Supplementary Information

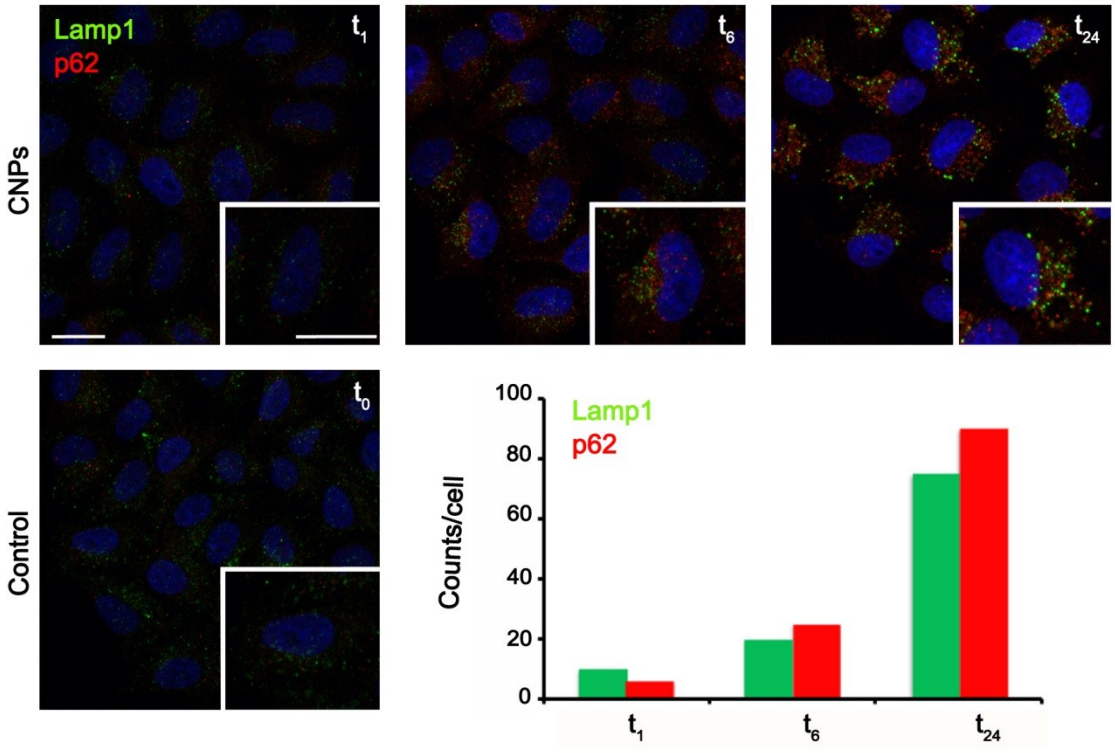

Figure S1. Confocal microscopy showing the increase in lysosomes (Lamp1) and autophagic vesicles (p62) in cells incubated for 1, 6 and 24 h with CNPs. Increasing Lamp1 and p62 correlated with CNP exposure time, as shown in the histogram. Images are from one single confocal section. Bars, 50 μm.

Cells previously incubated with CNPs for 1, 6 and 24 h were processed for immunofluorescence as previously described (1). Lysosome-specific mouse-monoclonal anti-Lamp1 primary antibody (BD Biosciences, San Jose, CA, USA), mouse monoclonal anti-p62 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), fluorescent secondary antibody Texas-Red-labeled anti-mouse IgG (Jackson Immunoresearch, West Grove, PA, USA) and Alexa-488-labeled anti-mouse IgG were used. Samples were analyzed with a TCS SP5II confocal laser scanning microscope equipped with PL APO 40×/1.25 NA and 63×/1.40 NA oil-

immersion objectives (Leica, Heidelberg, Germany). ImageJ software and related plugins (National Institutes of Health) were used for image processing, intracellular NP quantification, and NP co-localization.

(1) Ferraro D, Anselmi-Tamburini U, Tredici IG, Ricci V, Sommi P. Overestimation of nanoparticles-induced DNA damage determined by the comet assay. Nanotoxicology. 2016 Sep;10(7):861–70.

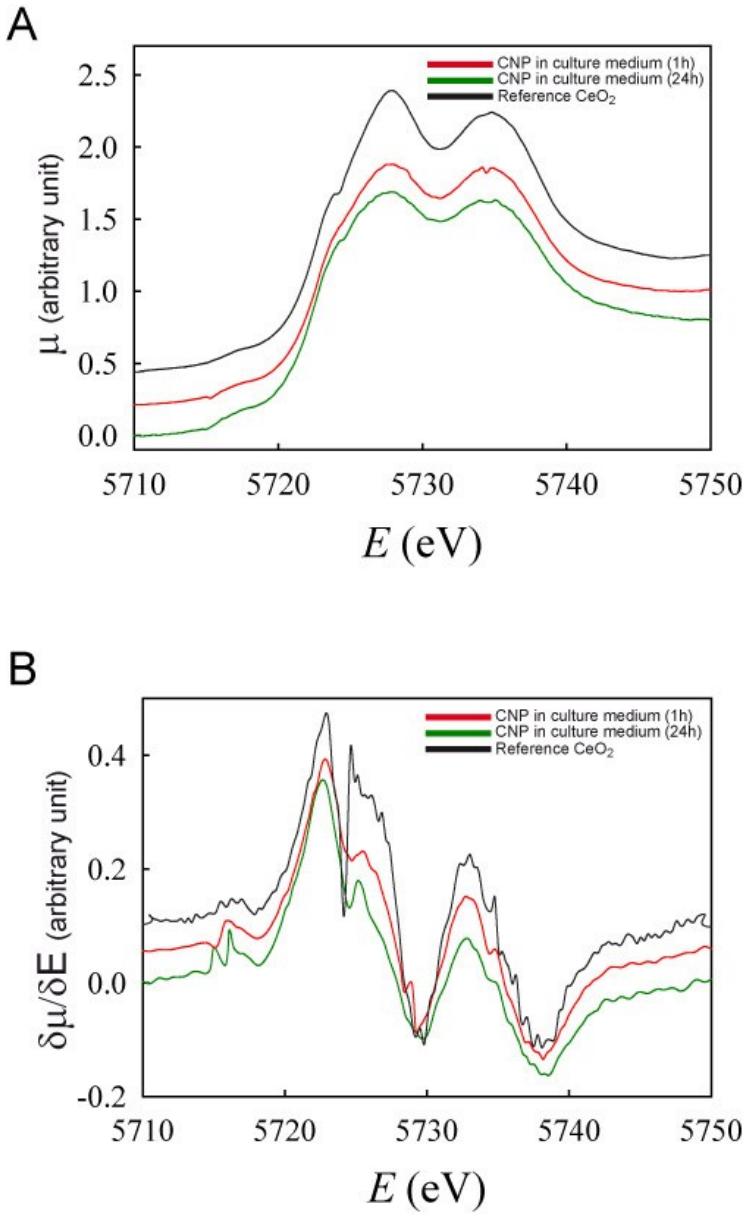

Figure S2. XANES spectra (A) and their respective derivatives (B) of CNPs incubated with culture medium for 1 and 24 h.

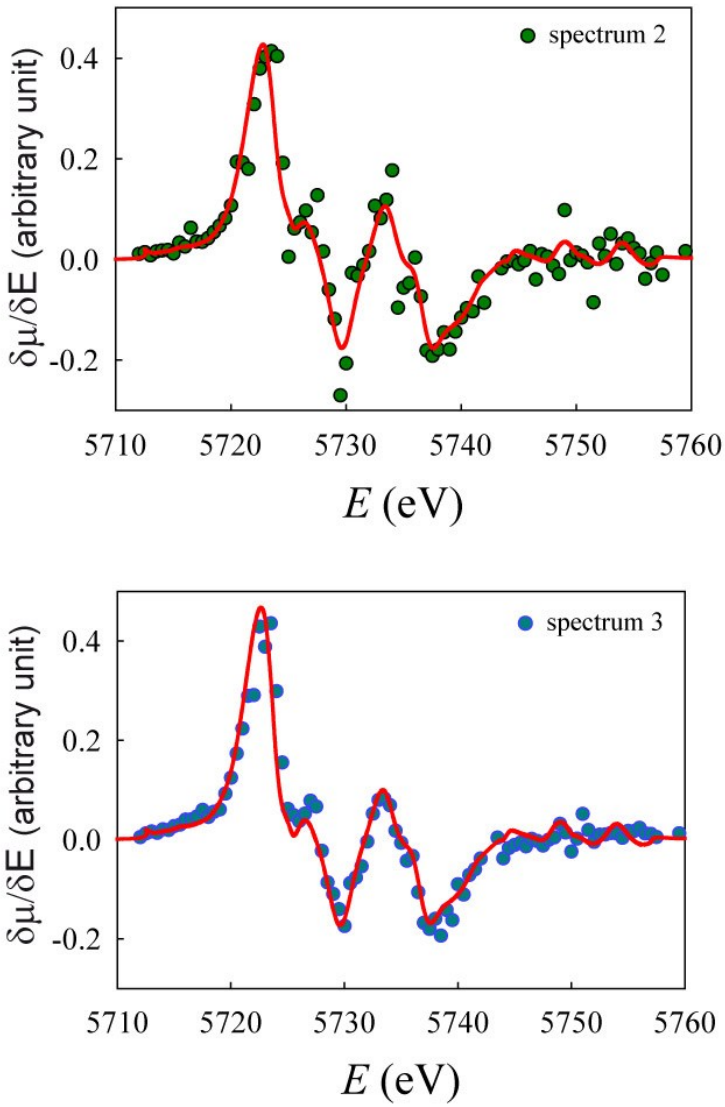

Figure S3. Fits of Spectra 2 and 3 of Figure 6 according to the linear combination. Circles: experimental data; red line: fit.