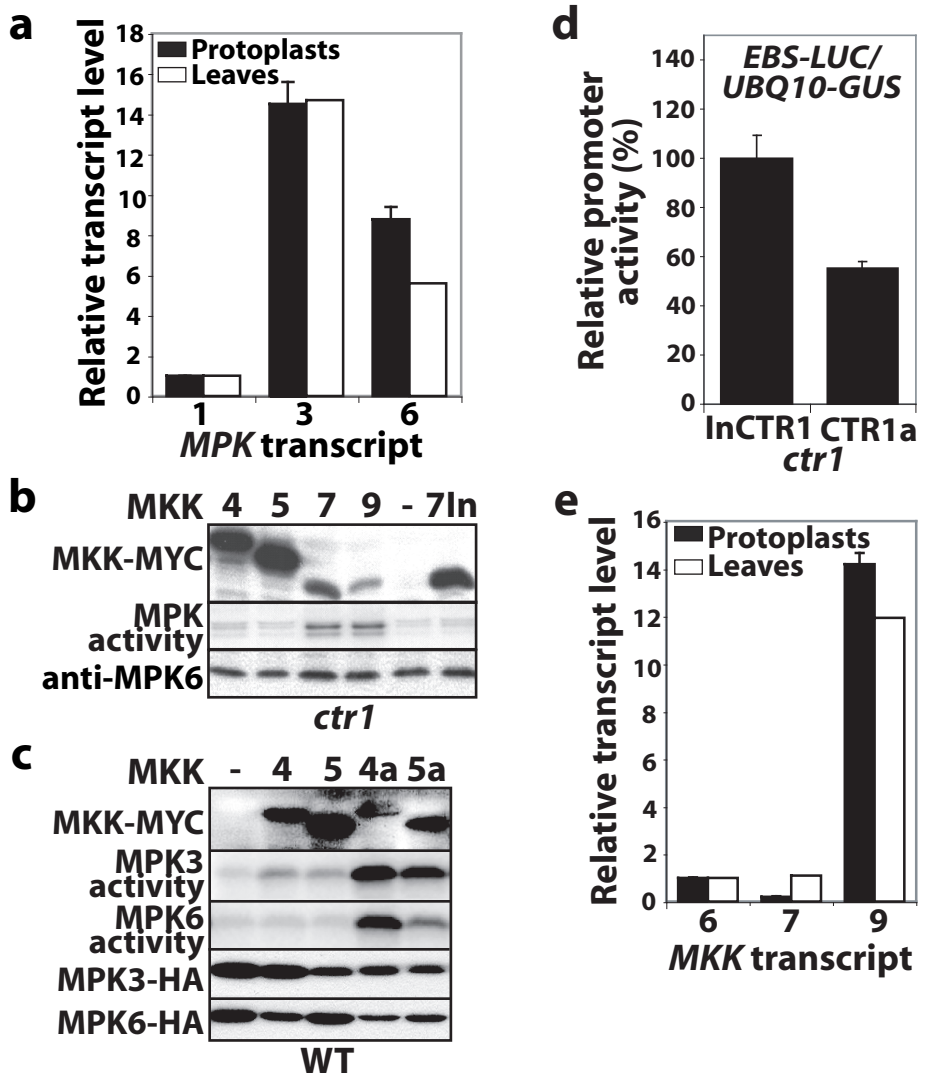
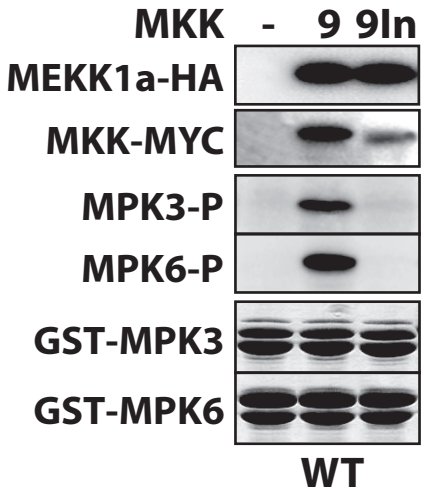
SUPPLEMENTARY INFORMATION



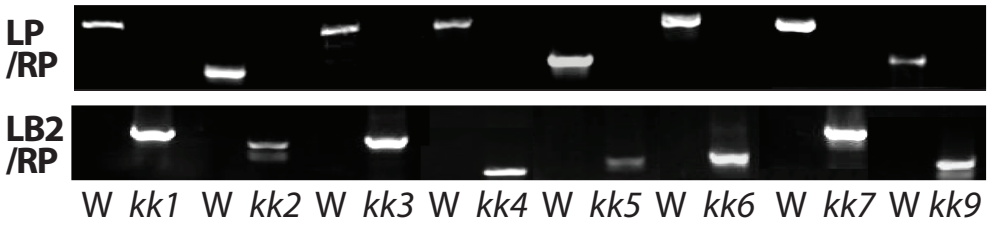
Supplementary Figure S1 | Specific *MPK*, *MKK* and *CTR1* expression and function in ethylene signalling. **a**, *MPK3* and *MPK6* are expressed in protoplasts and leaves. Quantitative RT-PCR (black, protoplasts) and ATH1 GeneChip data (white, mature leaves) retrieved using Genevestigator[50] are shown. Error bars, s.d. (n=3). **b,** Transient expression of WT MKK7 or MKK9 preferentially activates endogenous MAPKs in *ctr1* protoplasts. Empty vector (-) or an inactive form of MKK7^{K64M} (7In) was used as a control. *In-gel* MAPK assay[34-36] was performed to reveal endogenous protein kinase activities. Endogenous MPK6 protein level (anti-MPK6) was shown as a loading control. Experiments were repeated at least three times with similar results. **c**, WT MKK4 and MKK5 did not activate MAPKs in WT protoplasts. **d,** *EBS-LUC* activity is suppressed by expression of the CTR1a in *ctr1* protoplasts. Inactive CTR1 (InCTR1) was used as a control. **e,** Transcript level of *MKK9* is relatively more abundant than those of *MKK6* and *MKK7* in protoplasts and adult leaves. Error bars, s.d. (n=3).

Figure S1 Yoo et al.



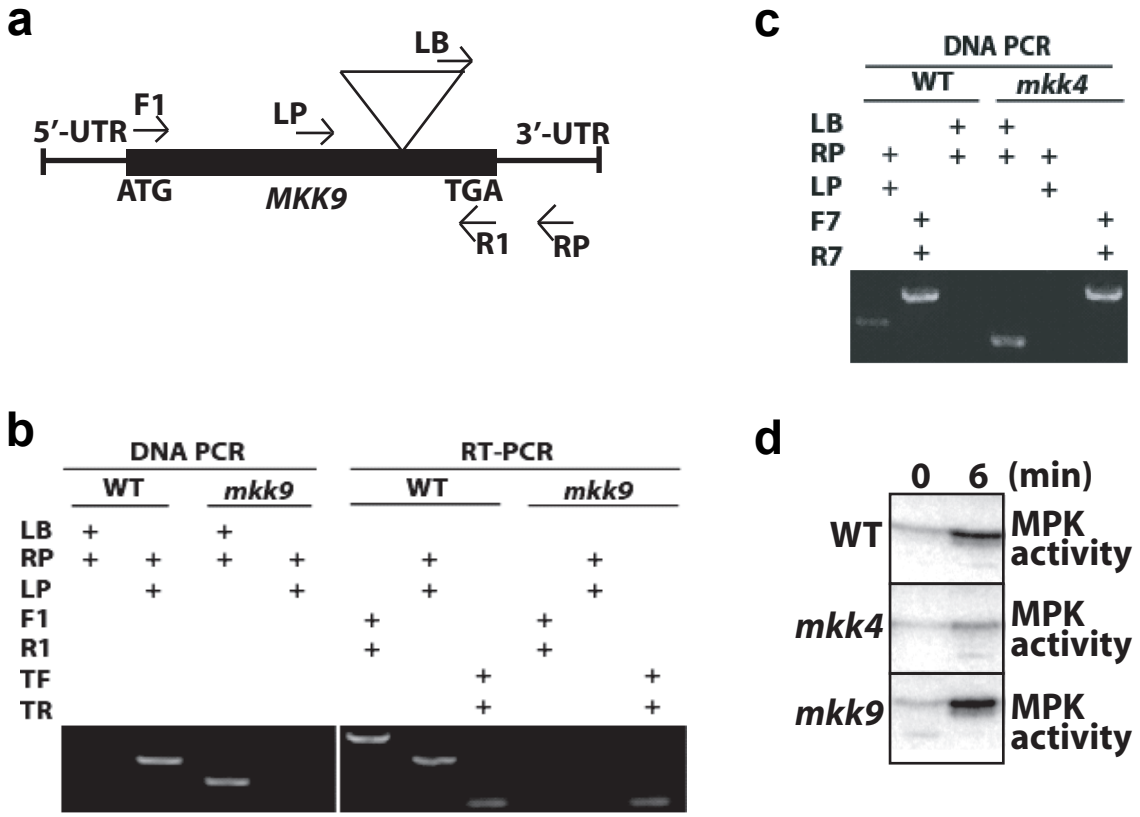
Supplementary Figure S2 | Active MKK9 directly phosphorylates MPK3 and MPK6. MKK9-MYC was activated by constitutively active MEKK1a-HA[36] in WT protoplasts and immunoprecipitated for an *in vitro* phosphorylation assay with GST-MPK3$^{K67R, K68R}$ or GST-MPK6$^{K92M, K93M}$, generated from *E. coli* with no autophosphorylation activity as previously described[36]. Empty vector (-) or an inactive mutant MKK9$^{S195A, S201A}$ (9In) served as a control. Expression of proteins was detected by immunoblot analysis using anti-HA (MEKK1a-HA) and anti-MYC (MKK-MYC) antibodies. Levels of GST-MPK3$^{K67R, K68R}$ and GST-MPK6$^{K92M, K93M}$ were determined by Commassie Blue staining.

Figure S2 Yoo et al.



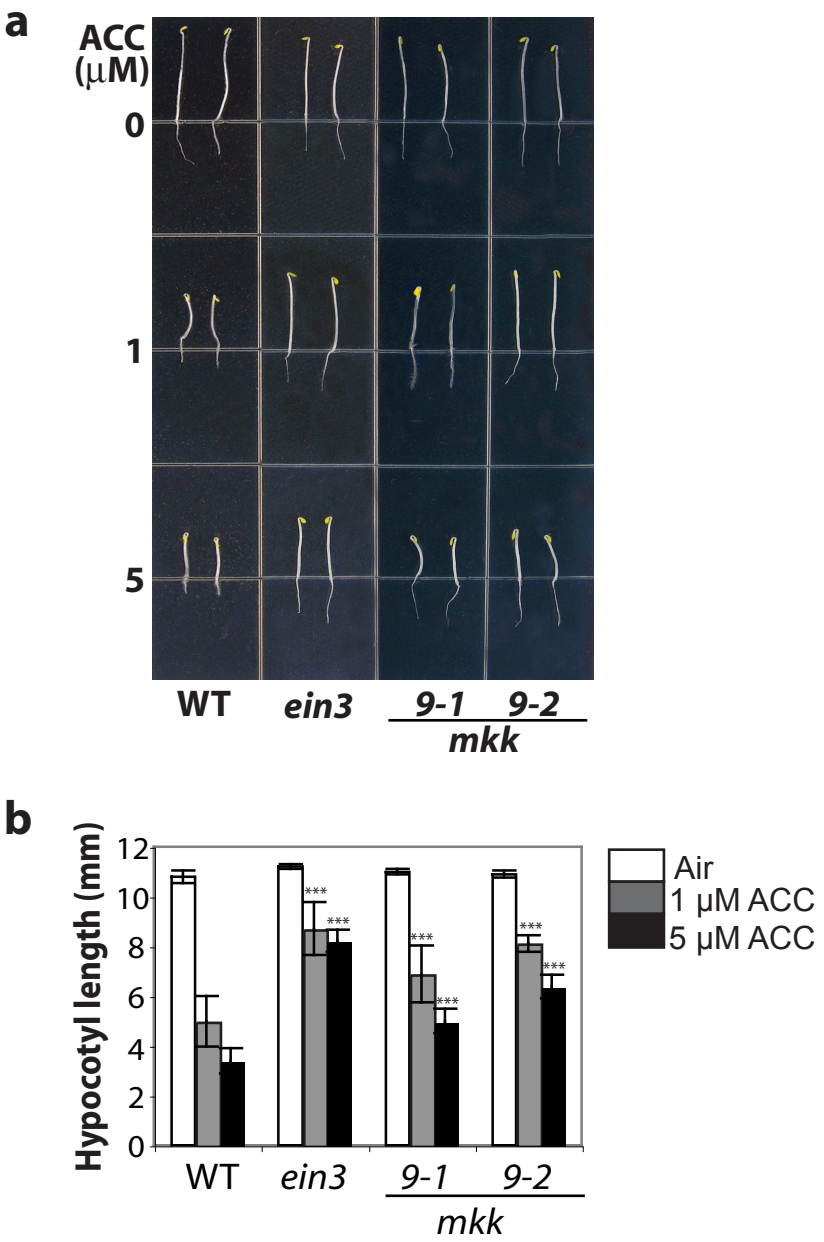
Supplementary Figure S3 | T-DNA insertion of the *mkk* mutants. Genomic DNA analyses of *mkk* (*kk1-9*) mutants and WT (W) by PCR. LB: T-DNA left border primer, LP/RP: primer sets for genomic DNA analyses of *MKKs*. The detailed primer sequence information is provoded in Supplementary Table S1. The *mkk4, mkk5* and *mkk6* mutants are not null alleles.

Figure S3 Yoo et al.



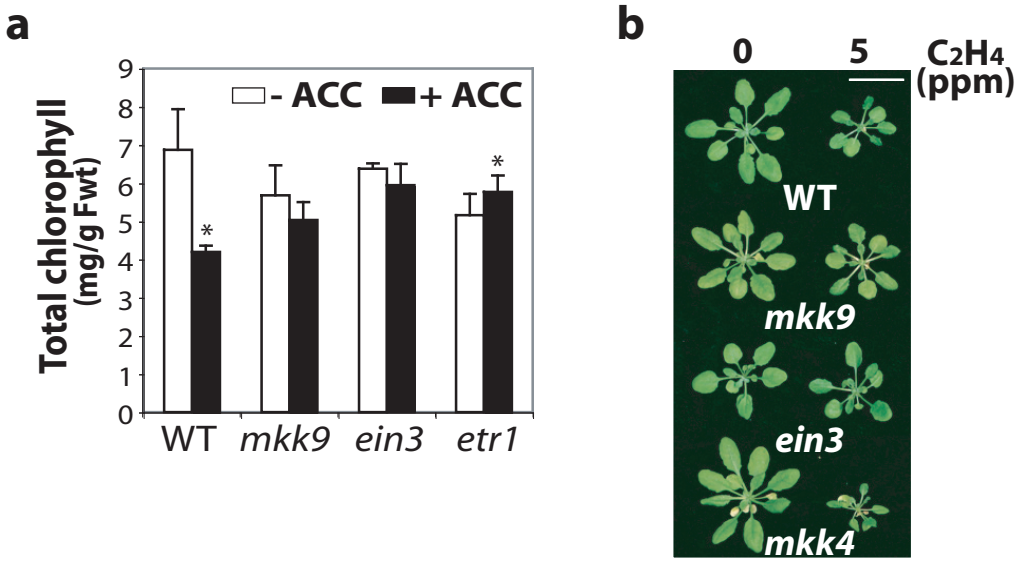
Supplementary Figure S4 Analysis of *mkk9-1* and *mkk4*. a, Schematic presentation of the *mkk9-1* gene structure with the T-DNA insertion. **b,** Genomic DNA PCR and RT-PCR analyses of WT and *mkk9-1*. RP/LP: Right and left primer set for *MKK9* DNA and RNA analyses. F1/R1: forward and reverse primer set for RT-PCR analysis of *MKK9*. TF/TR: forward and reverse primer set for RT-PCR analysis of *Tubulin4* as a positive control. The primer sequence information is provided in Supplementary Table S1. **c,** Genomic DNA PCR analysis of *mkk4*. A PCR control (At1g18350) was included using F7: AAGCACACGACGCACATTAAC and R7: CAGGCTCCTCACTTAAATCCC. **d,** Specific MAPK activation defect in *mkk4*. Transient mechanical stress activates strong endogenous MAPKs in WT and *mkk9*, but not in *mkk4*. *In-gel* MAPK assay was performed with minimal manipulation[35,36] .

Figure S4 Yoo et al.



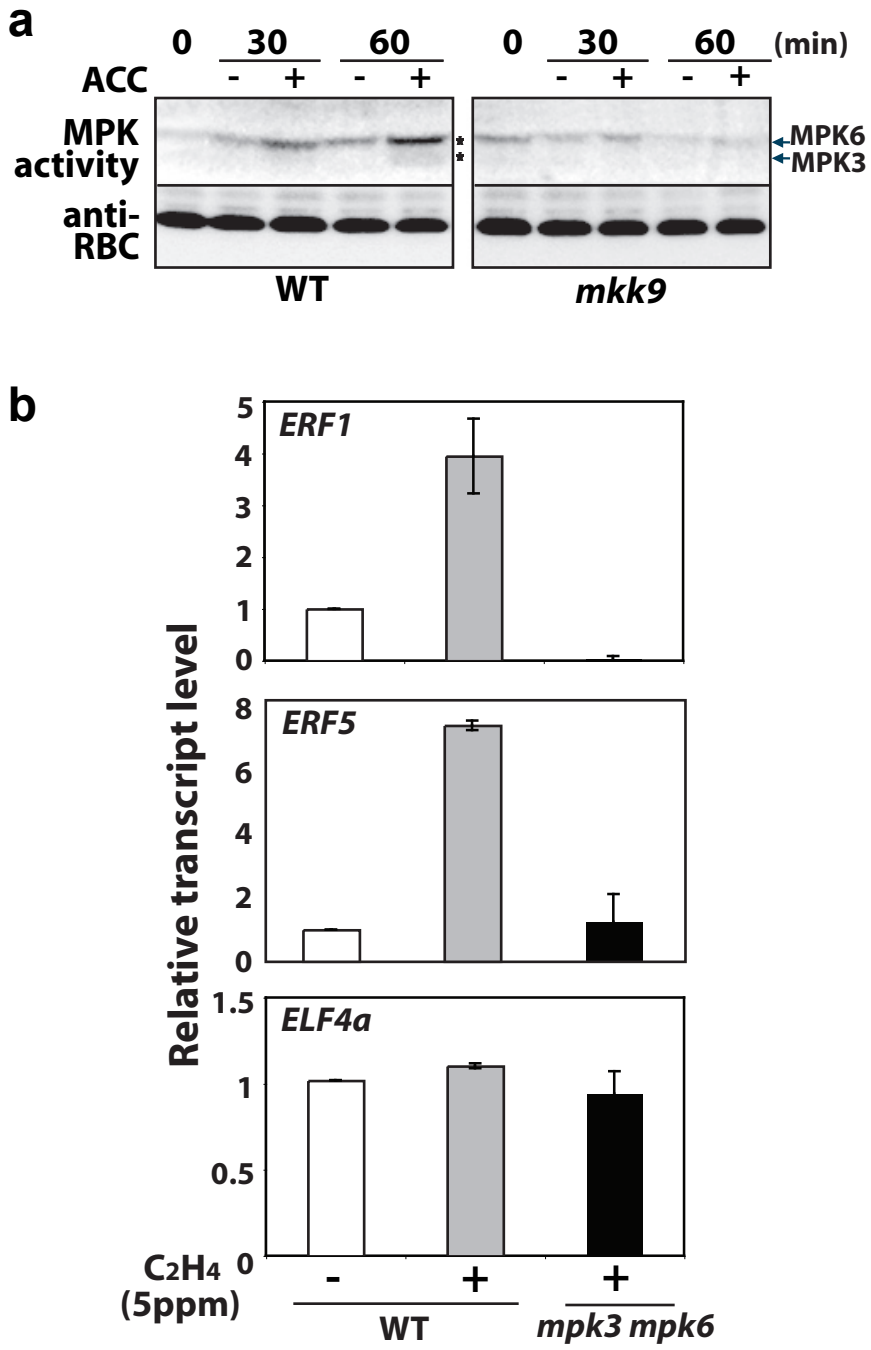
Supplementary Figure S5 | Analysis of ACC inhibition of hypocotyl elongation in *mkk9-1* and *mkk9-2*. a, Two independent *mkk9* alleles (3-d-old) show ACC insensitivity. Representative seedlings from each genotype population (n=20) are shown. Experiments were repeated five times with reproducible results.
b, Quantitative analysis of hypocotyl elongation in the presence of ACC. Error bar, s.d. (n=20). Experiments were repeated three times with similar results. Asterisks indicate differences between WT and mutant with statistical significance at *P<0.05, **P<0.01 and ***P<0.001 (t-test).

Figure S5 Yoo et al.



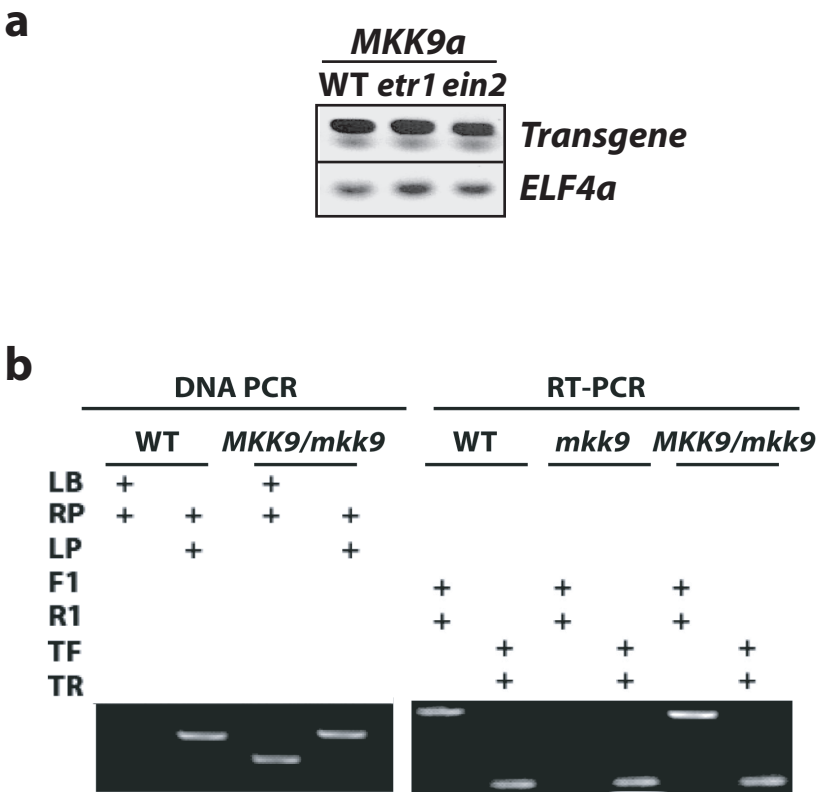
Supplementary Figure S6 | Diverse ethylene insensitive phenotypes of the *mkk9* mutant. a, Reduced chlorophyll degradation by 1 μM ACC in the detached *mkk9* leaves. Error bars, s.d. (n=3). The experiments were repeated twice with similar results. Asterisks over bars indicate differences between WT and mutant with statistical significance at *P<0.05 (t-test). **b,** Rosette development in *mkk9* and *ein3* is relatively resistant to C_2H_4 inhibition. Scale bar, 10 mm. Representative seedlings from each genotype population (n=10) are shown. Experiments were repeated three times with reproducible results.

Figure S6 Yoo et al.



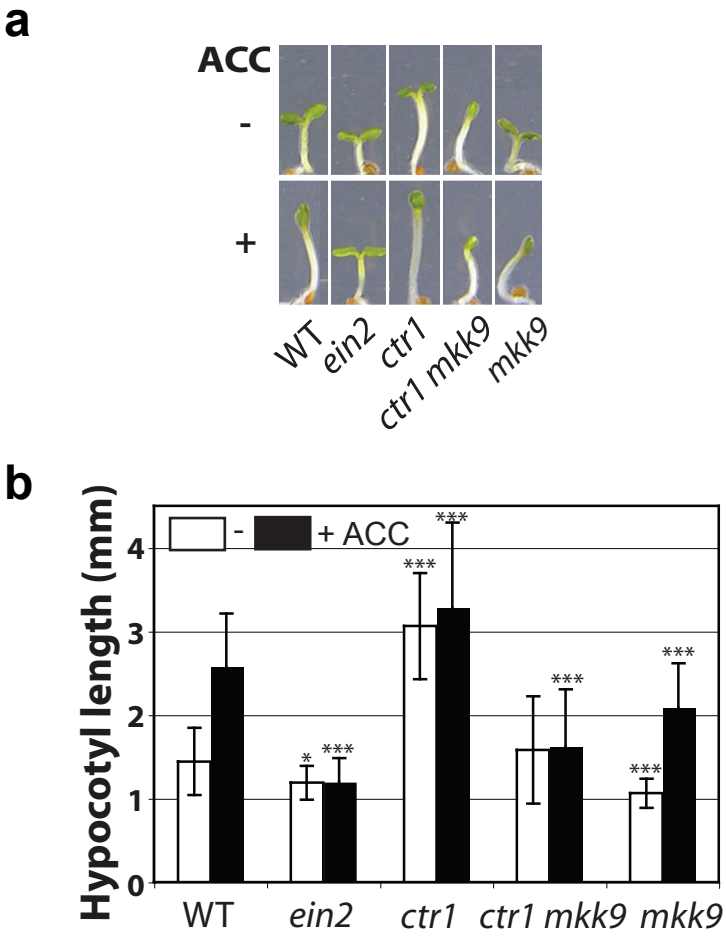
Supplementary Figure S7 | MAPKs and immediate early gene induction by ethylene in leaves. **a,** ACC (200 μM) was fed through the leaf petiole for 30 or 60 min. *In-gel* MAPK assay was performed with minimal manipulation. Endogenous RBC (anti-RBC) was used as a loading control. **b,** The transcript levels of *ERF1, ERF5* and *ELF4a* (Elongation Initiation Factor as control) were measured by qRT-PCR after 1h C_2H_4 treatment. The conditional *mpk3 mpk6* double mutant plants were generated by using virus-induced gene silencing[44] of *MPK3* in the *mpk6* T-DNA insertion mutant[29]. Error bars, s.d. (n=3).

Figure S7 Yoo et al.



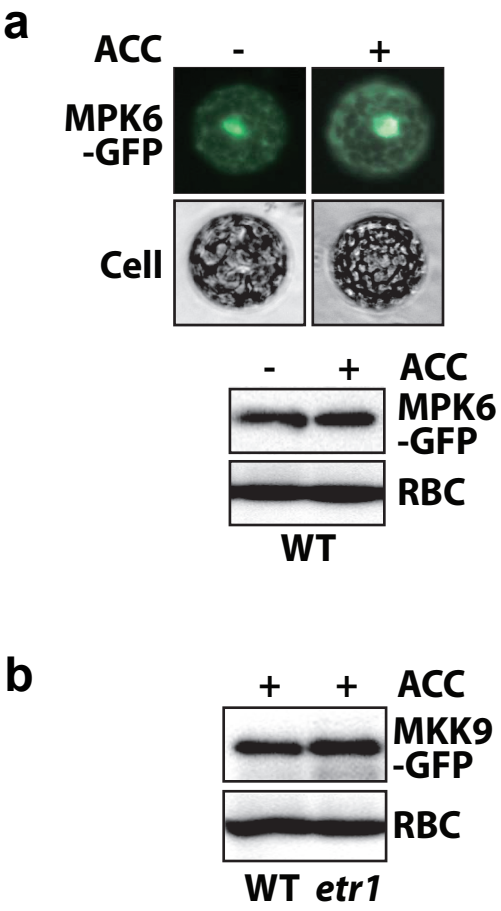
Supplementary Figure S8 | Transgenic plant analysis and complementation of *mkk9-1*. **a**, Similar levels of *MKK9a* transgene expression by RT-PCR. The transcript of *ELF4a* is an endogenous gene control. **b,** Genomic DNA PCR and RT-PCR analyses of WT and transgenic *mkk9* lines are shown. The *mkk9-1* mutant was complemented (*MKK9/mkk9*) with a 4.2kb genomic fragment containing 1.5kb upstream of the translation start site and 1.4kb downstream of the translation stop site using a binary vector *pBin19*. The detailed primer sequence information is provided in Supplememtary Table S1.

Figure S8 Yoo et al.

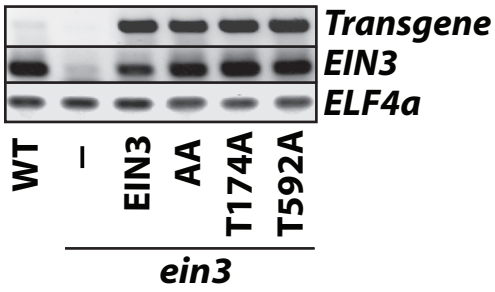


Supplementary Figure S9 | Ethylene responses in the *ctr1 mkk9* double mutant. a, Seedlings (5-d-old) germinated in constant light on water-agar plates (no salts or sugars) containing no (upper panels) and 50 μM ACC (lower panels). Ethylene insensitivity is shown in *ein2*, *mkk9* and *ctr1 mkk9*. **b,** Quantitative analysis of hypocotyl elongation promoted by ACC. Error bar, s.d. (n=20). The experiments were repeatedtwice with similar results. Asterisks over bars indicate differences between WT and mutants with statistical significance at *P<0.05, **P<0.01 and ***P<0.001 (t-test).

Figure S9 Yoo et al.



Supplementary Figure S10 | Analysis of MPK6-GFP and MKK9-GFP.
a, MPK6-GFP localizes in both the cytosol and nucleus without (-) or with (+) of 200 μM ACC. Immunoblot analysis of MPK6-GFP shows similar protein abundance.Endogenous RBC was used as a loading control.
b, MKK9-GFP protein abundance is similar in WT and *etr1* protoplasts in the presence of ACC(200 μM) despite its differential nuclear localization in WT.



Supplementary Figure S11 | Transgenic plant analysis. Similar RNA expression of EIN3 and EIN3 mutants in transgenic *ein3* lines. The transcript of *ELF4a* is an endogenous gene control.

Supplementary Table S1 | Oligonucleotides used in this study

Oligo name	Oligonucleotide (5'- 3')
	qRT-PCR primers for *MAPK*s
MPK1_f:	ATATGACCCGAAT GCAAACC
MPK1_r:	TTCCATGGCATCACTATTCG
MPK3_f:	AATGGCCATTGATCTTGTTGA
MPK3_r:	GTTACAAGATTA CATATGTGGAA
MPK6_f:	CCCGACAGTGCATCCTTTAG
MPK6_r:	GTTCCTTCATCTGCTCCTCTG
MKK6_f:	ACAACTAT ATGTCGCCTGAGAGG
MKK6_r:	CCCAAACTCCAAATGTCACTG
MKK7_f:	GTCGTGTGCTTTGGAGAACC
MKK7_r:	AAGGGTGACCGAGAAGCTG
MKK9_f:	AGGAGCTTCGTTGAGTGTTG
MKK9_r:	TCCCCTAACATTCTGGAGTATA
	qRT-PCR primers for ethylene response genes
ERF1_f:	GAGGAAACACTCG ATGAGACG
ERF1_r:	GGAGCGGT GATCAAAGTCAC
ERF5_f:	TGGA GAGACGTTTCCGTTTG
ERF5_r:	TGAGGAGATAACGGCGACAG
ACS2_f:	AATGGACGCAGACCAATCTT
ACS2_r:	GCTCGGAGAAGAGGTGAGTG
	qRT-PCR primers for controls
TF (*Tubulin4*):	AGGGAAAGGAAGAGAGGAAG
TR (*Tubulin4*):	GCTGGCTAATCCTACCTTTGG
ELF4F:	TCATAGATCTGGTCCTTGAAAC
ELF4R:	GGCAGTCTCTTCGTG CTGAC
	T-DNA screening primers (Salk)
T-DNA_LB:	TGGTTCACGTAGTGGGCCATCG
MKK1 (Salk_027645)	
MKK1_LP:	GACAAGTCTCTTAAGTCATAACATCTCG
MKK1_RP:	AACATGCTATCTGCCATCTGC

MKK3 (Salk_051970)

MKK3_LP: GAACAAACGTTTTCTCATGTGTG

MKK3_RP: AGAAGGATCCAGATGCTCGAC

MKK5 (Salk_067321)

MKK5_LP TAACCAGGCAACCATCTCAAG

MKK5_RP: TGGAAAGAGCGTGGAATACAC

MKK6 (Salk_084332)

MKK6_LP: CGCAGTCCTGTTTTCAAATTC

MKK6_RP: CAAAAGCTTCGTTAAAGCTCTCTC

MKK9 (Salk_146400)

MKK9.2_LP: GAAACTCAACGTTCTCGGATG

MKK9.2_RP: CCCAAAACTTATGTACACGATTG

T-DNA screening primers (SAIL)

T-DNA_LB: GCTTCCTATTATTCTTCCCAAATTACCAATACA

MKK2 (SAIL_511_H01)

MKK2_LP: TCACTGGAAGGTAAAACAAGAAATC

MKK2_RP: GTTAAAGCCATCCCTGACTCC

MKK4 (SAIL_565_A12)

MKK4_LP: CATCAACCTGATTAGGTTTGAG

MKK4_RP: CAGAGAGAACCGGGGAAAAG

MKK9 (SAIL_060_H06)

MKK9.1_LP: TCCCCTAACATTCTGGAGTATA

MKK9.1_RP: TCAAACCGGCGAATCTTCTTC

T-DNA screening primers (SM)

T-DNA_LB: TACGAATAAGAGCGTCCATTTTAGAGTGA

MKK7 (SM_3_17177)

MKK7_LP: AAGCACACGACGCACATTAAC

MKK7_RP: CAGGCTCCTCACTTAAATCCC

RT-PCR primers

MKK9_F1: ATGGCTTTAGTACGTGAACG

MKK9_R1: AAGATCTTCCCGGAGAAAA

TS_r: CGTATGGGTAACCAGCGTA

Supplementary reference

50. Zimmermann, P., Hirsch-Hoffmann, M., Hennig, L. & Gruissem, W. GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox. *Plant Physiol.* **136**, 2621-2632 (2004).