Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

Biocompatible BSA-MnO_2 nanoparticles for in vivo timely permeability imaging of blood brain barrier and prediction of hemorrhage transformation in acute ischemic stroke

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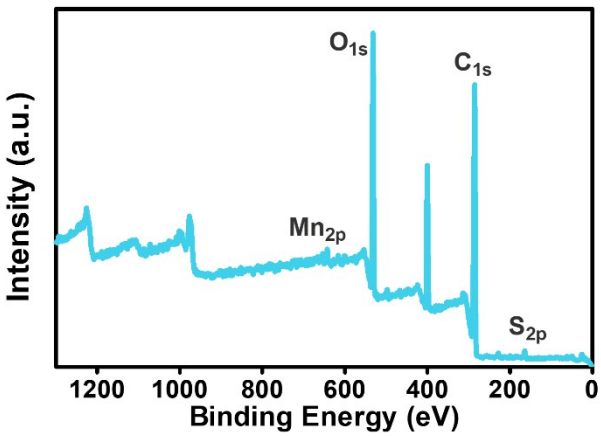

Fig. S1 XPS spectrum of major elements in BM NPs.

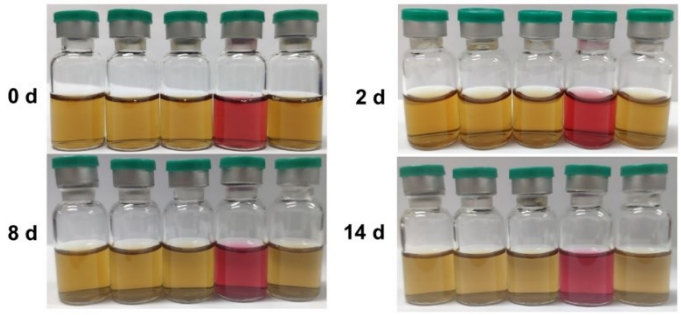

Fig. S2 Long-term colloidal stability of BM NPs dispersed in different media at concentration of 1 mg mL^{-1} (from left to right in each line were water, PBS, NS, DMEM, and FBS) for 14 days.

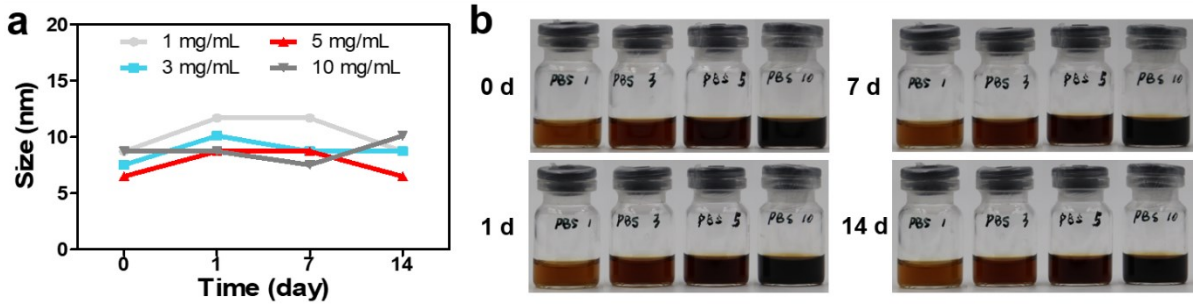

Fig. S3 Long-term colloidal stability of BM NPs dispersed in PBS (pH 7.4) with different concentrations (1, 3, 5, and 10 mg/mL) for 14 days. Hydrodynamic sizes (a) and photographs (b) of BM NPs dispersed in PBS during 14 days.

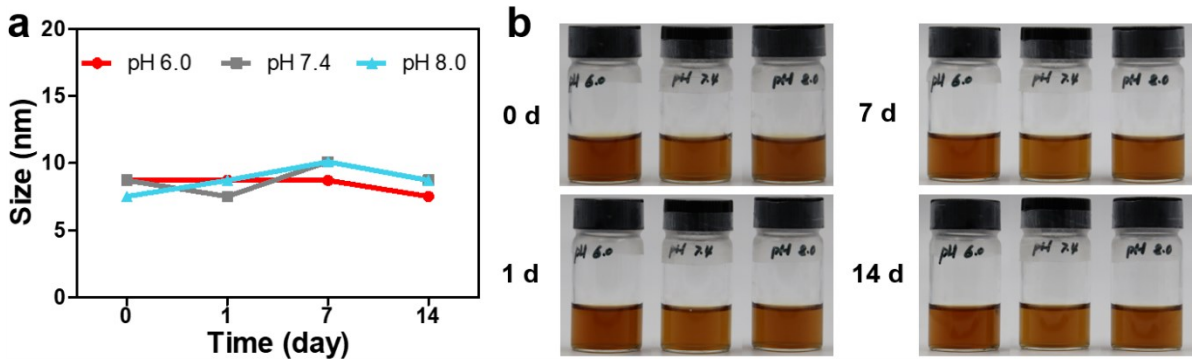

Fig. S4 Long-term colloidal stability of BM NPs dispersed in PBS with different pH values (6.0, 7.4, and 8.0) for 14 days. Hydrodynamic sizes (a) and photographs (b) of BM NPs dispersed in PBS during 14 days.

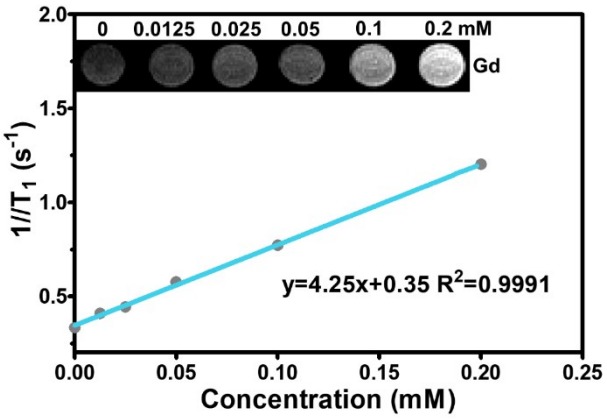

Fig.S5 Linear fitting of $1/T_1$ of Gd-DTPA in water as a function of Gd concentrations.

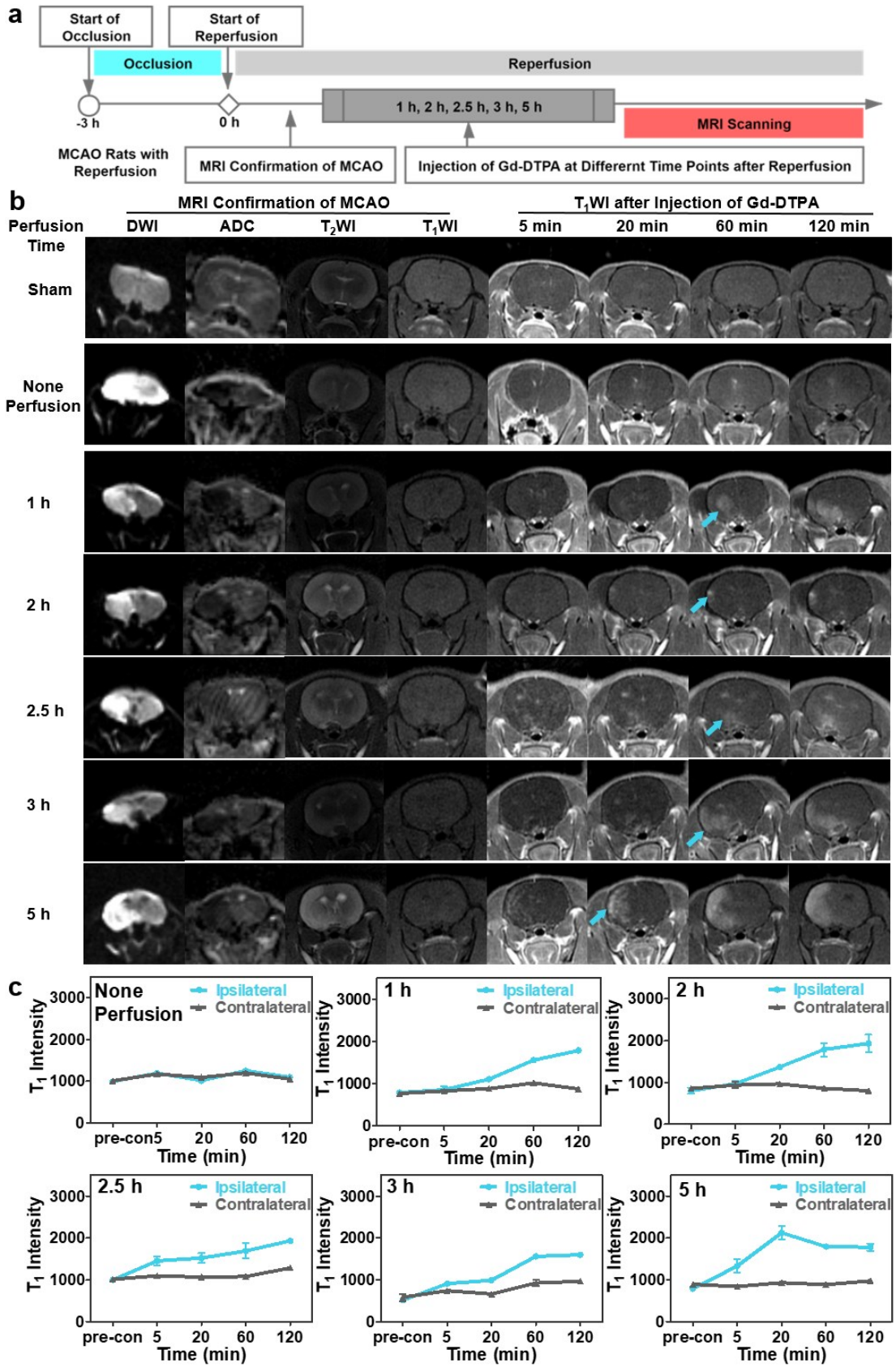

Fig. S6 MR imaging of BBB permeability in MCAO rats without perfusion or with different perfusion time (1, 2, 2.5, 3, and 5 h) post injection of Gd-DTPA. (a) A flow diagram of the

imaging assay. (b) MR imaging of sham-operated rat, MCAO rats without perfusion, and MCAO rats with different perfusion time before and after injection of Gd-DTPA at different time points. The enhanced regions were pointed by the blue arrows. (c) The T_1WI signal intensity curves of ipsilateral and contralateral regions in MCAO rats without perfusion and MCAO rats with different perfusion time before and after injection of Gd-DTPA at different time points.

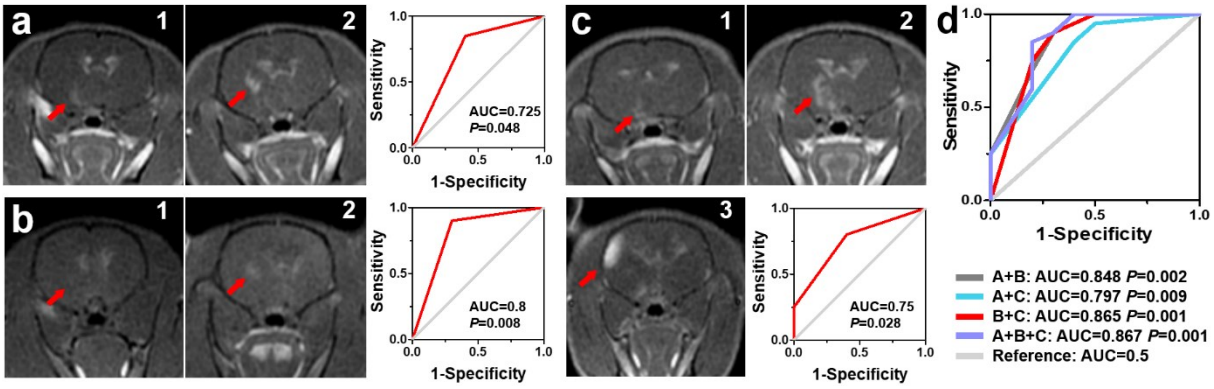

Fig. S7 ROC curves for quantitative prediction of HT after acute ischemic stroke via diverse imaging patterns from BM NPs-enhanced MR imaging. (a) Two categories of peak intensity (cut-off: enhanced intensity value ≥ 280 when compared with the contralateral normal region at 20 min post injection of BM NPs) and corresponding ROC curve for prediction of HT. Image 1 and image 2 showed the typical conditions of lower peak intensity (< 280) and increased peak intensity (≥ 280) when compared with the contralateral normal region, respectively. (b) Two categories of imaging duration (cut-off: 2 h post injection of BM NPs with enhanced intensity value > 100 when compared with the contralateral normal region) and corresponding ROC curve for prediction of HT. Image 1 and image 2 showed the typical conditions of shortened imaging duration (< 2 h) and extended imaging duration (≥ 2 h) when compared with the contralateral normal region, respectively. (c) Three categories of imaging region (cerebral parenchyma near the skull base, corpus striatum, and cortex) and corresponding ROC curve for prediction of HT. Image 1, image 2, and image 3 showed the typical imaging regions in cerebral

parenchyma near the skull base, corpus striatum, and cortex, respectively. For (a-c), the enhanced sites were pointed by the red arrows. (d) ROC curves of combined imaging patterns (A: increased peak intensity; B: extended imaging duration; C: expanded imaging region) for prediction of HT.

Table S1 ROC analysis of imaging patterns indicated by BM NPs-enhanced MR imaging for prediction of HT in acute ischemic stroke

Imaging patterns	AUC	*P*	Sensitivity	Specificity	95% CI
A	0.725	0.048	0.85	0.6	0.517 - 0.933
B	0.800	0.008	0.9	0.7	0.611 - 0.989
C	0.750	0.028	0.8	0.6	0.57 - 0.93
A+B	0.848	0.002	0.9	0.7	0.676 - 1
A+C	0.797	0.009	0.85	0.6	0.625 - 0.97
B+C	0.865	0.001	0.9	0.7	0.718 - 1
A+B+C	0.867	0.001	0.85	0.8	0.714 - 1

Pattern A: increased peak intensity; pattern B: extended imaging duration; pattern C: expanded imaging region

CI: confidence interval

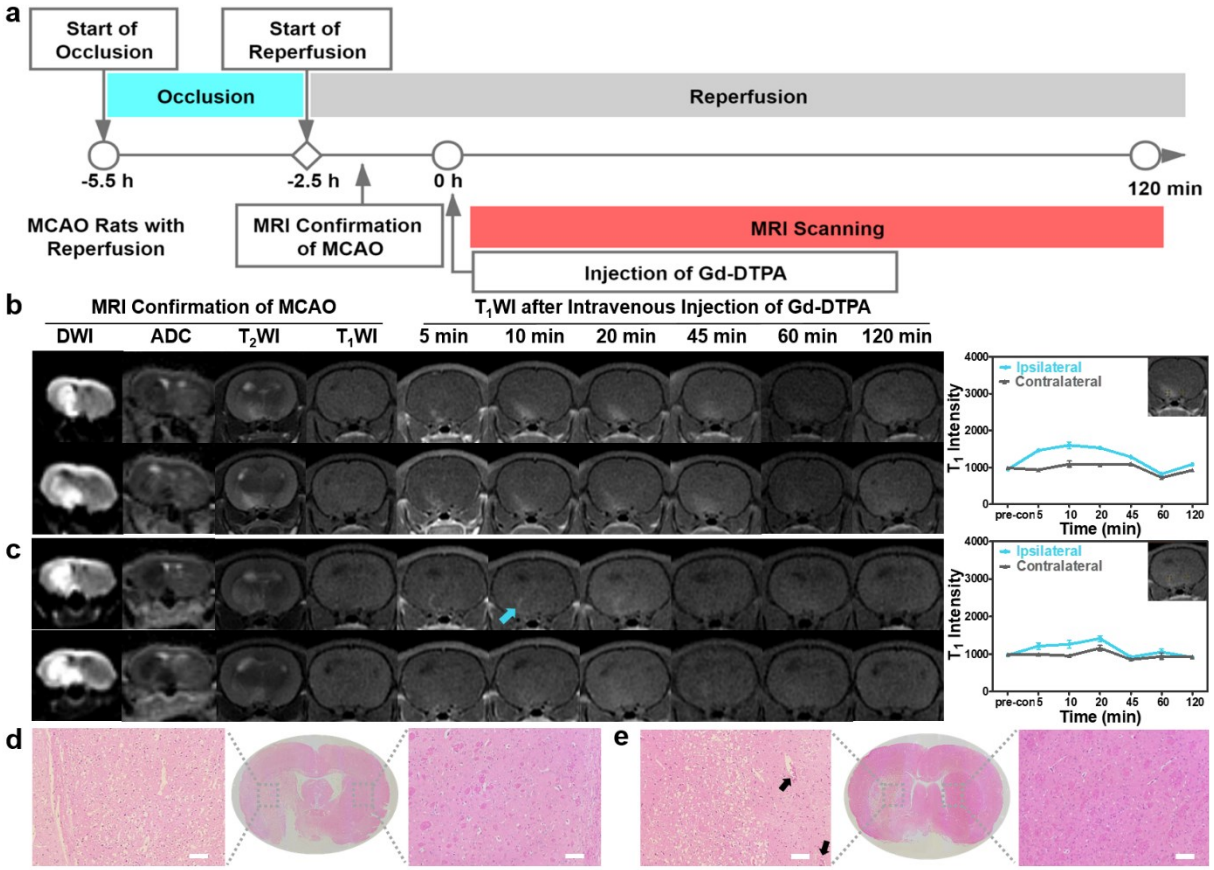

Fig. S8 The BBB permeability imaging indicated by the isodose Gd-DTPA in MCAO rats with or without HT. (a) A timeline for MRI acquisition after injection of isodose Gd-DTPA. (b) MR imaging and signal intensity curves of ipsilateral and contralateral regions in MCAO rats without HT before and after injection of isodose Gd-DTPA. (c) MR imaging and signal intensity curves of ipsilateral and contralateral regions in MCAO rats with HT before and after injection of isodose Gd-DTPA. (d, e) H&E staining images of brain tissues (both ipsilateral and contralateral side) corresponding to the MCAO rats in b and c, respectively (scale bar=100 μm). The hemorrhage region was pointed by the black arrows.

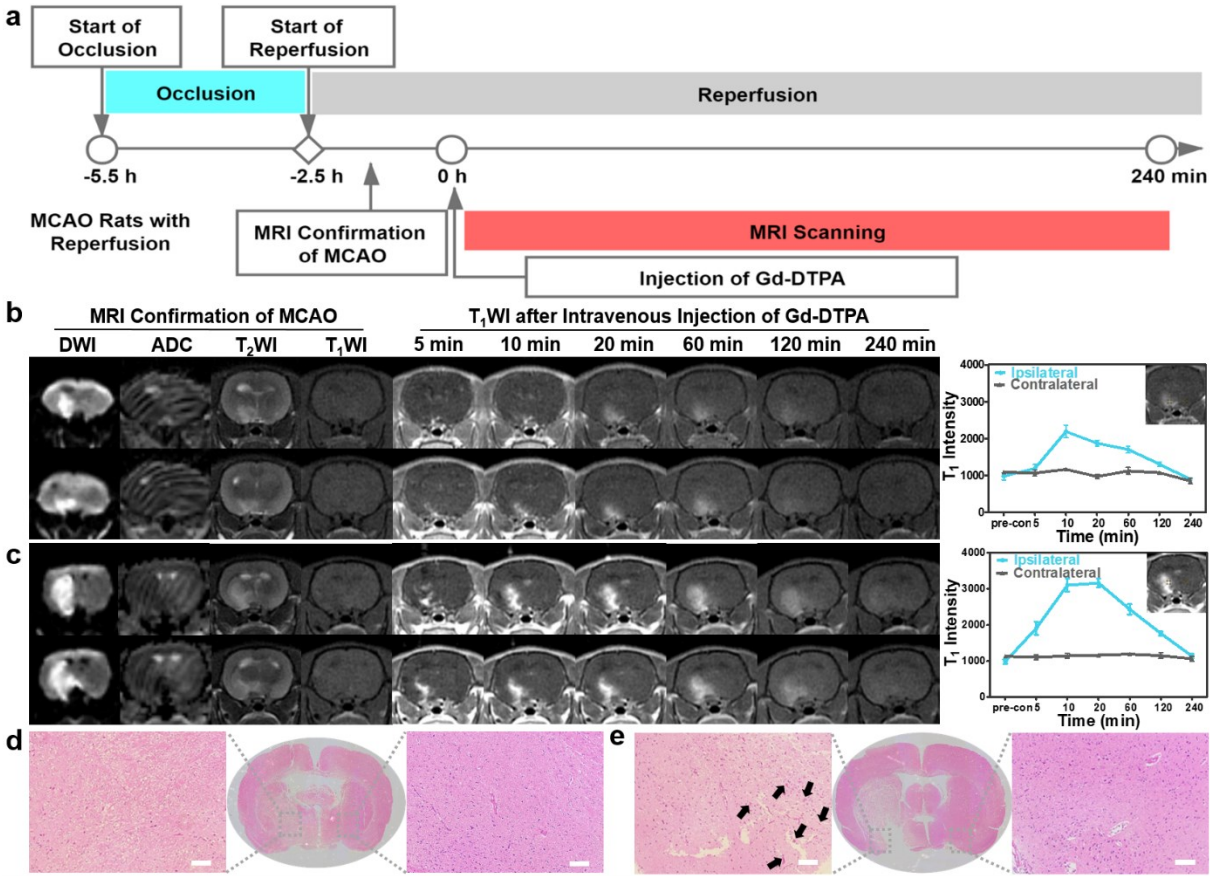

Fig. S9 The BBB permeability imaging indicated by the high dose Gd-DTPA in MCAO rats with or without HT. (a) A timeline for MRI acquisition after injection of high dose Gd-DTPA. (b) MR imaging and signal intensity curves of ipsilateral and contralateral regions in MCAO rats without HT before and after injection of high dose Gd-DTPA. (c) MR imaging and signal intensity curves of ipsilateral and contralateral regions in MCAO rats with HT before and after injection of high dose Gd-DTPA. (d, e) H&E staining images of brain tissues (both ipsilateral and contralateral side) corresponding to the MCAO rats in b and c, respectively (scale bar=100 μm). The hemorrhage region was pointed by the black arrows.

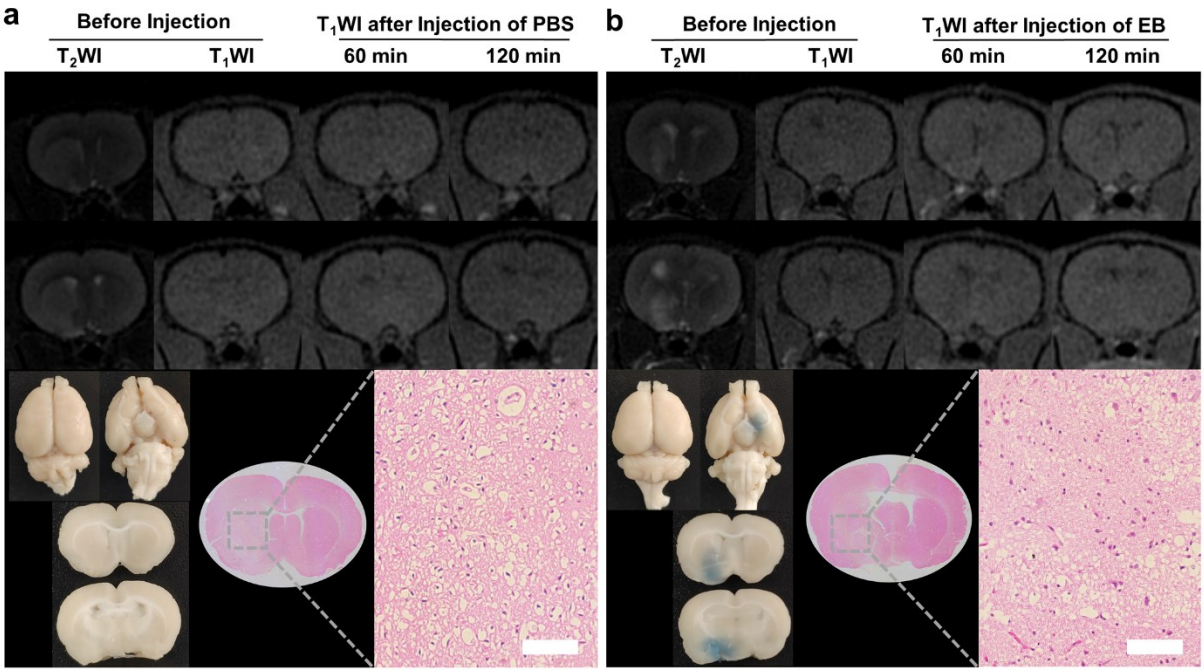

Fig. S10 MR imaging and histological analysis of MCAO rats after injection of PBS and EB, seperately. (a) MR imaging in vivo and H&E staining ex vivo of brain tissues in MCAO rats after injection PBS. (b) MR imaging in vivo, EB staining ex vivo, and H&E staining ex vivo of brain tissues in MCAO rats after injection of EB. Scale bar=100 μm.

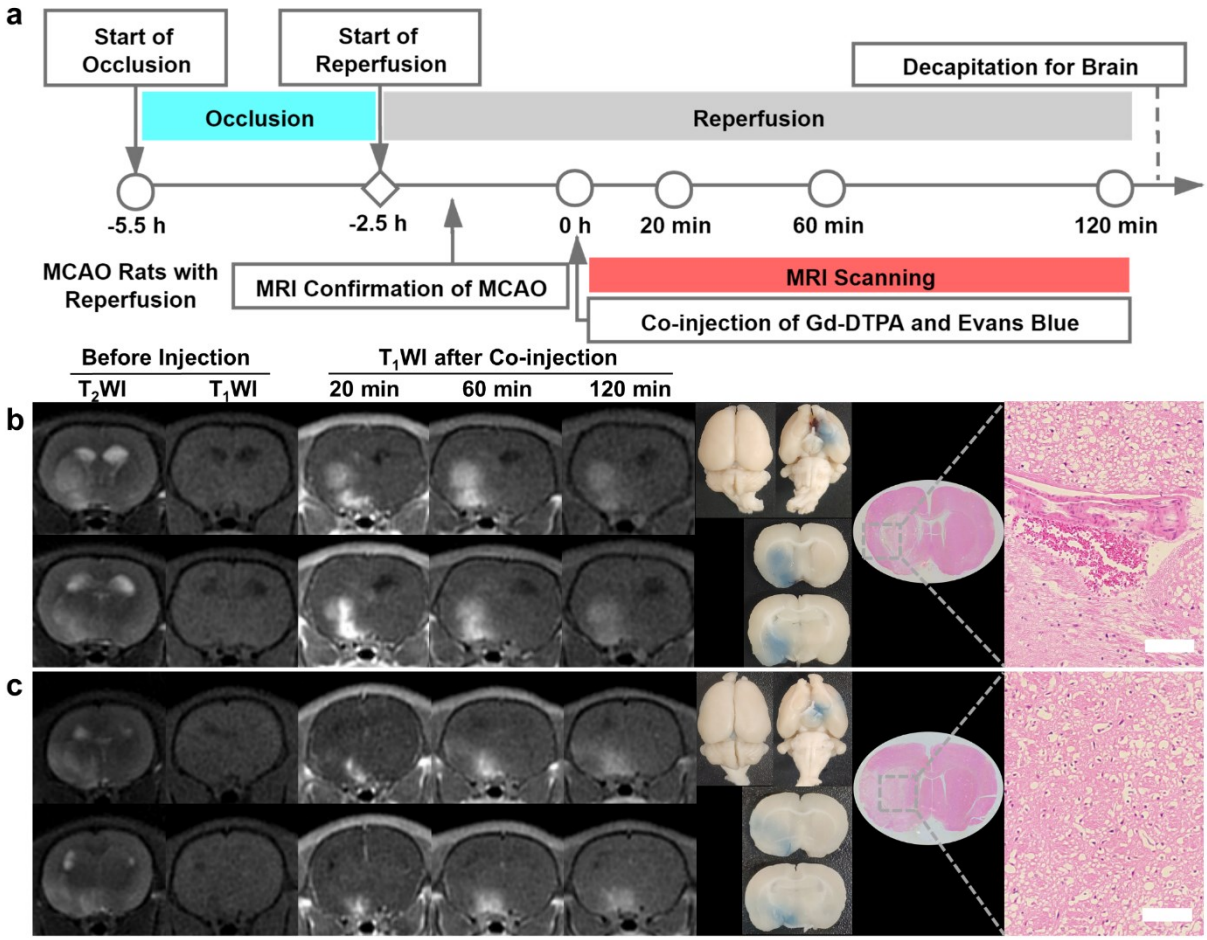

Fig. S11 Comparison of permeability assessment of BBB between high dose Gd-DTPA-enhanced MR imaging in vivo and EB-assisted staining ex vivo. (a) A timeline for MRI acquisition after co-injection of high dose Gd-DTPA and EB. MR imaging in vivo, EB staining ex vivo, and H&E staining ex vivo of brain tissues in MCAO rats with HT (b) or without HT (c) after co-injection of high dose Gd-DTPA and EB. Scale bar=100 μm.

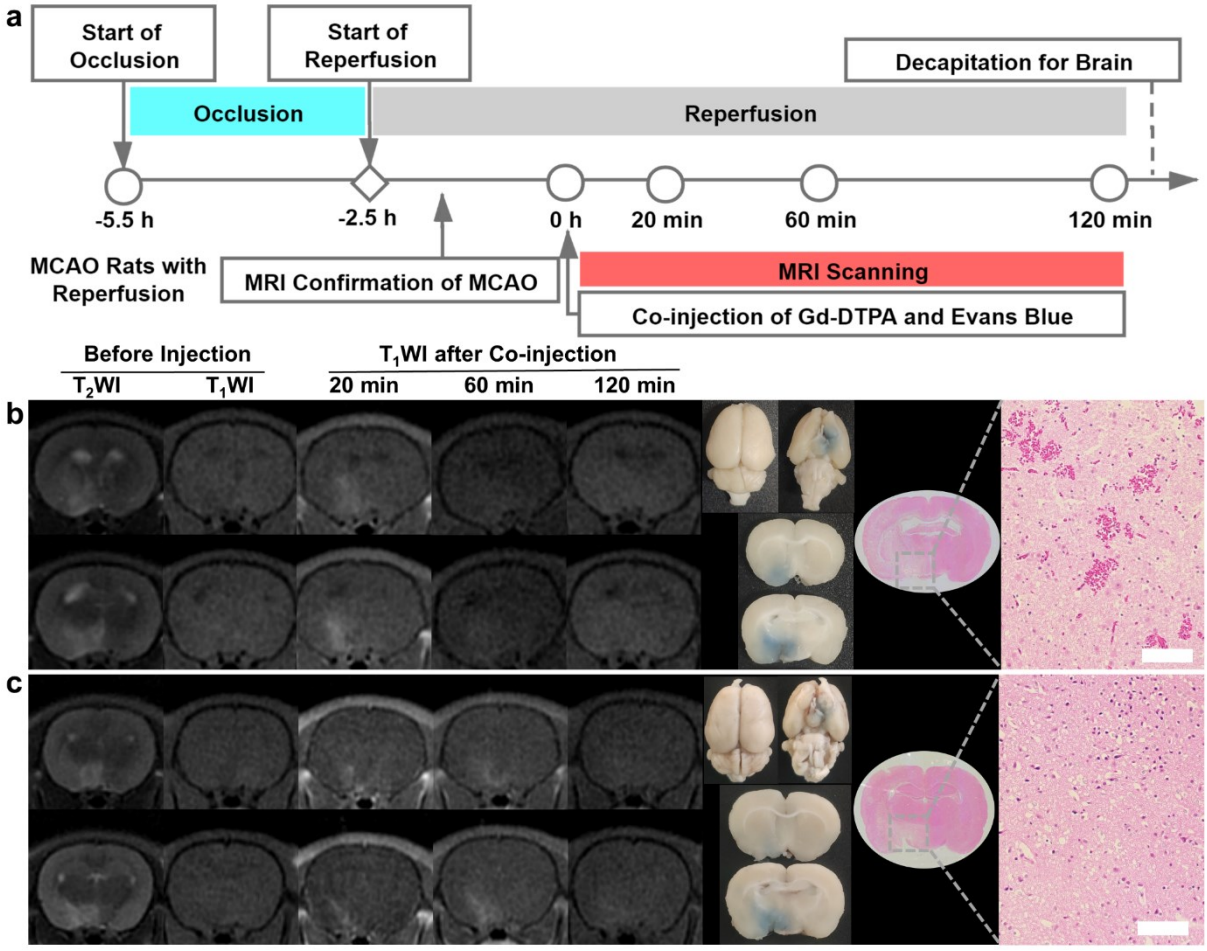

Fig. S12 Comparison of permeability assessment of BBB between isodose Gd-DTPA-enhanced MR imaging in vivo and EB-assisted staining ex vivo. (a) A timeline for MRI acquisition after co-injection of isodose Gd-DTPA and EB. MR imaging in vivo, EB staining ex vivo, and H&E staining ex vivo of brain tissues in MCAO rats with HT (b) or without HT (c) after co-injection of isodose Gd-DTPA and EB. Scale bar=100 μm.

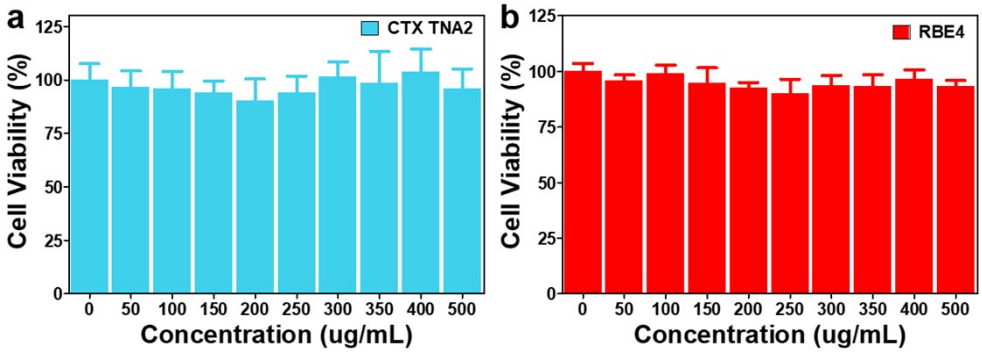

Fig. S13 Cell viabilities of BBB-related cells (a, CTX TNA2 cells; b, RBE4 cells) when incubated with BM NPs at different concentrations (0-500 μg mL^{-1}).

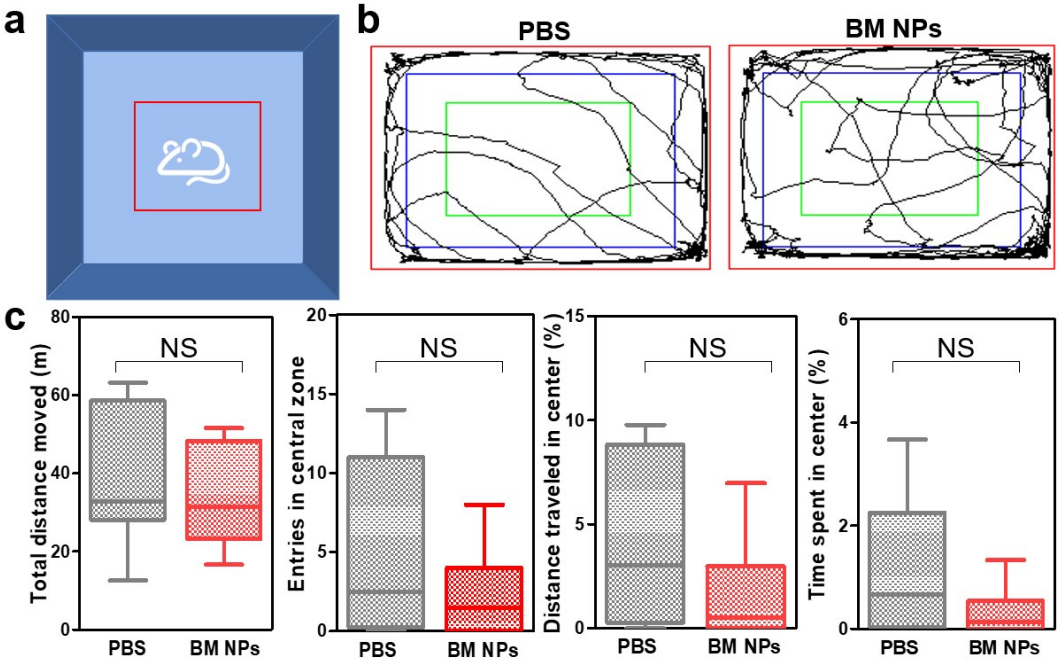

Fig. S14 Effect of BM NPs on the locomotor activity and mental state of rats evaluated by the open field test (OFT). Schematic diagram (a) and movement trace of rats (b) in OFT. The locomotor activity of rats in an OFT was measured by the following indicators: total distance moved (m), entries in central zone, distance traveled and time spent in center. (c) The results of OFT on rats at 7 days post administration of BM NPs. Rats with administration of PBS were set as the control group. The box shows the medians (solid bar), interquartile ranges (box), and min to max (whiskers). Independent Student's t-test was used for comparing variables with normal distribution, and the Mann-Whitney U test was used for the comparison of non-parametric variables. Data were analyzed by IBM Statistical SPSS 22.0 software. $P<0.05$ was considered statistically significant (n=8 for each group; NS, no significance).

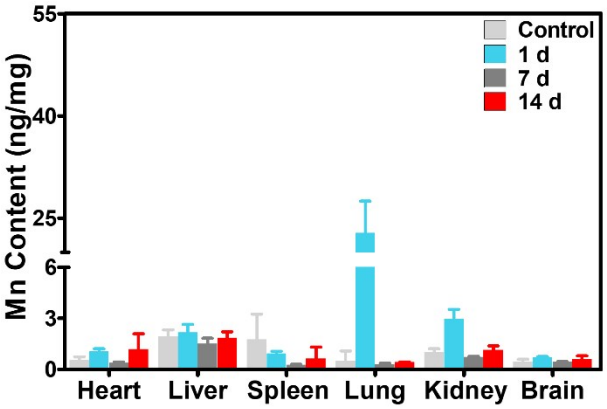

Fig. S15 The manganese level in vital organs of normal rats at different time points (1 d, 7 d, and 14 d) post intravenous injection of BM NPs (0.136 mmol Mn kg^{-1}, n=3).