Support Information

Iron Oxide Nanoparticles for Targeted Imaging of Liver Tumor with Ultralow Hepatotoxicity

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1. Standard curves of GAEN and iron

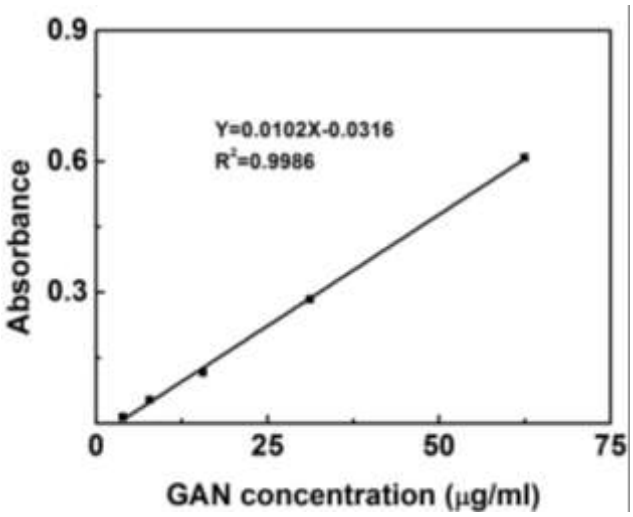

Fig. S1 Standard curve of GAEN solutions in different concentrations.

2. Morphology of Fe_3O_4 nanoparticles

We added 10 μl of Fe_3O_4 nanoparticles on copper grid, and dry them in the air at room temperature. We observed the morphology of nanoparticles by TEM (T20, FEIC) at 200 kV.

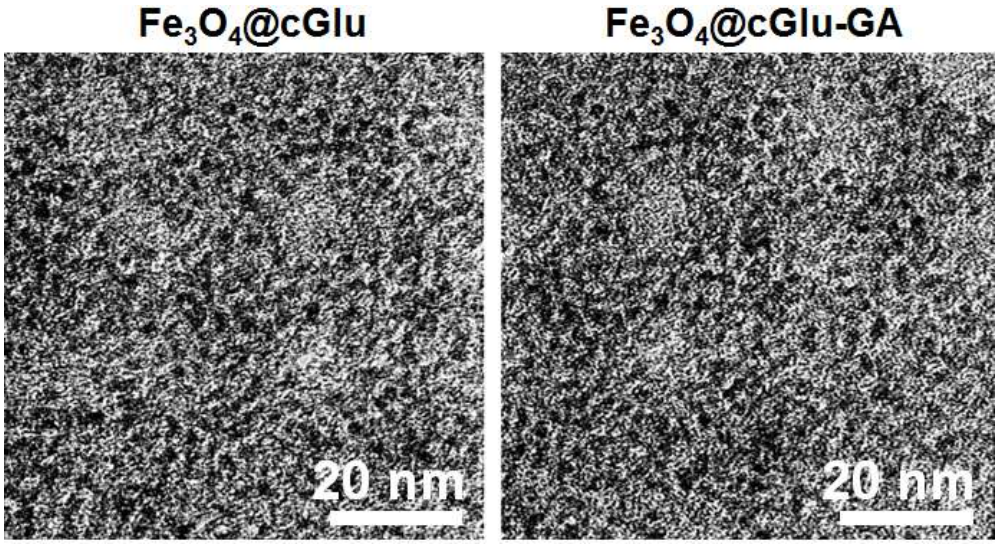

Fig. S2 TEM images of Fe_3O_4@cGlu and Fe_3O_4@cGlu-GA nanoparticles. We measured the average size of the nanoparticles by software (Nanomeasure 1.2).

3. Hydrodynamic size of Fe_3O_4 nanoparticles

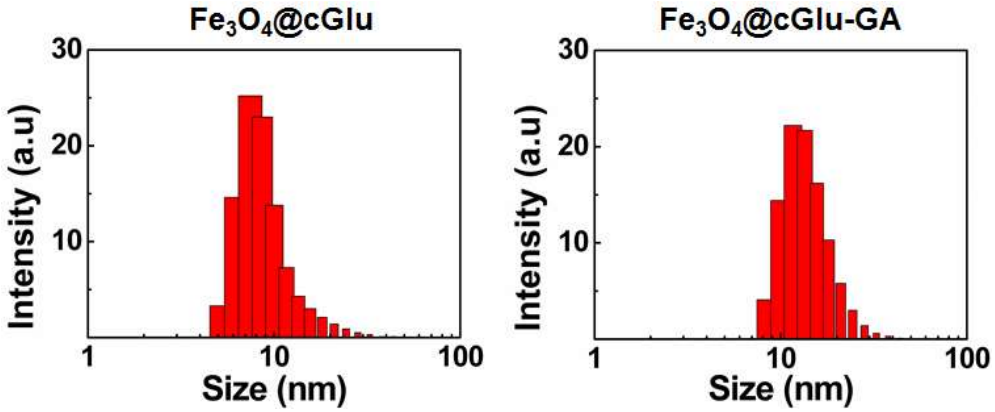

Fig. S3 Hydrodynamic size of Fe_3O_4 nanoparticles.

4. Cell viability of Fe_3O_4 nanoparticles

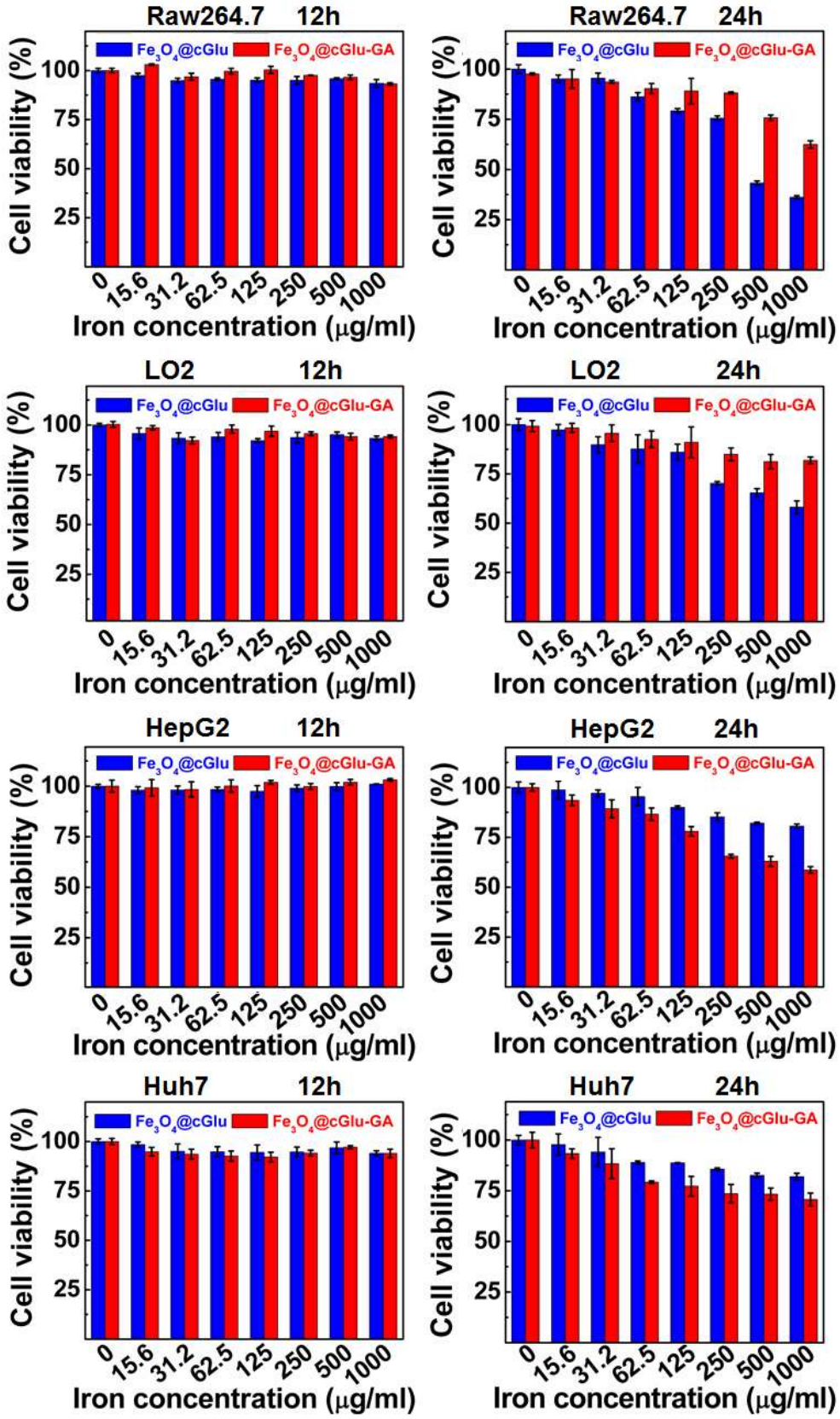

Fig. S4 Cell viability of Fe_3O_4 nanoparticles.

5. Binding ability of Raw264.7, LO2, HepG2, and Huh7 with GA

Specific binding of cells

We synthesized the GA-FITC according to literature.[1, 2] We seeded 2×10^6 of Raw264.7, LO2, HepG2, and Huh7 cells in 6-well culture plates, and treated the cells with FITC-GA for 2 h at 37 °C. We measured the fluorescence intensity (λex= 490 nm, λem= 525 nm) of 1×10^5 cells after they were washed with PBS by multimode reader, which was the total binding (TB).

We seeded 2×10^6 of Raw264.7, LO2, HepG2, and Huh7 cells, and treated the cells with 1 μM of GA for 0.5 h at 37 °C. We treated the cells with FITC-GA for 2 h at 37 °C, and measured the fluorescence intensity (λex= 490 nm, λem= 525 nm) of 1×10^5 cells, which was the non-specific binding (NB). We calculated the specific binding (SB) of these cells according to the follow equation.

SB=TB-NB

Confocal

We seeded 2×10^5 of Raw264.7, LO2, HepG2, and Huh7 cells in confocal dish, and treated them with FITC-GA (10 μM, green fluorescence) for 2 h. We observed the cells under confocal microscope (Zeiss 710, Zeiss) after they were washed by PBS.

Flow cytometry analysis

We seeded Raw264.7, LO2, HepG2, and Huh7 cells in 6-well plates (1×10^6 cells/well), and incubated them overnight at 37 ^{o}C. We treated the cells with GA for 0.5 h and FITC-GA for 2 h at 37 °C (GA/GA-FITC), or treated the cells with FITC-GA for 2 h. We washed and suspended the cells in medium, and counted the cells (2×10^5 cells) in flow cytometer (BD Biosciences, USA).

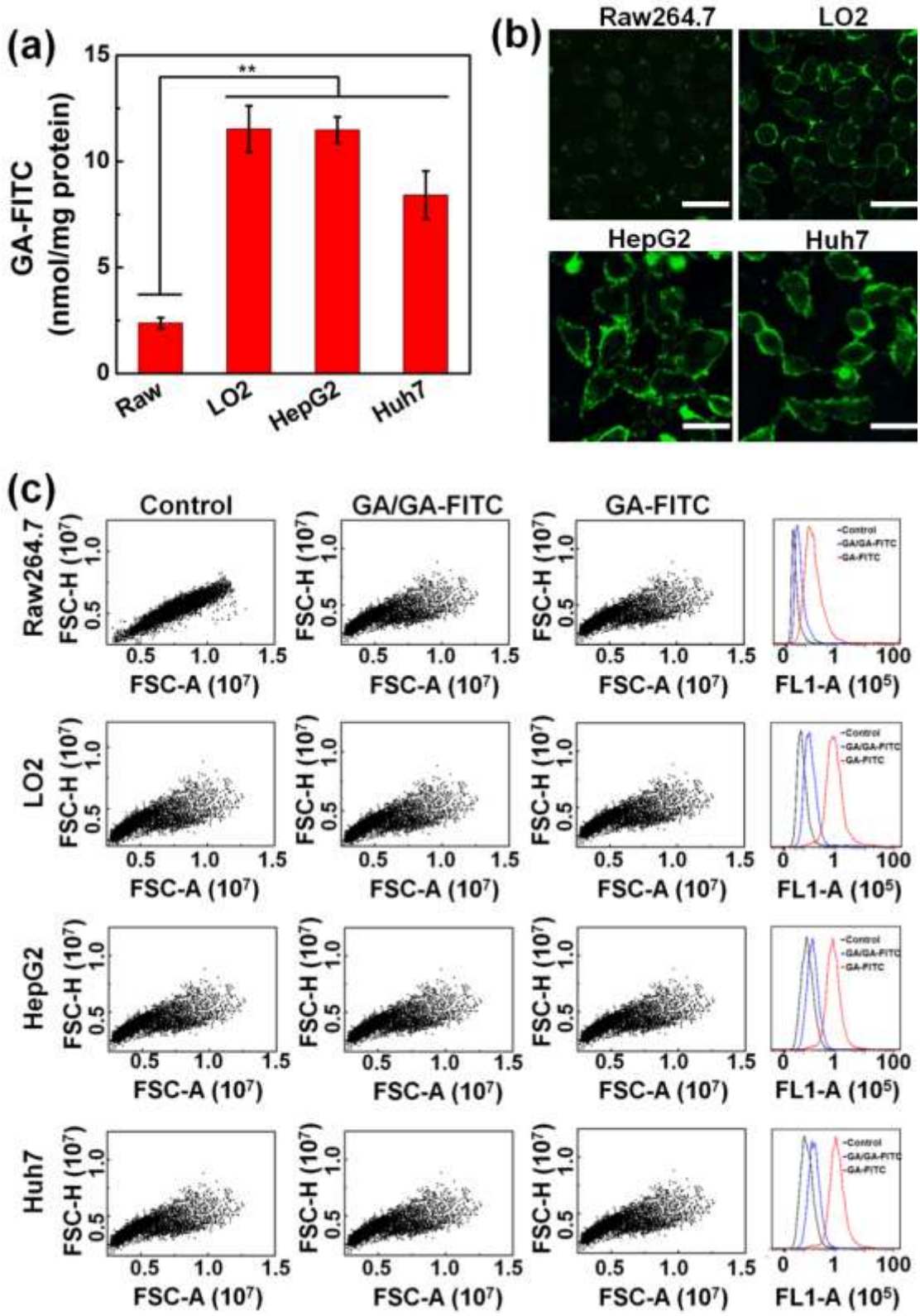

Fig. S5 Binding ability of Raw264.7, LO2, HepG2, and Huh7 with GA. (a) Specific binding of GA on cells. (b) Confocal images of cells after treated by GA-FITC. (c) Flow cytometry results of cells after treated by GA-FITC.

6. TEM images of cells after treated by Fe_3O_4 nanoparticles

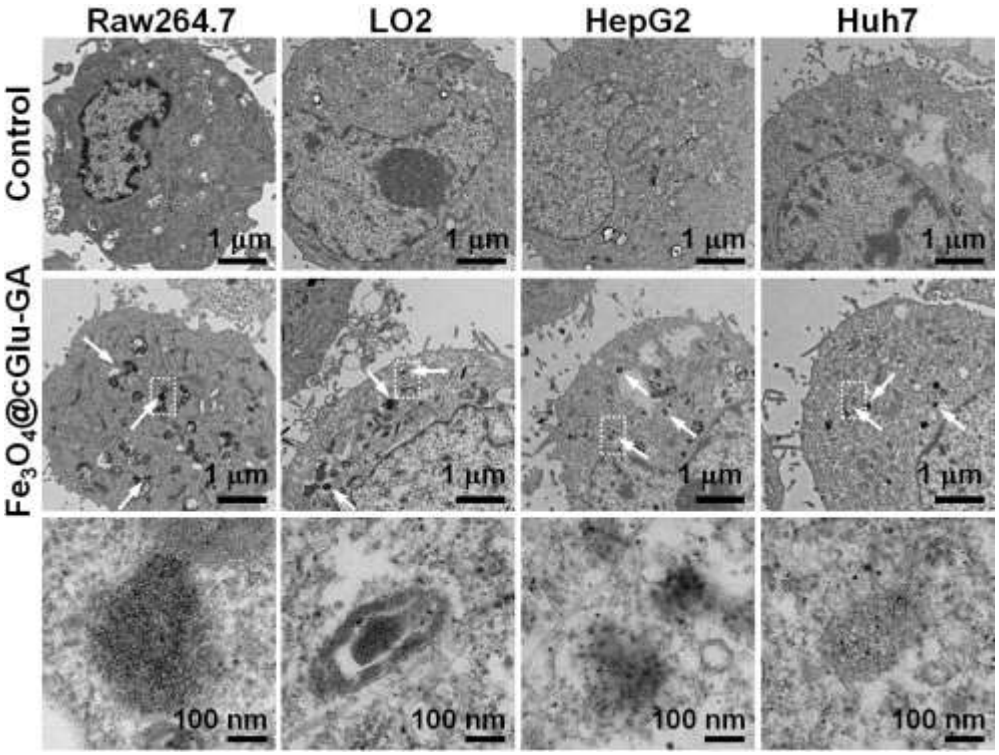

Fig. S6 TEM images of Raw264.7, LO2, HepG2, and Huh7 cells after treated by Fe_3O_4@cGlu-GA nanoparticles.

7. Prussian blue staining of livers, kidneys, and orthotopic tumors

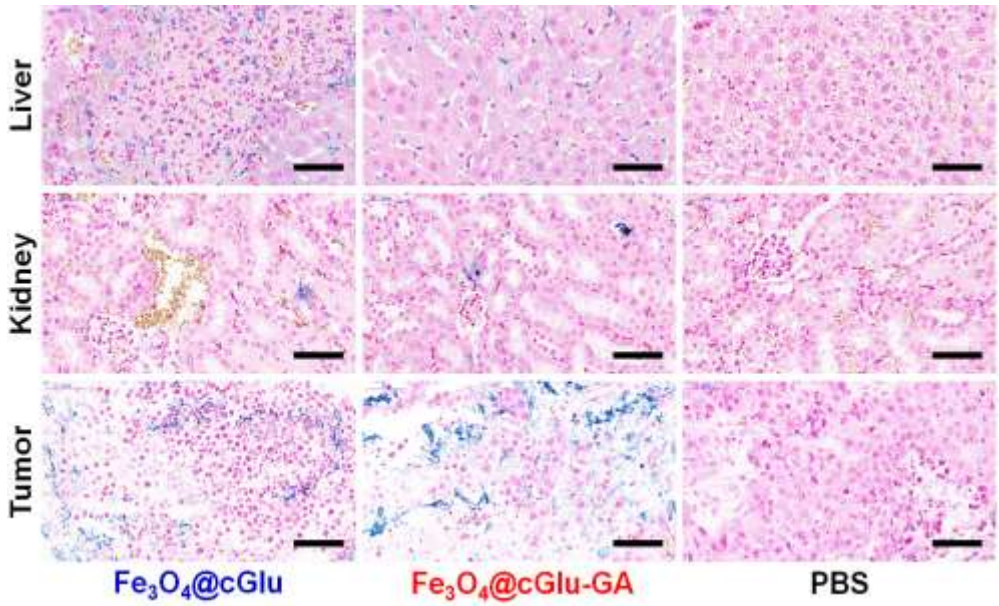

Fig. S7 Prussian blue staining of livers, kidneys, and orthotopic tumors. The scale bar is 50 μm.

8. Tunel staining of orthotopic tumor

We did the Tunel staining for the apoptosis of tumors. We washed the paraffin sections of tumor tissue by PBS for 5 min, and added proteinase K solution (50 μl; 20 μg/ml) to the tissue sample at 37 ˚C for 20 min. We added 20 μL of labeling buffer to tissue samples after they were washed three times with DI-water. We added 20 μl of labeling buffer containing terminal deoxynucleotidyl transferase and biotin-11-dUTP (1:8) to the sample tissue at 37 ˚C for 60 min. After immersed the labeled tissue samples in 2X SSC at room temperature for 15 min, we added confining liquid (50 μl) to them at room temperature for 30 min. We put 50 μl of confining liquid containing avidin-horseradish peroxidase (dilution, 1:50) to sample tissues at 37 ˚C for 60 min, and added DAB for coloration for 3 min. We used serial dilutions of ethanol to dehydrate (70, 80, 90, 95 and 100%), and observed under microscope.

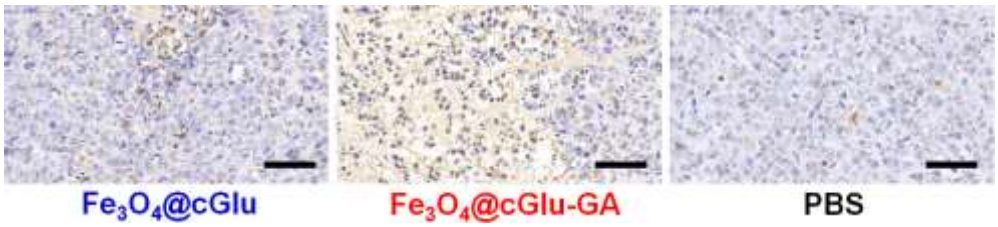

Fig. S8 Tunel staining of orthotopic tumors. The scale bar is 50 μm.

9. The composition of Fe_3O_4 nanoparticles

We measured the composition of the particles by ICP-MS (NexION 300X, PekinElmer) and element analyzer (Vario MACRO cube). We digested the nanoparticles by HNO_3, and diluted the solution by DI-water. We used iron in 2% HNO_3 as standard, and measured the iron concentration in samples.

We put freeze-dried nanoparticles (50 mg) in combustion tube of element analyzer (Vario MACRO cube). We measured the content of C, H, and N element of nanoparticles after complete burning, which used CO_2 and He as reference in thermal conductivity cell.

Table S1 The composition of Fe_3O_4 nanoparticles.

	Fe (%)	C (%)	H (%)	N (%)	O (%)
Fe_3O_4@cGlu	9.2	35.2	5.3	8.2	42.1
Fe_3O_4@cGlu-GA	8.6	38.6	5.6	7.8	39.4

The Fe_3O_4@cGlu and Fe_3O_4@cGlu-GA nanoparticles were composed of Fe, C, H, N, and O (Table S1). The ratio of Fe, C, H, N, and O in Fe_3O_4@cGlu nanoparticles was 9.2, 35.2, 5.3, 8.2, and 42.1%, and that in Fe_3O_4@cGlu-GA nanoparticles was 8.6, 38.6, 5.6, 7.8, and 39.4. The modification of GAEN on Fe_3O_4@cGlu increased the ratio of C and H, and decreased the ration of Fe, N, and O.

Reference

1. Y. Q. Sun, C. M. Dai, Y. Zheng, S. D. Shi, H. Y. Hu and D. W. Chen, *Life Sci.*, 2017, **188**, 186-191.

2. Y. Q. Sun, J. H. Lu, D. X. Yan, L. P. Shen, H. Y. Hu and D. W. Chen, *Environ. Toxicol. Pharmacol.*, 2017, **53**, 46-56.