Supplementary Information for:

Ultrathin carbon layer coated MoO_2 nanoparticles for high-performance near-infrared photothermal cancer therapy

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Experimental Details.

Preparation of the carbon coated MoO_2 Nanoparticles. Molybdenum dioxide powder (AR), Molybdenum powder (AR), H_2O_2 (AR) and acetone were commercially available from Shanghai Chemical Reagent Co. Ltd. Methoxypolyethylene glycolsthiol (mPEG-SH) was purchased from Jenkem Technology Co. Ltd. All reagents were of analytical grade and used as received without further purification. Hydrothermal reaction was used to synthesize C/MoO_2 nanoparticles in a sealed autoclave system. In a typical synthetic procedure, 1 mmol of molybdenum metal powder was added to a Teflon vessel (45mL). Then 0.8 mL of H_2O_2 was introduced and magnetically stirred for about 30 min to obtain the transparent yellow solution. As followed, 30 ml acetone was slowly added into the solution and stirred for 10 min. Then the Teflon vessel having mixed solution was first sealed in Teflon-lined stainless steel, autoclaved, heated and maintained at 200°C for 48 h. After cooling down to room temperature naturally, the product was collected by centrifugation, rinsed with ethanol for several times and finally dried at 60°C under vacuum.

Conjugation of PEG with C/MoO_2 nanoparticles. In order to enhance the biocompatibility, PEGylated treatment was done on C/MnO2 nanoparticles. Typically, 10.0 mg of mPEG-SH was added into 10 mL of C/MoO_2 aqueous solution (1 mg/mL) and incubated overnight at room temperature. The excess mPEG-SH was removed by centrifugation at 10, 000 rpm for 5 min and washed three times with ultra-purified H_2O.

Material's characterizations. Samples were characterized by X-ray powder diffraction (XRD) by a Philips X'Pert Pro Super diffractometer equipped with Cu Kα radiation (λ=1.54178 Å). Field emission scanning electron microscopy (FE-SEM) images were taken on a JEOL SEM (JSM-6700F). JEM-2100F field emission electron microscopy (TEM) with an acceleration voltage of 200 kV was used to collect high-resolution TEM images. UV-Vis-NIR absorption spectra were recorded with a Perkin Elmer Lambda 950 UV-Vis-NIR spectrophotometer. Raman spectra were detected by a Micro-Raman system (Renishaw, RM3000) with a 514.5 nm Ar laser. The size distribution profile in the aqueous solution was determined by Malvern Zetasizer Nano ZS90 dynamic light scattering (DLS) instrument with a He-Ne laser (633 nm) and 90^{o} collecting optics.

In vitro photothermal experiments. MDA-MB-231 cells and HepG2 cells were obtained from the American type culture collection (ATCC) and cultured in the Dulbecco's Modified Eagle Medium (DMEM, Gibco, Grand Island) supplemented with 10% fetal bovine serum (FBS, Hyclone, Thermo Scientific, USA). BxPC-3 cells were obtained from ATCC and cultured in the RPMI-1640 Medium (Gibco, Grand Island) supplemented with 10% FBS. To determine the cytotoxicity of PEGylated C/MoO_2 nanoparticles, cells were seeded into 96 well plates and incubated with different concentrations of nanocrystals for 24 h. Relative cell viabilities were determined by MTT assay according to previously reported methods.[31] To assess the effect of photothermal therapy, MDA-MB-231 cells were seeded in 96 cell plates and incubated with the hybrid nanoparticles for 6 h, followed by irradiation with an 808 nm laser for 10 min.

In vivo photothermal ablation of cancer cells. BALB/c nude mice were obtained from the Beijing HFK Bioscience Co., Ltd. and used at 6 weeks of age. Experiments were performed under ethical rules and all animals received care in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals. The procedures were approved in advance by the University of Science and Technology of China Animal Care and Use Committee. The xenograft tumor model was generated by subcutaneous injection of 42×10^{6} MDA-MB-231 cells suspended in 100 μL

phosphate buffered saline (PBS, with 30% Matrigel, BD Bioscience) into the second right mammary fat pad of nude mice. When the tumor volume grew to approximately 100 mm^3, the mice were randomly divided within 4 groups. Mice bearing MDA-MB-231 tumors were intratumorally injected with 40 μL of 1.0 mg ml^{-1} PEGylated C/MoO_2 nanoparticle and then immediately irradiated with an 808 nm NIR laser (BWT Beijing Co. Ltd., China) at the power density of 0.6 W cm^{-2} for 10 min. The other mice groups were treated only with the same volume/power density of sole PBS, PEGylated C/MoO_2 nanoparticles (NPs) and laser irradiation. The temperature of the tumor sites was recorded by an IR 7320 thermal camera and analyzed with the IR Flash Software (*Infrared Cameras. Inc.*). Tumor sizes and weights were monitored after every three days. The tumor volume was calculated with the formula of $V=0.5\times length\times width^2$. For H&E analysis, samples of tumors were sent to the first Affiliated Hospital of the Anhui Medical University.

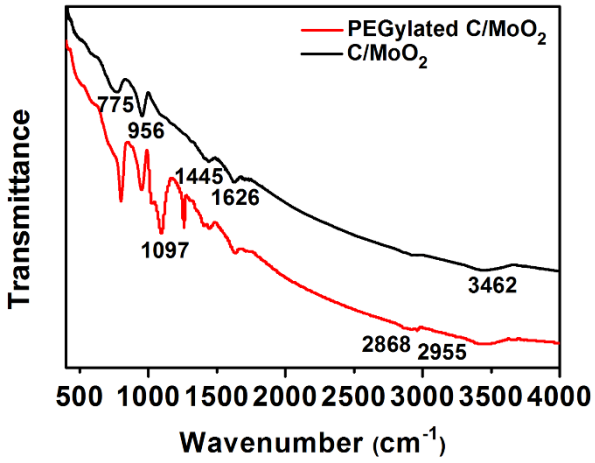

Figure S1. FT-IR curves of C/MoO_2 nanoparticles.

The sample exhibits a broad band at about 3462 cm^{-1}, due to the O–H stretching vibration of adsorbed water. The 1626 cm^{-1} transmission band is assigned to the stretching vibrations of C=C of sp^2 structure.[1] The bands at about 1097 cm^{-1} is corresponding to C-S stretching vibration absorbed on coated carbon, which shifts about 10 cm^{-1} to lower wavenumber comparing to the IR spectrum of pure PEG-SH, suggesting the formation of chemical bonds between PEG-SH and inorganic components.[2] The 2955 and 2868 cm^{-1} transmission bands are respectively assigned

to the asymmetric and symmetric stretching vibrations of methylene (CH_2) units in PEG-SH chain. Based on the above results, it is concluded that there are PEG-SH absorbed on the surface of C/MoO_2 nanoparticles, indicating the formation of PEGylated C/MoO_2 nanoparticles.

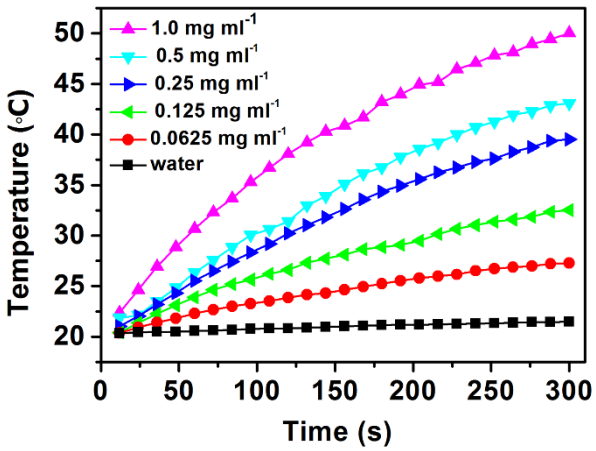

Figure S2. The temperature increase of the aqueous pure MoO_2 nanoparticles dispersions with different concentrations vs. time under NIR laser irradiation at the power density of 0.6 W cm^{-2}.

The mass extinction coefficient of the C/MoO_2 nanoparticles is 10.2 $Lg^{-1}cm^{-1}$ (λ=800 nm), which is comparable to gold nanorods (13.9 $Lg^{-1}cm^{-1}$). [3]

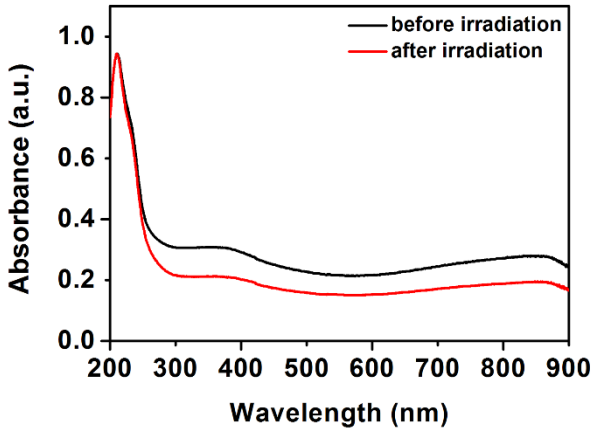

Figure S3. UV-vis-NIR spectra of aqueous pure MoO_2 nanoparticles dispersions before and after laser irradiation at the power density of 0.6 W/cm^2 for 1 hour.

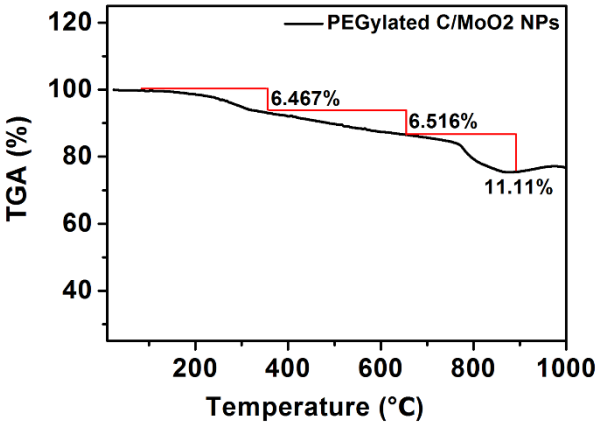

Figure S4. TGA curve of PEGylated C/MoO_2 nanoparticles.

The weight loss from room temperature to 350°C in the TG curve is attributed to the existence of water in the composite. In the TG curve, the large weight loss about 6.516 wt% in the range of 350 to 650°C may result from the surface modified PEG-SH.

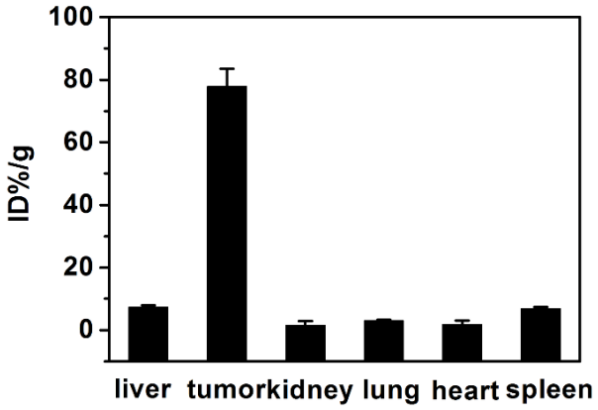

Figure S5. Biodistribution of PEGylated C/MoO_2 in mice determined by ICP-MS measured Mo concentrations. Error bars were based standard deviations (SD) of three mice per group.

In order to confirm the in vivo biodistribution of PEGylated C/MoO_2 nanoparticles, MDA-MB-231 tumor-bearing BALB/c mice intratumorally injected with PEGylated C/MoO_2 (1 mg/mL, 40 μ L) were scarified at 24 h. Major organs of mice (n = 3) were collected and solubilized by aqua regia for ICP-MS measurement of Mo element. High levels of Mo element were observed in the tumor, as well as reticuloendothelial

systems (RES) such as liver and spleen, which was believed as the clearance system in vivo for nano-sized materials.

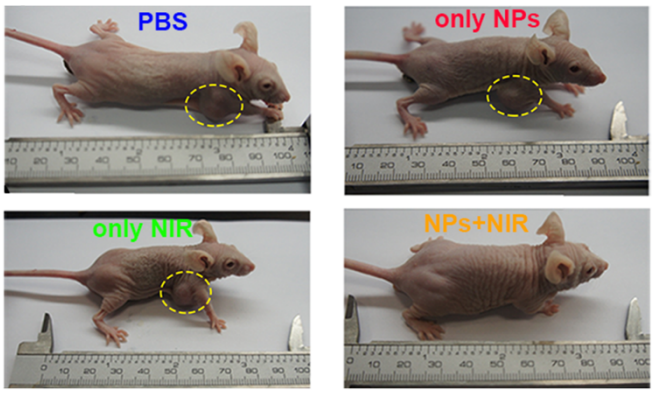

Figure S6. Representative photos of mice taken after various treatments within 7 days.

Fig. S6 show representative photos of mice after various treatments within 7 days. In contrast to the big-size tumors in control groups, the MDA-MB-231 tumors in mice for NPs+NIR group clearly disappeared after 7 days treatment.

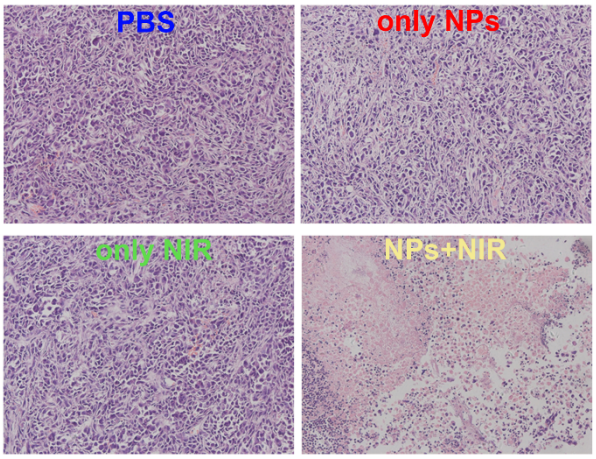

Figure S7. H&E analysis of tumors collected after two days of the initial treatment.

Moreover, for further clarification of mechanism of photothermal therapy, we collected tumors for the H&E stain after two days of NIR irradiation treatment. Fig. S7 shows that significant cell damage can be clearly observed in the NPs group undergoing NIR laser irradiation in contrast with the control groups. These

observations indicate that the elimination of tumors originates from the excellent photothermal ablation of C/MoO_2 nanoparticles, not due to the material's toxicity itself.

Reference:

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