Supplementary Information

A cancer cell membrane-camouflaged nanoreactor for enhanced radiotherapy against cancer metastasis

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1. Experimental section

1.1 Materials and reagents. Potassium permanganate, dimethyl sulfoxide, ammonia and anhydrous ethanol were purchased from Shanghai National Pharmaceutical Group Chemical Reagent Co.,Ltd. (3-aminopropyl) triethoxysilane (APTES) was purchased from Tianjin Heowns Biochemical Technology Co.,Ltd. 1-ethyl-3-(3-dimethlyaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Alfa Aesar Chemical Co.,Ltd. Anatase crystal titanium dioxide NPs and glucose oxidase were purchased from Sigma-Aldrich. The water used was Mill-Q secondary ultrapure water (18.2 MΩ/cm). Mouse skin melanoma (B16-F10) and mouse breast cancer cell line (4T1-Luc) were purchased from Shanghai Aolu Biotechnology Co., Ltd.

1.2 Instruments. Low resolution transmission electron microscope (HT7700, Japan). High speed refrigerated centrifuge (Sigma 3K 15, USA). X-ray powder diffractometer (Bruker D8, Germany). FLS-980 fluorescence photometer (Edinburgh Instruments Ltd, UK). Laser confocal fluorescence microscopy (Leica TCS SP8, Germany). Small animal imaging device (IVIS Lumina III, USA). Enzyme marker (Synergy 2, Biotek, USA). Homogenizer (IKA, Germany, T10 basic ultra-turrrax). Medical linear accelerator (Siemens Primus HI, Germany).

1.3 Synthesis of TiO_2-NH_2. First, 6 mg of TiO_2 NPs were dissolved in 10 mL of anhydrous ethanol, and then 150 μL of water and 70 μL of ammonia were added, under stirring for 30 minutes. After, 50 μL of APTES was added to the above solution. After stirring overnight at room temperature, the mixture was centrifuged (10,000 rpm, 10 min), and the precipitate was washed with anhydrous ethanol for 3 times and water for 2 times. Finally, TiO_2-NH_2 was dispersed in 10 mL of water. Amino-functionalized TiO_2 (TiO_2-NH_2) NPs were obtained.

1.4 Synthesis of TiO_2@MnO_2. $KMnO_4$ aqueous solution (300 mg in 10 mL H_2O) was added dropwise into TiO_2-NH_2 (40 mg in 15 mL H_2O) under ultrasonic condition, and then the mixed solution were reacted for 2 hours to obtain TiO_2@MnO_2 nanoparticles. The mixture was

centrifuged (10,000 rpm, 10 min), and the precipitate was washed with water for 3 times. Finally, the mixed was dispersed in water. TiO_2@MnO_2 NPs were obtained.

1.5 Synthesis of TiO_2@MnO_2-NH_2. 15mg of TiO_2@MnO_2 NPs was dispersed in a mixed solution of ethanol (20 mL) and water (200 μL) , mix evenly, then to join 200 μL ammonia adjust the pH value of solution, stirring for 30 min, and then 80 μL APTES was added to the above solution. After stirring overnight , the mixture was centrifuged (10,000 rpm, 10 min), and the precipitate was washed with anhydrous ethanol for 3 times and water for 2 times. Finally, TiO_2@MnO_2-NH_2 was dispersed in water. TiO_2@MnO_2-NH_2 NPs were obtained.

1.6 Synthesis of TiO_2@MnO_2-GOx. The carboxyl group of GOx was linked to the amino group on the surface of TiO_2@MnO_2 by an amide bond. 1 mg of GOx, 25 mg of EDC and 15 mg of NHS were dissolved in 1.0 mL, 0.5 mL and 0.5 mL PBS, respectively. Then, the mixture was mixed, and the activated GOx was obtained by standing in the dark for 30 min. The activated GOx was added to TiO_2@MnO_2-NH_2 solution and stirred for 24 h under low temperature and dark conditions. The mixture was centrifuged (10,000 rpm, 10 min), and the precipitate was washed with water for 3 times, and finally dispersed in PBS (pH=7.4) to obtain TiO_2@MnO_2-GOx NPs.

1.7 Cell culture. Two cell models were used throughout the experiement, the B16-F10 cells used were cultured in high-glucose DMEM medium containing 10% FBS and 100 U/mL 1% antibiotics (penicillin/streptomycin). 4T1-Luc cells were cultured in RPMI 1640 medium containing 10% FBS and 100 U/mL 1% antibiotics (penicillin/streptomycin).

1.8 Synthesis of TiO_2@MnO_2-GOx@C. The two cell models used in the experiment were coated with different cell membranes to achieve homologous targeting. First, the cells are digested and centrifuged for cell precipitation. Cells were washed twice with tris, $MgCl_2$ (pH=7.4) buffer, and resuspended with tris buffer with protease inhibitor. The cells were broken by homogenizer every 2 min for duration of 17 s for a total of 8 times. The whole

process was performed in an ice water bath. The cell membrane fragments were obtained by differential centrifugation of the broken cells. The cell membrane fragments were first centrifuged at 500 rpm for 10 min, then the precipitate was discarded and the supernatant was retained. Then the supernatant was centrifuged for 10 min at a speed of 10000 rpm. The precipitate was discarded again and the supernatant was taken. Then the supernatant was mixed with nanomaterials and stirred at a low temperature for 24 h at a slow speed, the membrane coating material was successfully obtained by centrifugation.[3]

1.9 Verification of the activity of GOx in TiO_2@MnO_2-GOx NPs. The activity of GOx in TiO_2@MnO_2-GOx NPs were tested by Diammonium 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS). TiO_2@MnO_2-GOx solution was divided into two groups (1 mM of glucose + peroxidase + ABTS, ABTS only), after reaction for 2 hours, centrifugation was carried out, and the supernatant was taken to measure the ultraviolet absorption spectrum.

1.10 Verification of the generation of reactive oxygen species. Firstly, the ability of producing O_2 by hydrogen peroxide catalyzed by manganese dioxide shell was tested. The H_2O_2 solution of 100 μM can be divided into three groups: TiO_2@MnO_2 NPs group, TiO_2 NPs group, and the control group. The content of oxygen in the solution was measured at different times by using a dissolved oxygen meter.

The ability of TiO_2@MnO_2-GOx to produce reactive oxygen species was tested using DCFH, a reagent that detects reactive oxygen species. Six control groups were set up: TiO_2@MnO_2-GOx group with glucose, TiO_2@MnO_2-GOx group without glucose, TiO_2@MnO_2 group, TiO_2 group, control group, X-rays only. Then, DCFH, a reagent for the detection of reactive oxygen species, was added to all the five groups and incubated for 4 h. Then, the first five groups were irradiated by 4 Gy X-ray, while the last group was not irradiated. After centrifugation, the supernatant was taken to measure the fluorescence spectrum.

1.11 MTT assays. B16-F10 cells and 4T1-Luc cells were inoculated into 96-well plates (about

104 cells in each well) and divided into 7 groups, and then the cells were allowed to stick to the wall overnight. TiO_2@MnO_2@C was diluted with culture medium to different concentrations (5, 10, 20, 50, 100 and 200 μg/mL), and then added to the 96-well plate for incubation for 24 hours. Then it was sucked out and discarded. 0.5 mg/mL MTT solution 150 μL was added for incubation for 4 h. Finally, the MTT was sucked out and discarded, and 150 μL DMSO was added. The absorbance of each group at 490 nm was measured by a microplate reader.

1.12 Cell cloning experiment. Cloning experiments were conducted using two cell models, B16-F10 cells and 4T1-Luc cells, using the same method. Will first vaccination in 60 mm cells in a petri dish, each number about 1000 cells in a petri dish, divided into 6 groups, and cells at 37 ℃ and 5% CO_2 incubator in the training, so that it is stick to the wall. Then the cells in the group were treated differently: the control group, the separate X-ray irradiation group, the TiO_2@MnO_2-GOx@C (0.1 mg/mL) group without X-ray irradiation, the TiO_2@C (0.1 mg/mL) group with X-ray irradiation, the TiO_2@MnO_2@C (0.1 mg/mL) group with X-ray irradiation, and the TiO_2@MnO_2-GOx@C group with X-ray irradiation. The 6 groups of cells in a lack of oxygen environment to cultivate 8 h, then, with 4 Gy doses of X ray to light, and then washing cells with PBS buffer to remove residues of NPs, and add fresh culture medium containing 10% fetal bovine serum and cells at 37 ℃ and 5% CO_2 incubator in 12 days. The cells were immersed in 4% paraformaldehyde for 15 min, and then stained with 0.2% crystal violet. The cell communities with more than 50 cells were counted. The surviving fraction = (surviving colonies) / (cells seeded × plating efficiency).[1]

1.13 Oxygen detection experiment. In order to verify that TiO_2@MnO_2-GOx@C can improve the hypoxic environment of tumor site, $[Ru(DPP)_3]Cl_2$ probe was used for oxygen detection experiment. First, the cells were divided into four groups and incubated overnight to adhere to the wall. Then, the four groups of cells were incubated with PBS, TiO_2@C, TiO_2@MnO_2@C

and TiO_2@MnO_2-GOx@C, respectively, and placed in hypoxic environment for incubated 4 h. Then, probe $[Ru(DPP)_3]Cl_2$ was added to the four groups of cells for incubated 6 h. The cells were washed with PBS 3 times and observed through CLSM.[2]

1.14 Active oxygen detection experiment. In order to verify that TiO_2@MnO_2-GOx@C can generate more reactive oxygen species under illumination, DCFH-DA probe is used to detect reactive oxygen species. First, the cells were divided into four groups and incubated overnight to adhere to the wall. Then, the four groups of cells were incubated with PBS, TiO_2@C, TiO_2@MnO_2@C and TiO_2@MnO_2-GOx@C, respectively, and placed in hypoxic environment for incubated 4 h. Then, probe $[Ru(DPP)_3]Cl_2$ was added to the four groups of cells for incubated 15 min and irradiated with an X-ray of 4 Gy. The cells were washed with PBS 3 times and observed through CLSM.

1.15 Animal tumor models. The cells were digested with trypsin, redispersed in serum-free medium, and then intravenously injected into Balb/c mice. Then B16-F10 metastatic tumor model was successfully established 7 days later, and 4T1-Luc metastatic tumor model was successfully established 4 days later.[3]

1.16 The metabolism products of nanoreactor. The mice B16-F10 metastatic tumor was intravenously injected with 150 μL TiO_2@MnO_2-GOx@C nanoparticles solution (1.2 mg/mL), and their excretions in 48 h were collected. The urine was washed with water for three times (the total volume was about 30mL) and the feces was dissolved in water. These solutions were centrifuged, and the precipitate and supernatant were collected. The centrifuged product of excretions was used in TEM and XRD tests. The supernatant and precipitate of urine and feces were used for the ICP-AES test.

1.17 Verification of the targeting of cell membrane. B16-F10 metastatic tumor mice were divided into two groups, one group was intravenous injected with TiO_2@MnO_2-GOx NPs, and the other group was intravenous injected with TiO_2@MnO_2-GOx@C NPs. After 24 hours, the

mice were dissected, and the organs and tumor tissues were respectively dissolved with aqua regia for ICP test.

1.18 Treatment of mice. The metastatic tumor mice were divided into six groups: PBS control group, single X-ray irradiation group, TiO_2@MnO_2-GOx@C treatment without X-ray irradiation group, TiO_2@C treatment and X-ray irradiation group, TiO_2@MnO_2@C treatment and X-ray irradiation group, and TiO_2@MnO_2-GOx@C treatment and X-ray irradiation group. The mice were fed for 14 days, during which they were weighed once every two days. After 14 days, B16-F10 metastatic tumor mice were dissected to observe the growth of lung tumors and photographed. Fluorescein was intraperitoneally injected into 4T1-Luc metastatic tumor mice, and imaging in vivo was performed 10 min later. Then, the mice were dissected to observe the light intensity in the lungs. Histological analysis was performed using hematoxylin - eosin staining (H&E) of the tumor, heart, liver, spleen, lung and kidney. Another group of mice were taken, and after treatment, the six groups of mice were fed separately until day 40, and the survival of the mice were recorded.

2. Supplementary figures

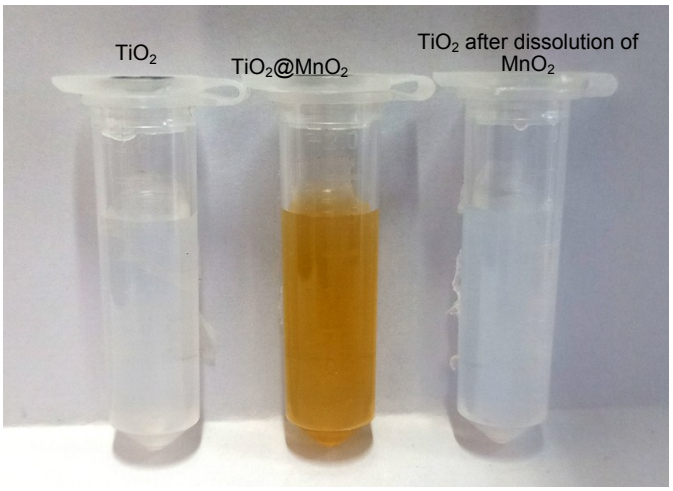

Fig. S1 Photograph of TiO_2, TiO_2@MnO_2 and TiO_2 after dissolution of MnO_2 in aqueous solution.

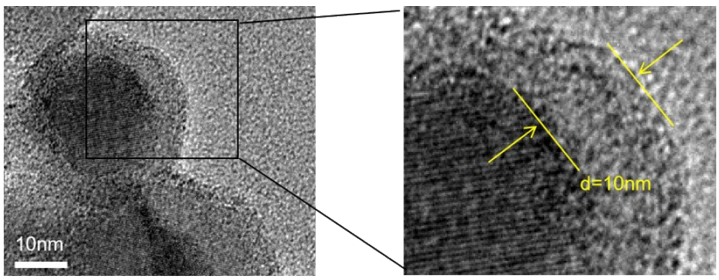

Fig. S2 HRTEM images of TiO_2@MnO_2-GOx@C nanoparticles.

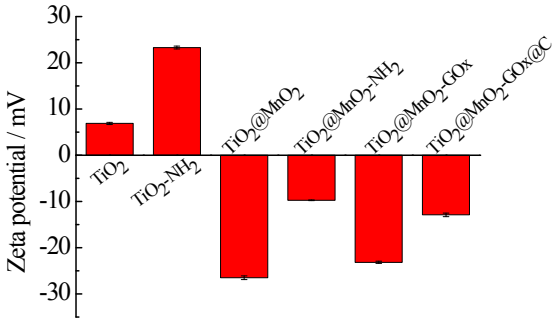

Fig. S3 Zeta potential of different nanoparticles.

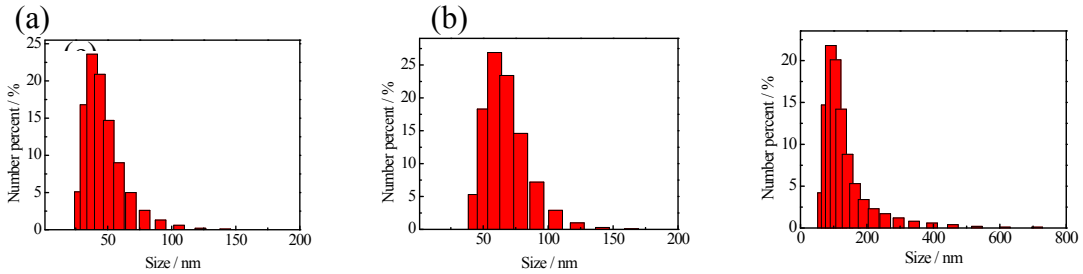

Fig. S4 Hydrodynamic diameter of (a) TiO_2, (b) TiO_2@MnO_2, (c) TiO_2@MnO_2-GOx@C nanoparticles.

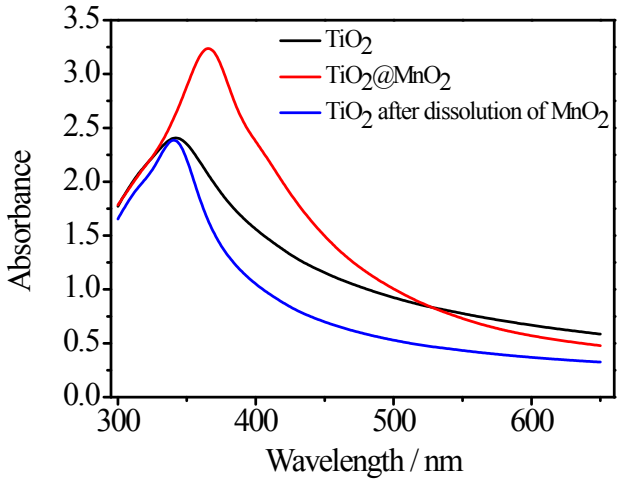

Fig. S5 Absorption spectra of TiO_2, TiO_2@MnO_2 and TiO_2 after dissolution of MnO_2.

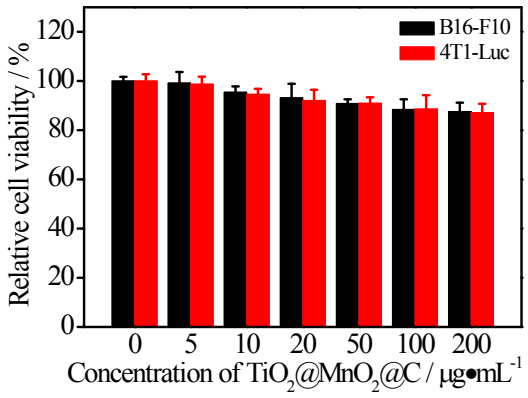

Fig. S6 The viability of B16-F10 cells and 4T1-Luc cells when incubated with different nanoparticles.

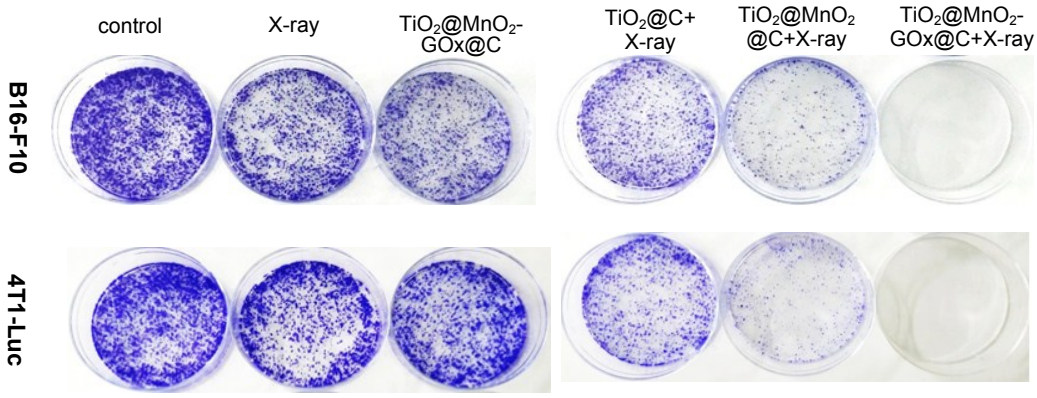

Fig. S7 Photographs of the surviving fractions B16-F10 cells and 4T1-Luc cells after different treatments. From left to right: PBS, X-ray only, TiO_2@MnO_2-GOx@C, TiO_2@C with X-ray, TiO_2@MnO_2@C with X-ray, TiO_2@MnO_2-GOx@C with X-ray.

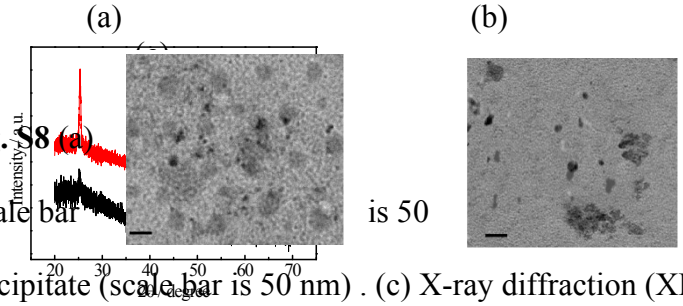

Fig. S8 (a) TEM image of urine precipitate (scale bar is 50 nm). (b) TEM image of fecal precipitate (scale bar is 50 nm) . (c) X-ray diffraction (XRD) diffractograms of TiO_2 and metabolite.

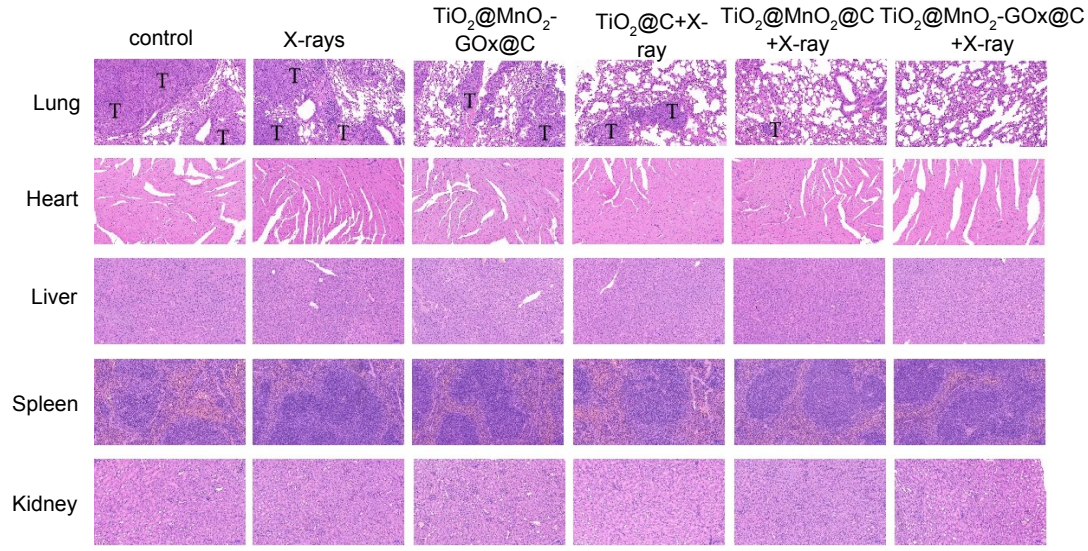

Fig. S9 Hematoxylin & eosin (H&E)-stained lung slices from B16-F10 tumor mice after different treatments. From left to right: PBS, X-ray only, TiO_2@MnO_2-GOx@C, TiO_2@C with X-ray, TiO_2@MnO_2@C with X-ray, TiO_2@MnO_2-GOx@C with X-ray.

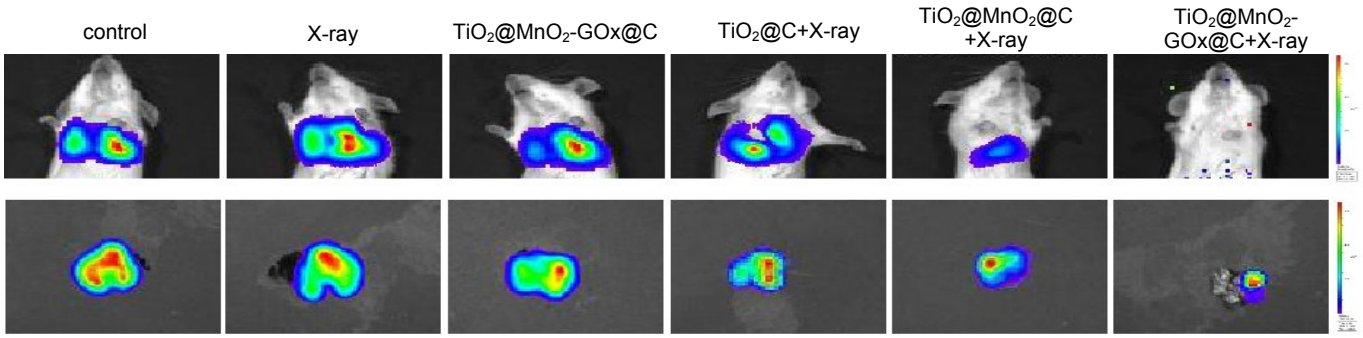

Fig. S10 The fluorescence images of ex and in vivo biodistribution of different treatment groups in lungs. From left to right: PBS, X-rays only, TiO_2@MnO_2-GOx@C, TiO_2@C with X-rays, TiO_2@MnO_2@C with X-rays, TiO_2@MnO_2-GOx@C with X-rays.

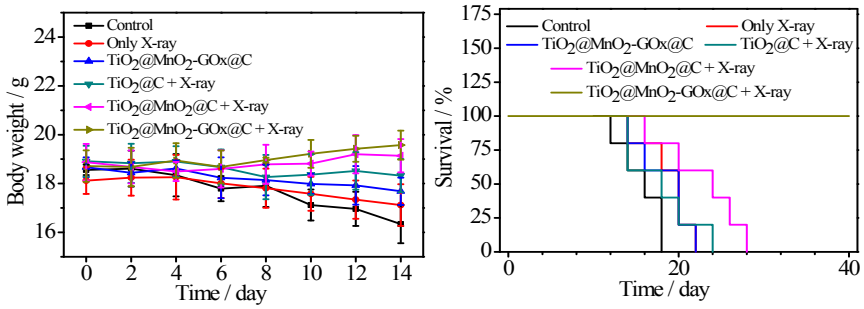

Fig. S11 Mouse body weight curves and survival profiles with different treatments.

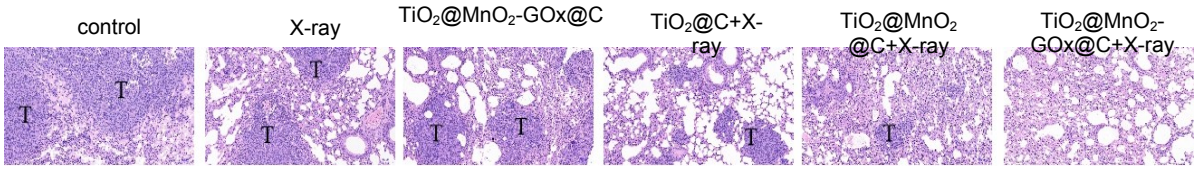

Fig. S12 Hematoxylin & eosin (H&E)-stained lung slices from 4T1-Luc tumor mice after different treatments. From left to right: PBS, X-ray only, TiO_2@MnO_2-GOx@C, TiO_2@C with X-ray, TiO_2@MnO_2@C with X-ray, TiO_2@MnO_2-GOx@C with X-ray.

3. References

1. N. Li, L. Yu, J. Wang, X. Gao, Y. Chen, W. Pan, B. Tang, *Chem. Sci*., 2018, **9**, 3159-3164.
2. W. Pan, Y. Ge, Z. Yu, P. Zhou, B. Cui, N. Li, B. Tang, *Chem. Commun.,* 2019, **55**, 5115-5118.
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