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Supporting Information for

Sakuranetin protects rice from brown planthopper attack by depleting its beneficial endosymbionts

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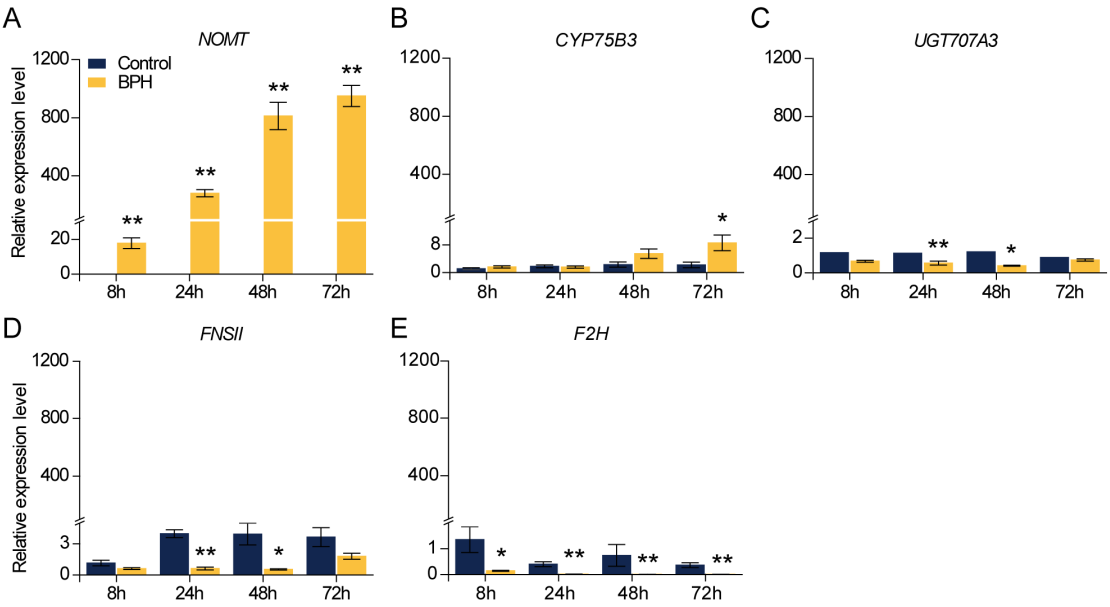

Figure S1 Transcript levels of flavonoid biosynthetic genes in BPH-attacked and control plants

Mean transcript levels (± SE, n = 5) of *NOMT* (A), *CYP75B3* (B), *UGT707A3*(C), *FNSII* (D), *F2H* (E) in BPH-treated plants and control plants. Asterisks indicate significant differences between treatments and controls (*, $P<0.05$; **, $P<0.01$; Student's t-test).

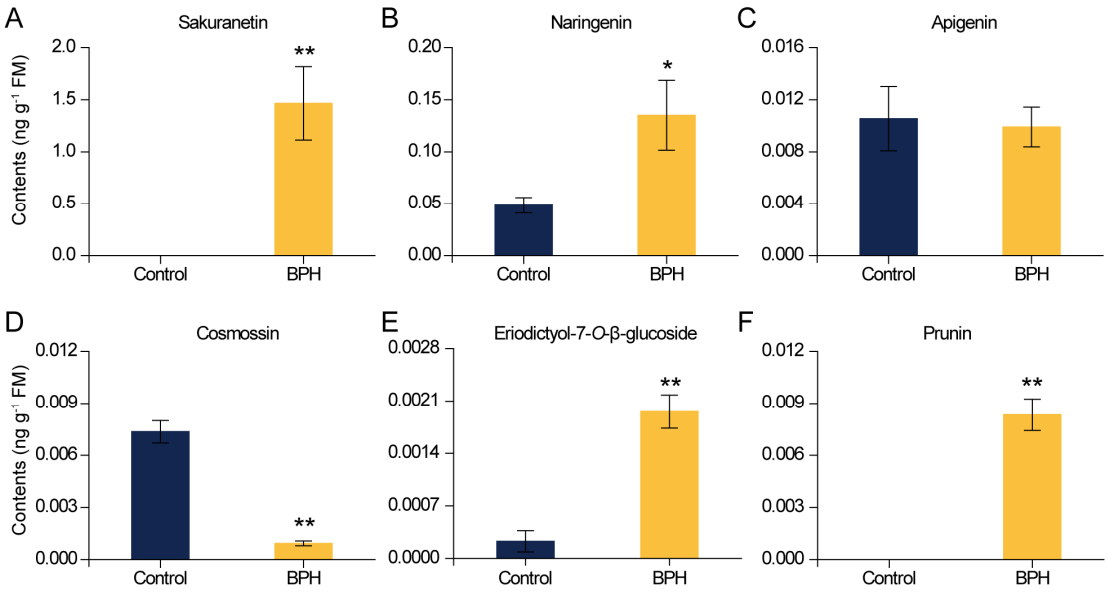

Figure S2 Concentrations of flavonoids in the phloem sap of BPH-attacked and control plants

Mean concentrations (± SE, n = 5) of sakuranetin (A), naringenin (B), apigenin (C), cosmossin (D), eriodictyol-7-*O*-β-glucoside (E), and prunin (F) in the phloem sap of BPH-attacked and control plants. Asterisks indicate significant differences between treatments and controls (*, $P < 0.05$; **, $P < 0.01$; Student's t-test).

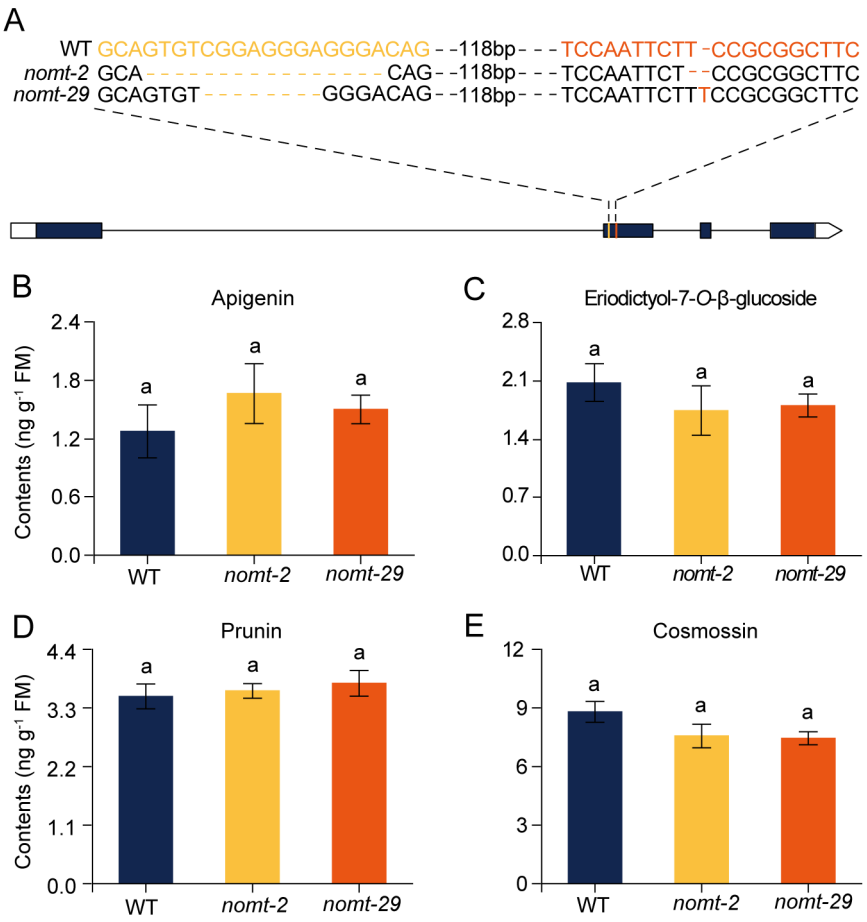

Figure S3 Flavonoids and transcript levels of their biosynthetic genes in *nomt* mutants and WT plants

(A) Mutation of the *NOMT* gene by CRISPR-Cas9-based genome editing. Two target sequences of single-guide RNAs are highlighted in yellow and red fonts. "-" indicates deletions. Mean concentrations (± SE, n = 5) of apigenin (B), eriodictyol-7-*O*-β-glucoside (C), prunin (D) and cosmossin (E) in *nomt* mutants and WT plants at 48 h after BPH infestation. Letters indicate significant differences among *nomt* mutants and WT plants ($P < 0.05$, one-way ANOVA followed by LSD test).

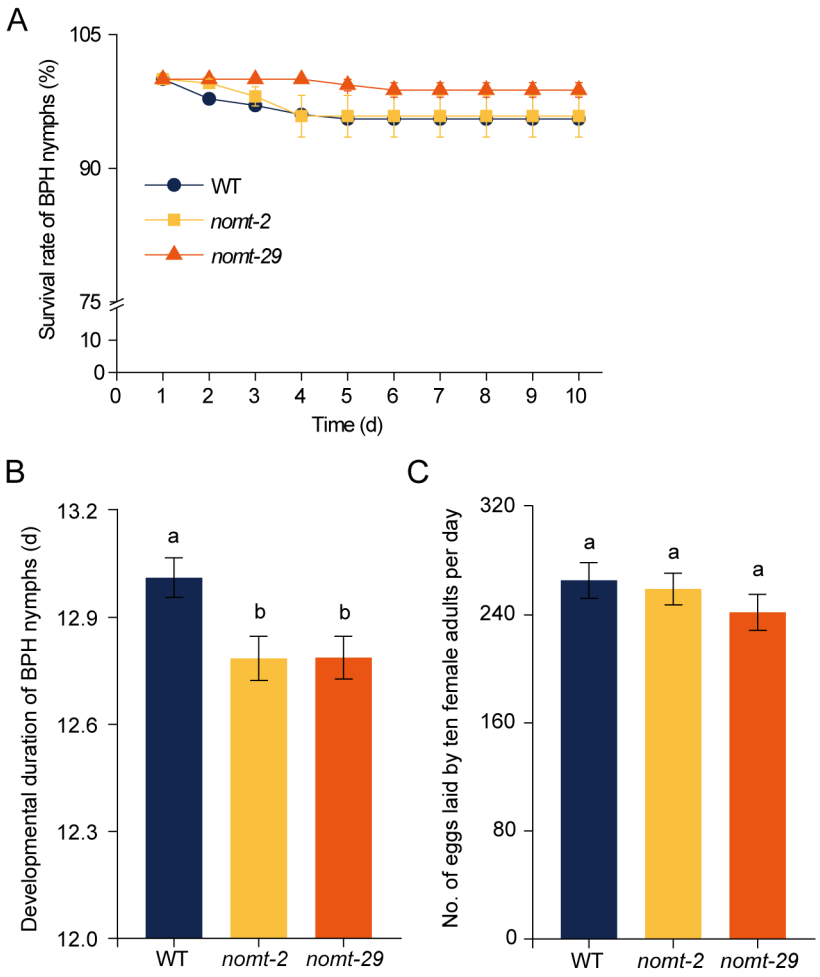

Figure S4 BPH performance on *nomt* mutants and WT plants

(A) Mean survival rate (± SE, n = 14) of fifteen newly hatched BPH nymphs fed on *nomt* mutants and WT plants. Fifteen newly hatched BPH nymphs were allowed to feed on each plant. (B) Mean developmental durations (± SE, n = 14) of BPH nymphs that fed on *nomt* mutants and WT plants. (C) Mean number (± SE, n = 12) of eggs laid by ten female adults per day on *nomt* mutants and WT plants. Ten gravid BPH females were allowed to oviposit on each plant for 24 h. Letters indicate significant differences among *nomt* mutants and WT plants ($P < 0.05$, one-way ANOVA followed by LSD test).

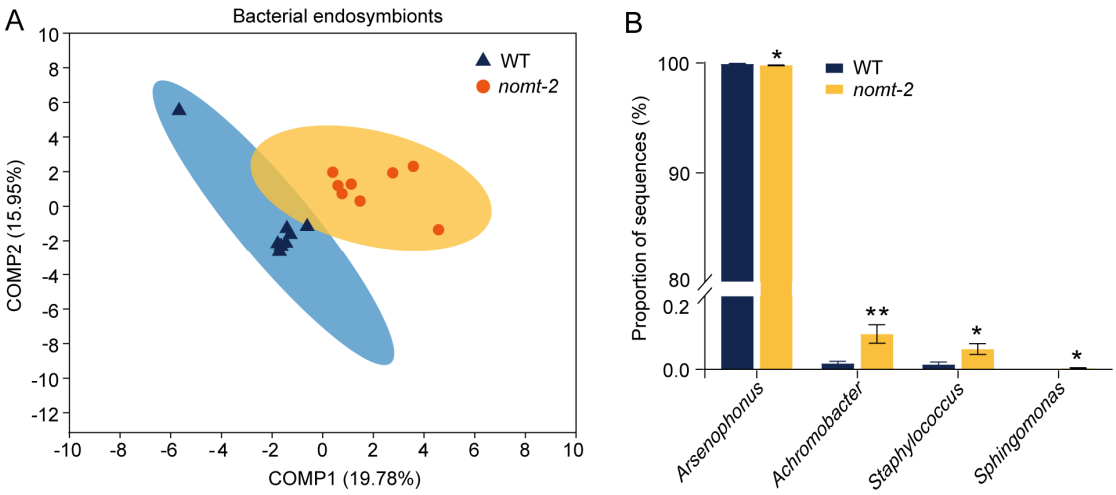

Figure S5 Differences in bacterial endosymbionts of BPH fed on *nomt* mutants and WT plants

(A) Partial least squares discriminant analysis (PLS-DA) of bacterial endosymbionts in BPHs fed on *nomt* mutants and WT plants. Eight biological replicates were used. (B) The differentially enriched bacteria at the genus level in BPHs fed on *nomt* mutants compared with that on WT plants (± SE, n = 8). Asterisks indicate significant differences between BPHs fed on *nomt* mutants and WT plants (*, $P < 0.05$, Wilcoxon rank-sum test).

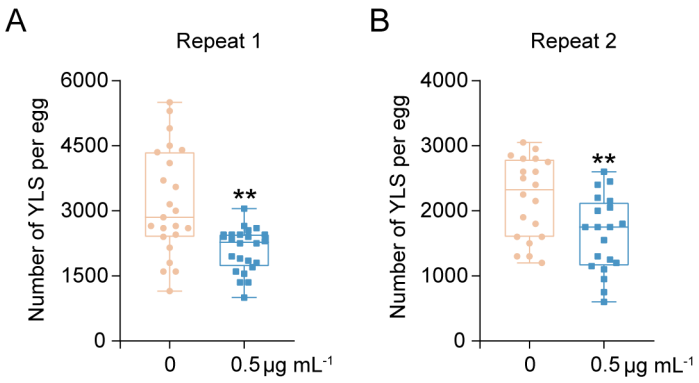

Figure S6 The effect of sakuranetin on YLS of fresh BPH eggs

Mean number (± SE, n = 20-23) of YLS in intact BPH egg placed on the filter paper socked with 0.5 μg mL^{-1} sakuranetin solution and control solution after 4 d. The fresh BPH eggs (within 3 h after oviposition) were used. The YLS in normal developing eggs (with embryonic red eye spots) were counted. The assays were repeated two times. Asterisks indicate significant differences between different treatments (**, $P < 0.01$; Student's t-test).

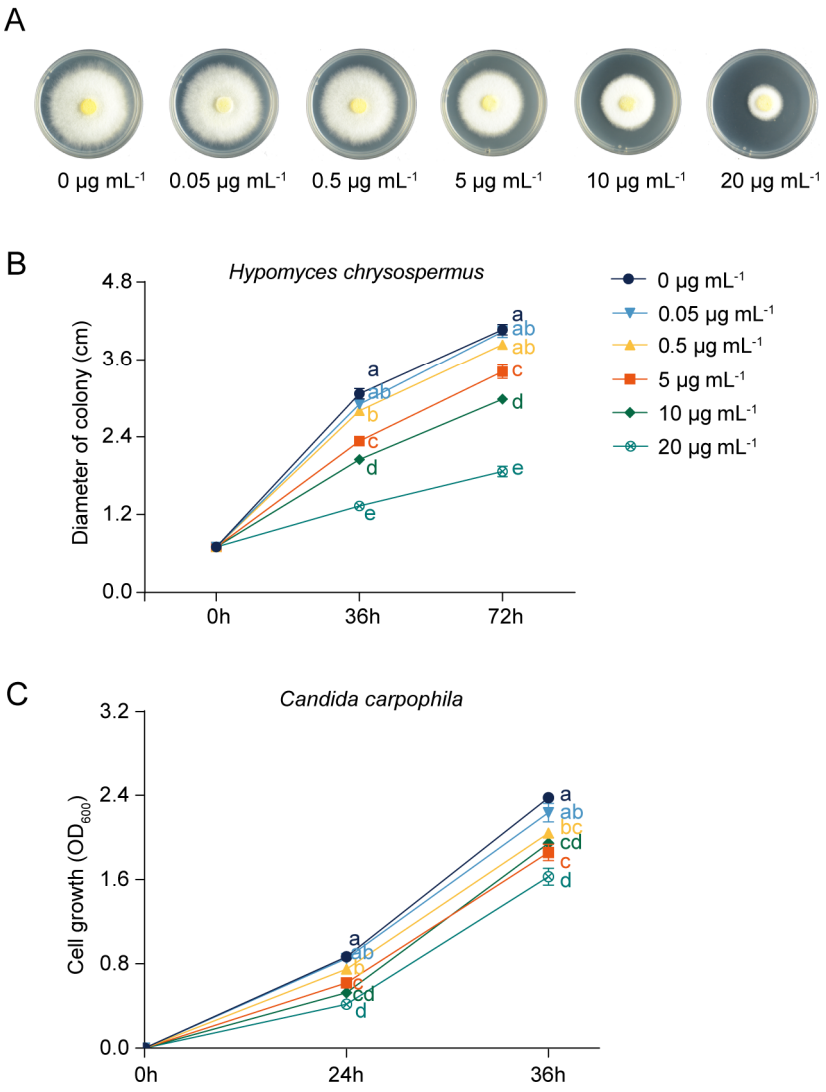

Figure S7 Anti-fungal activity of sakuranetin on *Hypomyces chrysospermus* and *Candida carpophila*

Growth (A) and mean (± SE, n = 6) diameter (B) of mycelium colonies of *H. chrysospermus* on PDA containing different concentrations of sakuranetin (from 0 to 20 μg mL^{-1}) at 72 h post-inoculation. Petri dish diameter is 6 cm. (C) The effects of sakuranetin on the growth of *C. carpophila*. Sakuranetin was added to liquid cultures of *C. carpophila* at different concentrations (from 0 to 20 μg mL^{-1}), and their growth was evaluated by measuring OD_{600} at 24 and 36 h after cultivation (± SE, n = 8). Asterisks indicate significant differences between sakuranetin treatments and controls ($P < 0.05$; one-way ANOVA followed by Tukey HSD post-hoc test).

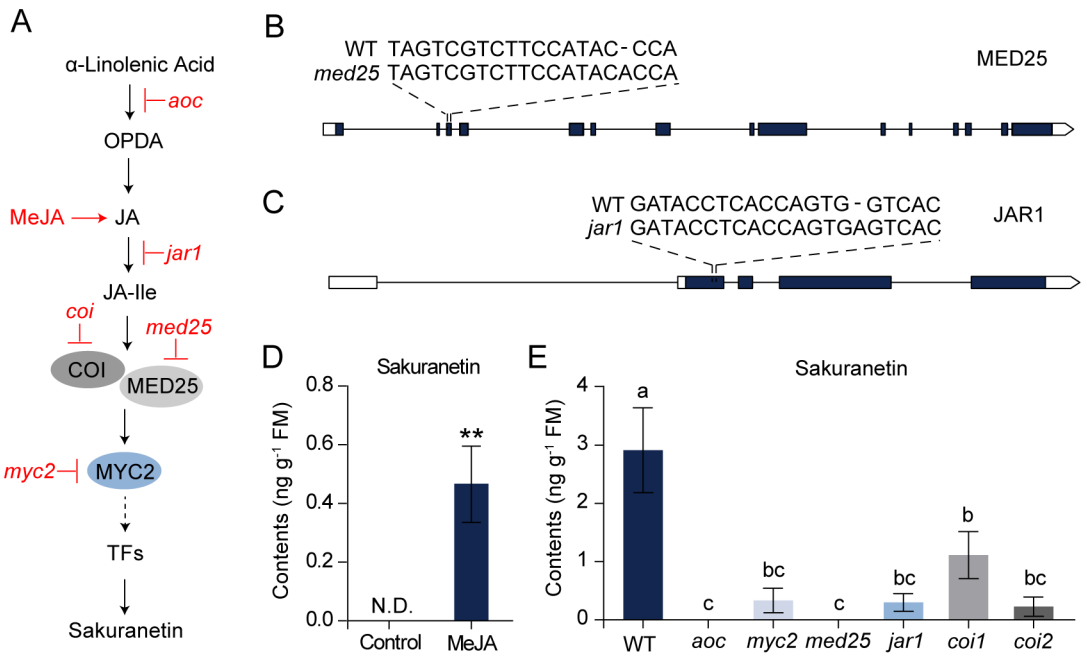

Figure S8 BPH-elicited sakuranetin biosynthesis is regulated by JA signaling

(A) Perturbations (shown in red) of JA signaling including exogenous MeJA treatment and mutations in JA biosynthetic genes (*AOC*, *JAR1*) and signaling genes (*COI*, *MED25*, *MYC2*). Mutation of *MED25* (B) and *JAR1* gene (C) by CRISPR-Cas9 based genome editing. "-" indicates deletions. (D) Mean sakuranetin concentrations (± SE, n = 6) in leaf sheaths of MeJA-treated plants and control plants. N.D., not detected. Asterisks indicate significant differences between treatments and controls (**, $P < 0.01$; Student's t-test). (E) Mean sakuranetin levels (± SE, n = 5) in JA-deficient and WT plants. Letters indicate significant differences among different concentrations of sakuranetin treatments ($P < 0.05$, one-way ANOVA followed by LSD test).

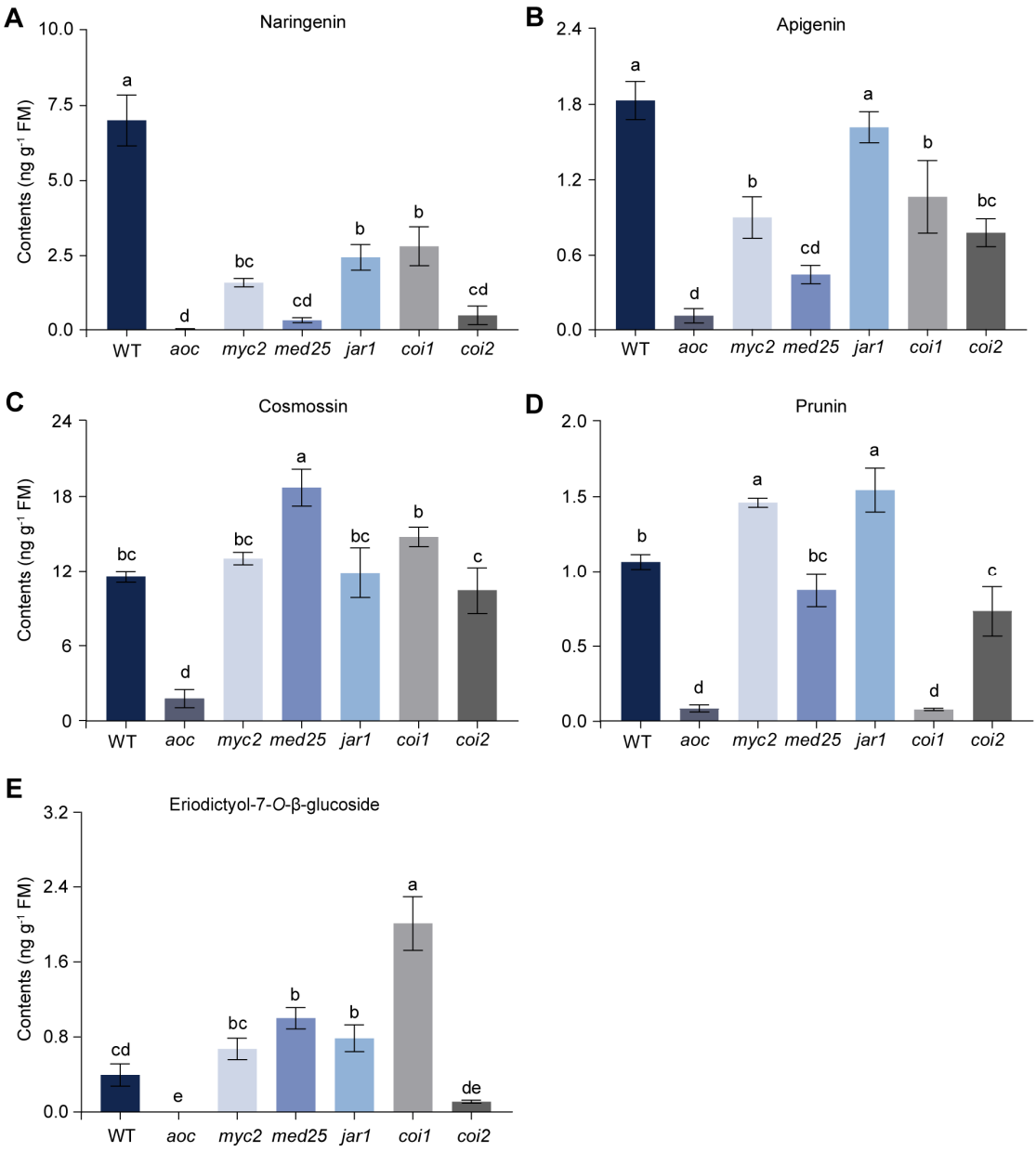

Figure S9 Concentrations of flavonoids in JA-deficient lines and WT plants

Mean concentrations (± SE, n = 5) of naringenin (A), apigenin (B), cosmossin (C), prunin (D) and eriodictyol-7-*O*-β-glucoside (E) in JA-deficient lines and WT plants. Letters indicate significant differences among JA-deficient lines and WT plants ($P < 0.05$, one-way ANOVA followed by LSD test).

Table S1. Primers used in this study.

Gene	Sequence (5'-3')	Purpose
NOMT-RT-F	CTACCTACATCTTCACCAACGT	RT-qPCR
NOMT-RT-R	GAGACTGAGAAGAGGAAACGAA	RT-qPCR
F2H-RT-F	CGCTTGTGCAGTGCTTTGACTG	RT-qPCR
F2H-RT-R	AGTAGAAGGAAGGGAGCGGTTG	RT-qPCR
CYP75B3-RT-F	AACGACCTTCTAAGCGTGCTG	RT-qPCR
CYP75B3-RT-R	CCGCAGTGAATAGGTTCAGGAG	RT-qPCR
UGT707A3-RT-	TGGAGTTCGAGGAGATGGA	RT-qPCR
UGT707A3-RT-	AACCACGTGTAGTTCGGGTT	RT-qPCR
FNS II-RT-F	CAGTGCTTCGATTGGCAGT	RT-qPCR
FNS II-RT-R	GCGTTACAGGGACAGGAAAG	RT-qPCR
Ubi-RT-F	AACCAGCTGAGGCCCAAGA	RT-qPCR
Ubi-RT-R	ACGATTGATTTAACCAGTCCATGA	RT-qPCR
16S-338F	ACTCCTACGGGAGGCAGCAG	16S rRNA sequencing
16S-806R	GGACTACHVGGGTWTCTAAT	16S rRNA sequencing
ITS-1F	CTTGGTCATTTAGAGGAAGTAA	ITS sequencing
ITS-2R	GCTGCGTTCTTCATCGATGC	ITS sequencing
Ascomycetes symbionts-RT-F	CGTAGGGAGAGCAGCAAAC	RT-qPCR
Ascomycetes symbionts-RT-R	CGATGCCAGAGCCAAGAG	RT-qPCR
Candida carpophila-RT-F	TTGGCTGCAAAAAGGTCGTG	RT-qPCR

Candida carpophila-RT-R	CTACCCTCCTACCACTCTACC	RT-qPCR
Actin-RT-F	GATGAGGCGCAGTCAAAGAG	RT-qPCR
Actin-RT-R	GTCATCTTCTCACGGTTGGC	RT-qPCR