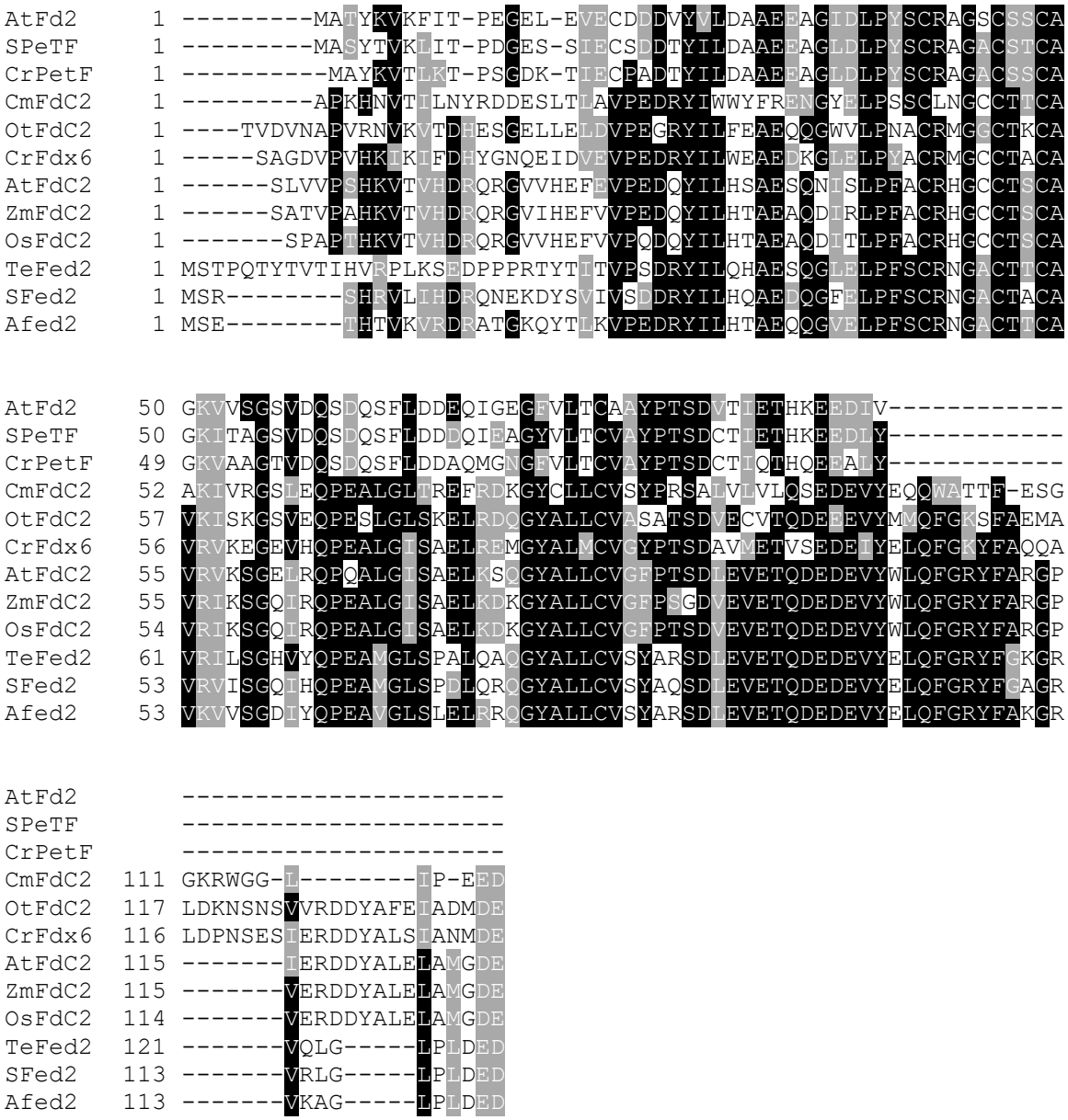

Fig. S1. Fed2/FdC2 proteins contain conserved features across photosynthetic organisms. (A) Alignment of Fed2/FdC2 proteins from various cyanobacterial, algal and higher plant species against representative photosynthetic Fd proteins (PetF) from model cyanobacteria, algae and higher plant species. Over 50% conservation in black, over 50% similarity in grey. The alignment was generated in Clustal Omega (Sievers et al. 2011). Transit peptide amino acids were removed from eukaryotic proteins for accurate comparison of mature proteins. Due to the variable numbers of Fd genes in the algae *Ostreochoccus tauri* (3) and *Cyanidioschyzon merolae* (3), their Fed2 homologues have been named FdC2 after the higher plant homologue rather than the algal homologue from *Chlamydomonas reinhardtii* (Fdx6). Fd isoproteins from different species: *Synechocystis* PCC 6803 - SFed2 sll1382, SPetF ssl0020; *Anabaena* PCC7120 - AFed2 all2919; *Thermosynechococcus elongatus* - TeFed2 WP_011057493.1; *C. reinhardtii*: CrFdx6 ABC88605.1, CrPetF AAA33085.1; *O. tauri* - OtFdC2 CAL53412.1; *C. merolae* - CmFdC2 CMR278c; *Arabidopsis thaliana*: AtFdC2 AT1G32550.1, AtFd2 AT1G60950; *Oryza sativa* - OsFdC2 Os03g0685000; *Zea mays* - ZmFdC2 ACG28100.1

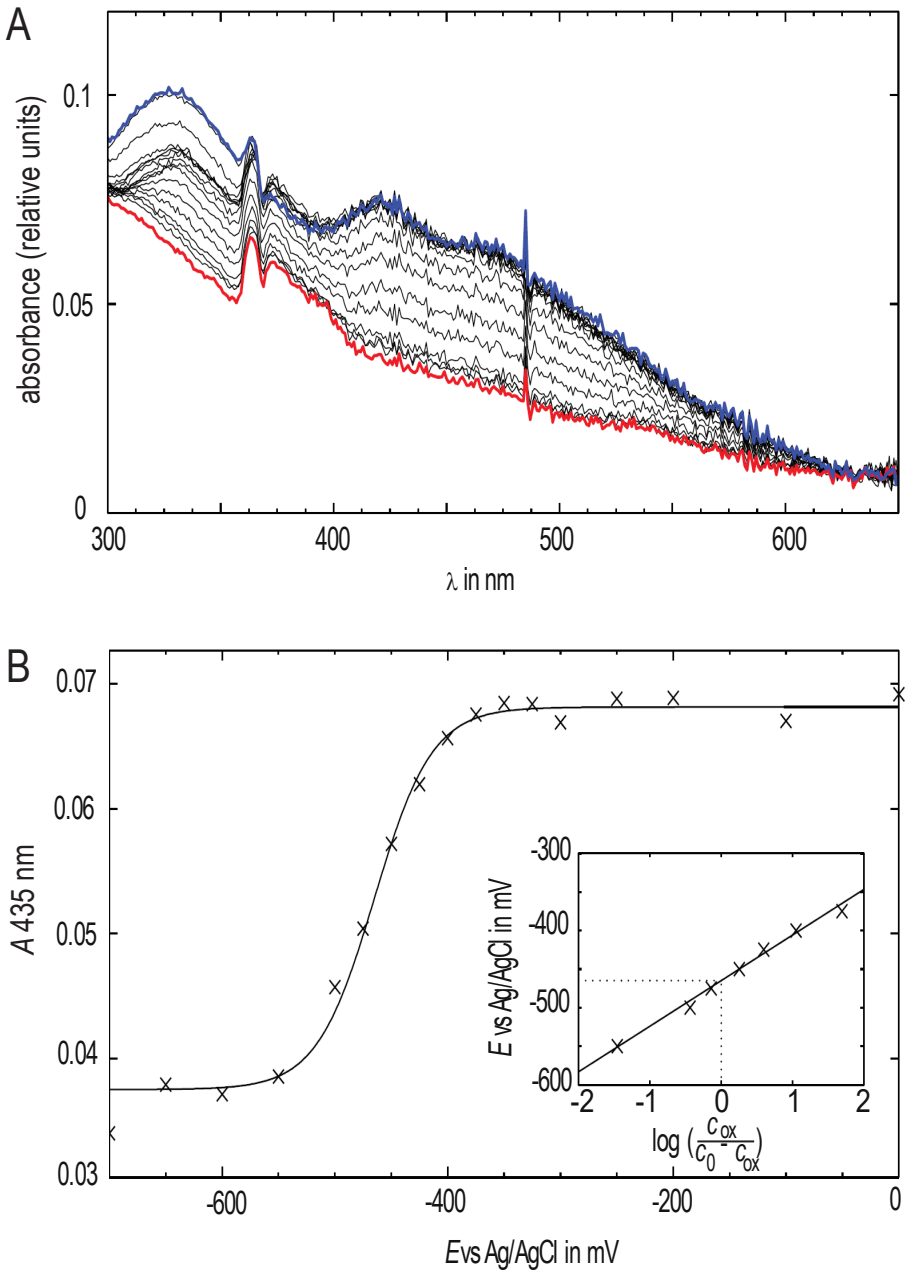

Fig. S2. Evaluation of the midpoint reduction potential Eo'FdC2 vs Ag/AgCl by spectroelectrochemistry of Fed2 in the presence of mediators (see Materials and Methods). *A*) UV-Vis contribution of the protein was obtained by subtracting the absorbance of mediators, over the potential range of 0 mV (blue) to -700 mV (red); *B*) Nernst plot derived from the absorbances in *A*) for λ = 437nm, inset: linearized Nernst plot, dotted lines indicate Eo'_{Fed2}.

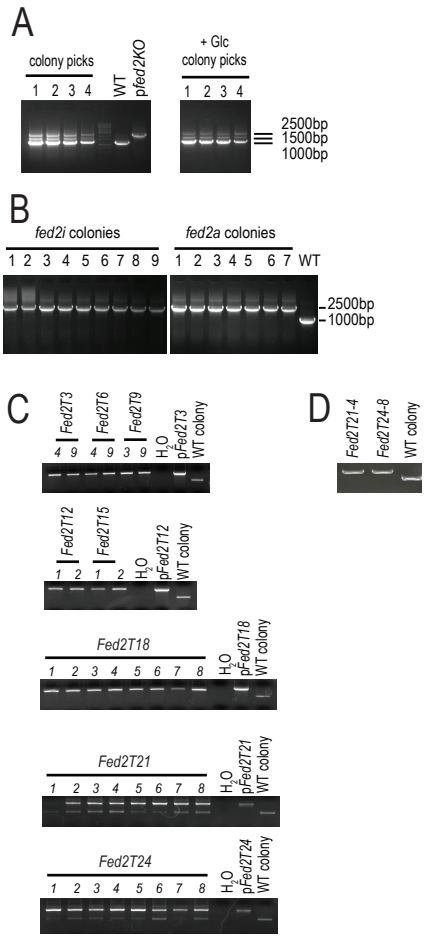

Fig. S3. Segregation of genetically manipulated strains. Agarose gel of DNA products from PCR reactions, using primers from either side of the *fed2* gene,on colony picks of *Synechocystis* following homologous recombination to integrate: (*A*) *Fed2* gene sequence disrupted by insertion of the gene for kanamycin resistance (*fed2KO*, see Fig 3), re-streaked 10 times on normal BG11 (left), or on BG11 supplemented with glucose (right). WT *Synechocystis* and plasmid controls indicate the size of WT and mutated gene products. (*B*) *Fed2* gene sequences driven by the inducible *nirA* promoter disrupted by insertion of the gene for kanamycin resistance (*fed2i* see Fig 3), or under control of the native promoter, but with the inducible *nirA* promoter 3' of *fed2*, driving expression in the antisense direction (*fed2a* see Fig 3). (*C*) *fed2* gene sequence with stop codons inserted to result in amino acid trunctations of 3,6,9,12,15,18, 21 and 24 (see Fig 4) at the C-terminal following 3 rounds of restreaking. (*D*) 21 and 24 amino acid C-terminal truncations following 20 rounds of re-streaking.

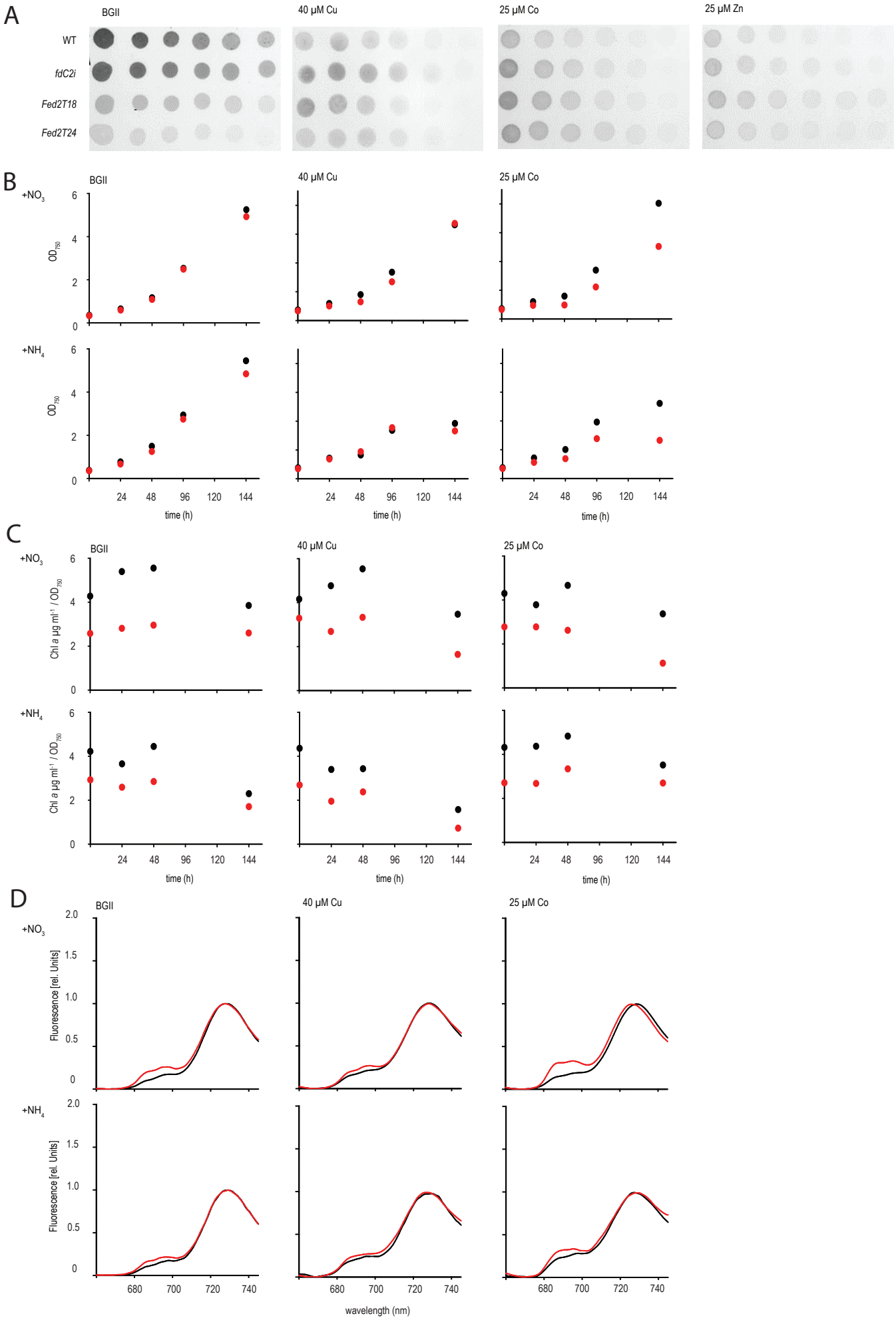

Fig. S4. Screening phenotypes of mutants expressing truncated versions of Fed2 on elevated trace metals. (*A*) Two fold dilution series of *Synechocystis* WT (same data as in Fig. 5A), *fed2i* inducible knock-down and truncated *fed2* lines *fed2T18* and *fed2T24* . Cell suspensions of 2 µg/mL chlorophyll were spotted onto BG11 with the indicated high metal concentrations. Plates were scanned after 7 days of growth. (*B*) Growth and (*C*) pigment analysis comparing WT and the Fed2 truncation line *fed2T24* cultivated in liquid media containing either NO_3 or NH_4 as a N source at the indicated high metal concentrations. WT (black), *fed2T24* (red). Precultured cells (grown in NH_4 containing media) were washed in N free BG11 media and subsequently diluted to an OD_{750} of 0.2 in media containing either NO_3 or NH_4 with the indicated metal concentrations. Growth rate was monitored for five days. Chlorophyll *a* content is expressed as a function of the cell density. (*D*) 77K fluorescence emission spectra of *Synechocystis* WT (black) and truncation line *fed2T24* (red) grown for 7 days in media containing either NO_3 or NH_4 as N source at the indicated metal concentrations. Cells were adjusted to a chlorophyll a content of 5 µg/ml. The excitation wavelength was 430nm. Experiments performed twice with basically the same result

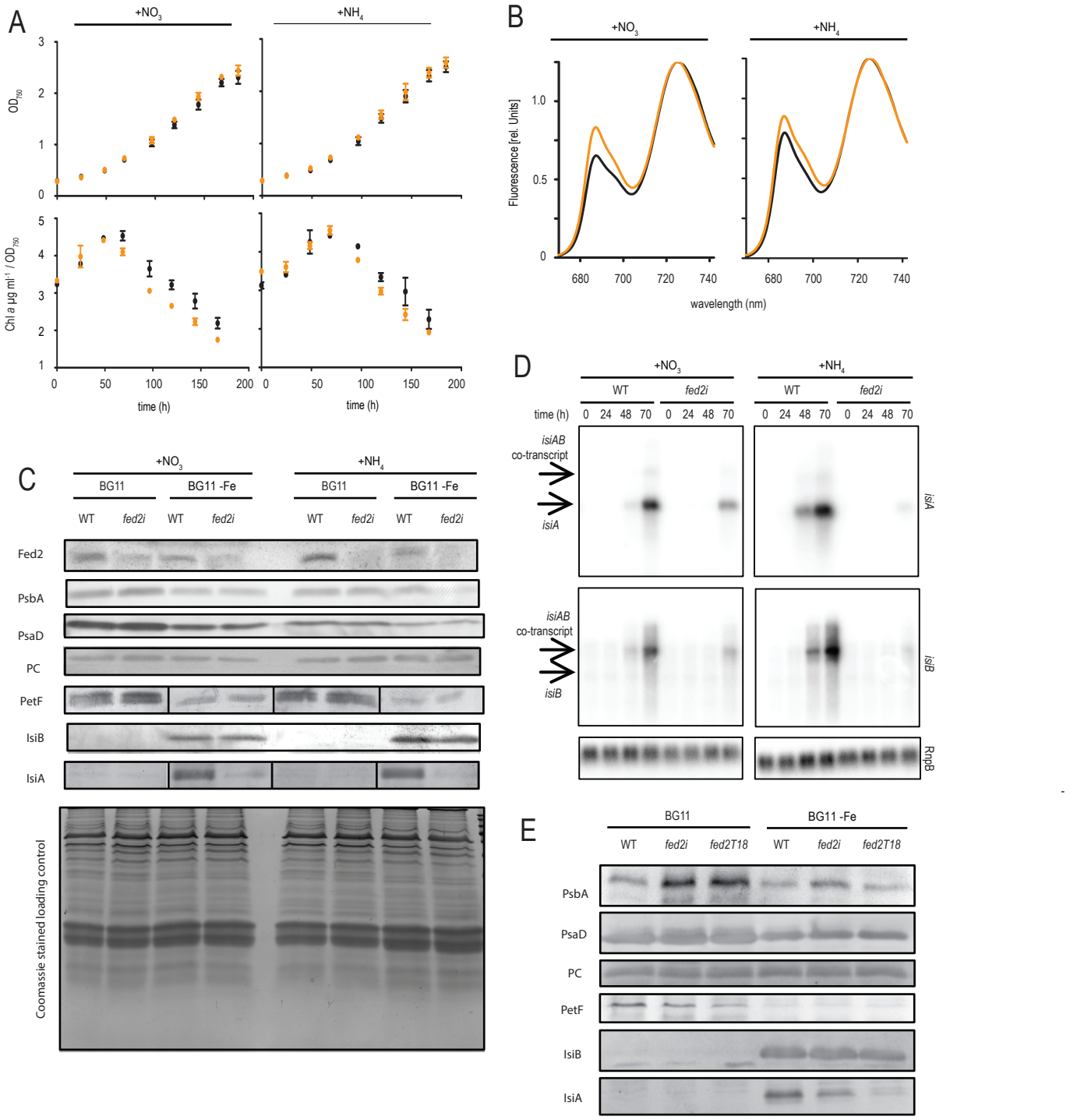

Fig. S5. Impact of iron depletion on the inducible knock down of Fed2. (*A*) Growth and pigment analysis comparing WT and the inducible Fed2 knockdown line *fed2i* cultivated in liquid media lacking iron and containing either nitrate or ammonium as a N source. WT (black), *fed2i* (orange). Precultured cells (grown in ammonium containing media) were washed in nitrogen free BG11 media and subsequently diluted to an OD_{750} of 0.2 in media containing either nitrate or ammonium in the indicated iron concentrations. Growth rate was monitored for five days. Chlorophyll *a* content is expressed as a function of the cell density. Results typical of three independent experiments. (*B*) 77K fluorescence emission spectra of *Synechocystis* WT (black) and inducible knock-down line *fed2i* (orange) grown for 8 days in media containing either nitrate or ammonium as N source in the indicated iron concentrations. Cells were adjusted to a chlorophyll a content of 5 µg ml^{-1}. The excitation wavelength was 430 nm. Experiments were performed three times with basically the same result. (*C*) Western Blot analysis of photosynthetic components. WT and *fed2i* lines were grown in regular BG11 and BG11 containing no iron with the indiated N source. Cells were diluted to the same OD in fresh BG11 and washed once before harvesting. Subsequently cell pellets were treated with SDS loading buffer and boiled for 5min before applying to an SDS PAGE gel. After electrophoresis proteins were subject to Western blotting and the indicated proteins were visualized using specific primary antibodies raised against PsbA, PsaD, PetF, IsiB and IsiA, with a secondary antibody conjugated to alkaline phosphatase. (D) *IsiA* and *isiB* transcript responses detected by Northern blot analysis following transfer of the cells to no iron containing media. Arrows indicate the expected sizes of transcripts. Below RnpB detection as control.(*E*) As for (C), except that only NH_4 medium was used and the genotypes WT, *fed2i* and *fed2T18* were compared

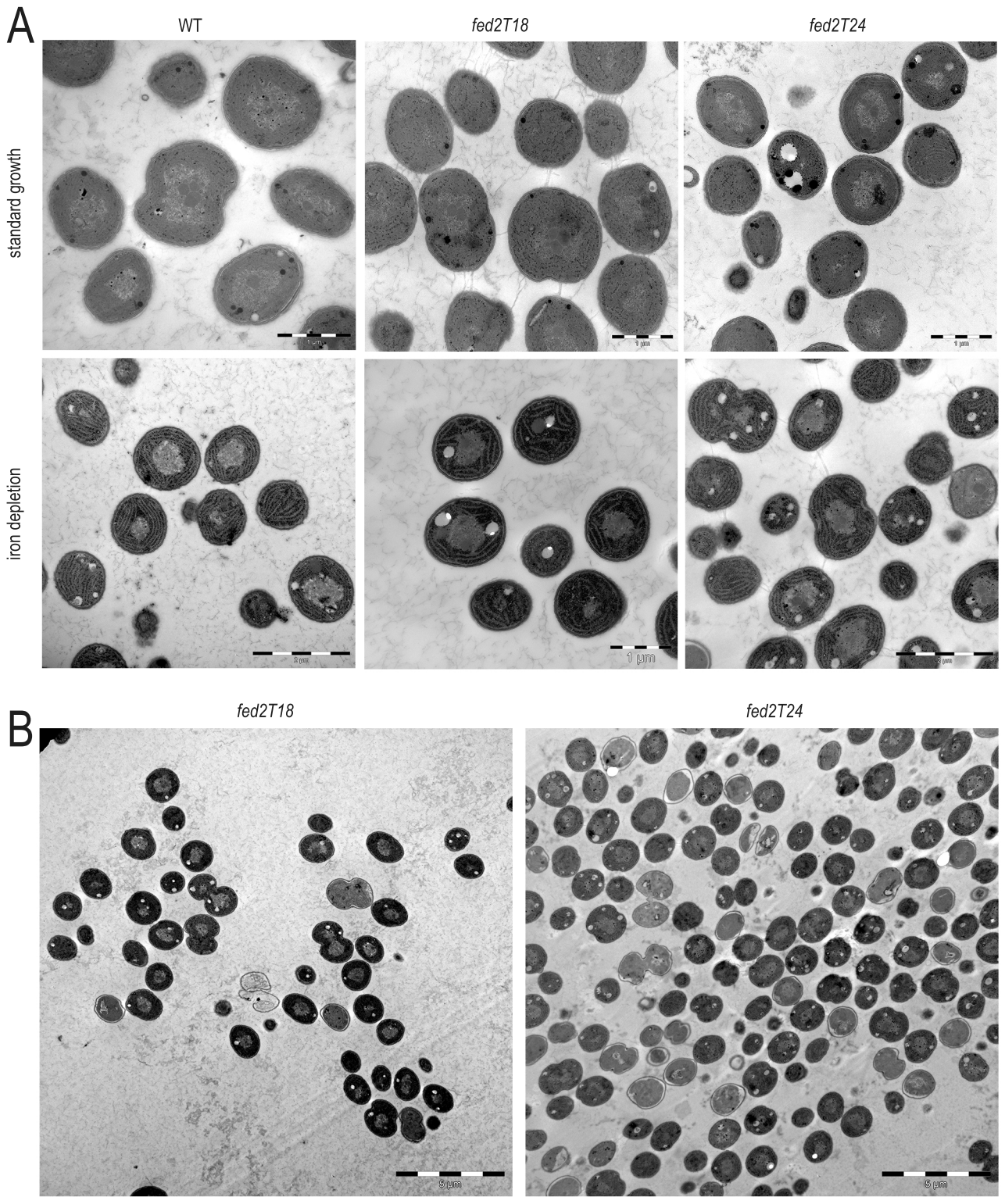

Fig. S6. Impact of Fed2 truncation on cyanobacterial cell structure. *Synechocystis* WT, *fed2T18* and *fed2T24* lines were transfered to BG11 with NO_3 as an N source and either with iron (top) or in the absence of iron (bottom) for 7 days prior to fixation and treatment for t.e.m. (*A*) representative groups of cells from WT, *fed2T18* and *fed2T24* lines in both iron replete media (top) and following transfer to media containing no iron (bottom). (*B*) Representative groups of *fed2T18* and *fed2T24* cells following removal of iron from the media, to show the proportion of cells lacking classical membrane structure and high density staining.

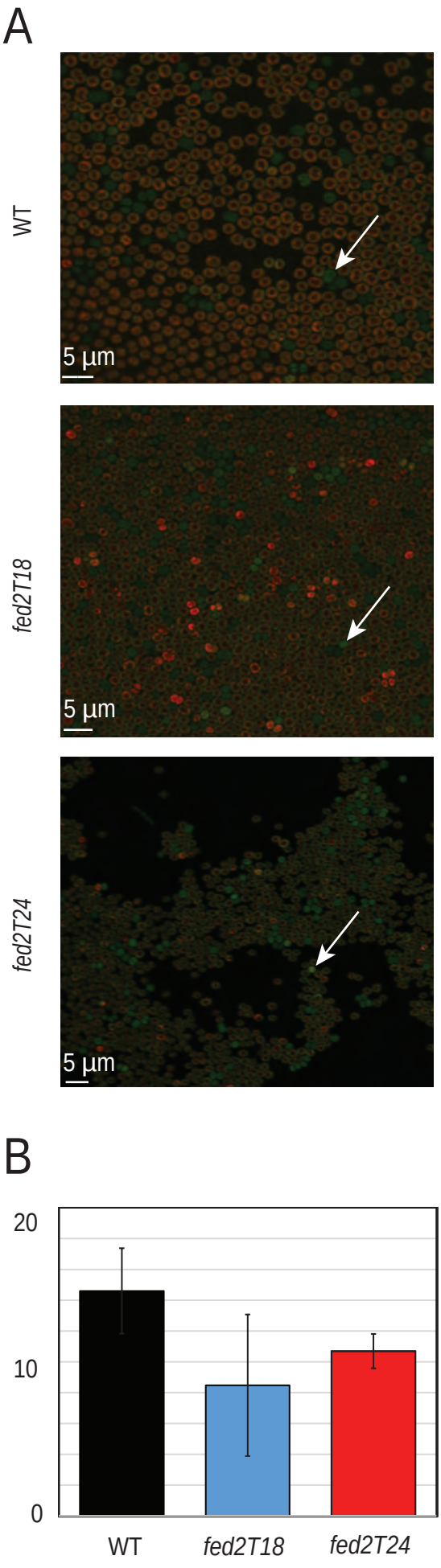

Fig. S7. Determination of cell viability by confocal microscopy. (*A*) *Synechocystis* cells of WT, *fed2T18* and *fed2T24* genotypes were grown in iron free media for 7 days before visualisation of chlorophyll autofluorescence by confocal microscopy. Dead cells are identified by characteristic green fluorescence following chlorophyll breakdown. (*B*) Average percentage of dead (green fluorescent) cells in three different samples of between 600 and 1024 cells of each genotype treated as for those shown in (*A*). Average values ± s.d.

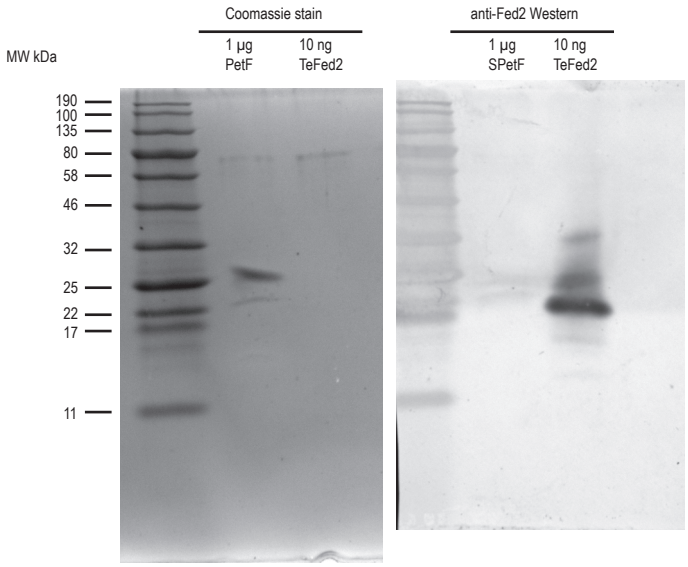

Fig. S8. Specificity of the antibody raised against TeFed2. Comparison of detection by Coomassie brilliant blue (left) and Western blotting using the anti-Fed2 antibody (right) of TeFed2 and a 100X concentration of *Synechocystis* PetF (the most abundant ferredoxin in *Synechocystis*), following separation on by Tris-Tricine SDS-PAGE. Due to their high negative charge ferredoxin proteins repel SDS, and migrate to higher position (20-25 kDa) than their actual molecular weight (11-15 kDa) in SDS-PAGE.

Table S1. Mass Spectrometry for identification of purified recombinant TeFdC2

Experiment 1 top band

Row	Cmpd.	m/z meas.	Mr calc.	Scores	P	Sequence	Range	Accession	Protein
1	69	5,495,930	10,975,605	73.3 (M:73.3)	0	R.VQLGLPLDED.-	121 - 130	Q8DID4	FdC2 *Thermosynechococcus elongatus*
2	1	5,550,750	11,084,754	39.4 (M:39.4)	0	R.NGACTTCAVR.I	53 - 62	Q8DID4	FdC2 *Thermosynechococcus elongatus*
3	2	5,555,840	11,094,594	49.3 (M:49.3)	0	R.NGACTTCAVR.I	53 - 62	Q8DID4	FdC2 *Thermosynechococcus elongatus*
4	17	5,765,930	11,515,823	65.6 (M:65.6)	0	R.TYTITVPSDR.Y	25 - 34	Q8DID4	FdC2 *Thermosynechococcus elongatus*
5	30	6,561,800	13,106,830	60.0 (M:60.0)	1	K.GRVQLGLPLDED.-	119 - 130	Q8DID4	FdC2 *Thermosynechococcus elongatus*
6	38	10,744,390	21,470,470	117.8 (M:117.8)	0	R.YILQHAESQGLELPFSCR.N	35 - 52	Q8DID4	FdC2 *Thermosynechococcus elongatus*
7	81	12,009,900	24,000,605	148.5 (M:148.5)	0	R.SDLEVETQDEDEVYELQFGR.Y	95 - 114	Q8DID4	FdC2 *Thermosynechococcus elongatus*

Experiment 1 bottom band

Row	Cmpd.	m/z meas.	Mr calc.	Scores	P	Sequence	Range	Accession	Protein
1	76	5,496,240	10,975,605	64.8 (M:64.8)	0	R.VQLGLPLDED.-	121 - 130	Q8DID4	FdC2 *Thermosynechococcus elongatus*
2	1	5,550,650	11,084,754	43.1 (M:43.1)	0	R.NGACTTCAVR.I	53 - 62	Q8DID4	FdC2 *Thermosynechococcus elongatus*
3	2	5,555,700	11,094,594	54.7 (M:54.7)	0	R.NGACTTCAVR.I	53 - 62	Q8DID4	FdC2 *Thermosynechococcus elongatus*
4	19	5,765,980	11,515,823	65.4 (M:65.4)	0	R.TYTITVPSDR.Y	25 - 34	Q8DID4	FdC2 *Thermosynechococcus elongatus*
5	39	6,561,850	13,106,830	71.2 (M:71.2)	1	K.GRVQLGLPLDED.-	119 - 130	Q8DID4	FdC2 *Thermosynechococcus elongatus*
6	52	10,744,360	21,470,470	114.0 (M:114.0)	0	R.YILQHAESQGLELPFSCR.N	35 - 52	Q8DID4	FdC2 *Thermosynechococcus elongatus*
7	93	12,009,860	24,000,605	151.7 (M:151.7)	0	R.SDLEVETQDEDEVYELQFGR.Y	95 - 114	Q8DID4	FdC2 *Thermosynechococcus elongatus*

Experiment 2 top band

Row	Cmpd.	m/z meas.	Mr calc.	Scores	P	Sequence	Range	Accession	Protein
1	46	5,495,940	10,975,605	68.0 (M:68.0)	0	R.VQLGLPLDED.-	121 - 130	Q8DID4	FdC2 *Thermosynechococcus elongatus*
2	2	5,550,740	11,084,754	41.2 (M:41.2)	0	R.NGACTTCAVR.I	53 - 62	Q8DID4	FdC2 *Thermosynechococcus elongatus*
3	20	5,766,160	11,515,823	67.9 (M:67.9)	0	R.TYTITVPSDR.Y	25 - 34	Q8DID4	FdC2 *Thermosynechococcus elongatus*
4	27	6,561,560	13,106,830	73.9 (M:73.9)	1	K.GRVQLGLPLDED.-	119 - 130	Q8DID4	FdC2 *Thermosynechococcus elongatus*
5	34	7,165,190	21,470,470	63.8 (M:63.8)	0	R.YILQHAESQGLELPFSCR.N	35 - 52	Q8DID4	FdC2 *Thermosynechococcus elongatus*
6	52	12,009,260	24,000,605	149.3 (M:149.3)	0	R.SDLEVETQDEDEVYELQFGR.Y	95 - 114	Q8DID4	FdC2 *Thermosynechococcus elongatus*

Experiment 2 bottom band

Row	Cmpd.	m/z meas.	Mr calc.	Scores	P	Sequence	Range	Accession	Protein
1	67	5,495,990	10,975,605	62.3 (M:62.3)	0	R.VQLGLPLDED.-	121 - 130	Q8DID4	FdC2 *Thermosynechococcus elongatus*
2	1	5,550,780	11,084,754	34.9 (M:34.9)	0	R.NGACTTCAVR.I	53 - 62	Q8DID4	FdC2 *Thermosynechococcus elongatus*
3	25	5,766,130	11,515,823	60.7 (M:60.7)	0	R.TYTITVPSDR.Y	25 - 34	Q8DID4	FdC2 *Thermosynechococcus elongatus*
4	35	6,561,520	13,106,830	72.7 (M:72.7)	1	K.GRVQLGLPLDED.-	119 - 130	Q8DID4	FdC2 *Thermosynechococcus elongatus*
5	46	10,744,240	21,470,470	136.4 (M:136.4)	0	R.YILQHAESQGLELPFSCR.N	35 - 52	Q8DID4	FdC2 *Thermosynechococcus elongatus*
6	76	12,009,860	24,000,605	149.0 (M:149.0)	0	R.SDLEVETQDEDEVYELQFGR.Y	95 - 114	Q8DID4	FdC2 *Thermosynechococcus elongatus*

Both 15 kDa (lower) and 30 kDa (upper) bands were cut from SDS-PAGE gels before cysteine carbamidomethylation, digestion with trypsin, extraction and analysis by HPLC followed by orbitrap (Q Exactive Plus). Data were processed in Mascot (Perkins et al. 1999). Data from two independent experiments are shown.

Perkins DN, Pappin DJ, Creasy DM, Cottrell JS (1999) Probability-based protein identification by searching sequence databases using mass spectrometry data. Electrophoresis 20 (18):3551-3567.

Table S2. Nucleotide primers used in this work	
P1	5′- TGGCAGCCCGAAATAATTCC-3′
P2	5′- GAGTTCACGCCTACCACAAG-3′
P3	5′- TAGGCCTTATGGGCTGGTTTGAATCC-3
P4	5′-TACCGGTTATGTCCCGTTCCCACCGAGTTC-3’
P5	5′- TACCGGTTCCTAGTCCTCATCTAAAGGC-3
P6	5′- TAGGCCTTGGCTACAACTACAACCTGAT-3′
P7	5′- TAGGAACTGATTACGAATTCCC-3′
P8	5′- TAAAGGCAAACCTAATCGCACC-3′
P9	5′- ACCTAATCGCACCCGACCAGC-3′
P10	5′- CACCCGACCAGCCCCAAAGTAG-3′
P11	5′- AGCCCCAAAGTAGCGGCCAAAC-3′
P12	5′- GTAGCGGCCAAACTGTAACTC-3″
P13	5′- AAACTGTAACTCATAAACCTCGTCCT-3′
P14	5′- CTCATAAACCTCGTCCTCATCTTG-3′
P15	5′- CTCGTCCTCATCTTGGGTTTCC-3′
P16	5′- GATAACCATAAATAACCAAGGATC-3′
P17	5′- CAGTTGTCTTTCCGCTTTGG-3′
P18	5′- GAGTTAGGGAGGGAGTTGCGG-3′
P19	5′- TAATACGACTCACTATAGGGGCACTGTCCTCACGCTCGC-3′
P20	5’-CTCCAGAGTGATTGGGAAGG-3’
P21	5’-TAATACGACTCACTATAGGGCGGTGGGCCAAAACC-3’