Supporting Information

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SI Materials and Methods

Construction of Δ*flv1::flv1* Complementation Strain. For complementation, the Δ*flv1* mutant was transformed with the plasmid containing Flv1 ORF under the control of the *psbA2* promoter. The construct was integrated into the *psbA2* locus of the *Synechocystis* genome by replacing the *psbA2* gene. Transformants were selected on agar plates supplemented with spectinomycin.

Construction of *Anabaena* Mutants. Transformation of *Anabaena* was achieved by triparental mating as described (1). Selection of double recombinants was based on the presence of the *sacB* gene in pRL271 and performed as described in ref. 2.

Construction of Proton Gradient Regulation 5 (*pgr5*)-*like* Mutant. Upstream and downstream noncoding regions of the *Synechocystis pgr5* homolog (*ssr2016*) were amplified from genomic DNA. The PCR product containing the upstream fragment was digested with KpnI and NsiI, whereas the one containing the downstream fragment was digested with NsiI and NotI. The fragments were ligated into pBleuscript digested with KpnI and NotI. This process resulted in a plasmid that contained an NsiI site, in place of the 198-base *ssr2016* ORF, surrounded by upstream and downstream sequences of 350 bases each. A 1.2-kb DNA fragment containing *aphI* (Km^r) was extracted from pUC4K by PstI and ligated into the NsiI site of previous plasmid. The final plasmid contained the *aphI* gene instead of *ssr2016* ORF, in the same orientation. This plasmid was used to transform the WT strain of *Synechocystis*, and the transformants were selected on plates with 50 $\mu g/mL^{-1}$ Km.

MIMS. A 1.5-mL cell suspension was injected into a thermostated (30 °C) measuring chamber covered with a Teflon membrane at the bottom, and the chamber was attached to a vacuum line of a mass spectrometer (Prima-B; Thermo Fisher Scientific). The suspension was continuously mixed by a magnetic stirrer. Different intensity of actinic light was applied by a LED-powered fiber optic illuminator (PerkinElmer Life Sciences) by using a neutral density filter when it was needed. Before measurements, a bubble of $^{18}O_2$ (mass 36, 99% $^{18}O_2$ isotope content; Euriso-Top) was injected to the top of cell suspension in the closed chamber. Sample was stirred until the level of $^{18}O_2$ and $^{16}O_2$ (mass 32) were equilibrated. After removing air bubbles, gas-exchange rates were measured. Determination of light-induced oxygen uptake was based on the fact that the cells produce $^{16}O_2$ from water by photosynthetic activity, whereas the amount of $^{18}O_2$ in the suspension reflects O_2 uptake.

Acridine Yellow (AY) Fluorescence. AY fluorescence changes were detected by the AEO module of DUAL-PAM-100 as described before (3). After 20-min dark adaptation, the cells were illuminated with the light intensity of 2,000 μmol photons·m^{-2}·s^{-1}. The concentration of AY was 5 μM, and Chl was 20 $\mu g/mL^{-1}$.

Protein Isolation, Electrophoresis, and Immunodetection. Harvested cells were suspended in a resuspension buffer containing 50 mM Hepes-NaOH, pH 7.5, 30 mM $CaCl_2$, 800 mM sorbitol, and 1 mM *e*-amino-*n*-caproic acid. The cells were broken by vortexing 6 × 1 min at 4 °C in the presence of glass beads under dim light. The total cell extract was obtained by centrifugation twice at 1,500 × *g* for 2 and 5 min to remove the glass beads and unbroken cells. The membrane and soluble fraction was then separated by centrifugation at 18,000 × *g* for 25 min.

Oxyblot Analysis. The protein samples from total cell extract (16 μg in each well) were separated on a 12% SDS/PAGE and immunoblotted onto the PVDF membrane. Carbonylated proteins were detected by using an OxyBlot Protein Oxidation Detection Kit (Millipore).

P700 Measurement. The acceptor side limitation of PSI, *Y*(NA), was calculated as $Y(NA) = (P_M - P_M')/P_M$, where P_M was defined as a maximal change of the P700 signal upon transformation of P700 from the fully reduced to the fully oxidized state and P_M' value represents the maximal change of the P700 signal at steady-state light upon application of a saturating pulse. For determination of P_M, a saturating pulse (5,000 μmol photons·m^{-2}·s^{-1}, 300 ms) was applied on samples preilluminated with far-red light (720 nm, 75 W/m^2, 10 s).

1. Elhai J, Wolk CP (1988) Conjugal transfer of DNA to cyanobacteria. *Methods Enzymol* 167:747–754.
2. Cai YP, Wolk CP (1990) Use of a conditionally lethal gene in *Anabaena* sp. strain PCC 7120 to select for double recombinants and to entrap insertion sequences. *J Bacteriol* 172(6):3138–3145.
3. Teuber M, Rögner M, Berry S (2001) Fluorescent probes for non-invasive bioenergetic studies of whole cyanobacterial cells. *Biochim Biophys Acta* 1506(1):31–46.

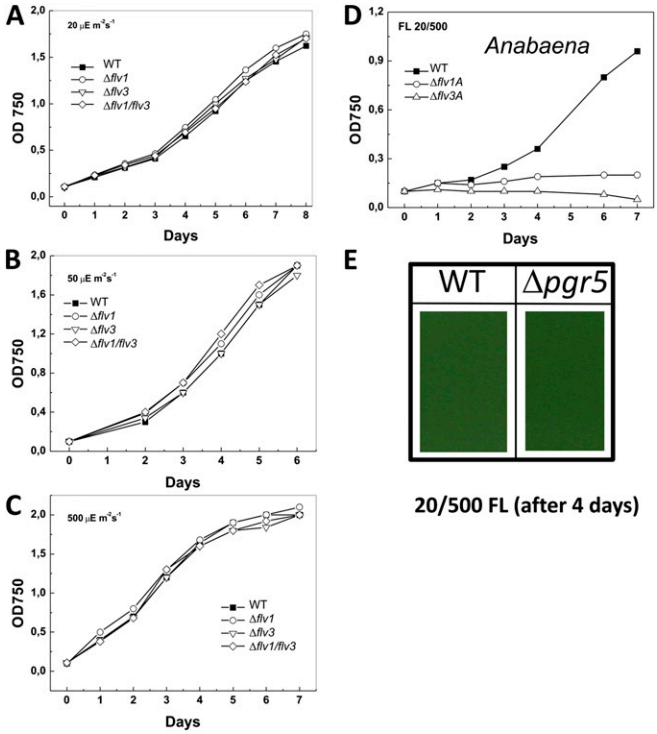

Fig. S1. Growth phenotype of the WT and mutant strains. (*A*–C) *Synechocystis* WT and Δ*flv1*, Δ*flv3*, and Δ*flv1/3* mutant strains were grown under constant light intensities of 20 μmol photons·m^{-2}·s^{-1}(*A*), 50 μmol photons·m^{-2}·s^{-1} (*B*), and 500 (*C*) μmol photons·m^{-2}·s^{-1}. (*D*) *Anabaena* WT and Δ*flv1*A and Δ*flv3*B mutants were grown under FL 20/500 regime for 4 d followed by adjustment of OD_{750} to 0.1 and shifting to constant growth light. (*E*) *Synechocystis* WT and Δ*pgr5*-like strains were grown under FL 50/500 regime for 5 d.

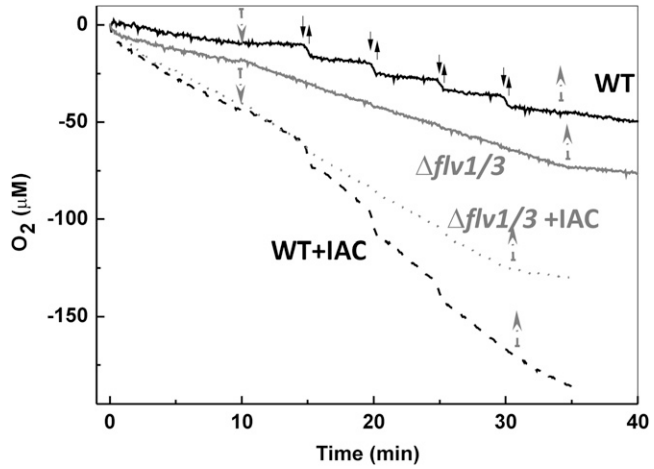

Fig. S2. Effect of KCN on O_2 uptake in the WT and Δ*flv1*/Δ*flv3* cells. O_2 uptake was recorded from the WT (black line) and Δ*flv1*/Δ*flv3* (gray line) cells during 10-min darkness and upon exposure of them to FL in the absence (solid line) and in the presence (dashed line) of 8 mM IAC. Down and up black arrows show turning on and off high-light pulses, respectively. Grey arrows show turning on and off background light.

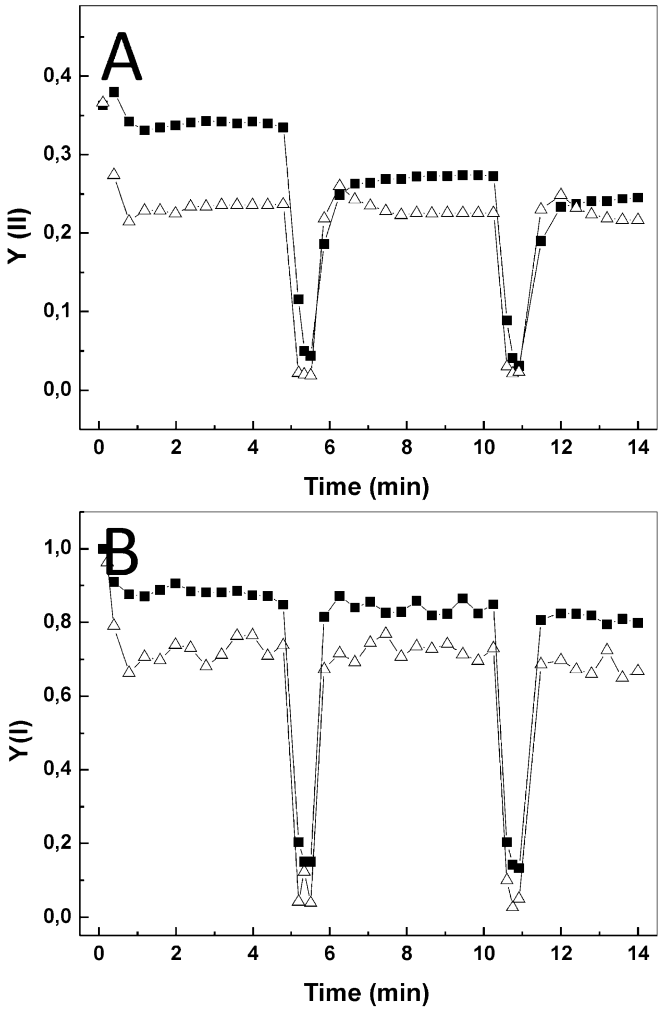

Fig. S3. PSII and PSI properties of the WT and Δ*flv1*/Δ*flv3* cells from FL. Chl fluorescence and P700 oxidoreduction were recorded by DUAL-PAM-100 (Fig. 5). *Y*(II) (*A*) and *Y*(I) (*B*) values in WT (■) and Δ*flv1*/Δ*flv3* (△) were calculated and plotted as a function of time.

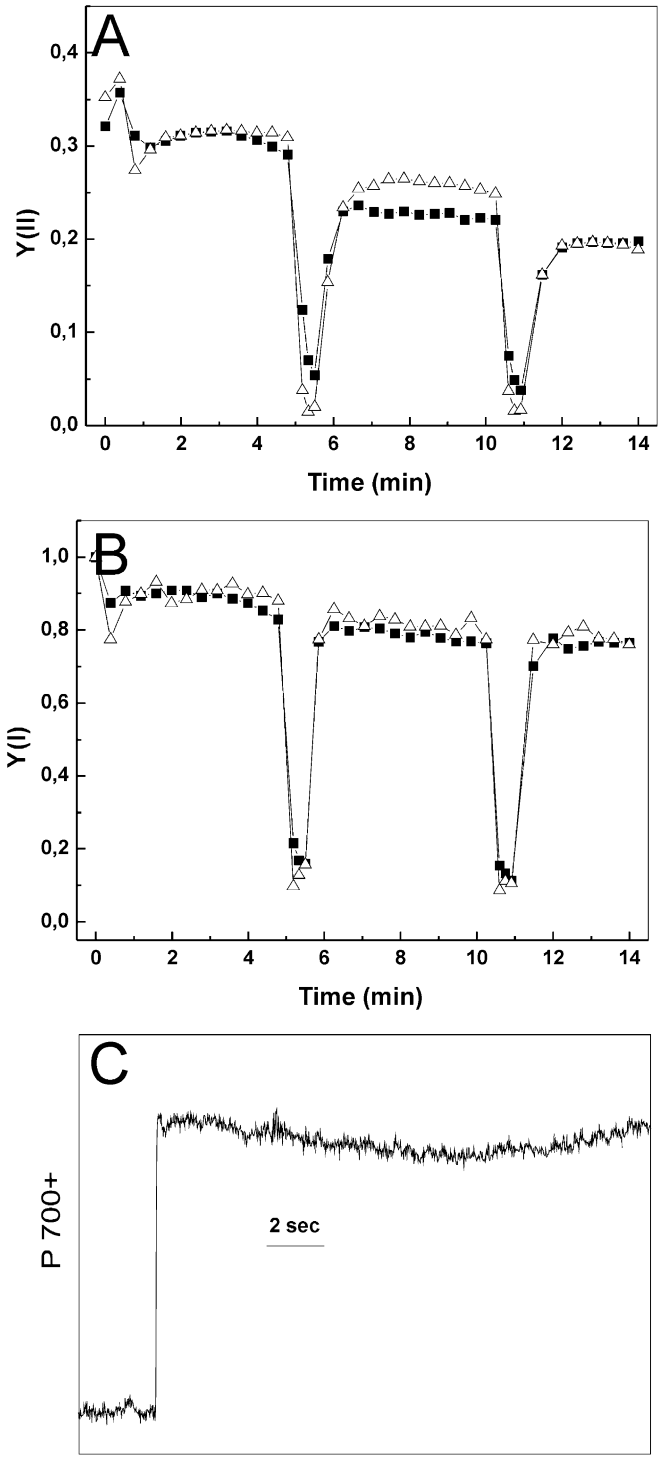

Fig. S4. PSII and PSI properties of the WT and Δ*flv1*/Δ*flv3* cells grown at constant growth light. Chl fluorescence and P700 oxidoreduction were recorded by DUAL-PAM-100 (Fig. 6). *Y*(II) (*A*) and *Y*(I) (*B*) values and acceptor-side limitation of PSI, *Y*(NA), (*C*) in WT (■) and Δ*flv1*/Δ*flv3* (△) were calculated and plotted as a function of time.

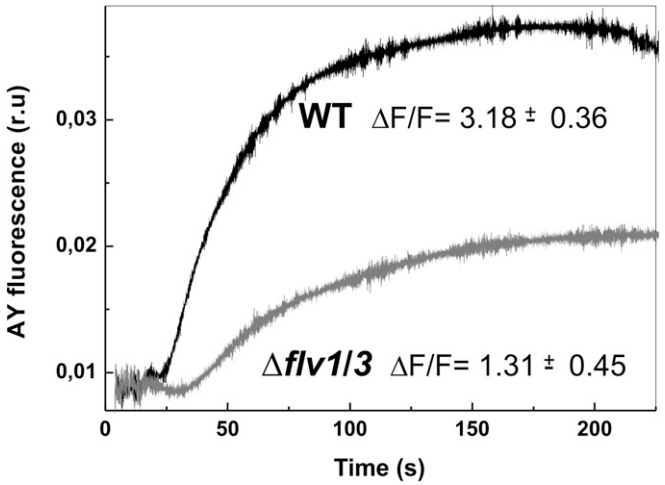

Fig. S5. Changes in AY fluorescence induced by illumination of dark-adapted cells. The WT and Δ*flv1*/Δ*flv3* mutant cells were grown at constant light and shifted to FL 50/500 for 3 d before the experiment. The relative changes of AY fluorescence (Δ*F*/*F*) is expressed as mean ± SD ($n = 4$).

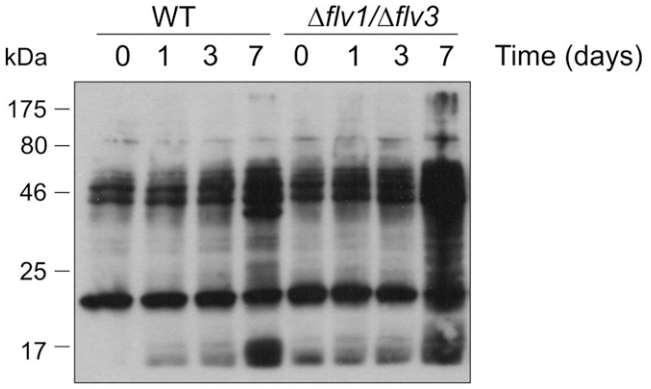

Fig. S6. Immunoblot analysis of carbonyl derivatives of proteins in the WT and Δ*flv1*/Δ*flv3* mutant cells. The cells were grown at constant light (day 0) and shifted to FL 50/500 for 1, 3, and 7 d.

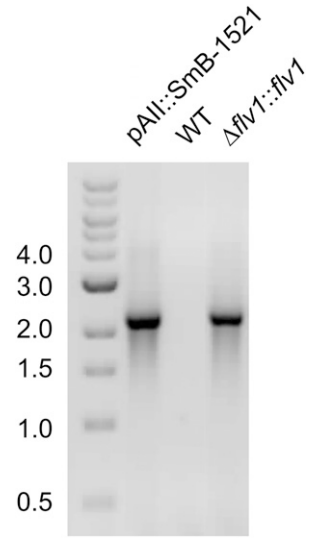

Fig. S7. Verification of complementation strain Δ*flv1*::*flv1* in *Synechocystis*. PCR was performed with vector pAII::SmB-1521 used in transformation and isolated chromosomal DNA from *Synechocystis* WT and Δ*flv1*::*flv1*strain. Specific primers recognized *psbA2* promoter and *sll1521* gene. Expected fragment length for pAII::SmB-1521 plasmid and mutant *psbA2* allele of Δ*flv1*::*flv1* strain is 2.2 kb.