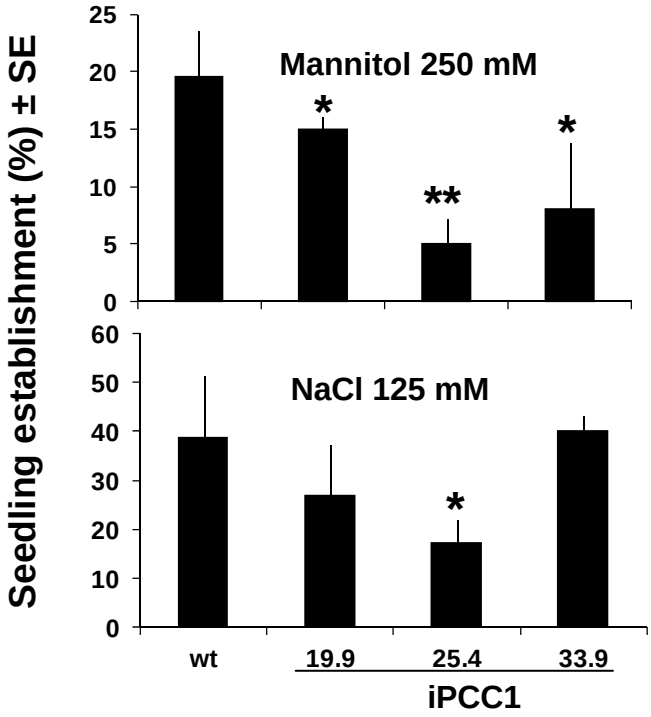


Figure S1. Seedling establishment in osmotic media supplemented with mannitol or NaCl. Values are the mean ± standard error of three replicates in each panel. Between 60 and 80 seeds per genotype were counted in every experiment. * and ** Mean significantly different in iPCC1 seeds when compared to a similar condition for wild type seeds with p-value < 0.05 and < 0.01, respectively, in Student´s t-test.

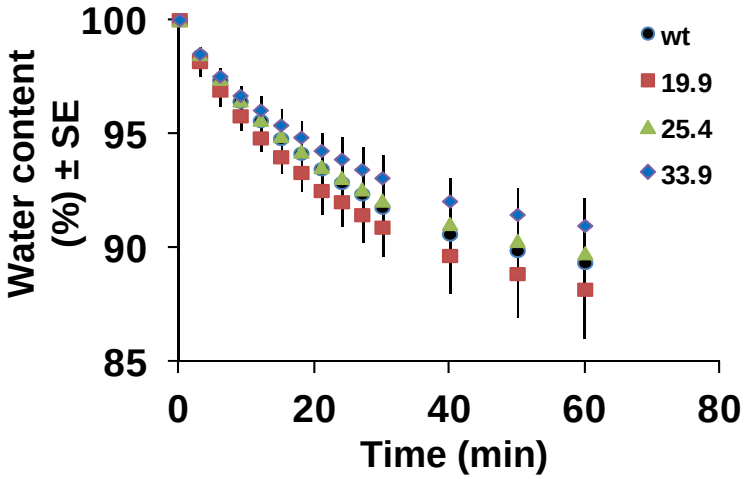


Fig. S2 Transpiration-mediated water loss. Curves show the water content of seedlings of the indicated genotypes left at room temperature for the times indicated after being removed from Petri dishes and softly blotted onto absorbent paper. Sets of five seedlings per genotype were weighed with an analytical balance, and values are the mean ± standard error of three independent experiments.

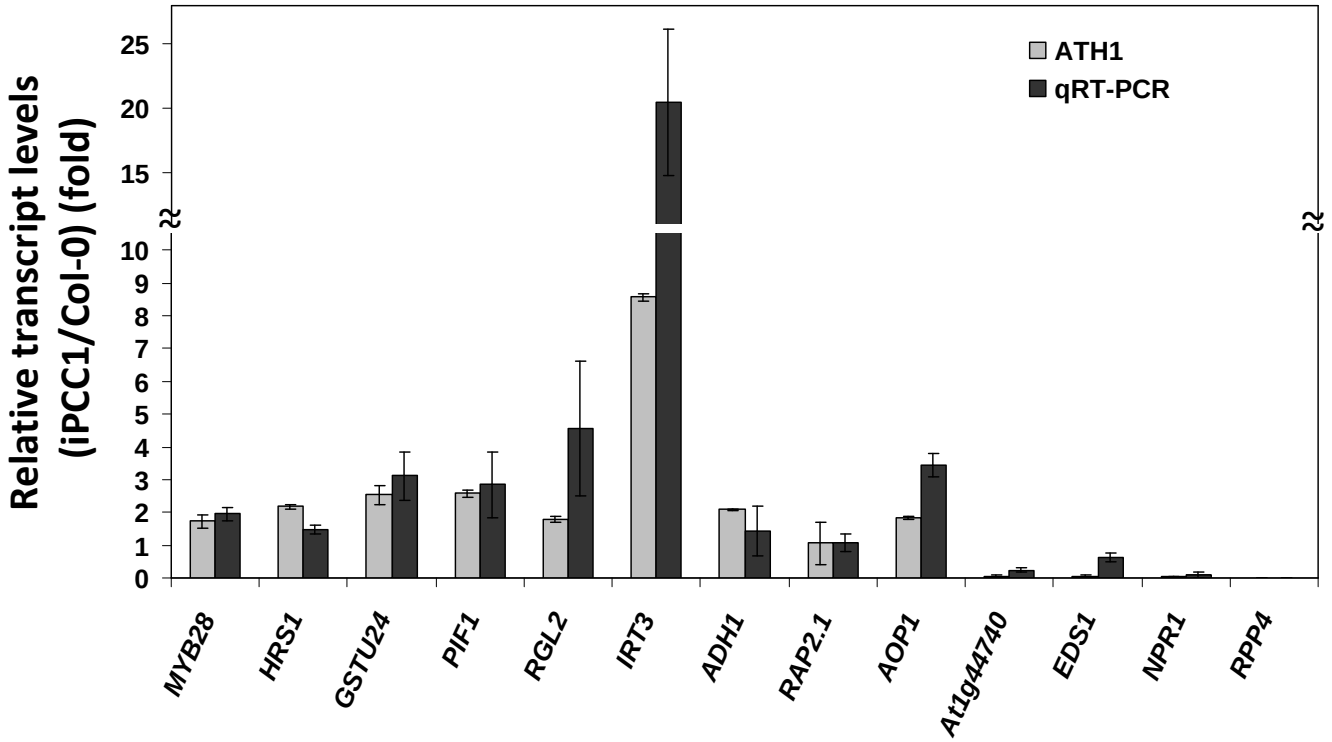


Figure S3. Comparison of transcript levels detected by microarray and qRT-PCR analyses. The ratio of the indicated transcripts in iPCC1/Col-0 obtained from the ATH1 microarray experiment and from independent biological replicates by qRT-PCR with the specific oligonucleotides as shown in Materials and Methods. Values are the mean of three replicates for both techniques ± standard error. Ratio values above 1 corresponded to up-regulated genes and below 1 to down-regulated genes in iPCC1 seedlings.

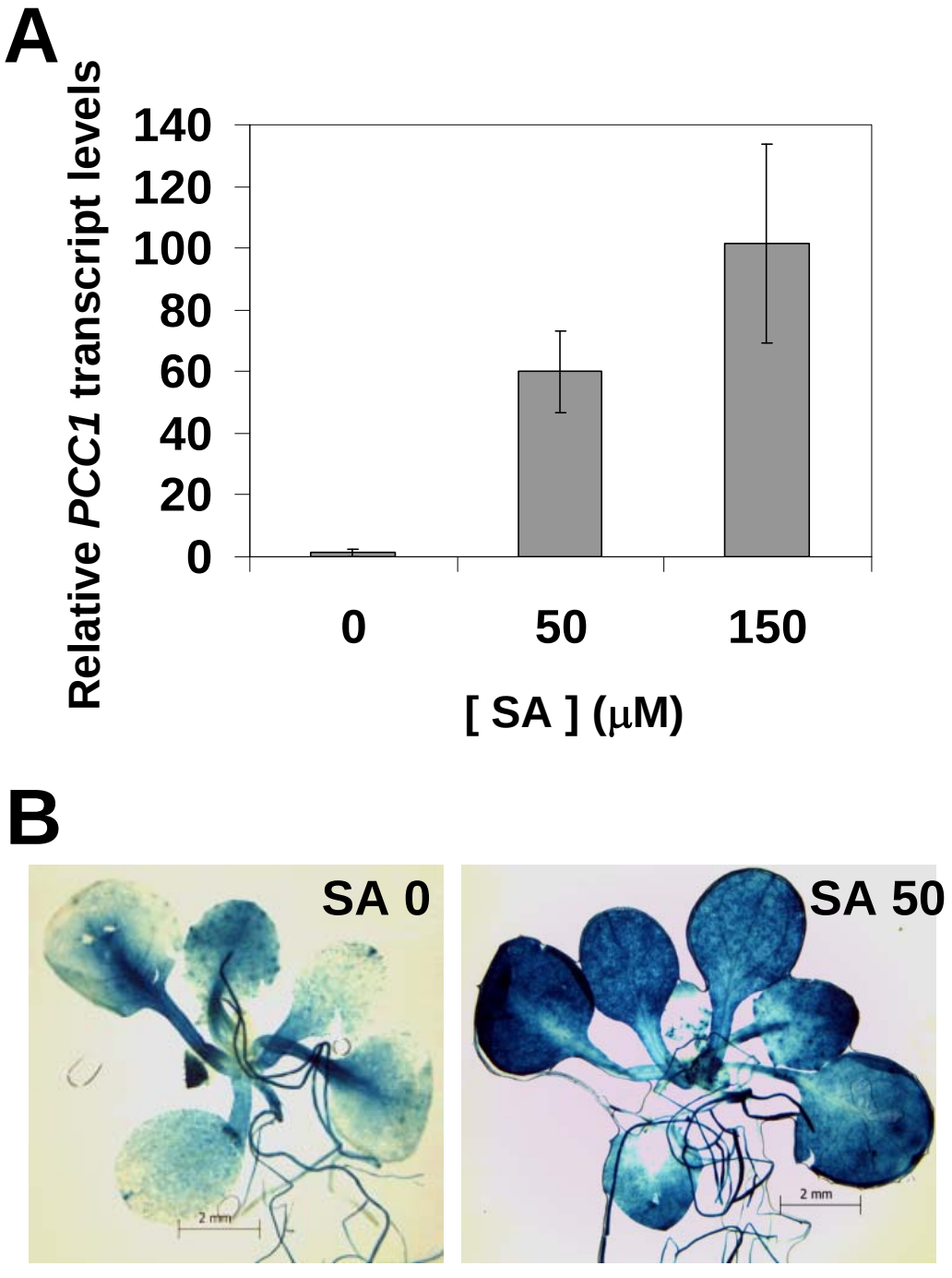
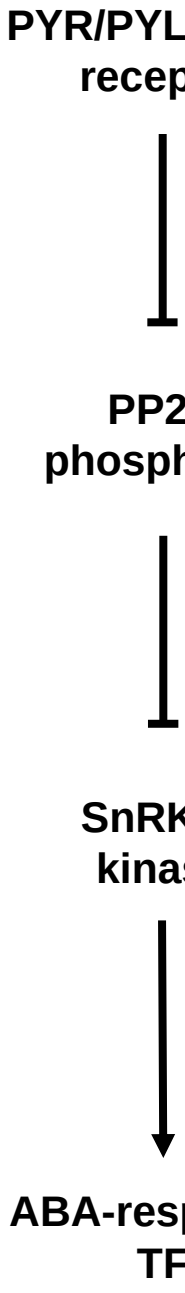


Figure S4. *PCC1* expression is induced by SA. (A) Levels of *PCC1* transcripts were quatified by RT-qPCR from total RNA extracted from Col-0 seedlings treated with the indicated SA concentration. Values are the mean of three biological replicates ± SD and they were normalized with the endogenous *ACTIN2/8* (*ACT2/8*) transcript levels. (B) β-glucuronidase staining of control untreated (left panel) and 50 μM SA treated *pPCC1::GUS* seedlings.



	Receptor	Fold	FDR
At4g17870	PYR1	-1.09	0.6170
At5g46790	PYL1	-1.23	0.3262
At2g26040	PYL2	-1.08	0.5633
At1g73000	PYL3	-1.00	0.9813
At2g38310	PYL4	-1.30	0.4487
At5g05440	PYL5	-1.03	0.9551
At2g40330	PYL6	1.22	0.4077
At4g01026	PYL7	1.12	0.4756
At5g53160	PYL8	1.04	0.9059
At1g01360	PYL9	1.16	0.1841

	Phosphatase	Fold	FDR
At4g26080	ABI1	-1.07	0.7767
At5g57050	ABI2	1.55	0.0431
At1g72770	HAB1	1.94	0.0488
At1g17550	HAB2	1.13	0.4927
At5g59220	HAI1	3.71	0.0174
At1g07430	HAI2	1.65	0.1827
At2g29380	HAI3	1.17	0.2439
At5g51760	AHG1	1.19	0.1796
At3g11410	AHG3	1.46	0.1240

	Kinase	Fold	FDR
At5g08590	SnRK2.1	-1.02	0.8884
At3g50500	SnRK2.2	-1.53	0.0089
At5g66880	SnRK2.3	1.20	0.1252
At1g10940	SnRK2.4	-1.07	0.5659
At5g63650	SnRK2.5	1.06	0.8278
At4g33950	SnRK2.6/OST1	1.24	0.0843
At4g40010	SnRK2.7	1.60	0.038
At1g78290	SnRK2.8	1.11	0.6625
At2g23030	SnRK2.9	-1.52	0.1106
At1g60940	SnRK2.10	-1.04	0.7768

	TF	Fold	FDR
At1g49720	ABF1	1.24	0.1040
At3g56850	ABF2	1.15	0.3031
At4g34000	ABF3	1.75	0.0586
At3g19290	ABF4	1.21	0.1505
At2g36270	ABI5	1.39	0.0328

Figure S5. Core ABA signalling module in Arabidopsis. Levels (Fold) and statistical significance (FDR) of the gene transcripts coding for ABA-related receptors, phosphatases, kinases and transcription factors in the microarrays analysis of iPCC1 versus wild type plants. Up-regulated genes were highlighted in orange and red and down-regulated genes in green.

Table S2. *In silico* analysis of motifs over-represented in the promoter sequences of genes that were up-regulated in iPCC1 vs Col-0 plants

Motif	Found on promoter sequences of up-regulated genes in iPCC1 plants (%)	Most over-represented GO term	Description	FDR
	397 of 484 (82%)	GO:0010876	lipid localization	8.1e-08
	177 of 484 (37%)	-	-	-
	132 of 484 (27%)	GO:0010876	lipid localization	5.7e-06
	101 of 484 (21%)	GO:0042592	homeostatic process	0.0086
	75 of 484 (15%)	GO:0009737	response to ABA stimulus	0.023
	74 of 484 (15%)	GO:0009628	response to abiotic stimulus	0.029
	22 of 484 (5%)	-	-	-

Table S3. *In silico* analysis of motifs over-represented in the promoter sequences of genes that were down-regulated in iPCC1 vs Col-0 plants

Motif	Found on promoter sequences of up-regulated genes in iPCC1 plants (%)	Most over-represented GO term	Description	FDR
	296 of 475 (62%)	GO:0006952	defense response	0.011
	263 of 475 (55%)	GO:0002376	immune system process	0.00039
	236 of 475 (50%)	GO:0006952	defense response	2.0e-05
	192 of 475 (40%)	GO:0008219	cell death	5.0e-05
	167 of 475 (35%)	GO:0006952	defense response	5.9e-05
	166 of 475 (35%)	GO:0008219	cell death	0.035
	151 of 475 (32%)	GO:0008219	cell death	0.018
	147 of 475 (31%)	-	-	-
	74 of 475 (16%)	-	-	-

Table S4. Lipid-related genes that were up- and down-regulated in iPCC1 compared to wild type plants.

Fold	FDR	Affymetrix probe	AGI loci	Description
Lipid Localization And Transport				
4.1	0.09870984	263098_at	At2g16005	MD-2-Related Lipid Recognition Domain-Containing Protein
4.08	0.00348602	247718_at	At5g59310	LTP4__Lipid Transfer Protein 4
3.58	0.06393555	248062_at	At5g55450	Bifunctional Inhibitor/Lipid-Transfer Protein/Seed Storage 2S Albumin Superfamily Protein
2.91	0.00180781	245349_at	At4g16690	ATMES16_MES16__Methyl Esterase 16
2.73	0.00022516	262748_at	At1g28610	GDSL-Like Lipase/Acylhydrolase Superfamily Protein
2.43	0.00535262	256145_at	At1g48750	Bifunctional Inhibitor/Lipid-Transfer Protein/Seed Storage 2S Albumin Superfamily Protein
2.38	0.06048593	248683_at	At5g48490	Bifunctional Inhibitor/Lipid-Transfer Protein/Seed Storage 2S Albumin Superfamily Protein
2.24	0.04496028	266098_at	At2g37870	Bifunctional Inhibitor/Lipid-Transfer Protein/Seed Storage 2S Albumin Superfamily Protein
2	0.003288	262740_at	At1g28590	GDSL-Like Lipase/Acylhydrolase Superfamily Protein
1.92	0.00265422	251968_at	At3g53100	GDSL-Like Lipase/Acylhydrolase Superfamily Protein
1.77	0.02825179	258038_at	At3g21260	GLTP3__Glycolipid Transfer Protein (GLTP) Family Protein
1.76	0.00366901	250519_at	At5g08460	GDSL-Like Lipase/Acylhydrolase Superfamily Protein
1.72	0.00560242	245215_at	At1g67830	ATFXG1_FXG1__Alpha-Fucosidase 1
1.63	0.00876914	253344_at	At4g33550	Bifunctional Inhibitor/Lipid-Transfer Protein/Seed Storage 2S Albumin Superfamily Protein
1.5	0.02634205	259070_at	At3g11670	DGD1__UDP-Glycosyltransferase Superfamily Protein
-1.53	0.02852046	263359_at	At2g15230	ATLIP1_LIP1__Lipase 1
-1.53	0.01109532	265646_at	At2g27360	GDSL-Like Lipase/Acylhydrolase Superfamily Protein
-1.56	0.01957738	254959_at	At4g10955	Alpha/Beta-Hydrolases Superfamily Protein
-1.57	0.05502735	248684_at	At5g48485	DIR1__Bifunctional Inhibitor/Lipid-Transfer Protein/Seed Storage 2S Albumin Superfamily Protein
-1.63	0.07504839	254894_at	At4g11840	Phospholipase D Gamma 3
-1.69	0.0102429	248808_at	At5g47510	Sec14p-Like Phosphatidylinositol Transfer Family Protein
-1.71	0.00921163	259934_at	At1g71340	PLC-Like Phosphodiesterases Superfamily Protein
-1.75	0.01383144	264501_at	At1g09390	GDSL-Like Lipase/Acylhydrolase Superfamily Protein
-1.81	0.01892646	254846_at	At4g11835	Phospholipase D Gamma 2
-1.79	0.01589309	263558_at	At2g16380	Sec14p-Like Phosphatidylinositol Transfer Family Protein
-1.91	0.00926823	252525_at	At3g46450	SEC14 Cytosolic Factor Family Protein / Phosphoglyceride Transfer Family Protein
-1.92	0.0146897	262733_s_at	At1g28670	ARAB-1__GDSL-Like Lipase/Acylhydrolase

				Superfamily Protein
-2.12	0.00799464	254313_at	At4g22460	Bifunctional Inhibitor/Lipid-Transfer Protein/Seed Storage 2S Albumin Superfamily Protein
-2.23	0.00048024	262561_at	At1g34340	Alpha/Beta-Hydrolases Superfamily Protein
-2.36	0.00338706	267096_at	At2g38180	SGNH Hydrolase-Type Esterase Superfamily Protein
-2.37	0.01666758	260153_at	At1g52760	Lysopl2__Lysophospholipase 2
-2.75	0.00211792	252937_at	At4g39180	ATSEC14_SEC14__Sec14p-Like Phosphatidylinositol Transfer Family Protein
-2.85	0.00198931	261201_at	At1g12850	Phosphoglycerate Mutase Family Protein
-3.84	0.00491101	265111_at	At1g62510	Bifunctional Inhibitor/Lipid-Transfer Protein/Seed Storage 2S Albumin Superfamily Protein
-5.07	0.00002192	263482_at	At2g03980	GDSL-Like Lipase/Acylhydrolase Superfamily Protein
-5.71	0.00014492	258071_s_at	At3g26070	Plastid-Lipid Associated Protein PAP / Fibrillin Family Protein
-14.04	0.00007334	249812_at	At5g23830	MD-2-Related Lipid Recognition Domain-Containing Protein
Lipid Storage				
2.98	0.00926451	249353_at	At5g40420	Ole2__Oleo2 (Oleosin 2)
2.02	0.01882996	258240_at	At3g27660	Ole3__Oleo4 (Oleosin 4)
1.79	0.19292396	248050_at	At5g56100	Glycine-Rich Protein / Oleosin
1.61	0.07375038	254095_at	At4g25140	Ole1__Oleo1 (Oleosin 1)
1.51	0.11350032	266654_at	At2g25890	Glycine-Rich Protein / Oleosin
-2.74	0.00800202	254798_at	At4g13050	Acyl-(Acyl Carrier Protein) Thioesterase
Phosphatidylinositol-Related Lipids				
4.22	0.00169001	249780_at	At5g24240	Phosphatidylinositol 3- And 4-Kinase Family Protein / Ubiquitin Family Protein
3.6	0.00018835	263433_at	At2g22240	Atips2 (Myo-Inositol-1-Phostpate Synthase 2)
1.8	0.00299373	262003_at	At1g64460	Phosphatidylinositol 3- And 4-Kinase Family Protein
1.6	0.00892487	258613_at	At3g02870	VTC4; 3'(2'),5'-Bisphosphate Nucleotidase/ L-Galactose-1-Phosphate Phosphatase/ Inositol Or Phosphatidylinositol Phosphatase/ Inositol-1(Or 4)-Monophosphatase
1.53	0.04265754	262540_at	At1g34260	Phosphatidylinositol-4-Phosphate 5-Kinase Family Protein
1.44	0.04662868	248155_at	At5g54390	AHL (Arabidopsis HAL2-LIKE); 3'(2'),5'-Bisphosphate Nucleotidase/ Inositol Or Phosphatidylinositol Phosphatase
-1.39	0.04956149	264927_at	At1g60490	PI3K_VPS34 1-Phosphatidylinositol-3-Kinase
-1.42	0.10517469	260466_at	At1g10900	Phosphatidylinositol-4-Phosphate 5-Kinase Family Protein
-1.62	0.10095718	252863_at	At4g39800	Atmips1 Myo-Inositol-1-Phostpate Synthase 1

Description of microarray experiments according to MIAME

Investigation Design Format (IDF)	
Investigation title	IBMCP JLeon lab iPCC1 versus wt Col -0
Experimental designs	iPCC1 transgenic lines vs wild type Col-0 seedlings
Person Last Name	León
Person First Name	José
E-mail	jleon@ibmcp.upv.es
Telephone	(+)3496387782
Affiliation & Address	IBMCP (CSIC-UPV), CPI Edificio 8E, Ingeniero Fausto Elio s/n, 46022 Valencia (Spain)
Person role	Investigator, submitter
Replicate types	3 independent biological replicates per genotype
Experiment description	1. Type of experiment: Compared analysis of the transcriptomes of 12-day old seedlings from 3 different transgenic lines expressing an RNA interference construct for PCC1 gene versus wild type background Col-0.
	2. Experimental factors: iPCC1 plants correspond to RNAi lines (iPCC1 19.9, 25.4 and 33.9, as previously reported in Segarra et al 2010 Plant, Cell & Environment 33, 11-22). Wild type seedlings were Col-0 (abbreviated as Col). Samples were harvested 12 h after dawn of day 12 after sowing and seedlings were grown under long days (16 h light / 8 h darkness) photoperiodic conditions.
	3. Number of hybridizations to Arabidopsis Genechip ATH1 microarrays: 6 distributed as 3 independent biological replicates of wild type Col-0 plus 3 replicates corresponding to the RNAi lines (iPCC1 19.9, 25.4 and 33.9)
	4. Goals of proposed experiments: Identification of the differential transcriptome affected by the reduced functionof PCC1 gene in iPCC1 plants

Sample and Data Relationship Format (SDRF)

1. Hybridization design:

#	Label	Genotype	Growth conditions	Tissue
1	Col 1	Col-0	MS media plus 1 % sucrose, 12 days	Seedlings
2	Col 2	Col-0	MS media plus 1 % sucrose, 12 days	Seedlings
3	Col 3	Col-0	MS media plus 1 % sucrose, 12 days	Seedlings
4	19.9	iPCC1 19.9	MS media plus 1 % sucrose, 12 days	Seedlings
5	25.4	iPCC1 25.4	MS media plus 1 % sucrose, 12 days	Seedlings
6	33.9	iPCC1 33.9	MS media plus 1 % sucrose, 12 days	Seedlings

2. RNA extraction:
Total RNA from wild type and iPCC1 plants was isolated and purified by the Micro-to-Midi Total RNA Purification System from Invitrogen (Carlsbad,

3. Quality controls:
RNAs from every genotype were checked by RT-PCR for mRNA levels of the *PCC1 and ACT2* genes. Moreover, total RNAs used for further preparation of hybridization probes were analysed to check integrity and purity by nanocapilar electrophoresis in Bioanalyzer Agilent 2100.

4. Labeling and hybridization protocols:
RNAs were ligated to an RNA oligonucleotide adaptor (Invitrogen) using T4 RNA ligase (Ambion, http://www.ambion.com/). The RNAs were extracted once with phenol-chloroform and non-ligated adapter was removed by chromatography with MicroSpin S-300 HR columns (GE Healthcare, http://www.gehealthcare.com/). Purified ligation products were precipitated in ethanol and used as templates for reverse transcription with Superscript III (Invitrogen) for 3 h at 46°C, using oligonucleotide oligo(dT) as primer. Template RNA was removed by alkaline hydrolysis and first-strand cDNA purified with S.N.A.P. columns (Invitrogen). Second-strand synthesis was performed with Taq DNA polymerase (Roche, http://www.roche.com/) for 5 min at 94°C, 5 min; 58°C, 1 min; 72°C, 10 min. A forward oligonucleotide T7-Adap primer, which was complementary to the RNA adapter and contained the sequence of bacteriophage T7 promoter, was used. Double-stranded cDNA was then purified with MinElute columns (Qiagen) and in vitro transcribed with T7 RNA polymerase, using a MessageAmp aRNA kit (Ambion). Amplified RNA was treated with DNase I (Roche) to remove cDNA templates, purified with an aRNA Purification Module (Ambion) and then used as template for single-stranded cDNA synthesis, according to Affymetrix instructions (http://www.affymetrix.com/) as dolows: aRNA was reverse transcribed with SuperScript II (Invitrogen) for 1 h at 42°C with oligo(dT) as primer. After alkaline hydrolysis of aRNA and purification (MinElute columns, Qiagen), cDNA was fragmented with 1.5 units of DNase I (GE Healthcare) into fragments in the 50–200 bp range. Finally, 3' ends of fragmented cDNA were biotin-ddUTP labeled with terminal deoxynucleotidyl transferase (Promega, http://www.promega.com/) and GeneChip DNA labeling reagent (Affymetrix).
Three biological replicates and their corresponding negative controls were independently hybridized to ATH1 microarrays (Affymetrix), containing 22 500 transcript variants from 24 000 well-characterized Arabidopsis genes. Each sample was added to a hybridization solution containing 100 mm 2-(N-morpholino) ethanesulfonic acid, 1 m Na+, and 20 mm of EDTA in the presence of 0.01% Tween-20. Hybridization was performed for 16 h at 45°C. Each microarray was washed and stained with streptavidin–phycoerythrin in a Fluidics station 450 (Affymetrix) and scanned at 2.5-µm resolution in a GeneChip® Scanner 3000 7G system (Affymetrix). Data analyses were performed using genechip operating software (GCOS), to generate the corresponding CEL files.
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5. Sample comparisons:
iPCC1 vs Col plants

6. Statistical analysis:
Linear model methods (LiMMA) were used for determining differentially expressed genes. To control the false-discovery rate, P-values were corrected using the method of Benjamini and Hochberg (1995). Criteria for selection of genes were fold value >1.5 and false-discovery rate ≤ 0.05. Statistical analysis and graphical visualization of data were performed with the interactive tool fiesta (http://bioinfogp.cnb.csic.es/tools/FIESTA/).