```
NaMYB8    MGRAPCCEKLGLKRGPWSKEEDDLLINYINKNGHPNWRALPKLAGLLRCGKSCRLRWTNYLRPD
NtMYBJS1  MGRAPCCEKLGLKRGPWSKEEDDLLINYINKHGHPNWRALPKLAGLLRCGKNCRLRWTNYLRPD
          ******************************:******************* ************

NaMYB8    IKRGNFTPEEENTIIKLHQVLGNRWSGIAARLPGRTDNEIKNIWHTRLKKRADDKSQPQQTQEI
NtMYBJS1  IKRGNFTPEEENTIIKLHQVLGNRWSGIAARLPGRTDNEIKNIWHTRLKKRVDDKSQPQETQDI
          ***************************************************** ***:****:**:*

NaMYB8    QDQQTME-TSKSEENLVTHSSEPEN----SEEISSPKTNFQIQQHPNSSSSTSFSGDSCSNTTA
NtMYBJS1  QDQQPMEITSKSEENFETHSSEPENSKDNSEEISSPKTNFQIQEHPNSSSSTSSSRDSCSNTTA
           ****.** *******: ********    **************:********* * ********

NaMYB8    TSMSTSSLADESRDQILLDNLLEVDDNFWSEVLWAPAETNDYNLSDLSFSSLEENYELNSSLND
NtMYBJS1  TS--TNSLADESRDQIMLDNLIEVDDNFWSEVLWAPAETNDYNVSDLSLSSLEENYELNSSLND
          ***.**********:****:************************:****:***************

NaMYB8    NWFWDDLFSTANELMLELPEL
NtMYBJS1  YWFWDDLFSTANELMLELPEL
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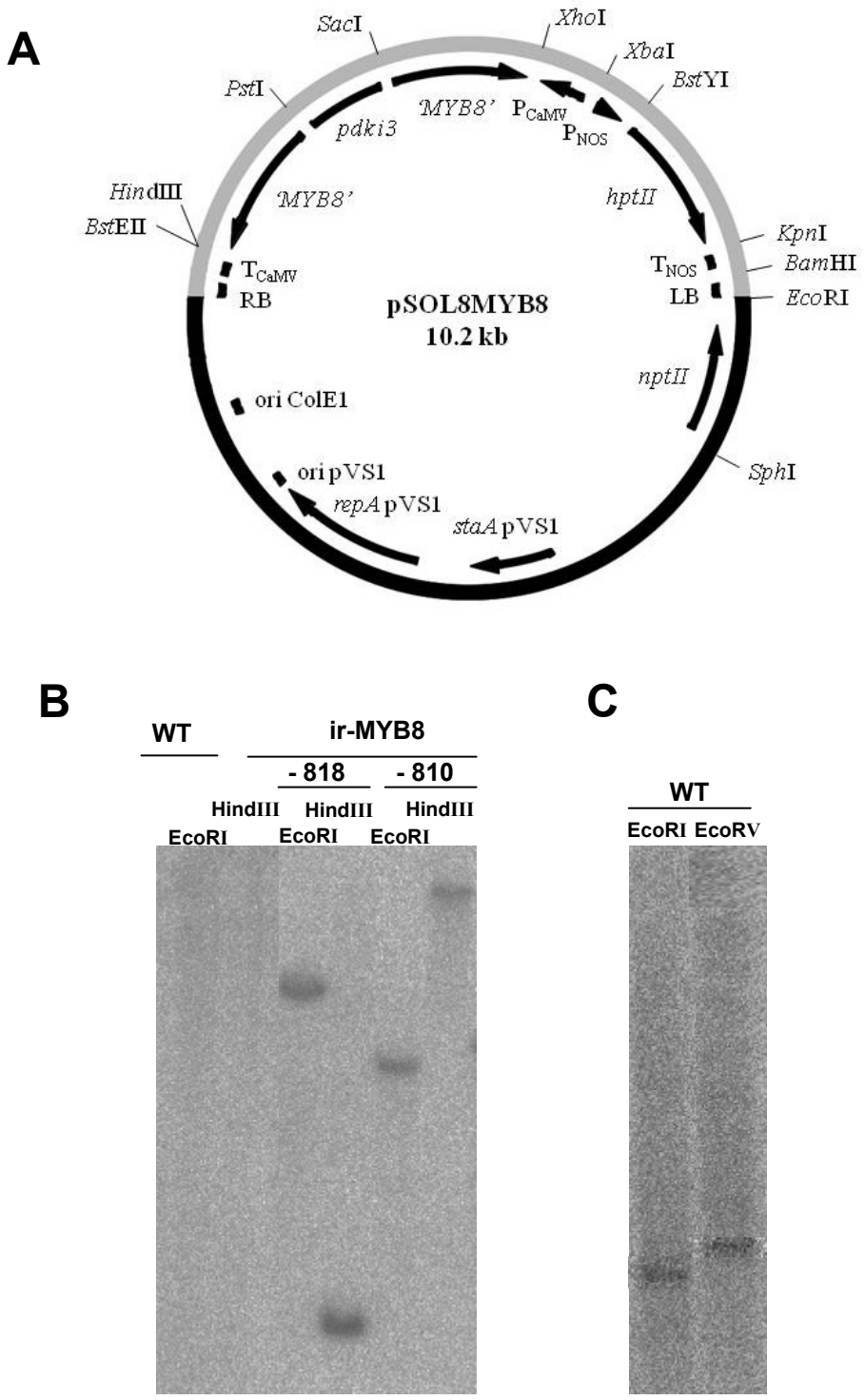
Supplemental Figure S1

Deduced NaMYB8 protein sequence aligned with its homolog from *N. tabacum* (NtMYBJS1).

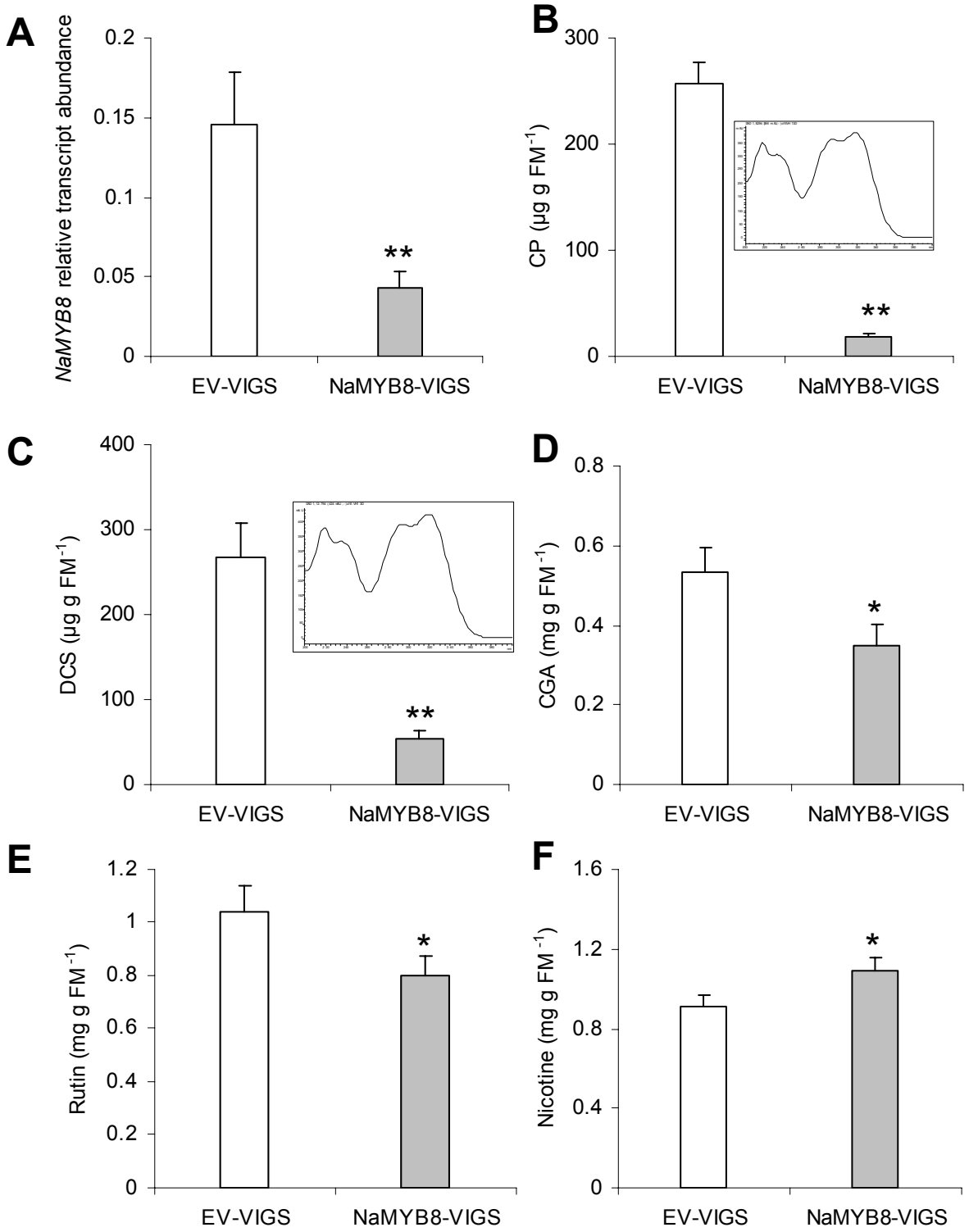
Sequence of NaMYB8 gene was originally obtained using PCR and cDNA template from *N. attenuata*, and primers designed according to NtMYBJS1 coding sequence. 3'-end of NaMYB8 gene was obtained by 3'RACE, and sequence was finally verified using 454-new generation sequencing of cDNAs from *N. attenuata*. The two R2R3-MYB repeats are highlighted in gray with dashed outlined boxes.



Supplemental Figure S2

The map of NaMYB8 transformation vector pSOL8MYB8 and Southern blot analysis of the two independently transformed ir-MYB8 lines.

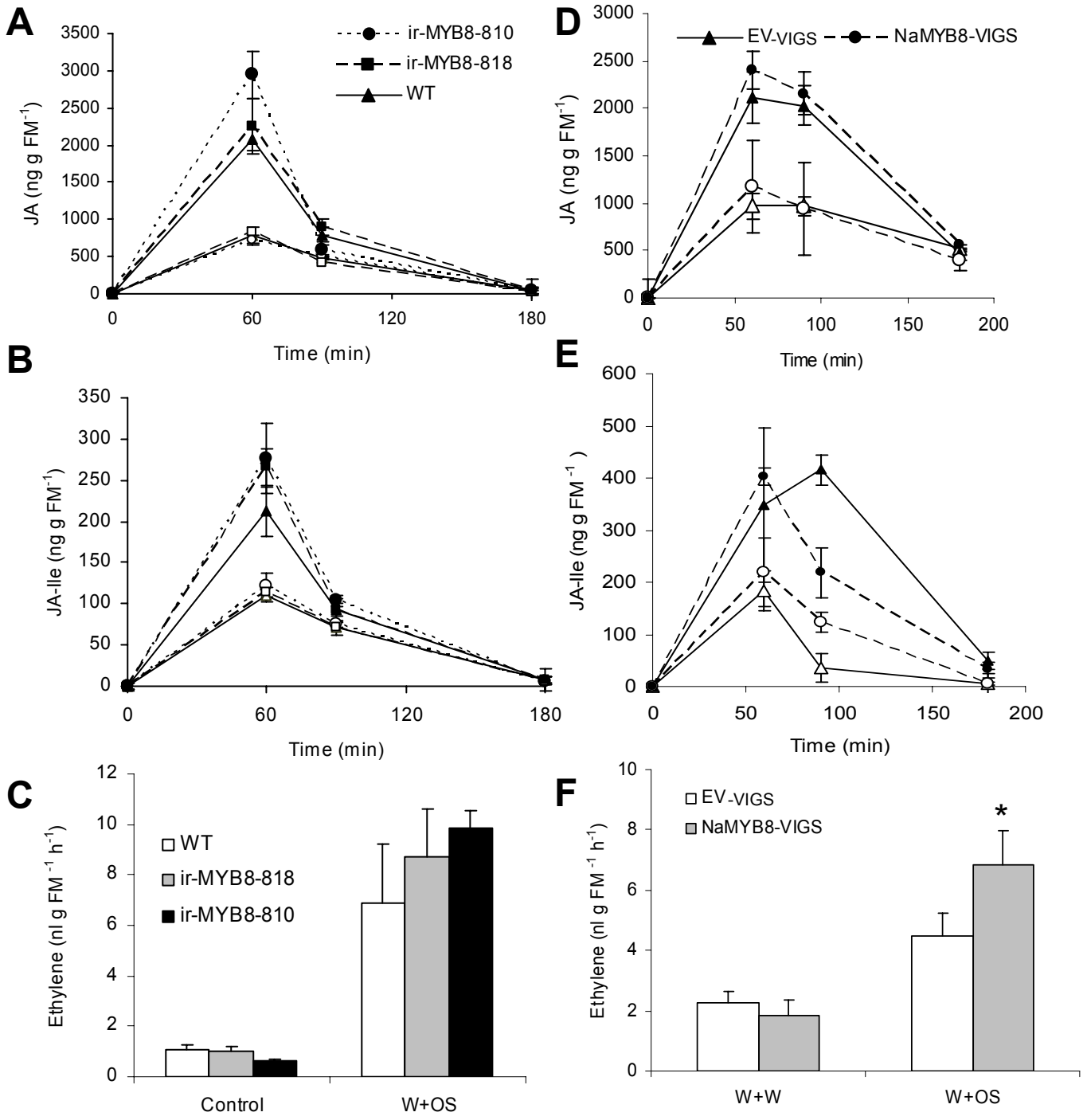
(A) Map of the transformation vector pSOL8MYB8. (B) Southern blot analysis of two independently transformed ir-MYB8 lines. A 10 µg of genomic DNA from each genotype was digested with EcoRI and Hind III and the hybridization was performed with 32P-labeled *hptII* (*h*ygromycin *p*hospho*t*ransferase) gene probe. Both lines harbored a single insertion of *hptII* gene, indicating that these transformed lines contained only a single copy of the T-DNA insertion. (C) Southern blot analysis with NaMYB8-specific probe shows that NaMYB8 is present as a single copy gene in *N. attenuata*'s genome.



Supplemental Figure S3

NaMYB8 is required for the accumulation of CP and DCS in *M. sexta* attacked leaves.

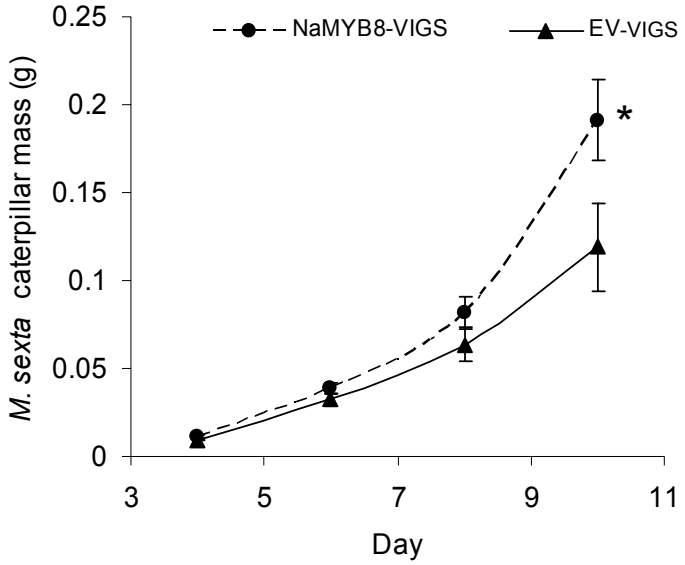
(A) Mean (± SE) silencing efficiency of NaMYB8 in VIGS-silenced plants. The concentrations of CP (B), DCS (C), CGA (D), rutin (E), nicotine (F) in the EV-VIGS and NaMYB8-VIGS leaves that were directly attacked by *M. sexta* caterpillars for 4 days were determined by HPLC coupled to a PDA detector. Asterisks represent significantly different concentrations of secondary metabolites accumulated between the EV-VIGS and NaMYB8-VIGS plants after similar treatments at $P < 0.05$ (*) and $P < 0.01$ (**; n = 5; FM, fresh mass). Inset represents the UV spectra of CP and DCS.



Supplemental Figure S4

Silencing NaMYB8 does not change W+OS-elicited phytohormone concentrations in the leaves.

A rosette leaf in (+1) position was wounded with a pattern wheel and 20 µL of either water or OS was applied to the wounds. Means (± SE) represent JA levels in (A) stably-silenced ir-MYB8 plants and (D) transiently-silenced NaMYB8-VIGS plants; JA-Ile levels in (B) stably-silenced and (E) transiently-silenced NaMYB8-VIGS plants, in the leaves harvested at indicated time points determined with LC-MS/MS (n = 5). The closed symbols represent W+W treatments and open symbols represent W+OS treatments. Mean (± SE) ethylene (ET) emissions from W+OS treated leaves of (C) stably-silenced ir-MYB8 plants and (F) transiently-silenced NaMYB8-VIGS plants, enclosed in 250-mL flasks and measured after 5 h with a photoacoustic laser spectrometer (n = 3; FM, fresh mass).



Supplemental Figure S5

***M. sexta* caterpillars perform better on NaMYB8-VIGS relative to the EV-VIGS plants.**

M. sexta neonates were placed on the stem leaf of either NaMYB8-VIGS or EV-VIGS plants and were allowed to feed without restricting the movement of caterpillars. Initial *M. sexta*'s caterpillars mass was recorded on 4th day of feeding, followed by recording mass every second day of the experiment ($P < 0.05$ (*); n = 15).