Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Biomimetic Smart Nanoplatform for Dual Imaging-Guided Synergistic Cancer Therapy

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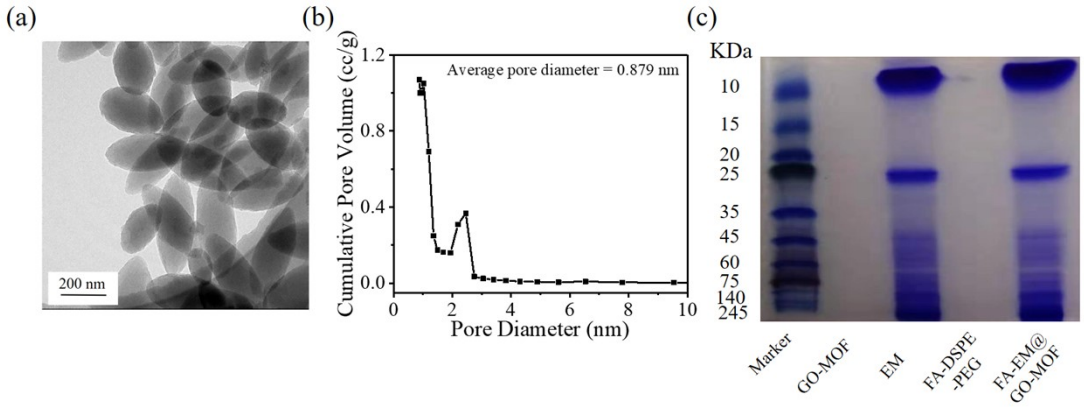

Figure S1. (a) TEM image of MOF. (b) Pore size distribution of GO-MOF using the data measured with N_2 at 77 K. (c) Protein analysis of protein marker, GO-MOF, EM, FA-DSPE-PEG and FA-EM@GO-MOF by using SDS-PAGE. Samples were stained with Coomassie Brilliant.

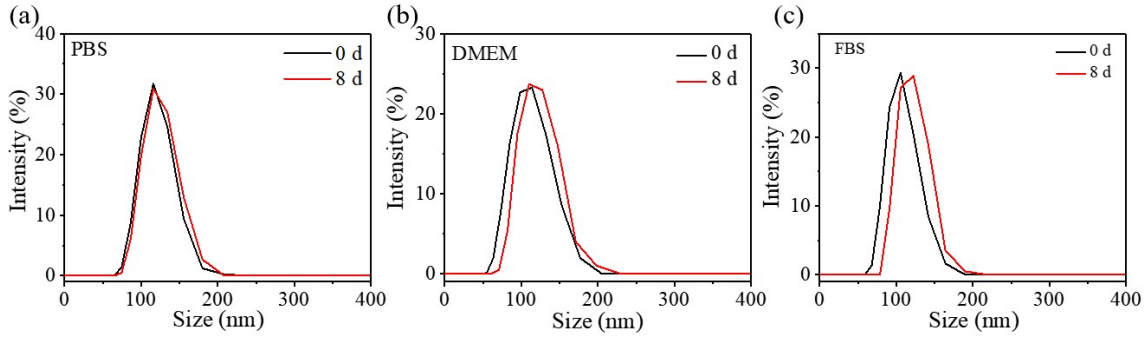

Figure S2. Particle size distribution of FA-EM@GO-MOF in PBS, DMEM, and FBS aqueous solution after a storage of 0 day and 8 days.

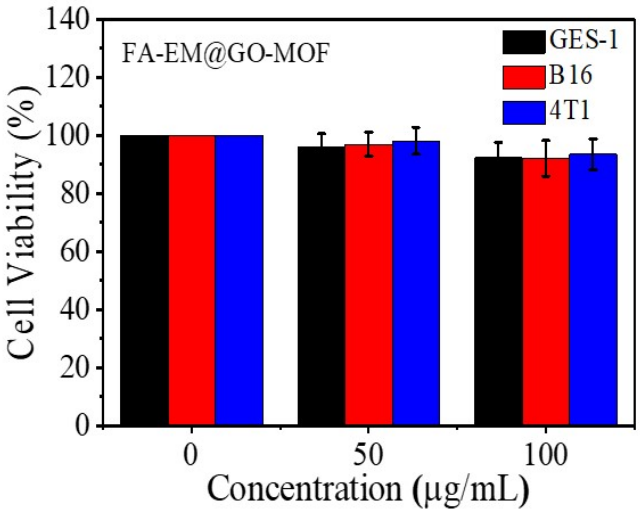

Figure S3. The effects of FA-EM@GO-MOF suspension with different concentrations on the viability of GES-1, B16 and 4T1 as determined by CCK-8 assay.

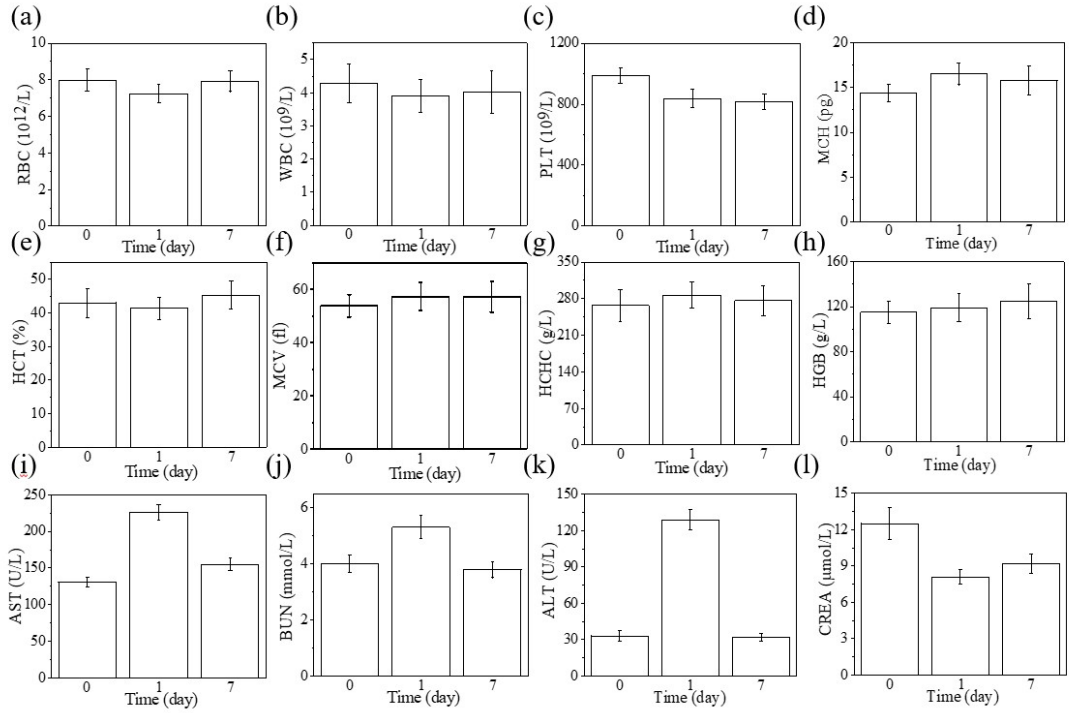

Figure S4. The blood tests (a-h) and the serum biochemistry index (i-l) of the mice on 0, 1, and 7 days post-injection of FA-EM@GO-MOF.

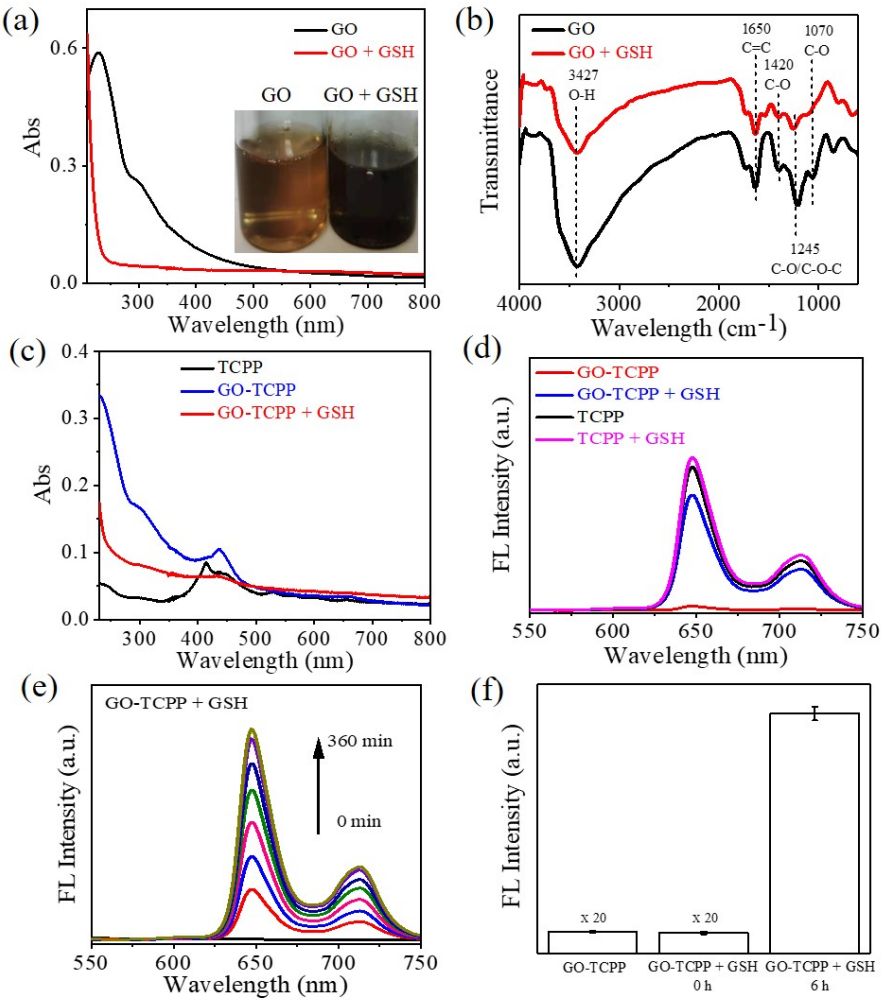

Figure S5. (a) UV absorption spectra of GO and GO+ GSH. The concentration of GO and GSH is 16 μg/mL and 10 mM, respectively. The inset shows the corresponding photos. (b) FTIR spectra of GO and GO + GSH. (c) UV absorption spectra of TCPP, GO-TCPP, and GO-TCPP + GSH. The concentration of TCPP, GO-TCPP and GSH is 16 μg/mL, 16 μg/mL and 10 mM, respectively. (d) Fluorescence spectra of GO-TCPP, GO-TCPP + GSH, TCPP, and TCPP + GSH. The concentration of GO-TCPP, TCPP and GSH is 100 μg/mL, 100 μg/mL and 10 mM, respectively. (e) Fluorescence spectra of GO-TCPP over time after the addition of GSH. (f) Fluorescence intensity of GO-TCPP, and the GO-TCPP after the addition of GSH (GO-TCPP + GSH) at 0 h and 6 h. The concentration of GO-TCPP and GSH is 100 μg/mL and 10 mM, respectively. The FL intensity of the GO-TCPP and GO-TCPP + GSH at 0 h groups were magnified 20 times.

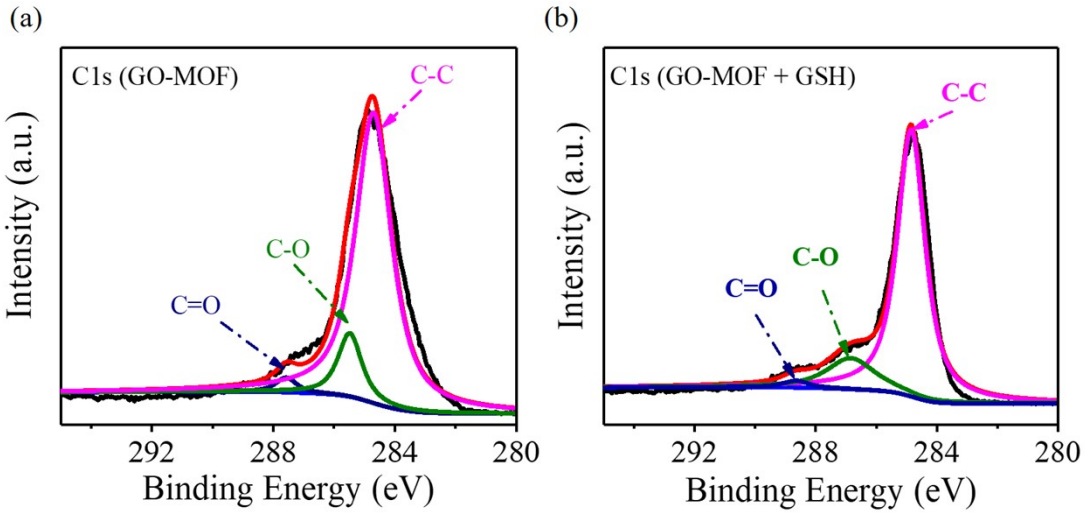

Figure S6. Narrow XPS scan spectra of C1s in GO-MOF (a) and GO-MOF + GSH (b).

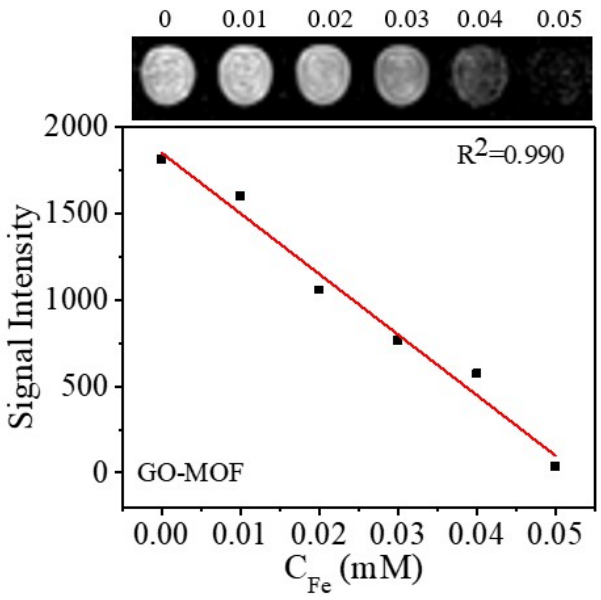

Figure S7. T_2-weighted MR signal intensity for GO-MOF in PBS as a function of Fe concentration (inset: T_2-weighted MRI of GO-MOF in PBS with different Fe concentrations).

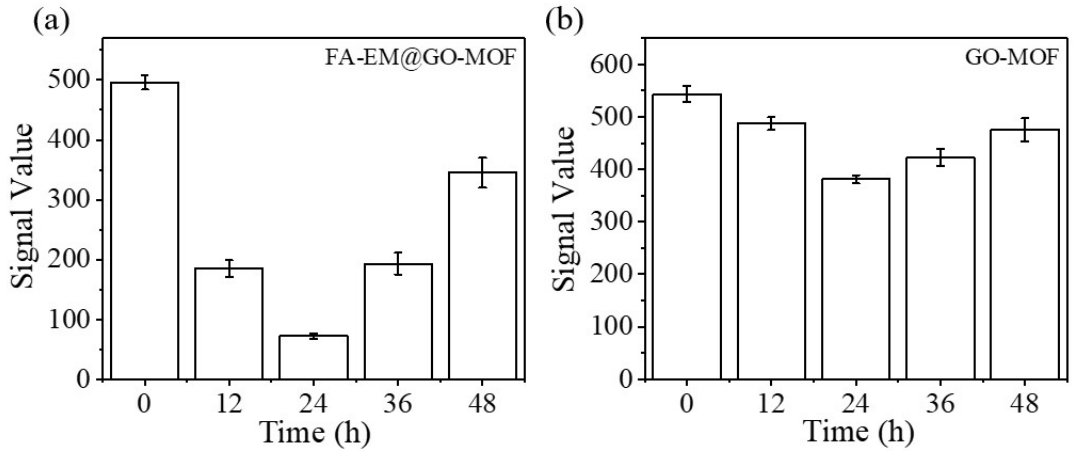

Figure S8. T_2-weighted MR signal intensity for the tumor tissues of (a) FA-EM@GO-MOF and (b) GO-MOF at designed time points.

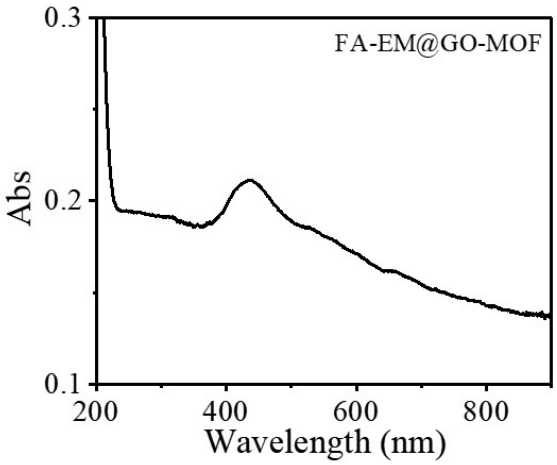

Figure S9. UV-vis absorption spectra of FA-EM@GO-MOF.

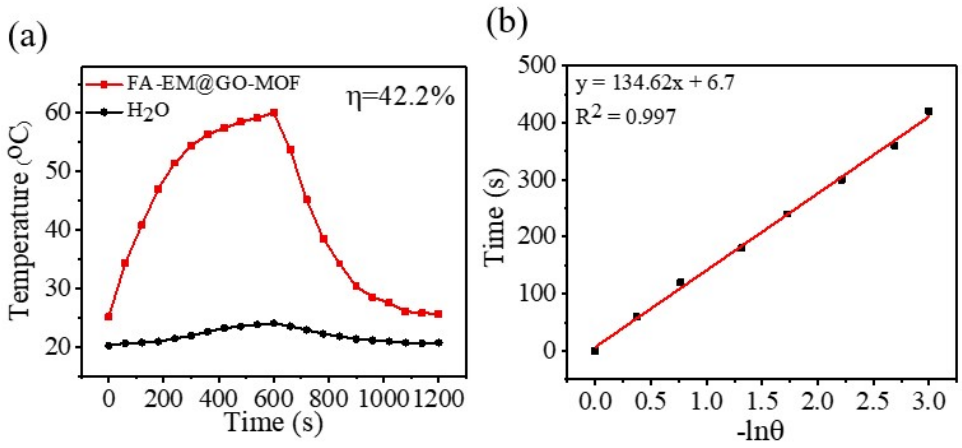

Figure S10 (a) Photothermal response of FA-EM@GO-MOF solution and H_2O treated with an NIR laser (808 nm, 1.5 W/cm^2) for 600 s and then the laser was shut off. (b) Linear relationship between time and –lnθ obtained from the cooling stage in Figure S10a.

The photothermal conversion efficiency (η) was determined by the following equations:

$$\eta = \frac{hS\,(\Delta T_{max,\,mix} - \Delta T_{max,\,H2O})}{I\,(1 - 10^{-A})} \quad (1)$$

$$t = -\frac{\sum_i miC_{p,i}}{hS}\ln\theta \quad (2)$$

$$\theta = \frac{T - T_{surr}}{T_{max,\,mix} - T_{surr}} \quad (3)$$

In Equation ***1***, h is the heat transfer coefficient. S refers to the surface area of the sample well. $\Delta T_{max,mix}$, the temperature change of FA-EM@GO-MOF dispersion at the maximum steady-state temperature, is 34.9 °C. $\Delta T_{max,\,H2O}$, the temperature change of pure water at the maximum steady-state temperature, is 3.8 °C. I, the laser power, is 1.64 W, where the area of light spot is 1.09 cm^2. A, the absorbance of FA-EM@GO-MOF (100 μg/mL) at 808 nm in aqueous solution, is 0.14.

In Equation ***2***, t is the time change during the laser on-off cycle. $\frac{\sum_i miC_{p,i}}{hS}$ can be

determined by the linear relationship of time vs. -lnθ. Since the mass of FA-EM@GO-MOF was too little compared with that of water solvent, the mass of FA-EM@GO-MOF were neglected. Thus, $\sum_i m_i C_{p,i}$ in Equation ***2*** is the product of mass and heat capacity of water solvent, where m_{H2O} is 0.2 g and C_{H2O} is 4.2 $J \cdot g^{-1} \cdot K^{-1}$. θ in Equation ***2*** is the dimensionless driving force temperature, as defined by Equation ***3***.

In Equation ***3,*** T is the solution temperature, which changes with the irradiation time. $T_{max,mix}$ and T_{surr} are the maximum steady-state temperature of FA-EM@GO-MOF dispersion and the ambient temperature of the surrounding, which are 60.1 ^oC and 25.2 ^oC, respectively, in this work.

According to these equations, the η value of FA-EM@GO-MOF was calculated to be 42.2%.

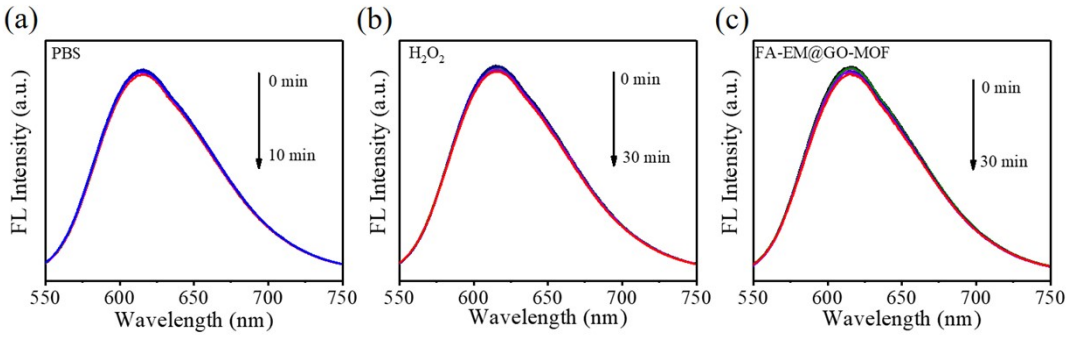

Figure S11. Fluorescence intensity of RDPP in PBS without (a), and with H_2O_2 (b) and FA-EM@GO-MOF (c) at different time points.

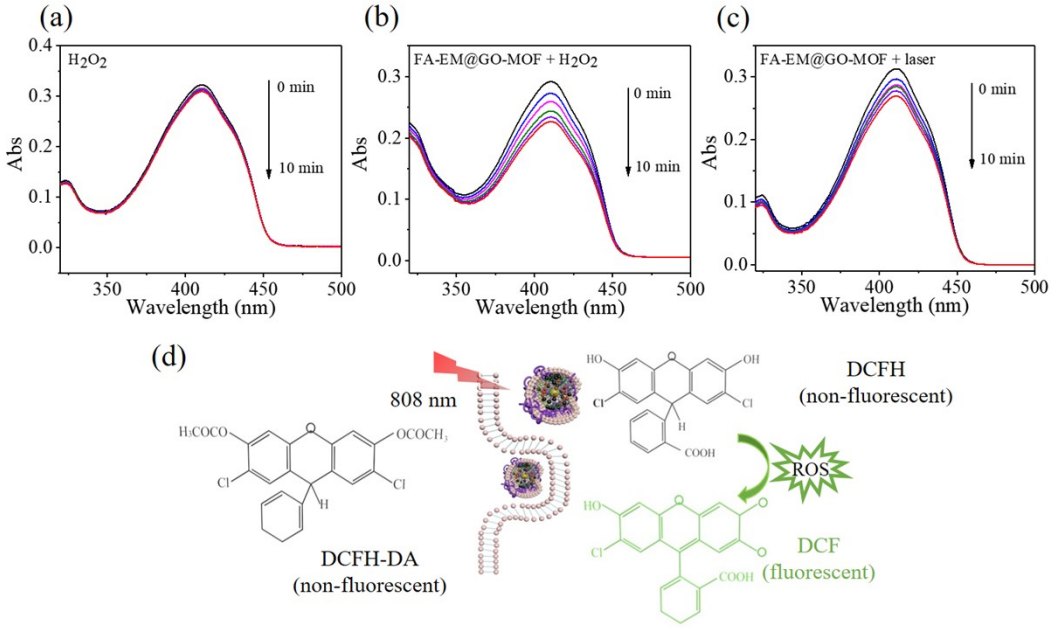

Figure S12. UV−vis absorption of DPBF in PBS containing H_2O_2 (a), FA-EM@GO-MOF + H_2O_2 without laser irradiation (b), and FA-EM@GO-MOF with 808 nm laser irradiation (c) at different time points. (d) Schematic illustration of the reaction mechanism of DCFH-DA oxidation by ROS.

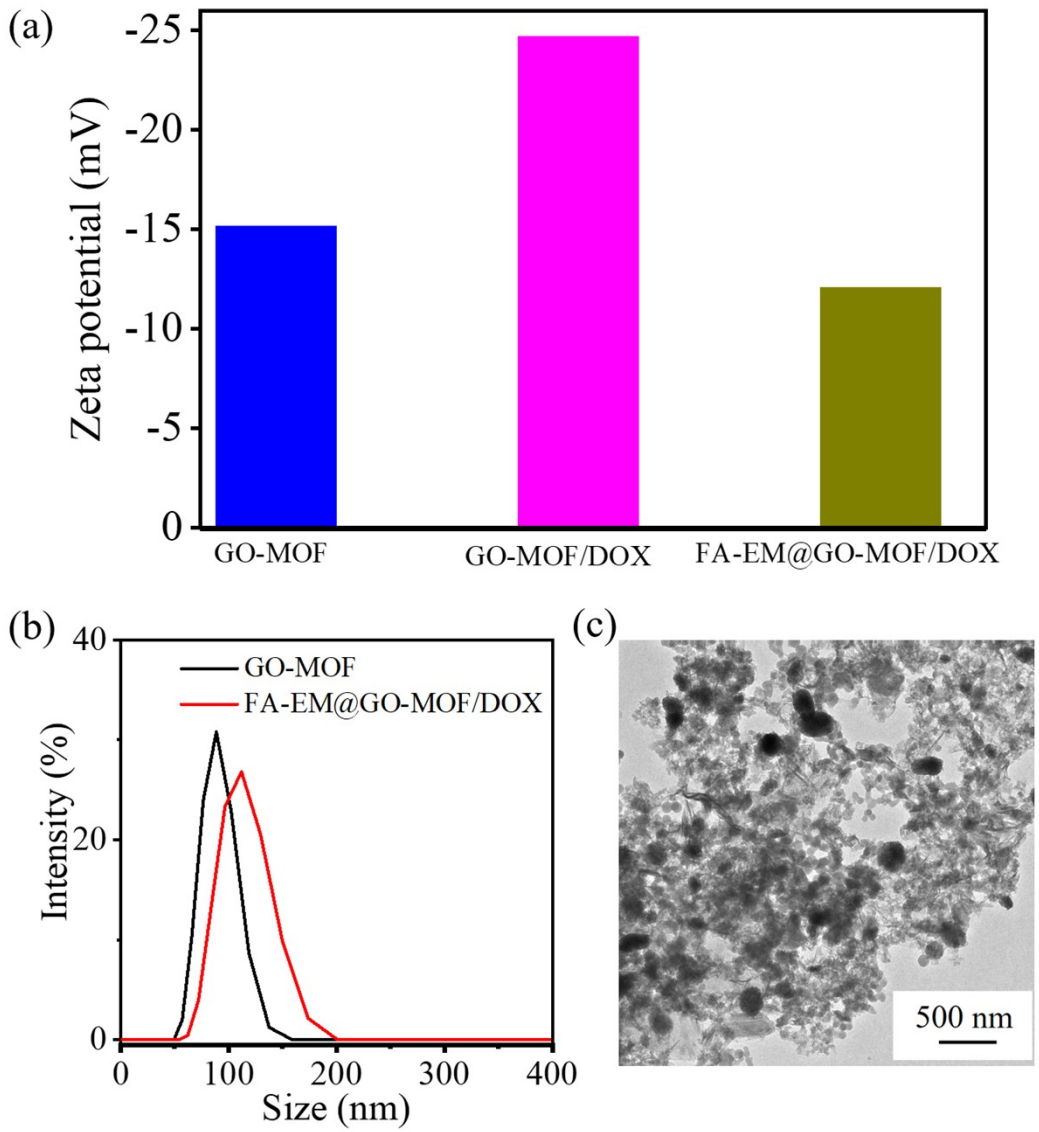

Figure S13. (a) Zeta potential of GO-MOF, GO-MOF/DOX and FA-EM@GO-MOF/DOX. (b) Particle size distribution of GO-MOF and FA-EM@GO-MOF/DOX. (c)TEM image of GO-MOF after 24 h-incubation with GSH.

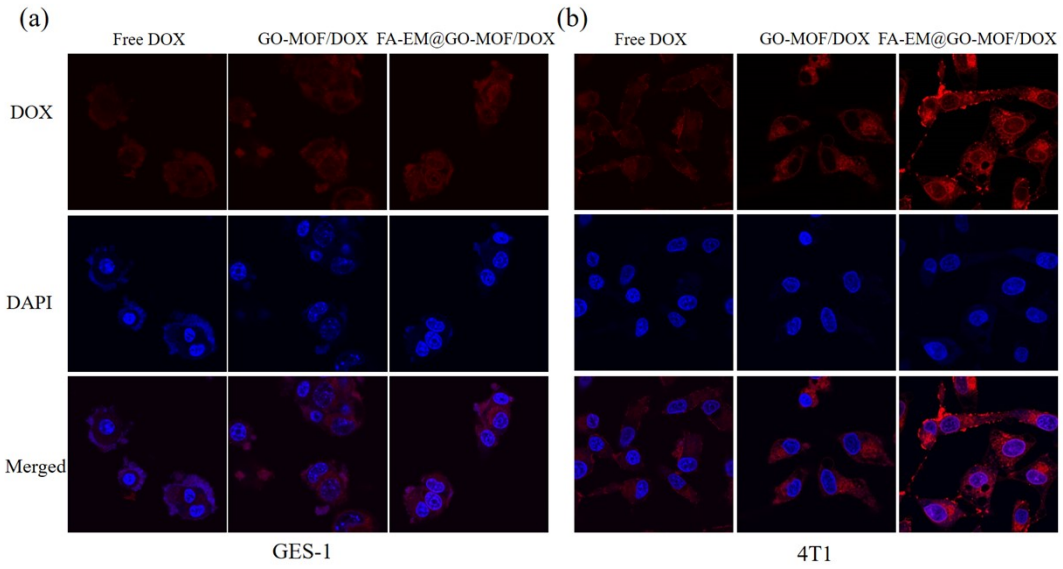

Figure S14. CLSM images of (a) GES-1 and (b) 4T1 cells after 3h-incubation with free DOX, GO-MOF/DOX and FA-EM@GO-MOF/DOX. Images shown cell nuclei stained by DAPI (blue), DOX fluorescence in cells (red) and the merged overlap of the two images. Scale Bar: 30 μm.

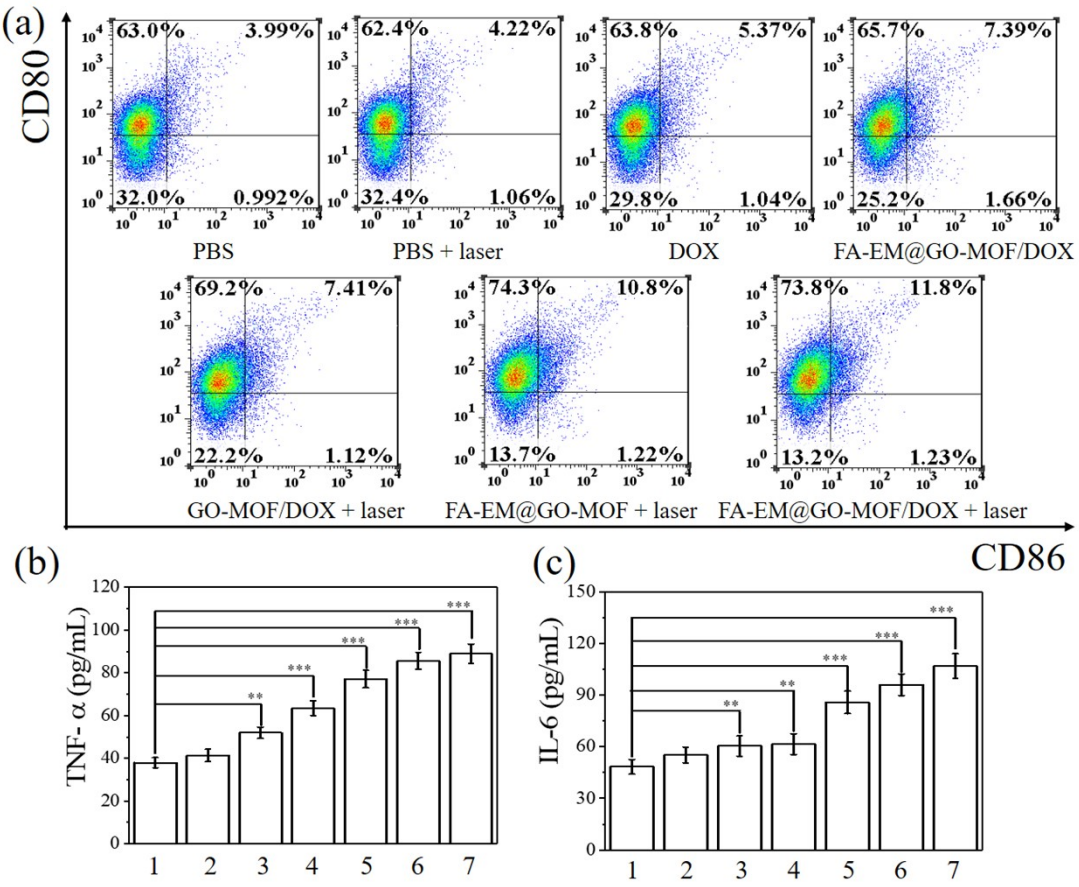

Figure S15. (a) Mature DCs ($CD80^+$ and $CD86^+$) induced from BMDCs upon 48 h-incubation with 4T1 cells from different treatments: PBS, PBS + laser, DOX, FA-EM@GO-MOF/DOX, GO-MOF/DOX + laser, FA-EM@GO-MOF + laser and FA-EM@GO-MOF/DOX + laser. TNF-α (b) and IL-6 (c) cytokine levels in supernatants from BMDCs upon 48 h-incubation with 4T1 cells from different treatments of PBS (column *1*), PBS + laser (column *2*), DOX (column *3*), FA-EM@GO-MOF/DOX (column *4*), GO-MOF/DOX + laser (column *5*), FA-EM@GO-MOF + laser (column *6*), FA-EM@GO-MOF/DOX + laser (column *7*). Significant differences (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$) among different groups are shown.

Experimental Section

Materials and Reagents

N, N-Dimethylformamide (DMF) and methanol were purchased from Sinopharm Chemical Reagent (Beijing, China). 4,4',4'',4'''-(porphine-5,10,15,20-tetrayl) tetrakis(benzoic acid) (TCPP), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[folate (polyethylene glycol)-2000] (DSPE-PEG-FA), 4',6-diamidino-2-phenylindole (DAPI), glutathione (GSH, reduced), $[Ru(dpp)_3]Cl_2$ (RDPP), 1, 3-diphenylisobenzofuran (DPBF), 2′, 7′-dichlorofluorescein diacetate (DCFH-DA), Iron (III) nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$), sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$), doxorubicin (DOX), cell counting kit-8 (CCK-8) and formic acid were bought from Aladdin (Shanghai, China). Fetal bovine serum (FBS), 1640 medium, dulbecco's modified eagle medium (DMEM), enzyme-linked immunosorbent assay (ELISA) kits, mouse interleukin-4 (IL-4), and mouse granulocyte-macrophage colony-stimulating factor (GM-CSF) were purchased from Gibco (Shanghai, China). All chemicals were used without further purification.

Apparatus and Procedures

Transmission electron microscopy (TEM) images were obtained from a transmission electron microscope (JEOL 2100, Japan). Fluorescence spectra were obtained from a FluoroMax-4 spectrofluorometer (Japan). Raman spectra were collected from a Perkin-Elmer Raman spectroscopy (Horiba evolution) with a diode laser operating system at 532 nm. 3.0 T clinical magnetic resonance imaging (MRI) scanner (GE Echospeed Signa, USA) were used to measure the transverse relaxation times (T_2) of Fe. Fourier transform infrared (FTIR) spectra were acquired from a NicoletNexus 470 spectrometer (USA). UV–vis absorption spectra were obtained from a Cary100 UV–vis spectrophotometer (USA). X-ray powder diffraction (XRD) measurements were performed on an X-ray diffractometer (GBC MMA Instrument). Brunauer-Emmett-Teller (BET) surface area and pore size distribution were measured by N_2 adsorption-desorption isotherms (NovaWin 1000e, USA). The ζ-potential measurements were performed by a NanoBrook Omni (Brookhaven, USA). Irradiation laser (808 nm) was

performed by a fiber-coupled NIR laser (MDL-N-808 nm-10W, Beijing Laserwave OptoElectronics Technology Co., Ltd., Beijing, China). The infrared thermal camera (HT-19, Guangzhou, China) was used to take the infrared thermal photos and record the temperature changes. Confocal laser scanning microscopy (CLSM, Nikon, Japan) was used to analyze the cells uptake behaviors.

Preparation of MOF

MOF was synthesized according to previous reports with slight modifications.[1] Briefly, $Fe(NO_3)_3 \cdot 9H_2O$ (100 mg) and $C_2H_3NaO_2 \cdot 3H_2O$ (400 mg) were dispersed in 30 mL of methanol and stirred for 30 min to obtain an orange solution. Then, the solution was refluxed for 12 h. After centrifugation and washing with methanol for three times, $[Fe_3O(CH_3COO)_6(H_2O)_3](CH_3COO)$ was dried in vacuum. Next, 100 mg of $[Fe_3O(CH_3COO)_6(H_2O)_3](CH_3COO)$ and 40 mg of TCPP were added to 20 mL of DMF to obtain a homogenous solution. After adding 2 mL of formic acid, the solution was heated at 80 °C for 24 h in an oven. After cooling to room temperature, the final MOF was obtained by centrifugation at 15000 rpm for 30 min and washing with DMF for three times. The final product of MOF was obtained after drying at 60 °C for 12 h in vacuum.

Cell culture

Anthropogenic gastric mucosa epithelial cells (GES-1 cells), anthropogenic melanoma cancer cells (B16 cells), murine breast cancer cells (4T1 cells) and macrophage cells (RAW264.7) were obtained from Nanjing Kebai Biological Technology Co., Ltd. The cells were incubated with fresh DMEM media containing 10% heat-inactivated FBS. All the cell lines were approved by the Southeast University.

Cell viability assay

CCK-8 assay was used to assess the cell viability after being incubated with the FA-EM@GO-MOF suspension with different concentrations. GES-1 cells, B16 cells and 4T1 cells were seeded into 96-well plates at a density of 1×10^4 cells per well and

incubated at 37°C in a 5% CO_2 incubator. After 12 h, the medium was replaced by fresh DMEM media containing 10% heat-inactivated FBS and different concentrations of FA-EM@GO-MOF (0 μg/mL, 50 μg/mL, and 100 μg/mL) for 12 h. Then, the medium was replaced by fresh DMEM media containing 10% FBS and 10% CCK-8 solution, and incubated for another 3 h in the dark. Synergy HT Multi-Mode Microplate Reader (Bio Tek, Winooski, VT, USA) was applied to measure the absorbance at 450 nm. Untreated cells were used as a control for the evaluation of the cell cytotoxicity of FA-EM@GO-MOF.

Cellular uptake

The internalization and intracellular distribution of FA-EM@GO-MOF/DOX in GES-1 cells and 4T1 cells were observed by CLSM. 5 mg/mL FA-EM@GO-MOF/DOX was incubated with GES-1 cells and 4T1 cells for 3 h, respectively.[2] The cells were fixed with 4% paraformaldehyde for 15 min and washed with PBS (6.7 mM, pH = 7.4) for three times. Subsequently, cells were stained with DAPI at room temperature for 15 min and observed with CLSM.

5×10^5 macrophage cells (RAW264.7) were planted into cell-culture dish and incubated for 12 h. Then, MOF, EM@MOF and FA-EM@MOF were added to each cell-culture dish and incubated for another 3 h. Subsequently, the cells were fixed with 4% paraformaldehyde for 15 min and washed with PBS (6.7 mM, pH = 7.4) for three times. After that, cells were stained with DAPI at room temperature for 15 min and washed with PBS (6.7 mM, pH = 7.4) for three times. The cellular uptake of MOF, EM@MOF and FA-EM@MOF by macrophage cells was observed by CLSM.[3, 4]

Animal welfare

All animal experiments were performed based on the protocol approved by the Institutional Animal Care and Use Committee of Southeast University (No.20200327004). 6-week-old BALB/c mice were purchased from Yangzhou University (Yangzhou, China) and feed in specific pathogen-free-conditions. All efforts

were made to minimize the animals' suffering and reduced the frequency of animal use.

Bone marrow-derived dendritic cells Isolation

Bone marrow-derived dendritic cells (BMDCs) were obtained from the bone marrow of six-week-old BALB/c mice according to the established methods with some modification.[5, 6] The mice were euthanized and immersed into 75% alcohol for 5 min. Then tibia and femur were dissected from the mice under aseptic environment. The bone marrow was flushed by 1640 medium. After filtering, red blood cells were lysed and washed with 1640 medium for three times, and bone marrow cells were obtained and incubated with fresh 1640 medium containing 10% FBS, 10 ng/mL IL-4, and 10 ng/mL GM-CSF. Half of the culture medium was discarded and replaced by equal volume of fresh culture medium for every two days. 7 days later, BMDCs were obtained.

***In vitro* BMDCs maturation and cytokine analysis assays**

4T1 cells (2×10^5 cells/well) were seeded into transwell upper chamber and incubated for 12 h. DOX, GO-MOF/DOX, FA-EM@GO-MOF, and FA-EM@GO-MOF/DOX were added into the 4T1 cells, respectively. After 3 h-incubation, 4T1 cells were treated with or without laser, and cocultured with BMDCs (5×10^4 cells/well) in lower chamber for 48 h. The BMDCs were collected, and the expression of maturation markers ($CD80^+$ and $CD86^+$) were analyzed by flow cytometry. Culture medium were collected from different groups and centrifuged for cytokine analysis. ELISA kits was used to determine TNF-α and IL-6 in supernatants from 4T1 cells-cocultured BMDCs via a standard protocol.[7, 8]

References:

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