

**RNAi-mediated silencing of a pyruvate dehydrogenase kinase enhances triacylglycerol biosynthesis in the oleaginous marine alga *Nannochloropsis salina***

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**Supplementary Table 1** Primer pairs used in the present study.

| Gene name                    | Primer sequence                            | Use                              | GenBank<br>accession no. |
|------------------------------|--|----------------------------------|--------------------------|
| PDK                          | PDKf: ATGATAGCACGTCGTGTACA                 | Full-length amplification        | XM_005855411             |
|                              | PDKr: TTAGACCAATGGCTCCTTGC                 |                                  |                          |
|                              | PDK_KpnI_f: GGGGTACCATGATAGCACGTCGTGTAC    | PDK-GFP fusion construction      |                          |
|                              | PDK_KpnI_r: GGGGTACCGACCAATGGCTCCTTGCA     |                                  |                          |
|                              | kd1_anti_f:                                | Sense sequence amplification     |                          |
|                              | AAAAGTGCAGACGCGTCAGCACCTTCACGA             |                                  |                          |
|                              | kd1_anti_r:                                |                                  |                          |
|                              | CTAGACTAGTGCGACCTTCTTGCACCGAGA             |                                  |                          |
|                              | kd1_sense_f: CCCATCGATGCGACCTTCTTGCACCGAGA | Antisense sequence amplification |                          |
|                              | kd1_sense_r:                               |                                  |                          |
|                              | CCGGAATTCACGCGTCAGCACCTTCACGA              |                                  |                          |
|                              | PDK_qf: CTCGACGAACTCCATCTGAA               |                                  |                          |
| PDK_qr: CCGTCTCGATCACATCTTGA |  |                                  |                          |

**Supplementary Table 1** Primer pairs used in the present study. (Continued)

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|                  |   |                             |          |
|------------------|---|-----------------------------|----------|
| eGFP             | eGFP_ KpnI_f:<br>GGGGTACCGGACCTAGGGGAGGAGGAGGAGGAATGGTGAGCA<br>AGGGCGAG   | PDK-GFP fusion construction |          |
| $\beta$ -tubulin | eGFP_XbaI_r: GCTCTAGATTACTTGTACAGCTCGTCCATGC<br>TUBf: CGGGGTACCACTGCGCATGGATTGACCGA<br>TUBr:<br>CCGCTCGAGTGCTTCACAAAAAAGACAGCTTCTTGAT | Promoter                    |          |
| RbcS1            | RbcS1_f: GCTGTTAGCACAGCACTTGC<br>RbcS1_r: CCATCCTTTCTGTACTGCTT  | Terminator                  | AY530155 |
| GUS              | GUS_f: GATATCTACCCGCTTCGCGT<br>GUS_r: CACCGAAGTTCATGCCAGTC  | Genomic PCR detection       |          |
| Bleomycin        | Bleo_f: TCGAGTTCTGGACCGACCGGCT<br>Bleo_r: TCCTGCTCCTCGGCCACGAAGT  | Genomic PCR detection       |          |
| Sense-GUS        | Sense-GUS_f: ACCTTCTTGACCCGAGAGCT<br>Sense-GUS_r: ACGGCGCTGACGCGATCAAA  | Genomic PCR detection       |          |

**Supplementary Table 1** Primer pairs used in the present study. (Continued)

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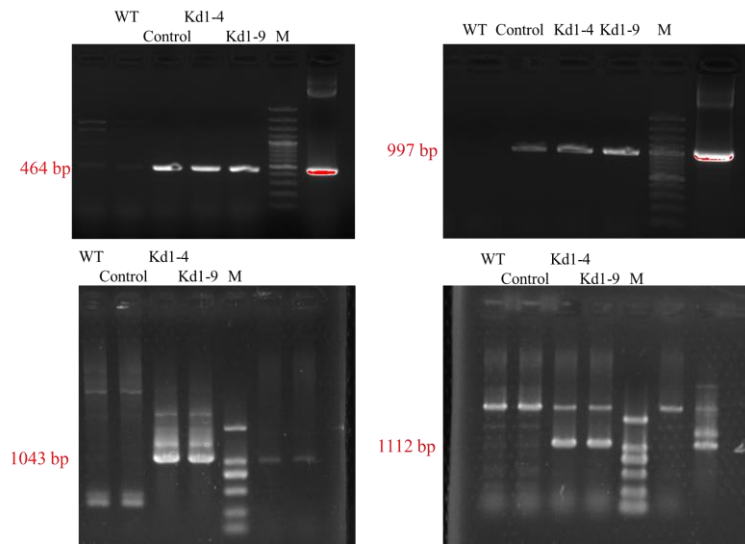
|               |  |                       |                    |
|---------------|--|-----------------------|--------------------|
| GUS-antisense | GUS-antisense_f: GCGTGGCAAAGGATTCGATAACG<br>GUS-antisense-r: GACCTTCTTGCACCCGAGAGC | Genomic PCR detection |                    |
| Actin         | Actin_f: ATTTTCGTGCGCCGGAGG<br>Actin_r: GTCGCACTTCATGATCGTCTG                      | Real time PCR         | XM_005852283       |
| PDHC_E1       | PDHCe1_f: TTGGTCAATCGCAGTATGGT<br>PDHCe1_r: CACCTCCTCAAGCTGTTTCA                   | Real time PCR         | AZIL01000171.1     |
| PDHC_E2       | PDHCe2_f: AGCCACTGAAACAACACCAG<br>PDHCe2_r: AGCCACTGAAACAACACCAG                   | Real time PCR         | XM_005854674.<br>1 |
| ICDH          | ICDH_f: CTCACTGGGCCTTATGACCT<br>ICDH_r: GGCTTGCCTTTCTGGTACTC                       | Real time PCR         | AZIL01000117       |
| CS            | CS_f: ATGGAAACAGCCTTCTTGCT<br>CS_r: CCGTCTGATGATGGTAAACG                           | Real time PCR         | AZIL01001543       |
| ME            | ME_f: ACTCCAAGGGCTTGATTGTC<br>ME_r: AATTCGTCCGACCTCTATGC                           | Real time PCR         | AZIL01000703.1     |

**Supplementary Table 1** Primer pairs used in the present study. (Continued)

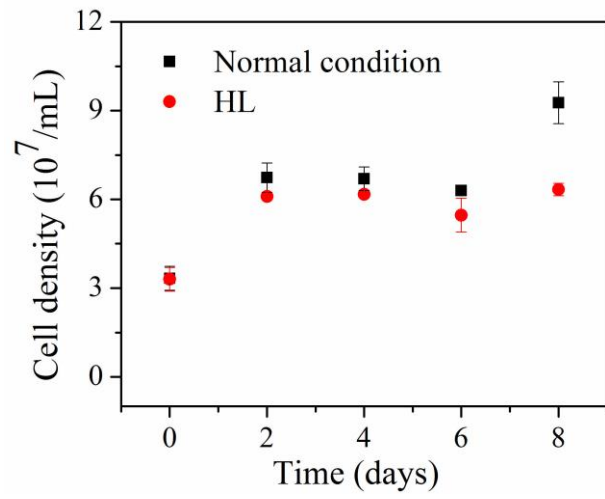
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|       |  |               |                |
|-------|--|---------------|----------------|
| G6PDH | G6PDH_f: CGAATTACGAGACCAGCTCA<br>G6PDH_r: CCCGACTTGTACATGCACTT | Real time PCR | AZIL01000829.1 |
| PDC   | PDC_f: AACAACCCTGACACAGTGGA<br>PDC_r: CGCACCTTCACACCTATGAC     | Real time PCR | XM_005854810   |
| ALDH  | ALDH_f: CGAAGCAGAGGCAGACATAA<br>ALDH_r: TCCATTCATGGAGGTGCTTA   | Real time PCR | AZIL01001205   |
| ACS   | ACS_f: CAGCCGCTTGTTGAGTGTAT<br>ACS_r: CCCAGCATATTCGAGCCTAT     | Real time PCR | JU980159.1     |

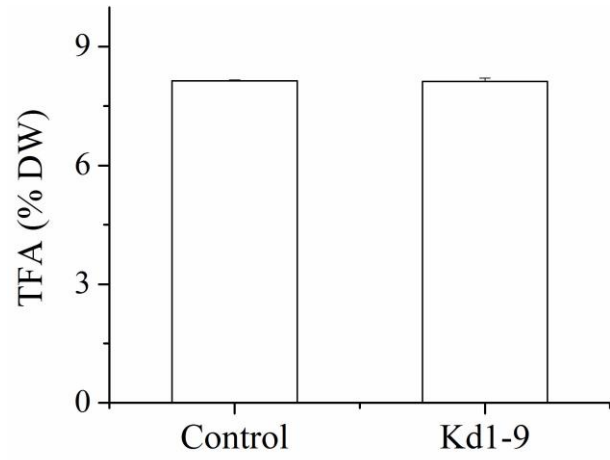
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**Supplementary Figure 1.** Detection of bleomycin resistant fragment (464 bp), GUS region (997 bp), fragment crossing sense and GUS sequence (1043 bp), fragment crossing GUS and antisense sequence (1112 bp) from transgenic *N. salina* (shown in full-length gels).

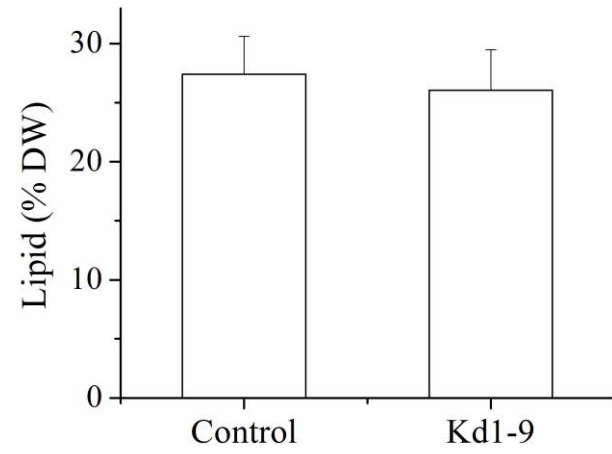


**Supplementary Figure 2.** The time curve of cell numbers in *N. salina* under normal conditions ( $50 \mu\text{mol}/\text{m}^2/\text{s}$ ) and high light conditions ( $250 \mu\text{mol}/\text{m}^2/\text{s}$ ).



**Supplementary Figure 3.** Total fatty acid content in control and Kd1-9 cells on day 0 before transferred into HL conditions. DW, dry weight.





**Supplementary Figure 4.** Total lipid content in control and Kd1-9 cells on day 2 under nitrogen deficiency conditions. DW, dry weight.

**Supplementary Data 1** Coding sequence of putative mitochondrial pyruvate dehydrogenase kinase from *Nannochloropsis salina*

ATGATAGCACGTCGTGTACAGTTTTTTGAAAACAAATTTATATCAGTATGCGTCAAAGCGTAGATTTGCACAGATCGCCACGTCTTCTGCCCTTTTTTCCCC  
ATCAGTCGCATCTCCAGCAGCACCAGCAACACGGACAAGTAGGGCTGCAGGAAGCTCCACGCCTTCTTTTTTGGCAACAGCAGACTCTTAAAGCGGAT  
AAAATACCTACGCCTACGAAAATAGCAGGTGCATCCGTGCCTTGGTCAGATTCAGCTCACAGCGTAAGCGAGTCGAAATCGGAAATCCTCCGAGACGA  
AGAAGCGACTATACTTACATATGCGAAGAAACGGTCGGAACGGGTGTCTCTGCGCAGTCTGGCGGAAGTGGGCTTGGGAACGCGACCTTGGTTGTCG  
AGTCTAAGAGATGCCATGTCCCTGGAAAATCCCCTCTGTTCCCTGAACGTGCGTCTCTGCAGGGTGTCTGACCCCACTCCCCGGCCAGAAAGGGGCT  
TCTGCGCATGGCGACCTTCTTGCACCGAGAGCTCCCGATTTCGCTTCGCACGCGGCATCACTTTCATCGACAAATTAGATTCAAGTCGACAGGCGCCCA  
GTTTGCCTGTGGTTCGAGAGTGGTACAGGGAATCCTTTCGAGACGTGGTGTCAAGCCCGTGCCCGTCACCGACGGCTGCGAGGAGAGCTTCGTGAA  
GGTGCTGACGCGTGTCCGGGACAGGCACGCGGACGAACTCTTGCTGGTTCGCGGAGGGGTCTTTGAACTGCGAGCCAAGCTAGGTATGGACGGTTTG  
GACGGGAGGGGCGGTTCGGAAGCTCTCCACGCGCAACTCGACGAACTCCATCTGAAGCGCATAGCCCTCCGCATCCTCGTGGGTTCATTACCTGGCTCT  
GCACCAGCCCCCTCGCCCAATTACGTGGGGATAATCTGCACGCGCACAAAACCTCAAGATGTGATCGAGACGGCGGCTGCGGACGCACGCTGGATTT  
GCAAGCAACGCTTCGACGGTTGCGCCCCGCGAGTGGAGGTGATTGGGGGGGAGGGTATGGTATGGCCTGCATTCCGGAAAGCTTATACTATCTGTCTG  
ATGGAACATCAAAAATTCATTGCGGGCAGTAGCAGAGCGCTACAGTGAGGCTTTGTTTCGTGACGGATCAATGGCACATGGCGCGGGCGGTGACGA  
AGGGTGTGTGCCCTCGATCAAAGTCATTYTCAGCCGAGAGTACAGTTTGACAGAAGGGCAACAGGTGGTATCGAGGTGCGGGACGAAGGGGGGGG  
CATTCCGCCGGAGGATCTAGGTAAGGTTTTCTGTTACCTTTTTTCCACTGCGGCTGATGCGGATGTGCAGCAACTCGTTATGGACCGTACGCCTCGGG  
GCTTGGGGGGGAAAGGAGTAAGCACAAACAGAGGGAACCTCAAAGAAAGTGCTCTCGCAGGTCTGGGGTATGGTTTGGGCATTGCCAAGTCATATGCT  
TTGTATTTGGGGGGGAGTTGGAATCAAATCGGCCGGGAGATGGCTGCTCGGTGTTTGTGACTTTGAGCCGGCTTGGTGAGTGCAAGGAGCCATT  
GGTCTAA