Supporting Information

Serum Protein Corona-Responsive Autophagy Tuning in Cells

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Supporting Text.

Particle Size and Zeta Potential Measurements

Particle size and zeta potential measurements were performed using the Delsa NanoC particles Analyzer (BECKMAN COULTER, America). The mean hydrodynamic diameter was determined via cumulative analysis. Determination of the zeta potential was based on electrophoretic mobility of the nanoparticles in the aqueous medium, which was performed using folded capillary cells in automatic mode.

Immunostaining

After treatment, cells were fixed using 4% paraformaldehyde in PBS for 20 min at room temperature, washed twice in PBS, and blocked for 45 min at room temperature in PBS containing 6% BSA and 0.25% Triton X-100. Cells were then stained with rabbit anti-LC3 antibody (1: 200 dilution, Novus) and FITC-labeled secondary antibody (1: 2000 dilution, KPL). The stained cells were visualized under a laser confocal microscope (Leica TCS SP5).

Western Blotting Analysis

After treatment, cells were washed twice with PBS and harvested by SDS-loading sample buffer. Protein samples were analyzed by 15% SDS-PAGE and blotted to PVDF membranes. The blots were blocked for 1 h using 6% nonfat milk in PBST (PBS containing 0.1% Tween20) buffer and then incubated overnight at 4°C with the primary antibodies as required:

anti-LC3 (1:1000 dilution, Novus), Beclin-1 (1:1000 dilution, Cell Signaling Technology) or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:1000 dilution, Abcam). After extensive washing, the blots were probed with a goat anti-rabbit horseradish peroxidase-conjugated antibody (1:10000 dilution, KPL) for 1 h. The blots were then developed by incubation with chemiluminescence (ECL) plus and exposed to X-ray film. The densities of all bands were quantified with a computer densitometer (AlphaImagerTM 2200 System Alpha Innotech Corporation, San Leandro, GBBOX-chemi-XL1.4). The expression of GAPDH was used as the protein loading control.

Transmission Electron Microscopy

After treatment, cells were washed twice with PBS, and prefixed with a few of 2.5% glutaraldehyde in 0.1 M PBS. Then, the cells were collected using a cell scraper and centrifuged at 2000 rpm for 10 min. Cell aggregates were fixed in 2.5% glutaraldehyde for at least 2 h. Following a further wash with PBS, the cells were then dehydrated in a graded gradient ethanol series and embedded in Epon618. Ultrathin sections of the embedded cells were examined by transmission electron microscope (TEM, JEOL-1230; JEOL).

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

After treatment, cells were washed three times with PBS, trypsinized, collected and transferred to Eppendorf tubes. Aliquots of 100 μL were taken from each sample to determine the number of cells, and the remainder of the cell suspensions was sonicated for 3-5 min. The cell supernatant after sonication was digested overnight by 70% nitric acid and then analyzed the Fe or Au concentration by inductively coupled plasma optical emission spectrometry (ICP-OES, Spectro Arcos Sop, Spectro, Germany).

Circular dichroism (CD) Measurements

CD measurements were performed on Chirascan Circular Dichroism Spectrometer with 1 mm path length quartz cuvette at 20 °C. The spectra were recorded from 190 - 250 nm (far-UV region for protein secondary- structure analysis) and 250 - 350 nm (near-UV region for protein side-chain tertiary structure analysis), and each spectrum was repeatedly scanned three times. The secondary structure of each protein (α-helix, β-sheet, turn and random coil) was obtained using CDNN software.

Cytotoxicity Analysis

Cell viability was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma-Aldrich, Shanghai, China) assay and expressed as a percentage of ODtest/ODcontrol. All of the viability assessment data was based on three independent measurements.

Caspase-3 Activity Determination

The activity of caspase-3 was determined using the Caspase-3 activity kit (Beyotime, China) according to the manufacturer's protocol. Briefly, a reaction mixture of 10 μL cell lysate, 80 μL reaction buffer (20 mM Tris-HCl, pH 7.5, 1% NP-40), and 10 μL caspase-3 substrate (Ac-DEVD-pNA) (2 mM) were prepared and incubated at 37 °C for 4 h. Absorbance at 405 nm was measured using a microplate reader (Bio-Rad 680, USA).

Supporting tables and figures.

Table S1. Molecular weight, size and isoelectric point of four kinds of representative serum proteins.[1-4]

	Molecular weight (kDa)	Molecular size (nm)	Isoelectric point
BSA	66	6.1 × 8.5 × 7.0	4.7 – 4.9
BTf	78	13.2 × 8.9 × 11.8	5.2 – 5.7
BIg	160	13.7 – 27.4 × 16.6 × 6.3	5.8 – 7.3
BFG	340	25.3 × 6.5 × 53.5	5.5 – 5.8

Table S2. Characterization of NP-protein complexes with different surface binding protein types and cell uptake and cell autophagy responses of them. For size and ζ potential determination, the particles were suspended in MiliQ water (Concentration: 10 μg/mL). All of the NPs exhibited a narrow size distribution, with polydispersity indices ranging from 0.1 to 0.3.

		Fe_3O_4			
	Fe_3O_4	@BSA	@BTf	@BIg	@BFG
Mean particle size (nm)	20.8 ± 0.3	31.9 ± 2.8	38.1 ± 0.8	62.1 ± 1.1	86.7 ± 0.5
Polydispersity index	0.11	0.24	0.25	0.20	0.27
Mean zeta potential (eV)	- 20.7 ± 1.9	- 16.1 ± 0.8	- 13.6 ± 0.4	- 12.4 ± 2.1	- 10.7 ± 1.1
Protein on Fe_3O_4 (μg/μg)	—	0.028	0.033	0.094	0.16
Molecular number on one Fe_3O_4	—	7.1	7.0	9.8	8.0
Thickness of protein corona (nm)	—	6.4 ± 1.5	11.3 ± 2.6	20.5 ± 2.3	34.2 ± 8.2
Intracellular Fe (μg /10^6 cells)	3.67	3.28	2.99	1.64	0.89
Number of LC3-puncta/cell	107.6 ± 3.1	101.3 ± 1.2	76.3 ± 0.8	51.7 ± 0.5	42.8 ± 1.5

Table S3. Calculation of proteins secondary structures before and after incubation with Fe_3O_4 NPs for 60 min.

Protein	Secondary structures (%)				
	α-helix	β-sheet	Turn	Random	Total
BSA	63.0	6.6	12.9	17.5	100.0
BSA + Fe_3O_4	59.2	6.3	13.4	21.1	100.0
BTf	13.9	33.8	20.0	32.3	100.0
BTf + Fe_3O_4	13.8	35.1	20.2	30.9	100.0
BIg	10.0	38.0	17.0	35.0	100.0
BIg + Fe_3O_4	10.1	38.5	16.6	34.8	100.0
BFG	9.0	39.4	18.7	32.9	100.0
BFG + Fe_3O_4	8.7	39.9	19.0	32.4	100.0

Table S4. The average number of green punctate LC3 in the cytoplasm (at least counting ~20 cells) after treatment indicated in Figure 18a. Standard deviations were given from three independent experiments.

	Fe_3O_4					AuNPs
	Sphere			Cube	Rod	
	5 nm	10 nm	20 nm			
FBS (-)	18.3 ± 1.8	72.2 ± 2.8	107.6 ± 3.1	59.3 ± 1.4	32.0 ± 0.3	35.5 ± 0.6
FBS (+)	14.0 ± 1.2	42.3 ± 1.5	49.0 ± 1.0	102.8 ± 2.0	92.5 ± 1.2	108.3 ± 1.2
@BSA	16.2 ± 1.0	67.2 ± 1.9	101.3 ± 1.2	58.5 ± 0.5	35.5 ± 0.5	39.0 ± 0.7
@BTf	15.8 ± 0.6	51.8 ± 0.8	76.3 ± 0.8	66.7 ± 1.3	42.3 ± 0.9	60.8 ± 1.4
@BIg	14.8 ± 1.5	45.5 ± 2.6	51.7 ± 0.5	79.8 ± 2.1	69.3 ± 1.3	89.5 ± 1.8
@BFG	12.5 ± 0.9	39.3 ± 1.2	42.8 ± 1.5	104.3 ± 1.3	100.5 ± 2.1	113.0 ± 0.9

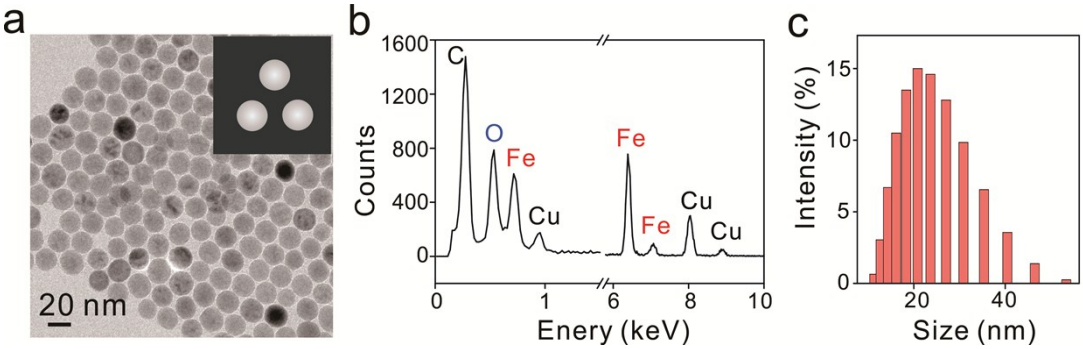

Figure S1. Characterization of 20 nm Fe_3O_4 nanoparticles (NPs). (a) TEM image of Fe_3O_4 NPs. (b) EDX analysis of TEM images. Sample was placed on a copper TEM grid for examination. (c) DLS analysis of Fe_3O_4 NPs.

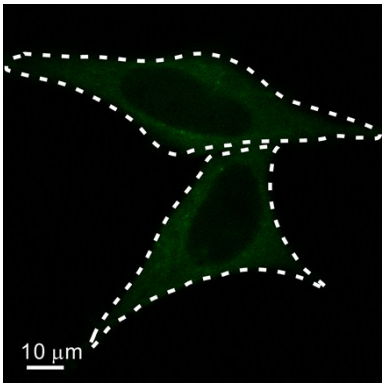

Figure S2. Immunostaining of LC3 in untreated cells. They induced 14 or less punctuates in cells, representing basal level of cell autophagy.

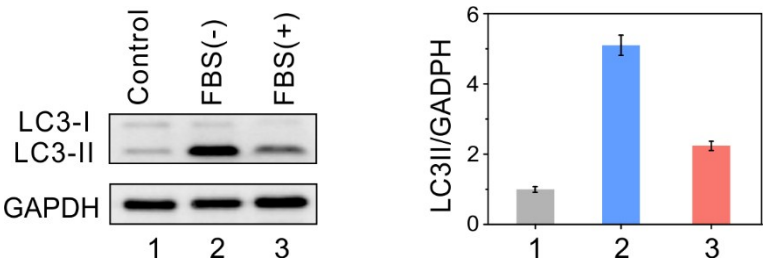

Figure S3. Representative immunoblots of autophagy related proteins LC3-II (left) and semi-quantified analysis (n＝3) in 200 μg/mL Fe_3O_4 NPs with or without serum treated cells (right). GAPDH was used as the loading control.

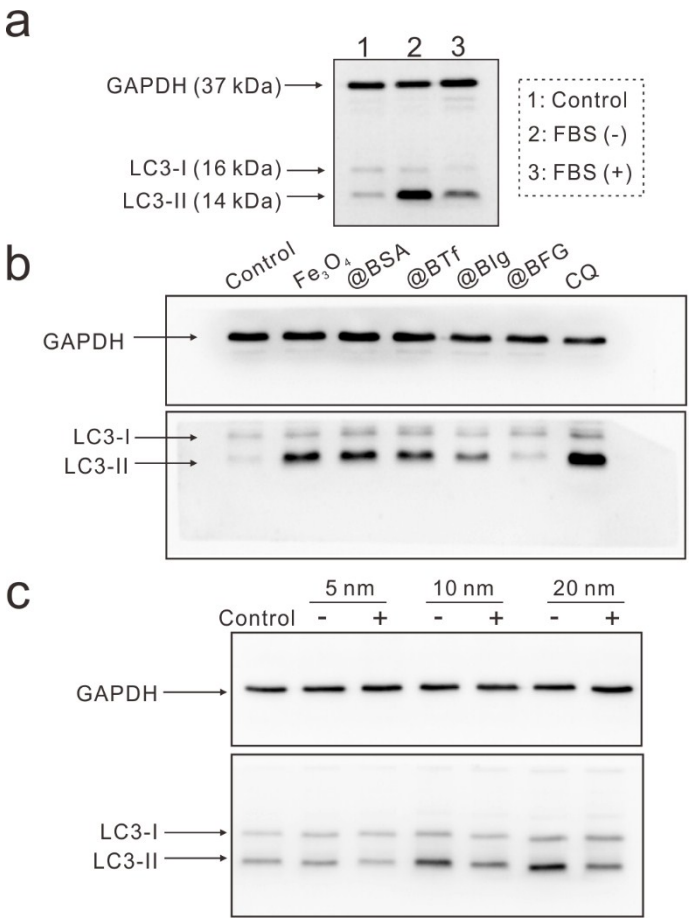

Figure S4. The original images of immunoblots of figure 1 (a), 2c (b) and 3b (c).

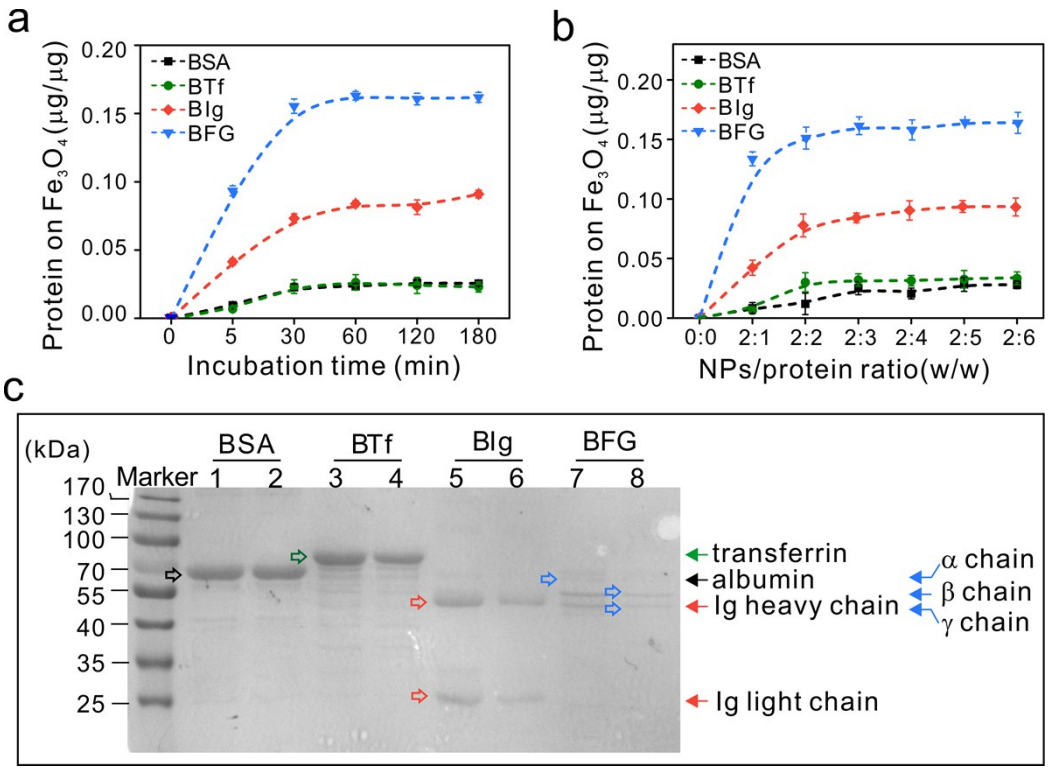

Figure S5. Adsorption of different species of proteins on Fe_3O_4 NPs. (a) The adsorption kinetics curves of each protein on Fe_3O_4 NPs. The concentration of NPs is 200 μg/mL and that of proteins is 500 μg/mL. Data represented as mean ± SD (n = 3). (b) The adsorption isotherm curves of different proteins on NPs at various complexation ratios (NPs vs proteins). Data represented as mean ± SD (n = 3). (c) SDS-PAGE of supernatant from incubation Fe_3O_4 NPs respectively, with BSA, BIg, BTf and BFG. The concentration of NPs is 200 μg/mL and that of proteins is 500 μg/mL. Lanes: 1, 3, 5 and 7 are controls of each proteins; 2, 4, 6 and 8 are supernatants from incubation Fe_3O_4 NPs with each proteins for 60 min. Lane 5 and 6, BIg has been divided into two monoers: heavy chain (60 kDa) and light chain (25 kDa). Lane 7 and 8, BFG has been divided into three monomers: □α-chain (63.5 kDa), □β-chain (56 kDa), and γ-chain (47 kDa).

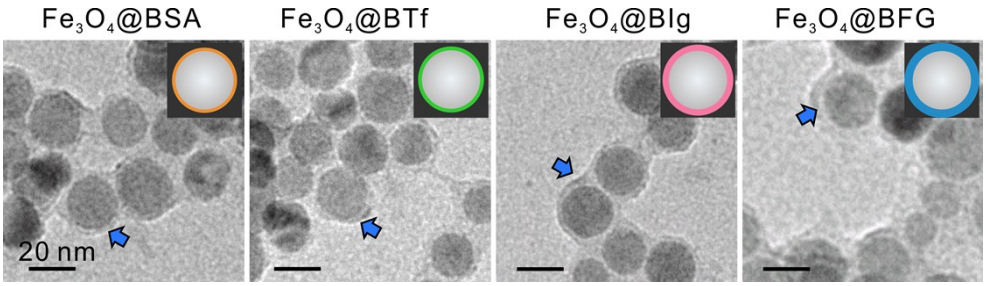

Figure S6. TEM images of Fe_3O_4 NPs coated with different proteins. Proteins adsorbed on NPs are indicated with blue arrows.

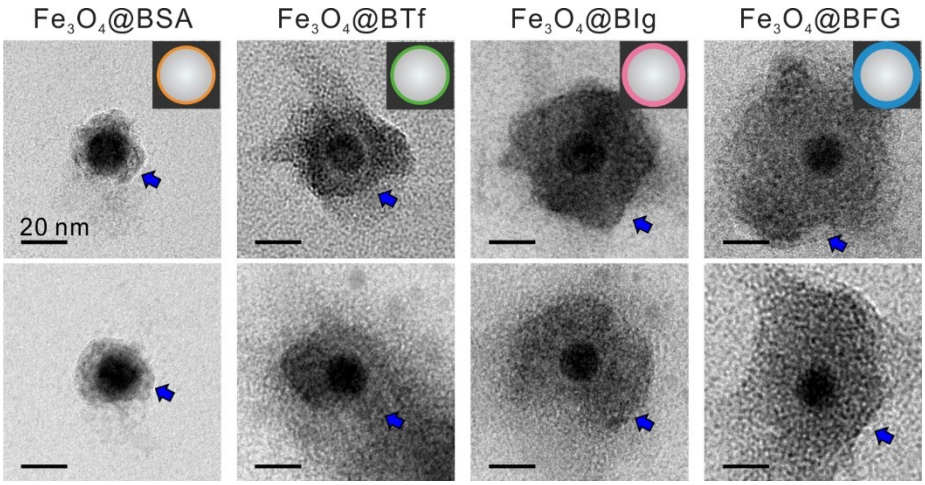

Figure S7. TEM images of Fe_3O_4 NPs coated with different proteins. Proteins adsorbed on NPs are indicated with blue arrows. Samples were stained with 0.75% uranyl acetate.

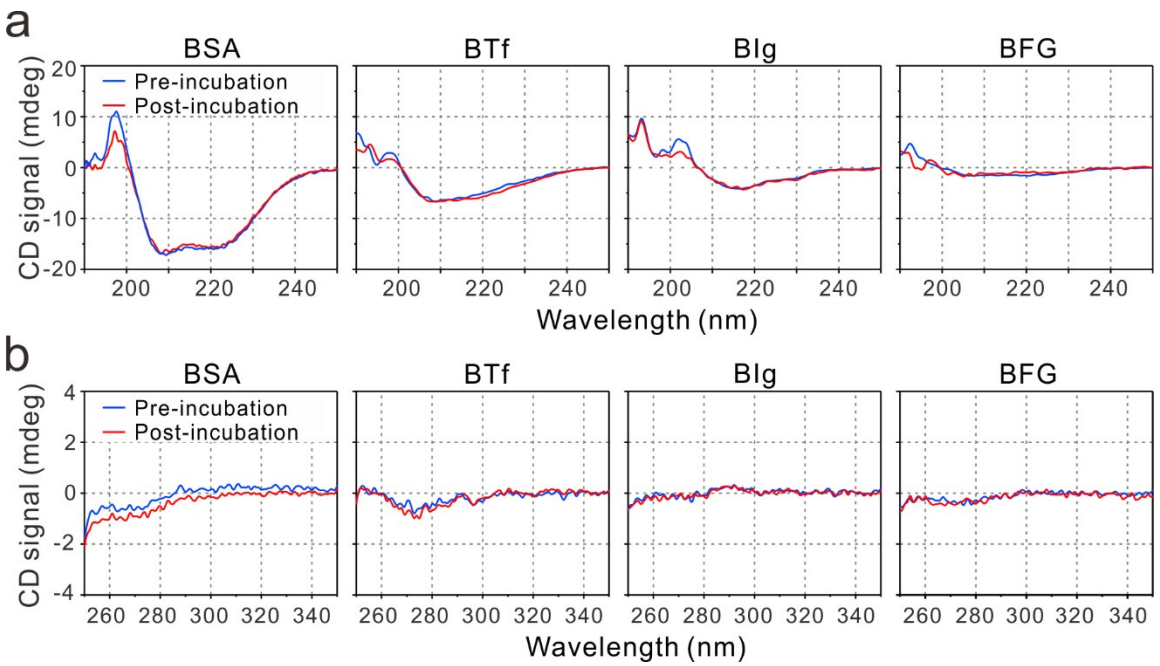

Figure S8. The CD spectra of proteins after incubation with Fe_3O_4 NPs. (a) The far-UV CD spectra. (b) The near-UV CD spectra.

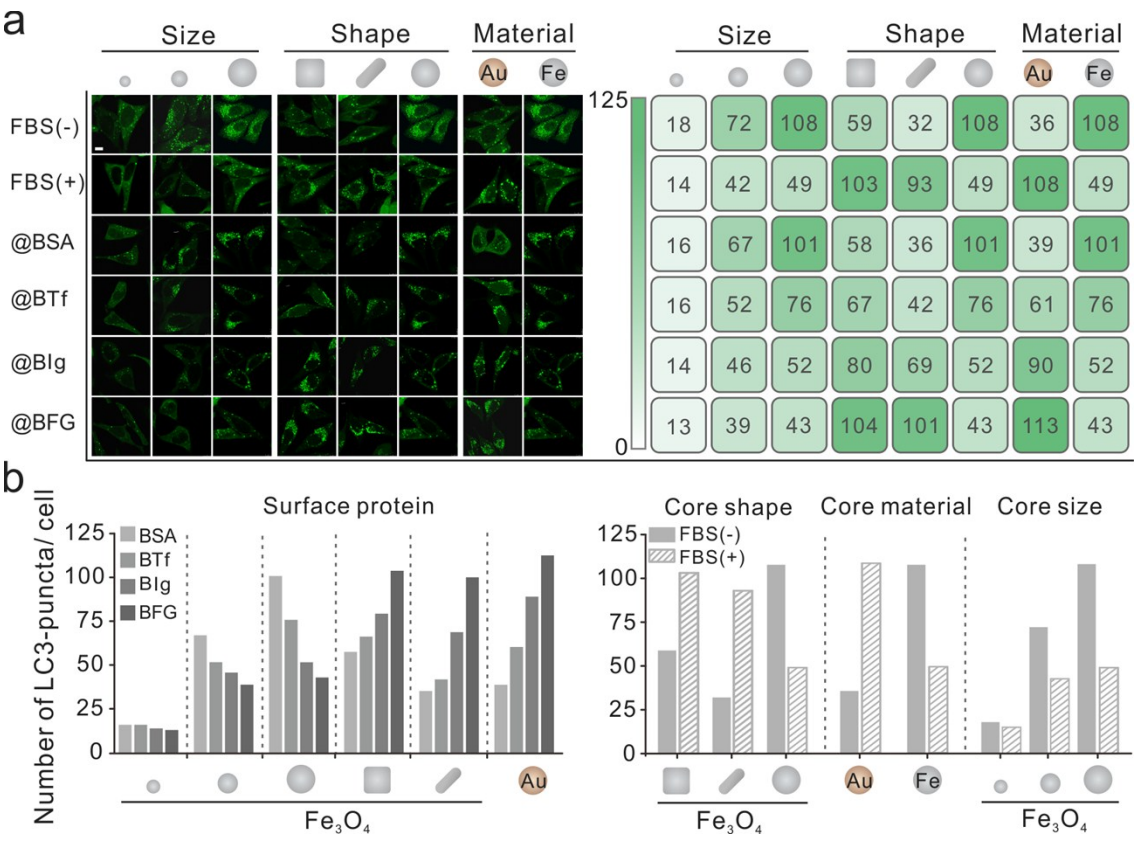

Figure S9. Autophagy modulation governed by NP-protein corona complexes. Cells were treated with 12 nM NPs in medium with or without serum or NPs coated with different proteins for 24 h. (a) Autophagy induction by NP-protein complex library in cells. Left: Representative immunostaining imaging of LC3 in cells. Scale bar = 20 μm. Right: The average number of green punctate LC3 in the cytoplasm (at least counting ~20 cells). The autophagy induction capability was shown by gradient of green colors. (b) The autophagy induction capability was plotted.

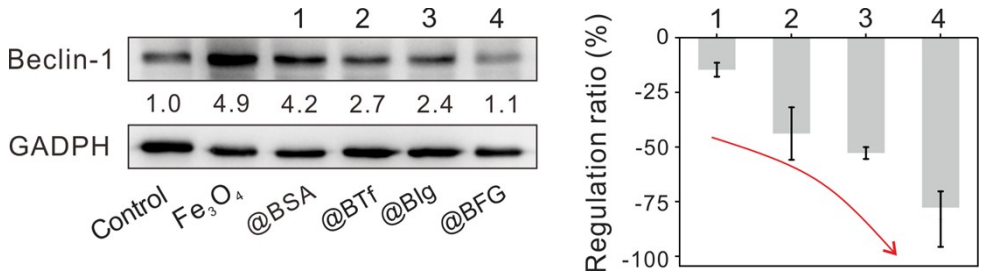

Figure S10. NP surface protein type - associated modulation on cell autophagy. Cells were treated with 200 μg/mL Fe_3O_4 NPs or Fe_3O_4 NPs coated with different species of representative serum proteins for 24 h. Autophagy levels by immunoblotting of Beclin-1 proteins (left, GAPDH was used as the loading control. Normalized band densities were shown below each band. No NPs treatment was defined as 1.0.) and semi-quantified analysis (n＝3) of Beclin-1 protein regulation ratio (right).

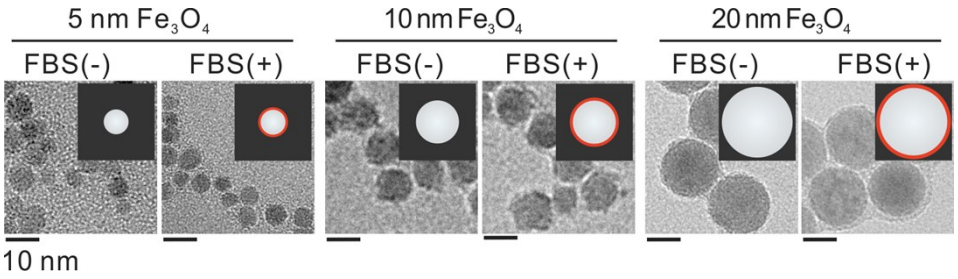

Figure S11. TEM images of different sized- Fe_3O_4 NPs with or without pre-incubation with complete cell culture medium for 1 h.

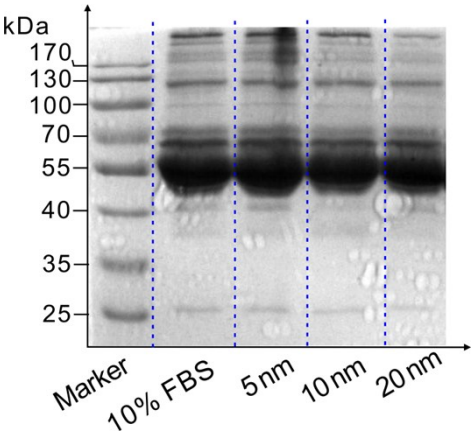

Figure S12. Adsorption of proteins on different sized- Fe_3O_4 NPs. Fe_3O_4 NPs (5, 10, 20 nm, 12 nM) were pre-incubated in medium with serum (10% FBS) for 1 h. SDS-PAGE analysis of supernatant after centrifugation.

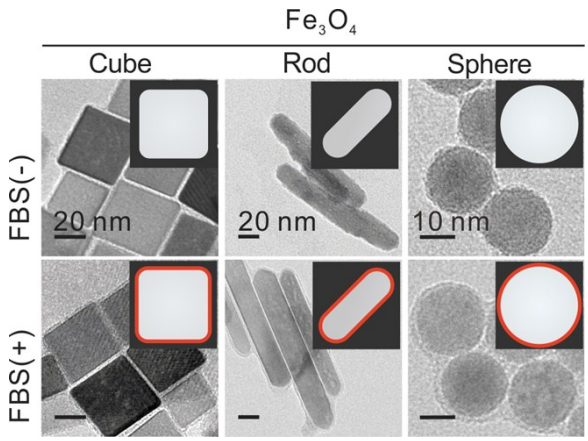

Figure S13. TEM images of different shaped- Fe_3O_4 NPs with or without pre-incubation with complete cell culture medium for 1 h.

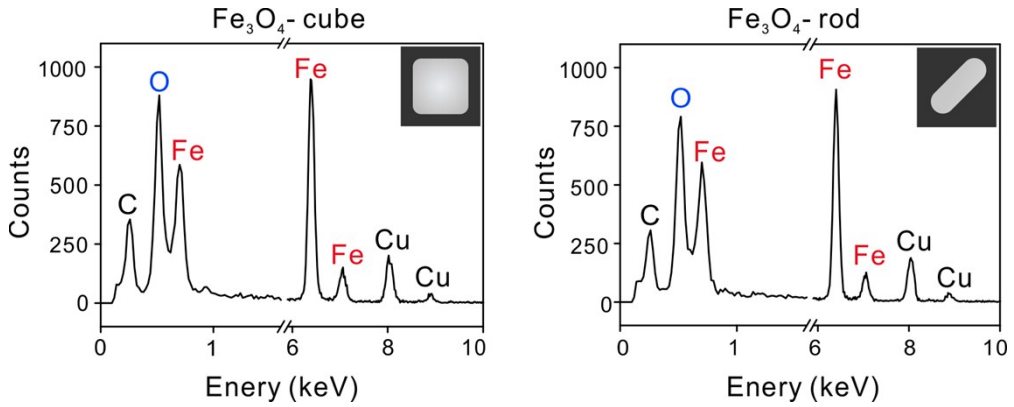

Figure S14. EDX analysis of TEM images in Figure S8 (cubic and rod Fe_3O_4 NPs). Sample was placed on a copper TEM grid for examination.

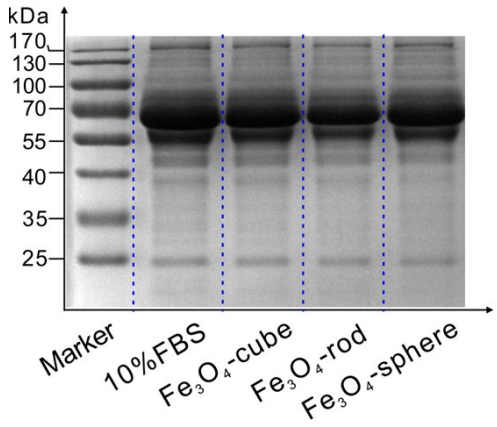

Figure S15. Adsorption of proteins on different shaped- Fe_3O_4 NPs. Fe_3O_4 NPs (spherical, cubic and rod, 200 μg/mL) were pre-incubated in medium with serum (10% FBS) for 1 h. SDS-PAGE analysis of supernatant after centrifugation.

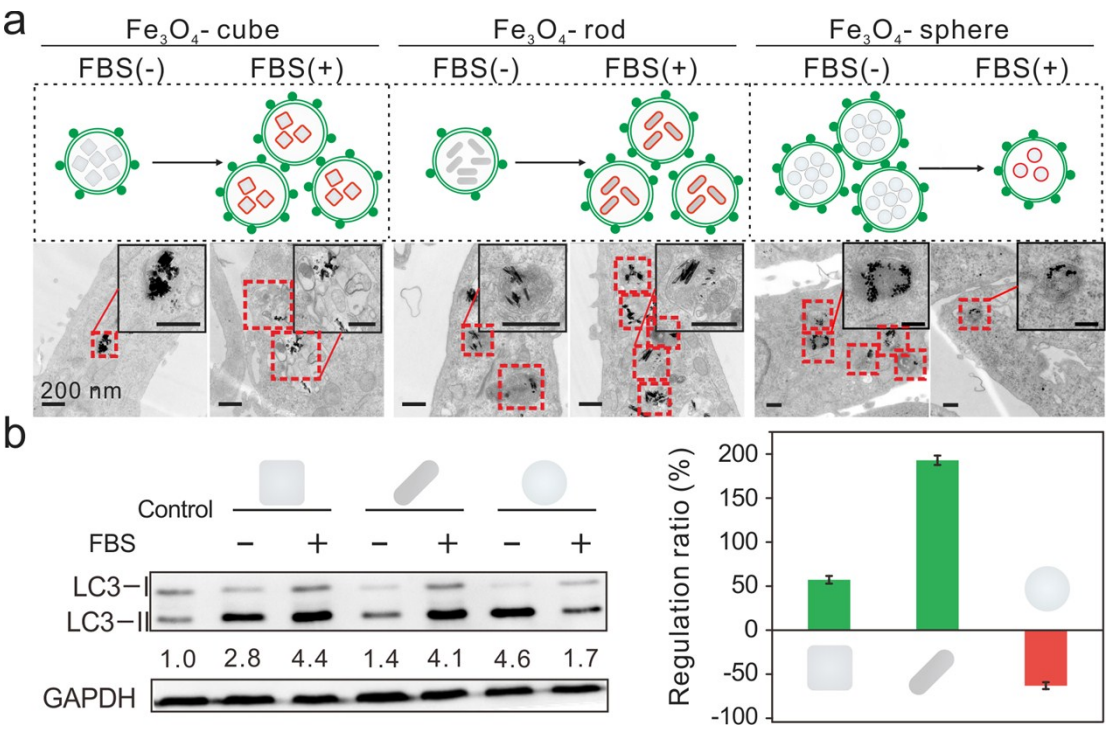

Figure S16. NP core shape- associated modulation on cell autophagy. Cells were treated with 200 μg/mL different shaped- Fe_3O_4 NPs in medium with or without serum for 24 h. (a) TEM imaging of cells (autophagosome containing NPs are indicated with red squares. scale bar = 200 nm). (b) Autophagy levels by immunoblotting of LC3 proteins (left, GAPDH was used as the loading control. Normalized band densities were shown below each band. No NPs treatment was defined as 1.0.) and semi-quantified analysis (n＝3) of LC3 protein regulation ratio (right).

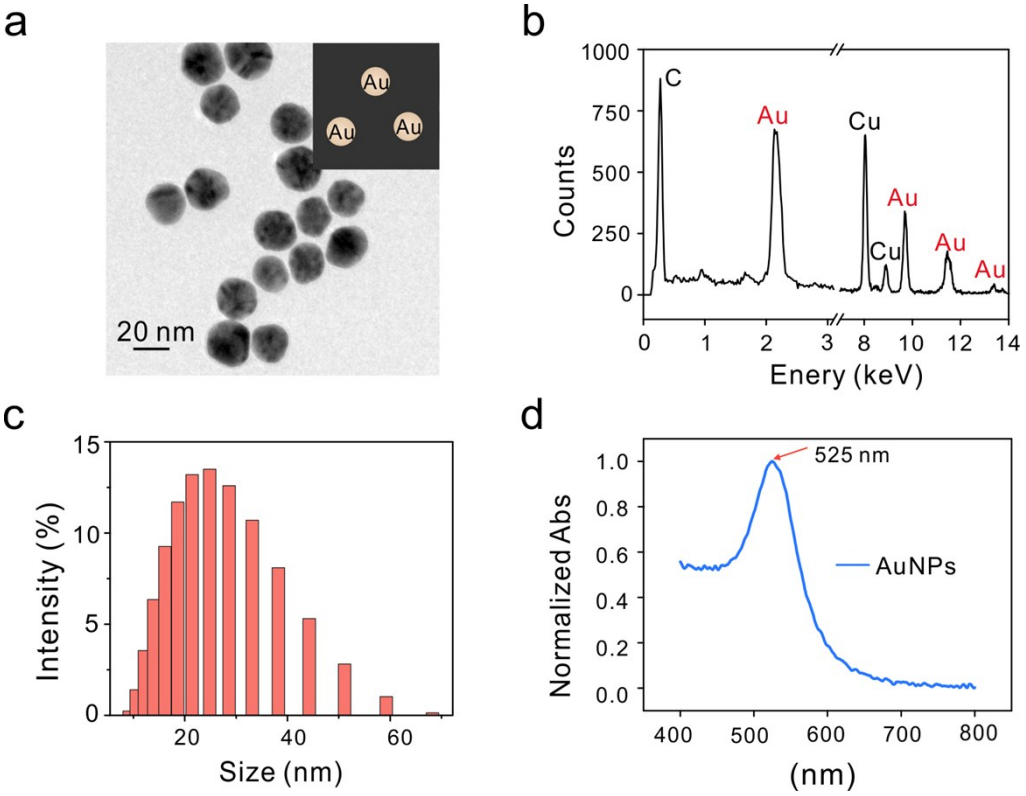

Figure S17. Characterization of 20 nm AuNPs. (a) TEM image of AuNPs. (b) EDX analysis of TEM images. Sample was placed on a copper TEM grid for examination. (c) DLS analysis of AuNPs. (d) UV-vis spectrum of AuNPs.

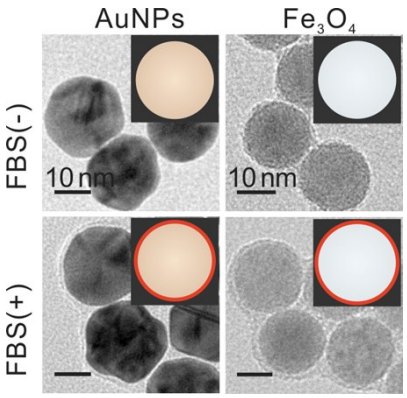

Figure S18. TEM images of different core composition NPs with or without pre-incubation with complete cell culture medium for 1 h.

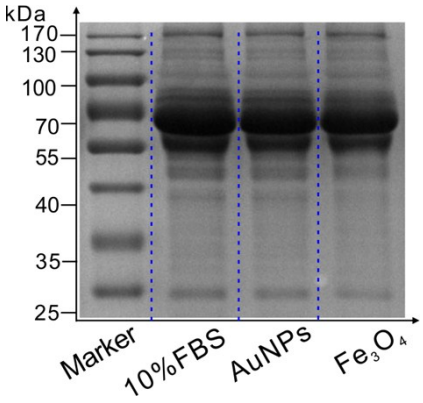

Figure S19. Adsorption of proteins on different core composition NPs. AuNPs and Fe_3O_4 NPs were pre-incubated in medium with serum (10% FBS) for 1 h. SDS-PAGE analysis of supernatant after centrifugation.

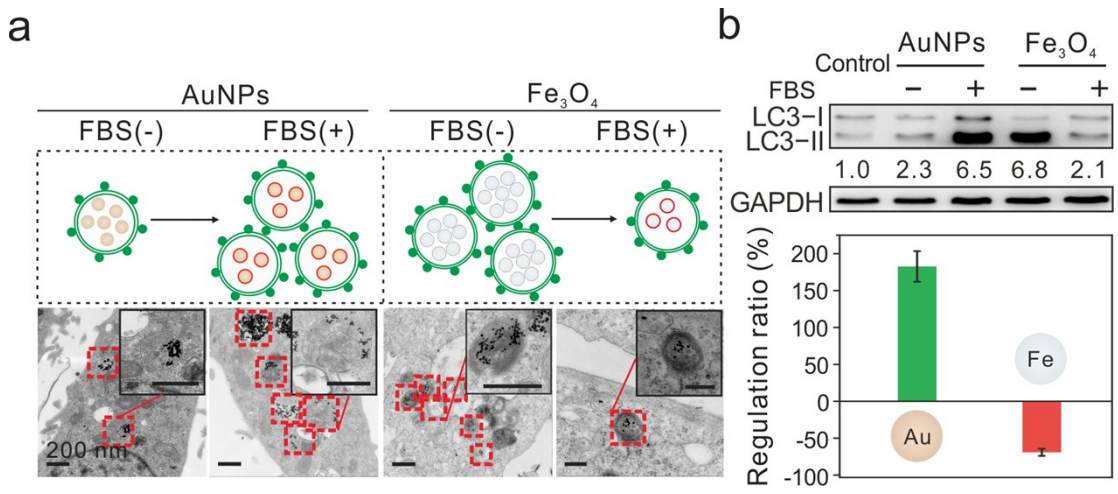

Figure S20. NP core material - associated modulation on cell autophagy. Cells were treated with 200 μg/mL different different core composition NPs in medium with or without serum for 24 h. (a) TEM imaging of cells (autophagosome containing NPs are indicated with red squares. scale bar = 200 nm). (b) Autophagy levels by immunoblotting of LC3 proteins (upper, GAPDH was used as the loading control. Normalized band densities were shown below each band. No NPs treatment was defined as 1.0.) and semi-quantified analysis (n＝3) of LC3 protein regulation ratio (bottom).

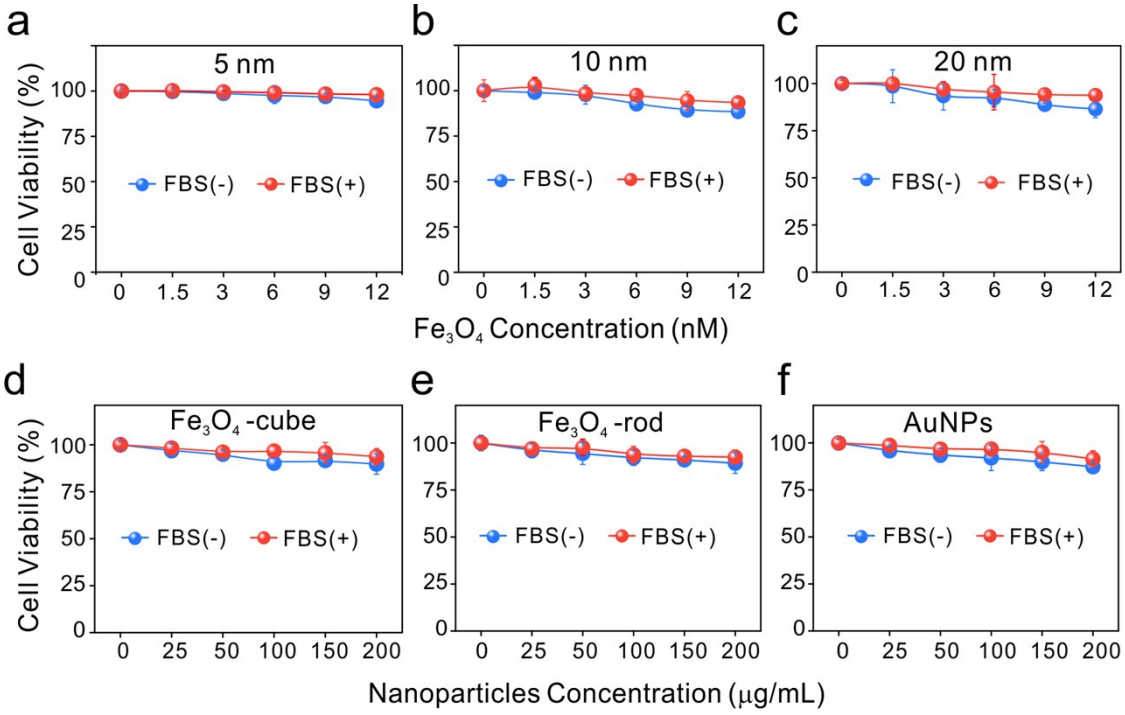

Figure S21. Biocompatibility assessment of numerous NPs and NP-protein complexes. (a - f) Cells were treated with NPs in medium with or without serum at indicated concentration for 24 h. Cell viability assessment (n=3; error bars are SD).

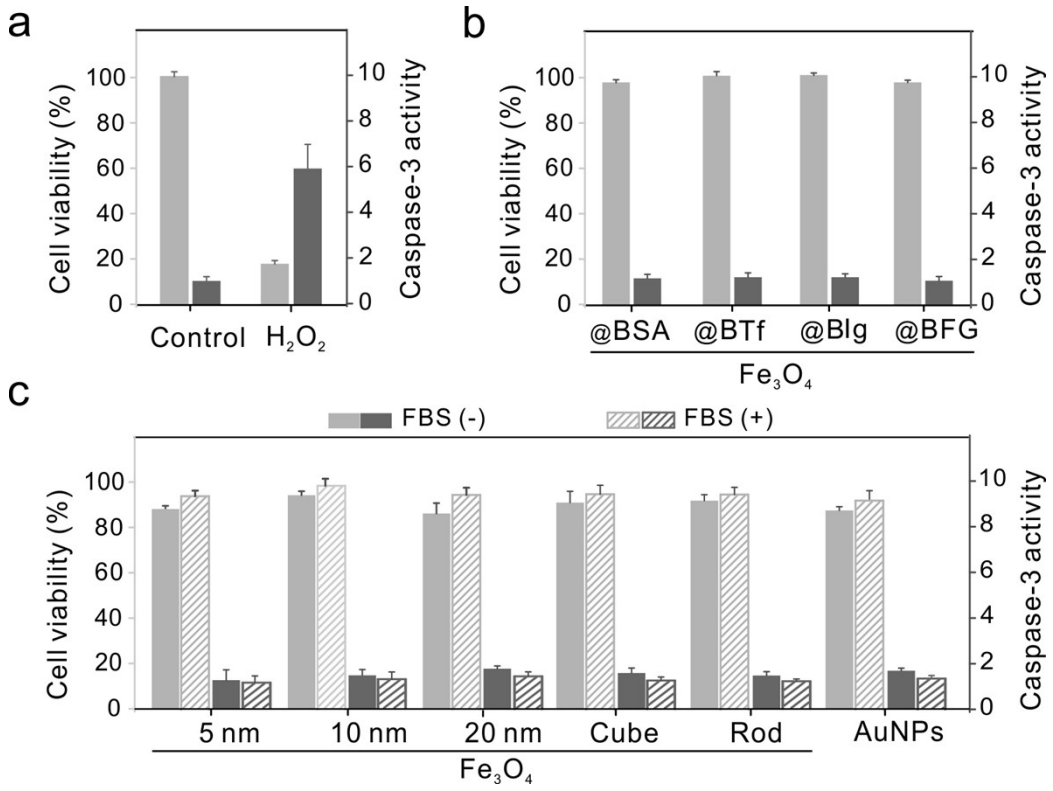

Figure S22. Biocompatibility assessment of numerous NPs and NP-protein complexes. Cells were treated with 100 μM H_2O_2 (a, positive control), 12 nM NPs coated with different proteins (b) or NPs in medium with or without serum (c) for 24 h. Cell viability and Caspase-3 activity assessment (n=3; error bars are SD).

References:

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