Supporting Information

Highly Selective Microglial Uptake of Ceria–Zirconia Nanoparticles for Enhanced Analgesic Treatment of Neuropathic Pain

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Experimental

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Inductively Coupled Plasma – Mass Spectroscopy analysis.

Determination of cerium content in primary microglial was performed by ICP-MS analysis (NexION 350D, Perkin-Elmer, USA). Cells were washed with PBS twice. The resulting solutions were diluted in HNO_3.

Table S1. Diffusion time and coefficient of four samples (FITC, Antibody-FITC, 7CZ-FITC NPs, and 7CZ-Ab-FITC NPs).

Samples	Diffusion Time (10^{-6} s)	Diffusion Coefficient (10^{-12} m^2/s)
FITC	7.156 ± 3.86	605.958 ± 212.467
Antibody-FITC	183.301 ± 1.712	28.4 ± 0.126
7CZ-FITC	354.379 ± 134.7	15.678 ± 5.533
7CZ-Ab-FITC	1282.649 ± 142.568	4.047 ± 0.456

[Counts per molecule (kHz)]

I. Antibody-FITC: 0.99
II. 7CZ-FITC: 0.86
III. 7CZ-Ab-FITC: 2.8

[Total amount (counts) of antibodies on a single 7CZ NP]

= 2.8 / 0.86 = 3.26

[Number of antibodies on a single 7CZ NP]

= 3.26 / 0.99 = 3.29

Our hydrodynamic diameter (HD) of 7CZ-Ab NPs is 18 nm, so they have 56.52 (= 2*π*9) nm of outermost radius.
Assuming that the antibodies (CD11b) formed a uniform monolayer,[1,2]

[Maximum number of antibodies in the monolayer of a 7CZ-Ab NP]

= 56.52 nm / 12 nm (transverse length of IgG) per antibody = 4.71 antibodies

[Percentage of actual area occupied by antibodies]

= (3.29 / 4.71) * 100% = 70%

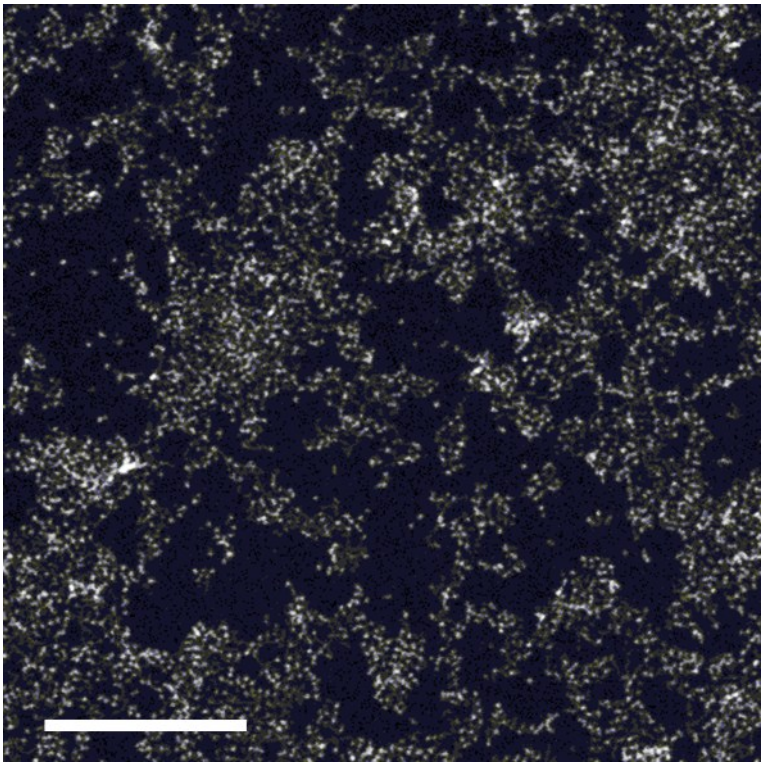

Figure S1. STEM image of 7CZ NPs. Scale bar: 100 nm.

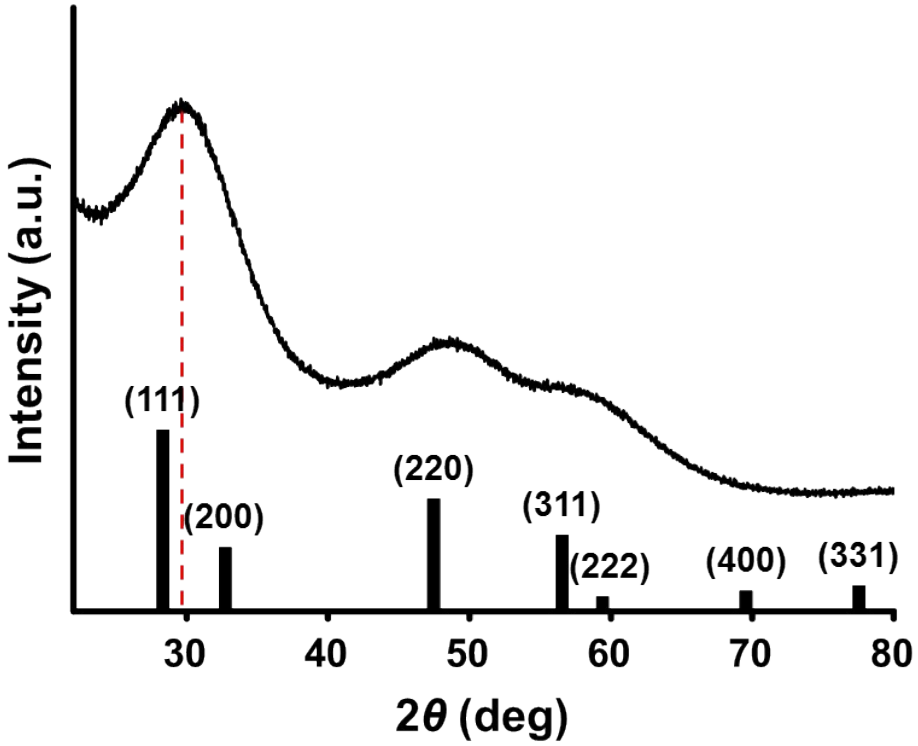

Figure S2. XRD pattern of 7CZ NPs. The black bars indicate the reference peaks of ceria. The red dashed line represents that fluorite peak (111) of 7CZ NPs is shifted.

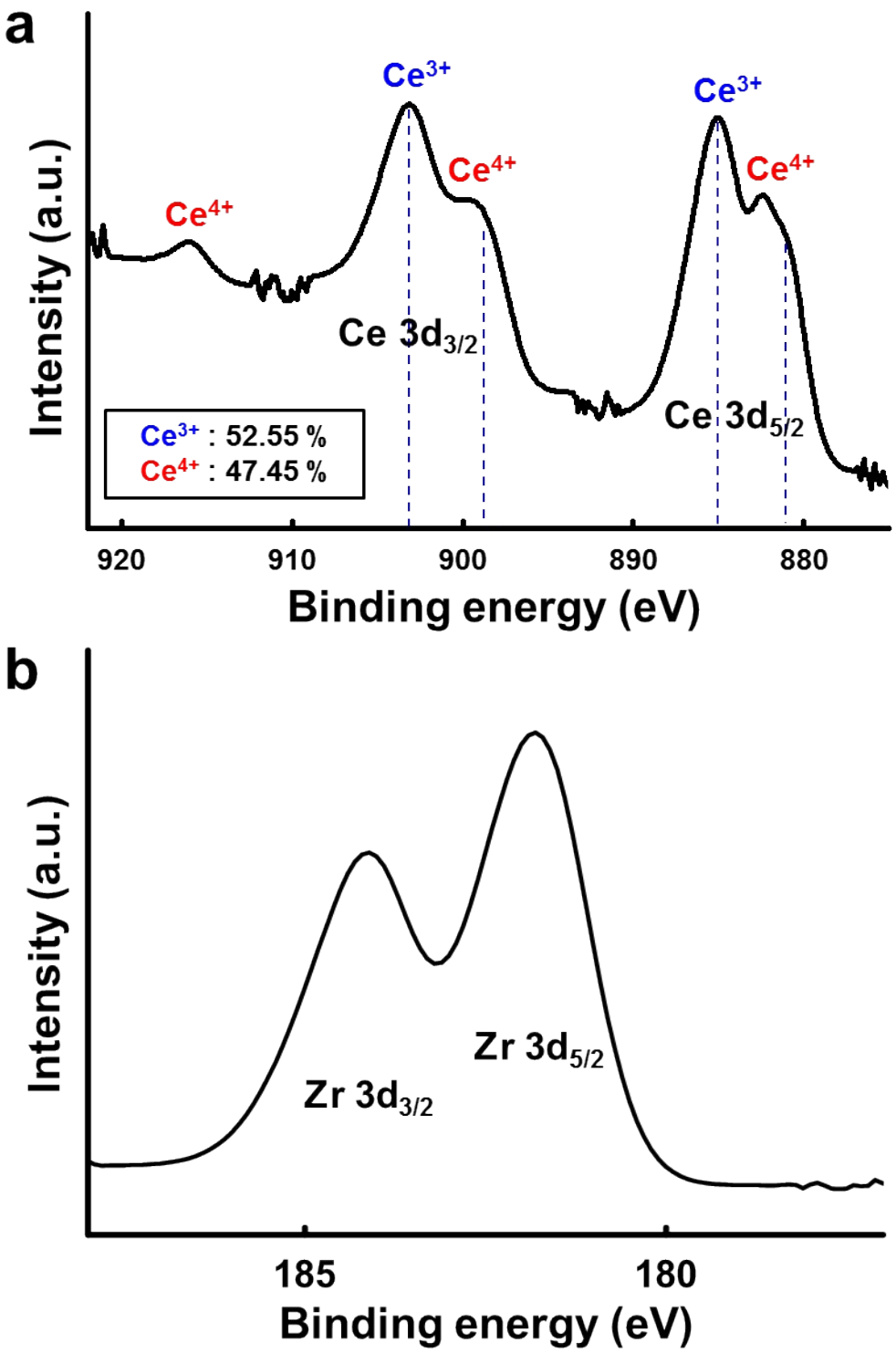

Figure S3. XPS analysis of (a) Ce (Ce^{3+}: 52.55% and Ce^{4+}: 47.45%) and (b) Zr ions in 7CZ NPs.

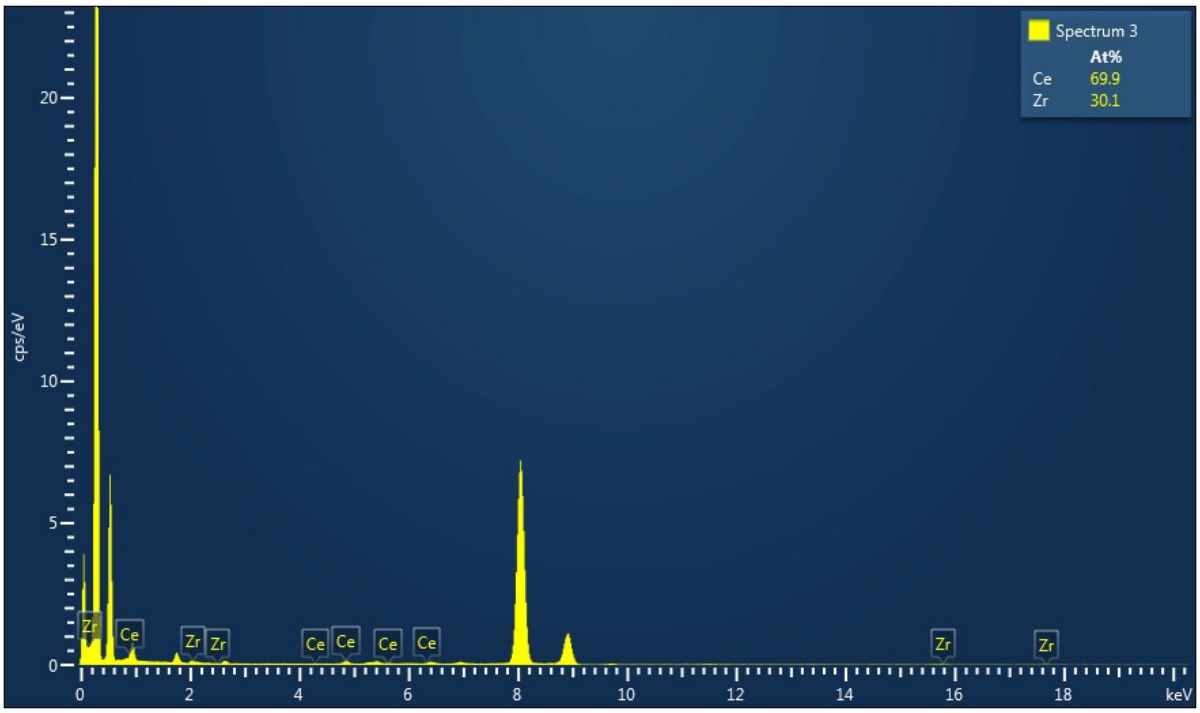

Figure S4. EDS spectra, confirming atomic compositions of 7CZ NPs (69.9 at% Ce and 30.1 at% of Zr).

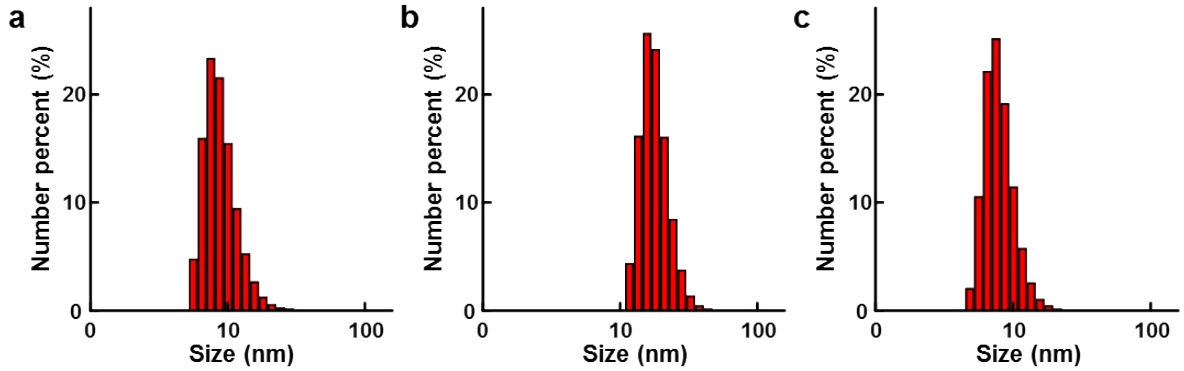

Figure S5. Hydrodynamic diameters of (a) 7CZ NPs, (b) 7CZ-Ab NPs, and (c) free Ab dispersed in PBS.

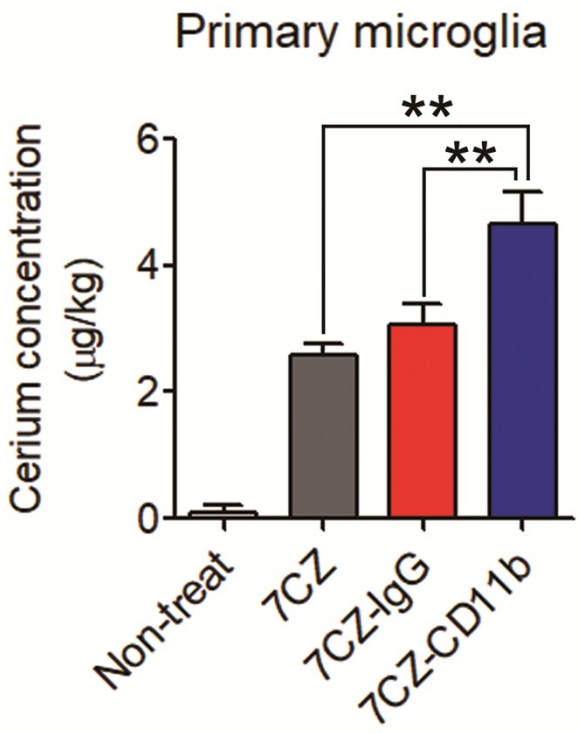

Figure S6. Cellular uptake of 7CZ NPs increases upon conjugation with CD11b antibody. Primary microglia were treated with 7CZ (0.02 mM) NPs, or 7CZ NPs conjugated with isotype control IgG (7CZ-IgG) or CD11b antibody (7CZ-CD11b) for 15 h. Upon trypsinization, cells were lysed and the intracellular cerium concentration was detected by ICP-MS. (n = 3 per each group, $^{**}p < 0.01$)

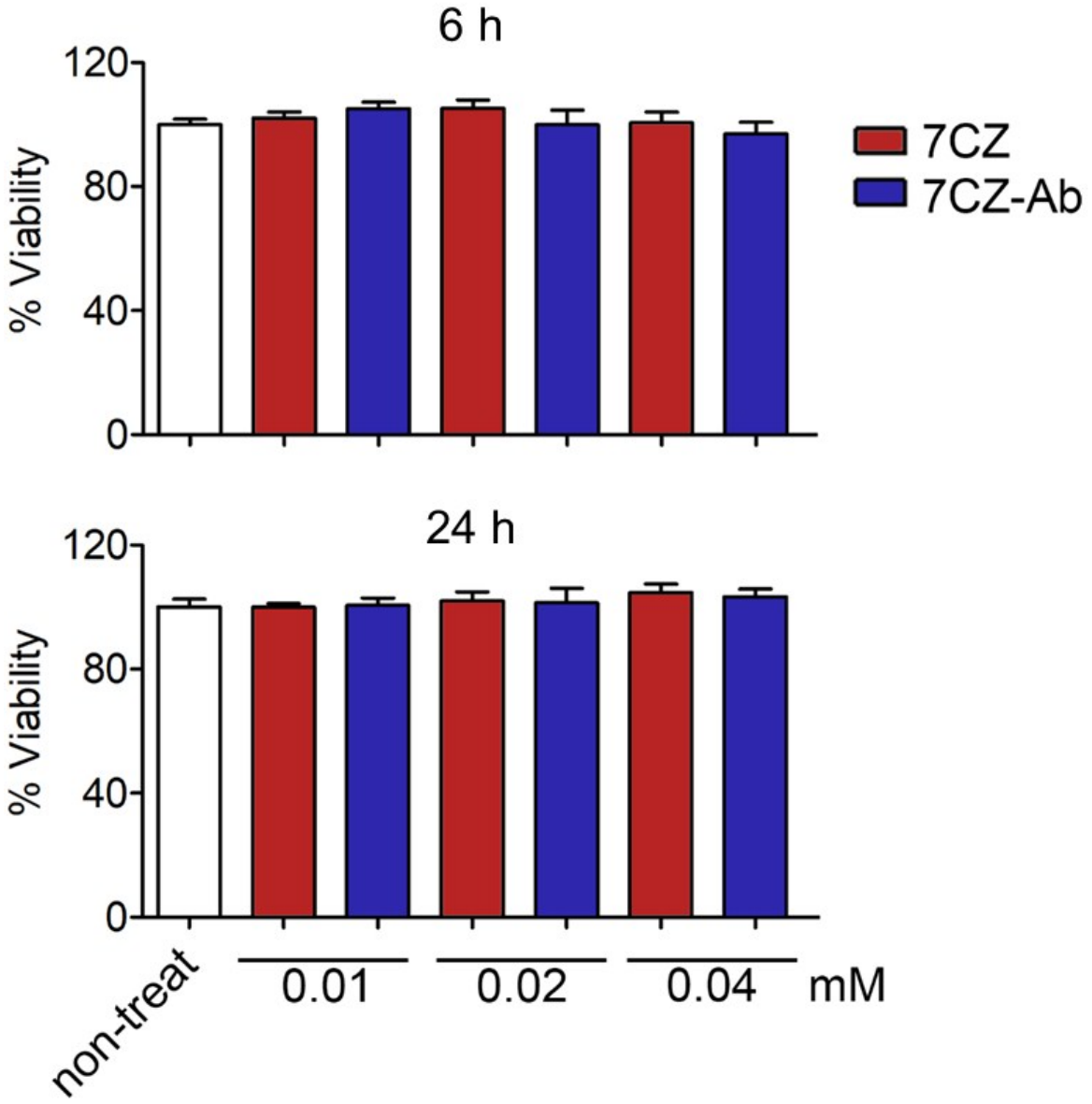

Figure S7. Primary mixed glia cells were treated with NPs for 6 and 24 h (n = 3 per each group). MTS reagent (20 µl) was added and incubated for 1 h at 37 °C. Absorbance was measured at 492 nm. Mean ± standard error of mean (SEM) is shown.

References

1 G. U. Nienhaus, P. Maffre and K. Nienhaus, *Methods in Enzymology* 2013, **519**, 115.
2 D. V. Sotnikov, A. V. Zherdev and B. B. Dzantiev, *Int. J. Mol. Sci.* 2015, **16**, 907.