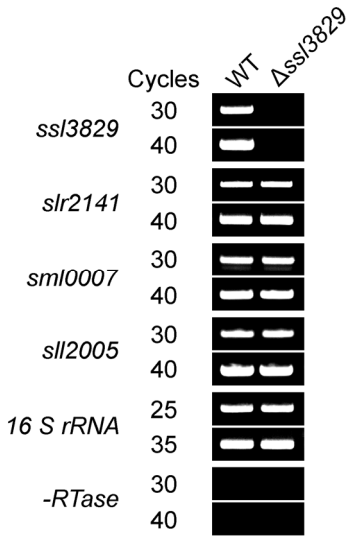
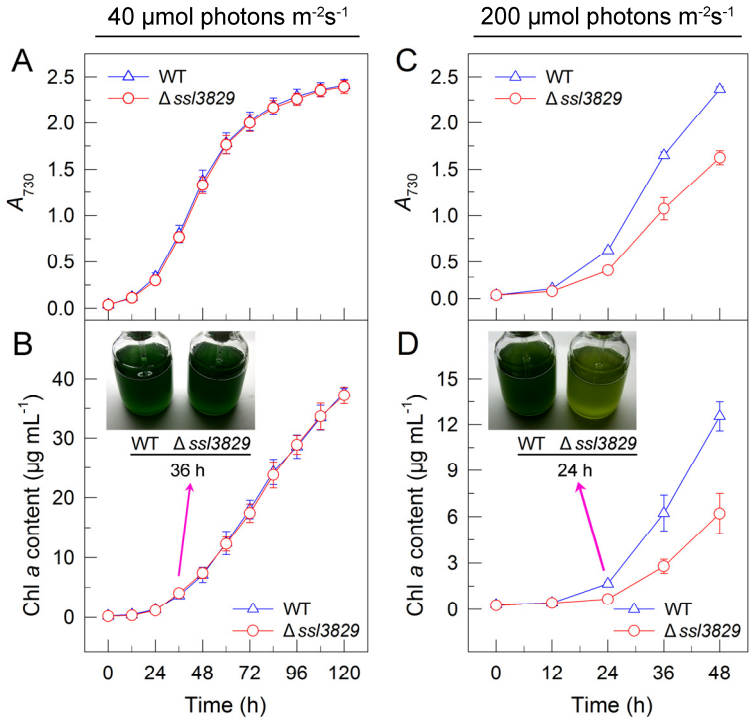
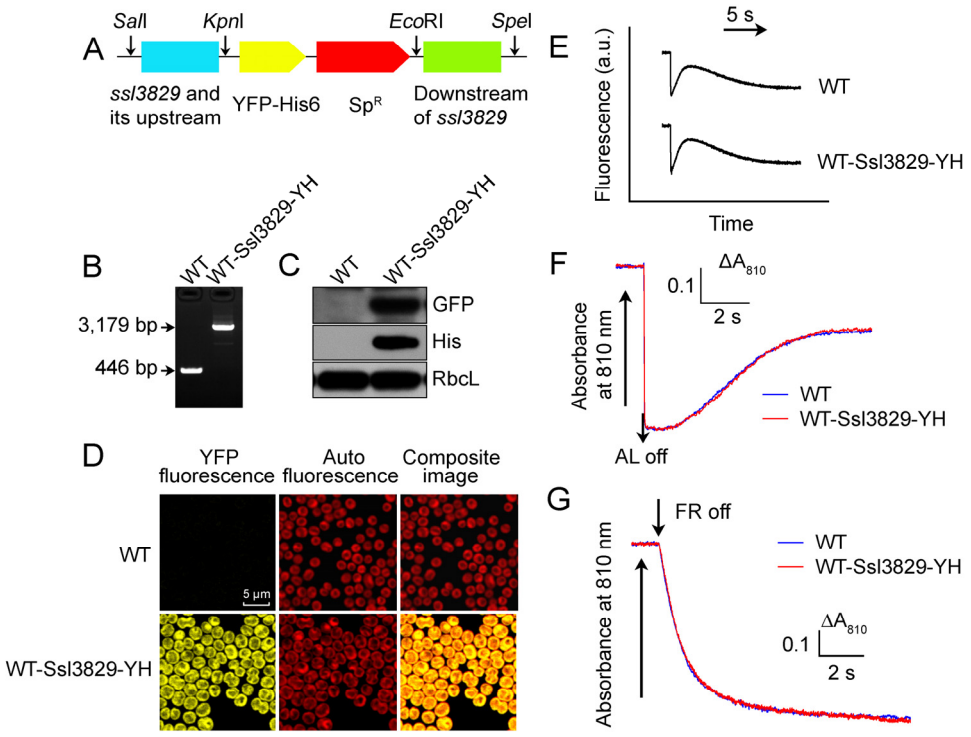
Supplemental Data



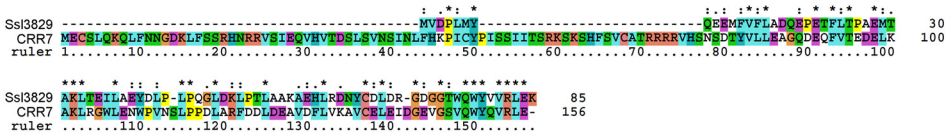
Supplemental Figure S1. RT-PCR analysis of *ssl3829* and its neighboring genes in the WT and Δ*ssl3829* strains. WT loci of *ssl3829* and its neighboring genes are indicated in Fig. 1C. The transcript level of *16 S rRNA* in each sample is shown as a control. The absence of contamination of DNA was confirmed by PCR without reverse transcriptase.



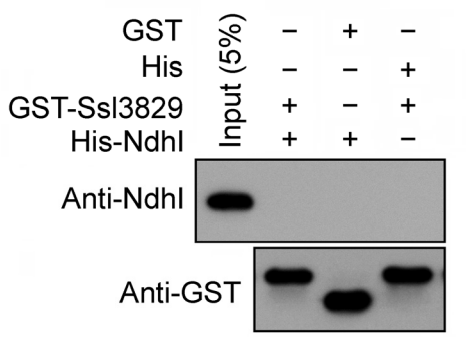
Supplemental Figure S2. Growth of WT and Δ*ssl3829* cells under different light intensities. Cell density (A) and Chl *a* content (B) were monitored under normal light (40 µmol photons $m^{-2}s^{-1}$). Cell density (C) and Chl *a* content (D) were monitored under high light (200 µmol photons $m^{-2}s^{-1}$). Values are means ± SD (n = 5).



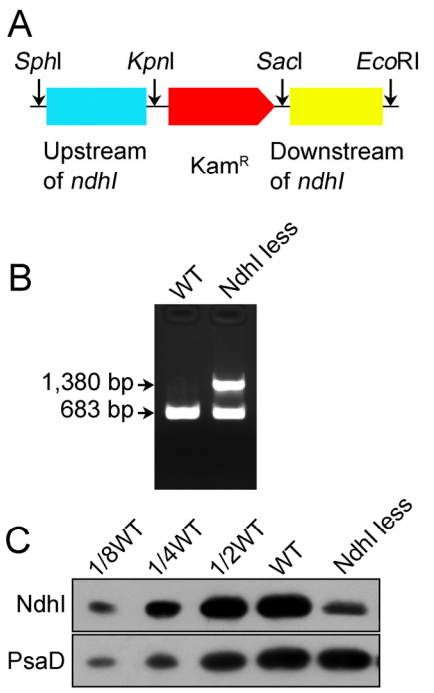
Supplemental Figure S3. Construction and characterization of WT-Ssl3829-YH strain. A, Construction of plasmid to generate WT-Ssl3829-YH mutant. A DNA fragment containing *ssl3829* and its upstream region amplified by PCR was ligated to the *Sal*I and *Kpn*I sites of the pEYFP-His6-Sp^R plasmid and a fragment downstream of *ssl3829* was ligated between *Eco*RI and *Spe*I sites. The plasmid thus constructed was used to transform WT cells to generate the tagged mutant. B, PCR segregation analysis of the WT-Ssl3829-YH mutant using the *ssl3829-yfp-his6*-E and -F primer sequences (Supplemental Table S1). C, Western analysis of proteins from the WT and WT-Ssl3829-YH strains using GFP and His antibodies. Total protein corresponding to 1 µg Chl *a* was loaded onto each lane, and detection of RbcL was detected as a loading control. D, Confocal microscopy analysis of WT and WT-Ssl3829-YH cells. The scale bar indicates 5 µm. E, Monitoring of NDH-CET activity by Chl fluorescence. F, Redox kinetics of P700 after termination of actinic light (AL) illumination (800 µEm^{-2}s^{-1} for 30 s) under a background of far-red light (FR). G, Kinetics of P700$^+$ re-reduction in darkness after turning off FR with a maximum at 720 nm in the presence of 10 µM DCMU. The Chl *a* concentration was adjusted to 20 µg/mL before measurement, and curves are normalized to the maximal signal. See Experimental Procedures for details.



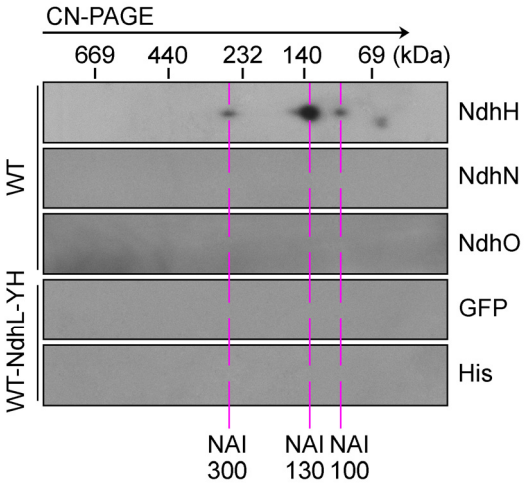
Supplemental Figure S4. Sequence comparison between Ssl3829 (*Synechocystis* sp. strain PCC 6803) and CRR7 (*Arabidopsis thaliana*) (At5g39210). The sequences were aligned using ClustalX 1.83. Asterisks indicate identical amino acids; colons and dots indicate conserved amino acid substitutions.



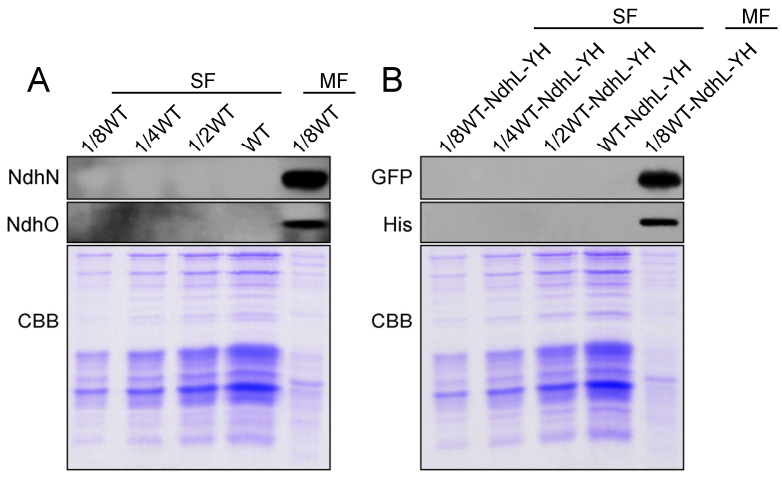
Supplemental Figure S5. GST-pulldown assay shows no interaction between Ssl3829 and NdhI. The expressed proteins were mixed and incubated with Glutathione Sepharose 4B beads on a rotating shaker at 4°C overnight. After washing, interaction of GST-Ssl3829 with His-NdhI was detected using the antibody against NdhI. The interactions of GST (without Ssl3829) with His-NdhI and of His (without NdhI) with GST-Ssl3829 were used as negative controls. The left lane represents input (5%) control.



Supplemental Figure S6. Deletion mutation and molecular identification of *ndhI* gene. A, Construction of plasmid used to generate the *ndhI* deletion mutant (Δ*ndhI*). B, PCR segregation analysis of the Δ*ndhI* mutant using the *ndhI*-G and *ndhI*-H primer sequences (Supplemental Table S1). C, Immunoblotting analysis of NdhI from the total protein of WT (including indicated serial dilutions) and NdhI-less mutant. Total protein corresponding to 1 μg Chl *a* was loaded onto each lane, and PsaD was detected as a loading control.



Supplemental Figure S7. Analysis of NdhL, NdhN and NdhO in NAIs in the cytoplasm. Cytoplasmic protein complexes isolated from the wild type (WT) and WT-NdhL-YH were separated by CN-PAGE followed by 2D SDS-PAGE. Proteins were immunodetected with the specific antibodies indicated. Dashed lines indicate the positions of NAIs.



Supplemental Figure S8. Comparison of NdhL, NdhN and NdhO protein abundances between soluble and membrane fractions. Cytoplasmic proteins were isolated from the WT (A) and WT-NdhL-YH (B) strains, and immunoblotting analyses were performed with the indicated antibodies. Cytoplasmic proteins (80 μg) were loaded on each well (100%), and total membrane proteins (10 μg) were also analyzed. In the lower panel, a replicate gel strained with Coomassie Brilliant Blue (CBB) was used as a loading control.

Supplemental Table S1. Primers used in this study.

Primers used for identifying the sites of transposon insertion.

Name	Primer sequence (5'–3')	Purpose
Transprimer-FP	ACCTACAACAAAGCTCTCATCAACC	Identifying the transposon insertion sites
Transprimer-RP	GCAATGTAACATCAGAGATTTTGAG	

Primers used to construct the pUC-Δ*ssl3829* vector.

Name	Primer sequence (5'–3')	Purpose
ssl3829-A	CGGAATTCCCATCCACCAAAGGAGC	Amplification of upstream region
ssl3829-B	GGGGTACCGTACATCAGTGGATCGAC	
ssl3829-C	CGGGATCCGGGGGGGGGGGGAAAG	Amplification of kanamycin gene
ssl3829-D	GGGGTACCGGGGGGGGGGGGCG	
ssl3829-E	CGGGATCCTTGGCAATGGTATGTGG	Amplification of downstream region
ssl3829-F	GCGTCGACTCCCAAGAGAGTATCAC	
ssl3829-G	CGGAATTCATGGTCGATCCACTG	Segregation analysis
ssl3829-H	CCCTCGAGCTATTTTTCCAGACG	

Primers used to construct pUC-Δ*ndhI* vector.

Name	Primer sequence (5'–3')	Purpose
ndhI-A	GGCATGCGAAGCTGTTTTGGATTCC	Amplification of upstream region
ndhI -B	GGGTACCCCTACCTGTTTGAGAATG	
ndhI-C	GGGGTACCGGGGGGGGGGGGAAAG	Amplification of kanamycin gene
ndhI-D	AACGAGCTCGGGGGGGGGGGGCGC	
ndhI-E	CGAGCTCTGAAAGCAGAATAGGTTG	Amplification of downstream region
ndhI-F	CGGAATTCTAGCACGATCGCCAGAC	
ndhI-G	CTTCTAACTTTGGCCGACTTAAACC	Segregation analysis
ndhI-H	ACGAGTTTACTTTAACGGGTAAAGC	

Primers used for the pEYFP-Ssl3829-YFP-His6 plasmid construction.

Name	Primer sequence (5'–3')	Purpose
ssl3829-yfp-his6-A	GCGTCGACAATCTAACCCCTGGTTG	Amplification of *ssl3829* and its upstream region
ssl3829-yfp-his6-B	GGGTACCCCTTTTTCCAGACGTACCAC	
ssl3829-yfp-his6-C	GGAATTCCTTGATCGATCCCATCTC	Amplification of downstream region
ssl3829-yfp-his6-D	GGACTAGTATACGCATCCACACACC	
ssl3829-yfp-his6-E	CAGTGCCAATCTCCACCTC	Segregation analysis
ssl3829-yfp-his6-F	CGTGGTCGTCATGGTCAC	

Primers used for identifying the segregation of *ssl3829-yfp-his6* in Δ*slr1097* mutant.

Name	Primer sequence (5'–3')
ssl3829-yfp-his6-E	CAGTGCCAATCTCCACCTC
ssl3829-yfp-his6-F	CGTGGTCGTCATGGTCAC

Primers used for RT-PCR.

Name	Primer sequence (5'–3')	Purpose
ssl3829-FP	GGTCGATCCACTGATGTACC	*ssl3829* transcript
ssl3829-RP	CATTGCCAAGTACCGCCATC	
slr2141-FP	GCAGCAACGGGGTATTGAAGCCA	*slr2141* transcript
slr2141-RP	GGAAAAATTGTGCTCCCGCTGTTAG	
sml0007-FP	ATGGATTGGCGTGTAATTGTAGTTG	*sml0007* transcript
sml0007-RP	CTAGGCTTCGCGACCTAAAACGTCC	
sll2005-FP	CAAGGTTACGTTGGACAATGGTGAG	*sll2005* transcript
sll2005-RP	CGCTCATTTTCCGTCGAAACTCAGG	
slr1097-FP	CAGAGATTCCCGAAGTTC	*slr1097* transcript
slr1097-RP	CCAAATCGTAGCCCAACAG	

16 S rRNA-FP	CGACTGCTAATACCCAATGTGC	*16 S rRNA* transcript
16 S rRNA-RP	GTCCCTCAGTGTCAGTTTCAGC	

Primers used to construct vectors to express NdhN protein to raise antibody.

Name	Primer sequence (5'–3')	Purpose
ndhN-FP	CGGGATCCATGTTGCCATTGCCA	NdhN antibody
ndhN-RP	CGGAATTCCTAGGCCGCCTGCAAG	

Primers used to construct the yeast two-hybrid vector.

Name	Primer sequence (5'–3')	Purpose
ssl3829-FP	CGGAATTCATGGTCGATCCACTG	Ssl3829 bait
ssl3829-RP	CCCTCGAGCTATTTTTCCAGACG	
ndhO-FP	CGGAATTCATGGCCGCTAAAATGAAAAAGG	NdhO bait
ndhO-RP	CCCCTCGAGCTAAGCCAGGGCTTCGATTTGG	
slr1097-FP	CCTCGAGATGGTCACCATCACCGTTAC	Slr1097 prey
slr1097-RP	CCTCGAGCTACAACTGGGCCAAAAAATTG	
ndhB-FP	CCCCTCGAGATGGACTTTTCTAGTAACGTTGCA	NdhB prey
ndhB-RP	CCCCTCGAGCTAGGGTAAATCATGGGAAATGGC	
ndhI-FP	CCCCTCGAGATGTTTAACAACATTCTCAAACAG	NdhI prey
ndhI-RP	CCCCTCGAGCTATTCTGCTTTCACCAAATCTTC	

Primers used to construct the fusion protein expression vector.

Name	Primer sequence (5'–3')	Purpose
ssl3829-FP	CGGAATTCATGGTCGATCCACTG	Purifying Ssl3829 using GST-tag
ssl3829-RP	CCCTCGAGCTATTTTTCCAGACG	
slr1097-FP	CGGGATCCATGGTCACCATCACC	Purifying Slr1097 using His-tag
slr1097-RP	GCGTCGACCTACAACTGGGCCAAAAAATTG	
ndhI-FP	CGGGATCCATGTTTAACAACATTC	Purifying NdhI using His-tag
ndhI-RP	CGAGCTCCTATTCTGCTTTCACCAAATC	