

SUPPLEMENTAL MATERIAL

Regulation of cyclic electron flow by chloroplast NADPH-dependent thioredoxin system

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Short title: Redox-regulation of the thylakoid NDH complex

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Molecular Plant Biology

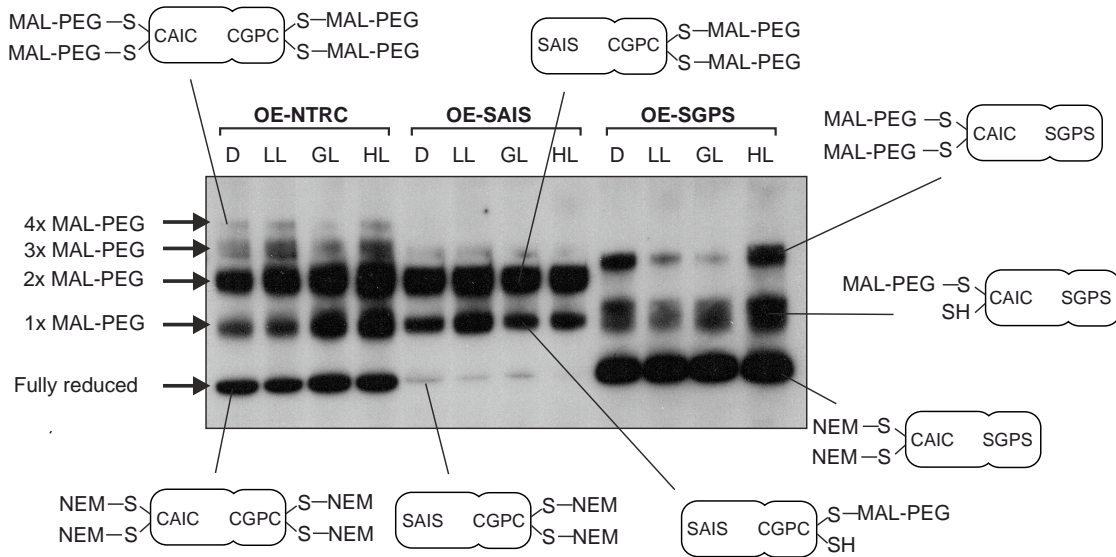
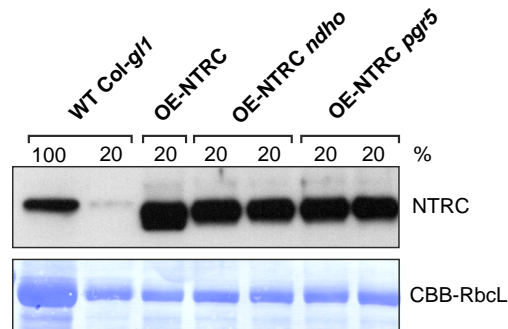
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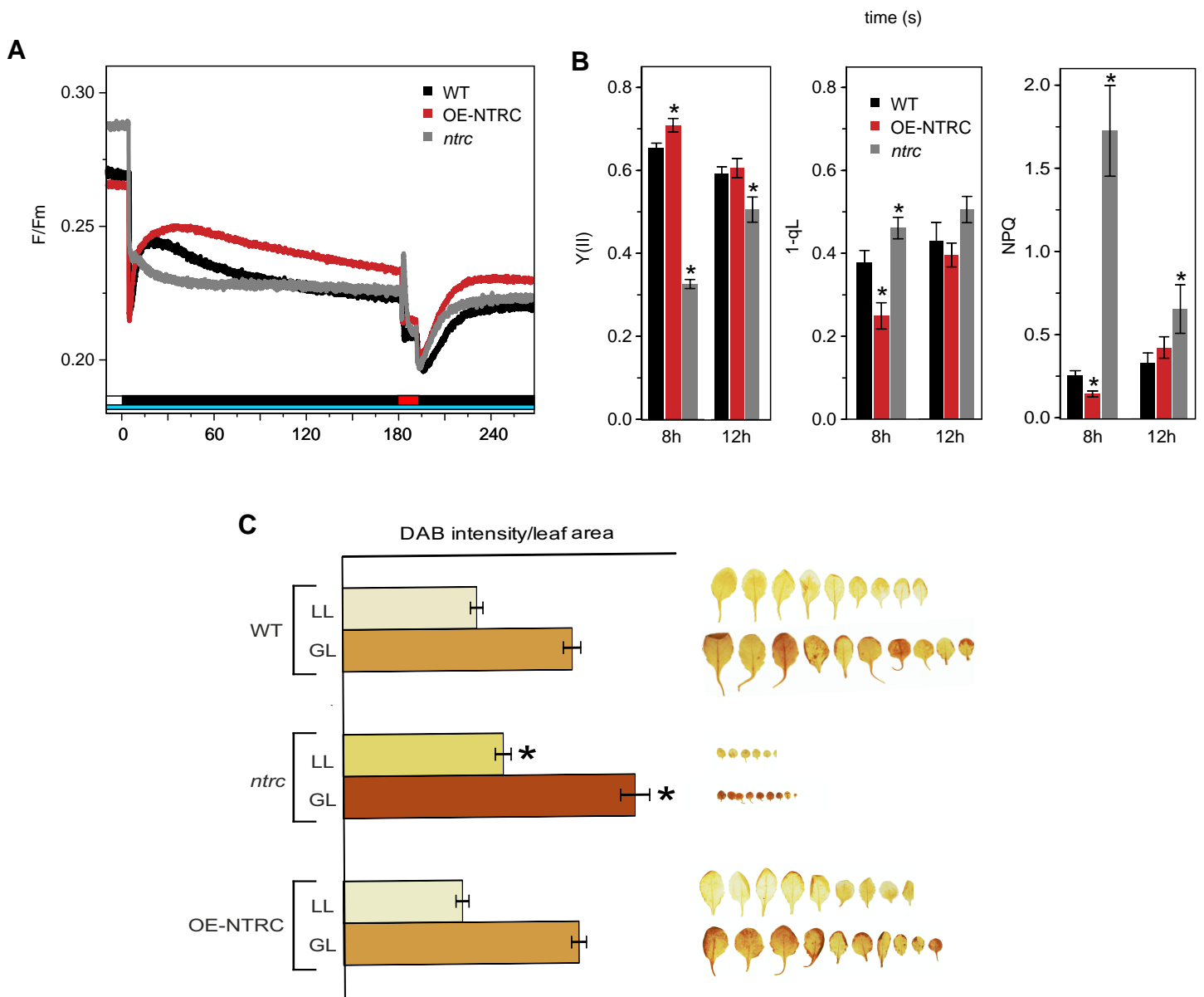
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A**B**

Supplemental Figure S1. Redox state and expression of NTRC in transgenic lines.

(A) Redox-state pattern of NTRC in leaves overexpressing NTRC. Total leaf proteins were extracted from leaves overexpressing NTRC (OE-NTRC) and from lines overexpressing mutated forms of NTRC where the redox-active cysteines in either the reductase domain (OE-SAIS) or in the TRX domain (OE-SGPS) have been mutated to serines (Toivola et al. 2013). In the mutated forms only 2 cysteine residues are available for alkylation by MAL-PEG molecules. The schematic drawings depict possible locations of MAL-PEG binding in specific bands. Leaves were incubated in darkness (D), or illuminated for 2 h in low light (LL, 40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), growth light (GL, 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) or high light (HL, 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$).

(B) Level of NTRC overexpression in OE-NTRC *ndho* and OE-NTRC *pgr5* plants detected by immunoblotting with an NTRC-specific antibody. 10 and 2 μg of soluble protein was loaded for WT and 2 μg for OE-NTRC, OE-NTRC *ndho* and OE-NTRC *pgr5*. Two individual plants were sampled for OE-NTRC *ndho* and OE-NTRC *pgr5*. Coomassie brilliant blue (CBB) staining was used as loading control.

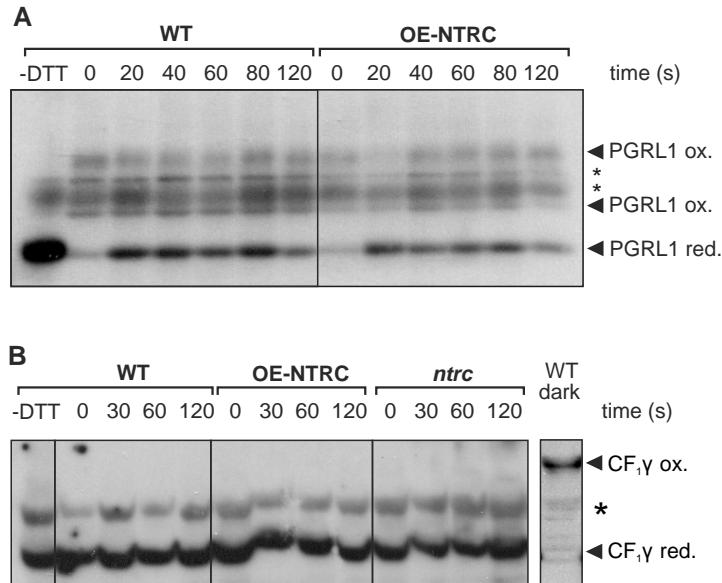


Supplemental Figure S2. Post-illumination fluorescence rise (PIFR) in plants grown in a 12h/12h photoperiod.

(A) PIFR in dark-adapted WT, OE-NTRC and *ntrc* plants grown in a 12h/12h photoperiod under 120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The cyan bar indicates exposure to a 480 nm measuring light of 0.28 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, the white bar depicts illumination with 67 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ white light and the red bar shows the duration of a pulse of far red light. The curves are averages of measurements from 3–4 individual leaves.

(B) Quantum yield of PSII (Y(II)), redox state of the PQ pool (1-qL) and non-photochemical quenching (NPQ) at Fm' (after 7 min illumination) in plants grown either in an 8h or 12h photoperiod. The values are averages of 3–10 measurements \pm SE, and statistically significant differences to WT according to Student's T-tests ($P < 0.05$) are marked with *.

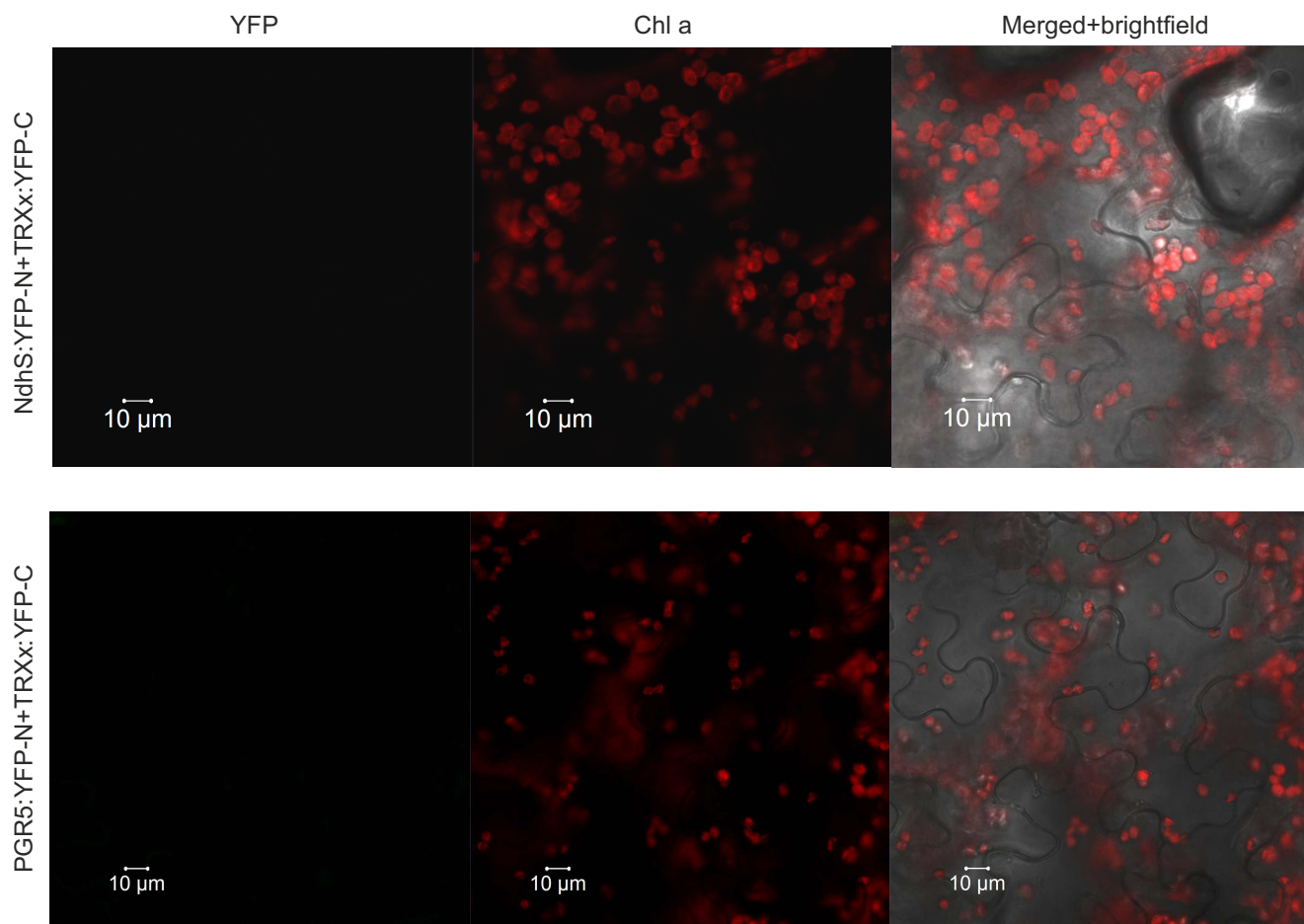
(C) Estimation of H_2O_2 content in WT, *ntrc* and OE-NTRC leaves after 1h illumination in low (LL) or growth light intensity (GL) based on the intensity of DAB staining. Values are represented as weighted (based on leaf area) averages of quantified DAB intensity from 12–19 individual leaves from 2 biological replicates \pm SE, and statistically significant differences to WT according to Student's T-tests ($P < 0.05$) are marked with *. Representative photographs of DAB-stained leaves are shown on the right.



Supplemental Figure S3. *In vivo* redox states of PGRL1 and CF₁γ during changes in light conditions.

(A) MAL-PEG assay to determine the *in vivo* redox state of PGRL1 during dark to light transitions. Samples were collected after illumination of dark-adapted leaves for 20, 40, 60, 80 and 120 seconds under growth light. -DTT is a control sample where free thiols were blocked with NEM but DTT was not added thereafter, preventing any alkylation by MAL-PEG.

(B) *In vivo* redox state of the ATP synthase γ subunit (CF₁γ) during transitions from low light (40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) to high light (600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) in WT, OE-NTRC and *ntrc* leaves. Prior to the light intensity shift plants grown under 200 $\mu\text{mol photons l m}^{-2} \text{s}^{-1}$ were kept 30 min under low irradiance. Samples were taken after illumination for 0, 30, 60 and 120 seconds in high light. A dark-incubated control (WT dark) is seen in the right panel.* marks unspecific bands.



Supplemental Figure S4. Bimolecular fluorescence complementation (BiFC) tests between TRX-x and NdhS and PGR5.

The left panel shows yellow fluorescent protein (YFP) fluorescence in green, the middle panel Chlorophyll a autofluorescence in red and the right panel a merged image of YFP, chlorophyll and brightfield images. YFP-N and YFP-C indicate expression of fusion proteins including the N-terminal and C-terminal parts of YFP, respectively, in tobacco (*Nicotiana benthamiana*) leaves.

Supplemental Table S1. Parameters determined from OJIP transients of chlorophyll a fluorescence.

Apparent F_0 values (F/F_m after 20 μ s of light), integrated area above O-J phase (0-3 ms) (A_{O-J}) and the initial slopes of the O-J transients ($m_{50-150 \mu s}$) in dark-adapted leaves of WT, OE-NTRC, *ntrc*, *pgr5* and *ndho* were calculated from the averaged curves in Figure 3.

line	F_0 ($F_{20\mu s}$)	A_{O-J}	$m_{50-150 \mu s}$
WT	0.18	1.62	0.58
OE-NTRC	0.21	1.46	0.74
<i>ntrc</i>	0.26	1.29	1.20
<i>pgr5</i>	0.21	1.84	0.47
<i>ndho</i>	0.17	1.76	0.57

Supplemental Table S2. Screening of putative NTRC target proteins by Co-IP/MS.

One hundred chloroplast proteins present in WT and/or OE-NTRC eluates but absent from *ntrc* eluates are listed in this table in the order of their abundance in the Co-IP eluates (Supplemental Dataset 1). At least two unique peptides were detected of each protein. Previously published TRX targets are highlighted with blue (articles 3, 5, 6, 7, 8, and 9 in Suppl. references) and established NTRC interactors with red colour (articles 1, 2, 4, 10, and 11 in Suppl. references), while proteins involved in CEF are in bold. Functions of the proteins are provided based on the annotation in the TAIR database.

#	AGI code	Annotation	<i>ntrc</i>	WT	OE-NTRC	Function
1	ATCG01110.1	NAD(P)H dehydrogenase subunit H	-	+	+	Cyclic electron flow
2	AT1G15980.1	NDH-dependent cyclic electron flow 1 (Ndh48)	-	+	+	Cyclic electron flow
3	AT3G11630.1	2-Cysteine peroxiredoxin A/B ⁽¹⁾	-	+	+	Redox regulation
4	AT4G25080.4	magnesium-protoporphyrin IX methyltransferase ⁽²⁾	-	+	+	Chl biosynthesis
5	AT5G43780.1	sulfate adenyltransferase, ATP sulfurylase (APS4, ATPS4)	-	+	+	Sulfate assimilation, AA synthesis
6	AT3G27925.1	DegP protease 1 ⁽³⁾	-	+	+	proteolysis
7	AT2G42220.1	Rhodanese/Cell cycle control phosphatase superfamily protein	-	+	+	Cell cycle
8	AT2G40100.1	LHCb4.3, LIGHT HARVESTING COMPLEX PHOTOSYSTEM II	-	+	+	Photosynthesis
9	ATCG01120.1	chloroplast ribosomal protein S15	-	+	+	Translation
10	AT4G17090.1	chloroplast beta-amylase ⁽⁴⁾	-	+	+	Starch degradation
11	AT1G69830.1	alpha-amylase-like 3 ⁽⁵⁾	-	+	+	Starch degradation
12	AT5G53580.1	ATPLR1, PLR1, PYRIDOXAL REDUCTASE 1	-	+	+	Carbon metabolism
13	AT5G03880.1	Thioredoxin family protein	-	+	+	Redox regulation
14	AT3G55610.1	delta 1-pyrroline-5-carboxylate synthase 2	-	+	+	AA biosynthesis
15	AT1G70820.1	phosphoglucomutase, putative	-	+	+	Carbon metabolism
16	AT4G22710.1	cytochrome P450, family 706, subfamily A, polypeptide 2	-	+	+	Secondary metabolism
17	AT3G54110.1	plant uncoupling mitochondrial protein 1	-	+	+	Photorespiration
18	AT4G05180.1	photosystem II subunit Q-2	-	+	+	Photosynthesis
19	AT2G04842.1	threonyl-tRNA synthetase, putative / threonine--tRNA ligase, putative	-	+	+	Translation
20	AT5G37510.1	NADH-ubiquinone dehydrogenase, mitochondrial, putative	-	+	+	Mt respiration
21	AT5G65220.1	Ribosomal L29 family protein	-	+	+	Translation
22	AT4G29670.1	ACHT2, ATYPICAL CYS HIS RICH THIOREDOXIN 2	-	+	+	Redox regulation
23	AT4G23650.1	CALCIUM-DEPENDENT PROTEIN KINASE 6, CDPK6, CPK3	-	+	+	Photosynthesis
24	AT1G58290.1	Glutamyl-tRNA reductase family protein; GLUTR, HEMA1 ⁽²⁾	-	+	+	Chl biosynthesis
25	AT1G58080.1	ATP phosphoribosyl transferase 1	-	+	+	AA biosynthesis
26	AT3G24430.1	HCF101	-	+	+	Photosynthesis
27	AT2G32730.1	26S proteasome regulatory complex, Rpn2/Psm1 subunit	-	+	+	Proteolysis
28	AT5G11880.1	Pyridoxal-dependent decarboxylase family protein	-	+	+	aa synthesis
29	AT1G63970.2	2C-METHYL-D-ERYTHRITOL 2,4-CYCLODIPHOSPHATE SYNTHASE, MECPS	-	+	+	Carbon & secondary metabolism
30	AT5G40950.1	ribosomal protein large subunit 27	-	+	+	Translation
31	AT5G03290.1	isocitrate dehydrogenase V, IDH-V	-	+	+	Carbon metabolism
32	AT5G65840.1	Thioredoxin superfamily protein	-	+	+	Redox regulation
33	AT4G23890.1	CHLORORESPIRATORY REDUCTION 31, CRR31, NdhS	-	+	+	Cyclic electron flow
34	ATCG00330.1	chloroplast ribosomal protein S14	-	+	+	Translation
35	AT1G34000.1	OHP2, ONE-HELIX PROTEIN 2	-	+	+	Photosynthesis
36	AT1G18500.1	IPMS1, ISOPROPYLMALATE SYNTHASE 1, MAML-4,	-	+	+	AA synthesis
37	AT2G28800.4	ALB3, ALBINO 3	-	+	+	Cplast biogenesis & senescence
38	AT5G08540.1	unknown protein	-	+	+	Unknown
39	AT1G08520.1	CHLD, ALB-1V, ALB1, ALBINA 1, PDE166	-	-	+	Chl biosynthesis
40	AT1G06680.2	photosystem II subunit P-1	-	+	+	Photosynthesis
41	AT5G26830.1	Threonyl-tRNA synthetase	-	+	+	Translation
42	AT4G15110.1	cytochrome P450, family 97, subfamily B, polypeptide 3	-	+	-	Carbon metabolism
43	AT5G66420.1	unknown protein	-	+	-	Unknown
44	AT1G33780.1	unknown protein	-	+	+	Unknown
45	AT1G45201.2	triacylglycerol lipase-like 1	-	+	+	Lipid metabolism
46	AT1G48350.1	Ribosomal L18p/L5e family protein	-	+	+	Translation
47	AT1G09620.1	leucine-tRNA ligase	-	+	+	Translation

48	AT1G64190.1	6-PHOSPHOGLUCONATE DEHYDROGENASE 1, PGD1 ⁽⁶⁾	-	+	-	Carbon metabol.
49	AT5G14320.1	Ribosomal protein S13/S18 family	-	+	-	Translation
50	AT5G19940.2	Plastid-lipid associated protein PAP / fibrillin family protein	-	+	+	Lipid metabolism
51	AT1G50450.1	Saccharopine dehydrogenase	-	+	+	
52	AT3G20680.1	unknown protein	-	+	+	unknown
53	AT1G77060.1	Phosphoenolpyruvate carboxylase family protein	-	+	+	Carbon metabolism
54	AT5G27380.1	glutathione synthetase 2, GSH2	-	+	+	Redox regulation
55	AT1G51110.1	Plastid-lipid associated protein PAP / fibrillin family protein	-	+	+	AA biosynthesis
56	AT3G10940.1	dual specificity protein phosphatase (DsPTP1) family protein	-	+	+	Starch degradation
57	AT5G47890.1	NADH-ubiquinone oxidoreductase B8 subunit, putative	-	+	+	Mitochondrial respiration
58	AT1G10760.1	GWD, GWD1, SEX1, SOP, SOP1, STARCH EXCESS 1 ⁽⁷⁾	-	+	-	Starch degradation
59	AT4G26900.1	HIS HF, HISN4	-	+	+	AA synthesis
60	AT4G21280.1	photosystem II subunit QA	-	-	+	Photosynthesis
61	ATCG00820.1	ribosomal protein S19	-	+	+	Translation
62	AT2G34460.1	NAD(P)-binding Rossmann-fold superfamily protein	-	+	+	unknown
63	AT1G56500.1	SOQ1, SUPPRESSOR OF QUENCHING 1	-	+	+	Redox regulation
64	AT5G54600.1	PLASTID RIBOSOMAL PROTEIN L24, RPL24	-	+	+	translation
65	AT2G21170.1	triosephosphate isomerase, TPI ⁽⁶⁾	-	+	-	Carbon metabolism
66	AT4G35630.1	phosphoserine aminotransferase	-	+	-	AA synthesis
67	AT4G31780.2	monogalactosyl diacylglycerol synthase 1, MGD1, MGDA ⁽⁸⁾	-	+	-	Lipid metabolism
68	AT3G04870.1	zeta-carotene desaturase, ZDS	-	+	+	Carbon metabolism, secondary metabolism
69	AT5G14060.1	lysine-sensitive aspartate kinase CARAB-AK-LYS	-	-	+	AA synthesis
70	AT1G80480.1	plastid transcriptionally active 17	-	-	+	unknown
71	AT1G75460.1	ATP-dependent protease La (LON) domain protein	-	-	+	Proteolysis
72	AT1G18060.1	unknown protein	-	+	+	Unknown
73	AT1G50200.2	Alanyl-tRNA synthetase	-	+	+	Translation
74	AT2G30790.1	photosystem II subunit P-2	-	+	+	Photosynthesis
75	AT3G54210.1	Ribosomal protein L17 family protein	-	+	-	Translation
76	AT3G01440.1	PsbQ-like 2	-	+	-	Cyclic electron flow
77	AT1G02560.1	nuclear encoded CLP protease 5	-	+	+	Proteolysis
78	AT2G15620.1	nitrite reductase 1, NIR1 ⁽⁹⁾	-	+	+	Nitrogen metabolism
79	AT5G18660.1	PALE-GREEN AND CHLOROPHYLL B REDUCED 2, PCB2	-	+	+	Chl biosynthesis
80	AT3G59780.1	Rhodanese/Cell cycle control phosphatase superfamily protein	-	+	+	Cell cycle
81	AT1G74880.1	NAD(P)H:plastoquinone dehydrogenase complex subunit O	-	+	+	Cyclic electron flow
82	AT5G36170.2	high chlorophyll fluorescent 109, HCF109	-	+	+	
83	AT1G16880.1	ACR11, ACT DOMAIN REPEATS 11	-	+	+	AA metabolism
84	AT5G24300.1	ATSS1, SS1, STARCH SYNTHASE 1 ⁽¹⁰⁾	-	+	-	Starch synthesis
85	AT1G78140.1	S-adenosyl-L-methionine-dependent methyltransferases superfamily	-	+	-	
86	AT4G25450.3	non-intrinsic ABC protein 8	-	+	-	unknown
87	AT5G21430.2	NADH dehydrogenase-like complex U, NdhU	-	+	-	Cyclic electron flow
88	AT1G16410.2	BUS1, BUSHY 1, CYP79F1, CYTOCHROME P450 79F1, SPS1	-	+	-	Secondary metabolism
89	AT2G05620.1	PROTON GRADIENT REGULATION 5, PGR5	-	+	-	Cyclic electron flow
90	AT3G06050.1	peroxiredoxin IIF	-	+	+	Redox regulation
91	AT2G32920.1	PDI-like 2-3, Protein disulfide-isomerase 2-3	-	+	+	Redox regulation
92	AT4G12830.1	alpha/beta-Hydrolases superfamily protein	-	+	+	unknown
93	AT3G55250.1	Putative calcium homeostasis regulator	-	-	+	Signalling
94	AT3G53900.1	uracil phosphoribosyltransferase	-	-	+	
95	AT1G77490.1	thylakoidal ascorbate peroxidase	-	+	+	Redox regulation
96	AT5G11450.1	PsbP domain-containing protein 5, PPD5	-	-	+	
97	AT4G34120.1	Cystathionine beta-synthase (CBS) family protein, CBSX2 ⁽¹¹⁾	-	-	+	Redox regulation
98	AT1G31800.1	LUTEIN DEFICIENT 5	-	+	+	Carotenoid biosynthesis
99	AT3G02780.1	isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase 2	-	+	+	Chl biosynthesis
100	AT4G37200.1	Thioredoxin superfamily protein HCF164	-	+	+	Redox regulation

Synechocystis ECYDELDWEVQYETAGDCFARYLVRIEMRESVKIIRQALKAMPGGPYENLEAKRLQEGK
Physcomitrella ECYDELDWQIQWQKEGDSLARYLVRIEMKESIKIIQQALKSIPGGPYENLEARRLQRGK
Sorghum ESYNQFDWKVQWQKEGDSLARYLVRVGEMRESIKIIQQAVEKIPGGPYENLEARRFKKAK
Brachypodium ESYNQFDWKVQWQKEGDSLARYLVRIEMRESIKIIQQAVEKIPGGPYENLEVRRFKKEK
Oryza ESYNQFDWKVQWQKEGDSLARYLVRIEMRESIKIIQQAVEKIPGGPYENLEVRRFKKAK
Glycine ECYEFDWEVQWQKEGDSLARYLVRIEMMESIKIIQQALEGIPGGPYENLEIRCFDREK
Arabidopsis **ESYDEFWEIQWQKQGDSLARYLVRLSEMTESIKIIQQALEGLPGGPYENLESRGFDRKR**
Solanum ESYDEFDWQVQWQREGDSLARYLVRIEMTESIKIIQQALEGIPGGPYENLEMRRFDRLK
Vitis ECYDEFDWEVQWQKEGDSLARYLVRIEMVASIKIIQQALEGIPGGPYENLEIRCFDRAR
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Synechocystis KSEWPDFQYQYIAKKVAPTFKIPAGEHYVRLESKGKELGIFIQGNDDVFPWRWKIRSADF
Physcomitrella KSEWNNFEYQFISKKPSPTFKLKPQEHYIRVEAPKGEIGVFLIGDDSVFPWRWKIRPPGF
Sorghum NPEWPDFEYRFLGKKPSPNFELSKQELYVVEAPKGEIGIYLVGDDSLFPWRWKIRPPGF
Brachypodium NSEWPDFEYRFLGKKPSPNFELSKQELYVRIEAPKGEIGIYLVGDDGLFPWRWKIRPPGF
Oryza NSEWPDFEYRFLGKKPSPNFELSKQELYARVEAPKGEIGIYLVGDDSLFPWRWKIRPPGF
Glycine EPEWNEFEYRFISKKPSPTFELPKQELYVRIEAPKGEIGIFLIGDQNGFPWRWKIRPPGF
Arabidopsis **NPEWPDFEYRFISKKPSPTFELSKQELYVVEAPKGEIGIFLIGDQSGFPWRWKIRPPGF**
Solanum DPEWPDFEYRFISKKPSPTFELSKQELYVVEAPKGEIGIFLIGDQSVFPWRWKIRPPGF
Vitis DPELNDFEYRFISKKPSPTFELSKQELYVVEAPKGEIGIFLIGDQNVFPWRWKIRPPGF
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Synechocystis NNLQILPHILKGVKVADIMAILGSIDIIMGSVDR
Physcomitrella INLQILPQLVKMKLADIMTILGSIDIIMGEVDR
Sorghum INLQILPQLVKMKLADIMTILGSIDIIMGEVDR
Brachypodium INLQILPQLVKMKLADIMTILGSIDIIMGEVDR
Oryza INLQILPQLVKMKLADIMTILGSIDIIMGEVDR
Glycine INLQILPQLVKRMKLADIMTILGSIDIIMGEVDR
Arabidopsis **INLQILPELVKRMKLADIMTILGSIDIIMGEVDR**
Solanum INLQILPQLVKRMKLADIMTILGSIDIIMGEVDR
Vitis INLQILPQLVKRMKLADIMTILGSIDIIMGEVDR
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Supplemental Table S5. Multiple alignment of Ndh48 (PNSB1) amino acid sequences in embryophytes. Sequences from *Physcomitrella patens* (PHYPADRAFT_106212), *Oryza sativa* (Os08g0276100), *Brachypodium distachion* (BRADI_3g19630), *Populus trichocarpa* (POPTR_0001s03600g), *Arabidopsis thaliana* (AT1G15980) and *Glycine max* (GLYMA_08G218800) were obtained and aligned as described in the legend for Supplemental table 2.

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Physcomitrella MSAMAAQVVL-----GAGLANCPQCSICVTRATSSLSHNSPKLQLRAFGTRTNVRLNA
Oryza          MQTPTMSTSMATPAKLP---SPPS--LPIARQ-----CCCHL----LQLGRRG--G
Brachypodium  MQTPTMYTSMARATSA--AANLP--APPRQ-----CYYHL----LPPAGR--S
Populus       ----MASTLPLPKTISPFLTNPPP--LPSTHFTSKPSFFNPSADHH----LPCTRKP---
Arabidopsis   ---MASSLPLPKPISPFFKTPPF--STSKPLV-----FL---NFQ---TRLTSRSSDV
Glycine       MAATYYLLPTSPKTFSPFLRNPPS--IPSGHHVSLGSL---DYP----FHCSSRRGNN

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Physcomitrella SS--VVVSAKKGFDDPFDDYGADEEDTMGELMSQGPQGAEDPRPARDPDSESYLDFPA
Oryza          AGVARASAKKNPWLDPFDDGPDDEFDY-RGAFSGG-KQEEDPRPPEDPANPYGFLRFPA
Brachypodium  VWALSASPKNPWLDPFDDGPDDEFDY-TGVYSGG-KQEEDPRPPEDPSNPYGFLRFPM
Populus       -SILTPYAKKNPWIDIFDDGEDLDMEY-GSLFVDG-KQEDTRPVDNPNPNPYGFLKFPK
Arabidopsis   S---VNLKKNPWLDPFDSGEDPDNEY-GSLFADG-KQEEDPRPPDNDPNPYGFLKFPK
Glycine       SVEANCNAKKNPWLDPFDDGEDPEMEY-GSLFADG-KQEEDPRPPDNDPNPYGFLKFPK
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Physcomitrella GFMPEVASLGILIRNDVRRCLCMISGGVYENLLFFPVIQLLKNRYPGVRIDVMATPRGKQ
Oryza          GYNPELDSLASKVRRDVRRAQVWSGGVYENLVFFPVVQLLRDRYPGVVDVVASARGKQ
Brachypodium  GYNPELDSLASKVRGDVRRACVWSGGVYENLVFFPVVQLLKDRYPGVLIDVVASARGKQ
Populus       GYNVEVAQLGLKIRGDVRRCCCMISGGVYENLLFFPVIQMLKDRYPGILVDVLASDRGKQ
Arabidopsis   GYTVELASLPLKIRGDVRRCCCVISGGVYENLLFFPVIQMLKDRYPGVQVDILTTERGKQ
Glycine       GYSVEIASLALKVRGDVRRCCCVISGGVYENLLFFPAIQMLKDRYPGVQIDVVASERGKQ
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Physcomitrella AYEMNKNVRKAWVHPVDDQFLRPVDFTEVVGKIKGEYYDLLVSTKLAGLGQSIFFWLASV
Oryza          VYEMCKNVR YADVDPDDWPEPAEYTHQLGVLKNRYDILIISTKLAGIGHALFLFMSSA
Brachypodium  VYEMCKNVR YANVDPDDWPEPAEYTHQLGVLKNRYDMLILSTKLAGTGHALFLFMSSA
Populus       CYELNKNVRWANVYDPDG-DPEPAIYTDMI GVLKNRYDVLVSTKLAGLGHASFMFMSSA
Arabidopsis   TYELNKNVRWANVYDPDDWPEPAEYTDMI GLLKGRYDMVLSTKLAGLGHAAFLFMTTA
Glycine       TYELNKNVRWANAYDPDEFPEPAEYTD MVGVLKNRYYDMVLSTKLAGLGHAAFLFMTTA
                ** : * * * * * . : * . . * . : * : * . * * * * : * * * * * :

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Physcomitrella RNKVS YTYPDVNAAGAAKFLDIAIKAPQLELAESGFNMYAEMIEELSQMGNVPKTEVPP
Oryza          RDKVGYVYPNVNSAGAGLFLTEMF RPTTNLADSGYNMYQDMLEWIGRPAKGVPEHPVPP
Brachypodium  REKVG YVYPNVNGAGAGLFLTEMF K PPTTNLSDGGYNMYQEMLEWIGRPGKGVPPQIPPP
Populus       RDKVSYIYPNVNAAGAGLLLTFTEFTPDSANLSEGGYHMYHQMLDWLGRPIYNVPRQPVP
Arabidopsis   RDRVSYIYPNVNSAGAGLMLSETFTAENTNLSELGYSMYTQMEDWLG RPF RSVPRTPLLP
Glycine       RDRVSYIYPNVNAAGAGLLLSETFVPDSQNLSDGGYNMYHQMDWLGLKPFREVP RQPVP
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Physcomitrella LEVGIGSKVKAYVEAKYREAGVREGEFLVFHGI ECDSSASMTSKGDKDCLLPLSMWAEIA
Oryza          LRVSISKKLRAFVEDKYSRAGVEKGFVVVHGIASDSFANMR SRGDDCLLPLEHWAEIA
Brachypodium  LRVSISKKL R GIVEDKYSRAGVEKGFVVVHGIASDSVANMTSRGDDCLLPLEQWAEIA
Populus       LRVSLSRK LKQYVEAKYRAGAEKGYIVIHGIESDSKASMQSRGDTDSLPLEVWDQIA
Arabidopsis   LRVSISRKVKEVVAAKYRNAGAVTGK FIVIHGIESDSKASMQSGDADSLLSLEKWAKII
Glycine       LRVSISKKLKEVVEAKYKAGAKKGYVVIHGIKSDSKASMQSRGDPDSLPLEVWAEIA
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Physcomitrella KSTS-----DKVVFVIPNEKWRRKVKEI-CGENAHIVFITTPGQLGALINASKGVVTTNT
Oryza KEISSEDNGLKPLFVIPHQKHREEVEET-VGKDTNILFITTPGQLTCLINDSVGVVATNT
Brachypodium KAISSQGNGLRPLFVMPHQKHREEIEDI-VGRETSYLFITTPGQLTCLINDSAGVVATNT
Populus DAIS----GFKPVFVIPHEKERENVEEIIYNEDIGILFITTPGQ-ATLINDSAGVIATNT
Arabidopsis **KGVR----GFKPVFVIPHEKERENVEDF-VGDDTSIVFITTPGQLAALINDSAGVIATNT**
Glycine DVIR----DVTPLFVIPHEKERENVEEI-FSEDASIVFITTPGQLAALINDSAGVIATNT
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Physcomitrella AALQIAIALKKSTVALFASQEKANLFIIPDYAK-DACAMVASKTGKLCGLDLKAATMAVST
Oryza AAVQLANARDKPCVALFSSKEKARLFLPYLEEKKGCTVVASSETGKLDIDVEAVKKAVKE
Brachypodium AAVQLANARDRPCVALFSSKEKAKLFLPYAEDKKSCTVVASATGKLDADIDIEAVKNAVKE
Populus AAIQLANAREKPSIALFGSEEKGVFVPNAEE-KKCIIVSSKTGKLDIDVGAVKQAMQI
Arabidopsis **AAIQLANARDKPCIGLFSSEEKGLFVPYAEKSNCVIASKTGKLDADIDIGTVKNAMQV**
Glycine TAVQLANAREKPCIALFCSEEKGNKFVPRAEE-KKCIIVSSKTGKLDIDVEAVKNAVQT
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Physcomitrella IAKEALVAA---
Oryza FKAAPSVALAQT
Brachypodium FEPAPSFALAQK
Populus FDMSLALV----
Arabidopsis **FEGSLALV----**
Glycine FNLSPALV----
: ..

Supplemental Table S6. Forward and reverse primers used for cloning of bimolecular fluorescence complementation (BiFC) constructs.

Restriction enzyme sites are underlined and translational start codon is marked by bold italic. Frw = forward primer, rev = reverse primer. In order to get proper fusion to C-terminal YFP half, reverse primer lacks a stop codon. RE = restriction enzyme. The cDNA clones were obtained from the Arabidopsis Biological Resource Center (ABRC). All primers were purchased from Sigma Aldrich.

Construct(s)	AGI-code	Primer sequences	RE-sites
pSPYCE.NDH-S	AT4G23890.1	Frw 5'- CTGCAGAGGATCC <i>ATGG</i> CGACTTCTTCGATCAC -3' Rev 5'- CTGCAGACCCGGGTGGTGCCTCTTCCTTTT -3'	BamHI XmaI
pSPYNE.NDH-S	AT4G23890.1	Frw 5'- CTGCAGAGGATCC <i>ATGG</i> CGACTTCTTCGATCAC -3' Rev 5'- CTGCAGACCCGGGTGGTGCCTCTTCCTTTT -3'	BamHI XmaI
pSPYCE.PGR5	At2g05620.1	Frw 5'- CTGCAGAGGATCC <i>ATGG</i> GCTGCTGCTTCGATTTTC -3' Rev 5'- CTGCAGACCCGGGAGCAAGGAAACCAAGCCTCT -3'	BamHI XmaI
pSPYNE.PGR5	At2g05620.1	Frw 5'- CTGCAGAGGATCC <i>ATGG</i> GCTGCTGCTTCGATTTTC -3' Rev 5'- CTGCAGACCCGGGAGCAAGGAAACCAAGCCTCT -3'	BamHI XmaI
pSPYCE.PGRL1A	At4g22890	Frw 5'- CTGCAGAGGATCC <i>ATGG</i> GTAGCAAGATGTTGTTTA -3' Rev 5'- CTGCAGACCCGGGAGCTTGGCTTCCTTCTGGC -3'	BamHI XmaI
pSPYNE.PGRL1A	At4g22890	Frw 5'- CTGCAGAGGATCC <i>ATGG</i> GTAGCAAGATGTTGTTTA -3' Rev 5'- CTGCAGACCCGGGAGCTTGGCTTCCTTCTGGC -3'	BamHI XmaI

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