Electronic Supplementary Material (ESI) for ChemComm.


# Electronic Supporting Information (ESI)

## Mn-Fe layered double hydroxides nanosheets: a new photothermal nanocarrier for $O_2$-evolving phototherapy

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## Experimental Section

***Reagents and chemicals:*** $MnCl_2$, $FeCl_3$, NaOH, $CH_3CH_2OH$ were purchased from Beijing Chemical Reagents Company (Beijing, China). Methylene blue (MB) were purchased from Aladdin Reagents Company (Shanghai, China). $[Ru(dpp)_3]Cl_2$ (RDPP), dicyandiamide, 1,3-diphenylisobenzofuran (DPBF), calcein acetoxymethyl ester (calcein AM), and propidium iodide (PI) were obtained from Sigma-Aldrich Co (St. Louis, MO, USA). Lactate Dehydrogenase Cytotoxicity Assay Kit and Hydrogen Peroxide Detection Kit were purchased from Beyotime (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) were obtained from Life Technologies Corporation (Los Angeles, CA, USA). Standard fetal bovine serum (FBS) was purchased from Tianjin Haoyang Biological Manufacture Co., Ltd. (Tianjin, P. R. China). The deionized (D.I.) water was used throughout the experiments. All chemicals were used without additional purification.

***Synthesis of FeMn-LDH:*** A simple co-precipitation method was performed to synthesize FeMn-LDH by dropping NaOH aqueous solution (40 mL, 0.15 M) into 10 mL of aqueous solution including 30 mM $MnCl_2$ and 10 mM $FeCl_3$ at room temperature under stirring.[1] After 90 min, the product was aged in an oven at 90 $^oC$ for 16 h, and then washed with water for three times. The precipitate was dispersed in water and dialyzed for 24 h to obtain FeMn-LDH.

***Synthesis of MgAl-LDH:*** Similarly, NaOH aqueous solution (40 mL, 0.15 M) was dropped into 10 mL of aqueous solution including 30 mM $MgCl_2$ and 10 mM $AlCl_3$ at room temperature under stirring.[1] After 90 min, the product was aged in an oven at 90 $^oC$ for 16 h, and then the LDH was obtained by centrifugation following three times washing by deionized water.

***Synthesis of FeMn-LDH/MB:*** To prepare FeMn-LDH/MB, MB (500 μg $mL^{-1}$, 100 uL) was added to a suspension of FeMn-LDH (200 μg $mL^{-1}$, 10 mL), and the mixture was stirred at room temperature for 12 h in the dark. After loading, the product was obtained by centrifugation at 10000 rpm for 10 min and the precipitate was washed for three times by deionized water.

***Synthesis of MgAl-LDH/MB:*** To prepare MgAl -LDH/MB, MB (400 μg $mL^{-1}$, 100 uL) was added to a suspension of MgAl-LDH (200 μg $mL^{-1}$, 10 mL), and the mixture was stirred at room temperature for 12 h in the dark. After loading, the product was obtained by centrifugation at 10000 rpm for 10 min and the precipitate was washed for three times by deionized water.

***Characterization:*** Transmission electron microscopy (TEM) measurements were performed on HITACHI H-8100EM (Hitachi, Tokyo, Japan). The samples for TEM characterization were prepared by placing 20 μL of colloidal solution on carbon-coated copper grid and dried at room temperature. X-ray photoelectron spectroscopy (XPS) measurements were performed on an ESCALAB 250 X-ray photoelectron spectrometer with a monochromatic X-ray source. The elemental mapping images were obtained by a FEI TECNAI G2 high-resolution transmission electron microscope operating with a field-emission gun at 200 kV. UV–vis spectra were

recorded on a VARIAN CARY 50 UV-vis spectrophotometer. Dynamic laser scattering (DLS) measurement was performed by ZEN3690 zetasizer. Concentrations of Fe and Mn were measured by inductively coupled plasma (ICP, ThermoScientific Xseries II, USA). The fluorescence of cells was imaged by using confocal laser scanning fluorescence microscope (CLSM, Leica TCS SP2, Leica Microsystems, Mannheim, Germany). The Lactate Dehydrogenase Cytotoxicity Assay was measured using a Versamax microplate reader (Bio-Tek Instruments, Inc., Winooski, VT, USA). Photothermal image of all samples were measured with an infrared imaging device (FLIR E40 of FLIR Systems, Inc., USA). Electron paramagnetic resonance (EPR) mearsurement was conducted by Bruker EMXNANO (Germany).

***Extracellular Generation of $O_2$ from FeMn-LDH:*** The generation of $O_2$ was quantified using a dissolved oxygen meter. Briefly, The detector of the dissolved oxygen meter was inserted into a 50 mL centrifuge tube containing 30 mL of FeMn-LDH dispersion and then $H_2O_2$ (100 μL, 30 mM) was added. And the group without the addition of FeMn-LDH and the group of water only were served as controls.

***Extracellular consumption of $H_2O_2$ with FeMn-LDH:*** the $H_2O_2$ was detected by Hydrogen Peroxide Detection Kit. Briefly, $H_2O_2$ (100, 30 mM) was added into a 50 mL centrifuge tube containing 30 mL of FeMn-LDH dispersion. After reacting for 10 min, the dispersion was centrifuged (10000 rpm, 10 min) and 50 uL of the supernatant was placed in a 96-well plate, and reacted with 100 uL of hydrogen peroxide detection reagent for 30 minutes, and the absorbance at 560 nm was measured. The 100 μM $H_2O_2$ solution was used as contral.

***Extracellular ROS Detection:*** DPBF was used to detect ROS by measuring its UV-vis absorption at 410 nm. Typically, 10 μL of ethanol solution of DPBF was added to 1 ml of ethanol water mixed solution (ethanol:water=1:1) containing FeMn-LDH/MB suspension (20 μg $mL^{-1}$) and the mixture was transferred into a 1 cm cuvette. The cuvette was kept in dark and irradiated by a 650-nm laser for 10 min (100 mW $cm^{-2}$) after the addition of $H_2O_2$ (0, 40 ,60, 80 μM). The absorption at 410 nm was recorded every 2 min and the group without irradiation was served as control.

***Internalization of FeMn-LDH/MB and MgAl-LDH/MB:***

1) ***CLSM Observing:*** HeLa cells were first seeded in culture dishes ($2.5 \times 10^4$ cells per dish) and cultured in a 5% $CO_2$ incubator at 37 °C for 24 h. Then, the cells were treated with 1 mL of DMEM including FeMn-LDH/MB (100 μg $mL^{-1}$) or MgAl-LDH/MB (100 μg $mL^{-1}$) for 24 h. After washing the cells three times with PBS, fresh medium was added to each well. Fluorescence of MB was then observed using CLSM with emission bands of 650-700 nm and excitation wavelengths of 633 nm and the internalized nanomaterials were observed by bright field.

2) ***ICP-MS Detection:*** HeLa cells were first seeded in culture bottles ($2.5 \times 10^5$ cells per bottle) and cultured in a 5% $CO_2$ incubator at 37 °C for 24 h. Then, the cells were treated with 5 mL of DMEM including FeMn-LDH/MB (100 μg $mL^{-1}$) or MgAl-LDH/MB (100 μg $mL^{-1}$) for 24 h at 37 °C or 4 °C and the group treated with 5 mL of DMEM as control. The groups treated at 4 °C were pre-cooled at 4 °C for 2 h before adding the materials. After washing the cells three times with PBS, 1 mL trypsin (2.5 mg/mL) was added. After treating for 3 min, 5 ml of PBS was added and repeatedly

blown for about 40 times, and then the cells were transferred to a 7 ml centrifuge tube. After centrifugation for 5 min (3000 rpm), the supernatant was removed as much as possible, and the centrifuged cells were redispersed into 1 mL of PBS and disrupted by a cell disrupter for 5 min. Finally, the volume of each group was made up to 1 ml, and measured by ICP-MS.

***In Vitro Photothermal Evaluation of FeMn-LDH:*** To test the photothermal performance of FeMn-LDH, different concentrations of FeMn-LDH dispersion were added to 1 mL tubes and then irradiated by 808 nm laser (1.0 W $cm^{-2}$) for 10 min. The temperature changes were recorded with a digital thermocouple and an infrared thermal imaging camera, respectively. To test the photothermal stability of FeMn-LDH, FeMn-LDH suspension (400 μg $mL^{-1}$) was irradiated with an 808 nm laser (1.0 W $cm^{-1}$) for 10 min and then the laser was switched off. The cycle was repeated five times.

***Intracellular Generation of $O_2$:*** The intracellular generation of $O_2$ was observed by CLSM imaging using RDPP as a probe. HeLa cells were incubated with 1 mL of DMEM including 5 μM RDPP for 4 h. After the cells were washed for three times with PBS, the cells were further incubated with 1 mL of DMEM including FeMn-LDH (100 μg $mL^{-1}$) for 0, 4, 8, 24 h, and the cells further incubated with 1mL of DMEM only were used as control. Excitation wavelength was chosen as 488 nm and emission was collected between 600 and 700 nm to observe the fluorescence of RDPP.

***Measurement of Intracellular $^1O_2$ Production:*** The intracellular $^1O_2$ production was observed by CLSM imaging using Singlet oxygen sensor green (SOSG) as a probe. The cells were first seeded in culture dishes ($2.5 \times 10^4$ cells per dish) and cultured in a 5% $CO_2$ incubator at 37 °C for 24 h. Then, the cells were treated with 1 mL of DMEM including free MB (2 μg $mL^{-1}$), MgAl-LDH/MB (100 μg $mL^{-1}$, equal to 2 μg $mL^{-1}$ MB) or FeMn-LDH/MB (100 μg $mL^{-1}$, equal to 2 μg $mL^{-1}$ MB) for 24 h. After the cells were washed three times with PBS, SOSG in DMEM (10 μM, 1 mL) was used to treat the cells for 15 min. After washing the cells three times with PBS, fresh medium was added to each well and the cells were irradiated with 650 nm laser for 15 min (100 mW $cm^{-2}$). Untreated cells served as the control group. Fluorescence was then observed using CLSM with emission bands of 510-560 nm and excitation wavelengths of 488 nm.

***In vitro Dark Cytotoxicity of FeMn-LDH/MB:*** The HeLa cells seeded in a 96-well plate were cultured for 24 h in an incubator containing 5% $CO_2$ at 37 °C. Different concentrations of FeMn-LDH/MB (0, 10, 20, 50, 100, 200 μg $mL^{-1}$) in DMEM (200 μL) were then added into each well of the 96-well plate and culture for another 24 h. The cell viability was evaluated with Lactate Dehydrogenase Cytotoxicity Assay Kit.

***In vitro Photodynamic Therapy or/and Photothermal Therapy Effect:*** The HeLa cells seeded in a 96-well plate were cultured for 24 h in an incubator containing 5% $CO_2$ at 37 °C. MB (2 μg $mL^{-1}$), MgAl -LDH/MB (100 μg $mL^{-1}$, equal to 2 μg $mL^{-1}$ MB) and FeMn-LDH/MB (100 μg $mL^{-1}$, equal to 2 μg $mL^{-1}$ MB) in 200 μL of DMEM were then added to the cells respectively. After 24 h, the cells were washed three times with PBS solution, fresh medium was added to each well, and the cells were irradiated with 650 nm laser (100 mW $cm^{-2}$, 15 min) or/and 808 nm laser (1.0 W

$cm^{-2}$, 10 min) for PDT or/and PTT, and further incubated for another 12 h. The cell viability was evaluated with Lactate Dehydrogenase Cytotoxicity Assay Kit and observed by CLSM imaging using calcein AM/PI co-staining. Untreated cells served as the control group.

**Animal Tumor Model and in vivo PDT or/and PTT Treatment by Intratumoral Injection and Intravenous Injection**: All animal-related experiments were conducted with the support and guidance of Jilin University Laboratory Animal Center and China-Japan Union Hospital of Jilin University. Female Kunming mice aged 6-8 weeks and weighing 20-25 g were selected as model mice. Tumor models were established by subcutaneous injection of around $10^6$ murine cervical cancer cells (U14) into the right alar area of mice. When the tumor volume reached 60 $mm^3$, mice were randomly divided into seven groups (ten mice per group) for each injection method and were treated with 0.9 % saline, 0.9 % saline with 808 nm and 650 nm laser irradiation (1.0 W $cm^{-2}$, 10 min; 100 mW $cm^{-2}$, 15 min), FeMn-LDH/MB without irradiation, MB with 650 nm laser irradiation (100 mW $cm^{-2}$, 15 min), FeMn-LDH/MB with 650 nm laser irradiation (100 mW $cm^{-2}$, 15 min), FeMn-LDH/MB with 808 nm laser irradiation (1.0 W $cm^{-2}$, 10 min), and FeMn-LDH/MB with 808 nm and 650 nm laser irradiation (1.0 W $cm^{-2}$, 10 min; 100 mW $cm^{-2}$, 15 min). Injection doses for MB and FeMn-LDH/MB were 2 μg $mL^{-1}$ and 100 μg $mL^{-1}$, respectively, with a total volume of 200 μL. Tumor length and width were measured every two days using a digital vernier caliper. The volume of the tumor was calculated by the following formula: Volume = tumor length×(tumor width)$^2$/2 and the relative tumor volume was calculated as $V/V_0$ (where $V_0$ was the tumor volume before the treatment). On the 15th day (for Intratumoral method) or 11th day (for intravenous injection method) of the experiment, the mice were sacrificed, the tumor was dissected out and weighed to evaluate the therapeutic efficiency.

***Histology and HIF-1α Staining:*** After the mice were sacrificed, the tumor was peeled off and fixed with 4% formaldehyde solution and paraffin. Then the tumor tissues were prepared into 4-μm-thick sections and subjected to hematoxylin & eosin staining and immunostaining for HIF-1α, and then observed with an electron microscope.

***In Vivo Thermal Imaging:*** For infrared thermography, tumor-bearing mice were divided into two groups and then irradiated with 808 nm laser for 10 min after intravenous injection of FeMn-LDH (500 μg $mL^{-1}$, 0.5 mL) and saline (0.9%) for 24 h followed by PT-imaging using an infrared (IR) thermal camera.

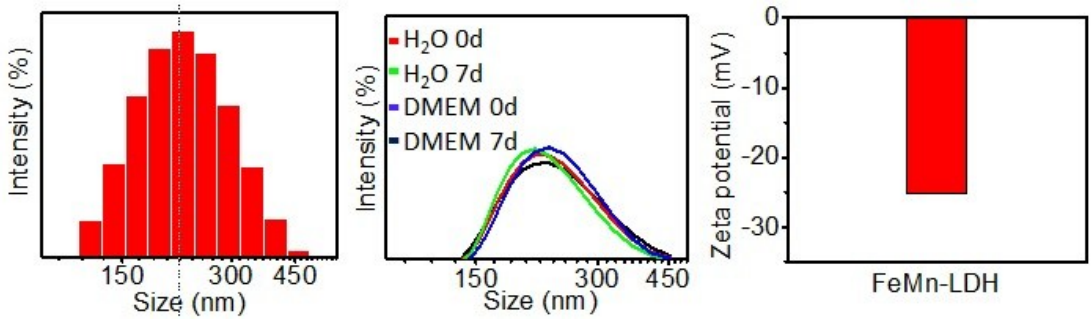


Figure S1. (a) The average hydrated particle size of FeMn-LDH in water. (b) Size distributions of the as-prepared FeMn-LDH nanosheets before and after standing in $H_2O$ and DMEM for 7 days. (c) Zeta potential of FeMn-LDH aqueous solution after fresh preparation

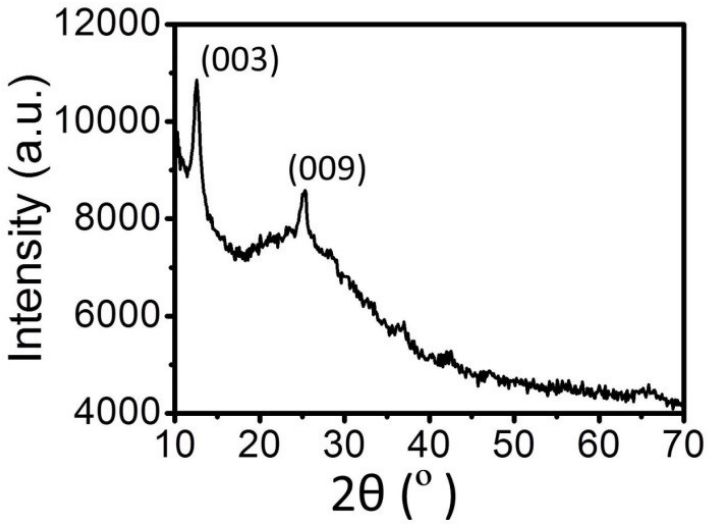


Figure S2. XRD spectrum of FeMn-LDH.

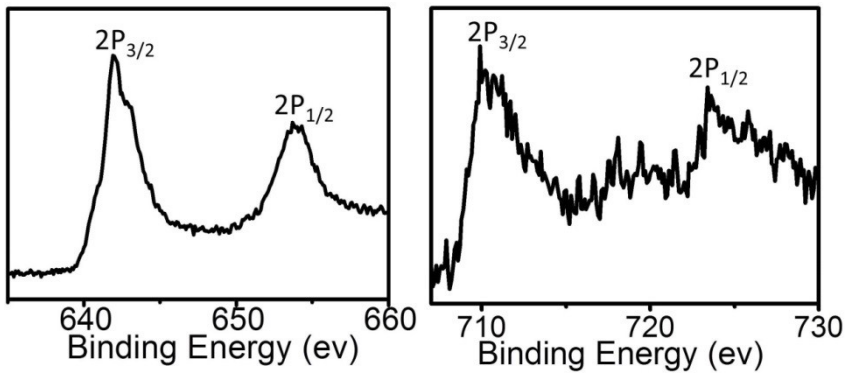


Figure S3. (a) Mn 2p and (b) Fe 2p X-ray photoelectron spectroscopy (XPS) spectra of FeMn-LDH.

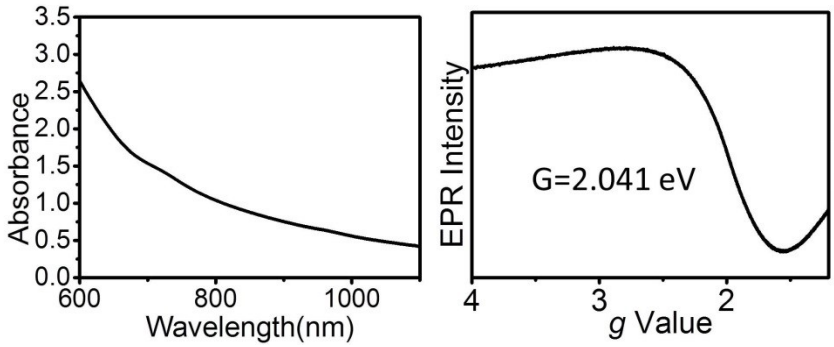


Figure S4. (A) Absorption spectrum of FeMn-LDH. (B) EPR spectrum of FeMn-LDH NPs on a common g-factor scale, suggesting the presence of oxygen deficiency.

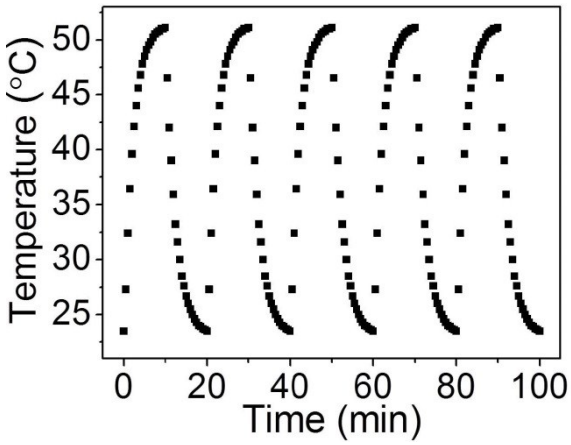


Figure S5. Heating curves of FeMn-LDH (400 ug $mL^{-1}$) for five laser on/off cycles.

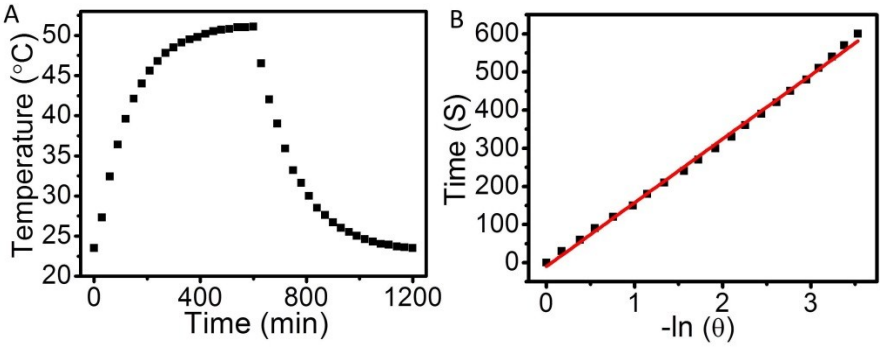


Figure S6. (A) The photothermal response of FeMn-LDH aqueous solution (400 μg $ml^{-1}$) for 10 min with an NIR laser (808 nm, 1.0 W $cm^{-2}$) and then the laser was shut off. (B) Linear time data versus −lnθ obtained from the cooling period of Figure S6A.

The photothermal transduction efficiency ($\eta$) is calculated as follows:[2]

$$\eta=\frac{hA(T_{max}-T_0)-Q_0}{I(1-10^{-A_\lambda})}\times 100\% \quad (1)$$

where $h$ is the heat transfer coefficient. $A$ is the surface area of the sample well. $T_{max}$ representing the equilibrium temperature is 51.1 °C. $T_0$ representing the ambient temperature is 23.5 °C. $Q_0$ is the heat energy of the quartz cell and solvent without nanoparticles, which is measured independently to be 20.0 mW. $A_{808}$ representing the absorption intensity of FeMn-LDH (400 μg mL$^{-1}$) at 808 nm is 1.01 (Figure S4A), and $\eta$ is the photothermal conversion efficiency.

The thermal time constant should be the same for either heating or cooling of the solution. $hA$ can be determined by applying the linear time data from the cooling period *vs* –lnθ (Figure S6B).

$$hA=\frac{m_sC\ +m_wC_w}{k} \quad (2)$$

where the mass of the sample solution ($m_s$) is 0.5 g, and its heat capacity value ($C_s$) is approximated to be 4.2 J g$^{-1}$ K$^{-1}$. The mass of quartz cell ($m_w$) is 0.773 g, and its heat capacity value ($C_w$) is approximated to be 0.839 J g$^{-1}$ K$^{-1}$. The slope of the linear equation in Figure S6B ($k$) is 168.

$$hA=\frac{0.773\times 0.839+4.2\times 0.5}{168}=0.0163 \quad (3)$$

Finally, substituting $hA$ value into Equation 1, the $\eta$ can be calculated as follow:

$$\eta=\frac{hA(T_{max}-T_0)-Q_0}{I(1-10^{-A_\lambda})}\times 100\%$$

(1)

$$=\frac{0.0163\times(51.1-23.5)-0.02}{1.0\times(1-10^{-1.1})}$$

$$=47.6\%$$

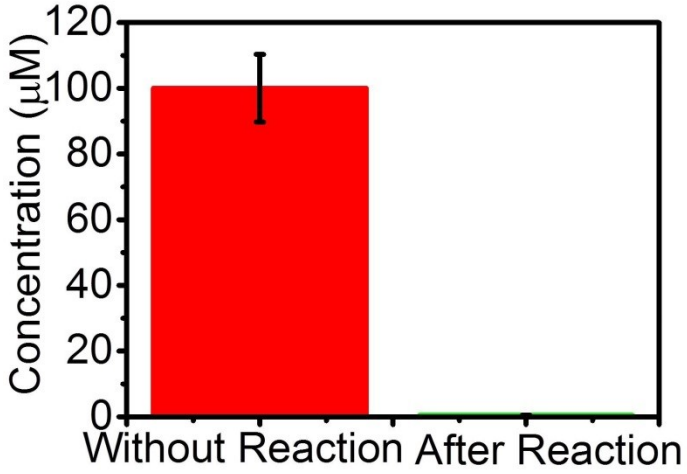


Figure S7. Concentration of $H_2O_2$ reacting without/with FeMn-LDH.

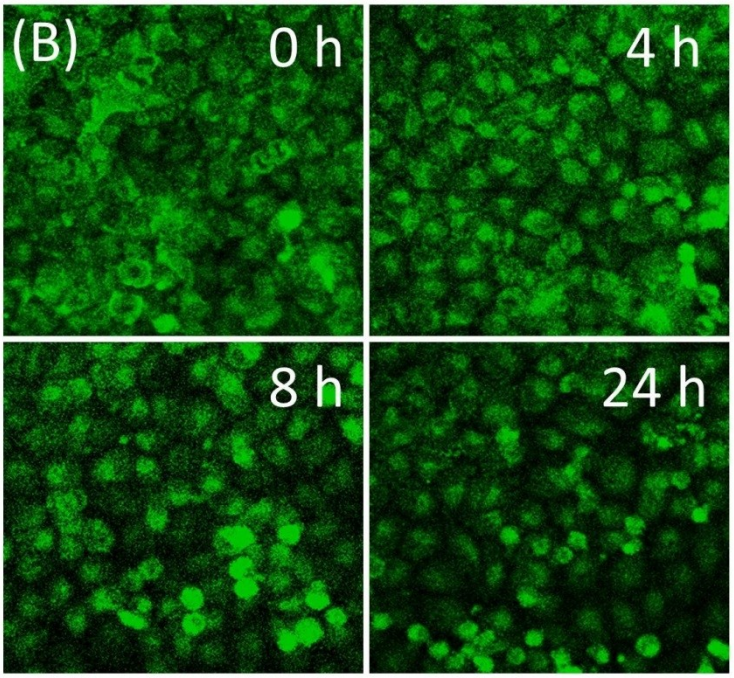


Figure S8. Fluorescence change of RDPP without FeMn-LDH at different time points (0, 4, 8, and 24 h).

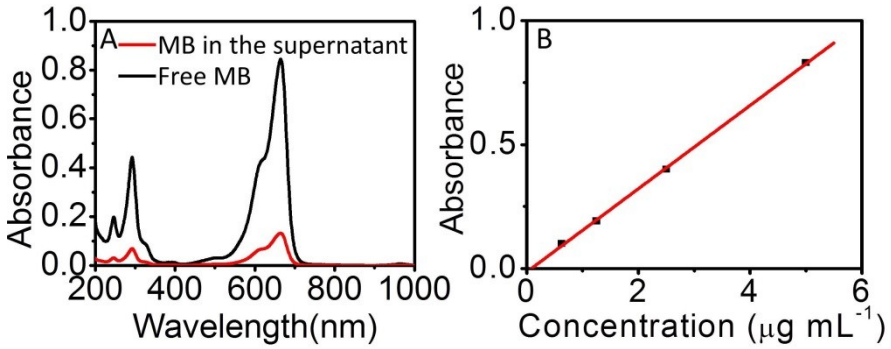


Figure S9. (A) UV-vis absorption spectra of MB before and after adsorption on FeMn-LDH. (B) Standard curve of aqueous MB solution drawn from the peak values of the UV-vis adsorption spectra.

The concentration of MB loaded onto FeMn-LDH was determined by measuring the characteristic absorbance of MB at 665 nm (Figure S9A). The loading concentration can be calculated according to the following formula:

Loading amount=$(C_i-C_s)/C_F$ (μg $mg^{-1}$)

Where $C_i$ representing the concentration of MB initially added is 5 μg $mL^{-1}$; $C_s$ representing the concentration of MB in the supernatant after loading is 0.9 μg $mL^{-1}$; $C_F$ representing the concentration of FeMn-LDH initially added is 200 μg $mg^{-1}$. So

Loading amount=(5-0.9)/0.2=20.5 μg $mg^{-1}$

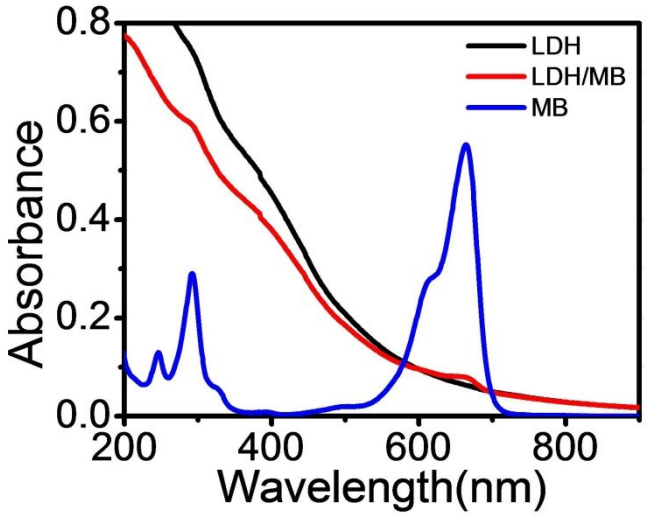


Figure S10. The UV-vis absorption spectra of FeMn-LDH, FeMn-LDH/MB, and MB.

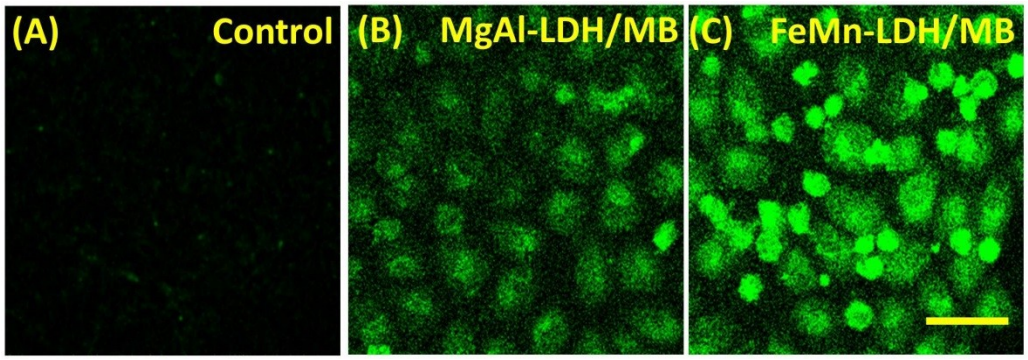


Figure S11. Intracellular ROS induced by MgAl-LDH/MB (B) and FeMn-LDH/MB (C) Scale bars: 50 μm.

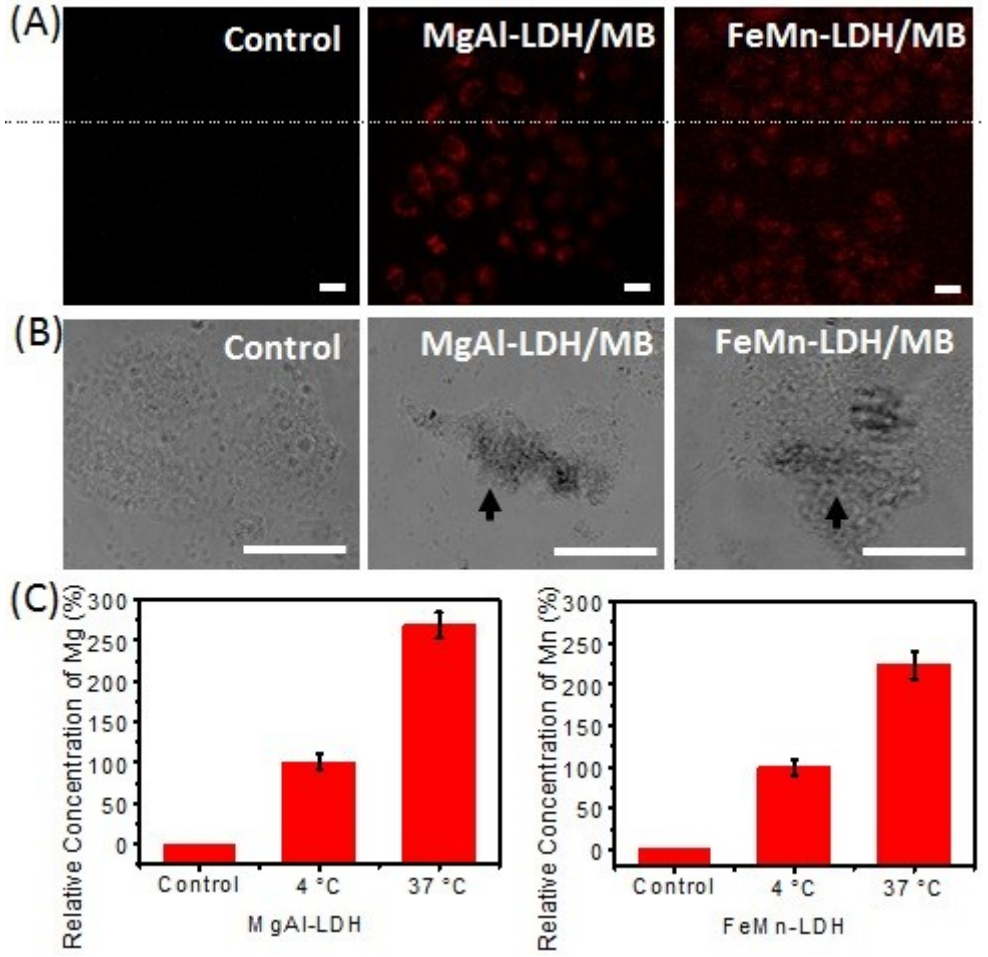


Figure S12. Internalization of FeMn-LDH/MB and MgAl-LDH/MB observed by (A) fluorescence of MB, (B) bright field of CLSM, and (C) ICP-MS. Scale bars: 20 μm.

The Internalization of FeMn-LDH/MB and MgAl-LDH/MB was observed by fluorescence of MB (Figure S12A) and bright field of CLSM (Figure S12B), which shown that both FeMn-LDH/MB and MgAl-LDH/MB can be internalized by HeLa cells. In addition, we compared the endocytosis of cells with FeMn-LDH/MB and MgAl-LDH/MB under conditions of 37 °C and 4 °C (inhibition of endocytosis)[3] by measuring the amount of element Mn, and found that the amount of uptake at 4 °C was much lesser than that at 37 °C, which indicated that the materials could be endocytosed by the cells at 37 °C (Figure S12C).

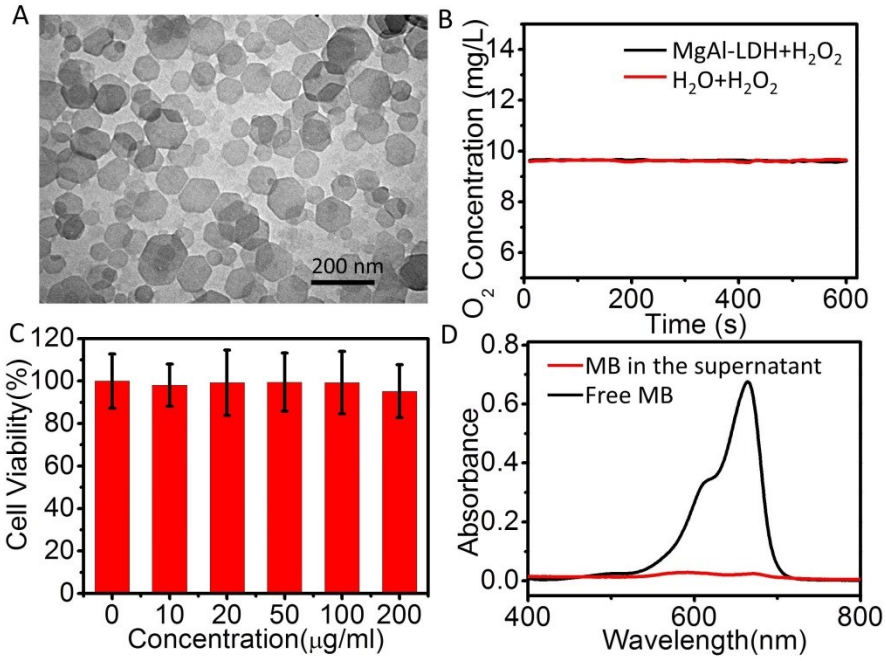


Figure S13. (A) TEM imaging of MgAl-LDH. (B) Oxygen generation between MgAl-LDH and $H_2O_2$. (C) Viability of the cells treated with at various concentrations of MgAl-LDH (0, 10, 20, 50, 100, and 200 μg $mL^{-1}$). (D) UV-vis absorption spectra of MB before and after absorption on MgAl-LDH.

The concentration of MB loaded onto MgAl-LDH was determined by measuring the characteristic absorbance of MB at 665 nm (Figure S13D). The loading concentration can be calculated according to the following formula:

Loading amount=$(C_i-C_s)/C_F$ (μg $mg^{-1}$)

Where $C_i$ representing the concentration of MB initially added is 4 μg $mL^{-1}$; $C_s$ representing the concentration of MB in the supernatant after loading is 0.01 μg $mL^{-1}$; $C_F$ representing the concentration of MgAl-LDH initially added is 200 μg $mg^{-1}$. So

Loading amount=(4-0.01)/0.2=19.95 μg $mg^{-1}$

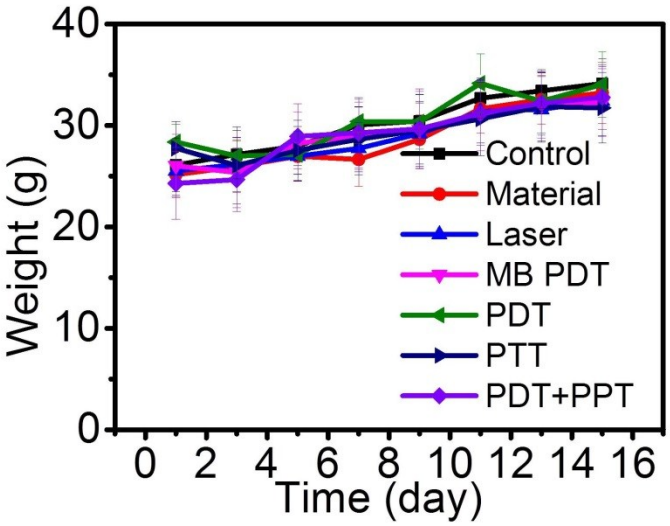


Figure S14. Body weight of mice at different treatment groups.

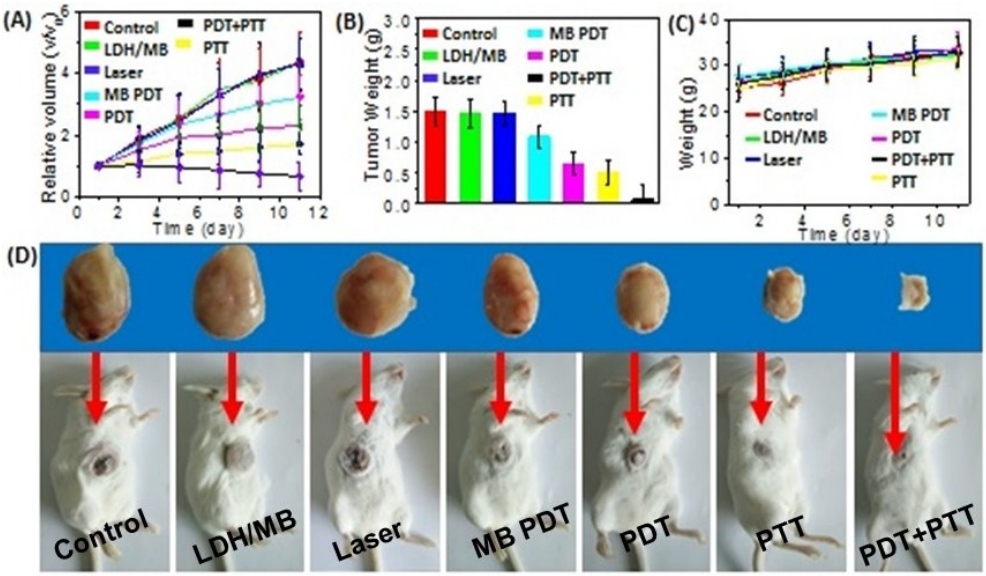


Figure S15. (A) Relative tumor volume, (B) mean tumor weights, and (C) body weight of tumor-bearing mice at different treatment groups. (D) Representative photographs of different groups of tumor-bearing mice after treatment.

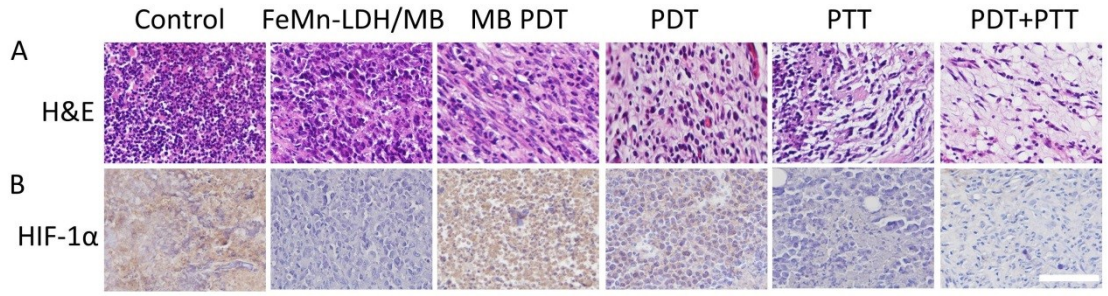


Figure S16. H&E staining (A) and HIF-1α staining (B) of the excised tumor sections after different treatment: saline (control), FeMn-LDH/MB, FeMn-LDH/MB+650 nm laser (PDT), FeMn-LDH/MB+808 nm laser (PTT), and FeMn-LDH/MB+808/650 nm (PDT+PTT). Scale bar = 50 μm.

After the treatments, hematoxylin and eosin (H&E) staining was used for the tumor histological analysis. It can be seen that in the control group, FeMn-LDH/MB group and the single-mode treatment groups, only little or partial necrosis appears, and most cells retain their regular morphology. In the dual-mode group, however, most cells showed necrosis and apoptosis with cell shrinkage and nuclei lysis, which confirm the effect of PDT/PTT synergistic therapy (Figure S16A).Then hypoxia-inducible factor (HIF-1α) staining assay was used to prove the ability of FeMn-LDH/MB to overcome hypoxia of tumor in vivo (Figure S16B). It can be seen that in the control group, the tumor tissues were stained brown and blue, and the MB PDT group were stained dark brown, indicating a lower $O_2$ concentration as the PDT process consuming $O_2$. In the FeMn-LDH/MB and PTT group, the tumor tissues were stained blue, suggesting that, as the presence of FeMn-LDH/MB, the tumor hypoxia was likely prevented. In the PDT and PDT/PTT groups, more brown color, compared to FeMn-LDH/MB group, was observed also due to the PDT process consuming $O_2$.

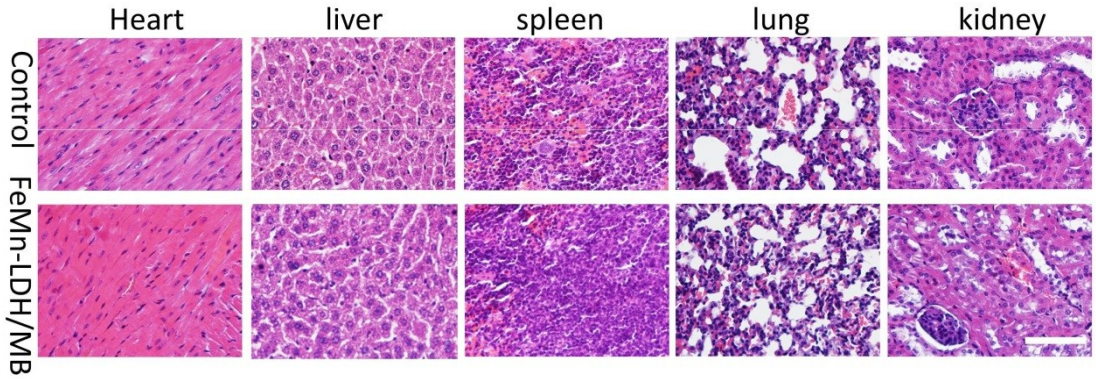


Figure S17. H&E staining of major organs from mice treated with or without FeMn-LDH/MB+808/650 nm laser irradiation. Scale bars: 50 μm.

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