Electronic Supplementary Information (ESI)

A novel FRET-based dendritic hybridization chain reaction for tumour-related mRNA imaging

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Experimental Section

Chemicals and Reagents.

Lipofectamine 3000 and Opti-Mem were obtained from Invitrogen (Thermo Fisher Scientific, USA). All Oligonucleotides were custom-designed by NUPACK (http://www.nupack.org/), then synthesized and HPLC purified by Sangon Biotechnology Co., Ltd (Shanghai, China). They were used as provided and diluted in SPSC buffer (0.75M NaCl, 50 mM NaH_2PO_4, pH 7.4) to give stock solution of 50 μM. Hoechst 33342 was obtained from Invitrogen Life Technologies Corporation. Loading buffer was purchased from TaKaRa Bio Inc. (Dalian, China). SYBR Gold was purchased from Invitrogen (U.S.A.). All the chemicals were of analytical grade and used without further purification. All cell lines were purchased from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). All the solutions were prepared by sartorius ultrapure water (18.2 MΩ cm, Milli-Q, Millipore). The sequences of the oligonucleotides are listed in Table S1.

Apparatus. All fluorescence spectra were carried out on a QM40-NIR (Switzerland). Gel images were obtained from multifunctional molecular imaging analysis system Azure C600 (America). Cells were incubated in a humidified HF90 CO_2 incubator (Shanghai Lishen Scientific Equipment Co.Ltd), and were visualized under Nikon A1+Ti2 laser confocal scanning system. The Atomic Force Microscope (AFM) was performed using Bioscope system (Bruker, USA).

Agarose Gel Electrophoresis. For DNA polymers produced by B-HCR or L-HCR, 100 nM of initiator were incubated with DNA hairpins (the concentration of each kind of DNA hairpins was 1μM) in SPSC buffer for 2h at 37 °C. To verify the successful assembly of the DNA nanostructures produced by two kinds of Hybridized Chain Reaction, the 10 μL DNA solution was mixed with 2 μL 6×loading buffer and 2 μL SYBR Gold, and then subjected to the 3% agarose gel electrophoresis. The electrophoresis was conducted in 1 × TBE buffer (89 mM Tris, 89 mM boric acid, 2.0 mM EDTA 8.3) at constant voltage of 100 V for 1 h. To test the feasibility of Tk1 mRNA detection by B-HCR, 200 nM target were incubated with hairpin mixture solution (B1+B2+H1+H2) for 2h at 37 °C. The electrophoresis process was as same as the above.

AFM Characterization. For AFM characterization of two kinds of HCR products, the sample was diluted in SPSC buffer that contained H1+H2or LH2(200 nM each) and initiator I (20 nM). Freshly cleaved mica was incubated with 20 μL 100 mM $NiCl_2$ buffer about 15 minutes to bear positive charges on its surface, for loading samples and imaging. DNA samples were diluted and deposited on the surface of the modified mica for 10 minutes to be totally combined with mica surface, followed by rinsing with ultrapure water and drying under a stream of nitrogen. Then the well-prepared samples were imaged by a Multimode 8 Atomic Force Microscope in SCANASYST-AIR Mode.

***In Vitro* Fluorescence Assay.** All assays were prepared in SPSC buffer containing (0.75M NaCl, 50 mM NaH_2PO_4, pH 7.4). All DNA hairpins (5μM) were heated to 95 °C for 5 min and then allow cool down to room temperature for at least 2h before use. Different concentrations of initiator were added to DNA nanoprobe (H1+H2 or H1+LH2) solutions. After incubation for 5h, the fluorescence emission spectrum of the mixture was collected from 550 nm to 800 nm, using the maximal excitation

wavelength at 530 nm. The slit width of the excitation and the emission light path was set to 10 nm. In the kinetic study, 20 nM Tk1 mRNA was employed at 37 °C. For the mRNA selectivity test, certain concentrations of Survivin, K-ras, HER-2, GalNAc-T, C-myc were mixed with the amplification system at the same concentration as Tk1 mRNA. The following steps were the same as mentioned above. All experiments were repeated at least three times.

Cell Culture and Transfection. MCF-7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), besides HepG2 cells and L02 cells were cultured in RPMI-1640 medium, which were both supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin at 37 °C in a humidified 5% CO2-containing atmosphere. The cells were grown to 80% confluence for 24 h before transfection with probes. The cell density was determined by using a hemocytometer. Probe transfection method was performed under the guidance of manufacture's protocol. Briefly, 1.25μl lipofectamine 3000 and certain concentration of DNA probes were mixed in 86μl Opti-MEM, then diluted into 200 μl with certain kind of culture medium and incubated at 37°C for 15 minutes.

Confocal Fluorescence Imaging. All cells were separately seeded in 35nm confocal dishes for 24 h. After washing 3 times with D-PBS, cells were incubated with 200 nM DNA nanoprobes in a 200 μl culture medium mixture for 2 to 10 hours respectively to obtain the best reaction response time. After incubation, dishes were washed by D-PBS twice and subjected to confocal imaging. To determine the variations of TK1 mRNA, cell dishes were incubated with 200 nM TK1 mRNA mimics or anti-TK1 mimics for 3h before incubation with DNA imaging probes. The image of all cells was observed under Nikon confocal scanning system. Cy3 fluorescence was excited by 561 nm and the emission was recorded in green channel from 570 to 620 nm; while Cy5 fluorescence image was received in red channel with 561 nm excitation and emission recorded from 663 to 738 nm. All cells were observed under 100X oil immersion objective. The fluorescence images were presented after handling by Image Proplus 6.0 software.

Cell Co-localization Assays. MCF-7 cells were seeded in a 35-mm confocal dish for 24 h at 37 ℃, and then incubated with DNA probes (concentrations were same with the probes used in cell imaging experiments) for 8 h. After incubation, D-PBS buffer was employed to rinse the cells 3 times for removing the remaining probes. Subsequently, the cells were treated with 5μg /mL hoechst-33342 for 15 min. Then, the cells were washed 2 times by D-PBS and subjected to confocal microscope imaging. The fluorescence of hoechst was carried out at the excitation of 405 nm and emissions were collected in blue channels. The excitations and emissions of Cy3 and Cy5 were collected in green and red channels.

Reverse transcriptase-PCR analysis. Total cellular mRNAs were extracted from MCF-7 cells, HepG2 cells and L02 cells by using Trizol reagent (Sangon Biotechnology Co., Ltd., Shanghai, China) under the guidance of manufacturer's protocol. The cDNA samples were prepared by using the reverse transcription (RT) reaction with AMV First Strand cDNA Synthesis Kit (BBI, Toronto, Canada). qRT-PCR analysis of mRNA was performed with SG Fast qPCR Master Mix (2X) (BBI) on a

LightCycler480 Software Setup (Roche). The TK1 mRNA expression level was calculated by normalizing to the expression of GAPDH and using the 2–ΔΔCt method.

Supporting Tables:

Oligo	Sequence (5'-3')
Initiator	AGTGGATTCGGCGTGGTTTAG
H1	CTA AACCACGCCGAATCCACT(Cy3)CAAGTA AGTGGATTCGGC GTG
H2	AGTGGATTCGGCGTGGTTTAGAGTGGATTCGGCGTGGTTTAGCC GAATCCACTCACGCCGAATCCACTTACTTG(Cy5)
L-H2	AGTGGATTCGGCGTGGTTTAGCACGCCGAATCCACTTACTTG(Cy 5)
B1	TTCGGCGTGGTTTAGAGACGAATACACCAGGGAGAACAGAAAC TGTATTCGTCTCTAAA
B2	GGTGCTGGCACAGGCTTCTTTGTATTCGTCTCTAAACCACGCCG AAAGTGGATTCGGCGTGGTTTAG
TK1	GUU UCU GUU CUC CCU GGG AAG CCU GUG CCA GCA CC
Anti-TK1 mimics	GGUGCUGGCACAGGCUUCCCAGGGAGAACAGAAAC(Inverted dT)
TK1 mimics	GUU UCU GUU CUC CCU GGG AAG CCU GUG CCA GCA CC(Inverted dT)
Survivin	GACCACCGCAUCUCUACAUUCAAGAACUGGCCCUU
K-ras	AUGACUGAAUAUAAACUUGUGGUAGUUGGAGCUGG
C-myc	AAUAGGGGGCUUCGCCUCUGGCCCAGCCCUCCCGC
GalNac-T	GCUUUGCCCCGGGGCTGGGGUGCAACA GACUCUUA

Table S1. Oligonucleotide sequences used in this work.

Supporting Figures:

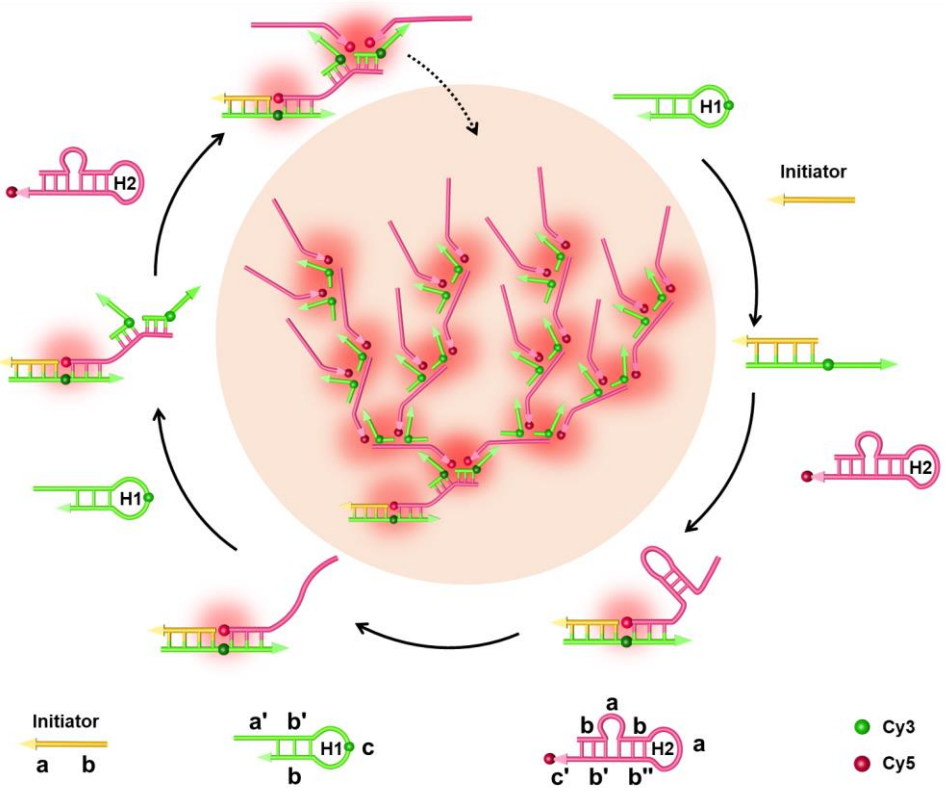

Figure S1. Schematic illustration of the proposed D-HCR. Here, H1 and H2 are labelled with fluorescence donor (Cy3) and acceptor (Cy5) at appropriate positions, respectively.

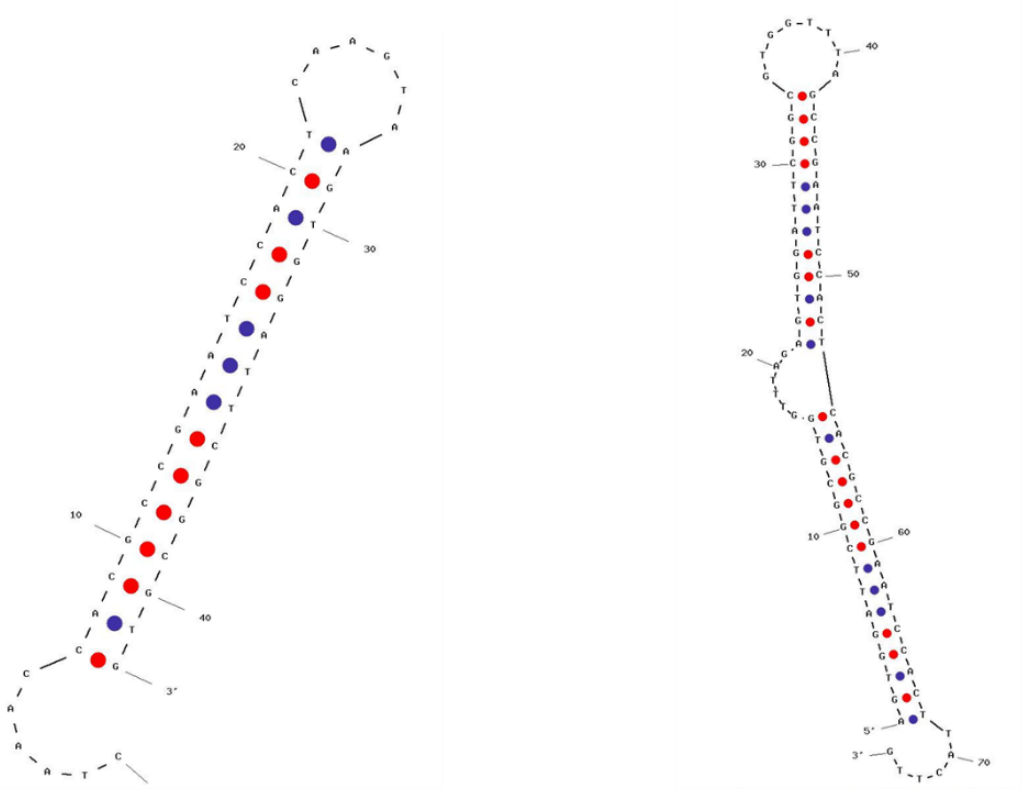

Figure S2. Secondary structure of H1 and H2, which were simulated by IDTDNA software (www.idtdna.com).

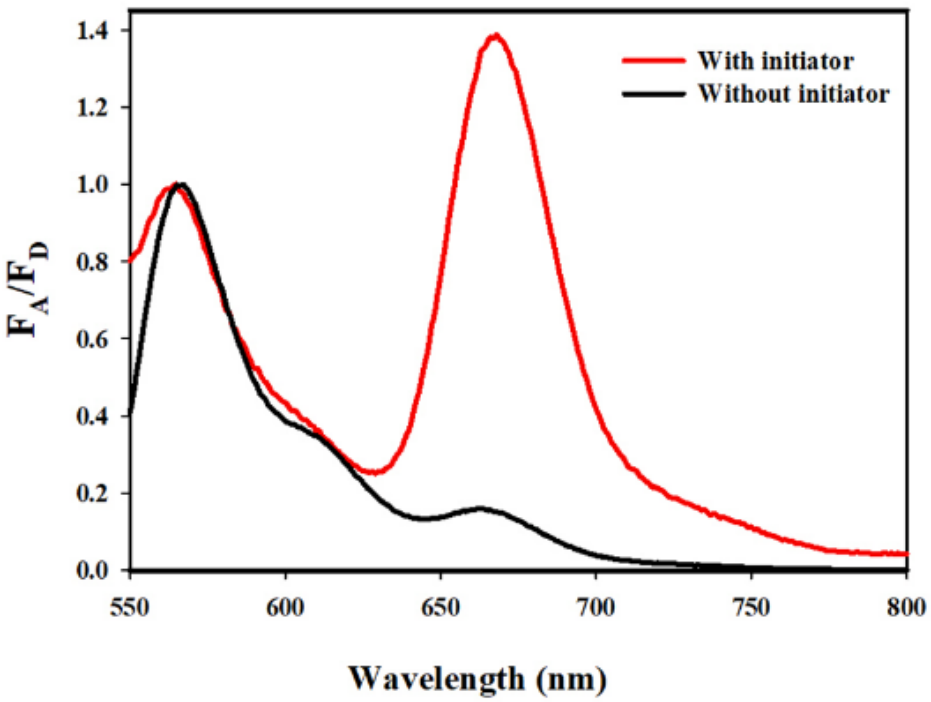

Figure S3. FRET ratios (Cy3 and Cy5) of D-HCR system were recorded with or without initiator, respectively.

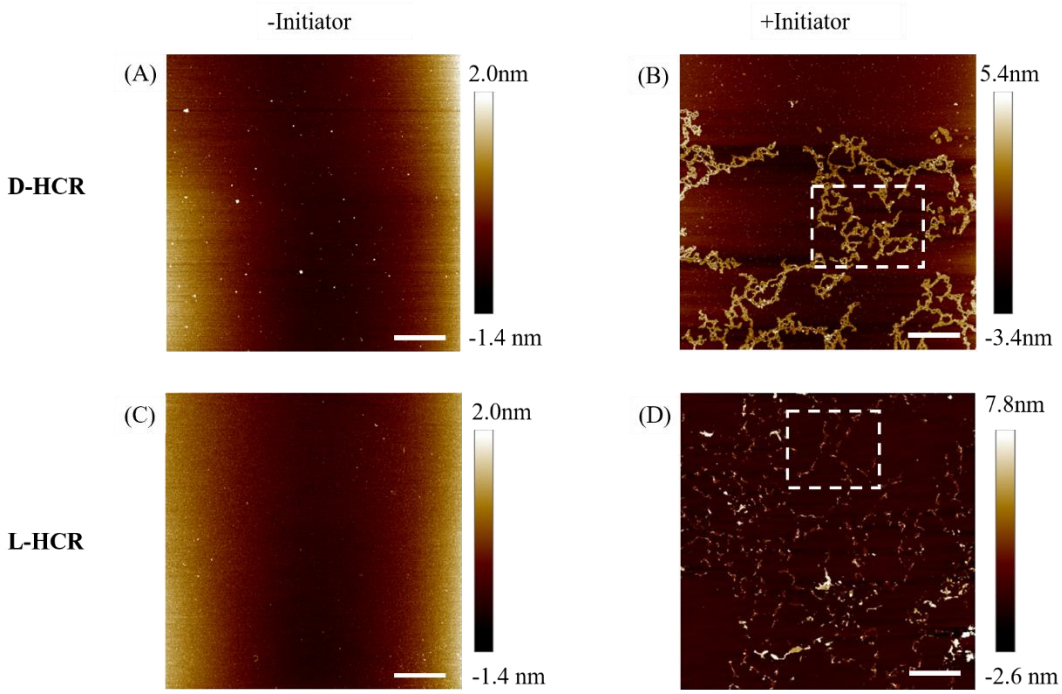

Figure S4. AFM characterization of HCR products. (A) and (B) were D-HCR products with or without initiator. (C) and (D) were L-HCR products with or without initiator. The scale bar is 2 μm.

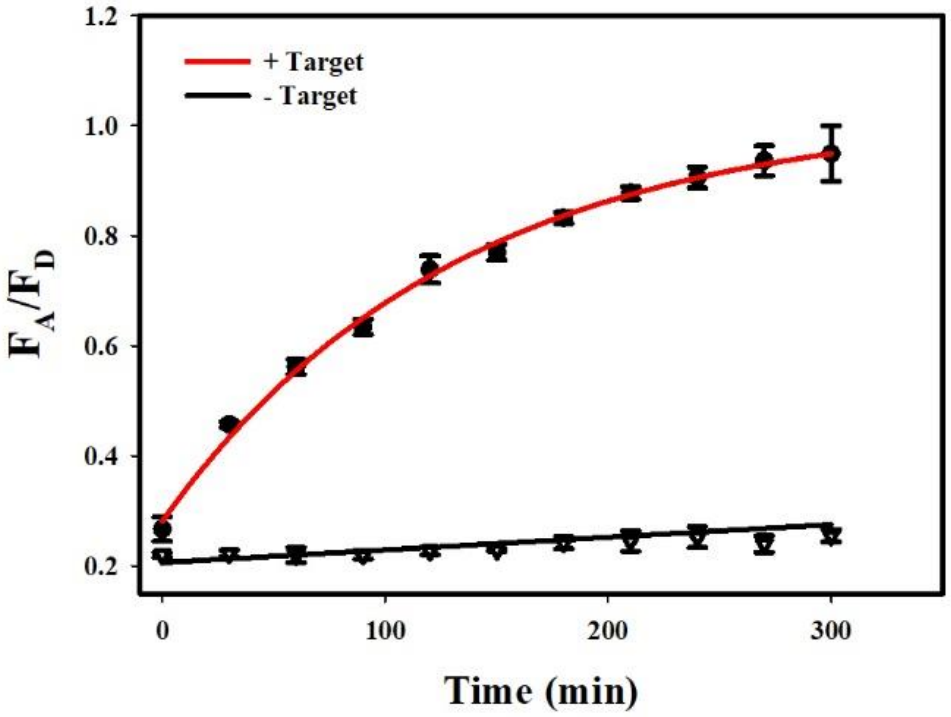

Figure S5. Kinetics analysis of the D-HCR system with or without target TK1 mRNA, respectively.

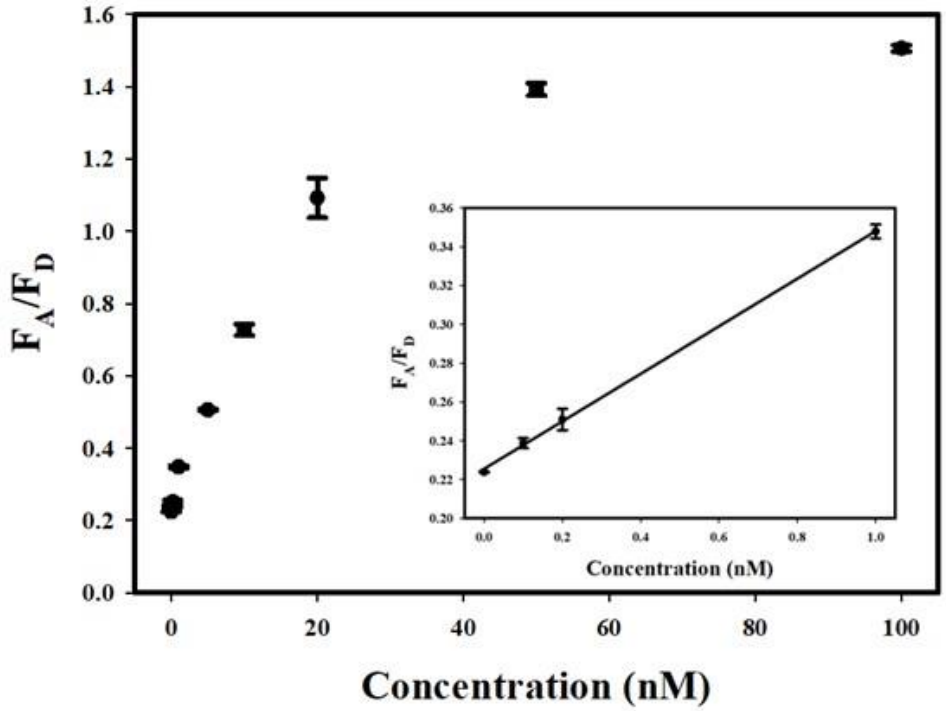

Figure S6. Response of the D-HCR system to different concentrations of target TK1 mRNA. Corresponding calibration curves for TK1 mRNA at a low concentration range.

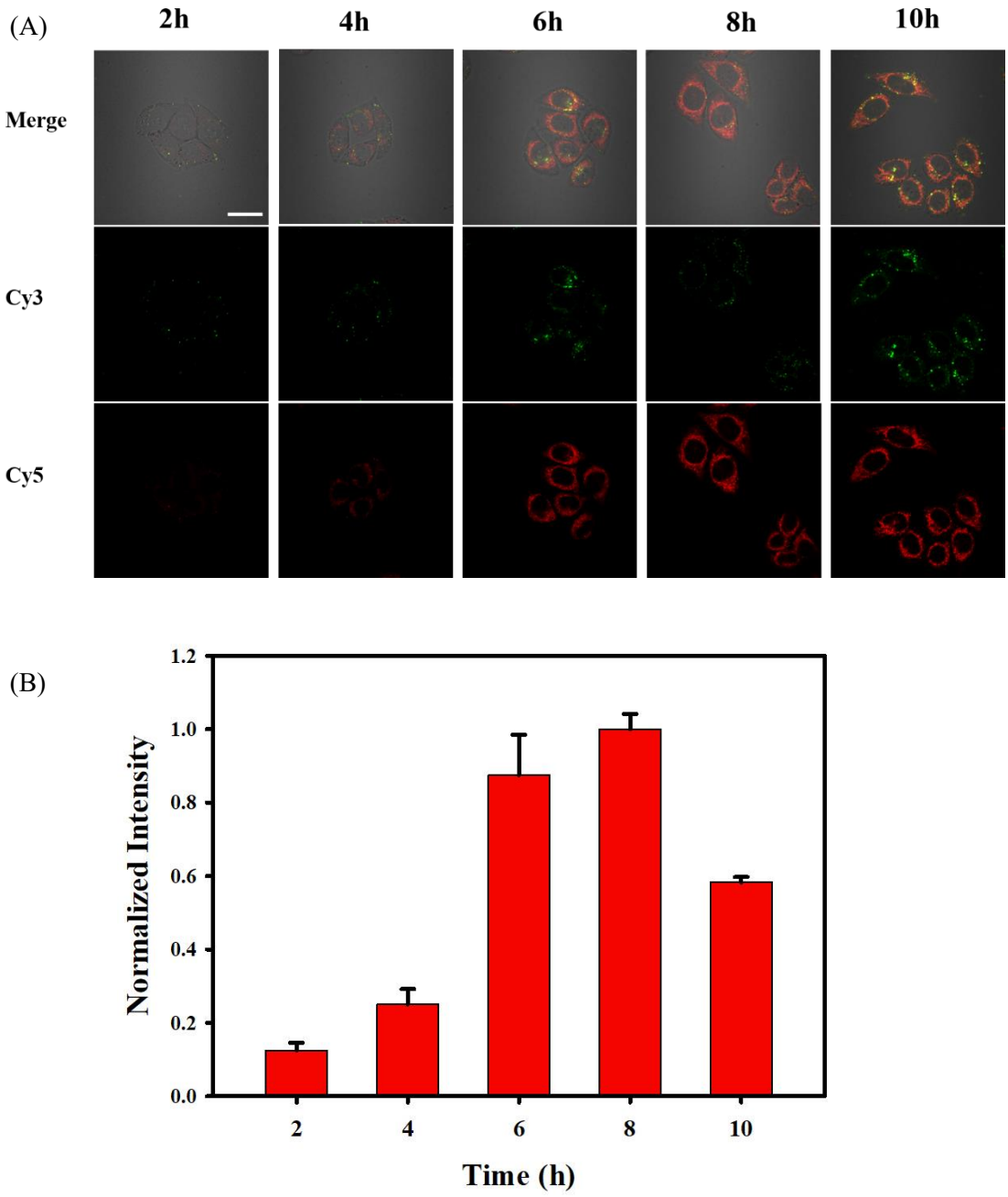

Figure S7. (A) Confocal fluorescence images of MCF-7 cells incubated with nanoprobes (B1+B2+H1+H2) for different times at 37 ℃. (B) Normalized intensity of FRET ratio at different time. The scale bar is 20 μm.

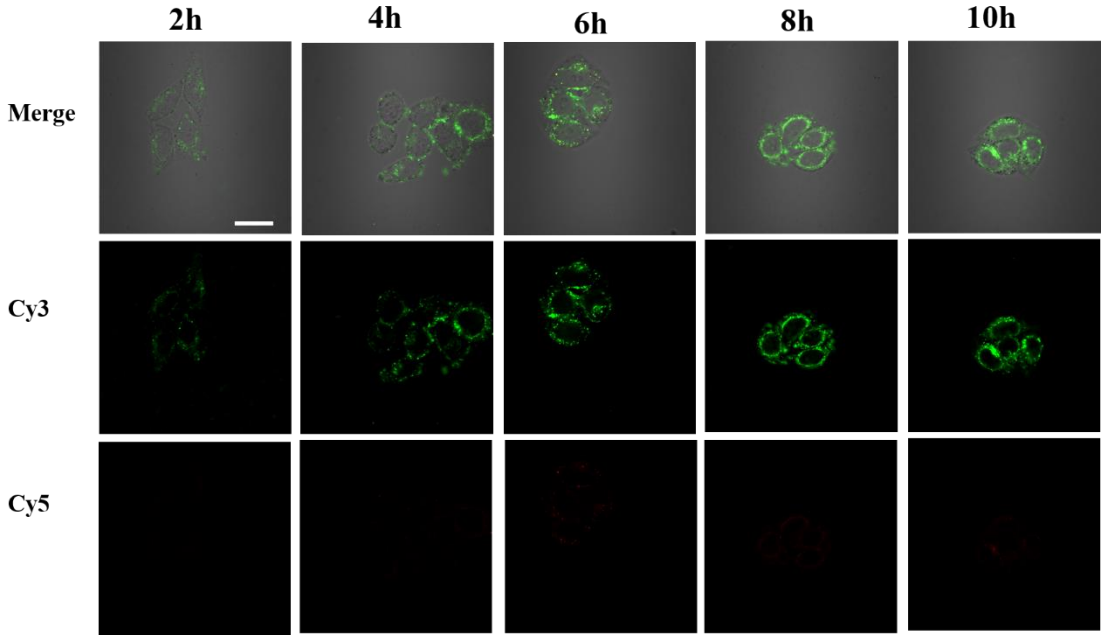

Figure S8. Confocal fluorescence images of MCF-7 cells incubated with nanoprobes (H1+H2) for different times at 37 ℃. The scale bar is 20 μm.

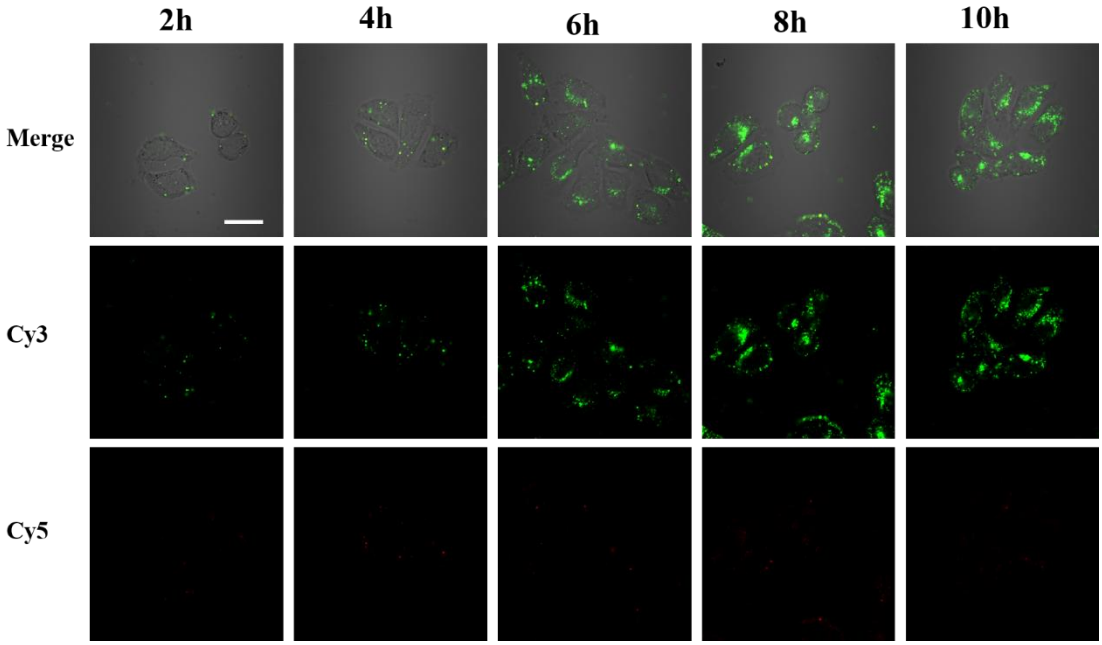

Figure S9. Confocal fluorescence images of L02 cells incubated with nanoprobes (B1+B2+H1+H2) for different times at 37 ℃. The scale bar is 20 μm.

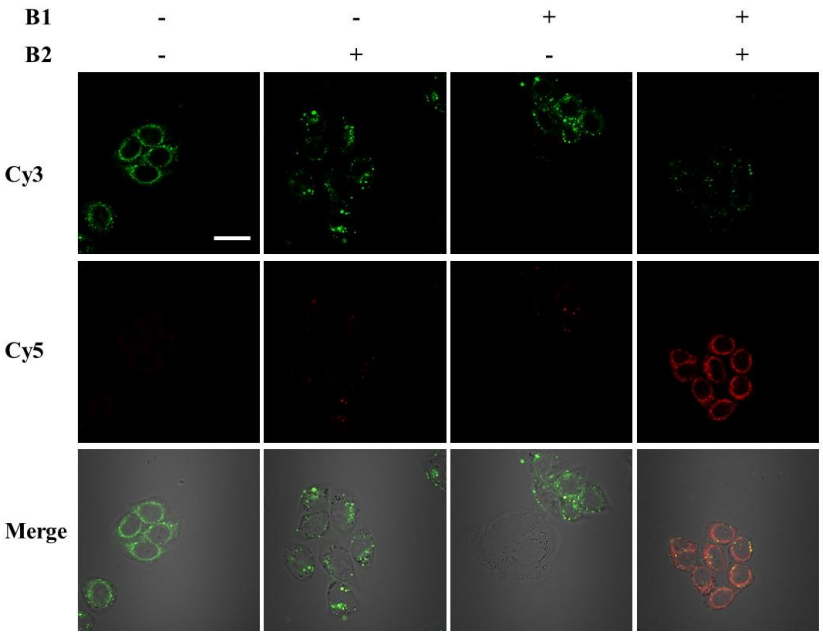

Figure S10. Confocal fluorescence images of MCF-7 cells incubated with nanoprobes for 8h at 37 ℃. The scale bar is 20 μm.

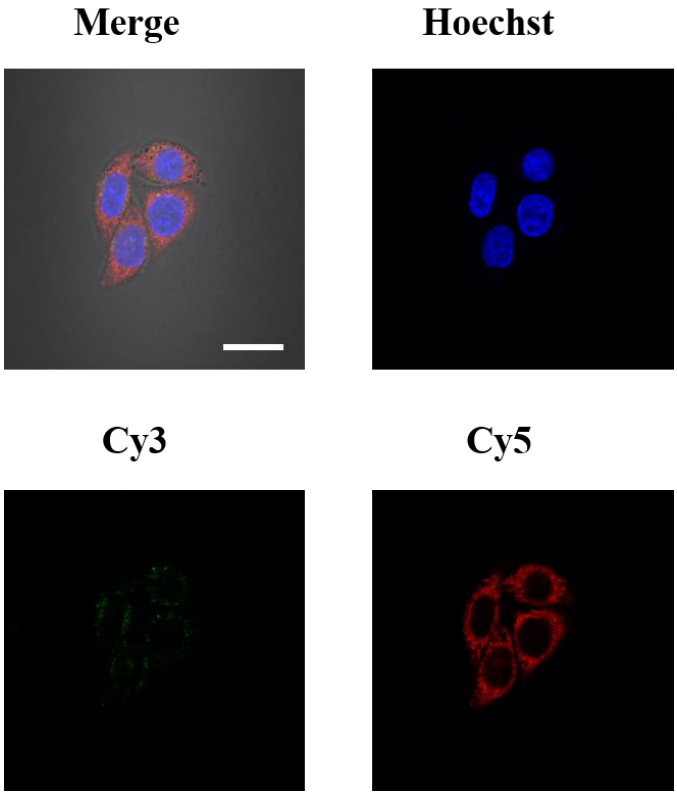

Figure S11. The fluorescence colocalization imaging of MCF-7 by D-HCR. The blue fluorescence represents that cell nuclei was stained by Hoechst. Scale bar is 20 μm.

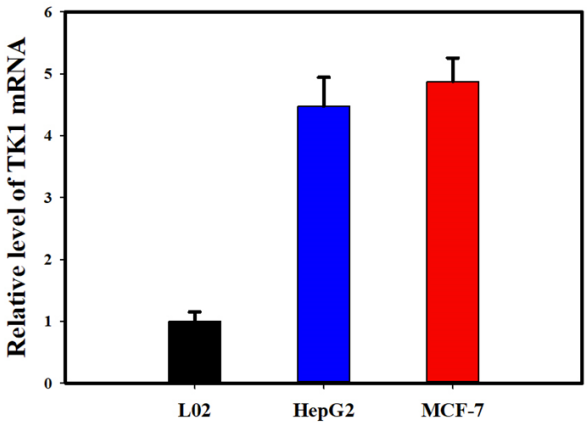

Figure S12. qRT-PCR analysis of relative expression levels of TK1 mRNA in L02 cells, HepG2 cells and MCF-7 cells.