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Supplementary Materials for

A fungal effector targets a heat shock–dynamin protein complex to modulate mitochondrial dynamics and reduce plant immunity

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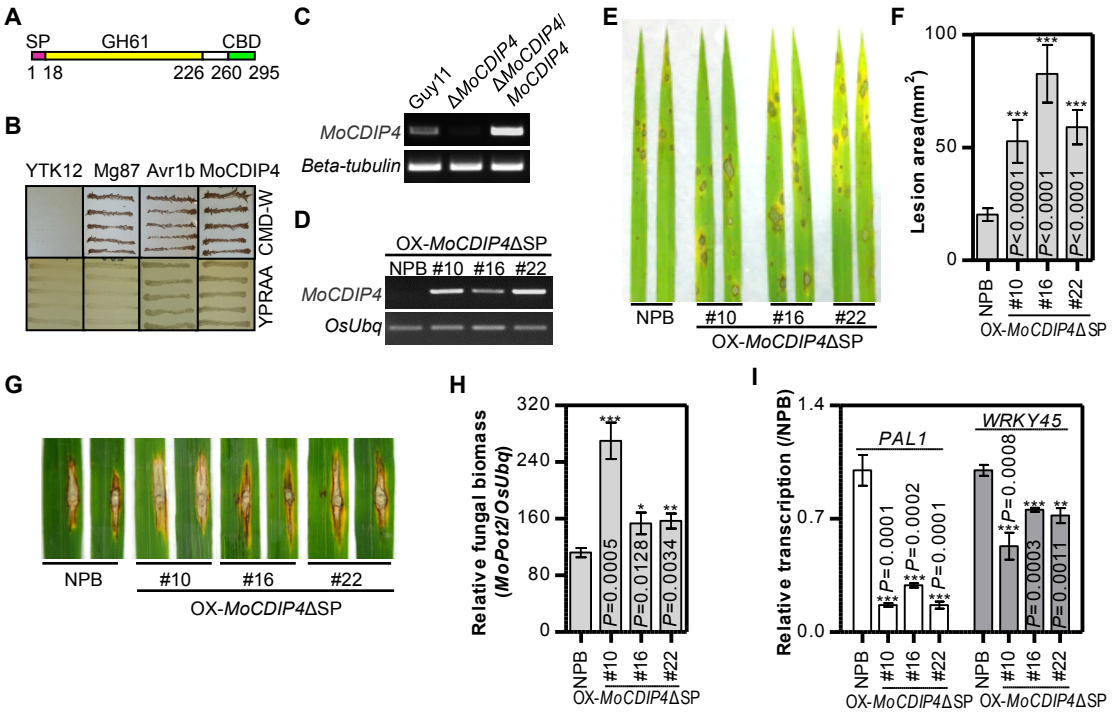

Fig. S1. MoCDIP4 is a virulent effector of *M. oryzae*. (**A**) The structure of MoCDIP4. (**B**) Secretory ability of the MoCDIP4 signal peptide. Avr1b and Mg87 were used as a positive and a negative control, respectively. (**C**) *MoCDIP4* transcript levels in the *MoCDIP4* mutant. Guy11 was the wild-type strain, Beta-tubulin was used as the loading control. (**D**) *MoCDIP4* transcript levels in *MoCDIP4* transgenic rice. Rice ubiquitin gene *OsUbq* was used as the loading control. (**E**) Spray inoculation of *MoCDIP4* overexpression plants with *M. oryzae*. (**F**) Lesion area of spray-inoculated leaves in (**E**). Bars represent means ± SD, n = 5. Asterisks indicate significant differences between the transgenic line and NPB according to a Student's *t*-test. (**G**) Punch inoculation of *MoCDIP4* overexpression plants with *M. oryzae*. (**H**) Relative fungal biomass of punch-inoculated leaves in (**G**). (**I**) Transcript levels of PR genes in *MoCDIP4* transgenic rice at 2 days after inoculation. Bars (H, I) represent means ± SD, n = 3. Asterisks (H, I) indicate significant differences between the transgenic line and NPB according to a Student's *t*-test.

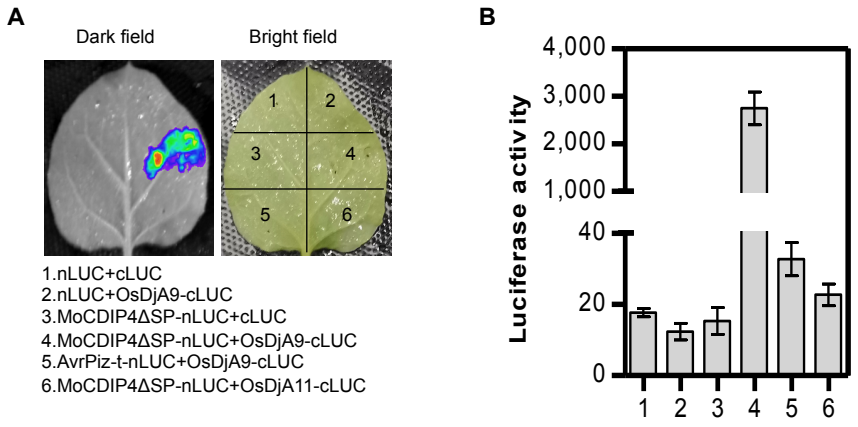

Fig. S2. MoCDIP4 interacts with OsDjA9 in LCI assay. (**A**) Fluorescent image of the MoCDIP4-OsDjA9 interaction in LCI assay. AvrPiz-t and OsDjA11 were used as the specific control. (**B**) Quantification of luciferase activity in the leaves shown in (**A**). Bars represent means ± SD, n = 3.

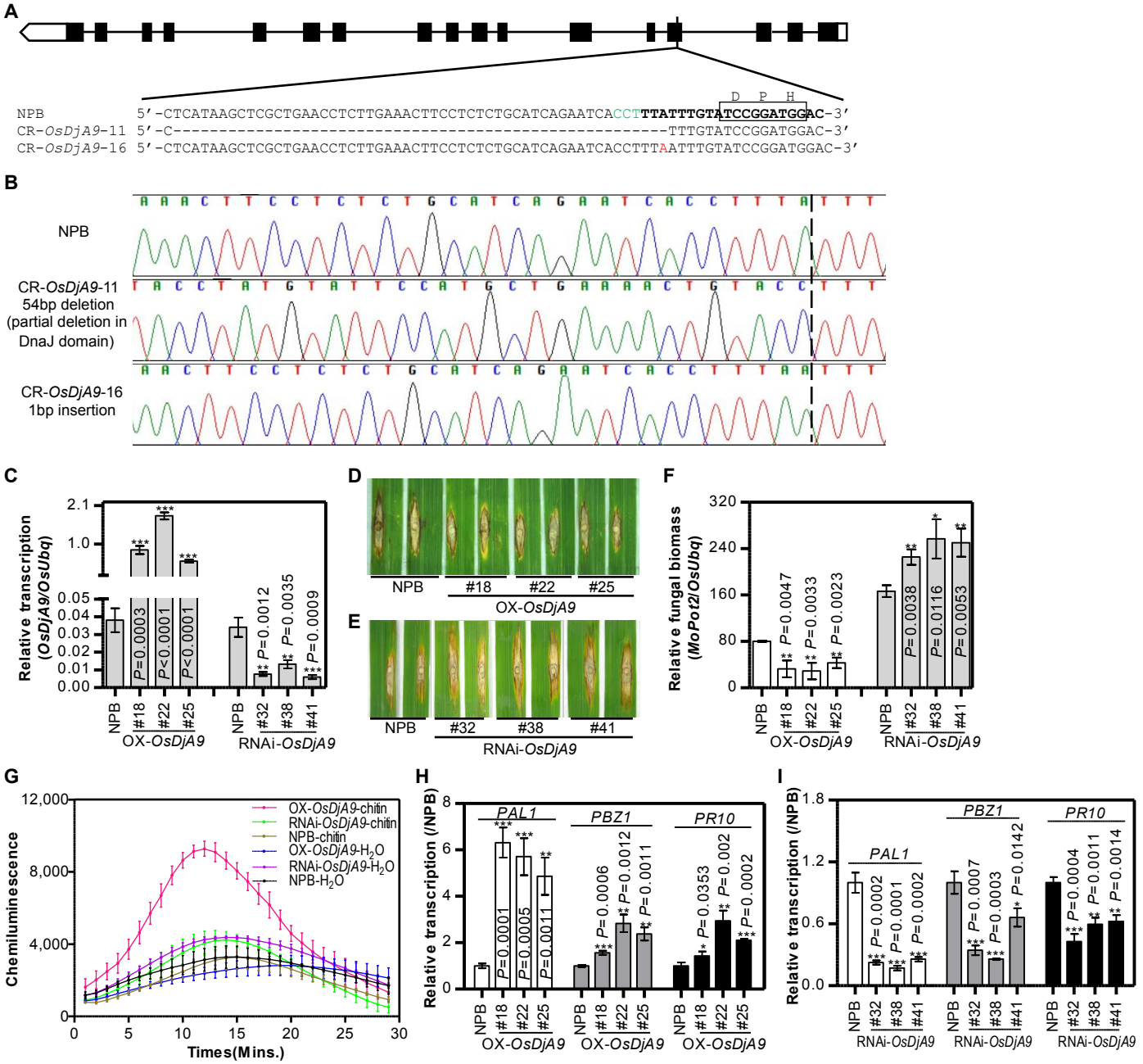

Fig. S3. OsDjA9 positively regulates rice immunity against *M. oryzae*. (**A**) Schematic representation of the *OsDjA9* structure and gene editing sites. Bold letters indicate the target sequences of single guide RNA, green letters indicate PAM, - indicates nucleotide deletion, and red letter indicates nucleotide insertion. (**B**) Sanger sequencing chromatograph of the target site in *OsDjA9*. (**C**) *OsDjA9* transcript levels in *OsDjA9* transgenic plants. Punch inoculation of *OsDjA9* (**D**) overexpression and (**E**) RNAi plants with *M. oryzae*. (**F**) Relative fungal biomass of punch-inoculated leaves in (**D, E**). (**G**) ROS accumulation in *OsDjA9* transgenic plants after chitin treatment. Transcript levels of PR genes in *OsDjA9* (**H**) overexpression and (**I**) RNAi plants at 2 days after inoculation. Bars (C, F-I) represent means ± SD, n = 3. Asterisks (C, F, H, I) indicate the significant differences between the transgenic line and NPB according to Student's *t*-test.

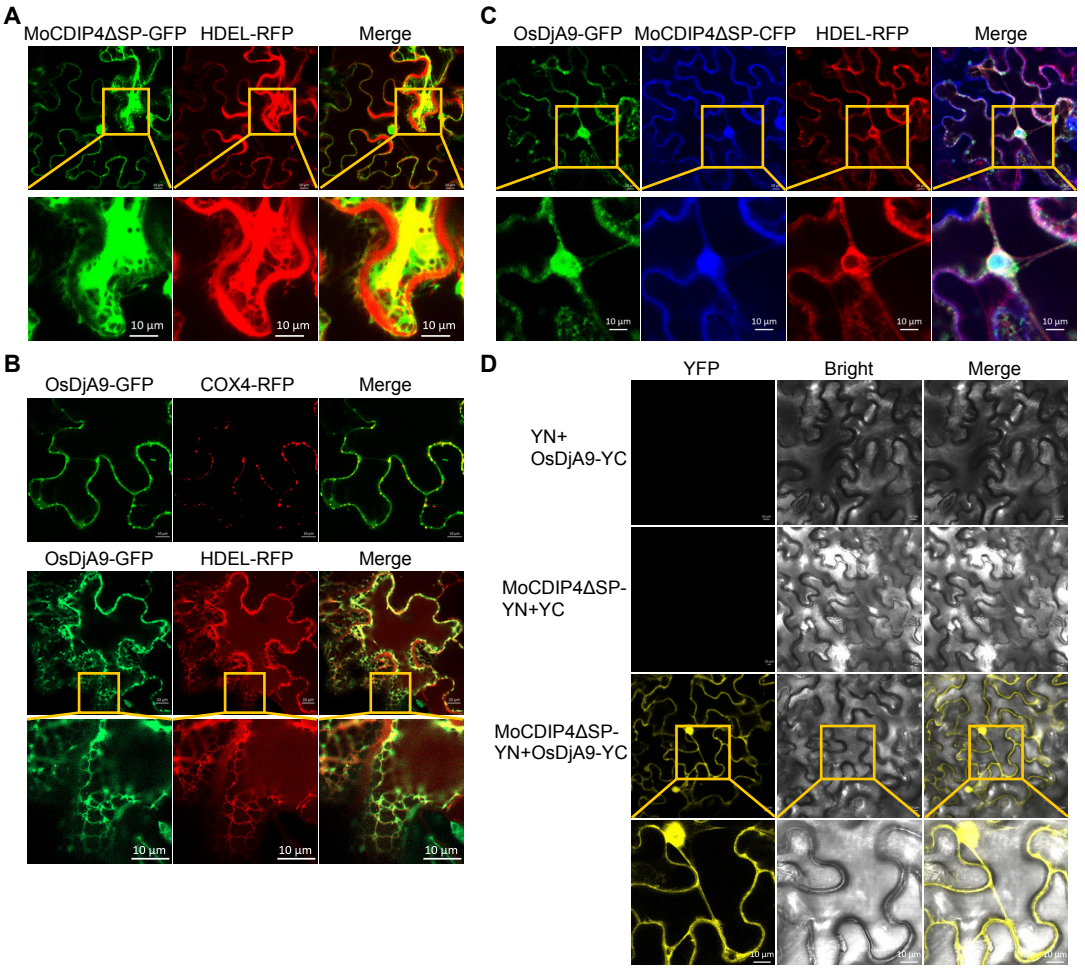

Fig. S4. MoCDIP4 and OsDjA9 are co-localized in the ER in *N. benthamiana*. (**A**) Subcellular localization of MoCDIP4 in *N. benthamiana*. HDEL was used as the ER marker. (**B**) Subcellular localization of OsDjA9 in *N. benthamiana*. COX4 was used as the mitochondrial marker; HDEL was used as the ER marker. (**C**) Co-localization of MoCDIP4 and OsDjA9 in *N. benthamiana*. HDEL was used as the ER marker. (**D**) BiFC analysis of the MoCDIP4-OsDjA9 interaction in *N. benthamiana*. Yellow squares (A-D) show the enlarged ER localization. Scale bars (A-D) represent 10 μm.

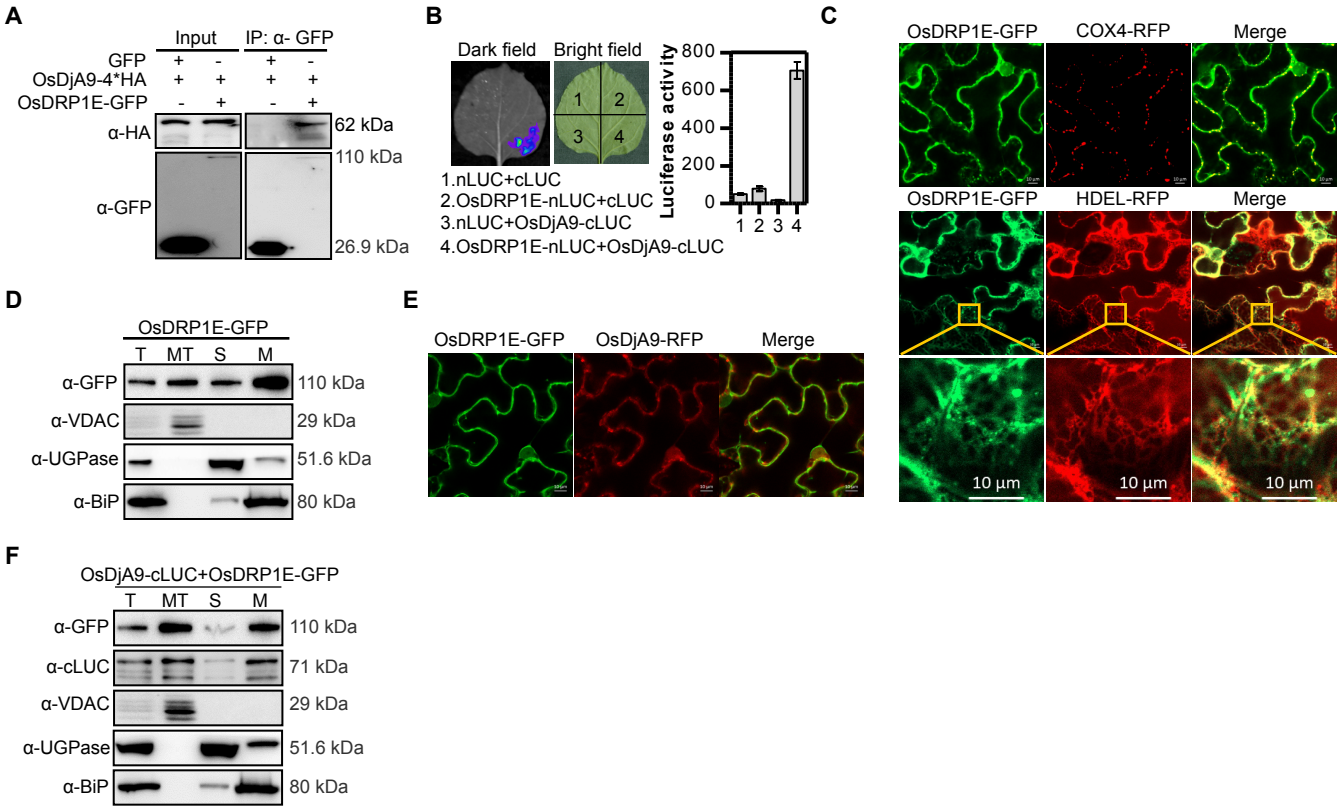

Fig. S5. OsDjA9 interacts with the dynamin-related protein OsDRP1E. (**A**) Interaction between OsDjA9 and OsDRP1E in Co-IP assay. 4*HA was a fusion of four HA tags. (**B**) Interaction between OsDjA9 and OsDRP1E in LCI assay. Bars represent means ± SD, n = 3. (**C**) Subcellular localization of OsDRP1E in *N. benthamiana*. COX4 was used as mitochondrial marker; HDEL was used as the ER marker. Yellow squares show the enlarged ER localization. (**D**) Detection of OsDRP1E in different cell fractions extracted from (**C**). T, total extract; MT, mitochondria; S, soluble fraction; M, membrane fraction. (**E**) Co-localization of OsDjA9 and OsDRP1E in *N. benthamiana*. (**F**) Detection of OsDjA9 and OsDRP1E in different cell fractions extracted from tobacco leaves co-infiltrated with OsDjA9 and OsDRP1E. Scale bars (C, E) represent 10 μm.

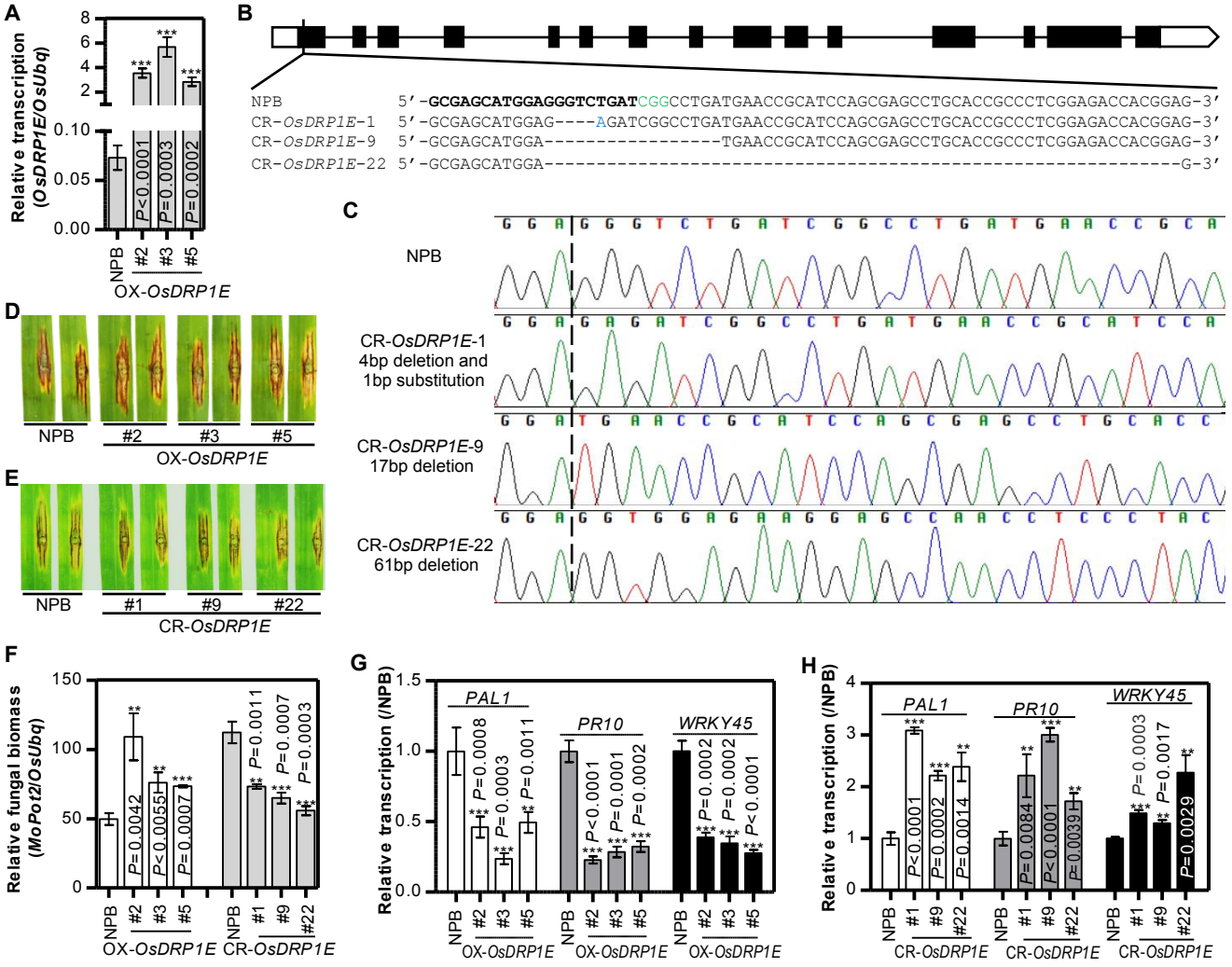

Fig. S6. OsDRP1E negatively regulates rice immunity. (**A**) *OsDRP1E* transcript levels in *OsDRP1E* overexpression plants. (**B**) Schematic representation of the *OsDRP1E* structure and gene editing site. Bold letters indicate the target sequences of single guide RNA, green letters indicate PAM, - indicates nucleotide deletion, blue letter indicates nucleotide substitution. (**C**) Sanger sequencing chromatograph of the target site in *OsDRP1E*. Punch inoculation of *OsDRP1E* (**D**) overexpression and (**E**) CRISPR/Cas9-edited plants with *M. oryzae*. (**F**) Relative fungal biomass of punch-inoculated leaves in (**D, E**). Transcript levels of PR genes in *OsDRP1E* (**G**) overexpression and (**H**) CRISPR/Cas9-edited plants at 2 days after inoculation. Bars (A, F-H) represent means ± SD, n = 3. Asterisks (A, F-H) indicate the significant differences between the transgenic line and NPB according to Student's *t*-test.

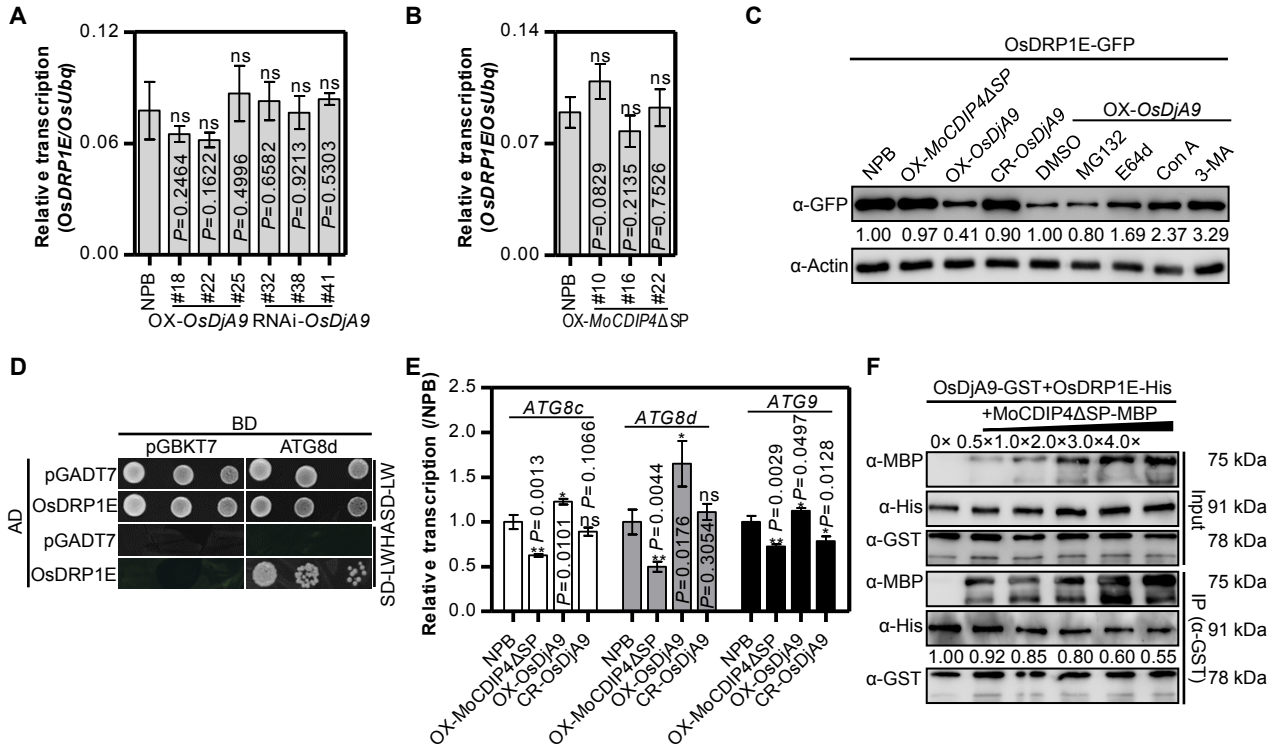

Fig. S7. MoCDIP4 stabilizes OsDRP1E by decreasing the association of OsDjA9 with OsDRP1E in a competitive manner. *OsDRP1E* transcript levels in (**A**) *OsDjA9* transgenic plants and (**B**) *MoCDIP4* transgenic plants. Bars represent means ± SD, n = 3. ns indicates no significant difference between the transgenic line and NPB according to Student's *t*-test. (**C**) OsDRP1E protein levels when expressed in NPB and transgenic rice protoplasts. Inhibitors were added at 12 h after transfection, respectively. Protoplasts were sampled at 12 h after the treatment. (**D**) Interaction between OsDRP1E and ATG8d in yeast. (**E**) Transcript levels of autophagy components in transgenic rice protoplasts when transformed with OsDRP1E-GFP plasmids. Bars represent means ± SD, n = 3. Asterisks indicate significant differences between the transgenic line and NPB according to a Student's *t*-test. (**F**) The dosage dependent effect of MoCDIP4 on the OsDjA9-OsDRP1E interaction in GST pull-down assay. Relative band intensity of each lane below the panel is determined by ImageJ.

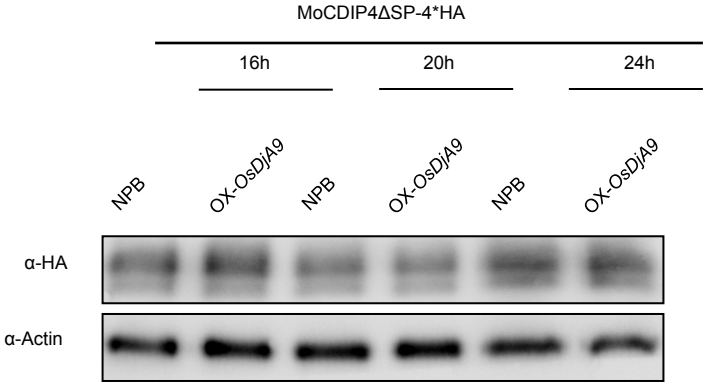

Fig. S8. OsDjA9 does not affect the protein levels of MoCDIP4. MoCDIP4 was expressed in NPB and *OsDjA9* overexpression rice protoplasts. Protoplasts were sampled at 16 h, 20 h, and 24 h after the transfection. The actin protein was used as the internal control.

Table S1. Primers are used in this study.

Primers name	Sequence (5'-3')	Purpose
PAL1-QF	AGCACATCTTGGAGGGAAGCT	qRT-PCR
PAL1-QR	GCGCGGATAACCTCAATTTG	
OsUbq-QF	CGCAAGAAGAAGTGTGGTCA	qRT-PCR
OsUbq-QR	GGGAGATAACAACGGAAGCA	
WRKY45-QF	ACGACATTATGGGTTTGAGCTT	qRT-PCR
WRKY45-QR	GAGACGACACATCAACAAGGAA	
PBZ1-QF	CCCTGCCGAATACGCCTAA	qRT-PCR
PBZ1-QR	CTCAAACGCCACGAGAATTTG	
PR10-QF	ATGAAGGAGAGGCTGGAGTTC	qRT-PCR
PR10-QR	CCTTAGCCTTGGTGATCTCGT	
OsUbq-GQF	TTCTGGTCCTTCCACTTTCAG	Fungal biomass
OsUbq-GQR	ACGATTGATTTAACCAGTCCATGA	
MoPOT2-GQF	ACGACCCGTCTTTACTTATTTGG	Fungal biomass
MoPOT2-GQR	AAGTAGCGTTGGTTTTGTTGGAT	
OsDjA9-QF	ATCCGACTCAACATAGTGCCTG	qRT-PCR
OsDjA9-QR	TTGCCTGAGTCACGTTCAATAC	
OsDRP1E-QF	CTATCCGTGCAAAATGTGAAGA	qRT-PCR
OsDRP1E-QR	TTCCTCACCAAGTCCCTTAAAA	
MoCDIP4-RT-F	TACATCTTCAGCATCGTCTTCG	RT-PCR
MoCDIP4-RT-R	CTCGATGAACTCGTTGTACCAG	
pRHV-*MoCDIP4*ΔSP-F	CGGGATCCATGCACTACATCTTCAGCATCG	pRHV, pRTVcHA
pRHV-*MoCDIP4*-R	CGAGCTCCAAGCACTGGCTGTAGTACTG	
pRHV-*OsDjA9*-F	CGAGCTCATGCGGCTCCCCGGCGACGCT	pRHV, pRTVcHA, pRTVcMyc
pRHV-*OsDjA9*-R	GGGGTACCTCCCGATGCTCCTGCTGCCTTT	
pANDA-*OsDjA9*-F	CGGGATCCGCGGCGGTGGAATGAATG	pANDA
pANDA-*OsDjA9-R*	CCGCTCGAGCCTTCACAGTCTTCCCAC	
U6a-*OsDjA9*-F	GCCGGTCCATCCGGATACAAATAA	pYLCRISPR/Cas 9P_{ubi}-H
U6a-*OsDjA9*-R	AAACTTATTTGTATCCGGATGGAC	
U6a-*OsDRP1E*-F	GCCGCGAGCATGGAGGGTCTGAT	pYLCRISPR/Cas 9P_{ubi}-H
U6a-*OsDRP1E*-R	AAACATCAGACCCTCCATGCTCG	
pRHV-*OsDRP1E*-F	CGGGATCCATGGCGAGCATGGAGGGTCT	pRHV, pSPYNE(R)173
pRHV-*OsDRP1E*-R	GGGGTACCCCTGGTCCATGCGACAGAGT	
pMAL-c2-*MoCDIP4*-F	AGAATTCATGAAGTCGACAACCTTCCT	pMAL-c2
pMAL-c2-*MoCDIP4*-R	GCTCTAGACTACAAGCACTGGCTGTAGTA	
pMAL-c2-*MoCDIP4*ΔSP-F	AGAATTCATGCACTACATCTTCAGCATCG	pMAL-c2
pGEX-6p-1-*OsDjA9*-F	TCCCCCGGGTATGCGGCTCCCCGGCGACGCT	pGEX-6p-1
pGEX-6p-1-*OsDjA9*-R	CCCTCGAGCTATCCCGATGCTCCTGCTGC	
pGADT7-*OsDjA9*-R	CCCTCGAGCCTATCCCGATGCTCCTGCTGC	pGADT7
pGADT7-*OsDjA11*-F	GGAGGCCAGTGAATTCATGGCGCGCGCCGCCGCCTC	pGADT7
pGADT7-*OsDjA11*-R	TCGAGCTCGATGGATCCCTCATCCGGAGGCAGCTGCAAC	
pCAMBIA1300-cLUC-*OsDjA11*-F	TACGCGTCCCGGGGCGGTACCATGGCGCGCGCCGCCGCCTC	pCAMBIA1300-cLUC
pCAMBIA1300-cLUC-*OsDjA11*-R	ACGAAAGCTCTGCAGGTCGACTCATCCGGAGGCAGCTGCAAC	
pCAMBIA1300-cLUC-*OsDjA9*-F	GGGGTACCATGCGGCTCCCCGGCG	pCAMBIA1300-cLUC
pCAMBIA1300-cLUC-*OsDjA9*-R	GCGTCGACCTATCCCGATGCTCCTGC	
pCAMBIA1300-nLUC-*MoCDIP4*ΔSP-F	CGGGATCCAATGCACTACATCTTCAGCATCG	pCAMBIA1300-nLUC, pCAMBIA1300-
pCAMBIA1300-nLUC-	GCGTCGACCAAGCACTGGCTGTAGTAC	

*MoCDIP4*ΔSP-R		cLUC
pGBKT7-*MoCDIP4*ΔSP-F	CATGGAGGCCGAATTCATGCACTACATCTTCAGCATCG	pGBKT7
pGBKT7-*MoCDIP4*ΔSP-R	TGCAGGTCGACGGATCCCCTACAAGCACTGGCTGTAGTAC	
MoCDIP4-pro-F	CATAGTCTATATAAGGCACGCTCATTACCATG	*MoCDIP4* knockout strain
MoCDIP4-pro-R	TTGACCTCCACTAGCTCCAGCCAAGCCACTTCATGATGGGCGGGGGGA	
MoCDIP4-3UTR-F	GAATAGAGTAGATGCCGACCGCGGGTTGGCTGGGAAGATTGAGGATGTGTG	
MoCDIP4-3UTR-R	AGAGCTTTGGGGCAGTAAGATA	
pKNGT-*MoCDIP4*-pro-F	GGGAACAAAAGCTGGGTACCTCAAACAGATCAAGAGGGATCA	*MoCDIP4* complementation strain
pKNGT-*MoCDIP4*-CDS-R	CTGCAGGCATGCAAGCTTCAAGCACTGGCTGTAGTACTGG	
pSPYCE(M)-*OsDjA9*-F	GCTCTAGAATGCGGCTCCCCGGCGACGCT	pSPYCE(M)
pCAMBIA1300-nLUC-*OsDRP1E*-F	ACGGGGGACGAGCTCGGTACCATGGCGAGCATGGAGGGTCTG	pCAMBIA1300-nLUC
pCAMBIA1300-nLUC-*OsDRP1E*-R	CGCGTACGAGATCTGGTCGACCCTGGTCCATGCGACAGAGTC	
pGADT7-*OsDRP1E*-F	GGAATTCCATATGATGGCGAGCATGGAGGGTCT	pGADT7, pGBKT7
pGADT7- *OsDRP1E*-R	CGGGATCCCCTACCTGGTCCATGCGACAGA	
pSUC2-SP-F	AATTCATGAAGTCGACAACCTTCCTCAGCCTGCTGGCGGCTCCGCTGGCCGCGCAGGCGC	pSUC2
pSUC2-SP-R	TCGAGCGCCTGCGCGGCCAGCGGAGCCGCCAGCAGGCTGAGGAAGGTTGTCGACTTCATG	