

Supplemental Figures

NaMYC2 transcription factor regulates a subset of plant defense responses in *Nicotiana attenuata*

Woldemariam et al.

A

>MYC2

ATACACACACAAACACTTCGCTACCCAAACAACTCTCTCCATTTTCACTC
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 CATTTTCTCTCTCTGATCAAGAATCAAAACAGATCTGAATTGATTTATCTGTTTTTTTC
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>MYC2L

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 CCGCACACCCCTTTTACCCCATTTCTCTCTCTATATATATATATATATCTTTT
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 CACTGCTGGAGATGCAATTTATATATTAATGAGCTGAAGTTGAAGCTTCAAAATACAG
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 AAGACTCATGGCGCCCTGGTCCCTCCACCAATCAAGATCACAAGATGTCTAGCCATACTG
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B

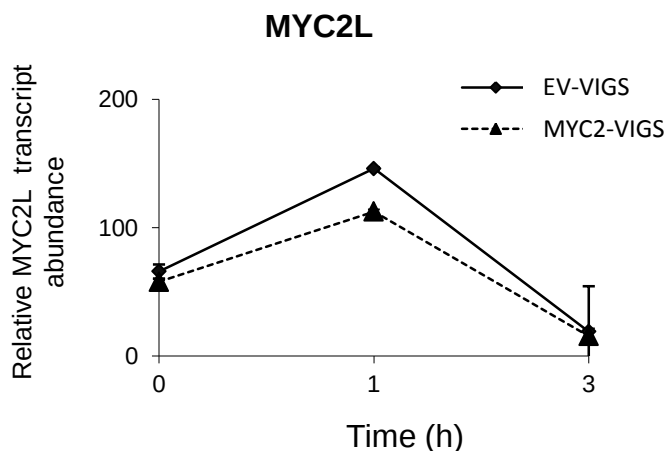


Figure S1. Nucleotide sequences and transcript abundances of MYC2-like transcription factor in MYC2-VIGS silenced *N. attenuata* plants. (A) Nucleotide sequences of *MYC2* and *MYC2-like* (*MYC2L*) genes in *N. attenuata*. (B) Transcript accumulation of NaMYC2 transcription factors was suppressed by Virus Induced Gene Silencing (VIGS; Fig. 2C) and the relative mean $\pm$ SE accumulation of *MYC2-like* transcripts (*MYC2L*, n=5) were determined before (0 h) and after WOS treatment (1 h, 2 h) in EV (solid line) and MYC2-VIGS (dashed) plants by qRT-PCR. GenBank Accession numbers: NaMYC2 (KC832837); NaMYC2-like (KC906192).

MYC2		1 ATACACACACAAACACTTCGCTACCCAAACAACACTCTCTCCATTTTCACT 	50
MYC2L		1 AT-CTTTCT---ACCTTCCGCTACCTT-----CTCTCTCCGCCG-CACA	39
MYC2		51 CACTCCTTATCACCAAACAATTCTTGGGTTTTTGAATATATACCCTAAAA 	100
MYC2L		40 CCCTC-TTTTCAC----CCATTATTCTCTCTCTCTATATATA-----	76
MYC2		101 AAGTTCCTCTCCATTTTCTCTCTGTATCAAGAATCAAACAGATCTGAA 	150
MYC2L		77 -----TATCTATATATCTTTCACG---CCACCAC TTCC-AA	108
MYC2		151 TTGATTTATCTGTTTTTTTTTCTGTGTTTTTGTTATATGGAATGACGGACT	200
MYC2L		109 CTG-----TTTGTGCTGGGTT-----TATGGACTGACTGATT	140
MYC2		201 ATAGAATACCAACGATGAATAATATATGGAGCAATACT---ACATCAGAC 	247
MYC2L		141 ACAGCTTACCCACCATGAAT---TTGTGGAATACTAGTGGTACTACCGAT	187
MYC2		248 GATAAC-----ATGATGGAAGCTTTTTTATCTTCTGATCCGTCGTCGTT 	291
MYC2L		188 GACAACGTTTCTATGATGGAAGCTTTTATGTCTTCTGATCTCACTTTCATT	237
MYC2		292 TTGGCCCGGAACAAC TACTACACCAACTCCCCGGACTTCAGTTTCT---- 	337
MYC2L		238 TTGGGCTACTTCTAATTCTACTGCTGCTGCTGTACCTCTAATTCTGATC	287
MYC2		338 -----CCGG-----CGCCGCCG-----CCG-----	352
MYC2L		288 ATATTCAGGTTAATACCCAACGGTTCTTCTCCGTCTTCTTGTGCTTCT . . .	337
MYC2		353 ---GTGACGAGTATTGCCGGAGACCCATTAAAGTCTATGCCATATTTTAA 	399
MYC2L		338 ACTGTCTACAGCTGTGGCTGTCTGATGCTTCAAAATCCATGCCTTTTTCAA	387
MYC2		400 CCAAGAGTCGCTGCAACAGCGACTTCAGACTTTAATCGACGGGGCTCGTG 	449
MYC2L		388 CCAAGAAACCCTTCAACAGCGCTTCAAAACCCTCATTGATGGTGCTCGCG	437
MYC2		450 AAGCGTGGACGTATGCCATATTTTGGCAATCGTCTGTGTGTGGATTTCGCG 	499
MYC2L		438 AGACATGGACCTATGCCATCTTTTGGCAGTCATCCGTCGTTGATTTAACG	487
MYC2		500 AGCCCCTCTGTGTGGGGTGGGGGAGATGGGTATTATAAAGGTGAAGAAGA 	549
MYC2L		488 AGTCCATTGTGTGGGGTGGGGGAGATGGTTACTACAAAGGTGAAGAAGA	537
MYC2		550 TAAAAATAAGCGTAAAACGGCGTCGTTTTTCGCTGATTTTATCACGGAGC 	599
MYC2L		538 TAAAGCCGGTCGGAAATTAGCTGTTTCTCTCCTGCTTATATAGCTGAGC	587
MYC2		600 AAGCACACCCGGA AAAAGGTTCTCCGGGAGCTGAATTCTTTAATTTCCGGC 	649
MYC2L		588 AAGAACACCCGGA AAAAGGTTCTCCGGGAGCTGAATTCGTTGATCTCCTGC	637
MYC2		650 ACACAAACTGGTGGTGAAAATGATGCTGTCTGATGAAGAAGTAACGGATAC 	699
MYC2L		638 ACGCAAACCGGGCACTG---ATGATGCCGTCGATGAAGAAGTTACCGACAC	687

MYC2	700	TGAATGGTTTTTCTGATTTCAATGACTCAATCGTTTGTTAACGGAAGCG 	749
MYC2L	685	TGAATGGTTCCTTATTTCCATGACCCAATCGTTTGTTAACGGAAGTG	734
MYC2	750	GGCTTCGGGGCTGGCGATGTACAGCTCAAGCCGATTTGGGTACTGGA 	799
MYC2L	735	GGCTTCGGGTGAGGCCTTATACAATTCCAGCCCCATTGGGTAGCCGGA	784
MYC2	800	GCAGAGAGATTAGCTGCTTCGCACTGCGAACGGGCCGACAAGCCCAAGG 	849
MYC2L	785	GCAGAGAAATTGGCAGCTTCCCACTGCGAACGGGCTCGGCAGGCCAGGG	834
MYC2	850	ATTCGGGCTTCAGACTATTGTTTGTATTCTTCAGCTAATGGTGTGTG 	899
MYC2L	835	ATTCGGGCTTCAGACGATGGTTGTATTCTTCAGCAAACGGCGTGGTTG	884
MYC2	900	AGCTCGGGTCAACTGAGTTGATATTCCAGACTGCTGATTTAATGAACAAG 	949
MYC2L	885	AATTGGGCTCCACGGAGTTGATTATTCAGAGTTCTGATCTCATGAACAAG	934
MYC2	950	GTTAAAGTTTTGTTTAAATTTAATATTGATATGGGTGCGACTACGGGCTC 	999
MYC2L	935	GTTAGAGTATTGTTTAACTTCAATAATGATTGGG-----CTC	972
MYC2	1000	AGGATCGGGCTCATGTGCTATTACGCTGAGCCCGATCCTTCGGCCCTTT . .	1049
MYC2L	973	TGGTTCG-----TGGGCTGTGCAGCCCGAGAGCGACCCGTCCGCTCTTT	1016
MYC2	1050	GGCTGACGGATCCGGCTTCCTCAGCTGTGGAAGTCAAGGATTCGAATACA 	1099
MYC2L	1017	GGCTCACTGATCCATCGCCTGCAGCTGTACAAGTCAAAGATTTAAATACA	1066
MYC2	1100	GTTCTTCAAGTAATAGTAGTAAGCAACTTGTGTTTGGAAATGAGAATTC 	1149
MYC2L	1067	GTTCCATCAAGTAATAGTAGTAAGCAAGTTGTGTTTGATAATGAGAATAA	1116
MYC2	1150	CGAAAA-----TGGTAATCA--AA-----ATTCTCAGCAA---ACAC . .	1181
MYC2L	1117	TGGTCACATTTGTGATAATCAGCAACAGCACCATTCTCAGCAACAACAC	1166
MYC2	1182	AAGGATTTTTCACTAGGGAGTTGAATTTTTCCGAATATGGATTTGATGGA 	1231
MYC2L	1167	AAGGATTTTTCACAGGGAGTTGAACCTTTTCAGAATTCGGGTTTGTGGA	1216
MYC2	1232	AGTAATACTCGGAATGGGAATGCAAATCTTCGCGTTCTTGCAAGCCCGA . .	1281
MYC2L	1217	AGTAGTAAT---AATAGGAATGGGAATTCATCAGTTCTTGCAAGCCAGA	1263
MYC2	1282	GTCTGGTGAAATCTTGAATTTTGGTGATAGTACTAAGAGAAGTGCTTCAA 	1331
MYC2L	1264	GTCGGGGGAAATCTTGAATTTTGGTGATAGCACTAAGAA-----AA	1304
MYC2	1332	GTGCAAATGGGAGCTTGTTTTCGGGCCAATCACAGTTCGGGCCCGGGTCT 	1381
MYC2L	1305	GTGCAAATGGGAACCTTATTTTCGGGTGAGTCCCATTTTGGGCGAGGG---	1351
MYC2	1382	GCGGAGGAGAACAAGAACAAGAACAAGAAAGGTCACCTGCATCAAGAGG .	1431
MYC2L	1352	---GAGGAGAATAAGAACAA-----GAAAAGGTCACCTGCTTCCAGAGG	1392

MYC2	1432	TAGCAACGATGAAGGGATGCTTTCATTTGTTTCGGGTGTGATCTTGCC--	1479
		. .	
MYC2L	1393	AAGCAATGAAGAAGGAATGCTTTCATTTGTTTCAGGTACAATCTTGCCCTG	1442
MYC2	1480	-AAGTTCAAACACGGGGAAGTCCGGTGGAGGTGGCGAT-----TCG	1519
		. .	
MYC2L	1443	CAGCTTCTGGTGCATGAAGTCAAGTGGATGTGTCGGTGAAGACTCCTCT	1492
MYC2	1520	GATCAATCAGATCTCGAGGCTTCGGTGGTGAAGGAGCGGATAGTAGTAG	1569
		. .	
MYC2L	1493	GATCATTCGGATCTTGAGGCCTCAGTGGTGAAAGAAGCTGAAAGTAGTAG	1542
MYC2	1570	AGTTGTAGACCCTGAGAAGAAGCCGAGGAAACGAGGAGGAAACCCGCTA	1619
		. .	
MYC2L	1543	AGTTGTAGAACCCGAAAAGAGGCCAAAGAAGCGAGGAAGCAAGCAGCAA	1592
MYC2	1620	ACGGGAGAGAGGAGCCGTTGAATCATGTGGAGGCAGAGAGACAAAGGAGG	1669
		. .	
MYC2L	1593	ATGGACGTGAGGAACCTTTGAATCACGTCAAGCAGAGAGGCAAGGAGA	1642
MYC2	1670	GAGAAATTAATCAAAGATTCTATGCACTTAGAGCTGTTGTACCAAATGT	1719
		. .	
MYC2L	1643	GAGAAATTAACCAAAGGTTCTACGCTTTAAGAGCTGTTGTTCCGAATGT	1692
MYC2	1720	GTCAAAAATGGATAAAGCATCACTTCTTGGTGATGCAATTGCATTATCA	1769
		. .	
MYC2L	1693	GTCCAAGATGGACAAGGCATCACTGCTTGGAGATGCAATTCATATATTA	1742
MYC2	1770	ATGAGTTGAAATCAAAGGTTCAGAATTCTGACTCAGATAAAGAAGATTG	1819
		. .	
MYC2L	1743	ATGAGCTGAAGTTGAAGCTTCAAAATACAGAAACAGATAGAGAAGACTTG	1792
MYC2	1820	AGGAACCAAATTGAATCTTTAAGGAAAGAATTAGC <u>CAACAAGGGATCAAA</u>	1869
		. .	
MYC2L	1793	AAGAGCCAAATAGAAGATTTGAAGAAAGAATTAGCTAGTGAAGACTCATG	1842
MYC2	1870	<u>CTATACCG</u> GTCTCCACCG--TCAA-ATCA--AGATCTC-----	1903
			
MYC2L	1843	GCGCCCTGGTCTCCACCAAATCAAGATCA <u>CAAGATGTCTAGCCATACTG</u>	1892
MYC2	1904	-----AAAATTCTAGATATGGATATCGACGTTAAGGTGATCGGATGGGAT	1948
		. .	
MYC2L	1893	GAAGCAAGATTGTAGATGTGGATATAGATGTTAAGATAATTGGATGGGAT	1942
MYC2	1949	GCTATGATTCTGTATACAATCTAATAAAAAGAACCATCCAGCCGCGAGGTT	1998
		. .	
MYC2L	1943	GCGATGGTTCTGTATACAATGTAATAAAAAGAACCATCCAGCTGCAAGGTT	1992
MYC2	1999	AATGGCCGCTCTCATGGAATTGGACTTAGATGTGCACCATGCTAGTGTTT	2048
		. .	
MYC2L	1993	AATGGTAGCCCTCAAGGAGTTAGATCTAGAAGTGCACCATGCCAGTGTTT	2042
MYC2	2049	CAGTGGTCAACGAGTTGATGATCCAACAAGCAACTGTGAAAATGGGAGT	2098
		. .	
MYC2L	2043	CAGTGGTGAACGATTTGATGATCCAACAAGCCACAGTGAAAATGGGTAGC	2092
MYC2	2099	CGGCTTTACACGCAAGAACAACCTCCGGATATCATTGACATCCAGAATTGC	2148
		. .	
MYC2L	2093	AGACTTTACACGGAAGAGCAACTTAGGATAGCATTGACATCCAGAGTTGC	2142

MYC2	2149	TGAATCGCGA TGAAGAGAAAT-ACAG-TAAATGGAAATTATCATAGTGAG	2196
			
MYC2L	2143	TGAAACACGCTAAAAACACTT GAGATCTCAATTGTAGG CTTTGAGTTAG	2192
MYC2	2197	CTCTGAATAATGTTATCTTTTCATTGAGCTAT-TTTAAGAGAATTTCTCCT	2245
			
MYC2L	2193	CCTTGTAATTTGTTCCAG-ACTATGCTAAATTTAAGAGC-TCTGTCTA	2240
MYC2	2246	ATAAGTTAGATCTTGAGTTTAAGGCTACTTAAAGTGGAAGCTGATTGAG	2295
			
MYC2L	2241	AT-----GTTTTGTGTCATCAAG-----TTAGAGATTAA---GATCAAG	2275
MYC2	2296	CTTCCCTCTTAGTTTTTGGGTATTTTCAACTTTTATATCTAGTTTGT	2345
			
MYC2L	2276	G---CTCTT---TTTGTGTTATTTTCCACTTGTA--CCTAGGA-----	2311
MYC2	2346	TCCACATTTTC-TGTACATAAATGTGAAACCAATACTAGATCTC AAGTTA	2394
			
MYC2L	2312	-----ATATTTCGTGTACATGATTGTTAAAAAAAAGTCGGGTGGTAGT--	2354
MYC2	2395	TGCTTTTTCATGTAATTAGAAATAAATATGCAGTTTCATCTTTTTC	2444
			
MYC2L	2355	-----TCTAGTTCATGTAA-----AAATTTGCAGCTCCATCCTCTCC	2391
MYC2	2445	TGT--TAAGGTCTGCTCC----ATCTATTTGTATTCTTTATCCAGAATA-	2487
			
MYC2L	2392	TTCAGTATTCTTTGTTTCAGGATATATATATGAAGAAGAAATTGATGGTTG	2441
MYC2	2488	-- 2487	
MYC2L	2442	GT 2443	

Red highlighted sequence was used for MYC2 silencing by VIGS

MYC2 primer pair is **underlined in bold blue**

MYC2L primer pair is **underlined in bold green**

Figure S2. Nucleotide alignment of MYC2 (KC832837) and MYC2-Like (MYC2L; KC906192) transcription factors from *N. attenuata* plants. The nucleotide sequences were aligned by EMBOSS Stretcher program (<http://www.ebi.ac.uk/Tools/psa/>). Sequence regions used for silencing of *MYC2* gene in MYC2-VIGS plants and qRT-PCR determination of transcript abundances were highlighted in color.]

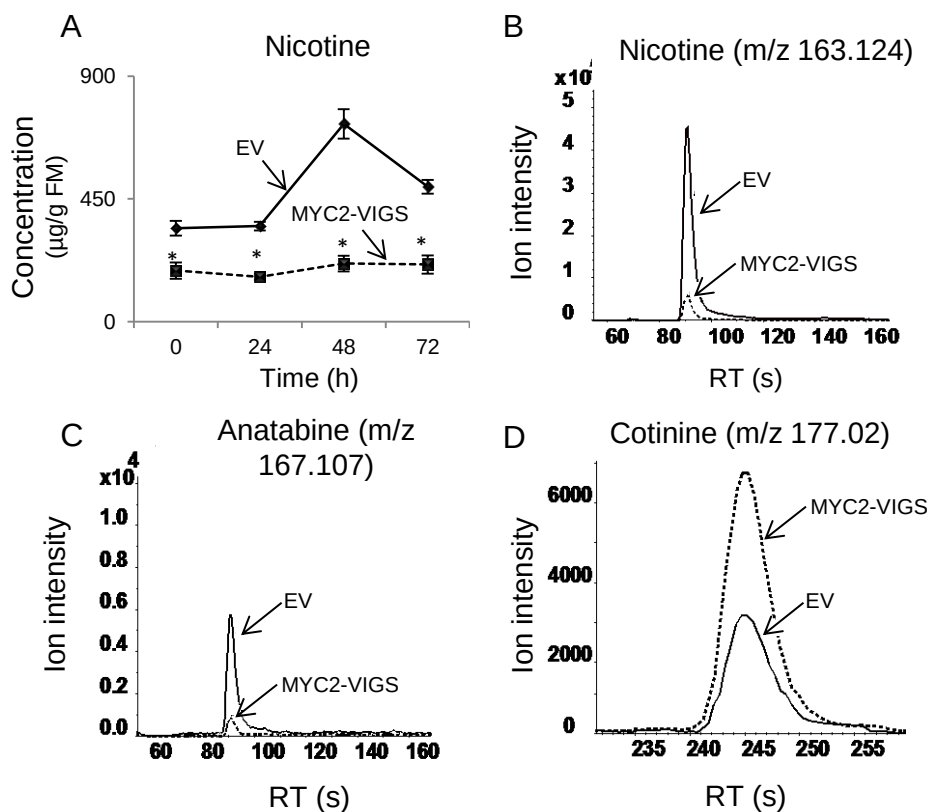


Figure S3. Targeted and untargeted analysis of nicotine and related alkaloids in EV and MYC2-VIGS plants. (A) Control and WOS-treated leaves ($n = 5$) of EV and MYC2-VIGS plants were collected and used to analyze the accumulation (mean $\pm$ SE) of nicotine. Statistically significant differences are indicated by asterisk. (B) to (D). Leaves ($n = 3$) of EV and MYC2-VIGS plants that were attacked by neonates of *M. sexta* for 4 d were collected to extract metabolites which were subsequently analyzed by HPLC-TOF-MS. The extracted ion chromatographs (EIC) for nicotine (B), anatabine (C) and cotinine (D) were overlaid to compare the regulation of alkaloid biosynthesis by MYC2.

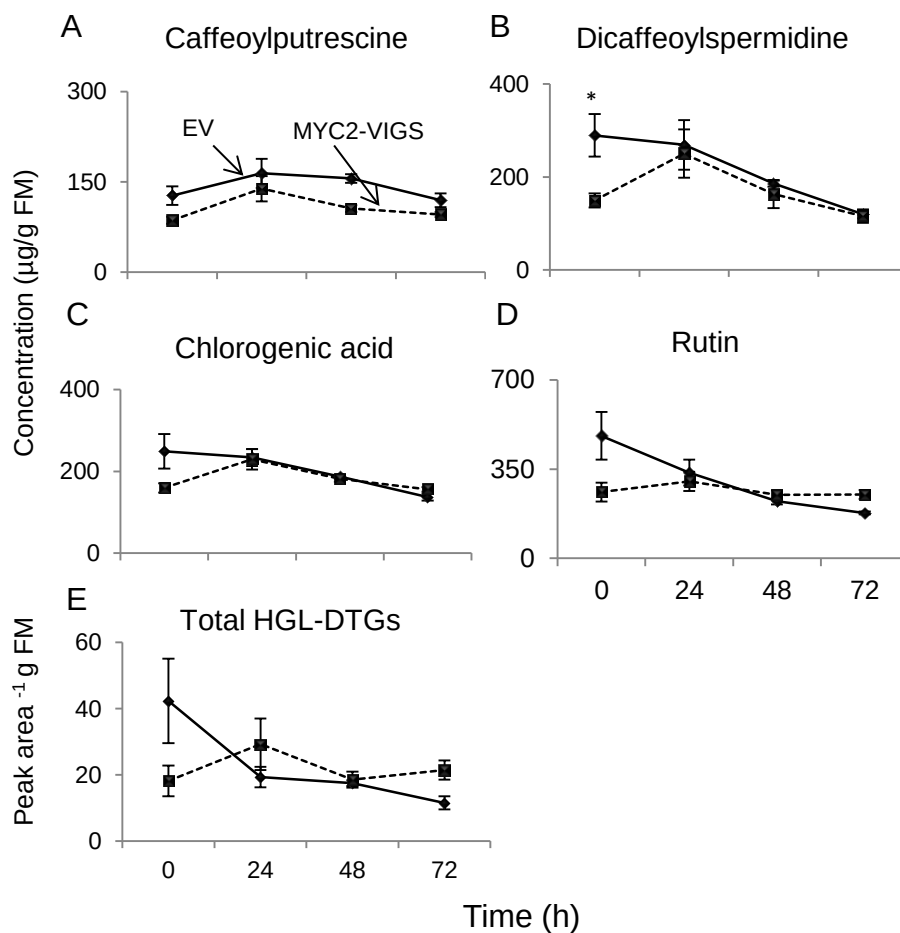


Figure S4. Secondary metabolites accumulation in EV and MYC2-VIGS plants before (0 h) or 24 , 48 and 72 h after WOS treatment. Control and WOS-treated leaves ($n = 3$) of EV and MYC2-VIGS plants were collected and used to analyze the accumulation (mean $\pm$ SE) of caffeoylputrescine (A), dicaFFEoylspermidine (B), chlorogenic acid (C), rutin (D) and total HGL-DTGs (E) on HPLC-PDA.

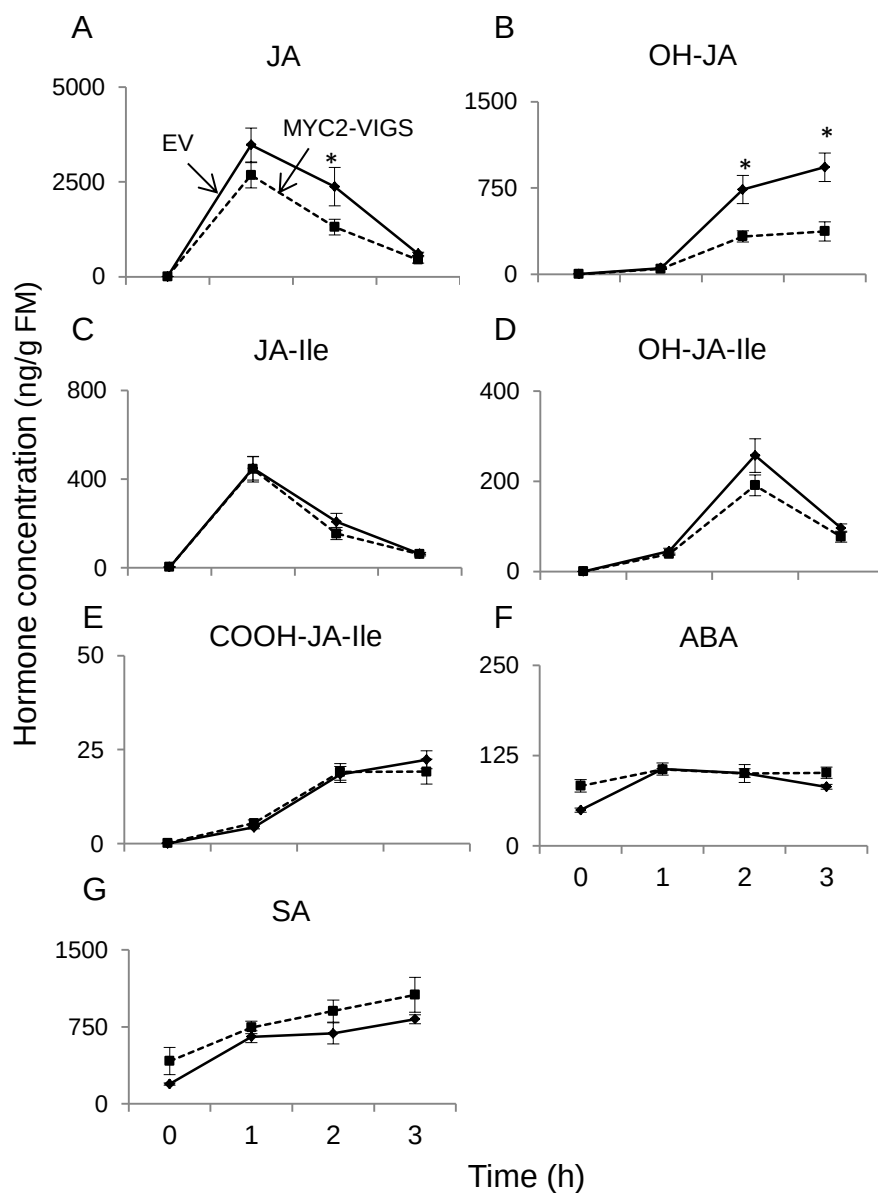


Figure S5. Accumulation of phytohormones in untreated and WOS-treated EV and MYC2-VIGS plants. Fully elongated leaves of EV and MYC2-VIGS plants were treated with WOS after 1, 2, and 3 h or collected without treatment and the accumulation of JA (A), OH-JA (B), JA-Ile (C), OH-JA-Ile (D), COOH-JA-Ile (E), ABA (F) and SA (G) was measured on LC-MS³. Differences that are statistically significant are indicated by asterisk (P < 0.05).