

Figure S1: Example of primary screen plate images using EMS mutagenized *PDH1_{pro}:LUC2*.

 M_2 seeds were plated on half-strength MS media (no sugar, with MES buffer) overlaid with nylon mesh, stratified for 4 days and plates placed vertically in a growth chamber and seedlings allowed to grow seven days. Plates were them imaged and the seedlings transferred to PEG-agar plates using the nylon mesh. Seedlings were then imaged again at 96 h after transfer. Seedlings having either low PDH1_{pro}:LUC2 activity before stress (not seen in this example plate) or high PDH1_{pro}:LUC2 after stress (as seen here for mutant 4255-1) were transferred to soil. All such putative mutants were rescreened in the subsequent generation and those having reproducible alterations in PDH1_{pro}:LUC2 activity retained for further characterization.



Figure S2: Identification of *CYP86A2* and *LACS2* as the causative mutated genes in the 4442-2 and 4255-1 EMS mutants

- A. Next Generation Mapping (NGM) output for 4442-4 showing four candidate genes.
- B. Analysis of proline accumulation at -1.2 MPa for two of the candidate genes identified in A found no significant increase in proline level compared to wild type. The multiple data points for each mutant are for seed of individual homozygous plants and stocks of Col-0 wild type grown at the same time as the mutants. Data are means ± S.E. (n = 6-9).
- C. NGM output for 4255-1 showing *LACS2* as the main candidate gene.



Figure S3: *PDH1_{pro}:LUC2* crossed to *cyp86a2-1* and *lacs2-1* T-DNA mutants shows the same high *PDH1_{pro}:LUC2* phenotype as the 4244-2 and 4255-2 EMS mutants



Figure S4: Expression of proline synthesis and catabolism genes in *cyp86a2* mutants. Data are means \pm S.E. (n=3). Significant differences (p \geq 0.05) compared to wild type in the same treatment are marked (*).



Figure S5: NADP⁺ and NADPH analysis of wild type, *cyp86a2-1* and *lacs2-1*. Data are means ± S.E. (n=2-6). There were no significant differences between mutants and wild type.



Figure S6: ROS levels of wild type, *cyp86a2-1* and *lacs2-1*. To investigate whether the mutants had greater overall ROS levels than wild type, plants were stained with the ROS sensitive dye H2DCFDA and root tips imaged. No substantial differences in H2DCFDA fluorescence was observed between wild type and mutants.





Figure S7: MapMan analysis of photosynthesis-related gene expression in stress and control treated wild type as well as *p5cs1-4* compared to wild type in unstressed and low water potential (-1.2 MPa, 96 h) treatments.

Knockout of *p5cs1-4* resulted in a distinctive effect of up and down regulation of genes involved in the light reactions and electron transport while no differences were seen in Calvin Cycle or Photorespiration-related genes. This pattern differed substantially from low water potential stress in wild type where genes in all branches of photosynthetic related metabolism were affected and most changes seen were down-regulation.







Unstressed p5cs1-4/W.T.



Figure S8: MapMan analysis of mitochondrial electron transport-related gene expression in stress and control treated wild type as well as *p5cs1-4* compared to wild type in unstressed and low water potential (-1.2 MPa, 96 h) treatments.

Knockout of p5cs1-4 resulted in similar upregulation of many Complex I genes in both control and stress treatments. This pattern differed substantially from the effect of low water potential stress in wild type.

Stressed p5cs1-4/W.T.





Figure S9: MapMan analysis of amino acid metabolism-related gene expression in *p5cs1-4* compared to wild type in unstressed and low water potential (-1.2 MPa, 96 h) treatments.

- A. Wild type stress versus wild type control. The significantly up or down regulated genes (Tables S2 and S3) were used for analysis but using a 2fold change for differentially expressed genes. This included 1981 genes with 411 metabolic related on the genes plotted general metabolic diagram shown here. Red boxes indicate metabolic processes of particular interest for comparison to data p5cs1-4 (light reactions, mitochondria electron transport, amino acid metabolism).
- B. Unstressed *p5cs1-4* compared to unstressed wild type. For MapMan analysis, all genes having fold change greater than 2-fold were included along with genes with reads in on sample but not the other which were found to be significant in the statistical analysis (Fig. 7A, Table S4 and S5). This included 3466 genes with 296 having metabolic functions displayed on this diagram.
- C. Stressed *p5cs1-4* compared to stressed wild type. Genes for plotting on MapMan were selected as described for B and included 2672 genes with 284 metabolism-related plotted on this diagram.

For both B and C, note the limited number of amino acid metabolism genes found compared to the wild type stress versus control plot in A. Conversely, a larger number of genes related to light reactions and mitochondrial electron transport were found to have altered expression in p5cs1-4 and the overall effect of p5cs1-4on their expression was dramatically different than the effect of stress on wild type.

Dataset: 3203 perturbations from data selection: AT_MPYY_AT#1-1 212/bonecists from gane selection: p5cs1 control		
Lugi unit 28 00 18 12 05 08 55 13 15 28 20 Development	Α	
		 most posts most posts most posts most posts most posts most posts
Your Signate		
Arabitropis Ballana (Top 80 meet similar Perturbations) icos deficiency / potopieting / icos deficiency study 8 (2011)		Sector Sector
Net1-1 (We-0 () (Pe/K 4, 17) G(4-0		
heat study 7 / unitwated cell suspension samples ceant-4 / Co40 D		
peti2 petil (Col-0 petil (Col-0 petil stark 2 (200 MP3.48) / retrasted 200 MP3.48 section service (201)		
C857900/ Bay-Openint polies tube provid carrier a since/ polies tube provid-(in-vitra)		
desarred sector study 10 (Col) 1 attracted seading samples (Col) cost of 1 Cold (0)		
C507301 / Bay-Opener#		
stratification (1%)/ and desicration		
cap1-4.1 Col-0 D		
Incompose RHB shally 2 (3.5 percel en.2 en 1) / RHB shally 2 (3.5 percel en.2 en 1) His school tel / non-influented most spengies		
CS07945 / Bay-Operant		
Georgan / Bay-cyaware Be 0 / Mir Offie 0		
C657E70 / Bay-Operant C65e 5 de prodit la dest 1 //e parter solitet service		
CS07332 / Bay-Opament		
C607805/ Bay-Commit C607866/ Bay-Commit		
MYBELL Cold O		
spet (Git-0		
- document/secone study 10 (355:GR-REV*) / antroaded seading samples (355:GR-RE CSI/2982 / Bay-Boarevit		
vez+1pet/CoH		
CS078X2 / Bay-Openert		
C657733/Bay-Operant descentionary study 13 (Col.) unterchard section strategies (Col.		
C557917 / Bay-Openerit		
G802771 / Gat G		
2.4.0 + bichratelin A (Ler) / 2.4.0 study 2.5.er)		
CS57530/Bay-0parat		
L-AOPP + UAA study 2 (4d)-1 mech treated Col-8 shoet aploal meristem samples (4d) CRONARI / Reactionment		
C55/540/ Bay-Operant		
Te-0 / Co+0 @		
C857725/ Bay-Epartert		
C557700 / Bay Content		
		INSISTAND OF A CONTRACTOR

B	Relative
Arabidopsis thaliana (Top 50 most similar Perturbations)	Similarity
iron deficiency / protoplasting / iron deficiency study 8 (24h)	1.060
lec1-1 / Ws-0 🚯	1.038
phyA'-L17 / Col-0	1.036
heat study 7 / untreated cell suspension samples	1.035
cop1-4 / Col-0 🚯	1.033
pab2 pab8 / Col-0 🕔	1.031
drought study 9 (35S::ABF3-48) / untreated 35S::ABF3-48 see	edling samples (24h 1.031
CS57560 / Bay-0 parent	1.031
pollen tube growth (semi in vivo) / pollen tube growth (in vitro)	1.030
dexamethasone study 10 (Col) / untreated seedling samples (Col) 1.030
cop1-4 / Col-0 📵	1.030
CS57561 / Bay-0 parent	1.028
low light / 4°C (220-280min) / low light / 21°C (220-280min)	1.028
stratification (1h) / seed desiccation	1.028
cop1-4 / Col-0 📵	1.028
cop1-4 / Col-0 📵	1.027
lincomycin+R+B study 2 (0.5µmol m-2 s-1) / R+B study 2 (0.5	µmol m-2 s-1) 1.027
H. schachtii / non-infected root samples	1.027
CS57645 / Bay-0 parent	1.027
CS57631 / Bay-0 parent	1.027
Se-0 / Mir-0/Se-0	1.026
CS57670 / Bay-0 parent	1.026
pollen tube growth (semi in vivo) / dry mature pollen samples	1.026
CS57533 / Bay-0 parent	1.025
CS57865 / Bay-0 parent	1.025
CS57658 / Bay-0 parent	1.025
MYB61 / Col-0 👀	1.025
CS57824 / Bay-0 parent	1.025
sps1 / Col-0	1.024
dexamethasone study 10 (35S:GR-REV*) / untreated seedling	samples (35S:GR- 1.024
CS57592 / Bay-0 parent	1.024
mur4-1prl1 / Col-0	1.024
CS57901 / Bay-0 parent	1.024
CS57693 / Bay-0 parent	1.024
CS57703 / Bay-0 parent	1.024
dexamethasone study 12 (Col) / untreated seedling samples (Col) 1.024
CS57917 / Bay-0 parent	1.024
axr2-1 / Col 👀	1.023
CS57771 / Bay-0 parent	1.023
2,4-D + trichostatin A (Ler) / 2,4-D study 2 (Ler)	1.023
toc1-1 / C24_CAB2::LUC_CaMV35S::AEQ 🚺	1.023
CS57530 / Bay-0 parent	1.023
L-AOPP + IAA study 2 (4d) / mock treated Col-0 shoot apical	meristem samples (~ 1.023
CS57896 / Bay-0 parent	1.023
CS57640 / Bay-0 parent	1.022
Mir-0 / Mir-0/Se-0	1.022
Te-0 / Col-0 🚺	1.022
CS57723 / Bay-0 parent	1.022
CS57823 / Bay-0 parent	1.022
CS57769 / Bay-0 parent	1.022

Relative Genevestigator Similarity Experiment #

AT-00168

AT-00627

AT-00026

AT-00616 AT-00667

AT-00422

AT-00216 AT-00466

AT-00669

AT-00616 AT-00216

AT-00467

AT-00490 AT-00616

AT-00616

AT-00501 AT-00024

AT-00216

AT-00216 AT-00102

AT-00216

AT-00466 AT-00216 AT-00216

AT-00216

AT-00015 AT-00216

AT-00627

AT-00627 AT-00216

AT-00352 AT-00216

AT-00216 AT-00216

AT-00669

AT-00216

AT-00604 AT-00216

AT-00525

AT-00398 AT-00216 AT-00658 AT-00216 AT-00216 AT-00102 AT-00678 AT-00216 AT-00216

Experiment # AT-00286

Fig S10: Differentially expressed genes in *p5cs1-4* control vs. W.T. control compared to public microarray data using the Genevestigator Signature analysis tool.

A. Heat map with the *p5cs1-4* expression data along the top and 50 most similar datasets below. The most similar data sets were identified using the Euclidean Distance option in Genevestigator signatures comparison tool. The *p5cs1-4* data was compared to 3283 "perturbations" (experiments involving mutants, stress and hormone treatments).

B. The 50 samples most similar to *p5cs1-4* listed in the same order (top to bottom) and in A. The Genevestigator experiment number (AT-XXXXX) is listed for each.

Several of the samples with expression pattern most similar to *p5cs1-4* came from experiments involving light signaling mutants and different light treatments (AT-00627, AT-00616) or salicylic acid (SA) treatment (AT-00216).
 Dateset:
 3283 perturbations from data selection: AT_AFFY_ATH1-0

 141 transcripts from gene selection: p5cs1 stress

 45
 4.8
 -10
 -0.9
 0.9
 1.0
 1.5
 2.8

			10-17-12-11
	0002.00 09911.00 09911.00 09911.00 09901.00 09001.00 09000.00 00 09000.00 00000.00 00000.00 00000.00 00000.00 00000.00 00000.00 00000.00 00000.00 00000.00 00000.00 00000.00 00000.00 00000.00 00000.00 000000	6, 1000 6, 1010 6, 1010 6, 1000 6, 100	 A. Unsequence of the second sec
Very Beach	2011-2211-000 2011-2011-2011-2011-2011-2	AT 1021200 AT 102110 AT 102100 AT 10204040 AT 102040 AT 1020400 AT 102000 AT 102000 AT	Contraction of the contractio
Achtdopeis Italians (Top 50 nost sinitiz Perturbations) aguars1 / aguars1 · @ aguars1 / act 0 ·			Note Gray
agurot 1 agurot 1 @ mil 2 lagurot 1 @ agurot 3 (cab @ StadPyleade (solt 2) angeland @			
nersis (Crés B) Harris (Le formation per semple and 61 (Crés B) Es 1 (Ban danne B)			
HSC Cold 0 HSC Value HSC V			
457 / Jopen J. 0 457 / José 0 452 / José 0 556 / José 464 - 1 J J55 / Pőlekit 0			
hud71-de352/hud71- 150 / Res 10 st0 / Res 0			
kk/ kot kp3/tg6-0 TG4e-rCP4 gPP Me/ Ne/ G6-0 gr4/1 rei			
Aut Cade 0 Cod 1 2020 ignef 1 ant welf 4 wid 1 muth1			
er 11 (Job) of pr1 (Lao) diought subg 2 (Sci 4) (untreated teal samples (Sci 4) model 1 (Fac)			
om/AC/979/30 (GAO p=0 (CA) e02 (CA) € e02 (CA) €			
+c0 / μc2 EX (flag μc) paret θ μc2 / μc2 / μc2 / μc2 μc2 / μc2			
agr1 est as2-1/apr1 est 0 mdT std i std / Cdd Tamb. / Etach € otf / Cdd			
her study Epitoprofess 20% in bobo() ont 22% to spread temperature.	Relative	Conquestigat	1.00 Lucr Outline With OPREVENTION
Arabidopsis thaliana (Top 50 most similar Perturbations)	milarity	Experiment #	⁵⁷ Fig S11: Differentially expressed genes
atgsnor1-3 / atgsnor1-1 0	1.055	AT-00393	in <i>p5cs1-4</i> stress vs. W.T. stress
atgsnor1-3 / Col-0 1	1.052	AT-00393	compared to public microarray data
sid2 / atgsnor1-1 0	1.048	AT-00393	using the Genevestigator Signature
atgsnor1-3 / Col-0 🕕	1.045	AT-00393	analysis tool.
35S:RPS4-HS eds1-2 / 35S:RPS4-HS (1)	1.045	AT-00665	Λ Heat map with the $n5cs1_{-}1$
eds16-1 / Col-0 🕕	1.044	AT-00421 AT-00614	A. Heat map with the pocsi-4
heat study 4 / untreated plant samples	1.043	AT-00387	expression data along the top and so
eds16-1 / Col-0 0	1.043	AT-00614	most similar datasets below. The most
sid2 / Col-0	1.040	AT-00407 AT-00393	similar data sets were identified using
high light study 7 (Col-0) / untreated leaf samples (Col-0)	1.039	AT-00618	the Euclidean Distance option in
sid2 / Col-0 🚯	1.039	AT-00313	Genevestigator signatures comparison
nigh light study / (AtWRKY63 OE1) / untreated leaf samples (AtWRKY63 (sid2 / atosnor1-1 1	0E1) 1.039 1.038	AT-00818 AT-00393	too. The <i>p5cs1-4</i> data was compared to
sid2-1 / Col-0 🚯	1.037	AT-00641	3283 "perturbations" (experiments
sid2-2 / Col-0 🚯	1.036	AT-00641	involving mutants, stress and hormone
355:RPS4-HS eds1-2 / 355:RPS4-HS 1	1.036	AT-00665 AT-00421	treatments)
Tsu-1 / Kas-1 🟮	1.036	AT-00512	treatments).
sid2 / ein2	1.035	AT-00406	B The 50 samples most similar to
BI-1 / Sha 😈 sid2 / coi1	1.034	AT-00407 AT-00406	p_{2} n_{2} n_{2} n_{3} n_{3
tcp2/tcp4ko / TCP4p::rTCP4:GFP	1.034	AT-00444	hottom) and in A The Consustingtor
Mir-0 / Mir-0/Se-0	1.034	AT-00102	bottom) and in A. The Genevestigator
npr1-1 / ein2 sid2 / Col-0	1.033	AT-00406 AT-00406	experiment number (AI-XXXXX) is listed
Col-2 / SQ-8 ()	1.033	AT-00612	for each.
npr1-1 / coi1	1.033	AT-00406	
nudt7-1 sid2-1 / nudt7-1 efr-1 / Col-0 🚯	1.032	AT-00421 AT-00597	The samples most similar to p5cs1-4
ga1 / Ler-0	1.032	AT-00131	were from experiments involving
drought study 2 (Col-0) / untreated leaf samples (Col-0)	1.032	AT-00292	Psuedomonas syringae innoculation of
atcesA4 / atcesA4 het.	1.031	AT-00426 AT-00406	atgsnor (S-nitrosoglutathione
oxt6:AtCPSF30 / Col-0	1.030	AT-00242	reductase) mutants (Genevestigator
jaw-D / Col-0	1.030	AT-00445	experiment number AT-00393. Other
sid2-2 / Col-0 🟮	1.030	AT-00581	experiments with some similarity in
sid2 / pad2	1.030	AT-00406	expression nattern compared to n5cs1-
BI-1 / Bay-0 parent 🕕	1.029	AT-00407	4 involved mutants involved in soliculis
ssi2-1 sid2-1 / ssi2-1	1.029	AT-00630	4 involved mutants involved in salicylic
npr1-1 sni1 ssn2-1 / npr1-1 sni1 🕕	1.029	AT-00001 AT-00471	acid (SA) synthesis (<i>sid2</i>) or SA-related
nudt7-1 sid2-1 eds1-2 / Col-0	1.029	AT-00421	signaling (<i>ssi2, npr1</i>) as well as other
Tamm-2 / Eden-1 🚯	1.028	AT-00613	pathogen-related signaling mutants or
oxt6 / Col-0 heat study 9 (photosynthesis 30% inhibited) / shift 22°C to optimal tempera	1.028 ature 1.027	AT-00242 AT-00500	overexpression lines (eds1, RPS4,
IAA study 7 (BI-1) / untreated seedling samples (BI-1)	1.027	AT-00407	nudt7).

und



Figure S12: RT-PCR check of lipid- and cuticle-related T-DNA mutants.

- A. RT-PCR analysis of T-DNA lines. Mutants used, and size of the fragment amplified are indicated. Primers used for the RT-PCR analysis are given in Supplemental Table S12.
- B. Amplification of Actin8 reference gene in all samples used in A.