Supplemental Data. Bartels et al. (2009). MAP KINASE PHOSPHATASE1 and PROTEIN TYROSINE PHOSPHATASE1 Are Repressors of Salicylic Acid Synthesis and SNC1-Mediated Responses in *Arabidopsis*.



### SALK-PTP1 gDNA: (2382 bp)

CTTCAATTAACCAGAAAAGGTCAGATTCTCCGCCGCCTCACACGACGGGCAACTTCAGTT CCTGCCGTCGCGTTAGATCCCGCCGTCAATCGGTCATCGGTCACCCGGTAAAACCTCTTCCG CAAAACTCTCTCTCCCCCGATCAGCTCAACCACTGCCACCAAGCTCTCGGCGTTTTCC GGGGAAAGATCCAAAATCCTGACTCGATCGCTCATGAGTTTACCGGTTTACAGGTTATAG ATCCATCCTCTAAATTGAGAATATTCCTCTTCCTAGTGTTTCTTCCTTAAAAACAAATTG GATCATAATACACTCTCTTCGAACGCAGGCTAATAGGATGTGGCCATCGGAGCTGCTGCT AAACAGTACAGTGGCTATGAACAGTGTCAATGTTGAGAAAAACAGATACAGTGATGTTGT GATTTTGATTTTGCTTTGGATATAGTTGACAAGAACAGGATTGTTCTGAATCCATGTAAA TGTTTATCTGTATCTTTTTGTGGTGATAGATTTTTTGAAGGTTTTACAATGTGTTAATGTT ATGTAGACGTCGGAGTCTGAGAGTATTTCTCAGTTCATAGCTACGCAAGGTCCT<mark>TTA</mark>CCA CACACGATGGAGGACTTCTCGGGAGATGGTTATTCAGCAGCATTGCCCCAATCATAGTGATG CTCTTTTTAGACGATTGGATATTAATATGATCTGTTGCAAATATACTAGTACAAGCGAAG TTGTTGTGAGCATTTGTTTCTCAAGTTAAATTTCTTCGTATTAAATTTTAGCAAGTTTTA ATCTTGTTTGTTTTTCATGCTAACTTATCTAATTACTTGTTACAGACTGTTAAATGCGG GGACTATTTTCAAGACGAAGATGGACCTAGAGAATTTGGCAACATATCTCTTACAACAAA GTGGATAAAGACTACTGACACTTCATTGATGTACGGAATCTTGAGGTTAACTACAAGGA GGTAAGAATCTTGTATCAGTTTTCTTCTTCTGTTTATTTTACCAGATCAAGTTATTTCTTCG CCGTTTTGCATATTCAGTATCCAGAATGGCCTGATCATGGAGTTCCCAAGGATACAGTGG CTGTCCGTGAAATTCTAAAAAGACTATATCAAGTACCACCTAGTCTCGGCCCAATCATTG TGCACTGCAGGTACGATGCATGTCAATGAGGGATACAAGTGTTGTTCAACGTCTCCTGAG AGAATTGTTTTTTTTCTCTCTCAGTGCAGGTATAGGAAGAACTGGAACATACTGTGCGATA CATAACACAATCCAAAGAATTCTTGCTGGCGATATGTCTGCGTTGGATCTTGCTAAAACC GTGGCACTATTTCGCAAGCAACCCATTGGCATGGTTCAAACCATGGTAAATCTTTCAGCG TTACAATTTTTAGCTACCAAATTTCTGTATCTCACCACATAATTTGTTCTGAATTGTTCA ATTCCTTACTCTTTTTGAAGGATCAATACTTCTTTTGCTACAATGCTATTGTTGATGAA TTAGAAGATCTAACCGCGGGGGACAAATGCTGGAACGAGTTCCTAAAGGTACGCAATTTCA ACAATCAAATCTGAGTGAGCCATACCTGTTATAATCTATGAATACTCTTATCTTGTCTAT CTTTTGATCCAGCTGAAGGGTTCGTCTGCTTAGAGAGGGAAAAAGGCTTCTACCCAACTA TATCAATTTCGTAATGTGATTCAAGAAGAAACCCGCGTGAATCTCTACATTCAAGACTTT TCAATTTCTGTAAATTTCCATATCTGATAGGTTTCATTGTTACTTTTGTTGTGGAAATGC **TGATTAAAAAGCAAATGAAGTCGCCAATGCGAGATTCTTCAAAGACTATGAATTCGGTTT** GATTTGGTTTGAAGTGTAAAAGAACCGAAGATTAATTTAATGGTTTAGACTTAACCGAGT TTCTTTCGAAAATAAATAAATCAGAAGCGGTTAAAATTGGCG

20 bp deletion in exon 4

## **Supplemental Figure 1**

- (A) The pROK2 T-DNA is inserted into exon 4 of PTP1 (At1g71860) in the ptp1-1 (SALK 118658) mutant. The vellow blocks represent the exons and the connecting black lines the introns.
- (B) The T-DNA insertion is exactly between the indicated residues (green and yellow) and is associated with a 20-bp deletion (underlined).RB = right border of the T-DNA, LB = left border.
- (C) The *ptp1-1* mutant does not show any phenotype compared to Col wild type under standard growth conditions. Photographs of 22-d-old plants are shown. Bars = 1 cm.
- (D) RNA gel blot analysis shows absence of wild-type PTP1 mRNA in the ptp1-1 mutant. The blot was sequentially probed with PTP1 and PHS1 cDNA (loading control). Note that the larger transcript detectable in ptp1-1 due to the T-DNA insertion cannot produce a functional PTP1 protein.
- (E) Fresh weight of 20-d-old plants. The means ± SE (n=47) are shown. The difference between Col and mkp1(Col) is statistically significant, as indicated by \*\*\* (Student's t test, P < 0.0001). The difference between Col and *ptp1* is not significant (ns).



- (A) Growth of *mkp1*(Ws) is comparable to the Ws wild type under standard growth conditions. A comparison of *mkp1*(Col) to the Col wild type is shown as contrast. Photographs of inflorescences were taken of 40-d-old plants, of rosettes of 28-d-old plants. Bars = 0,5 cm (inflorescence) and 1cm (rosettes)
- **(B)** The *mkp1*(Col) growth phenotype is complemented by expression of a polyoma(Pyo)-tagged MKP1. Pictures of 33-d-old plants are shown. Bars = 2 cm.
- (C) The PR-gene misexpression phenotype of mkp1(Col) is complemented by expression of a polyoma(Pyo)tagged MKP1.Quantitative RT-PCR data for PR1 and PR5 expression are shown. Error bars represent SD of technical triplicates.
- (D) An *MKP1*-co-suppression line (*mkp1-cs*) in the Col background shows aberrant growth phenotypes comparable to the *mkp1*(Col) T-DNA insertion line. Photographs of 22-d-old plants are shown. Bars = 1 cm.
- (E) Quantitative RT-PCR analysis of *MKP1* expression shows reduced *MKP1* mRNA levels in the *mkp1-cs* line, normal levels in *ptp1-1* and overexpression in the *mkp1/Pro35S:Pyo-MKP1* line. Error bars represent SD of three biological repetitions.
- (F) The *mkp1-cs* phenotype is associated with elevated *PR1* gene expression as determined by qRT-PCR. Error bars represent SD of three biological repetitions.
- (C, E, F) Samples from 22-d-old plants grown on soil under standard conditions.



**Supplemental Figure 3** Phenotypes of mkp1(Col) and mkp1 ptp1 appear late in development. Photographs of plants at the indicated number of days after germination are shown. Bars = 1 cm.



Photographs of 40-d-old plants grown on soil under standard conditions are shown. Aberrant phenotypes of mkp1(Col) are partially suppressed by mpk6-3. Bars = 2 cm.



Free (A, C) and conjugated (B, D) salicylic acid levels of 22-d-old soil-grown plants were measured by HPLC. (A, B) and (C, D) represent two different experiments. The average of three independent biological samples is shown for each genotype in each experiment. Error bars represent SD (n = 3).



Growth phenotypes of mkp1(Col) and mkp1 ptp1 are suppressed by elevated temperature, including the phenotypes of early senescence (**A**, **B**) and aberrant inflorescences (**C**). Photographs of 25-d-old plants are shown. Bars = 1 cm.



Co-correlation scatter plot according to the *Arabidopsis* Co-expression Tool (ACT). Pearson correlation coefficients are shown for *MKP1* and *PTP1* probes using 322 ATH1 arrays. The correlation *r*-values of each of 21891 genes with each of the two driver genes are plotted as blue squares. The values for the dual-specificity phosphatase genes are highlighted in red.

*MKP1* shows similar expression patterns with *PTP1*, giving a positive correlation against the other genes in the database. The two query genes always have correlation values of 1.0 with themselves. If they are strongly correlated with each other, they are located at the top right of the figure.

ACT: http://www.arabidopsis.leeds.ac.uk/act/coexpanalyser.php#CO3 Jen et al. (2006) Plant J. **46:** 336–348; Manfield et al. (2006) Nucleic Acids Res. **34:** W504-509.



**Supplemental Figure 8** The *mpk6* mutation does not suppress the *mpk4* dwarf growth phenotype. Photographs of 22-d-old plants are shown. Bars = 2 cm.

**Supplemental Table 1**: Primer sequences used for mutant genotyping. The left-border-specific primer used to genotype SALK insertion lines is LBa1 (5'-TGGTTCACGTAGTGGGCC-3')

eds1-22		
S_071051_fw	5'-CTAACTCAGCTCTCTTGACG-3'	
S_071051_rev	5'-TACGCTCAATGACCTTGGAG-3'	
WT: S_071051_fw/S_071051_rev = 0.45 kb; S_071051_rev/LBa1 = none		
<i>eds1-22</i> : S_071051_fw/ S_071051_rev = none; S_071051_rev/LBa1 = 0.69 kb		
mkp1		
MKP1 fw	5'-ACAAGTCTATGGAAGAAGC-3'	
MKP1 rev	5'-TGTCTTTCGCCACAGCATC-3'	
pGKB5 GusRb1	5'-ACGCAGCACGATACGCTGG-3'	
WT: MKP1_fw/MKP1_rev = 0.62 kb; MKP1_rev/pGKB5_GusRb1 = none		
<i>mkp1</i> : MKP1_fw/MKP1_rev = none; MKP1_rev/pGKB5_GusRb1 = 0.68 kb		
mnk3-1		
S 151594 fw	5'-CTTCTGTTGAACGCGAATTGCG-3'	
S 151594 rev	5' -TCCGTTGATGCAAGTTGAGCC-3'	
WT S 151594 fw/S	151594 rev = 1.3 kb <sup>-</sup> S 151594 fw/l Ba1 = none	
<i>mpk3-1</i> : S_151594_fv	v/S_151594_rev = none; S_151594_fw/LBa1 = 1.0 kb	
mpk4		
S_056245_fw	5'-TGACTGAATATGTTGTTACACG-3'	
S_056245_rev	5'-ACTCACCAAAGCCGTACC-3'	
WT: S_056245_fw/S_	_056245_rev = 1.0 kb;	
mpk4: S_056245_fw/s	S_056245_rev = none; S_056245_rev/LBa1 = 0.66 kb	
mpk6-2		
S 073907 fw	5'-GATCTTTTCCATCTGCGTCAAG-3'	
S 073907 rev	5'-CACTGTCGGGAACTTATCAGTGA-3'	
WT: S 073907 fw/S 073907 rev = 0.9 kb; S 073907 fw/LBa1 = none		
<i>mpk6-</i> 2: S_073907_fw/S_073907_rev = none; S_073907_fw/LBa1 = 0.8 kb		
NahG transgene		
NahG_fw	5'-GAAAAACAATAAACTTGGCTTGCG-3'	
NahG_rev	5'-ACCTTCCAGCACATGACTACG-3'	
WT: NahG_fw/NahG_rev = none		
NahG transgenic line: NahG_fw/NahG_rev = 0.52 kb		
pad4-1		
PAD4 fw	5'-TCGCATAAGACTAGCTAAGTTTTG-3'	
PAD4 rev	5'-TAAGTCTCCATTGCGTCACTC-3'	
pad4-1 fw	5'-TCGCATAAGACTAGCTAAGTTTTA-3'	
WT: PAD4_fw/PAD4	rev = 0.57 kb; pad4-1_fw/PAD4_rev = none	
pad4-1: PAD4_fw/PAD4_rev = none; pad4-1_fw/PAD4_rev = 0.57 kb		
ptp1-1		
S 118658 LP	5'-ACCTTTACCCTTTCTTCTCG-3'	
S 118658 RP	5'-AAATAGTCCCCGCATTTAAC-3'	
WT: S 118658 LP/S 118658 RP = 1.2 kb; S 118658 RP/LBa1 = none		
<i>ptp1-1</i> : S_118658_LP/S_118658_RP = none; S_118658_RP/LBa1 = 0.75 kb		
S 047058 fw	5'-TCTGTTGCTTTAACCTTTGCTCC-3'	
S 047058 rev	5'-TGGTGATTCCGATTTTCTTCCAC-3'	
WT: S 047058 fw/S	047058 rev = 0.64 kb; S 047058 rev/LBa1 = none	
snc1-11: S_047058_fw/S_047058_rev = none; S_047058_rev/LBa1 = 0.58 kb		

**Supplemental Table 2**: Primer sequences used for molecular cloning. *attB* recombination sites facilitating Gateway-based cloning are indicated in italic. Start ATG codons are indicated in blue, Stop codons in red.

MKP1	
attB1-MKP1	5'- <i>GGGGACAAGTTTGTACAAAAAAGCAGGCTTG<mark>ATG</mark>GTGGGAAGAGAGGATGCG-3'</i>
attB2-MKP1	5'- <i>GGGGACCACTTTGTACAAGAAAGCTGGGTC</i> GCTTGCGGGTGGGTG <b>TTA</b> TAG-3'
attB2-MKP1 <sup>-STOP</sup>	5'- <i>GGGGACCACTTTGTACAAGAAAGCTGGGTC</i> TAGCGCGCTCAGCAGTGC-3'
PTP1	
attB1-PTP1	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTGATGGCGACCGGTAAAACC-3'
attB2-PTP1	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAGGAACTCGTTCCAGC-3'
attB2-PTP1 <sup>-stop</sup>	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCGGAACTCGTTCCAGCATTTG-3'
MPK1	
attB1-MPK1	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTGATGGCGACTTTGGTTGATCCTC-3'
attB2-MPK1	5'- <i>GGGGACCACTTTGTACAAGAAAGCTGGGTC<b>TCA</b>GAGCTCAGTGTTTAAGGTTG-3'</i>
MPK2	
attB1-MPK2	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTGATGGCGACTCCTGTTGATCC-3'
attB2-MPK2	5'- <i>GGGGACCACTTTGTACAAGAAAGCTGGGTC<b>TCA</b>AAACTCAGAGACCTCATTGTTG-3'</i>
МРК3	
attB1-MPK3	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTGATGAACACCGGCGGTGGC-3'
attB2-MPK3	5'- <i>GGGGACCACTTTGTACAAGAAAGCTGGGTC<mark>CTA</mark>A</i> CCGTATGTTGGATTGAGTG-3'
MPK4	
attB1-MPK4	5'- <i>GGGGACAAGTTTGTACAAAAAAGCAGGCTTG<b>ATG</b>TCGGCGGAGAGTTGTTTC-3'</i>
attB2-MPK4	5'- <i>GGGGACCACTTTGTACAAGAAAGCTGGGTC</i> TCACACTGAGTCTTGAGGATTG-3'
MPK6	
attB1-MPK6	5'- <i>GGGGACAAGTTTGTACAAAAAGCAGGCTTG<b>ATG</b>GACGGTGGTTCAGGTCAAC-3'</i>
attB2-MPK6	5' - GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATTGCTGATATTCTGGATTGAAAG-3'