Supporting Information

Chemical and Materials

Silver nitrate (99+%), sodium borohydride ($NaBH_4$, powder, 98%), disodium hydrogen phosphate, sodium chloride, magnesium chloride, calcium chloride and glucose were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Dulbecco's phosphate buffered saline was purchased from Sigma. Cell cultures including HDMEM, Ham's F12 nutrient medium and DHanks were obtained from Life Technologies Corporation (Shanghai, China). Cell lysis solution and fetal bovine serum (FBS) were purchased from Xuzhou Combio Biotech Co., Ltd (Xuzhou, China). All DNA oligos were synthesized and purified by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The DNA sequences are as follows:

NC-Sgc8c-L5T-cTK1: 5'-CCC CCC CCC CCC TTT TTG GA ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GAA GTG TCT TTG GCA TAC TT -3'

NC-Sgc8c-L5T-cTK1-2: 5'-CCC CCC CCC CCC TTT TTG GA ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GAG CGA GTG TCT TTG GCA TAC TT-3'

cTK1-NC-Sgc8c-L5T: 5'-GCG AGT GTC TTT GGC ATA CTT CCC CCC CCC CCC TTT TTG GA ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GA-3'

C-myc-NC-Sgc8c-L5T: 5'-TTG GTG AAG CTA ACG TTG AGG CCC CCC CCC CCC TTT TTG GA ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GA-3'

cTK1-related target DNA: 5'-AAG TAT GCC AAA GAC ACT CGC-3'

single base mutated cTK1-related target DNA: 5'-ACG TAT GCC AAA GAC ACT CGC-3'

mutated cTK1-NC-Sgc8c-L5T: 5'-GCG AGT GTC TTT GGC ATA CTC CCC CCC CCC CCC TTT TTG GA ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GA-3'

Survivin target: 5'-TGG CAG CCC TTT CTC AAG-3'

K-ras target: 5'-TGG AGC TGG TGG CGT AG-3'

HER-2/neu target: 5'-CAC AGA CAT GAA GCT GC-3'

C-myc target: 5'-CCT CAA CGT TAG CTT CAC CAA-3'

GalNAc-T target: 5'-GCT TTC ACT ATC CGC ATA AGA-3'

All other chemicals involved in this work were analytical-grade. All aqueous solutions were prepared in DEPC treated ultrapure water (≥18MΩ, Milli-Q, Millipore).

Apparatus and Characterization

Fluorescence measurements were carried out using a LS-45/55 Fluorescence/Phosphorescence Spectrometer (PerkinElmer, USA). The excitation/emission wavelengths were set at 480 nm/595 nm and 555 nm/620 nm. Fluorescence imaging was performed with a confocal laser microscope (TCS SP5, Leica, Germany). Flow cytometric analysis was carried out using Cytomics FC 500 MCL (Beckman Coulter, USA). The secondary structure of DNA oligos were analyzed by OligoAnalyzer 3.1 (free online software from IDT).

Cells and Cell Culture Conditions

HeLa cells (human cervical carcinoma) and NIH-3T3 mouse fibroblast cells were obtained from Nanjing KenGen Biotech Co. Ltd. (Nanjing, China) and seeded in HDMEM medium (Gibco, Grand Island, NY) supplemented with 10% FBS, penicillin (100 μg/mL), and streptomycin (100 μg/mL) and incubated under 5% CO_2, 37℃. Human lung fibroblast HLF-1 cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, Chian) and cultured in Ham's F12 nutrient medium (Gibco, Grand Island, NY) supplemented with 10% FBS, penicillin (100 μg/mL), and streptomycin (100 μg/mL) and incubated under 5% CO_2, 37℃. The cells were harvested from 90% confluent cell culture plates and the cell density was determined by use of a Petroff-Hausser

cell counter prior to experiments. The binding buffer used for aptamer internalization was 4.5 g/L glucose and 5 mM $MgCl_2$ in Dulbecco's phosphate buffered saline with calcium chloride and magnesium chloride.

Preparation of Silver Nanoclusters

DNA stabilized Ag NCs were synthesized according to procedures described elsewhere with slight modifications.[1-3] Briefly, 27 μL of 250 μM cTK1-NC-Sgc8c-L5T was mixed with 56.1 μL 20 mM phosphate (pH 7.0) buffer, and then 27.7 μL 10 mM $AgNO_3$ was added to reach a nucleobase to Ag^+ molar ratio of 2:1. After chilled on ice for 15min, the mixture was reduced by quickly adding 8.1 μL of 4 mM $NaBH_4$, followed by vigorous shaking for 1min. The reaction was kept at 4℃ before use. (Note: The $NaBH_4$ solution must be freshly prepared prior to use.) The amounts of $AgNO_3$ and $NaBH_4$ for NC-Sgc8c-cTK1-2 stabilized Ag NCs and C-myc-NC-Sgc8c-L5T stabilized Ag NCs were changed accordingly to reach a nucleobase to Ag^+ molar ratio of 2:1. Mutated cTK1-NC-Sgc8c-L5T stabilized Ag NCs were prepared with the same process.

***In Vitro* Detection of DNA Target**

For the detection of DNA target, 500 nM cTK1-NC-Sgc8c-L5T stabilized Ag NCs and varied amounts of DNA target were added into binding buffer (final volume was 300 μL) and incubated at 37°C for 30 min. Then the fluorescence emission spectra of the obtained solution were directly recorded. For specific test, DNA target was substituted by Survivin target, K-ras target, HER-2/neu target, C-myc target, GalNAc-T target, single mutation of cTK1-related target DNA, and mutated probe with target mRNA, accordingly.

MTT Assay

HeLa cells and HLF-1 cells were plated in 96-well plates at a density of 1×10^4 cells per well in 200μL of culture medium and incubated for 24h at 37℃ before exposure to cTK1-NC-Sgc8c-L5T stabilized Ag NCs. Prior to the exposure, synthesized Ag NCs were centrifuged for 100 min at 10,000 rpm through a 3KDa cutoff filter to remove unbound Ag^+ from Ag NCs. Cells were treated with a range of Ag NCs concentrations (5 nM, 50 nM, 100 nM, 250 nM, 500 nM and 1 μM) for 24h and media were removed after treatment. Then the cells were washed twice with PBS buffer before the addition of HDMEM containing MTT (5mg/mL) (HeLa cells) or Ham's F12 nutrient medium containing MTT (5mg/mL) (HLF-1 cells) and further incubated at 5% CO_2, 37℃ for another 4h. At last, media containing MTT were replaced by 100 μL of dimethyl sulfoxide (DMSO) to solubilize the formazan crystals precipitates. The plate was shaken for 15min at 37℃ before measurements of optical absorbance at 490 nm on BioTek Epoch microplate reader. The absorbance of MTT at 490 nm is dependent upon the degree of activation of the cells. The cell viability was determined relative to the untreated control cells.[4]

Flow Cytometric Analysis

To quantify the sensing ability of cTK1-NC-Sgc8c-L5T stabilized AgNCs to intracellular TK1 mRNA, flow cytometry assay was performed. cTK1-NC-Sgc8c-L5T stabilized AgNCs were incubated with HeLa cells cultured in 6-well plate for 5h at 37℃. After incubation, cells were washed with PBS, lysed and redispersed in PBS buffer for the following flow cytometry analysis. NIH-3T3 mouse fibroblast cells were used as control.

Confocal Laser Microscopy Assay

The intracellular optical imaging of TK1 mRNA was examined with confocal laser microscopy by the fluorescence response of cTK1-NC-Sgc8c-L5T templated Ag NCs. HeLa cells (1×10^4 cells) were seeded in special petri dish. After 24 h, cells were transfected with 500 nM cTK1-NC-Sgc8c-L5T templated Ag NCs for 5h at 37℃ after washing for three times with phosphate-buffered saline (PBS). The cells were then washed with cold PBS for three times and placed above a 20×objective on the confocal microscope. The Ag NCs were excited with 488 nm or 543 nm. NIH-3T3 cells were cultured in special petri dish and treated with the same concentration of Ag NCs as

control. Additionally, the intracellular optical imaging of TK1 mRNA with mutated probe was also performed as control.

For nucleus staining, NIH-3T3 cells (1×10^4 cells) were seeded in special petri dish. After 24h, cells were transfected with 500 nM cTK1-NC-Sgc8c-L5T templated Ag NCs for 5h at 37℃ after three times wash with phosphate-buffered saline (PBS). The cells were then washed with cold PBS for three times. After fixed for 20min in 200 μL of 3.7% paraformaldehyde, nucleus staining was performed with 4'6-diamidino-2-phenylindole dihydrochloride (DAPI). The cells were further washed for three times with PBS buffer for confocal laser scanning after 10 min.

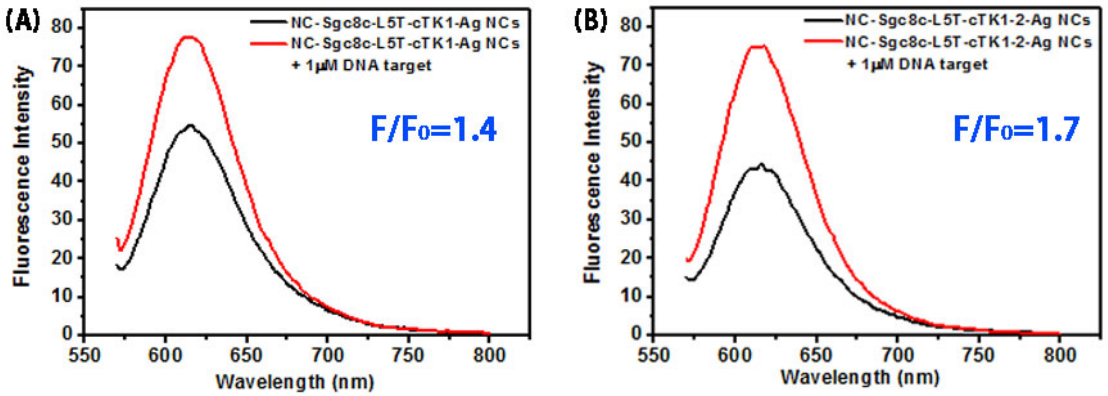

Fig. S1 Fluorescence response of NC-Sgc8c-L5T-cTK1 stabilized Ag NCs (A) and NC-Sgc8c-L5T-cTK1-2 stabilized Ag NCs (B) to DNA target. Excitation wavelength: 555nm.

Possible Mechanism of the "Light-up" and "Spectrum-shift" Response of Aptamer-Functionalized Ag NCs to TK1 mRNA

There are mainly two proposed mechanisms in the literature for DNA-stabilized Ag NCs hybridized with complementary sequence: (1) complementary oligonucleotides produce new clusters when they hybridize with the cluster-based reporter strand [5,6], and (2) considered static quenching in which base pairing blocked the favored binding sites for a near-infrared emitting species [7]. Additionally, Petty et al reported a hybridization-induced structural changes which resulted in the maximum absorption of DNA-templated Ag NCs from 400 nm to 720 nm. They speculated without the complement, the folded DNA host produced Ag NCs with 400 nm absorption and upon association of the target with its recognition site, the sensor unfolded to reveal the binding site for a relatively highly emissive species with near-infrared absorption at 720 nm [8]. In our study, we found the enhancement emission at 595 nm did not quench the original emission at 620 nm with target DNA concentrations increased (Fig. S2). Furthermore, with the time passing, the fluorescence emissions at 595 nm increased gradually, which was in agreement with the growth of Ag NCs (Fig. S3). Thus we ascribed such "light-up" and "spectrum-shift" to the formation of new Ag NCs after hybridization. In order to testify our hypothesis, Ag NCs stabilized with the duplex of cTK1-NC-Sgc8c-L5T and cTK1-related target DNA were synthesized. As shown in Fig. S4, the hybridization of cTK1-NC-Sgc8c-L5T and cTK1-related target DNA favored the formation of Ag NCs emitted at 595 nm. There was no obvious difference of emissions at 620 nm in these two groups.

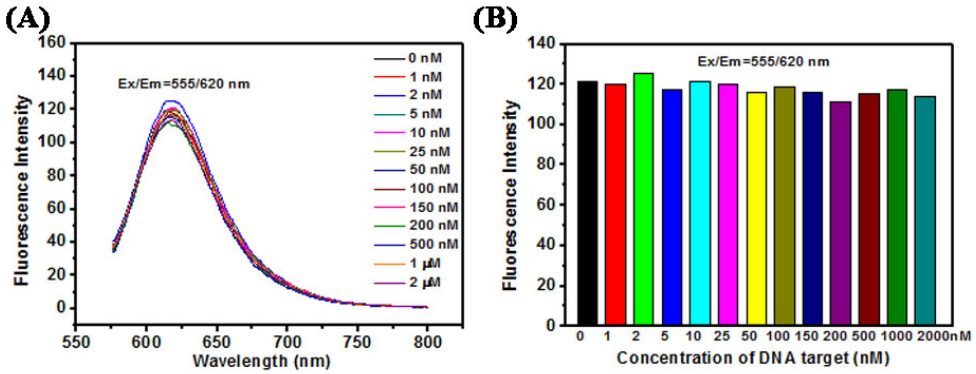

Fig. S2 Fluorescence emission spectra (A) and bar graph (B) of cTK1-NC-Sgc8c-L5T stabilized Ag NCs in the absence and presence of DNA target excited at 555 nm.

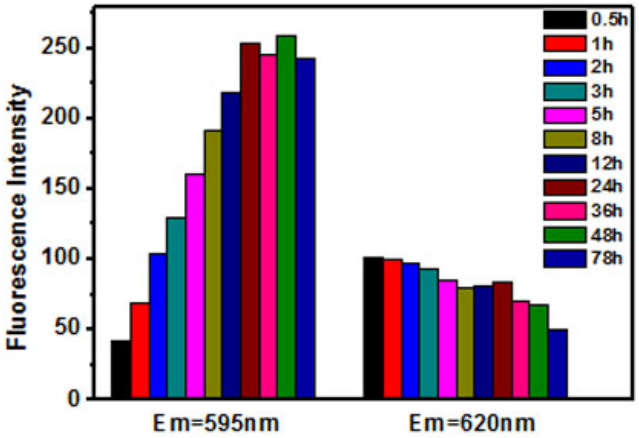

Fig. S3 Fluorescence emission intensity of cTK1-NC-Sgc8c-L5T stabilized Ag NCs after hybridization with DNA target with time passing.

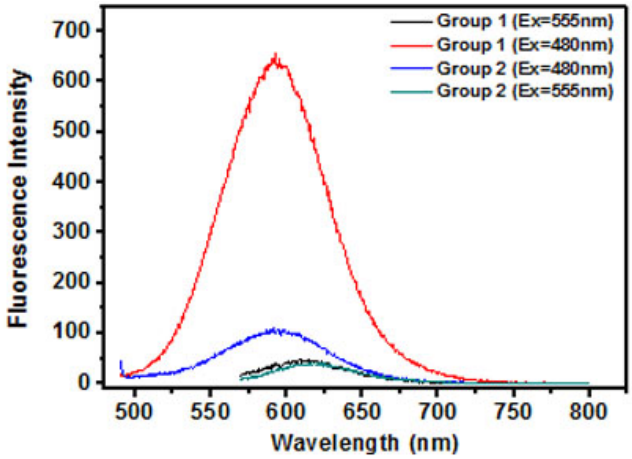

Fig. S4 Fluorescence spectra of Ag NCs excited at 480 nm and 555 nm, respectively. Group 1: cTK1-NC-Sgc8c-L5T was hybridized with cTK1-related target DNA first. And then the duplex was used as template to synthesize Ag NCs. Group 2: cTK1-NC-Sgc8c-L5T stabilized Ag NCs was incubated with cTK1-related target DNA. The concentrations of DNA oligos, $AgNO_3$ and $NaBH_4$ were the same in these two groups.

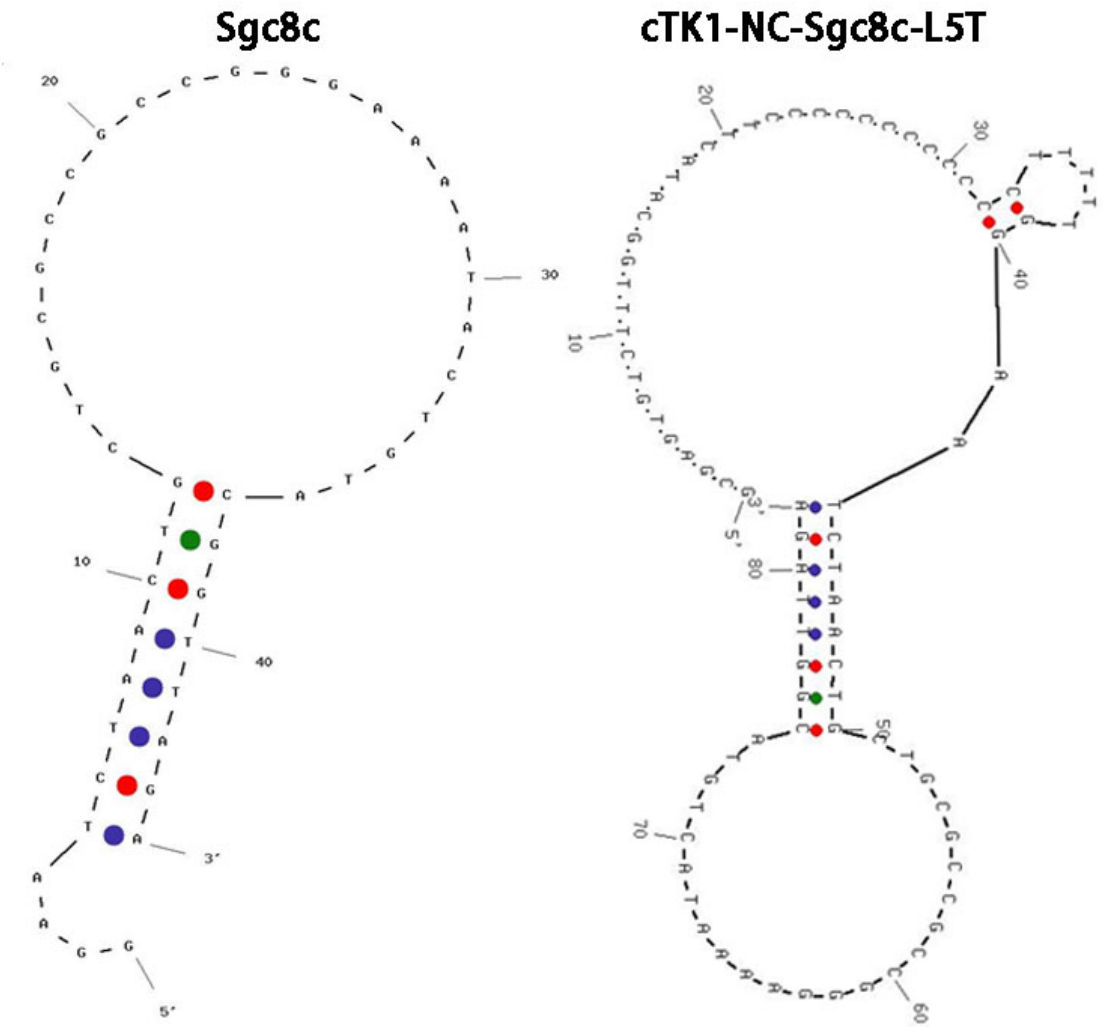

Fig. S5 The secondary structures of Sgc8c (left) and cTK1-NC-Sgc8c-L5T (right), analyzed by the free software Oligo Analyzer 3.1 from IDT. Parameter settings were 50 nM oligo and 50 mM Na+.

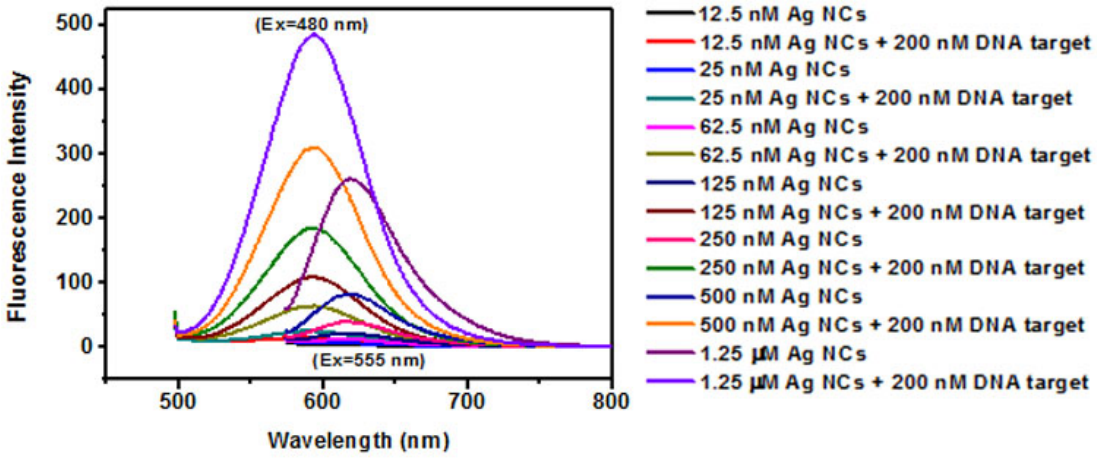

Fig. S6 Fluorescence emission spectra of cTK1-NC-Sgc8c-L5T stabilized Ag NCs with varied concentrations in the presence of 200 nM DNA target. Excitation wavelengths were 480 nm and 555 nm.

Table S1 Fluorescence intensity of cTK1-NC-Sgc8c-L5T stabilized Ag NCs with varied concentrations in the presence of 200 nM DNA target when excited at 480 nm and 555 nm, respectively.

	Ex/Em=480/595 nm	Ex/Em=555/620 nm	F(595nm)/F(620nm)
12.5 nM Ag NCs	N/A	N/A	N/A
12.5 nM Ag NCs + 200 nM DNA target	15.1	N/A	
25 nM Ag NCs	N/A	N/A	N/A
25 nM Ag NCs + 200 nM DNA target	25.1	N/A	
62.5 nM Ag NCs	N/A	11.8	5.40
62.5 nM Ag NCs + 200 nM DNA target	63.7	13.0	
125 nM Ag NCs	N/A	21.3	5.07
125 nM Ag NCs + 200 nM DNA target	108.0	21.4	
250 nM Ag NCs	N/A	39	4.76
250 nM Ag NCs + 200 nM DNA target	185.5	41.5	
500 nM Ag NCs	N/A	82.1	3.79
500 nM Ag NCs + 200 nM DNA target	311	80.8	
1.25 μM Ag NCs	18.9	261.1	1.87
1.25 μM Ag NCs + 200 nM DNA target	489.4	274.5	

The chosen of cTK1-NC-Sgc8c-L5T stabilized AgNCs concentration to detect DNA target

There are three reasons for choosing 500 nM of cTK1-NC-Sgc8c-L5T stabilized AgNCs for the detection of DNA target. (1) As shown in Table S1, although 62.5 nM Ag NCs presented the best S/N response to target DNA (5.4), the fluorescence intensity at 595 nm was too weak even 5.4-fold enhanced after hybridization. Considering the relative low expression of mRNA in cells, this weak signal would hamper the following intracellular imaging. (2) Confocal laser scanning microscopy images of HeLa cells incubated with different concentrations of cTK1-NC-Sgc8c-L5T stabilized Ag NCs (125 nM, 250 nM and 500 nM) were also performed. Obvious fluorescence was observed from 500 nM cTK1-NC-Sgc8c-L5T stabilized AgNCs group (Fig. S7). (3) The obvious cytotoxicity of 1 μM cTK1-NC-Sgc8c-L5T stabilized Ag NCs limited its cellular imaging at this concentration. Thus, 500 nM cTK1-NC-Sgc8c-L5T stabilized AgNCs was chosen for the intracellular imaging.

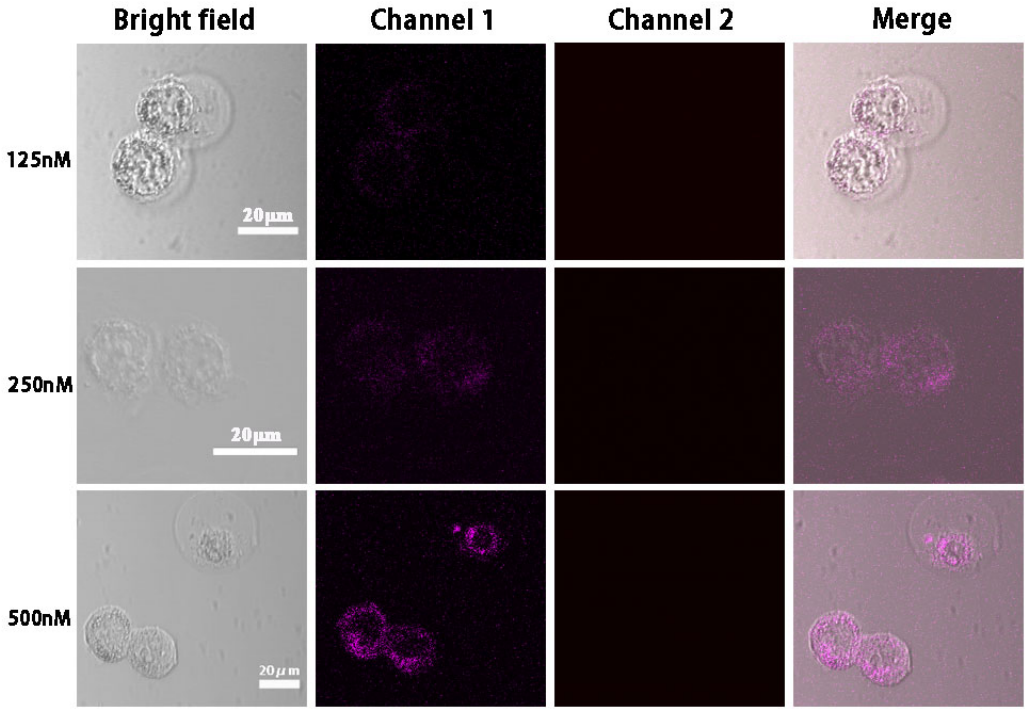

Fig. S7 Confocal laser scanning microscopy images of HeLa human cervical carcinoma cells incubated with different concentrations of cTK1-NC-Sgc8c-L5T stabilized Ag NCs (125 nM, 250 nM and 500 nM). Channel 1: excited at 488 nm. Channel 2: excited at 543 nm. Scale bar, 20 μm.

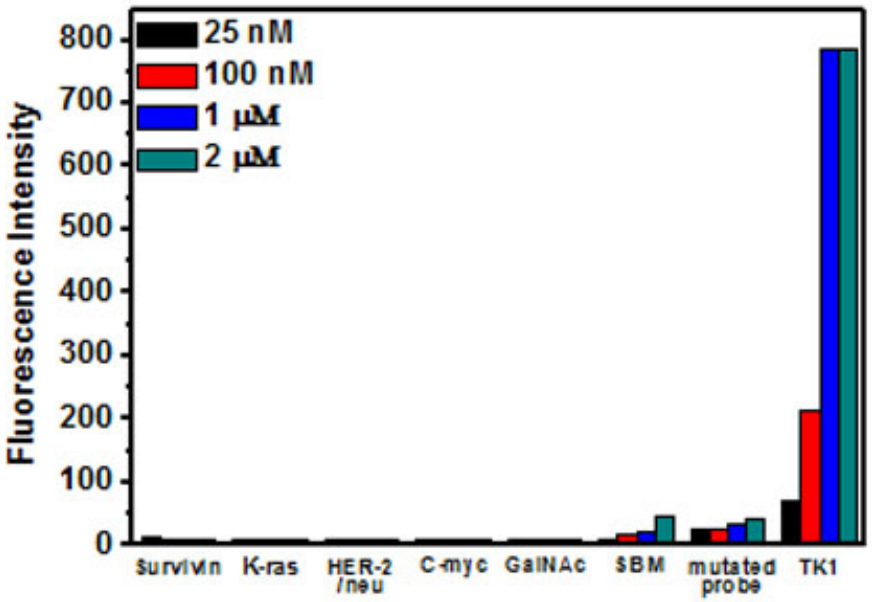

Fig. S8 Specificity test showing fluorescence response from the target DNA and other synthetic DNA sequences (Survivin, K-ras, HER-2/neu, C-myc, and GalNAc) as well as single base mutated target DNA (SBM). The fluorescence response of mutated cTK1-NC-Sgc8c-L5T stabilized Ag NCs to target DNA was also tested.. The experimental conditions were the same as those for target DNA. Concentrations varied from 25 nM to 2 μM.

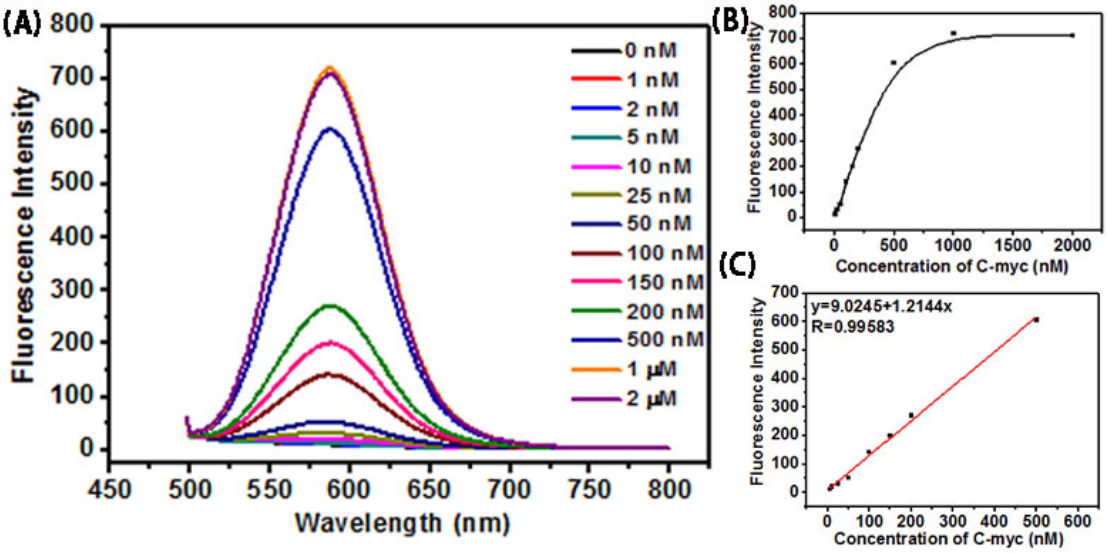

Fig. S9 Fluorescence response of C-myc-NC-Sgc8c-L5T stabilized Ag NCs to target DNA with concentrations ranging from 0 to 2 μM (A). (B) Intensity of fluorescence (emissions at 595 nm) increased with the concentration of target DNA and reached platform in the presence of 1 μM target DNA. (C) The linear range was from 0 to 500 nM.

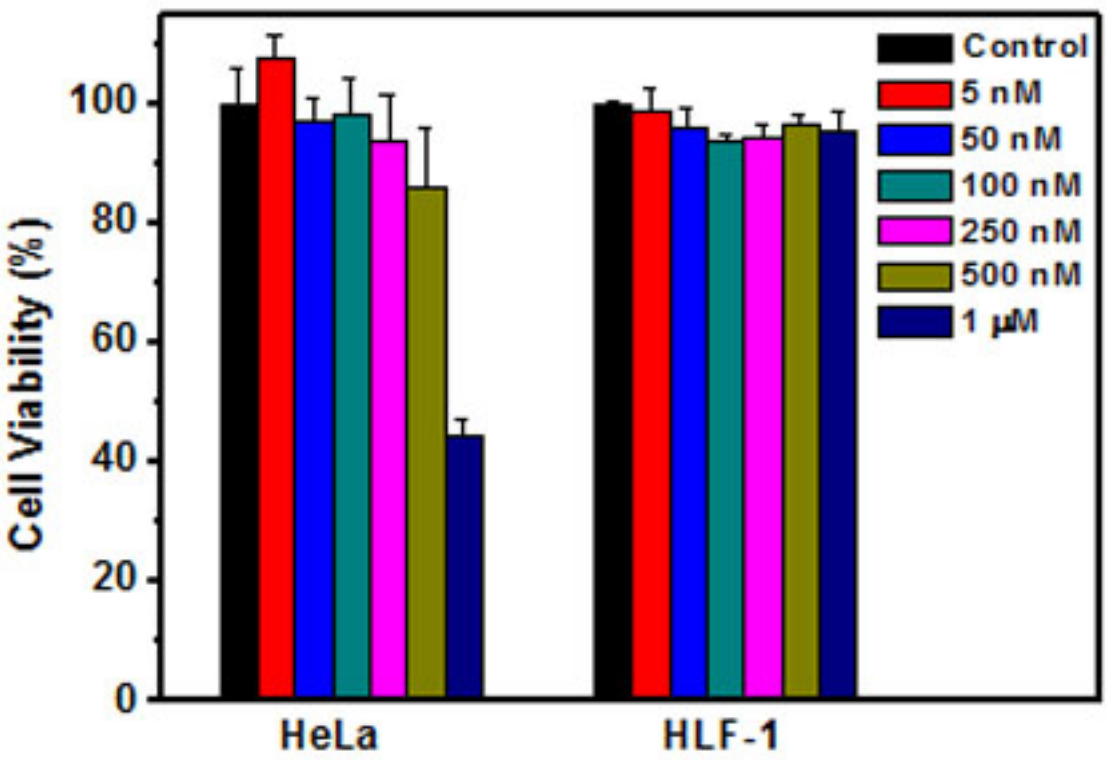

Fig. S10 Cytotoxicity assay of HeLa and HLF-1 cells treated with cTK1-NC-Sgc8c-L5T stabilized Ag NCs.

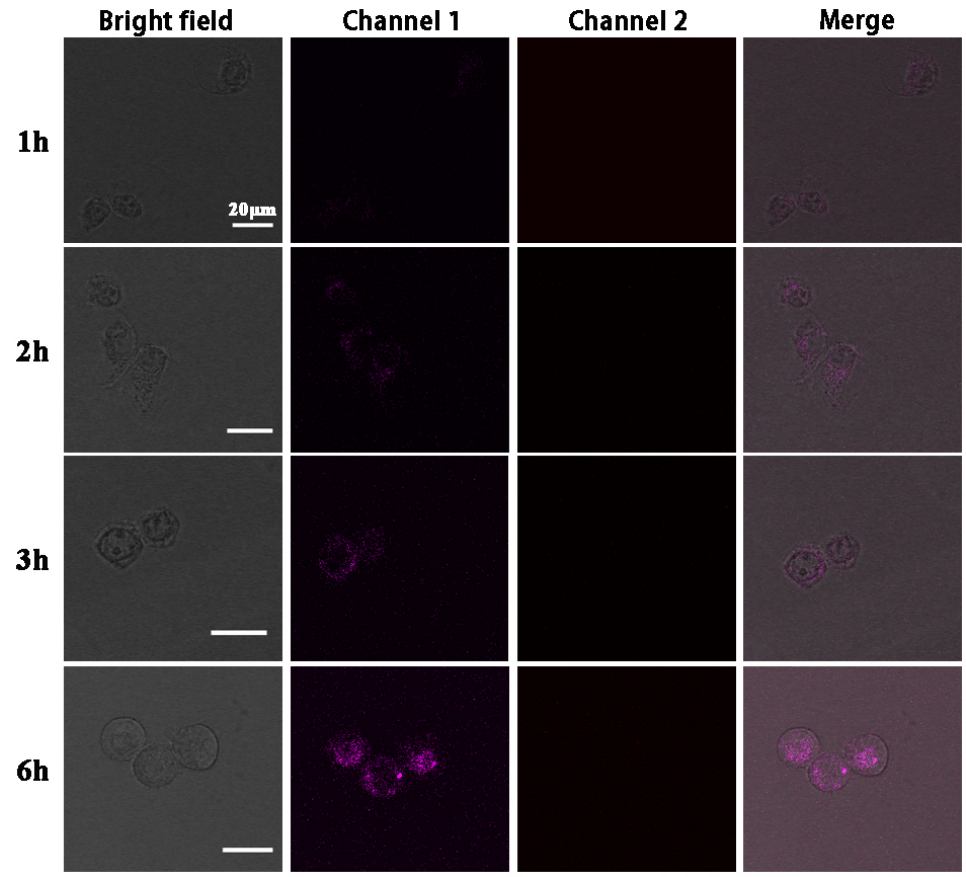

Figure S11. Confocal laser scanning microscopy images of HeLa human cervical carcinoma cells incubated with 500 nM cTK1-NC-Sgc8c-L5T stabilized Ag NCs at 37℃ at different time points: 1h, 2h, 3h and 6h. Channel 1: excited at 488 nm. Channel 2: excited at 543 nm. Scale bar, 20 μm.

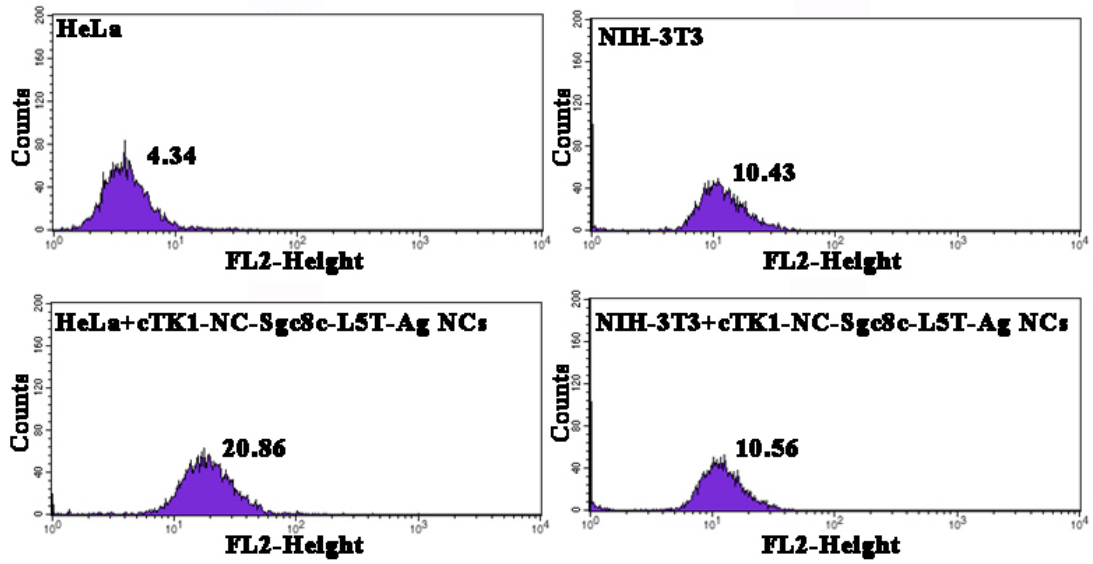

Figure S12. Flow cytometry assay to quantify the sensing ability of cTK1-NC-Sgc8c-L5T stabilized AgNCs to intracellular TK1 mRNA. NIH-3T3 was used as the control cells. Excitation: 488nm.

TK1 mRNA detection in cellular extracts

Since the designed probe was aimed for intracellular mRNA imaging, it is good to know if the probe can be used for quantitative study of mRNA with cellular samples. We prepared cellular extracts according to the reported method[9]. The cellular extracts were incubated with cTK1-NC-Sgc8c-L5T stabilized Ag NCs. However, no fluorescence emission at 595 nm was observed with 488 nm excitation and the fluorescence emission at 620 nm of cTK1-NC-Sgc8c-L5T stabilized Ag NCs disappeared after the addition of cellular extracts (shown in Fig. S13). The reason could be the high salt concentrations in cell lysate solution. Based on the results we currently have, TK1 mRNA in the cellular extracts can not be detected by this method. For living cell samples, the fluorescent emission when excited at 488 nm could be detected by flow cytometer (Fig. S12). But no fluorescent emission could be detected using fluorescence spectrometer. This might be due to the sensitivity of our proposed method is not high enough. We expect to improve the sensitivity of our method and photostability to salt of Ag NCs in the future.

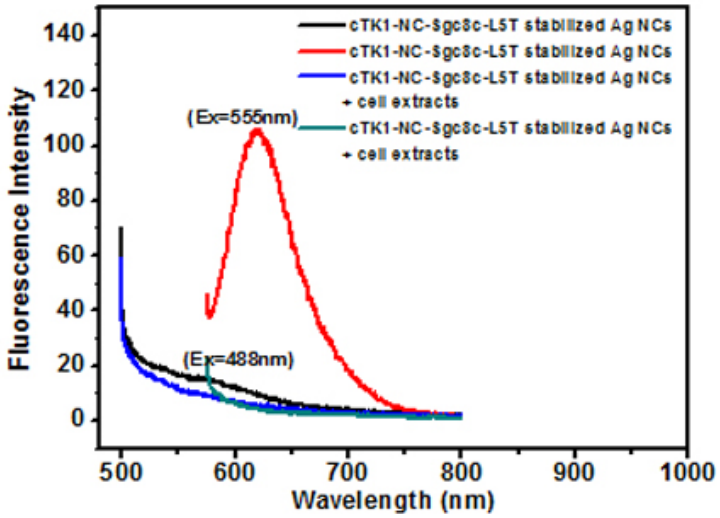

Figure S13. Fluorescence emissions of cTK1-NC-Sgc8c-L5T stabilized Ag NCs in the presence and absence of cellular extracts.

Reference

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