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Electronic Supporting Information for

Phenylboronic acid-modified polyamidoamine-mediated delivery of short GC rich DNA for hepatocarcinoma gene therapy

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Experimental section

1. Boron content analysis

The boron content of carrier was determined by an inductively coupled plasma optical emission spectrometer (ICP-OES, Agilent 725, Foster City, CA). The carrier PP and PBA were digested with HNO_3 solution to obtain samples with final concentrations of 2.84 mg/mL and 10.0 μg/mL, respectively. The final concentration of HNO_3 was kept at 1% v/v.

2. Gel retardation assay

The GCD condensation ability of PP was determined by gel retardation assay. The PP/GCD nanoparticles were formed by mixing these two components at different N/P ratios (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8). The nanoparticles were incubated at 37 °C for 30 min, and then loaded to 1% agarose gel electrophoresis in Tris-acetate-EDTA buffer (120 V, 20 min). Further, to detect the stability of nanoparticles, PP/GCD (N/P ratio of 30) was treated with DNase I solution (5 U) at 37 ^{o}C for 20 min, and then kept at 80 ^{o}C for 30 min to inactivate DNase I. Finally, the samples were subjected to the incubation with heparin sodium solution at a final concentration of 5.0 μg/mL for 20 min and then loaded to 1% agarose gel electrophoresis as described above. The gel was stained with ethidium bromide and captured by Bio-Rad Universal Hood II imaging system (Hercules, CA).

3. *In vitro* transfection assay of PP/GCD nanoparticles

The HepG2 cells (1.5×10^5 cells/well) were seeded in 6-well plates and cultured overnight. Then the cells were incubated with nanoparticles at different N/P ratios of 10, 20, 30 and 40 (5 μg/mL FAM-GCD) in serum-free medium for 4 h. Finally, the cells were collected and analyzed by Calibur flow cytometry (BD Bioscience Mountain View, USA).

4. Intracellular distribution analysis of PP/GCD nanoparticles

The HepG2 cells were inoculated in a 6-well plate with a sterilized coverslip (initial density of 1.2×10^5 cells/well) and cultured at 37 ^{o}C for 24 h. Subsequently, the cells were incubated with 1 mL FBS-free medium containing PP/Cy3-GCD nanoparticles (N/P ratio of 30, 5.0 μg/mL Cy3-GCD) for 2, 4 and 6 h, respectively. Afterwards, the cells were stained with LysoTracker Green for 10 min and washed with PBS three times. Finally, the coverslip was taken from the well, and the intracellular distribution of nanoparticles was analyzed by LSM 710 confocal laser scanning microscope (Carl Zeiss Microscopy LLC, Jena, Germany).

5. Cytotoxicity analysis of PP and PAMAM

The cell cytotoxicity of PP and PAMAM was evaluated by MTT methods. Briefly, HepG2 cells and human hepatocyte L02 cells were seeded into 96-well plates at a density

of 8.0×10^3 cells/well and incubated in DMEM containing 10% FBS at 37 °C overnight. The cells were treated with PP and PAMAM of different concentrations for 48 h. Next, 20 μL of MTT solution (5 mg/mL) was added into individual wells and incubated at 37 °C for 4 h. The formazan crystal was dissolved in 200 μL dimethyl sulfoxide (DMSO), and the absorbance at 492 nm was detected on a GF-M3000 microplate reader (CAIHONG, Shandong, China). The cell viability was calculated as the ratio of the absorbance values of treated and untreated cells.

6. Live/dead cell staining

The HepG2 cells (2.0×10^5 cells/well) were seeded into 6-well plates and incubated overnight. Then the cells were treated with PP/GCD and PAMAM/GCD nanoparticles (N/P ratio of 30, 5 μg/mL GCD) in serum-free medium for 6 h, and incubated in 10% FBS-containing DMEM for 48 h. After washing with PBS three times, the cells were stained by live/dead reagents for 20 min according to the manufacturer's instructions. Finally, the cells were washed with PBS twice and observed by Olympus IX73P1F fluorescence microscopy (Tokyo, Japan).

7. Inhibition of colony formation

The HepG2 cells were seeded into 6-well plates at a density of 2.0×10^5 cells/well and incubated overnight. The cells were treated with different nanoparticles (N/P ratio of 30, 5 μg/mL GCD) as described above. The treated cells were digested using 0.25% trypsin and re-seeded into individual wells of 6-well plates at a density of 2.0×10^4 cells/well for 7 days. Afterwards the cells were fixed with 75% (v/v) cold ethanol at 4 °C for 20 min and stained with 0.2% crystal violet solution for 15 min. Finally, the colony formation was observed and photographed on an Olympus IX73P1F fluorescence microscopy (Tokyo, Japan).

8. Western blotting analysis

The treated cells were harvested, washed with PBS three times and lysed in the RIPA lysis buffer supplemented with 1 mM PMSF on ice for 1 h. Then the supernatants were collected by the centrifugation at 12,000 rpm for 15 min, and the total protein concentration was determined by BCA assay kit. Equal amounts of proteins were subjected to 12% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membrane by electroblotting. Further, the membrane was blocked with PBST solution (0.1% (v/v) Tween in PBS buffer) containing 5% (w/v) fat-free milk power at room temperature for 2 h, and then incubated with the corresponding primary antibody (β-actin, procaspase-3, procaspase-8, procaspase-9, p53, β-tubulin) at 4 °C overnight. The membrane was washed with PBST three times and incubated with HRP-labeled secondary antibody at room temperature for 1 h. Finally, the

membrane was rinsed with PBST three times and visualized by enhanced chemiluminescence on Tanon 2500 chemiluminescence imaging system (Shanghai, China).

9. Caspase-3, -8 and -9 activities assay

The HepG2 cells were cultured and treated with different nanoparticles as described above. The activities of caspase-3, -8 and -9 in the transfected HepG2 cells were measured using the corresponding assay kits. Briefly, the treated cells were collected and suspended in the lysis buffer. The cell lysates were incubated with their individual substrates at room temperature for 2 h. The luminescence signal of caspase activities was detected by TECAN Infinite F200 Pro plate reader (Mannedorf, Switzerland).

10. Mitochondrial membrane potential analysis

The HepG2 cells were plated into 6-well plates at a density of 2.0×10^5 cells/well and treated with different nanoparticles as described above. The transfected cells were stained with JC-1 dye at 37 °C for 30 min and washed with PBS twice, and the mitochondrial membrane potential was measured on an Olympus IX73P1F fluorescence microscopy (Tokyo, Japan).

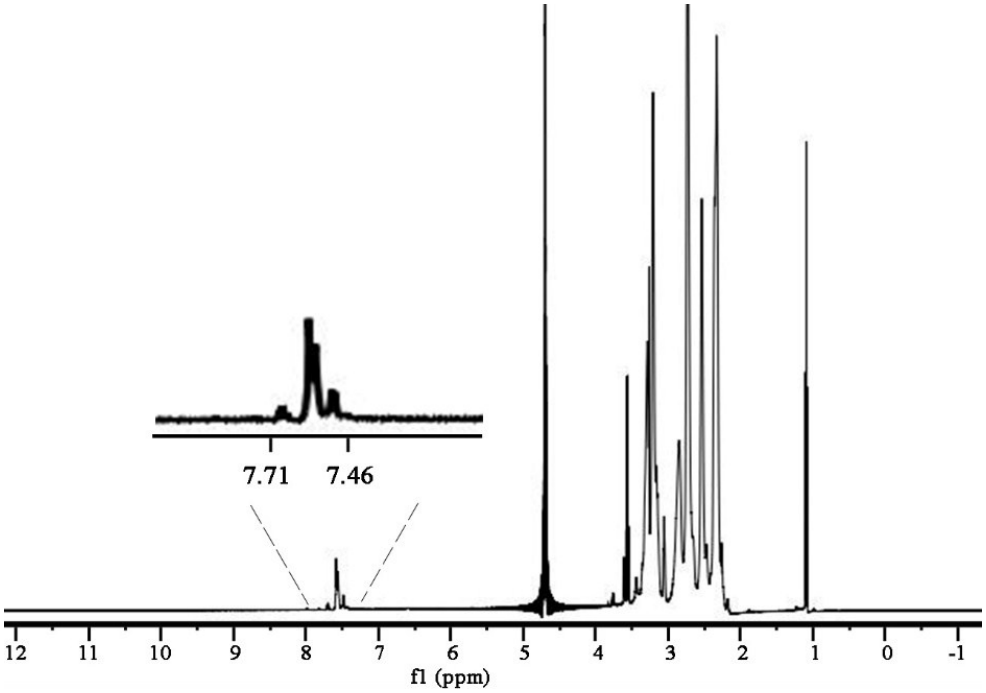

Fig. S1 The ^{1}H NMR spectrum of PP in D_2O.

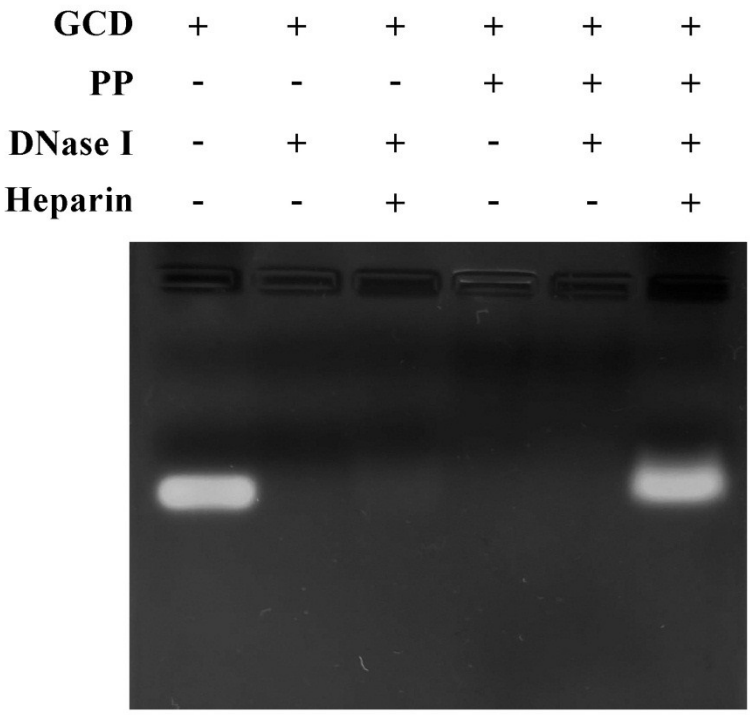

Fig. S2 Gel retardation assay of PP/GCD nanoparticles at an N/P ratio of 30 after the DNase I treatment (5 U for 20 min).

Table S1. The hydrodynamic diameter and zeta potential of free PP and PP/GCD nanoparticles at different N/P ratios. Data were presented as mean ± SD of triplicate experiments.

Nanoparticles	N/P ratio	Size (nm)	PDI	Zeta potential (mV)
free PP		307.17 ± 19.1	0.569	+12.53 ± 0.23
PP/GCD	10	280.70 ± 2.77	0.189	+27.20 ± 0.42
	20	210.70 ± 3.50	0.112	+29.17 ± 0.72
	30	188.47 ± 1.68	0.116	+30.83 ± 0.95
	40	211.90 ± 3.21	0.124	+34.50 ± 3.08

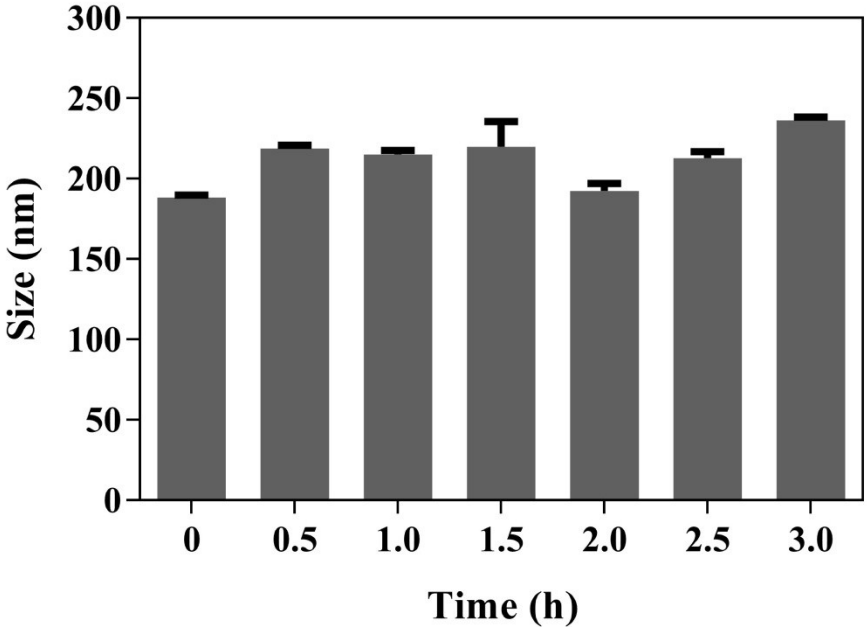

Fig. S3 The particle size change of PP/GCD nanoparticles (N/P ratio of 30) with the elongation of incubation time in PBS.

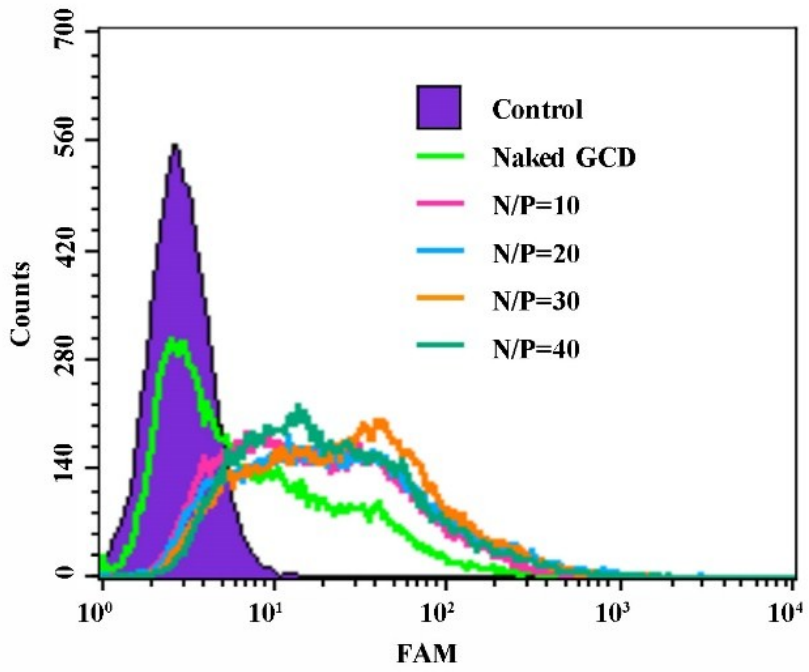

Fig. S4 Transfection efficiency analysis of PP/GCD nanoparticles in HepG2 cells at different N/P ratios using flow cytometry.

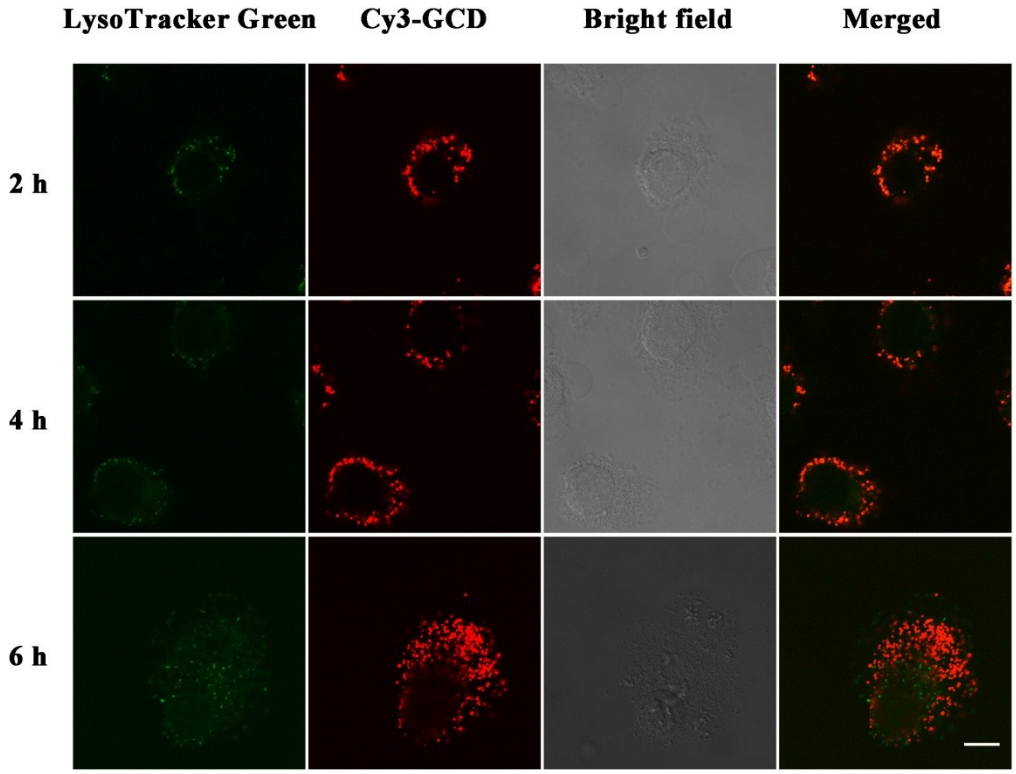

Fig. S5 Confocal laser scanning microscopic images of HepG2 cells exposed to PP/GCD nanoparticles for 2, 4 and 6 h. Green (LysoTracker Green) and red (Cy3-labeled GCD). The scale bar is 10 μm.

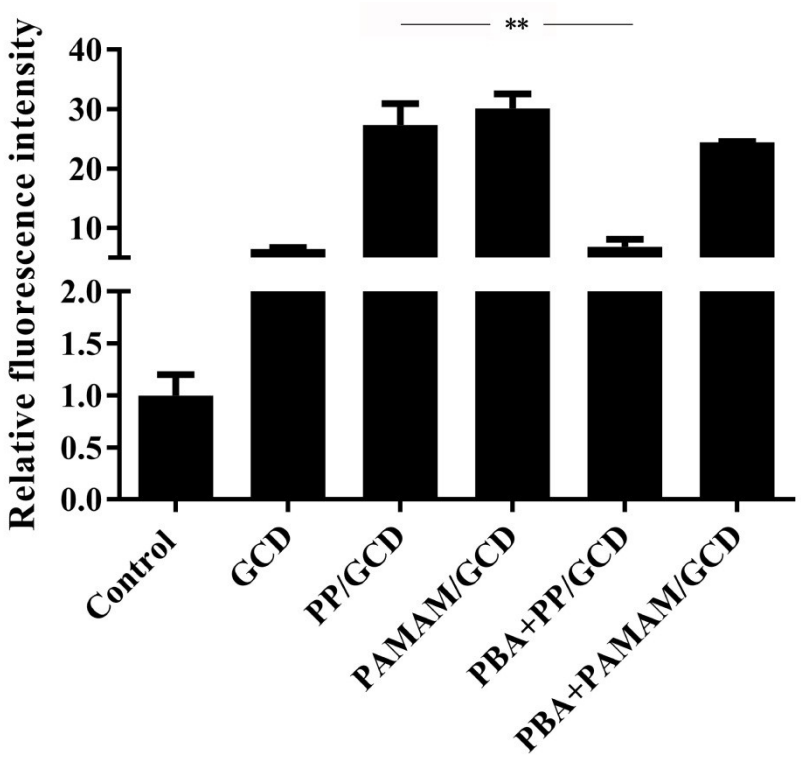

Fig. S6 Relative fluorescence intensity analysis of the confocal laser scanning microscopic images of HepG2 cells treated with different nanoparticles in Fig. 2 using Image J software (**p<0.01).

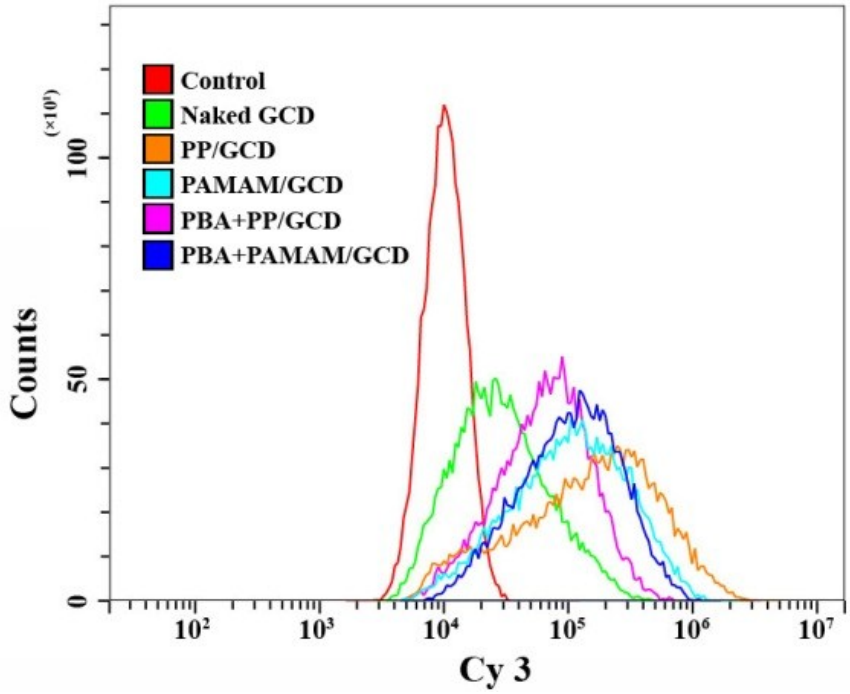

Fig. S7 The cellular uptake efficiency of different nanoparticles in HepG2 cells.

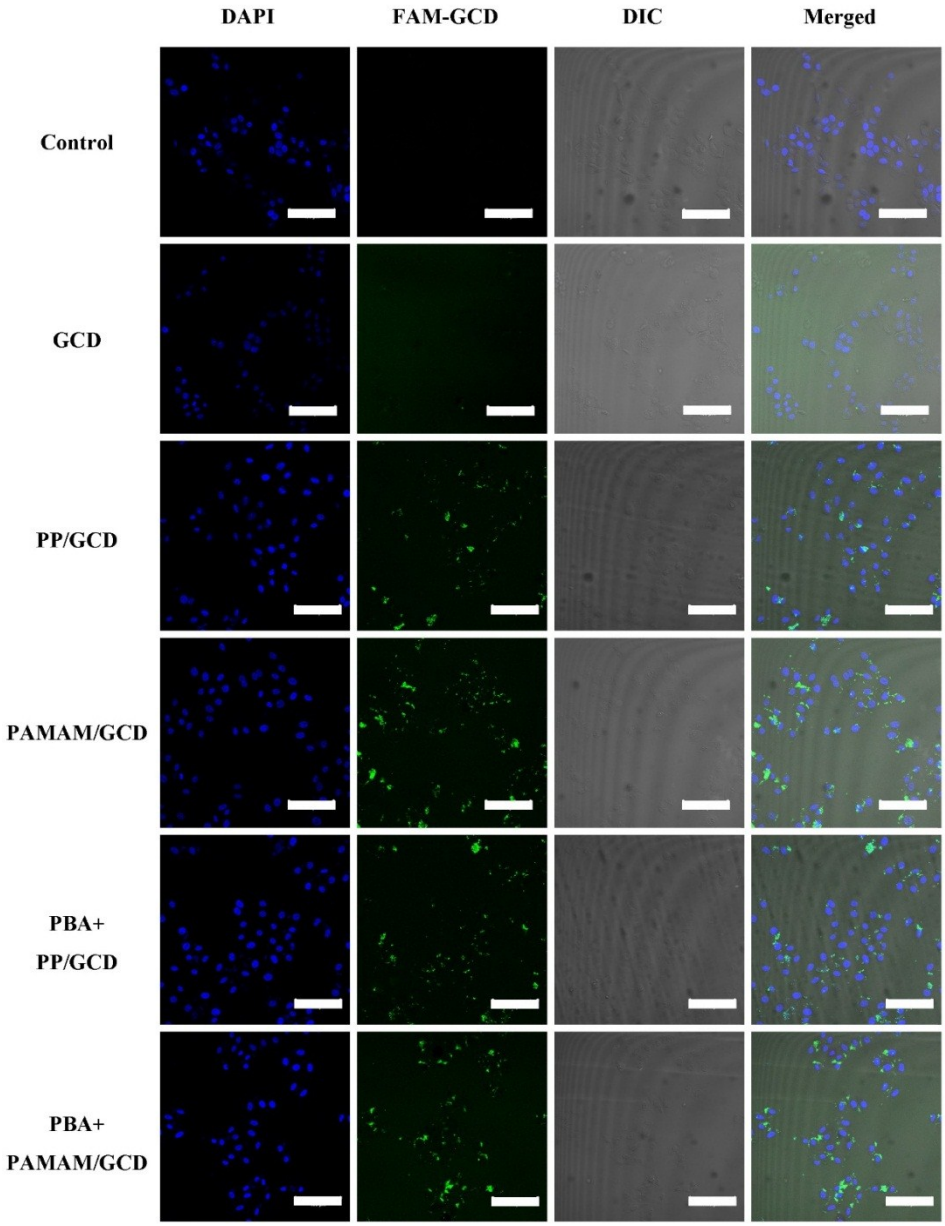

Fig. S8 The confocal laser scanning microscopic images of L02 cells treated with different nanoparticles: blue (DAPI, nuclei) and green (FAM-labeled GCD). The scale bar is 100 μm.

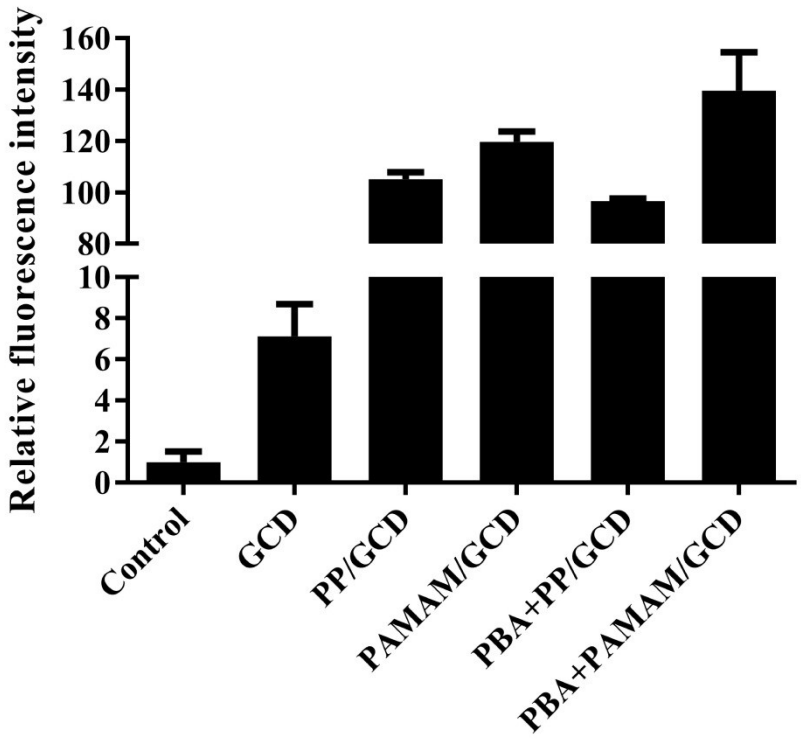

Fig. S9 Relative fluorescence intensity analysis of the confocal laser scanning microscopic images of L02 cells treated with different nanoparticles in Fig. S8 using Image J software.

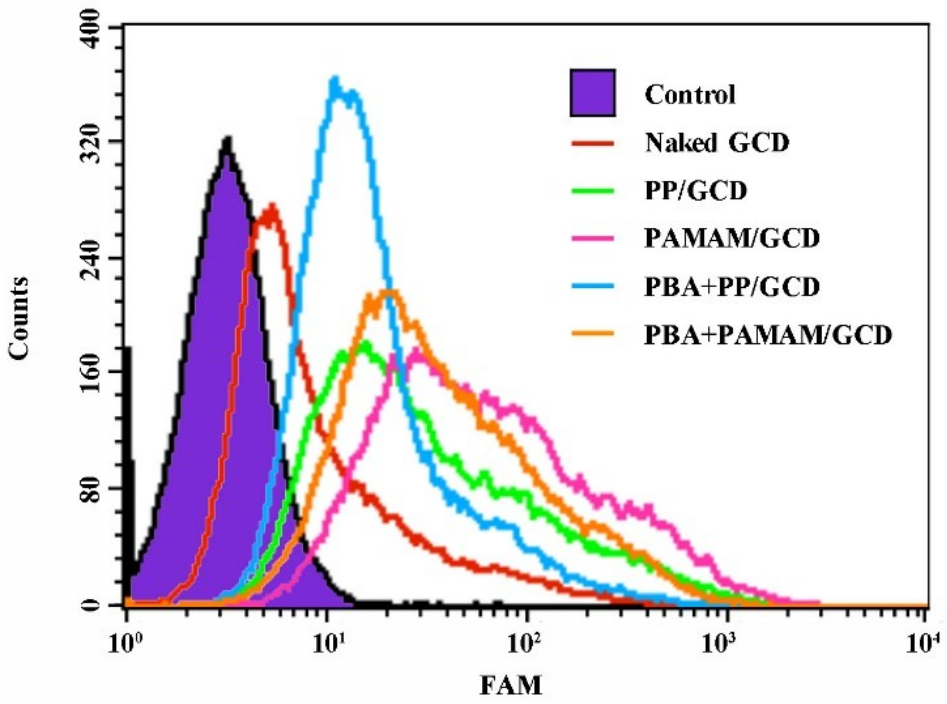

Fig. S10 The cellular uptake efficiency of different nanoparticles in L02 cells.

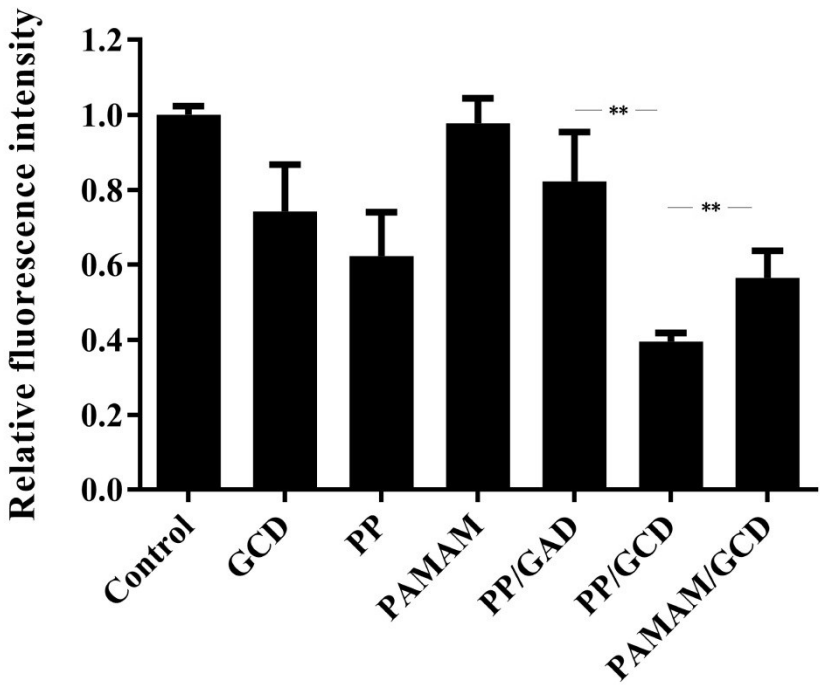

Fig. S11 Relative fluorescence intensity analysis of the β-tubulin immunofluorescence in HepG2 cells treated with different nanoparticles in Fig. 3A using Image J software (**p<0.01).

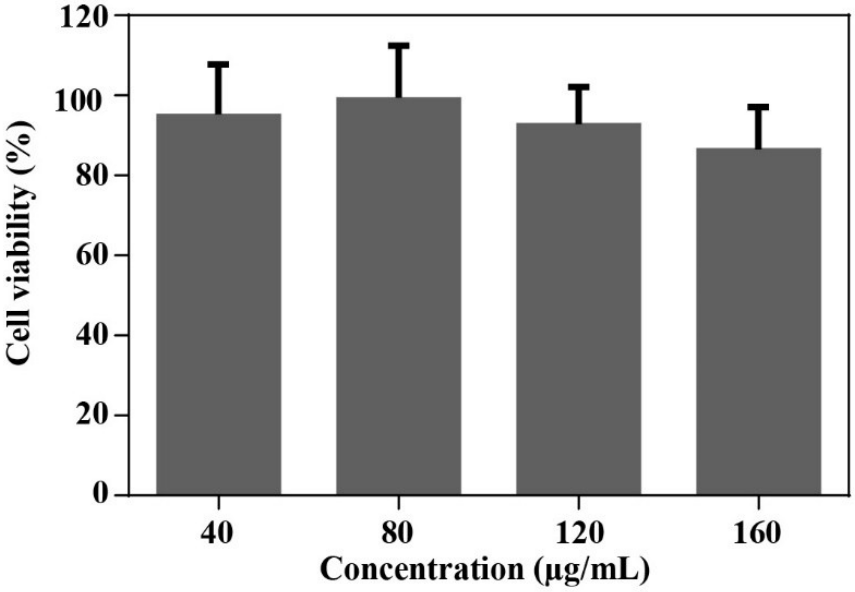

Fig. S12 The cytotoxicity analysis of PP at different concentrations in L02 cells.

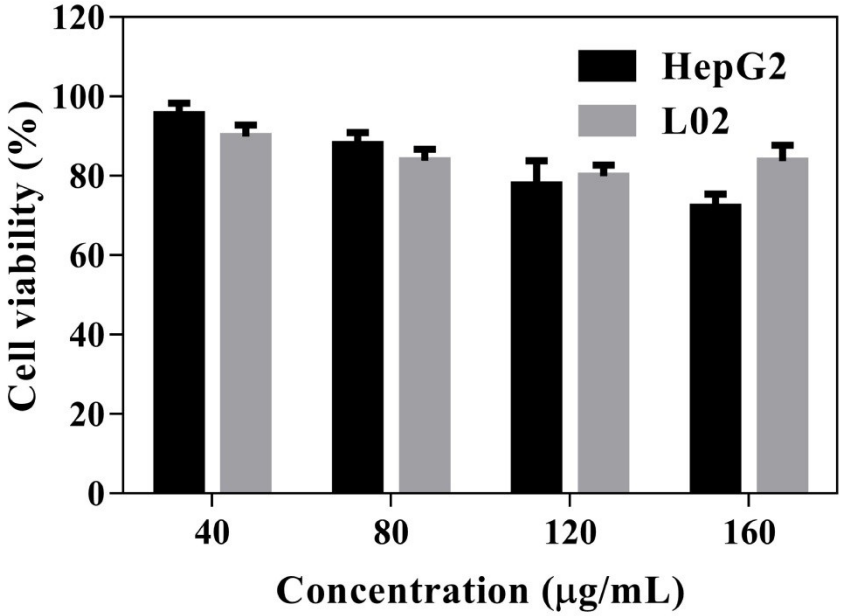

Fig. S13 The cytotoxicity analysis of PAMAM at different concentrations in HepG2 and L02 cells.

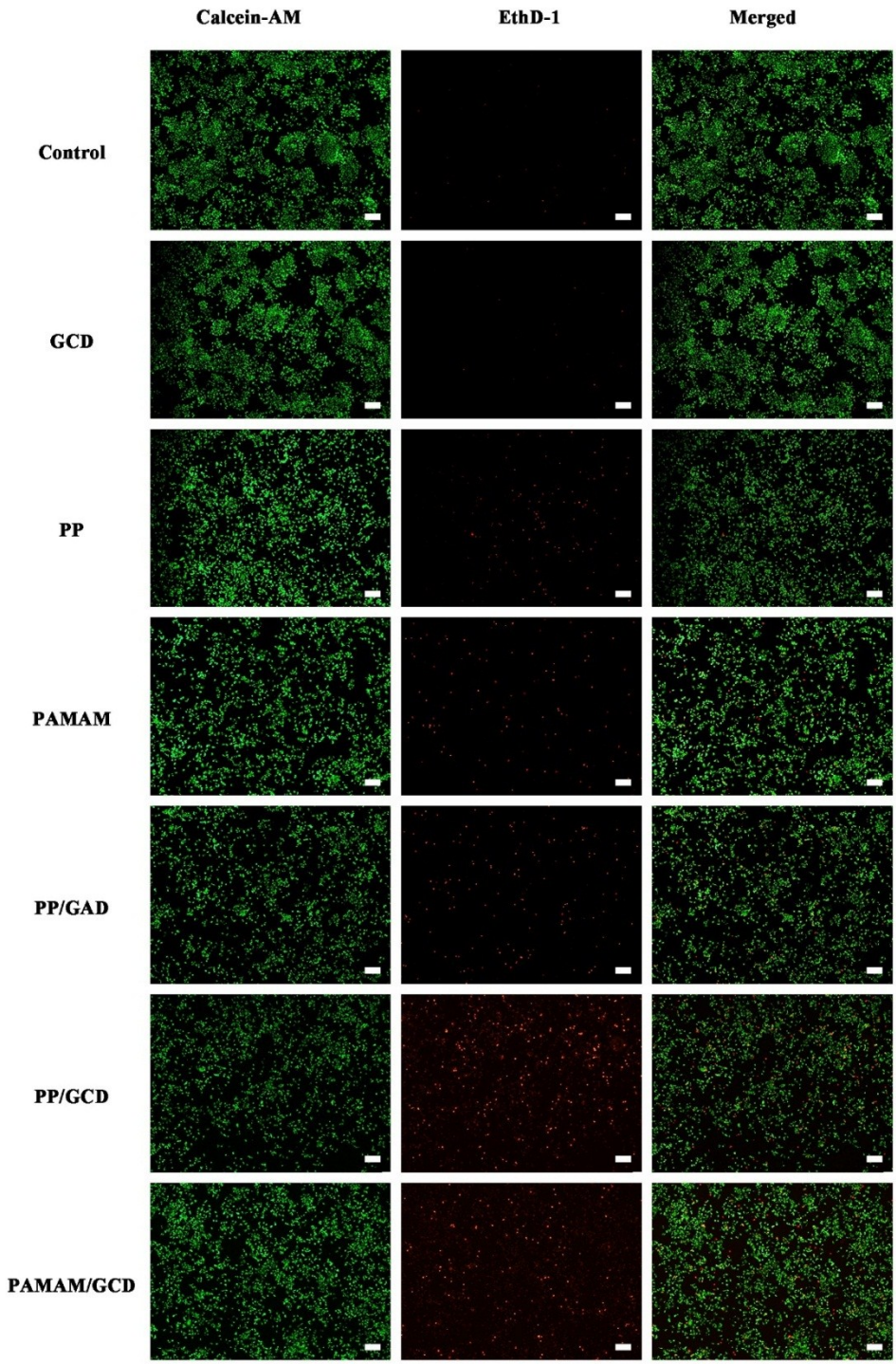

Fig. S14 Live/dead staining assay of HepG2 cells after the GCD transfection mediated by different carriers. The scale bar is 200 μm.

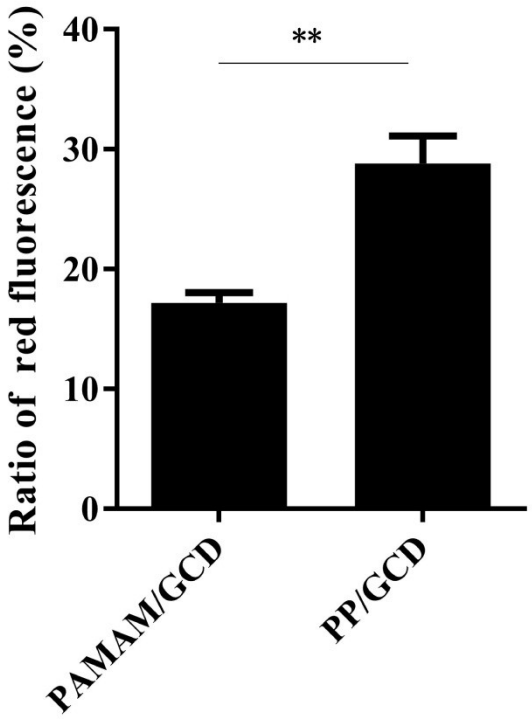

Fig. S15 The quantitative ratio analysis of red fluorescence in the total fluorescence in Live/dead staining of HepG2 cells after the PAMAM/GCD and PP/GCD transfection in Fig. S14, using Image J software (**p<0.01).

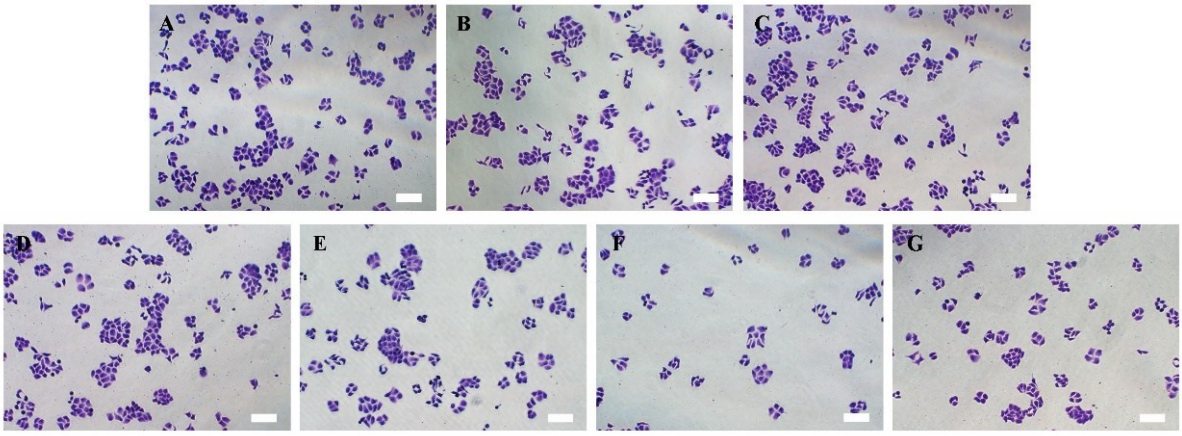

Fig. S16 Colony formation assay of HepG2 cells treated with different nanoparticles: (A) control; (B) GCD; (C) PP; (D) PAMAM; (E) PP/GAD; (F) PP/GCD; and (G) PAMAM/GCD. The scale bar is 200 μm.

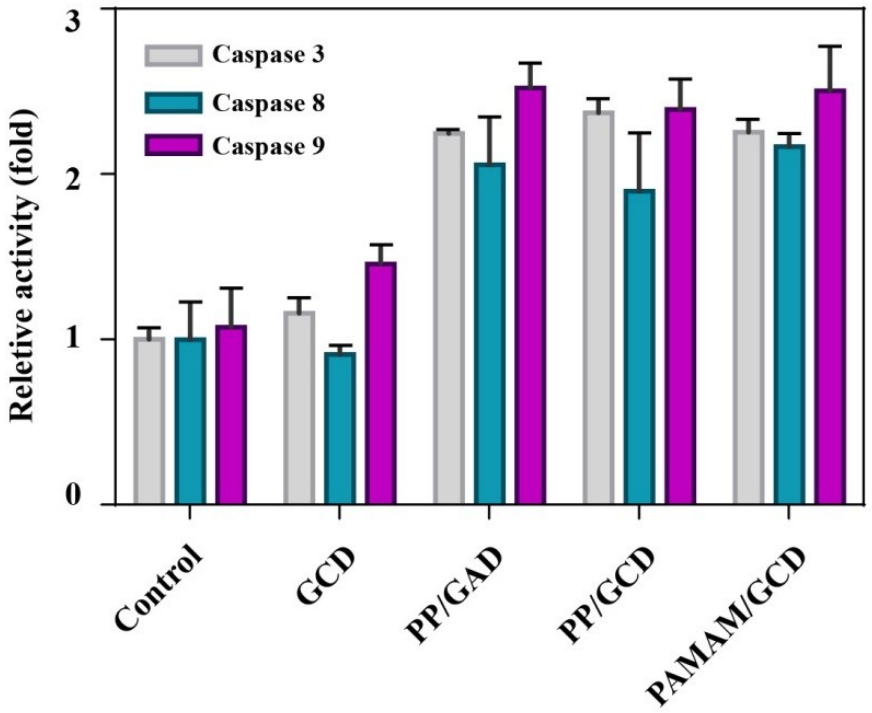

Fig. S17 The relative activities of caspase-3, -8 and -9 in HepG2 cells after the treatment with different nanoparticles.

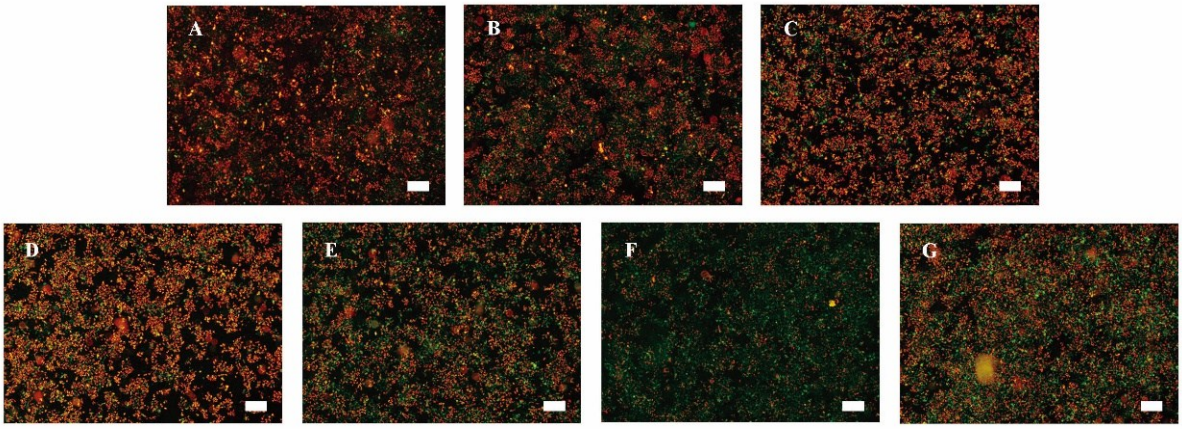

Fig. S18 The mitochondrial membrane potential of HepG2 cells treated with different nanoparticles: (A) control; (B) GCD; (C) PP; (D) PAMAM; (E) PP/GAD; (F) PP/GCD and (G) PAMAM/GCD. The scale bar is 200 μm.

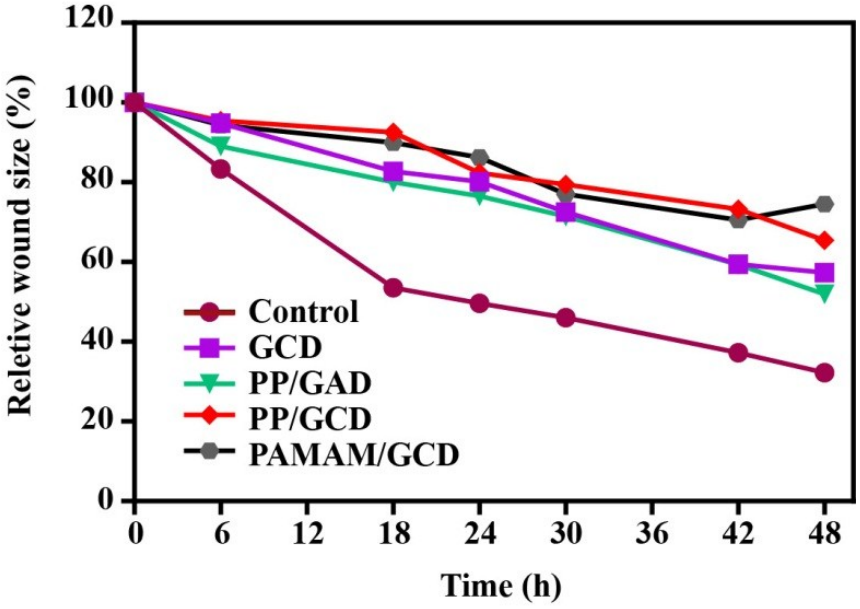

Fig. S19 Quantitative analysis of wound healing size in HepG2 cells after the treatment with different nanoparticles.

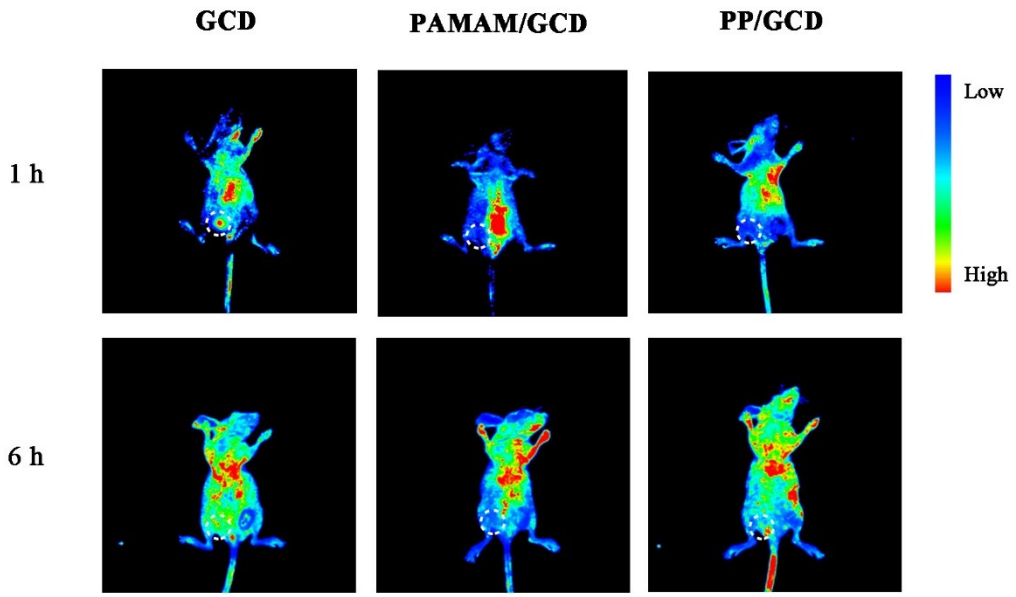

Fig. S20 The biodistribution of Cy3-labled GCD nanoparticles after the HepG2 tumor-bearing nude mice were intravenously injected with different nanoparticles for 1 and 6 h. (n=3 per group).

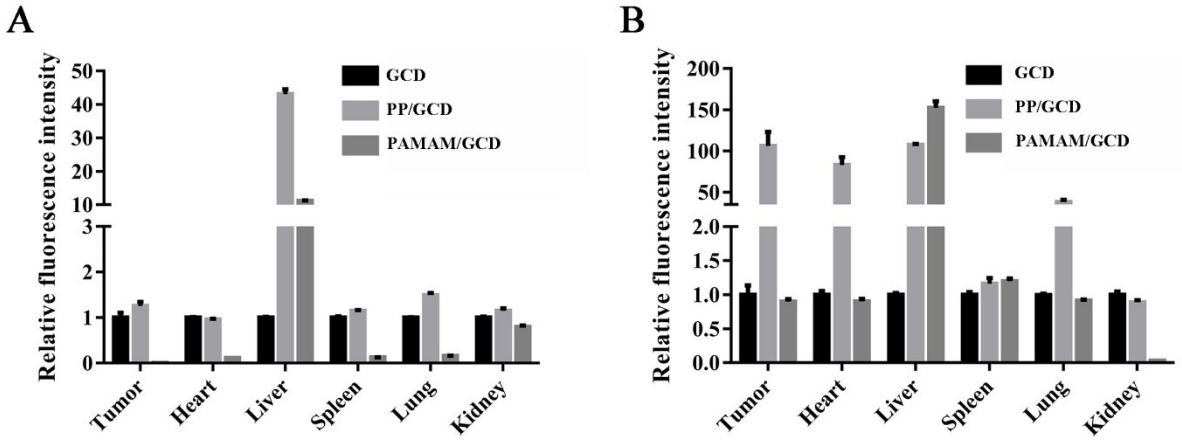

Fig. S21 Quantitative analysis for the biodistribution of different nanoparticles in major organs after the intravenous injection for 1 h (A) and 6 h (B) in Fig. 6, using Image J software.