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Supporting Information

A DNAzyme-mediated target-initiated rolling circle amplification strategy based on microchip platform for the detection of apurinic/apyrimidinic endonuclease 1 at single-cell level

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1. Experimental section

2. Supplementary tables and Figures

Experimental section

1.1 Reagents and materials

The apurinic/apyrimidinic endonuclease 1 (APE1), Exonuclease I (ExoI), ExonucleaseIII (Exo III), T4 DNA ligase, T7 Exonuclease (T7 Exo)，Uracil-DNA glycosylase (UDG), 8-oxoguanine DNA glycosylase (hOGG1), phi29 DNA polymerase and their corresponding buffers, the RNase inhibitor, bovine serum protein (BSA), deoxyribonucleotides mixture (dNTPs) were purchased from New England Biolabs Co., Ltd. (Beijing, China). DNA Marker, 5×TBE, diethyl pyrocarbonate treated water (DEPC water), Tris-HCl buffer was purchased from Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). Spanish agarose (Agarose G-10) was purchased from Biowest, Spain. DMEM medium and RPMI 1640 medium were purchased from Thermo Fisher Scientific Inc. Fetal bovine serum, Trypsin-EDTA digestive juice (0.25%), penicillin-streptomycin solution were purchased from Sigma (USA). NP-40 lysate was purchased from Biyun Biotechnology Co., Ltd. The DNA sequences were synthesized by Sinoponics Group Chemical Reagent Co., Ltd. (Shanghai, China). All synthetic DNA strands are graded by HPLC, and the sequence is shown in Table S1. Human normal liver cells (HL-7702), human cervical cancer cells (Hela) and human breast cancer cells (MCF-7) were obtained from cell/stem cell bank of the Chinese Academy of Sciences. Electrophoresis buffer was 25 mM borax solution (pH 8.8) containing 25 mM SDS. All other chemicals are analytically pure, and the water used in the preparation of the solution is ultrapure water treated with paraformaldehyde and diethyl pyrocarbonate (DEPC). All solutions were filtered through a 0.45 μm membrane filter.

1.2 Apparatus and microfluidic chip

Model 5702, 5424R centrifuges were purchased from Eppendorf China Ltd. The microchip electrophoresis laser-induced fluorescence detection (MCE-LIF) system was designed and constructed by our laboratory.[1] It consisted of an intelligent 8-

channel high voltage power supply (0~5000 V), a microfluidic glass chip（Fig. S2）, a laser at 473 nm, a the photomultiplier tube (PMT) and a data acquisition system (chromatography workstation HW-2000). The microchip was purchased from Dalian Tuo Microchip Technology Co., Ltd. The chip design was the same as the previous reported.[2]

1.3 Activation of microchip channels

The microchip channels were activated before use by using methanol, ultrapure water, 1 M HCl solution, ultrapure water, 1 M NaOH solution and ultrapure water cleaned sequentially. Before each sample injection, the microfluidic channel was rinsed sequentially with ultrapure water for 6 min, 1M NaOH solution for 10 min, ultrapure water for 6 min and electrophoresis buffer for 5 min. Finally, all reservoirs were filled with the electrophoretic buffer solution. Vacuum was applied to the reservoir BW in order to fill the separation channel with the electrophoretic buffer solution for MCE-LIF assay.

1.4 Preparation of the circular template probe

A 10 μL volume of phosphorylated DNA (P-DNA, 50 μM), 20 μL of link DNA (L-DNA, 50 μM), 10 μL of 10×T4 DNA ligase buffer (pH 7.4, 500 mM Tris HCl, 100 mM $MgCl_2$, 100 mM DTT, 10 mM ATP) and 56 μL of DEPC water were added into a 200 μL centrifuge tube, which was heated to 75 °C and reaction for 30 min, and then cooled down to room temperature. After that, 1.5 μL of 400 U/μL T4 DNA ligase water was added and reacted at 16 °C for 12 h. Then the reaction solution was heated to 85 °C and reaction for 20 min to inactivate the T4 DNA ligase. Next, 1.5 μL of exonuclease I (200 U/μL) and 1μL of exonuclease III (1000 U/μL) were added and reacted at 37 °C for 24 h to digest the leftover ssDNA and dsDNA to yield closed DNA. The enzymes were denatured by heating at 95 °C for 20 min. The prepared circular template probes named “CT” were then stored at -20 °C until use.

1.5 Agarose gel electrophoresis

The prepared sample was added into 2 μL 6×DNA loading buffer solution and mixed evenly. Then, the sample solution was added to the sample hole of the agarose gel. The concentration of the agarose gel used is 4%, the voltage was 100 V, and the electrophoresis time was 80 min. Finally, the Omega16ic gel imaging system was used for imaging.

1.6 Detection of APE1 activity

A 2.5 μL volume of 10×RCA reaction buffer solution (500 mM Tris−HCl, pH 7.5, 200 mM $MgCl_2$, and 10 mM DTT), 1 μL of CT solution, 5 μL of dNTPs (10 mM) solution, 0.5 μL of BSA (10 mg/mL) solution, 0.5 μL of RNase inhibitor (40 U/μL) solution, 2.5 μL of APE1 with different concentrations, 0.5 μL of phi29 DNA polymerase (10 U/μL), 2.5 μL of substrate probe SP (1 μM) and appropriate amount of DEPC treated water were mixed to make the total volume 25 μL of reaction solution. The reaction was carried out at 37 °C for 2 h, and then heated to 80 °C and incubated for 10 min to terminate the amplification reaction. When the reaction is over, 5 μL of substrate fluorescent probe F-DNA (1 μM) and 20 μL of DEPC water were added, and incubated at 37 °C for 1 h. Then the reaction mixture solution was used directly for MCE-LIF analysis.

Prior to MCE-LIF analysis, all reservoirs in microchip were filled with the electrophoretic buffer solution. Vacuum was applied in the reservoir BW to fill the separation channel with the electrophoretic buffer solution. Then, electrophoretic buffer solution in reservoir S was replaced by 10 μL reaction mixture solution, and different voltages were applied to respective reservoirs to complete sample injection, separation and detection. During the sample injection period, the sample reservoir S was applied 400 V, SW reservoir 0 V, B reservoir 200 V, and BW reservoir 250 V. After sample injection 20 s, the potential was switched to the separation and detection period immediately. During this period, S and SW reservoirs were applied 2000 V, B reservoir 2400 V, and BW reservoir 0 V.

1.7 Cells culture and cell lysate preparation

HL-7702 and MCF-7 cells were cultured in a humidified environment (37°C, 5% CO_2) with 100 IU/mL penicillin-streptomycin and 10% fetal calf serum in DMEM medium. Under similar culture conditions, Hela cells were cultivated in RPMI 1640 medium. The cell lysate was prepared according to the method in the literature.[2] 1.0×10^4 cells were collected in a 1.5 mL centrifuge tube, washed three times with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4), and centrifuged at 1300 rpm for 3 min. Then the cells were suspended in 100 μL NP-40 lysis buffer (10 mM Tris-HCl, pH 8.0, 1 mM $MgCl_2$, 1 mM EDTA, 1% NP-40, 0.25 mM sodium deoxycholate, 10% glycerol, 150 mM NaCl, 5 mM β-mercaptoethanol, and 0.1 mM AEBSF), and incubated for 30 min on ice. Next, the sample was centrifuged at 12000 rpm at 4 °C for 20 min. After further dilution, the supernatant can be directly used for APE1 detection or stored at -80 °C for further research.

2. Supplementary Tables and Figures

Table S1 Oligonucleotide sequences used in this work*

Name	Sequence (5'-3')
Substrate probe (SP)	GTGGACGATTAACCCA***GTCTAGGATTCGGC GTGGGTTAA*** XCGTCCAC
Non-substrate probe (NSP)	GTGGACGATTAACCCA***GTCTAGGATTCGGC GTGGGTTAA***TCGTCCAC
Phosphorylated DNA (P-DNA)	*p*- CGA ATC CTA GAC CCT AGC ATA GCC TCC CAA AAT ATC CTA TAT TTC GGC CCC GAC CTG GTT CGA TAT CTT AAC CCA CGC
Link DNA (L-DNA)	GTC TAG GAT TCG GCG TGG GTT AA
Substrate fluorescent probe (F-DNA)	FAM-ACT CTT CCT AGCT rA TGG TTC GAT CAA GA

*Substrate probe (SP) and non-substrate probe (NSP) include the initiator sequence (italic bold letters), underline sequences indicate complementary regions of the probes to form hairpin structure, the X is AP site. Phosphorylated DNA (P-DNA) and link DNA (L-DNA) were used to prepare circular template probe (CT), *p* indicates phosphate group. The rA denotes adenosine ribonucleotide at that position while all others are deoxyribonucleotides, FAM represents 6-carboxyfluorescein in substrate fluorescent probe (F-DNA).

Table S2 Comparison of methods for APE1 activity assay

Strategy	Dynamic range（U/mL）	Detection limit（U/mL）	Reference
Fluorescence method based on host-guest interaction of β-cyclodextrin polymer and pyrene	0.05-0.25	0.05	3
Unimolecular chemically modified DNA fluorescent probe	0.1-5	0.1	4
An electrochemical biosensor integrating immunoassay and enzyme activity analysis	0.01-4	0.00518	5
SERS biosensor	0.002-20	0.001	6
Based on isothermal amplified-generation of G-quadruplex	0.01-1.0	0.006	7
A DNA structure-mediated fluorescent biosensor	0.000002-0.004	1.7×10^{-6}	8
DNA-nanoprobe	0.01-1.0	0.01	9
Biodegradable MnO_2 nanosheet mediated hybridization chain reaction	0.001-10	0.0005	10
Bipedal DNA nanowalker fueled by catalytic assembly	0.02-1.0	0.01	11
Fluorescence amplification strategy	0-800 (cells)	Lower than a Hela cell (0.0005 U/mL)	This work

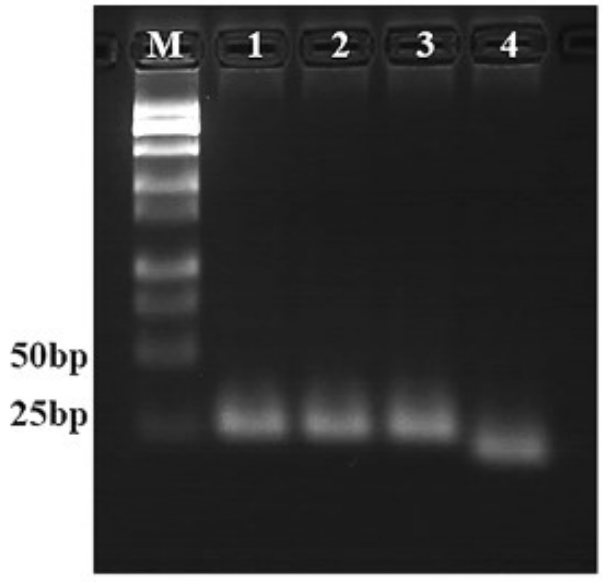

Figure S1. The agarose gel electrophoresis for characterization of hairpin substrate probe (SP). Lane M: DNA marker; lane 1: NSP; lane 2: NSP+APE1; lane 3: SP; lane 4: SP+APE1.

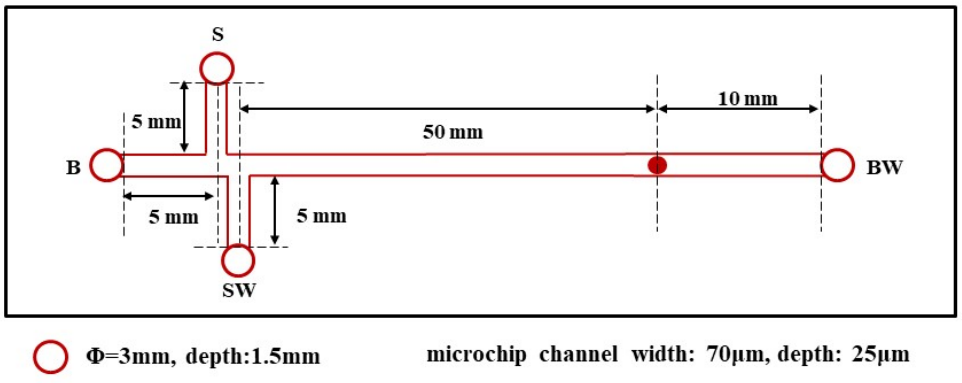

Figure S2. The structure of microchip for MCE-LIF. S: sample reservoir; SW: sample waste reservoir; B: buffer reservoir; BW: buffer waste reservoir.

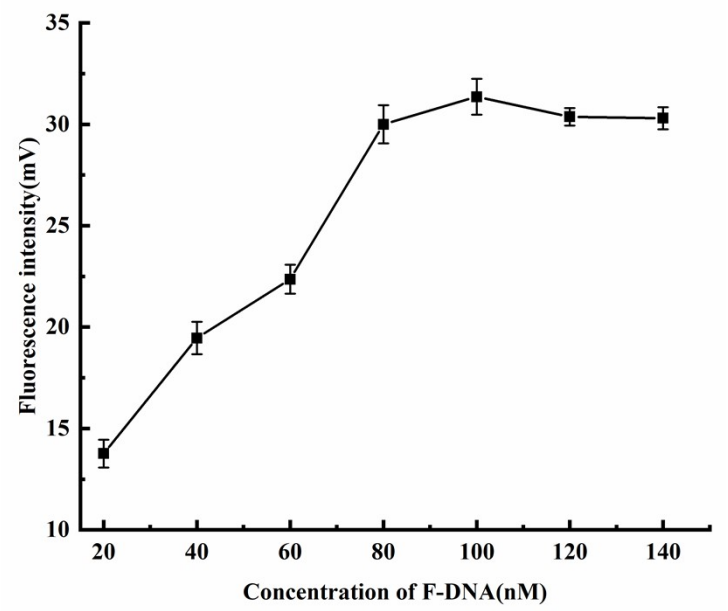

Figure S3.. The changes of fluorescence intensity of APE1 with the concentration of substrate fluorescent probe (F-DNA). Error bars show the standard deviation of three experiments.

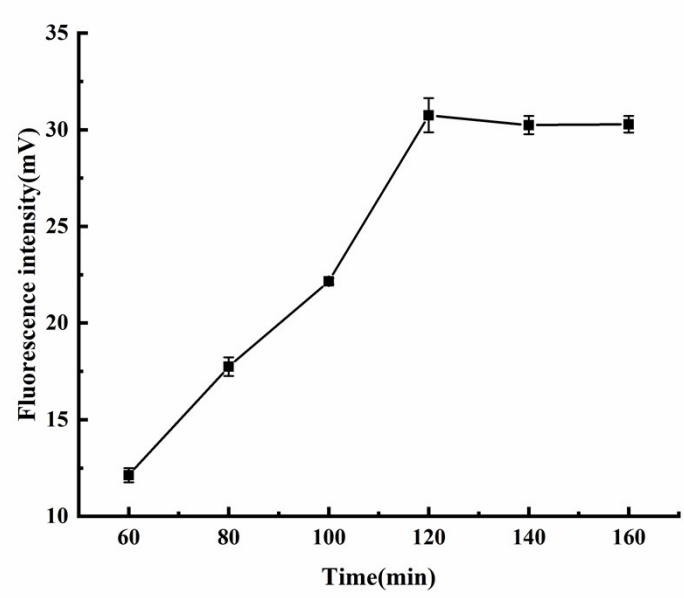

Figure S4. The changes of fluorescence intensity of APE1 with the reaction time. Error bars show

the standard deviation of three experiments.

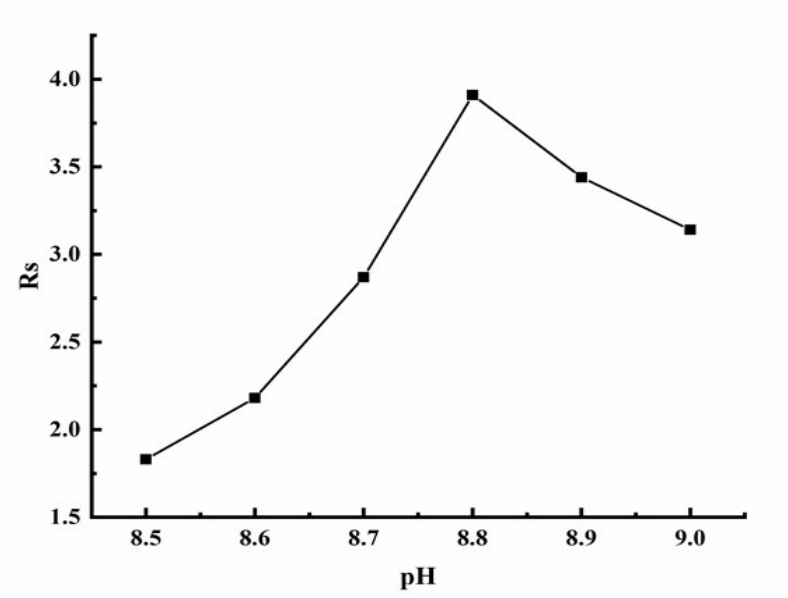

Figure S5. The effect of the pH of the electrophoresis buffer on the resolution.

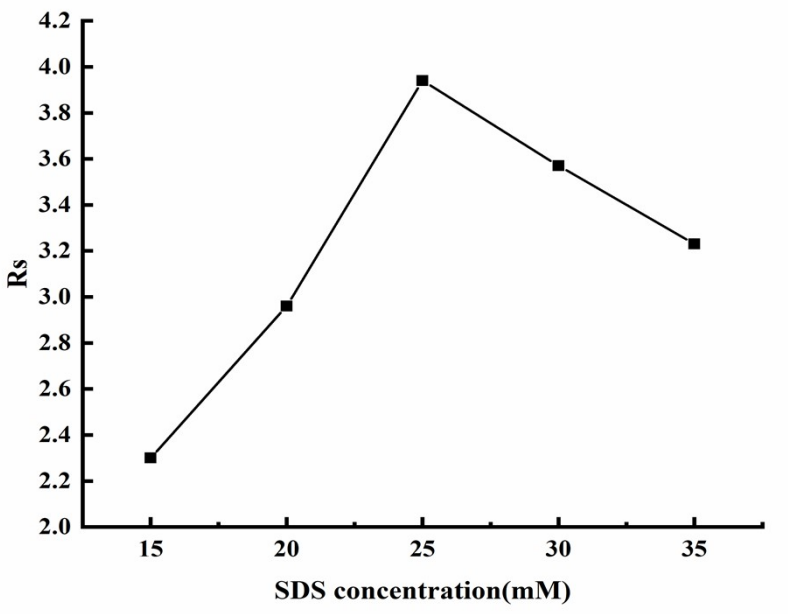

Figure S6. The effect of SDS concentration on resolution.

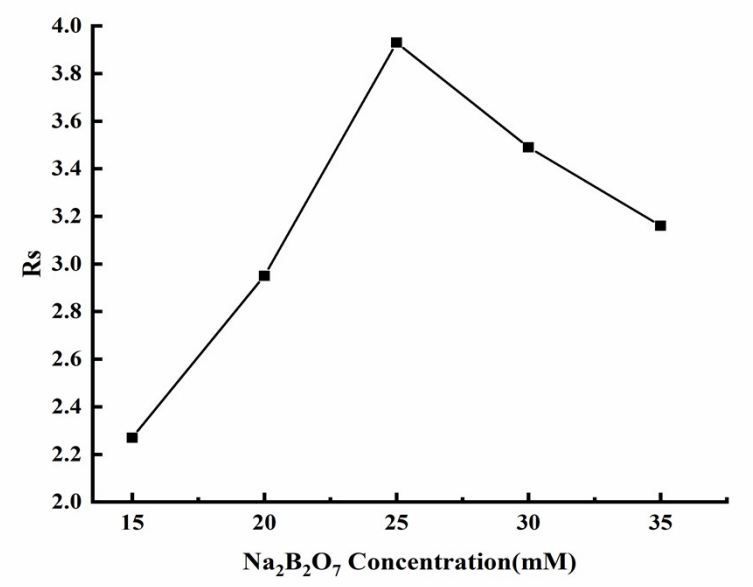

Figure S7. The effect of borax concentration on resolution.

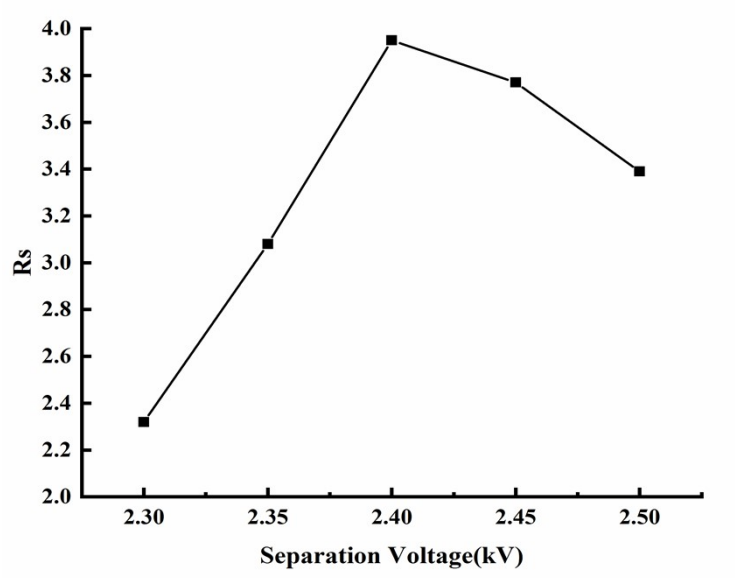

Figure S8. The effect of separation voltage on separation

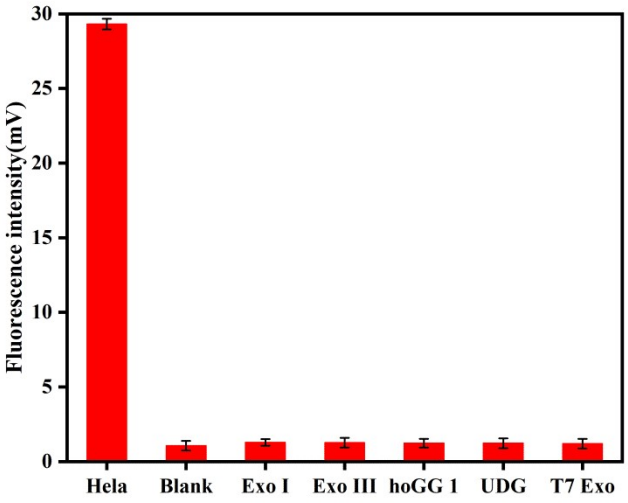

Figure S9. The specificity of method for APE1 detection. The concentrations of the different substances are Hela: 200 cells, blank: 0 cell, other: 1 U/mL. Error bars show the standard deviation of three experiments.

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