Electronic Supplementary Information

Highly narrow nanogap-containing Au@Au core-shell SERS nanoparticles: Size-dependent Raman enhancement and applications in cancer cell imaging

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Experimental Section

Chemicals and Materials

All the chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated. HPLC-purified ssDNA (5'-SH-polyA$_{30}$-3') were purchased from TaKaRa Biotechnology Co. Ltd (DaLian, China). Hydrogen tetrachloroaurate (III) tetrhydrate ($HAuCl_4 \bullet 4H_2O$) and sodium citrate were purchased from Sinopharm Chemical Reagent Co. Ltd (China). Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) medium were obtained from Gibco Co. (NewYork, USA). DAPI was purchased from Molecular Probes, Inc. (California, USA). The HCT 116 cell line was provided by the Cell Bank of Chinese Academy of Science (Shanghai, China). Other chemicals employed were of analytical reagent grade and were used without further purification. NANO pure water (>18.0 MΩ Milli-Q) was used in all experiments.

Apparatus

Field emission scanning electron microscope (SEM) images were taken with a Hitachi S-4800 field emission scanning electron microscope at an accelerating voltage of 200 kV. Transmission electron microscopy (TEM) imaging was taken with a JEOL-2100F instrument using an accelerating voltage of 200 kV. Confocal fluorescence microscope (CFM) images were captured with a Leica TCS SP5 II confocal laser scanning microscope equipped with an Andor EMCCD camera (Germany). The dark-field measurements were carried out on an inverted microscope (Olympus IX71, Japan) equipped with a dark-field condenser (0.8<NA<0.95) and a 60× objective lens (NA=0.8). UV–vis absorption spectra were obtained with a PerkinElmer Lambda 950 UV-vis spectrophotometer. Fourier transform infrared spectroscopy (FT-IR) spectra were recorded on a Nicolet 6700 FT-IR Spectrometer. Hydrodynamic radius measurements were performed using a Malvern Zetasizer Nano ZS instrument model ZEN3600 (Malvern, UK) equipped with a standard 633-nm laser. Raman measurement was performed on the XPLORA (HORIBA, Jobin Yvon, France) Raman microscope system.

Synthesis of Au core nanoparticles

AuNPs of about 15 nm were synthesized by citrate reduction of HAuCl4.[1, 2]

DNA modification of Au core nanoparticles

The modification of AuNPs (15 nm in diameter) with DNA was followed according to previous work. 100 μL AuNP (10 nM) solution was mixed with 3 μL of 100 μM purified DNA (5'-SH-polyA$_{30}$-3'). The resulting solution was placed at room temperature overnight. The mixture was then added to 10 mM sodium phosphate buffer (pH 7.4, 0.3 M NaCl) and was allowed to stand for 40 h. Afterward, the particles were washed three times in a 10 mM phosphate buffer (pH 7.4) using centrifugation (12000 rpm, 20 min, 4 ℃) to remove any excess DNA and were then redispersed in 1 mL of 10 mM sodium phosphate buffer with 0.3 M NaCl (pH 7.4).

Attachment of Raman reporters on Au-polyA conjugates

The SH-polyA-anchored AuNPs (500 μL, 1 nM) were mixed with 100 μL of small Raman reporter molecule (0.1 M 44DP, DTNB) solution, and the mixture was incubated for 3 days at room temperature with gentle shaking. The particles were then washed three times in 10 mM phosphate buffer (pH 7.4) using centrifugation (12000 rpm, 20 min, 20 ℃) to remove any excess Raman reporters and were then resuspended in 500 μL of 10 mM sodium phosphate buffer with 0.1 M NaCl (pH 7.4).

When prepared GCNPs including two kinds of small Raman reporters (44DP and DTNB), these two were mixed with a same concentration (0.05M) at first. After the mixture (polyA, 44DP and DTNB) was incubated for 3 days at room temperature with gentle shaking, they were co-encoded on the surface of Au cores simultaneously.

Synthesis of GCNPs

To form gold shells around these Au nanocores, 100 μL of the above obtained solution was mixed with 50 μL of 1% polyvinylpyrrolidone (PVP) solution. The mixture was mixed with 10 mM hydroxylamine hydrochloride solution (NH_2OH-HCl) and 5 mM chloroauric acid solution ($HAuCl_4$), the ratio of which was 1:1. The reaction mixtures were fiercely vortexed for 1 min and washed three times using centrifugation (8000rpm, 6min, and 20 ℃). The precipitate was redispersed in 100 μL of Milli Q water and stored at 4 ℃. With the different amount of $HAuCl_4$ added (10,

20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130 μL), GCNPs in different sizes (mean dimeters) (40.70, 45.10, 50.30, 53.25, 56.18, 62.17, 69.14, 76.79, 76.87, 81.23, 85.76, 89.21, and 99.64 nm) were prepared.

Synthesis of GCTs

SH-C_2H_4-NH_2-HA (SH-Mercaptoethylamine hydrochloride- hyaluronic acid) was first synthesized following the reported protocol[3]. 1mL of 1 mM SH-C_2H_4-NH_2 was mixed with excess HA (6000-8000 MW), and then stirred at room temperature overnight after adding NHS/EDC powder. 100 μL DMEM (cell culture media) was added into AuNPs solution (100 μL, 1 nM), GCNPs solution (100 μL, 1 nM) to prevent aggregation. After that, 100 μL SH-C_2H_4-NH_2-HA (1 mM) was added into two mixed solution mentioned above separately. The reaction mixtures were shaked gently overnight and washed three times using centrifugation. The precipitates were redispersed in 100 μL of DMEM and stored at 4 ℃ for further use.

Characterizations

TEM: The TEM samples were prepared by dropping the above solution (5 μL) onto a copper-coated grid. After drying in a far-infrared fast dryer, the samples were imaged using HRTEM.

SEM: The SEM samples were prepared by dropping the above solution (5 μL) onto a piece of 0.5x0.5cm Aluminium foil. Then, they were drying and fixed onto special sample platform, and finally were imaged using SEM.

UV: Samples for UV-vis absorption were diluted from 30 μL to 100 μL using NANO pure water, and shifted into colorimetric wares. Then the spectra were investigated on PerkinElmer Lambda 950 UV-vis spectrophotometer.

FTIR: Fourier transform infrared spectroscopy (FT-IR) was recorded on a Nicolet 6700 FT-IR Spectrometer. Samples were prepared by tableting freeze-dried samples (freeze drying in vacuum) and KBr powder mixture.

DLS：Particle sizes were obtained using a Malvern Zetasizer Nano ZS (Malvern Instrument, UK) based on dynamic light scattering. Zeta potential was measured using the same instrument. Before each measurement, the above solution was diluted into 1/7 of the original concentration using NANO pure water.

Cellular imaging in Laser Scanning Confocal Microscope (LSCM) reflection mode

HCT116 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated Fetal bovine serum (FBS, Gibco), 1% penicillin/streptomycin at 37 ℃ in humidified air containing 5% CO_2. Cells were then seeded on 24-well culture plates at a density of 5×10^5 cells/mL and cultured for 24 h. Afterwards, HCT116 cells were seeded in a 35 mm glass bottom dish at a density of 2×10^5 cells/mL and incubated at 37 ℃ for 12 h. They were then washed twice with phosphate buffer (PBS) and incubated with HA, GCNPs, and GCTs diluted in fresh culture medium (the final concentration was 0.2nM) for 0.5 h at 4 ℃. After that, cells were washed for three times with PBS and fixed by 4% formaldehyde. Then cells were washed again for three times with PBS, and then the cells were dyed by DAPI (5 ug/mL) for 5 minutes. After being washed,Images of these cells were then obtained using a Laser Scanning Confocal microscope (Leica TCS SP5) with a 633 nm He-Ne laser as the excitation light source.

Cellular imaging under Dark Field Microscope (DFM)

HCT 116 cells were cultured in 35 mm glass bottom dishes and incubated respectively with pure medium, medium with GCNPs, and medium with GCTs for 0.5 h at 4 ℃. They were then washed for three times with phosphate buffered saline (PBS). After that, cells were fixed by 4% formaldehyde and imaged under a dark-field microscopy.

Cellular imaging under Raman confocal microscope

HCT 116 cells and HEK 293 cells were incubated respectively with GCTs for 0.5 h at 4℃. The final concentrations of them were 1 nM. After washing with phosphate buffered saline (PBS) for three times, cells were fixed by 2.5% glutaraldehyde and imaged under a Raman confocal microscope (Renishaw inVia-reflex) with a 633 nm laser (80 mW) as the excitation light source. SERS measurements were performed using XPLORA (HORIBA, Jobin Yvon, France) Raman microscope system. The laser power of 1 mW was provided by the excitation source of a Melles Griot He-Ne laser operating at λ= 633 nm. Raman scattering was collected using a charge-coupled device (CCD) camera at a spectral resolution of 4 $cm^{-1.}$ An additional CCD camera

was fitted to an optical microscope to obtain optical images. All of the Raman spectra reported here were collected for 10s exposure times in the range of 600-2300 cm^{-1}. A 50× objective lens (~1 μm laser spot size) was used, and the pinhole was selected as 400 μm. Each Raman spectroscopic map contains at least 50 × 50 spectra, with 0.1 s integration time for each spectrum. Step sizes of 8 μm and 1.5 μm were used for Raman mapping to obtain large area images and high resolution images, respectively.

SERS enhancement factor (EF) calculations

The EF value of the GCNPs was calculated as 0.93×106. The calculation was based on the electromagnetic enhancement theory and approximately scales Raman scattering as E4 where |E| was got from the FDTD simulation [4].

However, the experimental EF value can reach to 1.0×108 which was consistent to the previous result [5], two orders of increase might be ascribed to the chemical enhancement effect. And the experimental SERS enhancement was calculated using the following equation:

$$EF = \frac{I_{SERS} N_{BULK}}{I_{SERS} N_{GCNPs}}$$

Where NBULK and NGCNPs are the number densities of the fluorescent ROX in solution and in GCNPs, respectively. Because the ROX were labeled on the DNA, the number of DNA on a gold seeds was equal to the number of the ROX. As substantiated by our previous work (Gold Nanostructures Encoded by Non-fluorescent Small Molecules in PolyA-mediated Nanogaps as Universal SERS Nanotags for Recognizing Various Bioactive Molecules), there was about 50 DNA strand adsorbed on a 15 nm gold seeds. After detected the Raman intensity of pure ROX (30 mM) in the solution and ROX labeled on DNA in the GCNPs (1 nM), respectively, we calculated the experimental EF was approximate to 1.0×10^8.

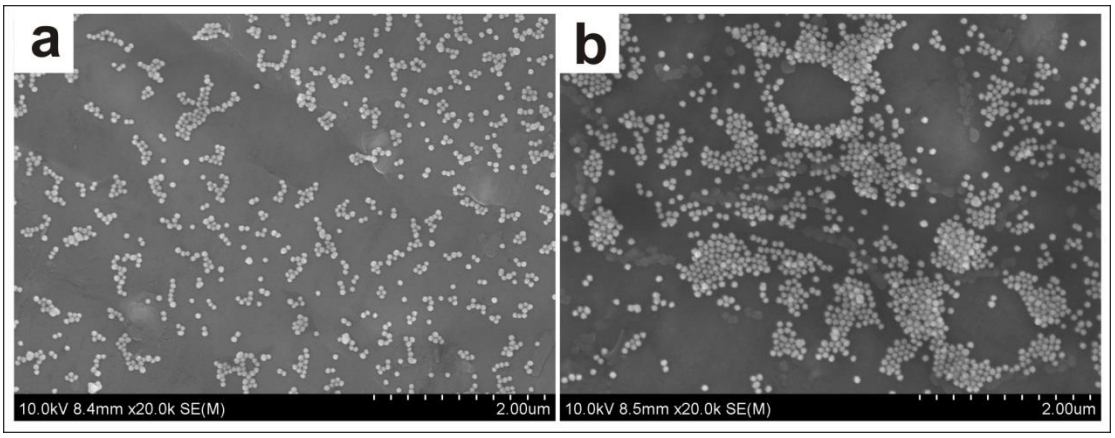

Fig. S1 SEM images of GCNPs encoded with 44DP (a), and DTNB (b) separately in the interior nanogaps.

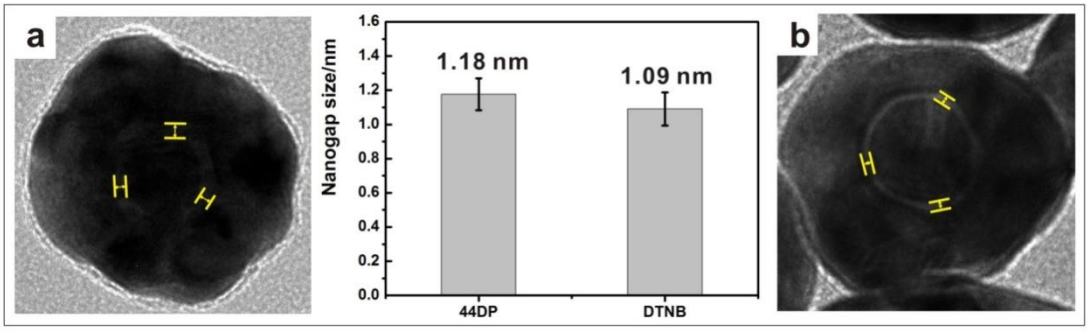

Fig. S2 Magnified TEM imgaes GCNPs encoded with 44DP (a), and DTNB (b) seperately in the interior nanogaps, and the corresponding gap sizes. Error bars represent the standard deviation of three independent measurements of seldom positions marked in the figures.

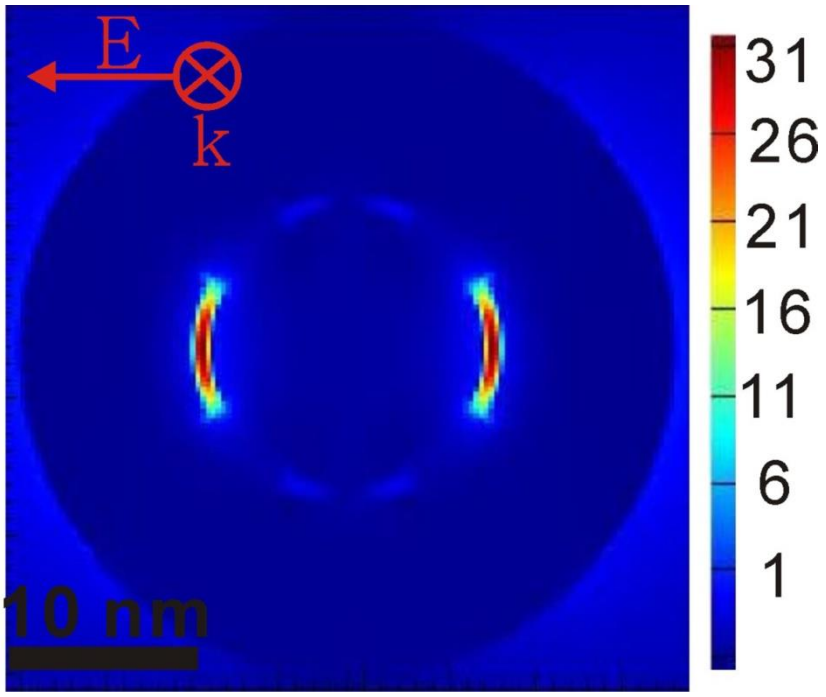

Fig. S3 FDTD simulation of electromagnetic field distribution of GCNPs. Scale bar, 10 nm. The incident excitation polarization is directed along the horizontal axis (λex=633 nm).

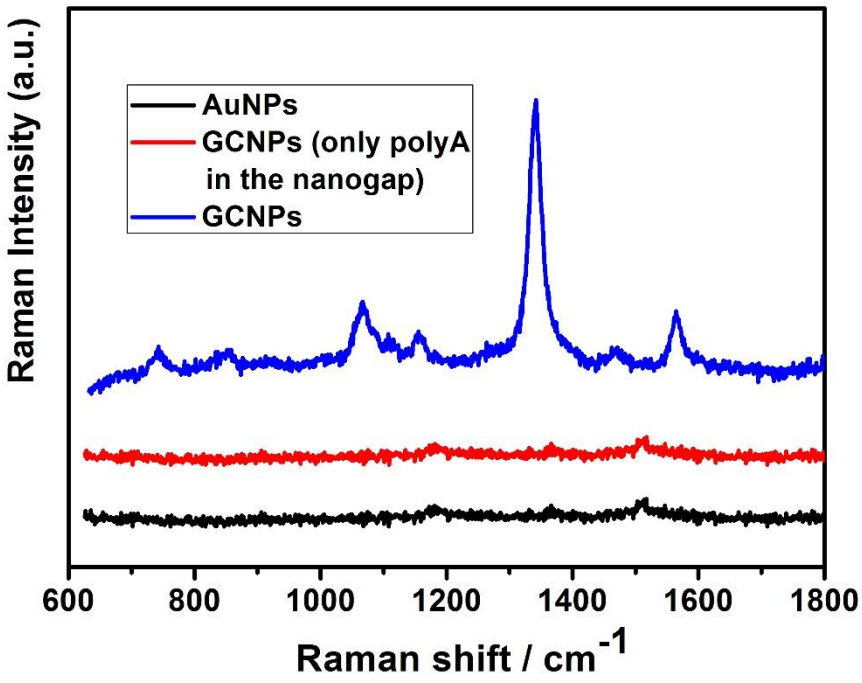

Fig. S4 Raman spectrum of AuNPs, GCNPs (only polyA in the nanogap without Raman molecules) and GCNPs of the same concentration.

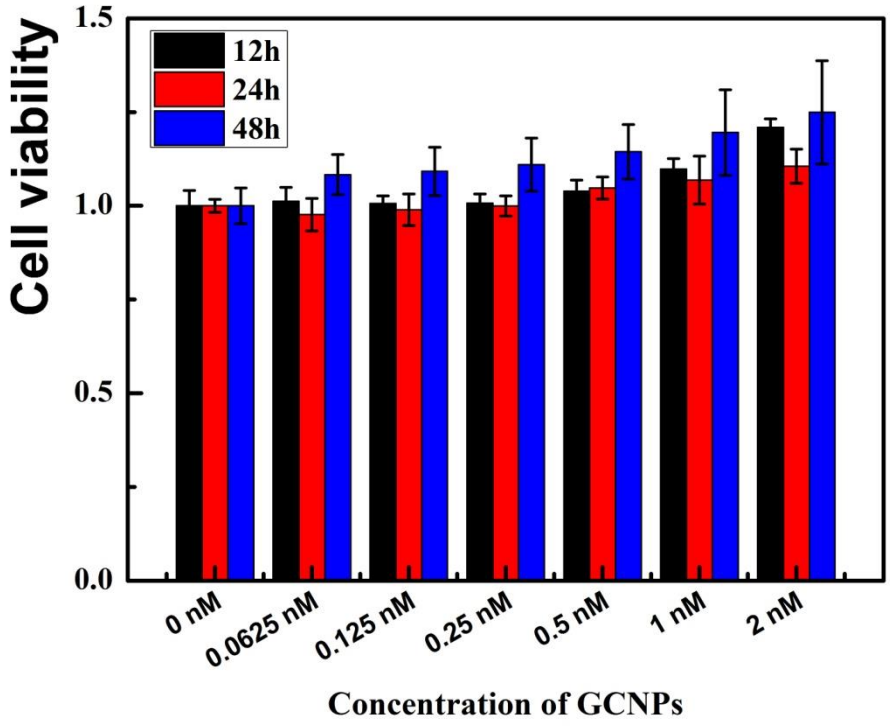

Fig. S5 In vitro cellular toxicity assay: relative cell viability of HCT116 cells incubated with different concentration of GCNPs for 48 hours. Each bar represents the mean (SD) of triplicate determinations.

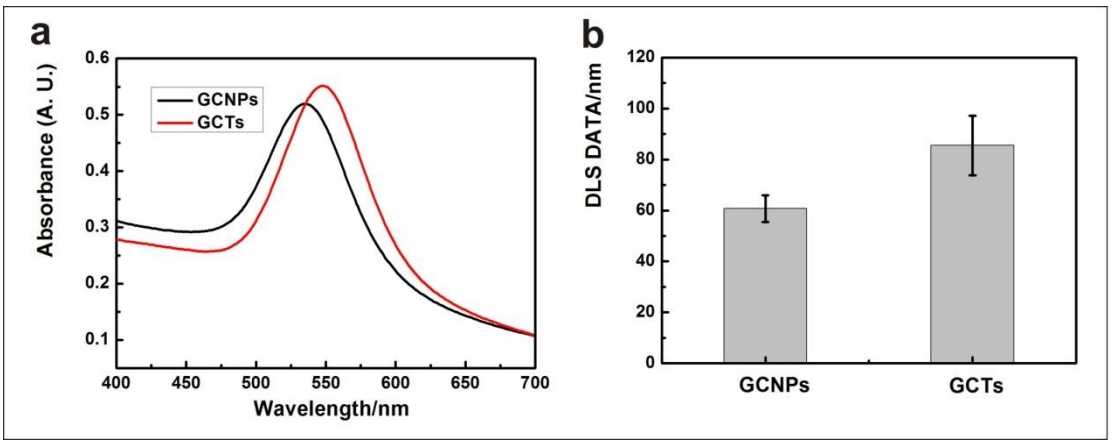

Fig. S6 Characterization of structures before and after modified with HA. a, UV-vis spectra for the nanoparticles. b, Hydrodynamic radii of those two nanoparticles (GCNPs, and GCTs) determined using dynamic light scattering (DLS). Error bars represent the standard deviation of three independent measurements.

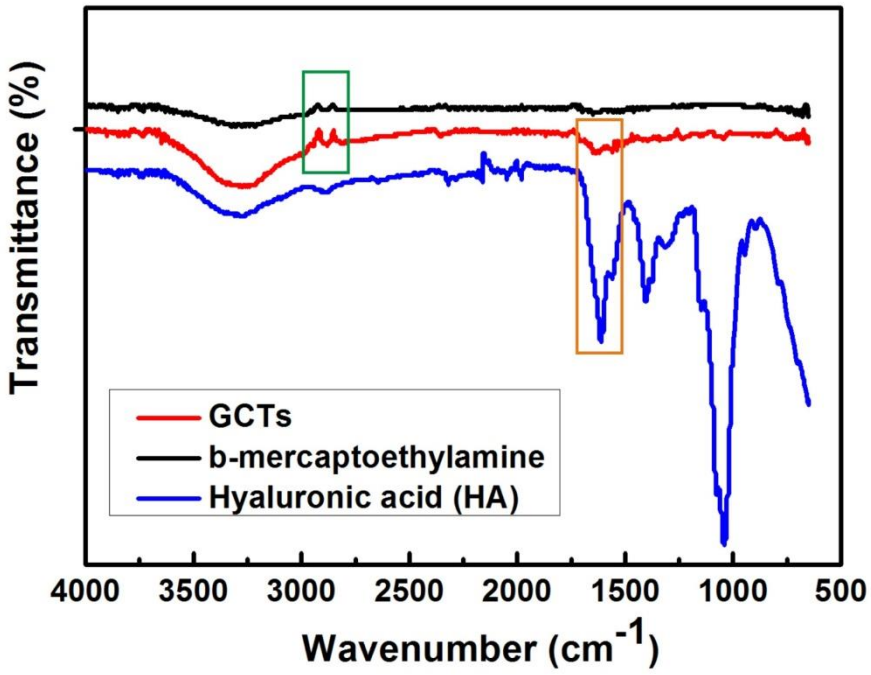

Fig. S7 FT-IR spectra of HA, β-mercaptoethylamine and GCTs.

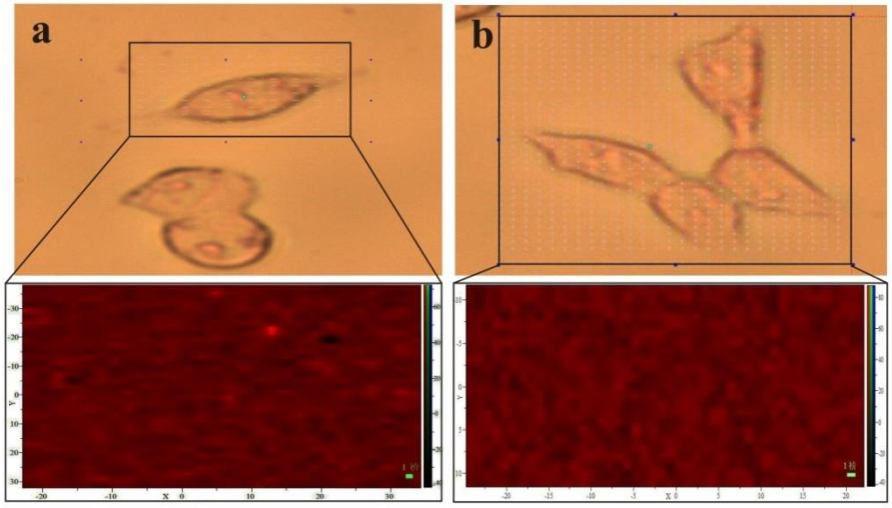

Fig. S8 Raman mapping images of GCNPs-treated HCT116 cells (a) and GCTs-treated healthy cells (b) under the characteristic peak of 1612 cm^{-1} for 44DP.

References

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