Supporting Information

Bioinspired Framework Nucleic Acid Capture Sensitively and Rapidly Resolves MicroRNAs Biomarkers in Living Cells

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Experimental Procedures

Materials and Reagents. The purified DNA sequences and DNA Marker were purchased from Sangon Biological Engineering Technology & Co., Ltd (Shanghai, China). All the RNA sequences were purchased by Gene-Pharma Co., Ltd. (Shanghai, China). The DNA and RNA sequences were listed in Table S1. Phosphate buffer saline (PBS, pH 7.4), Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) was obtained from Life Technologies Corporation (Los Angeles, CA, USA). The trypsin-EDTA was purchased from Gibco Life Technologies (AG, Switzerland). The streptavidin was purchased from Yeasen Biotech. Co., Ltd. (Shanghai, China). The DNase I was provided by Novoprotein Scientific Co., Ltd. (Shanghai, China). Lipofectamine 2000 was purchased from Invitrogen (MA, USA). 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H- tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (China). The miRNA primer were purchased from Tiangen (China). All other reagents were used as analytical grade. The ultrapure water (⩾18 MΩ·cm-1) was obtained from a Millipore water purification system and used in all experiments.

Apparatus. The native PAGE images was imaged on an Alliance Ld2 (Uvitec, Cambridge, U.K.). The fluorescence analysis were carried out on a Hitachi F-4500 fluorescence spectrofluorometer (Tokyo, Japan). The MTT assays were performed on an Anthos 2010 microplate reader (biochrom, USA). Flow cytometric analysis was carried out on a BD FACSAria (Becton, Dickinson and Company, USA). CLSM imaging was carried out on a LSM710META confocal laser scanning microscope (Zeiss, Germany). The qRT-PCR was performed on a CFX96 touch RT-PCR detection system (Bio-Rad, USA).

Electrophoresis analysis. The 12% and 5% PAGE was prepared by adjusting the concentration of 30% acrylamide/bis-acrylamide gel solution (29:1). The loading sample was prepared by mixing 5 μL DNA sample and 1 μL 6×DNA loading buffer. The PAGE was performed in bath of ice and stained in 3×GelRed (NaCl, 100 mM) for 30 min.

Cell Culture. The cells were grown in DMEM culture supplemented with 10% FBS and 1% penicillin/streptomycin and cultured at 37 ^{o}C in a humidified atmosphere containing 5% CO_2 and 95% air.

CLSM Imaging and flow Cytometric Assay. The cells were seeded into confocal dish for 24 h and then added 1 mL culture medium containing 100 nM SA-DTP for 4 h at 37 °C. The resulting cells were washed twice by PBS buffer (10 mM, pH7.4) and fresh DMEM medium (1 mL) was added. The cells were imaged using a CLSM system

(FV1200, Olympus, Japan). The free-CHA probes and H1/H3 probes also were transfected under the same conditions. The cells were treated with trypsin and dissolved PBS buffer (10 mM, pH7.4) for flow cytometric assay.

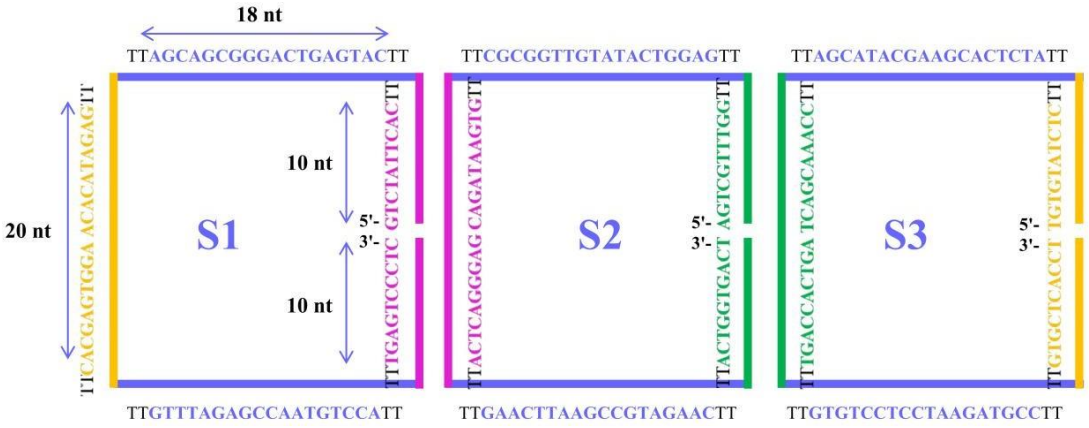

Figure S1. Strand diagram of the DTP.

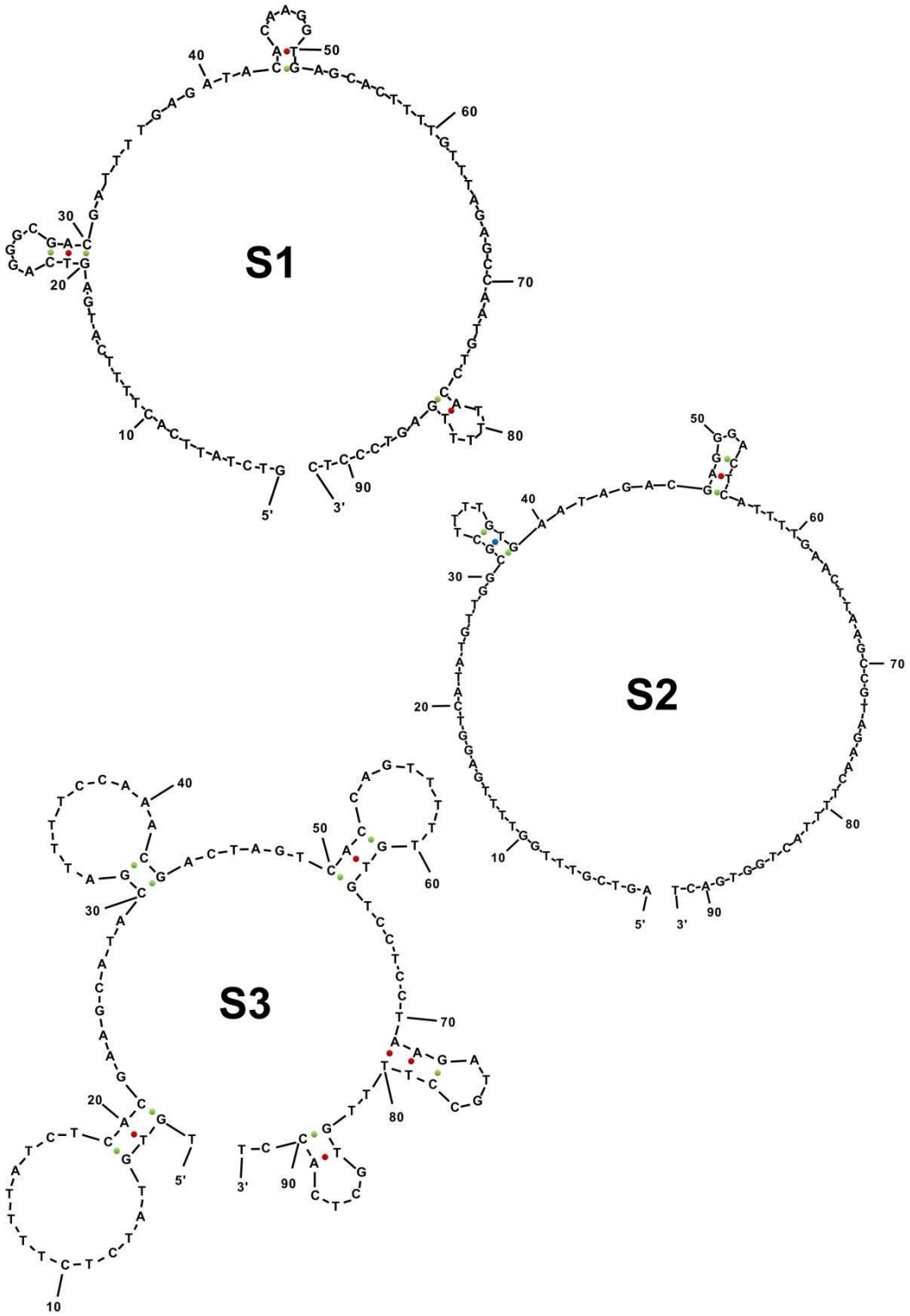

Figure S2. The most stable structure of S1, S2 and S3 strand.

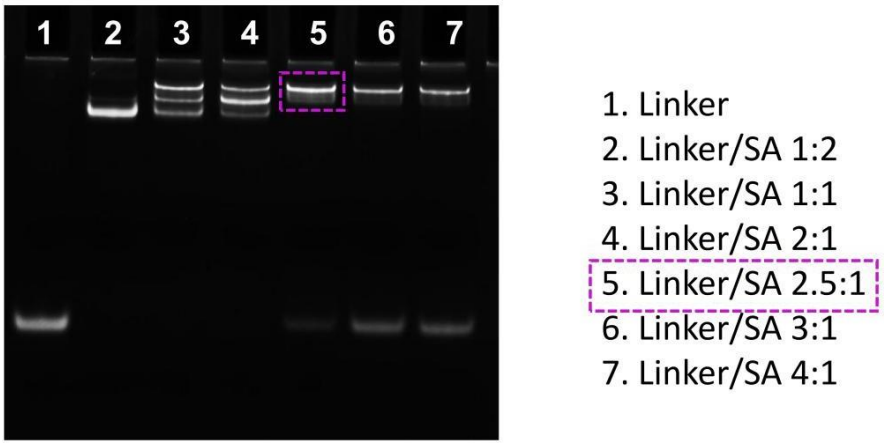

Figure S3. The PAGE imaging for the concentration optimization of streptavidin.

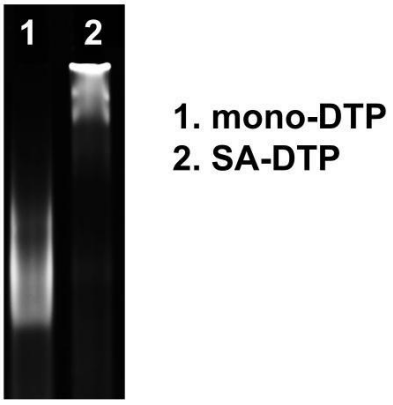

Figure S4. The PAGE imaging for the formation of SA-DTP.

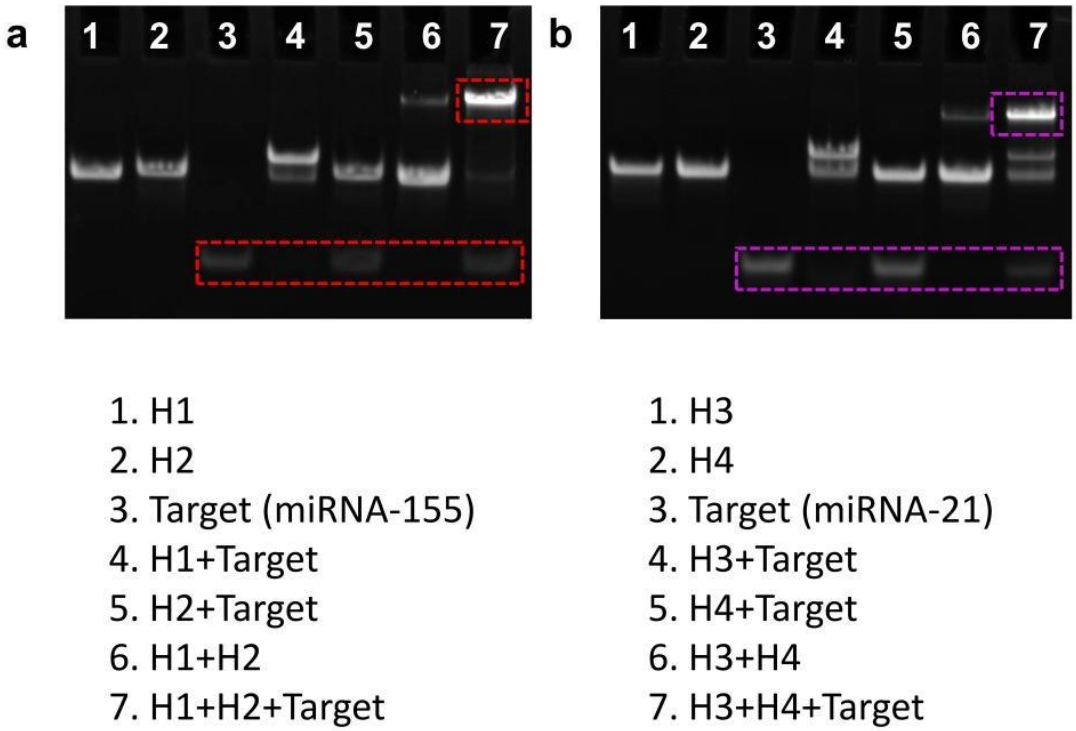

Figure S5. The native polyacrylamide gel electrophoresis (12.5%) imaging. (a) miRNA-155 and (b) miRNA-21 triggered CHA reaction.

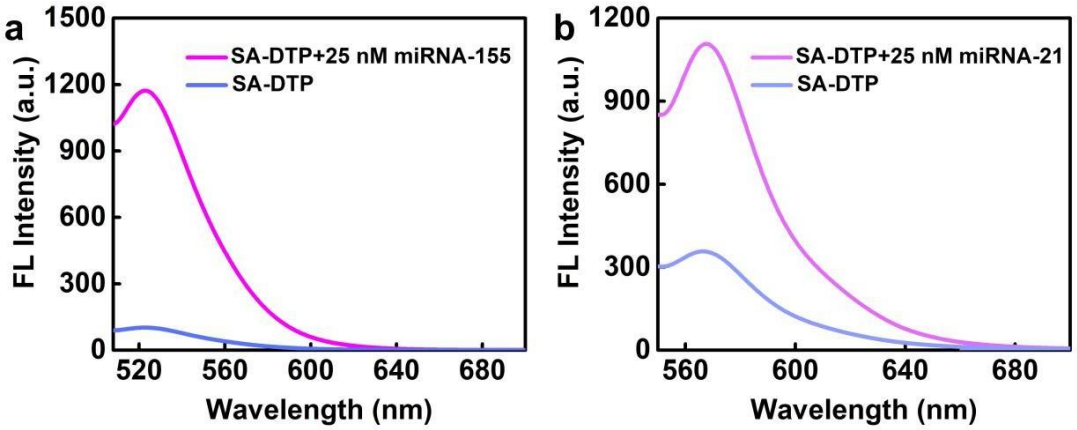

Figure S6. Fluorescence analysis of miRNAs triggered SA-DTP. The fluorescence intensity of (a) miRNA-155 and (b) miRNA-21response to SA-DTP system.

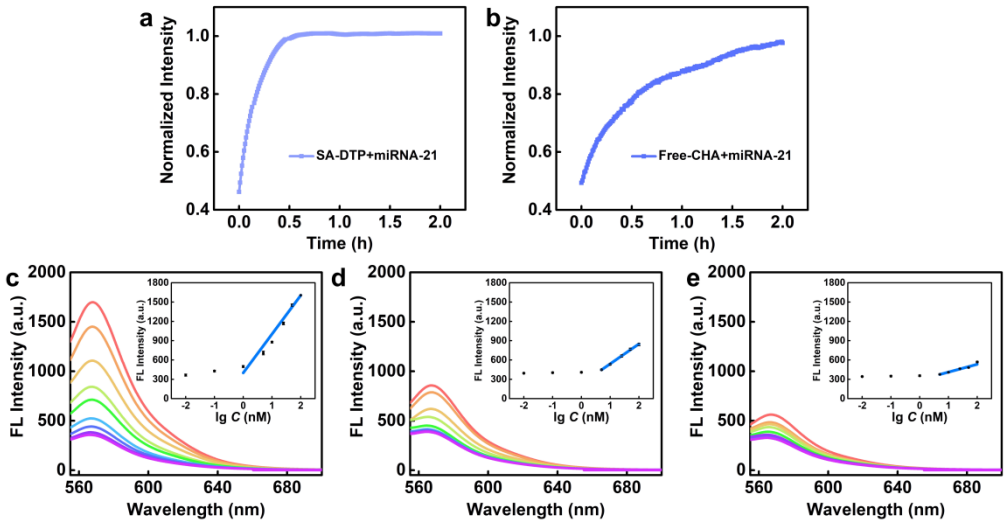

Figure S7. Fluorescence analysis of miRNA-21 triggered SA-DTP, free-CHA and H3. Time-dependent fluorescence spectra of CHA reaction in (a) SA-DTP (100 nM) and (b) a homogeneous solution containing free CHA probes (Free-CHA, 100 nM) in response to 25 nM miRNA-21. Fluorescence intensity corresponding to (c) SA-DTP, (d) Free-CHA and (e) H3 response to different concentration of miRNA-21 (100, 50, 25, 10, 5, 1, 0.1, 0.01 nM) and their corresponding calibration curves (Insert). The data error bars indicate means ± SD (n=3)

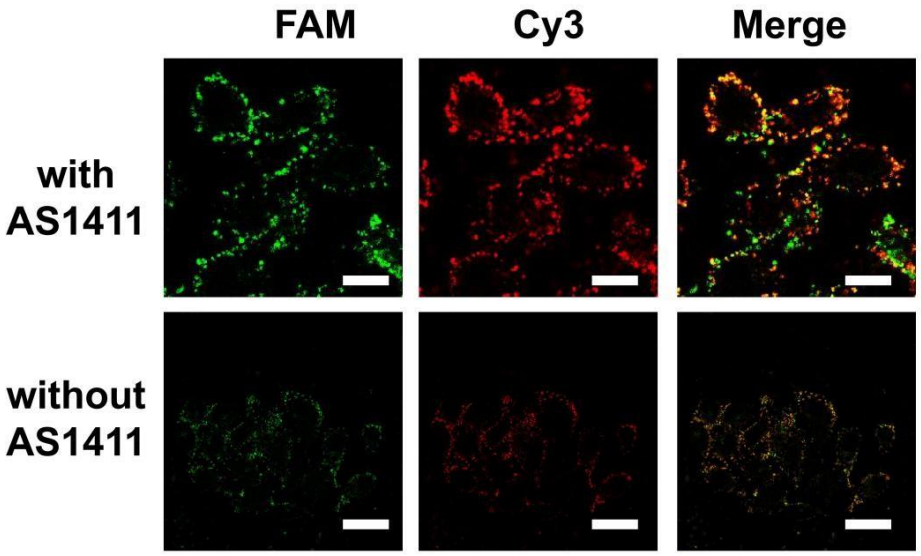

Figure S8. The cellular uptake ability of the SA-DTP system. Confocal fluorescence images of MCF-7 cells incubated with the SA-DTP system with and without AS1411 aptamer modified. Scale bar was 40 μm.

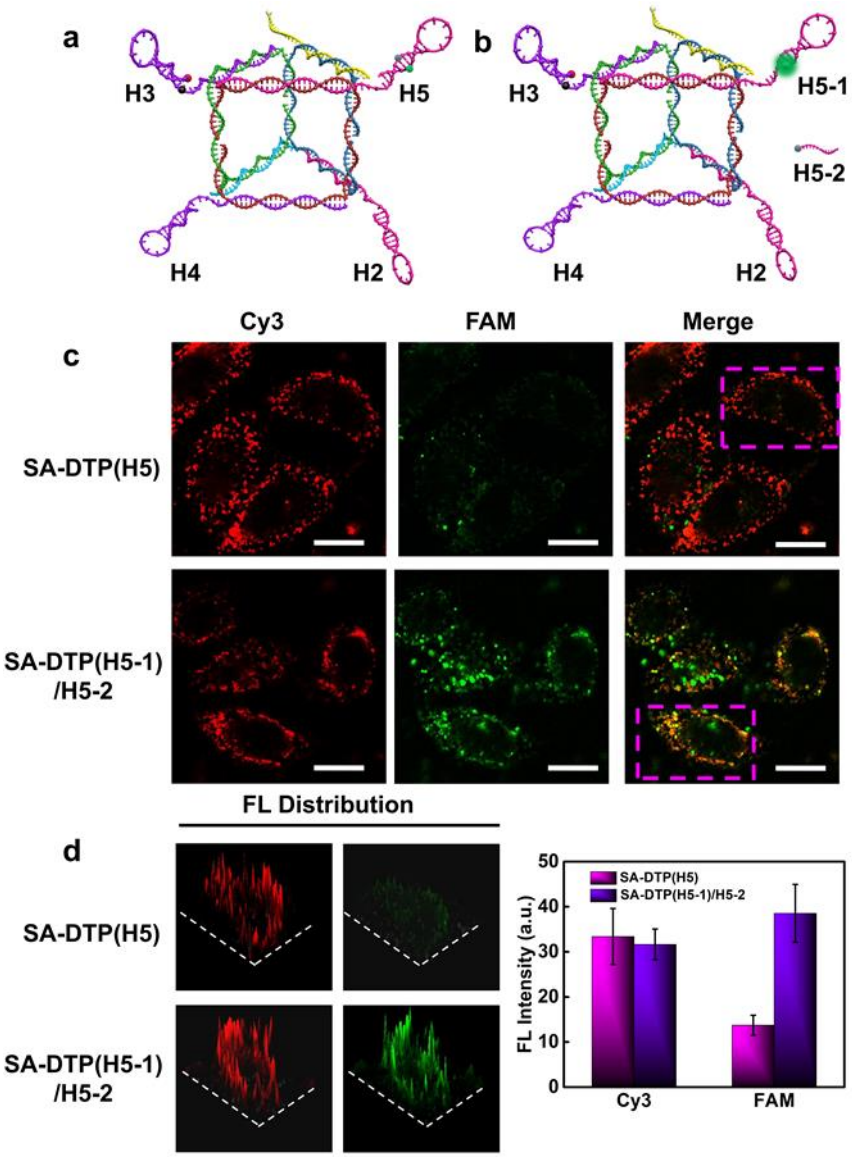

Figure S9. DNase degradation assay. Schematic illustration of (a) SA-DTP (H5) and (b) SA-DTP(H5-1)/H5-2 for DNase degradation assay. (c) CLSM images of MCF-7 cells incubated with SA-DTP(H5) (100 nM) and SA-DTP(H5-1)/H5-2 (100 nM) for 4 h, respectively. The scale bar was 40 μm. (d) Corresponding fluorescence distribution and intensities of these cells.

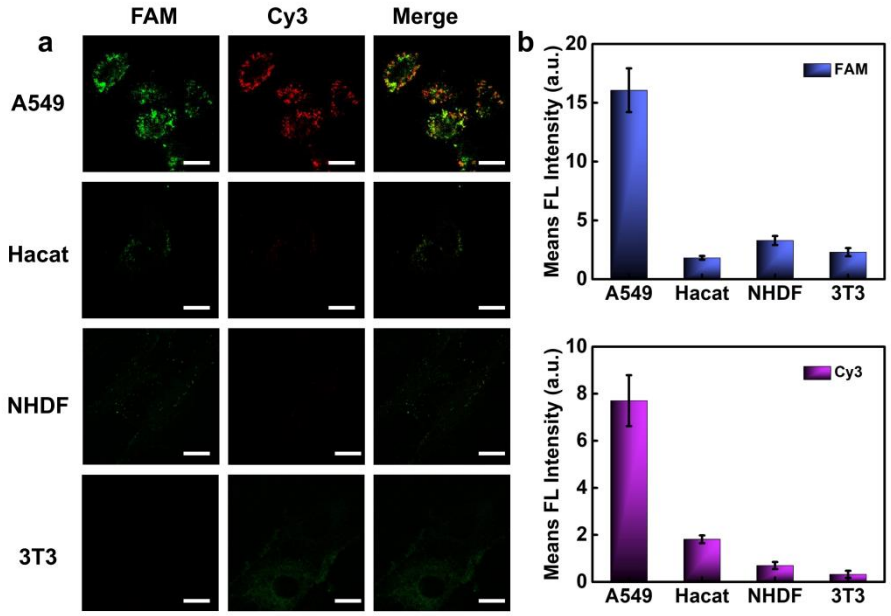

Figure S10. Different cells analysis using SA-DTP system. (a) Confocal fluorescence images of A549, Hacat, NHFD and 3T3 cells incubated with the SA-DTP system. Scale bar was 40 μm. (b) Corresponding fluorescence intensities of these cells.

Table S1. DNA and RNA sequences used in this work.

Name	Oligonucleotide sequences (5’—3’)
S1	GTCTATTCACTTTTCATGAGTCAGGGCGACGATTTTGAGATACACAAGGTGAGCACTTTTGTTTAGAGCCAATGTCCATTTTTGAGTCCCTC
S2	AGTCGTTTGGTTTTGAGGTCATATGTTGGCGCTTTTGTGAATAGACGAGGGACTCATTTTGAACTTAAGCCGTAGAACTTTTACTGGTGACT
S3	TGTGTATCTCTTTTATCTCACGAAGCATACGATTTTCCAAACGACTAGTCACCAGTTTTTGTGTCCTCCTAAGATGCCTTTTGTGCTCACCT
H1	**FAM**-TAATCGTGATAGGGGTCAACACTAACCTACCCCTATCACGAT/**iBHQ1dT**/AGCATTAATTTTTTTCGTCGCCCTGACTCATG
H2	CAACACTAACCTTAATCGTGATAGGGGTAGGTTAGTGTTGACCCCTATTTTTTTGGCATCTTAGGAGGACAC
H3	**Cy3**-TCAGACTGATGTTGAGCAAAGCTCTATTCAACATCAGTC/**iBHQ2dT**/GATAAGCTATTTTTTGCGCCAACATATGACCTC
H4	GCAAAGCTCTATTCAGACTGATGTTGAATAGAGCTTTGCTCAACATTTTTTTTGGACATTGGCTCTAAAC
AS1411	GGTGGTGGTGGTTGTGGTGGTGGTGGTTTTTTGTTCTACGGCTTAAGTTC
Linker	Biotin-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCGTATGCTTCGTGAGAT
H5	TAGCGTGC/**iBHQ1dT**/ATCCGGACAACACTAACCTTCCGGA/**i6FAMdT**/AGCACGCTACGTAGATTTTTTTTCGTCGCCCTGACTCATG
H5-1	ATCCGGACAACACTAACCTTCCGGA/**i6FAMdT**/AGCACGCTACGTAGATTTTTTTTCGTCGCCCTGACTCATG
H5-2	TAGCGTGCT-**BHQ1**
miRNA-155	UUAAUGCUAAUCGUGAUAGGGGU
miRNA-21	UAGCUUAUCAGACUGAUGUUGA