

## Supporting Information (SI)

### **Gold-Nanodot-Decorated Hollow Carbon Nanospheres Based Nanoplatform for Intracellular miRNA Imaging in Colorectal Cancer Cells**

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## **MATERIALS AND METHODS**

**Chemicals and Materials.** Tetraethyl orthosilicate (TEOS), dopamine hydrochloride, potassium and gold (III) chloride (KAuCl<sub>4</sub>) were purchased from Sigma-Aldrich. Hydrofluoric acid and N-hydroxy succinimide (NHS) were purchased from Sinopharm chemical reagent Co., Ltd (Shanghai, China). Ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC) was purchased from Aladdin Industrial Corporation (Shanghai, China). CCK-8 reagent was purchased from Dojindo (Japan). Anti-Human/Mouse CD44 FITC was purchased from eBioscience (USA). All other reagents and solvents were of analytical grade and without further purification. Lipofectamine™ 2000 transfection reagent was purchased from ThermoFisher Scientific (USA). All cell lines were obtained from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All of the samples of scrambled DNA probe, miR-21-1, miR-21-5p, miR141 and target miR-21 were synthesized by Sangon Biotech, Shanghai, China and purified by high-performance liquid chromatography (HPLC). The sequences are described as follows:

scrambled DNA probe (FAM-5'-TCAACATCAGTCTGATAAGCTA-3'),

miR-21-1 (5'-UAGCUUAUCATACUAAUCUUGA-3'),

miR-21-5p (5'-UCAACAUCAGUCUGAUAAGCUA-3'),

miR141 (5'-UAACACUGUCUGGUAAGAUGG-3'),

target miR-21 sequence (5'-UAGCUUAUCAGACUGAUGUUGA-3').

All of the synthesized DNA sequences were dissolved in ultrapure water (18.2 MΩ, Milli-Q Direct-8 water purification system, Millipore) as stock solution and kept at -20 °C.

**Apparatus.** The transmission electron microscopic (TEM) images were carried out on a TECNAI G2 20 U-Twin instrument (FEI, USA). Thermal gravimetric analysis (TGA) was performed using TGA/DSC 1 (METTLER TOLEDO, Switzerland). Particle size and zeta potential were determined using a Nano-ZS ZEN3600 (Malvern Instruments, UK). The UV-Vis absorption spectra were collected on a UV-2550 (SHIMADZU, Japan). The fluorescence spectra were measured on RF-5301PC (SHIMADZU, Japan). The fluorescence ratio of experiments in vitro were determined on Infinite®F50 microplate reader (TECAN, Switzerland). The concentration of Au ions were determined by an PlasmaQuant®MS inductively coupled plasma mass spectrometer (ICP-MS) (Analytik Jena, Germany). Confocal laser scanning microscopy (CLSM) imaging was performed using a Ti-U microscope equipped with a CSU-X1 spinning-disk confocal unit (Nikon,

Japan) and an EM-CCD camera (iXon<sup>+</sup>; Andor, USA). The qRT-PCR was performed on ABI StepOne Plus Detector System (Applied Biosystem, USA). Cell viability was measured by Infinite@F50 microplate reader (TECAN, Switzerland). Flow cytometry analysis was measured by Canto II (BD Company, USA). Gel electrophoresis assay was measured by ChemiDoc™ MP Imaging System (Bio-Rad, USA).

**Development of ssDNA–AuHCNs–HA FRET Nanoplatfom.** In a typical synthesis, ssDNA–AuHCNs–HA nanoplatfoms were prepared via the immobilization of FAM-labeled single strand DNA on the surface of AuHCNs–HA. For this purpose, AuHCNs and Dopamine modified hyaluronic acid (HA-DA) were synthesized following a literature procedure that we previously reported.<sup>1</sup> AuHCNs were synthesized using SiO<sub>2</sub> as template and polydopamine as carbon precursor. Typically, 1.5 mL ddH<sub>2</sub>O and 5 mL ammonium hydroxide solution (28%) were dissolved in ethanol (100 mL), then 1.5 mL TEOS was added dropwise. After stirring for 4 h, the SiO<sub>2</sub> was collected by centrifugation, washed with ethanol, and dried by vacuum. Then, 200 mg prepared SiO<sub>2</sub> were dispersed in 60 mL dopamine solution (3 mg·mL<sup>-1</sup> in 10 × 10<sup>-3</sup> m Tris buffer, pH 8.5) and allowed to proceed for 48 h under stirring at room temperature to forming SiO<sub>2</sub>@PDA composite. The resultant product was washed with ddH<sub>2</sub>O and dried by lyophilization. After that, the as-synthesized SiO<sub>2</sub>@PDA composite (200 mg) was dispersed in ddH<sub>2</sub>O (50 mL), then KAuCl<sub>4</sub> (50 mg) was added and the mixture was stirred for 30 min in an ice bath and the resulting Au nanodots decorated SiO<sub>2</sub>@PDA was collected by centrifugation, and subsequently washed with ddH<sub>2</sub>O and dried by lyophilization. The resulting Au-dispersed SiO<sub>2</sub>@PDA was carbonized at 500 °C for 3 h under Ar atmosphere. Finally, the SiO<sub>2</sub> core was removed by hydrofluoric acid (4%) to obtain AuHCNs. AuHCNs–HA was obtained by coating HA-DA on AuHCNs following the previous protocol<sup>1</sup> with a minor modification. AuHCNs (10 mg) was dispersed in PBS (20 mL, 10 mM, pH 7.4) containing 35 mg HA-DA and stirred at room temperature for 24 h, followed by centrifuging and washing with ddH<sub>2</sub>O for 3 times to remove residual HA-DA. The final product AuHCNs–HA was collected and lyophilized.

In quenching efficiency experiment, FAM-labeled scrambled DNA probe with a fixed concentration of 50 nM was incubated with AuHCNs–HA nanospheres in a series of concentrations (20-150 µg/mL) for 30 min at room temperature. Then, the fluorescence emission intensity at 520 nm was recorded with 488 nm excitation wavelength using a fluorescent microplate reader. The fluorescence quenching efficiency was calculated as  $Q_e = (F_o - F_q)/F_o$ , where  $Q_e$ ,  $F_o$  and  $F_q$  are the fluorescence quenching efficiency, original fluorescence intensity of ssDNA probe and fluorescence intensity of ssDNA probe after quenching by AuHCNs–HA.

The stabilities of ssDNA probe carried by AuHCNs and AuHCNs–HA under DNase I

treatment were investigated through polyacrylamide gel electrophoresis assay. Briefly, ssDNA probe (FAM-labeled at the 5'-end), ssDNA–AuHCNs, and ssDNA–AuHCNs–HA were incubated with DNase I (0.005 U/ $\mu$ L) at 37 °C for 5 and 20 min, respectively. ssDNA probe, ssDNA–AuHCNs and ssDNA–AuHCNs–HA without nuclease treatment were used as control groups. Native polyacrylamide gel (12%) was employed to verify the reaction at 220 V for 90 min. The bands were visualized by Gel Imaging System.

**In Vitro Fluorescence Experiments.** For target detection, ssDNA–AuHCNs–HA nanoplateforms (50 nM FAM-labeled scrambled DNA probe, 30  $\mu$ g/mL AuHCNs–HA) were incubated with different concentrations of target miR-21 from 0.05 to 50 nM. After 2 h incubation at 37 °C in the dark environment, the fluorescence emission intensity at 520 nm of each sample was measured with 488 nm excitation wavelength using microplate reader. The fluorescence intensity exhibits a linear correlation with the logarithm of miR-21 concentration in the range from 0.5 nM to 100 nM. The correlation equation is  $Y = 378.95 + 65.323x$  with a correlation coefficient of 0.987, where  $Y$  is the fluorescence intensity of detection system and  $x$  is the miR-21 concentration (nM). The limit of detection was determined calculated to be 0.68 pM, based on  $3\sigma/K$ , where  $\sigma$  is the standard deviation of the control group and  $K$  is the slope of the linear regression curve.

To investigate the selectivity, ssDNA–AuHCNs–HA nanoplateforms was firstly dispersed in PBS (20 mL, 10 mM, pH 7.4). Then a certain concentration of miR-21-1, miR-21-5p, miR141 and miR-21 stock solution were added into the solution system with a final concentration of 100nM. After 30 min incubation at 37 °C, the measuring processing was as the same as above.

**Cell Culture.** Human colorectal adenocarcinoma cells (SW48, LoVo), human colon epithelial cells (FHC) and mouse fibroblasts (NIH/3T3) were cultured in a Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) at 37 °C in 5% CO<sub>2</sub> atmosphere. Serum-free medium (Opti-MEM) was used during the transfection process.

**Cytotoxicity Assay.** SW48 cells were dispersed in a 96-well plate a density of  $8 \times 10^3$  cells/well. After overnight incubation, new medium containing the AuHCNs–HA (1.56, 3.13, 6.25, 12.5, 25, 50, 100 and 200  $\mu$ g/mL) were added to each well after the culture medium was removed. After incubation for 24 h, the medium was replaced by fresh medium containing CCK-8 reagent. After 2 h incubation at 37 °C, the cell viability was recorded by measuring the absorbance at 450 nm.

**Hemolysis Assay.** To isolate red blood cells (RBCs), 5 mL of human blood sample (from

a health volunteer) anticoagulated with EDTAK<sub>2</sub> was centrifuged at 1000 rpm for 5 min. The supernatant was removed and RBCs were dispersed in PBS (2%, v/v). Then 1 mL of RBCs suspension and 1 mL PBS solution containing different concentration of AuHCNs–HA nanoparticles (25, 50, 100, and 200 µg/mL) were mixed together and incubated at 37 °C for 2 h. The mixture was centrifuged at 3,000 rpm for 5 min and the absorbance of the supernatant was detected at 545 nm using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA). 1% (v/v) TritonX-100 and PBS were used as the positive and negative controls, respectively. The percentage of hemolysis was calculated as  $\text{Hemolysis}\% = (\text{OD}_{\text{sample}} - \text{OD}_{\text{negative}}) / (\text{OD}_{\text{positive}} - \text{OD}_{\text{negative}}) \times 100\%$ , where  $\text{OD}_{\text{sample}}$ ,  $\text{OD}_{\text{negative}}$ , and  $\text{OD}_{\text{positive}}$  are the absorbance intensities of the sample, PBS, and TritonX-100, respectively.

**Analysis of Cellular Uptake.** To investigate the effect of HA modification on cellular uptake of nanoplatforms, two different kinds of cell lines (LoVo and SW48) were seeded in 6-well plates with a density of  $3 \times 10^5$ /well. The cells were incubated with AuHCNs and AuHCNs–HA (100 µg/mL) at 37°C for 12 h. Then the cells were washed with PBS three times, trypsinized and collected with centrifugation. After collection, newly prepared aqua regia (2 mL) was added to the cells, and the solution was boiled for 12 h. The samples were diluted with ddH<sub>2</sub>O to 10 mL, and the concentration of Au ions was determined using ICP-MS.

**Confocal Fluorescence Imaging.** All cell lines were cultured in DMEM medium. For endogenous miR-21 expression monitoring, SW48 cells and LoVo cells were first seeded into confocal dishes (35 mm) and cultured overnight at 37 °C and 5% CO<sub>2</sub> environment. Then, prepared ssDNA–AuHCNs–HA nanoplatfrom solution (50 nM FAM-ssDNA, 30 µg/mL AuHCNs–HA) were added into the confocal dishes. After incubation, the cells were washed twice with PBS to remove the excellular nanoparticles. Then fluorescence images were collected using confocal microscope. The excitation laser was set at 488 nm, and the green fluorescence signal was collected within the range of 505-540 nm under the same conditions. Cell images in bright field, fluorescence, and overlap of the two fields were captured and analyzed. Cell fluorescence images were then analyzed with ImageJ software.

To determine the expression levels of miR-21 in different cells, SW48 cells, LoVo cells, HCT116 cells and NIH/3T3 cells were prepared. All cells were incubated with prepared nanoprobe solution (50 nM FAM-ssDNA, 30 µg/mL AuHCNs–HA) for 12 h, then washed and imaged.

For investigation of the various levels of miR-21 in living cells, SW48 cells were cultured overnight before use. Then the cells were incubated with different concentration

of miR-21 using Lipofectamine™ 2000 transfection reagent to increase the intracellular miR-21 levels. After transfection, the cells were incubated with prepared nanoprobe solution (50 nM FAM-ssDNA, 30 µg/mL AuHCNs-HA) for 12 h, then washed and imaged.

**RT-qPCR.** Total cellular RNA samples were extracted from SW48 cells, LoVo cells, HCT116 cells and NIH/3T3 cells respectively, using Trizol reagent according to the manufacturer's instructions. The cDNA synthesis was carried out by using the reverse transcription (RT) reaction with RevertAid Reverse Transcriptase (Thermo Scientific, USA). qPCR analysis of miRNA was performed with AceQ qPCR SYBR Green Master Mix (Vazyme biotech Co. Ltd., China) in a StepOne Plus (Applied Biosystems, USA). The primers used in this experiment were listed as follows:

miR-21 stemloop 5'-  
GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATAC  
GACTCAACA -3',

miR-21 forward 5'- TAGCTTATCAGACTGATG -3',

miR-21 reverse 5'- GTGCAGGGTCCGAGGT -3',

U6 stemloop 5'-  
GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGA  
CAAAATA -3',

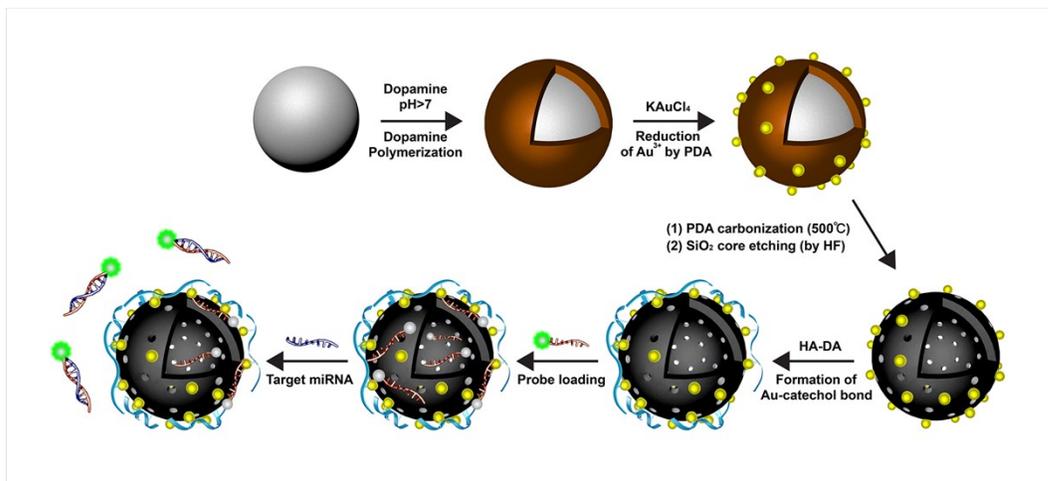
U6 forward 5'- GCTTCGGCAGCACATATACTAAAAT -3',

U6 reverse 5'- CGCTTCACGAATTTGCGTGTCAT -3'

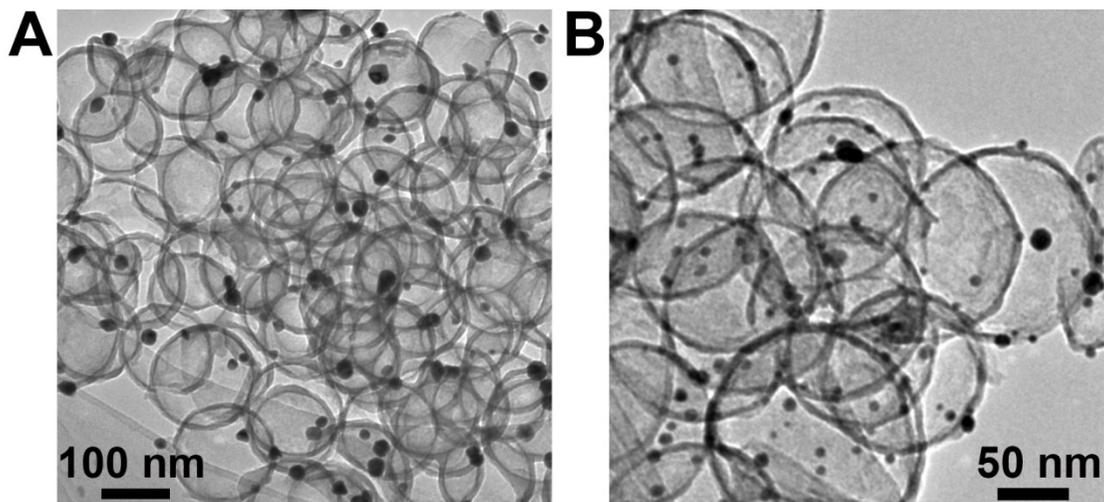
**Quantification of miR-21 in Transfected Cells.** The miR-21 in the transfected SW48 cells was extracted using Trizol reagent according to the manufacturer's instructions. Then the amount of miR-21 was quantified with the TaqMan® Small RNA Assay and ABI StepOne Plus Detector System.

**Flow cytometry analysis.** SW48 cells, LoVo cells, HCT116 cells and NIH/3T3 cells were separated from culture plate by Trypsin-EDTA solution. After centrifuge at 2000 rpm for 5 min and washing with PBS two times, the cells were resuspended in 100 µL PBS with FITC-labeled CD44 antibody (0.5 µg) for 20 min. After washing with PBS for three times, the cells were subjected to flow cytometry analysis with a 488 nm excitation laser.

## SUPPLEMENTARY RESULTS



**Figure S1.** Schematic of the preparation process of DNA–AuHCNs–HA nanoplateforms and the working mechanism for miRNA detection.



**Figure S2.** TEM micrograph of AuHCNs.

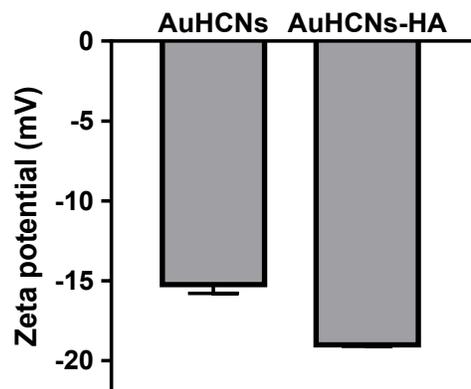


Figure S3. Zeta potential of AuHCNs and AuHCNs-HA.

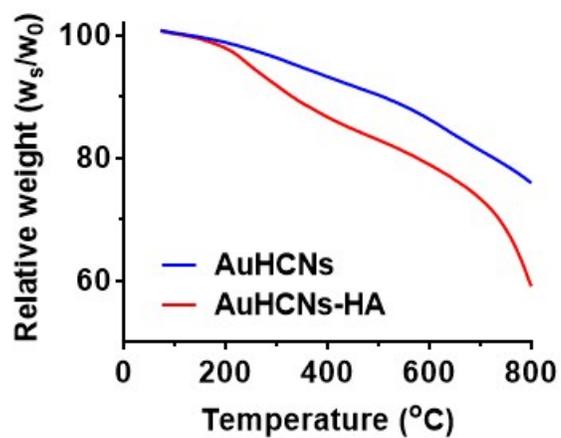
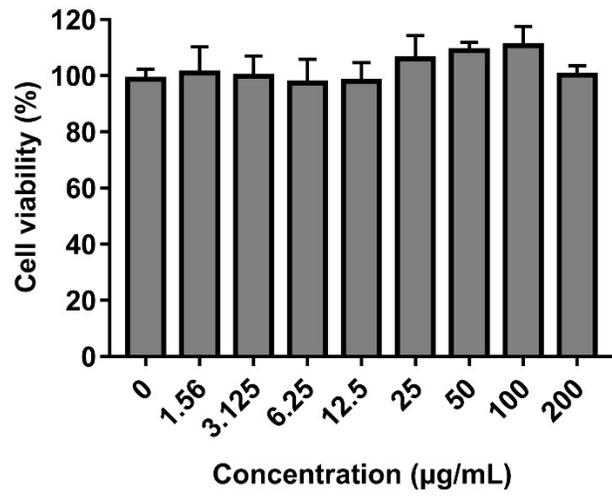
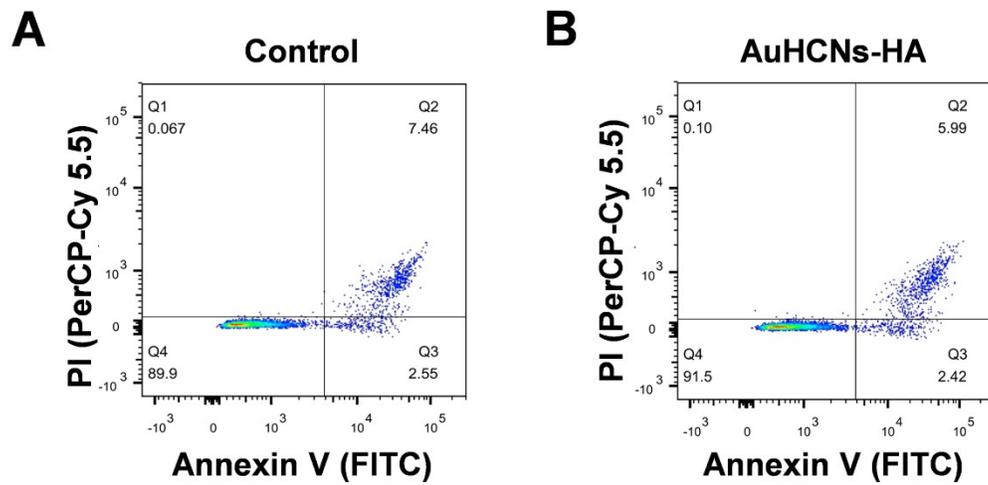


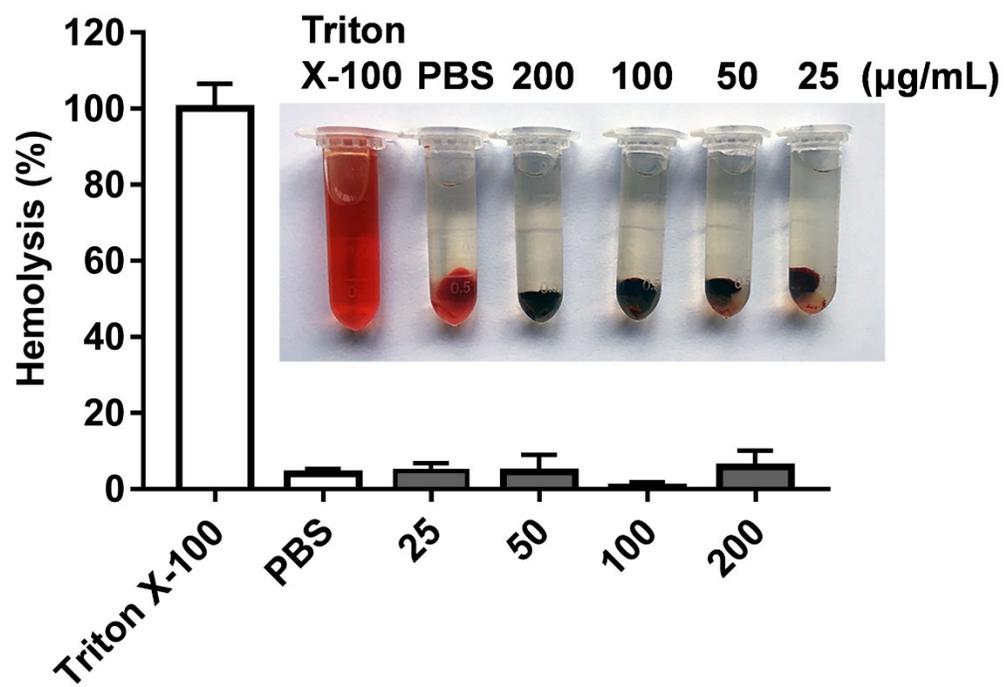
Figure S4. Thermogravimetric analysis (TGA) of AuHCNs and AuHCNs-HA.



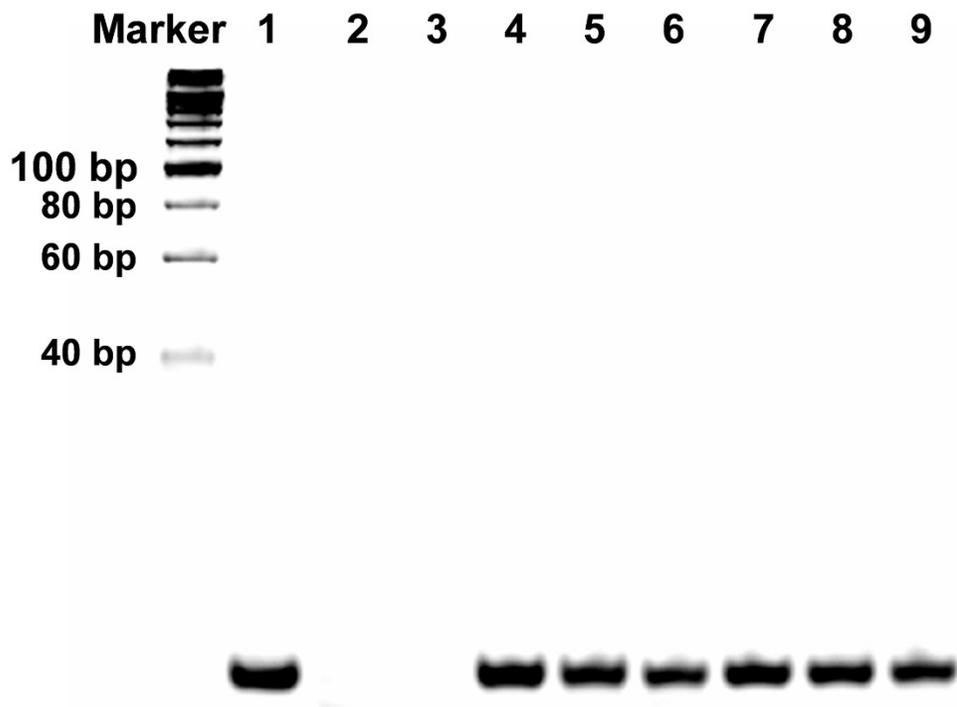
**Figure S5.** Cytotoxicity of AuHCNs–HA in SW48 cells.



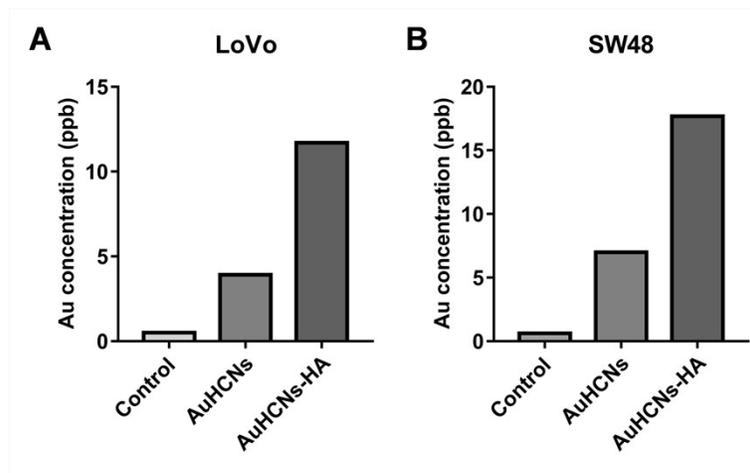
**Figure S6.** Flow cytometric analysis of apoptosis of SW48 cells and AuHCNs–HA treated SW48 cells.



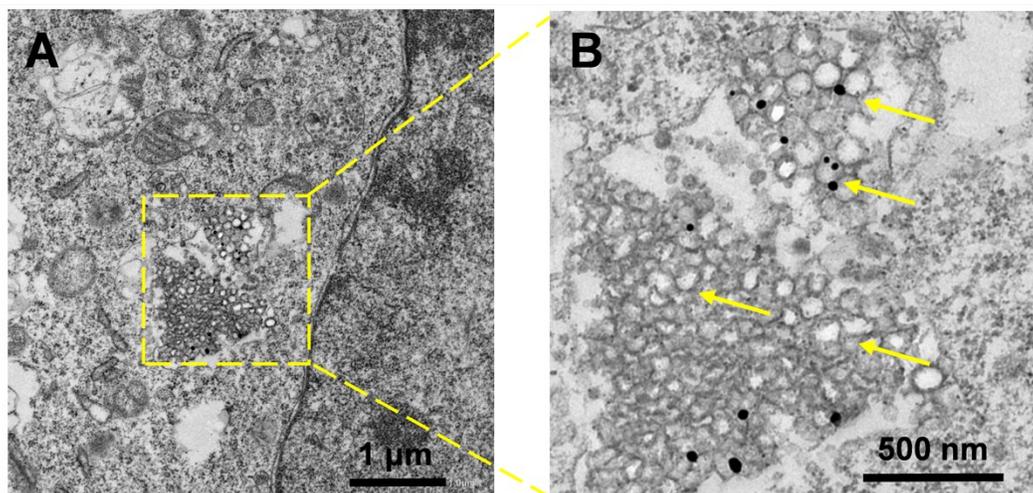
**Figure S7.** Hemolysis activity of AuHCNs-HA.



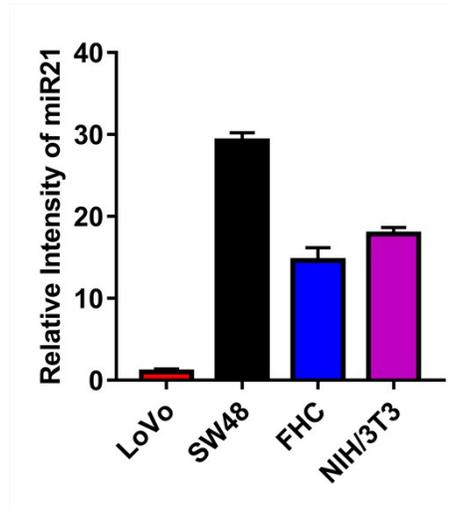
**Figure S8.** Gel electrophoresis of ssDNA, ssDNA–AuHCNs, and ssDNA–AuHCNs–HA with and without DNase I treatment. lanes 1–3: ssDNA without DNase I, ssDNA treated with DNase I for 5 and 20 min; lanes 4–6: ssDNA–AuHCNs–HA without DNase I, ssDNA–AuHCNs–HA treated with DNase I for 5 and 20 min; lanes 7–9: ssDNA–AuHCNs without DNase I, ssDNA–AuHCNs treated with DNase I for 5 and 20 min..



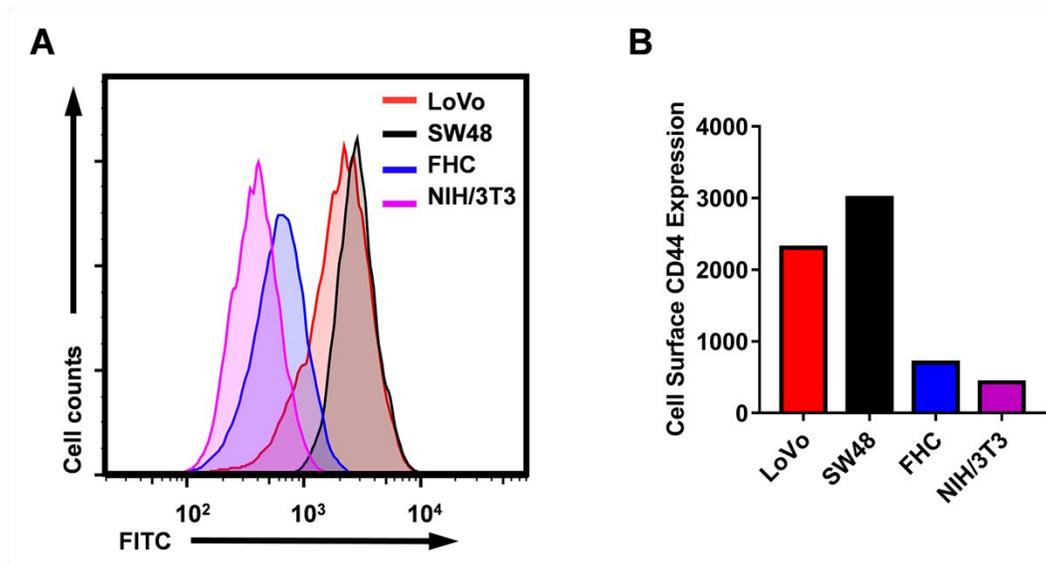
**Figure S9.** Cellular uptake of AuHCNs and AuHCNs-HA in CD44-positive LoVo (A) and SW48 (B) cells.



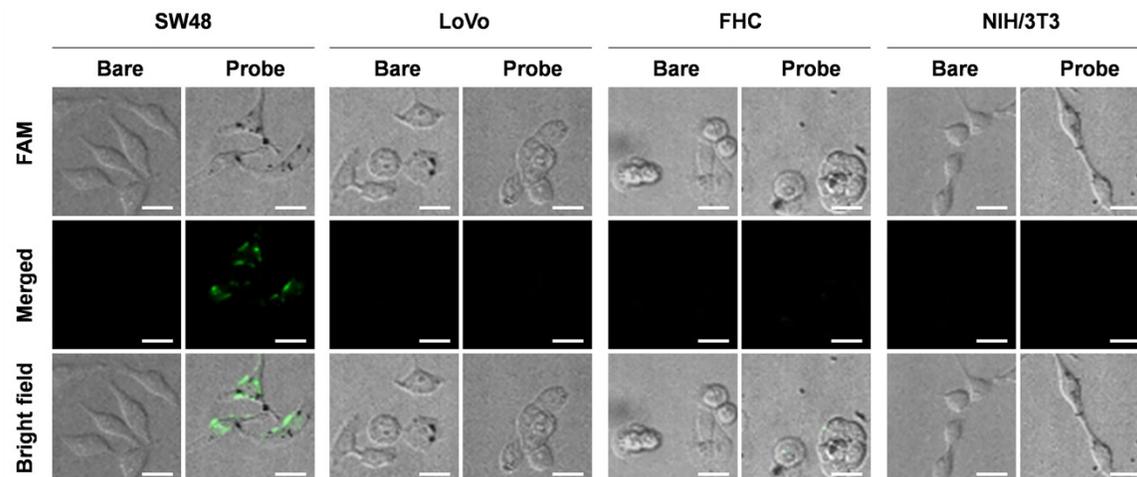
**Figure S10.** TEM images of cellular uptake of DNA-AuHCNs-HA. (A) TEM micrographs of SW48 cells incubated with DNA-AuHCNs-HA for 24 h. (B) is the enlargement of boxed area of (A).



**Figure S11.** RT-PCR results of miR21 expression for different cells.



**Figure S12.** Flow cytometry analysis (left) and the corresponding quantification (right) of cell surface CD44 expression.



**Figure S13.** Confocal microscopy images of LoVo cells, SW48 cells, FHC cells and NIH/3T3 cells incubated with ssDNA–AuHCNs–HA for 12 h. All scale bars are 20  $\mu\text{m}$ .

## Reference

1. L. Xu, J. Liu, J. Xi, Q. Li, B. Chang, X. Duan, G. Wang, S. Wang, Z. Wang and L. Wang, *Small*, 2018, 14, 1800785.