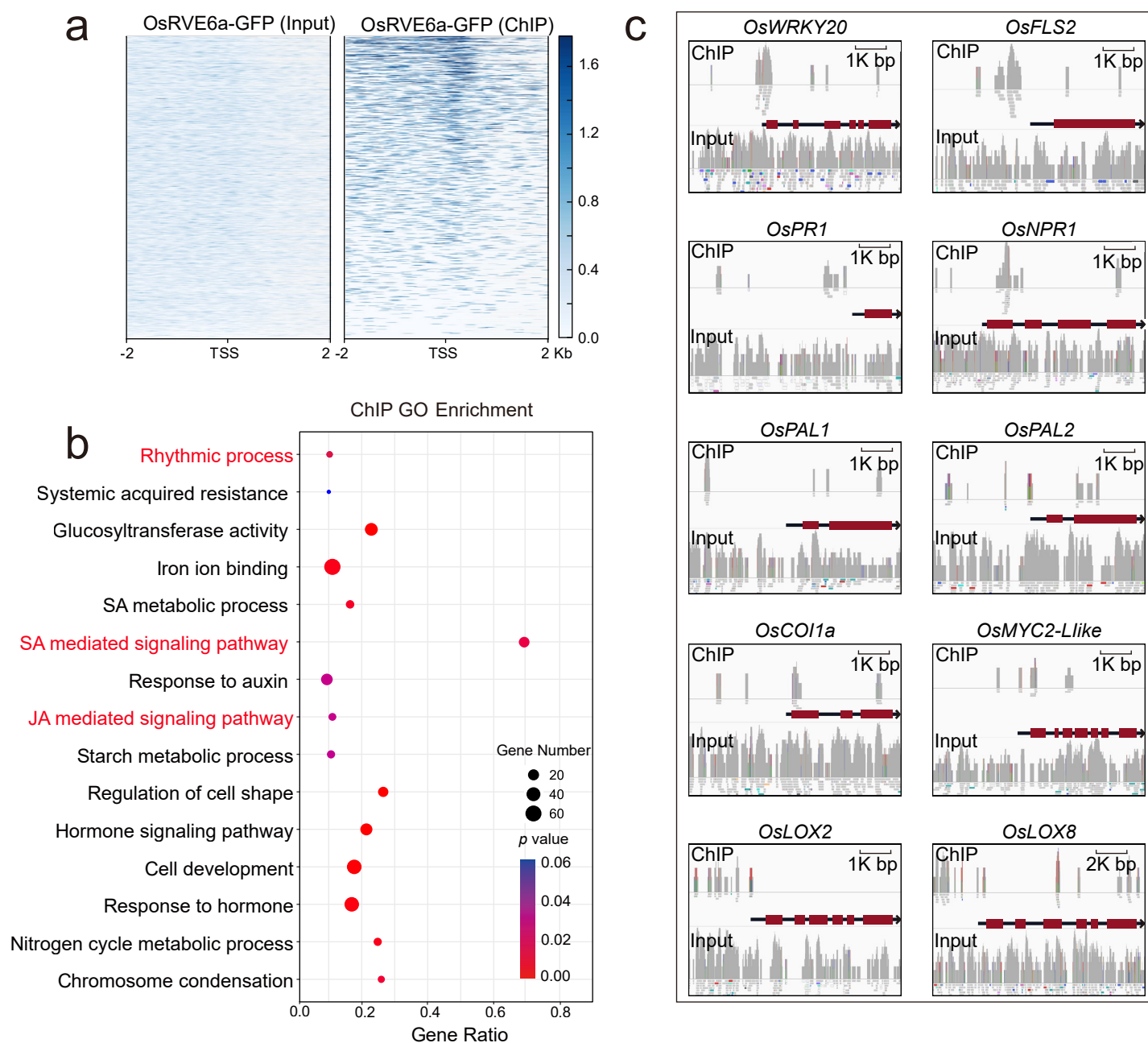


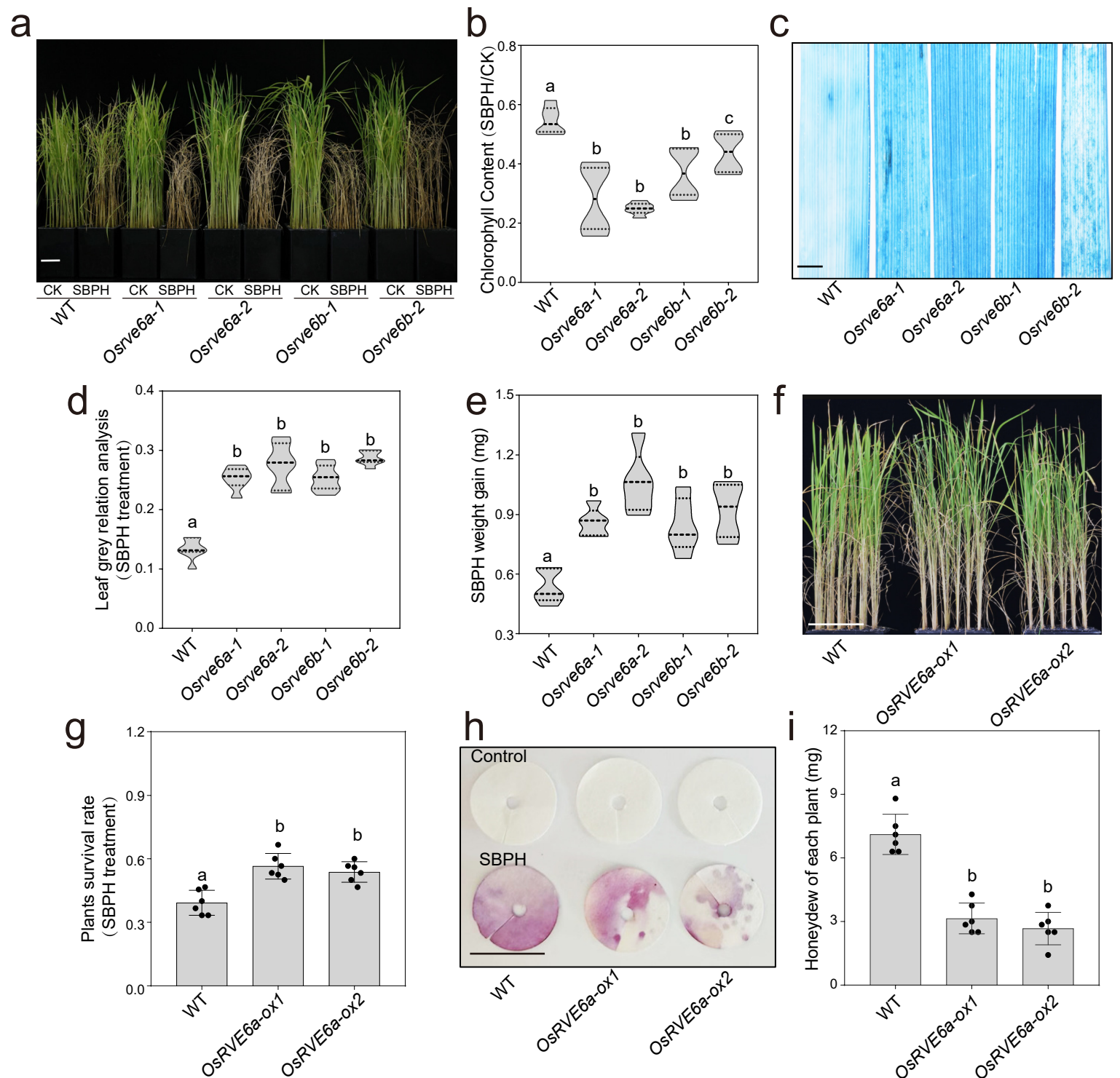
**Figure S1. Identification of *OsRVE6a/b* and generating relative transgenic seedlings**

**(a)** The phylogenetic tree of RVE proteins in rice and *Arabidopsis* was constructed using the MEGA11 program. The numbers indicated at the branch points represent bootstrap values based on 1,000 replicates, with homologues accurately listed adjacent to the tree. **(b)** Rhythmic expression patterns of *OsRVE6s* of two-week-old seedlings. Transcript levels were quantified using qPCR and normalized to the level of *OsUBIQUITIN* (n=3, biological replicates), Left Y-axis (black) represents *OsRVE6a/c* transcript levels and right Y-axis (red) represents *OsRVE6b* transcript level. **(c)** Expression of *OsRVE6a* in transgenic plant expressing *OsRVE6a*-GFP under control of *ZmUBIQUITIN* promoter (*OsRVE6a-ox1/2*). Data are presented as mean  $\pm$  SD (n=3, biological replicates), Statistical analysis was conducted using one-way ANOVA with post hoc Tukey's honest significant difference (HSD) test, Significant difference between samples are shown by different letters ( $p < 0.05$ ). **(d)** Confocal microscopic image of GFP fluorescence in *OsRVE6a-ox1* seedling (Scale bars, 20  $\mu$ m). **(e)** Generation of *OsRVE6a* loss-of-function mutants *Osrve6b-1* and *Osrve6b-2* in Nipponbare background using CRISPR/Cas9 technology. **(f)** and **(j)** *OsRVE6a* loss-of-function mutants were generated in the Zhonghua11 background using CRISPR/Cas9 technology and designated as *ZOsrve6a-1/2* and *ZOsrve6b-1/2*.



**Figure S2. Identification of biotic stress-related genes bound by OsRVE6a**

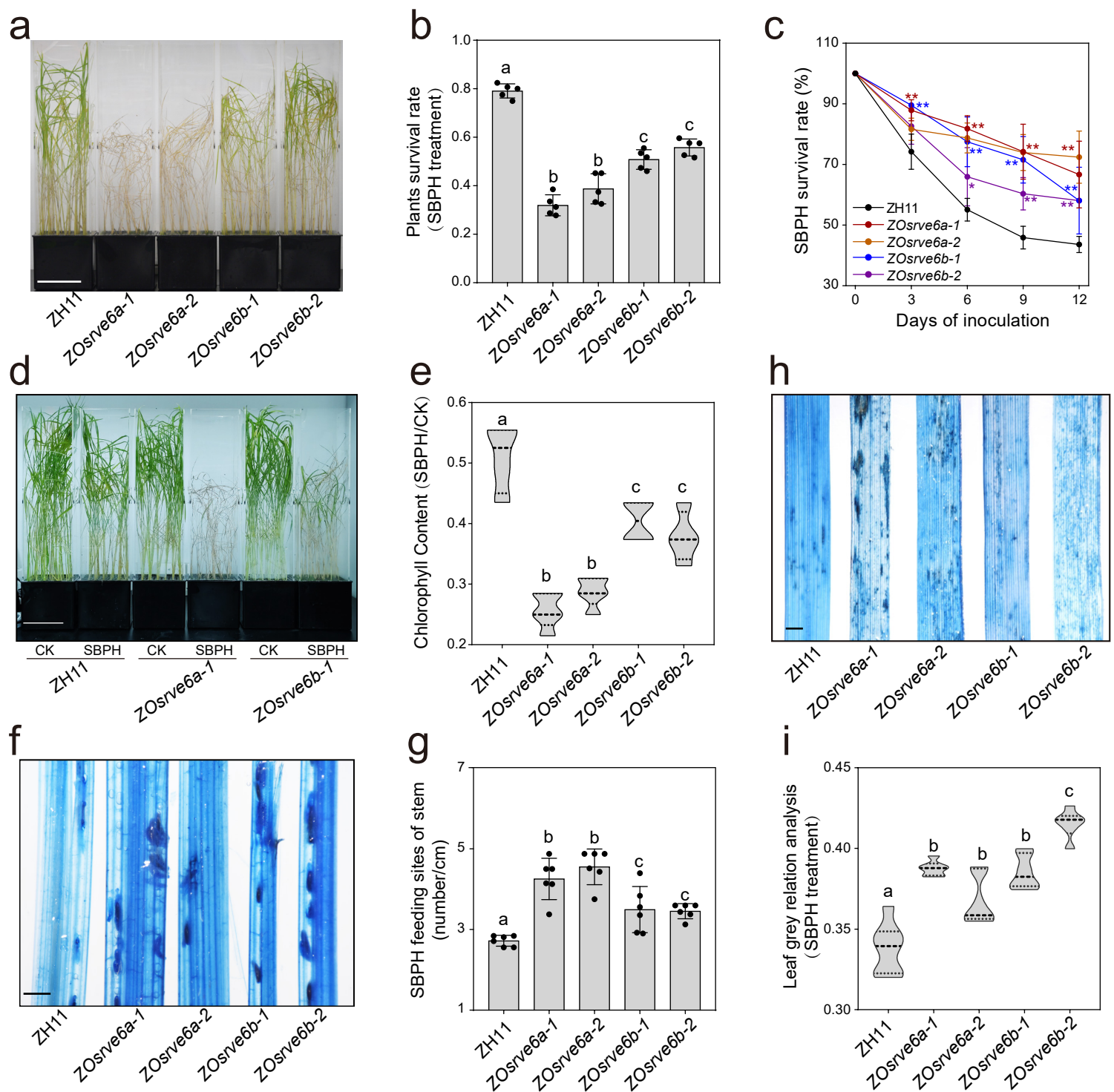
**(a)** Heatmaps representing ChIP-seq signals of OsRVE6a-GFP (IP and input). **(b)** Gene Ontology (GO) analysis showing the enriched GO categories of OsRVE6a putative target genes, and OsRVE6a is involved in various of biological processes ( $p < 0.05$  represents significantly enriched GO terms). **(c)** OsRVE6a-occupied regions identified in the promoters of defense genes in rice from ChIP-seq analysis, such as *OsWRKY20*, *OsFLS2*, *OsPR1* and key genes of salicylate acid and jasmonic acid pathways.



**Figure S3. OsRVE6a/b play a crucial role in regulating rice defense against SBPH**

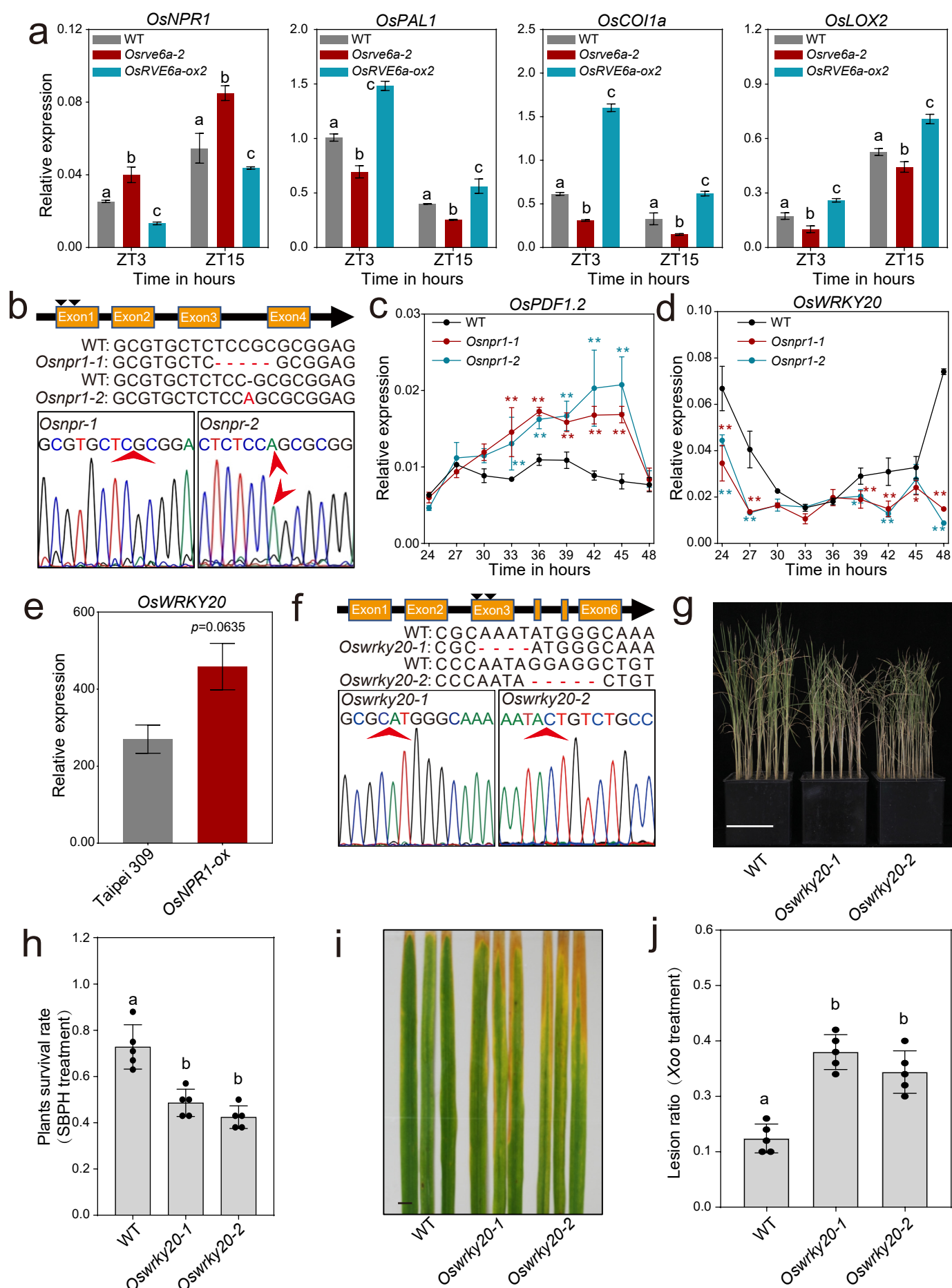
**(a)** The morphology of *Osrve6a/b* mutants and WT with and without SBPH treatment (Control check, CK) for 14 days (Scale bar, 10 cm). **(b)** Measurement of chlorophyll content in *Osrve6a/b* seedlings after SBPH treatment. The chlorophyll content ratio of SBPH to CK is presented as mean  $\pm$  SD (n=6, biological replicates). **(c)** and **(d)** Comparison of feeding sites generated by SBPH on WT and *Osrve6a/b* mutant leaves. The assessment was conducted via trypan blue staining, and the relationship between leaf color and greying was analyzed using ImageJ. Data are presented as mean  $\pm$  SD (n=6, biological replicates). **(e)** Weight gain of SBPH feeding on *Osrve6a/b* mutants and WT. Data are presented as mean  $\pm$  SD (n=6, biological replicates). **(f)** The morphology of *OsRVE6a-ox1/2* and WT observed under SBPH treatment (Scale bar, 10 cm). **(g)** The plant survival rate of *OsRVE6a-ox1/2* and WT seedling evaluated under SBPH treatment. Data are presented as mean  $\pm$  SD (n=6). **(h)-(i)** Honeydew measurement from *OsRVE6a-ox1/2* and WT seedlings under SBPH treatment. The honeydew secreted by SBPH was collected on filter papers that were soaked in 0.1% (w/v) ninhydrin in acetone solution, and the amino acid contents of honeydew were stained until purple blots appeared (Scale bar, 1.5 cm). Honeydew secreted by SBPH was weighed. Data are presented as mean  $\pm$  SD (n=6, biological replicates). **(b), (d), (e), (g)** and **(i)** Statistical analysis was conducted using one-way ANOVA with post hoc Tukey's honest significant difference (HSD) test, Significant difference between samples are shown by different letters (p < 0.05).





**Figure S4. OsRVE6a/b play a crucial role in regulating rice defense against SBPH in ZH11 background**

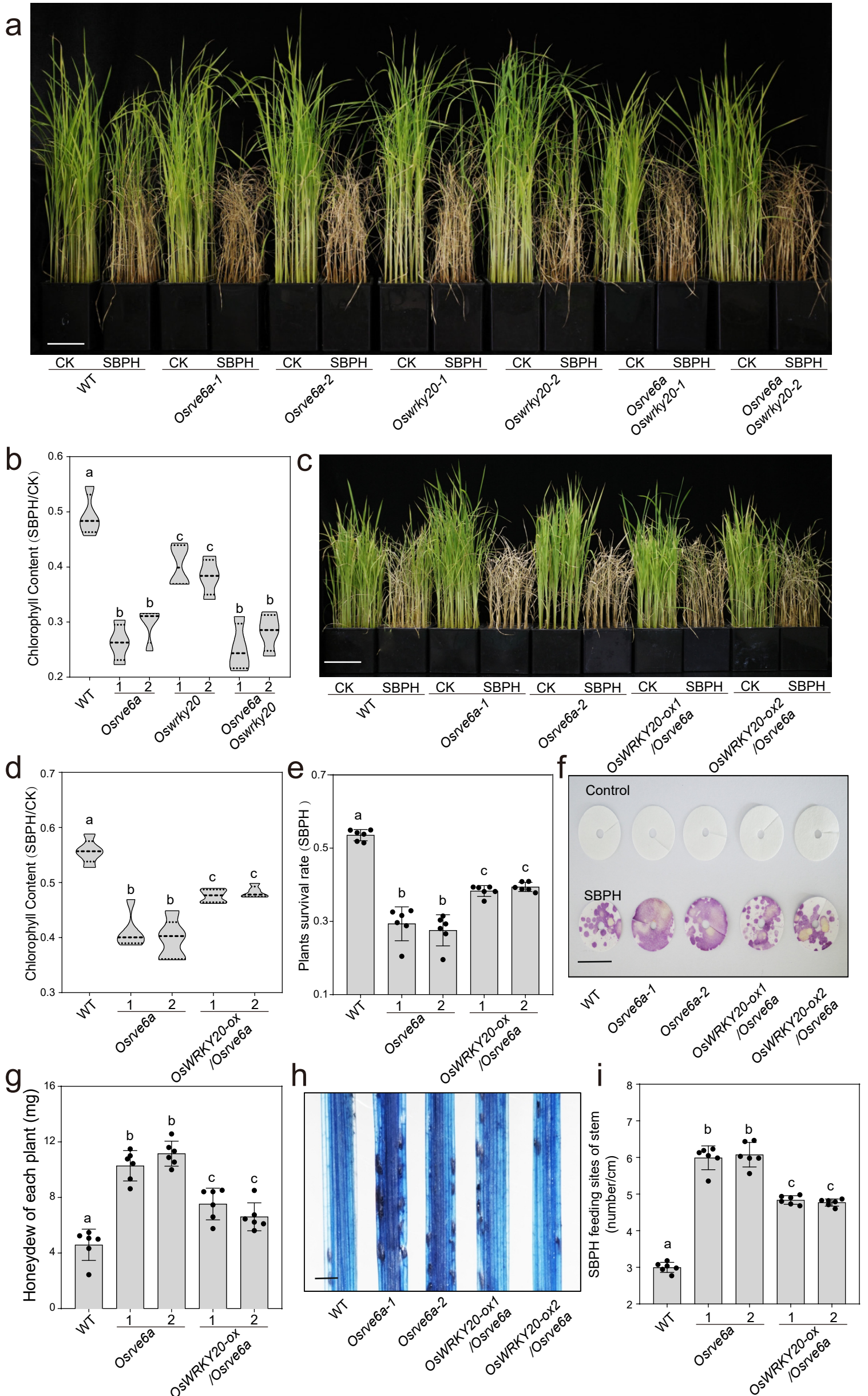
(a) The morphology of ZOs $rve6a/b$  mutants and WT under SBPH treatment for 14 days (Scale bar, 10 cm). (b) Survival rate of ZOs $rve6a/b$  mutants and WT seedling evaluated under SBPH treatment. Data are presented as mean  $\pm$  SD (n=5, biological replicates). (c) Comparison of survival rates of SBPH feeding on WT and ZOs $rve6a/b$  mutants. The survival rate of SBPH was assessed every three days, starting three days post-inoculation for a total of four assessments. Data are presented as mean  $\pm$  SD (n=6, biological replicates). Statistical analysis was conducted using two-way ANOVA with post hoc Tukey's honest significant difference (HSD) test, with significant differences indicated by asterisks (\* $p$  < 0.05, \*\* $p$  < 0.01). (d) and (e) Morphology of ZOs $rve6a/b$  mutants and WT seedlings examined with and without SBPH treatment for 14 days (Scale bar, 10 cm) (Control check, CK). The chlorophyll content ratios of SBPH/CK presented as mean  $\pm$  SD (n=6, biological replicates). (f) and (g) Analysis of feeding sites generated by SBPH on ZOs $rve6a/b$  mutants and WT seedling stem. The assessment was conducted via trypan blue staining (Scale bar, 2 mm), and feeding sites number are presented as mean  $\pm$  SD (n=6, biological replicates). (h) and (i) Feeding sites on ZOs $rve6a/b$  mutants and WT leaf assessed via trypan blue staining (Scale bar, 2 mm), with the relationship between leaf color and greying analyzed using ImageJ. Data are presented as mean  $\pm$  SD (n=6, biological replicates). (b), (e), (g) and (i) Statistical analysis was conducted using one-way ANOVA with post hoc Tukey's honest significant difference (HSD) test, Significant difference between samples are shown by different letters ( $p$  < 0.05).



**Figure S5. Validation of biotic stress-related genes bound by OsRVE6a**

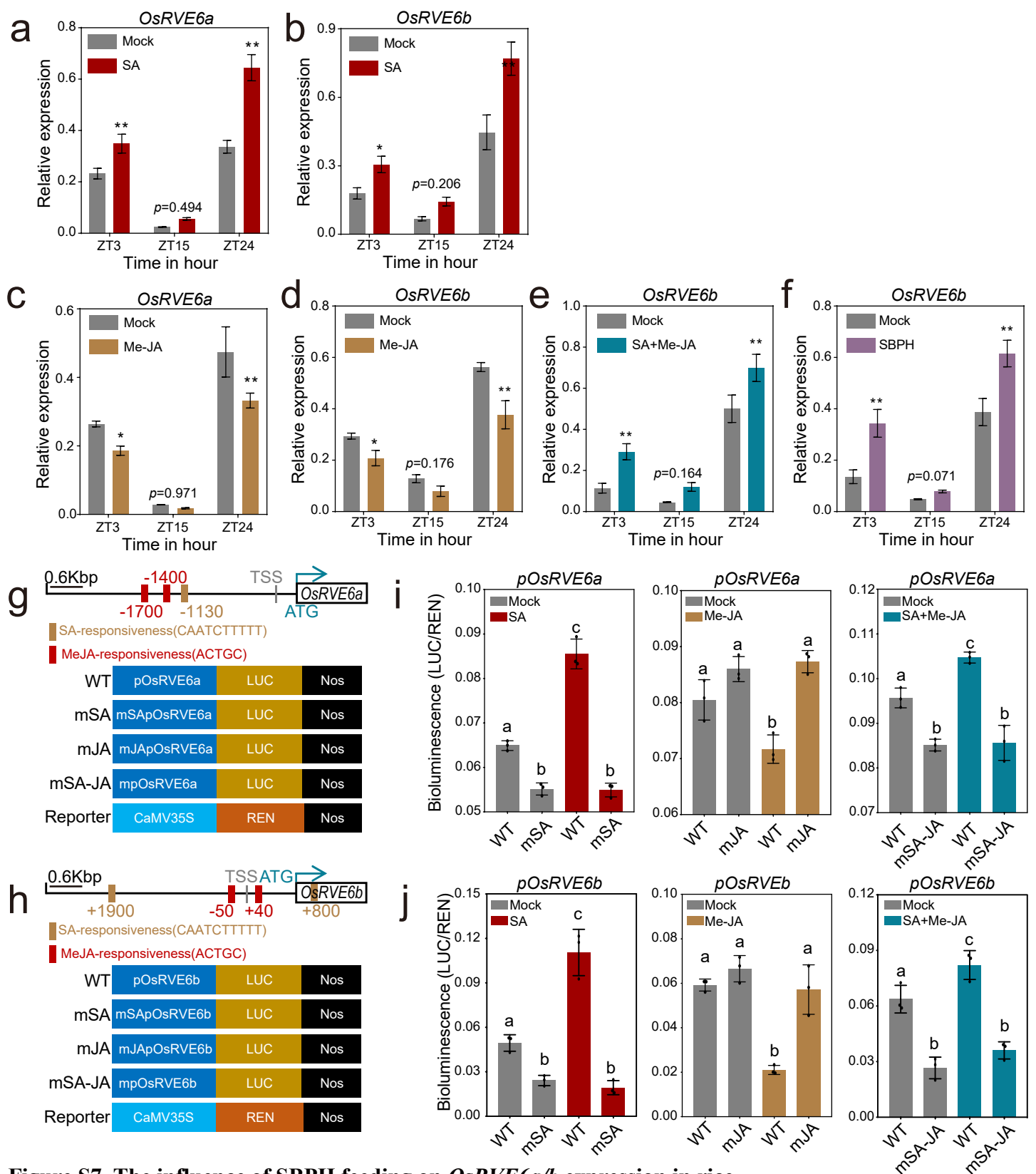
**(a)** Expression of *OsNPR1*, *OsPAL1*, *OsCOI1a* and *OsLOX2* in WT, *OsRve6a-2* and *OsRVE6a-ox2* seedlings were quantified by qPCR at ZT3 and ZT15. Data are presented as mean  $\pm$  SD ( $n=3$ , biological replicates), Statistical analysis was conducted using two-way ANOVA with post hoc Tukey's honest significant difference (HSD) test, Significant difference between samples are shown by different letters ( $p < 0.05$ ). **(b)** Sequencing analysis of two independent *Osnpr1-1/2* mutants generated by CRISPR/Cas9 technology in *Nipponbare* background. **(c)** and **(d)** Time course analysis of *OsPDF1.2* and *OsWRKY20* expression in *Osnpr1-1/2* and WT seedlings under LD conditions. Data are presented as mean  $\pm$  SD ( $n=3$ , biological replicates), Statistical analysis was conducted using two-way ANOVA with post hoc Tukey's honest significant difference (HSD) test, Significant difference between samples are shown by asterisks ( $*p < 0.05$ ,  $**p < 0.01$ ). **(e)** Comparison of *OsWRKY20* expression between Taipei309 and the *OsNPR1* overexpression line (*OsNPR1-ox*) from previously published microarray data. Data are presented as mean  $\pm$  SD ( $n=3$ , biological replicates), Statistical analysis was performed using two-sided paired Student's t-test, with significant differences indicated by asterisks ( $*p < 0.05$ ,  $**p < 0.01$ ). **(f)** *OsWRKY20* loss-of-function mutants *Oswrky20-1/2* were generated in *Nipponbare* background by CRISPR/Cas9 technology. **(g)** Morphology of *Oswrky20-1/2* mutants and WT under SBPH treatment for 14 days (Scale bar, 10 cm). **(h)** The plant survival rate of *Oswrky20-1/2* mutants and WT seedling evaluated under SBPH treatment ( $n=5$ , biological replicates). **(i)** Bacterial pathogen resistance phenotypes of *Oswrky20-1/2* mutants and WT, Plants were infected by scissors dipped in *Xanthomonas oryzae* (Xoo) solution at the tiller stage (Scale bar, 1 cm). **(j)** Mean lesion ratio (lesion/full leaf length) of *Oswrky20-1/2* mutants and WT ( $n=5$ , biological replicates). **(h)** and **(j)** Data are presented as mean  $\pm$  SD, Statistical analysis was conducted using one-way ANOVA with post hoc Tukey's honest significant difference (HSD) test, Significant difference between samples are shown by different letters ( $p < 0.05$ ).

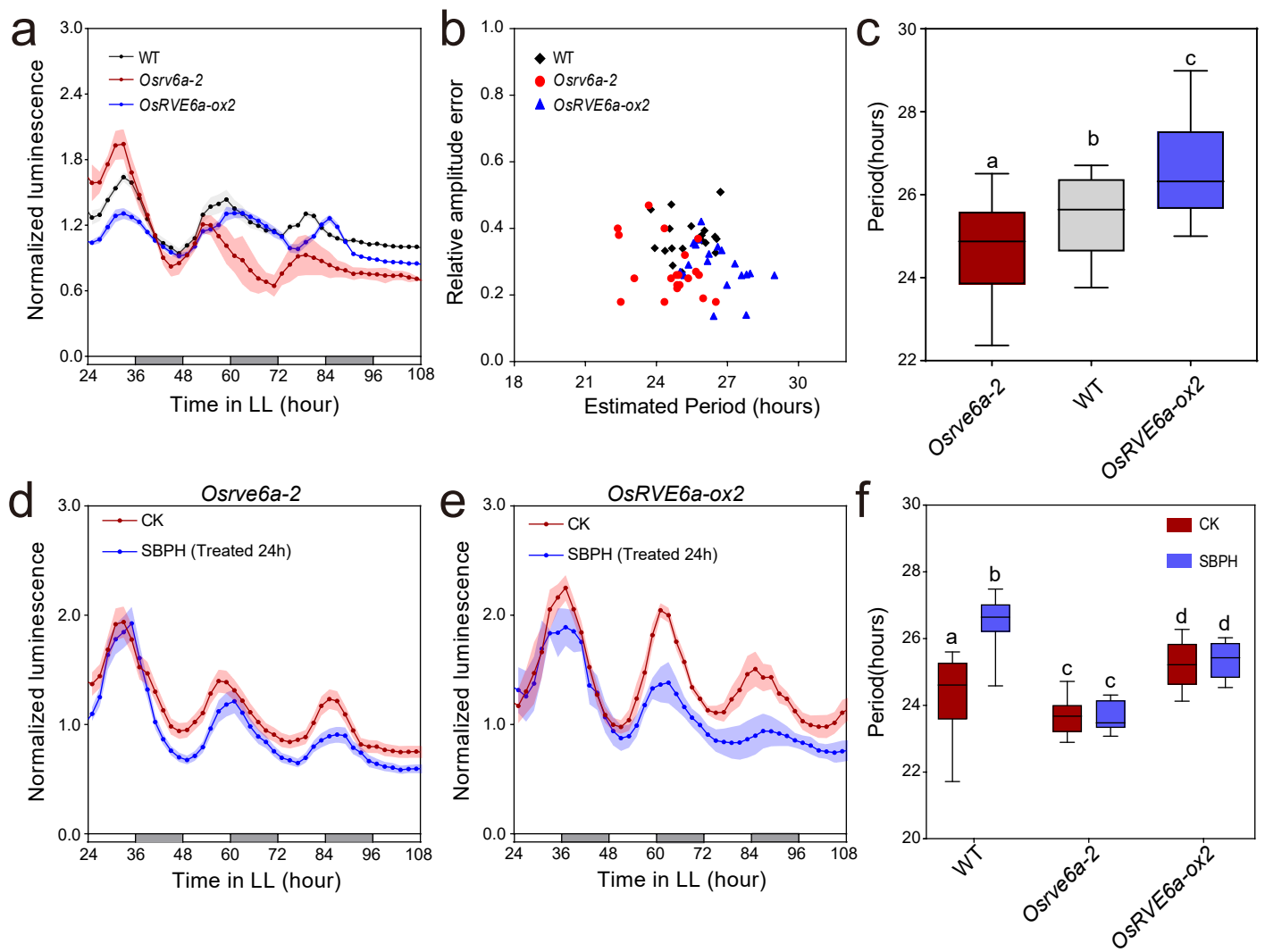




**Figure S6. *OsRVE6a* acts upstream of *OsWRKY20* to regulate rice resistance to SBPH**

(a) Images of WT, *Osrve6a*, *Osrwky20* single mutants and *Osrve6a Osrwky20* double mutants with and without SBPH treatment (Control check, CK) for 14 days (Scale bar, 10 cm). (b) Comparison of chlorophyll content among WT, *Osrve6a*, *Osrwky20* single mutants, and *Osrve6a Osrwky20* double mutants under SBPH treatment, The chlorophyll content ratio of SBPH/control. (c) and (d) Morphology of plants under SBPH treatment, including WT, *Osrve6a* and overexpression of *OsWRKY20* in *Osrve6a-1* mutant background (*OsWRKY20-ox/Osrve6a*) (Scale bar, 10 cm), Chlorophyll content ratio of SBPH/CK. (e) The plant survival rate of WT, *Osrve6a* and *OsWRKY20-ox/Osrve6a* seedlings evaluated under SBPH treatment. (f) and (g) Measurement of honeydew secreted by SBPH feeding on WT, *Osrve6a* and *OsWRKY20-ox/Osrve6a* seedlings, Honeydew collected onto filter papers, filter papers were soaked with 0.1% (w/v) ninhydrin in acetone solution. the amino acid contents of honeydew were stained until purple blots appeared (Scale bar, 1.5 cm). Honeydew secreted by SBPH were weighed. (h) and (i) Examination of SBPH feeding sites on stems of WT, *Osrve6a*, and *OsWRKY20-ox/Osrve6a* seedlings via trypan blue staining (Scale bar, 2 mm). (b), (d), (e), (g) and (i) Data are presented as mean  $\pm$  SD (n=6, biological replicates), Statistical analysis was conducted using one-way ANOVA with post hoc Tukey's honest significant difference (HSD) test, Significant difference between samples are shown by different letters ( $p < 0.05$ ).

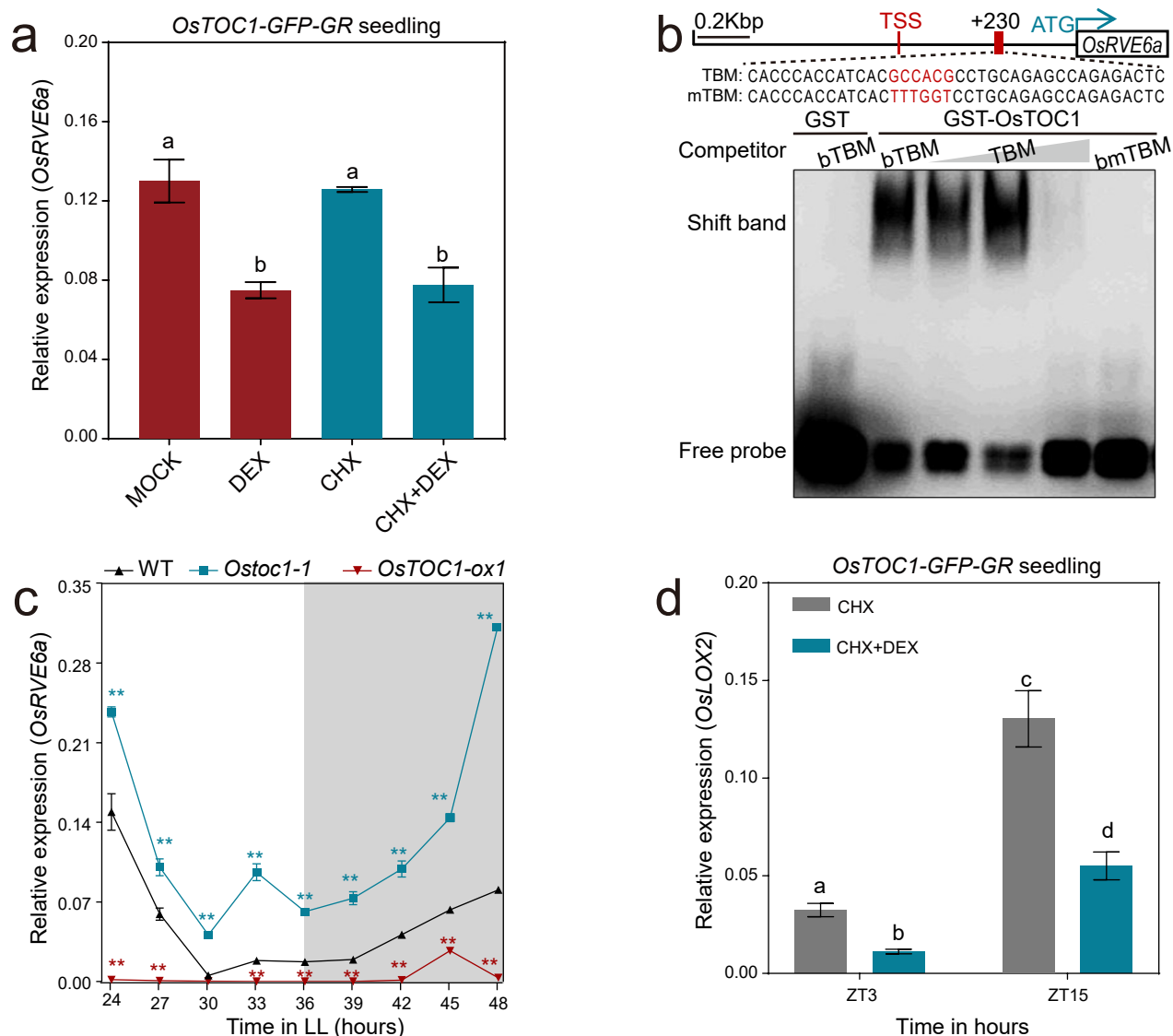




**Figure S8. SBPH feeding behavior influences rice circadian rhythm through OsRVE6a**

**(a)** Analysis of circadian rhythms of *CABR: LUC* in the WT, *Osrve6a-2* and *OsRVE6a-ox2* seedlings under LL conditions ( $n > 18$ , biological replicates). Shaded areas show the standard deviation. **(b)** Periods and relative amplitude error values of individual transgenic seedlings ( $n > 16$ , biological replicates). **(c)** Analysis of periods among different transgenic lines analyzed by FFT-NLLS algorithm. Data are presented as mean  $\pm$  SD ( $n > 16$ , biological replicates). Statistical analysis was performed using one-way ANOVA with post hoc Tukey's honest significant difference (HSD) test. Significant differences between samples are shown by distinct letters ( $p < 0.05$ ). **(d)-(e)** Circadian rhythms of *CABR: LUC* in the WT, *Osrve6a-2* and *OsRVE6a-ox2* seedlings under SBPH treatment. Shaded areas represent the standard deviation ( $n > 16$ , biological replicates). **(f)** Periods of different transgenic lines analyzed by FFT-NLLS algorithm. Data are presented as mean  $\pm$  SD. Statistical analysis was conducted using two-way ANOVA with post hoc Tukey's honest significant difference (HSD) test. Significant difference between samples are shown by different letters ( $p < 0.05$ ).





### Figure S9. OsRVE6 may indirectly regulate nocturnal signaling of SA and JA through OsTOC1

**(A)** The expression of *OsRVE6a* in *OsTOC1-GFP-GR1* seedlings treated with mock or DEX in the presence or absence of CHX at ZT10, and leaves were collected at ZT15. Data are presented as mean  $\pm$  SD (n=3, biological replicates), Statistical analysis was conducted using one-way ANOVA with post hoc Tukey's honest significant difference (HSD) test, Significant difference between samples are shown by different letters ( $p < 0.05$ ). **(B)** Verification of OsTOC1 interact with *OsRVE6a* promoter using EMSA, the competitors contain OsTOC1 binding motif (TBM) in *OsRVE6a* promoter, bTBM represents a biotin-labeled probes and bmTBM represents a biotin-labeled mutant probes. **(C)** Time course analysis of *OsRVE6a* expression in WT, *Ostoc1-1* and *OsTOC1-ox1* seedlings under LL condition. Data are presented as mean  $\pm$  SD (n=3, biological replicates), Statistical analysis was conducted using two-way ANOVA with post hoc Tukey's honest significant difference (HSD) test, Significant difference between samples are shown by asterisks (\* $p < 0.05$ , \*\* $p < 0.01$ ). **(D)** Expression of *OsLOX2* in *OsTOC1-GFP-GR1* treated with mock or DEX in the presence or absence of CHX at 2 hours before ZT0. Leaves were collected at ZT3 and ZT15. The expression of *OsLOX2* was quantified using qPCR. Data are presented as mean  $\pm$  SD (n=3, biological replicates). Statistical analysis was performed using one-way ANOVA with post hoc Tukey's honest significant difference (HSD) test. Significant differences between samples are shown by distinct letters ( $p < 0.05$ ).