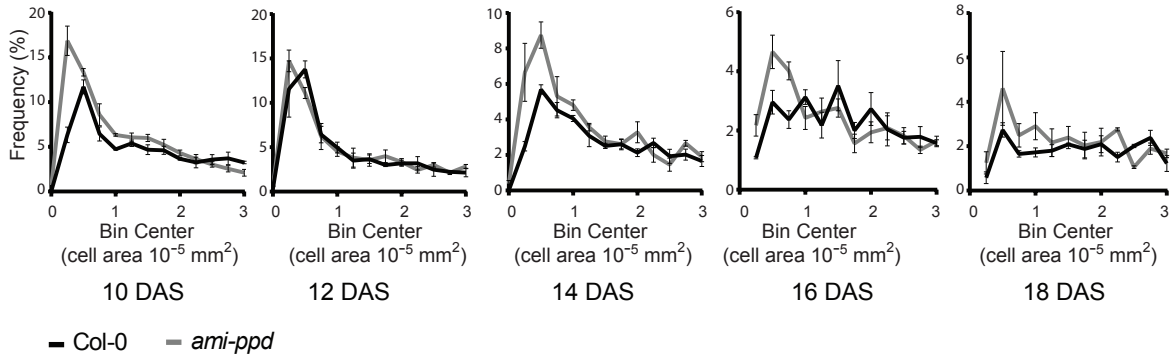
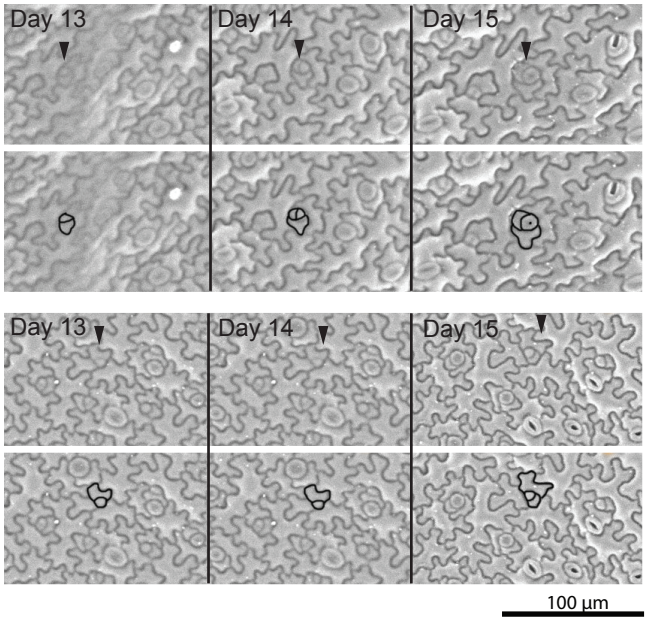
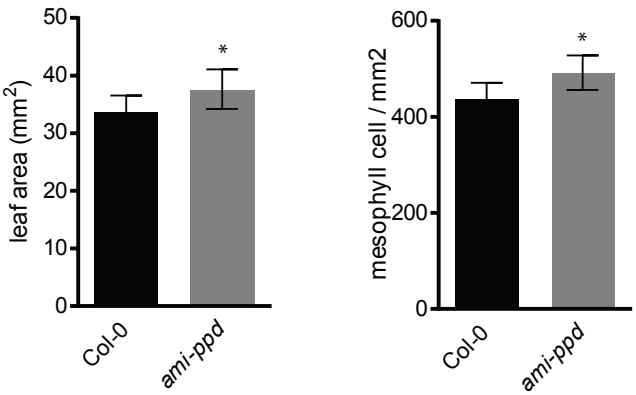
A



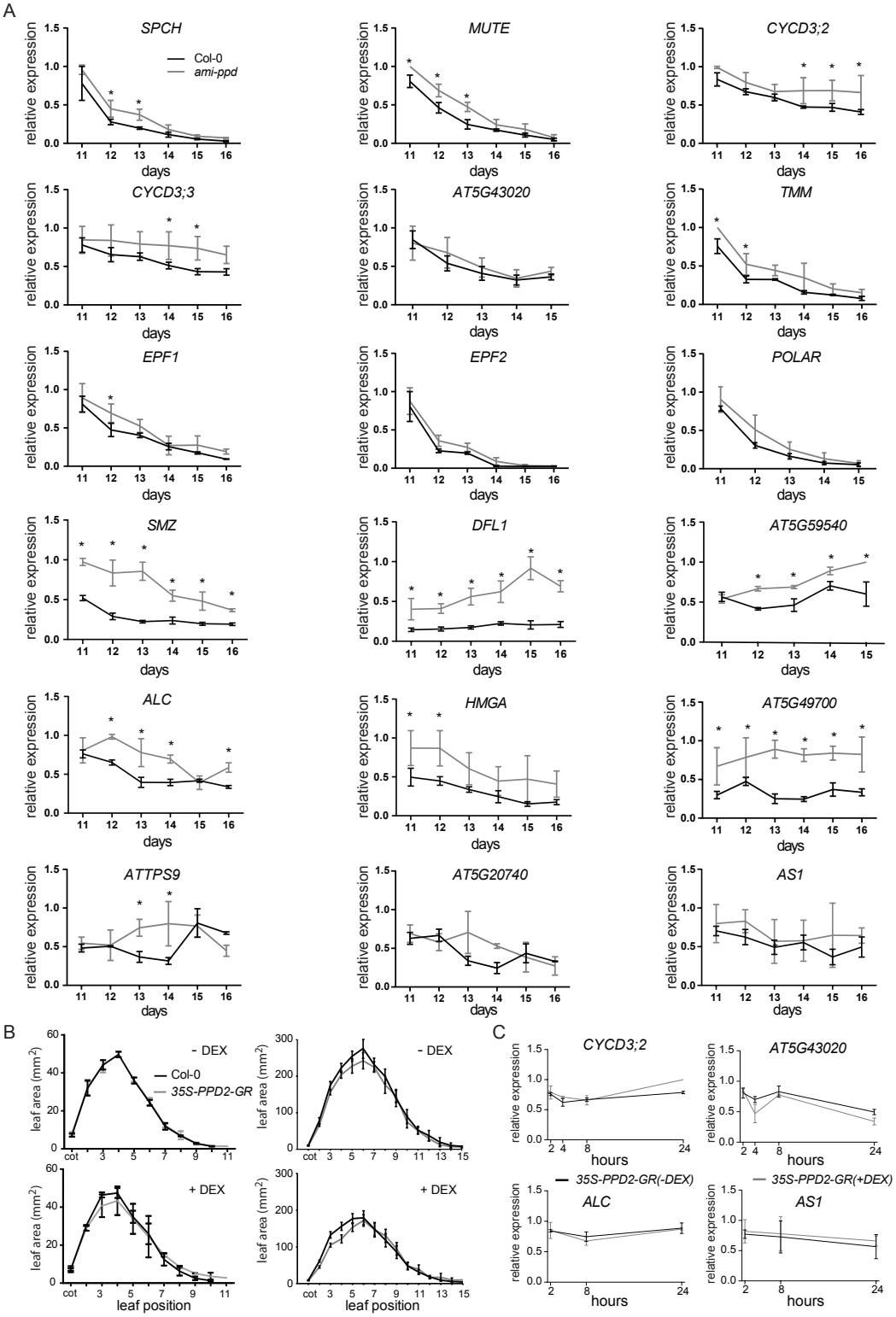

B




C

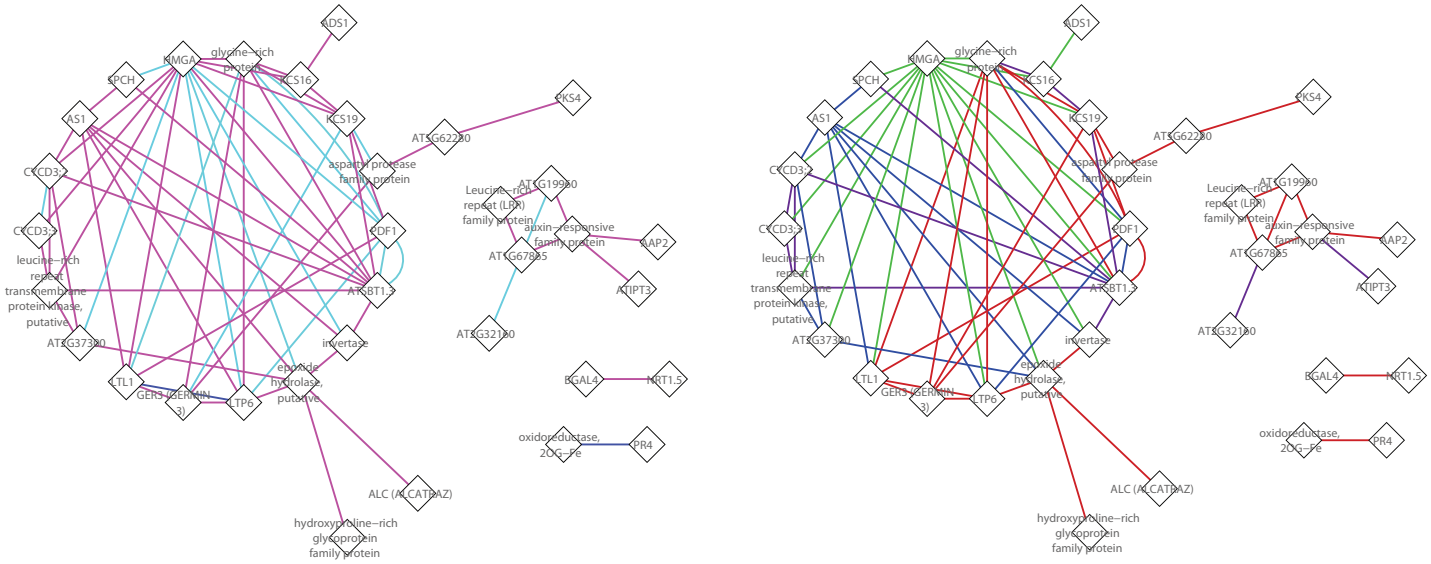



**Supplemental Figure 1. Pavement, Meristemoid and Mesophyll Cell characteristics in *ami-ppd* Leaves.**
(A) The frequency of cells as a function of their area is represented. Each bin represents a cell area of 0.25 $10^{-5}$ $mm^2$. Three to five leaves were analyzed for the cell-related parameters measurements.
(B) Two examples of images in the three time points used to estimate the proportion of cell types originating from meristemoid division are represented. Two events scored as asymmetric division are labeled (arrow and contour on the cells).
(C) Leaf area at 17 DAS and mesophyll cell density in Col-0 and *ami-ppd* first leaf pair. (n=3; error bars represent ±SE *: significant difference from wild type at p-value<0.05).




**Supplemental Figure 2. Expression of Known Stomatal Development and Patterning Genes, Cell Cycle-Related Genes and PPD2 Target Genes in Growing *ami-ppd* Leaves and Expression of PPD2 Target Genes in the *35S-PPD2-GR* Line Treated with or without Dexamethasone.**

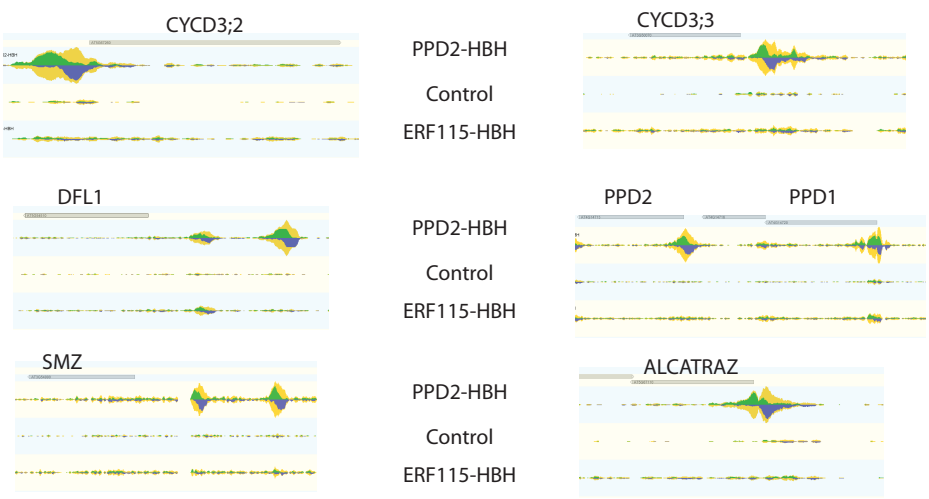
(A) The first leaf pair from *ami-ppd* or wild-type plants was harvested from 11 to 16 DAS and used for RNA extraction, followed by qRT-PCR. The expression of known stomatal development and patterning genes, cell cycle-related genes and putative targets of PPD2 was analysed (n = 3, error bars represent ± SE *: significant difference from wild type at p-value < 0.05). (B) Area of individual leaves of wild-type and *35S-PPD2-GR* plants grown *in vitro* for 21 DAS (left) or in soil (right) with (+DEX) or without (-DEX) Dexamethasone (n=3; error bars represent ±S E *: significant difference from the wild type at p-value<0.05). (C) Time-course analysis of PPD2 target gene expression after induction of *PPD2* in the *35S-PPD2-GR* line treated with DEX (n = 3, error bars represent ± SE, *: significant difference from the *35S-PPD2-GR* without DEX at p-value<0.05).




**Supplemental Figure 3. Co-Expression Networks Generated with the Differentially Expressed Genes in *ami-ppd* Using CORNET.**

Four predefined sub-sets of microarray expression data corresponding to four types of experiments (experiments in which leaf tissues are sampled (leaf), hormone treatment series (hormone), microarray experiments oriented toward growth, development and cell cycle studies (compendium1) or microarray experiments for which very similar experiments were removed (compendium2)) were used to perform co-expression analysis using the CORNET tool (De Bodt et al. 2010; De Bodt et al. 2012). Left panel: edges are colored as a function of the correlation coefficient (coef). Pink: 0.7<coef<0.8; blue: coef>0.7. Right panel: edges are colored in function of the experiment: Leaf: green, hormone: red, compendium 1: blue, compendium 2: purple.

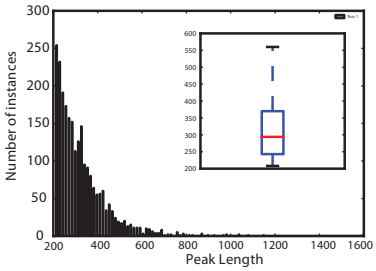
A



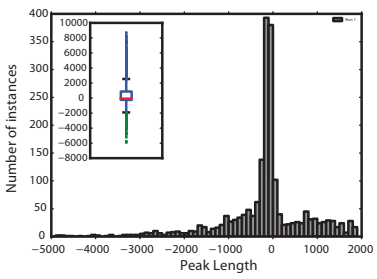

B

| Category and sub-category | # genes | p-value | Fold enrichment |
|---|---|---|---|
| hormone metabolism | 60 | 1.32E-06 | 1.93 |
| RNA.regulation of transcription | 230 | 5.53E-14 | 1.64 |
| RNA.regulation of transcription. WRKY domain transcription factor family | 15 | 1.75E-05 | 3.5 |
| RNA.regulation of transcription. MYB-related transcription factor family | 13 | 1.93E-06 | 4.7 |

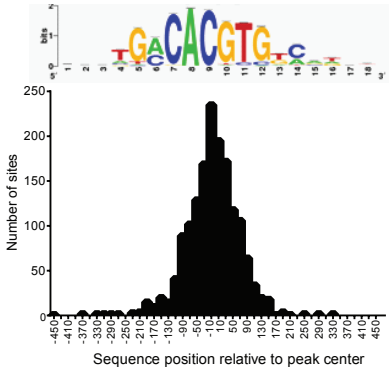
C



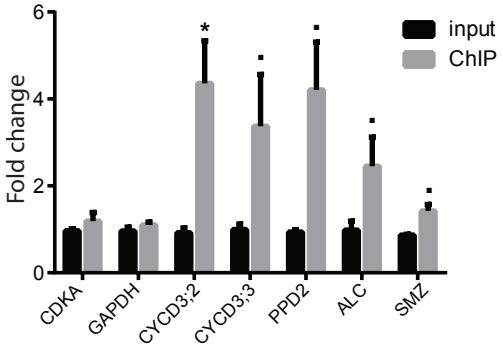

D




E




F



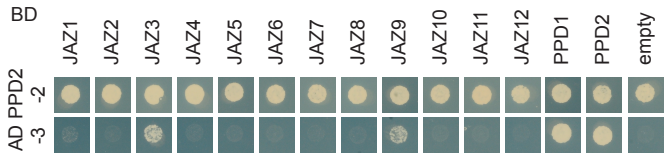

**Supplemental Figure 4. Genome-Wide Determination of PPD2 Binding Sites by TChAP-Seq**

(A) Genome view representation of the peaks identified by TChAP-Seq for *CYCD3;2*, *CYCD3;3*, *DFL1*, *PPD1*, *PPD2*, *SMZ* and *ALC* in the *35S-PPD2-HBH*, *35S-ERF115-HBH* (Heyman et al, 2013) and the control cell culture. Forward reads are represented in green, reverse reads in blue and total coverage in yellow. (B) Overrepresented gene ontology categories in the list of genes identified by TChAP-Seq. (C) Distribution of the peak lengths in base pairs of the 2,042 peaks. The boxplot in the inset shows the median peak length. (D) Distribution of peak summit located as a function of the translation start site (TSS). (E) GmCACGTGkC motif, containing the G-box sequence (CACGTG) identified by RSAT peak-motifs (Thomas-Chollier et al., 2012) and location as a function of the peak summit. (F) Validation of PPD2 target genes by ChIP-qPCR using a cell culture expressing $GS_{yellow}$-tagged PPD2. Enrichment was determined with qPCR by comparing the input and anti-GFP purified samples of the *35S-$GS_{yellow}$ -PPD2* to the control *35S-$GS_{yellow}$* cell culture. Asterisks indicate significant difference to the input. (n=4, error bars represent ±SE, * $p < 0.05$, ▪ $p<0.1$).

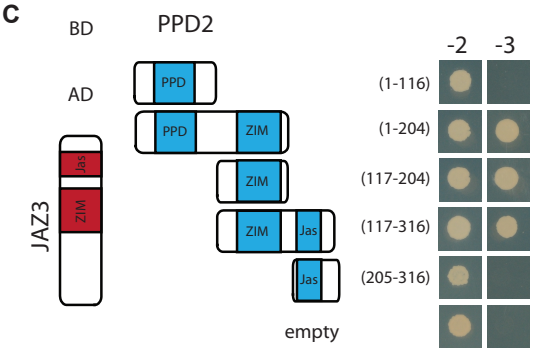
A

| Prey | | | | |
|---|---|---|---|---|
| **AGI** | **Protein** | **PPD2-GS** | **KIX8-GS** | **GS-KIX8** |
| **TIFY proteins** | | | | |
| AT4G14720 | PPD2 | 2 | 2 | 2 |
| AT4G32570 | TIFY8 | 2 | | 2 |
| AT3G17860 | JAZ3 | 2 | | |
| AT5G13220 | JAZ10 | 2 | | |
| **JAZ related proteins** | | | | |
| AT4G28910 | NINJA | 2 | | 2 |
| AT1G15750 | TPL | | | 2 |
| **KIX proteins** | | | | |
| AT3G24150 | KIX8 | 2 | 2 | 2 |
| AT4G32295 | KIX9 | 2 | | |
| **Other** | | | | |
| AT5G54770 | THI1 | 1 | 2 | |
| AT5G06290 | 2CPB | 1 | 2 | |
| AT1G49670 | NQR | 2 | | |
| AT1G50600 | SCL5 | | 2 | |
| AT4G21710 | NRPB2 | | 2 | |

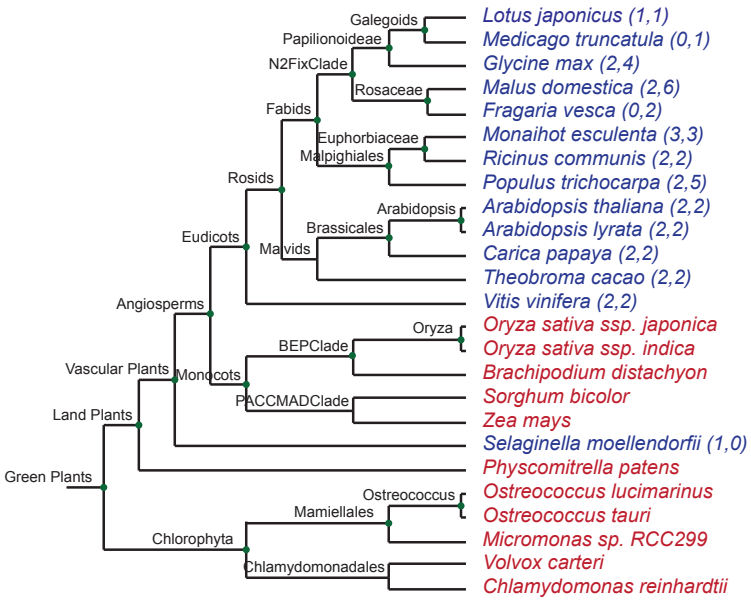
B

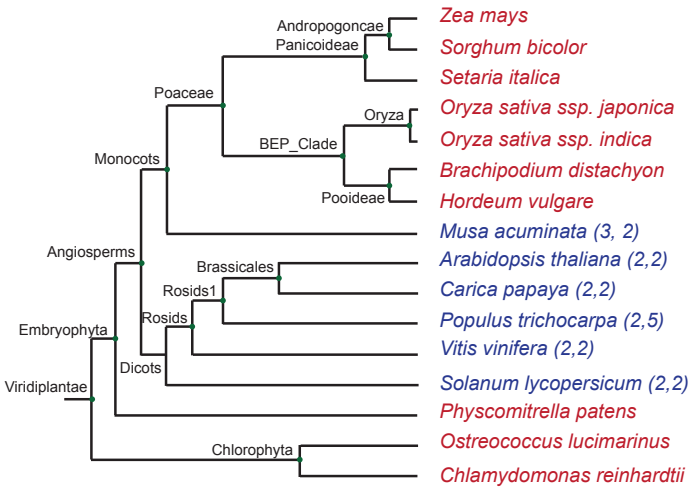



C



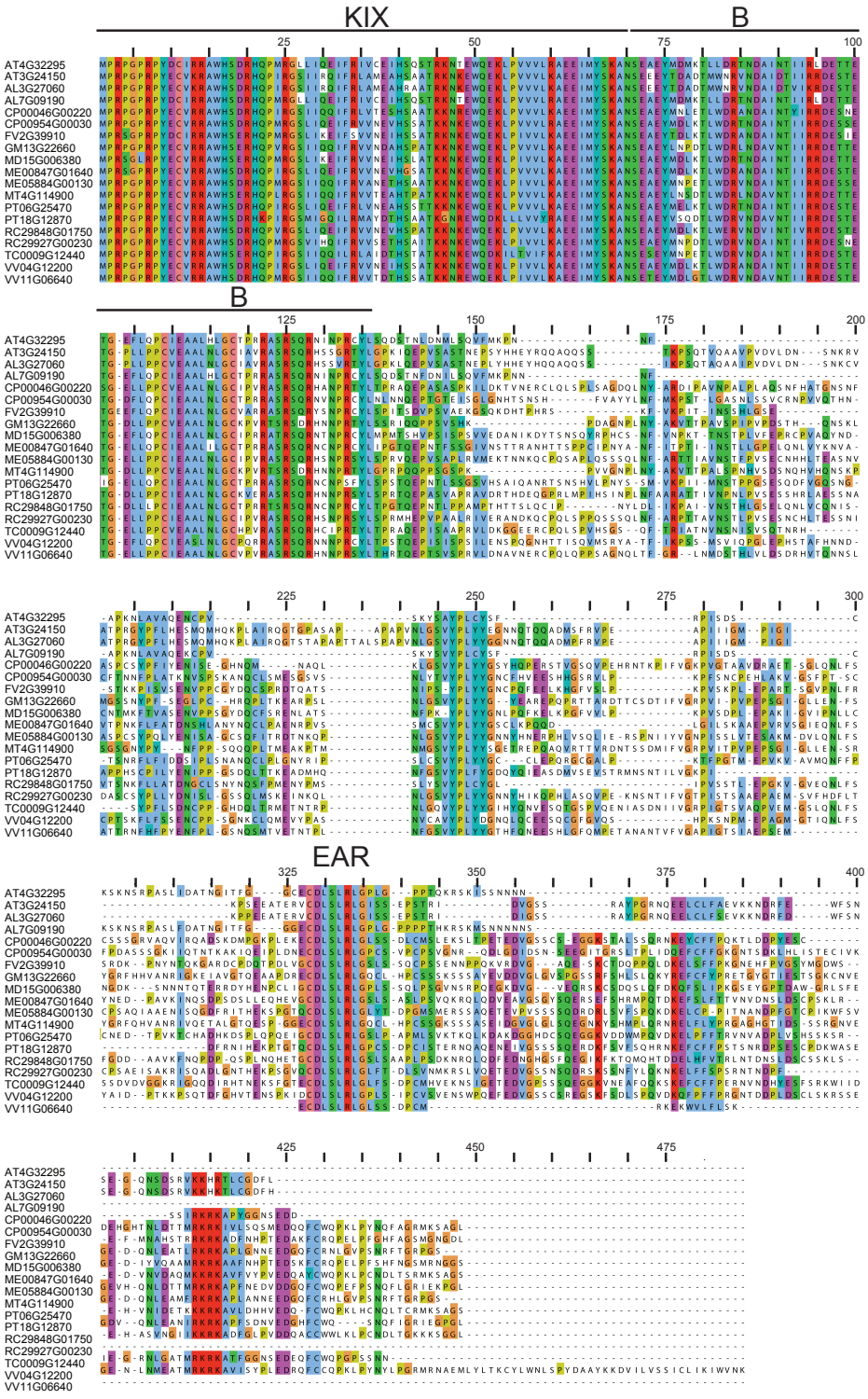

D



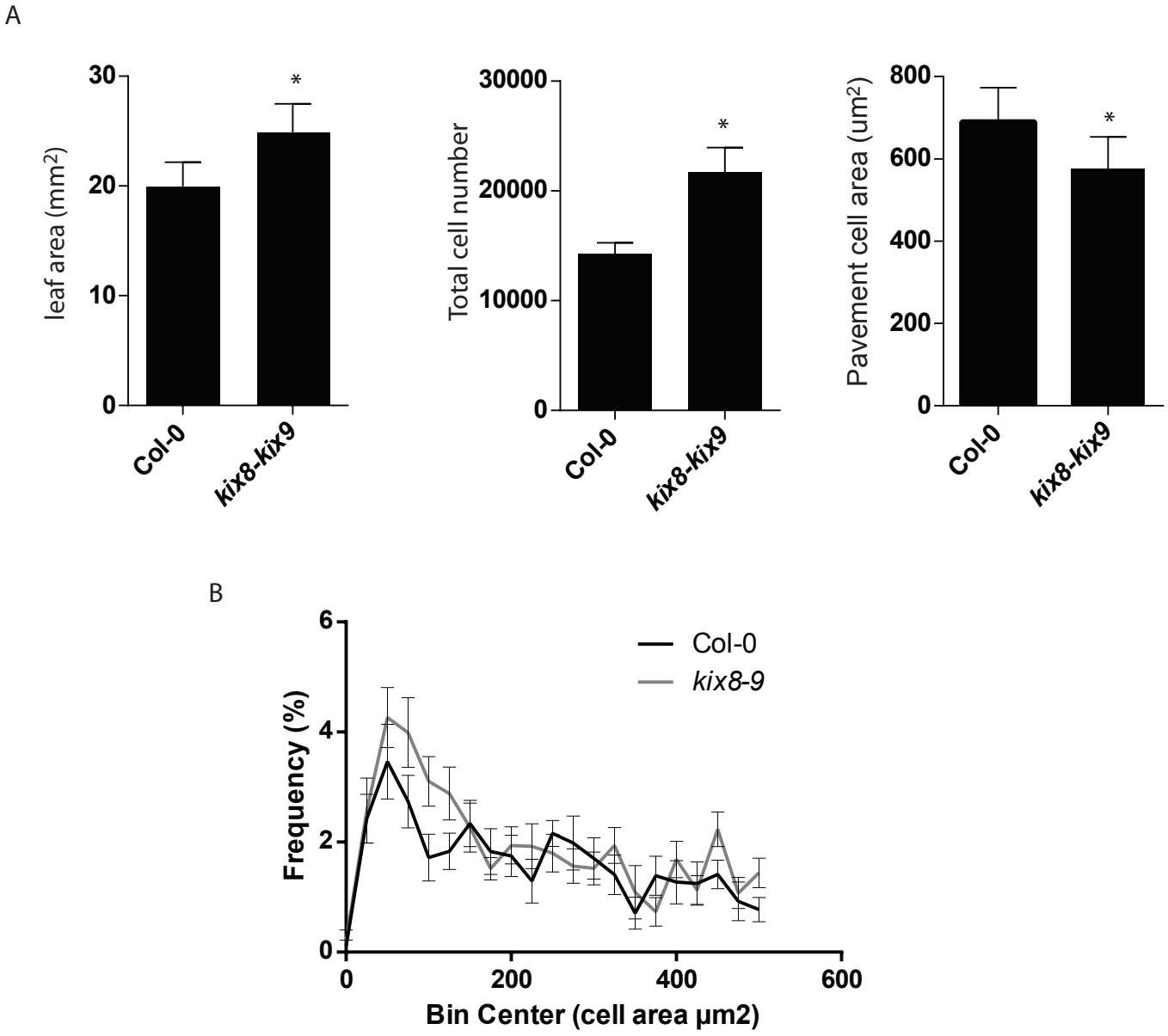

**Supplemental Figure 5. Proteins Purified by Tandem Affinity Purification Using PPD2 and KIX8 as Bait and Heterodimerization of PPD2 with JAZ Proteins.**

(A) Overview of the proteins purified by Tandem Affinity Purification using PPD2 and KIX8 as bait. TAP was performed on *Arabidopsis* cell cultures expressing PPD2 tagged with a GS-tag at the C-terminal region or KIX8 tagged with a GS tag at the C-terminal (KIX8-GS) region and N-terminal (GS-KIX8) region. Proteins were identified using peptide-based homology analysis of MS data. Background proteins identified in control experiments were withdrawn. The number indicates whether the prey was identified once or twice in 2 experiments with each bait protein. Abbreviations: AGI, *Arabidopsis* Genome Identifier; PPD2, PEAPOD2; NINJA, NOVEL INTERACTOR OF JAZ; TPL, TOPLESS; JAZ, JASMONATE ZIM DOMAIN; THI1, THIAMINE1; 2CPB, 2-CYS PEROXIREDOXIN B; NQR, NAD(P)H: QUINONE OXIDOREDUCTASE; SCL5, SCARECROW-like 5; NRPB2, NUCLEAR RNA POLYMERASE B2. (B) Yeast two-hybrid was done as in Figure 3 to test the interaction between PPD2 and all 12 JAZ proteins, PPD1 and PPD2. (C) Truncations of PPD2 were tested via yeast two-hybrid to identify the JAZ3 interaction domain. (D) PPD2, KIX8 and KIX9 orthologs in other plant species. Blue and red colors represent the existence or absence, respectively, of putative *Arabidopsis* PPD2, KIX8 and KIX9 orthologs in different species covered by the comparative genomics resource PLAZA 2.5 (upper tree) and PLAZA 3.0 Monocots (bottom tree). Numbers in brackets indicate the number of putative orthologs of PPD2 or KIX8/9 in each species (http://bioinformatics.psb.ugent.be/plaza).

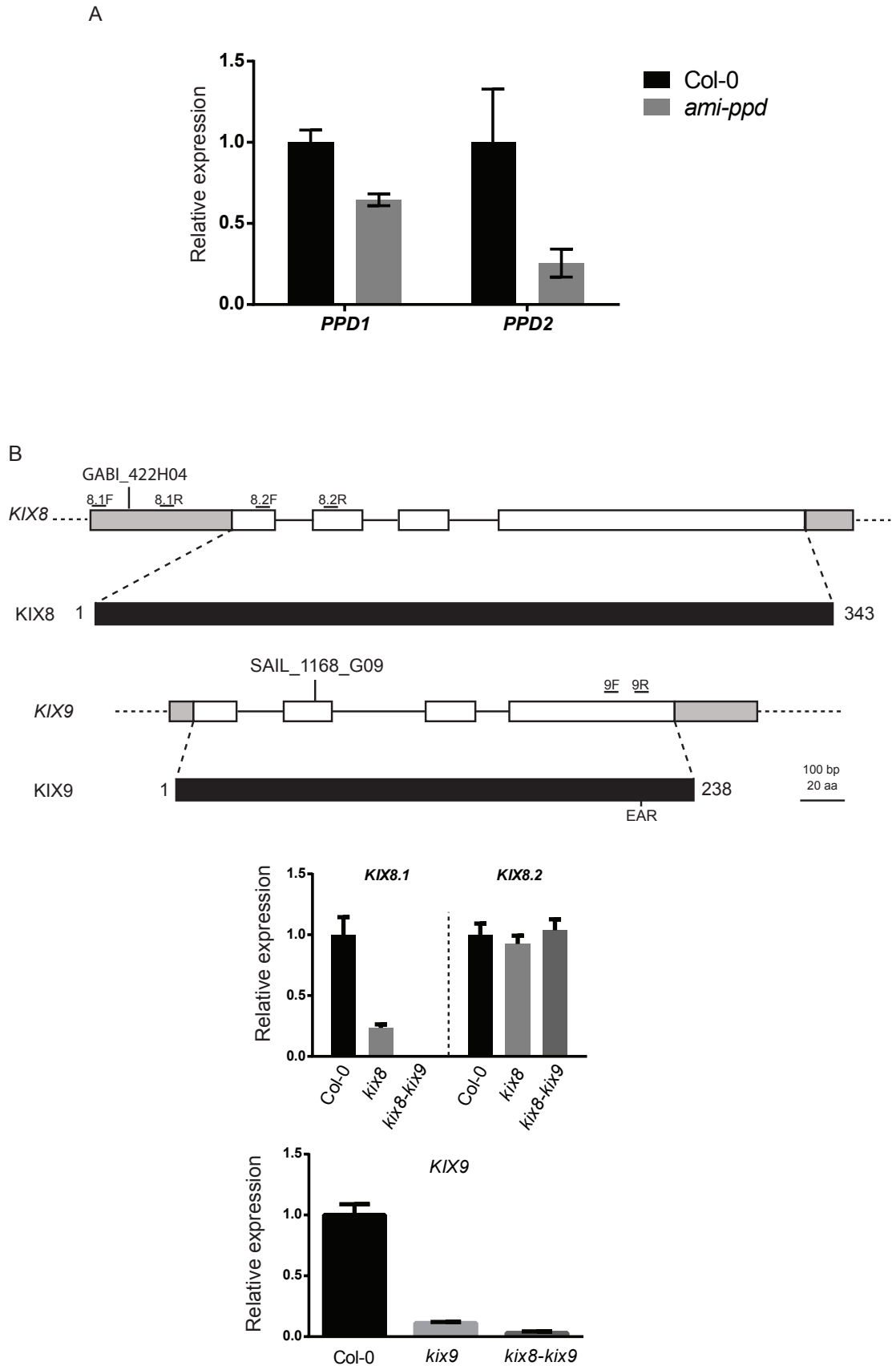



**Supplemental Figure 6. Alignment of Different KIX Protein Orthologs from Eudicots.**

Four conserved regions can be identified: an N-terminal and highly conserved KIX domain (1-69), a conserved B domain (aa 70-137 in KIX9), a C-terminal less conserved ERF-associated amphiphilic repression (EAR) motif (212-220) and a putative nuclear localization signal (NLS, 228-231). *Arabidopsis thaliana*: AT4G32295, AT3G24510; *Arabidopsis lyrata*: AL3G27060, AL3G09190; *Carica papaya*: CP0046G00220, CP00954G00030; *Fragaria vesca*: FV2G39910; *Glycine max*: GM13G22660; *Malus domestica*: MD15G006380; *Manihot esculenta:* ME00847G01640, ME05884G00130; *Medicago truncatula*: MT4G114900; *Populus trichocarpa*: PT06G25470, PT18G12870; *Ricinus communis*: RC29848G01750, RC29927G00230; *Theobroma cacao*: TC0009G12440; *Vitis vinifera*: VV04G12200, VV11G06640.




**Supplemental Figure 7. Cellular Phenotype of *kix8-kix9* double mutant at 14 DAS**. (A) Leaf (first pair) area, cell area and cell number of 14 day old *kix8-kix9* mutants compared to wild type (n=3; error bars represent ±SE *: significant difference from the wild type at p-value<0.05). (B) The frequency of cells in function of their area is represented. Eac h bin represents a cell area of 25 $\mu m^2$. Fifteen leaves were analyzed for the cell-related parameters measurements.




**Supplemental Figure 8. Expression of *PPD1* and *PPD2* in the *ami-ppd* line and expression of *KIX8* and *KIX9* in the T-DNA Insertion Lines for *KIX8* (GABI_422H04) and *KIX9* (SAIL_1168_G09), and in the double mutant *kix8-kix9*.**

Expression of *PPD1* and *PPD2* in the *ami-ppd,* analysed by qRT-PCR. (B) KIX8 and KIX9 gene structure (grey box: UTR, white box: exon, black line: intron) encoding for proteins of 343 and 238 amino acids, respectively (black box) and the location of the T-DNA insertions are indicated. Expression of *KIX8* and *KIX9* in *kix8*, *kix9* and *kix8-kix9* mutants, analyzed by qRT-PCR.

## Supplemental Methods

### Leaf Growth Parameter Analysis

For the rosette leaf area measurements, 8-12 seedlings were grown in soil or under *in vitro* conditions for 21 d. Individual leaves (cotyledons and rosette leaves) were dissected and their area was measured with the ImageJ software (http://rsb.info.nih.gov/ij/).

For the leaf area analysis over time, leaves were harvested daily from 8-10 plants grown *in vitro*. The leaves were cleared with 100 % ethanol, mounted in lactic acid on microscopic slides, and photographed. The leaf area was determined with the ImageJ software.

Abaxial epidermal cells (40-100 cells) were drawn for 3-5 leaves with a DMLB microscope (Leica) fitted with a drawing tube and a differential interference contrast objective. Photographs of leaves and drawings were used to measure the leaf area with the ImageJ software and to calculate the average cell area as previously described (Andriankaja et al., 2012). Leaf and cell areas were subsequently used to calculate cell numbers.

Dental resin imprints (Kagan et al. 1992) were taken daily from the abaxial surface of the first leaves from day 13 to day 15. Nail polish copies prepared from the dental resin imprints were analyzed by scanning electron microscopy. The fate of meristemoid cells was analyzed by evaluating whether, over time, a meristemoid became a guard mother cell, a stoma, whether it divided asymmetrically or whether it did not change.

### Gene expression Analysis

Total RNA from three biological repeats was extracted with an RNeasy plant mini kit (Qiagen) from the first leaf pair.

For genome-wide expression analysis, RNA from leaves harvested at 13 DAS were hybridized to the ATH1 array (Affymetrix). Expression data were analyzed as previously described (Gonzalez et al. 2010). The online tool CORNET (De Bodt et al. 2010; De Bodt et al. 2012) was used to perform co-expression analysis, with the differentially expressed genes identified by using predefined sets of microarray expression data. Four predefined subsets of microarray expression data corresponding to four types of experiments (experiments in which leaf tissues are sampled (leaf), hormone treatment series (hormone), microarray experiments oriented toward growth, development and cell cycle studies (compendium1) or microarray experiments for which very similar experiments were removed (compendium2)) were used.

For qRT-PCR analysis, total RNA was extracted from the first leaf pair harvested at 11 to 16 DAS from wild-type and *ami-ppd*, at 11, 13 and 15 DAS from *kix8*, *kix9* and *kix8-kix9* plants, or at 11 DAS from *35S-PPD2-GR* plants, 2, 4, 8 and 24 hours after transfer to medium supplemented or not with 5 µM of dexamethasone (DEX). For cDNA synthesis, 500 ng or 1 µg of RNA was used for the iScript cDNA Synthesis Kit (Bio-Rad) according to the instructions of the manufacturer. Relative expression levels were determined with the Light-Cycler 480 Real-Time SYBR green PCR System (Roche). The data were first normalized to the expression level of the housekeeping genes (AT5G44200 (GAGCATCTACAACGGTTTACATTGG, TAGGCCGGTCATCGAGAATAGTC) and AT3G48750 (ATTGCGTATTGCCACTCTCATAGG,

TCCTGACAGGGATACCGAATGC)) for each RNA sample. The primer sequences used in the qRT-PCR experiments are listed here (AGI code (forward primer, reverse primer)): AT5G59540 (TCACGTGAAGCCATCGTAGA, GCATCACGCGAGAAGAATGA), AT5G67110 (GAGACTCTTCTCGCTGCTCC, GCTGTGGAAAAGCACATTCCT), AT2G37630 (GTCCAGGGGGACTTGTGTTA, ACACTCCACAAGCTCTGACA), AT1G23870 (CGAAGACATGTTTGAGAGCA, GATACTCTGGCTTCGAGCTG), AT5G67260 (GTTGGAGCACAATCCAAGCA, AGTGGAGACATTCCAGGAGC), AT3G50070 (GATTTTCAAGTGGAAGAAGCAAG, CGAAAAACGAGATTGGAGTCA), AT5G54510 (ACACTAGCTATGCGGACACA, AGGGAGGAATTGGCGTGTTA), AT5G49700 (CGGAAGAAGAGCAAAAACAC, GTAGGGGCCCAAATAACATC), At2g20875 (ACCAACATCCTCCCATCCAAGTC, ACAAGACGGCATGGAGAACACG), At1g34245 (AGCTCAAACGCACCACAAGAAG, CACGTTTACACGGCGAACATGC), At5g07180 (AACCCCACAGTGGTTTGTTCA, CTTAAAAACCTGCCCCTCCGA), AT1G14900 (ACCAGATCCAGATGCTCCTC, CCAGATCCGGTAATGACCTT), AT5G20740 (CAGATGAGCAATGCACAGAC, CACGTGTCTCATCTAGCTGGT), AT5G43020 (AACAGAGACCGACGATGTGG, ACACTCTTCCATCACAGCCG), At3g06120 (AACGTCGAAAGACCCTAAACCG, TGGATGGCTCGATTGTCTGGTG), AT4g31805 (TTCGATACACCGCACCAGTT, CACCAAACAGGGGTAGTGGC), AT4G14713 (GACTATTTCTCCCAGAAGCCC, TACCGTTTTCAGGCAAATCA), AT3G54990 (GAGCCATGAAGTTTGGTGCC, CCCATCGTGGACCGATTGAT), At5g53210 (TCCTTCACCGCCTGTTCTAAGC, TGAATCTGGTGGTGGTTGATGCG), At1g80080 (AACAGTCTTCGGGTCCTTCACC, TTCACGTCCCGGAACTCCAAAG, AT4G14720 (CAAATGCGGTGGTTGGGCA, TTCAGGTGGTACTCCATCATA, AT3G24150 (TGACCGGTATGAGCTCAAGT, GAGTTCGATGTACGAGTCGC and ATGCCGAGGCCAGGACCA, TGCTTCCATTGCGAGTCTGA), AT4G32295 GCATTACCTTTGGTGGGTGT, TTGAGTAGGAGGTCCAAGAGGA)).

Transient expression assays were performed as described previously (Cuéllar Pérez et al., 2014). For investigating transcriptional repression potential, protoplasts were co-transfected with a reporter plasmid containing the firefly luciferase (*fLUC*) reporter gene driven by a promoter containing GAL4-binding sites, a normalization construct expressing Renilla luciferase (rLUC) under the control of the *35S* promoter, and a p2GAL4DBGW6 effector construct (Pauwels et al., 2010). For transient activation assays, promoters (*pCYCD3;2* and *pCYCD3;3*) were cloned with fLUC in pm42GW7 by MultiSite Gateway LR reaction and co-transfected with p2GW7 overexpression effector constructs (PPD2, KIX8 and/orKIX9). Protoplasts were prepared from a BY-2 tobacco cell culture. For each experiment, 2 µg of each plasmid were used. After transfection, protoplasts were incubated overnight in the dark, at room temperature and with gentle agitation. The next day, protoplasts were lysed, and fLUC and rLUC activities were determined with the Dual-Luciferase reporter assay system (Promega). Variations in transfection efficiency and technical error were corrected by normalization of fLUC by rLUC activities. All transactivation assays were conducted in an automated experimental set-up (Vanden Bossche et al., 2013).

**TChAP-Sequencing**

TChAP from cell suspension cultures was done according to Verkest et al. (2014).

For the TChAP-sequencing, *35S-PPD2-HBH* and wild-type PSB-D TChAP DNA libraries were prepared according to the protocol of Illumina and sequenced on a Genome II Analyzer (www.illumina.com/sequencing/). The quality of the raw data was evaluated with FASTQC (v0.10.0; http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/), and adaptors and other overrepresented sequences were removed using the fastx-toolkit (v0.0.13; http://hannonlab.cshl.edu/fastx_toolkit/). The reads were mapped to the unmasked TAIR10 reference genome of Arabidopsis (TAIR10_chr_all.fas; ftp.arabidopsis.org) using BWA with default settings for all parameters (v0.5.9; Li and Durbin (2009)). Reads that could not be assigned to a unique position in the genome were removed using samtools (v0.1.18; Li et al. (2009)) by setting the mapping quality threshold (-q) to 1. Redundant reads were removed, retaining only one read per start position, using Picard tools (v1.56; http://picard.sourceforge.net). Peak calling was performed using MACS (v2.0.10, Zhang et al. (2008); default parameters except –g 1.0e8 and FDR < 0.05).

Peak regions were annotated based on the location of their summits. A peak was assigned to the closest gene as annotated in the TAIR10 release represented in the PLAZA2.5 database (Van Bel et al. 2012); peaks can be assigned both 5' and 3' of a gene. Each assignment is considered as a potential TF-target interaction. The peak locations were categorized by assigning a peak to one of the following genomic regions: intergenic, 1-kb promoter (1 kb upstream of Transcription Start Site), 5' UTR, coding, intron, 3' UTR, and 1 kb downstream of the Transcription Stop Site. Visualiation of the location of the peaks along the genome was done by using the GenomeView representation tool (Abeel et al. 2012).

The sequences of the complete peak regions were masked for coding sequence and submitted to the Peak-Motifs algorithm using default settings (Thomas-Chollier et al. 2012). Motifs that could be aligned with a correlation score ≥ 75 % were collapsed. For each returned DNA motif, enrichment was defined as the ratio of the peak set frequency over the frequency in 1,000 random sets of peaks of the same size and length distribution sampled without replacement from the complete non-coding genome space (intergenic + UTR). The motifs from Peak-Motifs were mapped using matrix-scan (Turatsinze et al. 2008) using the same parameters as used by Peak-Motifs.

**ChIP-qPCR**

Chromatin affinity purification, done according to Kaufmann et al. (2010), was performed by using *Arabidopsis* transgenic plants overexpressing a GFP-tagged PPD2 and 35S-GFP transgenic plants as control. 0.8 g of plant tissue was used from 21 day old *in vitro*-grown *Arabidopsis* seedlings. Briefly, after plant material fixation in 1% (v/v) formaldehyde, tissues were homogenized, nuclei isolated, and lysed. Following sonication (15 s on/45 s off pulses; 3 cycles) of the crosslinked chromatin with the Bioruptor UCD-200 (Diagenode), a preclearing step was performed. The DNA-protein complexes were immunoprecipitated with anti-GFP antibody (Abcam) for 1 h at 4°C while gentle shaking and subsequently incubated for 50 min at 4°C with Protein-A agarose beads (Santa Cruz). The immunoprecipitated DNA was then reverse crosslinked with Proteinase K and recovered using the Qiaquick PCR purification Kit (Qiagen) and analyzed by qPCR.

Chromatin affinity purification, done according to a protocol adapted from Morohashi et al. (2012) was performed by using cell cultures overexpressing a GSyellow-tagged PPD2 and a 35S-GSyellow cell culture as control. This TAP tag is a derivative of the TAP tag GSrhino (Van Leene et al., 2015), replacing the IgG binding domains in the latter by YFP. Briefly, crosslinking was done by adding 0,75% formaldehyde to the cell culture and quench reaction was done by adding 250mM Glycine for 10 minutes. After washing with ice-cold PBS, material was stored at -80. 1g of material was ground in N2, and nuclei isolated according to the protocol of Sigma Aldrich using the plant nuclei isolation/extraction kit. Nuclear-enriched extracts were resuspended in 1,6 mL lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% deoxycholate, 0.1% SDS, 1 mM phenylmethylsulfonyl fluoride, and 10 mM sodium butyrate) and plant proteinase inhibitor cocktail (Sigma-Aldrich), followed by sonication with a Bioruptor (Diangenode) to ~200-400 bp of average fragment size, estimated by agarose electrophoresis. Precipitation was carried out by addition of ab290 anti-GFP (abcam) and Protein A Dynabeads (Magnetic).

The qPCR was performed with the Roche LightCycler 480 system and the B-R SYBRGreen SuperMix for iQ (Quanta Biosciences). Measurements were taken for the input (untreated chromatin), and the purified DNA for five or four biological repeats and three technical repeats. The data were normalized against two reference genes and converted into relative expression values. Significant DNA enrichment was determined for five PPD2 target genes by comparing the obtained values of the ChIP and input DNA samples and of the values obtained for PPD2 compared to the negative control sample (*35S-GFP* or *35S-GS$_{yellow}$* ), using an ANOVA test.

The primer sequences used in the qPCR experiments are listed here (AGI code (forward primer, reverse primer)): AT3G48750 (ATTGCGTATTGCCACTCTCATAGG, TCCTGACAGGGATACCGAATGC), AT1G13440 (ATTCCCAGCAGCACTGGAGC, TCAACGGTTGGGACACGGAA), AT5G67260 (GACCACAGAATCTGCAAAGC, CATATTTCTACATACGGTATG), AT3G50070 (AGTATCTTTACTCGCATCCAACG, ACTCACGTGCTTATTGAATGTGT), AT3G54990 (CTTCGCGTCGCAATGGTATC, TGGAAAAGGGTTGTCCTTTGG), AT5G67110 (TCGTCGGAAGAAGATGGAGG, GCGCTTCTCTGAAGAACGTG) and AT4G14720 (GAGAGAGCAGTGGCGTTTTC, TAGGATGCTTACGTGGCAGT).

**Protein-protein interaction assays**

Tandem affinity purification experiments were done by using *Arabidopsis* cell cultures overexpressing tagged PPD2 and KIX8. The bait ORFs (*PPD2* and *KIX8*) were cloned for CaMV 35S promoter-driven expression and C-terminal fusion to the GS-TAP tag (Bürckstümmer et al. 2006) in entry clones that were subsequently recombined with the pKCTAP destination vectors (Van Leene et al. 2008) by Multisite Gateway LR reactions. *Arabidopsis* cell suspension cultures (PSB-D) were transformed without callus selection as previously described (Van Leene et al. 2007). Tandem affinity purification of protein

complexes was done using the GS tag followed by a downscaled purification protocol as described (Van Leene et al. 2014). TAP eluates were analyzed on an LTQ Orbitrap Velos. Only proteins with at least two matched high confident peptides were retained. To obtain the final list of interactors, background proteins were filtered out based on frequency of occurrence of the co-purified proteins in a large dataset of 543 TAP experiments using 115 different bait proteins (Van Leene et al. 2014).

The web tools Plaza (Van Bel et al. 2012; Proost et al. 2014) and WoLF PSORT (Horton et al. 2007) were used for comparative genomics and organelle protein localisation analysis of the KIX proteins identified by TAP.

Yeast two- and three-hybrid assays were performed as described by Cuéllar Pérez et al. (2014). The ORFs of the proteins of interest were cloned into pGADT7 and pGBKT7 (or pGBT9) yeast two-hybrid vectors, by Gateway recombination reactions, generating bait and prey constructs. The *Saccharomyces cerevisiae* PJ69-4A yeast strain was co-transformed with bait and prey expressed from pGADT7 and pGBKT7 vectors. Transformants in yeast two-hybrid (Y2H) assays were selected on SD media lacking Leu and Trp (-2). Several independent colonies for each bait-prey combination were grown overnight in liquid (-2) media. The next day, 10- and 100-fold dilutions of the cultures were dropped on control (-2) and selective (-3) media, the latter additionally lacking His. Cotransformation with the empty pGADT7 and pGBKT7 (or pGBT9) vectors was used as negative controls. In yeast three-hybrid (Y3H) assays, the MultiSite pMG426 vector was used for expression of the third protein of interest, driven by the GDP promoter and C-terminally fused to the SV40 NLS-3xFLAG-6xHis tag (Nagels Durand et al. 2012Nagels Durand et al. 2012). The assay was performed as previously described for Y2H assays with modifications in the SD media used. For transformant selection and culturing in control media, SD media lacking Leu, Trp and Ura was used (-3), whereas selective media additionally lacked His (-4). Both in Y2H and Y3H assays, plates were allowed to grow for 2 days at 30°C, and interaction was scored in terms of growth on selective media.

## Sequence Alignment

The sequence alignment of the KIX proteins (Supplemental Data Set 3) was done using MUSCLE (**MU**ltiple **S**equence **C**omparison by **L**og- **E**xpectation, http://www.ebi.ac.uk/Tools/msa/muscle/) with default settings in JalView 2.8.2

**Supplemental references**

Abeel T, Van Parys T, Saeys Y, Galagan J, Van de Peer Y. 2012. GenomeView: a next-generation genome browser. *Nucleic Acids Res* **40**: e12.

Bürckstümmer T, Bennett KL, Preradovic A, Schütze G, Hantschel O, Superti-Furga G, Bauch A. 2006. An efficient tandem affinity purification procedure for interaction proteomics in mammalian cells. *Nat Methods* **3**: 1013-1019.

Cuéllar Pérez A, Nagels Durand A, Vanden Bossche R, De Clercq R, Persiau G, Van Wees SCM, Pieterse CMJ, Gevaert K, De Jaeger G, Goossens A et al. 2014. The non-JAZ TIFY protein TIFY8 from *Arabidopsis thaliana i*s a transcriptional repressor. *PLoS ONE* **9**: e84891.

De Bodt S, Carvajal D, Hollunder J, Van den Cruyce J, Movahedi S, Inzé D. 2010. CORNET: a user-friendly tool for data mining and integration. *Plant Physiol* **152**: 1167-1179.

De Bodt S, Hollunder J, Nelissen H, Meulemeester N, Inzé D. 2012. CORNET 2.0: integrating plant coexpression, protein-protein interactions, regulatory interactions, gene associations and functional annotations. *New Phytol* **195**: 707-720.

Gonzalez N, De Bodt S, Sulpice R, Jikumaru Y, Chae E, Dhondt S, Van Daele T, De Milde L, Weigel D, Kamiya Y et al. 2010. Increased leaf size: different means to an end. *Plant Physiol* **153**: 1261-1279.

Horton P, Park K-J, Obayashi T, Fujita N, Harada H, Adams-Collier CJ, Nakai K. 2007. WoLF PSORT: protein localization predictor. *Nucleic Acids Res* **35**: W585-587.

Kagan ML, Novoplansky N, Sachs T. 1992. Variable cell lineages form the functional pea epidermis. *Ann Bot* **69**: 303-312.

Kaufmann, K., Muiño, J.M., Østerås, M., Farinelli, L., Krajewski, P., and Angenent, G.C. (2010). Chromatin immunoprecipitation (ChIP) of plant transcription factors followed by sequencing (ChIP-SEQ) or hybridization to whole genome arrays (ChIP-CHIP). Nat. Protoc. 5: 457-472.

Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**: 1754-1760.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**: 2078-2079.

Morohashi, K., Casas, M.I., Falcone Ferreyra , L., Mejía-Guerra, M.K., Pourcel, L., Yilmaz, A., Feller, A., Carvalho, B., Emiliani, J., Rodriguez, E., Pellegrinet, S., McMullen, M., Casati, P., Grotewolda, E. (2012) A Genome-Wide Regulatory Framework Identifies Maize Pericarp Color1 Controlled Genes. Plant Cell 24: 2745–2764.

Nagels Durand A, Moses T, De Clercq R, Goossens A, Pauwels L. 2012. A MultiSite Gateway™ vector set for the functional analysis of genes in the model *Saccharomyces cerevisiae*. *BMC Mol Biol* **13**: 30.

Proost S, Van Bel M, Vaneechoutte D, Van de Peer Y, Inzé D, Mueller-Roeber B, Vandepoele K. 2015. PLAZA 3.0: an access point for plant comparative genomics *Nucleic Acids Res* **43** 974-81

Thomas-Chollier M, Herrmann C, Defrance M, Sand O, Thieffry D, van Helden J. 2012. RSAT peak-motifs: motif analysis in full-size ChIP-seq datasets. *Nucleic Acids Res* **40**: e31.

Turatsinze J-V, Thomas-Chollier M, Defrance M, van Helden J. 2008. Using RSAT to scan genome sequences for transcription factor binding sites and *cis*-regulatory modules. *Nat Protoc* **3**: 1578-1588.

Van Bel M, Proost S, Wischnitzki E, Movahedi S, Scheerlinck C, Van de Peer Y, Vandepoele K. 2012. Dissecting plant genomes with the PLAZA comparative genomics platform. *Plant Physiol* **158**: 590-600.

Van Leene, J., Eeckhout, D., Cannoot, B., De Winne, N., Persiau, G., Van De Slijke, E., Vercruysse, L., Dedecker, M., Verkest, A., Vandepoele, K., Martens, L., Witters, E., Gevaert, K., De Jaeger, G. (2015). An improved toolbox to unravel the plant cellular machinery by tandem affinity purification of Arabidopsis protein complexes. Nat. Protoc. 10: 169-87

Van Leene J, Stals H, Eeckhout D, Persiau G, Van De Slijke E, Van Isterdael G, De Clercq A, Bonnet E, Laukens K, Remmerie N et al. 2007. A tandem affinity purification-based technology platform to study the cell cycle interactome in *Arabidopsis thaliana*. *Mol Cell Proteomics* **6**: 1226-1238.

Van Leene J, Witters E, Inzé D, De Jaeger G. 2008. Boosting tandem affinity purification of plant protein complexes. *Trends Plant Sci* **13**: 517-520.

Verkest A, Abeel T, Heyndrickx KS, Van Leene J, Lanz C, Van De Slijke E, De Winne N, Eeckhout D, Persiau G, Van Breusegem F et al. 2014. A generic tool for transcription factor target gene discovery in Arabidopsis cell suspension cultures based on tandem chromatin affinity purification. *Plant Physiol* **164**: 1122-1133.

Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, Nusbaum C, Myers RM, Brown M, Li W et al. 2008. Model-based analysis of ChIP-Seq (MACS). *Genome Biol* **9**: R137.