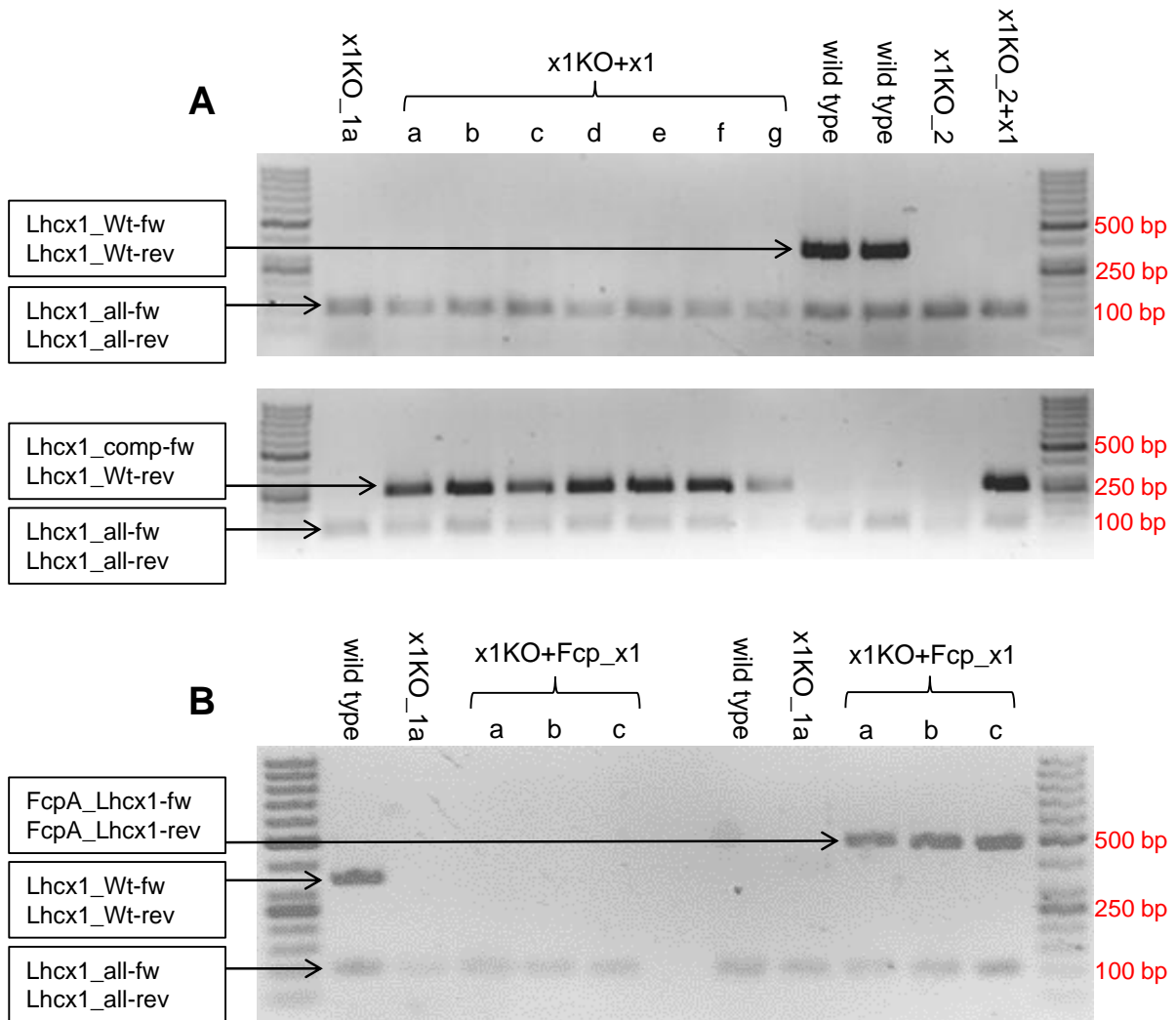


Supplementary Information



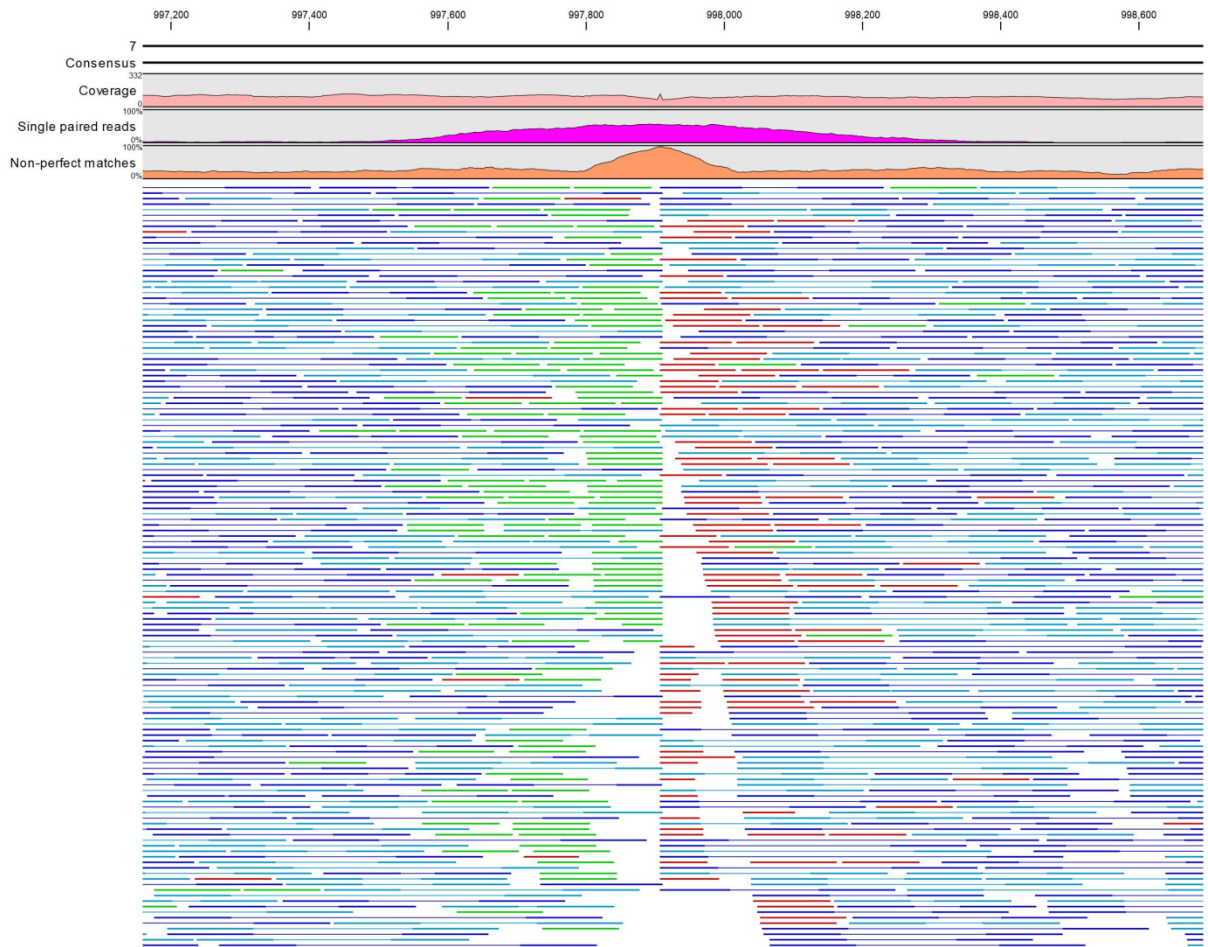
Supplementary Figure 1: Screening of *P. tricornutum* x1KO and complemented lines via PCR

A) Strains are characterized where the x1KO_1a/2 was complemented with an *LhcX1* gene under the control of the *LhcX1* promoter and terminator. B) Strains are characterized where x1KO was complemented with an *LhcX1* gene under the control of an *FcpA* promoter and terminator.

The primer combination LhcX1_all-fw and LhcX1_all-rev amplifies 102 bp at the 3'-end of the *LhcX1* gene which is still intact in the x1KO lines. The primer combination LhcX1_Wt-fw and LhcX1_Wt-rev amplifies a 347 bp fragment only in the Wt *LhcX1* gene but not in the x1KO lines, despite spanning the target sites for both TALEN pairs used to produce the x1KO lines. Using the primer combination LhcX1_comp-fw/LhcX1_Wt-rev yields a product of 262 bp, which is only present in the complemented x1KO+x1 lines, where an *LhcX1* gene without introns and with synonymous codon exchanges of the TALEN binding sites had been introduced under the control of the *LhcX1* promoter and terminator. The primer combination FcpA_LhcX1-fw and FcpA_LhcX1-rev amplifies a 479 bp fragment, which includes the whole *FcpA* promoter and the first part of the modified *LhcX1*. FcpA_LhcX1-rev primer is only able to bind to the first modified TALEN site of the complementation construct, while it cannot bind to the natural *LhcX1* gene. In B, the PCR for the five DNA samples on the left had been performed with the primer combination LhcX1_Wt-fw/rev, while for those five samples on the right the primer combination FcpA_LhcX1-fw/rev was used. The size of the corresponding marker bands is indicated in red. Source data are provided as a Source Data file.

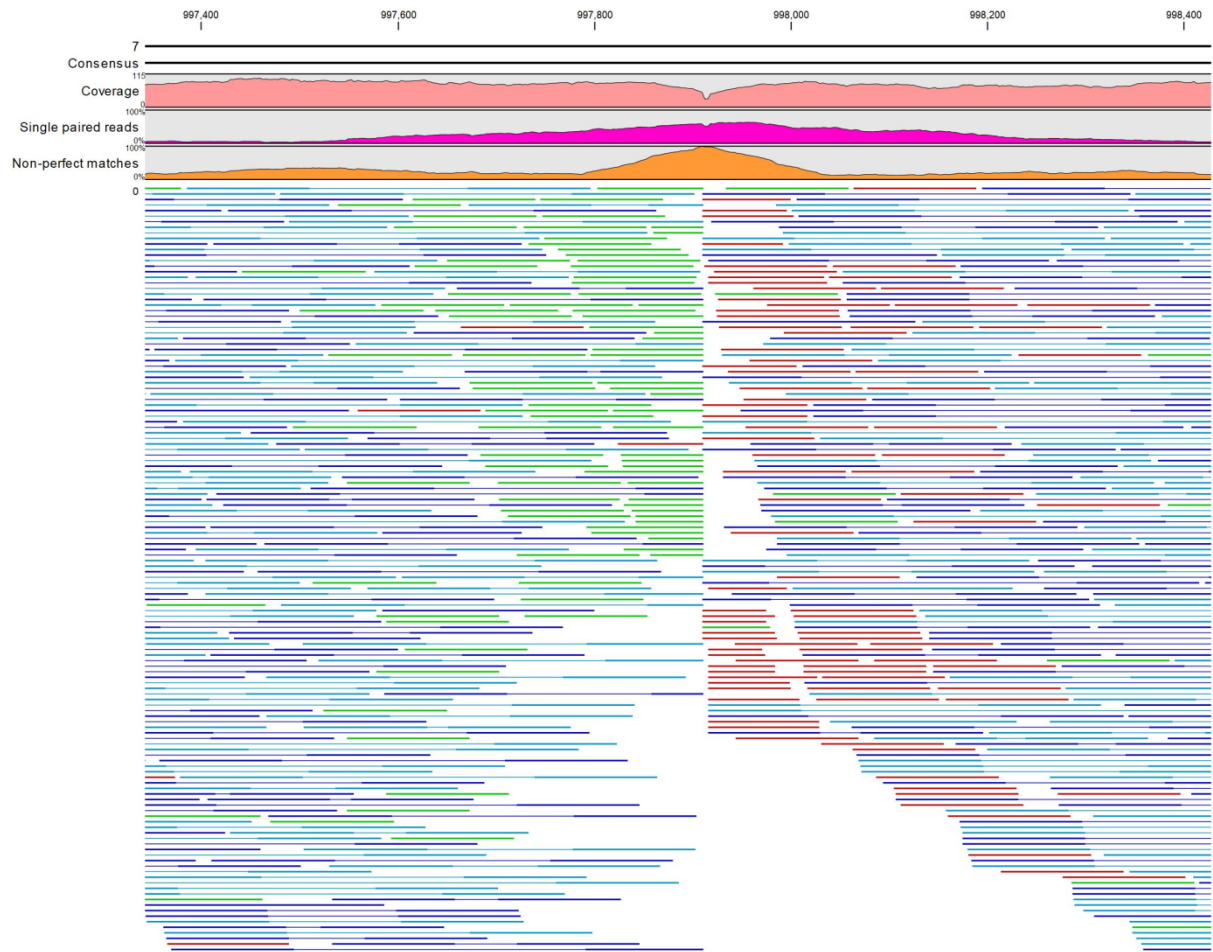
Supplementary Information

x1KO_1a



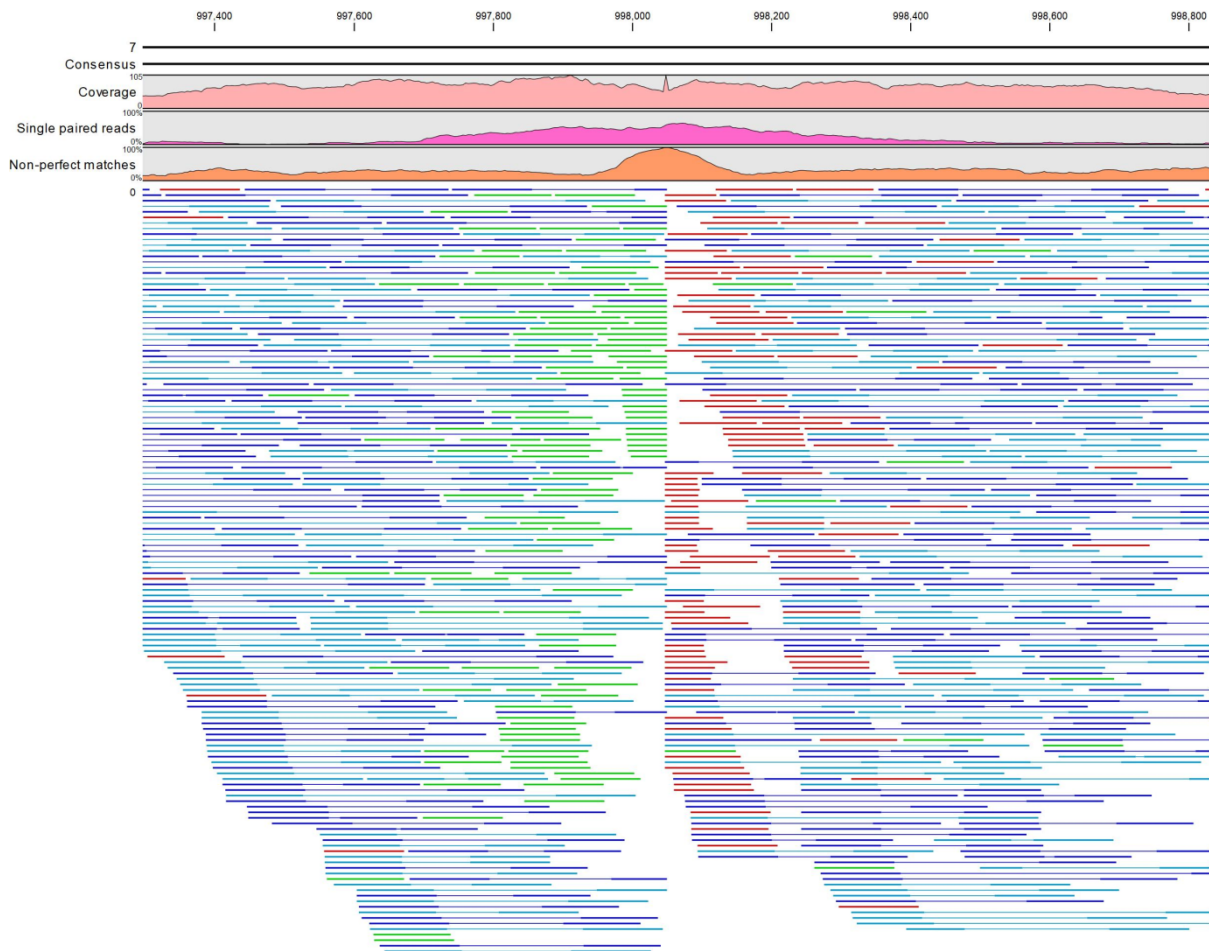
Supplementary Information

x1KO_1b



Supplementary Information

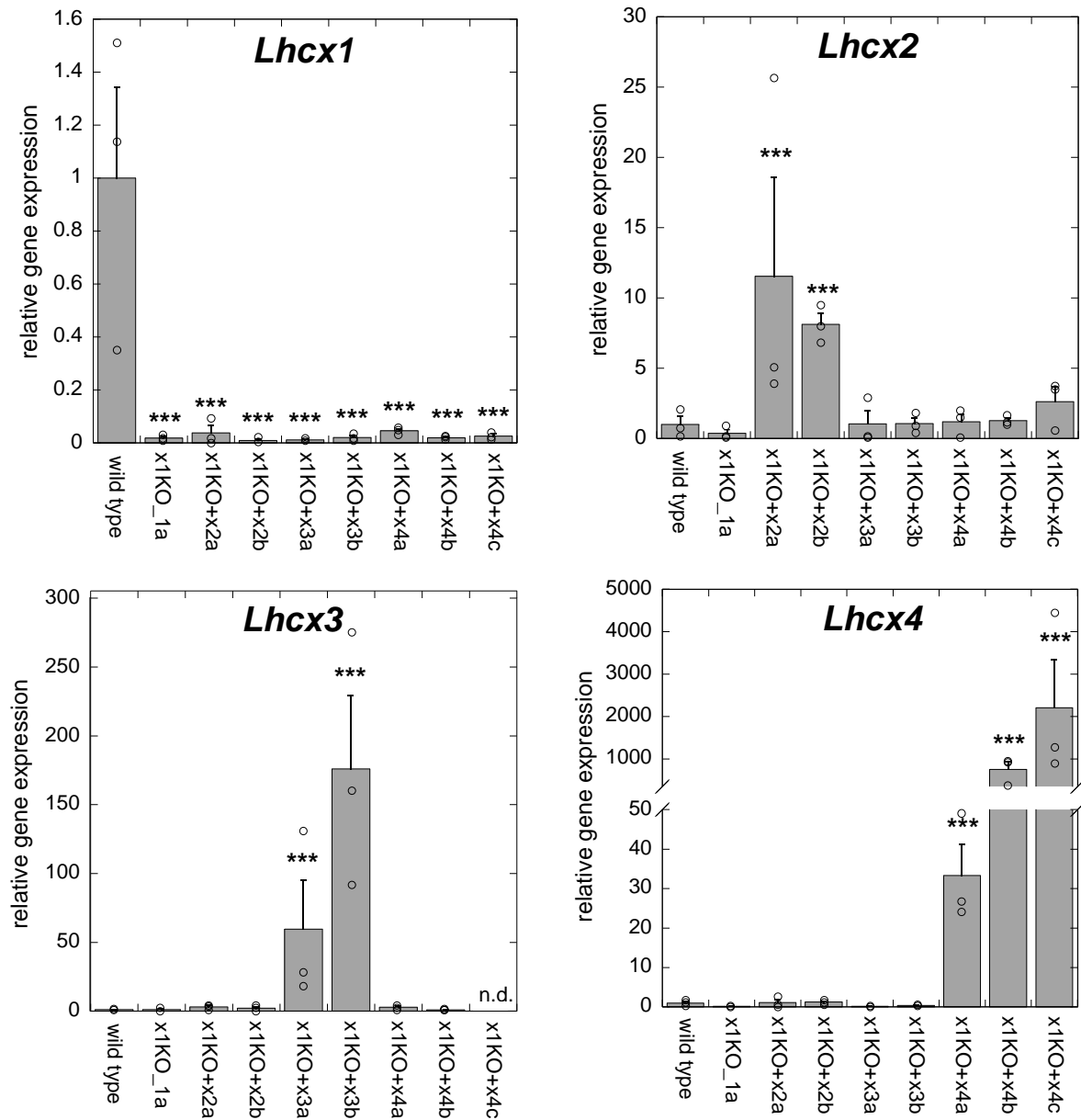
x1KO_2



Supplementary Figure 2: DNA sequencing results of the three *P. tricornutum* x1KO lines in the region of the *Lhcx1* gene

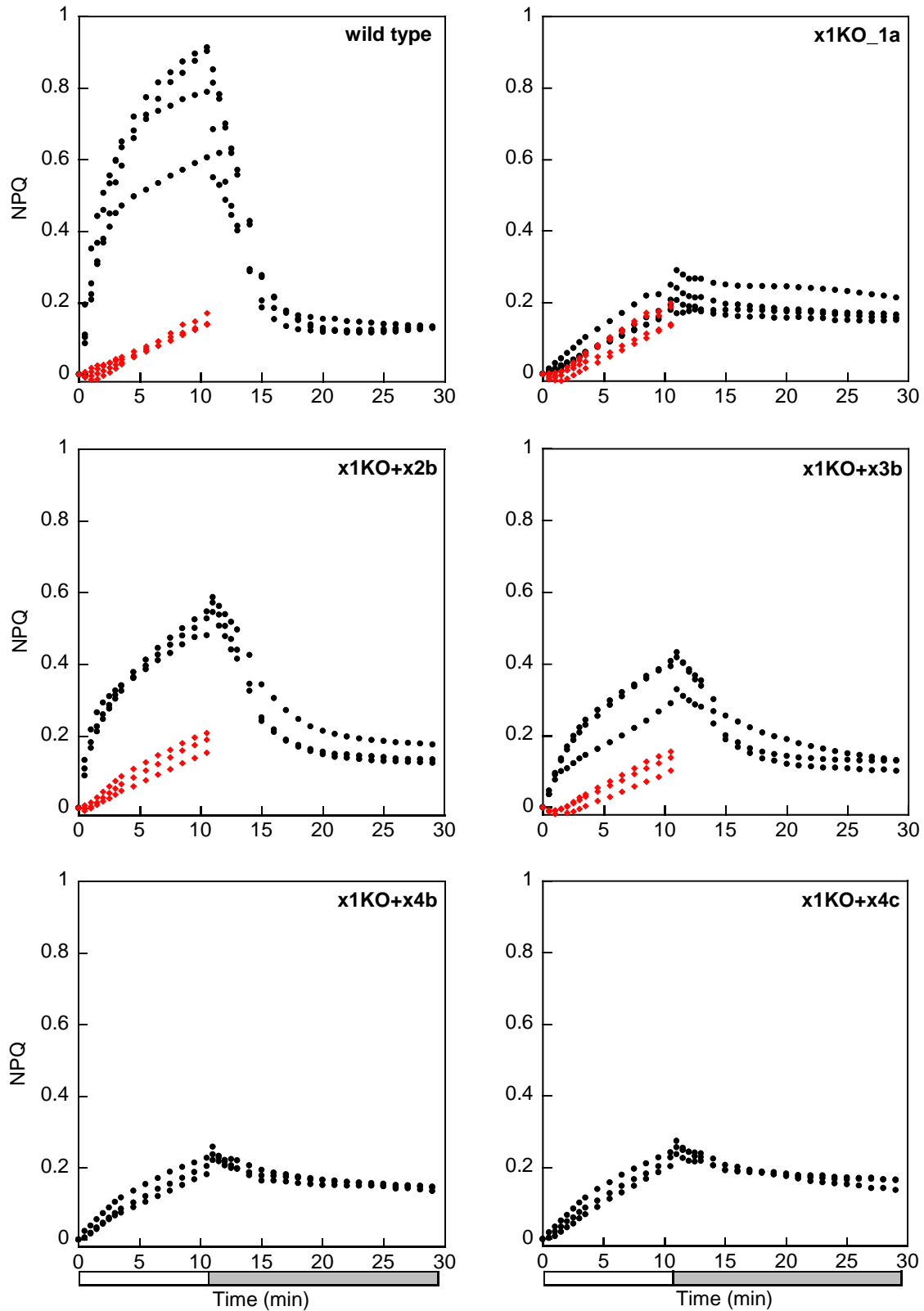
Illumina 125 bp paired end sequencing was performed. We obtained 23.4, 30.9, 20.1, and 12.6 million reads for the Wt4, x1KO_1a, x1KO_1b and x2KO strain, respectively. Graphical illustration was done using CLC Genomics (Qiagen, Germany). The blue/cyan bars indicate paired reads, with thicker lines depicting the sequenced reads and the thinner lines in between representing the region of the Wt4 (UTEX646) genome to which this part fits. Green bars indicate forward reads where the paired reverse read could not fit to the reference genome of Wt4. Red bars indicate reverse reads where the paired forward read could not be mapped to the reference genome. For all three x1KO lines, the region around the TALEN cutting site (~997900 on chromosome 7 for the TALEN pair 1, ~998050 for TALEN pair 2) was only covered by single reads and never any paired read spanning this region could be observed, likely due to large insertions. This is also supported by the orange labelled non-perfect matches chart, reaching 100% in this region in three x1KO lines. Raw sequencing reads can be obtained from the ENA depository under the accession code “PRJEB33825[<https://www.ebi.ac.uk/ena/data/view/PRJEB33825>]”.

Supplementary Information



Supplementary Figure 3: *Lhcx* expression of modified *P. tricornutum* strains cultivated under low light. Relative expression was normalized to expression of the *18s* gene, and then gene expression of the mutated strains was normalized to that of the wild type. Three biological replicates, each measured in technical triplicates, were analyzed and the mean + SE is indicated. Mean values of the biological replicates were tested for statistical significance using a randomization test performed by the REST algorithm. *** indicates $p < 0.001$ compared to wild type expression. n.d., not detected. Source data are provided as a Source Data file.

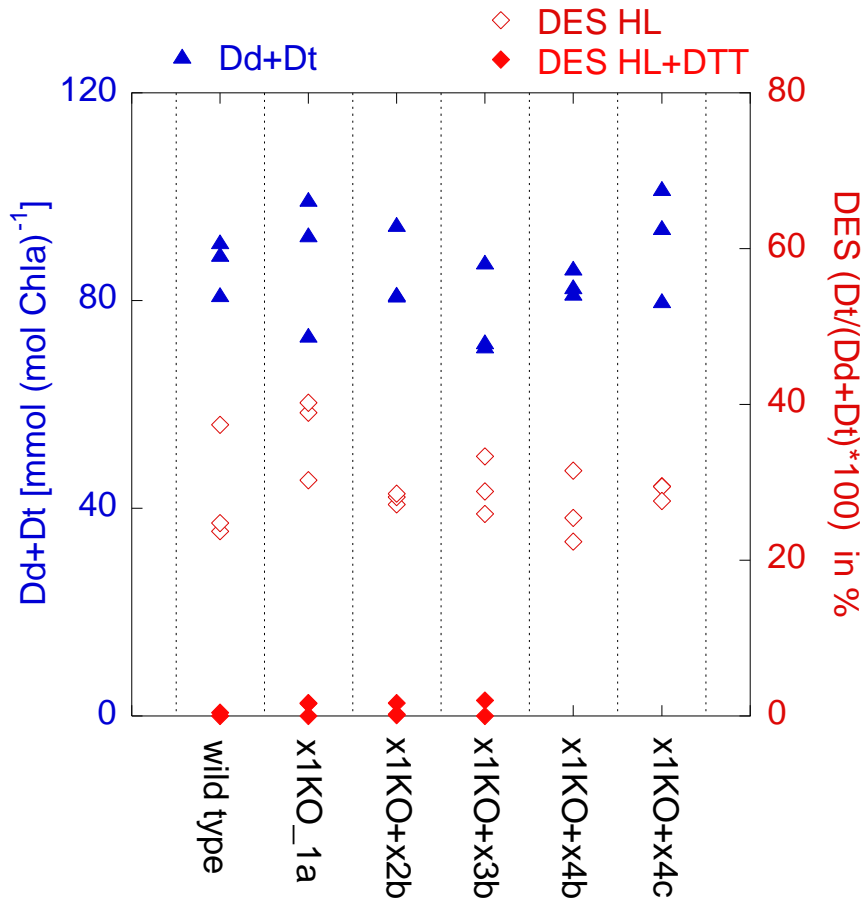
Supplementary Information



Supplementary Figure 4: NPQ capacity of wild type and mutants

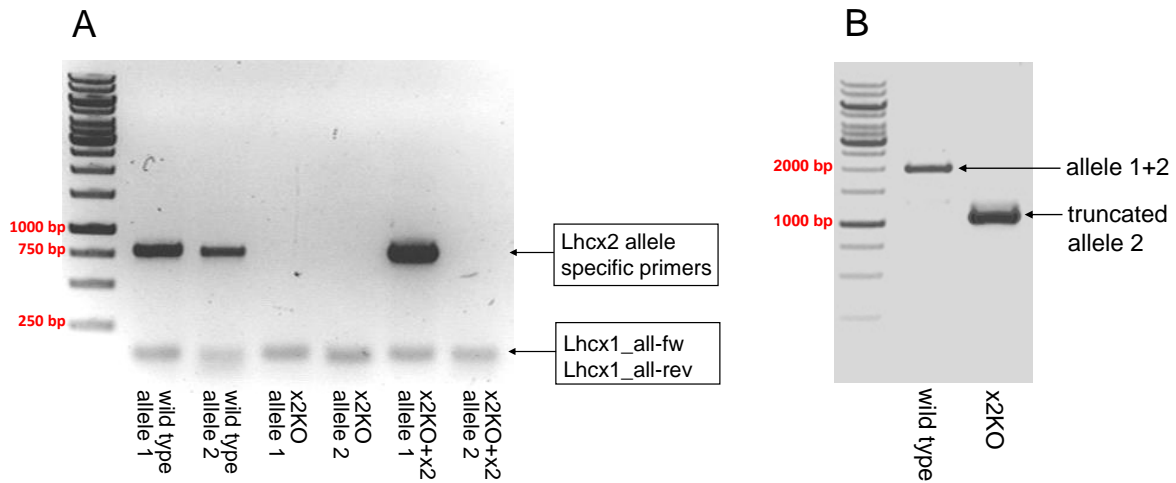
Wild type (4 biological replicates (BR)), x1KO_1a (4 BR), x1KO+x2b (3 BR), x1KO+x3b (3 BR), x1KO+x4b (3 BR) and x1KO+x4c (3 BR) cells were exposed to $1700 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (10 min, white bar), followed by 18 min of low light (grey bar). Strains were concentrated to $10 \text{ mg chlorophyll } a \text{ L}^{-1}$. Red points indicate samples which had been incubated with DTT prior to high light exposure in order to prevent diatoxanthin formation. Source data are provided as a Source Data file.

Supplementary Information



Supplementary Figure 5: Pool size of diadinoxanthin+diatoxanthin (Dd+Dt) and de-epoxidation state (DES)

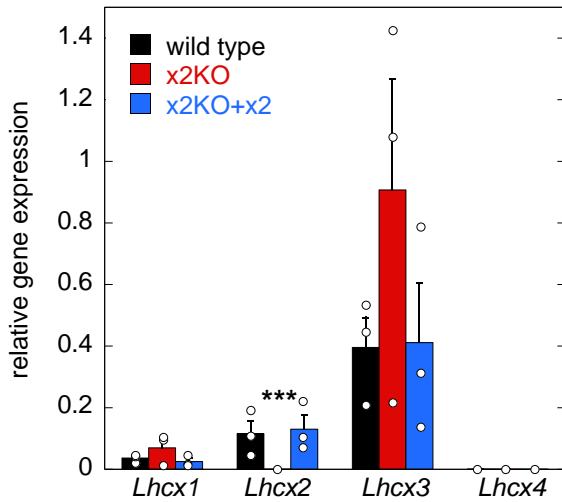
Cells were exposed to 10 min of 1700 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ without and with prior application of DTT. Three biological replicates each were measured. Source data are provided as a Source Data file.



Supplementary Figure 6: Characterization of *P. tricornutum* x2KO and complemented lines via PCR and gel electrophoresis

A) The two *Lhc2* alleles were amplified via allele specific primers (primer combinations *Lhc2*_allele1-fw/*Lhc2*_allele1-rev and *Lhc2*_allele2-fw/ *Lhc2*_allele2-rev, respectively). While in the wild type both alleles could be successfully amplified, they could not be detected in the x2KO strain. In the complemented x2KO+x2 line, the introduced allele 1 was detected. The primer combination *Lhc1*_all-fw and *Lhc1*_all-rev amplifies 102 bp at the 3'-end of the *Lhc1* gene, serving as a PCR positive control. B) PCR using the primer combination *Lhc2*_prom-fw and *Lhc2*_term-rev which spans the entire coding region of the *Lhc2* gene. A band could be amplified in the x2KO strain which, after Sanger sequencing, was identified as a truncated allele 2 of *Lhc2*, with a 750 bp deletion of the gene sequence and additional ~170 bp deletion of the terminator. In red, the size of the corresponding marker bands are indicated. Source data are provided as a Source Data file.

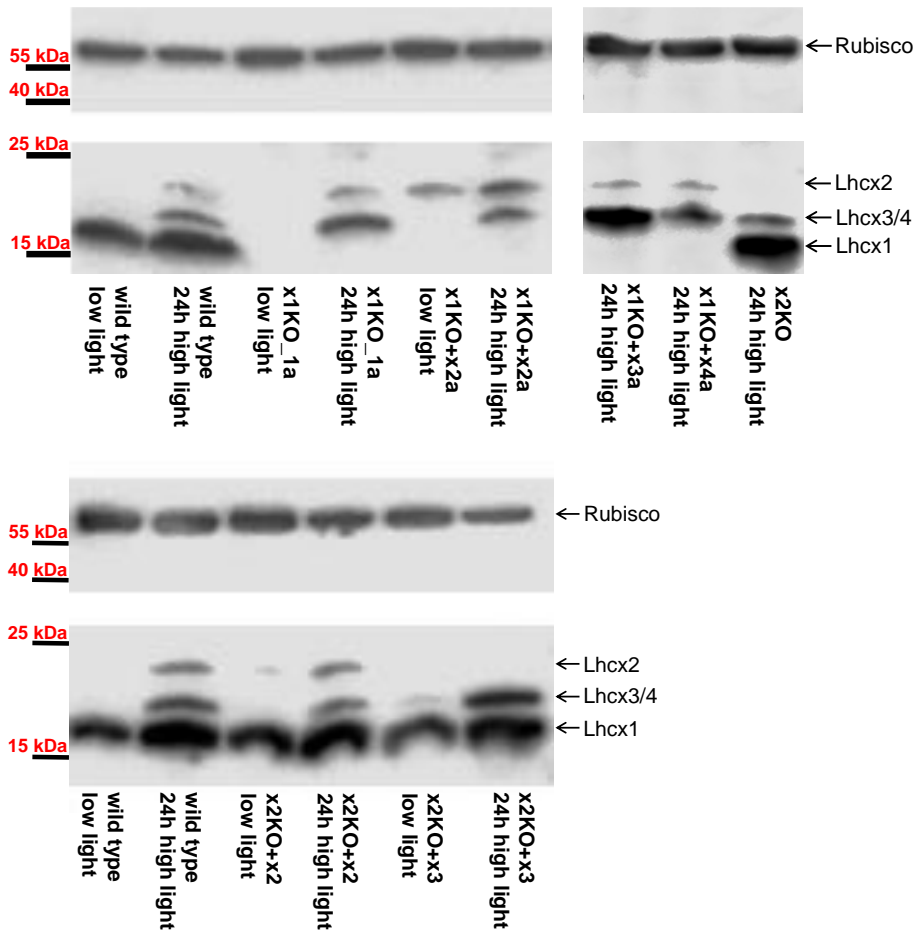
Supplementary Information



Supplementary Figure 7: *Lhcx* expression of wild type, x2KO and x2KO+x2 upon exposure to 2 hours of $\sim 700 \mu\text{mol photons m}^{-2} \text{s}^{-1}$

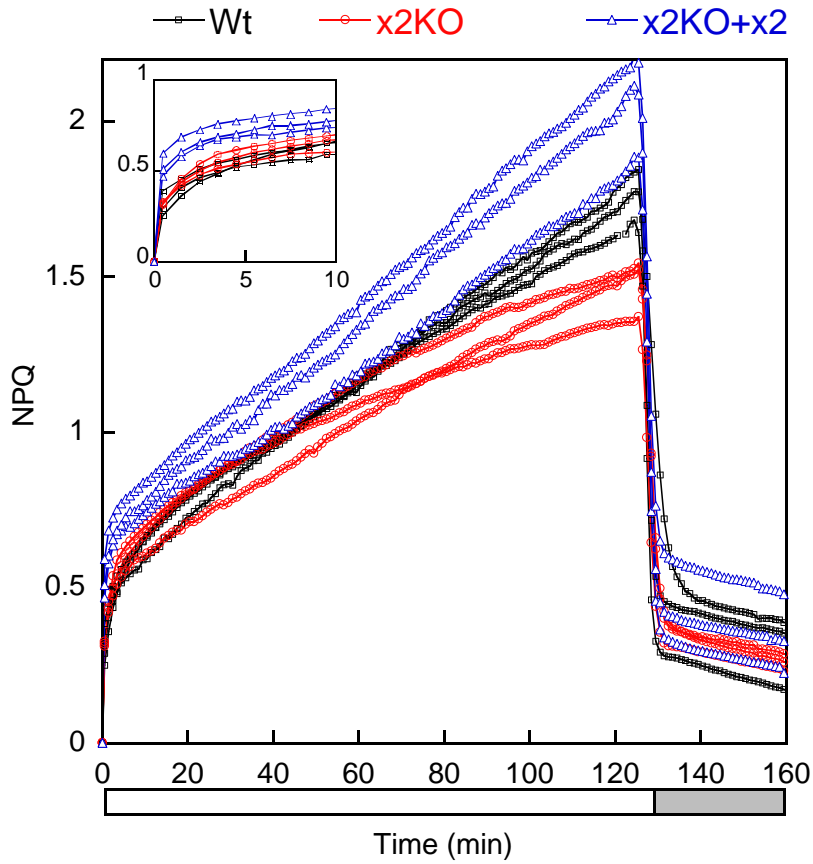
Relative expression was normalized to expression of *18s* gene. Three biological replicates, each measured in technical triplicates, were analyzed and tested for statistical significance using the REST algorithm. *** indicates $p < 0.001$ compared to wild type expression. SE is indicated. A different light intensity compared to Supplementary Figure 9 had been used, because we needed to use another sample setup, as the cuvette in the Dual-PAM did not allow harvesting enough cells for RNA isolation. Source data are provided as a Source Data file.

Supplementary Information



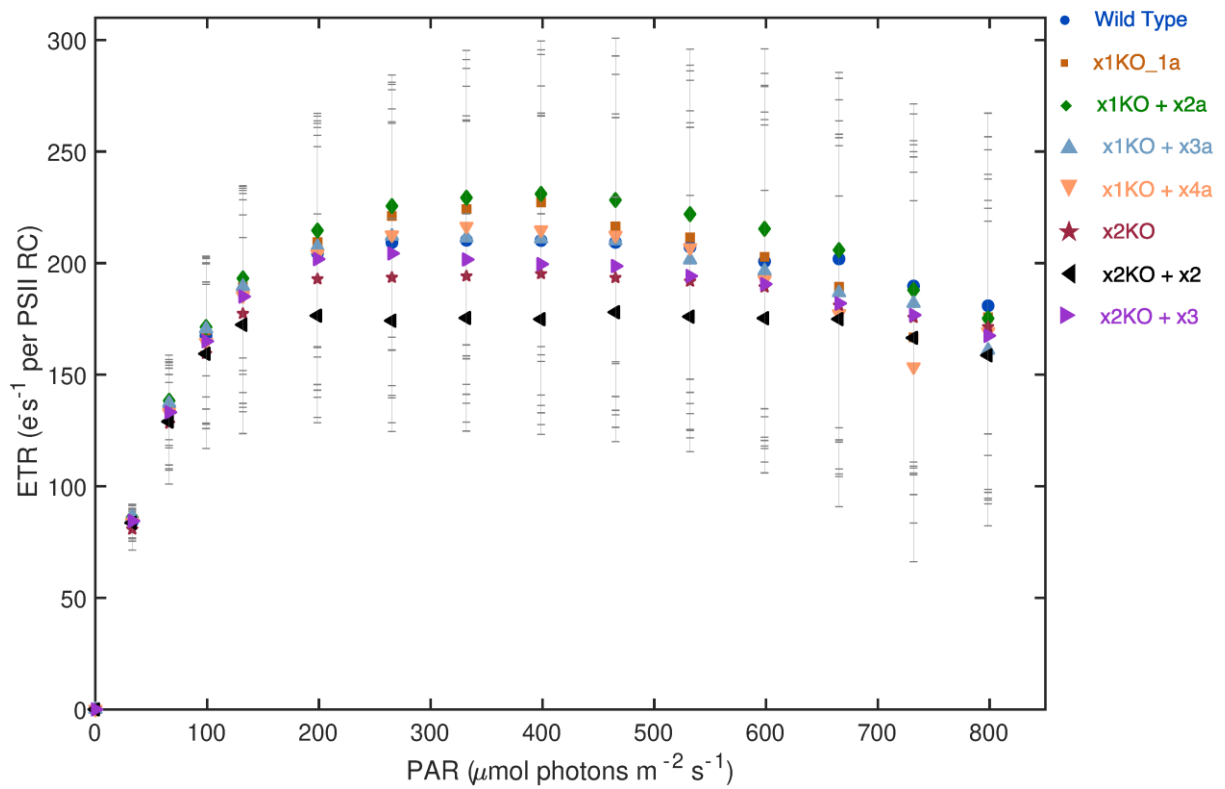
Supplementary Figure 8: Western blots of wild type and mutants from 24 h high light ($\sim 400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) grown cultures

The upper blots show the wild type, x1KO_1a, the supplemented x1_KO lines and the x2KO line, while the x2KO complemented with Lhc2 and supplemented with Lhc3 is shown in the lower blot. For comparison, some low light grown cultures were also analyzed. Three different blots (left, right and bottom) are shown. After blotting, the blots were cut and the upper half was incubated with a Rubisco antibody, while the lower half was incubated with the Lhcx antibody. Lhcx1 has the lowest, Lhcx2 the highest, and Lhcx3 and Lhcx4 have a molecular weight in between. Lhcx2 protein is not detectable under low light cultivation in the wild type, but only after prolonged high light cultivation. The x2KO line does not express Lhcx2 even after high light cultivation (upper right blot) as the x2KO+x3 line does not, too (lower blot). Source data are provided as a Source Data file.



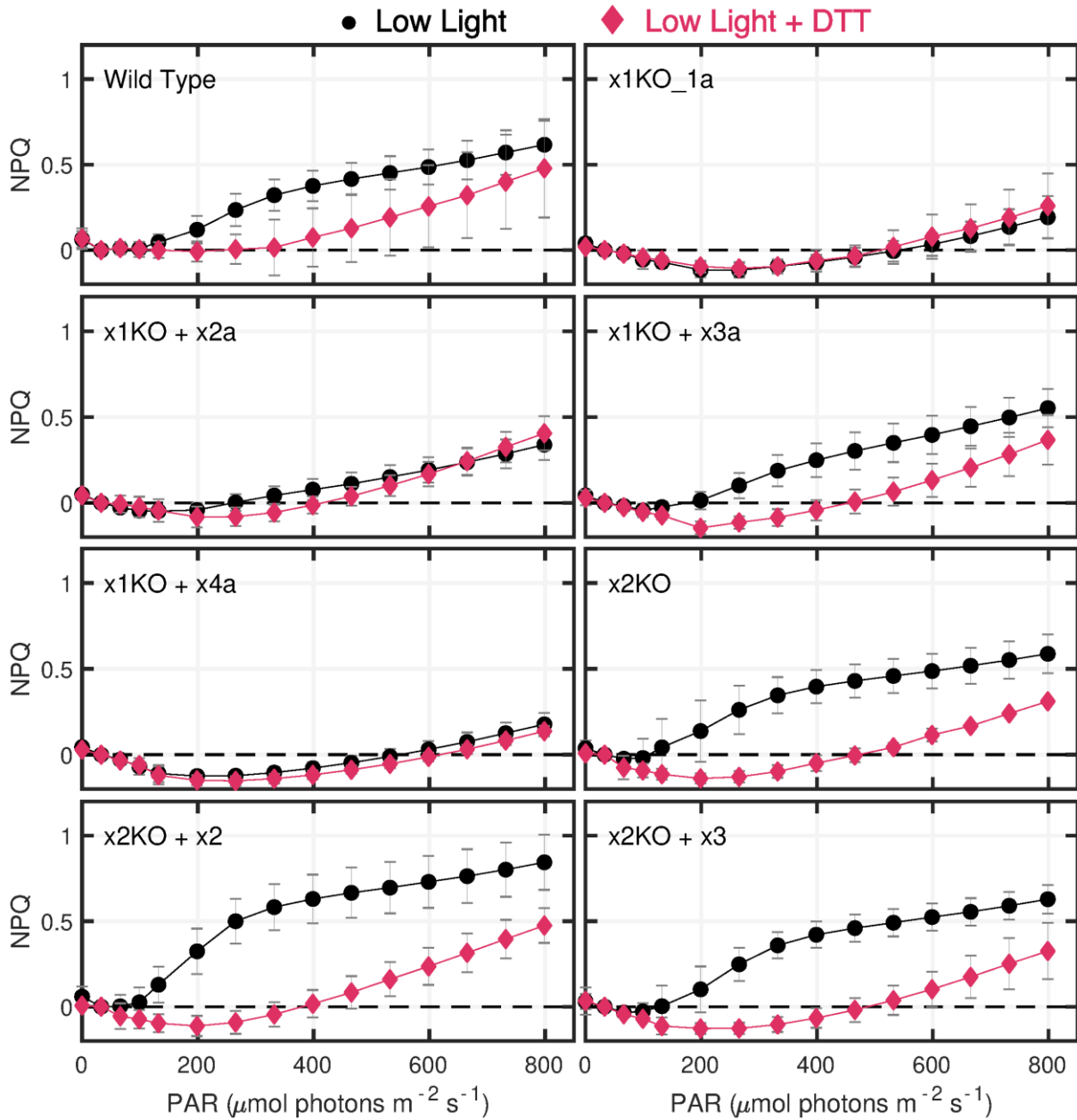
Supplementary Figure 9: NPQ capacity in wild type, x2KO and x2KO+x2 strains upon exposure to 130 min of $1700 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (white bar), followed by 30 min of low light recovery (grey bar). Three biological replicates are indicated. The inset shows the first 10 min of NPQ development in the three strains. Note that there are no major differences in NPQ capacity between the Wt and the x2KO line during the first hour of illumination, while during the second hour the Wt develops a higher NPQ capacity. The x2KO+x2 has a somewhat higher NPQ capacity right from the beginning. Source data are provided as a Source Data file.

Supplementary Information



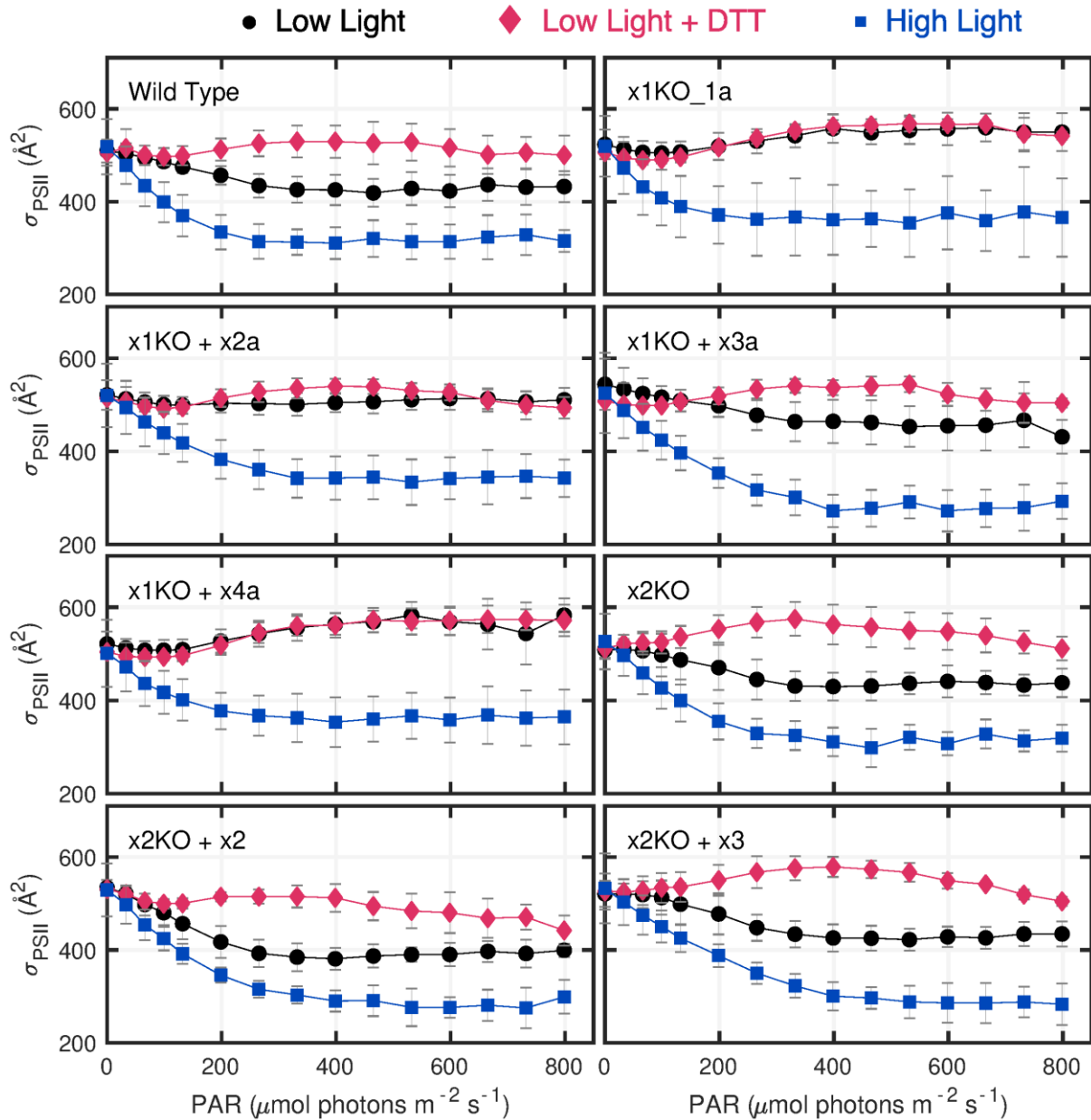
Supplementary Figure 10: Electron transport rates during rapid light curves of Wt and mutants grown under low light

Values are the mean of six biological replicates. SD is given. Source data are provided as a Source Data file.



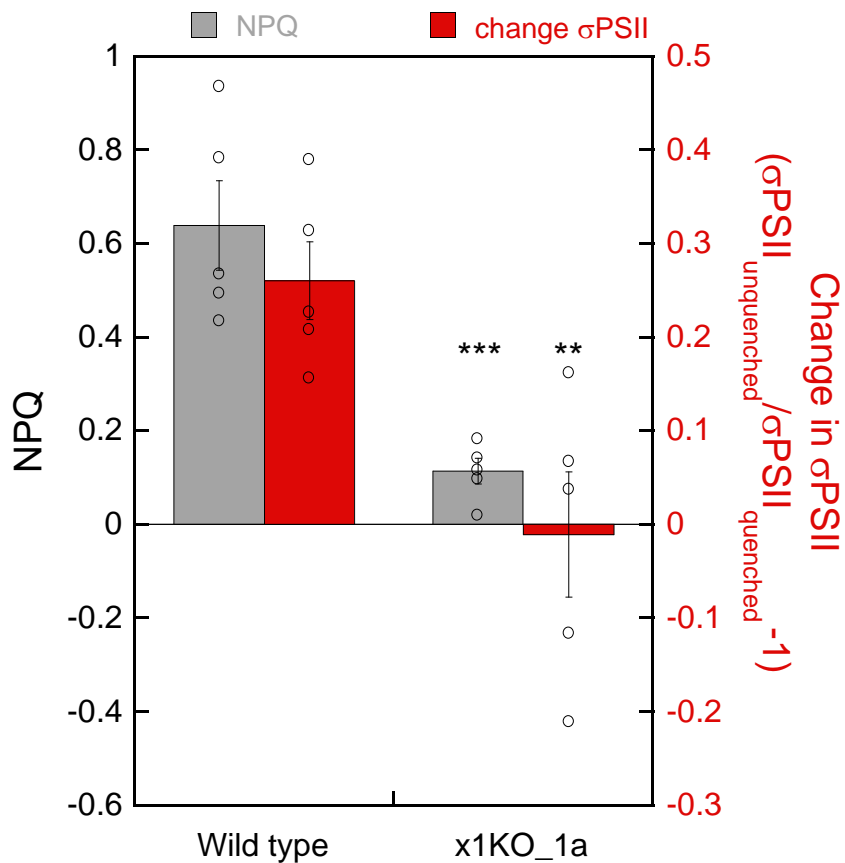
Supplementary Figure 11: NPQ development during rapid light curves of Wt and mutants grown under low light

Samples were exposed to increasing light intensities without or with prior incubation with DTT. Values are the mean of six (-DTT) or three (+DTT) biological replicates. SD is given. Dashed black line denotes zero NPQ. Source data are provided as a Source Data file.



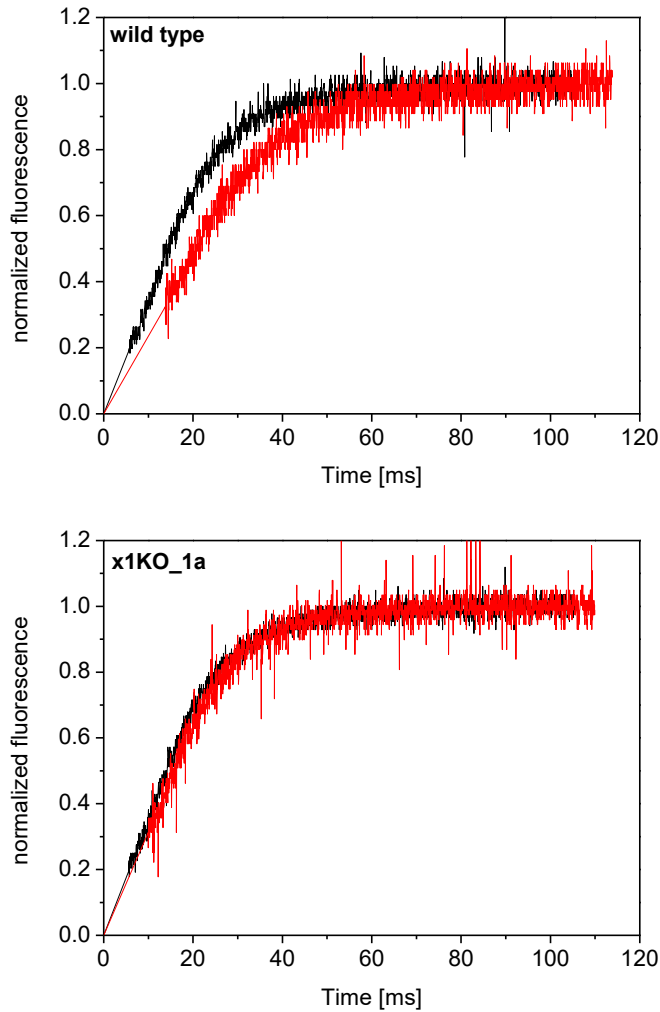
Supplementary Figure 12: Changes in σ_{PSII} during rapid light curves of Wt and mutants cultivated under low light and 24 h of high light ($\sim 400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$)

Low light grown samples were additionally incubated with DTT. Values are the mean of six (low light cultures), five (high light cultures) or three (low light + DTT) biological replicates. SD is given. Source data are provided as a Source Data file.



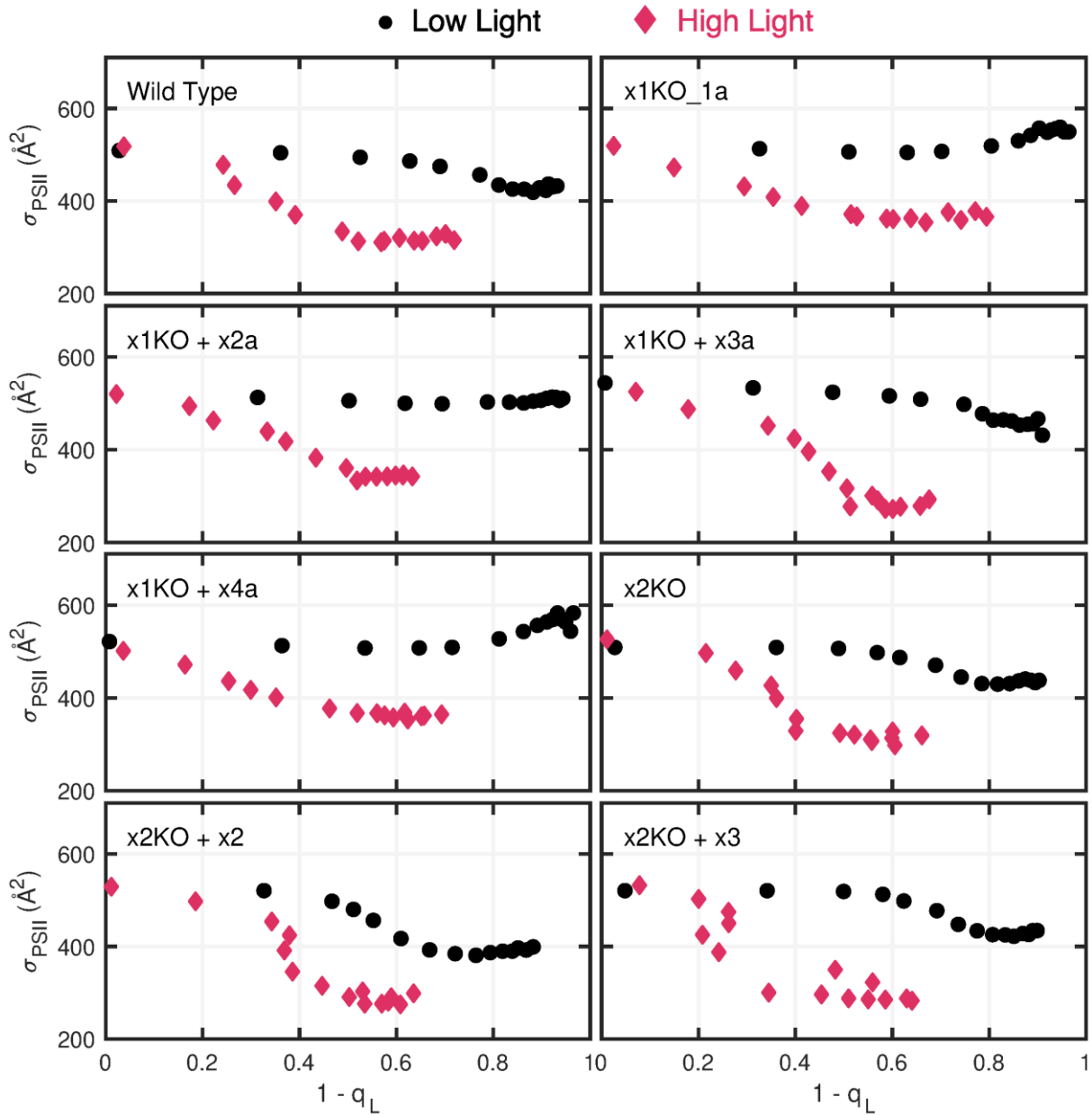
Supplementary Figure 13: Changes in NPQ and σ_{PSII} of wild type and x1KO cells upon three minutes of supra-optimal light exposure

The calculation of the change in σ_{PSII} here followed the approach of Tian et al. (2019)¹. In order to induce qE, cells were exposed for 3 min to 1700 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (blue+red light), after which light was switched off and DCMU was added 5 s later, followed by exposure to a weak red light flash. To record σ_{PSII} of unquenched cells, DCMU was added to dark acclimated cells, after which they were also exposed to a weak red light flash. The resulting fluorescence rises were normalized according to¹, the reciprocal of the areas above the normalized fluorescence rise curve were determined as the relative functional absorption cross sections, and the changes in functional absorption cross sections were determined - in analogy to the Stern Volmer equation of NPQ - as $\sigma_{PSII_{\text{unquenched}}}/\sigma_{PSII_{\text{quenched}}} - 1$. More methodological details can be found in Supplementary Figure 14. Values are the mean of five independent biological replicates and SE is given. Statistical significance between Wt and x1KO cells was tested using a two-tailed unpaired Student's t-test with 8 degrees of freedom. ** $p < 0.01$; *** $p < 0.001$. Source data are provided as a Source Data file.



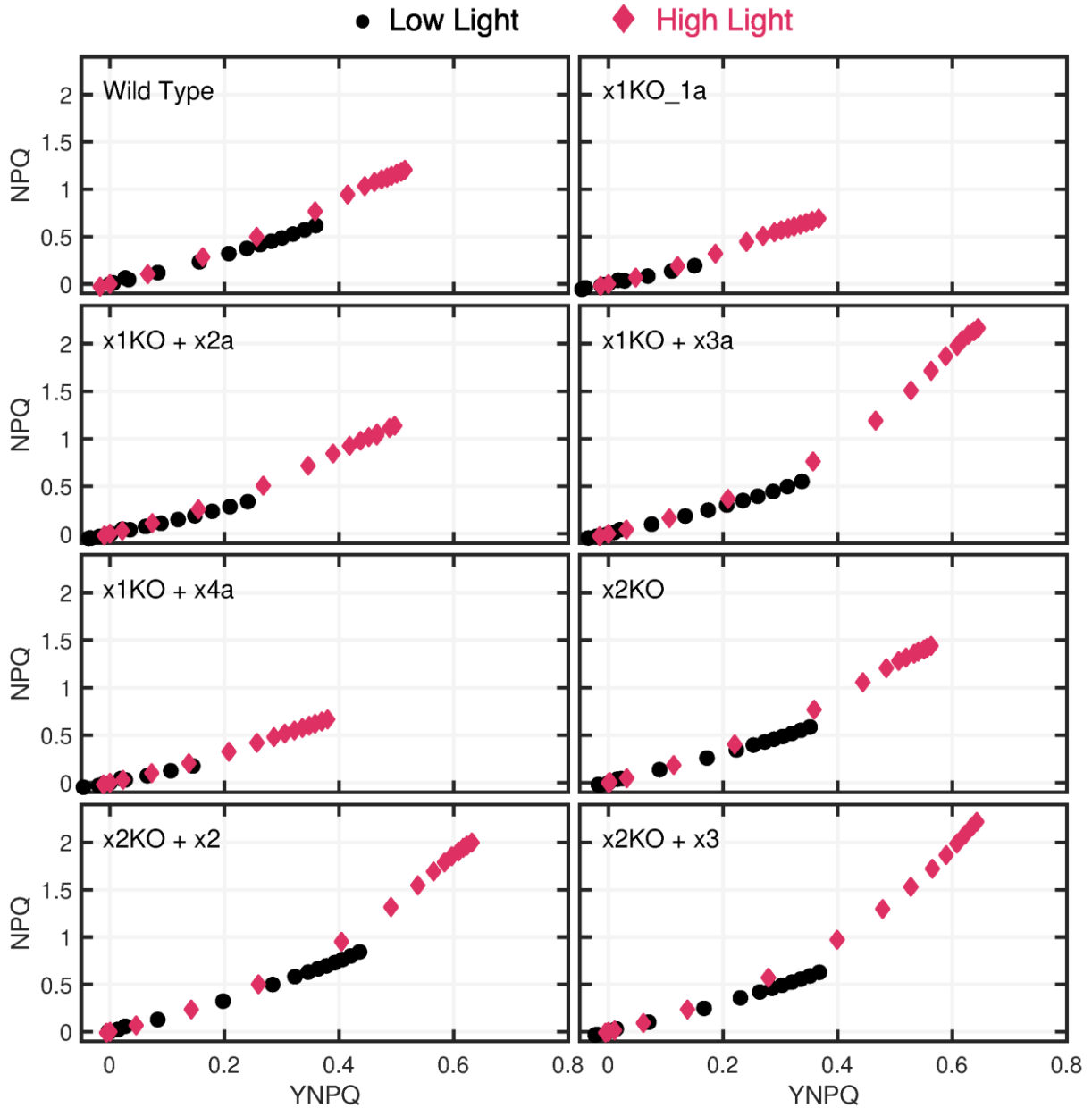
Supplementary Figure 14: Exemplary fluorescence induction traces of DCMU poisoned wild type and x1KO cells before and after supra-optimal light exposure

Cells were concentrated to 10 mg L^{-1} chlorophyll *a* and $10 \text{ }\mu\text{M}$ DCMU was added in the dark, in order to record the fluorescence induction curve of unquenched cells (black trace). In order to induce qE, cells were exposed to $1700 \text{ }\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (blue+red light) for 3 min, then shifted to darkness for 5 s after which DCMU was added (red trace). This time span was sufficient to allow a substantial re-oxidation of Q_A^- without a pronounced relaxation of qE. Fluorescence induction curves were recorded in the fast acquisition mode with a Dual-PAM (Walz, Germany) by applying a 300 ms red light flash with an intensity of $41 \text{ }\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, 15 s after DCMU application. F_m was reached roughly 60 – 100 ms after flash onset. Note that DCMU treatment strongly affects the F_o values and therefore has to be corrected, as described in Tian et al. (2019)¹. For the unquenched cells, F_o values were directly determined before treatment with DCMU, while for the quenched cells F_o' was calculated as $F_o' = F_o/[1+(F_o \cdot \text{NPQ}/F_m)]$ ¹. F_m and F_m' were obtained from averaging the fluorescence values recorded during the 90-100 ms time span of the red light flash after DCMU application. The first 500 measured points (first 15 ms) of each fluorescence induction trace were linearly fitted and extrapolated to the corresponding F_o/F_o' value using Origin (the straight line of the fluorescence trace). The fluorescence values were then normalized to values between 0 (F_o and F_o') and 1 (F_m and F_m') and to the positive time range. The area above the fluorescence induction curve is inversely proportional to the functional absorption cross section of PSII, thus the reciprocal of this area is taken as the functional absorption cross section. Consequently, the functional absorption cross section in Wt is lower after 3 min exposure to supra-optimal light, while it is unchanged in the x1KO strain.



Supplementary Figure 15: Changes in σ_{PSII} vs. $1 - q_L$ during rapid light curves in Wt and mutants grown under low light and 24 h of high light ($\sim 400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$)

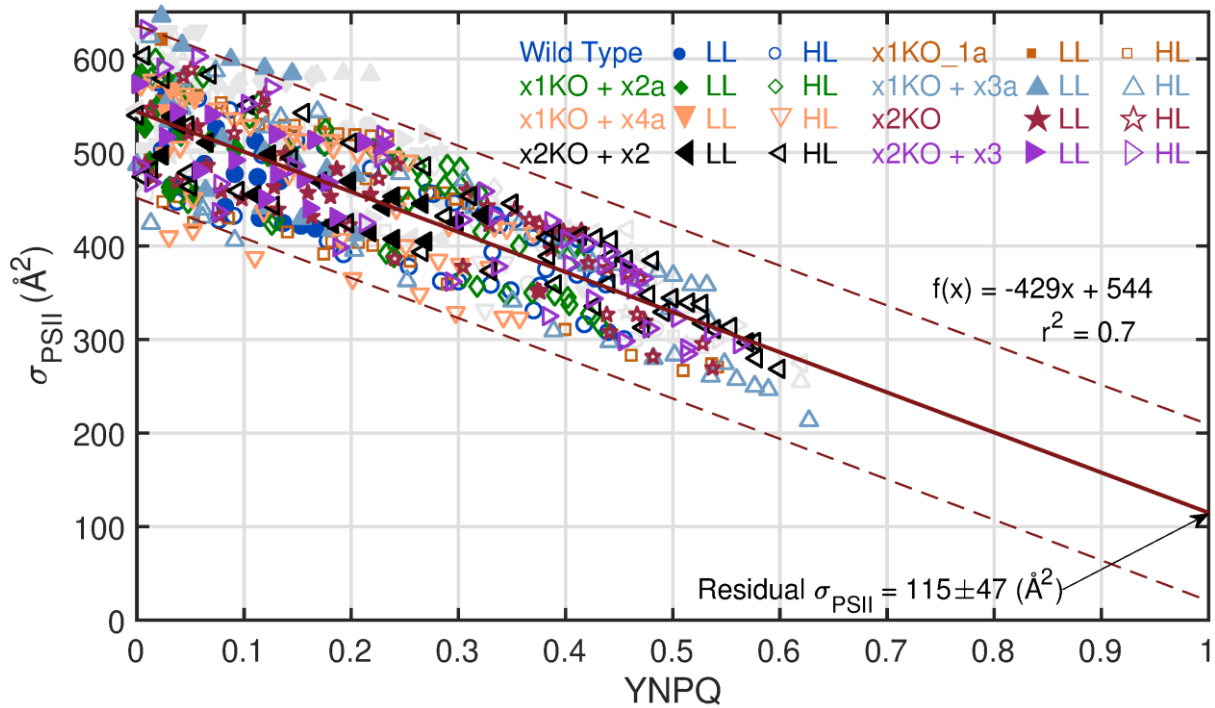
$1 - q_L$ is a proxy for the reduction state of the plastoquinone pool and thus a proxy for excitation pressure on PSII. Values are the mean of three (low light cultures) or five (high light cultures) biological replicates. Negative values were omitted. $1 - q_L$ was calculated as $1 - [(F_m' - F') / (F_m' - F_o')] * (F_o' / F')$. Source data are provided as a Source Data file.



Supplementary Figure 16: NPQ vs. Y(NPQ) during rapid light curves in Wt and mutants cultivated under low light and 24 h of high light ($\sim 400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$).

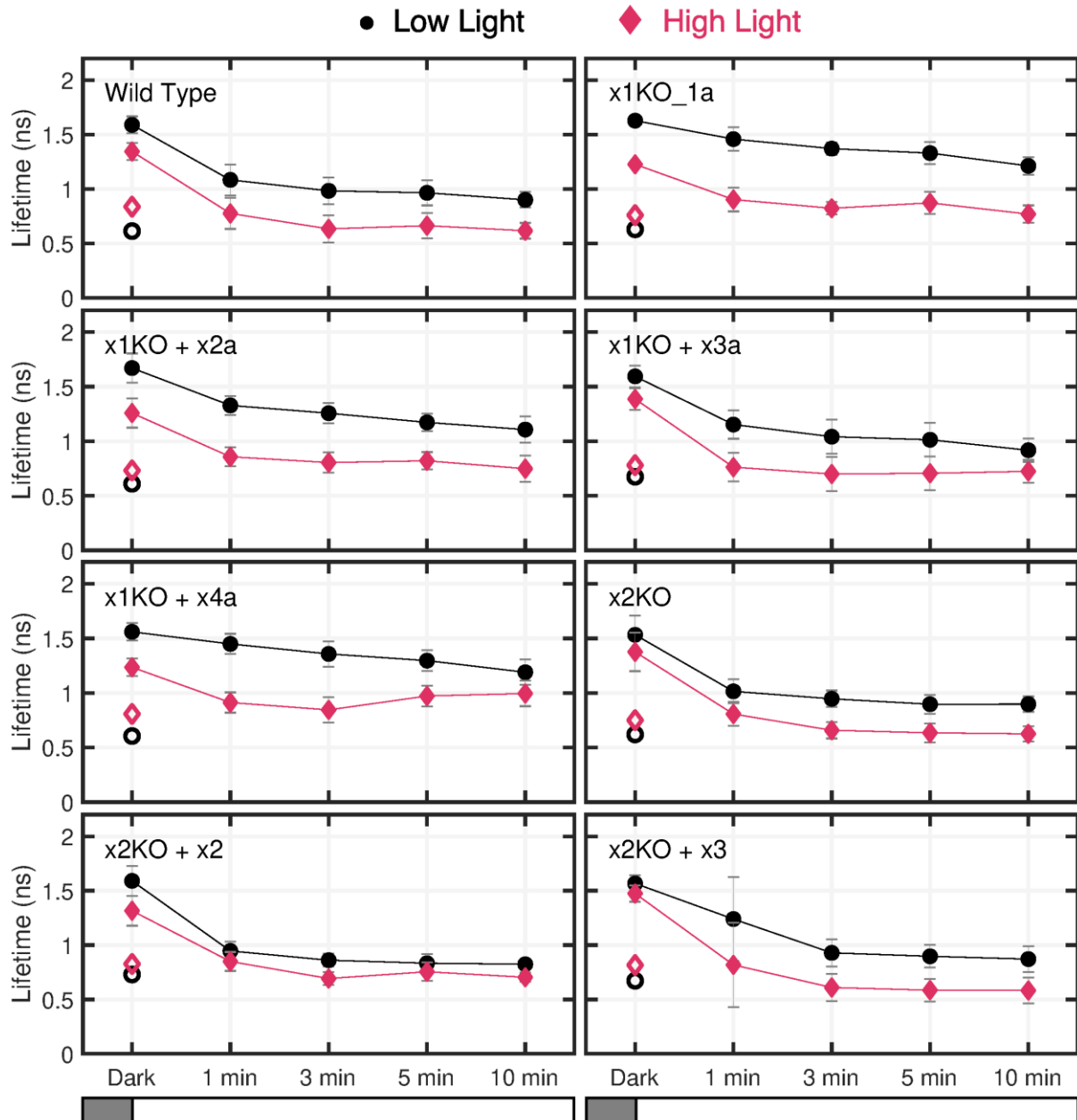
Plotted points represent the mean of six (low light cultures) or five (high light cultures) biological replicates. NPQ is calculated as $F_m/F_m' - 1$, and Y(NPQ) is calculated as $F/F_m' - F/F_m$. Source data are provided as a Source Data file.

Supplementary Information



Supplementary Figure 17: $\sigma_{PSII_{1s}}$ vs. $Y(NPQ)_{1s}$ from a second measurement at each light step, following 1 second of darkness, and the corresponding linear regression

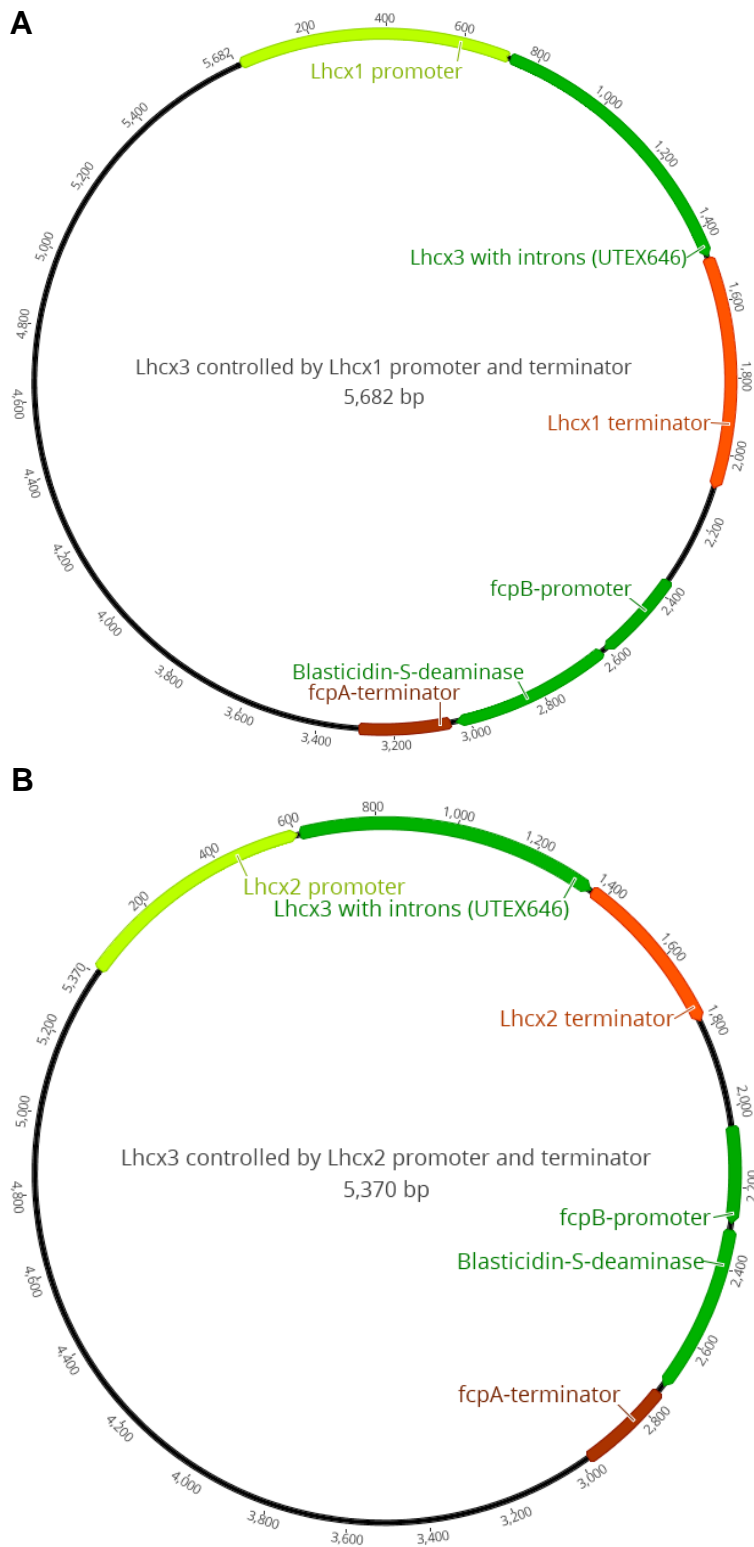
Individual data points of all measured strains cultivated both under low light (three biological replicates each), indicated by closed symbols, and high light (five biological replicates each), indicated by open symbols. Data points where an increase of $Y(NPQ)_{1s}$ did not lead to a further down-regulation of $\sigma_{PSII_{1s}}$ are not included in the regression calculation, but indicated in light grey. This was determined by calculating the percent change of each $\sigma_{PSII_{1s}}$ from its previous light step. If $\sigma_{PSII_{1s}}$ decreased by less than 5% of the total measured decrease for that curve while $Y(NPQ)_{1s}$ increased, it was omitted. Data points above $600 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and data points with a negative $Y(NPQ)_{1s}$ were also removed. A linear regression line, the 95% confidence interval, the regression equation and the r^2 are indicated. Source data are provided as a Source Data file.



Supplementary Figure 18: Average fluorescence lifetimes during 10 min of supra-optimal light exposure in low and high light cultivated Wt and mutant strains

Open symbols in the dark represent measurements under F_0 conditions. Subsequently, filled symbols represent data collected under F_m conditions. Plotted points represent the mean of six (low light cultures) or five (high light cultures) biological replicates. SD is given. Source data are provided as a Source Data file.

Supplementary Information



Supplementary Figure 19: Exemplary transformation vectors for the *P. tricornutum* x1KO_1a strain (A) and the x2KO strain with *Lhc3* (B)

The vectors contain a Blastidicin-S resistance cassette. In A) the *Lhc3* gene is under control of the *Lhc1* promoter and terminator, while in B) it is controlled via the *Lhc2* promoter and terminator.

Supplementary Information

GTTCTTCGTC/AAACACACT/CGCAAAG/AAGATA/TTACGTCCAATTCCAA/GCCCACTACGTACA
CCATGAAATTATCCTTGGCTATCCTTGGCCTTTGCGCCAGCACTAGTGCCGCTTTCGCTCC
TTCTGTTTCCCAGAGGACGTCTGTCTCTCTCCGAGAATCATTGGACCCCACGGAC/ATCCA
TGTCGGAAGTGGAAGGCGCCGTGAAAGACGCGGCTCCCAAAGTCTCCGACCCTTTCGACA
GCCCTCGTGATCTTGCCGGAGTCGTGCTCTACC GGCTTTTTTCGATCCGGCAGGCTTCGC
TGCCCCGAGCCGATGCCGGA/TACCATGAAGCGTTACCGGGAAGCGGAAGTTACTCACGGA
CGTGTGGGCATGATGGCCGTTGTGCGGCTTCTTTCGCGGGCGAAGCCGTTCGAGGGATCGTCC/
GTTTCTCTTTGACTCGCAAGTCAGCGGACCCGCCATTACTCACCTCAACCAGATTCCCTTCC
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GCGTTCTTTTCTTTCCATTTCGTTTTCCAGGTTGAACCCGAGAACGTCCCACCGGGCAAGC
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GCCTTCCGACGCGCAGGCTCTCAAATCGATCCAGACCAA/GGAACTGCAGAGTAAGTCA
TTTGCTGTTGT/CTGCTGTTACTGTCGTCCT/ATGGTACTATCGTGTTTACAGTTAGTTCACT
GCGTCAGAGTGTACCGTGTCACTGTTAATCCCACCAAATAACCGATTGAATATAACT
CGTTTTCTTTGGCTAGACGGACGTCTCGCCATGTTGGCGGCGGCTGGGTGCATGGCTCAG
GAATTGGCCAACGGAAAGGGCATTCTCGAAAACCTTGGT/GCTCTAAGGAATATA/GACGTT
CCAGTGTTTTGAACACTAGGCGCGATGGACAACAGAACGATTAGGCTAGCAACGAAAAGAAGCA
AAATACAGTAGAAATCAAGTAATTCTCCCACTTTGTT/GCAA.

Supplementary Figure 20: *Lhcx2* gene sequence of *P. tricornutum* strain 4

Bases before and after “/”, underlined and in bold, indicate alternating bases in the two alleles (in total 16, the first base always referring to allele 1), and the untranslated region is indicated in italics. Green letters indicate introns, while blue letters indicate the TALEN binding site.

Supplementary Information

WT	ATGAAATTATCCTTGGCTATCCTTGGCCTTTGCGCCAGCACTAGTGCCGCTTTCGCTCCT	60
x2KO	ATGAAATTATCCTTGGCTATCCTTGGCCTTTGCGCCAGCACTAGTGCCGCTTTCGCTCCT *****	60
WT	TCTGTTTCCCAGAGGACGTCTGTCTCTCTCCGAGAATCATTGGACCCACGGAATCCATG	120
x2KO	TCTGTTTCCCAGAGGACGTCTGTCTCTCTCCGAGAATCATTGGACCCACGGAATCCATG *****	120
WT	TCGGAAGTGAAGGCCCGTGAAAGACGCGGCTCCCAAAGTCTCCGACCCTTTCGACAGC	180
x2KO	TCGGAAGTGAAGGCCCGTGAAAGACGCGGCTCCCAAAGTCTCCGACCCTTTCGACAGC *****	180
WT	CCTCGTGATCTTGCCGGAGTCGTCTGCTCCTACCGCTTTT TCGATCCGGCAGGCTTCGCT	240
x2KO	CCTCGTGATCTTGCCGGAGTCGTCTGCTCCTACCGCTTTT TCGATCCGGCAGGCTTCGCT *****	240
WT	GCCCGAGCCGATGCCGGT ACCATGAAGCGTTACCGGGA AGCGGAAGTTACTCACGGACGT	300
x2KO	GCCCGA----- *****	246
WT	GTGGGCATGATGGCCGTTGTCTGGCTTTCTTGCGGGCGAAGCCGTCGAGGGATCGTCGTTT	360
x2KO	-----	246
WT	CTCTTTGACTCGCAAGTCAGCGGACCCGCCATTACTCACCTCAACCAGATTCTTCCATC	420
x2KO	-----	246
WT	TTTTGGATTCTCCTCACGGTGGGCATTGGTGCTTCCGAAATCACGCGGGCTCAAATTGGT	480
x2KO	-----	246
WT	TGGGTACGTATCGGGAGTTGTTGGTTCCAACAGTTTCGCTTTGTCCGTCTCTCACGCGTT	540
x2KO	-----	246
WT	CTTTTCCTTTCCATTTCGTTTTCCAGGTTGAACCCGAGAACGTCCCACCGGGCAAGCCGGG	600
x2KO	-----	246
WT	TCTCTCCGCGACGATTACGTCCCGGGTGACATTGGCTTTGATCCTCTCGGCTTGAAGCC	660
x2KO	-----	246
WT	TTCCGACGCGCAGGCTCTCAAATCGATCCAGACCAAGGAACTGCAGAGTAAGTCATTTGC	720
x2KO	-----	246
WT	TGTTGCTGCTGTTACTGTCTCCATGGTACTATCGTGTTTACAGTTAGTTCACTGCGTCA	780
x2KO	-----	246
WT	GAGTGTAACCGTGTCTACTGTTAATCCACCAAACCTAACCGATTGAATATAACTCGTTTT	840
x2KO	-----	246
WT	CTTTGGCTAGACGGACGTCTCGCCATGTTGGCGGCGGCTGGGTGCATGGCTCAGGAATTG	900
x2KO	-----	246
WT	GCCAACGAAAGGGCATTCTCGAAAACCTTGGGCTCTAAGGAATATGACGTTCCAGTGTT	960
x2KO	-----	246
WT	TTGAACACTAGGCGCGATGGACAACAGAACGATTAG	996
x2KO	-----	246

Supplementary Figure 21: Alignment of *Lhcx2* allele 2 amplified from wild type and the x2KO strain

In the x2KO strain, the *Lhcx2* gene is deleted from base pair 247 up to the end of the gene. The same deletion can also be found in the x2KO+x2 line. The binding sites for the forward and reverse TALEN construct used to induce the knockout in the *Lhcx2* gene are indicated in blue and bold.

Supplementary Information

Supplementary Table 1: TALENs used to target *Lhcx1* and *Lhcx2*

Gene	RVD forward TALEN	Backbone vector forward TALEN	RVD reverse TALEN	Backbone vector reverse TALEN	+ strand sequence (in minuscule: spacer sequence)
<i>Lhcx1</i>	HD NN HD NG NN HD HD NI HD HD NI NG HD HD NG NG NN HD NG	pM9_fcpA_NG (#90420)	NG NN NG NG NG NN NN NN HD HD NN NN NI NN HD NN NI NI NI	pM9_NR_NI (#90422)	CGCTGCCACCATCCTTGCT cttatcggctctgcccgtgc TTTCGCTCCGGCCCAAACA
<i>Lhcx1</i>	NI HD NI HD NN NI HD HD NN NG NN NN NG HD HD NG NG HD NG	pM9_fcpA_NG (#90420)	NN NN HD NG HD HD NI NI HD NG NI NN NI NG HD NG NG HD NG	pM9_NR_NG (#90423)	ACACGACCGTGGTCCTTCT tacataaacctgcagaa AGAAGATCTAGTTGGAGCCA
<i>Lhcx2</i>	HD NH NI NG HD HD NH NH HD NI NH NH HD NG NG HD NH HD NG	pM9_fcpA_NG (#90420)	HD HD HD NH NH NG NI NI HD NH HD NG NG HD NI NG NH NH NG	pM9_NR_NG (#90423)	CGATCCGGCAGGCTTCGCT gcccgagccgatgccggg ACCATGAAGCGTTACCGGG

Indicated are the repeat variable di-residues (single letter amino acid code) characteristic for the respective TALE monomers, the used backbone vectors (available at Addgene with the indicated vector code) and the targeted DNA sequence.

Supplementary Information

Supplementary Table 2: Cloned DNA sequences in order to complement the x1KO and x2KO lines

Cloned DNA sequence	Bases
<i>Lhcx1</i> promoter, used for <i>Lhcx2</i> , <i>Lhcx3</i> and <i>Lhcx4</i>	CCTTAACTGGGTCAGTACAGCGTGTGATTGTGAACAAAACCAATGGCCAGACGGATTTCGCAAGTTCACGACAGCTTTCGACGGTGAGAATGATGGTATCGCTTCGGGTCGGGTCGGTACTTTGCGGGACTGGCAACGTCCGCAGAGG GACTCACGGTCACTTCACTGTCTGTATCGACTGACGACTGACTCGTCTGATTCACTGTCAGGTTTCTGATCTAAGT TTATCAAATGCTACTACTTGGAGGTAGTCGTATCATTGAACTGCAACTTACAAAATCGAAGGTCCTTGCAACAT TTCTGACATGGGTAATTTTCATAGGATGTTACAATGTCGAGCTTGTATCACTCGGATACAACAGACCACCTTTAGGA AAGGGATTTTTTTTGTGCGAAAATAGGATGACATCAACAGGAAGTACGTTTCATAGCCCTTTATTTATAAATTGGAT GGGTCAATCCGACGTACGCATGTGTCTTTTGACCTTGTATCCATAAACCAGATTGACCCGTCACGTGCAATACG TCACGCCAATGAAAGACCCCGGATAAAGGGCCTAAAATTCAGTCTCGTGCAAAAACGCAGGATGATGCACCT GAACAGGTGACGTGTTCCGCGCAGTCGAATGAGTCCGAAGCCATCAGAGGCGAATTTATTTGCAACTGTTTGA CCCCATAAAACATTCTAGGAGATTTTGATAAAACACTTGAAAATAACG
<i>Lhcx1</i> terminator	AGAGTGCATCCATCCAAAACACTGGAGGATGCGTAGTCACATGCACAGAGTTAACAACCAATCCCTTTTCAAGCG AAGCTCGCATGGTCTGCCGAACAAAATTTAGTTGATGCCACCACATAGAACGCTGTAACATAGCCTAAAGCGC AGGTTTTATAATCAAATGTATGGAAGCAACTTTGCTCTTTGAAGCGGCTTTCTCAACGTTTGGAGATAGACAA TATCTTGGAGATTTGTGCGTCTGGGTTGATTGGTTGCAATCCTTGTATAGCATTACCAATTGTGCCGAAGTCG ACCGAACTGTTGTTCCGATTATACCCATGGGTGCGATACAAAAGAGAAATCCATGGAACAAGACTTTCGAAA ATTCGTGGTAGTCCGAAGGAACGCACACTGACTGTGACTGTGACTGACTGTCATAGCTAGCACGCACACTGACTGTG ACTTGTGACTGACTGTCATAACTAGCTAAAACAGGCATCACAGTCAGGCGGTTGACTGATAGGGCAGGCAAAG TACAGGCACAAAATTCGGACGAAACAACGCGACAAGATCAGTACCGCGTACGGCTTTTTGTTTACGGAACAT TCCCACCTTATCTGGACCATG
<i>Lhcx1</i> promoter for modified <i>Lhcx1</i> gene	CCTTAACTGGGTCAGTACAGCGTGTGATTGTGAACAAAACCAATGGCCAGACGGATTTCGCAAGTTCACGACAGCTTTCGACGGTGAGAATGATGGTATCGCTTCGGGTCGGGTCGGTACTTTGCGGGACTGGCAACGTCCGCAGAGG GACTCACGGTCACTTCACTGTCTGTATCGACTGACGACTGACTCGTCTGATTCACTGTCAGGTTTCTGATCTAAGT TTATCAAATGCTACTACTTGGAGGTAGTCGTATCATTGAACTGCAACTTACAAAATCGAAGGTCCTTGCAACAT TTCTGACATGGGTAATTTTCATAGGATGTTACAATGTCGAGCTTGTATCACTCGGATACAACAGACCACCTTTAGGA AAGGGATTTTTTTTGTGCGAAAATAGGATGACATCAACAGGAAGTACGTTTCATAGCCCTTTATTTATAAATTGGAT GGGTCAATCCGACGTACGCATGTGTCTTTTGACCTTGTATCCATAAACCAGATTGACCCGTCACGTGCAATACG TCACGCCAATGAAAGACCCCGGATAAAGGGCCTAAAATTCAGTCTCGTGCAAAAACGCAGGATGATGCACCT GAACAGGTGACGTGTTCCGCGCAGTCGAATGAGTCCGAAGCCATCAGAGGCGAATTTATTTGCAACTGTTTGA CCCCATAAAACATTCTAGGAGATTTTGATAAAACACTTGAAAATAACGGAATTCAGGCCCT
<i>Lhcx2</i> promoter	CTCACAGTAACATAGCATTGTGCGAACTAACCTGTAATACTAACGTGACGTATGAATGAATCGTGTATGACGTTCC GGTGTCGAGTCACTATACACCATTGGTGATACGATATCACGTGAGATATCACTGATATCTCACGTCGAATAGACA AGCTGCTTTTCAATGGGTGAAAACAACCAACACGGAACAAGTTCCTGTAACCTAACTATTGATTGTAGTTGG TGAAGAAAGCATTTCCTGACTGTGGGTACATGGAATACATCCTTTTTTGATAAATGTACAAAACAAGACTCATT TAGTCATAGTCAAGCGCAGTGCCTACTTGCCTGTGAAAAAATCGAGCTCTGAAAGGGTTAAAGTTCGAAGTTGTA CAAGGTAGAGCTGGTTTGCCTGTGTCAGGGCGTGGCCTACCTGACTCATGCATTCCGCGTGCCACAAAAACTCA CAGTCAGAGAGCCACTCCGAGAATCCTCCAGAATTCGTGGAAGATTTTTTCGTCACTTTTTCCAGTTCTCCCT TAGCTCTCATAGTTTCGTTTGCCTTCTTCGTAACACACTGCAAAGAGATTTACGTCCAATTCCAACCCACTACGT ACACC
<i>Lhcx2</i> terminator	GGAATATAACGTTCCAGTGTGTTTGAACACTAGCGCGATGGACAACAGAACGATTAGGCTAGCAACGAAAAGA AGCAAAAATACAGTAGAAATCAAGTAATCTCCACTTTGTTCAACACGCTGACAATCTTTATGTTAGTGTAGC CCTTTAATCGTGCACGTGTTCCGAGTCATTGCCCGGGACGTTTGTACAGCACATGAAACGAGAAACTTACCA GACAGTTGGCTCACAAATCAATTCATGAGTTTCTACATTGATTGACTACCAAGCTCCGTCATCGTTGCAGATT CATGCGTATGGAATCATTGAATCAAGTATCGTGAAGTTTCCAACGAGGAACCTGCGGTCACACTATTCTTCGTAC CAACCACGGAAGCTCCTGGTCCATCATGGCAGATCTCACGCTGGTAG
<i>Lhcx1</i> gene modified without introns	ATGAAGTTTCCGCAACTATATTGGCCCTTATCGGCTCTGCCGCTGCGTTTGGCCTGCACAGACGAGCCGTGCG TCTACTAGCCTCAGTACGCGAAGGAGGACTTGGTGGGGGCTATCCTCCGGTTCGATTCTTCGACCTCTTGGAT TTCCGCTGACAAGGCCGATTCCCCCACTTTGAAGCGATACCGTGAAGCTGAGCTACCCACGGACGTGTTGCCATG CTTGCCGTCGTTGGATTCTTGTCCGCGAGGCGGTAGAAGTTTCGTCGTTCTCTTCGATGCTTCTATCTCTGGCC CGGCCATCACCCACTTTCTCAAGTCCCGGCCCTTCTGGGTCCTCCTCACTATTGCTATCGGTGCTTCCGAACA GACCCGTGCCGTGATCGGCTGGGTGGATCCCGCGATGCCCGGTTGACAAGCCCGGTTCTTCCGTGACGACTA CGTCCCGGTGACCTCGGATTCGACCTCTCGGCCTCAAGCCTTCTGACCCGGAAGAAGTATCACTCTCCAGAC GAAGGAACCTCAGAACGGACGCTTGTATGCTTCCGCTGCCGGTTTCATGGCTCAGGAGCTTGTCAACGGGA AGGGAATCCTTGAGAATCTCAGGGTAA

Supplementary Information

<i>Lhcx2</i> gene	ATGAAATTATCCTTGGCTATCCTTGCCTTTCGCGCCAGCACTAGTGCCGCTTTCGCTCCTTCTGTTTCCCAGAGGA CGTCTGTCTCTCCGAGAATCATTGGACCCACCGAATCCATGTCGGAAGTGAAGGCGCCGTGAAAGACGCG GCTCCCAAAGTCTCCGACCCCTTCGACAGCCCTCGTGATCTTGCCGGAGTCGTCGCTCCTACCGCTTTTTCGATC CGGCAGGCTTCGCTGCCGAGCCGATGCCGGTACCATGAAGCGTTACCGGGAAGCGGAAGTACTCACGGACGT GTGGGCATGATGGCCGTTGTTCGGCTTCTTTCGCGGGCAAGCCGTCGAGGGATCGTCGTTTCTTTGACTCGCAA GTCAGCGGACCCGCCATTACTACCTCAACCAGATTCTTCCATCTTTTGGATTCTCCTCACGGTGGGCATTGGTG CTTCCGAAGTACGCGGGCTCAAATTGGTTGGGTACGTATCGGGAGTTGTTGGTTCCAACAGTTTCGCTTTGTCC GTCTCTACGCGTCTTTTCTTTCCATTCTGTTTCCAGGTTGAACCCGAGAACGTCCCACCGGGCAAGCCGGGTC TCCTCCGCGACGATTACGTCCCGGTGACATTGGCTTTGATCCTCTCGGCTTGAAGCCTTCCGACGCGCAGGCTC TCAAATCGATCCAGACCAAGGAAGTGCAGAGTAAGTCAATTGCTGTGCTGCTGTTACTGTGCTCCATGGTACTA TCGTGTTTACAGTTAGTTCACTGCGTCAGAGTGTACCGTGTACATACTGTTAATCCCACCAAATAACCGATTGAA TATAACTCGTTTCTTTGGCTAGACGGACGTCTCGCCATGTTGGCGGGCTGGGTGCATGGCTCAGGAATTGGC CAACGGAAAGGGCATTCTCGAAAACCTTGGTCTCTAA
<i>Lhcx2</i> gene modified	ATGAAATTATCCTTGGCTATCCTTGCCTTTCGCGCCAGCACGTCCGAGCATTTCACCAAGCGTCAGTCAACGA ACAAGCGTTAGCTTGGAGGAGAGCCTTGATCCAACAGAGAGTATGTCGGAAGTGAAGGCGCCGTGAAAGACG CGGCTCCCAAAGTCTCCGACCCCTTCGACAGCCCTCGTGATCTTGCCGGAGTCGTCGCTCCTACCGCTTTTGTGA CCCAGCTGGATTGACAGCCGAGCCGATGCCGGAACAATGAAAAGATATAGAGAAGCGGAAGTACTCACGGA CGTGTGGGCATGATGGCCGTTGTTCGGCTTCTTTCGCGGGCAAGCCGTCGAGGGATCGTCCTTTCTTTGACTCG CAAGTCAGCGGACCCGCCATTACTACCTCAACCAGATTCTTCCATCTTCTGGATCTTGTGACAGTGGGCATT GGTGCTCTGAGGTTACAGTGCACAGATTGGTTGGGTACGTATCGGGAGTTGTTGGTGCACAGTTTCGCTTT GTCGCTCTCACGCGTCTTTTCTTTCCATTCTGTTTCCAGGTTGAACCCGAGAACGTCCCACCGGGCAAGCCG GGTCTCCTCCGCGACGATTACGTCCCGGTGACATTGGCTTTGATCCTCTCGGCTTGAAGCCTTCCGACGCGCAG GCTCTCAAATCGATCCAGACCAAGGAAGTGCAGAGTAAGTCAATTGCTGTGTTGCTGTTACTGTGCTCCTGGT ACTATCGTGTTCAGATTAGTTCACTGCGTCAGAGTGTACCGTGTACATACTGTTAATCCCACCAAATAACCGAT TGAATATAACTCGTTTCTTTGGCTAGACGGACGTCTCGCCATGTTGGCGGGCTGGGTGCATGGCTCAGGAAT TGGCAACGGAAAGGGCATTCTCGAAAACCTTGGTCTCTAA
<i>Lhcx3</i> gene	ATGAAGCGCATCGCCGCTATCGCCCTTTCGCGCACTACGGCGTCCGCTTTAACGCATTCCGTTGCCGCAAGAAG GCTGCGCCAAAAAGCCGGTACGTCTACGCGAACCTTGGCATTTCACAGTACCACCGTGTGCGTGTGTACAGC TAGTGTGTCCGCGATTGCAATTCCTACGAACCACACTCGTTCCGCTTTGTCCCGCAGGTATTCTCGATCGAAA CGATCCCCGGTGCCTCGCTCCCGTTGGTATCTTTGATCCCTCGGTTTCGCGCCAAAGCCGACGAGTCCACCC TGAAGCGATACCGGAAGCCGAGCTCACCCACGGACGGTGGCCATGCTCGCTACCGTTGGCTTCTTGGTCCGT GAAGCCGTGAAGGATCTTCTTCTCTTTGATGCTTCTATCAAAGGACCTGCTATTTCTATCTCGCCCAAGTGC CGACTCCGTTCTGGGTTCTTTGACATTTTCATCGGGCCCGGAACAGACCCGTGCCGTATCGGCTGGCGGG ATCCTTCCGACTACCCTTCGACAAGCCCGTCTTTGAACGAGGACTACACCCCGGTGACATTGGCTTTGATC CTCTCGGACTCAAGCCAAAGGATGCGGAAGAACTAGGGTTCTACAGACCAAGGAAGTCCAGAACGGACGCTTG GCCATGCTTGCTGCCGCTGGATTCTGCGCAGGAAGTCTGGACGGCAAGGGAATCCTGGAACACCTCTCTA A
<i>Lhcx4</i> gene	ATGAAATTGTTACAGATCTTCTTGCCTTGGTTTATGTTGGAACCGCCGCTGGCTTTGCTCTGCCCCGTTCTCTA AGAAGACTTCTCCGTCACCAGTAAGTTGTCTCACACCGAGACCAATCTCATGTCCACTTGACGAAAGCATTGTCT TATGATCATAATTTCTCGTTTACTCTGCAGGAGGTATCAATTGAAAGTATGCTGTTGATCGTGGCGCCACTGGA TTCTTTGATCCACTCCGCTTCGCTGAAAGGGCCCGTCGAACACACTTAAGCGCTACCGCGAGTGTGAACTAACG CATGGCCGCTTGTATGTTGGCAACCGTGGGTTTCTCGCCGGCAAGCGGTTCAAATAACGAACTTTCTATGG AACGCCAAAGTTTCGGGGCTGTATAACGCATATTCCACAGATTCCAGCAACTTTTTGGGTTGCTCACCTG TTTATCGGTGTGGCCGAATTGTCACGTGCGCAAAGTCCATGGTTCCCCCAGTGACATCCCGTGGGTAAGGCT GGCCGAATGCGTGAAGATTACAATCCTGGGGACATTGGATTTCGACCTCTCAATTTAATGCCCGAAAGTTCCGA GGAGTTCTATAGTTGCAGACTAAAGAACTACAGAATGGGCGTTTGGCCATGCTGGGTGCTGCAGGTTTCTTGG CTCAAGAAGCAGTTAATGGGAAGGGTATTTTGGAGAATTTGTTGGCTAG

For the modified *Lhcx1* gene, 12 bases coding for the restriction enzymes recognition sites of *EcoRI* and *StuI* had been introduced before the start of the coding sequences (i.e. at the end of the promoter) due to cloning strategy reasons.

Supplementary Information

Supplementary Table 3: Primers used in this study

Name	Forward primer sequence	Reverse primer sequence
<i>Lhcx1_all</i> -fw	5'-CTCTCCAGACGAAGGAAC-3'	
<i>Lhcx1_all</i> -rev		5'-GATTCTCAAGGATTCCC-3'
<i>Lhcx1_Wt</i> -fw	5'-CTGCCACCATCCTTGCTCTT-3'	
<i>Lhcx1_Wt</i> -rev		5'-GACGAACCTTCTACCGCCTC-3'
<i>Lhcx1_comp</i> -fw	5'-GCCGCAACTATATTGGCCCT-3'	
<i>Lhcx2_allele1</i> -fw	5'-AAGAGATATACGTCCAATTCCAA-3'	
<i>Lhcx2_allele1</i> -rev		5'-AAGGACGACAGTAACAGCAA-3'
<i>Lhcx2_allele2</i> -fw	5'-CGTAAACACACCGCAAAA-3'	
<i>Lhcx2_allele2</i> -rev		5'-AAATGACTTACTCTGCAGTTCC-3'
<i>Lhcx2_prom</i> -fw	5'-CTCACAGTAACATAGCATTGTCTG-3'	
<i>Lhcx2_term</i> -rev		5'-CTACCAGCGTGAGATCTGC-3'
<i>FcpA_Lhcx1</i> -fw	5'-GGCTGCAGGACGCAATG-3'	
<i>FcpA_Lhcx1</i> -rev		5'-AGGGCCAATATAGTTGCGGC-3'
<i>Lhcx1_qPCR</i> _fw	5'-AAGGTTTCGTCGTTCTCTTCG-3'	
<i>Lhcx1_qPCR</i> _rev		5'-CGGAAGCACCGATAGCAATAGT-3'
<i>Lhcx2_qPCR</i> _fw	5'-CGCCATTACTCACCTCAACCAG-3'	
<i>Lhcx2_qPCR</i> _rev		5'-TCAACCCAACCAATTTGAGC-3'
<i>Lhcx3_qPCR</i> _fw	5'-TCTTGAACGAGGACTACACCCC-3'	
<i>Lhcx3_qPCR</i> _rev		5'-TCCGTTCTGGAGTTCCTTGGT-3'
<i>Lhcx4_qPCR</i> _fw	5'-ATGGCCGCGTTGCTATGTT-3'	
<i>Lhcx4_qPCR</i> _rev		5'-TATAGCAGGCCCCGAAACTTG-3'
<i>18s_qPCR</i> _fw	5'-TGCCCTTGTACACACCGC-3'	
<i>18s_qPCR</i> _rev		5'-AAGTTCTCGCAACCAACACCA-3'

Primers use for qPCR analyses are indicated as “*gene name*_qPCR_fw/rev”.

Supplementary Reference:

1: Tian, L. *et al.* pH dependence, kinetics and light-harvesting regulation of nonphotochemical quenching in *Chlamydomonas*. *Proc Natl Acad Sci USA* **116**, 8320-8325, doi:<https://doi.org/10.1073/pnas.1817796116> (2019).