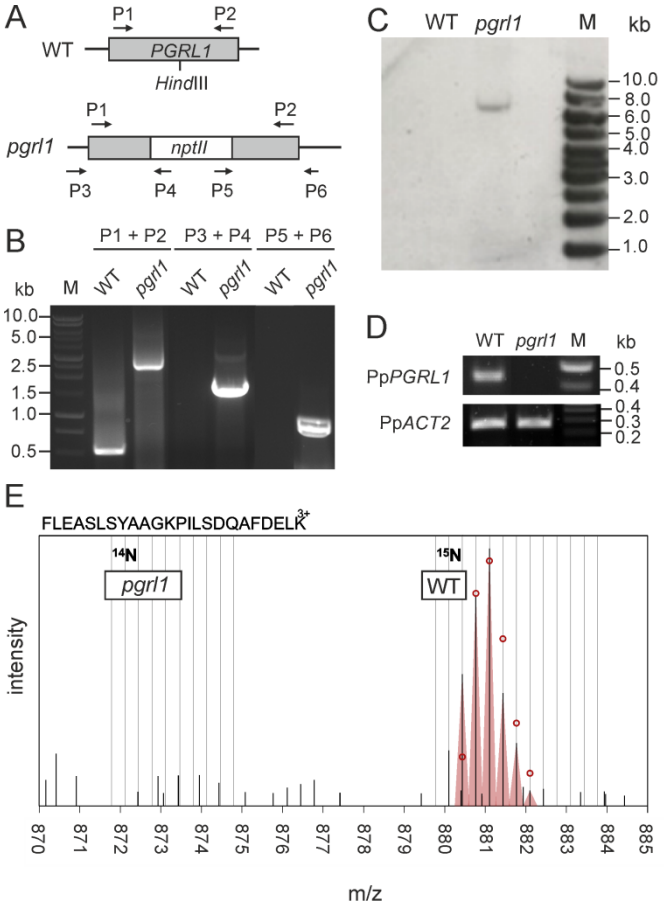
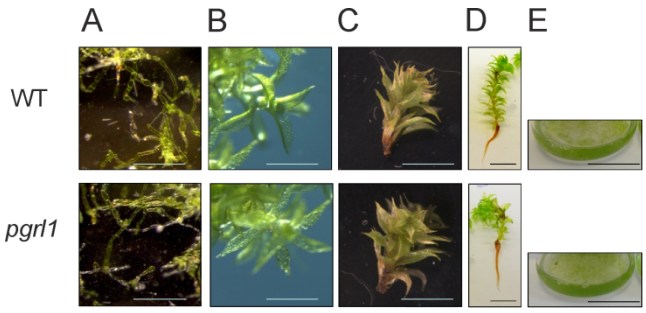
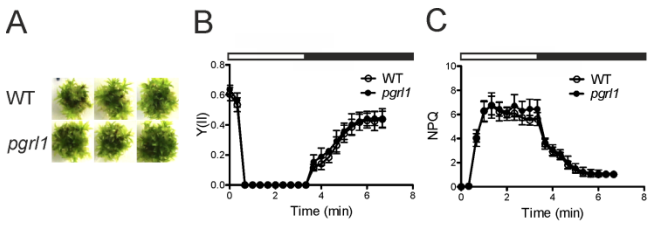


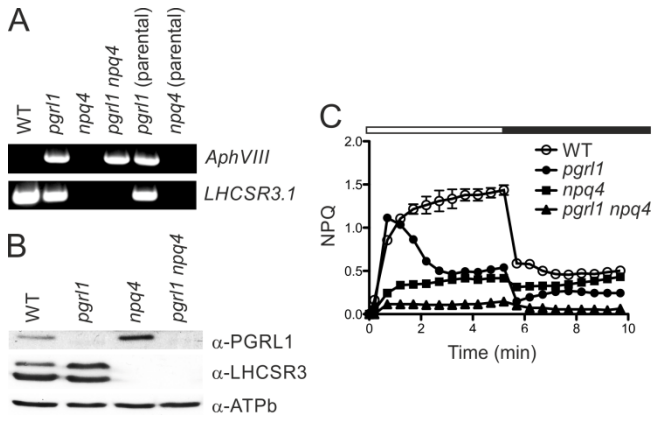
Supplemental Figure S1. The Physcomitrella *pgrl1* knockout mutant harbors a single copy of the disruption construct in the genome resulting in the lack of Pp*PGRL1* transcripts and protein expression. (**A**) Schematic representation of the wild type Pp*PGRL1* gene and the disruption construct used for generation of the knockout mutant. The Pp*PGRL1* genomic DNA sequence (grey box) was digested at a unique *Hind*III restriction site and the *nptII* selection marker cassette (white box) inserted to obtain a knockout construct. The annealing positions of the primers used to confirm gene targeting are indicated by the arrows. (**B**) PCR genotyping analysis of wild type and *pgrl1* mutant confirming the correct integration of the disruption construct into the Pp*PGRL1* gene locus. All amplification products were sequenced. (**C**) Southern blot analysis of wild type and *pgrl1* mutant excluding additional integration sites of the Pp*Δpgrl1-nptII* disruption construct in the genome of the *pgrl1*. (**D**) RT-PCR expression analysis of the Pp*PGRL1* and housekeeping (actin; Pp*ACT2*) genes in wild type and the *pgrl1* mutant strains. The molecular weight marker (M) is shown; kb: size in kilo base pairs. (**E**) PGRL1 protein detection via mass spectrometry supporting the absence of PGRL1 at the protein level in the Physcomitrella *pgrl1*. In the ^{15}N metabolically labeled WT the PGRL1-peptide FLEASLSYAAGKPILSDQAFDELK can be quantified by its isotopologue in the MS1 spectrum, but no corresponding isotopologue is present in the ^{14}N labeled mutant.



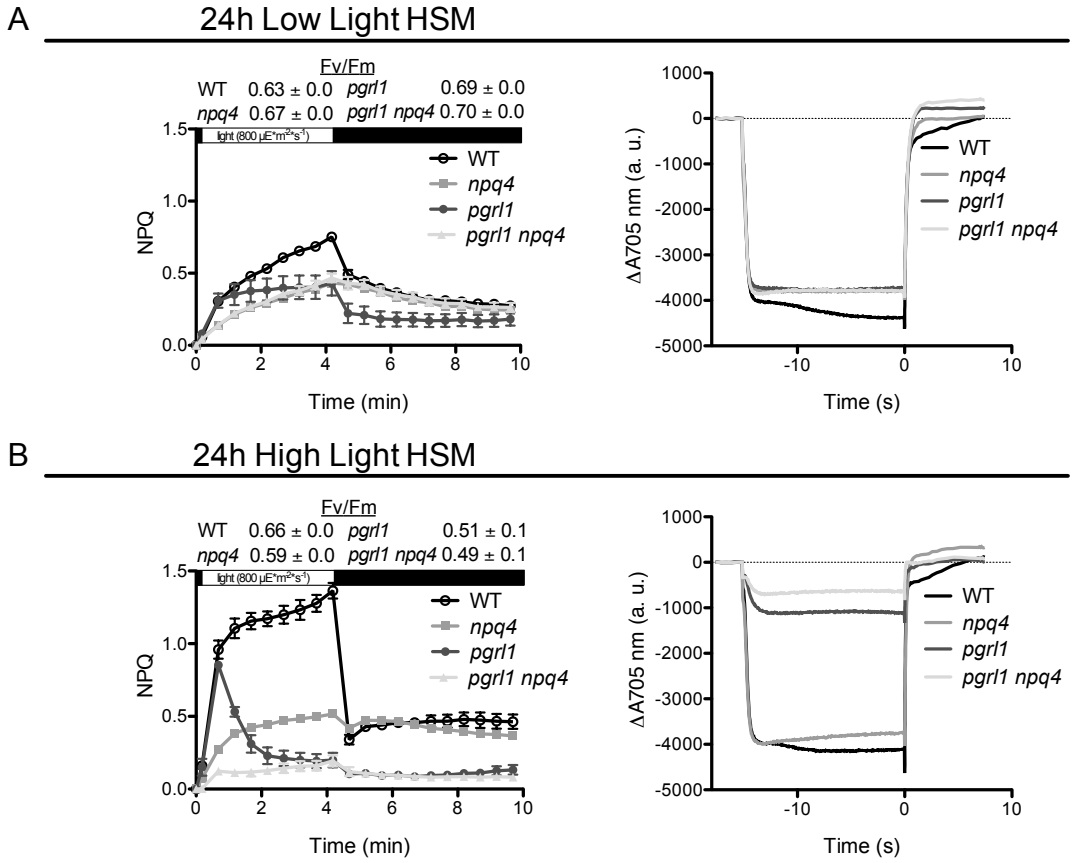
Supplemental Figure S2. Phenotypic comparison of the Physcomitrella wild type and the *pgrl1* during the developmental stages of the haploid gametophore grown under optimal conditions. (**A**) Nine days old protonemal filaments. Scale bar: 100µm. (**B**) 20 days old culture of protonema and gametophores. The gametophores develop from several subapical caulonemal cells Scale bar: 2mm. (**C** and **D**) 30 days old gametophore with the originated from the base rhizoids. Scale bar: 2mm. (**F**) 10 days old protonema grown in liquid KNOP media. Scale bar: 5 cm.



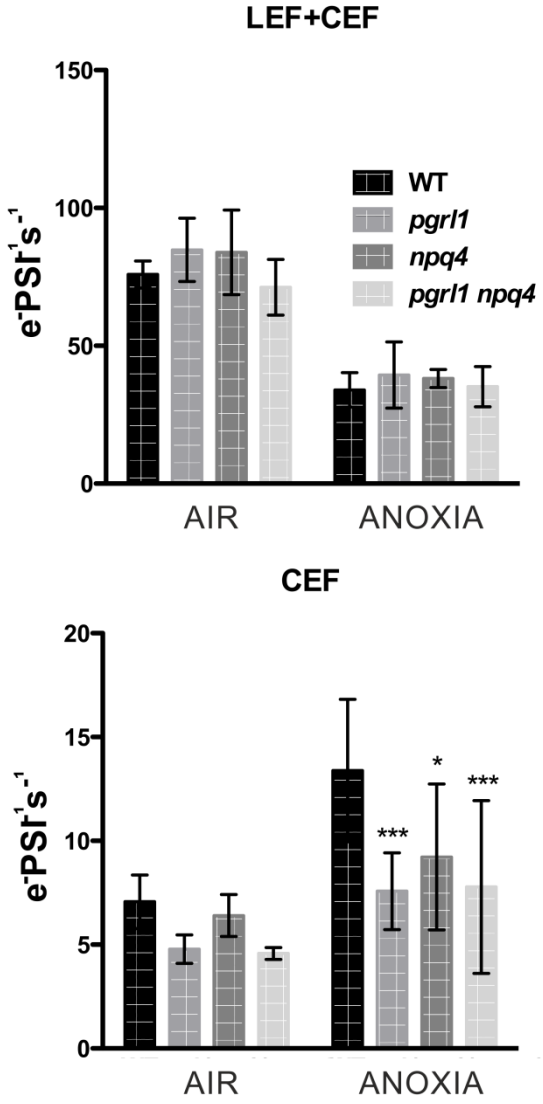
Supplemental Figure S3. Phenotype, time course of NPQ and Y(II) of Physcomitrella wild type and *pgrl1* gametophores grown for 1 month under optimal conditions (as control for long-term anaerobiosis experiment).



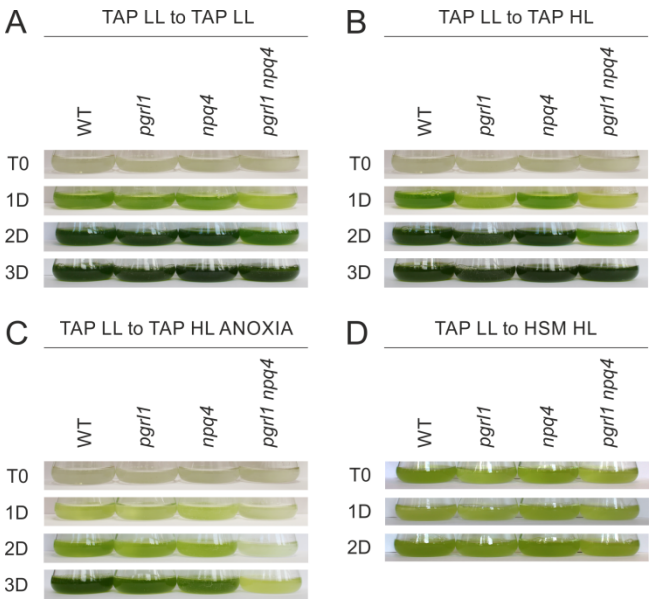
Supplemental Figure S5. Generation of the Chlamydomonas *pgrl1 npq4* double mutant. (**A**) the *pgrl1* (Tolleter et al., 2011) and *npq4* (Peers et al., 2009) mutants were first backcrossed four times to the wild type strain CC124 (WT) and then crossed to produce the double mutant. Insertion of the *AphVIII* disruption cassette within *PGRL1* and absence of *LHCSR3.1* were confirmed by colony PCR in both parental and backcrossed strains. (**B**) Western blot analysis confirming absence of the PGRL1 and LHCSR3 proteins in the selected strains. Cells were grown mixotrophycally under low light conditions and then shifted to high light autotrophic conditions for 24 hours. (**C**) Time course of NPQ were measured in 20 min dark-adapted Chlamydomonas cultures subjected to high light autotrophic conditions for 24 h, thus allowing full expression of LHCSR3. White bar indicate irradiation with light at 800 μE m^{-2} s^{-1}, whereas black bar indicate darkness. Data are means of three replicates ±SD.



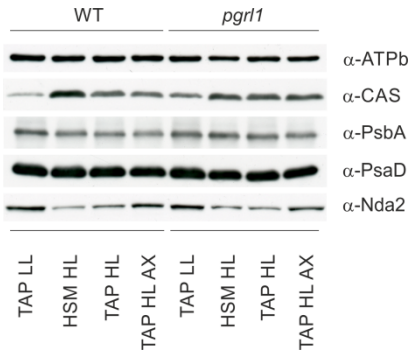
Supplemental Figure S6. Acceptor side inhibition of PSI in *pgrl1 npq4* and *pgrl1* cells under high light conditions accounts for impairment of NPQ. Cells were acclimated to low light conditions (TAP medium) before incubation in Minimal medium at 4 µg chl/ml at low light for 24h (**A**) or at high light intensities for 24h (**B**),.. Left panel: Time course of NPQ were measured in 20 min dark-adapted Chlamydomonas cultures. Right panel: P700 oxido-reduction kinetics were measured under aerobic conditions in the presence of 40 µM DCMU to block PSII. Using an actinic light intensity of 3300 µE m^{-2} s^{-1} gives access to the full amount of oxidizable P700. Data are means of three replicates ± SD.



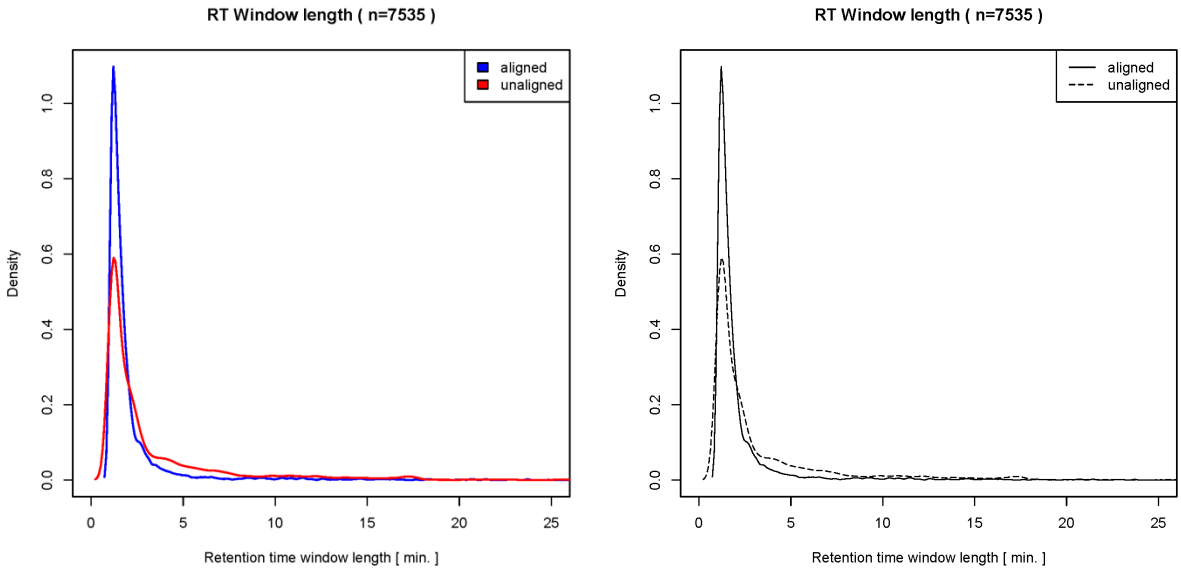
Supplemental Figure S7. CEF activity is impaired in the *pgrl1*, *npq4* and *pgrl1 npq4* strains. Relative amount of CEF under aerobic and anaerobic conditions in wild type, *pgrl1*, *npq4* and double mutants. CEF activity measurements were performed by following relaxation of the carotenoid electrochromic bandshift at 520 nm in dark-adapted tissues without (ctrl) and after addition of DCMU to the samples. Each data set represents the mean ±SD of 3 independent measurements. Asterisks indicate statistically significant differences according to Students t-test (*** = $P < 0.0001$).



Supplemental Figure S8. Susceptibility of *pgrl1, npq4* and *pgrl1 npq4* mutants to high light conditions and anaerobiosis in liquid media. Chlamydomonas strains were grown in liquid TAP medium under low light conditions, their concentration equalized to 0.1 x 10^6 cells mL^{-1} (1 x 10^6 cells mL^{-1} in **D**) and then allowed to growth under TAP low light (**A**, TAP LL, control), TAP high light (**B**, TAP HL), TAP high light anoxia (**C**, TAP HL ANOXIA) or HSM high light (**D**, HSM HL) conditions for 2-3 days. Repetition of the experiment with two independent biological replicates yielded essentially identical results.



Supplemental Figure S9: Protein expression analyses of Chlamydomonas wild type and *pgrl1* thylakoids under high light TAP anoxia, high light TAP and high light HSM conditions. 8 µg (80 µg for CAS and Nda2 blots) protein from isolated thylakoid membranes were loaded on each lane of a 13% polyacrylamide SDS-PAGE and blotted on nitrocellulose membranes. Levels of expression for CAS, PsbA, PsaD and Nda2 were assessed by incubation with specific antibodies. α-ATPb antibodies were used as a loading control.



Supplemental Figure S10: Retention time quality assessment shown as density plots for all observed retention time windows, i.e. (n=7535) before (red) and after (blue) alignment. Alignment procedure was performed using piqDB similar to the work of (Hohner et al., 2013; Barth et al., 2014).