Electronic Supplementary Information (ESI)

Amplified AND Logic Platform for Cell Identification

Jiaoli Wang, Jing Li, Shiyuan Liu, Xiangxian Meng, Xiaohai Yang, Jin Huang* and Kemin Wang*

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

*Email: jinhuang@hnu.edu.cn; kmwang@hnu.edu.cn

Experimental Section

Chemicals and Materials. All DNA oligonucleotides were synthesized and HPLC purified by Sangon Biotech Co., Ltd. (Shanghai, China) and dissolved in phosphate buffered saline (PBS) solution (10 mM phosphate buffer, 137 mM NaCl, 2.7 mM KCl, 5 mM $MgCl_2$, pH 7.4). The sequences of the oligonucleotides are described in Table S1. All cell lines were purchased from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Sartorius ultrapure water (18.2 MΩ cm) was used throughout the experiments. Washing buffer contained glucose (4.5 g/L) in PBS solution. Binding buffer was prepared by adding yeast tRNA (0.1 mg/mL) and BSA (1 mg/mL) to the washing buffer.

Cell Culture. CEM cells, Ramos cells, HeLa cells, and L02 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 100 U/mL 1% antibiotics penicillin/streptomycin and maintained at 37 °C in a 100% humidified atmosphere containing 5% CO_2.

Preparation of Sgc8c-A. H1, H2, H3 and H4 were dissolved with PBS to a common final concentration of 100 μM, heated to 95 °C for 5 min, then cooled to room temperature. A mixture of sgc8c-T probe, H1 and H2 (molar ratio is 1:10:10) left on 25 °C for 24 h in PBS, then H3 and H4 (equivalent concentration with H1 and H2) added, continued at 37 °C for 2 h. The formation of sgc8c-A was confirmed by 2% agarose gel electrophoresis.

Agarose Gel Electrophoresis. In the gel electrophoresis assay, a sample containing 10

μL of each reaction sample, 2 μL 6 × Loading buffer, and 2 μL of SYBR Gold was subjected to the 2% agarose gel electrophoresis. The electrophoresis was conducted in 1 × TBE buffer at constant voltage of 80 V for 1 h. followed by imaging under UV irradiation.

Fluorescence Analysis. The fluorescence of FAM was monitored in PBS on F-7000 fluorescence spectrometer (HITACHI, Japan). the maximal excitation wavelength at 488 nm, and collected between 510 and 650 nm. The slit width was set to 5 nm either the excitation or the emission light path.

Flow Cytometry Assay. To obtain the fluorescence by incubated sgc8c-A and sgc4f/C with the corresponding cells in 200 μL binding buffer for 1 h at 37 °C. CEM cells incubated with sgc8c-A as a control. After washing three times with washing buffer, the cells were analyzed with a flow cytometer (Gallios, Beckman Coulter, USA) by counting 10000 events. The FAM fluorescence signal was collected in FL1 with a 488 nm laser and a 505 to 525 nm band-pass filter.

Flow cytometry was used to evaluate incubation temperature. The CEM cells incubated with sgc8c-A and sgc4f/C in 200 μL binding buffer at 37 °C or 4 °C for 1 h, After being washed with washing buffer twice, the cells were analyzed with a flow cytometer by counting 10000 events. The FAM fluorescence signal was collected in FL1 with a 488 nm laser and a 505 to 525 nm band-pass filter.

To optimize the incubation time, the CEM cells incubated with sgc8c-A and sgc4f/C in 200 μL binding buffer at 37 °C for 0.5 h, 1 h, 1.5 h, 2 h, 4 h, After being washed with binding buffer twice, the cells were analyzed with a flow cytometer by counting 10000 events. The FAM fluorescence signal was collected in FL1 with a 488 nm laser and a 505 to 525 nm band-pass filter.

Confocal Fluorescence Imaging. Cells were plated on 15 mm confocal dish, then incubated with sgc8c-A and sgc4f/C in binding buffer at 37 °C for 1 h or various time (for time optimization), and then the cells were washed three times with washing buffer before imaging. All cells were observed under an Nikon A1 confocal laser scanning fluorescence microscope (Japan), and a 515 nm (±10 nm) bandpass filter was used for fluorescence detection. The fluorescence images were taken under 100× oil-immersion objective and an Ar^+ laser (488 nm) as an excitation source. The FAM fluorescence image was recorded in green channel with 488 nm excitation.

Supporting Tables:

Table S1. Oligonucleotide sequences used in this work.

Name	Sequence (from 5'-3')
Sgc8c-T	ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA-TTT-TGCTGCTGCTGCTGCTGCACGACG
Lib-T	$(N)_{41}$-TTT-TGCTGCTGCTGCTGCTGCACGACG
Lib-FAM	$(N)_{41}$-TTT-FAM
H1	***CGAAACGGAAGGTCATGCTTTAGGA***CGTCGTGCAGCAGCAGCAGCAG CAACGGCTTGCTGCTGCTGCTGCTG
H2	***TTAGACCGACGCGAACTTTGATGC***TGCTGCTGCTGCTGCTGCACGACG GCAGCAGCAGCAGCAGCAAGCCGT
L2	***TTAGACCGACGCGAACTTTGATGC***TTTTTTTTTTTTTTTTTTTTACGGC AGCAGCAGCAGCAGCAAGCCGT
C(Catalyst)	AATGGATTTTATCTCCTGTC
H3	***GCATCAAAGTTCGCGTCGGTCTAA***ACAGGAGAT(FAM)AAAATCCATT GTGGTGAAATGGATTTTAT-BHQ
H4	***TCCTAAAGCATGACCTTCCGTTTCG***TCCATTTCACCACAATGGATTTT ATTGTGGTGA
Sgc4f	ATCACTTATAACGAGTGCGGATGCAAACGCCAGACAGGGGGACAGG AGATAAGTGA
Sgc8c-FAM	ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA-FAM
Sgc4f-FAM	ATCACTTATAACGAGTGCGGATGCAAACGCCAGACAGGGGGACAGG AGATAAGTGA-FAM
BHQ-C	(BHQ)AATGGATTTTATCTCCTGTC

The linkage parts are shown in bold and italics, the toeholds and loops of hairpin structures for H1 and H2, H3 and H4 are labelled in the same color. The trigger part of HCR is labelled red color. The hybridization parts of sgc4f and C are labelled in the same color.

Supporting Figures:

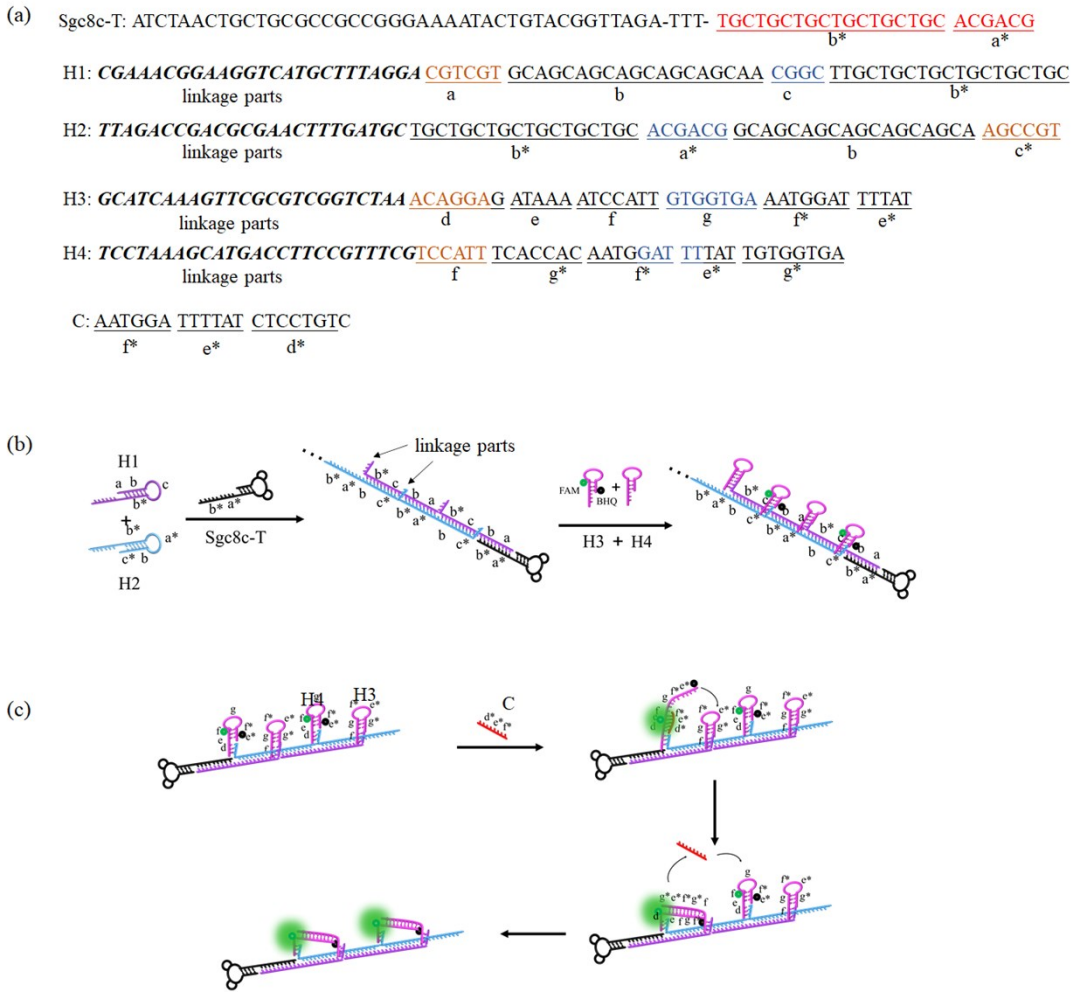

Figure S1. (a) The sequence of sgc8c-T, H1, H2, H3 and H4. (b) The formation process of sgc8c-A in detail. (c) the process of LCHA in detail. Letters in the (b) and (c) stand for different sequence domains in (a), in which letters marked with * are complementary to the corresponding unmarked letters.

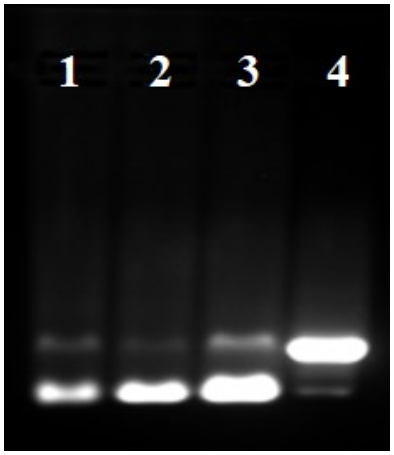

Figure S2. 2% agarose gel electrophoresis analysis of the CHA reaction. Lane 1: 1 μM H3, lane 2: 1 μM H4, lane 3: 1 μM H3 + 1 μM H4, lane 4: 1 μM H3 + 1 μM H4 + 100 nM C.

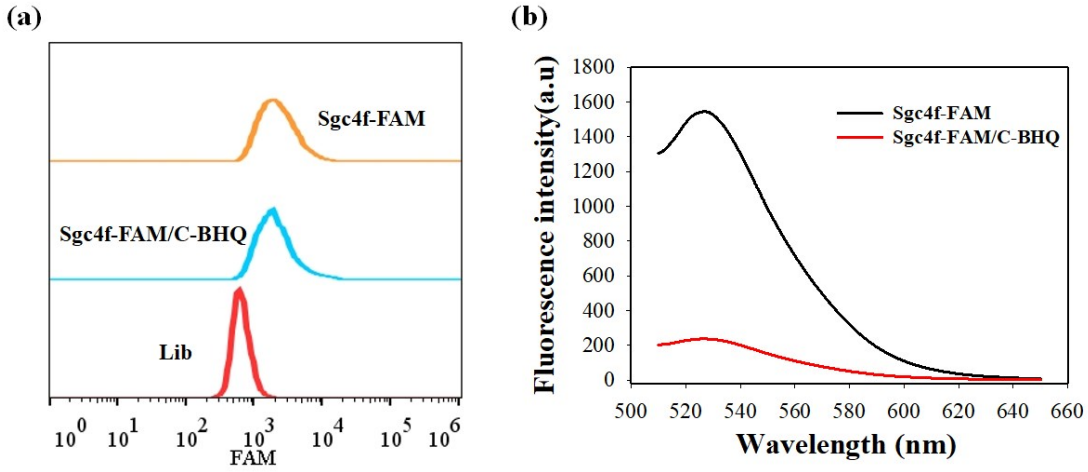

Figure S3. (a) Flow cytometry analysis of sgc4f-FAM or sgc4f-FAM/C-BHQ incubated with CEM cells. (b) Fluorescence analysis of sgc4f-FAM and sgc4f-FAM/C-BHQ.

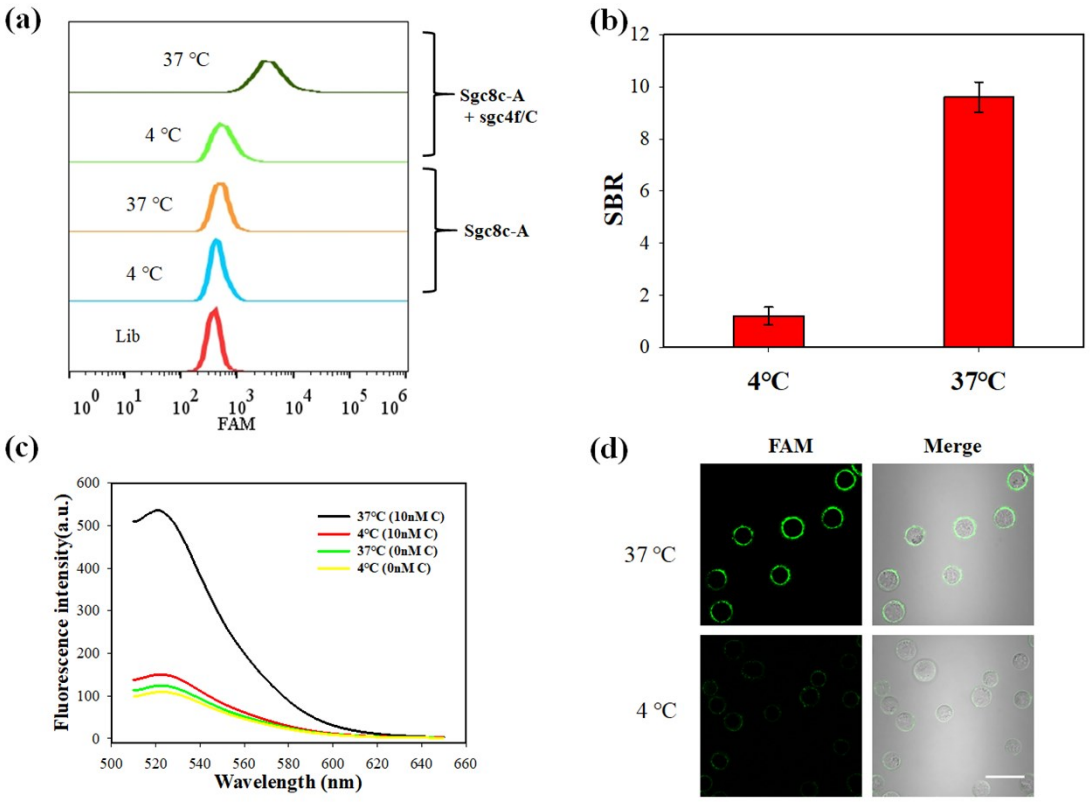

Figure S4. (a) Flow cytometry results of mixture of sgc8c-A and sgc4f/C incubated with CEM cells at 37 °C or 4 °C. (b) Flow cytometry quantification of the fluorescence by CEM cells in (a). Corresponding SBR ((sgc8c-A + sgc4f/C) to sgc8c-A) of different probes incubated with CEM cells. (c) Fluorescence analysis of 100 nM sgc8c-A with 10 nM C at 37 °C or 4 °C. (d) Confocal images of mixture of sgc8c-A and sgc4f/C incubated with CEM cells at 37 °C or 4 °C. The data error bars indicate mean ± SD (n =3). The scale bar is 20 μm.

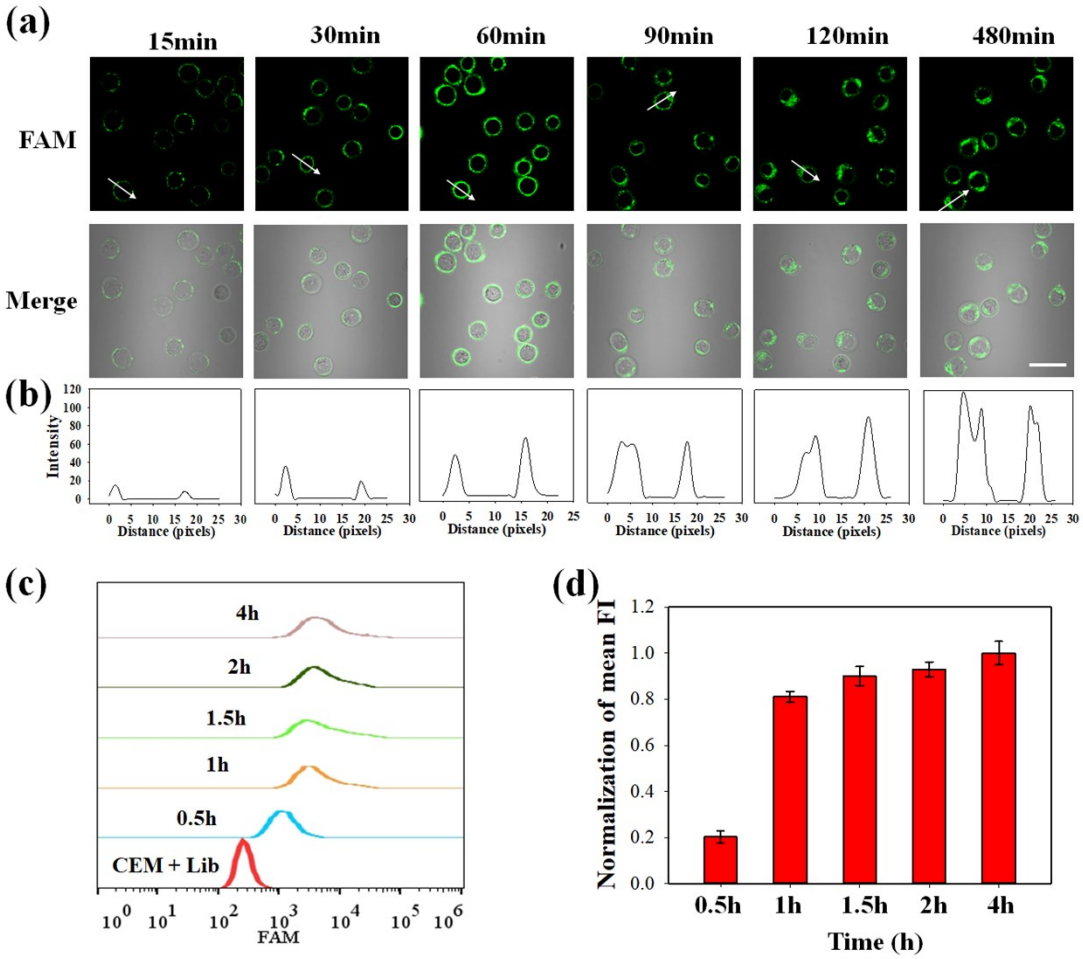

Figure S5. (a) Real-time confocal fluorescence imaging of CEM cells by AALP. The scale bar is 20 μm. (b) Spatial-intensity profiles of CEM cells along the white arrows in panel (a). (c) Flow cytometry analysis of real-time of CEM cells incubated with AALP. (d) Flow cytometry quantification of the fluorescence by CEM cells at different time points. The data error bars indicate mean ± SD (n =3).

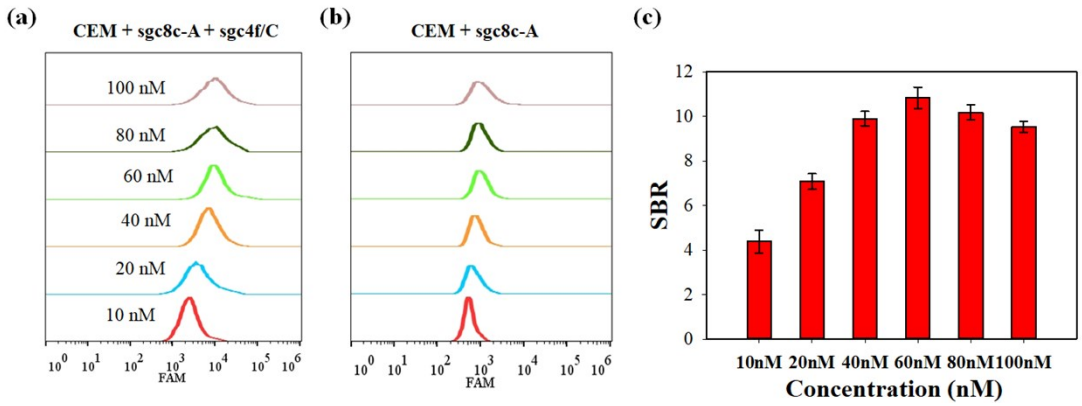

Figure S6. Optimization of the concentrations of sgc8c-A + sgc4f/C (a) and sgc8c-A only (b) incubated with CEM cells. (c) Quantification of the fluorescence in (a) and (b). Corresponding SBR ((sgc8c-A + sgc4f/C) to sgc8c-A) of different probes incubated with CEM cells. The data error bars indicate mean ± SD (n =3).

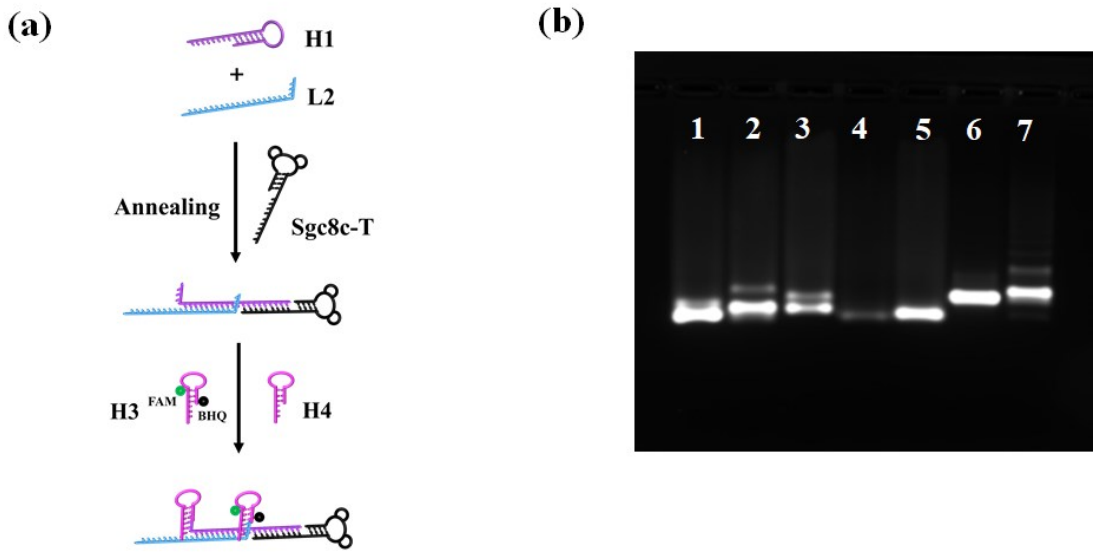

Figure S7. (a) Assembly process diagram of the nanostructure without signal amplification function. (b) 2% agarose gel electrophoresis confirm the formation of the nanostructure. Lane 1: 1 μM sgc8c-T, Lane 2: 1μM H1, Lane 3: 1μM L2, Lane 4: 1μM H3, Lane 5: 1μM H4, Lane 6: 1μM sgc8c-T + 1μM H1 + 1μM L2, Lane 7: 1μM sgc8c-T + 1μM H1 + 1μM L2 + 1μM H3 + 1μM H4.

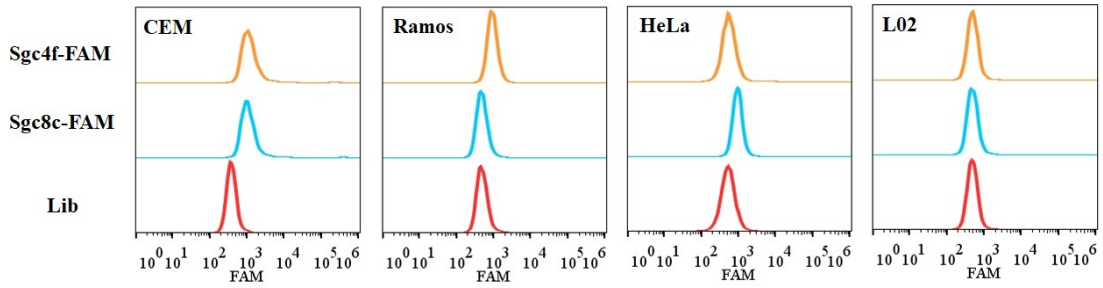

Figure S8. Aptamer binding affinity with CEM, Ramos, HeLa and L02 cells. The concentration of aptamers was 200 nM and the incubation time was 30 min.

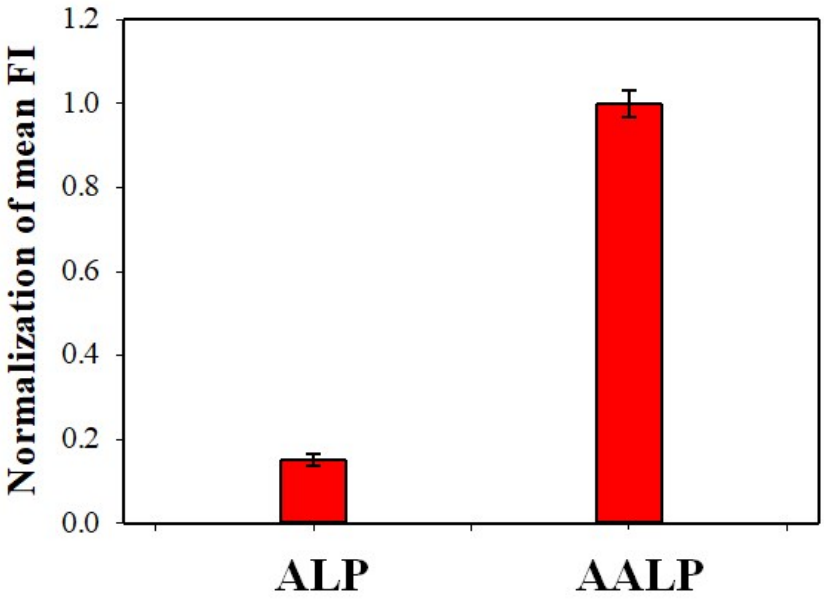

Figure S9. Flow cytometry quantification of the fluorescence in Figure 5c. The data error bars indicate mean ± SD (n =3).