Supplementary Figure 1 | Relation tree of Arabidopsis CPKs.
The complete protein sequences were aligned and analyzed with ClustalX and Treeview algorithms. The CPK genes in subgroup I involved in flg22 signalling are highlighted.
Supplementary Figure 2 | Flg22 activation of endogenous CDPKs requires FLS2 and calcium signalling. Endogenous CDPK activity was analyzed in protoplasts using an in-gel kinase assay with histone as an artificial substrate. a, Protoplasts from WT (Ler) or fsl2 mutant were treated with 100 nM flg22 for 20 min. b, Protoplasts from WT (Col-0) were treated with 2 mM LaCl₃, 4 mM BAPTA or H₂O control (ctl) for 15 min prior to elicitation with 100 nM flg22.
Supplementary Figure 3 | Schematic representation of CPK expression in mesophyll protoplasts. The expression level of 34 CPK genes was monitored by qRT-PCR. The values were normalized based on the control gene TUB4 and represented using a color scale for easy visualization (upper panel). The quality of the primers was confirmed using gDNA or cDNA from other plant organs (lower panel). The genes represented with white boxes are barely or not detectable in mesophyll protoplasts. Six of them are predicted to be pollen-specific (CPK14, 16, 17, 20, 24 and 34).
**Supplementary Figure 4 | Truncated CPKs are constitutively active.**
CPKac-FLAG were expressed in protoplasts and immunoprecipitated with anti-FLAG antibody. Kinase activity was measured in vitro, using histone as an artificial substrate, in the presence of EGTA. The expression level of CPKac-FLAG was monitored by immunoblot.
Supplementary Figure 5 | Ca\(^{2+}\)-dependent kinase activity of CDPKs is required for flg22 signalling. **a**, Kinase activity is required for NHL10-LUC induction. The constitutively active (ac) and the inactive kinase mutant (KM) of CDPKs are compared for NHL10-LUC activation, histone phosphorylation activity and protein expression (anti-FLAG). Error bars, s.d. (n=6). **b**, Full-length CPK11 requires Ca\(^{2+}\) for activation. The kinase activity and protein expression of full-length (FL) and constitutively active (ac) CPK11 are shown. Ca\(^{2+}\) chelater EGTA abolished the phosphorylation activity and Ca\(^{2+}\)-dependent mobility shift of CPK11 FL while CPK11ac lacking the EF-hand Ca\(^{2+}\)-binding domain and the pseudosubstrate inhibitory region was constitutively active, even in the presence of EGTA, as predicted\(^{2,4}\). **c**, Over-expression of full-length CDPKs cannot induce NHL10-LUC. Promoter activity and CPK protein expression are shown. Error bars, s.d. (n=6). **d**, Full-length CPK5 activity can be enhanced by Ca\(^{2+}\) ionophore A23187 in vivo. NHL10-LUC induction was measured in protoplasts treated with 1 mM Ca\(^{2+}\) without or with 100 or 200 nM ionophore A23187 for 3 h to partially mimic flg22 signalling. Constitutively active CPK5 (ac) is Ca\(^{2+}\)-independent but endogenous CDPKs are also activated by A23187. Error bars, s.d. (n=3).
Supplementary Figure 6 | Volcano plots of CPK5ac and CPK11ac target genes.
Graphic representation of CPK5ac and CPK11ac target genes using RMA or dChip analysis of microarray data. FC: fold change (Significant activation, FC = 2; significant repression, FC = 0.5), p-value = 0.05.
Supplementary Figure 7 | Calcium signalling inhibitors only attenuate MAPK activation by flg22. Protoplasts untransfected (top) or transfected with MPK6-HA (bottom) were treated with 4 mM BAPTA, 2 mM LaCl₃ or H₂O control (ctl) for 15 min prior to elicitation with 100 nM flg22 for 15 min. Endogenous MAPK activity was analyzed using an in-gel kinase assay with untransfected protoplasts (top). MPK6-HA was immunoprecipitated from transfected protoplasts with an anti-HA antibody and analyzed for in vitro kinase activity using MBP as a substrate (bottom).
Supplementary Figure 8 | Constitutively active CPKs do not activate MAPKs.
Protoplasts co-transfected with MPK3-HA or MPK6-HA and CPKac-FLAG, M KK4a-MYC or control DNA (-) were incubated for 6 h. Immunoprecipitated MAPK-HA was analyzed for in vitro kinase activity using MBP as a substrate. The expression level of MAPK-HA, M KK4a-MYC and CDPKac-FLAG was monitored by immunoblot.
**Supplementary Figure 9 | Analysis of cpk single mutants.**

**a**, T-DNA insertion in the *CPK5* gene (sail_657C06) is in the first intron. As no PCR product could be obtained with LP5 primer, the DNA organization at the RB site could not be determined. In the *cpk6* mutant (salk_025460), 2 copies of T-DNA were inserted in tandem in opposite direction in the first exon, generating a deletion of 45 bp from 68 to 113 bp downstream of the translation start codon (ATG). In the *cpk11* mutant (salk_054495), a single copy of T-DNA was inserted in the first exon, generating a deletion of 40 bp from 320 to 358 bp downstream of the ATG. Lines represent introns while dark and light grey boxes represent exons and UTR regions, respectively. Arrows indicate the primers used for genotyping. Primer sequences are listed in Supplementary Table 14. **b**, *CPK* expression in higher order *cpk* mutants. The *cpk5,6* double and *cpk5,6,11* triple mutants were generated by genetic crosses. The quadruple mutant was generated by introducing a 500-bp *CPK4*-specific construct into the *cpk5,6,11* triple mutant (*cpk5,6,11,4*<sup>VT35</sup>) using VIGS<sup>3,6</sup>. A *GFP* gene sequence was used as a control in the other plants (WT, *cpk5,6* and *cpk5,6,11*) to avoid non-specific effect of VIGS. Expression level of *CPK* genes in WT and *cpk* mutants was analyzed by RT-PCR. The T-DNA insertion in *CPK5*, 6 and 11 abolished *CPK* expression while *CPK4*-specific VIGS strongly reduced *CPK4* expression. Neither T-DNA insertion or VIGS affected the expression of the closest homolog *CPK26*. The expression of *UBQ5* was used as a control.
Supplementary Figure 10 | Single cpk mutants are not impaired in defense responses. **a**, Flg22-induced oxidative burst is similar in single cpk mutants and WT plants. ROS production was measured in relative light units (RLU) on leaf disks from wild-type (WT), cpk5, cpk6 and cpk11 mutants after treatment with 100 nM Flg22. Error bars, s.d. (n=9). **b**, Single cpk mutants display the same sensitivity to the pathogen *Pst* DC3000 as WT plants. Leaves from wild-type (WT), cpk5, cpk6 and cpk11 mutants were infiltrated with *P. syringae* DC3000 at 5x10^4 cfu/ml. Bacterial growth was measured 4 days after inoculation (means ± s.d. of three replicates). The experiments were repeated three times with similar results.
Supplementary Figure 11 | Flg22 activation of 60 kD CDPKs is diminished in cpk mutants. CDPK activity was determined by in-gel kinase assay in WT and cpk mutant protoplasts treated with 10 nM Flg22 for 15 min. The remaining 72 kD CDPK served as a control for specificity. Histone was used as an artificial in vitro substrate for CDPKs. Coomassie staining: protein loading control.
Supplementary Figure 12 | MAPK activation by flg22 is independent of CDPKs. MAPK activity was determined by in-gel kinase assay in seedlings treated with 1 μM flg22 for 15 min. MBP was used as an artificial in vitro substrate for MAPKs. Coomassie staining: protein loading control.
Supplementary Figure 13 | The cpk mutants are more susceptible to the bacterial pathogen Pst DC3000. Leaves from WT (Col-0), cpk, fIs2 and bak1 mutants were infiltrated with Pst DC3000 at 5x10^4 cfu/ml and bacterial growth was measured at indicated times. Error bars, s.d. (n=3). The differences are significant with p < 0.03 (***) or p < 0.05 (*) when compared with data from WT at the same time point based on the results of an unpaired Student’s t-test.
Supplementary Figure 14 | The four CPK-GFP are localized in both cytoplasm and nucleus. **a**, Subcellular localization of CPK-GFP fusion proteins. Specific constructs were expressed in protoplasts from Arabidopsis suspension cells for clear organelle visualization. GFP: GFP fluorescence, DIC: Differential interference contrast microscopy. The arrows indicate the nucleolus inside the nucleus. Scale bars are 15 μm. **b**, CDPK-GFP are not processed in transfected protoplasts. Proteins from protoplasts transfected with free GFP or CPK-GFP were analyzed by immunoblot with an anti-GFP antibody. Free GFP was not detected in protoplasts expressing CPK-GFP, confirming that the CPK-GFP localization observed using confocal microscopy corresponds to the fusion proteins.
SUPPLEMENTARY TABLES

Supplementary Table 1 | Expression level of CPK genes relative to TUB4 in mesophyll cell protoplasts. The expression level of CPK genes was monitored by qRT-PCR and was assigned the value « 0 » when no PCR product was obtained from mesophyll cell protoplasts. The quality of the primers was checked on genomic DNA or cDNA from other organs (Supplementary Fig. 3).

| Gene  | AGI Number | Expression level   |
|-------|------------|--------------------|
| CPK1  | At5g04870  | 0.33742 ± 0.12665 |
| CPK2  | At3g10660  | 0.01268 ± 0.00540 |
| CPK3  | At4g23650  | 3.56092 ± 1.83776 |
| CPK4  | At4g09570  | 0.61783 ± 0.16329 |
| CPK5  | At4g35310  | 0.71715 ± 0.24428 |
| CPK6  | At2g17290  | 0.36733 ± 0.06499 |
| CPK7  | At5g12480  | 0.62047 ± 0.24023 |
| CPK8  | At5g19450  | 0.19490 ± 0.02657 |
| CPK9  | At3g20410  | 2.15086 ± 1.20276 |
| CPK10 | At1g18890  | 1.13954 ± 0.36701 |
| CPK11 | At1g35670  | 0.42881 ± 0.15127 |
| CPK12 | At5g23580  | 0.07002 ± 0.01054 |
| CPK13 | At3g51850  | 0.29619 ± 0.09337 |
| CPK14 | At2g41860  | 0.00010 ± 0.00004 |
| CPK15 | At4g21940  | 0.54500 ± 0.24620 |
| CPK16 | At2g17890  | 0.00027 ± 0.00009 |
| CPK17 | At5g12180  | 0.00004 ± 0.00002 |
| CPK18 | At4g36070  | 0.00183 ± 0.00077 |
| CPK19 | At1g61950  | 0.00008 ± 0.00002 |
| CPK20 | At2g38910  | 0.00021 ± 0.00020 |
| CPK21 | At4g04720  | 0.13906 ± 0.01492 |
| CPK22 | At4g04710  | 0.00567 ± 0.00163 |
| CPK23 | At4g04740  | 0.03803 ± 0.01307 |
| CPK24 | At2g31500  | 0.00359 ± 0.00059 |
| CPK25 | At2g35890  | 0.00009 ± 0.00004 |
| CPK26 | At4g38230  | 0.06429 ± 0.01271 |
| CPK27 | At4g04700  | 0.02331 ± 0.00449 |
| CPK28 | At5g66210  | 2.41219 ± 0.87689 |
| CPK29 | At1g76040  | 0.29004 ± 0.16224 |
| CPK30 | At1g74740  | 0.37729 ± 0.15709 |
| CPK31 | At4g04695  | 0.05700 ± 0.01844 |
| CPK32 | At3g57530  | 1.54108 ± 0.62045 |
| CPK33 | At1g50700  | 0.05899 ± 0.01538 |
| CPK34 | At5g19360  | 0.00192 ± 0.00035 |
Supplementary Table 2 | CPK5 and CPK11 target gene list. The Supplementary Table 2 is available as a Microsoft Excel file. Shared target genes between CDPK and flg22 are marked in green.

Supplementary Table 3 | Shared CPK5 and CPK11 target genes overlapping with early flg22 responsive genes. The Supplementary Table 3 is available as a Microsoft Excel file.

Supplementary Table 4 | Specific CPK5 target genes overlapping with early flg22 responsive genes. The Supplementary Table 4 is available as a Microsoft Excel file.

Supplementary Table 5 | Specific CPK11 target genes overlapping with early flg22 responsive genes. The Supplementary Table 5 is available as a Microsoft Excel file.

Supplementary Table 6 | CPK5 and CPK11 target genes overlapping with early flg22 responsive genes in Arabidopsis protoplasts, leaves and seedlings. The data in Supplementary Table 6 is visualized in Fig. 2a and available as a Microsoft Excel file.

Supplementary Table 7 | CPK5 and CPK11 target genes overlapping with early MAMP responsive genes in Arabidopsis leaves and seedlings. The data in Supplementary Table 7 is visualized in Fig. 2b and available as a Microsoft Excel file.
Supplementary Table 8 | CPK5 and CPK11 target genes overlapping with microbe responsive genes in Arabidopsis leaves. The data in Supplementary Table 8 is visualized in Fig. 2c and available as a Microsoft Excel file.

Supplementary Table 9 | Sequences of oligonucleotide primers for CPKac cloning. The cloning sites (BamHI, BglII, StuI) are italicized. F: forward; R: reverse.

| Gene | AGI Number | Primer |
|------|------------|--------|
| CPK1 | At5g04870  | F: GAAGATCTATGGGTAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
| CPK2 | At3g10660  | F: CCGGATCCATGGGAATGCTGGAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
| CPK3 | At4g23650  | F: CCGGATCCATGGGGAATGCTGGAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
| CPK4 | At4g09570  | F: CCGGATCCATGGGGAATGCTGGAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
| CPK5 | At4g35310  | F: CCGGATCCATGGGGAATGCTGGAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
| CPK6 | At2g17290  | F: CCGGATCCATGGGGAATGCTGGAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
| CPK7 | At5g12480  | F: CCGGATCCATGGGGAATGCTGGAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
| CPK8 | At5g19450  | F: CCGGATCCATGGGGAATGCTGGAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
| CPK9 | At3g20410  | F: CCGGATCCATGGGGAATGCTGGAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
| CPK10| At1g18890  | F: CCGGATCCATGGGGAATGCTGGAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
| CPK11| At1g35670  | F: CCGGATCCATGGGGAATGCTGGAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
| CPK12| At5g23580  | F: CCGGATCCATGGGGAATGCTGGAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
| CPK13| At3g51850  | F: CCGGATCCATGGGGAATGCTGGAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
| CPK15| At4g21940  | F: CCGGATCCATGGGGAATGCTGGAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
| CPK21| At4g04720  | F: CCGGATCCATGGGGAATGCTGGAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
| CPK22| At4g04710  | F: CCGGATCCATGGGGAATGCTGGAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
| CPK23| At4g04740  | F: CCGGATCCATGGGGAATGCTGGAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
| CPK26| At4g38230  | F: CCGGATCCATGGGGAATGCTGGAATACCTGAGTTGTCGAAC  |
|      |            | R: AAAAGGCTTCACACGCAGTCTACTGTTGTCGAAC  |
Supplementary Table 10 | Sequences of oligonucleotide primers for site-directed mutagenesis of CPKac to generate the kinase mutant (KM). F: forward; R: reverse.

| Gene   | Mutation | Primer                                             |
|--------|----------|----------------------------------------------------|
| CPK4   | K54M     | F: CTAATTACGCTTTGCATGTCAATCCAAAACAG              |
|        |          | R: CGTTTTGGGATTGACATGCAAGCGTAATTAG               |
| CPK5   | K126M    | F: TTGACTACGCTTTGTATGTCAATATTGCGAAGAG            |
|        |          | R: CTCTTGGATATTGACATACAGCGTAAATTG                 |
| CPK6   | K114M    | F: TTGACTATGCTTTGTATGTCAATATTGCGAAGAG            |
|        |          | R: CTCTTGGATATTGACATACAGCGTAAATTG                 |
| CPK11  | K55M     | F: CTAATTACGCGCTCATGTCAATCCAAAACAG                |
|        |          | R: CGCTTCGGGATCGACATGCAGCACGCATCGAATTAG           |

Supplementary Table 11 | Sequences of oligonucleotide primers for FL CPK cloning. The cloning sites (BamHI, StuI) are italicized. F: forward; R: reverse.

| Gene   | AGI Number | Primer                                             |
|--------|------------|----------------------------------------------------|
| CPK4   | At4g09570  | F: CGCAGATCCATGGGAGAAACGACAAAACCT                 |
|        |            | R: GAAAGGCCCTTTTGGGTGAAATCAGAACGGA                |
| CPK5   | At4g35310  | F: CGCAGATCCATGGGCAATTTACGACCAAAGGA               |
|        |            | R: GAAAGGCCCTTGGATCTTGAATCAGGCACGGA               |
| CPK6   | At2g17290  | F: TCCCCCGGATCCATGGGCAATTTAGCTTGCAGA             |
|        |            | R: GAAAGGCCCTTGGATCTTGAATCAGGCACGGA               |
| CPK11  | At1g35670  | F: CGCAGATCCATGGGAGAAACGACAAAACCT                 |
|        |            | R: GAAAGGCCCTTGGATCTTGAATCAGGCACGGA               |
## Supplementary Table 12 | Sequences of oligonucleotide primers for qRT-PCR.

F: forward; R: reverse.

| Gene | AGI Number | Primer | Primer | Primer | Primer |
|------|------------|--------|--------|--------|--------|
| CPK1 | At5g04870  | F: AGACAAATGACGGGCAGAATTAG | R: TGATGCTTCCCTTTCTGACATC | | |
| CPK2 | At3g10660  | F: CAAGACAAAGTGACGGCGAATTAG | R: CCATTATGCTCCCTTTCTGAC | | |
| CPK3 | At4g23650  | F: TGACAACAGCGGCTACATAAC | R: ACGATCGGTTGCTACTCTGACG | | |
| CPK4 | At4g09570  | F: CTTGATAATGACGGCGAATTAG | R: AGTTCTGCTCTCCTCAACTC | | |
| CPK5 | At4g35310  | F: TATGGAAGCTGCTGATGTTG | R: GCCTCTTCCCTGCACTCTTTG | | |
| CPK6 | At2g17290  | F: AGACAAAGTGACGGCGAATTAG | R: CTCCTACACAGGATCATTTCC | | |
| CPK7 | At5g12480  | F: CGACAAGGATGACGGCGAATTAG | R: TCCAATCTGCTCCCTGATTTT | | |
| CPK8 | At5g19450  | F: GATGGAAGCTGCTGATGTTG | R: GCACAGAGACGGCAACAC | | |
| CPK9 | At3g20410  | F: CGACTCCGATAACGATGTTG | R: CGAGTGTGTGTTGTGTTG | | |
| CPK10| At1g18890  | F: GGGAAGCTGCTGCTGATGTTG | R: TGACGCCCTCCTGATTCAG | | |
| CPK11| At1g35670  | F: GCCATTACCGGTAAATTGCTGAG | R: GTTCCTGCTGTTGCTGAC | | |
| CPK12| At5g23580  | F: GTGCGGCTGATGTTGATG | R: TTGTCAATGCTTGATGTTG | | |
| CPK13| At3g51850  | F: TGAACCGCTGATGATTG | R: TGCAGGTTGAGGAGACACTG | | |
| CPK14| At2g41860  | F: GATGTCGATACCAATAAGGATGG | R: TTTCATTGCAATTGCAAGTCC | | |
| CPK15| At4g21940  | F: GTCGATAACGATAACGATGTAAG | R: TTGGTGTGGCAGTGTGCCAG | | |
| CPK16| At2g17890  | F: AACACCGATTCCAGAAGTGG | R: CAGATATGCAATTGCAAGTTCC | | |
| CPK17| At5g12180  | F: TGCATTACCGGCTGATGTTG | R: TTGACTACCCGCTTGACATTC | | |
| CPK18| At4g36070  | F: TCCACCGGATGATAGCCAC | R: CAATTGATGACTGCTGAC | | |
| CPK19| At1g61950  | F: TTGATGCTGATGATTG | R: TTGATGATGTGATTGAGAC | | |
| CPK20| At2g38910  | F: GTCGATAAAAGCAACGATGGAAG | R: CAGTGTCTTGCATACATCCAAC | | |
| CPK21| At4g04720  | F: CTGTGATTTCCGATGGAAG | R: TCCACGATAACCCCTGGTAC | | |
| CPK22| At4g04710  | F: AACACCGGACATAACGAG | R: TTCATCTCCCATACCATGAC | | |
CPK23  At4g04740  F:  TGAAGCGTCTGATGTGGATG
           R:  TTGTGTACGTGCTCATCGTG
CPK24  At2g31500  F:  CAACAGGAATGGACGGGATAAG
           R:  TTCTGATGCTCATGTTTATGC
CPK25  At2g35890  F:  TGCAAAATAACACGGATGTT
           R:  CACGATCATTTGCTTTCTCTTC
CPK26  At4g38230  F:  CGACAGGACACATGATGGAAG
           R:  TCCGCCTGTAGTCGATTTCG
CPK27  At4g04700  F:  GTGAAGAACCTGAGGGACAG
           R:  CCTCGCTGTAGTCGATTTCC
CPK28  At1g74740  F:  GGAAGTGGCGGATGTTAATG
           R:  CTGATTCGATGTATCCACTTCC
CPK29  At1g76040  F:  ATGGAAAGCTGCTGATGTGG
           R:  AGTACGATGCATTGTTGCAG
CPK30  At1g74740  F:  GGAAGTGGCGGATGTTAATG
           R:  CTGATTCGATGTATCCACTTCC
CPK31  At4g04695  F:  ACAATGAGCGGGGCACATAACC
           R:  GCTAATCTCATCTCCACACC
CPK32  At3g57530  F:  GATGCTGGAGACATCGATAGAG
           R:  AGAACGCAAAACGCTTTCTTG
CPK33  At1g50700  F:  CGCTGATAATGACGGTAGAATC
           R:  TTCCCACTTCTCATACAGCAC
CPK34  At5g19360  F:  AGCTGAGCAAGCCCTTCCG
           R:  AGTTTATCCGGCCCATCATTG
TUB4   At5g44340  F:  AGGGAAACGAAGACAGCAAG
           R:  GCTCGCTATCTCCCTTTGG
EIF4a  At3g13920  F:  TCATAGATCTGGTCCTTGAAACC
           R:  GGCAGTCTCTTCGTGCTGAC
PHII   At1g35140  F:  TTGGTTTAGACGGGATGGTG
           R:  AGTACGATGCATTGTTGCAG
FRK1   At2g19190  F:  CGGTCAGATTTCAACAGTTGTC
           R:  AATAGCGATGGGGCCTTGACTTAC
NHL10  At2g35980  F:  TTCCGTGTCCGTAACCCAAAC
           R:  CCCTCGTGTAGGCTGCGATTACG
PER62  At5g39580  F:  GGCATCCTGTCTCACTTGTG
           R:  CGTGATACCAAACCCATGAGC
PER4   At1g14540  F:  CGTTTACGCTATGCAGAC
           R:  ACGTGAGGAGTTGCGTTTGG
CYP82C2 At4g31970  F:  AATCTACCTGCTGCTGGACTG
           R:  GAGAAAATGCGCCATGTAAGG
CYP81F2 At5g57220  F:  AAATGGGAGAGCAACAACAAATG
           R:  ATCGCCATCTCAATGTTAC
WAK2   At1g79680  F:  CGTGTGAGTACACAAATCATCG
           R:  TGGTTAACCCTCTTTTGCTTTC
FOX    At1g26380  F:  GGCTGACCTTCAACCCTTAC
           R:  TTACTCTCTTGCGTTTGG
### Supplementary Table 13 | Sequences of oligonucleotide primers for semi-quantitative RT-PCR.

F: forward; R: reverse.

| Gene | AGI Number | Primer |
|------|------------|--------|
| **NHL10** | At2g35980 | F: ACCTAGCCCTCACTGTTCCTGT  
R: TAAACCTAACCTAAGCCTGAA |
| **UBQ5** | At3g62250 | F: GTGGTGCTAAGAAGAGGAAGA  
R: TCAAGCTTCAACTCTTTCTTT |
| **CPK4** | At4g09570 | F: TCCATACGAACACCAAGA  
R: GTTCCTCATAGTTCTGCTCC |
| **CPK5** | At4g35310 | F: GACGAAGGCGATAAACAAATA  
R: CCGCTCTAGTTGTTGAGAT |
| **CPK6** | At2g17290 | F: AAATCCACCACCACACTACTGT  
R: ACTGAAATGCAAGGACAGAT |
| **CPK11** | At1g35670 | F: GAGACGAAGCCAAACACCTA  
R: GCTGTGAAACTCCGAGAAATC |
| **CPK12** | At5g23580 | F: CAGATGGGGTCCTCTCCTACA  
R: CTCATAGTTCTCCGACCAAT |
| **CPK26** | At4g38230 | F: CTCTACTGTTGGGACACAGA  
R: CCGTAGTCAATCTTCCCA |

### Supplementary Table 14 | Sequences of oligonucleotide primers for genotyping cpk mutants.

LP: left primer; RP: right primer; LB: left border primer; RB: right border primer.

| Gene | AGI Number | Primer |
|------|------------|--------|
| **CPK5** | At4g35310 | LP5: TCGTTCCAAATGACCTTGAC  
RP5: GAGAGAAGACCGAGAGAGAC |
| **CPK6** | At2g17290 | LP6: CTCGCAACTAACGCTTACCTG  
RP6: TTTTGGAACATCTAATAGATCGATG |
| **CPK11** | At1g35670 | LP11: AATGATGGAAGGTGTTTATTTATGTAAG  
RP11: AAGCAATATTAGGGGACAGAACC |
| T-DNA sail | | LBa: TTCATAACACCAATCTCGATACAC  
RBa: GCGTGGACCGCTTGCTGCAACT |
| T-DNA salk | | LBb: GCGTGGACCGCTTGCTGCAACT  
RBb: GTTTCTGACGTATGCTCTAGC |
SUPPLEMENTARY METHODS

DNA constructs. All primers used for cloning and mutagenesis are listed in Supplementary Tables 9-11 and PCR products were checked by sequencing. Effector constructs of full-length (FL) or constitutively active CDPKs (CPKac) were generated by inserting the cDNA (including the first ATG) in a plant expression vector containing a FLAG epitope tag or GFP at the C-terminus, between a 35S-derived promoter and NOS terminator\textsuperscript{4,7-10}. The sequence between the promoter and the first ATG of each CDPK contains 8 nucleotides, including the BamHI restriction site for cloning and no additional ATG, which supports the translation to start at the first ATG of CDPKs. Sequencing validated that no additional amino acids were added to the N-terminus of CDPKs by the vector. The cDNA was amplified from Arabidopsis plants Col-0. The constructs for inactive kinase mutant (KM) forms of CDPK were generated using site-directed mutagenesis to replace the conserved lysine residue of the ATP-binding domain with a methionine. The effector constructs FLS2-HA, MKK4a-MYC, MPK3-HA and MPK6-HA have been previously described\textsuperscript{7}. For VIGS, the \textit{CPK4} construct was generated using a gene specific 500-bp fragment of the coding region that was cloned with the primers 5’-GGAATTCAACACCAAGATTAAGAGA-3’ and 5’-GGGGTACCACAGATACTGCCCTGGCT-3’.

In-gel protein kinase assays. For in-gel kinase assay of endogenous MAPKs, 10-day-old seedlings were ground in liquid nitrogen and homogenized in 150 \(\mu\)l extraction buffer (50 mM HEPES-KOH pH 7.5, 5 mM EDTA, 5 mM EGTA, 2 mM DTT, 25 mM \(\beta\)-glycerophosphate, 2 mM Na\textsubscript{3}VO\textsubscript{4}, 10 mM NaF, 1 mM PMSF, 5
µg/ml antipain, 5 µg/ml leupeptin). The protein extract was recovered after centrifugation at 17600 g for 15 min at 4°C. Equal amounts of proteins were loaded on 10% SDS-polyacrylamide gel embedded with 0.25 mg/ml myelin basic protein (MBP, Invitrogen). The gel was washed 3 times with washing buffer (25 mM Tris-HCl pH=7.5, 0.5 mM DTT, 5 mM NaF, 0.1 mM Na₃VO₄, 0.5 mg/ml BSA, 0.1% Triton X-100), then incubated for 16 h with 3 changes in renaturation buffer (25 mM Tris-HCl pH 7.5, 0.5 mM DTT, 5 mM NaF, 0.1 mM Na₃VO₄) at 4°C. After a 30 min preincubation at room temperature in the reaction buffer (25 mM Tris-HCl pH 7.5, 1 mM DTT, 0.1 mM Na₃VO₄, 12 mM MgCl₂, 1 mM EGTA), the kinase reaction was performed for 1 h in the reaction buffer supplemented with 25 µM cold ATP and 50 µCi [³²P] or [³³P] ATP. The reaction was stopped by extensive washes in 5% TCA and 1% sodium pyrophosphate for 6 h. The protein kinase activity was detected on dried gel by the Typhoon imaging system (GE Healthcare).

For in-gel kinase assay of endogenous CDPKs, protoplasts were directly resuspended in SDS sample buffer. Proteins were loaded on 10% SDS-polyacrylamide gel embedded with 0.25 mg/ml histone III-S (Sigma). The gel was treated for washing, renaturating and reaction steps as described above, with the following modifications: the reaction buffer contains 25 mM Tris-HCl pH 7.5, 1 mM DTT, 0.1 mM Na₃VO₄, 12 mM MgCl₂, 1 mM CaCl₂ and no cold ATP was added during the kinase reaction.

**In vitro protein kinase assays.** For in vitro kinase assay, protoplasts were lysed in 200 µl immunoprecipitation (IP) buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM EDTA, 1 mM DTT, 2 mM NaF, 2 mM Na₃VO₄, 1X cocktail inhibitor [Roche], 1% Triton X-100). To immunoprecipitate CPK-FLAG, protein extracts were incubated with anti-FLAG antibody at 4°C for 2 h, and additional 1 h after adding 15
µl protein G sepharose beads (GE Healthcare). The immunoprecipitated kinases were washed twice with IP buffer and once with kinase buffer (20 mM Tris-HCl pH=7.5, 15 mM MgCl₂, 1 mM CaCl₂ or 5 mM EGTA, 1 mM DTT). Kinase reaction was performed for 30 min in 15 µl kinase buffer containing 1 µg histone (Sigma), 50 µM cold ATP and 2 µCi [³²P] or [³³P] ATP. The reaction was stopped by adding SDS-PAGE loading buffer. After separation on 10% SDS-PAGE, radiolabelled histone was detected on dried gel by the Typhoon imaging system (GE Healthcare). For MAPK activity, immunoprecipitation was carried out with anti-HA antibody, and the kinase reaction was performed with 5 mM EGTA and 1 µg MBP.

**Bacterial growth assays.** The bacterial growth assays were performed as previously described in leaf⁸ and seedlings¹¹ with some modifications. For leaf assay, an overnight culture of *Pseudomonas syringae pv tomato* DC3000 was washed and resuspended to 5x10⁴ cfu/ml in H₂O. Arabidopsis leaves of 4-week-old plants were infiltrated with bacteria using a needleless syringe. Two and four days after inoculation, leaf disks were ground in 100 µl H₂O and serial dilutions were plated on KB medium. Bacterial cfu were counted 2 days after incubation at 28°C. For seedling assay, seedlings were grown in 6-well plates containing 1 ml of liquid medium (0.5 x MS, 0.5% sucrose) for 5 days under continuous light at 22°C. Seedlings were treated with 100 nM flg22 for 1 day prior to inoculation with *Pst* DC3000 at a final concentration of 1x10⁷ cfu/ml. After 3 days of co-cultivation, seedlings were surface sterilized with 70% ethanol and rinsed with H₂O before grinding tissues for bacterial counting as above. The experiments were repeated three times with similar results.
Microarray quality assessment. For microarrays processed at The Partners HealthCare Center for Personalized Genetic Medicine Microarray Facility, the array data quality was initially assessed in R using the BioConductor package arrayQualityMetrics. In addition, the BioConductor package Harshlight was used to provide assessment of possible large scale artefacts, and R code for SmudgeMiner was used for assessment of possible spatial bias. Affymetrix ATH1 GeneChip data were generated from duplicate or triplicate biological samples.

Comparison of various data processing methods. To define CPK5ac and CPK11ac target genes with high stringency and statistical significance, we processed and compared the ATH1 GeneChip data using various microarray analysis and statistic algorithms. Affymetrix probe level data for control, CPK5ac and CPK11ac in the form of .CEL files were loaded into FlexArray and examined and preprocessed with various algorithms, including RMA, dChip (both with adjustment of PM values, PMMM, and without adjustment to PM values, PM-only), and MAS 5.0. Each of the resulting analysis was then further processed on a gene level with regard to probability of change with respect to variability across duplicate arrays. This was done using the t-test as well as an adaptation of the t-test, SAM (Significance Analysis of Microarrays), and a Bayesian version of the t-test, cyber-T. Cyber-T is a version of the t-test that uses a Bayesian estimate of the treatment variance (window size of 101 and confidence ratio of 10 was used). In SAM, the statistic test computed for each gene is slightly different from the t-test in that it includes a factor in the denominator selected to minimize the coefficient of variation across arrays, and is also intended to guard against unrealistically small estimates of variability occasionally observed due to low sample size. Also, SAM does not use the
hypothetical t-distribution to assess significance of observed effects, but permutations of the data set generate an empirical null distribution. Significance is evaluated using this alternative null as a reference (100 permutations and a random number seed of 0 was used).

Affymetrix probe level data for control, CPK5ac and CPK11ac were also analyzed using Gene Chip Operating Software (GCOS)\textsuperscript{25}. Each Affymetrix array hybridization resulting from CPK5ac or CPK11ac RNA samples was compared to two separate Affymetrix arrays with control RNA from different biological experiments. A target signal of 1500 was used with default GCOS parameters\textsuperscript{25}. Regulated genes were then determined by removing genes consistent with the following criteria: a change call of ‘no change’; a signal log\textsubscript{2} ratio between -0.99 and 0.99 (less than two-fold change in expression); a detection call of absent in both control and treatment arrays. Because three separate algorithms are used in GCOS to determine detection, change, and signal value\textsuperscript{25}, there can be occasional discrepancies between the results of these algorithms. Genes showing these discrepancies were eliminated under the two additional criteria: a change call of ‘increase’ or ‘marginal increase’ combined with either a signal log\textsubscript{2} ratio less than 0, an absent call in the CPK5ac or CPK11ac array, or a signal value in the control RNA array that was greater than the signal value in the CPK5ac or CPK11ac array; a change call of ‘decrease’ or ‘marginal decrease’ combined with either a signal log\textsubscript{2} ratio greater than 0; an absent call in the control RNA array, or a signal value in the control that was less than the signal value in the CPK5ac or CPK11ac array.

After comparing the results from the extensive analyses of control, CPK5ac and CPK11ac arrays, it was found that RMA\textsuperscript{19,20} with cyber-T\textsuperscript{24} and a p-value cutoff of 0.05 was most in agreement with our qRT-PCR data for the validation of eight
marker genes (n=6) in triplicate biological samples (Fig. 3). The combination of RMA\textsuperscript{19,20} followed by cyber-T\textsuperscript{24} (a p-value cutoff of 0.05) was chosen to analyze arrays for 30 min and 60 min fgl22 treatment in protoplasts and seedlings (Fig. 2a) as well as arrays for MAMP signals (Fig. 2b) and microbial infection (Fig. 2c) downloaded from the public domain.

**Identification of CPK5ac-specific, CPK11ac-specific, and common CPK5ac and CPK11ac target genes.** Arrays for CPK5ac and CPK11ac along with respective control arrays were analyzed in FlexArray\textsuperscript{18} using RMA\textsuperscript{19,20} followed by cyber-T\textsuperscript{24} as well as dChip\textsuperscript{21} followed by cyber-T\textsuperscript{24}. Candidate target genes were chosen from each of the two analysis based on a p-value of 0.05 or less (Supplementary Fig. 6). Significant target genes separately showed a p-value of 0.05 and log\textsubscript{2} ratio >1 or < -1 in both RMA and dChip processing (Supplementary Table 2). The p-values of majority (93\%) of these target genes were below 0.03. The p-value of 0.05 was accepted to cover the validated marker gene CYP82C2 (Fig. 3, 4a), which had relatively low expression level, thus high variance. They were categorized as CPK5ac-specific (72 genes), CPK11ac-specific (59 genes), or common CPK5ac and CPK11ac (113 genes) target genes (Supplementary Table 2). The functional description of the listed genes is based on TAIR 8.0. Functional descriptions were downloaded from the TAIR website (ftp://ftp.arabidopsis.org/Genes/TAIR8_genome_release/TAIR8_functional_descriptions), Mapman\textsuperscript{26} and the Sheen lab annotation\textsuperscript{6} (http://genetics.mgh.harvard.edu/sheenweb/search_affy.html).
Identification of CPK5ac and CPK11ac target genes as early flg22 responsive genes. Duplicate or triplicate microarray data from three separate conditions of flg22 treatments in protoplasts (30 min and 60 min) (see online METHODS), seedlings (30 min and 60 min) (see online METHODS) and 5-week-old adult leaves (60 min) from Columbia ecotype (http://arabidopsis.org/info/expression/ATGenExpress.jsp, and see below) were collected. Resulting array data was assessed for quality as described above. Affymetrix .CEL files for protoplasts, seedlings and leaves were then imported into FlexArray\textsuperscript{18} and processed with RMA\textsuperscript{19,20} followed by cyber-T\textsuperscript{24}. Flg22 responsive genes were selected by the criteria of p-value of 0.05 or less after processing with RMA\textsuperscript{19,20} followed by cyber-T\textsuperscript{24}.

After processing, all data were imported into Excel. VBA scripts were then used to extract CDPK target genes from the protoplast, leaf, and seedling data for early flg22 responsive genes. The resulting list of candidate flg22-CDPK early target genes (Supplementary Table 2) was then selected manually by eliminating genes that met the following criteria: genes not regulated in any of the flg22 conditions; genes with a CDPK signal log\textsubscript{2} ratio of between -1 and 1; genes that were reverse regulated between CDPK and flg22. Three gene lists resulted; common CPK5ac and CPK11ac target genes that were also early flg22 responsive genes (81 genes) (Fig. 2a, Supplementary Table 3); CPK5ac-specific genes that were also early flg22 responsive genes (59 genes) (Fig. 2a, Supplementary Table 4), CPK11ac-specific genes that were also early flg22 responsive genes (31 genes) (Fig. 2a, Supplementary Table 5). The resulting gene lists were presented using Hierarchical Clustering analysis in Cluster 3.0\textsuperscript{27} and Java TreeView 1.1.3\textsuperscript{28} (Fig. 2a, Supplementary Table 6) as described below.
Comparison of flg22-CDPK early target genes with early MAMP responsive genes. Duplicate or triplicate microarray data from various MAMP treatments (elf26\textsuperscript{29}, chitin\textsuperscript{30}, harpin\textsuperscript{31}, NPP1\textsuperscript{32,33} and LPS\textsuperscript{34}) for 30 min, 60 min or 4 h in seedlings or leaves were downloaded from various public domain sources while data for PGN\textsuperscript{35} treatment was kindly provided by A. Gust and T. Nürnberger (see below). ABA early (60 min) genes in seedlings were used as a microarray control. Affymetrix .CEL files were imported into FlexArray\textsuperscript{18} and processed with RMA\textsuperscript{19,20} followed by cyber-T\textsuperscript{24}. Early MAMP responsive genes were selected by the criteria of p-value of 0.01 or less. After processing, all data were imported into Excel. VBA scripts were then used to extract genes from the RMA-cyber-T processed MAMP data using the list of 171 flg22-CDPK early target genes (Supplementary Tables 2-5). The resulting gene lists were presented using Hierarchical Clustering analysis in Cluster 3.0\textsuperscript{27} and Java TreeView 1.1.3\textsuperscript{28} (Fig. 2b, Supplementary Table 7) as described below.

Comparison of flg22-CDPK early target genes with genes regulated by microbial infection. Microarray data from microbial exposure to \textit{Pseudomonas syringae pv. phaseolicola}, \textit{P. infestans}, and \textit{B. cinerea}\textsuperscript{36} were downloaded from various public domain sources (see below). Affymetrix .CEL files were imported into FlexArray\textsuperscript{18} and processed with RMA\textsuperscript{19,20} followed by cyber-T\textsuperscript{24}. Regulated genes were selected by the criteria of p-value of 0.01 or less. After processing, data were imported into Excel. VBA scripts were then used to extract genes from the RMA-cyber-T processed microbial infection data using the list of 171 flg22-CDPK early target genes (Supplementary Tables 3-5). The resulting genes lists were presented using Hierarchical Clustering analysis in Cluster 3.0\textsuperscript{27} and Java TreeView 1.1.3\textsuperscript{28} (Fig. 2c, Supplementary Table 8) as described below.
Clustering of data

Gene lists with signal log ratio values, log₂ (fold-change), were imported into Cluster 3.0 \(^{27}\) for Hierarchical Clustering analysis using binary, agglomerative, hierarchical centroid linkage clustering with a Pearson correlation similarity metric for genes. More specifically, Correlation (uncentered), a modified Pearson correlation in which the mean is assumed to be 0, was the similarity metric chosen. The resulting clustering was viewed with Java TreeView 1.1.3 \(^{28}\) (Fig. 2).

**ATH1 GeneChip data from public domains**

All the original data for 18 ATH1 GeneChips generated for this project were submitted to Gene Expression Omnibus (GEO) microarray database at National Centre for Biotechnology Information (NCBI) for public access.

**Flg22**

Leaf data for flg22 (1 µM, 60 min) were obtained from the AtGenExpress project: B20-ATGEN, B34-ATGEN, and B6-ATGEN (F. Brunner and T. Nürnberger).

**Elf26**

Seedling data for elf26 (1 µM, 60 min) obtained in A. thaliana Landsberg ecotype were downloaded from ArrayExpress, European Bioinformatics Institute (EBI): E-MEXP 547, H elf wt 60 min (2 arrays) (G. Kunz).

**Chitin**

Leaf data for chitin (1 µM, 30 min) were downloaded from Gene Expression Omnibus, [http://www.ncbi.nlm.nih.gov/projects/geo/](http://www.ncbi.nlm.nih.gov/projects/geo/), at NCBI, dataset record
GDS1531. Crab-shell chitin: GSM48125, GSM48126, GSM48127; control: GSM48128, GSM48129, GSM48130 (K. Ramonell, GSE2538 Chitin Oligomer Experiment).

**Harpin**

Leaf data for harpin (4 h) were downloaded from AtGenExpress at TAIR website, TAIR accession: ExpressionSet:1008080727. HrpZ, 4 h: B11-ATGEN, B25 ATGEN, B39-ATGEN (AtGenExpress, F. Brunner and T. Nürnberger).

**NPP1**

Leaf data for NPP1 (4 h) were downloaded from AtGenExpress at TAIR website, TAIR accession: ExpressionSet:1008080727. NPP1, 4 h: B12-ATGEN, B26-ATGEN, B40-ATGEN (AtGenExpress, F. Brunner and T. Nürnberger). For these arrays, the controls used were 4 h: B10-ATGEN, B24-ATGEN, B38-ATGEN.

**LPS**

Leaf data for LPS (4 h) were downloaded from AtGenExpress at TAIR website, TAIR accession: ExpressionSet:1008080727. LPS 4 h: B14-ATGEN, B28-ATGEN, B42-ATGEN (AtGenExpress, F. Brunner and T. Nürnberger).

**PGN**

Leaf data for PGN (4 h) were obtained from A. Gust and T. Nürnberger in the form of Affymetrix .CEL files that were used for data analysis.
ABA

Data for ABA treatment (10 µM, 60 min) in seedlings were downloaded from AtGenExpress at TAIR website, TAIR accession: ExpressionSet: 1007964750. ABA 1 h: RIKEN-GODA13ARIKEN-GODA13B, RIKEN-GODA1A, RIKEN-GODA1B (AtGenExpress, H. Goda, S. Yoshida, Y. Shimada).

P. s. phaseolicola

Data for P. s. phaseolicola infection (6 h) were downloaded from AtGenExpress at TAIR website, TAIR accession: ExpressionSet:1007966202. Pseudomonas syringae pv. phaseolicola, 6 h: A45-ATGEN-31-1_6hr_PSPH, A46-ATGEN-31-2_6hr_PSPH, A48-ATGEN-31-4_6hr_PSPH (AtGenExpress, B. Kemmerling and T. Nürnberger).

B. cinerea

Data for B. cinerea infection (18 h) were downloaded from AtGenExpress at TAIR website, TAIR accession: ExpressionSet:1007967417. Botrytis cinerea, 18 h: BC181-1, BC181-2, BC181-3 (AtGenExpress, C. Denoux, F. Ausubel, J. Dewdney, S. Ferrari).

P. infestans

Data for P. infestans infection (12 h) were downloaded from AtGenExpress at TAIR website, TAIR accession: ExpressionSet:1007966021. Phytophthora infestans, 12 h: C13-ATGEN-1-PI-12, C14-ATGEN-2-PI-12, C15-ATGEN-3-PI-12 (AtGenExpress, D. Scheel, F. Brunner, L. Westphal).
1. Zimmermann, P., Hennig, L. & Gruissem, W. Gene-expression analysis and network discovery using Genevestigator. *Trends Plant Sci.* **10**, 407-409 (2005).

2. Harper, J. F. & Harmon, A. Plants, symbiosis and parasites: a calcium signalling connection. *Nat. Rev. Mol. Cell Biol.* **6**, 555-66 (2005).

3. Cheng, S. H., Willmann, M. R., Chen, H. C. & Sheen, J. Calcium signaling through protein kinases. The *Arabidopsis* calcium-dependent protein kinase gene family. *Plant Physiol.* **129**, 469-485 (2002).

4. Sheen, J. Ca$^{2+}$-dependent protein kinases and stress signal transduction in plants. *Science* **274**, 1900-1902 (1996).

5. Burch-Smith, T. M., Schiff, M., Liu, Y. & Dinesh-Kumar, S. P. Efficient virus-induced gene silencing in *Arabidopsis*. *Plant Physiol.* **142**, 21-27 (2006).

6. Baena-Gonzalez, E., Rolland, F., Thevelein, J. M. & Sheen, J. A central integrator of transcription networks in plant stress and energy signalling. *Nature* **448**, 938-942 (2007).

7. Asai, T. et al. MAP kinase signalling cascade in *Arabidopsis* innate immunity. *Nature* **415**, 977-983 (2002).

8. He, P. et al. Specific bacterial suppressors of MAMP signaling upstream of MAPKKK in Arabidopsis innate immunity. *Cell* **125**, 563-575 (2006).
9. Shan, L. et al. Bacterial effectors target the common signaling partner BAK1 to disrupt multiple MAMP receptor-signaling complexes and impede plant immunity. *Cell Host Microbe* 4, 17-27 (2008).

10. Yoo, S. D., Cho, Y. H. & Sheen, J. Arabidopsis mesophyll protoplasts: a versatile cell system for transient gene expression analysis. *Nat. Protoc.* 2, 1565-1572 (2007).

11. Schreiber, K., Ckurchumova, W., Peek, J. & Desveaux, D. A high-throughput chemical screen for resistance to Pseudomonas syringae in Arabidopsis. *Plant J.* 54, 522-531 (2008).

12. Kauffmann, A., Gentleman, R. & Huber, W. arrayQualityMetrics--a bioconductor package for quality assessment of microarray data. *Bioinformatics* 25, 415-416 (2009).

13. Suarez-Farinas, M., Pellegrino, M., Wittkowski, K. M. & Magnasco, M. O. Harshlight: a "corrective make-up" program for microarray chips. R package version 1.12.0. (2007).

http://asterion.rockefeller.edu/Harshlight/

14. Reimers, M. & Weinstein, J. N. Quality assessment of microarrays: visualization of spatial artifacts and quantitation of regional biases. *BMC Bioinformatics* 6, 166 (2005).

15. Choe, S. E., Boutros, M., Michelson, A. M., Church, G. M. & Halfon, M. S. Preferred analysis methods for Affymetrix GeneChips revealed by a wholly defined control dataset. *Genome Biol.* 6, R16 (2005).

16. Cheng, C. & Pounds, S. False discovery rate paradigms for statistical analyses of microarray gene expression data. *Bioinformation* 1, 436-446 (2007).
17. Murie, C., Woody, O., Lee, A. Y. & Nadon, R. Comparison of small n statistical tests of differential expression applied to microarrays. *BMC Bioinformatics* **10**, 45 (2009).

18. Blazejczyk, M., Miron, M. & Nadon, R. FlexArray: a statistical data analysis software for gene expression microarrays (Genome Quebec, Montreal, Canada, 2007).
URL http://genomequebec.mcgill.ca/FlexArray

19. Irizarry, R. A. et al. Summaries of Affymetrix GeneChip probe level data. *Nucleic Acids Res.* **31**, e15 (2003).

20. Irizarry, R. A. et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4**, 249-264 (2003).

21. Li, C. & Hung Wong, W. Model-based analysis of oligonucleotide arrays: model validation, design issues and standard error application. *Genome Biol.* **2**, 0032 (2001).

22. Hubbell, E., Liu, W. M. & Mei, R. Robust estimators for expression analysis. *Bioinformatics* **18**, 1585-1592 (2002).

23. Tusher, V. G., Tibshirani, R. & Chu, G. Significance analysis of microarrays applied to the ionizing radiation response. *Proc. Natl. Acad. Sci. U S A* **98**, 5116-5121 (2001).

24. Baldi, P. & Long, A. D. A Bayesian framework for the analysis of microarray expression data: regularized t-test and statistical inferences of gene changes. *Bioinformatics* **17**, 509-519 (2001).

25. Affymetrix: Microarray suite user guide. Affymetrix 2001, version 5: http://www.affymetrix.com/support/technical/manuals.affx
26. Thimm, O. et al. MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *Plant J.* **37**, 914-939 (2004).

27. de Hoon, M. J., Imoto, S., Nolan, J. & Miyano, S. Open source clustering software. *Bioinformatics* **20**, 1453-1454 (2004).

28. Saldanha, A. J. Java Treeview--extensible visualization of microarray data. *Bioinformatics* **20**, 3246-3248 (2004).

29. Zipfel, C. et al. Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation. *Cell* **125**, 749-760 (2006).

30. Ramonell, K. et al. Loss-of-function mutations in chitin responsive genes show increased susceptibility to the powdery mildew pathogen Erysiphe cichoracearum. *Plant Physiol.* **138**, 1027-1036 (2005).

31. Lee, J., Klessig, D. F. & Nurnberger, T. A harpin binding site in tobacco plasma membranes mediates activation of the pathogenesis-related gene HIN1 independent of extracellular calcium but dependent on mitogen-activated protein kinase activity. *Plant Cell* **13**, 1079-1093 (2001).

32. Fellbrich, G. et al. NPP1, a Phytophthora-associated trigger of plant defense in parsley and Arabidopsis. *Plant J.* **32**, 375-390 (2002).

33. Qutob, D. et al. Phytotoxicity and innate immune responses induced by Nep1-like proteins. *Plant Cell* **18**, 3721-3744 (2006).

34. Zeidler, D. et al. Innate immunity in Arabidopsis thaliana: lipopolysaccharides activate nitric oxide synthase (NOS) and induce defense genes. *Proc. Natl. Acad. Sci. U S A* **101**, 15811-15816 (2004).
35. Gust, A. A. et al. Bacteria-derived peptidoglycans constitute pathogen-associated molecular patterns triggering innate immunity in Arabidopsis. *J. Biol. Chem.* **282**, 32338-32348 (2007).

36. Ferrari, S. et al. Resistance to Botrytis cinerea induced in Arabidopsis by elicitors is independent of salicylic acid, ethylene, or jasmonate signaling but requires PHYTOALEXIN DEFICIENT3. *Plant Physiol.* **144**, 367-379 (2007).