Supporting Information

A multimodal therapeutic nano-prodrug as oxidative stress amplifier induces apoptosis and ferroptosis for cancer therapy

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Experimental Section

Materials. 1,3,5-trimethylbenzene (TMB), dopamine hydrochloride, ammonia hydroxide (NH_4OH, 28-30 wt%), Tris-HCl buffer (pH 8.5), methylene blue (MB), glutathione (GSH), dihydroartemisinin (DHA), hydrogen peroxide (H_2O_2, 30 wt%), and Rhodamine B (RhB) were purchased from Aladdin Biochemical Technology Co. Ltd. (Shanghai, China). Pluronic F127, Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), tannic acid (TA), hyaluronate (HA), 1,3-Diphenylisobenzofuran (DPBF), and indocyanine green (ICG) were acquired from Sigma-Aldrich Trading Co. Ltd. (Shanghai, China). RPMI-1640 medium, fetal bovine serum (FBS), phosphate-buffered saline (PBS), JC-1 Apoptosis Detection Kit and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Jiangsu KeyGen Biotech. Co. Ltd. (Nanjing, China). Calcein-AM/propidium iodide (PI) stain kit, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), Annexin V-FITC/PI apoptosis detection kit, GSH assay kit and Hoechst 33342 Staining Solution for Live Cells were purchased from Beyotime Biotechnology (Nanjing, China). Malondialdehyde (MDA) Colorimetric Assay Kit was purchased from Elabscience Biotech. Co. Ltd. Iron Assay Kit was obtained from Beijing Leagene Biotech. Co. Ltd. All animal experiments were conducted in accordance with the protocol approved by the Laboratory Animal Ethics Committee of Southeast University.

Measurement of DHA Loading Rate. All supernatants after centrifugation were collected for measuring the DHA loading rate. DHA can be converted to a UV-absorbing substance through hydrolysis by 0.2% NaOH solution at 60℃ for 30 min, and can be detected by the UV absorption peak at 236 nm. The drug loading content (LC) was calculated through the following formula:

$$LC(\%) = \frac{W_{Feed} - W_S}{W_{MPDA} + W_{Feed} - W_S} \times 100\%$$

Where W_{Feed} is the total amount of fed DHA, W_S is the DHA mass in the collected supernatant and W_{MPDA} is the mass of MPDA added in the initial loading process.

In addition, LC was also investigated by TGA analysis at a heating rate of 10 °C min^{-1} in a nitrogen flow from 100 to 800°C.

In vitro GSH Depletion. Various concentrations of MDTF (25, 50, 100, 150 and 200 μg mL^{-1}) were dispersed in PBS solution (pH 5.5), followed by mixing with GSH solution (1 mM) and co-incubated for 2 h at 37°C. The supernatants were treated with 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) solution and the GSH levels were measured by UV-vis spectroscopy.

Cell Culture. Murine breast cancer 4T1 cell line and human normal liver cell line (LO2) were obtained from the Cell Bank of the Chinese Academy of Science (Shanghai, China). The 4T1 and LO2 cells were cultured in RPMI-1640 medium, containing 10% FBS and 1% penicillin/streptomycin at 37°C under 5% of CO_2.

Intracellular GSH Depletion. 4T1 cells were seeded into 6-well plates at a density of 1×10^5 cells per well for 24 h. Then the cells were treated with PBS, DHA, MDH + L, MTFH, MDTFH, and MDTFH + L for 4 h, respectively. After that, the contents of intracellular GSH were determined by the GSH assay kit.

Animals and Tumor Model. Female BALB/c nude mice (4-5 weeks) were purchased from Jiangsu KeyGen Biotech. Co. Ltd (Nanjing, China). To establish the tumor model, 5×10^6 4T1 cells in saline were injected subcutaneously into the fourth pair of mammary glands on the right. The mice were used for further experiments when the tumor volume reached 100 mm^3.

Supplementary Figures

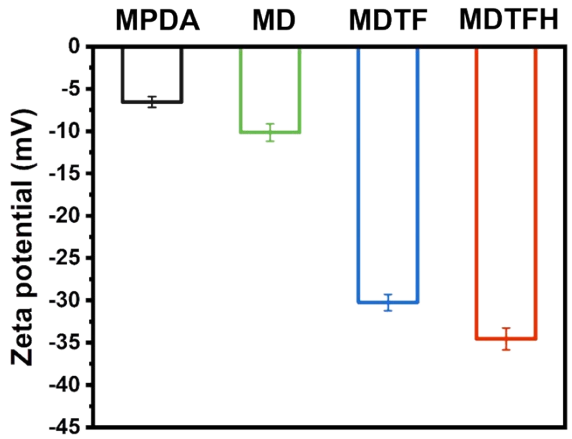

Figure S1. The zeta potentials of MPDA, MD, MDTF and MDTFH.

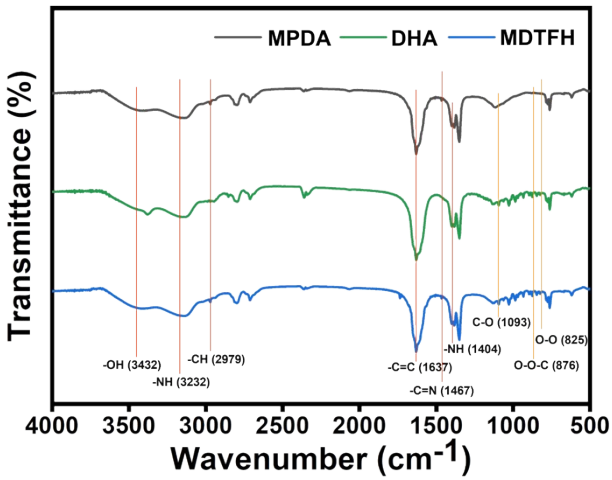

Figure S2. FT-IR spectra of MPDA, DHA and MDTFH.

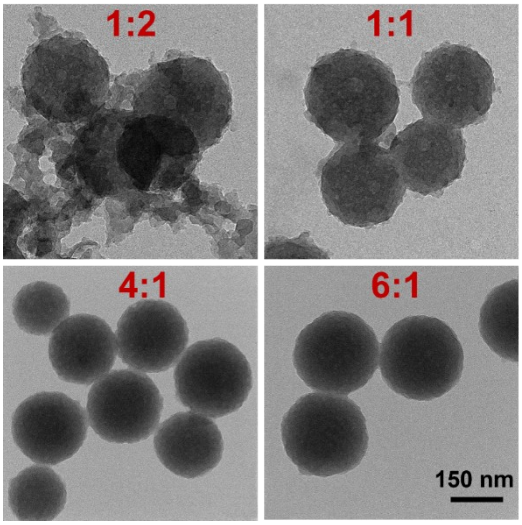

Figure S3. TEM images of MDTF prepared at different mass ratios of Fe to TA.

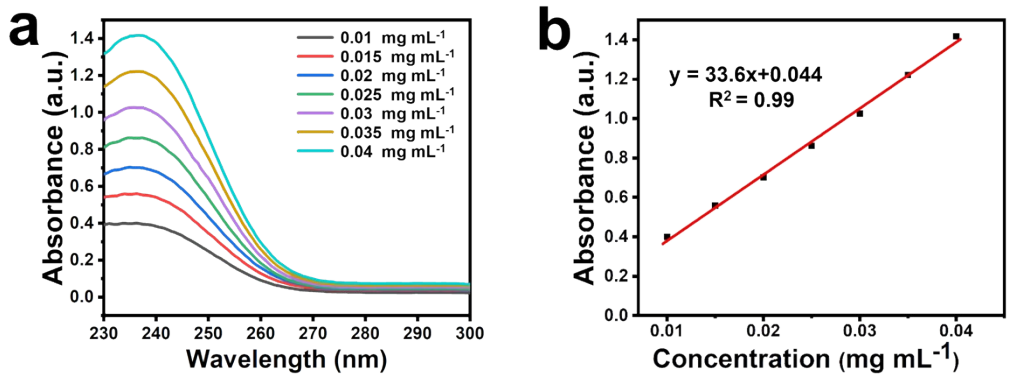

Figure S4. (a) UV-vis absorption curves of DHA solutions with different concentrations. (b) The standard curve for DHA absorbance values at 236 nm.

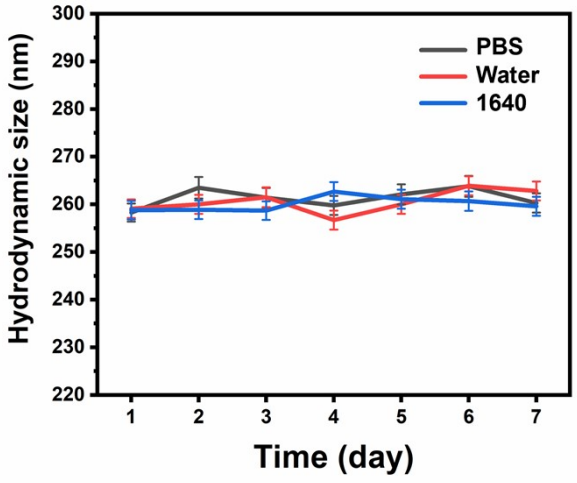

Figure S5. The hydrodynamic size changes of MDTFH in different simulated media for 7 days.

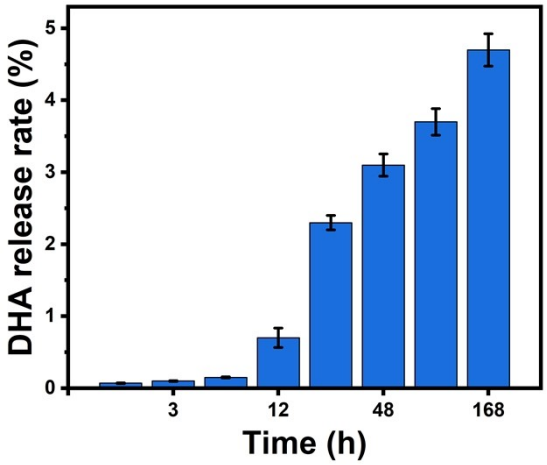

Figure S6. The release rate of DHA from MDTFH stored in PBS for 7 days

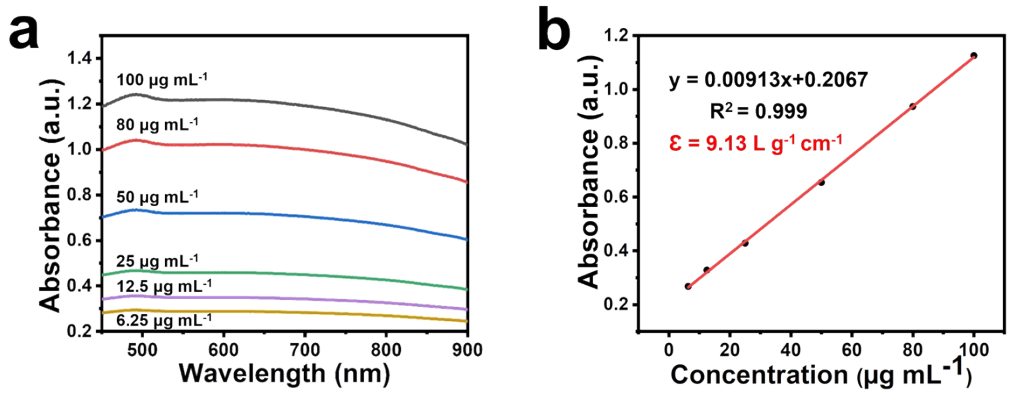

Figure S7. (a) UV-vis absorption curves of MDTFH solutions with various concentrations. (b) The mass extinction coefficient of MDTFH at 808 nm.

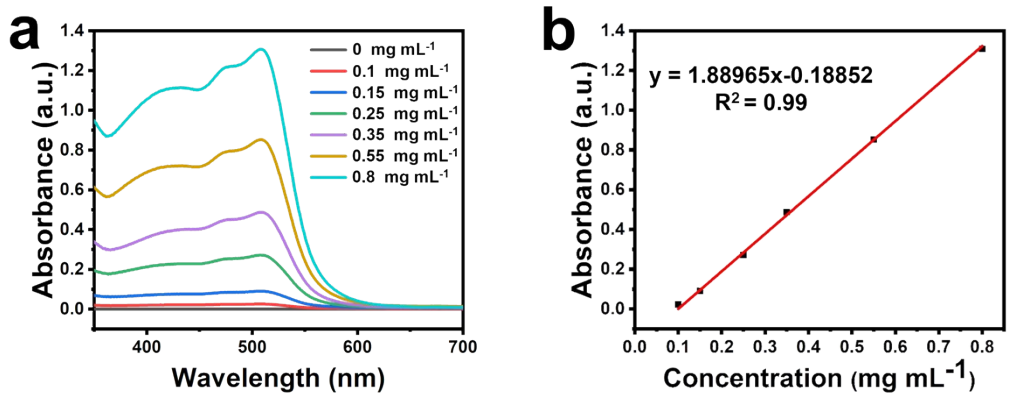

Figure S8. (a) UV-vis absorption curves of Fe (III) solutions at different concentrations. (b) The standard curve for absorbance values at 512 nm

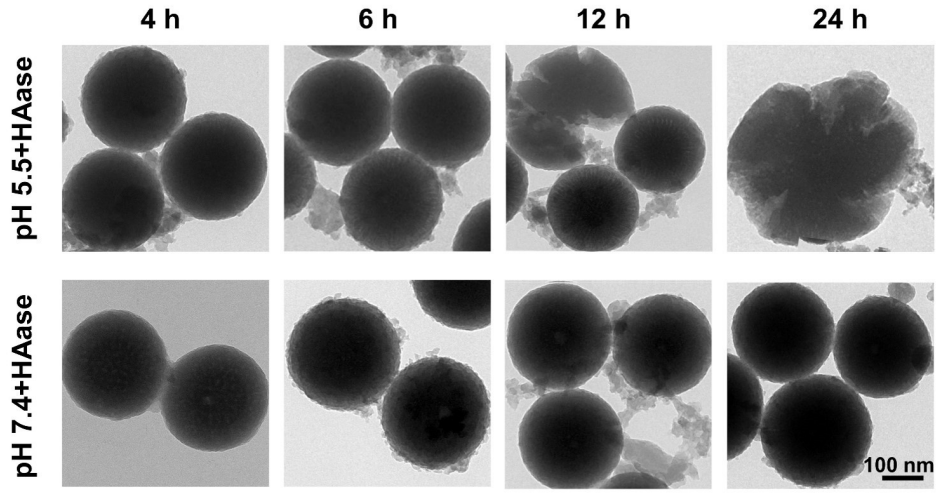

Figure S9. TEM images of MDTFH after incubation in PBS at different pH for different times.

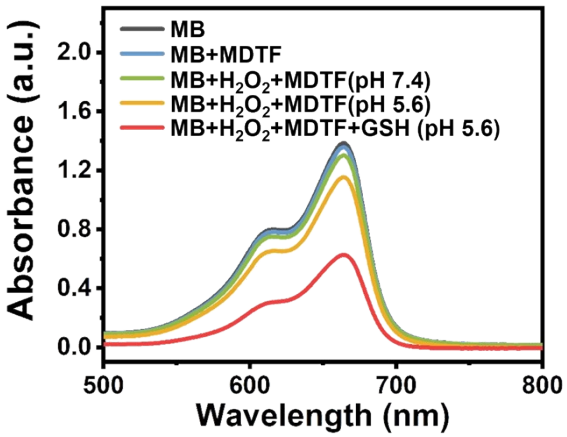

Figure S10. UV-vis absorption spectra of MB after different treatments.

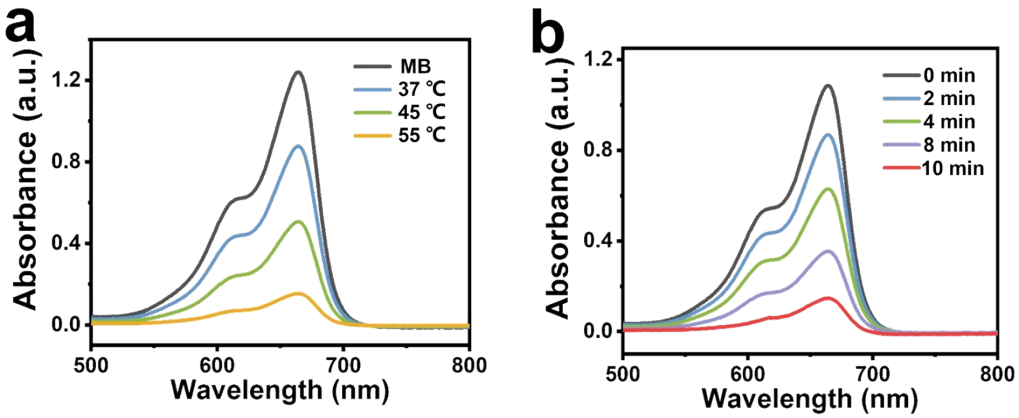

Figure S11. (a) UV-Vis absorption spectra of MB with MDTF at different temperatures and (b) after various times of NIR irradiation.

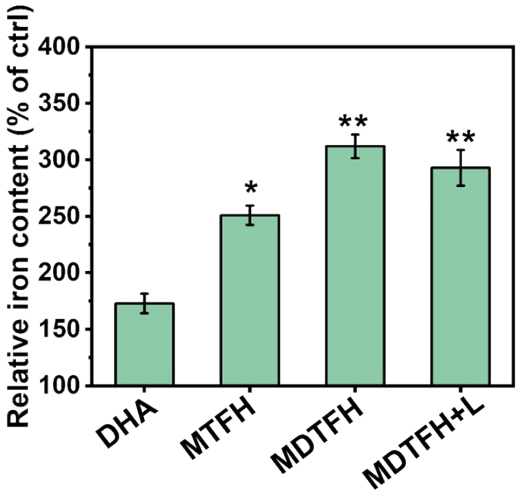

Figure S12. The relative iron contents in 4T1 cells after different treatments.

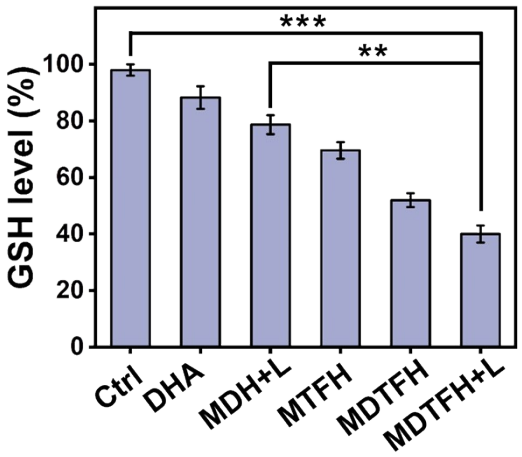

Figure S13. The intracellular GSH levels in different groups.

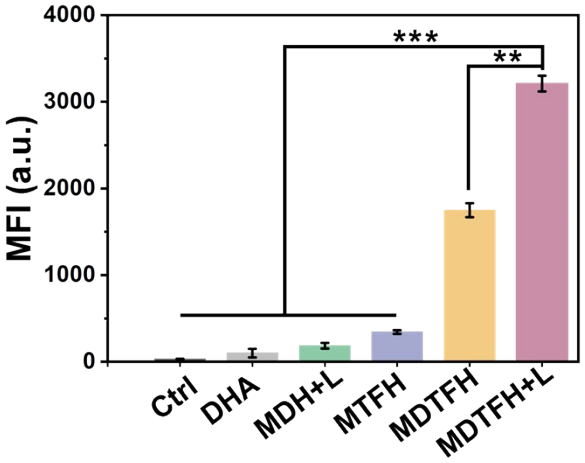

Figure S14. Quantitative analysis of the mean ROS fluorescence intensity.

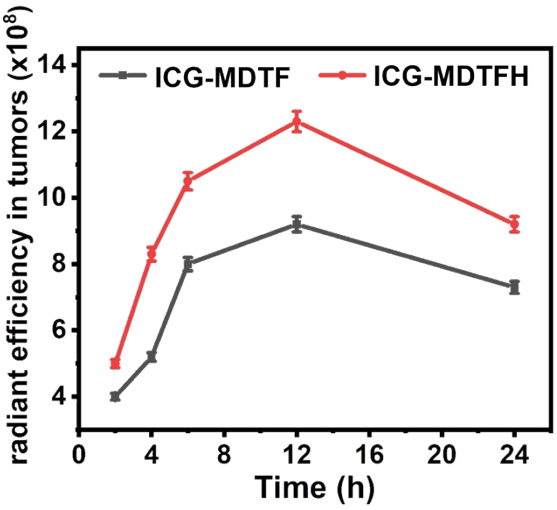

Figure S15. Relative fluorescent intensity of tumors at different time points.

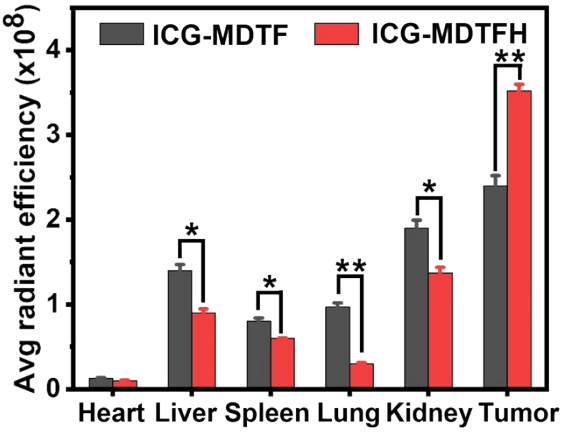

Figure S16. Fluorescent intensity of excised tumors and organs at 24 h after i.v. injection.

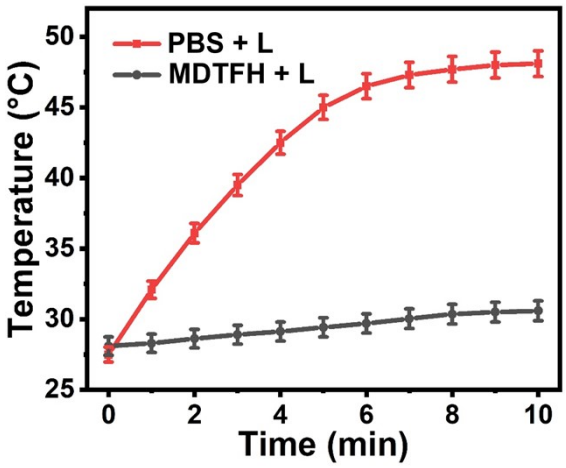

Figure S17. Temperature increasing curves of tumor sites after different treatments.

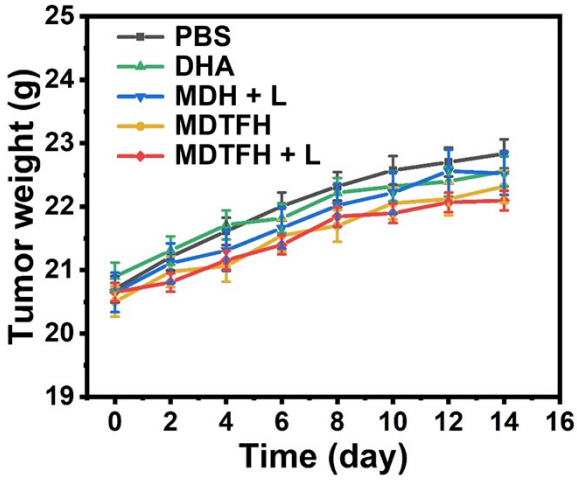

Figure S18. Body weight variation curves during the therapeutic process.

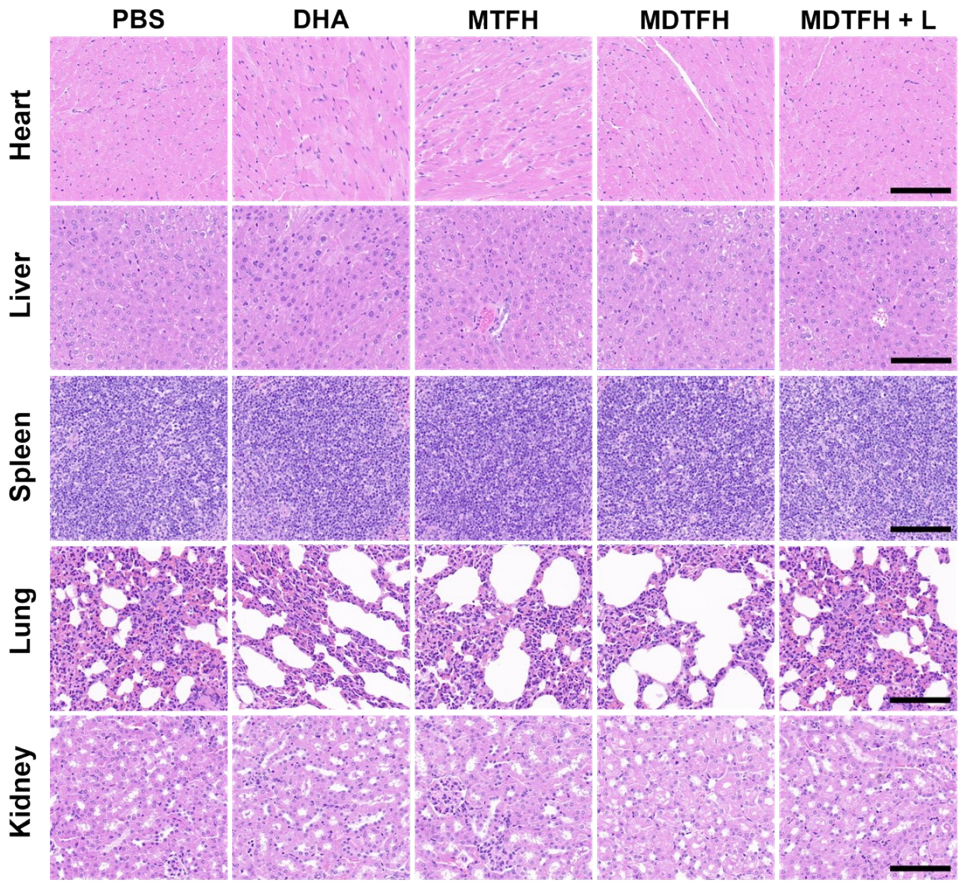

Figure S19. H&E staining images of the major organ sections of mice after different treatments (Scar bar: 100 μm).