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Supplementary Information

Digital quantification and selection of high-lipid-producing microalgae through a lateral dielectrophoresis-based microfluidic platform

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Supplementary Figure S1	Calculated real part of the Clausius-Mossotti factor of microalgal cells having different lipid content
Supplementary Figure S2	Calculated x-directional pDEP force and lateral displacement per single electrode
Supplementary Figure S3	Calculated lateral displacement of microalgal cells having different size and lipid content per single electrode
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Supplementary Figure S5	Flow cytometry measurement of lipid content of 12 mutants selected from EMS-mutated CC-406 cells
Supplementary Figure S6	Flow cytometry measurement of lipid content of 22 mutants selected from EMS-mutated #5
Supplementary Figure S7	Analysis of lipid content of cells through flow cytometry measurement
Supplementary Figure S8	Growth analysis of 1st-mutant #5 and 2nd-mutant #16

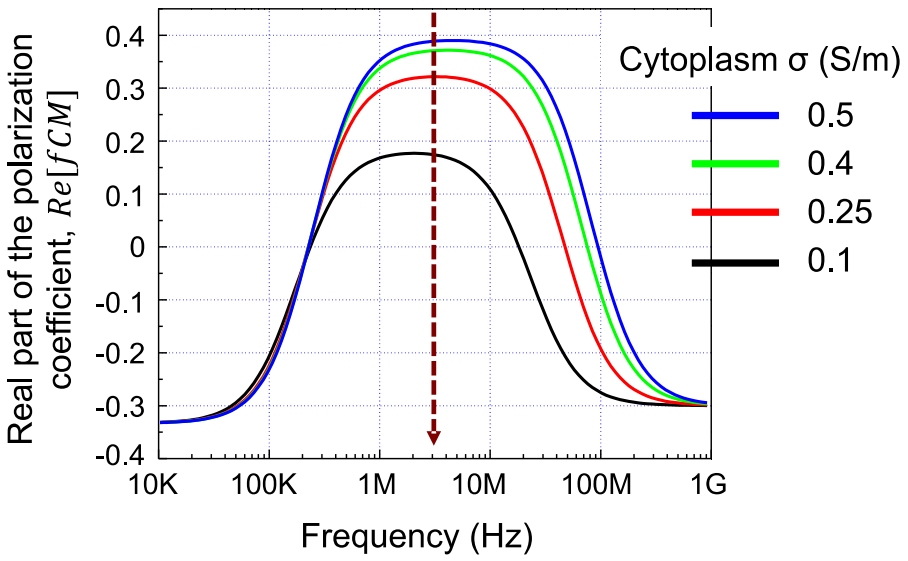

Figure S1. Calculated real part of the Clausius-Mossotti factor ($^{Re[f_{CM}]}$) of microalgal cells having different lipid content, when medium conductivity is set to 0.04 S/m. The measured average cell size of 7.6 $\pm$ 0.6 µm was used, and the cytoplasm conductivity was set to 0.5 S/m in the case of no intracellular lipid (1). The cytoplasm conductivity was calculated to be 0.4, 0.25, and 0.1 when intracellular lipid content increased to 25, 50, and 80% of cell volume, respectively. This resulted in decreased magnitude of $Re[f_{CM}]$ at the frequency of 3 MHz.

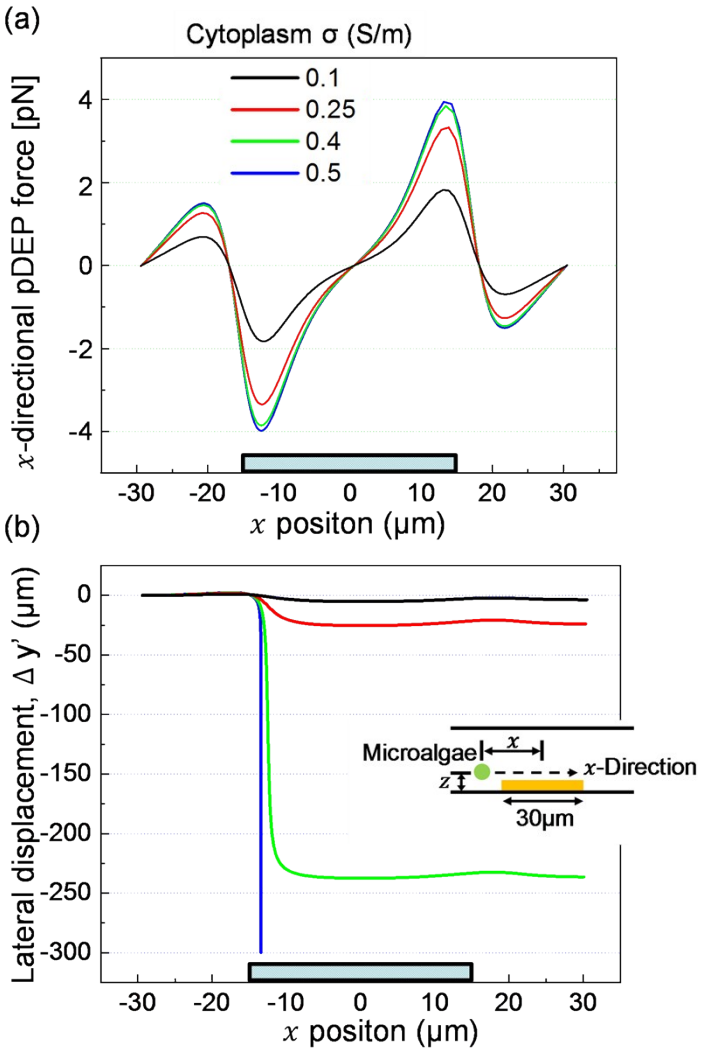

Figure S2. Calculated (a) x-directional pDEP force and (b) lateral displacements per single electrode positioned at an angle of 5.7° to the direction of flow, with cell's cytoplasm conductivities varying from 0.1 (—) to 0.25 (—), 0.4 (—), and 0.5 (—). The calculated x-directional pDEP force The hatched bar on the x-axis represents a single electrode in x-axis as shown in Fig. 1 (also see image in the inset). Based on the simplified line charge model represented by equation (1) in the main document, the pDEP force and lateral displacements were numerically calculated using a program written in C language. The graphs show that decreased cytoplasm conductivity results in decrease of the magnitude of pDEP force at the edge of electrode, and thus decrease of lateral displacement. The diameter of microalgae was set to 7.6 μm and $Re[f_{CM}]$ was set to 0.17, 0.32, 0.37, and 0.39 calculated in Fig. S1 when cytoplasm conductivities were 0.1, 0.25, 0.4, and 0.5, respectively. Other parameters used were $z = 7.6\,\mu m$, $v_a = 5\,V_{rms}$, n = 1 to 4, and $\eta = 0.96 \times 10^{-3}\,N \cdot s/m^2$.

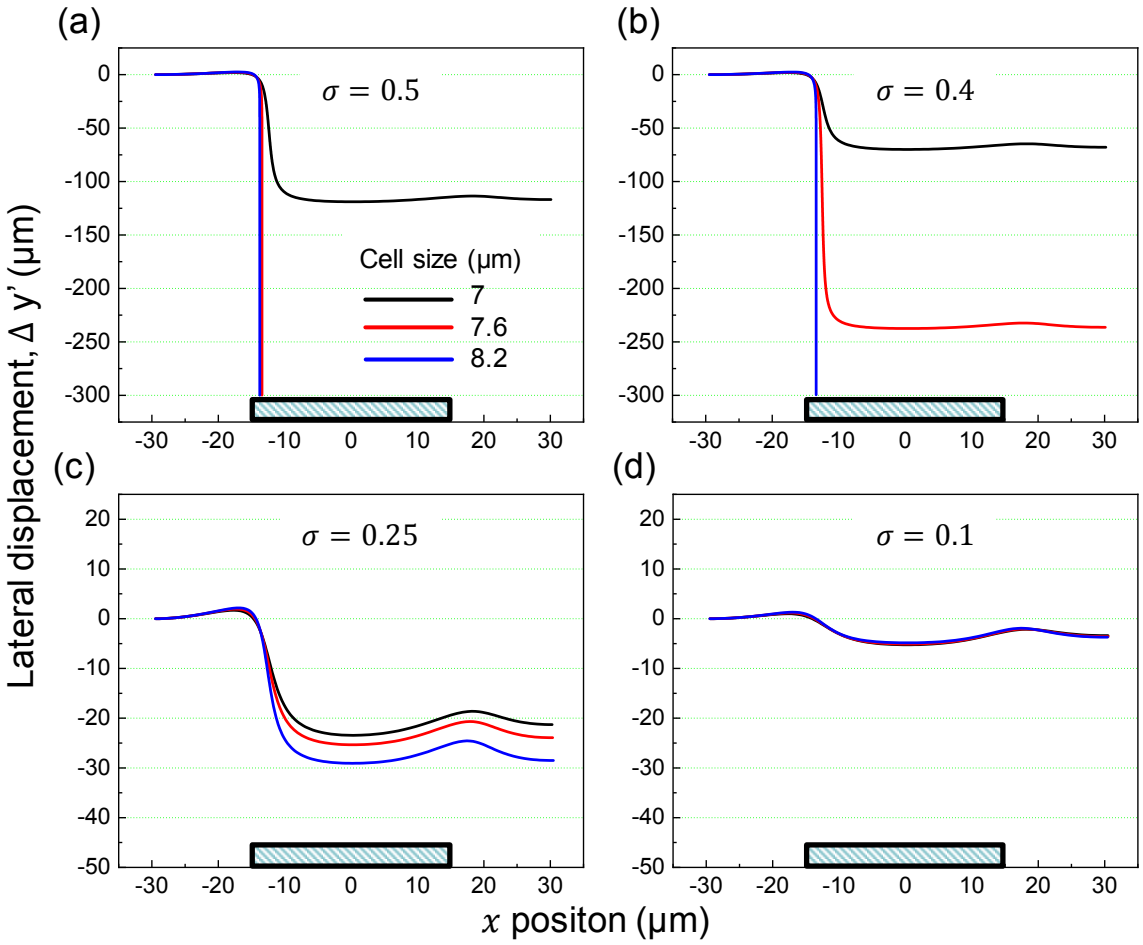

Figure S3. Calculated lateral displacements per single electrode positioned at an angle of 5.7° to the direction of flow, with different cell sizes (7 (—), 7.6 (—), and 8.2 (—) μm) and different cytoplasm conductivities (σ) (0.5, 0.4, 0.25, and 0.1 S/m). The graphs show that as the cytoplasm conductivity decreases, the lateral displacement is dominated by cytoplasm conductivity, rather than cell size. Other parameters used were z = cell diameter, $v_a = 5\, V_{rms}$, n = 1 to 4, and $\eta = 0.96 \times 10^{-3}\, N \cdot s/m^2$.

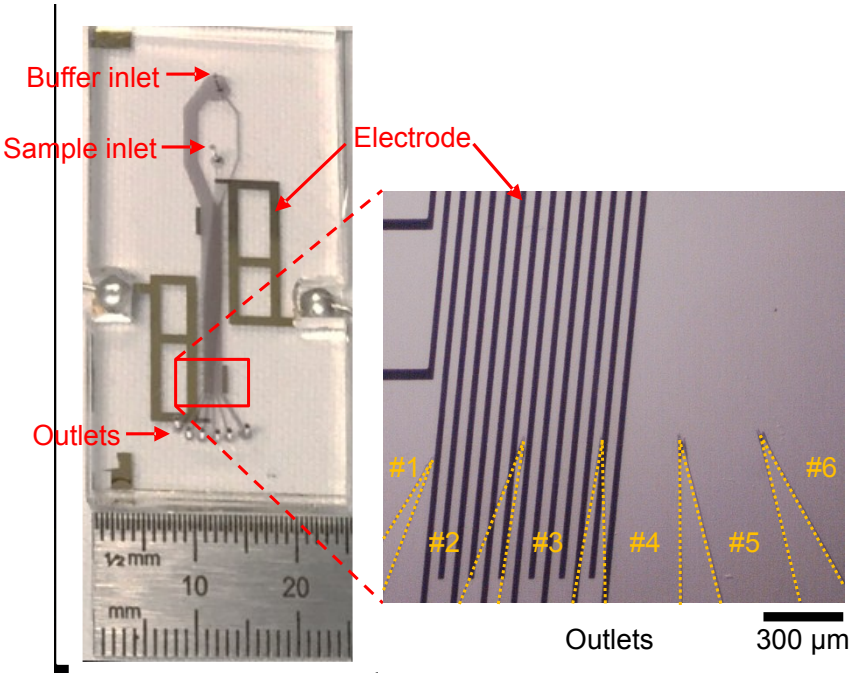

Figure S4. The fabricated lateral DEP microfluidic platform.

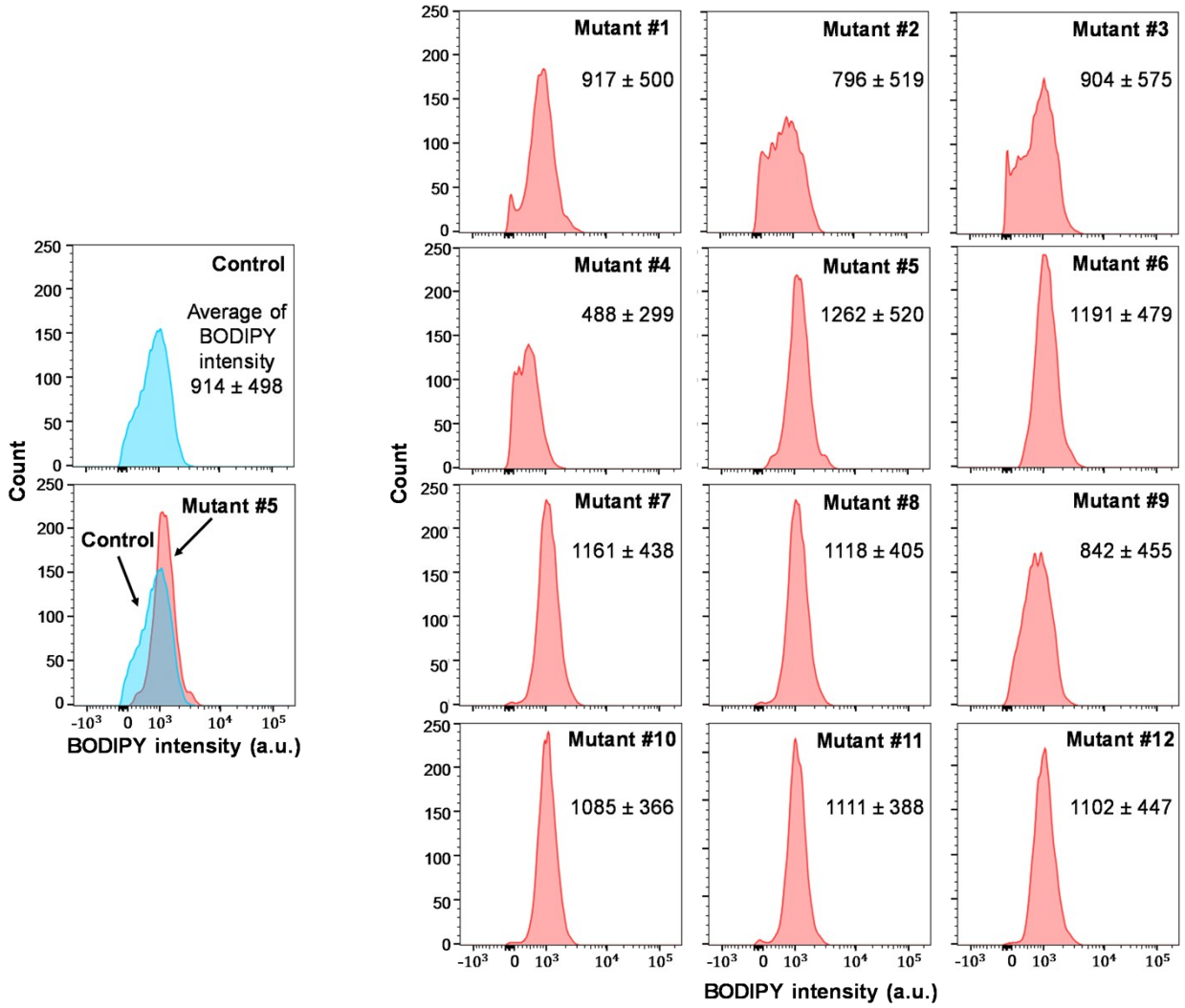

Figure S5. Flow cytometry characterization of lipid level of 12 mutants randomly selected from the first round of EMS mutant screening process, compared to the beginning population (CC-406 culture in TAP-N media for 72 hr). The average of BODIPY fluorescence intensity of each mutant was indicated in each graph with standard deviation. Among the 12 mutants, mutant #5 showed the highest average BODIPY fluorescence intensity, which was 1.4 times higher (1262 ± 520 (a.u.)) compared to that of wild-type CC-406 cells (914 ± 498 (a.u.)).

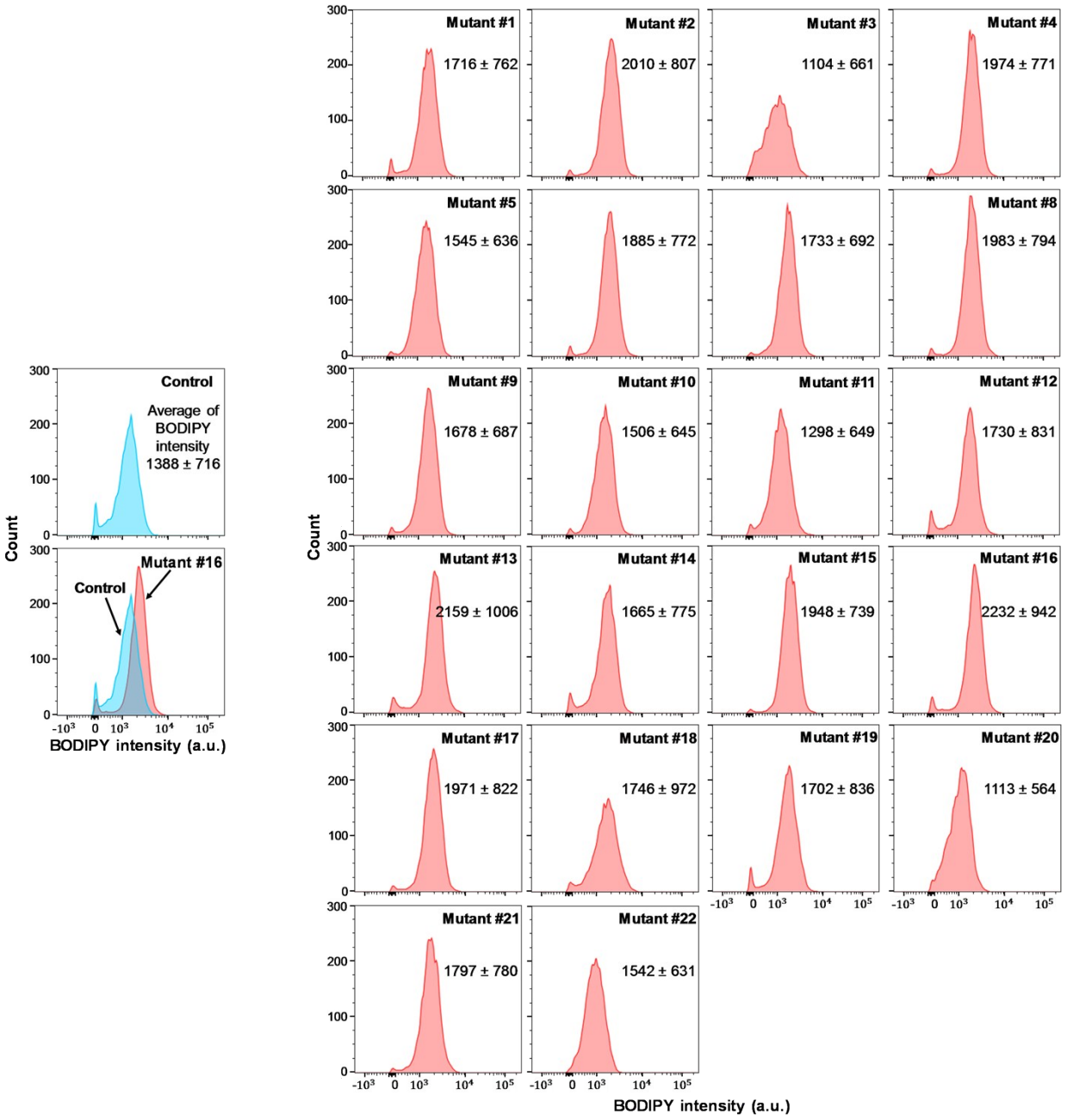

Figure S6. Flow cytometry characterization of lipid level of 22 mutants randomly selected from the screening process using EMS-mutated 1st-mutant #5, compared to the beginning population (CC-406 culture in TAP-N media for 72 hr). The average BODIPY fluorescence intensity of each mutant was indicated in each graph with standard deviation. Among the 22 mutants, mutant #16 showed the highest average BODIPY fluorescence intensity, which was 1.6 times higher (2232 ± 942 (a.u.)) compared to that of wild-type CC-406 cells (1388 ± 716 (a.u.)).

As shown in the flow cytometry analysis in **Fig. S7**, the average BODIPY-stained lipid fluorescence intensity of control (CC-406 cells with no lipid) was 6.0 ± 3.0 (× 10^4 a.u.). As expected, increased incubation time in TAP-N media resulted in increased intracellular lipid level, thus the fluorescence intensities of each sample cultured in TAP-N media for 12, 24, 36, and 48 hr were respectively 12.5 ± 7.2, 27.7 ± 19.1, 46.0 ± 23.4, and 65.6 ± 41.3 (× 10^4 a.u.). The intracellular lipid amount was close to saturation after 36 hr of incubation in TAP-N media, thus the histograms of 36 and 48 hr overlapped quite a bit, but the average BODIPY fluorescence intensity of the cells cultured for 48 hr was still 1.4 times higher than that for 36 hr. Based on this result, these five samples were used as a model microalgal population having five different intracellular lipid levels, and were used to test and demonstrate the feasibility of the lateral DEP microfluidic platform for microalgae separation depending on the lipid content.

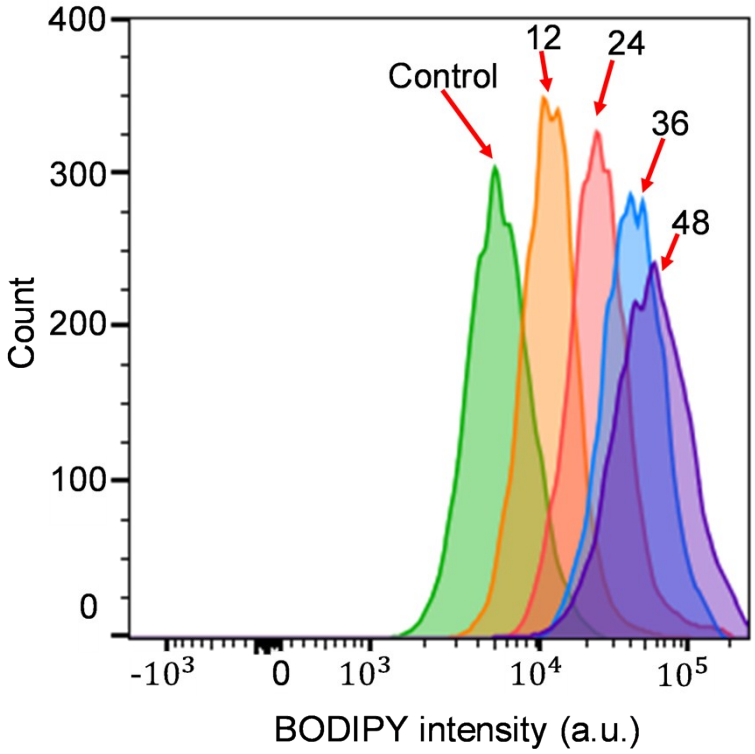

Figure S7. Flow cytometry analysis of lipid content of *C. reinhardtii* CC-406 cells cultured in TAP-N media for 12, 24, 36, and 48 hr. CC-406 cells with no lipid induction was used as a negative control.

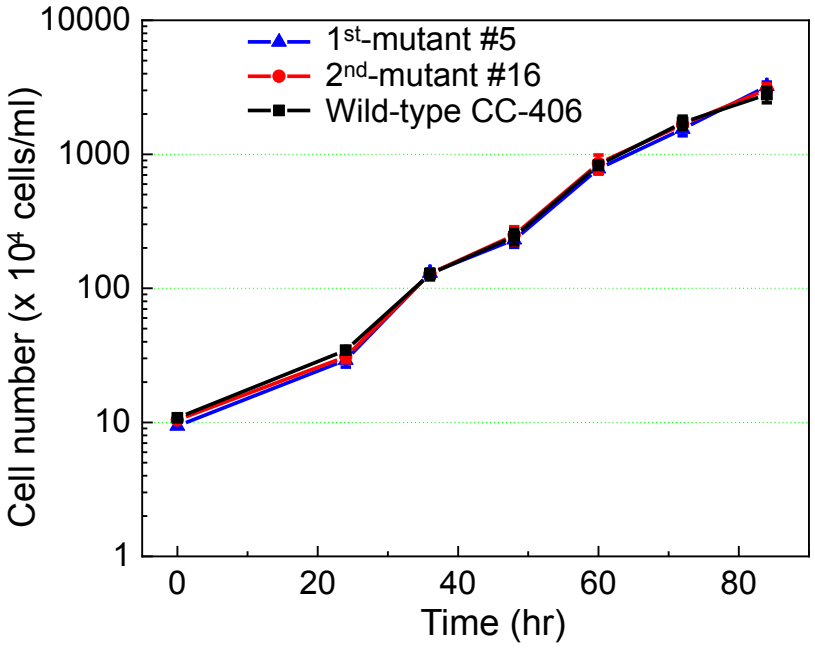

Figure S8. Growth comparison of 1st-mutant #5, 2nd-mutant #16, and wild-type CC-406 cultured in TAP media.

Reference

1. Y.-L. Deng, M.-Y. Kuo, Y.-J. Juang, Development of flow through dielectrophoresis microfluidic chips for biofuel production: Sorting and detection of microalgae with different lipid contents. Biomicrofluidics 8, 064120 (2014).