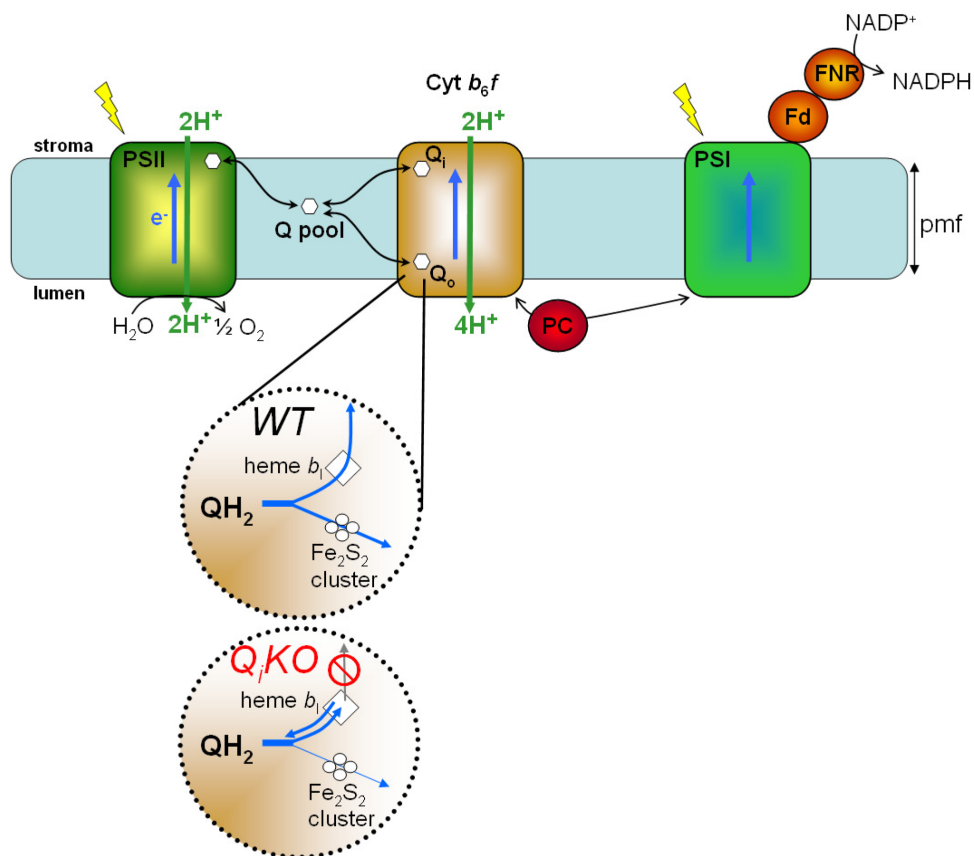
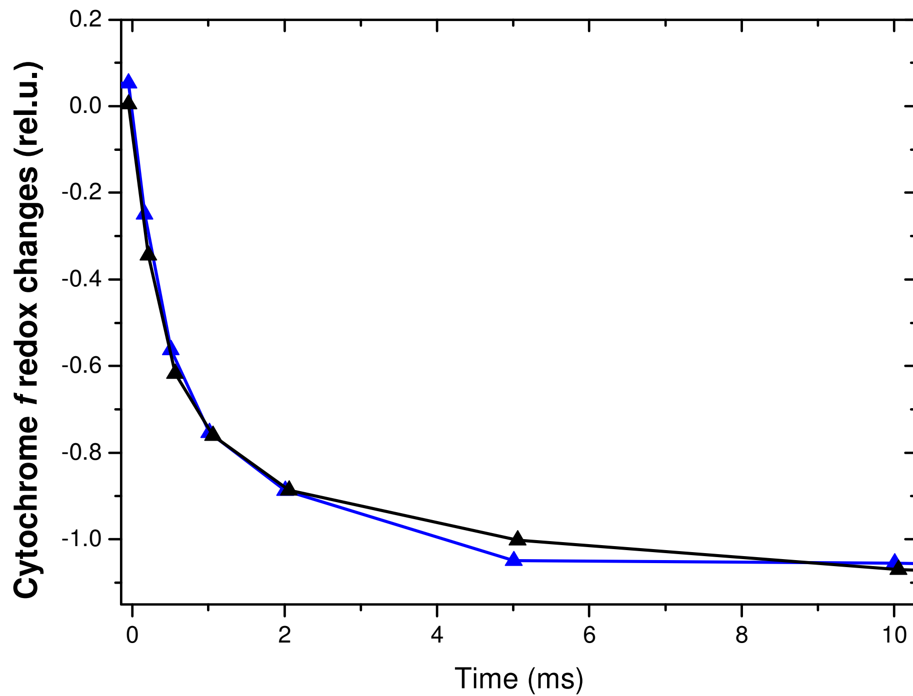
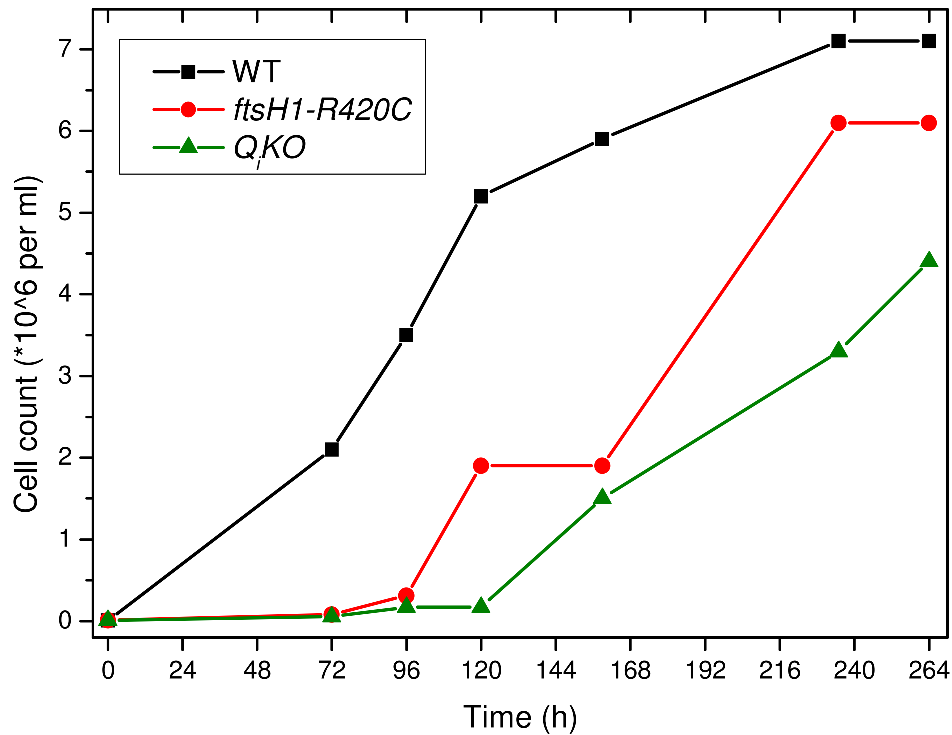


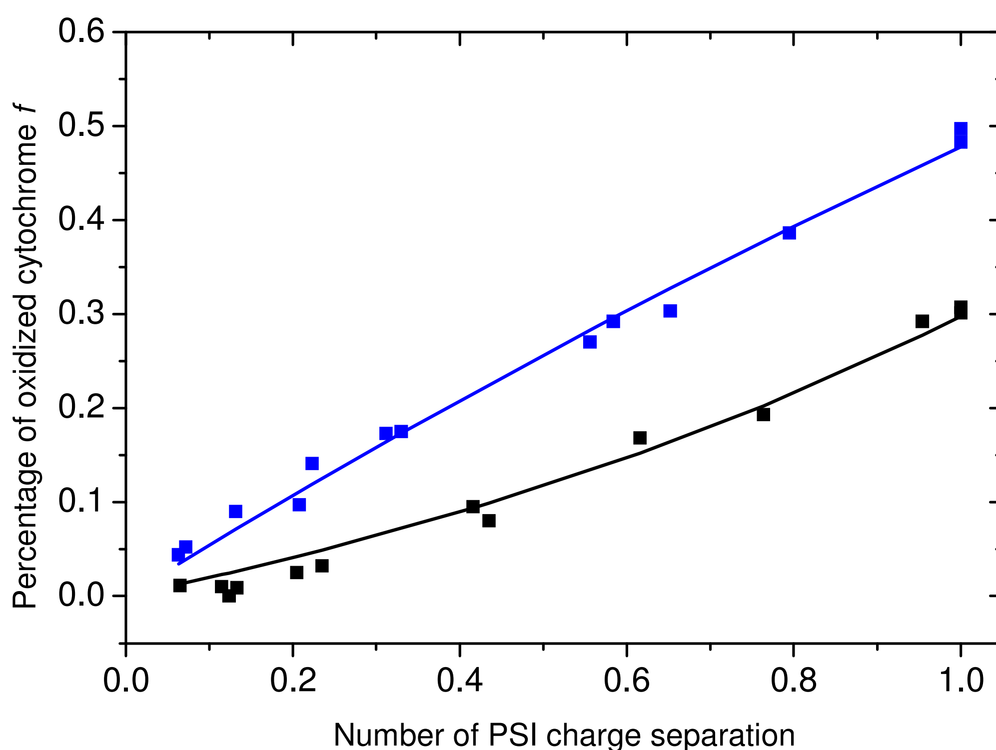
Supplementary Figure S1 | A schematic representation of the photosynthetic chain. The light-induced charge separation occurs in Photosystems I and II, triggering the turnover of the cytochrome b_6f complex which acts as a quinol plastocyanin oxidoreductase, which contributes, via the Q-cycle, to building up the proton motive force (pmf). As indicated, 6 H^+ are translocated per 2 electrons transferred from water to $NADP^+$. (Plastocyanin (PC), Ferredoxin (Fd), Ferredoxin NADP Reductase (FNR)). As highlighted, this process involves the bifurcated electron transfer (blue arrows) from the quinol (QH_2) to the Fe_2S_2 cluster, on the one hand, and to the heme b_l on the other hand. This bifurcated electron transfer step is widely considered as being mechanistically mandatory and thus expected to be impeded when the heme b_l is not available as an electron acceptor. The inactivation of the Q_i site in the Q_iKO case promotes this situation yet the present finding that the strain bearing this mutation can grow phototrophically reveals that the blockage can be alleviated.



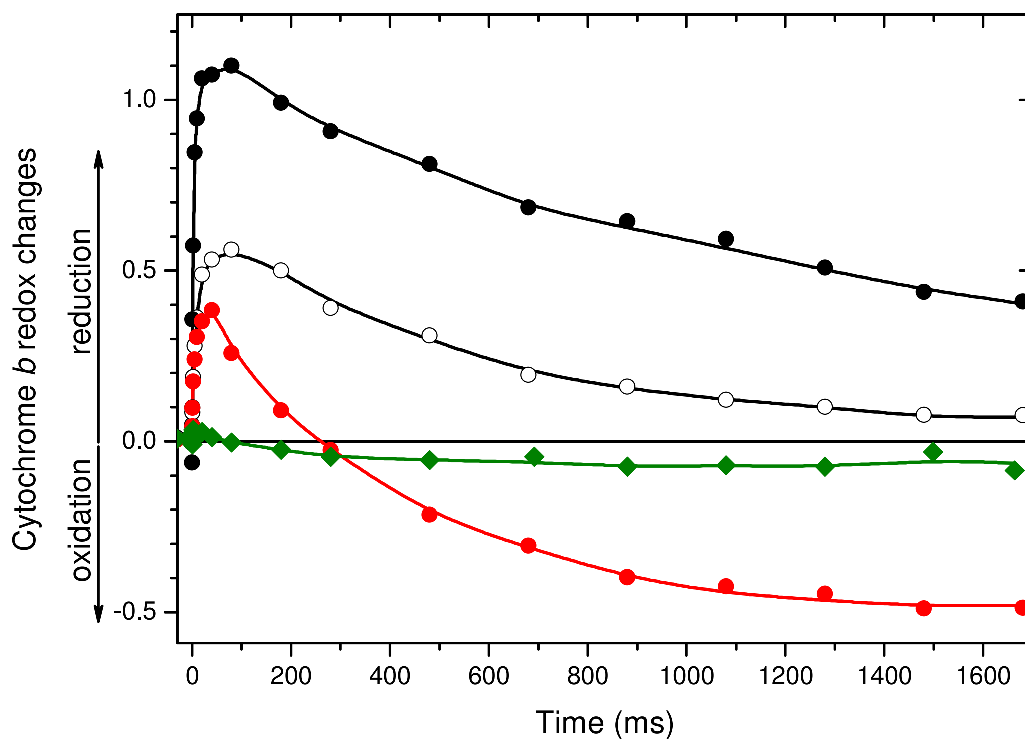
Supplementary Figure S2 | Light-induced redox changes of cytochrome *f*. Signal at 554 nm with a baseline drawn between 546 and 573 nm. The two kinetics were normalized on the PSI amount. Black, *WT*; Blue, *QiKO*. Cytochrome *f* oxidation in the presence of 100 µM TDS.



Supplementary Figure S3 | Phototrophic growth of the *WT*, Q_iKO and *ftsH1-R420C* strains in anaerobic conditions. Cell growth curves of *WT* (black squares), *ftsH1-R420C* (red circles), Q_iKO (green triangles) in minimal medium under 40 $\mu E \cdot m^{-2} \cdot s^{-1}$ of light and bubbled with a controlled atmosphere of 2% CO_2 and 98% N_2. Cells grown in heterotrophy were inoculated in 500 ml minimal medium at a concentration of 10^4 $cells \cdot ml^{-1}$. The cell density was determined by using a Malassez counting chamber. As the Q_iKO strain results from the combination of the *petB-H202Q* and *ftsH1-R420C* mutations, its growth behavior should be compared to that of the *ftsH1-R420C* strain rather than to that of the *WT*. Both the *ftsH1-R420C* and Q_iKO strains showed a similar lag phase, after which the growth rate of the latter was slightly slower than that of the former.



Supplementary Figure S4 | DNP-INT enables Rieske Fe_2S_2 protein movement while preventing quinol binding and oxidation in Q_o site. The figure shows the relative efficiency of the light-induced oxidation of cytochrome *f* as a function of the amount of light-induced charge separation in Photosystem I. Blue, TDS (20 µM); Black, DNP-INT (20 µM). Whereas both inhibitors prevent the oxidation of a quinol at the Q_o site, the amount of oxidized cytochrome *f* is lower in the presence of DNP-INT than of TDS. The latter is known to lock the head of the Rieske protein in the so-called proximal configuration[37] thereby preventing electron transfer between its Fe_2S_2 cluster and cytochrome *f*. The lower cytochrome *f* oxidation yield observed in the presence of DNP-INT shows that, at variance with TDS, this inhibitor allows the redox equilibration between the Rieske protein and cytochrome *f* and thus does not prevent the Rieske head movement.



Supplementary Figure S5 | DNP-INT inhibits the oxydation of b_l. Light-induced redox kinetics of cytochrome *b* at 564 nm with a baseline drawn between 546 and 573 nm in *Q_iKO*. Black, filled symbols, mildly reducing conditions; open symbols, after preillumination to get similar contents of pre-reduced and pre-oxidized heme *b*; Red, strongly reducing conditions. Green, DNP-INT (20 µM) in strongly reducing conditions. As expected by its quinone analog nature[38], DNP-INT inhibits reduction of heme b_l but also prevents the reoxidation of pre-reduced heme b_l. Since DNP-INT does not impede the Rieske protein movement (Supplementary Fig. S4), this demonstrates that reoxidation of heme b_l does not occur through the Rieske protein but rather via the reduction of the semiquinone intermediate.

Supplementary References

37. Zhang, Z. et al., Electron transfer by domain movement in cytochrome bc_1. *Nature* **392**, 677-684 (1998).
38. Delosme, R., Joliot, P., and Trebst, A., Flash-induced oxidation of cytochrome b_{563} in algae under anaerobic conditions - Effect of Dinitrophenylether of iodonitrothymol. *Biochim Biophys Acta* **893**, 1-6 (1987).