Electronic Supplementary Material (ESI) for Nanoscale.

Nano-ZnO leads to tubulin macrotube assembly and actin bundling triggering cytoskeletal catastrophe and cell necrosis

Lorena García-Hevia[a], Rafael Valiente[ab], Rosa Martín-Rodríguez[ac], Carlos Renero-Lecuna[ab], Jesús González[ad], Lidia Rodríguez-Fernández[e], Fernando Aguado[d], Juan C. Villegas[a] and Mónica L. Fanarraga[a]

SUPPLEMENTARY INFORMATION

Supplementary Figure 1

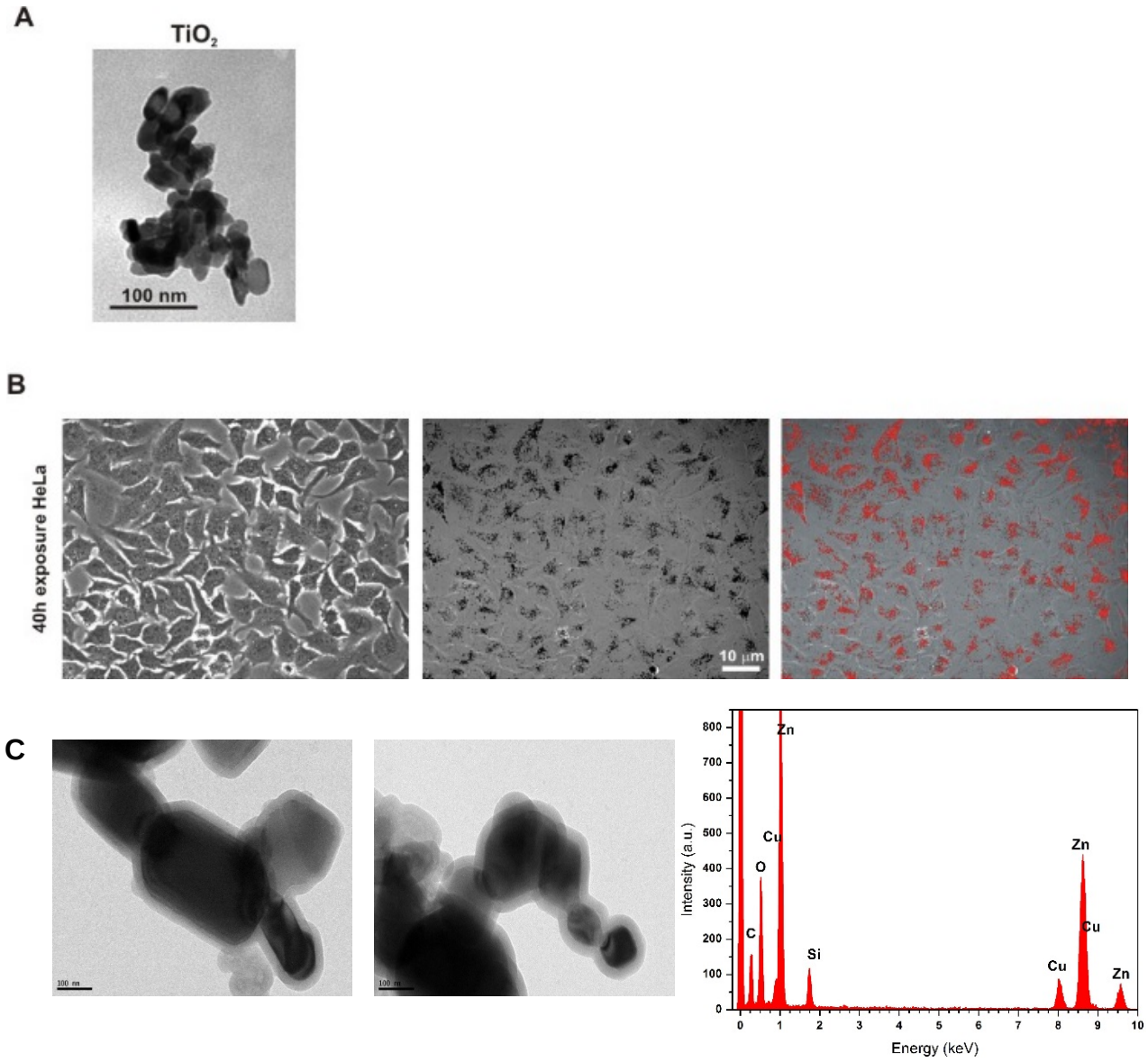

Figure S 1: (A) TEM image of representative TiO_2 nanoparticles. (B) Phase-contrast image of human epithelial cells exposed to 100 µg/mL of anatase TiO_2 NP during 40 h. (center). Same image in bright field microscopy showing the intracellular nanoparticles as black intracellular spots. (right) Same image processed with the NIS-Elements Advanced Research software highlighting the TiO_2 nanoparticles colored in red for better identification.(C) Transmission electron micrograph of the SiO_2-coated ZnO nanoparticles. (Right) EDX spectrum of the coated ZnO nanoparticles.

Supplementary Figure 2

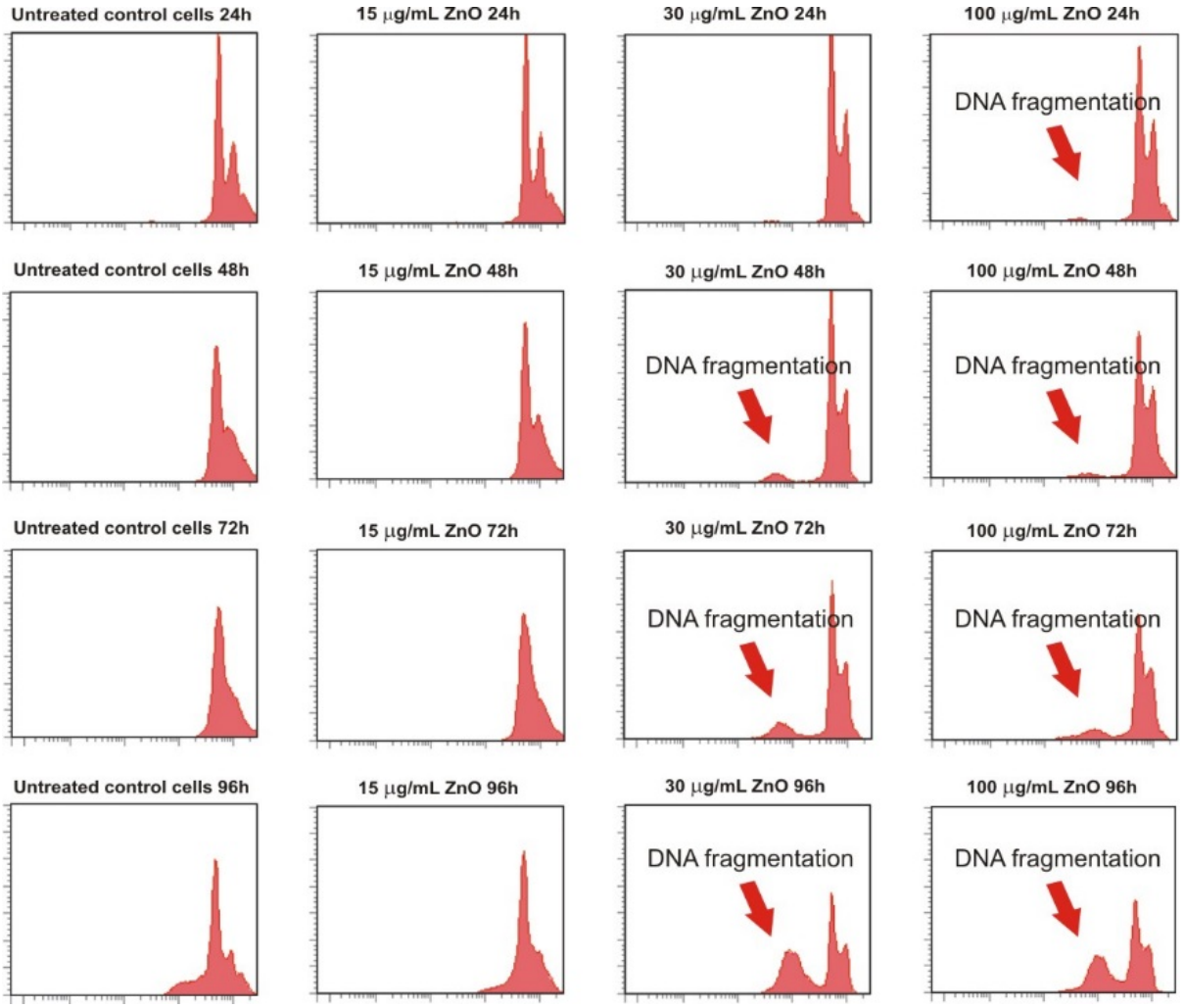

Figure S 2: Original flow cytometry charts used to quantify apoptosis and to elaborate the histograms represented in Figure 2D (red bars). These charts are histograms obtained for approximately 10.000 cells. The amount of DNA per cell is represented in the X axis, and the total number of cells in the Y axis. Arrows indicate the region of the chart where apoptosis is markedly observed.

Supplementary Figure 3

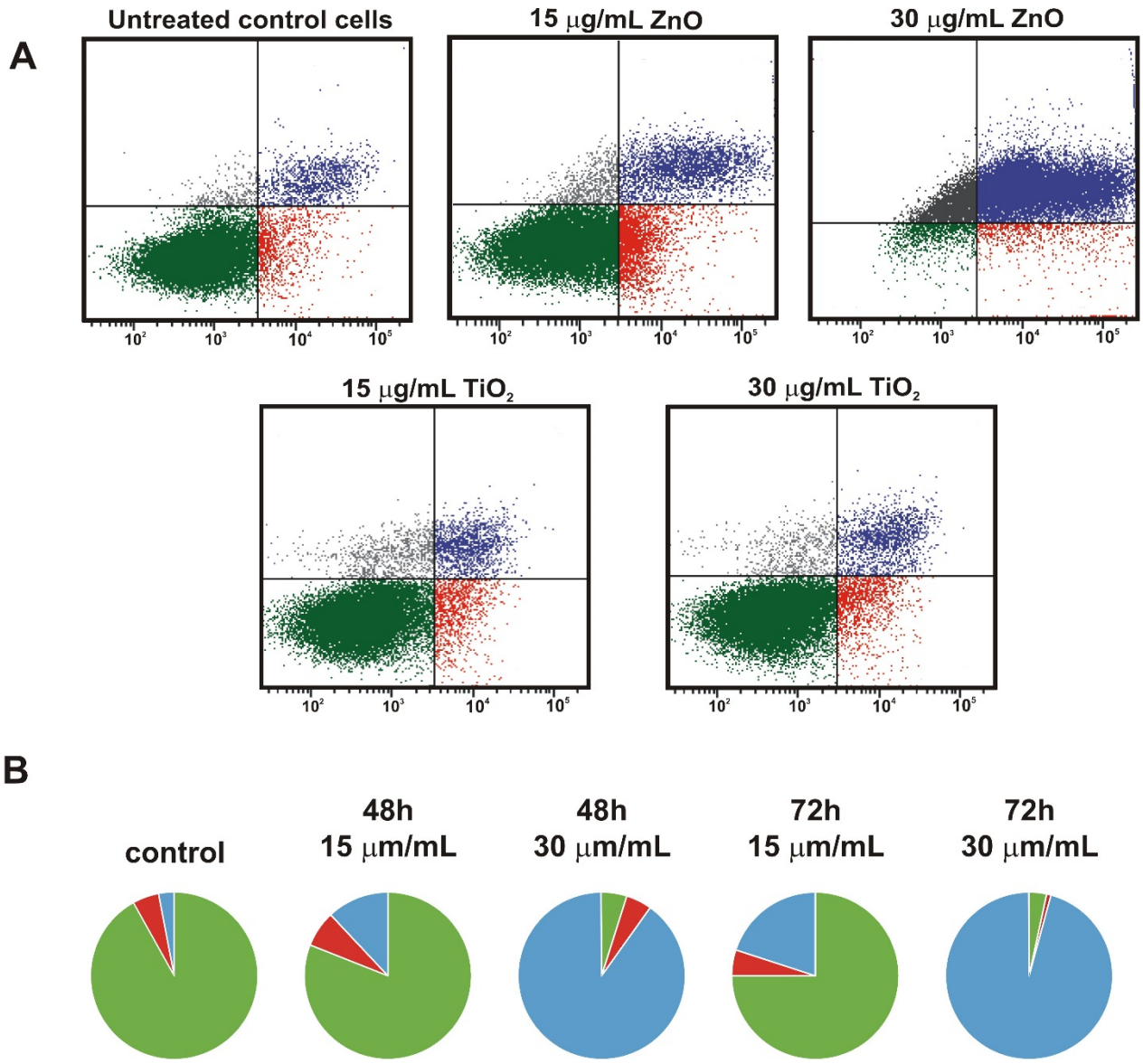

Figure S 3: Flow cytometry charts displaying viable cells (green), apoptotic cells (red), and necrotic cells (blue) obtained for cells exposed to ZnO for 24h. These graphs are the original flow cytometry data represented as pie charts in Figure 2E. (B) Time course pie charts representing results obtained for cells exposed to ZnO during 48 and 72h.

Supplementary Figure 4

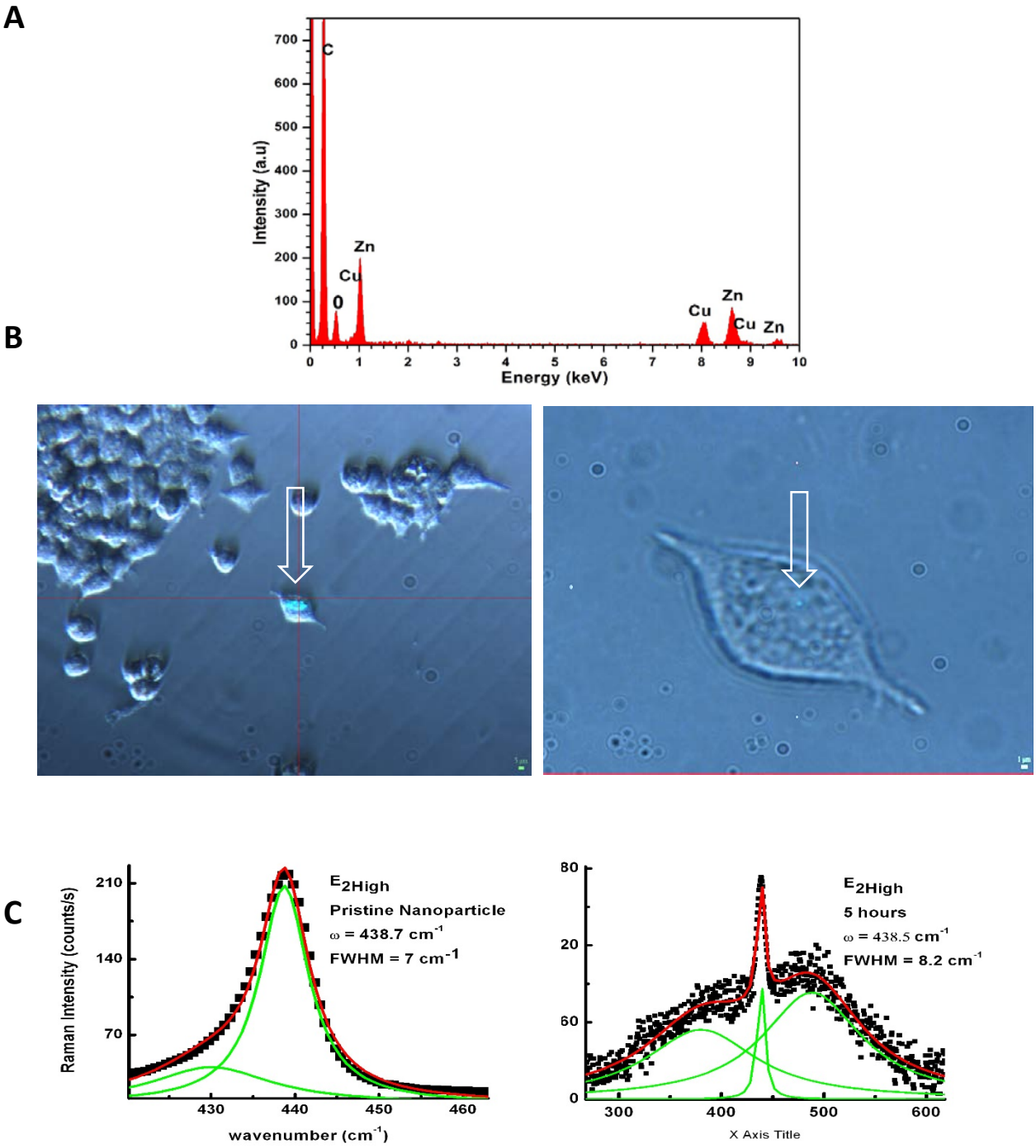

Figure S 4: (A) EDX spectrum of the "digested" ZnO nanoparticles in Figure 4B extracted from inside the keratinocytes. (B) Phase contrast image of HaCat cells captured through 20x (left) and 100x (right) objectives of the confocal Raman microscope during spectroscopic analysis. Arrows point at the laser spots. The sizes of the laser spots can be calculated with the scale bars (right, bottom corner). (C) Lorentzian fit of the Raman spectra of ZnO nanoparticles in the spectra domain of E_{2High} modes. A slight increase of the full wide at half-maximum (FWHM) Raman mode can be observed while the frequency remains practically constant.

Supplementary Figure 5

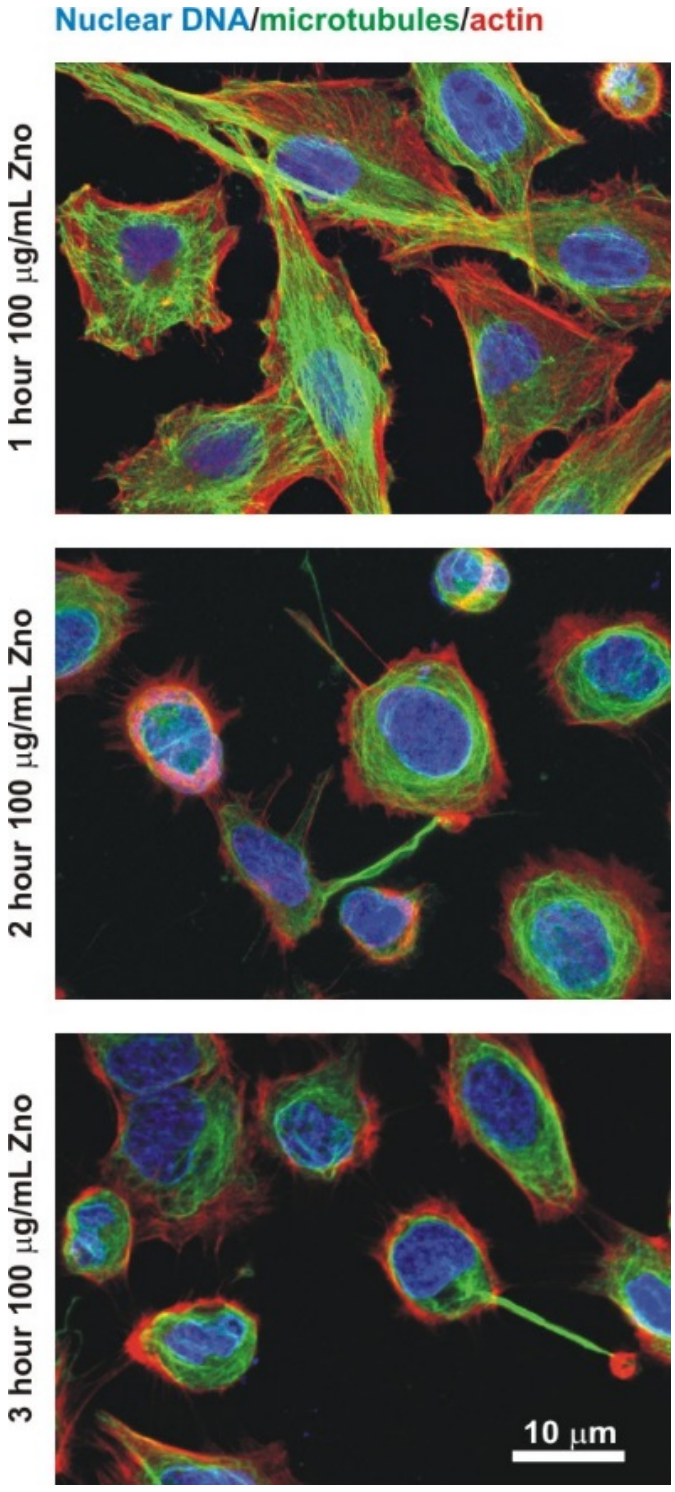

Figure S 5: Confocal microscopy images of cells exposed ZnO for the indicated times. These figures are high-resolution individual time points that complement those at Fig. 4. Aberrations in the organization of the actin microfilaments (red channel) and microtubules (green channel) progressively increase during exposure to 100 µg/mL ZnO nanoparticles.

Supplementary Figure 6

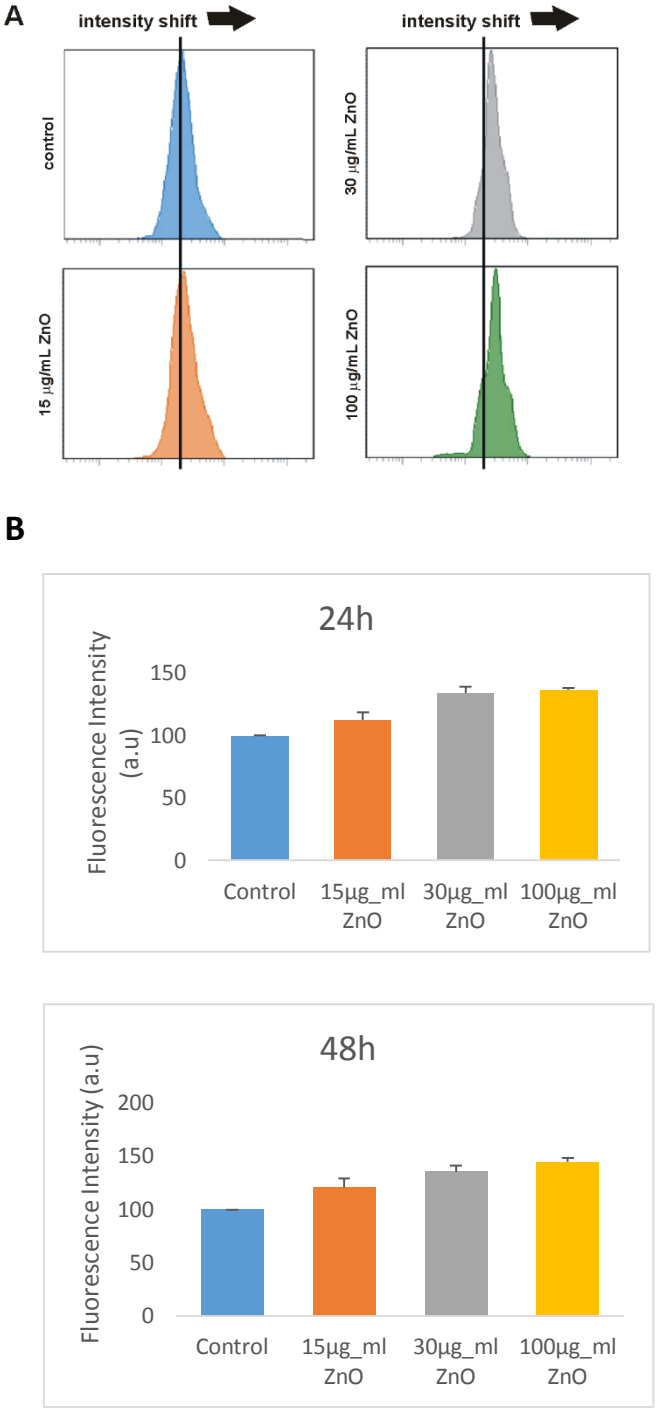

Figure S 6: ROS quantification in nano-ZnO exposed cells. (A) Intensity shift, representing the fluorescence levels per cell indicative of the intracellular ROS levels (24 h) quantified by Flow cytometric analysis. The amount of ROS per cell is represented in the X axis, and the total number of cells in the Y axis. Each histogram chart represent the lecture in approximately 10.000 cells. (B) Simplified graphs as histograms representing the intracellular ROS levels for cells exposed to the indicated amounts of ZnO NPs during 24 and 48 h (Error bars obtained for 3 replicates).

Supplementary Figure 7

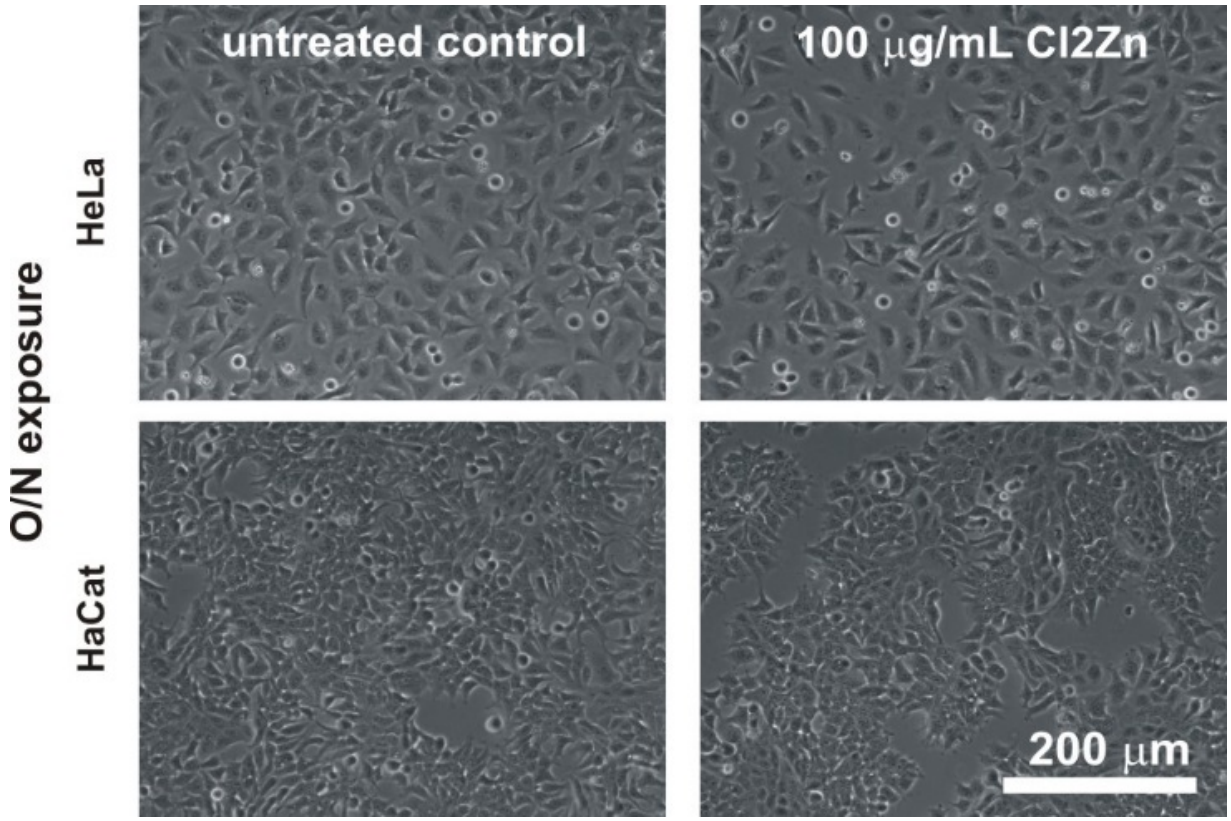

Figure S 7: Phase contrast images of cells exposed CL_2Zn. No evident toxicity sings are evident after O/N exposure to soluble Zn^{2+}.

Supplementary Figure 8

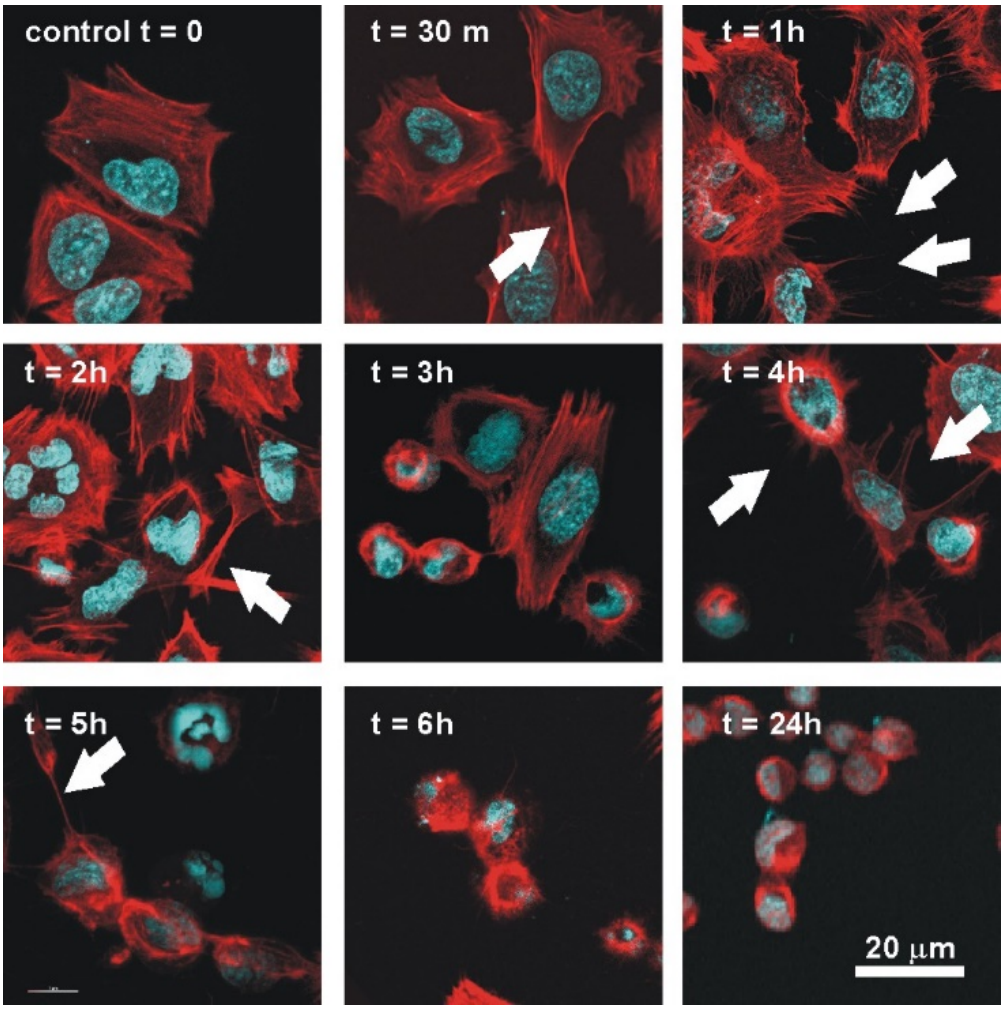

Figure S 8: Confocal microscopy images of The actin bundling changes in cells exposed to 100 μg/mL ZnO nanoparticles for the indicated times. A progressive actin microfilament bundling (red channel) is concomitant to cell collapse and nuclear compaction.

Supplementary Video:

Video S1: Phase contrast time-lapse video microscopy of HeLa and HaCat cells exposed to 100 µg/mL nano-ZnO during 260 min. Cell contraction is clearly observed in these cell cultures.