

## Supplementary data

# Knocking Out *OsRLK7-1* Impairs Rice Growth and Development but Enhances Its Resistance to Planthoppers

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**Figure S1.** Sequences of nucleotides and deduced amino acids of *OsRLK7-1*.

**Figure S2.** Alignment of nucleotide sequences of *OsRLK7* and *OsRLK7-1*.

**Figure S3.** Phylogenetic analysis of *OsRLK7-1* and its orthologs from different plant species.

**Figure S4.** Sanger sequencing results of potential off-target sites.

**Figure S5.** Mean transcript levels (+SE, n = 5) of *OsMPK6* in WT and *ko-rlk* plants 0, 1 and 3 h after they were infested with gravid BPH females.

**Figure S6.** Mean content (+SE, n = 5) of SA in WT and *ko-rlk* plants at 0, 3, 8, 12 and 24 h after infestation of gravid BPH females (a) or gravid WBPH females (b).

**Figure S7.** Knocking out *OsRLK7-1* does not influence the survival and development of BPH and WBPH nymphs.

**Figure S8.** Experimental setups for plant growth and herbivore bioassays.

**Figure S9.** Vectors used in this study.

**Table S1.** Primers used for qRT-PCR.

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5' ATGCGCGCGCCTCCCTTCTCCTCCCTCCTCCTCCTCCTCGGCCCTCCATGCCGCGCGCGCGCGCTCCGAGGTGTCGGCCCTCATGGCCTTCAAGAACGCCCTCACCATCCCTCCCACCGCGCGCGCTTCTTCGCCCGGTGGGACGCCGCGCGCTTCCCC 170  
M P P P S L L L L P P L L L L L L G L H A A A A A V S E V S A L M A F K N A L T I P P T A A A F F A R W D A A A A S P

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C N F T G V D C A N S G G G G V T A V A V E G L G V A A T S V P F D V L C G S L P S L V K L S L P S N T L A G G

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CACGGCGTGGTGGTGGCGGAGTTCGACTCGGAGGTGGGACGCTGAGCTCGATCCGGCACGTGAACGTGGTGAAGCTCTGTGCGAGCATCACAGCGACGCGCGCGGAGCCTGCTGGTGTACGAGCACCTCCCAATGGCAGCCTTACGAGCGCTGCACG 2380  
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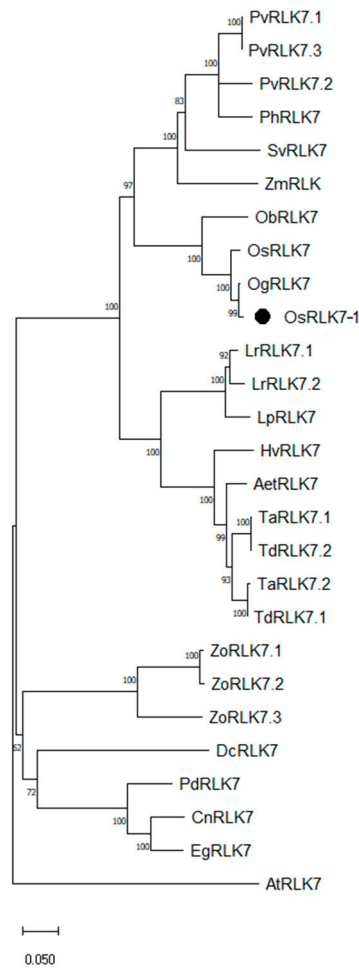
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GGTACGCGGCGGAGCGGATCATGCGGAGTACGGGAGGGCAGGACATCGTGGAGTGGGTGTTTCGCGGTTAGATAGCGGAGCAAGGTGATGTCCTCTCGACGCGAGCATCGCGGAGGATGGGAGAAGGAGGCGGTGAGGTGCTCCGTGTCGCGTGG 2890  
V T G R T A I M A E Y G E G R D I V E W V F R R L D S R D K V M S L L D A S I A E E W E K E E A V R V L R V A V

TGTGCACGACGAGCGCGTCTGATGAGGCGTCTGATGCGCTCCGTCTGTCGATGCTCGAGGCGCGGATCGGCGGGAGTTGGCATGGTCACTTCGGTGAAGGTCAAGGTGATCGCCTAG 3'  
V C T S R T P S M R P S M R S V V Q M L E A A G I G R E L A M V T S V K V K V I A \*

Figure S1. Sequences of nucleotides and deduced amino acids of *OsRLK7-1*.

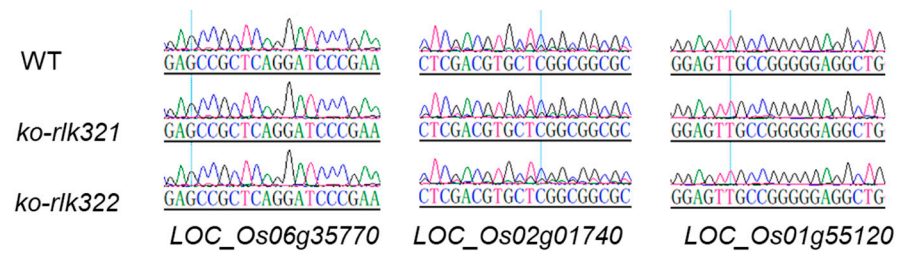




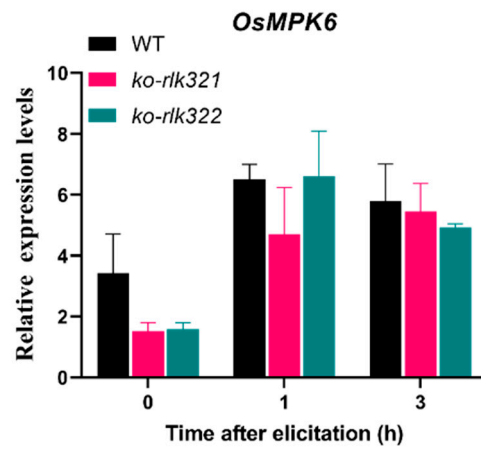
**Figure S3.** Phylogenetic analysis of OsRLK7-1 and its orthologs from different plant species.

The unrooted tree was constructed with a neighbor-joining method based on the alignment of protein sequences using MEGA11 software (default settings except the replicates of the bootstrap value, 1,000 replicates). The bootstrap values for the branches are shown. Plant species acronyms are included before its protein name: Os, *Oryza sativa*; Aet, *Aegilops tauschii* subsp. *strangulate*; At, *Arabidopsis thaliana*; Cn, *Cocos nucifera*; Dc, *Dioscorea cayenensis* subsp. *rotundata*; Eg, *Elaeis guineensis*; Hv, *Hordeum vulgare* subsp. *vulgare*; Lp, *Lolium perenne*; Lr, *Lolium rigidum*; Ob, *Oryza brachyantha*; Og, *Oryza glaberrima*; Pd, *Phoenix dactylifera*; Ph, *Panicum hallii*; Pv, *Panicum virgatum*; Sv, *Setaria viridis*; Ta, *Triticum aestivum*; Td, *Triticum dicoccoides*; Zm, *Zea mays*; Zo, *Zingiber officinale*. Sequence data in the phylogenetic tree can be found in the GenBank with accession numbers as below: OsRLK7-1(OR472548), OsRLK7(XP\_015620235.1), AetRLK7(XP\_020194737.1), AtRLK7(NP\_850942.1), CnRLK7(KAG1348101.1), DcRLK7(XP\_039139905.1), EgRLK7(XP\_010943231.1), HvRLK7(XP\_044948214.1), LpRLK7(XP\_051209176.1), LrRLK7.1(XP\_047050302.1), LrRLK7.2(XP\_047050623.1), ObRLK7(XP\_040385827.1), OgRLK7(XP\_052139509.1), PdRLK7(XP\_038970452.1), PhRLK7(XP\_025808423.1), PvRLK7(XP\_039840382.1), PvRLK7.1(XP\_039803010.1), PvRLK7.2(XP\_039803012.1), SvRLK7(XP\_034586844.1), TaRLK7.1(XP\_044386491.1), TaRLK7.2(XP\_044379179.1), TdRLK7.1(XP\_037439029.1), TdRLK7.2(XP\_037431991.1), ZoRLK7.1(XP\_042463340.1), ZoRLK7.2(XP\_042427680.1), ZoRLK7.3(XP\_042416481.1), ZmRLK(ONM32713.1).

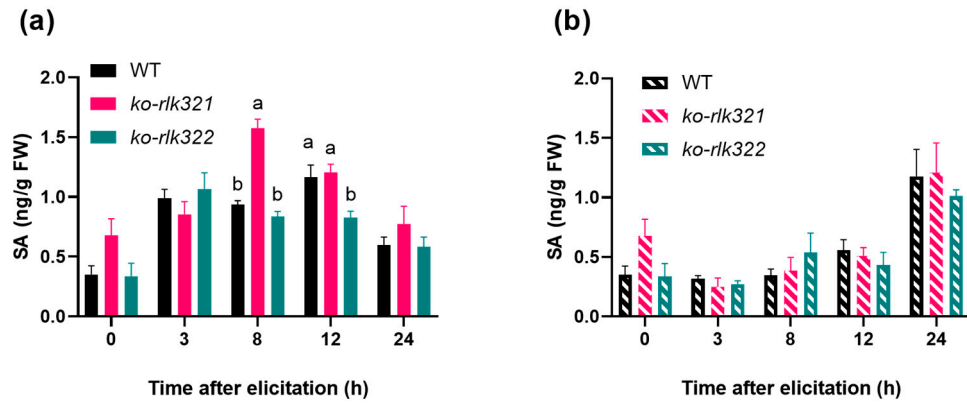




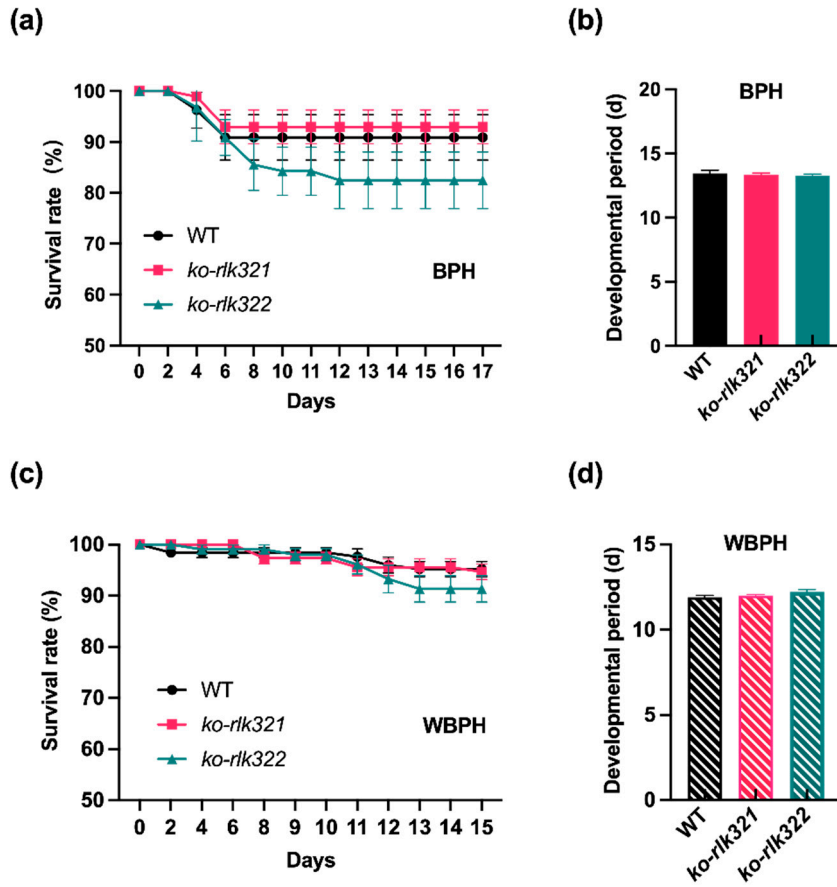
**Figure S4.** Sanger sequencing results of potential off-target sites.



**Figure S5.** Mean transcript levels (+SE, n = 5) of *OsMPK6* in WT and *ko-rlk* plants 0, 1, and 3 h after they were infested with gravid BPH females.



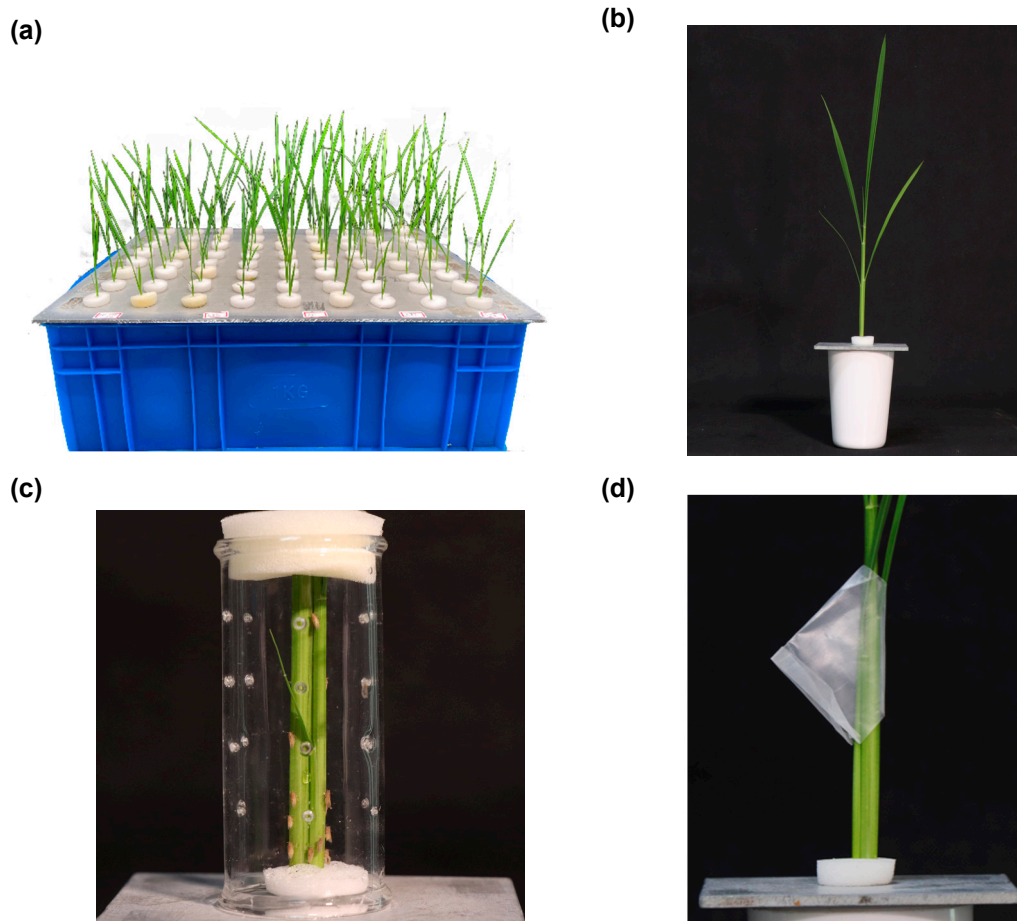
**Figure S6.** Mean content ( $\pm$ SE,  $n = 5$ ) of SA in WT and *ko-rlk* plants at 0, 3, 8, 12, and 24 h after infestation of gravid BPH females (a) or gravid WBPH females (b). Different letters indicate significant differences among lines at the same time point ( $p < 0.05$ , Tukey's post-hoc test)..



**Figure S7.** Knocking out *OsRLK7-1* does not influence the survival and development of BPH and WBPH nymphs.

(a) Mean survival rate ( $\pm$ SE,  $n = 15$ ) of newly hatched BPH nymphs on WT and *ko-rlk* (*ko-rlk321* and *ko-rlk322*) plants 0-17 d after they were released. (b) Mean developmental duration ( $\pm$ SE,  $n = 15$ ) immature stage of BPH on WT and *ko-rlk* plants. (c) Mean survival rate ( $\pm$ SE,  $n = 15$ ) of newly hatched WBPH nymphs on WT and *ko-rlk* (*ko-rlk321* and *ko-rlk322*) plants 0-15 d after they were released. (d) Mean developmental duration ( $\pm$ SE,  $n = 15$ ) of immature stage of WBPH on WT and *ko-rlk* plants.



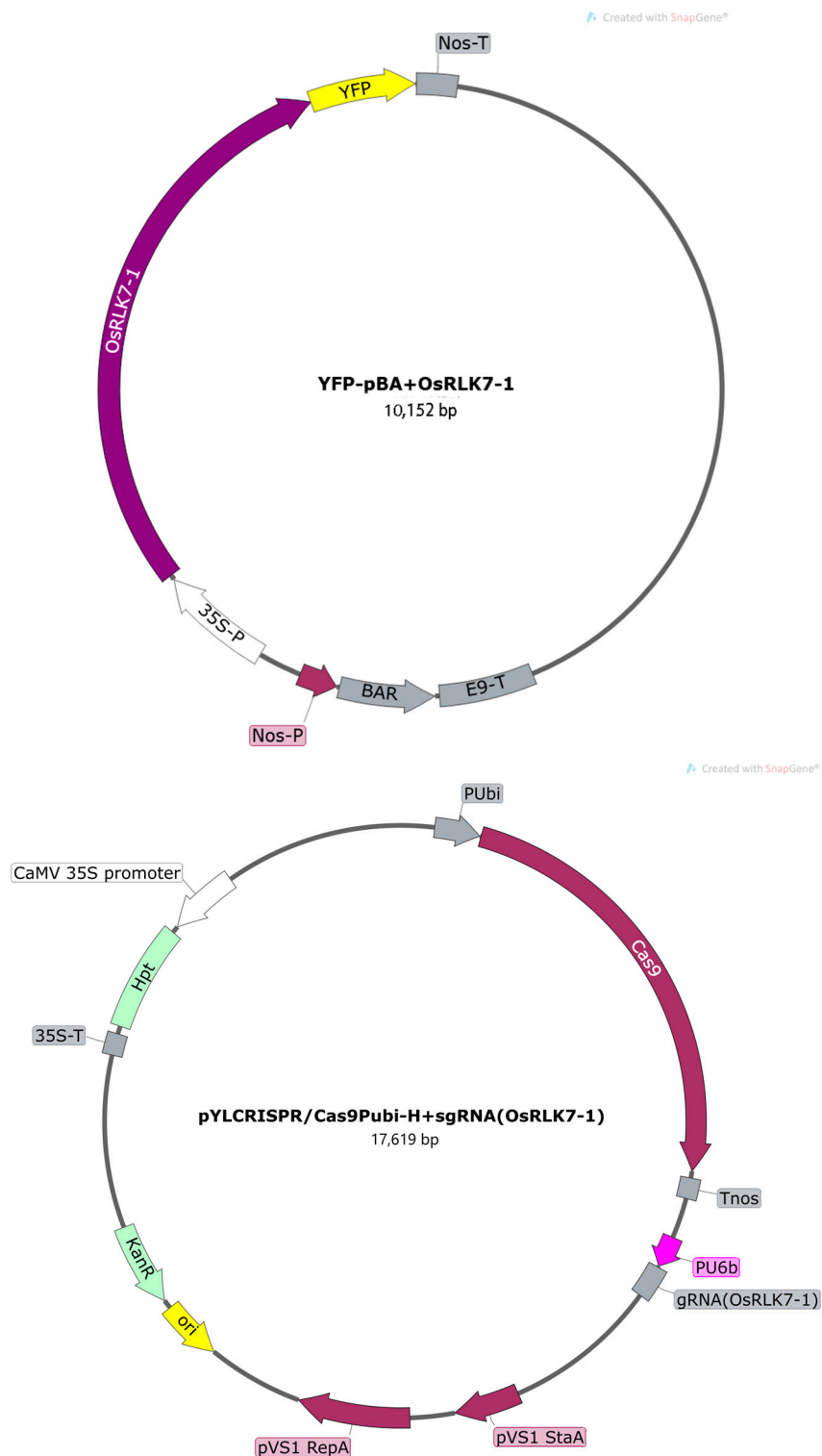


**Figure S8.** Experimental setups for plant growth and herbivore bioassays.

(a) Rice seedlings were planted in a hydroponic box with hydroponic solution. (b) Rice seedlings were individually transferred into single plastic cups. (c) The setup for planthopper bioassays. (d) The setup for planthopper feeding bioassays.

(a)

(b)



**Figure S9.** Vectors used in this study.

(a) The vector used for subcellular localization of *OsRLK7-1*. (b) The vector used for obtaining transgenic plants by pY-LCRISPR/Cas9Pubi-H.

**Table S1.** Primers used for qRT-PCR

Name	TIGR ID	Forward primer (5'-...-3')	Reverse primer (5'-...-3')
<i>OsACTIN</i>	<i>LOC_Os03g50885</i>	TGGACAGGTTATCAC CATTGGT	CCGCAGCTTCCATT CCTATG
<i>OsMPK3</i>	<i>LOC_Os03g17700</i>	CGACTTCGAGCAGA AGGCTCTA	G TTCATCTCGATCG CTTCGTT
<i>OsMPK6</i>	<i>LOC_Os06g06090</i>	CGCACGCTCAGGGA GATC	GGTATGATATCCCT TATGGCAACAA
<i>OsWRKY30</i>	<i>LOC_Os01g61080</i>	AGGCAAGCACAGCC ATGAC	GAAGACGATACGTT GGCATTAGC
<i>OsWRKY33</i>	<i>LOC_Os01g53040</i>	AACGGCTGCTCCATG AAGAA	TTGTGTGCGCCCTT GTAGAC
<i>OsWRKY45</i>	<i>LOC_Os05g25770</i>	CGACCGGAAGGCCA ACT	GAAGTAGGCCTTTG GGTGCTT

**Table S2.** Primers used for PCR

Name	TIGR ID	Forward primer (5'-...-3')	Reverse primer (5'-...-3')
<i>OsRLK7-1</i>	/	ATGCCACCGCCCTCC CTTCT	CTAGGGGATCACCTTGA CCTTCACCGA
<i>KO-RLK7-1</i>	<i>LOC_Os12g43</i> 640	ATGCCACCGCCCTCC CTTCT	CTAGGGGATCACCTTGA CCTTCACCGA
Fusion RLK7-1&YFP	/	ACGCGTTTAATTAAG ATGCCACCGCCCTCC CTTCT	TGACGTCCCGGGATCCG GGGATCACCTTGACCTTC ACCGA
<i>Cas9-test</i>	/	GTTGAGCCGCACAG AACGTCGAA	AAACTTCGACGTTCTGTG CGGCT
<i>KO-RLK7-1-Cas9</i> region check	<i>LOC_Os12g43</i> 640	AGGCTGCTGACCTAC TTACTACC	CCGTCTTCTCGAAGAACC CAT
Off-target test 1	<i>LOC_Os06g35</i> 770	AGGAGGAGGAGGAG GAGGAGGAGGA	TCGGAGAAGGTGAACCC GAGGTCTT
Off-target test 2	<i>LOC_Os01g55</i> 120	GCCGCATTTCCTGTCT CCATCC	CCCGCTCACCAAATCCAT CTTCA
Off-target test 3	<i>LOC_Os02g01</i> 740	GTCTACAAGCCCCAG ACCAA	AATTCAACAGCAACGCC GAT
Target site of <i>RLK7-1</i> for overlapping PCR	/	AGCCGCACAGAACG TCGAACAACACAAG CGGCAGC	AAACTTCGACGTTCTGTG CGGCTGTTTTAGAGCTAG AAAT
sgRNA expression cassette assembling	/	CTCCGTTTTACCTGTG GAATCG	CGGAGGAAAATTCCATC CAC
Pps / Pgs	/	TTCAGAGGTCTCTCT CGACTAGTATGGAAT CGGCAGCAAAGG	AGCGTGGGTCTCGACCG ACGCGTATCCATCCACTC CAAGCTC