

# PNAS

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Supplementary Information for

## **Chloroplast Sec14-like 1 (CPSFL1) is essential for normal chloroplast development and affects carotenoid accumulation in *Chlamydomonas reinhardtii***

José G. García-Cerdán<sup>1,2§\*</sup>, Eva M. Schmid<sup>3</sup>, Tomomi Takeuchi<sup>4</sup>, Ian McRae<sup>2</sup>, Kent McDonald<sup>5</sup>, Nichakarn Yordduangjun<sup>6</sup>, Ahmed M. Hassan<sup>11</sup>, Patricia Grob<sup>1,6</sup>, C. Shan Xu<sup>7</sup>, Harald F. Hess<sup>7</sup>, Daniel A. Fletcher<sup>3,8,9,10</sup>, Eva Nogales<sup>1,6,11</sup>, and Krishna K. Niyogi<sup>1,2,11\*</sup>

<sup>1</sup>Howard Hughes Medical Institute, University of California, Berkeley, CA 94720

<sup>2</sup>Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102

<sup>3</sup>Department of Bioengineering, University of California, Berkeley, CA 94720

<sup>4</sup>MSU-DOE Plant Research Laboratory and Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824

<sup>5</sup>Electron Microscope Lab, University of California, Berkeley, CA 94720

<sup>6</sup>Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

<sup>7</sup>Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA.

<sup>8</sup>UC Berkeley/UC San Francisco Graduate Group in Bioengineering, Berkeley, CA 94720

<sup>9</sup>Division of Biological Systems and Engineering, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

<sup>10</sup>Chan Zuckerberg Biohub, San Francisco, CA 94158

<sup>11</sup>Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

§Present address: Molecular, Cellular and Developmental Biology, University of Colorado at Boulder, Boulder, CO 80309.

\*To whom correspondence may be addressed: Email: [Jggarcia.cerdan@gmail.com](mailto:Jggarcia.cerdan@gmail.com) and [niyogi@berkeley.edu](mailto:niyogi@berkeley.edu)

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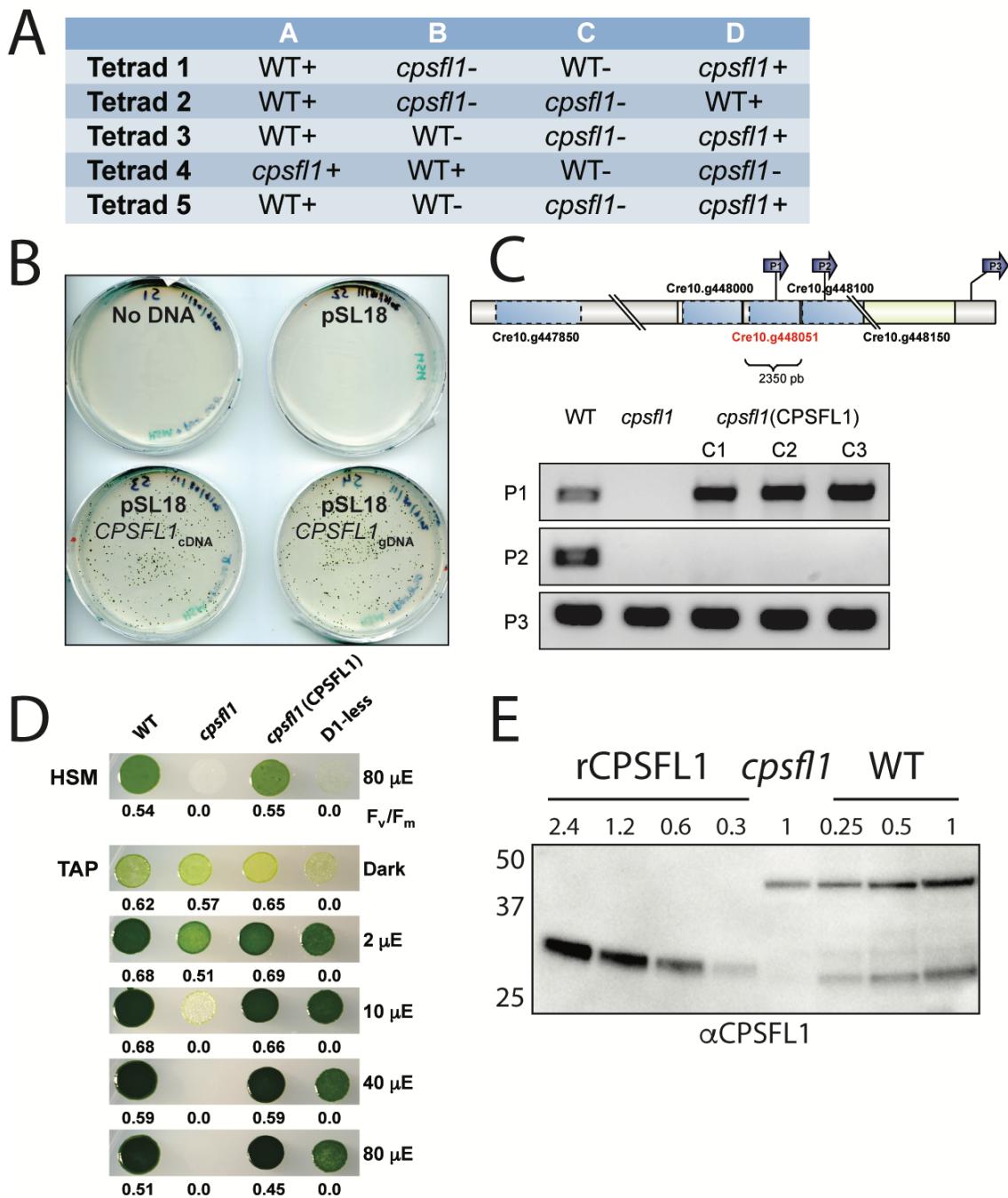
## Supplementary Materials and Methods

**Chemical reagents.** HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), DTT (Dithiothreitol), NaCl (Sodium chloride) and sucrose, were purchased from ThermoFisher Scientific. Atto 390-DOPE was purchased from ATTO-TEC. DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine), and POPA (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphate) were purchased from Avanti Polar Lipids (Alabaster, AL). All purchased chemical reagents were used without further purification.

**Lipid analysis.** Lipid abbreviations are: MGDG, Monogalactosyldiacylglycerol; DGDG, Digalactosyldiacylglycerol; DGTS, Diacylglyceryltrimethylhomoserine; PE, Phosphatidylethanolamine; PG, Phosphatidylglycerol; SQDG, Sulfoquinovosyldiacylglycerol; PI, Phosphatidylinositol; PA, Phosphatidic acid. For each biological replicate, 100 ml of cultures were grown in TAP liquid medium to an OD<sub>750</sub> of ~0.4 in the dark at 24°C. For both constant dark and light treated (100 μmol photons m<sup>-2</sup> s<sup>-1</sup>) samples, total lipids were extracted from 7 X 10<sup>8</sup> cells in methanol: chloroform: 88% formic acid (2: 1: 0.1, v/v/v), and the separation of the organic phase was achieved by the addition of 0.5 volume of extraction buffer (1M KCl; 0.2M H<sub>3</sub>PO<sub>4</sub>) followed by centrifugation. The organic phase was dried to completion under nitrogen gas, resuspended in 125 μl of chloroform, and 25 μl of lipid extracts were separated by thin layer chromatography (TLC) on Silica Gel 60 plates (EMD chemicals) using the following solvent systems: for MGDG and DGTS, chloroform: acetone: methanol: acetic acid: water (80: 32: 16: 3: 3, v/v) ; for PE, PG, DGDG, SQDG and PI, chloroform, methanol, acetic acid: water (75:13:9:3, v/v); for PA, chloroform: methanol: ammonium hydroxide (65: 25: 5, v/v); and for neutral lipids, petroleum ether: diethyl ether: acetic acid (80: 20: 1, v/v). For the separation of PA, TLC plates were pretreated with ammonium sulfate(1). The identity of individual lipid was confirmed based on the separation of known lipid standards and the use of dyes specific to the lipid head group. Following the visualization of lipids by brief iodine staining, fatty acid methyl esters (FAMEs) of each lipid as well as total cellular lipids were prepared in 1 M methanolic HCl by heating the samples at 80°C for 20 min. 5 μg pentadecanoic acid was used as an internal standard. Following the phase separation with hexane and 0.9 % (w/v) NaCl, the organic phase containing FAMEs was completely dried under nitrogen gas, resuspended in 60 μl of hexane, and quantified by gas chromatography with flame ionization detection (Agilent Technologies, 7890A GC system) using a temperature and run profile previously described in (2) with minor modifications.

**Phylogenetic tree construction.** CRAL-TRIO domain containing proteins were retrieved by BLAST search (blastp) against the reference protein yeast phosphatidylinositol transfer protein (Sec14p) (3). Retrieved sequences were curated manually against *Arabidopsis* and *Chlamydomonas* genome annotations (Phytozome v12). CRAL-TRIO and GOLD protein domains were annotated by scanprosite tool ([prosite.expasy.org](http://prosite.expasy.org)). A maximum likelihood phylogenetic analysis

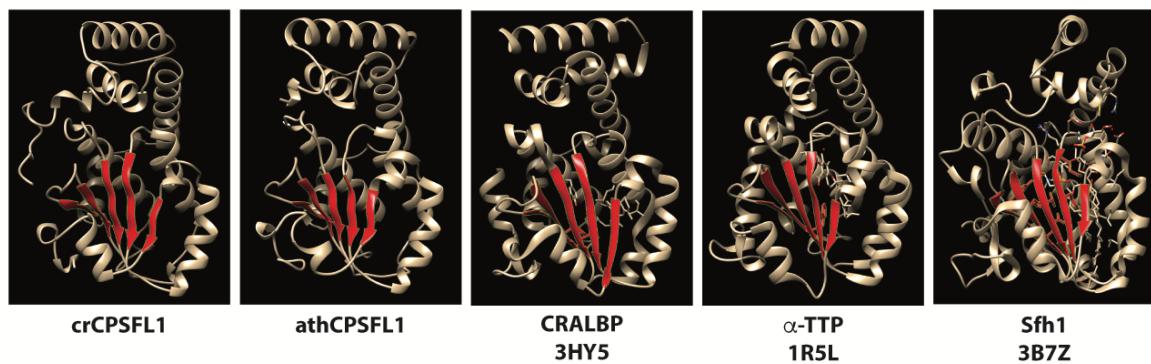
was performed following the server [http://phylogeny.lirmm.fr/phylo\\_cgi/index.cgi](http://phylogeny.lirmm.fr/phylo_cgi/index.cgi) mode “a la carte” (4). Fig Tree v1.4.2 was used to view the phylogenetic tree. CPSFL1 secondary structure prediction were analyzed with I-TASSER (5) . I-TASSER compares its protein structure simulation to all structures in the Protein Database Bank (PDB) library, by using a TM-align algorithm for sequence-order independent protein structure comparisons. The C-score is a confidence score for estimating the quality of predicted models ranging from low confidence (-5) to high confidence (+2). Image renders were performed with UCSF Chimera (6).



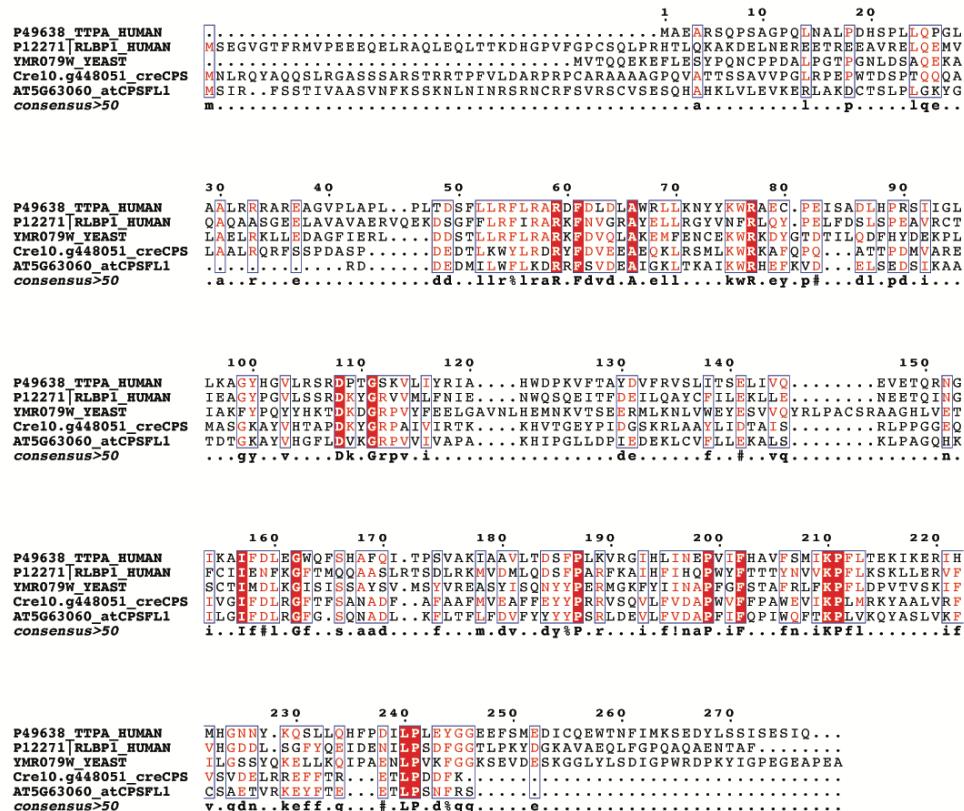
**Fig. S1. Genetic analysis and complementation of *cpsfl1*.** (A) Summary of tetrad analysis from a backcross of the mutant (mating type +) to the WT (mating type -), showing mating type (+/-) and *cpsfl1* phenotypes of five tetrads progeny. A-D are the four progeny of a selected tetrad. (B) Complementation of *cpsfl1* mutant was performed with either *CPSFL1* cDNA or genomic DNA (gDNA) under the control of *PSAD* promoter. pSL18 is the empty vector control. (C) Schematic representation of the location of primers P1, P2, and P3 within chromosome 10, and PCR analyses from WT, *cpsfl1*, and three complemented lines. (D) Growth

phenotypes of WT, *cpsf1*, *cpsf1*(CPSFL1), and D1-less (*fud7* mutant) cells spotted onto minimal (HSM) and TAP solid agar media, grown under different light irradiances, as indicated. PSII activity was assessed by measuring PSII maximum quantum efficiency ( $F_v/F_m$ ). The term  $\mu E$  represents  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . ( $E$ ) CPSFL1 protein quantification from WT and *cpsf1* mutant dark-grown whole cells. Loading of recombinant purified CPSFL1 protein dilutions are indicated in ng and whole cells loading of 1 corresponds to  $\sim 1 \times 10^6$  cells.

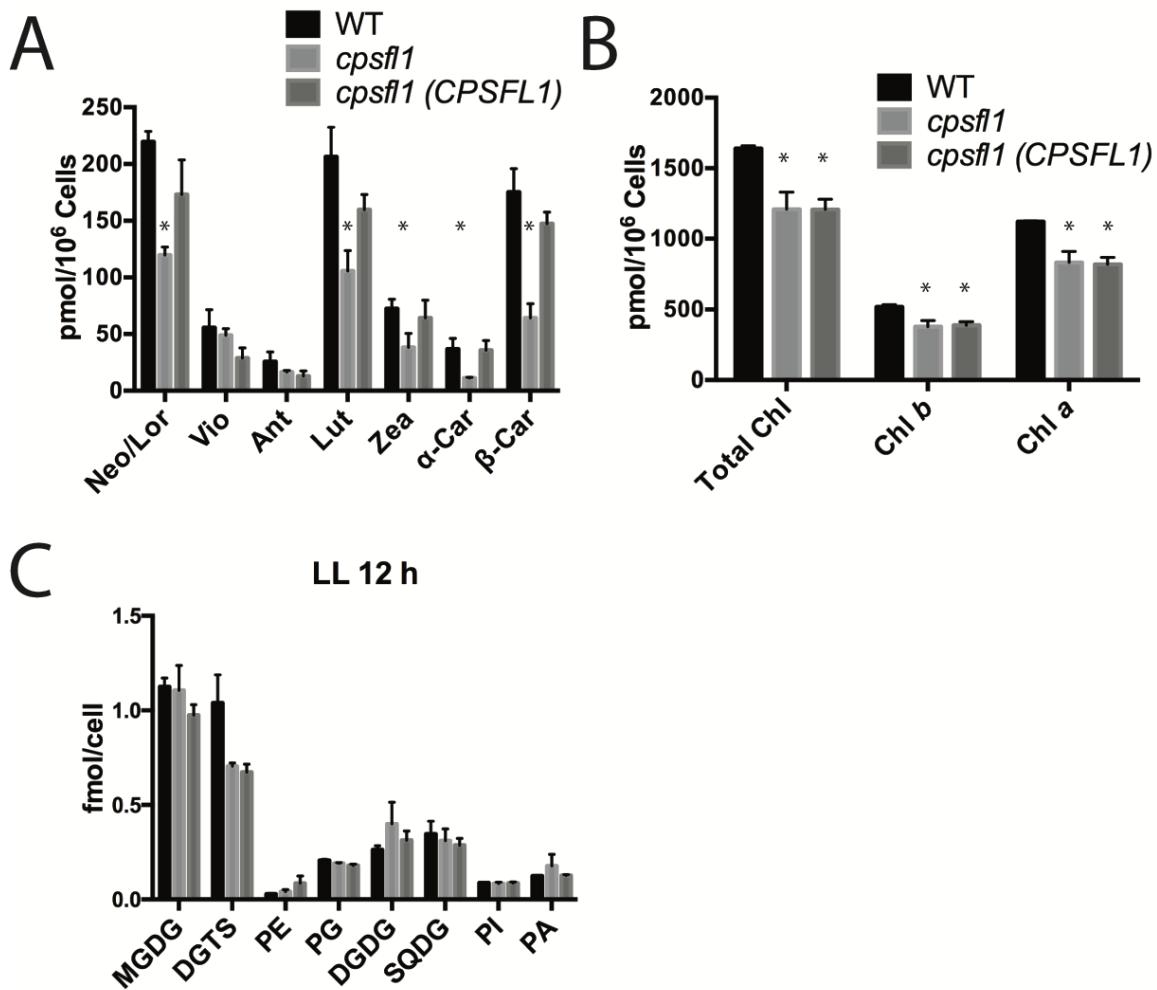
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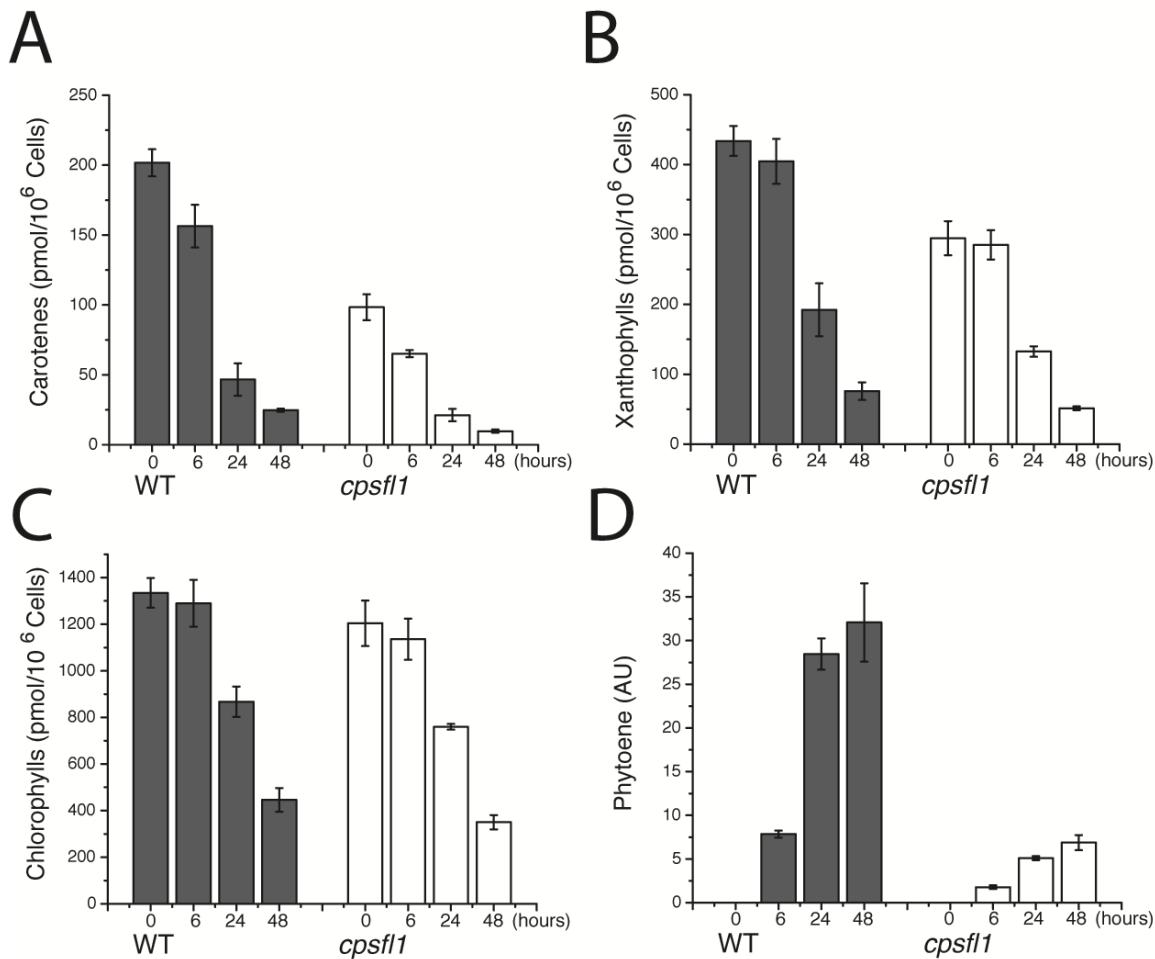
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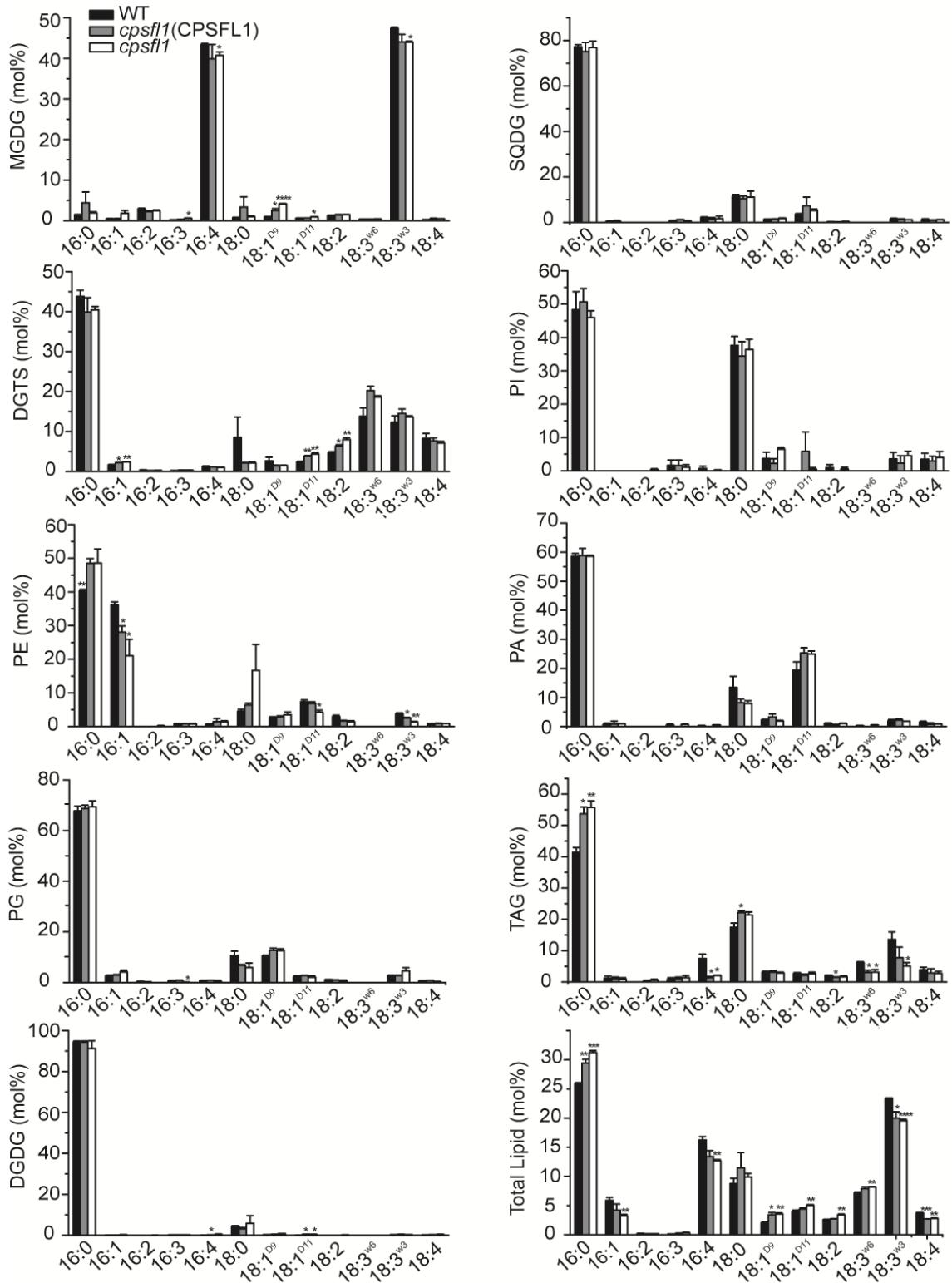
**Fig. S2. CRAL-TRIO domain proteins exhibit conserved predicted protein structure.** (A) Predicted protein structures of Arabidopsis and Chlamydomonas CPSFL1 and protein crystal structures of human cellular retinaldehyde-binding protein (CRALBP), human alpha-tocopherol transfer protein (α-TTP), and yeast Sec14 homolog (Sfh1); PDB: 3HY5, 1R5L, and 3B7Z, respectively. The predicted β-sheets of the CRAL-TRIO domain are depicted in red. (B) Multiple protein sequence alignment between Arabidopsis CPSFL1, Chlamydomonas CPSFL1, human CRALBP, human α-TTP, and yeast Sec14 homolog (Sfh1).



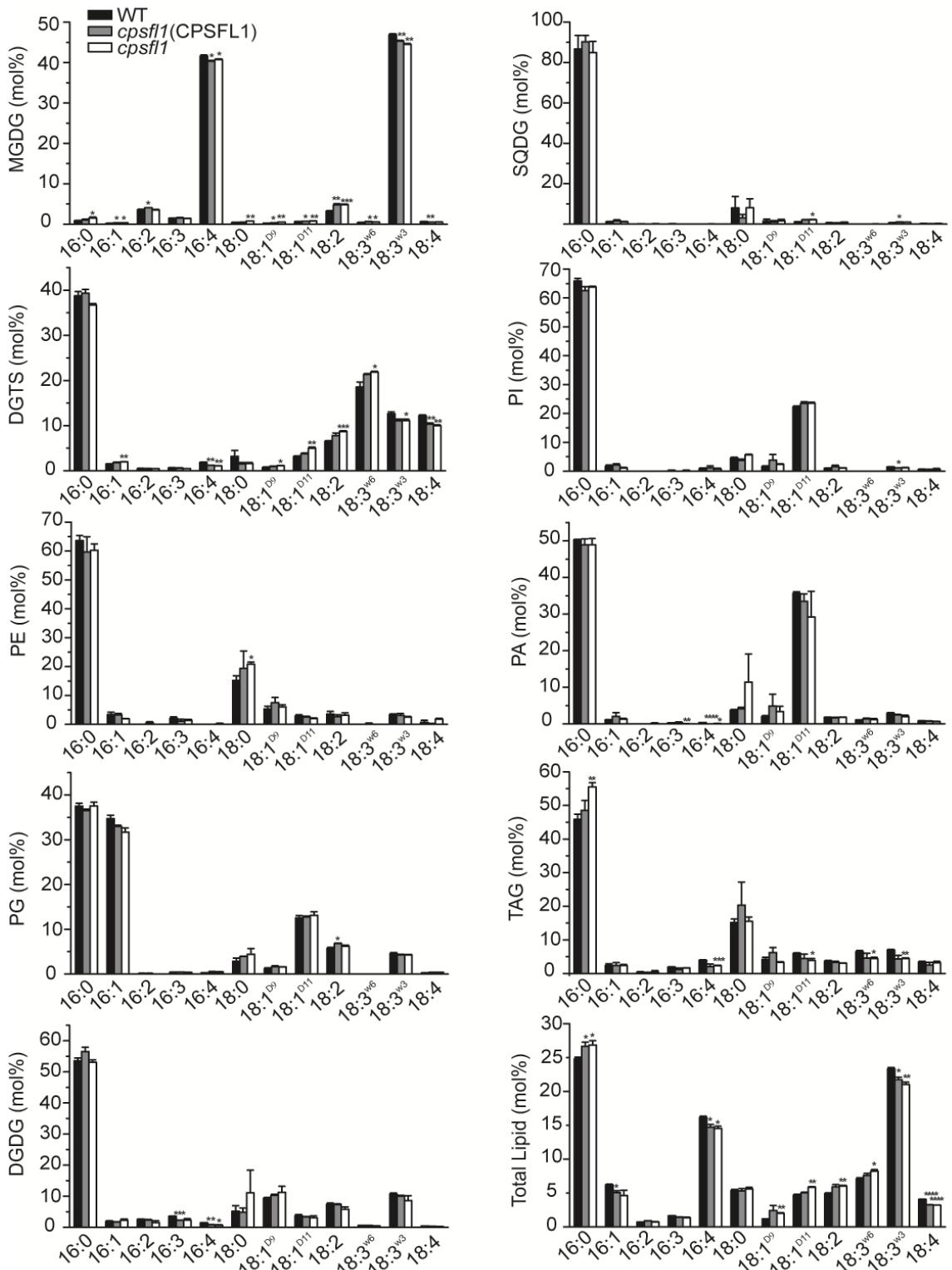
**Fig. S3. HPLC pigment analysis of WT, *cpsf1*, and *cpsf1(CPSFL1)* complemented cells.** (A) Carotenoids. (B) Chlorophylls. (C) Lipids. Cells for pigment analysis were treated with HL for 1 h. Cells for lipid analysis were grown in the dark and then shifted to low light for 12 h. Abbreviations for the different pigments are found in Methods. Data represent means  $\pm$  SD (n=3). Significantly changed pigments relative to the WT (two-tailed Student's t-test; P<0.05) are marked with asterisks.



**Fig. S4. HPLC pigment analysis of dark-grown WT and *cpsf1* mutant cells treated with the herbicide norflurazon for 48 h. (A) Carotenes. (B) Xanthophylls. (C) Chlorophylls. (D) Phytoene. Data represent means  $\pm$  SD (n=2).**



**Fig. S5. Acyl group compositions of different lipid species of dark-grown WT, *cpsfl1*, and *cpsfl1(CPSFL1)* complemented line.** Data represent means  $\pm$  SD ( $n=3$ ).



**Fig. S6. Acyl group compositions of different lipid species of WT, cpsfl1, and cpsfl1(CPSFL1) complemented line grown in the dark and then shifted to low light for 12 h. Data represent means  $\pm$  SD (n=3).**

**Table 1.** Oligonucleotides used in this study.

Primers name	Use of Primer	Orientation	Sequence 5'- 3'
gDNA_CPSFL1 F	genomic DNA cloning	Forward	CGCTAATGAGATGGACGTGA
gDNA_CPSFL1 R	genomic DNA cloning	Reverse	AATTACGTGCCCTGTTCCA
cDNA_CPSFL1 F	cDNA cloning	Forward	TCACCGAACTCATCGCAATG
cDNA_CPSFL1 R	cDNA cloning	Reverse	AATTACGTGCCCTGTTCCA
CPSFL1g/cDNA F	gDNA/cDNA cloning into pSL19	Forward	ACAGAATTCAATAGCGCTCGTCAGCATT
CPSFL1g/cDNA R FLAG	gDNA/cDNA cloning into pSL19	Reverse	ACAGGATCCTCACTTGTGTCATCGTCCCTGTAGTCCTTGAAGTCGTCAAGGCAGAG
oeCPSFL1 F	pET28(a+) cloning	Forward	GGAATTCATATGGCTGCTGCTGCCGGGCCGAG
oeCPSFL1 R FLAG	pET28(a+) cloning	Reverse	ACAGGATCCTCACTTGTGTCATCGTCCCTGTAGTCCTTGAAGTCGTCAAGGCAGAG
oeGFP_F	pET28(a+) GFP	Forward	AGCCATATGGCCAAGGGCGAGGAGCTGTT
oeGFP_R	pET28(a+) GFP	Reverse	ACAGGATCCTACTTGTACAGCTCGTCCA
P1F	Genotyping	Forward	ATTGTTGGCATCTTCGACCT
P1R	Genotyping	Reverse	AACTCTCTCCGCAAGCTCATC
P2F	Genotyping	Forward	CTTCGCTTGTGTTGTGA
P2R	Genotyping	Reverse	ACCCTTGCTGTTGCAATAC
P3F	Genotyping	Forward	CAGCTGACATTGCCGACTT
P3R	Genotyping	Reverse	CAGGTAGCGTTGCTTGAGTG
(MT+)F	Mating type	Forward	GATTGCTCTGCGTTGCAGA
(MT+)R	Mating type	Reverse	CCGCACATGAGACGTTACAG
(MT-)F	Mating type	Forward	GCCACGAAGGCAGTTACATT
(MT-)R	Mating type	Reverse	TGGCGTACCTTCTGTAGGG

## SI References

1. Benning C & Somerville CR (1992) Isolation and genetic complementation of a sulfolipid-deficient mutant of *Rhodobacter sphaeroides*. *J Bacteriol* 174(7):2352-2360.
2. Zauner S, Jochum W, Bigorowski T, & Benning C (2012) A cytochrome b5-containing plastid-located fatty acid desaturase from *Chlamydomonas reinhardtii*. *Eukaryot Cell* 11(7):856-863.
3. Sha B, Phillips SE, Bankaitis VA, & Luo M (1998) Crystal structure of the *Saccharomyces cerevisiae* phosphatidylinositol-transfer protein. *Nature* 391(6666):506-510.
4. Dereeper A, et al. (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res* 36(Web Server issue):W465-469.
5. Yang J, et al. (2015) The I-TASSER Suite: protein structure and function prediction. *Nat Methods* 12(1):7-8.
6. Pettersen EF, et al. (2004) UCSF Chimera--a visualization system for exploratory research and analysis. *J Comput Chem* 25(13):1605-1612.

**Movie S1.** FIB-SEM 3D reconstruction video of WT cell.

**Movie S2.** FIB-SEM 3D reconstruction video of *cpsf1* mutant cell.

**Dataset S1.** Flanking DNA insertion sequence.

```
>CAL028_01_06
AAGGAACCGCCTGGCGGCTCTGCAGCACAGCACCGCGCTATGACGAGGCCAGGAGT
GGCAGAGGCGTCTGGCGCCGTGGCCGCTGGAGGACAGCATGTTCAACCCGTGGGGCG
CCGTAATAGCAGCAGATGGTGCCGGCAACATGGCGCAG
```

**Dataset S2.** *CPSFL1* Genomic DNA, CDS and *CPSFL1* protein sequences.

Genomic *CPSFL1* DNA sequence. The blue uppercase letters represent UTR regions, the red uppercase letters represent exons, and the black uppercase letters represents introns.

```
CGCCAGAGCGCTCGCGCTGCGCAGGCTTACACGTAATGTCATTGCTACTT
CATTGCTATCCTTCGCATATGTATCGCTTGAACAAATAACAATCTATGTTAGT
TAGCGCGCTCAGCAGCGATGCCATCGAAAAAACACAATGCCCATGTAA
CTGAGAACTTGAAGCTTACAAACGCTTATCATGATTATTGCAGTTATAATAGA
CTTACATACATTATCACTGTTCTCTGGGCTGGGGTGGCGAGGTAGC
GGCAAGCACGTCGTTGTCACCGAACATCGCAATGAACTTGAGACAGTAC
GCGCAGCAGAGCTGGGGAGCTAGCTCTGCGCTTACAAGGCG
CACGCCGTTGTGCTTGATGCGCACCAGCGCCATGCGCTCGTGCTGCTG
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GTGTGGCTATTGGTCGTGCCCTGCCACAATGGTATTCCCCAAAGGTCGGA
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#### CPSFL1 CDS:

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#### CPSFL1 protein sequence:

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**Dataset S3.** Protein sequences and accession numbers.

>P49638\_TTPA\_HUMAN Alpha-tocopherol transfer protein  
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RFLRARDFDLDLAWRLLKNYYKWRAECPEISADLHPRSIIGLLKAGYHGVLRSR  
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Q

>P12271|RLBP1\_HUMAN Retinaldehyde-binding protein 1 OS=Homo sapiens  
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>NP\_195629.2 (AT4G39180)

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>NP\_199562 (AT5G47510)

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>NP\_189128 (AT3G24840)

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>NP\_568006.1 (AT4G36490)

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>NP\_173669 (AT1G22530)

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EEEKPAVVTIEKAFAAADQEEETKTVEAVEESIVSITLPETAAYVEPEEVSIWGIPLL  
EDERSDVILLKFLRARDFKVKEAFTMLKNTVQWRKENKIDDLVSEDLEGSEFEK  
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WWYIPYYKTFGSIITS PRTRS KMVLSGPSKSAETIFKYVAPEVVPVKYGGLSKDS  
PFTVEDGVTEAVVKSTS KYTIDL PATEG STLSWELRVLGADVS YGAQFEP SNEA  
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>NP\_200427 (AT5G56160)

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GKAHPGKLM EVTTIERYLK YHVQEFERTLQEKLPAC SVA AKRRVTTTTILDVE  
GLGMKNFTPTAANLLATIAK VDCNYY PETLHRMFIVNAGIGFRSFLWPAAQKLL  
DPMTI AKIQVLEPRSLSKLLEAIDSSQLPEFLGGLCKCPNEGGCLRSNKGPWND  
PEI VELVHHMEVNNVPQTTTAPLHVRDYDSTTCTISP KETLKEEPEPEEYYSST  
GSRSSMHTCIVPPLSDKASTSDGDKITTVESIESAQSQLLDADTENTFANTS VR  
EGGQILRGALREKINSENIFHLVKILLVFPKL FVLF GFLLPGYWQRQNTVVVPD  
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>NP\_565387.1 (AT2G16380)

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>NP\_177361 (AT1G72160)

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>NP\_177360.1 (AT1G72150)

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ESAVAPVVVETVAVAEEAEPVEPEEVSIWGVPLLQDERSDVILTCKFLRARDFKVK  
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LGKRALWQFIRRAVKQFEDNYPEFAAKELFINVPWWYIPYYKTFGSIITSRTRS  
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>NP\_192655.2 (AT4G09160)

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AEDSEQTHEVTPEKETVKSEFLNHVAEDSEQTHEVTPETETVKSEVLNHAED  
SEQPRGVTPPTPETETSEADTLLVTSETTEPNHAAEDYSETEPSQKLMLEQR  
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