

Two mitogen-activated protein kinase kinases, MKK1 and MEK2, are involved in wounding- and specialist lepidopteran herbivore *Manduca sexta*-induced responses in *Nicotiana attenuata*

Maria Heinrich, Ian T. Baldwin, and Jianqiang Wu

Supplementary material

Supplementary Table S1 Primers used to clone the open reading frames of *NaMKK1* and *NaMEK2* in *N. attenuata*

Primers	Sequences (5'-3')
NaMKK1 ORF 1	ACCCGACCCTGTTTACCCATC
NaMKK1 ORF 2	AATTAAGCTTGCACGAACTGTC
NaMEK2 ORF 1	CAATCAATCAATCATGCGACC
NaMEK2 ORF 2	CCCATCATCCCCATTAATAGA

Supplementary Table S2 Primers used to clone partial *NaMKK1* and *NaMEK2* into pTV00 to obtain VIGS constructs

Primers	Sequences (5'-3')
NaMKK1-VIGS-BamHI	CTGAG <u>GGATCC</u> GCAGCGGATATTTGGAGT
NaMKK1-VIGS-HindIII	GTCAA <u>AGCTT</u> CTGTAAACAACACTCGATAA
NaMEK2-VIGS-HindIII	GTCAA <u>AGCTT</u> CCTCGTGGTTACCGTAGA
NaMEK2-VIGS-ClaI	ATCG <u>ATCGAT</u> CTCCGCCCTTCTTCCT

Note: bold and underlined nucleotides are restriction sites for cloning into pTV00 vector

Supplementary Table S3 GenBank accession numbers or Swiss-Prot accession numbers of the MAPKKs for phylogenetic analysis

Gene names	Accession numbers	Gene names	Accession numbers
NaMEK2	HQ023234	OsMKK1	EF5296231
NaMKK1	HQ023235	OsMEK2	Os06g05520
AtMKK1	At4g26070	OsMKK3	Os06g27890
AtMKK2	At4g29810	OsMKK4	Os02g54600
AtMKK3	At5g40440	OsMKK5	Os06g09180
AtMKK4	At1g51660	OsMEK1	Q5QN75.1
AtMKK5	At3g21220	NtMEK2	BAE97401
AtMKK6	At5g56580	NtSIPKK	AAF67262
AtMKK7	At1g18350	NtNPK2	BAA06731
AtMKK8	At3g06230	NbMKK1	AB243987
AtMKK9	At1g73500	NbMEK2	AB360636
AtMKK10	At1g32320		

Note: the species of origin of the MAPKKs are indicated by the two letters in front of the protein names; At, *Arabidopsis thaliana*; Na, *Nicotiana attenuata*; Nb, *Nicotiana benthamiana*; Nt, *Nicotiana tabacum*; Os, *Oryza sativa*.

Supplementary Table S4 Primers used for qPCR

Primers	Sequences (5'-3')
NaMKK1-Forward	ATC CCG AGA TTC GCC GTC AA
NaMKK1-Reverse	TCG AGT GTG CCG GAG TTC AT
NaMEK2-Forward	GAG AGT GCT GGC ACA AAC TAT
NaMEK2-Reverse	TCC ATA TAT CTC CAG CAT ACC C
NaEF1a-Forward	CCCACTTCCCACATTGCTG
NaEF1a-Reverse	CGCATGTCCCTCACAGCAA

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1 -----MALVRERRQLNLRLPLLPEPSER-RPR- ---FPLLPILP-----PSSI C NbMKK1
1 MRPLQPPPPAAAAATT-SSSTTASMPPPPSRNRRRRRTDLTLPLLPQRDPALAVPLLPLLPIL NtMEK2
1 MRPLQPPPPAAAAATTTSSSTTASMPPPPSRNRRRRRTDLTLPLLPQRDPALAVPLLPLLPIL NaMEK2
1 -----MALVRERRQLNLRLPLLPEPSER-RPR- ---FPLLPILP-----PSSI S NaMKK1

37 TTNSTANTATTTASTTTIS SELEKLKVLCHCNCGTVYEVRHKRTSSAIYALKVVHCDSDP NbMKK1
60 SAPSSSSSSSSSSPLPILPLNFSELERINRICSCACGTVYKVLHRPTIGRLYALKVIYCNHED NtMEK2
61 SAPSSSSSSSSSSPLPILPLNFSELERINRICSCACGTVYKVLHRPTIGRLYALKVIYCNHED NaMEK2
37 TTNSAAANTTTTTAPTTTIS SELEKLKVLCHCNCGTVYKVRHKRTSSAIYALKVVHCDSDP NaMKK1

97 EIRRCLREISLLRRTDSPYVIKCFGVIDMPCGDIGILMEYMNVCTLESLLKSQATFSEL NbMKK1
120 SVRLCMCREIELLRDVDNPNVRCHDMFDHNC-EIQVLLEFMDKCSLEGIHIPK----ES NtMEK2
121 SVRLCMCREIELLRDVDNPNVRCHDMFDHNC-EIQVLLEFMDKCSLEGIHIPK----ES NaMEK2
97 EIRRCVLREISLLRRTDSPYIIKCFGVIDMPCGDIGILMEYMNSCTLESLLKSHATFTEL NaMKK1

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175 ALSDLTRCVLSLGLYLLRRKIVHRDIKPSNLLINSRREVKIADFGSRVLACTLDPCNSS NtMEK2
176 ALSDLTRCVLSLGLYLLRRKIVHRDIKPSNLLINSRREVKIADFGSRVLACTLDPCNSS NaMEK2
157 SLAKIAKCVLSLGLDYLLNHKIIHRDLKPSNLLVNREMEVKIADFGVSKIMCRTLDPCNSY NaMKK1

217 VGTCAYMSPERFDPGTYGVNYNGYAADIMSLGLTLMELYICHFFPLPPCQRPDWATLMCA NbMKK1
235 VGTIAYMSPERINTDLNHGQYDGYAGDIMSLGVSILEFYLGRFFPS-VCRSGDWASLMCA NtMEK2
236 VGTIAYMSPERINTDLNHGQYDGYAGDIMSLGVSILEFYLGRFFPS-VCRSGDWASLMCA NaMEK2
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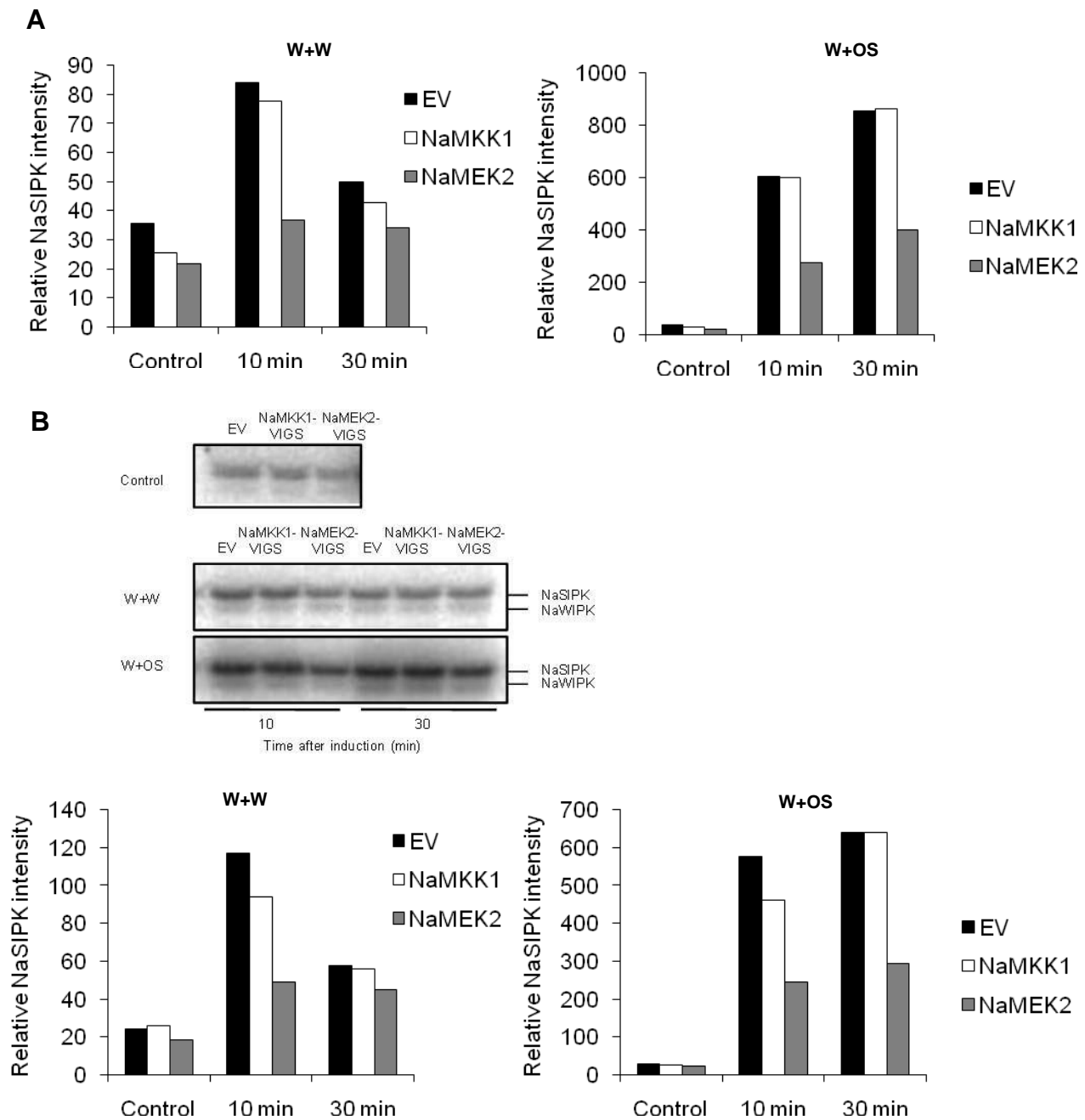
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294 ICMSQPEAPANASREFFRDFIACCLCQRDPARRVTAVQLLRHPFITQNSPAATTT-GNMMP NtMEK2
295 ICMSQPEAPANASREFFRDFIACCLCQRDPARRVTAVQLLRHPFITQNSPATTTTTGNMMP NaMEK2
277 ICFGEPPSLPEGTSGNFKDFIECCLCKESSKRVSAQCLLQHPFILSNLK. NaMKK1

325 LPNQVHQPAHQLLPPPHFSS NbMKK1
353 LPNQVHQPAHQLLPPPHFSS NtMEK2
355 LPNQVHQPAHQLLPPPHFSS NaMEK2
326 LPNQVHQPAHQLLPPPHFSS NaMKK1

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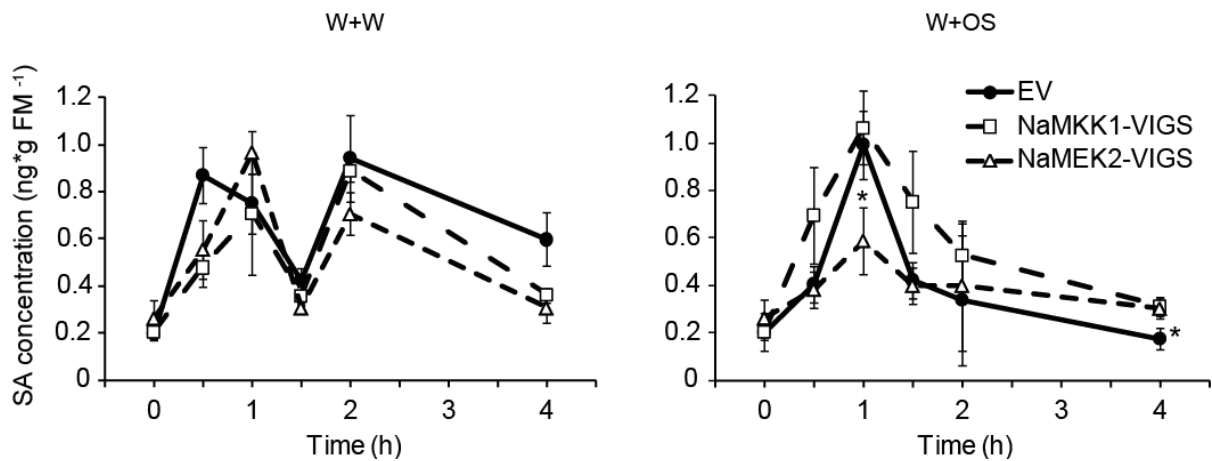
Supplementary Fig. S1 Alignment of the protein sequences of *N. attenuata* NaMKK1 and NaMEK2 with *N. benthamiana* NbMKK1 and *N. tabacum* NtMEK2.

Protein sequences of NaMKK1, NaMEK2, NbMKK1, and NtMEK2 were aligned using ClustalW algorithm. Shaded amino acid sequences represent residues that match the consensus sequence. The conserved motif of plant MAPKKs [S/TxxxxxS/T] are highlighted with green frames (two consecutive motives).



Supplementary Fig. S2 Wounding- and simulated herbivory-induced MAPK activity in EV, NaMKK1-VIGS, and NaMEK2-VIGS plants.

N. attenuata plants were wounded with a fabric pattern wheel and 15 μ l of water or *M. sexta* oral secretions (OS) were applied immediately to wounds (W+W and W+OS respectively); untreated plants served as controls. In-gel kinase activity assays were performed to determine the activity of NaSIPK and NaWIPK. (A) The band intensities of NaSIPK in Fig. 3B. (B) In-gel MAPK activity assay (upper panel) and NaSIPK band intensities (lower panel) obtained from an independently repeated experiment.



Supplementary Fig. S3 Salicylic acid accumulation in EV, NaMKK1-VIGS, and NaMEK2-VIGS plants after W+W and W+OS treatment.

Rosette leaves of EV, MKK1-VIGS, and MEK2-VIGS plants were wounded with a fabric pattern wheel and 15µl water or *M. sexta* oral secretions (OS) were applied to wounds. Samples were harvested after indicated times and SA levels were analyzed with LC/MS method. Asterisks indicate significant differences between EV and NaMKK1-VIGS or NaMEK2-VIGS plants (*t*-test; *, $P < 0.05$; $N = 5$).