

A sequential MAP kinase cascade regulates mechanical signalling

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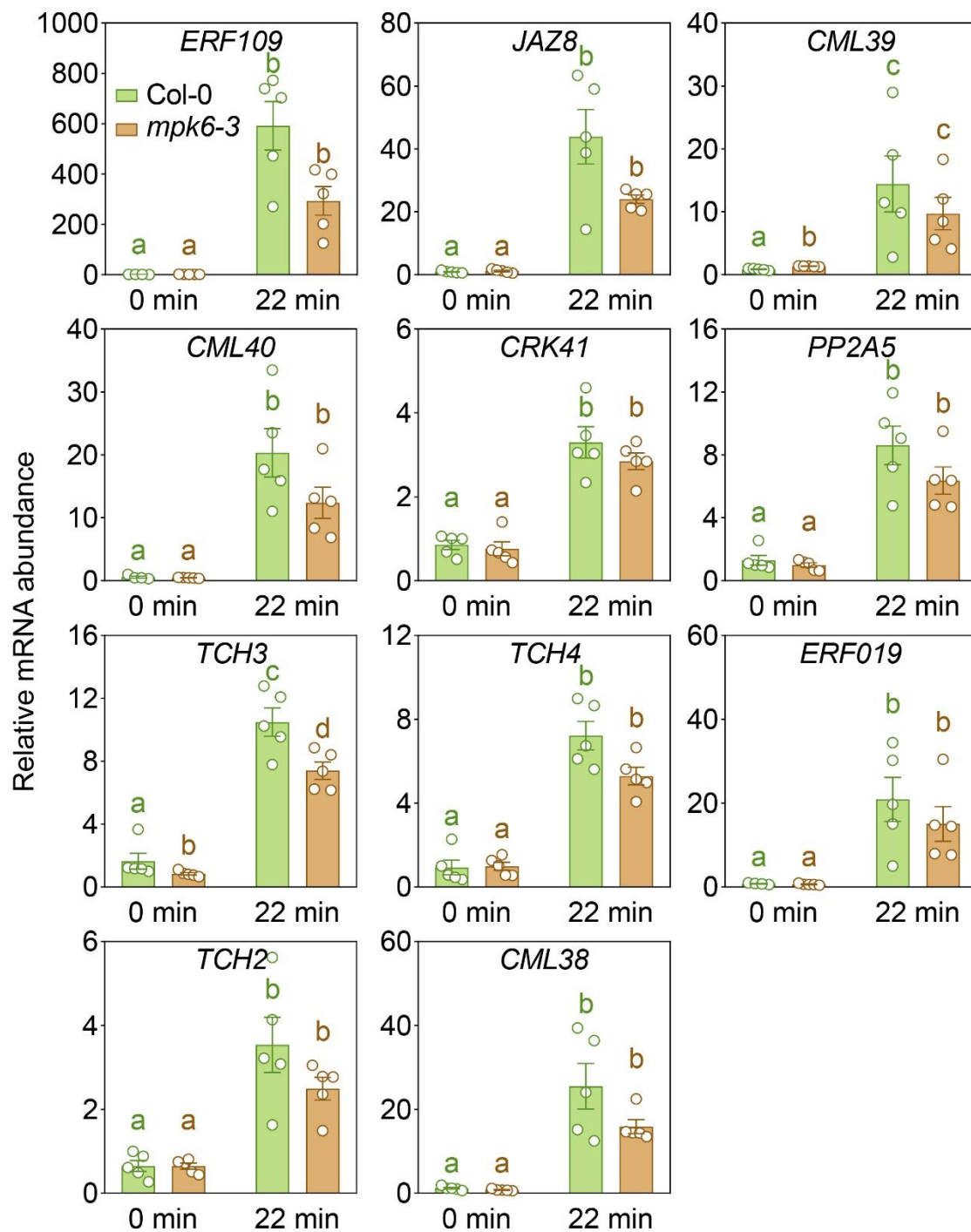
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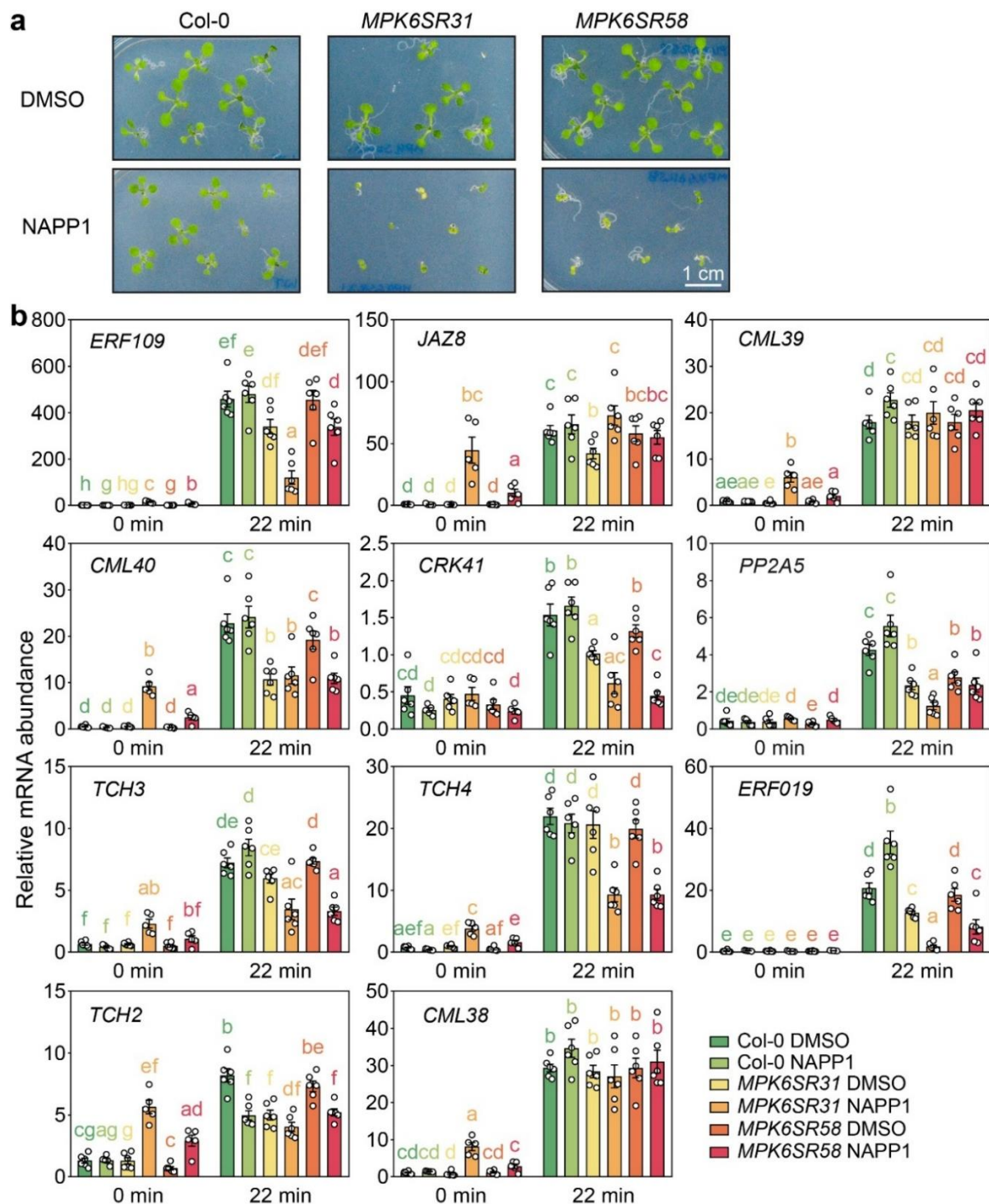
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SUPPLEMENTARY FIGURES

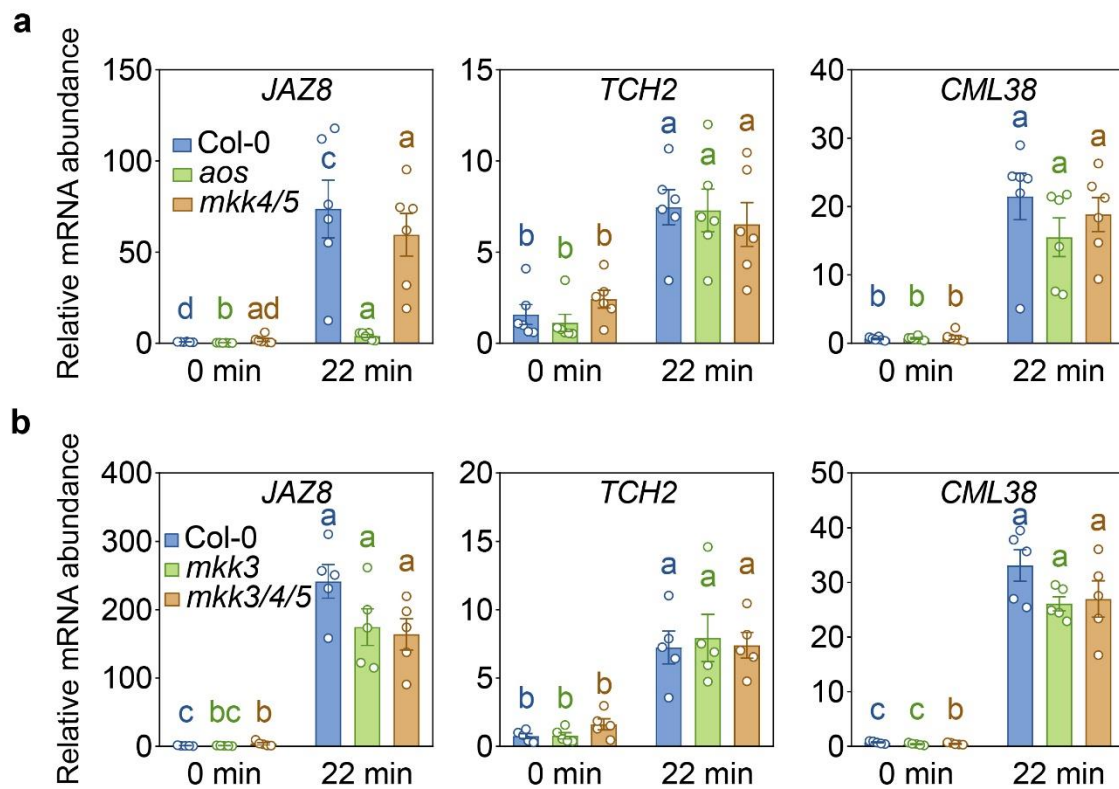


Supplementary Fig. 1 Analysis of touch marker gene expression in the *mpk6-3* mutant. 12-day-old seedlings before (0 min) and 22 min after touching by gentle brushing were collected for qRT-PCR. The expression of selected touch marker genes was measured in Col-0 (WT) versus the *mpk6-3* mutant. The y axis represents the relative mRNA level. Data are presented as mean $\pm$ SE. $n=5$ biologically independent samples. Each dot indicates a biological replicate. Statistical significance was based on Kruskal-Wallis test followed by Wilcoxon rank sum tests. Different letters represent the significant differences between genotypes ($p < 0.05$).

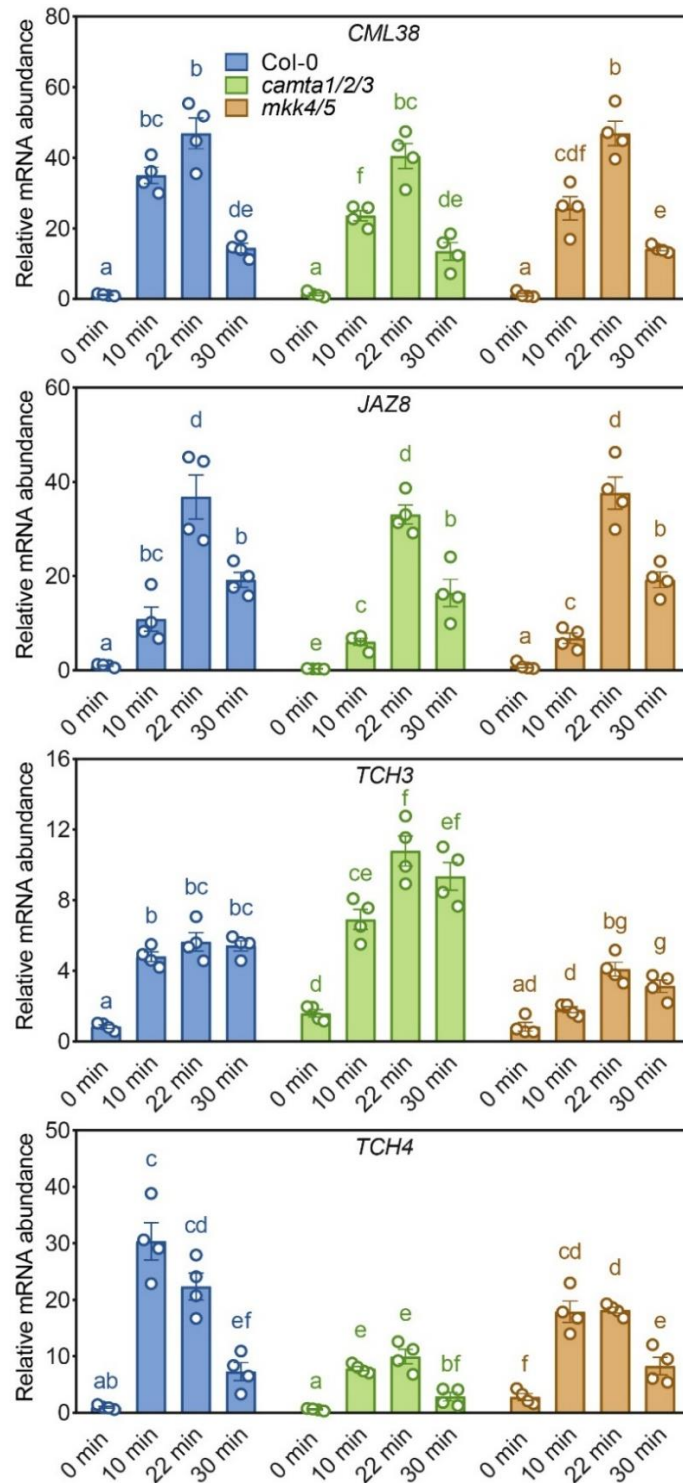


Supplementary Fig. 2 Analysis of touch marker gene expression in the *MPK6SR* lines.

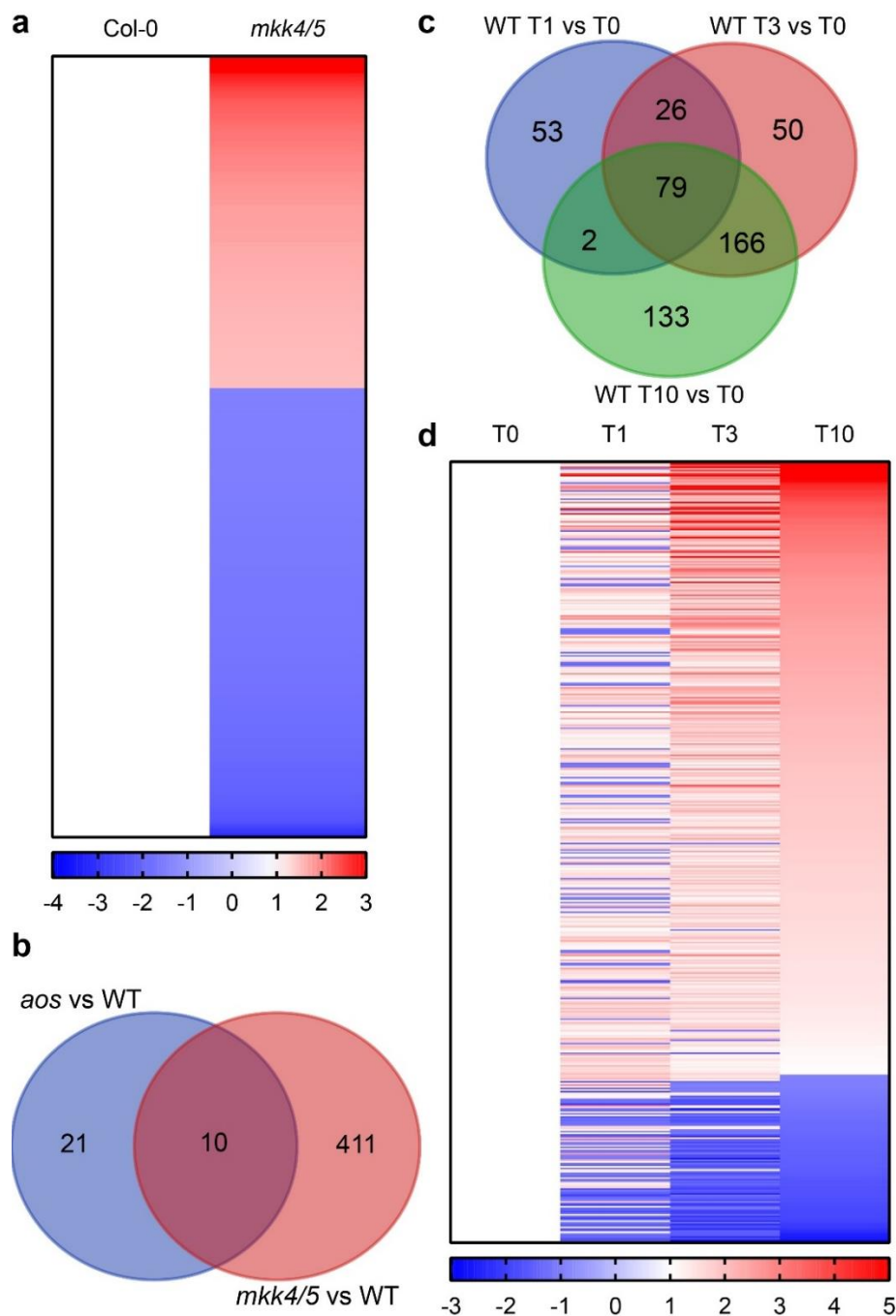
a A representative picture of 10-day-old WT and *MPK6SR* seedlings grown on half-strength MS containing either DMSO (mock) or 1 μ M NAPP1. Scale bar = 1 cm. **b** Touch marker gene expression in the *MPK6SR* lines. 12-day-old seedlings before (0 min) and 22 min after touching by gentle brushing were collected for qRT-PCR. The expression of selected touch marker genes was measured in Col-0 (WT) versus the *MPK6SR* lines (*MPK6SR31* and *MPK6SR58*). The y axis represents the relative mRNA level. Data are presented as mean $\pm$ SE. $n=6$ biologically independent samples. Each dot indicates a biological replicate. Statistical significance was based on Kruskal-Wallis test followed by Wilcoxon rank sum tests. Different letters represent the significant differences between genotypes ($p < 0.05$).



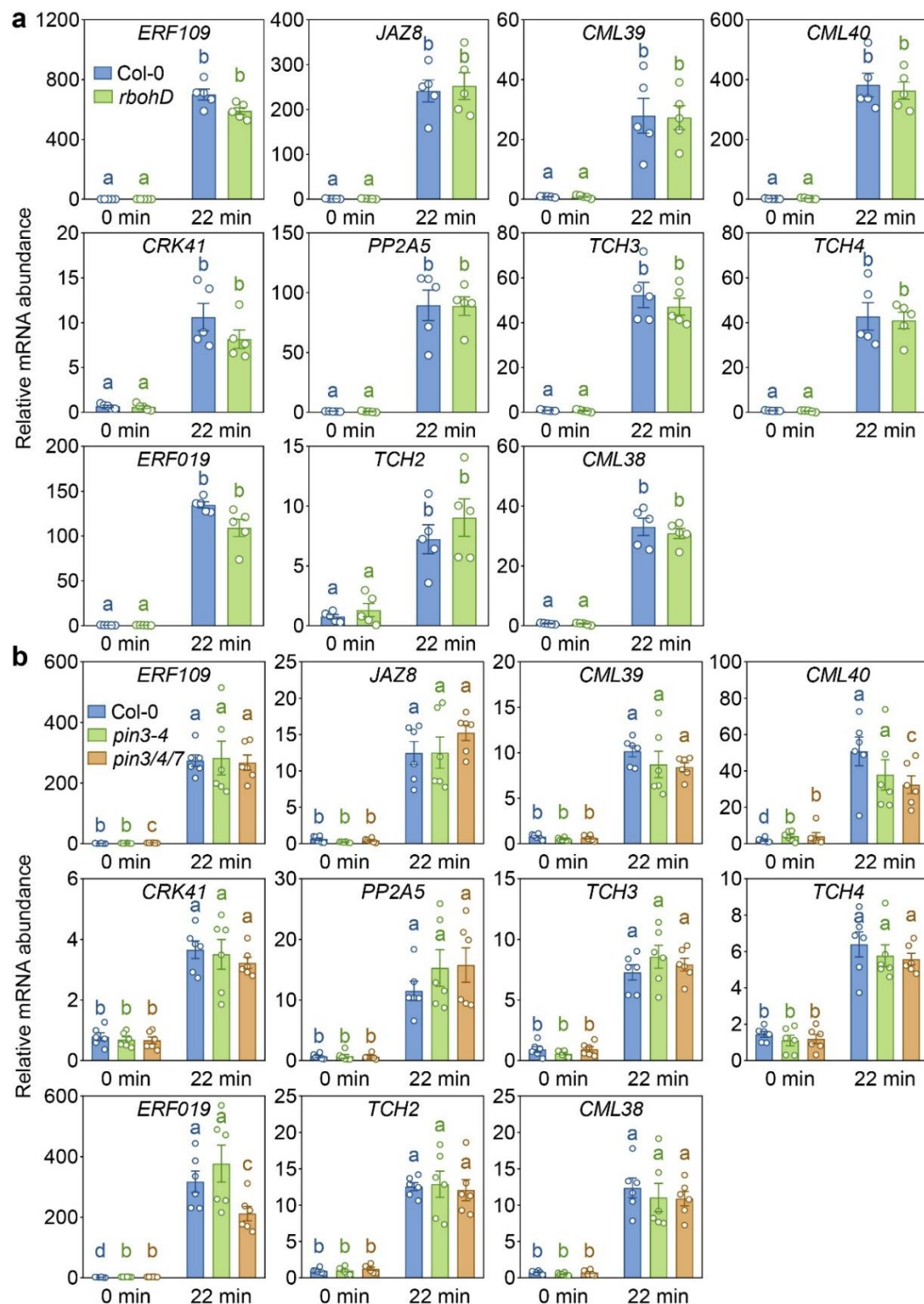
Supplementary Fig. 3 Analysis of touch marker gene expression in the mutants *aos*, *mkk4/5*, *mkk3* and *mkk3/4/5*. 12-day-old seedlings before (0 min) and 22 min after touching by gentle brushing were collected for qRT-PCR. The expression of selected touch marker genes was measured in Col-0 (WT) versus the *aos* and *mkk4/5* mutants (**a**), and WT versus the *mkk3* and *mkk3/4/5* mutants (**b**). The y axis represents the relative mRNA level. Data are presented as mean $\pm$ SE. $n=6$ (**a**) and $n=5$ (**b**) biologically independent samples. Each dot indicates a biological replicate. Statistical significance was based on Kruskal-Wallis test followed by Wilcoxon rank sum tests. Different letters represent the significant differences between genotypes ($p < 0.05$).



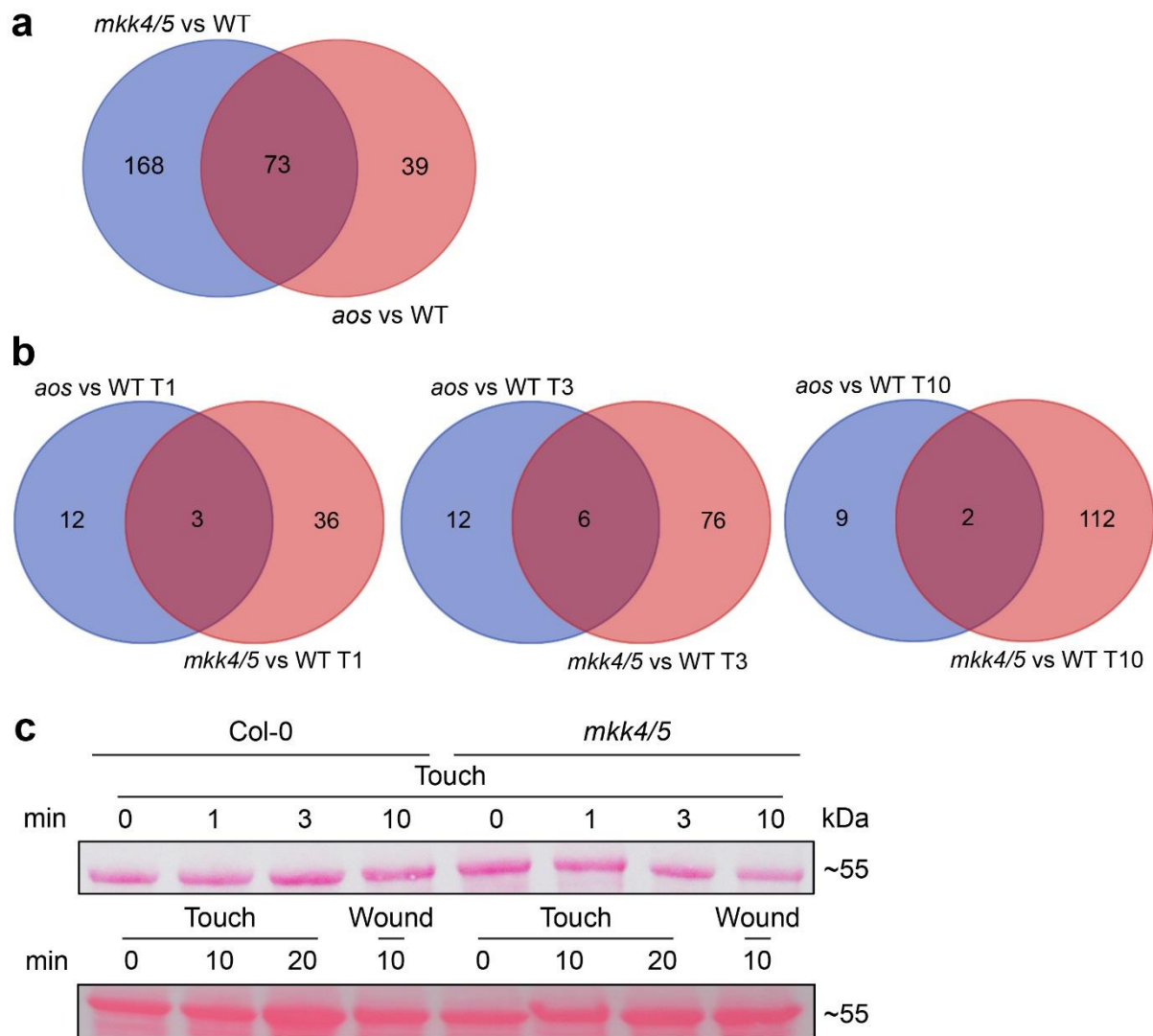
Supplementary Fig. 4 Analysis of touch marker gene expression at different time points in the mutants *camta1/2/3* and *mkk4/5*. 12-day-old seedlings before (0 min) and 10, 22 and 30 min after touching by gentle brushing were collected for qRT-PCR. The expression of selected touch marker genes was measured in Col-0 (WT) versus *camta1/2/3* and *mkk4/5*. The y axis represents the relative mRNA level. Data are presented as mean $\pm$ SE. $n=4$ biologically independent samples. Each dot indicates a biological replicate. Statistical significance was based on Kruskal-Wallis test followed by Wilcoxon rank sum tests. Different letters represent the significant differences between genotypes ($p < 0.05$).



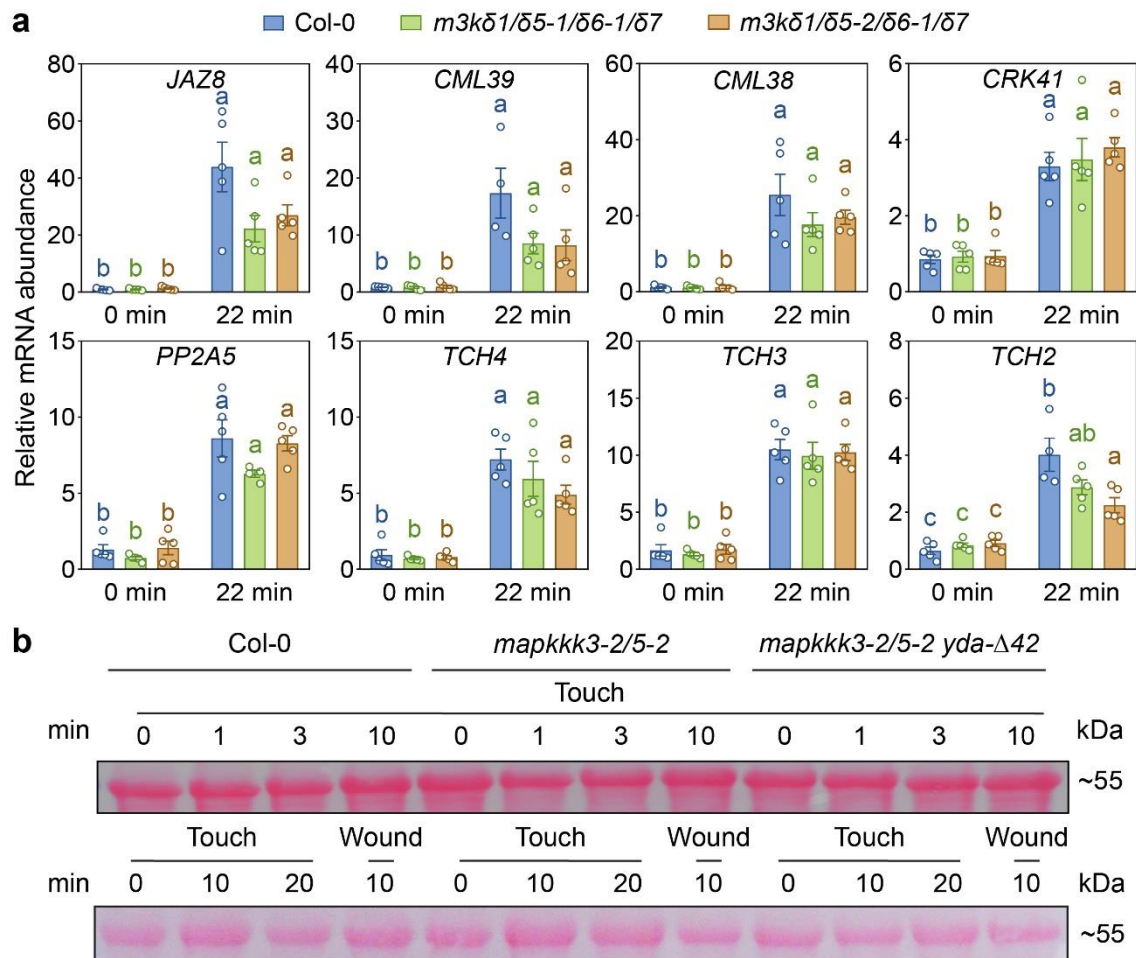
Supplementary Fig. 5 Dynamic changes in protein phosphorylation due to genotype effect and time effect. **a** Heatmap represents all the differentially phosphorylated sites due to genotype effect in *mkk4/5* compared to in *Col-0*. Color bar indicates linear fold changes. **b** Venn diagram represents the number of phosphosites due to genotype effect between the *aos* and *mkk4/5* mutants (FDR_{genotype} < 0.05, p_{COMP1} < 0.05 for WT versus *aos*, p_{COMP2} < 0.05 for WT versus *mkk4/5*, 1.5x FC (FC > 1.5 or < 0.6666)). **c** Venn diagram representing the number of differentially phosphorylated sites due to time effect in WT at 1, 3 and 10 min after touching compared to untouched condition. **d** Heatmap represents all the differentially phosphorylated sites due to time effect in WT at 1, 3 and 10 min after touching compared to untouched condition. Color bar indicates linear fold changes.



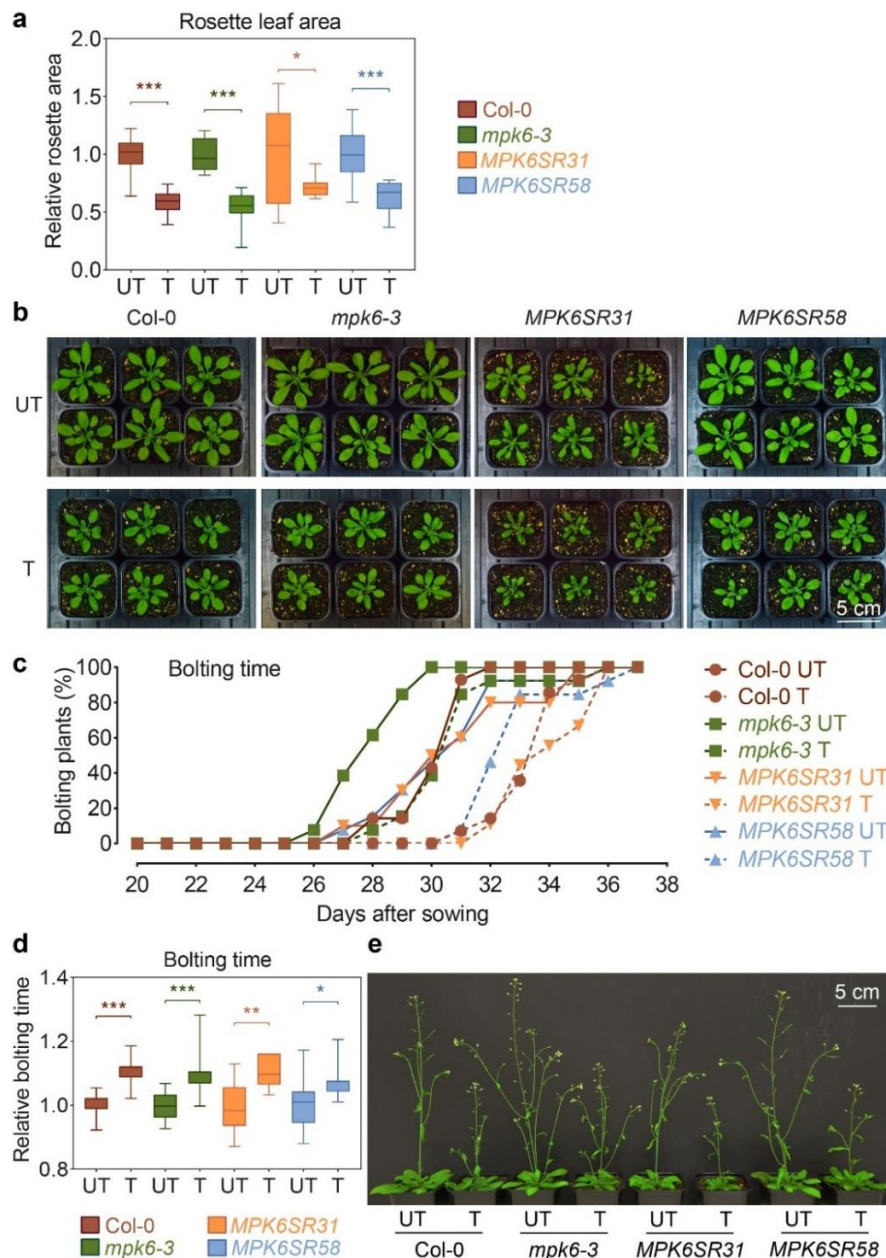
Supplementary Fig. 6 Analysis of touch marker gene expression in the *rbohD*, *pin3-4* and *pin3/4/7* mutants. 12-day-old seedlings before (0 min) and 22 min after touching by gentle brushing were collected for qRT-PCR. The expression of selected touch marker genes was measured in Col-0 (WT) versus the mutants *rbohD* (a) and *pin3-4* and *pin3/4/7* (b). The y axis represents the relative mRNA level. Data are presented as mean $\pm$ SE. $n=5$ (a) and $n=6$ (b) biologically independent samples. Each dot indicates a biological replicate. Statistical significance was based on Kruskal-Wallis test followed by Wilcoxon rank sum tests. Different letters represent the significant differences between genotypes ($p < 0.05$).



Supplementary Fig. 7 Comparison of protein phosphosites due to interaction effect between the *aos* and *mkk4/5* mutants and immunoblot analysis of phosphorylated MPKs in *mkk4/5* before and after touch or wounding treatment. **a** Venn diagram represents the number of phosphosites due to interaction effect over the time course of touch treatment between the *aos* and *mkk4/5* mutants (FDR_{int} < 0.05, p_{COMP1_time} for *aos* versus WT, p_{COMP2_time} < 0.05 for *mkk4/5* versus WT). **b** Venn diagram represents the number of phosphosites due to interaction effect at each time point of touch treatment (1, 3 and 10 min) between the *aos* and *mkk4/5* mutants (FDR_{int} < 0.05, p_{COMP1_time} for *aos* versus WT, p_{COMP2_time} < 0.05 for *mkk4/5* versus WT). A cutoff in FC was applied for each time point as followed: *aos* T1/WT T1 >1.5 or <0.66666 and *mkk4/5* T1/WT T1 >1.5 or <0.66666; *aos* T3/WT T3 >1.5 or <0.66666 and *mkk4/5* T3/WT T3 >1.5 or <0.66666; *aos* T10/WT T10 >1.5 or <0.66666 and *mkk4/5* T10/WT T10 >1.5 or <0.66666. **c** Ponceau staining of immunoblots using an antibody against phosphorylated MAPKs in WT and *mkk4/5* before and after touch or wounding treatment. Similar results were obtained in three independent experiments.



Supplementary Fig 8 Analysis of touch marker gene expression in the $m3k\delta1/\delta5-1/\delta6-1/\delta7$ and $m3k\delta1/\delta5-2/\delta6-1/\delta7$ mutants and immunoblot analysis of phosphorylated MPKs in $mapkkk3-2/5-2$ and $mapkkk3-2/5-2 yda-\Delta42$ before and after touch or wounding treatment. **a** 12-day-old seedlings before (0 min) and 22 min after touching by gentle brushing were collected for qRT-PCR. The expression of selected touch marker genes was measured in Col-0 (WT) versus the $m3k\delta1/\delta5-1/\delta6-1/\delta7$ and $m3k\delta1/\delta5-2/\delta6-1/\delta7$ mutants. The y axis represents the relative mRNA level. Data are presented as mean $\pm$ SE. $n=5$ biologically independent samples. Each dot indicates a biological replicate. Statistical significance was based on Kruskal-Wallis test followed by Wilcoxon rank sum tests. Different letters represent the significant differences between genotypes ($p < 0.05$). **b** Ponceau staining of immunoblots using an antibody against phosphorylated MAPKs on WT, $mapkkk3-2/5-2$ and $mapkkk3-2/5-2 yda-\Delta42$ mutants before and after touch or wounding treatment. Similar results were obtained in three independent experiments.



Supplementary Fig. 9 Analysis of the *mpk6-3* mutant and *MPK6SR* lines on thigmomorphogenesis. **a** Box and whisker plot representing relative rosette leaf area of 28-day-old plants, either untouched or after 14 days of regular touching. $n=9-14$ plants ($*=p<0.05$, $***=p<0.001$, $****=p<0.0001$). Box plot shows the minimum and maximum values (whiskers), the 25th and 75th percentiles (box bounds), and the median (center line). **b** A representative picture of 28-day-old untouched plants or plants in which touch treatment had been applied twice-daily for 14 days. Scale bar = 5 cm. **c** Line graph showing the percentage of bolting plants over the growth period (days after sowing). **d** Box and whisker plot represents relative bolting time under untouched and touched conditions. $n=9-14$ plants ($*=p<0.05$, $***=p<0.001$, $****=p<0.0001$). Box plot shows the minimum and maximum values (whiskers), the 25th and 75th percentiles (box bounds), and the median (center line). **e** A representative picture of 38-day-old untouched plants or plants in which touch treatment had been applied twice-daily for 24 days. Scale bar = 5 cm. UT=untouched, T=touched.