Supplemental Data

A

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NaAOX1          -MMIRGATRMTRTVMGHMGPRYFSTAILRNDAGTGVMTGAAGFMHGVPVNPSEKVLW--- 56
NaAOX2          -MMIRGATRMTRTVMGHMGPRYFSTAILRNDAGTGVMTGAAGFMHGVPVNPSEKALVTWV 59
AtAOX1a         MMITRGGAKAAKSLLVAAGPRLFST--VRTVSSHEALSASHILKPGVTS------AWIWT 52
                 *: **.:: :::::   *** ***  :*. :.  .::.:  :  **.

NaAOX1          -HIPVMGSRSASTMALNDKQ-------HDKKVENGGT-AASGGGDGGDEKSVVSYWGVPP 107
NaAOX2          RHIPVMGSRSASTMALNDKQ-------HDKKVENGGT-AASGGGDGGDEKSVVSYWGVPP 111
AtAOX1a         RAPTIGGMRFASTITLGEKTPMKEEDANQKKTENESTGGDAAGGNNKGDKGIASYWGVEP 112
                   .: * * ***::*.:*        ::**.** .* . :.**:. .:*.:.***** *

NaAOX1          SKVTKEDGTEWKWNCFRPWETYKADLTIDLTKHHAPTTFLDKFAYWTVKALRYPTDIFFQ 167
NaAOX2          SKVTKEDGTEWKWNCFRPWETYKADLTIDLTKHHAPTTFLDKFAYWTVKALRYPTDIFFQ 171
AtAOX1a         NKITKEDGSEWKWNCFRPWETYKADITIDLKKHHVPTTFLDRIAYWTVKSLRWPTDLFFQ 172
                .*:*****:****************:****.***.******::******:**:***:***

NaAOX1          RRYGCRAMMLETVAAVPGMVGGMLLHCKSLRRFEQSGGWIKALLEEAENERMHLMTFMEV 227
NaAOX2          RRYGCRAMMLETVAAVPGMVGGMLLHCKSLRRFEQSGGWIKALLEEAENERMHLMTFMEV 231
AtAOX1a         RRYGCRAMMLETVAAVPGMVGGMLLHCKSLRRFEQSGGWIKALLEEAENERMHLMTFMEV 232
                ************************************************************

NaAOX1          AKPNWYERALVFAVQGVFFNAYFVTYLVSPKLAHRIVGYLEEEAIHSYTEFLKELDKGNI 287
NaAOX2          AKPNWYERALVFAVQGVFFNAYFVTYLVSPKLAHRIVGYLEEEAIHSYTEFLKELDKGNI 291
AtAOX1a         AKPKWYERALVITVQGVFFNAYFLGYLISPKFAHRMVGYLEEEAIHSYTEFLKELDKGNI 292
                ***:*******::***********: **:***:***:************************

NaAOX1          ENVPAPAIAIDYWRLPKDSTLRDVVLVVRADEAHHRDVNHFASDIHYQGQQLKDSPAPIG 347
NaAOX2          ENVPAPAIAIDYWRLPKDSTLRDVVVVVRADEAHHRDVNHFASDIHYQGQQLKDSPAPIG 351
AtAOX1a         ENVPAPAIAIDYWRLPADATLRDVVMVVRADEAHHRDVNHFASDIHYQGRELKEAPAPIG 352
                **************** *:******:************************::**::*****

NaAOX1          YH 349
NaAOX2          YH 353
AtAOX1a         YH 354
                **
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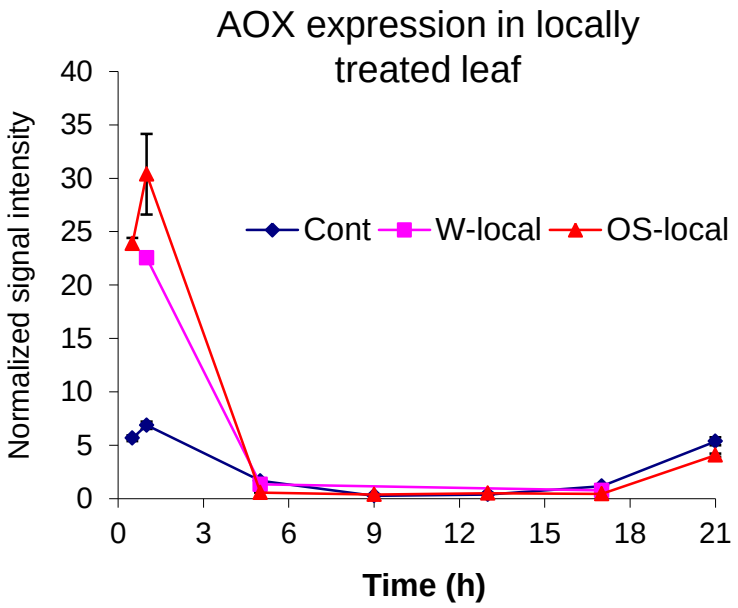
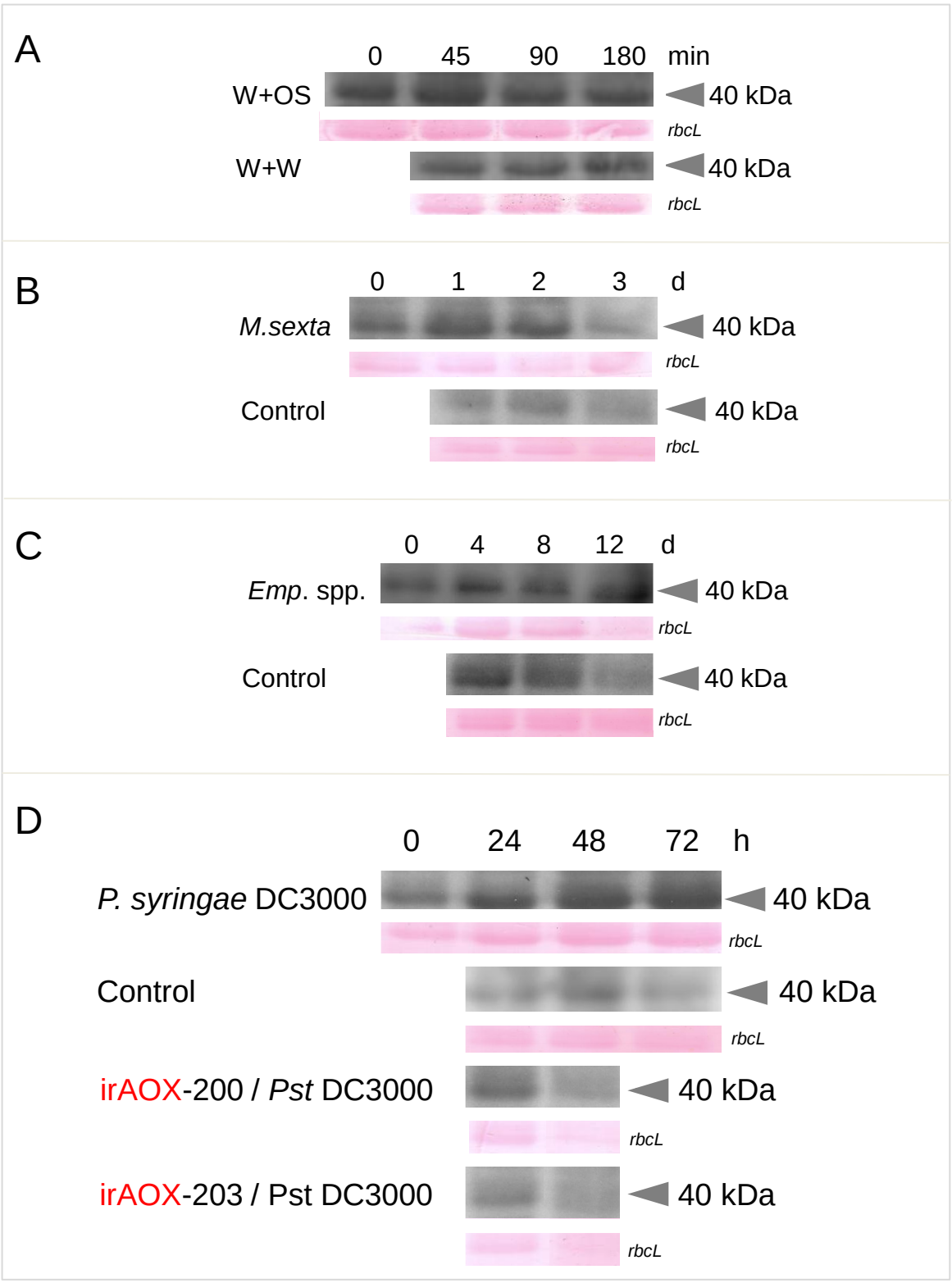
B



Supplemental Figure S1

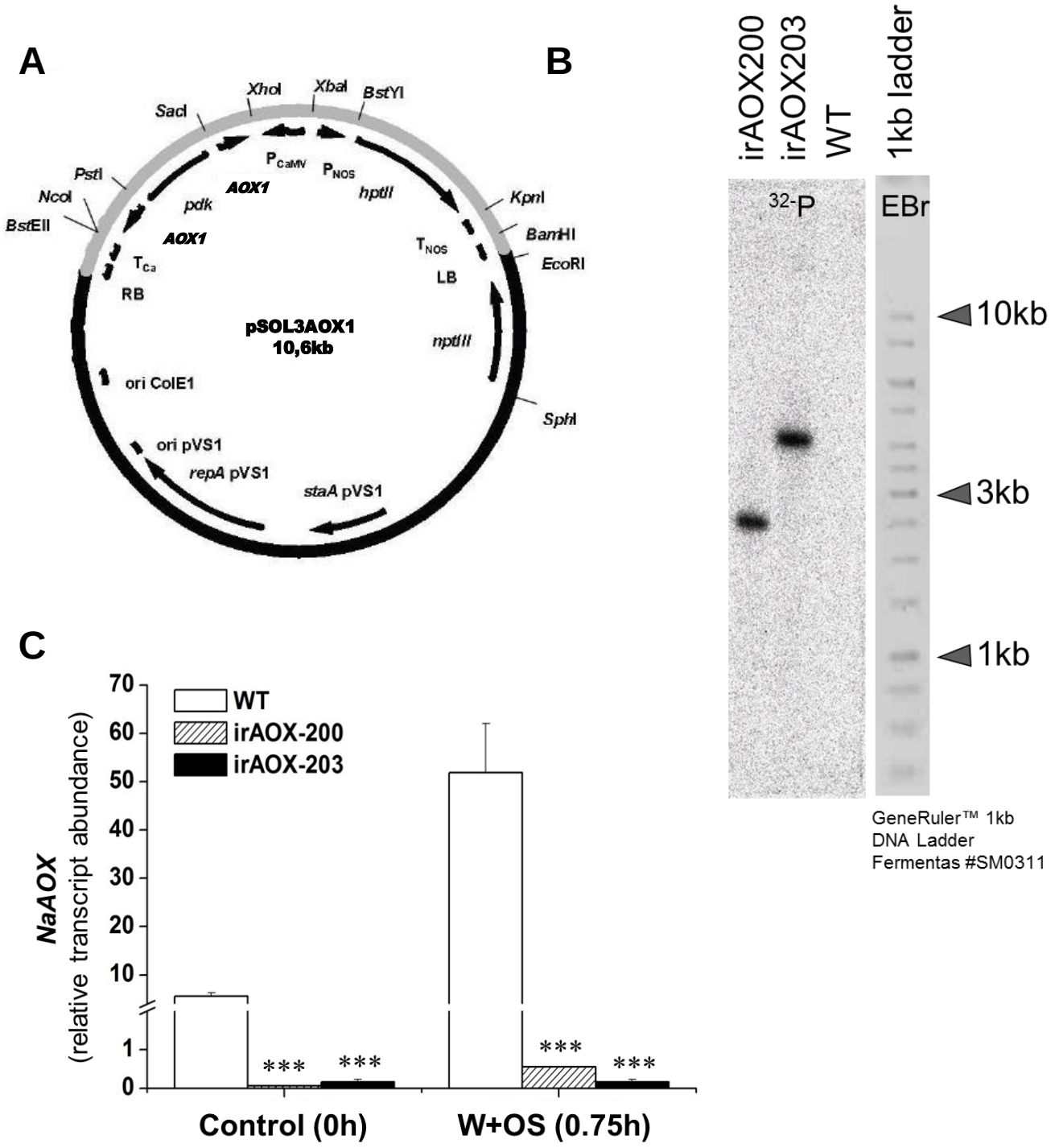
AOX genes from *N. attenuata* are highly homologous to AOX1a gene from *Arabidopsis thaliana*. (A) Deduced NaAOX1 (AY422688) and NaAOX2 (AY422689) protein sequences were aligned with AtAOX1a protein from *A. thaliana* (AT3G22370.1). Asterisks show conserved amino acids in all three proteins. (B) The accumulation of AOX transcripts in local treated leaves was determined by microarrays (*n=3*) after elicitation of the leaves with wounding (W+W) or simulated herbivory (W+OS); control plants remained untreated. Control and W+OS samples were harvested at 0.5, 1, 5, 9, 12, 17 and 21 h post treatment; samples from W+W treatment were collected at 1, 5 and 17 h post treatment.

S2



Supplemental Figure S2

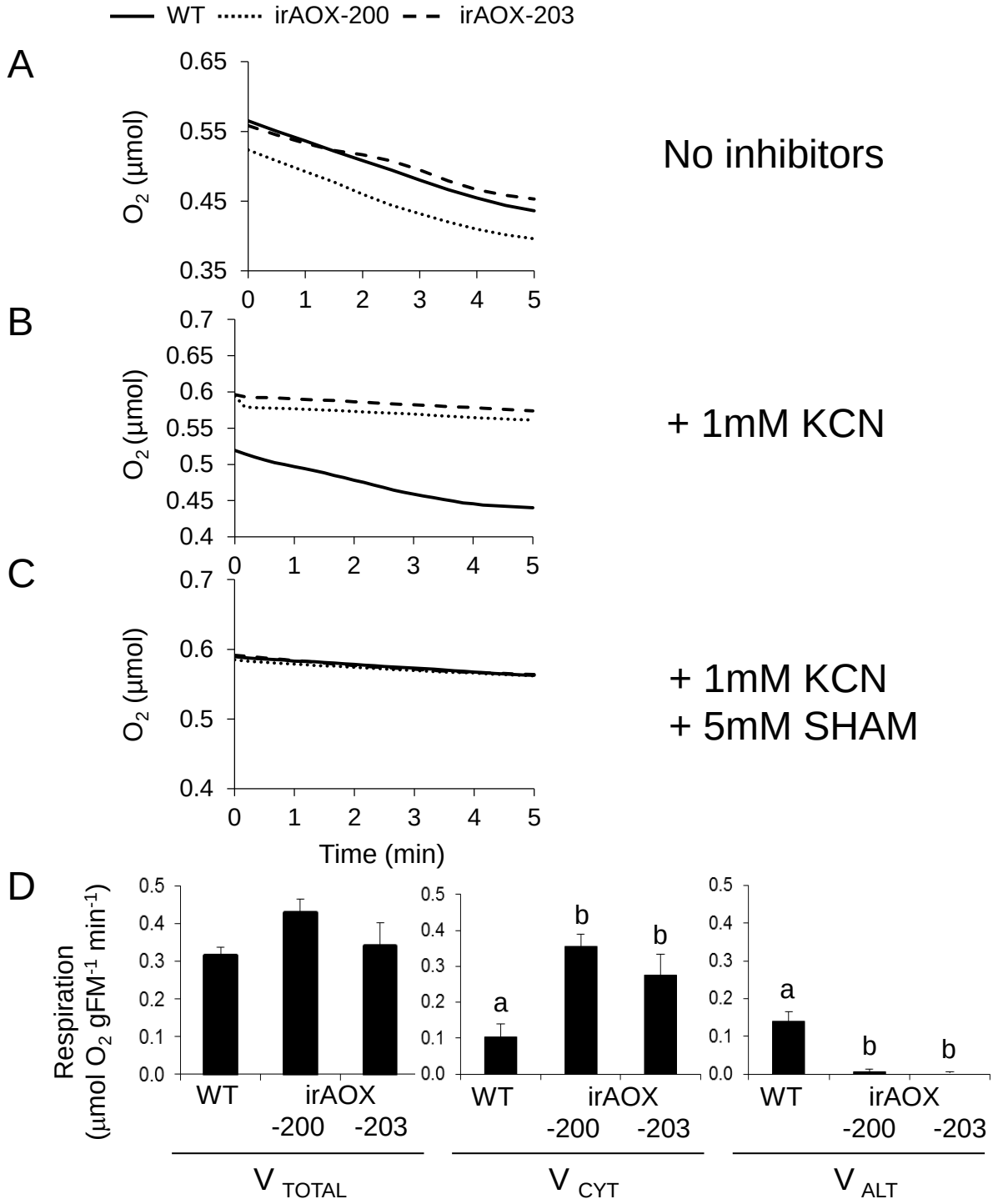
***NaAOX* proteins increase in response to simulated herbivory, feeding of *M. sexta* caterpillars, *Empoasca* spp. attack and *P. syringae* DC3000 infection in *N. attenuata*.** (A) NaAOX protein levels after simulated herbivory as shown in Fig. 1A determined by western blotting with specific antibody raised against conserved AOX protein peptide sequence; (B) AOX levels after *M. sexta* caterpillar feeding as in Fig. 1B; AOX levels after *Empoasca* spp. attack as in Fig. 4; and (D) AOX levels during *Pst* infection as in Fig. 7. *rbcL* shows the RuBisCo large subunit (≈50 kDa) in a Ponceau S-stained membrane prior to western blotting (reddish pink stain).



Supplemental Figure S3

Structure of plant transformation vector pSOL3AOX used for silencing of *AOX* genes in *N. attenuata* and characterization of irAOX transgenic lines. (A) A 511bp of *NaAOX1* gene (position 338-848 in AY422688) was inserted into pSOL3 vector as an inverted-repeat construct with *hptII* used as plant selection marker gene. (B) Two *Agrobacterium*-transformed lines irAOX-200 and irAOX-203 were subjected to Southern blot analysis using genomic DNA digested with XbaI restriction enzyme and hptII-radiolabeled probe. Both lines showed single insertion of T-DNA fragment into the genome. (C) Mean (± SE) levels of NaAOX transcripts in unelicited and 0.75h W+OS-elicited leaves of two independently transformed homozygous irAOX lines and wild-type plants. Asterisks indicate significant differences between WT and individual irAOX lines (one-way ANOVA, *** P<0.001; *n=5*). Note the break in the Y-axis.

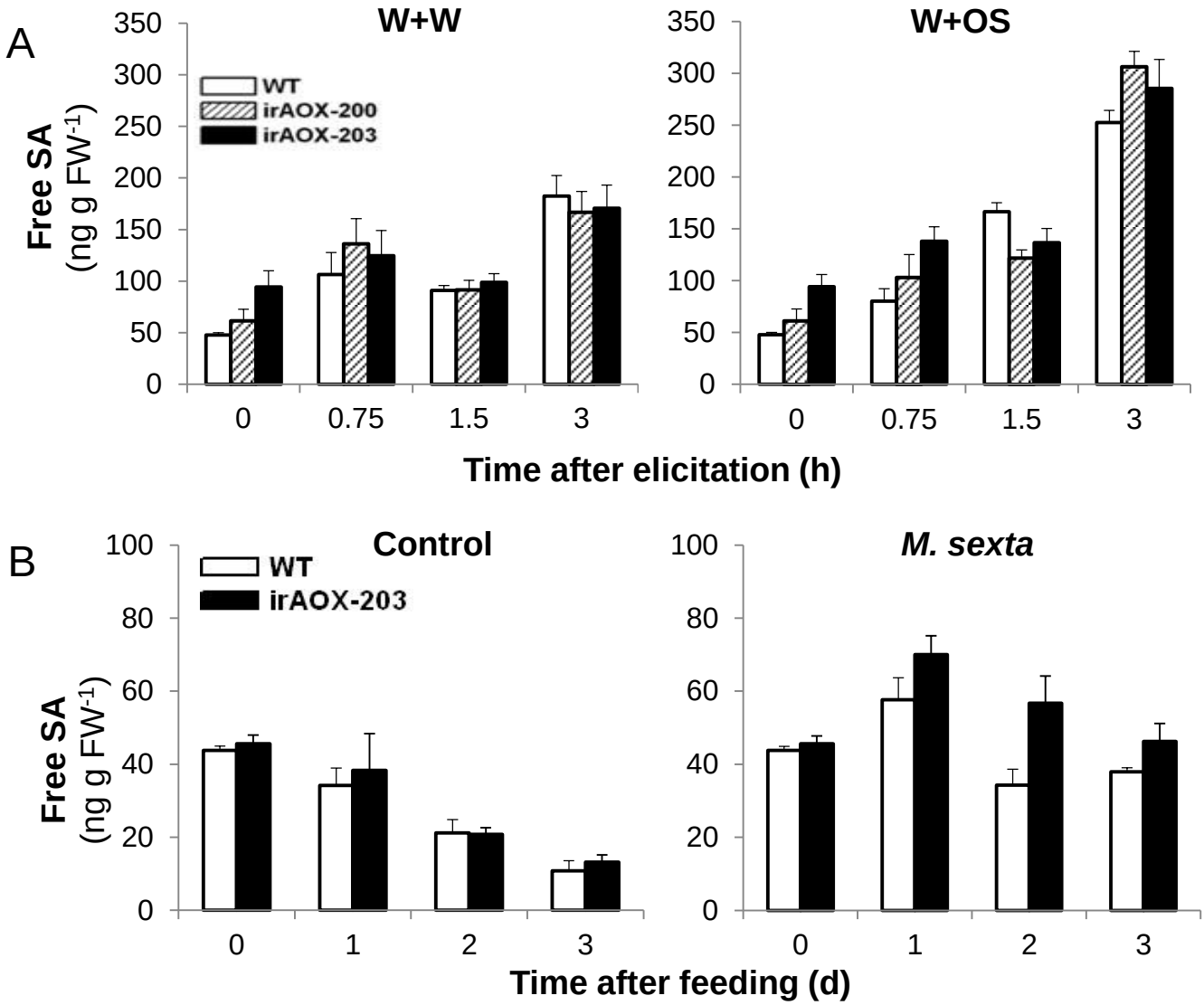
S4

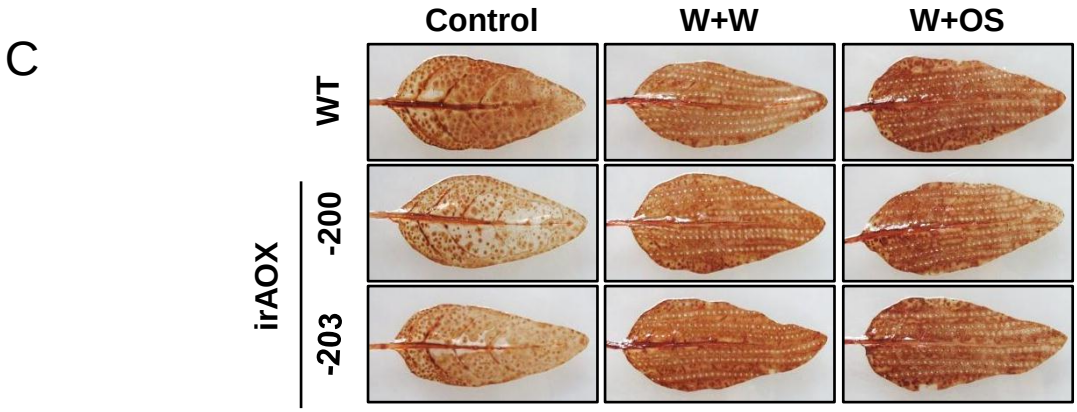


Supplemental Figure S4

Functional characterization of irAOX lines. Oxygen consumption in the leaves was determined with a miniaturized oxygen Clark-type electrode (Oxygen microsensor, Unisense, Denmark) after incubation of leaf discs in oxygen-saturated buffer and application of (A) no inhibitors to determine total respiration (B) 1 mM KCN to determine cyanide-sensitive cytochrome c respiration, (C) 1 mM KCN and 5mM SHAM to determine SHAM-sensitive alternative respiration. (D) irAOX plants, supported by efficient silencing of *AOX* genes shown in Supplemental Figure S3C, showed very low alternative respiration rates demonstrated as reduced portion of SHAM-sensitive respiration capacity. In contrast, irAOX plants showed higher activity of cytochrome c pathway, most likely due to reduced competition by AOX pathway and higher availability of reduced ubiquinone pool. Experiment was conducted with 3 biological replicates using WT and 2 transgenic lines, irAOX-200 and irAOX-203. Statistically significant differences were determined by one-way ANOVA ($P < 0.01$, $n=3$).

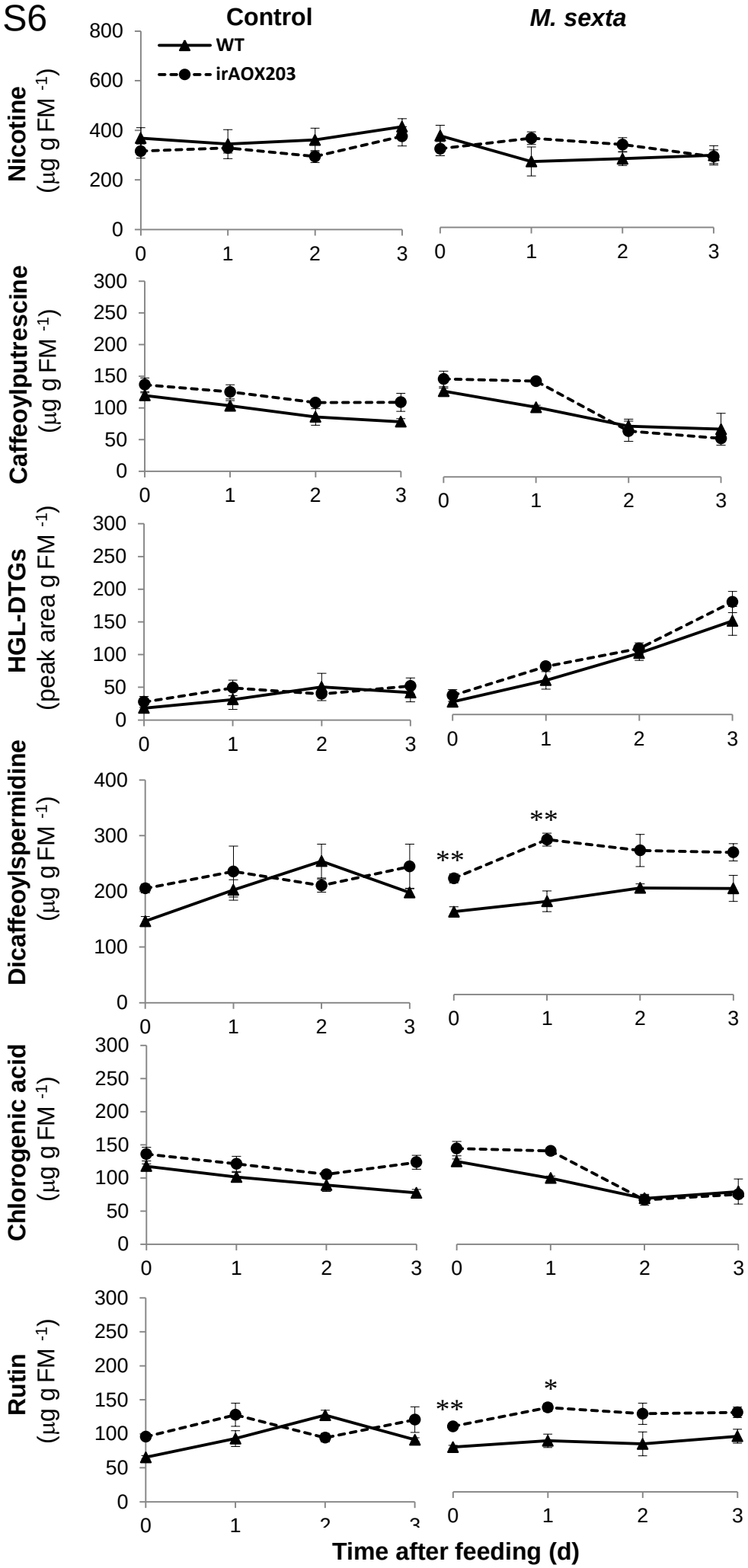
S5





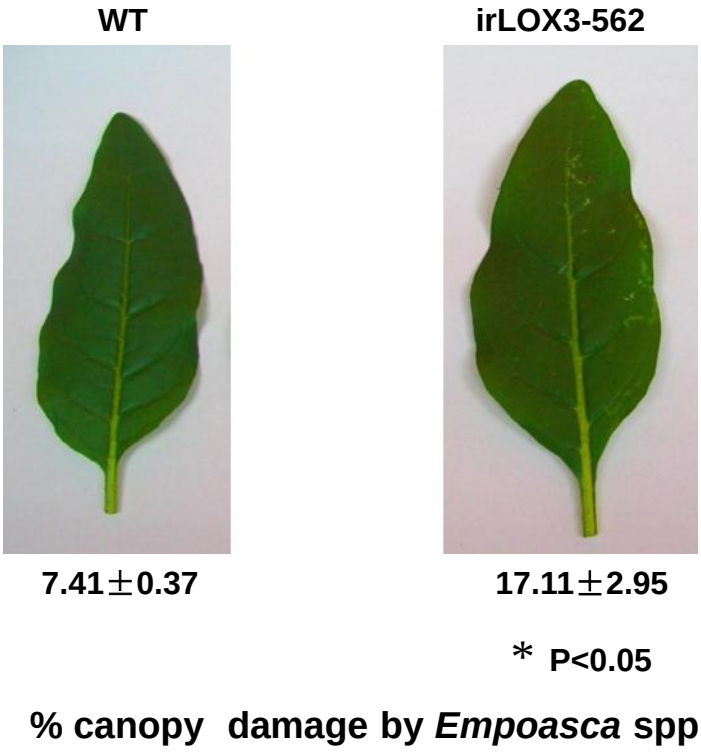
Supplemental Figure S5

irAOX WOS- and herbivory-induced plants have salicylate levels and hydrogen peroxide (H_2O_2) levels comparable to WT plants. (A) Mean (± SE) levels of SA in the samples collected at 0, 0.75, 1.5, and 3 h after W+W (*n=5*, left panel) and W+OS (*n=5*, right panel) treatments. (B) Mean (± SE) levels of SA in samples collected in control plants (*n=3*, left panel) and plants after 0, 1, 2, and 3 d of *M. sexta* feeding (*n=3*, right panel). (C) Intact leaves for the DAB (H_2O_2**)** staining were treated with W+W, W+OS or remained untreated (control) and they were detached from the plants 45 min after treatment. The entire excised leaf was floated in the DAB staining solution for 24 h in the dark and de-stained to visualize brown precipitate of DAB indicating the presence of H_2O_2.



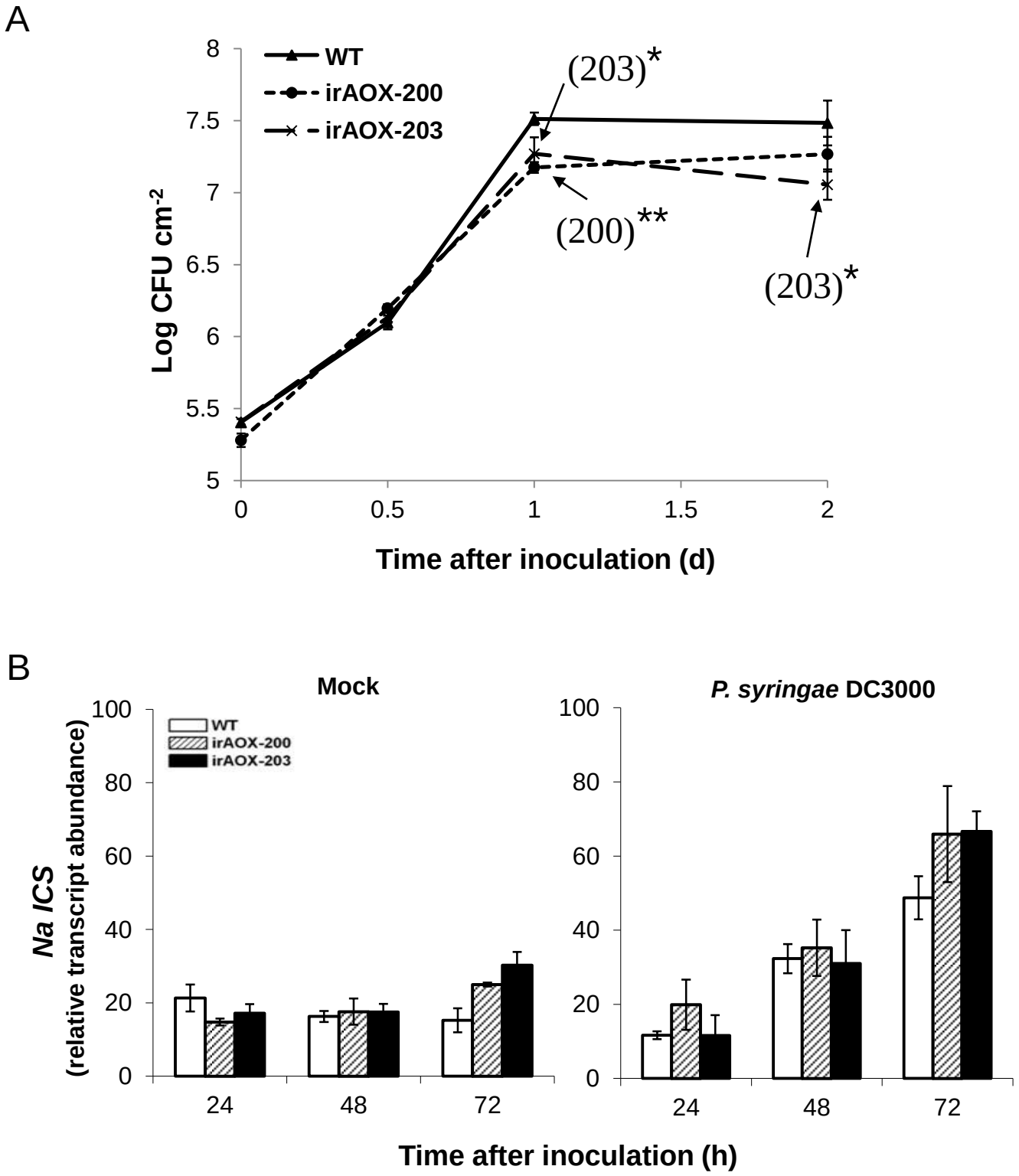
Supplemental Figure S6

Exposure to *M. sexta* feeding does not change a majority of the secondary metabolite profiles in irAOX plants compared to WT. Mean (± SE) levels of secondary metabolites from WT and irAOX plants collected after 0, 1, 2, and 3 d of *M. sexta* feeding (*n=3*). Samples were extracted in acidified 40% methanol and subjected to HPLC separation and detection based on UV absorbance of the compounds (17-hydroxygeranyllinalool diterpene glycosides (HGL-DTGs), detected at 210 nm; nicotine, 254 nm; chlorogenic acid, caffeoylputrescine, dicaffeoylspermidine, 320 nm; rutin, 360 nm).



Supplemental Figure S7

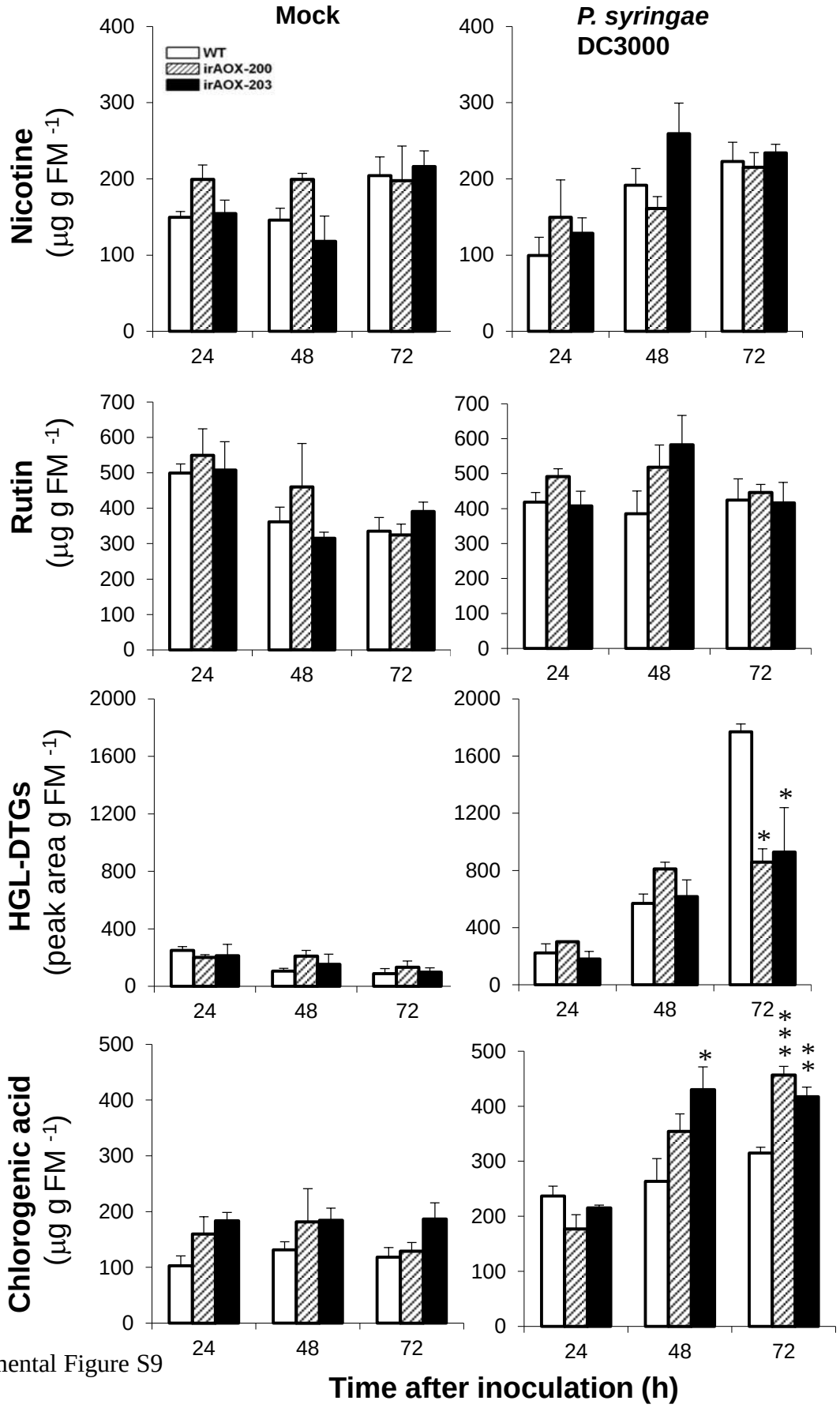
Silencing of NaLOX3 compromises defense of *N. attenuata* against *Empoasca spp.* Leaves from WT and irLOX3 plants with reduced JA levels after 12 d of exposure to *Empoasca* spp. leafhoppers. Numbers show mean (± SE) average leaf damage by *Empoasca* spp. estimated at the end of experiment (12 d). Asterisks indicate significant differences between WT and irLOX3 genotypes determined by unpaired Student's *t*-test (* P<0.05; *n=6*).



Supplemental Figure S8

Multiplication of *Pst* DC3000 is slightly suppressed in irAOX plants and *NaICS* transcript levels are not differentially regulated in WT and irAOX plants infected with *Pst* DC3000. (A) Mean (±SE) Log_{10} of colony forming units (CFU) determined in *Pst* DC3000-infected WT and irAOX leaves at indicated time points. Significant differences at respective time points were determined by one-way ANOVA (*P<0.05; **P<0.01; *n=5*). (B) Mean (±SE) relative transcript levels of *N. attenuata ISOCHORISMATE SYNTHASE* (Na*ICS*) gene in mock and *Pst* DC3000-inoculated leaves of WT and two irAOX lines. No significant differences between genotypes at respective time points were determined by one-way ANOVA.

S9



Supplemental Figure S9

Secondary metabolites levels are altered in irAOX plants relative to WT after *Pst* DC3000 infection. Mean (± SE) levels of secondary metabolites from samples collected 24, 48, and 72 h after infection. Significantly higher chlorogenic acid (CGA) levels correlated with the increased SA levels and higher PAL transcripts in irAOX plants, while significantly lower 17-hydroxygeranyllinalool diterpene glycosides (HGL-DTG) levels correlated with decreased JA levels in these plants. Asterisks indicate significant differences between WT and irAOX plants at respective time points determined by one-way ANOVA (* P< 0.05; *** P< 0.001; *n=6*).

T1

Supplemental tables

Gene	Forward primer(5'->3')	Reverse primer(5'->3')
NaAOX1*	TCGAAATGACGCCGGAAC	TTTCTCCGATGGATTCACCG
NaPAL1	TTTGCATACGCTGATGACGC	TGGAAGATAGAGCTGTTCGCG
NaPAL2	ACTTGTTCGCCTACGCTGATG	TCTTCGAAAGCTCCAATCTTTTG
NaActin	GGTCGTACCACCGGTATTGTG	GTCAAGACGGAGAATGGCATG
NaHIN1	GCGTCCAGTATTCAAAGGTCA	CGCATGTAAAGCTTCACTTCC
NaRbohD	CAATCATACTCTATGCTAGT	CTCAAATGGAGAAACTGCA
NaICS	TTTGCAACCTCCCCAGTC	ACCCCTAGCCCGTGTTC

* Note：NaAOX1 Taqman probe：TTATGACTGGCGCCGCCGGTTT）

Supplemental Table 1

Real time PCR primers and probes

The primers and Taqman probe sequences used for Taqman qPCR, and primer sequences used for SYBR Green-based RT-qPCR assays.