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# Metabolic engineering of lipid catabolism increases microalgal lipid accumulation without compromising growth

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12/16/13

# Why I Think You Should Know About This Work

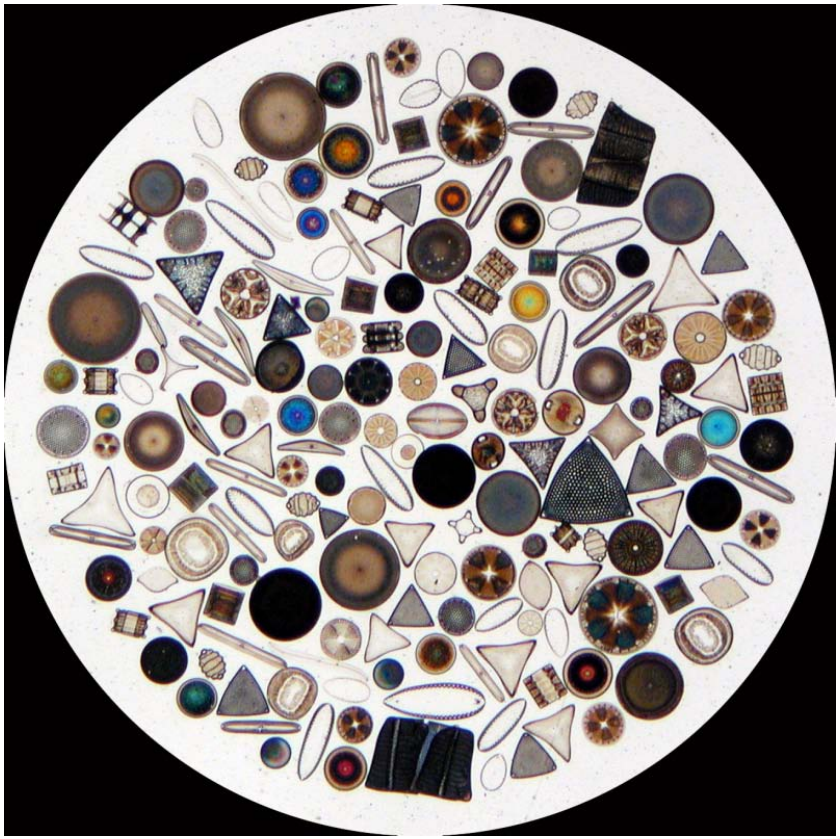
- Maintaining high growth rates and high biomass accumulation is imperative for algal biofuel production on large economic scales

- Disrupting lipid catabolism is a practical approach to increase lipid yields in microalgae without affecting growth or biomass

# Why I am Interested in This Work

- I have been working on the lipid matabolism in the microalgae Chlamydomonas. The Chlamy cells began to acuumulate TAG under N starvation, however in this situations, the growth was almost stopped.
- the authors hypothesized that knockdown of lipid catabolism would have less impact on the primary carbon pathways associated with growth.

# *Thalassiosira pseudonana*



From Wikipedia

Diatom

- **>200 genus, 100000 species, responsible for approximately 20% of global carbon fixation in the land and 45% in the ocean**

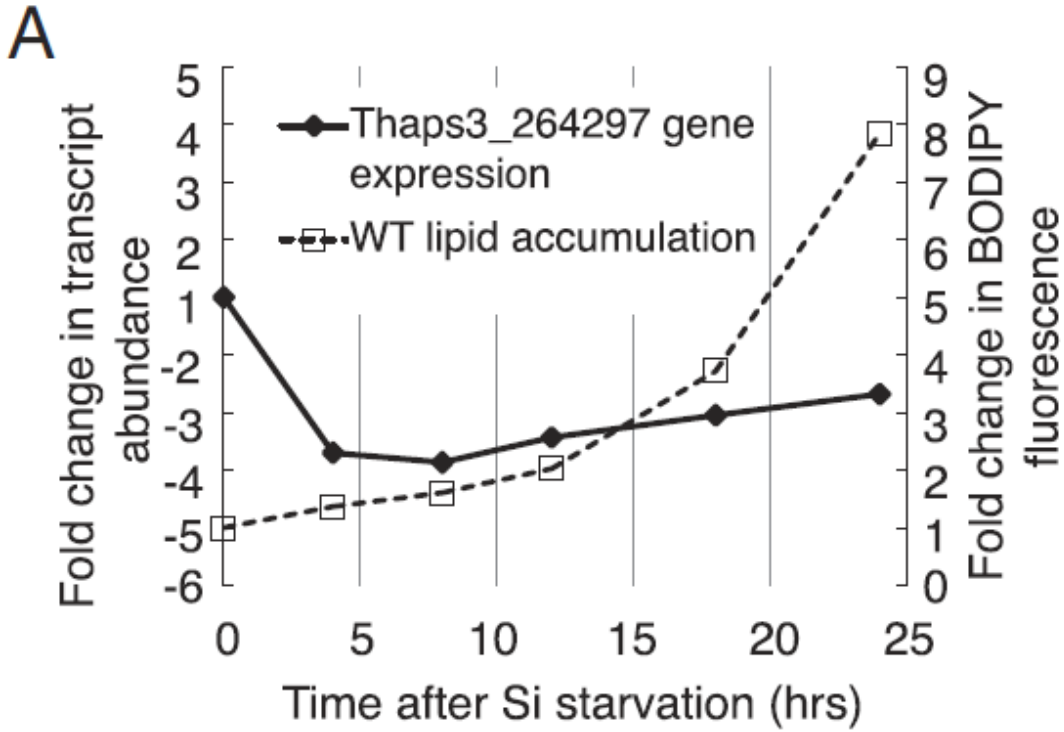
- **Enclosed within a cell wall made of silica (hydrated silicon dioxide) called a frustule**

- **Plastids acquired by secondary endosymbiosis.**

**Nuclear genome: 34Mbp**
**Plastid genome: 129kbp**
**Mitochondrial genome: 44kbp**

*Science 306, 79 (2004)*

# Transcriptomics-Guided Identification of Lipase Thaps3_264297




**(A) Fold change in abundance of Thaps3_264297 transcript (primary axis) during silicon starvation-induced lipid accumulation in WT T. pseudonana (secondary axis). Transcriptomic data from a previously generated Affymetrix whole genome tiling microarray was obtained from S.R.S. and M.H.**

# Characterization of Thaps3_264297

A

HG lipid binding domain

```
CGI-58          TPLVLIHGFGGGLGLWALNFGDLCTNRP--VYAFDLLGFGRSSRPRFDS----------- 122
ABHD4           TPLVMVHGFGGGVGLWILNMDSLSARRT--LHTFDLLGFGRSSRPAFPR----------- 115
At4g24160       PTLVMVHGYGASQGFFFRNFDALASRFR--VIAIDQLGWGGSSRPDFTCR---------- 168
Thaps3_264297   APLVLIHGYANGSLYFYRNLMGLSHFHFGSIYALDMLGWGLSSRPTFDLQLLGDDNGD-- 158
Ict1p           TPTVLIHGYAASSMAFYRTFENLSDNIK-DLYAIDLPANGASEAPALQVNKTKKIKSLRF 129
```

catalytic serine lipase motif GXSXG

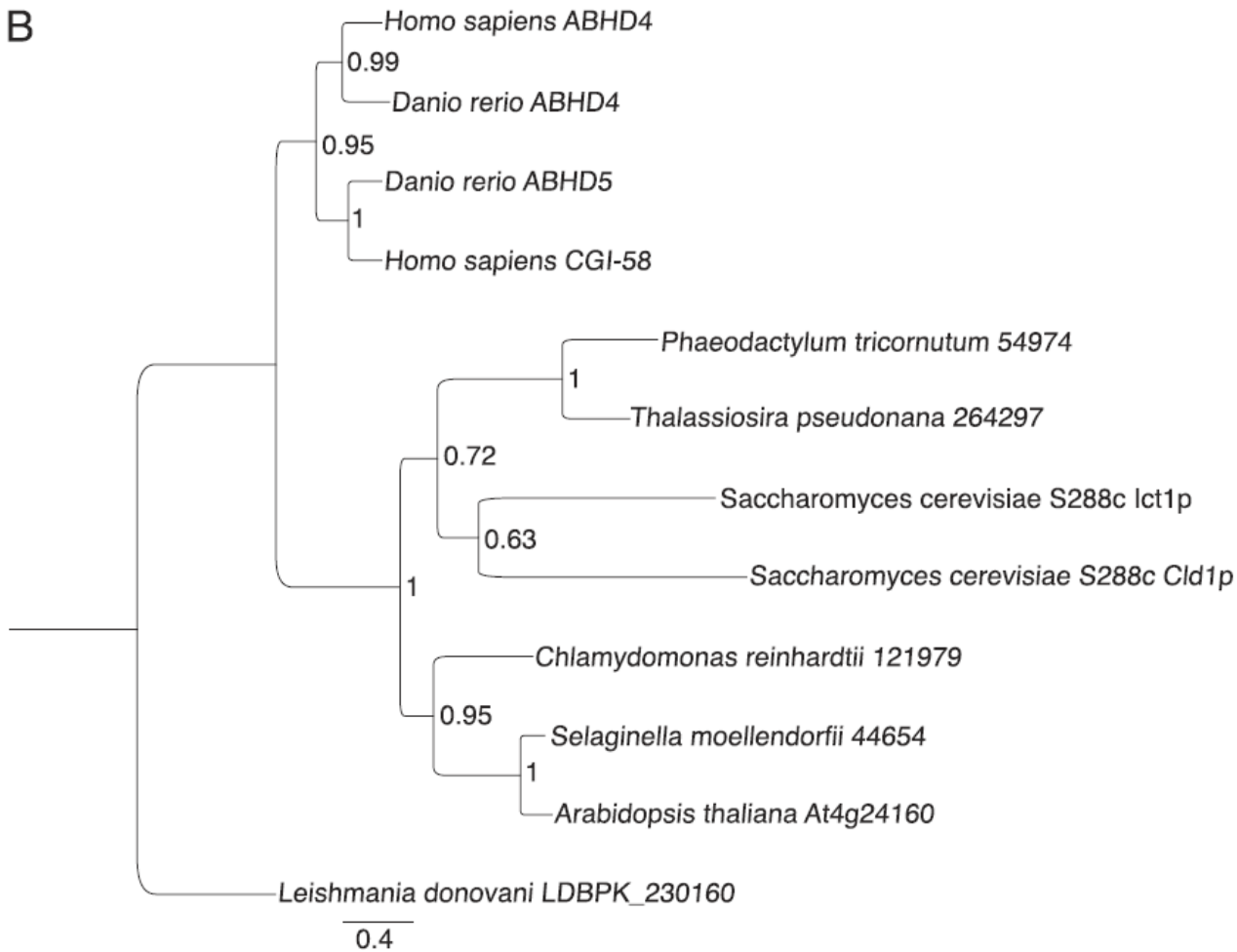
```
CGI-58          -----------------------DAEEVENQFVESIEEWRCALGLDKMILIGHNLGGFLA 159
ABHD4           -----------------------DPEGAEDEFVTSIETWRETMGIPSMILIGHSLGGFLA 152
At4g24160       -----------------------STEETEAWFIDSFEEWRKAQNLSNFILIGHSFGGYVA 205
Thaps3_264297   ------------TNDDKRSNEHKQVASAEHFFVESLESWRKQHDLPKITLAGHSMGGYLS 206
Ict1p           KHIEDDVVIPVIEKRPPAEDIKSHLEQYESYFVDRIEQWRKDNKLRKINVVGHSFGGYIS 189
```

$H(X)_4D$ acyltransferase motif

```
CGI-58          -------------RPHSYVKTIAILGACHYVYAD------------------QPEEFNQK 340
ABHD4           -------------RPDSYVRDMEIKGASHHVYAD------------------QPHIFNAV 333
At4g24160       -------------SMKVPCEIIRVPQGCHFVFID------------------NPIGFHSA 392
Thaps3_264297   KRTEWEKQKNNNESPPPKVFLHGVRNACHLLMLDNYEEFNSALIIAAGGEDRLPSNFPRP 433
Ict1p           -----------MLKNKAKASYVEVPDACHNLFLD------------------NPQHFASS 387
```

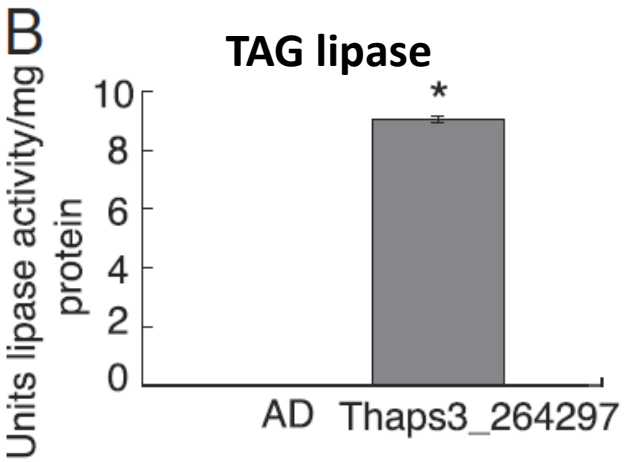
**Partial alignment of Thaps3_264297 amino acid sequence with CGI-58 and orthologs Abhydrolase domain-containing 4 (Homo sapiens), At4g24160 (Arabidopsis thaliana), and Ict1p (Saccharomyces cerevisiae).**

# Characterization of Thaps3_264297

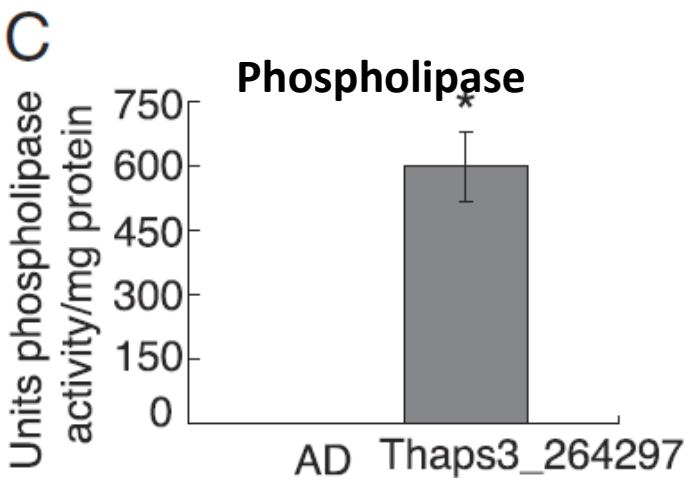


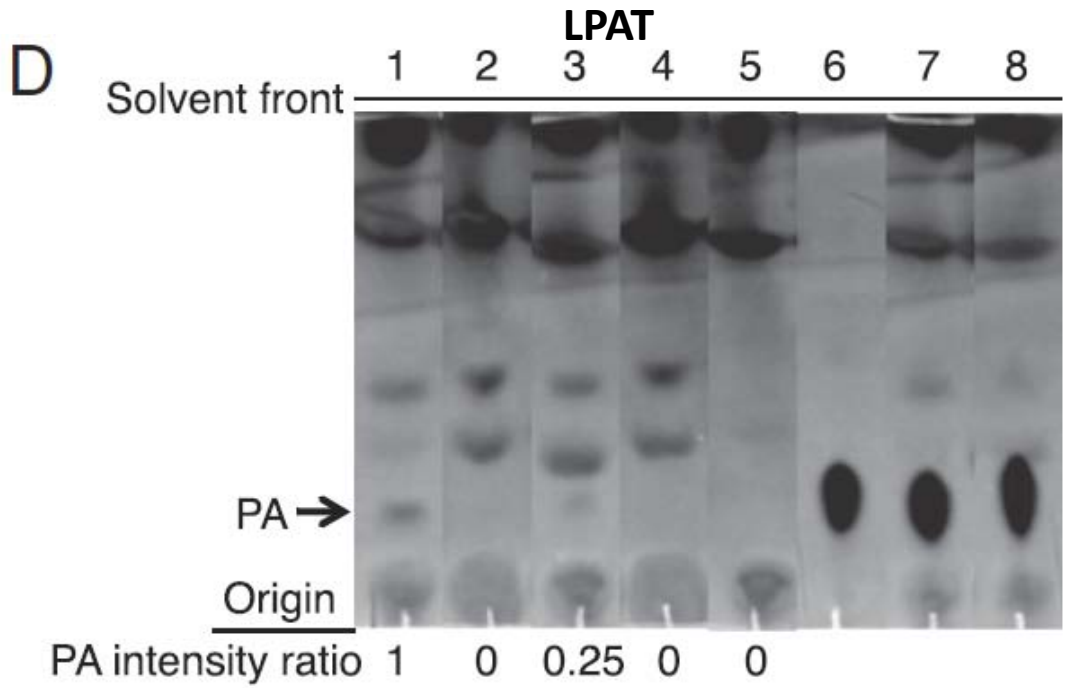

**Phylogenetic tree of CGI-58 homologs.**

# Thaps3_264297 is involved in lipid catabolism



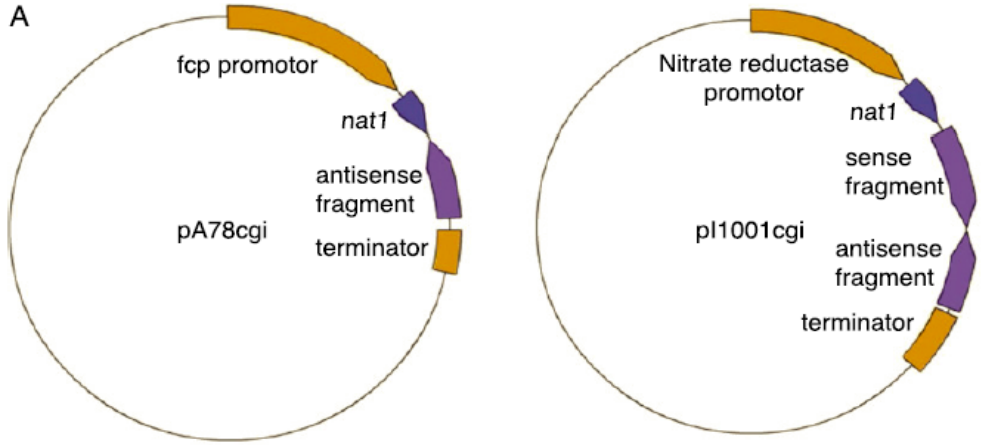

alcohol dehydrogenase (AD)

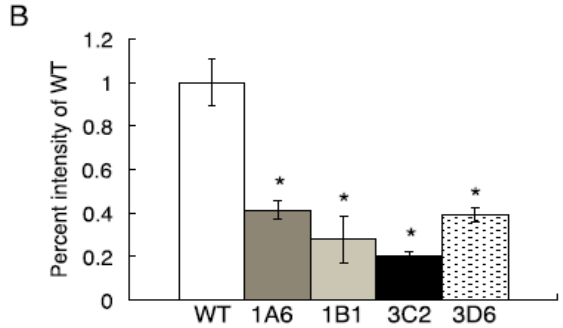


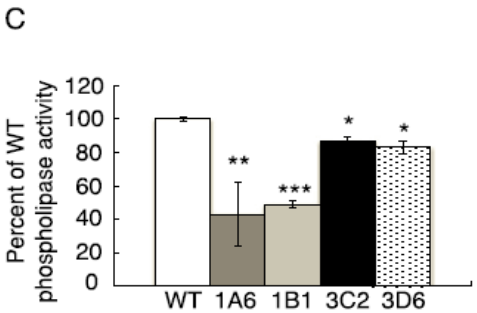



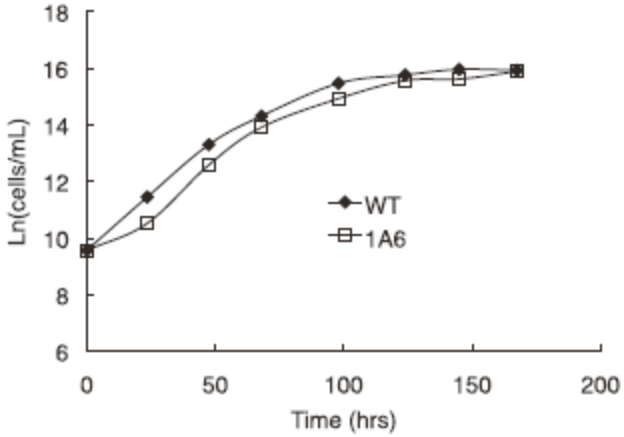

**(D)Lanes from TLC plate exhibiting lipid extracts from lysophosphatidic acid acyltransferase activity assay reactions using oleoyl-CoA and lysophosphatidic acid as substrates. TLC plate was stained with primuline and analyzed under UV. Activity is demonstrated by production of phosphatidic acid (PA). Reactions consisted of 1, substrates with lysates of E. coli expressing recombinant Thaps3_264297; 2, lysates with no substrate; 3, substrates with lysates of E. coli expressing an empty control vector; 4, control lysates with no substrates; and 5, substrates with no lysates. Standard PA is shown in 6, cospotted in 7, with extracts from reaction 1 to show comigration, and cospotted in 8, with extracts from reaction 3 to show comigration.**

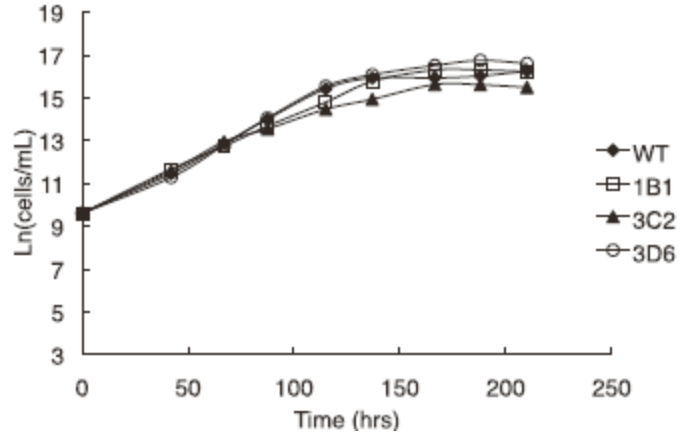
# Knockdown of Thaps3_264297 using antisense and interfering RNA



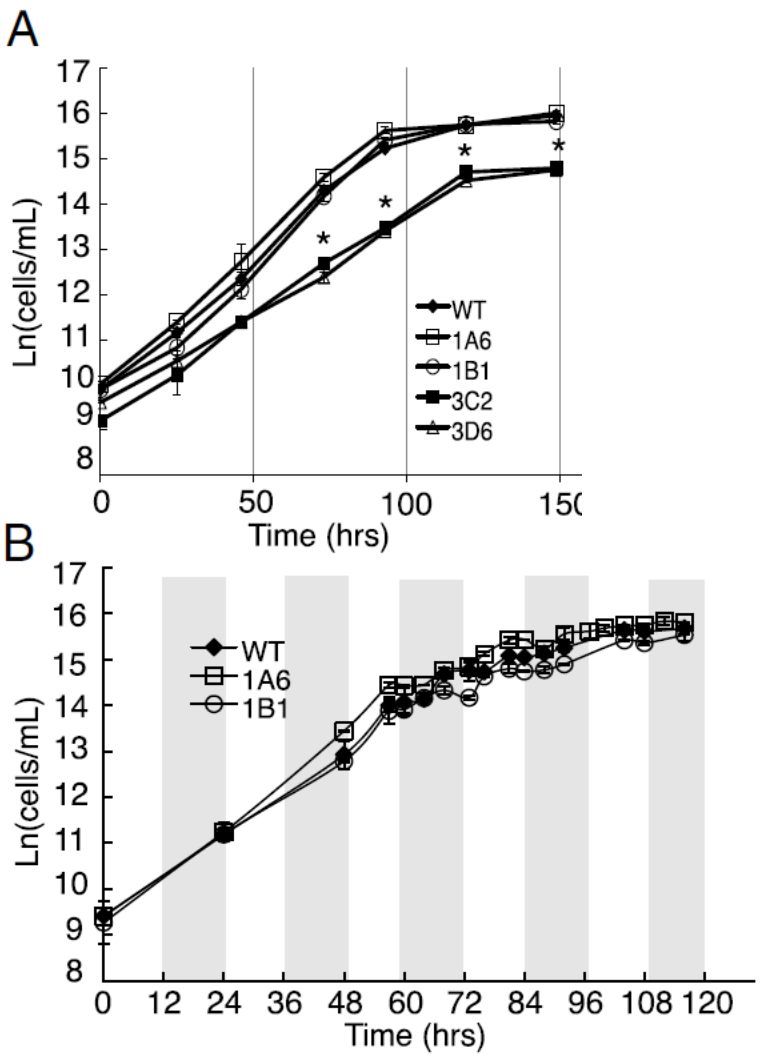




**(B) Relative intensities of Thaps3_264297 protein from immunoblots of transgenic strains compared with WT using anti-Thaps3_264297 antibodies.**
**(C) Functional enzyme assays of phospholipase activity in soluble cell lysates of WT and transgenic strains (n = 3).**

# Growth of knockdown strains under nutrient-replete conditions



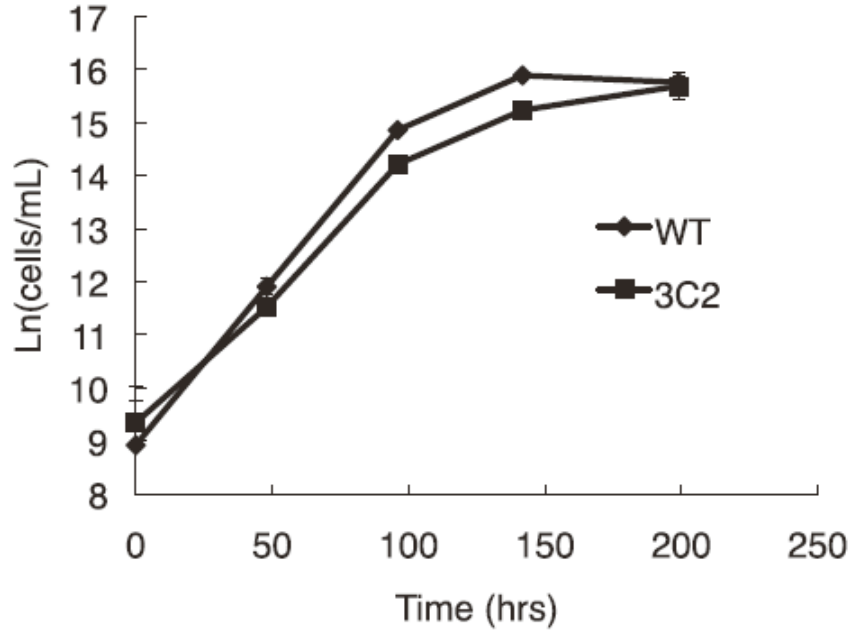
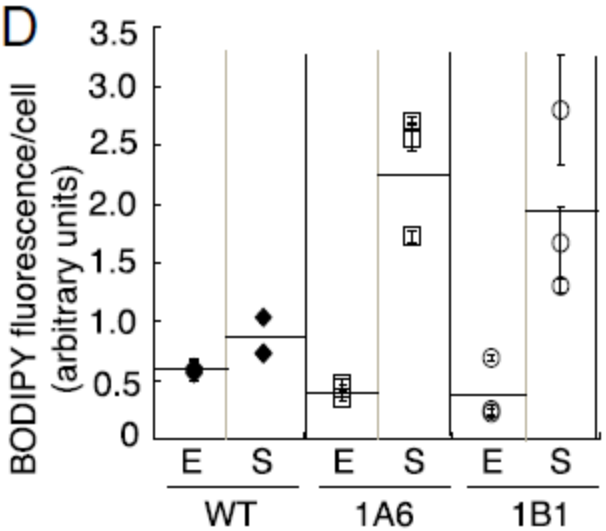
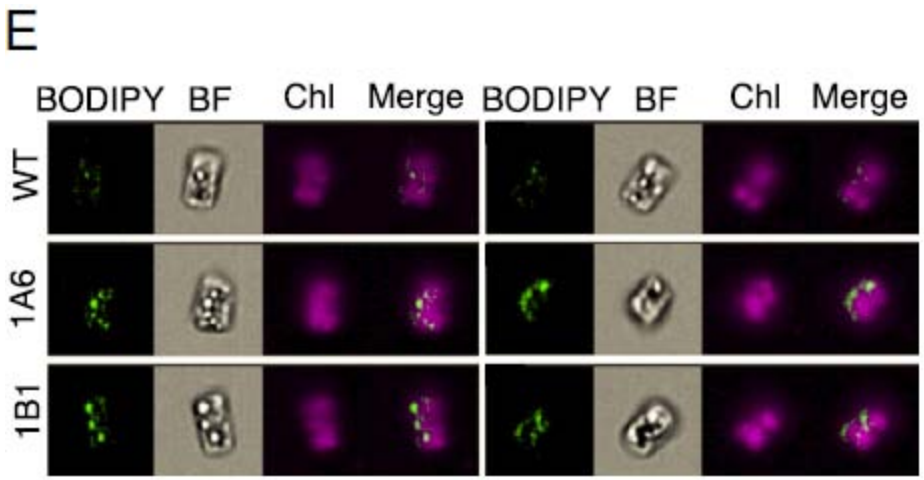
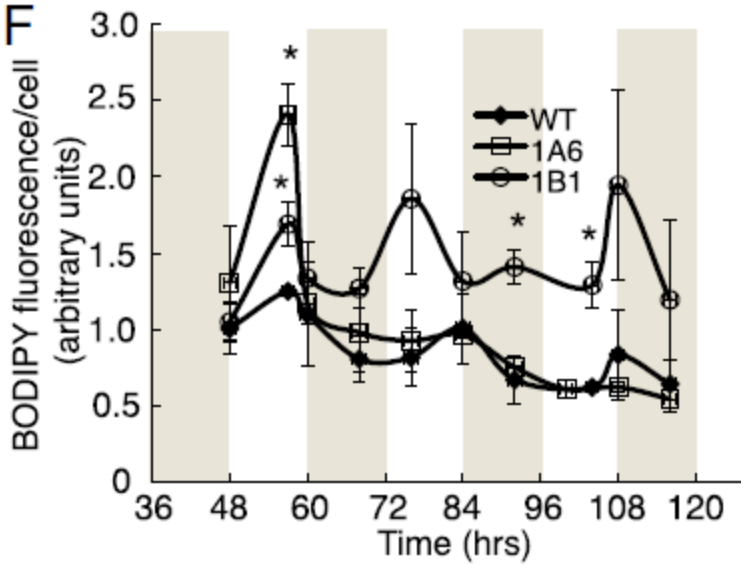




**Fig. S5. Repression of RNAi construct in 3C2. Growth of WT and strain 3C2 grown in repressive media (conditions under which knockdown construct is not expressed) as determined by cell density (n = 3).**

# Lipid accumulation of knockdown strains under nutrient-replete conditions



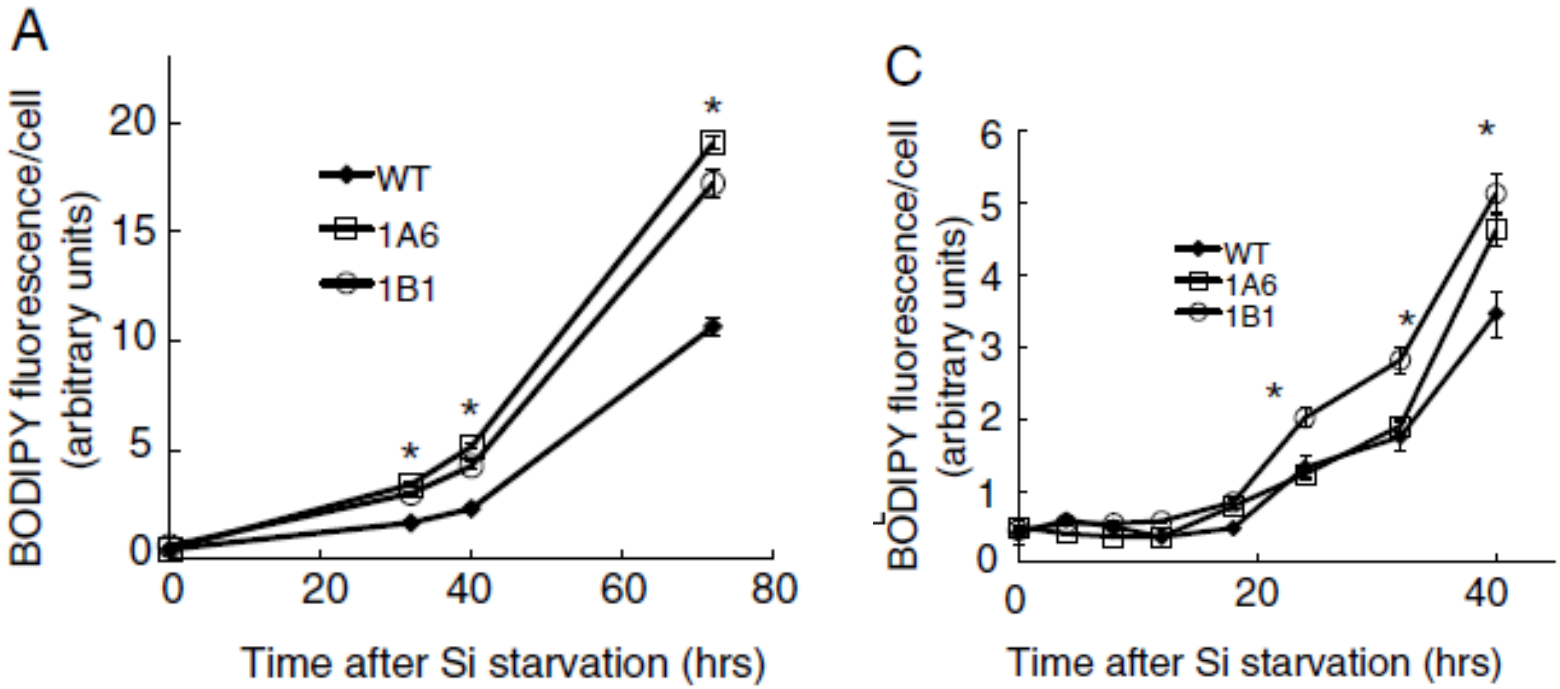




**(D) Increased BODIPY fluorescence and TAG accumulation of 1A6 and 1B1 over WT in stationary phase. E, exponential phase; S,stationary phase. Error bars are SEM from imaging flow cytometry (n > 100). Horizontal lines depict average of three graphed populations.**

**(E) Imaging flow cytometry images of representative cells selected because their BODIPY fluorescence per cell was the average of the population for three replicates of WT, 1A6, and 1B1 in stationary phase. BODIPY and chlorophyll fluorescence (Chl) are both shown.**

**(F) TAG levels in 1A6, 1B1 and WT over light:dark cycle monitored using BODIPY fluorescence. Shaded regions denote dark periods.**

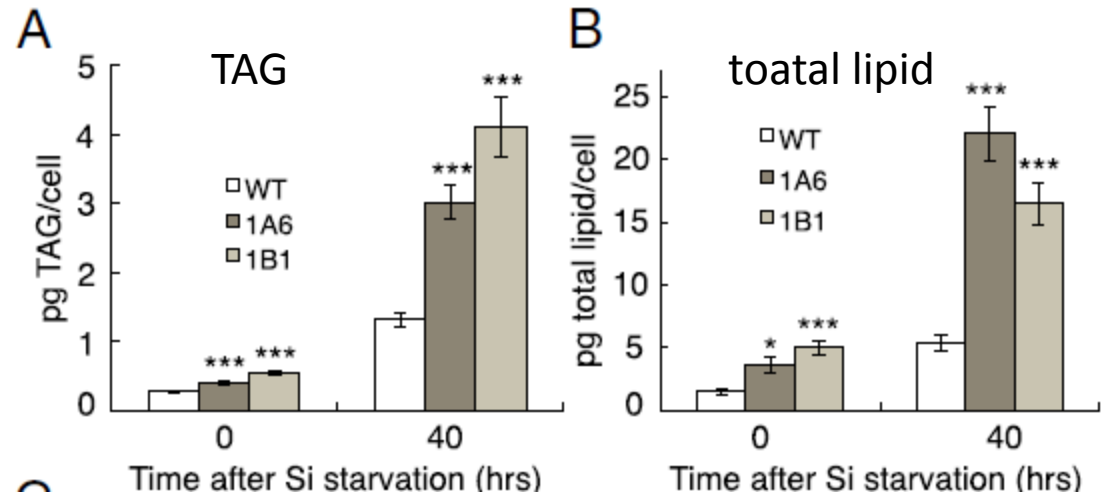
# Lipid accumulation of knockdown strains under nutrient-deplete conditions




**(A) Increased BODIPY fluorescence in 1A6 and 1B1 over WT during silicon starvation-induced lipid accumulation.**

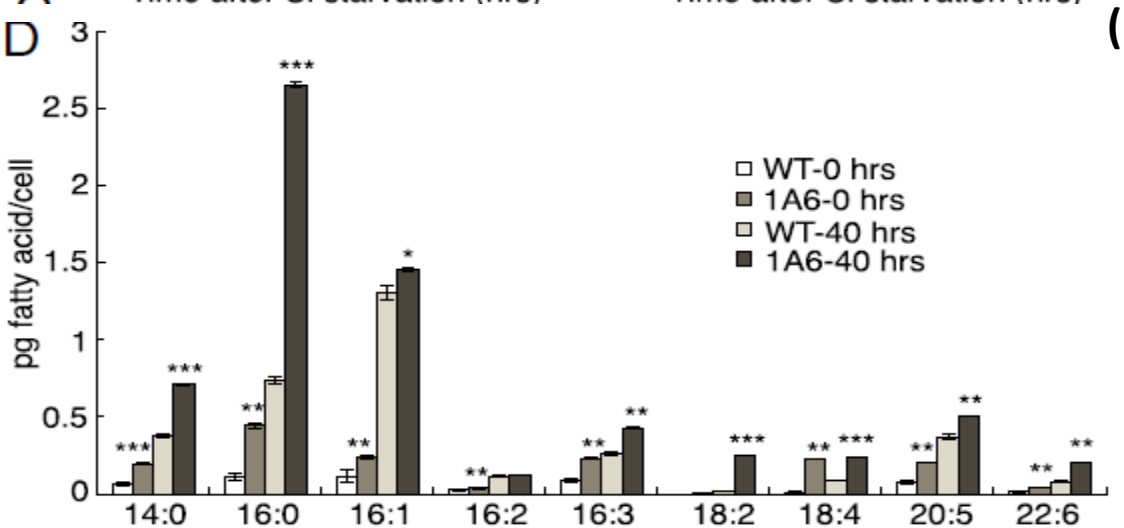
**(C) High-resolution time series of TAG accumulation in WT, 1A6, and 1B1 during silicon starvation.**

# Lipid accumulation of knockdown strains under nutrient-deplete conditions




**(A)Quantification of picograms of TAG per cell in WT, 1A6, and 1B1 at 0 and 40 h after silicon starvation (n = 8).**

**(B) Quantification of picograms of total lipid per cell in WT, 1A6, and 1B1 at 0 and 40 h after silicon starvation (n = 8).**



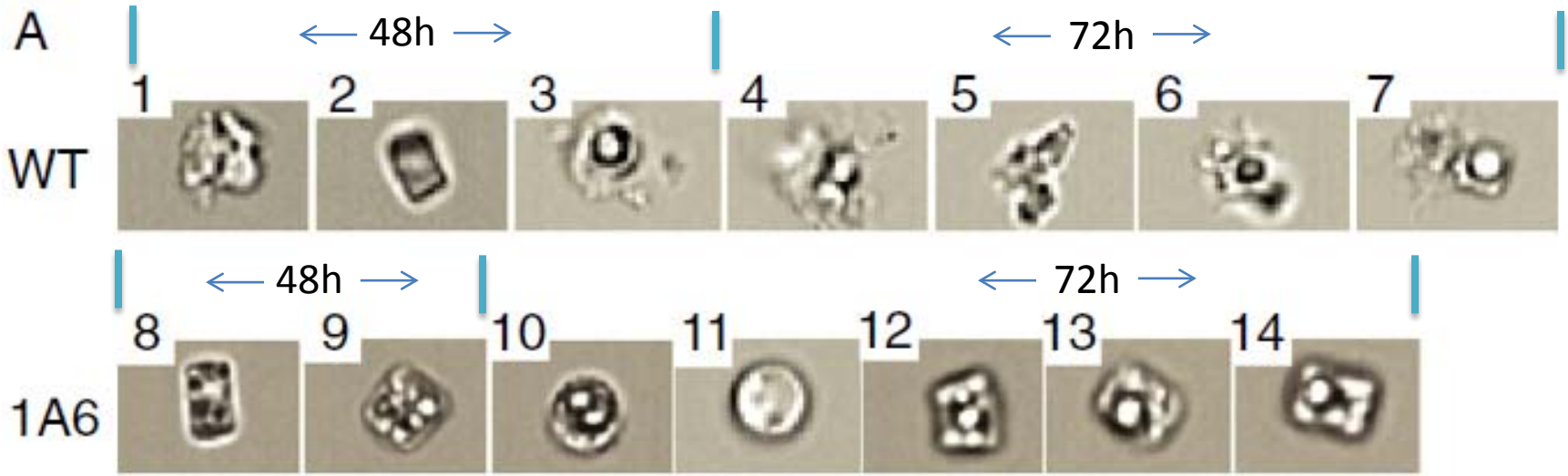

**(D) FAME profiling of 1A6 and WT during silicon starvation (n = 2).**

A

| | WT | 1A6 | 1B1 |
|---|---|---|---|
| Biomass (mg/L) | 9.98 | 20.1 | 26.7 |
| Lipid yield (mg/L) | 1.52 | 3.63 | 5.03 |

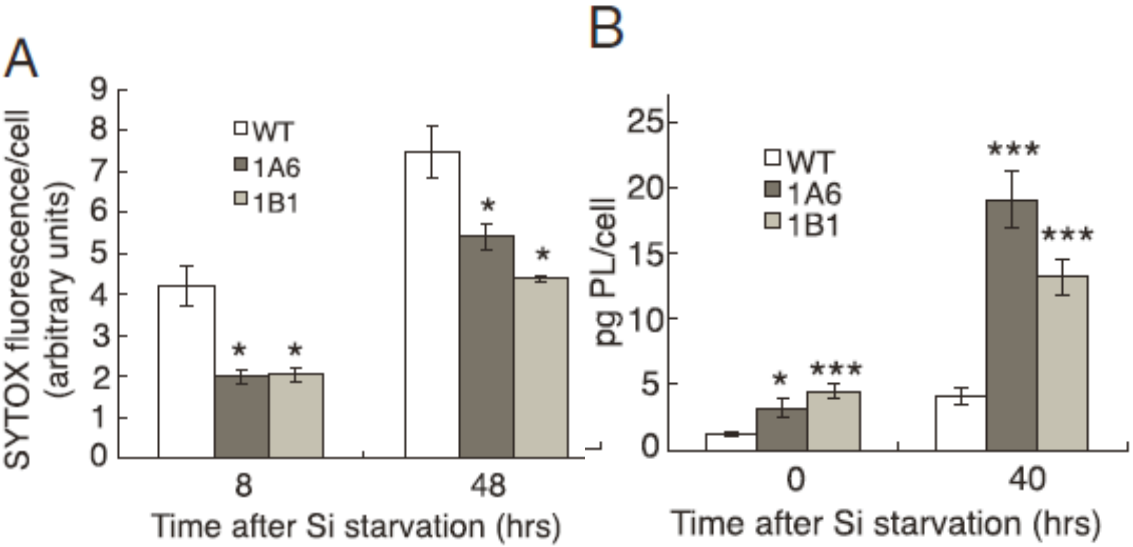
**Biomass and lipid yields from exponentially growing cultures of 1A6, 1B1, and WT.**

# Increased intactness of transgenic strains during silicon limitation



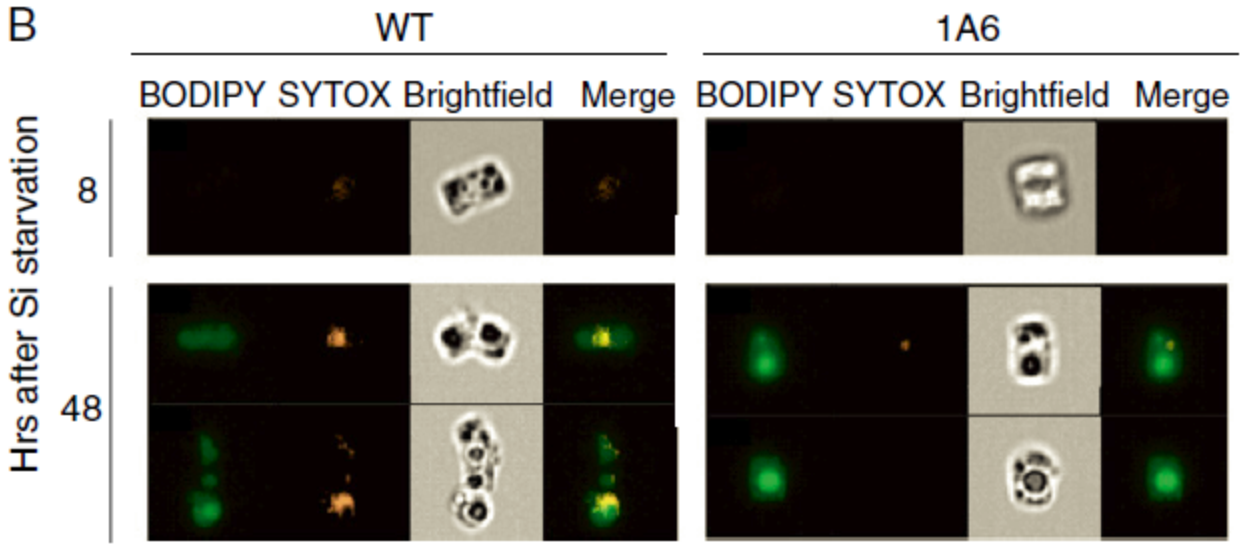

**(A) Images represent randomly selected cells of WT and 1A6 after 48 h (cells 1 and 2 and 8–10) and 72 h (cells 4–7 and 11–14) of silicon starvation. Cells of 1A6 seem to be more intact overall, whereas the majority of WT cells are either partially (cells 3, 6, and 7) or completely (cells 1, 4, and 5) degraded.**

# Increased intactness of transgenic strains during silicon limitation



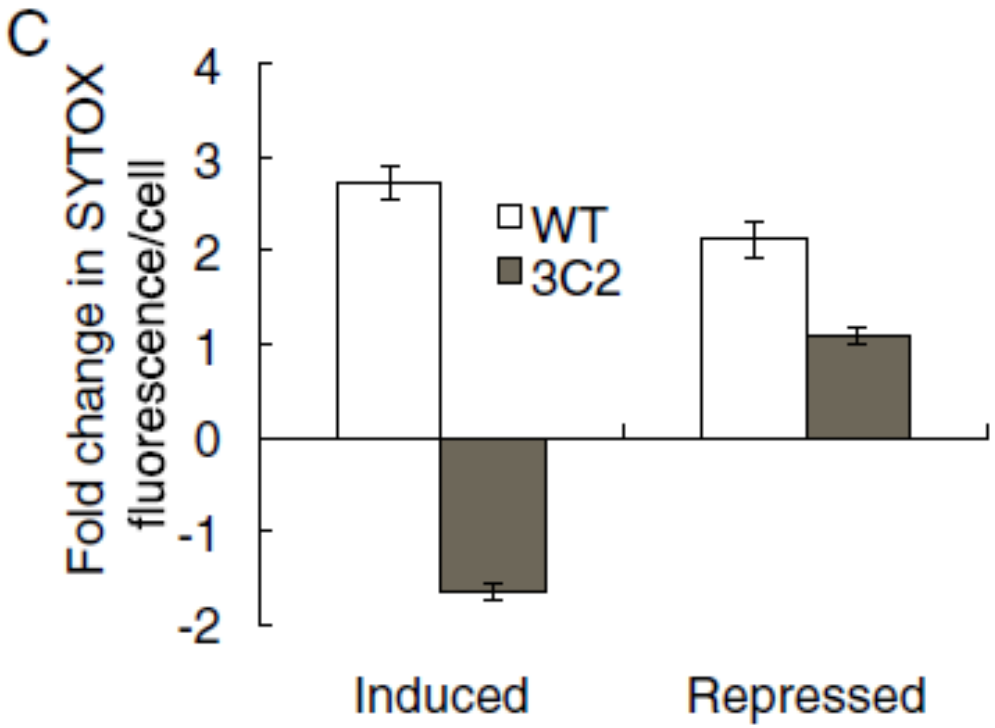

A) SYTOX fluorescence per cell in WT, 1A6, and 1B1 at 8 and 40 h of silicon starvation. Error bars are SEM from imaging flow cytometry (n >100)

B) Quantification of picograms of polar lipids (PLs) per cell in WT, 1A6, and 1B1 at 0 and 40 h of silicon starvation (n = 8).




(B) Representative images of cells of 1A6 and WT after 8 and 48 h of silicon starvation exhibiting the average SYTOX fluorescence of the population for each strain.

# Decreased membrane degradation was a consequence of Thaps3_264297 knockdown




**(C) Decreased membrane permeability is correlated to expression of the knockdown construct.**
**Fold change in SYTOX fluorescence after 48 h of silicon starvation in WT and strain 3C2 when RNAi construct is under induced or repressed conditions.**

# Conclusions

- **Knockdown of the CGI-58 homolog in T. pseudonana results in increased accumulation of TAG droplets, total lipid production, and lipid yields without negatively affecting growth, cell division, or biomass under continuous light and light:dark cycling.**
- **Thaps3_264297 may not be involved in mobilizing TAG for rapid growth, but rather, like A. thaliana's homolog, in lipid homeostasis or remodeling, therefore allowing the knockdown to grow similarly to WT.**

# Take-Home Message

- **Disrupting lipid catabolism can be used to produce strains with favorable characteristics for biofuel production.**

# Thanks