

## Supplementary Information

**Table S1.** Fatty acid composition of *Ostreococcus RCC809* (*O. RCC809*) and *F. cylindrus* cultures in stationary phase. Values are the average of three independent experiments ( $\pm$ standard deviation). ND—not detected.

**Figure S1.** Multiple sequence alignment of Ost809D6 with  $\Delta$ 6-desaturases from *Mantoniella squamata* (*M.squaD6*, CAQ30479.1) [1], *Micromonas pusilla* (*M.pusD6*, XP\_002502445.1) [2], *Ostreococcus lucimarinus* (*O.lucD6*, DAA34893.1) [3] and *Ostreococcus tauri* (*O.tauriD6*, XP\_003082578.1) [4]. Conserved histidine boxes are shaded in grey. Conserved amino acid residues are indicated with an asterisk. The position of conserved cytochrome *b*<sub>5</sub> domain motif is marked with a solid line. The alignment was obtained using CLUSTAL W.

**Figure S2.** Multiple sequence alignment of Ost809D4 with  $\Delta$ 4-desaturases from *Pavlova lutheri* (*P.lutD4*, AAQ98793.1) and *Pavlova salina* (*P.salD4*, AY926606.1) [4], *Isochrysis galbana* (*I.galD4*, AY630574) [5], *Ostreococcus lucimarinus* (*O.lucD4*, XM\_001415706.1) [6], and *Emiliana huxleyi* (*E.huxD4*, [7]). Conserved histidine boxes are shaded in grey. Conserved amino acid residues are indicated with an asterisk. The position of conserved cytochrome *b*<sub>5</sub> domain motif is marked with a solid line. The alignment was obtained using CLUSTAL W. A related  $\Delta$ 4-desaturase from *Thalassiosira pseudonana* (*TpD4*, AAX14506.1) [8] was omitted from this line-up on the basis of poor alignment.

**Figure S3.** Multiple sequence alignment of FcElo6 with  $\Delta$ 6-elongase from *Thalassiosira pseudonana* (*TpElo6*, AY591337.1) and *Ostreococcus tauri* (*OtElo6*, AY591335) [5]. Conserved amino acid residues are indicated with an asterisk, whereas conserved motifs are framed. The alignment was obtained using CLUSTAL W.

**Figure S4.** Codon-optimised nucleotide sequences. (A), Ost 809D6; (B), Ost809D4; (C) Fc ELO6.

**Table S1.** Fatty acid composition of *Ostreococcus RCC809* (*O. RCC809*) and *F. cylindrus* cultures in stationary phase. Values are the average of three independent experiments ( $\pm$ standard deviation). ND—not detected.

Fatty Acid	Composition (Molar %) $\pm$ SD	
	<i>O. RCC809</i>	<i>F. cylindrus</i>
<b>16:0</b>	20.12 $\pm$ 0.6	12.1 $\pm$ 0.2
<b>16:1<i>n</i>-7</b>	18.1 $\pm$ 0.3	24.5 $\pm$ 0.1
<b>16:4<i>n</i>-3</b>	5.08 $\pm$ 0.4	ND
<b>18:0</b>	1.24 $\pm$ 0.5	2.4 $\pm$ 0.3
<b>18:1<i>n</i>-9</b>	8.17 $\pm$ 0.2	ND
<b>18:1<i>n</i>-7</b>	8.03 $\pm$ 0.1	ND
<b>LA</b>	9.12 $\pm$ 0.2	0.7 $\pm$ 0.3
<b>GLA</b>	3.29 $\pm$ 0.3	1 $\pm$ 0.4
<b>ALA</b>	3.98 $\pm$ 0.4	0.8 $\pm$ 0.3
<b>SDA</b>	19.13 $\pm$ 0.6	5.7 $\pm$ 0.5
<b>20:2<i>n</i>-6</b>	0.65 $\pm$ 0.4	1 $\pm$ 0.2
<b>20:4<i>n</i>-3</b>	ND	7.4 $\pm$ 0.1
<b>EPA</b>	1.26 $\pm$ 0.4	31.4 $\pm$ 0.3
<b>DHA</b>	1.83 $\pm$ 0.5	2.5 $\pm$ 0.1
<b>24:0</b>	ND	1.4 $\pm$ 0.2

**Figure S1.** Multiple sequence alignment of Ost809D6 with  $\Delta 6$ -desaturases from *Mantoniella squamata* (*M.squaD6*, CAQ30479.1) [1], *Micromonas pusilla* (*M.pusD6*, XP\_002502445.1) [2], *Ostreococcus lucimarinus* (*O.lucD6*, DAA34893.1) [3] and *Ostreococcus tauri* (*O.tauriD6*, XP\_003082578.1) [4]. Conserved histidine boxes are shaded in grey. Conserved amino acid residues are indicated with an asterisk. The position of conserved cytochrome *b*<sub>5</sub> domain motif is marked with a solid line. The alignment was obtained using CLUSTAL W.

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Ost809D6      -----MRVETEDDNVPTVTVGLSEESDGMKGARNPGARAWKSTLEP
O.lucD6      MCVETTEGTSRMTANERTSSSSSLEGGTPTVTVGMGSE-DAGKKTRNASVTAWTKELEP
O.tauriD6    -----MCVETENNDGIPTVEIAFDGE----RERAEANVKLSAEKMEP
M.squaD6    -----MCPPKESTRKNAGGPLTRGKLSADLAKLEP
M.pusD6      -----MTRGNKAKLDNSKLAKLEP
                                     : **

Ost809D6      HAVAKSFDRRWVKVDGVEYDVTDFKHPPGGSVIYYMLSN TGADATEAFKEFHYSRKKARKA
O.lucD6      HAIAKTFERRYVTIEGVEYDVTDFKHPPGGSVIYYMLSN TGADATEAFKEFHYSRKKARKA
O.tauriD6    AALAKTFARRYVVEGVEYDVTDFKHPPGGSVIYYMLSN TGADATEAFKEFHHSRKKARKA
M.squaD6    HKLAQTFDTRWVRVGDVEYDVTNFKHPPGGSVIYYMLSN TGADATEAFNEFHMSRPAKWKM
M.pusD6      HKLAQTFEQRWVRIDDVEYDVTNFKHPPGGSVIYYMLSN TGADATEAFKEFHMSRPAKWKM
                                     : * : * * : . * : * : * : * : * : * : * : * : * : * : * : * : *

Ost809D6      LAALPQREPEDAS--PVEDANMLKDFAKWRKDLEREGFFKPSPAHVAYRFAELAAMFALG
O.lucD6      LAALPHKPVDAATREPIEDEAMLKDFAQWRKELEREGFFKPSPAHVAYRFAELAAMFALG
O.tauriD6    LAALPSRP---AKTAKVDDAEMLQDFAKWRKELEREGFFKPSPAHVAYRFAELAAMYALG
M.squaD6    LKALPNRPAETPRSQ-DPDGPMLEDFAKWRAQLEKEGFFKPSIAHVAYRIAELAAMFALG
M.pusD6      LKALPQRPAETPRSA-DPDAPMLQDFARWRAELEKEGFFEPSRLHLAYRCLELCATFALG
* * * * : . * * : * : * : * : * : * : * : * : * : * : * : * : *

Ost809D6      TALMYARWHATSVFVTACFFGARCGWVQHEGGHSSLTGSIWWDKRIQAFTAGFGLASSGD
O.lucD6      TALMHARWHVASVIVYSCFFGARCGWVQHEGGHNSLTGNIWWDKRIQAFAAGFGLASSGD
O.tauriD6    TYLMYARYVVSSVLVYACFFGARCGWVQHEGGHSSLTGNIWWDKRIQAFTAGFGLAGSGD
M.squaD6    CYIMSLGYPVVASIVFGAFFGARCGWVQHEGGHNSLTGNIWWDKRIQAATCGFGLSTSGD
M.pusD6      TFLMYIGRPLLASIVYGAFFGARCGWVQHEGGHNSLTGSIWWDKRIQAATCGFGLSTSGD
: * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *

Ost809D6      MWNLMHNKHHATPQKVRHDMDLDTT PAVAFFNTAVEENRPRKFSKWLWRVQAWT FVPVTS
O.lucD6      MWNNMHNKHHATPQKVRHDMDLDTT PTVAFFNSAVEENRPRGFSKWLWRLQAWT FVPVTS
O.tauriD6    MWNSMHNKHHATPQKVRHDMDLDTT PAVAFFNTAVEDNRPRGFSKYWRLQAWT FIPVTS
M.squaD6    MWNQMHNKHHATPQKVRHDMDLDTT PAVAFFKTAVEDNRPRGFSRAWSRAQAWT FVPVTS
M.pusD6      MWNLQMHNKHHATPQKVRHDMDLDTT PAVAFFDTAVEDNRPRGFSKTWARAQAWT FVPITS
* * * * * : * * : * : * : * : * : * : * : * : * : * : * : * : *

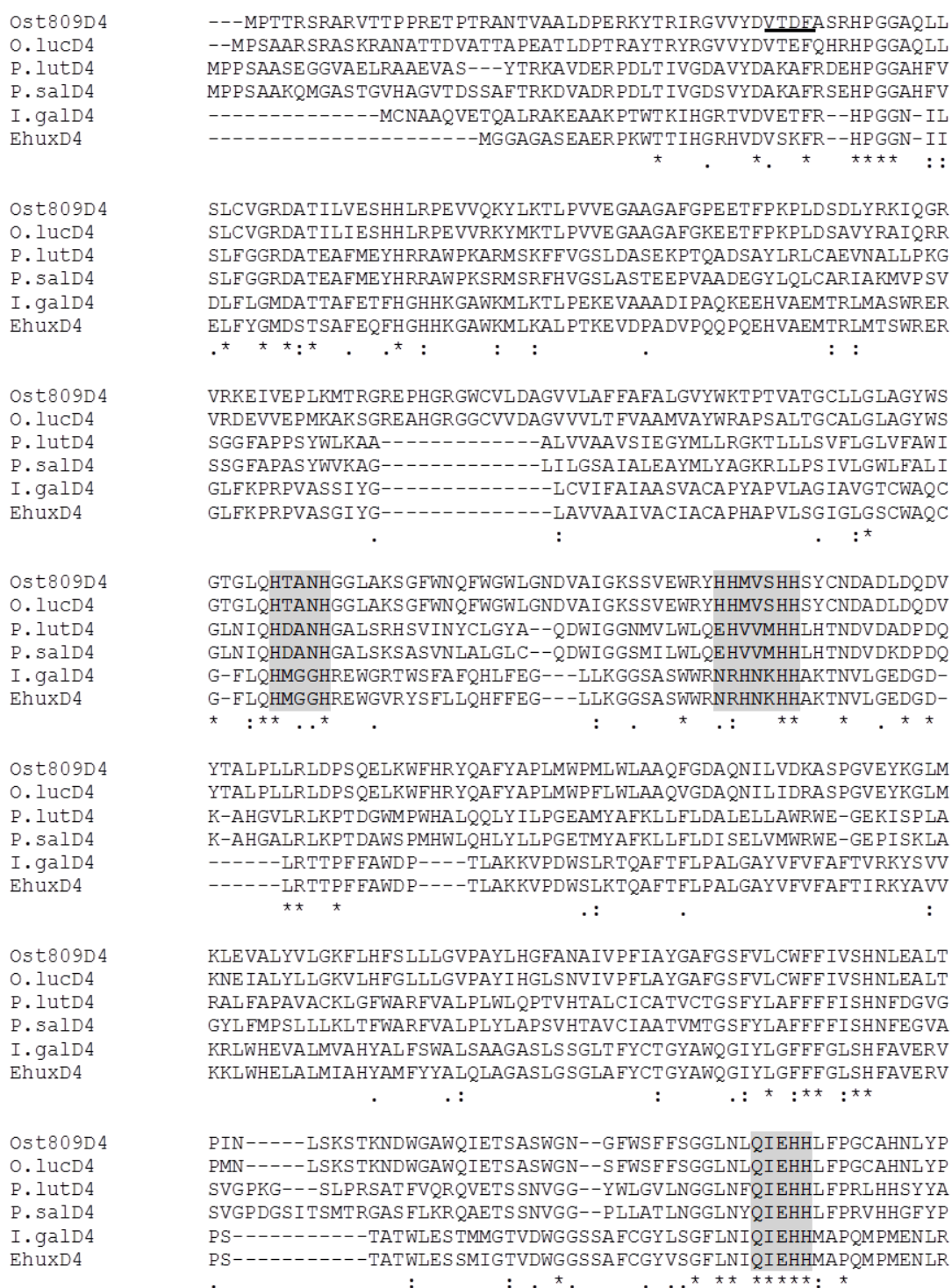
Ost809D6      -GLVLLAWMYLLHPRHIARRKNEYEEAAWIVAAHVIRT SVIKAVTGYSWITCYGLFLSTMW
O.lucD6      -GMVLFWFVFLHPRNALRRKSFEEAAWMFSAHVIRTAVIKAVTGYSWIASYGLFAATMW
O.tauriD6    -GLVLLFWMFLLHPSKALKGKYEELVWMLAAHVIRTWTIKAVTGFTAMQSYGLFLATSW
M.squaD6    GLLVQMFWIYVLHPRQVARKKNEYEEASWMLSHVLRATIKYAGGYSWPVAYLWFSFGNW
M.pusD6      GVLVQMFWIYVLHPRQVLRKKNEYEEASWMLSHVVRTAVIKLALGCGTAEAYGWFWVGNW
: * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *

Ost809D6      VSGCYLFAHFSTSHTHLDVVP SDKHLSWVRYAVDHTIDIDP SKSVVNWLMGYLNCQVIHH
O.lucD6      ASGCYLFAHFSTSHTHLDVVP SDKHLSWVRYAVDHTIDINPNNSVNWLMGYLNCQVIHH
O.tauriD6    VSGCYLFAHFSTSHTHLDVVP ADEHLSWVRYAVDHTIDIDPSQGVNWLMGYLNCQVIHH
M.squaD6    IAYMYLFAHFSTSHTHLEVP SDKHLSWVNYAVDHTVDIDP SKGYVNWLMGYLNCQVIHH
M.pusD6      IAYMYLFAHFSTSHTHLDVVP SDKHLSWVNYAVDHTVDINPRNSIVNWLMGYLNCQVIHH
: * * * * * : * * : * : * : * : * : * : * : * : * : * : * : *

Ost809D6      LFPDMPQFRQPEVSRRFVSAFKKWNLNYKVMSYYGAWKATFGNLNEVGKHYIYIQQSQITK
O.lucD6      LFPDMPQFRQPEVSRRFVSAFKKWNLNYKVLTYYGAWKATFGNLNDVGKHYVHGSQRVK
O.tauriD6    LFPDMPQFRQPEVSRRFVSAFKKWNLNYKVMTYAGAWKATLGNLNDVGKHYVHGHQSHGK
M.squaD6    LFPDMPQFRQPEVSRRFVSAFKKWNLNYKVLTYYGAWKATFTNLDTVGQHYKHKGAHAH
M.pusD6      LFPDMPQFRQPEVSRRFVSAFAEKWNLNYKVLTYYGAWKATFNLDRVQGHYVNGKAKAH
* * * * * : * * : * : * : * : * : * : * : * : * : * : * : *

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**Figure S2.** Multiple sequence alignment of Ost809D4 with  $\Delta 4$ -desaturases from *Pavlova lutheri* (*P.lutD4*, AAQ98793.1) and *Pavlova salina* (*P.salD4*, AY926606.1) [4], *Isochrysis galbana* (*I.galD4*, AY630574) [5], *Ostreococcus lucimarinus* (*O.lucD4*, XM\_001415706.1) [6] and *Emiliania huxleyi* (*E.huxD4*, [7]). Conserved histidine boxes are shaded in grey. Conserved amino acid residues are indicated with an asterisk. The position of conserved cytochrome *b*<sub>5</sub> domain motif is marked with a solid line. The alignment was obtained using CLUSTAL W. A related  $\Delta 4$ -desaturase from *Thalassiosira pseudonana* (*TpD4*, AAX14506.1) [8] was omitted from this line-up on the basis of poor alignment.



**Figure S3.** Multiple sequence alignment of FcElo6 with Δ6-elongase from *Thalassiosira pseudonana* (TpElo6, AY591337.1) and *Ostreococcus tauri* (OtElo6, AY591335) [5]. Conserved amino acid residues are indicated with an asterisk, whereas conserved motifs are framed. The alignment was obtained using CLUSTAL W.

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FcElo6      -----MDEYKATLESVGDALIQWADPESQFTGFTKGWFLTDFTS
TpElo6      -----MDAYNAAMDKIGAAIIDWSDPDGKFRADREDWWLCDFRS
OtElo6      MSGLRAPNFLHRFWTKWDYAISKVVFTCADSFQWDIGPVS SSTAHLPAIESPTPLVTSLL
              .   :   ...   *   ...   ...   .   .   *

FcElo6      AFSIALVYVLFVVIIGSQVMKVLPAIDPYPIKFFYNVSQIMLCAYMTIEACLLAYRNGYTI
TpElo6      AITIALIYIAFVILGSAVMQSLPAMDYPPIKFLYNVSQIFLCAYMTVEAGFLAYRNGYTV
OtElo6      FYLVTVFLWYGRLTRSSDKKIREPTWLRRFIICHNAFLIVLSLYMCLGCVAQAYQNGYTL
              :::   :   *   :   .   :   :   *   *   *   *   :   .   **   :   :   :

FcElo6      MPCVGYNRDDPAIGNLLWLFYVSKVWDFWDTLFIIVLGKKWRQLSFLHVIYHHTTIFLFYWL
TpElo6      MPCNHFNVDPPVANLLWLFYISKVWDFWDTLFIIVLGKKWRQLSFLHVIYHHTTIFLFYWL
OtElo6      WG-NEFKATETQLALYIYIFVYSKIYEFVDTYIMLLKNNLRQVSLHVIYHHTTISFIWWI
              ::   ..   .   .   :   :   :   :   :   :   *   *   :   :   *   :   :   :   :   :   :   :

FcElo6      NANVFYDGGDIYLTIALNGFIHTVMYTYYFICMHTKDCKTGKSLPIWWKSLTLLQLLQFFI
TpElo6      NANVLYDGGDIFLTILLNGFIHTVMYTYYFICMHTKDSKTGKSLPIWWKSLTAFQLLQFFI
OtElo6      IARRAPGGDAYFSAALNSWVHVCMYTYYLLSTLIGKEDPKRSNYLWWGRHLTQMOMLQFFI
              *   .   **   :   :   **   :   :   *   :   :   *   :   :   *   :   :   *   :   :   *

FcElo6      TMMSQGLYLIIFGCESLSIRVTATYVVYIILSLFFLFAQFFVASYMQPKKSKTA-ELGTLLI
TpElo6      IMMSQATYLVFHGCDKVS LRITIVYFVSLLSLFFLFAQFFVQSYMAPKPKKSA-----
OtElo6      FNVLQALYCASF--STYPKFLSKILLVYMMSSLGLFGHFYYSKHIAAAKLQKKQQ-----
              :   *   *   .   .   .   :   .   *   :   :   :   *   :   :   :   :   :   :
    
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**Figure S4.** Codon-optimised nucleotide sequences. (A), Ost 809D6; (B), Ost809D4; (C) Fc ELO6.

**A**

1 ATGCGTGTGGAAACCGAAGACGATAATGTGCCAACTGTTACTGTGGGATTGTCAGAGGAG  
61 TCCGATGGAATGAAGGGAGCAAGGAACCCCGGAGCACGTGCTTGGAAGTCGACGTTGGAG  
121 CCGCACGCCGTGGCAAAGTCATTTCGATCGTAGGTGGGTAAAGGTTGACGGAGTCGAATAC  
181 GACGTAAGTATTTCAGCATCCCGGAGGATCAGTTATCTACTATATGCTTTCTAACACC  
241 GGAGCTGATGCCACTGAGGCTTTCAGGAATTTCACTATCGTAGTAAGAAGGCCAGGAAG  
301 GCACTTGCTGCCCTCCACAACTGAGCCTGAAGACGCTTCGCCAGTCGAGGATGCCAAT  
361 ATGCTCAAGGACTTCGCAAAGTGGCGTAAGGATTTGGAGAGGGAAGGATTCTTTAAGCCA  
421 AGTCCTGCTCACGTGGCCTACCGTTTTCGCCGAAGTTCGAGCTATGTTTGCTTTGGGAACT  
481 GCCCTTATGTATGCACGTTGGCATGCTACGTCTGTCTTCGTAACAGCCTGTTTCTTTGGA  
541 GCAAGGTGTGGATGGGTGCAACACGAGGGAGGACATTCTTCCTTGACCGGATCCATCTGG  
601 TGGGATAAGCGTATTTCAGGCATTCCTGCTGGATTTGGACTTGCCAGTTCGGGAGACATG  
661 TGGAACCTCATGCACAATAAGCACCATGCAACGCCACAAAAGTTAGGCATGATATGGAC  
721 CTCGATAACCACTCCTGCAGTGGCTTTCTTTAACACAGCTGTTGAGGAAAATCGTCCTAGG  
781 AAGTTCTCTAAGTTGTGGCTTCGTGTCCAGGCCTGGACCTTTGTGCCCGTTACTTCCGGA  
841 TTGGTACTCTTGGCATGGATGTACCTTCTCCACCCGCGTCATATCGCTCGTAGGAAGAAC  
901 TATGAGGAAGCCGCATGGATTGTGGCTGCCCATGTTATCAGGACCTCCGTCATTAAGGCT  
961 GTAACGGGATACAGTTGGATCACATGTTATGGACTCTTCTTGTCGACTATGTGGGTCTCA  
1021 GGATGCTACCTCTTCGCTCACTTTTCAACGTCTCACACACATTTGGACGTGGTTCCATCT  
1081 GATAAGCACCTTTCCTGGGTGCGTTACGCCGTTGATCATAACCATCGACATTGATCCTTCC  
1141 AAGAGTGTTCGTAAGTGGCTCATGGGATATTTGAACTGTCAGGTTATCCACCATTTGTTC  
1201 CCCGACATGCCGCAATTTTCGTCAGCCCGAAGTCAGTCGTAGGTTTCGTATCGTTTGCCAAG  
1261 AAGTGGAACCTTAATTACAAGGTCATGTCTTACTATGGAGCCTGGAAGGCAACCTTCGGA  
1321 AATCTCAACGAAGTCGGAAGCACTACTACATCCAAGGAAGTCAAATCACAAAGAAGACG  
1381 GTTTAG



Figure S4. Cont.

**B**

1 ATGCCAACTACTCGTTCTCGTGCTCGTGTTACTACTCCACCTCGTGAAACTCCTACTCGT  
61 GCTAATACTGTTGCTGCTTTAGATCCAGAACGTAAATATACACGTATTCGAGGTGTTGTA  
121 TATGATGTTACTGATTTTGCTAGTCGACATCCAGGTGGTGCACAATTATTATCTTTATGT  
181 GTTGGTCGTGATGCTACAATTTTAGTAGAATCACATCATTACGACCAGAAGTTGTACAA  
241 AAATATTTAAAAACATTACCTGTTGTAGAAAGGTGCTGCTGGTGCATTTGGTCCAGAAGAA  
301 ACTTTTCAAAACCTTTAGATAGTGATTTATATCGTAAAATTCAAGGTCGTGTTCGAAAA  
361 GAAATTGTAGAACCATTAAAAATGACACGTGGTGCAGAACCTCATGGTCGTGGTTGGTGT  
421 GTTTTAGATGCTGGTGTGTATTAGCTTTCTTTGCTTTTGCATTAGGTGTTTATTGGAAA  
481 ACACCAACTGTAGCTACTGGTTGTTTATTAGGTTTAGCAGGTTATTGGTCTGGTACAGGT  
541 TTACAACATACTGCTAATCATGGTGGTTTAGCAAAATCAGGTTTTTGAATCAATTTTGG  
601 GGTGGTTAGGAAATGATGTTGCTATTGGTAAATCAAGTGTAGAATGGCGTTATCATCAT  
661 ATGGTTTCACATCATAGTTATTGTAATGATGCTGATTTAGATCAAGATGTTTATACAGCA  
721 TTACCATTATTACGTTTAGATCCTTCACAAGAATAAAATGGTTTCATCGTTATCAAGCA  
781 TTTTATGCACCTTTAATGTGGCCTATGTTATGGTTAGCTGCACAATTTGGTGATGCTCAA  
841 AATATTTTAGTTGATAAAGCAAGTCCAGGTGTAGAATATAAAGGTTTAAATGAAATTAGAA  
901 GTTGCTTTATATGTATTAGGAAAATTTTACATTTTCTTTATTATTAGGTGTTCTGCA  
961 TATTTACATGGTTTTGCTAATGCAATTGTACCATTTATTGCTTATGGTGCATTTGGTTCA  
1021 TTTGTTTTATGTTGGTTTTTCATTGTAAGTCATAATTTAGAAGCATTAAACACCAATTAAT  
1081 TTATCTAAATCAACTAAAAATGATTGGGGTGCTTGGCAAATTGAAACTAGTGCATCTTGG  
1141 GGTAATGGTTTTTGGTCATTTTCTCAGGTGGTTTAAATTTACAAATTGAACATCATTTA  
1201 TTTCTGGTTGTGCTCATAATTTATATCCAAAAATGGTTCCTATTATTAAAGAAGAATGT  
1261 GAAAAAGCAGGTGTTACATATACTGGTTATGGTGGTATTTTGGTTTATTACCAATTACT  
1321 CGTGATATGTTTGCTTATTTATATAAAAATGGGTCGTCAATCTAAAAAATCTGCTTAA

Figure S4. Cont.

C

1 ATGGATGAGTACAAGGCCACTTTGGAGTCAGTAGGAGACGCTATTATTCAATGGGCAGAC  
61 CCCGAGTCACAGTTCACCGGATTCACCTAAGGGATGGTTCCTCACCGATTTTACTTCAGCT  
121 TTCTCTATCGCCCTCGTCTACGTATTGTTTCGTGATCATTGGATCCCAAGTGATGAAGGTT  
181 CTCCCTGCTATCGACCCATACCCTATCAAGTTCCTTTTATAACGTTAGTCAGATCATGTTG  
241 TGTGCCTATATGACCATTGAGGCATGCCTCTTGGCTTACCCTAATGGATATACTATCATG  
301 CCCTGTGTCGGATACAACAGGGATGACCCGGCAATTGGAAATCTTCTCTGGCTTTTCTAT  
361 GTCTCGAAAGTATGGGATTTTTGGGACACGATCTTCATTGTGTTGGGAAAGAAGTGGCGT  
421 CAATTGTCATTTCTTCACGTTTACCACCATAACCACTATCTTCCTCTTCTACTGGCTCAAC  
481 GCCAATGTCTTCTACGATGGAGACATCTATTTGACCATTGCACCTAACGGATTTATCCAC  
541 ACGGTAATGTACACATACTACTTTCATCTGTATGCATACCAAGGATAAGAAGACTGGAAAG  
601 AGTCTCCCAATCTGGTGGAAAGTCTTCCCTCACGTTGCTTCAATTGTTCCAGTTCATCACA  
661 ATGATGTCTCAGGGACTCTACTTGATCATTTTTCGGATGCGAAAGTTTGTGATCAGGGTG  
721 ACGGCCACATACGTGGTTTATATTCTTTCTCTCTTCTTTTTGTTGCCCCAGTTTTTCGTC  
781 GCCTCTACATGCAGCCCAAGAAGTCCAAGACAGCCTGA

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