Supporting Information for

Hierarchical Nanocarrier for Precisely Regulating Therapeutic Process via Dual-Mode Controlled Drug Release in Target Tumor Cells

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General Techniques. UV-vis spectra were recorded on an UV-3600 spectrophotometer (Shimadzu, Kyoto, Japan). Fluorescence spectra were obtained from an F-7000 spectroflurophotometer (Hitachi). Transmission electron micrographs (TEM) were performed on a JEOL 2010 transmission electron microscope, using an accelerating voltage of 200 kV. Atomic force microscopy (AFM) images were obtained on a SPI3800 controller operated in tapping mode with an acquisition frequency of 1.5 Hz and line density of 512.2×2 μm scans. N_2 adsorption-desorption isotherms were carried out on a Micromeritics ASAP 2020M automated sorption analyzer. The samples were degassed at 100 °C for 12 h. The specific surface areas were calculated from the adsorption data in low pressure range using the Brunauer-Emmett-Teller (BET) modal and pore size was determined following the Barret-Joyner-Halenda (BJH) method. Confocal laser scanning microscopy (CLSM) studies were performed using a Leica TCS SP5 microscope (Germany) with excitation at 405 nm or 488 nm. Dynamic light scattering (DLS) experiments were taken at 25 °C using a Brookhaven 90 Plus instrument. Flow cytometric analysis was conducted using Cytomics FC 500 MCL (BECKMAN COULTER, U.S.A.). MTT assay was recorded at 490 nm using a ThermoFisher Scientific Varioskan Flash multifunctional microplate reader.

Synthesis of GQDs. The GQDs were synthesized from graphene oxide (GO) by our previous reported method. Briefly, GO (10 mg) synthesized from natural graphite powder by a modified Hummers method was firstly mixed carefully with concentrated HNO_3 (20 mL) and H_2SO_4 (10 mL), thus the concentrations of HNO_3

and H_2SO_4 were 8 M and 4.5 M respectively. Then the mixture was ultrasonication for 3 h, followed by heating and refluxing under microwave irradiation for 3 h in microwave oven operating at a power of 240 W. The product was brown transparent solution. After cooling to room temperature, the pH value of the solution was tuned to 8 with NaOH in an ice-bath under mild ultrasonication. The solution was filtered through a 0.22 μm microporous membrane to remove the large tracts of GO and a deep yellow solution was separated. The above obtained deep yellow filter solution was reduced with $NaBH_4$ (1 g) under vigorous stirring at room temperature for 3 h. The solution color faded to yellow. Then HNO_3 solution was added dropwise to terminate the reaction and tune the pH to 8. The suspension was filtered through a 0.22 μm microporous membrane and further dialyzed to obtain brightly blue fluorescent GQDs.

Synthesis of Gold Nanorods. The seed solution for gold nanorods growth was prepared as follows: 100 μL of 1% $HAuCl_4$ was mixed with 10 mL of 0.1 M CTAB solution in a 10 mL beaker. 0.6 mL of fresh 0.01 M $NaBH_4$ was then injected to the Au(III)-CTAB solution under vigorous stirring (1200 rpm). The solution color changed from yellow to brownish yellow and the stirring was stopped after 2 min. The seed solution was aged at room temperature for 30 min before use.

To prepare the growth solution, 7.0 g (0.037 M in the final growth solution) of CTAB and 1.234 g of NaOL were dissolved in 250 mL of warm water (~50 °C) in a 1L Erlenmeyer flask. The solution was allowed to cool down to 30 °C and 12 mL of 4 mM $AgNO_3$ solution was added under vigorous stirring. After 30 s, the mixture was

kept undisturbed at 30 °C for 15 min after which 250 mL of 1 mM $HAuCl_4$ solution was added under vigorous stirring. The solution became colorless after 90 min of stirring (100 rpm) and 2.1 mL of HCl (37 wt. % in water, 12.1 M) was then introduced to adjust the pH. After another 15 min of slow stirring at 100 rpm, 1.25 mL of 0.064 M ascorbic acid (AA) was added and the solution was vigorously stirred for 30 s. Finally, 0.75 mL of seed solution was injected into the growth solution. The resultant mixture was stirred for 30 s and left undisturbed at 30°C for 12 h for nanorod growth. The final products were isolated by centrifugation at 8500 rpm for 25 min followed by removal of the supernatant.

Docetaxel (Doc) Loading and Release Experiments. The Doc molecules were loaded into the P/R-DMSGRs@GQD by incubating the nanoparticles (1.0 mg mL^{-1}) with different concentrations of Doc stock solutions in methanol for 24 h. By centrifugation, the P/R-DMSGRs@GQD-Dox and the supernatant were separated and collected. Then the P/R-DMSGRs@GQD-Dox was washed repeatedly with PBS buffer (pH 5.0). The Dox loading capacities were quantified by high performance liquid chromatography (HPLC).

The Doc loaded nanocarriers were incubated in Krebs solution for different amounts of time. Meanwhile, the samples were exposed or not to the NIR laser (300 mw, 10-min, 30-s on and 30-s off) repeatedly by every 2 h intervals. After the treatment, the Doc released from the nanocarriers was collected by centrifugation at 10000 rpm. The amount of released Doc in the supernatant solutions was measured by HPLC.

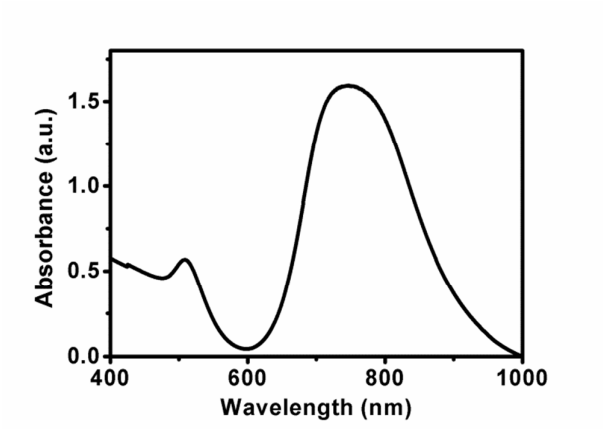

Figure S1 The UV-Vis spectra of DMSGRs-NH_2.

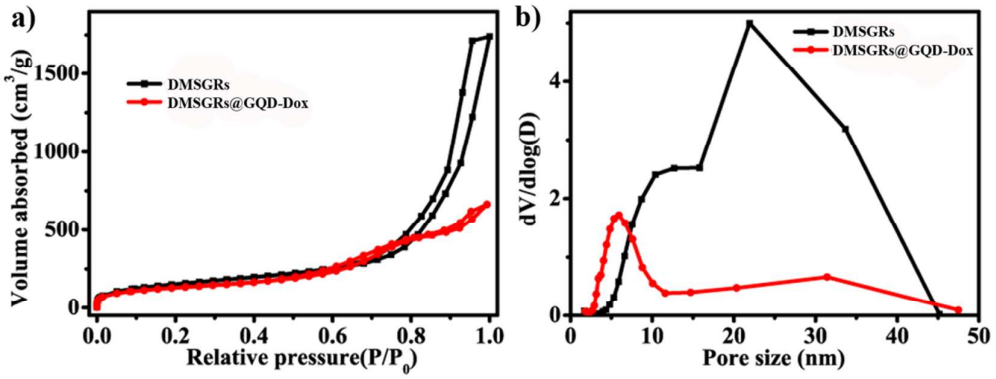

Figure S2 a) Nitrogen adsorption-desorption isotherms of DMSGRs, and DMSGRs@GQD-Dox. b) Pore sizes distributions of DMSGRs, and DMSGRs@GQD-Dox. (BET specific surface values, pore volumes, and pore sizes calculated from the nitrogen adsorption-desorption isotherms were 536.5 m^2 g^{-1}, 2.6 cm^3 g^{-1}, 22.7 nm for DMSGRs, 457.9 m^2 g^{-1}, 0.9 cm^3 g^{-1}, 5.7 nm for DMSGRs@GQD-Dox)

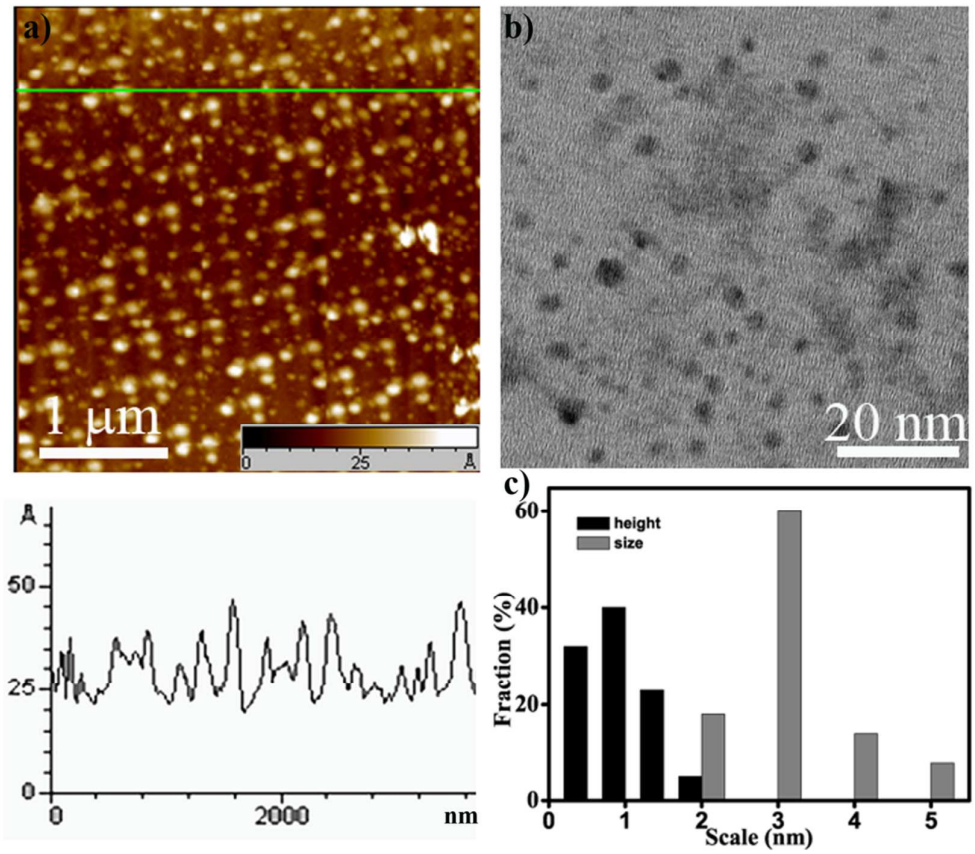

Figure S3 a) AFM image and its corresponding height profiles of GQDs. b) HRTEM image of GQDs. c) Size and height distributions of GQDs.

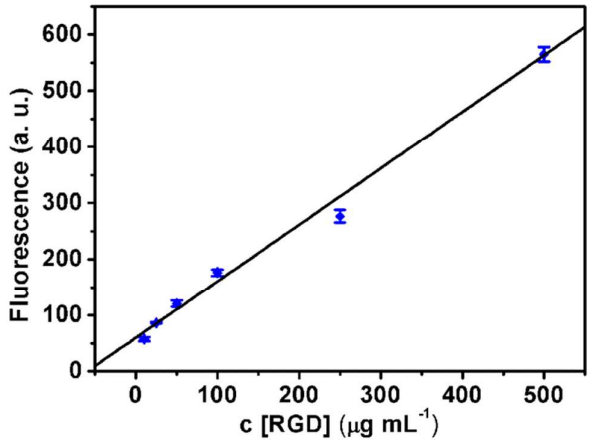

Figure S4 Calibration curve for arginine determination by fluorescence using RGD peptide.

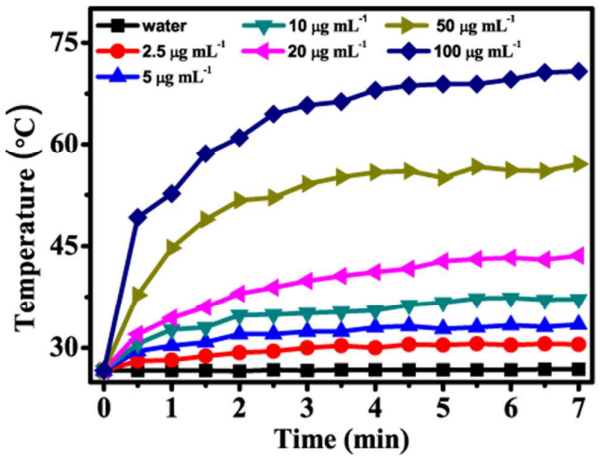

Figure S5 The rate of temperature rise and the final temperature were proportional to particle concentration at the constant laser function power (795 nm, 300 mW)

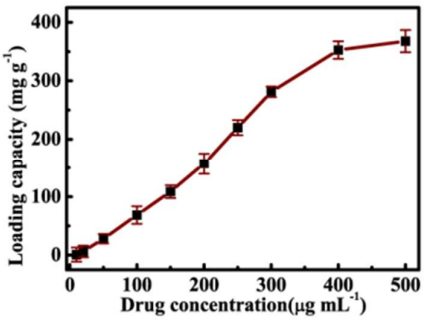

Figure S6 The Dox loading capacity of the nanocarriers at different concentrations of Dox stock solutions.

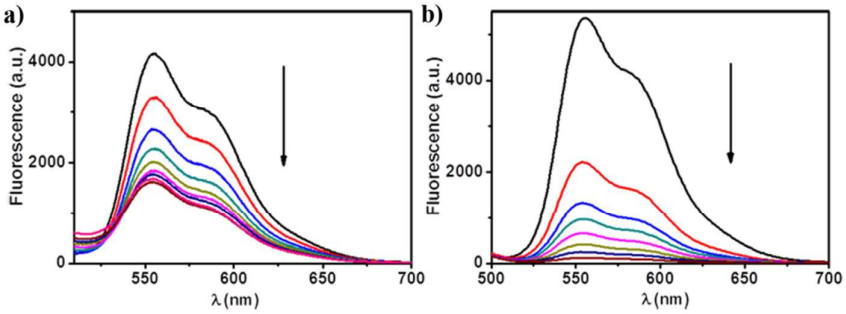

Figure S7 Fluorescence emission spectra of Dox with the addition of GQDs (a) or DMSGRs@GQD (b).

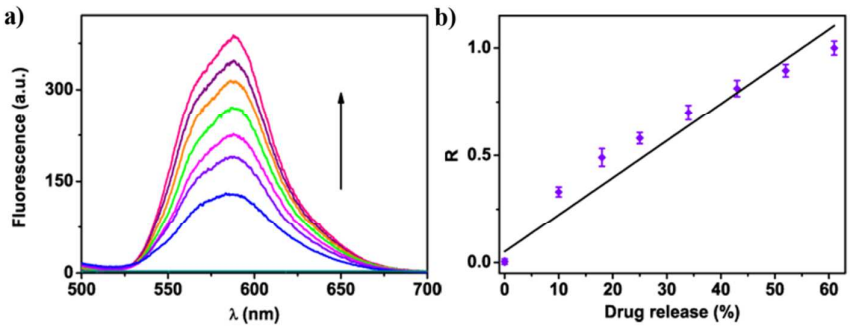

Figure S8 (a) Changes in Dox fluorescence of the nanocarriers upon exposure in NIR laser for different times. (b) Linear relationships between the amount of Dox released and the signal (R) of the Dox, the $R=F-F_0/F_{max}-F_0$, where F_0 is the intensity at 0 h, F_{max} is the intensity at 7th irradiation.

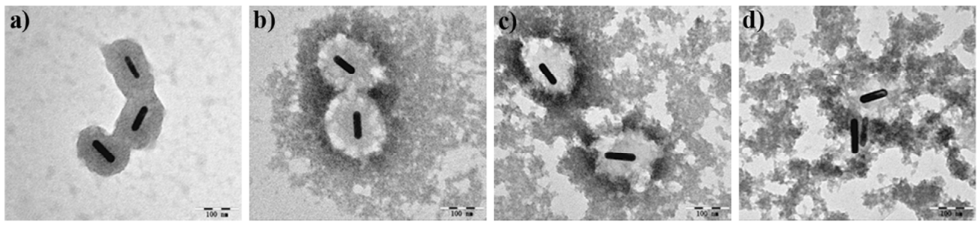

Figure S9 TEM characterization for the biodegradation of the nanocarriers in cell culture medium containing 10% FBS: (a) 24 h, (b) 48 h, (c) 72 h, (d) 96 h. After 24 h, the serum proteins adsorbed onto the surface of the nanoparticles and accelerated the drug release *via* hydrophobic interaction.

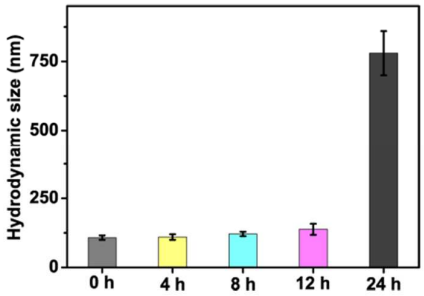

Figure S10 Long-term stability of the nanocarriers in cell culture medium containing 10% FBS. The DLS measurements were performed after the solutions were shaken at a speed of 200 rpm for 0 h, 4 h, 8 h, 12 h, and 24 h. The error bars were obtained from three independent measurements. The nanocarriers exhibited excellent stability within 12 h. Significant changes in hydrodynamic sizes were observed after 24 h owing to the aggregation of nanoparticles, which may result from the shedding of PEG due to the degradation of the nanocarrers.

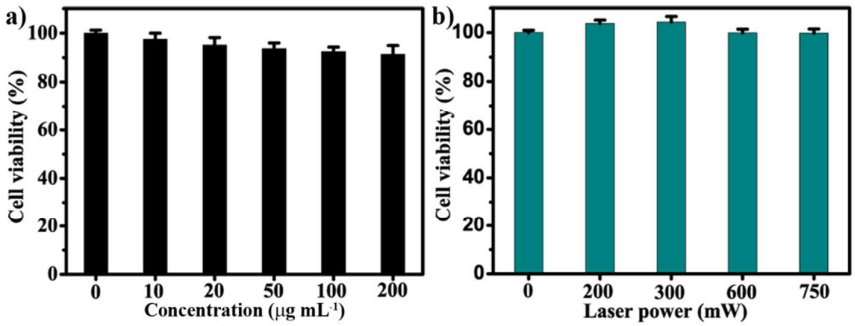

Figure S11 (a) Cell viability of the cells incubated with different concentrations of nanocarriers. (b) Cell viability of the cells irradiated with different laser powers.

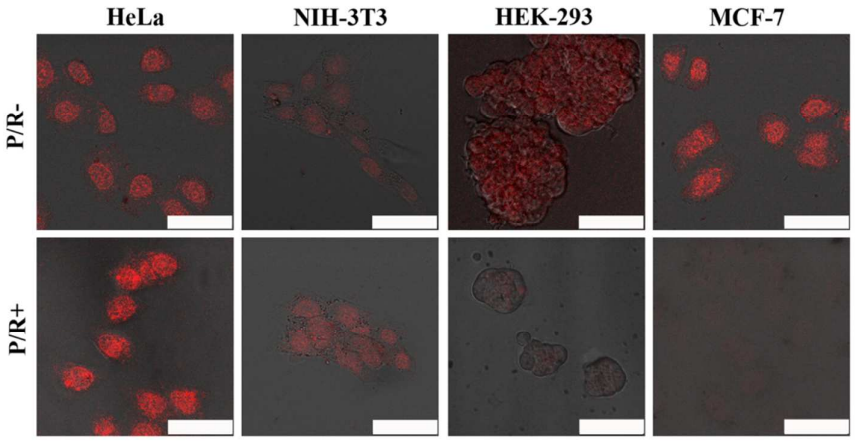

Figure S12 CLSM images of four different cells after incubating with P/R-DMSGRs@GQD-Dox or DMSGRs@GQD-Dox.

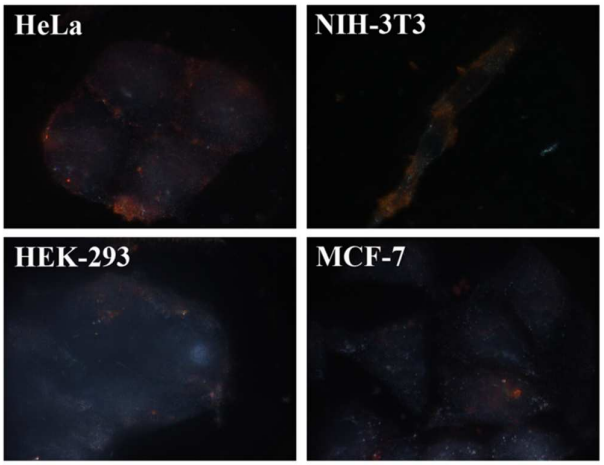

Figure S13 Dark-field images of four different cells after incubating with P/R-DMSGRs@GQD-Dox or DMSGRs@GQD-Dox.

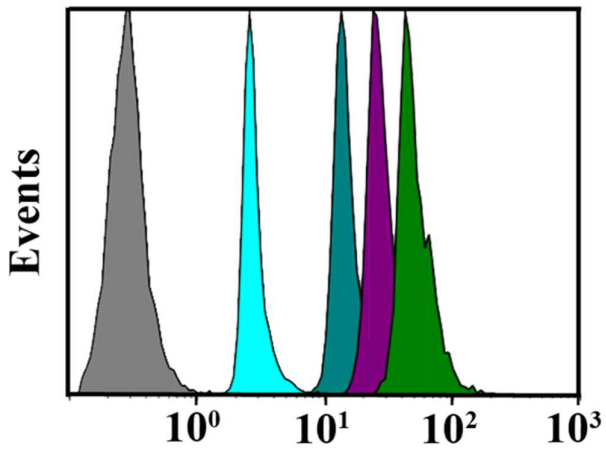

Figure S14 Flow cytometry results of Dox release measured at 0 h, 24 h, 48 h, 72 h, 96 h (from left to right), respectively.

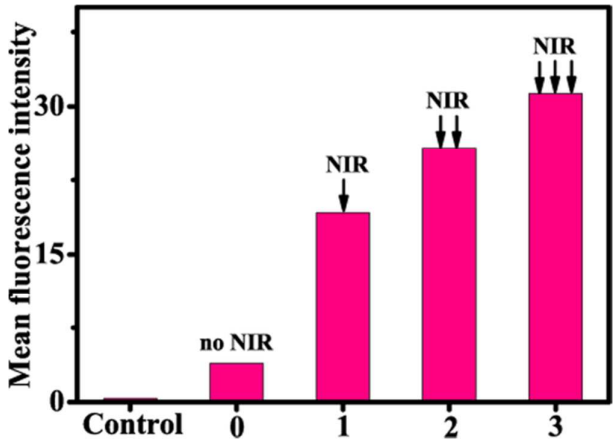

Figure S15 Mean fluorescence intensity of released Dox with NIR laser irradiating for different times. The cells pre-incubated with the nanocarriers were exposed under NIR laser with the power of 300 mW for 3 min per time, and the laser was repeatedly turned on and off.

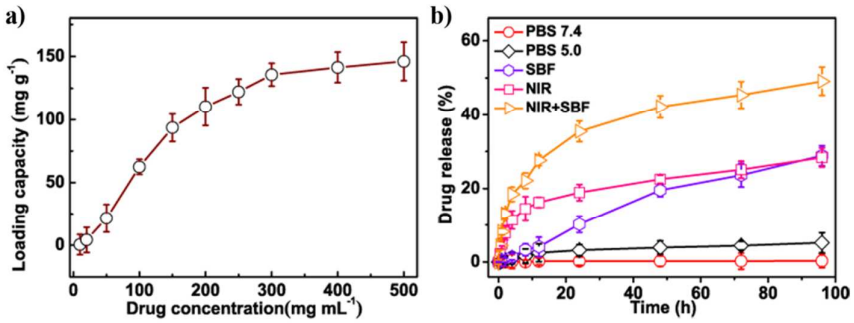

Figure S16 (a) The Doc loading capacity of the nancarriers at different concentrations of Doc stock solutions. (b) The controlled drug release profiles of the nanocarriers with different conditions.

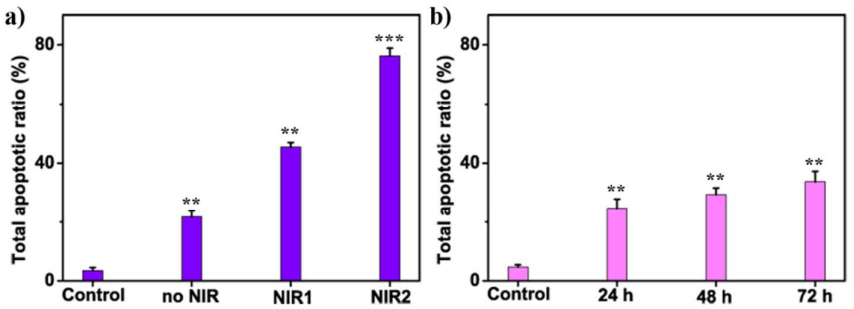

Figure S17 Total apoptotic ratio of the HeLa cells treated by the nanocarriers with NIR irradiation (a) or with different incubation time (b). Error bars indicate s. d. (n=3). $*P < 0.05$, $**P<0.01$, $***P<0.001$ compared with the control group. (two-tailed Student's *t*-test)

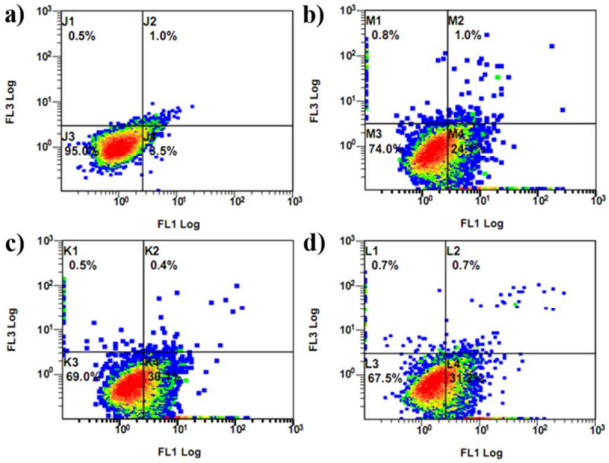

Figure S18 Flow cytometric analysis of HeLa cell apoptosis after incubation with Doc loaded nanocarriers with different time (a, control; b, 24 h; c, 48 h, d, 72 h) using Annexin V-FITC/PI staining.

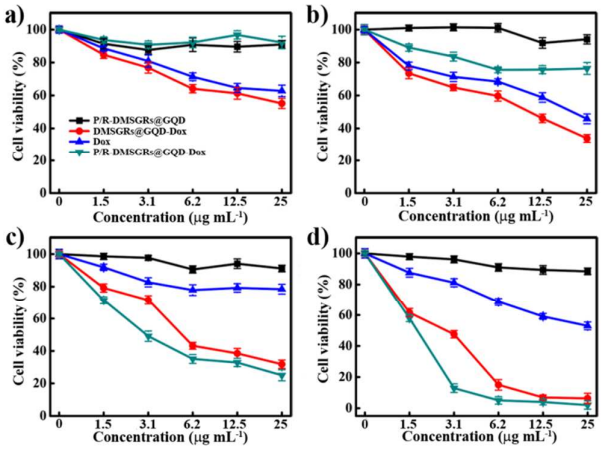

Figure S19 Cell viability of HEK cells (a, b) and HeLa cells (c, d) incubated with different concentrations of P/R-DMSGRs@GQD, DMSGRs@GQD-Dox, Dox, and P/R-DMSGRs@GQD-Dox for 24 h and 48 h, respectively and irradiated with NIR laser of 300 mW for twice.