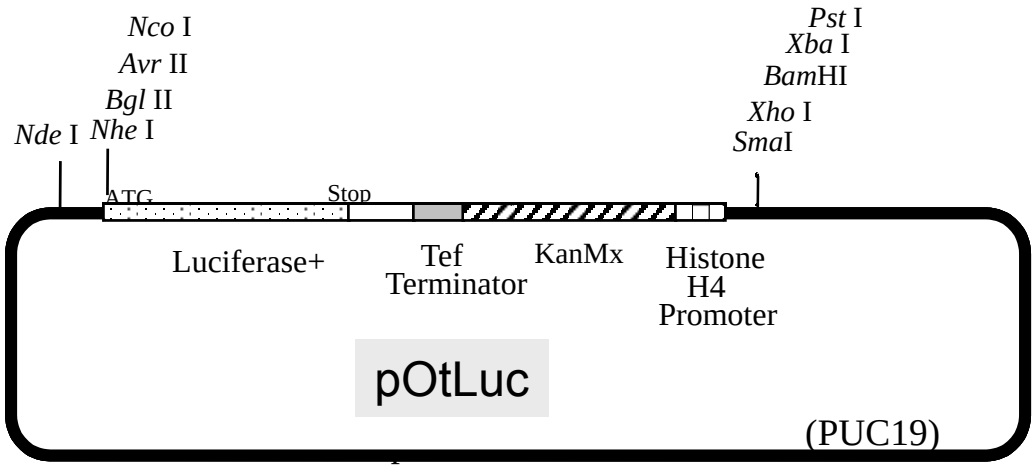
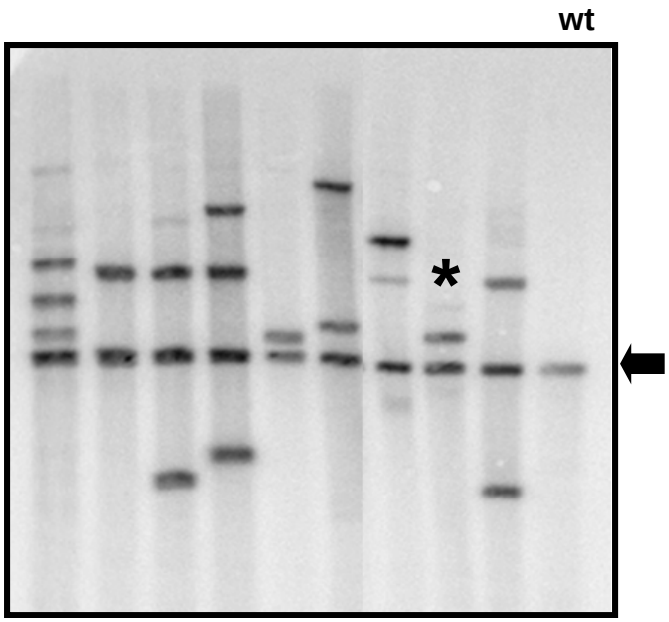
Supplemental Data. Corellou et al. (2009). Clocks in the green lineage: Comparative functional analysis of the circadian architecture of the picoeukaryote *Ostreococcus* . Plant Cell. 10.1105/tpc.109.068825

Supplemental Figure 1



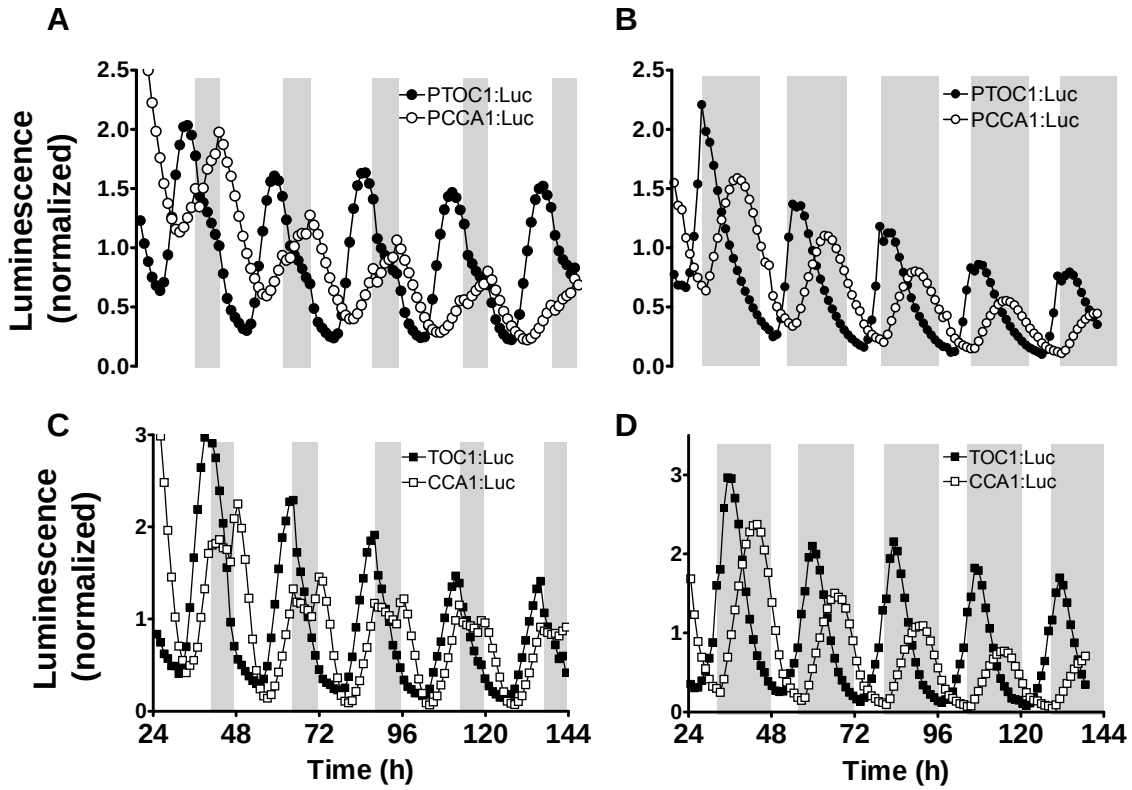
Supplemental Figure 1. POtLuc expression vector designed to generate promoter or full gene luciferase fusion in *Ostreococcus*. Schematic map of the pOtLuc vector used to introduce *Ostrecoccus* sequences fused to luciferase. The KanMx resistance gene was used to select stable transformants (resitance to G418).

Supplemental Figure 2



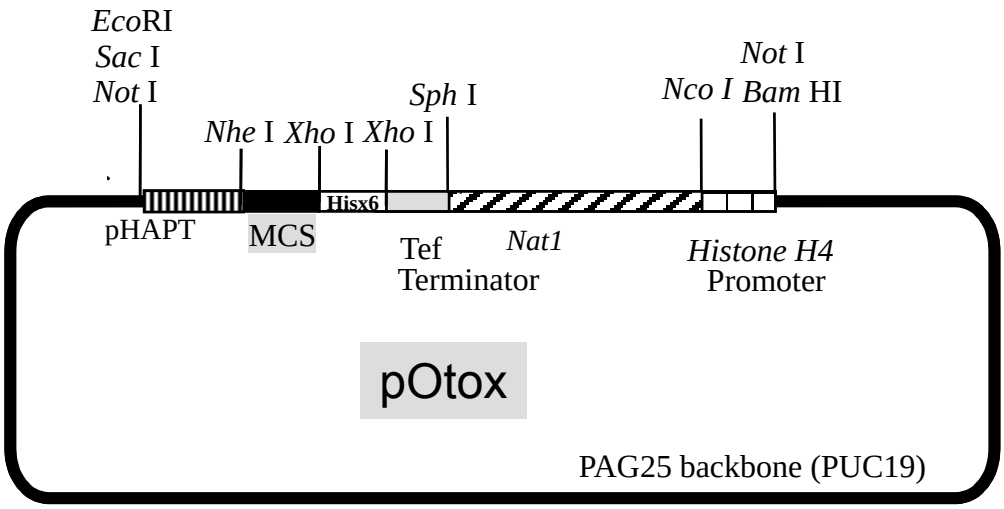
Supplemental Figure 2. Analysis of TOC1:Luc transformants by *Southern* blot analysis. Linearised DNA from a pOtluc vector containing the full *TOC1* gene fused in frame to luciferase was introduced by electroporation to *Ostreococcus*. DNA from stable transformants, resistant to G418, was digested with *Apa*I (single restriction site in the *TOC1* gene at 1.4 kb from the promoter), and hybridized to *Ostreococcus TOC1*. The endogenous *TOC1* gene copy present in untransformed cells (wild type wt) is indicated by the black arrow and the single inserted copy of *TOC1* in the TOC1:Luc-8 line reference line is indicated by a star. Note, at most three insertions with an average of 1.5 copy per line. All transformants contained the full TOC1:Luc sequence as confirmed by PCR with flanking primers.

Supplemental Figure 3



Supplemental Figure 3. Relative phase adjustments of *TOC1* and *CCA1* promoter activity and protein synthesis to photoperiod. Night is represented by plain grey areas**.** Bioluminescence of representative lines carrying *TOC1* and *CCA1* transcriptional (promoter fused to luciferase) or translational (full gene fused to luciferase) fusions. Means of triplicates (SEM smaller than symbols) of representative transcriptional and translational fusions are shown under LgD (LD: 16,8) (A,C) and ShD (LD: 8,16) (C,D).

Supplemental Figure 4



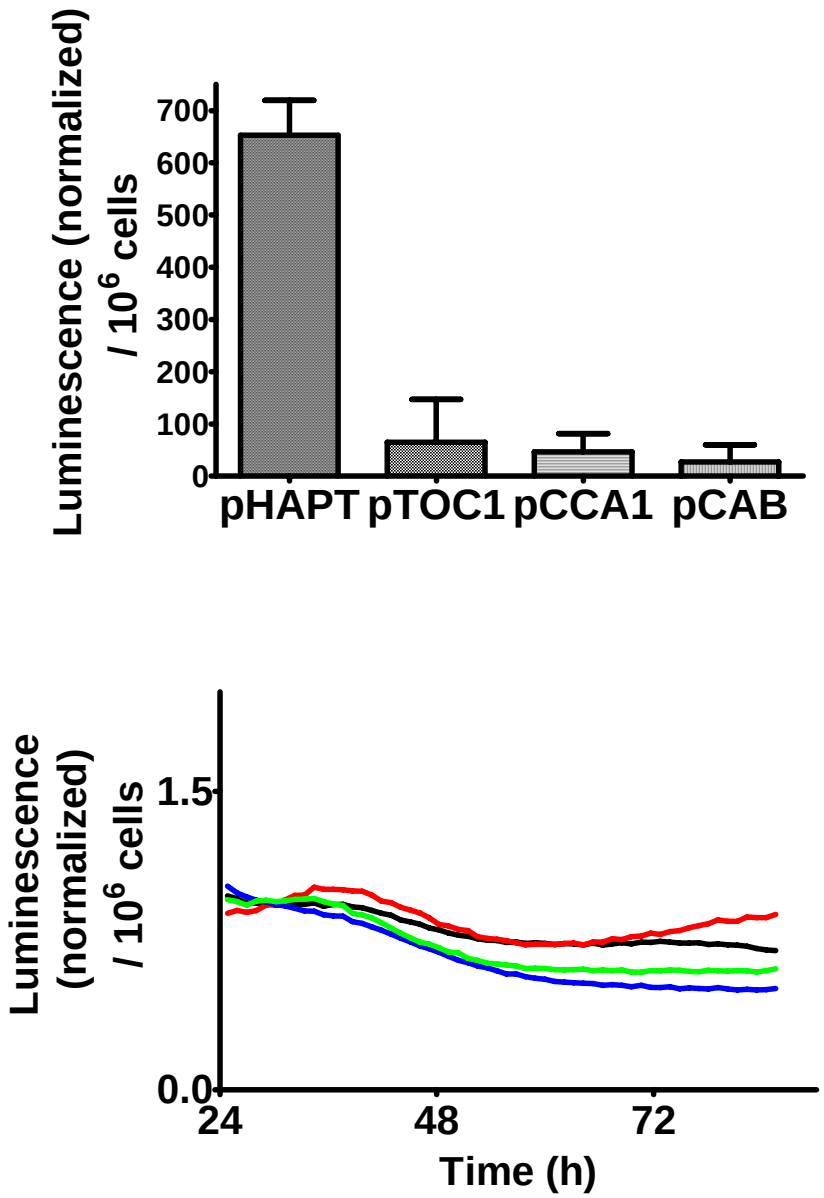
pOtox MCS:

*Psp*OM I *Sna*B I *Avr* II *Hind* III *Xho* I His His His His His His *Xho I*

```
CG GGC CCT TAC GTA CCT AGG AAG CTT CTC GAG CAC CAC CAC CAC CAC CAC TGA CTC GAG TGA
GC CCG GGA ATG CAT GGA TCC TTC GAA GAG CTC GTG GTG GTG GTG GTG GTG ACT GAG CTC ACT
```

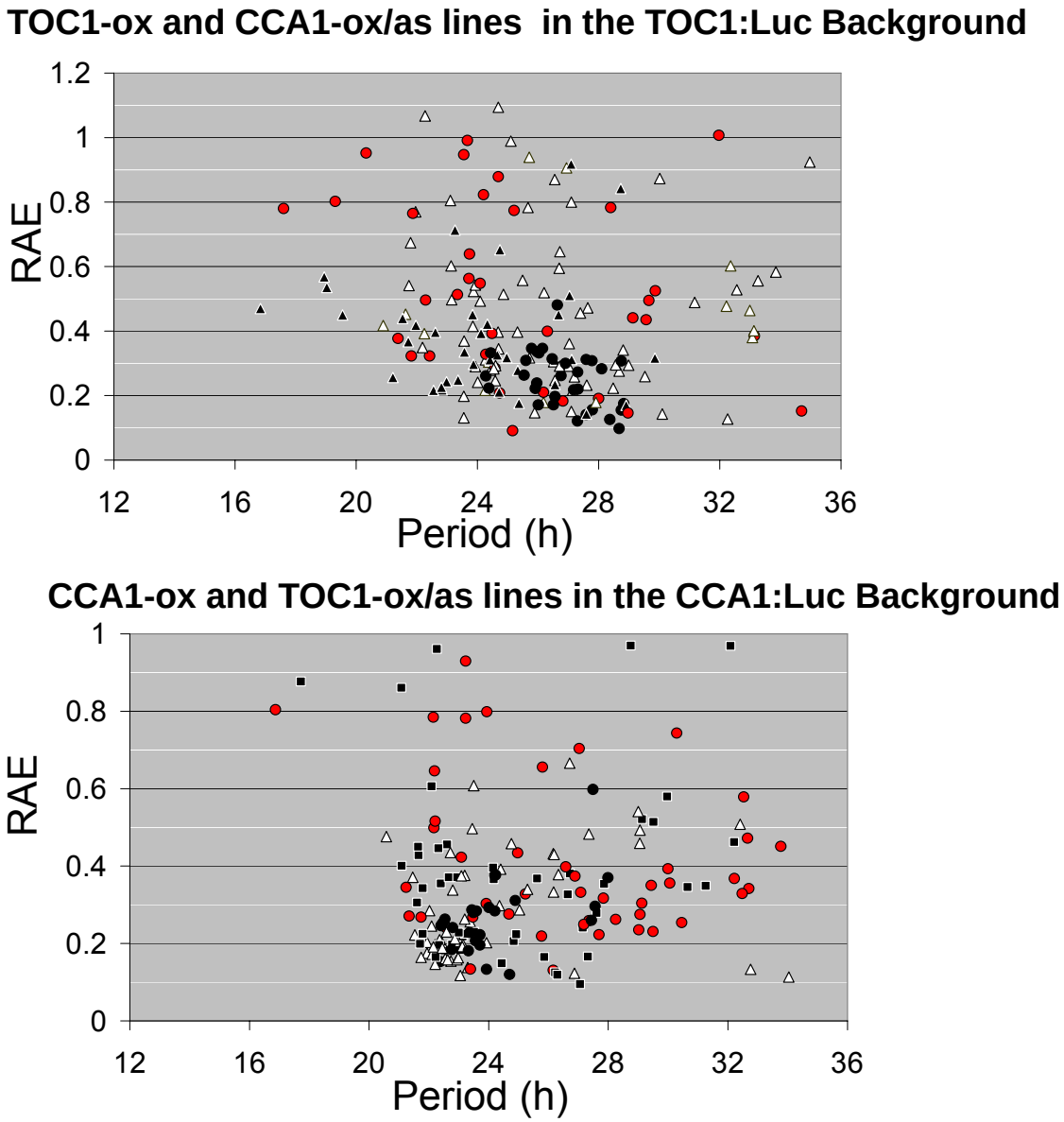
Supplemental Figure 4. Schematic map of the overexpression/antisense pOtox vector. Overexpression or knock-down of the gene of interest is achieved by expressing the sequence of interest in sense or antisense orientation under control of the strong High Affinity Phosphate Transporter (HAPT) promoter

Supplemental Figure 5



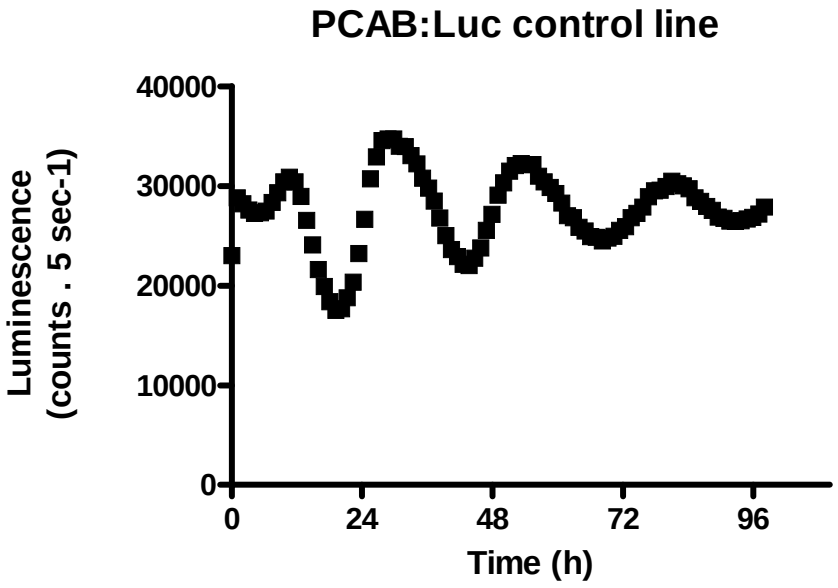
Supplemental Figure 5. Analysis of the promoter activity of the high affinity phosphate transporter (PHAPT). A, Relative strength of PHAPT (n=11), PTOC1 (n=7), PCCA1 (n=6) and PCAB (n=6) promoters. Luciferase transcriptional fusions were plated at the same density and luminescence was recorded under LL. Luminescence values over the last four hours were averaged for each line. Mean luminescence values/10^6 cells (±SD). **B,** Luminescence profiles of four representative PHAPT:Luc lines under constant light. Cells were submitted to six hours of darkness and released into constant light. The luminescence signal is shown from 24 hours after lights on.

Supplemental Figure 6



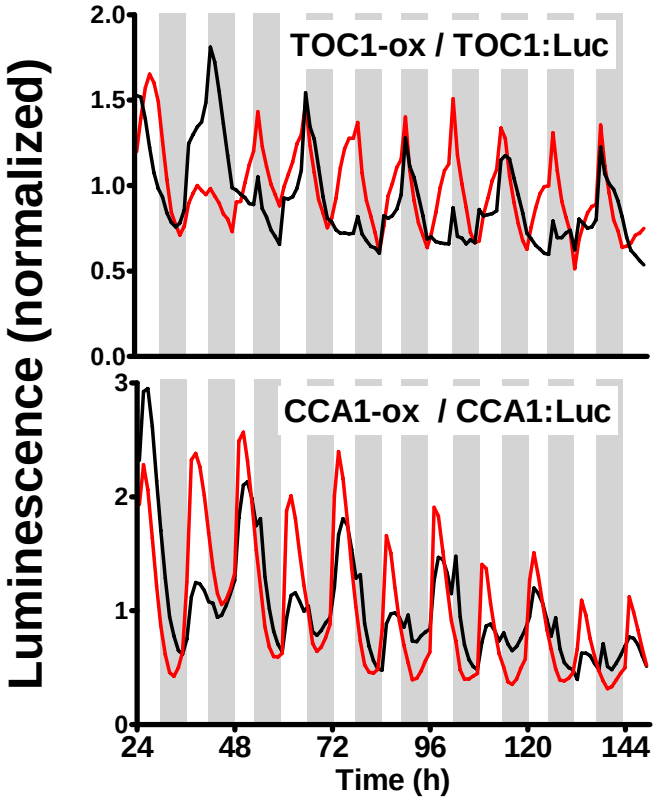
Supplemental Figure 6. Screening for altered rhythmicity in *TOC1* and *CCA1* overexpression/antisense lines in the CCA1:Luc and TOC1:Luc background. Lines were screened for alterations of rhythmicity in reporter genes as judged by observation of bioluminescence traces under constant light after release from LD: 12,12 entrainment. To assess observations relative amplitude error estimation was further performed over at least 3 days recording (excluding noisy traces) using FFT-NLLS. Graphs represent all single RAE values from all screening. Screens were performed several times so that defective cell lines could be plotted more than once. Period variation may be attributable to differences of cell concentration and should not be considered. For the great majority of the lines judged as arrhythmic, no period could be estimated (so no RAE) and the data are not plotted. A good correlation was found between severely altered patterns of oscillation and RAEs above 0.5. A line was therefore considered as arrhythmic if the estimated RAE was consistently above 0.5, dampened rhythmic if the estimated RAE was consistently above 0.3 and rhythmic otherwise, from at least three biological triplicates in each experiment. Black circle: Control lines. Red circle: TOC1-ox lines. White triangle: CCA1-ox lines. Black square: TOC1-as lines. Black triangle: CCA1-as lines.

Supplemental Figure 7



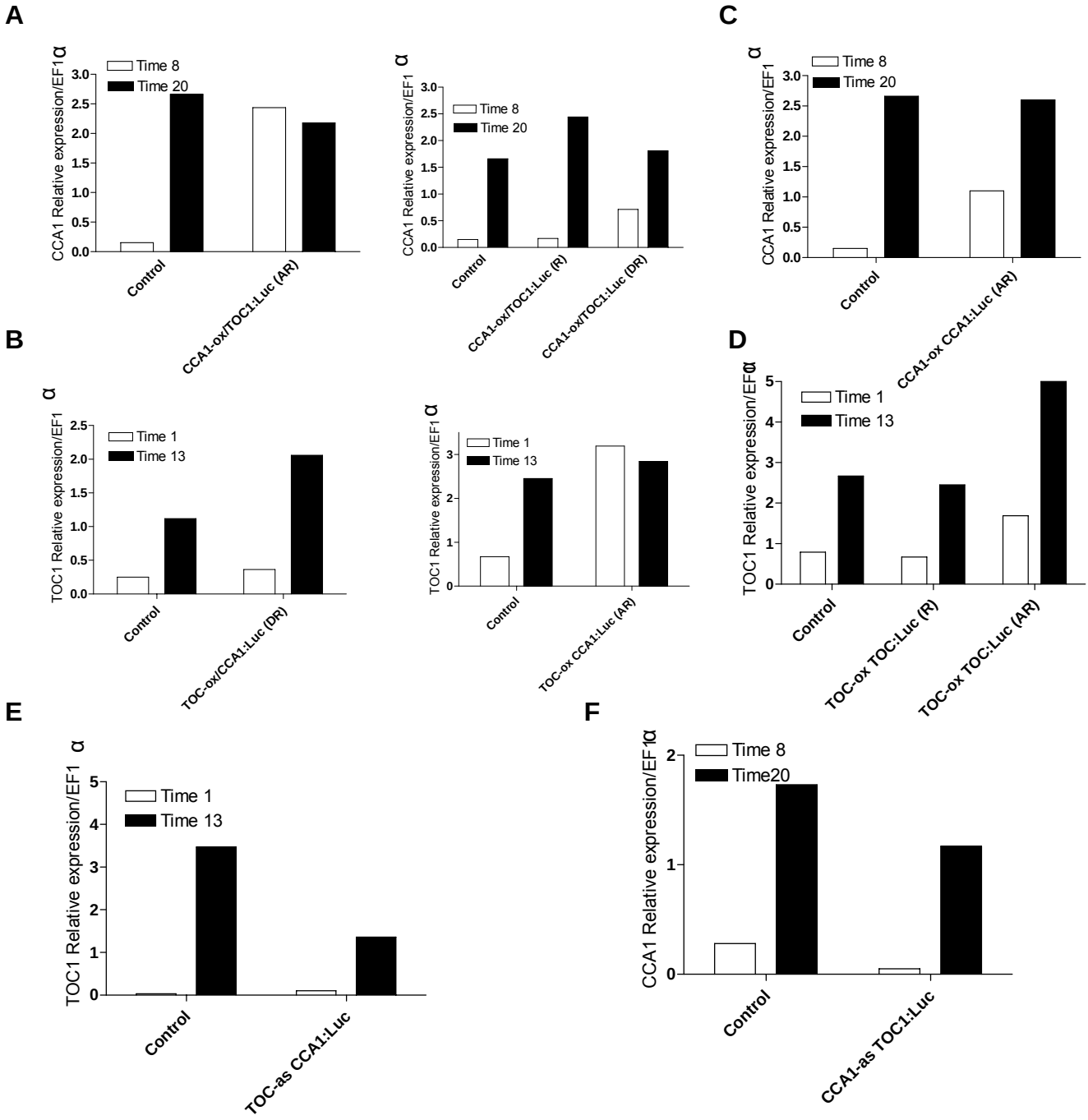
Supplemental Figure 7. Luminescence recording of the PCAB:Luc control line in constant line, corresponding to the experiment presented in Figure 4.

Supplemental Figure 8



Supplemental Figure 8. Overexpression of *TOC1* and *CCA1* disrupts circadian regulated expression of CCA1 and TOC1. Bioluminescence traces of representative CCA1 :Luc and TOC1:Luc ox lines under LD: 6,6. Lines grown under constant light were transferred to new medium at the same cell density, with luciferin, from LL to LD: 6,6. Time Zero corresponds to the first lights ON. TOC1:Luc and CCA1:Luc control lines are represented in black, ox lines are plotted in red. Monitoring of luciferase is monitored from 24 hours after addition of luciferin. TOC1:luc and CCA1:luc display a biphasic 24 hour module In contrast ox lines respond directly to each LD: 6,6 cycle. Data sets are representative at least of three trials.

Supplemental Figure 9



Supplemental Figure 9. RT-PCR analysis of overexpression and antisense lines with rhythmicity defects in TOC1:Luc and CCA1:Luc backgrounds. Transcripts were extracted at times of maximal and minimal transcript levels of *TOC1* (Time 13- Time 1) and *CCA1* (Time 20- Time 8) in controls (Light is switched on at Time 0). Transcript levels were determined in ox-lines showing varied degrees of altered rhythmicity (A-D). *TOC1* or *CCA1* transcript levels were similar between control and rhythmic lines (R) (A and D). In arrhythmic lines, the maximal and minimal levels tended to equalize, whereas intermediate levels were observed in lines with dampened rhythmicity (DR). Transcript levels were moderately reduced in TOC1-as CCA1:Luc (AR in LL) and CCA1-as lines (6:6 LD phenotype) (E and F).

Supplemental Table 1

		A. thaliana	*O. tauri*
family name	function (domains)	"No copies" (Accession)	"No copies" (Accession)
CCA1/LHY REVEILLE	oscillator (MYB)	11 (At2g46830, At1g01060, At5g17300, At1g18330, At3g10113, At5g37260, At4g01280, At5g52660, At3g0960, At1g01520, At5g02840)	1 (AY740076)
PRR (TOC1, PRR3, PRR5, PRR7, PRR9)	oscillator (REC, CCT)	5 (At5g61380, At2g46790, At5g60100, At5g24470, At5g02810)	1 (AY740079)
Cryptochromes (CRY, CRY DASH)/ photolyase	blue light perception	3 (At4g08920, At1g04400, At5g24850)	2 (AY740085) (AY740084)
Phototropins	blue light perception	2	1 (CAL58288)
SRR1	red light input	1 (At5g59560)	1 (AY740080)
Constans like (CO/COL1/ COL2)	light input (B box, CCT)	>20 (At5g15840, At5g15850, At3g02380, ...)	1 (AY740087)

Supplemental Table 1. Identification of putative homologues of *Arabidopsis thaliana* clock related genes and photoreceptors in the green alga *Ostreococcus tauri*. No homologues of *ELF3*, *ELF4*,*Gigantea and Phytochromes* were found.

Supplemental Table 2

Cloning primers				
sequence amplified	**Forward primer**	**Reverse primer**	**destination vector**	**cloning sites**
TOC1 (full gene)	TTTGCTAGCACCTCGAGCCGGGACCAAAAA	TTTCCATGGCGCTCGCGTCTCGAGACC	pOtLuc	NheI/NcoI
TOC1 (CDS)	GCTCTAGAATGTCCGACGCGGAACGCGCTC	CTGCAGAACCAGAGCCTGGTCACTTGGAGCCATCGCGAGACGA	pOtOx	XbaI/BstXI
TOC1 (promoter)	TTTGCTAGCACCTCGAGCCGGGACCAAAAA	TTTCCATGGCGCTCGCGTCTCGAGACC	pOtLuc	NheI/NcoI
CCA1 (promoter)	GCTAGCACGCCGCGTACATCATCATCATC	CCATGGCGCGCCCGTACGAATCGATCCGCCGACTCG	pOtLuc	NheI/NcoI
CCA1 (CDS)	CTTAAGATGGGAGATCAAGGCGAGGCCCCATCGAGT	CCATGGTTATTTGGTTTCACCACCCGCCCGCTGTT	pOtOx	pspOM I
CCA1 (CDS)	CGCGGATCCATGGGAGATCAAGGCGAGGC	GCTCTAGATGATTATGGTTTCACCACCCGCC	pGex	EcoRI
CCA1 (full gene)	GCTAGCACGCCGCGTACATCATCATCATC	CCATGGTTTTGGTTTCACCACCCGCCCGCTGTTTATG	pOtLuc	NheI/NcoI
CAB (promoter)	AATGCTAGC GCGTCACCGCGCCTCGAGA	AATCCATGGCGCGGCGTCGAGGTGTGC	pOtLuc	NheI/NcoI
HAPT (promoter)	TTTAGATCTAAATACTTTTCCAGGAAAAGCAG	CCATGGCGAGACGGGTAAAGCGCGA	pOtLuc	BglII/NcoI
Histone H4 (promoter)	GTACCGCCCGGGTACCGTTGCGCTC	ACACGTTCGAAAACAACCATGC	pOtLuc	none
Checking primers				
sequence amplified	**Forward primer**	**Reverse primer**		
NAT1 (internal sequence)	ACTGGATGGGTCCTTCACC	CTCTACATGAGCATGCCCTG		
KanMx i(nternal sequence)	GGTCTGCGATTCCGACTCGTCC	GCCCGATGCGCCAGAGTTGT		
Quantitative RT PCR Primers				
sequence amplified	**Forward primer**	**Reverse primer**	**Target**	**Efficiency**
CCA1 (CDS)	CTAGTACGTCGTCGAGC	CCACGAACGGACTCAT	CCA1-ox lines	2.01
CCA1 (3' UTR)	TCTTCGATGGGCGATACTT	TCTTCGATGGGC6GATACTT	CCA1-as lines	1.81
TOC1 (CDS)	GGCTGAGCGTGCAGCT	CTGCGTTAGATCTAGGC	TOC1-ox lines	2.01
TOC1 (3' UTR)	GGCAGAGAGGAACAATACG	GTGCCTAGAGGTTGAATTCTT	TOC1-as lines	1.81
EF1 alpha (CDS)	GACGCGACGGTGGATCAA	CGACTGCCATCGTTTTACC	reference gene	1.99
EMSA probes				
EE Ostreococcus	CCGATCAAATATGTTTATCGTGAAAATATCTCAAAGACGATCACTTTTCACC			
Generic EE Arabidopsis	GTCCCGTAAAATATCTCGCGTCCA			
mEE Ostreococcus	CCGATCAAATATGTTTATCGTGAAAAGCTTTCAAAGACGATCACTTTTCACC			

Supplemental Table 2. List of oligonucleotides used for cloning, checking recombinant lines, quantitative RTPCR and EMSA.For RT-PCR, prime efficiencies are given. For EMSA, the probes and competitor were generated by annealing two complementary oligonucleotides together. Only the forward sequence is given.