



Figure S1. Seedling establishment in osmotic media supplemented with mannitol or NaCl. Values are the mean \pm 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 \pm 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 \pm standard error. Ratio values above 1 corresponded to up-regulated genes and below 1 to down-regulated genes in iPCC1 seedlings.



B



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 \pm 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 μ M 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
PTR/PTL/RCAR	At2g38310	PYL4	-1.30	0.4487
receptor	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
		Phoenhatasa	Fold	FDR
	At4a26080	ABI1	-1 07	0 7767
–	At5q57050	ABI2	1.55	0.0431
	At1q72770	HAB1	1.94	0.0488
5540	At1g17550	HAB2	1.13	0.4927
PP2Cs	At5g59220	HAI1	3.71	0.0174
nhosnhatase	At1g07430	HAI2	1.65	0.1827
phoophataoo	At2g29380	HAI3	1.17	0.2439
_	At5g51760	AHG1	1.19	0.1796
	At3g11410	AHG3	1.46	0.1240
	AVE 00500	Kinase	Fold	FDR
L	At5g08590	SnRK2.1	-1.02	0.8884
—	At3g50500	SNRK2.2	-1.53	0.0089
	At3966880	SNRKZ.3	1.20	0.1252
SnRK2s	ALTG10940	SHRKZ.4 SpRK2.5	-1.07	0.0009
kinggo	At/a33950	SnRK2 6/0ST1	1.00	0.0270
Kinase	At4q40010	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
Ļ		TF	Fold	FDR
*	At1g49720	ABF1	1.24	0.1040
ABA-responsive	At3g56850	ABF2	1.15	0.3031
	At4g34000	ABF3	1.75	0.0586
IFS			1 21	0 1505
	At0 = 0.0070		1.21	0.1000

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
AAAAGAAA	397 of 484 (82%)	GO:0010876	lipid localization	8.1e-08
TAAATA&A	177 of 484 (37%)	-	-	-
CTTCITCI	132 of 484 (27%)	GO:0010876	lipid localization	5.7e-06
AGATAGAT	101 of 484 (21%)	GO:0042592	homeostatic process	0.0086
CTCTCTCT	75 of 484 (15%)	GO:0009737	response to ABA stimulus	0.023
CACCITCIC	74 of 484 (15%)	GO:0009628	response to abiotic stimulus	0.029
CCCCCCCC	22 of 484 (5%)	-	-	-

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

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

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

	Fold	FDR	Affymetrix	AGI loci	Description
			probe		
	Lipid L	ocalization And	Transport		
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	4.1	0.09870984	263098_at	At2g16005	MD-2-Related Lipid Recognition Domain-
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $					Containing Protein
3.58 0.06393555 248062_{at} At5g5540 Bifunctional Inhibitor/Lipid-Transfer Protein/Secd Storage 2S Albumin Superfamily Protein 2.91 0.00180781 245349_{att} At4g16690 ATMES16_MES16_Methyl Esterase 16 2.73 0.00022516 262748_{att} At1g28610 GDSL-Like Lipase/Acylhydrolase 2.43 0.00535262 256145_{att} At1g48750 Bifunctional Inhibitor/Lipid-Transfer Protein/Secd Storage 2S Albumin 2.38 0.06048593 248683_{att} At5g48490 Bifunctional Inhibitor/Lipid-Transfer Protein/Secd Storage 2S Albumin 2.24 0.04496028 266098_{att} At2g37870 Bifunctional Inhibitor/Lipid-Transfer Protein/Secd Storage 2S Albumin 2.24 0.04496028 266098_{att} At1g28590 GDSL-Like Lipase/Acylhydrolase Superfamily Protein 1.92 0.00265422 251968_{att} At1g28590 GDSL-Like Lipase/Acylhydrolase 1.92 0.00265422 25198_{att} At3g21260 GDSL-Like Lipase/Acylhydrolase 1.77 0.02825179 258038_{att} At2g07830 GDSL-Like Lipase/Acylhydrolase 1.76 $0.$	4.08	0.00348602	247718_at	At5g59310	LTP4_Lipid Transfer Protein 4
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$\begin{array}{c c c c c c c c c c c c c c c c c c c $	1.50	0.005.000.00	0.4504.5	4.4.65020	Superfamily Protein
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Image: Protein/Seed Storage 2S Albumin Superfamily Protein1.5 0.02634205 259070_{at} $At3g11670$ $DGD1_UDP-GlycosyltransferaseSuperfamily Protein-1.530.02852046263359_{at}At2g15230ATLIP1_LIP1_Lipase 1-1.530.01109532265646_{at}At2g27360Superfamily Protein-1.560.01957738254959_{at}At4g10955Alpha/Beta-Hydrolases Superfamily Protein-1.570.05502735248684_{at}At5g48485Superfamily Protein-1.630.07504839254894_{at}At4g11840Phospholipase D Gamma 3-1.690.0102429Sec14p-Like Phosphatidylinositol TransferFamily Protein-1.710.00921163259934_{at}At1g09390-1.750.01383144264501_{at}At2g16380-1.790.0192646254846_{at}At2g16380-1.790.00926823Sec14p-Like Phosphatidylinositol TransferFamily Protein-1.710.00926823Sec14p-Like Phospholipase D Gamma 2-1.790.0146897263558_{at}At2g16380-1.910.00926823Sec14p-Like Phospholipase D Gamma 2-1.920.0146897267733 s atAt1g28670-1.920.0146897267733 s atAt1g28670$	1.63	0.00876914	253344_at	At4g33550	Bifunctional Inhibitor/Lipid-Transfer
1.50.02634205259070_atAt3g11670DGD1_UDP-Glycosyltransferase Superfamily Protein-1.530.02852046263359_atAt2g15230ATLIP1_LIP1_Lipase 1-1.530.01109532265646_atAt2g27360Superfamily Protein-1.560.01957738254959_atAt4g10955Alpha/Beta-Hydrolases Superfamily Protein-1.570.05502735248684_atAt5g48485Superfamily Protein-1.630.07504839254894_atAt4g11840Phospholipase D Gamma 3-1.690.0102429Sec14p-Like Phosphodiesterases Superfamily-1.710.00921163259934_atAt1g71340PLC-Like Lipase/Acylhydrolase-1.750.01383144264501_atAt1g09390Superfamily Protein-1.790.01589309263558_atAt2g16380Superfamily Protein-1.910.00926823252525_atAt3g46450Phospholipase D Gamma 2-1.920.0146897262733 s. atAt1g28670ARAB-1-1.920.0146897262733 s. atAt1g28670ARAB-1					Protein/Seed Storage 25 Albumin
1.5 0.02634205 $2590/0_at$ At3g11670DGDT_OD-Glycosyltransferase Superfamily Protein-1.53 0.02852046 263359_at At2g15230ATLIP1_LIP1_Lipase 1-1.53 0.01109532 265646_at At2g27360Superfamily Protein-1.56 0.01957738 254959_at At4g10955Alpha/Beta-Hydrolases Superfamily Protein-1.56 0.01957738 254959_at At4g10955Alpha/Beta-Hydrolases Superfamily Protein-1.57 0.05502735 248684_at At5g48485Superfamily Protein-1.63 0.07504839 254894_at At4g11840Phospholipase D Gamma 3-1.69 0.0102429 Sec14p-Like Phosphatidylinositol Transfer Family Protein-1.71 0.00921163 PLC-Like Phospholiesterases Superfamily Protein-1.75 0.01383144 264501_at At1g09390 263558_at At2g16380Superfamily Protein-1.79 0.01589309 Sec14p-Like Phosphatidylinositol Transfer Family Protein-1.91 0.00926823 263558_at At2g16380-1.92 0.0146897 262733_5 atAt1g28670ARAB-1-1.92 0.0146897 262733_5 atAt1g28670ARAB-1GDSL-Like Lipase/Acylhydrolase	15	0.02(24205	250070	A (2 - 11 C70	Superfamily Protein
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	1.5	0.02634205	259070_at	At3g11670	DGDIUDP-Glycosyltransferase
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	1.52	0.02952046	262250 at	A+2~15220	ATLID1 LID1 Liness 1
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	-1.55	0.02832040	203339_at	At2g15250	ATLIFI_LIFI_LIPase I
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	1.52	0.01100522	265616 at	A+2~27260	Superfamily Protein
-1.300.01937738234339_atAt4g10933Atplia/Beta-Hydrolases Superfamily Protein-1.510.019377382348684_atAt5g48485DIR1_Bifunctional Inhibitor/Lipid-Transfer Protein/Seed Storage 2S Albumin-1.570.05502735248684_atAt5g48485Superfamily Protein-1.630.07504839254894_atAt4g11840Phospholipase D Gamma 3-1.690.0102429Sec14p-Like Phosphatidylinositol Transfer Family Protein-1.710.00921163PLC-Like Phosphodiesterases Superfamily Protein-1.750.01383144264501_atAt1g71340-1.750.01383144264501_atAt1g09390-1.810.01892646254846_at-1.790.01589309Sec14p-Like Phospholipase D Gamma 2-1.790.00926823SEC14p-Like Phosphatidylinositol Transfer Family Protein-1.910.00926823SEC14 Cytosolic Factor Family Protein-1.920.0146897262733 s atAt1g28670-1.920.0146897262733 s atAt1g28670	-1.55	0.01109332	203040_at	At2g27300	Alpha/Pata Hudrolasas Superfemily Protein
-1.570.05502735248684_atAt5g48485Superfamily Protein-1.630.07504839254894_atAt4g11840Phospholipase D Gamma 3-1.690.0102429Sec14p-Like Phosphotiesterases Superfamily-1.710.00921163PLC-Like Phosphodiesterases Superfamily-1.710.00921163PLC-Like Phosphodiesterases Superfamily-1.750.01383144264501_atAt1g09390-1.810.01892646254846_atAt4g11835-1.790.01589309Sec14p-Like Phospholipase D Gamma 2-1.910.00926823SEC14 Cytosolic Factor Family Protein-1.920.0146897262733 s atAt1g28670-1.920.0146897262733 s atAt1g28670ARAB-1	-1.50	0.01937738	234939_at	At4g10955	DID1 Difunctional Inhibitar/Linid Transfer
-1.570.05502735248684_atAt5g48485Superfamily Protein-1.630.07504839254894_atAt4g11840Phospholipase D Gamma 3-1.690.0102429Sec14p-Like Phosphatidylinositol Transfer-1.710.00921163PLC-Like Phosphodiesterases Superfamily-1.710.00921163PLC-Like Phosphodiesterases Superfamily-1.750.01383144264501_atAt1g71340-1.810.01892646254846_atAt4g11835-1.790.01589309Sec14p-Like Phospholipase D Gamma 2-1.910.00926823SEC14 Cytosolic Factor Family Protein-1.920.0146897262733 s atAt1g28670-1.920.0146897262733 s atAt1g28670ARAB-1					DIKIBIUICUOIIAI IIIIIDIUOI/LIPIU-IIAIISIEI Drotoin/Sood Storago 25 Albumin
-1.57 0.03302733 $248044at$ $At3g48483$ $Supertaining Hotem-1.630.07504839254894_atAt4g11840Phospholipase D Gamma 3-1.690.0102429248808_atAt5g47510Sec14p-Like Phosphatidylinositol Transfer-1.710.00921163259934_atAt1g71340PLC-Like Phosphodiesterases Superfamily-1.750.01383144264501_atAt1g09390Superfamily Protein-1.810.01892646254846_atAt4g11835Phospholipase D Gamma 2-1.790.01589309Sec14p-Like Phosphatidylinositol Transfer-1.910.00926823SEC14 Cytosolic Factor Family Protein /-1.920.0146897262733 s atAt1g28670ARAB-1GDSL-Like Lipase/Acylhydrolase$	1.57	0.05502735	248684 at	At5a18185	Superfamily Protein
-1.690.01304839234894_atAttg11840Thispholipase D Gamma 3-1.690.0102429248808_atAt5g47510Sec14p-Like Phosphatidylinositol Transfer Family Protein-1.710.00921163259934_atAt1g71340PLC-Like Phosphodiesterases Superfamily Protein-1.750.01383144264501_atAt1g09390Superfamily Protein-1.810.01892646254846_atAt4g11835Phospholipase D Gamma 2-1.790.01589309263558_atAt2g16380Family Protein-1.910.00926823SEC14 Cytosolic Factor Family Protein-1.920.0146897262733 s atAt1g28670ARAB-1GDSL-Like Lipase/Acylhydrolase	-1.57	0.03502735	240004_at 254804_at	At/g11840	Phospholipase D Gamma 3
11.090.0102429248808_atAt5g47510Family Protein-1.710.00921163259934_atAt1g71340PLC-Like Phosphodiesterases Superfamily Protein-1.750.01383144264501_atAt1g09390Superfamily Protein-1.810.01892646254846_atAt4g11835Phospholipase D Gamma 2-1.790.01589309Sec14p-Like Phosphatidylinositol Transfer Family Protein-1.910.00926823SEC14 Cytosolic Factor Family Protein / 252525_at-1.920.0146897262733 s atAt1g28670-1.920.0146897262733 s atAt1g28670ARAB-1	-1.05	0.07304839	234894_at	Al+g11040	Sec1/n Like Phosphatidylinositol Transfer
-1.710.00921163PLC-Like Phosphodiesterases Superfamily-1.710.00921163PLC-Like Phosphodiesterases Superfamily-1.750.01383144264501_atAt1g71340-1.750.01383144264501_atAt1g09390-1.810.01892646254846_atAt4g11835-1.790.01589309Sec14p-Like Phospholipase D Gamma 2-1.910.00926823SEC14 Cytosolic Factor Family Protein-1.920.0146897262733 s atAt1g28670-1.920.0146897262733 s atAt1g28670	-1.09	0.0102429	2/18808_at	At5g/17510	Family Protein
-1.71 0.00921103 259934_at At1g71340 Protein -1.75 0.01383144 264501_at At1g09390 Superfamily Protein -1.81 0.01892646 254846_at At4g11835 Phospholipase D Gamma 2 -1.79 0.01589309 263558_at At2g16380 Family Protein -1.91 0.00926823 SEC14 Cytosolic Factor Family Protein / 252525_at SEC14 Cytosolic Factor Family Protein / Phosphoglyceride Transfer Family Protein -1.92 0.0146897 262733 s at At1g28670 ARAB-1 GDSL-Like Lipase/Acylhydrolase	-1 71	0.00921163	240000_at	Al.5g+7510	PLC-Like Phosphodiesterases Superfamily
-1.750.01383144264501_atAt1g09390Superfamily Protein-1.810.01892646254846_atAt4g11835Phospholipase D Gamma 2-1.790.01589309263558_atAt2g16380Sec14p-Like Phosphatidylinositol Transfer-1.910.00926823252525_atAt3g46450Phosphoglyceride Transfer Family Protein-1.920.0146897262733 s atAt1g28670ARAB-1GDSL-Like Lipase/Acylhydrolase	-1./1	0.00721105	25993/ at	$\Delta t 1 \sigma 7 1340$	Protein
-1.75 0.01383144 264501_at At1g09390 Superfamily Protein -1.81 0.01892646 254846_at At4g11835 Phospholipase D Gamma 2 -1.79 0.01589309 263558_at At2g16380 Sec14p-Like Phosphatidylinositol Transfer -1.91 0.00926823 252525_at At3g46450 Phosphoglyceride Transfer Family Protein -1.92 0.0146897 262733 s. at At1g28670 ARAB-1 GDSL-Like Lipase/Acylbydrolase			23773+_dt	1115/15+0	GDSL-Like Linase/Acylhydrolase
-1.81 0.01892646 254846_at At4g11835 Phospholipase D Gamma 2 -1.79 0.01589309 263558_at At2g16380 Sec14p-Like Phosphatidylinositol Transfer -1.91 0.00926823 252525_at At3g46450 Phosphoglyceride Transfer Family Protein -1.92 0.0146897 262733 s. at At1g28670 ARAB-1 GDSL-Like Lipase/Acylhydrolase	-1 75	0.01383144	264501 at	At1009390	Superfamily Protein
-1.79 0.01589309 263558_at At2g16380 Sec14p-Like Phosphatidylinositol Transfer -1.91 0.00926823 252525_at At3g46450 Phosphoglyceride Transfer Family Protein -1.92 0.0146897 262733 s. at At1g28670 ARAB-1 GDSL-Like Lipase/Acylbydrolase	-1.81	0.01892646	254846 at	ΔtΔσ11835	Phospholinase D Gamma 2
1.17 0.01307307 263558_at At2g16380 Family Protein -1.91 0.00926823 SEC14 Cytosolic Factor Family Protein / 252525_at SEC14 Cytosolic Factor Family Protein / Phosphoglyceride Transfer Family Protein -1.92 0.0146897 262733 s at At1g28670 ARAB-1 GDSL-Like Lipase/Acylhydrolase	-1 70	0.01580300	207070_ai	Al - g11055	Sec14n-Like Phoenbatidylinositol Transfor
-1.91 0.00926823 At3g46450 Family From -1.92 0.0146897 262733 s at At1g28670 ARAB-1 GDSL-Like Lipse/Acylhydrolase	-1./2	0.01505505	263558 at	At2016380	Family Protein
-1.92 0.0146897 262733 s at At1g28670 ARAB-1 GDSL-Like Lipse/Acylhydrolase	-1 91	0.00926823		112510500	SEC14 Cytosolic Factor Family Protein /
-1.92 0.0146897 262733 s at At1g28670 ARAB-1 GDSL-Like Lipse/Acylhydrolase	1.71	0.00720025	252525 at	At3946450	Phosphoglyceride Transfer Family Protein
	-1.92	0.0146897	262733 s at	At1928670	ARAB-1 GDSL-Like Linase/Acylhydrolase

				Superfamily Protein
				Bifunctional Inhibitor/Lipid-Transfer
				Protein/Seed Storage 2S Albumin
-2.12	0 00799464	254313 at	At4922460	Superfamily Protein
_2.12	0.007779404	262561 at	$\Delta t 1 \sigma 3 / 3 / 0$	Alpha/Beta-Hydrolases Superfamily Protein
-2.23	0.000+002+	202301_dt	Aligototo	SGNH Hydrolase Type Esterase Superfamily
236	0.00338706	267006 at	A +2 a 3 8 1 8 0	Protoin
-2.30	0.00558700	260153 at	At1g52760	Lycopl2 Lycophospholipasa 2
2.37	0.01000738	200133_at	Alig52700	ATSEC14 SEC14 Sec14p Like
-2.15	0.00211792	252027 at	A + 4 = 20180	Phosphatidulinosital Transfor Family Protain
2.95	0.00108021	232937_at	Al4g59160	
-2.65	0.00198931	261201 at	At1a12850	Phoenhoglycorata Mutasa Family Protain
		201201_at	Atig12850	Pifunctional Inhibitor/Linid Transfer
				Brutein/Saed Storage 2S Albumin
2.01	0.00401101	265111 of	A +1 ~62510	Superfemily Protein
-3.84	0.00491101	203111_at	At1g02510	CDSL Like Linese/Aculhudrolose
5.07	0.00002102	262492 at	A +2 ~02080	GDSL-Like Lipase/Acylnydrolase
-5.07	0.00002192	203482_at	At2g03980	Superiamity Protein
5 71	0.00014402	259071	A 12 - 2 CO 7 O	Plastid-Lipid Associated Protein PAP /
-5./1	0.00014492	S_at	At3g26070	Fibrillin Family Protein
14.04	0.00007224	240012	4.5.00000	MD-2-Related Lipid Recognition Domain-
-14.04	0.00007334	249812_at	At5g23830	Containing Protein
Lipid S	torage	0.400.50	4.5.40400	
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
Phosph	atidylinositol-R	elated 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

ription of microarray experiments ac ng to MIAME

investigation Design	Tornat (IDT)	
Investigation title	IBMCP JLeon lab iPCC1 ve	rsus wt Col -0
Experimental designs	iPCC1 transgenic lines vs w	ild 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 F	dificio 8E, Ingeniero Fausto Elio s/n, 46022 Valencia (Spain)
Person role	Investigator, submitter	
Replicate types	3 independent biological rep	licates per genotype
	1. Type of experiment:	
Experiment description	Compared analysis of the tra	nscriptomes 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	a 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 independe	at biological replicates of wild type Col-0 plus 3 replicates corresponding to the RNAi lines (iPCC1 19.9, 25.4 and 33.9)
	Goals of proposed experimental	nents:
	Identification of the different	ial transcriptome affected by the reduced function of PCC1 gene in iPCC1 plants

Sample and Data Relationship Format (SDRF)

1. nybruization 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:					

Cotal RNA from wild type and iPCC1 plants w Midi Ta al RNA P 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.

Labeling and hybridization protocols:

4. Locenting and hybridization protects.
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 oligonicleotide 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 \$ paintee win 15-7-7-7 sonning (urmeign), become and spinusses and performed win req. Deter joynessis (notes), independent jois min at 94°C, 5 min; 58°C, 1 min; 72°C, 10 min. A forward oligonucleotide 17-Adap primer, which was complementary to the RNA adapter and contained the sequence of bacteriophage 17 promoter, was used. Double-stranded cDNA was then purified with MinElute columns (Qiagen) and in vitro transcribed with 17 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 GDrA reinpartes, punited with an arXiVA runnation involute (rinnound) and uten beeu as reinparte to single-standard CDVA syminesis, according to Affymetrix instructions (http://www.affymetrix.com) as dolows: aRNA was reverse transcribed with SuperScript II (Invitregen) for 1 bat 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 (GE Healthcare) into fragments in the 50–200 bp range. Finally, 3' ends of fragmented cDNA were biotin-ddUTP labeled with terminal

(concentration of the second o GeneChip® Scanner 3000 7G system (Affymetrix). Data analyses were performed using genechip operating software (GCOS), to generate the

Concernso of a system (Anymetrix). Data analyses were periodined using generality operating software (SCOS), to generate the corresponding CEL files. 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 nm 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 seanned 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 prresponding CEL files.

5. Sample comparisons: iPCC1 vs Col plants

. Statistical analysis

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