Supplementary Information

**PPP1, a plant-specific regulator of transcription controls Arabidopsis development and PIN expression**

René Benjamins¹²⁺, Elke Barbez¹, Martina Ortbauer¹, Inez Terpstra³, Doris Lucysyn¹†, Jeanette Moulinier-Anzola¹‡, Muhammad Asaf Khan¹⁻, Johannes Leitner¹, Nenad Malenica¹#, Haroon Butt¹, Barbara Korbei¹, Ben Scheres², Jürgen Kleine-Vehn¹ and Christian Luschnig¹∗

1) Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna (BOKU), Muthgasse 18, 1190 Wien, Austria
2) Plant Developmental Biology, Wageningen University Research, 6708 PB Wageningen, The Netherlands
3) Swammerdam Institute for Life Sciences, Faculty of Science, University of Amsterdam, 1090 GE Amsterdam, The Netherlands

§) present address: Syngenta Seeds B.V., Westeinde 62, 1601 BK Enkhuizen, The Netherlands

$) present address: Gregor Mendel Institute of Molecular Plant Biology, Dr. Bohr-Gasse 3, 1030 Vienna, Austria

#) present address: University of Zagreb, Faculty of Science, Department of Molecular Biology, Horvatovac 102a, 10000 Zagreb, Croatia

°) present address: Department of Bioinformatics and Biotechnology (BNB), Government College University, Faisalabad (GCUF), Allama Iqbal Road, Faisalabad, 38000, Pakistan.

†) equal contribution

Running title: PIN transcriptional control

*) for correspondence: rene.benjamins@syngenta.com
christian.luschnig@boku.ac.at
**Supplementary Fig. 1. PPP1 domain prediction and expression in yeast.**

(a) Residue plot of PPP1 amino acid sequence showing the compactness (black) and secondary structure (red) values on a per residue basis. Negative values indicate extended conformations (beta-sheets), whereas positive values are indicative of alpha-helical structure elements. The residue-specific compactness value represents a quantitative parameter describing the structural complexity of an individual residue in the context of 3-D protein fold. Large values are found for residues located in stable parts of the protein, small values are found for flexible loop regions or unfolded segments of the polypeptide chain. Calculations of meta-structural parameters are based on statistical distribution functions of 3-D atomic coordinates extracted from PDB.

(b) Residue secondary structure of PPP1 (residues 575-615) displaying a potential helix-turn-helix (HTH) structure (GGFRRIALMMNLSLAYKHRPKGYWDNLENLQEEIGRFQQS). As a sequence motif, the HTH is poorly conserved. Alpha helix 1 typically starts with a basic amino acid residue (F-RR) and has a terminal basic residue (Y-KHRKPG). Turns typically are rich in Lysines (KHRKPKGYW). Basic amino acid residues (G-R-FQQ) were identified at the C-terminal end of alpha helix 2.

(c) Y-1-H analysis performed with AD-PPP1 and a PIN1 promoter fragment fused to HIS3. Dilution series of these yeast cells were plated on complete SC medium (SC+His) and on SC lacking histidine supplemented with 20 mM 3-AT (SC-His/3-AT).

(d) Activation of a GAL-promoter driven ADE2 marker upon expression of AD-PPP1 in yeast strain Y187. “GAD424”: empty vector control. “AD-PPP1”: Y187 expressing PPP1 fused to the GAL4 activation domain. Right panel is a 10-fold dilution of the sample on the left. Incubation was at 22°C on SC-medium prepared with 1/10th of adenine found in standard SC-medium (SC-Ade).
Supplementary Fig. 2. Root growth of eir1-4 PIN2p::PIN2:VENUS and eir1-4 PIN2pm::PIN2:VENUS. (a-c) eir1-4 PIN2p::PIN2:VENUS controls (a) were compared to eir1-4 PIN2pm::PIN2:VENUS seedlings exhibiting weak (b) or strong (c) root tropism defects. Left: representative seedlings at 5 DAG. Right: Orientation of root growth, when germinated on vertically oriented agar plates. (n = number roots analyzed). Bars: a-c = 3 mm.
Supplementary Fig. 3. GFP signals in transgenic *Arabidopsis* stably expressing 35S::*GFP:PPP1* (green). (a) Portion of a root meristem. (b) Root meristem epidermis cells. Red signals: propidium iodide staining to visualize cell walls. Bars: a = 20 μm; b = 10 μm.
Supplementary Fig. 4. Complementation analysis of ppp1 alleles. (a) Comparison of wild type ("wt"), ppp1-476 and ppp1-476 transformed with a genomic copy of PPP1 (ppp1-476/genPPP1) at 18 DAG. (b) Genotyping performed with genomic DNA of wild type (PPP1), wild type homozygote for cPPP1 (full-length PPP1 cDNA fused to a PPP1 promoter fragment; wt/cPPP1), ppp1-411 (homozygous) and ppp1-411 homozygous for cPPP1 (ppp1-411/cPPP1). All ppp1-411/cPPP1 lines tested (n = 6) are indistinguishable from wild type and produce viable progeny, whereas homozygous ppp1-411 exhibits growth arrest at early developmental stages (n = 90 individuals tested). Arrows to the left indicate migration of a wild type genomic PPP1 fragment (PPP1; upper band) and of a PPP1 cDNA fragment (cPPP1; lower band), amplified by PCR.
Supplementary Fig. 5. PIN2 expression and analysis of directional root growth defects in ppp1 loss-of-function lines. (a,b) Expression of PIN2::PIN2:VENUS (yellow signal) in Col-0 wild type (a) and ppp1-476 (b) root meristems at 8 DAG. (c) Semi-quantitative RT-PCR performed with cDNA isolated from Col-0 wild type seedlings ("WT") and three independent ami-ppp1 silencer lines ("1,2,3"). An ACTIN-specific PCR performed with these cDNAs (ACT) and a negative control lacking cDNA ("-"") are displayed. (d-g) Representative seedlings of wild type (d) and of the three different ami-ppp1 lines analyzed in "c" (e-g) after growth on vertically oriented plates at 6 DAG. Bars: a,b = 50 μm; d-g = 2.5 mm.
**Supplementary Table 1.** Primer combinations used for qRT-PCR analysis.

| Gene | Forward primer | Reverse primer |
|------|----------------|---------------|
| PIN1 | 5'-TACTCCGAGACCTCCAACCTACG-3' | 5'-TCCACCGCCACCACCTCC-3' |
| PIN2 | 5'-ATTTCCTCCTCACGACAACCTC-3' | 5'-GAGACAAAGGCCAAAGCA-3' |
| PIN3 | 5'-GAGGGAGAGGAAGGAAAGGAAAAC-3' | 5'-CTTGGCTTTGAATGTTGGGACATCAG-3' |
| PIN4 | 5'-GGAACCTCTGTGCCACGTTTG-3' | 5'-ACTATCCTGTAGGCAACGCAG-3' |
| PIN7 | 5'-GTCCGGATTAGACATTCCCTTTACCC-3' | 5'-TCAAGGCAGTTGCAAAAAGAGATTCG-3' |
| PILS2 | 5'-GTGAT GTTGTACCTGATG-3' | 5'-AAGTTGACATTCCATGCTGAG-3' |
| PILS3 | 5'-AGGCGACCATGGAAGGGTTG-3' | 5'-GGTGTAACGTAGTACAGATGAGAG-3' |
| PILS4 | 5'-CTTGGGAATAGTGCTTCTGCGGT-3' | 5'-GCACTGACATTCGTCTTGAG-3' |
| AUX1 | 5'-TTACATATTTGGCGCGGC-3' | 5'-GATGGAGGGAAGGTAACT-3' |
| LAX3 | 5'-GATTACCCGTCGCCGTTG-3' | 5'-GGAGCCGGCAAAAGGTAAG-3' |
| ABCB4 | 5'-GGAAGACATTCCATGCTCA-3' | 5'-CCAAGGAACATGGTTCTTCC-3' |
| PPP1 | 5'-AGTCACCACACTTCTCAAAGTATACCC-3' | 5'-CGCATAAATTGGACGGAAGATCTTC-3' |
| Elf4a | 5'-CTGGAGGGTTGGAGGCTGCTAT-3' | 5'-CCAAGGGTAAAGGCAAGGA-3' |
| TUB | 5'-ACTCGTTGGGAGGAGGACT-3' | 5'-ACACCAGACATAGTGCGGAAATCAAG-3' |
| ACT2 | 5'-GCTGGAGATCAGATGCCTCA-3' | 5'-GTGGATTCCAGCATTCAT-3' |