

The *Nicotiana attenuata* NaHD20 plays a role in leaf ABA accumulation during water stress, benzylacetone emission from flowers and the timing of bolting and flower transitions

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Supplementary Material

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NtHD20 KVVNNKNTRRRDEQIKSLIMFENETKLEP RIQLAREIGLQPRQVAI
NaHD20 KVVNNKNTRRRDEQIKSLPMFENETKLEP RIQLAREIGLQPRQVAI

NtHD20 FQNKRARWKQLERDYNIKSNFDNLASVNSLKKENQSLLQLOKLN
NaHD20 FQNKRARWKQLERDYNIKSNFDNLASVNSLKKENQSLFQ.LOKLN

NtHD20 DLMQKQREGEQYCSIGFDSYNRIENTIKNKEMEGKPSFDLSRGV
NaHD20 DLMQKEKEGCQYCSIGFDSYNRINNTIKNKEMEGKPSFDLSHGV

NtHD20 NGVISDDDSEKADYFC
NaHD20 NGVISDDDSEKADYFC

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Figure S1. Alignment of partial HD20 amino acid sequences from *N. tabacum* and *N. attenuata*.

ClustalW was used for pair-wise alignments using the Mega4 software. The parameters used were: gap open penalty: 10; gap extension penalty: 0.1; protein weight matrix: Gonnet; gap separation distance: 4. Only a partial coding sequence for NtHD20 is available (ET048311). NaHD20: *N. attenuata* HD20 (HM107874).

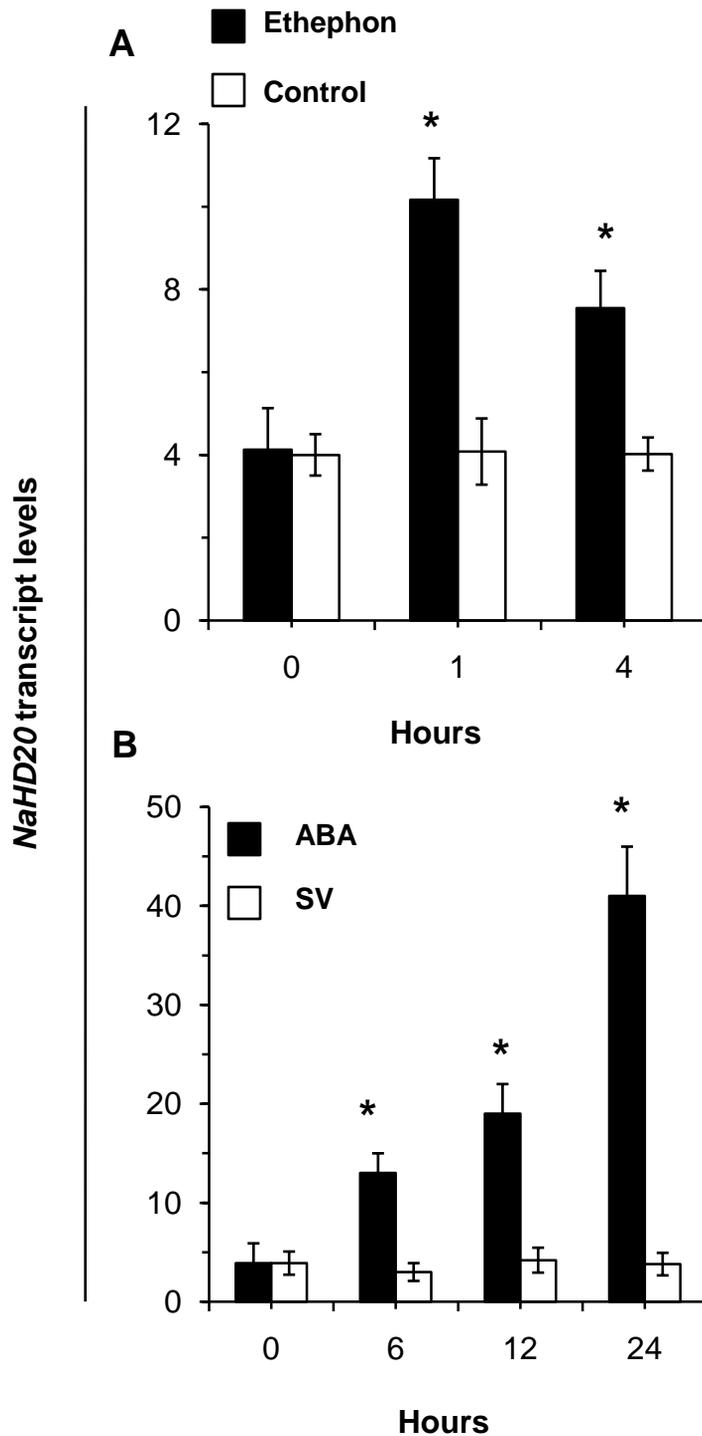


Figure S2. Analysis of *NaHD20* transcript levels in leaves after ethephon and ABA application.

Total RNA was extracted from leaves of rosette stage WT *N. attenuata* plants either treated with **(A)** 1 mM ethephon or 1 mM phosphoric acid (control) or **(B)** 300 μ M ABA or solvent (SV; 0.02% aqueous Tween-20) at different times. After cDNA synthesis, *NaHD20* mRNA levels were quantified by qPCR. *: Student t-test, $P < 0.05$ (control vs treatment); ($n=3$, bars denote \pm SE).

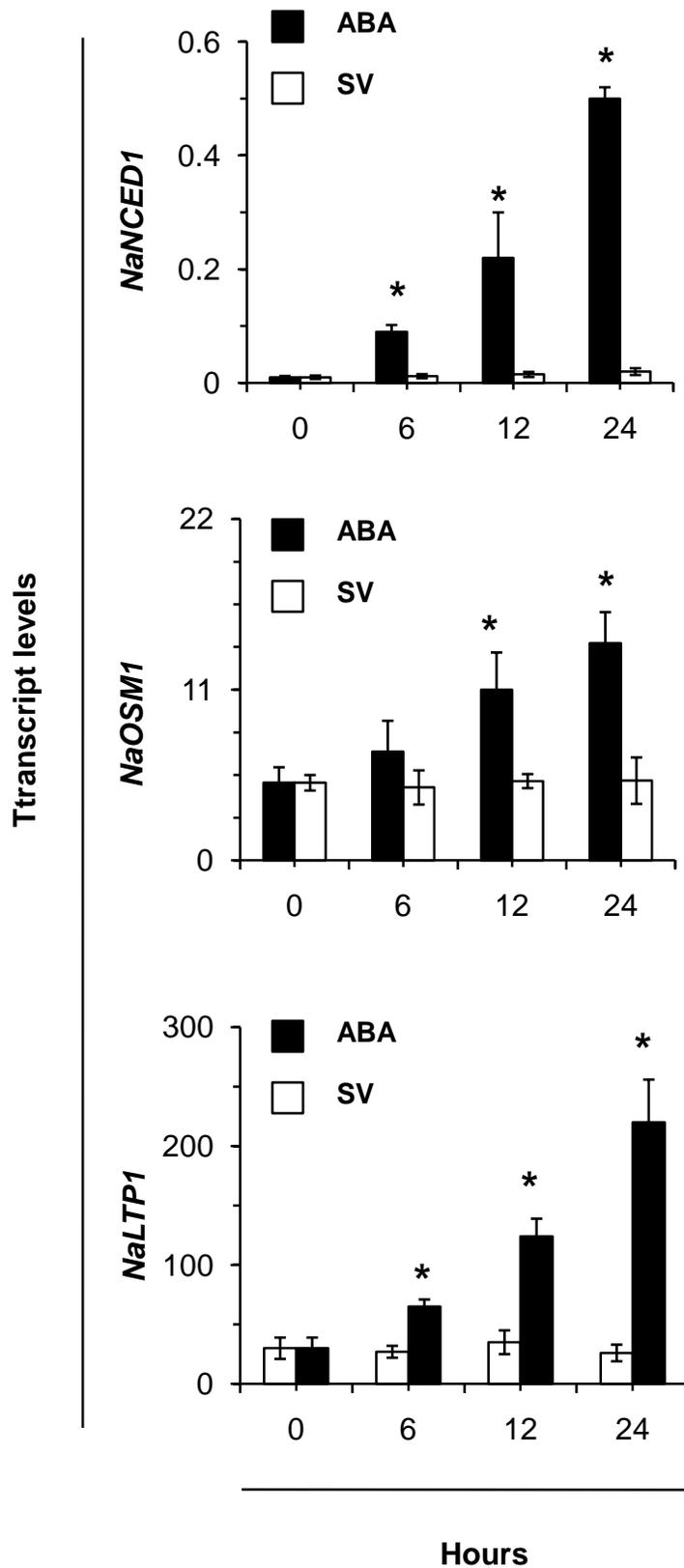


Figure S3. Leaf levels of *NaNCED1*, *NaOSM1* and *NaLTP1* after ABA treatment. Total RNA was extracted from leaves of rosette stage WT *N. attenuata* plants treated with 300 μ M ABA or solvent (SV; Fig. S2) for different times. After cDNA synthesis, *NaNCED1*, *NaOSM1* and *NaLTP1* mRNA levels were quantified by qPCR. *: Student t-test, $P < 0.05$ (control vs treatment); (n=3, bars denote \pm SE).

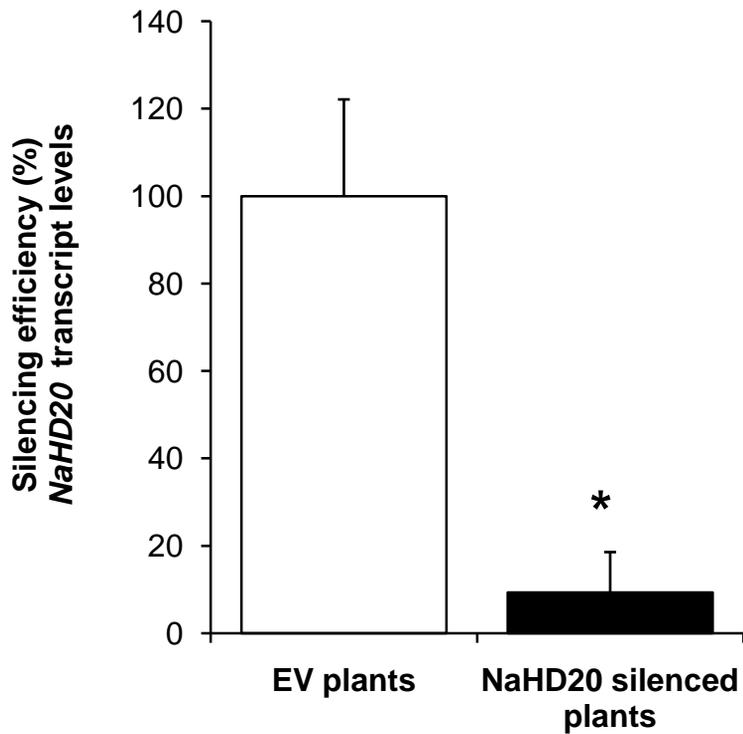


Figure S4. Silencing efficiency of *NaHD20* expression in *NaHD20*-VIGS-silenced plants.

Total RNA was extracted from leaves of EV and *NaHD20*-silenced plants and used for cDNA synthesis. *NaHD20* mRNA levels were quantified by qPCR. *: Student t-test, $P < 0.05$ (control vs treatment); (n=3, bars denote \pm SE).

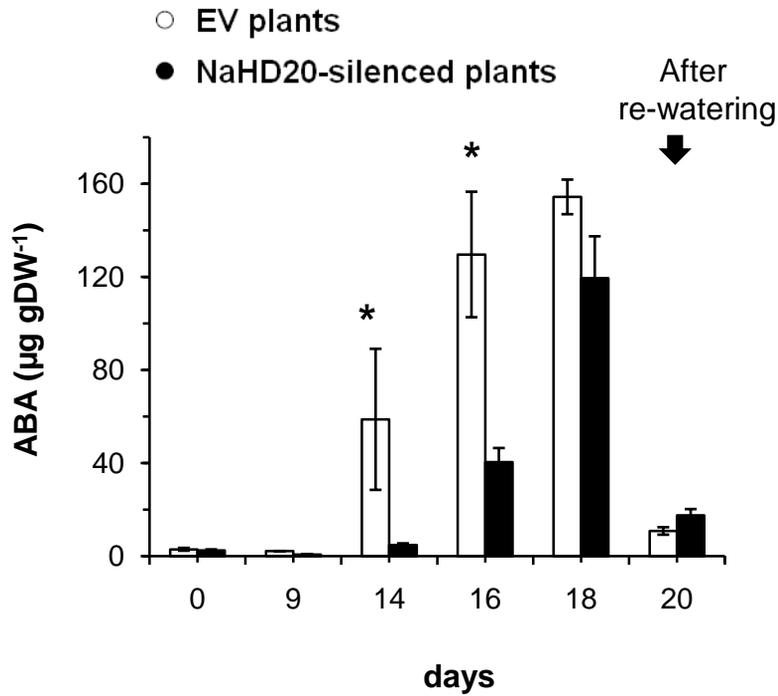


Figure S5. ABA accumulation in EV and *NaHD20*-silenced plants (gram dry weight⁻¹).

Rosette stage EV and *NaHD20*-silenced plants were water-stressed for several days and leaf tissue harvested at different times, extracted and ABA levels quantified by LC-MS/MS (see legend of Figure 4). The arrow marks the day of re-watering (day 18 after the beginning of the experiment). *: one-way ANOVA with Fisher's PLDS, $P < 0.05$ ($n=5$; bars denote \pm SE). gDW: gram dry weight obtained after freeze-drying the sample.

--○-- EV -○- EV (W+OS)

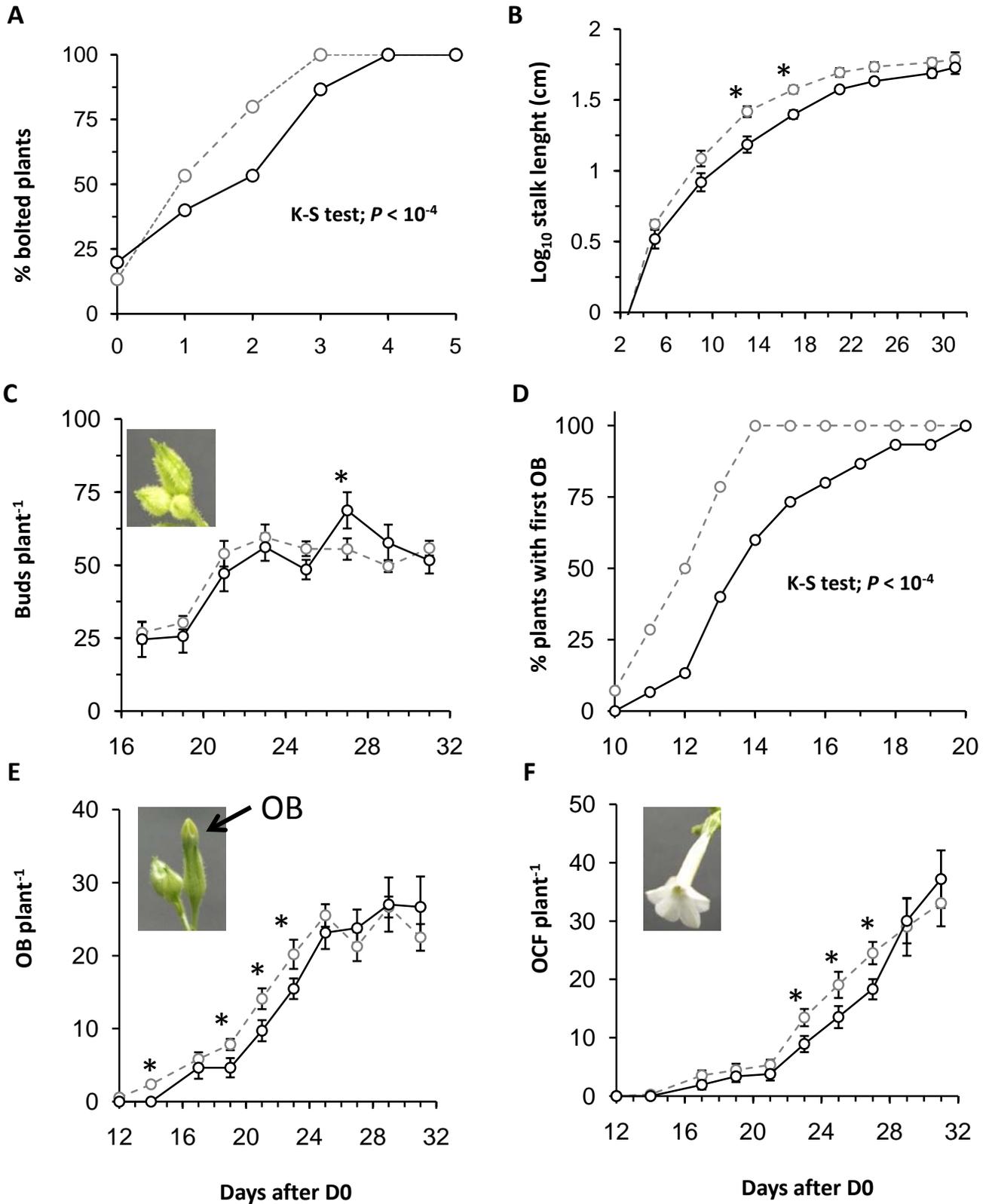


Figure S6. Analysis of reproductive phase change and flower transition in EV plants after OS elicitation.

EV and *NaHD20*-silenced plants were used for analysis but only the data for EV plants is shown. Plants were elicited daily with *M. sexta* OS for 11 consecutive days starting 3 days before bolting. Abbreviations: OB, opened buds; OCF, opened-corolla flowers. Distributions of bolted plants and plants with first OB were compared by two-sample Kolmogorov-Smirnov (K-S) test; $P < 10^{-4}$. *: Mann-Whitney *U*-test; $P < 0.05$ (control vs treatment). Each data point corresponds to data collected for 15 plants per genotype .

--o-- EV plants -o- EV plants (*P. syringae*)

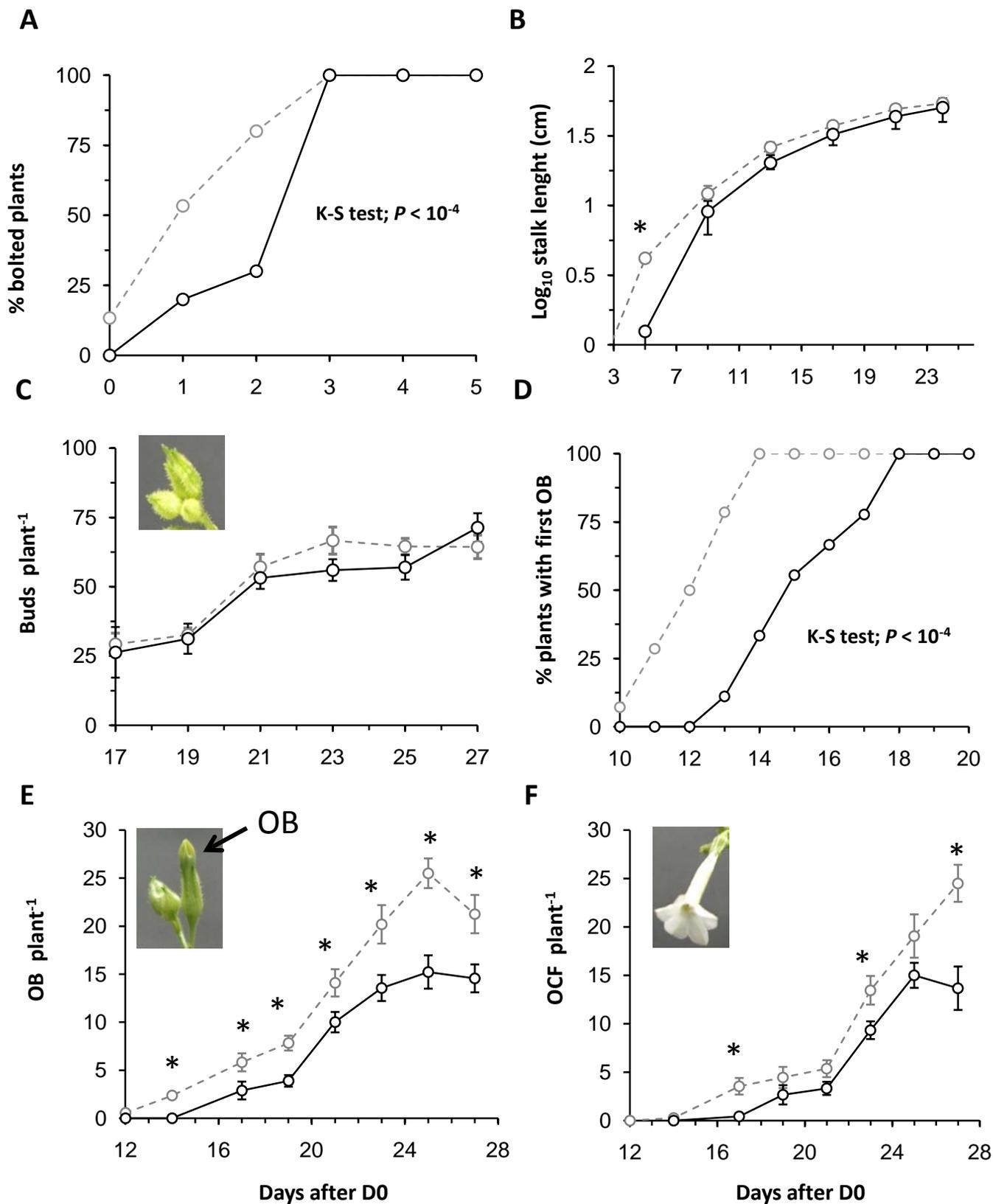


Figure S7. Analysis of reproductive phase change and flower transition in EV plants after *Pseudomonas syringae* infection.

EV and NaHD20-silenced plants were used for analysis but only the data for EV plants is shown. Plants were infected with *P. syringae* (see Materials and Methods) before bolting and subsequently analyzed. Abbreviations: OB, opened buds; OCF, opened-corolla flowers. Distributions of bolted plants and plants with first OB were compared by two-sample Kolmogorov-Smirnov (K-S) test; $P < 10^{-4}$. *: Mann-Whitney *U*-test; $P < 0.05$ (control vs treatment). Each data point corresponds to data collected for 15 plants per genotype.

Supplementary Table S1. Primer sequences used for cloning and real time qPCR.

NaHD20	cloning	F	AGAAGGTTAATAATAAGAATACGAGG
	cloning	R	AGCCGAATAATCAGCCTTTATGC
	qPCR	F	CCGAGAAAGAAGGTGGACAGTATTG
	qPCR	R	AGCCGAATAATCAGCCTTTATGC
	VIGS cloning	F	GCGGCGGTGACAGCAACTCGAGAGGGATTACAAC
	VIGS cloning	R	GCGGCGGGATCCCTGTCATCATCTGAAATTACACC
	silencing efficiency	F	GAGCAGATTAATCATTAGAACCC
	silencing efficiency	R	GGGAAGCAAGATTGTCAA AATTGG
	3'RACE	F	CCGAGAAAGAAGGTGGACAGTATTG
	5'RACE	R	GGGAAGCAAGATTGTCAA AATTGG
NaHD48	cloning	F	CATGCAATCTTGAATATCAGAGC
	cloning	R	ATCAGATTCTGTCCCAAGTAGTC
	qPCR	F	ATGAATCCCTCCTCATCCAGTTGC
	qPCR	R	ATCAGATTCTGTCCCAAGTAGTC
NaHD47	cloning	F	CATTTTCATGCCCTTTTGCTGTC
	cloning	R	CCTTGAACTCATTGTATTTGTATC
	qPCR	F	CCGAACAGCACAATTTCAAGCG
	qPCR	R	AGGATTGAGGGTGCTGTGCTC
NaHDkl	cloning	F	AGTCTTAGCCTCAGTTTTCCAG
	cloning	R	ACACGTTACATGAAGGGCAC
	qPCR	F	AGGGCAAGGACAAAGTTGAAGC
	qPCR	R	ACACGTTACATGAAGGGCAC
NaHD49	cloning	F	ACTAGTGATTCTAATGCTAGCG
	cloning	R	GATTCAGACTTATAATAGGATC
	qPCR	F	CCTTCACCAATCCATCAGCAGCTTG
	qPCR	R	TGATTGATTAGTGACCTTAATTC
NaNCED1	qPCR	F	ACAGCCGACCCACGTGTCCA
	qPCR	R	CGACAAGCGTAACTTGCGGAGC
NaOSM1	qPCR	F	CTGCGACTATCGAGGTCCGAAAC
	qPCR	R	GTACCTCGTGGTGCATTGATCAC
NaLTP1	qPCR	F	GCTAACGCCCCATGCAGACGCT
	qPCR	R	GTGCACCGTGGAGCAGTCAATGG
SMART II A	Clontech 5'RACE	F	AAGCAGTGGTATCAACGCAGAGTACGCGGG
5'RACE CDS	Clontech 5'RACE	R	5'-(T) ₂₅ V N-3' (N = A, C, G, or T; V = A, G, or C)
LUPM	Clontech 5'RACE	F	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT
SUPM	Clontech 5'RACE	F	CTAATACGACTCACTATAGGGC

Supplementary Table S2. Partial cDNA sequences of additional HD-Zip type I and II transcripts expressed in *N. attenuata* leaves.

NaHD20 (Subfamily I)	GATATGTTTGATGGAGGGGAATTTCTTGTACTTCTTCAGCAGCTGCTCTTAATTCTGCAGAATGTTTCAGTAGTAGCAGCTTTAGCAGT TTACCATCCTCAAAGAAGAAGAAGGTTAATAATAAGAACACGAGGAGGTTTCAGCGATGAGCAGATTAAATCATTAGAAACCATGTTTCGAG AACGAGACTAAATTTGGAACCAAGAAAGAAACAGCAGTTAGCACGAGAACTGGGGTTACAACCTCGTCAGGTTGCAATTTGGTTTCAGAAT AAGAGAGCTCGATGGAAATCAAAGCAACTCGAGAGGGATTACAACATACTTAAGTCCAATTTTGACAATCTTGCTTCCAGTACAACCTCC TTAAAGAAAGAAAACCAATCCTTGCTTTTCCAGTTGCAAAAGCTGAATGATCTGATGCAGAAATCCGAGAAAGAAGGTGGACAGTATTGT TCAATTTGGCTTTGATCAGGAGTCGTATAACAGAGACAATAACTATTAAGAATAAGGAAATGGAGGGGAAGCCAAGTTTGTCAATTTGAC TTATCAGAGCATGGAGTTAATGGTGTAATTTTCAGATGATGACAGTAGCATAAAAGCTGATTATTTCCGGCTTGGATGAAGAACTGTATCAT TTACTGAAAATGGTAGAAGCAGGGGATAGTTCTTTAACTTCCCCTGAAAACCTGGGGTACCCTAGAGGATGATGGTCTCTTGAACCCAGCAG CCTAATGGTTGTAATTATGATCAGTGGTGGGATTTCTGGCTTGAACCATAATTAATTATTGCACCATAGACAAAAATATACCCATCTAAA CGTTGGCTTTGAGGGGAAGTTTATAACATATGATCAGATGCCAGCGTCTGTACATTTGAGCACCTCAACGTCCACCAATCTGTACCAT AAGTGGCTGAGTGCATAGTTGAGTTTGCCCTCAGCTTAGGGTCATAGCACTACTATGGAGAGAAAAAACTGAATGCTTTTACCATAATAG AGGCTTTGCTATCAGAAGCCCTTTTCTACTGGAGATTTGTAGTATCGCTCAATCTTGT
NaHD48 (Subfamily I)	CATGCAATCTTGAATATCAGAGCAGNAAAACAAAGGAAAAGAAGAAATGCTGTAGGAGAAAAGGCTTCCAACAAACTCCAGCATGTGGAGT CAATCATCCTTATTAGGAAAGGATCTAAATCTGCACCTCTATGTCAAGTATTAAGAATTGCAAGGACAACATGCGAAGAAGATTCAACGAC GAGCAAATTCGATCACTAGAACACATGTTTGAGACAGAAGCAAGACCTGAACTGAGAACAAAAACAGCAGTTGGCTAAAAGCTTAGGCCTA CAGCCGCGCCAAGTTGCTATATGGTTTTCAGAACAGAGAGCTAGATCAAAGTCCAAGCAACTTGATATGGAGTATAGAATGCTTAAAAATC AGTTATGATAACCTTGGTTCCAAGTATGAAATGCTCAAAAAAGAGAATGAATCCCTCCTCATCCAGTTGCAGAGACTTAGAAAAGTTAACT GAAAAGGGTGGCAATGAAGAGATTCAGAATGAAGGCAATAAATTAGACACAGAGGTGATCAAGTCAGAGTTCATACCAGAAAACCTTCGAGC CCTGAATTTGGCTTACCTTTGTGTGAAGACAGTAGGGAATTGACTACTTGGGGACAGAATCTGAT
NaHD47 (Subfamily II)	CATTCATGCCCTTTTGTCTGTCTGTCAATTTTAGTAACCAAAAGTGAAGGAAATTAATTATATTAATAAATGACCTTTTCCCACCTTT AAATCTTTTGATATGATTGCAGATCGGAACCTGGAGACATGCAGAGTGGAGACGAGGACTTTTCTAAAGGGAATCGACGTGAACCGGCT GCCGGCAACGCGGATGCAGAGGAAGAAGCCGGCGTATCGTCTCCGAACAGCACAATTTCAAGCGTGAGTGGCAATAAGCGAAAATGACAG AGAACCAAACCTGTGACGAGCATGAAATGGAAAGGGCTTGCTCTAGAGGCATCAGCGACGAAGAGGACGGAGAAAACCTGTAGAAAAAACT CCGTTTAACTAAGGAACAGTCCGGCCGTTCTTGAAGATAACTTCAAAGAGCACAGCACCCCTCAATCCTGTAAGTTAACTATTTAATTAGAC CATCATTATTAGATACAATAACAATGAGTTCAAGG
NaHDkl (Subfamily II)	AGTCTTAGCCTCAGTTTTCAGNATAATAACAAGNAATAGTACTACAACCAATAATCCTTTGCATCATCAGCTTAATCTCATCCCCTCCAT CTTACCTTTTAAATCTCTTTTCAAAAACCTTCTTGGACTGATCTTTCCCTTCTCAGATCGGAACTCGGAGACATGCAGAGTGGAGACGA GGACATTTCTGAAGGGAATAGACGTGAACCGGCTGCCGGCAGCGGCGACCGCATGAAGAAGCCGGCGTATCATCACCTAACAGCACCA TTTCGAGTGTGAGTGGAAATAAGAGAAGCGAAAGAGAGACAAATAACTGTGAGGAGCATGAAATGGAAAGAGCTTCTGATGAAGAAGATG GAGAAAACCTTAGAAAAGAACTTCGGCTTTTCGAAAAGACCAATCTGCTGTTCTTGGAGGACTTTCAAAGAACACAACACTCACAACTCTA AACAAAAGCTGGCTTTGGCTAAAAGACTGGGATTGAGACCTAGGCAAGTAGAGGCTGTTTTCAGAACAGGAGGGCAAGGACAAAAGTTGA AGCAAACAGAAGTTGACTGTGAATTTTGAAGAGATGTTGTGAGAATTTGACAGAGAAAACAGGAGATTGCAGAAGGAAGTTCAAGAGT TGAGGGCACTTAAGCTTTTCACTCAATTTTACATGCAAATGACTCCTCCTACCACCTTACCATGTGCCCTTCATGTGAACGTGT
NaHD49 (Subfamily II)	ACTAGTGATCTAATGCNAGCGTAAAAAGGAGAGAGNCGCTGGTAGTGAAGAGACANCGACTGAGGTAGAGAGACTTTCCTCCAGAGTT AGCGATGAAGATGATGATGGTTCTAACGCTAGGAAGAACTTAGGCTCAGTAAAGCACAATCTGCTCTTTTAGAGGAAAGCTTCAAGCAA CACAGCACTCTCAATCNTAAGCAAAAACAGGACTTAGCCAGGGATCTGAATCTAAGGCCTCGTCAGGTTGAAGTCTGGTTCCAGAACAGA AGAGCCAGAACAAAGCTGAAGCAAACAGAGGTAGACTGTGAGTTCTGAAAAAATGTTGCGAGACGCTAACAGAGGAAAACAGGAGGCTT CACAAAGGAATTGCAAGAAGTGAAGGCTGTAATAATAGCGCAACCTTTGTACATGCAGCTGCCGGCGCCACGTTAACCATGTGTCTTCT TGCGAGAGGATCGCGCGCTCGGAGAAAATCCATCGAAGAATCCATTCACCTTTGGCTCAGAAGCCTCACTTCTACAATTCCTTACCAAT CCATCAGCAGCTTGTAAAGTAGAGGGCATATATAAATAATATACTCTCTAATAGTATATGATATCAATATCATCTTTAGAATATCTCCA AAGCCGCCACTTTTGGTTAAGGCCTTGTATGAAATCAACTGACTAGTGCAGTTGTATGTGTAGAGTTAGAATTAAGGTCACATAATCA ATCATTTTAAATTTGTTTCTTATGATCCTATTATAAGTCTGAATC