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Immunolocalization of a Netrin-3 Like Peptide in *Tetrahymena thermophila* Using Antibodies Against the N- and C-terminus of the Protein

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Abstract

Tetrahymena thermophila are free-living, unicellular, eukaryotic protozoans that live in a variety of aquatic environments. These organisms interact with their environment by responding to chemorepellents and chemoattractants which direct them toward favorable stimuli, such as food, and away from unfavorable stimuli, such as predators. We have previously described two netrin-like proteins, a netrin-1 like protein, and a netrin-3 like protein, which are secreted from *Tetrahymena*. Both of these proteins act as chemorepellents, and may allow cells to communicate with each other regarding population density, preventing them from outgrowing the available environmental resources. In our current study, we used antibodies against the N- and C-terminal of netrin-3 to show the distribution of this protein throughout the cell. We find that netrin-3 is highly colocalized with the endoplasmic reticulum and colocalizes with tubulin to a lesser extent. This is to be expected for a protein that is secreted from cells and trafficked on microtubules.

Methods and Materials

Immunofluorescence was carried out using a modified protocol obtained from cellsignal.com. Briefly, cells were washed twice in PBS, reconstituted in 3.7% formaldehyde in PBS, and allowed to fix for 15 min at room temperature. After fixation, cells were rinsed three times in PBS before being blocked in blocking buffer for 60 minutes. After washing off blocking buffer, cells were incubated overnight at room temperature in primary antibody at a dilution of 1:100. After rinsing three times in PBS, cells were incubated in fluorochrome-containing secondary antibody for 1–2 hours at room temperature in the dark. Cells were then rinsed three times in PBS. 5 ml of cell suspension was then applied to a slide and mixed with 5 ml of DAPI. Cell suspension was then covered with a coverslip and observed under a fluorescence microscope at 400X.

Results

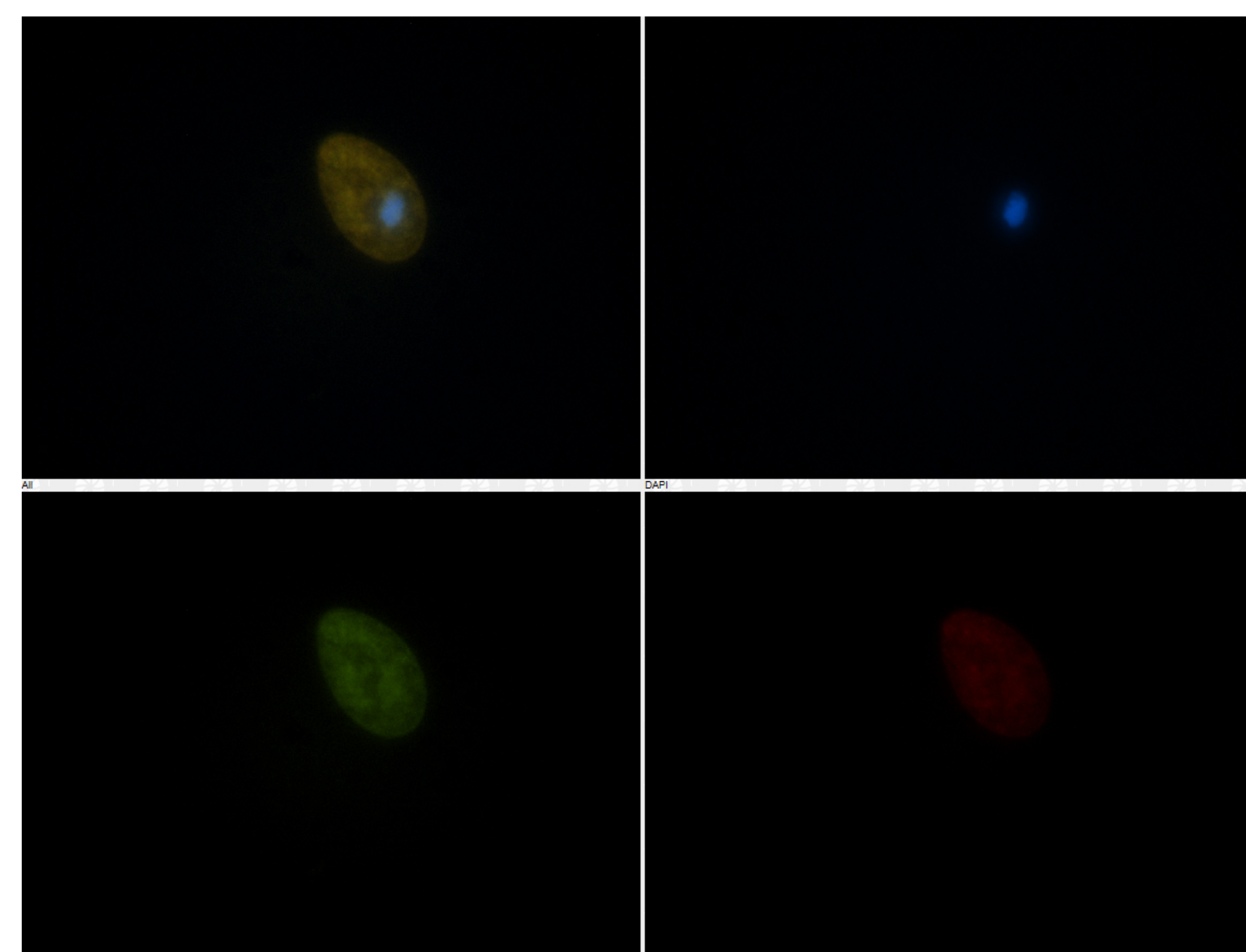


Figure 1. Comparison of Netrin-1 localization (green) with Netrin-3 localization (red). DAPI (nuclear) staining is shown in blue. Both netrin-1 and netrin-3 appear to localize to the cytosol, where one would expect to find ER, Golgi, and other components of the secreted protein trafficking pathway.

Figure 2. Comparison of Netrin-3 C terminal (top) staining and Netrin-3 N terminal staining (bottom) when compared to tubulin staining. Netrin-3 staining is in red, tubulin staining is in green, DAPI (nuclear) staining is in blue. Netrin-3 shows cytosolic distribution, while tubulin is localized to the cilia, the oral groove, and the centrosome as well as to the cytosol.

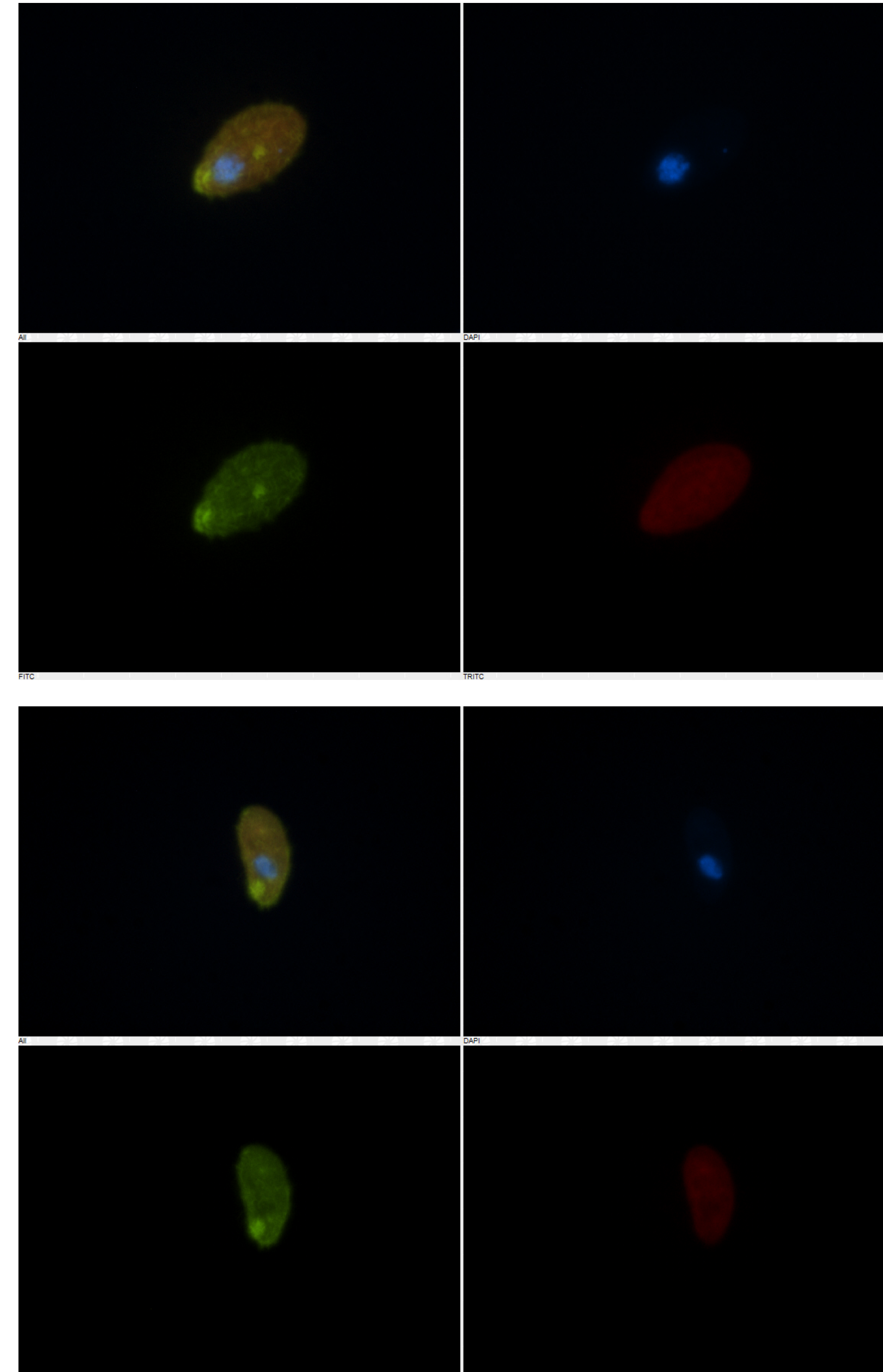
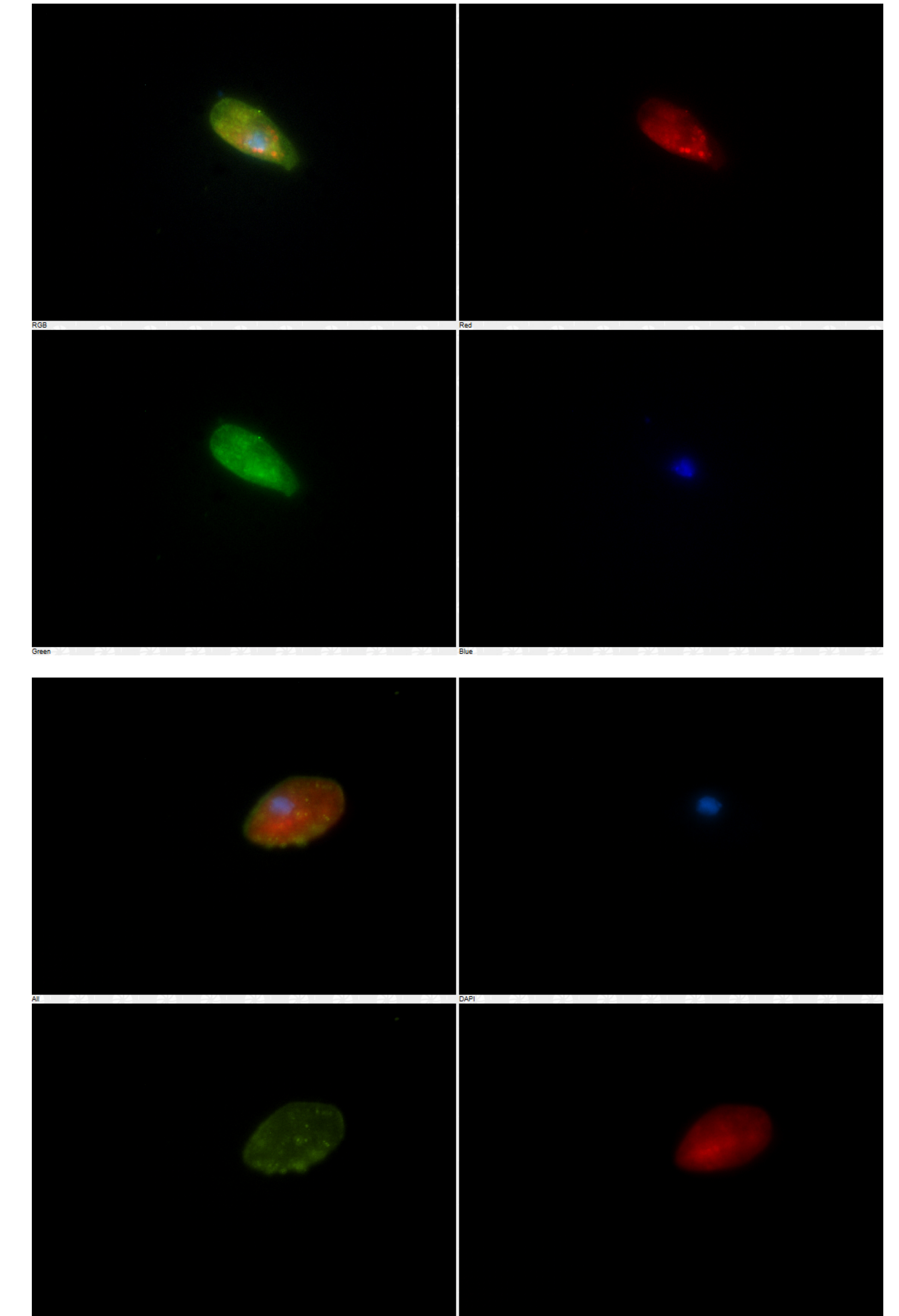


Figure 3. Comparison of Netrin-3 C terminal (top) staining and Netrin-3 N terminal staining (bottom) when compared to ER staining. Netrin-3 staining is in green, ER staining is in red, DAPI (nuclear) staining is in blue. Netrin-3 shows cytosolic distribution and some co-localization with ER tracker, as would be expected for a secreted protein. The N3C antibody appears to bind mainly to cytosolic targets, while the N3N antibody binds targets on the plasma membrane as well as in the cytosol.



Conclusions

1. Netrin 3 shows cytosolic staining which colocalizes with ER Tracker™, which is what one would expect for a secreted protein.
2. Anti N3C and anti N3N antibody staining overlaps; however, N3N antibody appears to bind to a plasma membrane or ciliary target protein as well as to cytosolic proteins.

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