

## **Mitochondrial Dysfunction and Loss of Glutamate Uptake in Primary Astrocytes Exposed to Titanium Dioxide Nanoparticles**

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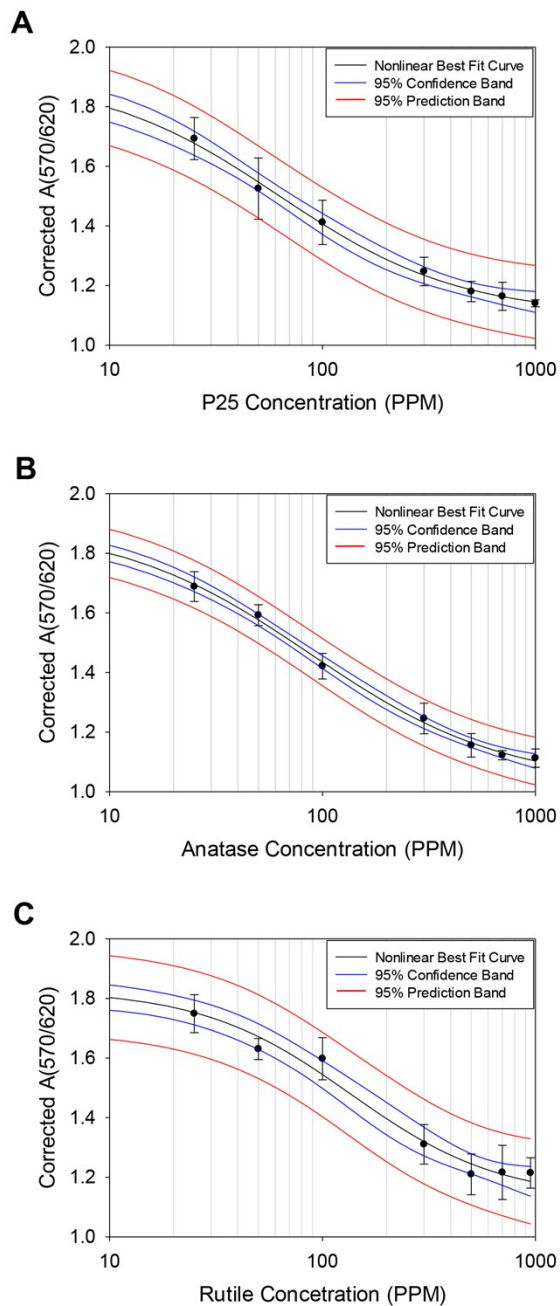
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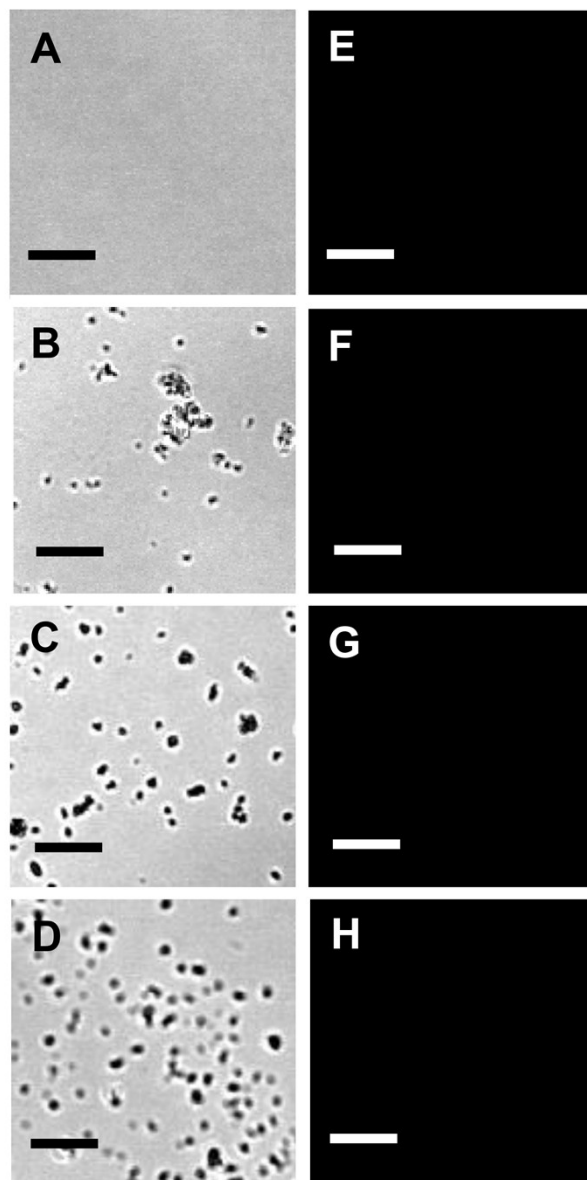
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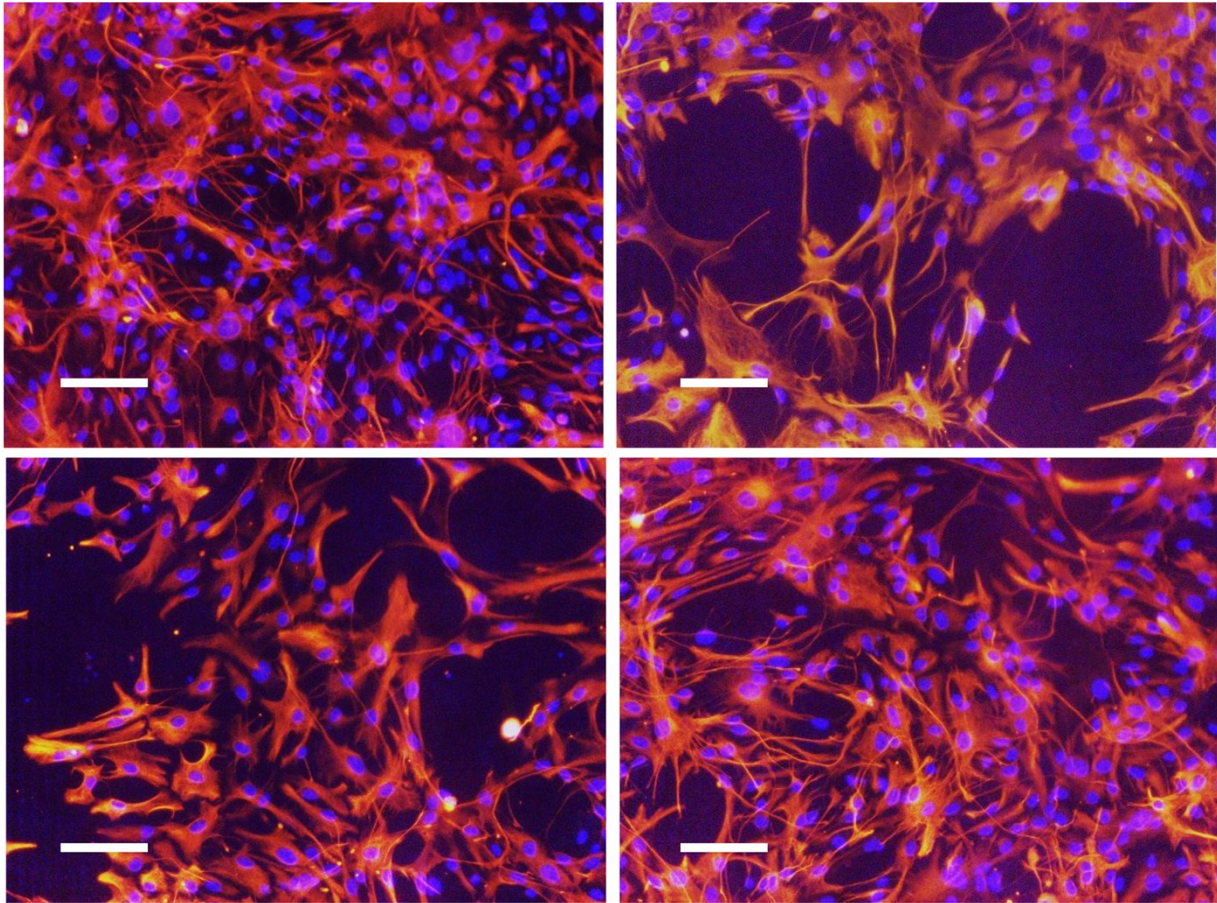
## SUPPLEMENTARY INFORMATION



**Suppl Fig 1. Lethal Concentration.** Lethal concentration was quantified at 0ppm, 25ppm, 50ppm, 100ppm, 300ppm, 500ppm, 700ppm and 1000ppm nanoparticle. P25 (A) was observed to be the most lethal nanoparticle after 24 hr treatment followed by anatase (B) and rutile (C) as determined by calculating the  $LC_{50}$  utilizing sigma plot analysis. (N = 6).



**Suppl Fig 2.** Acellular stained images of  $\text{TiO}_2$  nanoparticles with MitoTracker Red. Confocal phase (A-D) and florescent (E-H) images of no nanoparticles (A,E), 100 ppm P25 (B,F), 100 ppm Anatase (C, G) and 100 ppm Rutile (D,H) nanoparticles treated with 300 nM MitoTrackerR Red CMXROS dye as described in the experimental section. Scale bar 10  $\mu\text{m}$ .



**Suppl Fig 3.** Representative images of the untreated astrocyte culture. The astrocyte culture utilized for experiments was characterized by immunostaining with anti-glial fibrillary acidic protein (GFAP, red) and DAPI nuclear staining (blue). Cells were fixed with 4% paraformaldehyde for 20 min, permeabilized in 0.1% Triton X-100 for 15 min and background blocked with 1% bovine serum albumin (BSA) in PBS for 1 hr at room temperature. Cells were stained in primary antibody solution (1:1000 anti-GFAP [DAKO] in 1% BSA in PBS) at 4 °C overnight, secondary antibody solution (1:500 anti-Rabbit rhodamine [Millipore] in PBS) for 2 hr at room temperature and DAPI staining solution (1 µg/ml DAPI in PBS) for 5 min at room temperature. Images were obtained using Axiovert 40 CFL [Zeiss] and images taken with a Progres C3 [Jenoptick] camera with an X-Cite series 120Q [Lumen Dynamics] lamp and a CY3 or DAPI filter [Chroma]. Culture purity was determined to be > 90 % astrocytes by counting the number of GFAP positive nuclei over the total nuclei using Image J cell counter [NIH.gov].