Supporting Information

Tokutsu and Minagawa 10.1073/pnas.1222606110

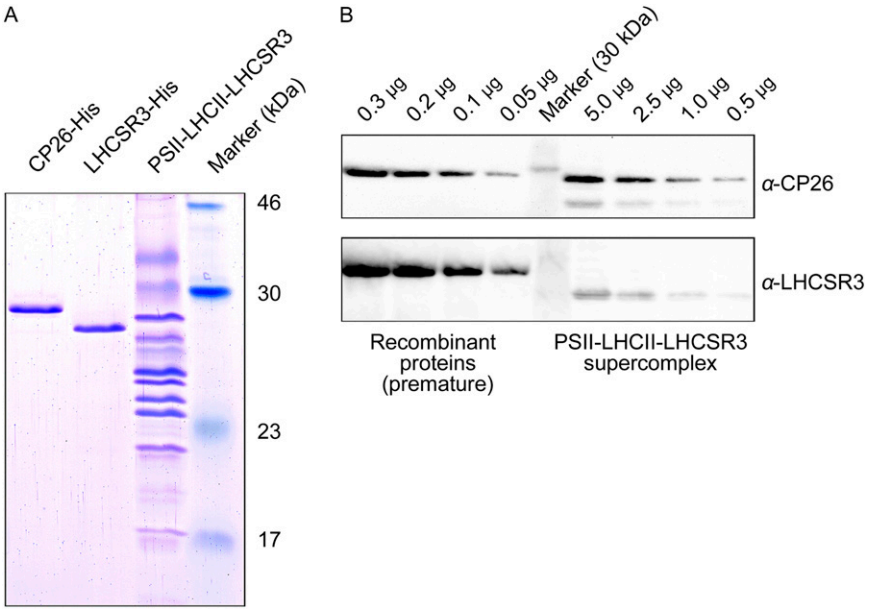

Fig. S1. Quantification of the light-harvesting complex stress-related protein 3 (LHCSR3) in the photosystem II (PSII)–light-harvesting complex II (LHCII)-LHCSR3 supercomplex. (*A*) Recombinant proteins [0.5 μg of CP26 (a minor monomeric LHCII protein)-His and LHCSR3-His proteins] purified from the *Escherichia coli* and PSII-LHCII-LHCSR3 supercomplex (5.0 μg protein) from the high-light (HL)-grown WT were analyzed by SDS/PAGE and stained with Coomassie brilliant blue R-250. (*B*) The recombinant proteins (0.05–0.3 μg protein) and proteins in the PSII-LHCII-LHCSR3 supercomplex (0.5–5.0 μg protein) were detected immunologically with the indicated antibodies.

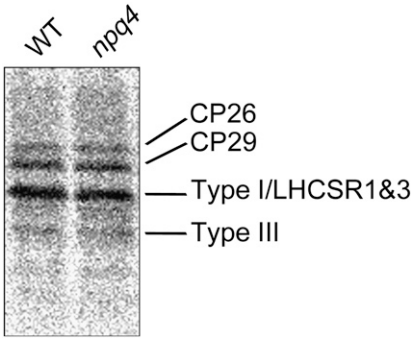

Fig. S2. Dicyclohexylcarbodiimide (DCCD) binding to the PSII supercomplexes isolated from the HL-grown cells. The PSII-LHCII-LHCSR3 supercomplex from the HL-grown WT and the PSII-LHCII supercomplex from the HL-grown nonphotochemical quenching 4 (*npq4*) mutant were treated with [^{14}C]-DCCD under the same conditions as for the DCCD-inhibited energy-dependent quenching (qE) activation. The radiolabeled polypeptides were visualized by autoradiography after separation by SDS/PAGE. Type I, major LHCII type I (LHCBM3/4/6/8/9); type III, major LHCII type III (LHCBM2/7).

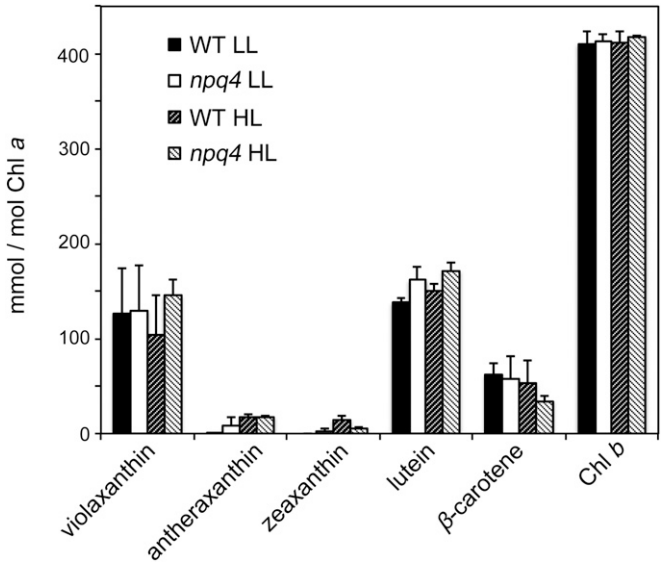

Fig. S3. Pigment composition of the PSII supercomplexes. Pigment composition of the PSII supercomplexes from WT and the *npq4* mutant grown under either low-light (LL) or HL conditions were analyzed by ultraperformance liquid chromatography (UPLC). Each data point represents the mean of three individual preparations ($n = 3$).

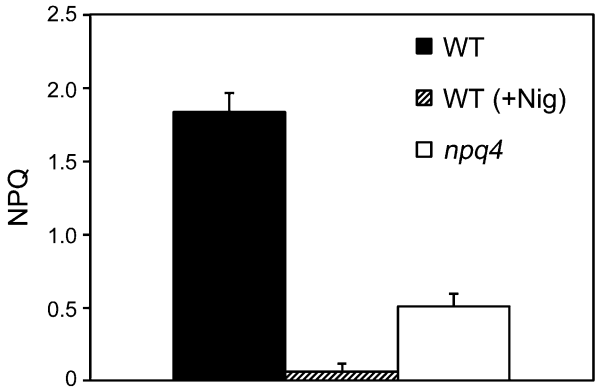

Fig. S4. Nonphotochemical quenching (NPQ) of *Chlamydomonas reinhardtii* strains. Cells were cultured at 500 μE/m^2/s to the midexponential phase. NPQ was measured with an actinic light at 900 μE/m^2/s after 20 min of dark adaptation. WT cells were treated with 10 μM nigericin for 5 min before the measurements when indicated. Each data point represents the mean of three individual measurements ($n = 3$). Error bars indicate SEs.

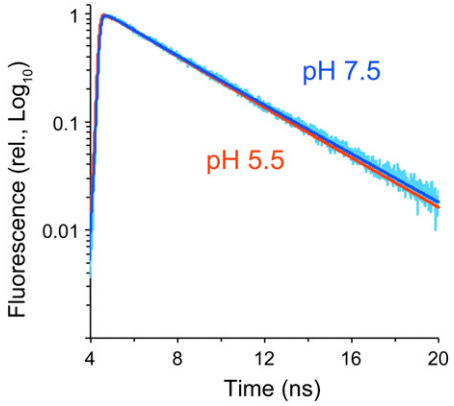

Fig. S5. Time-resolved fluorescence analysis of the free LHCII fraction. Single photon counting of the fluorescence kinetics of the free LHCII fractions from HL-grown WT. The data were recorded at 682 nm at pH 7.5 and 5.5. Decay curves were fitted using three exponential functions. The light-cyan trace represents the actual data points, and the red and dark-blue lines are the fitted curves.

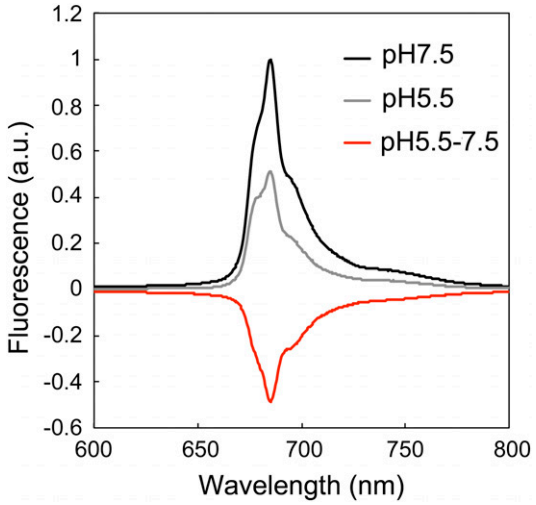

Fig. S6. Low-temperature fluorescence emission spectra of the unquenched (pH 7.5) and quenched (pH 5.5) PSII-LHCII-LHCSR3 supercomplex. The fluorescence emission spectra of the PSII-LHCII-LHCSR3 supercomplex at pH 7.5 (black) and at pH 5.5 (gray) were measured at 77 K. The difference spectrum (pH 5.5–7.5) is shown as a red line. Protein samples were excited at 440 nm. The spectra were normalized to the photon counts of fluorescence at 23 °C.

Table S1. Stoichiometry of LHCSR3 in the PSII-LHCII-LHCSR3 supercomplex

Protein (kDa)	Protein amount, ng protein/μg PSII-LHCII-LHCSR3
LHCSR3 (28.9)	5.9 ± 1.1
CP26 (31.0)	43.9 ± 4.0

A minor monomeric LHCII CP26 is associated with the PSII supercomplex at a one-to-one ratio. Protein content was determined by densitometric analysis of the immunoblots shown in Fig. S1. Protein molecular weights were deduced from their premature amino acid sequences. LHCSR3/CP26 = 0.14. LHCSR3/PSII-LHCII-LHCSR3 supercomplex = 0.28.

Table S2. Chlorophyll fluorescence lifetimes of the free LHCII fractions from WT

LHCII fraction (pH)	A_1, %	τ_1, ns	A_2, %	τ_2, ns	τ_{AVE}, ns
HL (pH 7.5)	4.0	1.2	96.0	3.8	3.7
HL (pH 5.5)	6.2	1.9	93.8	3.9	3.7
LL (pH 5.5)	13.2	2.2	86.8	4.0	3.8

Average fluorescence lifetimes (τ_{AVE}) and the three lifetime components after fitting are shown. A, relative amplitude; τ, lifetime.

Table S3. NPQ calculated from total photon counts obtained during time-resolved fluorescence measurements of the PSII-LHCII-LHCSR3 supercomplex from WT and the PSII-LHCII supercomplex from the *npq4* mutant

Variable	WT	*npq4*
Photon count at pH 7.5 ($PC_{7.5}$)*	11,432	11,718
Photon count at pH 5.5 ($PC_{5.5}$)*	5,552	9,168
NPQcalc†	1.06	0.28

*Photons/μg chlorophyll/s.
†NPQcalc = ($PC_{7.5}$ − $PC_{5.5}$)/$PC_{5.5}$.