Electronic Supplementary Information

In vivo MRI tracking and therapeutic efficacy of transplanted mesenchymal stem cells labeled with ferrimagnetic vortex iron oxide nanorings for liver fibrosis repair

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Cell culture

Human umbilical cord mesenchymal stem cells (hMSCs) were provided by Nanjing Drum Tower Hospital (Nanjing, China). The hMSCs (passage 4-10) were cultured with DMEM/F12 basal medium supplemented with penicillin-streptomycin (1%) and fetal bovine serum (10%), and then placed into a carbon dioxide incubator (Thermo 3111).

Proliferation and apoptosis examination

For proliferation hMSCs were incubated with FVIO@PEG nanotracers (35 μg mL^{-1} Fe) for 4 hours. The hMSCs without labeling were set as control. Next, the labeled and unlabeled hMSCs were digested and re-cultured into a 24-well plate at a density of 1×10^4 cells per well, respectively. After 1, 3, 5, and 7 days of culture, the relative number of cells was detected by CCK-8, and the absorbance at 450 nm was measured on a microplate analyzer.

Annexin V-EGFP apoptosis detection kit (Beyotime Biotechnology) was applied to evaluate the apoptosis induction ability of the FVIO@PEG labeled hMSCs. Specifically, hMSCs were seeded into a 6-well plate at a density of 1×10^5 per well, and then treated with FVIO@PEG nanotracers (35 μg mL^{-1} or 50 μg mL^{-1} Fe). After 4-hour culturing, the collected hMSCs were washed with PBS and resuspended into 195 μL Annexin V-EGFP binding solution. After that, the cell suspension was sequentially added with 5 μL Annexin V-EGFP and 10 μL propidium iodide (PI) staining solution, and incubated for 20 minutes at room temperature in dark. Flow cytometry was used to analyze the fluorescence intensity of EGFP and PI in the labeled hMSCs. The unlabeled

hMSCs were taken as control.

Osteogenic and adipogenic differentiation assay

The labeled hMSCs were seeded into a 24-well plate at a density of 1×10^4 cells per well. After 24-hour culturing, the medium was replaced by osteoinductive differentiation complete medium (Cyagen Biosciences Inc.), which was prepared according to the manufacturer's instructions. After changing the medium every 3 days for 21 days, the cells were fixed with 4% paraformaldehyde solution for 20 min, and then stained with Alizarin Red S. The red-stained calcium nodules in the cells were observed under a microscope. For quantitative analysis, DMSO was used to dissolve the dye in the cells and the absorbance at 550 nm was recorded by a microplate analyzer.

The preparation of adipogenic differentiation medium A (induction medium Inc.) and medium B (maintenance medium Inc.) referred to the manufacturer's instructions. The labeled hMSCs were seeded into a 24-well plate at a density of 1×10^4 cells per well. After culturing for 24 hours, the medium was replaced with adipogenic differentiation medium A and B for 3 and 1 days, respectively. After 15 days of alternate culturing, medium B was used for continuous culture for 4-7 days. Subsequently, the cells were fixed with 4% paraformaldehyde and stained with Oil Red O. The formation of lipid droplets was observed under a microscope. For quantitative analysis, the intracellular dye was dissolved in DMSO, and the absorbance at 490 nm was measured with a microplate analyzer.

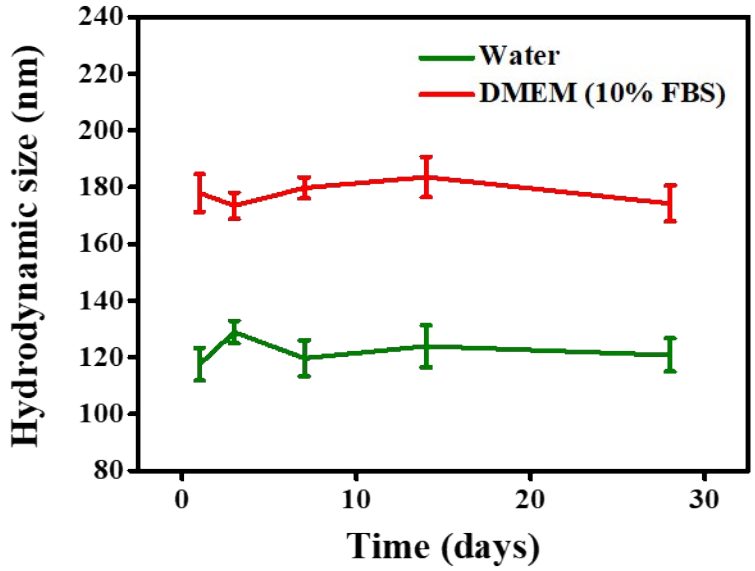

Fig. S1 Hydrodynamic size of FVIO@PEG measured as a function of time upon incubation in deionized water and DMEM containing 10% FBS, respectively.

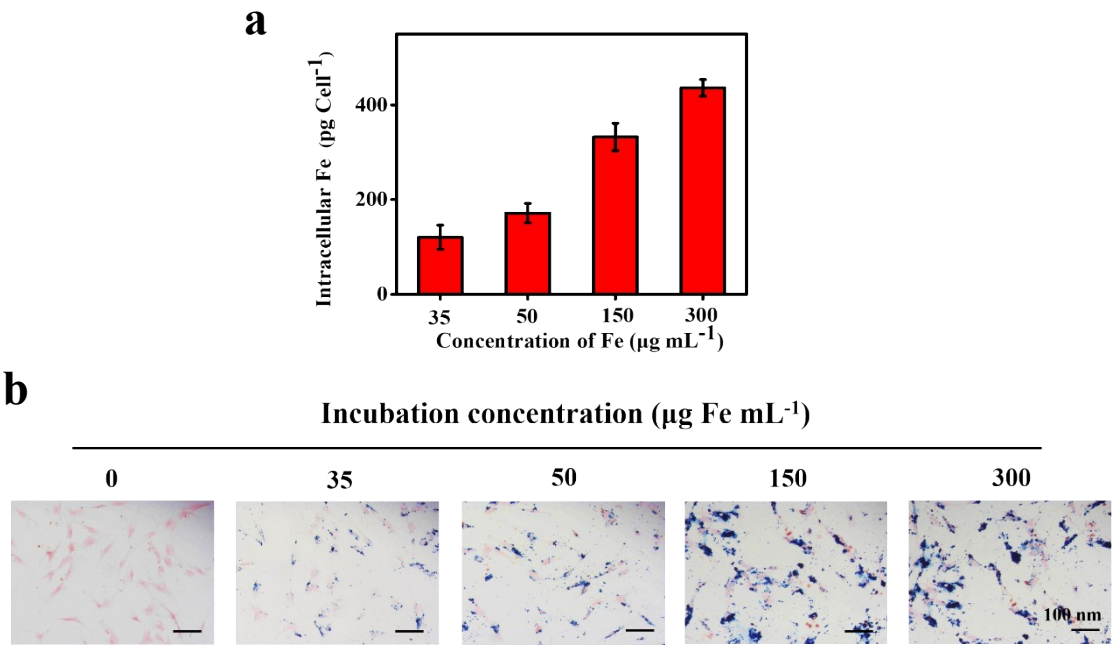

Fig. S2 (a) Intracellular Fe content after incubation with Ruicun at various Fe concentrations for 4 hours. (b) Optical images of Prussian blue-stained hMSCs after incubating with Ruicun at different iron concentrations.

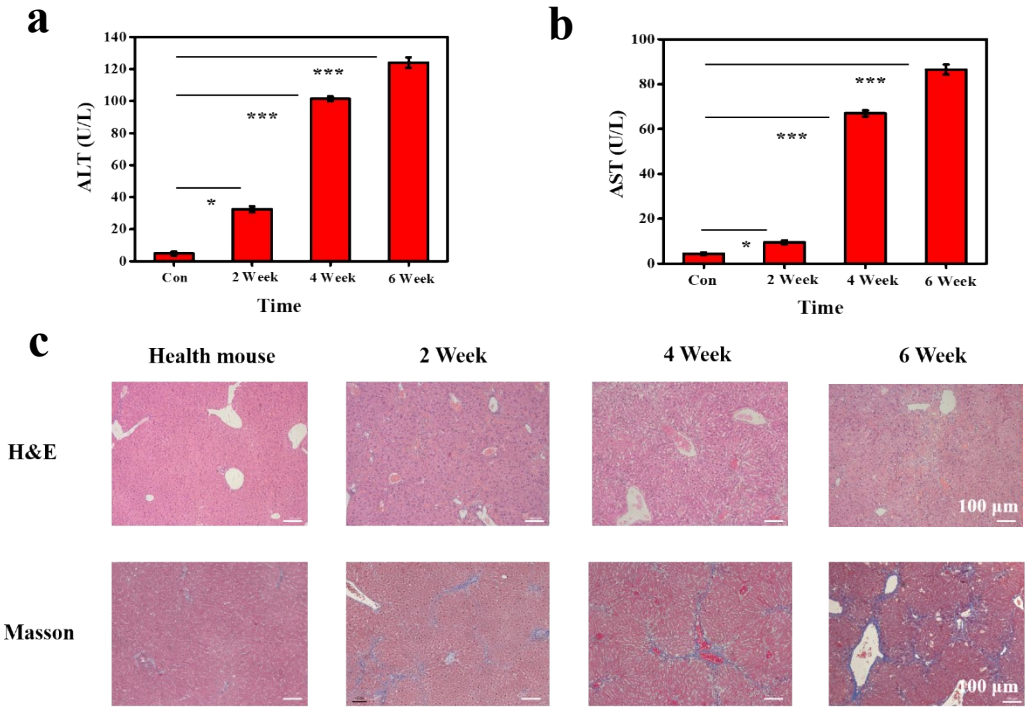

Fig. S3 (a) Plasma ALT and (b) AST levels of mice after CCl_4 treatment in the 2nd week, 4th week and 6th week. (c) H&E and Masson's trichrome staining of the liver sections at 2, 4, and 6 weeks after administration of CCl_4. The mice treated with PBS were taken as control.