

Supplementary File

The effects of lanthanide-doped upconverting nanoparticles on cancer cell biomarkers

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Figure S1

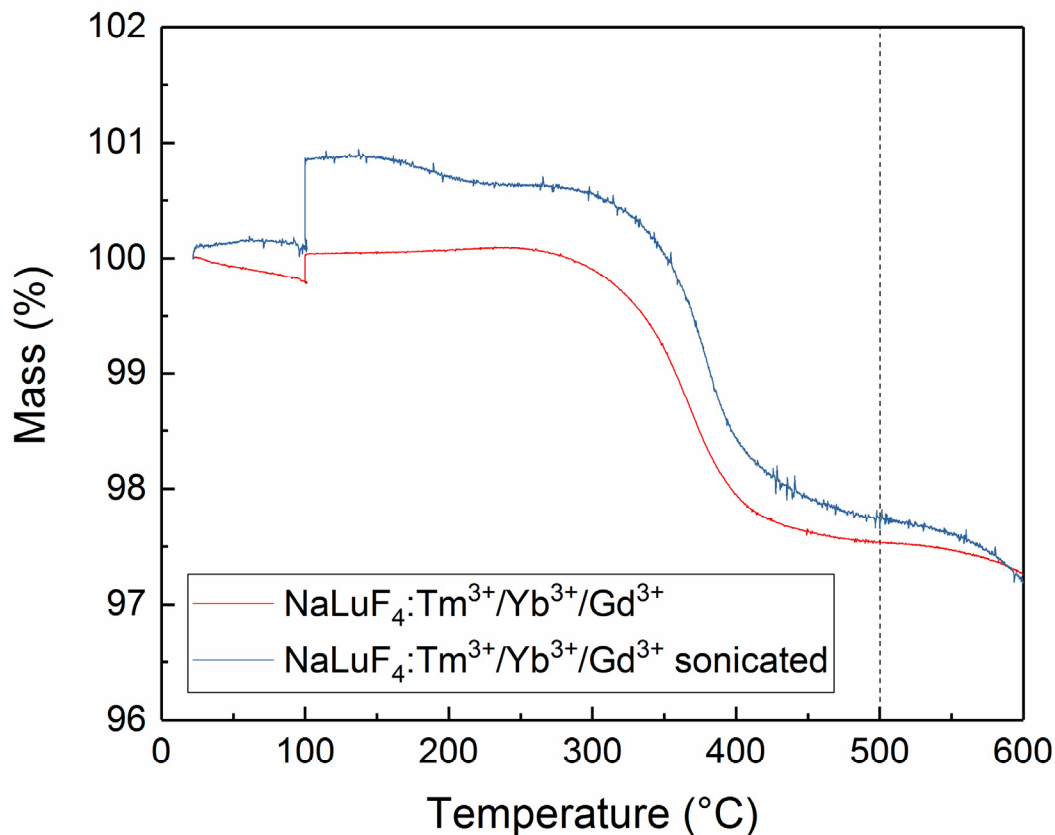


Figure S1. Thermogravimetric analysis indicates that sonication reduced the UCNP surface coating ~10% by mass. The surface coating comprises 2.2% of the total sample weight as compared to 2.5% for the unsonicated UCNPs. These values correspond to a surface coverage of 86.8% before sonication and 76.1% after sonication, assuming spherical UCNPs and an area of 0.4 nm^2 occupied by each oleate molecule. The reduction in organic content was assessed by measuring the mass loss when the sample reached 500 °C .

The apparent abrupt increase in mass at $\sim 100 \text{ °C}$ is due to an equilibration step during which the temperature is held constant for 30min and the mass increases gradually. The source of this increase is likely organic contaminants in the instrument that accumulate during this period, and thus do not represent a true contribution to the surface coating. Therefore, the initial mass prior to heating was defined as 100%. This effect was more pronounced in the sonicated sample due to a lower starting mass of the UCNP sample.

Figure S2

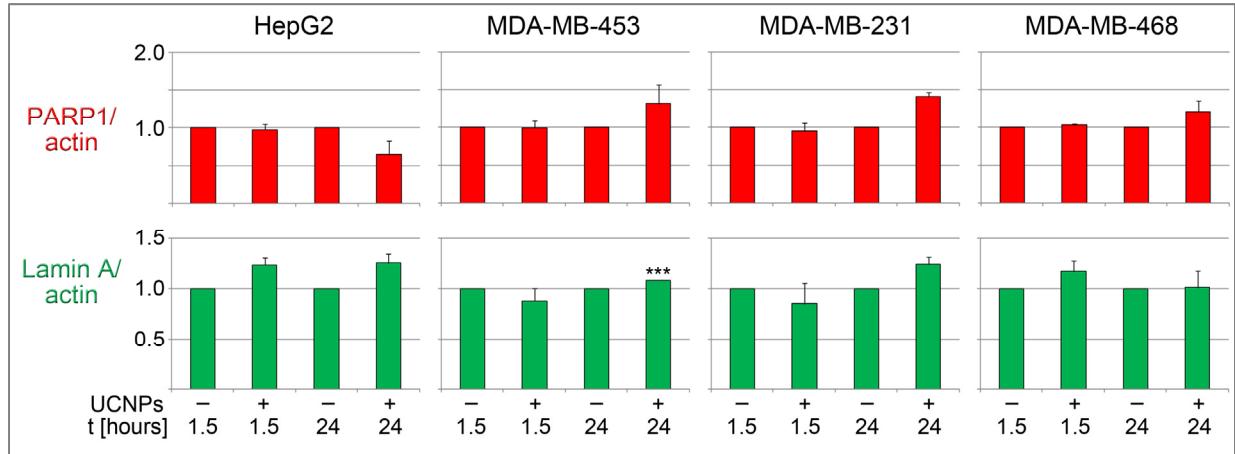


Figure S2. Evaluation of PARP1 and lamin A abundance in HepG2, MDA-MB-453, MDA-MB-231 and MDA-MB-468 cells. Samples were treated and processed for Western blotting as described for Fig. 3. Results were normalized to actin. Data are shown as average +SEM for at least two independent experiments. Student's t-test identified significant differences, ***, $p < 0.001$.

Figure S3

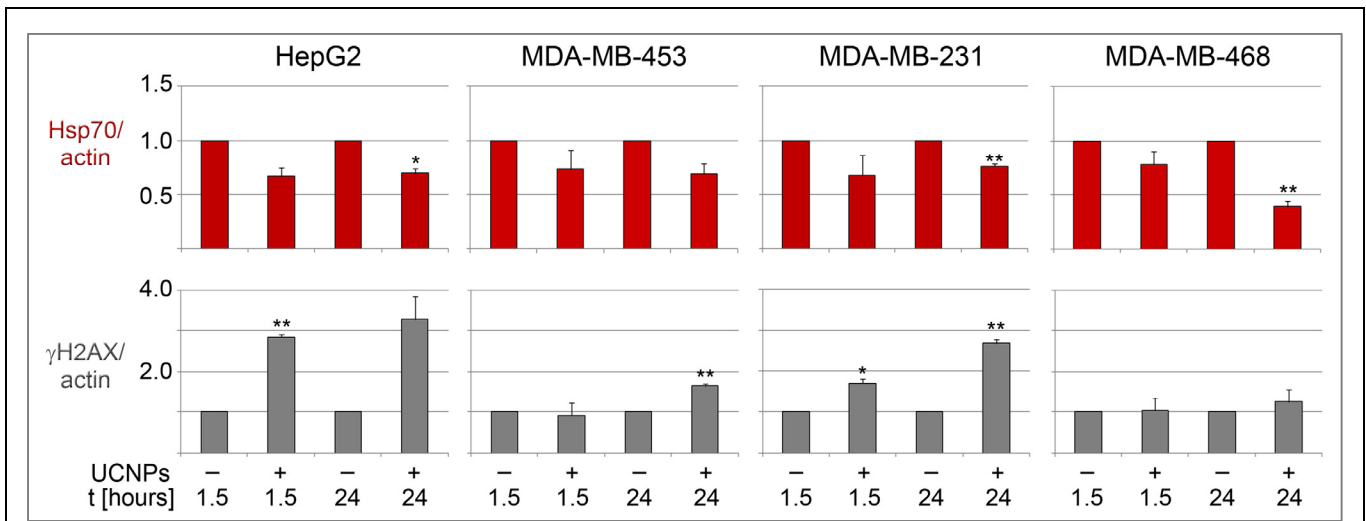


Figure S3. Evaluation of different cellular stress indicators in cancer cell lines. HepG2, MDA-MB453, MDA-MB-231 and MDA-MB-468 cells were treated with vehicle or Ln-UCNPs as in Fig. 4. The abundance of hsp70s and γ H2AX were quantified. Results are presented for two independent experiments as average +SEM. Significant statistical differences, determined by Student's t-test, are indicated by *, $p < 0.05$ or **, $p < 0.01$.

Figure S4

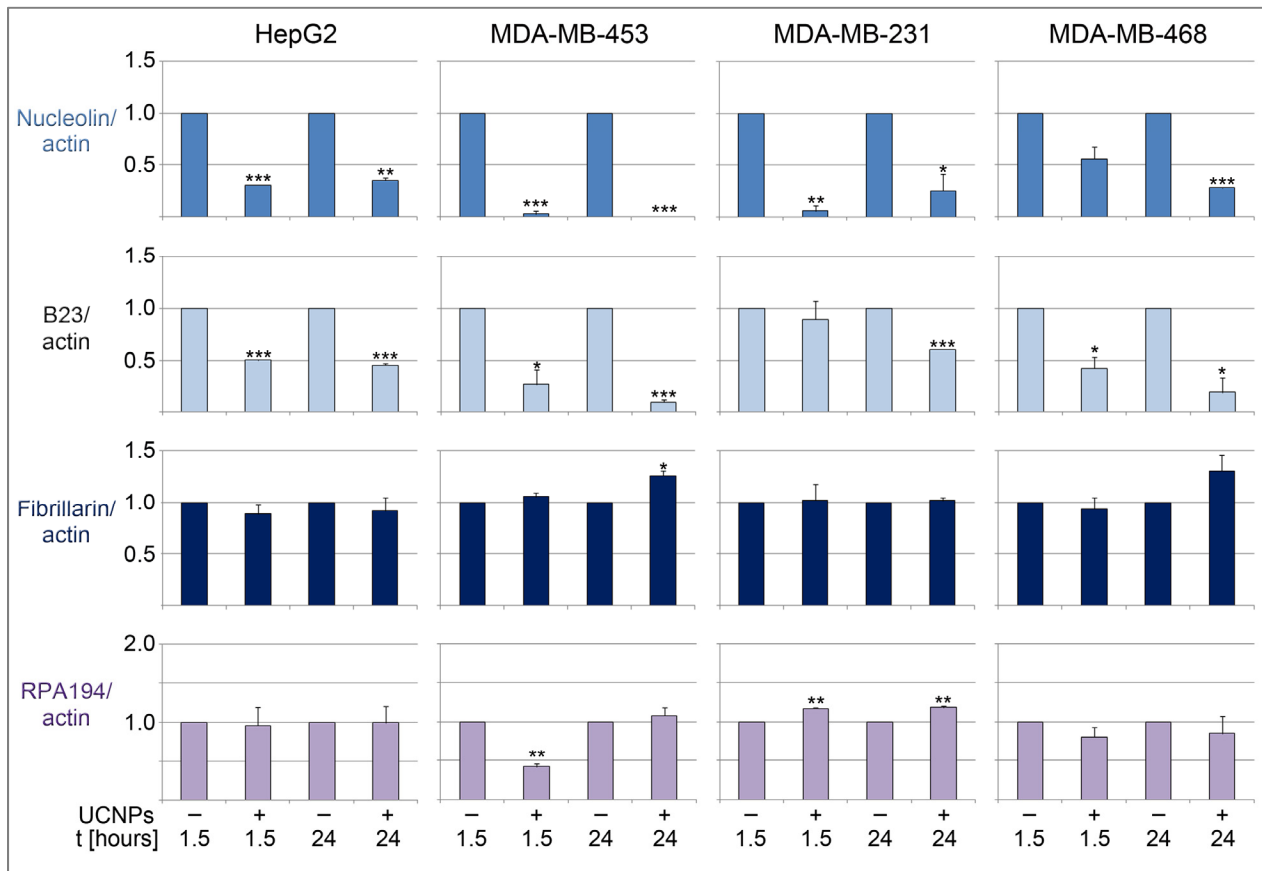


Figure S4. Effects of Ln-UCNPs on the abundance of nucleolar proteins in HepG2, MDA-MB-453, MDA-MB-231 and MDA-MB-468 cells. Treatment of culture cells, preparation of crude extracts and Western blot analyses are as described for Fig. 3. Two independent experiments were conducted for each data point. Results are presented as average +SEM. Student's t-test identified significant differences between vehicle controls and Ln-UCNP treated samples. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure S5

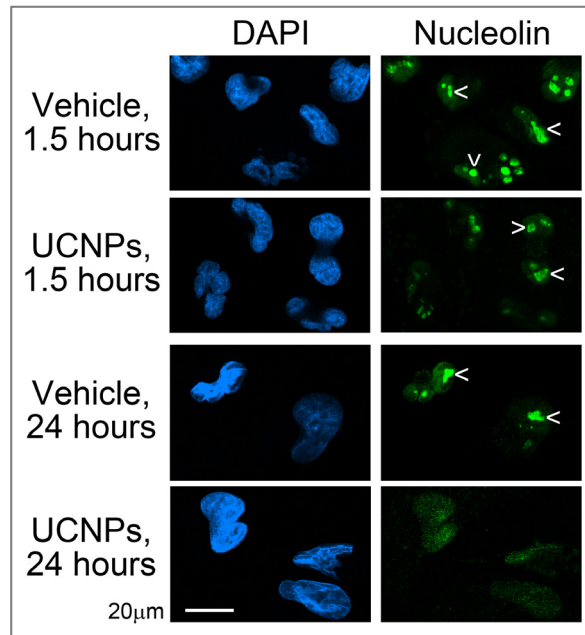


Figure S5. Ln-UCNPs alter the organization of nucleoli. U251N cells were incubated for 1.5 or 24 hours with vehicle or Ln-UCNPs. Cells were fixed and processed for immunofluorescence with antibodies against nucleolin. Images were acquired at identical settings of the laser. Several nucleoli are marked by arrowheads. DAPI demarcates the nucleus. Scale bar is 20µm. Note that after 24 hours nucleolin signals are low, and the protein no longer concentrates in nucleoli.

Figure S6

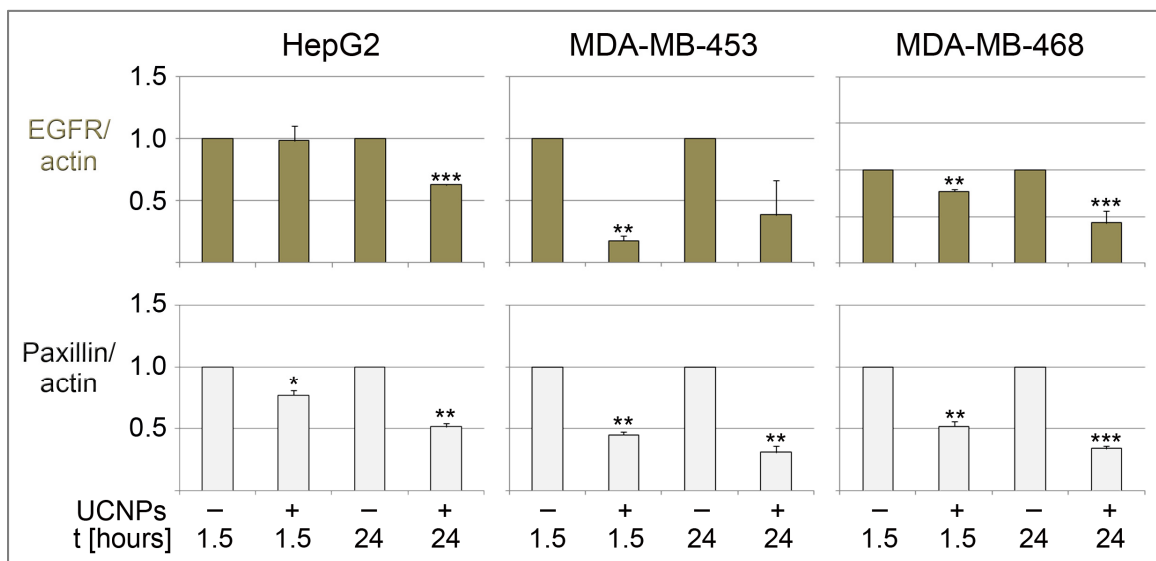


Figure S6. Effect of Ln-UCNPs on EGFR and paxillin abundance in different cancer cell lines. Cells were treated with Ln-UCNPs for 1.5 or 24 hours. Crude extracts were evaluated for EGFR and paxillin concentrations as described for Fig. 6. Results were normalized to vehicle controls. Data for two independent experiments depicted as average +SEM. Student's t-test identified significant differences between vehicle controls and treatment groups; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure S7

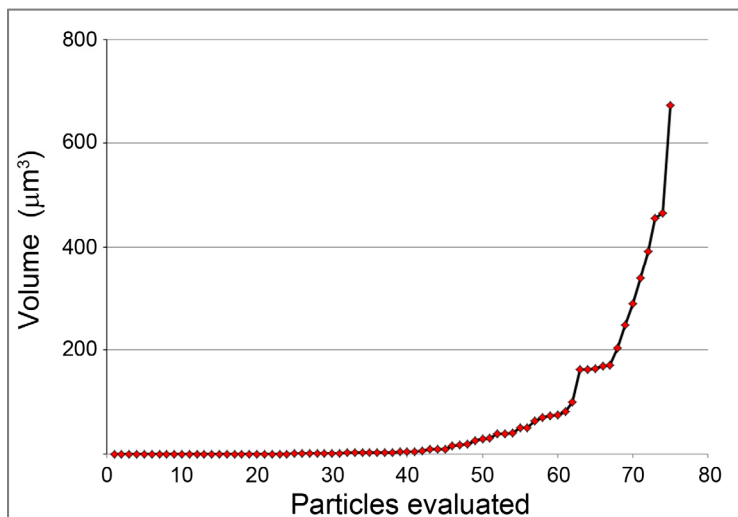


Figure S7. During incubation with growing cells, Ln-UCNPs form agglomerates that widely differ in volume. Culture cells were treated for 24 hours with Ln-UCNPs, as shown in Fig. 8. Volume measurements were performed with Imaris software for a randomly selected area of the cover slip. The graph depicts the volume distribution for Ln-UCNPs that were identified as spherical objects.