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Supplementary Materials for

A novel nanoprobe for visually investigating the controversial role of miRNA-34a as oncogene or tumor suppressor in cancer cells

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1. Reagents and Instruments

1.1 Reagents. Tris (hydroxymethyl) aminomethane (Tris), dimethyl sulfoxide (DMSO), $CaCl_2$, NaCl, $MgCl_2$, ethanol was purchased from China National Pharmaceutical Group Corporation (Shanghai, China); Dopamine hydrochloride and lipopolysaccharide (LPS) were purchased from Solarbio company (Beijing, China); Genistein was purchased from Aladdin Biochemical Technology company (Shanghai, China). Caspase-3 was purchased from Abcam company; 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich. DMEM, fetal bovine serum, antibiotics penicillin/streptomycin, trypsin were purchased from Biological Industries; Glutathione, hemoglobin and bovine serum albumin were purchased from Sangon Biotech (Shanghai, China); Phosphate buffer solution (PBS), A549 (human lung cancer cell line), HepG-2 (human liver cancer cell line), Hela (human cervical cancer cell line) and MCF-7 (human breast cancer cell line) were purchased from Procell (Wuhan, China); Caspase-3 activity and Annexin V apoptosis detection kit for live cell was purchased from Beyotime (Shanghai, China). All aqueous solutions were prepared using ultrapure water of 18.2 mΩ•cm.

1.2 Instruments. The ultraviolet-visible absorption spectra (UV-vis) were recorded with UV-visible spectrometer (UV 1920, Shanghai Prism Technology Co., Ltd.); All fluorescence spectra were collected on a fluorescence spectrometer (F97 Pro, Shanghai Prism Technology Co., Ltd.); Absorbance of MTT assays was recorded by a microplate reader (Thermo Fisher Scientific); All pH measurements were measured with a digital pH-meter (pH-3c, Shanghai LeiCi, China). Transmission electron microscope (TEM) images were taken from the emission of a JEM-2100 transmission electron microscope; Scanning electron microscopy (SEM) images were acquired using a Regulus 8220 field-emission scanning electron microscope; Fourier transform infrared (FT-IR) spectra were obtained on a Nicolet 10 spectrometer (Thermo Fisher, Waltham, MA, USA); Confocal fluorescence images were accomplished with a confocal laser scanning microscopy (Leica SP8, Germany); Flow cytometry analysis was performed on BD FACSCalibur (BD Bioscience, USA).

1.3 Synthesis of oligonucleotides and peptide

All oligonucleotides used in this study were purified by HPLC and synthesized by Shanghai Sangon

Biological Engineering Co., Ltd. (Shanghai, China). All peptides were synthesized and purified by Kingsray Biotechnology Co., Ltd. (Jiangsu, China). The DNA and peptide sequences used in the experiment are shown in Table S1.

Table S1. Sequences of oligonucleotides and peptide employed in this work.

Oligonucleotide	Sequences (from 5′ to 3′)
MB	5′-Texas Red-CGTGCACAACCAGCTAAGACACT GCACG-3′
miRNA-34a	5′-GCAGTGTCTTAGCTGGTTGT-3′
mismiRNA-34a	5′-GCAGTGTCTTATCTGGTTGT-3′
peptide	Cy5-Ahx-Gly-Gly-Asp-Glu-Val-Asp-Gly-Gly-Cys
miRNA-67	5′-TCTGCACACCTCTTGACACTCCG-3′
miRNA-21	5′-TAGCTTATCAGACTGATGTTGA-3′
miRNA-221	5′-AGCTACATTGTCTGCTGGGTTTC-3′

1.4 Investigation the stability of nanoprobes

To study the stability of the nanoprobes, the fluorescence signal changes of the two dyes in different pH solutions were first investigated. The prepared nanoprobes were dispersed in Tris-HCl solutions with different pH (2, 3, 7, 7.4, 8, 9, 10) solutions and incubated at room temperature for 6 h. Secondly, the two fluorescence signals of the nanoprobes were detected after incubating the nanoprobes with cell culture medium for different time (0, 4, 24, 48, 72 h). The fluorescence intensities of Texas Red were collected with $\lambda_{ex}/\lambda_{em}$ = 595/615 nm and the fluorescence intensities of Cy5 were collected with $\lambda_{ex}/\lambda_{em}$ = 650/670 nm.

1.5 Cell culture

All cells used in the experiment were cultured in DMEM (1% antibiotics penicillin/streptomycin and 10% fetal bovine serum) and incubated at 37°C in a 5% CO_2 humidified incubator.

1.6 MTT assay

In order to study the biological toxicity of the nanoprobes, MTT assay was carried out to detect the cell viability of the nanoprobes in four cell lines A549, HepG-2, Hela and MCF-7 cells. The cells were seeded in a 96-well plate at a density of 10^6 cells/well and cultured for 24 h. Afterwards,

nanomaterials (0, 2, 4, 6, 8, 10 μg/mL) were added to the cells and further incubated for 6 h, respectively. Then, the cell medium was discarded and 10 μL of MTT solution (5 mg/mL) was added to each well for 4 h. The formed formazan crystals were dissolved in 150 μL of dimethyl sulfoxide (DMSO). The absorbance at a wavelength of 490 nm was measured on a Multiskan FC. Cell viability was calculated based on the absorbance normalized to the data from untreated cells.

1.7 Apoptosis kit detection

To further investigate the apoptosis of A549, HepG-2, Hela and MCF-7 cells after treatment with different drugs, we selected the apoptosis kit (Caspase-3 activity and Apoptosis Detection Kit for Live Cell) to stain the four cell lines and the cell apoptosis was analyzed by CLSM. Each type of cells was divided into three groups in parallel. Group I was the untreated control group; group II and III were treated with genistein (0.1 μM) LPS (10 μg/mL) for 12 h, respectively. Then, the cells were stained according to the provided illustration of apoptosis detection kit and the fluorescence signals were collected at the excitation wavelength of 500 nm and 587 nm, respectively.

1.8 Flow cytometry analysis

We first divided A549, HepG-2, Hela and MCF-7 into three parallel groups and inoculated them in petri dishes for 24 h. Then two group of cells were treated with genistein (0.1 μM) and LPS (10 μg/mL) for 12 h, respectively, and the group without drug treatment served as the control. The prepared nanoprobes (6 μg/mL) were further added to the cells and incubated for 6 h. Finally, the fluorescence signals of Texas Red and Cy5 were collected by flow cytometry at appropriate excitation wavelengths.

2. Supplementary Figures

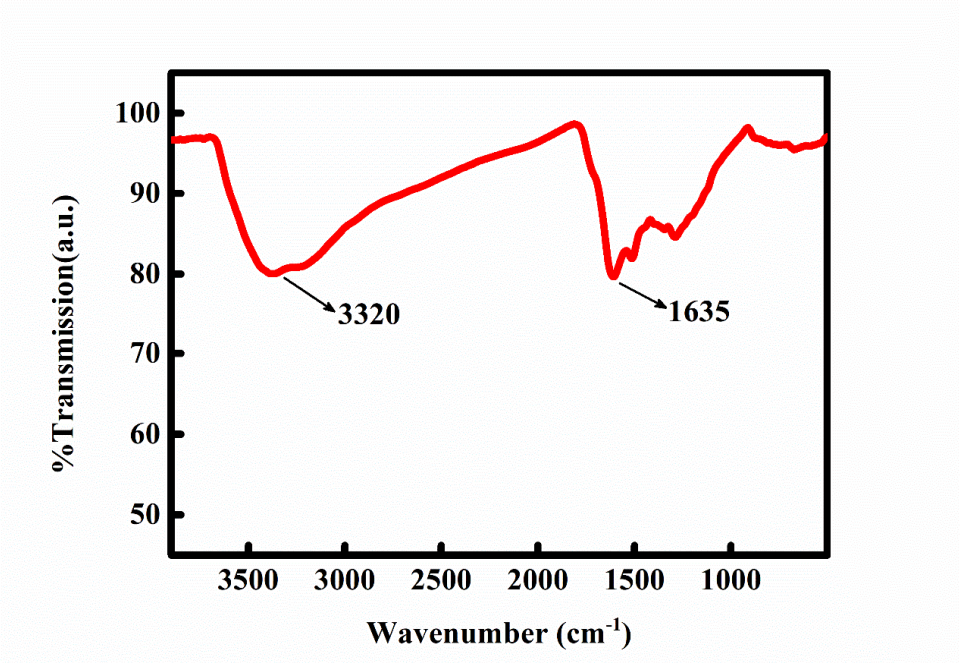

Figure S1. FTIR spectra of PDA NPs.

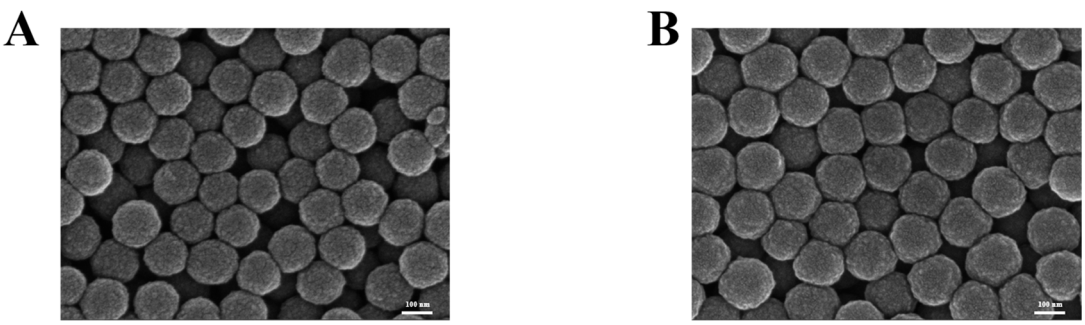

Figure S2. SEM of PDA NPs (A) and nanoprobes (B) (Scale bar is 100 nm.).

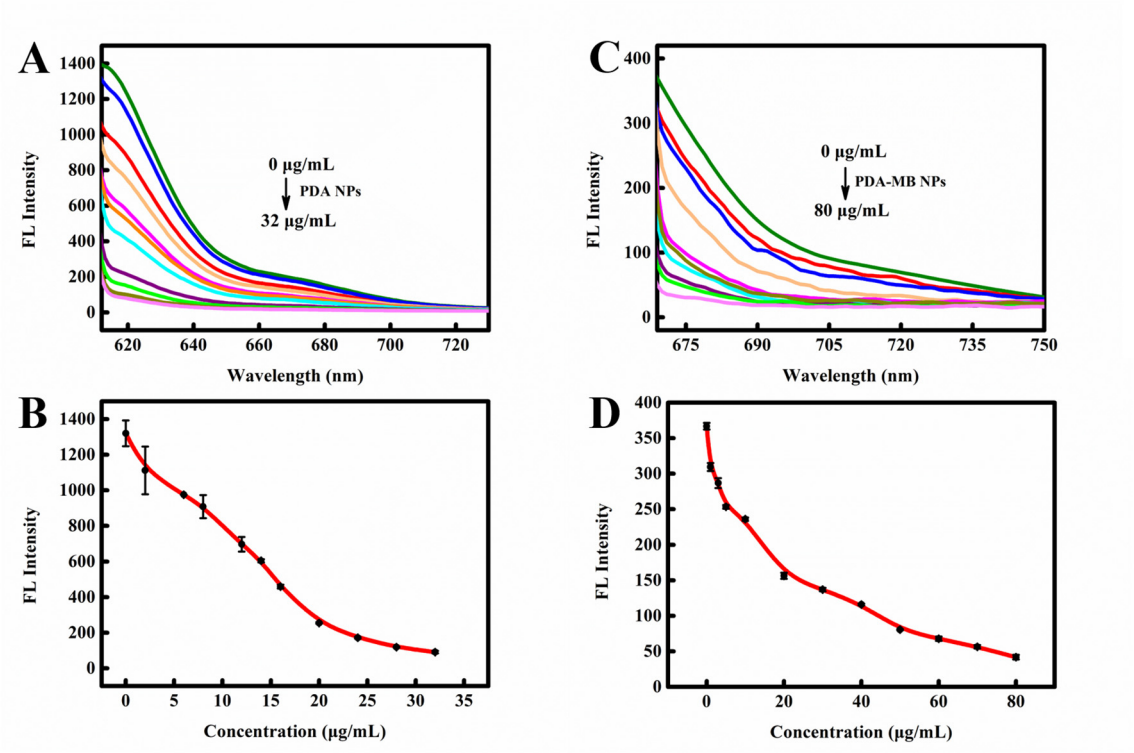

Figure S3. Fluorescence quenching spectra (A) and fluorescence intensity at 615 nm (B) of Texas Red-labeled MB (150 nM) with various concentrations of PDA NPs (0-32 ug/mL). Fluorescence quenching spectra (C) and fluorescence intensity at 670 nm (D) of Cy5-labeled peptide with various concentrations of PDA-MB NPs (0-80 μg/mL).

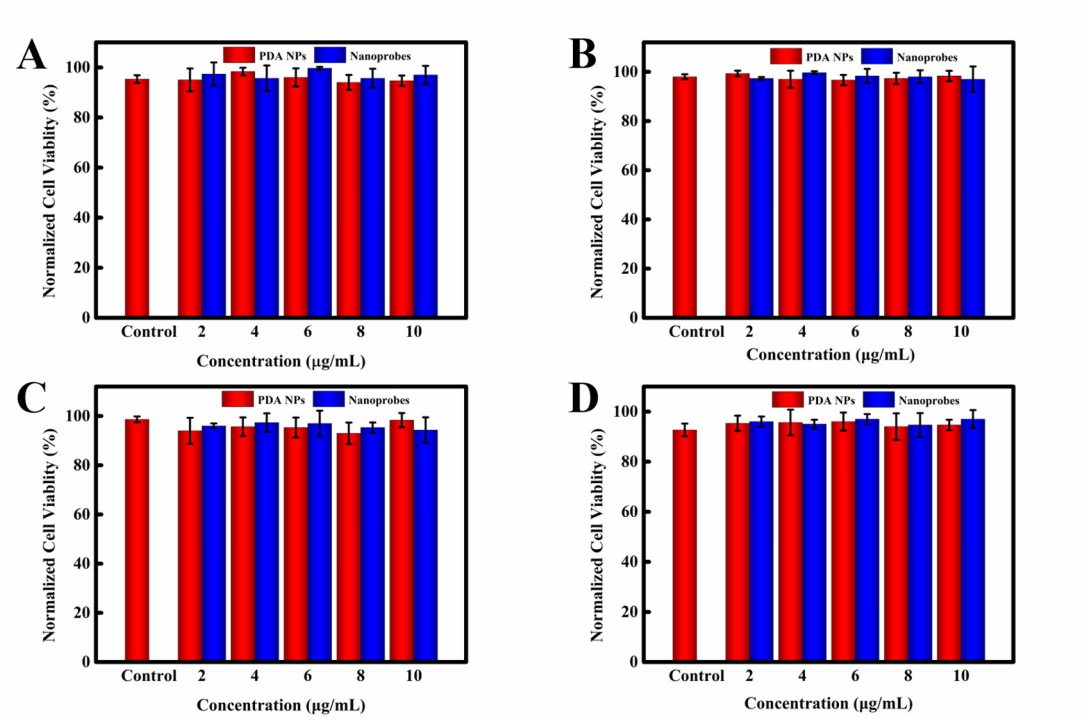

Figure S4. MTT assay. (A) A549 cells, (B) HepG-2, (C) Hela and (D) MCF-7 cells were incubated with different concentrations of PDA NPs and nanoprobes for 6 h, respectively.

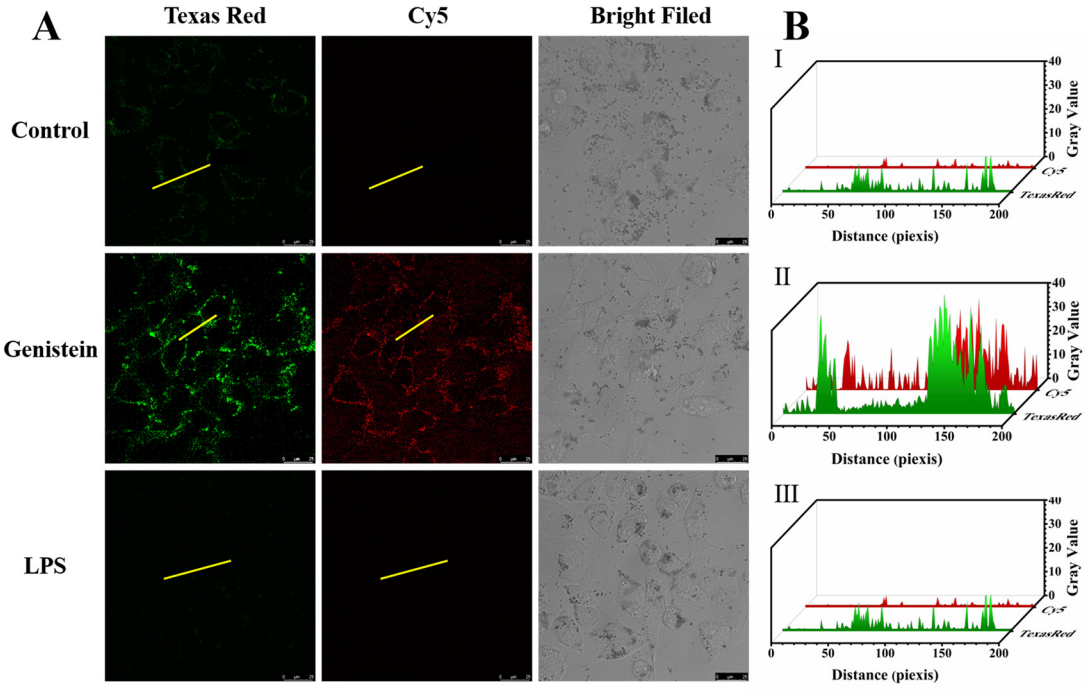

Figure S5. (A) CLSM imaging of miRNA-34a and Caspase-3 in HepG-2 cells. (B) Quantification of fluorescence intensity (yellow line) in confocal imaging of HepG-2 cells. Scale bars: 25 μm.

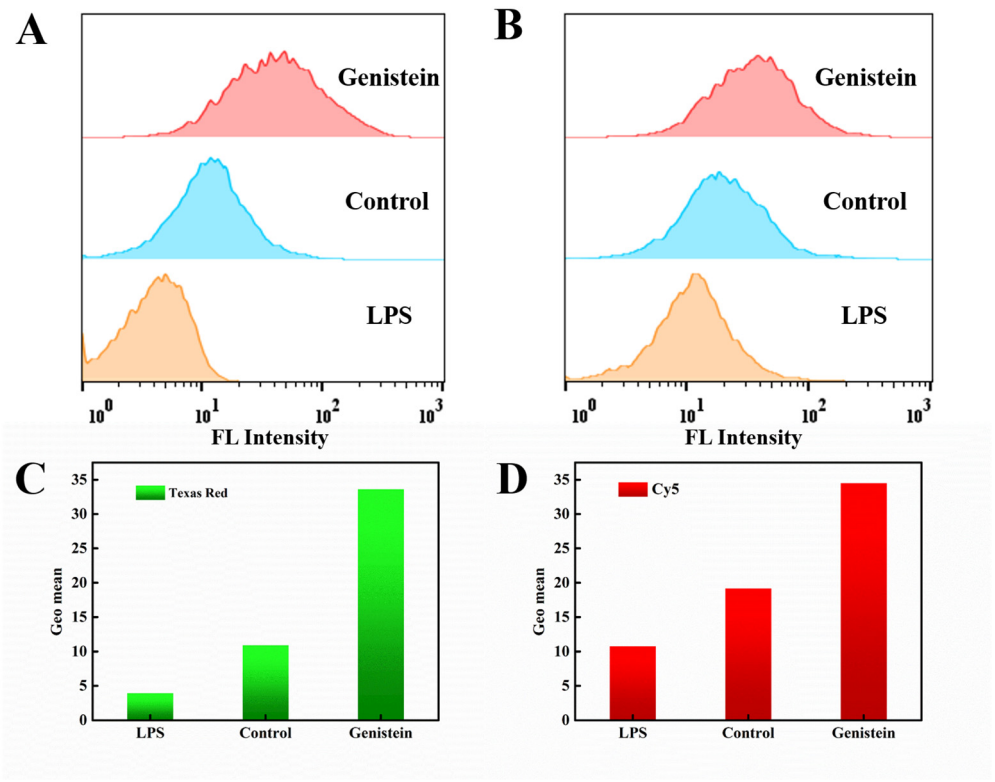

Figure S6. Flow cytometry of Texas Red-labeled MB (A) and Cy5-labeled peptide (B) in HepG-2 cells. (C and D) Geo mean quantification of flow cytometric results.

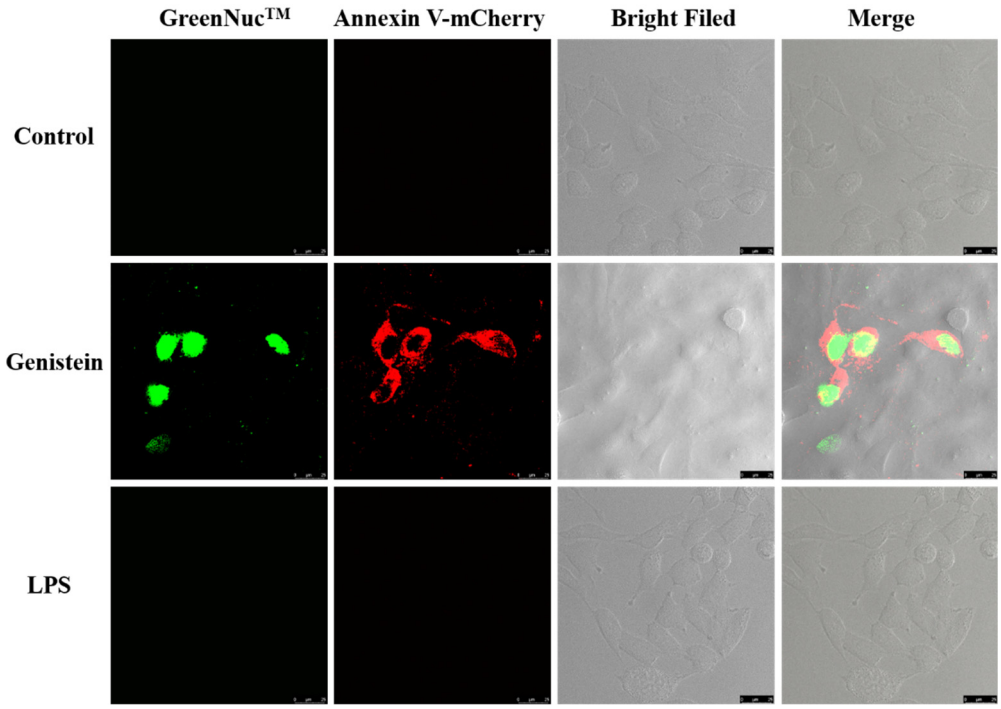

Figure S7. HepG-2 cells were stained with the apoptosis detection kit and analyzed by confocal microscopy. Scale bars: 25 μm.

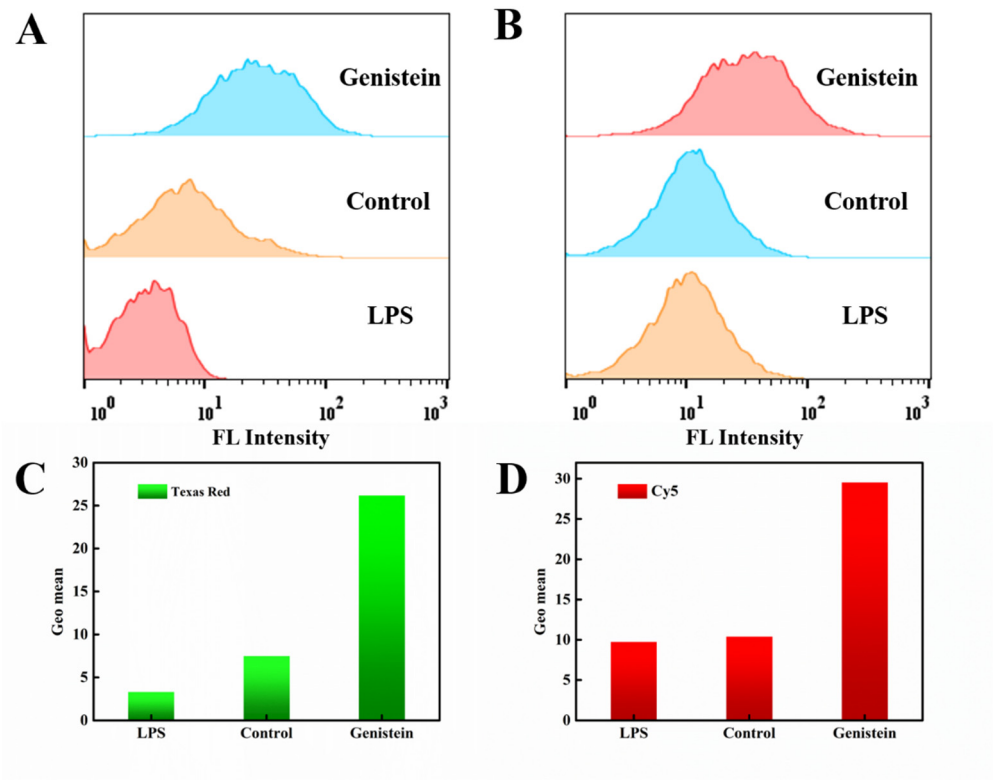

Figure S8. Flow cytometry of Texas Red-labeled MB (A) and Cy5-labeled peptide (B) in Hela cells. (C and D) Geo mean quantification of flow cytometric results.

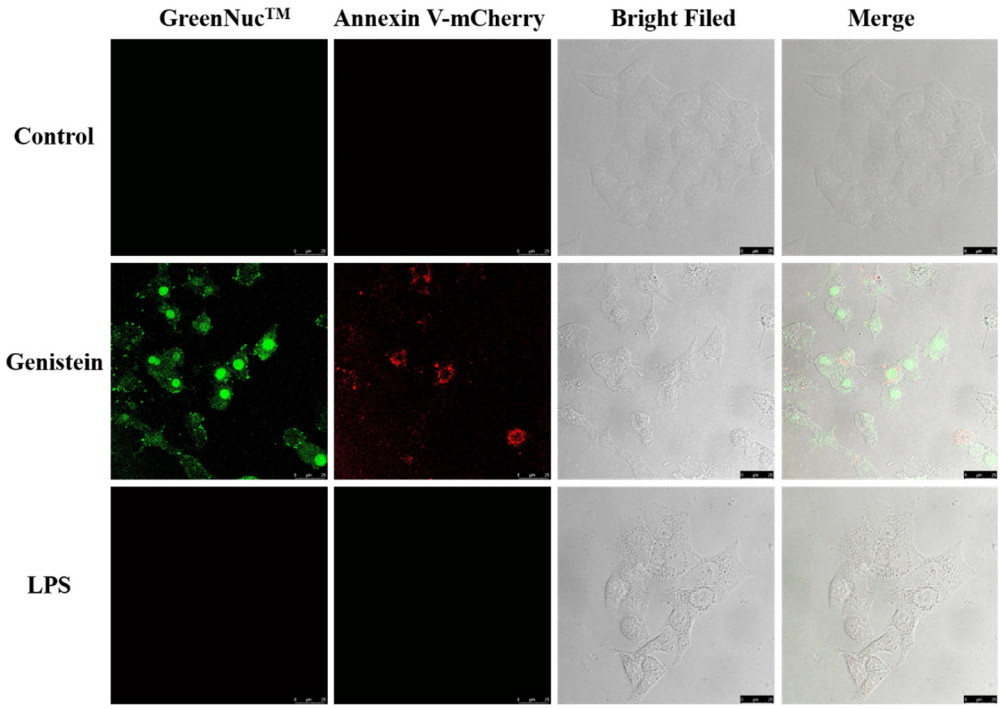

Figure S9. Hela cells were stained with the apoptosis detection kit and analyzed by confocal microscopy. Scale bars: 25 μm.

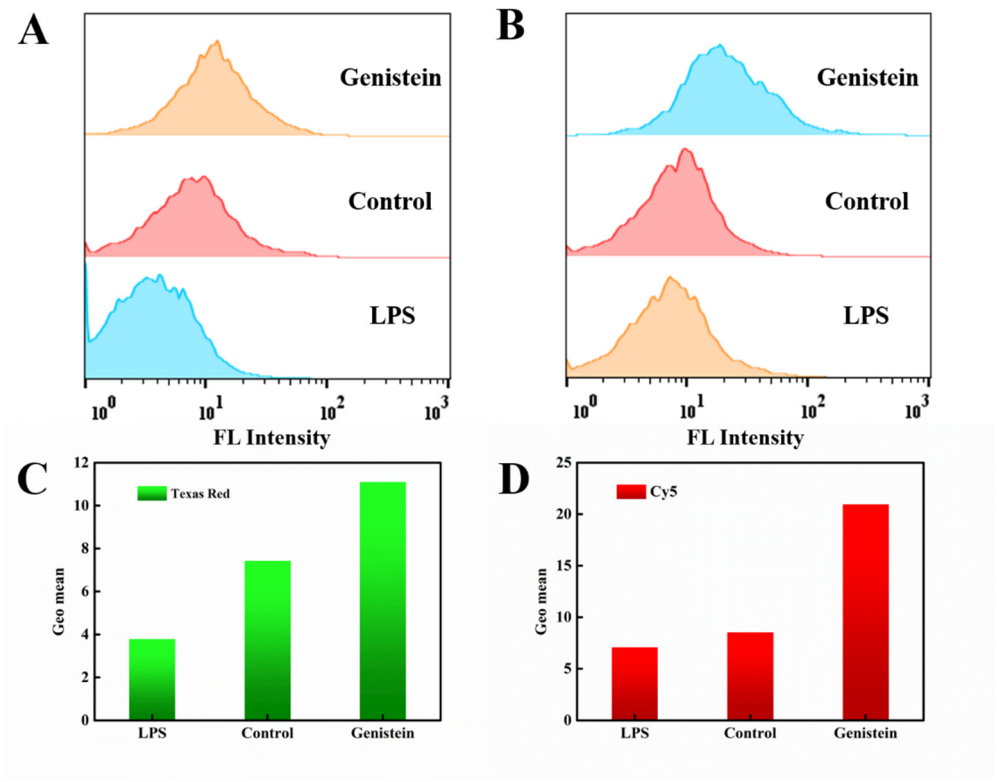

Figure S10. Flow cytometry of Texas Red-labeled MB (A) and Cy5-labeled peptide (B) in MCF-7 cells. (C and D) Geo mean quantification of flow cytometric results.

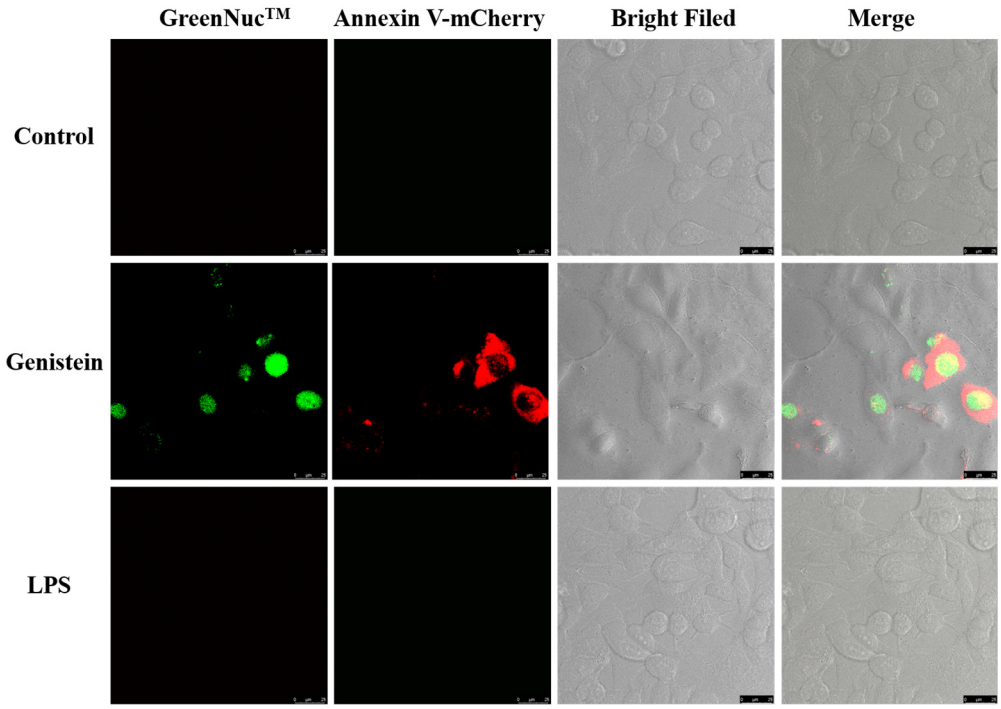

Figure S11. MCF-7 cells were stained with the apoptosis detection kit and analyzed by CLSM. Scale bars: 25 μm.