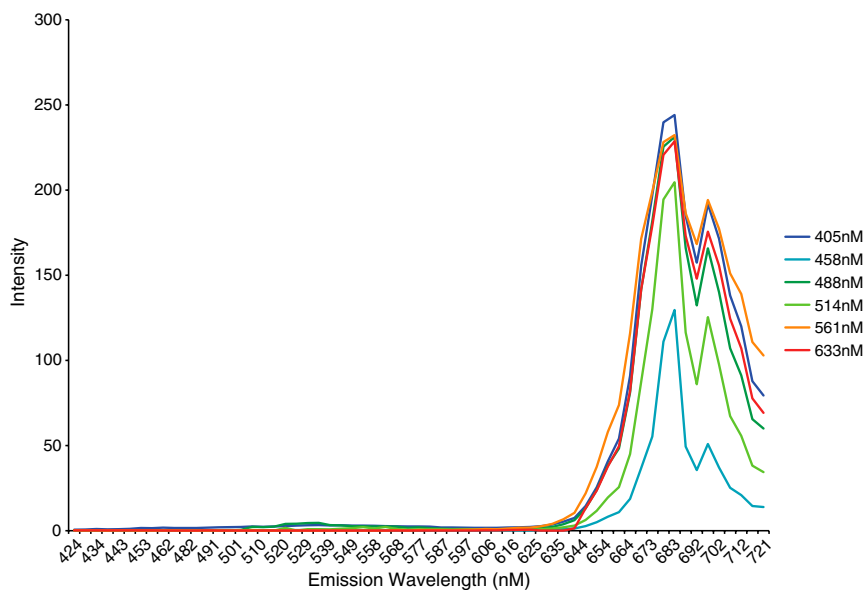
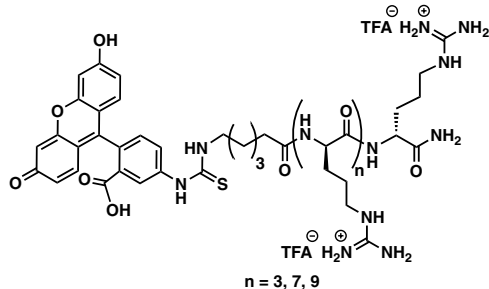


# Supporting Information

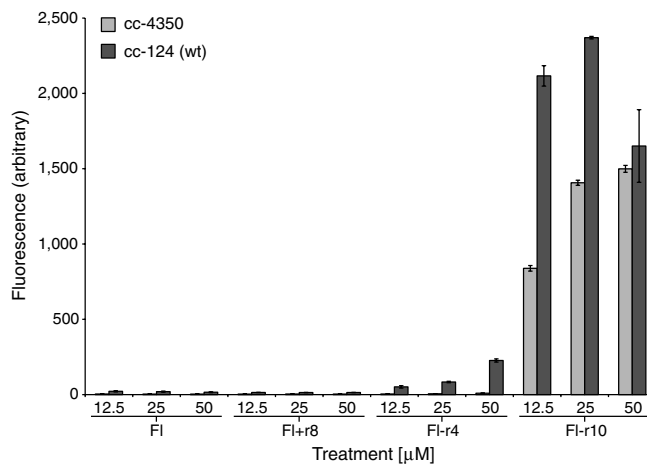
Hyman et al. 10.1073/pnas.1202509109



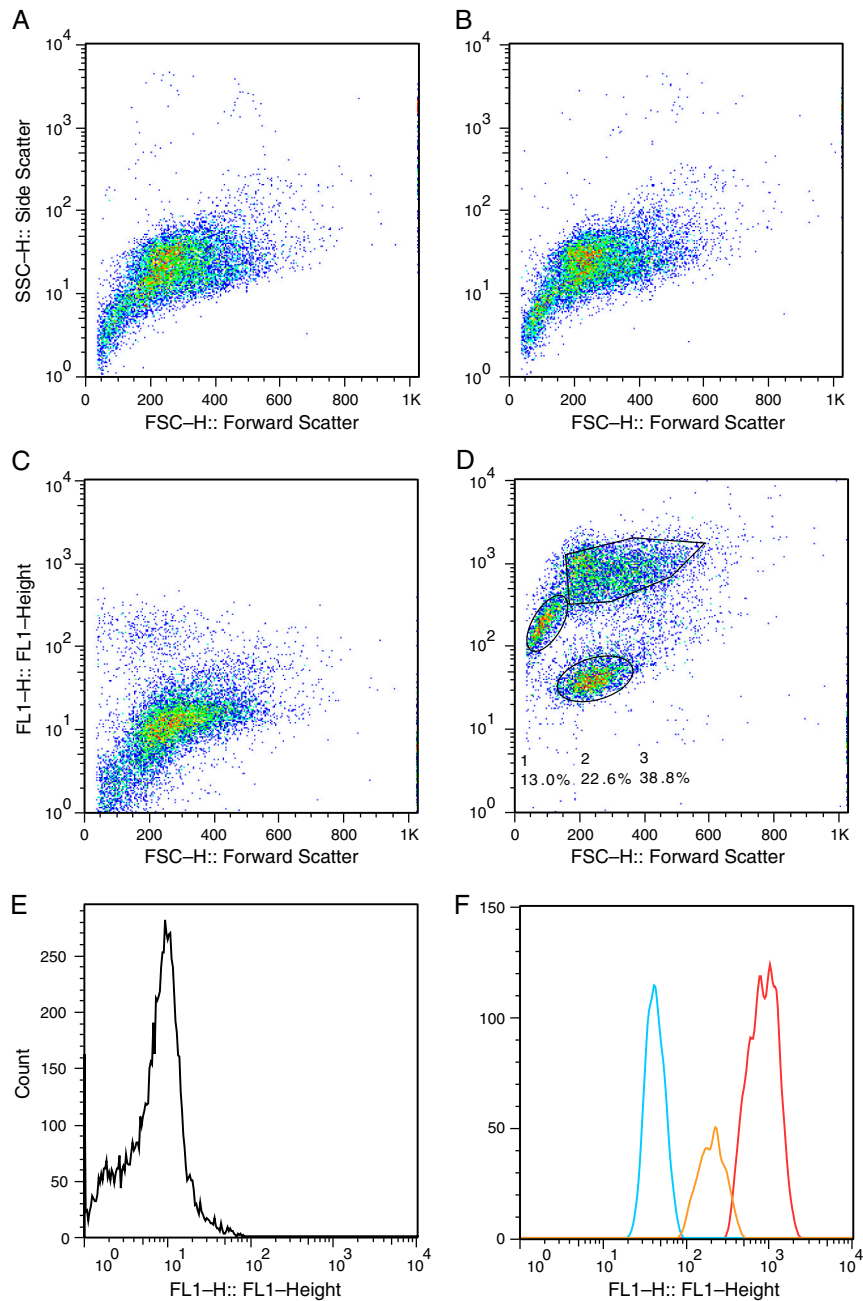
**Fig. S1.** Wavelength scans of wild-type *C. reinhardtii* at several excitation wavelengths. When illuminated with laser light at 405, 458, 488, 514, 568, or 633 nm, the *C. reinhardtii* chloroplast presents strong autofluorescence starting around 650 nm and going up past 720 nm. There is also a much weaker, yet still visible, autofluorescence from approximately 450 nm to almost 600 nm. These scans indicate that bright fluorophores emitting at <650 nm can be used as probes without great concern about overlapping autofluorescence, whereas the native fluorescence from the chloroplast at approximately 700 nm can be used to track and image cells without the use of other dyes.



**Fig. S2.** Chemical structure of oligo-(D)-arginine covalently attached to fluorescein. Molecules used in this paper have  $n = 3, 7, \text{ or } 9$ .

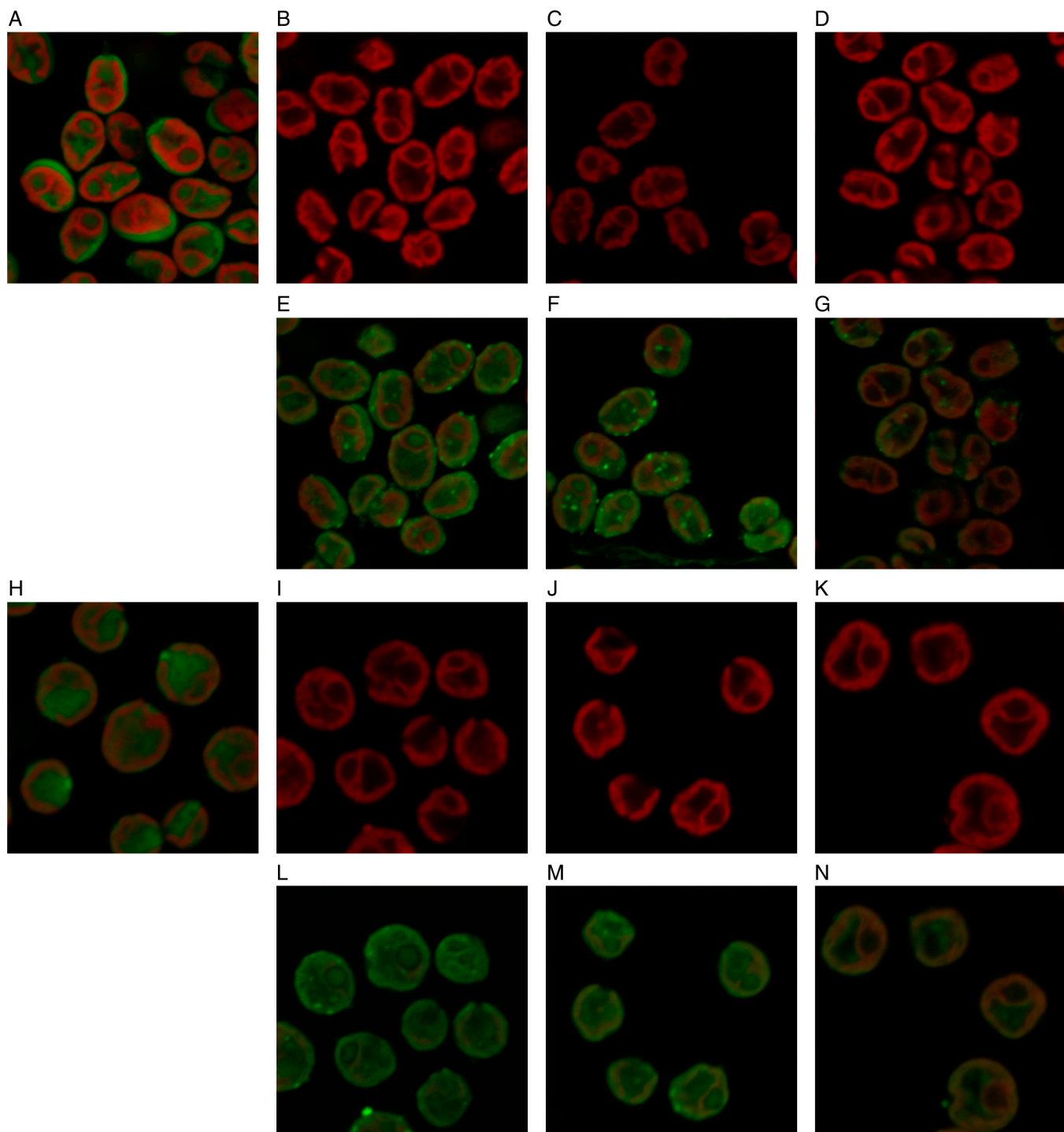


**Fig. S3.** Flow cytometry data for FI-r4 and FI-r10 in *C. reinhardtii*. Graph showing FI-r4 and FI-r10 treatments compared to controls of FI alone and the non-covalent mixture of FI+r8 in (light gray bars) cell wall mutant cc-4350 cells and (dark gray bars) wild-type cells. In both cases, FI-r10 treated cells show dramatic uptake; however, the increased number of arginines in FI-r10 caused difficulty in handling of the samples.



**Fig. S4.** Use of fluorescently-labeled Gr-MoTrs along with flow cytometry allows for differentiation of species in a mixture. Flow cytometry of different algae in the family Chlorophyceae using the autofluorescence from the chloroplast does not allow differentiation of species in a mixture. The mixture is 1:1:1 of wild-type *C. reinhardtii*, *S. dimorphus*, and *N. oleabundans*. Wild-type cells (A) untreated and (B) treated with Fl-r8 and examined using forward (FSC) and side scatter (SSC) do not present any means of differentiation. (C) Untreated cells are still not differentiable when examined using FSC and Fl-1 (530/20 nm); however, (D) Fl-r8 treated cells are now distinctly separated and can be gated for sorting or analysis. (E, F) The same result is shown as a histogram indicating ease of separation.





**Fig. S6.** Additional fluorescence microscopy images of wild-type *C. reinhardtii* and fluorescence microscopy images of cc-4350 cells showing delivery of an active enzyme. Wild-type cells were treated with (A) HRP-streptavidin:biotin-R9 complex, (B, E) HRP-streptavidin and R9 mixture, and (C, F) HRP-streptavidin alone and incubated with a chemical detection reagent for HRP (HRP substrate) that produces a fluorescent product. As a control, (D, G) wild-type cells were treated with HRP-streptavidin:biotin-R9 complex but not exposed to the HRP substrate. (E–G) The lower panels are images taken with a higher exposure to show background fluorescence in the control images. Cell wall mutant cc-4350 cells were treated with (H) HRP-streptavidin:biotin-R9 complex, (I, L) HRP-streptavidin:R9 mixture, and (J, M) HRP-streptavidin alone and incubated with the HRP substrate. As a control, (K, N) cc-4350 cells were treated with HRP-streptavidin:biotin-R9 complex but not exposed to the HRP substrate. (L–N) The lower panels are images taken with high laser power to show background fluorescence in the control images.