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 $b_{6}f$ 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$_{2}$S$_{2}$ cluster, on the one hand, and to the heme $b_{1}$ 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 $b_{1}$ is not available as an electron acceptor. The inactivation of the Q$_{i}$ site in the Q$_{i}$KO 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, QKO and ftsH1-R420C strains in anaerobic conditions.** Cell growth curves of WT (black squares), ftsH1-R420C (red circles), QKO (green triangles) in minimal medium under 40 µE·m⁻²·s⁻¹ of light and bubbled with a controlled atmosphere of 2% CO₂ and 98% N₂. Cells grown in heterotrophy were inoculated in 500 ml minimal medium at a concentration of 10⁴ cells·ml⁻¹. The cell density was determined by using a Malassez counting chamber. As the QKO 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 QKO 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$_2$S$_2$ 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$_2$S$_2$ 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 oxidation of $b_1$. Light-induced redox kinetics of cytochrome $b$ at 564 nm with a baseline drawn between 546 and 573 nm in $Q_i$KO. 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 $b_1$ but also prevents the reoxidation of pre-reduced heme $b_1$. Since DNP-INT does not impede the Rieske protein movement (Supplementary Fig. S4), this demonstrates that reoxidation of heme $b_1$ does not occur through the Rieske protein but rather via the reduction of the semiquinone intermediate.
Supplementary References
