Supporting Information

NIR-II light powered hydrogel nanomotor for intravesical instillation with enhanced bladder cancer therapy

Wei Chen,[1#] Yingfei Wang,[2,4#] Hao Hu,[1] Yu Zhu,[2] Hongxia Zhao,[2] Jie Wu,[2] Huangxian Ju,[2] Qing Zhang,[1*] Hongqian Guo,[1*] and Ying Liu[2,3*]

[1]*Nanjing Drum Tower Hospital Clinical College of Nanjing University of Chinese Medicine, Nanjing 210008, PR China.*

[2]*State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, PR China.*

[3] *Chemistry and Biomedicine Innovation Center, Nanjing University, Nanjing 210023, PR China.*

[4] *State Key Laboratory of Digital Medical Engineering, School of Biological Science and Medical Engineering, Southeast University, Nanjing 210096, PR China.*

[#]*Equal contribution.*

[*]Corresponding author (Phone/Fax: +86-25-89681918. E-mail: yingliu@nju.edu.cn)

Experimental Section

Materials & Reagents

Copper(II) acetylacetonate ($Cu(acac)_2$ 97%), iron(III) acetylacetonate ($Fe(acac)_3$ 95%), and oleylamine (80−90%) were purchased from Aladdin Ltd. (Shanghai, China). Sulfur powder (99%) was purchased from Alfa Aesar Co., Ltd. (Shanghai, China). Poly(vinylpyrrolidone) (PVP, MW 10000), copper (II) chloride dihydrate ($CuCl_2{\cdot}H_2O$), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2, 30%), cholesterol, poly(ethylene glycol) diacrylate (PEG-DA, MW 575), 2-hydroxy-2-methyl-propiophenone (photo initiator), and poly(2-hydroxyethyl methacrylate) (pHEMA) were purchased from Sigma-Aldrich Co., Ltd. (Shanghai, China). DSPE-PEG-acrylate (MW 2000), DSPE-PEG-folic acid (DSPE-PEG-FA, MW 2000) and DSPE-PEG (MW 2000) were purchased from ToYong Biotech. Inc. (Shanghai, China). Lecithin (analytical standard, 98%) and saline were purchased from Yuanye Biotech Co., Ltd. (Shanghai, China). SG-2506 borosilicate glass was purchased from Shaoguang Chrome Blank Co., Ltd. (Changsha, China). The dechroming liquid was prepared with 20% (m/v) $Ce(NH_4)_2(NO_3)_6$ and 3.5% (v/v) CH_3COOH. The etching solution contained 1.86% (m/v) NH_4F, 4.64% (v/v) HNO_3 and 5.00% (v/v) HF. SYLGARD™ 184 Silicone Elastomer Kit for polydimethylsiloxane (PDMS) was purchased from Dow Corning (Midland, MI).

Dulbecco's modified Eagle's media (DMEM), PBS, pancreatic ferment, and MTT cell proliferation and cytotoxicity assay kit were obtained from KeyGEN Biotech (Nanjing, China). Lyso-Tracker Green, reactive oxygen species assay kit, and Annexin APC/PI apoptosis detection kit were obtained from Beyotime Institude of Biotechnology Co., Ltd. (Shanghai, China). The bladder cancer cell line murine MB49 was obtained from National Collection of Authenticated Cell Cultures. Luc-MB49 cells were obtained by institute of urology Nanjing Drum Tower Hospital following a reported protoco.[41] D-Luciferin potassium salt and BODIPY^{TM} 581/591 C11 were purchased from Thermo Fisher Scientific (Shanghai, China). Female C57BL/6 mice (8 weeks) were purchased from GemPharmatech Co.,Ltd. (Nanjing, China).

Apparatus

UV-vis absorption spectra were recorded on a UV-3600 UV-vis-NIR spectrophotometer

(Shimadzu, Japan). Transmission electron microscopic (TEM) images were obtained from a JEM-2100 transmission electron microscope (JEOL Ltd., Japan). Scanning electron microscopic (SEM) images were obtained by a JSM-7800F field emission scanning electron microscope (JEOL Ltd., Japan) at 10 kV. The dynamic light scattering (DLS) and zeta potential analysis was conducted on a ZetaPlus 90 Plus/BI-MAS (Brookhaven, U.S.A.). FTIR spectra were measured with a Nicolet iS50 FT-IR Spectrometer (Thermo Fisher Scientific, U.S.A.). Glass slides were cleaned and activated by an Oxygen Plasma Cleaner (Model PDC-MG, Chengdu MINGHENG Science & Technology Co., Ltd., China). The lipid bilayer encapsulated hydrogel nanodroplets (LipGel-NDs) was prepared by a home-made PDMS microfluidic chip pumped by FlOW-EZ (Fluigent, France). UV-induced polymerization of LipGel-NDs was achieved via a UVLED (AVENTK, Shanghai, China) to obtain lipid bilayer encapsulated hydrogel nanomotors (LipGel-NMs). Photothermal performance of LipGel-NMs was tested by an IR thermal camera (Fortic 225-1, Fotric, China), and the motion of LipGel-NMs was recorded by an inverted microscope (Leica DMI 3000B, Leica, Germany). The fluorescence imaging of cells was performed on a TCS SP8 STED 3X confocal laser scanning microscope (CLSM) (Leica, Germany). MTT assay was performed on a microplate reader (Biotek CBM, USA). Flow cytometric analysis was performed on a CytoFLEX flow cytometer (Beckman-Coulter, U.S.A.). *In vivo* mice imaging was performed on the IVIS Lumina XRMS III *in vivo* imaging system (PerkinElmer, USA). Concentration of copper was measured by the inductively coupled plasma atomic emission spectrometer (ICPE-9810, Shimadzu, Japan).

Synthesis of copper peroxide (CuO_2) NPs

CuO_2 NPs were synthesized according to a previously reported procedure[42-44], 0.5 g PVP was dissolved in 5 mL aqueous solution containing 0.01 M $CuCl_2{\cdot}2H_2O$. 5 mL of 0.02 M NaOH aqueous solution and 100 μL of 10 M H_2O_2 were added sequentially to the above mixture solution. After stirring at 800 rpm for 20 min, the as-obtained CuO_2 NPs were collected by centrifugation at 10000 rpm for 10 min and washed with water three times.

To verify peroxo groups in CuO_2, $KMnO_4$ (50 μg mL^{-1}) was dissolved in an aqueous solution containing H_2SO_4 (0.1 M) and treated with H_2O_2, Cu^{2+}, CuO_2 NPs for 15 min, respectively. The mixtures were centrifuged subsequently, and corresponding UV-Vis spectra of supernatants were measured at 400-650 nm.

***In vitro* verification of ·OH generation**

Aqueous solution containing 10 μg mL^{-1} MB and 10 mM H_2O_2 was treated with various concentrations of CuO_2 NPs and LipGel-NMs (with equivalent CuO_2 NPs concentrations of 0.2, 0.4, 0.6, 0.8, 1.0 mM), and stirred at 150 rpm at 37°C. The reaction mixtures were centrifuged after 2 h incubation, and UV-vis spectra of their supernatants were collected from 400-800 nm. Aqueous solution treated with 1.0 mM Cu^{2+} was set as positive control group.

Synthesis of Fe_3O_4@Cu_9S_8 NPs

Fe_3O_4@Cu_9S_8 NPs were synthesized according to a previously reported procedure.[45] 15 mL of oleylamine was added to a three-necked flask, stirred at 400 rpm under nitrogen atmosphere and heated to 300°C at a heating rate of 2°C min^{-1}. 0.5 mmol of $Fe(acac)_3$ was dissolved in 3 mL of oleylamine and 2 mL of N-methyl-2-pyrrolidone, and subsequently injected into the hot flask. The reaction mixture was kept at 300°C for 10 min and cooled down to 60°C slowly, which immediately resulted in a dark color. 30 mL of ethanol was then poured into the flask, which obtained a black precipitate. The as-obtained Fe_3O_4 NPs were collected by centrifugation at 10000 rpm for 10 min and re-dispersed in 10 mL of cyclohexane for further use.

To prepare core-shell structured Fe_3O_4@Cu_9S_8 NPs, the above-obtained cyclohexane dispersed Fe_3O_4 NPs was heated to 70°C slowly in a three-necked flask under nitrogen atmosphere while stirring at a speed of 800 rpm. 3 mL of oleylamine containing 1 mmol of sulfur was rapidly injected into the flask and reacted at 70°C for 10 min. Subsequently, 0.5 mmol of $Cu(acac)_2$ dissolved 4 mL chloroform / 1 mL oleylamine mixture was injected to the reaction mixture and stirred at 70°C for another 30 min, which gradually showed dark green color for the reaction mixture. The reaction mixture was centrifuged at 10000 rpm for 10 min and washed three times with ethanol and chloroform to obtain Fe_3O_4@Cu_9S_8 NPs with oleylamine as stabilizing ligand (Fe_3O_4@Cu_9S_8-OA NPs), which were kept in 10 mL chloroform at room temperature for further use.

To disperse the above-obtained Fe_3O_4@Cu_9S_8-OA NPs in aqueous solution,[45-47] it was dispersed in 10 mL chloroform (1 mg mL^{-1}), mixed with 20 mg DSPE-PEG-acrylate (MW 2000), and stirred overnight at a speed of 800 rpm at room temperature. The chloroform was then

removed under nitrogen flow, and the as-obtained water dispersed Fe_3O_4@Cu_9S_8 NPs was kept at room temperature for further use.

Preparation of LipGel-NMs

Lipid precursor solution for lipid bilayed encapsulated hydrogel nanodroplets was prepared by mixing lecithin, cholesterol, DSPE-PEG (MW 2000) and DSPE-PEG-folic acid (DSPE-PEG-FA, MW 2000) in a molar ratio of 5:3:1:1 in chloroform, dried under a stream of nitrogen at room temperature to form a dry lipid film on the bottom of a glass vial. The vial was then placed into a vacuum desiccator and kept at 50^{o}C overnight to ensure complete removal of chloroform. The dried lipid film was dissolved in isopropyl alcohol (IPA) with 5 mM of total lipid concentration to make lipid precursor solution.

Hydrogel precursor solution was prepared by mixing 10% (v/v) PEG-DA-575, 0.1% (w/v) Fe_3O_4@Cu_9S_8 NPs, 1.0% (w/v) CuO_2 NPs, and 1.0% (v/v) photo initiator in DI water.

The lipid bilayer wrapped hydrogel nanomotors (LipGel-NMs) were prepared with a flow-focusing microfluidic chip according to our previously reported method.[40] To fabricate the microfluidic chip, a glass substrate mould imparted with designed microchannel structure was prepared via photolithograph. A photomask (Shenzhen Newway Photomask Making Co., Ltd., China) was used to transfer the designed microchannel pattern (100 μm wide) onto the borosilicate glass substrate by 15 seconds UV exposure. The borosilicate glass substrate was then sequentially immersed in developer for 30 seconds, dechroming liquid for 3 minutes and etching solution for 90 minutes at room temperature, washed with ultrapure water and dried at 80^{o}C for 1 h to complete a mould. A homogeneous, degassed mixture of PDMS oligomer and curing agent at a ratio of 10:1 (w/w) was poured over the as-prepared mould and kept at 80^{o}C for 90 min.

The as-obtained PDMS replica was sealed on a glass slide via oxygen plasma treatment to produce LipGel-NMs generation system, which contained two inlets for importing the lipid precursor phase and the hydrogel precursor phase respectively. The lipid precursor was imported into the central channel and flowed at a lower speed. The hydrogel precursor was imported into the side channels at a higher flow rate to hydro-dynamically focus the central lipid precursor stream, generating hydrogel nanodroplets that contained CuO_2 NPs, Fe_3O_4@Cu_9S_8 NPs and wrapped with lipid bilayer (LipGel-NDs).

After separating non-encapsulated CuO_2 NPs and Fe_3O_4@Cu_9S_8 NPs by centrifugation at 7500 rpm for 15 min, LipGel-NDs were collected in a vial and carefully placed on top of a magnet overnight until all of the Fe_3O_4@Cu_9S_8 NPs were gathered to a point at the bottom of each LipGel-NDs, and polymerized under 365 nm UV irradiation with 75 mW cm^{-2} for 50 s to get LipGel-NMs, which were stored in DI water at 4°C for further use.

LipGel-NMs-Cy3 were prepared following the above procedure with Cy3 labelled PEG-DA (PEG-DA-Cy3) for intracellular imaging of LipGel-NMs.

Two control nanomotors were set as following: NonFA-LipGel-NMs (NF-LipGel-NMs), which didn't contain FA targeting groups was prepared with lipid precursor of lecithin, cholesterol, and DSPE-PEG (MW 2000) in the absence of DSPE-PEG-FA. Noncurative-LipGel-NMs (NC-LipGel-NMs), which didn't contain CuO_2 NPs for CDT was prepared with hydrogel precursor of PEG-DA, Fe_3O_4@Cu_9S_8 NPs, and 1.0% (v/v) photo initiator.

Photothermal performance testing

2 mg mL^{-1} CuO_2 NPs dispersed aqueous solution, 0.2 mg mL^{-1} Fe_3O_4@Cu_9S_8 NPs dispersed aqueous solution and LipGel-NMs dispersed aqueous solution containing equivalent concentration of Fe_3O_4@Cu_9S_8 NPs were irradiated respectively under 1064 nm NIR-II irradiation (1 W cm^{-2}) for 10 minutes, and the solution temperatures were recorded with an IR thermal camera (Fortic 225-1, Fotric, China).

Movie capture of LipGel-NMs motion

10 μL LipGel-NMs dispersed aqueous solution was transferred onto a glass slide, and irradiated with 1064 nm NIR-II light. An inverted optical microscope equipped with a 20 × objective was used for video capture of LipGel-NMs motion, and movies of 60 frames at a rate of 10 frames per second (FPS) were recorded. Movement trajectories were traced and analyzed with Leica MM AF 1.5 system and Origin software. Based on the extracted trajectories, the motion speed of LipGel-NMs was calculated according to the equation: speed=χ/t, where χ and t represent travelling distance and time of LipGel-NMs respectively.

Corresponding mean square displacement (MSD) was determined according to the equation: MSD=$(x_{\Delta t}-x_0)^2+(y_{\Delta t}-y_0)^2$,[48] where (x_0, y_0) and $(x_{\Delta t}, y_{\Delta t})$ refer to the positions of LipGel-NMs at

time point of t_o and after time interval of Δt, respectively.

Cell culture

HUVEC cell line and MB49 cell line were cultured at 37°C in DMEM media (KeyGEN KGM12800-500) supplemented with 10% FBS in a humidified incubator containing 5% CO_2 and 95% air. Cell numbers were determined with a Petroff-Hausser cell counter (U.S.A.).

Intracellular imaging of LipGel-NMs-Cy3

200 μL of MB49 cells suspension (~5 × 10^4) were seeded in a 4-well confocal dish per well and cultured at 37°C for 24 h. Each well was incubated with LipGel-NMs-Cy3 (200 μL, 200 μg mL^{-1}) with 1064 nm NIR-II light irradiation (1 W cm^{-2}) for 10 min, subsequently washed with PBS thoroughly for three times to remove uninternalized LipGel-NMs-Cy3. MB49 cells were subsequently stained with Lyso-Tracker Green (200 μL, 250 nM) for 5 min to indicate cell lysosomes, and the co-localization of Cy3 fluorescence and Lyso-Tracker Green fluorescence were monitored with TCS SP8 STED 3X confocal laser scanning microscope (CLSM) (Leica, Germany).

Penetration capability characterization of LipGel-NMs-Cy3

Transwell assay. To evaluate the penetration capability of LipGel-NMs-Cy3 at tumor boundary, 1 × 10^5 of HUVEC cells were seeded in the upper chamber of a 12-well Transwell per insert (polycarbonate filter, 3 μm pore, Corning) to mimic the vascular barrier of tumor tissue. HUVECs monolayer was treated with 500 μL LipGel-NMs-Cy3 (200 μg mL^{-1}) in the presence and absence of 10 min 1064 nm laser irradiation (1 W cm^{-2}) respectively, and co-cultured with MB49 cells at the lower chamber for another 12 h. The lower MB49 cells were stained with Lyso-Tracker Green (200 μL, 250 nM), and colocalized with LipGel-NMs-Cy3 via CLSM characterization. To evaluate the penetration capability of LipGel-NMs-Cy3 at tumor position, similar experiment was conducted by replacing the upper chamber HUVECs monolayer with tumor cells (MB49) to mimic the interior of tumor tissue.

3D multicellular tumor spheroids (MTSs) model. 3D MTSs model of MB49 cells was constructed according to previously reported literatures.[49-51] A thin film of poly(2-

hydroxyethyl methacrylate) (pHEMA) was coated on the bottom of a T-25 cell culture flask with subsequent exposure to ultraviolet light over night to sterilize. 5×10^5 MB49 cells suspended in 5 mL DMEM containing 10% FBS were seeded in the above-prepared pHEMA coated T-25 cell culture flask, and incubated at 37°C in a humidified incubator containing 5% CO_2 and 95% air. The culture medium was replaced every other day. MB49 cells 3D MTSs (~350 μm in diameter) were formed spontaneously in 10 days. To investigate the penetration capability of LipGel-NMs in 3D MTSs, 200 μg mL^{-1} LipGel-NMs-Cy3 were incubated with 3D MTSs in the presence and absence of 10 min 1064 nm laser irradiation (1 W cm^{-2}) respectively. After continuous incubation for 12 h, MB49 cells 3D MTSs were washed and re-suspended in PBS, and imaged with a CLSM imaging system.

MTT assay

MTT assay was performed to quantitatively evaluate the therapeutic effect of LipGel-NMs *in vitro*. MB49 cells were seeded in a 96-well plate at a density of 5000 cells per well and incubated at 37°C for 24 h. Subsequently, 100 μL of PBS, NC-LipGel-NMs, CuO_2 NPs and LipGel-NMs (with equivalent CuO_2 concentration of 10 μg mL^{-1}) were added into each well and co-cultured for 48 h in the presence of 10 min 1064 nm laser irradiation (1 W cm^{-2}), respectively. PBS treated MB49 cells without 1064 nm laser irradiation were set as control. Afterwards, 50 μL of 1× MTT solution was added to each well and incubated with MB49 cells for 4 h to test cell viability. After removing the remained MTT solution, the produced formazan precipitates were dissolved in 150 μL DMSO and the absorbance was measured at 490 nm with a microplate reader.

Verification of intracellular ROS generation

DCFH-DA was used as a fluorescent ROS probe to quantify intracellular ·OH generation. 5×10^5 MB49 cells were seeded in a 6-well plate per well and incubated at 37°C overnight, subsequently treated with NC-LipGel-NMs, CuO_2 NPs and LipGel-NMs (with equivalent CuO_2 concentration of 10 μg mL^{-1}) respectively in the presence of 10 min NIR-II light irradiation, and further incubated for 6 h. MB49 cells in each well were then collected and stained with 10 μM DCFH-DA for 30 min. After washing with PBS for three times, intracellular fluorescence for MB49 cells were tested with flow cytometry.

***In vitro* lipid peroxidation analysis**

5×10^5 MB49 cells were seeded in a 6-well plate per well and incubated at 37°C overnight, subsequently treated with NC-LipGel-NMs, CuO_2 NPs and LipGel-NMs (with equivalent CuO_2 concentration of 10 μg mL^{-1}) respectively in the presence of 10 min NIR-II light irradiation and further incubated for 12 h. MB49 cells in each well were then collected and stained with 1 μg mL^{-1} C11-BODIPY$^{581/591}$ solution for 30 min. After washing with PBS for three times, intracellular fluorescence for MB49 cells were tested with flow cytometry.

Cell apoptosis analysis

Cell apoptosis induced by LipGel-NMs was evaluated by Annexin V-APC/PI apoptosis detection kit using flow cytometry. 5×10^5 MB49 cells were seeded in a 6-well plate per well and cultured at 37°C for 24 h, subsequently treated with PBS, NC-LipGel-NMs, CuO_2 NPs and LipGel-NMs (with equivalent CuO_2 concentration of 10 μg mL^{-1}) respectively for another 24 h in the presence of 10 min NIR-II irradiation. Afterwards, MB49 cells in each well were collected and washed three times with PBS, subsequently stained with 5 μL Annexin V-APC and PI at room temperature for 15 min in dark condition, followed by flow cytometry to identify the apoptotic cells.

Verification of LipGel-NMs *in vivo* anti-tumor effect

Female C57BL/6 mice (8 weeks) were purchased from GemPharmatech Co.,Ltd (Nanjing, China). All *in vivo* experiments were performed in accordance with the NIH guidelines for the care and use of laboratory animals (NIH Publication No. 85-23 Rev. 1985) by qualified operators (Certificate Number of 220212754) in Institutional Animal Use and Care Regulations approved by the Model Animal Research Center of Nanjing University (MARC), and the ethical approval for related animal experiments were obtained from the IRB of Nanjing Drum Tower Hospital with approval number of 2021AE01059.

Verification of LipGel-NMs *in vivo* anti-tumor effect

Orthotopic bladder tumor model was established by implanting Luc-MB49 cells (2×10^6) into the

bladder wall. After tumor cell inoculation, C57BL/6 mice were treated with D-Luciferin potassium salt (10 mg kg^{-1}) for bioimaging to monitor the development of tumor size. After the tumor volumes reached the desired size, these mice were stochastically divided into four groups (n=5). Then randomly grouped mice were administrated with 50 μL of (1) saline, (2) CuO_2 NPs (10 mg kg^{-1}), and (3,4) LipGelNMs (the equivalent CuO_2 concentrations in LipGelNMs were 10 mg kg^{-1}) through intravesical instillation under anesthetics (injection anesthesia with zoletil/xylazine (60 mg/kg/10 mg/kg) for 60 min. Immediately after bladder instillation, the mice in group (4) were exposed to 1064 nm laser (1 W cm^{-2}) for 30 min (10 min break for each 10 min exposure), while groups (1)-(3) were not irradiated. The intravesical instillation and 1064 nm NIR-II light irradiation were operated at Day 1, 5, 9, and 13 repeatedly, and bioimaging was operated to monitor the tumor size at Day 1, 11, and 16 repeatedly. On Day 17, all the mice were sacrificed, and photos of tumors were taken.

Histopathology analysis

For Hematoxylin and Eosin (H&E) staining, the heart, liver, spleen, lung, kidney, and tumor of mice from the four groups were harvested and fixed in 4% paraformaldehyde, then embedded in paraffin. The paraffin-embedded tissues were then cut into slices and stained with hematoxylin and eosin for observation with an optical microscope.

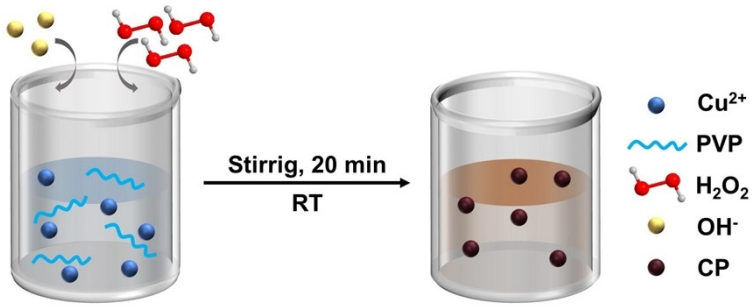

Figure S1. Schematic illustration of the synthetic process of CuO_2 NPs.

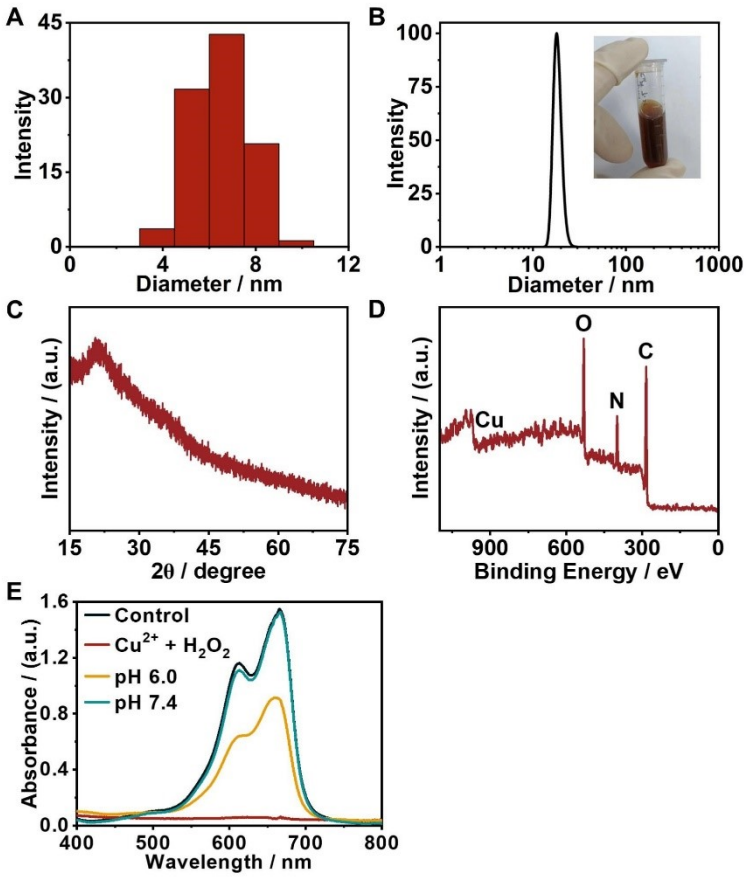

Figure S2. (A) Size distribution and (B) DLS analysis of CuO_2 NPs. Inset in (B): photograph of water dispersed CuO_2 NPs. (C) Powder XRD pattern and (D) survey XPS spectrum of CuO_2 NPs. (E) UV-vis absorption spectra of MB aqueous solution (Control) and incubating MB aqueous solution with Cu^{2+}/H_2O_2 (Cu^{2+} + H_2O_2), CuO_2 NPs at acidic solution (pH 6.0) and neutral solution (pH 7.4), respectively.

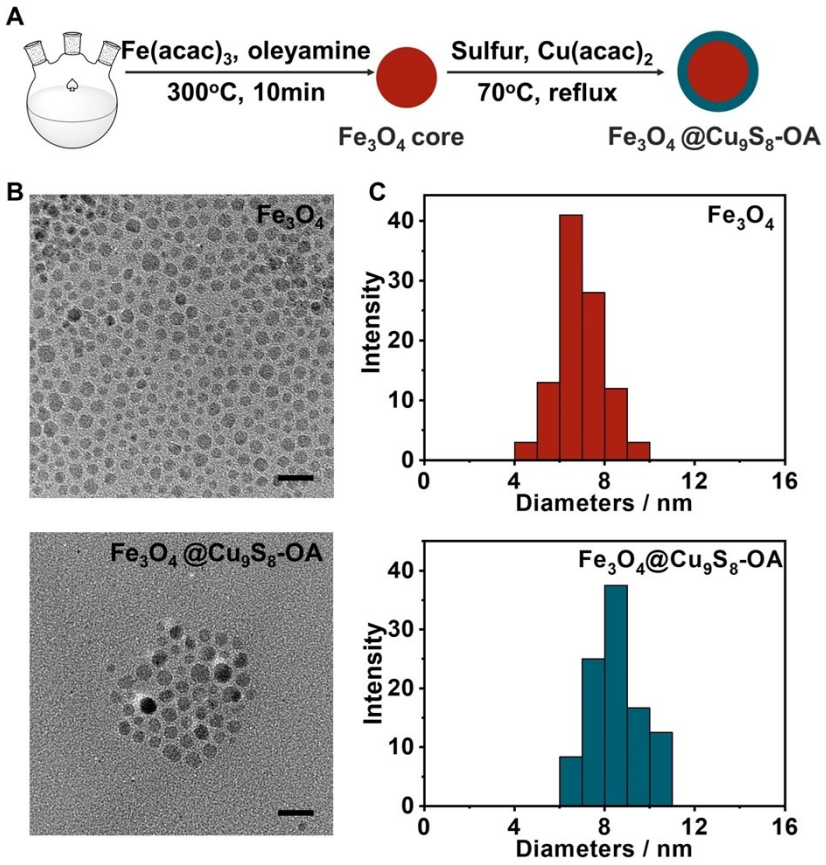

Figure S3. (A) Schematic illustration of Fe_3O_4@Cu_9S_8-OA synthesis, (B) TEM images and (C) size distributions of Fe_3O_4 core and Fe_3O_4@Cu_9S_8-OA NPs (scale bar in (B): 20 nm).

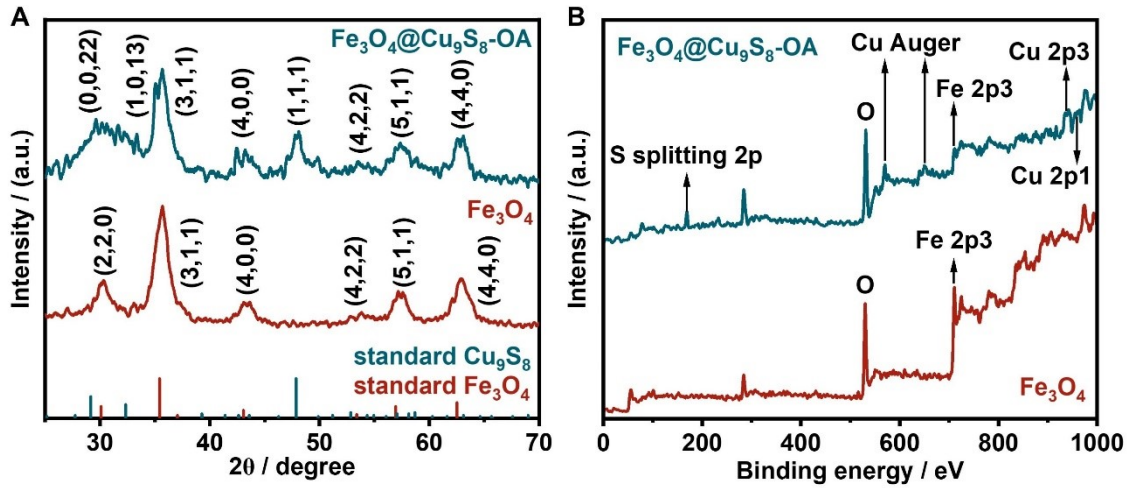

Figure S4. (A) Powder XRD patterns and (B) XPS spectra of Fe_3O_4 (brown line) and Fe_3O_4@Cu_9S_8-OA NPs (blue line). Standard Fe_3O_4 (PDF#19-0629) and Cu_9S_8 (PDF#36-0379) phases were referenced in (A).

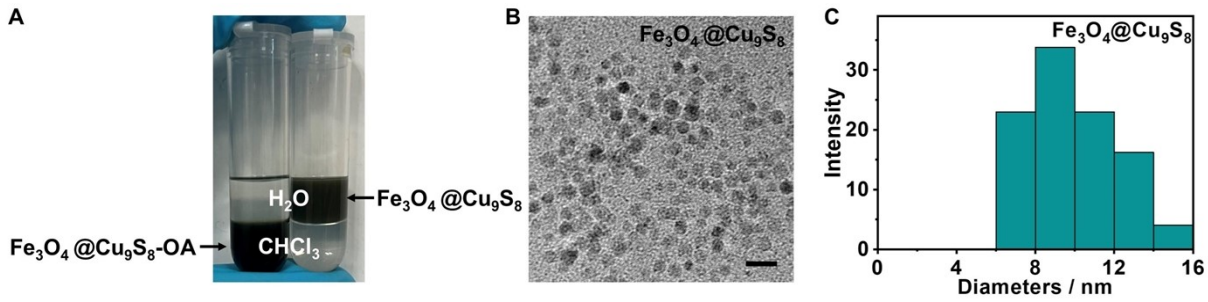

Figure S5. (A) Photograph of Fe_3O_4@Cu_9S_8-OA NPs and Fe_3O_4@Cu_9S_8 NPs. (B) TEM image and (C) Size distribution of Fe_3O_4@Cu_9S_8 NPs. Scale bar in (B): 20 nm.

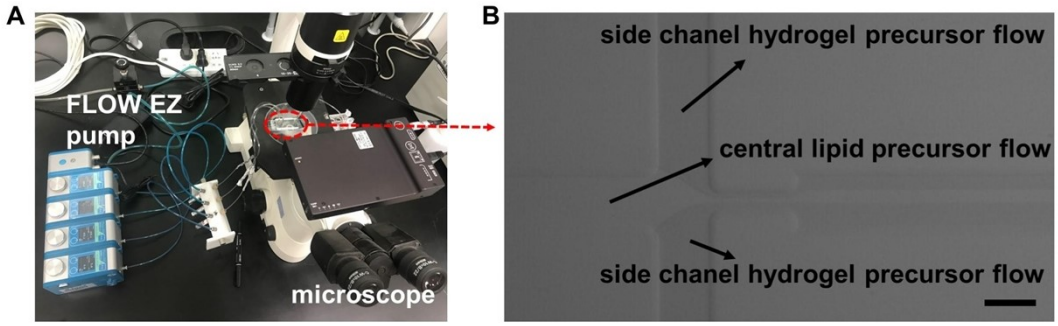

Figure S6. (A) Set up of microfluidic system equipped with a flow-focusing configured microfluidic chip, FLOW-EZ pumps for LipGel-NDs generation, and microscope for observation. (B) Bright field microphotograph of microfluidic channel with hydrodynamic flow focusing for LipGel-NDs generation (scale bar: 100 μm).

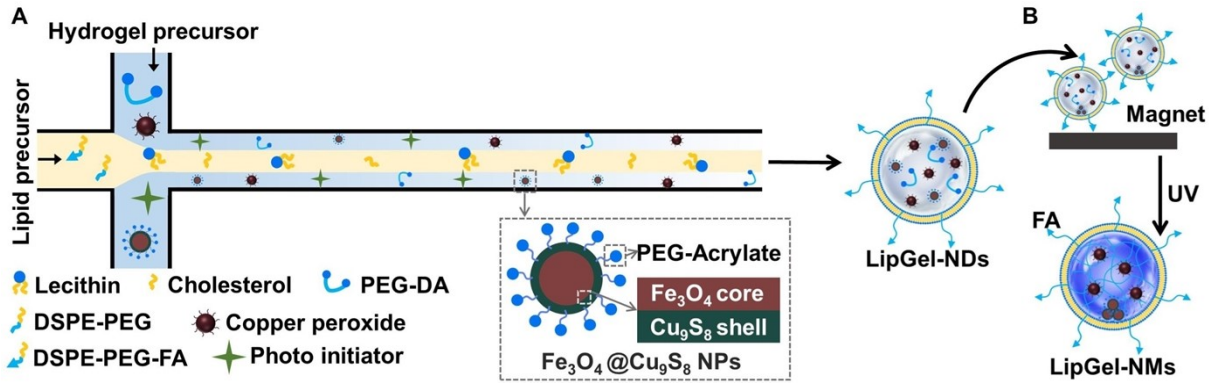

Figure S7. Schematic illustrations for synthesis of (A) LipGel-NDs and (B) LipGel-NMs.

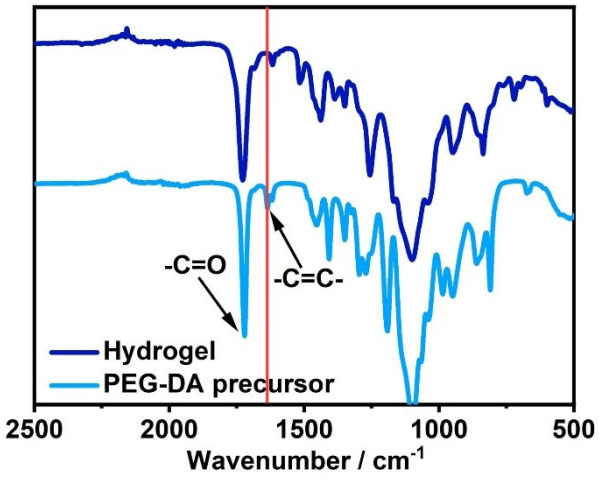

Figure S8. FTIR spectrum of PEG-DA precursor and hydrogel.

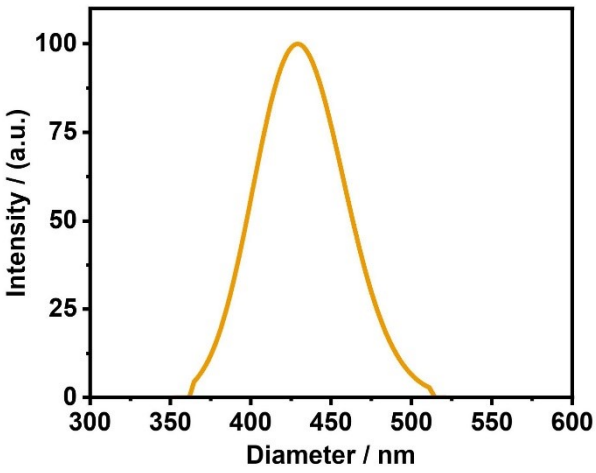

Figure S9. DLS analysis of LipGel-NMs.

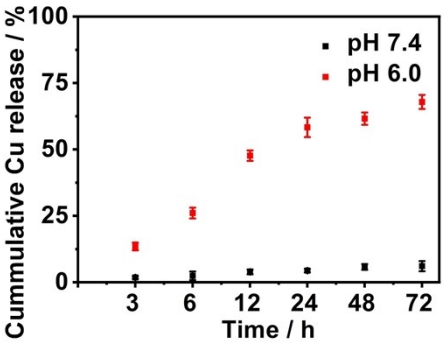

Figure S10. Cumulative release of Cu^{2+} from LipGel-NMs in PBS (pH 6.0 and pH 7.4) after 10 min 1064 nm laser irradiation. The data error bars indicated means ± SD (n=3).

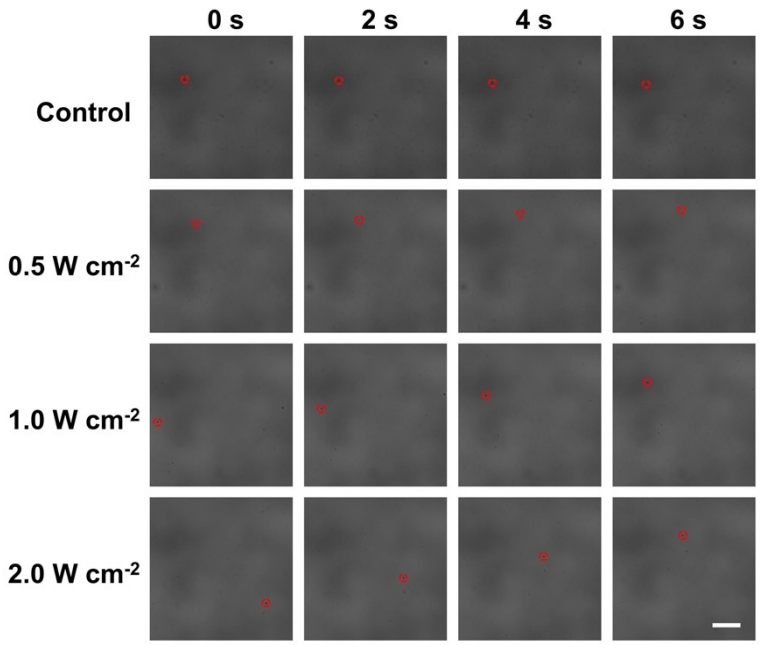

Figure S11. Time-lapsed images of LipGel-NMs in PBS (control) and under NIR-II irradiations of 0.5, 1.0, and 2.0 W cm^{-2}, respectively (scale bar: 100 μm).

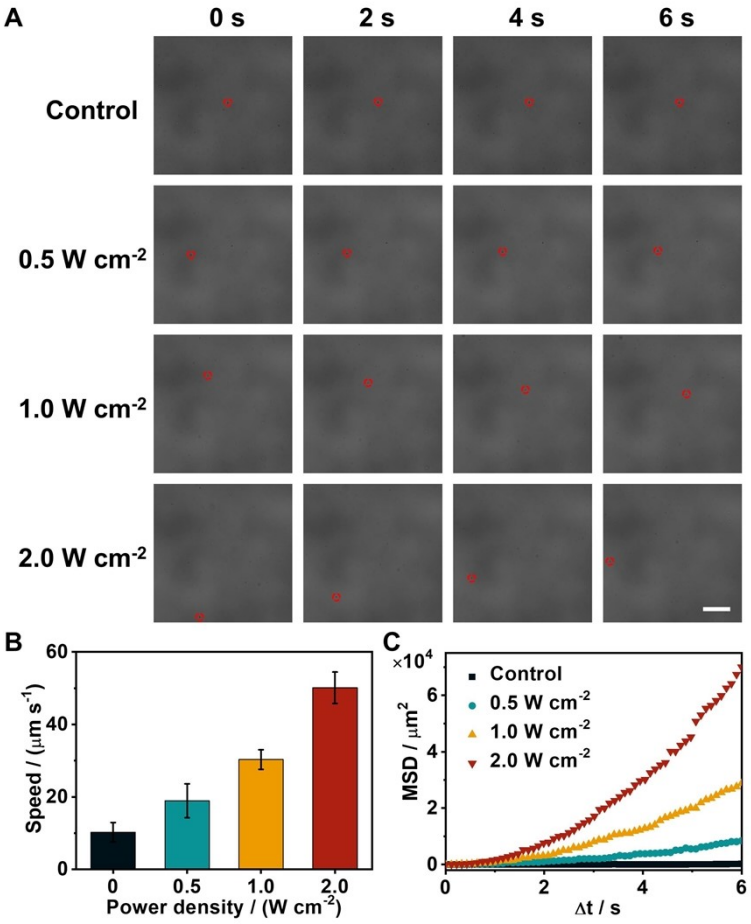

Figure S12. (A) Time-lapsed images of LipGel-NMs in cell culture media supplemented with 10% FBS (control) and under NIR-II irradiation of 0.5, 1.0, and 2.0 W cm^{-2}, respectively (scale bar: 100 μm), and corresponding (B) motion speeds and (C) MSD plots (6 s). Error bars indicated means ± SD (n=10).

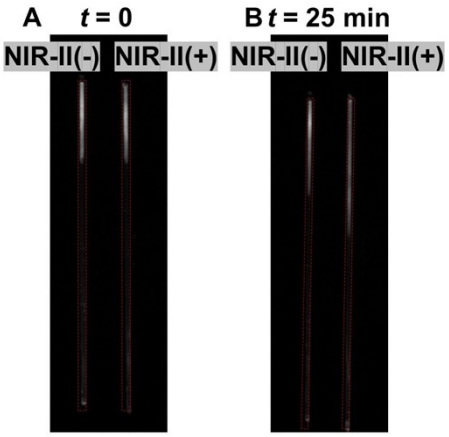

Figure S13. Fluorescent images obtained at (A) t = 0 and (B) t = 25 min after seeding of LipGel-NMs-Cy7.5 in horizontally positioned glass capillary in the presence (NIR-II(+)) and absence (NIR-II(-)) of 10 min NIR-II light irradiation.

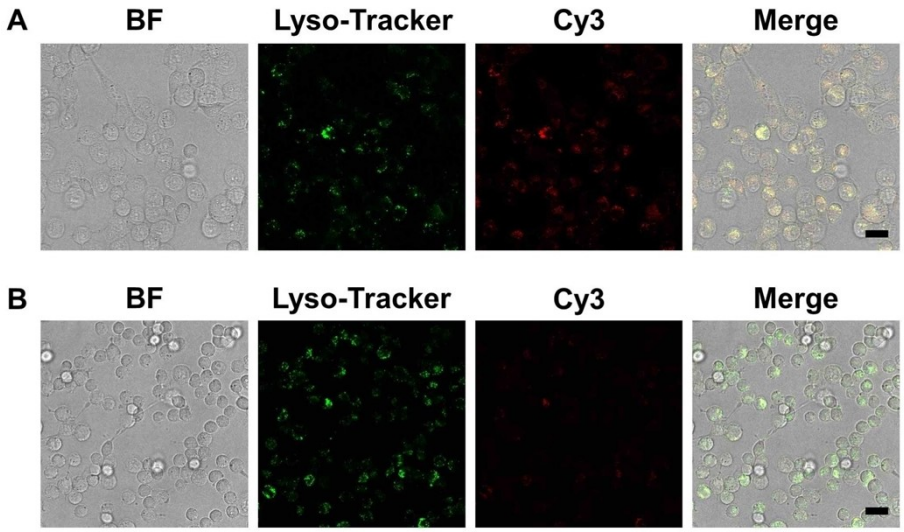

Figure S14. CLSM images of MB49 cells incubated with (A) LipGel-NMs-Cy3 for 180 min in the absence of NIR-II light irradiation and (B) NF-LipGel-NMs-Cy3 for 25 min with 10 min NIR-II irradiation, and stained with Lyso-Tracker Green. Red channel (Cy3) fluorescence was collected at 555-605 nm with 543 nm excitation, and green channel (Lyso-Tracker Green) fluorescence was collected at 505-555 nm with 488 nm excitation (scale bar: 20 μm).

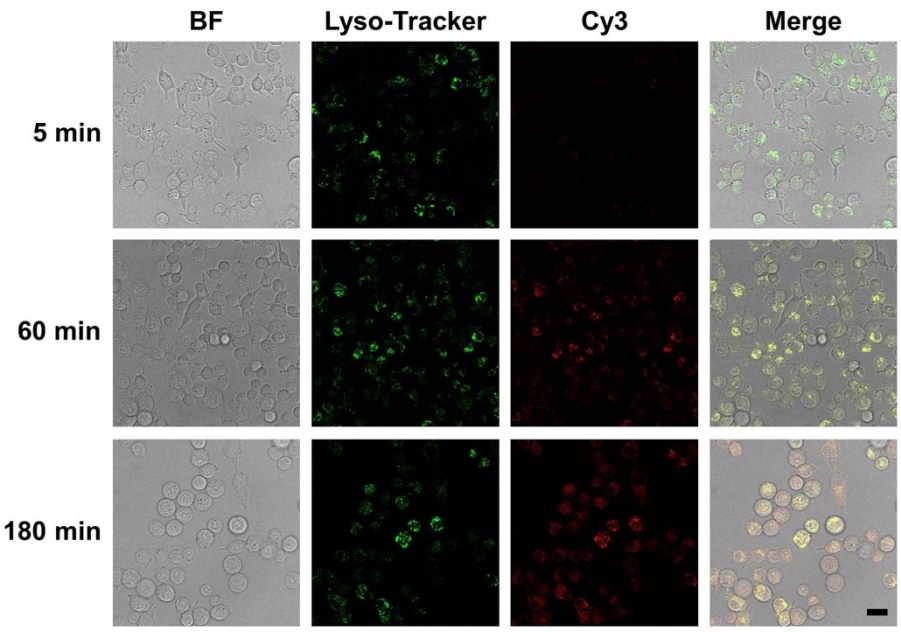

Figure S15. Time-dependent CLSM images of MB49 cells incubated with LipGel-NMs-Cy3 for different times after 10 min NIR-II irradiation and stained with Lyso-Tracker Green. Red channel (Cy3) fluorescence was collected at 555-605 nm with 543 nm excitation, and green channel (Lyso-Tracker Green) fluorescence was collected at 505-555 nm with 488 nm excitation (scale bar: 20 μm).

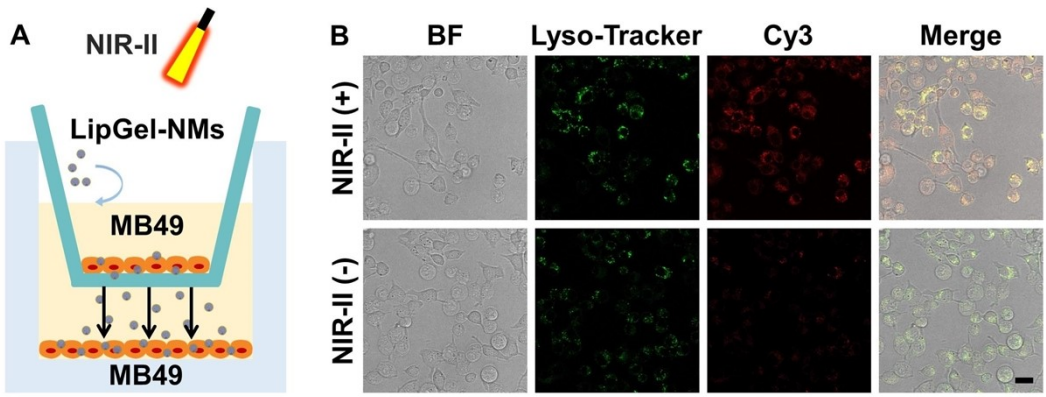

Figure S16. (A) Schematic illustration and (B) CLSM images for intratumoral penetration of LipGel-NMs-Cy3 in the presence (NIR-II (+)) and absence (NIR-II (-)) of NIR-II irradiation (scale bar: 20 μm). Red channel (Cy3) fluorescence was collected at 555-605 nm with 543 nm excitation, and green channel (Lyso-Tracker Green) fluorescence was collected at 505-555 nm with 488 nm excitation (scale bar: 20 μm).

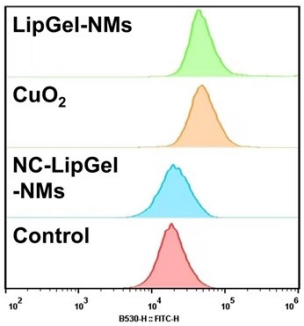

Figure S17. Flow cytometry analysis of intracellular DCF fluorescence intensities for MB49 cells incubated with NC-LipGel-NMs, CuO_2 NPs, and LipGel-NMs respectively.

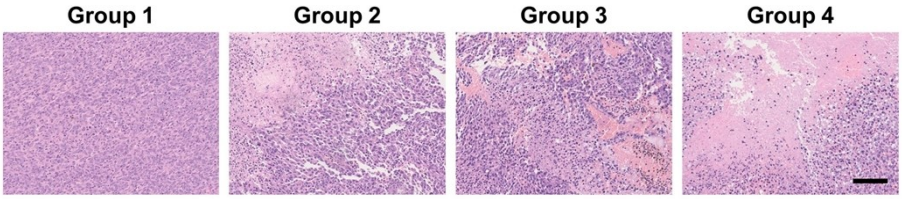

Figure S18. Representative H&E staining images of tumor tissues on Day 17 after various treatments (scale bar: 100 μm). Group 1-4 represented 1: saline instillation, 2: CuO_2 NPs instillation, 3: LipGel-NMs instillation without NIR-II light irradiation, and 4: LipGel-NMs instillation with NIR-II light irradiation.

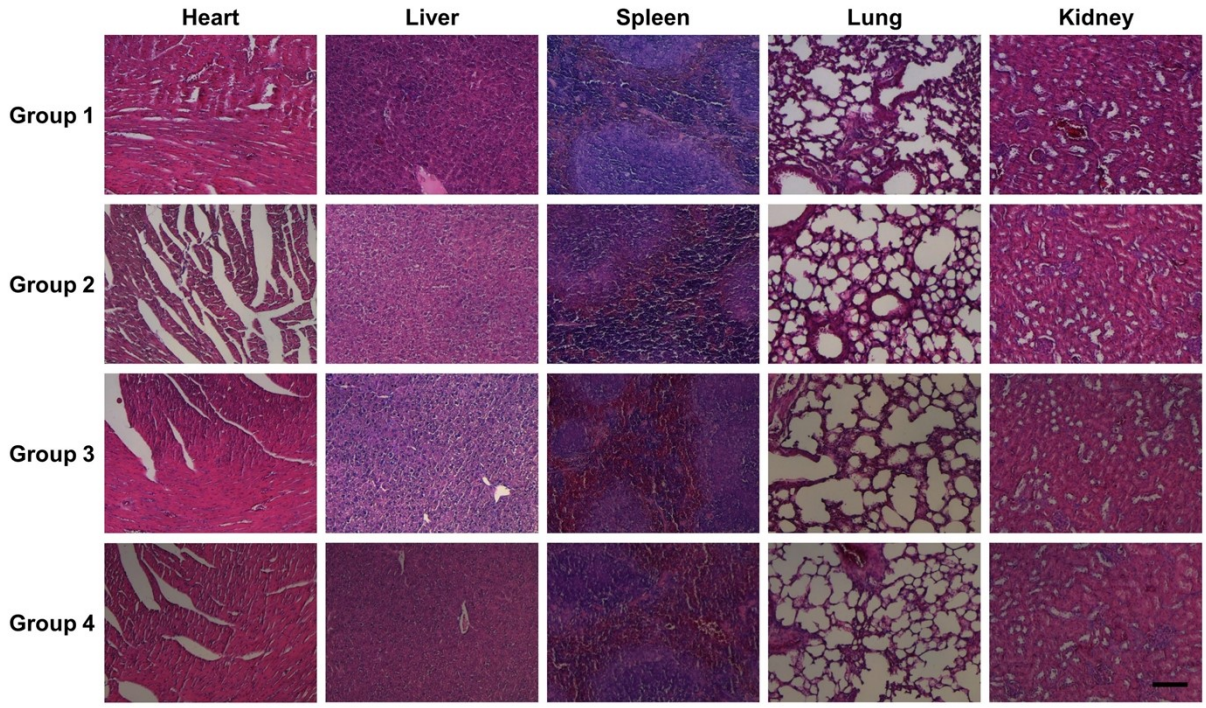

Figure S19. H&E staining of heart, liver, spleen, lung, and kidney of mice on Day 17 after various treatments (scale bar: 100 μm). Group 1-4 represented 1: saline instillation, 2: CuO_2 NPs instillation, 3: LipGel-NMs instillation without NIR-II light irradiation, and 4: LipGel-NMs instillation with NIR-II light irradiation.