

Electronic Supplementary Information

Shape-Dependent Cell Uptake of Iron Oxide Nanorods: Mechanisms of Endocytosis and Implications on Cell Labeling and Cellular Delivery

*Anbu Mozhi Thamizhchelvan^a, Hedi Ma^b, Tianhe Wu^a, Darlene Nguyen^a, Jonathan Pedelford^b,
Ted J. Whitworth^c, Yuancheng Li^{a,b}, Lily Yang^{d,e} and Hui Mao^{a,e,*}*

^a Department of Radiology and Imaging Sciences, Emory University School of Medicine,
Atlanta, Georgia 30322, USA.

^b 5M Biomed, LLC, Atlanta, Georgia 30303, USA.

^c Robert P. Apkarian Integrated Electron Microscopy Core, Emory University School of
Medicine, Atlanta, Georgia 30322, USA.

^d Department of Surgery, Emory University School of Medicine, Atlanta, Georgia 30322, USA.

^e Winship Cancer Institute, Emory University School of Medicine, Atlanta, Georgia 30322, USA.

Corresponding Author

*Hui Mao, PhD, Department of Radiology and Imaging Sciences, Emory University School of
Medicine, Atlanta, GA 30329, USA, E-mail: hmao@emory.edu

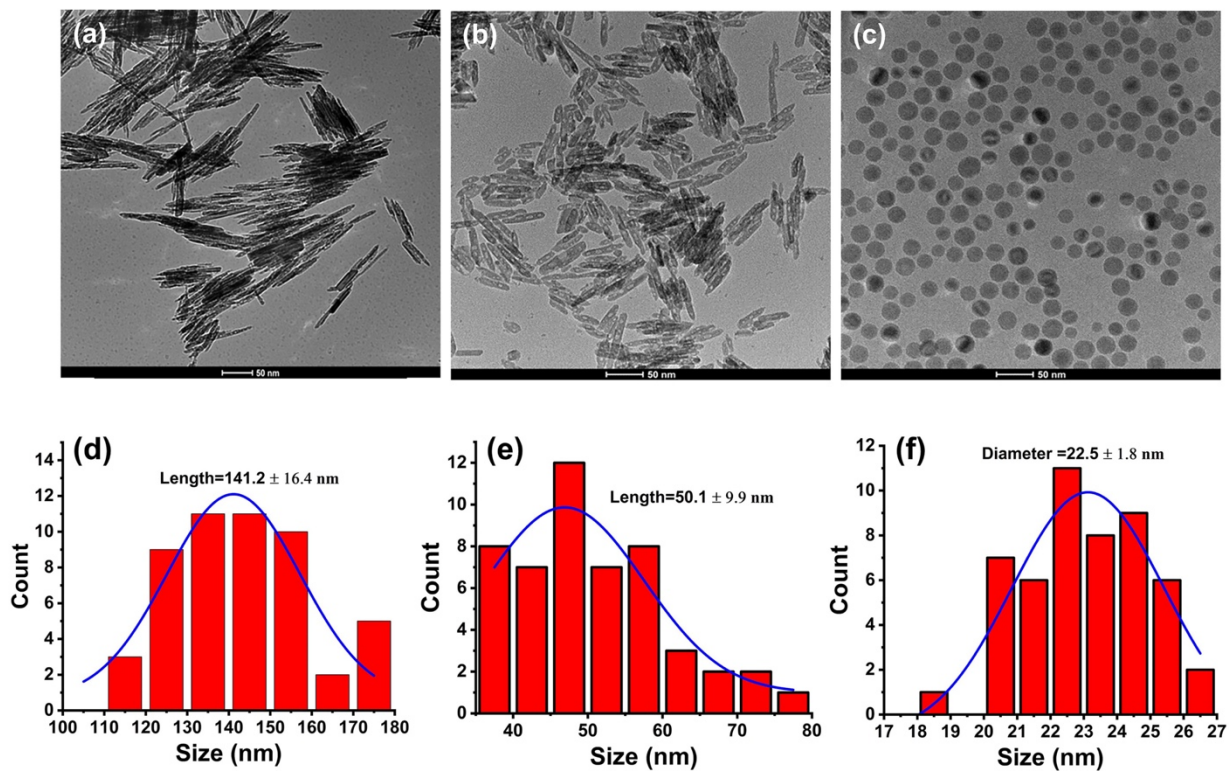


Fig. S1 Transmission electron microscopy (TEM) images of oleylamine coated (a) IONR_(L), (b) IONR_(S), and (c) IONP. The size distributions of oligosaccharide coated (d) IONR_(L), (e) IONR_(S), and (f) IONP were obtained after measuring 50 particles in the field of view.

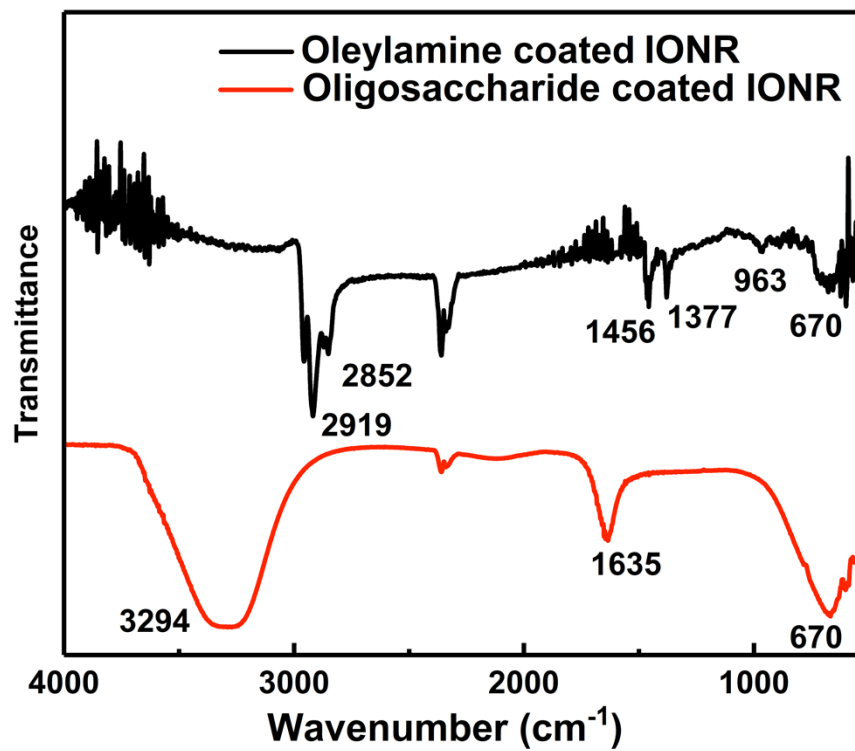


Fig. S2 Fourier transform infrared (FTIR) spectra of oleylamine coated IONR (black) and oligosaccharide-coated IONR (red).

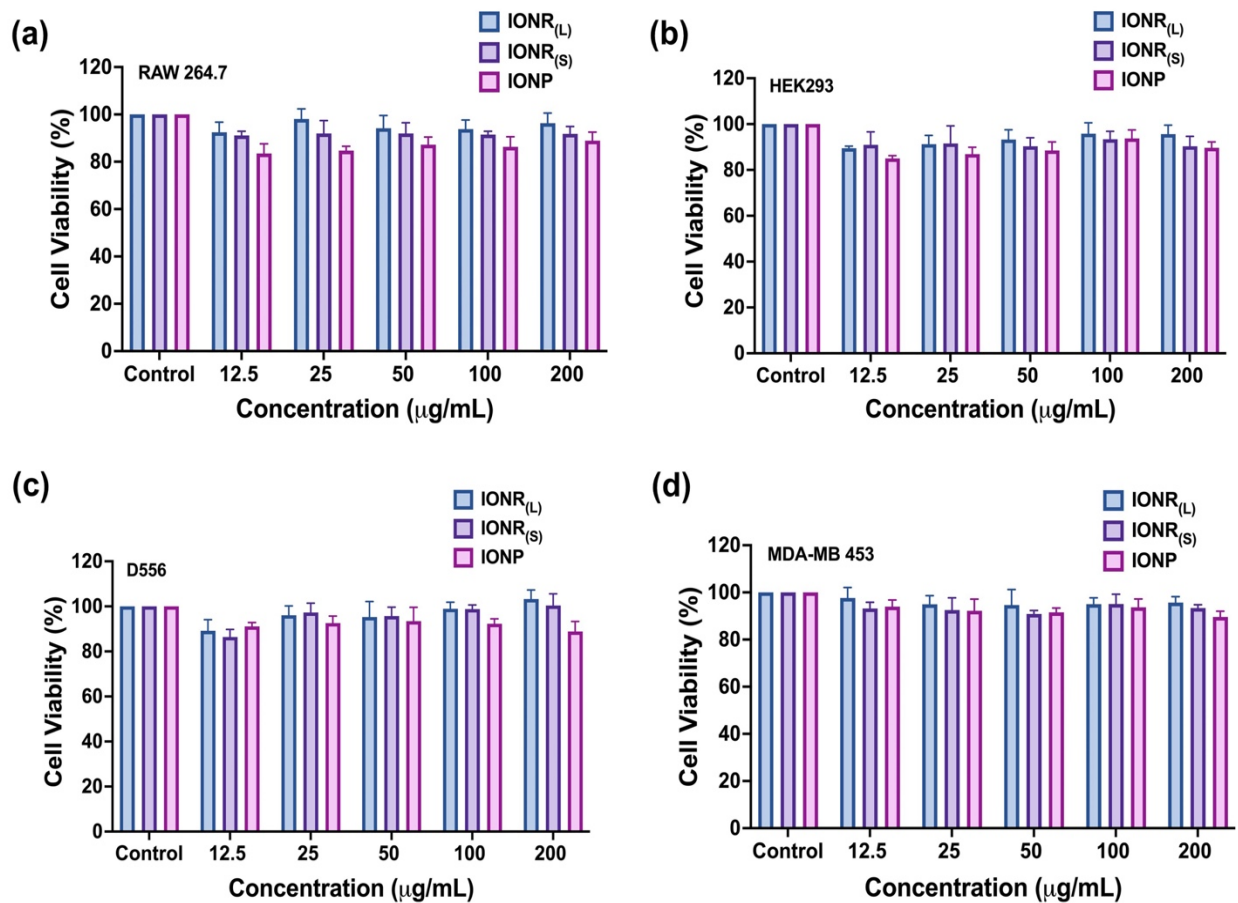


Fig. S3 Comparison of cytotoxicity of oligosaccharide coated IONR_(L), IONR_(S), and IONP on different cell lines measured by MTT assay. (a) RAW264.7 (murine macrophage cells), (b) HEK293 (Normal embryonic kidney cells) (c) D556 (Medulloblastoma cells), and (d) MDA-MB-453 (triple negative breast cancer cells) were treated by IONR_(L), IONR_(S), and IONP at different concentrations for 48 h. Data are presented as mean values (n = 3) with the standard deviations.

RAW264.7 Cell Line

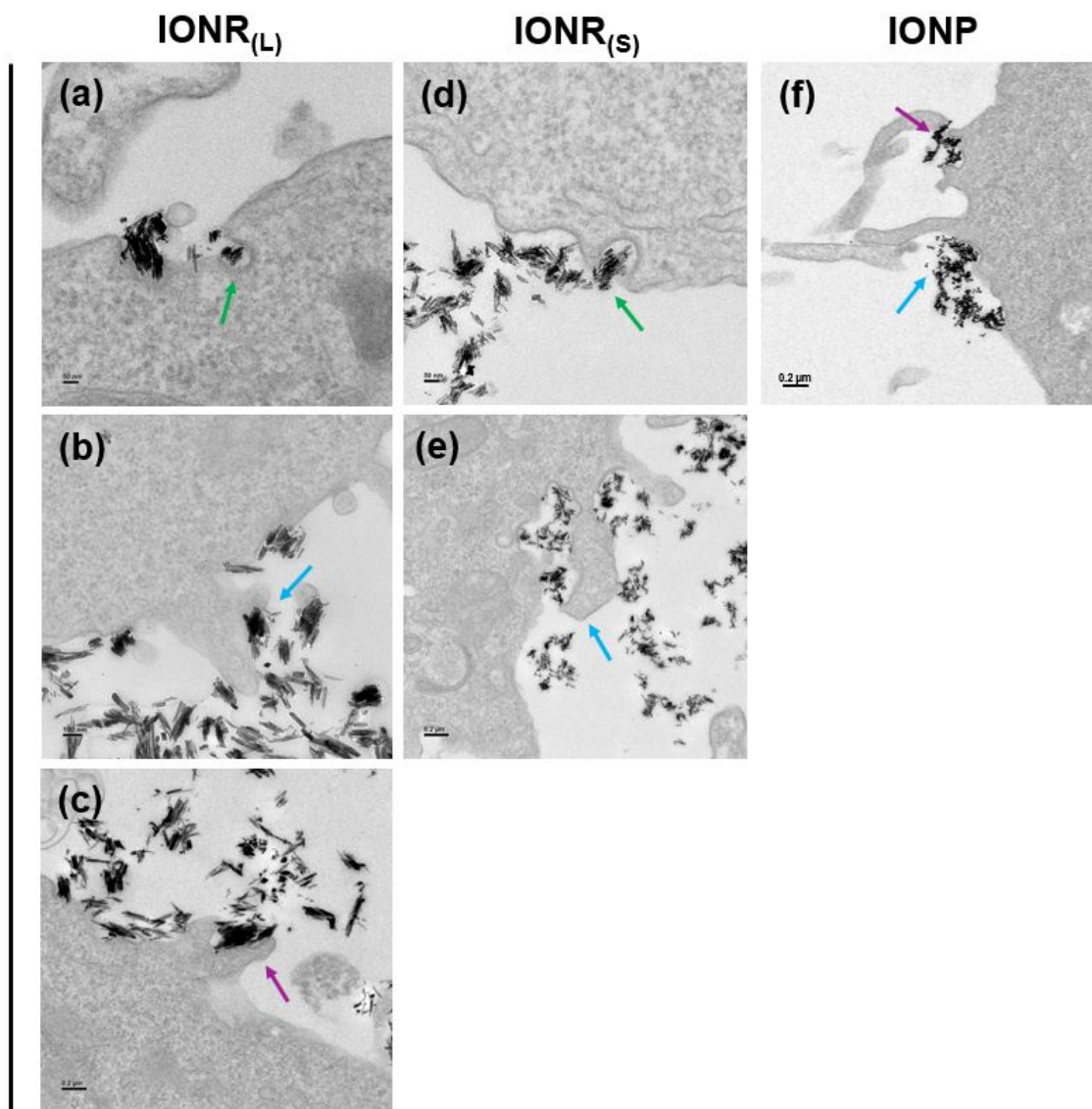


Fig. S4 TEM images of Raw264.7 (murine macrophage cells) collected after 2 h hours treatment with oligosaccharide-coated (a-c) IONR_(L) and (d, e) IONR_(S), and (f) IONP at the concentration of 50 μg Fe/mL. Green Arrow – Clathrin-mediated (clathrin-coated pits); Blue arrow – Macropinocytosis (macropinosomes); Magenta arrow – Phagocytosis; Red arrow – Sinking Phagocytosis. Scale bar indicates 100 nm, 0.2 μm, and 0.1 μm.

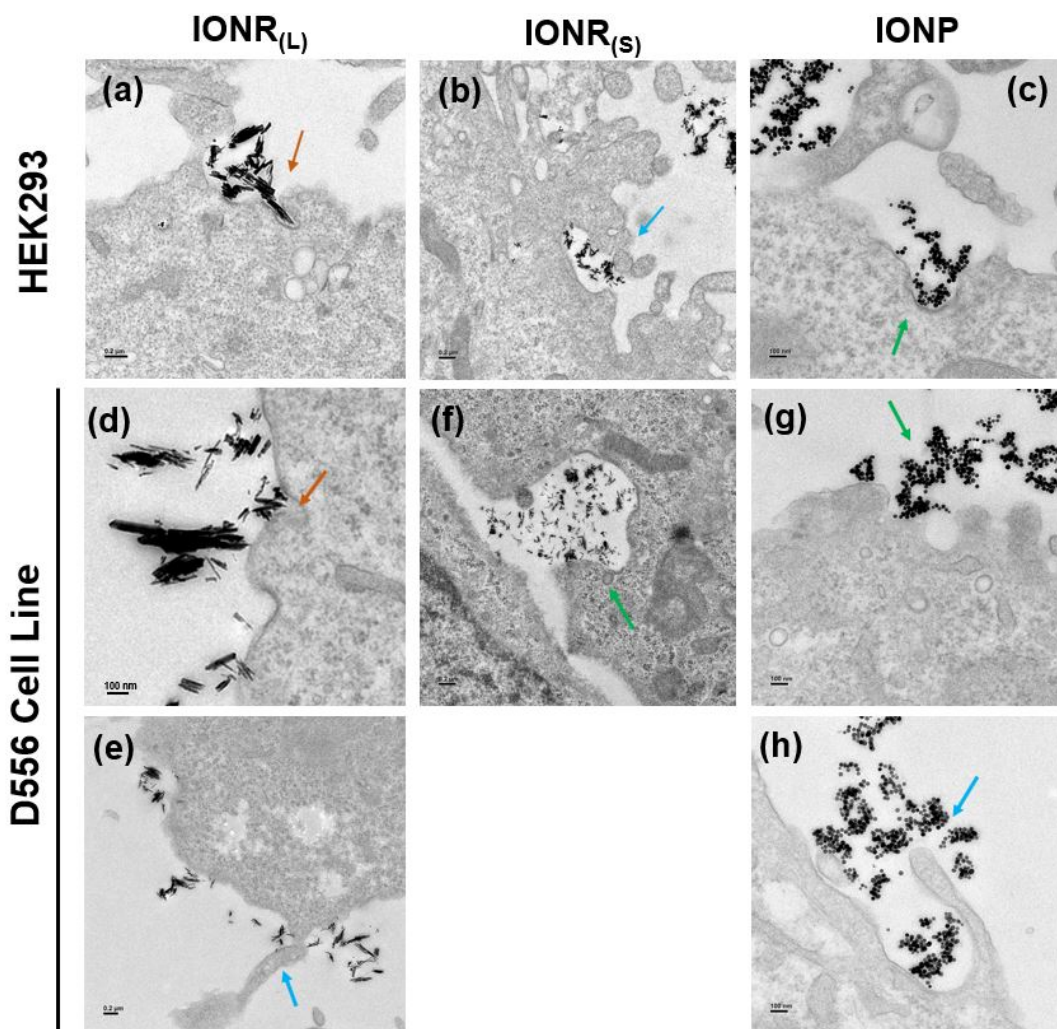


Fig. S5 TEM images of different cells at 2 h time point of treatment with different nanoparticles at the concentration of 50 $\mu\text{g Fe/mL}$. HEK293 embryonic kidney cells treated with oligosaccharide-coated (a) $\text{IONR}_{(L)}$ (b) $\text{IONR}_{(S)}$ and (c) IONP ; D556 human medulloblastoma cells treated with (d, e) $\text{IONR}_{(L)}$, (f) $\text{IONR}_{(S)}$, and (g, h) IONP ; Green arrow – Clathrin-mediated (clathrin-coated pits); Brown arrow – Caveolae-mediated (flask-shaped structures); Blue arrow – Macropinocytosis (macropinosomes); Scale bar indicates 50 nm, 100 nm, and 0.2 μm .

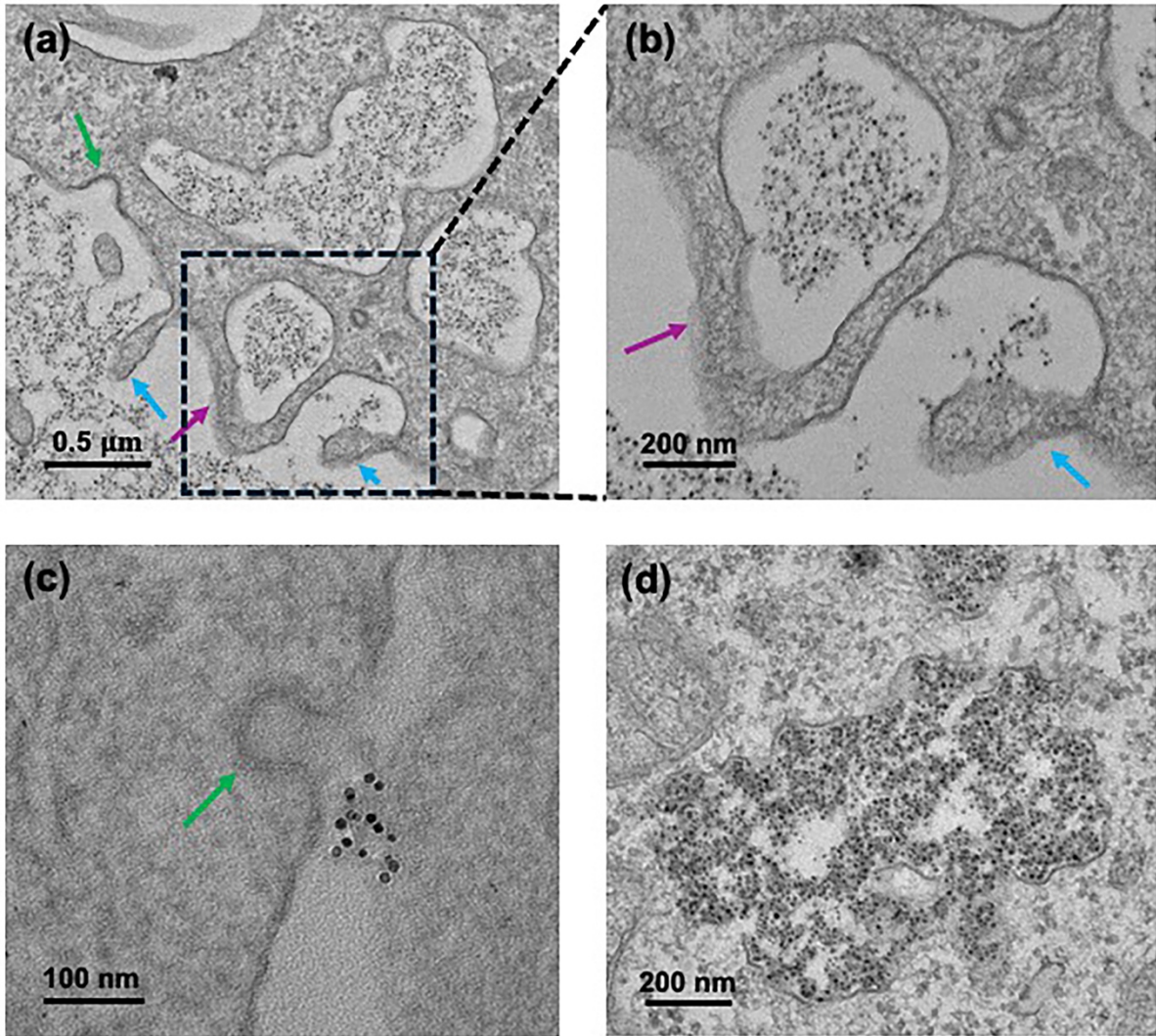


Fig. S6 TEM images of D556 human medulloblastoma cells collected at the 2 h time point of treatment with spherical nanoparticle SHP-10 (IONP with the core size of 10 nm) at the concentration of 50 $\mu\text{g Fe/mL}$. (a) Magenta arrow - phagosome sealing and blue arrow – macropinocytosis; (b) Enlarged region selected from the field of view in the image (a) showing SHP-10 internalized in the phagocytic cup, leading to phagosome sealing; (c) Green arrow – Clathrin-mediated (clathrin-coated pits); and (d) localization of SHP-10 inside the endolysosomal compartment at 4 h time point of the treatment. Scale bar indicates 100 nm, 200 nm, and 0.5 μm .

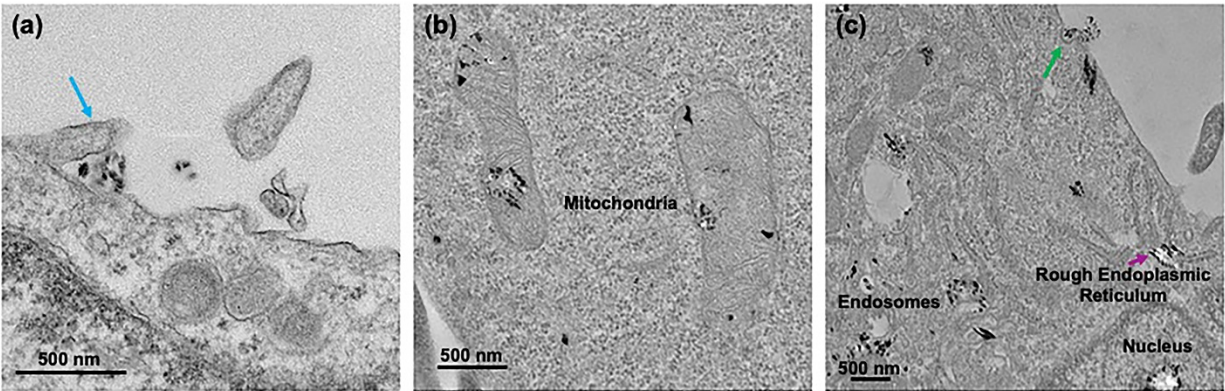
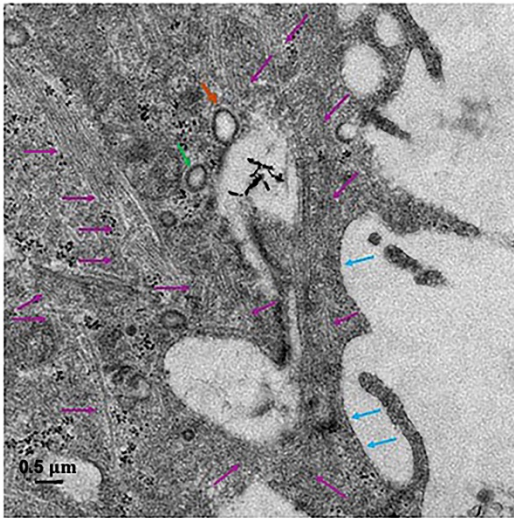


Fig. S7 TEM images of D556 human medulloblastoma cells treated with spherical nanoparticle Ferumoxytol (core size 7-10 nm) at the concentration of 50 $\mu\text{g Fe/mL}$ after 2 hours. (a) Blue arrow – macropinocytosis; (b) Ferumoxytol accumulated in the mitochondria; (c) Ferumoxytol accumulated in the endosomes, nucleus and rough endoplasmic reticulum (magenta arrow) at 4 h time point. Green arrow – Clathrin-mediated (clathrin-coated pits). Scale bar indicates 500 nm.

(a)



(b)

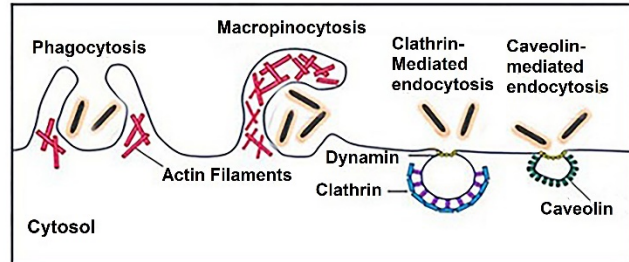


Fig. S8 A TEM image of a D556 human medulloblastoma cell (a) treated with oligosaccharide-coated IONR_(L) at the concentration of 50 $\mu\text{g Fe/mL}$. (b) Schematic illustration of different endocytosis pathways of internalization of IONR_(L) by D556 cells. Green arrow – Clathrin-mediated (clathrin-coated pits); Brown arrow – Caveolae-mediated (flask-shaped structures); Blue arrow – Macropinocytosis (macropinosomes); Magenta arrow – Actin filaments; The scale bar indicates 0.5 μm .

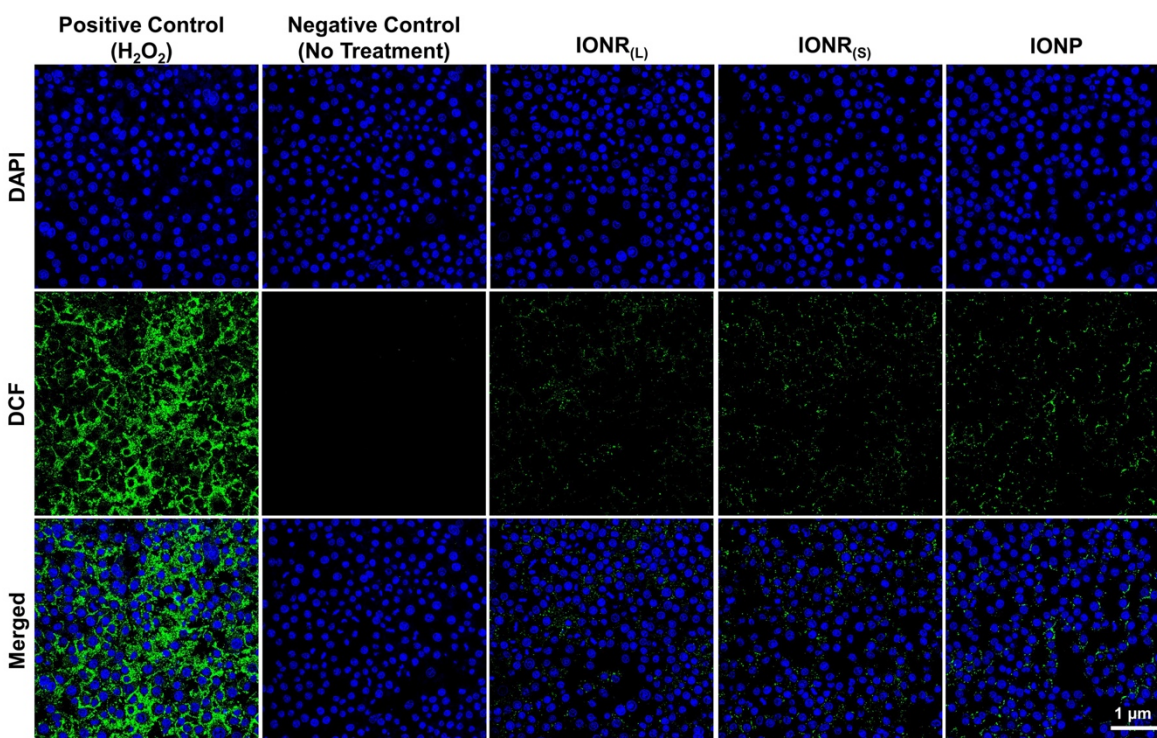


Fig. S9 CLSM images with dichlorodihydrofluorescein (DCF) staining for detection of intracellular reactive oxygen species (ROS) levels in RAW 264.7 cells after being treated with different oligosaccharide coated IONR_(L), IONR_(S), and IONP for 24 h. The ROS (green signal from DCF) level was measured using DCFH-DA. The positive control was treated with 30% hydrogen peroxide (H₂O₂) in a serum-free medium at a ratio of 1:1000 for 20 min. Nucleus stained with Hoechst – blue. The cells without nanoparticle treatment were used as a control group. The scale bar indicates 1 μm and 40 × objective lens.

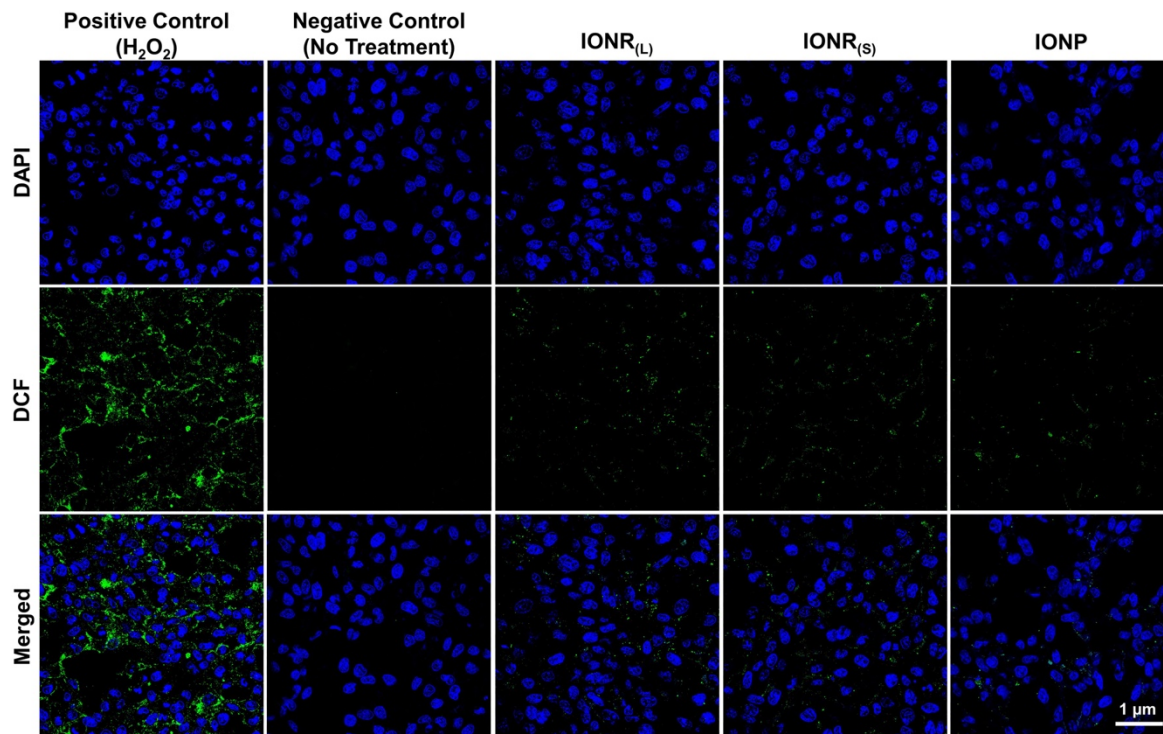


Fig. S10 CLSM images with DCF for detection of intracellular ROS levels in D556 cell lines after being treated with different oligosaccharide coated IONR_(L), IONR_(S), and IONP for 24 h. The positive control was treated with 30% hydrogen peroxide (H₂O₂) in a serum-free medium at a ratio of 1:1000 for 20 min. The ROS (green signal from DCF) level was measured using DCFH-DA. Nucleus stained with Hoechst – blue. The cells without nanoparticle treatment were used as a control group. The scale bar indicates 1 μm and 40 × objective lens.

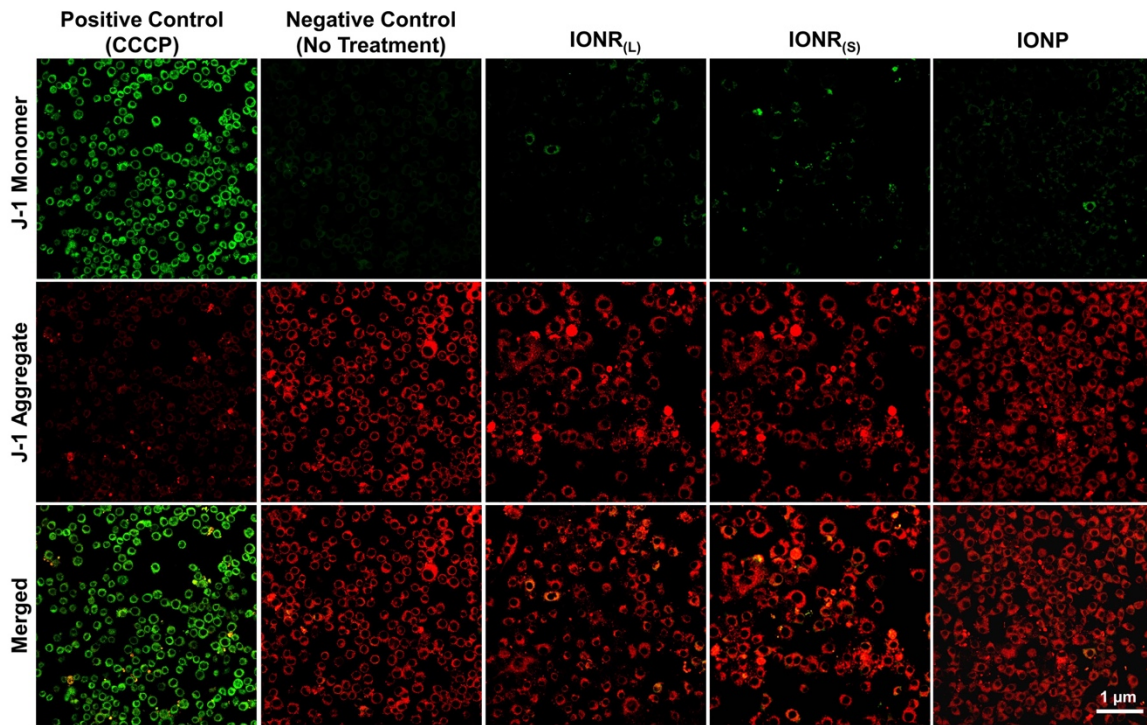


Fig. S11 CLSM images show changes in mitochondrial membrane potential ($\Delta\psi_m$) in RAW 264.7 cell lines after being treated with different oligosaccharide coated IONR_(L), IONR_(S), and IONP for 24 h. The scale bar indicates 1 μm and 40 \times objective lens.

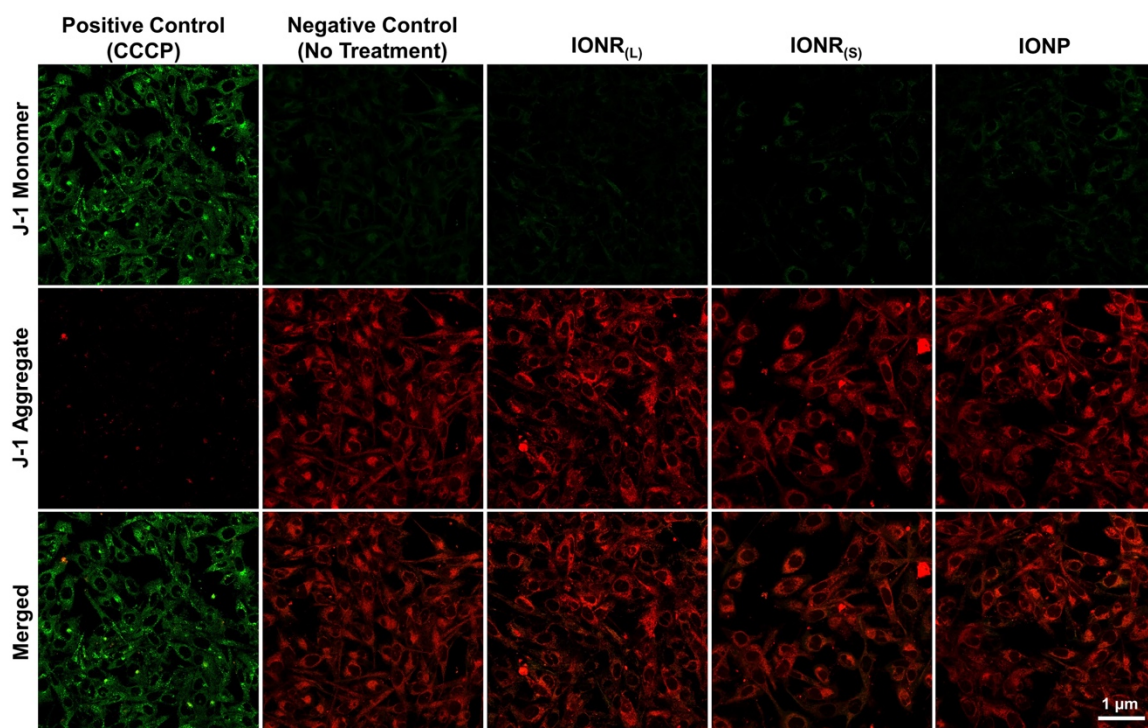


Fig. S12 CLSM images show changes in mitochondrial membrane potential ($\Delta\psi_m$) in D556 cell lines after being treated with different oligosaccharide coated IONR_(L), IONR_(S), and IONP for 24 h. The scale bar indicates 1 μm and 40 \times objective lens.