Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplemental data: Table S1, Figure S1–S4.

Table S1. Characterization of the NPs used in this study.

Nanoparticles	Hydrodynamic diameter (nm)	Zeta potential (mV)	Polydispersion index (PDI)
Fe_3O_4 NPs (negatively charged)	**53.4 ± 1.2**	**-56.34 ± 1.34**	**0.20**
Fe_3O_4 NPs (nearly neutral charged)*	**36.9 ± 1.8**	**-9.6 ± 1.07**	**0.19**
Au NPs**	**35.3 ± 0.2**	**-20.99 ± 0.91**	**0.26**

*Stabilized by polyethylene glycol (PEG).

**Stabilized by sodium citrate.

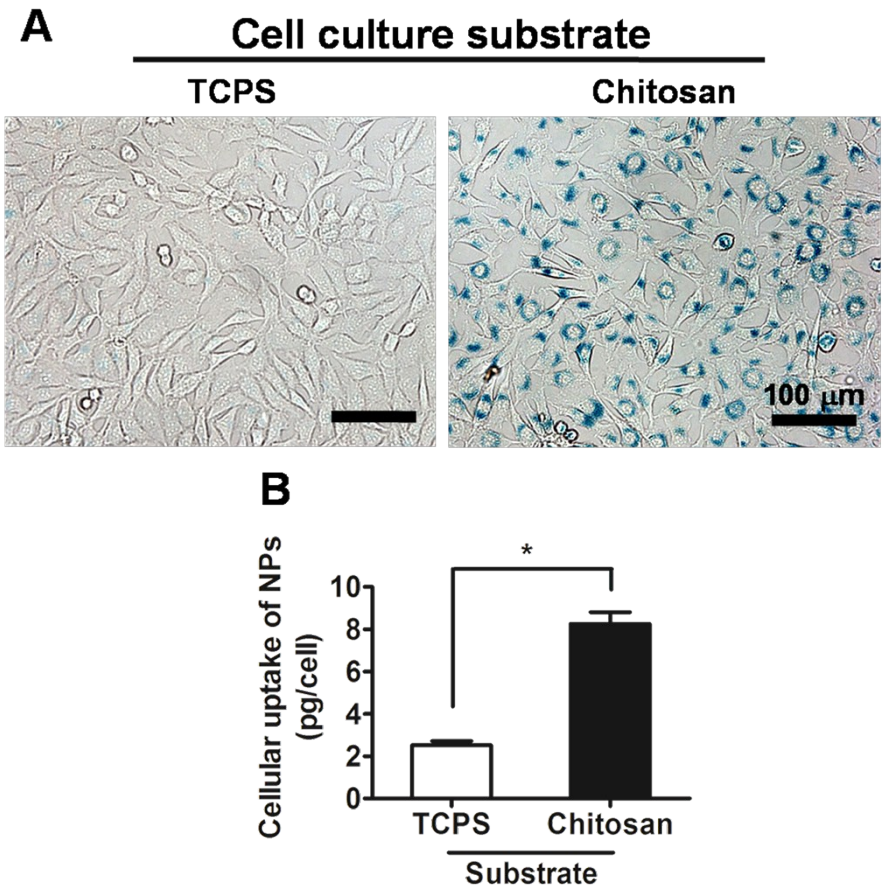

Figure S1. Cellular uptake of nearly neutral charged Fe_3O_4 NPs on TCPS or chitosan. (A) Prussian blue staining for L929 fibroblasts co-cultured with Fe_3O_4 NPs (25 μg/ml) on different substrates (TCPS or chitosan) for 24 h. (B) The amount of Fe_3O_4 NP uptake by cells (pg per cell, based on the mass of iron) grown on different substrates, quantified by ICP-MS (Agilent 7500ce, Japan). Cells were washed with PBS, detached with 0.25% trypsin, and collected by centrifugation. Cell pellets were dissolved in 3% HNO_3 and the iron concentration was determined using the ICP-MS. The cell number was measured by the DNA Hoechst 33528 dye stain assay The average amount of uptake per cell (pg/cell) was then calculated. *$p < 0.05$ among the indicated groups.

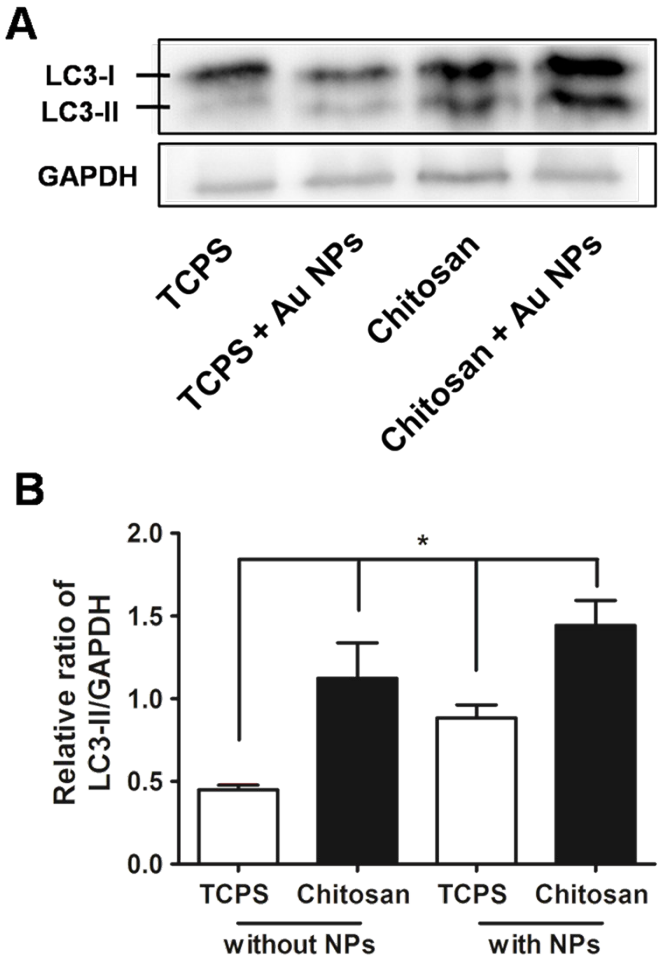

Figure S2. The autophagy responses for fibroblasts on TCPS or chitosan with or without Au NP treatment. Fibroblasts were cultured on the substrates for 12 h before the medium was replaced with that containing 25 μg/ml Au NPs and cultured for another 6 h. (A) The expression of LC3-II (autophagy-associated protein) analyzed by Western blot. (B) The semi-quantification of LC3-II protein levels from Western blot.

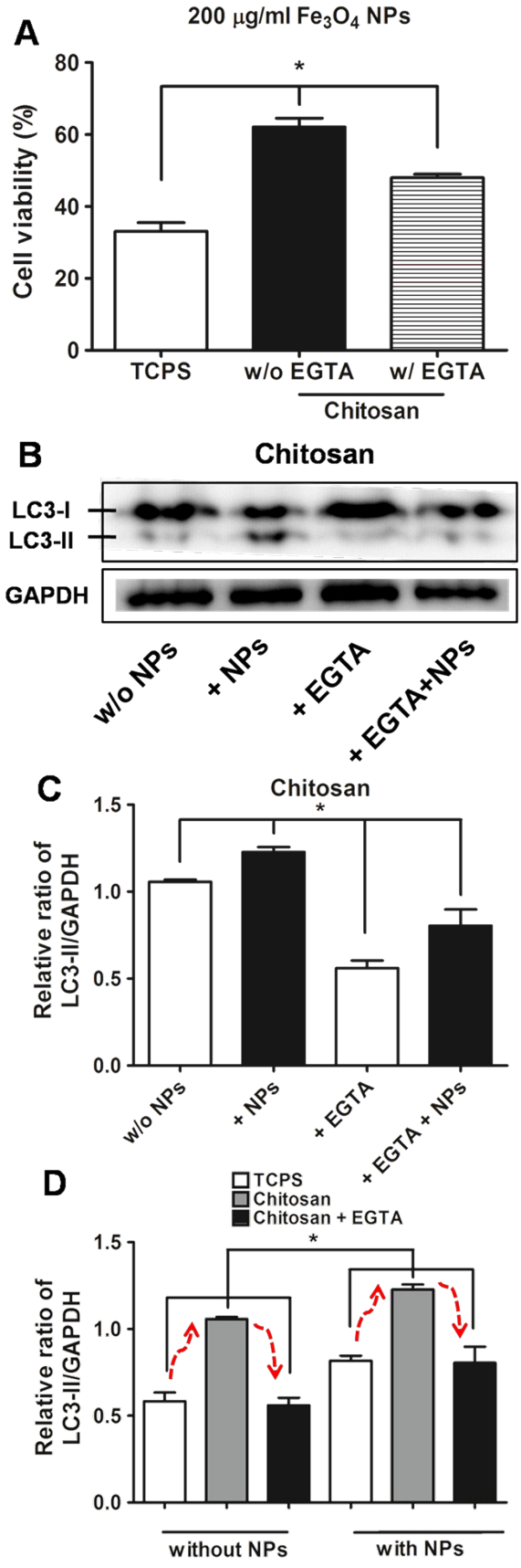

Figure S3. The cell viability and autophagy response of fibroblasts on chitosan

substrates upon NP treatment. (A) The viability of fibroblasts cultured on chitosan, treated with or without EGTA, and exposed to Fe_3O_4 NPs (200 μg/ml) for 48 h. (B) The protein expression of LC3-II (autophagy) for fibroblasts on chitosan substrates treated with NPs and/or EGTA, analyzed by Western blot. (C, D) The semi-quantification of LC3-II protein levels from Western blot. *$p < 0.05$ among the indicated groups.

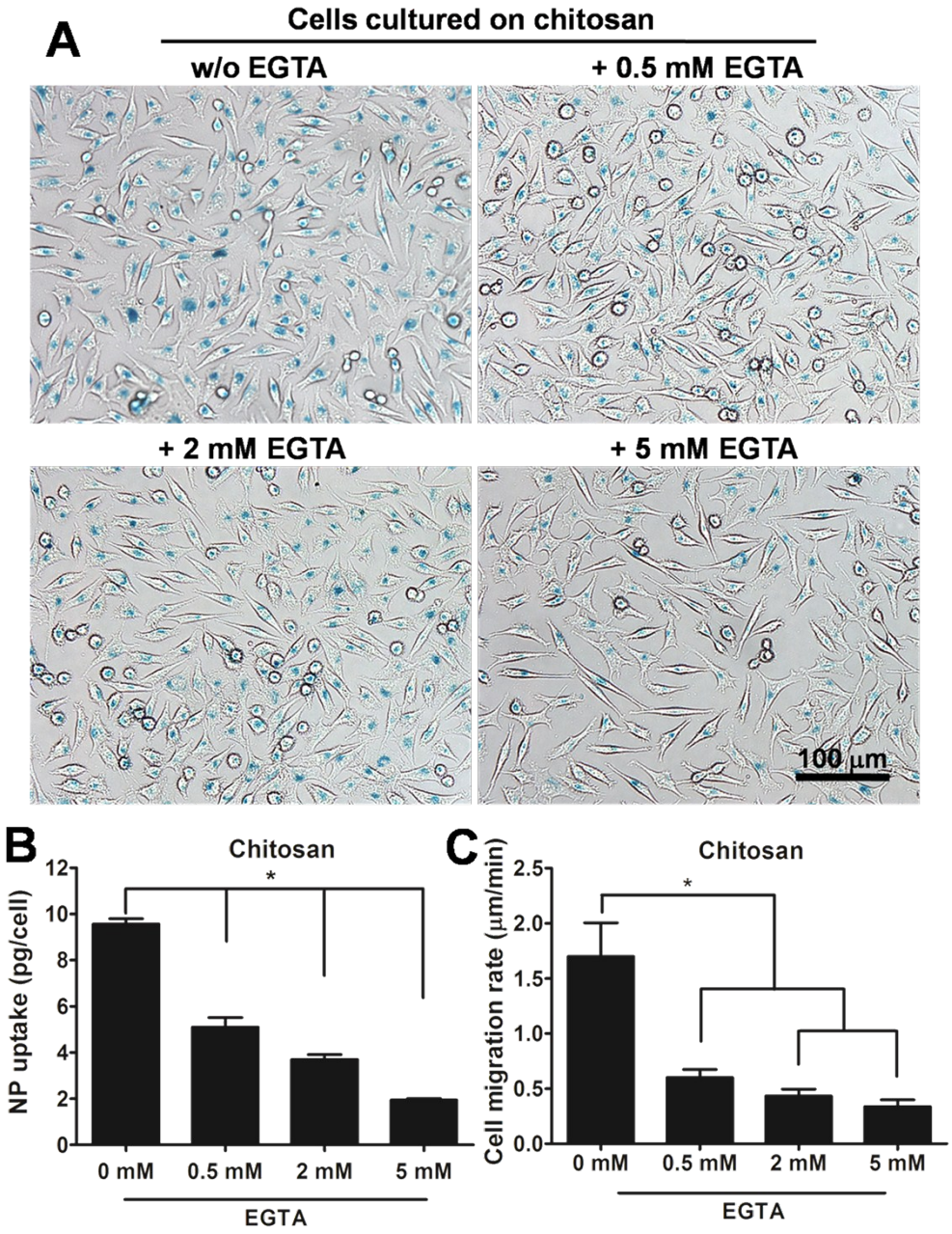

Figure S4. The effect of calcium ion on the cell migration and uptake of Fe_3O_4 NPs. L929 fibroblasts exposed to Fe_3O_4 NPs (25 μg/mL) and treated with different concentrations of EGTA on chitosan materials for 24 h. (A) Prussian blue staining for cells treated with EGTA. (B) The amount of NP uptake for cells under EGTA treatment, quantified by AA. (C) The average migration rate for cells grown on chitosan with EGTA treatment.