

Supplemental figure 1

Local sequence alignment of *Arabidopsis* 20 MAPKs.

Alignment was performed using ClustalX. Homologous position of MPK6 Y144 residue is shown in orange. Homologous positions of MPK6 D218 and E222 residues are shown in blue. MPK6 sequence is in red.

Accession number for the sequences: At1g10210 (MPK1), At1g59580 (MPK2), At3g45640 (MPK3), At4g01370 (MPK4), At4g11330 (MPK5), At2g43790 (MPK6), At2g18170 (MPK7), At1g18150 (MPK8), At3g18040 (MPK9), At3g59790 (MPK10), At1g01560 (MPK11), At2g46070 (MPK12), At1g07880 (MPK13), At4g36450 (MPK14), At1g73670 (MPK15), At5g19010 (MPK16), At2g01450 (MPK17), At1g53510 (MPK18), At3g14720 (MPK19), and At2g42880 (MPK20).

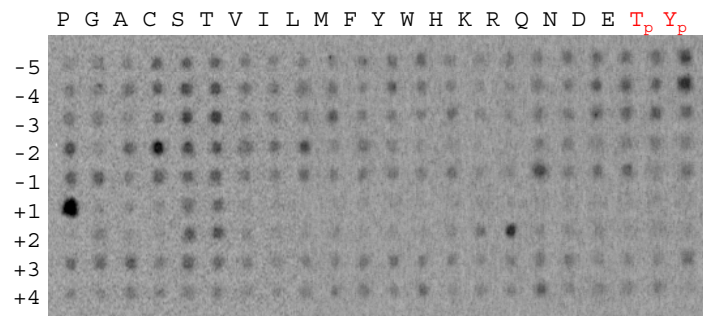
Group C	MPK1	...DYYLVYELMDTDLHQQIKSSQVL... ANCDLKICDFGLARASNT---KGQFMTEYVWTRWYRAPELLL...
	MPK2	...DYYLVYELMDTDLHQQIKSSQVL... ANCDLKICDFGLARTSNT---KGQFMTEYVWTRWYRAPELLL...
	MPK7	...DYYLVYELMDTDLHQQIKSSQSL... ANCDLKICDFGLARTSQG---NEQFMTEYVWTRWYRAPELLL...
	MPK14	...DYYLVYELMDSDLNQQIKSSQSL... ANCDLKICDFGLART-----YEQFMTEYVWTRWYRAPELLL...
Group B	MPK4	...DYYIVYELMDTDLHQQIIRSNQPL... ANCDLKLGFGLARTKS----ETDFMTEYVWTRWYRAPELLL...
	MPK11	...DVHIVYELMDTDLHQQIIRSNQPL... ANCDLKIGDFGLARTKS----ETDFMTEYVWTRWYRAPELLL...
	MPK12	...DYYIVYELMDTDLQRILRSNQTL... SKNELKIGDFGLARTTS----DTDFMTEYVWTRWYRAPELLL...
	MPK5	-----MDTDLHQQIIRSNQSL... SNCDLKITDFGLARTTS----ETEYMTEYVWTRWYRAPELLL...
	MPK13	...DYYIVYELMDTDLHQQIIRSTQTL... TNCDLKICDFGLARTSN----ETEIMTEYVWTRWYRAPELLL...
Group A	MPK10	...DYYIVNELMEFDLYRTLKSDQEL... TQCDLKICDFGLARATP----ESNLMTEYVWTRWYRAPELLL...
	MPK3	...DYYISTELMDTDLHQQIIRSNQSL... ANCDLKICDFGLARPTS----ENDFMTEYVWTRWYRAPELLL...
	MPK6	...DYYIAYELMDTDLHQQIIRSNQAL... ANCDLKICDFGLARVTS----ESDFMTEYVWTRWYRAPELLL...
Group D	MPK8	...DIYVVFELMESDLHQQVIKANDDL... ADCKLKICDFGLARVSFNDAPTAIFWTDYVATRWRAPELCG...
	MPK15	...DYYVVFELMESDLHQQVIKANDDL... ADCKLKICDFGLARVSFNDAPTAIFWTDYVATRWRAPELCG...
	MPK9	...DIYVVFELMESDLHQQVIKANDDL... SDCKLKICDFGLARVSFNDAPSAIFWTDYVATRWRAPELCG...
	MPK17	...DIYVVFELMESDLHVVLVKVNDDL... ADCKIKICDLGLARVSFTDPSAVFWTDYVATRWRAPELCG...
	MPK16	...DIYVVFELMESDLHQQVIKANDDL... ADCKLKICDFGLARVAFNDTPTAIFWTDYVATRWRAPELCG...
	MPK18	...DIYVVFELMESDLHQQVIKANDDL... ANCKLKVCFGLARVAFNDTPPTTVFWDYVATRWRAPELCG...
	MPK19	...DIYVVFELMESDLHQQVIKANDDL... ANCKLKVCFGLARVSFNDTPPTTVFWDYVATRWRAPELCG...
	MPK20	...DIYVVFELMESDLHQQVIKANDDL... ANCKLKICDFGLARVAFNDTPPTTVFWDYVATRWRAPELCG...
		*: ** : : : * :. :*: *:**** *:***.*****

Supplemental figure 2

Substrate preference of some MAPKs.

Phosphorylation of the semi-degenerated peptide array by MPK3^{WT}, MPK3^{T129C} and MPK4^{Y124C}.

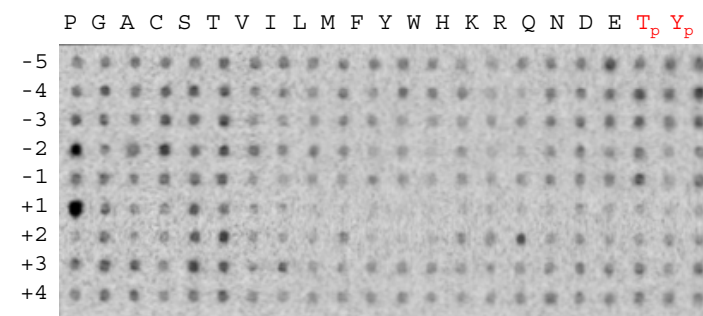
A MPK3^{wt}



B MPK3^{T119C}

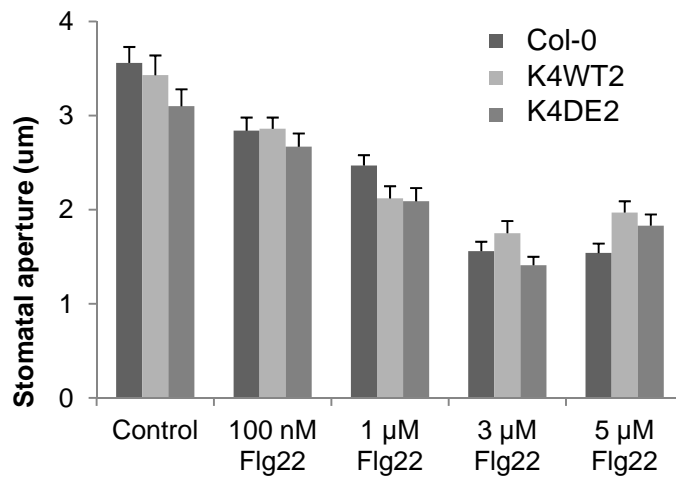


C MPK4^{Y124C}



Supplemental figure 3

Stomatal closure induced by flg22 at indicated concentrations in Col-0, K4WT2 and K4DE2 lines. Data are mean \pm SE of two independent experiments.



Supplemental table 1

List of MPK6 mutations identified in the yeast complementation screen.

Clone number	Protein mutation
1	G43R;K112M;Y144F;S217G
2	Y144C;L387H;Q394L
3	Y144C
4	C78S;Y144C
5	D118N;Y144F;F219I
6	P68L;Y144C
7	P37L;Y144F;C360Y;H371Y
8	A9V;D218G;E222A
9	R109C;L133I;Y144F;F364L
10	F98L;R274H;P319H;F364I
11	S5P;A136P;Y144C
12	D103Y;Y144C
13	K112R;Y144C
14	H42Q;D240N;R274H;L276H;C360R
15	H116N;N237I;R274H
16	E34V;N138S;Y144F;V250L;F364L
17	K112T;Y144F
18	D268E;R274H;N294K;T361I
19	A97T;H116L;Y144C
20	E34V;D99A;Y144C;N349D;H371Q;I382S
21	Y144C;I299M;S309P
22	I69L;R267Q;D268N;E287K;H318N;F366L
23	N54I;Y144F;C360Y
24	A25T;I47V;I51M;I111V;Y144F;H165Y
25	F21Y;Y144F;K327R;P363S;F366I
26	N96T;Y144C;F366S
27	C78S;Y144C

Supplemental table 2

Pairs of primers used in this work.

Pairs of primers used for GateWay cloning.	
Clone name	Sequence of primer couple
pDNR207-MPK3-END/STOP	GGA GAT AGA ACC ATG AAC ACC GGC GGT GGC TCC ACC TCC GGA TCM ACC GTA TGT TGG ATT GAG TGC T
pDNR207-MPK4-END/STOP	GGA GAT AGA ACC ATG TCG GCG GAG AGT TGT TTC TCC ACC TCC GGA TCM CAC TGA GTC TTG AGG ATT GAA C
pDNR207-MPK6-END/STOP	GGA GAT AGA ACC ATG GAC GGT GGT TCA GGT CA TCC ACC TCC GGA TCM TTG CTG ATA TTC TGG ATT GAA AGC
pDNR207-MPK7-END/STOP	GGA GAT AGA ACC ATG GCG ATG TTA GTT GAG CCA C TCC ACC TCC GGA TCM GGC ATT TGA GAT TTC AGC TTC AG
pDNR207-MKK1-END/STOP	GGA GAT AGA ACC ATG AAC AGA GGA AGC TTA TGC C TCC ACC TCC GGA TCM CCA TTG CGA GAT GAA GGA GC
pDNR207-MKK4-END/STOP	GGA GAT AGA ACC ATG AGA CCG ATT CAA TCG CCT C TCC ACC TCC GGA TCM TGT GGT TGG AGA AGA CGA G
pDNR207-ERF104-END/STP	GGA GAT AGA ACC ATG GCA ACT AAA CAA GAA GCT TCC ACC TCC GGA TCM AGT GAC GGA GAT AAC GGA AAA
pDNR207-VIP1-END/STOP	GGA GAT AGA ACC ATG GAA GGA GGA GGA AGA GGA TCC ACC TCC GGA TCM GCC TCT CTT GGT GAA ATC CAT
pDNR207-MKS1-END/STOP	GGA GAT AGA ACC ATG GAT CCG TCG GAG TAT TTT TCC ACC TCC GGA TCM ATC TTG ATC CCA AAT ATG ACT AAA
Pairs of primers used for point mutations.	
Primer name	Sequence of primer couple
<i>MPK3</i> ^{T119Y}	F: ATC CAT CAA CTC ACA AAC AAT ATA TAC R: GTA TAT ATT GTT TGT GAG TTG ATG GAT
<i>MPK3</i> ^{D193G/E197A}	F: GAG AAT GGT TTT ATG ACT GCG TAT GT R: ACA TAC GCA GTC ATA AAA CCA TTC TC
<i>MPK3</i> ^{I119C}	F: GTTTATATCTCTTGTGAATTAATGGAT R: ATCCATTAATTCACAAGAGATATAAAC
<i>MPK4</i> ^{Y124C}	F: GTC CAT AAG CTC ACA AAC AAT GTA AAC R: GTT TAC ATT GTT TGT GAG CTT ATG GAC
<i>MPK4</i> ^{D198G/E202A}	F: CGA GAC TGG CTT TAT GAC TGC ATA TGT TG R: CAA CAT ATG CAG TCA TAA AGC CAG TCT CG
<i>MPK6</i> ^{Y144C}	F: GAT GTT TAC ATC GCG TAT GAG TTA ATG GAC AC R: GTG TCC ATT AAC TCA TAC GCG ATG TAA ACA TC
<i>MPK6</i> ^{D218G}	F: TTC TGA GAG TGG TTT CAT GAC TGA A R: TTC AGT CAT GAA ACC ACT CTC AGA A
<i>MPK6</i> ^{E222A}	F: TTC ATG ACT GCA TAT GTT GTC ACG AGA R: TCT CGT GAC AAC ATA TGC AGT CAT GAA
<i>MPK6</i> ^{D218G/E222A}	F: GAG AGT GGT TTC ATG ACT GCA TAT GTT R: AAC ATA TGC AGT CAT GAA ACC ACT CTC
<i>MPK7</i> ^{Y114C}	F: ATC CAT TAG CTC ACA AAC CAA ATA AAC R: GTT TAT TTG GTT TGT GAG CTA ATG GAT
<i>MPK7</i> ^{Q188G/E192A}	F: GGT AAT GGA CAG TTC ATG ACT GCG TAT GTG GTT R: AAC CAC ATA CGC AGT CAT GAA CTG TCC ATT ACC
Other primers	
DR_F/DR_R	TTT CTC TTT CTT TCC TAT AAC ACC AAT AG TG GTG TCA ACA ACG TAT CTA CCA ACG ATT TGA CC
PC2_F/PC2_R (BglII underlined)	<u>AGA TCT</u> CCA GCT TTC TTG TAC AAA GTG GTG ATC <u>AGA TCT</u> TCA CTT CTC GAA CTG AGG ATG AGA C
GFP_S/GFP_R (BamH1 underlined)	<u>GGA TCC</u> ATG GTA GAT CTG ACT AGT AAA GGA G <u>GGA TCC</u> TCA CAC GTG GTG GTG GTG GTG GCT AG

Supplemental table 3

Plasmids used in this study.

Name	Description	Origin
pDR195gtw	Yeast strong expression vector	Oomen <i>et al.</i> , 2009. <i>New Phytol.</i> 181:637-50
pDEST17	Expression of His tagged protein in Coli	Invitrogen
Peri-HIS-MBP	Expression of Peri-His-MBP tagged protein in Coli	Nallamsetty <i>et al.</i> , 2005. <i>Protein Sci.</i> 14:2964-71
pDEST22	Yeast 2 hybrid vector	Invitrogen
pDEST32 modified	Yeast 2 hybrid vector	Modified from invitrogen to carry a Kanamycin Resistant gene

Supplemental text 1

DNA sequence of the PC2 Tag.

CCAGCTTTCTTGTACAAAGTGGTGATCATGGGTGAACAAAAGTTGATTTCTGAAGAAGATTT
GAACGGTGAACAAAAGCTAATCTCCGAGGAAGACTTGAACGGTGAACAAAATTAATCTCA
GAAGAAGACTTGAACGGATCCTCTAGAGGTGAACAAAAGTTGATTTCTGAAGAAGATTTGA
ACGGTGAACAAAAGCTAATCTCCGAGGAAGACTTGAACGGTGAACAAAATTAATCTCAGA
AGAAGACTTGAACGGATCCTCTAGAGGTGAACAAAAGTTGATTTCTGAAGAAGATTTGAAC
GGTGAACAAAAGCTAATCTCCGAGGAAGACTTGAACGGTGAACAAAATTAATCTCAGAAG
AAGACTTGAACGGATCCACTAGTGGATCCCCCGGGCTGCAGCCTAGGGATTACGATATCC
CAACGACCGCCAGTCATCACCATCACCATCACCATCACTGGTCTCATCCTCAGTTCGAGAA
GTGA