## **Supporting Information**

Comparison of nanomedicine-based chemotherapy, photodynamic therapy and photothermal therapy using

## reduced graphene oxide as the model system

Jingjing Liu, Kai Liu, Liangzhu Feng, Zhuang Liu, Ligeng Xu\*



**Supporting Figure S1. (a)** Cumulative release profiles of DOX from rGO-PEG/DOX at pH 5.5 and pH 7.4 PBS. **(b)** Cumulative release profiles of Ce6 from rGO-PEG/Ce6 at pH 7.4 PBS. **(c)** Fluorescence spectra of rGO-PEG/Ce6 and free Ce6 at the same concentration of Ce6. **(d)** Fluorescence spectra of rGO-PEG/DOX and free DOX at the same concentration of DOX.



**Supporting Figure S2.** The effects of rGO-PEG and its conjugates on the viabilities of U87 cells using 2D and 3D culture models. **(a-c)** Relative viabilities of 2D U87 cells incubated with rGO-PEG (a), rGO-PEG/Ce6 and free Ce6 (b) rGO-PEG/DOX and free DOX (c) for 4 h. Cells were washed with fresh culture medium and irradiated with 808 nm laser at the power density of 2 W/cm<sup>2</sup> for 5 min (a), or 660 nm laser at the power density of 5 mW/cm<sup>2</sup> for 30 min (b). Then, cells were further incubated for 24 h before MTT assay. Cells without laser irradiation were regarded as control. **(d-f)** Relative viabilities of 3D U87 tumor spheroids incubated with fresh culture medium and irradiated with fresh culture medium and irradiated with 808 nm laser at the power density of 2 more further incubated for 24 h before MTT assay. Cells without laser irradiation were regarded as control. **(d-f)** Relative viabilities of 3D U87 tumor spheroids incubated with rGO-PEG (d), rGO-PEG/Ce6 and free Ce6 (e) rGO-PEG/DOX and free DOX (f) for 4 h. Cells were washed with fresh culture medium and irradiated with 808 nm laser at the power density of 2 W/cm<sup>2</sup> for 10 min (d), or 660 nm laser at the power density of 5 mW/cm<sup>2</sup> for 60 min (e). Then, cells were further incubated for 24 h before APH assay. Cells without laser irradiation were regarded as control.



**Supporting Figure S3.** The therapeutic efficacy comparison of three rGO-based treatment modalities at the same concentration of rGO on A549 cells. (a), (b) The effects of three rGO-based treatment modalities on the cell viability under 2D and 3D models, respectively. The cells (2D) or tumor spheroids (3D) were incubated with rGO-PEG/DOX, rGO-PEG/Ce6 and rGO-PEG for 4 h. After replacing with fresh culture medium, except rGO-PEG/DOX treatment group, cells of other two groups were irradiated by 661 nm or 808 nm laser. Finally, the cell viability was evaluated using MTT assay (for 2D model) or APH assay (for 3D model) at 24 h post irradiations.



Supporting Figure S4. TUNEL assay image of untreated U87 tumor spheroid.