

Supplemental Figure 1. Influence of O_2 on levels of PSI and PSII reaction centres, PsaA and PsbA, respectively, as indicated by western blotting of *C. reinhardtii* wild-type (WT) 4A and *npq4* grown under 17% and 35% O_2 . (A) Various protein amounts were loaded for checking suitable protein amounts for western blotting of PsbA and PsaA in cells grown under 35% O_2 . (B) Protein extracts from cells grown under 17% and 35% O_2 were from three independent experiments conducted on three separate occasions, but loaded and blotted together on the same 15-well gel. Each lane contained 10µg protein for PsaA and 1µg protein PsbA. (C) Densitometric quantification of band intensity of data shown in (B) for WT-4A (white) and *npq4* (black).



Supplemental Figure 2. Effect of high light in the presence and absence of lincomycin on PSII activity, as measured via net O_2 production under saturating light in the presence of 1 mM NaHCO₃. Closed symbols without lincomycin from two independent experiments measured at different time points. Open symbols with 2.5 mM lincomycin, n=3±SD. Orange and blue symbols represent wild type (WT)-4A and *npq4*, respectively.



Supplemental Figure 3. Effect of high light on NPQ and LHCSR1 levels in WT-4A and *npq4*. NPQ at 1000 μ mol quanta m⁻² s⁻¹ as indicated by the white bar on the x-axis. Wild-type 4A (WT; squares) and *npq4* (circles) were measured before (filled symbols) or after a high light-treated for 2 h (+1.5 h recovery) n=3±SD. The inset shows a western blot of the NPQ protein LHCSR1 in the same cells used for NPQ measurements, with PsbA (PSII reaction centre) shown as loading control.



Supplemental Figure 4. Changes in PsbA protein levels in *npq4* and WT during high light treatment in the presence of lincomycin with N_2 or O_2 gas purging. Wild-type (WT)-4A and *npq4* were high light-treated for 1.5 h in the presence of 2.5 mM lincomycin and 5mM NaHCO₃ while purging with N_2 or O_2 gas. 1µg of protein was loaded per lane. The Coomassie-stained band of the large RuBisCo subunit (*c*. 50kD) is provided for loading controls. Shown are typical blots from three independent experiments of proteins extracted from the same cells used for data shown in Figure 4.



Supplemental Figure 5. The effect of low light during recovery of high light-treated cultures on quantum yields of PSII (Φ PSII) and levels of PsbA, the PSII reaction centre. WT-D66, WT-4A and *npq4* were treated for 16 h under high light (500 µmol m⁻² s⁻¹) in photoautotrophic liquid media and then recovered in the dark (black) or low light (25 µmol quanta m⁻² s⁻¹; white), n = 3 ± SD. The difference in Φ PSII between dark and low light recovered cells is indicative of photoinhibition. The inset in (A) shows PsbA levels of WT-D66 and *npq4* during recovery under low light. Coomassie-stained gel of the major LHC proteins (*c*. 20-28kD) is shown for loading control.



Supplemental Figure 6. Effect of acrolein, bromoxynil and DCMU on P700dependent absorption changes, indicating maximum P700+ levels. (A) Cells were cultivated on TAP agar under low light before treatment with 0-1800 ppm (atmospheric) concentrations of volatile acrolein for 4 h, as indicated by the key. Kinetics are averages of three biological replicates each measured three times around a saturating pulse starting a 0 ms. Cells were cultivated on TAP agar under low light before treatment with 0-1800 ppm (atmospheric) concentrations of volatile acrolein for 4 h, as indicated by the key. (B) Photoautotrophic cells were cultivated in liquid media (control; grey dotted line) and high light-treated at 300μ E for 3 h in the presence of 50μ M bromoxynil (orange line) or 10μ M DCMU (blue line). All measurements were made in the presence of 10μ M DCMU.



Supplemental Figure 7. High light-treated *npq4* has higher tolerance to ${}^{1}O_{2}$ than WT, and high light treatment in a high O_{2} atmosphere induces greater tolerance than in ambient O_{2} . (A) WT-4A and *npq4* cells were treated with high light (300 µmol quanta m⁻² s⁻¹) for 0, 2 or 4 h and tolerance to ${}^{1}O_{2}$ was tested by adding various concentrations of Rose Bengal (RB) for 24 h at 50 µmol quanta m⁻² s⁻¹. Cell survival was monitored by chlorophyll content of cultures relative to non-treated cells, n=3±SD. (B) Photoautotrophic WT-4A and *npq4* cells on agar were pre-treated with high light for 4 h, either at ambient O_{2} (high light) or 80 % O_{2} (high light + O_{2}). Subsequently, cells were re-suspended in liquid medium at 10 µg mL⁻¹ total chlorophyll with 10 µM RB and treated with high light for up to 16 min. Every 4 min, 10µL of RB-treated culture was spotted onto TAP agar and cultivated for 5 d under low light before imaging.