

Supplementary Materials

# Silencing a Simple Extracellular Leucine-Rich Repeat Gene *OsI-BAK1* Enhances the Resistance of Rice to Brown Planthopper *Nilaparvata lugens*

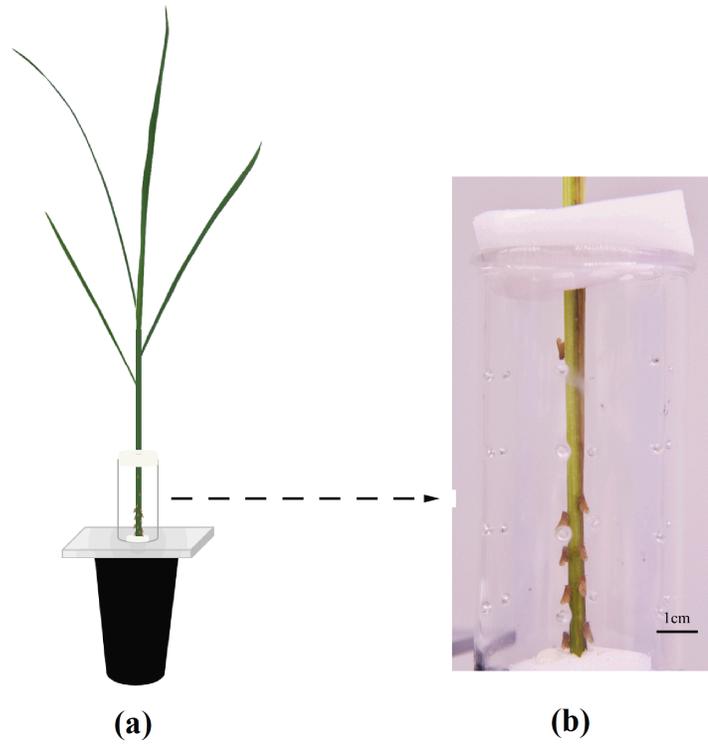
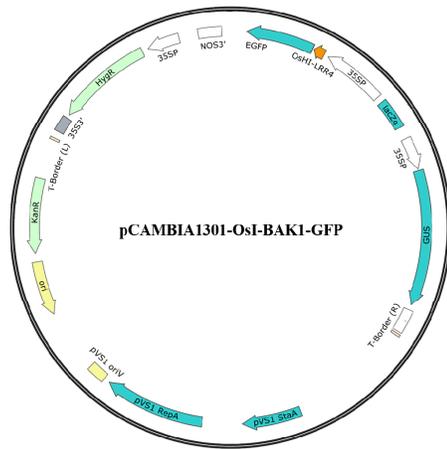
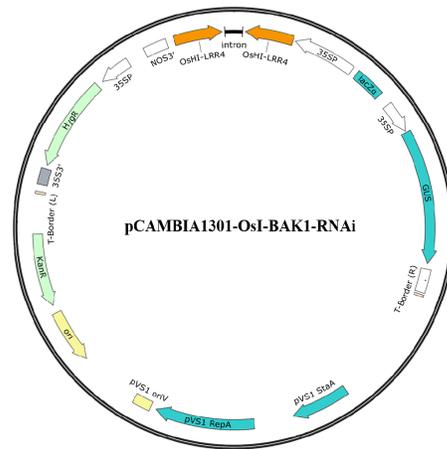


Figure S1. Setups for herbivore bioassay or treatment.

(a)



(b)



**Figure S2.** Vectors used in this study. (a) The vector used for subcellular localization of *OsI-BAK1*. (b) The vector used for obtaining transgenic plants by silencing *OsI-BAK1*.

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1      ATGGCGCCCCGCGTGTCCCTGCTCGCCCGCGGGCGCCGTTGGCCGTGGCCGTGGTCCTTGCTT
1      M A P R V S L L A A G A V A V A V V L L

61     CTGCGCCGCGCCGCGCGGGCGTCCAACGACGAGGGGGACGCGCTGTACGCGCTGCGGACG
21     L A A P A R A S N D E G D A L Y A L R T

121    AGGCTGTGCGGATCCCAACGGCGTGTGCGAGCTGGGACCCGACCCTCGTCAACCCCTGC
41     R L S D P N G V L Q S W D P T L V N P C

181    ACCTGGTTCCATGTACCTGCGACCAGCCAGCCGCGTCCGCGCTGGATTTAGGAAAC
61     T W F H V T C D H A S R V V R L D L G N

241    TCCAACATCTCCGGCTCGATTGGCCCTGAGCTAGGCCGTCTTGTGAACCTCCAATACCTG
81     S N I S G S I G P E L G R L V N L Q Y L

301    GAGCTCTACAGGAACAATCTTAACGGTGAGATCCCAAAAGAATTGGGCAATCTCAAGAAT
101    E L Y R N N L N G E I P K E L G N L K N

361    TTGATCAGCTTGGATTTGTATGCCAACAAGCTCACTGGAACAATCCCCAAGTCGCTTTCC
121    L I S L D L Y A N K L T G T I P K S L S

421    AAGCTCGGCTCGCTGAGATTGATGCGGTTGAACAATAACAAGCTTGCTGGATCAATTCCA
141    K L G S L R F M R L N N N K L A G S I P

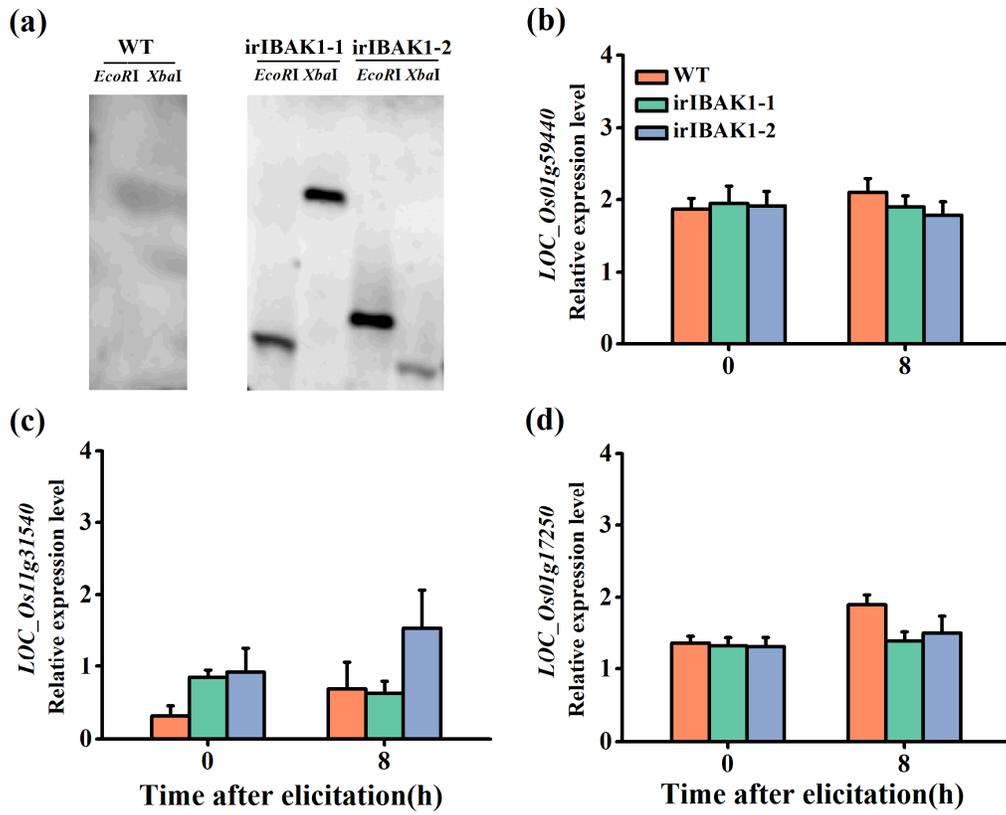
481    AGGGAGCTGGCCAAACTATCCAACCTGAAAGTCATTGACTTGTCTAACAATGACCTCTGT
161    R E L A K L S N L K V I D L S N N D L C

541    GGAACTATTCTGTGACGGTCCCTTCTCAACCTTCCCTCTTCGAAGCTTTGAGAACAAC
181    G T I P V D G P F S T F P L R S F E N N

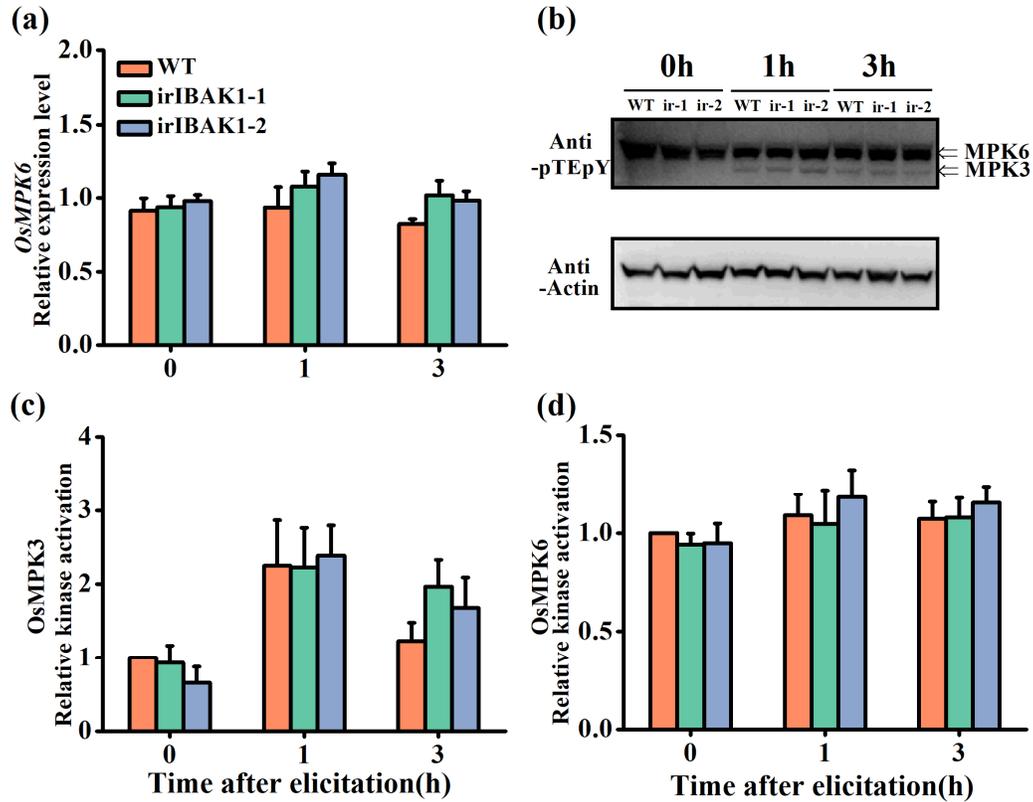
601    AACAGGCTCAACGGCCAGAGCTGCAAGGTTTGGTTCTTATGACTTTGGATGTTAA
201    N R L N G P E L Q G L V P Y D F G C *

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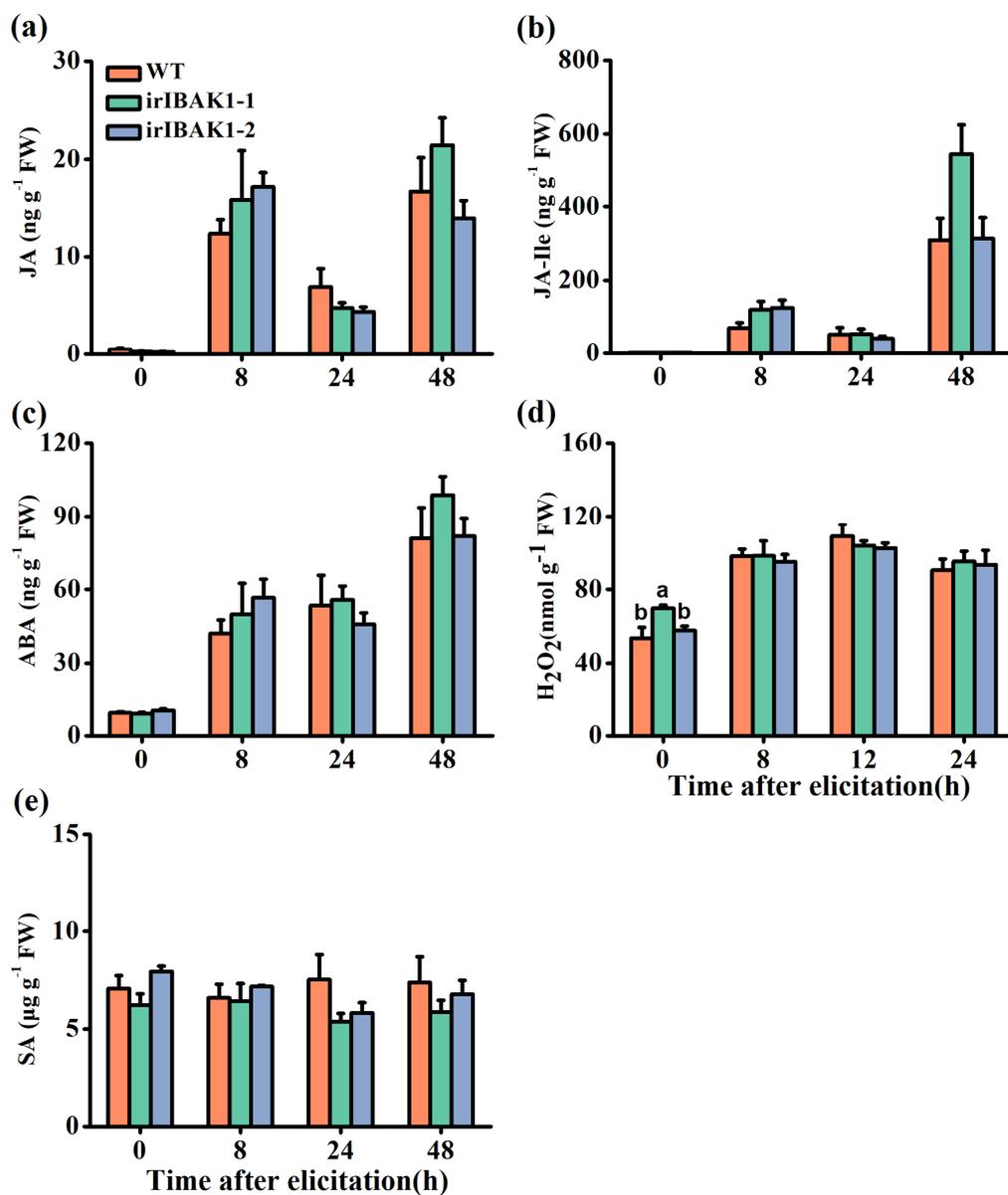
Figure S3. Sequences of nucleotides and deduced amino acids of OsI-BAK1.



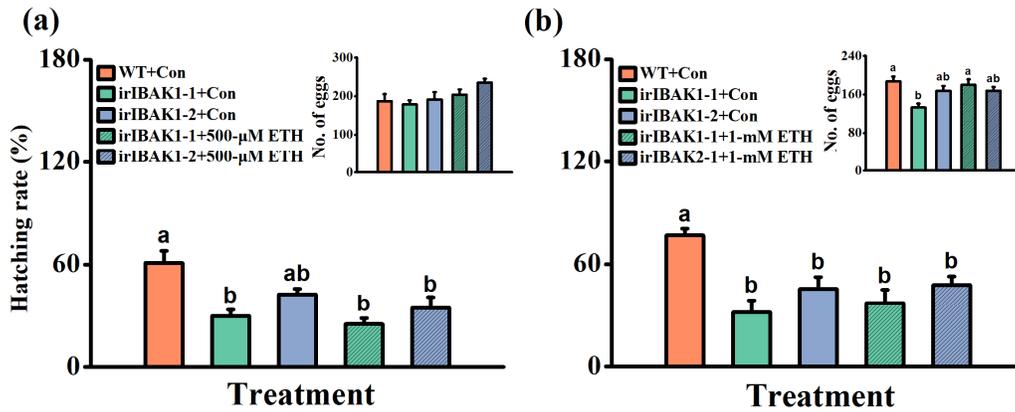
**Figure S4.** DNA gel-bolt analysis of wild type (WT) and *ir-ibak1* plants and the transcript level of genes sharing high similarity with *Osl-BAK1* in *ir-ibak1* plants. (a) Genomic DNA was digested with *EcoRI* or *XbaI*. The blot was hybridized with a probe specific for reporter gene GUS. (b–d) Mean expression levels (+ SE, n = 5) of *LOC\_Os01g59440* (b), *LOC\_Os11g31540* (c) and *LOC\_Os01g17250* (d) at 0 and 8 h after BPH infestation.



**Figure S5.** OsI-BAK1 does not affect BPH-induced transcript levels and kinase activation of OsMPK3 and OsMPK6. (a) Mean transcript level (+ SE, n = 5) of *OsMPK6* in WT and *ir-ibak1* plants after BPH infestation. (b) MPK activity in wild type (WT) and *ir-ibak1* plants at 0, 1 and 3 h after gravid BPH female infestation. *ir-1* represents *irIBAK1-1*; *ir-2* represents *irIBAK1-2*. The rice leaf sheaths from five replicate plants were harvested at the indicated times. Immunoblotting was performed using either anti-pTEpY antibody (first section) to detect phosphorylated MPKs or actin antibodies (second section) as a loading control. This experiment was replicated 4 times with similar results. (c and d) The quantitative relative MPK kinase activity for OsMPK3 (c) and OsMPK6 (d) versus OsACTIN, respectively. Quantitative analysis (+SE, n = 4) of each line at each time point was calculated taking WT sample without BPH infestation (at 0h) as 1 for normalization.



**Figure S6.** Silencing *Osl-BAK1* does not influence basal and BPH-induced levels of JA, JA-Ile, ABA, H<sub>2</sub>O<sub>2</sub> and SA. (a-c) Mean contents (+ SE, n = 5) of JA (a), JA-Ile (b) and ABA (c) in wild type (WT) and *ir-ibak1* plants at 0, 8, 24 and 48 h after infestation of gravid BPH females. (d) Mean contents (+ SE, n = 5) of H<sub>2</sub>O<sub>2</sub> in WT and *ir-ibak1* plants at 0, 8, 12 and 24 h after infestation of gravid BPH females. (e) Mean contents (+ SE, n = 5) of SA in WT and *ir-ibak1* plants at 0, 8, 24 and 48 h after infestation of gravid BPH females.



**Figure S7.** Spraying ethephon does not recover the hatching rate of BPH eggs on *ir-ibak1* plants. (a) Mean hatching rate (+ SE, n = 8) of BPH eggs on wild type (WT) and *ir-ibak1* plants after they were sprayed thoroughly with 500-µM ethephon or not. Inserts: Mean numbers of BPH eggs (+ SE, n = 8) on WT and *ir-ibak1* plants. (b) Mean hatching rate (+ SE, n = 8) of BPH eggs on WT and *ir-ibak1* plants after they were sprayed thoroughly with 1-mM ethephon or not. Inserts: Mean number of BPH eggs (+ SE, n = 8) on WT and *ir-ibak1* plants. Con, control solution. ETH, ethephon. Different letters represent significant difference among treatments ( $p < 0.05$ ; Tukey's HSD post-doc tests).

**Table S1.** Primers and probes used for real time-qPCR

<b>Gene names</b>	<b>TIGR ID</b>	<b>Forward primer (5'---3')</b>	<b>Reverse primer (5'---3')</b>	<b>Probe (5'---3')</b>
<i>OsI-BAK1</i>	LOC_Os03g32580	TTGCTGGATCAATTCCAAGG	GAAGGTTGAGAAGGGACCGT	CTGGCCAAACTATCCAACCTGAAAG
<i>OsACTIN</i>	LOC_Os03g50885	TGGACAGGTTATCACCATTGGT	CCGCAGCTTCCATTCCATATG	CGTTTCCGCTGCCCTGAGGTCC
<i>OsWRKY24</i>	LOC_Os01g61080	AAGAGATGGAGGAAAGACGGTG	TGTCGATGTCGCTCATGGTT	AGGGGATCTCCATGGCTGGCAA
<i>OsWRKY53</i>	LOC_Os05g27730	AACGGCTGCTCCATGAAGAA	TTGTGTGCGCCCTTGTAGAC	CTCGCCGACGGCCGCATC
<i>OsWRKY70</i>	LOC_Os05g39720	CCGCTGCTGTTTTGATCATCT	GGAGCTAAGCTAACTCACTCCACA	ATCGGGCCGTCAATTTGATCAGCA
<i>OsMPK3</i>	LOC_Os03g17700	CGACTTCGAGCAGAAGGCTCTA	GTTTCATCTCGATCGCTTCGTT	ACGAGGACCAAATGAAGCAGCTGAT
<i>OsMPK6</i>	LOC_Os06g06090	CGCACGCTCAGGGAGATC	GGTATGATATCCCTTATGGCAACAA	CTCCGCCACATGGACCACGAGAA
<i>OsACO1</i>	LOC_Os09g27750	CCAGCTGGTGAAAGAGAGGA	CATGTCGATGATCGGGAACG	
<i>OsACS1</i>	LOC_Os03g51740	GCTCAGGTCTCTCCAGTGTT	ACTTGGCTCACCATCTCCAA	
<i>OsERF2</i>	LOC_Os06g08340	GTGGACCAGATGATCGAGGAG	GATGAACAGGGCACATCAGC	
	LOC_Os01g59440	GCCTGGATCTTGGAATTT	TTCCGATGGGATCGTTCCTT	
	LOC_Os11g31540	AGTTGGGATCCAACCCTTGT	CCAGCTGTGGAATCAGAGGA	
	LOC_Os01g17250	GAGCTCGGGAGTCTGAAGAA	CCTCGGGATTGGACCGTTTA	