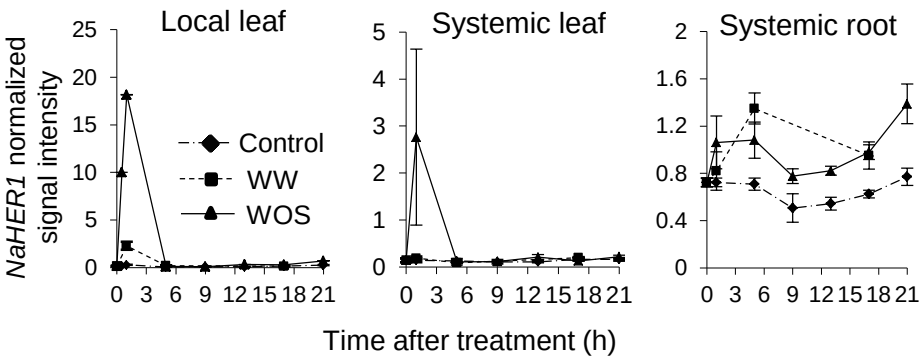

Supplemental Figure 1. *NaHER1* is systemically regulated after WOS treatment. Patterns of local and systemic normalized signal intensity of *NaHER1* transcript abundances (± SE, n=3) after WW and WOS treatments, and in control untreated plants extracted from a previously published microarray data set (Kim et al., 2011). WT leaves were wounded and immediately treated with 20 µL of water (WW), or 1:5 water-diluted oral secretions (WOS) from *M. sexta* larvae, and collected at the designated time points.

A

```
  1 ATGGAGATAG AGGTAATGAT GCCATCTCCA GCGGTGGATT TCAACGTCGA CAGTGGTTGC ACAACTCCTT
 71 ATATGAGTGC ACCTTCAAGT CCTCCACGTG TCGCCACCTT GTTCTACAGT GCACCCGCTA GTCCCACCCG
141 CATTTCCTCA CTTTACGATG AAATTAACTT TGACCCAATT GAAGATGATG ATTTTGCTTT TGATTTCAGT
211 GGTCAATTAG AGAGGATTTC TTTTTCAGCA GCTGATGAAC TCTTTGATGG TGGAAAAATT AAGCCTTTAA
281 AACCACCACC TCGGTTTCAG TACGAAGGAA AACACATTGA TTCTCCAAAA TCACCAAAAA AATTGTTCAA
351 AGAAGCATTT TCGCCCCGAC ATAAAAAGAA AGATTTTGAC CCATTGGCTG CAGCTTTACA AAAACAGAGT
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491 CATTTAGAGT TTCTGATTTA TTATATGATC AAGAAAGCAA TCAACAAAAC CCAAAAAAGT CAGCCTCTGT
561 TTCATCATCG TCATCTTCTT CTTCCTCCTC TTCAACTTCT TCAGTTTCTT CAATGATCTC ACTCTGGTCC
631 AAAAAATGGA AACTCAAAGA CTTGTTTCTA TTCAGAAGTT CTTCAGAAGG TCGAGCAAGT AGTACGGAAC
701 AATTAAATAA GTACGAATTA TTGAAGAAGA GTCATCAAGA AGATGTGAAA AATTCCAGCT TTAGGTCAAC
771 AGACAGTGTT AGATCGAGAA AGAAAGGACC GGTTTCGGCT CATGAGTTGC ATTATACAAT GAACAGGGCA
841 GTTTCAGAAG AGATGAAGAA GAAAACATAT TTGCCATACA AACAGGGTTT ATTGGGTTGC TTAGGTTTCA
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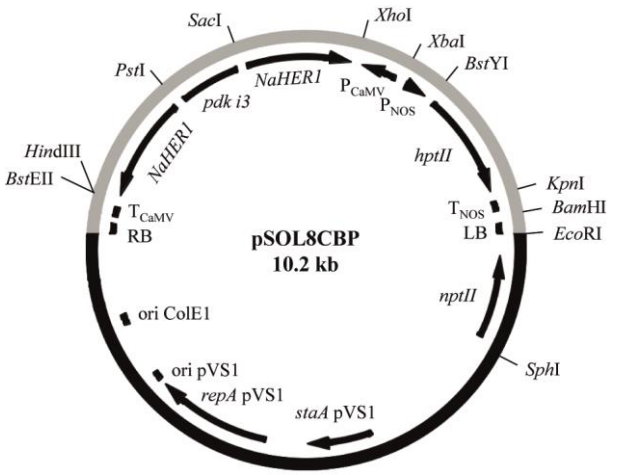
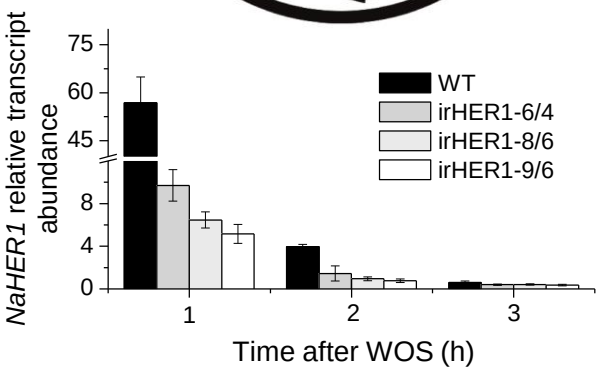
B

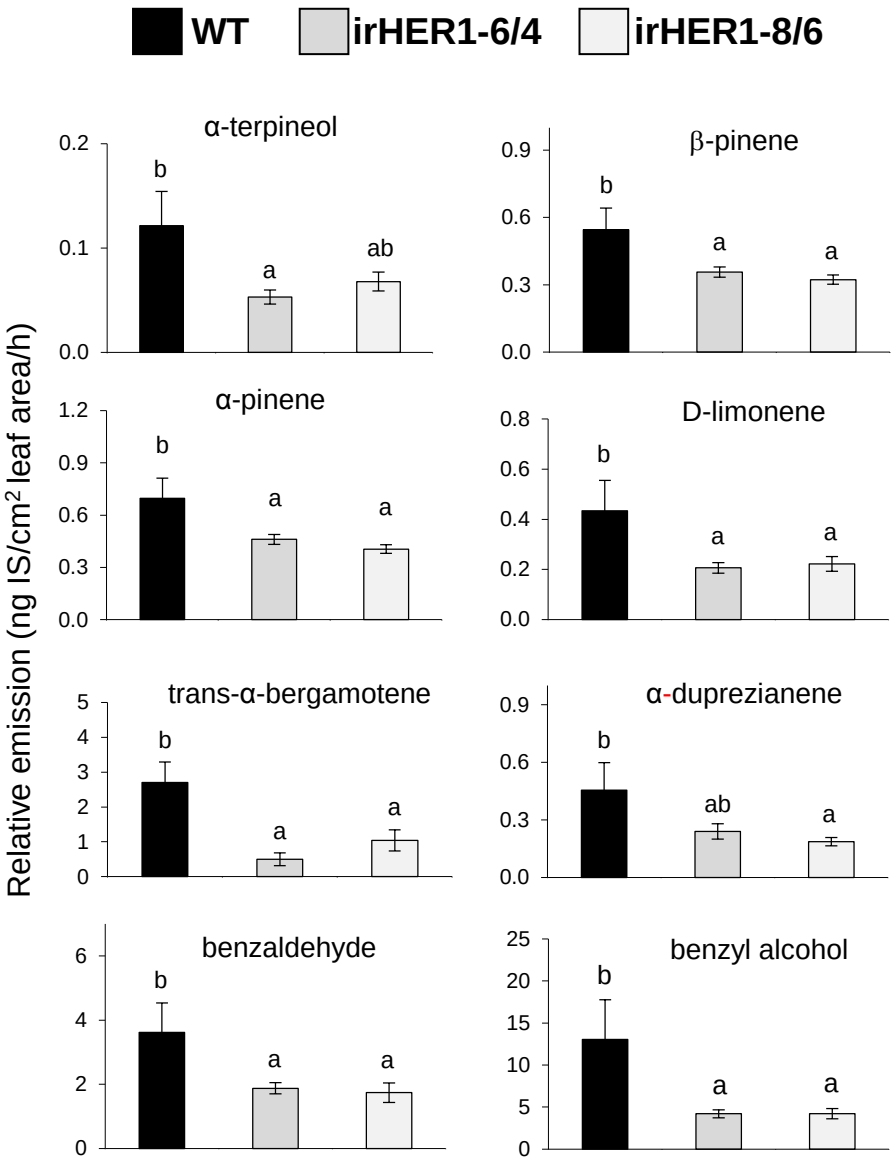


C

D



Supplemental Figure 2. Sequences, silencing construct and transgenic plant information. (A) Full length mRNA sequence of *NaHER1*; bold underlined sequence was used to create inverted repeat structure (hairpin) and independently silence *NaHER1* by VIGS and RNAi. (B) Physical map of pSOL8CBP plant binary transformation vectors used to generate irHER1 plants. The pSOL8CBP vector contains a strong constitutive cauliflower mosaic virus 35S (CaMV) promoter. cDNA fragment shown in (A) was cloned into the two multiple cloning sites in reverse orientation, which was separated by a *pyruvate orthophosphate dikinase* (*pdk i3*) intron to form an inverted repeat construct. (C) Southern blot analysis confirming the presence of a single T-DNA insertion in the *N. attenuata* genome of each transgenic line used in the study. A 10 µg aliquot of genomic DNA prepared from each of three independently transformed lines (irHER1-6/4, -8/6 and -9/6) was digested with XbaI restriction enzyme and separated by gel electrophoresis. Radioactively-labeled DNA fragment of the *hygromycin phosphotransferase* gene (*hptII*) was used as a hybridization probe. (D) Silencing efficiency of *NaHER1* in three independent *NaHER1*-silenced lines determined by qPCR.



Supplemental Figure 3. *NaHER1* silencing reduces emissions of several isoprenoid and phenylpropanoid/benzenoid volatiles from local WOS-treated *N. attenuata* leaves. Mean (± SE; n≥6) releases of volatile compounds from locally treated leaves of WT and irHER1 plants. A single local leaf in 35 d-old WT or irHER1 plants was mechanically wounded and treated with 20 µL of diluted OS from *M. sexta* (WOS). 18 h after treatment, volatiles were collected from the headspace of the same leaf for 7 h and analyzed by GC-MS. Different letters show significant differences between samples determined by one-way ANOVA, followed by a Fisher PLSD *post hoc* test (P ≤0.05). The volatile blends emitted from irHER1 leaves were strongly suppressed in the production of terpenoids (α-pinene, α-terpineol, β-pinene, D-limonene, *(E)*- α-bergamotene, α-duprezianene), and phenylpropanoid/benzenoids (benzaldehyd, benzyl alcohol). SE, standard error.

>Na_454_00400 (*NaMYC2*)

```
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 241 TCAGACGATA ACATGATGGA AGCTTTTTTA TCTTCTGATC CGTCGTCGTT TTGGCCCGGA
 301 ACAACTACTA CACCAACTCC CCGGACTTCA GTTTCTCCGG CGCCGCCGCC GGTGACGAGT
 361 ATTGCCGGAG ACCCATTAAA GTCTATGCCA TATTTTAACC AAGAGTCGCT GCAACAGCGA
 421 CTTCAGACTT TAATCGACGG GGCTCGTGAA GCGTGGACGT ATGCCATATT TTGGCAATCG
 481 TCTGTTGTGG ATTTCGCGAG CCCCTCTGTG TTGGGGTGGG GAGATGGGTA TTATAAAGGT
 541 GAAGAAGATA AAAATAAGCG TAAAACGGCG TCGTTTTCGC CTGATTTTAT CACGGAGCAA
 601 GCACACCGGA AAAAGGTTCT CCGGGAGCTG AATTCTTTAA TTTCCGGCAC ACAAACTGGT
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 781 CGATTTGGGT TACTGGAGCA GAGAGATTAG CTGCTTCGCA CTGCGAACGG GCCCGACAAG
 841 CCCAAGGATT CGGGCTTCAG ACTATTGTTT GTATTCCTTC AGCTAATGGT GTTGTTGAGC
 901 TCGGGTCAAC TGAGTTGATA TTCCAGACTG CTGATTTAAT GAACAAGGTT AAAGTTTTGT
 961 TTAATTTTAA TATTGATATG GGTGCGACTA CGGGCTCAGG ATCGGGCTCA TGTGCTATTC
1021 ACGCTGAGCC CGATCCTTCG GCCCTTTGGC TGACGGATCC GGCTTCCTCA GCTGTGGAAG
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1981 ATCCAGCCGC GAGGTTAATG GCCGCTCTCA TGGAATTGGA CTTAGATGTG CACCATGCTA
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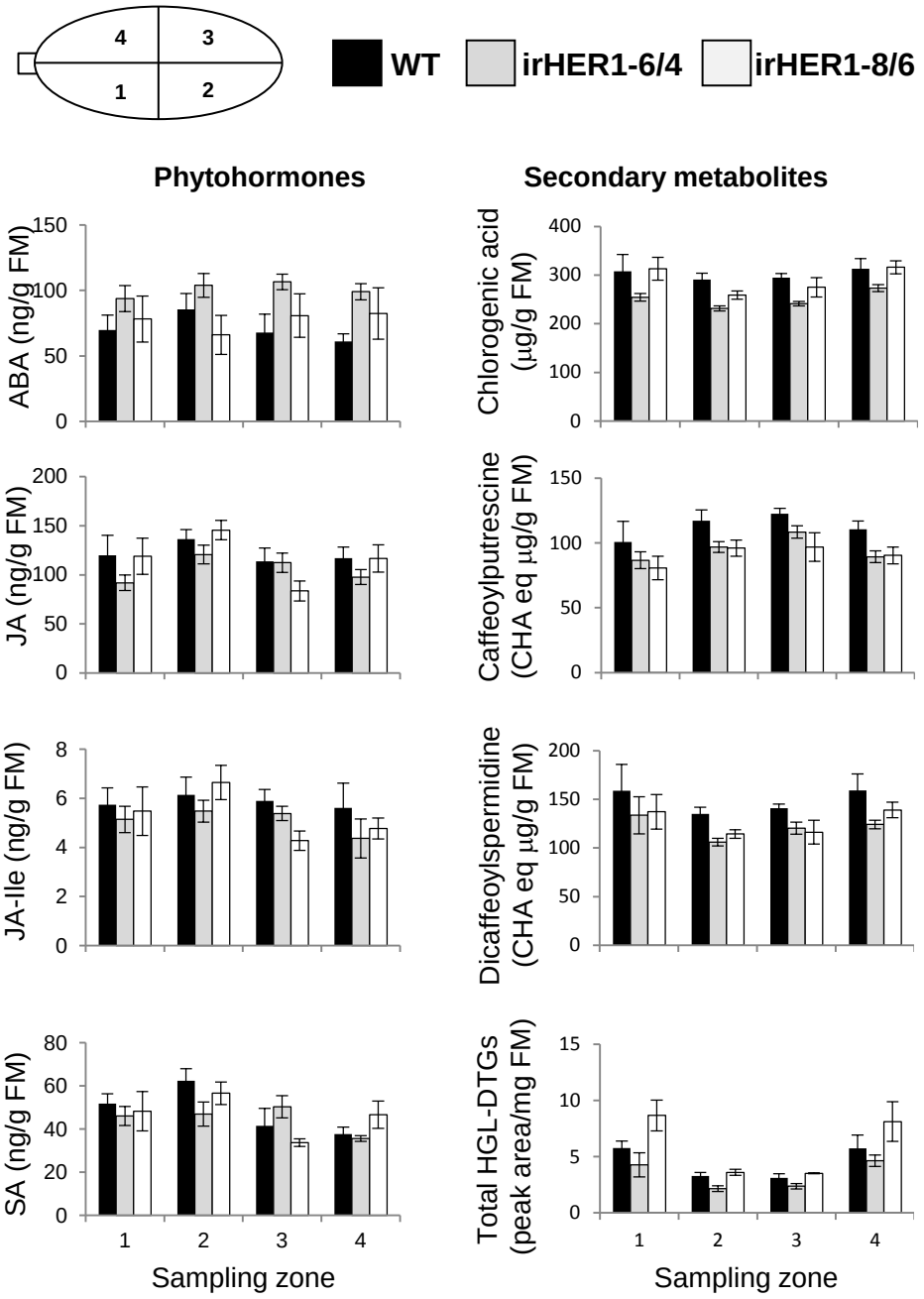
2461 TCTATTTGTA TTCTTTATCC AGAATA
```

Supplemental Figure 4. The sequence of the *NaMYC2*. The cDNA sequence of bHLH-domain *NaMYC2* was obtained after searching cDNA libraries of *N. attenuata* using authentic *A. thaliana* (AB000875) and *N. benthamiana MYC2* (GQ859153) sequences.



Supplemental Figure 5. Silencing of *NaHER1* does not affect constitutive levels of phytohormones (ABA, JA, JA-Ile and SA) and defense metabolites (chlorogenic acid, caffeoylputrescine, dicaffeoylspermidine, and total HGL-DTGs). Untreated rosette stage *N. attenuata* leaf samples were collected and analyzed by LC-MS/MS: ABA, JA, JA-Ile, SA (± SE, n=4) and HPLC: chlorogenic acid (CHA), caffeoylputrescine (CP), dicaffeoylspermidine (DCS), HGL-DTGs (± SE, n=4), respectively. Leaves were divided into four equal parts during sampling and each part was analyzed separately. SE, standard error; FM, fresh mass; CHA eq., chlorogenic acid equivalents.

>Na_454_17098 (*NaPYL4*)

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  1 CAAAAGAGCC CCATTAGCGA CCACTCTTAC AGTTTACGAC ATAAGAATCA TCAGCTCTTC
 61 TTTCATTTCA GATCAAAATC TAACAACACT TGGCCAAAAA AAAATGCCTC CTAGTTCTCC
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181 ACAATCTCAG CAATTACAAA GGCGTACTAT GCCGATACCT AGTACGACAC AAGTCCCAGA
241 TTCCGTTGTC CGATTCCATA CTCACCCGGT GGGTCCCAAC CAGTGCTGCT CCGCCGTGAT
301 CCAGCGGATT TCCGCCCCCG TCTCCACCGT ATGGTCAGTG GTCCGCCGTT TCGACAACCC
361 TCAAGCATAC AAGCACTTTG TCAAGAGCTG CCACGTCATC GTAGGGGATG GTGACGTCGG
421 CACTCTCCGC GAGGTCCGAG TGATCTCGGG CCTTCCAGCT GCGTCCAGCA CGGAAAGACT
481 CGAGATCCTC GACGACGAGC GACATGTCAT TAGCTTTAGC GTAGTCGGTG GAGACCACCG
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661 TGTTTTCGTT GATACCATCG TCAAATGCAA CCTCACATCG TTATCGCAGA TCGCAGTAAA
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901 GATAAGTTCT TGGTTACGGA ATTAATTTCA TTCTCTTTAA TTATATCAAG TACTTGCTTC
961 TTTTTTTGTC CTCATTGCTC TATGTTTAAC TTTATCTGTG TATTTGTGAA AATAA
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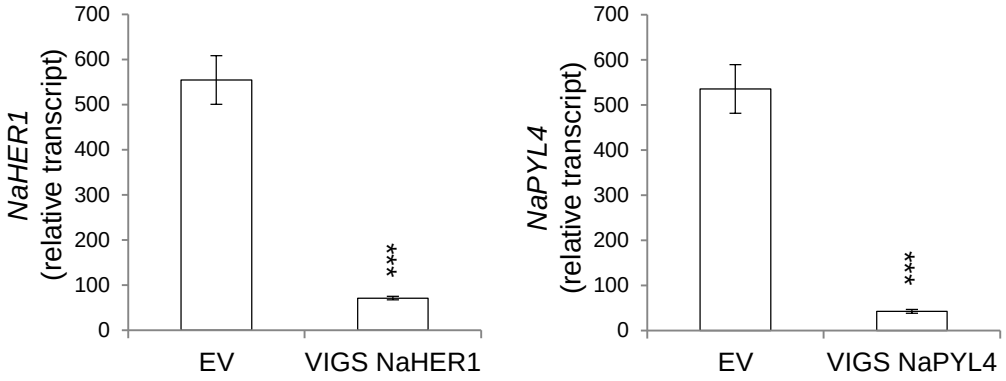
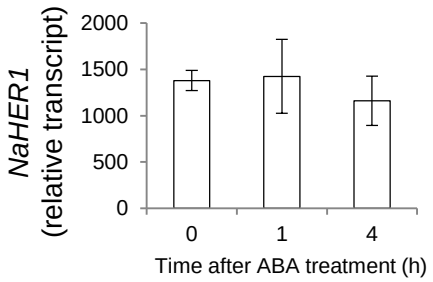
Supplemental Figure 6. cDNA fragment of *NaPYL4* gene. The cDNA sequence of a putative *N. attenuata* ABA receptor *NaPYL4* was obtained after searching cDNA libraries of *N. attenuata* using authentic *N. tabacum PYL4* (AJ966358) sequence. The bold and underlined sequence was used to design VIGS construct and silence *NaPYL4*.



Supplemental Figure 7. *NaHER1* and *NaPYL4* silencing efficiency by VIGS. Relative transcript abundances (± SE, n=4) of *NaHER1* and *NaPYL4* determined by qPCR. *NaHER1* and *NaPYL4* were silenced by VIGS method (see Material and Method for more details). Leaves were wounded and immediately treated with 20 µL of 1:5 water-diluted oral secretions (WOS) from *M. sexta* larvae. Samples were collected after 1 h and analyzed by qPCR. Asterisks represent significant differences determined by ANOVAs, followed by Fisher's PLSDs *post hoc* test (* $P \leq 0.05$), *** $P \leq 0.001$; SE, standard errors.



Supplemental Figure 8. ABA does not induce *NaHER1* expression. Relative transcript abundances (± SE, n=5) of *NaHER1* determined by qPCR. The leaves of *N. attenuata* (one per plant) were treated with 20 μL of 100 μM ABA in lanolin paste. Samples from treated leaves were collected at designated time points and analyzed by qPCR.

>Na_454_00091 (*NaABA1*)

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 241 AATTCTTTAC CATGCAGTAG AAGCTTGGAA AATGGTAAAA TCAAGAAAGT CAAAGGAGCA
 301 GTAAAAGCCA CAATAGCTGA AGCTCCAGCT ACTACTCCAA CAACTAATTT GAAGAAGAAG
 361 TTGAAAGTAC TAGTAGCAGG TGGTGGGATT GGAGGATTAG TGTTTGCATT GGCTGCAAAG
 421 AAAAGGGGAT TTGAGGTGTT GGTATTTGAG AGGGATTTAA GTGCTATTAG AGGAGAAGGG
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1981 CTTTCCTACT CGCTTTCATC CATCAGATAT TATTGAATTT GGTTCTGATA AGAAGGCAGC
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2341 TATGTATATA CAATCACAGA GGCCATAATG TTAAAAAGTG TTTTACCTTC AATAAATAAA
2401 AAAAAAAAA
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Supplemental Figure 9. The sequence of *NaABA1* gene. The cDNA sequence of a putative *N. attenuata NaABA1* was obtained after searching cDNA libraries of *N. attenuata* using authentic *A. thaliana* (AT5G67030) and *Nicotiana plumbaginifolia* (X95732) ABA1 sequence.

>Na_454_00067 (*NaPDR12*)

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 241 CTGGTTACAA GATGGAGCCA GCAAATTTAA GTAATTTGCG AGGGAGTAGT TTAAGAGGAA GTACGAGAGG GAGTTTAAGG
 321 GCAAATAGCA ATTCAATATG GAGAAATAAT GGTGTAGAAA TATTCTCACG TTCATCTAGA GATGAAGATG ATGAAGAGGC
 401 ACTTAAATGG GCTGCTCTTG AAAAACTTCC AACTTTTGAT AGATTAAGAA AAGGTCTTTT GTTTGGTTCA CAAGGTGCTG
 481 CTGCTGAAGT TGACATTAAT GATCTTGGTT TTCAAGAAAG GAAGAATTTG CTTGAGAGAC TTGTTAAAGT TGCTGATGAA
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3681 GCGTCCCTCG CCCTGGTTCA AAGGACTTGC ATTTTGAAAC TCAATACTCA CAATCATTCT GGACGCAATG TATGGCTTGC
3761 CTATGGAAGC AACACTGGTC ATACTGGCGT AATCCAGCTT ACACCGCAGT CAGATTCATC TTCACGACAT TCATAGCACT
3841 AATCTTCGGG ACAATGTTCT GGGATCTTGG TACTAAAGTG AGCAAGAGCC AAGATCTTTT AAACGCCATG GGATCAATGT
3921 ATGCTGCTGT TCTCTTCCTT GGTGTACAAA ATGCATCATC AGTGCAACCA GTTGTAGCCG TTGAGCGTAC AGTATTTTAC
4001 AGAGAAAGAG CTGCTGGAAT GTACTCTGCC ATCCCCTATG CCTTTGGACA GGTTTCCATT GAAATCCCTT ACATATTTGT
4081 ACAATCTGTG TTTTATGGTA TCATTGTCTA TGCTATGATT GGATTCGAAT GGGATGTTGG CAAGTTCTTT TGGTACTTGT
4161 TCATCATGTT TTTCACCCTT TTGTACTTTA CATTCTACGG TATGATGAGT GTGGCTGTCA CACCAAATCA AAACGTGGCT
4241 TCAATTGTTG CTGCCTTCTT CTATGGTGTA TGGAATCTCT TCTCAGGATT CATCATTCCA CGACCTCGTA TGCCTGTATG
4321 GTGGAGATGG TACTACTGGG CTAACCCGGT TGCATGGACC TTGTATGGTT TGGTTGCATC ACAATTCGCA GACATCCAAA
4401 CAAAACTATC TGATAATGAA ACTGTGGAAC AATTCTTGAG ACGTTACTTT GGCTTTAAGC ATGATTTTCT TGGAGTTGTT
4481 GCAGCTATGC TCACTGCATA TGTCTTTGTG TTTGCCTTTT TACATTTGCT TTTTGCCATC AAGGCGTTCA ATTTTCCAGA
4561 GAAGATAAGT GAATTTAATC AACTGAAGAA CTTTGAGGAA GAAGTGGTTA CAAAATTAGT ATGTATAGGT TACTACTTTG
4641 AGAAAGTCTT TCTTTTCAAC TTTTTGAATA CTAGGTGTAT GTTGTTGTAA TATTCATTGG AAATCCTAAA CTTTAATTTA
4721 AGTTAAAAAA AAAAAA
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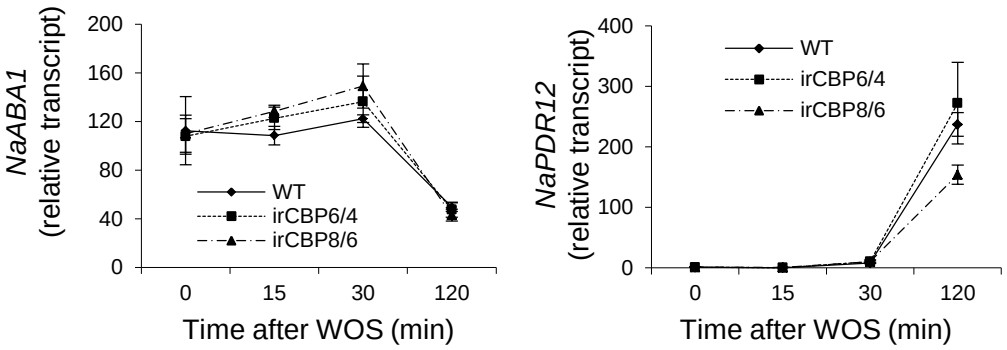
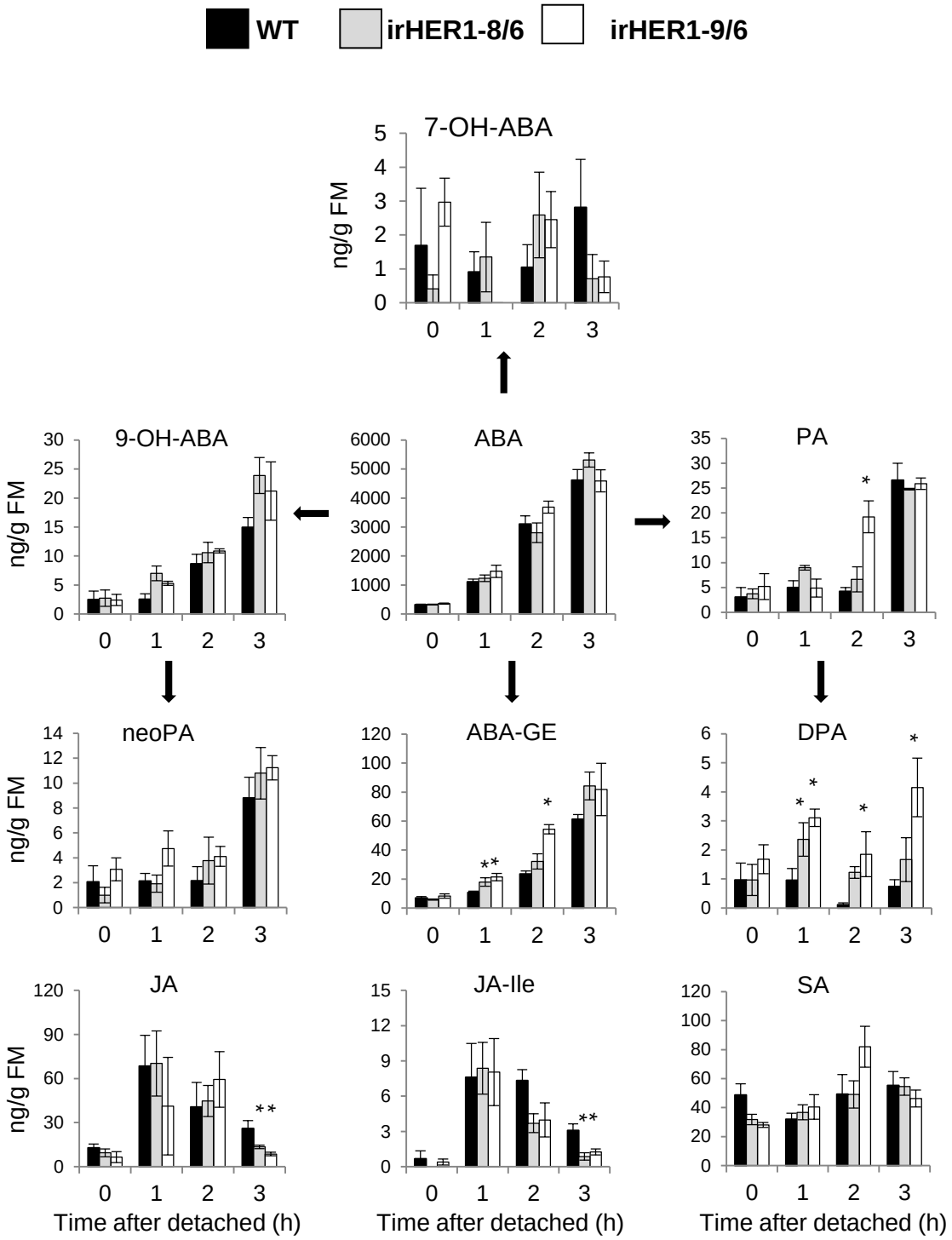
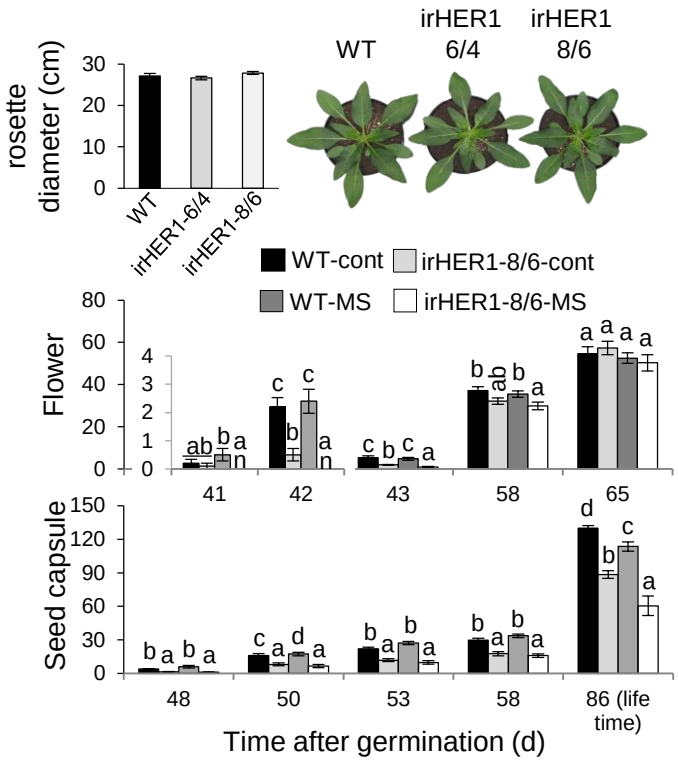
Supplemental Figure 10. The sequence of *NaPDR12.* The cDNA sequence of a putative *N. attenuata* ABA transporter *NaPDR12* was obtained after searching cDNA libraries of *N. attenuata* using authentic *A. thaliana PDR12* (NM_101421) sequence.



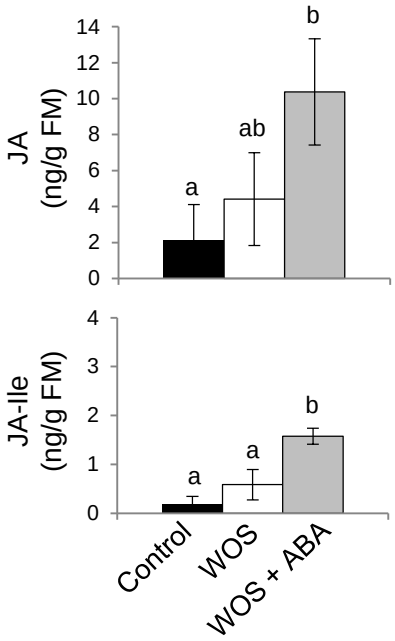
Supplemental Figure 11. Silencing of *NaHER1* gene does not affect expression of the ABA biosynthetic gene *NaABA1* or expression of the ABA transporter *NaPDR12*. Rosette stage *N. attenuata* plants were treated with WOS in zone 2. Samples were collected at designated time points, extracted and analyzed by real-time PCR for *NaABA1* and *NaPDR12* transcripts.



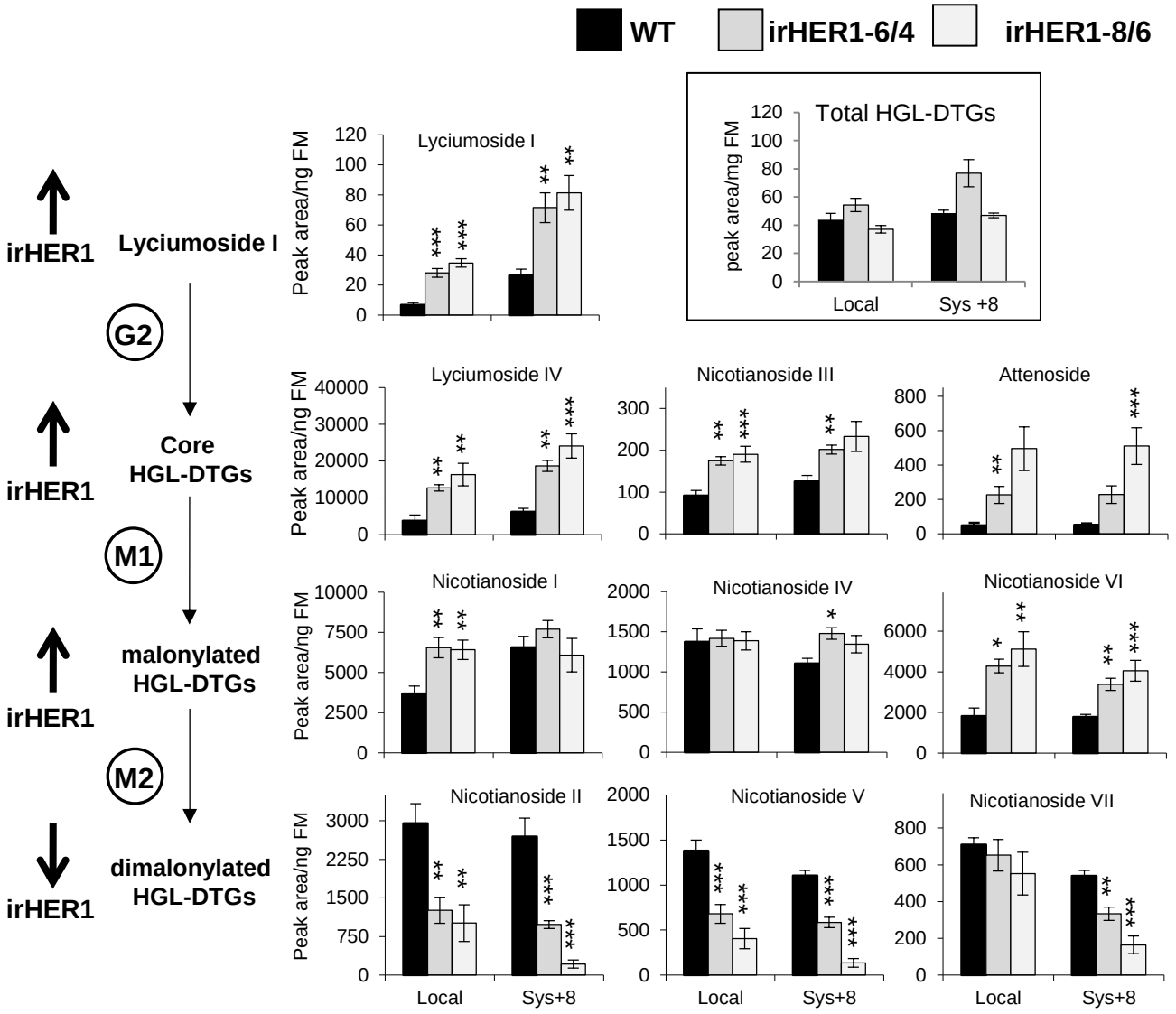
Supplemental Figure 12. *NaHER1* silencing alters ABA metabolism, JA and JA-Ile accumulation in detached leaf. Mean (± SE, n=4) levels of ABA metabolites, JA, JA-Ile, and SA in detached *N.attenuata* leaves collected at designated time points and analyzed by LC-MS/MS. Metabolite levels were compared by ANOVAs, followed by Fisher's PLSDs *post hoc* test (* $P \leq 0.05$). SE, standard error.



Supplemental Figure 13. *NaHER1* silencing plants does not affect rosette diameter but delays flowering time and reduces seed capsule numbers in *N. attenuata*. Plants were maintained under continuous attack from *M. sexta* neonates (MS) or left untreated (Cont), and flower and seed capsule numbers (± SE, n=10) were counted at designated time points. Different letters show significant differences between samples determined by Mann-Whitney U test ($P \leq 0.05$). Note that the flower numbers at the day 41 and 42 were present with their own y-axis.



Supplemental Figure 14. ABA affects JA accumulation of OS-elicited plants. Leaves of *N. attenuata* (one per plant) were mechanically wounded and immediately treated with 20 µL of diluted OS from *M. sexta* (WOS) or WOS supplemented with 100 mM ABA. Samples were collected after 4 d and analyzed by LC-MS/MS for phytohormone contents (± SE, n=4). Samples were compared by one-way ANOVA, followed by a Fisher PLSD *post hoc* test; different letters show significantly differences between samples ($P \leq 0.05$). FM, fresh mass.



Supplemental Figure 15. **Accumulation of individual HGL-DTGs in WT and irHER1 plants.** Mean (± SE, n=4) accumulation of total and 10 individual HGL-DTGs determined by HPLC and LC-MS/MS, respectively. One leaf in each of the 47-d-old WT and irHER1 plants was treated with 20 µL diluted OS from *M. sexta* (WOS) and local and systemic samples [8-th leaf above the local WOS leaf (sys+8 in figure)] were collected after 48 h and analyzed by HPLC and LC-MS/MS. Asterisks represent significant difference between WT and irHER1 plants determined by one-way ANOVA, followed by a Fisher PLSD *post hoc* test; * $P \leq 0.05$, ** $P < 0.01$, *** $P < 0.001$. SE, standard error.

NaEF1a	forward primer: 5’-CCACACTTCCCACATTGCTGTCA-3’
NaEF1a	reverse primer: 5’-CGCATGTCCCTCACAGCAAAAC-3’
NaMYC2	forward primer: 5’-CCTCCACCGTCAAATCAAGA-3’
NaMYC2	reverse primer: 5’-GACTCCCCATTTTCACAGTTGCTTG-3’
NaMYB8	forward primer: 5’-AACCTCAAGAAACTCAGGACATACAA-3’
NaMYB8	reverse primer: 5’-GATGAATGTGTGACCAAATTTTCC-3’
NaHER1	forward primer: 5’-CCACGTGTCGCCACCTGTT-3’
NaHER1	reverse primer: 5’-GAAACGCAGGTGGTGGTTT-3’
NaABA1	forward primer: 5’-GAAGGCAGCATTTCGCGT-3’
NaABA1	reverse primer: 5’-CTTCAGAATTCTCAAGCTG-3’
NaPDR12	forward primer: 5’-ACTGAAGAACTTTGAGGAAG-3’
NaPDR12	reverse primer: 5’-GAATATTACAACAACATACACC-3’
NaPYL4	forward primer: 5’-CCATTAGCGACCACTCTTAC-3’
NaPYL4	reverse primer: 5’-GGAGCTTATTCTTTGGAG-3’

Supplemental Figure 16. Specific primer sequences used in qPCR (SYBR) analyses.