SUPPORTING INFORMATION:

Hybrid Graphene-Gold Nanoparticle-based Nucleic Acid Conjugates for Cancer-Specific Multimodal Imaging and Combined Therapeutics

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A. METHODS

Synthesis of cysteamine functionalized nanoparticles (Au NP). 40 nm (TEM size) cysteamine functionalized gold nanoparticles were synthesized using cysteamine as a ligand from $HAuCl_4$ salt. 20.0 ml of 0.53 mg/ml $HAuCl_4$ solution was prepared, and 2.4 mg cysteamine hydrochloride salt was added into the solution in a 50-ml round bottom flask and stirred in the dark at a speed of 1,200 rpm using a magnetic bar at room temperature. The solution turned from yellow to orange instantly. After one hour, 10.0 µl of 0.38 mg/ml $NaBH_4$ solution was rapidly injected into the $HAuCl_4$ solution, and a faint purple color appeared after the injection of $NaBH_4$ solution. The solution continued to be stirred for 2 hours at a speed of 1,200 rpm and then stirred overnight at a speed of 500 rpm. Then the nanoparticles were centrifuged down at 4,000 rpm for 5 minutes using a microcentrifuge and washed with water twice to remove free cysteamine molecules. The concentration of cysteamine functionalized gold nanoparticles were estimated by drying 1.0 ml solution in an oven and then correlated to the absorption at 527 nm in the UV-Vis spectrum.

Synthesis of CTAB functionalized gold nanorods (Au NR). Au NR is prepared using a previously established protocol.[1] Briefly, 10 mL hexadecyltrimethylammoniumbromide (CTAB, Sigma) solution was prepared at a concentration of 0.2 M followed by the addition of 10 mL HAuCl4 solution at a concentration of 0.5 mM. After vigorous stirring, 1.2 mL 10 mM $NaBH_4$ solution (ice-cold) was added. A dark-yellow solution will appear immediately. After stirring at room temperature for 5 minutes, the solution containing the seeds was formed. To further grow into nanorods, 10 mL CTAB (concentration of 200 mM) was slowly added to 0.20 mL of 4 mM AgNO3 solution at room temperature, followed by the addition of 10 mL 1 mM HAuCl4 solution. Afterwards, 140 µL of 80 mM ascorbic acid (AA) solution was added, and the growth solution became colorless. Lastly, into the colorless solution, 24 µl seed solution initially prepared was quickly added at room temperature. The final solution was purified by centrifugation at 5,000 rpm for 5 minutes and washed by ultrapure water twice. Note that over two times of washing was found to lead to permanent aggregations of Au NR.

Synthesis of nano-sized negatively charged Graphene oxide (GO). Graphene oxide with sizes around 100 nm was synthesized based on a previously reported method with minor modifications.[2-3] 1.0 g of graphite flakes were added to a mixture of 12.0 ml concentrated sulfuric acid (98%), 2.5 g P_2O_5, and 2.5 g $K_2S_2O_8$, then stirred and heated at 83 ºC for 6 hours to get the pre-oxidized graphite oxide. After washing with 500 ml distilled water, the pre-oxidized graphite oxide was dried under vacuum overnight and used for exfoliation. In the ice water bath, 1.0 g pre-oxidized oxide was added into 120 ml concentrated H_2SO_4, then 15.0 g $KMnO_4$ was slowly added in 10 minutes with the temperature kept below 20 °C. After another 20 minutes of stirring, the temperature of the reaction was gradually raised to 35 °C and continuously kept for 3.5 hours. Then 250 ml

distilled water was added with caution, and the temperature was kept below 50 °C. After 3 hours, the reaction was quenched by adding 700 ml distilled water followed by 20 ml 30% H_2O_2 aqueous solution, with the appearance of a bright yellow color in the solution. The as-synthesized graphite oxide was washed with 10% HCl solution and distilled water for three times. After exfoliated under ultrasonication for 3 hours underwater bath, the graphene oxide solution was centrifuged at 13,300 rpm for 1 hour twice, and the supernatant solution was used.

Synthesis of Au@GO NP, Au@rGO, Au@GO NR, and Au NP-GO assembly. Into 5 ml highly concentrated GO solution (3.0 mg/ml), 1.0 ml cysteamine functionalized gold nanoparticles, CTAB-functionalized gold nanorod solution (0.1 mg/ml) was added drop by drop (10 µl for each drop) under vigorous stirring (800 rpm). After the addition, the solution was transferred to a 10-ml glass vial, sealed, sonicated, and vortexed at 2,000 rpm for 1 hour, and a dark red solution was obtained. Solutions containing Au@GO NP or Au@GO NR were centrifuged at 8,000 rpm and washed with distilled water three times to get rid of any suspended GO. Au@rGO NP was obtained by incubating Au@GO NP at 1.0 mg/mL ascorbic acid solution for 24 hours, followed by purification. To synthesize the gold nanoparticle decorated graphene, 0.1 ml cysteamine functionalized gold nanoparticles solution was added into a 1.0 ml of 0.1 mg/ml GO solution followed by 20 minutes of bath sonication. The solution turned purple 1 minute after the addition of gold nanoparticles. After sonication, the gold nanoparticle decorated graphene was then washed with distilled water three times.

Characterizations of GO, Au NP, Au NR, GO-encapsulated Au NP (Au@GO NP), GO-encapsulated. Zeta potentials and hydrodynamic sizes of the nanoparticles were recorded using a Malvern Nano ZS instrument. 100 µg/ml of GO, 10 µg/ml Au NP, Au NR, Au@GO NP, Au@GO NR, and 20 µg/ml Au NP-GO aqueous solutions were used for the zeta potential and zeta size measurement. TEM was performed on all nanoparticles at a concentration of 50 µg/ml. Raman spectrum of Au NP-GO nanoparticles was performed using a Renishaw inVia Raman microscope at a wavelength of 633 nm and a 10% power output. A GO encapsulated Au NP solution with a concentration of 100 µg/ml was drop cast on a cover glass, dried overnight and then measured with the Raman spectrum. UV-Vis absorption spectrum of 40 nm citric capped gold nanoparticles (40 nm Au-citric), GO (100 nm GO), 40 nm cysteamine functionalized Au NP, and 40 nm Au@GO NP was recorded in an Agilent Cary 60 UV-Vis spectrometer. The citric capped gold nanoparticles were purchased from TED Pella®.

Biocompatibility test of the Au@GO NPs on different types of cells. All cancer cells (HUH7.5, Panc-1, A431, ASPC1, MCF7, A549, B16, MDA-MB-231, U87-EGFP, U87-ERFP) and somatic cells (fibroblasts and astrocytes) were cultured in DMEM (Dulbecco's modified Eagle's medium) cell media supplemented with high

glucose, 10% FBS (fetal bovine serum), 1% streptomycin-penicillin, and 1% glutamax unless mentioned otherwise. Twenty-four hours before the cell viability test, 2×10^4 cells with a volume of 100 µl were seeded into each well of 96 well plates to attain 60%-80% cell confluency during the cell viability test. Au@GO NPs and Au@GO NRs were first dissolved in 0.5% FBS cell media and then added to get different concentrations (0 µg/ml, 5 µg/ml, 10 µg/ml, 20 µg/ml, 50 µg/ml, and 100 µg/ml) for cell uptake. After 3 hours of incubation, the cell media was changed to 10% FBS DMEM and after 48 hours, the cell viability was measured using a standard Presto blue assay. A fluorescence peak at 590 nm under excitation of 570 nm was used to quantify the cell viability, by assuming the cell viability of control cells without any nanoparticles is 100%.

Evaluation of photothermal effect from Au@GO NP, Au@rGO, Au@GO NR, and Au NP-GO assembly. 2.0 µg/ml GO, 20 µg/ml cysteamine functionalized nanoparticles, 20 µg/ml citric acid-functionalized nanoparticles, and 20 µg/ml Au@GO NP, Au@rGO, Au@GO NR, and Au NP-GO assembly were measured with photothermal effect under identical conditions. Concentrations were normalized based on their UV-Vis absorption intensity (227 nm for GO and 527 nm for gold nanoparticles). 1.0 ml nanoparticle solution was added to a pre-cleaned quartz cuvette with a thin metal thermal probe. A NIR laser (808 nm wavelength) was fixed with laser in proximity with the quartz surface at 45 °C. The temperature of the solution was first stabilized for 5 minutes, then the laser with a pre-set power density of 6.6 W/cm^2 was turned on, and then the temperature of the solution was recorded every one minute for 8 minutes. The setup remained the same during the photothermal test for all four solutions and water only was used as a control.

Dark-field imaging of the glioblastoma cells. For dark-field imaging and SERS imaging, we used a glioblastoma U87-EGFP cell line. The cells were seeded into a 24 well plate with cover glass with a cell density of 6×10^4 cells per well. After 24 hours incubation with cell confluency percentage of 30%-50%, the Au@GO NP nanoparticles and gold nanoparticle decorated graphene were delivered using Opti-MEM cell media at a concentration of 50 µg/ml followed by 6-hour incubation. Then the cell media was changed to 10% FBS DMEM and continued with incubation for 12 hours. For dark-field imaging, the cells were then washed with PBS and fixed using formalin. An Olympus IX83 inverted motorized microscope was used to obtain the dark-field images.

Mild PT hyperthermia performed in glioblastoma cells. U87-EGFR VIII cells were seeded in a 96 well plate at a cell density of 20k per well. Au@GO NP nanoparticles in Opti-MEM were then delivered into the cells at a concentration of 50 µg/ml and incubated for 3 hours. The cell media was later changed back to 10% FBS DMEM cell media. Different laser powers were applied to the cells vertically with a distance of 0.5 cm for

5 minutes in each well. After laser exposure, the cell media was changed, and the cells were incubated for 48 hours before measuring the cell viability using a standard Presto blue assay. For the qRT-PCR analysis, we extracted RNA from cells using a standard TRIzol procedure. Hyperthermia stimulated HSP family and cellular apoptotic family Caspase 3 was analyzed. The designed sequences for HSP and Caspase 3 genes are listed as below: Caspase3: forward primer, 5'-AGAGGGGATCGTTGTAGAAGTC; reverse primer, 3'-ACAGTCCAGTTCTGTACCACG. HSP27: forward primer, 5'-TGGACCCCACCCAAGTTTC; reverse primer, 3'-CGGCAGTCTCATCGGATTTT. HSP70: forward, 5'-TTTTACCACTGAGCAAGTGACTG; reverse, 3'-ACAAGGAACCGAAACAACACA. HSP72: forward primer, 5'-TGCTGATCCAGGTGTACGAG; reverse primer, 3'-CGTTGGTGATGGTGATCTTG. HSP90: forward primer, 5'-CTTGGGTCTGGGTTTCCTC; reverse primer, 3'- GGGCAACACCTCTACAAGGA

Gene Expression Analysis: TRIzol Reagent from Life Technologies was used to extract cell mRNAs, which were transcribed to cDNA for the quantitative PCR (qPCR) analysis. Afterward, cDNA converted from 1 μg of total RNA by the Superscript III First-Strand Synthesis System (Life Technologies) will be used for the qPCR reactions through StepOnePlus Real-Time PCR System (Applied Biosystems) using Power SYBR Green PCR Master Mix (Applied Biosystems) with the primers designed for each of the target mRNAs. The Ct (times of cycling) values were then normalized to the control GAPDH gene. We used Standard cycling conditions for all the qPCR reactions, which were performed at a melting temperature of 60℃.

Study and detection of the antisense loading and detachment using FRET and SERS. A 100 pmol/1.0 ml Cy5-labeled oligonucleotide (sequence: 5'-/Cy5/TGC GCT CCT GGA CGT AGC CTT -3') solution was first prepared and measured with fluorescence. Then 100 μl 0.5 mg/ml Au@GO NP solution was added into the oligonucleotide solution. After incubation at room temperature for 15 minutes, the fluorescence from the solution was measured. To prove the quenching was not from the extinction of Au@GO NP nanoparticles, 1 nmol/100 μl of a complementary DNA (sequence: 5'- AAG GCT ACG TCC AGG AGC GCA -3') was added to the solution and was incubated for 15 minutes. The fluorescence from the solution was then measured. For the detection using SERS, the solutions of Au@GO NP conjugated with Cy5-labeled ASON and after binding toward the complementary DNA, were dried and cast to a glass slide. Films formed afterward were performed with Raman measurement using a Renishaw inVia Raman microscope at laser wavelength at 633 nm and at a 40x magnification.

SERS-based detection of BCL2 genes in cells. The cells were seeded into 24 well plates with cover glass with a cell density of 6×10^4 per well. After 24 hours incubation with cell confluency percentage 30%-50%, Au@GO

NP conjugated with Cy5-labeled ASON targeting BCL2 mRNAs were delivered using Opti-MEM cell media at a concentration of 50 μg/ml followed by 6 hours incubation. Then the cell media was changed to 10% FBS DMEM and continued with incubation for 12 hours. For SERS imaging, Renishaw inVia Raman microscope was used. The cell media was changed to PBS during the measurement. At a laser wavelength at 633 nm and 20x magnification, the Raman signals from the selected spots were collected or underwent 20 by 20 μm mapping at a resolution of 1 μm per spot. Glioblastoma and astrocytes were used as individual experimental and control cells for the detection. Maps were created using OriginLab.

GFP & BCL2 knockdown in glioblastoma cells using Au@GO NP nanoparticles loaded with GFP antisense. Into 0.5 ml of 0.1 mg/ml Au@GO NP nanoparticles and 0.5 ml of 10 μg/ml GO, we added 10 μl aqueous solution with 1 nmol of GFP antisense (5'-TGC GCT CCT GGA CGT AGC CTT -3') and 0.1 ml of 10x PBS. After incubation and shaking for 24 hours, the nanoparticles and GO were filtered through a filter with 100k MW cut-off. Then they were washed with the enzyme-free water and re-dissolved in Opti-MEM cell media to get the desired concentrations (Au@GO NP nanoparticles at 50 μg/ml and GO at the concentration of 5 μg/ml). These two groups, with control groups: no addition, 50 μg/ml Au@GO NP nanoparticles, 5 μg/ml GO, and 100 pmol/1 ml antisense DNA, were then delivered into U87-EGFP cells in a 24 well plate, at a cell density of 60k cells/well. After 6 hours, the cell media was changed into 10% FBS DMEM cell media and cultured for another 48 hours. The GFP fluorescence of these experimental groups was then checked under the Nikon Ti Eclipse microscope. The GFP knockdown efficiency was calculated based on the average GFP fluorescence intensity in the fluorescent images. BCL2 antisense (sequence: 5'-TCT CCC AGC GTG CGC CAT-3' was loaded in Au@GO NP nanoparticles and delivered using the same method described for GFP antisense. After 48 hours of delivery, mRNA was extracted from the cells and analyzed with qRT-PCR following a standard procedure with the designed primer (forward primer: 5'-GGTGGGGTCATGTGTGTGG; reverse primer: 3'-CGGTTCAGGTACTCAGTCATCC).

Photothermal-gene co-sensitized chemotherapy on glioblastoma cells. 100 μg Au@GO NP nanoparticles were firstly loaded with excess BCL2 antisense (1 nmol) in PBS, then incubated for 24 hours, washed and re-dissolved in Opti-MEM cell media. Afterward, a stock doxorubicin aqueous solution at a concentration of 1 mg/ml was added into 1 ml Opti-MEM cell media to obtain different doxorubicin concentrations, 0, 0.1, 0.5, 1, 5, and 10 μg/ml. After incubation at room temperature for 4 to 6 hours, the nanoparticles loaded with doxorubicin were delivered into U87-EGFR VIII cells (doxorubicin is expected to release at lower PH when entered cancer cells). U87-EGFR VIII cells were seeded at a density at 20k cells/well in a 96 well plate. After incubation for 6 hours, the media was changed to 10% FBS. The cells were continued to be cultured for 24

hours, and then cells were exposed to an 808 nm NIR laser at a distance of 0.5 cm and laser intensity of 1.2 W/cm^2 for 5 minutes in each well (laser intensity measured with a Newport power meter). After the NIR laser exposure, the cell media was changed, and the Presto blue assay was performed after 48 hours of culturing. For control groups, nanoparticles with only doxorubicin loading, nanoparticles with only doxorubicin and BCL2 antisense, and nanoparticles with only doxorubicin and NIR laser exposure were performed in parallel under identical conditions.

Synthesis of iRGD functionalized Au@GO NP nanoparticles. 0.5 mg of DSPE-PEG2000-NH_2 (Avanti Polar Lipids) was dissolved in 0.5 ml 0.4 mg/ml Au@GO NP nanoparticle solution by careful pipetting, followed by extensive bath sonication for 1 hour below 25 °C (changing water in the sonicator every 10 minutes). Then the nanoparticles were centrifuged down at 8,000 rpm and washed with distilled water twice and then dissolved in PBS. To introduce the maleimide group for sulfhydryl conjugation, 0.5 mg sulfo-SMCC crosslinker dissolved in 50 µl DMSO was added into the lipid functionalized Au@GO NP nanoparticles, and the solution was shaken at room temperature for 2 hours to complete the reaction and then washed with PBS. Before conjugating the iRGD with cysteine functionalities to the maleimide group on the nanoparticle, 2.0 mg TCEP dissolved in 20 µl of 0.5 M $NaHCO_3$ (pH~6) was diluted to 0.2 mol/L by adding distilled water. After adding 25 µl of 0.2 M TCEP into 200 nmol iRGD (in the form of lyophilized powder), 0.5 ml of 0.4 mg/ml Au@GO NP nanoparticles functionalized with lipid and sulfo-SMCC were added into the iRGD solution. The reaction proceeded for 24 hours in the fridge at 4 °C. Finally, the iRGD functionalized nanoparticles were washed with distilled water.

Targeted delivery of Au@GO NP nanoparticles functionalized with iRGD peptides. MCF-7 and MDA-MB231 cells were seeded on cover glass in a 24 well plate at a cell density of 60k cells per well and cultured for 24 hours. When the cell reached 30%-50% confluency, iRGD-functionalized Au@GO NP nanoparticles were delivered to the two cell lines at a concentration of 20 µg/ml with 0.5 ml volume (Opti-MEM) each well. After 1 hour, the cell media was washed with PBS and changed to 10% FBS DMEM. After incubation for 6 hours, the cells were washed with PBS for dark field imaging analysis. To selectively knockdown of BCL2 in MDA-MB 231 cells, 20 µg/ml iRGD functionalized Au@GO NP nanoparticles, loaded with BCL2 antisense and doxorubicin as described in the combined therapy part, were delivered to the two cell lines cultured in a 96 well plate for 1 hour. After cell media change and incubation for 24 hours, the cells were exposed to NIR laser for 5 minutes at a laser density of 1.2 W/cm^2. Forty-eight hours after the laser exposure, the cell viability of the two cell lines under different concentrations of doxorubicin was measured using a standard Presto blue assay.

Fabrication of the microfluidic device: The microfluidic device was designed using Autocad software (Autodesk, USA) and produced through a two-step photolithography process. The two-step photolithography process was used to fabricate three chambers of 250 μm height for cell culture and bridge channels of 20μm height connecting each cell culture chambers. The bridge channel was fabricated with SU-8 2025 photoresist on a silicon wafer and spin-coat at 4000 rpm, and the chambers were coated with SU-8 100 photoresist on the silicon wafer and spin-coat 1000 rpm after the bridge channel was fabricated. After UV light exposure, a two-step pattern was produced on the silicon wafer through a developing process, and polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning Corp., USA) was poured thereon and placed in an oven at 80 ℃ for one hour. The PDMS is then carefully detached from the silicon wafer and attached onto the thin glass using oxygen plasma treatment (Femto Science, Korea). Finally, it was sterilized through 70% ethanol and UV light.

Cell culture of MCF-7 and MDA-MB-231 in microfluidic devices: MCF-7 and MDA-MB-231, breast cancer cells were cultured with Dulbecco`s Modified Eagle`s Medium (DMEM) and RPMI 1640 (Thermo Fisher Scientific, USA), respectively. Both medium containing 10 % fetal bovine serum (FBS, Thermo Fisher Scientific, USA) and 1 % penicillin-streptomycin (Thermo Fisher Scientific, USA). MCF-7 cells were stained with Far-red (Thermo Fisher Scientific, USA) and MDA-MB-231 cells were stained with carboxyfluorescein diacetate succinimidyl ester (CFSE, Thermo Fisher Scientific, USA). To co-culture MCF-7 and MDA-MB-231 cells in the microfluidic device, Gelatin methacrylate (GelMA) hydrogel was injected into the center chamber of the microfluidic device. 5% v/v GelMA hydrogel synthesis method was performed in the same manner as previously described. After crosslinked, 4×105 cells/mL MCF-7 cells were seeded into the left chamber of the microfluidic device and 4×105 cells/mL MDA-MB-231 cells were injected into the right chamber of the microfluidic device.

Photothermal therapy in the microfluidic co-culture platform: MCF7 and MDA-MB-231 cells were seeded into the microfluidic device and cultured for 3 days. After 3 days, Au@GO-DOX nanocomposite was injected through the center channel while GelMA hydrogel was filled. The Au@GO-DOX nanocomposite was treated for 6 hours, and the NIR laser was irradiated to the entire microfluidic device for 10 minutes. The images after NIR laser irradiation were obtained by a fluorescent microscope. Afterward, cell viability in the microfluidic device was analyzed through a live/dead assay kit (Thermo Fisher Scientific, USA). Quantitative analysis of cell viability survival was performed via Image J software J (National Institute of Health, USA).

SERS-based detection of BCL2 genes *in vivo*. A PBS solution of Au@GO NP-cy5-BCL2 ASON was injected into the tumor site and control site (skin and muscle tissues outside of the tumor region) at a concentration of

100 μg/10μL. 24-hour after the nanoparticle injection, mice were anethestized by isofluorane and then the SERS measurements were performed using a Renishaw In Viva Raman instruments. At a laser wavelength at 633 nm and 20x magnification, the Raman signals from the selected spots were collected or underwent 20 by 20 μm mapping at a resolution of 1 μm per spot. Glioblastoma and astrocytes were used as respective experimental and control cells for the detection. Maps were created using OriginLab.

***In vivo* tumor suppression in mice**: To examine anti-cancer effects of Au@GO NP based delivery system in the breast cancer tissue, 6-week-old BALB/c nude mice were purchased from RaonBio (Kayonggi-do, Yongin-si, South Korea) (control, n=3; xenograft, n=3). All animals were acclimatized to the animal facility for at least 48 hours before experimentation and maintained according to the Guide for the Care and Use of Laboratory Animals published by the NIH. The animals were housed in a barrier under HEPA filtration and provided with sterilized food and water ad libitum. The animal facility maintained 12-hour light/dark cycles at room temperature 21 ± 2°C with 30~40% humidity. Approximately 5.0×10^6 MDA-MB-231 cells were mixed with 354234-matrigel (BD, San Jose, California, USA) and subcutaneously injected in both shoulders and both thighs (4 sites in mice). Nanoparticle injection and anti-tumor studies were conducted when the tumors were around 4 mm in diameter. Specifically, PBS solutions of iRGD-Au@GO NP-Dox-BCL2 ASON was injected into the tumor site and control site (skin and muscle tissues outside of the tumor region) at a concentration of 100 μg/10μL and an injection frequency of 2 times per week. Tumor sizes were measured before each injection and recorded. For the *in vivo* gene expression analysis, tumor tissues from the control and experimental condition were extracted, homogenized and the RNAs were extracted by Trizol® for the qRT-PCR experiments. The designed primers for *in vivo* gene analysis are listed as below: BAX: forward primer, 5'-CCCGAGAGGTCTTTTTCCGAG; reverse primer, 3'-CCAGCCCATGATGGTTCTGAT. Caspase3: forward primer, 5'-AGAGGGGATCGTTGTAGAAGTC; reverse primer, 3'-ACAGTCCAGTTCTGTACCACG. Cytochrome c (Cyto-c): forward primer, 5´-TTTGGATCCAATGGGTGATGTTGAG; reverse primer, 5´-TTTGAATTCCTCATTAGTAGCTTTTTTGAG. BID: forward primer: TGGACTGTGAGGTCAACAACG; reverse primer: AGTCTGCAGCTCATCGTAGCC.

Analysis of *in vivo* particle distribution. One week after the last treatment, grafted tumor tissues and other organs (liver, spleen, and kidney) were dissected and embedded in aqua regia for the nanoparticle eluting from the tissue. After a one-week incubation, the concentration of gold ion from each solution was analyzed using an inductively coupled plasma-mass spectrometer (ICP-MS, ELAN 6100, Perkin-Elmer SCIEX) at the National Center for Interuniversity Research Facilities (NCIRF), Seoul National University.

B. SUPPLEMENTARY FIGURES

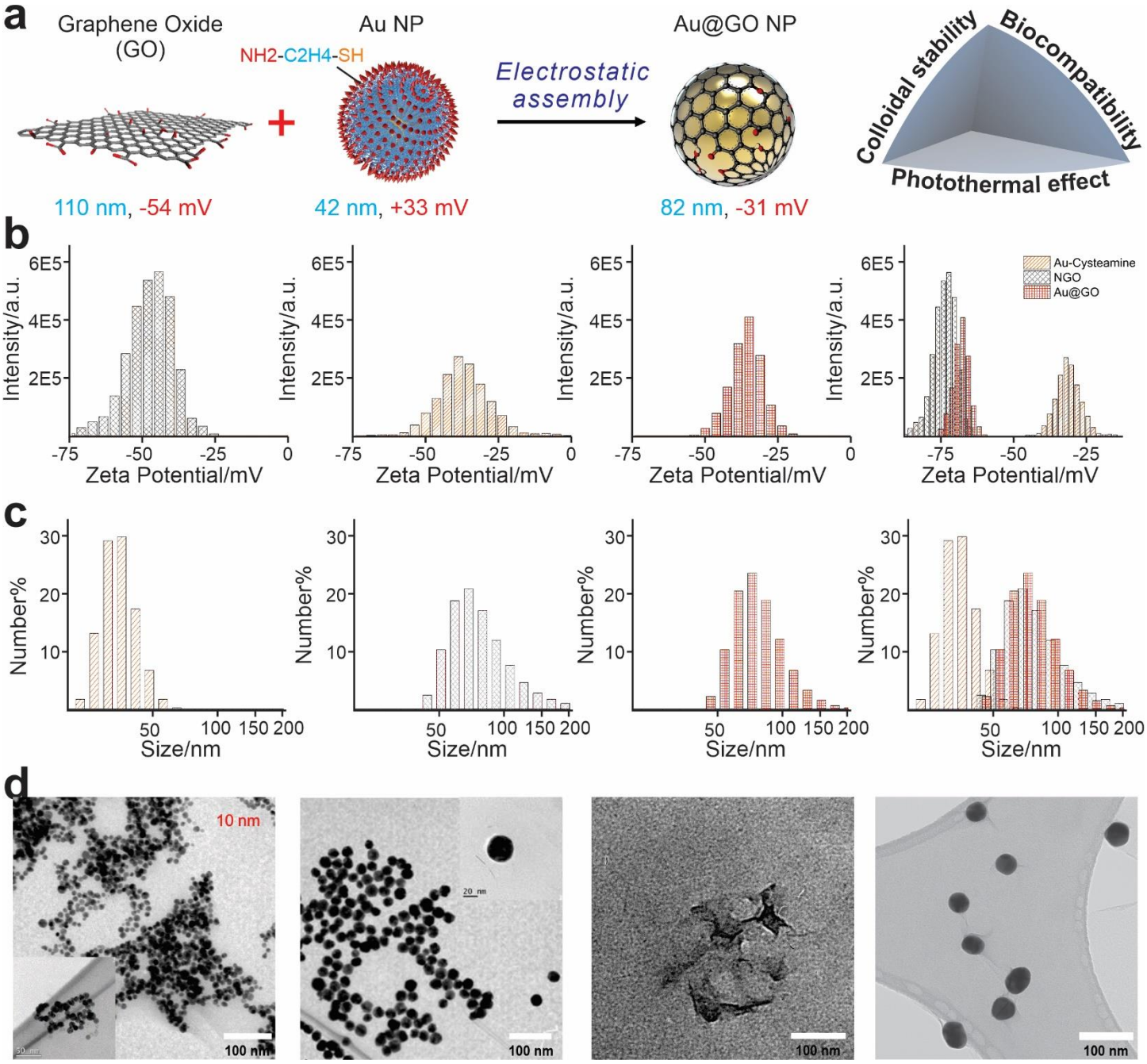

FIGURE S1. Additional characterizations of Au@GO NPs. **a**, Schematic diagram illustrating the synthesis of Au@GO NPs with high biocompatibility, excellent colloidal stability and robust photothermal effects. **b-d**, Zeta potential (**b**), size (**c**), and transmission electron microscope (TEM) measurements of the Au NP, GO, and Au@GO NP.

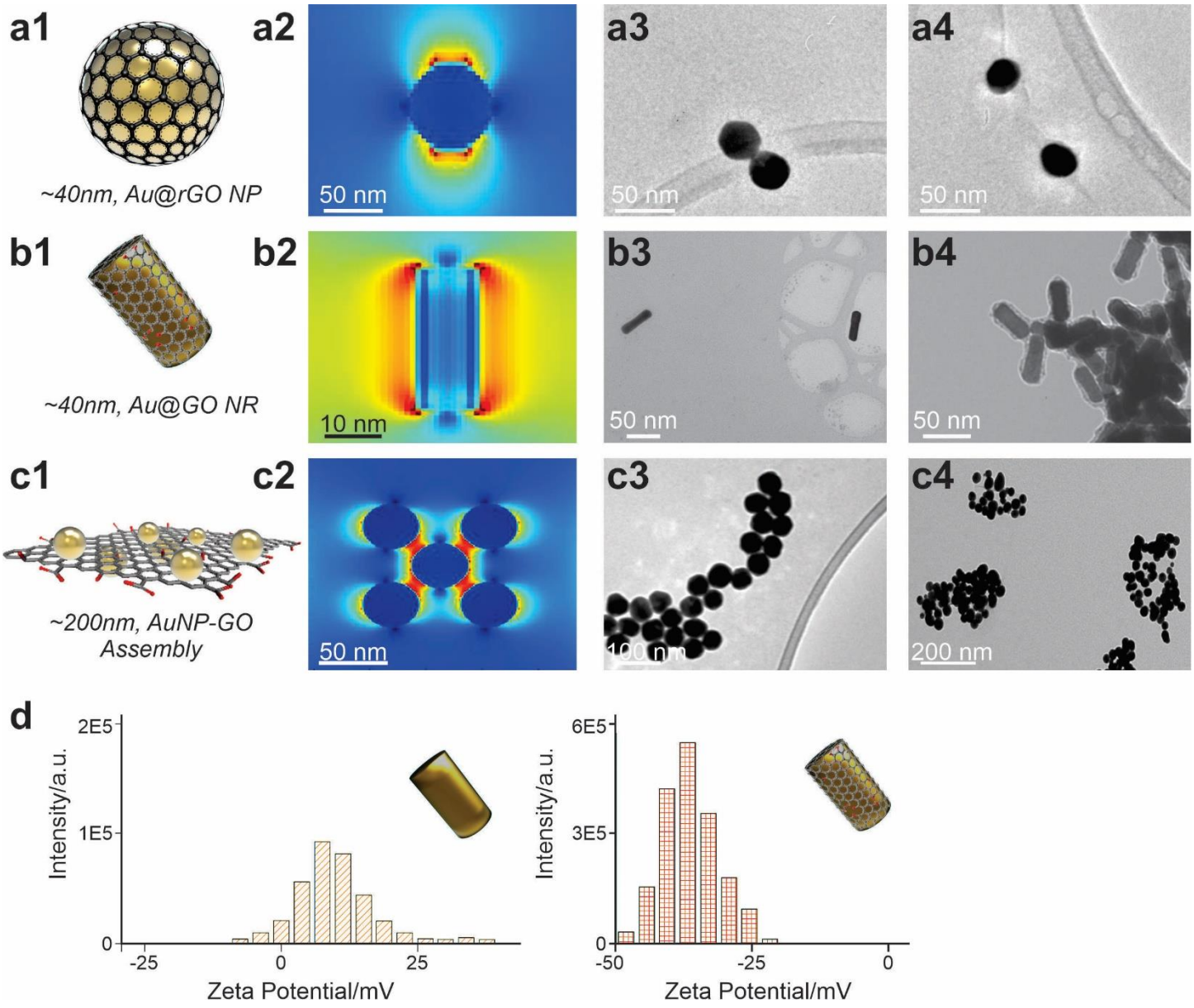

FIGURE S2. Characterizations of Au@rGO NP, Au@GO NR, and Au NP-GO assembly. **a-c**, Schematic diagrams (**a1-c1**), FDTD simulations (**a2-c2**), representative TEM images of the positively charge nanoparticle (**a3-c3**) and the assembled hybrid nanoparticle (**a4-c4**) of Au@rGO NP (**a**), Au@GO NR (**b**), and Au NP-GO assembly (**c**). **d**, Zeta potential characterizations of Au NR (capped by CTAB) before (graph on the left) and after (graph on the right) encapsulation by GO.

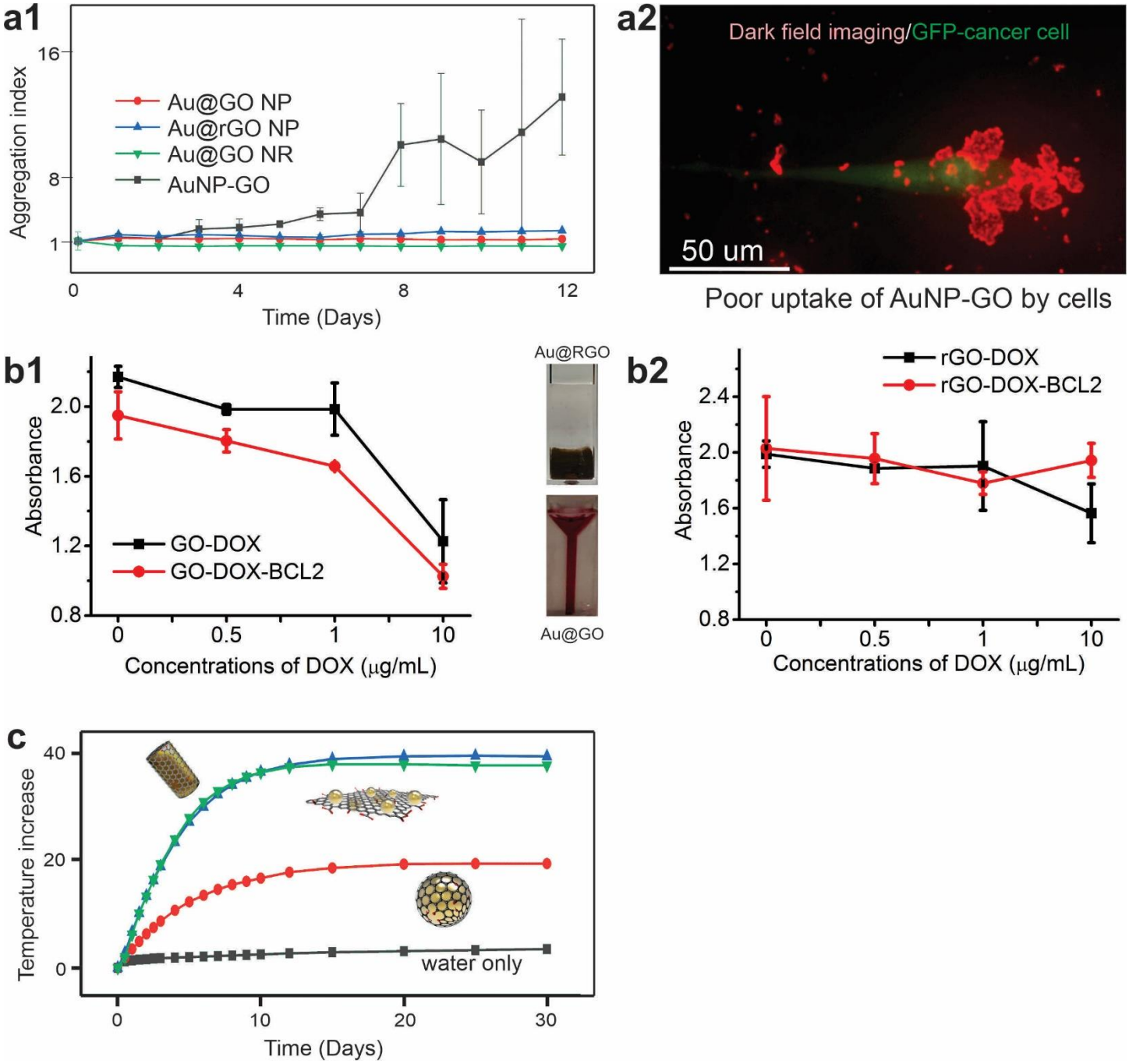

FIGURE S3. Comparison on colloidal stability, cellular uptake, therapeutic effect and photothermal hyperthermia between Au@GO NP and rGO NP, Au@GO NR, and Au NP-GO assembly. **a1**, A graph summarizing the aggregation index (a ratio of measured size normalized to the initial size at Day0) showing the better colloidal stability of the Au@GO NP compared to other hybrid nanoparticles. **a2**, A representative DFI image showing the poor cellular uptake of the Au NP-GO assembly which has a large size and poor colloidal stability. **b**, Relative cell viability of cancer cell (MDA-MB-231) treated by GO- (graph on the left) and rGO (graph on the right)-delivered chemotherapeutics (DOX) showing a higher hydrophilic surface of GO can facilitate the delivery of drugs. **c**, A graph confirming the robust photothermal effects from Au@GO NPs, Au@GO NRs, and Au NP-GO assembly.

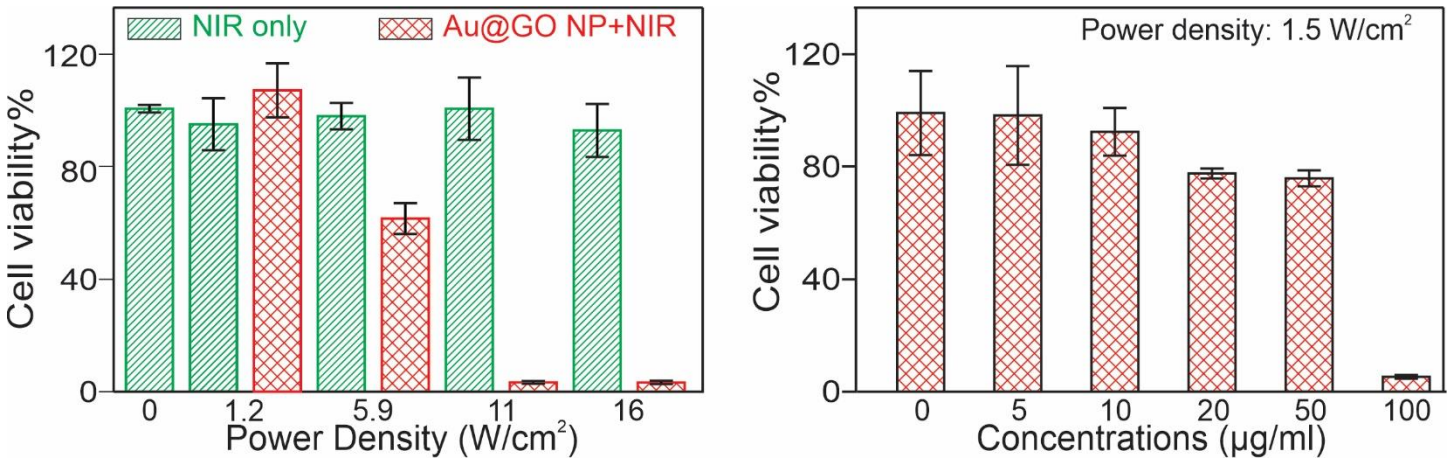

FIGURE S4. NIR photothermal effects of Au@GO NP on cancer cells. Left column indicates the cell viability of glioblastoma cells treated with 10 μg/mL Au@GO NP followed by NIR exposure at varying laser power densities. The right column indicates the cell viability of glioblastoma cells treated by varying concentrations of Au@GO NPs at a fixed NIR laser power density.

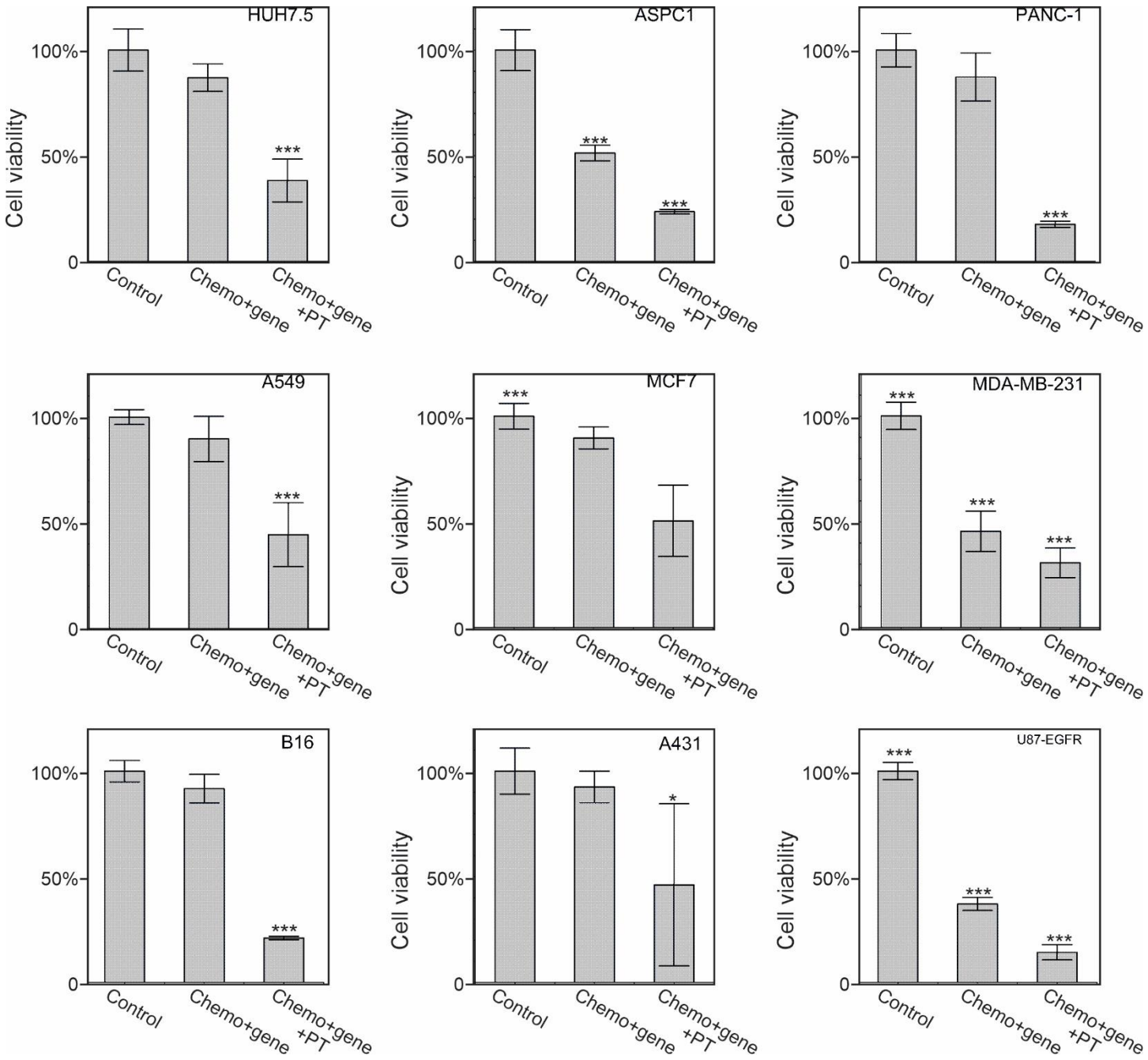

FIGURE S5. Combinatorial cancer suppression assay showing the Au@GO NP-based therapeutic platform for generalized cancer killing through combined chemotherapy (chemo), gene therapy (gene), and photothermal therapy (PT). *P<0.05, **P<0.01, and ***P<0.001, by one-way ANOVA with Tukey post-hoc analysis. n=3 technical replicates. Error bars are standard deviation around the mean.

a

Cell line	Species	Germ layer	Tissue origin	Patient information	Cell bank
HUH7.5	Human	Endoderm	Hepatoma	57 years adult, male, Japanese	JCRB
ASPC1	Human	Endoderm	Pancreas	62 years adult, female, Caucasian	CLS
Panc-1	Human	Endoderm	Pancreas	56 years adult, male, Caucasian	CLS
A549	Human	Endoderm	Lung	58 years adult, male, Caucasian	NCI-DTP
MCF7	Human	Ectoderm	Mammary gland	69 years adult, female, Black	JCRB
MDA-MB-231	Human	Ectoderm	Mammary gland	51 years adult, female, Black	NCI-DTP
B16	Mouse	Ectoderm	Skin	Age unknown, male	JCRB
A431	Human	Ectoderm	Skin	85 years adult, female	CLS
U87-EGFR	Human	Ectoderm	Brain	Age unknown, male	ATCC

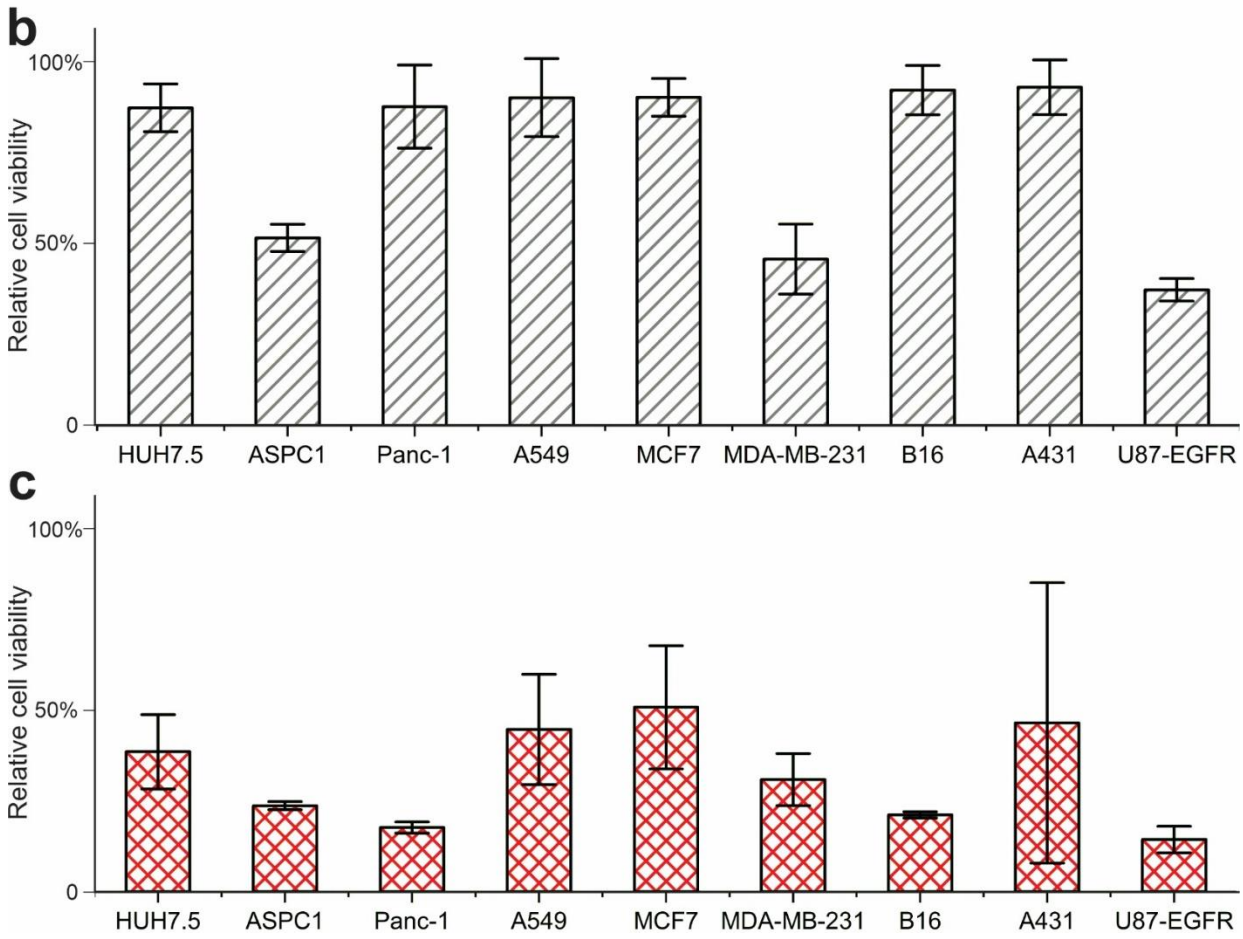

FIGURE S6. Cancer type-dependent responses to combined therapies. a, Descriptions on the 9 different cell lines tested in the combinatorial cancer suppression assay. **b-c**, Graphs summarizing the heterogeneous responses of the 9 different cancer cell lines to the combined treatment of chemotherapy and gene therapy (**b**), or chemotherapy, gene therapy, and photothermal therapy (**c**).

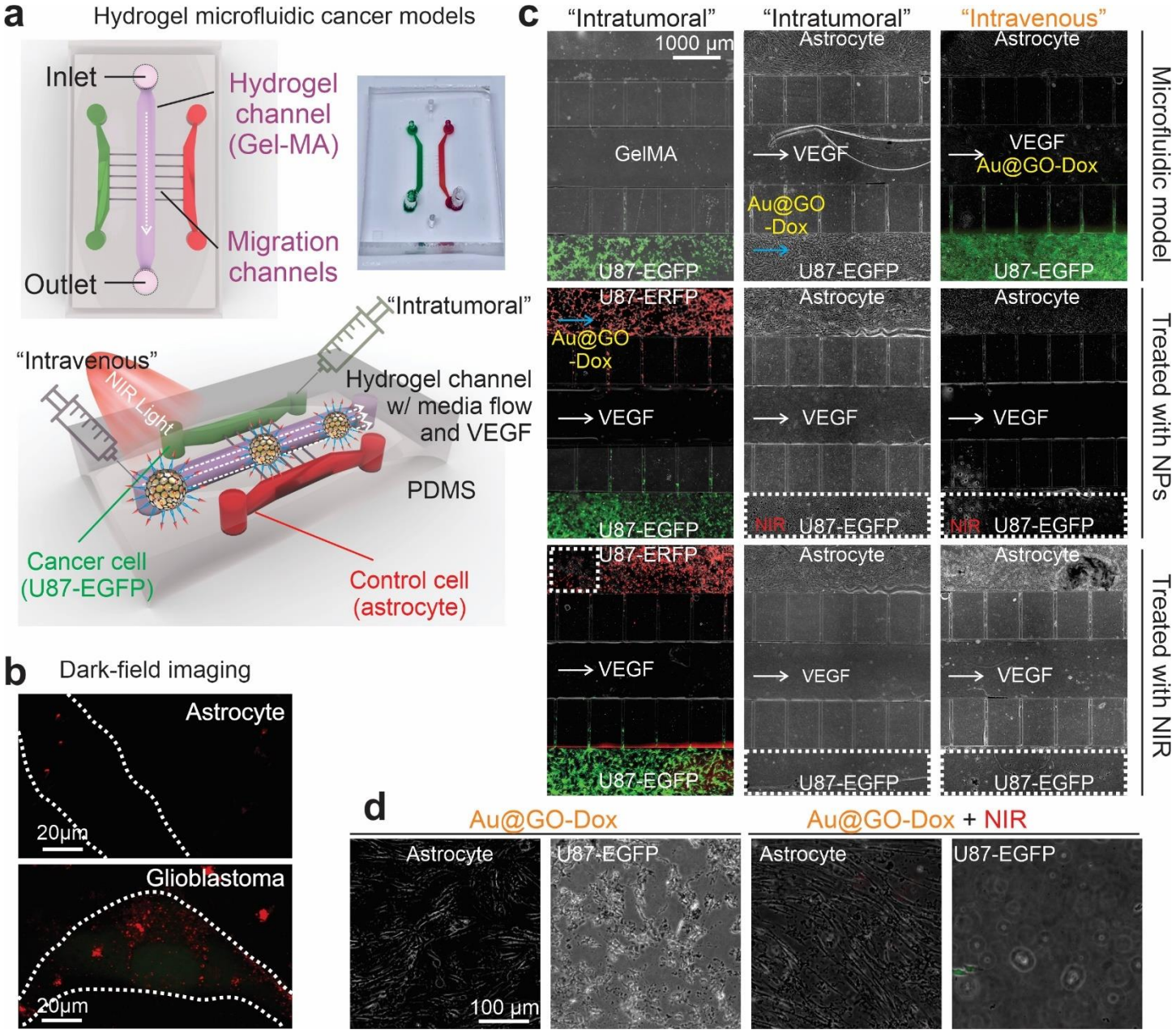

Figure S7. Microfluidic models-based evaluation of Au@GO NP-based therapeutic platform in the treatment of cancer cells of glioblastoma origins. a, Schematic diagrams (top left and bottom images) and a representative photograph (top right image) illustrating the overall design of the hydrogel-based microfluidic device for investigating the targeting and therapeutic effect of the iRGD-conjugated Au@GO NPs. **b**, DFI images showing the selective update of Au@GO NPs by glioblastoma cells (U87-EGFR cell line) compared to the normal glial cells (astrocyte). **c**, Fluorescent and bright-field images showing the general layout of the microfluidics used for evaluation of Au@GO NP-based therapeutic platform that mimics "intratumoral" (direct injection into the target cell channel) and "intravenous" injection (flow through the central hydrogel channel) of nanoparticles into the tumor sites. **d**, Representative bright-field images showing the selective killing of cancer cells while maintaining a high viability of the control non-cancerous cells.

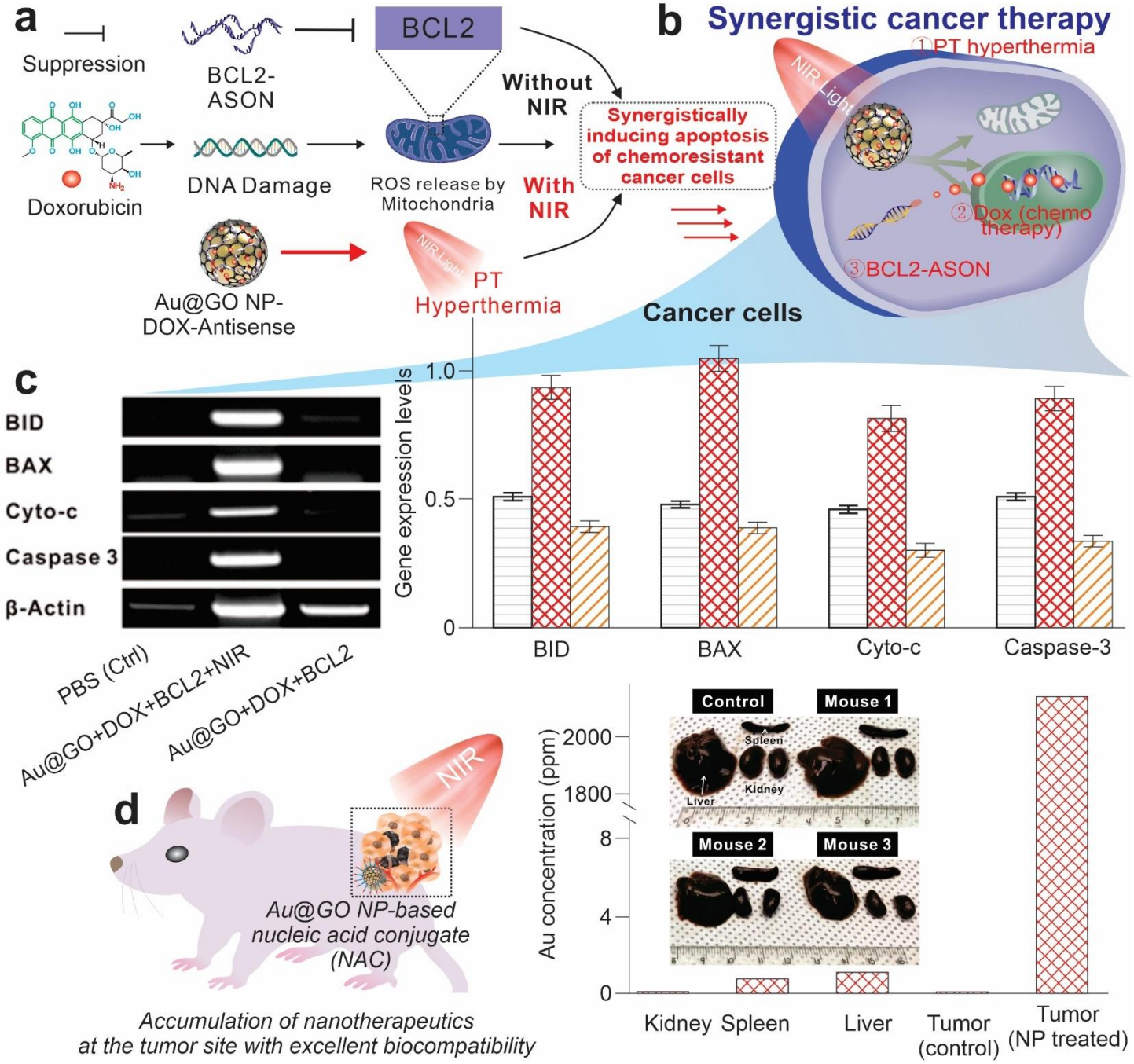

FIGURE S8. Supplementary data for *in vivo* tumor suppression assay. a-b, Schematic diagrams illustrating the molecular pathways (**a**) of the Au@GO NP-based synergistic cancer therapy (**b**). **c**, *In vivo* gene expression levels of apoptotic markers of MDA-MB-231 cells when treated by saline only [PBS control (Ctrl)], or by Au@GO NP-based therapeutic platform with or without NIR exposure. **d**, *In vivo* accumulations of Au@GO NP in different organs. Images inside the graph are showing organs harvested from four different mice control and experimental groups (mouse 1, mouse 2, and mouse 3) to confirm the *in vivo* biocompatibility of Au@GO NP.

C. REFERENCES CITED IN SUPPLEMENTARY INFORMATION

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