Supporting Information

Unraveling cell type-specific targeted delivery of membrane-camouflaged nanoparticles with plasmonic imaging

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Experimental Sections

Materials The 40 nm gold nanoparticles (AuNPs) were purchased from BBI solutions. The HeLa (human cervical cancer), A549 (human lung cancer), and MCF7 (human breast cancer) were obtained from the Type Culture Collection of the Chinese Academy of Sciences. The human umbilical mesenchymal stem cells (HUMSC) were purchased from Huaya stem cell corporation (Chongqing, China). MEM, RPMI1640, FBS were purchased from Invitrogen. DMEM and Ultroser G serum substitute for HUMSC culture were purchased from Lonza and PALL respectively. The DiI fluorescent dye for membrane staining was purchased from the Beyotime company (Suzhou, China). All other reagents, unless otherwise specified, were purchased from Sigma-Aldrich.

Preparation of membrane coated AuNPs Cell membrane materials were collected according to a previously reported approach[1]. Briefly, 1×10^9 cells were harvested and resuspended in ice-cold hypotonic lysing buffer (20 mM Tris-HCl pH = 7.5, 10 mM KCl, 2 mM $MgCl_2$) supplemented with 1 EDTA-free mini protease inhibitor tablet (Pierce). For the synthesis of AuNP@CCM^{Trypsin}, 0.25% Trypsin-EDTA solution were added to digest cells at 37 ℃ for 30 seconds before membrane collection. The solution was then added to a tissue grinder with tight-fitting pestle (Kimble) to disrupt cells, following extrusion, centrifugation and purification. Red blood cell membrane materials were collected from male SD mice (6-8 wks, Shanghai SLAC Laboratory) by hypotonic methods[2]. For liposome synthesis, the lipid in chloroform solution was mixed to have 85% dioleoylphosphatidylcholine (DOPC) and 15% dioleoylphosphatidylserine (DOPS) (w/w). The lipid mixture was evaporated to be film and thoroughly dried under a stream of nitrogen. The dried mixture was resuspended in the TM buffer (50 mM Tris HCl, 10 mM magnesium sulfate at pH 7.5) and followed by three repetitive freeze-thaw cycles. The total lipid concentration was 2 mg/mL. The lipid solution was kept at 4 ℃ until use. The cell membrane derived vesicles or synthesized liposomes were then co-extruded with 40 nm AuNPs through a

400 nm porous polycarbonate membrane for 5 - 10 times to achieve the coated particles. Then the mixture was centrifuged at 6000 *g* for 10 minutes and resuspended in the PBS buffer (pH=7.4) for purification. Note that AuNP@CCM should be freshly prepared before incubation with cells. The concentration of gold nanocores was determined by UV-Vis spectra absorbance[3].

Cell culture Cancer cells were cultured in MEM (HeLa) or RPMI 1640 (A549, MCF7) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin and cultured at 37 ℃ in a humidified atmosphere of 5% CO_2. HUMSC were grown in serum-free medium (DMEM) supplemented with Ultroser G serum substitute.

Cellular uptake Cells were seeded into glass bottom dish with 20 mm well (CellVis) to achieve approximately 50% confluency. After the cells fully attached to the glass surface, the fresh medium mixed with freshly synthesized nanoparticles at a final concentration of 50 pM was added. After incubation at 37 ℃ for indicated time, cells were washed twice in PBS and fixed with 4% paraformaldehyde solution (PFA) at room temperature for 10 minutes.

Western blotting HeLa, A549, MCF7 and HUMSC cells were extracted with RIPA lysis buffer (moderate, Beyotime) and protein concentrations were determined using a BCA assay kit (Beyotime). Samples with equal amount of protein were separated by SDS-PAGE and transferred to PVDF membranes. Expression level of integrin $\alpha_v\beta_3$ was detected using a primary antibody (D2N5H, CST), followed by fluorescent secondary antibody and imaged using an Odyssey CLx Imaging System. An anti-β-actin antibody (D6A8, CST) was included as control.

Fluorecence flow cytometry HeLa, A549, MCF7 and HUMSC cells were incubated with anti-integrin $\alpha_v\beta_3$ antibody (LM609, Abcam) for 1 hour and then fixed with 4% PFA before analysis by FACSCalibur (BD Biosciences).

Integrin inhibition HeLa and HUMSC cells were treated with 1 mM RGD[4] (sigma) or 100 nM Cilengitide[5] (Selleck) incubation with 50 pM of AuNP@CCMs or bare AuNP. After 1 hour, cells were examined using a DFM. To downregulate the expression of integrin $\alpha_v\beta_3$, cells were transfected with targeting siRNA (sc-29373, Santa Cruz Biotechnology) using lipofectamine 3000 (Invitrogen) 24 hours before cellular uptake experiments.

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) 20,000 cells were seeded in 6 wells plates (Coring) and cultured overnight. After treatment, cells were washed for

three times before collection. Cell pellet was digested with aqua regia (HCl: HNO_3=3:1). Gold content in the lysates was measured using an Optima 8000 ICP-AES spectrometer (PerkinElmer). Cellular uptake was normalized to cell number and reported as nanograms of gold per 1000 cells.

Cell imaging Confocal images were recorded with a Lecia SP8 confocal microscope and collected with a HC×PL APO 63×, 1.4 NA oil-immersion objective. For DFM imaging, images were acquired by an Olympus IX71 inverted microscope equipped with a 60× 0.5-0.9 NA oil-immersion objective, an Olympus DP70 camera, a 100 W halogen lamp, a NKT supercontinuum laser and optical filters.

Correlative dark field and scanning electron microscope (SEM) imaging A microslide glass (thickness, 1 mm) was cut to the size of 1.5 cm × 0.4 cm. The piranha solution (H_2SO_4:H_2O_2 = 3:1, v/v) was used to clean the glass and enhance its hydrophilic property. Then 20 μL AuNP@CCMs (2 pM) in ultra-pure water was added to the glass for 20 min. The glass with sample was then dried using nitrogen gas. A mark was made at the center of glass with a PILOT ultra-thin marker pen. DFM images and scattering light spectrum of sample on the dry glass near the mark were acquired with a 60×, 0.7 NA dry objective and a triple grating monochromator (Acton SP2300, Princeton Instruments). The glass surface was then sprayed by gold to enhance the electric conductivity (3 mA for 30 s, sputter coater, Quantum Design). The ex-situ SEM images of the field that was close to the mark were acquired at the EHT at 10 kV (LEO, 1350vp).

Image analysis A workflow shown in Figure S3 was used to analyze DFM images automatically. The first step is to find particles inside cell zone based on the built-in function "Find Maxima" of ImageJ software (version 1.52p), which will return the locations of each local maxima meeting preset parameter (prominence>10). Next, with the locations, the RGB values of each maxima could be extracted and transformed into HSI color coordinates according to equations below. As the color of AuNPs changed from green to yellow when they aggregated, it was simple to distinguish AuNP from cell background by setting color threshold (hue < 150, saturation > 0.1, intensity > 30) and classify the type of plasmonic signal with hue value (single > 70, cluster < 70). Further counting analysis on single and cluster on single cell level were based on the data frame exported by the workflow. A demo consisted of ImageJ macro scripts and single cell image were available in the supplementary data frame files.

$$H = \frac{360}{\pi} \times arccos\left(\frac{(R-G)+(R-B)}{2 \times \sqrt{(R-G) \times (R-G) + (R-B) \times (G-B)}}\right)$$

$$S = 1 - \frac{3 \times min(R,G,B)}{R+G+B}$$

$$I = \frac{R+G+B}{3}$$

Figures

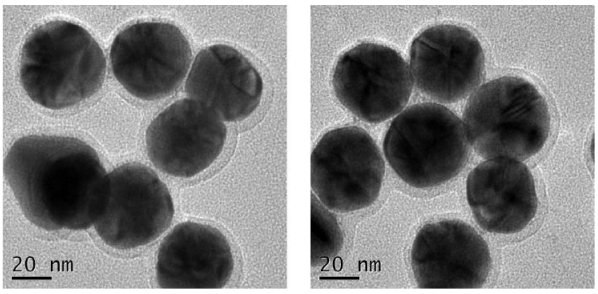

Figure S1. Representative TEM images of AuNP@CCMs, scale bar represents 20 nm.

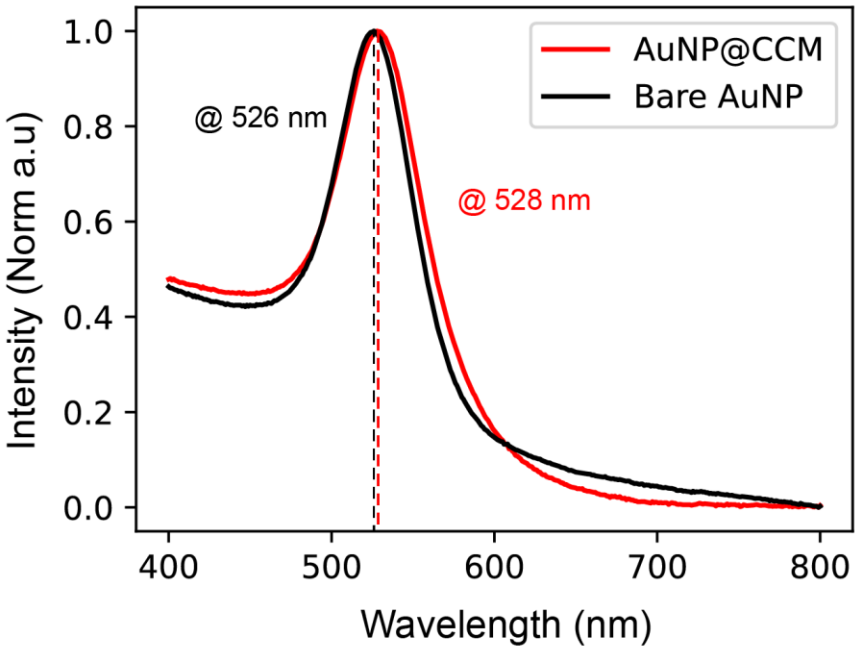

Figure S2. Absorbance spectrum of gold nanoparticles before (black) and after (red) cell membrane coating.

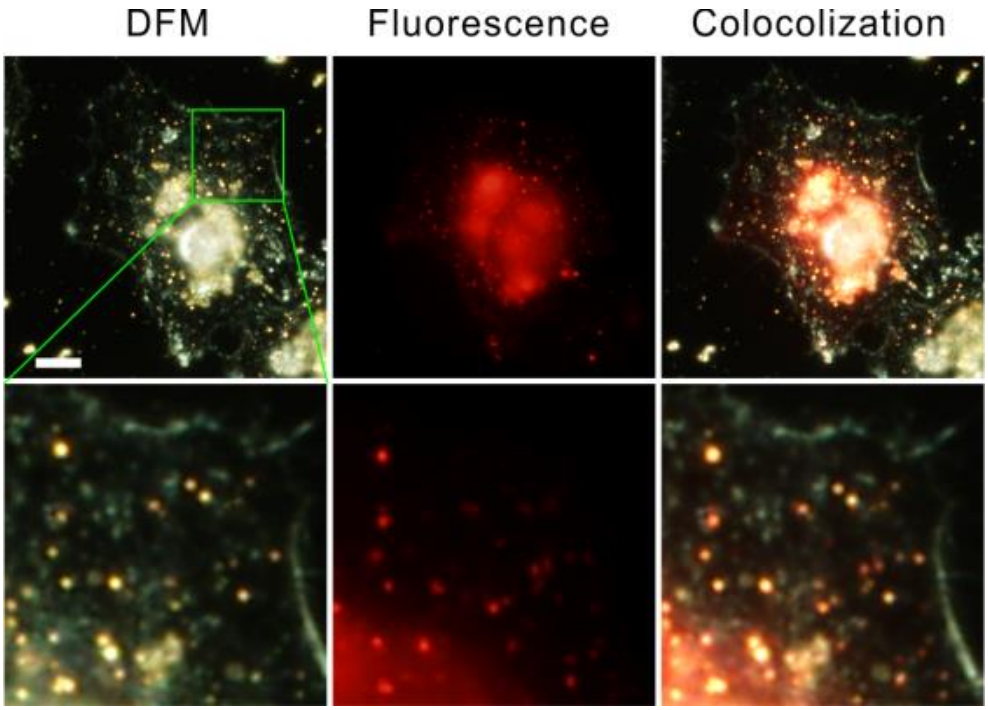

Figure S3. Representative DFM and FM images of intracellular AuNP@CCMs in the same field of view taken with a correlative microscope. Bottom: Enlarged view of the area in the green rectangles. Scale bar represents 5 μm.

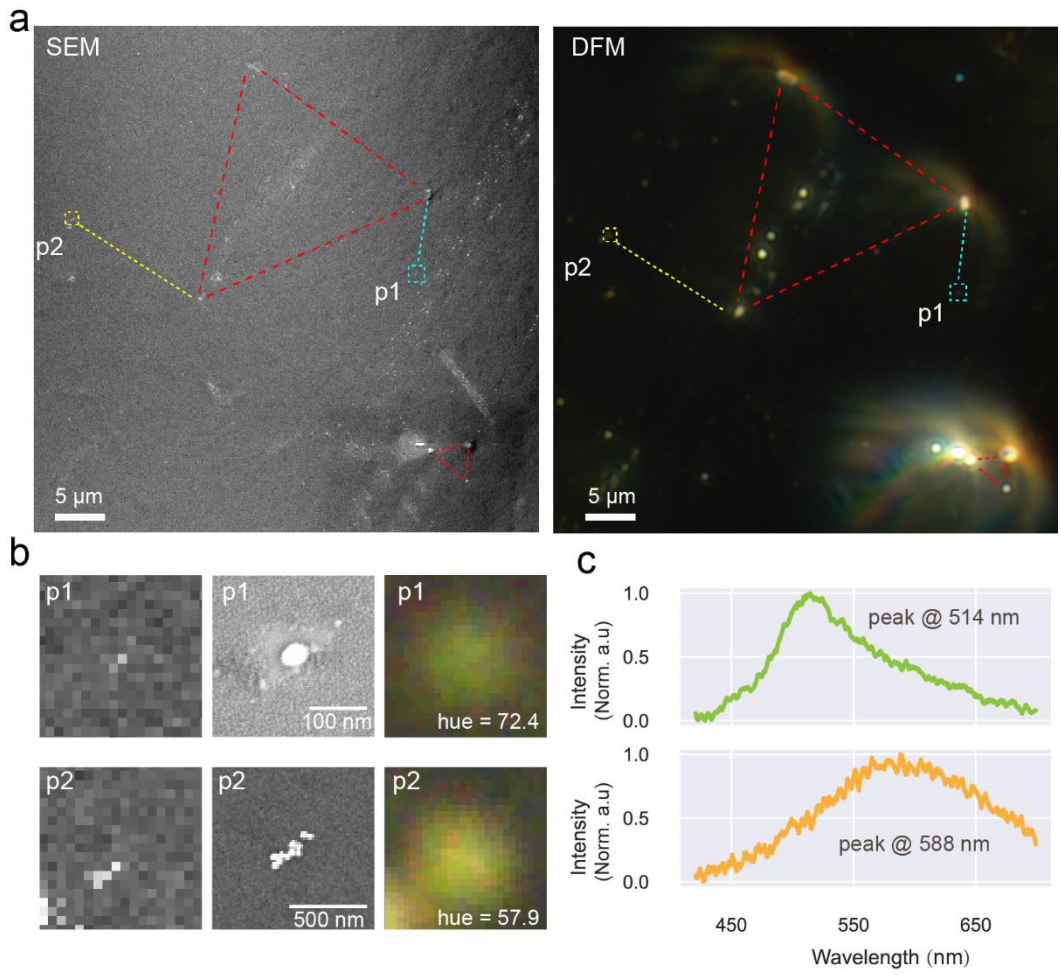

Figure S4. Correlative DFM/SEM imaging of AuNP@CCMs. (a) The SEM and DFM images of AuNP@CCMs in the same view. Two triangles for ex-situ localization are indicated in red dash line, the single (p1, in cyan rectangle) and the clustered (p2, in yellow rectangle) AuNP@CCMs are annotated. Scale bar is 5 μm. (b) The enlarged view of SEM and DFM images showing p1 and p2, high-resolution SEM images are shown in the middle column, scale bars are 100 nm for p1 and 500 nm for p2. (c) The scattering light spectrum of p1 (the upper, green line) and p2 (the bottom, orange line).

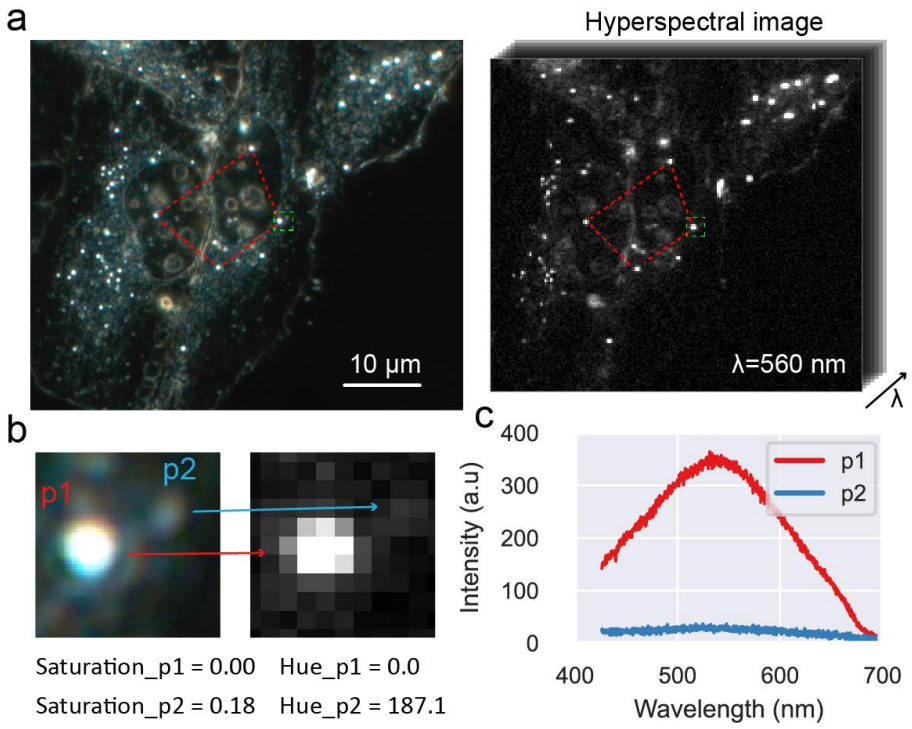

Figure S5. Grey spots represent background noise. (a) The DFM image and hyperspectral image of untreated HeLa cells. Four feature points are linked by red dash lines for colocalization. The hyperspectral layer at center wavelength (λ=560 nm, Δ λ =0.272 nm) is presented, Scale bar is 10 μm. (b) The enlarged view of the green rectangle in (a). Two representative spots are marked as p1 and p2. The hyperspectral image (right) is correlated to the dark field spots. (c) The scattering light spectrum of p1 (red line) and p2 (blue line).

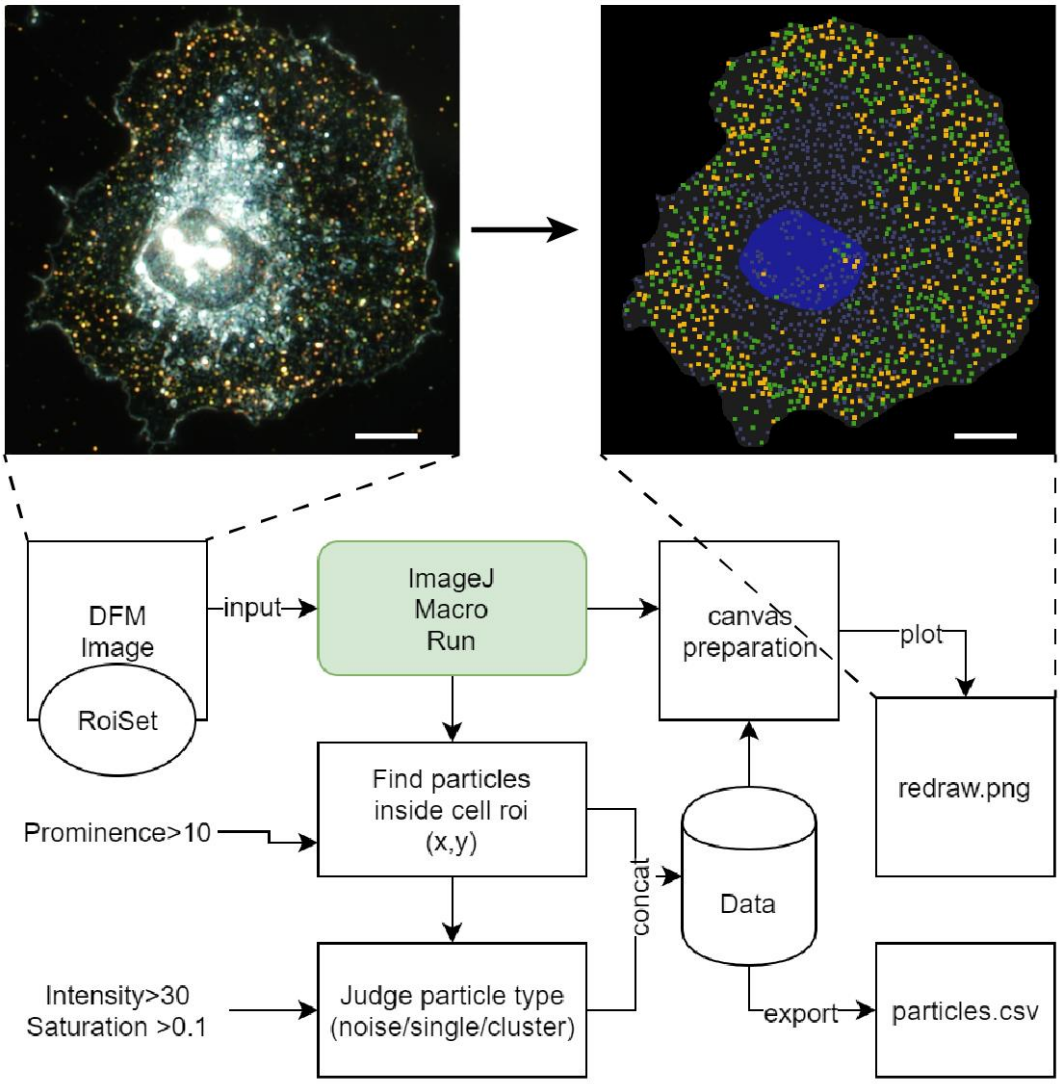

Figure S6. Workflow for data analysis. A typical dark-field image of single cell (scale bar=5 μm) and attached ROI (region of interest) records were input into the ImageJ software. Run the ImageJ macro to start the following procedures, including 'Find particles' and 'Judge particle type'. The particle location and type information were then concatenated into data. Based on the data, the original dark-field image could be transformed into the pseudo-color picture (redraw.png), where the cell nucleus is blue, cell plasma is grey and particles are in small rectangle of colors dependent on their type (single: green, cluster: yellow, noise: bluish grey). For further statistics, the data could be exported to data frame file (particles.csv).

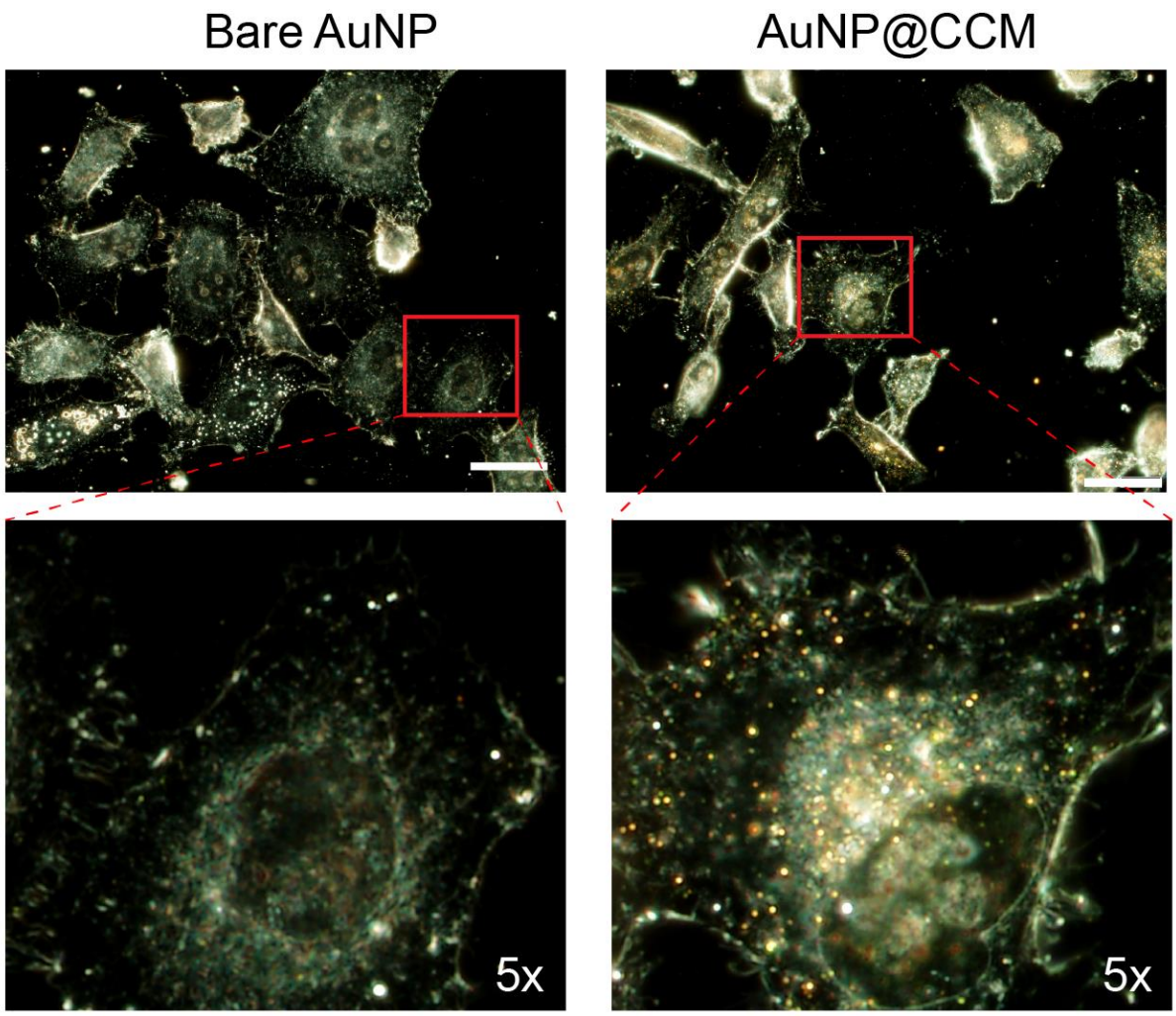

Figure S7. Representative DFM images of HeLa cells incubated with bare AuNPs or AuNP@CCMs for 2 hours at a final concentration of 50 pM. Scale bar is 20 μm.

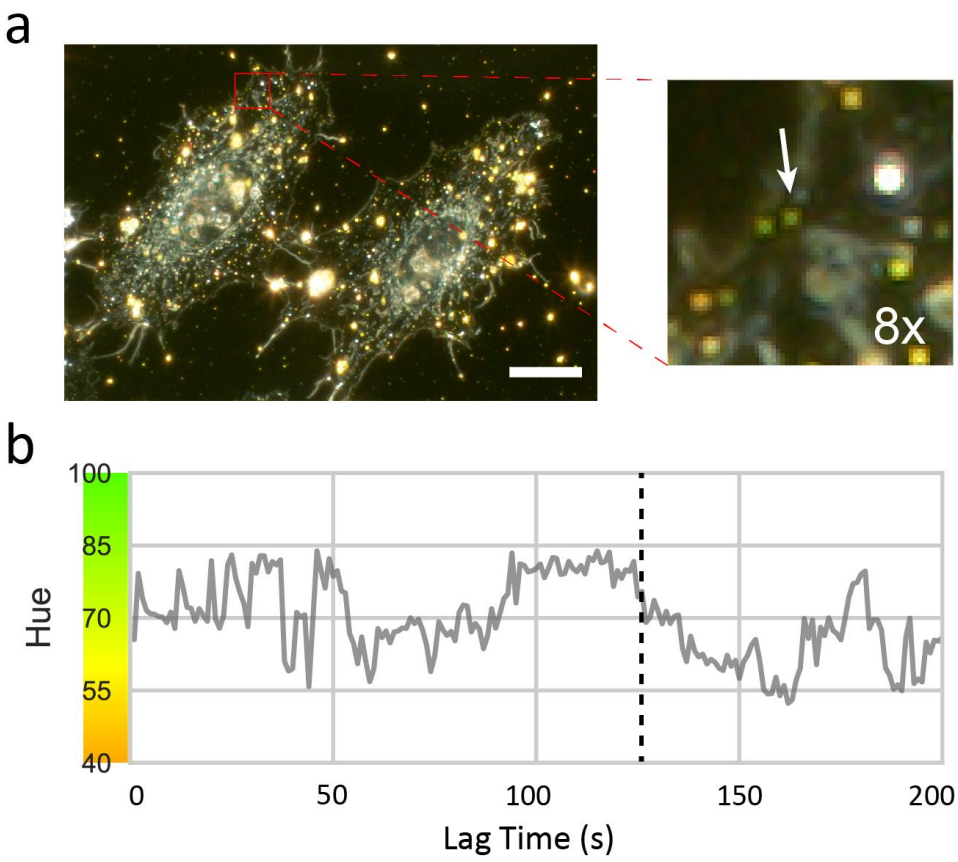

Figure S8. (a) A snapshot of the time-lapse imaging of the agglomeration process of two single AuNP@CCMs. Scale bar is 10 μm. (b) The hue value of the signal of selected spot (indicated with a white arrow) over time. At 125 seconds (marked by a dash line), the hue value decreased into the yellow color range (hue<70).

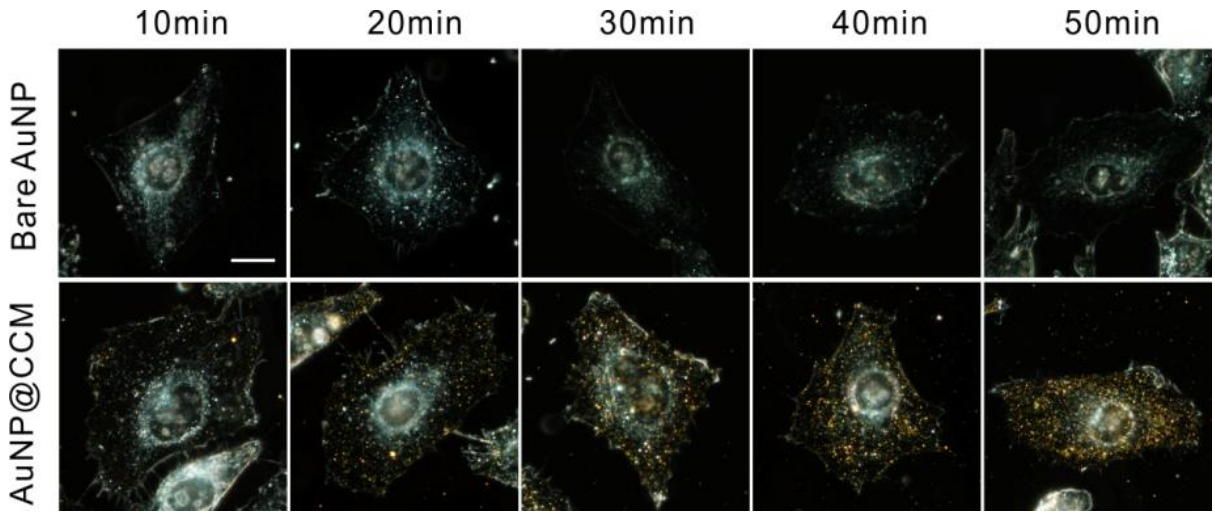

Figure S9. Representative DFM images of HeLa cells incubated with bare AuNPs or AuNP@CCMs for indicated time. Scale bar is 10 μm.

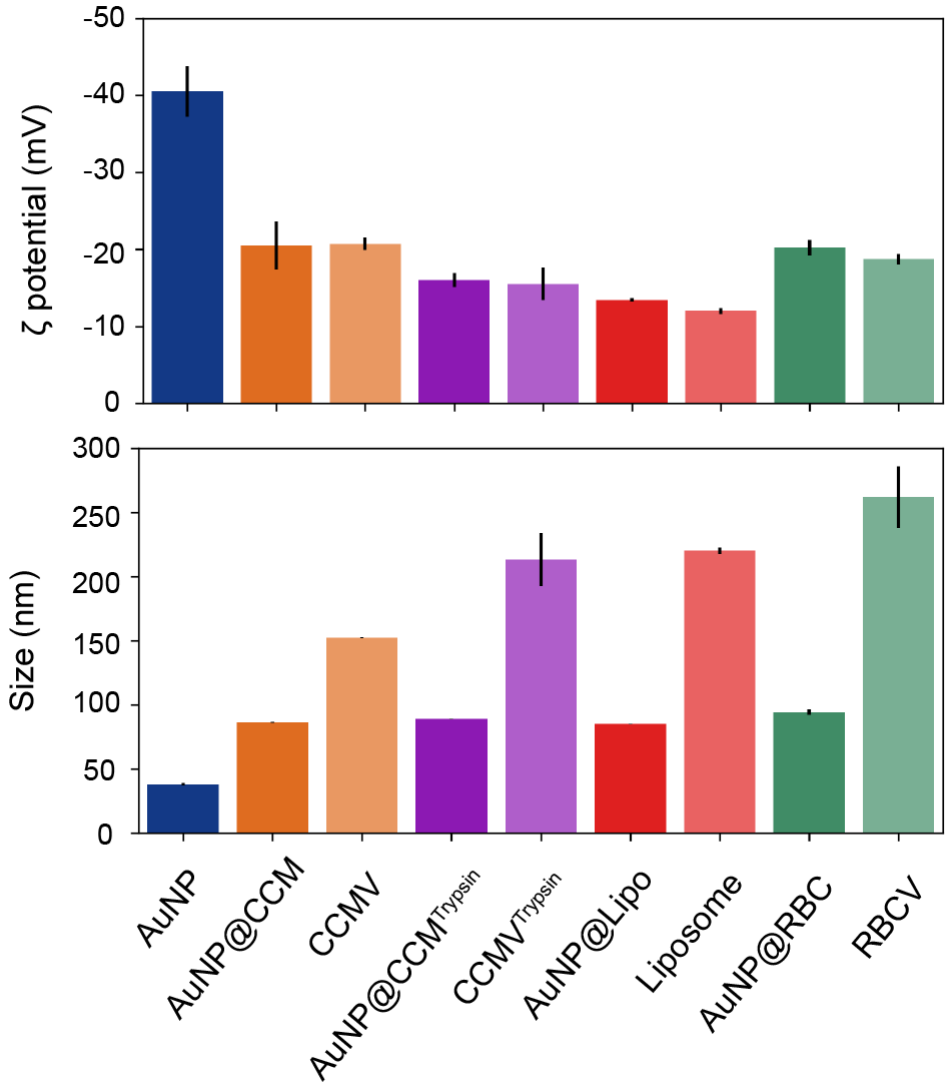

Figure S10. (a) Zeta potential and hydrodynamic size of AuNPs, liposome, cell membrane-derived vesicles, trypsin-treated vesicles, and AuNPs with indicated coating: AuNPs coated with HeLa membrane (AuNP@CCM), HeLa cell membrane derived vesicles (CCMV), AuNPs coated with trypsin-treated CCM (AuNP@CCM^{Trypsin}), trypsin-treated CCMVs (CCMV^{Trypsin}), AuNPs coated with liposomes (AuNP@Lipo), red blood cell membrane derived vesicles (RBCV), AuNPs coated with RBC membrane (AuNP@RBC). Bars represent means ± SD (n=3).

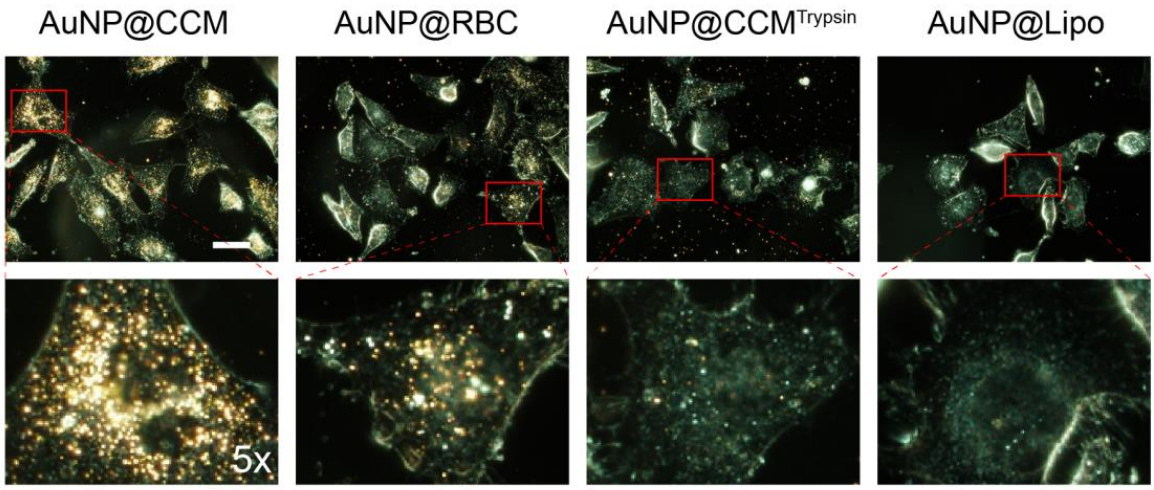

Figure S11. Representative DFM images of HeLa cells incubated with indicated particles. Scale bar is 20 μm.

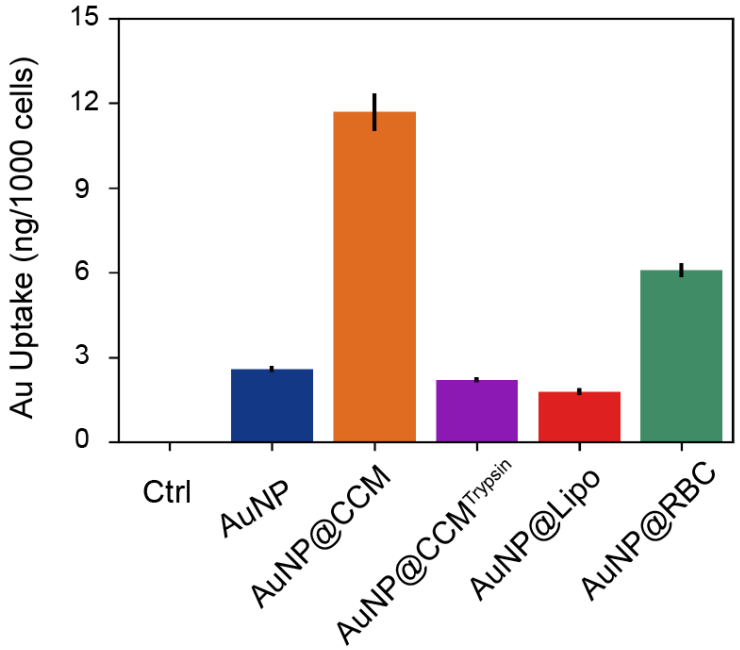

Figure S12. Cellular uptake of AuNPs with indicated surface fabrications are determined by ICP-AES measurements. Cells were incubated with 50 pM materials for 4 hours, Bars represent means ± SD (n=3).

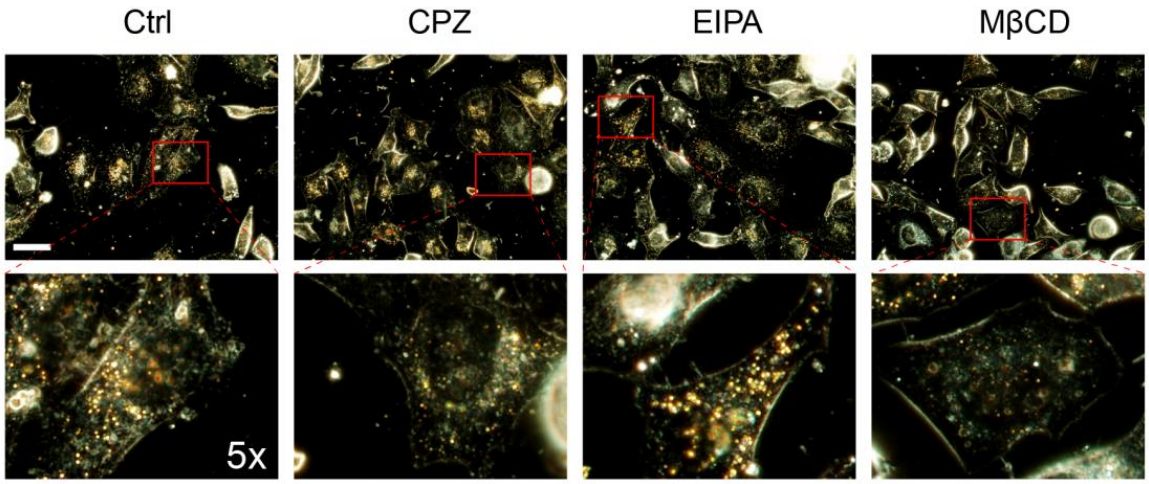

Figure S13. Representative DFM images for HeLa cells pretreated with indicated inhibitors before incubation with 50 pM AuNP@CCMs. Scale bar is 20 μm.

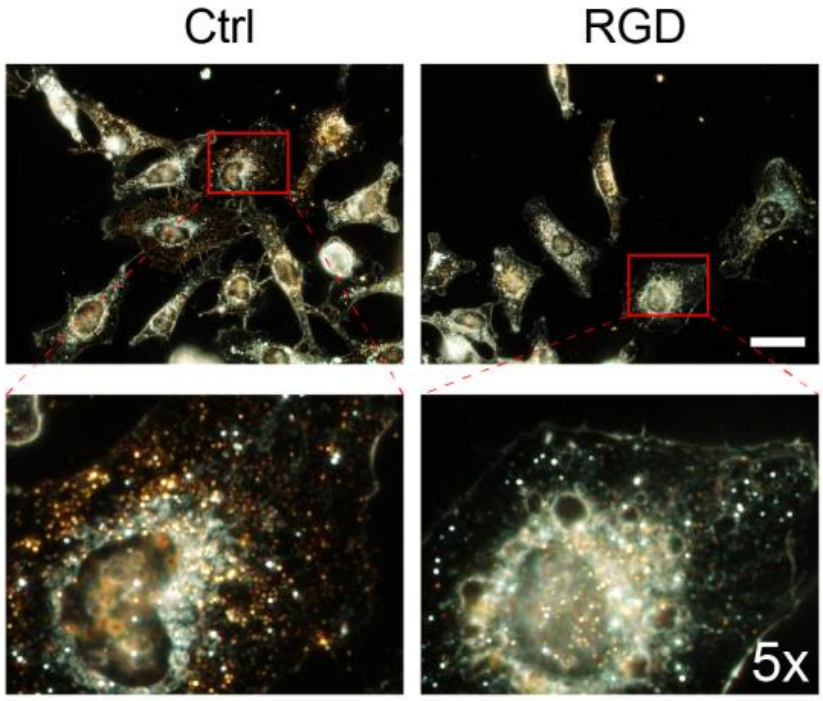

Figure S14. Representative DFM images for HeLa cells pretreated with RGD before incubation with 50 pM AuNP@CCMs for 2 hours. Scale bar is 20 μm.

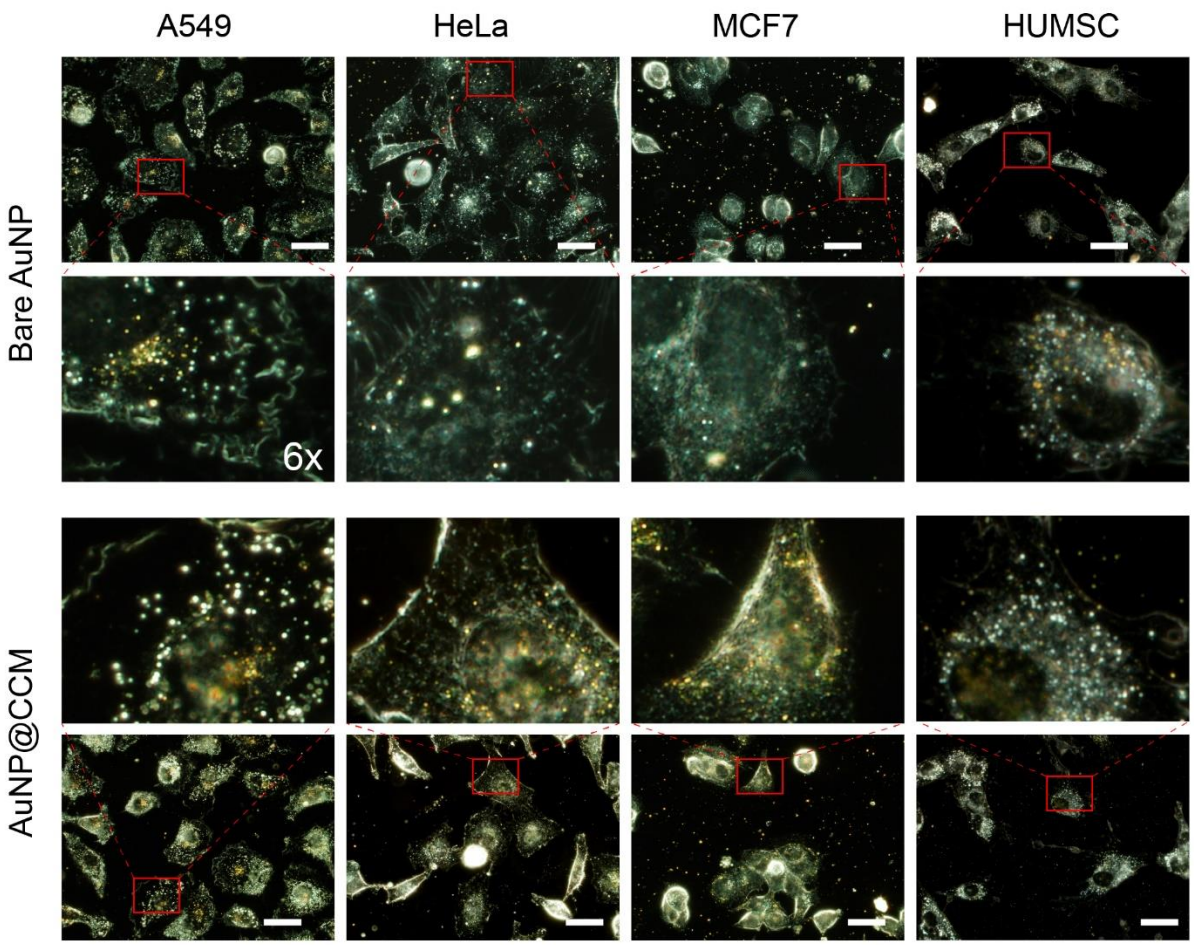

Figure S15. Representative DFM images showing cellular uptake of AuNPs and AuNP@CCMs (50 pM) in A549, HeLa, MCF7 and HUMSC cells. Incubation time is 4 hours. Scale bar is 20 μm.

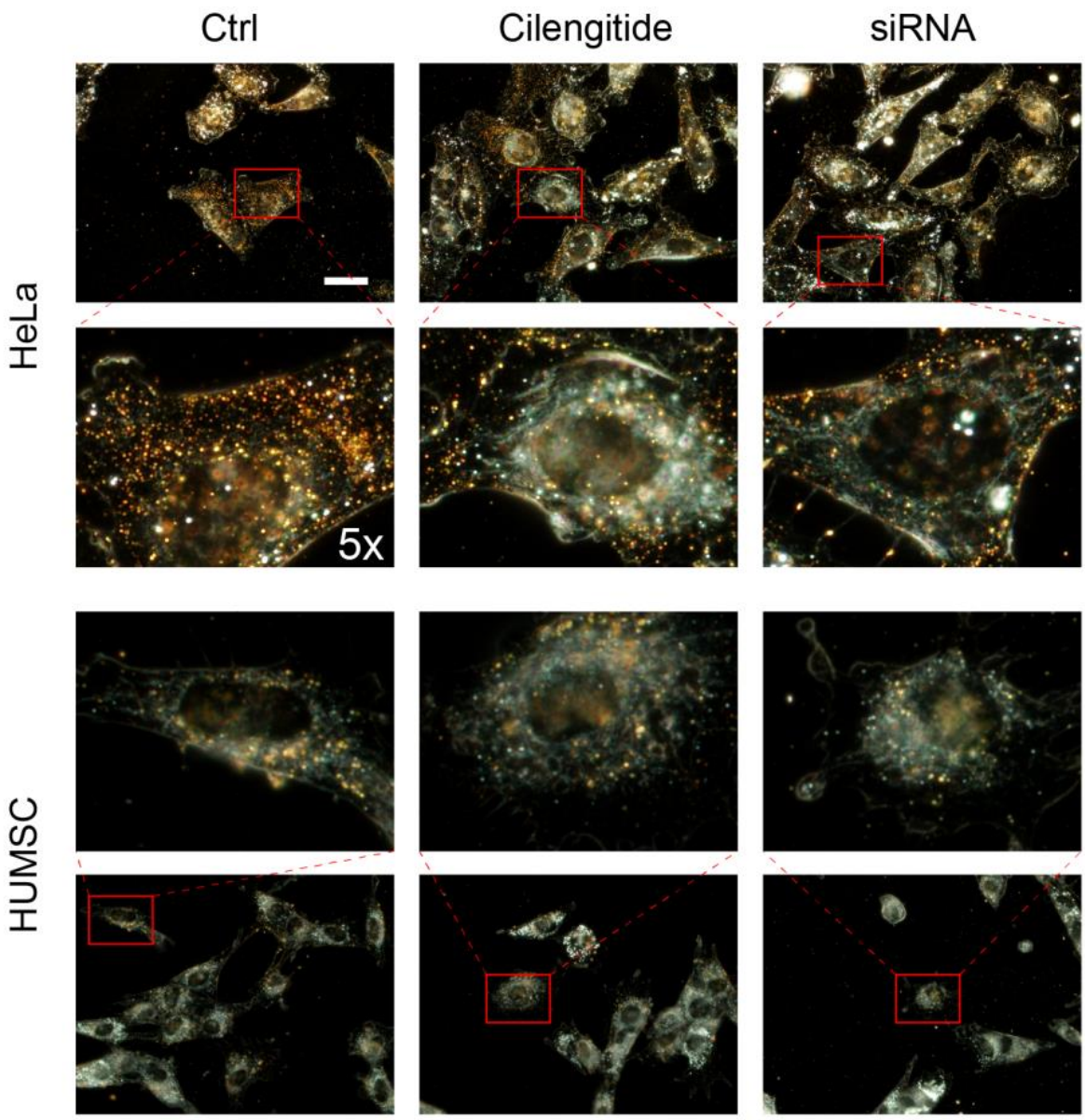

Figure S16. Representative DFM images of cellular uptake of AuNP@CCM (50 pM) by HeLa and HUMSC cells pretreated with cilengitide or siRNA against Integrin $\alpha_v\beta_3$. Scale bar is 20 μm.

Reference

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