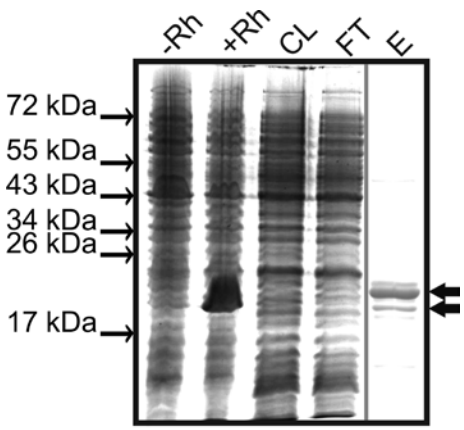
SUPPLEMENTARY MATERIAL

name	**sequence 5` to 3` (fw / rev)** ***[5`-modification]***
Primers used for cloning	
MOC1-MTS_A fw / rev	ggatccagctacaccactgggagcag / gaattcctacgcggcgctaaagctcttg
MOC1Ex fw / rev	acgggatcccagcagcagttgtaccgcc / ccgtaagcttctagttgcggcccatgc
Primers used for EMSA probe generation by PCR (EMSA screen)	
Probe 1 (1-542) Cy for / rev	actacgcatgcctaagtgc *[Cy-3]* / ttttgcattgacacacttttttaca
Probe 2 (518-1061) for / Cy rev	tgtaaaaagtgtgtcaatgcaaaa / aggtgttaacagccaaagcag *[Cy-3]*
Probe 3 (1035-1594) Cy for / rev	attaggctgctttggctgtt *[Cy-3]* / ggttctctagctggtatgatgc
Probe 4 (1573-2125) for / Cy rev	gcatcataccagctagagaacca / gcttcttgatgattgctcacg *[Cy-3]*
Probe 5 (2104-2646) Cy for / rev	ccgtgagcaatcatcaagaa *[Cy-3]* / gattggccgtgctccttac
Probe 6 (2628-3179) for / Cy rev	gtaaggagcacggccaatc / ctgagcagccatacgaacg *[Cy-3]*
Probe 7 (3157-3704) Cy for / rev	cctacgttcgtatggctgct *[Cy-3]* / caccactaagttgttctatcagtgc
Probe 8 (3679-4226) for / Cy rev	caaccataaagcactgatagaacaa / cgctactctggtcactgctg *[Cy-3]*
Probe 9 (4203-4742) Cy for / rev	acaccagcagtgaccagagt *[Cy-3]* / tgccggttggacttttaact
Probe 10 (4699-5239) for / Cy rev	ccatgttggcggttagtagg / caatagcatgataataccgtgacc *[Cy-3]*
Probe 11 (5216-5755) Cy for / rev	ggtcacggtattatcatgctattgt *[Cy-3]* / gacctcagggtgaccaaaga
Probe 12 (5733-6284) for / Cy rev	ggttctttggtcaccctgag / caccaatgaatagcaaccagaa *[Cy-3]*
Probe 13 (6260-6799) Cy for / rev	cacttctggttgctattcattgg *[Cy-3]* / gatgtgggccaaagacacat
Probe 14 (6780-7330) for / Cy rev	atgtgtctttggcccacatc / ccaagaagaagcccagaaag *[Cy-3]*
Probe 15 (7305-7860) Cy for / rev	tgttcactttctgggcttctt *[Cy-3]* / cccacctaaataacgaatggt
Probe 16 (7834-8385) for / Cy rev	ggttaaaccattcgttatttaggtg / tcaaaaccaggctgttcaaa *[Cy-3]*
Probe 17 (8366-8910) Cy for / rev	tttgaacagcctggttttga *[Cy-3]* / tgagccagcaccaaactcta
Probe 18 (8891-9450) for / Cy rev	tagagtttggtgctggctca / ttgcagtacggtattaataaaaagtaa *[Cy-3]*
Probe 19 (9428-9978) Cy for / rev	tttttattaataccgtactgcaaacc *[Cy-3]* / tcccagaaaagcttgaggat
Probe 20 (9956-10490) for / Cy rev	tcttatcctcaagcttttctgg / cagaacggctgtagcaagc *[Cy-3]*
Probe 21 (10464-11010) Cy for / rev	ccggtatcagtggcttgcta *[Cy-3]* / agcgtactcggcgataaaga
Probe 22 (10990-11530) for / Cy rev	ccctattctttatcgccgagt / tttacctgaatagatttcgcaga *[Cy-3]*
Probe 23 (11500-12094) Cy for / rev	ggttttctgcgaaatctattca *[Cy-3]* / gcatgatgggtaccaaaacc
Probe 24 (12030-12569) for / Cy rev	ggttttggtacccatcatgc / aactagcagctggtggtgttg *[Cy-3]*
Probe 25 (12538-13086) Cy for / rev	ctgtaaagctacaacaccacca *[Cy-3]* / aagcctttcgctgacaaaga
Probe 26 (13064-13604) for / Cy rev	tttctatctttgtcagcgaaagg / tcaccaggcgttatagatttga *[Cy-3]*
Probe 27 (13582-14131) Cy for / rev	ttcaaatctataacgcctggtg *[Cy-3]* / tgctcgaccgttcagaaaac
Probe 28 (14100-14668) for / Cy rev	ggtctttgccacgttttctg / tgccaaaaggcataaataggtt *[Cy-3]*
Probe 29 (14641-15194) Cy for / rev	tgcacaaacctatttatgccttt *[Cy-3]* / gctttgcgtaaattctgtgga
Probe 30 (15153-end) for / Cy rev	cactgccgatcatactggaa / actacgcatgcctaagtgc *[Cy-3]*
Primers used for EMSA probe generation by PCR	
SELEX-S3 long fw /rev	ttttatgaattgtttgtgaacacc / cctccgaatattacccaacg *[Cy-3]*
S2/nd1 Cy3 for / rev	tgaacccagcagtgttgaat / catagcgacgctcaacaaaa *[Cy-3]*
LHCBM6 NG Cy3 for	gacaagggctttgagagtgc *[Cy-3]*
LHCBM6 NG long rev	acgacatagcaaggctcacc
LHCBM6 NG short rev	acccatattgacgatgcaca
Small oligonucleotide probes for EMSA	
S3_1 / S3_1 rc	aattgtttgtgaacacccccaag / cttgggggtgttcacaaacaatt *[Cy-3]*
S3_2 / S3_2 rc	cgttaatggtgaacactttaaacg / cgtttaaagtgttcaccattaacg *[Cy-3]*
S3_2 Mut / S3_2 Mut rc	cgttaatgagtagccatttaaacg / cgtttaaatggctactcattaacg *[Cy-3]*
S3_1 Mut I / S3_1 Mut I rc	aattgtttgcgaacacccccaag / cttgggggtgttcgcaaacaatt *[Cy-3]*
S3_1 Mut II / S3_1 Mut II rc	aattgtttgtgaaccacccccaag / cttgggggtggttcacaaacaatt *[Cy-3]*
S3_1 Mut III / S3_1 Mut III rc	aattgtttttgaacacccccaag *[Cy-3]* / cttgggggtgttcaaaaacaatt
S3_1 Mut IV / S3_1 Mut IV rc	aattgtttctgaacacccccaag *[Cy-3]* / cttgggggtgttcagaaacaatt
S3_1 Mut V / S3_1 Mut V rc	aattgtttgtcaacacccccaag *[Cy-3]* / cttgggggtgttgacaaacaatt
S3_1 Mut VI / S3_1 Mut VI rc	aattgtttgttaacacccccaag *[Cy-3]* / cttgggggtgttaacaaacaatt
S3_1 Mut VII / S3_1 Mut VII rc	aattgtttgtaaacacccccaag *[Cy-3]* / cttgggggtgtttacaaacaatt
S3_1 Mut VIII / S3_1 Mut VIII rc	aattgtttgtgaacatcccccaag *[Cy-3]* / cttgggggatgttcacaaacaatt
S3_1 Mut IX / S3_1 Mut IX rc	aattgtttgtgaatacccccaag *[Cy-3]* / cttgggggtattcacaaacaatt
S3_1 Mut X / S3_1 Mut X rc	aattgtttgtgagcacccccaag *[Cy-3]* / cttgggggtgctcacaaacaatt
S3_1 Mut XI / S3_1 Mut XI rc	aattgtttgtgcacacccccaag *[Cy-3]* / cttgggggtgtgcacaaacaatt
S3_1 Mut XII / S3_1 Mut XII rc	aattgtttgtgaacaacccccaag *[Cy-3]* / cttgggggttgttcacaaacaatt
CAST (Cyclic amplification and selection of targets)	
oligonucleotide template	aagctttgaccatgcagc-$(n)_{20}$-cgtctagcatatggatcc
SELEX Cy-3 fw / rev	aagctttgaccatgcagc / ggatccatatgctagacg

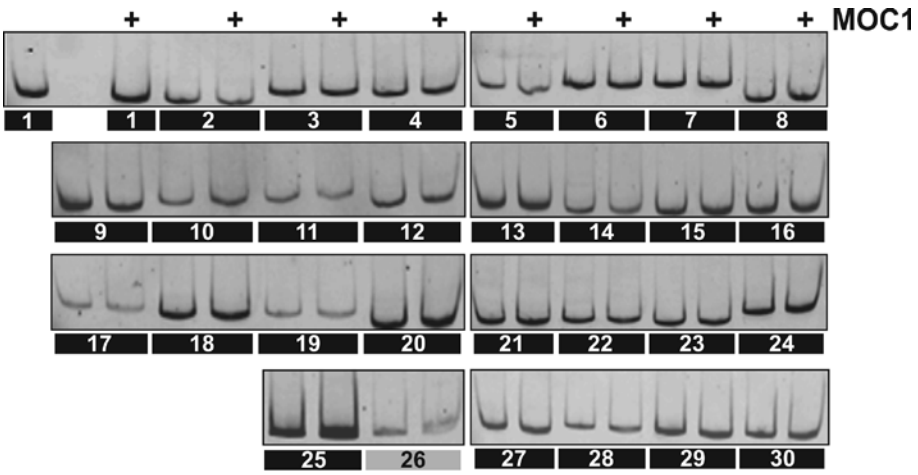
Q-PCR (relative mtDNA content)	
Chlamy nd1 fw / rev	acgcgacccatttgatctac / agcgaagaacagggcagtaa
TBP for / rev	tgccacgttcaaggagttta / tgcttcatgcggtagatgag
RT-Q-PCR (processed transcripts)	
CYN19-3 fw / rev	gccaacccttggtctactt / gaagttctccgcagtcttgg
Chlamy cob fw / rev	gcctacccaactccaatgaa / gtgagcgtaacgcaagatca
Chlamy nd4 fw / rev	acactatggccggttctttg / cactaccagcagttggagca
Chlamy nd5 fw / rev	ccccaattgctcgttttcta / ccggtaacggtgaatagcat
Chlamy cox1 fw / rev	tggtaatgccagccctattc / taagcggtccaaccagtacc
Chlamy nd2 fw / rev	ccaccatttgcaggtttctt / gcaggcagaggttagagtgg
Chlamy nd6 fw / rev	tattttgttgtgcgctttgc / tagctcagtggctgggatct
Chlamy rtl fw / rev	ctgccctgcttctaatggag / taccaaaaccaggacggaag
Chlamy L7 fw / rev	cgacagcgactgtttaccaa / acgccaattaaacgtcaagg
Chlamy L8 fw / rev	ttcagaccgtcgtgagacag / ggggtcattcggtgttattg
Chlamy L3 fw / rev	aacgtctattggacccgaaa / gatttcgcagaaaaccagcta
Chlamy L6 fw / rev	aacgttctggaacaattacgc / aaccgtattcactgtttgttatgc
Chlamy L5 fw / rev	ttgtcataggcgggcttc / tctgcgttgaacaccagaac
Chlamy S3 fw / rev	ctatcgctgccaattgcttt / gcttcaacgcgtaagtttgg
Chlamy S4 fw / rev	tggtatccaacttgtgggtgt / ttgcattgctgaaaagttcg
RT-Q-PCR (unprocessed transcripts)	
nd4-cob fw / rev	ccattgctaattggtgctgt / ttcattggagttgggtaggc
nd4-nd5 fw / rev	cggtttgctaagcgttggta / gcaaacaaaatcaacaaaacaga
cox1-nd2 fw / rev	ctgccagctctcactaatgc / ccaaaacacaagtcaagctca
nd2-nd6 fw /rev	cctagcttttgctccggtta / gcgcacaacaaaatagcaga
L5-L7 fw / rev	tatgtccggcactccaaat / gccgagttcctttaacatgg
L7-nd1 fw / rev	gcattaatgacccagggtaaca / ggtgcgctcagctagagtaaa
nd1-rtl fw / rev	tcgttacgaccaattcatgc / agtgtggaacaaatccaaaatg
rtl-L8 fw / rev	tgctacgtacattgggttgg / cgttgatcagggtaactgtgt
L8-S3 fw / rev	ttgtcatttacacggcatagc / cctccgaatattacccaacg
L4-S4 fw / rev	tagcgcaagcgaatggtaat / tggtatccaacttgtgggtgt
L1-L2b fw / rev	gggattgctgactgatagtgc / gatgtggggcttgtgagagt
L4-S4 as fw / rev	gtagcgcaagcgaatggta / cgaacttttcagcaatgcaa
L1-L2a as fw / rev	caccaatacgtgagcattcg / aacccataatgcaaaaggtacg
nd1-L3 fw / rev	tcgttacgaccaattcatgc /agccagtaaagtttctttagtattgga
RT-PCR (cob-L2b as)	
cob-L2b as N1 fw / rev	tacctacttgtttgtcaaccacttg / gagaggggccatcacagag
cob-L2b as N2 fw / rev	caaaccaactaatattttgcattga / aaatgttactaggaccccaacg

Supplementary Table 1.
Olignucleotides used in the study.



Supplementary Figure 1.

Coomassie Brilliant blue-stained 12.5% SDS-PAGE with different fractions collected during Ni-NTA purification of recombinant MOC1 (amino acids 46-255) from *E. coli* cells after induction of MOC1 expression with rhamnose (+Rh). The cleared lysate (CL) which was incubated with Ni-NTA resin is shown along with the column flow-through (FT) and pooled eluted fractions (E). Bands excised from the gel were analysed by peptide mass fingerprint. Black arrows indicate the position of MOC1.



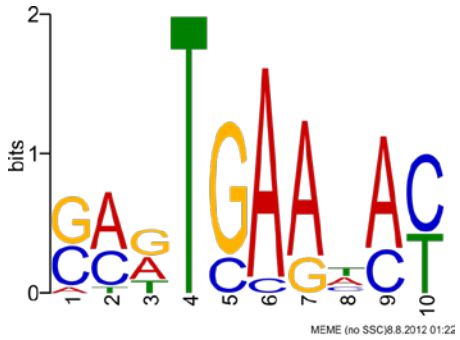
1: 1-542	11: 5216-5755	21: 10464-11010
2: 518-1061	12: 5733-6284	22: 10990-11530
3: 1035-1594	13: 6260-6799	23: 11500-12094
4: 1573-2125	14: 6780-7330	24: 12030-12569
5: 2104-2646	15: 7305-7860	25: 12538-13086
6: 2628-3179	16: 7834-8385	26: 13064-13604
7: 3157-3704	17: 8366-8910	27: 13582-14131
8: 3679-4226	18: 8891-9450	28: 14100-14668
9: 4203-4742	19: 9428-9978	29: 14641-15194
10: 4699-5239	20: 9956-10490	30: 15153-15758

Supplementary Figure 2.

EMSA-based screening of the *C. reinhardtii* mitochondrial genome to identify fragments containing MOC1 binding sites.

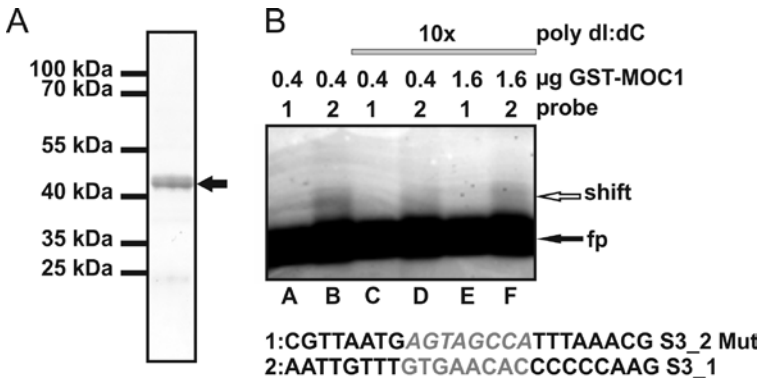
Upper part: EMSA results obtained with recombinant MOC1 and 30 distinct overlapping mtDNA fragments which were fluorescently labelled (Cy-3 dye). The full array of probes covered the entire mtDNA of *C. reinhardtii*. All reactions contained unspecific competitor in mass excess (40fold) relative to the probe and addition of recombinant MOC1 is indicated by ``+``.

Lower part: mtDNA regions represented by probes 1-30 relating to the publicly available mtDNA sequence of *C. reinhardtii* (NCBI Accession NC_001638).



Supplementary Figure 3.

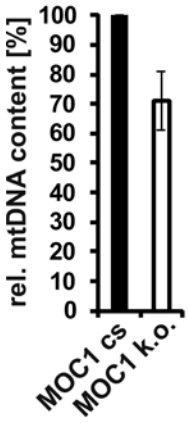
Sequence logo obtained by MEME analysis (46) of the CAST library after ten rounds of selection. Score bit values are indicated on the y-axis and the sequence position on the x-axis. Shown is the reverse complement of the motif identified by MEME. The expect value for this motif derived from 14 out of 29 sequences was 9.8 (information content 10.4 bits; relative entropy 10.2 bits).



Supplementary Figure 4

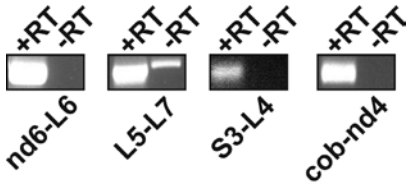
A) Purified GST-MOC1 (black arrow) after SDS-PAGE separation and Coomassie Brilliant Blue staining.

B) EMSA with short fluorescent oligonucleotide probes (bottom part; sequences of S3_2 Mut and S3_1) and different amounts (µg) of recombinant GST-MOC1 in the presence or absence of unspecific competitor (poly dI:dC) in mass excess relative to the amount of probe (10x). Numbers (upper part; probe) indicate which probe was added to the binding reaction and arrows the positions of free probe (black arrow; fp) and complexes (white arrow; shift) in the gel.



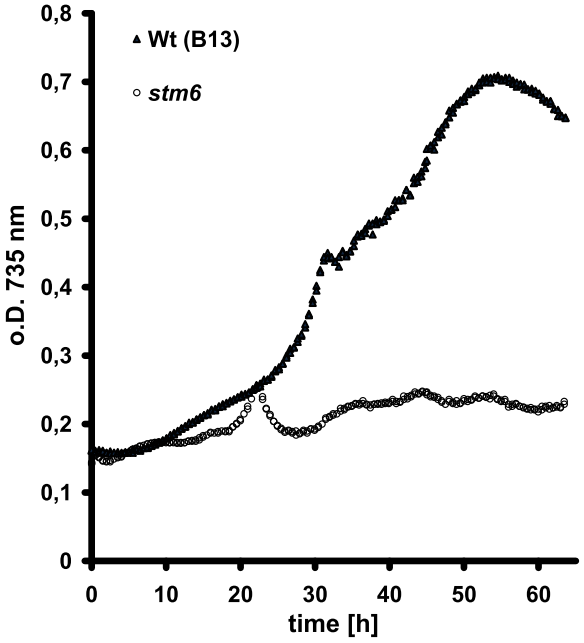
Supplementary Figure 5.

Relative mitochondrial DNA content assessed by quantitative PCR analyses. The mitochondrial *nd1* gene was used as a target gene and the nuclear gene encoding TATA-box binding protein (TBP) served as the reference gene. The mitochondrial DNA content in the MOC1-complemented strain (MOC1 cs) was set to 100%. Error bars indicate the standard deviation of two biological replicates each including three technical replicates (n=6).



Supplementary Figure 6.

Existence of a large precursor transcript covering the entire LTU and RTU. Based on results published previously by Gray and Boer (12,13) RT-PCR was used to determine if polycistronic transcripts detected before (12,13) could result from even larger polycistronic precursors. Primers were designed to bind in two different adjacent coding sequences and samples lacking reverse transcriptase (-RT) during the cDNA synthesis step were used to rule out that contaminating mtDNA in total RNA samples served as the template for subsequent PCR amplification.



Supplementary Figure 7.
Growth of the MOC1-free mutant *stm6* and the MOC1-complemented strain B13 under strictly heterotrophic conditions. The optical density (o.D. 735 nm) was monitored during cultivation to follow the growth of both strains.

SUPPLEMENTARY METHODS

Production of recombinant His-MOC1 in *E. coli* for binding studies

The pellet derived from 2L of LB-Ampicillin culture was resuspended in 30 ml ice-cold sonication buffer (50 mM HEPES pH 8.0; 2 mM $MgCl_2$; 20 mM NaCl; 25 mM imidazole; 5 mM β-mercaptoethanol; 1 mg/ml lysozyme (VWR); 3 tablets Complete Protease Inhibitor Cocktail (Roche); 15 µl Benzonase (Merck Millipore)) and cells lysed by sonication on ice. After adding NaCl to a final concentration of 1 M the lysate was incubated for 15 min on ice and subsequently cleared by centrifugation at 20,000 g_{max} and 4°C for 20 min. To the cleared lysate 1.5 ml equilibrated Ni-NTA resin (Quiagen) was added and incubated for 1 h at 4°C under constant slow rotation. The resin was first washed with 20 column volumes of binding buffer (sonication buffer containing 1M NaCl) followed by 20 column volumes of wash buffer (50 mM NaH_2PO_4 pH 8.0; 25 mM Imidazole; 300 mM NaCl; 5 mM β-mercaptoethanol) containing 1% (w/v) Triton X-100 and 10 column volumes of wash buffer supplemented with 25% (w/v) glycerol (Carl Roth). Two additional washing steps were carried out with 4 volumes of wash buffer containing 40 and 60 mM imidazole respectively prior to elution with an imidazole step gradient (100-500 mM imidazole in wash buffer). Directly after elution 2 mM EDTA (Carl Roth) and 1 mM Pefabloc® SC protease inhibitor (Carl Roth) were added to each fraction followed by pooling of protein-containing fractions and dialysis against EMSA buffer (10 mM HEPES pH 7.5; 100 mM KCl, 10% (w/v) glycerol; 5 mM β-mercaptoethanol). After dialysis and addition of 0.2 mg/ml BSA (Carl Roth) and 0.1 % (w/v) Tween20 (Carl Roth) aliquots were stored at -80°C.

Production of recombinant GST-MOC1 in *E. coli* for binding studies

A pellet derived from 1L of E. coli culture in LB-Ampicillin was resuspended in 20 ml ice-cold sonication buffer (PBS pH 7.4; 1 mg/ml lysozyme; 15 µl Benzonase; 2 tablets Complete Protease Inhibitor Cocktail) prior to cell lysis by sonication. The lysate was supplemented with 1mM DTT and 2 mM EDTA and subsequently centrifuged for 10 min at 20,000 x g and 4°C. After addition of Triton-X-100 to 0.1% (w/v) the cleared lysate was incubated with 1 ml glutathione-cellulose (Carl Roth) for 2h at 4°C under constant rotation. After several washing steps with PBS (+0.1% (w/v) Triton-X-100; 1 mM DTT; 2 mM EDTA) GST-MOC1 was eluted using elution buffer (100 mM Tris pH 8.0; 100 mM KCl; 1 mM DTT; 10 mM GSH). Pooled fractions were dialyzed against EMSA buffer