Supplementary Information for

Red emitting fluorescent carbon nanoparticles to track spatio-temporal dynamics of endocytic pathways in model neuroblastoma neurons

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Tables S1

Legends for Movies S1

SI References

Other supplementary materials for this manuscript include the following:

Movies S1

Supplemental information – Figures and Legends

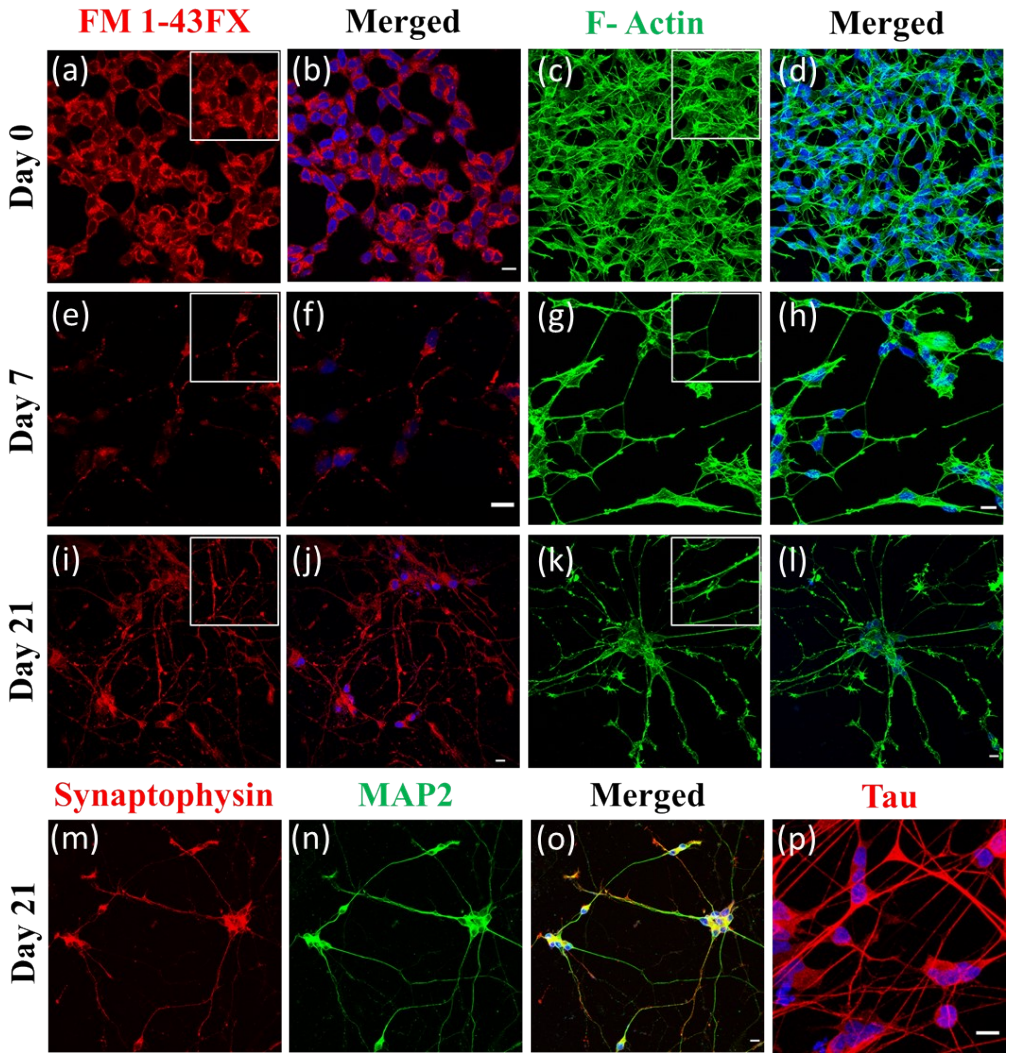

Figure S1. FM 1-43, Phalloidin (F-actin) and Immunofluorescence staining of SH-SY5Y cells during differentiation into mature neurons. a-l, show the FM 1-43, and Phalloidin (F-actin) staining images of SH-SY5Y cells during differentiation at day 0 **(a-d)**, day 7**(e-h),** and day 21 **(i-l)**. **Figure S3 a-b**, **e-f**, and **i-j** shows FM 1-43 staining at day 0, day 7, and day 21. **Figure S3 a,c,** inset shows the zoom images of SH-SY5Y cells at day 0 with epithelial morphology of cells. **Figure S3 e,g** inset shows the zoom images of the neurite processes formation at day 7 of differentiation. **Figure S3 i,k** inset shows the zoom images of the fully differentiated neurons showing the axons, neurite connections at day 21. **Figure S3 c-d**, **g-h**, and **k-l** show Phalloidin staining at day 0, day 7, and day 21. Immunofluorescence analysis on the differentiated neurons at day 21 with the specific markers such as Synaptophysin Monoclonal Antibody (SP11) **(Figure S3 m)**, MAP2 Monoclonal Antibody (M13) **(Figure S3 n)**, and Tau (5A6) monoclonal antibody **(Figure**

S3 p). **Figure S3 o** shows the merged image of the SP11 and MAP2 marker. All Scale bars are equal to 10 µm.

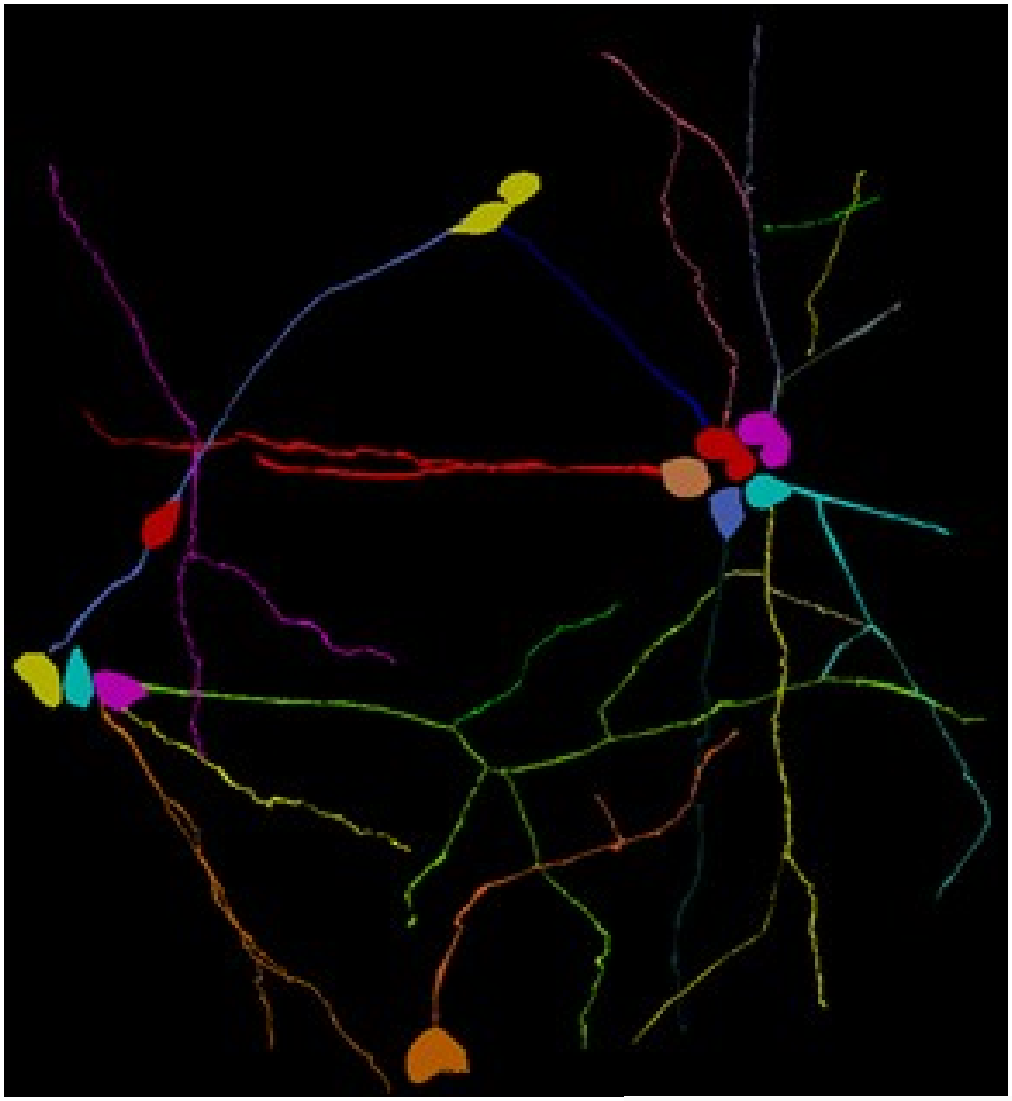

Fig. S2. The Automatic 3D neuron reconstruction of intricate neuronal structures in fully differentiated neurons using Neurolucida 360 software. The figure shows the specific neuronal structures of fully differentiated neurons such as axons, dendrites reconstruction for 3D modeling and for quantitative analysis.

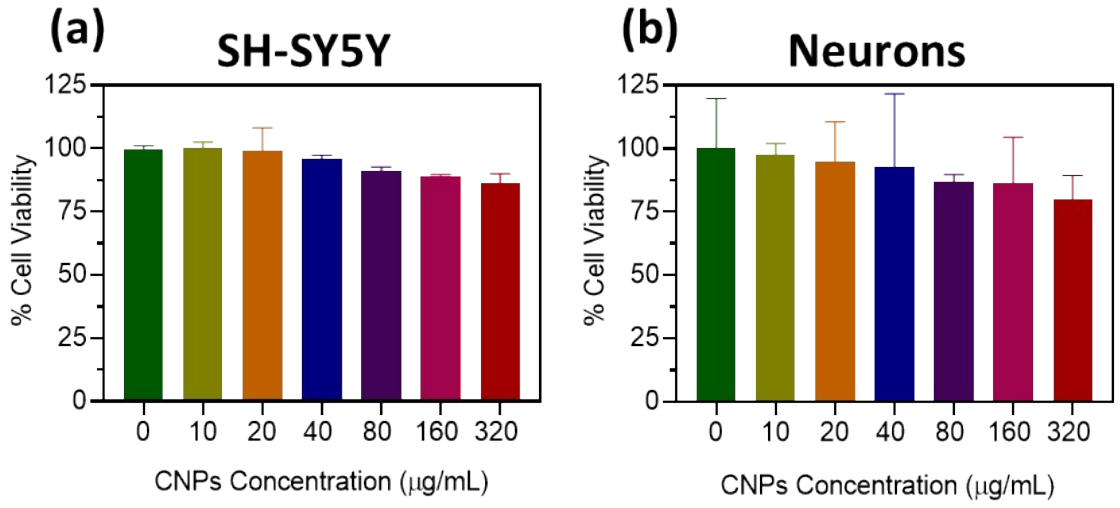

Fig. S3. The toxicity of the CNPs after 24 h treatment on SH-SY5Y cells and differentiated neurons as measured by using Alamar Blue assay.

The CNPs with varying concentrations (10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml, 160 µg/ml, and 320 µg/ml) incubated at 37 °C for 24 h in SH-SY5Y cells and differentiated neurons at day 21. After 24 h, media was removed and washed with PBS followed by incubation of 10% Alamar Blue Cell Viability Reagent (diluted with DMEM/F-12 medium only) for 4 hours at 37°C with 5% CO2. Following incubation with the reagent, the absorption measured at 570 nm using a microplate reader. All data were represented as mean with SD of three independent replicates.

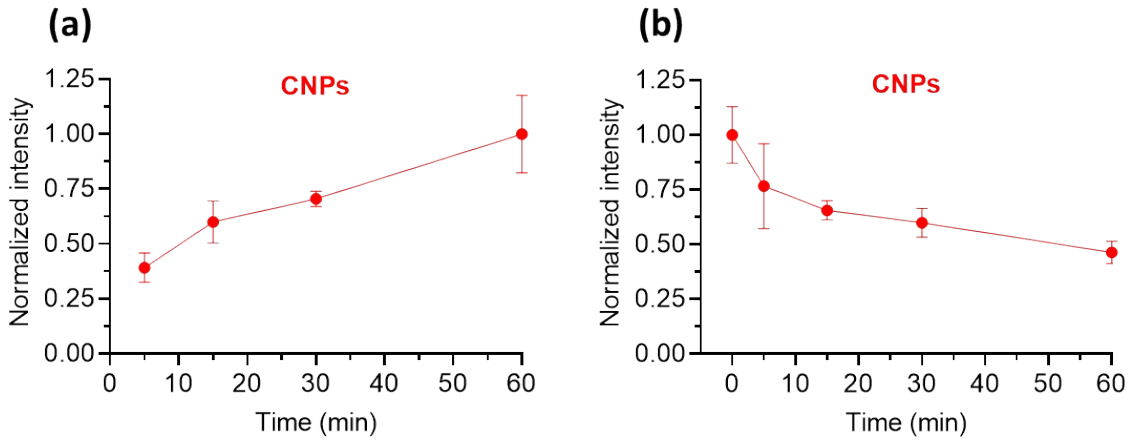

Fig. S4. Cellular uptake and recycling of carbon nanoparticles (CNPs) in SH-SY5Y cells.

a, Cellular uptake of CNPs in SH-SY5Y cells at different time intervals ranging from 5 min to 60 min at 37 °C. The CNPs (40 µg/ml) were incubated at various times and fixed and imaged. **Figure a,** shows the quantification of normalized intensity for CNPs from 25 different cells. **b,** Cellular recycling of CNPs in SH-SY5Y cells after cellular internalization of 15 min at different time intervals ranging from 5 min to 60 min at 37 °C. The CNPs (40 µg/ml) were incubated at various times for recycling, fixed, and imaged. **Figure b**, shows the quantification of normalized intensity for CNPs from 25 different cells. All error bars indicate standard errors.

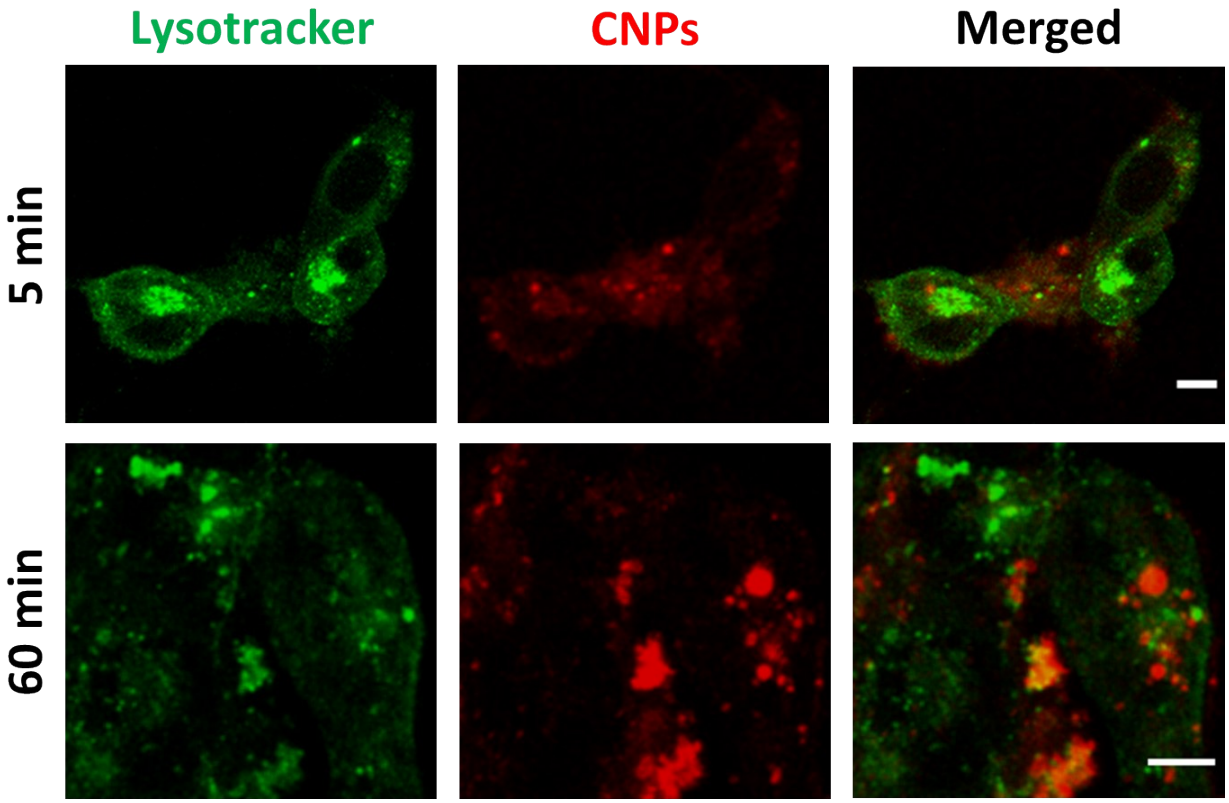

Fig. S5. The colocalization of the CNPs with the cellular organelle-specific dye in SH-SY5Y cells. Lysotracker green is used to label the lysosome. The image represents the cellular uptake of CNPs with the Lysotracker. All scale bars are equal to 5 µm.

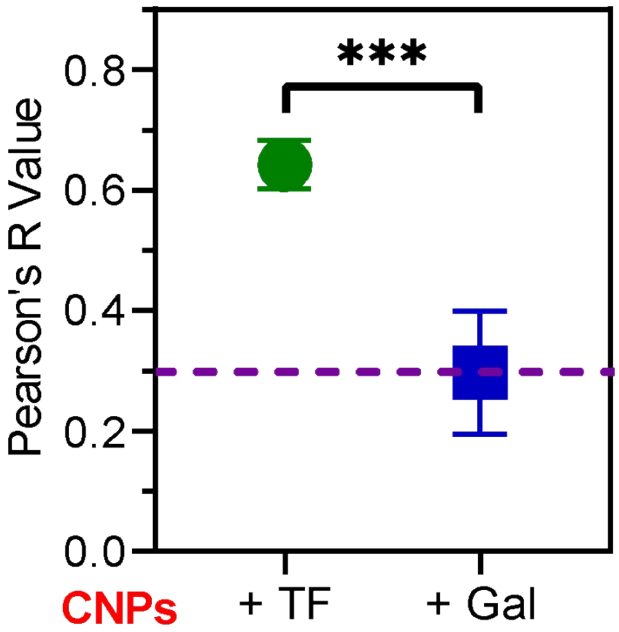

Fig. S6. Quantification of the colocalization of Tf^{A488} with CNPs, and $Gal3^{A488}$ with CNPs. The cellular uptake of TF, Gal3, and CNPs in SH-SY5Y cells for 15 min at 37°C. Pearson's R-value is calculated from a minimum of 25 cells. Error bars indicate standard errors in colocalization of transferrin and carbon nanoparticles and galectin3 and carbon nanoparticles. Two-tailed unpaired t-test, P value< 0.001 ***).

Table S1. Specific cellular endocytosis inhibitor molecules and the mechanism of inhibition

Cellular Pathway	Inhibitor	Mechanism of inhibition
Clathrin-mediated endocytosis (CME)	Pitstop (20 μM)	Pitstop blocks the endocytic ligand association with the terminal domain of clathrin protein inhibiting the CME pathway(1)
Clathrin-mediated endocytosis (CME)	Dynasore (80 μM)	Dynasore inhibits the GTPase activity of the dynamin1, dynamin2, and Drp1, and the mitochondrial dynamin inhibiting the CME pathway(2)
Clathrin-independent endocytosis (CIE)	Lactose (100 mM)	Lactose molecules bind to the glycans on the plasma membrane receptors blocking the lectin mediated CIE pathway(3)
Caveolae-mediated endocytosis (CvME)	Methyl-β-cyclodextrin (MβCD) (5 mM)	MβCD molecule disrupts the caveolae and lipid raft formation(4)

Movies S1. The reconstruction of 3D model of fully differentiated neurons using Neurolucida 360.

SI References

1. L. Von Kleist, *et al.*, Role of the clathrin terminal domain in regulating coated pit dynamics revealed by small molecule inhibition. *Cell* **146**, 471–484 (2011).

2. T. Kirchhausen, E. Macia, H. E. Pelish, Use of Dynasore, the small molecule inhibitor of dynamin, in the regulation of endocytosis. *Methods Enzym.* **438**, 77 (2008).

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4. Z. Hai Sui, *et al.*, Intracellular trafficking pathways of Edwardsiella tarda: From clathrin- and caveolin-mediated endocytosis to endosome and lysosome. *Front. Cell. Infect. Microbiol.* **7** (2017).