Electronic Supplementary Information (ESI)

Tumor Cell-Specific Split Aptamers: Target-Driven and Temperature-Controlled Self-Assembly on Living Cell Surface †

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Experimental Section

1. Materials.

All the DNA molecules 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), calcein-AM, Hoechst-33342 and propidium iodide (PI) were purchased from Sigma. Streptavidin-coated 96-well plates were obtained from Greiner Bio-One. Bovine serum albumin (BSA) was purchased from Dingguo Biotech. Co., Ltd. Yeast tRNA was obtained from Sinopharm Chemical Reagent Co., Ltd. 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.

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). SMMC-7721 cells (human hepatocellular cancer) were obtained from the Shanghai Institute of Cell Biology of the CAS. 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 °C in a humidified incubator containing 5% wt/vol CO_2.

3. Flow Cytometry Assays.

Generally, DNA molecules were incubated with 2×10^5 cells in 200 μL binding buffer for 90 min in the dark at a specified temperature. Then, cell samples without washing were directly detected with a FACSCalibur cytometer (BD Biosciences) by counting 10000 events. As for investigation of the temperature-controlled reversible assembly of split aptamers on cell surface, CCRF-CEM cells were firstly labeled with split Sgc8c (320 nM Sgc8c-3a and 40 nM Cy5-Sgc8c-2b) in binding buffer on ice for 90 min. Next, the labeled cells were incubated at 37 °C for 1 h and then on ice for 1 h. During the incubation, 100 μL cell samples were collected for flow cytometry assays every 10 min.

4. Modification of 96-Well Plates.

Streptavidin-coated 96-well plates were added with 50 μL/well Biotin-T_{10}-Sgc8c-3a or Biotin-T_{15}-ZY11b in Tris buffer (100 mM NaCl, 30 mM Tris-HCl, pH 7.4). After incubation at 37 °C for 1 h, the plates were washed with PBS for three times to remove unbound DNA molecules. Then, 50 μL/well 1% BSA solution was added for an 1 h-incubation at 37 °C to block unbound reactive sites. After washing, the plates were finally added with 50 μL/well binding buffer and stocked on ice in the dark for subsequent uses.

5. Cell Staining with Fluorescent Dyes.

CCRF-CEM cells were firstly stained with 6 μM calcein-AM (green light emission with blue light excitation) at 37 °C for 20 min in the dark and then suspended in binding buffer for subsequent uses. SMMC-7721 cells were firstly incubated with 5 μg/mL Hoechst-33342 (blue light emission with UV light excitation) at 37 °C for 10 min in the dark and then suspended in binding buffer for subsequent uses. As for cell viability analysis, a calcein-AM/PI-based double staining method was used to judge live or dead cells. Detailedly, cells were incubated in PBS containing both 6 μM calcein-AM and 4 μM PI (red light emission

with green light excitation) for 20 min at 37 °C. After washing, cells were suspended in binding buffer and observed on a fluorescent microscope equipped with a mercury lamp.

6. Temperature-Controlled Assembly of Cells on Microplate Well Surface.

Sgc8c-3a- or ZY11b-coated 96-well plates were added with 50 μL/well binding buffer containing 3×10^5 cells as well as 6.4 μL/well Sgc8c-2b or ZY11a (50 μM). After incubation on ice for 90 min, the plates were washed with precooled binding buffer for three times to remove unbound cells. Next, 100 μL/well binding buffer were added. And the cells assembled on well surface were observed and counted on an optical microscope. Then, the plates were incubated at 37 °C for 60 min and the supernatant was collected for observation. Also, the residual cells on well surface were observed and counted on an optical microscope after washing with prewarmed binding buffer for three times.

Figures

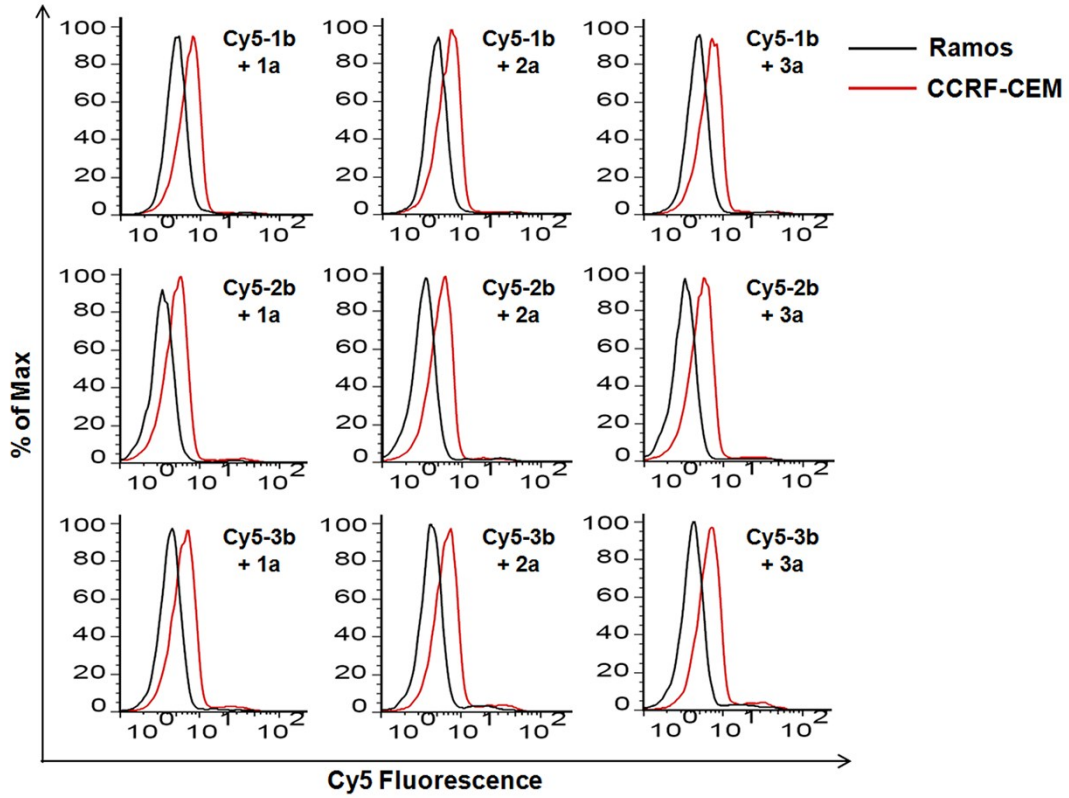

Figure S1. Flow cytometry assay results of target CCRF-CEM cells or nontarget Ramos cells incubated with 9 pairs of split aptamers respectively. The incubation was performed on ice in the dark for 60 min. The used DNA concentration was 25 nM for a and 200 nM for Cy5-b. The fluorescence of Cy5 was excited with a 633 nm laser and measured at the FL4 channel (653-669 nm). The corresponding histogram of the fluorescence ratios of CCRF-CEM cells to Ramos cells for every pair of split aptamers was presented in Figure 1B.

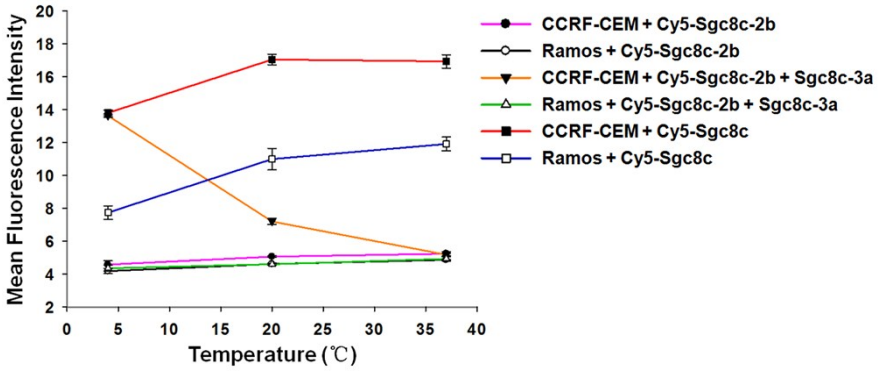

Figure S2. The corresponding line chart of mean fluorescence intensities in Figure 2A. (Error bars: standard deviations from three repeated experiments.)

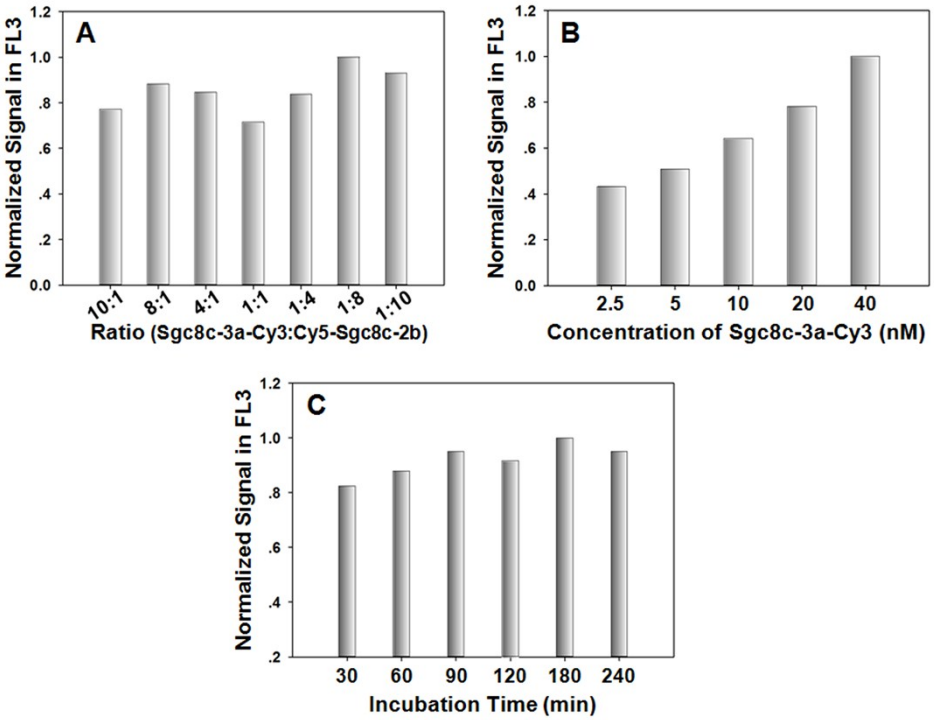

Figure S3. Effect of different conditions on the assembly of split aptamers (Sgc8c-3a-Cy3/Cy5-Sgc8c-2b) on target cell surface. The Cy3-Cy5 FRET signals were measured with a flow cytometer at the FL3 channel (488 nm excitation and 670 nm long pass emission). (A) CCRF-CEM cells were incubated with different ratios of Sgc8c-3a-Cy3 to Cy5-Sgc8c-2b on ice for 90 min. (The "1" was fixed to be 40 nM DNA.) (B) CCRF-CEM cells were incubated with different concentrations of Sgc8c-3a-Cy3/Cy5-Sgc8c-2b on ice for 90 min. (The ratio of Sgc8c-3a-Cy3 to Cy5-Sgc8c-2b was fixed to be 1:8.). (C) CCRF-CEM cells were incubated with Sgc8c-3a-Cy3/Cy5-Sgc8c-2b (40 nM/320 nM) on ice for different time.

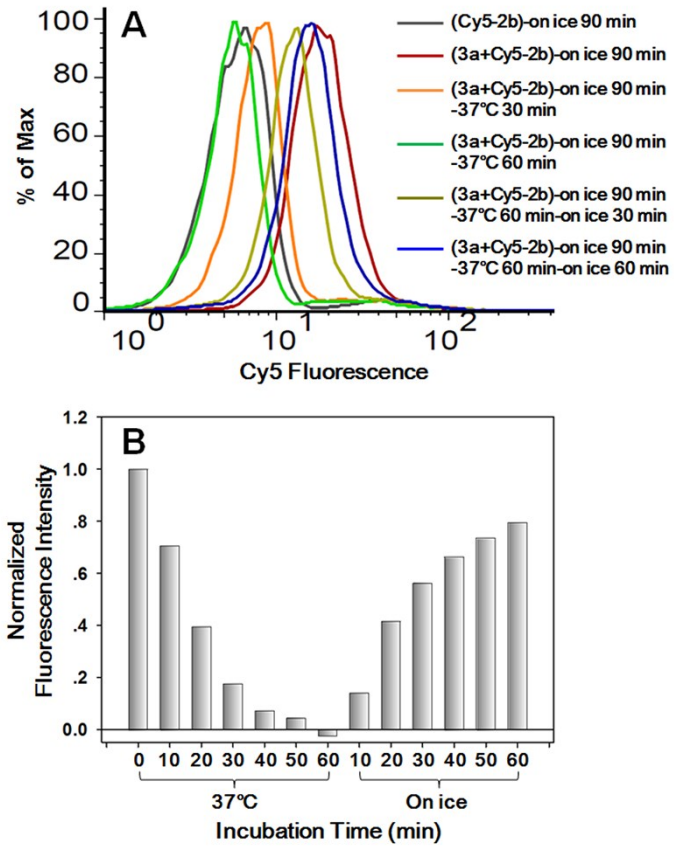

Figure S4. Temperature-controlled reversible assembly of split aptamers on cell surface. CCRF-CEM cells were firstly labeled with Sgc8c-3a/Cy5-Sgc8c-2b (320 nM/40 nM) on ice for 90 min. Then, the labeled cells were successively incubated at 37 °C for 60 min and on ice for 60 min, during which cells were detected every 10 min. (A) Representative flow cytometry results. (B) Histogram of the normalized background-subtracted mean fluorescence intensities.

Figure S5. Fluorescence images of the streptavidin-coated microplate well surface after modification with different concentrations of Biotin-T_{10}-Sgc8c-3a-Cy3 molecules.

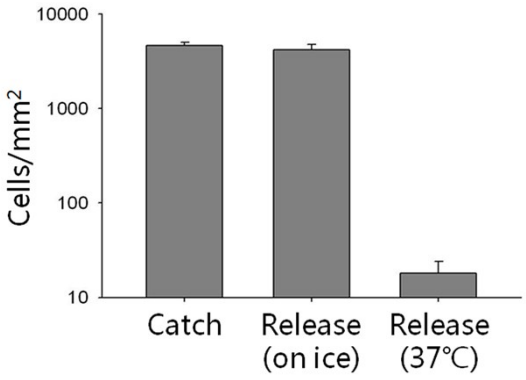

Figure S6. The corresponding histogram of the cell densities calculated for d, e and f groups in Figure 5B. (Error bars: standard deviations from nine images.)

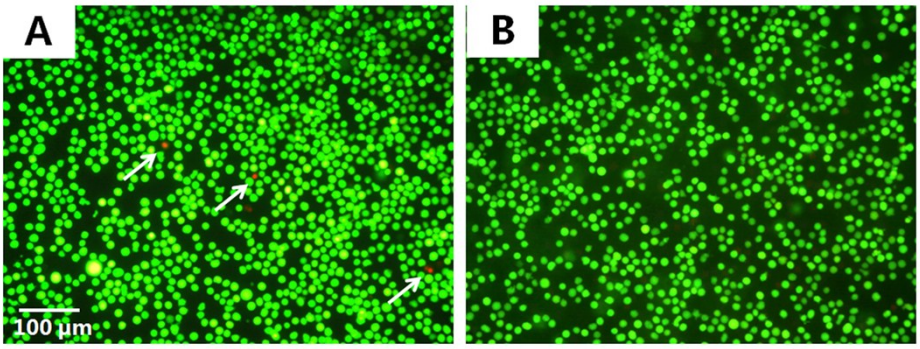

Figure S7. Fluorescence images of CCRF-CEM cells stained by both calcein-AM (green: live cells) and PI (red: dead cells), (A) before capture or (B) after capture and then release. (The arrows point to the dead cells.)

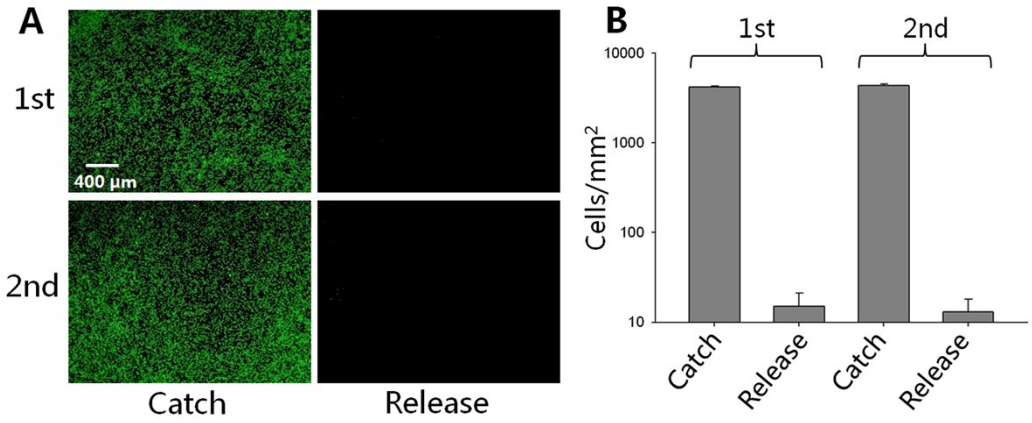

Figure S8. Recycling of the Sgc8c-3a-coated microplates for CCRF-CEM cell catch and release. (A) Fluorescence images of the calcein-AM-labeled CCRF-CEM cells (green) on the well surface. (B) The corresponding calculated cell densities. (Error bars: standard deviations from nine images.)

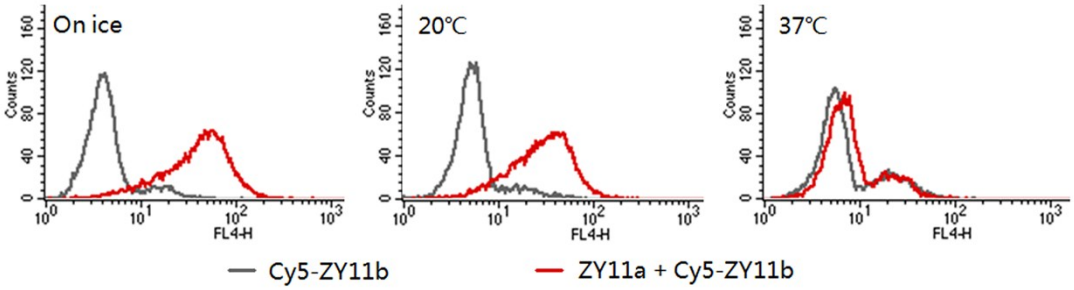

Figure S9. Flow cytometry assays of SMMC-7721 cells after a 90-min incubation with different probes at different temperatures. (DNA concentration: 25 nM.)

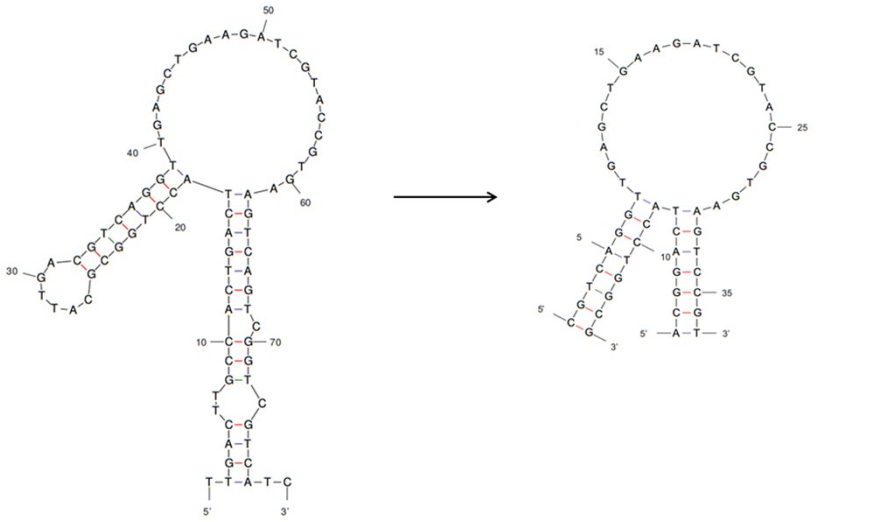

Figure S10. The calculated secondary structures of the intact ZY11 aptamer and the split ZY11.

Table S1. All of the oligonucleotides used in this work.

DNA	Sequence
Sgc8c-1a	5’-ATCTAACTGCTGCGCCGCCGGG-3’
Sgc8c-2a	5’-ATCTAACTGCTGCGCCGCCGGGAA-3’
Sgc8c-3a	5’-ATCTAACTGCTGCGCCGCCGGGAAAA-3’
Sgc8c-3a-Cy3	5’-ATCTAACTGCTGCGCCGCCGGGAAAA-Cy3-3’
Cy5-Sgc8c-1b	5’-Cy5-GTACGGTTAGA-3’
Cy5-Sgc8c-2b	5’-Cy5-CTGTACGGTTAGA-3’
Cy5-Sgc8c-3b	5’-Cy5-TACTGTACGGTTAGA-3’
Sgc8c-2b	5’-CTGTACGGTTAGA-3’
Cy5-Sgc8c	5’-Cy5-ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA-3’
Biotin-T_{10}-Sgc8c-3a	5’-Biotin-T_{10}-ATCTAACTGCTGCGCCGCCGGGAAAA-3’
Biotin-T_{10}-Sgc8c-3a-Cy3	5’-Biotin-T_{10}-ATCTAACTGCTGCGCCGCCGGGAAAA-Cy3-3’
ZY11a	5’-CGTCAGGTTGAGCTGAAGATCGTACCGTGAAGTCCGT-3’
Cy5-ZY11b	5’-Cy5-ACGGACTACCTGGCG-3’
Biotin-T_{15}-ZY11b	5’-Biotin-T_{15}-ACGGACTACCTGGCG-3’