# Electronic supplementary information for

# Tumor-targeting dual stimuli-responsive controllable release nanoplatform based on DNA-conjugated reduced graphene oxide for chemo-photothermal synergetic cancer therapy

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## 1. Experimental details

### 1.1 Other materials used in the experiment

Sodium nitrate ($NaNO_3$), sodium hydroxide (NaOH), sulfuric acid ($H_2SO_4$), potassium permanganate ($KMnO_4$), hydrogen peroxide ($H_2O_2$), hydrochloric acid (HCl), potassium chloride (KCl), sodium chloride (NaCl), magnesium chloride ($MgCl_2$) and graphite powder were purchased from Sinopharm Chemical Reagent Co. Ltd (China). Propidium iodide (PI) was obtained from J&K Chemical Ltd (China). Cell counting kit was offered by Dojindo Co. Ltd (Shanghai, China). Ultrapure water (18.2 MΩ·cm) was purified by Millipore Milli-Q purification system

### 1.2 Instruments used to characterize the prepared nano-composites

The reduction process of GO was monitored by UV-vis spectrophotometer (Perkin-Elmer, USA). The size and morphology of the prepared nano-composites were observed with a Nanoscope IIID atomic force microscopy (AFM) (Bruker, USA) with tapping mode. The X-ray photoelectron spectroscopy (XPS) results were obtained with Esca Lab 250 X-ray photoelectron spectrometer (XPS, Thermo Fisher Scientific Company, USA). Fourier transform infrared (FT-IR) spectra were investigated on a Nicolet iS50 FT-IR spectrometer (Thermo Nicolet Corporation, USA) after freeze drying all the samples. The photo-thermal heating effect of nano-composites was irridiated by an 808 nm laser (Laserwave Optoelectronics Technology Co., Ltd., China) and the temperature was monitored by a real time paperless-recorder (Sinomeasure CO., Ltd., China). An F-4600 fluorescence spectrometer (HITACHI, Japan) was used for fluorescent spectrometry experiment. A confocal laser scanning microscope (Nikon T2, Japan) and flow cytometer (BD Biosciences, USA) were employed for cellular uptake and apoptosis analysis.

### 1.3 Preparation of GO

Graphene oxide (GO) was firstly synthesized with a modified method of Hummers. In detail, about 3g graphite powder and 1.5g $NaNO_3$ were dispersed into 70mL concentrated $H_2SO_4$ at ice bath with vigorous stirring. Then, 9g $KMnO_4$ was slowly added in the solution. After the mixture became brownish by stirring for 30min at 35℃, it was diluted by adding 300mL $H_2O$. Subsequently, 20mL $H_2O_2$ (30%) was slowly added to reduce the remaining oxidant and make the solution become yellow. Then, the produced GO were separated by centrifuging at 10000rpm for 30min, and then was washed with HCl solution (v/v 10%) and Miili-Q water for five times respectively. The obtained GO was finally dialyzed with molecular weight cut off (MWCO) 14000Da membranes for one week to remove residual metallic ions, and then was dried in vacuum oven at 50℃ for next use.

**1.4 Preparation of rGO-PDA**

0.2g above prepared GO were re-dispersed in 100mL of 10 mM Tris-HCl buffer solution (pH 8.5), and the whole was ultrasonicated for 3h under 40KHz. Then, the whole was centrifuged at 30000rpm for 1h to remove any large size or aggregated GO sheets. The supernatant containing single layered GO sheets (2mg/mL) were collected for further use. About 25mL above prepared GO solution was dispersed in 75mL of 10mM Tris-HCl buffer solution (pH 8.5) under sonication, and then 50mg dopamine hydrochloride was added into the mixture and the whole was vigorously stirred at 70℃ for 24h to obtain dopamine-reduced GO nano-sheets (rGO-PDA). The produced rGO-PDA was finally dialyzed with MWCO 14000Da membranes for a week and then stored at 4 ℃ for next use.

**1.5 Monitoring of photo-thermal heating effect**

rGO-PDA and GO were respectively dispersed in PBS with the same concentration of 50μg/mL to compare the photo-thermal heating effect while PBS (pH

7.4) was used as the blank control. They were respectively irradiated by an 808 nm NIR laser (Beijing Laserwave Optoelectronics Technology Co., Ltd.) with the power density of 2.0 W/cm$^2$ for 10min. For investigating the concentration dependent photo-thermal heating effect, rGO-PDA solutions with varying concentrations of rGO-PDA (20-100μg/mL) were respectively irradiated by an 808 nm NIR laser with the power density of 2.0 W/cm$^2$ for 10min. Meanwhile, 50μg/mL of rGO-PDA solution was repeated exposed under the above irradiation condition for 5 cycles to evaluate the photothermal stability. Additionally, to assess the influence of laser power density on the photothermal effect, 50μg/mL rGO-PDA solutions were irradiated under different power densities (0.5, 1.0, 1.5 and 2.0W/cm$^2$). The temperature of solution was recorded by a digital thermometer in real time. The photo-to-thermal conversion efficiency was calculated according to the previous report as follows: [1, 2]

$$\eta = \frac{Q}{W} = \frac{cm\Delta T}{pt}$$

where η represents the thermal efficacy, Q is the heat released by rGO-PDA solution, W is the work induced by the input power, c is the heat capacity of the rGO-PDA solution, m is the mass of the rGO-PDA solution, ΔT is the average temperature change of the rGO-PDA solution, p is the input power of the laser to the solution, and t represents the irradiation time.

**1.6 Cell Culture:**

Human acute lymphoblastic leukemia T-cells (CEM) and acute lymphoblastic leukemia B-cells (Ramos) and human normal liver cells HL-7702 (L02) were incubated in 1640 medium supplemented with 10% FBS and 1% penicillin-streptomycin at 37 ℃ under 5% $CO_2$. The medium was routinely replaced with fresh culture medium every other day.

**1.7 Cytotoxicity assays about various concentration, time and laser powers**

CEM cells were plated on 96-well plates at a density of $1.0\times10^5$ cells/well respectively for 12h, following washed twice with PBS buffer. (1) In order to verify the biocompatibility of the nano-platform, the pretreated CEM cells were respectively incubated in complete culture medium containing rGO-PDA-dsDNA-sgc8 with various concentrations (0, 2, 4, 8, 12, 16, 20 μg/mL) for 24h. (2) To assess the influence of treatment time, the pretreated CEM cells were respectively cultured with 100μL complete culture medium of rGO-PDA-dsDNA-sgc8, DOX and rGO-PDA-dsDNA-sgc8/DOX (equivalent DOX, 20μM) with/without NIR irradiation (808nm, 1.5W/cm$^2$,10min) for 6h, 12h, 24h and 36h. (3) To evaluate the effect of laser power, the pretreated CEM cells were respectively cultured with complete culture medium including the same concentrations of rGO-PDA-dsDNA-sgc8 and rGO-PDA-dsDNA-sgc8/DOX for 24h, accepting 808nm laser irradiation at a power of 0.5, 1.0, 1.5 and 2.0W/cm$^2$ for 10min.

All of the experiments were performed in triplicate. Finally, the treated cells were washed with PBS buffer and stained with 100μL of Cell Counting Kit-8 (CCK-8) assay for 45min. The cell vitalities were measured with a microplate reader by detecting the absorbance at 450nm.

The cytotoxicity analysis for human normal liver cells HL-7702 (L02) was carried out in the same way.

**1.8 Cell apoptosis study**

CEM and Ramos cells were plated on 96-well plates at a density of $1.0\times10^5$ cells/well respectively for 12h, following washed twice with PBS buffer. To evaluate the chemo-therapy effect of our nano-platform, the pretreated cells were cultured with free DOX or rGO-PDA-dsDNA-sgc8/DOX (equivalent DOX, 20μM) in 100μL

complete culture medium for 24 h, respectively. To assess the photo-thermal treatment and synergistic therapeutic efficiency, the pretreated cells were respectively cultured with the same mass of nano-platforms: rGO-PDA-dsDNA-sgc8 or rGO-PDA-dsDNA-sgc8/DOX (equivalent DOX, 20μM) in 100μL complete culture medium for 12h. They subsequently accepted 808nm laser irradiation at a power of 1.5W/$cm^2$ for 10min and incubated for another 12h and then washed twice with PBS buffer. The treated cells were stained with propidium iodide (PI) for 15min and eventually determined by flow cytometric analysis.

**Reference**

[1] S.H. Su, J.L. Wang, E. Vargas, J.H. Wei, R. Martínez-Zaguilán, S. R. Sennoune, M. L. Pantoya, S.R. Wang, J. Chaudhuri, and J.J. Qiu, ACS Biomater. Sci. Eng. 2016, 2, 1357.

[2] D.L. Meng, S.J. Yang, L. Guo, G.X. Li, J.C. Ge, Y. Huang, C.W. Bielawskibc and J.X. Geng, Chem. Commun. 2014, 50, 14345.

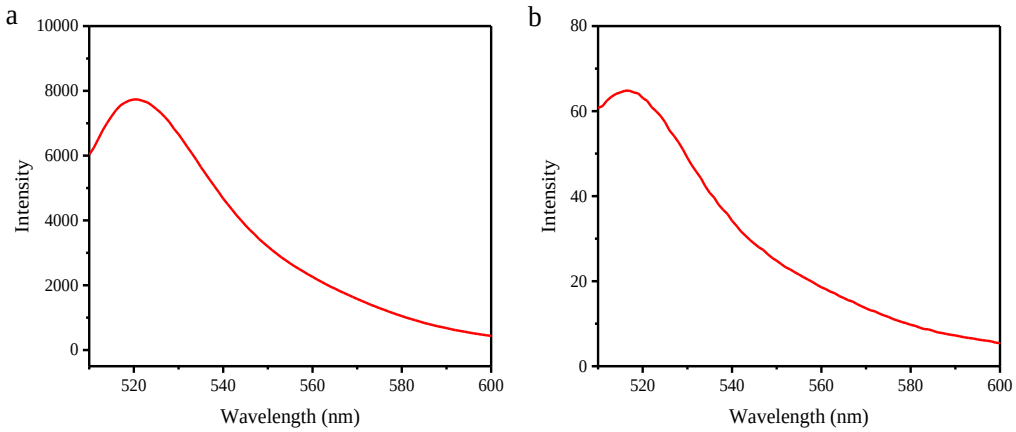


**Figure S1:** Fluorescence spectrum of DNA-FITC before conjugating onto rGO-PDA (a), and after conjugation (b).

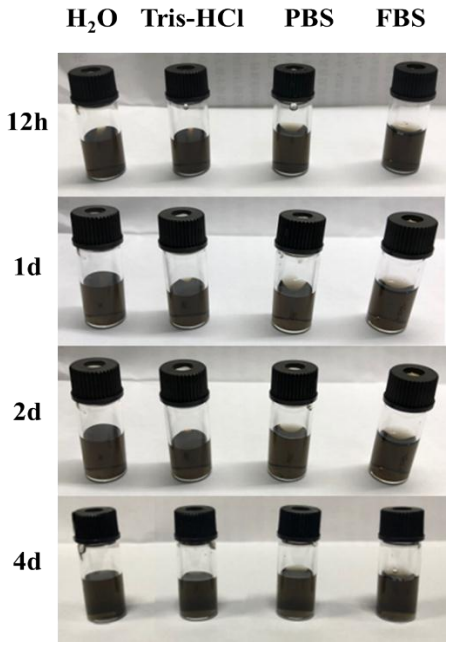


**Figure S2**: Photographs of 0.1mg/mL rGO-PDA-dsDNA-sgc8 nano-composites in various solvents ($H_2O$, Tris-HCl, PBS, FBS) at 12h, 1d, 2d and 4d, respectively.

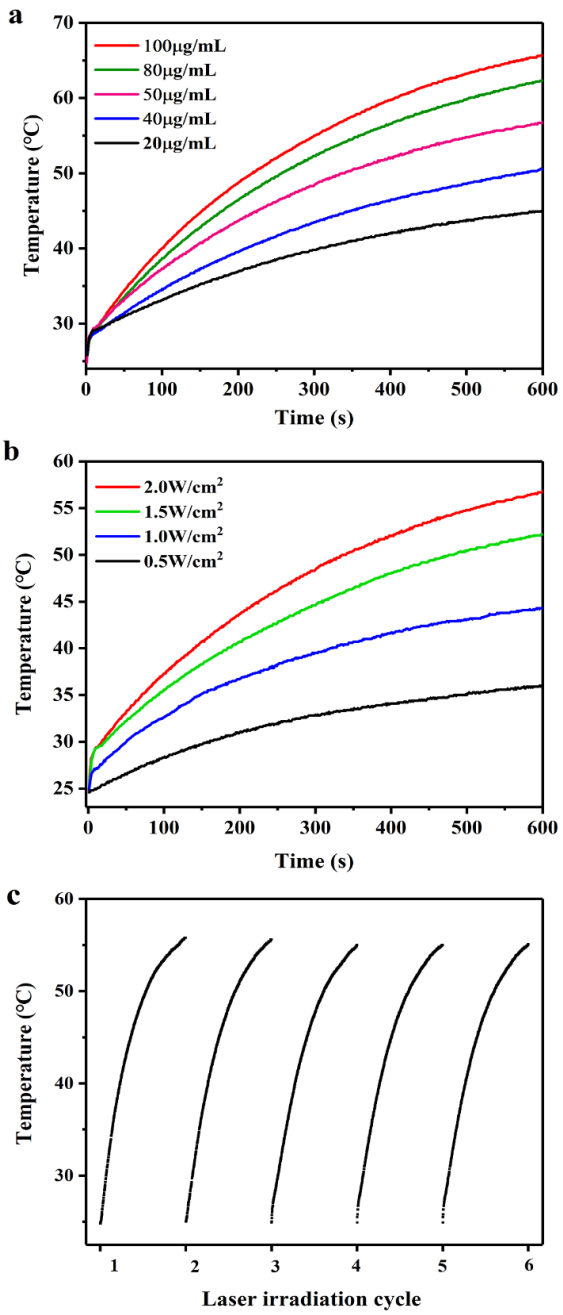


**Figure S3:** (a) Photo-thermal heating curves of the rGO-PDA dispersions with different concentrations. (b) Photo-thermal heating curves of the rGO-PDA dispersions under various irradiation power intensities. (c) Photo-thermal heating curves of the rGO-PDA dispersion (50μg/mL) under repeated laser irradiation with the power density of 2.0 W/cm$^2$ at 808 nm for 10min per cycle.

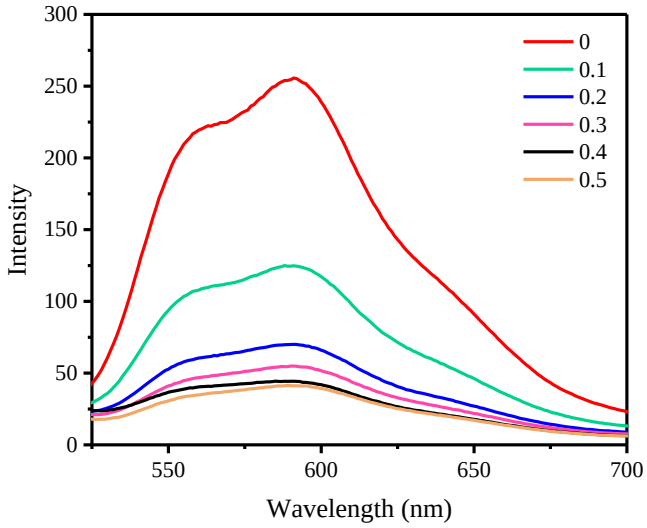


**Figure S4:** Fluorescence spectra of the DOX (2 μM) solution with increasing amount of dsDNA-sgc8 (from top to bottom: 0, 0.1, 0.2, 0.3, 0.4 and 0.5μM) at an excitation of 480 nm.

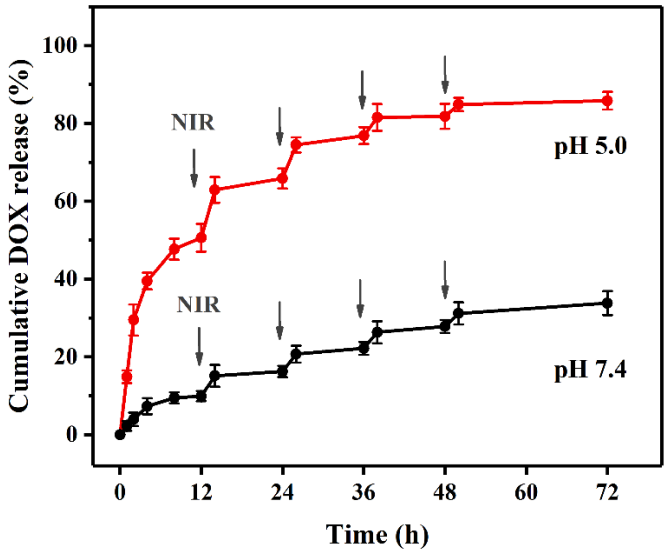


**Figure S5**: DOX release profiles from rGO-PDA-dsDNA-sgc8/DOX nano-composites at different pH with NIR laser irradiation (808 nm, 1.5 W/cm$^2$, 10min) at 12h, 24h, 36h and 48h.

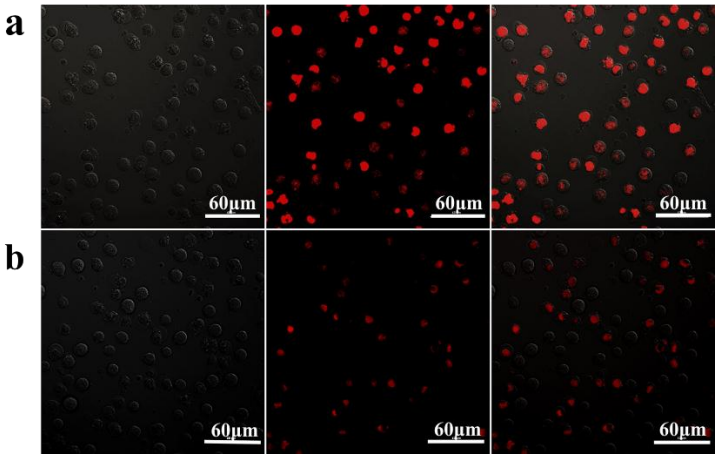


**Figure S6**: Confocal microscopy images of CEM cells incubated with rGO-PDA-dsDNA-sgc8/DOX nano-composites after pretreated with sgc8 aptamer (b) or not (a) (Images of red fluorescence originated from DOX, scale bar = 60 μm).

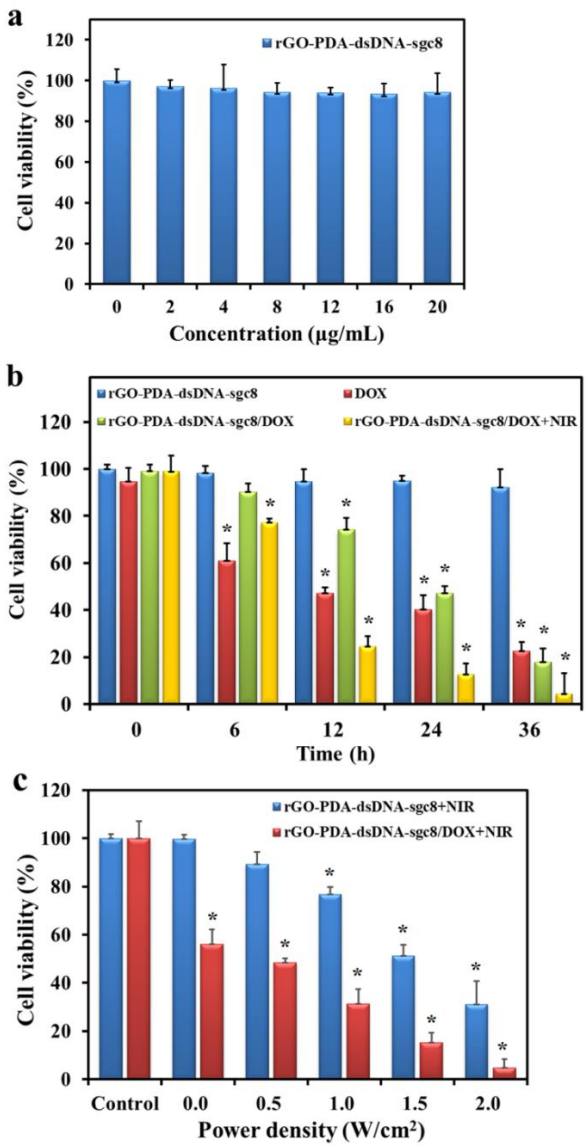


**Figure S7**: Cell viability of CEM cells treated: (a) with rGO-PDA-dsDNA-sgc8 at various concentrations; (b) with 20μM of free DOX, rGO-PDA-dsDNA-sgc8 and rGO-PDA-dsDNA-sgc8/DOX at different treating time with and without NIR laser irradiation, respectively; (c) with the same concentration of rGO-PDA-dsDNA-sgc8 and rGO-PDA-dsDNA-sgc8/DOX under various laser irradiation powers at 808nm. The statistical differences (P values) were calculated by student's t-test. The asterisks indicate $P<0.05$ versus control cells, suggesting differences were considered statistically significant.

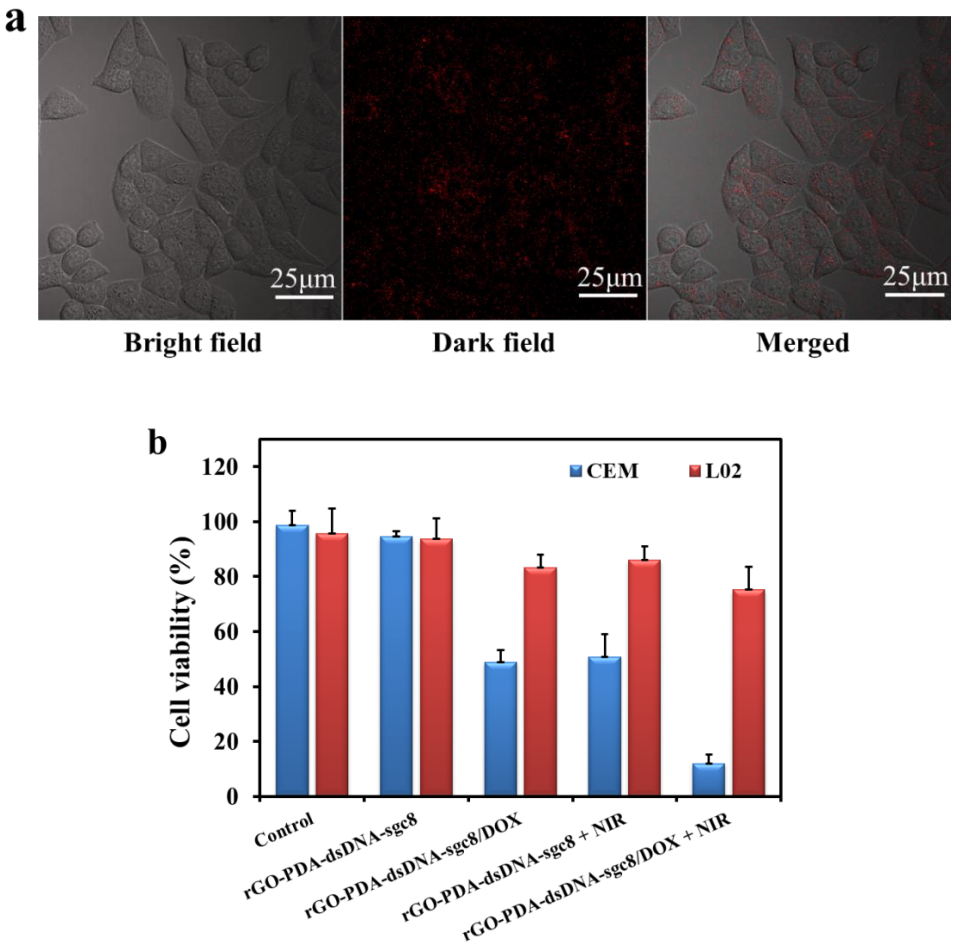


**Figure S8**: (a) Confocal microscopy images of L02 cells incubated with rGO-PDA-dsDNA-sgc8/DOX nano-composites (Images of red fluorescence originated from DOX). (b) Cell viability of L02 cells incubated with the same concentration of rGO-PDA-dsDNA-sgc8 and rGO-PDA-dsDNA-sgc8/DOX with or without NIR laser irradiation, respectively.