Electronic Supplementary Information (ESI)

An Intelligent Nanodevice Based on Telomerase-triggered Photodynamic and Gene-silencing Synergistic Effect for Precise Cancer Cells Therapy

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Table of Contents

Experimental Section

Apparatus.

The fluorescence spectra were recorded using Fluorescence Spectrometer FS5 (Edinburgh Instruments, UK). With a 900 V PMT voltage, the excitation and emission slit were set at 5.0 nm. Zeta potential and dynamic light scattering (DLS) were measured on the Malvern Zetasizer Nano ZS90 (USA) at room temperature. Atomic force microscopy (AFM) images of samples were measured on a Multimode 8 (Bruker, USA). Transmission electron microscope (TEM) was performed on a field emission high resolution 2100F transmission electron microscope (JEOL, Japan) at an acceleration voltage of 200 kV. The flow cytometric analysis was carried on the Cytoflex (Beckman, USA). The cell viability was evaluated by a microplate reader (ELx800, BioTek, USA). All fluorescence imaging was measured on a confocal laser scanning fluorescence microscope (Nikon, Japan). The concentration of Mn^{2+} was determined by the inductively coupled plasma mass spectrometry (ICP-MS NexION300x, USA).

Agarose Gel Electrophoresis.

For the gel electrophoresis of the telomerase-activated the conformation switching of the DNA duplex probe and the release of the block strand, 9 μL mixture solution containing 1.2 μL of 10 μM block strand, 1 μL of 10 μM DNAzyme, 2 μL 5×Tris-HCl and 4.8 μL DEPC water was annealed from 70℃ to 37℃, followed by adding 1 μL cell extract (1×10^7 cells) or other given reagents at 37℃ for another 2 h, the obtained samples were mixed with 2 μL of 6×loading bu□er and performed on an agarose gel (4%, w/v). The preparation of samples about DNAzyme catalysis the cleavage of the substrate, 10 μL mixture solution containing 1.2 μL of 10 μM substrate, 1 μL of 10 μM DNAzyme, 2 μL of 1 mM Mn^{2+}, 2 μL 5×Tris-HCl and DEPC water (3.8 μL) were incubated at 37 ℃ for 2 h. Then, the obtained samples were mixed with 2 μL of 6×loading bu□er and performed on an agarose gel (4%, w/v).

All the electrophoresis assays were performed in 1× Tris-borate-EDTA (TBE) bu□er (90 mM Tris-HCl, 2 mM EDTA, 90 mM boric acid and pH 8.0) at 110 V for 2 h at room temperature.

Cell Extract.

The cell extract was obtained by using Nuclear and Cytoplasmic Protein Extraction Kit. 2×10^6 cells were collected and dispensed in 1.5 mL EP tube, washed twice with PBS (0.1 M, pH 7.4), and resuspended in 200 μL of CHAPS lysis buffer (10 mM Tris-HCl, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 0.5% CHAPS, 10% glycerol and pH 7.5). The mixture was incubated on ice bath for 15 min and centrifuged at 16000 rpm at 4℃ for 5min. Then the supernatant was collected as cell extract and stored at -80℃ for further use.

Flow Cytometry Experiments.

In a typical experiment, HeLa cells (0.5 mL, 1×10^6 cells mL^{-1}) were seeded in a 35-mm dish and cultured in RPMI-1640 medium for 24 h at 37 ℃. Subsequently, the cells were incubated with the given reagents, then treated with 0.25% trypsin for 2 min and centrifuged at 1800 rpm for 2 min followed by washing with PBS twice. Finally, the cells were re-dispersed in 500 μL of 1×PBS for flow cytometric analysis on a Cytoflex flow cytometry system.

For apoptosis analysis, the cells (0.5 mL, 1×10^6 cells mL^{-1}) were incubated with the given reagents for 4 h at 37 ℃. Then the cells were washed twice with 1×PBS (6.7 mM PB, pH 7.4) and continue cultured in RPMI-1640 medium for 24 h. After that, the cells were treated with above reagents again. After 24 h incubation, the cells were treated with 0.2 mL 1×PBS (6.7 mM PB, pH 7.4) containing 2 μg mL^{-1} Alexa Fluor 488 annexin V, 10 μg mL^{-1} PI dead cell apoptosis kit for 15 min at room temperature. Then cells were re-suspended in 1 mL PBS followed by flow cytometry assay with PI channel and FITC channel.

Real-Time Reverse Transcriptase-PCR Analysis.

HeLa cells (0.5 mL, 1×10^6 cells mL^{-1}) were seeded in a 35-mm dish, cultured in 3 mL RPMI-1640 medium for 24 h at 37 ℃, and incubated with the given reagents for 4 h at 37 ℃. Then the

cells were washed twice with 1×PBS (6.7 mM PB, pH 7.4) and continue cultured in RPMI-1640 medium for 24 h. After that, the cells were treated with above reagents again. After 24 h incubation, the total cellular RNAs were extracted from HeLa cells by using the Uniq-10 column Trizol total RNA extraction kit (Sangon) according to the manufacturer's instructions. The cDNA samples were prepared with Revert Aid Premium Reverse Transcriptase (Thermo Fisher Scientific) and stored at -20 °C for future use. The analysis of cDNA was performed with SybrGreen PCR Master Mix (ABI, USA) on an ABI StepOnePlus qPCR instrument. The 20 μL reaction solution contained 2 μL cDNA sample, 10 μL of SG Fast qPCR Master Mix (High Rox, 2×), 0.4 μL of primer forward (10 μM), 0.4 μL of primer reverse (10 μM) and 7.2 μL of the nuclease-free water. The PCR conditions were as follows: an initial 95 °C for 3 min followed by 40 cycles for 15 s at 95 °C, for 20 s at 57 °C and for 30 s at 72 °C. The primers were used of survivin forward primer, 5'-ttctcaaggaccaccgcat-3'; survivin reverse primer, 5'-tctcagtggggcagtggat-3'.

Western Blot Analysis.

The previous steps were the same as the mentioned above, after 48 h incubation, cells were washed twice with cold PBS. All subsequent steps were performed at 4°C, cells were lysed for 30min in 75 μL of lysis buffer (50 mM Tris, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 5 mM EDTA, 1 mM PMSF, 0.1% SDS, 2 μg mL^{-1} each of sodium orthovanadate, sodium fluoride, leupeptin and pH 7.4). Then the lysate was centrifuged at 13000 rpm for 15min. The supernatant was collected at 1.5 mL EP tube and determined the concentration of total cellular proteins by using BCA protein assay kit. By SDS-PAGE, total cellular proteins were separated and transferred to PVDF membranes. After incubated with anti-survivin antibodies and secondary antibodies, the membranes were sent to detection on Gel Imaging System (Bio-RAD).

Table S1. The sequences of all oligonucleotide strands are listed in table S1.

Name	Sequence (5'-3')
Ce6-DNAzyme	tcggccaggctagctacaacgaccgctcccctaaccct(Ce6)aaccctaaccctaacccaatccgtcgagcagagtt
BHQ2-block	BHQ2- agggttaggggagaggt
Control Ce6-DNAzyme	tcggccaggctagctacaacgaccgctcccctaaccct(Ce6)aaccctaaccctaacccaatccttctagtagagat
Substrate	FAM-ggagcggraruggccga-Dabcyl
DNAzyme	tcggccaggctagctacaacgaccgctcccctaaccctaaccctaaccctaacccaatccgtcgagcagagtt
Block	agggttaggggagaggt
Control DNAzyme	tcggccaggctagctacaacgaccgctcccctaaccctaaccctaaccctaacccaatccttctagtagagat
Ce6-mistaken DNAzyme	tcggccaggcagtacaccaccgctcccctaaccct(Ce6)aaccctaaccctaaccctaacccaatccgtcgagcagagtt

[a]Compared to the Ce6-DNAzyme, the control Ce6-DNAzyme contained incorrect telomerase primer sequence which was marked in red underline, and Ce6-mistaken DNAzyme contained non-DNAzyme sequence that did not catalyze the cleavage of the survivin mRNA, which was marked in green underline.

Table S2. Intracellular Mn^{2+} concentration determined by inductively coupled plasma mass spectrometry (ICP-MS).

Sample	Mn^{2+} (μg/L)
Blank	0.091 ± 0.008
MnO_2	1.124 ± 0.006

[a]All values were obtained as the average of three repetitive determinations plus standard deviation.

HeLa cells (~3.65×10^5 cells) were incubated with 1mL of RPMI-1640 containing MnO_2 (25 μg/mL) for 4 h at 37℃. The cells were then collected and lysed by 500 μL of cell lysate, and were 1:40 diluted using ultrapure water. The ICP-MS determination was then performed to calculate the Mn^{2+} concentration in single cell, which was about 0.062 pg. The Mn^{2+} concentration in HeLa cells without treatment of MnO_2 nanosheets was about 0.005pg determined by using the same method.

Figure S1. The agarose gel electrophoresis image for the feasibility analysis of the telomerase-activated the conformation switching of the DNA duplex probe and the release of the block strand.

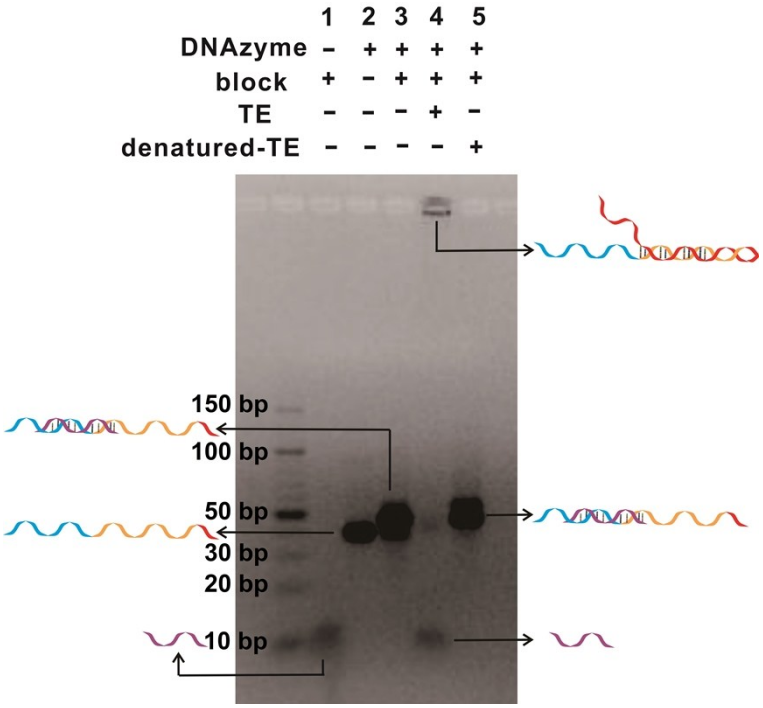

Figure S2. The telomerase detection in vitro by using DNA duplex probes. **(**A**)** The fluorescence spectra of the DNA duplex probe in response to the lysate (with different concentrations of telomerase) extracted from different numbers of HeLa cells. **(**B**)** The relationship between the fluorescence signal and cell numbers. Insert: Linear relationship between the fluorescence signal and cell numbers. Error bars represented the standard deviation of three parallel experiments.

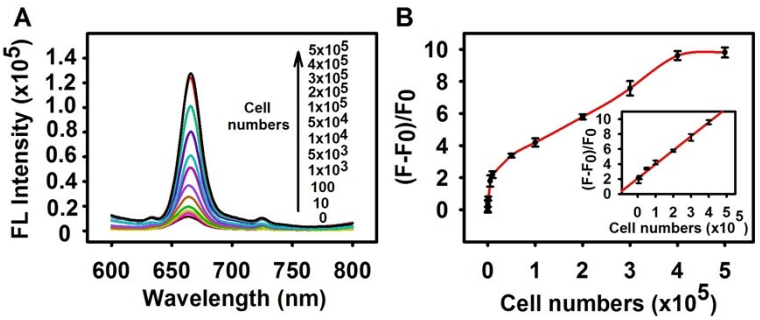

Figure S3. The agarose gel electrophoresis image for DNAzyme catalytic cleavage of substrate.

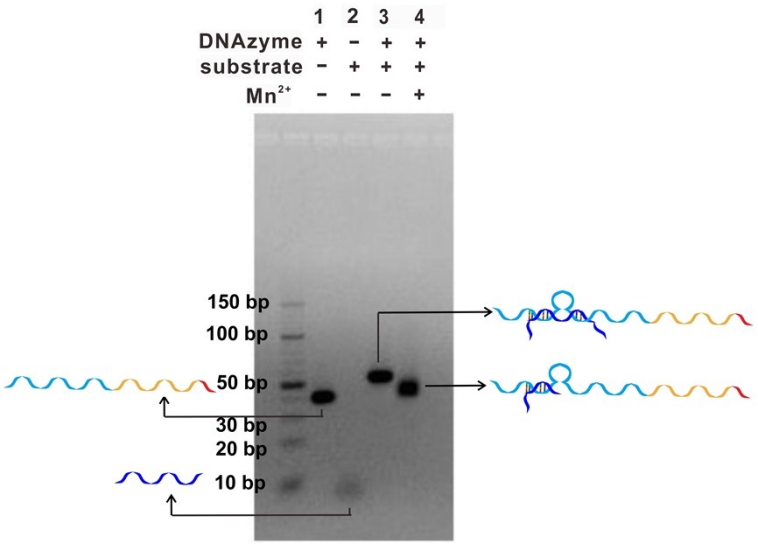

Figure S4. The characterization of MnO_2 nanosheets, (A) AFM, (B) The height profile for the labeled-section with the white line in AFM, (C) TEM, (D) UV-vis adsorption spectrum.

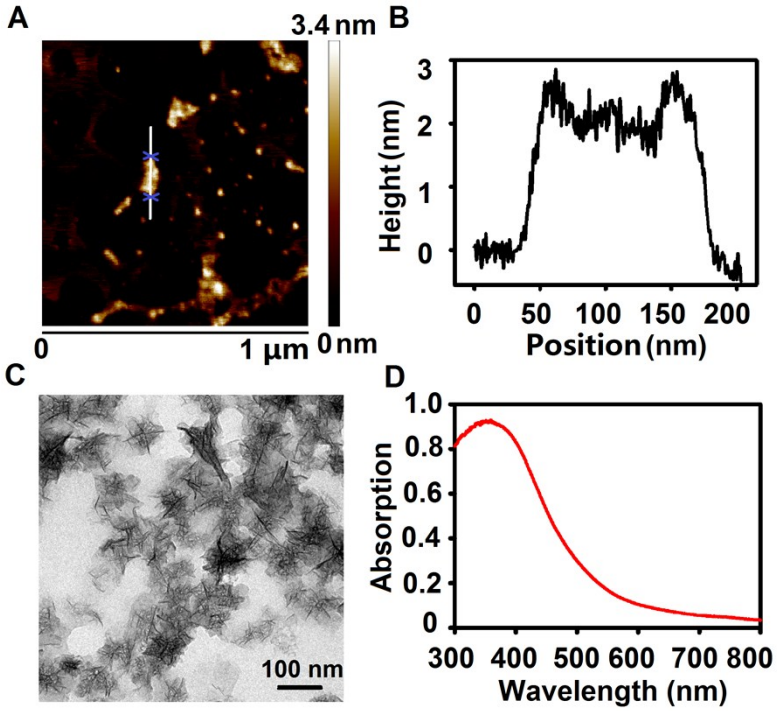

Figure S5. The characterization of DNA-MnO_2 nanodevice. (A) The fluorescence spectral of DNA duplex probes after adsorbing with different concentrations of MnO_2 nanosheets. (B) The fluorescence quenching efficiency of MnO_2 nanosheets with different concentration. (C) Zeta potential of MnO_2 nanosheets (blue) and DNA-MnO_2 nanodevice (red). (D) The dynamic light scattering (DLS) measurement of MnO_2 nanosheets (blue) and DNA-MnO_2 nanodevice (red). (E) Fluorescence-based stability of DNA-MnO_2 nanodevice (black line), compared with free probes (red line) in the presence of endonuclease DNase I.

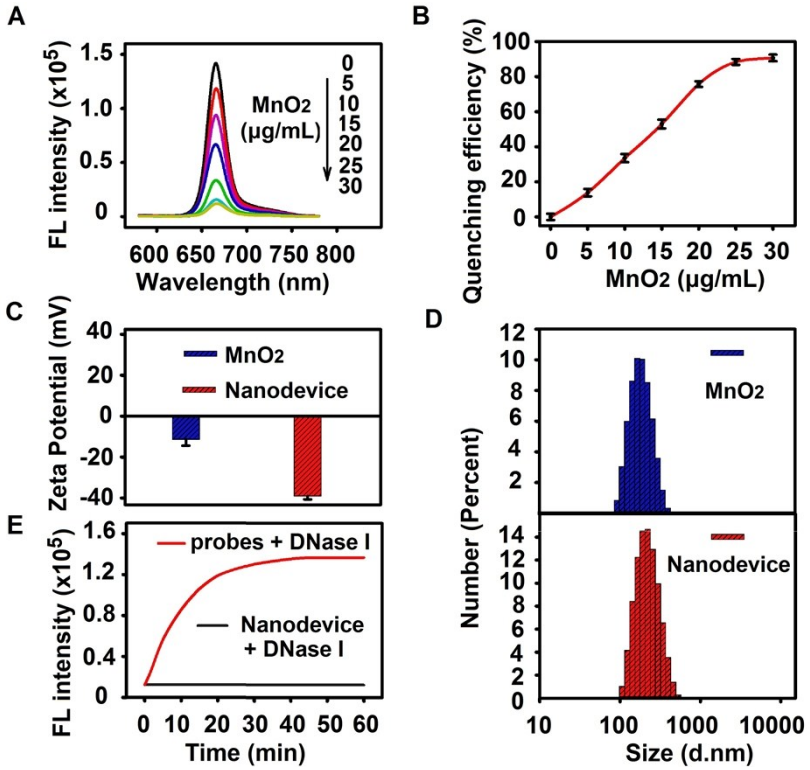

Figure S6. The UV-vis absorption spectra of MnO_2 nanosheets after degradation with GSH.

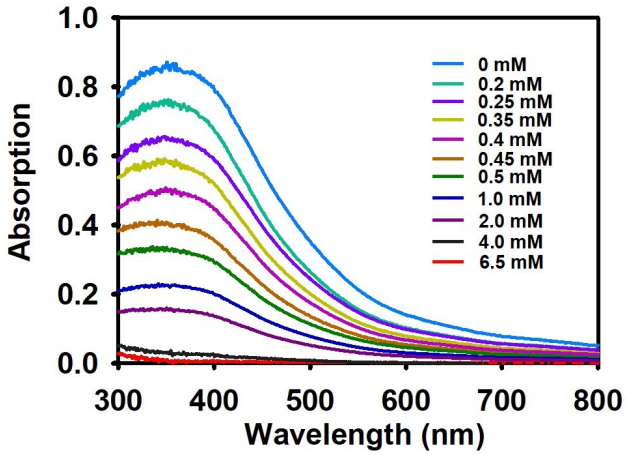

Figure S7. Fluorescence spectra responses of the substrate after different treatment. When the substrate was mixed with DNAzyme and MnO_2 nanosheets, an obvious fluorescence enhancement was observed after introduction with GSH (6.5mM GSH was added into the mixture of 50 nM DNAzyme, 50 nM substrate and 25 μg/mL MnO_2 nanosheets).

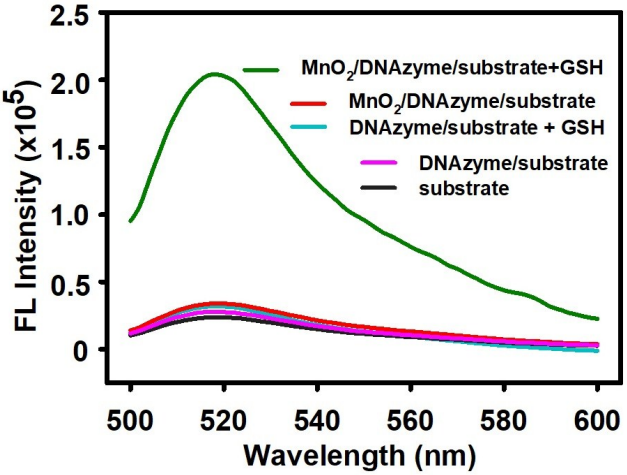

Figure S8. The CCK-8 assay of MnO_2 nanosheets. Hela cells were incubated with different concentrations of MnO_2 nanosheets for 48 h. Error bars were estimated from three replicate measurements.

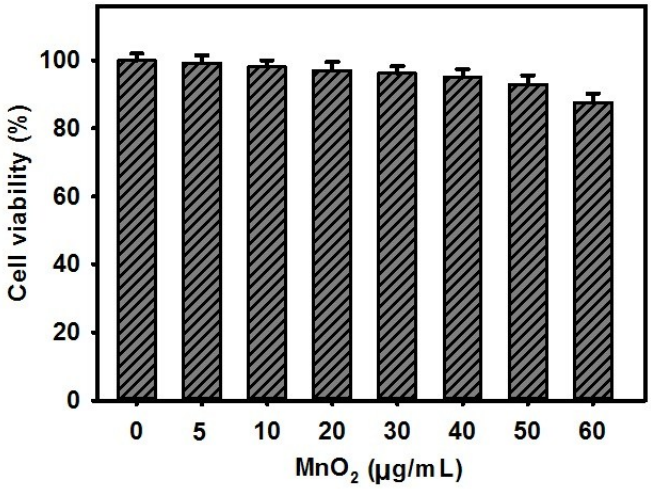

Figure S9. The DNA-MnO_2 nanodevice in response to the different concentrations of telomerase in living cells. (A) Fluorescence imaging for HeLa cells treated with different concentrations of telomerase inhibitor EGCG followed by incubation with DNA-MnO_2 nanodevice. Scale bar: 20 μm. (B) The histogram of the mean fluorescence intensity corresponding to the former fluorescence imaging. (C) The corresponding flow cytometric assays of the former fluorescence imaging.

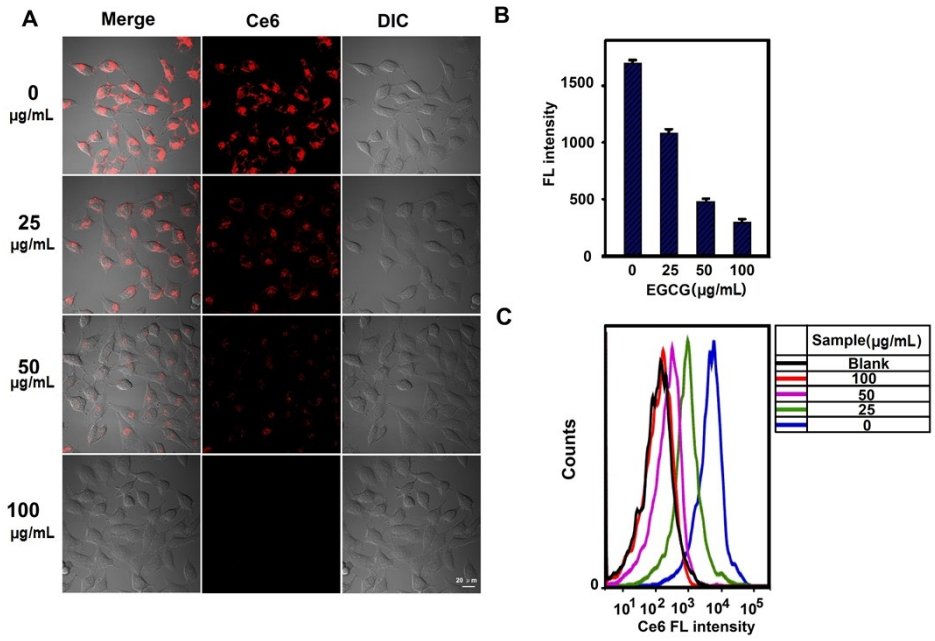

Figure S10. The DNA-MnO_2 nanodevice in response to the different living cells. (A) Fluorescence imaging of the different cells after incubation with DNA-MnO_2 nanodevice. Scale bar: 20 μm. (B) The histogram of the mean fluorescence intensity corresponding to the former fluorescence imaging. (C) The flow cytometric for different cells before (black line) and after (red line) incubated with the DNA-MnO_2 nanodevice.

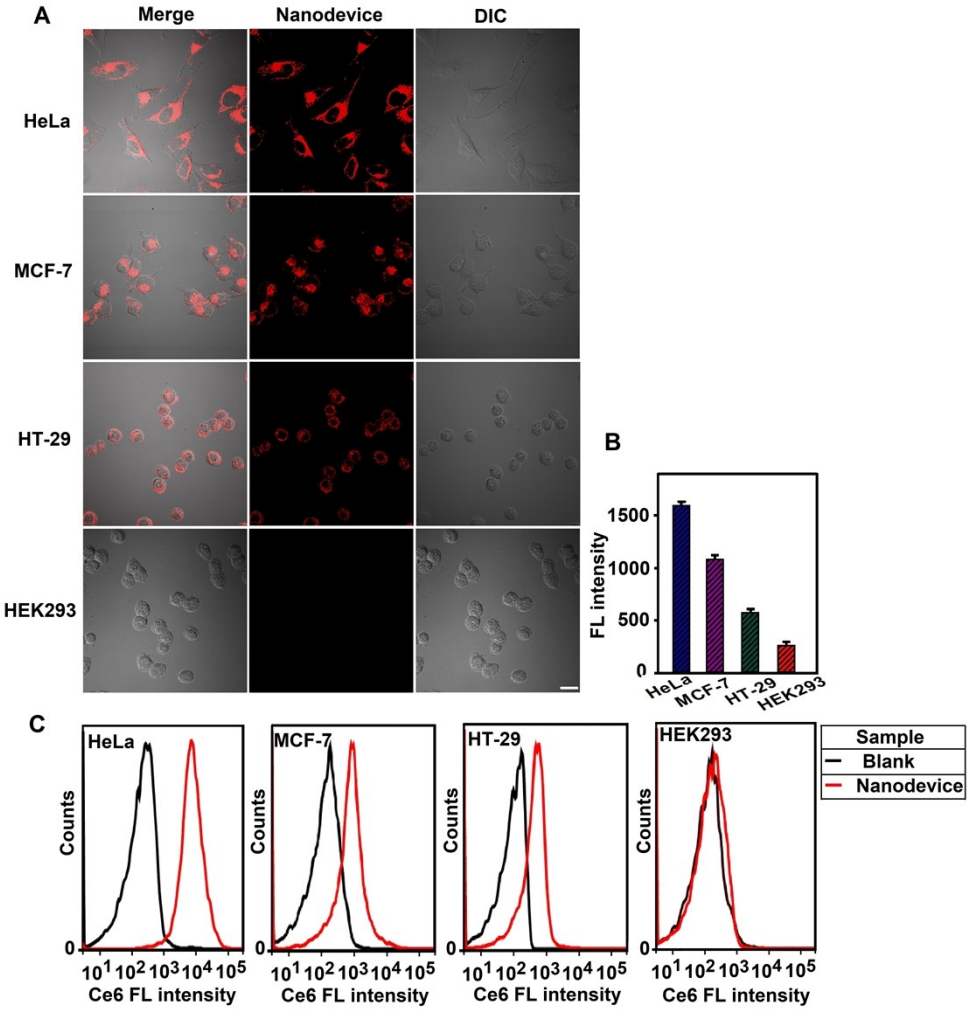

Figure S11. The study of hypoxia in HeLa cells. (A) Fluorescence imaging of the hypoxia in the HeLa cells before (blank) and after (MnO_2) incubated with MnO_2 nanosheets, using Green Hypoxia Reagent. Scal bar: 20 μm. (B) The histogram of the mean fluorescence intensity corresponding to the former fluorescence imaging.

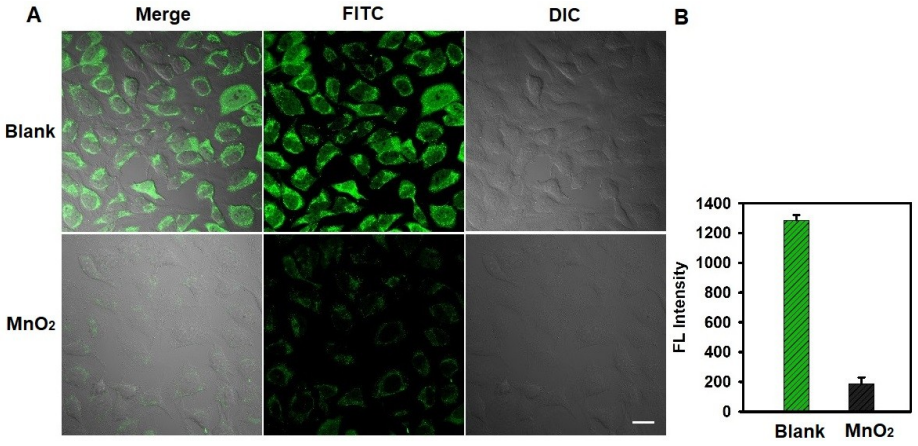

Figure S12. The study of ROS in HeLa cells. (A) Fluorescence imaging of the ROS in the HeLa cells after (MnO_2) and before (blank) incubated with MnO_2 nanosheets, using CellROX® Green Reagent. Scal bar: 20 μm. (B) The histogram of the mean fluorescence intensity corresponding to the former fluorescence imaging.