Electronic Supplementary Information

Intracellular Enzyme-powered DNA circuit with Tunable Amplifier for miRNA Imaging

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Experimental Section

Materials.

Human apurinic/apyrimidinic endonuclease I (APE1) and ThermoPol reaction buffer were obtained from NEB (Beverly, MA). TRIZOL Reagent was from Invitrogen. High Capacity cDNA Reverse Transcription Kits (4368814), FastStart Universal SYBR Green Master (ROX) (04914066001) were purchased from ABI and Roche, respectively. All of the oligonucleotides used in this work were synthesized by Sangon Co. (Shanghai, China) and their sequences are listed in Table S1. All modified oligonucleotides were purified by HPLC, while unmodified oligonucleotides were purified by PAGE. 2-Nitro-5-thiocyanobenzoic acid (NTCB) and tert-Butyl hydroperoxide (TBHP) were purchased from Aladdin Industrial Inc. All chemicals were used as received without additional purification. DNase/RNase free deionized water from Tiangen Biotech Co. (Beijing, China) was used in all experiments.

Design and characterization of the DNA circuits by gel.

The software Tiamat was used for DNA structure design and sequence generation. The DNA circuits were characterized by 23% native PAGE which was operated at 4 °C for 4 h at a constant voltage of 120 V. To confirm that DNA circuits worked, 500 nM of the prepared DNA circuits and 0.658 nM of APE1 were mixed in sealed 0.2 mL PCR tube and incubated at 37 °C for 1 h. All samples were then analyzed by denaturing PAGE that contains 20% polyacrylamide (29:1, acrylamide: bisacrylamide) and urea (8.3 M). Denaturing PAGE were run at room temperature for 1.5 h at a constant voltage of 200 V. The gel was subsequently stained with SYBR Gold.

Amplified miRNA detection by using fluorophore and quencher labeled DNA probe in homogeneous solution.

All reactions in homogeneous solution were carried out in 0.2 mL sealed PCR tube. 100 nM hairpin probe were annealed from 95 °C to 25 °C over 10 min in ThermoPol buffer (20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$ and 0.1% Triton X-100, pH 8.8). Next, the FAM and BHQ1 labeled probe, the target and APE1 were added simultaneously to reach final concentrations of 100 nM, 100 nM and 0.658 nM. Then fluorescence was recorded immediately in the FAM channel of a real-time PCR cycler (Rotor-Gene Q, QIAGEN, Germany) at 37 °C, using a gain of 8, with a time interval of 5 s.

Operating DNA circuits in living cells.

HeLa, A549 and MCF-7 cell lines were cultured in 1640 medium supplemented with 1% Penn/Strep and 10% fetal bovine serum and incubated at 37 °C in a humidified

atmosphere of 5% CO_2/95% air. The cells were transferred to a laser confocal culture dish for fluorescence imaging in an appropriate density. The cells were cultured with 5 μL NTCB (100 μM) in culture dishes for 2 h. After washed with PBS sufficiently, the cells were incubated with a mixture of Lipofectamine 2000 (Invitrogen, USA) and DNA circuits (labeled with FAM and BHQ1) in 1640 medium for 12 h. The nucleus were stained by Hoechst 33342 for 30 min. To adjust the amplification degree by tuning the amplifier, the cells were treated with 500 μM TBHP for 30 min to increase the overexpression of APE1 and incubated with a mixture of Lipofectamine 2000 and DNA circuits in 1640 medium for 6 h. Fluorescence imaging was carried out on an inverted fluorescence microscope equipped with a mercury light source (Nikon). The filter with 470 nm/ 510 nm and 360 nm/ 447 nm were used to detecting the emission of FAM and Hoechst33342 respectively. The images were acquired using a 40× or 100× objective and recorded by the EMCCD (50 ms, gain 3).

Total RNA extract of cells and real-time PCR detection of miRNA-21.

The cells were grown to 70-80% confluency, treated with 500 μM TBHP for 30 min. Followed by washed with PBS, total RNA were extracted with Trizol agent. After obtaining RNA in cells, a reverse transcription was carried on a thermal cycler to produce cDNA by using a stem-loop primer as the following procedure: 25 °C for 10 min, 37 °C for 120 min, 85 °C for 5 min and 4 °C hold. Then miRNA-21 in cells were detected by real-time PCR with a fast PCR mix (Fast start Universal SYBR Green Master, Roche) containing 500 nM forward and reverse primer respectively. The reactions were carried out on a thermocycler (Rotor Gene-Q, Qiagen, Germany) as the following procedure: 95 °C for 15 min, 5 cycles of enrichment at 94 °C for 20 s, 55 °C for 30 s and 72 °C for 34 s, 40 cycles of denaturation at 94 °C for 20 s, annealing and extension at 60 °C for 34 s.

Western bolt analysis of APE1 in different cells.

Cytoplasmic total protein of the cells were obtained using NE-PER Nuclear and Cytoplasmic Extraction kit (ThermoFisher, USA). The protein concentrations of cells were determined with BSA standard curve method from absorbance. For western blot analysis, 30 μg of proteins were resolved on 10% SDS-PAGE, transferred onto nitrocellulose membranes, and probed with anti-APE1 antibodies (1: 5000) (ab194, abcam). The membranes were incubated with goat anti-mouse IgG labeled with horseradish peroxidase (HRP) (1: 5000) (ab6789, abcam). Bands on blots were visualized using an enhanced chemiluminescence (ECL) detection system (5200, Tanon, China).

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

Name	Figures	Sequence (5'-3')
Hairpin Probe		
H1	1(C), 1(D) 1(E), S1(A), 2, 3(A-C), 4(A), S3, S5, S6	CTGATGTTGAATATGAGCCTACTCAACATCAGT CTGATAAGCTA
H1-FQ	3(D), S4-S6	**(FAM)**CTGATGTTGAATATGAGCCTACTCAACAT CAGTCTGATAAGCTA**(Dabcyl)**
H2	S1(B)	GACTGATGTTGACGAGGTGTAGCAGTTATGGT CAACATCAGTCTGATAAGCTA
H3	S1(C)	CTGATGTTGACGAGGTGTAGCAGTTATGATCAA CATCAGTCTGATAAGCTA
Reporter		
R1-X-FQ	1-4, S1(A), S3, S5-S6	GTAGGC**T(-FAM)**C**X**TAT**(-BHQ1)**TCA
R1-X	1(D)	GTAGGCTC**X**TATTCA
R1-FQ	3(B), S5-S6	GTAGGC**T(-FAM)**CATAT**(-BHQ1)**TCA
R1	S1(A)	GTAGGCTCTATTCA
R2	S1(B-C)	CATAACTGTACACCTCG
Input		
miR21-	1-2, S1-S3, S4-S6	UAGCUUAUCAGACUGAUGUUGA
miR214	2(C)	UGCCUGUCUACACUUGCUGUGC
let-7f	2(C)	UGAGGUAGUAGAUUCGUAUAGUU
dsDNA Substrate for APE1		
cR1	1(E)	TTTTTTGAATACGAGCCTACTTTTT
cR3	S2	GTGTGAGCAGGGATACTAGT
R3-X-FQ	S2	AC**T(-FAM)**AGTATC**X**CTGC**T(-BHQ1)**CACAC
cR1&I	1(E)	TGAATATGAGCCTACTCAACATCAGTCTGATAA GCTA
Primer		
RT-primer	4(B), S7	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCA CTGGATACGACTCAACA
FP	4(B), S7	CGGGGTAGCTTATCAGACTGA
RP	4(B), S7	GTGCAGGGTCCGAGGT

[a] X represents AP site.

Supplementary Figures

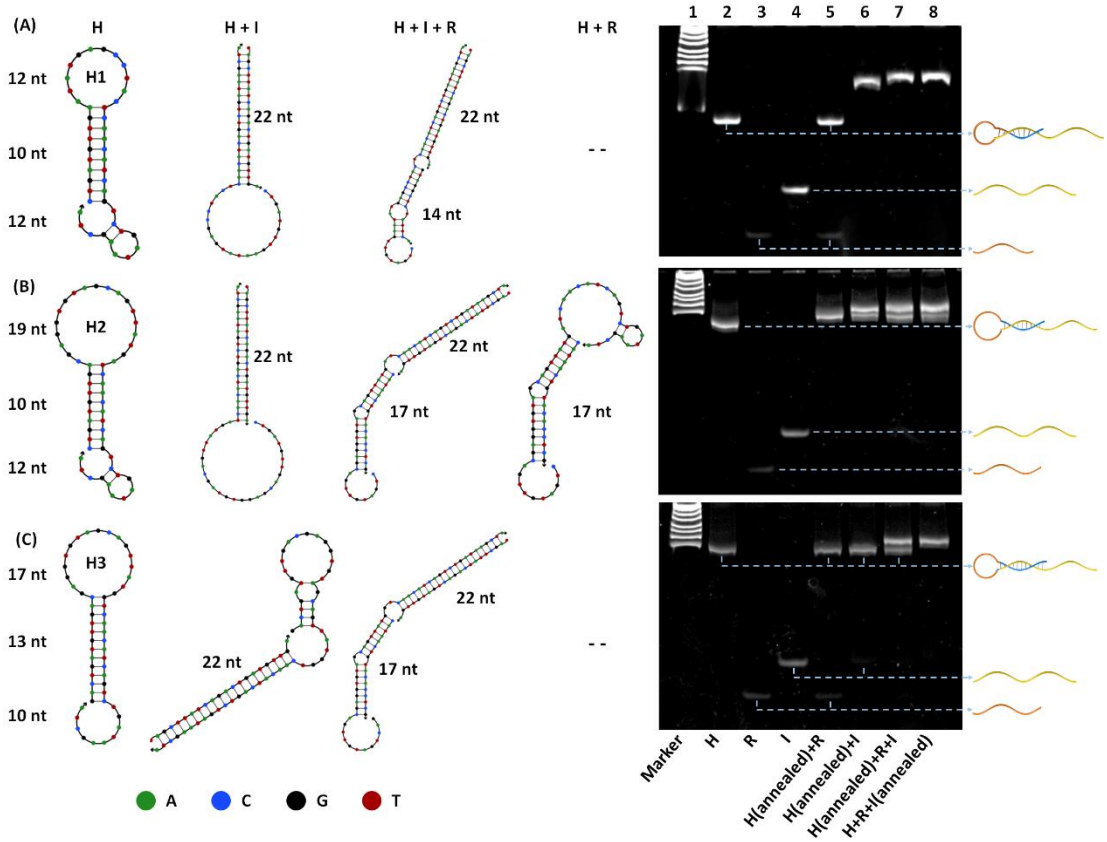

Figure S1. Structure prediction and characterization of the components of DNA circuits, hairpin (H), input (I), and reporter (R). (A) Predicted secondary structures of H, H+I, H+I+R, and H+R at 37 °C by NUPACK analysis tool.[1] The length of different domains are shown. Some structures are not presented because the formation probability is very low. (B) Characterization of the DNA circuits by native gel. Lane 1: 50-bp marker, Lane 2: H, Lane 3: R, Lane 4: Input, Lane5: annealed H was incubated with R, Lane 6: annealed H was incubated with I. Lane 7: annealed H was incubated with I and R, Lane 8: annealed H-R-I complex.

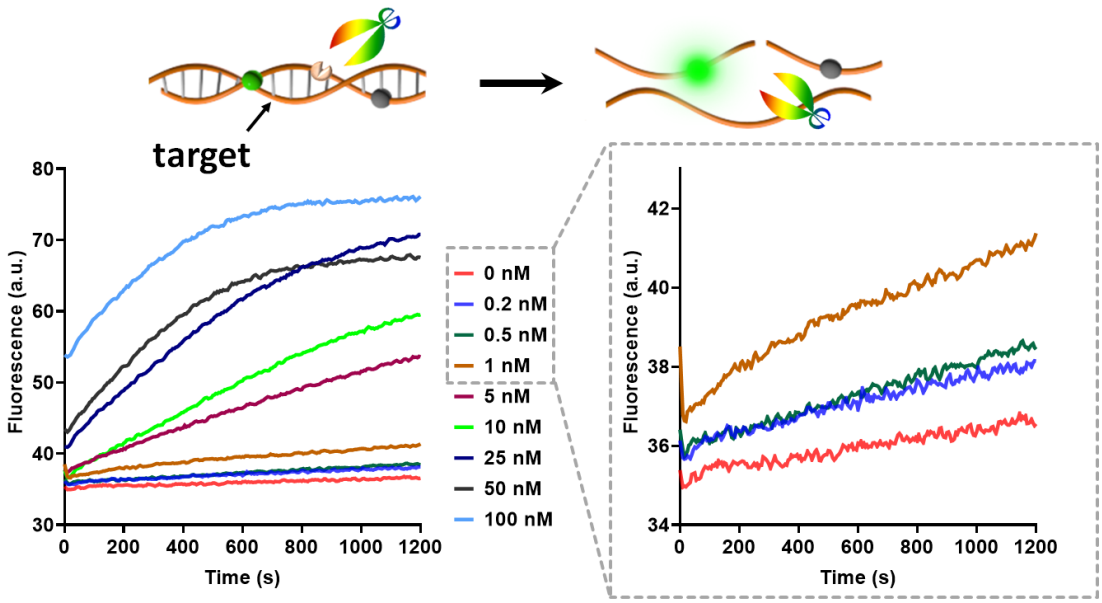

Figure S2. Fluorescence vs time traces of the enzymatic reactions of the double stranded substrate at different concentrations. The reporter strand (R3-FQ) was fixed at 100 nM, its complementary strand (cR3) varied from 0.2 to 100 nM.

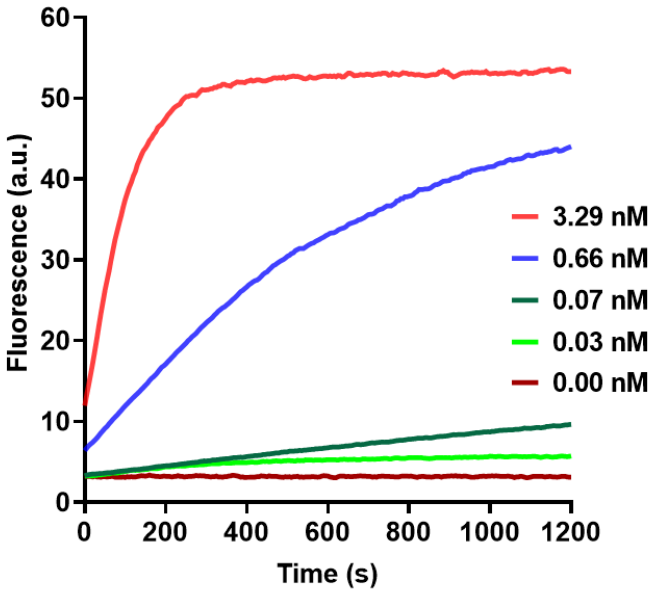

Figure S3. Fluorescence vs time traces of the circuits with varied APE1 concentrations.

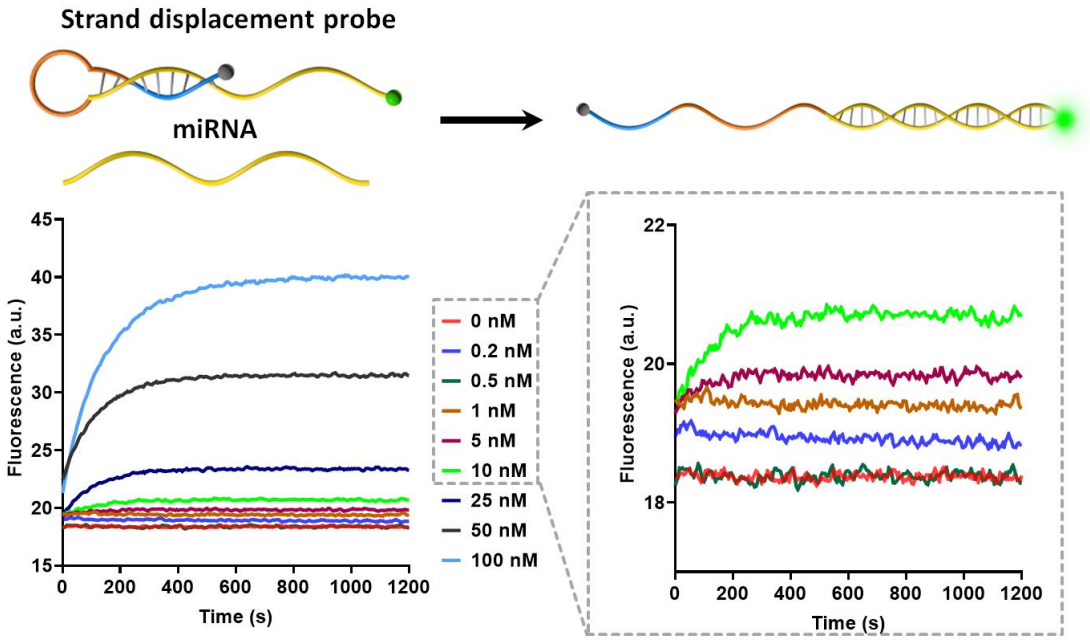

Figure S4. Fluorescence vs time traces of the in vitro detection of miR-21 by the strand displacement probe.

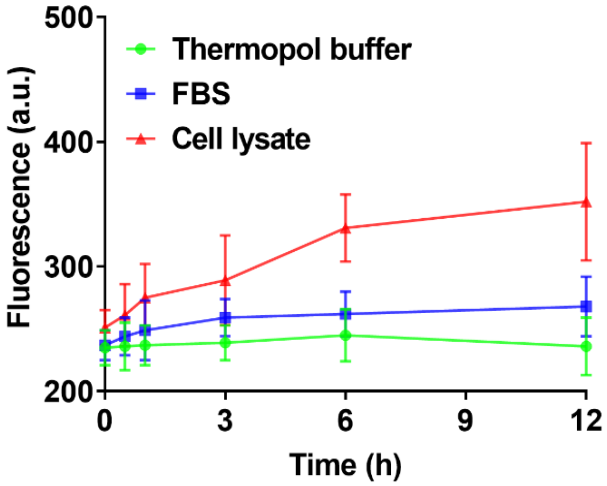

Figure S5. The stability assay of the probes over time in 1X Thermopol buffer, 10% fetal bovine serum (FBS) and HeLa cell lysate. The error bars indicate means ±SD (n=3).

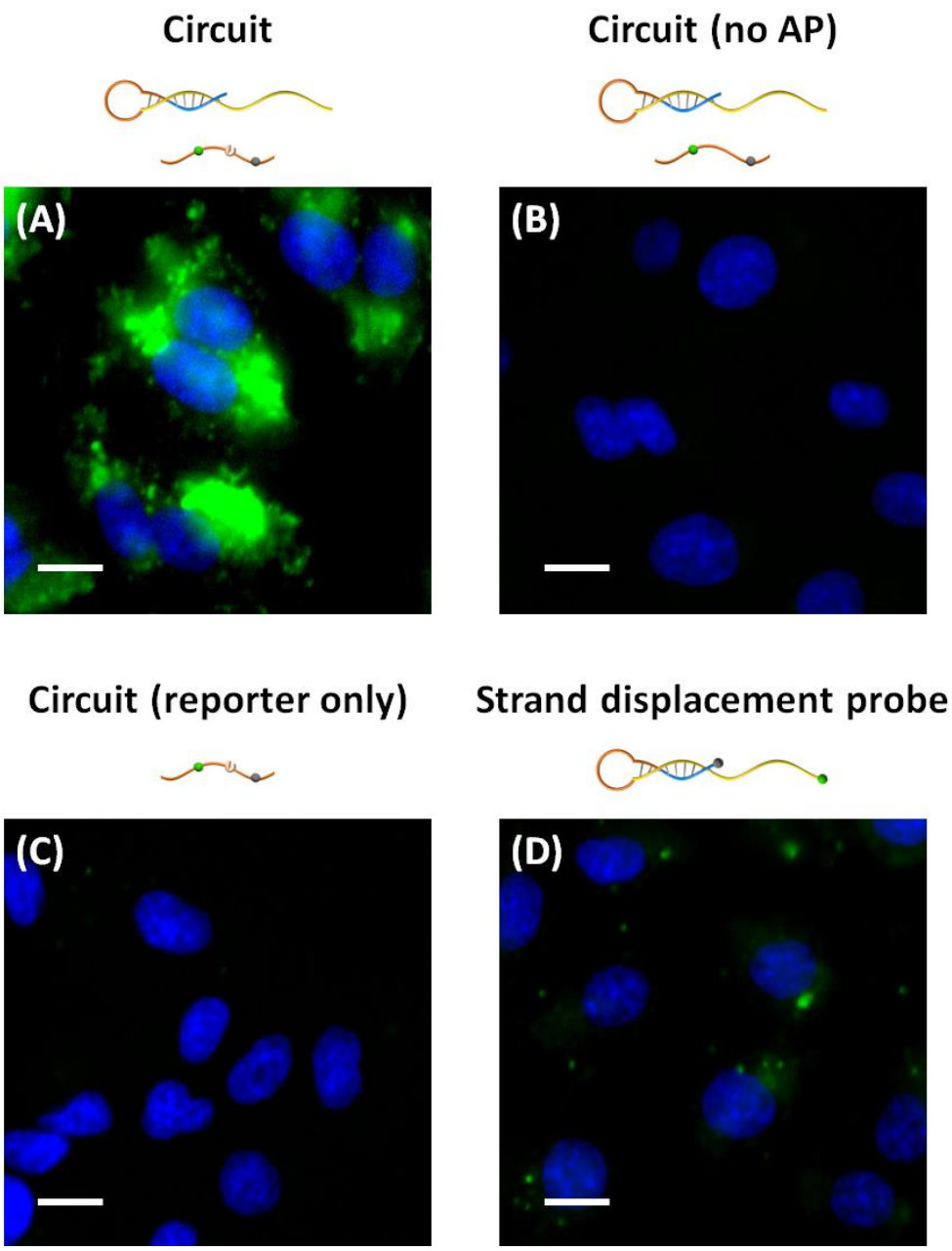

Figure S6. Fluorescence imaging of MCF-7 cells by using different probes, (A) the circuit probe, (B) the circuit probe without AP site, (C) the circuit probe without the hairpin, (D) the strand displacement probe (for the *in vitro* assay of this probe see **Figure S4**). Scale bar: 5 μm.

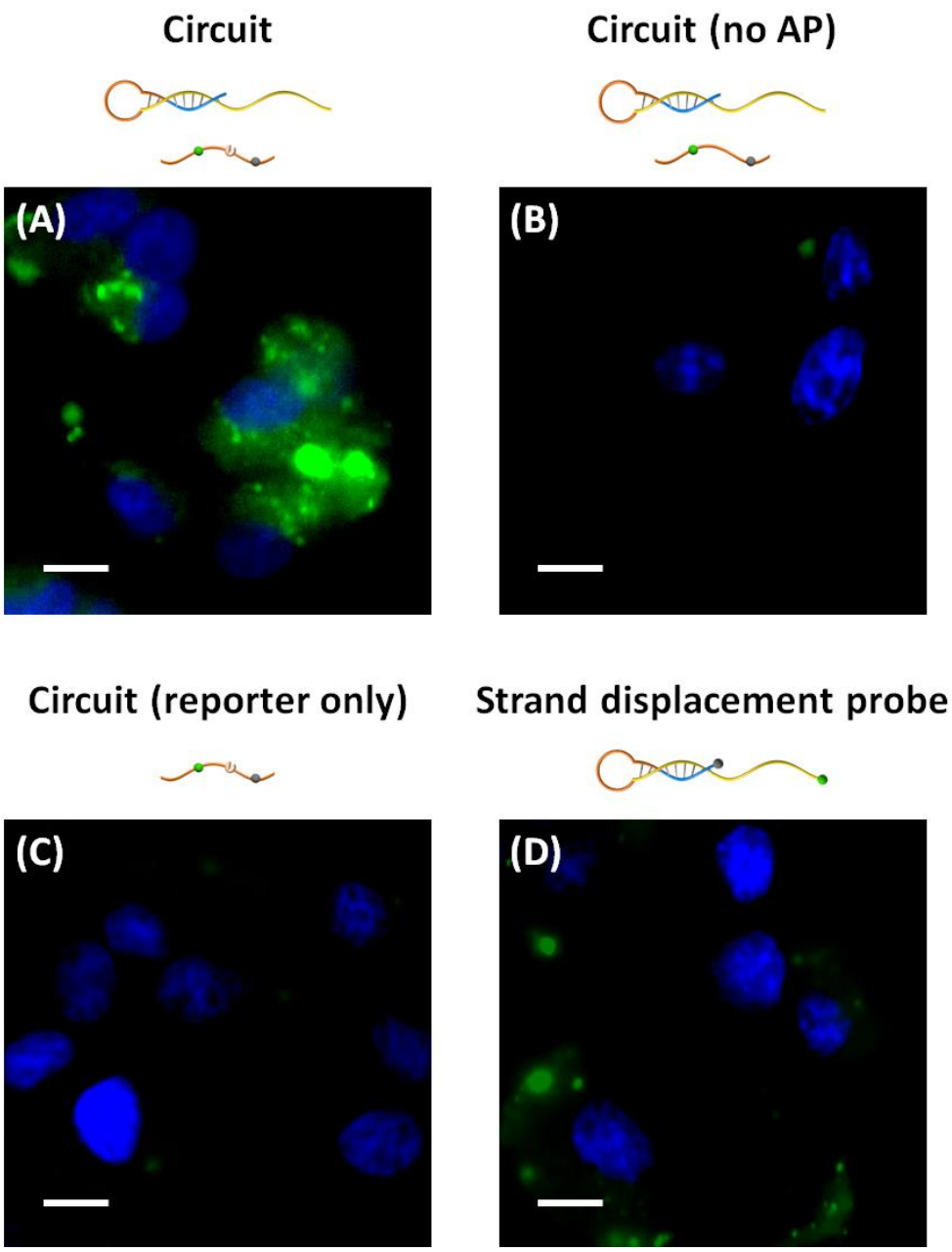

Figure S7. Fluorescence imaging of A549 cells by using different probes, (A) the circuit probe, (B) the circuit probe without AP site, (C) the circuit probe without the hairpin, (D) the strand displacement probe (for the *in vitro* assay of this probe see **Figure S4**). Scale bar: 5 μm.

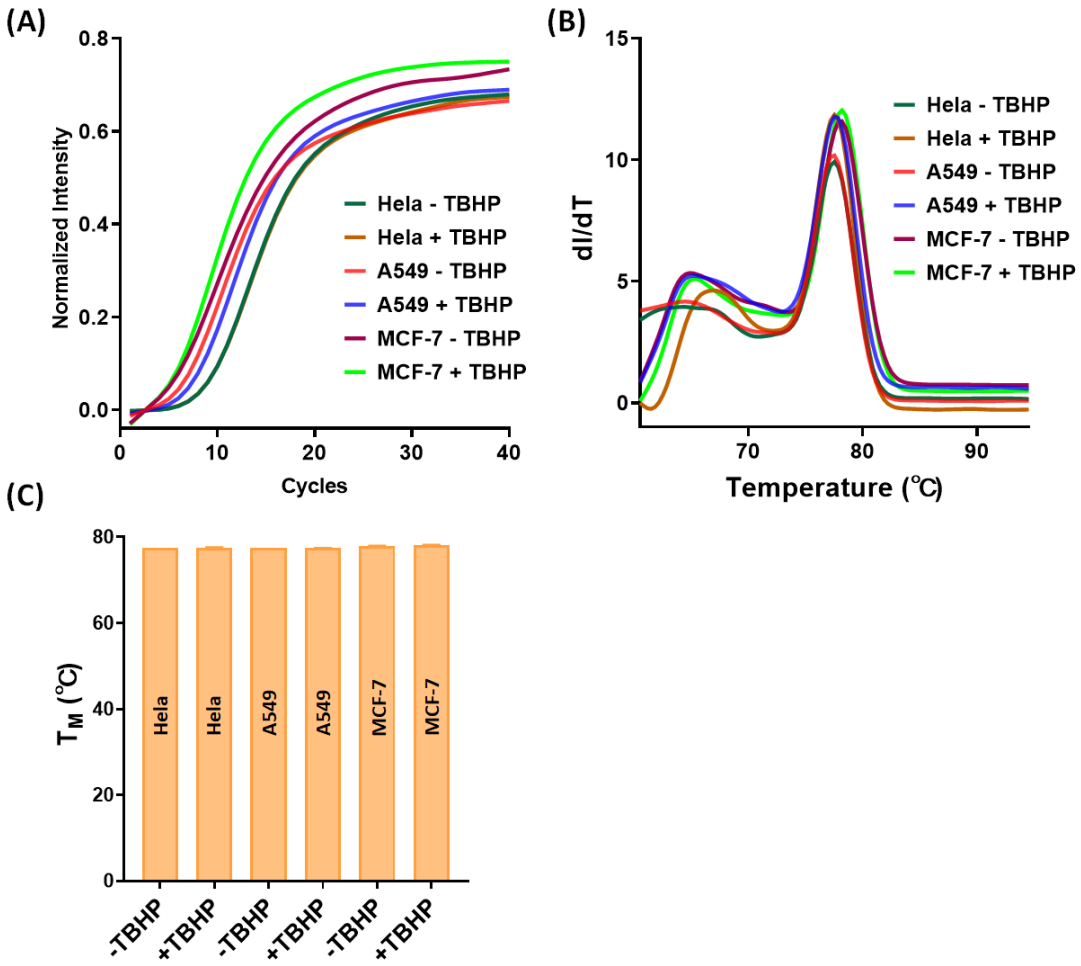

Figure S8. Quantification of miR-21 by qRT-PCR. (A) Raw qPCR curves of extracted miR-21 of the three cell lines under different conditions. (B) Melting curves of the PCR products. (C) Comparison of the melting temperatures.

Reference

(1) Zadeh, J. N.; Steenberg, C. D.; Bois, J. S.; Wolfe, B. R.; Pierce, M. B.; Khan, A. R.; Dirks, R. M.; Pierce, N. A. *J. Comput. Chem.* **2011**, *32*, 170-173.