-Supporting Information-

A multifunctional silver nanocomposite for the apoptosis of cancer cell and intracellular imaging

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Experimental Section

Reagents and Materials. Dulbecco's Modified Eagles Medium (DMEM), RPMI 1640 medium (1640), fetal bovine serum (FBS) and trypsin were purchased from Invitrogen. Tris (2, 2'-bipyridine) ruthenium (II) chloride hexahydrate (RuBpy), tetraethyl orthosilicate (TEOS) and (3-aminopropyl) triethoxysilane (APTES, 99%) were purchased from Sigma-Aldrich. AgNPs with the size of 3 nm were purchased from Nanjing XFNANO Materials Tech Co., Ltd. Human hepatocellular carcinoma cell lines (HepG2), liver-derived cell lines (QSG-7701) and Hoechst 33342 staining kit were purchased from Key GEN Biotech. ROS detection kit (DCFH-DA), Annexin V-FITC and PI were purchased from Thermo Fisher. All other reagents were of analytical reagent grade. The water (18.2 MΩ cm) used throughout the experiments was pretreated with Milli-Q (Millipore, Inc., Bedford, MA). RGD (RGDRGDRGDRGDPGC) and NLS (CGGGPKKKRKVGG) peptide chains were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai China).

Apparatus. Transmission electron micrographs were acquired by JEM-1011 and JEM-2100 transmission electron microscope (JEOL Ltd., Japan). The fluorescence emission spectra were obtained on a Shimadzu fluorescence S-3 spectrophotometer (RF-5301PC, Shimadzu Co., Japan). UV-vis absorption spectra were recorded using a nanodrop UV-vis spectrophotometer (Nanodrop-2000C, USA). The ζ potential was acquired with a Malvern (Nano-Z, Malvern Instruments Ltd., Britain) instrument. ICP-AES was conducted with Optima 5300 DV ICP-AES system (Perkin Elmer, USA). Cell confocal fluorescence images were acquired with a TCS SP5 confocal fluorescence microscopy (Leica, Germany). The flow cytometric analysis result was recorded with Cytomics FC500 (Beckman, USA).

Preparation of PEG/RGD/NLS modified RuBpy@SiO_2-Ag NPs (nanocomposite). RuBpy@SiO_2 was prepared according to a published method. Briefly, 22.5 mL cyclohexane, 5.3 mL Triton X-100 and 5.4 mL hexanol were mixed and stirred for 15 min. Then, 10.0 mg RuBpy dispersed in 1.5 mL water was added and stirred. Consequently, 0.35 mL $NH_3 \cdot H_2O$ was added and stirred. Finally, 0.3 mL TEOS was added and stirred for 24 h. After that, 15 mL acetone was added to precipitate the nanoparticles and the nanoparticles were washed by ethanol. Finally, RuBpy@SiO_2 NPs were dispersed in ethanol.

50 μL APTES was added into the aforementioned solution. The mixture was heated to 80 °C and kept for 12 h. Then, RuBpy@SiO_2-NH_2 was centrifuged and rinsed with ethanol for three times after the mixture was allowed to cool down naturally. 100 μL RuBpy@SiO_2-NH_2 were added in 5 mL tube and dilute to 1.6 mL. Then different amounts of Ag NPs (50 μL, 100 μL, 200 μL) were separately added in the aforementioned mixture drop by drop under ultrasound. After that the mixture was kept for another 30 min under ultrasound. Then RuBpy@SiO_2-Ag NPs were acquired after centrifugation and washed with water. The followed RuBpy@SiO_2-Ag NPs were prepared with the addition of 100 μL Ag NPs.

RuBpy@SiO_2-Ag NPs were dispersed and then mixed with 1 mM PEG. The mixture was incubated overnight. After that, PEG modified RuBpy@SiO_2-Ag NPs was acquired after centrifugation and washed with water. Then 1.2 mM RGD and 1.2 mM NLS were added to modify RuBpy@SiO_2-Ag NPs and the mixture was incubated overnight. At last, PEG/RGD/NLS modified RuBpy@SiO_2-Ag NPs were acquired after centrifugation and washed with water.

Calculation of the number of Ag NPs on RuBpy@SiO_2. The densities of Ag and SiO_2 of ρ_1=10.49 g/cm^3 and ρ_2=2.2 g/cm^3 are approximately adopted as the densities of Ag NPs and RuBpy@SiO_2, respectively. In a spherical approximation, the molecular weight of Ag NPs and RuBpy@SiO_2 named as M_1 and M_2 is roughly calculated $M=4/3\pi R^3\rho N_A$ (N_A, Avogadro constant). The concentrations of Ag NPs and RuBpy@SiO_2 as C_1 and C_2 (C_1=38.85 μg/mL, C_2=80 μg/mL) were acquired by ICP-AES and weigh, separately. Then, the weight of Ag NPs and RuBpy@SiO_2 (noted as m_1 and m_2) was calculated by the formula m=CV, when the volume was fixed as V. Finally, the number of Ag NPs on each RuBpy@SiO_2 was calculated as ca. 207 by the following equations 1 and 2.

$$M = V\rho = \frac{4}{3}\pi R^3\rho \quad (1)$$

$$N = \frac{N_1}{N_2} = \frac{N_A \times m_1 \div \left(\frac{4}{3}\pi R_1^{\,3}\rho_1\right)}{N_A \times m_2 \div \left(\frac{4}{3}\pi R_2^{\,3}\rho_2\right)} = \frac{N_A \times C_1 V \div \left(\frac{4}{3}\pi R_1^{\,3}\rho_1\right)}{N_A \times C_2 V \div \left(\frac{4}{3}\pi R_2^{\,3}\rho_2\right)} = \frac{C_1 \times R_2^{\,3}\rho_2}{C_2 \times R_1^{\,3}\rho_1} \quad (2)$$

Cell Culture. QSG-7701 cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 100 IU/mL penicillin and 100 IU/mL streptomycin at 37 °C in a 5% CO_2-95% air incubator MCO-15AC (Sanyo, Tokyo, Japan). HepG2 cells were respectively incubated with high-glucose DMEM supplemented with 10% FBS, 100 IU/mL penicillin and 100 IU/mL streptomycin.

Time course confocal images of HepG2 cells upon incubation. HepG2 cells were digested and seeded on confocal dishes for 24 h at 37 °C. Then the same concentration of nanocomposite (80 μg/mL) was added into the dishes and the cells were incubated for different time (0 h, 2 h, 4 h, 6 h, 8 h). Then confocal images were acquired after washes with PBS (pH=7.4). The cells were excited at 488 nm and the emission was collected from 550 to 600 nm.

Confocal images of HepG2 cells with incubation of different concentration of nanocomposite. HepG2 cells were digested and seeded on confocal dishes for 24 h at 37 °C. Then different concentrations of nanocomposite (5 μg/mL, 20 μg/mL, 40 μg/mL, 80 μg/mL) were respectively added into the dishes and the cells were incubated for 6 h. Then confocal images were acquired after washes with PBS (pH=7.4). The cells were excited at 488 nm and the emission was collected from 550 to 600 nm.

Intracellular location of nanocomposite. HepG2 cells were digested and seeded onto confocal dishes for 24 h at 37°C. Then, HepG2 cells were incubated for 6 h with the same concentration of nanocomposite (80 μg/mL). After that, cells were washed for three times with PBS (pH=7.4) and stained with Hoechst 33342 for 20 min. Finally, confocal imaging was conducted after cells were

rinsed with PBS for three times. Hoechst 33342 was excited at 405 nm, and the emission was collected from 450 to 500 nm. The nanocomposite was excited at 488 nm and the emission was collected from 550 to 600 nm.

Targeted imaging of nanocomposite with different cells. HepG2 cells or QSG-7701 cells were digested and seeded on confocal dishes and 6-well plates for 24 h, respectively. Then the cells were incubated with the same concentration of nanocomposite (80 μg/mL) for 6 h. After that, confocal imaging was conducted after cells on confocal dishes were rinsed with PBS (pH=7.4) for three times. The cells on 6-well plates were digested and concentrated (1000 rpm, 5 min), and then flow cytometry result was recorded to evaluate the specificity of the nanocomposite to cancer cells with flow cytometry FC500.

ROS detection. HepG2 cells were digested and seeded on cell culture dishes for 24 h. Then the cells were divided into four groups. The cells of four groups were incubated for 6 h with different concentrations of nanocomposite (5, 20, 40, 80 μg/mL). Then, the cells were incubated for another 12 h after the cells were rinsed for three times with PBS (pH=7.4). The cells of positive control group were pretreated with ROS positive reagent. After that, the cells were washed with PBS for three times and then stained with ROS detection kit for 20 min. At last, confocal imaging was conducted after cells were washed with PBS for three times. DCF was excited at 488 nm, and the emission was collected from 500 to 550 nm. The nanocomposite was excited at 488 nm and the emission was collected from 550 to 600 nm.

Cell Apoptosis Assay. HepG2 cells were seed into 6-well plates (1×10^5 per well) for 24 h at 37 °C. Then, the cells were divided for three groups, group I was cultured with pure cell culture medium, group II was incubated with nanocomposite for 6 h, then washed with PBS for three times and cultured with cell culture medium for another 24 h, group III was incubated with nanocomposite for 6 h, then rinsed with PBS for three times and cultured with cell culture medium for another 48 h. After that process, the cells were digested, concentrated (1000 rpm, 5 min) and washed with PBS for three times. Finally, cells were incubated with apoptosis analysis reagent (Annexin V-FITC and PI) according to the manufacturer's instructions, and then analyzed with flow cytometry.

Supplementary Figures

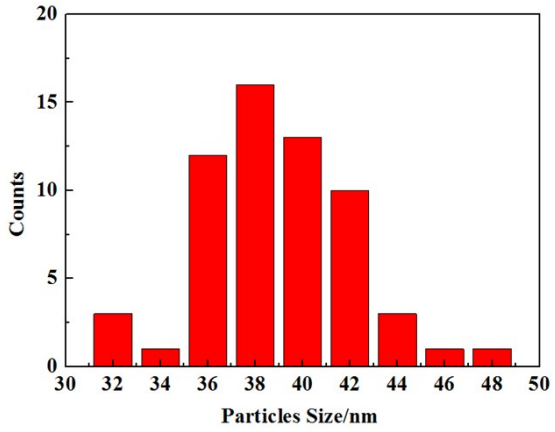

Fig. S1 Particles size distribution of RuBpy@SiO_2-NH_2.

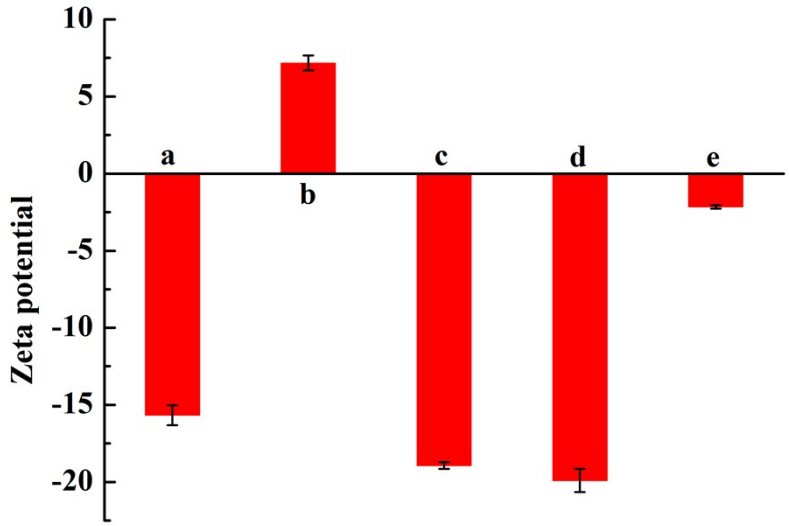

Fig. S2 The ζ potentials of RuBpy@SiO_2(a), RuBpy@SiO_2-NH_2(b), RuBpy@SiO_2-Ag(c), RuBpy@SiO_2-Ag-PEG (d) and PEG/RGD/NLS modified RuBpy@SiO_2-Ag (e), respectively.

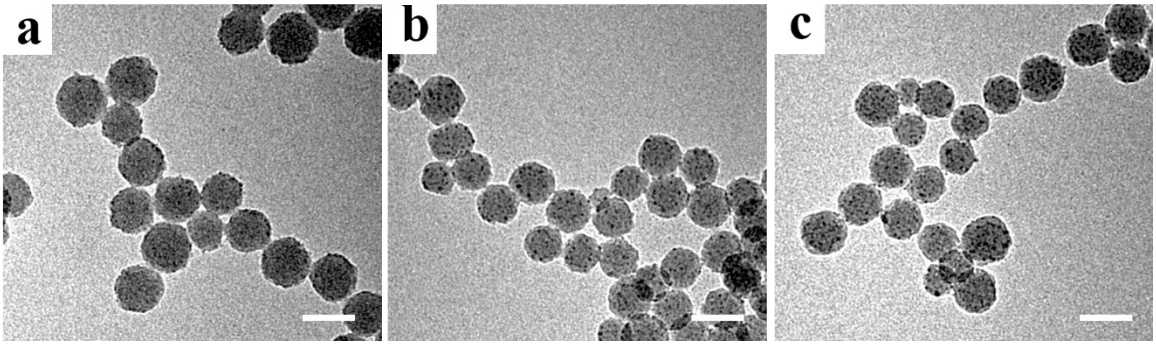

Fig. S3 TEM images of RuBpy@SiO_2-Ag (a), RuBpy@SiO_2-Ag-PEG (b), and PEG/RGD/NLS modified RuBpy@SiO_2-Ag (c), the scale bar is 50 nm.

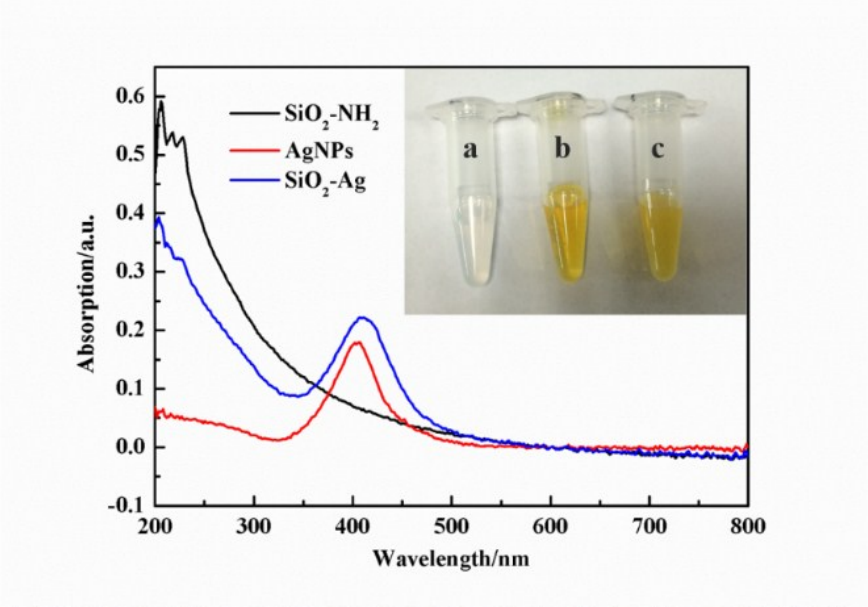

Fig. S4 UV-vis spectra of SiO_2-NH_2, Ag NPs, and SiO_2-Ag NPs. Inset figure: Image of SiO_2-NH_2 (a), Ag NPs (b) and SiO_2-Ag NPs solutions (c).

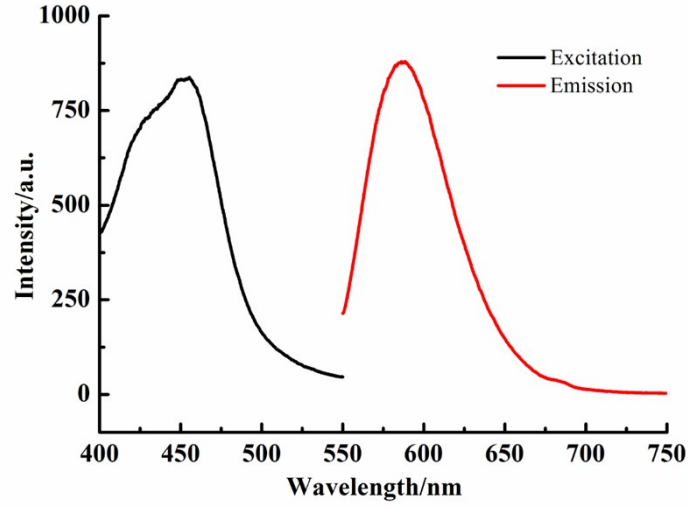

Fig. S5 The fluorescent excitation and emission spectra of RuBpy@SiO_2.

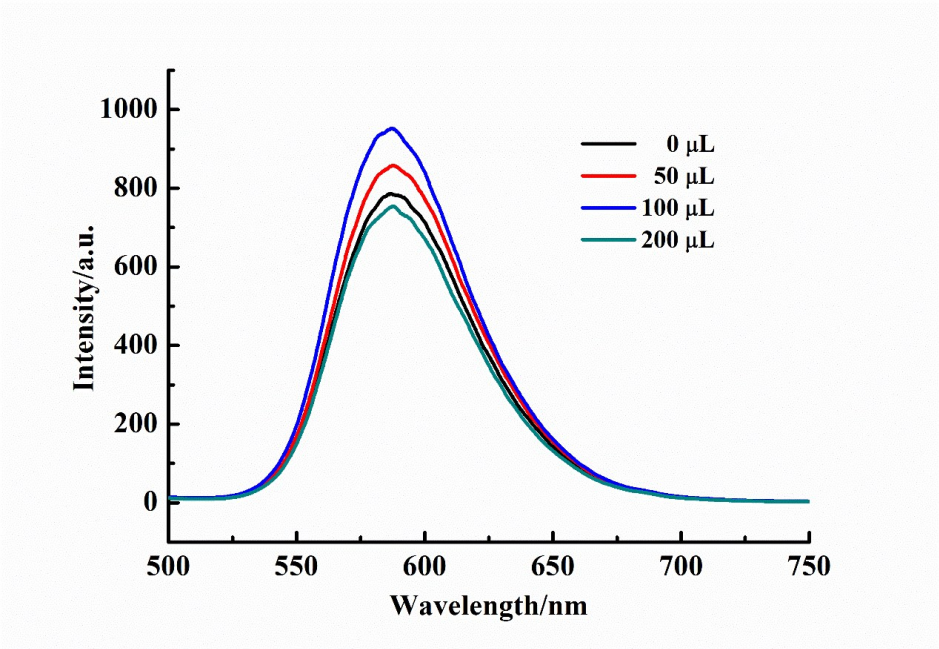

Fig. S6 The fluorescence intensities of RuBpy@SiO_2 after assembly of Ag NPs using Ag NPs bulk solutions with different volumes.

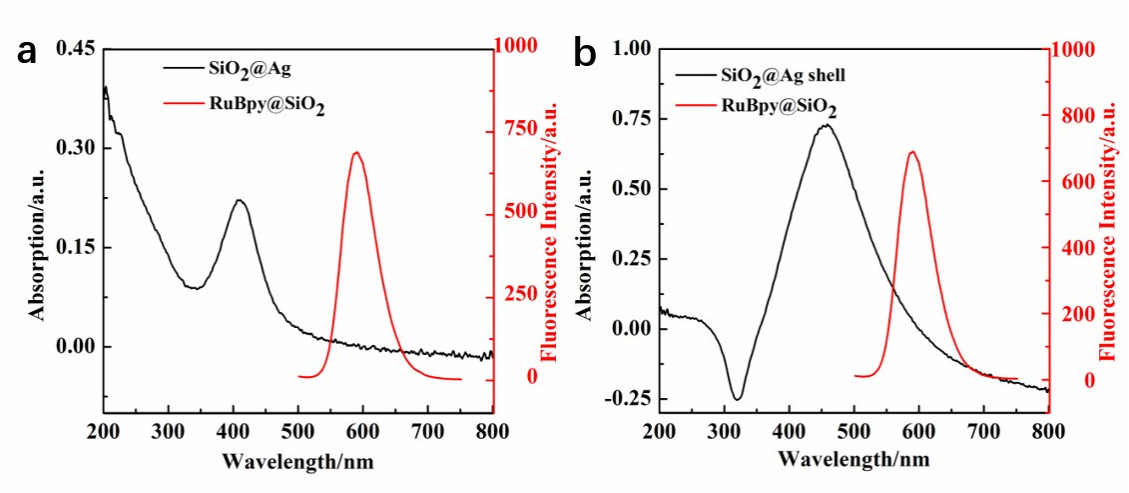

Fig. S7 UV-vis spectrum of SiO_2@Ag NPs and spectrum of RuBpy@SiO_2 (a), and UV-vis spectrum of SiO_2@Ag shell and fluorescence spectrum of RuBpy@SiO_2 (b).

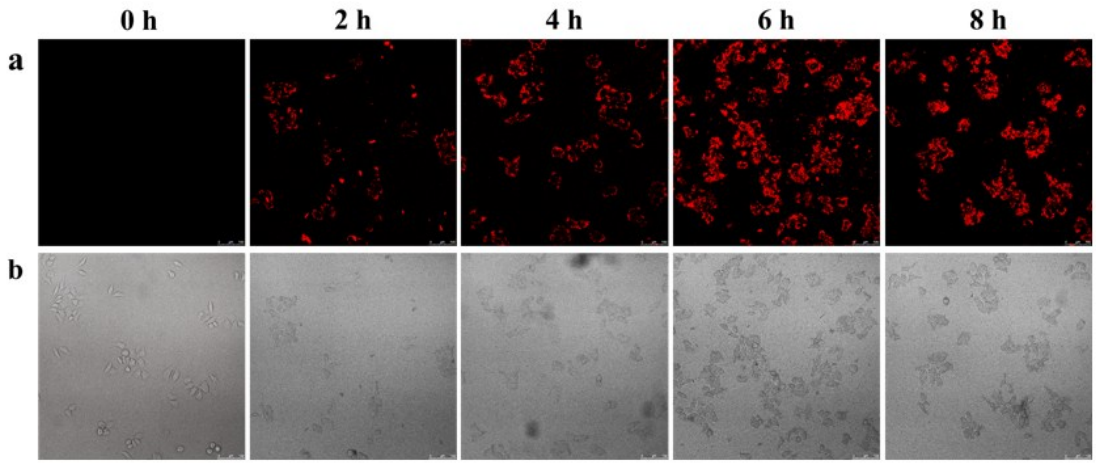

Fig. S8 Confocal images (a) and bright field images (b) of HepG2 cell after incubation with nanocomposite for different time (0 h, 2 h, 4 h, 6 h, 8 h).

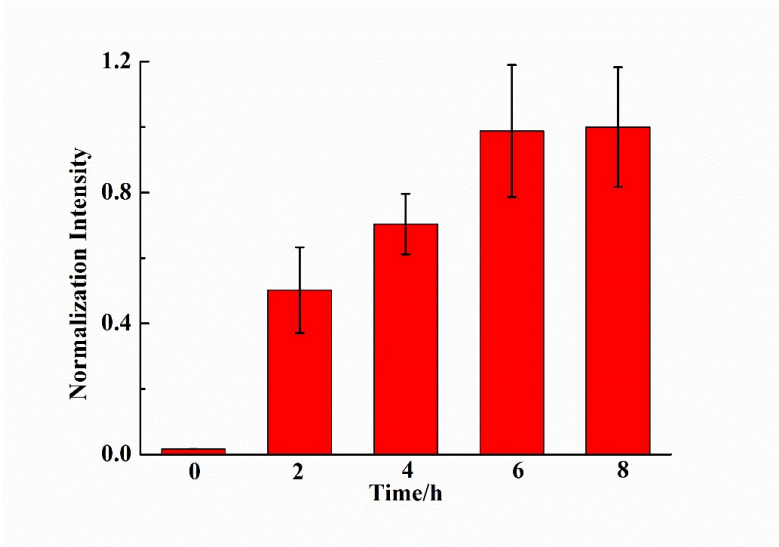

Fig. S9 The mean fluorescence intensity quantification of HepG2 cells after incubation with nanocomposite for 0 h, 2 h, 4 h, 6 h and 8 h in Fig. S6.

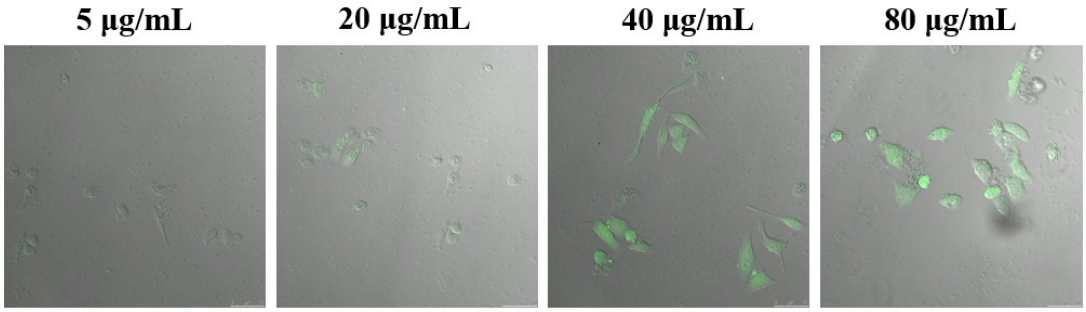

Fig. S10 Confocal images of HepG2 cells after incubation with different concentrations of nanocomposite for 12 h, then incubated with DCFH-DA for 20 min.