Increased biomass productivity in green algae by tuning Non-Photochemical-Quenching

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SUPPLEMENTARY INFORMATION

Table S1: Biomass productivity of WT, npq4 and npq4 lhsr1 strains*. Biomass productivity was calculated as the maximum of the first derivate of the curve obtained by sigmoidal fitting of the growth curves reported in Figure 2. The data significantly different from WT are indicated with *. Statistical significance were estimated by two-tailed non parametric Wilcoxon-Mann-Whitney test with n=5, α=0.05, and a p-value of 0.009 in all cases where reported data are significantly different from the WT. In the case of Light A and B the statistical significance of the differences observed between WT, npq4 and npq4 lhcsr1 were additionally confirmed performing the two-tailed non parametric Kruskal-Wallis test (n=5, α= 0.05 for all conditions, p=0.0013 and 0.0019 for Light A and B respectively).*

LIGHT A	**WT**	***npq4***	***npq4 lhscr1***
Max ΔOD/Day	0.48 ± 0.11	0.53 ± 0.08	0.48±0.04
Time (Day)	2.67 ± 0.18	1.37 ± 0.41*	2.26±0.09*
LIGHT B	**WT**	**npq4**	**npq4 lhscr1**
Max ΔOD/Day	0.40 ± 0.06	0.45 ± 0.10	0.34±0.04
Time (Day)	2.78 ± 0.24	1.95 ± 0.16*	2.41±0.08*
LIGHT C	**WT**	***npq4***	
Max ΔOD/Day	0.32 ± 0.01	0.57 ± 0.06*	
Time (Day)	2.05 ± 0.23	1.47 ± 0.17*	
LIGHT D	**WT**	***npq4***	
Max ΔOD/Day	0.17 ±0.04	0.25 ± 0.07	
Time (Day)	2.53 ± 0.34	1.14 ± 0.02*	
LIGHT E	**WT**	***npq4***	
Max ΔOD/Day	0.18 ± 0.06	0.25 ± 0.05	
Time (Day)	2.32 ± 0.47	1.57 ± 0.08*	

Table S2: WT and npq4 pigment profiling. *Pigments were extracted in acetone 80 % and analyzed by HPLC. Chl a and b, (chlorophyll a and b); Nx, neoxanthin; Vx, violaxanthin; Ax, antheraxanthin; Lute, lutein; Zx, zeaxanthin; β-car, β-carotene. The de-epoxidation state (DEP) was calculated from the concentration of the Violaxanthin cycle pigments as follows: DEP=(Zx+(0,5*Ax))/(Vx+Ax+Zx). Standard deviation are reported for each value (n=3).*

	WT										
	Chl / Cell	**Chl *a*/*b***	**Chl / Car**	**Nx**	**Vx**	**Ax**	**Lute**	**Zx**	**β-Car**	**Chl**	**DEP**
Light A	1,23E-06	2,18	2,16	94,69	81,16	81,55	106,23	94,18	78,91	100	0,525
s.d.	*8,38E-08*	*0,01*	*0,18*	*6,09*	*0,94*	*1,86*	*0,38*	*0,40*	*16,63*		*0,004*
Light B	1,22E-06	2,64	2,20	105,60	78,20	122,60	94,10	106,20	111,30	100	0,546
s.d.	*4,04E-07*	*0,01*	*0,62*	*0,00*	*0,80*	*1,90*	*0,30*	*0,50*	*24,20*		*0,003*
Light C	7,99E-07	2,50	2,24	76,22	85,72	109,94	124,09	128,44	110,74	100	0,566
s.d.	*2,42E-08*	*0,01*	*0,35*	*0,14*	*1,15*	*1,63*	*1,21*	*1,54*	*23,56*		*0,001*
Light D	1,66E-06	2,00	2,62	98,33	86,12	116,04	102,88	98,29	90,81	100	0,520
s.d.	*3,11E-07*	*0,01*	*0,13*	*3,87*	*2,95*	*9,51*	*2,98*	*2,20*	*26,81*		*0,001*
Light E	2,15E-06	1,93	2,55	114,35	104,61	82,32	112,81	55,50	84,54	100	0,399
s.d.	*6,87E-07*	*0,06*	*0,56*	*19,24*	*8,19*	*7,99*	*6,35*	*3,84*	*14,53*		*0,000*

	npq4										
	Chl / Cell	**Chl *a*/*b***	**Chl / Car**	**Nx**	**Vx**	**Ax**	**Lute**	**Zx**	**β-Car**	**Chl**	**DEP**
Light A	1,45E-06	1,91	2,55	100,19	75,90	87,04	102,32	108,53	90,71	100	0,560
s.d.	*2,9E-07*	*0,01*	*0,45*	*10,45*	*0,55*	*2,72*	*1,18*	*6,05*	*6,80*		*0,203*
Light B	1,11E-06	2,01	2,19	102,34	84,61	102,15	121,35	147,11	112,32	100	0,594
s.d.	*3,29E-07*	*0,00*	*0,48*	*1,60*	*2,94*	*7,44*	*5,07*	*4,00*	*30,57*		*0,181*
Light C	1,15E-06	2,06	2,24	83,80	77,16	110,52	123,08	189,14	109,97	100	0,649
s.d.	*1,15E-07*	*0,01*	*0,60*	*1,02*	*1,34*	*18,98*	*7,60*	*29,71*	*40,23*		*0,258*
Light D	1,59E-06	1,69	2,46	132,64	85,61	54,48	101,89	80,59	86,73	100	0,489
s.d.	*3,85E-07*	*0,00*	*0,02*	*39,98*	*25,84*	*10,35*	*1,73*	*0,09*	*32,00*		*0,195*
Light E	1,5E-06	1,89	2,64	102,27	82,52	39,81	102,05	62,25	94,92	100	0,445
s.d.	*1,97E-07*	*0,01*	*0,72*	*31,45*	*19,55*	*19,86*	*24,23*	*13,91*	*16,91*		*0,138*

Figure S1. *Cell size and shape of WT CC425, npq4 and npq4 lhcsr1 mutant grown for 4 days at 200 µmol photons* m^{-2} s^{-1}

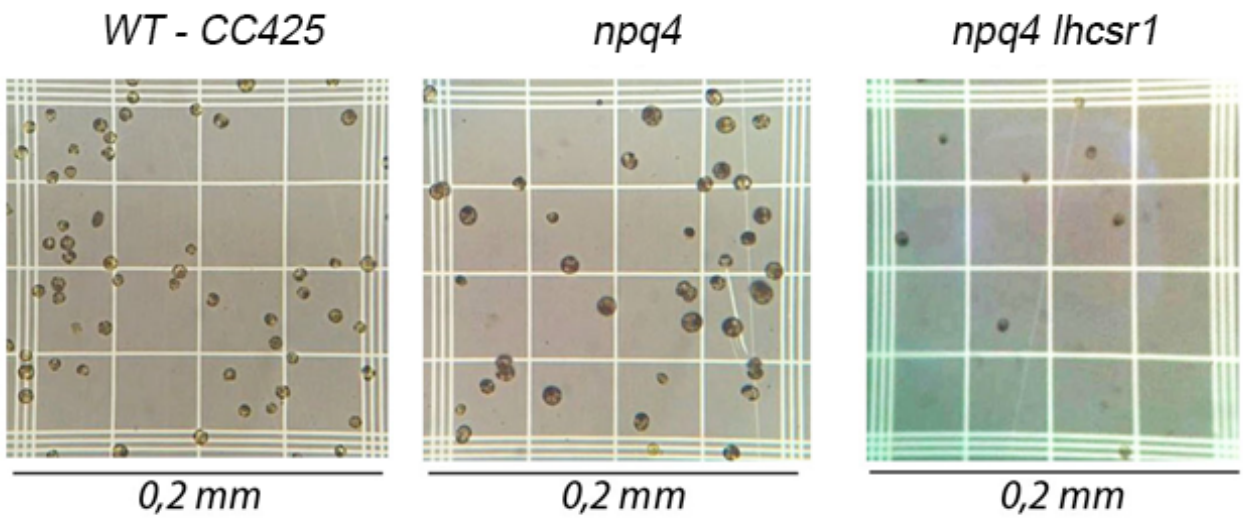

Figure S2. PSII quantum yield (Fv/Fm) of WT and NPQ4 genotypes at different days of growth. *Samples were measured in 96 well-plates using a videoimaging system. The mean value of 5 independent measurements is shown with standard deviation (n=5).*

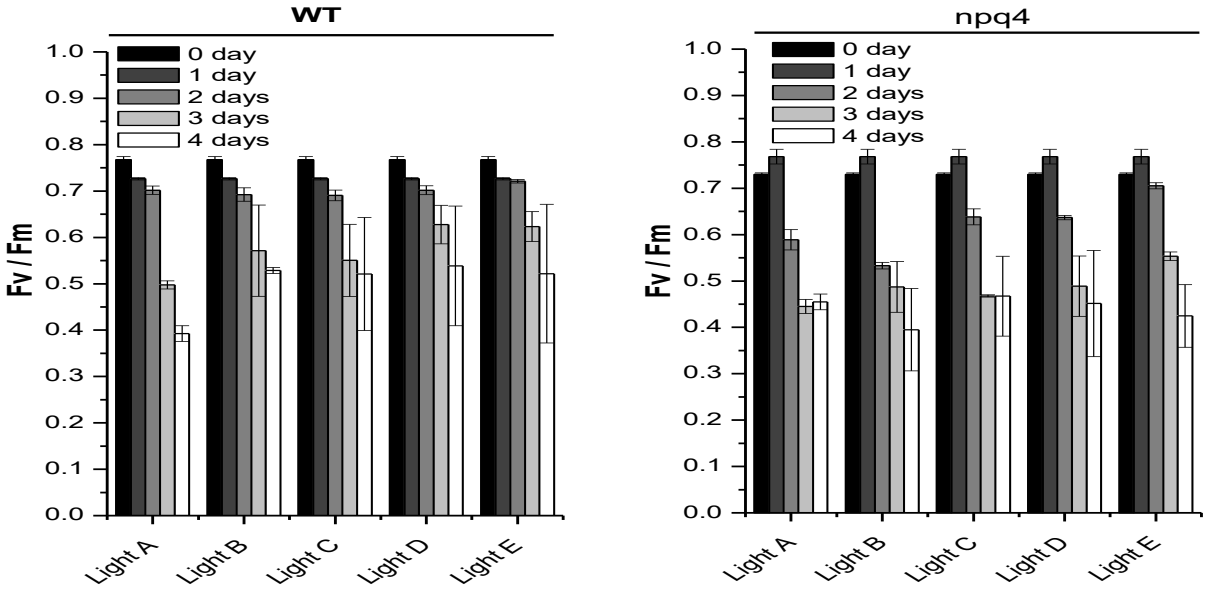

Figure S3. PSII quantum yield (Fv/Fm) of WT, npq4 and npq4 lhcsr1 genotypes at different days of growth. *Samples were measured in 96 well-plates using a videoimaging system. The mean values of 5 independent measurements are shown with standard deviation (n=5).*

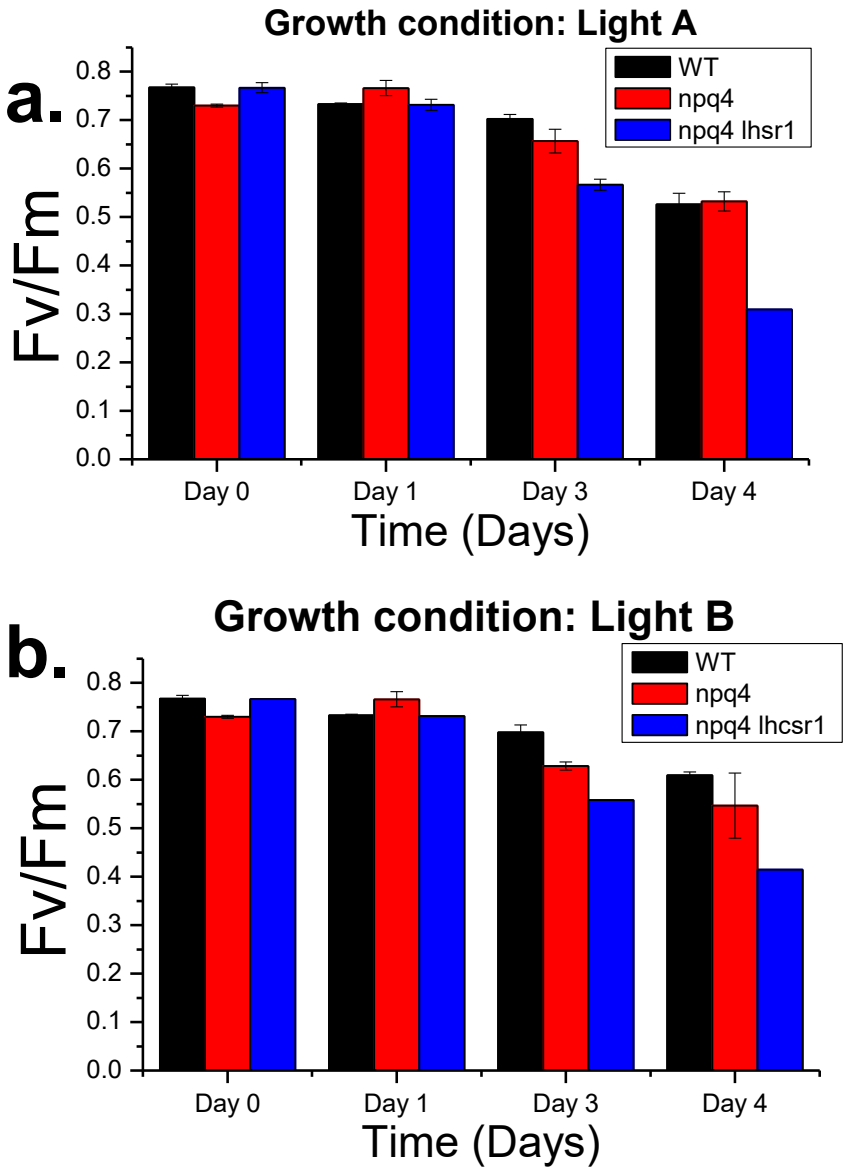

Figure S4. Immunological titration of LHCSR1, LHCSR3 and PSII in WT and npq4 total protein extracts. *Panel a.: Immunoblot analysis on protein extracts with specific anti-LHCSR and anti-CP43 antibody. White spaces were included were immunoblot signals were not in the same filter even if samples derive from the same experiment and blots were processed in parallel. Panel b.: LHCSR1 accumulation normalized to the amount of CP43. Panel c.: LHCSR3 accumulation normalized to CP43 ratio. Panel d: maximum NPQ value obtained for WT cells plotted as a function of LHCSR3/CP43 ratio. Panel e: maximum NPQ value obtained for WT cells plotted as a function of LHCSR1/CP43 ratio Panel f: maximum NPQ value obtained for npq4 cells plotted as a function of LHCSR1/CP43 ratio. The data reported are the mean value of 3 independent measurements (n=3) with standard deviation, as normalized to WT Light B conditions.*

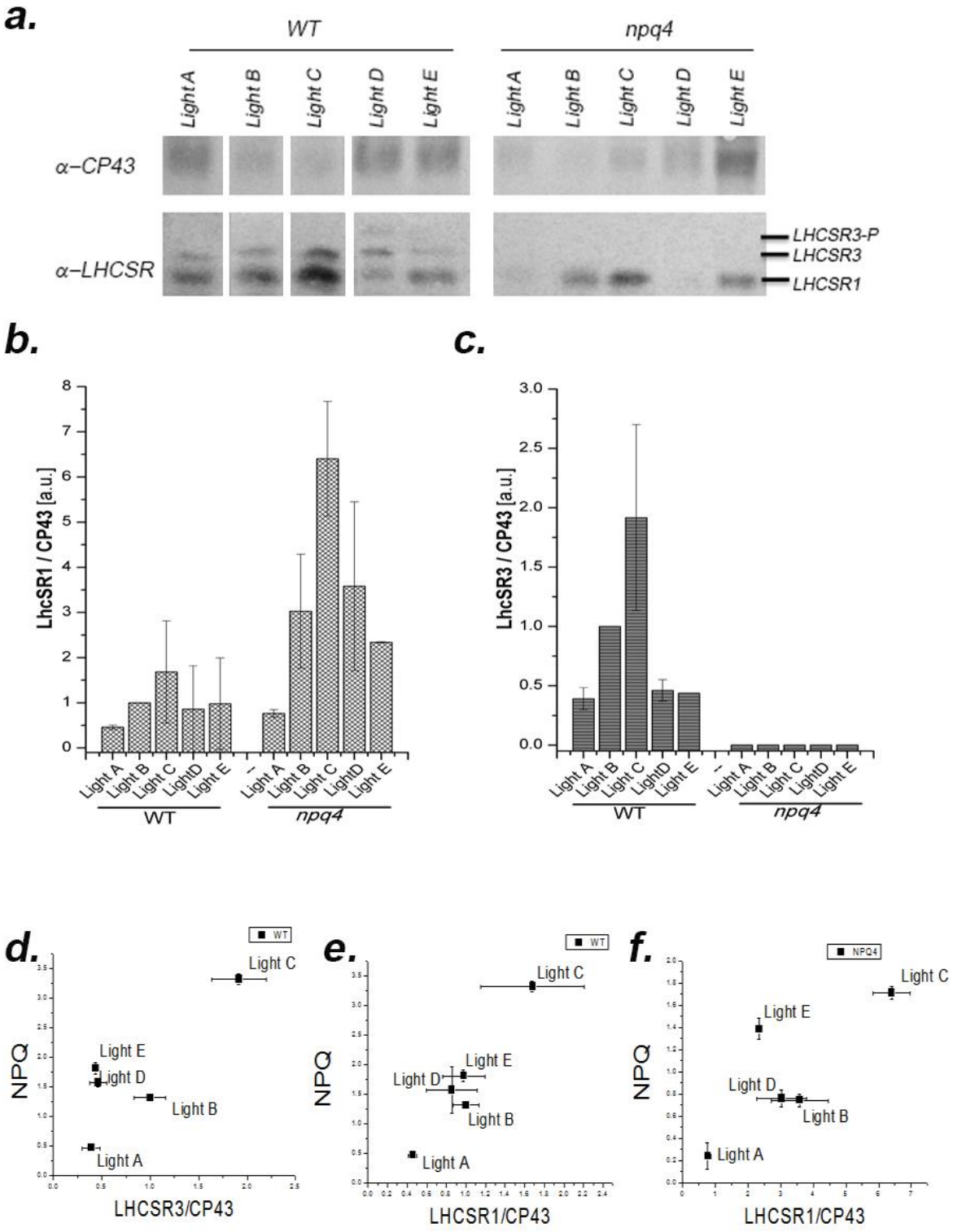

Figure S5. Correlation between PCE and heat dissipation of the light absorbed. *Panel a.: Photon Conversion Efficiency plotted as a function of NPQ for WT (black), npq4 (red) and npq4 lhcsr (blue) mutants. Panel b.: Photon Conversion Efficiency plotted as a function of variation in percentage of maximum fluorescence ((Fm – Fm')/Fm (%)) for WT (black), npq4 (red) and npq4 lhcsr (blue) mutants. Mean values with standard deviation are reported for both panel (n=5). Trend-lines (dashed lines) were calculated by exponential (Panel a.) or linear (Panel b.) fit not considering the values of npq4 lhcsr1 mutant. The equation for exponential fit used in Panel a is: y=a*exp(-x/b)+y0, where a= 5.63605E-4, b=0.55487, y0= 5.63605E-4 and adjusted R^2= 0.7. The equation for linear fit used in Panel b is: y=a+bx, where a= 3.93E-3 and b= -4.96004E-5 and adjusted R^2= 0.7.*

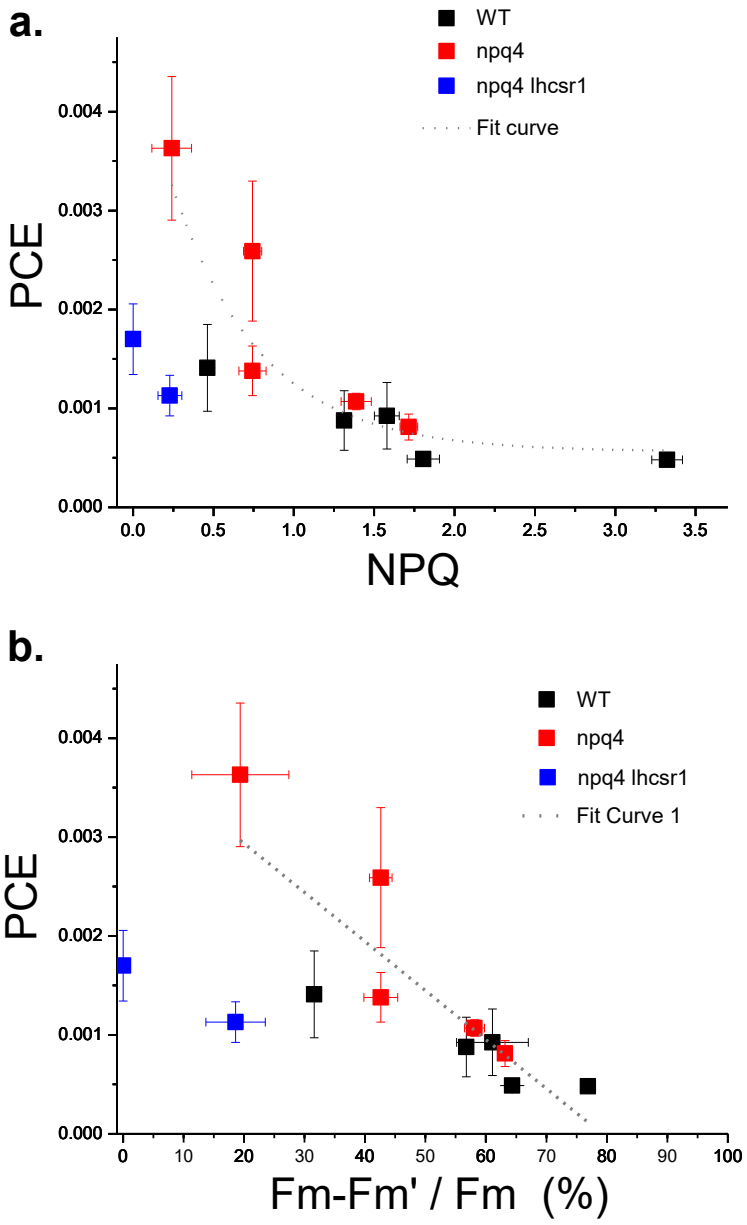

Figure S6. Proton motive force in WT and npq4 cells. *Total proton motive force (Δpmf) in WT and npq4 cells was measured as difference in absorption at 520 nm, due to electrochromic shift of carotenoid absorption spectrum (ECS). Results obtained were normalized by the chlorophyll concentration of the samples analyzed. The data reported are the mean value of 3 independent measurements (n=3) with standard deviation, as normalized to 100 in the case of WT Light A.*

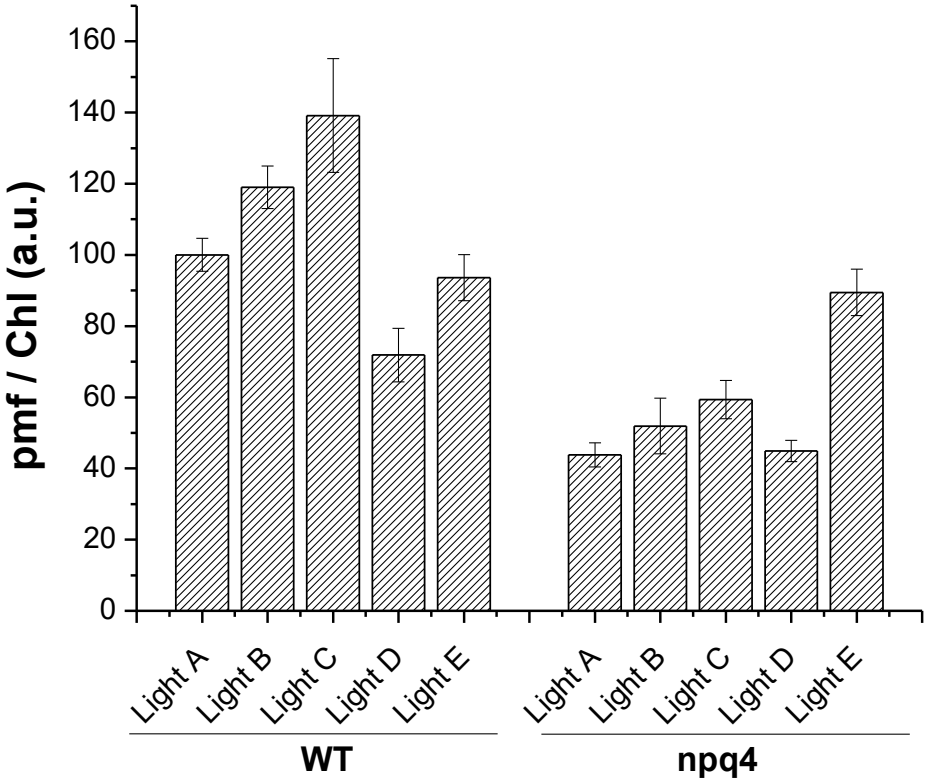

Figure S7. Immunoblot analysis of WT and npq4 mutant. *Panel A: immunoblot analysis performed on WT and npq4 proteins extract using specific antibodies for CP43, PsaA, Cyt f, ATPase β-subunit, LHCII and RUBISCO.*

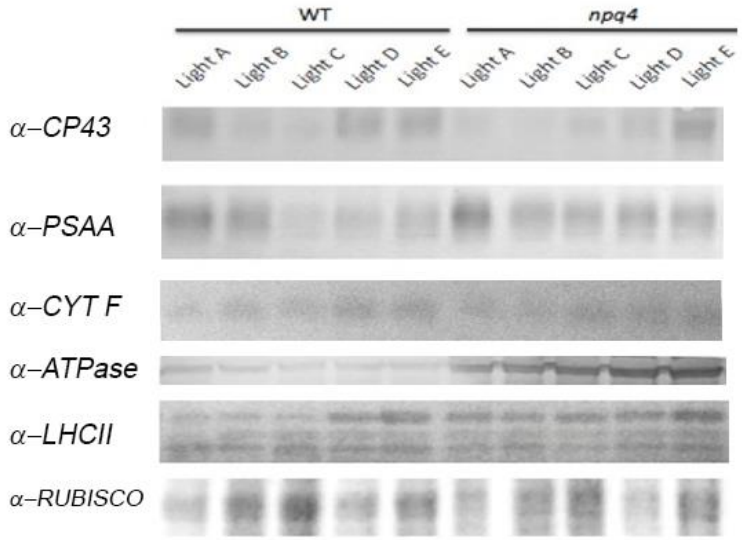

Figure S8: Densitometric analysis of immunoblot results*. Panel a.-f.: Immunoblot signal reported in Figure S7 were analyzed by densitometry in order to determine the relative protein abundance. Each protein level was normalized to the protein level in the case of WT cells grown at Light A. In WT cells the level of the CP43 subunit of PSII was similar under Light A, Light D and Light E, but strongly reduced at higher continuous irradiances (Light B and C); the pattern for npq4 was contrasting, with CP43 being reduced in all continuous light regimes and increased only in intermittent light conditions. Conversely, the level of PsaA was higher in npq4 mutant compared to WT. Panel g.: PSI to PSII ratio calculated as PSAA/CP43 ratio. In all conditions, PSI/PSII ratio was higher in npq4 compared to WT although the extent depended on conditions. The level of Cyt f was similar in all conditions with a moderate increase towards higher irradiances. The level of ATPase showed the most consistent differences between WT and npq4, with a clear overaccumulation in the mutant under all conditions. Panel h.: LHCII content per PSII reaction center estimated as LHCII/CP43 ratio. The plot shows the mean value of 3 independent measurements with standard deviation (n=3).*