Electronic Supplementary Information (ESI)

Tumor-selective catalytic nanosystem for activatable theranostics

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Reagents and Materials. Graphene oxide (GO) was supplied by Xianfeng Nanomaterials Co. Ltd. (Nanjing, China). Silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), sodium hypochlorite solution (NaClO), ferrous sulfate ($FeSO_4$) and hydrogen peroxide (H_2O_2, 30%, w/w) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). *tert*-Butyl hydroperoxide (TBHP), 3-morpholinosydnonimine hydrochloride (SIN-1), xanthine, cytochrome C, xanthine oxidase, Phorbol 12-myristate 13-acetate (PMA), N-acetyl-L-cysteine (NAC), 5, 5-dimethyl-1-pyrroline N-oxide (DMPO) and intracellular hydrogen peroxide assay kits were bought from Sigma-Aldrich (St. Louis, Mo, USA). CellTiter96® AQueous One Solution Cell Proliferation Assay kit was purchased from Promega (Madison, USA). Annexin V-FITC/PI apoptosis detection kit was purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, China). All other chemicals used in this work were of analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). The HPLC-purified and lyophilized DNA probes used in this work were provided by Sangon Biotech Co., Ltd. (Shanghai, China). Human serum samples were obtained from the Third Xiangya hospital (Changsha, China). Ultrapure water (electric resistance > 18.3 MΩ) obtained through a Millipore Milli-Q water purification system (Billerica, MA, USA) was used throughout the experiments.

Generation of ROS. $^1O_2$ was obtained by the addition of NaClO to H_2O_2 at a molar ratio of 10:1. ClO^-, $ONOO^-$ and •OtBu were separately supplied by NaClO, SIN-1 and TBHP. For the preparation of O_2^-, 500 μL xanthine (10 μM) was added to 500 μL cytochrome C (10 μM) and xanthine oxidase (1U/L) mixture at 37 °C for 20 min. Hydroxyl radical (•OH) was generated through ferrous ions mediated Fenton reaction by mixing with H_2O_2 at a molar ratio of 1:10 at 37 °C for 30 min.

Synthesis of TAMRA-DNA-templated AgNPs on GO nanosheets. The synthesis of TAMRA-DNA-templated AgNPs was accomplished through reduction reaction of $AgNO_3$ with $NaBH_4$. 1 μM DNA-1 (sequence: 5'-AAT GTG CTC CCC CAG CGC GCT T-(TAMRA)-3') and 1 μM DNA-2 (sequence: 5'-TGG GGG AGC ACA TT-3')

were mixed together to obtain duplex TAMRA-DNA by hybridization in 10 mM HEPES buffer (50 mM $NaNO_3$, pH 7.5) for 30 min. GO nanosheets at a final concentration of 20 μg/mL was then added to the duplex TAMRA-DNA solution for adsorption of duplex TAMRA-DNA on the surface of GO. After incubation for 30 min, the mixture was centrifuged, and excess TAMRA-DNA in the supernatant was removed. After being washed by 10 mM HEPES buffer for several times, the precipitated TAMRA-DNA@GO was redispersed in 1 mL of 10 mM HEPES buffer, followed by addition of $AgNO_3$ to the TAMRA-DNA@GO mixture under vigorous stirring (final concentration of $AgNO_3$ was 100 μM). After incubation for 5 min, 500 μM freshly prepared reducing agent solution of $NaBH_4$ was injected constantly into the mixture containing $AgNO_3$, TAMRA-DNA, and GO over 30 min under vigorous stirring. The resultant mixture was washed twice with 10 mM HEPES buffer and centrifuged at 12000 rpm for 15 min to remove free AgNPs. The final products were redispersed in 10 mM HEPES buffer for further characterization and use.

Preparation of the Serum Samples. Briefly, 20 μL serum was diluted with 20 μL double-distilled water and then 500 μL 80 mM $Ba(OH)_2$ and 500 μL 80 mM $ZnSO_4$ were added and mixed. The supernatant solutions were collected by centrifugation at 4000 rpm for 15 min and used for further experiments.

Instruments and Characterization. The fluorescence measurements were carried out on a FluoroMax-4 spectrofluorometer (HORIBA, NJ, USA) and the fluorescence emission spectra of TAMRA were collected from 550 nm to 660 nm at room temperature with an excitation wavelength of 530 nm. The transmission electron microscope (TEM) images and energy dispersive X-ray spectroscope (EDS) spectrum were obtained on a field-emission high-resolution 2100F TEM (JEOL, Japan) at an acceleration voltage of 200 kV. Agarose gel electrophoresis was visualized via a Tanon 4200SF gel imaging system (Tanon Science & Technology Co., Ltd., China). The X-ray photoelectron spectroscopy (XPS) analysis was performed using an X-ray photoelectron spectroscope (Thermo Fisher Scientific, USA). Electron spin resonance

(ESR) spectrum was measured by JEOL JES-FA200 spectrometer (JEOL, Japan). The Raman spectra were recorded using a Renishaw invia-reflex Raman microscope (Renishaw, England) with an excitation at 633 nm HeNe laser, and the corresponding laser was focused into the center of sample using a 1 mm slit width and 50× long working distance objective. The MTT assays were operated on the ELx800 Microplate Reader (BioTek, USA).

***In vitro* Detection of H_2O_2.** For the detection of H_2O_2, as-prepared AgNPs-TAMRA-DNA@GO (final concentration of 10 μg/mL) were mixed with different concentrations of H_2O_2 in 10 mM phosphate buffer (pH 6.0). The resulted solutions were incubated at 37 °C for 1 h to perform the reaction. Afterwards, the fluorescence spectra were acquired under the excitation at 530 nm.

Fluorescence Imaging of Living Cells. HeLa and L02 cells were cultured in the RPMI 1640 medium supplemented with 10% FBS, 100 IU/mL penicillin and 100 IU/mL streptomycin in a humidified atmosphere containing 5% CO_2.

For fluorescence imaging of H_2O_2 in living cells, HeLa and L02 cells were plated on 35-mm Petri dish with 10-mm bottom well in the culture medium for 24 h. To monitor endogenous H_2O_2, cells were stimulated with or without 5 μM PMA or 1.0 mM NAC at 37 °C for 1 h, followed by incubation with AgNPs-TAMRA-DNA@GO (20 μg/mL) or commercial H_2O_2 detection kits or TAMRA-DNA@GO (20 μg/mL) for 2 h, afterwards, the cells were subjected to fluorescence imaging after washing twice with PBS. For the investigation of time-dependent fluorescence imaging of intracellular H_2O_2, confocal microscopy images were acquired for a specified time (from 0 h to 3 h) after the incubation with 20 μg/mL AgNPs-TAMRA-DNA@GO at 37 °C. All fluorescence images were acquired on a Nikon TI-E+A1 SI confocal laser scanning microscope equipped with an oil immersion 60× objective. Under the excitation of 560 nm, the fluorescence signals were captured in red channel (570-620 nm).

For flow cytometric analysis of H_2O_2 in living cells, HeLa and L02 cells were

plated on 35 mm Petri dish at 1.0×10^6 cells in the culture medium and cultured for 24 h. Cells were pretreated with or without 5 μM PMA or 1.0 mM NAC for 1 h, followed by incubation with AgNPs-TAMRA-DNA@GO (20 μg/mL in the culture medium) for another 2 h, the culture medium were removed and washed twice with PBS, then, the cells were digested with 0.25% trypsin and redispersed in the culture medium. Flow cytometric analysis were performed on a FACScan cytometer (Beckman Coulter, USA).

Cellular Viability Assay. For cellular viability assay, HeLa and L02 cells were seeded in 96-well plates at 1.0×10^5 cells per well and incubated at 37 °C in 5% CO_2 atmosphere for 48 h. Then, the cells were treated with a given concentration of GO, TAMRA-DNA@GO and AgNPs-TAMRA-DNA@GO in RPMI 1640 medium supplemented with 10% FBS. After incubation at 37 °C for 24 h, 20 μL of CellTiter 96® AQueous One Solution Reagent was pipetted into each well of the 96-well plate containing 100 μL of culture medium. Subsequently, the plate were placed at 37 °C for 2 h in a humidified, 5% CO_2 atmosphere, the absorbance at 490 nm were recorded using a ELx800 Microplate Reader (BioTek, USA).

Apoptosis Analysis. For the apoptosis assay, HeLa and L02 cells were seeded in a 35-mm Petri dish at 1.0×10^6 cells in 2 mL of complete RPMI 1640 medium and cultured for 24 h. Subsequently, the culture medium were removed and washed twice with PBS, the cells were separately treated with GO, TAMRA-DNA@GO and AgNPs-TAMRA-DNA@GO for 2 h, HeLa and L02 cells without the treatment were used as control. Then the cells were harvested with 0.25% trypsin without containing EDTA and resuspended in 195 μL of 1×binding buffer. Afterwards, 5 μL of Annexin V-FITC and 10 μL of PI were added, and the cells were incubated in the dark for 15 min at room temperature. The fluorescence-activated cell sorter analysis (FACS) were analyzed by flow cytometry (Beckman Coulter, USA). The data was analyzed by CytExpert software (Beckman Coulter, USA).

Scheme S1 Illustration of synthesis of AgNPs-TAMRA-DNA@GO nanoprobe and H_2O_2-triggered Fenton-like reaction for lighting up fluorescence in vitro.

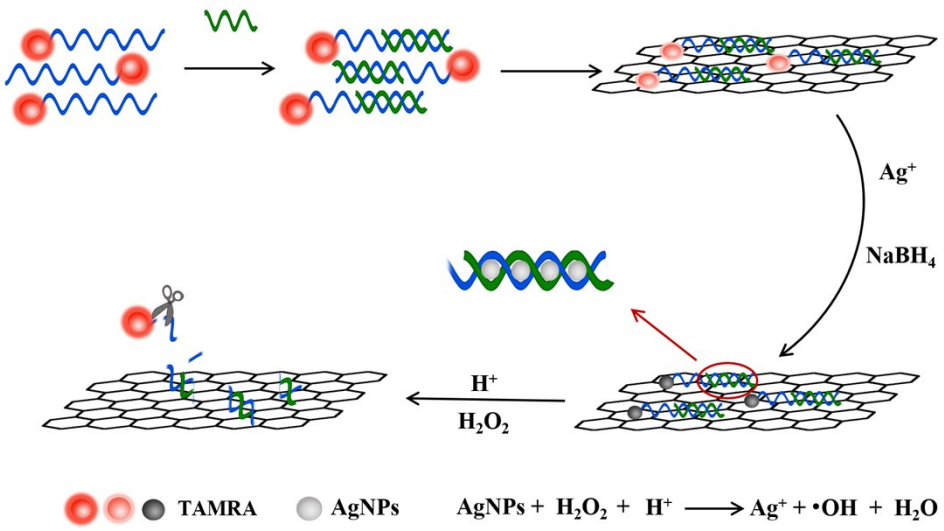

Fig. S1 Energy dispersive X-ray spectroscope (EDS) spectrum of AgNPs-TAMRA-DNA@GO. Peaks from Cu and Si were attributed to the copper grid that was employed to prepare EDS sample and glassware, respectively.

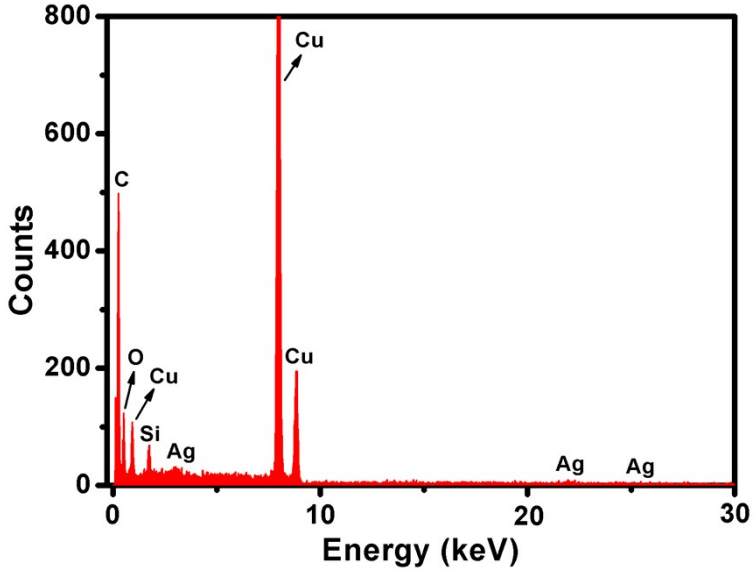

Fig. S2 X-ray photoemission spectroscopy (XPS) spectrum of AgNPs-TAMRA-DNA@GO.

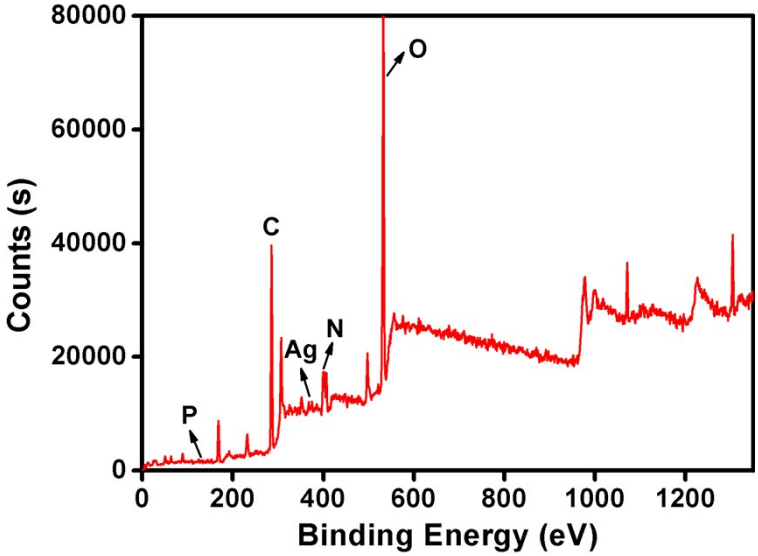

Fig. S3 Raman spectra of TAMRA-DNA@GO (black) and AgNPs-TAMRA-DNA@GO (red).

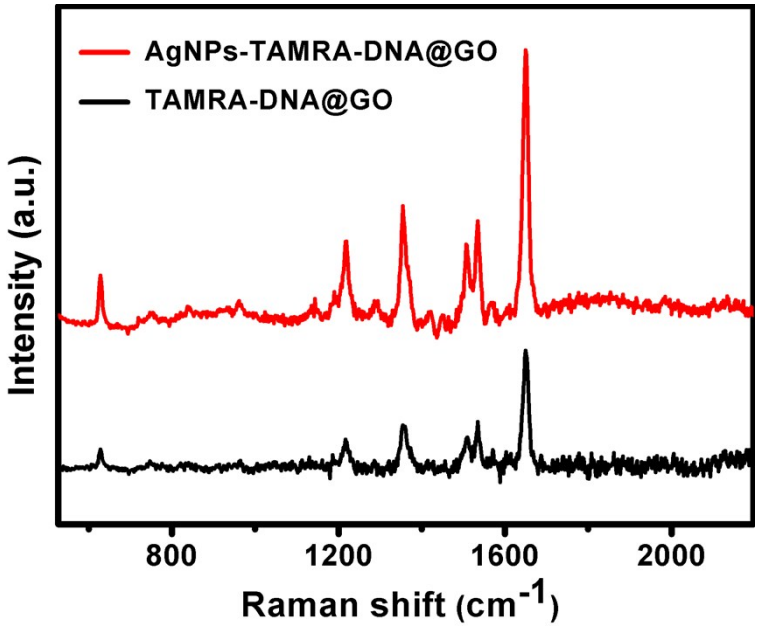

Fig. S4 TEM image of AgNPs@GO in the absence of TAMRA-DNA template.

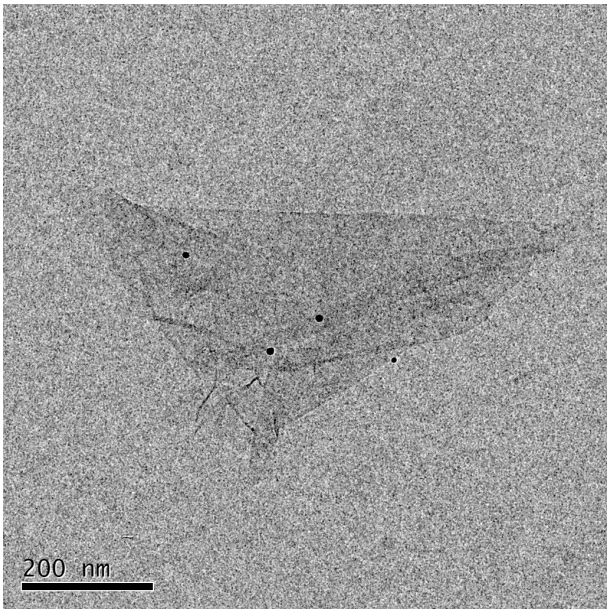

Fig. S5 Gel electrophoresis of reaction products of AgNPs-TAMRA-DNA@GO. M, DNA marker; lane 1, TAMRA-DNA@GO; lane 2, TAMRA-DNA@GO + 0.4 mM H_2O_2; lane 3, TAMRA-DNA@GO + 100 nM Ag^+ + H_2O_2; lane 4, AgNPs-TAMRA-DNA@GO + H_2O_2.

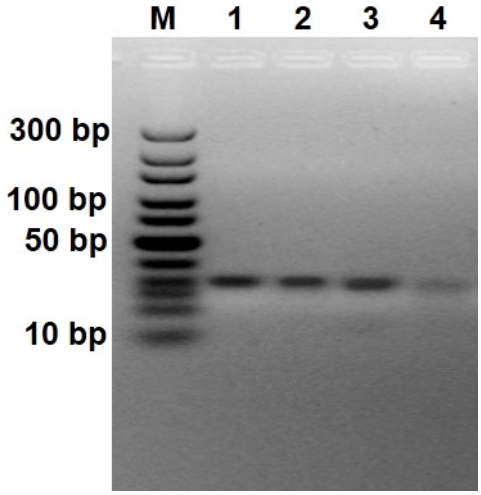

Fig. S6 X-ray photoemission spectroscopy (XPS) analysis of AgNPs-TAMRA-DNA@GO before (top) and after (bottom) reaction with 0.4 mM H_2O_2. The raw XPS spectra, Ag $3d_{5/2}$ (left) and Ag $3d_{3/2}$ (right), could be deconvolved into two distinct components corresponding to the binding energies for Ag(0) (red) and Ag(I) (green). The deconvolved Ag $3d_{5/2}$ spectra gave two binding energies of 367.5 eV and 368.2 eV, which corresponded to Ag(0) and Ag(I), respectively. The intensities of these two peaks indicated that the AgNPs-TAMRA-DNA@GO contained ~72.43% Ag(0) and ~27.57% Ag(I) before the reaction, while the percentage of Ag(I) increased to ~75.25% after the reaction with H_2O_2.

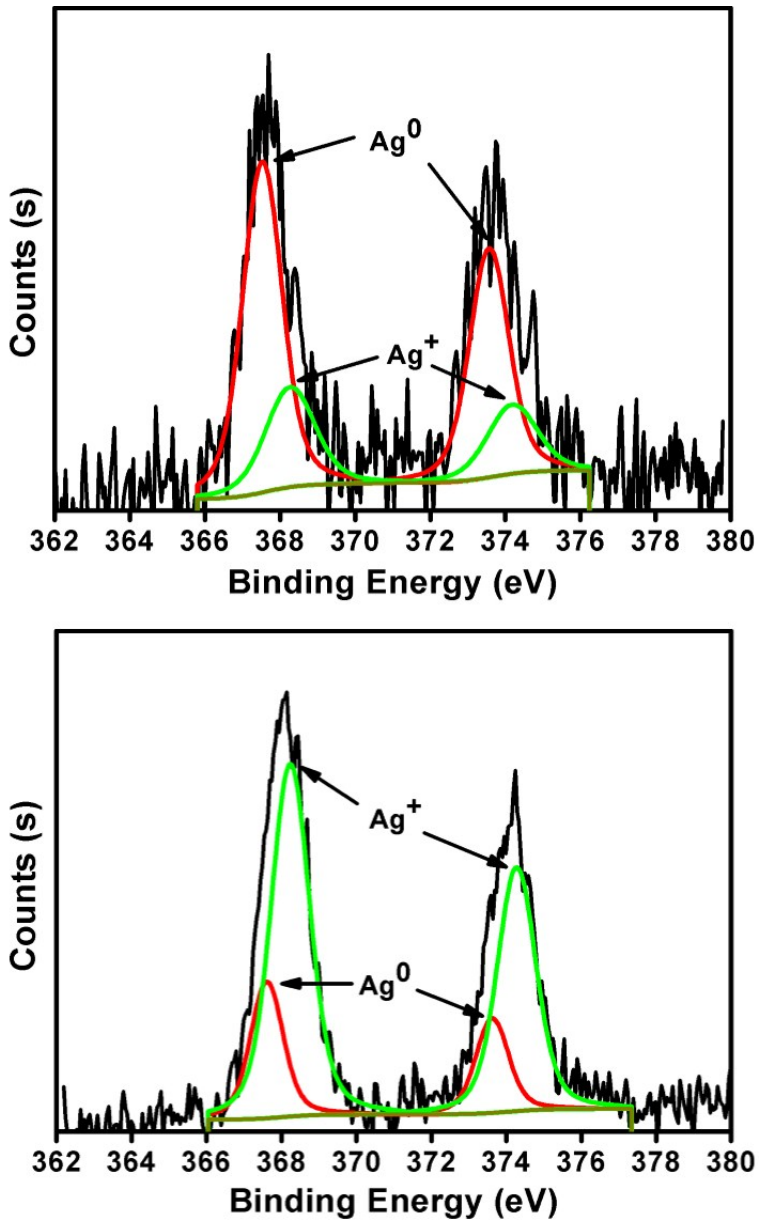

Fig. S7 Fluorescence assay for evaluating the stability of AgNPs-TAMRA-DNA@GO nanosystem in 10% human serum. Inset photographs showed corresponding solutions of AgNPs-TAMRA-DNA@GO mixed with 10% human serum for 1, 2, 3 and 4 weeks, respectively.

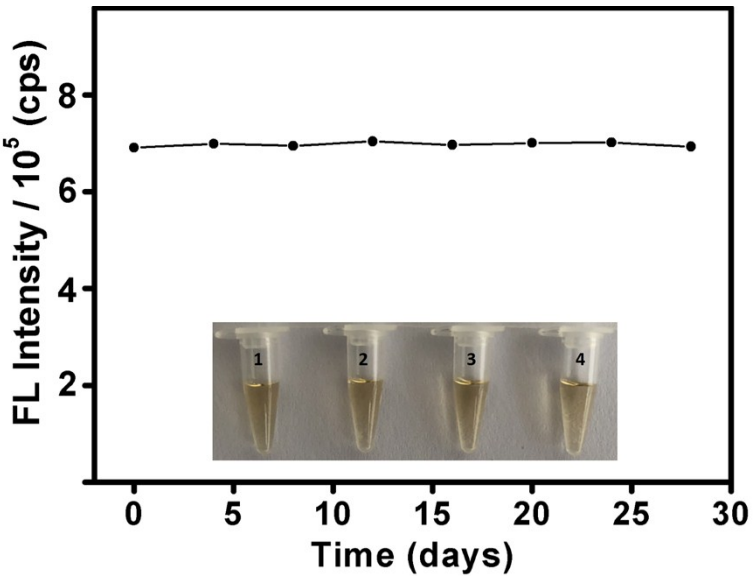

Fig. S8 Fluorescence assay of 50 nM TAMRA-DNA in the different concentration of H_2O_2.

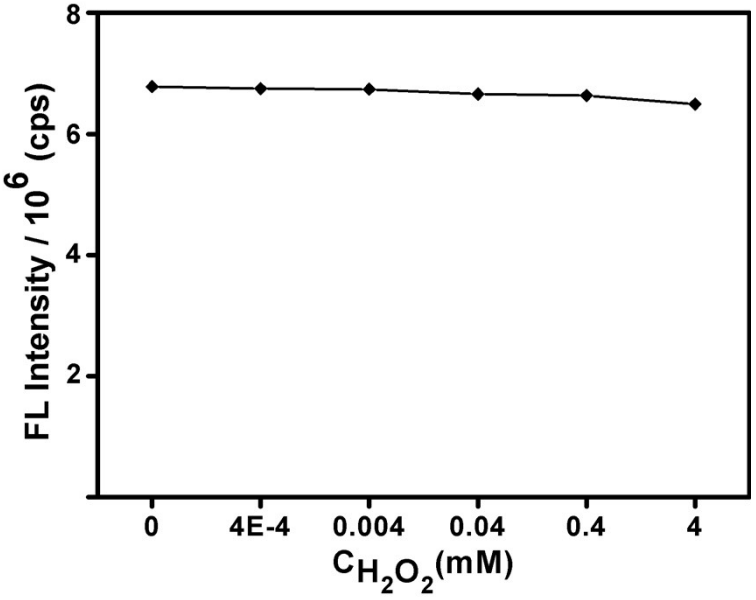

Fig. S9 Fluorescence responses of the AgNPs-TAMRA-DNA@GO nanosystem with the addition of 0.4 mM H_2O_2 at different pH. F and F_0 correspond to fluorescence intensities at 580 nm with excitation at 530 nm obtained in the presence or absence of H_2O_2, respectively.

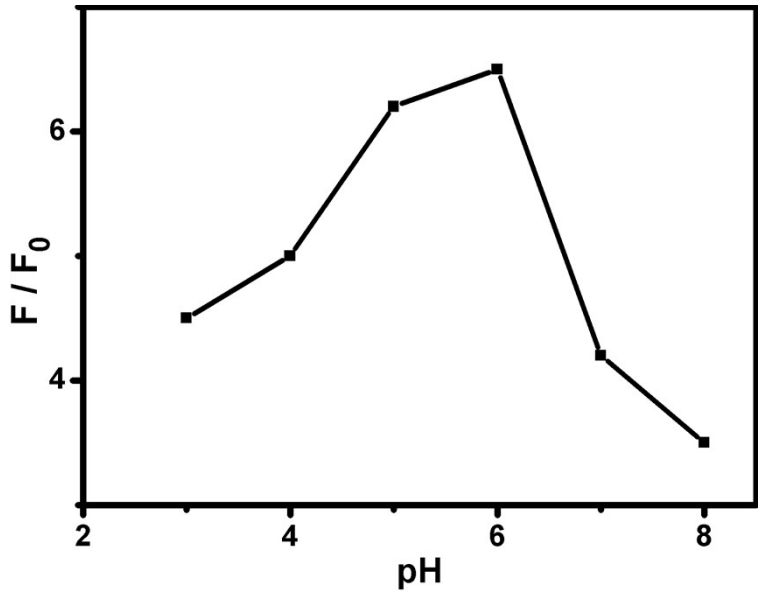

Fig. S10 (A) Fluorescence spectra of AgNPs-TAMRA-DNA@GO towards H_2O_2 of varying concentrations. (B) The fluorescence intensities at 580 nm versus different concentrations of target H_2O_2. Inset: The linear relationship between fluorescence peak intensities and H_2O_2 concentrations. Error bars indicated standard deviations of three repetitive experiments.

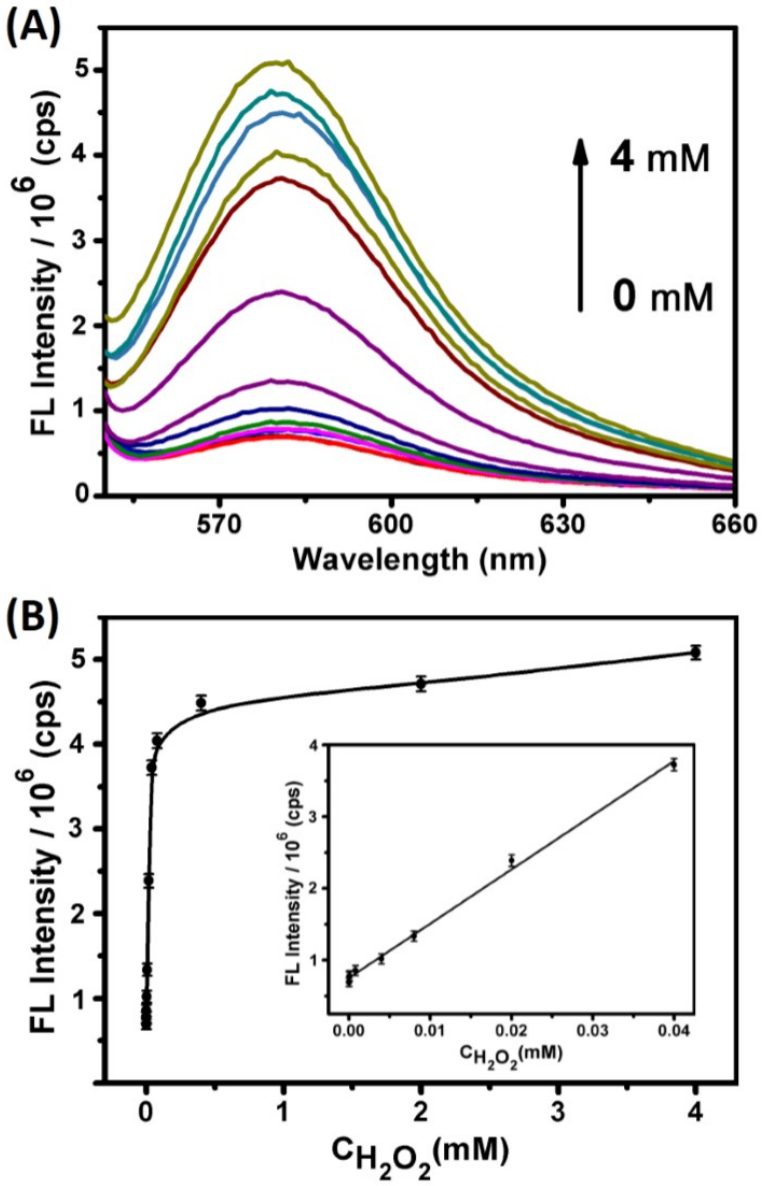

Fig. S11 Real-time fluorescence monitoring of the peak intensity at 580 nm of the AgNPs-TAMRA-DNA@GO nanosystem in the absence (a) or presence (b) of 0.04 mM H_2O_2, respectively.

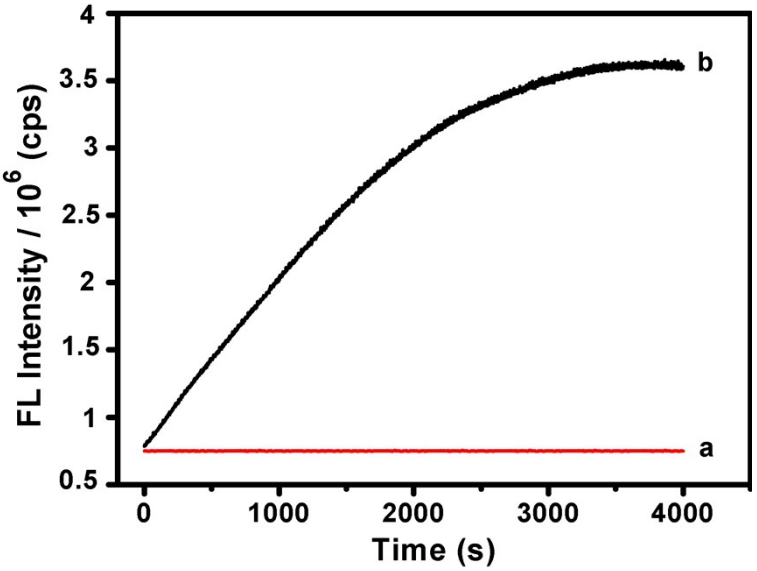

Fig. S12 Fluorescence responses of AgNPs-TAMRA-DNA@GO to H_2O_2 and ·OH (0.4 mM), as well as various ROS (2.0 mM). All reactions were performed in phosphate buffer (10 mM, pH 6.0) with 10 μg/mL AgNPs-TAMRA-DNA@GO at 37 °C. F and F_0 indicated fluorescence intensities at 580 nm with excitation at 530 nm acquired in the presence or absence of ROS, respectively.

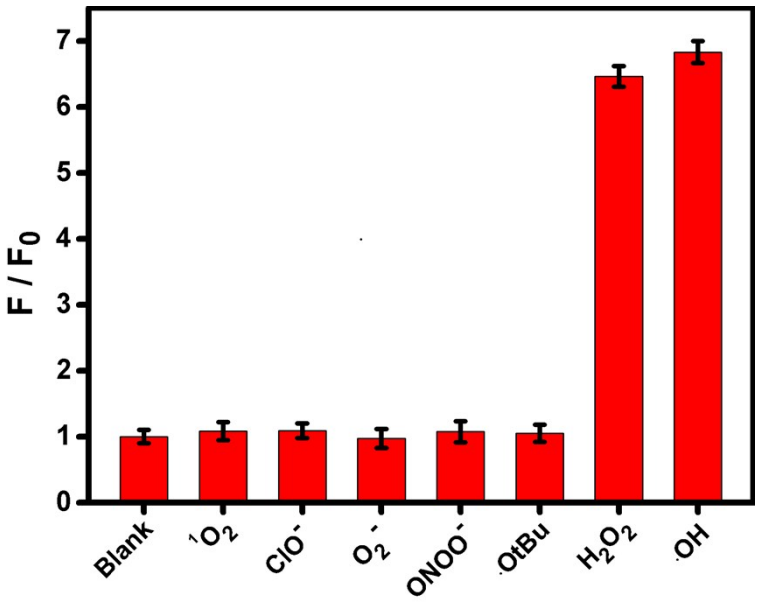

Fig. S13 (A) Confocal fluorescence images of intracellular H_2O_2 in HeLa and L02 cells with AgNPs-TAMRA-DNA@GO or commercial H_2O_2 detection kits. Before imaging, all cells were incubated with AgNPs-TAMRA-DNA@GO (20 μg/mL) or commercial H_2O_2 detection kits for 2 h. (B) The bar graph was the quantification of relative fluorescence intensity of these cells normalized to the HeLa cells treated with AgNPs-TAMRA-DNA@GO. (C) Flow cytometric assay of cells incubated with AgNPs-TAMRA-DNA@GO.

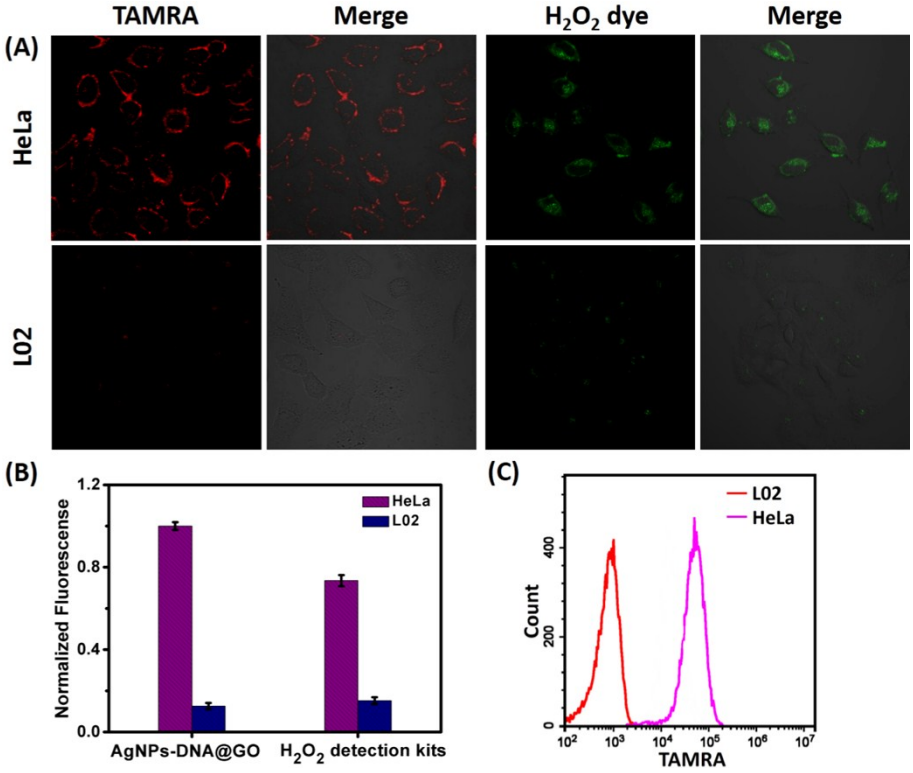

Fig. S14 Confocal fluorescence images of TAMRA-DNA@GO (20 μg/mL) incubated HeLa and L02 cells.

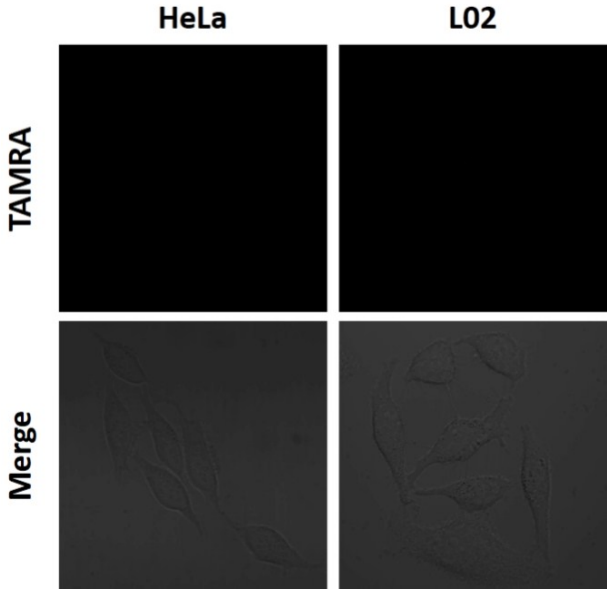

Fig. S15 (A) Confocal images of H_2O_2 in HeLa cells. (B) Normalized bar graph of relative fluorescence intensities of HeLa cells treated with AgNPs-TAMRA-DNA@GO. (C) Flow cytometric detection of intracellular H_2O_2. Control cells incubated with AgNPs-TAMRA-DNA@GO (green), cells pretreated with PMA followed by incubation with AgNPs-TAMRA-DNA@GO (pink), and cells pretreated with NAC followed by incubation with AgNPs-TAMRA-DNA@GO (red).

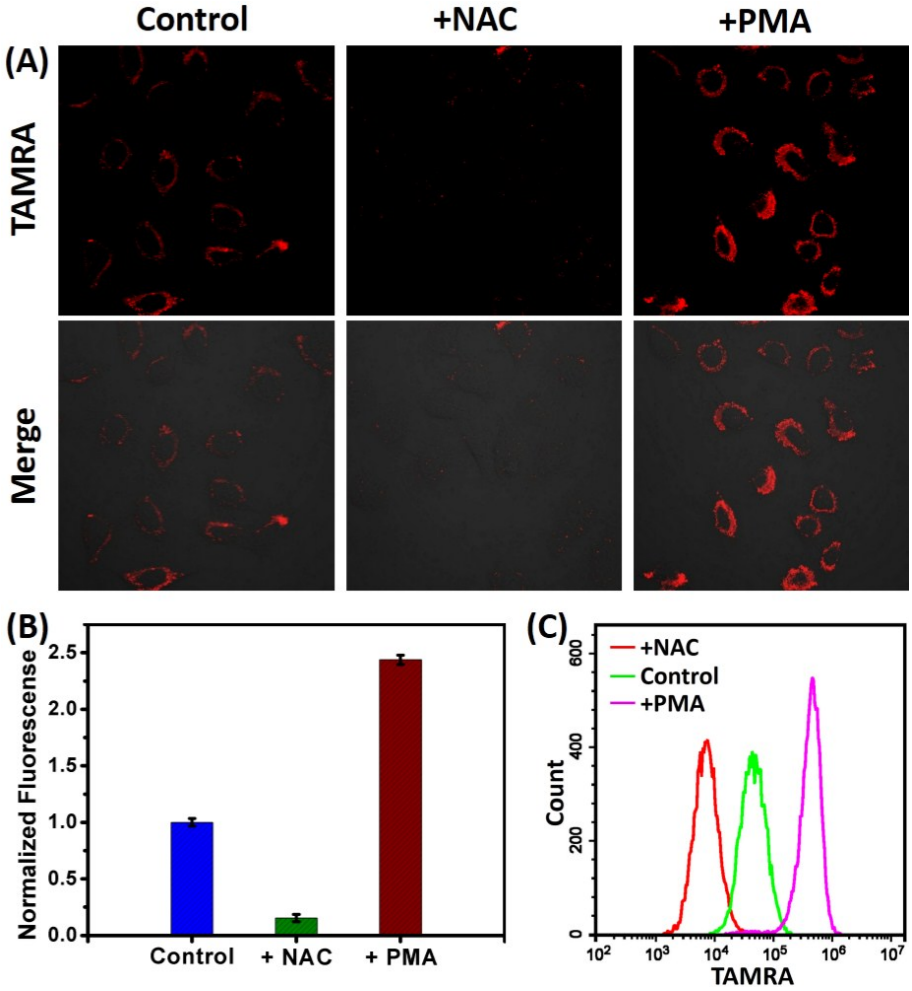

Fig. S16 Real-time confocal fluorescence imaging of HeLa cells incubated with 20 μg/mL AgNPs-TAMRA-DNA@GO.

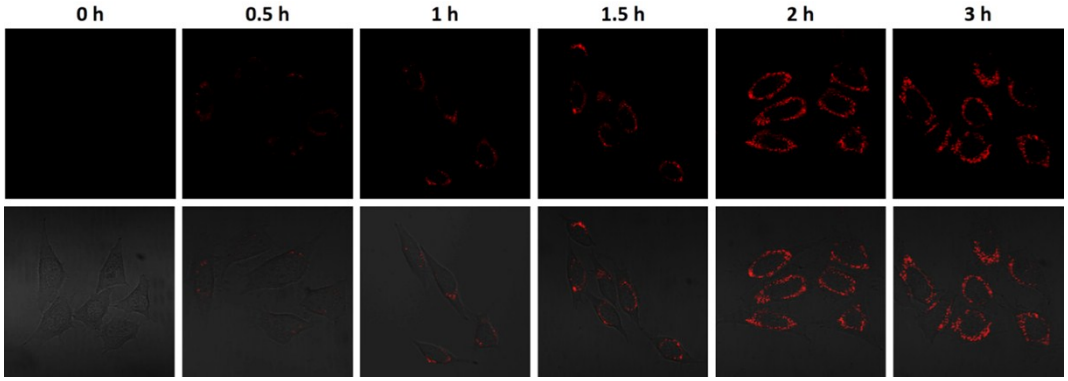

Fig. S17 Flow cytometry analysis of L02 and HeLa cells apoptosis with a combination incubation of Annexin V-FITC and PI after GO, TAMRA-DNA@GO and AgNPs-TAMRA-DNA@GO treatment.