Supplementary Information

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

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Running title: *PIN* transcriptional control

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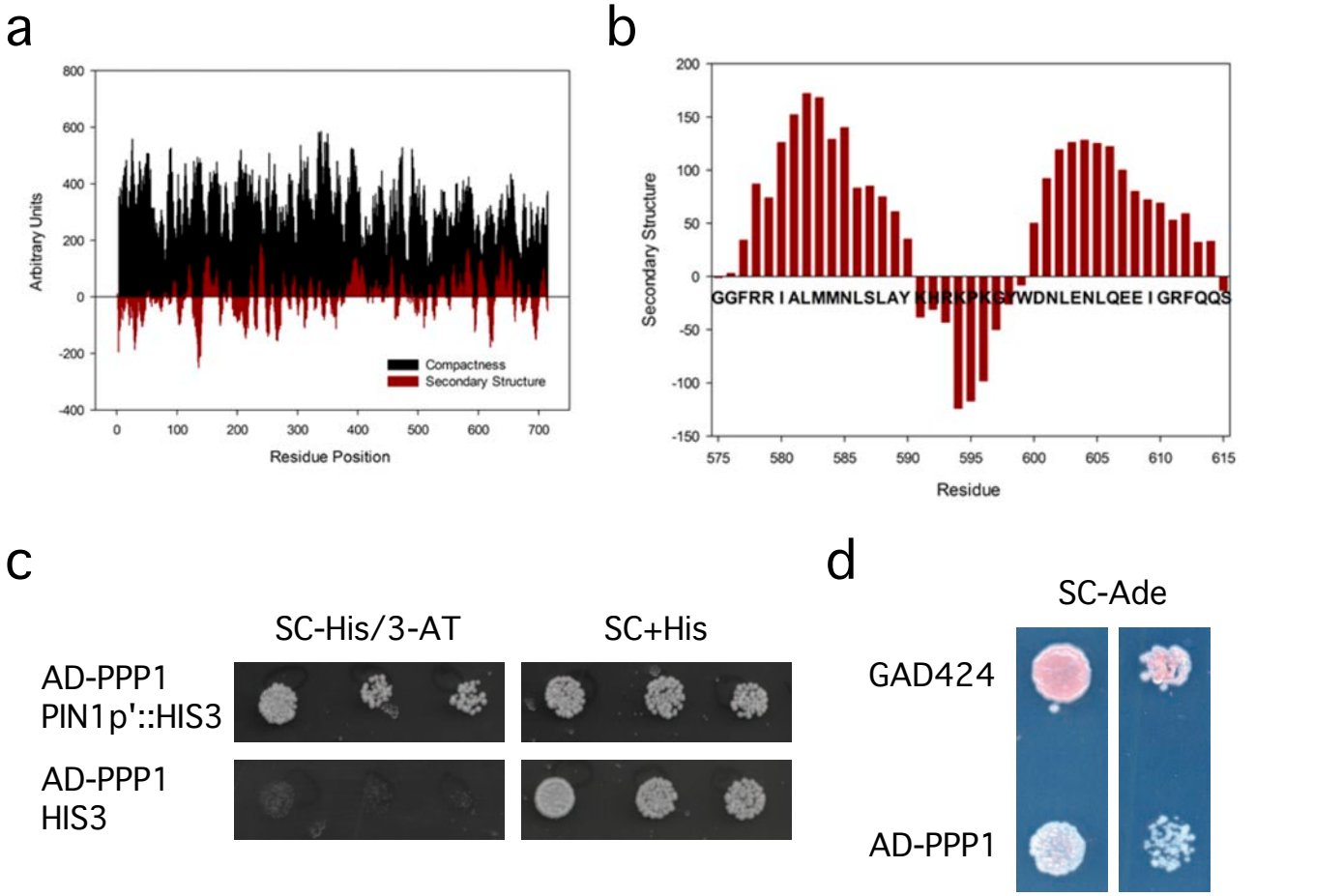
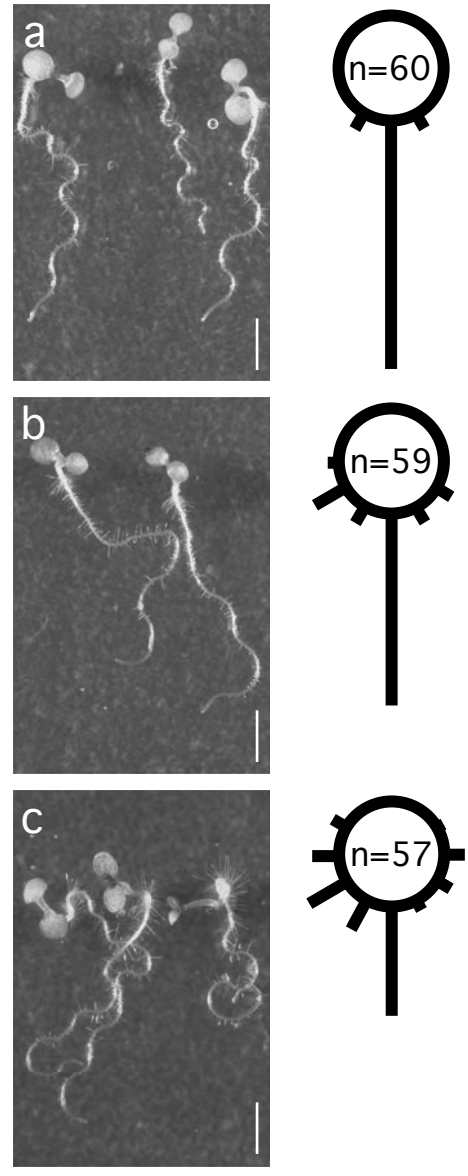
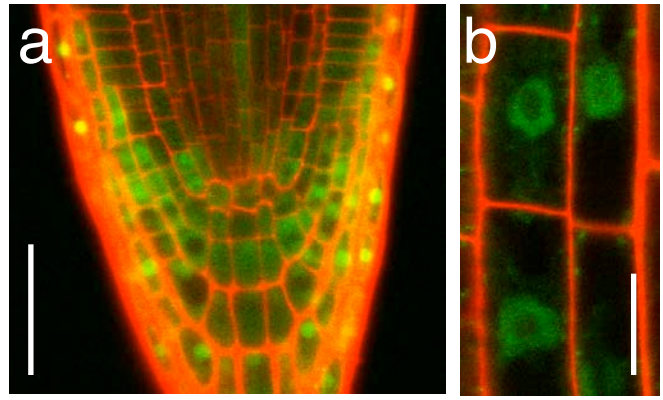


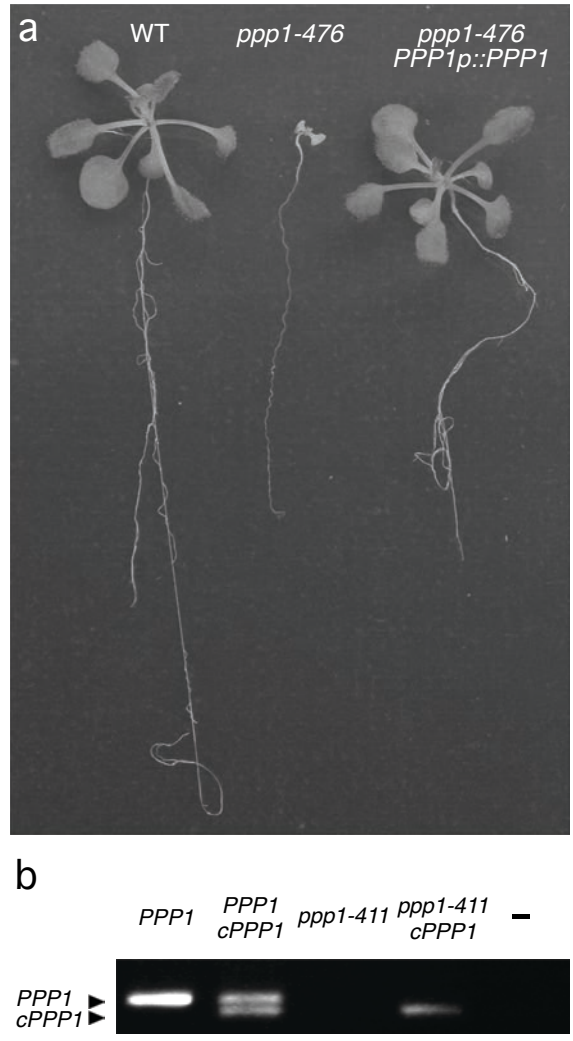
Supplementary Fig. 1. PPP1 domain prediction and expression in yeast. (a) Residue plot of PPP1 amino acid sequence showing the compactness (black) and secondary structure (red) values on a per residue basis. Negative values indicate extended conformations (beta- sheets), whereas positive values are indicative 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 (GGFRRIALMMNLSLAYKHRKPKGYWDNLENLQEEIGRFQQS). 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).



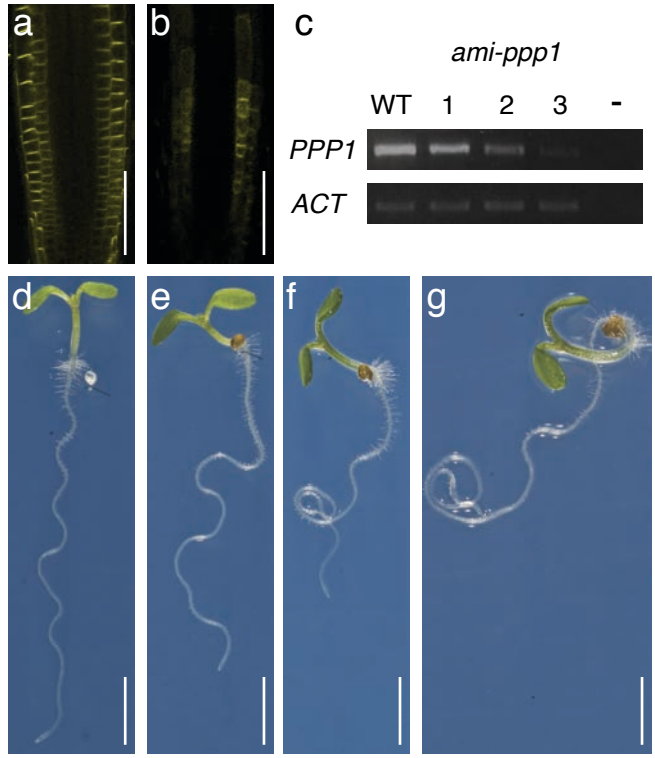
Supplementary Fig. 2. Root growth of *eir1-4 PIN2p::PIN2:VENUS* and *eir1-4 PIN2pm::PIN2:VENUS*. (**a-c**) *eir1-4 PIN2p::PIN2:VENUS* controls (a) were compared to *eir1-4 PIN2pm::PIN2:VENUS* seedlings exhibiting weak (b) or strong (c) root tropism defects. Left: representative seedlings at 5 DAG. Right: 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'-TACTCCGAGACCTTCCAACTACG-3'	5'-TCCACCGCCACCACTTCC-3'
PIN2	5'-ATTCCTCCTCACGACAACCTC-3'	5'-GAGACAAGGGACCAAGCAA-3'
PIN3	5'- GAGGGAGAAGGAAGAAAGGGAAAC -3'	5'- CTTGGCTTGTAATGTTGGCATCAG -3'
PIN4	5'- GGAACTCTGTCGCCACGTTTG -3'	5'- ACTATTCCTTGAGGCAACGCAG -3'
PIN7	5'- GTCCGTTAGGCACTTCCTTTACCC -3'	5'- TCAAGGCGGTGCAAAAGAGATTCG -3'
PILS2	5'-GTGATGCTTGTACTTGGTGGTATG-3'	5'-AACTTGAACATTGGATCTGCTGAG-3'
PILS3	5'- AGGCGACCATGCAAGTGTTG -3'	5'- GTGGTACAGCTAGATGACAGTGAG -3'
PILS5	5'- CTTGGAATAGTCTGTGTTCGGTAC -3'	5'- GCACTGAGCATTCGTCTTGAG -3'
AUX1	5'- TTACATATTTGGCGCGTGTT -3'	5'- GATGGAGGCAATGGCTAAGT -3'
LAX3	5'- GATTACCCGTGGTTGTACCC -3'	5'- GGAGCAGGAGCAAAGGTAAG -3'
ABCB4	5'- GGAGACATTGAGCTTCGTCA -3'	5'- CCAAAGCAACTGTCTTTCCA -3'
PPP1	5'-AGTCACCACACACTTCTCAAGTATACC-3'	5'-CGCATAAATTTGACGGAAGATCTTC-3'
EIF4a	5'-CTGGAGGTTTTGAGGCTGGTAT-3'	5'-CCAAGGGTGAAAGCAAGAAGA-3'
TUB	5'-ACTCGTTGGGAGGAGGAACT-3'	5'-ACACCAGACATAGTAGCAGAAATCAAG-3'
ACT2	5'-GCTGAGAGATTCAGATGCCCA-3'	5'-GTGGATTCCAGCAGCTTCCAT-3'