Supplemental Table 1. Oligonucleotide primers used in this study.

All primer sequences are written in 5' to 3' direction.

Name	Sequence
qRT-F	GAGCCTGAGTACGCCAAGTC
qRT-R	AATATCGTGCTTGGGAGTGG
RACK1-F	GTCATCCACTGCCTGTGCTT
RACK1-R	CCTTCTTGCTGGTGATGTTG
DGTT1-F	AGTCCCACGGAGGCTGAG
DGTT1-R	ACACCCTTGCCGTACTTGTC
4DF	ATGAGCAGCAAAATATCAGATCTTACATCTACACAAAATAAGTGAA GCTTGATATCGAAT
4DR	TTATTGAGTAAAACTGGTGGTGCTTTTGTGCTGCGTTGAAGATACGA CTCACTATAGGGC
KMF	TGAAGCTTGATATCGAATCGGATCCCCGGGTTAATTAA
KMR	TACGACTCACTATAGGGCGAATTCGAGCTCGTTTAAAC
3DF	ATGAAGGAAACGGCGCAGGAATACAAGGTGTCTGCTGTAATATGAA GCTTGATATCGAAT
3DR	CTACCTACTCCGTCTTGCTCTTATTATGTCGTCTAATTTAAATACGAC TCACTATAGGGC
595F	TATCCATATGACGTTCCAGATTACGCTGCTCAGTGCGGCCGCATGGG TTGCGGAGCGAGC
595R	GAATTTCGACGGTATCGGGGGGATCCACTAGTTCTAGCTAGACTAAC CGACCTCCTGCTT
LICF	AAAGAGGCGCGTNATGGGTTGCGGAGCGAGC
LICR	CGGAAGGCGCGTTACGCTGCCGGCGGCGG
P1	CTAGTAAGAGCCTCCTCATTGGGATATCTCGCTGATCGGCAC
P2	CATGGGGGTGGTGGTGATCAGCGCTATATCTTAATGAGGAGGCTCTT G
M1	CTAGCAAGAGCCTCCTCATTAAGATATAGCGCTGATCACCACCACC
M2	CCCATGGTGCCGATCAGCGAGATATCCCAATGAGGAGGCTCTTA

N represents a random nucleotide.

Supplemental Table 2. Putative lipase-encoding genes selected for expression in yeast.

Name	Protein ID in JGI *Chlamydomonas* 4.0 database	Reason for selection and reference	Result
LIP1	184308/519543	Down-regulation under nitrogen (N) deprivation (1)	519543 was the right model according to sequencing of the coding sequence amplified by PCR. This gene complemented the lipase null mutant of yeast
LIP2	191373	Up-regulation under N deprivation (1)	Not expressed (data not shown)
LIP3	160378	Found in lipid droplet proteomics (2)	No major effect on growth and TAG content
LIP4	319684	Down-regulation under N deprivation (1)	Not cloned (data not shown)
LIP5	141065	Down-regulation under N deprivation (1)	No major effect on growth and TAG content
LIP6	148864	Up-regulation under N deprivation (1)	No major effect on growth and TAG content
LIP7	157360	Up-regulation under N deprivation (1)	Not expressed
LIP8	344422	Up-regulation under N deprivation (1)	No major effect on growth and TAG content

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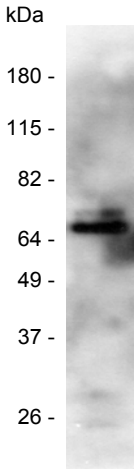
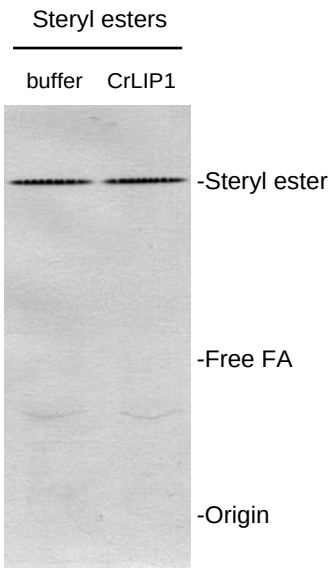
2. **Moellering, E. R. and C. Benning.** 2010. RNA interference silencing of a major lipid droplet protein affects lipid droplet size in *Chlamydomonas reinhardtii*. Eukaryot. Cell **9**:97-106



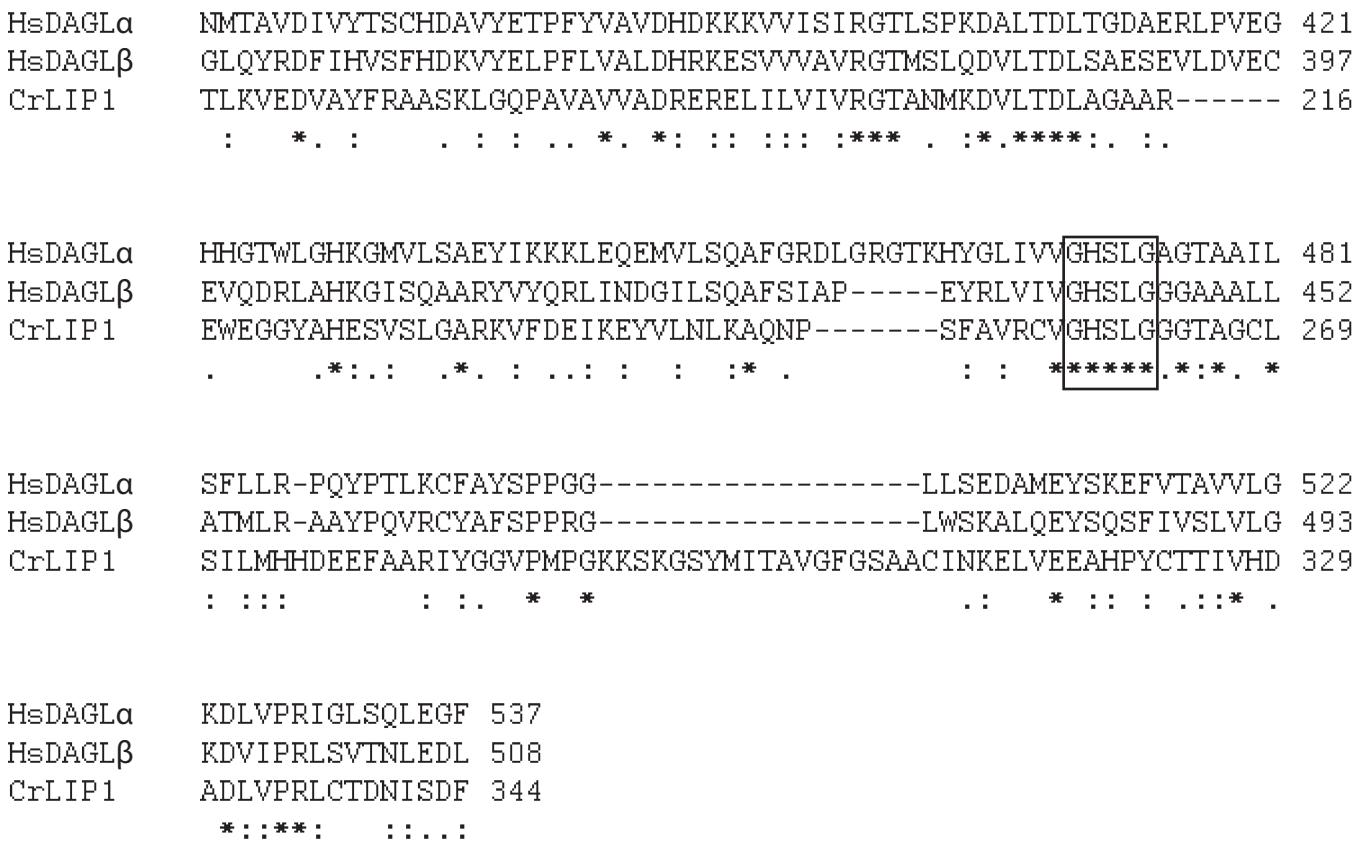
Supplemental FIGURE 1. Western blotting for hexahistidine-tagged CrLIP1 protein purified from *E. coli* cells.

Two µg purified protein was loaded for immunodetection. Note the doublets of CrLIP1 isoforms. The molecular reason is unknown.



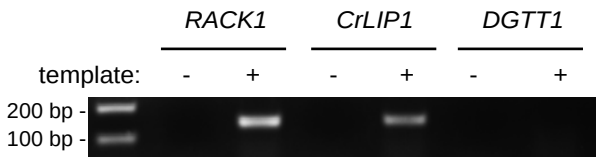
Supplemental FIGURE 2. Recombinant CrLIP1 fails to degrade steryl esters *in vitro*.

Thin-layer chromatographyresolution of lipids extracted from lipase assay mixtures with radioactive steryl esters derived from yeast cells as the substrate. TLC was developed in petroleum ether-diethyl ether-H2O (80:20:1 in volume). An autoradiograph is shown. The position of free fatty acids was marked by running oleic acids in the TLC and aligning the film to the TLC plate. Appearance of radioactive free fatty acids is considered as evidence for esterase activity on steryl esters.



Supplemental Figure 3. CrLIP1 shares sequence similarity to two human DAG lipases DAGLα and DAGLβ.

Shown are Clustal W2 results using the lipase domains of each of the three proteins. The “GXSXG” hydrolase motif is boxed. Part of the sequence for each protein was used as indicated by the residue numbers on the right side. Asterisk (*), colon (:) and period (.) denote conserved residues, conservative substitutions and semiconservative substitutions respectively.



Supplemental FIGURE 4. RT-PCR quantitative comparison of *RACK1*, *CrLIP1* and *DGTT1* transcripts in vegetatively growing cells.

No template control was performed for each gene. Calculated sizes for PCR products are: 139 bp for *RACK1*, 138 bp for *CrLIP1* and 98 bp for *DGTT1*.