Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary information for:

Biological interactions of ferromagnetic iron oxide-carbon nanohybrids with alveolar epithelial cells.

Vercellino S. *et al.*

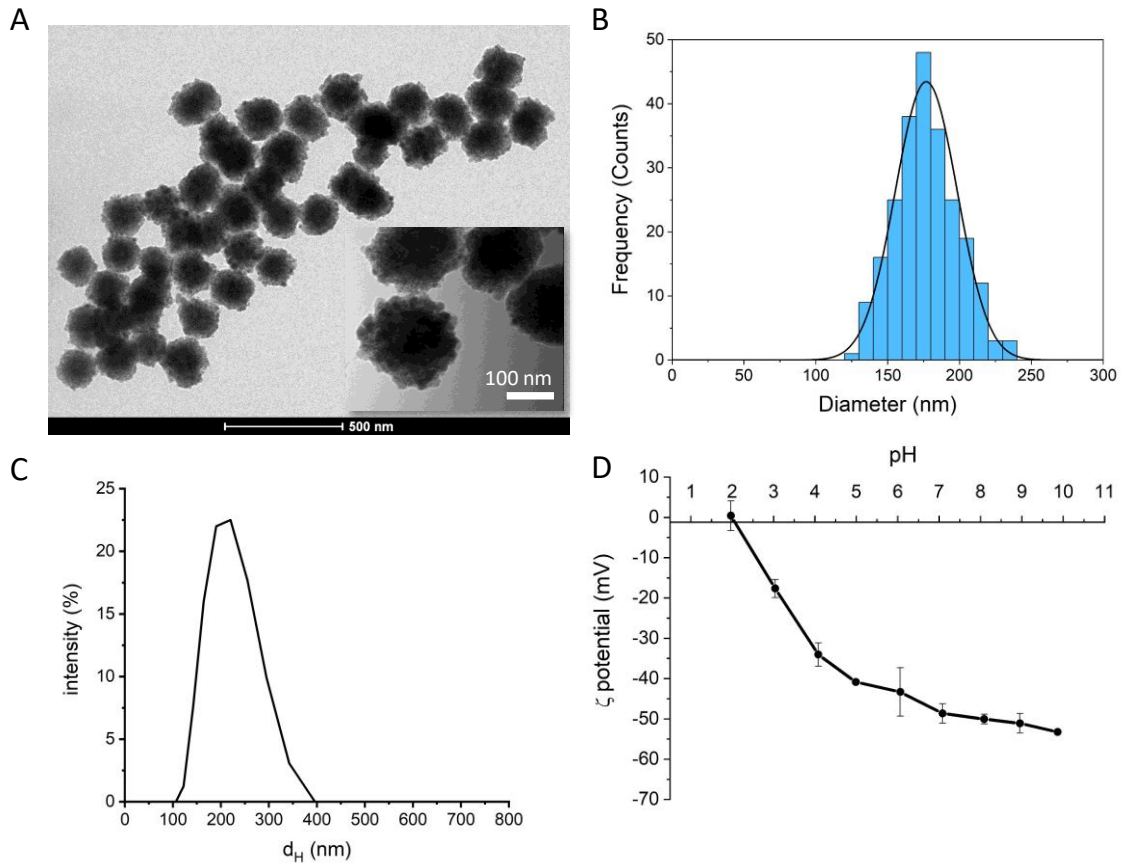

Figure S1. Synthesis of larger IONP@C following a reported methodology. A) Representative TEM micrographs show a nanoparticle structure where the iron oxide core is more spread into the carbonaceous matrix. B) Statistical size distribution from TEM imaging resulting in an average diameter of about 175 nm. C) Size distribution evaluated by DLS (PDI=0.12); D) ζ-potential vs. pH evaluated by ELS. For these nanoparticles, 10 times the amount of H_2O_2 was used with respect to the IONP@C presented in the main text.

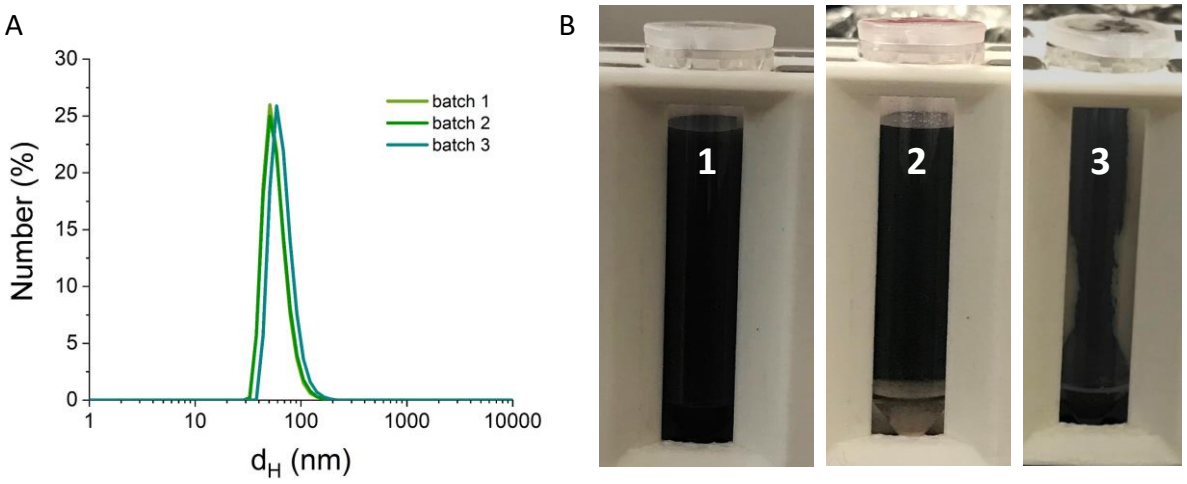

Figure S2. Further IONP@C characterization. A) Synthesis reproducibility assessment. The hydrodynamic diameter distribution measured by DLS and expressed as relative number of three different IONP@C batches. B) magnetic separation of the nanohybrids from the suspension: (1) IONP@C suspension at T= 0 on the magnetic rack (2) NPs suspension at T= 4 hours (note all the NPs are separated from the solvent – the solvent is clear at the bottom of the tube) (3) NPs after discarding the solvent, the IONP@C is on the tube wall.

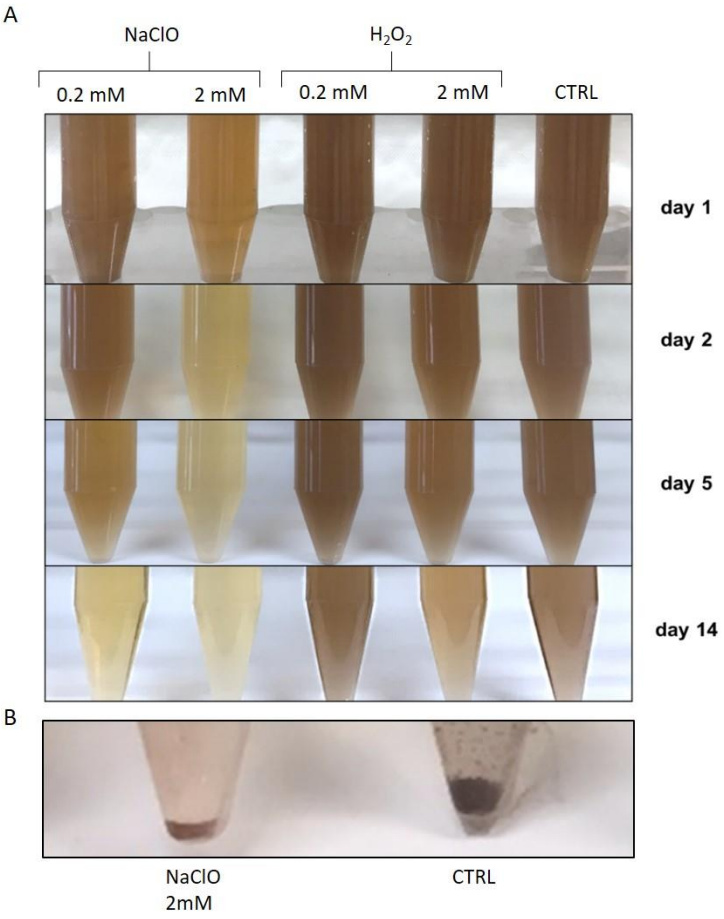

Figure S3. Degradation of Fe_3O_4@C. A) Appearance of the suspensions of IONP@C incubated in NaClO or H_2O_2 0.2 or 2 mM of at different time points. B) Appearance of the residue after incubation of the IONP@C in 2 mM NaClO. CTRL: IONP@C in PBS.

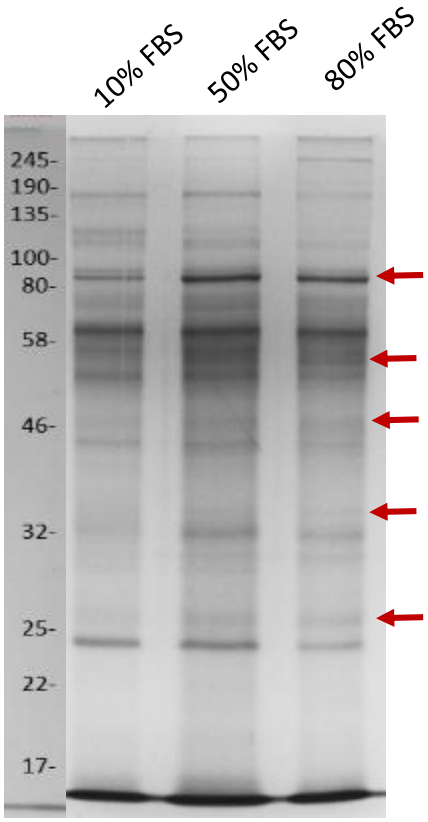

Figure S4. IONP@C protein corona composition. SDS PAGE gel of the protein corona formed after incubating the nanoparticles in different percentages of FBS. Differences in protein corona profile are highlighted with arrows.

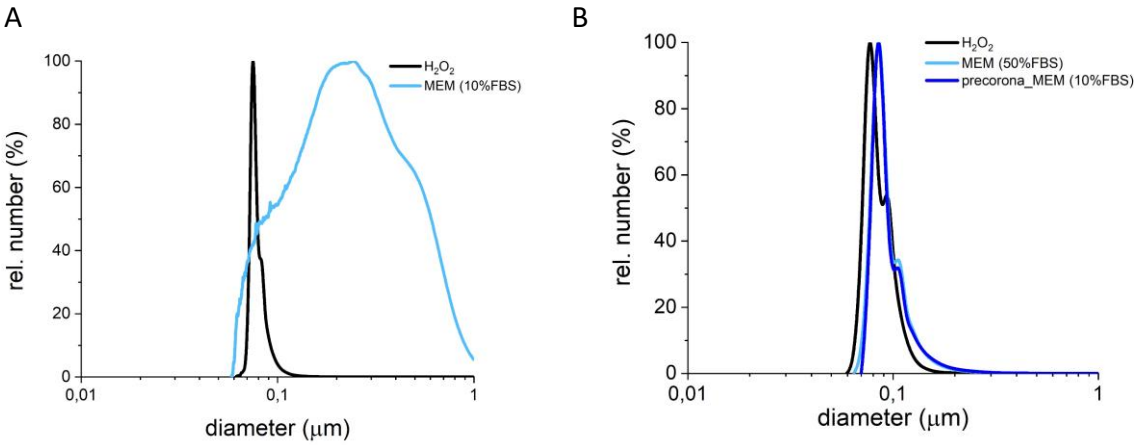

Figure S5. Assessment of IONP@C colloidal stability is complex media. All distributions are measured by DCS and expressed as relative number. The black curves represent IONPs in water. The blue curves indicate A) the size distribution of IONP@C resuspended in cMEM supplemented with 10% FBS for 24h; B) the size distribution of IONP@C resuspended in cMEM supplemented with 50% FBS for 24h and IONP@C with a precorona resuspended in cMEM supplemented with 10% FBS. Classic cMEM is not sufficient to maintain the colloidal stability in the absence of a preformed protein corona layer.

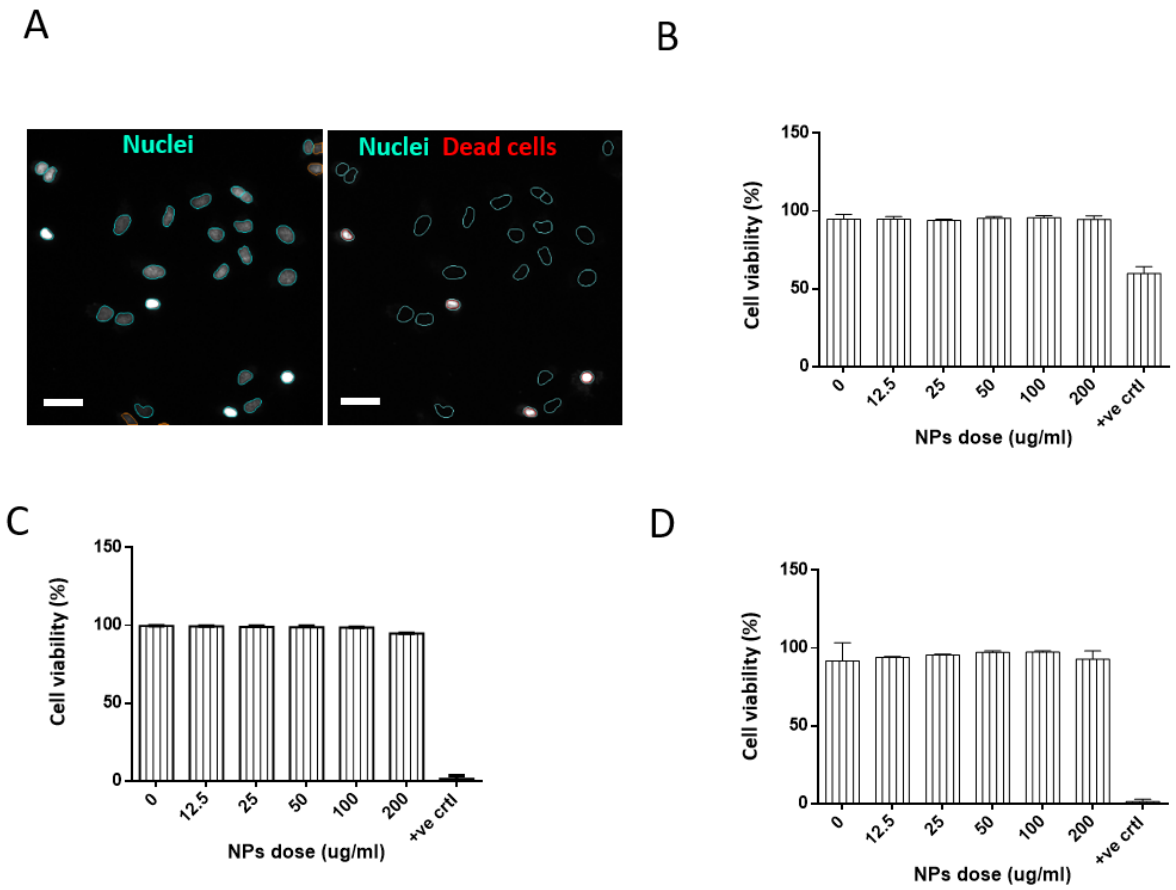

Figure S6. Live/dead count test of IONP@C nanohybrids treated A549 cells. A) High Content Analysis strategy for live/dead cell count. The cells were incubated with 200 µg/mL of IONP@C for 72 h. The masks are generated based on the fluorescence signal of subcellular organelles. For the live/dead count, the nuclei (turquoise) and the dead cells (red) are counted. The IONP@C with pre-corona resulted non-cytotoxic for all the concentrations tested at B) 24, C) 48, and D) 72 hours. The results are reported as mean ± SD from n=3 independent experiments in triplicate.

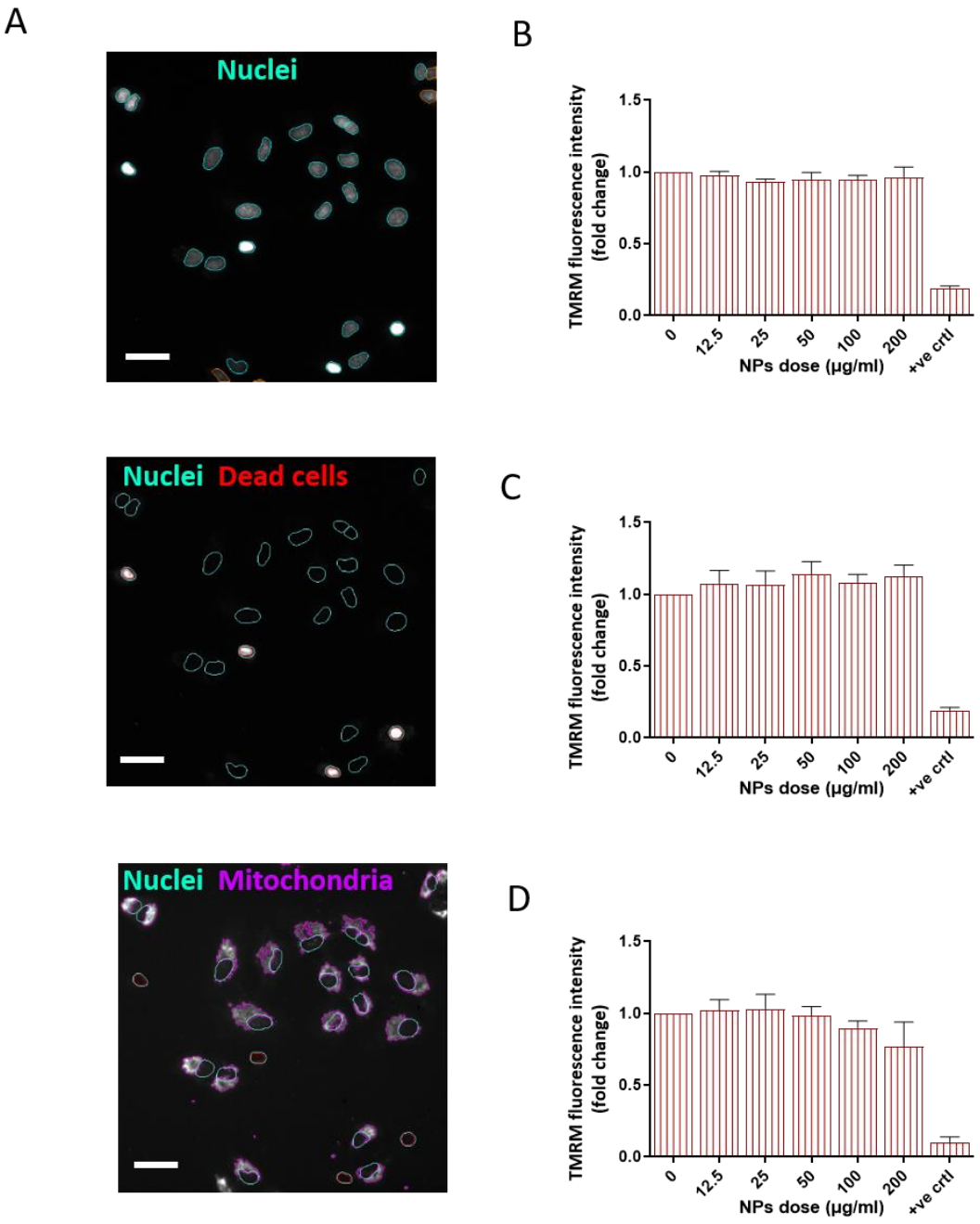

Figure S7. Mitochondrial health test after exposure to IONP@C nanohybrids. A) High Content Analysis strategy for the mitochondria health test. The cells were incubated with 200 µg/mL of IONP@C for 72 h. The masks are generated based on the fluorescence signal of subcellular organelles, and the intensity recorded within the mitochondrial mask (purple) represent the mitochondrial membrane potential status. As shown in the panel A, typically dead cells (highlighted in red), have little or no signal in the TMRM channel. The IONP@C with pre-corona resulted non-cytotoxic for all the concentrations tested at B) 24, C) 48, and D) 72 hours. The results are reported as mean ± SD from n=3 independent experiments in triplicate.

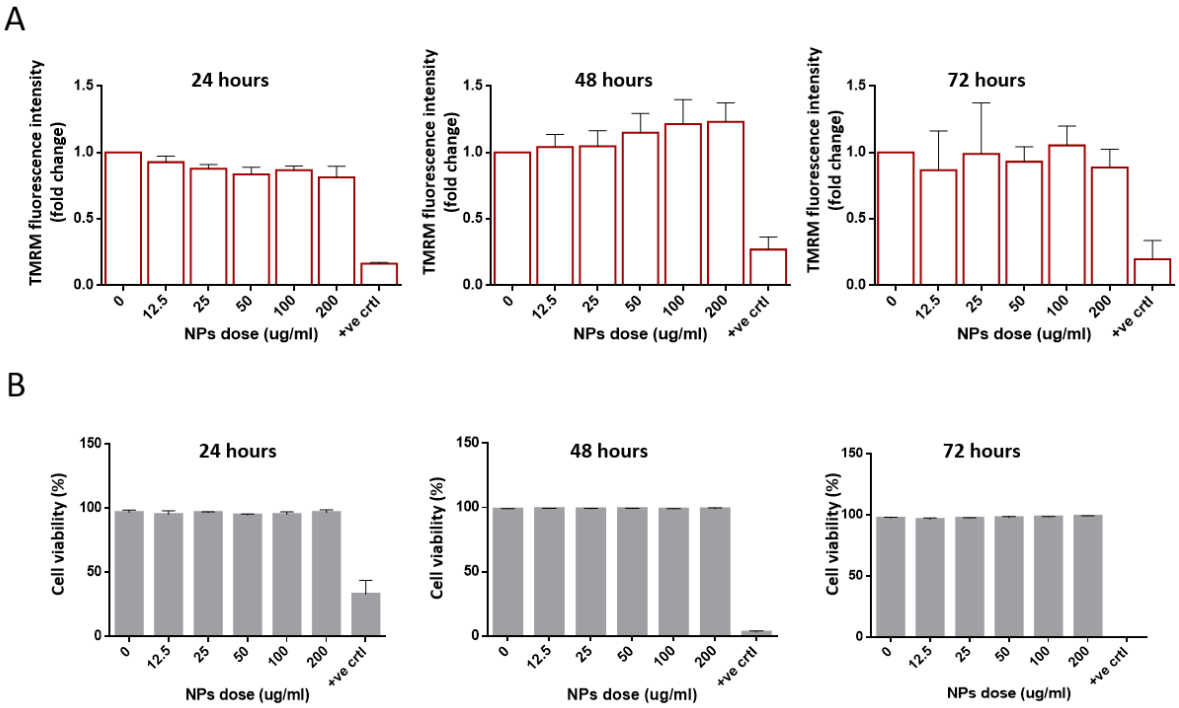

Figure S8. Nanohybrids viability in 10% FBS-supplemented medium. The IONP@C were precoated in full FBS, then washed and re-suspended in cMEM (10 % FBS) resulting non-cytotoxic for all the concentrations tested after 24, 48 or 72 hours. The evaluation was carried out by High Content Analysis, by TMRM fluorescence quantification (A) or live/dead count (B). The results are reported as mean ± SD from n=3 independent experiments in triplicate.

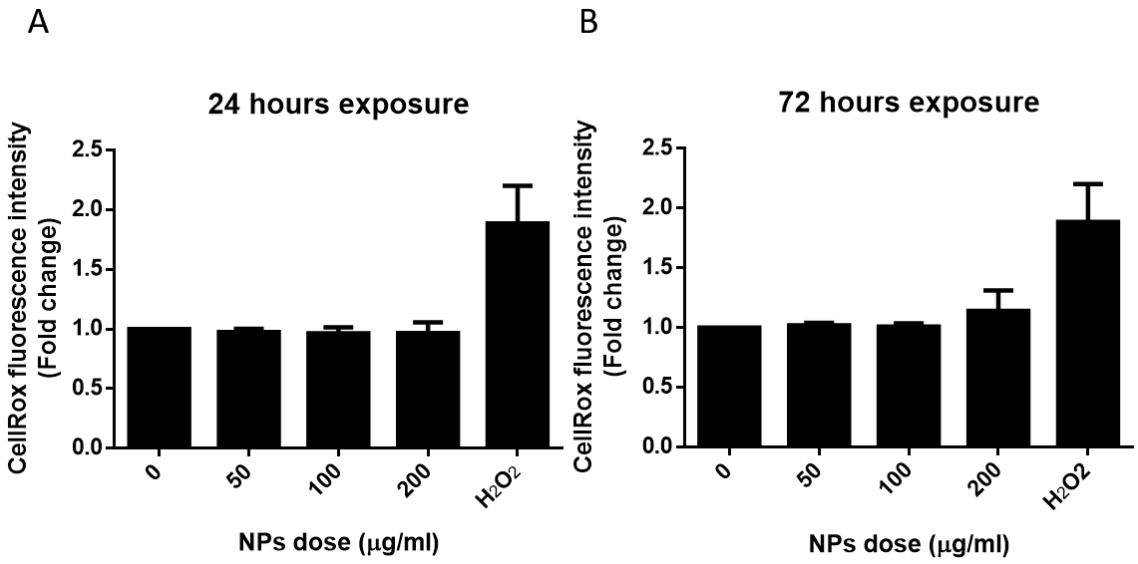

Figure S9. IONP@C NPs-induced oxidative stress. A549 cells were exposed to different IONP@C concentrations (0, 50, 100 and 200 μg/mL) for 24 or 72 hours (mean ± SD, n=1). Exposure to 200 μM H_20_2 was used as a positive control for ROS induction.

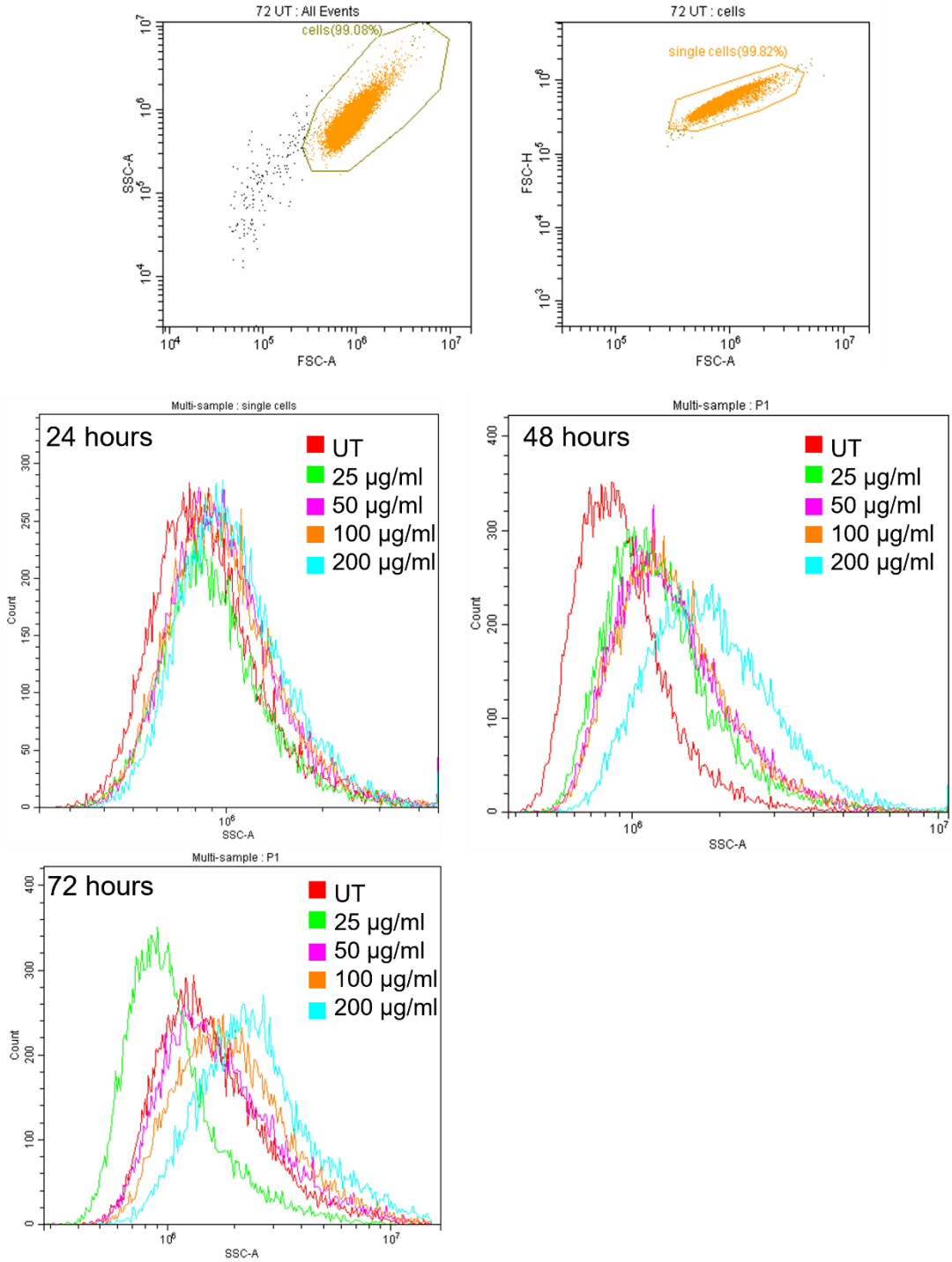

Figure S10. **Flow cytometry analysis of NPs uptake gating strategy**. Above, the cells were gated based on SSC-A vs FSC-A, and further gated to select the single cell population based on the FSC-H vs FSC-A. The side scattering histograms for the 3 different time points are shown in the bottom panels (one representative replicate). The distribution shifted to the right with increasing concentration of NPs, confirming that internalization.

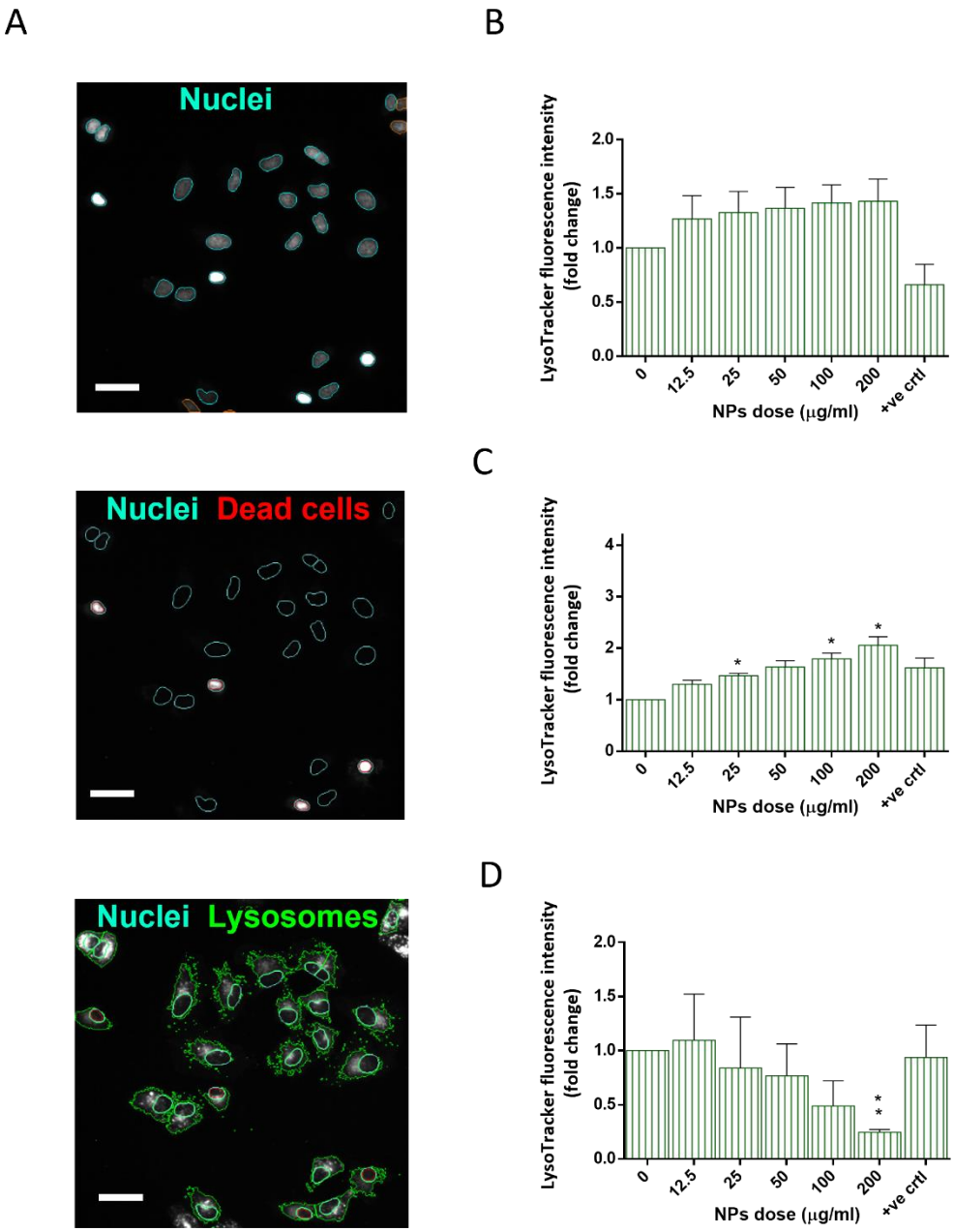

Figure S11. **Lysosomal state assessment of A549 cells after exposure to IONP@C nanohybrids.** A) High Content Analysis strategy for the lysosomes health test. The cells were incubated with 200 µg/mL of IONP@C for 72 h. The masks are generated based on the fluorescence signal of subcellular organelles, and the intensity recorded within the lysosomes mask (green) represents the lysosomes' pH status. The NPs induced lysosomes acidification in a dose-dependent fashion after incubation for B) 24 C) and 48 hours, while after D) 72 hours of continuous exposure to NPs, the lysosomes showed a dose-dependent de-acidification. The results are reported as mean ± SD from n=3 independent experiments in triplicate. Statistical analysis: **** $p<0.0001$, *** $p<0.001$, ** $p<0.01$, * $p<0.05$ one-way ANOVA/Tukey's tests.

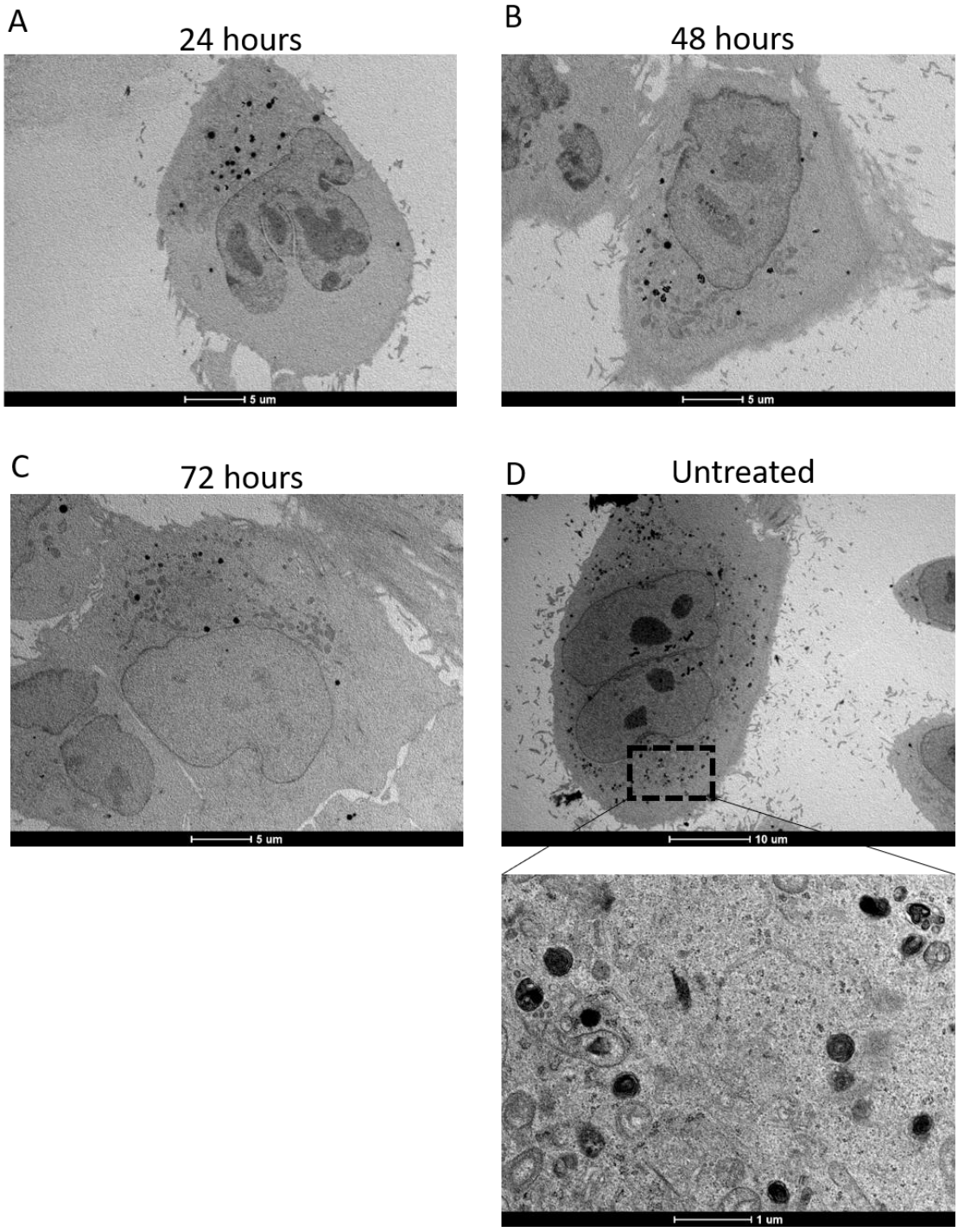

Figure S12. **Entire cell TEM images for the inset shown in the main figure 6**. Cells were incubated for A) 24, B) 48, and C) 72 hours with IONP@C, or were D) untreated (control, in 50% supplemented MEM).

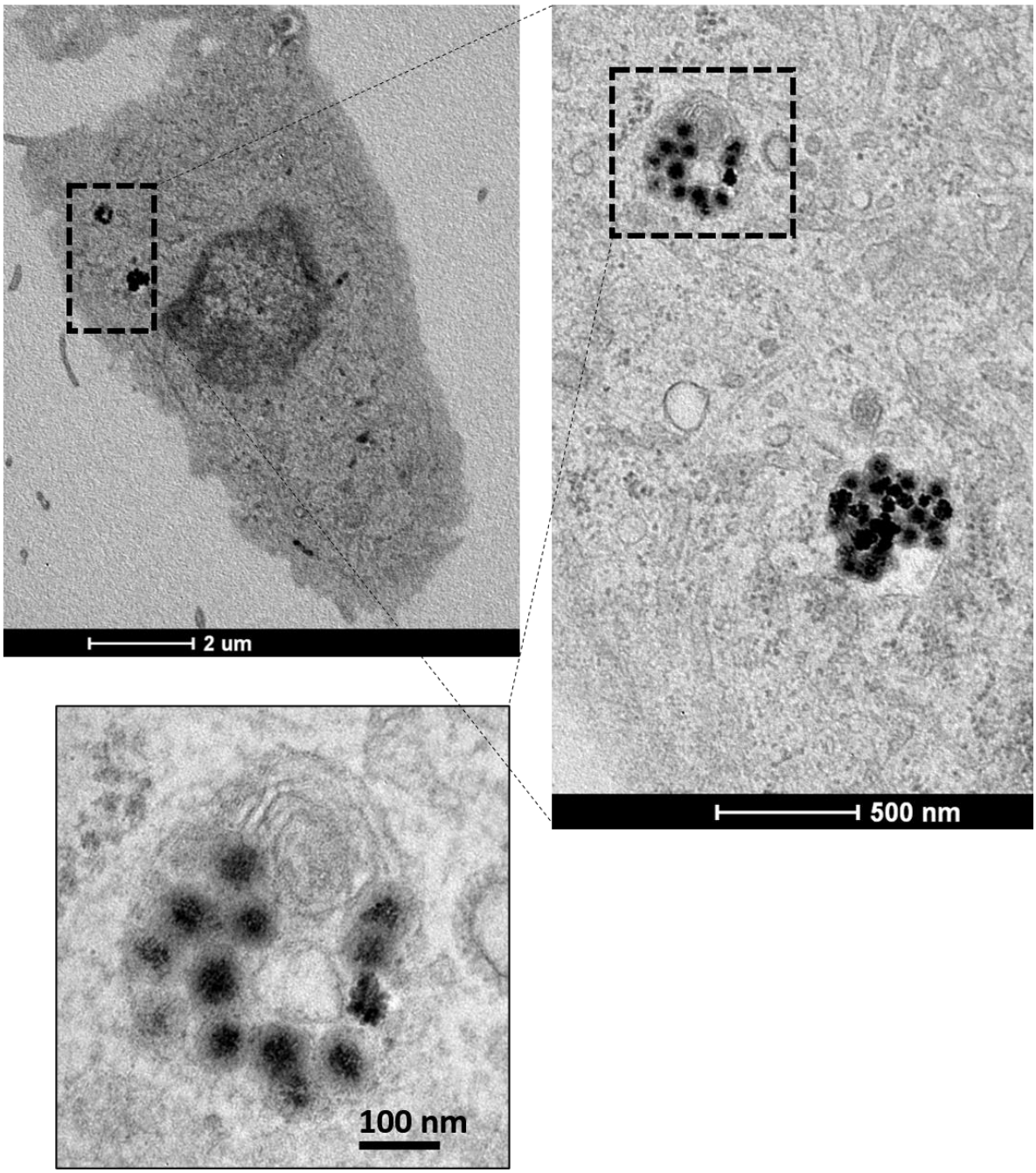

Figure S13. Example of rare NPs co-localised with a lamellar body after 48 hours of exposure to 100 µg/ml of IONP@C.