Supplementary Information

CRISPR-Cas13a-based dual-channel AND-logic gated biosensor for the simultaneous assay of APE1 and miRNA-224

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1. Experimental Section

Materials and reagents

All the oligonucleotides used in this work were synthesized and purified by Hippo Biotechnology (Huzhou, China) (Table S1). LbCas13a protein was purchased from Bio-lifesci (Guangzhou, China). Potassium chloride (KCl), magnesium chloride hexahydrate ($MgCl_2·6H_2O$), tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), potassium acetate (CH_3COOK), Tris-acetate ($C_6H_{15}NO_5$), Magnesium acetate $Mg(CH_3COO)_2·4H_2O$, dithiothreitol (DTT), and RNase-free water was purchased from Sangon Biotech. Co., Ltd. (Shanghai, China). Apurinic/apyrimidinic endonuclease 1 (APE1), NEBuffer 2.0, NEBuffer 2.1, NEBuffer 3.1, NEBuffer 4.0, and PCR buffer were purchased from New England Biolabs Ltd. (Beijing, China).

CRISPR–Cas13a fluorescence assay

In brief, 20 μL CRISPR-Cas13a reaction system contains LbCas13a (50 nM), crRNA (20 nM), FQ-reporter (400 nM), various concentrations of RNA target, and RNase-free water in 1× NEBuffer 4.0. Subsequently, the reaction system was transferred to a fluorescence plate reader and incubated at 37 °C for 60 min, with a fluorescence measurement taken every minute on Thermal Cycler Dice Real-Time System III (TaKaRa, Japan).

Preparation of blocked crRNA

To obtain the blocked crRNA, 10 μM DNA blocker without AP sites (DB) or with AP sites (ADB) was annealed with different concentrations of crRNA. The mixture was heated to 95 °C for 5 min, followed by gradual cooling down to 25 °C at a rate of 0.5 °C/min. Finally, the annealed products were stored at −20 °C for further use.

Buffer optimization of CRISPR-Cas13a and APE1 system

The 20 μL CRISPR-Cas13a reaction system contains LbCas13a (50 nM), crRNA (20 nM), FQ-reporter (400 nM), RNA target (1 nM), and RNase-free water in 1× PCR buffer, NEBuffer 4.0, NEBuffer 2.0, NEBuffer 2.1, or NEBuffer 3.1 (Table S2). Subsequently, the reaction system was transferred to a fluorescence plate reader and incubated at 37 °C for 60 min, with a fluorescence measurement taken every minute on Thermal Cycler Dice Real-Time System III (TaKaRa, Japan). The 20 μL APE1 reaction system contains 20 nM blocked crRNA (FAM) by ADB, 1 U/μL APE1

and RNase-free water in 1× PCR buffer, NEBuffer 4.0, NEBuffer 2.0, NEBuffer 2.1, or NEBuffer 3.1 (Table S2). Subsequently, the reaction system was transferred to a fluorescence plate reader and incubated at 37 °C for 60 min, with a fluorescence measurement taken every minute on Thermal Cycler Dice Real-Time System III (TaKaRa, Japan).

Fluorescence analysis using the blocked CRISRP-Cas13a system

In brief, 20 μL CRISPR-Cas13a reaction system contains LbCas13a (50 nM), crRNA-DB/crRNA-ADB (20 nM), FQ-reporter (400 nM), 1 nM of RNA target, and RNase-free water in 1× NEBuffer 4.0. Subsequently, the reaction system was transferred to a fluorescence plate reader and incubated at 37 °C for 60 min, with a fluorescence measurement taken every minute on Thermal Cycler Dice Real-Time System III (TaKaRa, Japan).

Single-channel detection of APE1

In brief, the 20 μL reaction system contains 20 nM crRNA (FAM/ROX)-ADB (BHQ1/BHQ2), various concentrations of APE1, RNase-free water in 1× NEBuffer 4.0. Subsequently, the reaction system was transferred to a fluorescence plate reader and incubated at 37 °C for 60 min, with a fluorescence measurement taken every minute on Thermal Cycler Dice Real-Time System III (TaKaRa, Japan).

CRISPR-Cas13a-based single-channel detection of APE1

The 20 μL CRISPR-Cas13a reaction system contains LbCas13a (50 nM), crRNA-ADB (20 nM), FQ-reporter (400 nM), various concentrations of APE1 and 10 nM miRNA-224, RNase-free water in 1× NEBuffer 4.0. Subsequently, the reaction system was transferred to a fluorescence plate reader and incubated at 37 °C for 60 min, with a fluorescence measurement taken every minute on Thermal Cycler Dice Real-Time System III (TaKaRa, Japan).

Dual-channel detection of APE1 and miRNA-224

In brief, the 20 μL reaction system contains LbCas13a (50 nM), crRNA (ROX)-ADB (BHQ2) (20 nM), FQ-reporter (400 nM), various concentrations of APE1 and miRNA-224, RNase-free water in 1× NEBuffer 4.0 or 1% human serum. Subsequently, the reaction system was transferred to a fluorescence plate reader and incubated at 37 °C for 60 min, with a fluorescence measurement taken every minute on Thermal Cycler Dice Real-Time System III (TaKaRa, Japan).

Ethical approval

The clinical blood samples used in this study were supplied by the Hunan Normal University School Hospital. All experiment followed institutional guidelines and conducted in accordance with the code of ethics of the World Medical Association. The study obtained informed consent from all subjects and approved by the Biomedical Ethics Committee of Hunan Normal University (2025-356).

2. Supporting Tables

Table S1. Oligonucleotide sequences used in this study.

Names	Sequences (5′→3′)
S20	CAAGTCACTAGTGGTTCCGT
S20R2	CAAGTCACTAGTGGTTCCGTGT
S20R4	CAAGTCACTAGTGGTTCCGTGTTT
S20R6	CAAGTCACTAGTGGTTCCGTGTTTTA
S20R8	CAAGTCACTAGTGGTTCCGTGTTTTAGT
S20-1AP	CAAGTCACT/idSp/GTGGTTCCGT
S20-2AP	CAAGTC/idSp/CTAGTGG/idSp/TCCGT
S20R2-1AP	CAAGTCACTA/idSp/TGGTTCCGTGT
S20R2-2AP	CAAGTCA/idSp/TAGTGG/idSp/TCCGTGT
S20R4-1AP-1	CAAGTCACTAGT/idSp/GTTCCGTGTTT
S20R4-1AP-2	CAAGTC/idSp/CTAGTGGTTCCGTGTTT
S20R4-2AP-1	CAAGTCAC/idSp/AGTGGTT/idSp/CGTGTTT
S20R4-2AP-2	CAAGTC/idSp/CTAGTGGTTC/idSp/GTGTTT
S20R4-2AP-3	CAAGT/idSp/ACTAGT/idSp/GTTCCGTGTTT
S20R4-3AP	CAAGT/idSp/ACTAGT/idSp/GTTCC/idSp/TGTTT
S20R6-1AP	CAAGTCACTAGTG/idSp/TTCCGTGTTTTA
S20R6-2AP	CAAGTCAC/idSp/AGTGGTTC/idSp/GTGTTTTA
S20R6-3AP	CAAGTC/idSp/CTAGTG/idSp/TTCCGT/idSp/TTTTA
S20R8-1AP	CAAGTCACTAGTG/idSp/TTCCGTGTTTTAGT
S20R8-2AP	CAAGTCACT/idSp/GTGGTTCC/idSp/TGTTTTAGT
S20R8-3AP	CAAGTCA/idSp/TAGTGGT/idSp/CCGTGTT/idSp/TAGT
crRNA-miRNA-224	GACCACCCCAAAAAUGAAGGGGACUAAAACACGGAACC ACUAGUGACUUG
crRNA- miRNA-lef-7f	GACCACCCCAAAAAUGAAGGGGACUAAAACAACUAUAC AAUCUACUACCUCA

crRNA-miRNA-10b	GACCACCCCAAAAAUGAAGGGGACUAAAACCACAAAUU CGGUUCUACAGGGUA
crRNA-miRNA-21	GACCACCCCAAAAAUGAAGGGGACUAAAACUCAACAUC AGUCUGAUAAGCUA
crRNA-miRNA-155	GACCACCCCAAAAAUGAAGGGGACUAAAACACCCCUAUC ACGAUUAGCAUUAA
crRNA-miRNA-17	GACCACCCCAAAAAUGAAGGGGACUAAAACCUACCUGCA CUGUAAGCACUUUG
crRNA-miRNA-221	GACCACCCCAAAAAUGAAGGGGACUAAAACGAAACCCA GCAGACAAUGUAGCU
crRNA-miRNA-222	GACCACCCCAAAAAUGAAGGGGACUAAAACAGGAUCUA CACUGGCUACUGAG
crRNA (FAM)	GACCACCCCAAAAAUGAAGGGGACUAAAACACGGAACC ACUAGUGACUUG-FAM
S20R4-2AP-1 (BHQ1)	BHQ1-CAAGTCACTAGT/idSp/GTTCC/idSp/TGTTT
crRNA (ROX)	GACCACCCCAAAAAUGAAGGGGACUAAAACACGGAACC ACUAGUGACUUG-ROX
S20R4-2AP-1 (BHQ2)	BHQ2-CAAGTCACTAGT/idSp/GTTCC/idSp/TGTTT
miRNA-224	CAAGUCACUAGUGGUUCCGU
miRNA-lef-7f	UGAGGUAGUAGAUUGUAUAGUU
miRNA-10b	UACCCUGUAGAACCGAAUUUGUG
miRNA-21	UAGCUUAUCAGACUGAUGUUGA
miRNA-155	UUAAUGCUAAUCGUGAUAGGGGU
miRNA-17	CAAAGUGCUUACAGUGCAGGUAG
miRNA-221	AGCUACAUUGUCUGCUGGGUUUC
miRNA-222	CUCAGUAGCCAGUGUAGAUCCU
FQ-reporter	FAM-UUUUUU-BHQ1

Table S2. Buffers used in this study.

Names	Components
PCR buffer	10 mM Tris-HCl (pH 8.9), 10 mM KCl, 15 mM $MgCl_2$
NEBuffer 4.0	20 mM Tris-acetate(pH 7.9), 50 mM Potassium Acetate, 10 mM Magnesium Acetate, 1 mM DTT
NEBuffer 2.0	10 mM Tris-HCI(pH 7.9), 50 mM NaCl 10 mM $MgCl_2$, 1mM DTT
NEBuffer 2.1	10 mM Tris-HCl (pH 7.9), 50 mM NaCl, 10 mM $MgCl_2$, 100 µg/mL BSA
NEBuffer 3.1	50 mM Tris-HCl (pH 7.9), 100 mM NaCl, 10 mM $MgCl_2$, 100 µg/mL BSA

3. Supporting Figures

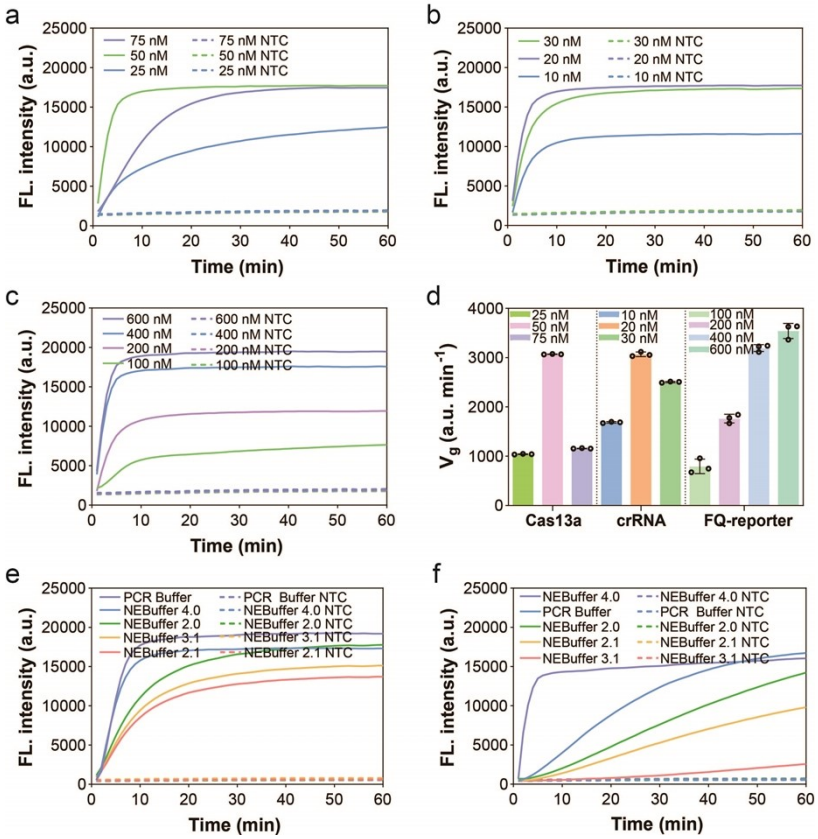


Fig. S1 (a–c) Optimization of experimental parameters, including the concentration of Cas13a (a), crRNA (b) and FQ-reporter (c). (d) Reaction rate V_g of the fluorescence data in (a–c). The fluorescence generation rate V_g ($V_g = \Delta F/\Delta t$, where V represents velocity, F represents fluorescence intensity, and t represents time, initial 5 min). (e,f) Screening of reaction buffer for the CRISPR-Cas13a (e) and APE1 system (f). RNA target: 1 nM, APE1: 1 U/μL. NTC: negative target control.

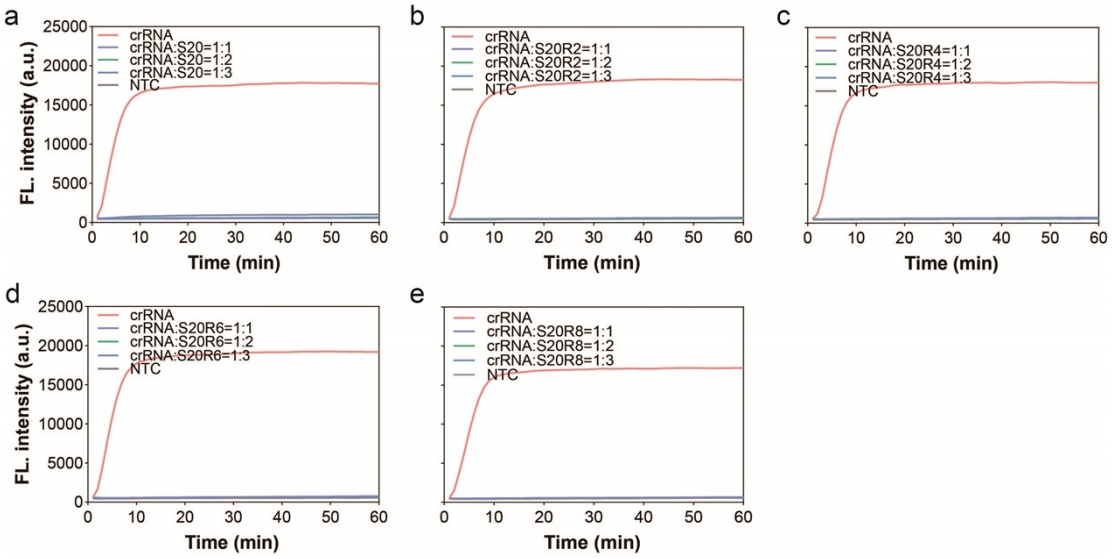


Fig. S2 Fluorescence kinetic curves of crRNA and different DBs, including S20 (a), S20R2 (b), S20R4 (c), S20R6 (d), S20R8 (e) with concentration ratios of 1:1, 1:2, 1:3. RNA target: 1 nM, APE1: 1 U/μL. NTC: negative target control.

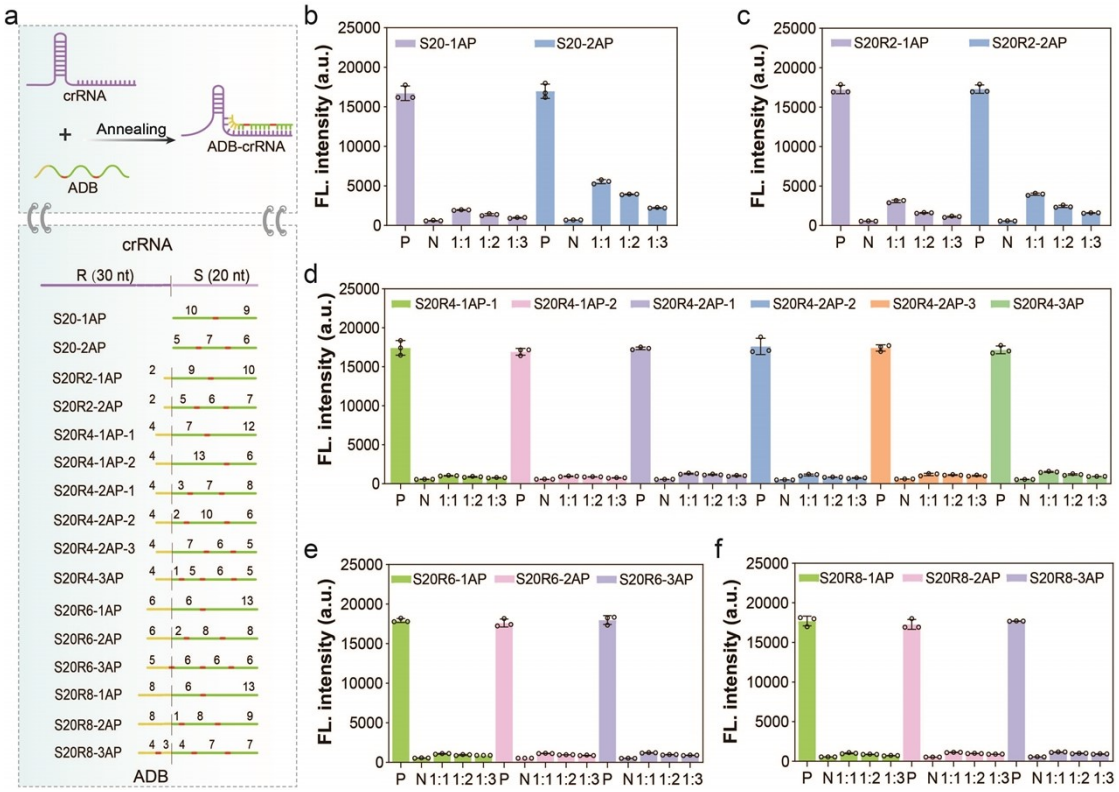


Fig. S3 (a) Schematic illustration of various ADBs with different lengths and positions of AP sites. The numbers on the ADB represent the number of bases for each DNA fragment separated by AP sites. (b–f) Evaluation of blocking efficiency of S20-AP (b), S20R2-AP (c), S20R4-AP (d), S20R6-AP (e), S20R8-AP (f) on crRNA with different ratios of 1:1, 1:2, and 1:3. All the fluorescence intensities represent the end point fluorescence at 60 min. APE1: 1 U/μL. Error bars represent three individual experiments. P: positive control, N: negative control.

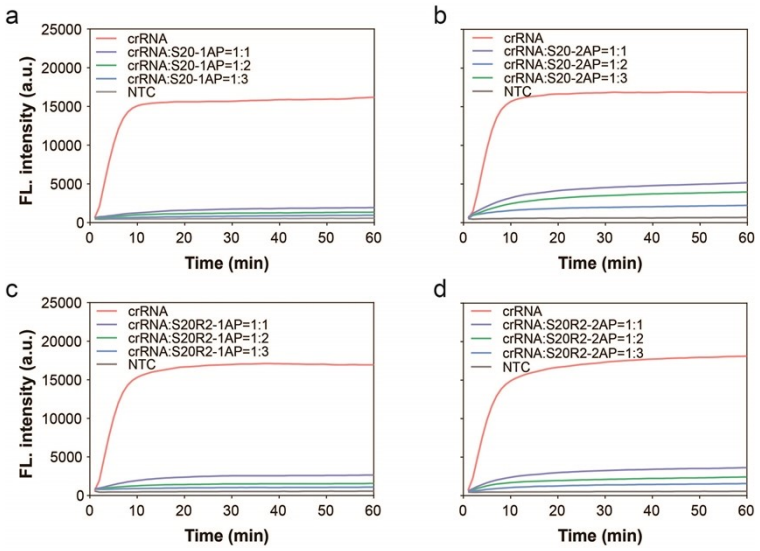


Fig. S4 Fluorescence kinetic analysis of the blocking efficiency of S20-AP (a), S20-2AP (b), S20R2-1AP (c), S20R2-2AP (d) on crRNA with different ratios of 1:1, 1:2, and 1:3. APE1: 1 U/μL. NTC: negative target control.

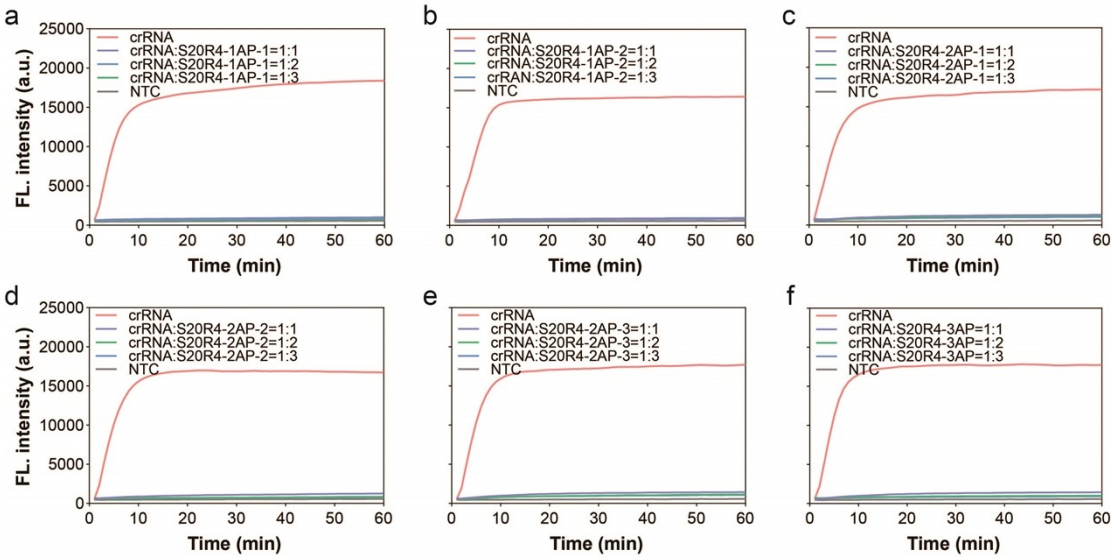


Fig. S5 Fluorescence kinetic analysis of the blocking efficiency of S20R4-1AP-1 (a), S20R4-1AP-2 (b), S20R4-2AP-1 (c), S20R4-2AP-2 (d), S20R4-2AP-3 (e), S20R4-3AP (f) on crRNA with different ratios of 1:1, 1:2, and 1:3. APE1: 1 U/μL. NTC: negative target control.

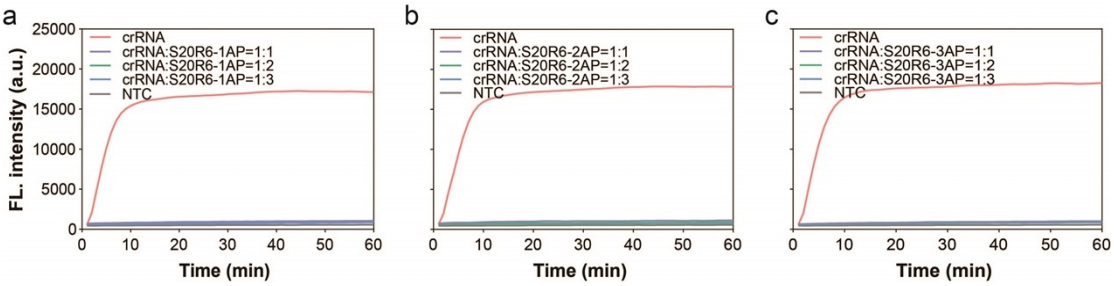


Fig. S6 Fluorescence kinetic analysis of the blocking efficiency of S20R6-1AP (a), S20R6-2AP (b), S20R6-3AP (c) on crRNA with different ratios of 1:1, 1:2, and 1:3. APE1: 1 U/μL. NTC: negative target control.

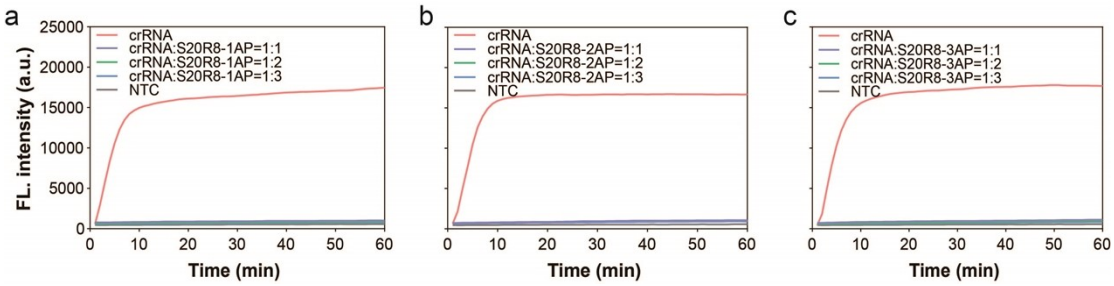


Fig. S7 Fluorescence kinetic analysis of the blocking efficiency of S20R8-1AP (a), S20R8-2AP (b), S20R8-3AP (c) on crRNA with different ratios of 1:1, 1:2, and 1:3. APE1: 1 U/μL. NTC: negative target control.

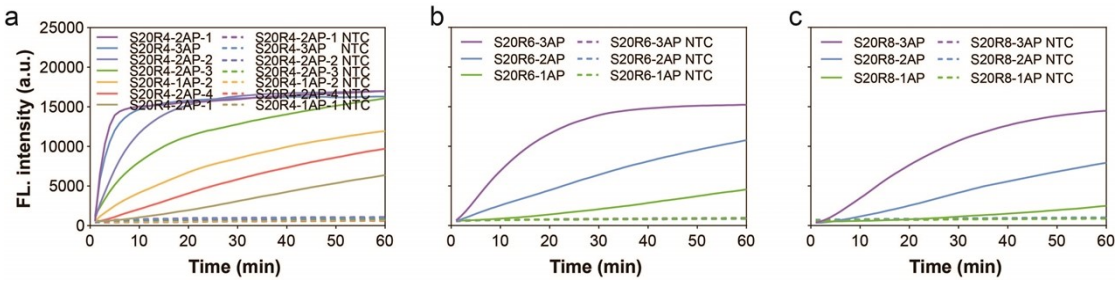


Fig. S8 Fluorescence kinetic curves of the recovery rate of crRNA from ADB-crRNA in the presence of APE1 (1 U/μL) using the FAM channel, including S20R4-AP (a), S20R6-AP (b), S20R8-AP (c). NTC: negative target control.

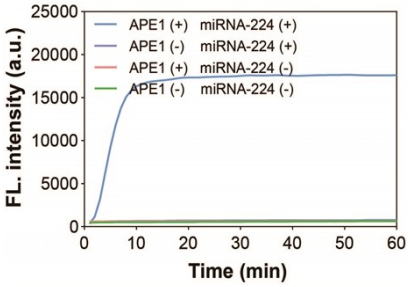


Fig. S9 Fluorescence kinetic analysis of the feasibility of the AND logic-gated biosensing platform based on the CRISPR-Cas13a system in the presence/absence of APE1 and miRNA-224.

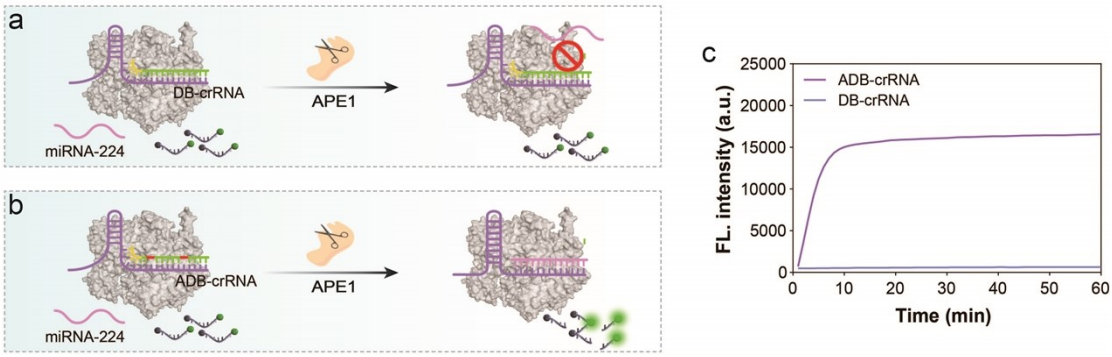


Fig. S10 (a) Schematic illustration of crRNA-DB-based CRISPR-Cas13a system for the detection of APE1. (b) Schematic illustration of crRNA-ADB-based CRISPR-Cas13a system for the detection of APE1. (c) Fluorescence kinetic analysis of crRNA-DB or crRNA-ADB-based CRISPR-Cas13a system for the detection APE1.

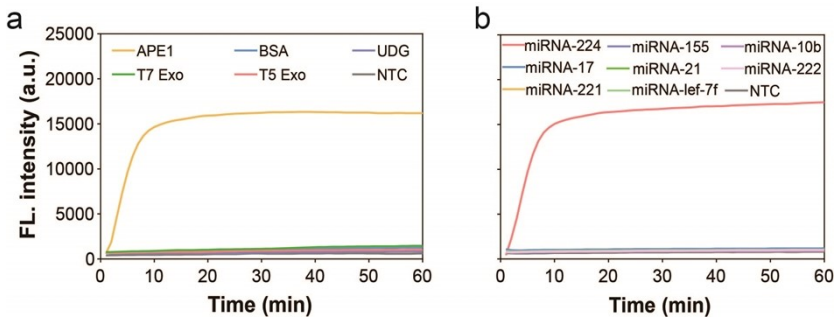


Fig. S11 (a) Selectivity analysis of the platform to T7 Exo, T5 Exo, UDG (Uracil-DNA glycosylase), BSA (Bovine albumin), APE1. (b) Selectivity analysis of the platform to different miRNAs. APE1: 1 U/μL, miRNA-224: 1 nM. NTC: negative target control.

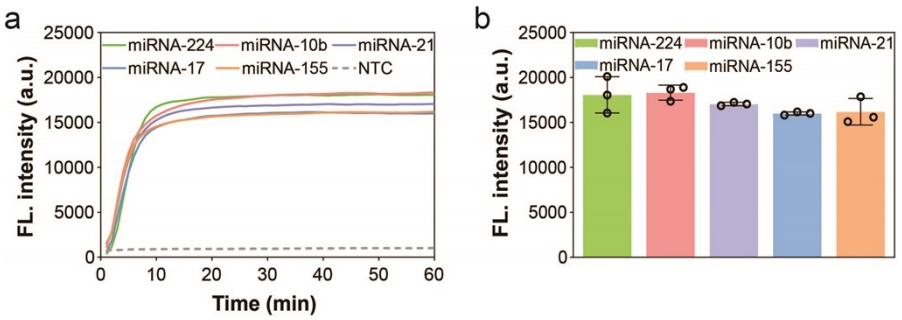


Fig. S12 (a) Fluorescence analysis of the universality of the CRISPR-Cas13a-based single-channel detection of different miRNAs in the presence of APE1. (b) Fluorescence intensities of the analysis in (a). All the fluorescence intensities represent the end point fluorescence at 60 min. Error bars represent three individual experiments. NTC: negative target control.

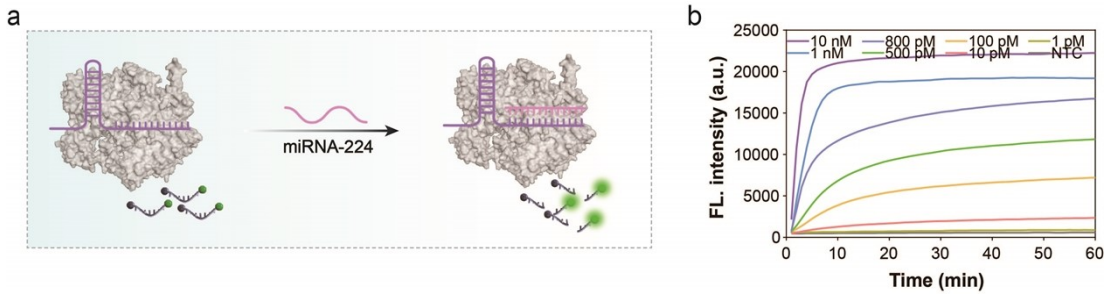


Fig. S13 (a) Schematic illustration of traditional CRISPR-Cas13a system for the miRNA-224 detection. (b) Sensitivity analysis of the traditional CRISPR-Cas13a system for the miRNA-224 detection ranging from 1 pM to 10 nM. Cas13a (50 nM), crRNA (20 nM), FQ reporter (400 nM). NTC: negative target control.

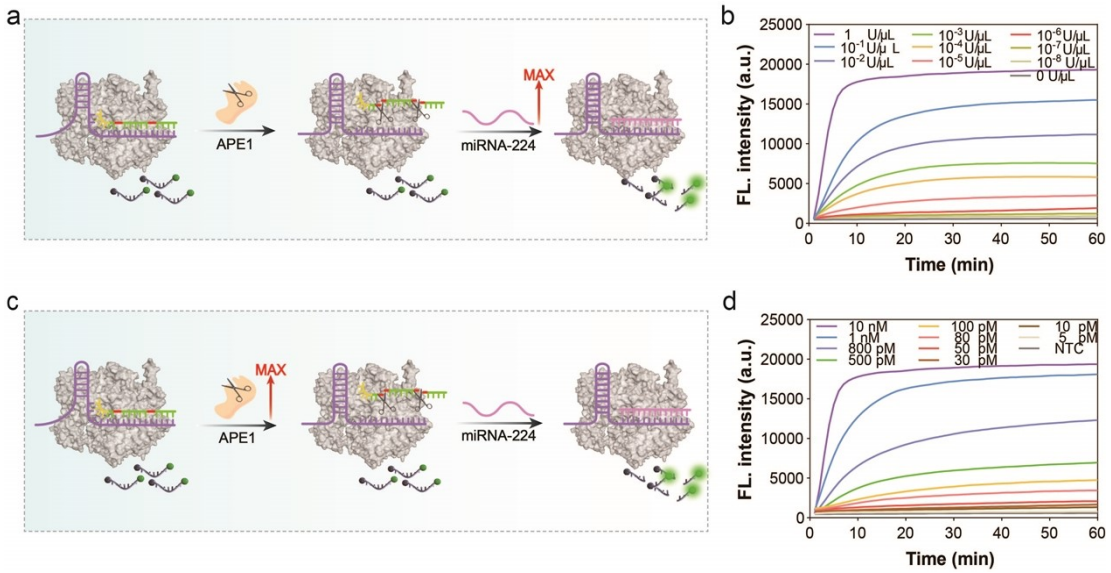


Fig. S14 (a) Schematic illustration of the detection of APE1 in the presence of miRNA-224 with fixed concentration. (b) Sensitivity analysis of APE1 with the concentration ranging from 0 to 1 U/μL in the presence of 10 nM miRNA-224. (c) Schematic illustration of the detection of miRNA-224 in the presence of APE1 with fixed concentration. (d) Sensitivity analysis of miRNA-224 with the concentration ranging from 5 pM to 10 nM in the presence of 1 U/μL APE1. NTC: negative target control.

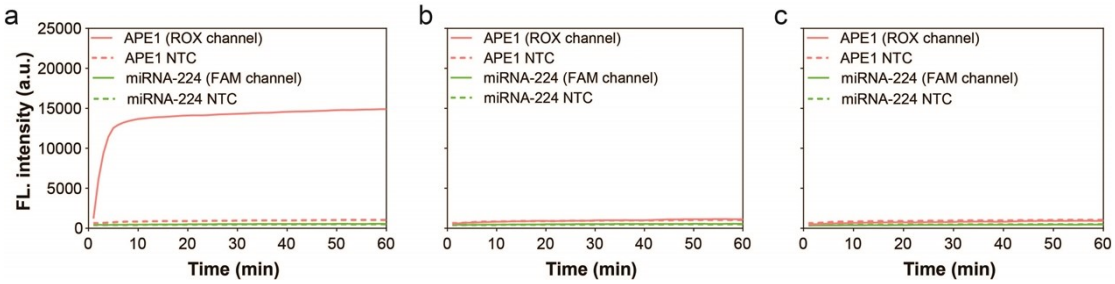


Fig. S15 Fluorescence kinetic analysis of the AND-logic gated dual-channel platform in the presence/absence of APE1 (1 U/μL) and miRNA-224 (1 nM). NTC: negative target control.

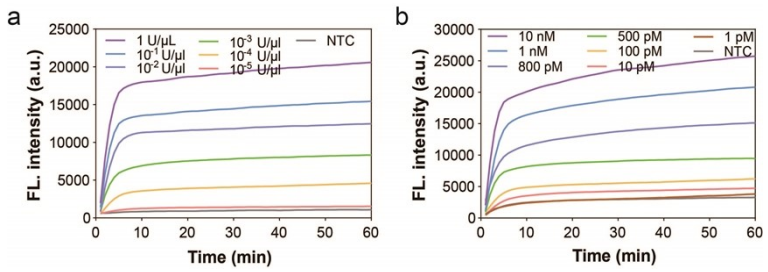
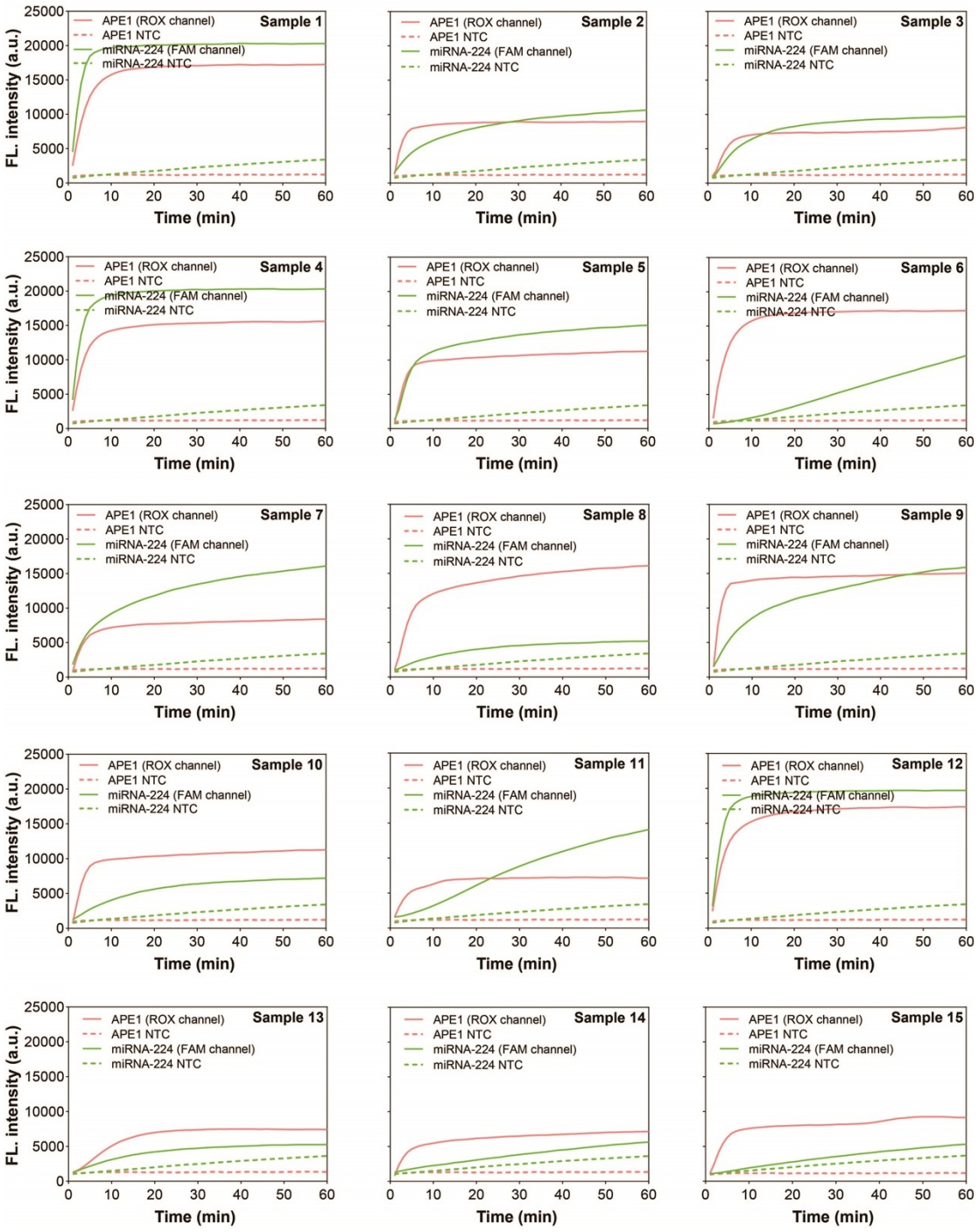


Fig. S16 (a) Fluorescence responses of the crRNA-ADB complex in the presence of APE1 ranging from 10^{-5} U/μL to 1 U/μL in serum. (b) Fluorescence responses of the traditional CRISPR-Cas13a system in the presence of miRNA-224 ranging from 1 pM to 10 nM in serum. NTC: negative target control.


Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6
Sample 7
Sample 8
Sample 9
Sample 10
Sample 11
Sample 12
Sample 13
Sample 14
Sample 15
APE1 (ROX channel)
APE1 NTC
miRNA-224 (FAM channel)
miRNA-224 NTC
FL. intensity (a.u.)
Time (min)
0
5000
10000
15000
20000
25000
0
10
20
30
40
50
60

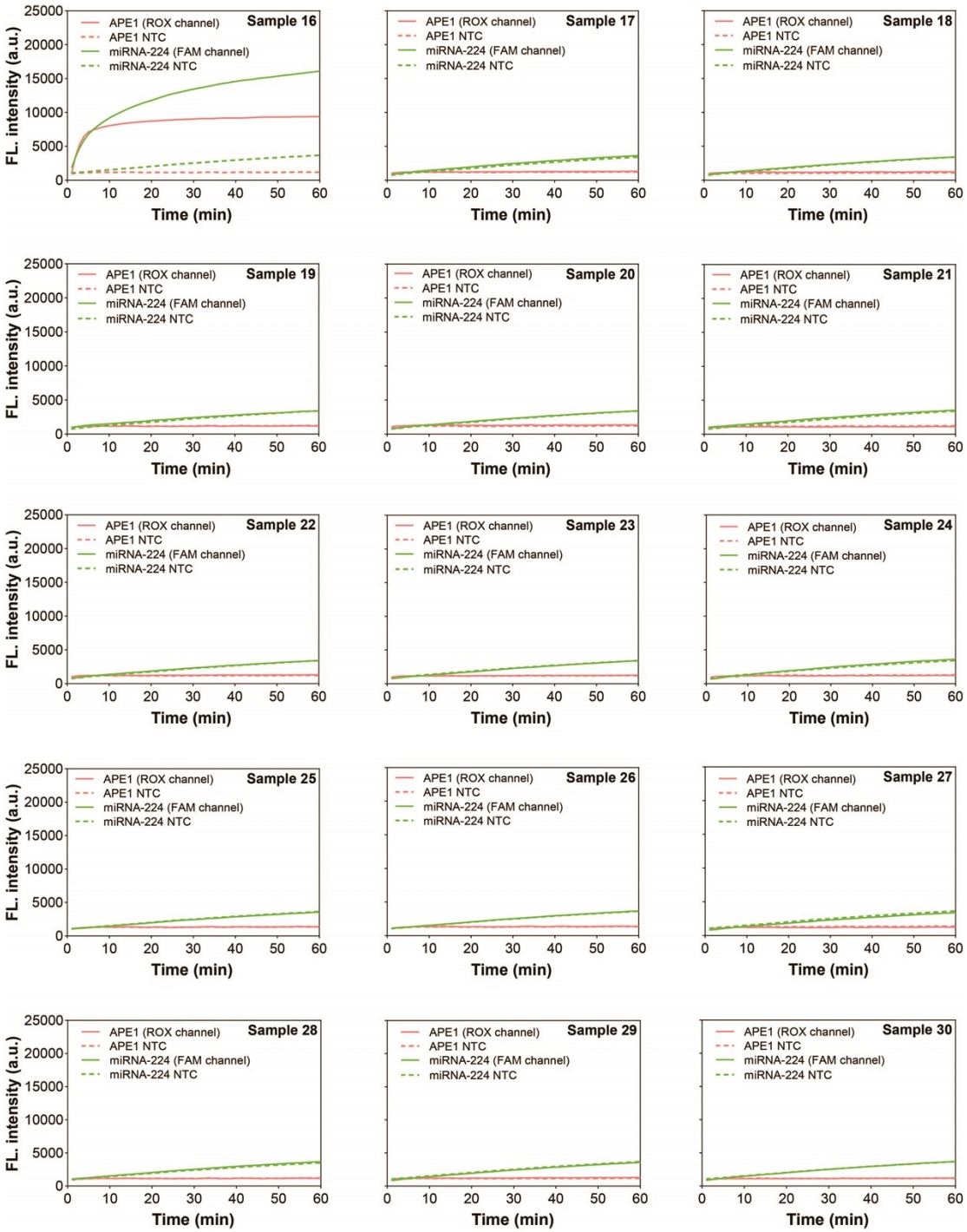


Fig. S17 Assay of the CRISPR-Cas13a-based dual-channel platform for APE1 and miRNA-224 in simulated serum samples. Positive sample ID: 1–16, Negative sample ID: 17–30. NTC: negative target control.