Supporting Information

Receptor-targeting fluorescence imaging and theranostics using a graphene oxide based supramolecular glycocomposite

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S1. Additional figures

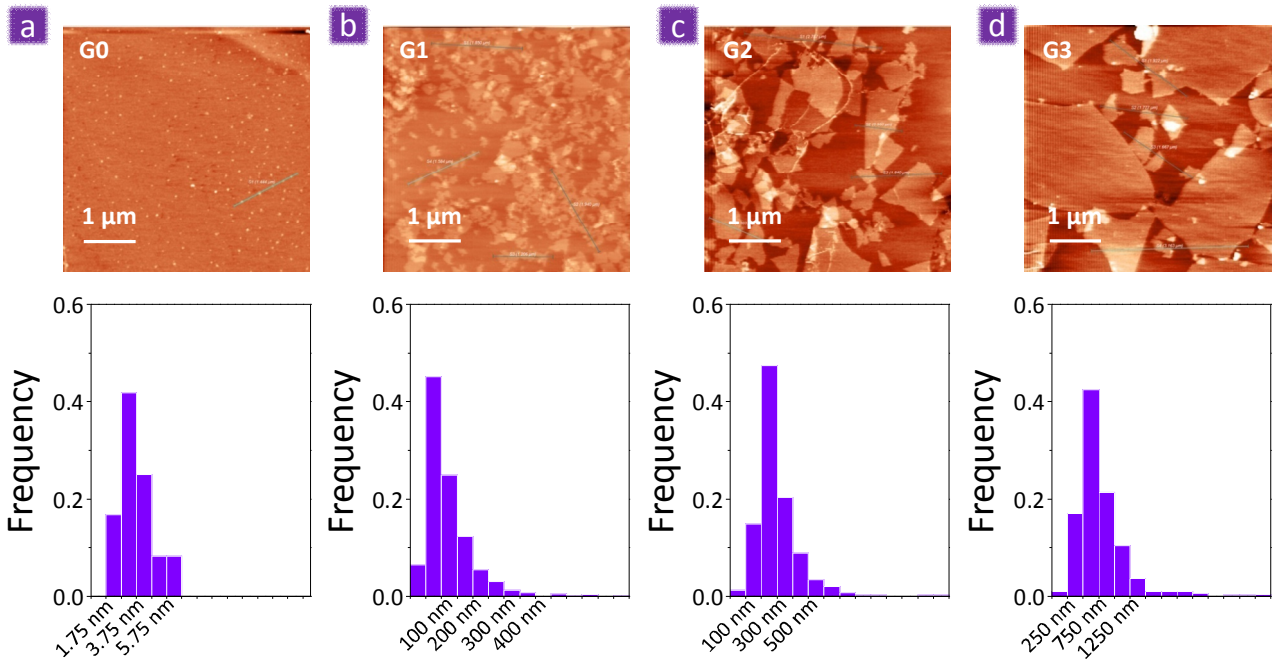

Figure S1. Size distribution of the GOs used. AFM images and the corresponding histogram depicting the size distribution of (a) **G0**, (b) **G1**, (c) **G2** and (d) **G3**. The histograms were produced by counting over 1000 GO sheets each.

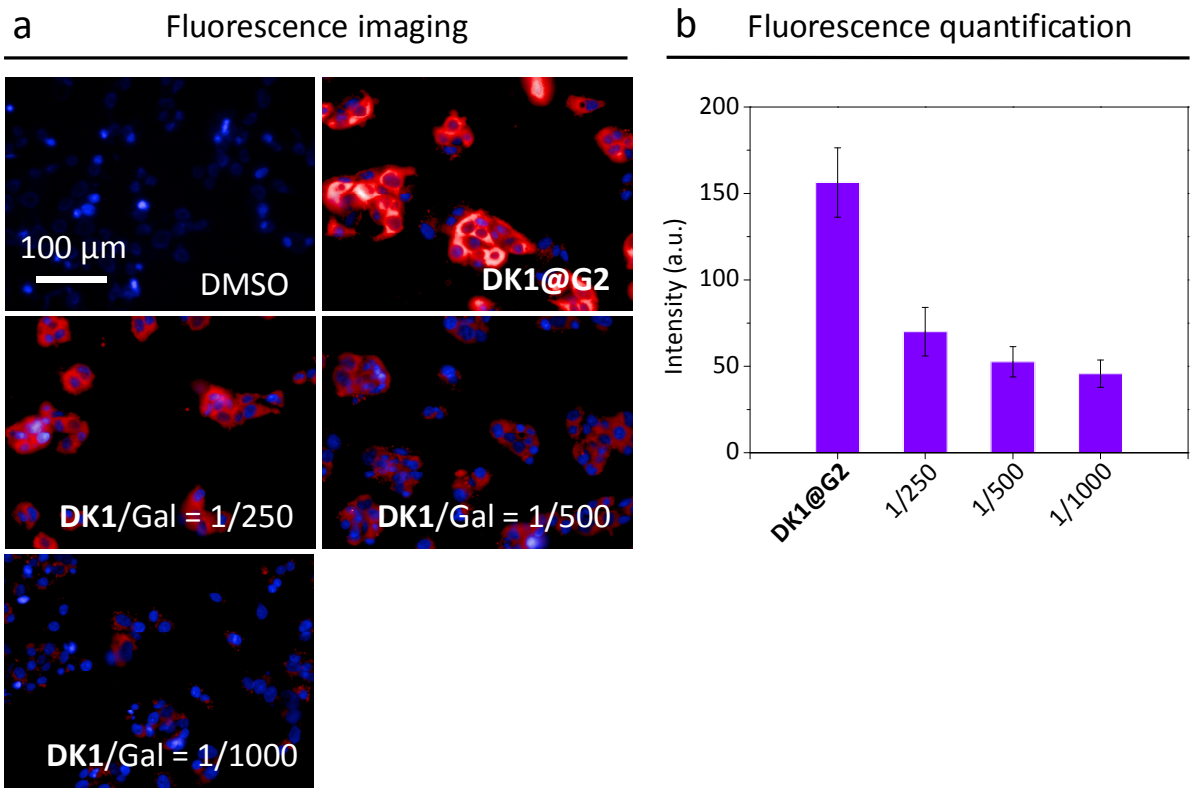

Figure S2. Competition assay of Hep-G2 staining using DK1@G2 with free D-galactose.

Fluorescence imaging (a) and fluorescence intensity quantification (b) of Hep-G2 cells using **DK1@G2** (40 µM/50 µg mL^{-1}) without or with pre-treatment of free D-galactose (the molar ratios of **DK1**/Gal are 1/250, 1/500 and 1/1000). The cell nuclei are stained with Hoechst 33342 (Scale bar 100 µm) (exitation channel: 520-550 nm, emission: 580-650 nm).

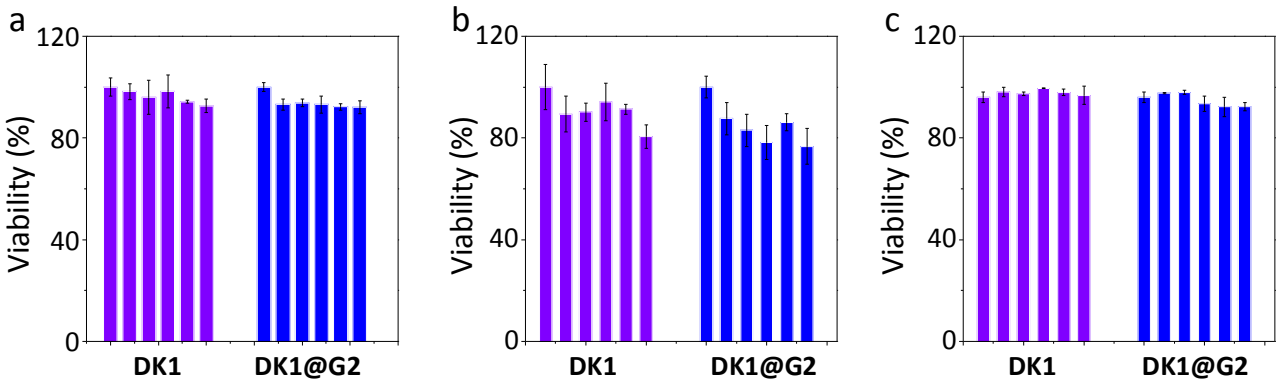

Figure S3. Cell viability assay. Cell viability of (a) HEK293 and (b) Hep-G2 determined by a kit-8 (CCK8) assay, and that of (c) Hep-G2 determined by a cell-nucleus counting assay in the presence of **DK1** (concentration from left to right column: 0, 40, 50, 200, 400 μM) or **DK1@G2** (concentration of **G2**: 50 μg mL^{-1}).

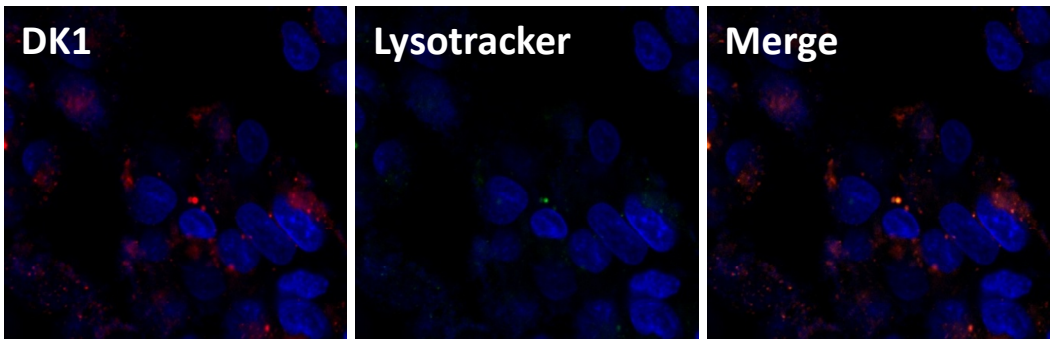

Figure S4. Cellular localization of DK1. Confocal microscopic imaging of **DK1** (20 μM) in the presence of lysotracker (20 μM) (exitation channel: 520-550 nm, emission: 580-650 nm).

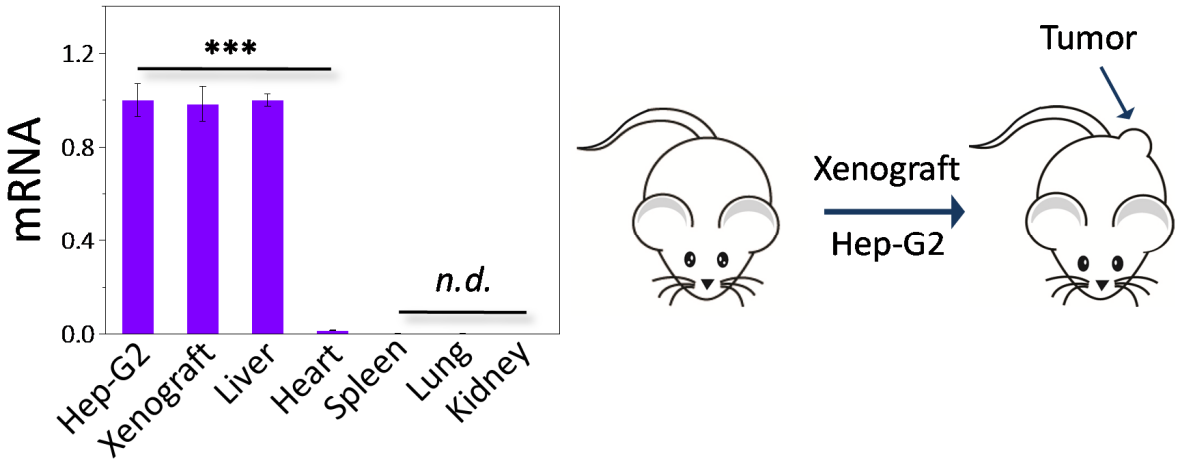

Figure S5. RT-qPCR of ASGPr expression on tissues. Left: Relative mRNA level of ASGPr encoded by Hep-G2 cells (as a control), the xenograft and different tissues measured by quantitative polymerase chain reaction (***P< 0.001; n.d. means not detectable). Right: Cartoon depicting the xenograft of Hep-G2 with mouse.

S2. Experimental Section

General remarks. Proteins were purchased from Sigma-Aldrich. Graphene oxide (2 mg mL^{-1}) was purchased from Xianfeng Nanotechnologies Co., Ltd (Nanjing, China). All chemicals used are of analytical grade. Ultrapure water was obtained from a Milli-Q integral Pure/Ultrapure Water Production unit.

Preparation of the supramolecular DK1@CPT@GO. 80 μL of **DK1** (1 mM DMSO solution) was added to 2 mL of GO (0.4 mg mL^{-1} water solution), and the resulting mixture was sonicated for 5 min and stirred for another 3 h. To the resulting crude mixture, different concentrations of **CPT** (DMSO solution) was added, and the mixture was stirred at room temperature for 12 h in the dark to produce the supramolecular system that can be used as is.

Atomic force microscope (AFM). Morphology study was performed with an AFM (AJ-III, Aijian nanotechnology Inc., China) in tapping mode to simultaneously collect height and phase data. A droplet of GO dispersion (0.1 mg/mL) was cast onto a freshly cleaved mica surface, followed by drying at room temperature before analysis.

Cell culture and transfection. Hep-G2 cells were maintained in Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% Fetal bovine serum (Gibco, Gland Island, NY, USA) and passaged every 3-4 days based on 90% confluency.

Establishment of the Hep-G2 knockdown stable cell line (sh-ASGPr). Plasmids encoding ASGP-R1 specific shRNA was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Lentiviral particles were generated according to the manufacturer's instructions. Briefly, 293T cells were seeded in a six-well tissue culture plate and were grown to 80-90% confluency in antibiotic-free normal growth medium supplemented with FBS. 3 μg shRNA plasmid was cotransfected with 1.8 μg pCAG-VSVG and 2.7 μg PAX2 into 293T cells using 15 μL lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After 6 h, the medium was changed to fresh DMEM with

10% FBS. After 72 h, the lentivirus-containing supernatant were collected, filtered, and then employed for analysis.

Cell imaging. Cells (Hep-G2, sh-ASGPr Hep-G2 and Hela) were cultured in DMEM (Dulbecco modified Eagle medium) supplemented with 10% FBS. A549 cells were cultured in F12 supplemented with 10% FBS, MCF-7 cells in EMEM (Eagle's minimal essential medium) supplemented with 10% FBS and HCT116 cells in 5A supplemented with 10% FBS (fetal bovine serum). Cells (1.5×10^5) were seeded on a black 96-well microplate with optically clear bottom (Greiner bio-one, Germany) overnight. The cells were incubated with **DK1** in the absence and presence of GOs, followed by addition of the nuclear staining reagent Hoechst (5 μg mL^{-1}) at 37 °C in a humidified atmosphere of 5% CO_2 in air for 5 min. Then, cells were washed with PBS (phosphate buffered saline) three times and fixed using 4% paraformaldehyde. The fluorescence images were recorded using an Operetta high content imaging system (Perkinelmer, US) and quantified and plotted by columbus analysis system (Perkinelmer, US).

Cell viability assay. Cell viability was measured using a cell counting kit-8 (CCK-8, Dojindo, Rockville, MD). 1×10^4 cells were seeded in a 96-well plate and cultured in HG-DMEM (high-glucose DMEM) supplemented with 10% FBS and 1% PS at 37 °C under 5% CO_2. After 10 h, the cells were washed with 100 μL of serum-free HG-DMEM (1% PS) two times and incubated with 100 μL of different concentrations of material composites in serum-free HG-DMEM (1% PS). After exposure for 24 h, the cells were washed twice with serum-free HG-DMEM and 10 μL of CCK-8 solution was added to each well containing 100 μL of serum-free HGDMEM, followed by a gentle shake. After incubation for 2 h at 37 °C under 5% CO_2, 80 μL of the mixture was transferred to another 96-well plate. The absorbance of the mixture was measured at 450 nm using an M5 microplate reader (Molecular Device, USA).

Tissue imaging. The xenograft, heart, liver, spleen and lung sections of the Hep-G2-bearing xenograft mice were fixed in 4% paraformaldehyde in PBS overnight. 12 μm sections were cut using

a rotary microtome. Sections were treated with PBS for 10 min, and then incubated with **DK1@G2**. Then, the sections were gently washed with PBS three times and mounted by glycerin. The fluorescence images were recorded using a fluorescence microscope (Olympus, Japan). The use and care of experimental animals was approved by the Institutional Animal Care and Use Committee (IACUC), East China Normal University (ECNU), with the certification number of AR201404020.