Title:

Overexpression of a novel *Arabidopsis* PP2C isoform, AtPP2CF1, enhances plant biomass production by increasing inflorescence stem growth

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Supplementary Method S1. PCR templates for plasmid construction.

Arabidopsis genomic DNA and cDNA, and RIKEN Arabidopsis full-length (RAFL) cDNA clones (Seki *et al*., 1998, 2002) were used as templates for PCR to generate plasmid constructs as described below.

Supplementary Method S2. Construction of plasmids for the establishment of transgenic *Arabidopsis* plants.

The two oligonucleotides (5′-AGCTTGGCGCGCCTTAATTAAACTAGTCTCGAGGTCGACT-3′ and 5′-CTAGAGTCGACCTCGAGACTAGTTTAATTAAGGCGCGCCA-3′) were designed to anneal to each other, and inserted into the *Hind*III/*Xba*I sites of the *pBI121* vector (Clontech, Mountain View, CA, USA) to produce *pBI101N2*. To construct plasmids expressing β-glucuronidase (GUS) reporter gene under the control of the *AtPP2CF1* promoter (*pAtPP2CF1:GUS*), a DNA fragment containing the *AtPP2CF1* promoter was obtained by PCR using primers 5′-AGTCGACTGACTCAAAATCACGTTCTTGAA-3′ and 5′-AGGATCCTTTGTCCAGAAAGTGAAAATATC-3′. This PCR fragment was inserted into the *pTOPO blunt* vector (Invitrogen, Carlsbad, CA) to produce *pAtPP2CF1pro*. The *Sal*I-*BamH*I fragment of *pAtPP2CF1pro* was inserted into the *Sal*I-*BamH*I sites of *pBI101N2*.

To construct plasmids expressing GUS reporter gene under the control of the *At3g16800* or *At5g27930* promoter (*pAt3g16800:GUS* and *pAt5g27930:GUS*, respectively), DNA fragments containing the *At3g16800* or *At5g27930* promoter were obtained by PCR. The following primers were used for PCR: *At3g16800* promoter, 5′-GCTATGACCATGATTACGCCAAGCTTAGACTGAATAATATAATGTCGG-3′ and 5′-AAGGGACTGACCACCCGGGGATCCTCTTTGATTCTCTTATGATCCTAC-3′; *At5g27930* promoter, 5′-GCTATGACCATGATTACGCCAAGCTTAAACAAATCACGGGACGGGT-3′ and 5′-AAGGGACTGACCACCCGGGGATCCCTTTTAAATACCCCAAAAGATTAA-3′. These PCR fragments were inserted into the *Hind*III/*BamH*I sites of the *pBI121* vector using the In-Fusion Cloning System (Clontech, Mountain View, CA, USA), according to the manufacturer's instructions.

To construct plasmids expressing *AtPP2CF1* under the control of the 35SΩ promoter (*p35SΩ:AtPP2CF1*), a DNA fragment containing the *AtPP2CF1* genome was obtained by PCR using primers 5′-ACGCGTCGACATGGGACATTTCTCTTCCATGTTCAACGG-3′ and 5′-TGTACATGTACACTATAGAGATGGCGACGACGATGAAGAATGG-3′. This PCR fragment was inserted into the *pCR2.1* vector (Invitrogen, Carlsbad, CA) to produce *pAtPP2CF1*. The *Hind*III-*Eco*RI fragment of *35SΩ:sGFP(S65T)* plasmid (Chiu *et al*., 1996) was inserted into the *Hind*III-*Eco*RI sites of *pBI121* to produce *p35SΩ:sGFP*. The *Sal*I-*BsrG*I fragment of *pAtPP2CF1* was inserted into the *Sal*I/*BsrG*I sites of *p35SΩ:sGFP*.

To construct plasmids expressing *ABI1* under the control of the 35SΩ promoter

(*p35SΩ:ABI1*), a DNA fragment containing the *ABI1* ORF was obtained by PCR using primers 5′-AATTACTATTTACAATTACAGTCGACATGGAGGAAGTATCTCCGGC-3′ and 5′-AGCCGGGCGGCCGCTTTACTTGTACATCAGTTCAAGGGTTTGCTCT-3′. This PCR fragment was inserted into the *Sal*I/*BsrG*I sites of the *p35SΩ:AtPP2CF1* using the In-Fusion Cloning System.

Supplementary Method S3. Construction of plasmids for yeast *ptc1* complementation test.

For *pYC2/CT AtPP2CF1*, a DNA fragment containing the *AtPP2CF1* ORF was obtained by PCR using primers 5′-ACCCCGGATCGGACTACTAGCAGCTGTAATGGGACATTTCTCTTCCATGT-3′ and 5′-CGGCCCTCTAGGATCAGCGGGTTTAAACCTATAGAGATGGCGACGACGATG-3′. This PCR fragment was inserted into the *Pyu*II/*Pme*I sites of the *pYC2/CT* vector (Invitrogen, Carlsbad, CA) using the In-Fusion Cloning System.

For *pYC2/CT ABI1*, a DNA fragment containing the *ABI1* ORF was obtained by PCR using primers 5′-ACCCCGGATCGGACTACTAGCAGCTGTAATGGAGGAAGTATCTCCGGC-3′ and 5′-CGGCCCTCTAGGATCAGCGGGTTTAAACTCAGTTCAAGGGTTTGCTCT-3′. This PCR fragment was inserted into the *Pyu*II/*Pme*I sites of the *pYC2/CT* vector using the In-Fusion cloning system.

For *pYC2/CT PTC1*, a DNA fragment containing the *PTC1* ORF was obtained by PCR from *Saccharomyces cerevisiae* S288C genome using primers 5′-ACCCCGGATCGGACTACTAGCAGCTGTAATGAGTAATCATTCTGAAATCTT-3′ and 5′-CGGCCCTCTAGGATCAGCGGGTTTAAACTTAGAGGAAGACAACCATGAC-3′. This PCR fragment was inserted into the *Pyu*II/*Pme*I sites of the *pYC2/CT* vector using the In-Fusion cloning system.

Supplementary Method S4. Construction of plasmids for purification of AtPP2CF1 and ABI1.

To construct plasmids for recombinant AtPP2CF1 with a glutathione *S*-transferase (GST) tag at the N-terminus (GST-AtPP2CF1), a DNA fragment containing the *AtPP2CF1* ORF was obtained by PCR using primers 5′-TCGGATCTGATCGAAGGTCGTGGGATCCTGATGGGACATTTCTCTTCCATGT-3′ and 5′-AGTCAGTCACGATGCGGCCGCTCGAGCTATAGAGATGGCGACGACG-3′. This PCR fragment was inserted into the *BamH*I/*Xho*I sites of the *pGEX-5X-2* vector (Amersham Biosciences/GE Healthcare, Piscataway, NJ) using the In-Fusion Cloning System to produce *pGEX GST-AtPP2CF1*.

To construct plasmids for recombinant ABI1 with a GST tag at the N-terminus (GST-ABI1), a DNA fragment containing the *ABI1* ORF was obtained by PCR using primers

5′-TCGGATCTGATCGAAGGTCGTGGGATCCTGATGGAGGAAGTATCTCCGGC-3′ and 5′-AGTCAGTCACGATGCGGCCGCTCGAGTCAGTTCAAGGGTTTGCTCT-3′. This PCR fragment was inserted into the *BamH*I/*Xho*I sites of the *pGEX-5X-2* using the In-Fusion cloning system to produce *pGEX GST-ABI1*.

Supplementary Method S5. Yeast two-hybrid (Y2H) assays.

The control plasmids *pGBKT7* (bait plasmid) and *pGADT7 AD* (prey plasmid) for yeast two-hybrid assay were provided with the Matchmaker Gold Two-Hybrid System (Clontech, Mountain View, CA, USA). For *pGBKT7 PYR1, PYL1–13, AtPP2CF1, or ABI1*, DNA fragments containing the *PYR1*, *PYL1-13*, *AtPP2CF1*, or *ABI1* ORF were obtained by PCR. The following primers were used for PCR: *PYR1*, 5′-tgatctcagaggaggacctgcatatgccttcggagttaacacc-3′ and 5′-gcaggtcgacggatccccgggaattctcacgtcacctgagaaccact-3′; *PYL1*, 5′-tgatctcagaggaggacctgcatatggcgaattcagagtcctc-3′ and 5′-gcaggtcgacggatccccgggaattcttacctaacctgagaagagttg-3′; *PYL2*, 5′-tgatctcagaggaggacctgcatatgagctcatccccggccgt-3′ and 5′-gcaggtcgacggatccccgggaattcttattcatcatcatgcatagg-3′; *PYL3*, 5′-tgatctcagaggaggacctgcatatgaatcttgctccaatcca-3′ and 5′-gcaggtcgacggatccccgggaattctcaggtcggagaagccgtgg-3′; *PYL4*, 5′-tgatctcagaggaggacctgcatatgcttgccgttcaccgtcc-3′ and 5′-gcaggtcgacggatccccgggaattctcacagagacatcttcttctt-3′; *PYL5*, 5′-tgatctcagaggaggacctgcatatgaggtcaccggtgcaact-3′ and 5′-gcaggtcgacggatccccgggaattcttattgccggttggtacttcg-3′; *PYL6*, 5′-tgatctcagaggaggacctgcatatgccaacgtcgatacagtt-3′ and 5′-gcaggtcgacggatccccgggaattcttacgagaatttagaagtgtt-3′; *PYL7*, 5′-tgatctcagaggaggacctgcatatggagatgatcggaggaga-3′ and 5′-gcaggtcgacggatccccgggaattctcaaaggttggtttctgtatg-3′; *PYL8*, 5′-tgatctcagaggaggacctgcatatggaagctaacgggattga-3′ and 5′-gcaggtcgacggatccccgggaattcttagactctcgattctgtcgt-3′; *PYL9*, 5′-tgatctcagaggaggacctgcatatgatggacggcgttgaagg-3′ and 5′-gcaggtcgacggatccccgggaattctcactgagtaatgtcctgaga-3′; *PYL10*, 5′-tgatctcagaggaggacctgcatatgaacggtgacgaaacaaa-3′ and 5′-gcaggtcgacggatccccgggaattctcatatcttcttctccataga-3′; *PYL11*, 5′-tgatctcagaggaggacctgcatatggaaacttctcaaaaata-3′ and 5′-gcaggtcgacggatccccgggaattcttacaactttagatgagccac-3′; *PYL12*, 5′-tgatctcagaggaggacctgcatatgaaaacatctcaagaaca-3′ and 5′-gcaggtcgacggatccccgggaattcttaagtgagctccatcatctt-3′; *PYL13*,

5′-tgatctcagaggaggacctgcatatggaaagttctaagcaaaaa-3′ and 5′-gcaggtcgacggatccccgggaattcttacttcatcattttctttgt-3′; *AtPP2CF1*, 5′-tgatctcagaggaggacctgcatatgggacatttctcttccatgt-3′ and 5′-gcaggtcgacggatccccgggaattcctatagagatggcgacgacg-3′; *ABI1*, 5′-tgatctcagaggaggacctgcatatggaggaagtatctccggc-3′ and 5′-gcaggtcgacggatccccgggaattctcagttcaagggtttgctct-3′. These PCR fragments were inserted into the *Nde*I/*EcoR*I sites of the *pGBKT7* using the In-Fusion Cloning System.

For *pGADT7 AD SnRK2.2, SnRK2.3, SnRK2.6, AtPP2CF1, or ABI1*, DNA fragments containing the *SnRK2.2*, *SnRK2.3*, *SnRK2.6*, *AtPP2CF1*, or *ABI1* ORF were obtained by PCR. The following primers were used for PCR: *SnRK2.2*, 5′-acgacgtaccagattacgctcatatggatccggcgactaattc-3′ and 5′-tatcgatgcccacccgggtggaattctcagagagcataaactatctc-3′; *SnRK2.3*, 5′-acgacgtaccagattacgctcatatggatcgagctccggtgac-3′ and 5′-tatcgatgcccacccgggtggaattcttagagagcgtaaactatctc-3′; *SnRK2.6*, 5′-acgacgtaccagattacgctcatatggatcgaccagcagtgag-3′ and 5′-tatcgatgcccacccgggtggaattctcacattgcgtacacaatctc-3′; *AtPP2CF1*, 5′-acgacgtaccagattacgctcatatgggacatttctcttccatgt-3′ and 5′-tatcgatgcccacccgggtggaattcctatagagatggcgacgacgatg-3′; *ABI1*, 5′-acgacgtaccagattacgctcatatggaggaagtatctccggc-3′ and 5′-tatcgatgcccacccgggtggaattctcagttcaagggtttgctct-3′. These PCR fragments were inserted into the *Nde*I/*EcoR*I sites of the *pGADT7 AD* using the In-Fusion Cloning System.

The Y2H assay was performed using the Matchmaker Gold Two-Hybrid System (Clontech, Mountain View, CA, USA), according to the manufacturer's instructions. Yeast cells were grown and maintained in SD or YPD medium. SD medium lacking adenine, His, Leu, and Trp (SD-AHLW) but supplemented with 125 ng ml^{-1} aureobasidin A and 40 ng ml^{-1} X-α-gal (5-bromo-4-chloro-3-indolyl-α-D-galactopyranoside) was used as a high-stringency selective medium for the assay.

Supplementary references

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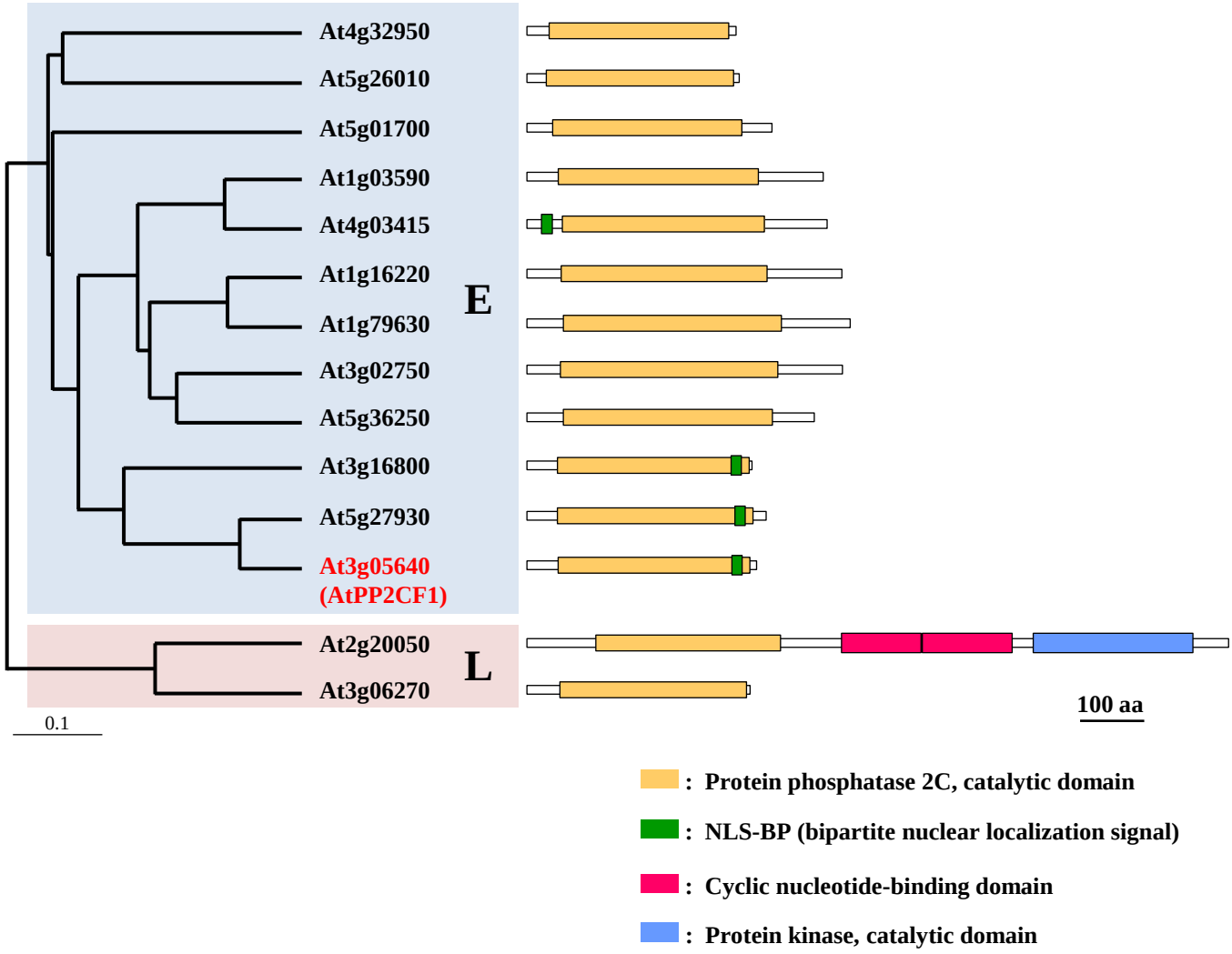
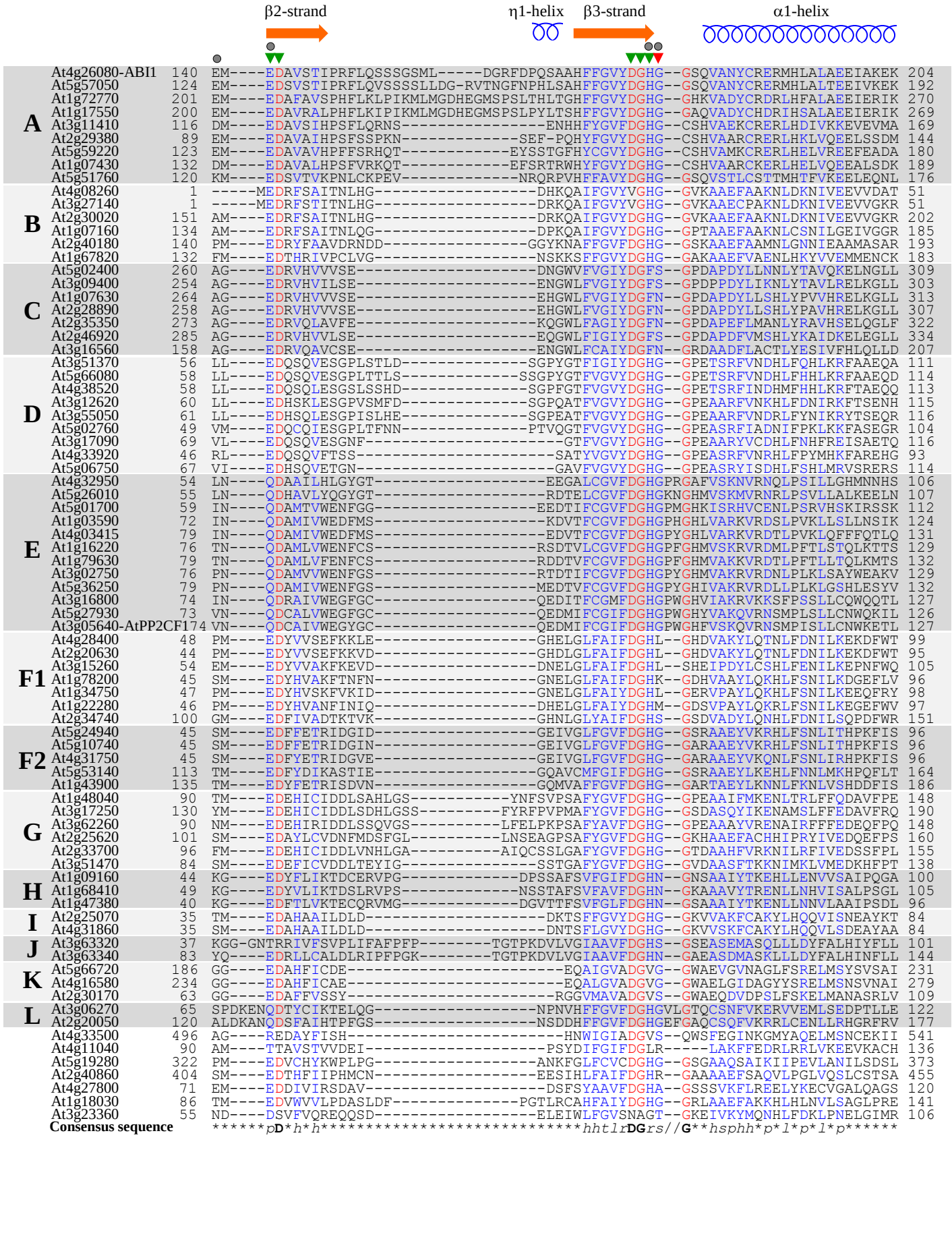
Supplementary Table S1. Gene-specific primers used in RT-qPCR.

At1g03590	5'- tcagggaaaaattgtcttctgc -3'	
	5'- tcatcaagcacagattttgagg -3'	
At1g16220	5'- cttgaagacacttcagcaggag -3'	
	5'- gttgaagcctcttccttcttgt -3'	
At1g79630	5'- atgccgacaaaaacacttcata -3'	
	5'- cagctaacatgaccaatctctca -3'	
At5g01700	5'- agttgcagctgagatgttggtt -3'	
	5'- cacgtttccttctcttgggtaa -3'	
At3g02750	5'- agatttgtgcctggaaagtgaa -3'	
	5'- gacacagtaccttgtcttgggt -3'	
At5g36250	5'- aggagctcaatgtctcctatgc -3'	
	5'- tacaatgagtcacacacgagca -3'	
At5g26010	5'- ctgcatggaagaaaaggcttaa -3'	
	5'- caacagctaagcttggaccata -3'	
At4g32950	5'- gtgatgaagtcagcaagtgagg -3'	
	5'- tggctgtggattatgcttcttg -3'	
At3g16800	5'- atcaatgtattttgggcgtcat -3'	
	5'- tatctctccattctcaatcactcc -3'	
At3g05640	5'- actagtacttttggaccgggca -3'	
(*AtPP2CF1*)	5'- ttgtgataggactctggtttgg -3'	
	5'- gaacccgataaagagctacagcg -3'	(for transgenic plant analysis)
	5'- cttcgtctgaaactgtggccaa -3'	
At5g27930	5'- ggaagaaaaagagacaaggatact -3'	
	5'- catcgctgatctatttacaacac -3'	
At4g03415	5'- atgcagaggatgagaaaacggt -3'	
	5'- attcttcaagatcagtcctcag -3'	
At4g27960	5'- tcacaatttccaaggtgctgc -3'	
(*UBC9*)	5'- tcatctgggtttggatccgt -3'	
At4g26080	5'- tgaatatgggtctctccaagaaa -3'	
(*ABI1*)	5'- tacaaggggctttttagaatgt -3'	
	5'- atccggaagtgacggctgtga -3'	(for transgenic plant analysis)
	5'- tcatccgcgagcaacgatgca -3'	



Supplementary Figure S1. Phylogenetic analysis of *Arabidopsis* PP2Cs belonging to subfamilies E and L. *Arabidopsis* subfamilies E and L PP2Cs constitute a sister clade, forming a monophyletic cluster (Xue *et al*., 2008). For phylogenetic analysis, entire PP2C protein sequences from *Arabidopsis* subfamilies E and L were identified by The Arabidopsis Information Resource (TAIR) database (http://www.arabidopsis.org/) and were aligned with the PROMALS3D program (Pei *et al*., 2008) using two PDB IDs, chain B of 3KDJ and chain A of 3JRQ, as reference 3D structures and secondary structures. The tree was generated using TreeView (Page, 1996). Functional domains were predicted using InterProScan (Zdobnov and Apweiler, 2001) and PROSITE (Falquet *et al*., 2002).

Supplementary Figure S2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η1-helix
β3-strand
α1-helix
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B
C
D
E
F1
F2
G
H
I
J
K
L
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At1g48040 90 TM----EDEHICIDDLSAHLGS----------YNFSVPSAFYGVFDGHG--GPEAAIFMKENLTRLFFQDAVFPE 148
At3g17250 130 YM----EDEHICIDDLSDHLGSS----------FYRFPVPMAFYGVFDGHG--GSDASQYIKENAMSLFFEDAVFRQ 190
At3g62260 90 NM----EDEHIRIDDLSSQVGS----------LFELPKPSAFYAVFDGHG--GPEAAAYVRENAIRFFFEDEQFPQ 148
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At3g51470 84 SM----EDEFICVDDLTEYIG----------SSTGAFYGVFDGHG--GVDAASFTKKNIMKLVMEDKHFPT 138
At1g09160 44 KG----EDYFLIKTDCERVPG----------DPSSAFSVFGIFDGHN--GNSAAIYTKEHLLENVVSAIPQGA 100
At1g68410 49 KG----EDYVLIKTDSLRVPS----------NSSTAFSVFAVFDGHN--GKAAAVYTRENLLNHVISALPSGL 105
At1g47380 40 KG----EDFTLVKTECQRVMG----------DGVTTFSVFGLFDGHN--GSAAAIYTKENLLNNVLAAIPSDL 96
At2g25070 35 TM----EDAHAAILDLD----------DKTSFFGVYDGHG--GKVVAKFCAKYLHQQVISNEAYKT 84
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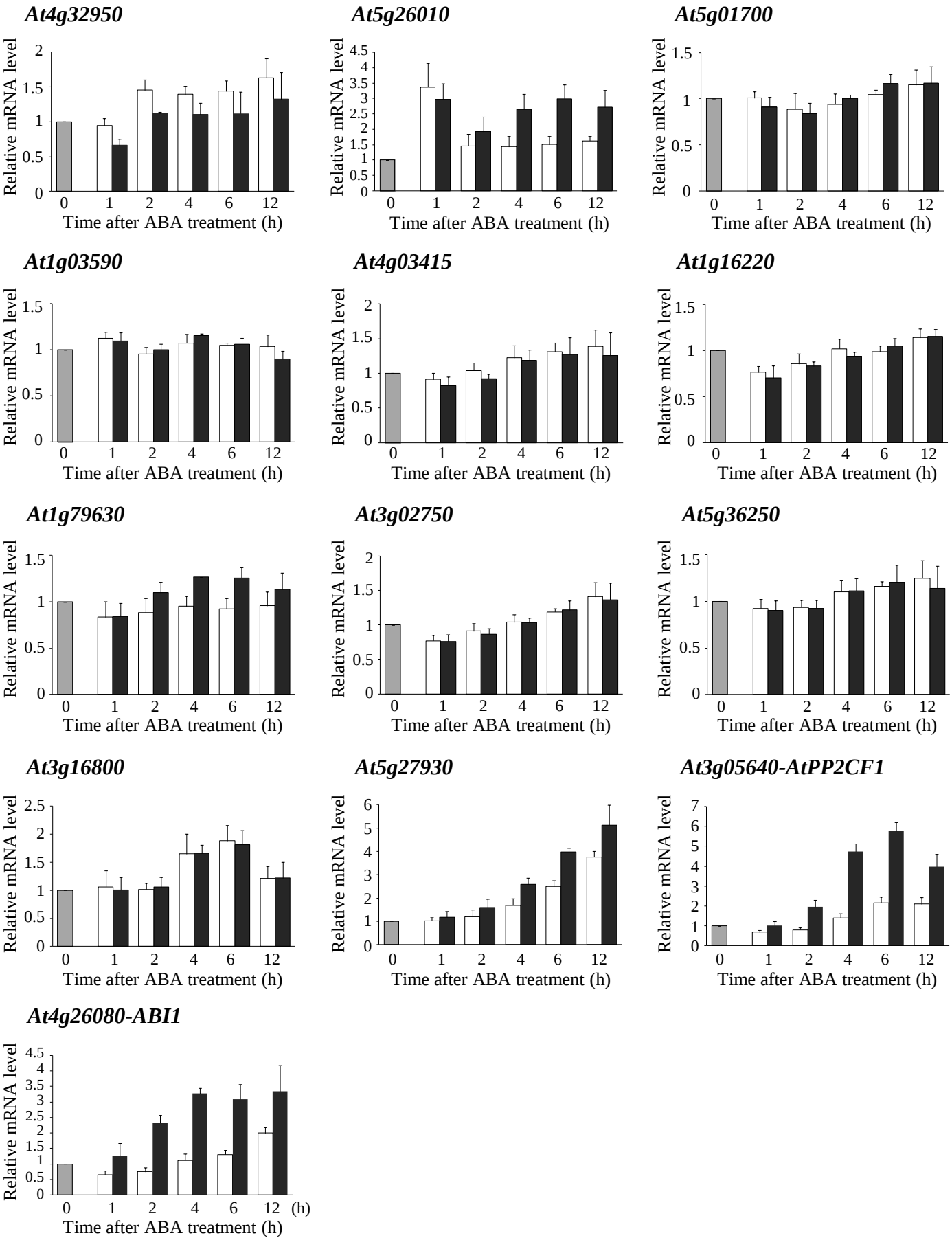
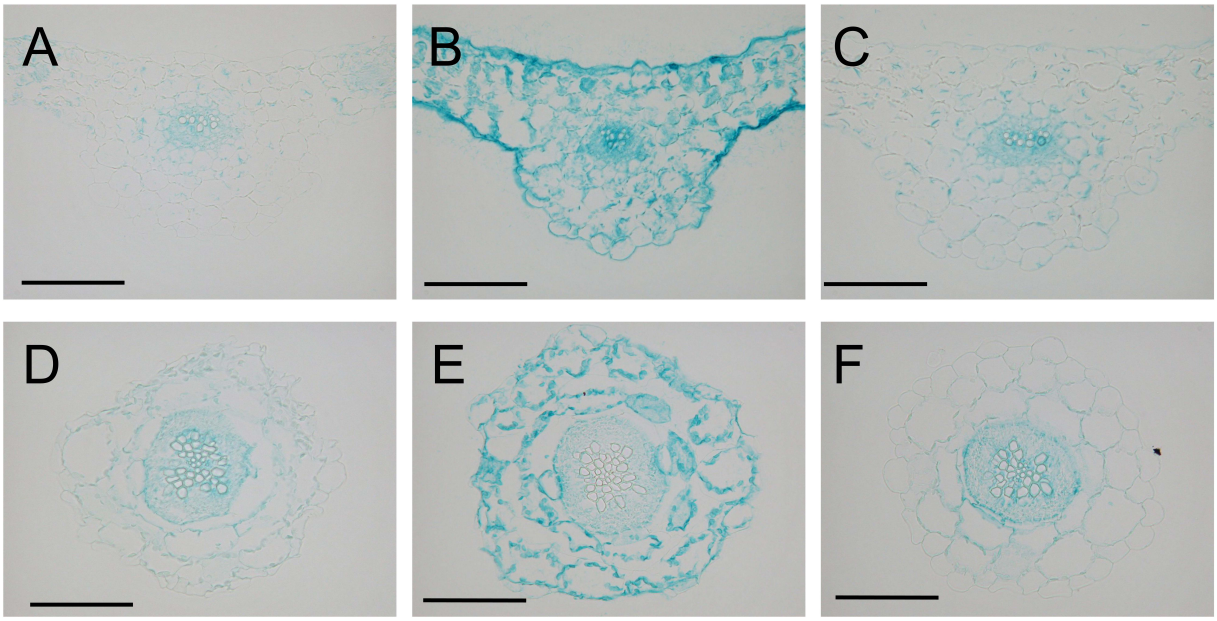
At3g63340 83 YQ----EDRLLCALDLRIPFPGK--------TGTPKDVLVGIAAVFDGHN--GAEASDMASKLLLDYFALHINFLL 144
At5g66720 186 GG----EDAHFICDE----------EQAIGVADGVG--GWAEVGVNAGLFSRELMSYSVSAI 231
At4g16580 234 GG----EDAHFICAE----------EQALGVADGVG--GWAELGIDAGYYSRELMSNSVNAI 279
At2g30170 63 GG----EDAFFVSSY----------RGGVMAVADGVS--GWAEQDVDPSLFSKELMANASRLV 109
At3g06270 65 SPDKENQDTYCIKTELQG----------NPNVHFFGVFDGHGVLGTQCSNFVKERVVEMLSEDPTLLE 122
At2g20050 120 ALDKANQDSFAIHTPFGS----------NSDDHFFGVFDGHGEFGAQCSQFVKRRLCENLLRHGRFRV 177
At4g33500 496 AG----REDAYFISH----------HNWIGIADGVS--QWSFEGINKGMYAQELMSNCEKII 541
At4g11040 90 AM----TTAVSTVVDEI----------PSYDIFGIFDGLR-----LAKFFEDRLRRLVKEEVKACH 136
At5g19280 322 PM----EDVCHYKWPLPG----------ANKFGLFCVCDGHG--GSGAAQSAIKIIPEVLANILSDSL 373
At2g40860 404 SM----EDTHFIIPHMCN----------EESIHLFAIFDGHR--GAAAAEFSAQVLPGLVQSLCSTSA 455
At4g27800 71 EM----EDDIVIRSDAV----------DSFSYAAVFDGHA--GSSSVKFLREELYKECVGALQAGS 120
At1g18030 86 TM----EDVWVVLPDASLDF----------PGTLRCAHFAIYDGHG--GRLAAEFAKKHLHLNVLSAGLPRE 141
At3g23360 55 ND----DSVFVQREQQSD----------ELEIWLFGVSNAGT--GKEIVKYMQNHLFDKLPNELGIMR 106
Consensus sequence ******pD*h*h**********hhtlrDGrs//G**hsphh*p*l*p*l*p******

Supplementary Figure S2. Sequence alignment of conserved consensus motif 4 in all *Arabidopsis* PP2Cs. The secondary structure of ABI1 was superimposed on top of the protein sequence alignment. Green arrowheads and gray circles indicate active-site residues and PYR/PYL/RCAR-binding residues of ABI1, respectively. The red arrowhead indicates the Gly residue converted to Asp in *Arabidopsis abi1-1* and *abi2-1* mutants (Gosti *et al.*, 1999; Merlot *et al.*, 2001; Rodriguez *et al.*, 1998). Entire protein sequences of all *Arabidopsis* PP2Cs were identified by The Arabidopsis Information Resource (TAIR) database (http://www.arabidopsis.org/) and were aligned in PROMALS3D (Pei *et al*., 2008) using two PDB IDs, chain B of 3KDJ and chain A of 3JRQ, as reference 3D structures and secondary structures. Consensus amino acids are represented as given below. Consensus symbols: conserved amino acids are in bold and uppercase letters; aliphatic (I, V, L): *l*; aromatic (Y, H, W, F): *r*; hydrophobic (W, F, Y, M, L, I, V, A, C, T, H): *h*; polar (D, E, H, K, N, Q, R, S, T): *p*; tiny (A, G, C, S): *t*; small (A, G, C, S, V, N, D, T, P): *s*.

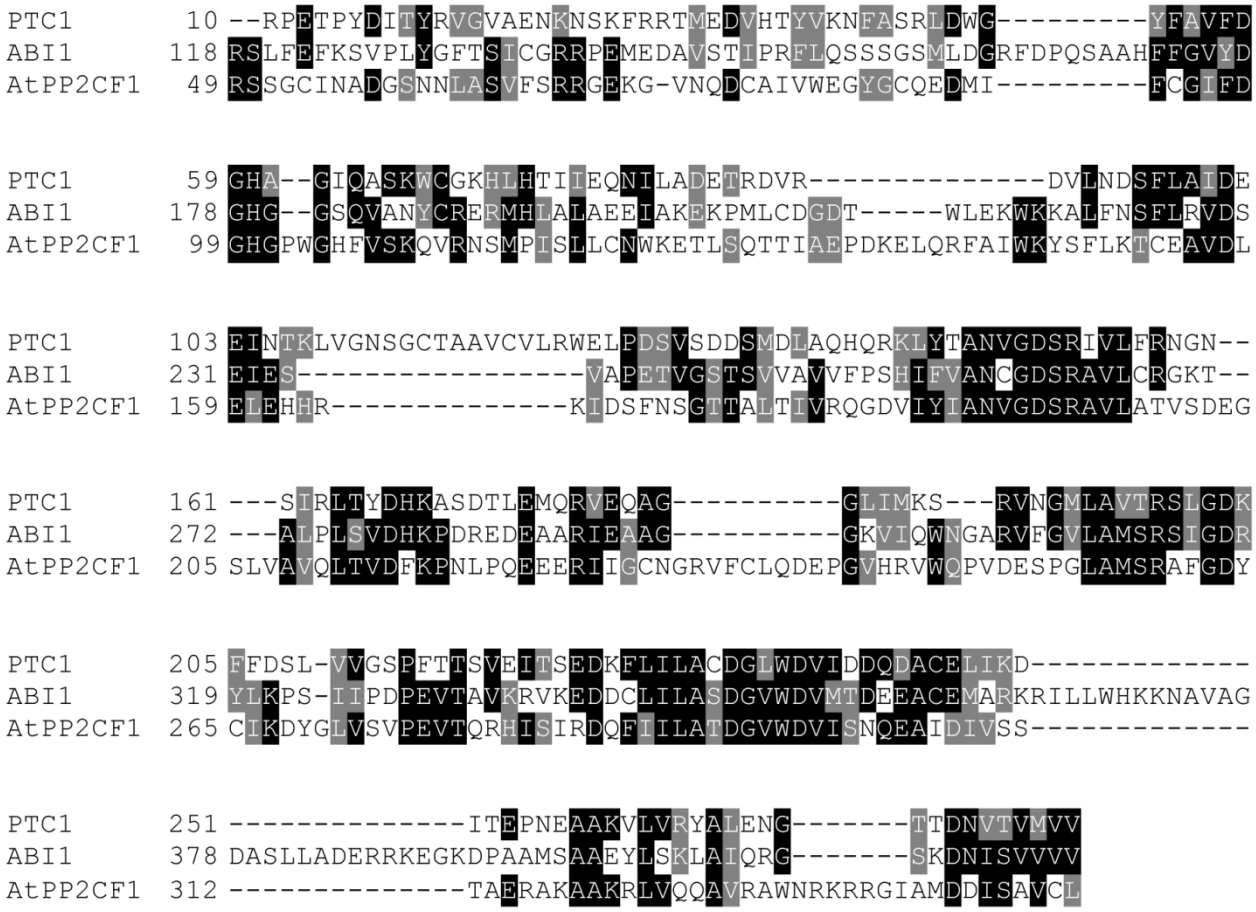
Supplementary Figure S3



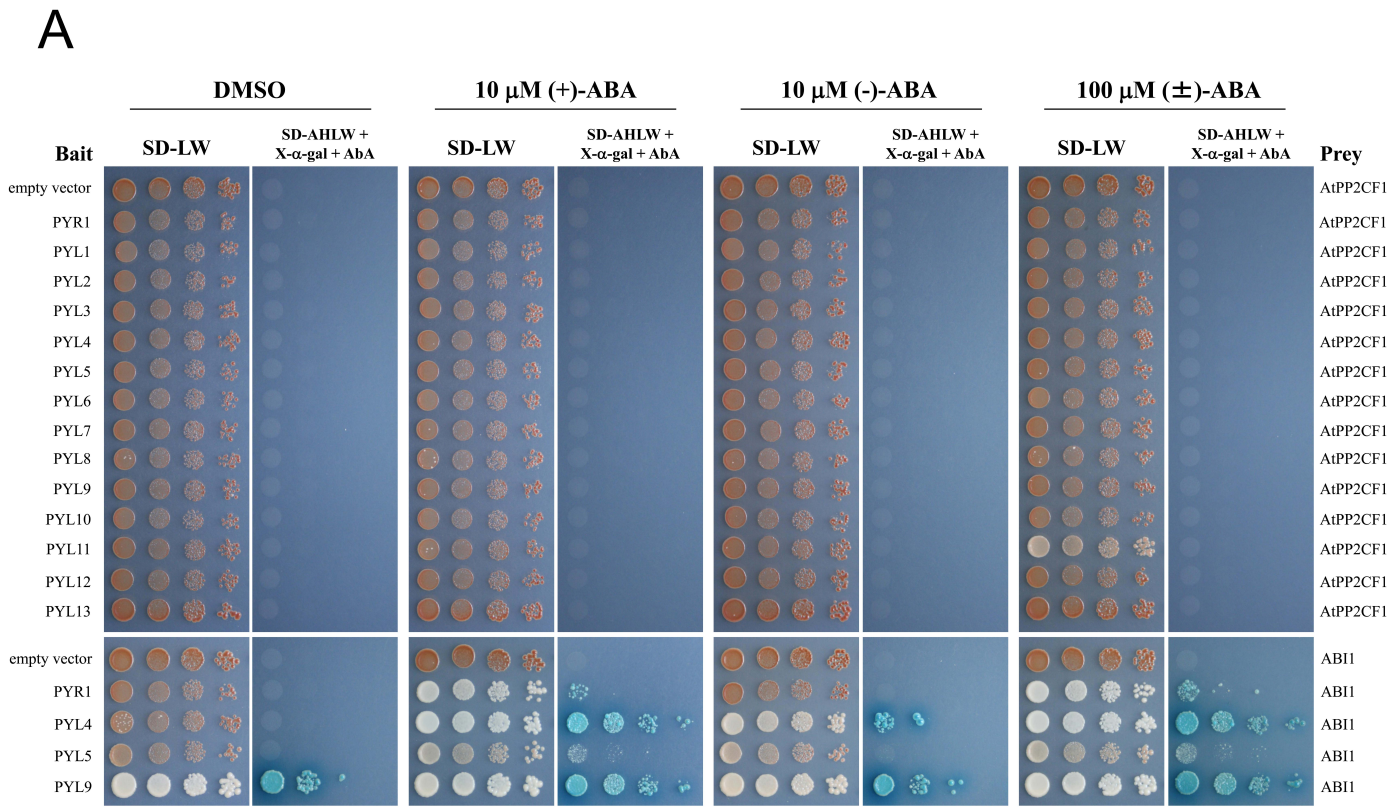
Supplementary Figure S3. Time course of subfamily E PP2C mRNA expression in aerial parts of wild-type *Arabidopsis* plants treated with ABA. Wild-type *Arabidopsis* plants were germinated aseptically on MS medium with gellan gum. Two-week-old seedlings were sprayed with 10 μM ABA or 0.1% DMSO (mock control). Total RNA from aerial parts was isolated at the indicated time points after ABA or mock treatment and subjected to RT-qPCR. The expression ratios of individual subfamily E PP2C genes to *UBC9* were calculated for each time point and for each treatment (ABA, black bars; mock control, white bars). The expression ratios before ABA treatment (0 h) were arbitrarily set to 1.0 and are shown as gray bars. Values represent the mean ± S.D. of three independent experiments.

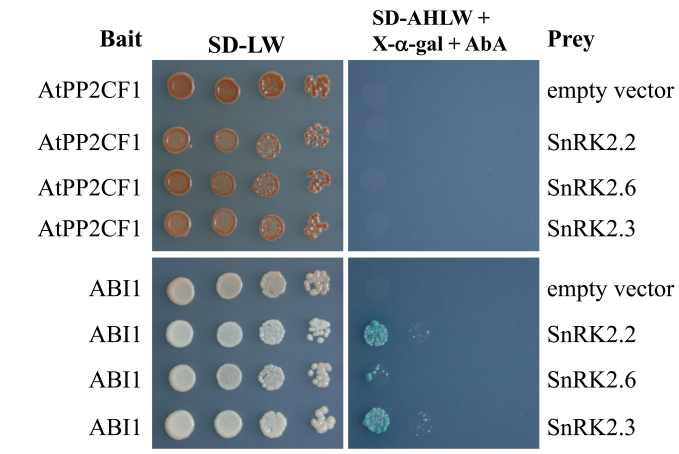


Supplementary Figure S4. Histochemical analysis of GUS activity in transgenic *Arabidopsis* plants *pAtPP2CF1:GUS*, *pAt3g16800:GUS*, and *pAt5g27930:GUS*. GUS-stained tissues were sectioned into 12-μm-thick slices after paraffin embedding (GenoStaff, Inc., Tokyo, Japan). (A–C) Transverse sections of the basal parts of rosette leaves of *pAtPP2CF1:GUS* (A), *pAt5g27930:GUS* (B), and *pAt3g16800:GUS* (C) transgenic plants. (D–F) Transverse sections of hypocotyls of *pAtPP2CF1:GUS* (D), *pAt5g27930:GUS* (E), and *pAt3g16800:GUS* (F) transgenic plants. All scale bars: 100 μm.

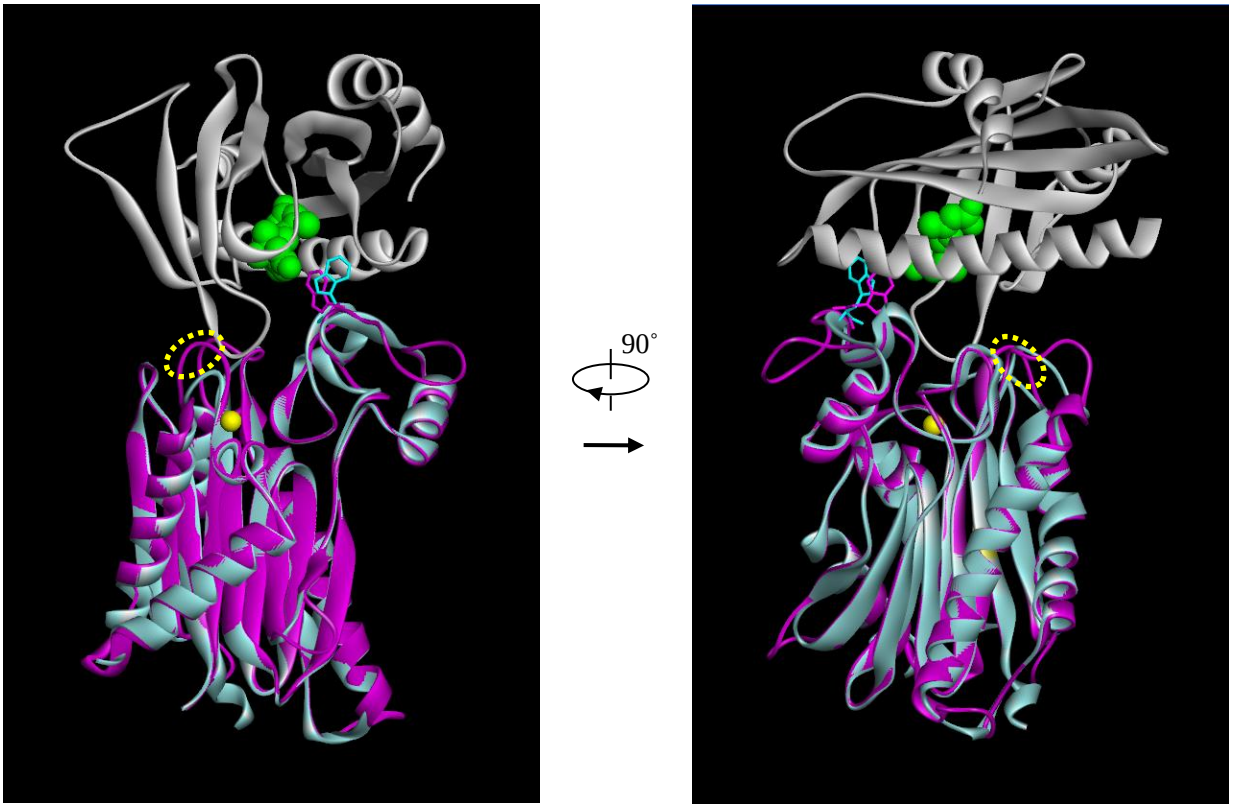


Supplementary Figure S5. Multiple sequence alignments of the predicted phosphatase catalytic domain in AtPP2CF1 with those in PTC1 and ABI1. Amino acid sequence alignment was performed using ClustalX (Thompson *et al*., 1997). Alignments include the phosphatase domains of AtPP2CF1 (residues 49–347) in *Arabidopsis thaliana* (accession No. BAH19512), ABI1 (residues 118–420) in *A. thaliana* (accession No. CAA54383), and PTC1 (residues 10–279) in *Saccharomyces cerevisiae* (accession No. CAA98562). The residues in black boxes are identical in at least two of the three polypeptides, and those in shaded boxes share similarity with conserved residues. Numbers in the left column indicate residue numbers in the polypeptides.

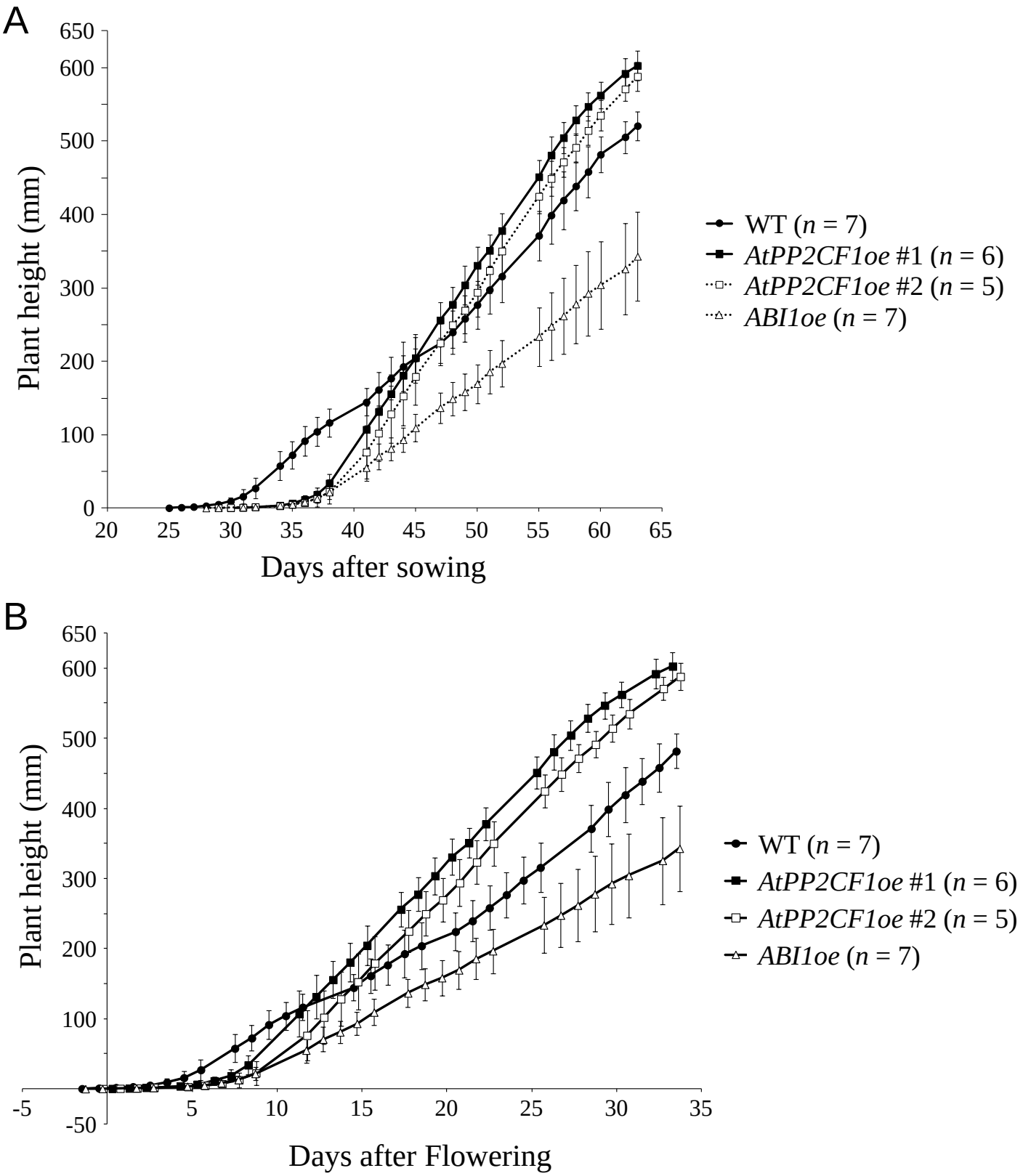




Supplementary Figure S6. AtPP2CF1 did not interact with PYR/PYL/RCAR receptors or with SnRK2 kinases in a Y2H assay. (A) Interaction assay using all PYR/PYL/RCAR receptor proteins as bait (fused to the Gal4 DNA-binding domain, DB) and either AtPP2CF1 or ABI1 as prey (fused to the Gal4 activation domain, AD). ABI1 was used as a positive control. Serial dilutions of exponentially growing yeast cell cultures were spotted on an SD plate lacking Leu and Trp (SD-LW) and an SD-AHLW plate containing aureobasidin A and X-α-gal (SD-AHLW+AbA+X-α-gal), supplemented with 0.01% DMSO (mock control), 10 μM (+)-ABA, 10 μM (-)-ABA or 100 μM (±)-ABA and growth was observed after 5 d at 30° C. (B) Interaction assay using either AtPP2CF1 or ABI1 as bait (fused to DB) and three SnRK2 kinase proteins as prey (fused to AD). ABI1 was used as a positive control. Serial dilutions of exponentially growing yeast cell cultures were spotted on an SD-LW plate and an SD-AHLW+AbA+X-α-gal plate, and growth was observed after 5 d at 30° C.

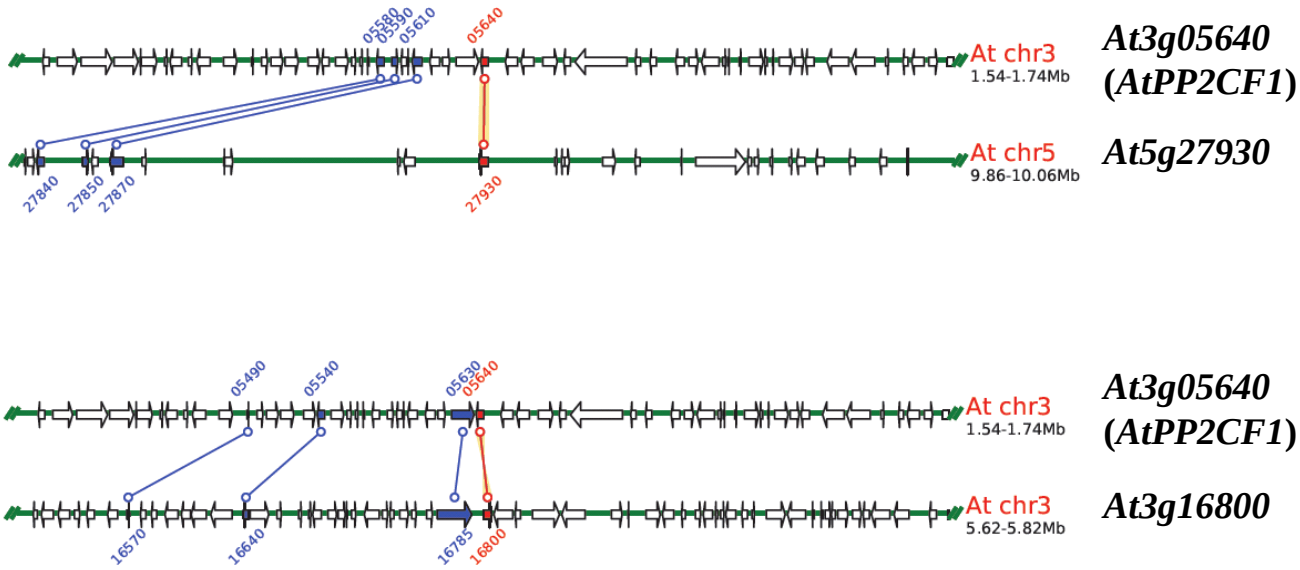


Supplementary Figure S7. Homology-based structural modelling of AtPP2CF1. A ribbon representation of AtPP2CF1 was generated. The hypothetical structure of AtPP2CF1 (magenta) was superimposed with the well-defined crystal structure of ABI1 (blue) in complex with PYL1 (gray) and (+)-ABA (green) (Protein Data Bank code 3kdj). (+)-ABA is shown as a green sphere. At least one metal ion in the putative active site of AtPP2CF1 is shown (yellow sphere). Trp246 of AtPP2CF1 and Trp300 of ABI1 are highlighted in the stick representation. The dotted yellow circle represents the β3-α1 loop of consensus motif 4 (Also see supplementary Fig. S2). Two perpendicular views are shown.



Supplementary Figure S8. Kinematic analysis of the height of wild-type (WT), *AtPP2CF1oe* (#1 and #2), and *ABI1oe* plants. (A) Comparison of plant height according to days after sowing. (B) Comparison of plant height according to days after flowering. This figure was modified from supplementary Fig. S8A. 'Day 0' was defined as the average flowering date after sowing (Also see Fig. 7A). Values represent the mean ± S.D.

Supplementary Figure S9. Safranin-stained epidermises peeled from primary inflorescence stems of wild-type (WT), *AtPP2CF1oe* (#1 and #2) and *ABI1oe* plants. Basal parts (20 mm from the base) of primary inflorescence stems in 9-week-old plants were used for observations. Scale bar: 100 μm.



Supplementary Figure S10. Segmental chromosomal duplication regions of *AtPP2CF1*. Segmental chromosome duplications of *AtPP2CF1* in the *Arabidopsis* genome were identified by the PGDD (Plant Genome Duplication Database) (Lee *et al*., 2012) with a threshold score of 200 or more. Segmental duplication maps display only ± 100 kb regions. Red arrows indicate the *AtPP2CF1*, *At5g27930*, and *At3g16800* loci. Blue arrows indicate the other paralogous gene pairs on each distinct chromosomal region.