Electronic Supplementary Information for

Enhancing APE1 detection through apurinic/apyrimidinic site inhibition of DNA polymerase: an innovative, highly sensitive approach

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SI 1. Sequences used in this work

Table S1.

Strand name	Sequence (from 5' to 3')
Template-1	CGCGGGACCCTCCAGAAGAGCGGCCGGCGCCGTGACTCAGCACTGGGGCGGAGCGGGGCGGGACCACCCTTATAAGGCTCGGAGGCCGCGAGGCCTTCGCTGGAGTTTCGCCGCCGCAGTC
FP-1	CGCGGGACCCTCCAGAAGA
RP-1	GACTGCGGCGGCGAAACT
FP-2	_G_GGGACCCTCCAGAAGA
RP-2	GACTGC_GC_GC_AAACT
Upstream-1	ACTGCTACGCTCGGTGACTGCGGCGGCGAAACT
Upstream-2	ACTGCT_CGCTCGGTGACTGCGGCGGCGAAACT
Upstream-3	TCTACCGAGCGTAGCAGT
Template-2	CAATGCTGACCTACGACTGAACAGATCCGATCTACGACAATGCTGACCTACGACTG
Primer-1	CAGTCGTAGGTC
Primer-2	CAGTCGTAGG_C
Primer-3	CAGTCGTAGG_CTTGCG
Primer-4	C_GTCGTAGGTC
Primer-5	CAGTCGT_GG_C
Template-3	(5'P)TACCCGAGCAATACCACTACCCGAGCAATACCACGACCTACGACTGCAG
Primer-6	CTGCAGTCGTAGG_C
Primer-7	CTGCAGTCGTAGG_CATGCG
Probe-1	5'FAM- TACCCGA_CAATACC -3'BHQ1

AP sites was denoted as "_". 5' Phosphorylation shown as "(5'P)".

SI 2. Materials and methods

1. Materials

All oligonucleotides (Table S1) were provided by Sangon Biotech (Shanghai, China). Taq PCR Master Mix (2X, without Dye) and deionized water (DNase/RNase free) were purchased from Sangon Biotech (Shanghai, China). ，EvaGreen (20X in water) was purchased from MaoKang Biotech (Shanghai, China) . APE1, Endo IV, Nt.Awli, Lambda exonuclease (λ exo), APE1 buffer and Theromopol Reaction Buffer were purchased from New England Biolabs (NEB, MA, USA). The inhibitor 7-nitroindole-2-carboxylicacid (NCA) was purchased from MedChemExpress (MCE, NJ, USA). The concentrations of DNA oligo-nucleotides were measured by NanoDrop 2000 UV–vis Spectrophotometer (ThermoFisher Scientific, MA, USA). The thermal cycling and fluorescence signal measurement were carried out by Q1000 Real-Time PCR system (LongGene, Hangzhou, China). Reactive oxygen species detection reagents (Cell Counting Kit-8(CCK-8)) used in cell experiments were purchased from bioswamp (Wuhan, China).

2. PCR using primers with AP sites

1 μL of Template-1 (10 fM, see its sequence in Table S1) and primers were mixed with EvaGreen and Taq PCR Master Mix, adding up to 25μL by deionized water finally. Then the PCR procedure was set up, including denaturing (95℃ for 15 seconds), annealing, and extending(72℃ for 20 seconds). Thermal cycling and fluorescence intensity was carried out by the Rotor-Gene Q real-time PCR instrument (QIAGEN, Hilden, Germany)

3. Verification the inhibitory effect of AP sites in different regions

Taq PCR Master Mix, EvaGreen were added to the system in 25μL consisting of 200nM Template-1 and 200nM primers with or without AP site outside the matching region of template and primer. Then fluorescence intensity was measured at 50℃ constantly.

4. Verification of the inhibiting effect of the AP site on Taq polymerase.

1 μL of Template-2 (10 μM, see its sequence in Table S1) and Primer-1 or Primer-2 with equal amounts were mixed with 1×ThermoPol to 10 μL before heating and annealing. Then 5μL of the mixture, EvaGreen, Taq PCR Master Mix and Endo IV were added with Deionized water

and the final volume of the solution was 25 μL. Fluorescence intensity was measured immediately.

5. Observation of the stability of AP site at a high temperature

Taq PCR Master Mix, λ exo, APE-1 and Probe-1 were added to the system containing 400nM Template-3 and Primer-6 in 20 μL incubated at different temperature before added. Fluorescence intensity was measured immediately

6. Application of the new amplification module

Taq PCR Master Mix, Lambda Exonuclease, APE-1 and Probe-1 were added to the system containing 400nM Template-3 and Primer-6 in 20 μL. Fluorescence intensity was measured immediately.

7. Optimization of the detection method

Taq PCR Master Mix, Lambda Exonuclease and APE-1 were added to the system in 20 μL consisting of 400nM template and 40nM primer. Then the system was incubated at a constant temperature for a period of time and the incubation time and temperature were subsequently changed. When incubation finished, APE-1 and Lambda Exonuclease were inactivated in condition of heated 75℃ for 15 min before the system was added Probe-1 and Endo IV and fluorescence intensity was measured.

Following the optimization of incubation time and temperature, Primer-6 was replaced by Primer-7 and the procedure was repeated as above.

8. Detection of APE-1 activity

The reaction system contained 400nM template, 400nM primer Taq PCR Master Mix, Lambda Exonuclease and multiple-concentration gradient APE-1. Then the reaction system was incubated at 40℃ for 40min and was heated next to inactive APE-1 and Lambda Exonuclease. Finally, Probe-1 and Endo IV were add to the system fluorescence intensity was measured at instant.

9. Assay of APE1 in cell lysates

Roughly 1.5×107 HeLa cells were lysed and resuspended by lysis buffer on the ice. The mixture solution was then centrifuged at 12,000 rpm for 5 min at 4 ℃ to remove insoluble substances. 1 μL of lysate and NCA with final concentration of 50 μM were added to the reaction system. The detection procedure was as same as Section (8. Detection of APE-1 activity).

SI 3. Comparison of different APE1 detection methods

Table S2

method	publish year	LOD	simplicity	reaction time
Han et al.[1]	2022	0.0026 U/mL	Low temperature required	>2h
Qi et al.[2]	2023	0.001 U/mL	Complex procedure	>3h
Liu et al.[3]	2022	0.006 U/mL	Complex template preparation	>2.5h
Li et al.[4]	2019	0.0005 U/mL	Difficult synthesis condition	~1.5h
Zhou et al.[5]	2020	0.01 U/mL	Relatively simple procedure	~2h
Huang et al.[6]	2017	0.006 U/mL	Operation-friendly	~2h

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SI 4. The inhibitory effect of AP site on Taq polymerase

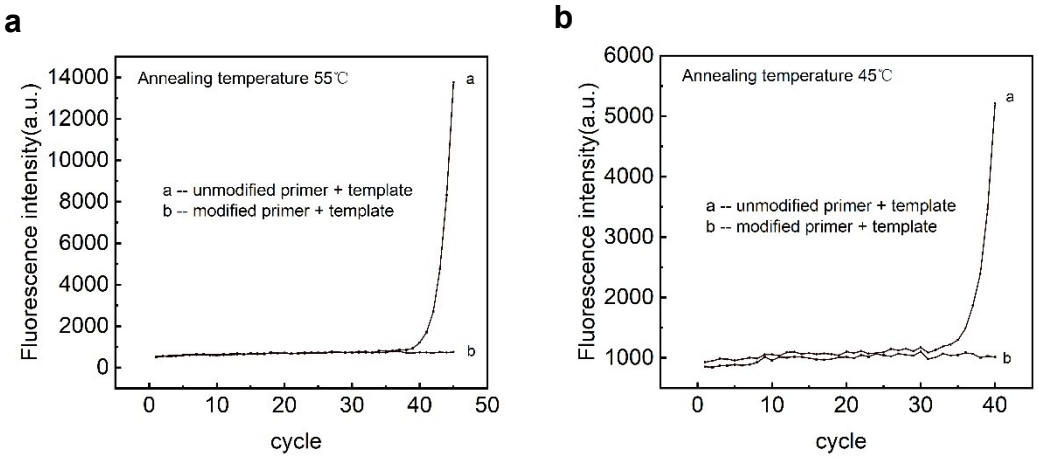

Figure. S1. (a) PCR signals of solutions added Template-1 and primers with AP sites(FP-1 and RP-1) or without the AP site(FP-2 and RP-2). (b) PCR signals of solutions added the Template-1 and primers with AP sites(FP-1 and RP-1) or without the AP site(FP-2 and RP-2) after decreasing the annealing temperature.

SI 5. Effect of the primers with the AP site in different regions

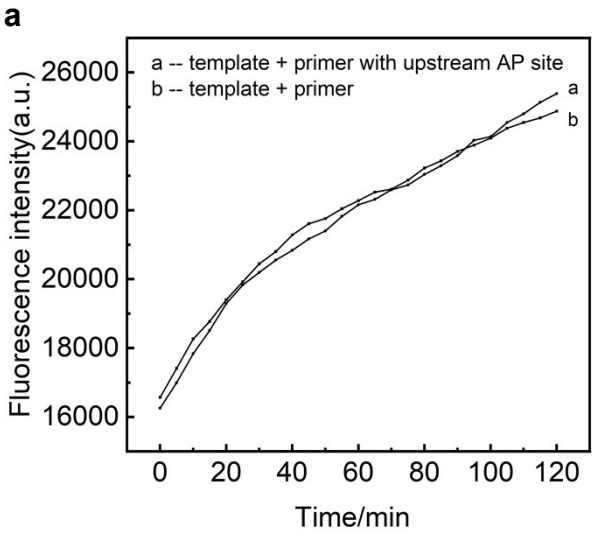

Figure S2. (a) Fluorescent signals of system comprising Taq PCR Master Mix, APE-1 ,Endo IV and primer.

SI 6. Effect of primers with different structure and number or position of the AP site

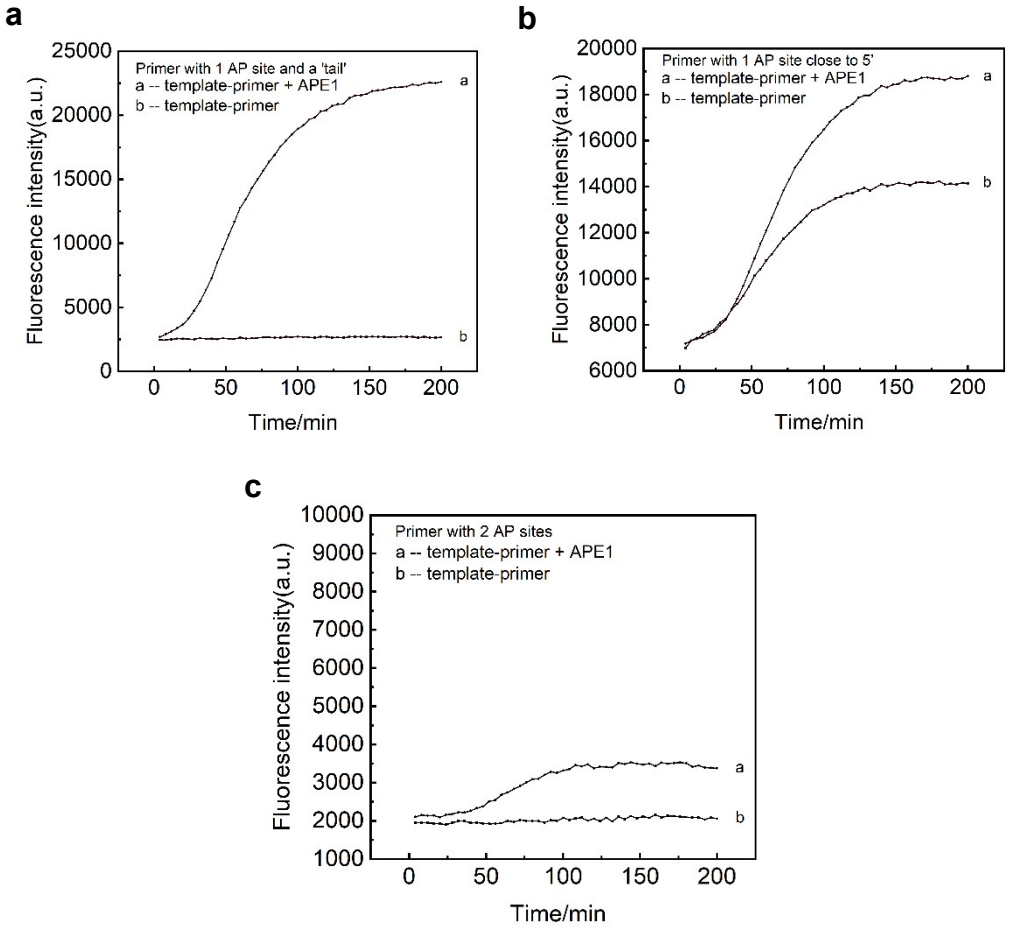

Figure S3. (a) Fluorescent signals of solutions comprising template-primer(Template-2, Primer-3), Taq PCR Master Mix, Nt.Alwi and with or without APE-1. (b) Fluorescent signals of solutions comprising template-primer(Template-2, Primer-4), Taq PCR Master Mix, Nt.Alwi and with or without APE-1. (c) Fluorescent signals of solutions comprising template-primer(Template-2, Primer-5), Taq PCR Master Mix, Nt.Alwi and with or without APE-1.

SI 7. The amplification and detection results of EXPAR-based module

For the signal amplification module, our initial design was based on the exponential amplification reaction (EXPAR). However, its amplification ability was not as expected. We attributed it to Taq polymerase which is rarely used in EXPAR.[1]

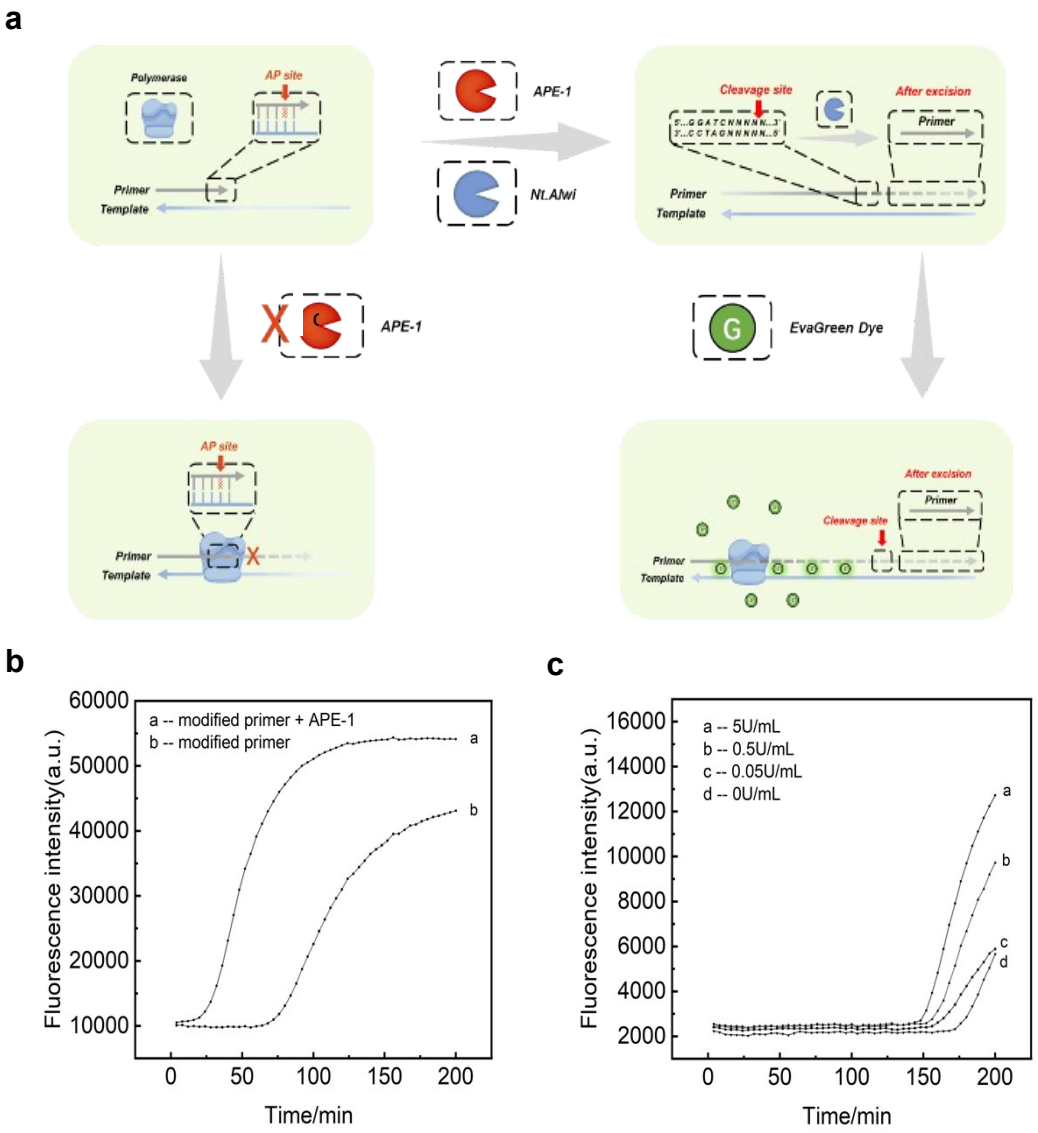

Figure S4. (a) The principle of the original amplification module based on EXPAR. (b) Verification of availability of the EXPAR-based amplification module in condition that sufficient APE-1 exists. (c) Result of the detection of limited amount of APE-1.

SI 8. The stability of AP site at a high temperature

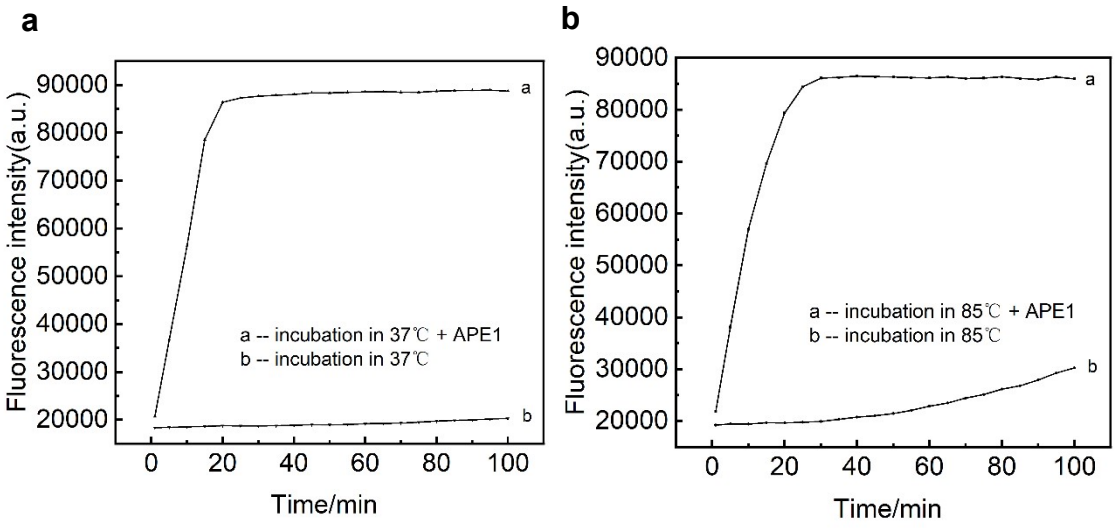

Figure S5. (a) Fluorescent signals of the system containing template-primer (Template-3, Primer-6, incubated at 37℃ in advance), Taq PCR Master Mix, λ exo and Probe-1 with or without APE-1. (b) Fluorescent signals of the system containing template-primer (Template-2, Primer-6, incubated at 85℃ in advance), Taq PCR Master Mix, λ exo and Probe-1 with or without APE-1.

SI 9. The structure optimization of primers in detection methods

A mismatch region 5-nt long at the 3' end of the primer. when the primer binds to the template, a "tail" structure will appear, which can further strengthen the inhibitory effect of AP site on the primer extension. However, when Primer-7 used, the one with a mismatch region, the detection ability of this method decreased obviously, especially in the range with low APE1 concentration. As the concentration of APE1 was reduced to 0.5 U/mL, the signal of the system was significantly reduced and could not be distinguished from the signal of the system without APE1.

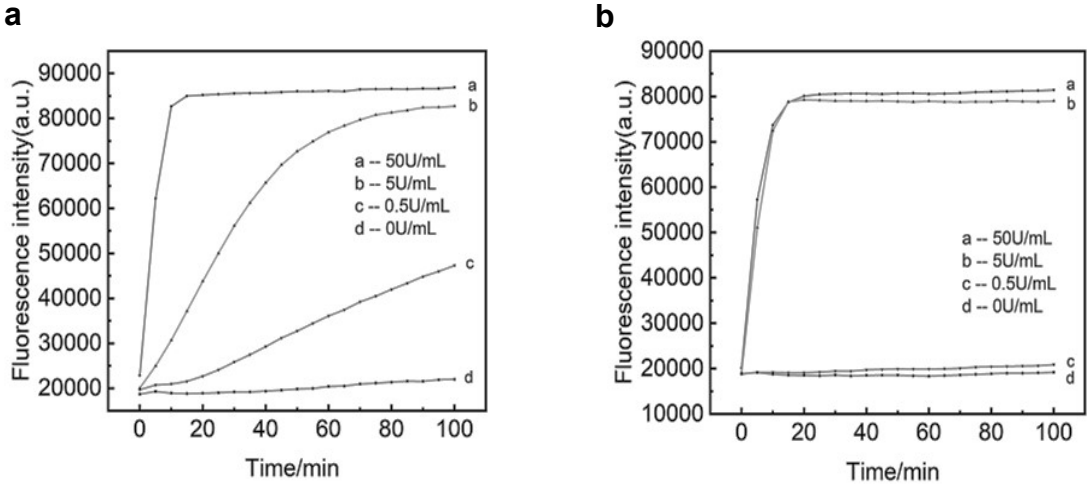

Figure S6. (a)The detection of limited amount of APE-1 using the primer without mismatch “tail”. (b)The detection of limited amount of APE-1 using the primer with mismatch “tail”.

SI 10. Spiked recovery test

It's reported the serum APE1 level in the colorectal cancer patients was about 0.005U/mL.[2] Based on this data, we tests two spiked concentrations, which varied 20% about 0.005U/mL.

Table S3.

spiked concentration	measured	%
0.004U/mL	0.004224U/mL	105.6%
0.006U/mL	0.006164U/mL	102.7%

Sl 11. Assay of APE1 in Siha cell and Skov3 cell

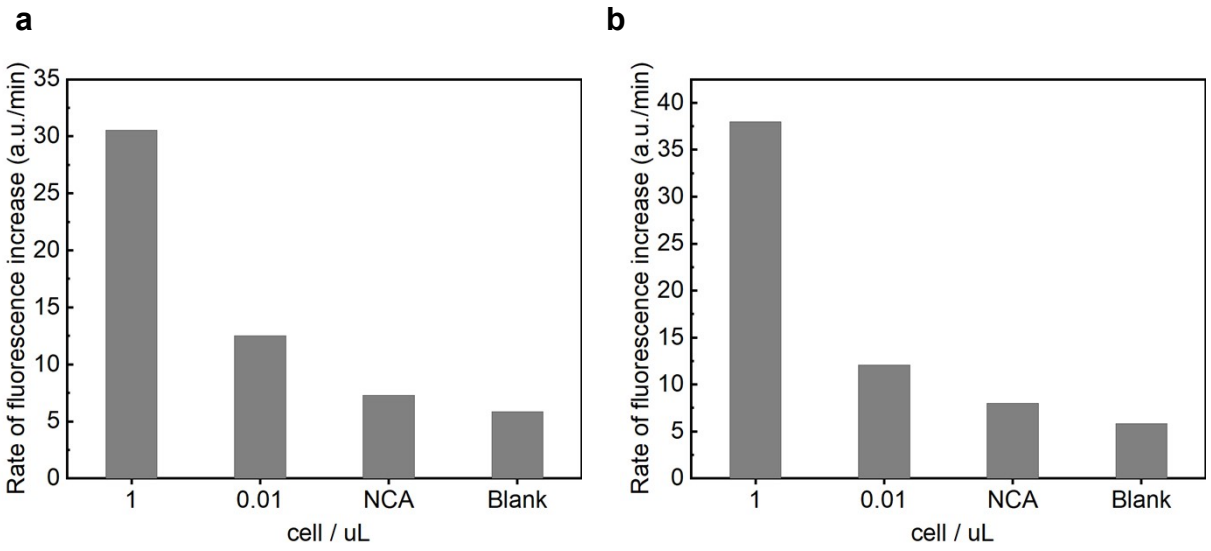

Figure S7. (a) The APE1 assay for SKOV3 cell lysate and NCA. (b) The APE1 assay for SiHa cell lysate and NCA.

SI 12. References

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