Supporting Information

Detection of UVA/UVC-Induced Damage of p53 Fragment by Rolling Circle Amplification with AIEgens

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1 Abbreviation list

Abbreviation	Full name
UV	Ultraviolet
UVA	Ultraviolet A
UVB	Ultraviolet B
UVC	Ultraviolet C
AIE	Aggregation-induced emission molecules
AIEgens	Aggregation-induced emission luminogens
TPE-Z	Quaternized tetraphenylethene salt
RCA	Rolling circle amplification
MB	Molecular beacon

2 Experimental section

2.1 Materials

Phi29 DNA polymerase, 10×Phi29 DNA Polymerase reaction buffer and BSA was purchased from New England Biolabs (Beijing, China) and used without further purification. The deoxynucleotide solution mixture (dNTPs) and DEPC-treated water were purchased from TaKaRa Biotech Company (Dalian, China; DEPC=diethylpyrocarbonate). Primer 1 (for polymerization reaction), primer 2 (for RCA reaction), p53 gene fragment, the fluorophore/quencher labeled nucleic acid probes, and the circular p53 gene fragment were synthesized by TakaRa Biotech Company (Dalian, China), purified by HPLC, and confirmed by mass spectrometry.

Table S1. The detailed information of DNA sequences

Name	Detailed sequence information (from 5’ to 3’)
primer 1	CCT GGG TCT TCA
primer 2	CCA TTG TTC AAT ATC GTC CG
p53	ATT TGA TGC TGT CCC CGG ACG ATA TTG AAC AAT GGT TCA CTG AAG ACC CAG G
MB	FAM- CCT GGG TCT TCA GTG AAC CAT TGT TCA ATA TCG TCC GGG GAC AGC ATC AAA T -Dabcyl
circular p53	phosphate-ATT TGA TGC TGT CCC CGG ACG ATA TTG AAC AAT GGT TCA CTG AAG ACC CAG G

2.2 Synthesis of TPE-Z

Quaternized tetraphenylethene salt (TPE-Z) was synthesized according to the previous literatures.[1,2]

2.3 Fluorescence measurement

Fluorescence spectra were measured using a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies) with the following settings: λex=350 nm, λem=400-600 nm, slit, 5 nm, PMT detector voltage = 600 V. The rolling circle amplification reaction was performed in 50 μL 1×phi29 DNA polymerase reaction buffer (500 mmol/L Tris-HCl, pH 7.5 at 25 ºC, 100 mmol/L $MgCl_2$, 100 mmol/L $(NH_4)_2SO_4$, 40 mmol/L DTT).

2.4 Image acquisition

Fluorescence imaging was performed using OLYMPUS biological confocal laser scanning microscope. The RCA products bound to the TPE-Z molecules in the solution were imaged using 20 × objective. The excitation wavelength was 405 nm.

3 Additional Figures

3.1 Electrophoresis of linear DNA before or after the head-to-tail ligation

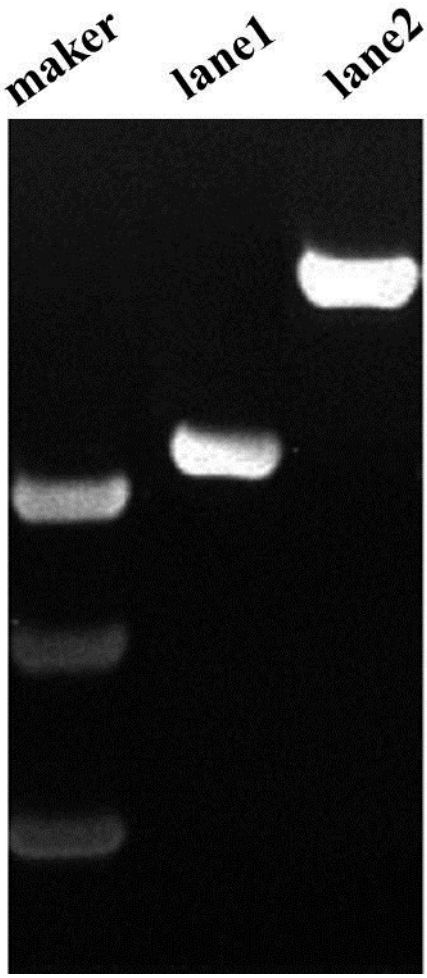

Figure S1. PAGE-QC electrophoresis of linear DNA before (lane1) or after (lane2) the head-to-tail ligation. The maker was 30-50bp.

3.2 Fluorescence emission spectra as time variation of the rolling circle amplification

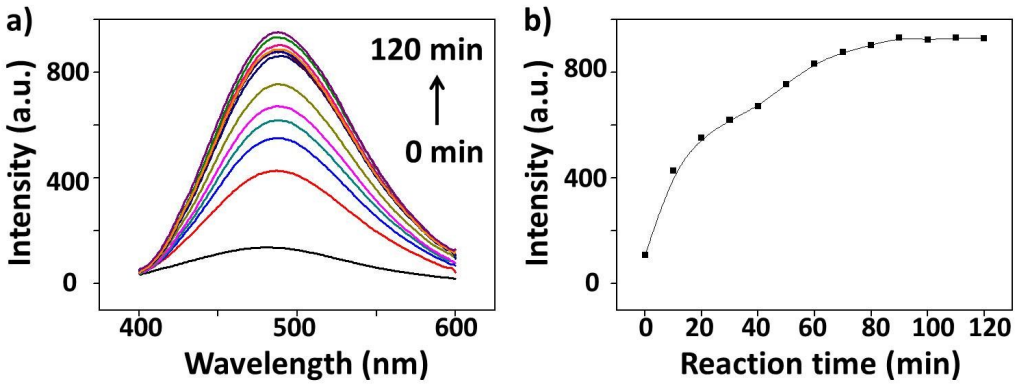

Figure S2. (a) Fluorescence emission spectra of TPE-Z in the presence of rolling circle amplification products with different reaction time in the range from 0 to 120 minutes. (b) Fluorescence emission intensity of TPE-Z at 488 nm vs reaction time. The concentration of TPE-Z was 100 μmol/L.

3.3 Fluorescence emission spectra as detection time variation of TPE-Z molecules

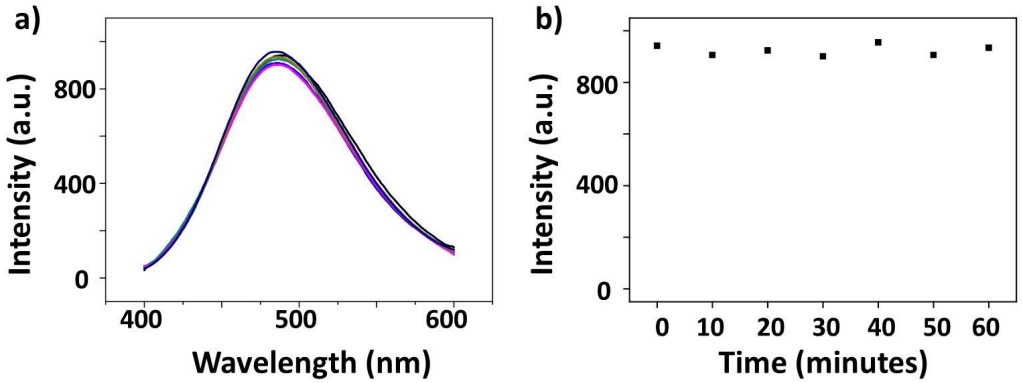

Figure S3. (a) Fluorescence emission spectra of TPE-Z in the presence of rolling circle amplification products with different detection time in the range from 0 to 60 minutes, when the p53 was intact. (b) Fluorescence emission intensity of TPE-Z at 488 nm vs detection time. The concentration of TPE-Z was 100 μmol/L.

3.4 Fluorescence emission spectra as concentration variation of the circular DNA

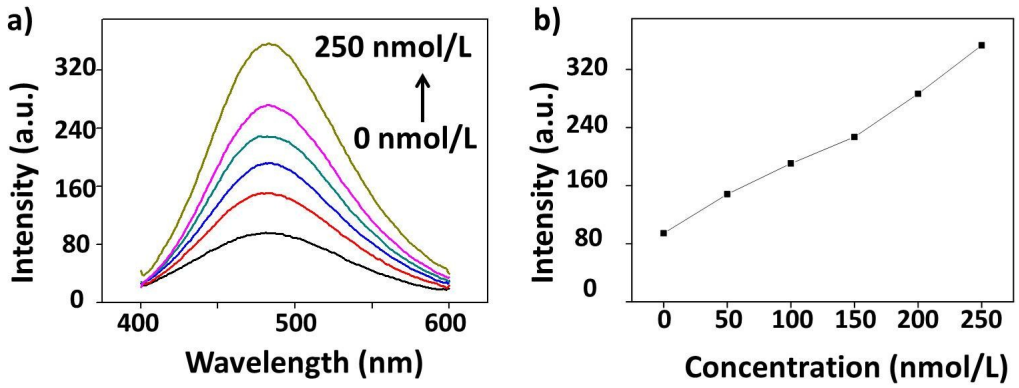

Figure S4. (a) Fluorescence emission spectra of TPE-Z in the presence of circular p53 and primer 2. (b) Fluorescence emission intensity of TPE-Z at 488 nm vs the concentration of circular p53. The concentration of TPE-Z was 100 μmol/L.

3.5 Fluorescence emission spectra of rolling circle amplification products in the present of different concentration of circular p53

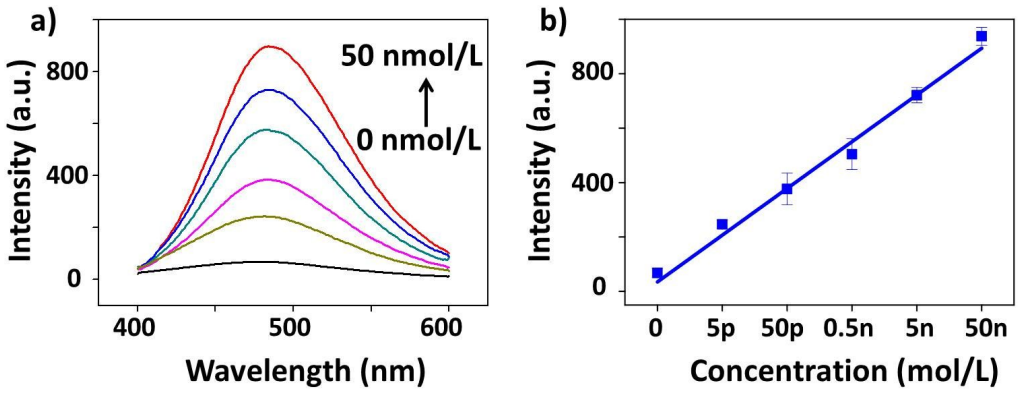

Figure S5. (a) Fluorescence emission spectra of rolling circle amplification products in the presence of different concentration of circular p53. (b) Fluorescence emission intensity of TPE-Z at 488 nm vs the concentration of circular p53. The concentration of TPE-Z was 100 μmol/L. Error bars indicate standard deviation of triplicate tests.

3.6 Fluorescence emission spectra as concentration variation of the TPE-Z

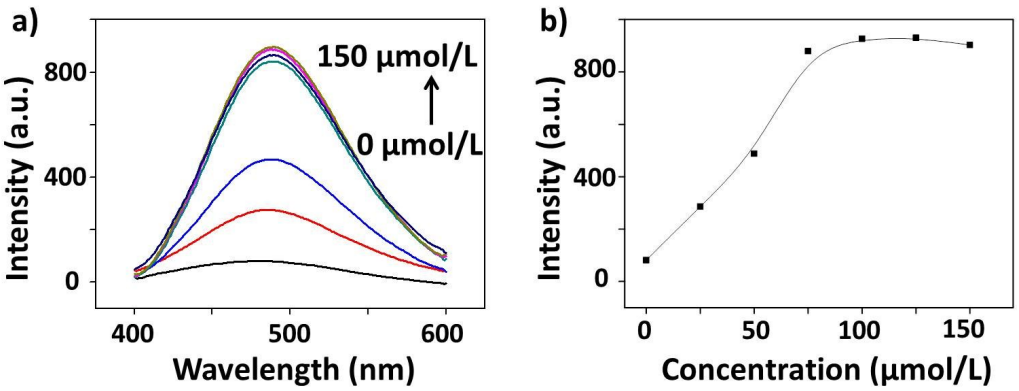

Figure S6. (a) Fluorescence emission spectra of rolling circle amplification product with different concentration of TPE-Z. (b) Fluorescence emission intensity of TPE-Z at 488 nm vs the concentration of TPE-Z.

3.7 Fluorescence emission intensity of TPE-Z in the present of different concentration of p53 (a) and as a function of UVA radiation time (b) in linear assay

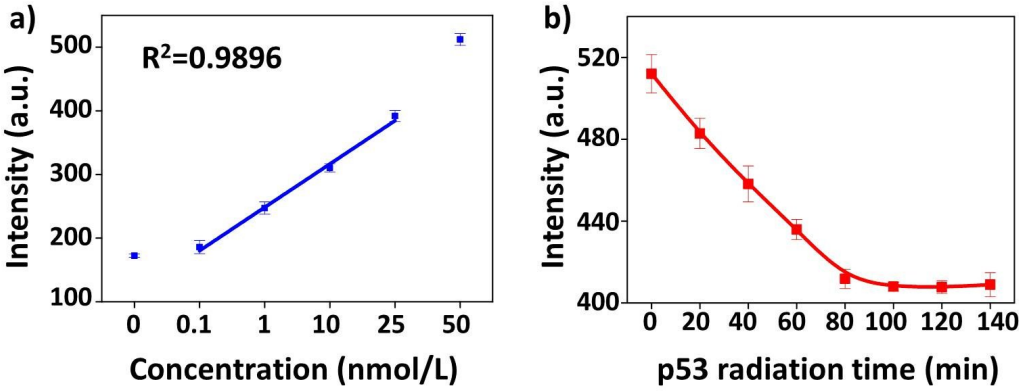

Figure S7. (a) Fluorescence emission intensity of TPE-Z at 488 nm vs the concentration of p53 in linear assay. (b) Fluorescent intensity as a function of UV radiation time. The p53 was radiated under ultraviolet rays (365 nm), with the intensity of 3.8 mW/cm^2. Error bars indicate standard deviation of triplicate tests.

3.8 Fluorescence emission intensity of TPE-Z in the present of different concentration of p53 (a) and as a function of UVA radiation time (b) in polymerization assay

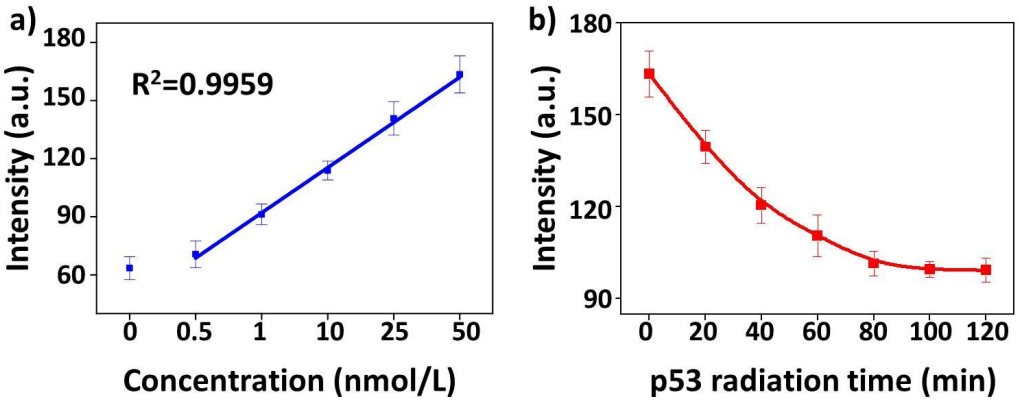

Figure S8. (a) Fluorescence emission intensity of TPE-Z at 488 nm vs the concentration of p53 in polymerization assay. (b) Fluorescent intensity as a function of UV radiation time. The p53 was radiated under ultraviolet rays (365 nm), with the intensity of 3.8 mW/cm^2. Error bars indicate standard deviation of triplicate tests.

3.9 Fluorescence imaging of rolling circle amplification product in the presence of TPE-Z with intact/damaged DNA

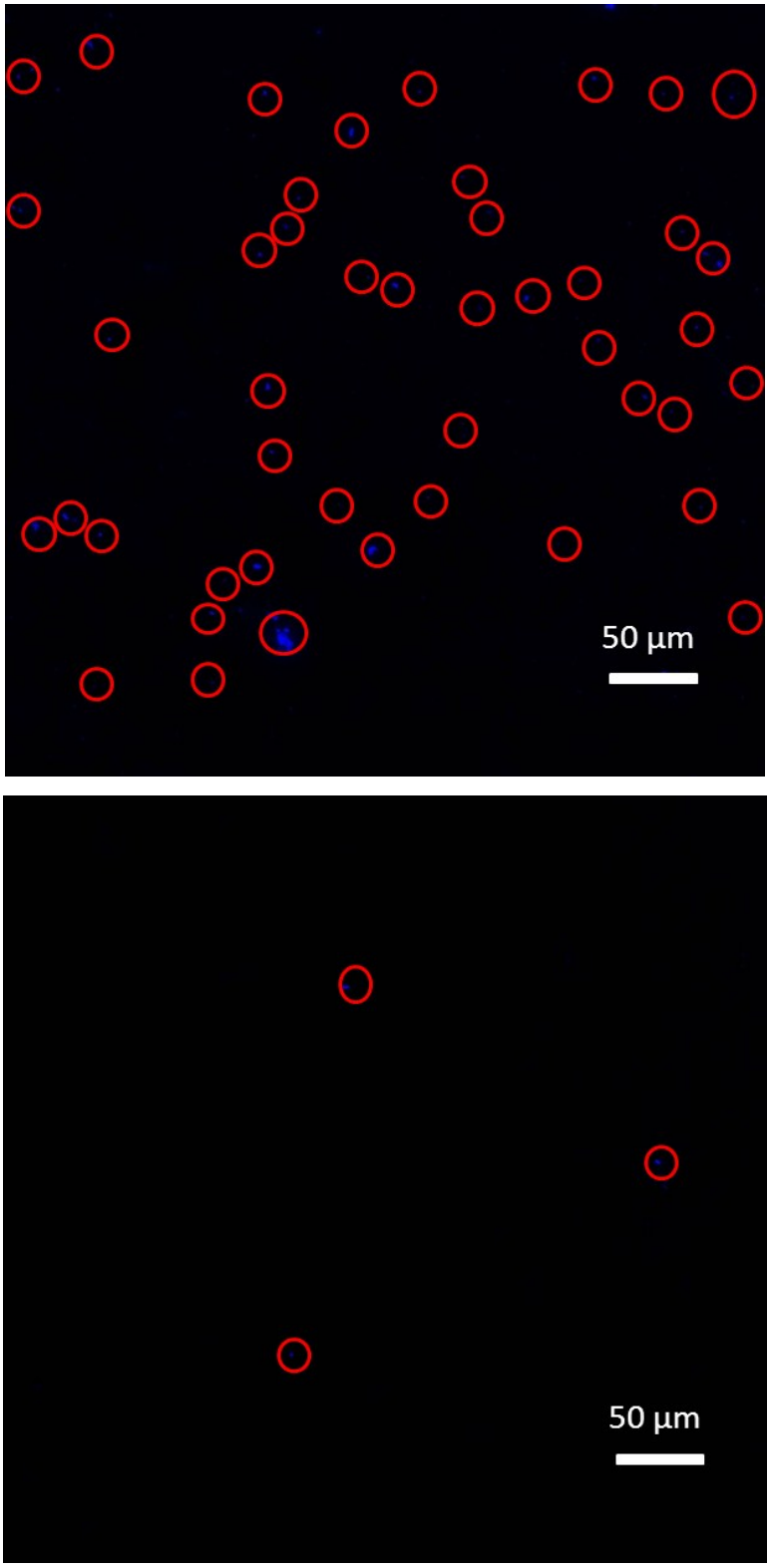

Figure S9. Fluorescence imaging of rolling circle amplification products in the presence of TPE-Z with intact circular p53 (Top) and damaged circular p53 (Bottom). The concentration of circular p53 and primer 2 were 50 nmol/L. Scale bar for all images is 50 µm. The excitation wavelength was 405 nm.

3.10 Specificity experiments of three assays

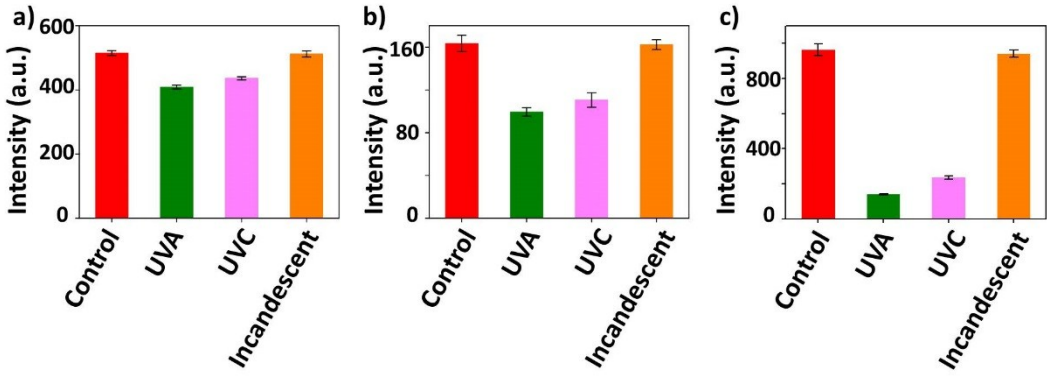

Figure S10. Fluorescence responses of p53 gene radiated by UVA, UVC, and incandescent light (IL) in linear assay (a), polymerization assay (b) and amplification assay (c).

3.11 HPLC analysis

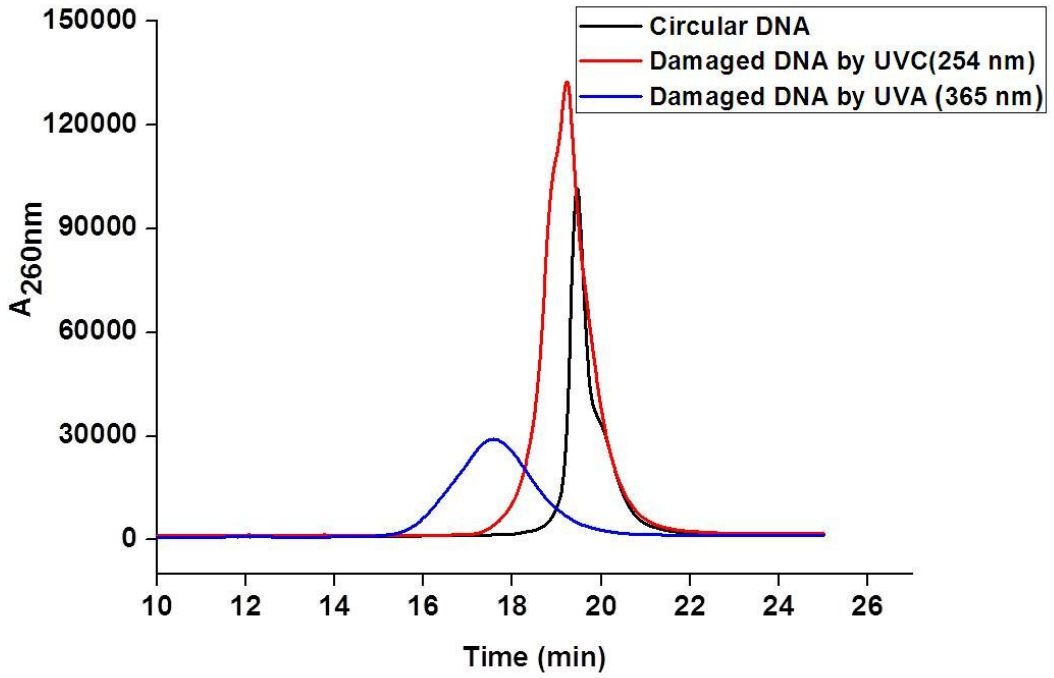

Figure S11. HPLC analysis of circular DNA before UV radiation (the black line) and after 254 nm UV radiation for 200 minutes (the red line), 365 nm UV radiation for 90 minutes (the blue line). The concentration of circular DNA was 10×10^{-6} mol/L.

3.12 Electrophoresis analysis

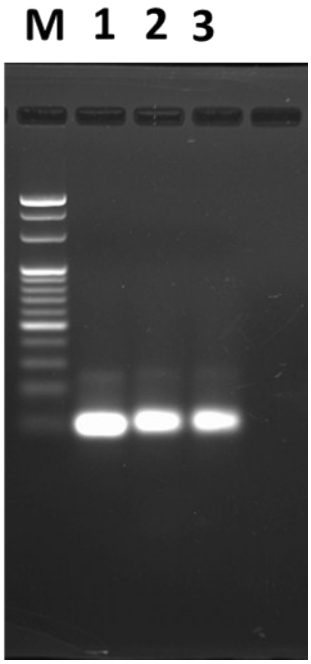

Figure S12. Electrophoresis analysis of p53 fragment before (lane 1) and after UVA (lane 2) and UVC (lane3) radiation for 200 minutes. The concentration of p53 fragment was 10 μmol/L.

3.13 The performance of three assays

Table S2 A summary of Figure S5, S7, S8 and S10

	Linear Assay	Polymerization Assay	Amplification Assay
I_{max}/I_{min}-1	20%	61%	598%
I_0/I_{R20}-1	6%	15%	397%
detection limit	0.1 nmol/L	0.5 nmol/L	5 pmol/L
linear range	0.1-25 nmol/L	0.5-50 nmol/L	0-50 nmol/L
specificity	acceptable	good	excellent

$I_{R20:}$ fluorescence intensity of detect system radiation by 20min

4. Reference

1 R. Hu, N. L. Leung and B. Z. Tang, *Chem. Soc. Rev.*, 2014, **43**, 4494-4562.
2 J.-P. Xu, Y. Fang, Z.-G. Song, J. Mei, L. Jia, A. J. Qin, J. Z. Sun, J. Ji and B. Z. Tang, *Analyst*, 2011, **136**, 2315-2321.