Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Information

DNA delivery by high aspect ratio nanomaterials to algal chloroplasts

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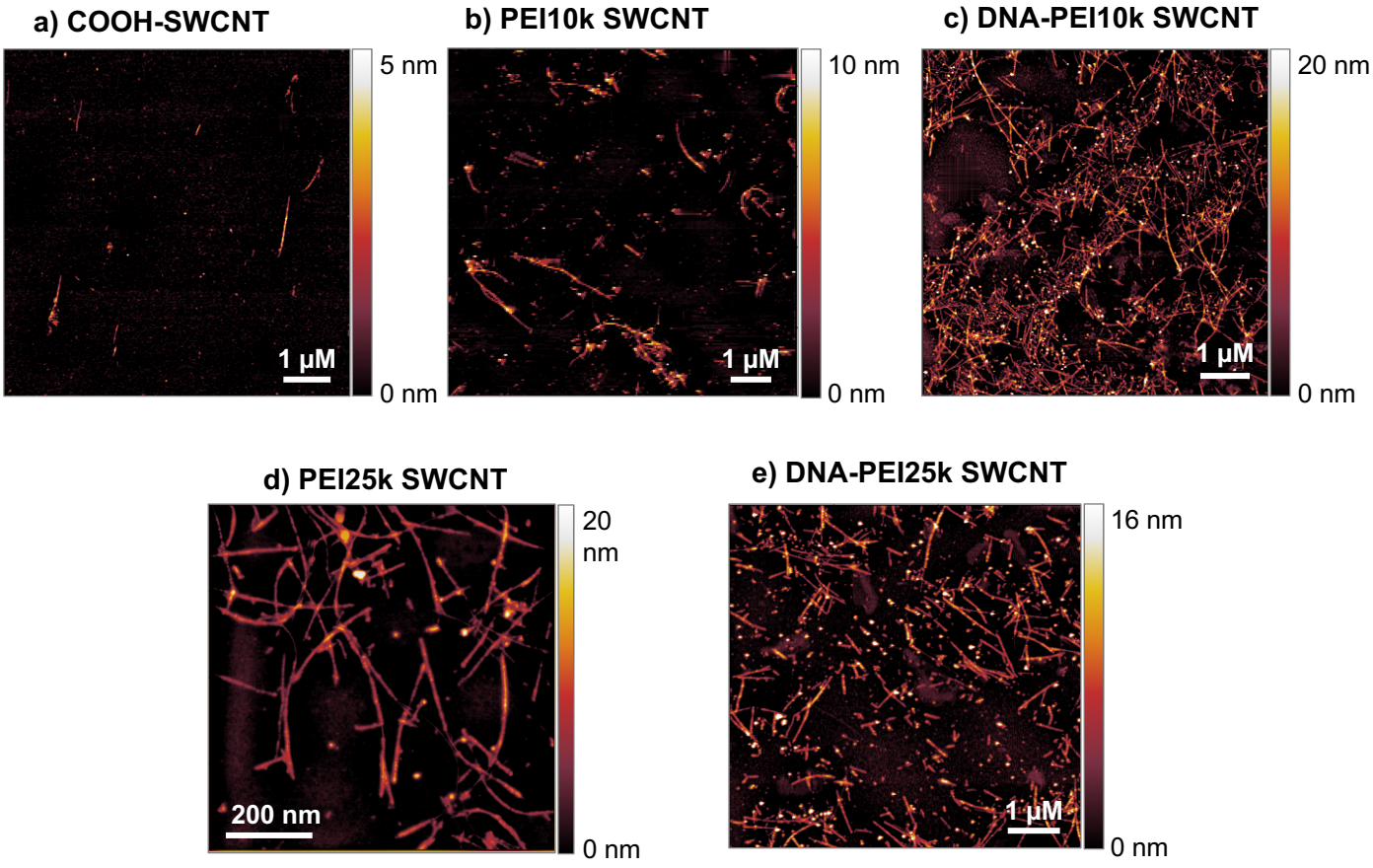
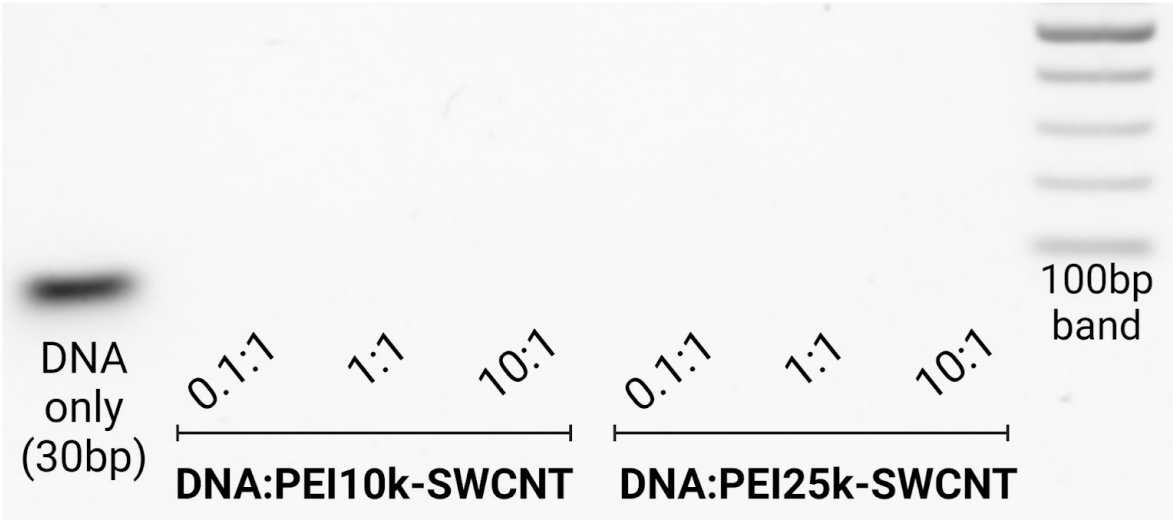
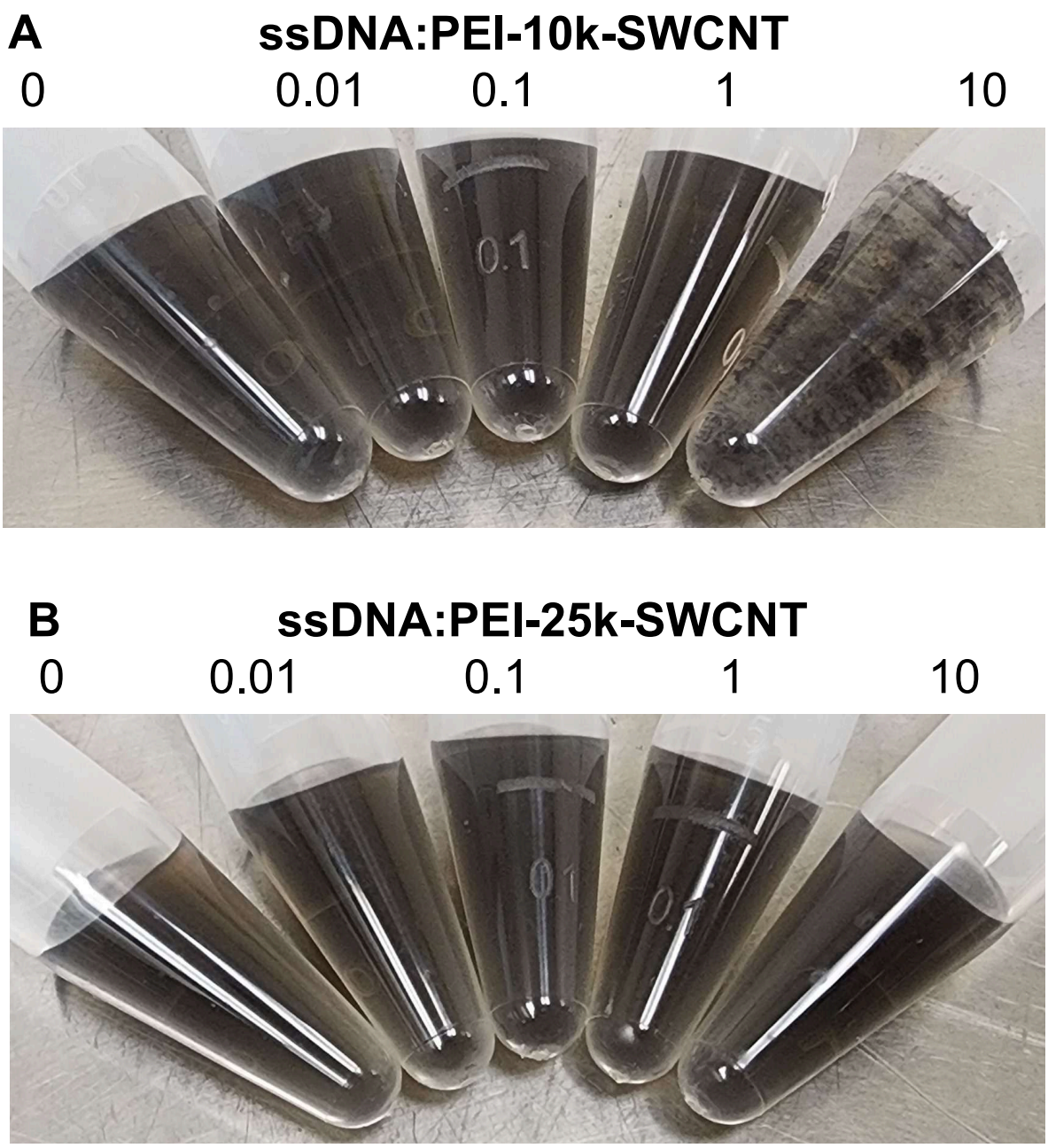


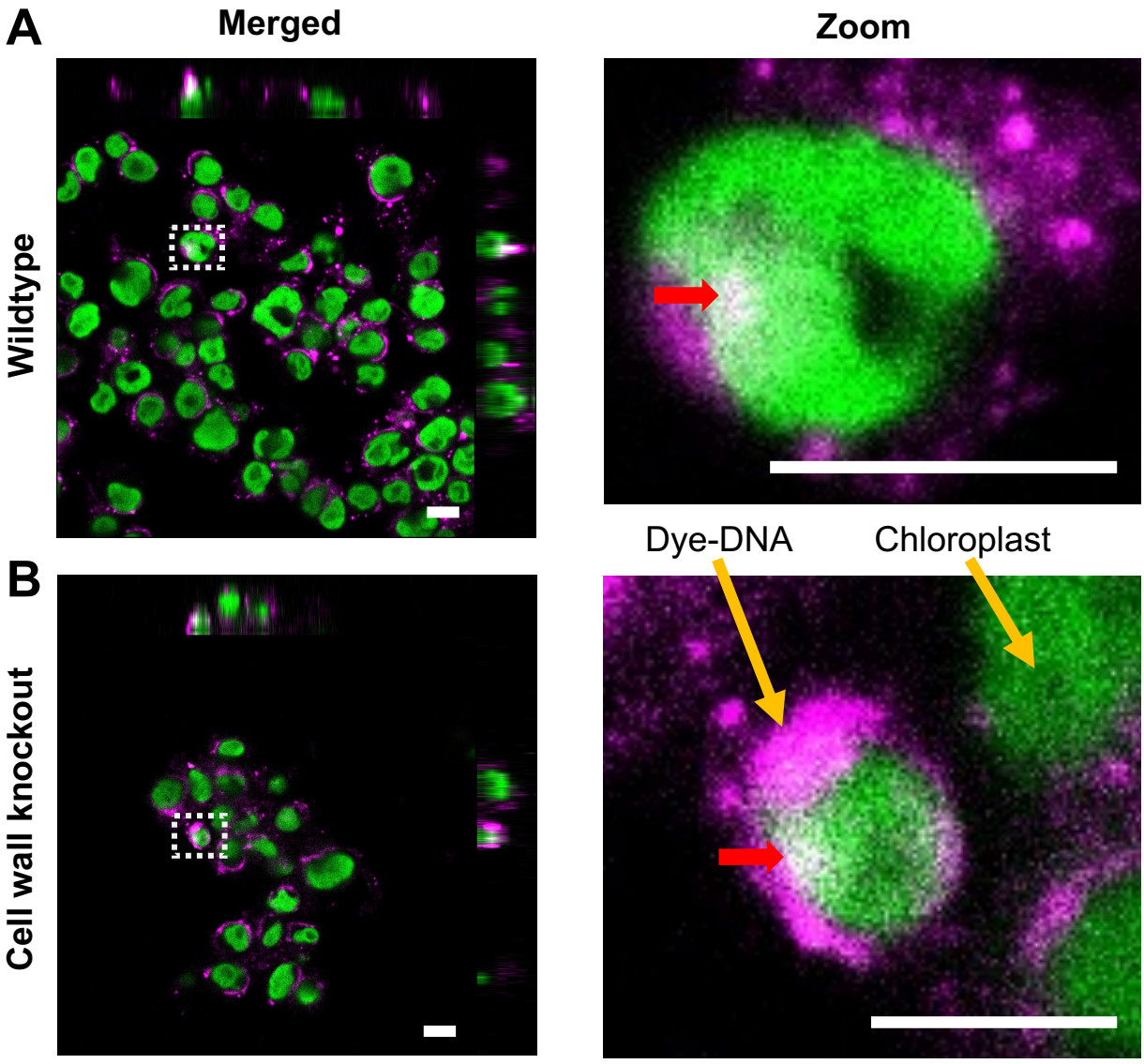
Supplementary Figure 1. AFM images of a) COOH-SWCNT, b) PEI10k SWCNT c) GT_{15} ssDNA coated PEI10k-SWCNT, d) PEI25k SWCNT, e) GT_{15} ssDNA coated PEI25k-SWCNT collected by tapping mode.



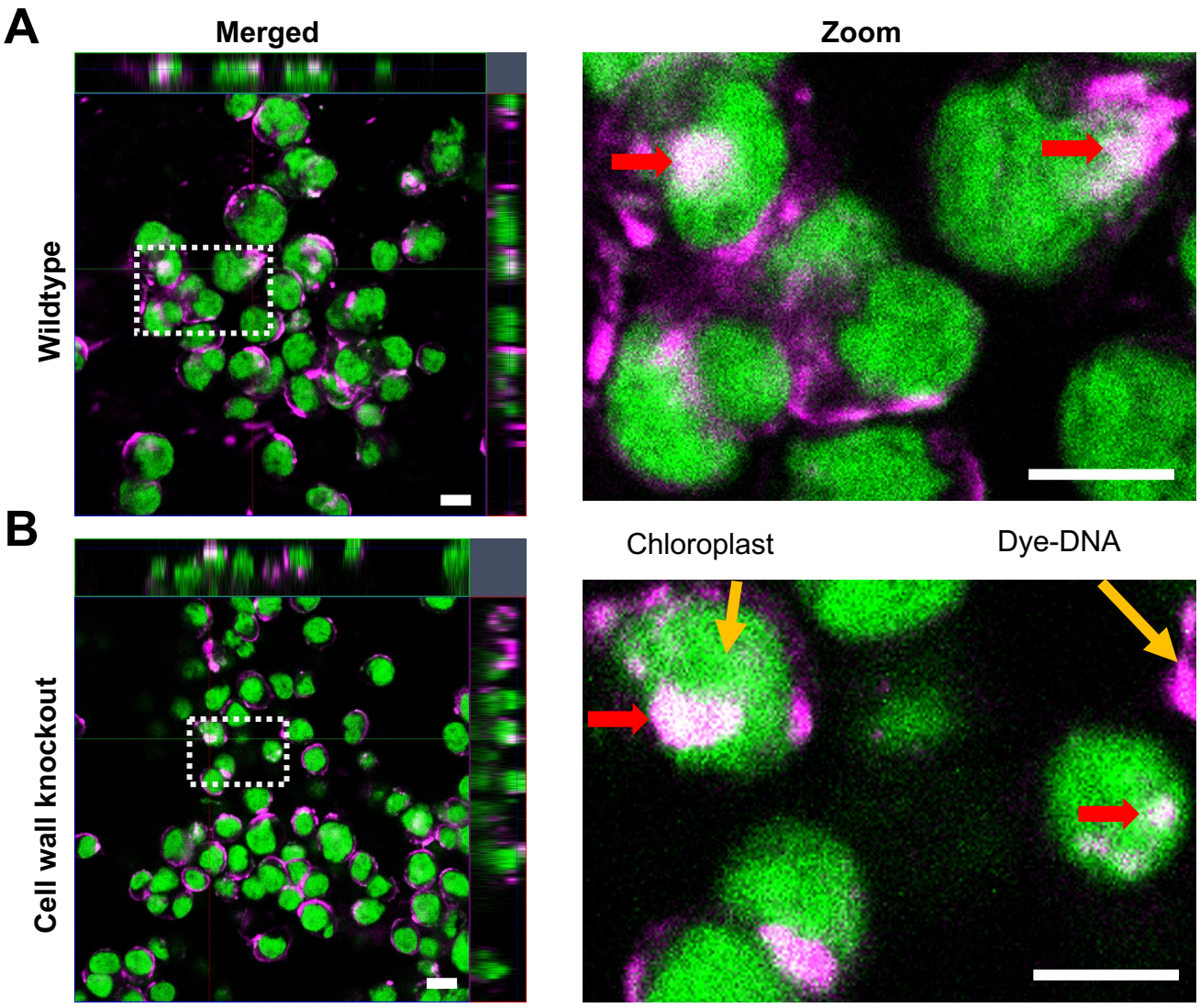
Supplementary Figure 2. Nanomaterial characterization of ssDNA binding efficiency. Gel electrophoresis with 1% TBE agarose gel of 0.1:1, 1:1 and 10:1 mass ratios of GT_{15}:PEI-SWCNT for DNA loading efficiency quantification after a 1 hour binding reaction shows 100% binding to PEI10k- and PEI25k-SWCNT.



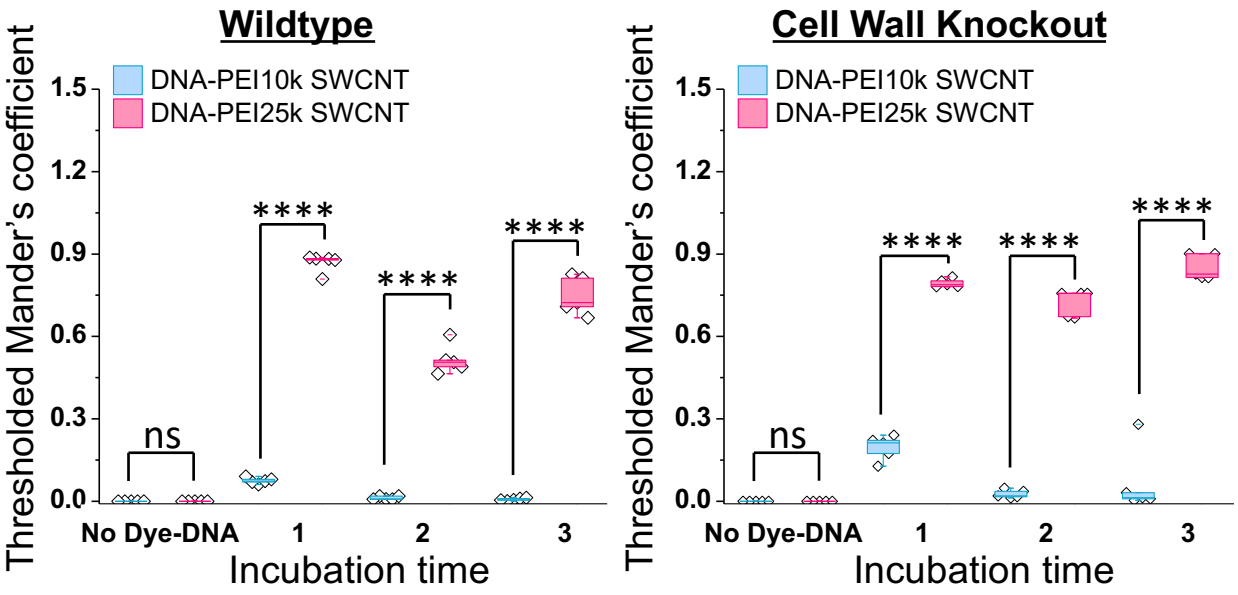
Supplementary Figure 3. Aggregation of ssDNA-PEI-SWCNT at high ssDNA:PEI-SWCNT ratio. a) PEI10k-SWCNT visibly aggregated at a 10:1 ratio of ssDNA:PEI-SWCNT, while b) PEI25k-SWCNT did not show any visible signs of aggregation at any of the testing ratios.



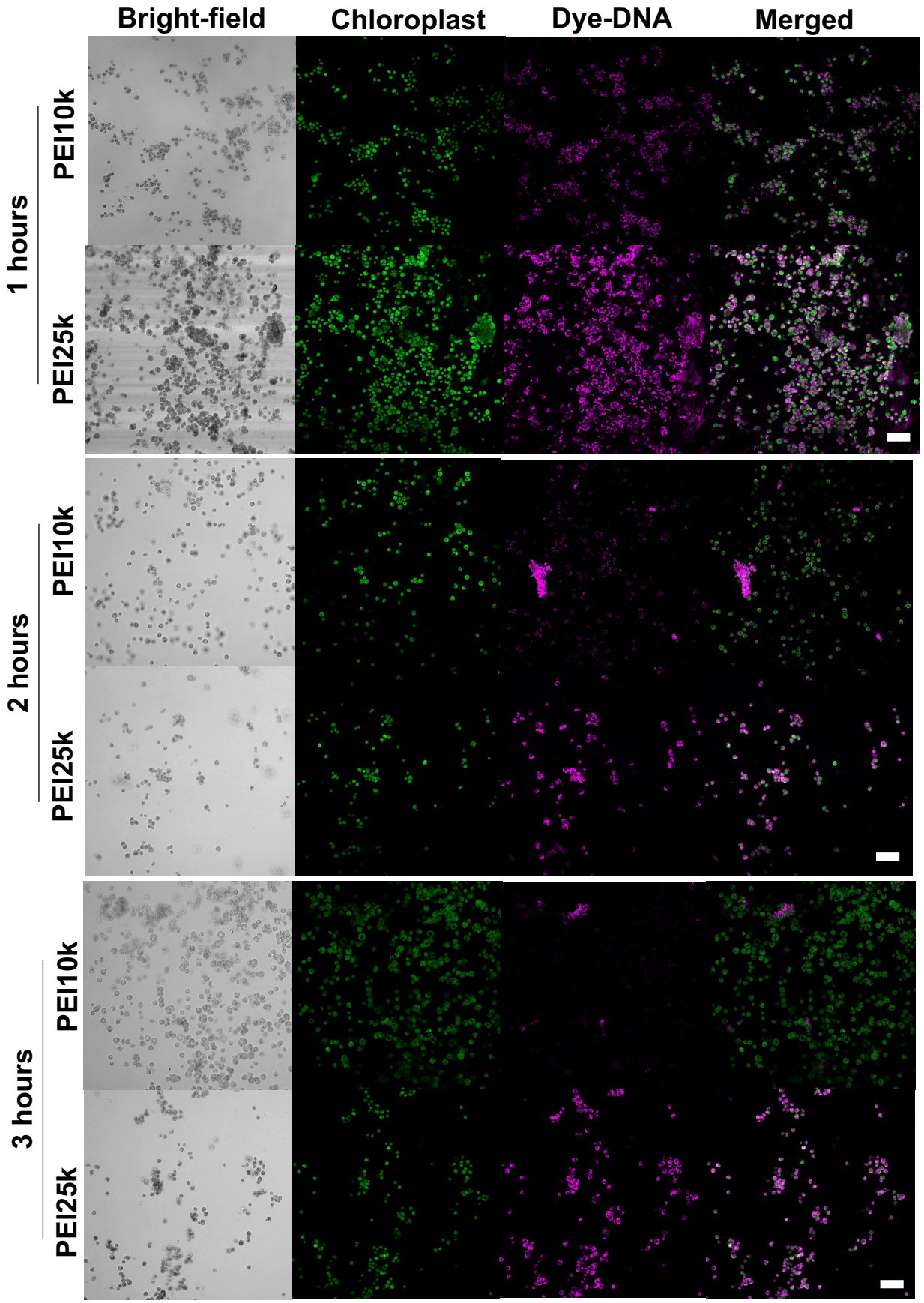
Supplementary Figure 4. Colocalization of Dye-DNA delivered by PEI10k-SWCNT within algae chloroplasts. Dye-DNA-PEI10k-SWCNTs colocalization with chloroplasts in the a) wildtype and b) cell wall knockout strain, after 1 hour incubation with 300 fg/cell of PEI10k-SWCNTs at a 1:1 Dye-DNA:SWCNT ratio (n=5).The scale bar is 10 μm. Overlap between Dye-DNA and chloroplasts is highlighted in the orthogonal views representing projections on the z-axis. Red arrows indicate areas of overlap.



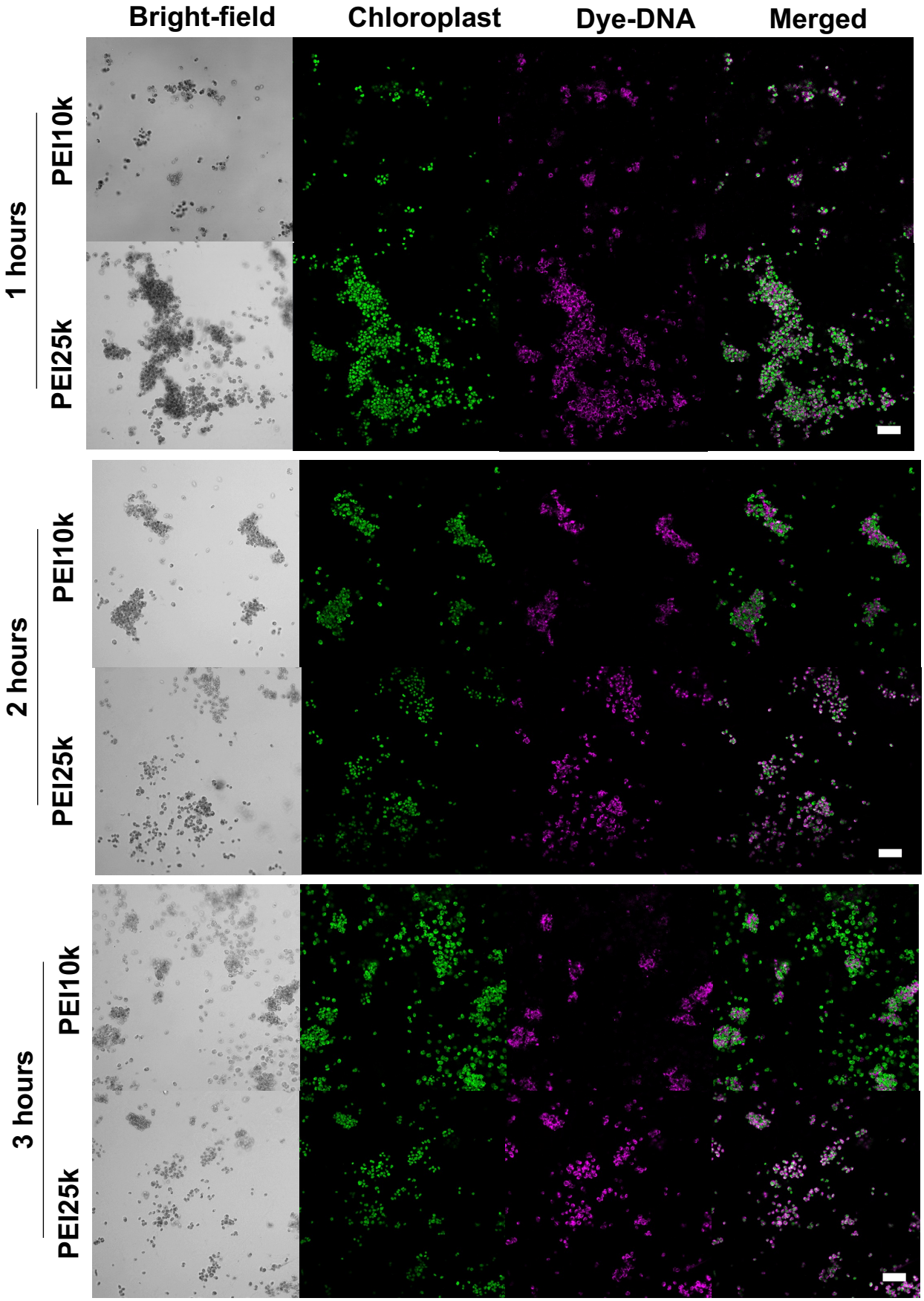
Supplementary Figure 5. Colocalization of Dye-DNA delivered by PEI25k-SWCNT within algae chloroplasts. Dye-DNA-PEI25k-SWCNTs colocalization with chloroplasts in the a) wildtype and b) cell wall knockout strain, after a 1 hour incubation with 300 fg/cell of PEI25k-SWCNTs with a 1:1 Dye-DNA:SWCNT ratio (n=5).The scale bar is 10 μm. Overlap between Dye-DNA and chloroplasts is highlighted in the orthogonal views representing projections on the z-axis. Red arrows indicate areas of overlap.



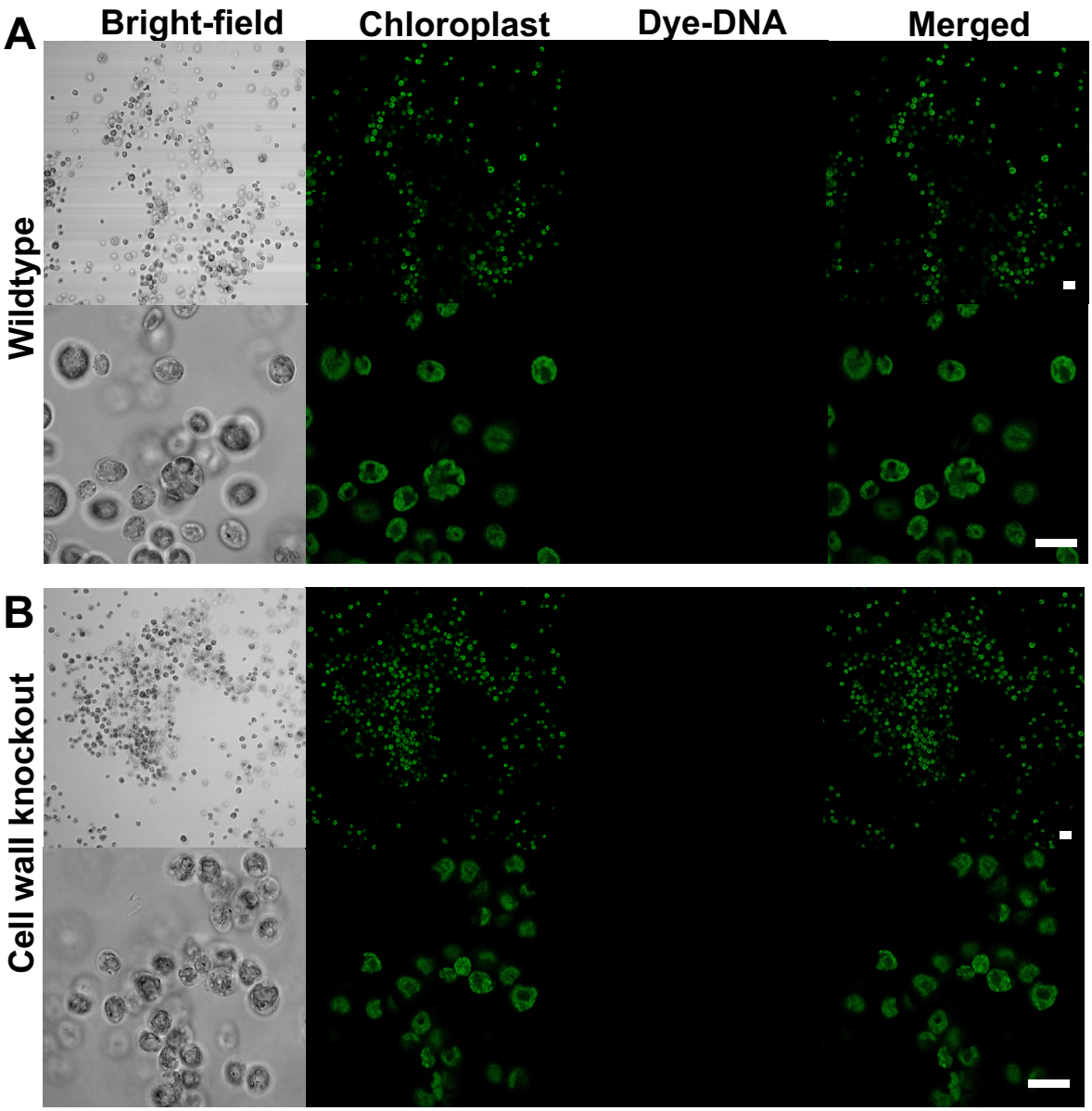
Supplementary Figure 6. Dye-DNA-SWCNT uptake into algae chloroplasts over time. DNA-PEI25k-SWCNT increased colocalization of Dye-DNA with chloroplasts (P*<0.05, ****<0.0001) to a larger extent than DNA-PEI10k-SWCNT in the wildtype and cell wall knockout strain after 1, 2 and 3 hour incubation (300 fg/cell of PEI-SWCNT with a 1:1 Dye-DNA:SWCNT ratio) (n=5; 1-way ANOVA analysis; box and whisker plot represents the minimum, 25th percentile, median, 75th percentile, and maximum)



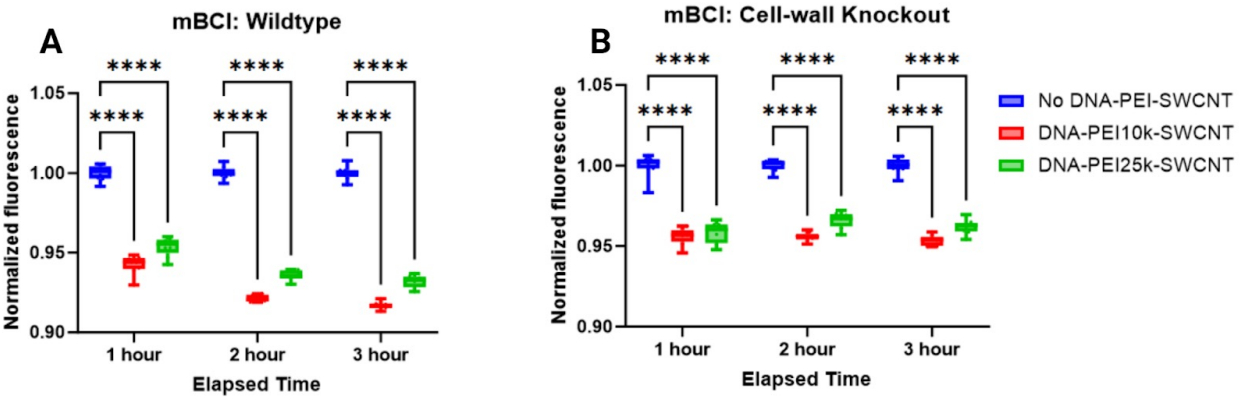
Supplementary Figure 7. Population-level wildtype algae with Dye-DNA-PEI10k-SWCNT and -PEI25k-SWCNT confocal microscopy across multiple time points. Higher colocalization between chloroplasts and dye-DNA is observed in PEI25k than in PEI10k SWCNT-treated algae at 300 fg/cell and 1:1 mass ratio of DNA:PEI-SWCNT. Representative population-level image; scale bar is 50 uM.



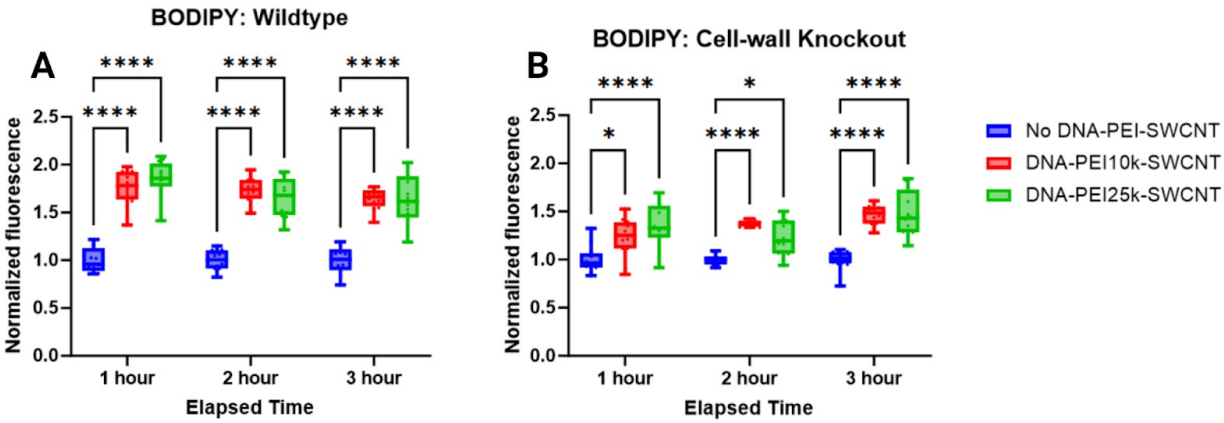
Supplementary Figure 8. Population-level cell wall knockout algae with Dye-DNA-PEI10k-SWCNT and -PEI25k-SWCNT confocal microscopy across multiple time points. Higher colocalization between chloroplasts and dye-DNA is observed in PEI25k than in PEI10k SWCNT-treated algae at 300 fg/cell and 1:1 mass ratio of DNA:PEI-SWCNT. Representative population-level image; scale bar is 50 uM.



Supplementary Figure 9. Dye-DNA without PEI-SWCNT does not associate with algae. No Dye-DNA fluorescence was found in either a) wildtype or b) cell wall knockout algae exposed to the negative control without PEI-SWCNTs. The scale bar is 20 μm.



Supplementary Figure 10. Reduced Glutathione (GSH) upon reaction with ROS generated after DNA-PEI-SWCNT exposure. Monochlorobimane (mBCl) assay indicated that intracellular reduced Glutathione (GSH) levels decrease within one hour in response to both DNA-PEI10k- and DNA-PEI25k-SWCNT in a) wildtype and b) cell wall knockout strain (ANOVA one way test, ****P < 0.0001)(n=3). Glutathione is an antioxidant molecule used by algae cells to regulate ROS levels.



Supplementary Figure 11. Lipid peroxidase assay detects damage to lipid membranes due to reaction with ROS. BODIPY C11 identified an increase in lipid oxidation in algae after exposure to 300 fg/cell DNA-PEI-SWCNT in 1:1 ratio to DNA:PEI-SWCNT by mass. a) Wildtype and b) cell-wall knockout strains both showed statistically significant higher levels of lipid peroxidation, *P< 0.05 and ****P < 0.0001, respectively, when exposed to PEI-10k-SWCNT and PEI-25k-SWCNT (ANOVA one-way test; n=3, technical triplicate) indicating ROS damage in lipid membranes and impact on membrane integrity.

Supplementary Table 1. Zeta potentials of PEI10k- and PEI25k-SWCNT in the presence of various ssDNA concentrations (10 mM final TE buffer, pH 8.0).

DNA:PEI-SWCNT	**PEI 10k**	**PEI 25k**
No DNA	14.5 ± 2.0	30.1 ± 2.0
0.01:1	14.7 ± 1.8	28.8 ± 2.0
0.1:1	14.6 ± 1.8	29.8 ± 1.7
1:1	17.0 ± 1.6	30.6 ± 2.9