Electronic Supplementary Information

Natural hyperoside based novel light-up fluorescent probe with AIE and ESIPT characteristics for on-site and long-term imaging of *β*-galactosidase in living cells

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1. Experimental section

1.1. Materials

Cysteine (Cys), homocysteine (Hcy), glutathione (GSH), tetrahydrofuran (THF), methylthiazolyldiphenyl-tetrazolium bromide (MTT), quercetin, dimethylsulfoxide (DMSO), chloroform, and methanol were supported by Aladdin Reagent Co., Ltd. (Shanghai, China). *β*-Glucosidase (*β*-Glu), glucose oxidase (GOD), *β*-galactosidase (*β*-Gal, 3.2.1.23), D-galactose, Golgi-Tracker Green, Lyso-Tracker Green, Mito-Tracker Green, and indocyanine green (ICG) were purchased from Macklin Reagent Co., Ltd. (Shanghai, China). All chemicals and reagents were of analytical grade without additional purification. The Milli-Q water purification system (Millipore, Bedford, MA, USA) provided ultrapure water (18.2 M Ω•cm).

1.2. Instruments

^{1}H-NMR and ^{13}C-NMR spectrum were obtained using Bruker spectrometer with chemical shifts reported in ppm and coupling constants (*J*) reported in Hz. High resolution mass spectra (HRMS) were recorded on Bruker compact QTOF-MS with an electrospray ionization (ESI) interface (Bruker Co., Bremen, Germany). TEM images were obtained using Tecnai G2 20S-Twin transmission electron microscope (TEM, FEI, Prague, Czech). Dynamic light scattering (DLS) measurements were carried out with a ZetaSizer Nano ZS type Zeta potential meter (Malvern Instrument Company, England). UV-vis spectra were measured with UV-2600 spectrophotometer (Shimadzu, Japan). The fluorescence measurements were carried out on a Perkin-Elmer LS-55 luminescence spectrometer (PerkinElmer, USA). Fluorescence lifetime measurements were carried out on Fluo Time 100 (PicoQuant, Germany). pH value was controlled using Five Easy plus pH Meter (Mettler Toledo, China). Cell imaging was studied by Snap cool HQ^{2} imaging system (Photometrics Inc., USA).

1.3. Preparative isolation of hyperoside from *Hedyotis diffusa*

Dried *Hedyotis diffusa* was bought from local drugstore, Hunan, China, which was identified by Prof. Shuihan Zhang, Research Institute of Chinese Medicine, Hunan Academy of Chinese Medicine, Changsha, China. After that, *Hedyotis diffusa* was ground and sieved, and materials between 180 and 250 μm was used for extraction. *Hedyotis diffusa* powder (50.0 g) was extracted with 60% ethanol (800 mL, three times) at 85 °C (each for 3 h). After filtration, the extract was combined and evaporated to dryness, which was then chromatographed on repeated silica gel columns using chloroform–methanol (1:0–0:1) as solvent systems to afford 32.7 mg of hyperoside.

Hyperoside, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.64 (s, 1H, 5-OH), 10.87 (s, 1H, 7-OH), 9.74 (s, 1H, 4'-OH), 9.16 (s, 1H, 3'-OH), 7.67 (dd, J = 8.5, 2.2 Hz, 1H, H-6'), 7.53 (d, J = 2.2 Hz, 1H, H-2'), 6.82 (d, J = 8.5 Hz, 1H, H-5'), 6.41 (d, J = 2.0 Hz, 1H, H-8), 6.20 (d, J = 2.0 Hz, 1H, H-6), 5.38 (d, J = 7.7 Hz, 1H, H-1''), 5.14 (d, J = 4.5 Hz, 1H, Glc-OH), 4.86 (d, J = 5.3 Hz, 1H, Glc-OH), 4.44 (d, J = 12.1, 4.9 Hz, 1H, Glc-OH), 4.43 (d, J = 4.9 Hz, 1H, Glc-OH), 3.65 (s, 1H, H-4''), 3.58 (m, 1H, H-2''), 3.45 (dd, J = 9.9, 5.6 Hz, 1H, H-6''a), 3.38 (d, J = 4.5 Hz, 1H, H-3''), 3.33-3.26 (m, 2H, H-5'' and H-6''b); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.0 (C-4), 164.6 (C-7), 161.7 (C-5), 156.8 (C-9), 156.7 (C-2), 148.9 (C-4'), 145.3 (C-3'), 133.9 (C-3), 122.5 (C-6'), 121.55 (C-1'), 116.4 (C-2'), 115.6 (C-5'), 104.4 (C-10), 102.2 (C-1''), 99.1 (C-6), 94.0 (C-8), 76.3 (C-5''), 73.6 (C-3''), 71.7 (C-2''), 68.4 (C-4''), 60.6 (C-6'').

1.4. Computation Methods

Time-dependent density functional theory (TD-DFT) calculations were used for the theoretical investigation of ESIPT mechanism. Local minima and transition state structures were obtained by optimization of geometry. Geometries were determined with the B3LYP/6-31+G (d) basis set, while energies were determined by single point

calculations with the B3LYP/6-311+G (d,p) basis set upon optimized structures. All calculations were performed using Gauss 9.0 package.

1.5. Responses of hyperoside to *β*-Gal

Hyperoside (100.0 μg) is dissolved in THF/PBS solution (20/80, *v*/*v*, pH 7.4, 50 mM, 12.5 mL). After that, mixtures were sonicated for 2 h at 25 °C and placed in a fume hood to volatilize all THF for preparation of hyperoside solution. Then, different concentrations of *β*-Gal in PBS solution (pH 7.4, 30 μL) were mixed with hyperoside solution (3.0 mL), and final concentrations of *β*-Gal were from 0 to 14 U mL^{-1}. Mixtures were incubated at 37 ^{o}C for 70 min, and then UV spectra and FL spectra were measured. The excitation wavelength, excitation and emission silts were set at 360, 5, and 20 nm, respectively.

1.6. Cytotoxicity and cellular imaging investigation

HeLa and SKOV-3 cells were supported by Xiangya Hospital of Central South University. Cytotoxicity experiments of hyperoside and quercetin were evaluated using MTT assay. SKOV-3 cells and Hela cells were seeded in 96-well plate with the density of 1×10^4 cells per each well and attached for 24 h in cell culture medium at 5 % CO_2 atmosphere. Then, cells were treated with hyperoside or quercetin with different concentrations (0–1.0 mg mL^{-1}), and cultured for another 24 h at 5 % CO_2 atmosphere. Freshly prepared MTT (150 μL, 0.5 mg mL^{-1}) was added to each well to form the violet formazan for 4 h. Supernatant was removed and DMSO (150 μL) was added and shaken for 15 min to dissolve the violet formazan. Control experiments were performed under the same conditions without the addition of hyperoside or quercetin. Optical density (OD) of each well was measured using Enzyme Linked Immunosorbent Assay reader at 490 nm. Cell viability was calculated as following: cell viability (%) = $(OD_{treated}/OD_{control}) \times 100\%$, where $OD_{treated}$ and $OD_{control}$ were obtained with and

without treatment with hyperoside or quercetin.

For cellular imaging, SKOV-3 cells were seeded in 6-well plate with density of 7 × 10^4 cells per well and attached for 24 h in cell culture medium at 5% CO_2 atmosphere. Then, SKOV-3 cells were treated with hyperoside (100 μg mL^{-1} in culture medium, 1.5 mL) at 37°C for 2h. Another group of SKOV-3 cells was preincubated with D-galactose solution (1.0 mM, 1.5 mL) at 37°C for 0.5 h. After that, medium was removed and SKOV-3 cells were treated with hyperoside (100 μgmL^{-1} in culture medium, 1.5 mL) at 37°C for 2h. HeLa cells were pretreated with *β*-Gal solution (12 U mL^{-1} in culture medium, 1.5 mL) at 37°C for 2h for the exogenous *β*-Gal group. After removal of culture medium, HeLa cells were incubated with hyperoside (100 μg mL^{-1} in culture medium, 1.5 mL) at 37°C for 2h. Finally, all cells were imaged by confocal laser scanning microscope.

For colocalization imaging, SKOV-3 cells were seeded in 6-well plate with the density of 7 × 10^4 cells per well and attached for 24 h in cell culture medium at 5 % CO_2 atmosphere. SKOV-3 cells were incubated with hyperoside (100 μg mL–1 in culture medium, 1.5 mL) at 37°C for 2 h, and then co-stained with Golgi-Tracker Green (0.3 mg mL^{-1}) for 30 min, Mito-Tracker Green (200 nM) for 30 min, or Lyso-Tracker Green (75 nM) for 60 min. Finally, SKOV-3 cells were imagined by confocal laser scanning microscope.

For long-term imaging, SKOV-3 cells were seeded in 6-well plate with the density of 7 × 10^4 cells per well and attached for 24 h in cell culture medium at 5% CO_2 atmosphere. Then SKOV-3 cells were incubated with hyperoside (100 μg mL^{-1} in culture medium, 1.5 mL), and imagined by confocal laser scanning microscope at different time intervals (0.5–24 h).

2. Supplementary Fig. S1-S15

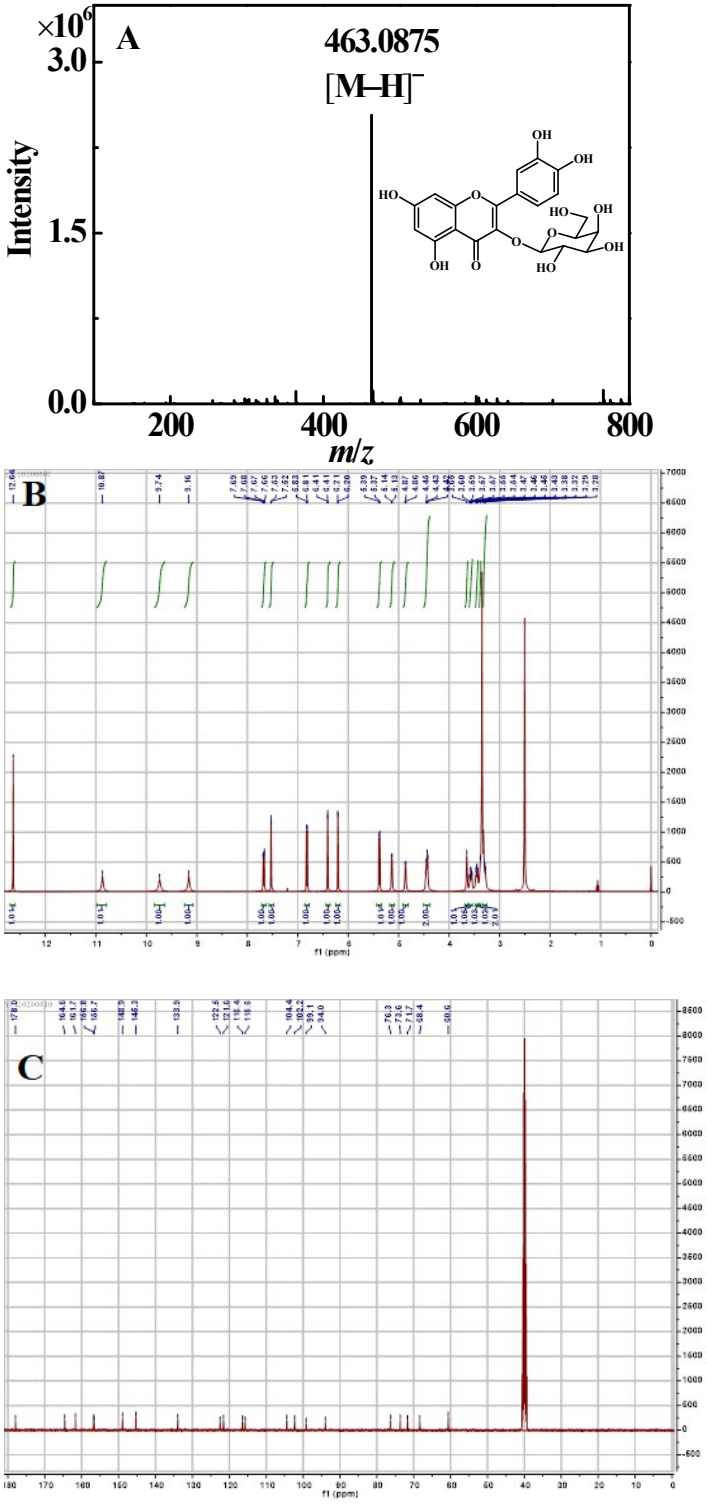


Fig. S1 High-resolution MS (A), ^{1}H NMR (B) and ^{13}C NMR (C) spectra of purified hyperoside.

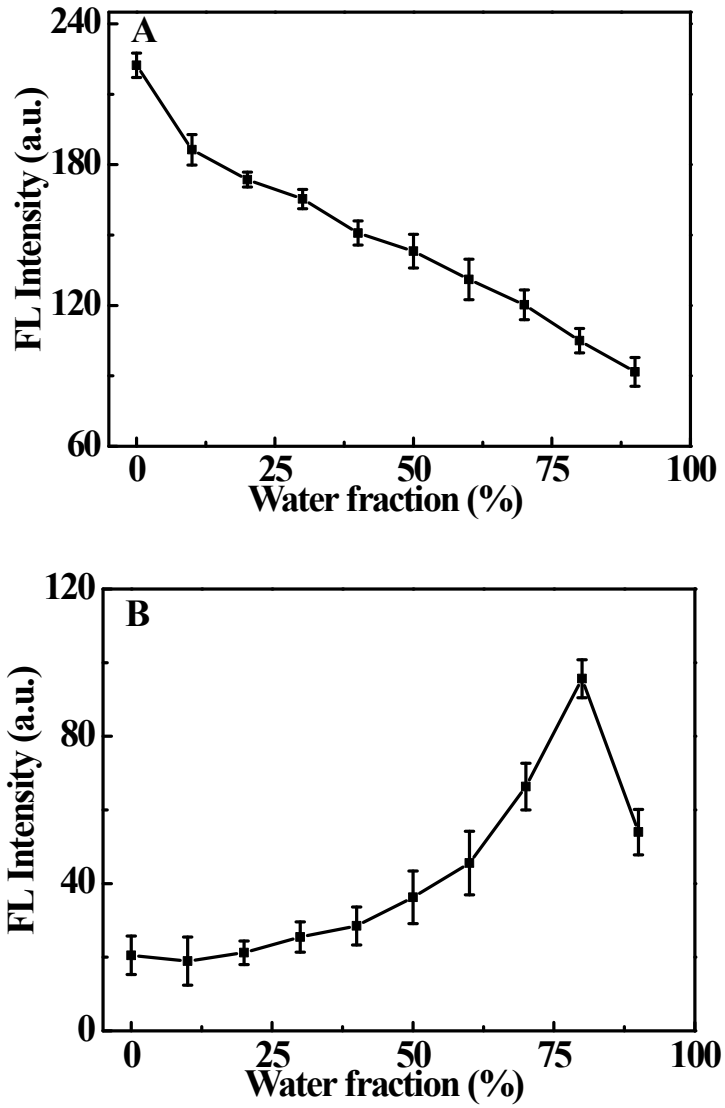


Fig. S2 FL intensities at 405 nm of hyperoside (10 μg mL^{-1}) (A) and at 530 nm of quercetin (10 μg mL^{-1}) (B) in THF with different water fractions.

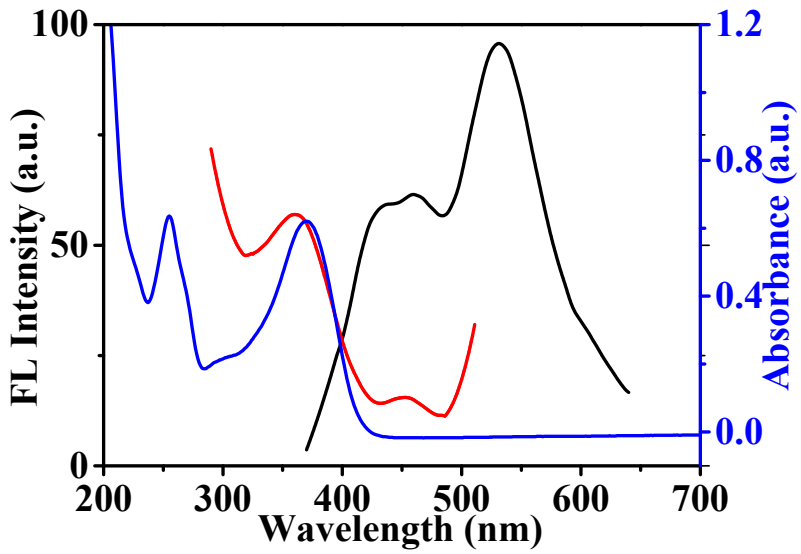


Fig. S3 Emission (black line), excitation (red line) and UV-vis (blue line) spectra of quercetin in THF/H_2O (20/80, *v*/*v*).

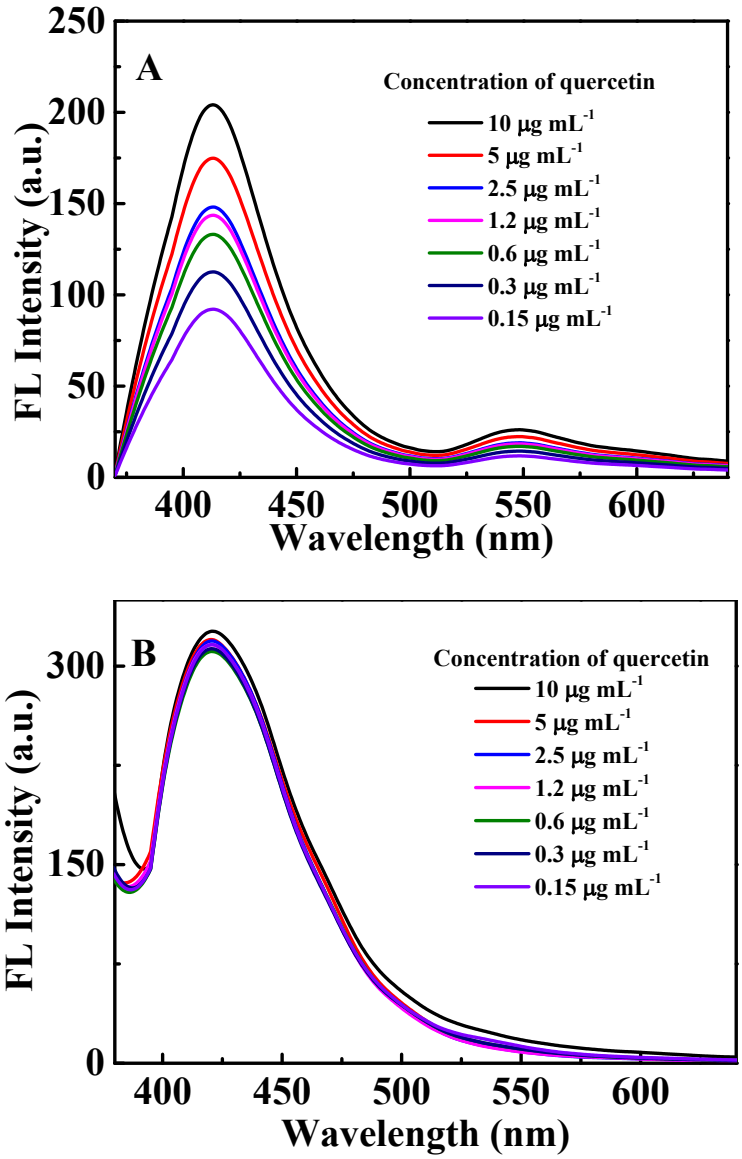


Fig. S4 FL spectra of quercetin in THF (A) and DCM (B) solutions.

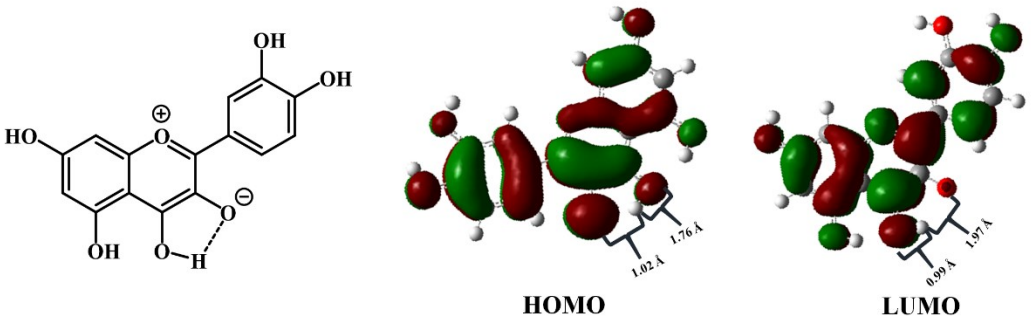


Fig. S5 HOMO (ground state) and LUMO (excited state) molecular orbitals of keto form of quercetin calculated with TDDFT at the level of B3LYP/6-31G* in THF solution.

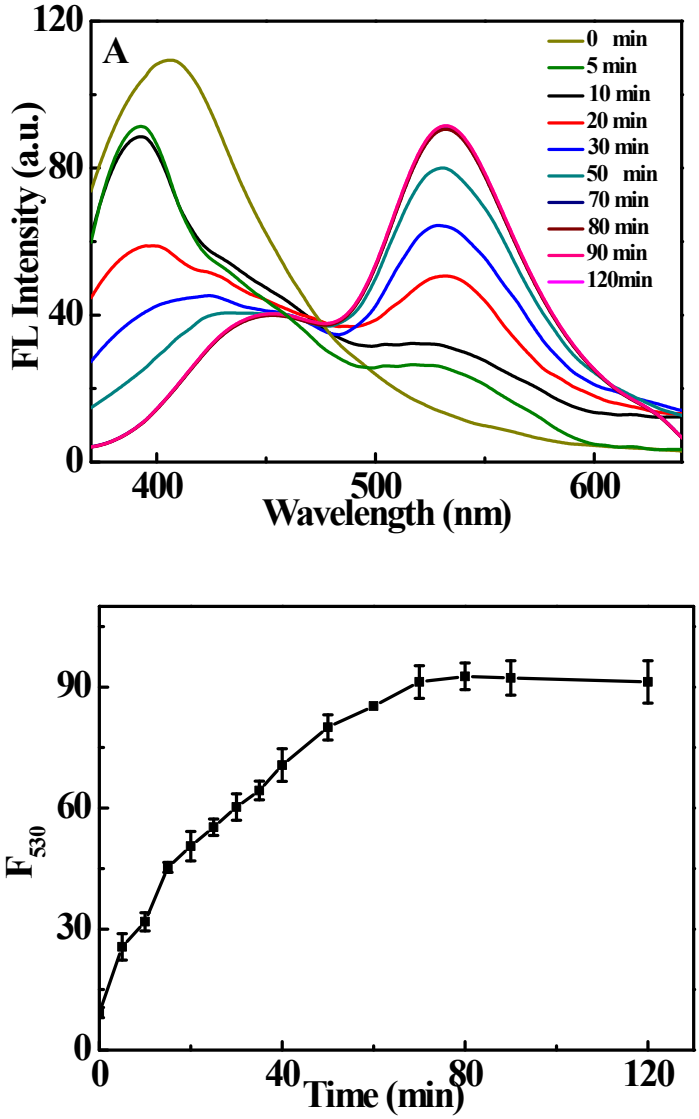


Fig. S6 FL spectra of hyperoside (10 μg mL^{-1}) after addition of *β*-Gal (12 U mL^{-1}) with different incubation time (A); FL intensity at 530 nm (F_{530}) for hyperoside after addition of *β*-Gal (12 U mL^{-1}) with different incubation time (B).

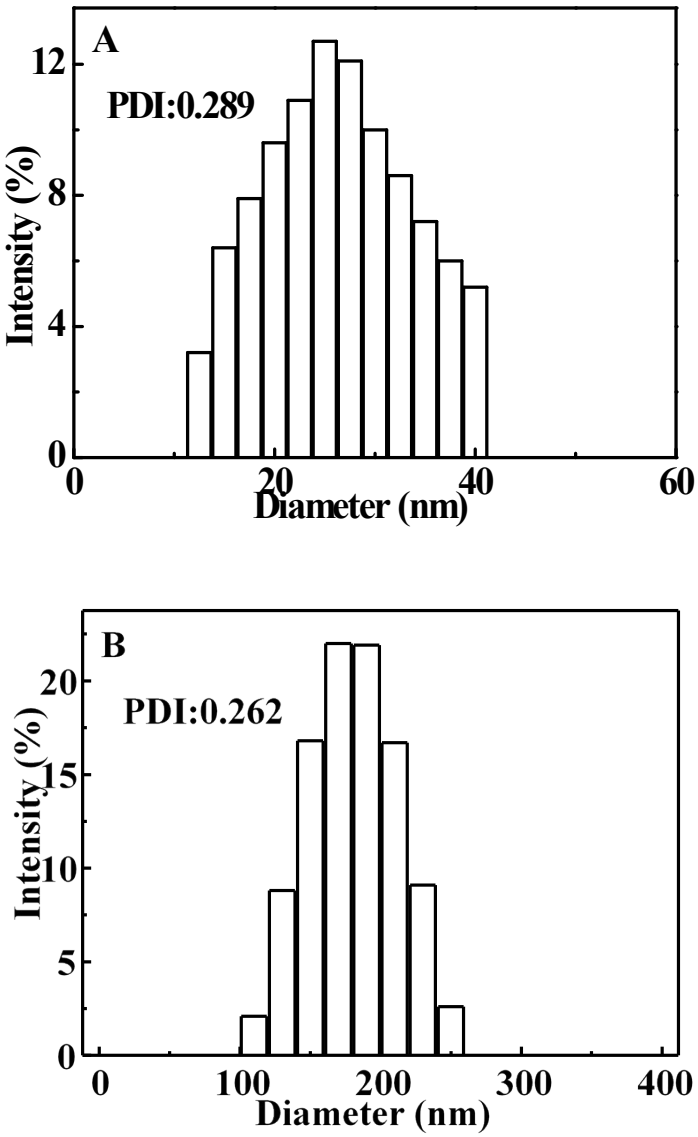


Fig. S7 DLS of hyperoside (10 μg mL^{-1}) before and after incubation with *β*-Gal (12 U mL^{-1}).

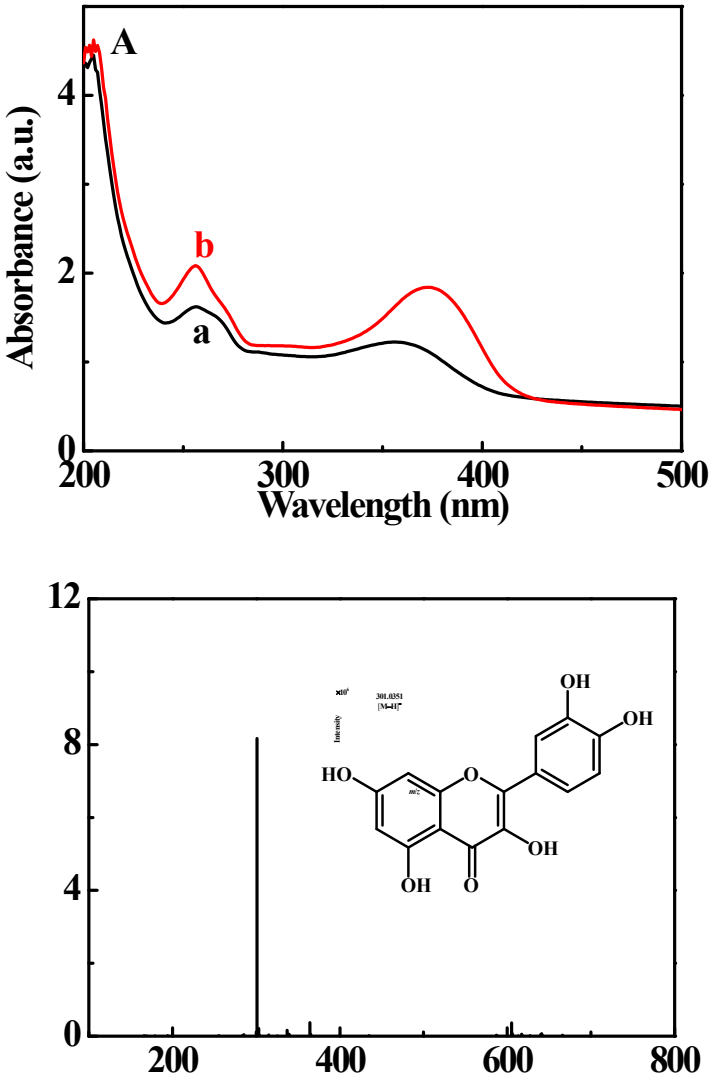


Fig. S8 UV-vis spectra of hyperoside (10 μg mL^{-1}) before (a) and after (b) addition of *β*-Gal (A); MS spectrum of the product of hyperoside after addition of *β*-Gal (12 U mL^{-1}) (B).

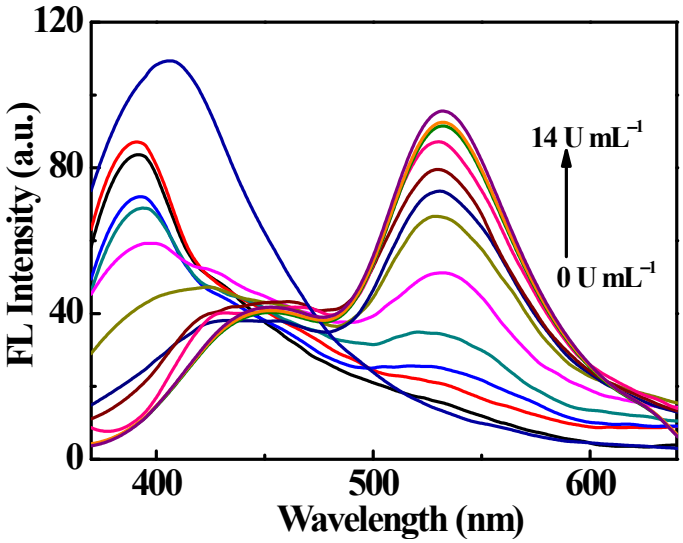
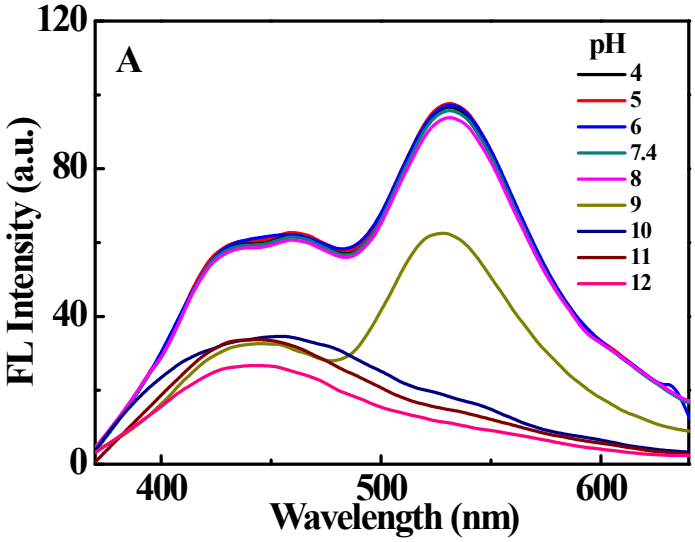
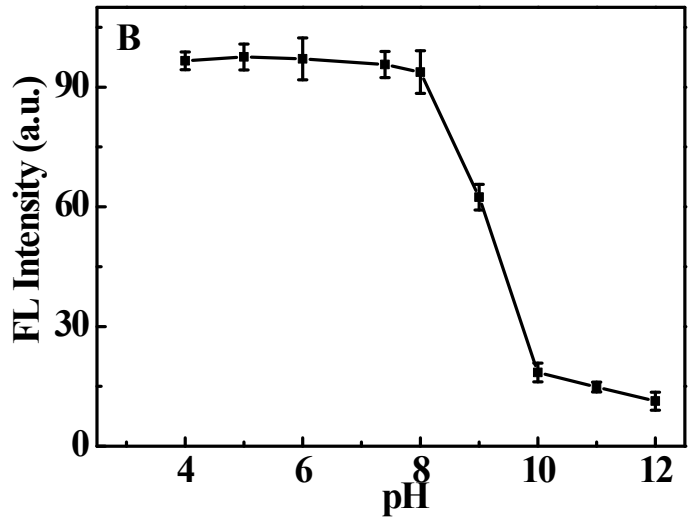
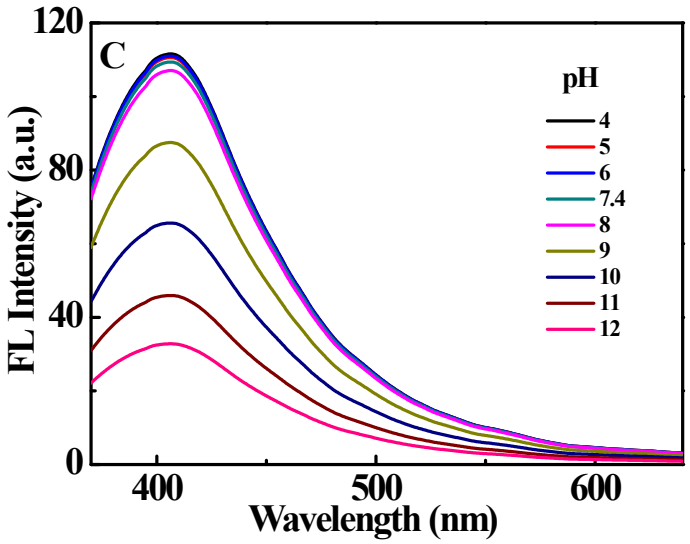


Fig. S9 FL spectra of hyperoside (10 μg mL^{-1}) after incubation with different concentrations of β-Gal (0–14 U mL^{-1}).


A
pH
4
5
6
7.4
8
9
10
11
12
FL Intensity (a.u.)
Wavelength (nm)
0
40
80
120
400
500
600


B
FL Intensity (a.u.)
pH
0
30
60
90
4
6
8
10
12


C
pH
4
5
6
7.4
8
9
10
11
12
FL Intensity (a.u.)
Wavelength (nm)
0
40
80
120
400
500
600

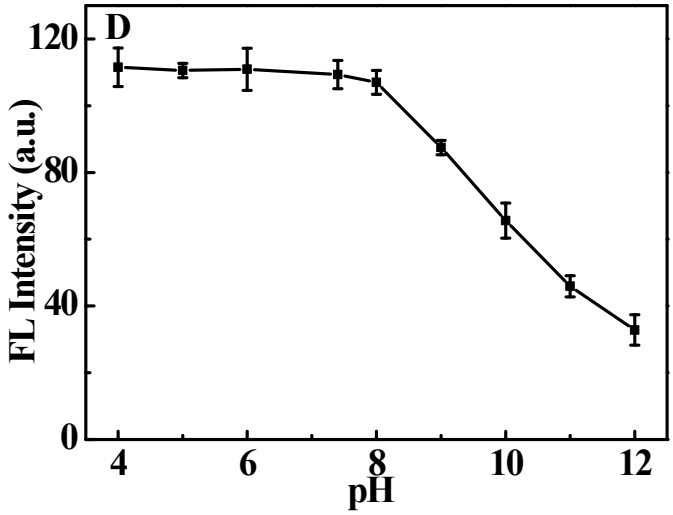


Fig. S10 FL spectra of quercetin (10 μg mL^{-1}) (A) and hyperoside (10 μg mL^{-1}) (C) at pH 4–12; FL intensity at 530 nm for quercetin (B) and FL intensity at 405 nm for hyperoside (D) at pH 4–12.

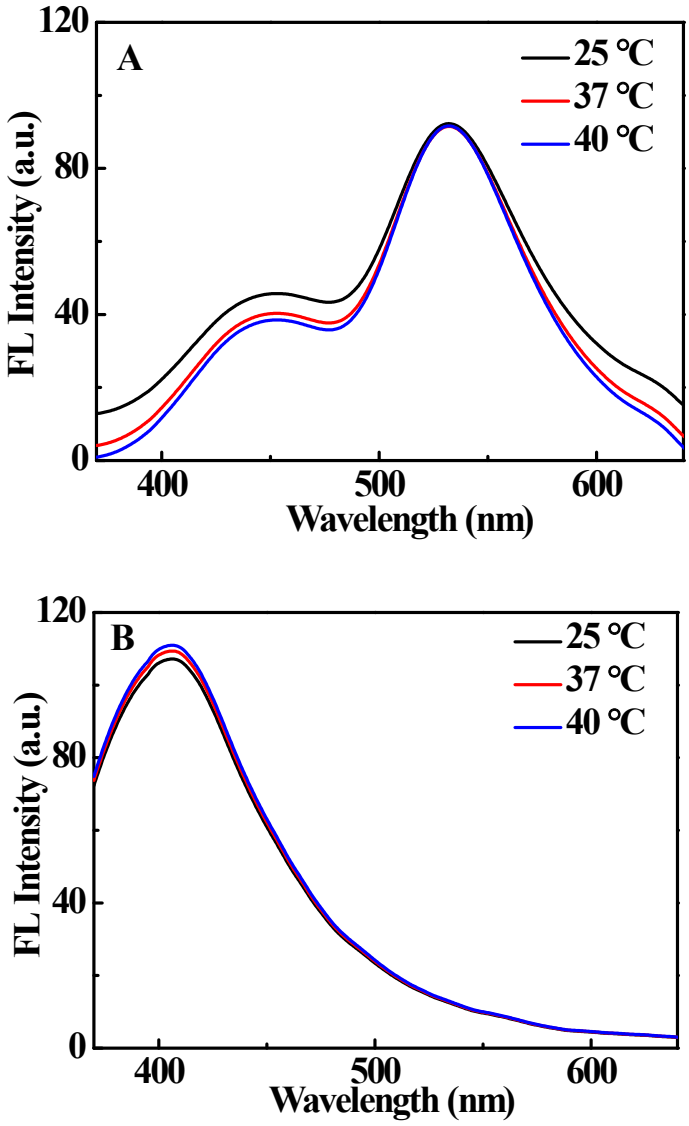


Fig. S11 FL spectra of quercetin (10 μg mL^{-1}) (A) and hyperoside (10 μg mL^{-1}) (B) at 25 ℃, 37 ℃ and 40 ℃.

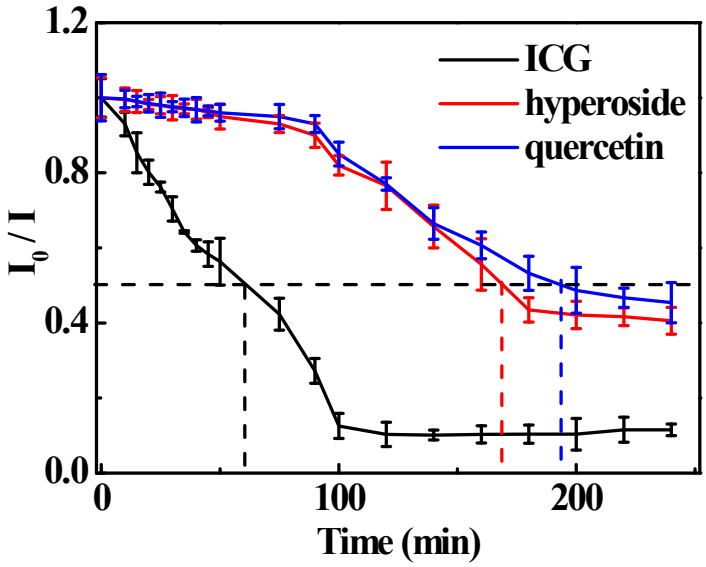


Fig. S12 Time-dependent fluorescence intensity of ICG (10 μg mL^{-1}, E_{ex} at 780 nm, E_m at 812 nm), hyperoside (10 μg mL^{-1}, E_{ex} 360 nm, E_m at 405 nm), and quercetin (10 μg mL^{-1}, E_{ex} 360 nm, E_m at 530 nm) under sustained UV irradiation.

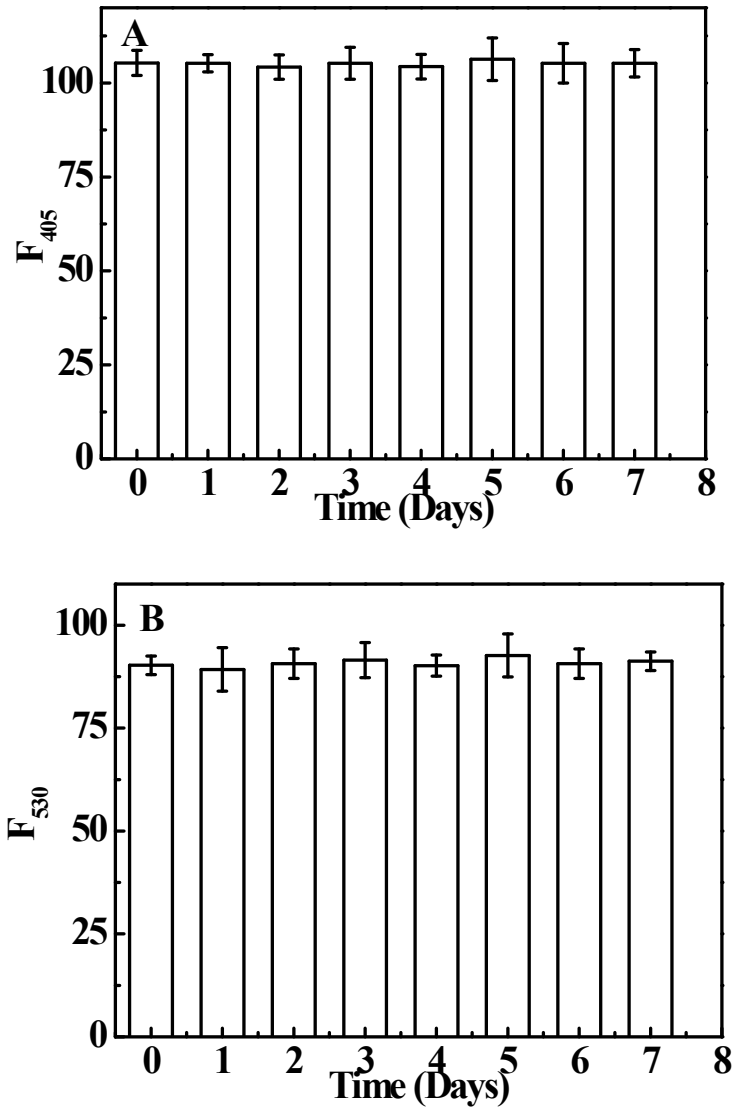


Fig. S13 FL intensity at 405 nm (F_{405}) of hyperoside (10 μg mL^{-1}) (A) and FL intensity at 530 nm (F_{530}) of quercetin (10 μg mL^{-1}) (B) in human serum for one weak.

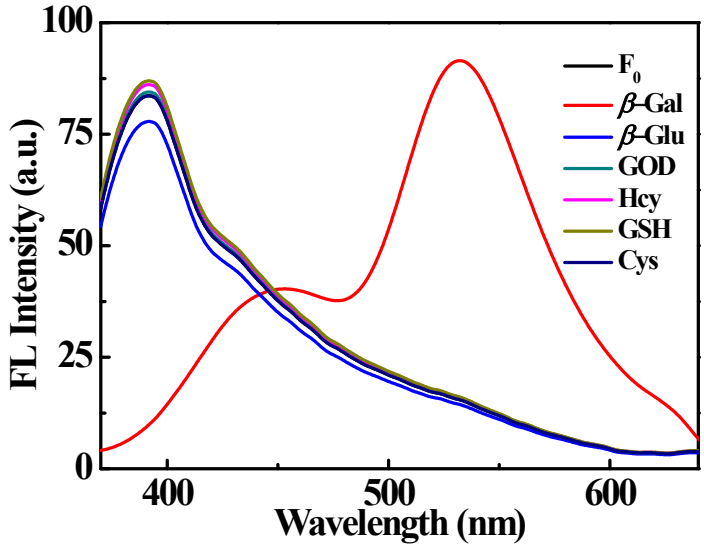
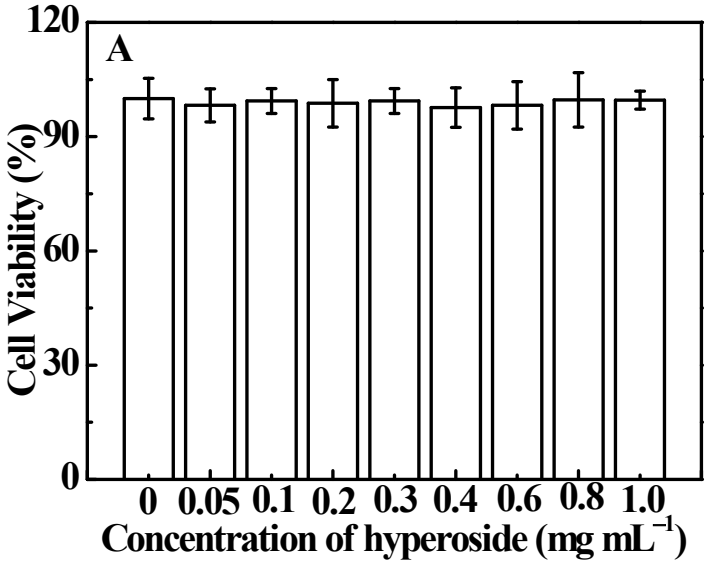
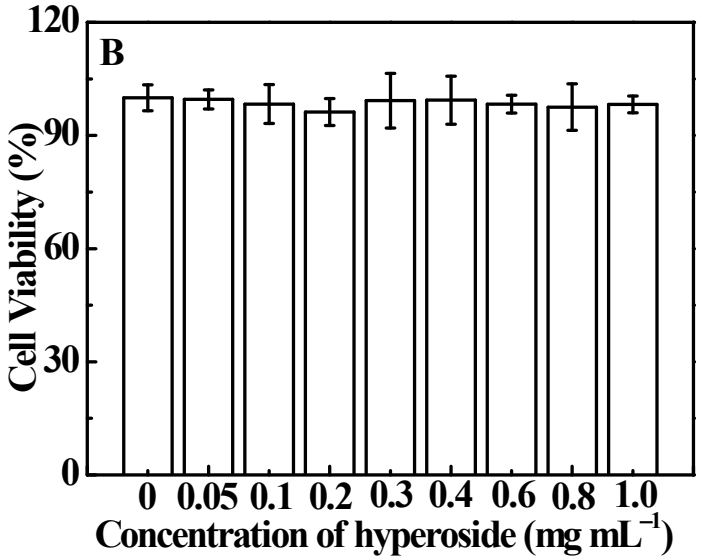
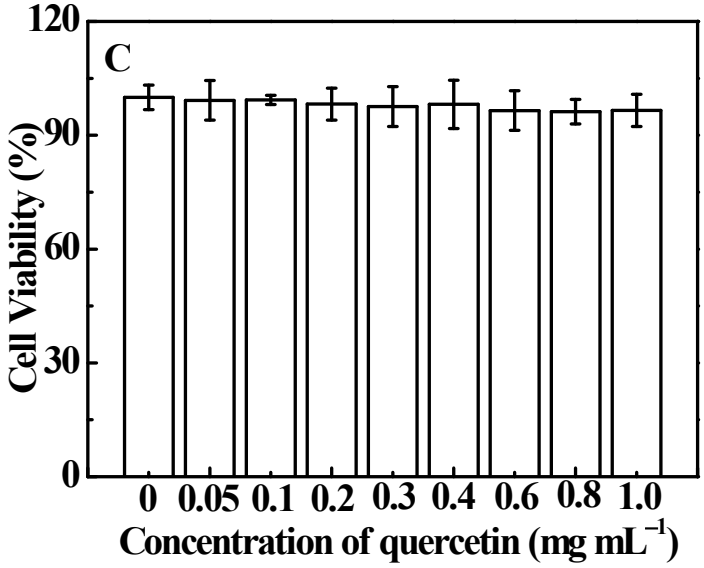


Fig. S14 FL spectra of hyperoside (10 μg mL^{-1}) after incubation with β-Gal (12 U mL^{-1}) and various other analytes (100 equiv.).


A
120
90
60
30
0
Cell Viability (%)
0 0.05 0.1 0.2 0.3 0.4 0.6 0.8 1.0
Concentration of hyperoside (mg mL^{-1})


B
120
90
60
30
0
Cell Viability (%)
0 0.05 0.1 0.2 0.3 0.4 0.6 0.8 1.0
Concentration of hyperoside (mg mL^{-1})


C
120
90
60
30
0
Cell Viability (%)
0 0.05 0.1 0.2 0.3 0.4 0.6 0.8 1.0
Concentration of quercetin (mg mL^{-1})

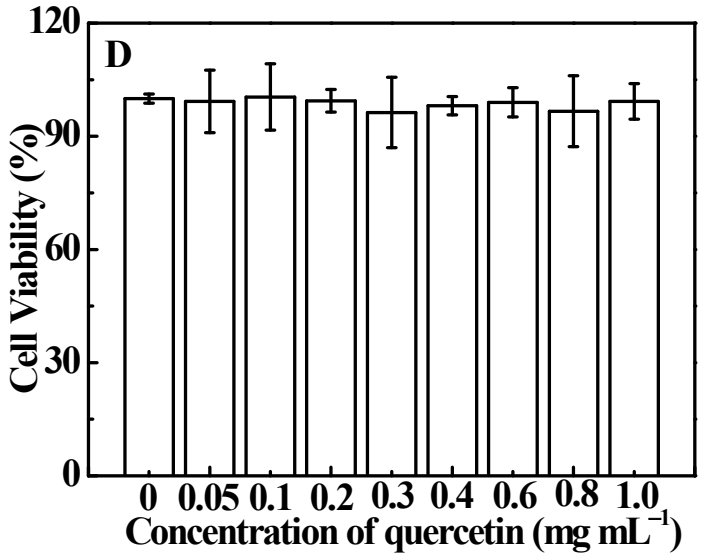


Fig. S15 Cell viabilities of HeLa (A) and SKOV-3 (B) cells treated with different concentrations of hyperoside; Cell viabilities of HeLa (C) and SKOV-3 (D) cells treated with different concentrations of quercetin.