Electronic Supplementary Information

A cancer cell membrane-encapsulated MnO_2 nanoreactor for combined photodynamic-starvation therapy

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Experimental section

Materials and Reagents. hexanol, Cyclohexane, Triton X-100, tetraethyl orthosilicate (TEOS), 1-(3-Diaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Alfa Aesar Chemical Ltd. (Tianjin, China); Potassium permanganate ($KMnO_4$), sodium carbonate (Na_2CO_3) and Hydrogen peroxide (H_2O_2) 30 wt% solution were purchased from Sinopharm Chemical Reagent Co., Ltd. (China); (3-aminopropyl)-triethoxysilane (APTES), Chlorin e6 (Ce6) was purchased from Frontier Scientific Co, Ltd. (USA); Protease inhibitors, glucose oxidase (GOx) were purchased from Sigma-Aldrich (USA).; Hypoxic bags were purchased from Mitsubishi Gas Chemical Co., Inc. (Japan); Tris(bathophenanthroline)ruthenium(II) chloride ($[Ru(dpp)_3]Cl_2$) was purchased from Heowns (China); 2′,7-dichlorodihydrofluorescein diacetate (DCFH-DA) was purchased from Beyotime (China). Murine melanoma cells (B16-F10) were purchased from Shanghai Aoluo Biotechnology Co. Ltd. The experimental water used was Mill-Q secondary ultrapure water (18.2 $M\Omega \cdot cm^{-1}$). The chemical reagents used in the experiment were analytical grade and without purification.

Instruments. Transmission electron microscopy (TEM, HT7700, Japan) was carried out to characterize the morphology of the nanoparticles. UV-Vis spectrophotometer (UV-1700, Shimadzu, Japan) was used to measure the UV-Vis absorption spectra. Fluorescence spectrometer (FLS980，Edinburgh Instruments Ltd., UK) was used to detect the fluorescence of nanoparticles. Fourier infrared spectrometer (Nicolet iS50 FT-IR) was used to measure the infrared spectrum. pH

were measured by a pH-3c digital pH-meter (Shanghai LeiCi, China). Confocal laser scanning microscope (LEICA TCS SP5, Germany) and live animal imaging system (IVIS Lumina III, US) were applied in vitro cell experiments and vivo imaging, respectively. MTT microplate reader (Rayto RT-6000, US) and inductively coupled plasma atomic emission spectrometer (ICP-AES，iCAP 7600，Thermo Fisher, US) were used in this work.

Synthesis of H-MnO_2-GOx-Ce6@C. Hollow manganese dioxide (H-MnO_2) nanoparticles were synthesized following the reported method.[1] First, Solid silica nanoparticles ($sSiO_2$) were synthesized through inverse microemulsion method.[2] 5.3 mL TX-100, 22.5 mL Cyclohexane and 5.4 mL n-hexanol were added to a 100 mL flask and stirred 5 min, then 0.75 ml ammonia and 1.0 mL water were added, then stirred for 30 min. Finally, 500 μL TEOS and 100 μL APTES were mixed and added to the above solution. The mixture was stirred for 24 h in room temperature. The $sSiO_2$ was obtained by centrifugation and washed three times with ethanol and water. Under ultrasonication, $KMnO_4$ solution (150 mg) was added drop by drop into the suspension of $sSiO_2$ (20 mg) and continuous ultrasound for 6 h. The mesoporous MnO_2-coated $sSiO_2$ was obtained by centrifugation. The as-prepared mesoporous MnO_2-coated $sSiO_2$ was dissolved in Na_2CO_3 solution (2 M) at 60 °C for 12 h and the H-MnO_2 was obtained by centrifugation at 14,800 rpm and washed several times. The obtained H-MnO_2 was functionalized with amino groups by using APTES. Ce6 (6 mg) was added to the solution containing above H-MnO_2 and stirred for 24 h in the darkness to form H-MnO_2-Ce6. The carboxyl group in GOx (4 mg) was activated by

EDC (95 mg) and NHS (57 mg) for 30 min, and then stirred with H-MnO_2-Ce6 for 24 h at 4 ^oC. The H-MnO_2-GOx-Ce6 was obtained by centrifugation and washed three times with water. The cell membrane of the B16-F10 cells was extracted via the reported method.[3] And the membrane was added to the H-MnO_2-GOx-Ce6 and the mixture was stirred overnight. The final H-MnO_2-GOx-Ce6@C was centrifuged and washed several times with water.

Detection of $^1O_2$. ABMD was used for $^1O_2$ detection. H-MnO_2-GOx-Ce6 were dispersed in oxygen-free PBS with glucose added or not, and then incubated with ABMD. And then irradiated with 655 nm laser light (0.1 $W \cdot cm^{-2}$ for 5 min). UV-Vis absorption of ABMD was determined after centrifugation.

Cell culture. B16-F10 cells were treated with high-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and 1% 100 U/mL penicillin/streptomycin and were incubated under 37 °C within 5% CO_2 atmosphere. Anaerobic culture condition was 5% CO_2, 1% O_2 and 94% N_2 at 37 °C.

MTT assay. For the cell toxicity assay, B16-F10 cells were seeded onto a 96-well plate (10^4 per well) and cultured for 24 h. Then the H-MnO_2 of different concentrations (1, 2, 4, 8, 10, 20, 40, 80, 100, and 200 μg/mL) in DMEM media were added into the well and co-incubated for 24 h. Then the media were removed and 150 μL of MTT solution (0.5 mg/mL) were added into each wells. After 4 h, MTT solution was removed and 150 μL DMSO were added and the absorbance was monitored at 490 nm by a microplate reader.

For PDT effect, B16-F10 cells were incubated with PBS, H-MnO_2-GOx@C, H-

MnO_2-Ce6@C and H-MnO_2-GOx-Ce6@C under anaerobic condition for 4 h, respectively. And then were treated with 655 nm laser light (0.1 W cm^{-2}, 5 min). After 24 h, their relative viabilities were tested.

Confocal imaging. To verify the O_2 generation in the cells, B16-F10 cells were cultured overnight in confocal dishes, and PBS， H-MnO_2@C and H-MnO_2-GOx-Ce6@C (20 μg/mL) were added for co-incubated under normal O_2 or anaerobic conditions for 4 h, then incubated with $[Ru(dpp)_3]Cl_2$ probe for 6 h. The cells were washed three times with PBS before confocal imaging.

To detect the ROS in the cells, B16-F10 cells were incubated a confocal dishes overnight, and then incubated with H-MnO_2-Ce6@C and H-MnO_2-GOx-Ce6@C under hypoxic conditions for 4 hours. Next the cells were incubated for 15 minutes with DCFH-DA probe, followed by 655 nm laser irradiation (0.1 W cm^{-2}, 5 min). The cells were washed three times with PBS before confocal imaging.

Animal model. All procedures of animal study were approved by Principles of Laboratory Animal Care (People's Republic of China) and the Guidelines of the Animal Investigation Committee, Biology Institute of Shandong Academy of Science, China. Female Balb/c mice (6-8 weeks) were housed under normal conditions. B16-F10 cells (10^6) were suspended in 100 μL DMEM media and subcutaneously injected into the back of the mice. The tumor volume was calculated by the formula: volume = length × $width^2/2$. The mice were treated when the tumor volumes were about 70 mm^3.

***In vivo* treatment.** The tumor-bearing mice were injected intravenously with PBS,

Ce6， H-MnO_2-GOx-@C, H-MnO_2-Ce6@C and H-MnO_2-GOx-Ce6@C (dose of MnO_2 = 10 mg kg^{-1}, Ce6 = 1.05 mg kg^{-1}), respectively. 24 h later, the tumor was treated with 655 nm laser light (0.1 W cm^{-2}, 15 min). Tumor volume and body weights were recorded every 2 days for 2 weeks. The H&E staining of the organs (heart, liver, spleen, lung, and kidney) were tested at 7 days post-injection and the tumors were tested at 12 h post-treatment.

***In vivo* antitumor efficacy of relapse.** When the B16-F10 tumors on the back of the mice reached about 300 mm^3, the tumors were excised and only a small amount of residual tissue was left. After the wound healing of the mouse, PBS, Ce6, H-MnO_2-GOx-@C, H-MnO_2-Ce6@C and H-MnO_2-GOx-Ce6@C (dose of MnO_2 = 10 mg kg^{-1}, Ce6 = 1.05 mg kg^{-1}) were injected intravenously into the mice, respectively. After 24 h, the tumor area was treated with a 655 nm laser light (0.1 W·cm^{-2}) for 15 minutes. The body weight was measured every other day for 20 days.

References

1. G. Yang, L. Xu, Y. Chao, J. Xu, X. Sun, Y. Wu, R. Peng and Z. Liu, *Nat. Commun.*, 2017, **8**, 902.
2. D. Chen, L. Li, F. Tang and S. Qi, *Adv. Mater.*, 2009, **21**, 3804-3807.
3. Z. Yu, P. Zhou, W. Pan, N. Li and B. Tang, *Nat. Commun.*, 2018, **9**, 5044.

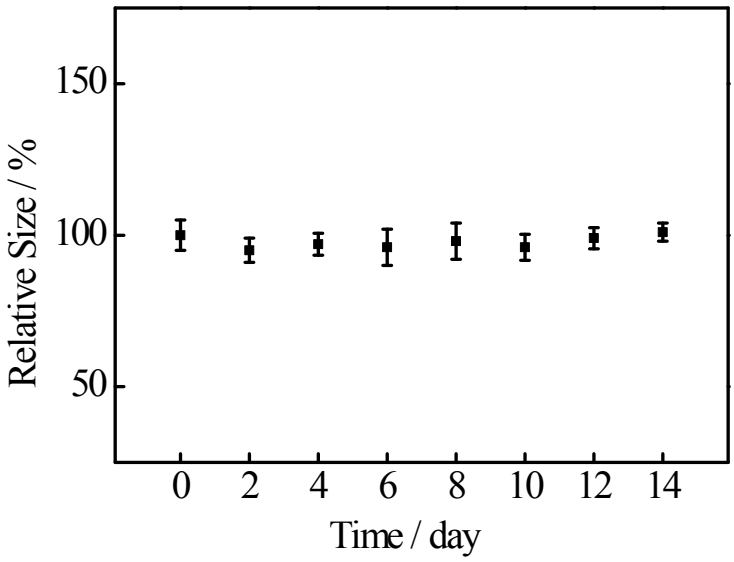

Fig. S1 Size change of H-MnO_2 within 14 days in water.

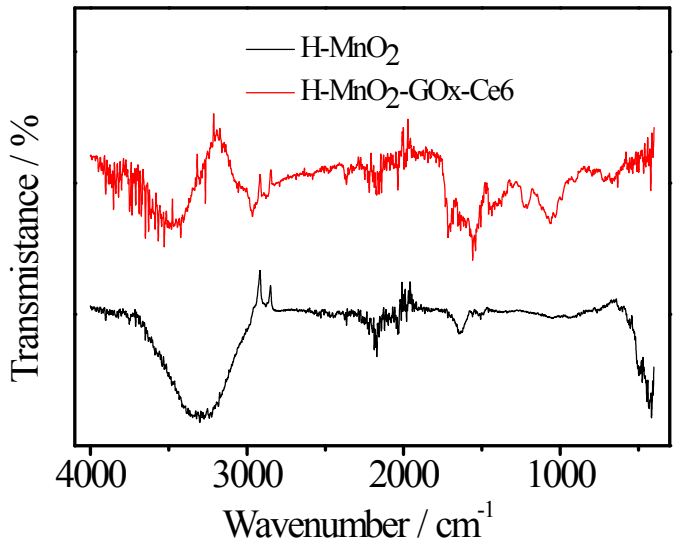

Fig. S2 Infrared spectra of H-MnO_2 and H-MnO_2-GOx-Ce6.

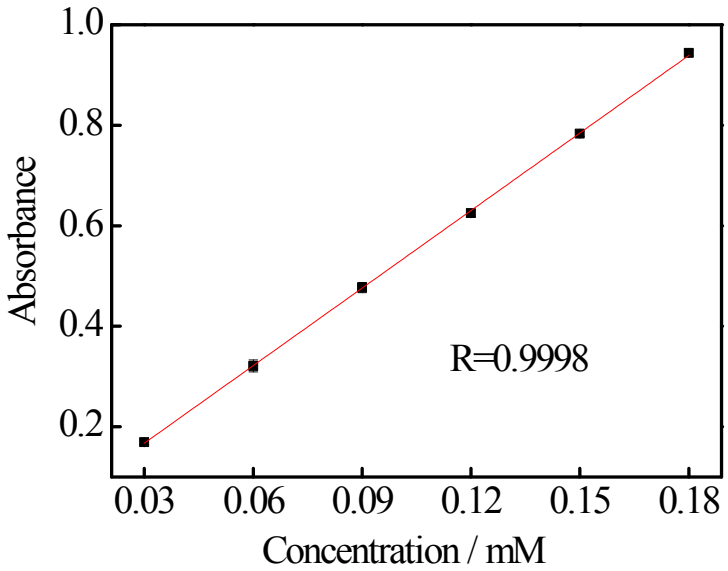

Fig. S3 UV absorption standard curve of Ce6 at 608 nm.

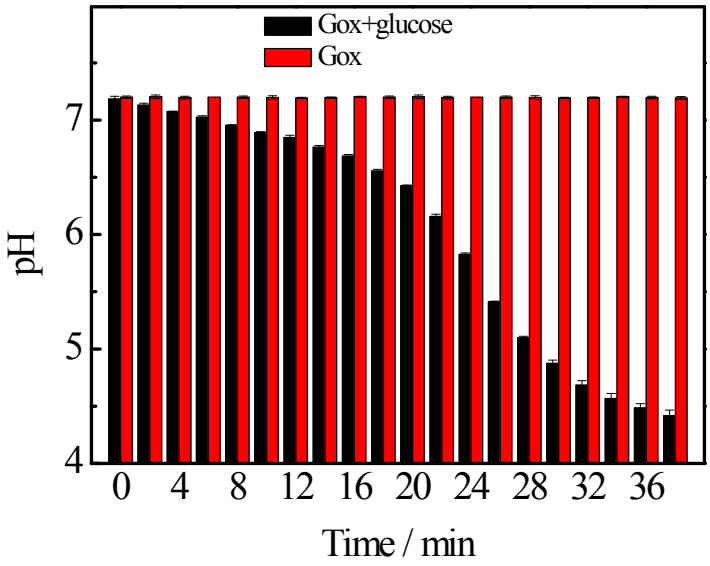

Fig. S4 The pH value changes of GOx solution in the presence or absence of glucose at 37 ^{o}C.

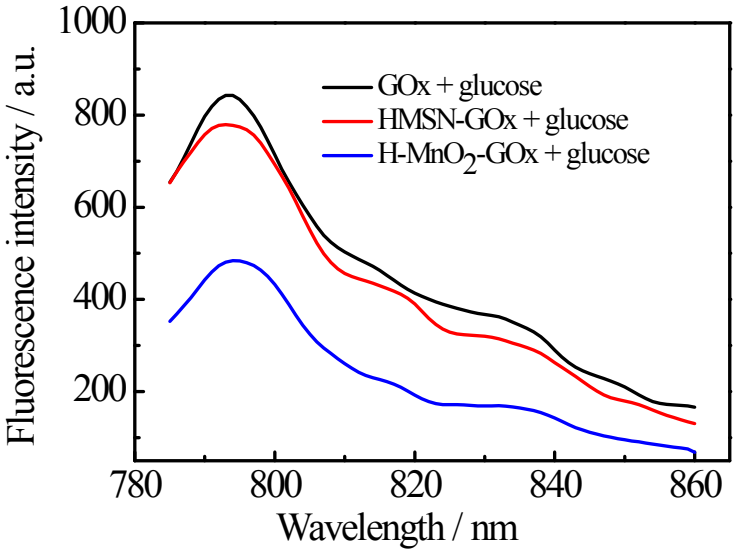

Fig. S5 Fluorescence spectra of Cy-O-Eb when GOx, HMSN-GOx and H-MnO_2-GOx incubated with glucose.

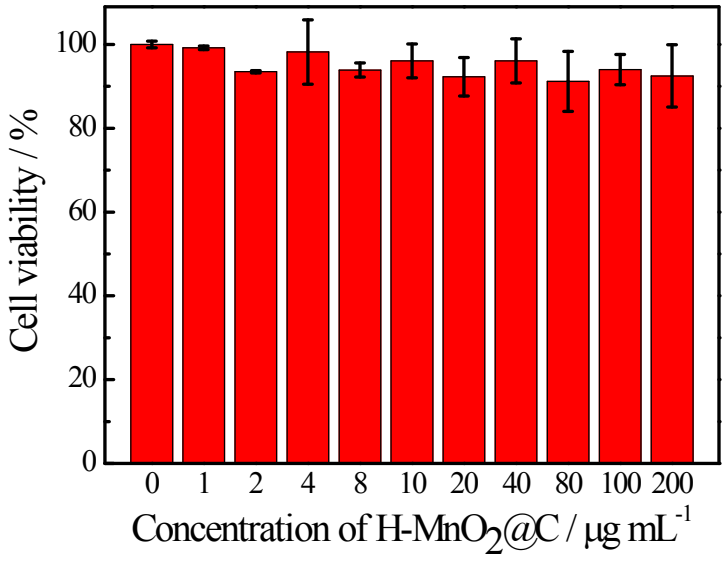

Fig. S6 Cell viability of B16-F10 cells incubated with various concentrations of H-MnO_2@C for 24 h.

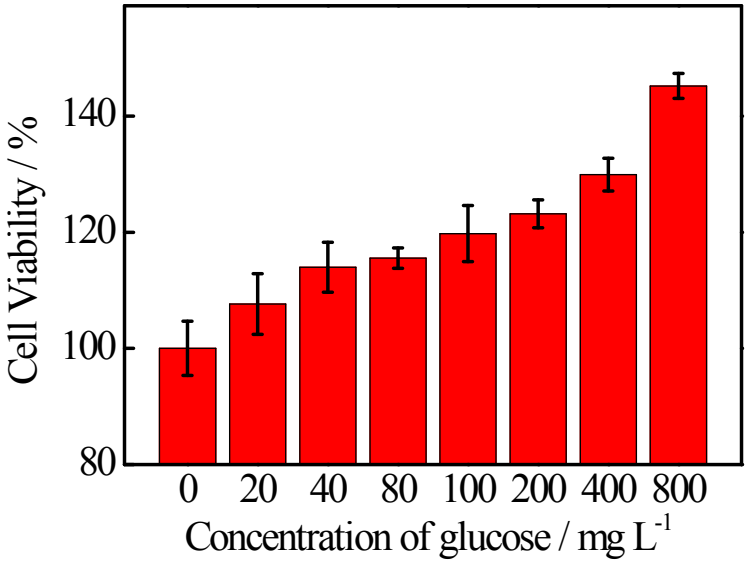

Fig. S7 Cell viability of B16-F10 cells treated with glucose for 24 h at various concentrations.

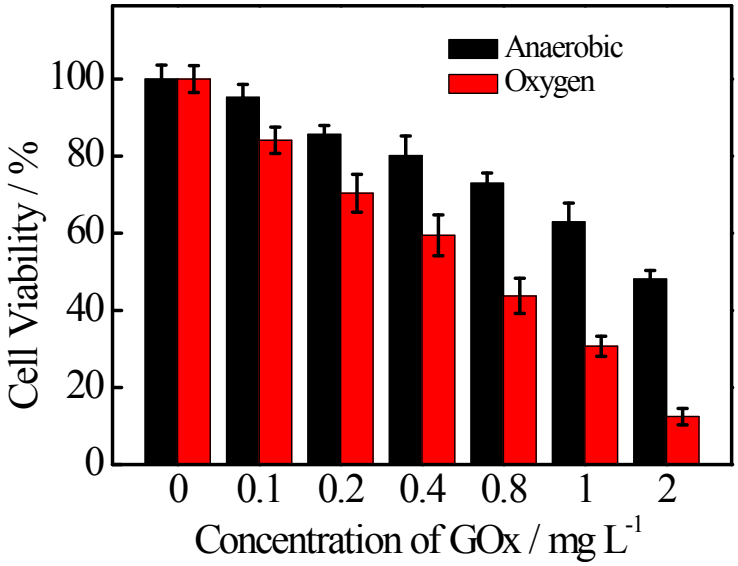

Fig. S8 Cells incubated with GOx separately under hypoxic and normal oxygen conditions.

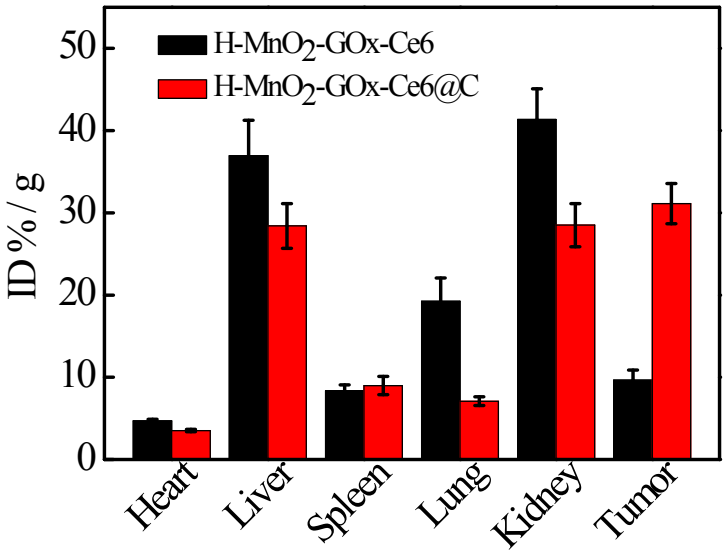

Fig. S9 Biodistribution of H-MnO_2-GOx-Ce6 and H-MnO_2-GOx-Ce6@C at 24 h post-injection by ICP-AES.

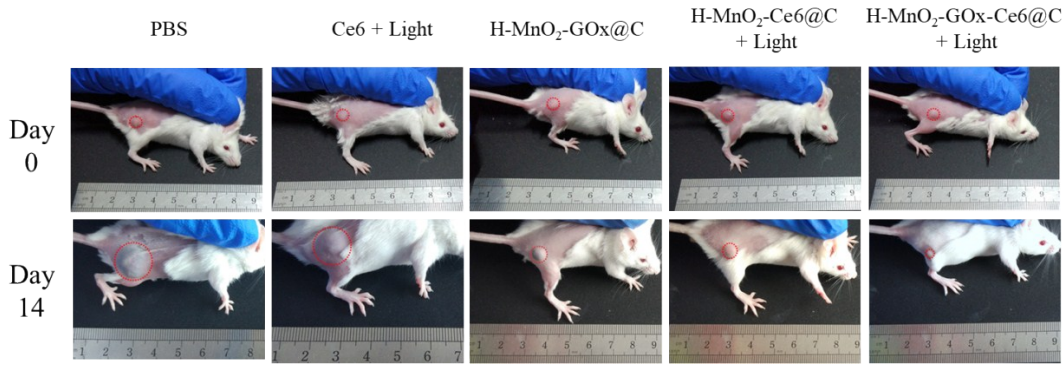

Fig. S10 Photographs of tumors in mice at day 0 and day 14.

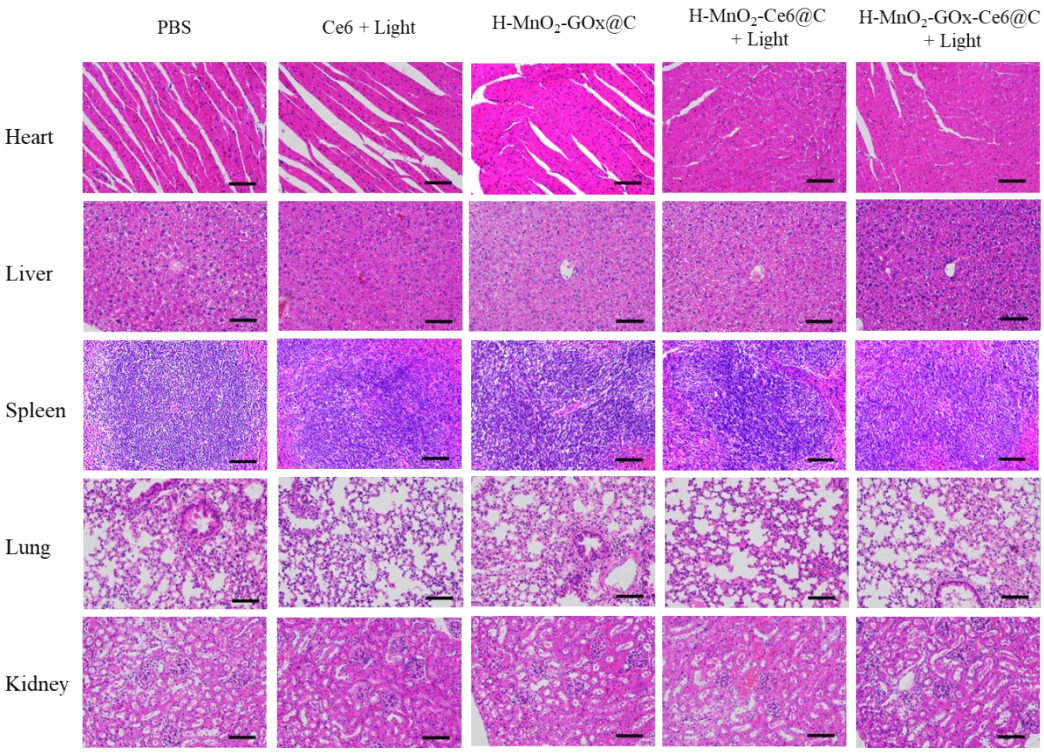

Fig. S11 H&E staining of heart, liver, spleen, lung and kidney in the mice from various groups (Scale bars are 100 μm).

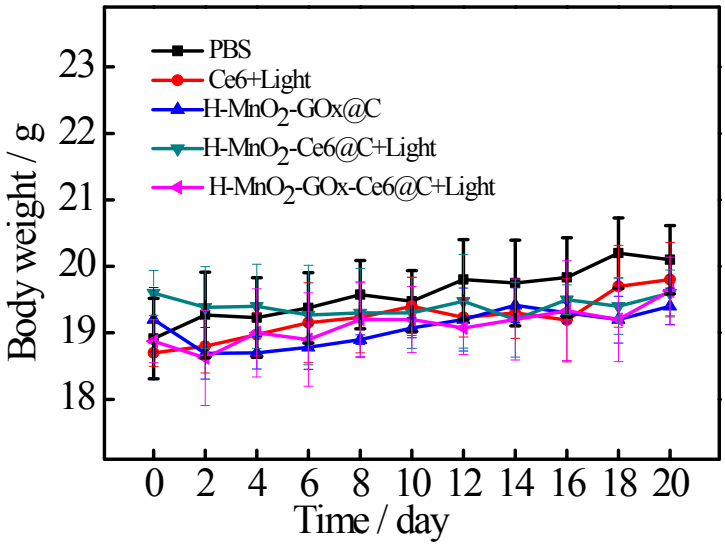

Fig. S12 Body weight changes of tumor-bearing mice. They were measured every other day for 20 days.