Electronic Supplementary Information for

mRNA-activated DNAzyme nanoprobe for tumor cell precise imaging and gene therapy

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Experimental section

Chemicals and reagents

Bovine serum albumin (BSA), and tris(2-carboxyethyl) phosphine (TCEP) were purchased from Sigma (St. Louis, MO, USA). Trisodium citrate and chloroauric acid (HAuCl4) were purchased from J&K (Beijing, China). Anti-vascular endothelial growth factor (VEGF) antibody was acquired from BD Biosciences (USA). Fetal bovine serum (FBS) were obtained from ExCell Biology (Shanghai, China). DAPI was purchased from Biyuntian Biotechnology Co., Ltd. (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) was acquired from Gibco BRL (Gaithersburg, MD, USA). SybrGreen PCR Master Mix was purchased from ABI (USA). The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) was ordered from Keygen Biotech Co., Ltd. (Jiangsu, China). AMV First Strand cDNA Synthesis Kit was purchased from BBI (Toronto, Canada). Human bladder cancer cells (T24 cells), human non-malignant urothelial cells (HCV-29 cells), human liver cancer cells (HepG2 cells), and human normal liver cells (7702 cells), were supplied by the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The nucleic acid chain was synthesized by Sangon Biotech (Shanghai, China) and purified by High Performance Liquid Chromatography (HPLC). The sequences for nucleic acid chain were shown in Table S1. All other reagents were of analytical grade and used without further purification.

Table S1. Oligonucleotide sequence (5'→3')

Oligonucleotides	Sequences
P1	HS-TTTTTGGGTGGGTGGCGTAGAGATGTAAAATGTAGA
P2	TTACATCTCTACGCCACCCA-Cy5- CCCGGCTAGCTACAACGA CGGACG
FQ DNA	GGGCCCGTCCGT-FAM-ATrGrGT-Dabcyl-GGGTGTGCTGGCT
Survivin mRNA	CAAAGACCACCGCATCTCTACATTTTACATCTCTACGCCA CCAGAAAC
TK1 mRNA	AGCAGACAAGTACCACTCCGTGTG
EGR-1 mRNA	ATCGTCCAGTGTGGCCGCGG
AKT1 mRNA	GCTTCACCCCAGCCATGAAGGA
VEGF forward	CGA AAC CAT GAA CTT TCT GC
VEGF reverse	CCTCAGTGGGCACACACTCC
β-actin forward	TGGCACCACACCTTCTACAATG
β-actin reverse	TCTCAAACATGATCTGGGTCATCT

Apparatus

FEI Tecnai G2 F-20 transmission electron microscope (FEI Company, Hillsboro, OR, USA); the Nano Zetasizer (Malvern Panalytical Ltd., Malvern, UK); H1650-W high-speed micro centrifuge (Hunan Xiangyi centrifuge instrument Co., Ltd.); FA604A electronic balance (Shanghai Jingtian Electronic instrument Co., Ltd.);

Cary60 Ultraviolet Spectrophotometer (Agilent Technologies Inc Company, USA); KQ5200B Ultrasonic Cleaner (Kunshan Ultrasonic instrument Co., Ltd.); BD FACSAriall flow Cytometer (BD Company, USA); LSM710 laser confocal microscope-living cell workstation (Zeiss company, Germany); LS-55 fluorescence spectrophotometer (Perkin-Elmer company, USA); El×800 enzyme labeling instrument (Bio Tekinstruments company, USA).

Synthesis of gold nanoparticles

First, prepare 20 nm Au NPs according to the method described in the literature.[1] In simple terms, add 100 μL of $HAuCl_4$ solution with a concentration of 28 mM to 10 mL of deionized water. Heat the solution to boiling and then add 350 μL of sodium citrate solution with a concentration of 38.8 mM. Continue heating the solution at a boiling temperature for 7 minutes, and then allow it to cool naturally to room temperature. Finally, filter the prepared Au NPs through a 0.45 μm Millipore membrane filter and store them at 4 ℃ for later use. Transmission electron microscopy (TEM) images (Fig. S1 A) revealed that the average size of the Au NPs is 20 nm.

Preparation of Au-DNAzyme nanoprobe

First, hybridize the P1 and P2 strands to form a P1/P2 hybrid. Then, modify the P1/P2 hybrid onto the surface of Au NPs using the freeze-thaw method as described by Liu.[2] In simple terms, mix the P1/P2 hybrid with tris (2-carboxyethyl) phosphine (TCEP) in a ratio of 1:20 and allow the reaction to proceed at room temperature for 1 hour. Next, add 20 μL of the TCEP-treated P1/P2 hybrid to 100 μL of AuNP solution.

Place the mixture at -20 °C for 2 hours, then thaw at room temperature and centrifuge to wash the Au-DNAzyme nanoprobe.

Quantification of P1/P2 hybrid loaded on the Au NPs

The quantitation of P1/P2 hybrid on Au NPs was as previous reported.[3] Reacting 1 nM Au NPs with 50 nM TCEP-treated P1/P2 hybrid using a freezing method.The reaction product of the Au-DNAzyme nanoprobe was centrifuged, and the supernatant was collected for fluorescence measurement. The fluorescence of Cy5 was excited at 633 nm and detected at 664 nm. The fluorescence intensity of Cy5, both before and after immobilization, was converted into the molar concentration of the P1/P2 hybrid. This conversion was done using interpolation from standard linear calibration curves that were prepared with known concentrations of the P1/P2 hybrid, using buffers with the same pH and ionic strength. The total amount of the P1/P2 hybrid immobilized on the Au NPs was then calculated. To determine the amount of the P1/P2 hybrid on each Au NPs, the molar concentration of the hybrid was divided by the original concentration of the Au NPs. This calculation allowed for the determination of the quantity of the P1/P2 hybrid present on a single Au NPs.

Fluorescence detection of survivin mRNA and DNAzyme production with the Au-DNAzyme nanoprobe in vitro

To detect survivin mRNA and monitor DNAzyme production, the substrate strand FQ DNA was labeled with FAM/Dabcyl. In a typical experiment, 5 μL of different concentrations of survivin mRNA or other mRNA molecules were added to 445 μL of Tris-HCl buffer (pH 7.4, 100 mM NaCl, 5 mM $MgCl_2$) containing 2.5 nM Au-

DNAzyme nanoprobe and 100 nM FQ DNA. The mixture was incubated at 37 °C for 160 minutes and then subjected to fluorescence measurement. Cy5 fluorescence was excited at 633 nm, and both the excitation and emission slits were set at 10 nm. All experiments were repeated 5 times, and each sample was measured three times.

Cell Culture

T24 cells, HCV-29 cells, HepG2 cells, 7702 cells, and MCF-7 cells were cultured in DMEM medium supplemented with 10% FBS and penicillin-streptomycin (100 U/mL). The cells were incubated at 37 °C in a humidified 5% CO_2 incubator. The culture medium was replaced every two days, and cells in the logarithmic growth phase were selected for subsequent experiments.

Fluorescence imaging of cells

Cell imaging at different incubation time: The mixture of Au-DNAzyme nanoprobe (final concentration of 2.5 nM) and DPAI (final concentration of 5 nM) was added to HepG2 cells, and the cells were incubated for different time periods. After incubation, fluorescence imaging was performed to visualize the cellular.

Different cells imaging: The mixture of Au-DNAzyme nanoprobe (final concentration of 2.5 nM) and DPAI (final concentration of 5 nM) was added separately to HepG2 cells, 7702 cells, T24 cells, and HCV-29 cells. After incubating for 6 hours, fluorescence imaging was performed to visualize the cellular .

Co-incubating normal cells with tumor cells: First, HepG2 cells and 7702 cells are co-cultured together and incubated continuously for 24 hours. Subsequently, a

mixture of Au-DNAzyme nanoprobe (final concentration of 2.5 nM) and DPAI (final concentration of 5 nM) is added to the mixed cells, and they are incubated for 6 hours. After that, fluorescence imaging is performed to observe the cells.

Surviving mRNA down-regulation cell imaging: After treating HepG2 cells with 2 nM YM155 for 24 hours, a mixture containing 2.5 nM of Au-DNAzyme nanoprobe and 5 nM of DPAI is added to the HepG2 cells. The cells are then incubated for 6 hours. Subsequently, fluorescence imaging is performed to observe the cells.

DNAzyme generation cell imaging: The mixture of Au-DNAzyme nanoprobe (final concentration of 2.5 nM) and DPAI (final concentration of 5 nM) is added to HepG2 cells and 7702 cells that have been transfected with FQ DNA (100 nM) using liposomes. The cells are then incubated for 6 hours before fluorescence imaging is performed.

Live/dead imaging of the cells: After incubating HepG2 cells with the Au-DNAzyme nanoprobe (final concentration of 2.5 nM) for 48 hours, the cells were treated with calcein AM and propidium iodide (PI) for imaging.

The excitation wavelength of DAPI was 405 nm, and the fluorescence signal with emission wavelength of 410 nm~480 nm was collected. The excitation wavelength of calcein AM and FAM were 488 nm, and the fluorescence signal with emission wavelength of 495 nm~540 nm was collected. The excitation wavelength of PI was 543 nm, and the fluorescence signal with emission wavelength of 550 nm~620 nm was collected. The excitation wavelength of Cy5 was 633 nm, and the fluorescence signal with emission wavelength of 640 nm~740 nm was collected.

Flow cytometry experiment

7702 cells with HepG2 cells: The mixture of Au-DNAzyme nanoprobe (final concentration of 2.5 nM) and DPAI (final concentration of 5 nM) was separately added to HepG2 cells and 7702 cells. After incubation for 6 hours, the cells were collected.

Surviving mRNA down-regulation: First, HepG2 cells were treated with 2 nM YM155 for 24 hours. Then, Au-DNAzyme nanoprobe (final concentration of 2.5 nM) were added to the HepG2 cells and incubated for 6 hours. After incubation, the cells were collected.

The fluorescence intensity of Cy5 was detected using flow cytometry. Cy5 was excited at a wavelength of 633 nm.

RT-PCR experiment

In the experiment on survivin mRNA expression levels, HepG2 cells are first treated with YM155 (2 nM). Then, Trizol reagent is used to extract total RNA from the treated HepG2 cells, and PrimeScript RT reagent is used to generate cDNA of survivin mRNA. Subsequently, RT-PCR is performed to detect the cDNA and calculate the level of survivin mRNA in the cells.

In the experiment on VEGF mRNA expression levels, HepG2 cells are first treated with Au-DNAzyme nanoprobe for 48 hours. Then, Trizol reagent is used to extract total RNA from the treated cells, and PrimeScript RT reagent is used to generate cDNA of VEGF mRNA. Finally, the cDNA is analyzed using RT-PCR to determine the expression level of VEGF mRNA in the cells.

Western blot experiment

First, treat HepG2 cells with Au-DNAzyme nanoprobe for 48 hours. Then, collect the total cellular proteins by incubating HepG2 cells in a lysis buffer.

After that, determine the protein concentration using the BCA Protein Assay Kit. Then, separate the total cellular proteins using SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis) and transfer them onto a polyvinylidene fluoride membrane. Next, block the membrane with 10% (w/v) non-fat dry milk powder in TBST buffer (20 mM Tris-HCl, 137 mM NaCl, 0.1% Tween-20, pH 7.4) for 2 hours at room temperature. Incubate the membrane overnight at 4 °C with anti-VEGF antibody (1:1000), anti-SUR antibody, and anti-Bcl-2 antibody (all at 1:1000). Afterwards, incubate the membrane at room temperature with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (1:2000) for 2 hours. Anti-β-actin antibody is used as a control. Finally, detect the proteins using enhanced chemiluminescence Western blotting reagents (NCI4106) and analyze them with ChemiDoc XRS+ and Image Lab software.

Determination by MTT test

A group of normal cells (7702 cells) and tumor cells HepG2 cells) were used for toxicity analysis. Using Au-DNAzyme nanoprobe (final concentration of 2.5 nM) and Au NPs (final concentration of 2.5 nM), treat HepG2 cells and 7702 cells separately for 48 hours. Subsequently, 20 μL of MTT was added into the plate and continued incubating for 5-6 h. After that, the culture medium in the plate was discarded and added 100 μL of DMSO. The above obtained products were shaken at a low speed,

and finally detected the absorbance of each well in the 96-well plate at the wavelength of 490 nm by using RT 6000 enzyme labeling instrument.

Results and discussion

Characterization of Au-DNAzyme nanoprobe

To verify the successful preparation of the Au-DNAzyme nanoprobe, we used transmission electron microscope (TEM) to characterize the synthesized nanoparticles. As shown in Fig. S1, the synthesized Au NPs and Au-DNAzyme nanoprobe has a well-dispersed spherical structure, the particle size is uniform, and the average size of Au NPs is about 20 nm (Fig. S1A); the average size of Au-DNAzyme nanoprobe is about 23 nm (Fig. S1B). We conducted EDX analysis on the Au-DNAzyme nanoprobe, and the results indicated the presence of elements including Au, N, C, O, S, and P (Fig. S1C). This finding serves as strong evidence that Au nanoparticles (Au NPs) have successfully loaded DNA, forming the Au-DNAzyme nanoprobe. The Au NPs was characterized by dynamic light scattering (DLS). As shown in Fig. S2, the average fluid diameter of Au NPs is about 31.4 nm; the average fluid diameter of Au-DNAzyme nanoprobe is about 38.8 nm. Due to DNA being immobilized on gold nanoparticles, the particle size of Au-DNAzyme nanoprobes will increase. Compared with the TEM results, the particle size increases, which is caused by the hydration particle size measured in solution. Zeta potential analysis was performed to confirm the assembly of nucleic acids on AuNPs (Fig. S3). The Zeta potential of Au NPs was measured as -22.82 mV. Upon modification with nucleic acids, the zeta potential shifted to -11.93 mV, indicating a decrease in the negative charge on the surface of

AuNPs. As shown in Fig. S4, the UV/Vis spectra of the Au-DNAzyme nanoprobe exhibit characteristic peaks corresponding to Au NPs, nucleic acids, and Cy5 dye. This observation indicates that the P1/P2 strands are immobilized on the surface of Au NPs. These results suggest the successful assembly of Au-DNAzyme nanoprobe.

Determination of surface coverage of oligonucleotides on Au NPs

According to the method described above, the Cy5-labeled P1/P2 hybrid was immobilized on the Au NPs. The quantity of P1/P2 hybrid immobilized on the Au NPs can be quantitatively determined by comparing the Cy5 fluorescence intensity before and after immobilization. The fluorescence intensity values were converted to molar concentrations of the P1/P2 hybrid using a standard linear calibration curve (Fig. S5). The fluorescence intensities before and after immobilization were 157.22 and 51.368, respectively. The concentration of the immobilized P1/P2 hybrid on the Au NPs was calculated using the equation I_{before}-I_{after}=I =2.64C+24.89, resulting in a value of approximately 40 nM for C P1/P2. The concentration of Au NPs was 1 nM. By dividing the molar concentration of the P1/P2 hybrid by the concentration of Au NPs, the surface coverage of the P1/P2 hybrid on each Au NP was estimated to be approximately 40.

DNAzyme production with the Au-DNAzyme nanoprobe in vitro

To validate that mRNA can activate the constructed Au-DNAzyme nanoprobes to generate DNAzyme and cleave VEGF mRNA, we used a substrate chain labeled with FAM and Dabcyl groups to verify the cleavage activity of the DNAzyme. The experimental results are shown in Fig. S6. In the absence of the target survivin mRNA,

the system exhibited low background fluorescence signal. However, upon the addition of the target survivin mRNA, the fluorescence signal of the system significantly increased. This indicates that under the stimulation of survivin mRNA, the displacement reaction of the Au-DNAzyme nanoprobes occurs, resulting in the generation of DNAzyme with cleavage activity. As a result, the substrate chain of VEGF mRNA is cleaved, leading to fluorescence recovery.

The influence of reaction time

To determine the optimal reaction time for the specific binding of Au-DNAzyme nanoprobe to the tumor marker survivin mRNA, we incubated the Au-DNAzyme nanoprobe with a target survivin mRNA concentration of 200 nM for different durations and performed fluorescence detection. As shown in Fig. S7, the fluorescence intensity of Cy5 increased with increasing reaction time. However, beyond 150 min, there was no significant change in the fluorescence signal intensity of Cy5. Therefore, we selected 150 min as the optimal reaction time for detecting the target in the solution system.

Fluorescence detection of survivin mRNA

We further investigated the detection performance of the Au-DNAzyme nanoprobe for the target survivin mRNA. We incubated the Au-DNAzyme nanoprobe with different concentrations of survivin mRNA for 150 min and performed fluorescence measurements. As the concentration of survivin mRNA increased, the fluorescence signal of the system gradually enhanced (Fig. S8A). This is because with

higher survivin mRNA concentration, more survivin mRNA molecules undergo strand displacement reaction with the P1 strand in the Au-DNAzyme nanoprobe, leading to the displacement of more Cy5-labeled P2 strands from the surface of Au NPs and resulting in increased fluorescence intensity. To assess the linear range and detection limit of this method, we performed linear regression analysis on the logarithmic values of Cy5 fluorescence intensity and survivin mRNA concentration. The results showed a good linear relationship between the Cy5 fluorescence intensity and the logarithmic value of survivin mRNA concentration in the range of 0.4 nM to 180 nM (Fig. S8B). The linear equation is: $I = 75.15\lg C + 48.83$ (R^2=0.992), where I represents the fluorescence intensity of Cy5 at 664 nm (a.u) and C represents the concentration of survivin mRNA (nM). The detection limit of this method for survivin mRNA is 0.28 nM.

Specificity test

To investigate the selectivity of the Au-DNAzyme nanoprobe for survivin mRNA detection, we conducted fluorescence determination using the Au-DNAzyme nanoprobe with target survivin mRNA, other endogenous mRNA (ATK1 mRNA, EGR-1 mRNA, and TK1 mRNA), and corresponding mixtures at the same concentration (200 nM). The experimental results are shown in Fig. S9. When the target survivin mRNA was introduced into the Au-DNAzyme nanoprobe, the fluorescence intensity of the system increased. Under the same experimental conditions, when other endogenous mRNA with the same concentration was added to the Au-DNAzyme nanoprobe, the fluorescence intensity of the system did not change

significantly compared to the control group. When mixtures containing target survivin mRNA and other endogenous mRNA were added to the Au-DNAzyme nanoprobe, the fluorescence intensity of the system was similar to the systems where only survivin mRNA was added. These experimental results demonstrate that the Au-DNAzyme nanoprobe exhibits good specificity in responding to survivin mRNA.

Incubation time of fluorescence imaging in HepG2 cells

The incubation time of Au-DNAzyme nanoprobe in cells can affect the binding degree between P1 strand and target survivin mRNA. Therefore, HepG2 cells were incubated with the Au-DNAzyme nanoprobe for 1 h, 2 h, 4 h, 6 h, and 8 h, and fluorescence imaging was performed using confocal laser scanning microscopy to explore the optimal incubation time. The results are shown in Fig. S10. After 1 hour of incubation, fluorescence signals of Cy5 were observed within the cells, and the fluorescence intensity gradually increased with the incubation time. This is because the Au-DNAzyme nanoprobe specifically binds to the target survivin mRNA, causing the P2 strand to detach from the Au NPs and resulting in fluorescence recovery of Cy5. At 6 hours of incubation, a strong fluorescence signal was observed, and there was no significant change in fluorescence signal with further incubation time, indicating that the binding between the nanoprobe and target survivin mRNA reached equilibrium after entering the cells. Therefore, we selected 6 hours as the optimal incubation time for the nanoprobe in subsequent cellular experiments.

Au-DNAzyme nanoprobe for fluorescence imaging analysis of survivin mRNA in

normal cells and tumor cells

To further validate the applicability of the constructed nanoprobe for survivin mRNA imaging and its ability to distinguish between normal and tumor cells, T24 cells and HCV-29 cells were co-cultured in the same cell culture dish. After incubation with the Au-DNAzyme nanoprobe at a concentration of 2.5 nM for 6 hours, confocal fluorescence imaging was performed. The experimental results are shown in Fig. S11. Only T24 cells exhibited fluorescence signals of Cy5, indicating specific binding of the nanoprobe to survivin mRNA in these cells. In addition, flow cytometry was used to measure the fluorescence intensity of Cy5 in 7702 cells and HepG2 cells (Fig. S12). The results showed that HepG2 cells had significantly higher Cy5 fluorescence intensity compared to 7702 cells, while the Cy5 fluorescence intensity in 7702 cells was similar to that of the control group. This is consistent with the results of different cells fluorescence imaging experiments (Fig. 2), further demonstrating the ability of the constructed Au-DNAzyme nanoprobe to distinguish between normal and tumor cells.

Analysis of survivin mRNA down-regulated cells

To validate the relationship between the signal output of the Au-DNAzyme nanoprobe and the expression level of survivin mRNA in cells, we introduced the Au-DNAzyme nanoprobe into YM155-treated HepG2 cells and performed cell imaging analysis and RT-PCR analysis. As shown in Fig. S13, the expression level of survivin mRNA was significantly reduced in YM155-treated HepG2 cells. Additionally, the

Cy5 fluorescence signal in YM155-treated HepG2 cells was noticeably decreased (Figs. S14-S15). This is attributed to the inhibitory effect of YM155 on the expression level of survivin mRNA. As a result, the activation of the Au-DNAzyme nanoprobe by the target decreased, leading to a significant reduction in fluorescence intensity compared to untreated cells. These experiments demonstrate that the fluorescence signal output of the Au-DNAzyme nanoprobe is associated with the expression level of survivin mRNA in cells, indicating its excellent specificity.

The expression level of VEGF mRNA and VEGF

To evaluate the inhibitory effect of Au-DNAzyme on VEGF mRNA and VEGF in cells, HepG2 cells were treated with 2.5 nM Au-DNAzyme nanoprobe for 48 hours, and the expression level of VEGF mRNA was measured using RT-PCR. As shown in Fig. S16, the expression of VEGF mRNA was significantly inhibited by 43.61% due to gene silencing. Furthermore, the impact of gene silencing on the corresponding protein level was assessed using the Western blot method, as shown in Fig. S17. After treatment with the Au-DNAzyme nanoprobe, the expression of VEGF protein was noticeably suppressed, consistent with the RT-PCR results. These findings indicate that VEGF mRNA-specific DNAzyme was generated in situ in HepG2 cells, resulting in the inhibition of VEGF mRNA and VEGF protein expression.

Reference:

1. H. Zhang, X. Xu and W. Jiang, *Chem. Sci*., 2020, **11**, 7415-7423.

2. B. Liu and J. Liu. *J. Am. Chem. Soc*., 2017, **139**, 9471-9474.

3. A. P. Alivisatos, K. P. Johnsson, X. Peng, T. E. Wilson, C. J. Loweth, M. P. Bruchez and P. G. Schultz, Nature, 1996, 382, 609-611.

Supporting Figures

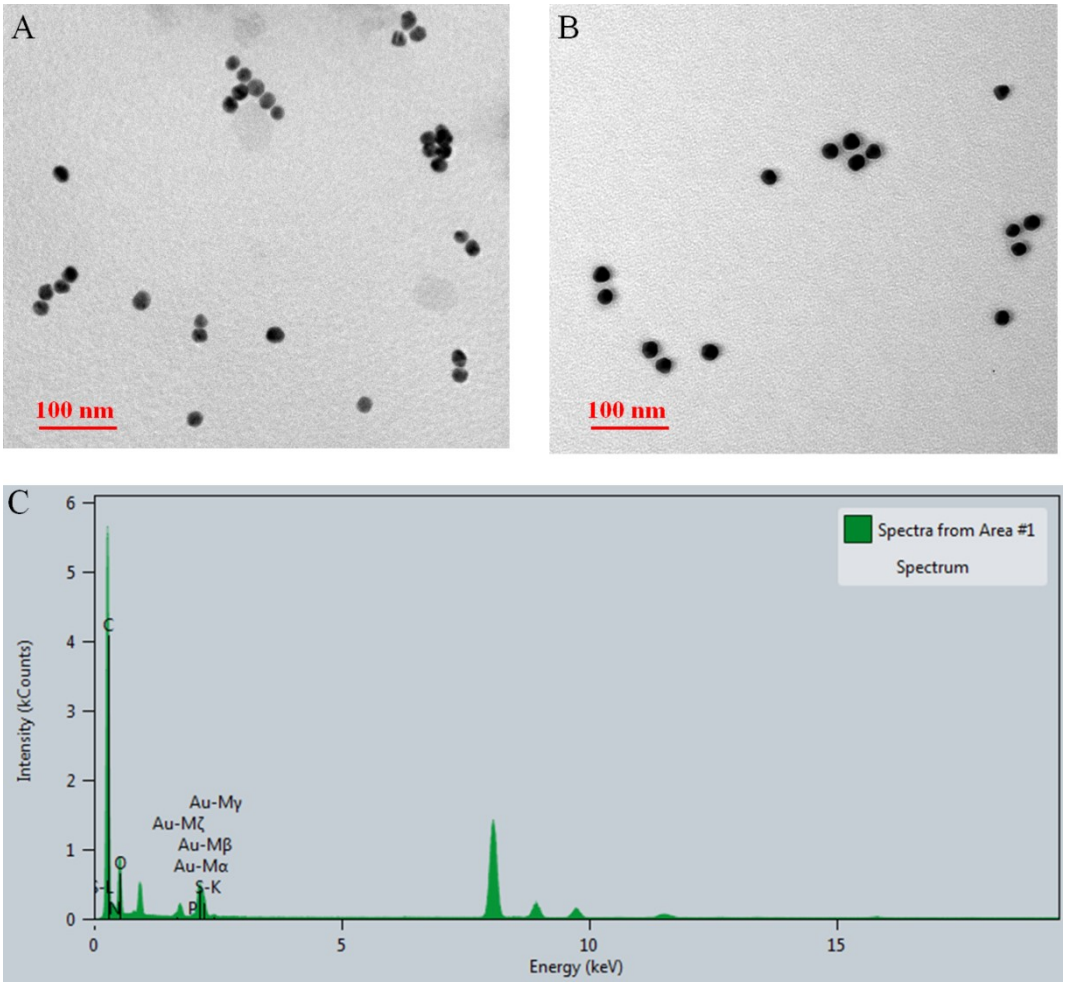

Fig. S1. (A) TEM of Au NPs; (B) TEM of Au-DNAzyme nanoprobe; (C) Energy Dispersive X-Ray Spectroscopy of Au-DNAzyme nanoprobe.

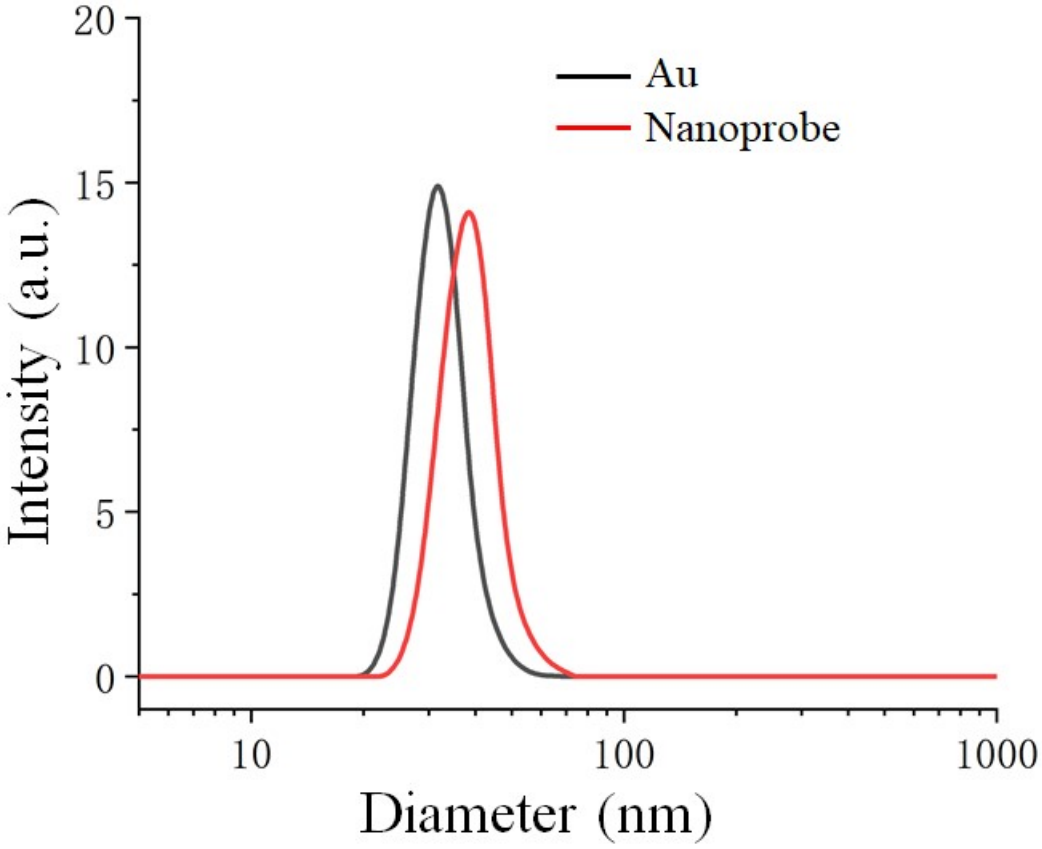

Fig. S2. DLS of Au NPs and Au-DNAzyme nanoprobe.

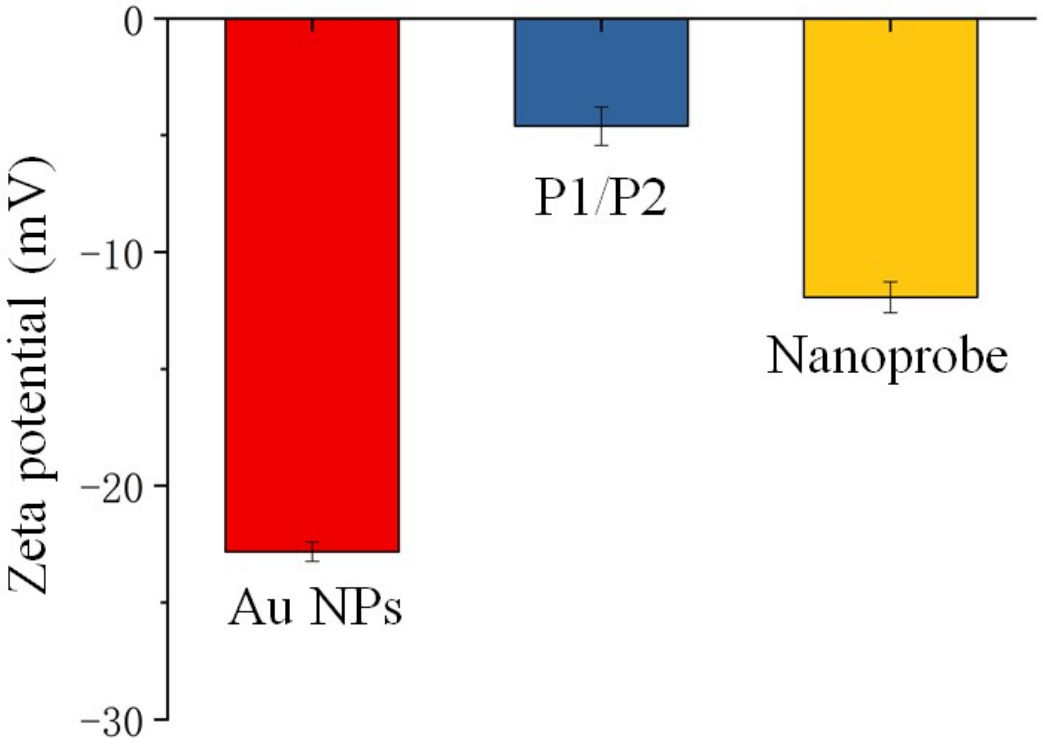

Fig. S3. Zeta potential analysis of Au NPs, P1/P2 hybrid and Au-DNAzyme nanoprobe. Error bars were derived from N=5 experiments.

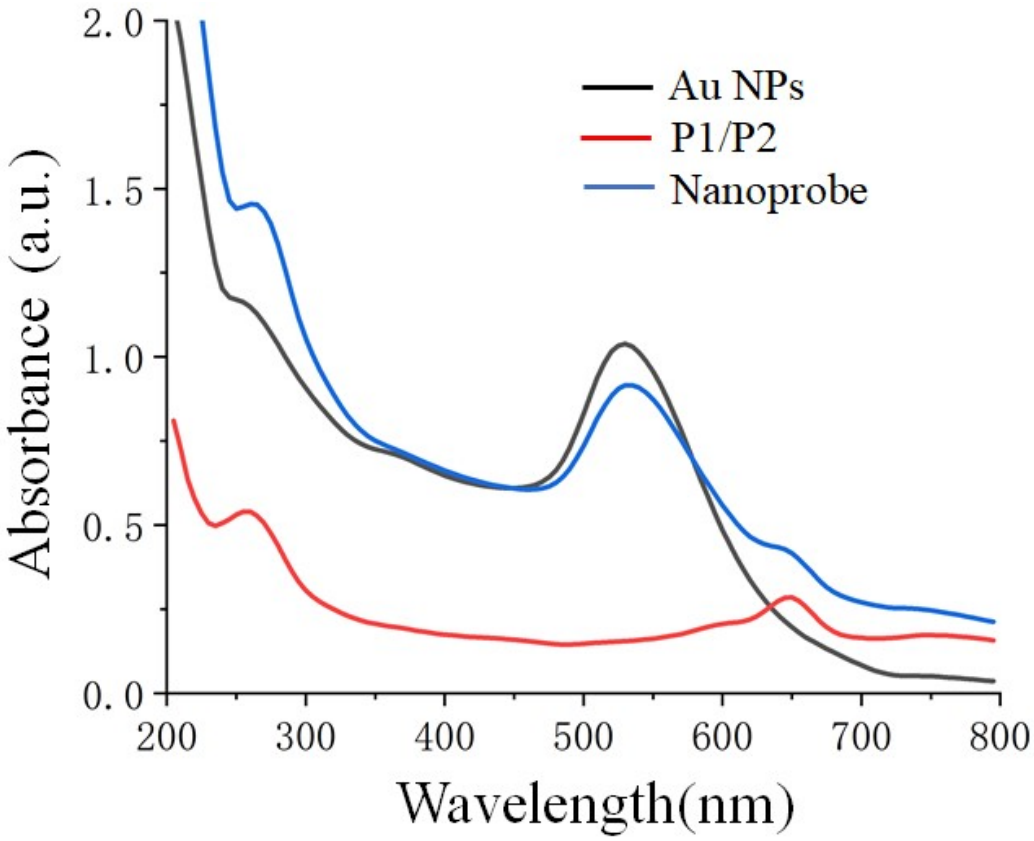

Fig. S4. The UV-vis spectrum of the Au NPs, P1/P2 hybrid and Au-DNAzyme nanoprobe.

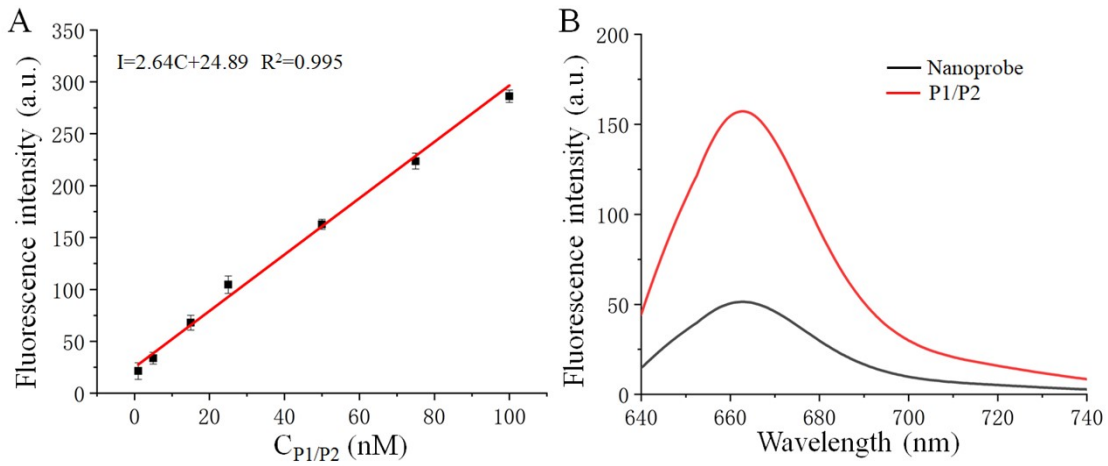

Fig. S5. (A) The standard linear calibration curves of the P1/P2 hybrid; (B) The fluorescence spectra of the P1/P2 hybrid before and after were immobilized on the Au NPs in liquid supernatant. Error bars were derived from n=5 experiments.

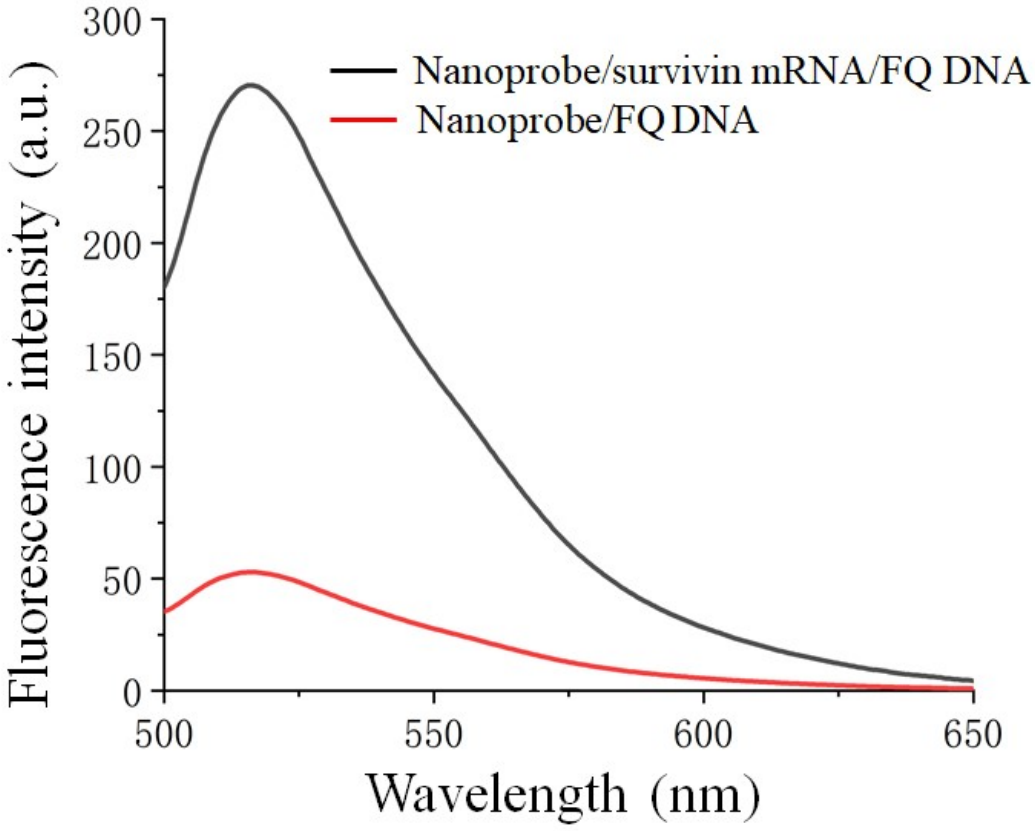

Fig. S6. Fluorescence spectra of the DNAzyme substrate chain FQ DNA upon the detection of survivin mRNA. All samples contained 5 mM Mg^{2+}.

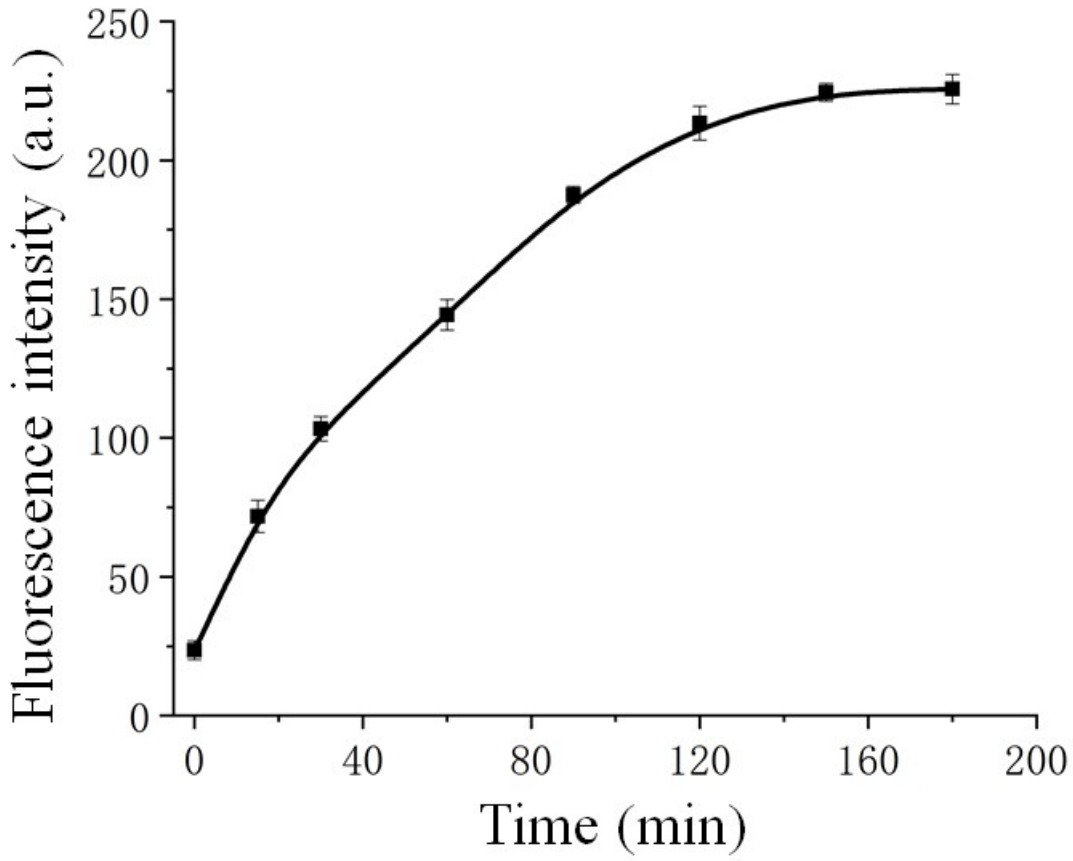

Fig. S7. The effect of different reaction time on survivin mRNA detection (the final concentration of survivin mRNA is 200 nM, the reaction time is 0 min, 15 min, 30 min, 60 min, 90 min, 120 min, 150 min, 180 min; Cy5 excitation wavelength is 633 nm, emission wavelength is 664 nm, excitation and emission gap width is 10 nm, voltage is 750 V). All samples contained 5 mM Mg^{2+}. Error bars were derived from n=5 experiments.

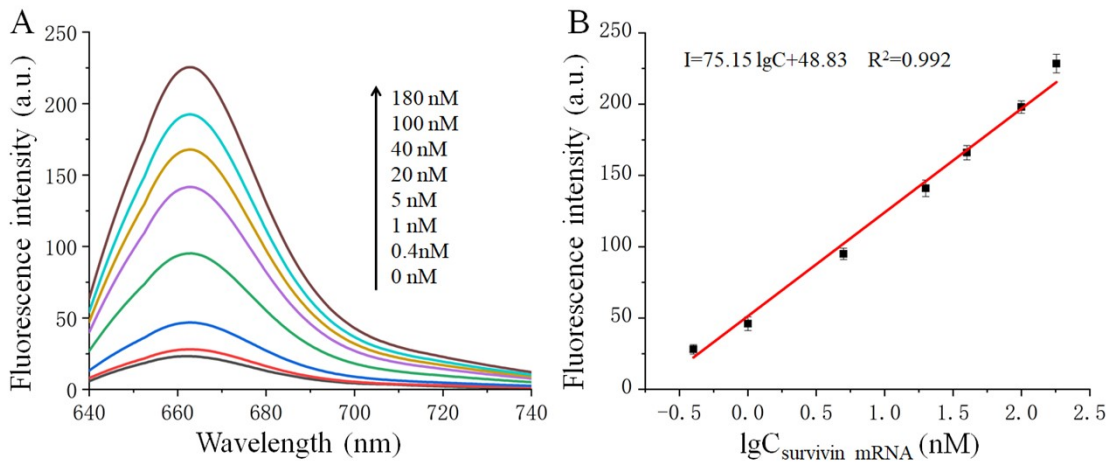

Fig. S8. (A) Fluorescence spectra of the proposed Au-DNAzyme nanoprobe upon the detection of survivin mRNA at different concentrations (0, 0.4, 1, 5, 20, 40, 100 and 180 nM). (B) Calibration curves corresponding to the Au-DNAzyme nanoprobe. All samples contained 5 mM Mg^{2+}. Error bars were derived from n=5 experiments.

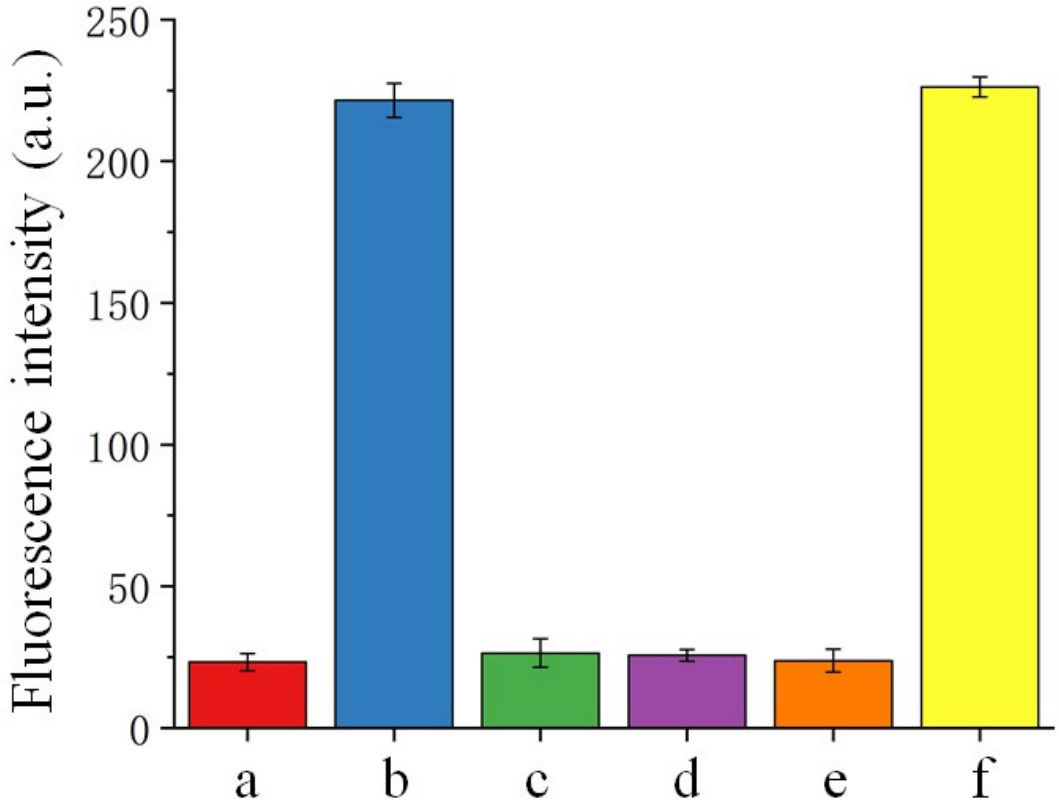

Fig. S9. Specificity response of Au-DNAzyme nanoprobe to different targets (a: control; b: survivin mRNA; c: TK1 mRNA; d: ATK1 mRNA; d: EGR-1 mRNA; d: mixture). All samples contained 5 mM Mg^{2+}. Error bars were derived from n=5 experiments.

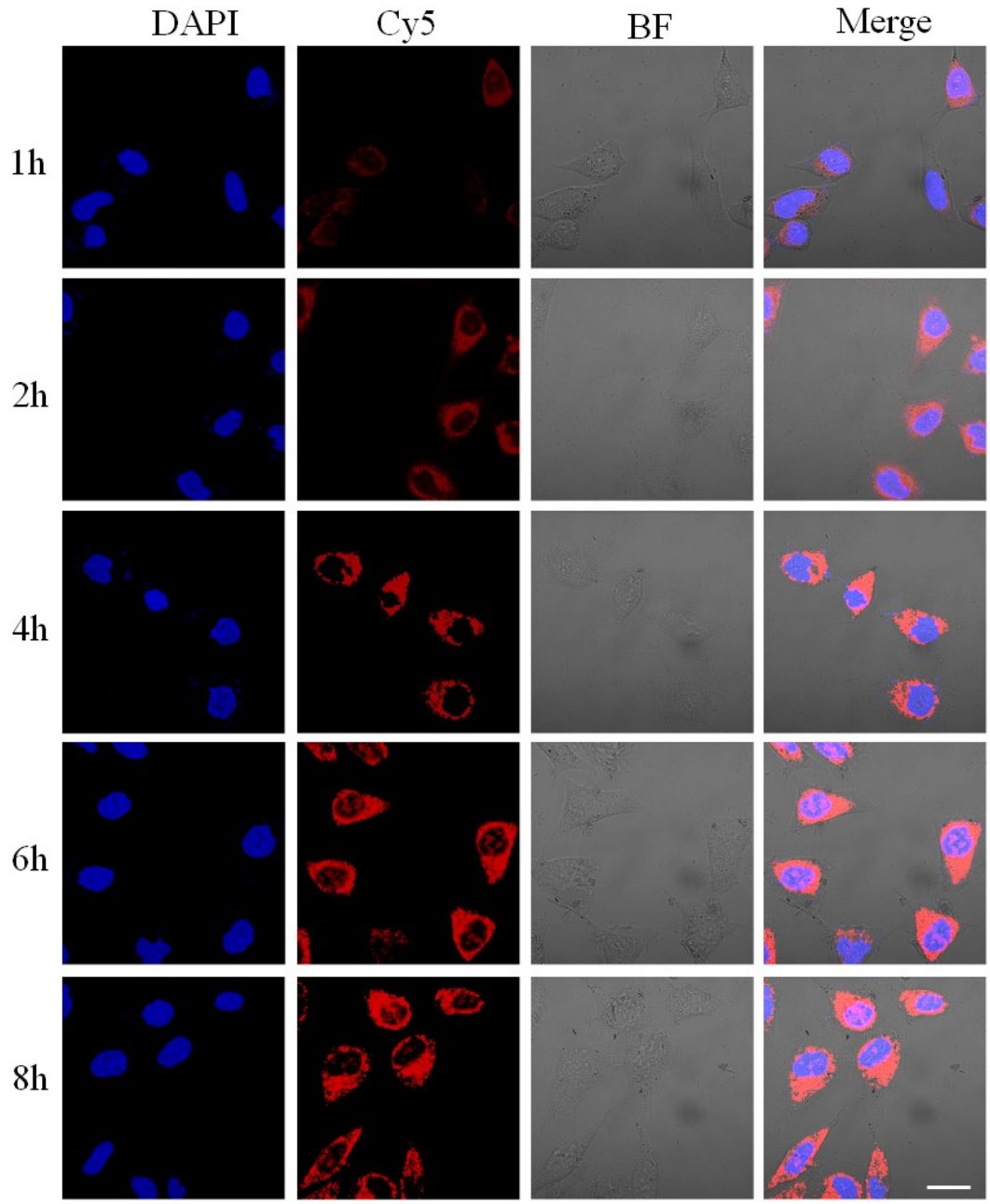

Fig. S10. Fluorescence imaging of survivin mRNA in HepG2 cells after incubation with the Au-DNAzyme nanoprobe (2.5 nM) for different time at 37 ℃. The fluorescence of DAPI and Cy5 was measured with the excitation wavelengths of 405 and 633 nm, respectively. Scale bar: 20 μm.

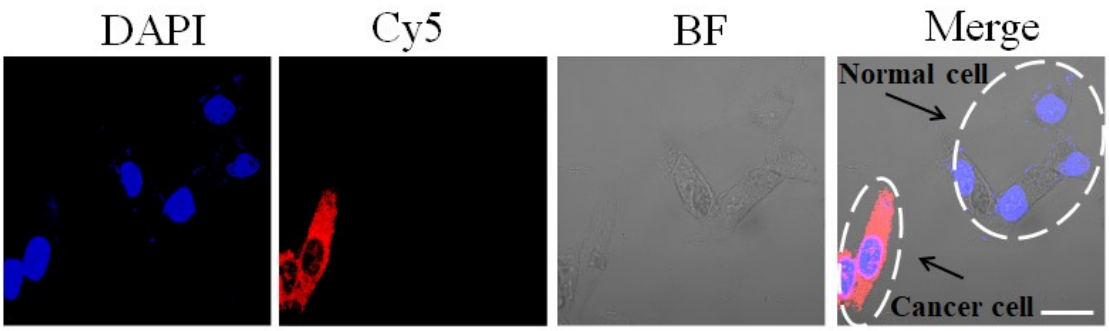

Fig. S11. Fluorescence targeting imaging analysis of survivin mRNA in co-cultured HepG2 cells and 7702 cells. Scale bar: 20 μm.

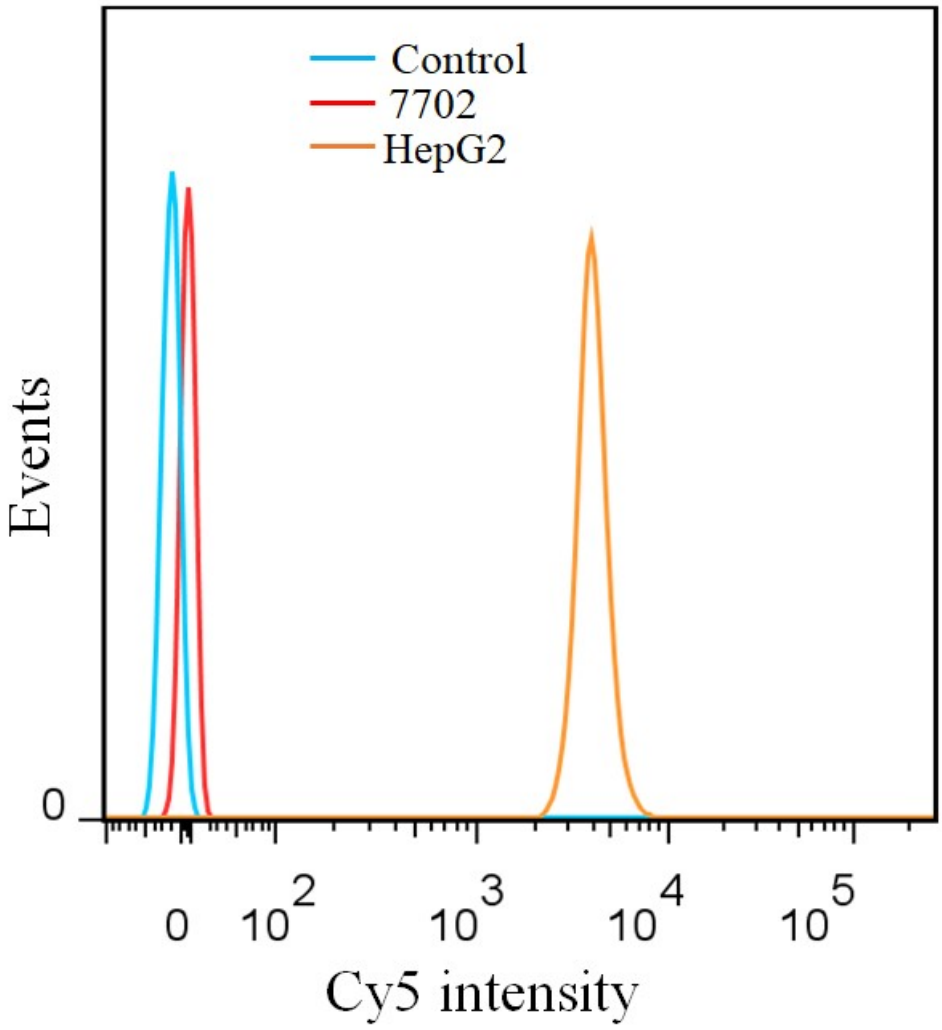

Fig. S12. Flow cytometry analysis of Cy5 fluorescence in HepG2 tumor cells and 7702 normal cells with the Au-DNAzyme nanoprobe.

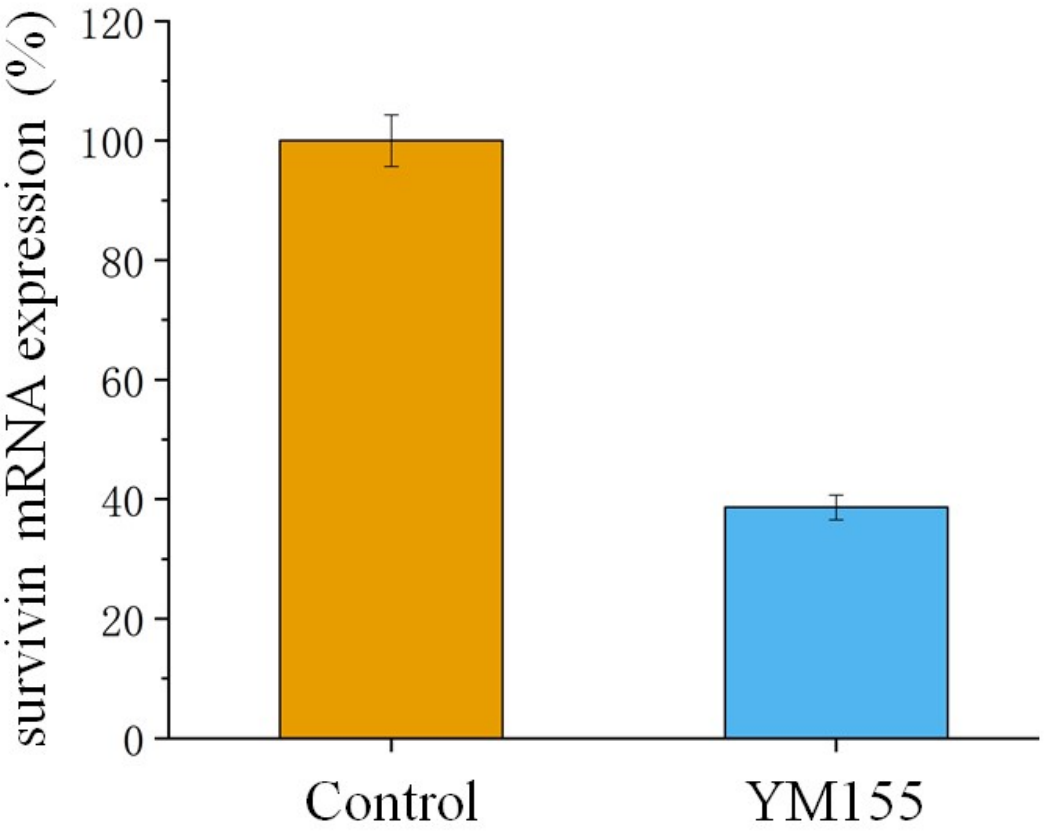

Fig. S13. The survivin mRNA expression in HepG2 cells treated with YM155 (2 nM).

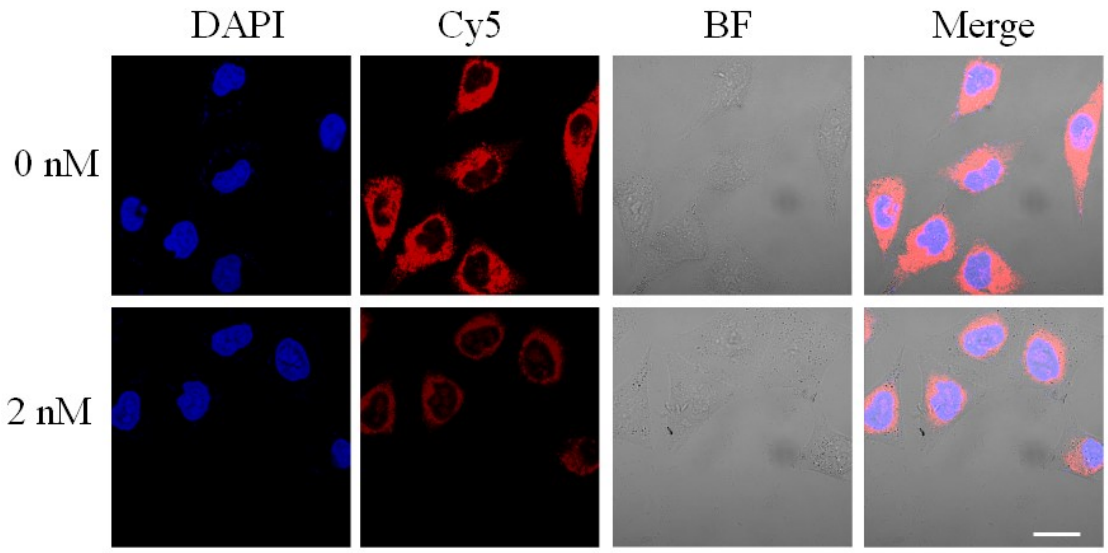

Fig. S14. Au-DNAzyme nanoprobe fluorescence imaging of HepG2 cells treated with YM155.

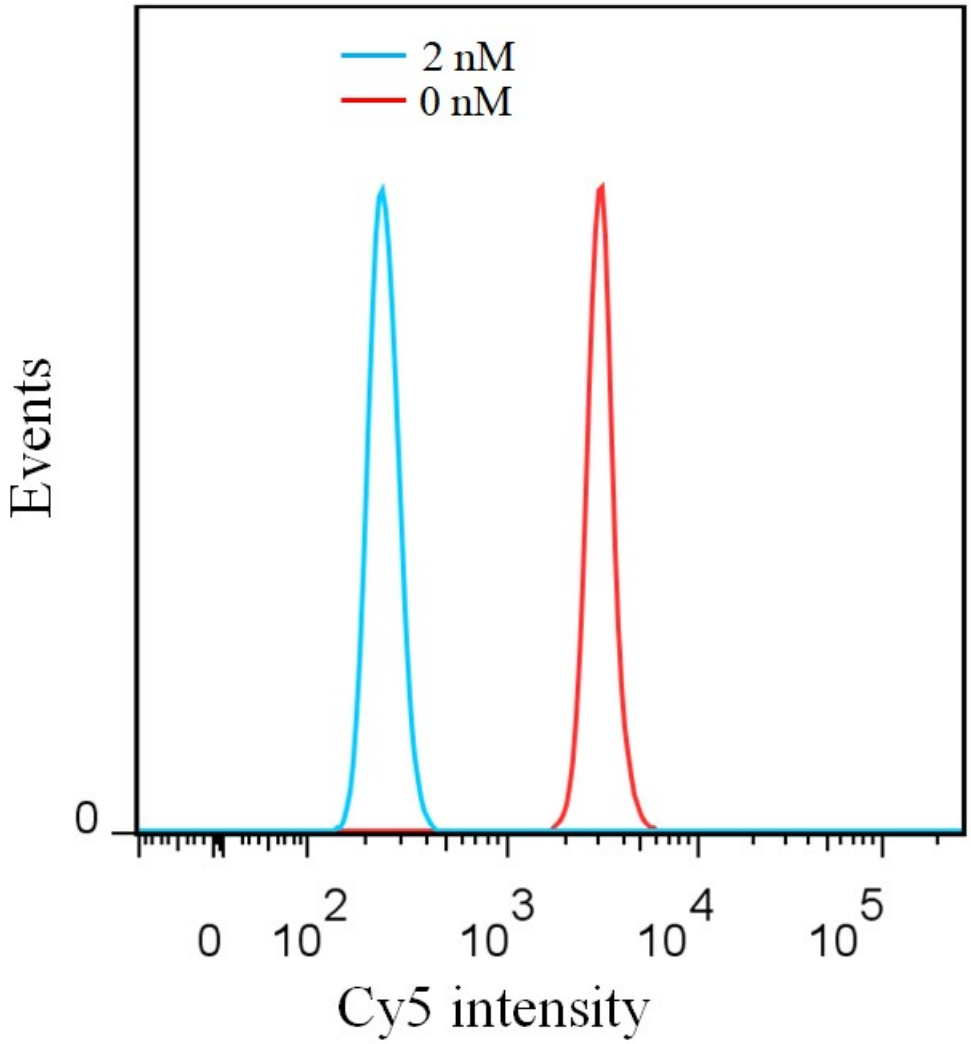

Fig. S15. Survivin mRNA in HepG2 cells treated with YM155 was analyzed by flow cytometry.

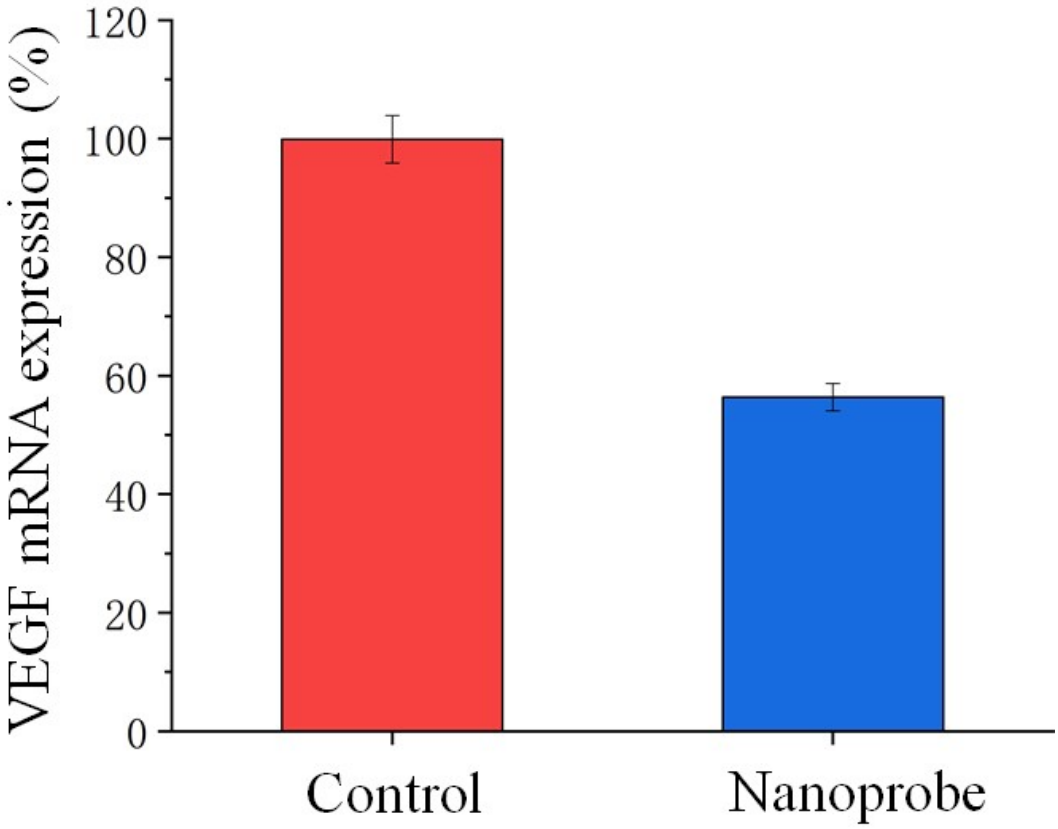

Figure S16.The VEGF mRNA expression in HepG2 cells treated with Au-DNAzyme nanoprobe (2.5 nM) for 48h.

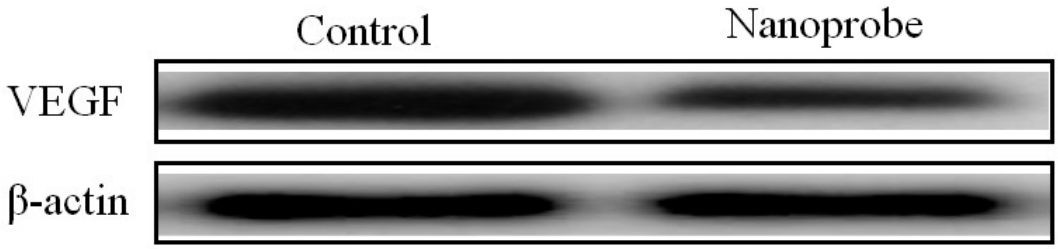

Fig. S17. Western blot assay results of the expressions of VEGF proteins in HepG2 cells after incubated with Au-DNAzyme nanoprobe (2.5 nM) for 48 h.