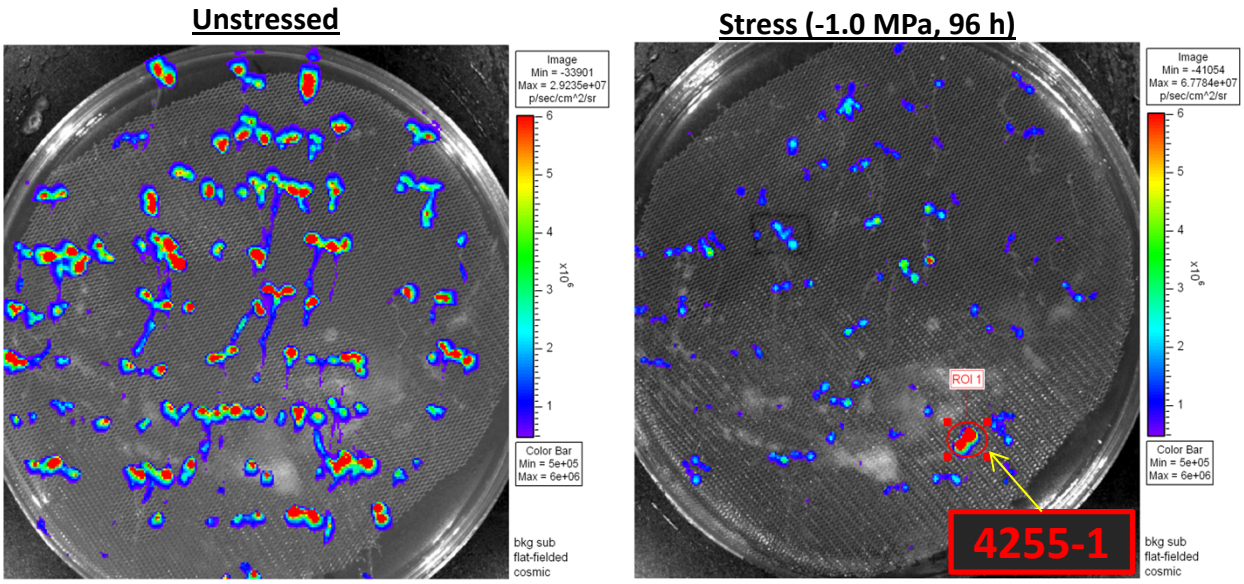
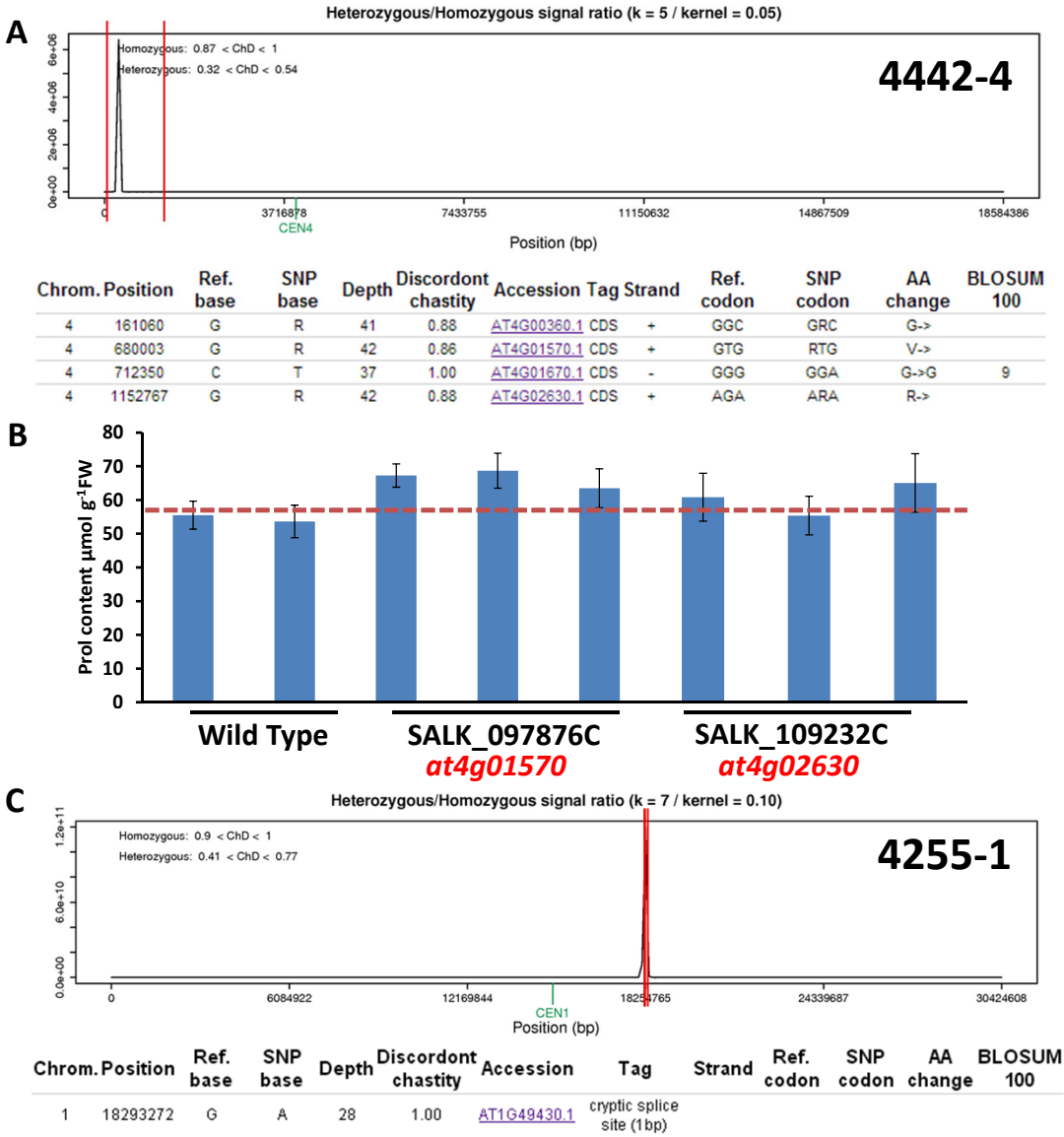


Figure S1: Example of primary screen plate images using EMS mutagenized *PDH1$_{pro}$:LUC2*.

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.



Chrom.	Position	Ref. base	SNP base	Depth	Discordont chastity	Accession	Tag	Strand	Ref. codon	SNP codon	AA change	BLOSUM 100
4	161060	G	R	41	0.88	AT4G00360.1	CDS	+	GGC	GRC	G->	
4	680003	G	R	42	0.86	AT4G01570.1	CDS	+	GTG	RTG	V->	
4	712350	C	T	37	1.00	AT4G01670.1	CDS	-	GGG	GGA	G->G	9
4	1152767	G	R	42	0.88	AT4G02630.1	CDS	+	AGA	ARA	R->	

Chrom.	Position	Ref. base	SNP base	Depth	Discordont chastity	Accession	Tag	Strand	Ref. codon	SNP codon	AA change	BLOSUM 100
1	18293272	G	A	28	1.00	AT1G49430.1	cryptic splice site (1 bp)					

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.

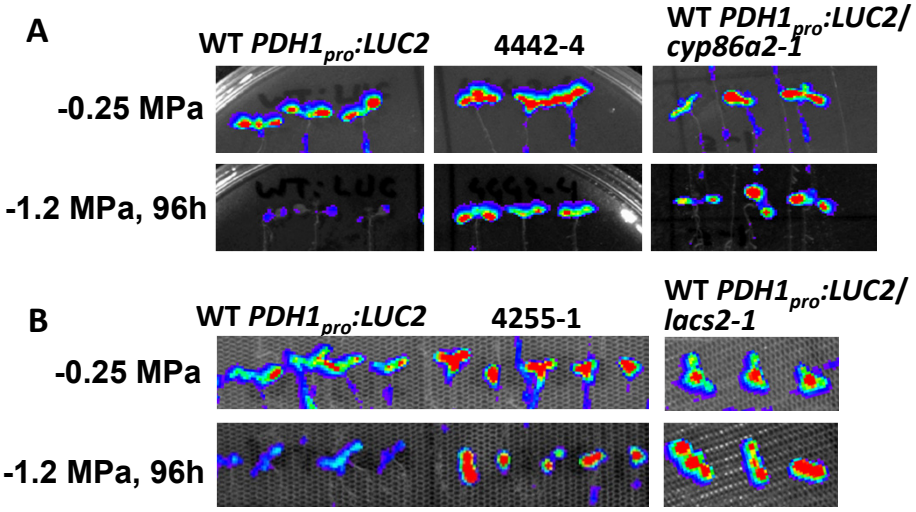


Figure S3: *PDH1*$_{pro}$*:LUC2* crossed to *cyp86a2-1* and *lacs2-1* T-DNA mutants shows the same high *PDH1*$_{pro}$*:LUC2* phenotype as the 4244-2 and 4255-2 EMS mutants

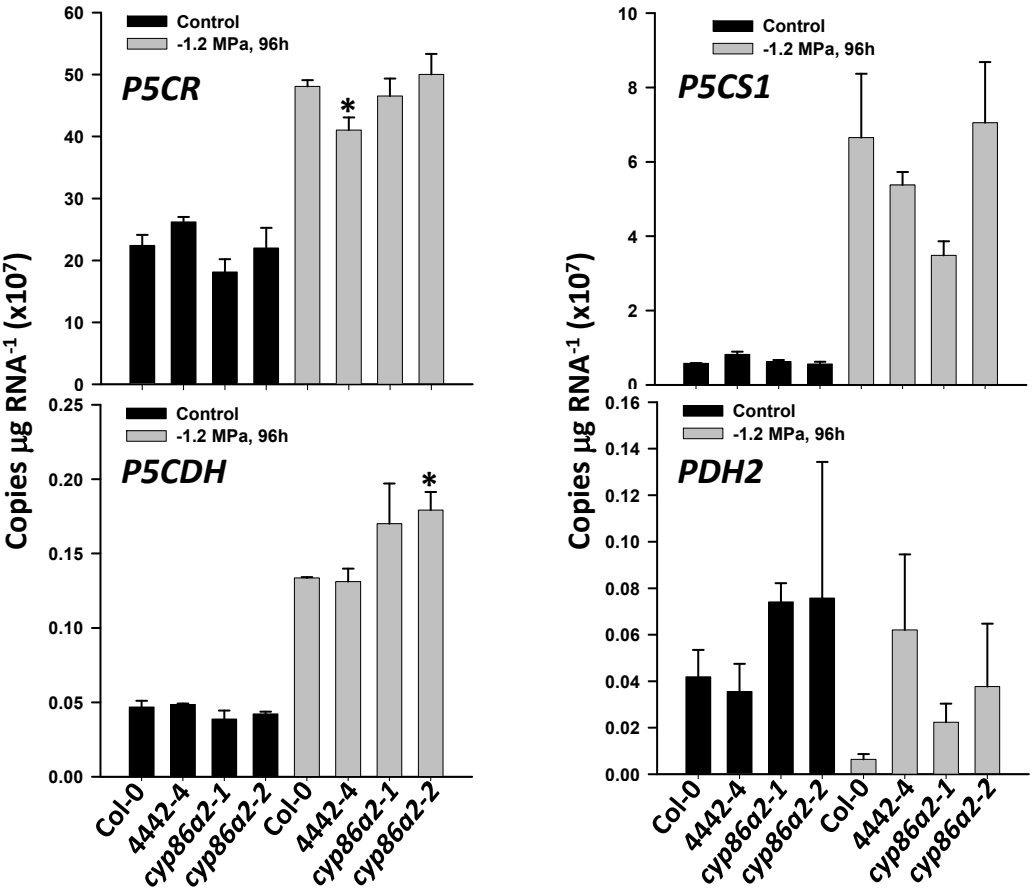


Figure S4: Expression of proline synthesis and catabolism genes in *cyp86a2* mutants. Data are means ± S.E. (n=3). Significant differences ($p \geq 0.05$) compared to wild type in the same treatment are marked (*).

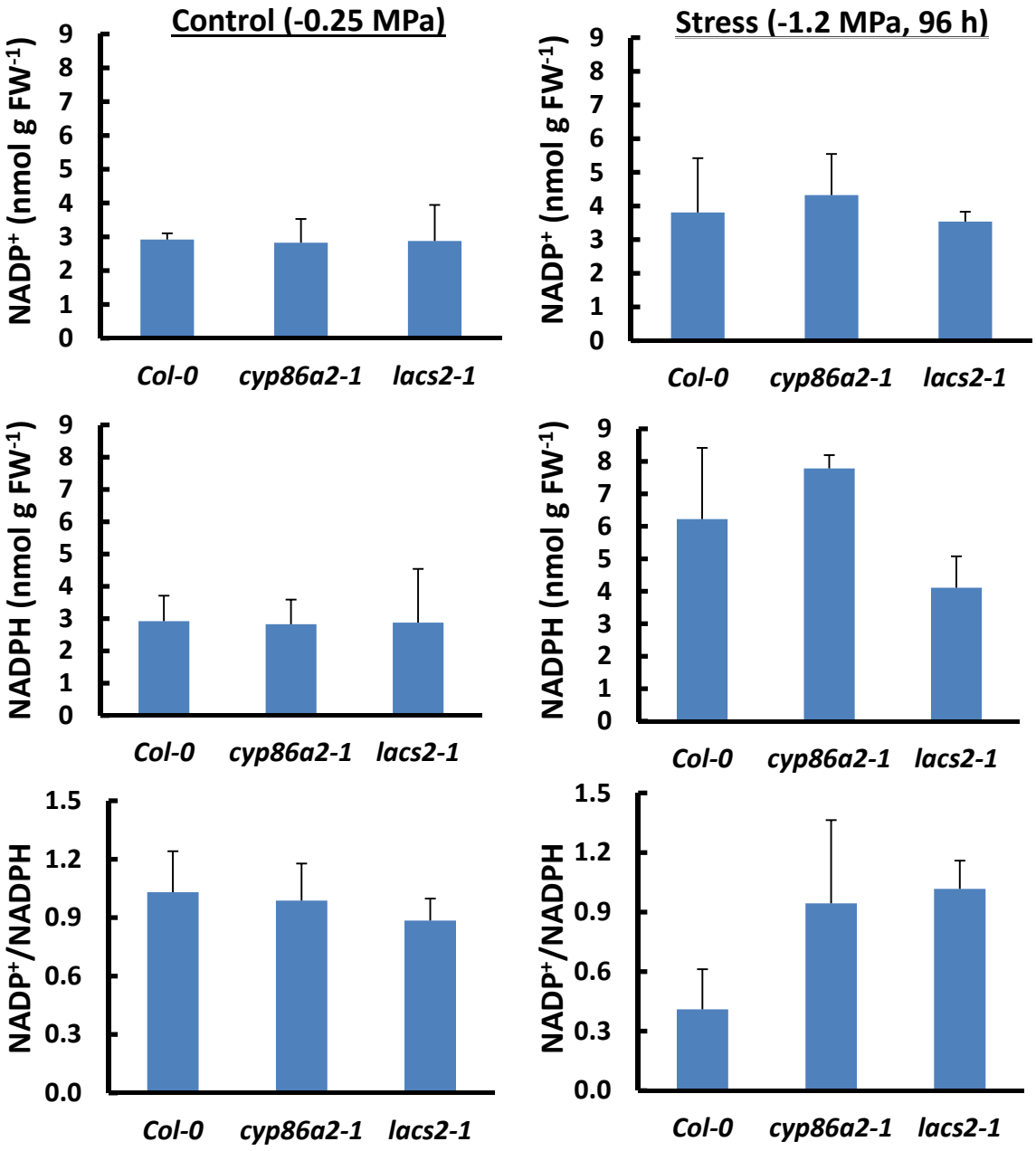


Figure S5: NADP⁺ and NADPH analysis of wild type, *cyp86a2-1* and *lacs2-1*. Data are means ± S.E. (n=2-6). There were no significant differences between mutants and wild type.

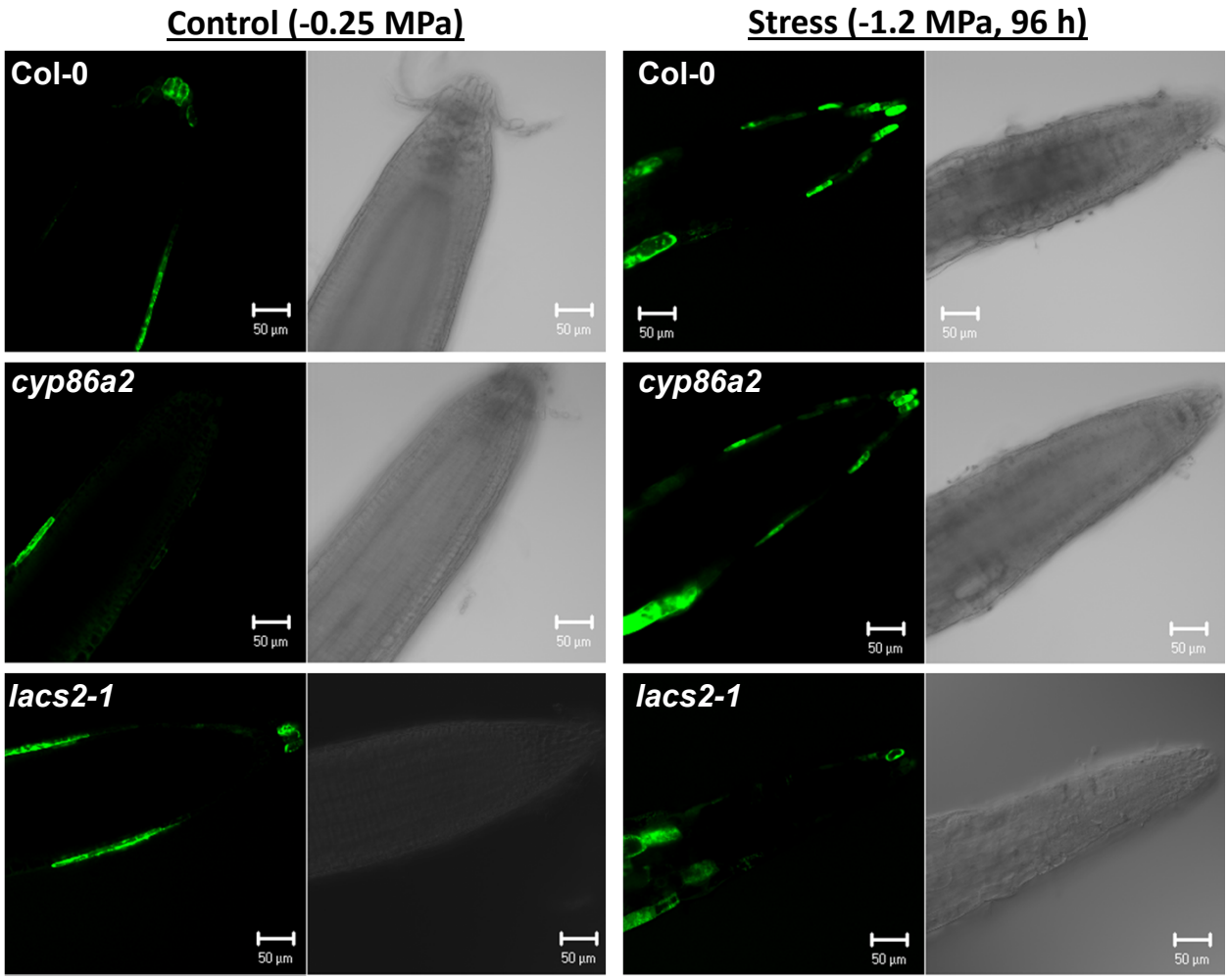


Figure S6: ROS levels of wild type, *cyp86a2-1* and *lacs2-1*. To investigate whether the mutants had greater overall ROS levels than wild type, plants were stained with the ROS sensitive dye H2DCFDA and root tips imaged. No substantial differences in H2DCFDA fluorescence was observed between wild type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

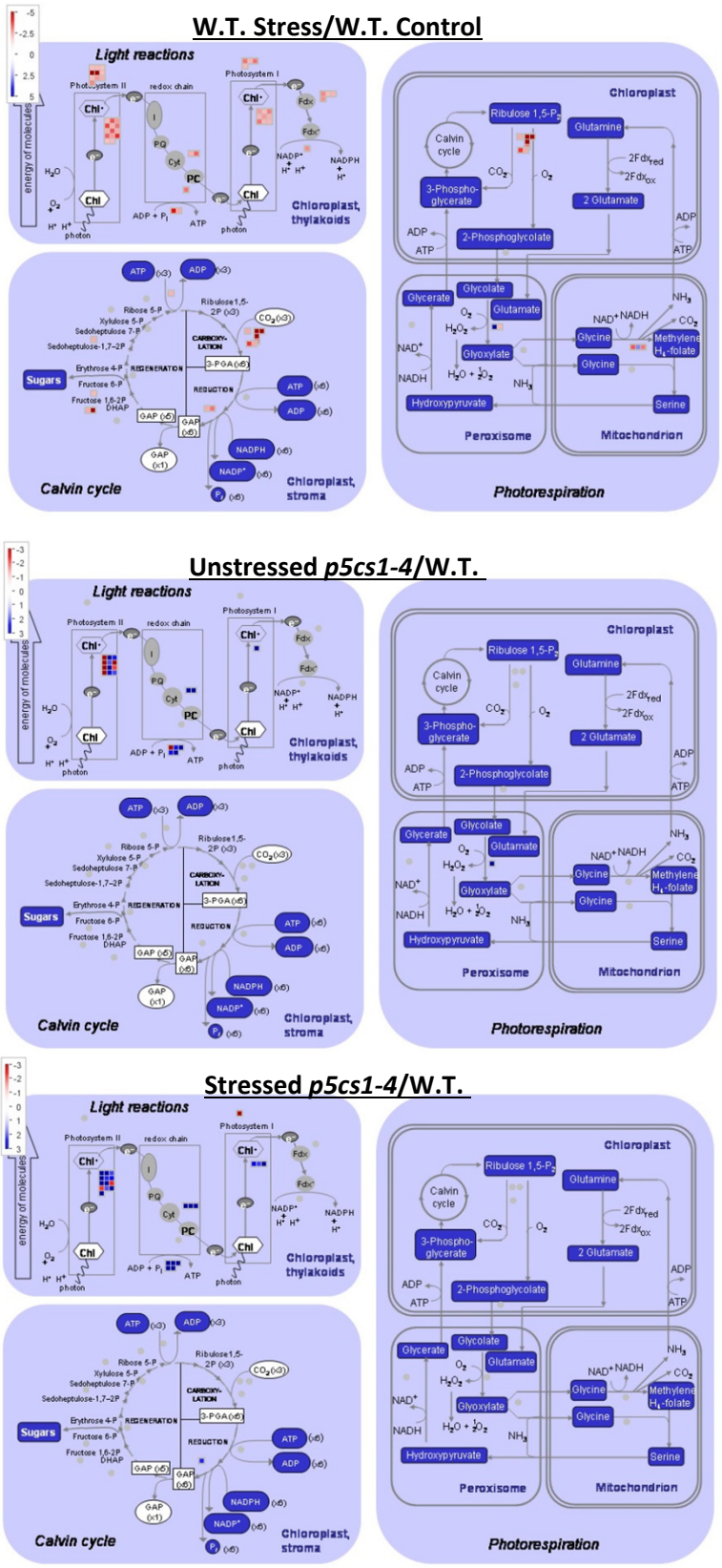
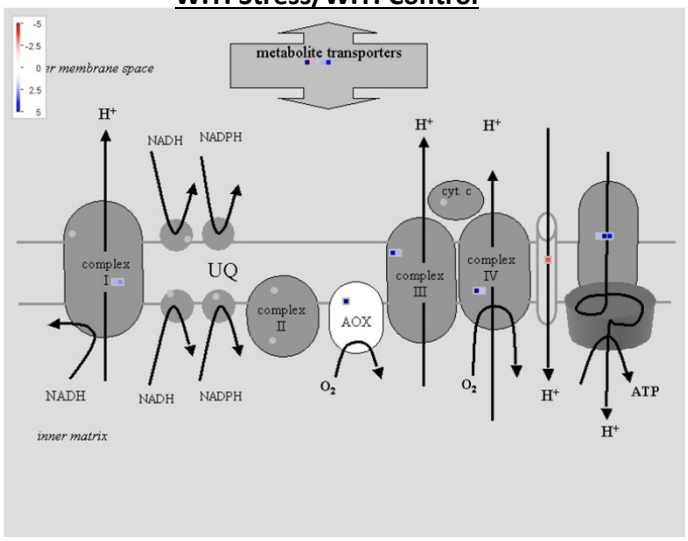


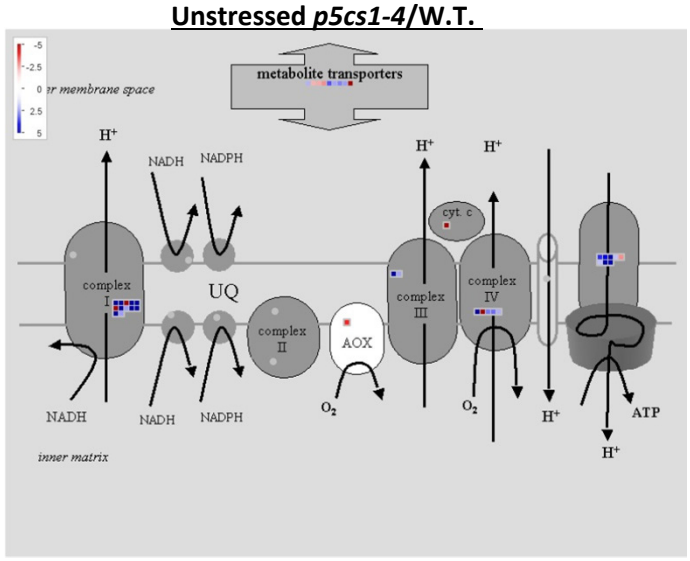
Figure S7: MapMan analysis of photosynthesis-related gene expression in stress and control treated wild type as well as *p5cs1-4* compared to wild type in unstressed and low water potential (-1.2 MPa, 96 h) treatments.

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.

W.T. Stress/W.T. Control



Unstressed *p5cs1-4*/W.T.



Stressed *p5cs1-4*/W.T.

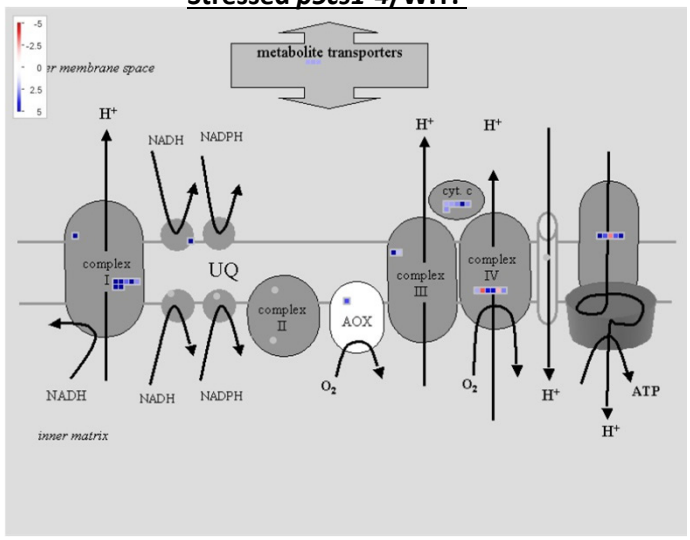


Figure S8: MapMan analysis of mitochondrial electron transport-related gene expression in stress and control treated wild type as well as *p5cs1-4* compared to wild type in unstressed and low water potential (-1.2 MPa, 96 h) treatments.

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.

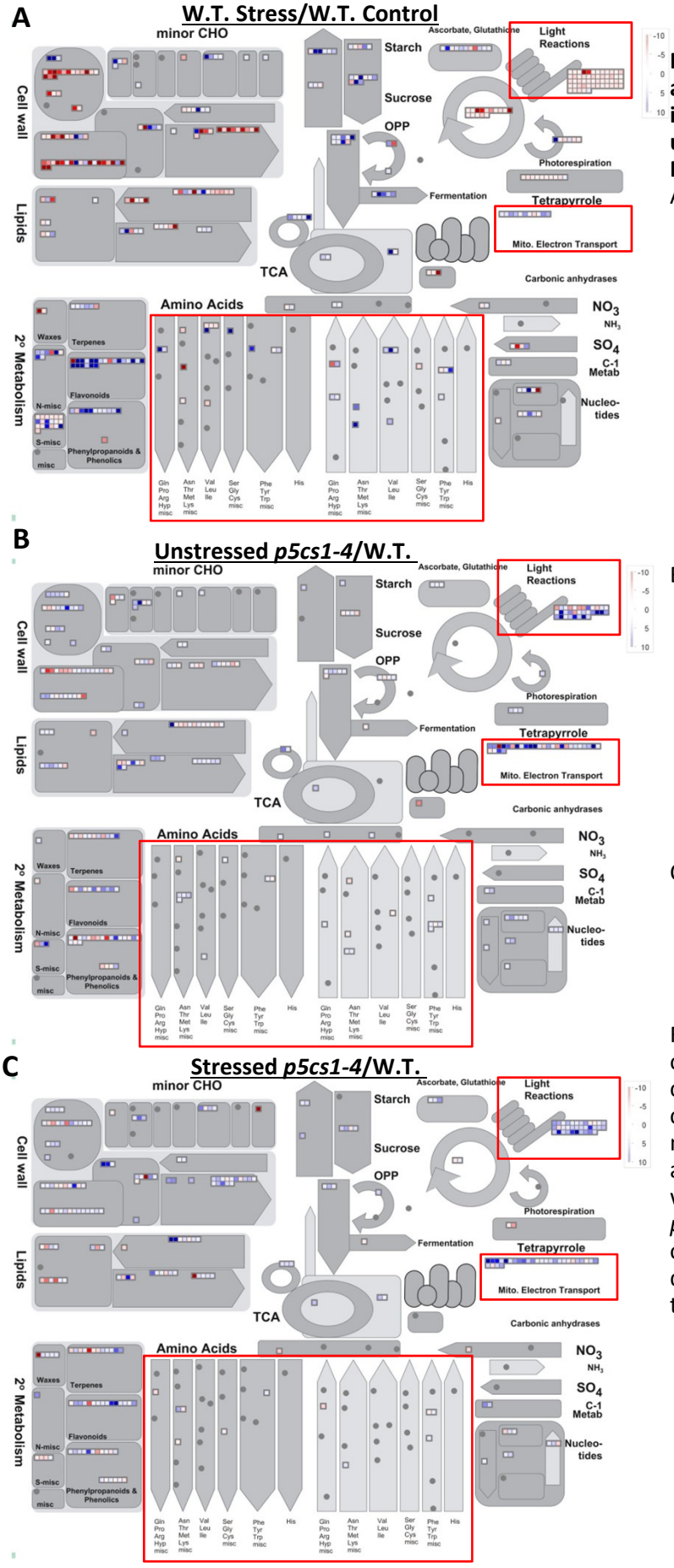


Figure S9: MapMan analysis of amino acid metabolism-related gene expression in *p5cs1-4* compared to wild type in unstressed and low water potential (-1.2 MPa, 96 h) treatments.

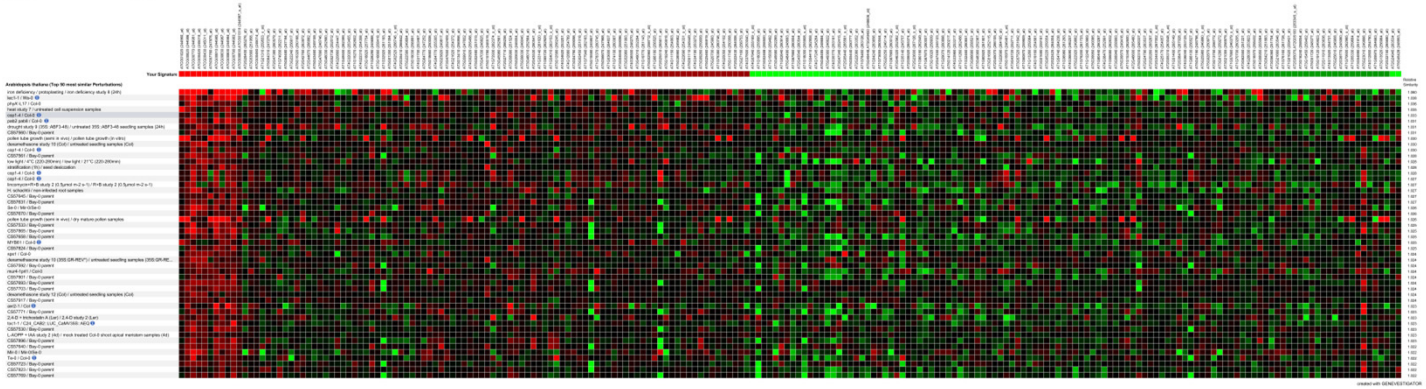
A. Wild type stress versus wild type control. The significantly up or down regulated genes (Tables S2 and S3) were used for analysis but using a 2-fold change for differentially expressed genes. This included 1981 genes with 411 metabolic related genes plotted on the general metabolic diagram shown here. Red boxes indicate metabolic processes of particular interest for comparison to *p5cs1-4* data (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 *p5cs1-4* on their expression was dramatically different than the effect of stress on wild type.

A



B

Arabidopsis thaliana (Top 50 most similar Perturbations)	Relative Similarity	Genevestigator Experiment #
iron deficiency / protoplasting / iron deficiency study 8 (24h)	1.060	AT-00286
lec1-1 / Ws-0	1.038	AT-00168
phyA'-L17 / Col-0	1.036	AT-00627
heat study 7 / untreated cell suspension samples	1.035	AT-00026
cop1-4 / Col-0	1.033	AT-00616
pab2 pab8 / Col-0	1.031	AT-00667
drought study 9 (35S::ABF3-48) / untreated 35S::ABF3-48 seedling samples (24h	1.031	AT-00422
CS57560 / Bay-0 parent	1.031	AT-00216
pollen tube growth (semi in vivo) / pollen tube growth (in vitro)	1.030	AT-00466
dexamethasone study 10 (Col) / untreated seedling samples (Col)	1.030	AT-00669
cop1-4 / Col-0	1.030	AT-00616
CS57561 / Bay-0 parent	1.028	AT-00216
low light / 4°C (220-280min) / low light / 21°C (220-280min)	1.028	AT-00467
stratification (1h) / seed desiccation	1.028	AT-00490
cop1-4 / Col-0	1.028	AT-00616
cop1-4 / Col-0	1.027	AT-00616
lincomycin+R+B study 2 (0.5µmol m-2 s-1) / R+B study 2 (0.5µmol m-2 s-1)	1.027	AT-00501
H. schachtii / non-infected root samples	1.027	AT-00024
CS57645 / Bay-0 parent	1.027	AT-00216
CS57631 / Bay-0 parent	1.027	AT-00216
Se-0 / Mir-0/Se-0	1.026	AT-00102
CS57670 / Bay-0 parent	1.026	AT-00216
pollen tube growth (semi in vivo) / dry mature pollen samples	1.026	AT-00466
CS57533 / Bay-0 parent	1.025	AT-00216
CS57865 / Bay-0 parent	1.025	AT-00216
CS57658 / Bay-0 parent	1.025	AT-00216
MYB61 / Col-0	1.025	AT-00015
CS57824 / Bay-0 parent	1.025	AT-00216
sps1 / Col-0	1.024	AT-00627
dexamethasone study 10 (35S:GR-REV*) / untreated seedling samples (35S:GR-	1.024	AT-00627
CS57592 / Bay-0 parent	1.024	AT-00216
mur4-1prl1 / Col-0	1.024	AT-00352
CS57901 / Bay-0 parent	1.024	AT-00216
CS57693 / Bay-0 parent	1.024	AT-00216
CS57703 / Bay-0 parent	1.024	AT-00216
dexamethasone study 12 (Col) / untreated seedling samples (Col)	1.024	AT-00669
CS57917 / Bay-0 parent	1.024	AT-00216
axr2-1 / Col	1.023	AT-00604
CS57771 / Bay-0 parent	1.023	AT-00216
2,4-D + trichostatin A (Ler) / 2,4-D study 2 (Ler)	1.023	AT-00525
toc1-1 / C24_CAB2::LUC_CaMV35S::AEQ	1.023	AT-00398
CS57530 / Bay-0 parent	1.023	AT-00216
L-AOPP + IAA study 2 (4d) / mock treated Col-0 shoot apical meristem samples (	1.023	AT-00658
CS57896 / Bay-0 parent	1.023	AT-00216
CS57640 / Bay-0 parent	1.022	AT-00216
Mir-0 / Mir-0/Se-0	1.022	AT-00102
Te-0 / Col-0	1.022	AT-00678
CS57723 / Bay-0 parent	1.022	AT-00216
CS57823 / Bay-0 parent	1.022	AT-00216
CS57769 / Bay-0 parent	1.022	AT-00216

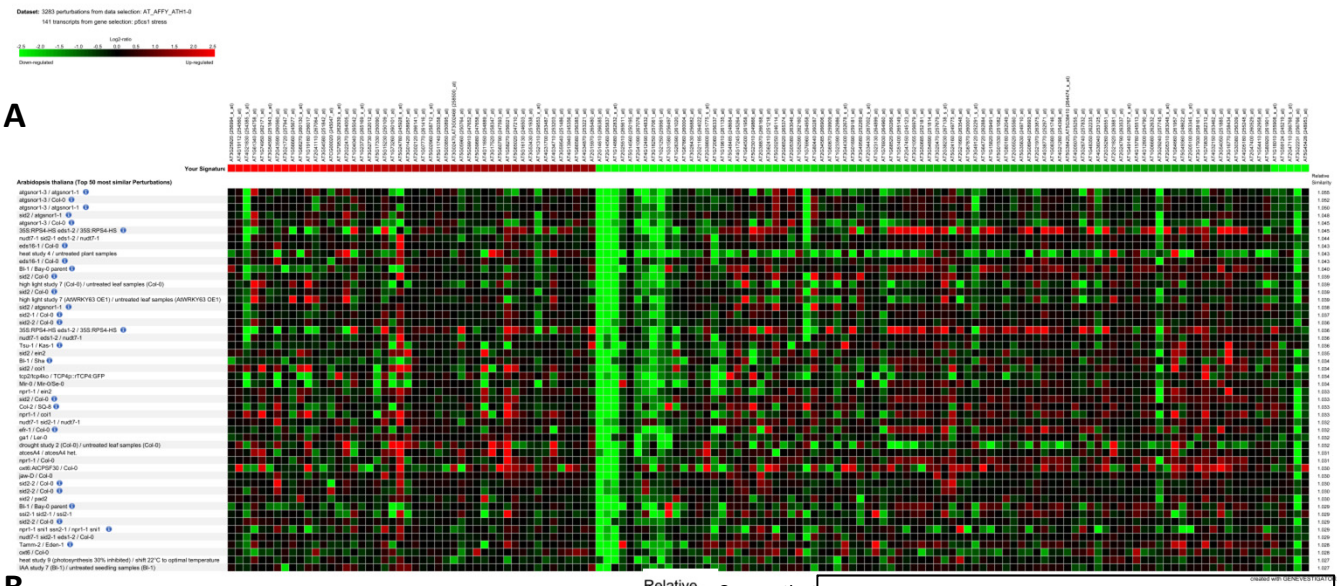
Fig S10: Differentially expressed genes in *p5cs1-4* control vs. W.T. control compared to public microarray data using the Genevestigator Signature analysis tool.

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).

A



B

Arabidopsis thaliana (Top 50 most similar Perturbations)

	Relative milarity	Genevestigator Experiment #
atgsnor1-3 / atgsnor1-1	1.055	AT-00393
atgsnor1-3 / Col-0	1.052	AT-00393
atgsnor1-3 / atgsnor1-1	1.050	AT-00393
sid2 / atgsnor1-1	1.048	AT-00393
atgsnor1-3 / Col-0	1.045	AT-00393
35S:RPS4-HS eds1-2 / 35S:RPS4-HS	1.045	AT-00665
nudt7-1 sid2-1 eds1-2 / nudt7-1	1.044	AT-00421
eds16-1 / Col-0	1.043	AT-00614
heat study 4 / untreated plant samples	1.043	AT-00387
eds16-1 / Col-0	1.043	AT-00614
Bl-1 / Bay-0 parent	1.040	AT-00407
sid2 / Col-0	1.039	AT-00393
high light study 7 (Col-0) / untreated leaf samples (Col-0)	1.039	AT-00618
sid2 / Col-0	1.039	AT-00313
high light study 7 (AtWRKY63 OE1) / untreated leaf samples (AtWRKY63 OE1)	1.039	AT-00618
sid2 / atgsnor1-1	1.038	AT-00393
sid2-1 / Col-0	1.037	AT-00641
sid2-2 / Col-0	1.036	AT-00641
35S:RPS4-HS eds1-2 / 35S:RPS4-HS	1.036	AT-00665
nudt7-1 eds1-2 / nudt7-1	1.036	AT-00421
Tsu-1 / Kas-1	1.036	AT-00512
sid2 / ein2	1.035	AT-00406
Bl-1 / Sha	1.034	AT-00407
sid2 / coi1	1.034	AT-00406
tcp2/tcp4ko / TCP4p::rTCP4:GFP	1.034	AT-00444
Mir-0 / Mir-0/Se-0	1.034	AT-00102
npr1-1 / ein2	1.033	AT-00406
sid2 / Col-0	1.033	AT-00406
Col-2 / SQ-8	1.033	AT-00612
npr1-1 / coi1	1.033	AT-00406
nudt7-1 sid2-1 / nudt7-1	1.032	AT-00421
efr-1 / Col-0	1.032	AT-00597
ga1 / Ler-0	1.032	AT-00131
drought study 2 (Col-0) / untreated leaf samples (Col-0)	1.032	AT-00292
atcesA4 / atcesA4 het.	1.031	AT-00426
npr1-1 / Col-0	1.031	AT-00406
oxt6:AtCPSF30 / Col-0	1.030	AT-00242
jaw-D / Col-0	1.030	AT-00445
sid2-2 / Col-0	1.030	AT-00581
sid2-2 / Col-0	1.030	AT-00581
sid2 / pad2	1.030	AT-00406
Bl-1 / Bay-0 parent	1.029	AT-00407
ssi2-1 sid2-1 / ssi2-1	1.029	AT-00630
sid2-2 / Col-0	1.029	AT-00661
npr1-1 sni1 ssn2-1 / npr1-1 sni1	1.029	AT-00471
nudt7-1 sid2-1 eds1-2 / Col-0	1.029	AT-00421
Tamm-2 / Eden-1	1.028	AT-00613
oxt6 / Col-0	1.028	AT-00242
heat study 9 (photosynthesis 30% inhibited) / shift 22°C to optimal temperature	1.027	AT-00500
IAA study 7 (Bl-1) / untreated seedling samples (Bl-1)	1.027	AT-00407

Fig S11: Differentially expressed genes in *p5cs1-4* stress vs. W.T. stress 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 too. 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.

The samples most similar to *p5cs1-4* were from experiments involving *Psuedomonas syringae* innoculation of *atgsnor* (S-nitrosoglutathione reductase) mutants (Genevestigator experiment number AT-00393. Other experiments with some similarity in expression pattern compared to *p5cs1-4* involved mutants involved in salicylic acid (SA) synthesis (*sid2*) or SA-related signaling (*ssi2, npr1*) as well as other pathogen-related signaling mutants or overexpression lines (*eds1, RPS4, nudt7*).

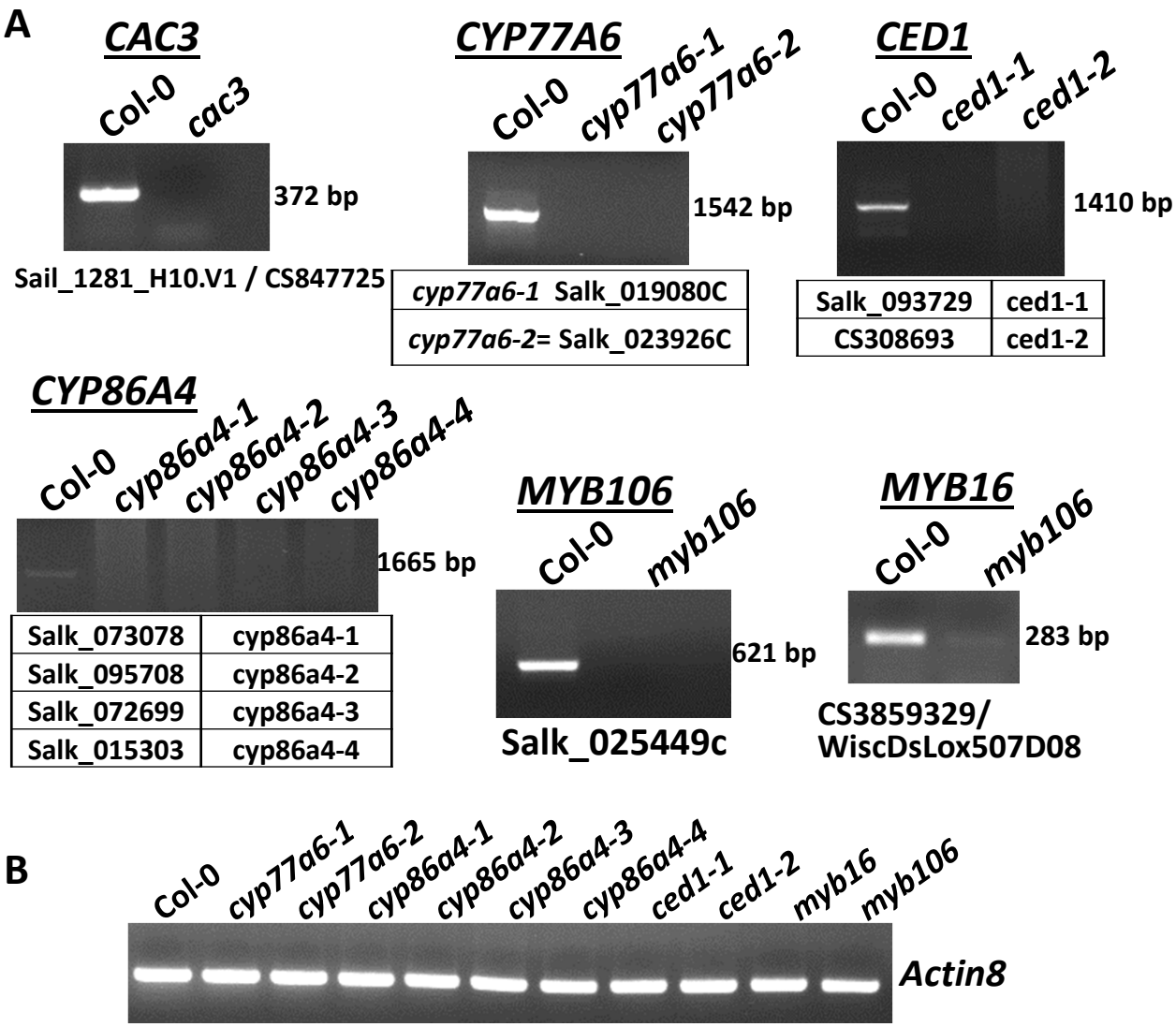


Figure S12: RT-PCR check of lipid- and cuticle-related T-DNA mutants.

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.