Supplemental data Figure S1

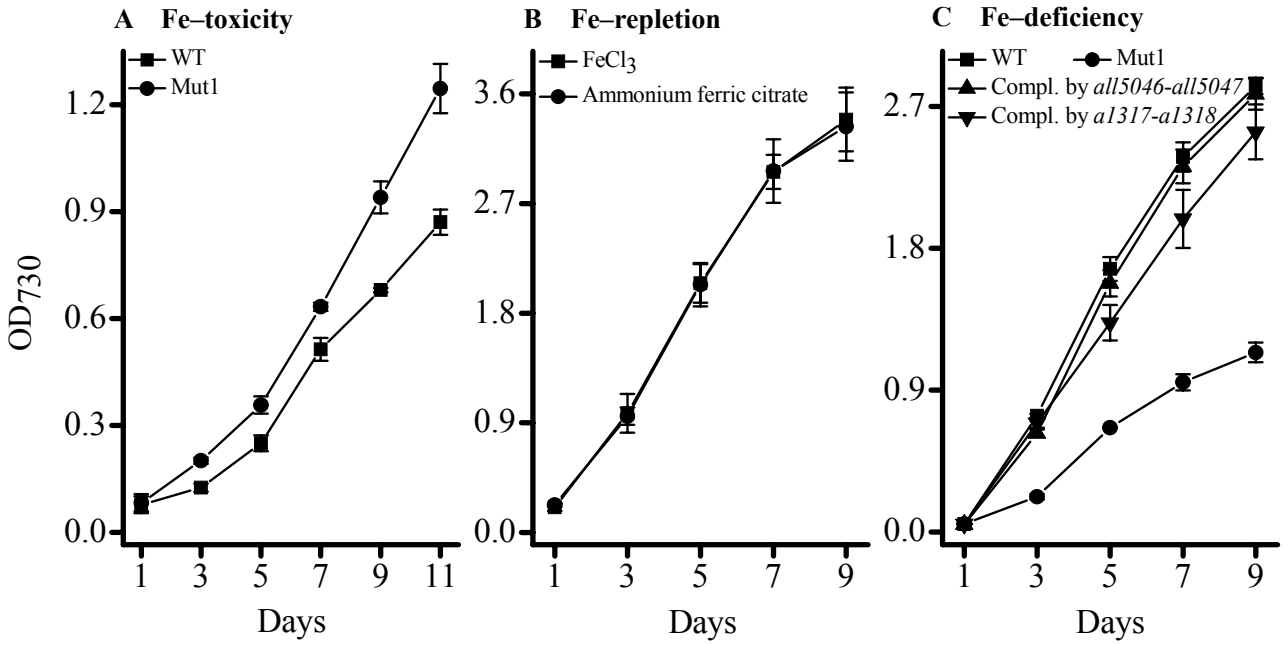

Figure S1 Growth characteristics of *Synechocystis* 6803 wild type (WT), *Δsll1404-1405* mutant (Mut1) and strains complemented with *Anabaena* sp. PCC 7120 *exbBD* homologs (*all5046-all5047*) or *Synechococcus* sp. PCC 7002 *exbBD* homologs (*a1317-a1318*). Strains were cultured at 30 °C under continuous illumination of 40 μmol photons·m^{-2}·s^{-1}. (A) WT and Mut1 cells were grown in BG11 medium supplemented with 1 mM ammonium ferric citrate. (B) WT cells were grown in YBG11 containing 2 μM ammonium ferric citrate or 2 μM $FeCl_3$ (replacing ammonium ferric citrate with the same concentration of $FeCl_3$ and omitting citric acid). (C) WT, Mut1 and complementation strains were cultured in iron-deficient BG11 medium (replacing ammonium ferric citrate with ammonium citrate).

Supplemental data Figure S2

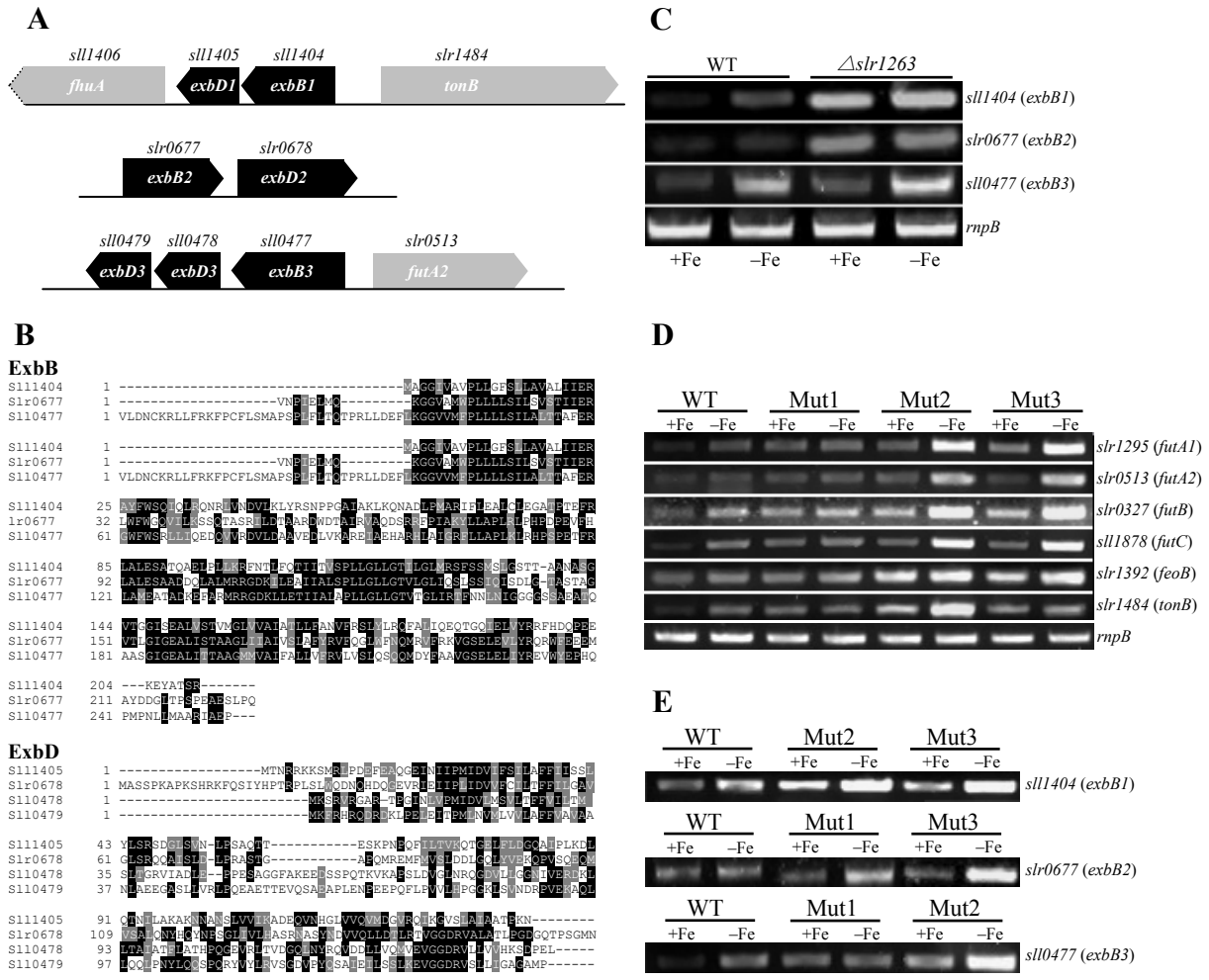

Figure S2 Putative components, amino acid sequence alignment, and transcriptional analysis of *tonB*, *exbB*, *exbD* genes in *Synechocystis* 6803. (A) The genomic organization of the *exbB-exbD* gene clusters in *Synechocystis* is shown based on the annotations deposited in Cyanobase (http://genome.kazusa.or.jp/cyanobase). (B) Alignment of the amino acid sequences of the putative ExbB and ExbD proteins in *Synechocystis* 6803. Identical and similar residues are shaded in black and gray, respectively. (C) Transcript levels of the putative *exbB* genes in *Synechocystis* wild type and *Δsll1263* mutant cultured under standard iron concentration (+Fe) or iron-limiting (–Fe) conditions for 24 h. The reference gene is the housekeeping gene *rnpB*. (D) Transcript levels of the known iron uptake genes in wild type and mutants cultured under standard iron concentration (+Fe) or iron-limiting (–Fe) conditions for 24 h. (E) Transcript levels of *exbB* genes in the single mutants of other *exbB-exbD* gene clusters cultured under standard iron concentration (+Fe) or iron-limiting (–Fe) conditions for 24 h.

Supplemental data Figure S3

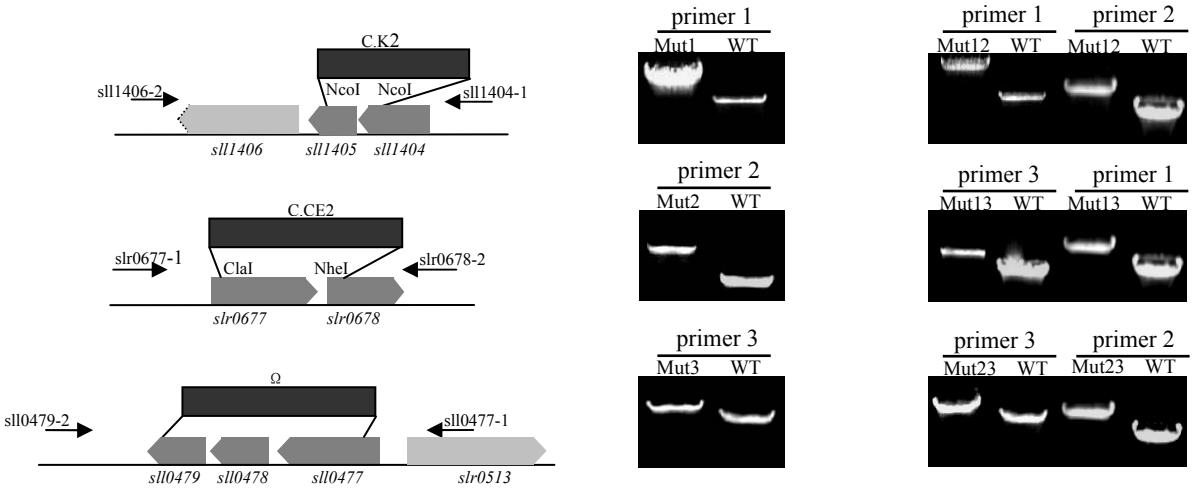

Figure S3 PCR results, showing complete segregation of the three single mutants and three double mutants. Primer 1: sll1404-1/sll1406-2; primer 2: slr0677-1/slr0678-2; primer 3: sll0477-1/sll0479-2. The primers used are listed in Table S2.

Supplemental data Table S1

Table S1 Strains, plasmids and primers used in this study.

Strains, plasmids and primers	Derivation and / or relevant characteristics[a]	Reference(s) or source
***Synechocystis* strains**		
PCC6803	Wild type	This study
Mut1	Km^r, *Synechocystis* 6803 mutant, result of transformation with pHS036	This study
Mut2	Em^r, *Synechocystis* 6803 mutant, result of transformation with pHS178	This study
Mut3	Sp^r, *Synechocystis* 6803 mutant, result of transformation with pHS205	This study
Mut12	$Km^r Em^r$, *Synechocystis* 6803 mutant, result of transformation with pHS036 and pHS178	This study
Mut13	$Km^r Sp^r$, *Synechocystis* 6803 mutant, result of transformation with pHS036 and pHS205	This study
Mut23	$Em^r Sp^r$, *Synechocystis* 6803 mutant, result of transformation with pHS178 and pHS205	This study
Mut123	$Km^r Em^r Sp^r$, *Synechocystis* 6803 mutant, result of transformation with pHS036, pHS178 and pHS205	This study
WT(Ω-PsbAII-Sll0477-flag)	Sp^r, Omega-P_{psbAII}-*sll0477*-flag integrated into *slr0168* in the genome of *Synechocystis* wild type	This study
WT(Ω-PsbAII-Slr0678-flag)	Sp^r, Omega-P_{psbAII}-*slr0678*-flag integrated into *slr0168* in the genome of *Synechocystis* wild type	This study
Mut1(Ω-PsbAII-Sll1404-Sll1405)	Sp^r, Omega-P_{psbAII}-*sll1404-sll1405* integrated into *slr0168* in the genome of *Synechocystis* Mut1	This study
Mut1(Ω-PsbAII-Slr0677-Slr0678)	Sp^r, Omega-P_{psbAII}-*slr0677-slr0678* integrated into *slr0168* in the genome of *Synechocystis* Mut1	This study
Mut1(Ω-PsbAII-Sll0477-Sll0478-Sll0479)	Sp^r, Omega-P_{psbAII}-*sll0477-sll0478-sll0479* integrated into *slr0168* in the genome of *Synechocystis* Mut1	This study
Mut2(Ω-PsbAII-Sll1404-Sll1405)	Sp^r, Omega-P_{psbAII}-*sll1404-sll1405* integrated into *slr0168* in the genome of *Synechocystis* Mut2	This study
Mut2(Ω-PsbAII-Slr0677-Slr0678)	Sp^r, Omega-P_{psbAII}-*slr0677-slr0678* integrated into *slr0168* in the genome of *Synechocystis* Mut2	This study
Mut2(Ω-PsbAII-Sll0477-Sll0478-Sll0479)	Sp^r, Omega-P_{psbAII}-*sll0477-sll0478-sll0479* integrated into *slr0168* in the genome of *Synechocystis* Mut2	This study
Mut1(Ω-PsbAII-All5046-All5047)	Sp^r, Omega-P_{psbAII}-*all5046-all5047* integrated into *slr0168* in the genome of *Synechocystis* Mut1	This study
Mut1(Ω-PsbAII-A1317-A1318)	Sp^r, Omega-P_{psbAII}-*a1317-a1318* integrated into *slr0168* in the genome of *Synechocystis* Mut1	This study
Yeast strains		
Y187	*MATα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, gal4△, met-, gal80, URA∷GAL 1*$_{USA}$*—GAL 1TATA—LacZ*	Harper et al. (1993)
Y187 (pGKT7-Sll1404/ pGADT7-Sll1405)	$Ap^r Km^r$, Y187 transformed with pHS635 and pHS636	This study
Plasmids		
pHS036	$Ap^r Km^r$, PCR fragment containing *sll1404-sll1405* cloned into pMD18T, and C.K2 inserted in its *Nco*I site	This study
pHS 175	$Ap^r Km^r$, PCR fragment containing *sll0477* cloned into pMD18T	This study
pHS 176	$Ap^r Km^r$, PCR fragment containing *sll0479* cloned into pMD18T	This study
pHS 178	$Ap^r Cm^r$, PCR fragment containing *slr0677-slr0678* cloned into pMD18T, which was excised by *Cla*I	This study

	and *Nhe*I and then inserted by C.CE2	
pHS 205	$Ap^r Sp^r$, an omega drug, linked to the *Hind*III endonucleases side of plasmid pHS175	This study
pHS 208	$Ap^r Sp^r$, the DNA fragment digested from the pHS176 with *Pst*I and *BamH*I, was cloned to the *Xba*I side of pHS205	This study
pHS 211	Ap^r, PCR fragment containing the psbAIIpromoter region was cloned into pBluescript II SK(－)	Jiang et al. (2012)
pHS 215	$Ap^r Sp^r$ omega cassette from pRL57 inserted into pHS 211 at the *Cla*I site	Jiang et al. (2012)
pHS 298	$Ap^r Sp^r$, P*psbAII* expression vector, Omega-P_{psbAII} from pHS215 inserted into Platform0168 at the *Eco*RI site	Jiang et al. (2012)
pHS 431	$Ap^r Sp^r$, the *sll0477*-flag fragment was cloned to the P*psbAII* expression vector, pHS298	This study
pHS 433	$Ap^r Sp^r$, the *sll0477-sll0478-sll0479*-flag fragment was cloned to the P*psbAII* expression vector, pHS298	This study
pBAD24	Ap^r, *araBAD* promoter, AraC	Guzman et al. (1995)
pHS 503	Ap^r, the PCR *sll1404-sll1405* the *Nde*I side of pBAD24	This study
pRL 446	Km^r, a cloning vector with a kanamycin resistance marker (C.K2)	Elhai and Wolk (1988)
pRL 57	Sp^r, cloning vector with a spectinomycin resistance cassette omega	Black et al. (1993)
pRL 598	$Cm^r Em^r$, cloning vector with a erythromycin resistance marker (C.CE2)	Elhai and Wolk (1988)
pHS 635	Km^r, the *sll1404* fragment was cloned into the pGKT7 vector	This study
pHS 636	Ap^r, the *sll1405* fragment was cloned into the pGADT7 vector	This study
pHS 791	$Ap^r Sp^r$, the *all5046-all5047* fragment was cloned to the P*psbAII* expression vector, pHS298	This study
pHS 792	$Ap^r Sp^r$, the *a1317-a1318* fragment was cloned to the P*psbAII* expression vector, pHS298	This study
pGADT7	Ap^r, Yeast two-hybrid expression vector with ADH1 promoter and a fusion of GAL4 AD	Clontech
pGBKT7	Km^r, Yeast two-hybrid expression vector a fusion of GAL4 DNA binding domain (DNA-BD).	Clontech

Primers

sll1404-1	5′-GTGTGACTTCTGGGATGGGAG-3′
sll1406-2	5′-TGGTGCTGGGATGCCTTCAT-3′
slr0677-1	5′-CCACTATTACCCACGCATTGGA-3′
slr0678-2	5′-TGCAAAGCCCGGTCAATGGC-3′
sll0477-1	5′-GTGAAGCCATACCATAGCCCAT-3′
sll0477-2	5′-AAGGGCAAGGATGGACAGCAGT-3′
sll0479-1	5′- TTGAAGGAAGTGGGCGGTGA-3′
sll0479-2	5′-GGACTGACAGCACCTTTGGC-3′
slr1484-1	5′-GAGGAGGGGCACCGCCACT-3′
slr1484-2	5′-AGGGATTGATGACCCAGGGAA-3′
slr0677com-1	5′-TGGTGAATCCCATTGAGTTGATGCAAAAGGG-3′
slr0678com-2	5′-TTACTTGTCGTCGTCGTCCTTGTAGTCCTGTTGGGGGGCACTGGG-3′
sll0477com-1	5′- TGCTGGATAATTGCAAGAG-3′

sll0479com-2	5′-TCACTTGTCGTCGTCGTCCTTGTAGTCAGGCATGGCCCCAGCACC-3′
exbB-1	5′-TGGGTAATAATTTAATGCAGACGGACC-3′
exbD-2	5′-TTACTTCGCTTTGGCGGTTTCTTCG-3′
all5046-1	5′-CATATGGGAATCCAAAATCTTTTTGC-3′
all5047-2	5′-CATATGTTAACGTTTTTGAGTGGCGAT-3′
a1317-1	5′-CATATGTGGCCCTTAGTACTCTTGTC-3′
a1318-2	5′-CATATG CTATTGCTGATTATTTTGTAACT-3′
psbAIIoe-1	5′-GCGTGCAAGGCCCAGTGATC-3′
psbAIIoe-2	5′-CATATGGTTATAATTCCTTAGTTCAGATTGGAACTGACTAAACTTAGTC-3′
sll1404Y2H-1	5′-CATATGGCCGGGGGCATAGTGG-3′
sll1404Y2H-2	5′-CTCGAGTCATCGGGAAGTCGCATACTC-3′
sll1405YH-1	5′-CATATGACCAACCGGAGGAAAAAGAG-3′
sll1405YH-2	5′-CTCGAGTTAGTTCTTGGGCGTGGCG-3′
rnpB-1	5′-TTAGTTCTTGGGCGTGGCG-3′
rnpB-2	5′-TTGCCCCTCCGACCTTGCTT-3′
sll1263-1	5′-CCAAGTTCGTCGCTTTATCCGC-3′
sll1263-2	5′-ACCACAGCCACCGCAATCAGC -3′
sll1404RT-1	5′-CGAGCGTGCCTATTTTTGGAGT-3′
sll1404RT-2	5′-TGTCCCCAGTAAGCCCAGGA-3′
slr0677RT-1	5′-AGAGGTTGTGGTTTTGGGGC-3′
slr0677RT-2	5′-TGGGTGGGGCAAACGCA-3′
sll0477RT-1	5′-CGGAGGCTACCCAGGC-3′
sll0477RT-2	5′-ATTCACTGCCCACTGCGG-3′
slr1484RT-1	5′-ACAGCCCGTATTTGGTCGC-3′
slr1484RT-2	5′-CTGGGAGTTGGTTTCGGTTT-3′
slr0513RT-1	5′-TCACGGCATTACAACACCGA-3′
slr0513RT-2	5′-TCTGGGTGAAGCCATACCAT-3′
slr0327RT-1	5′-TCTCACCGTTTCCTATGCCCA-3′
slr0327RT-2	5′-ACCACTCAGCAGAAGACCAA-3′
slr1295RT-1	5′-CCAAAAGTTATCCCGTCGCCT-3′
slr1295RT-2	5′-ACGAGCCAAATCCACTGTGA-3′
sll1878RT-1	5′-CGCTATCCCCACGAACTATCC-3′
sll1878RT-2	5′-GCCACAAAACGGGAAGCAGG-3′
slr1392RT-1	5′-GTGGTGGTGAACGCTGCCCA-3′
slr1392RT-2	5′-CACGGGGTGATCGGAAAGA-3′

[a] Ap, ampicillin; Km, kanamycin; Sp, spectinomycin; Cm, chloramphenicol; Em, erythromycin.

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