Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

A unique dual recognition hairpin probe mediated fluorescent amplification method for sensitive detection of uracil-DNA glycosylase and endonuclease IV activities

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Table S1. Sequences of oligonucleotides used in this study.

Name	Sequence (5’ - 3’)
Hairpin probe 1	GTGGTGAGGAGTGAGGTAGGTGGTATATTAGGATCGTGTGGT TUATACCACCTACCTCACTCCTCACCAC
Hairpin probe 2	GTGGTGAGGAGTGAGGTAGGTGGTATAATTAGGATCGTGTGG TTUATACCACCTACCTCACTCCTCACCAC
Hairpin probe 3	GTGGTGAGGAGTGAGGTAGGTGGTATAAACTTAGGATCGTGT GGTTUATACCACCTACCTCACTCCTCACCAC
Hairpin probe 4	GTGGTGAGGAGTGAGGTAGGTGGTATAAACCATTAGGATCGT GTGGTTUATACCACCTACCTCACTCCTCACCAC
Hairpin probe 5	GTGGTGAGGAGTGAGGTAGGTGGTATAAACCACATTAGGATC GTGTGGTTUATACCACCTACCTCACTCCTCACCAC
Padlock template	p-GATCCTAACCCAACCCGCCCTACCCAAAACCCAACCCGCCCT ACCCAAAACCCAACCCGCCCTACCCAACCACAC

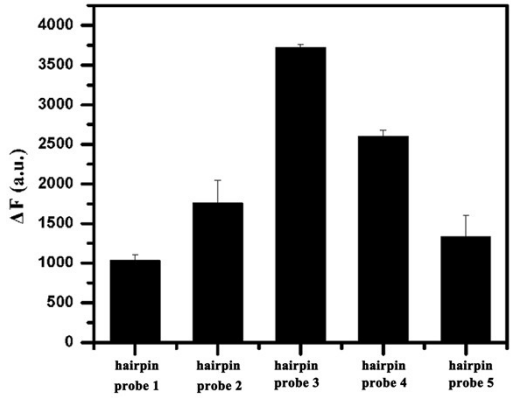

Fig. S1. Fluorescence response of the UDG sensing system to different hairpin probes (hairpin probe 1, hairpin probe 2, hairpin probe 3, hairpin probe 4 and hairpin probe 5). Condition: $C_{\text{hairpin probe}}$ = 80 nM, C_{UDG} = 0.50 U/mL, $C_{\text{Endo IV}}$ = 50 U/mL, $C_{\text{padlock template}}$ = 40 nM, C_{dNTPs} = 1.0 mM. Error bars represent the standard deviations of the results from three independent experiments.

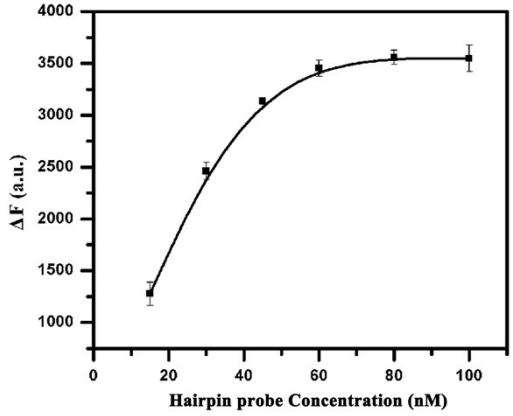

Fig. S2. Effect of different concentrations of hairpin probe on ΔF of the UDG sensing system. Condition: C_{UDG} = 0.50 U/mL, $C_{\text{Endo IV}}$ = 50 U/mL, $C_{\text{padlock template}}$ = 40 nM, C_{dNTPs} = 1.0 mM. Error bars represent the standard deviations of the results from three independent experiments.

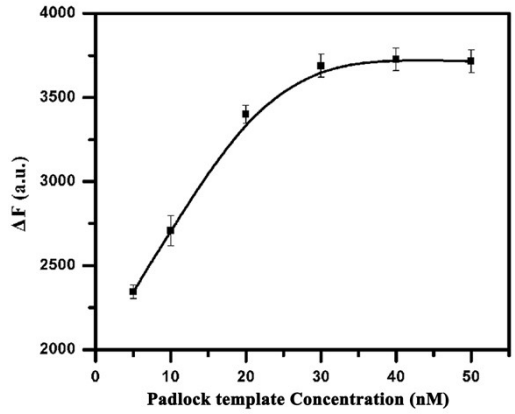

Fig. S3. Effect of different concentrations of padlock template on ΔF of the UDG sensing system. Condition: $C_{hairpin\ probe}$ = 80 nM, C_{UDG} = 0.50 U/mL, $C_{Endo\ IV}$ = 50 U/mL, C_{dNTPs} = 1.0 mM. Error bars represent the standard deviations of the results from three independent experiments.

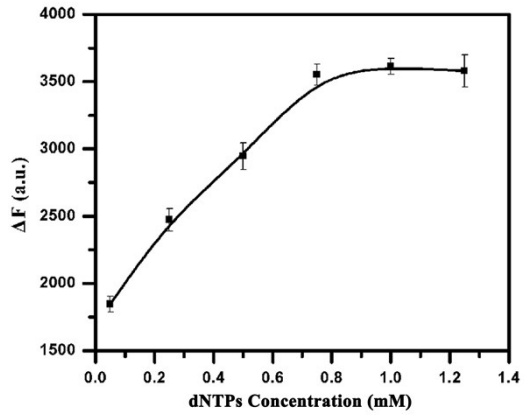

Fig. S4. Effect of different concentrations of dNTPs on ΔF of the UDG sensing system. Condition: $C_{hairpin\ probe}$ = 80 nM, C_{UDG} = 0.50 U/mL, $C_{Endo\ IV}$ = 50 U/mL, $C_{padlock\ template}$ = 40 nM. Error bars represent the standard deviations of the results from three independent experiments.

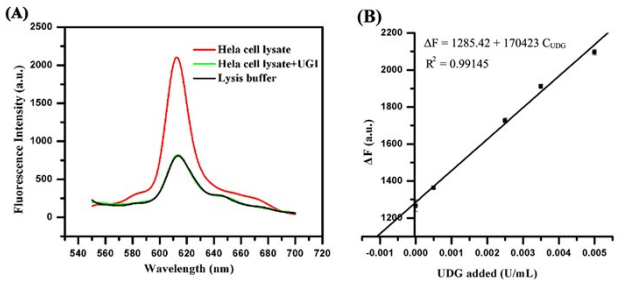

Fig. S5. (A) Fluorescence emission spectra of the UDG sensing system in the absence and presence of 1.0 μL HeLa cells lysate, and the inhibitory effect of 1.0 U/mL UGI on the UDG activity in the HeLa cells lysate. (B) Fluorescence measurement of UDG activity in HeLa cells lysate by the standard addition method. Error bars represent the standard deviations of the results from three independent experiments.

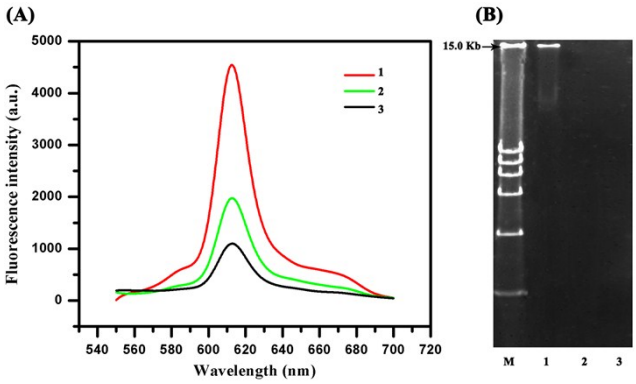

Fig. S6. (A) Fluorescence emission spectra for Endo IV activity assay under different conditions. (B) Agarose gel (0.7%) electrophoresis results of the Endo IV sensing system under different conditions. (1) Hairpin probe + UDG + Endo IV (50 U/mL) + Padlock template + T4 DNA ligase + phi29 DNA polymerase + dNTPS + NMM, (2) Hairpin probe + UDG + Padlock template + T4 DNA ligase + phi29 DNA polymerase + dNTPS + NMM, (3) Hairpin probe + UDG (with the inactivation of UDG after the uracil-excision reaction) + Padlock template + T4 DNA ligase + phi29 DNA polymerase + dNTPS + NMM .

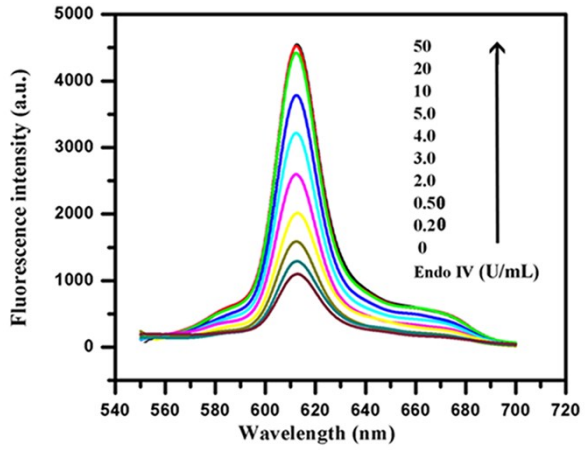

Fig. S7. Fluorescence emission spectra of the Endo IV sensing system at different concentrations of Endo IV.

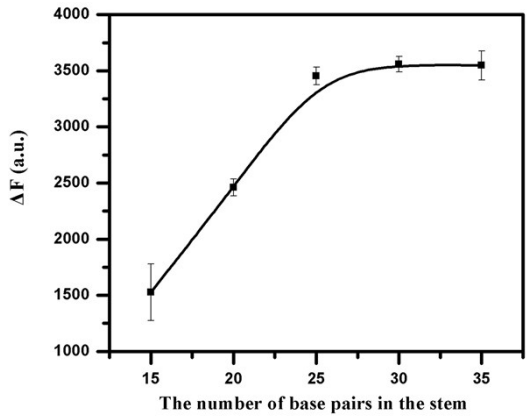

Fig. S8. The variation tendency of net fluorescence intensity ΔF with different lengths of stem in the hairpin probe.

The variation tendency of fluorescence responses with different lengths of stem in the hairpin probe was examined. As shown in Fig. S8, the net fluorescence intensity ΔF increased when the number of base pairs in the stem of hairpin probe increased from 15 to 25. And the ΔF reached the maximum when the number of base pairs in the stem was 30. The reason was that the hairpin probe was not stable when the number of base pairs in the stem was less than 25. And the unstable hairpin probe could trigger the subsequent rolling circle amplification in the absence of target, which resulted in the high background and the low net signal. The hairpin probe with 30 base pairs in the stem ensured a high net signal and could be beneficial for

sensitive detection of the target. Thus, the hairpin probe with 30 base pairs in the stem was selected through the experiment.

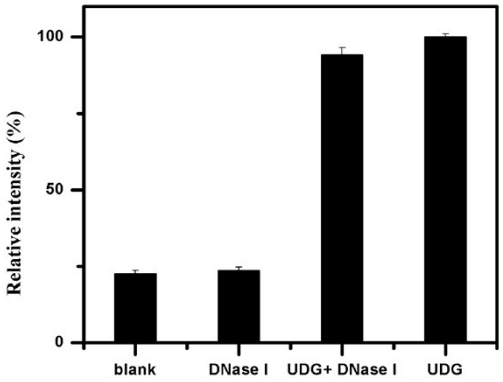

Fig. S9. Specificity of the proposed UDG sensing system to UDG by comparing it to DNase I at the 0.50 U/mL level.

As shown in Fig. S9, DNase I, which was an endonuclease that acted on both double-stranded and single-stranded DNA, could only give low relative intensity which was comparable to that in the blank solution. Additionally, the relative fluorescence intensity of the mixed sample consisting of UDG (0.50 U/mL) and DNase I (0.5 U/mL) was comparable to that of the sample without DNase I. This result was consisted with the report that DNase I was not active in buffers laking Ca^{2+} (http://www.thermofisher.com/cn/zh/home/references/ambion-tech-support/nuclease-enzymes/general-articles/dnase-i-demystified.html). And the reaction buffer in the proposed method was lack of Ca^{2+} , so the DNase I was not active in this UDG sensing system.

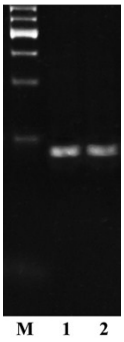

Fig. S10. Polyacrylamide gel (15%) electrophoresis analysis of the the products of the hairpin probe in the presence of DNase I. Lane 1 contains 200 nM hairpin probe without DNase I. Lane 2 contains 200 nM hairpin probe with 0.5 U/mL DNase I.

As shown in Fig. S10, when the sensing system containing hairpin probe incubated with DNase I, the bright band located in the same position as that without DNase I. And no band of the degradation product was observed. The result suggested that the probe in the proposed sensing system was not digested by DNase I.

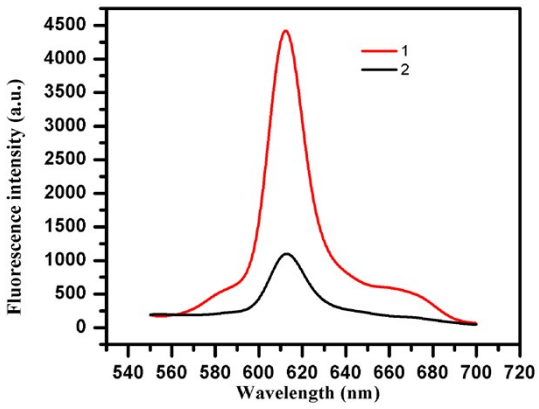

Fig. S11. Fluorescence emission spectra for APE1 activity assay under different conditions. (1) Hairpin probe + UDG + APE1 (40 U/mL) + Padlock template + T4 DNA ligase + phi29 DNA polymerase + dNTPS + NMM, (2) Hairpin probe + UDG + Padlock template + T4 DNA ligase + phi29 DNA polymerase + dNTPS + NMM.

As shown in Fig. S11, in the absence of APE1, the system showed very weak fluorescence intensity (curve 2). However, the fluorescence intensity had significant

enhancement in the presence of 40 U/mL APE1 (curve 1). This result demonstrated that the probe could be adopted for APE1 activity detection.

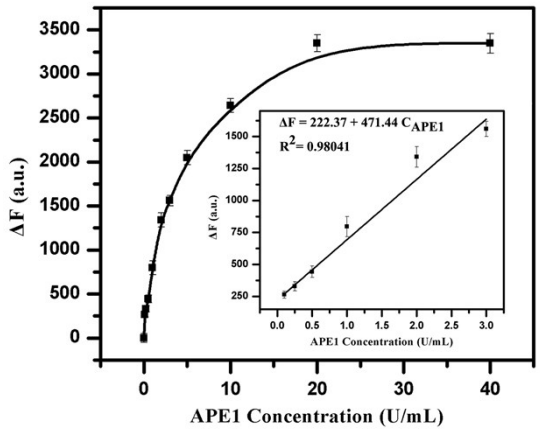

Fig. S12. Calibration curve of Δ F versus different concentrations of APE1 (0 U/mL, 0.10 U/mL, 0.25 U/mL, 0.50 U/mL, 1.0 U/mL, 2.0 U/mL, 3.0 U/mL, 5.0 U/mL, 10 U/mL, 20 U/mL, 40 U/mL). Inset shows the linear responses at low concentrations of APE1.

Fig. S12 showed the calibration curve for APE1 detection, and a linear working range from 0.10 to 3.0 U/mL was obtained. The detection limit for APE1 was found to be 0.10 U/mL, which was comparable to the finding in another report (Zhao et al., Anal. Chem., 2015, 87, 11952-11956).

Table S2. Comparison of different fluorescent methods for the detection of UDG or Endo IV activities.

Method	Target	Detection limit	Strategy	Ref.
Label-based fluorescence	UDG	0.033 U/mL	Real-time monitoring of uracil removal by uracil-DNA glycosylase using fluorescent resonance energy transfer probes	1
Label-based fluorescence	UDG	0.002 U/mL	A target-activated autocatalytic DNAzyme amplification strategy for the assay of base excision repair enzyme activity	2
Label-free fluorescence	UDG	0.05 U/mL	A Label-Free, Quadruplex-Based Functional Molecular Beacon (LFG4-MB) for Fluorescence Turn-On Detection of DNA and Nuclease	3
Label-free fluorescence	UDG	0.020 U/mL	Detection of base excision repair enzyme activity using a luminescent G-quadruplex selective switch-on probe	4
Label-free fluorescence	UDG	0.00044 U/mL	A DNA machine-based fluorescence amplification strategy for sensitive detection of uracil-DNA glycosylase activity	5
Label-free fluorescence	Endo IV	0.20 U/mL	Label-Free Luminescent Switch-on Detection of Endonuclease IV Activity Using a G-Quadruplex Selective Iridium(III) Complex	6
Label-free fluorescence	UDG and Endo IV	0.00017 U/mL for UDG and 0.11 U/mL for Endo IV	A unique dual recognition hairpin probe mediated fluorescent amplification method for sensitive detection of uracil-DNA glycosylase and endonuclease IV activities	This method

Table S3. Detection of Endo IV in spiked sample with 10% HeLa cell lysate using the proposed method.

Sample	Endo IV added (U/mL)	Detected by this method (U/mL)	Recovery (%)	RSD (%)
1	0.50	0.52	104.0	6.1
2	2.0	1.98	99.0	5.5
3	4.0	3.81	95.3	5.2

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