

Supplementary Information

A comprehensive study on ferredoxin isoforms in the cyanobacterium Synechocystis sp. PCC 6803

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Table S1: A comparison of the surface charges on all *Synechocystis* Fdxs studied in this work.

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Table S4: Primers used in this work.

Supporting Figures

Fdx1 (Ssl0020); length 97 aa; MW 10.4 kDa; calculated pI 3.75

>sp|P27320|FER_SYNY3 Ferredoxin-1 OS=Synechocystis sp. (strain PCC 6803 / Kazusa)
OX=1111708 GN=petF PE=1 SV=2
MASYTVKLITPDGESSIECSDDTYILDAAEEAGLDLPYSCRAGACSTCAGKITAGSVDQSDQSFL
DDDQIEAGYVLTCAVAYPTSDCTIETHKEEDLY

Fdx2 (Sll1382); length 122 aa; MW 13.8 kDa; calculated pI 4.65

>tr|P74159|P74159_SYNY3 Ferredoxin OS=Synechocystis sp. (strain PCC 6803 / Kazusa)
OX=1111708 GN=petF PE=3 SV=1
MSRSHRVLIHDRQNEKDYSVIVSDDRYILHQAEDQGFELPFSCRNGACTACAVRVISGQIHQPEA
MGLSPDLQRQGYALLCVSYAQSDLEVETQDEDEVYELQFGRYFGAGRVRLGLPLDED

Fdx3 (Slr1828); length 106 aa; MW 11.3 kDa; calculated pI 4.09

>tr|P73388|P73388_SYNY3 Ferredoxin OS=Synechocystis sp. (strain PCC 6803 / Kazusa)
OX=1111708 GN=petF PE=3 SV=1
MVNTYTAEIQHQGQTYTISVPEDKTVLQAADDEGIQLPTS CGAGVCTTCAALITEGTAEQADGMG
VSAELQAEGYALLCVAYPRSDLKIITEKEDEVYQRQFGGQG

Fdx4 (Slr0150); length 103 aa; MW 10.8 kDa; calculated pI 4.17

>tr|P74449|P74449_SYNY3 Ferredoxin OS=Synechocystis sp. (strain PCC 6803 / Kazusa)
OX=1111708 GN=petF PE=3 SV=1
MGAIYSVNLVNPATGSDVTIEVAEDELILEAAENQGLDLPYSCRAASCVAACAGRLLGTEHTDK
GSDFLKPEELAAGCVLLCAAYATSDCKILTHQEEALFG

Fdx5 (Slr0148; sequence is actually shorter than listed in the UniProt database (without the highlighted sequence in grey, the valine at the start is a methionine)); length 160 aa; MW 17.8 kDa; calculated pI 4.92

<http://alcoadb.jp/cyano/PCC6803/slr0148/network>

>tr|P74447|P74447_SYNY3 Ferredoxin OS=Synechocystis sp. (strain PCC 6803 / Kazusa)
OX=1111708 GN=slr0148 PE=3 SV=1
MTMPPLWNCVSVANRVNAIVASTKEDCMAKTIKLDPIDLKVAIETNDNLLSGLLGQDLRIMKECGG
RGMCATCHVYITAGMESLSPLNRREQRTLEVITTHNRYSLACQARVLDEGVVVELPAGMYVSE
IEDIEELIGRRAEENILNPRDGSILVEKGKLTIRSMISQLDDQLQAAKIQIVNDTDE

Fdx6 (Ssl2559); length 98 aa; MW 10.7 kDa; calculated pI 4.39

>tr|P73171|P73171_SYNY3 Ferredoxin OS=Synechocystis sp. (strain PCC 6803 / Kazusa)
OX=1111708 GN=ssl2559 PE=4 SV=1
MNNCVISFPQTKFLPLSLEFNACLAEYLTPDNSPILFGCRTGLCGTCLVKVVGELISPEAEEREILA
ILAPDDVQARLACQIKLTGDIAIRAYQSDEI

Fdx7 (Sll0662); length 133 aa; MW 15.1 kDa; calculated pI 4.85

>tr|Q55980|Q55980_SYNY3 Ferredoxin OS=Synechocystis sp. (strain PCC 6803 / Kazusa)
OX=1111708 GN=sll0662 PE=1 SV=1
MVIADLNFPDPHRSGLPELGGDWRNFDDRSGLPELGGELRERGVYVDEVTGIGCKNCAHVA
PNTFTIEQEHGRSRAFSQNGDDEAVIQEAIDTCPVDCIHWVPYDELKHLEEKRKHQIRPLGYPQ
INPHL

Fdx8 (Sssr3184); length 75 aa; MW 8.0 kDa; calculated pI 4.12

>tr|P73649|P73649_SYNY3 Ferredoxin OS=Synechocystis sp. (strain PCC 6803 / Kazusa)
OX=1111708 GN=ssr3184 PE=4 SV=1
MPHTIVTETCEGVADCEACPVACIHPGDGKNTIGTDWYWIDFATCIDCGICLQVCPVEGAILPEE
RPDLQKSPA

Fdx9 (Slr2059); length 134 aa; MW 14.8 kDa; calculated pI 5.42

>tr|P73811|P73811_SYNY3 Ferredoxin OS=Synechocystis sp. (strain PCC 6803 / Kazusa)
OX=1111708 GN=slr2059 PE=4 SV=1
MKKRVTLTFPRSAVQMPVTYRLAKDFNIAANIIRAQVAPNQVGKVVLELSGDIDQLEASLEWMRS
QSIEVSLASREIVDDQS CVD CGL CTGV CPTTEALSLDPDSFRLMFRRSR CVV CEQ CIPS CPVQAIA
TNF

Fdx10 (Sll1584); length 134 aa; MW 15.6 kDa; calculated pI 8.13

>tr|P73195|P73195_SYNY3 Sll1584 protein OS=Synechocystis sp. (strain PCC 6803 / Kazusa)
OX=1111708 GN=sll1584 PE=4 SV=1
MENPVPITPSETIVDS CQRLGLGRIQRHLFL CC DQTKPK CC SKEDSLATWDYLLKKRLPELGLD CT
QSSRDGNIFRTKAN CLRV CQ QGPILLVYPEGIWYRNVTPTVMEKILQEHLQNRPVVEEYRFFTHPL
SHL

Note: Without structural analysis it is not possible to predict which one of two C residues bind the Fe-S cluster.

Fdx11 (Ssl3044); length 99 aa; MW 10.8 kDa; calculated pI 9.18

>tr|P74283|P74283_SYNY3 Ferredoxin OS=Synechocystis sp. (strain PCC 6803 / Kazusa)
OX=1111708 GN=ssl3044 PE=3 SV=1
MTITFVKEQKDIVVAQGAGNLRKALQNGVDIYTLKGKLMN C GGYGQ C GT CIVEITAGMENLSPKT
DFENRVLRKKPDNFRLA C QTLVNGPVSNTKPKG

Fdx12 (Ssr1041); length 79 aa; MW 8.3 kDa; calculated pI 3.98

>tr|P74801|P74801_SYNY3 Ssr1041 protein OS=Synechocystis sp. (strain PCC 6803 / Kazusa)
OX=1111708 GN=ssr1041 PE=4 SV=1
MAVTIHFLPDDVTVAARVGEPILDVAERAGVFIPTG C LMGS C H A C EVELGDGTPI C A C ISAVPVG
VELEINLYDDLTW

Fig. S1. Amino acid sequences of Fdx1-Fdx12 from *Synechocystis* p. PCC 6803. Cysteines coordinating Fe-S clusters are highlighted in yellow, other cysteines are highlighted in cyan. Values for molecular weight and pI were calculated based on the sequences above using the Expasy website (https://web.expasy.org/compute_pi/).

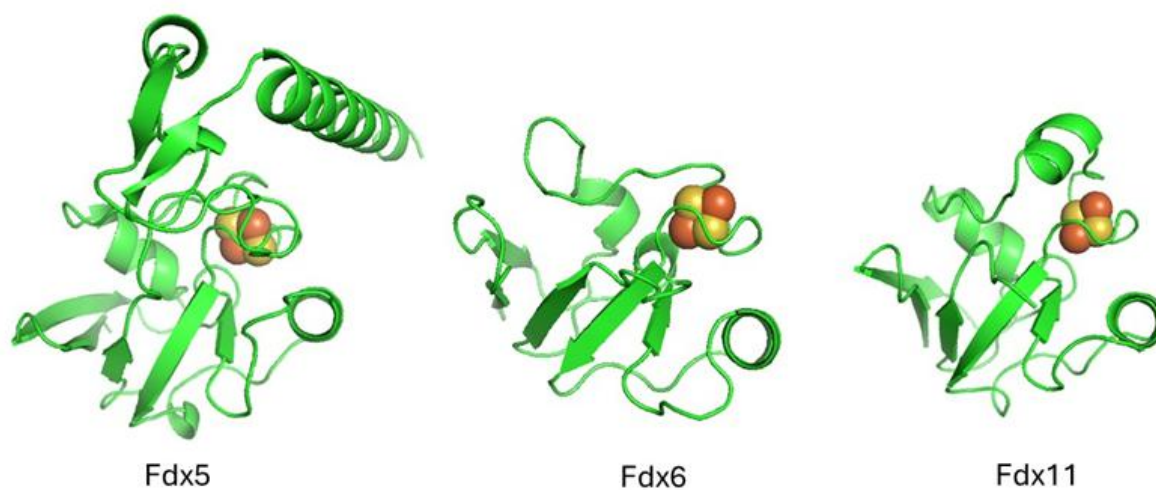


Fig. S2. Structures of Fdx5, Fdx6 and Fdx11 (generated in Pymol). The same models presented as in Figure 1, in a different orientation.



Fig. S3. AlphaFold-predicted structure of Fdx7, highlighting a poorly defined N-terminal region (yellow) with low confidence (taken from the UniProt website: <https://www.uniprot.org/uniprotkb/Q55980/entry>). The well-defined core, predicted with high confidence, is shown in blue.

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Fdx8      ETCGVA--DCVEACPVACIHPGDG--KNTIGTDWYWIDFATCIDCG-----TCLQVCPVEGAILPEERPDLOKSPA 75
7Fe-8SST6I DCEGLA--DCVNACPVACIHMNG--INKKGTNFYWIDFNTCIDCG-----TCLQVCPLENAILAEERSELQQIN- 74
2V2K      EPCVDVKDKACIEECPVDCIYEG-----ARMLYIHPDEGVDCG-----ACEPVCVVEAIYYEDDVFDQWSSYA 68
1H98      EPCIGVKDQSCVEVCPVECTYDG-----GDQFYIHPDECIDCG-----ACVPACPVNAIYPEEDVPEQWKSXI 68
1BD6      EPCIGTKDASVEVCPVDCIHEG-----EDQYYIDPDVDCIDCG-----ACEAVCPVSAIYHEDFVPEEWKSYI 68
1XER      DLCTADG--SCINACPVNVPQWYDTPGHPASEKKADPVNEQACIFCM-----ACVNVCPVAAIDVKFP----- 103
sp|P08813| DKCTGDG--ECVDVCPVEVYELQD-----GKAVPVNEEECLGCE-----SCVEVCEAGAITVEEN----- 62
6FD1      DNCIKCKYTDCEVCPVDCFYEG-----PNFLVIHPDECIDCA-----LCEPECPAQAIFFSEDEVPEDMQEF 68
1DUR      DSCITACG--ACKPECPVNCIQEG-----SIYAIADADSCIDCG-----SCASVCPVGPAPNPED----- 55
1CLF      DSCVSCG--ACASECPVNAISQG-----DSIFVIDADTCIDCG-----NCANVCPVGPAPVQE----- 55
2FDN      EACISCG--ACEPECPVNAISSG-----DDRYVIDADTCIDCG-----ACAGVCPVDAVPQA----- 55
1BLU      DECINCD--VCEPECPNGAISQG-----DETYVIEPSLCTECVGHYETSQCVEVCPVDCI IKDPSHEETEDELR 72
2FGO      DDGINCD--VCEPECPNGAISQG-----EEIYVIDPNLCTECVGHYDEPQCQVCPVDCIPLDDANVESKDQLM 72

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Fig. S4. Sequence alignment of Fdx8 with selected other [7Fe-8S] and [8Fe-8S] ferredoxins with available crystal structures. The figure displays a portion of the alignment, focusing on the key Fe-S binding sequence. Residues are color-coded based on their functional roles: cysteine residues coordinating the first Fe-S cluster are highlighted in black, those coordinating the second Fe-S cluster are in red, and conserved cysteine residues involved in hydrogen bonding one of the Fe-S clusters are highlighted in cyan. Residues Y (yellow) in position CX₂YX₂₋₄C determine the type of the first Fe-S cluster as [3Fe-4S] or [4Fe-4S], or exchangeable [3/4Fe-4S]. Conserved residues present in Fdx8 and across the sequences are shaded in gray. The ferredoxins sequences are derived from the following organisms: *Synechocystis* sp. PCC 6803 (Fdx8), *Paulinella chromatophore* (7Fe-8SST6), *Mycobacterium smegmatis* (2V2K), *Thermus aquaticus* (1H98), *Bacillus schlegelii* (1BD6), *Sulfolobus tokodaii* (1XER), *Desulfovibrio vulgaris* (sp|P08813|), *Azotobacter vinelandii* (6FD1), *Peptoniphilus asaccharolyticus* (1DUR), *Clostridium pasteurianum* (1CLF), *Clostridium acidurici* (2FDN), *Allochromatium vinosum* (1BLU), and *Pseudomonas aeruginosa* (2FGO). These organisms represent diverse phyla including *Cyanobacteria*, *Actinobacteria*, *Firmicutes*, *Deinococcus-Thermus*, *Archaea*, *Proteobacteria*, and *Peptostreptococcaceae*. Sequence alignment was performed using Clustal Omega.

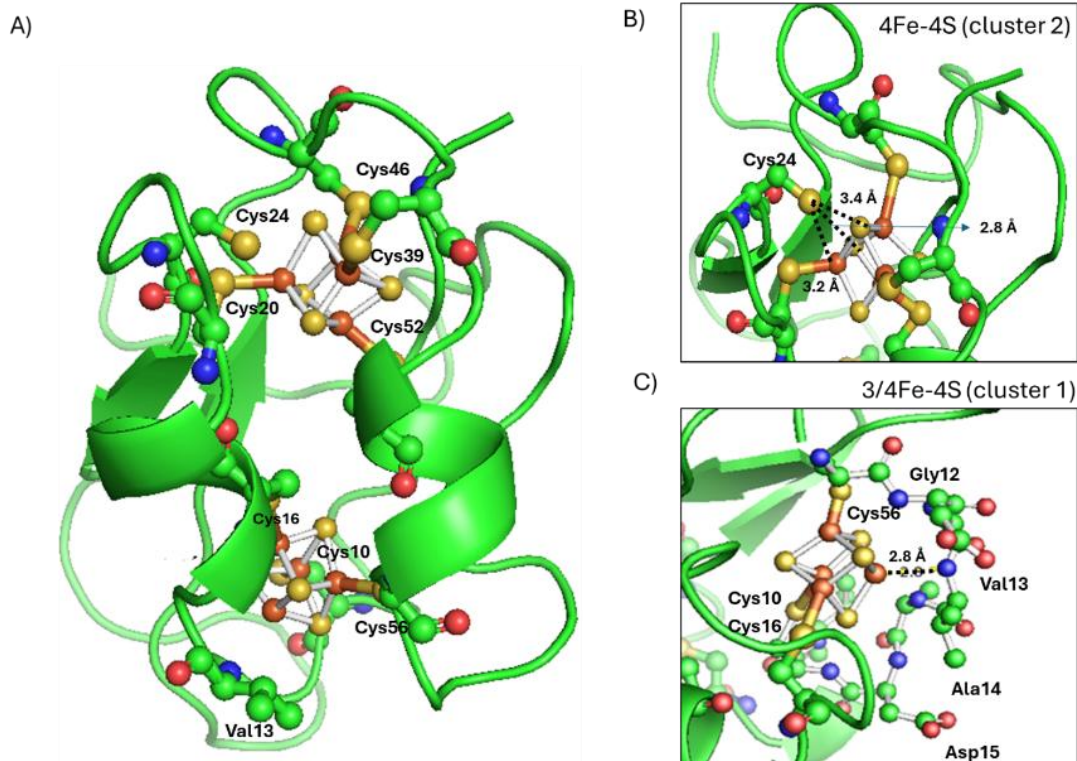


Fig. S5. Fdx8 structural model showing Cysteine residue distribution, as well as the position of Val13 in proximity to the [3/4Fe-4S] cluster (sequence coordinating the first cluster DCV₁₃EACPV; see Fdx8 sequence in Figure S4; Figure generated in Pymol). The position of the Fe-S cluster was determined based on comparison with known structures PDB ID 1H98 from *Thermus aquaticus* using Pymol. Green = peptide, nonpolar residues; orange = iron; yellow = sulfur; blue = nitrogen; red = oxygen; dashed lines are distance between Fe and key residues (A) whole protein, (B) and (C) zoom in on [4Fe-4S] and [3/4Fe-4S] clusters, respectively. The roles of cysteine residues were assigned based on their proximity to the Fe-S clusters in the model structure. Residues Cys10, Cys16, and Cys56 coordinate the iron atoms in Cluster 1 ([3/4Fe-4S]), while residues Cys20, Cys46, Cys49, and Cys52 coordinate the iron atoms in Cluster 2 ([4Fe-4S]). Notably, the orientation and distance of the sulfur atom in Cys24 relative to Cluster 2 suggest that it does not directly coordinate an Fe atom. Instead, it likely forms a hydrogen bond with a sulfur atom in the cluster, as indicated by the 2.8 Å distance. Furthermore, the position and orientation of Val13 in the model suggest that its backbone nitrogen may coordinate an Fe atom in Cluster 1, also at a distance of 2.8 Å.

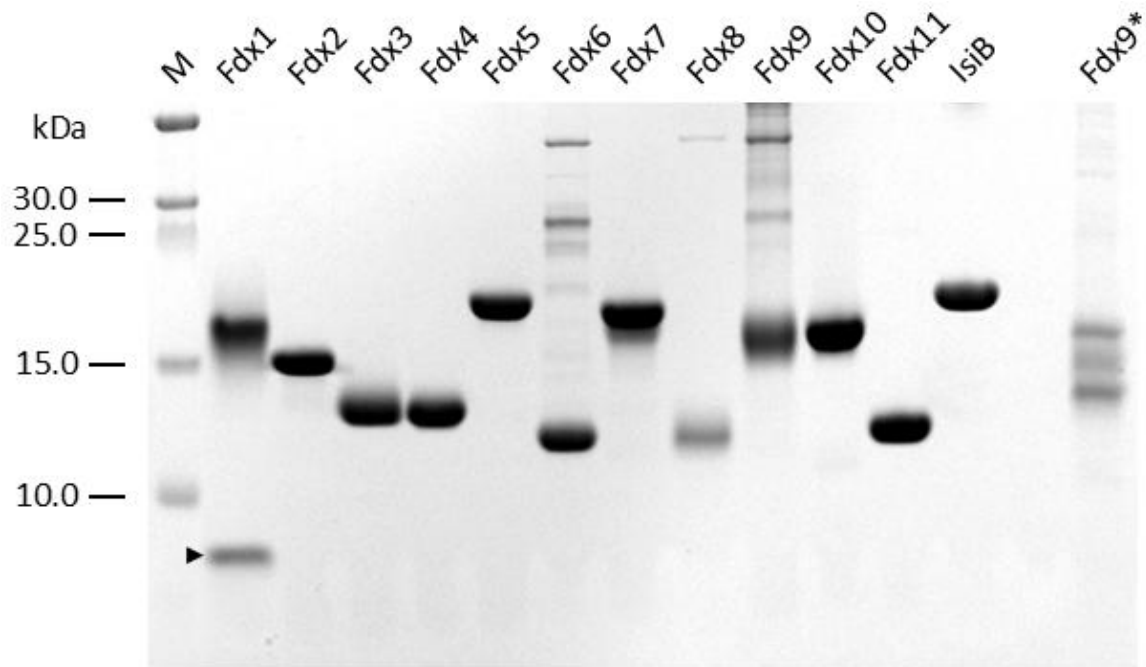


Fig. S6. SDS PAGE analysis of the purified *Synechocystis* Fdx1-Fdx11 and flavodoxin (IsiB) (see also Table S2). The arrowhead indicates the Fdx1 protein band. The other major band in that lane, just above 15 kDa, is maltose-binding protein (Accession # A0A384L126; detected by LC/MS, data not shown) which was introduced into the sample at the TEV cleavage step (see Materials and Method) (see Table S2 for more details). All of the proteins purified in this study were routinely checked for purity and quality by SDS PAGE analysis and the samples presented here are typical representatives for the proteins that were used in the subsequent analyses.

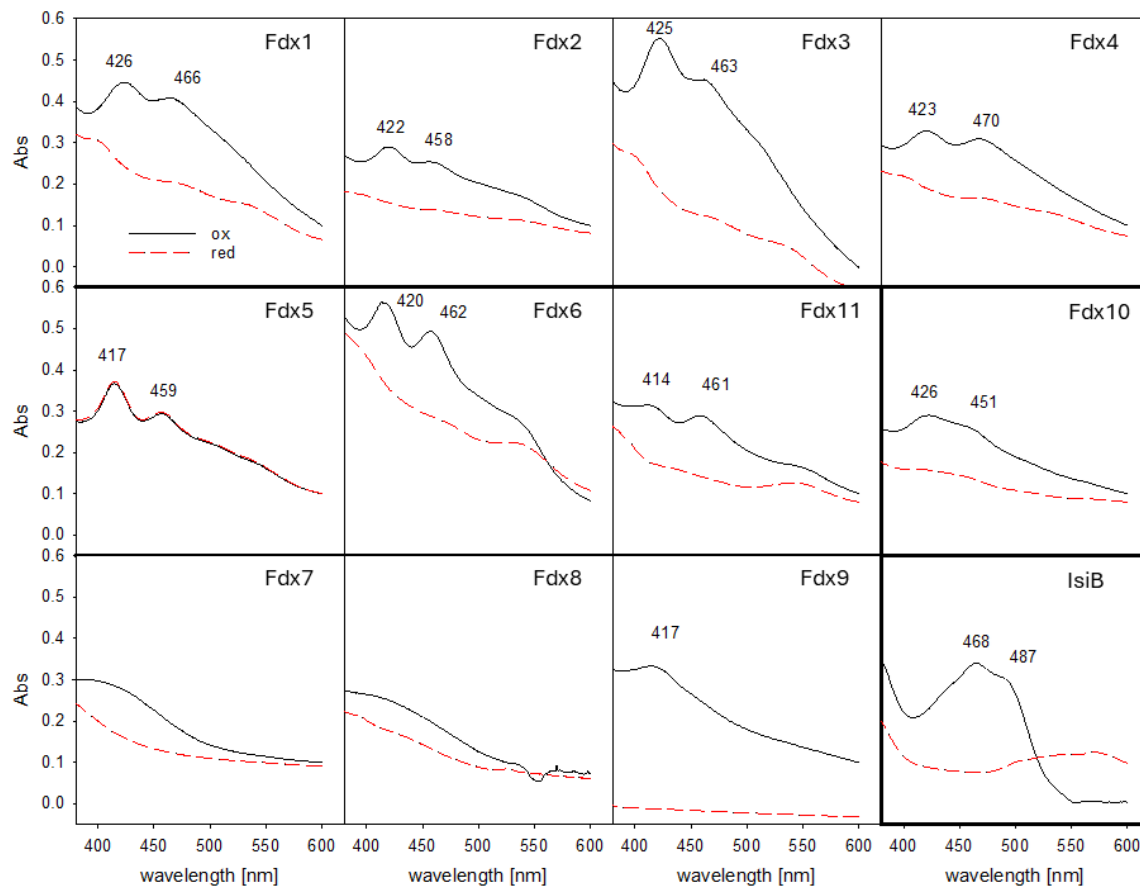


Fig. S7. UV-Vis absorption spectra of as purified Fdxs 1-11 and flavodoxin (black line), as well as spectra from reduced proteins (red dashed line) between 350 and 450 nm. Each sample was measured three times ($n = 3$) and the averaged absorption was plotted. Reduction conditions (a final concentration of 4 mM sodium dithionite) that for the other proteins lead to a change in absorption, did not change the spectrum for Fdx5. Buffer included 50 mM HEPES buffer, 0.1M NaCl and 5% glycerol at pH 8. Spectra belonging to one ferredoxin subtype (plant-type (Fdx1 to Fdx4), adrenodoxin-type (Fdx5, Fdx6 and Fdx11), thioredoxin-like (Fdx10) and bacterial-type (Fdx7 to Fdx9)) are grouped and boxed (thick black lines).

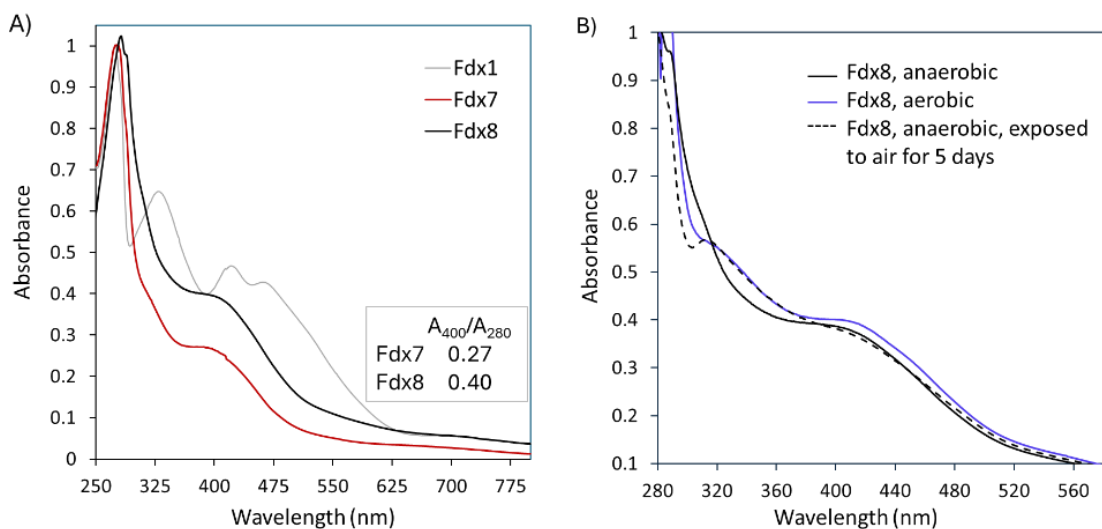


Fig. S8. (A) UV-Vis spectra of Fdx1, Fdx7, and Fdx8. Spectra were recorded using as-purified proteins. Fdx1 and Fdx7 were prepared under aerobic conditions, while Fdx8 was maintained strictly anaerobically. (B) UV-Vis spectra of Fdx8 under different preparation conditions: strictly anaerobic versus aerobic. The spectrum of anaerobically prepared Fdx8 suggests the presence of [4Fe-4S] clusters, whereas the aerobically prepared sample likely contains a mixture of [4Fe-4S] and [3Fe-4S] clusters. Upon exposure to air, the spectral feature centered at A_{400} that is characteristic of the presence of a [3Fe-4S]²⁺ clusters becomes increases. Fdxs were stored in 50 mM HEPES buffer, 0.1M NaCl and 5% glycerol at pH 8. The samples in both panels were measured several times at different concentrations ($n = 3$) and representative spectra are shown.

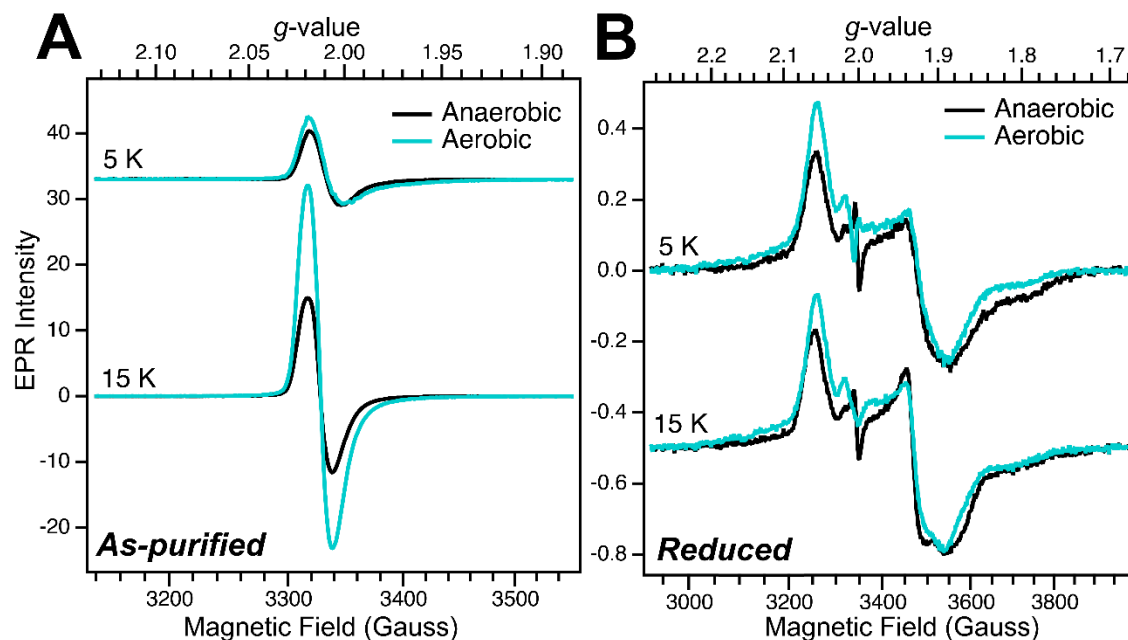


Fig. S9. EPR spectra of Fdx8 expressed and purified under aerobic (teal lines) or strictly anaerobic (black lines) conditions. EPR samples were prepared without any further treatment of the protein (i.e. no reconstitution was performed) and the as-purified spectra (A) collected. Samples were then thawed and treated with a high concentration stock of sodium dithionite (NaDT) to achieve a final concentration of 5 mM NaDT, re-frozen, and the reduced spectra (B) collected. Spectra collected using 1 mW of microwave power at either 5 K or 15 K sample temperature and are shown after cavity background subtraction and additional baseline correction using a polynomial spline ($n=1$; due to sample scarcity).

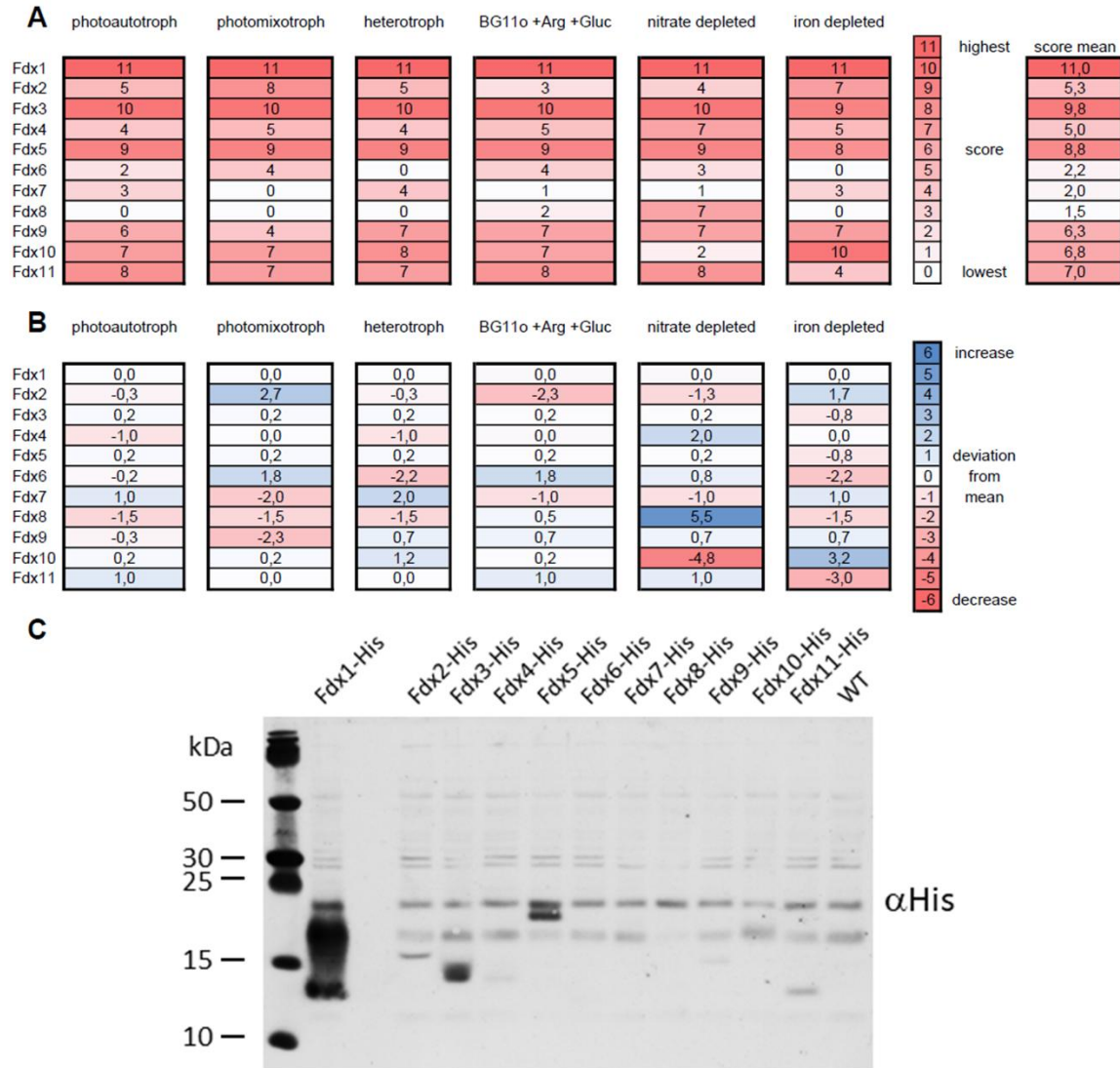


Fig. S10. Qualitative analysis of Fdx expression levels for the immunoblot analysis shown in Figure 4. (A) Expression levels were assessed and compared by evaluating the signal strengths in the immunoblot analysis shown in Figure 4 of the main text by eye. The score ranges from 0 to 11 (11 = highest expression, 0 = no detectable expression). When two signals appeared equally strong, they received the same score, and the next intense band was scored accordingly lower. In some cases, the assessment and scoring were hampered by nearby unspecific signals from the α His antibody. While a comparison within one panel is allowed, a comparison between two different panels may be flawed due to potentially different exposure times. Mean scores were calculated over all the tested conditions. (B) The calculated means were subtracted from the scores given in panel A. A positive value therefore indicates a relative increase in expression compared to the other conditions, while a negative value indicates a relative decrease. (C) Less exposed version of the immunoblotting analysis for the photoautotrophic samples (compare to Fig 4A of the main manuscript) to show the individual signal bands obtained for Fdx1.

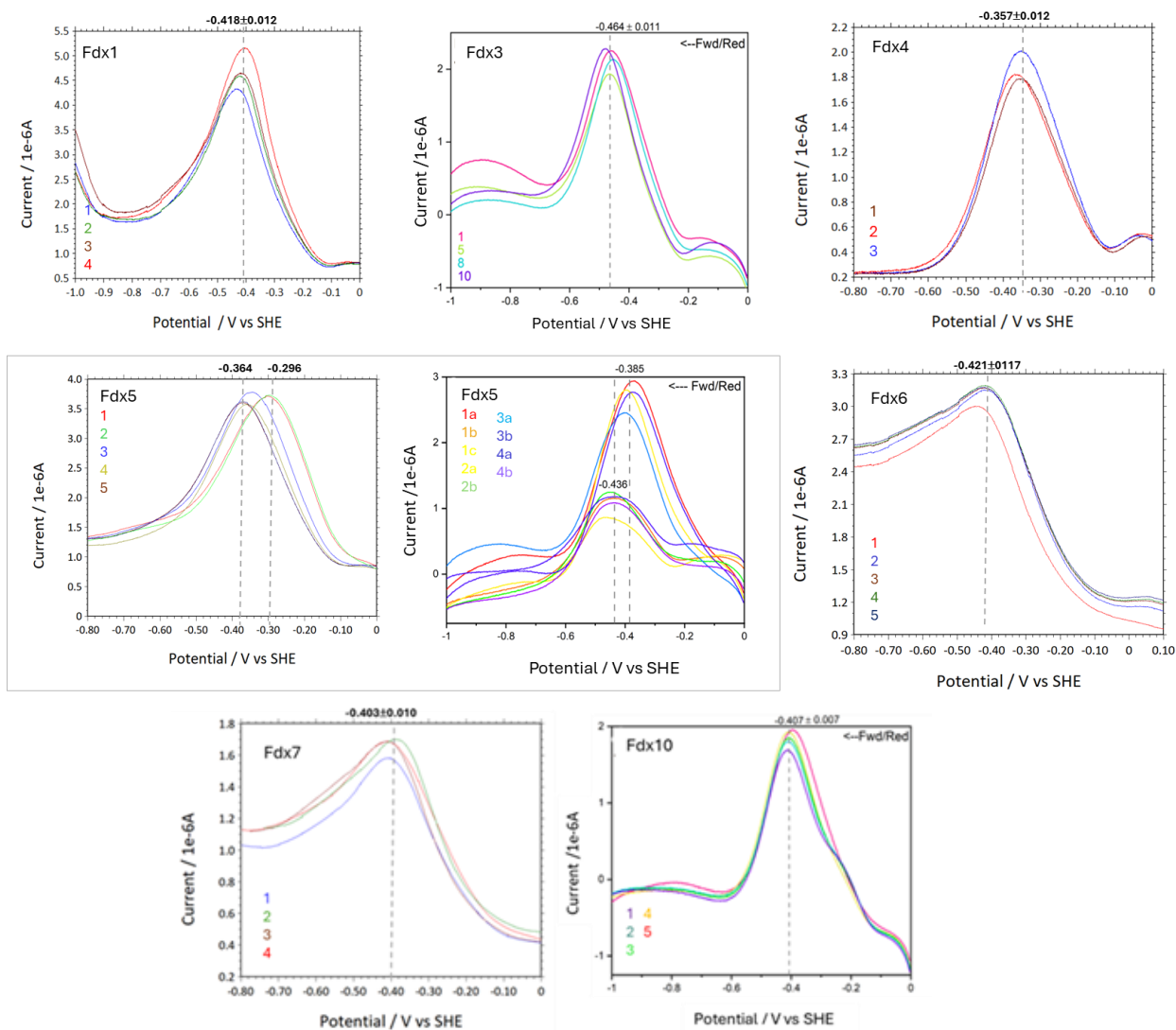


Fig. S11. Redox potentials were measured using square wave voltammetry with proteins immobilized on a polished pyrolytic graphite edge (PGE) electrode in HEPES buffer (pH 8.0). Potentials were converted to values vs. SHE by adding 199 mV to the Ag/AgCl reference. Numbers indicate subsequent scans measured for one or more Fdx electrodes. Fdx5 displays an increasing negative potential shift in the peak current over each scan from an unknown change in the Fdx5 FeS cluster. Two graphs for Fdx5 are boxed to indicate that they belong to the same protein.

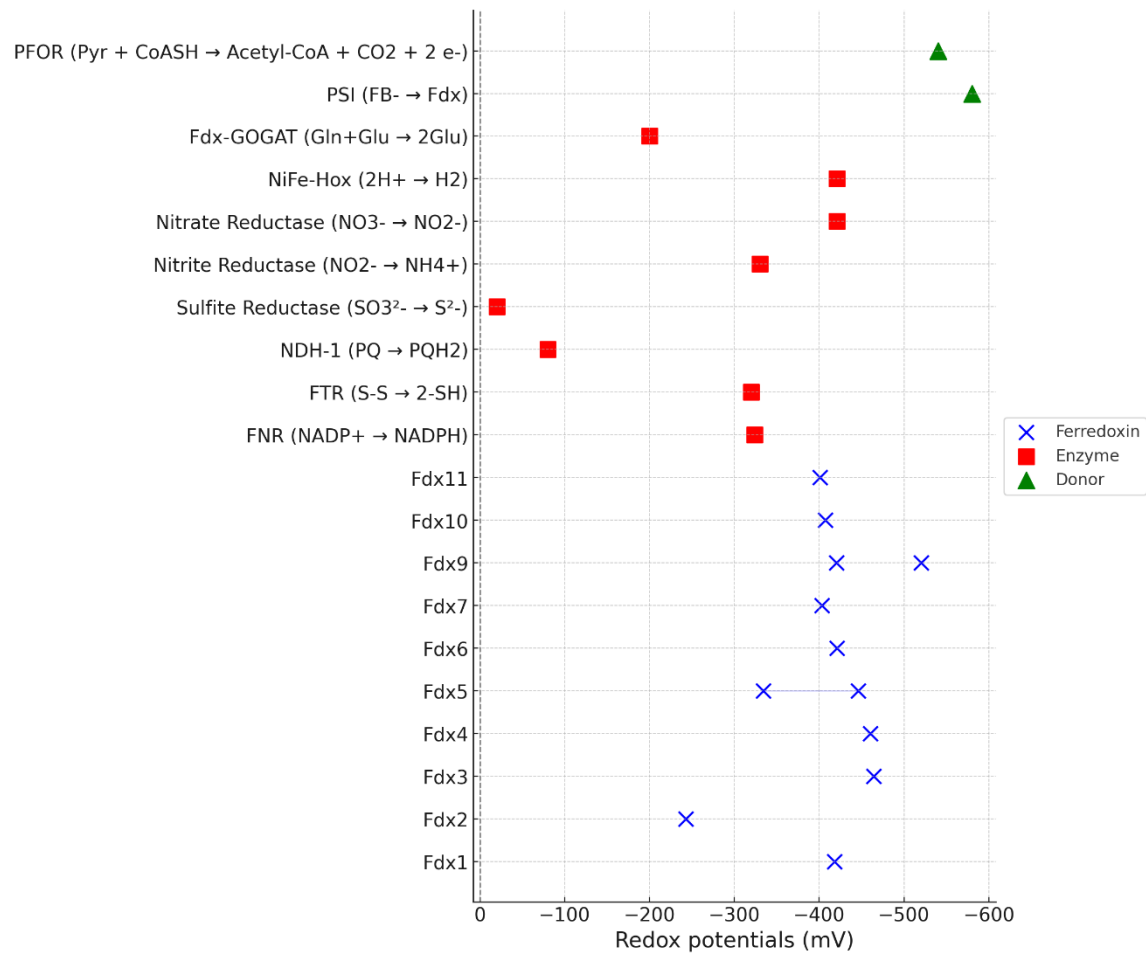


Fig. S12. Comparison of redox midpoint potentials (E_m) for *Synechocystis* Fdxs and redox potentials (E_m) of reactions catalyzed by relevant protein partners. For Fdx4 the E_m value from the literature [65] is included. There are two measured E_m 's for Fdx5, -280 and -430 mV (Table 1). The E_m values for the protein partners were obtained from the following references: FNR [66], FTR [67], Hox [66], Fdx-GOGAT [4], NDH1 [68], SR (sulfite reductase) [69], NR (nitrate reductase) [66], NirA (nitrite reductase) [66], PSI [70], and PFOR [42]. The value for Flv1/3 is +816 mV [66].

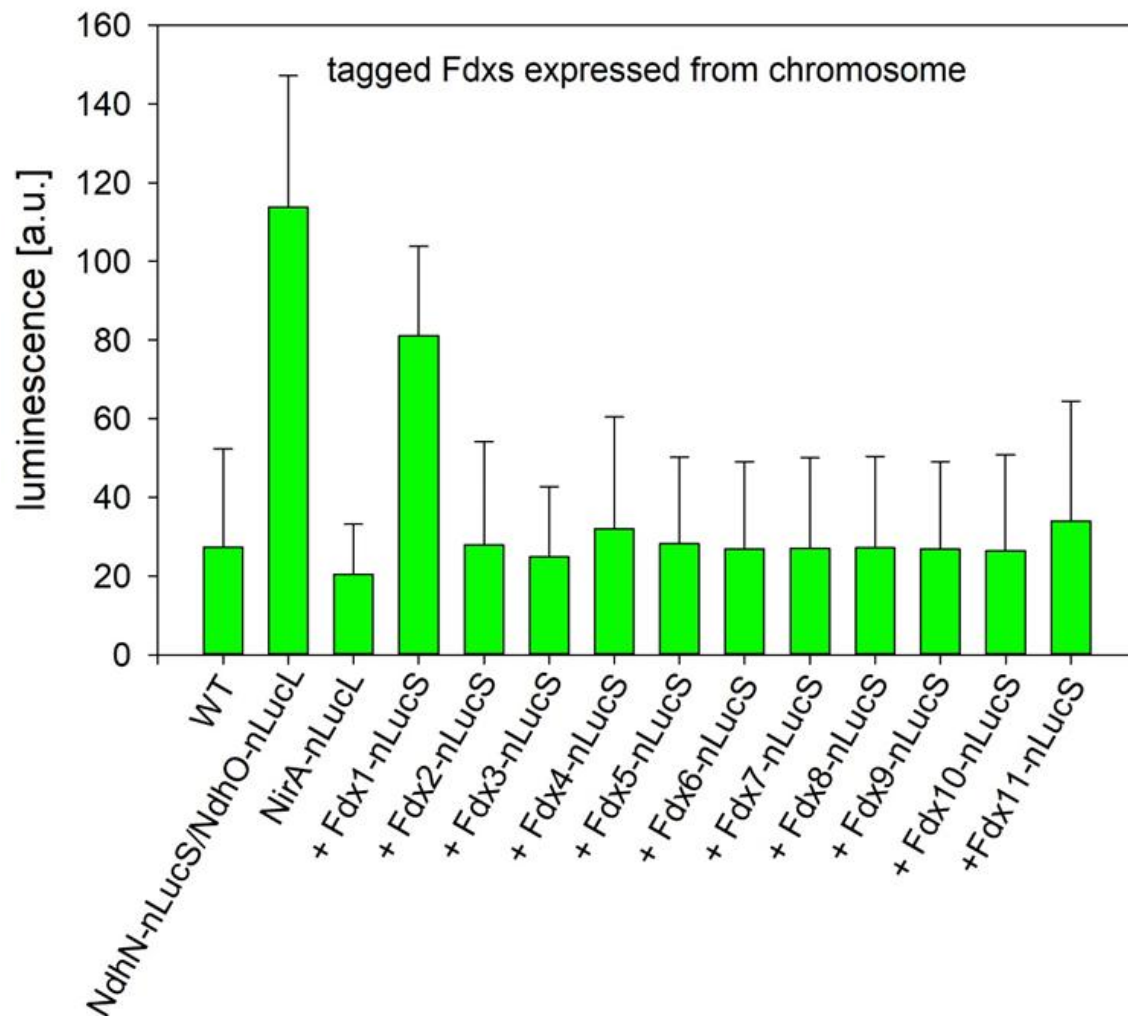


Fig. S13: *In vivo* interaction of nitrite reductase with Fdxs1-11 expressed under the control of their native promoters. In all strains the nitrite reductase and Fdxs were tagged with the LgBit subunit (nLucL) or the SmBit subunit (nLucS), respectively, at the genome level (integration via homologous recombination). The NdhO-nLucL / NdhS-nLucS expressing strain was measured as a positive control. Plots are the means and standard deviations of three independent experiments.

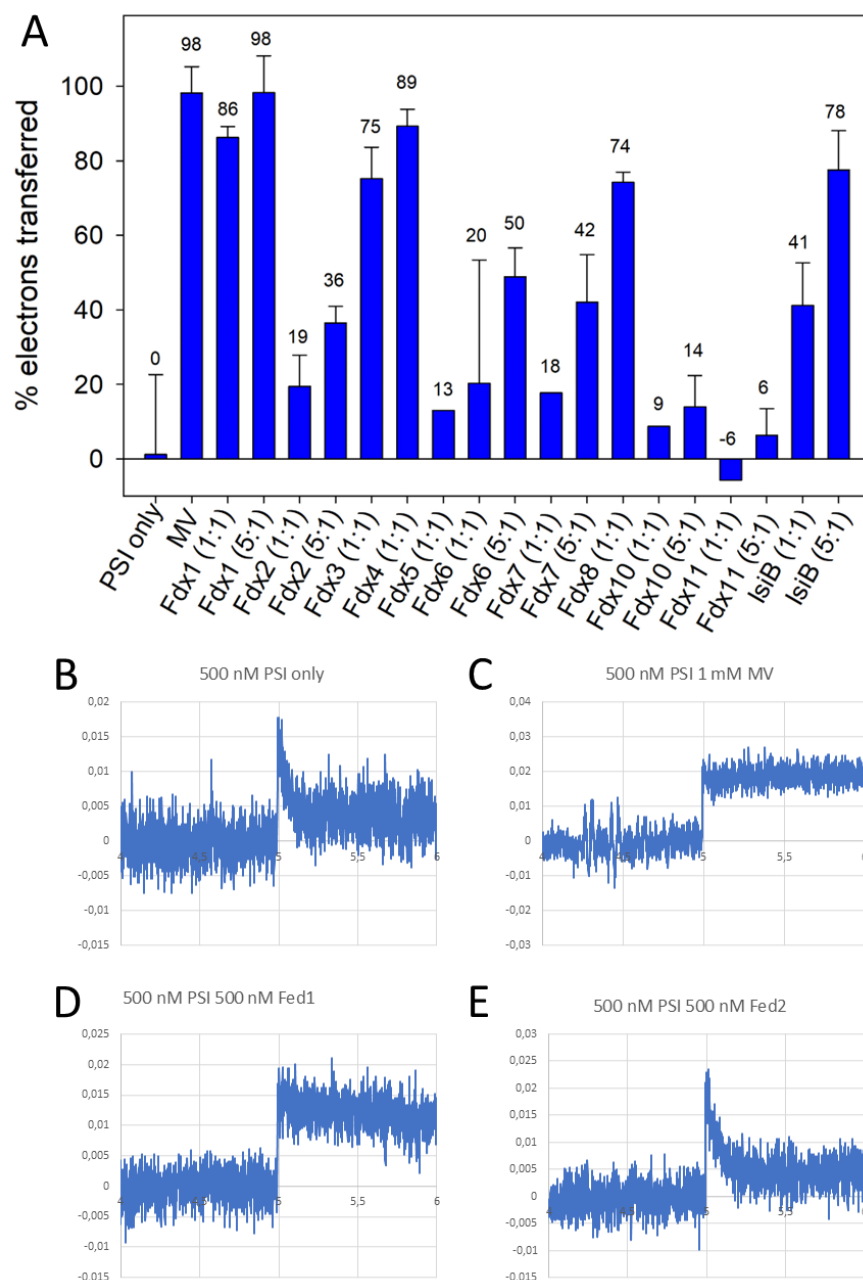


Fig. S14: Electron transfer from photosystem I (PSI) to purified Fdxs. (A) Electron transfer was assessed by P700⁺ reduction kinetics measurements with PSI at a concentration of 500 nM and Fdx in equal concentration (500 nM, 1:1) or in excess (2500 nM, 5:1). The numbers above the columns represent the calculated percentage of electron transfer. Plots are the means and standard deviations of three independent measurements. (B) PSI only and (C) PSI with added methyl viologen (1 mM) were measured as negative and positive controls, respectively. Fdx9-Strep (Fig. S6) was measured, however, due to the insufficient quality of this sample and its oxygen sensitivity, we denote “n.d.” in Table 2. A value of above 30% of electrons transferred was chosen as the cutoff and resulted in a “+” denotation in Table 2, when this value was observed for both ratios. When this threshold was only surpassed for the 5:1 ratio, this resulted in a “+/-” denotation for reactivity with PSI in Table 2. The P700⁺ reduction kinetics traces for one measurement for (D) Fdx1 and (E) Fdx2 are shown as examples. Fdx5 (1:1), Fdx7 (1:1), Fdx10 (1:1) and Fdx11 (1:1) were measured once (n=1), while all others were measured three times (n=3).

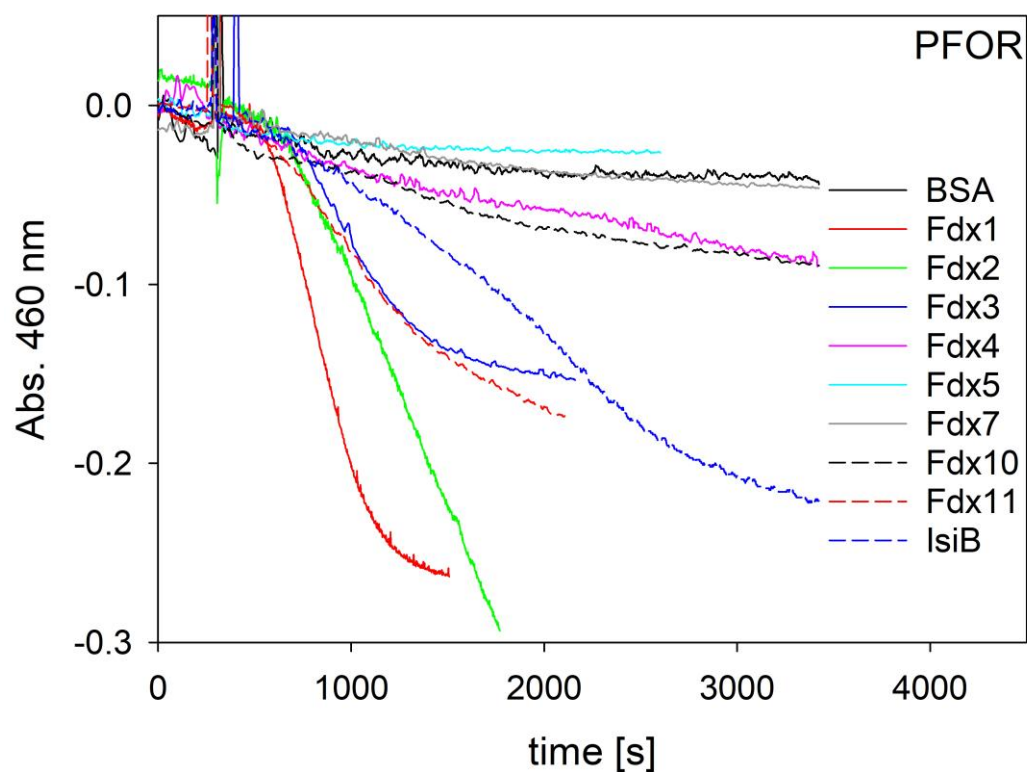


Fig. S15: Electron transfer from pyruvate ferredoxin oxidoreductase (PFOR) to purified Fdxs. To assess Fdx reduction, Fdx absorbance was monitored at 460 nm in the PFOR/Fdx reduction assay ($n=1$; due to sample scarcity). Fdx5 could not be determined, because there is no detectable change in absorption for this protein (Fig. S7). Fdx6 and Fdx8 could not be tested due to insufficient material. In these measurements the Fdx9* sample (Fig. S6) was measured. However, due to the insufficient quality of this particular sample and its oxygen sensitivity, we denote “n.d.” in Table 2.

GACTACTATAGGGCGAATTGGGTACCATATGAAGAAGCGTGTGACCCTGACTTTTCCCC
 GTAGTGCGGTGCAAATGCCCCTCACTTATCGGCTGGCCAAGGATTTTAATATTGCCGCC
 AATATTATCCGAGCCCAGGTGGCCCCCAATCAGGTAGGCAAAGTAGTACTGGAATTGTC
 CGGGGACATTGACCAACTGGAAGCGTCTTTGGAGTGGATGCGCTCCCAGAGTATTGAAG
 TTTCTTGGCTAGTCGGGAAATTGTCATTGATGACCAAAGTTGTGTAGATTGTGGCCTC
 TGTACCGGGGTTTGCCCTACGGAGGCTCTGAGTTTAGATCCGGATAGTTTTCGCTTGAT
 GTTCCGTCGTTCCCGTTGTGTGGTCTGTGAGCAATGTATTCCCTTCCTGCCCCGTCCAGG
 CGATCGCCACTAATTTCCCATCGGAGGATCCGAAAACCTGTATTTTCAGGGCGGCCT
 AGCCCCGGGCATCACCATCACCATCACTAAGATATCTAGGAATTCAGCTCCAGCTTTTG
 TTCCCTTTAGT

Fig. S16. Fragment ordered from Genscript for the generation of the pMB0122 vector. The following features are indicated: green highlight, sequence overlapping with the pBluescript SK+ vector; yellow highlight, coding sequence of *Synechocystis* Fdx9; blue highlight, a linker sequence containing a BamHI restriction site and TEV cleavage site; cyan highlight, NheI restriction site; gray highlight, SmaI restriction site; underlined yellow highlight, 6-His-tag coding sequence; purple highlight, TAA and TAG stop codons; red highlight, EcoRV restriction site; black highlight, EcoRI restriction site.

Fdx6 (ssl2559; codon-optimized):

CATATGAATAATTGCGTAATTTCTTTTCTCAAACCTAAATTTTTGCCCTATCACTAGAATTTAACGCTTG
TCTAGCGGAATATTTAACCCAGACAATTCGCCAATTTTATTCGGTTGTCGCACAGGATTATGTGGTACCT
GTCTAGTGAAAGTCGTGGGGGAAATCCTCTCCCCAGAGGCCGAGGAAAGGGAAATTTGGCTATTCTGGCT
CCTGATGATGTTTACGGCTAGATTGGCTTGTCAAATTAAGTTAACAGGAGATATTGCCATTAGAGCTTATCA
AAGTGATGAAATTCCCATCGGAGGATCC

Fdx8 (ssr3184; codon-optimized):

CATATGCCCCATACCATTGTTACGGAAACCTGCGAAGGCGTGGCCGACTGCGTTGAAGCCTGCCCCGTAGC
CTGCATTACCCCGGAGACGGGAAAAACACCATTTGGCACCATTGGTACTGGATTGACTTTGCCACCTGCA
TCGATTGCGGCATCTGCCTCCAAGTTTGCCCGGTGGAAGGGGCCATTCTGCCGGAGGAACGGCCTGATTTA
CAGAAAAGCCCTGCCCCCATCGGAGGATCC

Fdx9 (slr2059; codon-optimized):

CATATGAAGAAGCGTGTGACCTGACTTTTCCCCGTAGTGCGGTGCAAATGCCCGTCACTTATCGGCTGGC
CAAGGATTTTAATATTGCCGCCAATATTATCCGAGCCCAGGTGGCCCCCAATCAGGTAGGCAAAGTAGTAC
TGGAATTGTCCGGGGACATTGACCAACTGGAAGCGTCTTTGGAGTGGATGCGCTCCCAGAGTATTGAAGTT
TCCTTGGCTAGTCGGGAAATTGTCATTGATGACCAAAGTTGTGTAGATTGTGGCCTCTGTACCGGGGTTTG
CCCTACGGAGGCTCTGAGTTTAGATCCGGATAGTTTTTCGCTTGATGTTCCGTCGTTCCCGTTGTGTGGTCT
GTGAGCAATGTATTCCTTCCTGCCCCGTCCAGGCGATCGCCACTAATTTCCCATCGGAGATCC

Fdx10 (sl11584; codon-optimized):

CATATGGAAAACCCTGTCCCCATAACTCCCTCAGAAACCATTGTCGATAGTTGCCAACGTTTAGGCCTTGG
GCGCATTCAACGGCATCTGTTTCTCTGTTGTGACCAAACAAAACCGAAATGCTGTAGCAAAGAAGATAGCC
TCGCCACCTGGGATTATCTGAAAAGCGTTTGCCGGAGTTGGGGCTGGATTGCACTCAGTCCAGTCGTGAT
GGGAACATTTTCCGCACTAAGGCTAATTGTTTGCGGGTTTGCCAGCAGGGGCCAATTTTACTGGTATATCC
AGAGGGCATTGTGTACCGGAACGTGACCCCCACAGTAATGGAAAAAATTCTCCAAGAGCATATTTTGCAA
ACCGTCCGGTGGAGGAGTATCGCTTTTTTACCCACCCTCTTTCGCATTTACCATCGGAGATCC

Fig. S17. Codon-optimized sequences of various Fdxs (Genscript). Added restriction sites are indicated: **NdeI**, **BamHI**. Additional linker sequence is underlined.

Supporting Tables

Table S1. A comparison of the surface charges on all *Synechocystis* Fdxs studied in this work. The surface charge predictions were obtained in Pymol using the APBS surface charge calculation plugin.

Protein	Total surface residues	Positively charged aa	Negatively charged aa	Total charge @ pH 7	List of charged surface residues
Fdx1	80	5	20	-15.88	KDEEDDDEEDRKDDDDDEDEHKEED
Fdx2	103	14	21	-9.68	RHRDREKDDDRHEDERRHEDRDEEDEDEE RRRDED
Fdx3	91	6	16	-10.89	EHEDKDDEEEDERDKEKEDER
Fdx4	85	7	17	-11.79	DEEDEEDRREEHDKDKEEDKHEE
Fdx5	141	21	25	-5.78	KKDDKEDDRKERHERRRREHRRRDEEEDEE RREERDEKKRDDKDDE
Fdx6	85	7	12	-4.99	KEEDRKEEEREDDRKDRDE
Fdx7	82	14	14	-5.39	ERDKHEEHRDDEEDDHDEKHEEKRRHRH
Fdx8	70	5	12	-8.79	HEEDEHDKDDDEEERDK
Fdx9	118	14	15	-0.99	KKRRRKDRKEDDEEREREDDDEDDRRRRE
Fdx10	114	20	15	+2.31	EEDRRRHDKKKEDDKKREDRDRKREKER EERHH
Fdx11	68	2	12	-9.99	DDREDEREEDEEDD
Fdx12	81	13	8	+5	KEKDREKDKKEEKDRRKDKK

Table S2. Information on Fdx1 to Fdx12 and flavodoxin (IsiB) over-expression and purification. Fdx8 and Fdx9 were prepared using two different expression plasmids and strains (Δ iscR BL21 strain is described in [32]). Fdx8-Strep was not analyzed by SDS-PAGE (Fig. S6). The purification method for Fdx9-Strep performed as described in [32]. Fdx6, Fdx9 and Fdx10 were purified from combinations of separate independent expressions. Fdx12 could not be expressed as soluble protein and was therefore not purified in this study. Protein concentrations were determined by Bradford assay, and the relative abundance was determined by LC/MS (data not shown). Buffer A: 25 mM NaPO₄, pH=7.0; 50 mM NaCl; 5% (v/v) glycerol; Buffer B: 50 mM Tris pH 8.5, 50 mM NaCl, 5% glycerol. Abbreviations: Fed6co, Fdx6 codon optimized sequence; TEV, TEV protease recognition site; GST, glutathione-S-transferase affinity-tag; 6xHis, 6x histidine affinity-tag; MBP, maltose-binding protein; Strep, Strep affinity-tag; n.d., not determined.

Protein	Expression Plasmid	<i>E. coli</i> Expression strain	Protein [μM]	relative abundance	adjusted protein [μM]	buffer
Fdx1	Fed1-TEV-GST-6xHis	KRX	652	8.53%	56	A
Fdx2	Fed2-TEV-GST-6xHis	KRX	1326	90.64%	1202	A
Fdx3	Fed3-TEV-GST-6xHis	KRX	1086	69.57%	755	A
Fdx4	Fed4-TEV-GST-6xHis	KRX	1382	97.38%	1346	A
Fdx5	Fed5-TEV-GST-6xHis	KRX	1419	99.95%	1418	A
Fdx6	Fed6-TEV-GST-6xHis Fed6co-TEV-MBP-His	KRX C41 (DE3)	270	74.08%	200	A
Fdx7	Fed7-TEV-GST-6xHis	KRX	934	98.37%	919	A
Fdx8	Fed8co-TEV-MBP-His	KRX	955	n.d.	n.d.	B
Fdx8*	Fed8-Strep	Δ iscR BL21	n.d.	n.d.	n.d.	B
Fdx9	Fed9-TEV-GST-6xHis Fed9co-TEV-MBP-His	KRX KRX	247	n.d.	n.d.	A
Fdx9*	Fed9-Strep	BL21 (DE3)	116	84.93%	99	B
Fdx10	Fed10-TEV-GST-6xHis Fed10co-TEV-MBP-His	KRX KRX	518	98.16%	508	A
Fdx11	Fed11-TEV-GST-6xHis	KRX	1603	79.40%	1273	A
Fdx12	Fed12-TEV-GST-6xHis	KRX	No soluble protein expression in <i>E. coli</i>			
IsiB	IsiB-TEV-GST-6xHis	Rosetta 2 pLysS (DE3)	771	92.24%	711	A

Table S3. EPR properties of the reduced *Synechocystis* Fdx signals presented in Fig. 3.

Protein	<i>g</i> -values	Rhombicity (%) ^a	Fe-S cluster type	Reference
Fdx1	<i>g</i> = 2.048, 1.955, 1.877; <i>g</i> _{av} = 1.96	89	[2Fe-2S]	this work, [4]
Fdx2	<i>g</i> = 2.048, 1.957, 1.869; <i>g</i> _{av} = 1.96	98	[2Fe-2S]	this work
Fdx3	<i>g</i> = 2.044, 1.952, 1.868; <i>g</i> _{av} = 1.95	94	[2Fe-2S]	this work
Fdx4 ^b	<i>g</i> = 2.054, 1.963, 1.878; <i>g</i> _{av} = 1.97	96	[2Fe-2S]	this work, [45]
Fdx5 ^c	u.d.	u.d.	u.d.	this work
Fdx6	<i>g</i> = 2.040, 1.946, 1.883; <i>g</i> _{av} = 1.96	75	[2Fe-2S]	this work
Fdx7 ^d	<i>g</i> = 2.068, 1.926, 1.888; <i>g</i> _{av} = 1.96 <i>g</i> = 2.058, 1.932, 1.869; <i>g</i> _{av} = 1.95	35 60	[4Fe-4S]	this work
Fdx8	<i>g</i> = 2.068, 1.932, 1.871; <i>g</i> _{av} = 1.96 <i>g</i> = 2.058, 1.929, 1.890; <i>g</i> _{av} = 1.96	55 39	2x[4Fe-4S]	this work
Fdx9 ^f	<i>g</i> = 2.07, 2.05, 2.01, 1.99, 1.94	u.d.	2x[4Fe-4S]	[32]
Fdx10	<i>g</i> = 2.002, 1.948, 1.919; <i>g</i> _{av} = 1.96	64	[2Fe-2S]	this work
Fdx11	<i>g</i> = 2.016, 1.941, 1.930; <i>g</i> _{av} = 1.96	20	[2Fe-2S]	this work

^aCalculated according to the following equation: percent rhombicity = $300 \cdot ((g_y - g_x) / (2g_z - g_y - g_x))$, where $g_z > g_y > g_x$ [46]. ^bFdx4 is homologous to Fdx2 from *Thermosynechococcus elongatus* and displayed a similar EPR signal at *g* = 2.051, 1.964, 1.869 [45]. No EPR signal could be obtained for Fdx5. ^cWhile the overall signal intensity of Fdx5 was very weak, several resonances near *g* = 2 and *g* = 1.93 were observed. ^dThe spectrum of Fdx7 was simulated with two overlapping rhombic signals, with the total simulation consisting of 64% contribution of the *g* = 2.068 signal and 36% contribution of the *g* = 2.058 signal, along with a very minor contribution of a species at *g* = 2.003 (<1%). The spectrum of Fdx8 was simulated with 2 overlapping rhombic signals, with the total simulation consisting of 68% contribution of the *g* = 2.068 signal and 32% contribution of the *g* = 2.058 signal, along with a very minor contribution of a species at *g* = 2.003 (<1%). ^fThe *g*-values listed here for Fdx9 give the general positions of spectral features and were not obtained from simulation; initial attempts at simulating the complex spectrum of Fdx9 with and without the addition of spin-spin coupling are reported in [32]. Abbreviations: *g*_{av}, *g*-value average, u.d., undetermined.

Table S4. Primers used in this work.

#	primer name	internal reference	sequence	used for
1	Syn_ssl0020_Fed1-fw	MB01_B4	5'-GCGCATATGGCATCCTATACCGTTAAATTGATCACC-3'	cloning of expression construct
2	Syn_ssl0020_Fed1-rev	MB01_C8	5'-GCGGGATCCTCCGATGGGGTAGAGGTCTTCTTCTTTGTGGGTTTCAAT-3'	cloning of expression construct
3	Syn_sll1382_Fed2-fw	MB01_B5	5'-GCGCATATGTCCC GTTCCCACCGAGTTCT-3'	cloning of expression construct
4	Syn_sll1382_Fed2-rev	MB01_C9	5'-GCGGGATCCTCCGATGGGGTCTCATCTAAAGGCAAACCTAATCG-3'	cloning of expression construct
5	Syn_slr1828_Fed3-fw	MB01_B6	5'-GCGCATATGGTTAACACCTACACCGCCGAAAT-3'	cloning of expression construct
6	Syn_slr1828_Fed3-rev	MB01_D1	5'-GCGGGATCCTCCGATGGGACCTTGCCGCGCAACTGCC-3'	cloning of expression construct
7	Syn_slr0150_Fed4-fw	MB01_B7	5'-GCGCATATGGGTGCAATTTATTCCGTCAATTTAGTCAAT-3'	cloning of expression construct
8	Syn_slr0150_Fed4-rev	MB01_D2	5'-GCGGGATCCTCCGATGGGACCAAAACAACGCTTCTTGTGGTGAG-3'	cloning of expression construct
9	Syn_slr0148_Fed5-fw	MB01_B8	5'-GCGCATATGGCTAAAACATATTAAGCTCGACCCCAT-3'	cloning of expression construct
10	Syn_slr0148_Fed5-rev	MB01_D3	5'-GCGGGATCCTCCGATGGGTTCATCGGTATCGTTGACAATCTGAATTTTGG-3'	cloning of expression construct
11	Syn_ssl2559_Fed6-fw	MB01_B9	5'-GCGCATATGAATAATTGCGTAATTTCTTTCTCAAACATAAATTTTGG-3'	cloning of expression construct
12	Syn_ssl2559_Fed6-rev	MB01_D4	5'-GCGGGATCCTCCGATGGGAATTTATCAGCTTTGATAAGCTCTAATGGCAATATCT-3'	cloning of expression construct
13	Syn_sll0662_Fed7-fw	MB01_C1	5'-GCGCATATGGTGATAGCTGATCTAAATTTCTCTCCC-3'	cloning of expression construct
14	Syn_sll0662_Fed7-rev	MB01_D5	5'-GCGGGATCCTCCGATGGGCAATGGGGATTGATTGCGGGTAACC-3'	cloning of expression construct
15	Syn_ssr3184_Fed8-fw	MB01_C2	5'-GCGCATATGCCCCATACCATTTGTTACGGAAACC-3'	cloning of expression construct
16	Syn_ssr3184_Fed8-rev	MB01_D6	5'-GCGGGATCCTCCGATGGGGGCAGGGCTTTTCTGTAAATCAGGC-3'	cloning of expression construct
17	Syn_slr2059_Fed9-fw	MB01_C3	5'-GCGCATATGAAGAAGCGTGTGACCCTGACTTTT-3'	cloning of expression construct
18	Syn_slr2059_Fed9-rev	MB01_D7	5'-GCGGGATCCTCCGATGGGGAAATTAGTGGCGATCGCCTGGAC-3'	cloning of expression construct
19	Syn_sll1584_Fed10-fw	MB01_C4	5'-GCGCATATGGAAAACCTGTCCCCATAACTCC-3'	cloning of expression construct
20	Syn_sll1584_Fed10-rev	MB01_D8	5'-GCGGGATCCTCCGATGGGTAAATGCGAAAGAGGGTGGGTAAAAAAGC-3'	cloning of expression construct
21	Syn_ssl3044_Fed11-fw	MB01_C5	5'-GCGCATATGACCATTACCTTTGTTAAAGAGCAGAAGG-3'	cloning of expression construct
22	Syn_ssl3044_Fed11-rev	MB01_D9	5'-GCGGGATCCTCCGATGGGGCCTTTGGGTTGGTGTTCACACTCA-3'	cloning of expression construct
23	Syn_ssr1041_Fed12-fw	MB10_A2	5'-TTTAACTTTAAGAAGGAGATATACATATGATGGCAGTAACGATACACTTTTGC-3'	cloning of expression construct
24	Syn_ssr1041_Fed12-rev	MB10_A3	5'-CCTGAAAATACAGGTTTTTCGGATCCTCCGATGGGCCAGGTGAGATCATCATAAAGATTAATTCTAATTCCTG-3'	cloning of expression construct
25	Syn_sll0248_IsiB-fw	MB02_A3	5'-GTTTAACTTTAAGAAGGAGATATACATATGACAAAAATTGGACTTTTTTACGGTAC-3'	cloning of expression construct
26	Syn_sll0248_IsiB-rev	MB02_A4	5'-GCCGCCCTGAAAATACAGGTTTTTCGGATCCTCCGATGGGGGATTGCAAAATTGGTTTAATTCAC-3'	cloning of expression construct
27	MBP-fw	Common	5'-GCTAGCATGAAAATCGAAGAAGGTAAACTGGTAATCTG-3'	cloning of expression construct
28	MBP-rev	Common	5'-CCCGGGAGTCTGCGCGTCTTTCAGGGCTT-3'	cloning of expression construct
29	Fed1-His_1-fw	MB03_F1	5'-GACTACTATAGGGCGAATTGGGTACCTCGAGATGGCATCCTATACCGTTAAATTGATCACC-3'	generation of C-entry plasmid, mutant confirmation
30	Fed1-His_2-rev	MB03_F2	5'-GAATTCCTCGAGGGATCCTCCGATGGGGTAGAGGTCTTCTTCTTTGTGGGTTTCA-3'	generation of C-entry plasmid

#	primer name	internal reference	sequence	used for
31	Fed1-His_3-fw	MB03_F3	5'-CCCATCGGAGGATCCCTCGAGGAATTCGGGTAATAATGCTGGCCATGGGCTA-3'	generation of C-entry plasmid
32	Fed1-His_4-rev	MB03_F4	5'-ACTAAAGGGAACAAAAGCTGGAGCTCTGGAAATTGCTTTACTAAATGTGCCCC-3'	generation of C-entry plasmid, mutant confirmation
33	Fed2-His_1-fw	MB03_F5	5'-GACTACTATAGGGCGAATTGGGTACCTCGAGATGTCCCGTCCACCGAGTTCT-3'	generation of C-entry plasmid, mutant confirmation
34	Fed2-His_2-rev	MB03_F6	5'-GAATTCCTCGAGGGATCCTCCGATGGGGTCTCATCTAAAGGCAAACCTAATCG-3'	generation of C-entry plasmid
35	Fed2-His_3-fw	MB03_F7	5'-CCCATCGGAGGATCCCTCGAGGAATTCGTAGGCTACAACCTGATTTTCC-3'	generation of C-entry plasmid
36	Fed2-His_4-rev	MB03_F8	5'-ACTAAAGGGAACAAAAGCTGGAGCTGGGAATGTCTTGATCAGTAATCCAATG-3'	generation of C-entry plasmid, mutant confirmation
37	Fed3-His_1-fw	MB03_F9	5'-GACTACTATAGGGCGAATTGGGTACCTCGAGATGGTTAACACCTACACCGCCGAAATC-3'	generation of C-entry plasmid, mutant confirmation
38	Fed3-His_2-rev	MB03_G1	5'-GAATTCCTCGAGGGATCCTCCGATGGGACCTTGCCGCCGAACCTGCC-3'	generation of C-entry plasmid
39	Fed3-His_3-fw	MB03_G2	5'-CCCATCGGAGGATCCCTCGAGGAATTCATTTCGGCTGGAATTCTCCCTTCCC-3'	generation of C-entry plasmid
40	Fed3-His_4-rev	MB03_G3	5'-ACTAAAGGGAACAAAAGCTGGAGCTGCTGTTTTAGGTAGCCCTGCACTG-3'	generation of C-entry plasmid, mutant confirmation
41	Fed4-His_1-fw	MB03_G4	5'-GACTACTATAGGGCGAATTGGGTACCTCGAGATGGGTGCAATTTATTCCGTCATTTAGTCA-3'	generation of C-entry plasmid, mutant confirmation
42	Fed4-His_2-rev	MB03_G5	5'-GAATTCCTCGAGGGATCCTCCGATGGGACCAACAACGCTTCTTGGTGAGT-3'	generation of C-entry plasmid
43	Fed4-His_3-fw	MB03_G6	5'-CCCATCGGAGGATCCCTCGAGGAATTCTCAGCCACCGGCTACAACCTCCG-3'	generation of C-entry plasmid
44	Fed4-His_4-rev	MB03_G7	5'-ACTAAAGGGAACAAAAGCTGGAGCTGCAGCGGCTTGGAAGCTTCCAAC-3'	generation of C-entry plasmid, mutant confirmation
45	Fed5-His_1-fw	MB03_G8	5'-GACTACTATAGGGCGAATTGGGTACCTCGAGGTGGCTAAAACTATTAAGCTCGACCC-3'	generation of C-entry plasmid, mutant confirmation
46	Fed5-His_2-rev	MB03_G9	5'-GAATTCCTCGAGGGATCCTCCGATGGGTTTCATCGGTATCGTTGACAATCTGAATTTTG-3'	generation of C-entry plasmid
47	Fed5-His_3-fw	MB03_H1	5'-CCCATCGGAGGATCCCTCGAGGAATTCCTTTCCTGGCTGACTGTGGCGGTT-3'	generation of C-entry plasmid
48	Fed5-His_4-rev	MB03_H2	5'-ACTAAAGGGAACAAAAGCTGGAGCTAATAGACCCTCCCGCAGACGGT-3'	generation of C-entry plasmid, mutant confirmation
49	Fed6-His_1-fw	MB03_H3	5'-GACTACTATAGGGCGAATTGGGTACCTCGAGGCGTAATTTCTTTTCCTCAAACCTAAATTTTGCC-3'	generation of C-entry plasmid, mutant confirmation
50	Fed6-His_2-rev	MB03_H4	5'-GAATTCCTCGAGGGATCCTCCGATGGGAATTTTCATCACTTTGATAAGCTCTAATGGCAATATC-3'	generation of C-entry plasmid
51	Fed6-His_3-fw	MB03_H5	5'-CCCATCGGAGGATCCCTCGAGGAATTCAGATTTTGGTATGTGGTGGCTGAAAGTAG-3'	generation of C-entry plasmid
52	Fed6-His_4-rev	MB03_H6	5'-ACTAAAGGGAACAAAAGCTGGAGCTGCAGTTGGTGACATTATTAGCAAATCGATG-3'	generation of C-entry plasmid, mutant confirmation

#	primer name	internal reference	sequence	used for
53	Fed7-His_1-fw	MB03_H7	5'-GACTACTATAGGGCGAATTGGGTACCTCGAGATGGTGATAGCTGATCTAAATTTTCCTCCC-3'	generation of C-entry plasmid, mutant confirmation
54	Fed7-His_2-rev	MB03_H8	5'-GAATTCCTCGAGGGATCCTCCGATGGGCAAATGGGGATTGATTGCGGGTAAC-3'	generation of C-entry plasmid
55	Fed7-His_3-fw	MB03_H9	5'-CCCATCGGAGGATCCCTCGAGGAATTCCTAATTGGGTGATGGAATCTAATCGAAATCAG-3'	generation of C-entry plasmid
56	Fed7-His_4-rev	MB03_I1	5'-ACTAAAGGGAACAAAAGCTGGAGCTGGTATTTGAAACGGTGGTGTTATTTTGCTG-3'	generation of C-entry plasmid, mutant confirmation
57	Fed8-His_1-fw	MB03_I2	5'-GACTACTATAGGGCGAATTGGGTACCTCGAGGTTGAACGTCGCTACCCCTAGAAC-3'	generation of C-entry plasmid, mutant confirmation
58	Fed8-His_2-rev	MB03_I3	5'-GAATTCCTCGAGGGATCCTCCGATGGGGCAGGGCTTTTCTGTAAATCAGGC-3'	generation of C-entry plasmid
59	Fed8-His_3-fw	MB03_I4	5'-CCCATCGGAGGATCCCTCGAGGAATTCGGTTGGGAGGGGTCTAACTGC-3'	generation of C-entry plasmid
60	Fed8-His_4-rev	MB03_I5	5'-ACTAAAGGGAACAAAAGCTGGAGCTGGTAGGACCCTGGTGTAAGAAAG-3'	generation of C-entry plasmid, mutant confirmation
61	Fed9-His_1-fw	MB03_I6	5'-GACTACTATAGGGCGAATTGGGTACCTCGAGATGAAGAAGCGTGTGACCCTGACTTTT-3'	generation of C-entry plasmid, mutant confirmation
62	Fed9-His_2-rev	MB03_I7	5'-GAATTCCTCGAGGGATCCTCCGATGGGGAAATTAGTGGCGATCGCCTGGAC-3'	generation of C-entry plasmid
63	Fed9-His_3-fw	MB03_I8	5'-CCCATCGGAGGATCCCTCGAGGAATTCGTTCTCAAACGCGCATCGCCAAAATTG-3'	generation of C-entry plasmid
64	Fed9-His_4-rev	MB03_I9	5'-ACTAAAGGGAACAAAAGCTGGAGCTGTAAGCCAGGGCGGTCTAATTTCCC-3'	generation of C-entry plasmid, mutant confirmation
65	Fed10-His_1-fw	MB04_A1	5'-GACTACTATAGGGCGAATTGGGTACCTCGAGATGGAAAACCTGTCCCCATAACTCC-3'	generation of C-entry plasmid, mutant confirmation
66	Fed10-His_2-rev	MB04_A2	5'-GAATTCCTCGAGGGATCCTCCGATGGGTAAATGCGAAAGAGGGTGGGTAAAAAAGC-3'	generation of C-entry plasmid
67	Fed10-His_3-fw	MB04_A3	5'-CCCATCGGAGGATCCCTCGAGGAATTCATTATCAAAACCGCTATAGAAACGAGTCGG-3'	generation of C-entry plasmid
68	Fed10-His_4-rev	MB04_A4	5'-ACTAAAGGGAACAAAAGCTGGAGCTGAGGAAACCAACCTTTTAATCGCCGAAAA-3'	generation of C-entry plasmid, mutant confirmation
69	Fed11-His_1-fw	MB04_A5	5'-GACTACTATAGGGCGAATTGGGTACGTGACCATTACCTTTGTAAAGAGCAGAAG-3'	generation of C-entry plasmid, mutant confirmation
70	Fed11-His_2-rev	MB04_A6	5'-GAATTCCTCGAGGGATCCTCCGATGGGGCCTTTGGGTTTGGTGTTACACTCA-3'	generation of C-entry plasmid
71	Fed11-His_3-fw	MB04_A7	5'-CCCATCGGAGGATCCCTCGAGGAATTCGGGCGATCACCAGAGGCCA-3'	generation of C-entry plasmid
72	Fed11-His_4-rev	MB04_A8	5'-ACTAAAGGGAACAAAAGCTGGAGCTGTTGTACTTTATAGTGTGTAGTATCCCG-3'	generation of C-entry plasmid, mutant confirmation
73	Gent-fw	Common	5'-GGTTCGTGCCTTCATCCGTCGAC-3'	generation of toolkit vector
74	Gent-rev	Common	5'-CGCCACCTAACCAATTCGGTCGAC-3'	generation of toolkit vector
75	NirA-fw	MB05_H7	5'-GACGGTGCCTAAGCCCGTGC GGATCC-3'	mutant confirmation
76	NirA-rev	MB05_H6	5'-GTAAGTTACCTACCTATCCACGAACCAT-3'	mutant confirmation

#	primer name	internal reference	Sequence	used for
77	Fed1-nLuc-pSSR-fw	MB09_F2	5'-GAGTAGTGGAGGTTACCATATGGCATCCTATACCGTTAAATTGATCAC-3'	rhamnose inducible expression
78	Fed1-nLuc-pSSR-rev	MB09_F3	5'-CGGCAGCAGATCTCATGTAGAGGTCTTCTTCTTTGTGGGT-3'	rhamnose inducible expression
79	Fed2-nLuc-pSSR-fw	MB09_F4	5'-GAGTAGTGGAGGTTACCATATGTCCCGTCCACCGAGTTCT-3'	rhamnose inducible expression
80	Fed2-nLuc-pSSR-rev	MB09_F5	5'-CGGCAGCAGATCTCATGTCCTCATCTAAAGGCAAACCTAAT-3'	rhamnose inducible expression
81	Fed3-nLuc-pSSR-fw	MB09_F6	5'-GAGTAGTGGAGGTTACCATATGGTTAACACCTACACCGCC-3'	rhamnose inducible expression
82	Fed3-nLuc-pSSR-rev	MB09_F7	5'-CGGCAGCAGATCTCATACCTTGCCCGCCGAA-3'	rhamnose inducible expression
83	Fed4-nLuc-pSSR-fw	MB09_F8	5'-GAGTAGTGGAGGTTACCATATGGGTGCAATTATTCCGTCAATTTAGT-3'	rhamnose inducible expression
84	Fed4-nLuc-pSSR-rev	MB09_F9	5'-CGGCAGCAGATCTCATACCAACAACGCTTCCTCTTG-3'	rhamnose inducible expression
85	Fed5-nLuc-pSSR-fw	MB09_G1	5'-GAGTAGTGGAGGTTACCATATGGCTAAAACTATTAAGCTCGACCC-3'	rhamnose inducible expression
86	Fed5-nLuc-pSSR-rev	MB09_G2	5'-CGGCAGCAGATCTCATTTTCATCGGTATCGTTGACAATCTGAATTTTG-3'	rhamnose inducible expression
87	Fed6-nLuc-pSSR-fw	MB09_G3	5'-GAGTAGTGGAGGTTACCATATGAATAATTGCGTAATTTCTTTTCCTCAAACCTAAATTT-3'	rhamnose inducible expression
88	Fed6-nLuc-pSSR-rev	MB09_G4	5'-CGGCAGCAGATCTCATAATTTTCATCACTTTGATAAGCTCTAATGGCA-3'	rhamnose inducible expression
89	Fed7-nLuc-pSSR-fw	MB09_G5	5'-GAGTAGTGGAGGTTACCATATGGTGATAGCTGATCTAAATTTTCCTCC-3'	rhamnose inducible expression
90	Fed7-nLuc-pSSR-rev	MB09_G6	5'-CGGCAGCAGATCTCATCAAATGGGGATTGATTGCGG-3'	rhamnose inducible expression
91	Fed8-nLuc-pSSR-fw	MB09_G7	5'-GAGTAGTGGAGGTTACCATATGCCCATACCATTTGTTACGG-3'	rhamnose inducible expression
92	Fed8-nLuc-pSSR-rev	MB09_G8	5'-CGGCAGCAGATCTCATGGCAGGGCTTTTCTGTAAATCAG-3'	rhamnose inducible expression
93	Fed9-nLuc-pSSR-fw	MB09_G9	5'-GAGTAGTGGAGGTTACCATATGAAGAAGCGTGTGACCCTG-3'	rhamnose inducible expression
94	Fed9-nLuc-pSSR-rev	MB09_H1	5'-CGGCAGCAGATCTCATGAAATTAGTGGCGATCGCCTGGACGGG-3'	rhamnose inducible expression
95	Fed10-nLuc-pSSR-fw	MB09_H2	5'-GAGTAGTGGAGGTTACCATATGGAAAACCCTGTCCCCATAAC-3'	rhamnose inducible expression
96	Fed10-nLuc-pSSR-rev	MB09_H3	5'-CGGCAGCAGATCTCATTAAATGCGAAAGAGGGTGGGTAAAAAAG-3'	rhamnose inducible expression
97	Fed11-nLuc-pSSR-fw	MB09_H4	5'-GAGTAGTGGAGGTTACCATATGACCATTACCTTTGTAAAGAGCAGAAG-3'	rhamnose inducible expression
98	Fed11-nLuc-pSSR-rev	MB09_H5	5'-CGGCAGCAGATCTCATGCCTTTGGGTTTGGTGTTCAC-3'	rhamnose inducible expression
99	Fed12-nLuc-pSSR-fw	MB09_I9	5'-TAGAGTAGTGGAGGTTACCATATGGCAGTAACGATACACTTTTTC-3'	rhamnose inducible expression
100	Fed12-nLuc-pSSR-rev	MB10_A1	5'-CGCTGCGGCAGCAGATCTCATCCAGGTGAGATCATATAAAGATTAATTTCTAATTCC-3'	rhamnose inducible expression
101	Isib-nLuc-pSSR-fw	MB10_A8	5'-TAGAGTAGTGGAGGTTACCATATGACAAAAATTGGACTTTTTTACGGTACTCA-3'	rhamnose inducible expression
102	Isib-nLuc-pSSR-rev	MB10_A9	5'-CGCTGCGGCAGCAGATCTCATGGATTGCAAAATTGGTTTAATTTCACTTACC-3'	rhamnose inducible expression
103	pSSR_seq-fw	MB08_B9	5'-ATGGCCCATTTTCCTGTACGTAACGAG-3'	mutant confirmation
104	pSSR_seq-rev	MB08_C1	5'-TTTGCTTCAGATGTATGCTCTTCTGC-3'	mutant confirmation