domain of PUB13) as substrates in the presence of [γ-^{32}P] ATP. Phosphorylation is visualized by autoradiography (top panel). Loading are shown by CBB staining (bottom panel). – and + indicate absent or present of proteins labeled on the left. Molecular weights are labeled on the right. Black triangles indicate specific protein.

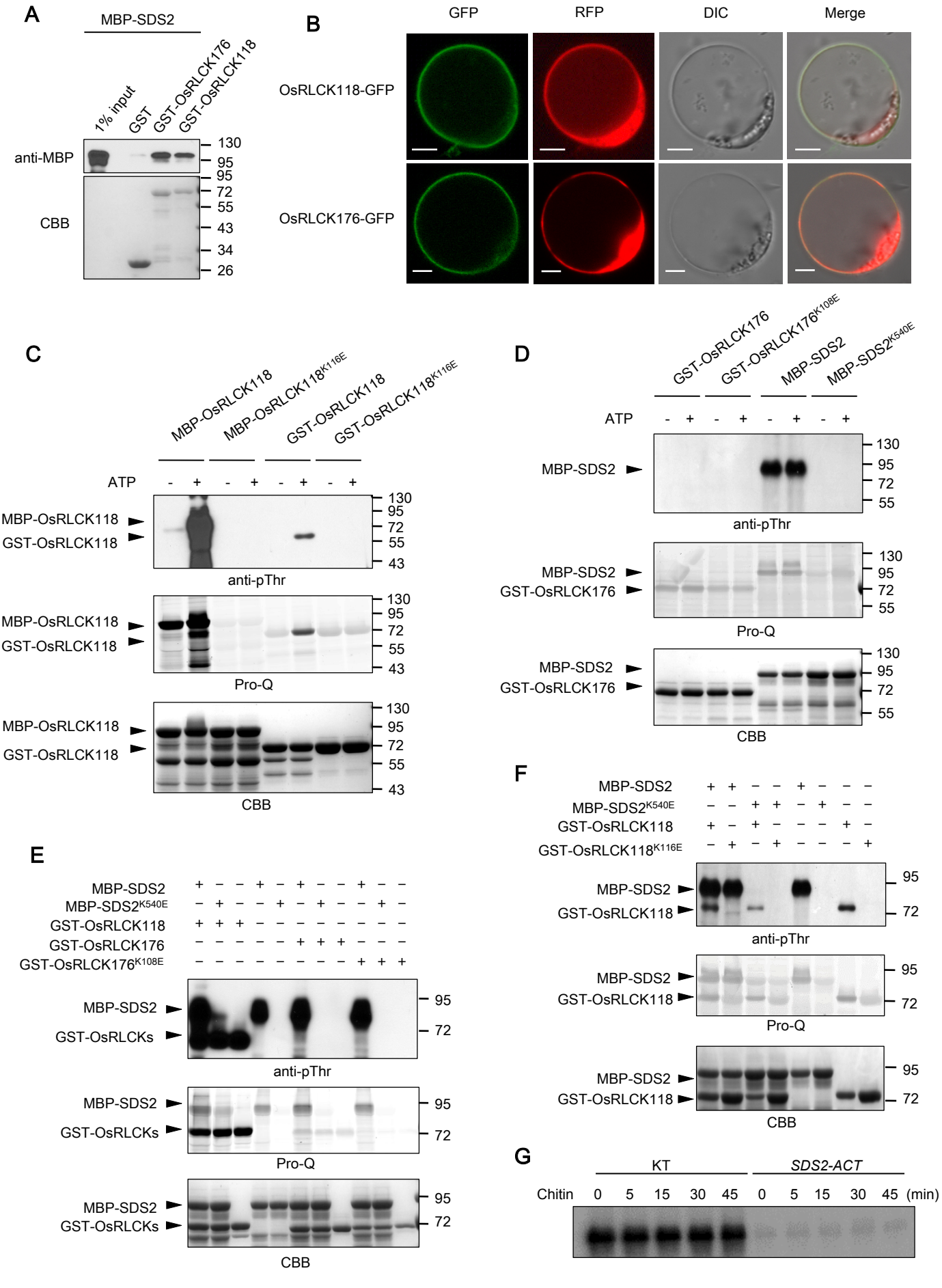
A
MBP-SDS2
1% input
GST
GST-OsRLCK176
GST-OsRLCK118
anti-MBP
CBB
130
95
95
72
55
43
34
26
B
GFP
RFP
DIC
Merge
OsRLCK118-GFP
OsRLCK176-GFP
C
MBP-OsRLCK118
MBP-OsRLCK118K116E
GST-OsRLCK118
GST-OsRLCK118K116E
ATP
MBP-OsRLCK118
GST-OsRLCK118
anti-pThr
Pro-Q
CBB
130
95
72
55
43
D
GST-OsRLCK176
GST-OsRLCK176K108E
MBP-SDS2
MBP-SDS2K540E
ATP
MBP-SDS2
GST-OsRLCK176
anti-pThr
Pro-Q
CBB
130
95
72
55
E
MBP-SDS2
MBP-SDS2K540E
GST-OsRLCK118
GST-OsRLCK176
GST-OsRLCK176K108E
MBP-SDS2
GST-OsRLCKs
anti-pThr
Pro-Q
CBB
95
72
F
MBP-SDS2
MBP-SDS2K540E
GST-OsRLCK118
GST-OsRLCK118K116E
MBP-SDS2
GST-OsRLCK118
anti-pThr
Pro-Q
CBB
95
72
G
KT
SDS2-ACT
Chitin
0
5
15
30
45
0
5
15
30
45
(min)

Figure S5. Related to Figure 5: OsRLCK118 and OsRLCK176 are Genuine RLCKs and transphosphorylation between them and SDS2. (A) Pull-down assay of the SDS2-OsRLCKs interaction. Baits: GST-OsRLCK176 and GST-OsRLCK118. Prey: MBP-SDS2. The prey was detected by immunoblotting (anti-MBP). Loading amount was detected by CBB staining. (B) Subcellular localization of OsRLCK118 and OsRLCK176. OsRLCK118-GFP and OsRLCK176-GFP were co-transfected with RFP into rice protoplasts. RFP was used as a cytoplasm marker. GFP, RFP, DIC and merge channels are labeled on the top of the pictures. Scale bars represent 10 μm. Autophosphorylation assay of OsRLCK118 (C) and OsRLCK176 (D). OsRLCK118^{K116E} and OsRLCK176^{K108E} are kinase-inactive mutants. Purified proteins were applied to phosphorylation assays at the present (+) or absent (-) of 100μM ATP. Protein samples after reaction were separated on a SDS-PAGE gel and detected by immunoblot or Pro-Q staining to determine the phosphorylation level. CBB staining is used to determine loading control. A same gel was used for Pro-Q staining and CBB staining. Molecular markers are labeled on the right of the picture. Black triangles indicate target proteins. MBP-SDS2 is used as positive control in (A). This is the same experiment as in Figure 1F. (E) and (F) Transphosphorylation between SDS2 and OsRLCK118/176. GST-OsRLCK176, GST-OsRLCK176^{K108E} (kinase-inactive) and GST-OsRLCK118 were combined with MBP-SDS2 or MBP-SDS2^{K540E} to apply to the phosphorylation assay. Those proteins alone were used as an autophosphorylation control. (D) Transphosphorylation between SDS2 and GST-OsRLCK118 or GST-OsRLCK118^{K116E} (kinase-inactive). Protein phosphorylation was detected by immunoblotting (anti-pThr) or staining (Pro-Q). CBB staining shows loading amounts. Pro-Q staining and CBB staining were performed with the same gel. – and + indicate absent or present of proteins labeled on the left. Molecular weights are labeled on right. Black triangles label specific protein. (G) Detection of OsRLCK118-HA *in vivo* phosphorylation in SDS2-ACT plants. Plasmids of OsRLCK118-HA are transfected into protoplasts of KT and SDS2-ACT plants separately. Transfected protoplasts are treated with 5μg/ml chitin for 0 min, 5min, 15min, 30min and 45min. Phosphorylation of OsRLCK118 is determined by band shift.

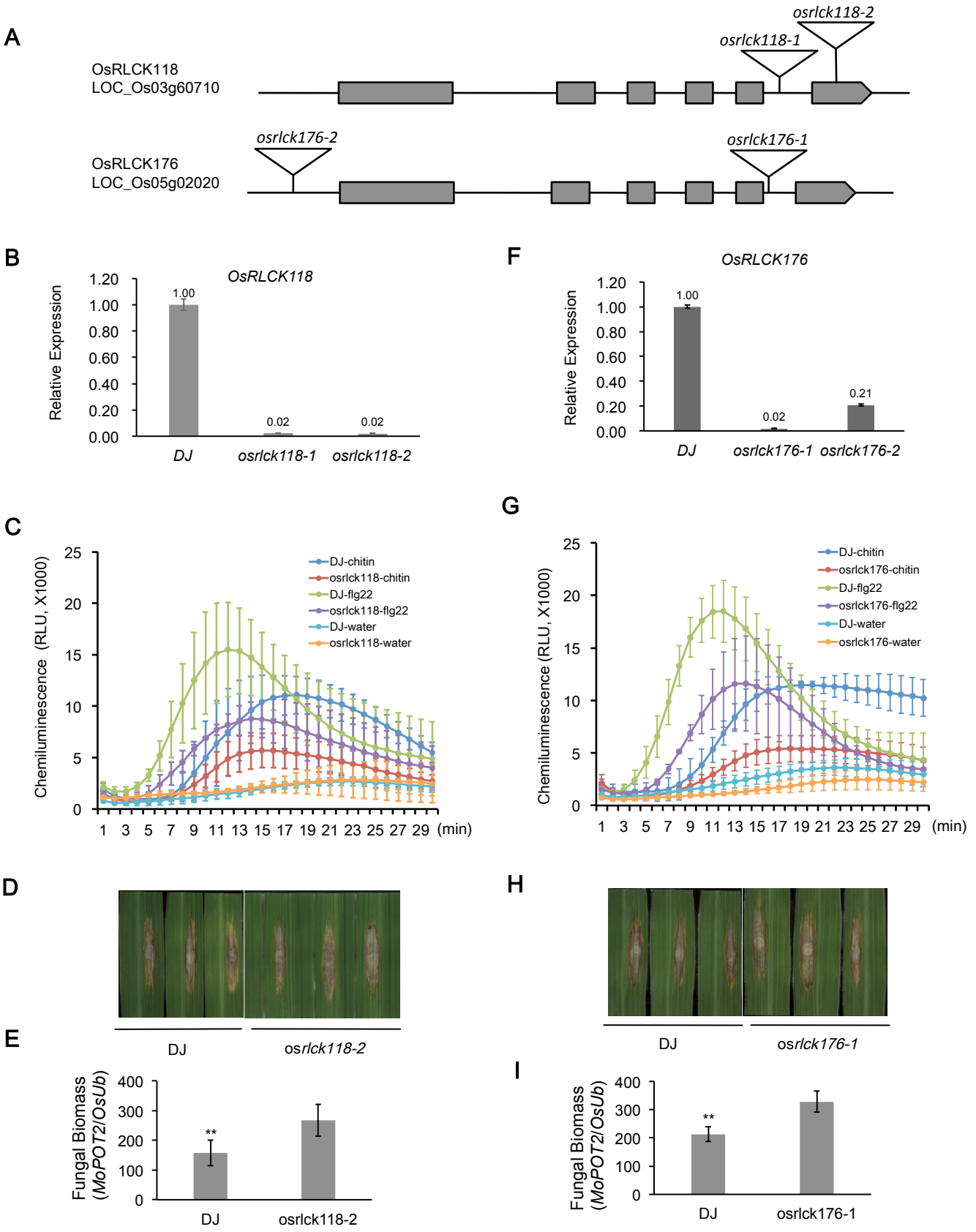

Figure S6. Related to Figure 6: OsRLCK118/176 Positively Regulate Rice Immunity. (A) Diagram of OsRLCK118 and OsRLCK176 gene structure and their T-DNA insertion mutants. Boxes and lines indicate exons and introns, respectively. Triangles indicate T-DNA insertions. (B) and (F) qRT-PCR analysis of OsRLCK118 and OsRLCK176 transcripts in the mutant lines. (C) and (G) ROS induction of *osrlck118* and *osrlck176* plants upon 10μg/ml chitin and 100μM flg22 treatments. Values are means ± SD, n = 3 (technological repeats). These assays are replicated twice with similar results.(D) and (H) Punch inoculation of *osrlck176* and *osrlck118* plants with blast isolate RO1-1 at 9 dpi. (E) and (I) Quantification of fungal biomass of inoculated leaves in (D) and (H). Values are means ± SD, n = 3 (technological repeats). Inoculations are replicated twice with similar results. Asterisks represent significant difference determined by Student's *t* test (** $p < 0.01$).

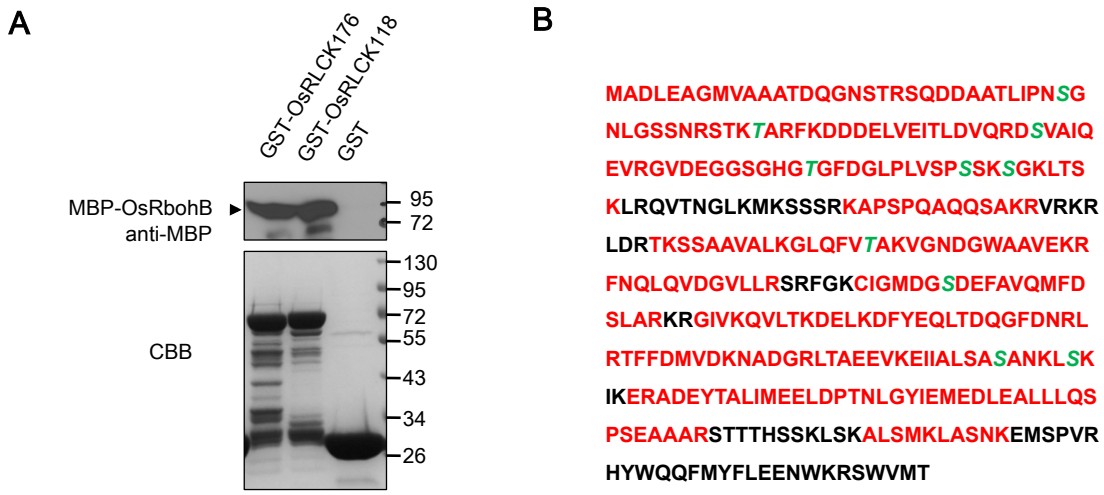

Figure S7. Related to Figure 7: OsRbohB interacts with OsRLCK118/ and OsRLCK176 and identification of OsRbohB phosphorylation sites. (A) Pull-down assay of the OsRLCK118/176-OsRbohB interactions. Baits: GST-OsRLCK176 and GST-OsRLCK118. Prey: MBP-OsRbohB-N (N terminal1-355aa). The prey was detected by immunoblotting (anti-MBP). CBB staining shows loading amounts of the baits. (B) Mass spectrum (MS) analysis of GST-OsRbohB (N-terminus) phosphorylation by MBP-OsRLCK118. MS coverage is 80.56%. Red and black sequences indicate covered and uncovered peptides in the MS data. Green italic letter highlights the detected phosphorylation sites.

Table S1. Related to Figure 1: Candidate Genes in the 28kb Interval

Candidate Genes	Annotation	Mutation
LOC_Os01g57460	Frataxin	No
LOC_Os01g57470	EF hand family protein	No
LOC_Os01g57480	serine/threonine-protein kinase, SD-1 receptor like kinase	G/A transition
LOC_Os01g57490	D-mannose binding lectin family protein, serine/threonine-protein kinase, SD-1 receptor like kinase	No
LOC_Os01g57500	Expressed protein	No
LOC_Os01g57510	receptor protein kinase, SD-1 receptor like kinase	No

Table S2. Related to Key Resource Table: Primers Used in This Study,

Primers name	Sequence (5'-3')	Purpose
SDS2-qRT-F	CCACTATGAGCGATGTCATCTC	
SDS2-qRT-R	CATCATGGGCATCCATTTCGGCTG	Quantative RT-PCR
SDS2-RT-F	GCAGGATATTGCTGTCAAGAGGCT	
SDS2-RT-R	GCAGGCTGCCTAGGATCAGGT	SDS2 variants
OsRLCK118-qRT-F	CACCGAGAATGGTTGGCAGA	
OsRLCK118-qRT-R	TGCGAACCTCTCCTAAACAGA	Quantative RT-PCR
OsRLCK176-qRT-F	AAAGGGCTCGCATTTCTCCA	
OsRLCK176-qRT-R	CTCTTGTCACCAGTCGGTCC	Quantative RT-PCR
OsPAL1-qRT-F	CTACAACAACGGGCTGACCT	
OsPAL1-qRT-R	TCTGGACATGGTTGGTGATG	Quantative RT-PCR
OsPR5-qRT-F	CAACAGCAACTACCAAGTCGTCTT	
OsPR5-qRT-R	CAAGGTGTCGTTTTATTCATCAAC	Quantative RT-PCR
OsUb-qRT-F	AACCAGCTGAGGCCCAAGA	
OsUb-qRT-R	CGATTGATTTAACCAGTCCATG	Quantative RT-PCR

MoPOT2-F	ACGACCCGTCTTTACTTATTTGG	
MoPOT2-R	AAGTAGCGTTGGTTTTGTTGGAT	Fungal biomass
OsUb-F	TTCTGGTCCTTCCACTTTCAG	
OsUb-R	ACGATTGATTTAACCAGTCCATGA	Fungal biomass