Supporting Information

A Fe (III) intercalated clay nanoplatform for combined chemo/chemodynamic therapy

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Experimental Section

Chemicals: Montmorillonite, $Fe(NO_3)_3 \cdot 9H_2O$, and DOX·HCl were obtained from Aladdin Reagent (Shanghai, China). Phosphate buffered saline (PBS), 2',7'-dichlorofluorescein diacetate (DCFH-DA), dimethyl sulfoxide (DMSO), and calcein acetoxymethyl ester (CalceinAM) were acquired from Sigma-Aldrich and Fisher (USA). 0.25% Trypsin-EDTA, fetal bovine serum (FBS), and Roswell Park Memorial Institute (RPMI) 1640 were obtained from Thermo Fisher Scientific (USA). Penicillin/streptomycin was purchased from ChemeGen Bio-tech Pioneer (Shanghai, China). MTT, 5,5',6,6'-tetrachloro-1,1'-3,3'-tetraethyl-benzimidazolylcarbocyanine iodide (JC-1), Lysotracker Green, and Hoechst33342 were acquired from Beyotime Biotechnology Co., Ltd (Shanghai, China). Ultrapure water was got from the Millipore water purification system.

Preparation of Fe-MMT. MMT (2 g) was added into 150 mL ultrapure water under vigorous stirring. 200 mg $Fe(NO_3)_3 \cdot 9H_2O$ was further added into the aqueous solution. Then, the mixture was heated at 85 °C for 2 h. After centrifugation, precipitates were collected and washed with water for several times. Fe-MMT were obtained after vacuum freeze-drying.

Preparation of Fe-MMT/DOX. Fe-MMT (30 mg) was dispersed in 10 mL of phosphoric acid buffer (pH = 7.4) and then 30 mg DOX·HCl was added. The mixture was shake at 37 °C for 24 h. Fe-MMT/DOX was collected through centrifugation and further washed with water until supernatant colorless.

Fe ions release study. 2mg of Fe-MMT were dispersed in 1 mL PBS buffer solution phosphate buffer at different pH (pH = 5.0, 6.5, or 7.4) and moved into dialysis tubes (MWCO: 3500 Da). Subsequently, each dialysis tube was immersed into 9 mL PBS buffer solution and incubated at 37 °C. The concentration of Fe ions in solution was recorded using ICP-OES at given time intervals.

Detection of reactive oxygen species. Terephthalic acid (TA) was used as an indicator to detect the ability of Fe-MMT to produce ROS. 20 μL of TA (1 mg/mL) were applied to the reaction system. 10 μL of H2O2 (30% w/w) was added as substrates. DI water was used to fill volume of 1 mL. Upon capturing •OH, negligibly fluorescent TA would generate 2-hydroxy terephthalic acid with unique fluorescence around 435 nm. After reaction, the pernatant was added onto an enzyme plate and the fluorescence intensity at 435 nm was recorded.

Cell culture. Mouse breast carcinoma cells (4T1) were incubated in DMEM medium containing 10% FBS and 1% antibiotics (penicillin-streptomycin, 10000 U mL^{-1}) at 37 °C in a humidified atmosphere containing 5% CO_2.

Cell uptake. 4T1 cells were seeded onto 6-well plates in 1 mL DMEM medium and allowed to grow for 24 h. The medium was replaced with DOX or Fe-MMT/DOX containing DMEM at the same DOX concentration. After incubation for 3 h at 37 °C, the cells were washed with PBS twice and stained by Lysotracker Green and Hoechst 33342. The stained cells were washed with PBS three times. Finally, each sample was quickly observed by a fluorescence microscope.

Intracellular ROS detection. Intracellular ROS generation was detected via CLSM after stained with DCFH-DA. 4T1 cells (50 000/well) were seeded in six-well plates and incubated with RPMI 1640 medium for 24 h. Then, different materials were added and re-incubated for another 10 h. The cells were further stained with DCFH-DA (10 μM) after washed with PBS and imaged via CLSM (Ex = 488 nm; Em = 525 nm).

In vitro cytotoxicity study. Cell viability was performed by using MTT assay. 4T1 cells (6 000/well) were seeded in the 96-well plates for 24 h. Subsequently, the cells were co-oncubated with MMT, Fe-MMT, or Fe-MMT/DOX with different concentrations. After co-incubation for 24 h, 20 μL of MTT (5 mg/mL) was added and incubated for another 4 h. Then the medium was replaced with 150 μL of DMSO in each well. Then, the absorbance at 570 nm was detected by a microplate reader . The relative cell viability was calculated according to the following equation: cell viability = $(OD570_{(samples)}-OD570_{(blank)})/(OD570_{(control)}-OD570_{(blank)})\times100\%$, where $OD570_{(samples)}$ was obtained in the presence of nanoparticles and $OD570_{(control)}$ was obtained without treatment, $OD570_{(blank)}$ was blank.

Animal model. All animal experiments were performed in accordance with the National Institute of Health Guidelines under the protocols, approved by the Ethics Committee of Hubei Provincial Center for Disease Control and Prevention. BALB/c mice (6 weeks old, female) were purchased from Hunan Silaikejingda Experimental Animal Co. Ltd (Changsha, China). The tumor model was established by subcutaneously injecting 4T1-luc cells (1×10^7 cells per mouse) into the BALB/c mice.

In Vivo Antitumor Assay. 4T1-luc tumor-bearing mice were separated into 5 groups, followed by PBS, MMT, Fe-MMT, Fe-MMT/DOX, and MMT/DOX administration. After 9 days of 4T1-luc cell injection into Balb/c mice, PBS, MMT (200 μL, 1mg/mL), Fe-MMT, Fe-MMT/DOX, and MMT/DOX were intravenously

administered into mice. During the therapy process, weights and tumor sizes of mice were monitored. 20 days later, the mice were sacrificed and the tumor tissues were obtained for further analyses.

Statistical analysis. All data were presented as mean ± standard deviation (s.d).

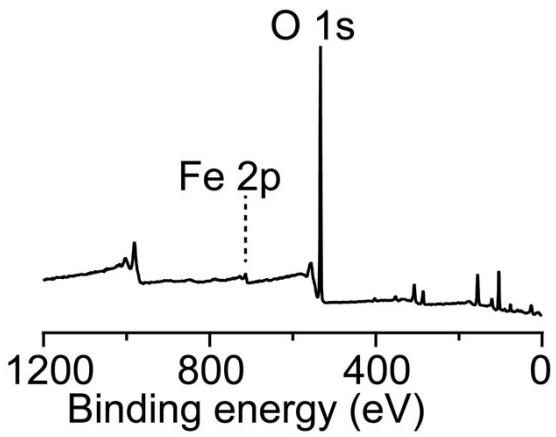

Figure S1. XPS spectrum of Fe-MMT.

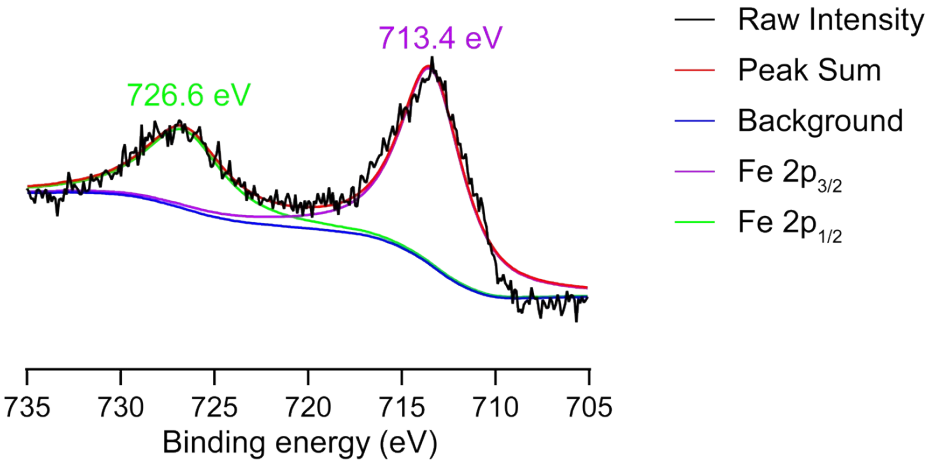

Figure S2. High-resolution Fe 2p spectrum of Fe-MMT.

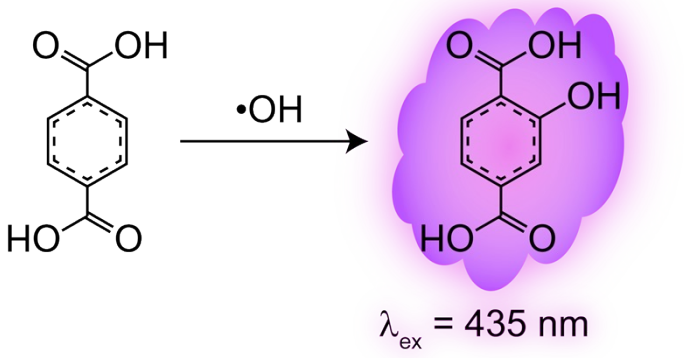

Figure S3. The illustration of the detection of •OH by TA.

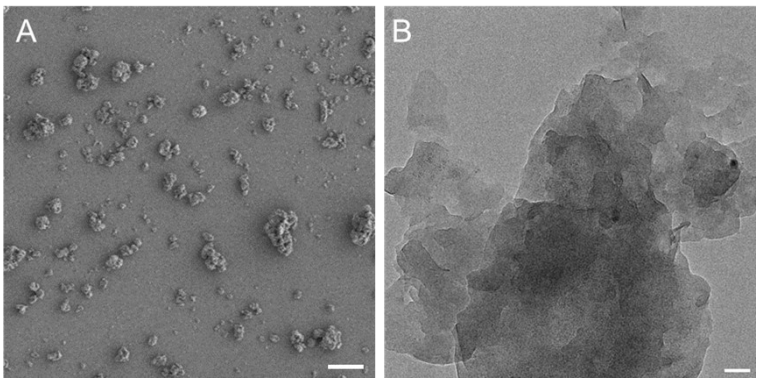

Figure S4. SEM (A) and TEM(B) images of Fe-MMT/DOX.

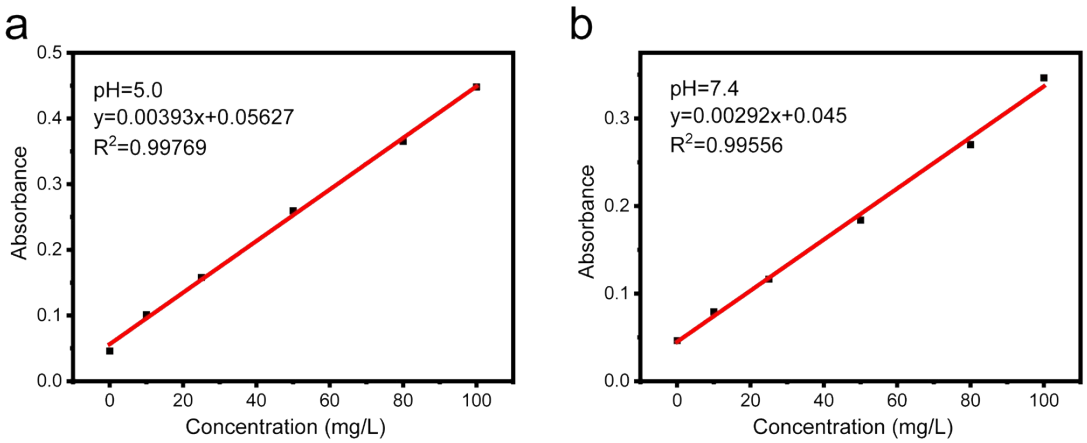

Figure S5. The standard curve of DOX.

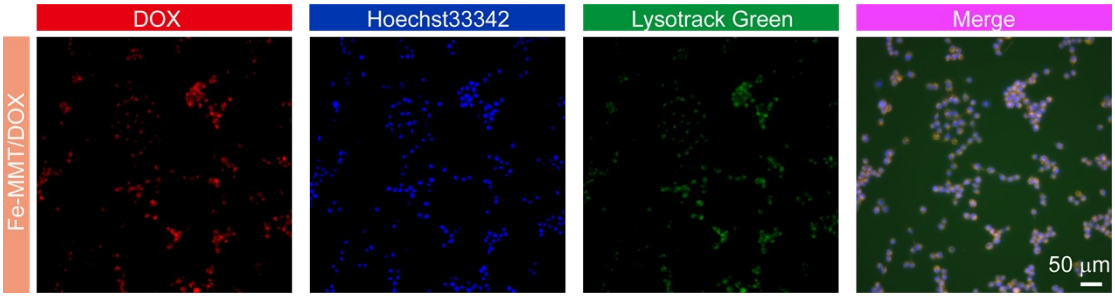

Figure S6. CLSM images of 4T1 cells after treatment with Fe-MMT/DOX stained with Hoechst 33342 (blue) and Lysotracker Green (green).

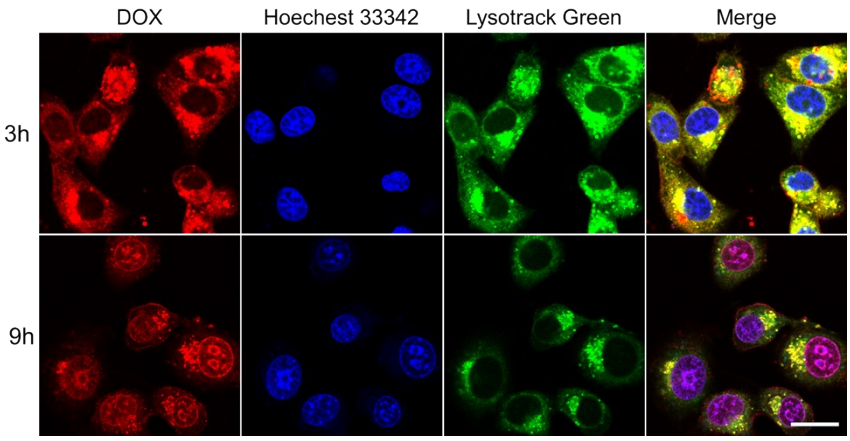

Figure S7. CLSM images of 4T1 cells after treatment with Fe-MMT/DOX for 3 h and 9 h. Scale bar = 18.4 μm**.**

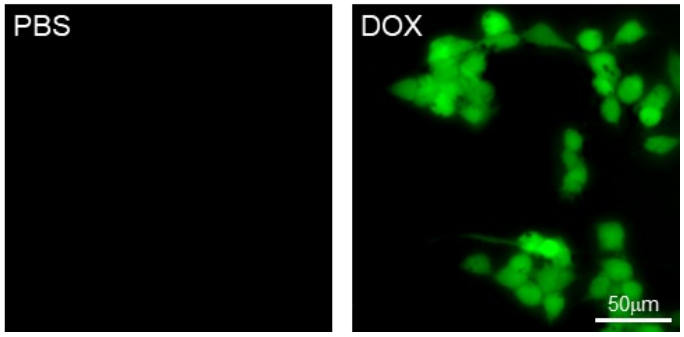

Figure S8. Intracellular ROS levels of 4T1 cells after different treatments.

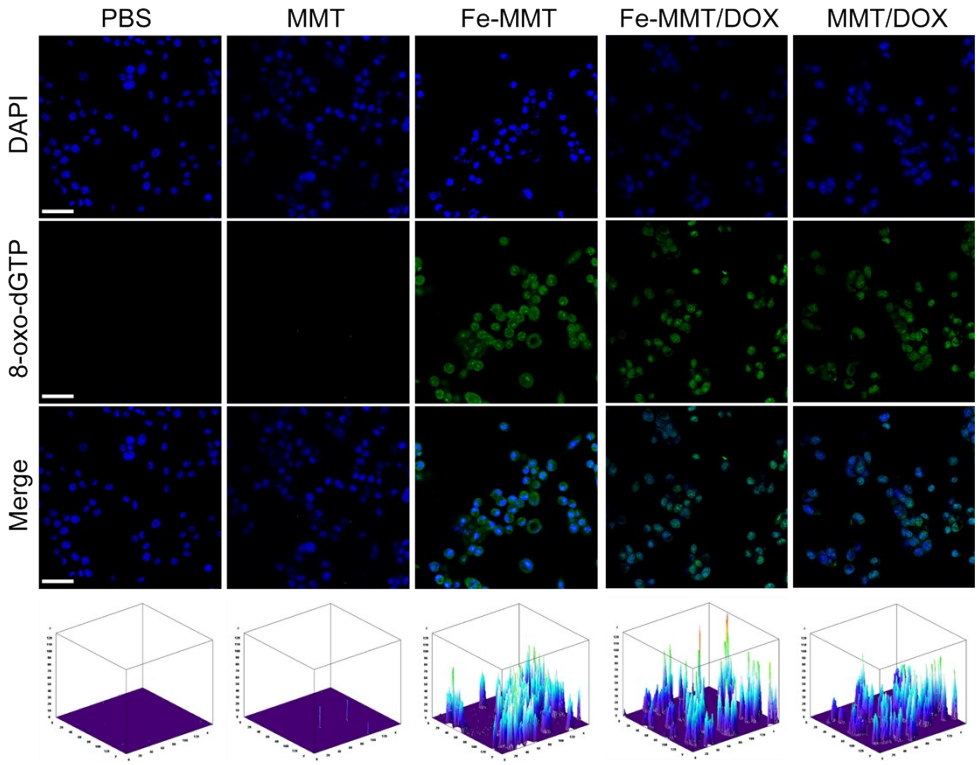

Figure S9. Immunofluorescence images of 8-oxo-dGTP and their corresponding surface plot images. Scale bar = 50 μm**.**

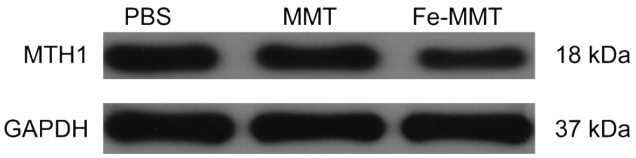

Figure S10. Western blotting analysis of MTH1 protein expression in 4T1 cells after different treatments.

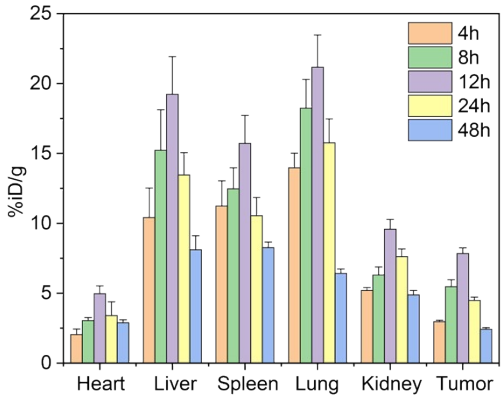

Figure S11. *In vivo* distribution of tumor-bearing mice after injection of Fe-MMT/DOX intravenously (n = 3).

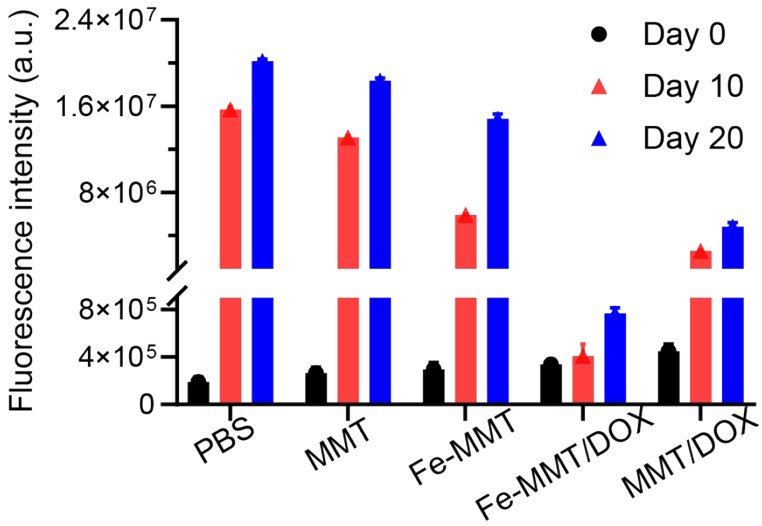

Figure S12. Quantification of the bioluminescenceintensityin orthotopic 4T1-luc tumor-bearing mice after different treatments during 20days (n = 6).

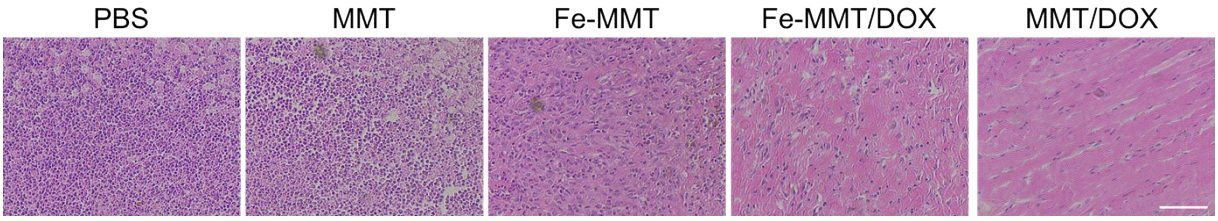

Figure S13. H&E staining images of tumor tissues after different treatments. Scale bar: 200 μm.

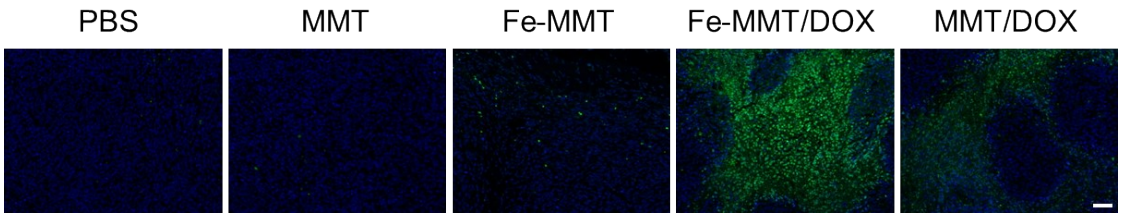

Figure S14. Immunofluorescence staining of TUNEL. Blue: cell nuclei; green: TUNEL.

Scale bar: 50 μm.

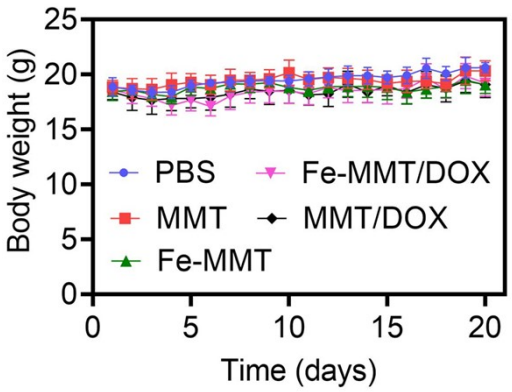

Figure S15. Body weight of Body weight curves of mice after different treatments during therapy (n = 6).

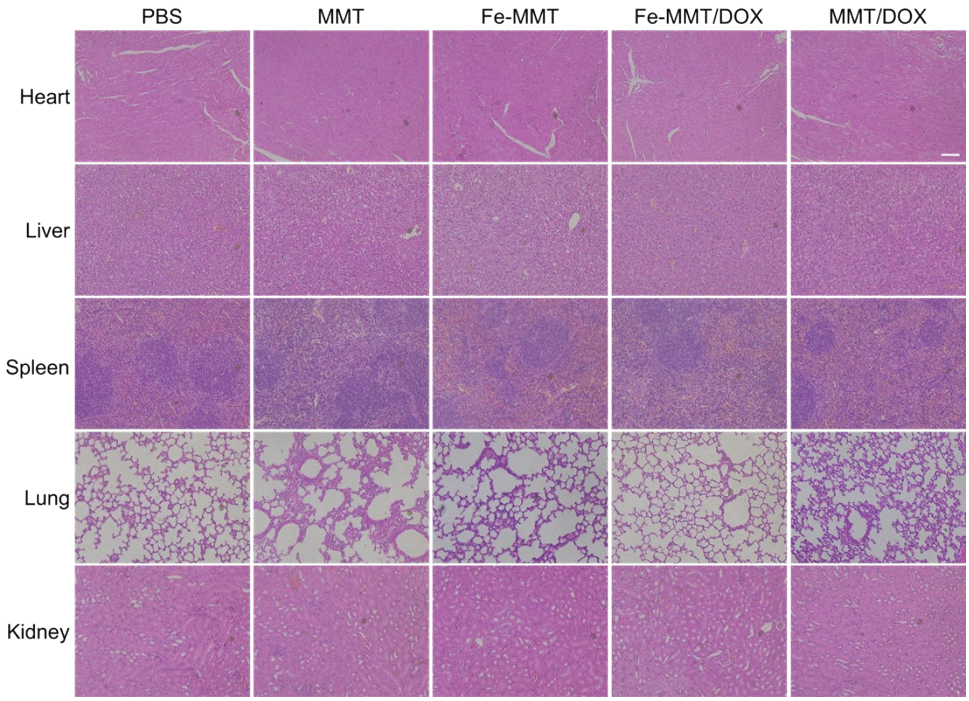

Figure S16. H&E staining of the main organs (scale bar, 200 μm).