Supporting information

A tumor microenvironment-mediated $Bi_{2-x}Mn_xO_3$ hollow nanospheres *via* glutathione depletion for photothermal enhanced chemodynamic collaborative therapy

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Live/Dead Cell Staining. Typically, 4T1 cells were seeded in a 24-well plate at 5×10^4 cells/well and then incubated with BM for 12 hours. Subsequently, the cells were irradiated with an 808 nm laser (1 W cm^{-2}) for 3 min. Then, calcein (AM) and propidium iodide (PI) were added for the staining of living and dead 4T1 cells and incubated to form different fluorescent substances. Digital fluorescence photographs of the cells were captured using a fluorescence microscope.

Apoptosis. Apoptosis quantitatively explored by flow cytometry. Usually, 4T1 cells were seeded into a 6-well plate at 1×10^5 cells/well and then incubated with BM for 12 hours. Subsequently, the cells were irradiated with an 808 nm laser (1 W cm^{-2}) for 3 min. After that, the cells were digested with trypsin and stained with Annexin V-FITC/PI, and the percentage of apoptotic cells was quantitatively determined by flow cytometry analysis.

Hydroxyl Radical (·OH) assessment. BM (100 μg mL^{-1}) was introduced to 10 mL of mixed solution containing 25 mM $NaHCO_3$ and 0.5 mM GSH, and the solution was stirred for 15 min at 37 ℃. The supernatant was obtained by centrifuging the mixed solution, 8 mM H_2O_2 and MB (20 μg mL^{-1}) were added to the supernatant, and the solution was incubated at 37 °C in the dark for 30 minutes. Then, the absorbance was measured at 665 nm to evaluate the CDT performance.

ROS Detection by Flow Cytometry. 4T1 cells were seeded in 6-well plates at a density of 4×10^5 cells/well and cultured for 24 h. Then the cells were co-incubated with BM or PBS for 4 h. In particular, the cells in BM+NIR and BM+H_2O_2+NIR groups were exposed to 808 nm laser for 5 min. Then, the cells in each group were incubated with 1 mL of FBS-free RMPI 1640 medium containing DCFH-DA (1:1000) for 20 min in the dark prior. After that, the cells in each group were collected after washing with 2 mL of PBS twice, and their DCF fluorescence intensity was assessed by flow cytometry.

Animal model. A tumor model was established by subcutaneously injecting 4T1 cells into the backs of female BALB/c mice (4 or 6 weeks old). The mice were injected with BM when the tumor volume reached 50-100 mm^3. All animal experiments were under the guidance and approved by the Institutional Animal Care of Hefei University of Technology and Use Committee.

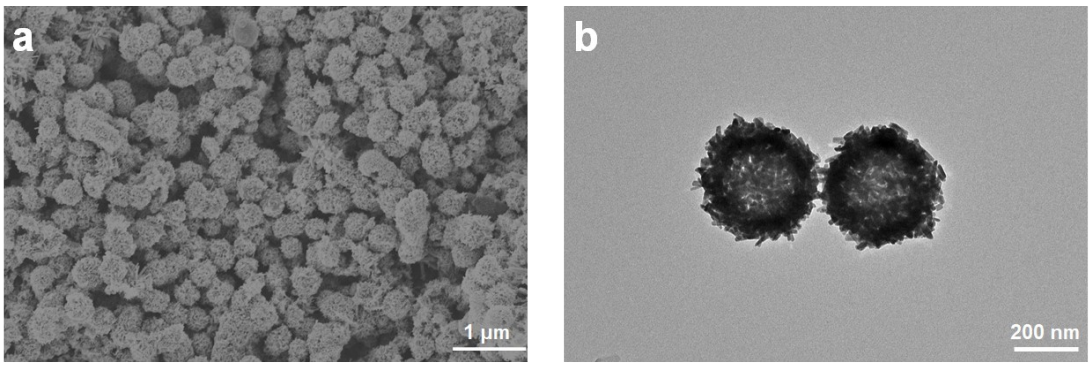

Fig. S1 (a) SEM image of Bi_2S_3 hollow nanospheres. (B) TEM image of Bi_2S_3 hollow nanospheres.

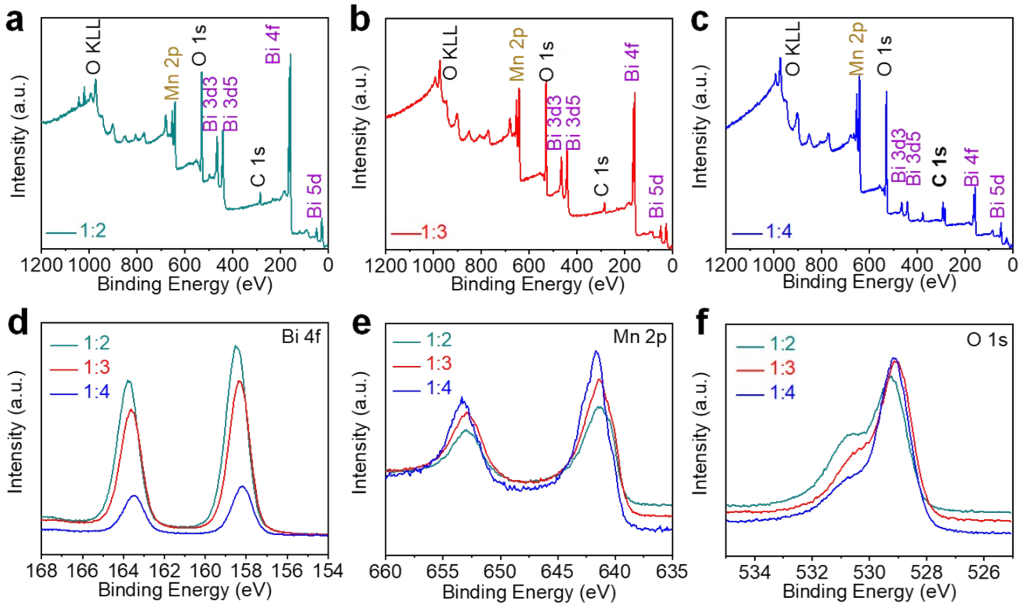

Fig.S2 (a) Full XPS of 1:2 BM nanospheres. (b) Full XPS of 1:3 BM nanospheres. (c) Full XPS of 1:4 BM nanospheres. (d) XPS spectra of Bi 4f. (e) XPS spectra of Mn 2p. (f) XPS spectra of O 1s.

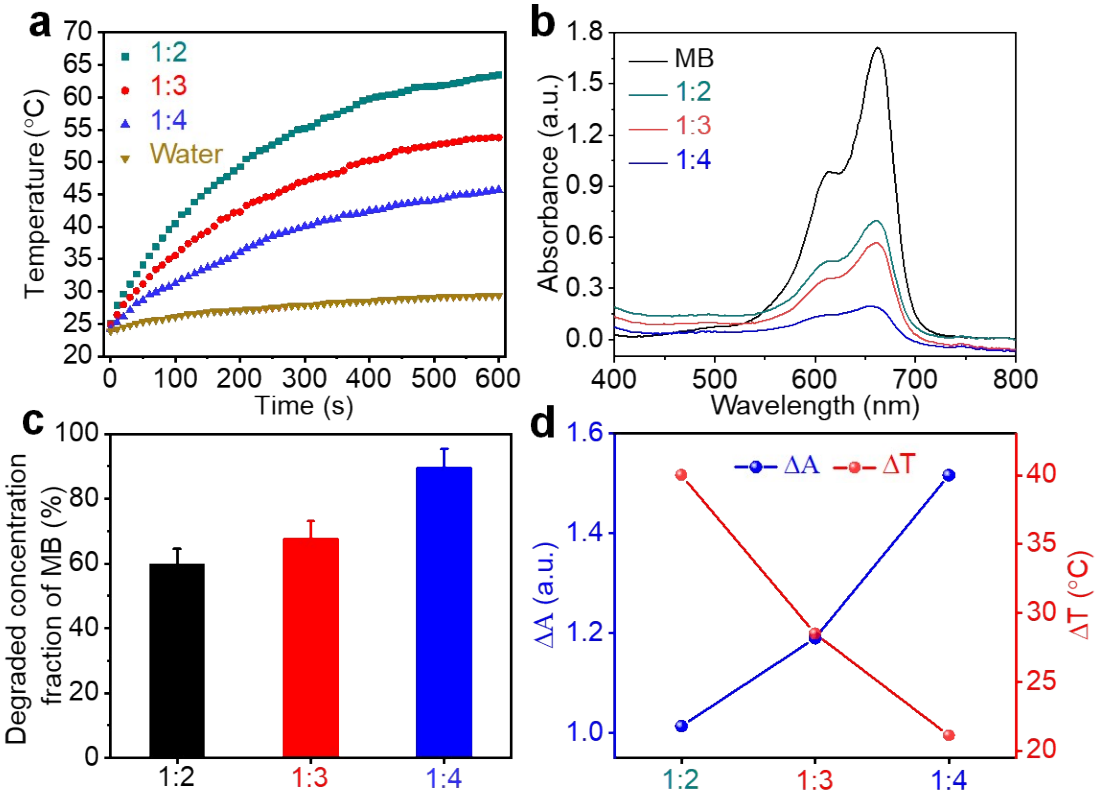

Fig. S3 (a) Temperature change in BM with different BM (1:2, 1:3, 1:4) under NIR laser irradiation. (b) Absorbance of MB treated with different BM (1:2, 1:3, 1:4). (c) Bar graph showing the degradation percent of MB after different BM (1:2, 1:3, 1:4) incubation. (d) Photothermal and MB degradation for different BM (1:2, 1:3, 1:4) groups.

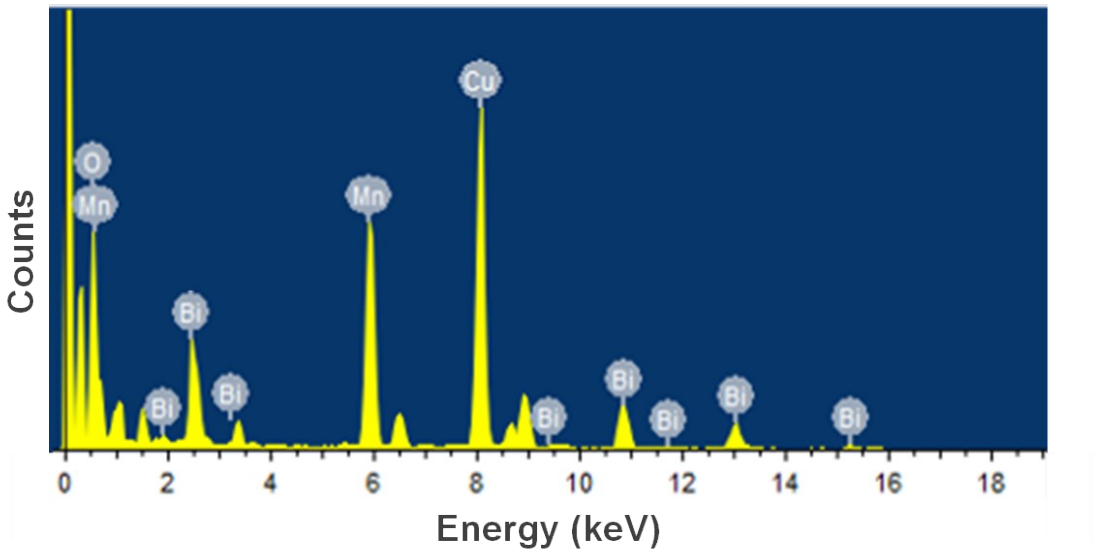

Fig. S4 Energy dispersive X-ray spectra of the as-prepared BM hollow nanospheres

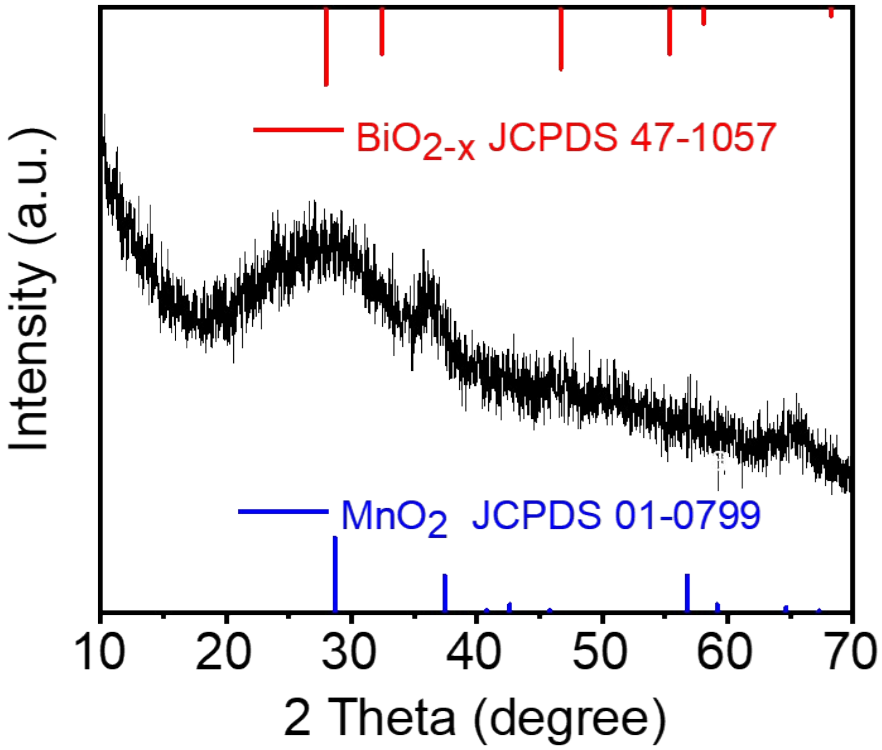

Fig. S5 XRD patterns of as-obtained BM

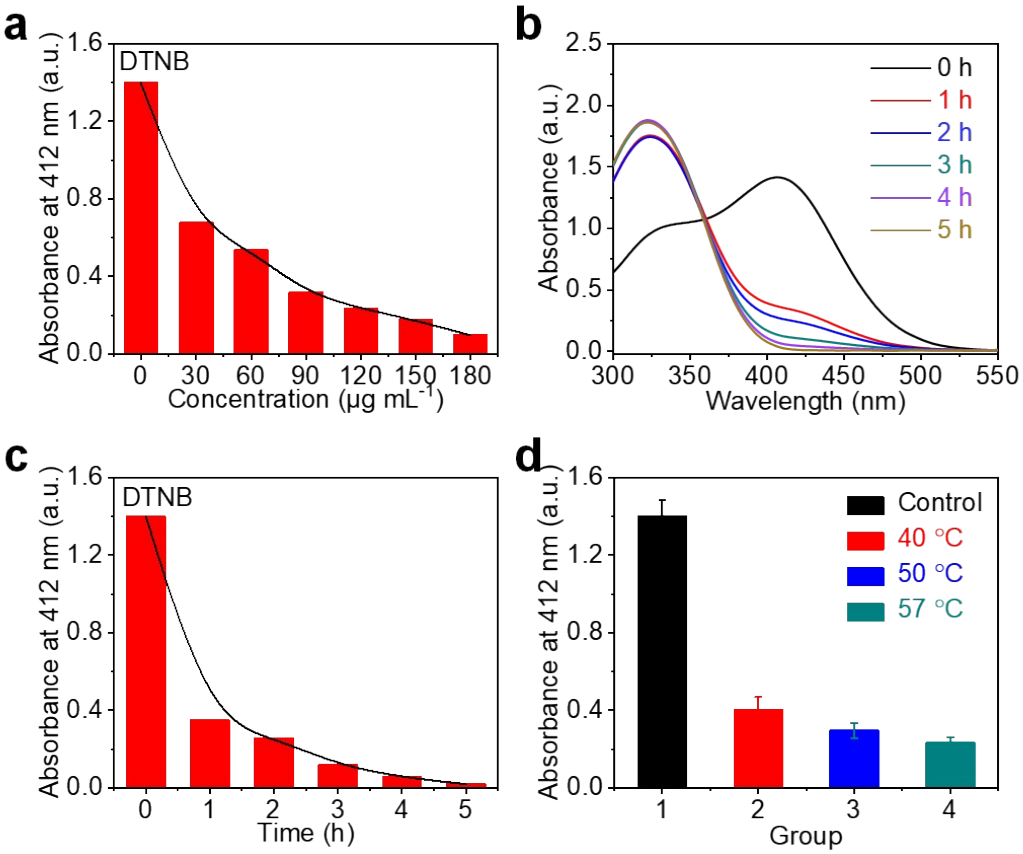

Fig. S6 (a) Histogram of GSH consumption for different concentrations of BM dispersions. (b) GSH depletion profile with BM dispersions at various time characterized by DTNB. (c) Histogram of GSH consumption of BM dispersions at different time points. (d) Histogram of GSH consumption of BM dispersions at different temperatures.

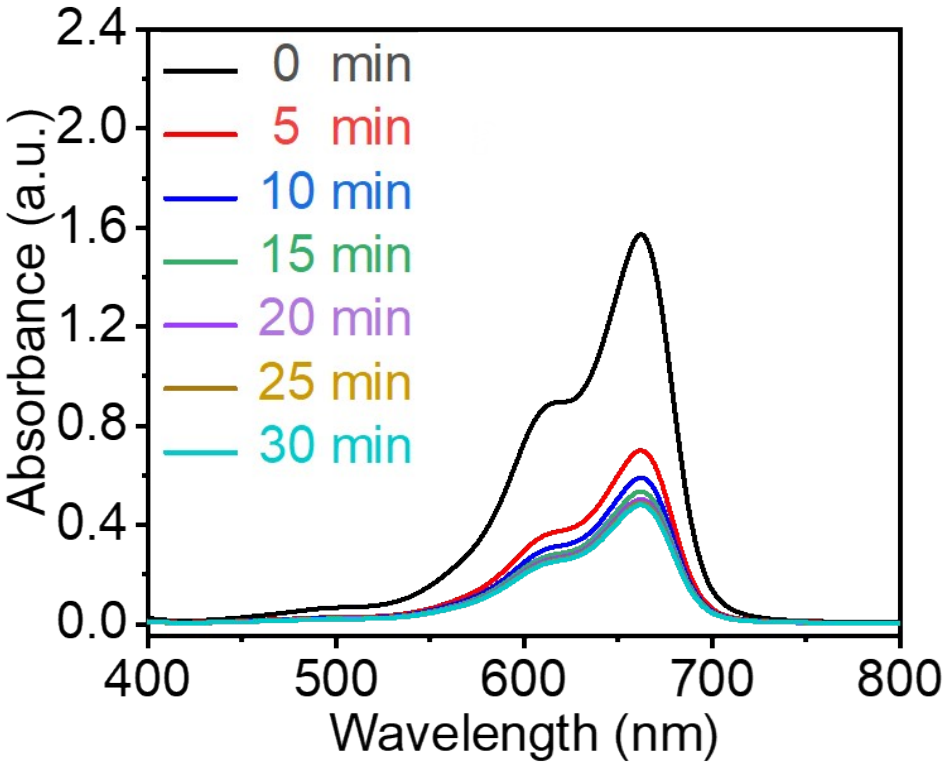

Fig. S7 UV–Vis-NIR absorption of MB at different time periods by BM hollow nanospheres mediated Fenton-like reaction.

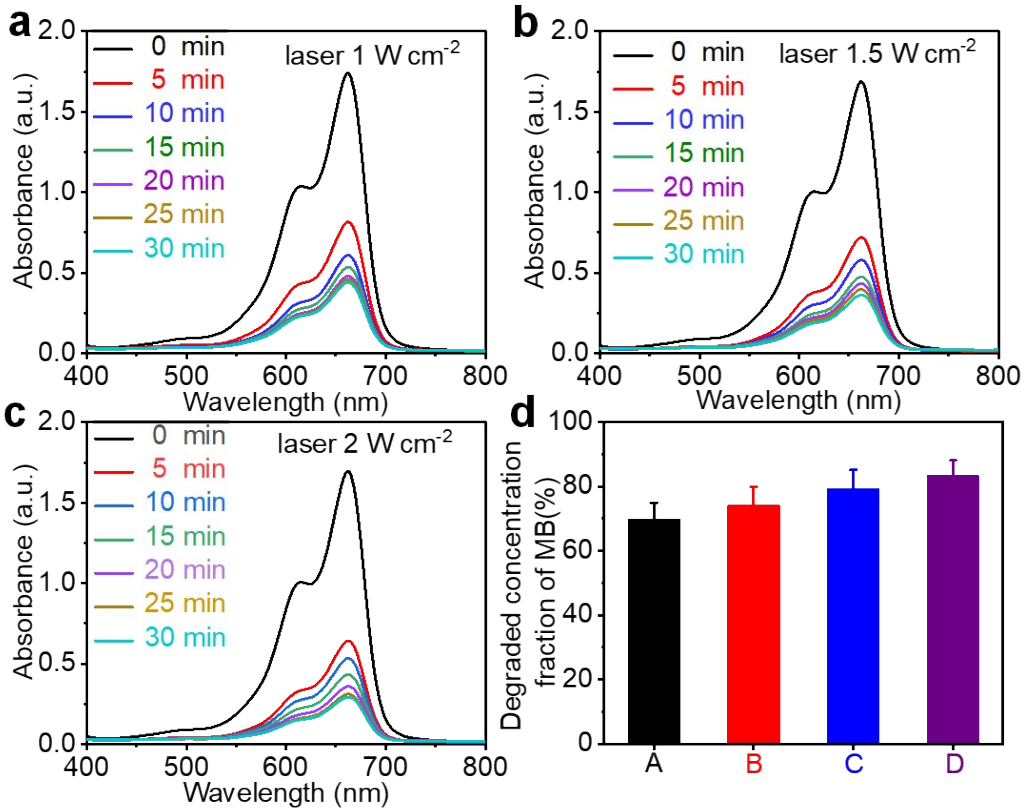

Fig. S8 The MB degradation by BM hollow nanospheres mediated Fenton-like reaction under laser irradiation with different power. (a) 1 W cm^{-2}, (b) 1.5 W cm^{-2}, (c) 2 W cm^{-2}; (d) Bar graph showing the degradation percent of MB under laser irradiation with different power: (A)without laser, (B) 1 W cm^{-2}, (C) 1.5 W cm^{-2}, (D) 2 W cm^{-2}

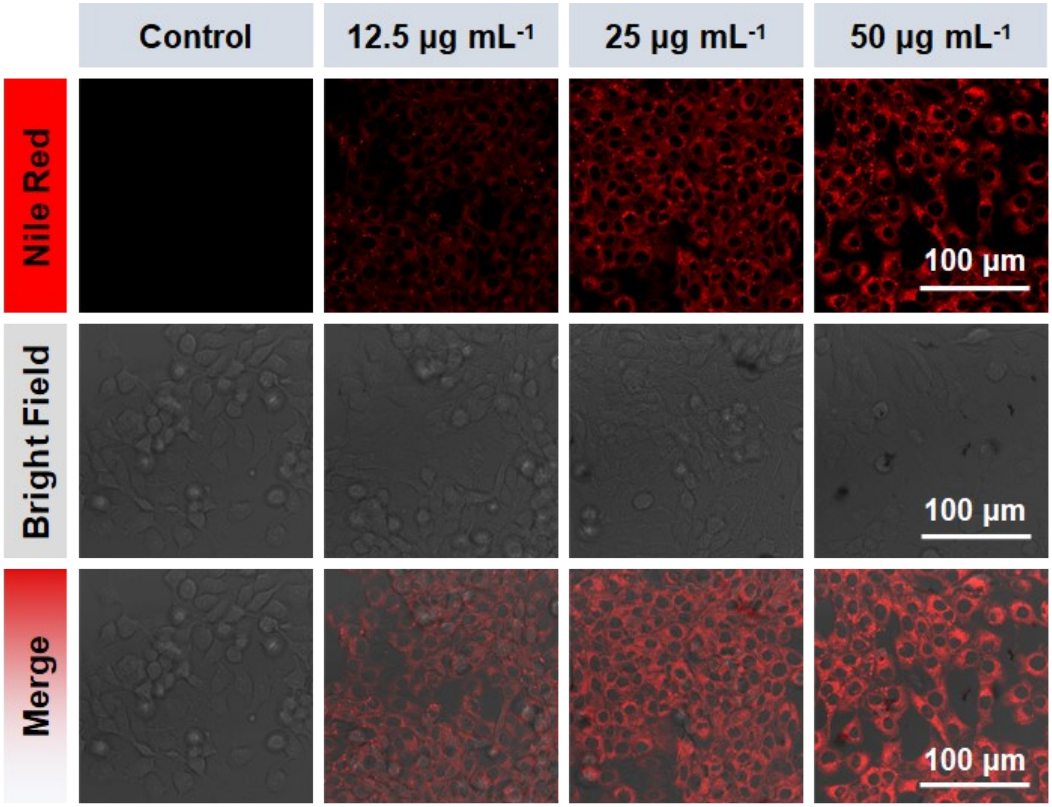

Fig. S9 CLSM images of 4T1 cells after 4 h incubation with BM at different concentration (0, 12.5, 25, 50 μg mL^{-1}). Scale bar: 100 μm.

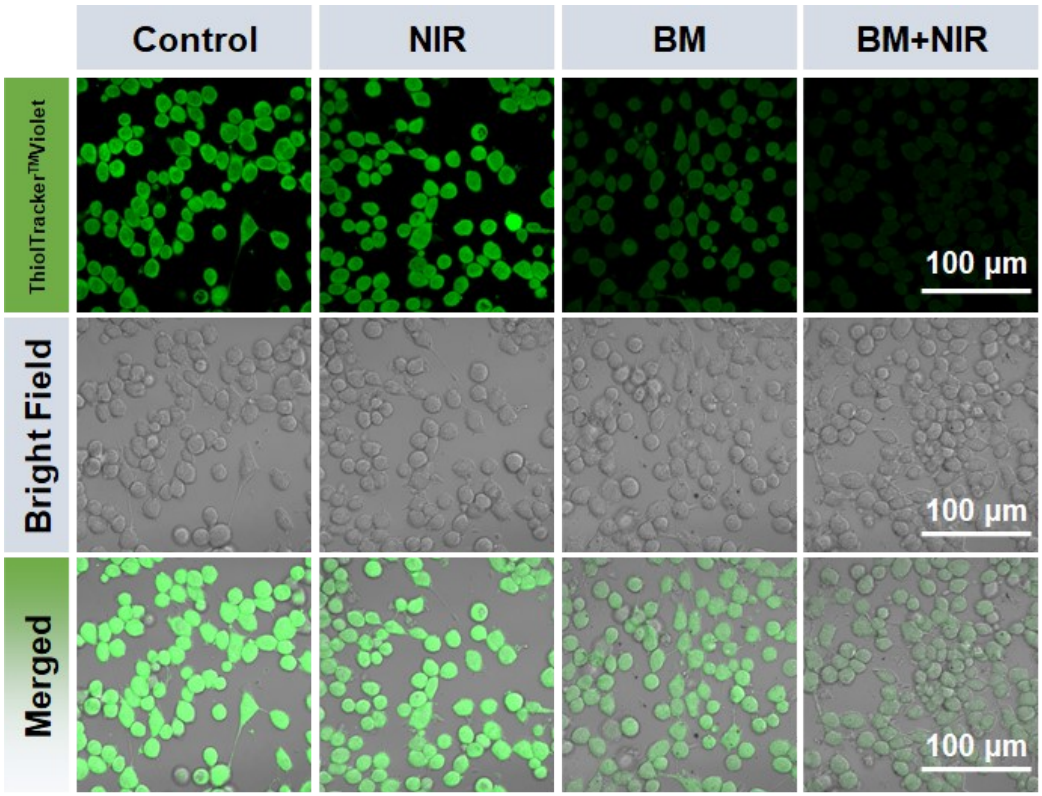

Fig. S10 CLSM images of 4T1 cells for GSH analysis stained by ThiolTracker™ Violet after different treatments. Scale bar: 100 μm.

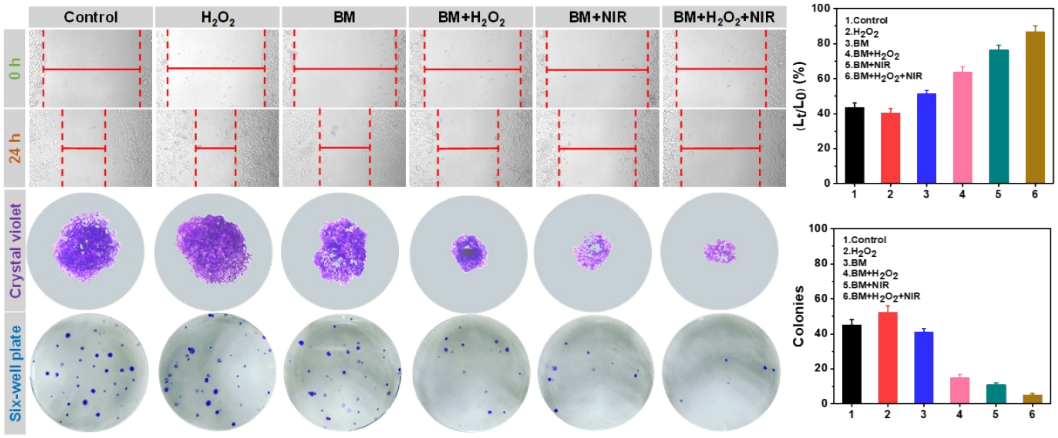

Fig.S11 Photographs and bar graphs of 4T1 cell migration and proliferation with different treatments.

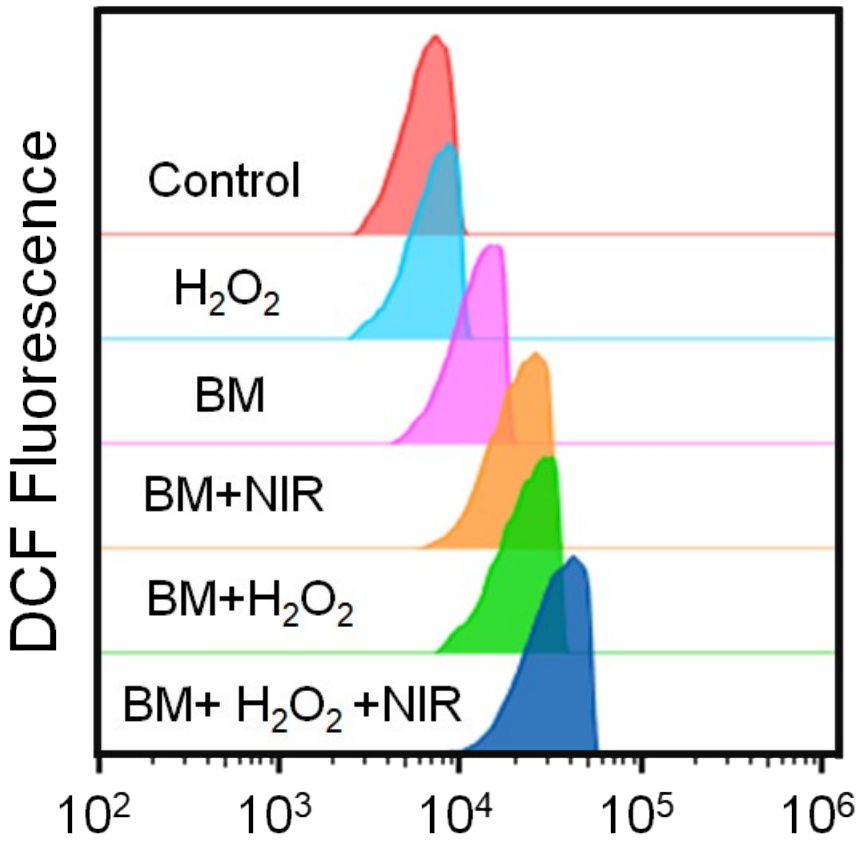

Fig.S12 Flow cytometry analysis of intracellular ROS.

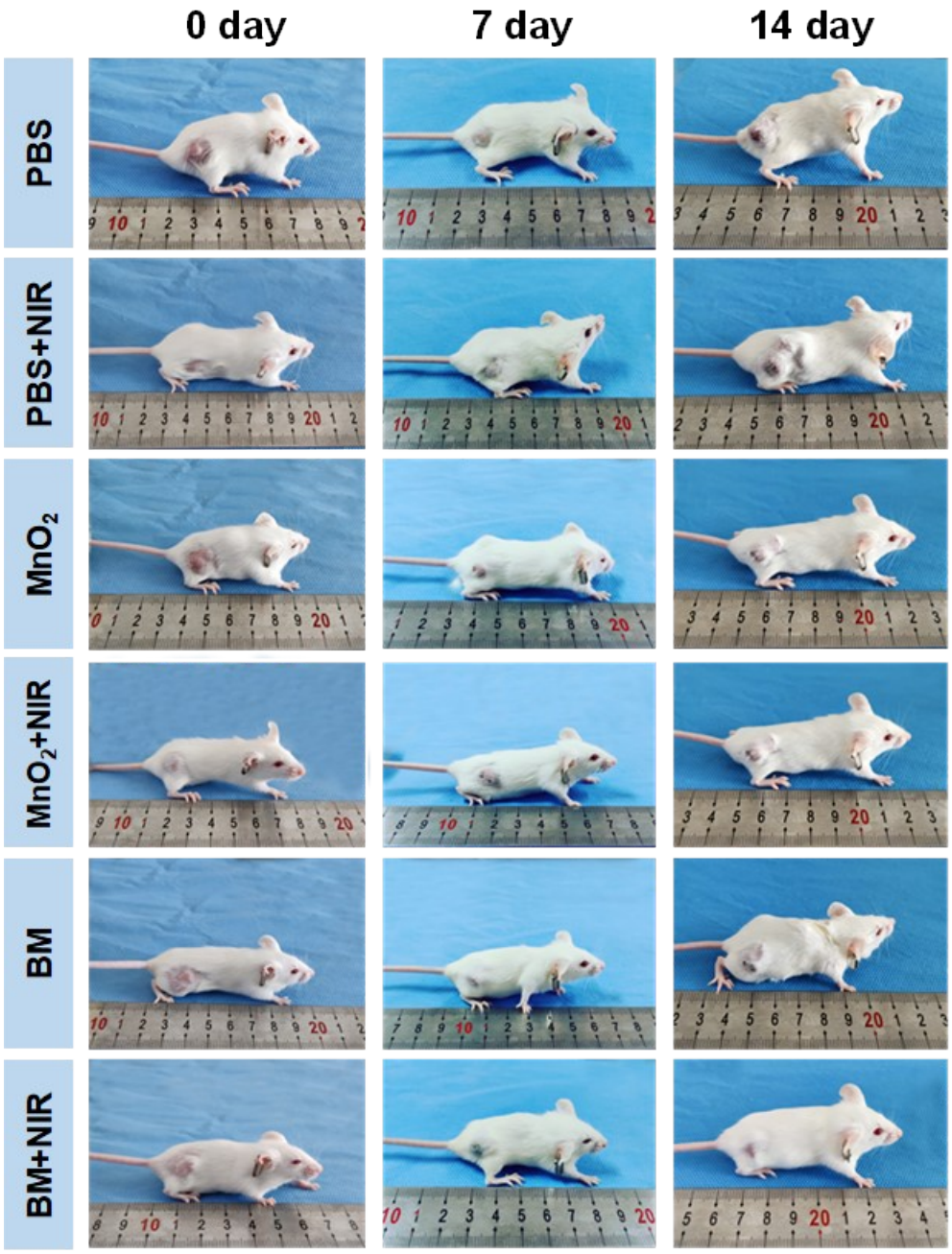

Fig. S13 Pictures of different treatment groups of mice on different days

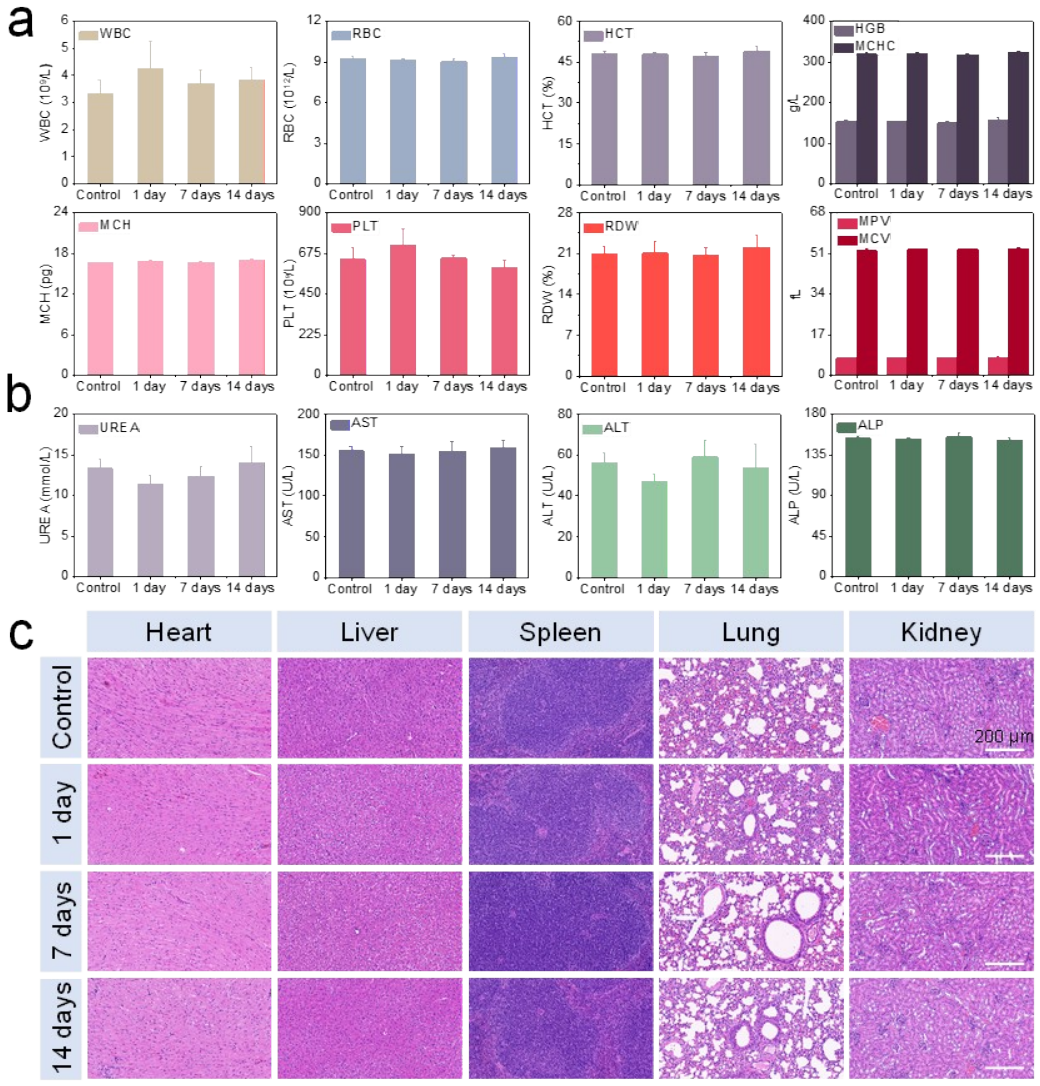

Fig. S14 In vivo toxicology assays of the BM NPs. (a) Blood panel analysis and (b) blood biochemistry test of healthy mice after intravenous injection of BM (10 mg kg-1) at different days. (c) H&E staining images of major organs (heart, liver, spleen, lung, kidney) of the mice after injection of BM NPs at different time points.

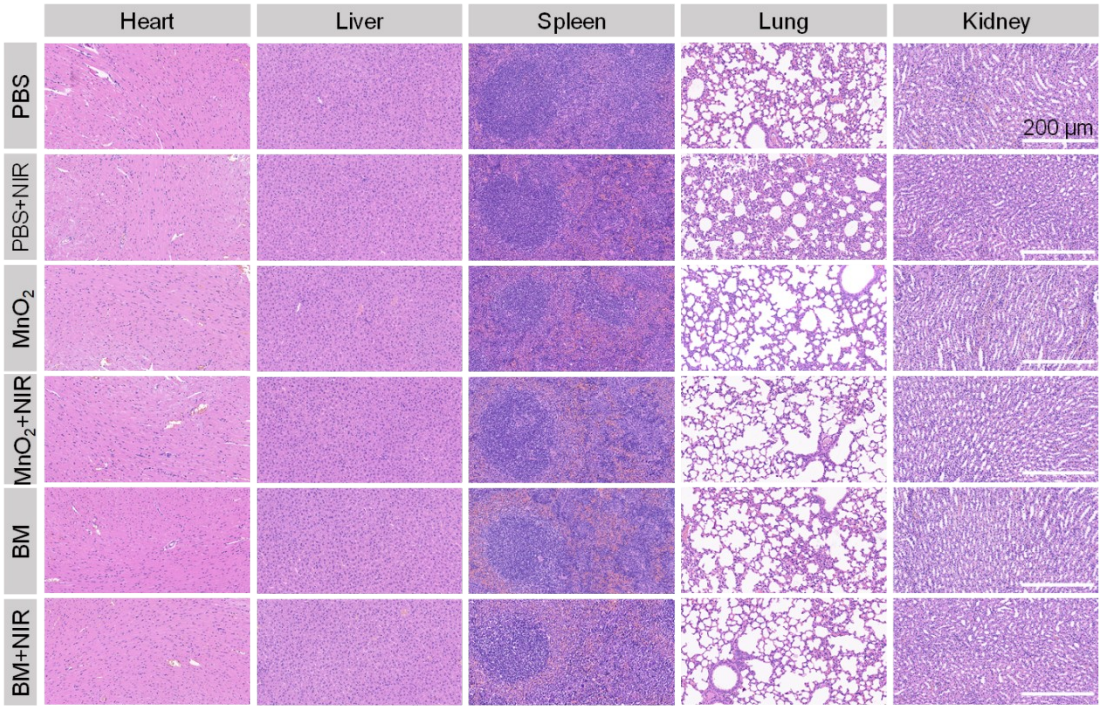

Fig. 15 In vivo toxicity evaluation of different treatment groups. H&E staining images of normal organs in each group.

Table S1. Element composition of BM hollow nanospheres after the reusability test from EDX analyses.

Element	Weight %	Automic %
Mn K	34.97	28.30
Bi L	42.48	9.04
O K	22.55	62.66
Total	100.00	100.00