A

OsImpα   OsImpα1a   OsImpα1b   MoImpα

■ MoHTR1_NLS   ■ Importin α

B

OsImpα   OsImpα1a   OsImpα1b   MoImpα

■ MoHTR1$^{\Delta SP,NLS}$ ■ Importin α

Supplementary figure 1. Predicted interaction structures between MoHTR1 and importin αs.
(A) Predicted interaction models of MoHTR1 NLS (MoHTR1_NLS) and importin αs of rice and rice blast fungus. **(B)** Predicted interaction models of signal peptide and NLS removed MoHTR1 (MoHTR1$^{\Delta SP,NLS}$)and importin αs of rice and rice blast fungus. Magenta color structures indicate MoHTR1_NLS and MoHTR1$^{\Delta SP,NLS}$ and cyan color structures indicate importin αs.
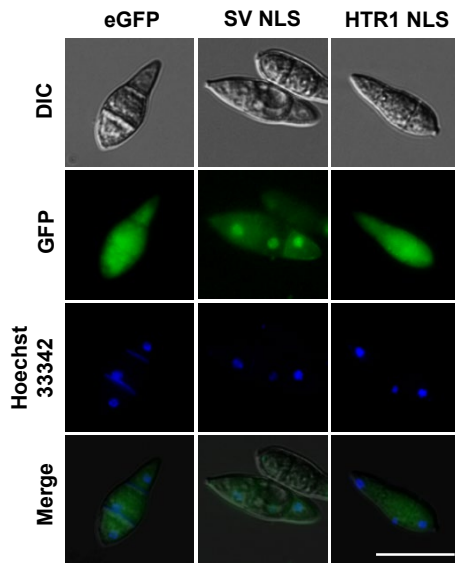
Supplementary figure 2. Cytoplasm-to-nuclear translocation role of native NLS of MoHTR1 in fungal cells. Subcellular localization of eGFP with native MoHTR1 NLS (HTR1 NLS) in the fungal conidia cells. SV NLS, well known cytoplasm to nuclear translocation associated NLS, was used to positive control. Nuclei was stained using Hoechst 33342. Scale bar; 20 μm. Representative data are shown from independently experiments and source data are provided as a Source Data file.
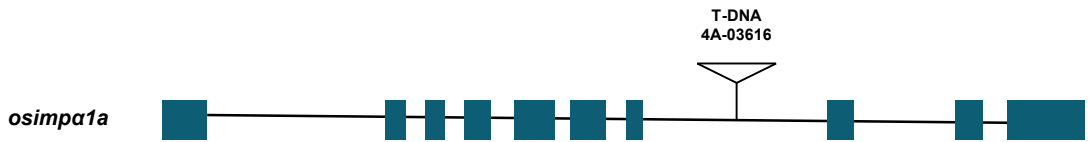
Supplementary figure 3. Rice importin α (Os01g14950.1, *OsImpα1a*) gene structure and insertion site of T-DNA. Green boxes indicate exon regions and black line indicates intron regions of *OsImpα1a.*
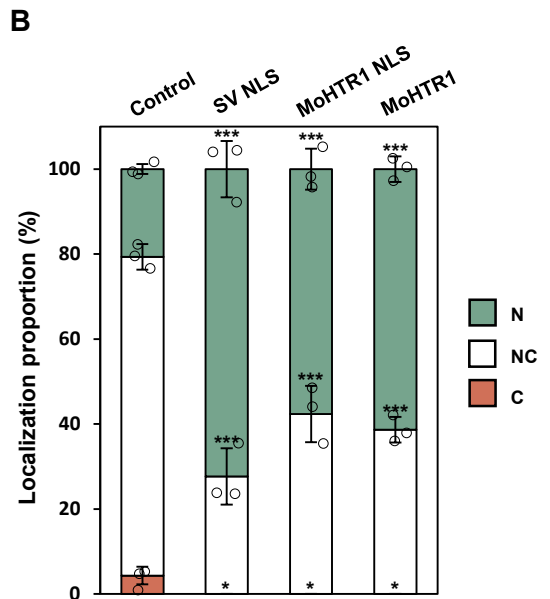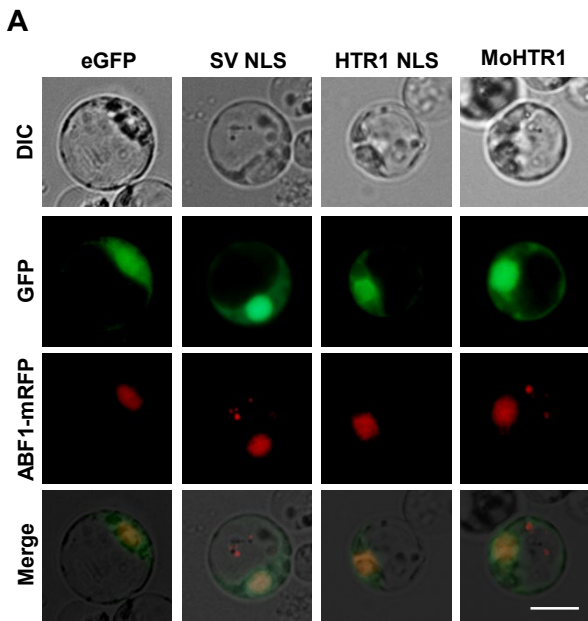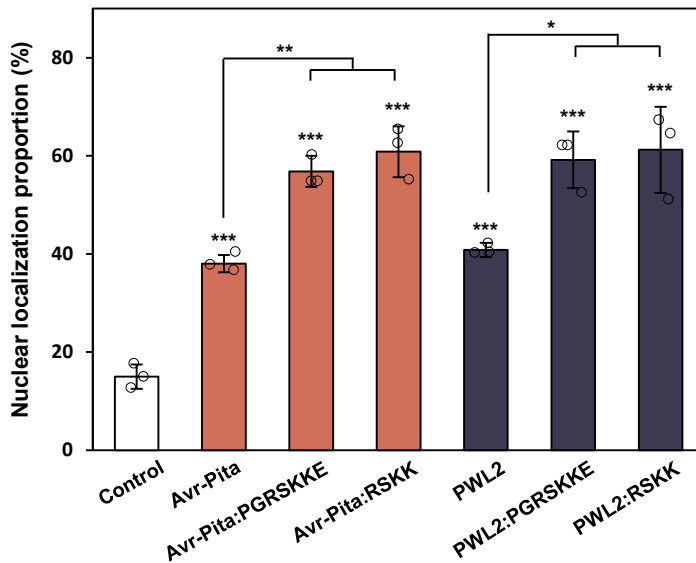
Supplementary figure 4. Cytoplasm-to-nuclear translocation role of native NLS of MoHTR1. (A) Subcellular localization of eGFP with Simian virus NLS (SV NLS), native MoHTR1 NLS (HTR1 NLS), and MoHTR1 in the rice protoplasts. SV NLS, well known cytoplasm to nuclear translocation associated NLS, was used to positive control. ABF1:mRFP was used for rice nuclei marker. Scale bar; 10 μm. **(B)** Subcellular localization proportion of SV NLS, MoHTR1 NLS, and MoHTR1 in the rice protoplasts. Mean ± SD, n = 3 independently transfected protoplasts, significance was determined by an unpaired two-tailed Student's t-test ((*p < 0.05 and ***p < 0.001). Representative data are shown from independently experiments and source data are provided as a Source Data file.

Supplementary figure 5. The nuclear localization proportion of the two cytoplasmic effectors by tagging MoHTR1 NLS and key sequence of MoHTR1 NLS. Intracellular localization of Avr-Pita and PWL2, two cytoplasmic effectors of M. oryzae, in the rice protoplast. Each cytoplasmic effectors were fused with MoHTR1 NLS (PGRSKKE) and key sequence of MoHTR1 NLS (RSKK), respectively and cloned into eGFP expressing plasmid under CaMV 35S promoter. The nuclear localization proportion of these cytoplasmic effectors were observed under fluorescence microscope. Mean ± SD, n = 3 independently transfected protoplasts, significance was determined by an unpaired two-tailed Student's t-test (*p < 0.05, **p < 0.01, and ***p < 0.001). Representative data are shown from independently experiments and source data are provided as a Source Data file.
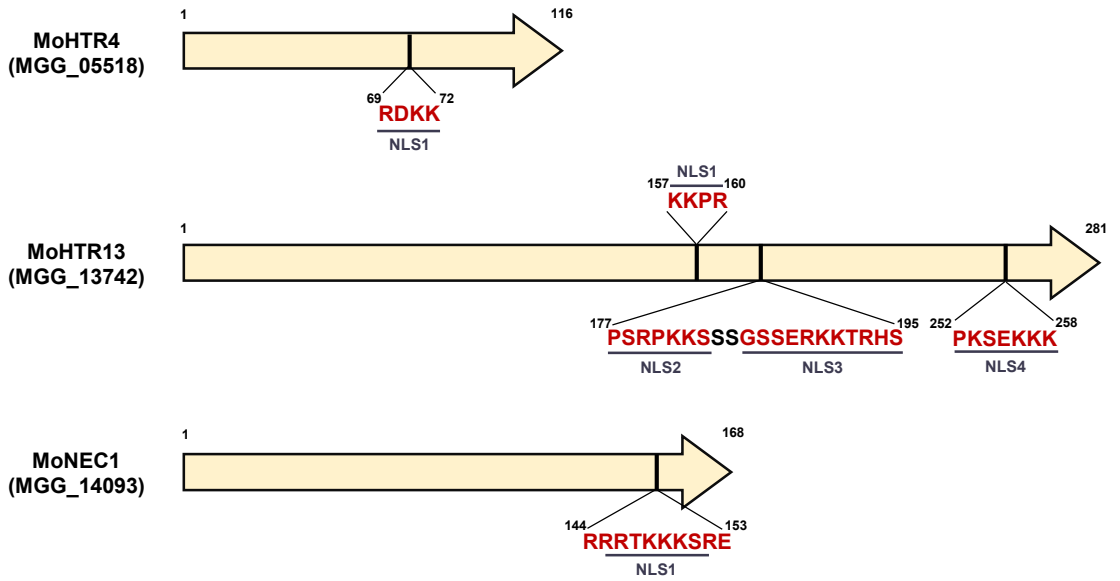
Supplementary figure 6. Predicted NLS in the three nuclear effector candidates. Black bar indicates location of predicted NLSs in each three nuclear effector candidates.
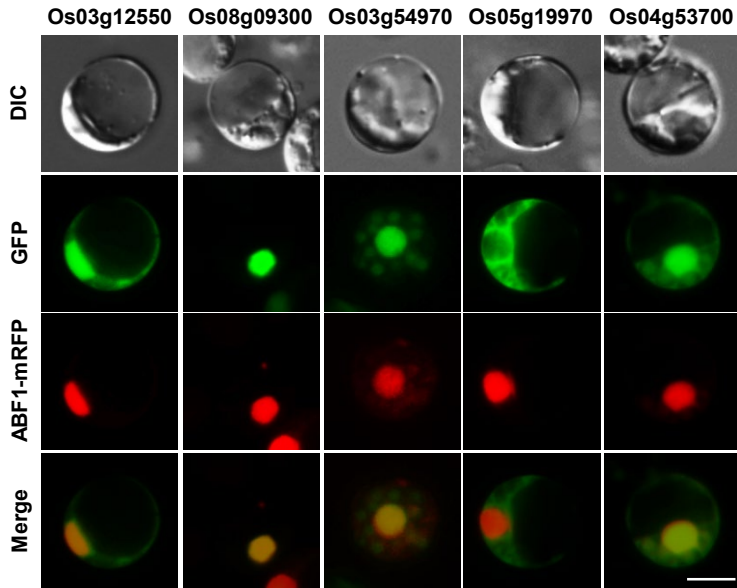
Supplementary figure 7. Localization of RxKK sequences containing rice proteins in the rice protoplast. Subcellular localization of eGFP with RxKK sequences containing rice proteins in the rice protoplasts. ABF1:mRFP was used for rice nuclei marker. Scale bar; 10 µm. Representative data are shown from independently experiments and source data are provided as a Source Data file.
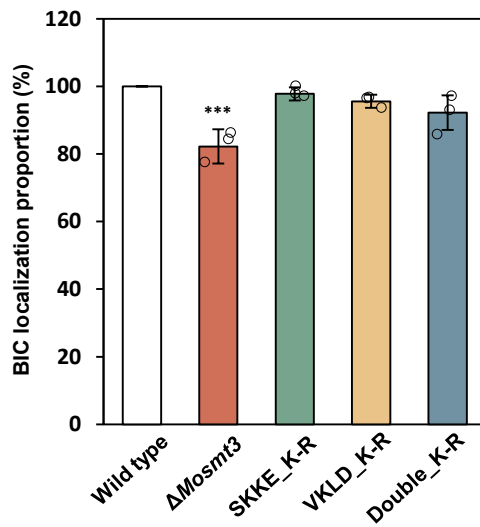
Supplementary figure 8. BIC localization of SUMOylation defected MoHTR1. The BIC localization proportion of SUMOylation site point mutants of MoHTR1 in the rice sheath cells. Significance was determined by *t*-test (***p < 0.001). Mean ± SD, n = 3 independently infected sheath cells, significance was determined by an unpaired two-tailed Student's t-test (*p < 0.05, **p < 0.01, and ***p < 0.001). Representative data are shown from independently experiments and source data are provided as a Source Data file.
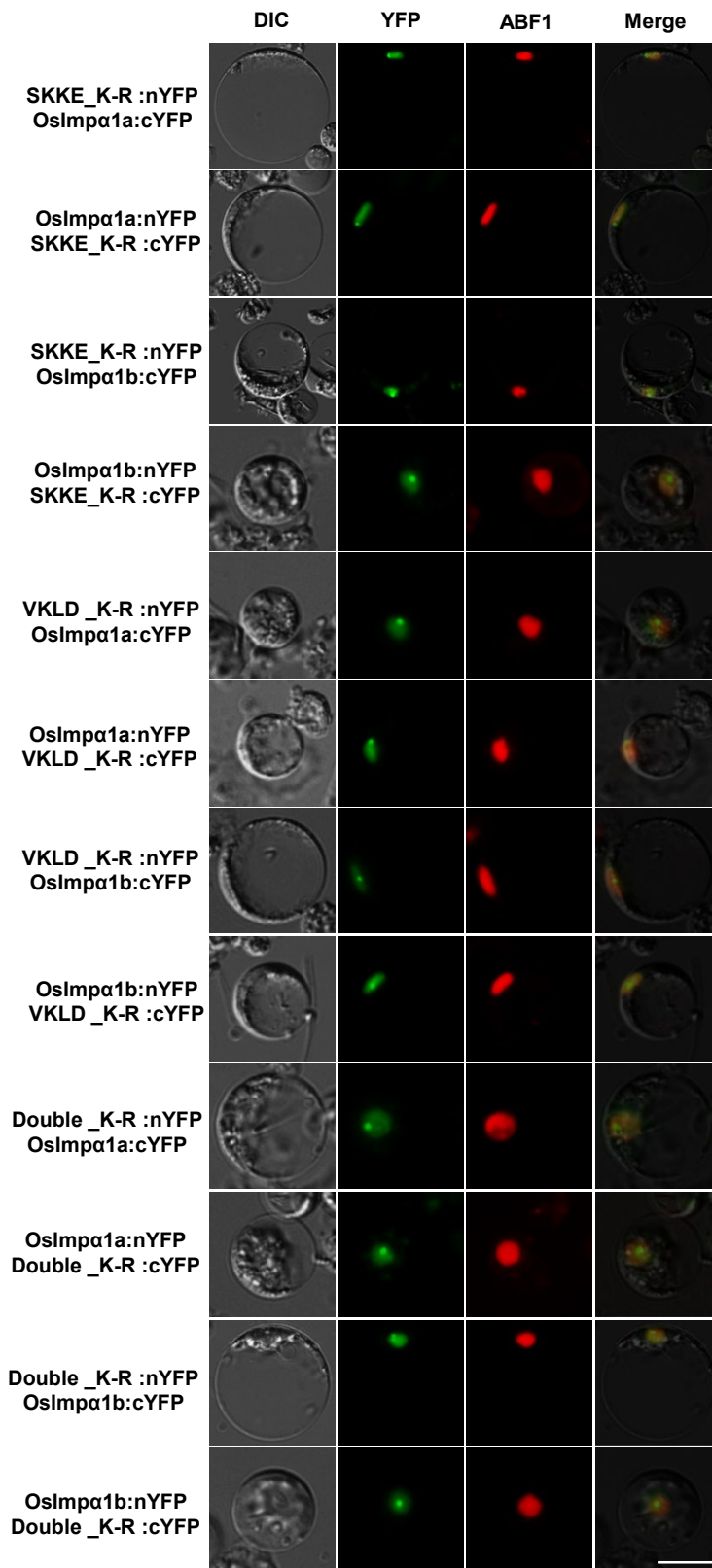
Supplementary figure 9. in vivo interaction between SUMOylation site-mutated variants and two rice importin αs. BiFC assay in the rice protoplasts. Three SUMOylation site-mutated variants (SKKE_K-R, VKLD_K-R, and Double_K-R) were interacted with OsImpα1a and OsImpα1b. ABF1:mRFP was used for rice nuclei marker. Scale bar; 10 μm. Representative data are shown from independently experiments and source data are provided as a Source Data file.

Supplement Table 1. Summary of AlphaFold analysis.

Protein A	Protein B	PAE[a] average	pLDDT[b] average	pTM[c]	ipTM[d]
MoHTR1_NLS	MoImpα	24.07	80.03	0.768	0.596
	OsImpα	26.51	78.40	0.744	0.507
	OsImpα1a	25.01	84.24	0.818	0.486
	OsImpα1b	25.03	82.07	0.793	0.491
MoHTR1$^{\Delta NLS}$	MoImpα	29.86	65.93	0.637	0.231
	OsImpα	30.10	65.04	0.620	0.218
	OsImpα1a	29.86	68.68	0.657	0.285
	OsImpα1b	29.96	68.06	0.643	0.230

[a] PAE (predicted aligned error) is the expected positional error. Scaled from 0.75 to 31.75, low score indicating low predicted error.

[b] pLDDT (predicted local distance difference test) is a per-residue measure of local confidence. Scaled from 0 to 100, high score indicating accurate prediction.

[c] pTM (predicted template modelling) means well predicated the overall structure.

[d] ipTM (interface predicted template modelling) means the accuracy of the predicted positions of the protein-protein complex.

Supplement Table 2. Primer sequence used in this study.

Name	Sequence 5'→3'
Primers for site-directed mutagenesis	
MoHTR1_NLS_mut_K/R-A	CATGTGAAGCCCGGTGCGTCAGCGGCGGAGATTGCCCAGAGG
MoHTR1_NLS_mut_K-R	AAGCCCGGTAGGTCAAGGAGGGAGATTGCCCAGAGG
MoHTR1_SUMO_VKLD_mut_K-R	TACGGAGCCGCGGTGAGACTCGATAAACCCACG
MoHTR1_SUMO_SKKE_mut_K-R	AAGCCCGGTAGGTCAAGGAAGGAGATTGCCCAG
05518_NLS_mut_K,R-A	GCCGAGGCGCAGGCCGACGCGGCGCCTTTCCAGTGT
13742_NLS_mut_K,R-A	GCAGGTCCGTCGGCTCCCGCAGCGTCGTCATCAGGA
14093_NLS_mut_K,R-A	GATTCTCGGCGGGCGACAGCGGCGAAGAGCAGGGAG
Primers for generation of MoHTR1 NLS deletion mutant	
HTR1_NLS_del_5'flnk_F	CACCATGGCCCCCATGCCTTCCGG
HTR1_NLS_del_5'flnk_R	CTTCACATGCCCCTGTTCCTG
HTR1_NLS_del_3'flnk_F	CAGGAACAGGGGCATGTGAAGATTGCCCAGAGGATCAGGAAC
HTR1_NLS_del_3'flnk_R	TTAGAGCTGGACCGGGTCGCCTTG
Primers for Luciferase assay	
MYB4pro HindIII F	AAGCTTAAATTTATTTGGTATATTATATAGT
MYB4pro BamHI R	GGATCCAAGGTTGGAACGGGGA
HTR1_CACC_F	CACCATGGCCCCCATGCCTTCCGGCT
HTR1_R	TTAGAGCTGGACCGGGTCGCCT
Primers for local observation of cytoplasmic proteins	
PWL2_promoter_F_attb	AAAAAGCAGGCTTAGCGTCAGTGAACAAACCTG
PWL2_ORF_R_attb	AGAAAGCTGGGTACATAATATTGCAGCCCTCTTCTCG
PWL2_ORF_R_PGRSKKE_attb	AGAAAGCTGGGTACTCCTTCTTTGACCTACCGGGCATAATATTGCAGCCCTCTTCTCG
PWL2_ORF_R_SVNLS_attb	AGAAAGCTGGGTAGACCTTCCTCTTCTTCTTGGGCATAATATTGCAGCCCTCTTCTCG
Pita_promoter_F_ attb	AAAAAGCAGGCTTAGCCGAGTCGTTCTGAGGGTAG
Pita_ORF_R_ attb	AGAAAGCTGGGTA ACAATATTTATAACGTGCACATTG
Pita_ORF_R_PGRSKKE_ attb	AGAAAGCTGGGTACTCCTTCTTTGACCTACCGGGACAATATTTATAACGTGCACATTG
Pita_ORF_R_SVNLS_ attb	AGAAAGCTGGGTAGACCTTCCTCTTCTTCTTGGGACAATATTTATAACGTGCACATTG
PWL2_CDS_F_cacc	CACCATGGGTGGCGGGTGGACTAACAAAC
PWL2_CDS_F_PGRSKKE_cacc	CACCATGCCCGGTAGGTCAAAGAAGGAGGGTGGCGGGTGGACTAACAAAC
PWL2_CDS_F_SV_cacc	CACCATGCCCAAGAAGAAGAGGAAGGTCGGTGGCGGGTGGACTAACAAAC
PWL2_CDS_R	CATAATATTGCAGCCCTCTTCTCG
Pita_CDS_F_cacc	CACCATGTTCACCAACATTGGCACCTTTTC
Pita_CDS_F_PGRSKKE_cacc	CACCATGCCCGGTAGGTCAAAGAAGGAGTTCACCAACATTGGCACCTTTTC
Pita_CDS_F_SV_cacc	CACCATGCCCAAGAAGAAGAGGAAGGTCTTCACCAACATTGGCACCTTTTC
Pita_ORF_R_	ACAATATTTATAACGTGCACATTG
MoHTR1 NLS_F_CACC	CACCATGCCCGGTAGGTCAAAGAAGGAG
MoHTR1 NLS_R	CTCCTTCTTTGACCTACCGGGCAT
SVNLS_F_CACC	CACCATGCCCAAGAAGAAGAGGAAGGTC
SVNLS_R	GACCTTCCTCTTCTTCTTGGGCAT
Primers for observation of nuclear effector candidates	
MGG_05518_qRT_F	GCTTGGAAAGCGGAATTTGCAG
MGG_05518_qRT_R	CTCCGTGGAATGCTTTGACATG
MGG_13742_qRT_F	GAAGGAGTCCGACCACGTTAATG
MGG_13742_qRT_R	GAAGGTGCCGTAGGTGCCTTAG
MGG_14093_qRT_F	CGTACGGTCAAGAGCGAGTAC
MGG_14093_qRT_R	CGAGAATCGCCAAAGCCCTTA C
MGG_14093_CACC_F	CACCATGGCCCCTGTGTCGGAATCTGT
MGG_14093_R	GTTCCAGACTTCATGCTCTGTG
MGG_05518_CACC_F	CACCATGCGCTCCGAGGTCGACAGCCGTGAT
MGG_05518_R	GCTATCGAATCTACGTTCATC
MGG_13742_CACC_F	CACCATGGCGCCTTTGCCATTCAATAGC
MGG_13742_R	ATTCCCCTTGGGCCTTCCTC
Primers for observation of RxKK containing rice proteins	
RxKK_03g12550_CDS_CACC_F	CACCATGAATGTACAAGTCCACAGAG
RxKK_03g12550_CDS_R	TCTCGATATAAATGAAAACATGCTG
RxKK_08g09300_CDS_CACC_F	CACCATGGATCTGGAGACGGAGA
RxKK_08g09300_CDS_R	AGAAAAAATGGACGCTACTAACC
RxKK_03g54970_CDS_CACC_F	CACCATGCAATCTGGGGCTCCTGA
RxKK_03g54970_CDS_R	GTTCAGGAGCGACAACGA
RxKK_05g19970_CDS_CACC_F	CACCATGGACGACAGCTGCGCGGTGT
RxKK_05g19970_CDS_R	CTTCCTTCCATTGCCCGTAAAG
RxKK_04g53700_CDS_CACC_F	CACCATGTTCATATCCACACCTCTC
RxKK_04g53700_CDS_R	TGGGCTTTTCTTCTTGGCA
Primers for qRT-PCR plant defense related genes	

Actin_F	GCGTGGACAAAGTTTTCAACCG
Actin_R	TCTGGTACCCTCATCAGGCATC
PR1a_RT_F	GGCACGAGTCGATCTCCA
PR1a_RT_R	ACCAGCAAGCAGCAGGAT
PR1b_RT_F	GGCAACTTCGTCGGACAGA
PR1b_RT_R	CCGTGGACCTGTTTACATTTT
PR3_RT_F	GGCGTTCTGGTTCTGGATGAC
PR3_RT_R	CGCCGTTGATGATGTTGGTC
PR4_RT_F	TGGGACCTGAACAAAGTGAGC
PR4_RT_R	TGGATACACTTGCCACACGAG
PR10a_RT_F	ACACTCGACGGAGACGAAGC
PR10a_RT_R	CAGGGTGAGCGACGAGGTA
PAL_RT_F	CCCTGCCAATCTGCTGAACTA
PAL_RT_R	GCCGCTATGCAACGAAGAAT
NPR1_RT_F	CACGCCTAAGCCTCGGATTA
NPR1_RT_R	TCAGTGAGCAGCATCCTGACTAG
LOX1_RT_F	CGATGGCCGGAACAAGGATA
LOX1_RT_R	TGGAGCGTTTTGTCTCATCA
ACS1_RT_F	TCGGCCAAGACCCTCGACG
ACS1_RT_R	CGAAAGGAATCTGCTACTGCTGC
EBP89_RT_F	TGACGATCTTGCTGAACTGAA
EBP89_RT_R	CAATCCCACAAACTTTACACA

Primers for qRT-PCR of MoHTR1 target candidates

SRZ1_qRT_F	CTTTGATGGTGGGGACATGTC
SRZ1_qRT_R	GTGCTCATTGCATCCAGACC
OsABIL2_qRT_F	CCTGAGGTCACAGTTGTCG
OsABIL2_qRT_R	CGCATTCTTCTTGTGCCATAG
OsBURP16_qRT_F	GTATACTACTGCCACTCGGTG
OsBURP16_qRT_R	CACCTAGTGCCACAAAAGC
OsbZIP39_qRT_F	CAATGAAGCGATTCCACTCAC
OsbZIP39_qRT_R	GATCGGCTAGCACCGAGACAAC
OsCCT11_qRT_F	CAGCAGCCATCCATTGAGCGAG
OsCCT11_qRT_R	GAGCAGTACTAGGTCCTCTTTG
OsHKT2_qRT_F	GTGCAGAACTTGGCATTTTCAC
OsHKT2_qRT_R	CCATATGCACTGATGATTTC
OsJAZ13_qRT_F	GCAGATGACCATCTTCTAC
OsJAZ13_qRT_R	CTTCCTCTTCTCCATGAAC
OsLH2_qRT_F	GGATCTTTCACCACCTGGTC
OsLH2_qRT_R	GAAATCGAGCCATGATCTTG
OsIsu1_qRT_F	GCAAGCAAATGGAGGAAGTAG
OsIsu1_qRT_R	CTTTGCTTCATAGTCTTTCACG
CRK10_qRT_F	CATCGAGTTGATCGATCCATC
CRK10_qRT_R	CCAGTACTACTAAGCATGGC
OsAOS2_qRT_F	GTTACATGGGAGCACTGGACTAG
OsAOS2_qRT_R	CTGACATCAATGGCCTATCAG
OsARD2_qRT_F	GATTGTTTTGCCTGCGGGAATG
OsARD2_qRT_R	CCTTTCTAGCTGGCAGATGG
Myb4_qRT_F	TCTGAATTCTGTGCTACGCAG
Myb4_qRT_R	TTCTTGATCTCGTTGTCCGTC
OsWRKY53_qRT_F	GTGATCACCACCTACGAGG
OsWRKY53_qRT_R	GAGCATCTCGAGGGTGTAG
Pi21_qRT_F	GAAGCCCAAGCCATGCGAGAAG
Pi21_qRT_R	CTCAGGCTTTGGGCAGCAC
OsiSAP7_qRT_F	GAAGAAGGTGGGGCTGAC
OsiSAP7_qRT_R	CGCTCTTGTAGTCGAAGC
OsRacB_qRT_F	GTGCTGTTCCTATCACCACTG
OsRacB_qRT_R	GCTGCAGCACCACCTTTATTG

Primers for yeast two hybrid assay

MoHTR1_CDS_F_CACC	CACC ATGGCCCCCATGCCTTC
MoHTR1_CDS_R	TCAGAGCTGGACCGGGTCGCCTT
MGG_15072_CDS_F_CACC	CACC ATG GCCGAGCGCTACATCCCCGA
MGG_15072_CDS_R	TTACATGTCCATCGACTCGCCAC
Os01g14950_CDS_F_CACC	CACCATGTCGCTGCGCCCGAGCGA
Os01g14950_CDS_R	TTATTTGAATTGAGCAGCACCAC
Os01g24060_CDS_F_CACC	CACCATGTCGCTCCGGCCGAGCGA
Os01g24060_CDS_R	CTACGGTGCATTTCCATCCAAATCG
Os05g06350_CDS_F_CACC	CACCATGTCGCTGCGGCCGAGCGA
Os05g06350_CDS_R	TCAGCCAAAGTTGAATCCACC

Primers for protein purification

HTR1_BamHI_F	GGATCCATGGCCCCCATGCCTTCCGGCT
HTR1_HindIII_R	AAGCTTTTAGAGCTGGACCGGGTCGCCT

Supplement Table 3. Summary of RNA sequencing quality and read counts for 9 libraries.

	Raw reads (bp)	Clean reads (bp)	Raw reads	Clean reads	Mapped reads
Wild type_rep1	10,718,392,532	9,409,940,107	70,982,732	62,471,990	53,461,781
Wild type_rep2	9,764,181,554	9,140,221,219	64,663,454	60,655,976	52,839,261
Wild type_rep3	10,573,746,612	9,082,276,147	70,024,812	60,338,330	49,384,131
Δ***Mohtr1*_rep1**	10,222,059,760	9,172,390,147	67,695,760	60,915,964	51,154,663
Δ***Mohtr1*_rep2**	10,531,879,446	9,227,505,722	69,747,546	61,292,176	51,107,624
Δ***Mohtr1*_rep3**	10,798,865,868	9,574,995,126	71,515,668	63,563,966	53,839,948
Δ*Mohtr1*::MoHTR1^{ΔNLS}_rep1	8,860,998,006	7,795,955,877	58,682,106	51,746,322	44,792,616
Δ*Mohtr1*::MoHTR1^{ΔNLS}_rep2	10,423,620,600	8,984,551,955	69,030,600	59,793,262	46,083,871
Δ*Mohtr1*::MoHTR1^{ΔNLS}_rep3	10,668,830,406	9,207,919,792	70,654,506	61,163,400	50,945,397

Supplementary References

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