Electronic Supporting Information

for

Aptamer modified-selenium nanoparticles for dark-field microscopy imaging of nucleolin

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Materials and methods

Materials. Selenium dioxide (SeO_2) is obtained from Aladdin Reagent Co., Ltd (Shanghai, China). Polyvinylpyrrolidone (PVP, MW=55000) and streptavidin (SA) are purchased from Sigma-Aldrich (USA). Ascorbic acid (Vc) is purchased from Dingguo Changsheng Biotechnology Co. Ltd. (Beijing, China). Bovine serum albumin (BSA) is purchased from Shanghai Biology Products Institute (Shanghai, China). Anti-NCL-specific siRNA (sc-29230), control siRNA-A (sc-37007), siRNA Transfection Reagent (sc-29528) and siRNA Transfection Medium (sc-36868) are from Santa Cruz Biotechnology (Shanghai, China). All aptamers are synthesized and purified through HPLC by Shanghai Songon Biotech. Specific sequences of aptamers are listed in Table S1. All water used is ultrapurified with an LD-50G-E Ultra-Pure Water System (Lidi Modern Waters Equipments Co., Chongqing, China).

Table S1 Aptamers sequences

Aptamer for in vitro tests	Sequences (5' to 3')
Apt0	Bio-GGTGGTGGTGGTTGTGGTGGTGGTGG
Apt1	Bio-(T6)GGTGGTGGTGGTTGTGGTGGTGGTGG
Apt2	Bio-(T15)GGTGGTGGTGGTTGTGGTGGTGGTGG
Apt3	Bio-(T25)GGTGGTGGTGGTTGTGGTGGTGGTGG
Apt4	Bio-(T35)GGTGGTGGTGGTTGTGGTGGTGGTGG
$PolyT_{41}$	Bio-(T15)TTTTTTTTTTTTTTTTTTTTTTTTTTT
Texas Red-Apt2	Bio-(T15)GGTGGTGGTGGTTGTGGTGGTGGTGG- Texas Red

Apparatus. Dark-field images are acquired with an Olympus BX-51 microscope (Tokyo, Japan), which is equipped with a highly numerical dark field condenser (UDCW). The colorful dark-field light scattering photographs are captured with an Olympus E-510 digital camera (Tokyo, Japan). The light scattering spectra of single particle are carried out through the Olympus BX-51 dark-field system integrated with an Acton Research MicroSpec 2300i monochromator and a Princeton Instruments PIMAX intensified charge coupled device (ICCD) (Trenton, USA). The size and morphology of the SeNPs are imaged by a Hitachi S-4800 scanning electron microscope (SEM, Tokyo, Japan). The UV absorption spectra are obtained from a Hitachi U-3010 spectrophotometer (Tokyo, Japan). The elemental compositions of SeNPs are measured with an ESCALAB 250 X-ray photoelectron spectroscopy (XPS). Fourier transform infrared (FT-IR) spectra are collected on a Hitachi FTIR-8400S Fourier Transform Infrared spectrometer (Tokyo, Japan). The high-resolution transmission electron microscope (HRTEM) image is performed on a Tecnai G2 F20 field emission transmission electron microscope (FEI, USA). The dark-field light scattering images are

quantitatively analyzed using the image J software.[1] Fluorescence imaging is operated on a DSU live-cell confocal microscope (Olympus, Japan) system.

Preparation and purification of SeNPs. Firstly, the PVP solution (8ml, 50 mg) is added into a clear drying round-bottom flask, refluxing condenser to 70 ^{o}C under vigorous stirring. Then 1 ml SeO_2 solution (0.5 mM) is poured into the round-bottom flask. After maintaining the solution for 2 min, 1 ml Vc solution (100 mg ml^{-1}) is added quickly into the mixed solution. After 12 h, the solution shows bright-red, indicating that PVP-stabilized SeNPs are produced. The obtained products are purified through a 3.5 kDa dialysis membrane for 1 day with 6 changes of distilled water in order to remove the small molecules. After being dried, a large scale of the red powder can be obtained. The products are finally stored in a 4 ^{o}C refrigerator.

Conjugation of Apt-SeNPs. SA is added into the SeNPs suspension, incubating at 37 ^{o}C for 3 h by using a rotary shaker. Then BSA solution (5 mg ml^{-1}) is added which is used to block the excessive binding sites on SeNPs surface. The mixture is centrifuged at 6000 rpm for 4 min. After that, the sediment is washed three times to gain SeNPs-streptavidin conjugates (SA-SeNPs). Biotin-aptamer, which can precisely target nucleolin (NCL) with high affinity and specificity[2], is added into the SA-SeNPs solution and incubated at 37 ^{o}C for 30 min. The final product (aptamer-based SeNPs; Apt-SeNPs) is acquired by centrifugation at 6000 rpm for 4 min.

To obtain obvious imaging results, influencing factors, such as the consumption of SA and aptamer, aptamer length, are taken into consideration. The mass ratios of SA and SeNPs are adjusted from 1:200 to 1:20 through keeping SeNPs concentration constant at 200 μg ml^{-1}. We also investigate the effects of usage (from 25 to 125 nM) and length (from Apt0 to Apt4) of aptamer on imaging. Eventually, the Apt-SeNPs are synthesized with 10 μg ml^{-1} SA and 62.5 nM Apt2.

Cellular toxicity test. The 1×10^5 cells per ml of human epidermoid cancer cells (HEp-2) in Roswell Park Memorial Institute 1640 medium (RPMI 1640) supplemented with 10 % fetal bovine serum (FBS) are added to each well of a 96-well plate (100 μl per well), the cells are cultured first for 24 h in an incubator (37 ^{o}C, 5% CO_2). Then the culture medium is replaced by a fresh culture medium with 2 % FBS 1640 medium containing the obtained SeNPs/SA-SeNPs/Apt-SeNPs probes with different concentration for another 24 h. A mixture solution of 10 μl of Cell Counting Kit-8 (CCK-8) solution and 90 μl RPMI 1640 is added to every well. After 30 min, the optical density (OD) of the mixture is measured at 450 nm with a Microplate Reader Model. The cell viability is estimated according to the following equation:

$$\text{Cell viability (\%)} = (OD_{\text{treated}} - OD_{\text{PBS}}) / (OD_{\text{control}} - OD_{\text{PBS}}) \quad (2)$$

OD_{control} is obtained in the absence of nanoparticles, OD_{PBS} is the absorption value of PBS around in 96 plate-well, and OD_{treated} is obtained in the presence of Apt-SeNPs/SA-SeNPs/PolyT_{41}-SeNPs.

Cellular imaging with dark-field microscopy. HEp-2 cells in RPMI 1640 supplemented with 2 % fetal bovine serum are added to imaging dishes (1 ml per well). Then cells are cultured for 24 h in an incubator (37 ^{o}C, 5 % CO_2). After 24 h incubated, the culture medium is replaced with 1 ml PRMI 1640 containing 5 μg ml^{-1} Apt-SeNPs (or the same concentration of SA-SeNPs/PolyT_{41}-SeNPs for a control) at 4 ^{o}C for 30 min, then rinsed with PBS buffer three times, fixed with 4 % paraformaldehyde, and transferred for dark-field microscopic (DFM) imaging. The statistics of light intensity are investigated using Image-Pro Plus (IPP) 6.0 software and mean light scattering intensity per cell is obtained by Image J software.

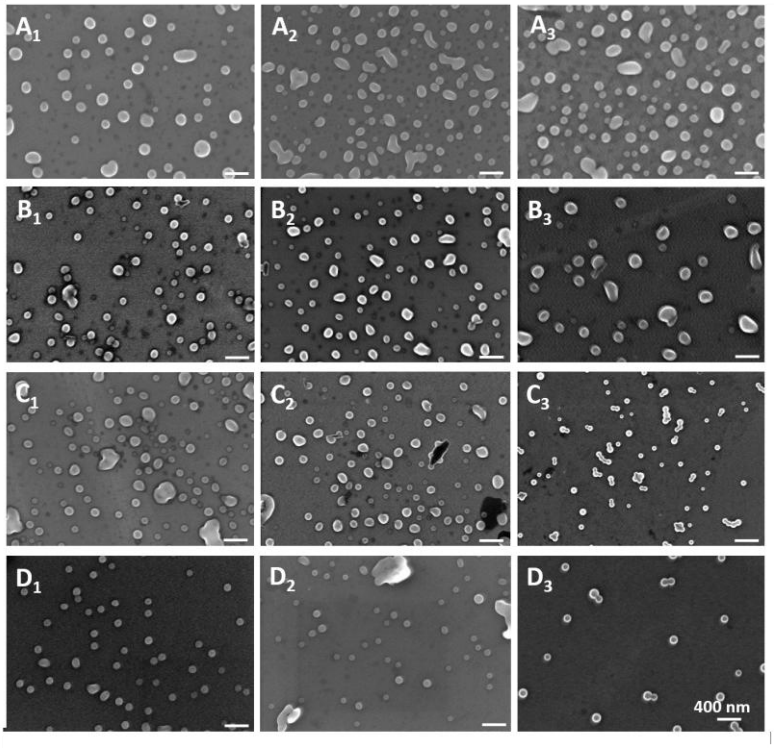

Fig. S1 Effects of the quality of PVP and Vc, reaction temperature and time on the synthesis of the as-prepared SeNPs. The quality of PVP is (A_1) 50, (A_2) 100, (A_3) 150 mg. The quality of Vc, reaction temperature and time are 100 mg, 90 ^{o}C and 8 h, respectively. The quality of Vc is (B_1) 100, (B_2) 300, (B_3) 500 mg. The quality of PVP, reaction temperature and time are 50 mg, 90 ^{o}C and 8 h, respectively. The reaction temperature is (C_1) 0, (C_2) 30, (C_3) 90 ^{o}C. The quality of PVP and Vc, reaction time are 50 mg, 100 mg and 8 h, respectively. The reaction time is (D_1) 4, (D_2) 8, (D_3) 12 h. The quality of PVP and Vc, reaction temperature are 50 mg, 100 mg and 90 ^{o}C, respectively. All the scale bar, 400 nm.

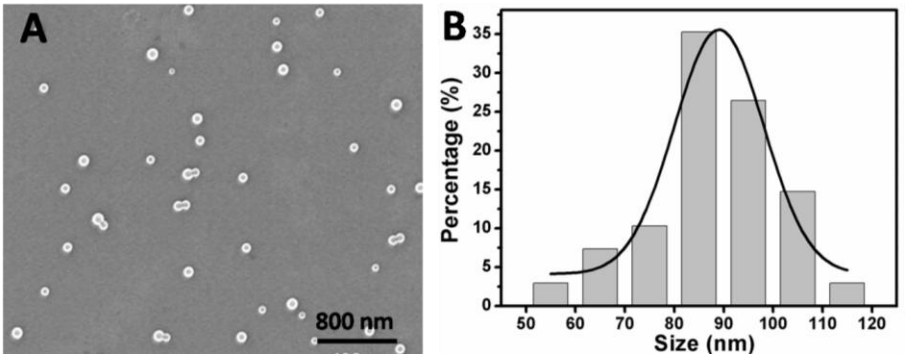

Fig. S2 The morphology and size distribution of the SeNPs. (A) A large-scale SEM image and (B) particle size distribution of the SeNPs.

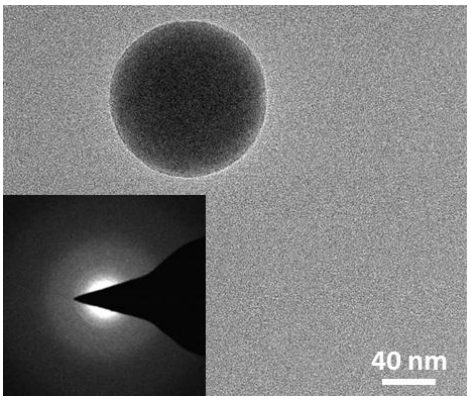

Fig. S3 SAED pattern of the SeNPs.

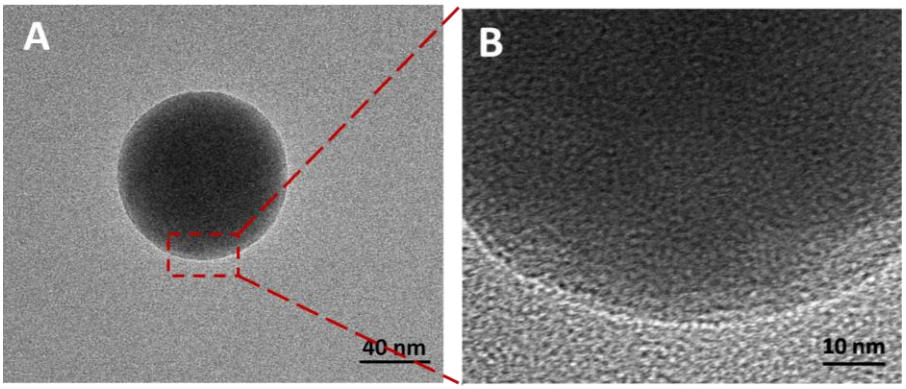

Fig. S4 The structural information of the as-prepared SeNPs. (A) The HRTEM image of the SeNPs and (B) the enlarged HRTEM image of the square area in (A).

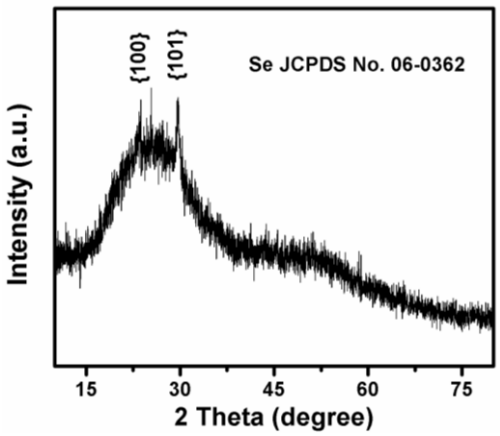

Fig. S5 The XRD pattern of the SeNPs.

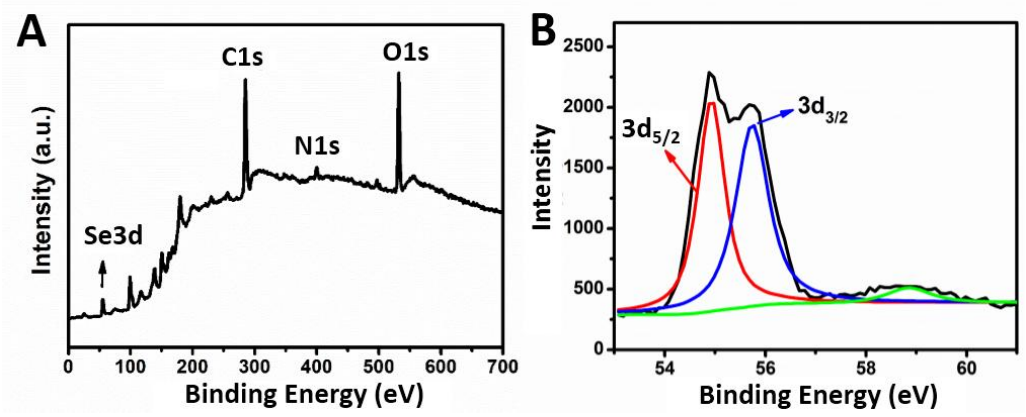

Fig. S6 Characterization of the as-prepared SeNPs. (A) XPS and (B) Se3d spectra of SeNPs.

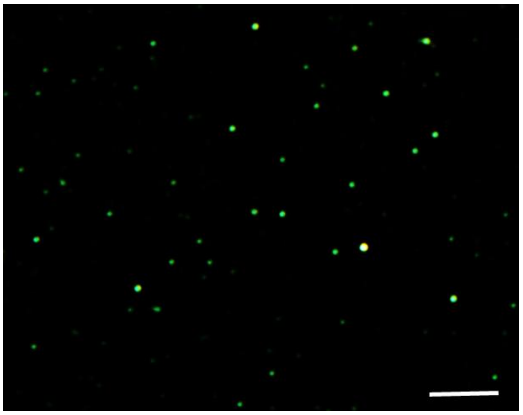

Fig. S7 A large scale dark-field light scattering image of the SeNPs. Scale bar, 10 μm.

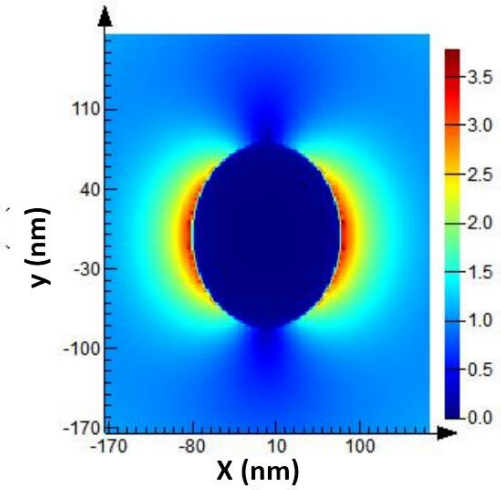

Fig. S8 Localized electric field distributions of a SeNP by FDTD simulation.

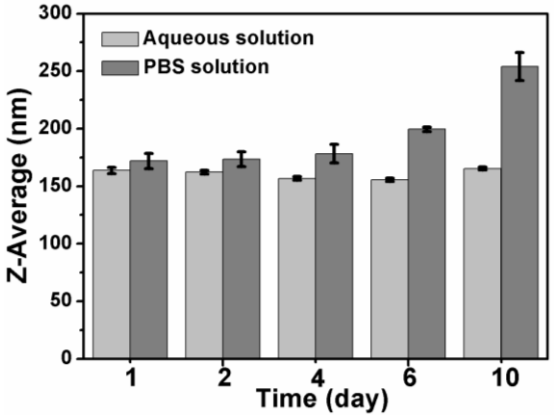

Fig. S9 Time course of the size distribution of SeNPs in aqueous and PBS solutions (pH = 7.4). Results are mean ± SD of the triplicate experiments.

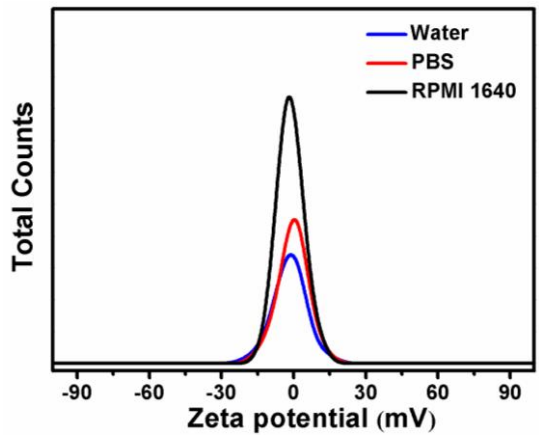

Fig. S10 Stability of SeNPs in water, PBS (pH 7.4) and RPMI 1640 medium.

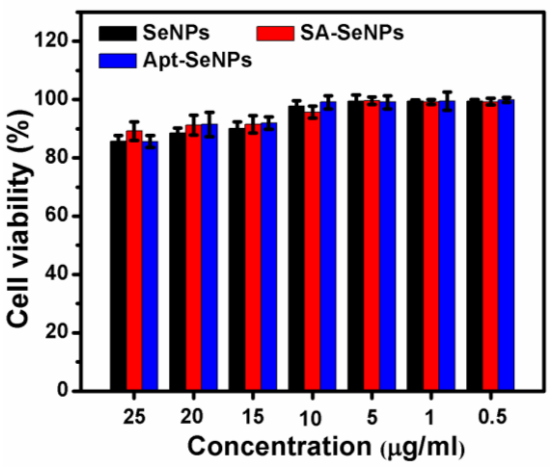

Fig. S11 Toxicity test of the SeNPs, SA-SeNPs and Apt-SeNPs on HEp-2 cells.

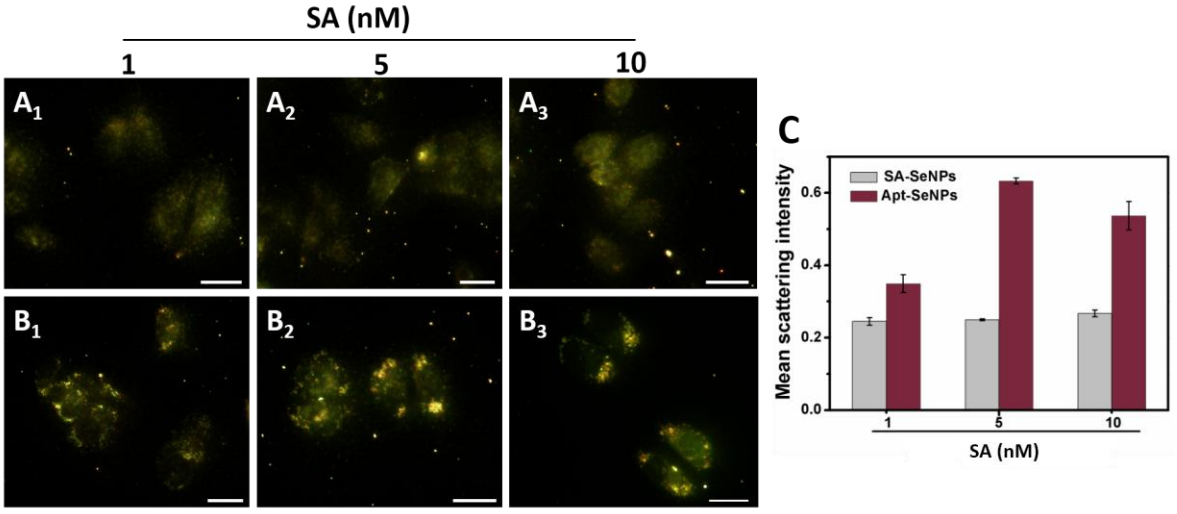

Fig. S12 DFM images and intensity analysis. DFM images of HEp-2 cells cultured with (A_1-A_3) SA-SeNPs as the control groups and (B_1-B_3) Apt-SeNPs as the experimental groups. The SA-SeNPs and Apt-SeNPs are prepared with different concentrations of SA (1, 5, 10 nM). HEp-2 cells: 1.0 $\times 10^5$ cells ml^{-1}. Nanoparticles: 5 μg ml^{-1}. Scale bar, 10 μm. (C) Mean light scattering intensity per cell versus the SA quantity by using Image J software. The error bars represent three replicated experiments.

The optimization process of the amount of SA is as following: SA solutions of different concentrations (1, 5, 10 nM) are added into SeNPs suspension (200 ug ml^{-1}) to synthesize SA-SeNPs and then synthesize Apt-SeNPs with the addition of Apt3 (51 bases). The same amount of SA-SeNPs (control groups) and Apt-SeNPs (experimental groups) are added into the monolayer HEp-2 cells substrate for 30 min at 4 ^oC, following by imaging under DFM.

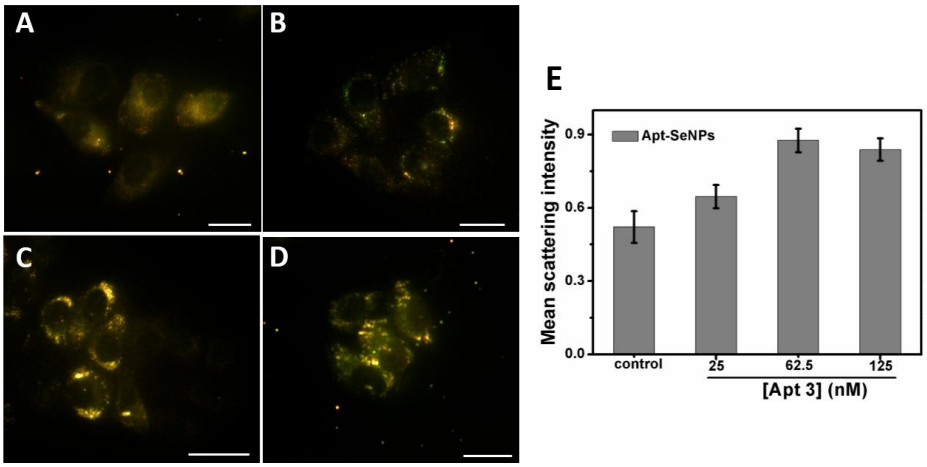

Fig. S13 DFM images and intensity analysis. DFM images of HEp-2 cells cultured with (A) SA-SeNPs and (B-D) Apt-SeNPs. The Apt-SeNPs are prepared with different quantities of Apt3 (25, 62.6 and 125 nM) from (B) to (D). HEp-2 cells: 1.0×10^5 cells ml^{-1}. Nanoparticles: 5 μg ml^{-1}. Scale bar, 10 μm. (C) Mean light scattering intensity per cell versus the Apt3 quantity using Image J software. Error bars represent three replicated experiments.

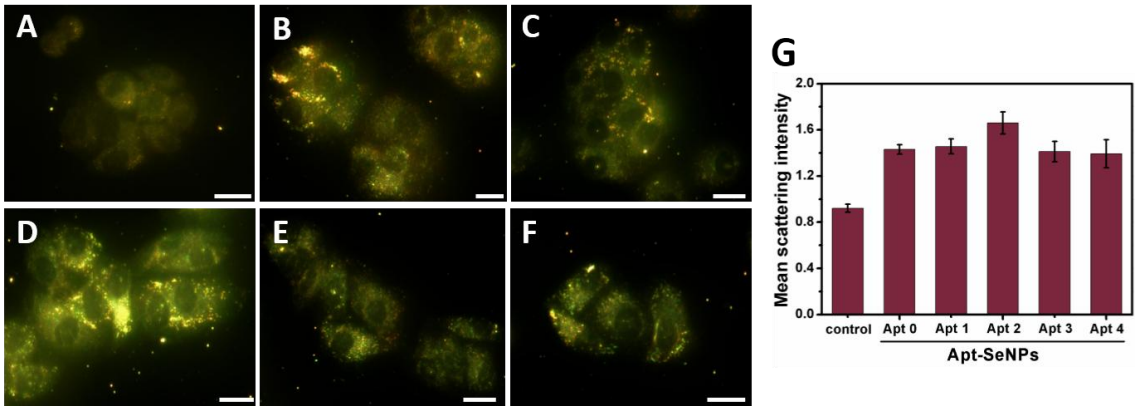

Fig. S14 DFM images and intensity analysis. DFM images of HEp-2 cells cultured with (A) SA-SeNPs and (B-F) Apt-SeNPs. The Apt-SeNPs are prepared with different length of aptamer (Apt0, Apt1, Apt2, Apt3, Apt4) from (B) to (F). HEp-2 cells: 1.0×10^5 cells ml^{-1}. Nanoparticles: 5 μg ml^{-1}. Scale bar, 10 μm. (G) Mean light scattering intensity per cell versus the aptamer length using Image J software. Error bars represent three replicated experiments.

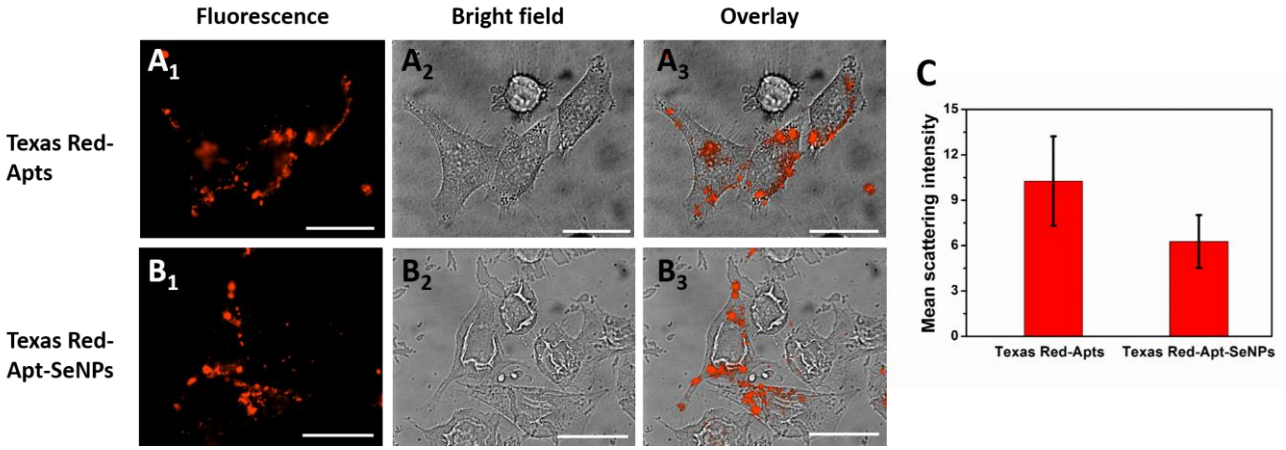

Fig. S15 Confocal fluorescence imaging and intensity analysis. Confocal fluorescence imaging of the HEp-2 cells cultured with (A_1-A_3) Texas Red-Apts and (B_1-B_3)Texas Red-Apt-SeNPs. Different imaging channels are displayed horizontally for each sample (from left to right): fluorescence (EX: 510-560 nm), bright field and overlay images. HEp-2 cells: 1.0×10^5 cells ml^{-1}. Texas Red-Apt-SeNPs: 5 μg ml^{-1}. Texas Red-Apts: 10 nM, Scale bar, 20 μm. (C) Mean light scattering intensity per cell versus different fluorescence probes using Image J software. Error bars represent three replicated experiments.

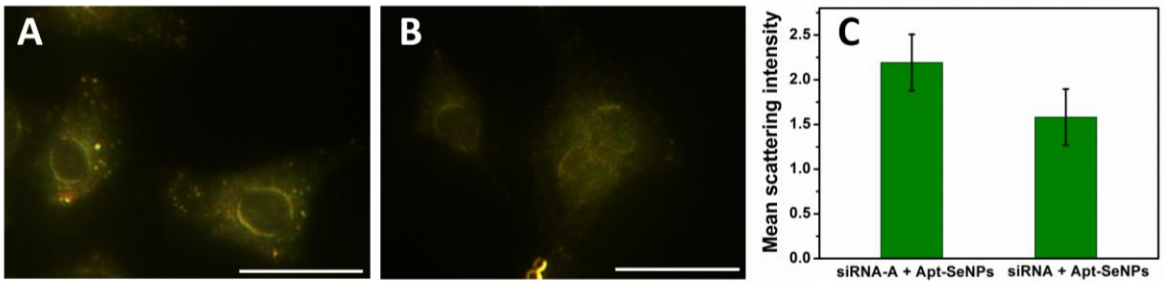

Fig. S16 DFM images and intensity analysis. DFM images of HEp-2 cells treated with Apt-SeNPs following (A) control siRNA-A or (B) anti-NCL-specific siRNA transfection. HEp-2 cells: 1.0×10^5 cells ml^{-1}. Apt-SeNPs: 5 μg ml^{-1}. Scale bar, 10 μm. (C) Mean light scattering intensity per cell using Image J software. Error bars represent three replicated experiments.

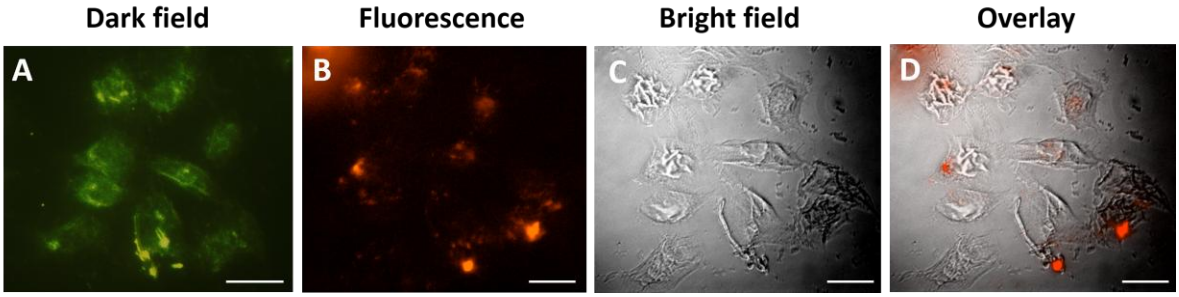

Fig. S17 Co-localization of the HEp-2 cells. (A) DFM and (B-D) confocal fluorescent microscopy images of the HEp-2 cells located at the same area. $EX_{\text{fluorescence channel}}$: 510-560 nm. HEp-2 cells: 1.0×10^5 cells ml^{-1}. Texas Red-Apt-SeNPs: 5 μg ml^{-1}. Scale bar, 10 μm.

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2. Wu, J. H.; Song, C. C.; Jiang, C. X.; Shen, X.; Qiao, Q.; Hu, Y. Q. *Mol. Pharm.* **2013,** 10, 3555-3563.