Supplemental Data

Supplemental Table S1 Primers used for preparation of stable transformation vectors and VIGS constructs

Primer name	Primer sequence (5'-3')	Construct
pRESC5-C4-1	ATGGGTAATAACTGTTTTTCCAGC	pRESC5-NaCDPK4
pRESC5-C4-2	TTGATGTTGGCTTCTGTGGTTATC	
pRESC5-C5-1	GTTAGTGGTTCAAATAGCAATACCCC	pRESC5-NaCDPK5
pRESC5-C5-2	CCACAAGGAATAACCCCTCCAC	
CDPK4-190-VIGS-1	GTCAGGATCCATGGGTAATAACTGTTTTTC	pTV-NaCDPK4
CDPK4-190-VIGS-2	GTCAAAGCTTTTCTGTGGTTATCTTTAACT	
CDPK5-190-VIGS-1	GTCAGGATCCATGGGCAGCTGTTTTTCTAG	pTV-NaCDPK5
CDPK5-190-VIGS-2	GTCAAAGCTTGGCTGTTTTTTGTATTAATAT	
CDPK4/5-VIGS-1	GCGGCGGTCGACCAACCTAGGAGTTCTCAGC	pTV-NaCDPK4/5
CDPK4/5-VIGS-1	GCGGCGGGATCCACATTCTCGTGACCAGC	
CDPK4-Prom-1	CGATGAATTCGAAATTATGCACATTTTTAGTAATA	pCAM-NaCDPK4Pro:GUS
CDPK4-Prom-2	AGTCAGATCTACCATATTTAGGATATGAATATAAAACC	
CDPK5-Prom-1	CGATGGATCCTTGTGCTTTCGCGTTGC	pCAM-NaCDPK5Pro:GUS
CDPK5-Prom-2	CTAGCCATGGATTGGTGAAAATGATGAAAAAGTG	

Supplemental Table S2 Sequences of primers used for quantitative real-time PCR (SYBR Green analysis)

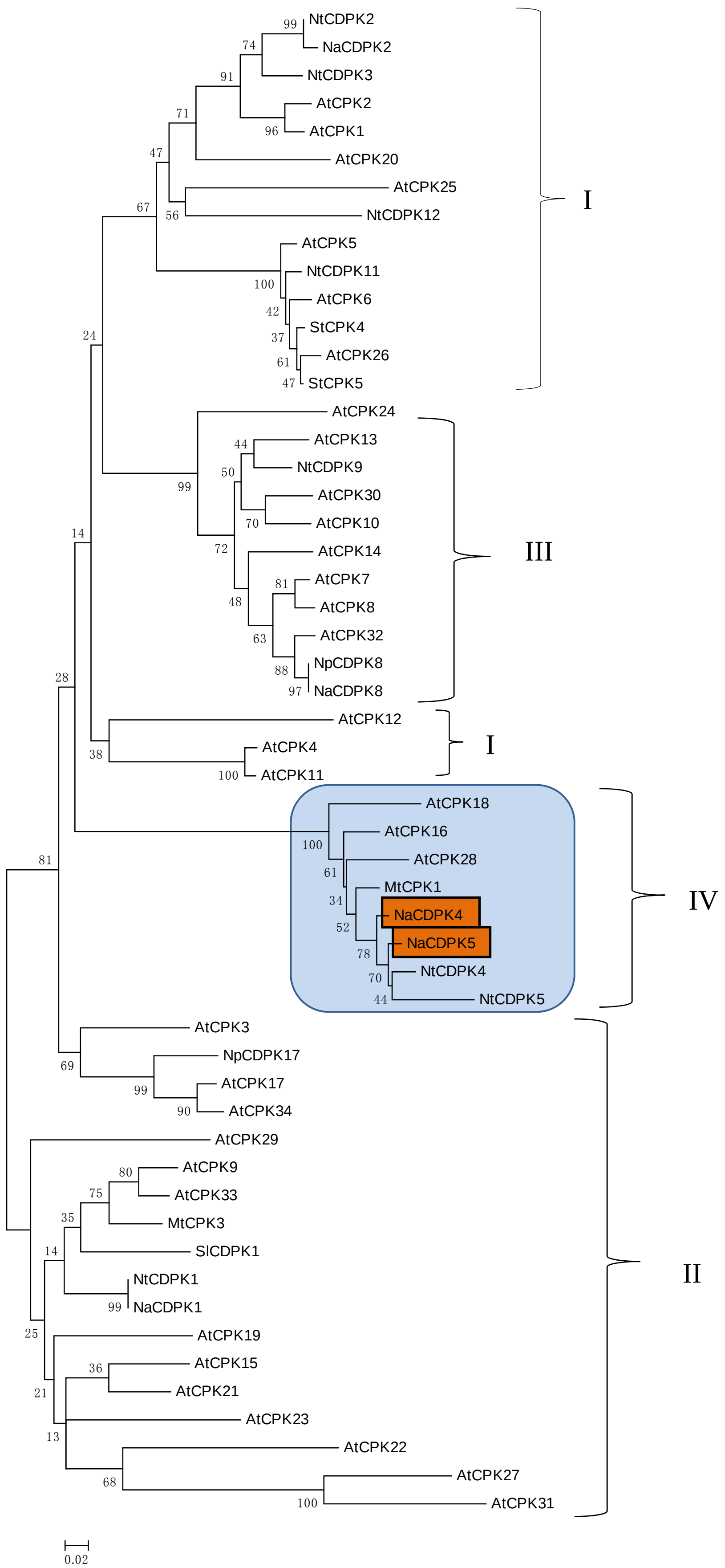
Gene	**Forward Primer (5'-3')**	**Reverse Primer (5'-3')**
Na*Actin2*	GGTCGTACCACCGGTATTGTG	GTCAAGACGGAGAATGGCATG
Na*CDPK1*	CCGTGCAGCCGATACAAAC	TTTCCCAAGTGTATATTGCTTCCTAA
Na*CDPK2*	GCAATGTGGCGAACTCGG	CTGCTGTTTCACCTCTGGCAC
Na*CDPK4*	CGTTGCCACCCACAAGTCTAA	TACATCCTCAACTGCAATCGTAG
Na*CDPK5*	GGGTATACATATGTTGCTACAGAT	ACCACATTCTCGTGACCAGC
Na*GLA1*	AGTAGCAGATGATGTTAGTACATGTA	ACATGTGAATATGCCCATGGCATACT
Na*LOX3*	GGCAGTGAAATTCAAAGTAAGAGC	CCCAAAATTTGAATCCACAACA
Na*AOS*	GACGGCAAGAGTTTTCCCAC	TAACCGCCGGTGAGTTCAGT
Na*AOC*	AACTACCTAACCCTCTCATTTCTCA	AAGCGAAGATAGGCAGGGC
Na*OPR3*	AATGGAGTTGGAGTTTGTTT	AGGTGGTTGAAGCAGTCGTT
Na*ACX1*	GAATGTCTGTTGCTTGTGCTCA	TACCGCAAAGCACCTCCAG

Supplemental Table S3 Accession numbers or locus numbers of genes whose deduced protein sequences were used for phylogenetic analysis

Gene	Accession No.	Gene	Accession No.	Gene	Accession No.
At*CPK1*	At5g04870	At*CPK18*	At4g36070	Nt*CDPK1*	AF072908
At*CPK2*	At3g10660	At*CPK19*	At1g61950	Nt*CDPK2*	AJ344154
At*CPK3*	At4g23650	At*CPK20*	At2g38910	Nt*CDPK3*	AJ344155
At*CPK4*	At4g09570	At*CPK21*	At4g04720	Nt*CDPK4*	AF435451
At*CPK5*	At4g35310	At*CPK22*	At4g04710	Nt*CDPK5*	AY971376
At*CPK6*	At2g17290	At*CPK23*	At4g04740	Nt*CDPK9*	HQ141792
At*CPK7*	At5g12480	At*CPK24*	At2g31500	Nt*CDPK11*	HQ158609
At*CPK8*	At5g19450	At*CPK25*	At2g35890	Nt*CDPK12*	GQ337420
At*CPK9*	At3g20410	At*CPK26*	At4g38230	Np*CDPK17*	AJ699161
At*CPK10*	At1g18890	At*CPK27*	At4g04700	Mt*CDPK1*	AY821654
At*CPK11*	At3g20410	At*CPK28*	At5g66210	St*CDPK1*	AF363784
At*CPK12*	At5g23580	At*CPK29*	At1g76040	St*CPK4*	AB279737
At*CPK13*	At3g51850	At*CPK30*	At1g74740	St*CPK5*	AB279738
At*CPK14*	At2g41860	At*CPK31*	At4g04695		
At*CPK15*	At4g21940	At*CPK32*	At3g57530		
At*CPK16*	At2g17890	At*CPK33*	At1g50700		
At*CPK17*	At5g12180	At*CPK34*	At5g19360		

Note: the first two letter of each gene indicate the species origins of these genes. At: *Arabidopsis thaliana*; Nt: *Nicotiana tabacum*; Np: *Nicotiana plumbaginifolia*; St: *Solanum tuberosum*; Mt: *Medicago truncatula*.

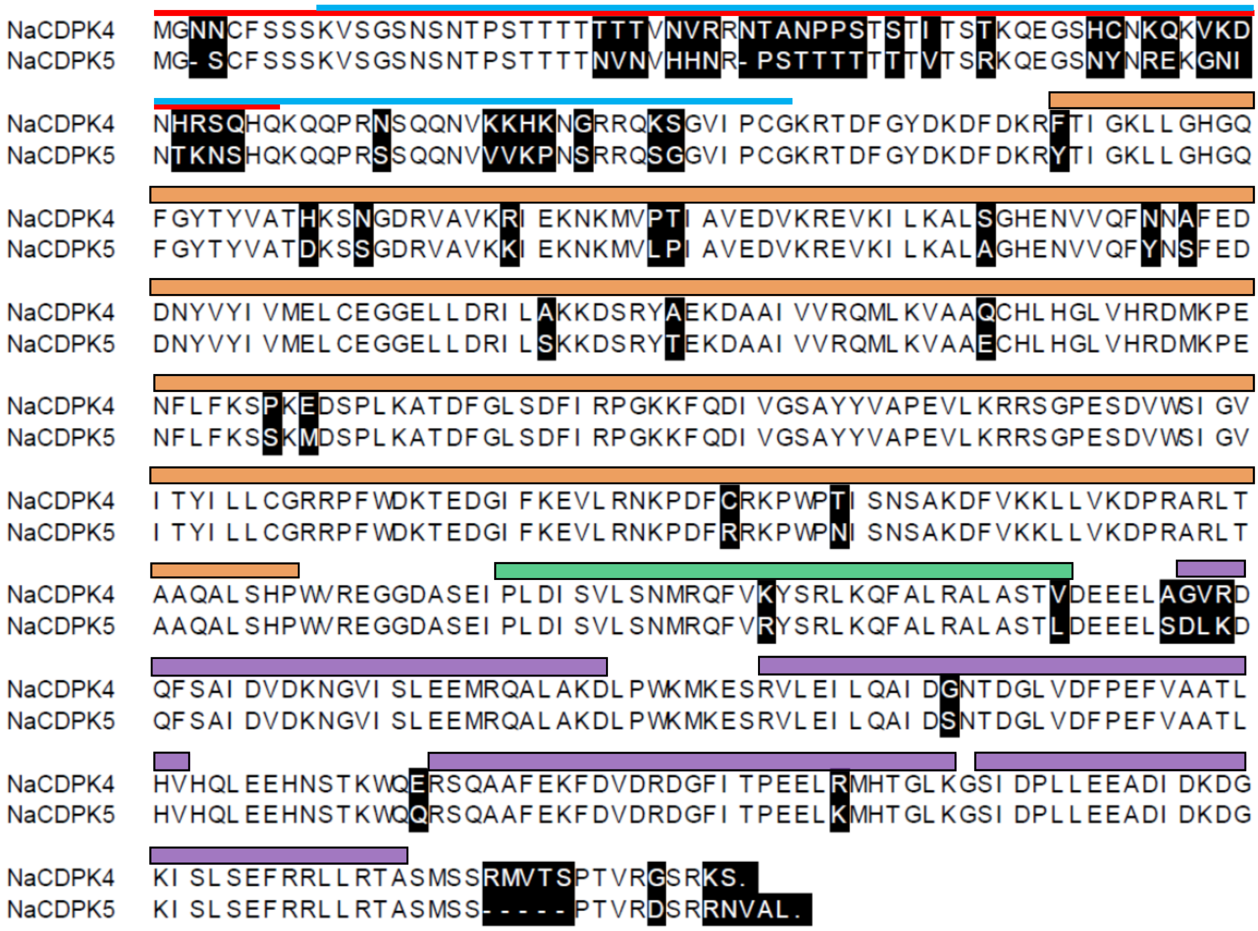
Supplemental Figures



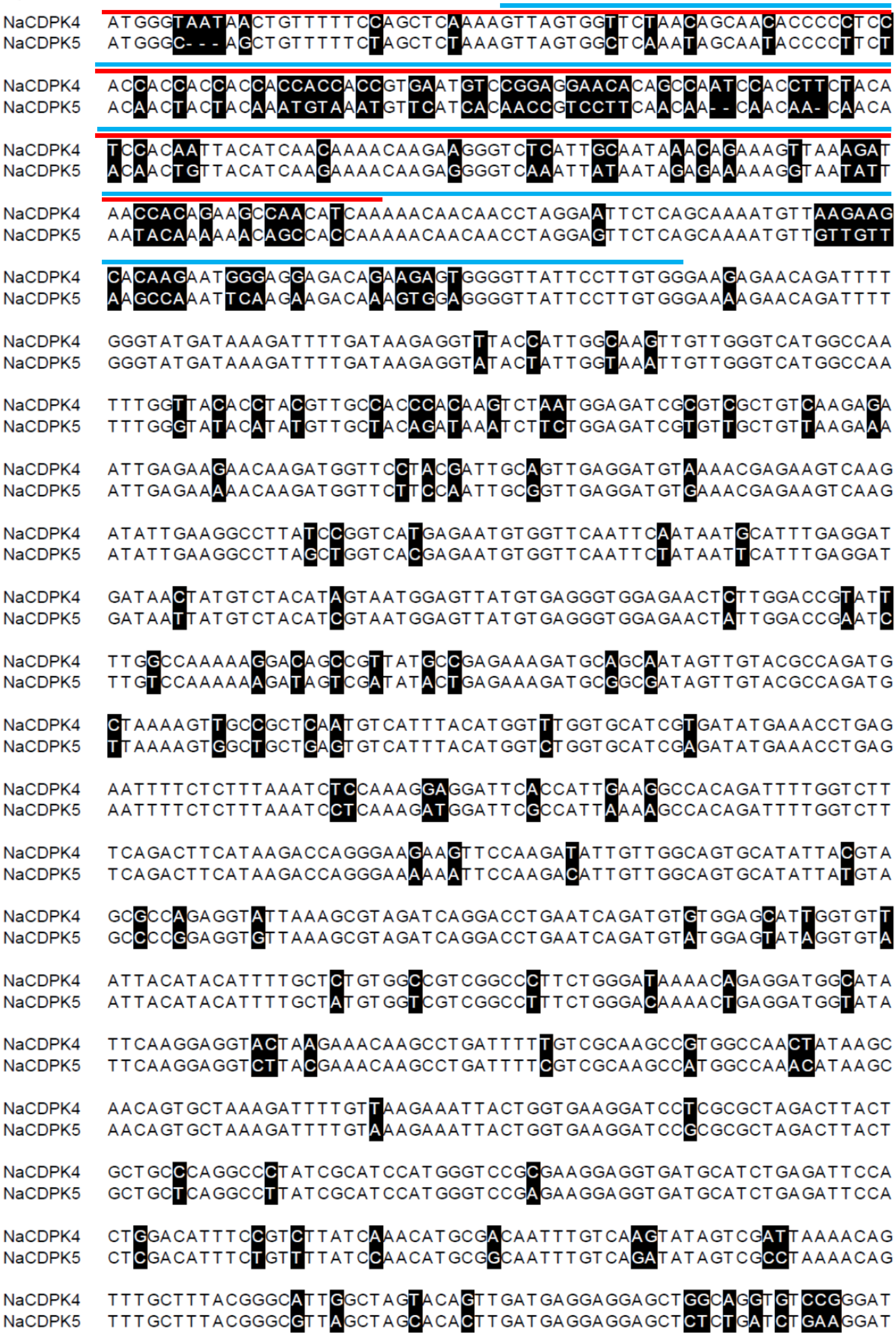
Supplemental Figure S1. Phylogenetic analysis of CDPKs.

Protein sequences of CDPKs from different plants were aligned using the Clustal W algorithm, and an unrooted Neighbor-Joining tree and bootstrap values were constructed using MEGA 4 software. The first two letters of protein names indicate species origins (At: *Arabidopsis thaliana*, Nt: *Nicotiana tabacum*; Np: *Nicotiana plumbaginifolia*; St: *Solanum tuberosum*; Mt: *Medicago truncatula*). Roman numbers indicate the subgroups of CDPKs according to Cheng et al., Plant Physiology, 2002, **129,** 469-485. NaCDPK4 and NaCDPK5 are highlighted in an orange background and CDPKs that cluster with Arabidopsis subgroup IV of CDPKs are indicated with a blue box.

A

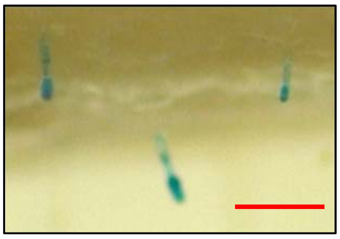

NaCDPK4 MGNNCFSSSKVSGSNSNTPSTTTTTTTVNVRRNTANPPSTSTITSTKQEGSHCNKQKVKD
NaCDPK5 MG-SCFSSSKVSGSNSNTPSTTTTNVNVHHNR-PSTTTTTTTVTSRKQEGSNYNREKGNI
NaCDPK4 NHRSQHQKQQPRNSQQNVKKHKNGRRQKSGVIPCGKRTDFGYDKDFDKRFTIGKLLGHGQ
NaCDPK5 NTKNSHQKQQPRSSQQNVVKPNSRRQSGGVIPCGKRTDFGYDKDFDKRYTIGKLLGHGQ
NaCDPK4 FGYTYVATHKSNGDRVAVKRIEKNKMVPTIAVEDVKREVKILKALSGHENVVQFNNAFED
NaCDPK5 FGYTYVATDKSSGDRVAVKKIEKNKMVLPIAVEDVKREVKILKALAGHENVVQFYNSFED
NaCDPK4 DNYVYIVMELCEGGELLDRILAKKDSRYAEKDAAIVVRQMLKVAAQCHLHGLVHRDMKPE
NaCDPK5 DNYVYIVMELCEGGELLDRILSKKDSRYTEKDAAIVVRQMLKVAAECHLHGLVHRDMKPE
NaCDPK4 NFLFKSPKEDSPLKATDFGLSDFIRPGKKFQDIVGSAYYVAPEVLKRRSGPESDVWSIGV
NaCDPK5 NFLFKSSKMDSPLKATDFGLSDFIRPGKKFQDIVGSAYYVAPEVLKRRSGPESDVWSIGV
NaCDPK4 ITYILLCGRRPFWDKTEDGIFKEVLRNKPDFCRKPWPTISNSAKDFVKKLLVKDPRARLT
NaCDPK5 ITYILLCGRRPFWDKTEDGIFKEVLRNKPDFRRKPWPNISNSAKDFVKKLLVKDPRARLT
NaCDPK4 AAQALSHPWREGGDASEIPLDISVLSNMRQFVKYSRLKQFALRALASTVDEEELAGVRD
NaCDPK5 AAQALSHPWREGGDASEIPLDISVLSNMRQFVRYSRLKQFALRALASTLDEEELSDLKD
NaCDPK4 QFSAIDVDKNGVISLEEMRQALAKDLPWKMKESRVLEILQAIDGNTDGLVDFPEFVAATL
NaCDPK5 QFSAIDVDKNGVISLEEMRQALAKDLPWKMKESRVLEILQAIDSNTDGLVDFPEFVAATL
NaCDPK4 HVHQLEEHNSTKWQERSQAAFEKFDVDRDGFITPEELRMHTGLKGSIDPLLEEADIDKDG
NaCDPK5 HVHQLEEHNSTKWQQRSQAAFEKFDVDRDGFITPEELKMHTGLKGSIDPLLEEADIDKDG
NaCDPK4 KISLSEFRRLLRTASMSSRMVTSPTVRGSRKS.
NaCDPK5 KISLSEFRRLLRTASMSS-----PTVRDSRRNVAL.

B

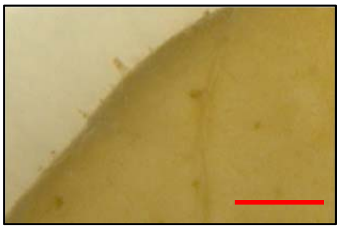

NaCDPK4 ATGGGTAATAACTGTTTTTCCAGCTCAAAAGTTAGTGGTTCTAACAGCAACACCCCCTCC
NaCDPK5 ATGGGC---AGCTGTTTTTCTAGCTCTAAAGTTAGTGGCTCAAATAGCAATACCCCTTCT
NaCDPK4 ACCACCACCACCACCACCACCGTGAATGTCCGGAGGAACACAGCCAATCCACCTTCTACA
NaCDPK5 ACAACTACTACAAATGTAAATGTTCATCACAACCGTCCTTCAACAA--CAACAA-CAACA
NaCDPK4 TCCACAATTACATCAACAAAACAAGAAGGGTCTCATTGCAATAAACAGAAAGTTAAAGAT
NaCDPK5 ACAACTGTTACATCAAGAAAACAAGAGGGGTCAAATTATAATAGAGAAAAAGGTAATATT
NaCDPK4 AACCACAGAAGCCAACATCAAAAACAACAACCTAGGAATTCTCAGCAAAATGTTAAGAAG
NaCDPK5 AATACAAAAAACAGCCACCAAAAACAACAACCTAGGAGTTCTCAGCAAAATGTTGTTGTT
NaCDPK4 CACAAGAATGGGAGGAGACAGAAGAGTGGGGTTATTCCTTGTGGGAAGAGAACAGATTTT
NaCDPK5 AAGCCAAATTCAAGAAGACAAAGTGGAGGGGTTATTCCTTGTGGGAAAAGAACAGATTTT
NaCDPK4 GGGTATGATAAAGATTTTGATAAGAGGTTTACCATTGGCAAGTTGTTGGGTCATGGCCAA
NaCDPK5 GGGTATGATAAAGATTTTGATAAGAGGTATACTATTGGTAAATTGTTGGGTCATGGCCAA
NaCDPK4 TTTGGTTACACCTACGTTGCCACCCACAAGTCTAATGGAGATCGCGTCGCTGTCAAGAGA
NaCDPK5 TTTGGGTATACATATGTTGCTACAGATAAATCTTCTGGAGATCGTGTTGCTGTTAAGAAA
NaCDPK4 ATTGAGAAGAACAAGATGGTTCCTACGATTGCAGTTGAGGATGTAAAACGAGAAGTCAAG
NaCDPK5 ATTGAGAAAAACAAGATGGTTCTTCCAATTGCGGTTGAGGATGTGAAACGAGAAGTCAAG
NaCDPK4 ATATTGAAGGCCTTATCCGGTCATGAGAATGTGGTTCAATTCAATAATGCATTTGAGGAT
NaCDPK5 ATATTGAAGGCCTTAGCTGGTCACGAGAATGTGGTTCAATTCTATAATTCATTTGAGGAT
NaCDPK4 GATAACTATGTCTACATAGTAATGGAGTTATGTGAGGGTGGAGAACTCTTGGACCGTATT
NaCDPK5 GATAATTATGTCTACATCGTAATGGAGTTATGTGAGGGTGGAGAACTATTGGACCGAATC
NaCDPK4 TTGGCCAAAAAGGACAGCCGTTATGCCGAGAAAGATGCAGCAATAGTTGTACGCCAGATG
NaCDPK5 TTGTCCAAAAAAGATAGTCGATATACTGAGAAAGATGCGGCGATAGTTGTACGCCAGATG
NaCDPK4 CTAAAAGTTGCCGCTCAATGTCATTTACATGGTTTGGTGCATCGTGATATGAAACCTGAG
NaCDPK5 TTAAAAGTGGCTGCTGAGTGTCATTTACATGGTCTGGTGCATCGAGATATGAAACCTGAG
NaCDPK4 AATTTTCTCTTTAAATCTCCAAAGGAGGATTCACCATTGAAGGCCACAGATTTTGGTCTT
NaCDPK5 AATTTTCTCTTTAAATCCTCAAAGATGGATTCGCCATTAAAAGCCACAGATTTTGGTCTT
NaCDPK4 TCAGACTTCATAAGACCAGGGAAGAAGTTCCAAGATATTGTTGGCAGTGCATATTACGTA
NaCDPK5 TCAGACTTCATAAGACCAGGGAAAAAATTCCAAGACATTGTTGGCAGTGCATATTATGTA
NaCDPK4 GCGCCAGAGGTATTAAAGCGTAGATCAGGACCTGAATCAGATGTGTGGAGCATTGGTGTT
NaCDPK5 GCCCCGGAGGTGTTAAAGCGTAGATCAGGACCTGAATCAGATGTATGGAGTATAGGTGTA
NaCDPK4 ATTACATACATTTTGCTCTGTGGCCGTCGGCCCTTCTGGGATAAAACAGAGGATGGCATA
NaCDPK5 ATTACATACATTTTGCTATGTGGTCGTCGGCCTTTCTGGGACAAAACTGAGGATGGTATA
NaCDPK4 TTCAAGGAGGTACTAAGAAACAAGCCTGATTTTTGTCGCAAGCCGTGGCCAACTATAAGC
NaCDPK5 TTCAAGGAGGTCTTACGAAACAAGCCTGATTTTCGTCGCAAGCCATGGCCAAACATAAGC
NaCDPK4 AACAGTGCTAAAGATTTTGTTAAGAAATTACTGGTGAAGGATCCTCGCGCTAGACTTACT
NaCDPK5 AACAGTGCTAAAGATTTTGTAAAGAAATTACTGGTGAAGGATCCGCGCGCTAGACTTACT
NaCDPK4 GCTGCCCAGGCCCTATCGCATCCATGGGTCCGCGAAGGAGGTGATGCATCTGAGATTCCA
NaCDPK5 GCTGCTCAGGCCTTATCGCATCCATGGGTCCGAGAAGGAGGTGATGCATCTGAGATTCCA
NaCDPK4 CTGGACATTTCCGTCTTATCAAACATGCGACAATTTGTCAAGTATAGTCGATTAAAACAG
NaCDPK5 CTCGACATTTCTGTTTTATCCAACATGCGGCAATTTGTCAGATATAGTCGCCTAAAACAG
NaCDPK4 TTTGCTTTACGGGCATTGGCTAGTACAGTTGATGAGGAGGAGCTGGCAGGTGTCCGGGAT
NaCDPK5 TTTGCTTTACGGGCGTTAGCTAGCACACTTGATGAGGAGGAGCTCTCTGATCTGAAGGAT

Supplemental Figure S2. Alignment of NaCDPK4 and NaCDPK5 amino acid and nucleotide sequences and regions used for RNAi constructs.

(A) Amino acid sequence alignment of NaCDPK4 and NaCDPK5. Regions used for constructing pRESC5-CDPK4 and pRESC5-CDPK5 are indicated with the red and blue bar, respectively. The sequences under the brown, green, and purple bars represent the kinase domain, autoinhibitory domain, and EF hands, respectively. **(B)** Nucleotide sequence alignment of *NaCDPK4* and *NaCDPK5*. Regions used for constructing pRESC5-CDPK4 and pRESC5-CDPK5 are below the red and blue bar, respectively.



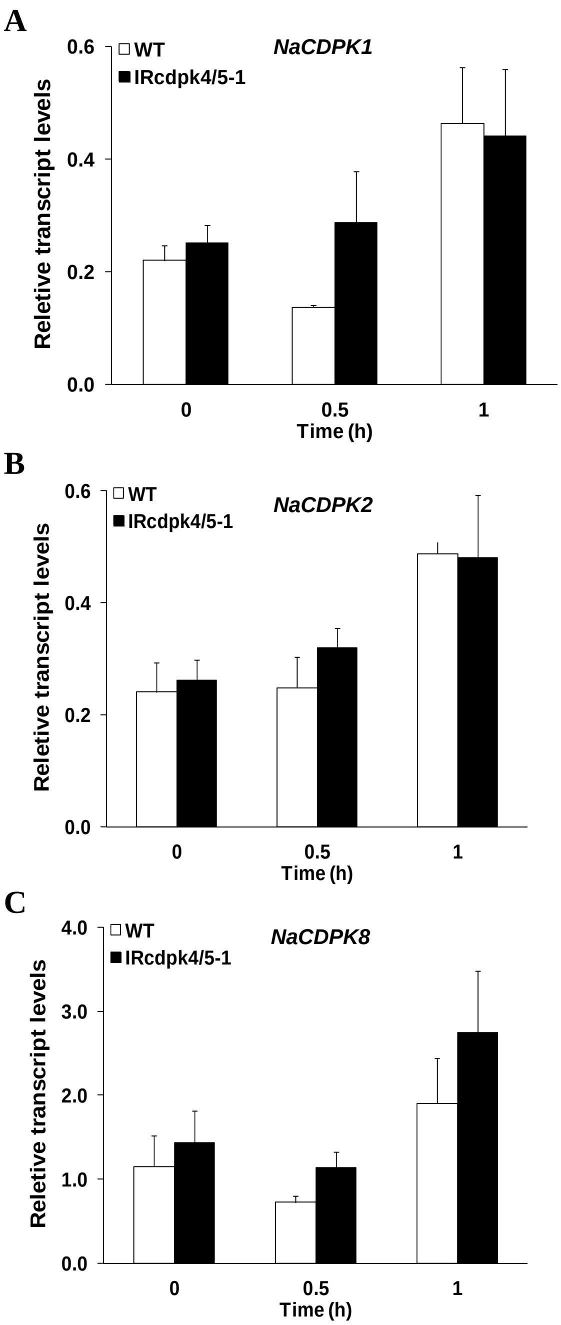
NaCDPK4Pro:GUS



NaCDPK5Pro:GUS

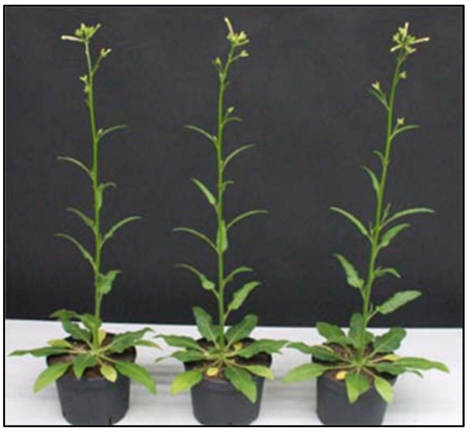
Supplemental Figure S3. *NaCDPK4* but not *NaCDPK5* is expressed in trichomes of *N. attenuata*.

Histochemical GUS assays were done for the leaves of *NaCDPK4Pro:GUS* and *NaCDPK5Pro:GUS* plants. Photographs were taken under a stereo microscope. Bar = 200 µm.



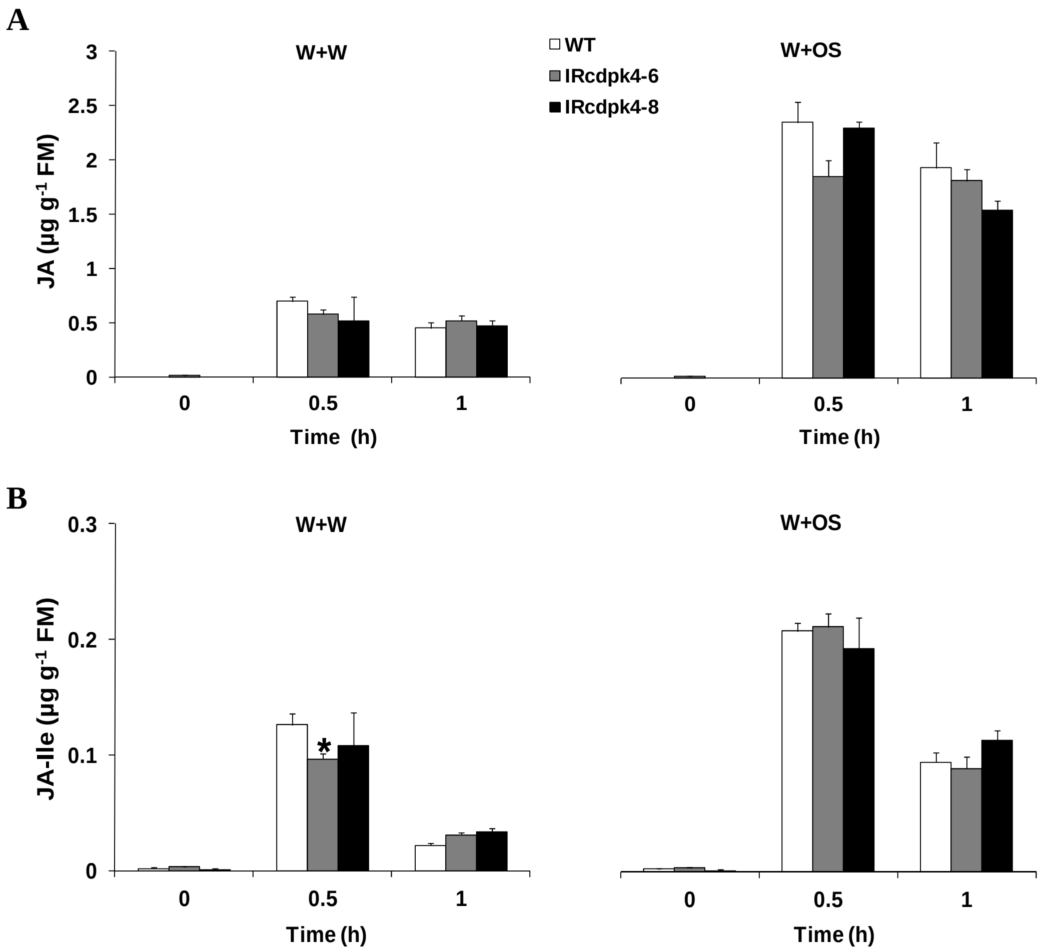
Supplemental Figure S4. Transcript levels of other CDPKs in IRcdpk4/5 plants.

Wild-type (WT) and IRcdpk4/5 (line IRcdpk4/5-1) plants were wounded with a pattern wheel and 20 µL of *M. sexta* OS were applied to wounds. Transcript levels (mean ± SE) of **(A)** *NaCDPK1*, **(B)** *NaCDPK2*, and **(C)** *NaCDPK8* were determined using qRT-PCR in samples collected at indicated times (N = 5).



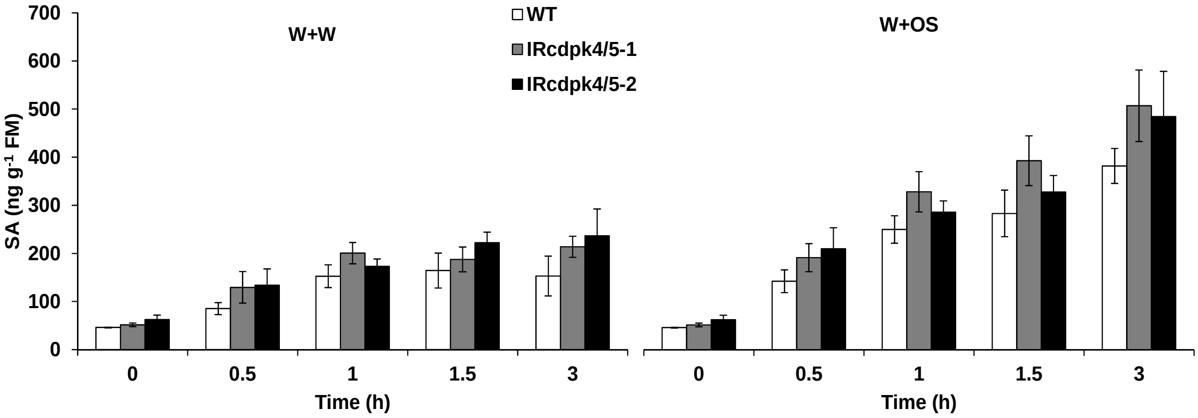
Supplemental Figure S5. Morphology of IRcdpk4 plants at early flowering stage.

Wild-type (WT) and IRcdpk4 (lines IRcdpk4-6 and IRcdpk4-8) plants were photographed 43 days after germination.



Supplemental Figure S6. JA and JA-Ile contents in IRcdpk4 plants.

Wild-type (WT) and IRcdpk4 (lines IRcdpk4-6 and IRcdpk4-8) were wounded with a pattern wheel and 20 µL of water or *M. sexta* OS were applied to wounds (W+W and W+OS, respectively). Contents (mean ± SE) of **(A)** JA and **(B)** JA-Ile were analyzed in samples collected after indicated times. Asterisks indicate significant differences between WT and IRcdpk4 plants (Student's *t*-test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).



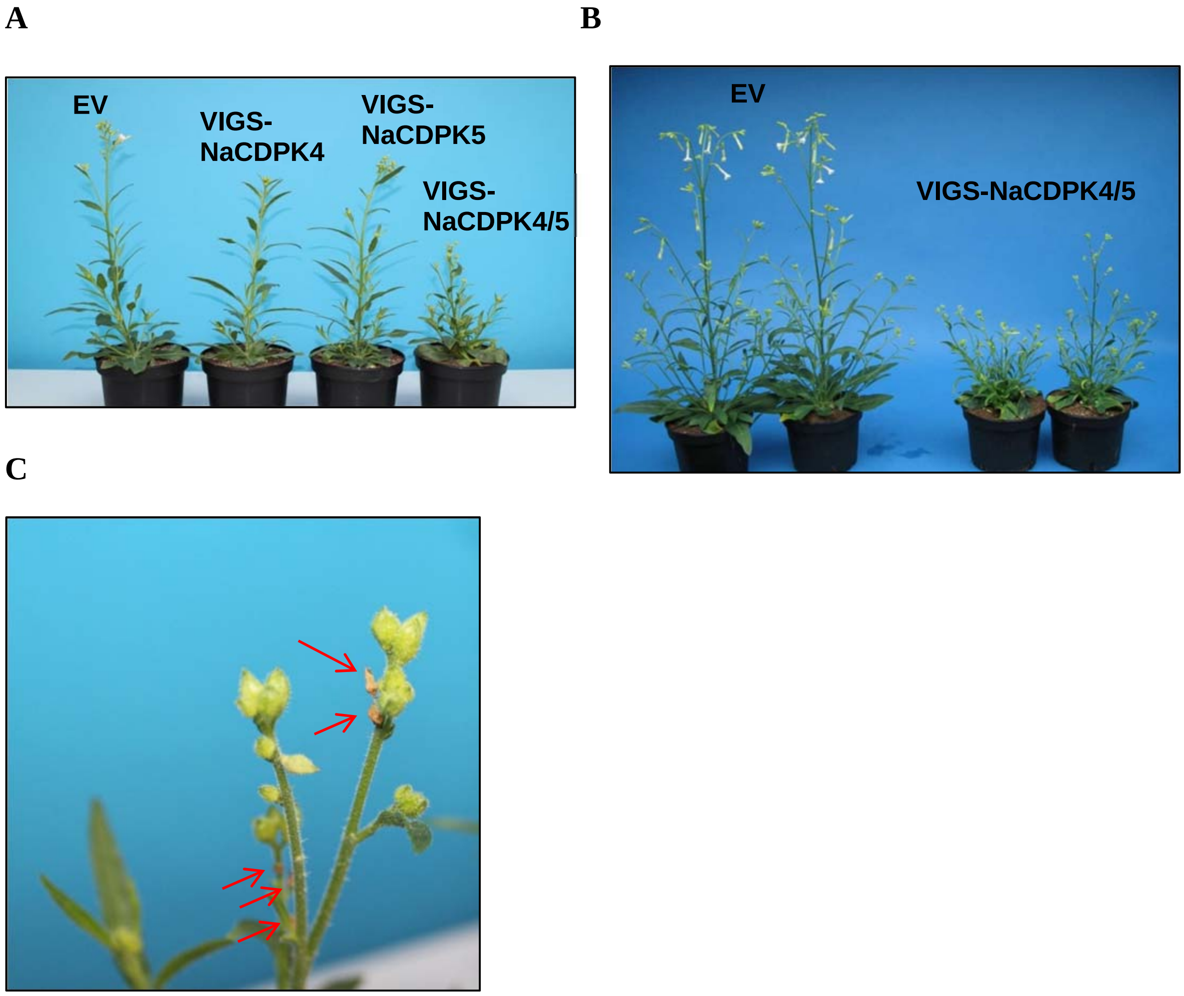
Supplemental Figure S7. SA contents in wild-type and IRcdpk4/5 plants.

Wild-type (WT) and IRcdpk4/5 (lines IRcdpk4/5-1 and IRcdpk4/5-2) were wounded with a pattern wheel and 20 µL of water or *M. sexta* OS were applied to wounds (W+W and W+OS, respectively). Contents (mean ± SE) of SA were analyzed in samples collected after indicated times. No significant differences between WT and IRcdpk4 plants (Student's *t*-test).

```
NaCDPK4  ATGGGTAATAACTGTTTTTCCAGCTCAAAAGTTAGTGGTTCTAACAGCAACACCCCCTCC
NaCDPK5  ATGGGC---AGCTGTTTTTCTAGCTCTAAAGTTAGTGGCTCAAATAGCAATACCCCTTCT

NaCDPK4  ACCACCACCACCACCACCACCGTGAATGTCCGGAGGAACACAGCCAATCCACCTTCTACA
NaCDPK5  ACAACTACTACAAATGTAAATGTTCATCACAACCGTCCTTCAACAA--CAACAA-CAACA

NaCDPK4  TCCACAATTACATCAACAAAACAAGAAGGGTCTCATTGCAATAAACAGAAAGTTAAAGAT
NaCDPK5  ACAACTGTTACATCAAGAAAACAAGAGGGGTCAAATTATAATAGAGAAAAAGGTAATATT

NaCDPK4  AACCACAGAAGCCAACATCAAAAACAACAACCTAGGAATTCTCAGCAAAATGTTAAGAAG
NaCDPK5  AATACAAAAAACAGCCACCAAAAACAACAACCTAGGAGTTCTCAGCAAAATGTTGTTGTT

NaCDPK4  CACAAGAATGGGAGGAGACAGAAGAGTGGGGTTATTCCTTGTGGGAAGAGAACAGATTTT
NaCDPK5  AAGCCAAATTCAAGAAGACAAAGTGGAGGGGTTATTCCTTGTGGGAAAAGAACAGATTTT

NaCDPK4  GGGTATGATAAAGATTTTGATAAGAGGTTTACCATTGGCAAGTTGTTGGGTCATGGCCAA
NaCDPK5  GGGTATGATAAAGATTTTGATAAGAGGTATACTATTGGTAAATTGTTGGGTCATGGCCAA

NaCDPK4  TTTGGTTACACCTACGTTGCCACCCACAAGTCTAATGGAGATCGCGTCGCTGTCAAGAGA
NaCDPK5  TTTGGGTATACATATGTTGCTACAGATAAATCTTCTGGAGATCGTGTTGCTGTTAAGAAA

NaCDPK4  ATTGAGAAGAACAAGATGGTTCCTACGATTGCAGTTGAGGATGTAAAACGAGAAGTCAAG
NaCDPK5  ATTGAGAAAAACAAGATGGTTCTTCCAATTGCGGTTGAGGATGTGAAACGAGAAGTCAAG

NaCDPK4  ATATTGAAGGCCTTATCCGGTCATGAGAATGTGGTTCAATTCAATAATGCATTTGAGGAT
NaCDPK5  ATATTGAAGGCCTTAGCTGGTCACGAGAATGTGGTTCAATTCTATAATTCATTTGAGGAT

NaCDPK4  GATAACTATGTCTACATAGTAATGGAGTTATGTGAGGGTGGAGAACTCTTGGACCGTATT
NaCDPK5  GATAATTATGTCTACATCGTAATGGAGTTATGTGAGGGTGGAGAACTATTGGACCGAATC

NaCDPK4  TTGGCCAAAAAGGACAGCCGTTATGCCGAGAAAGATGCAGCAATAGTTGTACGCCAGATG
NaCDPK5  TTGTCCAAAAAAGATAGTCGATATACTGAGAAAGATGCGGCGATAGTTGTACGCCAGATG

NaCDPK4  CTAAAAGTTGCCGCTCAATGTCATTTACATGGTTTGGTGCATCGTGATATGAAACCTGAG
NaCDPK5  TTAAAAGTGGCTGCTGAGTGTCATTTACATGGTCTGGTGCATCGAGATATGAAACCTGAG

NaCDPK4  AATTTTCTCTTTAAATCTCCAAAGGAGGATTCACCATTGAAGGCCACAGATTTTGGTCTT
NaCDPK5  AATTTTCTCTTTAAATCCTCAAAGATGGATTCGCCATTAAAAGCCACAGATTTTGGTCTT

NaCDPK4  TCAGACTTCATAAGACCAGGGAAGAAGTTCCAAGATATTGTTGGCAGTGCATATTACGTA
NaCDPK5  TCAGACTTCATAAGACCAGGGAAAAAATTCCAAGACATTGTTGGCAGTGCATATTATGTA

NaCDPK4  GCGCCAGAGGTATTAAAGCGTAGATCAGGACCTGAATCAGATGTGTGGAGCATTGGTGTT
NaCDPK5  GCCCCGGAGGTGTTAAAGCGTAGATCAGGACCTGAATCAGATGTATGGAGTATAGGTGTA

NaCDPK4  ATTACATACATTTTGCTCTGTGGCCGTCGGCCCTTCTGGGATAAAACAGAGGATGGCATA
NaCDPK5  ATTACATACATTTTGCTATGTGGTCGTCGGCCTTTCTGGGACAAAACTGAGGATGGTATA

NaCDPK4  TTCAAGGAGGTACTAAGAAACAAGCCTGATTTTTGTCGCAAGCCGTGGCCAACTATAAGC
NaCDPK5  TTCAAGGAGGTCTTACGAAACAAGCCTGATTTTCGTCGCAAGCCATGGCCAAACATAAGC

NaCDPK4  AACAGTGCTAAAGATTTTGTTAAGAAATTACTGGTGAAGGATCCTCGCGCTAGACTTACT
NaCDPK5  AACAGTGCTAAAGATTTTGTAAAGAAATTACTGGTGAAGGATCCGCGCGCTAGACTTACT

NaCDPK4  GCTGCCCAGGCCCTATCGCATCCATGGGTCCGCGAAGGAGGTGATGCATCTGAGATTCCA
NaCDPK5  GCTGCTCAGGCCTTATCGCATCCATGGGTCCGAGAAGGAGGTGATGCATCTGAGATTCCA

NaCDPK4  CTGGACATTTCCGTCTTATCAAACATGCGACAATTTGTCAAGTATAGTCGATTAAAACAG
NaCDPK5  CTCGACATTTCTGTTTTATCCAACATGCGGCAATTTGTCAGATATAGTCGCCTAAAACAG

NaCDPK4  TTTGCTTTACGGGCATTGGCTAGTACAGTTGATGAGGAGGAGCTGGCAGGTGTCCGGGAT
NaCDPK5  TTTGCTTTACGGGCGTTAGCTAGCACACTTGATGAGGAGGAGCTCTCTGATCTGAAGGAT
```

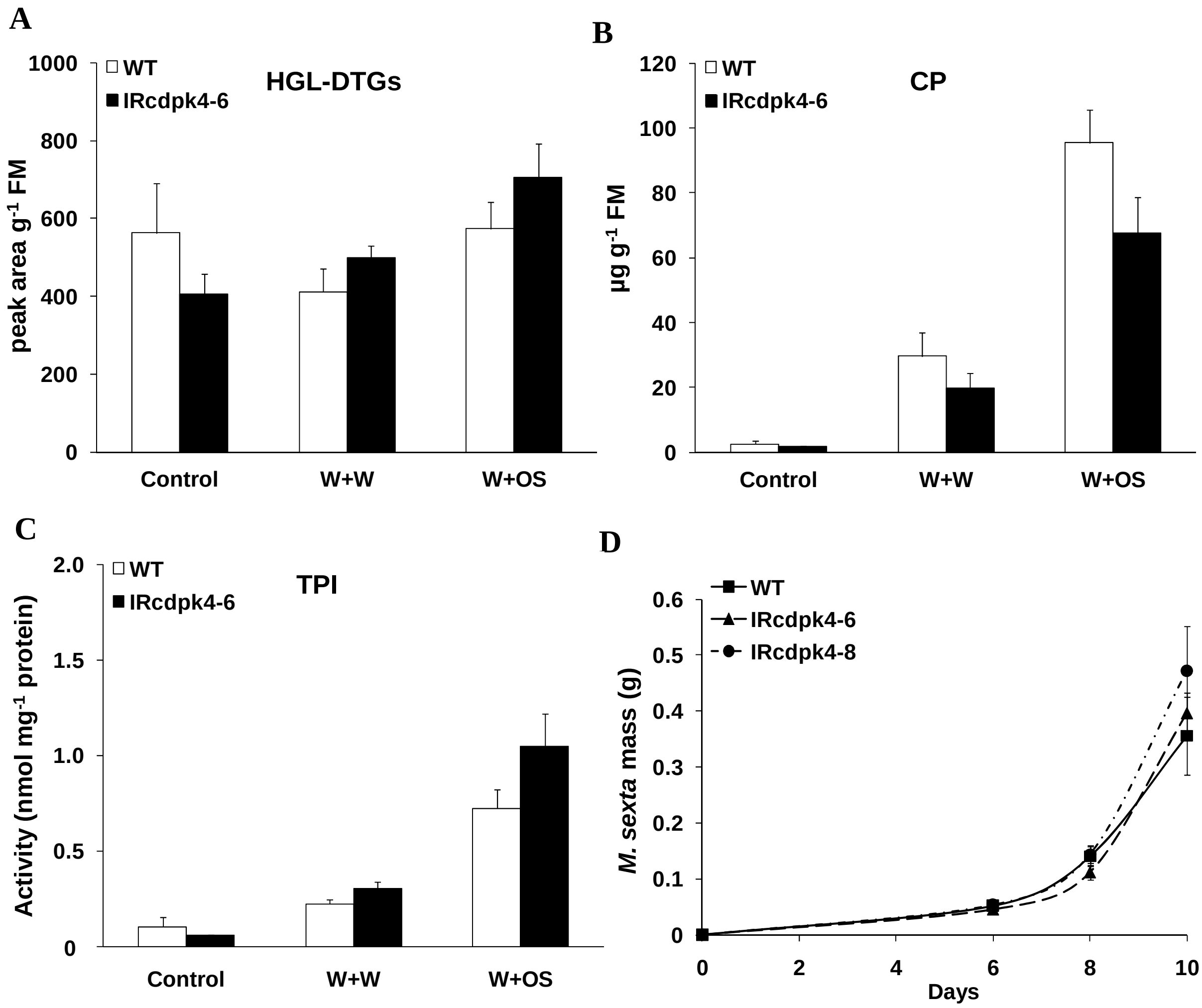
Supplemental Figure S8. Sequences used for preparing VIGS constructs.

The first 190 bp of *NaCDPK4* and *NaCDPK5* coding sequences were used to construct pTV-NaCDPK4 and pTV-NaCDPK5 (indicated by the yellow and green bar, respectively). The sequence of *NaCDPK5* under the purple bars was used to prepare pTV-NaCDPK4/5.



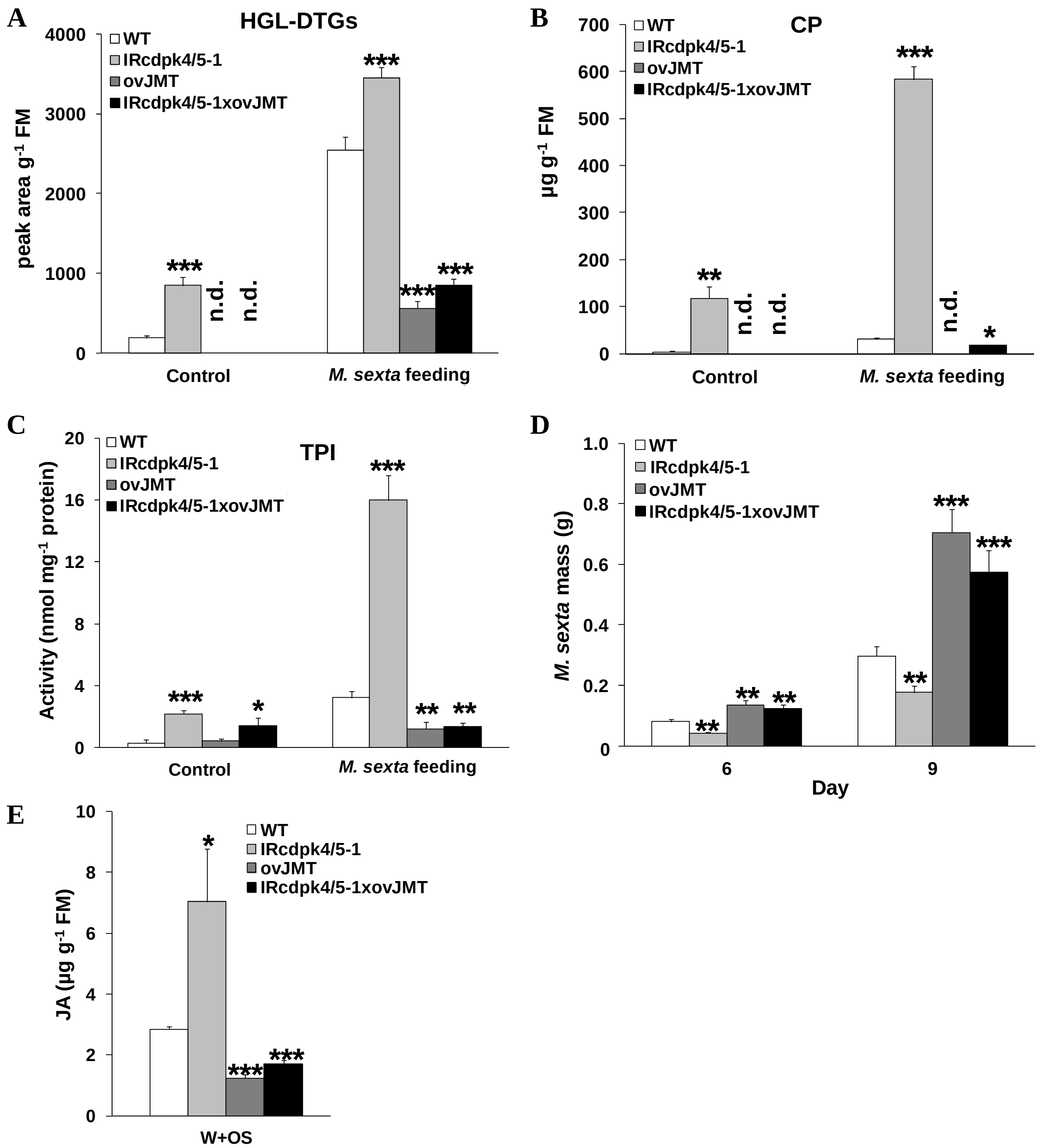
Supplemental Figure S9. VIGS-NaCDPK4/5 but not VIGS-NaCDPK4 or VIGS-NaCDPK5 shows developmental defects.

(**A**) Fifty-five days old EV, VIGS-NaCDPK4, VIGS-NaCDPK5, and VIGS-NaCDPK4/5 plants. **(B)** Sixty-five days old EV and VIGS-NaCDPK4/5. **(C)** Flower buds of VIGS-NaCDPK4/5 plants. Red arrows indicate aborted buds which had completely dried out.



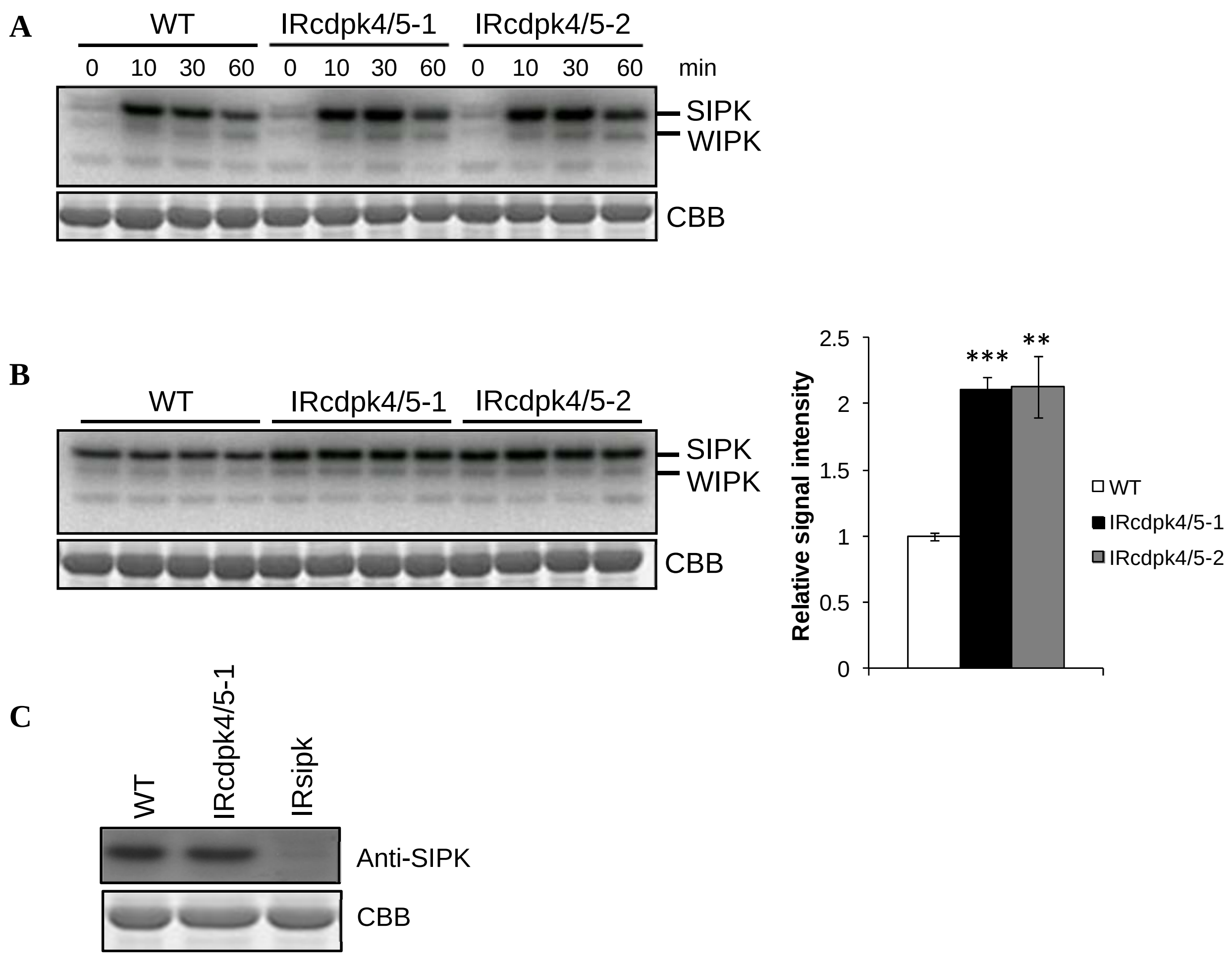
Supplemental Figure S10. Silencing *NaCDPK4* alone does not result in enhanced levels of wounding- and herbivory-induced defensive secondary metabolites and insect resistance.

Wild-type (WT) and IRcdpk4 (lines IRcdpk4-6 and IRcdpk4-8) were wounded with a pattern wheel and 20 µL of water or *M. sexta* OS were applied to wounds (W+W and W+OS, respectively). Concentration (mean ± SE) of **(A)** 17-hydroxygeranyllinalool diterpene glucosides (HGL-DTGs) and **(B)** caffeoylputrescine (CP), and **(C)** activity of trypsin proteinase inhibitors (TPIs) were quantified in plants 3 days after treatment; non-treated plants severed as controls. **(D)** Masses of *M. sexta* grown on WT and IRcdpk4 over 10 days (Student's *t*-test; P > 0.1, N =5).



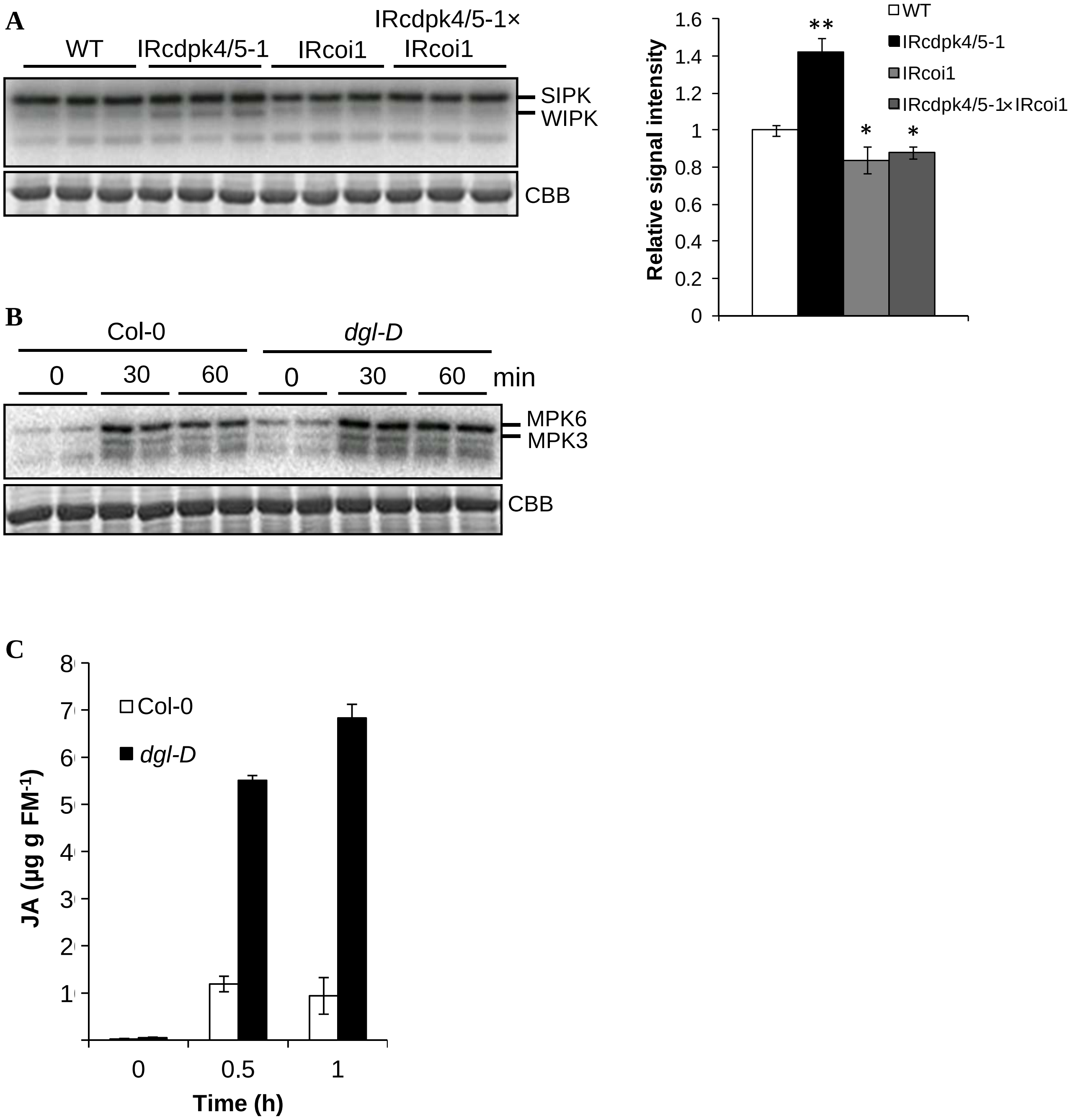
Supplemental Figure S11. Compromising the accumulation of JA in IRcdpk4/5 plants abolishes herbivore defenses.

M. sexta larvae were reared on wild-type (WT), IRcdpk4/5-1, ovJMT, and IRcdpk4/5-1×ovJMT. Concentration (mean ± SE) of **(A)** 17-hydroxygeranyllinalool diterpene glucosides (HGL-DTGs) and **(B)** caffeoylputrescine (CP), and **(C)** activity of trypsin proteinase inhibitors (TPIs) were quantified in plants that had been fed by *M. sexta* for 9 days. **(D)** The masses of *M. sexta* on day 6 and 9. **(E)** JA levels. Plants were wounded with a pattern wheel and 20 µL of *M. sexta* OS were applied to wounds immediately. JA contents were determined in samples collected 1 h after treatment. Asterisks indicate significant differences between WT and IRcdpk4/5-1, ovJMT, or IRcdpk4/5-1×ovJMT plants (Student's *t*-test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). n.d. = not detected.



Supplemental Figure S12. IRcdpk4/5 plants have increased levels of SIPK activity but not abundance.

(A) MAPK activity assay in W+OS-treated WT, IRcdpk4/5-1, and IRcdpk4/5-2 plants. Note: each sample was pooled from 5 biological replicates. **(B)** MAPK activity levels in WT, IRcdpk4/5-1, and IRcdpk4/5-2 samples 30 min after W+OS treatment (4 biological replicates each). Right panel: quantification of relative band intensities (mean ± SE) of SIPK (the average intensity of WT samples was designated as 1); asterisks represent significant difference between WT and IRcdpk4/5 plants (N = 4, Student's *t*-test; **, $P < 0.01$; ***, $P < 0.001$). **(C)** Protein blotting analysis of SIPK levels. Total proteins from wild-type (WT), IRcdpk4/5 (line IRcdpk4/5-1), and IRsipk were separated on a SDS-PAGE gel and were subsequently transferred to a PVDF membrane. Anti-SIPK antibody was used to detect the levels of SIPK protein. CBB: photograph of the RuBisCO large subunit that was visualized by staining the gel with Coomassie Brilliant Blue.



Supplemental Figure S13. SIPK/MPK6 and WIPK/MPK3 over-activation is dependent on the high JA levels and JA signaling.

(A) MAPK activity levels in wild-type (WT), IRcdpk4/5-1, IRcoi1, and IRcdpk4/5-1×IRcoi1 plants 30 min after being treated with W+OS (3 biological replicates each). Right panel: quantification of relative band intensities (mean ± SE) (the average intensity of WT samples was designated as 1); asterisks represent significant difference between WT and other plants (N = 3, Student's *t*-test; *, P < 0.05; **, P < 0.01). **(B)** MAPK activity in wounded

Arabidopsis Col-0 and *dgl-D* mutant. Col-0 and *dgl-D* were wounded with a pattern wheel and MAPK activity was determined in samples harvested at indicated times using an in-gel kinase assay (2 biological replicates each).Wild-type (Col-0) and *dgl-D* plants were wounded with a pattern wheel, and JA levels (mean ± JA) were determined in samples collected at indicated times (N = 5). **(C)** JA levels in wild-type and *dgl-D* Arabidopsis plants. Wild-type (Col-0) and *dgl-D* plants were wounded with a pattern wheel, and JA levels (mean ± JA) were determined in samples collected at indicated times (N = 5).