Electronic Supplementary Information (ESI)

Temperature-responsive split aptamers coupled with polymerase chain reaction for label-free and sensitive detection of cancer cells

Jinlu Tang, Xiaoxiao He, Yanli Lei, Hui Shi, Qiuping Guo, Jianbo Liu, Dinggeng He, Lv'an Yan, and Kemin Wang**

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, College of Biology, Hunan University, Key Laboratory for Bio-Nanotechnology and Molecular Engineering of Hunan Province, Changsha, 410082, China.

* E-mail: kmwang@hnu.edu.cn. Phone/Fax: +86-731-88821566.

* E-mail: huishi_2009@hnu.edu.cn. Phone/Fax: +86-731-88821566.

Experimental Section

1. Materials.

All the DNA probes reported in this article were custom-designed and then synthesized by Sangon Biotech. Co., Ltd. (Shanghai, China). Sequences of the oligos are listed in Table S1. Dulbecco's phosphate buffered saline (D-PBS) were purchased from Thermo Fisher Scientific (U.S.A.). PCR Mix were purchased from BioTeke Co., Ltd (Beijing, China), SYBR Green I were purchased from Sigma (U.S.A.). Bovine serum albumin (BSA) was purchased from Dingguo Biotech. Co., Ltd. (Beijing, China). Low molecular weight DNA ladder was purchased from New England Biolabs Ltd. (U.S.A.). Yeast tRNA was obtained from Sinopharm Chemical Reagent Co., Ltd.(Shanghai, China) All other reagents were of the highest grade available. Deionized water was obtained through the Nanopure Infinity ultrapure water system (Barnstead/Thermolyne Corp.). Binding buffer was prepared by adding 1 mg/mL BSA and 0.1 mg/mL yeast tRNA into D-PBS containing 4.5 g/L glucose and 5 mM $MgCl_2$.

2. Cells.

SMMC-7721 cells (human hepatocellular cancer), Bel-7404 cells (human hepatocellular cancer) and L02 cells (human normal hepatocytes) were obtained from the Shanghai Institute of Cell Biology of the CAS. HepG2 cells (human hepatocellular cancer) and HeLa cells (human cervical cancer) were purchased from American Type Culture Collection. CCRF-CEM cells (human acute lymphoblastic leukemia, T cell line) were obtained from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (CAS). Ramos cells (human Burkitt's lymphoma, B cell line) were purchased from the Cancer Institute and Hospital (Chinese Academy of Medical Sciences). All cell lines were cultured in RPMI 1640 medium supplemented with 15% fetal calf serum and 100 IU/mL penicillin-streptomycin, and incubated at 37 ℃ in a humidified incubator containing 5% wt/vol CO_2. Before experiment, the live cells is normally counted by cell-count board.

3. Flow Cytometry Assays.

Generally, DNA molecules were incubated with 2×10^5 cells in 200 μL binding buffer for 30 min in the dark at 37 ℃. Then, cell samples were detected with a Gallios cytometer (Backman company) by counting 20000 events after washed twice with D-PBS. As for investigation of the temperature-controlled reversible assembly of split aptamers on cell surface, SMMC-7721 cells were firstly labeled with split probe (20 nM split apt-a/FAM-split apt-b) in binding buffer on ice for 30 min. Next, the labeled cells were incubated at 37 ℃ for 30 min and then 20000 cells were collected for flow cytometry assays.

4. Gel Electrophoresis.

Analysis by electrophoresis was carried out on 3% agarose gels by SYBR Gold staining, cast and run in 0.5 × TBE buffer (4.5 mM Tris, 4.5 mM boric acid, 0.1 mM EDTA, pH 7.9) at room temperature. Electrophoresis was performed at a constant potential of 100 V for 60 min with loading of 10 μL of each sample into the lanes.

5. Cell Detection with the Split Apta-PCR Strategy.

SMMC-7721 cells were firstly incubated with split probe at 4 ℃ for 30 min in 200 μL binding buffer, and then suspended in D-PBS at 37 ℃ for 30 min after washed twice. After centrifugation, supernatant containing recovered split apt-a probes was used for PCR amplification. Specifically, 10 μL supernatant was added into 30 μL PCR mix solution contained 500 nM primers. To increase the efficiency of PCR

amplification, the annealing temperature was optimized. Finally, conditions used for PCR amplification were as following: 3 min at 94 ℃; then, 16 cycles of 0.5 min at 94 ℃, 0.5 min at 59 ℃, and 0.5 min at 72 ℃, followed by 3 min at 72 ℃. The PCR products would be used for fluorescence measurements.

6. Fluorescence Measurements.

10 μL PCR products were diluted to 100 μL with 2 × SRBY Green I solution. The mixture was displayed in dark at room temperature for 30 min. Then, fluorescence experiments were performed using a Hitachi F-7000 fluorescence spectrometer. The excitation was made at 485 nm with a recording emission range of 500-700 nm. Excitation slit was 2.5 nm and emission slit was set at 5 nm.

Table S1. All of the oligonucleotides used in this work. [α]

DNA	Sequence
Split apt-a	5'-AATGCTGGGATGCTCTCGCA*CGTCAGGTTGAGCTGAAGATC GTACCGTGAAGTCCGT*TACGCGTGGTTTGCTCGCTA-3'
Split apt-b	5'-*ACGGACTACCTGACG*-3'
Split ctrl-a	5'-AATGCTGGGATGCTCTCGCAN_{37}TACGCGTGGTTTGCTCGCTA-3'
Primer	5'-TAGCGAGCAAACCACGCGTA-3' 5'-AATGCTGGGATGCTCTCGCA-3'
FAM-split apt-b	5'-FAM-*ACGGACTACCTGACG*-3'
Cy3-split apt-b	5'-Cy3-*ACGGACTACCTGACG*-3'
Split apt-a-Cy5	5'-AATGCTGGGATGCTCTCGCA*CGTCAGGTTGAGCTGAAGATC GTACCGTGAAGTCCGT*(Cy5)TACGCGTGGTTTGCTCGCTA-3'
Split ctrl-a-Cy5	5'-AATGCTGGGATGCTCTCGCAN_{37}(Cy5)TACGCGTGGTTTGCTCGCTA-3'
Sgc8c-a	5'-TACCTCCGCACCCTCCTACA*ATCTAACTGCTGCGCCGCCGG GAAAA*-3'
Sgc8c-b	5'-*CTGTACGGTTAGA*-3'
Primer-Sgc8c	5'-TACCTCCGCACCCTCCTACA-3' 5'-TTTTCCCGGCGGCGC-3'
FAM-sgc8c-b	5'-FAM-*CTGTACGGTTAGA*-3'

[α] The sequences of primer binding sites are shown in brown and underlined. The aptamer sequence for target binding is marked in blue and italic.

Figures

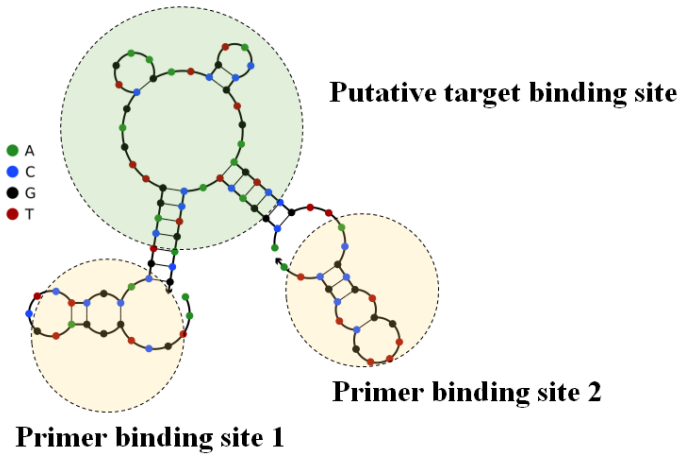

Figure S1. The predicted secondary structure of split apt-a/split apt-b designed for detecting SMMC-7721 cells, with expressly marked primer binding sites and putative target binding site.

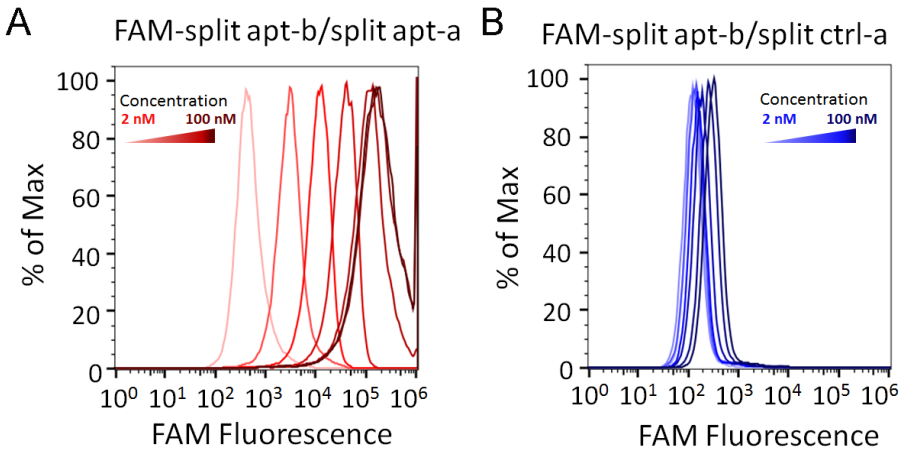

Figure S2. Flow cytometry assays of target SMMC-7721 cells incubated with (A) FAM-split apt-b/split apt-a probes or (B) FAM-split apt-b/split ctrl-a probes at different concentrations. The incubation was performed on ice in the dark for 30 min. The used DNA concentrations were 2 nM, 5 nM, 10 nM, 15 nM, 20 nM, 50 nM and 100 nM. The fluorescence of FAM was excited with a 488 nm laser and measured at the FL1 channel (505-545 nm).

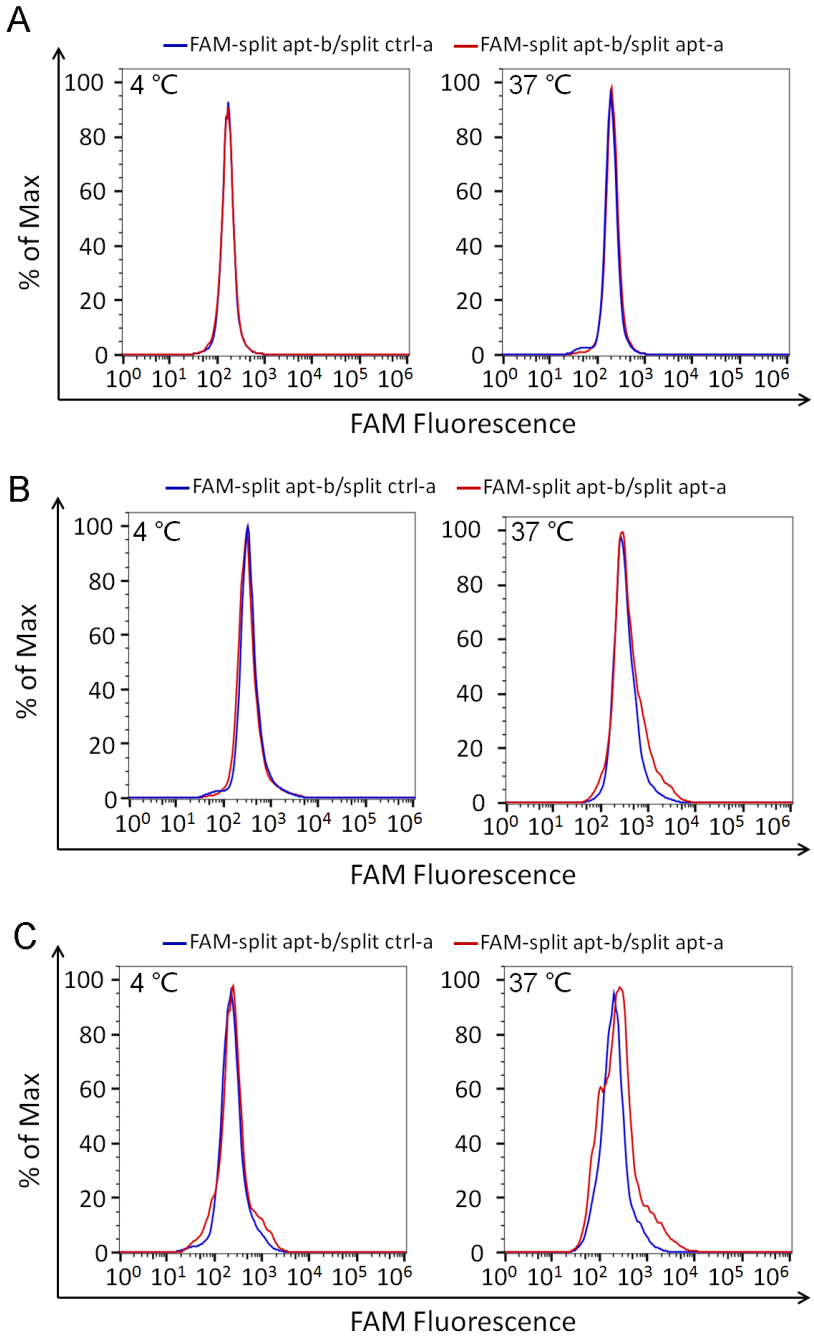

Figure S3. Flow cytometry assays of Bel-7404 cells (A), HeLa cells (B) or L02 cells (C) after incubation with different probes (20 nM) for 30 min at different temperatures.

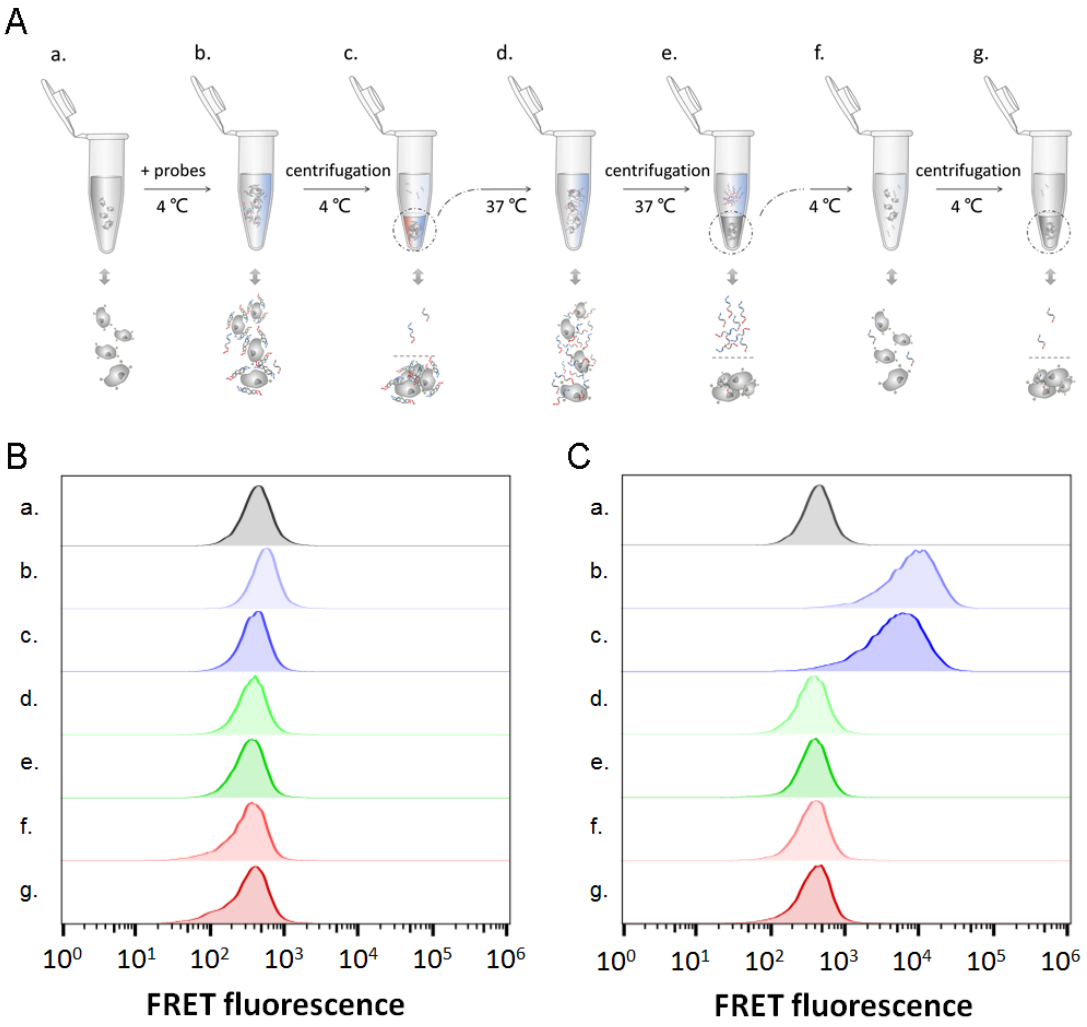

Figure S4. Fluorescence resonance energy transfer (FRET) signal monitoring of SMMC-7721 cells after sequential treatments by flow cytometry. (A) Schematic representation of the sequential treatments; (B) Flow cytometry assay results using Cy3-split apt-b/split ctrl-a-Cy5; (C) Flow cytometry assay results using Cy3-split apt-b/split apt-a-Cy5. (a) Pure cells; (b) Cells incubated with probes (20 nM) for 45 min at 4 ℃; (c) Cells centrifuged at 4 ℃; (d) Cells incubated at 37 ℃ for 30 min; (e) Cells centrifuged at 37 ℃; (f) Cells incubated at 4 ℃ for 45 min; (g) Cells centrifuged at 4 ℃.

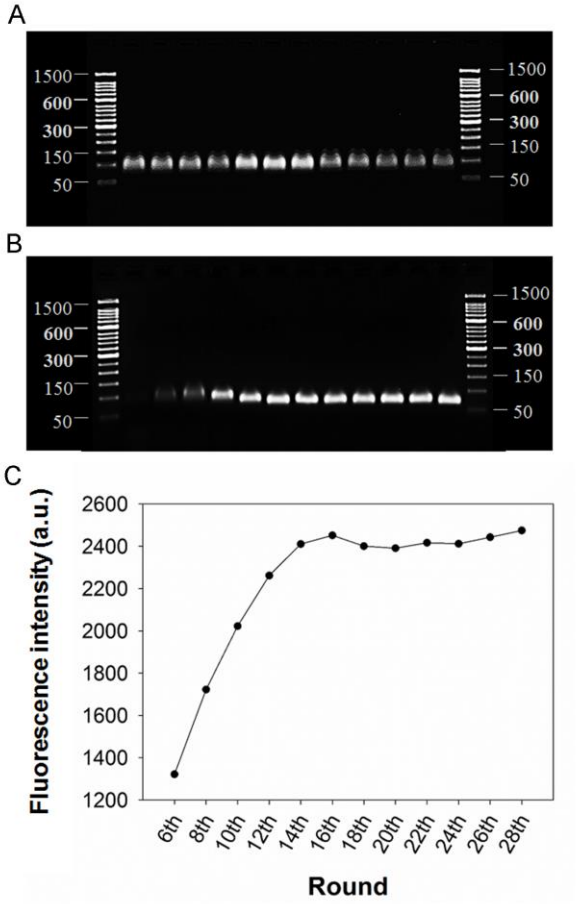

Figure S5. Agarose gel electrophoresis (3%) and fluorescence measurements of PCR products using split apt-a templates. (A) Optimization of annealing temperatures in PCR. From left to right: 53.9 ℃, 54.1 ℃, 54.9 ℃, 56.0 ℃, 57.4℃, 58.8 ℃, 60.5 ℃, 62.1 ℃, 63.6 ℃, 64.8 ℃, 65.6 ℃ and 66.0 ℃. (B) Optimization of the number of PCR rounds. From left to right: 6th, 8th, 10th, 12th, 14th, 16th, 18th, 20th, 22th, 24th, 26th and 28th rounds. (C) Fluorescence intensities of the SYBR Green I-colored PCR products under different rounds corresponding with (B).

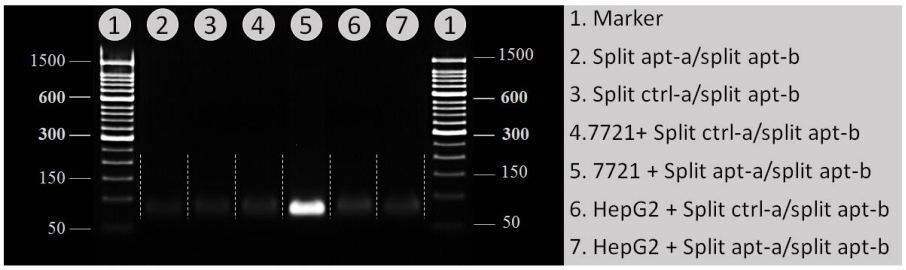

Figure S6. Characterization of the PCR products via different samples using 3% agarose gel electrophoresis, showing feasibility of the thermosensitive split apta-PCR strategy for detecting target SMMC-7721 cells.

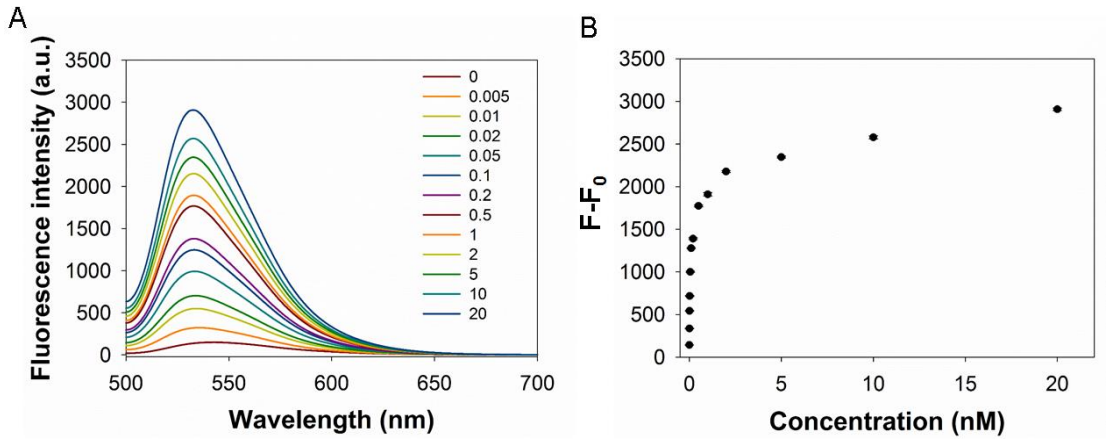

Figure S7. Fluorescence measurements of the SYBR Green I-stained PCR products prepared with different concentrations of split apt-a. (A) Fluorescence spectra recorded at various concentrations of split apt-a (nM). (B) Corresponding calibration curve illustrating the relationship between the detected fluorescence signal increase ($F-F_0$) and the concentration of split apt-a. (Error bars: standard deviations from three repeated experiments.)

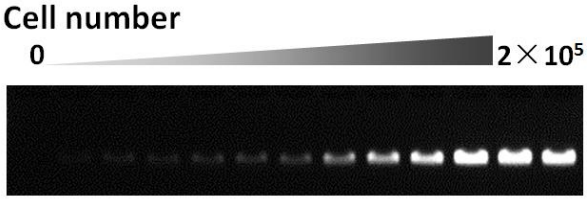

Figure S8. Characterization of the PCR products from SMMC-7721 cell samples with different cell numbers treated by split apt-a/split apt-b, using 3% agarose gel electrophoresis.

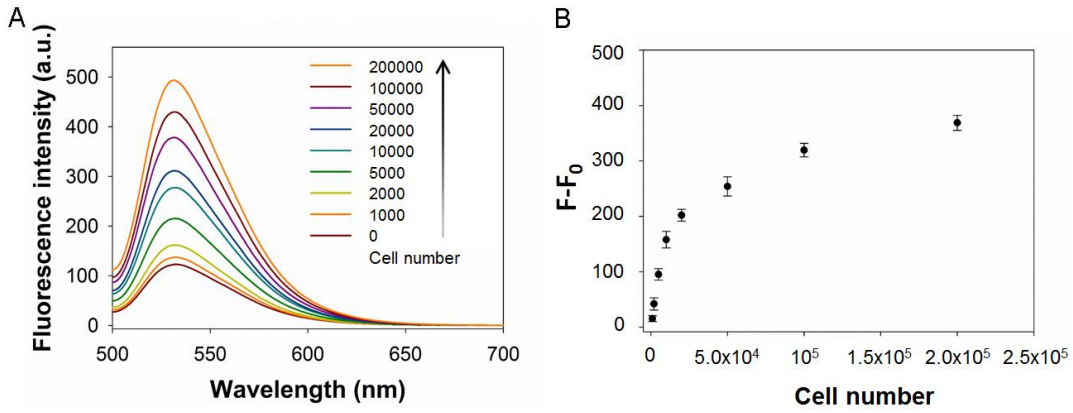

Figure S9. Quantitative analysis of target SMMC-7721 cells with the thermosensitive split apta-PCR strategy, in the presence of 10000 negative control Bel-7404 cells. (A) Fluorescence spectra recorded at various numbers of SMMC-7721 cells. (B) Corresponding calibration curve illustrating the relationship between the detected fluorescence signal increase ($F-F_0$) and the cell number. F and F_0 represented fluorescent intensities detected in the presence and absence of SMMC-7721 cells, respectively. Error bars: standard deviations from three repeated experiments.

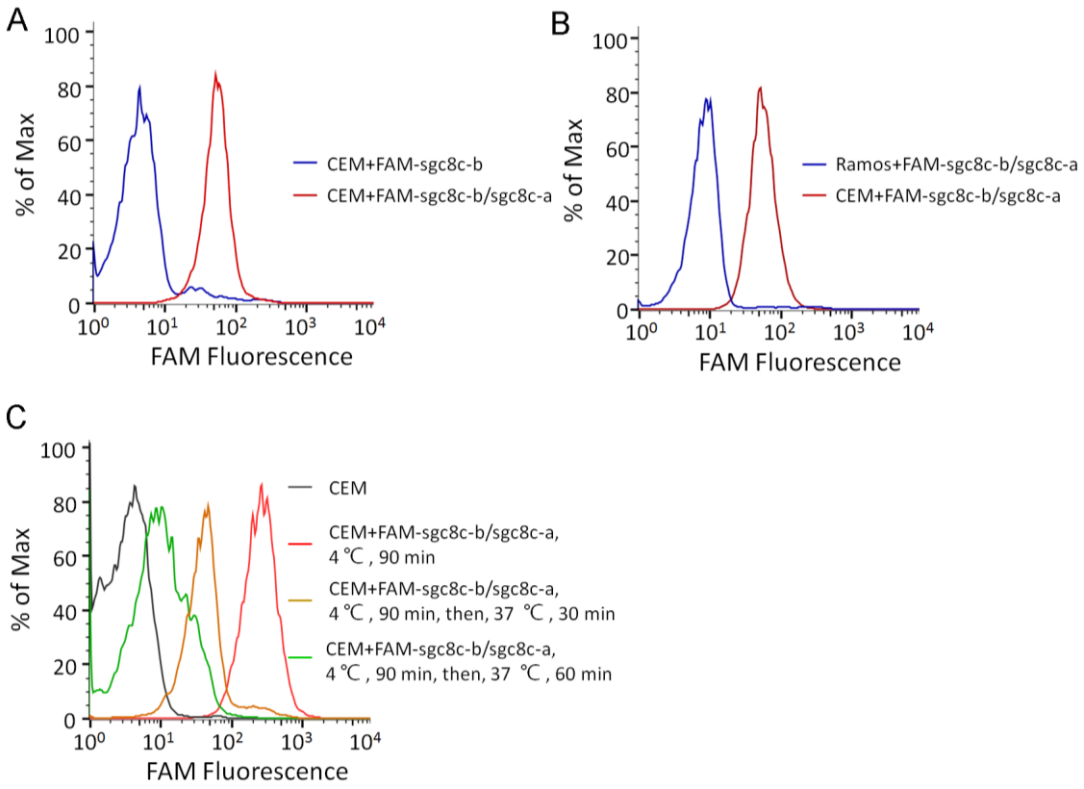

Figure S10. Flow cytometry investigation of the affinity (A), specificity (B) and thermosensitivity (C) of the reformed split aptamers (sgc8c-a and sgc8c-b) for detection of CCRF-CEM cells.

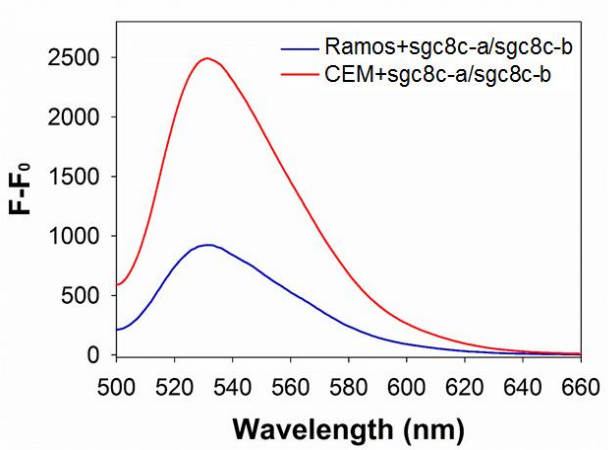

Figure S11. Background-subtracted fluorescence spectra of the SYBR Green I-stained PCR products from different cell samples treated with sgc8c-a/sgc8c-b, showing feasibility of the thermosensitive split apta-PCR strategy for specific detection of CCRF-CEM cells.