Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Figures

Multiparametric nanoparticle-induced toxicity readouts with single cell resolution in HepG2 multicellular tumour spheroids

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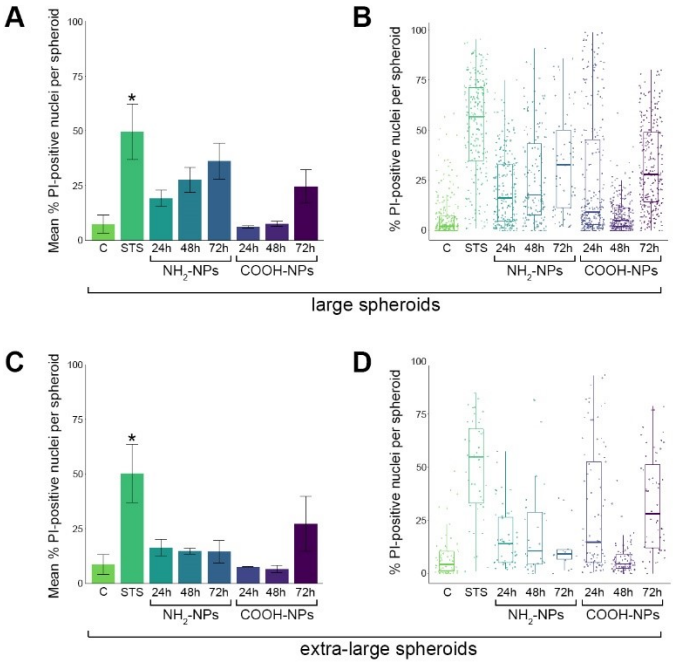

Figure S1. Single cell analysis of spheroid nuclei following treatment with NPs. (A) Graph showing mean % of PI-positive nuclei per large spheroid after the various treatments as indicated. (B) Boxplot showing median value of PI-positive nuclei value and quartiles in large spheroids. Data in (A, B) are from 6 replicate wells; total number of spheroids analysed was 1666. (C) Graph showing mean % of PI-positive nuclei per extra-large spheroid after the various treatments as indicated. (D) Boxplot showing median % of PI-positive nuclei value and quartiles in extra-large spheroids. Data in (C, D) are from 3 replicate wells per treatment; total number of spheroids analysed was 429. C, control; STS, staurosporine. Asterisks denote $p<0.01$ compared to control samples.

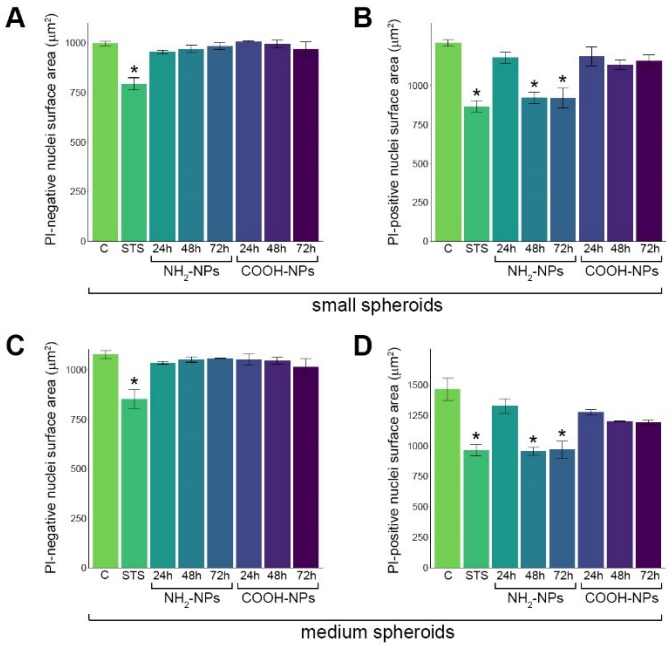

Figure S2. Single cell analysis of spheroid nuclei surface area following treatment with NPs. (A) Graph showing surface areas of PI-negative nuclei in small spheroids after the various treatments as indicated. (B) Graph showing surface areas of PI-positive nuclei in small spheroids after the various treatments as indicated. (C) Graph showing surface areas of PI-negative nuclei in medium spheroids after the various treatments as indicated. (D) Graph showing surface areas of PI-positive nuclei in medium spheroids after the various treatments as indicated. Data are from 6 replicate wells per treatment; total number of small spheroids analysed was 1515; total number of medium spheroids analysed was 1049. C, control; STS, staurosporine. Asterisks denote $p<0.01$ compared to control samples.

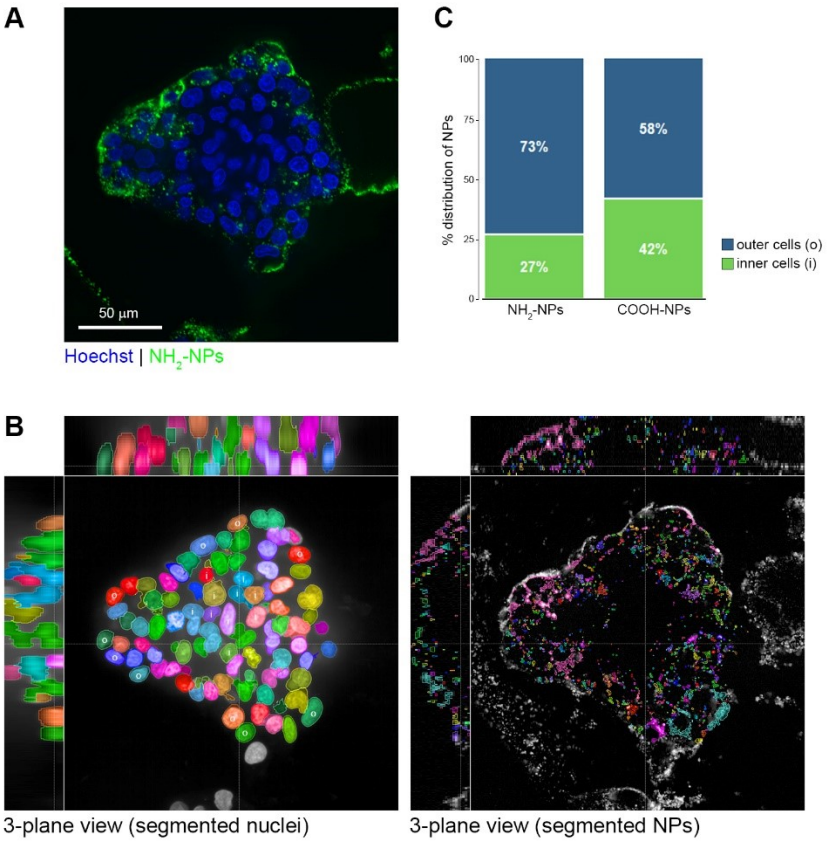

Figure S3. Analysis of NP penetration into medium spheroids. (A) Example image showing a single confocal slice of a medium spheroid treated with NH_2-NPs for 72 h. Nuclei are labelled with Hoechst 33342 and are shown in blue, NH_2-NPs are shown in green. (B) Three-plane views of the spheroid shown in (A) showing segmentation of individual cell nuclei (left) and NPs (right). Pseudo-colouring is used to indicate identification of distinct structures. Cells are classified as either inner (i) or outer (o) with respect to whether they have direct access to the surrounding cell culture medium. (C) Graph showing quantification of NPs (based on intensity) in the inner or outer cells. Data are from 10 spheroids incubated with NH_2-NPs and 10 spheroids incubated with COOH-NPs, with a total of 1824 cells analysed.