Supporting Information

Single-layer MoS_2 nanosheet grafted upconversion nanoparticles for near-infrared fluorescence imaging-guided deep tissue cancer phototherapy

*Jianyu Han,[a†] Hongping Xia,[b†] Yafeng Wu,[a†] Shik Nie Kong,[b] Amudha Deivasigamani,[b] Rong Xu,[*a] Kam M. Hui,[*b] Yuejun Kang[*c]*

[a.] *School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore 637459, Singapore. E-mail: rxu@ntu.edu.sg (R. Xu)*

[b.] *Division of Cellular and Molecular Research, National Cancer Centre, 11 Hospital Drive, Singapore 169610, Singapore. E-mail: cmrhkm@nccs.com.sg (K.M. Hui)*

[c.] *Faculty of Materials and Energy, Institute for Clean Energy and Advanced Materials, Southwest University, 2 Tiansheng Road, Beibei, Chongqing, 400715, China. E-mail: yjkang@swu.edu.cn (Y. Kang)*

Supplementary Methods

Materials: $RECl_3$ (RE=Y, Yb and Er), Chitosan (CS), 1,3-diphenylisobenzofuran (DPBF), 2',7'-dichlorfluorescein-diacetate (DCFH-DA), oleic acid (OA), 1-octadecen (ODE), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), N-hydroxy-succinimide (NHS), Poly(acrylic acid) (PAA), folic acid (FA) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich. Zinc(II) phthalocyanine (ZnPc) and Molybdenum(IV) sulfide (MoS_2) were obtained from Alfa Aesar. All chemicals were used as received without further purification. CS stock solution (0.05 wt %) was prepared by dissolving CS in glacial acetic acid.

Synthesis of $NaYF_4$: Yb, Er Up-conversion Nanoparticles: $NaYF_4$: Yb (18%), Er (2%) up-conversion nanoparticles were synthesized following a reported procedure with a minor modification.[1] YCl_3 (0.8 mmol), $YbCl_3$ (0.18 mmol), and $ErCl_3$ (0.02 mmol) were added to a 100 mL three-necked flask containing 12 mL oleic acid and 15 mL octadecene (ODE). The solution was heated to 160°C under a nitrogen atmosphere to obtain a homogeneous solution with vigorous stirring and then cooled down to room temperature. Thereafter, a solution of NaOH (2.5 mmol) and NH_4F (4 mmol) in 10 mL methanol solution was slowly added into the flask and stirred for another 30 min. Methanol was evaporated from the solution through slow heating, then the solution was degassed at 100 °C for 10 min, and subsequently heated to 300°C and maintained for 90 min under an argon atmosphere. After the solution was cooled down, nanoparticles were precipitated by adding ethanol and washed with cyclohexane and ethanol several times.

Synthesis of Carboxyl-Functionalized $NaYF_4$: Yb, Er Nanoparticles (PAA-UCNPs): To form carboxyl-functionalized upconversion nanoparticles, a ligand exchange method was used through Poly(acrylic acid) (PAA).[2] Typically, PAA (150 mg) was mixed with 15 ml diethylene glycol (DEG) in a three-necked flask, and the solution was heated to 110 °C to

obtain a homogeneous solution with vigorous stirring. 2.5 ml toluene solution containing 50 mg UCNPs was slowly added into the above solution at 110°C for 1 h under nitrogen atmosphere. The obtained solution was then heated to 240°C for 1.5 h. After cooling down to room temperature, excess dilute hydrochloric acid aqueous solution (0.1 M) was added, nanoparticles was obtained by centrifuging and washed several times with pure water.

Synthesis of Chitosan-Functionalized Single-Layer MoS_2 Nanosheets: Chitosan-functionalized MoS_2 nanosheets (denoted as MoS_2-CS) were synthesized by simple liquid-phase exfoliation method.[3] Briefly, MoS_2 flakes (20 mg) were ground with NaCl for 60 min, then washed with deionized water to remove NaCl. The obtained ground MoS_2 flakes were dried at 60°C for 24 h, then added to a flask containing 20 mL oleum. The mixture was heated to 90°C for 8 h with vigorous stirring, the oleum-treated MoS_2 flakes were obtained by centrifugation and repeated washing, after that, they were redispersed into 20 mL of deionized water, subsequently, 10 mL of chitosan solution (0.05 wt %) was added dropwise to the above solution under bath sonication treatment. After bath sonication for another 40 min, the mixture was further probe-sonicated for 2 h at power of 320 W. MoS_2-CS was collected by centrifuging and repeated washing with deionized water.

Synthesis of MoS_2-UCNPs: PAA-UCNPs (0.5 mg/mL) was mixed with a 2 mL aqueous solution of EDC (0.03 mmol) and NHS (0.03 mmol), then sonicated for 30 min at room temperature. Subsequently, MOS_2-CS (0.5 mg/mL) solution was added dropwise into above mixture and stirred vigorously for 24 h. MoS_2-UCNPs composites were obtained by centrifuging and washed several times with water.

Synthesis of MoS_2-UCNPs-FA: MoS_2-UCNPs (0.5 mg/mL) was mixed with a 2 mL aqueous solution of EDC (0.03 mmol) and NHS (0.03 mmol), then sonicated for 30 min at room temperature. Subsequently, FA (0.5 mg/mL) solution was added dropwise into above mixture and stirred vigorously for 24 h. MoS_2-UCNPs-FA composites were washed with water and freeze-dried.

Synthesis of MoS_2-UCNPs-FA/ZnPc: MoS_2-UCNPs-FA (5 mg) was dispersed by sonication in 10 mL 0.15 mg/mL ZnPc DMF solution. After stirring overnight, excess ZnPc was removed by centrifugation, the obtained precipitate (MoS_2-UCNPs-FA/ZnPc) was washed with PBS three times and freeze-dried. Different concentrations were prepared by dispersing weighted MoS_2-UCNPs-FA/ZnPc in PBS buffer for subsequent use.

Drug loading: ZnPc loading capacity in the MoS_2-UCNPs-FA were determined by fluorescence spectrophotometer with the excitation wavelength of 610 nm and the emission wavelength of 650-750 nm.[4] The concentration of ZnPc was calculated with calibration curve, which was carried out in ethanol. When MoS_2-UCNPs-FA/ZnPc was soaked in ethanol, ZnPc was extracted, then centrifugated and the concentration of ZnPc in the supernatant was obtained from fluorescence spectrophotometer. ZnPc loading capacity was calculated with the following equation: loading capacity (%) = [concentration of ZnPc in the MoS_2-UCNPs-FA]/[concentration of MoS_2-UCNPs-FA/ZnPc] ×100.

Cytotoxicity assays: FR+ MDA-MB-231 cells, HCC38 cells and FR− MCF-7 cells were cultured in normal RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 μg/mL penicillin and 100 μg/mL streptomycin at 37 ºC with 5% CO_2. For cell viability assay, MDA-MB-231, HCC38 and MCF-7 cells were seeded in 96-well plates at 5×10^4 cells per well for 24 h, then different concentrations of MoS_2-UCNPs (0, 20, 40, 80, 160 and 320 μg/mL) were added to the wells. After incubation for another 24 h, the cells were washed with PBS. Standard cell viability MTT assay was carried out to determine the cell viabilities.

Reactive oxygen species (ROS) detection: ROS product in physiological solution was detected by the chemical probe DPBF.[5] Typically, 30 μL DPBF (10 mmol/L) ethanol solution was mixed with 3 mL MoS_2-UCNPs-FA/ZnPc (160 μg/mL) solution and then transferred into a cuvette. The solution was irradiated by a 980 nm laser with a power of 0.2 W/cm^2 for 5 min in

the dark, and the DPBF absorption at 410 nm was recorded every 1 min. For the control experiments, all conditions were the same except without laser irradiation. ROS product inside cancer cells was tested with DCFH-DA Intracellular ROS Assay Kit.[6] MDA-MB-231 cells were cultured in 12-well plates at 5×10^5 cells per well for 24 h, 100 μL MoS_2-UCNPs-FA/ZnPc (160 μg/mL) was added to each well and incubated for 2 h, then DCFH-DA was diffused into the wells and incubated for 40 min. After washing thoroughly with PBS, the cells were irradiated by 660 or 980 nm laser with the power of 0.2 W/cm^2 for 5 min. To test ROS generation in deep tissue, 1 cm thick porcine tissue was placed on the cell dishes and then irradiated by 660 or 980 nm laser with the power of 0.2 W/cm^2 for 5 min. After irradiation, fluorescence images were recorded with a fluorescence microscope.

In vitro PTT and PDT treatments: For PTT treatment, cells were cultured in 96-well plates at density 5×10^4 cells per well for 24 h, then different concentration of MoS_2-UCNPs-FA/ZnPc was added to the wells and incubated for 2 h. After that, the cells were washed with PBS three times and irradiated using 808 nm NIR laser at a power density of 1 W/cm^2 for 6 min. Then, the cells were incubated for another 24 h. Thereafter, standard MTT assay was carried out to determine the cell viabilities. For PDT treatment, the method was the same with PTT treatment, except that 980 nm laser at a power density of 0.2 W/cm^2 was used. For combined treatment, cells were cultured in 96-well plates at 5×10^4 cells per well for 24 h, then 40 or 80 μg/mL MoS_2-UCNPs-FA/ZnPc was added to the wells and incubated for 2 h. After that, the cells were washed thoroughly and irradiated using 808 nm NIR laser at a power density of 1 W/cm^2 for 6 min. Subsequently, cells were irradiated with 980 nm laser at a power of 0.2 W/cm^2 for 5 min. After additional incubation for 24 h, cell viability was determined using standard MTT assay.

Upconversion luminescent imaging of cells: MDA-MB-231 cells were seeded on 14 mm glass coverslips and allowed to adhere for 24 h. The cells were incubated with 160 μg/mL

MoS_2-UCNPs-FA/ZnPc at 37°C for 2 h. Before imaging, the glass coverslips were washed three times with PBS to remove excess MoS_2-UCNPs-FA/ZnPc. Upconversion fluorescence imaging were then performed using an inverted microscope (Eclipse Ti-U, Nikon, Tokyo, Japan) equipped with C2-SHS Scanner and Controller under excitation of 980 nm laser at power density of 0.2 W/cm^2. Upconversion luminescence signals were detected in the green channel and red channel.

In vivo PTT and PDT treatments: Female Ncr nude mice (18~20 g, 6~8 weeks old) used in this study were purchased from the InVivos Pte. Ltd. (Singapore). All experiments were carried out in compliance with the IRB approved protocols. The HCC38 orthotopic tumor model was established by injection 5 million of HCC38 cells into the thoracic mammary fat pad of all mice to produce orthotopic breast tumor models. The mice were then grouped and investigated when the tumor growth to a diameter of around 5 mm. The mice were randomly assigned into five groups treated with different injections as follows: (1) the Vehicle Control; (2) MoS_2-UCNPs-FA/ZnPc injection only; (3) MoS_2-UCNPs-FA/ZnPc injection with 808 nm NIR laser irradiation; (4) MoS_2-UCNPs-FA/ZnPc injection with 980 nm NIR laser irradiation; (5) MoS_2-UCNPs-FA/ZnPc injection with 808 and 980 nm NIR laser irradiation. 100 μL of saline or MoS_2-UCNPs-FA/ZnPc (2 mg/ml) was injected into each HCC38 tumor-bearing mouse in intratumoral direct injection for PDT and PTT. For combined therapy, the tumor region was first irradiated by 980 nm NIR laser (0.2 W/cm^2, 5 min) for PDT and then 808 nm NIR laser (1 W/cm^2, 6 min) for PTT. After treatment, the tumor volume was examined and calculated as length × (width)^2 × 1/2 with a calliper for over 2 weeks.

Hematoxylin and Eosin (H&E) Staining Analysis. Histology analysis was performed at 2 weeks after treatment. The tumors and organs (liver and kidney) of the mice in the control group and each treated group were isolated from the mice, fixed with 10% neutral buffered formalin and embedded in paraffin. The sliced organs and tumor tissues (5 mm) were stained with Hematoxylin and Eosin (H&E) and examined by a microscope.

Supplementary Figures

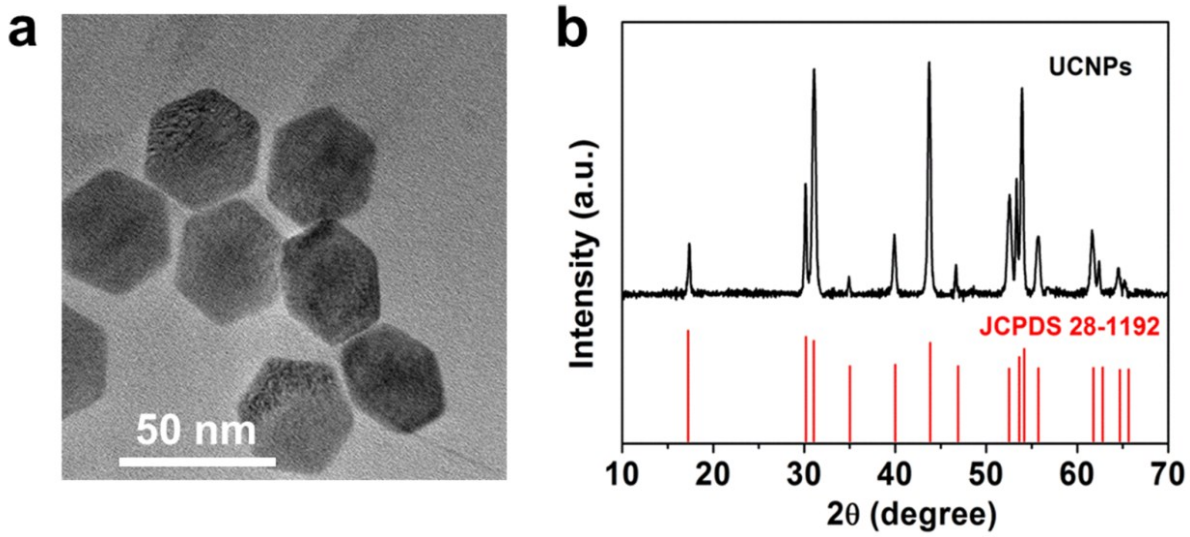

Figure S1. (a) TEM image of UCNPs; (b) XRD patterns of UCNPs. The standard pattern of hexagonal phases NaYF4 is used as reference (JCPDS 28-1192).

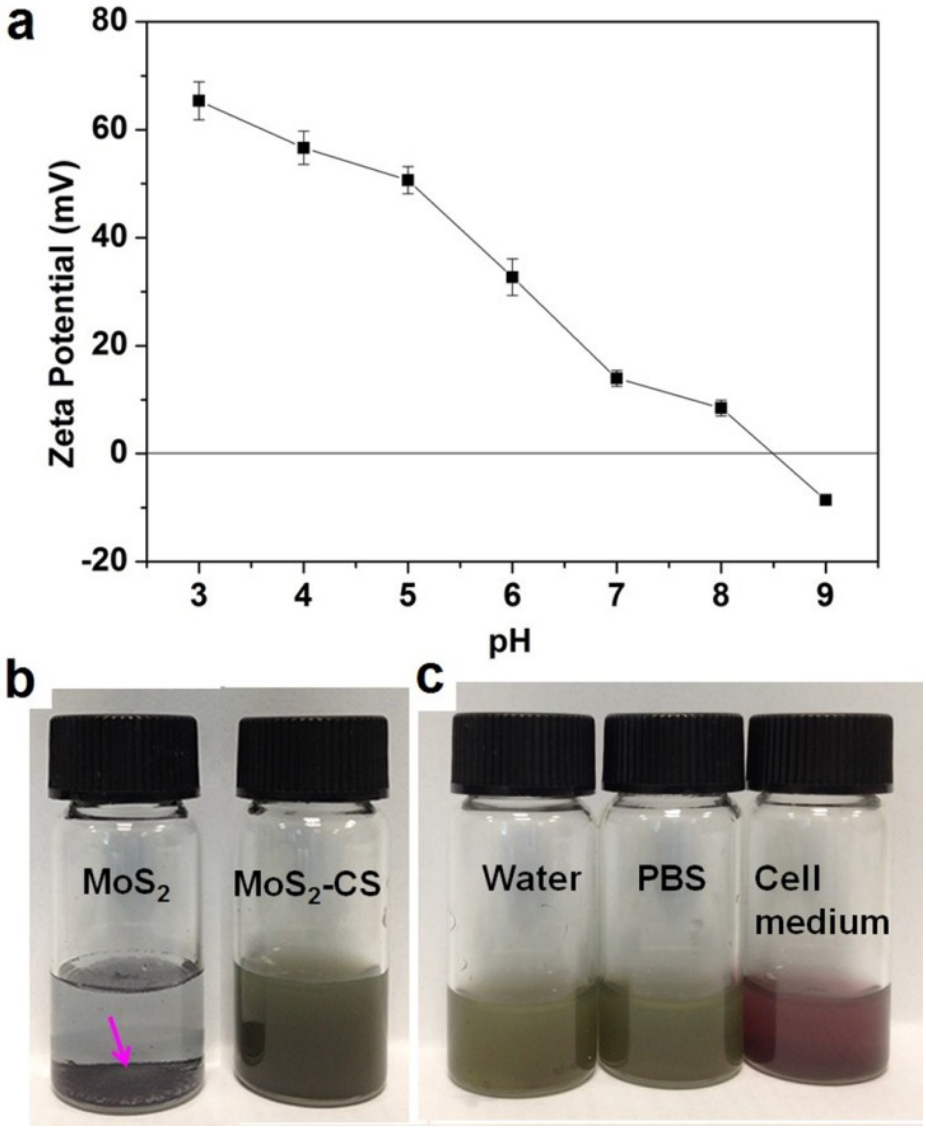

Figure S2. (a) Zeta-potential of MoS_2-CS in water with different pH values; (b) Digital photographs for the dispersion status of commercial MoS_2 flakes (left) and prepared MoS_2-CS nanosheets (right) after 1 week with a concentration of 300 μg/mL; (c) Digital photographs of MoS_2-CS nanosheets dispersion with concentration of 150 μg/mL in water, PBS and cell medium respectively after 1 week.

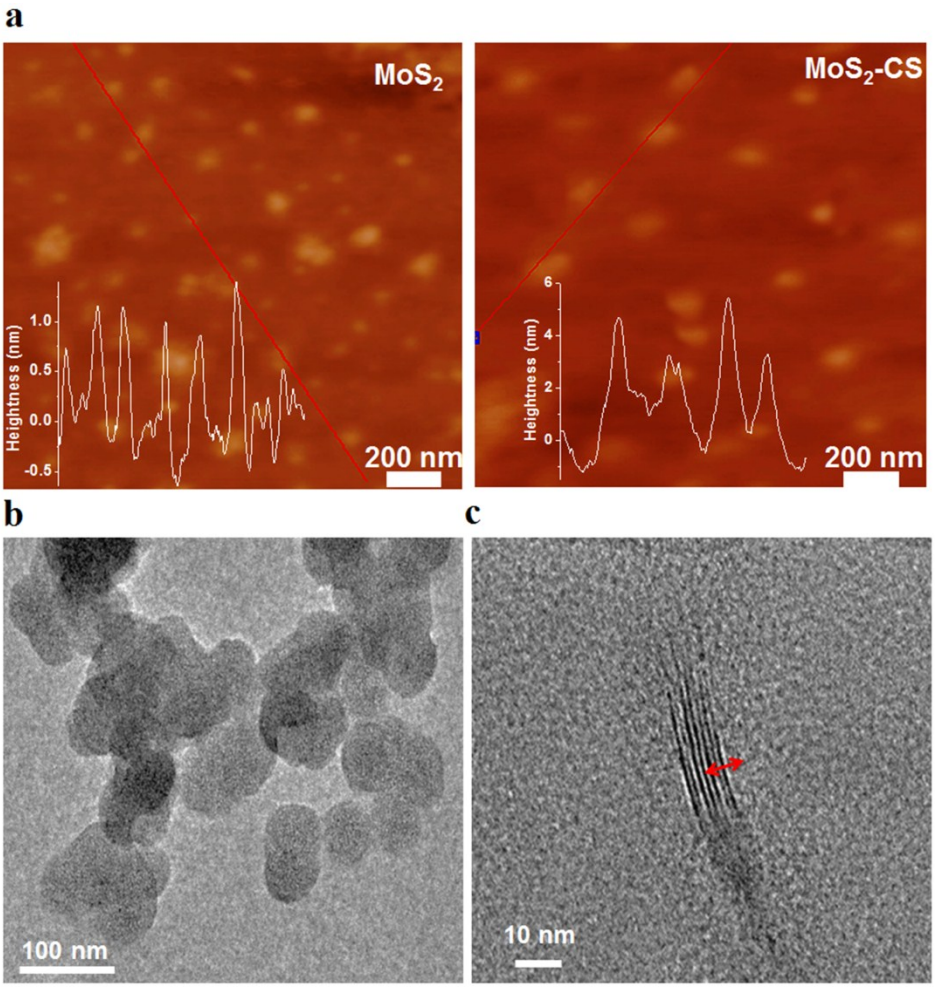

Figure S3. (a) AFM images of single-layer MoS_2 and prepared MoS_2-CS nanosheets with topographic heights of ~1.0 and ~5 nm, respectively; (b) TEM image of MoS_2-CS nanosheets; (c) High-resolution TEM images of single-layer MoS_2.

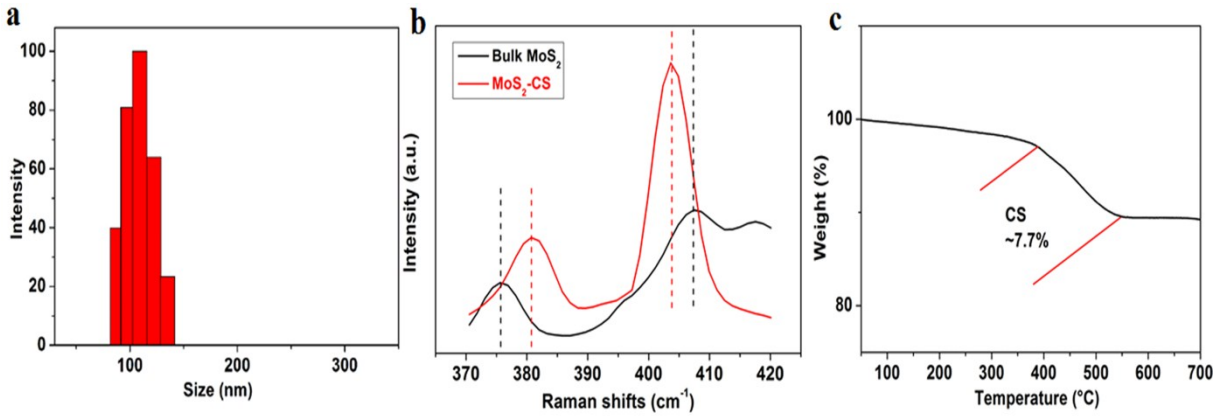

Figure S4. (a) DLS of prepared MoS_2-CS nanosheets; (b) Raman spectra of commercial MoS_2 flakes and prepared MoS_2-CS nanosheets; (c) Thermal gravimetric (TGA) analysis of the prepared MoS_2-CS nanosheets.

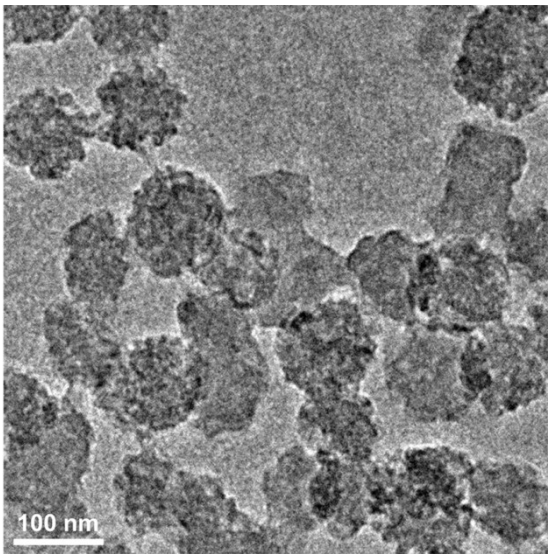

Figure S5. TEM image of MoS_2-UCNPs.

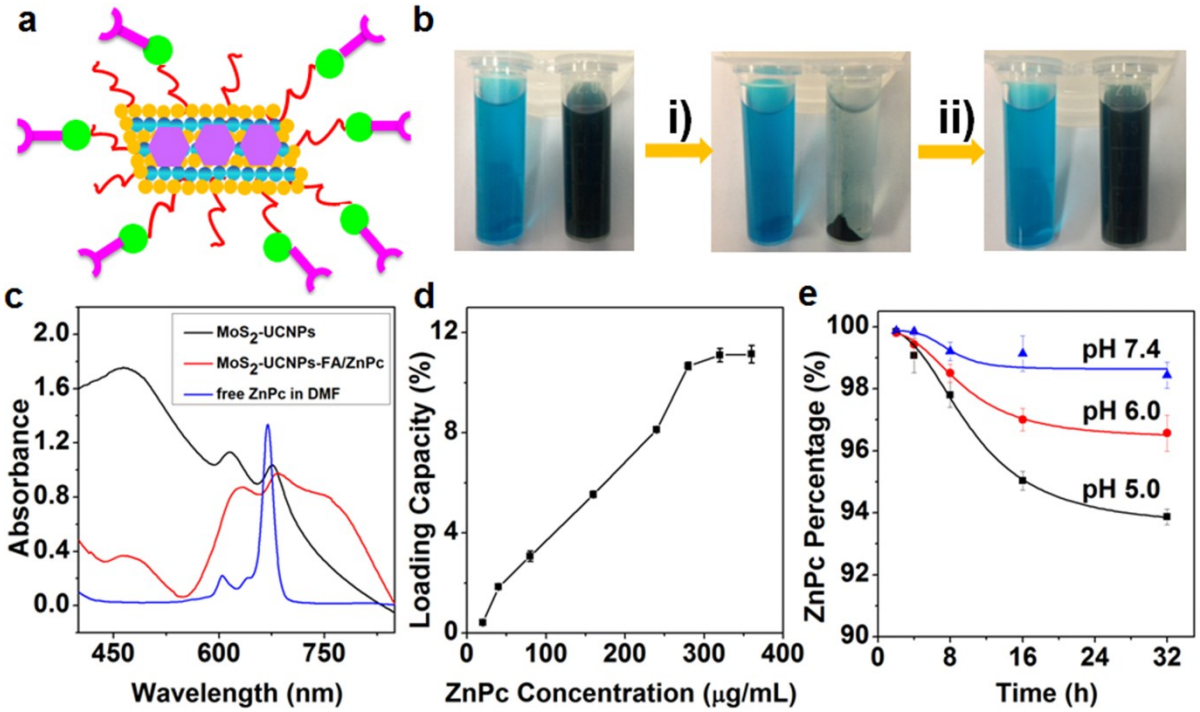

Figure S6. Drug loading and release. (a) Schematic of loading ZnPc onto the MoS_2-UCNPs-FA surface; (b) Photographs of free ZnPc (left, blue color, 300 μM) and MoS_2-UCNPs-FA/ZnPc (ZnPc = 300 μM) solutions (right, black color) after centrifugation (i) and sonication (ii); (c) Absorption spectra of free ZnPc in DMF, MoS_2-UCNPs and MOS_2-UCNPs-FA/ZnPc in aqueous solution; (d) Plots of ZnPc loadings capacity at various ZnPc concentrations; (e) Release profile of ZnPc as the function of time at pH 5.0, 6.0 and 7.4 PBS buffer.

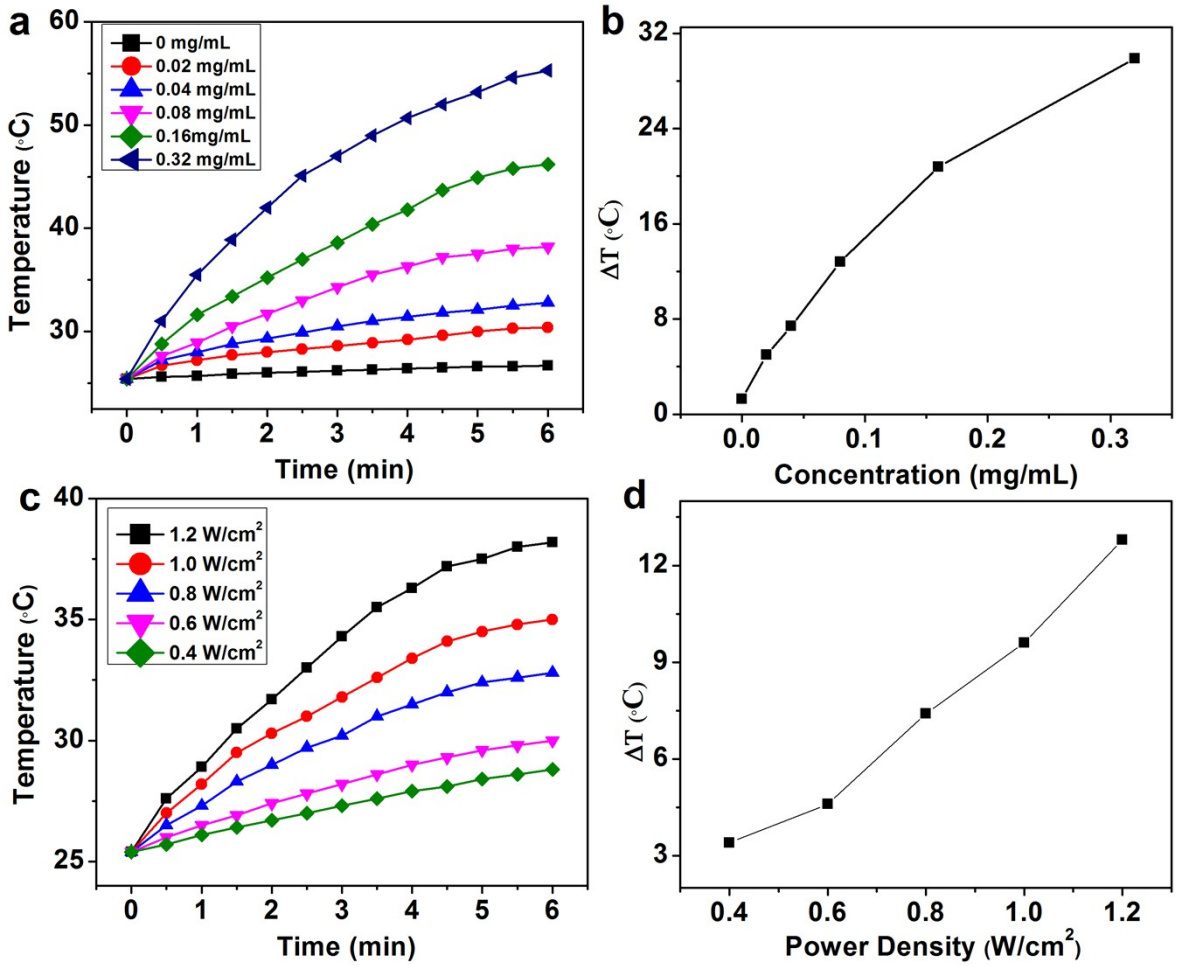

Figure S7. (a) Photothermal heating curves of pure water and MoS_2-UCNPs solution under 808 nm laser irradiation (1.2 W/cm^2) at different concentrations for 6 min; (b) Plot of temperature change versus MoS_2-UCNPs concentration after 6 min irradiation; (c) Photothermal heating curves of MoS_2-UCNPs solution (0.04 mg mL^{-1}) irradiated by 808 nm laser with different power densities for 6 min; (d) Plot of temperature change versus power density after 6 min irradiation.

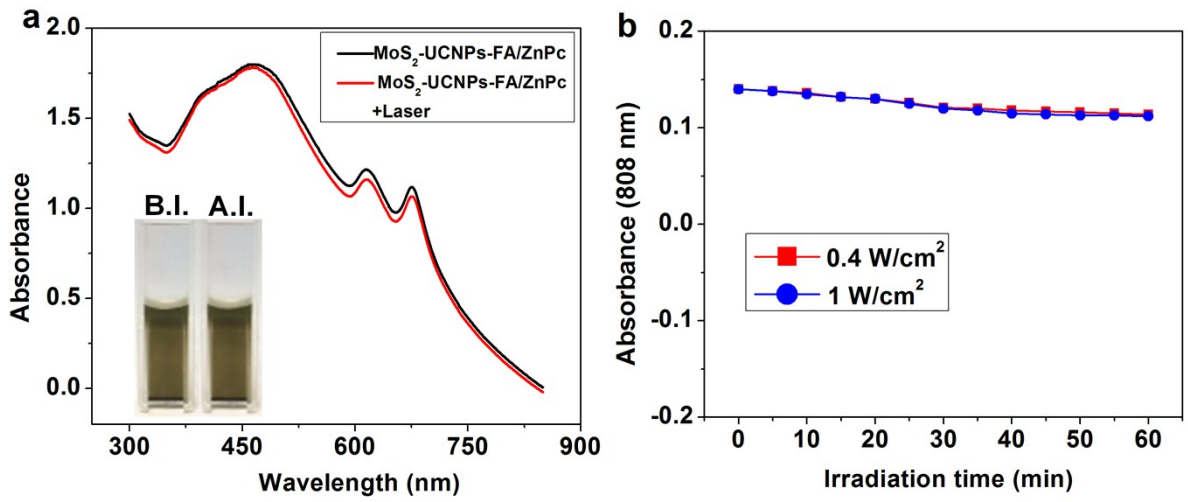

Figure S8. Photothermal stability study of MoS_2-UCNPs under 808 nm laser irradiation. (a) UV-vis-NIR absorption spectra of MoS_2-UCNPs before and after laser irradiation (1 W/cm^2 for 1 h). Inset: photos of MoS_2-UCNPs solutions before irradiation (B.I.) and after irradiation (A.I.) at 1 W/cm^2 for 1 h. (b) Change of absorbance at 808 nm for MoS_2-UCNPs solutions under laser irradiation at different power densities (0.4 and 1 W/cm^2) for 1 h.

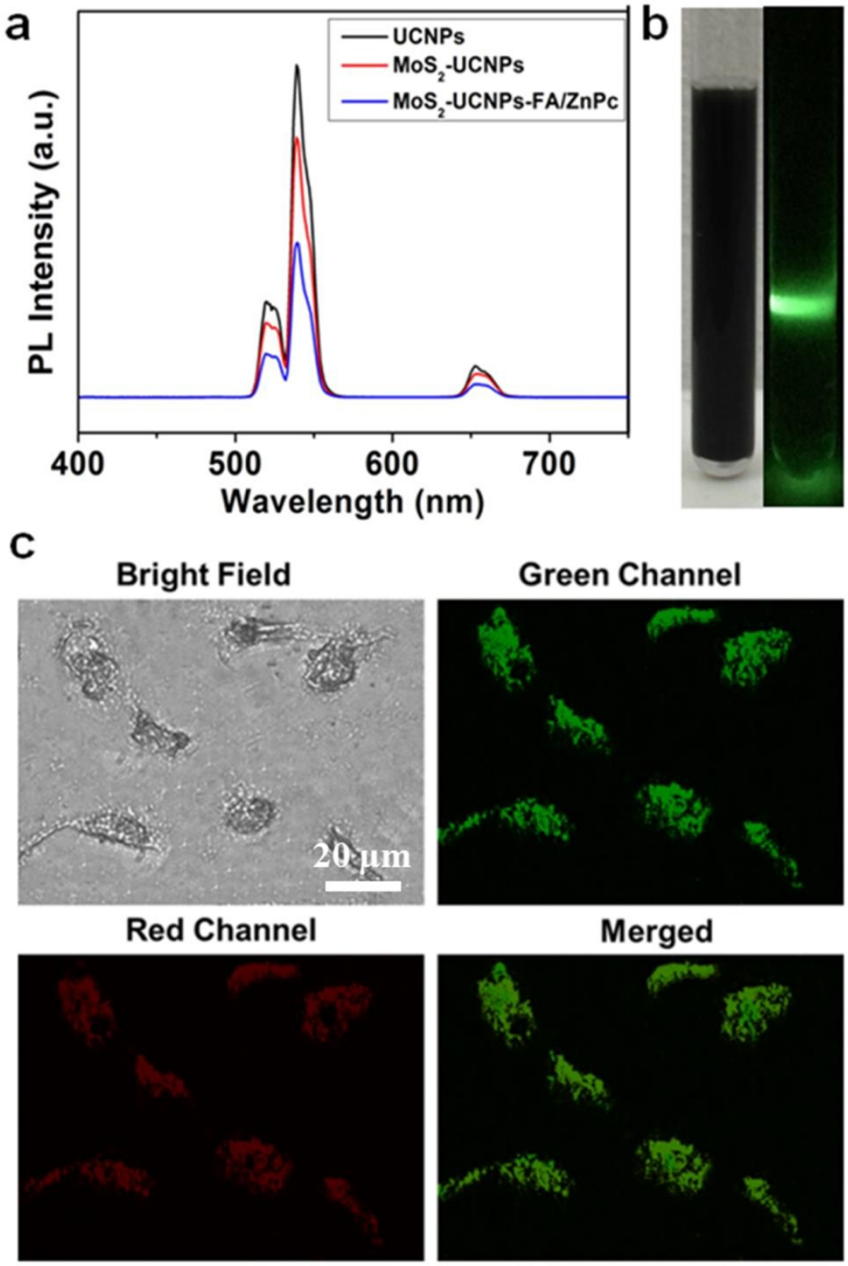

Figure S9. (a) Upconversion emission spectra of UCNPs, MoS_2-UCNPs and MoS_2-UCNPs-FA/ZnPc excited with a continuous wave 980 nm laser; (b) Photographs of MoS_2-UCNPs under ambient light (left) and under continuous wave 980 nm laser irradiation (right); (c) Upconversion luminescence imaging at green channel (λ_{em} = 520-560 nm) and red channel (λ_{em} =600-700 nm) of MDA-MB-231 cells after incubation with 160 μg/mL MoS_2-UCNPs-FA/ZnPc for 2 h. Scale bar = 20 μm.

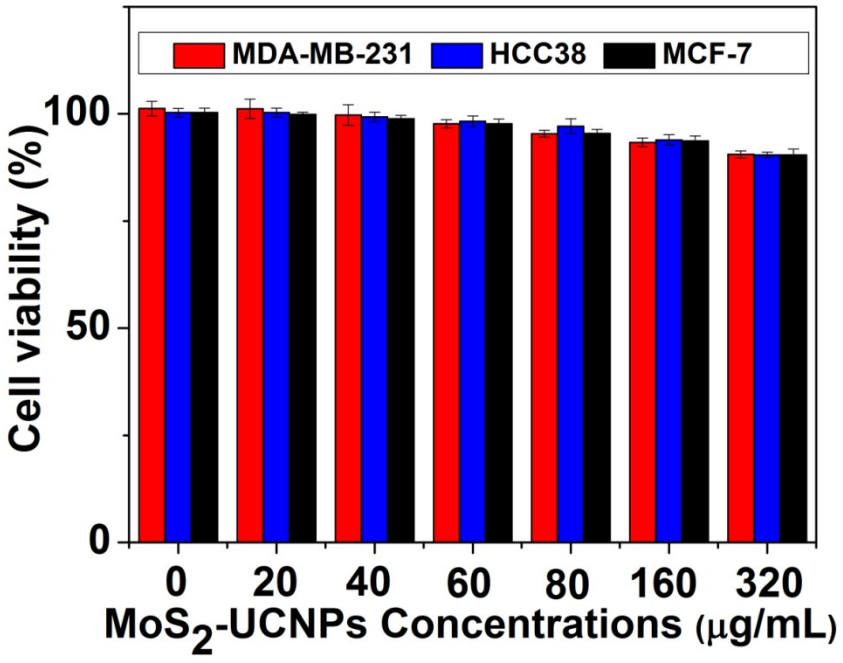

Figure S10. Cell viability of MDA-MB-231, HCC38 and MCF-7 cells incubated with MoS_2-UCNPs at different concentrations for 24 h, respectively.

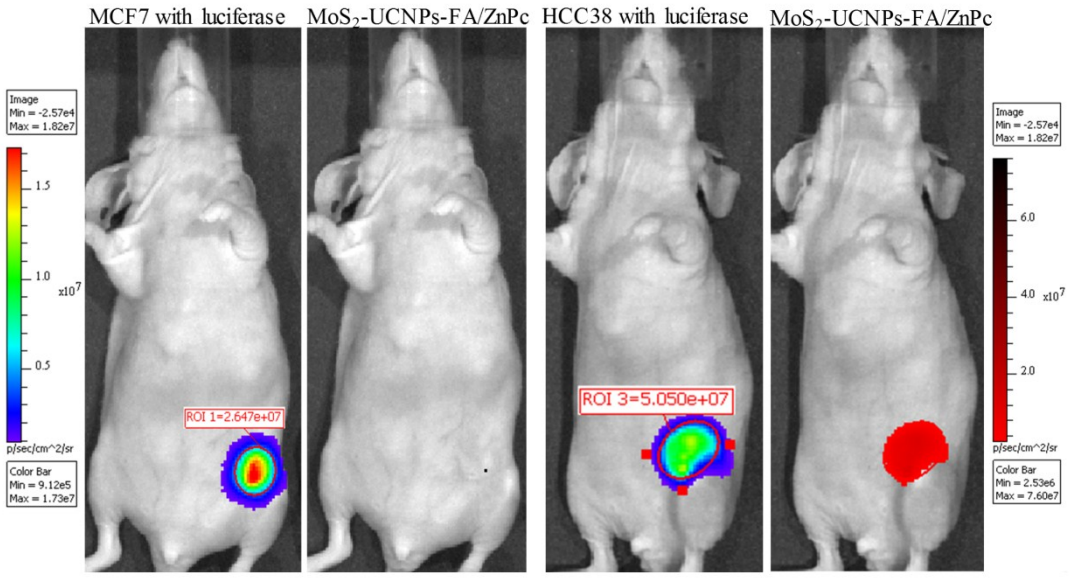

Figure S11. The fluorescence images of luciferase-labelled (blue) MCF7 and HCC38 tumor models and activated fluorescence signal of MoS_2-UCNPs-FA/ZnPc (red) in FR+ HCC38 tumor model only.

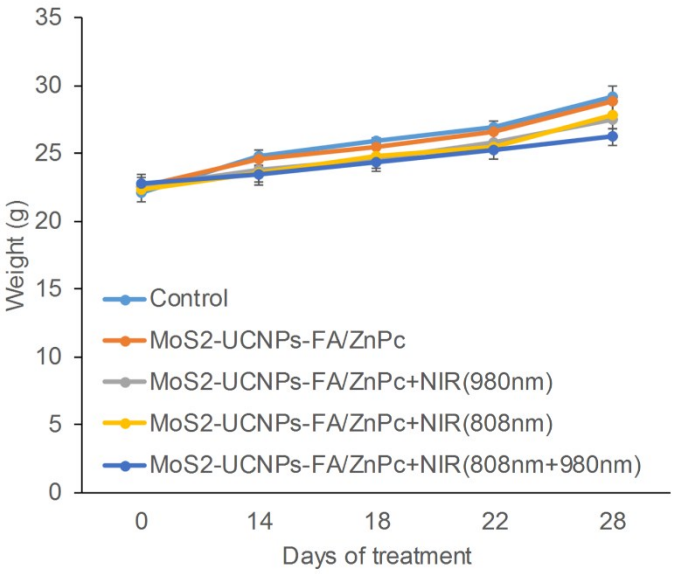

Figure S12. The body weight of mice in each group examined at different days after treatment.

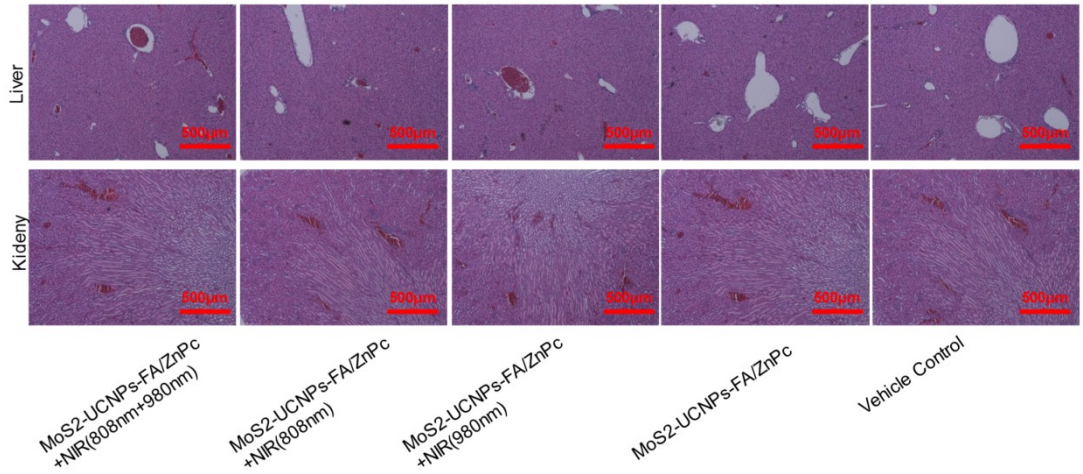

Figure S13. The representative H&E-stained images of the liver and kidney collected from the control group and different treatment groups. No liver and kidney damage was observed in each group. Scale bar = 500 μm.

Additional References

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