SUPPLEMENTAL MATERIAL

Supplemental Table 1. Primers used for amplification of the expression constructs used in this study.

Name	Sequence 5'-3'	Restriction Site
CrΔ4FAD_NdeI-F	TA **CATATG** ATGAACGCCACGATGCAG	NdeI
CrΔ4FAD_NdeI-R	CG **CATATG** GAACTTGGAGAGCATCAG	NdeI
CrΔ4FAD_XbaI-F2	TA **TCTAGA** ATGAACGCCACGATGCAG	XbaI
CrΔ4FAD_XhoI-R2	CG **CTCGAG** GAACTTGGAGAGCATCAG	XhoI
CrΔ4Cytb5_EcoRI-F	TA **GAATTC** ATG GTCGCTGAGCCCGTCGTTG	EcoRI
CrΔ4Cytb5_XhoI-R	TATA **CTCGAG** GGAGGCGGCAACCTCGG	XhoI
CrFAD13Cytb5_EcoRI-F	TA **GAATTC** ATGTGCAGGCCTACCGATTCC	EcoRI
CrFAD13Cytb5_XhoI-R	TA **CTCGAG** CGCGCTGATGGTCGACAC	XhoI
AtCytb5_EcoRI-F	GC **GAATTC** ATGCCGACACTCACAAAGC	EcoRI
AtCytb5_XhoI-R	GC **CTCGAG** AGTCTTGCGAGAGAAC	XhoI

Supplemental Table 2. Primers used for semi quantitative RT-PCR

Name	**Sequence**	**Size**	**No of cycles**
CrMGD1-int-F	5'- CAGCAGCACTTCTGGCAATGTC -3'	458 bp (genomic)	30
CrMGD1-int-R	5'- CTCCTGGAAAGCCGCTTTGATG -3'	290 bp (cDNA)	
CrDGD1-int-F	5'- GGTTCTTCGAGCAGTTCC -3'	756 bp (genomic)	30
CrDGD1-int-R	5'- GACGGCTTGTAGTCAGTG -3'	322 bp (cDNA)	
CrRACK1-int-F	5'- GTGCTGTCCGTGGCTTTCTC -3'	637 bp (genomic)	24
CrRACK1-int-R	5'- GGCCCACCAGGTTGTTCTTC -3'	253 bp (cDNA)	
CrΔ4FAD-int-F	5'- TGTGGGCGCTCTTCCCCTTC -3'	891 bp (genomic)	34
CrΔ4FAD-int-R	5'- GCGCTCTCGTCCAGGTTCTC -3'	325 bp (cDNA)	

References

Guschina, I.A., and Harwood, J.L. (2006) Lipids and lipid metabolism in eukaryotic algae. *Prog. Lipid Res.* 45, 160-186.

Meyer, A., Kirsch, H., Domergue, F., Abbadi, A., Sperling, P., Bauer, J., Cirpus, P., Zank, T.K., Moreau, H., Roscoe, T.J., Zähringer, U., and Heinz, E. (2004) Novel fatty acid elongases and their use for the reconstitution of docosahexaenoic acid biosynthesis. *J. Lipid Res.* 45, 1899-1909.

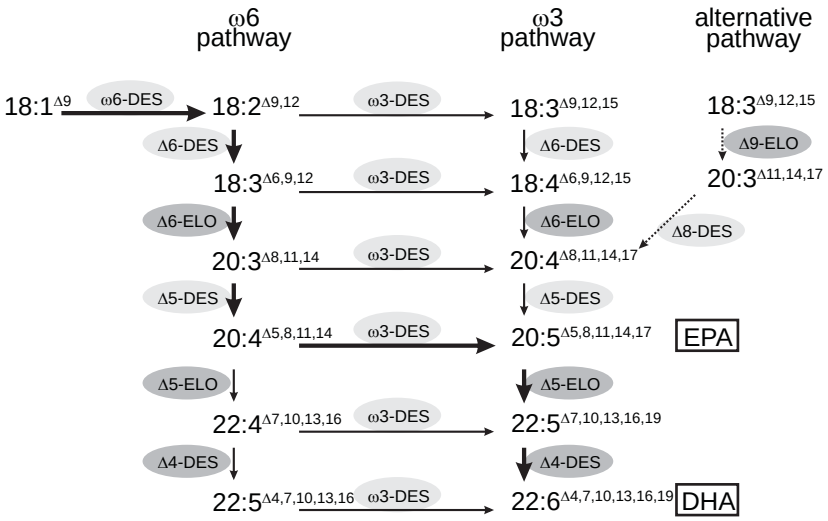
Supplemental Figure 1. Schematic representation of biosyntheses of polyunsaturated acyl groups.

Synthesis of PUFAs in most microalgae involves stepwise action of specific fatty acid desaturases and elongases (modified after (Meyer et al., 2004)). While the Δ6- and Δ5-elongases exclusively utilize acyl-CoAs, the substrates for most desaturases are lipids (mostly phosphatidylcholine, PtdCho or monogalactosyldiacylglycerol, MGDG). The dominant order of the steps in most microalgae is indicated with bold arrows. Some algae such as Euglena or Isochrysis synthesize EPA and DHA via an alternative pathway which is Δ6-desaturase- and Δ6-elongase independent. Instead $18{:}3^{\Delta 9,12,15}$ is elongated to $20{:}3^{\Delta 11,14,17}$ and then desaturated at the Δ8-position (Guschina and Harwood, 2006). The Δ4-desaturase independent pathway of DHA synthesis from EPA (the so-called “Sprecher pathway”) is displayed with gray arrows. It is found in mammals.

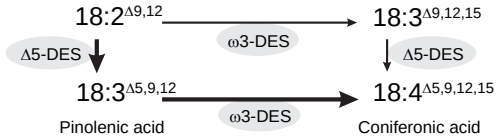
(b) Synthesis of pinolenic and coniferonic acids in Chlamydomonas. Chlamydomonas does neither contain a Δ6- nor Δ5-elongase and thus cannot synthesize C_{20} or C_{22} fatty acids. The alga also lacks a Δ6-desaturase. But the activity of a Δ5-desaturase results in the accumulation of $18{:}3^{\Delta 5,9,12}$ and $18{:}4^{\Delta 5,9,12,15}$ in ER lipids. These fatty acids are also known as pinolenic and coniferonic acid since they were first discovered in pine trees.

(c) Synthesis of hexadecatetraenoic acid (16:4) in the plastid of Chlamydomonas. The synthesis of $18{:}3^{\Delta 9,12,15}$ and $16{:}3^{\Delta 7,10,13}$ is accomplished in the same way as in vascular plants. All desaturation steps most likely occur on MGDG. The synthesis of $16{:}4^{\Delta 4,7,10,13}$ involves the activity of an additional Δ4-desaturase (named CrΔ4FAD). Since 16:4 is limited to MGDG, CrΔ4FAD is expected to be located in the plastid, using mainly $16{:}3^{\Delta 7,10,13}$ as substrate. Abbreviations: DES – desaturase, DHA – docosahexaenoic acid, ELO – elongase, EPA – eicosapentaenoic acid.

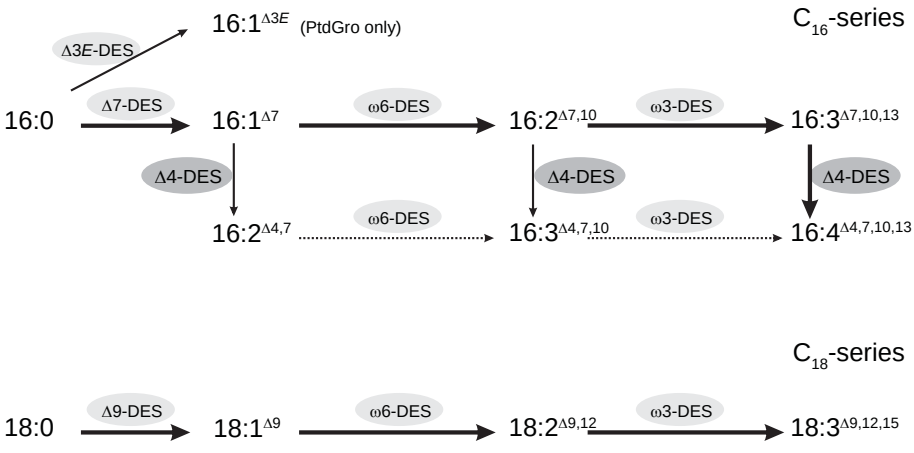
A Synthesis of EPA and DHA (in the ER of microalgae)



B PUFA biosynthesis in the ER of Chlamydomonas



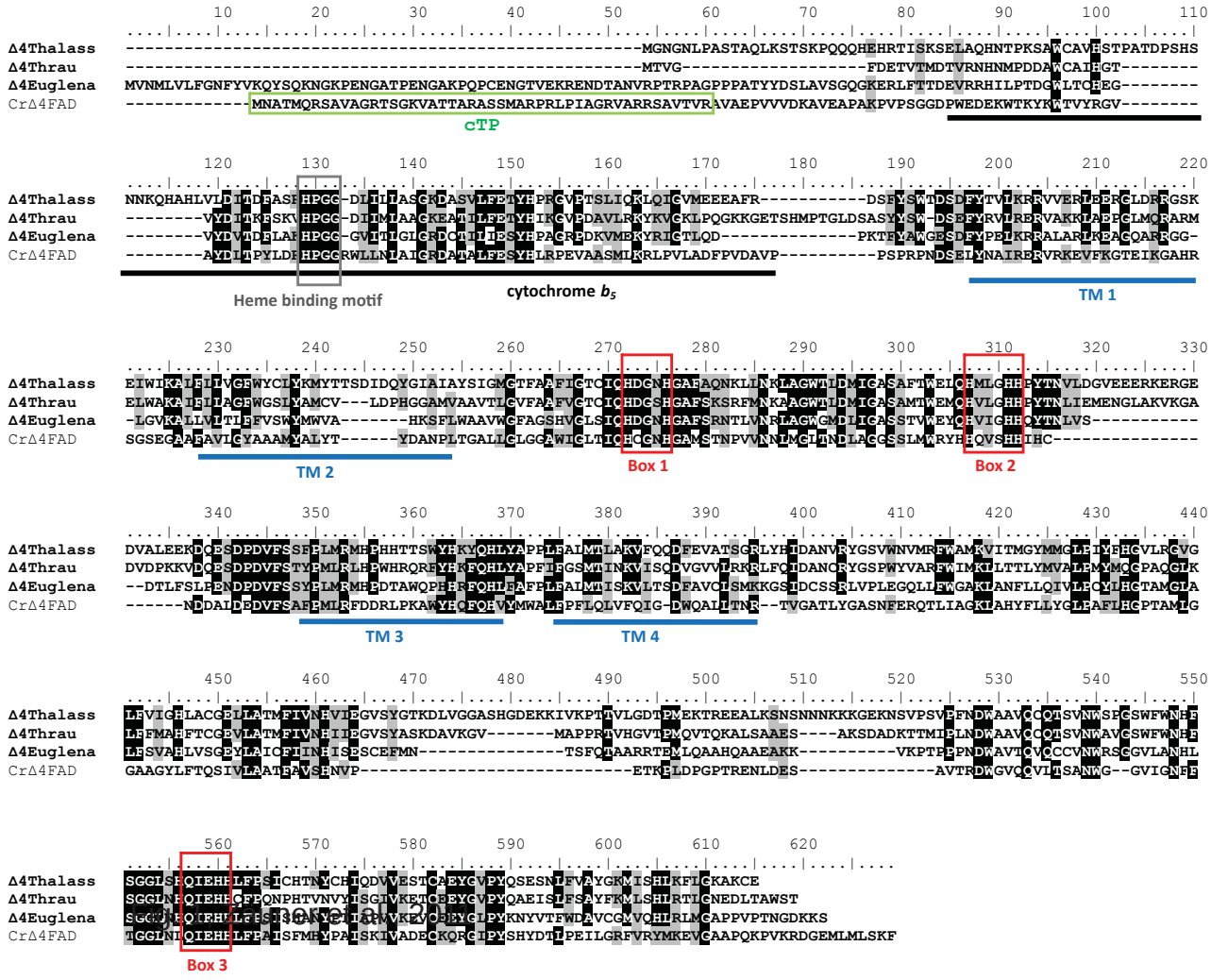
C PUFA biosynthesis in the plastid of Chlamydomonas



Supplemental Figure 1, Zauner et al, 2012

Supplemental Figure 2. Protein sequence alignment of CrΔ4FAD and known Δ4-desaturases.

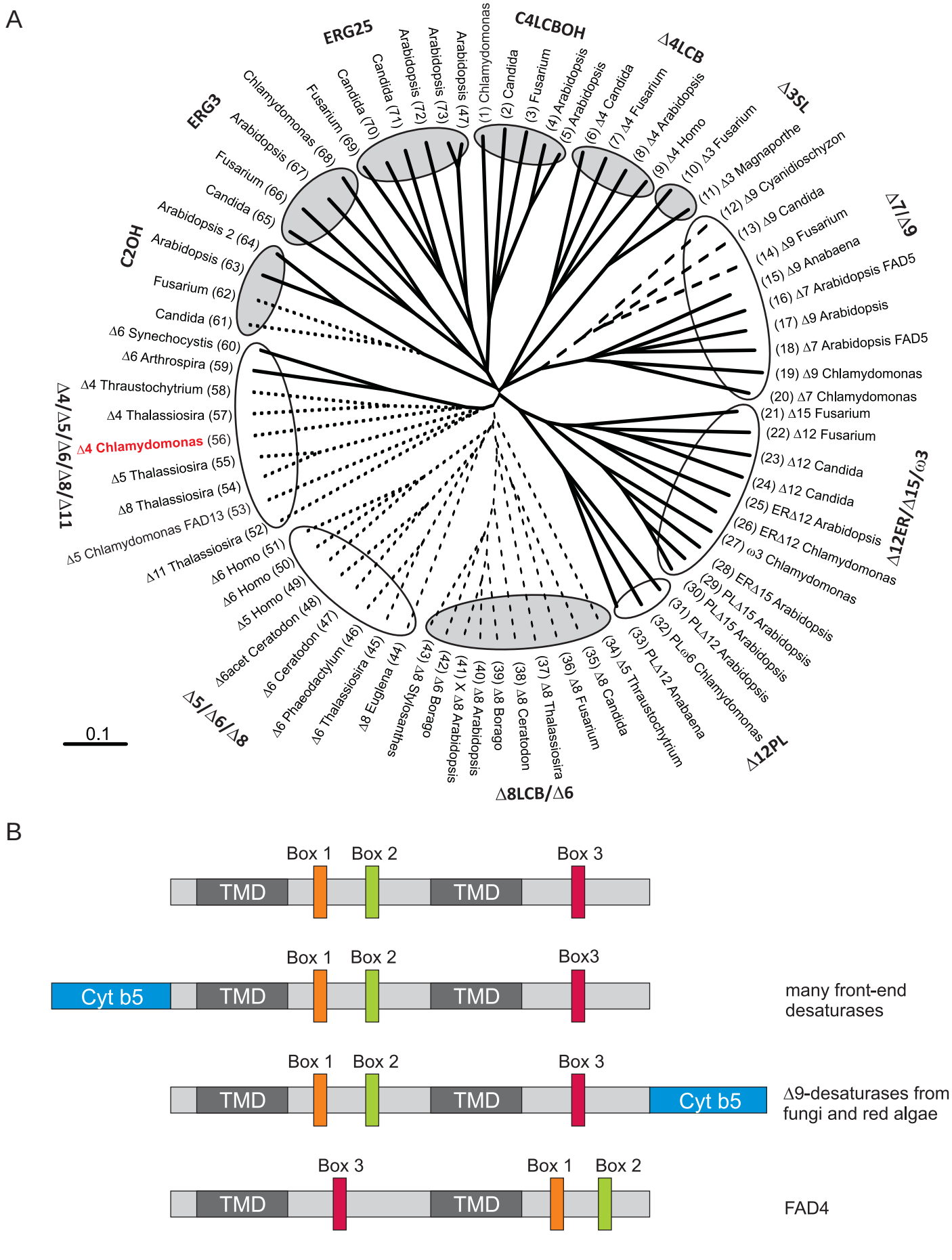
The Δ4-desaturases from *Thalassiosira pseudonana* (AAX14506), *Thraustochytrium tricornutum* (CAI58877) and *Euglena gracilis* (AAQ19605) were included in the ananlysis. Positions of the transmembrane helices (TM), histidine boxes, cytochrome b_5-domains, and the putative chloroplast transit peptide (cTP) of CrΔ4FAD are indicated. Note that the N-terminal sequence extension found in the sequence from Euglena was not recognized as transit peptide by any of the tested prediction programs.



Supplemental Figure 2, Zauner et al, 2012

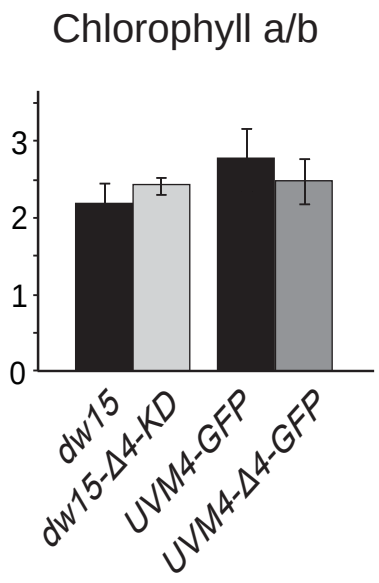
Supplemental Figure 3. Supplemental Figure. Comparison of different fatty acid desaturases. **(A)** Regioselectivity dendrogram of desaturase-like sequences. CrΔ4FAD is marked in red. Regioselectivities are marked by numbers (Δ- and ω-desaturases) and subcellular localization (PL=plastid, ER=Endoplasmatic Reticulum). The branches labeled C2OH and C4LCBOH refer to the sphingolipid modifying enzymes acyl amide α-hydroxylases and long chain base (LCB) C4-hydroxylases, whereas Δ4LCB and Δ8LCB refer to LCB desaturases. The branch containing acyl amide Δ3 desaturase sequences is labeled Δ3SL; ERG3 and ERG25 refer to C5-sterol desaturase and C4-sterol methyl oxidase sequences, respectively. All sphingolipid and sterol-modifying enzyme groups are highlighted by a grey background. Cytochrome b_5-fusion proteins are indicated by *dashed lines* (C-terminal fusion) and *dotted lines* (N-terminal fusion), respectively. GenBank™ protein accession numbers are as follows: (1) XP_001699995, (2) XP_715359, (3) XP_383758, (4) NP_563944, (5) NP_177122, (6) XP_722116, (7) XP_390550, (8) NP_192402, (9) AF466375, (10) XP_383758, (11) XP_368852, (12) AB006677, (13) XP_714854, (14) XP_386360, (15) BAA03434, (16) NP_565721, (17) NP_172098, (18) NP_172098, (19) XM_001701218, (20) XP_001694618, (21) XP_388066, (22) XP_385960, (23) XP_722258, (24) XP_714324, (25) NP_850139, (26) XP_001691669, (27) XP_001689663, (28) NP_180559, (29) NP_187727, (30) NP_196177, (31) NP_194824, (32) XP_001693068, (33) YP_324705, (34) AAM09687, (35) XP_719958, (36) XP_390021, (37) XP_002291331, (38) XP_001778179, (39) AAG43277, (40) NP_191717, (41) NP_182144, (42) AAC49700, (43) ABV23278, (44) AAD45877, (45) XP_002291529, (46) XP_002182901, (47) CAB94993, (48) CAB94992, (49) AAF29378, (50) NP_004256, (51) NP_068373, (52) AAS75335, (53) XP_001698534, (54) AAX14502, (55) XP_002296867, (56) JN089704, (57) AAX14506, (58) AAN75710, (59) CAA60573, (60) NP_441824, (61) XP_717303, (62) XP_385340, (63) NM_129030, (64) NM_11820, (65) XP_713612, (66) XP_382678, (67) NP_192948, (68) XP_001701457, (69) XP_390006, (70) XP_722703, (71) XP_713456, (72) NP_567670, (73) NP_850133, (74) NP_563789.

(B) Schematic structure of desaturase-like sequences. Positions of Cytochrome b_5 domains (Cyt b5), histidine boxes (Box1-3) and transmembrane domains (TM) are indicated. Note that the positions of the histidine boxes of FAD4 are different from that found in other sequences.



Supplemental Figure 3, Zauner et al, so12

Supplemental Figure 4. Chlorophyll a/Chlorophyll b ratio of transgenic Chlamydomonas lines. Pigments were isolated, their absorbance spectra detected in 80 % acetone and used for calculation of chlorophyll a and chlorophyll b content. Data were obtained from 6 independent experiments.



Supplemental Figure 4, Zauner et al, 2012