Supporting Information

Transmigration of magnetite nanoparticles across the blood-brain barrier in a rodent model: Influence of external and alternating magnetic field

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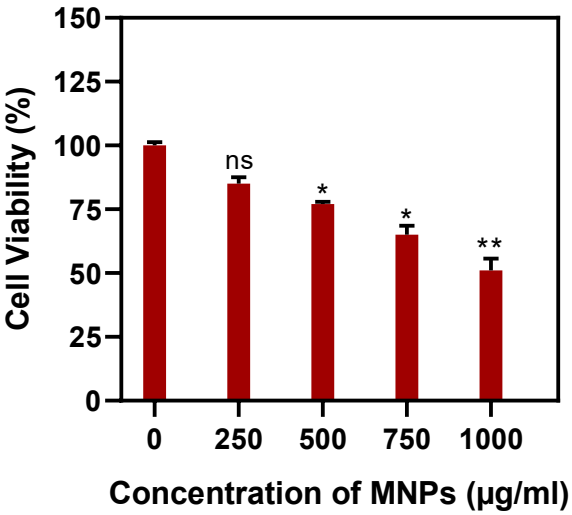

Figure S1: Biocompatibility of the developed MNPs on rat brain bEnd.3 cells (unpaired t test was performed; data represented as mean ± standard error of the mean; ns represents non-significant; *p<0.01, **p<0.05 were considered statistically significant compared to 0 µg/ml).

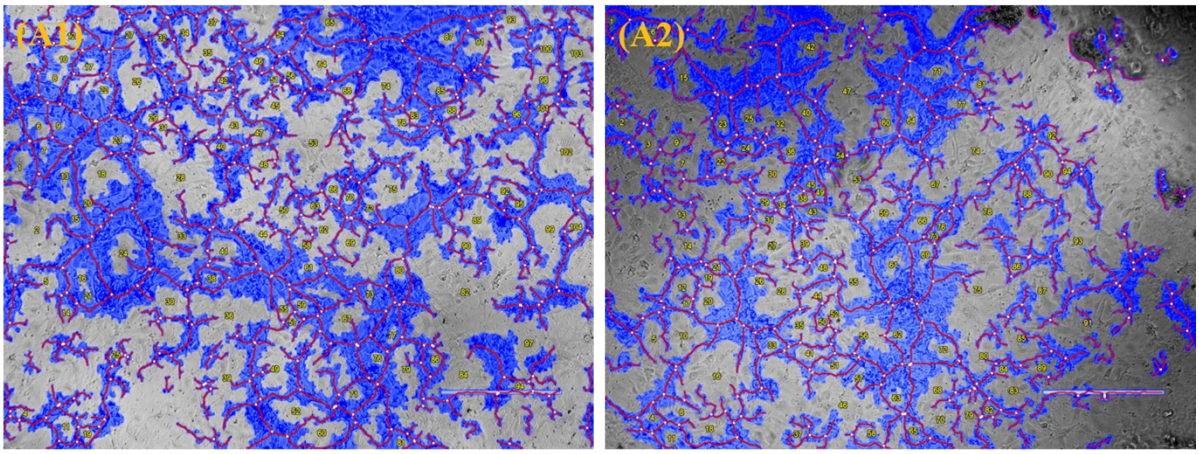

Figure S2. *In vitro* tube formation in (A1) PBS and (A2) MNPs treated bEnd.3 cells. Scale bar = 400 µm.

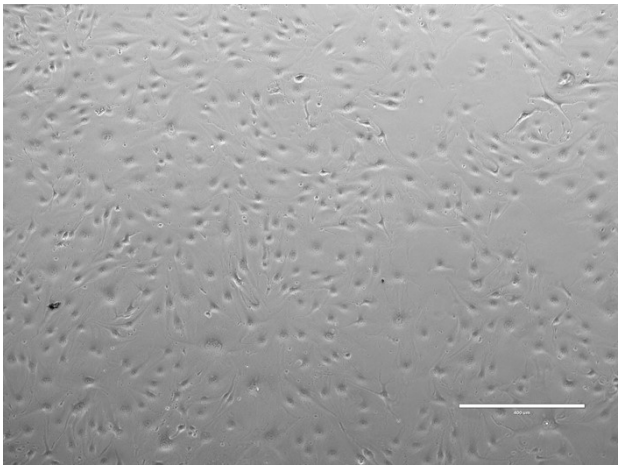

Figure S3. Morphology of bEnd.3 cells grown as monolayer. Scale bar = 400 μm.

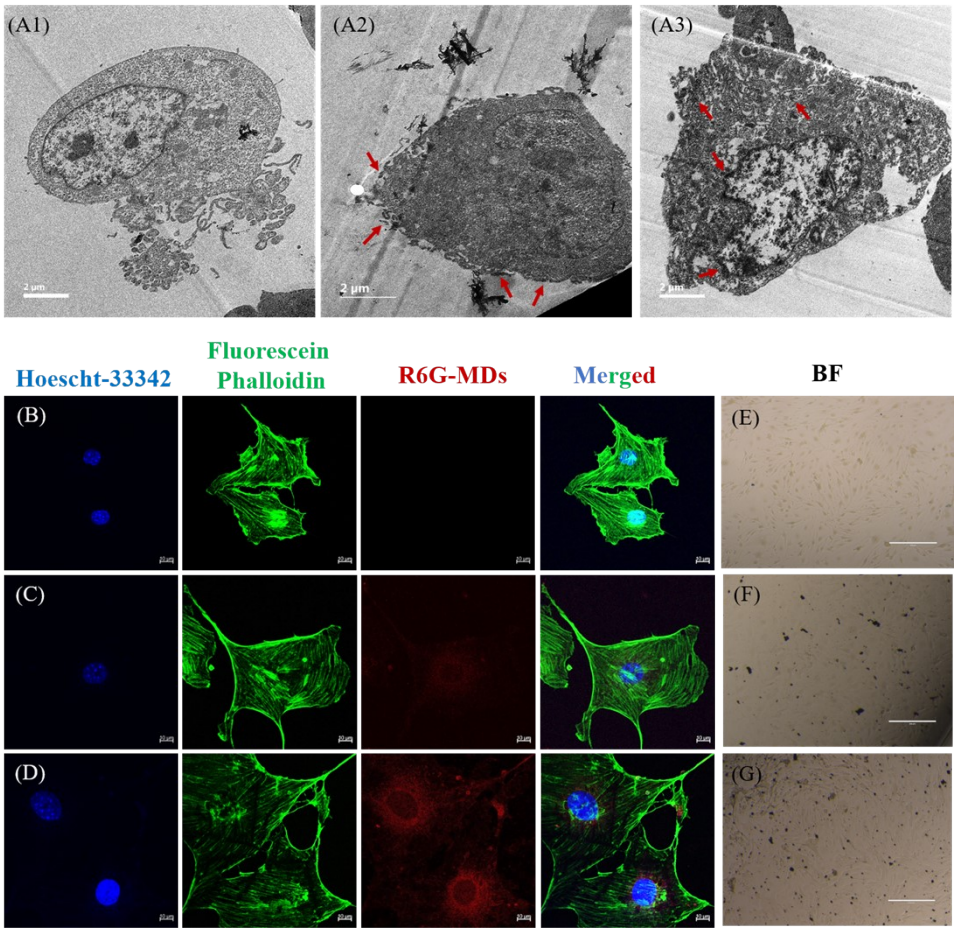

Figure S4. Cell association assay. (A) TEM image analysis of (A1) PBS (Control), (A2) Cells 1 h post-MNPs incubation and (A3) Cells 24 h post-MNPs incubation. Scale bar = 2 µm. Red arrows indicate the presence of MNPs. (B-D) Confocal microscopy (Scale bar = 10 µm) and (E-G) Prussian Blue staining (Scale bar = 200 µm) of bEnd.3 cells treated with (B, E) PBS (Control); (C,F) MNPs alone; (D,G) MNPs under the influence of 1.2 kOe EMF.

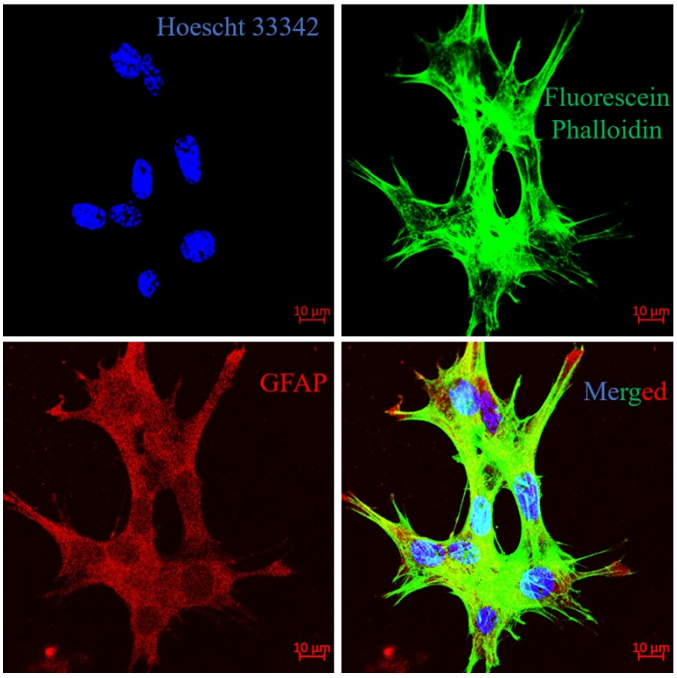

Figure S5. C8-D1A cells. Nuclei is stained blue using Hoescht-33342; cytoskeletal is stained green using Fluorescein phalloidin and astrocytic marker GFAP is presented in red. Scale bar = 10 µm.

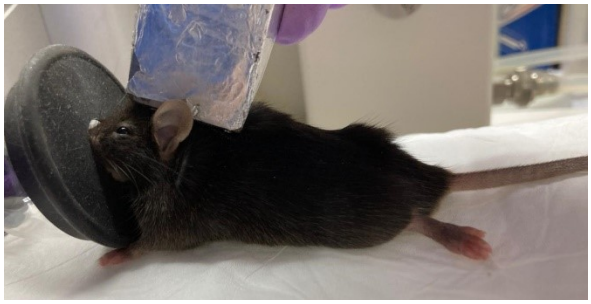

Figure S6. External magnetic field-guided delivery of MNPs across the BBB.

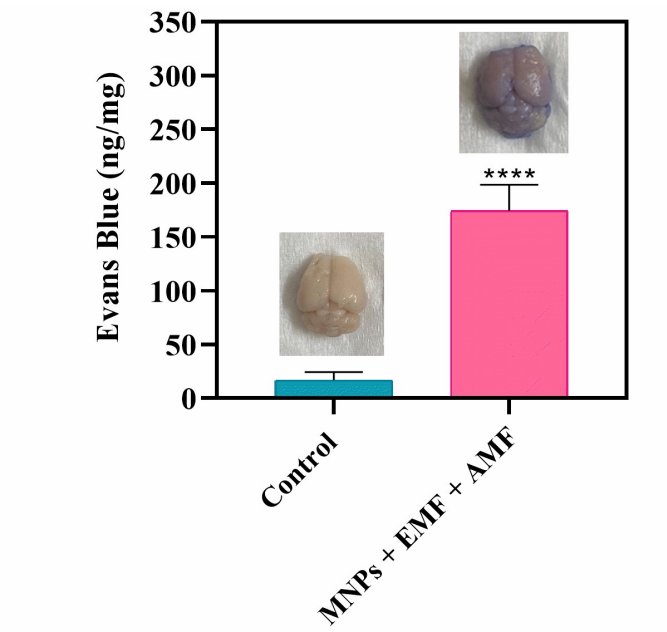

Figure S7. Evans Blue dye permeabilization across the blood-brain barrier following the dual-targeting strategy. ****p<0.0001 were considered statistically significant.

Table S1. Sequences of primers used for determining TJ proteins at BBB.

Gene	**Forward primer**	**Reverse primer**
ZO-1	5’ – TTTTGTCCCACTTGAATCCC - 3’	5’ - AGCTCTTGGGTCATGCACTT - 3’
Claudin-1	5’ – CTTGTTTGCAGAGACCCCAT - 3’	5’ - CAATGACAGCCATCCACATC - 3’
Caudin-5	5’ – CTGGACCACAACATCGTGAC - 3’	5’ - GTACTTGACCGGGAAGCTGA - 3’
GAPDH	5’ – AGACAGCCGCATCTTCTTGT - 3’	5’ - CTTGCCGTGGGTAGAGTCAT - 3’