Supporting Information

Manganese-Iron Layered Double Hydroxide: A Theranostic Nanoplatform with pH-Responsive MRI Contrast Enhancement and Drug Release

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EXPERIMENTAL SECTION

Chemicals and apparatus. Manganese(II) nitrate tetrahydrate ($Mn(NO_3)_2 \cdot 4H_2O$, 97.5%, Ourchem), Iron(III) nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$, AR), and Sodium hydroxide (NaOH, AR) were purchased from Sinopharm Chemical Reagent Co. Ltd (China). Methotrexate (MTX, ≥98%) was purchased from Aladdin (Shanghai, China).

Apparatus. Transmission electron microscopy (TEM) images were captured on a Tecnai G2 F20 at an accelerating voltage of 200 kV. X-ray powder diffraction (XRD) patterns were obtained on a Rigaku Ultima IV system. Atomic force microscopy (AFM) images were obtained in the tapping mode in air using a Bruker Multimode 8 atomic force microscope. The samples were prepared by dropping their dispersions on mica substrates. X-ray spectroscopy (EDS) and energy-dispersive X-ray (EDX) element mapping analyses were performed was on a JEM 2100F microscope at an accelerating voltage of 200 kV. The X-ray photoelectron spectroscopy (XPS) data were collected on a Thermo escalab 250Xi XPS spectrometer. The metal concentration of samples was determined by an Agilent 7500ce inductively coupled plasma mass spectrometry (ICP-MS). The high-performance liquid chromatography (HPLC) analyses was performed on a TriSep-2100. The dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer nano ZS instrument.

Synthesis of MnFe-LDH and MnFe-LDH-MTX. We synthesized MnFe-LDH by a simple co-precipitation strategy with hydrothermal treatment. In a typical procedure, 0.3765 g of $Mn(NO_3)_2 \cdot 4H_2O$ (1.5 mmol) and 0.202 g of $Fe(NO_3)_3 \cdot 9H_2O$ (0.5 mmol) were dispersed in 50 mL of H_2O at room temperature under vigorous stirring. Then the pH of the mixture was adjusted to about 13 by slowly dropping of NaOH solution (0.2 M, ~50 mL). After further stirring for 5 min,

the resulting suspension was transferred into a Teflon-lined stainless steel autoclave. After hydrothermal treatment at 120 °C for 3 h, the product was collected by centrifugation and washed with deionized water several times. The synthesis of MnFe-LDH-MTX is similar to that of MnFe-LDH except that the NaOH solution was mixed with 6 mg of MTX. The concentration of MTX was calculated according to the standard curve of known MTX concentrations (0.98-250 μg mL^{-1}) versus peak areas determined by HPLC. HPLC was performed under the following conditions: stationary phase, Symmetry C18 column (250 mm × 4.6 mm, 5 μm); temperature, 25 °C; elution flow rate, 1 mL/min; mobile phase, methanol/PBS (pH 6.0) in 40/60 ratio (v/v); detection wavelength, 306 nm.

In vitro MRI. Both relaxivity and phantom imaging studies were performed on a 0.5 T NMR120-Analyst NMR system (Niumag Corporation, Shanghai, China). The longitudinal relaxation times (T_1) were measured using an inversion recovery (IR) sequence. The longitudinal relaxivity (r_1) was determined from the slope of the plot of $1/T_1$ against the metal concentration ([Mn + Fe] in mM). T_1-weighted phantom images were acquired using a 2D multi-slice spin-echo (MSE) sequence with the following parameters: TR/TE = 100/2 ms, thickness = 1 mm, 512 × 512 matrices, slices = 1, and NS = 4. For cell imaging, 1×10^7 HeLa cells were incubated with MnFe-LDH (50 μg/mL) at 37 °C for 1 h, 2 h, 4 h, and 8 h, respectively. Then the cells were harvested and washed with PBS buffer three times to remove the free MnFe-LDH. Finally, the cells were concentrated at the button of small tubes by centrifugation and then used for T_1-weighted imaging.

Cytotoxicity assay. The cytotoxicity of the MnFe-LDH and MnFe-LDH-MTX was tested by 3-(4, 5-dimethylthiazol-2-y1)-2, 5-diphenyltetrazolium bromide (MTT) method. HeLa or Hep G2 cells were firstly seeded into a 96-well plate with a density of 1×10^4 cells/well in RPMI-1640, and

incubated in the atmosphere of 5% CO_2 at 37 °C for 24 h. The cells were then incubated with MnFe-LDH (or MnFe-LDH-MTX) at various concentrations for another 24 h (or 48 h). Subsequently, the culture medium was removed, each well was added with 100 μL new culture medium containing MTT (0.5 mg mL^{-1}) and the plate was incubated for 4 h at 37 °C. Then the medium was discarded and each well was added 200 μL DMSO. The OD_{490} value (Abs.) of each well was measured by a SH-1000 Lab microplate reader immediately. Cell viability was calculated from OD_{490} value of experimental group by subtracting that of blank group.

In vivo MRI. Animal experiments were executed according to the protocol approved by Institutional Animal Care and Use Committee of Fuzhou University. To induce a solid tumor, murine sarcoma S180 cells (5×10^6 in 100 μL PBS) were injected subcutaneously into the right thigh areas of the male BALB/c nude mice. The mice were used when the tumor grew to ~6 mm in diameter. T_1-weighted images of the mice were first collected on a 3 T MRI scanner (GE Discovery MR750) without injection. The mice were then intratumorally injected with 50 μL of MnFe-LDH-MTX (dosage of 5.0 mg [Mn + Fe] per kg of mouse body weight). The same slices were further acquired at different time points (1 h, 2 h, 4 h, 8 h and 24 h) after the injection. All the images were obtained using an FSE sequence under the following parameters: TR/TE = 513/14.5 ms, FOV = 8 × 8 cm, 320 × 224 matrices, thickness = 1 mm. To quantify the contrast enhancement, the signal-to-noise ratio (SNR) was measured by finely analyzing regions of interest (ROIs) of the images, and the contrast enhancement was defined as the increase of SNR after the injection, $\Delta SNR = (SNR_{post} - SNR_{pre})/SNR_{pre}$.

In vivo therapy. To assess the therapeutic performance of MnFe-LDH-MTX on solid tumor, the S180 tumor-bearing nude mice with a tumor diameter of ~6 mm were divided into three

groups (five mice per group). The mice of the treatment group were intratumorally injected with MnFe-LDH-MTX (5.0 mg [Mn + Fe] per kg of mouse body weight). Other two groups were used as controls: the mice of blank group were without any treatment; the mice of PBS group were intratumorally injected with 150 μL of PBS only. The sizes of tumors were measured by a caliper and the tumor volume was calculated according to equation: tumor volume = (tumor length) × (tumor width)^2/2. Relative tumor volumes were calculated as V/V_0 (V was the tumor volume calculated after treatment, while V_0 was the initiated tumor volume before treatment).

Supporting Figures

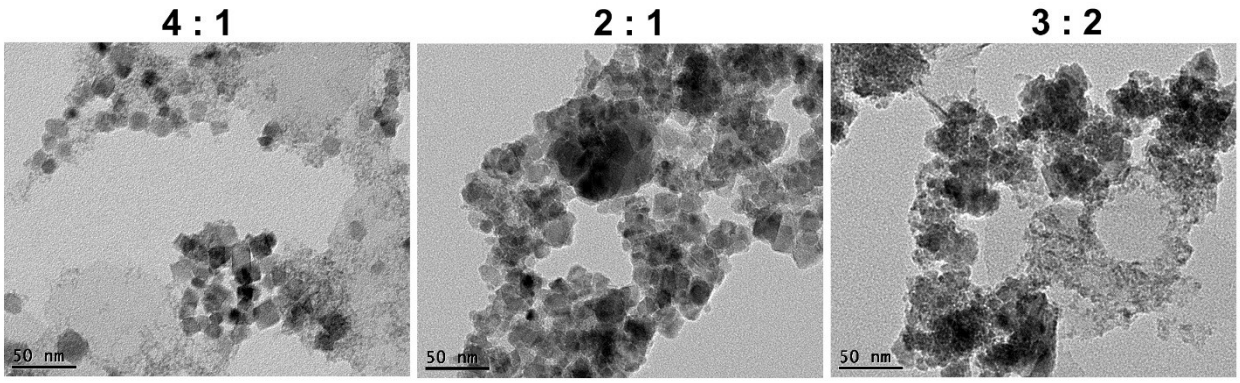

Figure S1. TEM images of MnFe-LDH synthesized from different Mn^{2+} : Fe^{3+} molar ratios of precursors.

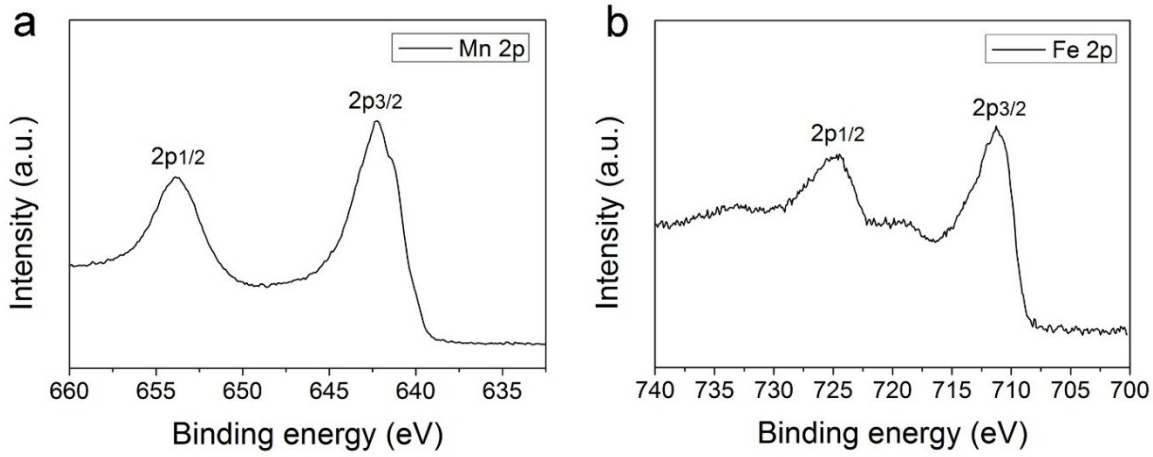

Figure S2. (a) Mn 2p and (b) Fe 2p X-ray photoelectron spectroscopy (XPS) spectrum of MnFe-LDH.

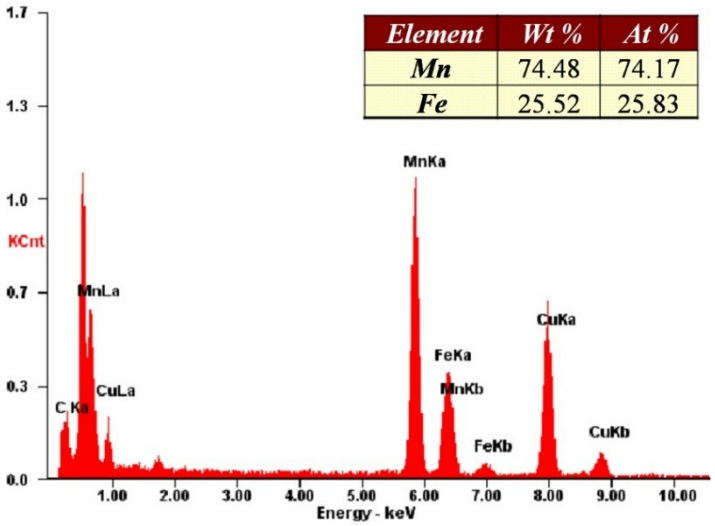

Figure S3. X-ray spectroscopy (EDS) analysis of MnFe-LDH.

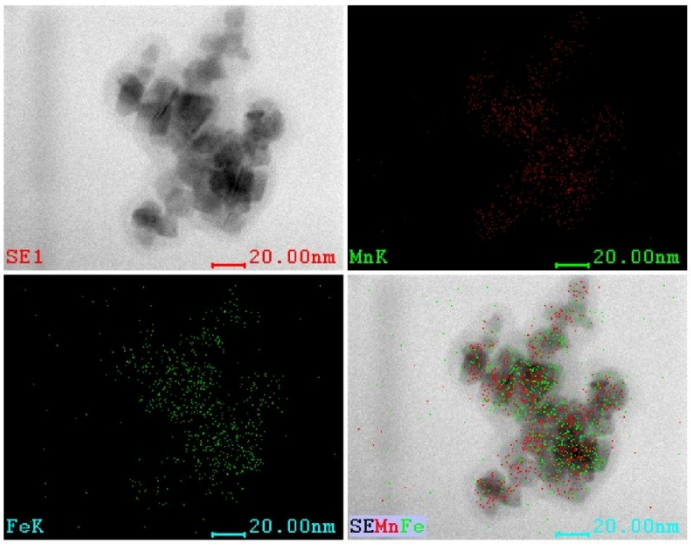

Figure S4. Energy-dispersive X-ray (EDX) element mapping analysis of MnFe-LDH.

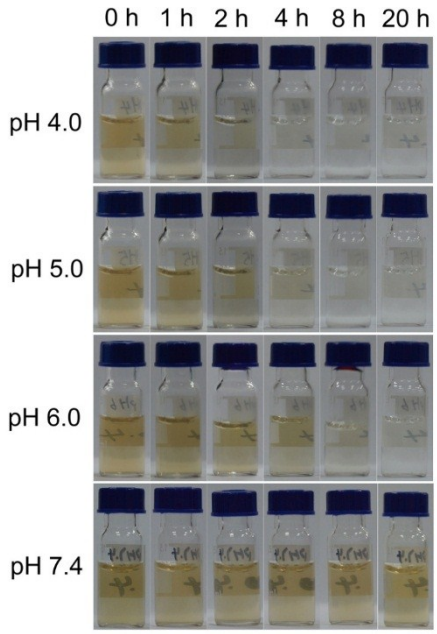

Figure S5. Photographs collected at different time points of MnFe-LDH in buffer solutions of various pH (0.4 mM [Mn + Fe], pH 4.0, pH 5.0, pH 6.0: citrate buffers; pH 7.4: phosphate buffer).

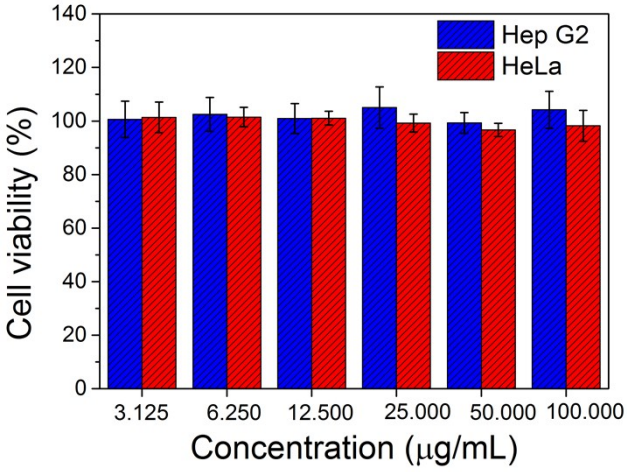

Figure S6. Cell viability of Hep G2 and HeLa cells after incubated with MnFe-LDH with different metal ion (Fe + Mn) concentrations at 37 °C for 24 h (n = 3).

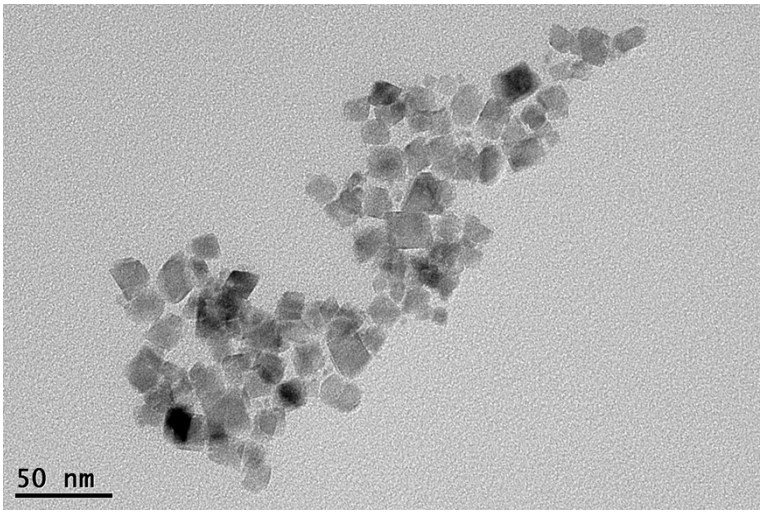

Figure S7. TEM image of MnFe-LDH-MTX.

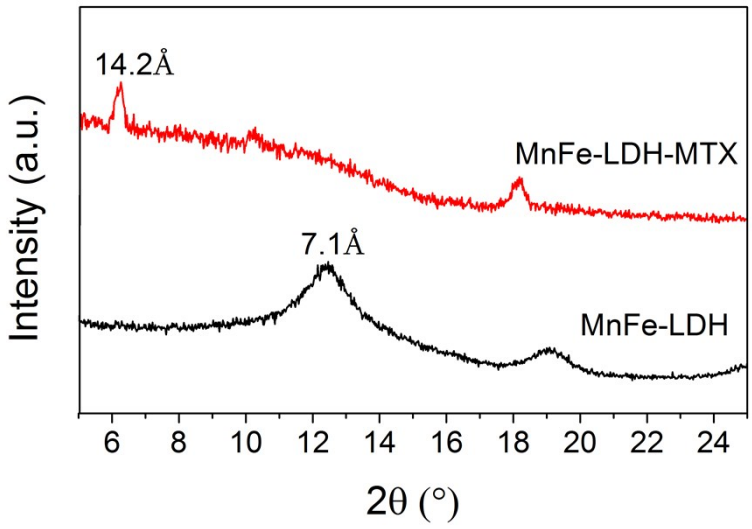

Figure S8. XRD patterns of MnFe-LDH and MnFe-LDH-MTX.

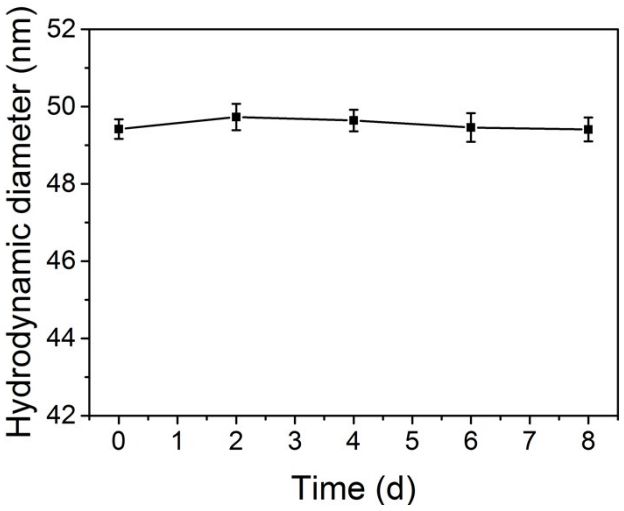

Figure S9. Hydrodynamic diameters of MnFe-LDH-MTX measured by DLS within 8 days (n = 3).

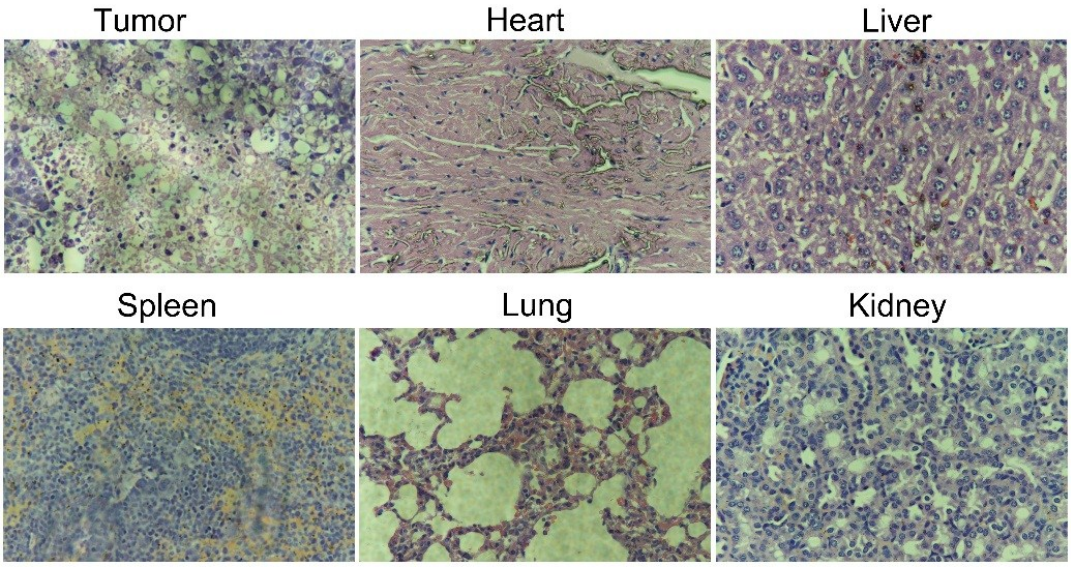

Figure S10. Hematoxylin and eosin (H&E) stained images of tumor and major organs of mouse 3 days after the treatment.