

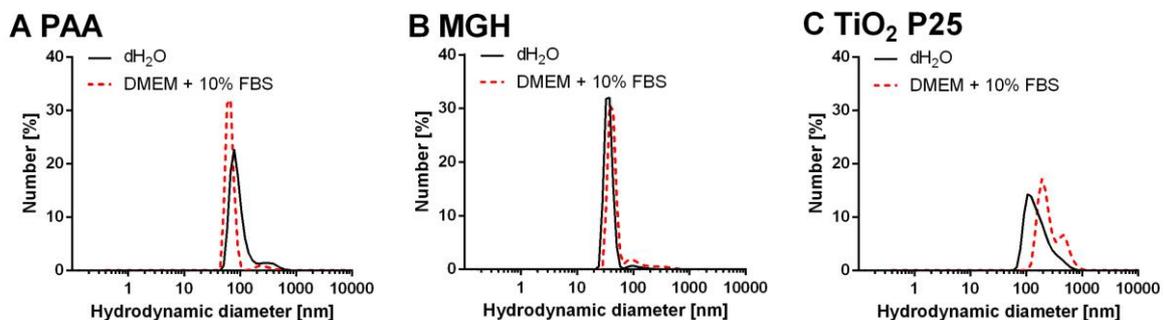
# Analysis of the Direct and Indirect Effects of Nanoparticle Exposure on Microglial and Neuronal Cells In Vitro

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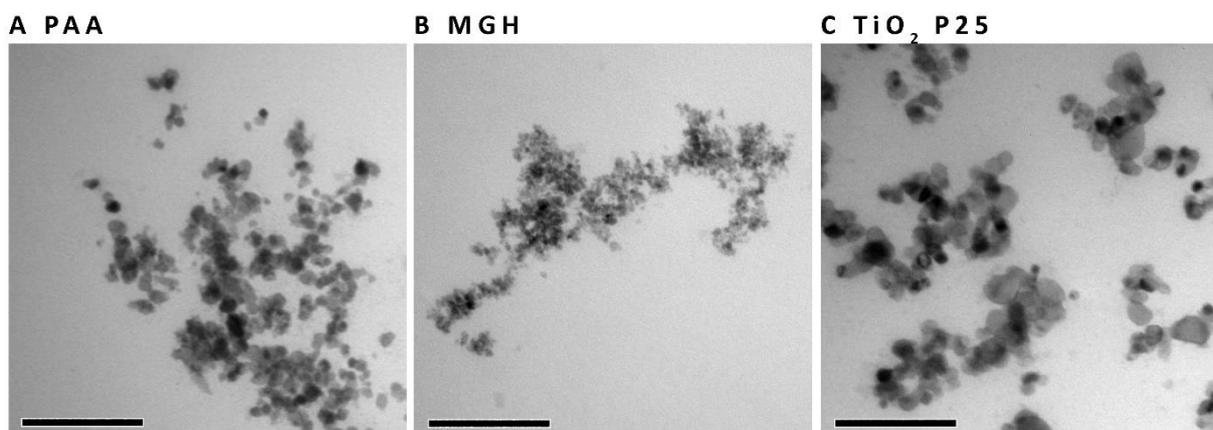
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## Supplementary information

### Nanoparticle characterization

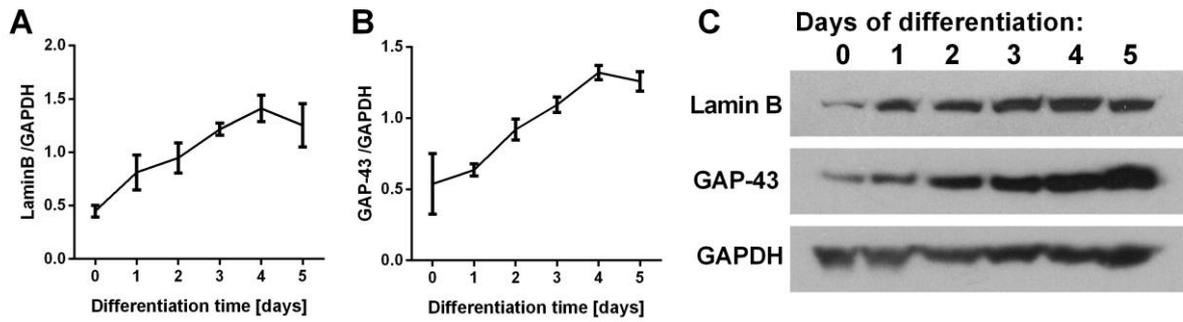


**Figure S1:** Particle number distribution for (A) polyacrylic acid (PAA) coated magnetic nanoparticles, (B) maghemite (MGH) nanoparticles and (C) industrial TiO<sub>2</sub> P25 nanoparticles analysed in distilled water (dH<sub>2</sub>O) and cell culture media supplemented with 10% FBS (medium + 10% FBS). The graphs represent one representative measurement.



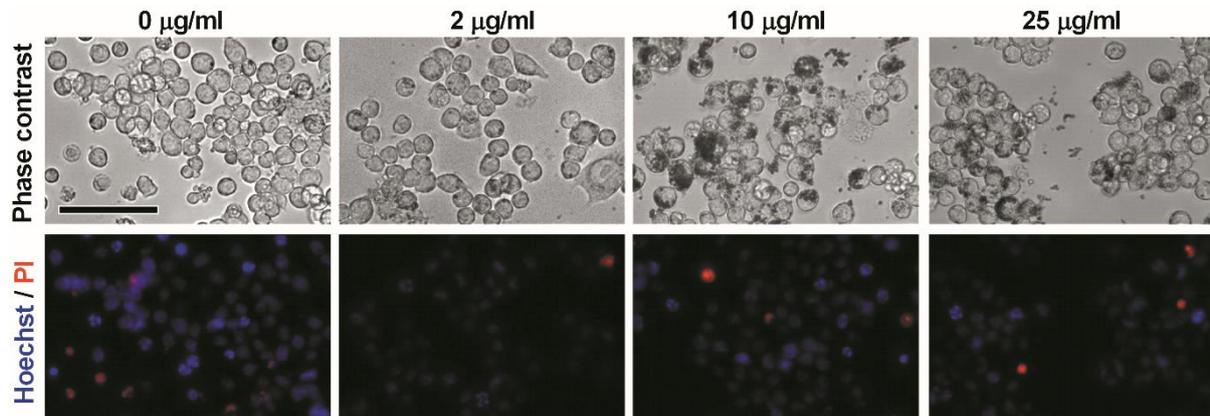
**Figure S2:** TEM micrographs of (A) polyacrylic acid (PAA) coated magnetic nanoparticles, (B) maghemite (MGH) nanoparticles and (C) industrial TiO<sub>2</sub> P25 nanoparticles in cell culture media with 10% FBS. Scale bars correspond to 200 nm.

### CAD cell differentiation

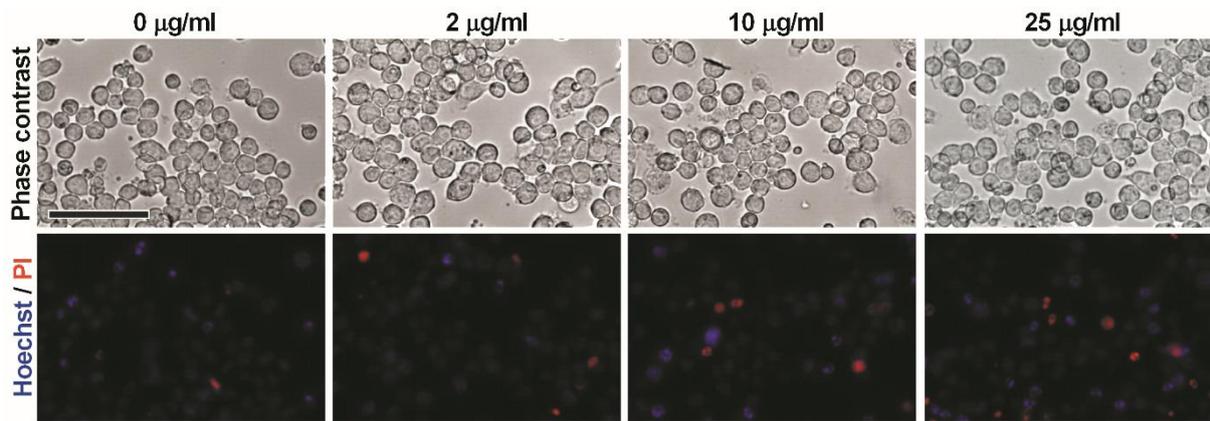


**Figure S3: Differentiation timeline of mouse neuronal CAD cells.** Cells were exposed to differentiation media at day 0 and differentiated up to 5 days. Each day, a sample was lysed to assess changes in neuronal differentiation markers (A) Lamin B and (B) GAP-43. Mean and standard error are shown for three biological repeats. Sample western blot is shown in (C).

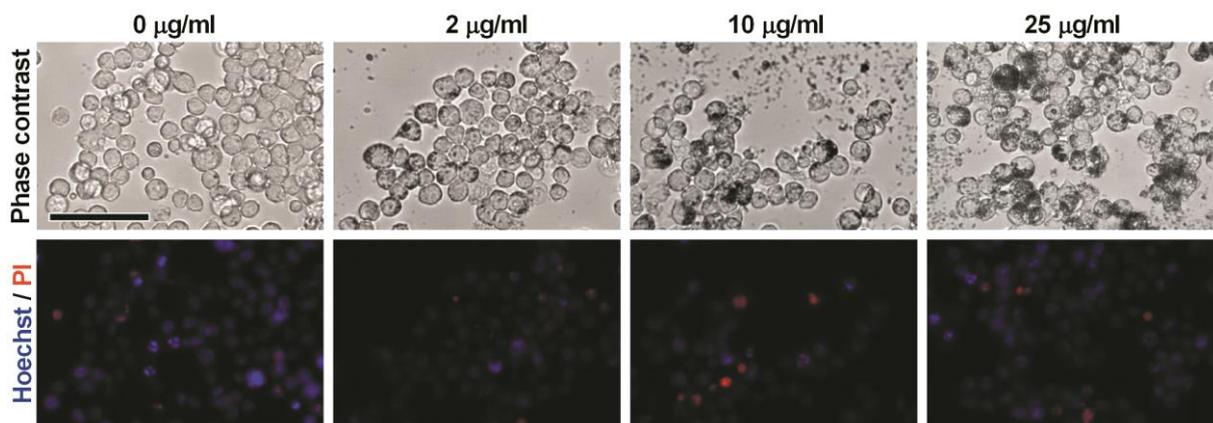
### Cell viability



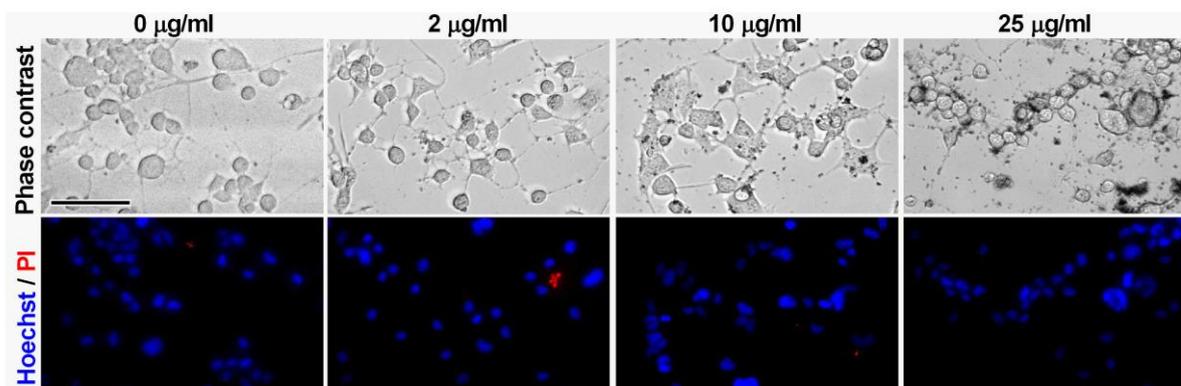
**Figure S4: Viability and morphology of mouse microglial cells following 24 h incubation with polyacrylic acid (PAA) coated magnetic NPs.** Viable cells are labelled with Hoechst 33342 (blue) and dead cells are labelled with propidium iodide (PI; red). Scale bar corresponds to 50 µm.



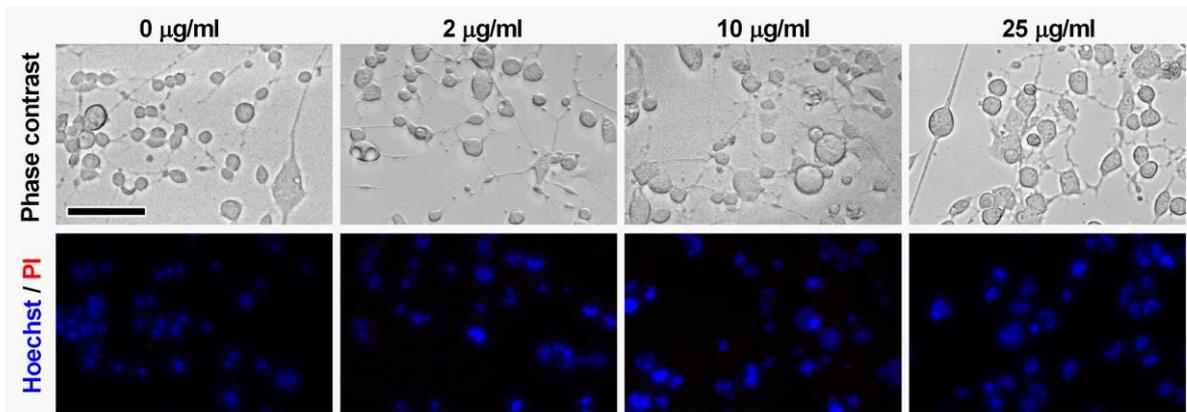
**Figure S5:** Viability and morphology of mouse microglial cells following 24 h incubation with uncoated maghemite (MGH) NPs. Viable cells are labelled with Hoechst 33342 (blue) and dead cells are labelled with propidium iodide (PI; red). Scale bar corresponds to 50  $\mu\text{m}$ .



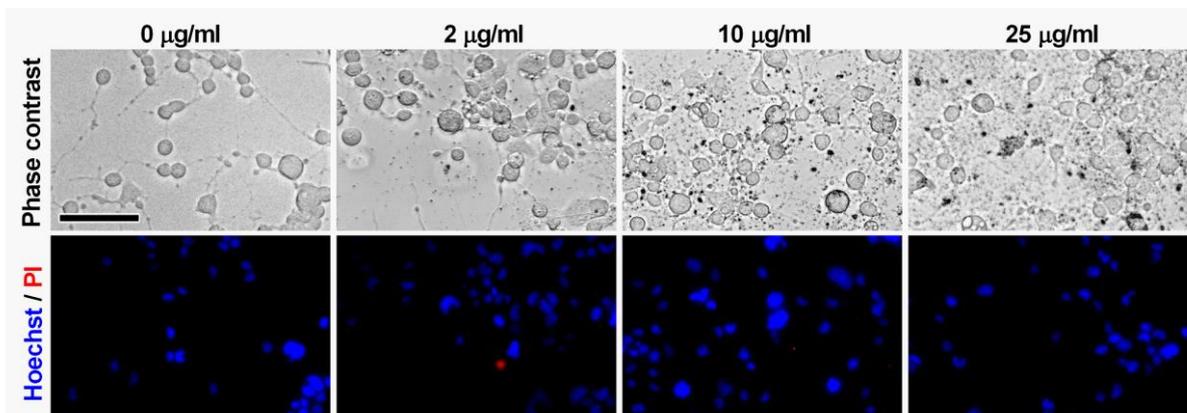
**Figure S6:** Viability and morphology of mouse microglial cells following 24 h incubation with industrial  $\text{TiO}_2$  P25 nanoparticles. Viable cells are labelled with Hoechst 33342 (blue) and dead cells are labelled with propidium iodide (PI; red). Scale bar corresponds to 50  $\mu\text{m}$ .



**Figure S7:** Viability and morphology of CAD neuronal cells following 48 h incubation with polyacrylic acid (PAA) coated magnetic nanoparticles. Viable cells are labelled with Hoechst 33342 (blue) and dead cells are labelled with propidium iodide (PI; red). Scale bar corresponds to 100  $\mu\text{m}$ .

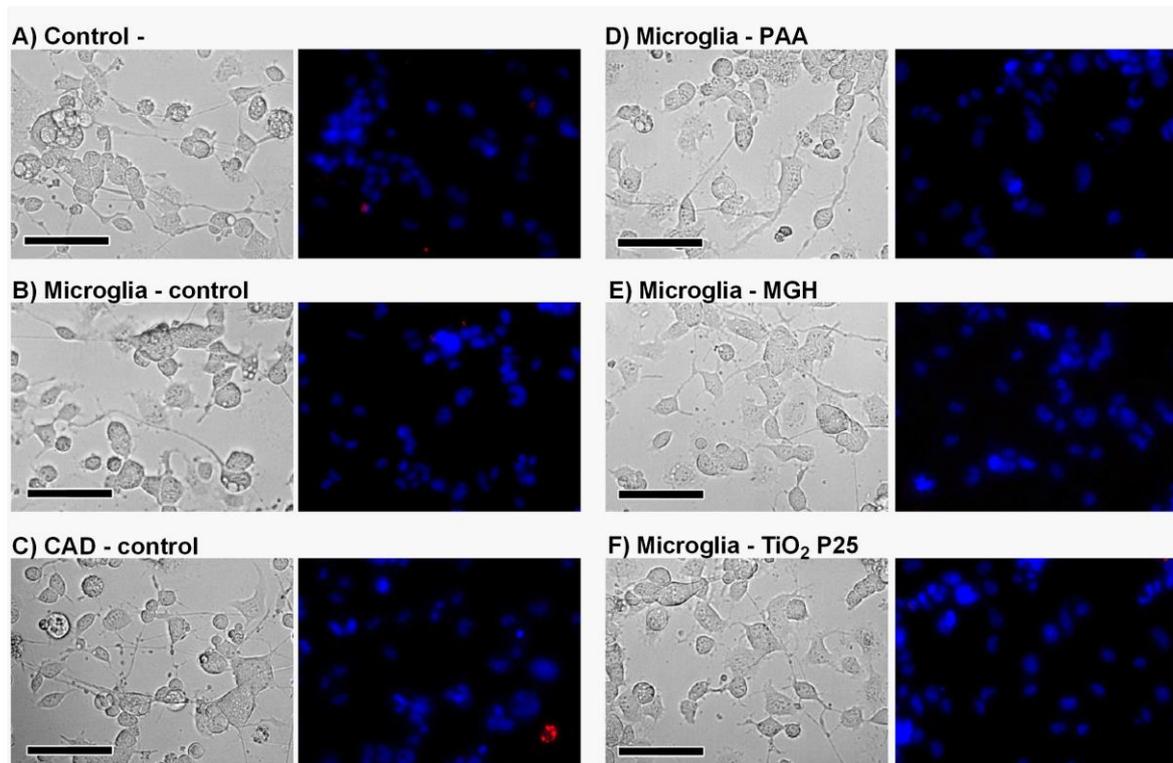


**Figure S8:** Viability and morphology of CAD neuronal cells following 48 h incubation with uncoated maghemite (MGH) nanoparticles. Viable cells are labelled with Hoechst 33342 (blue) and dead cells are labelled with propidium iodide (PI; red). Scale bar corresponds to 100  $\mu\text{m}$ .



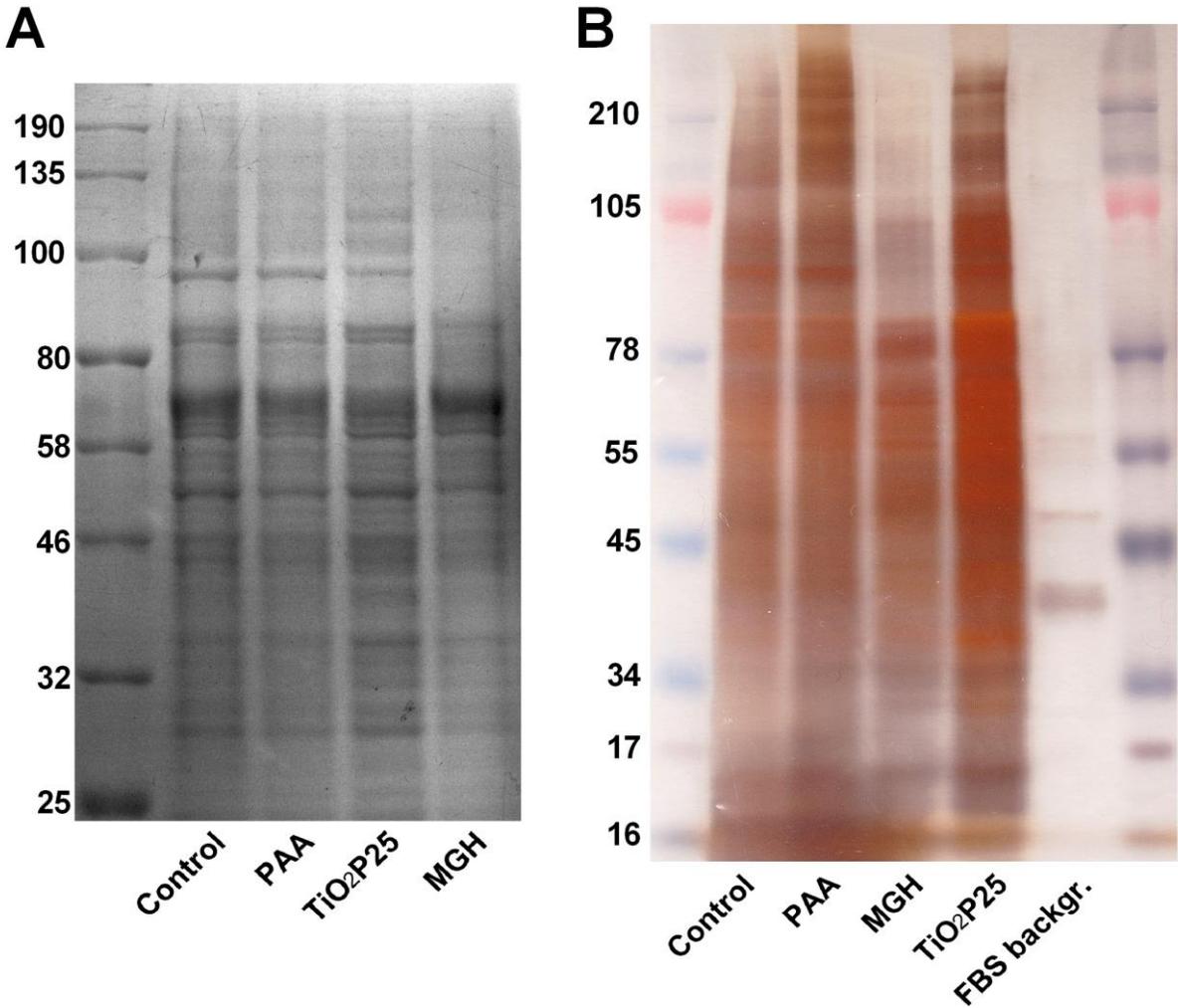
**Figure S9:** Viability and morphology of CAD neuronal cells following 48 h incubation with industrial  $\text{TiO}_2$  P25 nanoparticles. Viable cells are labelled with Hoechst 33342 (blue) and dead cells are labelled with propidium iodide (PI; red). Scale bar corresponds to 100  $\mu\text{m}$ .

## Co-culture exposure



**Figure S10:** The indirect effect of NPs on viability and morphology of CAD neuronal cells in co-culture with mouse microglial cells. Differentiated CAD cells were either (A) left untreated, (B) grown in co-culture with non-treated microglial or (C) CAD cells as a control, or exposed to microglial cells incubated with 25 μg/mL (D) polyacrylic acid (PAA) coated magnetic NPs (E) uncoated maghemite (MGH) or (F) industrial TiO<sub>2</sub> P25 NPs for 24 h in transwell system. Following 24 h incubation, transwell inserts were removed and CAD cells were left to rest for another 24 h before analysis. Viable cells are labelled with Hoechst 33342 (blue) and dead cells are labelled with propidium iodide (PI; red). Scale bar corresponds to 100 μm.

Analysis of secreted proteins



**Figure S11:** SDS-page analysis of proteins secreted from mouse microglial cells incubated with 25  $\mu\text{g}/\text{mL}$  of NPs for 24 h in FBS free medium. Polyacrylic acid (PAA) coated magnetic NPs, uncoated maghemite (MGH) or industrial TiO<sub>2</sub> P25 NPs were used. Untreated microglial cells were used as a control for protein secretion and an empty well with cell culture medium was used to determine FBS background after washing with FBS free medium. Proteins were concentrated and resolved on 4-12% polyacrylamide gels. Gels were stained with (A) Coomassie blue stain to obtain low sensitivity staining or with (B) silver nitrate staining to obtain high sensitivity staining.