Supplementary Information

CRISPR-Cas12a Detection of DNA Glycosylases via DNA Modification Switching

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S1. Chemicals and materials

All chemicals were sourced from Macklin (Shanghai, China) unless otherwise specified. Oligodeoxynucleotides (ODNs), including DNA and RNA, were synthesized and purified by Generary Biotechnology (Shanghai, China). The LbCas12a protein (Cat No. CAS12-010B, 10 μM) was expressed and purified from E. coli by EZassay Biotech (Shenzhen, China). Bovine Serum Albumin (BSA) (Cat No. 36101ES25) and the qPCR machine celemetor-96 (Cat No. 80520ES03) were bought from Yeasen Biotechnology Co., Ltd. (Shanghai, China). Uracil-DNA Glycosylase (UDG) (Cat No. M0280L), OGG1 (Cat. No. M0464S, 8,000 U/mL), and APE1 (Cat No. M0282S) were sourced from New England Biolabs, Inc. (USA). GelstainRed™ Nucleic Acid Dye (Cat No. S2009L) was purchased from US EVERBRIGHT (Suzhou, China). Enhanced Cell Counting Kit 8 (WST-8/CCK8) (Cat No. E-CK-A362) was bought from Elabscience Biotechnology Co., Ltd (Wuhan, China). Fetal bovine serum (Cat No. abs981-500mL) was sourced from Absin Biotechnology Co., Ltd (Shanghai, China). Lateral flow detection strips (Cat No. CAS-cmCSA01) were supplied from Keer Life (Suzhou, china).

S2. Methods

1. Gel Electrophoresis Verification of UDG Recognition and Cleavage of dU-DNA/RNA Hybrid

A mixture was prepared to achieve final concentrations of crRNA (2.5 μM), and crRNA complementary single-strand CS (2.875 μM) or dU-modified dU-CS (2.875 μM) in 1X annealing buffer (20 mM Tris-HCl, 5 mM KCl, 140 mM NaCl, pH 7.4 @ 25°C). This mixture was incubated at 95°C for 5 minutes and then slowly annealed to 4°C at a rate of 0.1°C/s using a PCR machine. Subsequently, a portion of the stable double-stranded DNA-RNA hybrid was prepared to a final concentration of 500 nM DNA-RNA hybrid, UDG (625 U/L), and 1X reaction buffer (2 mM Spermidine, 40 mM Tris-HCl, 6 mM $MgCl_2$, 1 mM DTT, 40 mM Glycine, 0.001% TritonX-100, 0.4% PEG20000; pH 8.5 @ 25°C), with a total volume of 20 μL. The reaction was incubated at 37°C for 1 hour, followed by the addition of 4 μL of 6X loading buffer. The samples were subjected to 20% non-denaturing PAGE at 130V for 1.5 hours and then analyzed using Gel Red DNA Dye staining.

2. Gel Electrophoresis Verification of OGG1-Mediated Release of crRNA from 8OG-DNA/crRNA Hybrid

A mixture was prepared to achieve final concentrations of crRNA (2.5 μM), and crRNA complementary single-strand CS (2.875 μM) or 8OG-modified 8OG-CS (2.875 μM) in 1X annealing buffer (20 mM Tris-HCl, 5 mM KCl, 140 mM NaCl, pH 7.4 @ 25°C). This mixture was incubated at 95°C for 5 minutes and then slowly annealed to 4°C at a rate of 0.1°C/s using a PCR machine. Subsequently, a portion of the stable

double-stranded DNA-RNA hybrid was prepared to a final concentration of 500 nM DNA-RNA hybrid, OGG1 (400 U/mL), and 1X reaction buffer, with a total volume of 20 μL. The reaction was incubated at 37°C for 1 hour, followed by the addition of 4 μL of 6X loading buffer. The samples were subjected to 20% neutral gel electrophoresis at 130V for 1.5 hours and then analyzed using Gel Red DNA Dye staining.

3. Validation of dU-CS or OG-CS Inhibition of CRISPR-Cas12a Activity

Various concentration ratios of crRNA and dU-CS or OG-CS were annealed in the aforementioned buffer to form crRNA-CS hybrid double strands with varying concentrations of dU-CS or OG-CS. These annealed mixtures were prepared to a final concentration of crRNA-CS hybrid double strand (1 nM, based on crRNA concentration). The reactions included FQ (250 nM), DNA activator (10 nM), and LbCas12a (50 nM) in a 1X reaction buffer. The samples were incubated at 37°C, and fluorescence measurements were taken every 30 seconds over a total of 60 cycles using qPCR.

4. Programmable CRISPR-Cas12a System for Selective Fluorescence Detection of UDG

A mixture was prepared to achieve final concentrations of crRNA (200 nM) and dU-CS (300 nM) in 1X reaction buffer and incubated at 95°C for 5 minutes, followed by annealing to 4°C. Subsequently, a portion of the annealed crRNA/dU-CS (1 nM) was incubated with varying concentrations of UDG or other target proteins, cell extracts in 1X reaction buffer, in a total volume of 10 μL. The samples were incubated at 37°C for 15, 30 or 45 minutes. Afterward, FQ (250 nM), DNA activator (10 nM), and LbCas12a (50 nM) were added to a total volume of 20 μL in 1X reaction buffer, and fluorescence measurements were taken every 30 seconds over a total of 60 cycles using qPCR.

5. Programmable CRISPR-Cas12a System for Selective Fluorescence Detection of OGG1

A mixture was prepared to achieve final concentrations of crRNA (200 nM) and OG-CS (300 nM) in 1X annealing buffer and incubated at 95°C for 5 minutes, followed by annealing to 4°C. Subsequently, a portion of the annealed crRNA/OG-CS (1 nM) was incubated with varying concentrations of OGG1 or other target proteins, cell extracts in 1X reaction buffer, in a total volume of 20 μL, including FQ (250 nM), DNA activator (10 nM), and LbCas12a (50 nM). The samples were incubated at 37°C, and fluorescence measurements were taken every 30 seconds over a total of 1 hour using qPCR.

6. Paper Strip Detection of UDG or OGG1

Under the same conditions as described for fluorescence detection of UDG or OGG1, the F-Q probe was substituted with an FAM-Biotin probe at a concentration of 250 nM. For UDG detection, dU-CS was used, and for OGG1 detection, OG-CS was used. Following a 1-hour incubation at 37°C, paper strips were

inserted and left undisturbed for 8 minutes. The results were then photographed using a smartphone. Image analysis was performed with ImageJ software, converting the images to 8-type, subtracting the background, setting measurement values for grayscale analysis, inverting colors, and using the rectangle tool to obtain the grayscale of the test line.

7. Inhibitor Verification for System Selectivity in UDG or OGG1 Detection

Under the same conditions described in section 3, for UDG, the added UDG was replaced with a mixture pre-incubated with various concentrations of UGI for 30 minutes. For OGG1, the added OGG1 was replaced with a mixture pre-incubated with various concentrations of $CdCl_2$ for 30 minutes.

8. Specificity Experiment for UDG or OGG1 Detection

Under the same conditions described in section 3, the added UDG or OGG1 was replaced with APE1, BSA, OGG1, and their mixtures.

S3. ODN sequences used in this study

Table S1. Sequences of DNA or RNA oligonucleotides used in this work.

Name	Sequence	Notes
crRNA	AAUUUCUACUCUUGUAGAUUUAUCGCAACUUUCUACUGAAUU	RNA
CS	AATTCAGTAGAAAGTTGCGATAAATCTACAAGAGTAGAAATT	DNA
dU-CS	AATTCAG/ideoxyU/AGAAAGTTGCGA/ideoxyU/AAATCTACAAGAG/ideoxyU/AGAAATT	DNA
OG-CS (OG257-CS)	AATTCAGTA/i8oxodG/AAAGTTGC/i8oxodG/ATAAATCTACAAGA/i8oxodG/TAGAAATT	DNA
OG7-CS	AATTCAGTAGAAAGTTGCGATAAATCTACAAGA/i8oxodG/TAGAAATT	DNA
OG5-CS	AATTCAGTAGAAAGTTGC/i8oxodG/ATAAATCTACAAGAGTAGAAATT	DNA
OG57-CS	AATTCAGTAGAAAGTTGC/i8oxodG/ATAAATCTACAAGA/i8oxodG/TAGAAATT	DNA
OG148-CS	AATTCA/i8oxodG/TAGAAAGTT/i8oxodG/CGATAAATCTACAAGAGTA/i8oxodG/AAATT	DNA
OG247-CS	AATTCAGTA/i8oxodG/AAAGTT/i8oxodG/CGATAAATCTACAAGA/i8oxodG/TAGAAATT	DNA
DNA activator-F	GTTGTAAAACGACGGCCAGTTTTGTTATCGCAACTTTCTACTGAATTCGG	DNA
DNA activator-R	CCGAATTCAGTAGAAAGTTGCGATAACAAAACTGGCCGTCGTTTTACAAC	DNA
F-Q	FAM-CCCCCCCC-BHQ1	DNA
FAM-Biotin	FAM-CCCCCCCC-Biotin	DNA

Scheme S1. Schematic representation of the duplexes formed by RNA and DNA strands, along with the Tm calculation of the respective segments post-cleavage.

S4. Extended Data

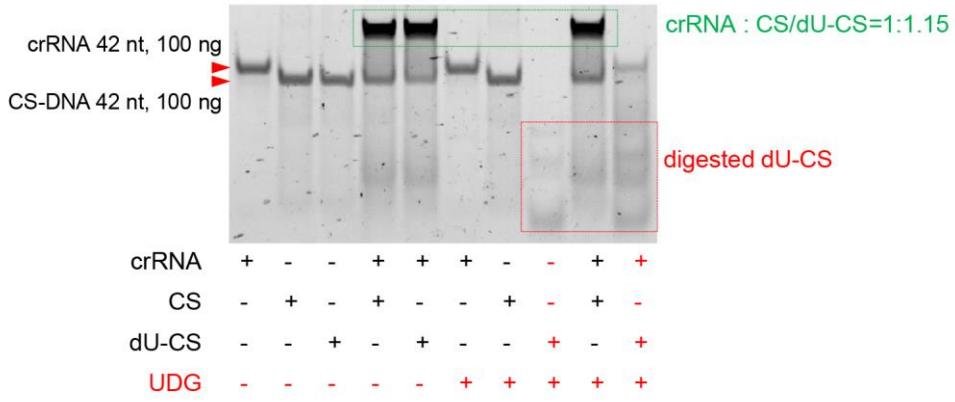

Fig. S1. Evaluation of crRNA, canonical complementary strand (CS), dU-modified strand (dU-CS), and their respective double-stranded structures using 20% non-denaturing polyacrylamide gel electrophoresis (PAGE). The results include reactions with and without Uracil-DNA Glycosylase (UDG). Each lane contained 100 ng of oligonucleotide, including DNA marker such as crRNA, CS, and dU-CS and their respective double-stranded structures. The ratio of RNA to DNA hybrid is 1:1.15.

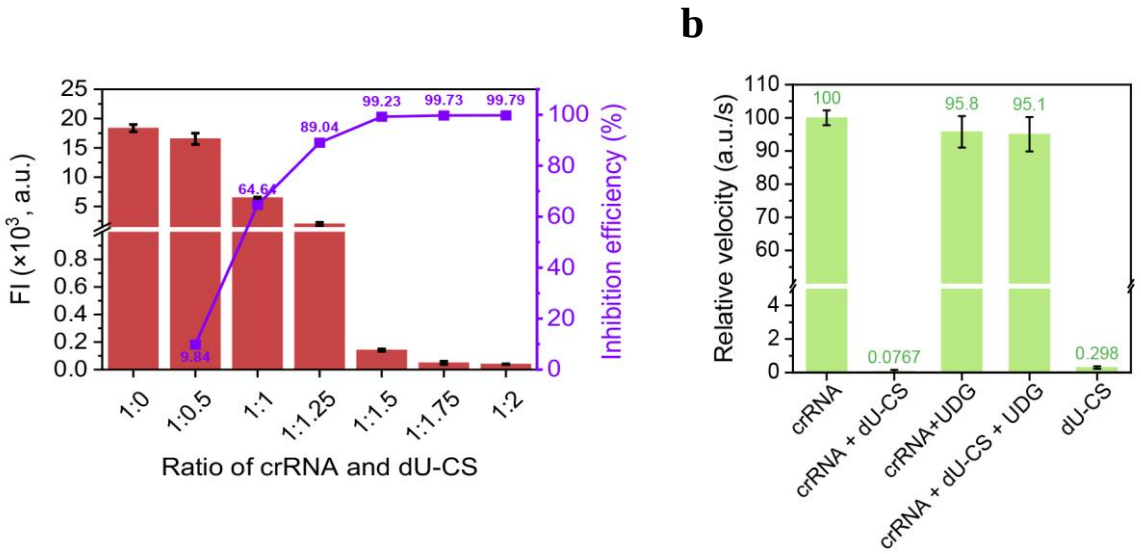

Fig. S2. (a) Fluorescence bar chart displaying the annealing products of varying ratios of crRNA to dU-CS, ranging from 1:0 to 1:2, incubated at 37°C with Cas12a, DNA activator, and F-Q probe. Curve plots depicting CRISPR-Cas12a function inhibition efficiency are also presented. (b) Detection recoveries of Cas12a cleavage activity, comparing the relative velocity of crRNA and annealing products of crRNA to dU-CS, with and without UDG treatment. Error bars are standard deviation (SD) (n = 3).

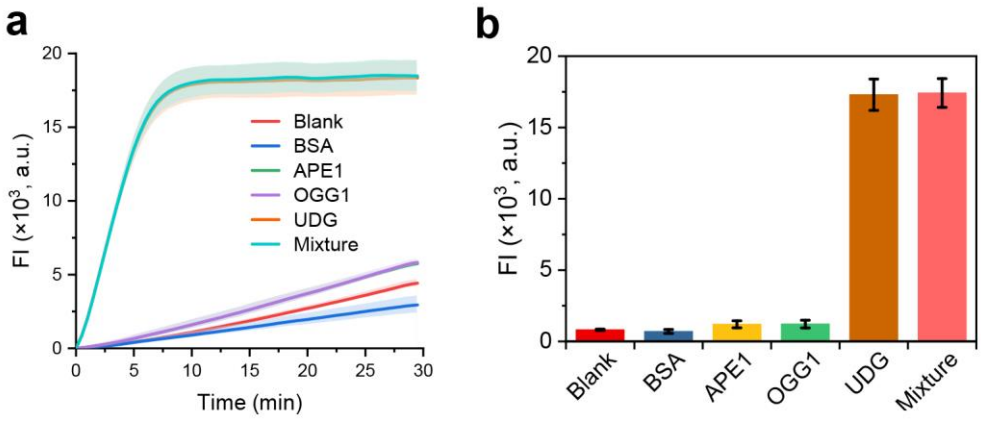

Fig. S3. Real-time fluorescence detection using the currently developed programmable CRISPR-Cas12a system for UDG. The system's response to BSA, APE1, OGG1, UDG, and their mixtures is illustrated in (a), accompanied by a bar chart of fluorescence quantification in (b). Error bars are standard deviation (SD) (n = 3).

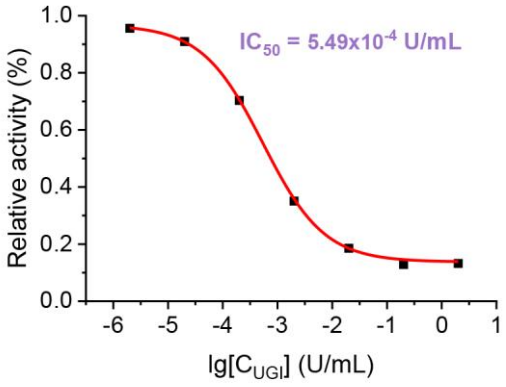

Fig. S4. Graph showing the relationship between UGI concentration and its inhibitory effects on system activity within the programmable CRISPR-Cas12a detection system for UDG. Error bars are standard deviation (SD) (n = 3).

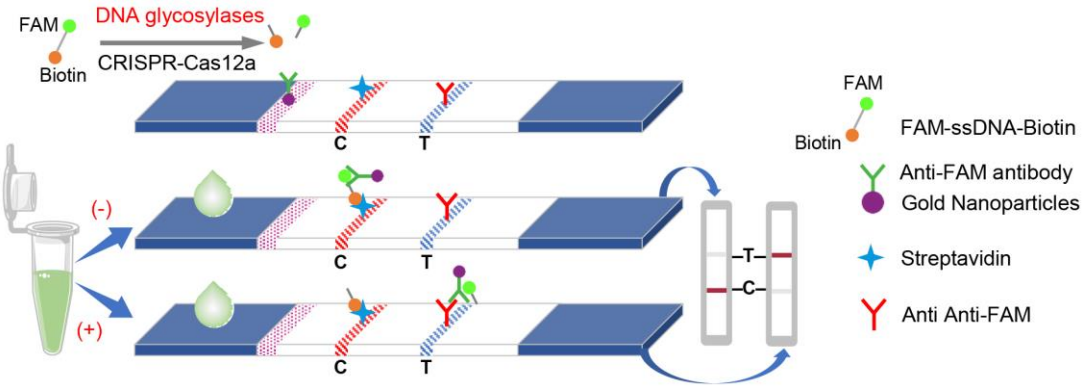

Fig. S5. Schematic representation depicting the integration of the programmable CRISPR-Cas12a system with commercial test strips for the detection of DNA glycosylases.

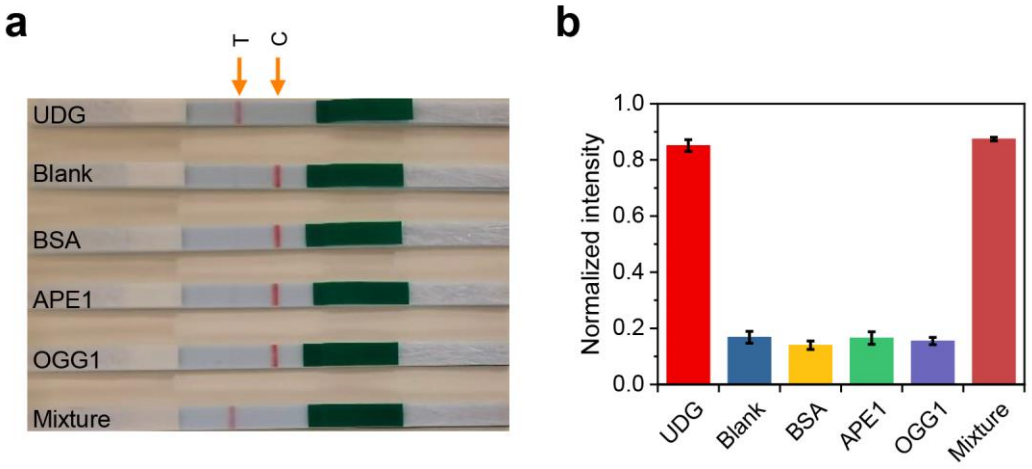

Fig. S6. Visual and quantitative results for the CRISPR-Cas12a test strip detection of UDG. Mobile phone images of test strips detecting BSA, APE1, OGG1, UDG, and their mixtures are shown in (a), with corresponding ImageJ grayscale quantification in (b). Error bars are standard deviation (SD) (n = 3).

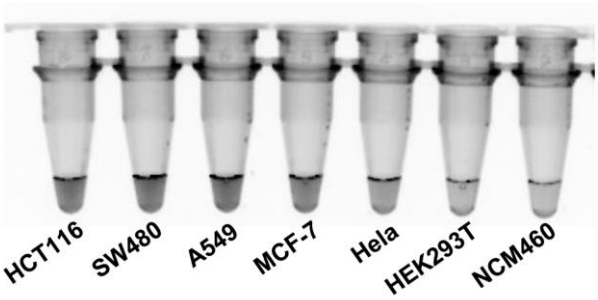

Fig. S7. Direct visual fluorescence images from the CRISPR-Cas12a system detecting UDG in nuclear protein extracts from various cultured cell lines.

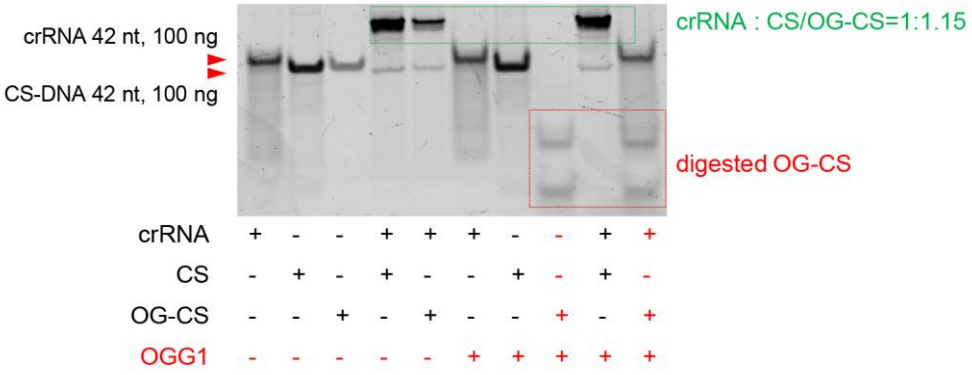

Fig. S8. Analysis of crRNA, canonical complementary strand (CS), 8-oxoguanine-modified strand (8OG-CS), and their respective double-stranded structures using 20% non-denaturing PAGE. Results are presented for reactions both with and without OGG1 enzyme. Each lane contained 100 ng oligonucleotide, including DNA marker such as crRNA, CS, and 8OG-CS and their respective double-stranded structures. The ratio of RNA to DNA hybrid is 1:1.15.

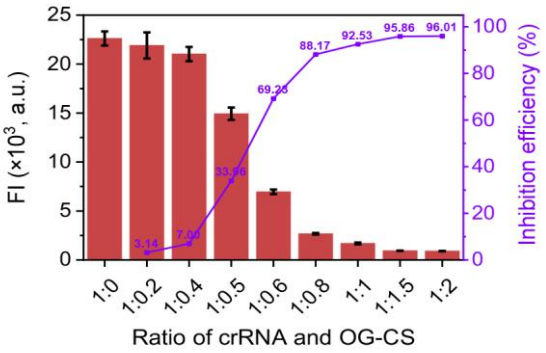

Fig. S9. Fluorescence bar chart showing the products of annealing varying ratios of crRNA to 8OG-CS, from 1:0 to 1:2, incubated at 37°C within a reaction system containing Cas12a, DNA activator, and F-Q probe. Additionally, curves illustrating CRISPR-Cas12a fluorescence inhibition are presented. Error bars are standard deviation (SD) (n = 3).

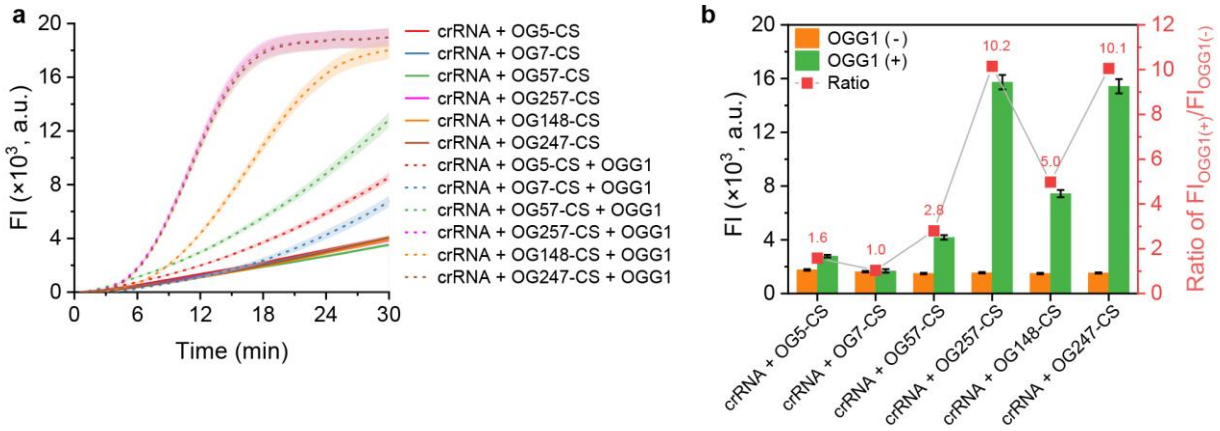

Fig. S10. (a) Real-time fluorescence data showing the inhibitory effects of 8-oxoguanine (8OG) modifications at various positions within complementary DNA strands on Cas12a enzyme activity, as well as the subsequent impact of OGG1 treatment. (b) Bar graph analysis of fluorescence intensity at the 15-minute mark from panel (a), including the comparison of fluorescence recovery pre- and post-OGG1 treatment.

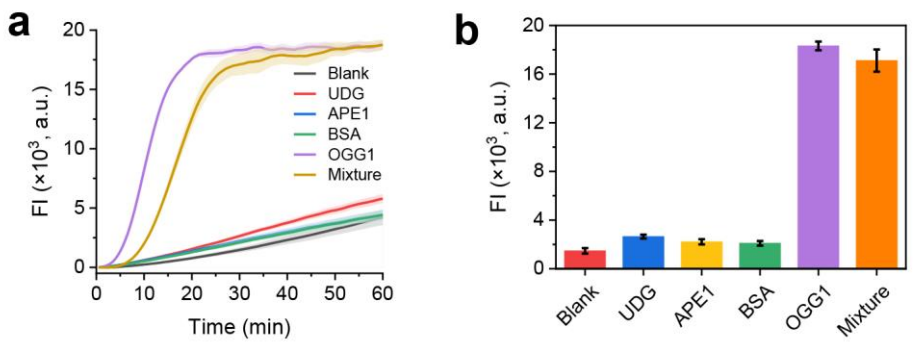

Fig. S11. Real-time fluorescence detection of OGG1 using the currently developed programmable CRISPR-Cas12a system. Response to UDG, APE1, BSA, OGG1, and their mixtures is depicted in (a), with corresponding fluorescence quantification displayed in (b). Error bars are standard deviation (SD) (n = 3).

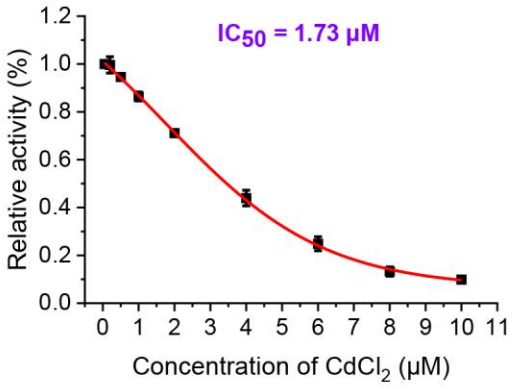

Fig. S12. Graph demonstrating the relationship between $CdCl_2$ concentration and its inhibitory effect on system activity within the programmable CRISPR-Cas12a detection system for OGG1. Error bars are standard deviation (SD) (n = 3).

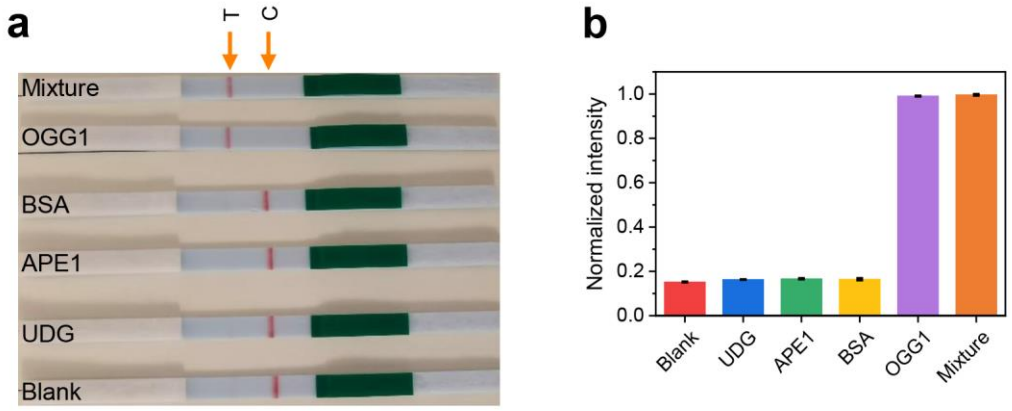

Fig. S13. Visual results and quantitative analysis for the CRISPR-Cas12a test strip detection of OGG1. Mobile phone images capturing test strips detecting UDG, APE1, BSA, OGG1, and their mixtures are displayed in (a), along with ImageJ grayscale quantification in (b). Error bars are standard deviation (SD) (n = 3).

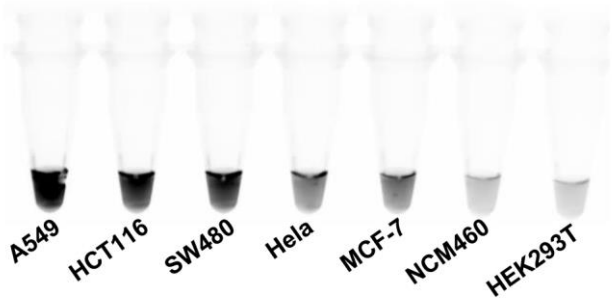

Fig. S14. Direct visual comparison of fluorescence results from the CRISPR-Cas12a system detecting OGG1 in nuclear protein extracts from various cultured cell lines.

Table S2. Comparison of the detection performance of the currently developed method for DNA glycosylases with other existing technologies.

Detection method	Target	LOD (U mL^{-1})	References
CRISPR-Cas12a direct detection	UDG	0.000 61	This work
G4 / FRET	UDG	0.087	Zhao et al. [1]
Hairpin / G4	UDG	0.01	Ma et al. [2]
silver nanorod / SERS	UDG	0.003	Huang et al. [3]
Cas12a / double-loop cascade signal amplification	UDG	0.001 25	Guo et al. [4]
Matrix assisted laser desorption/ionization time-of-flight mass spectrometry technique	UDG	0.001	Chang et al. [5]
Paper device / uracil-rich DNA hydrogel	UDG	0.000 64	Xue et al. [6]
Electrochemical biosensor / nanozyme /cascade signal amplification	UDG	0.000 48	Liu et al. [7]
RCA / CRISPR-Cas12a	UDG	0.000 5	Cheng et al. [8]
RCA / G-quadruplex	UDG	0.000 14	Yang et al. [9]
Electrochemical biosensor / nanozyme	UDG	0.000 66	Tian et al. [10]
isothermal CRISPR amplification	UDG	0.000 917	Han et al. [11]
SDA / G-quadruplex	UDG	0.000 027	Wu et al. [12]
E-RCA / G-quadruplex / Dumbbell	UDG	0.000 01	Fan et al. [13]
TdT-assisted formation / CuNCs	UDG	0.000 05	Liu et al. [14]
E-SDA / CRISPR/Cas12a /HEX	UDG	0.000 031	Chen et al. [15]
Enzyme / DNA Walker	UDG	0.000 04	Li et al. [16]
RCA / Cy3 and Cy5 / Magnetic	UDG	0.000 000 001 54	Li et al. [17]
RCA / signal amplification	UDG	0.000 000 002 41	Zhang et al. [18]
CRISPR-Cas12a direct detection	OGG1	0.09	This work
Graphene / gold nanoparticles hybrids	OGG1	1.6	Yuan et al. [19]
TdT extension amplification	OGG1	0.002	Wang et al. [20]
RCA / G4	OGG1	0.001 43	Sun et al. [21]
CdTe quantum dots	OGG1	0.001	Jie et al. [22]
Nanopore	OGG1	0.006 5	Shang et al. [23]
Functional DNA-Zn^{2+} coordination nanospheres	OGG1	0.000 042	Yang et al. [24]

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