Supplementary Materials

A multimodal system with synergistic effects of magneto-mechanical, photothermal, photodynamic and chemo therapies of cancer in graphene-quantum dot-coated hollow magnetic nanospheres

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SUPPORTING MATERIALS CONTENTS

Supplementary Section 1: Methods

(1) Measurement of the photothermal conversion

Temperature increase

Two hundred microliters of nanoparticle aqueous suspensions were placed in glass tubes (inner diameter, 6 mm) and the open ends were covered with sealing film after the solutions were added. The hollow magnetic nanosphere (HMNS), HMNS/SiO_2, and HMNS/SiO_2/graphene quantum dots (GQDs) suspensions all contained 0.5 mg/mL of HMNSs. The nanoparticles were coated with soybean phospholipid layers (liposomes). The concentration of GQDs and lipid were 0.2 and 5 mg/mL, respectively. Two lasers (Shanghai Inter-Diff Optoelectronics Tech. Co., Ltd., Shanghai, China) with wavelengths of 671 (power density, 0.2 W/cm^2) and 808 nm (power density, 0.25 W/cm^2) were used to irradiate the aqueous suspensions. A thermocouple thermometer (Shanghai Instrument Factory Co., Ltd., Shanghai, China) equipped with a probe was used for the temperature measurements. The original temperature was maintained at 25.3±0.10°C. The samples were continuously irradiated for 0–20 min, and the temperature at 0, 5, 10, and 20 min after irradiation was measured. The temperature of each sample was measured three times. As controls, the temperatures of distilled water and the aqueous solution containing soybean phospholipids (0.6 mg/mL) and polyvinylpyrrolidone (PVP) (20 mg/mL) under 671- and 808-nm laser irradiation were detected in the same manner as described above.

Photothermal conversion efficiency

The differential scanning calorimeter (DSC, Q2000, TA Company, USA) was used to measure the specific heat capacity of the HMNSs, GQDs and thermocouple probe. Briefly, a baseline of heat flow was established using an empty crucible for the first; a sapphire was then placed in the same crucible to establish a standard line; and at the last, the sample line was also measured using the same crucible.

The specific heat capacity (c_p) can be estimated based on the following equation:

$$C_p = \frac{m_{sapphire}}{m_{sample}} \times \frac{H_{sample} - H_{baseline}}{H_{sapphire} - H_{baseline}} \times c_{p(sapphire)}$$

Where $c_{p(sapphire)}$ = 0.7788 J/(g·K) when the temperature stays on 300 K. [Ditmars DA, Ishihara S, Chang SS, Bernstein G, West ED. Enthalpy and heat-capacity standard reference material: synthetic sapphire (alpha-Al2O3) from 10 to 2250 K. J Res Nat Bur Stand. 1982; 87: 159-63].

The results showed that the specific heat capacities of the GQDs, HMNSs and thermocouple probe were 1.5908, 0.7477 and 0.6048 J/(g·K), respectively.

For measuring photothermal conversion efficiency of the HMNSs and GQDs, 8.1 mg of HMNS or 5.1 mg of GQD dry powders were spread on a glass slide. The diameter of spreading area was 0.5 cm. The thermocouple thermometer probe was placed on the powder surface. The powder was then irradiated by the 671-nm laser for 30 s. As a control, the thermometer probe alone was also irradiated by the 671-nm laser. The temperature change of each group was measured three times.

The results showed that after 30 sec of irradiation, the temperatures of GQDs and HMNSs placed with thermocouple probe increased from 25.4±0.5°C to 76.3±0.4°C and 81.6±1.4°C, respectively. The temperatures of thermocouple probe alone increased from 26.3±0.3°C to 50.5±1.4°C after 30 sec of irradiation.

The photothermal conversion efficiency can be estimated based on the following equation:

$$\eta = \frac{cm\Delta T}{PtS} \times 100\% = \frac{(c_{probe}m_{probe} + c_{sample}m_{sample})\Delta T_{sample} - c_{probe}m_{probe}\Delta T_{probe}}{PtS} \times 100\%$$

where η is photothermal conversion efficiency, c and m are the massspecific heat capacity and mass of the sample, respectively. ΔT is the temperature increase, P is the laser energy density, t is the irradiation time, and S is the surface of sample that absorbs light.

(2) Measurement of the intracellular reactive oxygen species (ROS)

Human esophagus carcinoma cells were seeded in three wells, each in a 96-well plate at a density of ~1×10^4 cells/well. Twenty-four hours after culture, the medium was removed. One well was seeded with 100 μL of serum-free RPMI-1640 medium and 100 μL of fresh 2,7-dichlorofluorescin diacetate (DCFH-DA), while 100 μL of serum-free RPMI-1640 medium-dispersed nanoparticles and 100 μL of fresh DCFH-DA was added to two other wells. One of the wells containing the nanoparticles was irradiated with the 671-nm laser for 20 min. The LP-HMNS/SiO_2 and LP-HMNS/SiO_2/GQDs (LP: liposome) were dispersed by PVP using the same method as described in the paper. The GQD concentration was 0.2 mg/mL, and the HMNS concentration was 0.5 mg/mL. The cell fluorescence intensity was acquired at 488 nm excitation and 525 nm emission. The ROS level was expressed as the ratio of the fluorescence intensity of the cells containing nanoparticles to that of the cells alone without irradiation. Each experiment was repeated three times.

(3) Measurement of drug release

Drug release in aqueous solution

The 200 μL DOX-loaded HMNS/SiO_2/GQD aqueous suspension (HMNSs: 0.5 mg, GQDs: 0.2 mg) was placed in a glass tube (inner diameter, 6 mm) and the open end were covered with sealing film after the solution was added. The 671-nm laser (power density, 0.2 W/cm^2) was used to irradiate the aqueous suspension for 20 min. The precipitates were then collected by magnetic separation immediately. The supernatant solution was measured

through UV–Vis absorbance taken on a diode array spectrophotometer (UV-2102PC; Unico, Shanghai, China) with a deuterium lamp source. As a control, 200 μL DOX-loaded HMNS/SiO_2/GQD aqueous suspension with the same concentration as described above was placed at room temperature for 20 min in the dark room, and the precipitate was also collected by magnetic separation, and the supernatant solution was also measured through UV–Vis absorbance. The nanocomposites were stabilized with liposomes and PVP. All experiments were repeated in triplicate.

Intracellular drug release

The Eca-109 cells were cultured at a density of ~1×10^4 cells/well on a 96-well plate. Twenty-four hours after culture, the medium was removed and 100 μL of serum-free RPMI-1640 medium (containing 20 mg/mL of PVP) containing liposome-coated HMNS/SiO_2/GQDs-DOX were added. The cells were irradiated by the 671-nm laser for 5 min, 10 min or 20 min, respectively. As controls, the cells were incubated with the liposome-coated HMNS/SiO_2/GQDs-DOX in dark condition without laser irradiation for 5 min, 10 min or 20 min, respectively. The concentrations of the HMNSs in the cells were maintained at 0.5 mg/mL. Cell images were taken by an upright fluorescent microscope (Nikon Eclipse TE2000, Japan) under excitation wavelength of 365 nm.

(4) The toxicity of the nanocomposoites to cells

Investigating whether the nanosystems were uptaken by cells

Eca-109 cells were seeded into cover glasses (size: 18 × 18 mm) which were placed in dishes. After 24 h of incubation, the culture medium was removed and 200 μL of serum-free RPMI-1640 medium (containing 20 mg/mL of PVP)-dispersed LP-HMNS were added into cells. The concentration of HMNSs was 0.5 mg/mL. After 30 min of incubation, the cells

were washed with PBS and then imaged with a laser-scanning confocal fluorescent microscope (TCSNT: Leica, Nussloch, Germany) (Excitation: 488 nm, Emission: 500~600 nm).

Cytotoxicity

Eca-109 cells were cultured at a density of ~1×10^4 cells/well in 96-well plates. Twenty-four hours after culture, the medium was removed and 100 μL of serum-free RPMI-1640 medium (containing 20 mg/mL of PVP) –dispersed LP-HMNSs or LP-HMNS/SiO_2/GQDs were added into the cells. The concentration of HMNSs was 0.5 mg/mL. The cells were then incubated in 37°C for 24 h and 36 h, respectively. The cell viabilities were measured on SpectraMax M5 Multi-Mode Microplate Readers using CellTitre-Glo® reagent.

(5) Cell destruction induced by near-infrared (NIR) laser irradiation

Briefly, the cells were seeded in three wells, each in a 96-well plate at a density of ~1×10^4 cells/well. Twenty-four hours after culture, the medium was removed. The amount of 100 μL of serum-free RPMI-1640 medium (with 20 mg/mL PVP) containing magnetic nanoparticles (e.g., LP-HMNSs) was added to two wells and the remaining well was treated as a control, adding only 100 μL of serum-free RPMI-1640 medium. The concentration of HMNSs in cells was maintained at 0.5 mg/mL. One of the wells containing nanoparticles was irradiated with the 671- or 808-nm laser for 20 min and the remaining wells were not irradiated. As controls, the cells containing lipids (2.5 mg/mL) and PVP (20 mg/mL) were also irradiated with the laser for 20 min. After the treatment, the culture medium was removed and replaced with fresh medium. Cells were then continuously cultured at 37°C for 1 h. Each experiment was repeated six to eight times.

(6) Establishing of standard curve of GQDs and doxorubicin hydrochloride (DOX)

A series of standard GQDs aqueous solutions were prepared at concentrations of 25, 125, 6.25, 3.125, and 6.5625 μg/mL. A fluorescence spectrophotometer (F-2500, Hitachi, Japan) with a xenon lamp source was used to detect the emission spectrum of each concentration from 400 to 800 nm at 400 nm excitation. The standard curve was established according to the emission intensity at 453 nm. The DOX standard curve was established similarly as described above using an absorption spectrophotometer (UV-2102PC, Unico, Shanghai, China) with a deuterium lamp source, according to the absorption at 482 nm at the concentrations of 51, 25.5, 12.75, 6.375, and 3.1875 μg/mL.

Supplementary Section 2: Figures

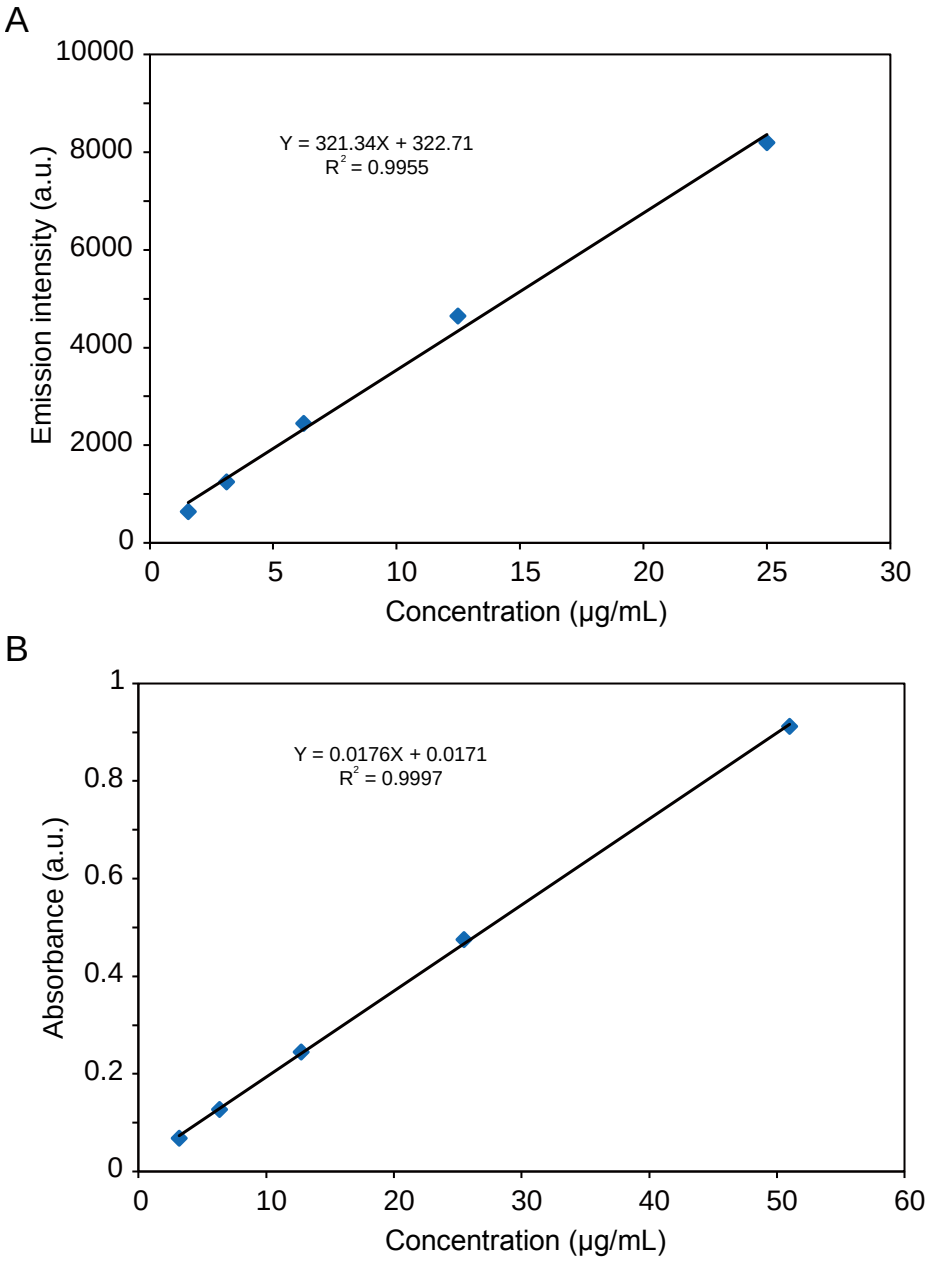

Figure S1. Standard curve of (A) GQDs and (B) DOX.

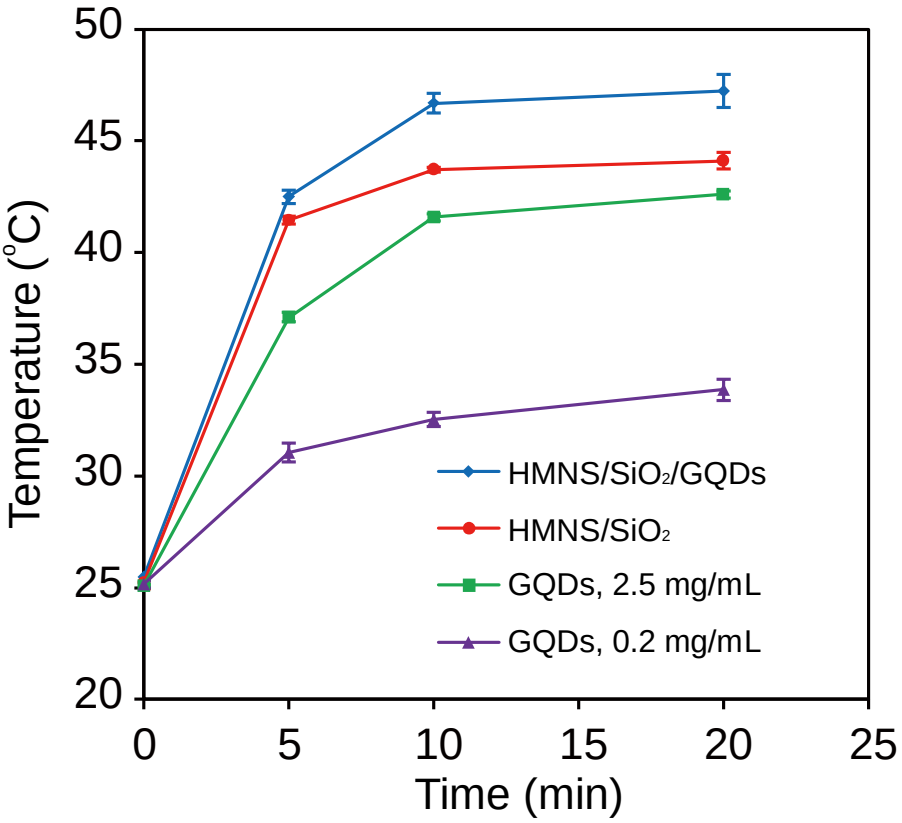

Figure S2. Temperature vs. irradiation time of the aqueous suspensions of GQDs and LP-HMNS/SiO_2 and LP-HMNS/SiO_2/GQDs (HMNSs: 0.5 mg/mL, lipid: 2.5 mg/mL, PVP: 20 mg/mL). Irradiation carried out at 671 nm. Data are expressed as the mean ± the standard deviation. The error bars are based on three experiments per group.

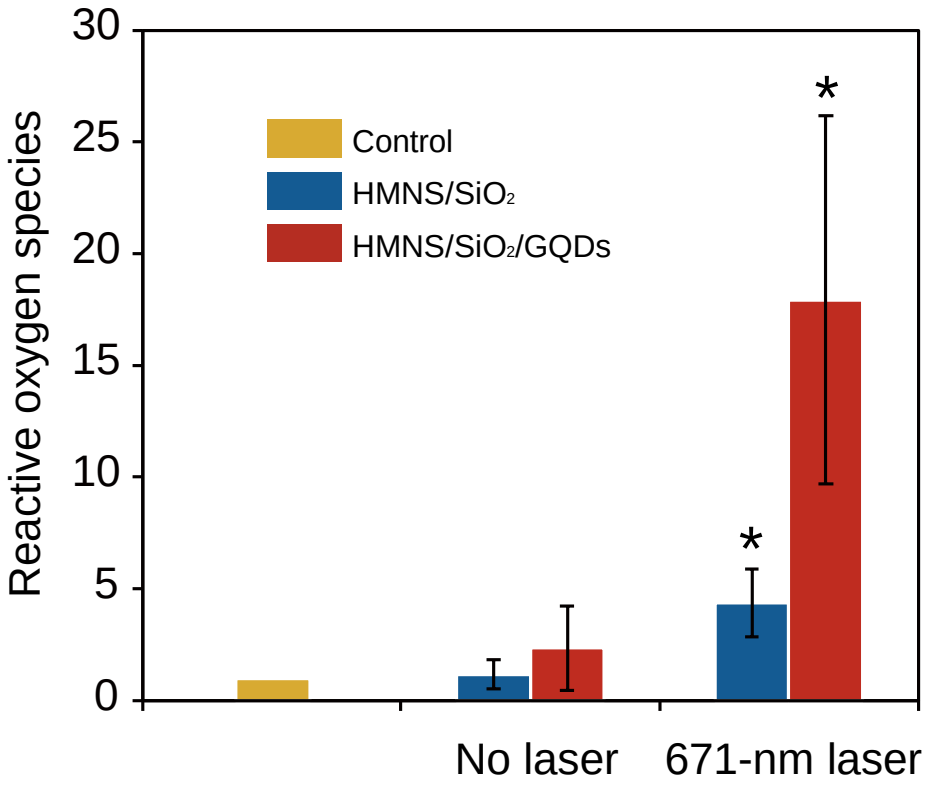

Figure S3. Intracellular reactive oxygen species levels (relative to control) generated by LP-HMNS/SiO_2 and LP-HMNS/SiO_2/GQDs (HMNSs: 0.5 mg/mL, GQDs: 0.2 mg/mL, lipid: 2.5 mg/mL, PVP: 20 mg/mL). The significance level observed was * $P<0.05$, in comparison with the group values of cells without nanoparticles and any treatment (control). Data are expressed as the mean ± the standard deviation. The error bars are based on five experiments per group.

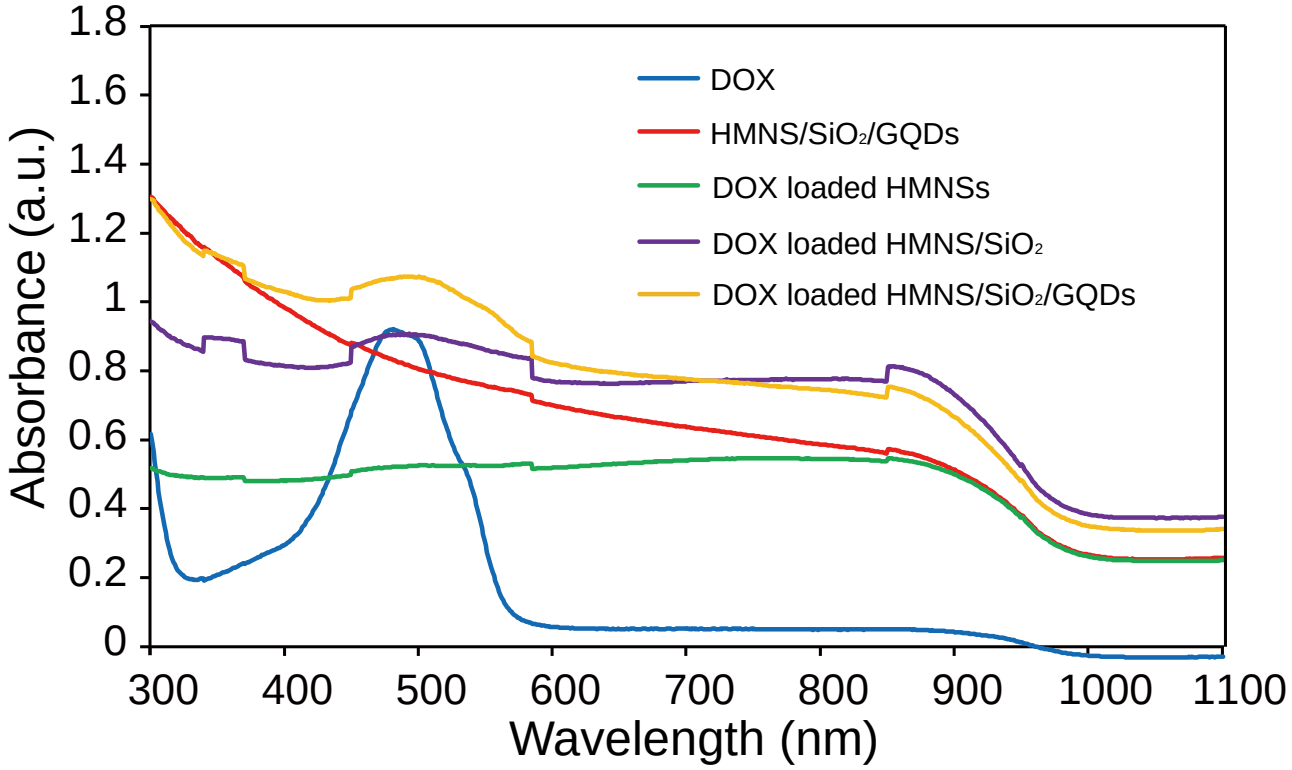

Figure S4. Absorption spectra of the doxorubicin hydrochloride (DOX, 0.05 mg/mL), HMNS/SiO_2/GQDs, DOX-loaded HMNSs, DOX-loaded HMNS/SiO_2, and DOX-loaded HMNS/SiO_2/GQDs (HMNSs: 0.25 mg/mL).

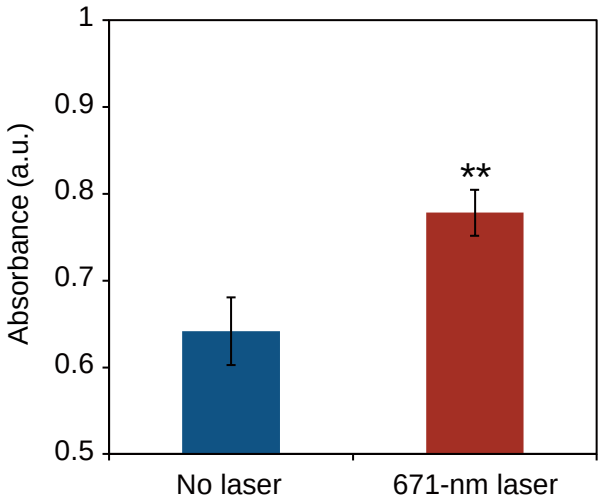

Figure S5. Absorption intensity (at 482 nm) of the supernatant solutions obtained from the DOX-loaded HMNS/SiO_2/GQDs (HMNSs: 6 mg/mL) with and without 671-nm laser irradiation. For the irradiation group, the samples were treated with the laser for 20 min. (The drug release under the magnetic field-mediated mechanical stimulation was similar with that under NIR laser irradiation, data not shown)

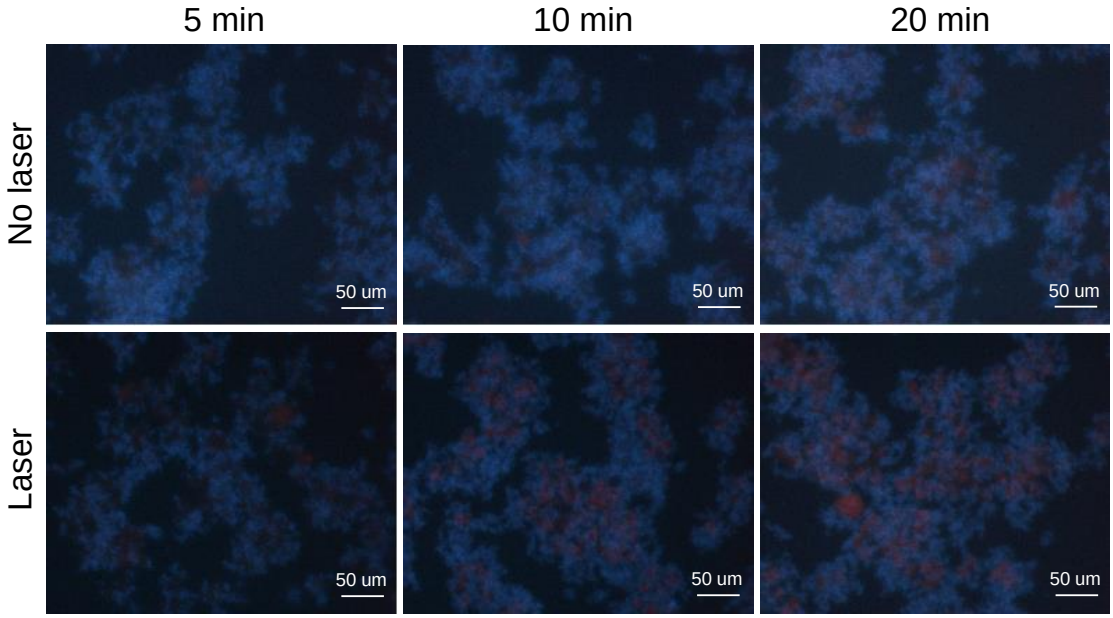

Figure S6. Intracellular drug release of the DOX-loaded HMNS/SiO_2/GQDs nanocomosites (HMNSs: 6 mg/mL) with and without 671-nm laser irradiation. Eca-109 cells were incubated with the nanocomposites for different time or irradiated with the laser for different time.

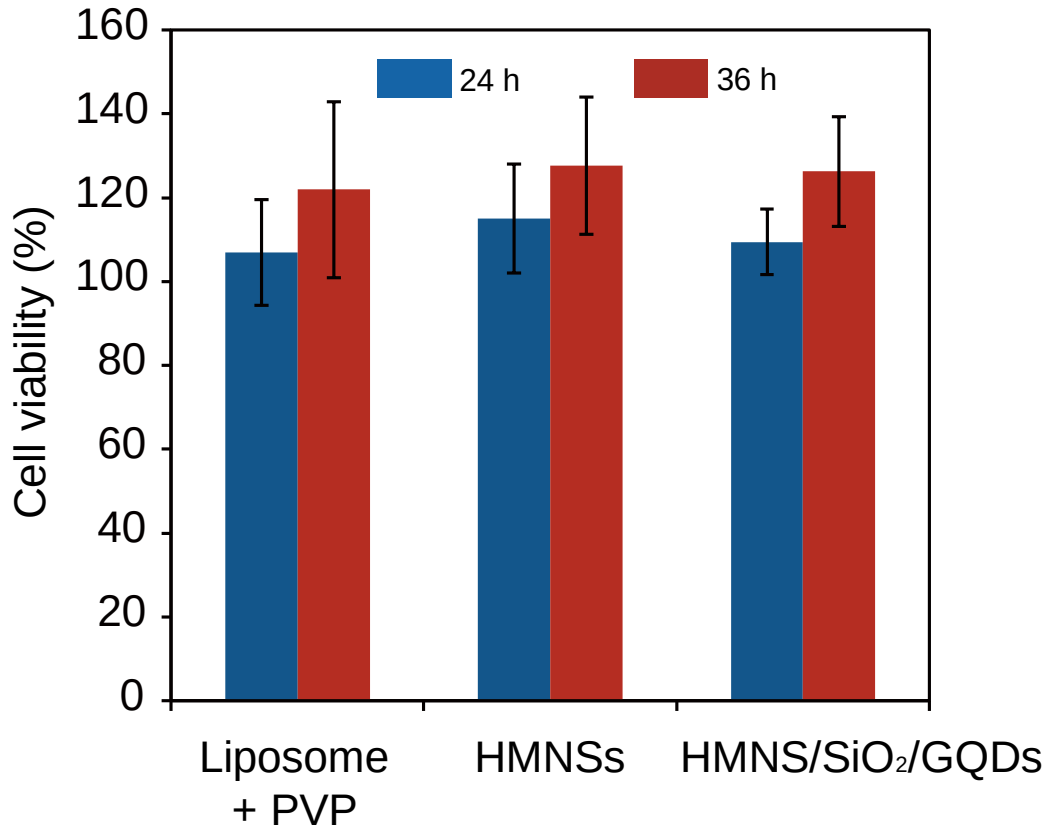

Figure S7. Viabilities of Eca-109 cells incubated with LP-HMNSs or LP-HMNS/SiO_2/GQDs or blank liposomes for 24 and 36 h, respectively. The nanoparticles were stablized by PVP. The concentration of the HMNSs, GQDs, lipid and PVP were 0.5, 0.2, 2.5 and 20 mg/mL, respectively.

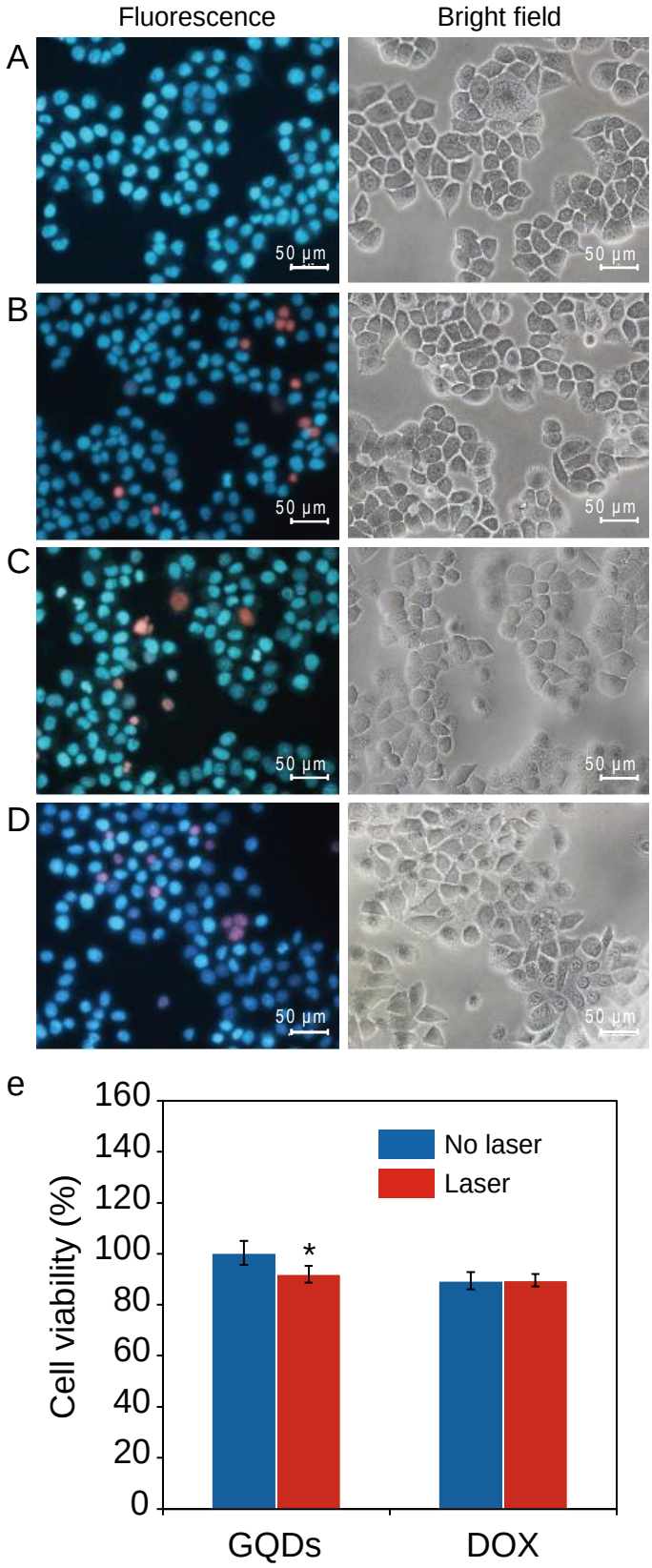

Figure S8. GQDs and DOX upon laser irradiation for killing cancer cells and control. The Eca-109 cells incubated with 0.2 mg/mL of GQDs and exposed to (A) no laser or (B) irradiation by a 671-nm laser for 20 min. The Eca-109 cells incubated with 0.3 mg/mL of DOX and exposed to (C) no laser or (D) irradiation by a 671-nm laser for 20 min. (E) The

quantitative analysis of the cell viabilities of the four samples in (a–d). The significance level observed was * $P<0.05$, in comparison with the group values of cells incubated with GQDs laser irradiation. Data are expressed as the mean ± the standard deviation. The error bars are based on five experiments per group.

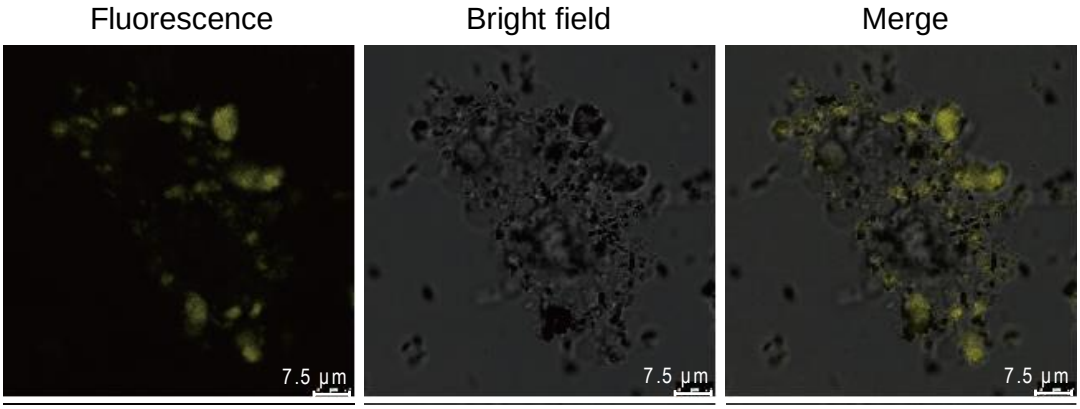

Figure S9. Confocal images of the Eca-109 cells incubated with the LP- HMNS/SiO_2/GQDs for 30 min.