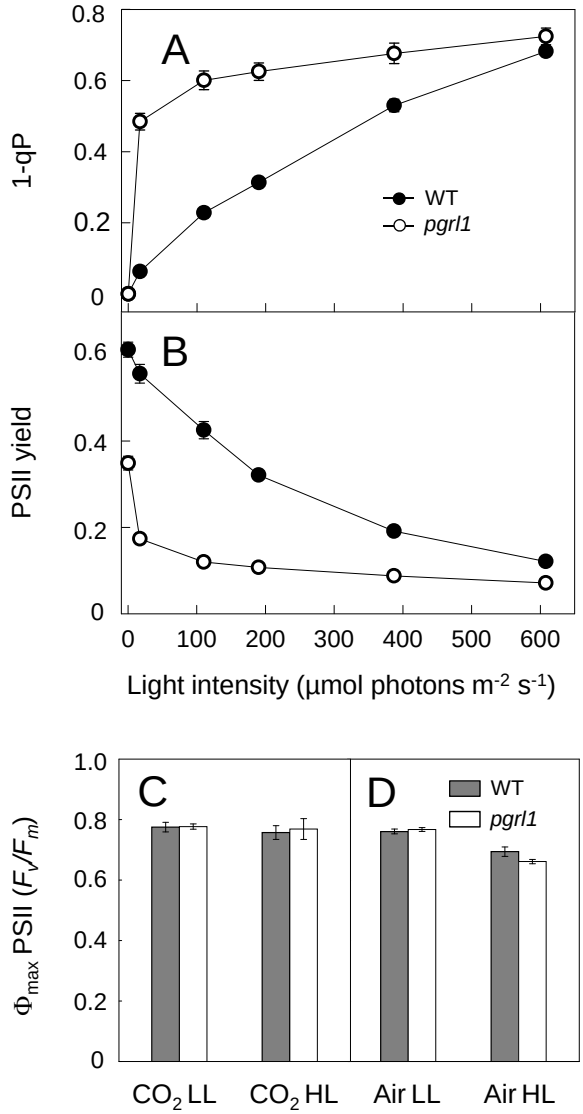
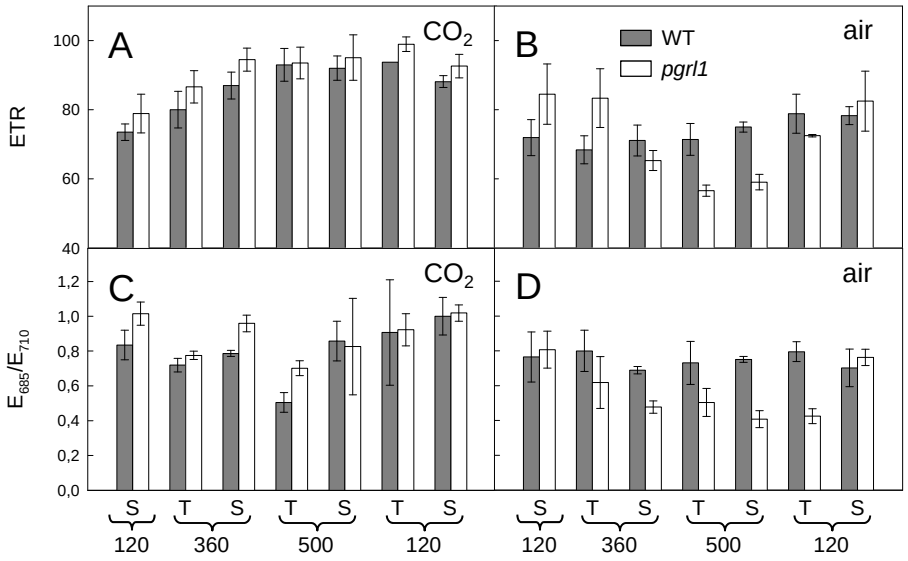
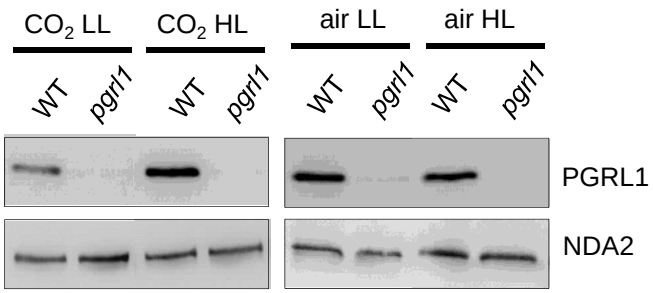


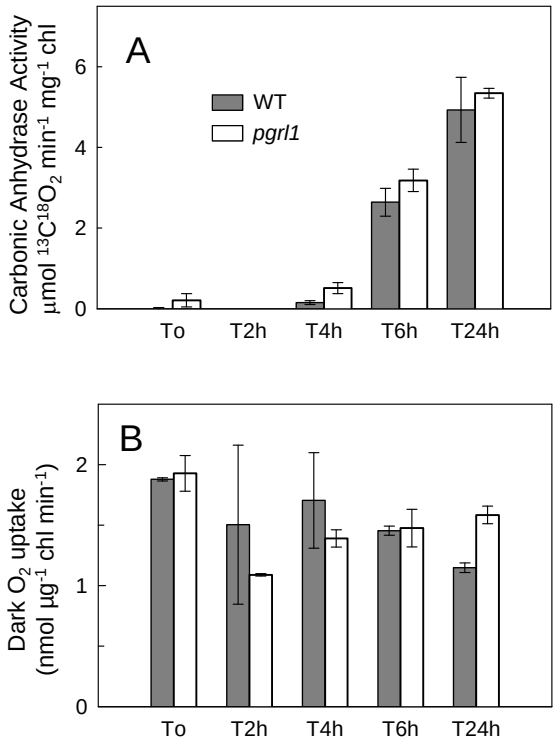
Supplemental Figure 1. Photosynthetic activity of *C. reinhardtii* wild-type and *pgrl1* mutant lines measured by chlorophyll fluorescence. (A, B) Cells were grown photoautotrophically in batch cultures for 24 h in air under 200 µmol photons m^{-2} s^{-1} light intensity ("air HL"). Chlorophyll fluorescence measurements were performed at increased light intensities after 30 min dark adaptation in dark, in the presence of 5 mM $NaHCO_3$. (A) chlorophyll fluorescence parameter (1-qP) related to the reduction of Q_A; (B) PSII yield measured in the light measured as *(Fm'-Fs)/Fm'*. Wild-type (closed circles), and *pgrl1* mutant (open circles) lines; (C, D) Maximal PSII yield measured as the chlorophyll fluorescence ratio *Fv/Fm* after 30 min dark adaptation from photoautotrophic batch cultures performed under LL (50 µmol photons m^{-2} s^{-1}) or HL (200 µmol photons m^{-2} s^{-1}) in the presence of 2% CO_2 enriched air or in the presence of air. Wild-type line (dark bars), *pgrl1* (white bars). Shown are means ± SDs (n = 3).



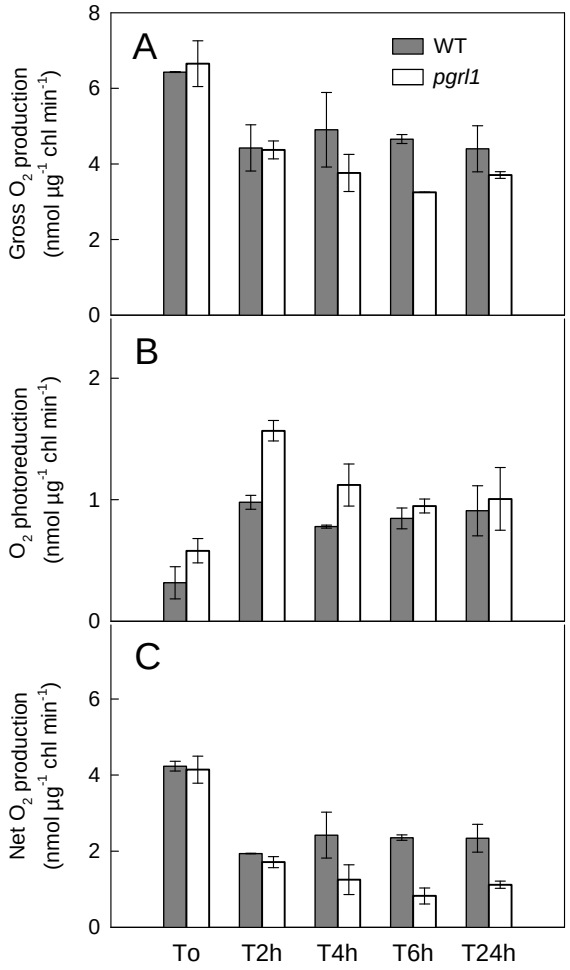
Supplemental Figure 2. ETR capacities and 77K chlorophyll fluorescence measured in wild-type and *pgrl1* during transients from moderate to high light performed under two different CO_2 concentrations. Wild-type (dark bars) and *pgrl1* (white bars) cells were grown photo-autotrophically in 1-L photobioreactors operated as turbidostats at a constant biomass concentration (≈ 4 x 10^5 cells mL^{-1}). After 48 h growth at 120 µmol photons m^{-2} s^{-1}, light intensity was sequentially changed to 360, 500 and to 120 µmol photons m^{-2} s^{-1}, the cell culture being stabilized for at least 24 h before each light change. Samples were taken for chlorophyll fluorescence measurements at 1 h (T for transitory) and 24 h (S for stabilized) after each light change. (A, B) ETR measurements performed at a light intensity of 400 µmol photons m^{-2} s^{-1} following addition of 5 mM $NaHCO_3$. (A, C) cells grown in the presence of 2% CO_2 in air. (B, D) cells grown in the presence of CO_2 air levels. (C, D) Ratio of 77K chlorophyll fluorescence emission peaks measured at 685 nm and 710 nm. Shown are means ± SDs (n=3).



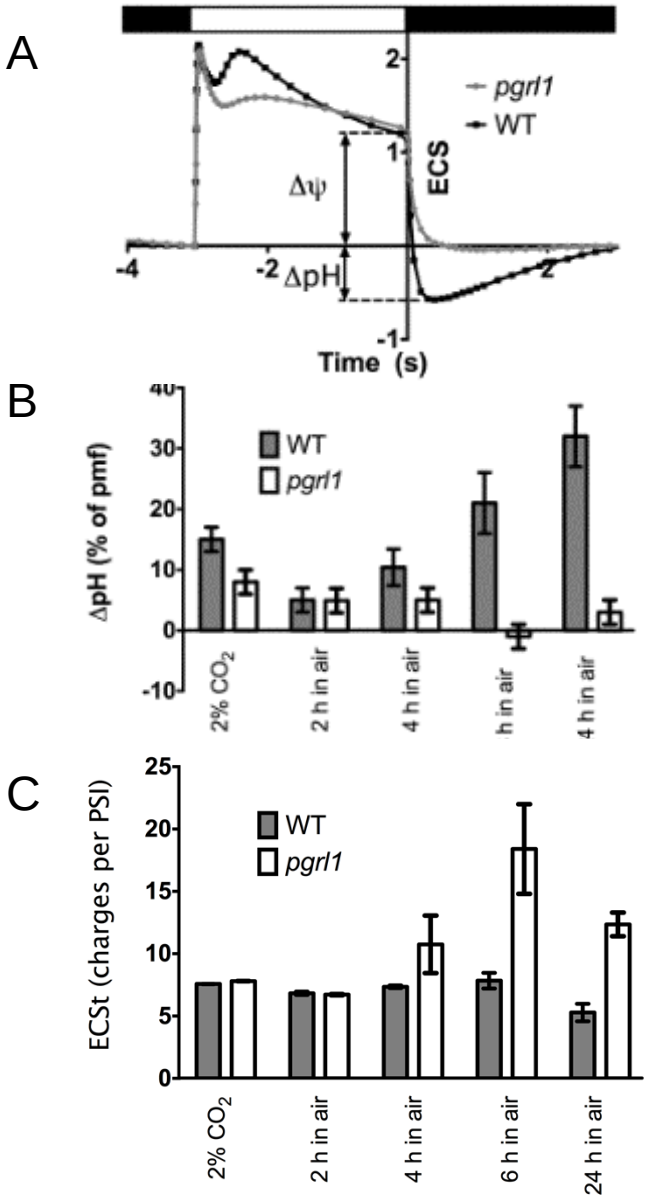
Supplemental Figure 3. Accumulation of NDA2, and PGRL1 proteins in wild-type and *pgrl1 C. reinhardtii* lines grown under different environmental conditions. The wild-type and *pgrl1* mutant lines were grown in photobioreactors operated as turbidostats at a constant biomass concentration (≈ 1.5 x 10^6 cells mL^{-1}). Cells were cultivated under 120 µmol photons m^{-2} s^{-1} (LL) or 500 µmol photons m^{-2} s^{-1} (HL) light intensity and in the presence of 2% CO_2 in air (CO_2) or in air (air). After a 24-h adaptation period in the indicated conditions, samples were taken from the photobioreactors. Proteins were extracted from total cells and separated under denaturing conditions (10% PAGE Bis-Tris SDS-MOPS). Samples were loaded at equal protein amounts based on Coomassie staining.



Supplemental Figure 4. Effect of a high-to-low (air) CO_2 concentration switch on carbonic anhydrase activity and mitochondrial respiration measured on intact cells. Cells were grown autotrophically in photobioreactors operated as turbidostats at a constant biomass concentration (≈ 1.5 x 10^6 cells mL^{-1}) in the presence of 2% CO_2-enriched air and under high light (500 µmol photons m^{-2} s^{-1}). At T_0, stabilized cultures were shifted to air at the same light intensity. Cells (1.5 mL) were sampled after 0, 2, 4, 6 and 24 h. (A) Induction of the carbon concentrating mechanism (CCM) was followed by measuring carbonic anhydrase activity of intact cells by Membrane Inlet Mass Spectrometry (MIMS). Upon addition of doubly labelled CO_2 [$^{13}C^{18}O_2$], added at a final concentration of 0.5 mM, the exchange activity of carbonic anhydrase between CO_2 and H_2O was followed in the dark by measuring the rate of decrease of the [$^{13}C^{18}O^{18}O$] species (m/e = 49). (B) O_2 uptake rates were measured in the dark using MIMS. Wild-type control (dark bars) and *pgrl1* (white bars) lines. Shown are means ± SDs (n = 3).



Supplemental Figure 5. Light-dependent O_2 exchange measured in wild-type and *pgrl1* cells shifted from high CO_2 to low CO_2 (air). *C. reinhardtii* cells were grown autotrophically in photobioreactors operated as turbidostats at a constant biomass concentration (≈ 1.5 x 10^6 cells mL^{-1}) in the presence of 2% CO_2-enriched air and under high light (500 µmol photons m^{-2} s^{-1}). The stabilized cultures were shifted to air, light intensity was not changed. Cells (1.5 mL) were sampled after 0, 2, 4, 6 and 24 h and O_2 uptake rates were measured using a membrane inlet mass spectrometer in the presence of [^{18}O] enriched O_2. O_2 consumption rates were first measured during a 5-min dark period, then light (600 µmol photons m^{-2} s^{-1}) was switched on and light-dependent O_2 exchange rates were measured during a 10-min period. (A) Gross O_2 production rate; (B) Light-induced O_2 uptake rate; (C) Net O_2 uptake rate calculated as the difference between gross O_2 production and O_2 uptake in the light. WT (dark bars), *pgrl1* (white bars). Shown are means ± SDs (n = 2).



Supplemental Figure 6. Electrochromic shift (ECS) and proton concentration (ΔpH) in *pgrl1* and wild-type *C. reinhardtii* cells to a switch from high to low CO_2. Cells were cultivated autotrophically in photobioreactors operated as turbidostats at a constant biomass concentration (≈ 1.5 x 10^6 cells mL^{-1}) in the presence of 2% CO_2-enriched air under a light intensity of 500 µmol photons m^{-2} s^{-1}. Upon 48 h stabilization, cultures were shifted to air CO_2 levels. Samples were taken before and 2, 4, 6 and 24 h after the shift in order to monitor electrochromic shift (ECS) of carotenoids. (A) ECS measured as an absorbance change at 520 nm in intact cells of *pgrl1* or WT after 24 h of shifting in continuous illumination of 3 s (white bar). The relative contributions of ΔΨ and ΔpH to the proton-motive force (Cruz et al., 2005) are shown by vertical arrows. The decay of ECS after a preillumination shows the decay of the proton-motive force (pmf) accumulated in the light. This pmf is parsed into a difference of c[Gilles2]tion of protons (ΔpH) and an electrical component (ΔΨ) (Cruz et al., 2001). (B) Fraction amount of ΔpH with regard to tota[Gilles1]-motive force (pmf). (C) Total ECS (ECSt). Error bars are means ± SDs (n = 3). After shifting into air, a increase of ΔpH in WT but not in *pgrl1* was observed. This difference is attributed to the loss of cyclic electron flow around PSI (absence of PGRL1) and to the loss of PSI itself (PSI photo inhibition in *pgrl1* HL).

Slide 6

Gilles1 If this refers to statistical significance, please describe the analysis. If it does not, please choose another word here.

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Gilles2 Please define the error bars in B and C.

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PELTIER Gilles 097083, 6/12/2014

Supplemental Literature Cited

Cruz, J. A., T. J. Avenson, A. Kanazawa, K. Takizawa, G. E. Edwards and D. M. Kramer (2005) Plasticity in light reactions of photosynthesis for energy production and photoprotection. ***J Exp Bot*** **56,** 395-406

Cruz, J. A., C. A. Sacksteder, A. Kanazawa and D. M. Kramer (2001) Contribution of electric field (Delta psi) to steady-state transthylakoid proton motive force (pmf) in vitro and in vivo. control of pmf parsing into Delta psi and Delta pH by ionic strength. ***Biochemistry*** **40,** 1226-1237