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

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## 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 | CTGA**GGATCC**GCAGCGGATATTTGGAGT |
| NaMKK1-VIGS-HindIII | GTCA**AAGCTT**CTGTAAACAACACTCGATAA |
| NaMEK2-VIGS-HindIII | GTCA**AAGCTT**CCTCGTGGTTACCGTAGA |
| NaMEK2-VIGS-ClaI | ATCG**ATCGAT**CTTCCGCCCCTTCTTCCT |

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

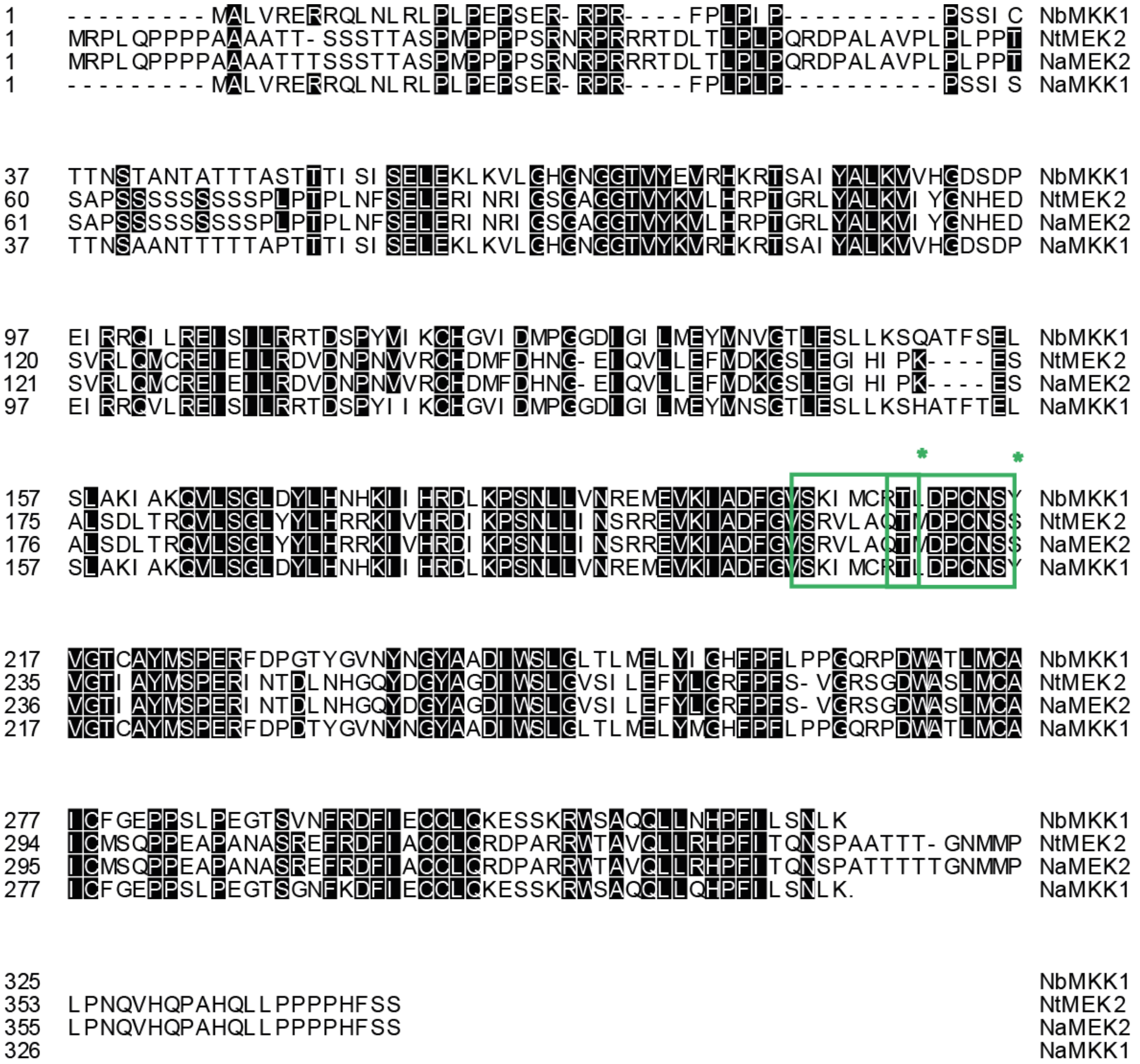
**Supplementary Table S3** GenBank accession numbers or Swiss-Prot accession numbers of the MAPKKs for phylogenetic analysis

| 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 | CCACACTTCCCACATTGCTG |
| NaEF1a-Reverse | CGCATGTCCCTCACAGCAAA |



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1     ---------MALVRERRQLNLRLPLPEPSER-RPR----FPLPIP----------PSSIC  NbMKK1
1     MRPLQPPPPAAAATT-SSSTTASPMPPPPSRNRPRRRTDLTLPLPQRDPALAVPLPLPPT  NtMEK2
1     MRPLQPPPPAAAATTTSSSTTASPMPPPPSRNRPRRRTDLTLPLPQRDPALAVPLPLPPT  NaMEK2
1     ---------MALVRERRQLNLRLPLPEPSER-RPR----FPLPLP----------PSSIS  NaMKK1

37    TTNSTANTATTTASTTTISISELEKLKVLGHGNGGTVYEVRHKRTSAIYALKVVHGDSDP  NbMKK1
60    SAPSSSSSSSSSPLPTPLNFSELERINRIGSGAGGTVYKVLHRPTGRLYALKVIYGNHED  NtMEK2
61    SAPSSSSSSSSSPLPTPLNFSELERINRIGSGAGGTVYKVLHRPTGRLYALKVIYGNHED  NaMEK2
37    TTNSAANTTTTTAPTTTISISELEKLKVLGHGNGGTVYKVRHKRTSAIYALKVVHGDSDP  NaMKK1

97    EIRRQILREISILRRTDSPYVIKCHGVIDMPGGDIGILMEYMNVGTLESLLKSQATFSEL  NbMKK1
120   SVRLQMCREIEILRDVDNPNVVRCHDMFDHNG-EIQVLLEFMDKGSLEGIHIPK----ES  NtMEK2
121   SVRLQMCREIEILRDVDNPNVVRCHDMFDHNG-EIQVLLEFMDKGSLEGIHIPK----ES  NaMEK2
97    EIRRQVLREISILRRTDSPYIIKCHGVIDMPGGDIGILMEYMNSGTLESLLKSHATFTEL  NaMKK1

                                                          *     *
157   SLAKIAKQVLSGLDYLHNHKIIHRDLKPSNLLVNREMEVKIADFGVSKIMCRTLDPCNSY  NbMKK1
175   ALSDLTRQVLSGLYYLHRRKIVHRDIKPSNLLINSRREVKIADFGVSRVLAQTMDPCNSS  NtMEK2
176   ALSDLTRQVLSGLYYLHRRKIVHRDIKPSNLLINSRREVKIADFGVSRVLAQTMDPCNSS  NaMEK2
157   SLAKIAKQVLSGLDYLHNHKIIHRDLKPSNLLVNREMEVKIADFGVSKIMCRTLDPCNSY  NaMKK1

217   VGTCAYMSPERFDPGTYGVNYNGYAADIWSLGLTLMELYIGHFPFLPPGQRPDWATLMCA  NbMKK1
235   VGTIAYMSPERINTDLNHGQYDGYAGDIWSLGVSILEFYLGRFPFS-VGRSGDWASLMCA  NtMEK2
236   VGTIAYMSPERINTDLNHGQYDGYAGDIWSLGVSILEFYLGRFPFS-VGRSGDWASLMCA  NaMEK2
217   VGTCAYMSPERFDPDTYGVNYNGYAADIWSLGLTLMELYMGHFPFLPPGQRPDWATLMCA  NaMKK1

277   ICFGEPPSLPEGTSVNFRDFIECCLQKESSKRWSAQQLLNHPFILSNLK             NbMKK1
294   ICMSQPPEAPANASREFRDFIACCLQRDPARRWTAVQLLRHPFITQNSPAATTT-GNMMP  NtMEK2
295   ICMSQPPEAPANASREFRDFIACCLQRDPARRWTAVQLLRHPFITQNSPATTTTTGNMMP  NaMEK2
277   ICFGEPPSLPEGTSGNFKDFIECCLQKESSKRWSAQQLLQHPFILSNLK.            NaMKK1

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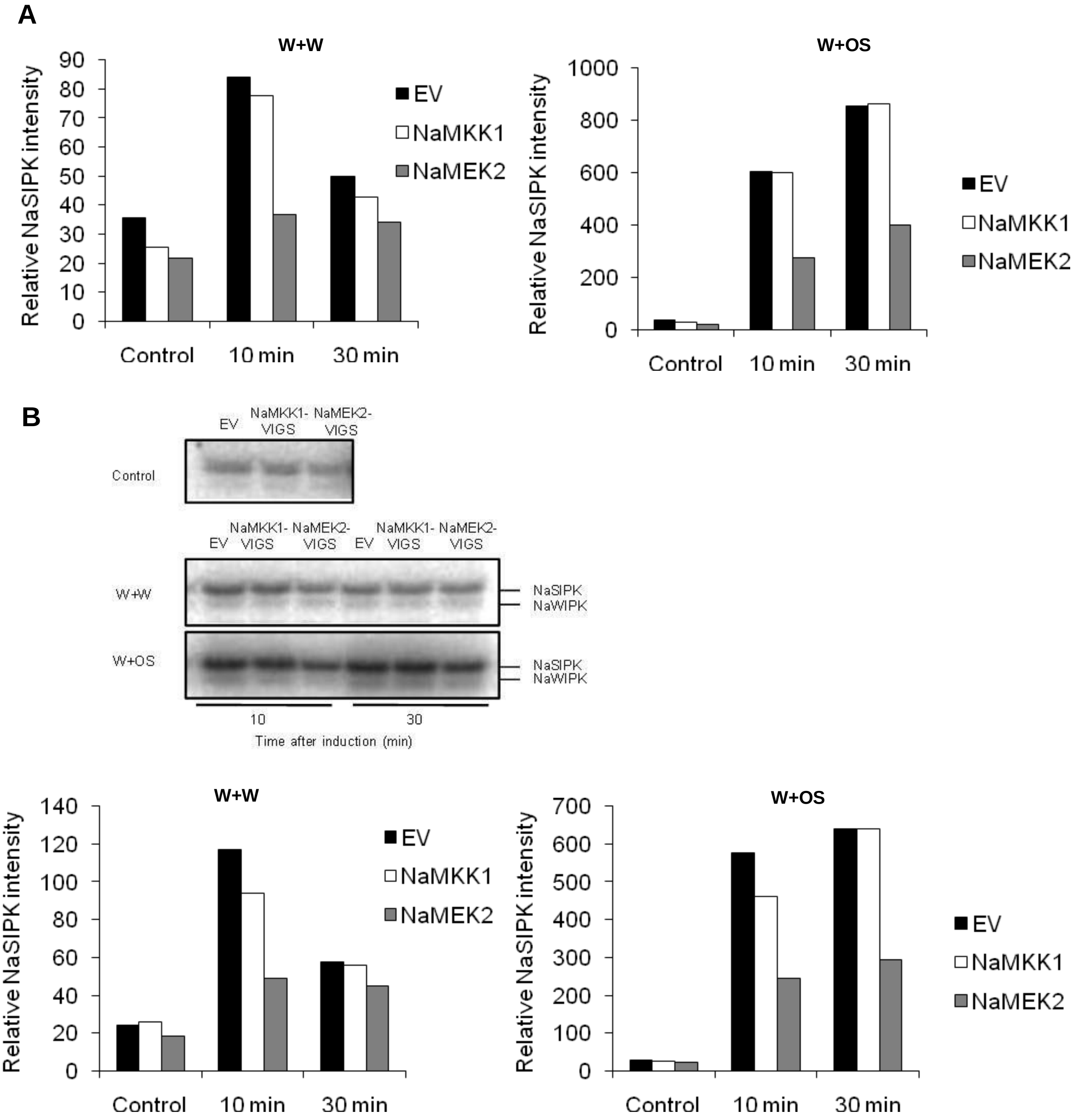
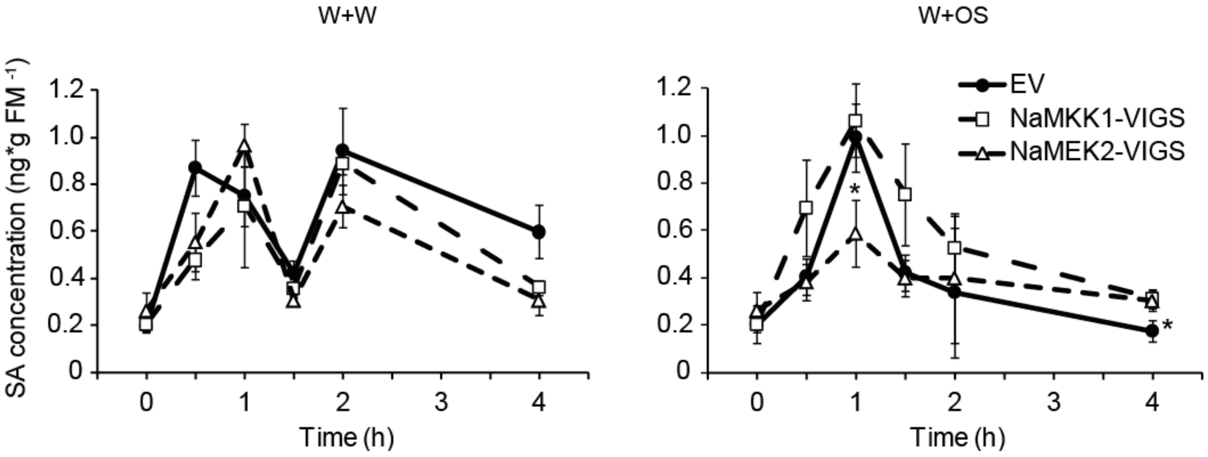
**Supplementary Fig. S1** Alignment of the protein sequences of *N. attenuata* NaMKK1 and NaMEK2 with *N. benthamiana* NbMKK1 and *N. tabacum* NtMEK2.

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 µl 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; *, $P < 0.05$; $N = 5$).