Supporting Information

MPK4 phosphorylation dynamics and interacting proteins in plant immunity

Tong Zhang[a,b], Jacqueline D. Schneider[a,c], Chuwei Lin[a,b,], Sisi Geng[b,d], Tianyi Ma[a,b], Sheldon R. Lawrence[b,d], Craig P. Dufresne[e], Alice C. Harmon[a,b,d], Sixue Chen[a,b,d,f,*]

[a] Department of Biology, University of Florida, Gainesville, FL 32610, USA

[b] Genetics Institute, University of Florida, Gainesville, FL 32610, USA

[c] Department of Chemical Engineering, University of Florida, Gainesville, FL 32610, USA

[d] Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL 32610, USA

[e] Thermo Fisher Scientific, 1400 Northpoint Parkway, West Palm Beach, FL 33407, USA

[f] Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL 32610, USA

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Supplemental Figure 1. Screening for *MPK4* overexpressing lines and primary root growth of the *MPK4* transgenic plants. (**A**) Identification of *MPK4* overexpressing lines using immunoblot. T1 seedlings geminated on selective ½ MS plate were considered as candidate lines, and protein samples from these lines were analyzed by immunoblot using anti-FLAG antibody. Candidates 3 and 4 were used as *MPK4* overexpressing line 1 and line 2, respectively. (**B**) Images of ten-day-old seedlings of the Col-0 and *MPK4* transgenic Arabidopsis lines. (**C**) Comparison of the primary root length between the *MPK4* transgenic lines and the Col-0. Data represented measurements of 40 individual plants in each genetic background, and the standard deviation was shown as the error bars. Student's t-test showed no statistical difference between the transgenic lines and the Col-0 at $p < 0.05$ level.

Supplemental Figure 2. Analysis of major phytohormones in the *MPK4* transgenic plants.
For each of the indicated hormone or their derivatives, the relative levels were presented as intensity (in thousands) using white bar (left) for Col-0, grey bar (middle) for *MPK4*-line 1, and black bar (right) for *MPK4*-line 2. **ABA**: absisic acid; **ABA-GE**: abscisic acid-glucose-ester conjugate; **BR**: epi brassinolide; **GA_3**: gibberelline A3; **GA_4**: gibberelline A4; **IAA**: indole-3-acetic acid; **I3CA**: indole-3-carboxylic acid; **IBA**: indole-3-butyric acid; : **LA**: linolenic acid; **PA**: phaseic acid; **TA**: traumatic acid; **tZTG**: trans-zeatin-glucoside conjugate; **tZTR**: Trans-zeatin riboside conjugate; **MeAc**: methyl indol-3-acetate.

Supplemental Figure 3. Transcript analysis of *PR1*, *PR2* and *WRKY40* in Col-0 and the *MPK4* transgenic lines. RT-PCR was performed with mRNA extracted from two-week-old seedlings. *Actin 8* was used as a loading control. Three biological repeats were performed with similar results.

Supplemental Figure 4. Bacterial growth assay of BnMPK4 overexpressing lines. Five-week old plants were inoculated with *Pst* DC 3000 at an OD600 of 0.2 by foliar spraying, and bacterial growth was

assayed at two days after the treatment. Three leaves from individual plants were combined as one replicate and six biological replicates were performed. Error bars represent standard deviation, and * indicates $p < 0.05$ statistically comparing to the WT.

Supplemental Figure 5. Phosphorylation of MPK4 at the tyrosine residue in the TEY motif. The MS/MS spectrum for determining the peptide sequence and phosphorylation site was annotated. Note that the peptide shown had a missed cleavage by trypsin, comparing to the shorter peptide in Figure 3A.

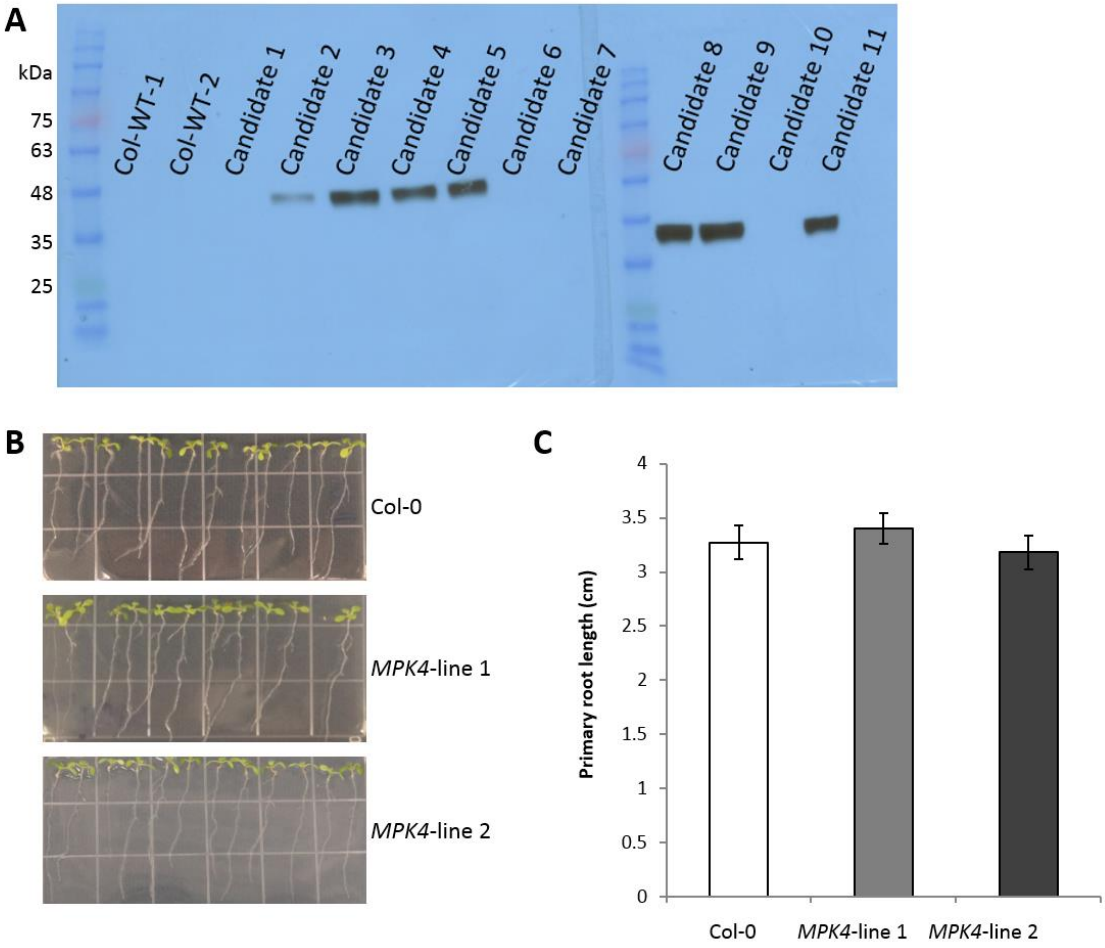
Supplemental Figure 6. Quality control metrics of label-free proteomics on flg22-induced MPK4-interacting protein network. Four biological replicates of transgenic plants overexpressing *MPK4* under mock- (MPK4_Mock) and flg22-treatment (MPK4_flg22) were performed. (**A**) Correlation of four biological replicates of the mock samples. R^2 values are displayed at the upper right and the distribution of the protein abundance is shown in blue. (**B**) Correlation of four biological replicates of the flg22-treated samples. R^2 values are displayed at the upper right and the distribution of the protein abundance is shown in blue. (**C**) Overlapping of proteins identified in the control and flg22-treated samples. (**D**) Clustering analysis of the proteins identified in the control and flg22-treated samples.

Supplemental Figure 7. Transcript analysis of genes encoding putative MPK4-interacting proteins in *B.napus* guard cells. RNA (1 µg) was extracted from guard cells and the gene expression was detected by RT-PCR using equal amount of cDNA. **GRT:** Glutaredoxin family protein; **MDG:** malate dehydrogenase; **ADK1:** Adenosine kinase 1; **RBOH D:** Respiratory burst oxidase homolog D; **RBOH F:** Respiratory burst oxidase homolog F; **CPN 60 α:** Chaperonin-60 alpha; **RPS5:** Ribosomal protein S5; **VIN3:** Villin 3. Red arrows indicate the expected band of PCR products. Note that RBOH D, but not RBOH F, has been shown to be expressed in guard cells.

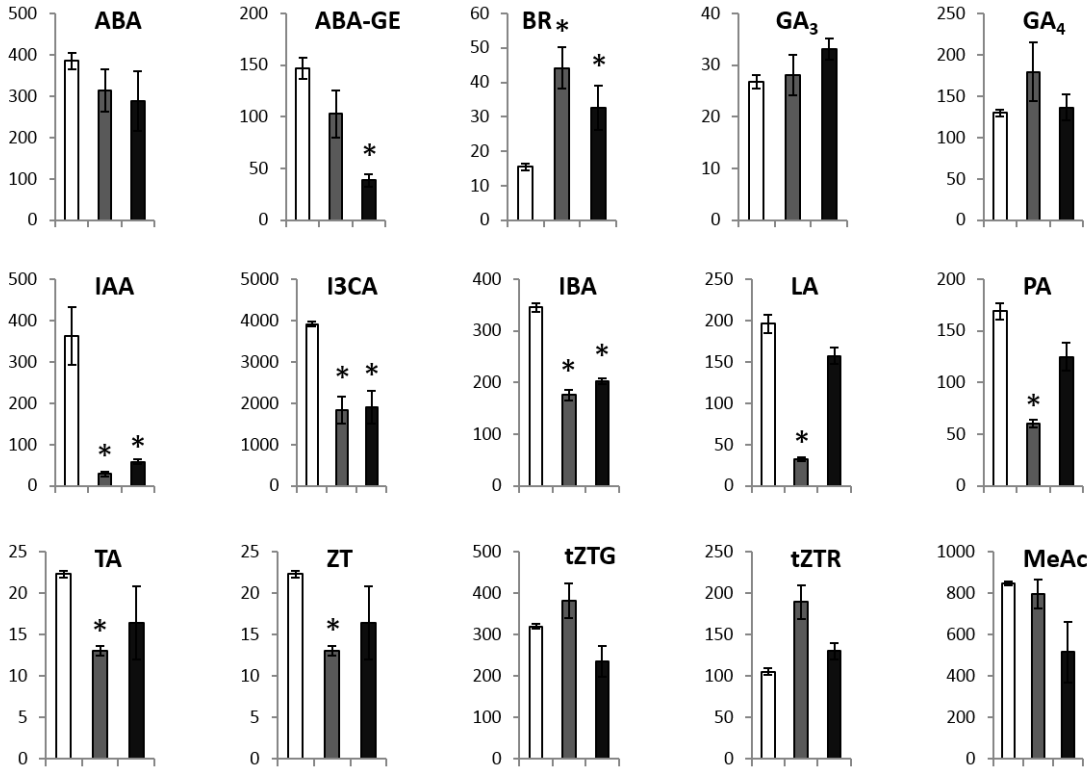
Supporting Data 1. List of proteins identified in the mock and flg22 treated Arabidopsis transgenic plants overexpressing the *B. napus MPK4*.

Supporting Data 2. List of proteins showing significant changes in the mock and flg22 treated transgenic plants overexpressing the *B. napus MPK4*.

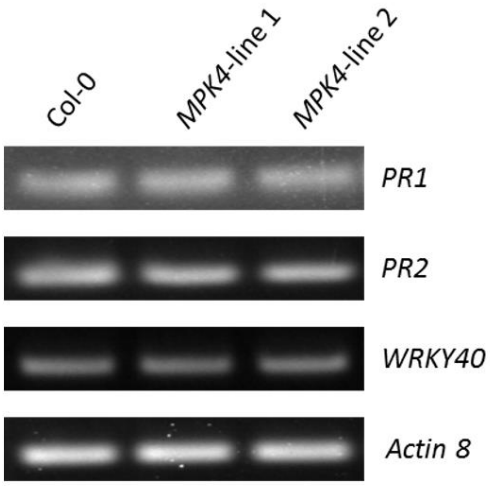
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



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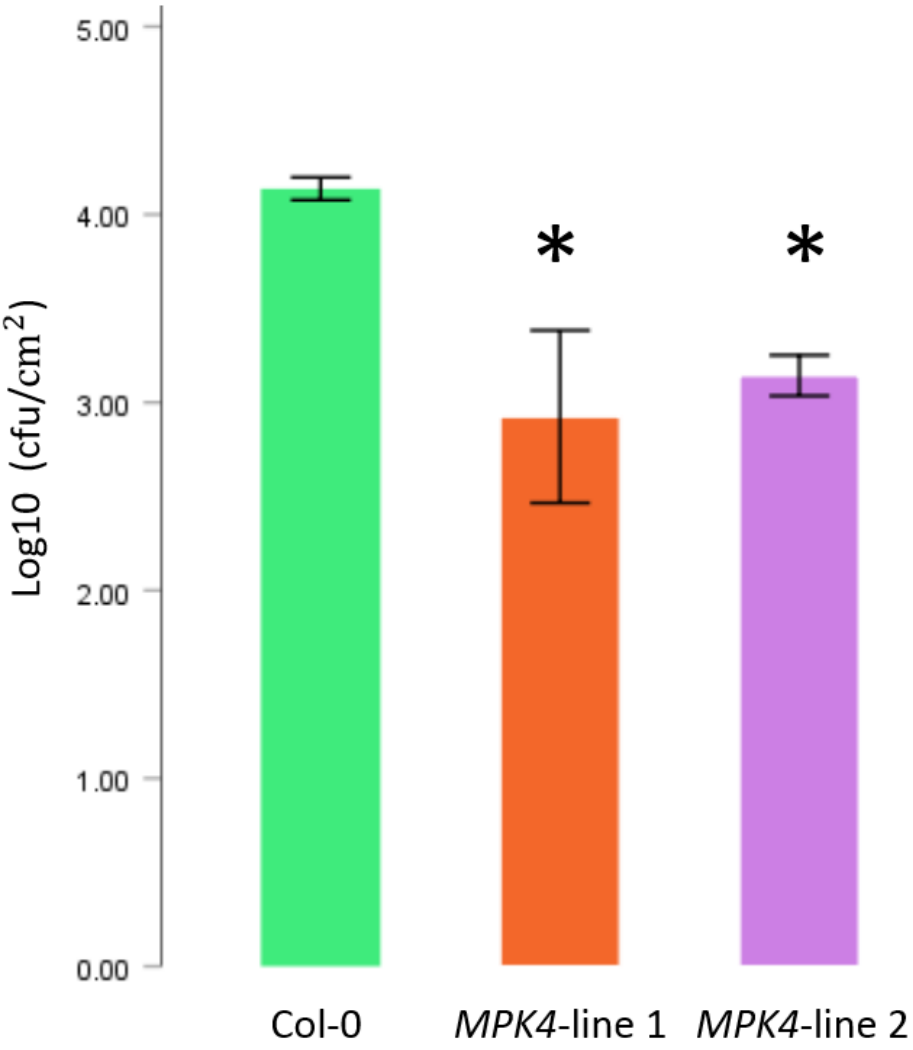

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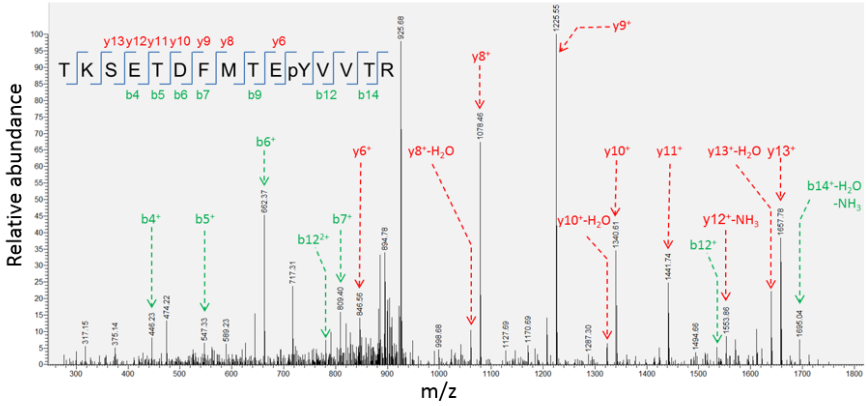

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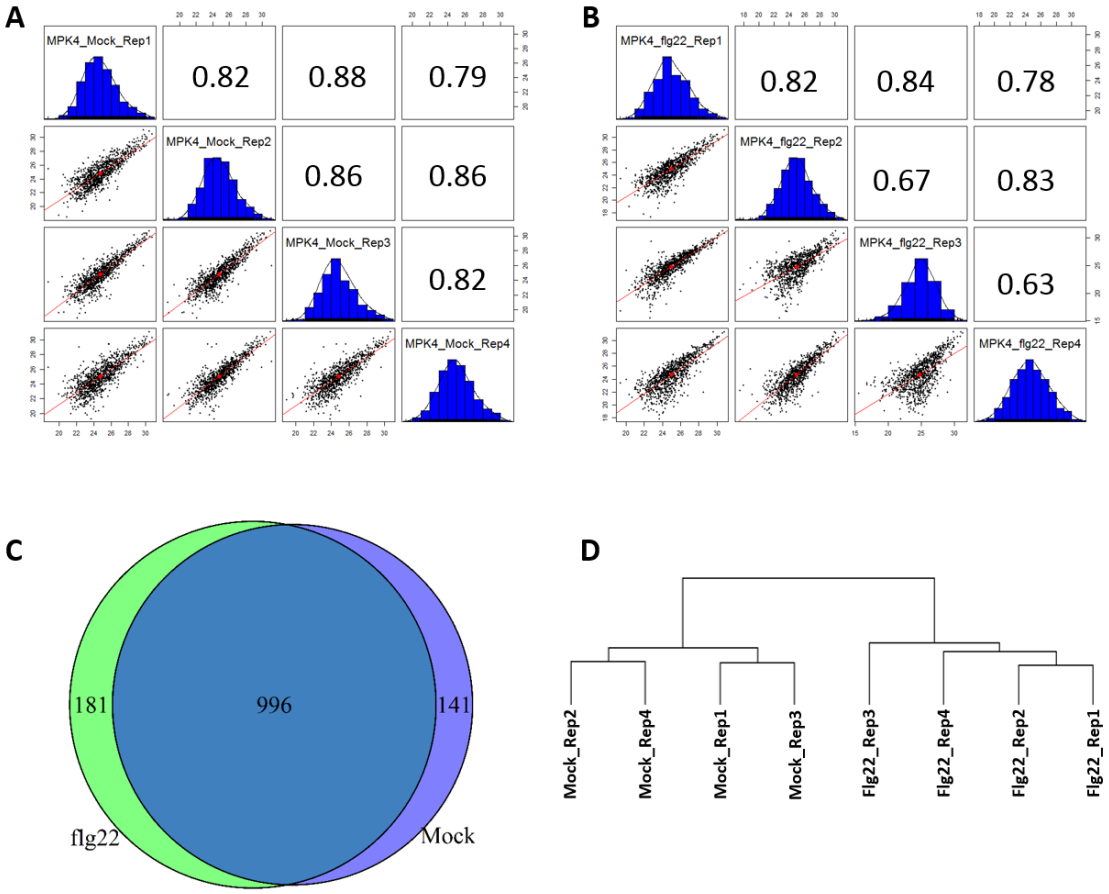
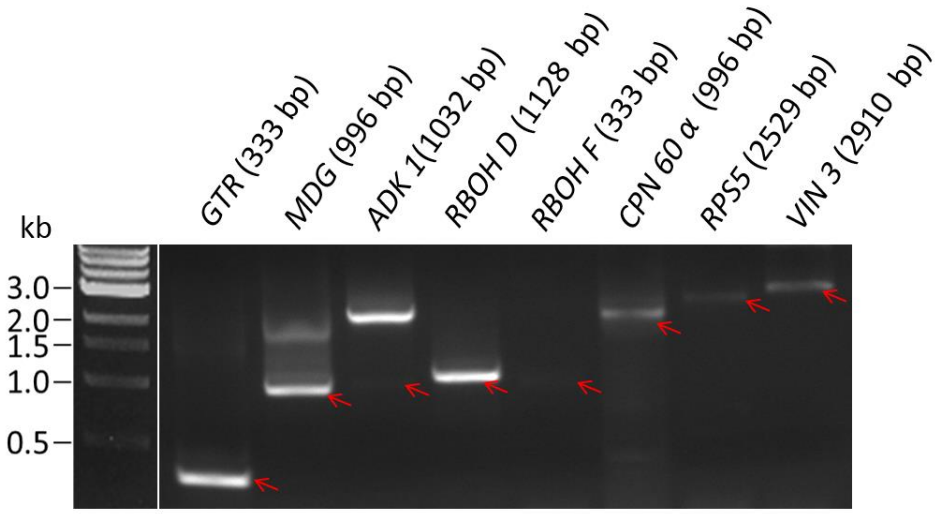


Supplemental Figure 7



Supporting Data 1

Supporting Data 2