Electronic Supplementary Information

Biodegradable MnO_2 Nanosheets mediated Hybridization Chain Reaction for Imaging of Human Apurinic/Apyrimidinic Endonuclease 1 activity in Living Cells

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Experimental section

Martials and apparatus. Manganese chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$), hydrogen peroxide (H_2O_2, 30 wt %), Tetramethylammonium hydroxide pentahydrate (TMA·OH) were purchased from Alfa Aesar (China). All the oligonucleotide sequences used in this study were synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China) and purified by HPLC. The sequence information was listed in Table S1. The thermodynamic parameters of oligonucleotides were calculated by using bioinformatics software (http://www.bioinfo.rpi.edu/applications/).[1] HeLa cells (human cervical carcinoma cell line) were collected from the cell bank of Central Laboratory of Xiangya Hospital (Changsha, China). Cell culture media was purchased from Thermo Scientific (MA, USA). LysoTracker Green DND-26 was obtained from Invitrogen (Carlsbad, CA). Cell Proliferation Assay kit (CellTiter96 AQueous One Solution) was obtained from Promega (Madison, WI). The APE 1 and 10 × NEBuffer 4 (50 mM KAc，20 mM Tris-Ac，10 mM $Mg(Ac)_2$，1 mM DTT) were purchased from New England Biolabs Ltd (Beijing, China). Deionized and sterilized water (resistance >18.2 MΩ) was used throughout the experiments.

Synthesis of MnO_2 nanosheets. MnO_2 nanosheets were prepared on the basis of the literature. The mixture of 20 mL 0.6 M TMA•OH and 3.0 wt% H_2O_2 was added to 10 mL 0.3 M $MnCl_2$•$4H_2O$ aqueous solution in 15 s, immediately formed a dark brown suspension. The product was then vigorously stirred overnight at room temperature.

The crude product was centrifuged for 40 min at a speed of 2000 rpm and washed several times with ethanol and ultra-pure water. Subsequently, the obtained bulk MnO_2 was then put in fine vacuum at -60°C for the removal of residual solvent. In order to obtain MnO_2 nanosheets, 5 mg dried MnO_2 crude products were dispersed in 10 mL ultra-pure water and crushed by ultrasonic cell crasher.

In vitro detection of APE 1 activity. The typical APE 1 activity was performed in 25 μL reaction mixture containing 200 nM substrate probe, 1 × NEBuffer 4 and different concentrations of APE 1 at 37 ºC for 60 min. Subsequently, 10 μL hybridization buffer (25 mM Tris-HCl, pH 8.0, 250 mM $MgCl_2$), 5 μL H1 (1 μM) and 5 μL H2 (1 μM) were added. After 1 h incubation time at 37 ºC, fluorescence spectrum of each sample was recorded at room temperature in a 100 μL quartz cuvette on a F-7000 fluorescence spectrophotometer (Hitachi, Japan). The excitation wavelength was 630 nm with a recording emission range from 650 to 750 nm and the excitation and emission slits were set at 10 nm.

Gel electrophoresis analysis. The resultant mixture was collected and analyzed using gel electrophoresis in 3% (w/w) agarose stained by 0.5 μg/mL goldview and 0.5 μg/mL ethidium bromide. Electrophoresis was performed at a constant voltage of 101 V for 120 min with a load of 10 μL of sample in each lane. The gel was visualized using a Tocan 240 gel imaging system (Shanghai Tocan Biotechnology Company, China).

Cellular toxicity assay. HeLa cells were implanted into a 96-well microplate with10,000 cells per well. 100 U/mL penicillin, 100 U/mL streptomycin, 10% fetal

bovine serum were added to the 200 μL RPMI 1640 culture medium then placed into the cells and cultured at 37 °C for 24 h. Subsequently, cells were treated with MnO_2 nanosheets of a given concentration (0 μg/mL, 20 μg/mL, 40 μg/ mL, 60 μg/mL, 100 μg/mL) in 200 μL RPMI 1640 medium at 37 °C for 24 h. The medium was removed, and the mixture of 20 μL of Cell Proliferation Assay reagent and 100 μL of fresh medium was added to each well followed by absorbance measurements at 490 nm using a VersaMax Tunable Microplate Reader (VWR International, USA).

Fluorescence imaging in living cells. HeLa cells were cultured on a 35 mm Petri dish in 2 mL RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 U/mL at 37 ^{o}C in a humidified incubator containing 5% wt / vol CO_2 for 24 h then removed the culture medium. For the physisorption of probes on the surface of MnO_2 nanosheets, 1 mL culture medium contained 100 nM SP, 100 nM H1 and 100 nM H2 were mixed with 75 μg/mL MnO_2 nanosheets for 30 min at room temperature. After that, the above mixture was added into the above mixture and incubated with HeLa cells at 37 °C for 3 h. Before imaging, the cells were washed three times with cold PBS and placed with 1 mL fresh medium at 37 ^{o}C.

The APE1 activity inhibition experiment were carried out by pre-treated HeLa cells for 48 h at 37 °C with 1 mL culture medium including 7-nitroindole-2-carboxylicacid (NCA) for different concentration. After that, HeLa cells were rinsed with PBS three times and incubated with 1 mL culture medium including 75 μg/mL MnO_2 nanosheets, 100 nM SP, 100 nM H1 and 100 nM H2 for 3 h at 37 °C. Subsequently, the cells were washed with cold PBS and added 1 mL fresh medium for

the next fluorescence imaging.

For the investigation of time-dependent APE 1 activity on cell surface, confocal microscopy images were acquired every 30 minutes. Cell suspension was dropped on poly-d-lysine coated 35 mm glass bottom dishes, and the fluorescence intensities measurements were performed after a settling time of 5 minutes.

All fluorescence images were obtained by using an oil-immersed objective (60×, NA 1.3) on a confocal laser scanning fluorescence microscope setup consisting of an Olympus FV1000 confocal scanning system (Olympus Co., Japan) with an Olympus IX81 inverted microscope. Red He-Ne laser (635 nm) was used as the excitation light source for the Cy5-labeled probe, and a long-pass filter of 655-755 nm was used for fluorescence detection. An Ar+ laser (488 nm) was used as the excitation source for the detection of LysoTracker Green DND-26, and a bandpass filter of 500-550 nm was used for fluorescence detection.

Flow cytometry assay of APE 1 activity. HeLa cells (10^5 cells) were incubated with 75 μg/mL MnO_2 nanosheets, 100 nM SP, 100 nM H1 and 100 nM H2 in 2 mL fresh medium at 37 ^oC for 3 h and washed three times with cold PBS. Then, the cells were detached with 50 μL of 0.25 % trypsin for 5 min and centrifuged for 5 min at 300 g followed by two washes with 500 μL PBS and re-suspension in 1 mL PBS for flow cytometry assay on a FACSVerseTM flow cytometer (BD Biosciences, USA).

Table S1. Sequences of DNA probes[a]

Name	Sequence (5′-3′)
Substrate probe(SP)	GTGGACGATTAACCCAGTCTAGGATTCGGCGTGGGTTAAXCGTCCAC
Non-substrate probe (NSP)	GTGGACGATTAACCCAGTCTAGGATTCGGCGTGGGTTAATCGTCCAC
Hairpin probe H1	TTAACCCACGCCGAAT(Cy5)CCTAGACTCAAAGTAGTCTAGGAT(BHQ2)TCGGCGTG
Hairpin probe H2	AGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCTAGACTACTTTG
Cy5-H2	Cy5-AGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCTAGACTACTTTG

[a] SP probe and NSP probe includes the initiator sequence (green). Underline sequences indicate complementary regions of the probes to form hairpin structure. X represents abasic sites.

Fig. S1. Agarose gel electrophoresis images of HCR products. Lane M: DNA marker (25-500 bp); lane 1: 1 μM SP; lane 2: 1 μM H1; lane 3: 1 μM H2; lane 4: 500 nM H1 + 500 nM H2; lane 5: 500 nM SP + 500 nM H1 + 500 nM H2; lane 6: 1000 U/mL APE 1 + 500 nM SP + 500 nM H1 + 500 nM H2; lane 7: 1000 U/mL inactive APE 1 + 500 nM SP + 500 nM H1 + 500 nM H2.

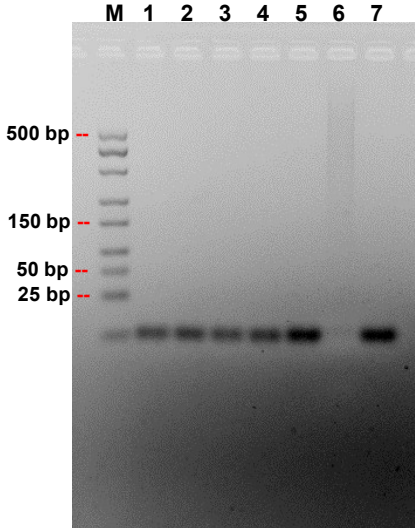


Fig. S2 Specificity of APE 1 assay. Bars represent the fluorescence intensity of the assay in the presence of varying enzyme: 10 U/mL DNase I, 10 U/mL UDG, 10 U/mL RNase A, 10 U/mL APE 1. Error bars are standard deviations of three repetitive experiments.

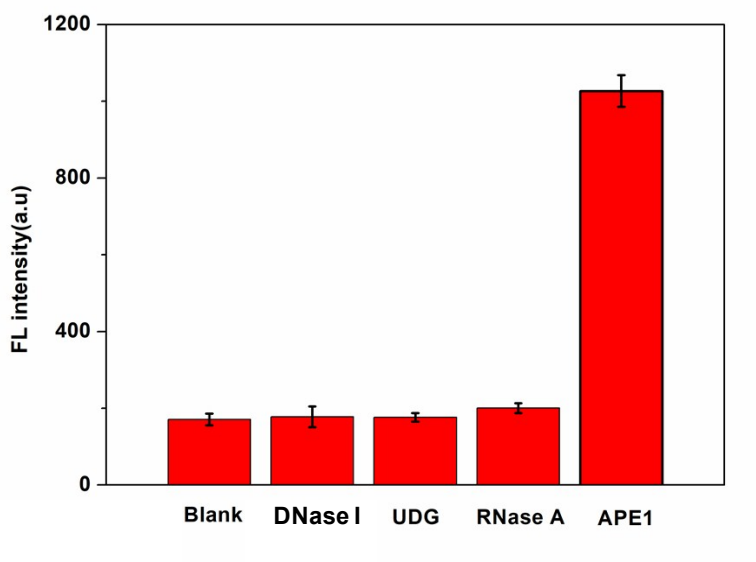


Fig. S3. The changes in fluorescence signal ratio with different concentration of SP. F and F_0 are the fluorescence signals in the presence and the absence of APE 1, respectively. The concentration of H1 and H2 are both 100 nM. Error bars are standard deviations of three repetitive experiments.

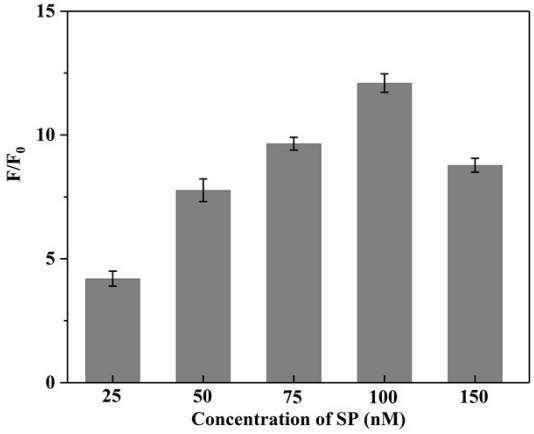
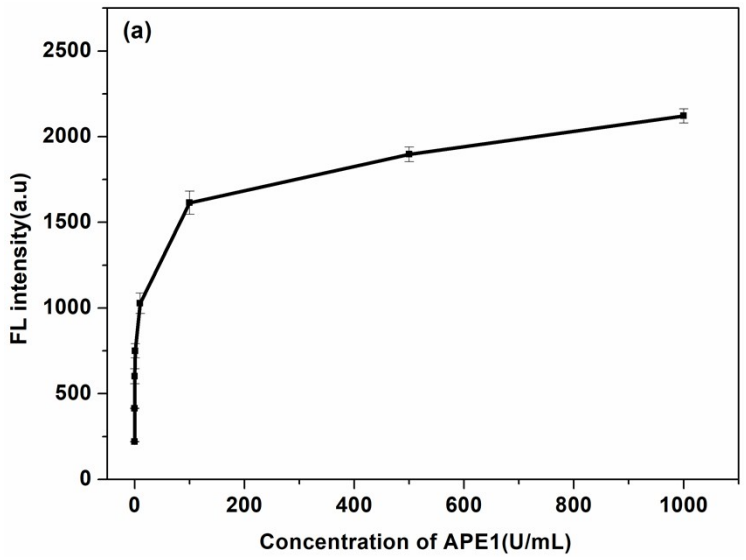


Fig. S4 (a) Plot of fluorescence peak intensities at 667 nm versus APE 1 concentration, (b) Fluorescence peak intensities versus logarithmic APE 1 concentrations. Error bars indicated standard deviations across three repetitive assays.



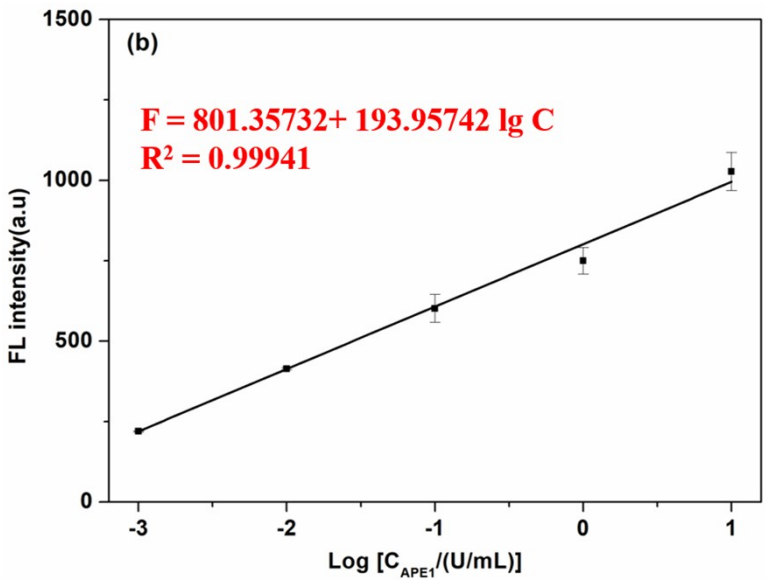


Fig. S5 (a) Fluorescence spectral responses obtained from amplification-free reactions of SP and H1 with APE 1 of varying concentrations. (b) Fluorescence peak intensities versus logarithmic APE 1 concentrations. Error bars indicated standard deviations across three repetitive assays.

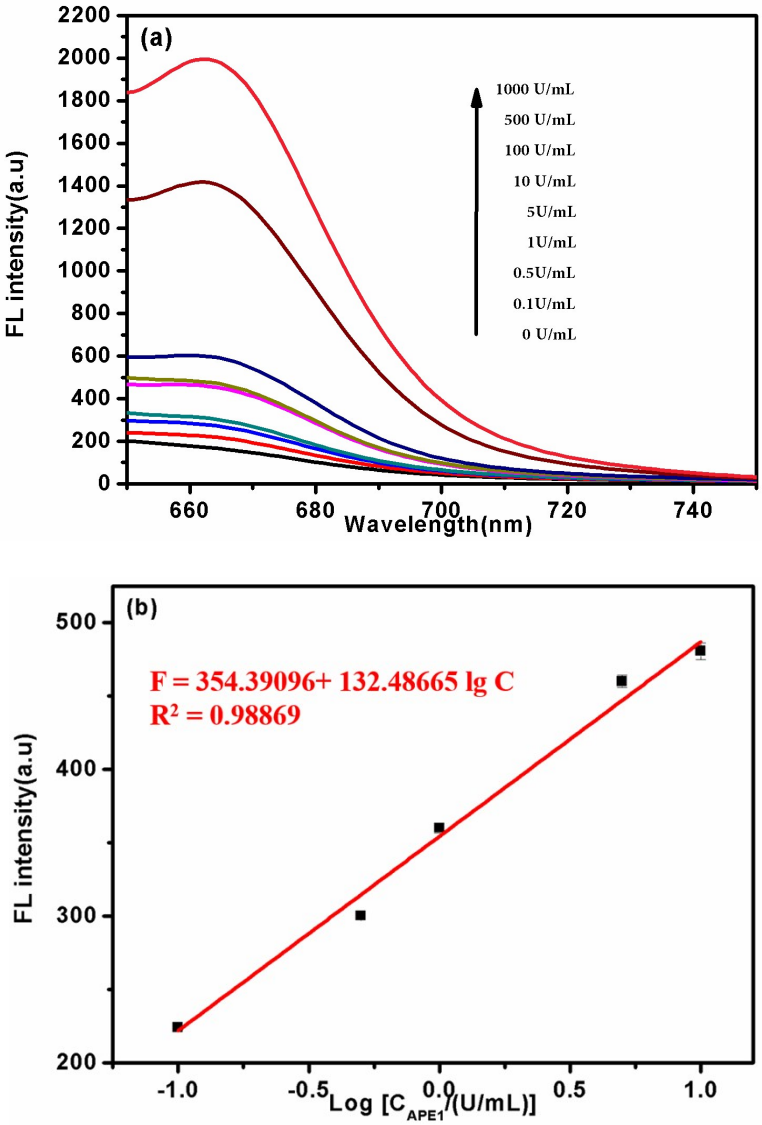


Fig. S6 Time-dependent fluorescence response curves obtained for different APE 1 concentrations. The concentrations of APE1 are 0, 0.001, 0.01, 10 U/mL, respectively.

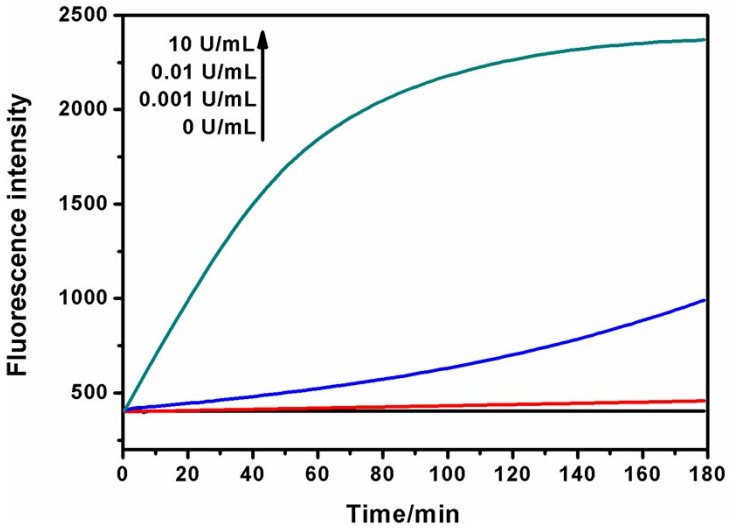


Fig. S7. (a) XPS spectra of MnO_2 nanosheets, (b) high-resolution Mn2p XPS spectra.

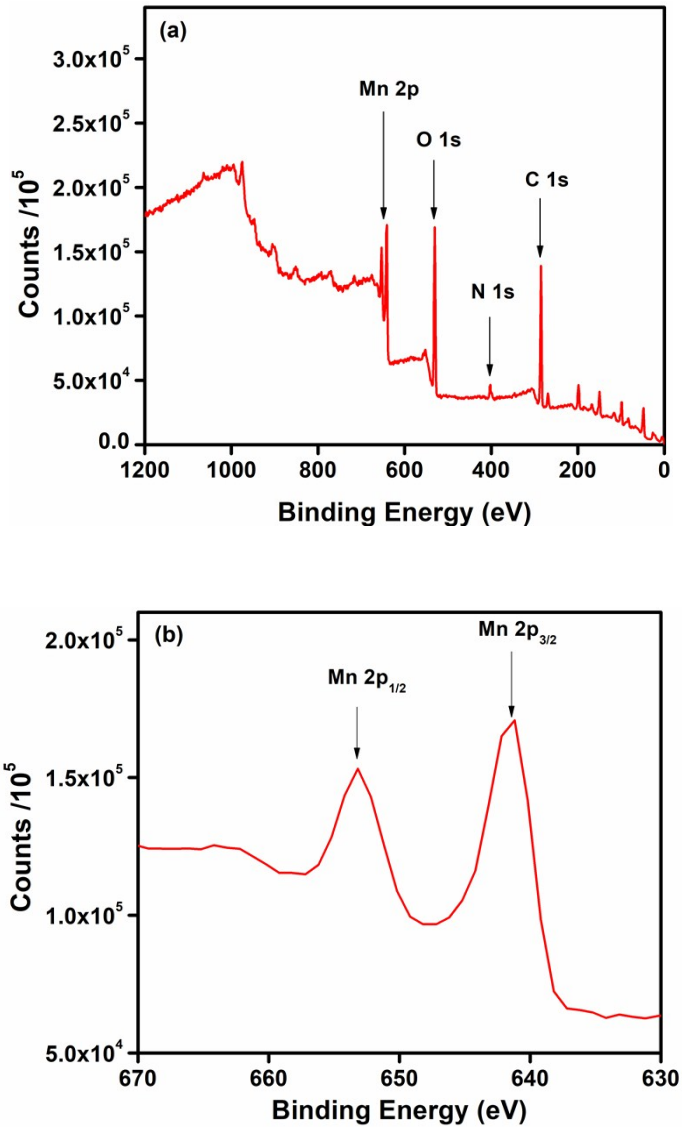
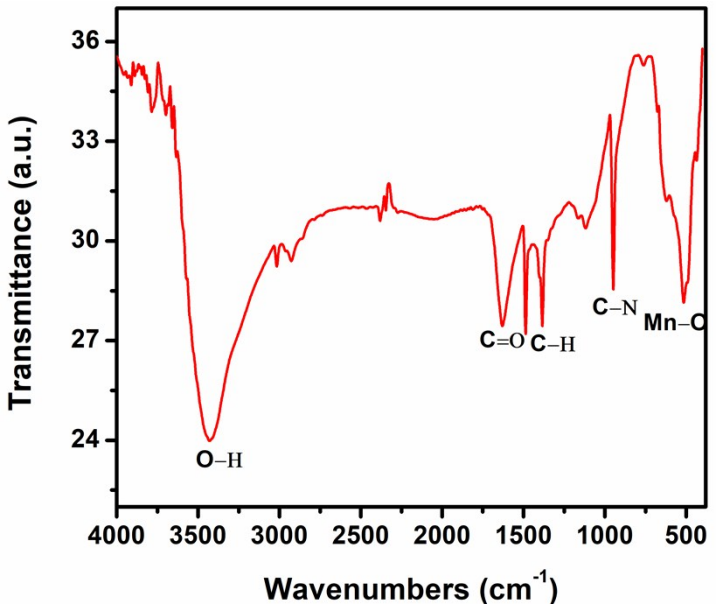


Fig. S8. Fourier-transform infrared spectroscopy (FT-IR) of MnO_2 nanosheets.



FT-IR spectrum of MnO_2 nanosheets presents that the broad band around 3440 cm^{-1} represented the O-H antisymmetric stretching vibration of the interlayer water molecules and framework hydroxyl groups. The sharp absorption peak at 1119 cm^{-1} is assigned to the coordination of Mn by the O–H. And the peaks at 507 cm^{-1} are considered as the main characteristic absorption bands of birnessite MnO_2, corresponding to Mn-O stretching modes of the octahedral layers in the birnessite structure.

Fig. S9. UV−vis absorption spectrum of MnO_2 nanosheets.

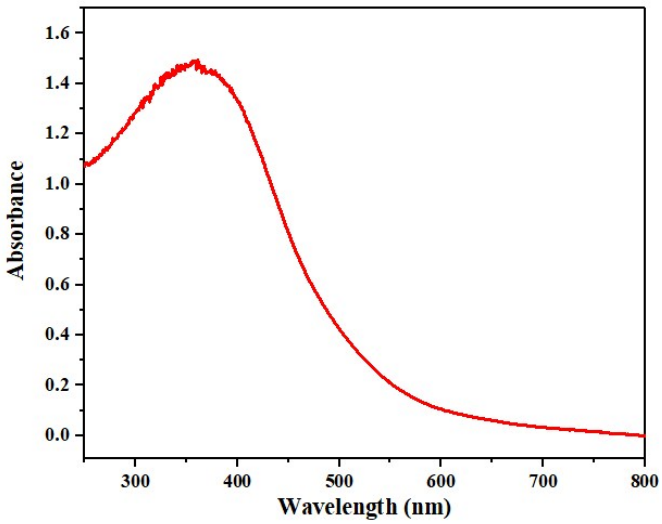


Fig. S10. (a) Transmission electron microscope (TEM) images of MnO_2 nanosheets, (b) Dynamic light scattering (DLS) of MnO_2 nanosheets.

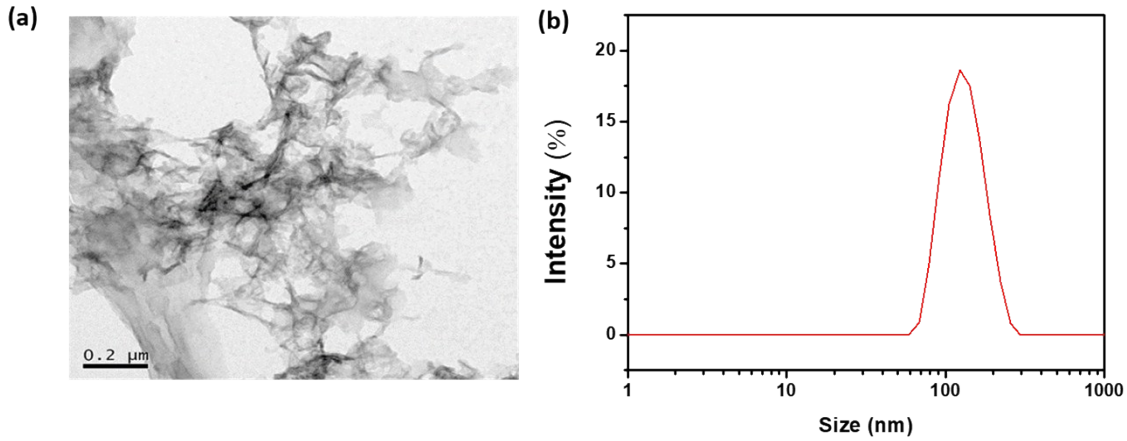


Fig. S11. (A) AFM image of MnO_2 nanosheets; (B) Height profile of the section in part A.

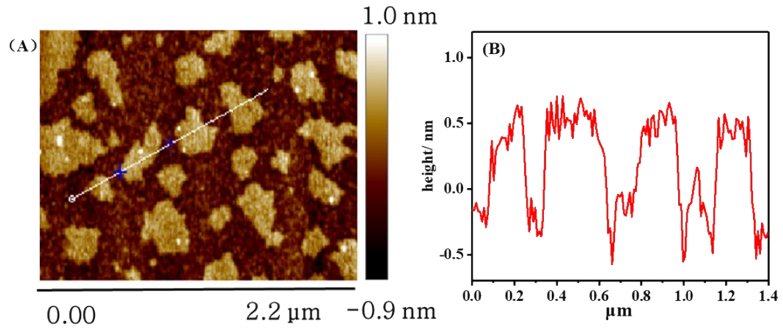


Fig. S12. (A) Fluorescence intensity of 100 nM Cy5-H2 when incubated with different concentrations of MnO_2 nanosheets (B) Fluorescence spectrum response of Cy5-H2/MnO_2 nanocomplex to different concentrations of GSH.

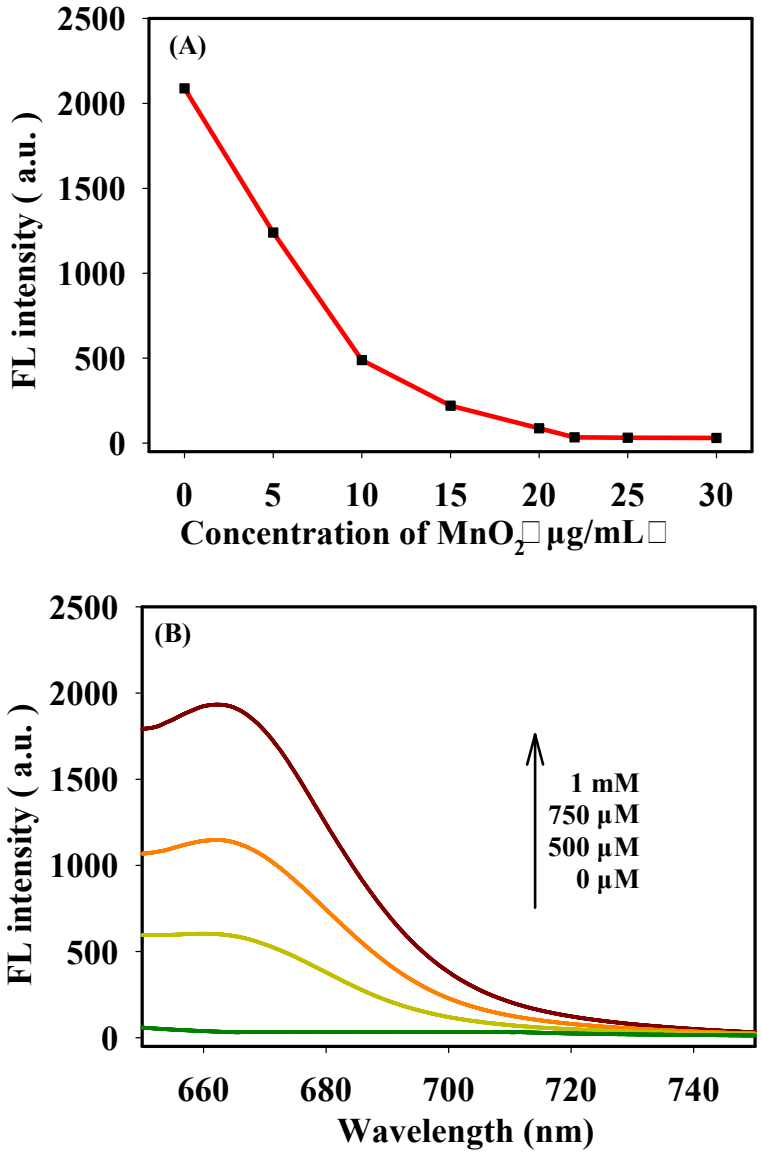


Fig. S13. Fluorescence-based stability of free H1, H1/MnO_2 nanocomplex after DNase I (1 unit) treatment for 1 h.

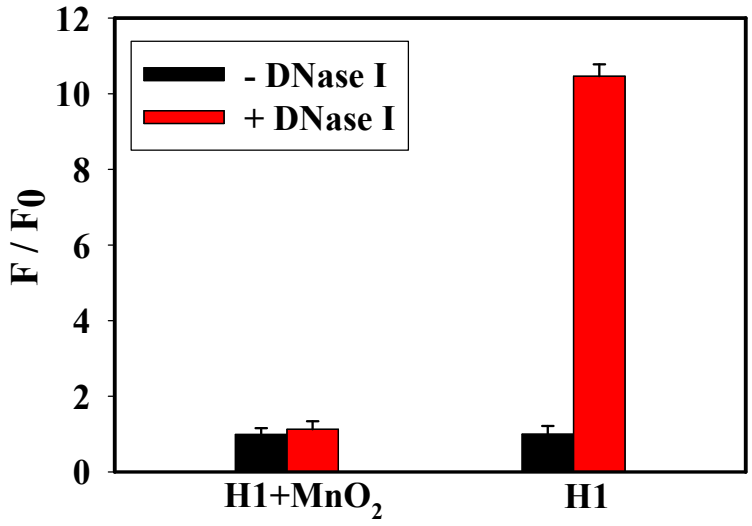


Fig. S14. Stability of SP, H1 and H2 in MnO_2 nanosheets upon cell medium. F are the fluorescence signals for the different incubation time, F_0 are the fluorescence signals when the probes mixed with MnO_2 nanosheets upon cell medium immediately. Error bars are standard deviations of three repetitive experiments.

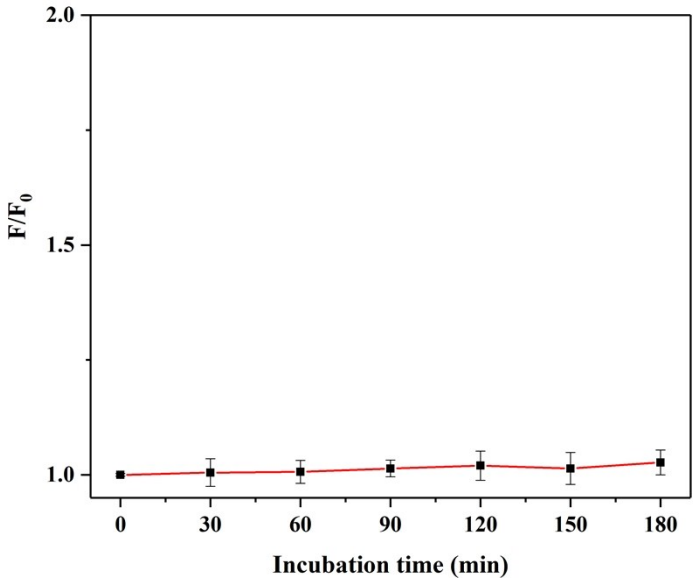


Fig. S15. Figure 1. (a) Typical fluorescence spectral in different reaction system. The concentration of SP, H1 and H2 are all 100 nM, the amount of MnO_2 nanosheets is 75 μg/mL, the concentration of GSH and APE 1 are 5 mM and 1000 U/mL. (b) Fluorescence spectral responses to APE 1 of different concentrations in the presence of MnO_2 nanosheets with GSH. All the reactions were performed at 37 ºC for 3 h in cell culture medium.

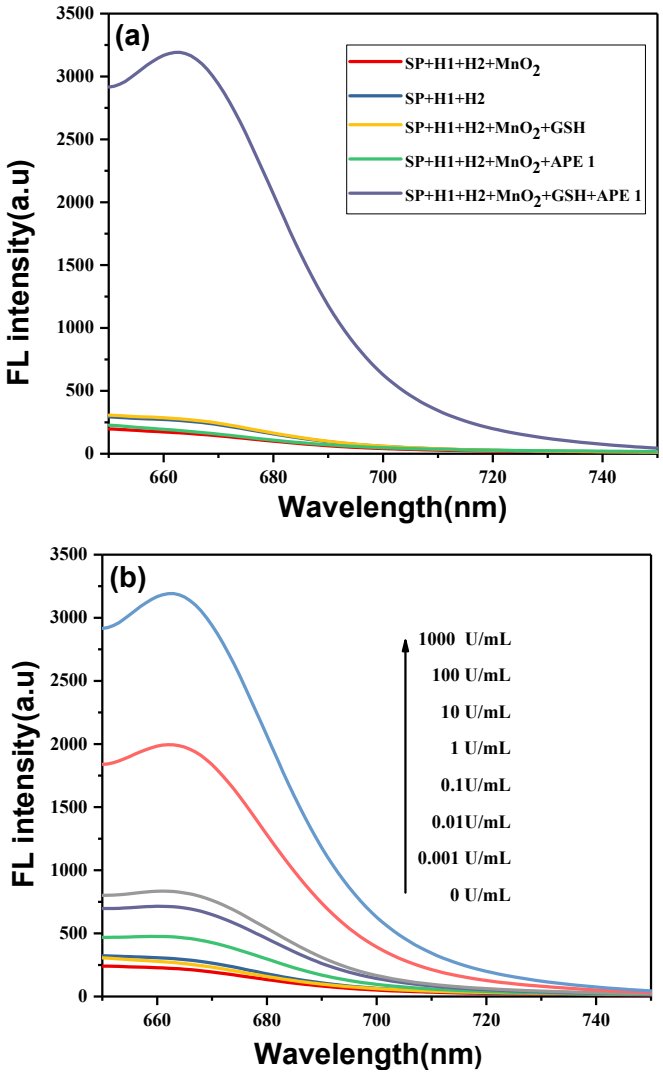


Fig. S16. Cytotoxicity of MnO_2 nanosheets incubated with HeLa cells at different concentrations in 24 h. Error bars indicated standard deviations across three repetitive assays.

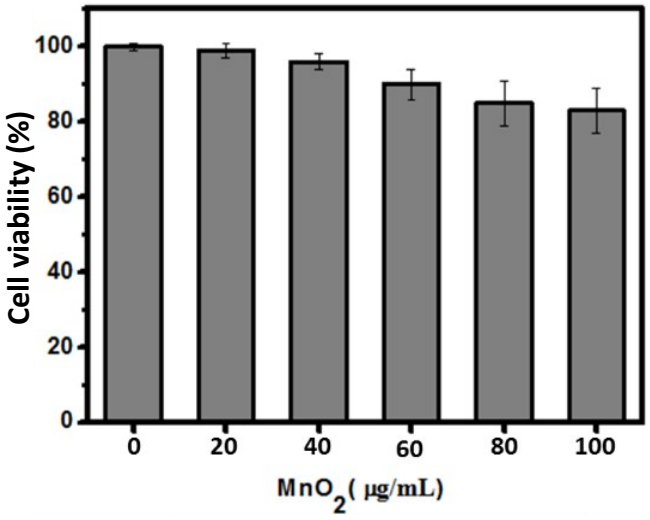


Fig. S17. Flow cytometric assay of HeLa cells. Cells incubated 100 nM SP, 100 nM H1 and 100 nM H2 delivered by 75 μg/mL MnO_2 nanosheets (green); cells 100 nM NSP, 100 nM H1 and 100 nM H2 delivered by 75 μg/mL MnO_2 nanosheets (red).

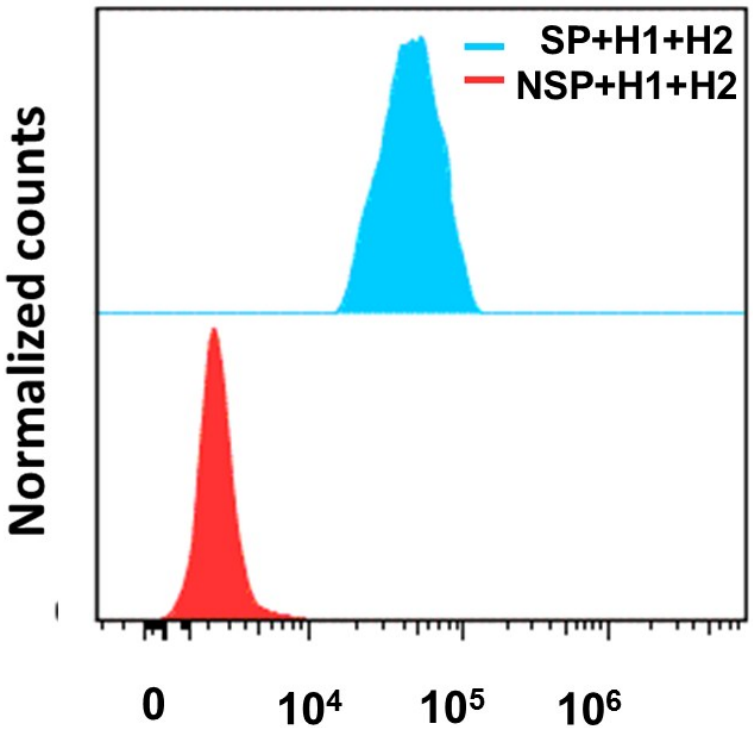


Fig. S18. Confocal microscopy image of HeLa cells after incubated for 3 h with 100 nM SP, 100 nM H1 and 100 nM H2 delivered by 75 μg/mL MnO_2 nanosheets followed by staining with a culture medium containing 50 nM Hoechst and LysoTracker Green DND-26 for 30 min.

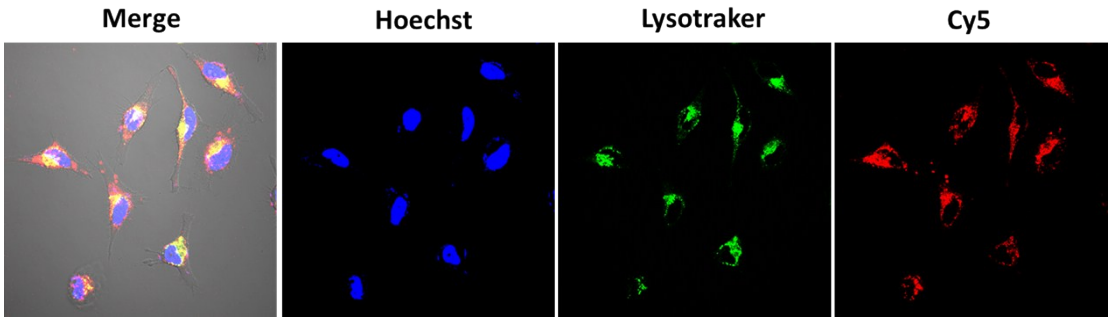
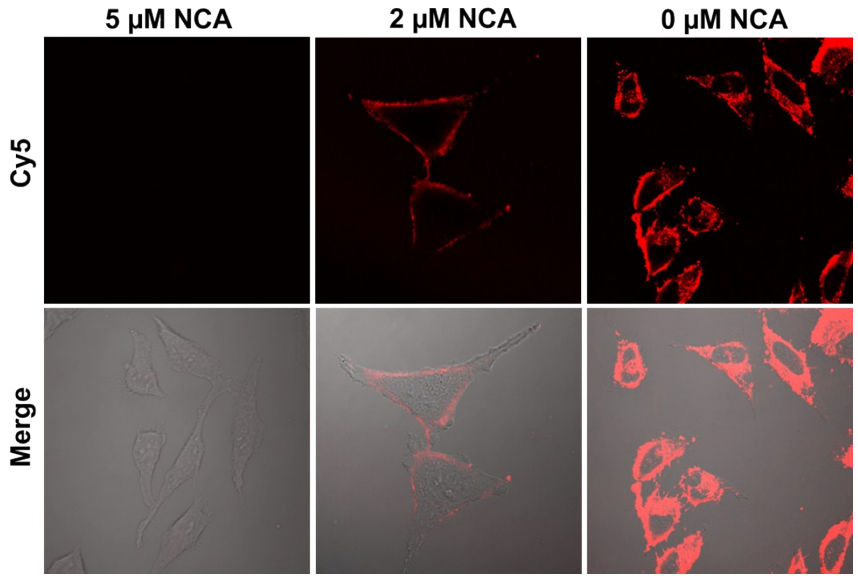


Fig. S19. Fluorescence images for HeLa cells treated with varying concentrations of an APE 1 activity inhibitor 7-nitroindole-2-carboxylicacid (NCA) followed by incubation with 100 nM SP, 100 nM H1 and 100 nM H2 delivered by 75 μg/mL MnO_2 nanosheets.



References:

1. M. Zuker, *Nucleic Acids Res.,* 2003, **31**, 3406–3415.