SUPPORTING INFORMATION

Albumin coating of magnetic nanoparticles for imaging, tracking and delivery through biological barriers

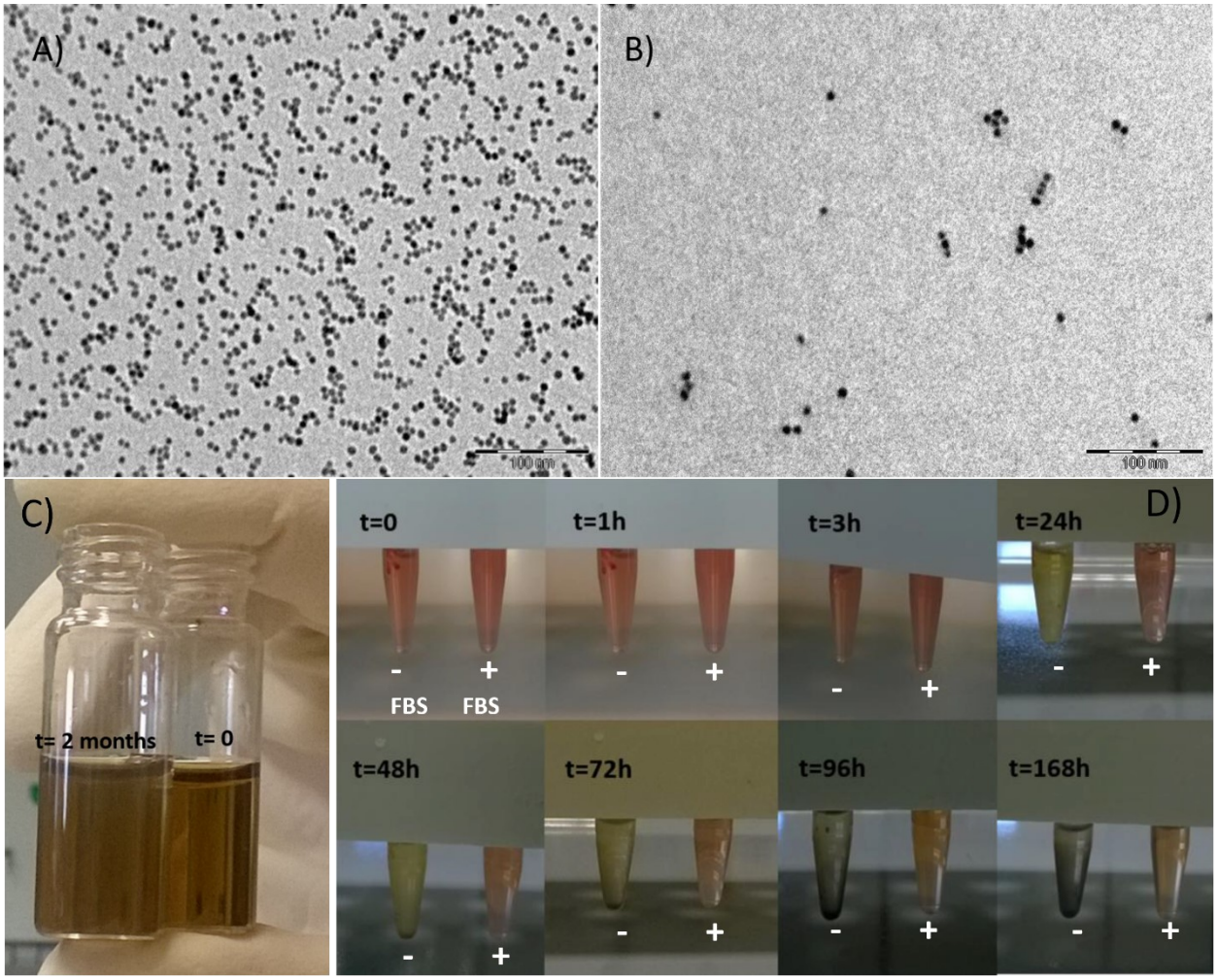

Figure S1. Low magnification TEM images of A) bare MNPs in organic solvent and B) in phosphate buffer after functionalization with BSA molecules. C) Picture of the colloidal solutions (in phosphate buffer) of the MNP@BSA at time 0 and after 2 months of storage at 4 °C. D) Pictures of the Eppendorf tubes containing the MNP@BSA dispersed in cell medium without and with 10% FBS acquired over time from t=0 up to one week of storage.

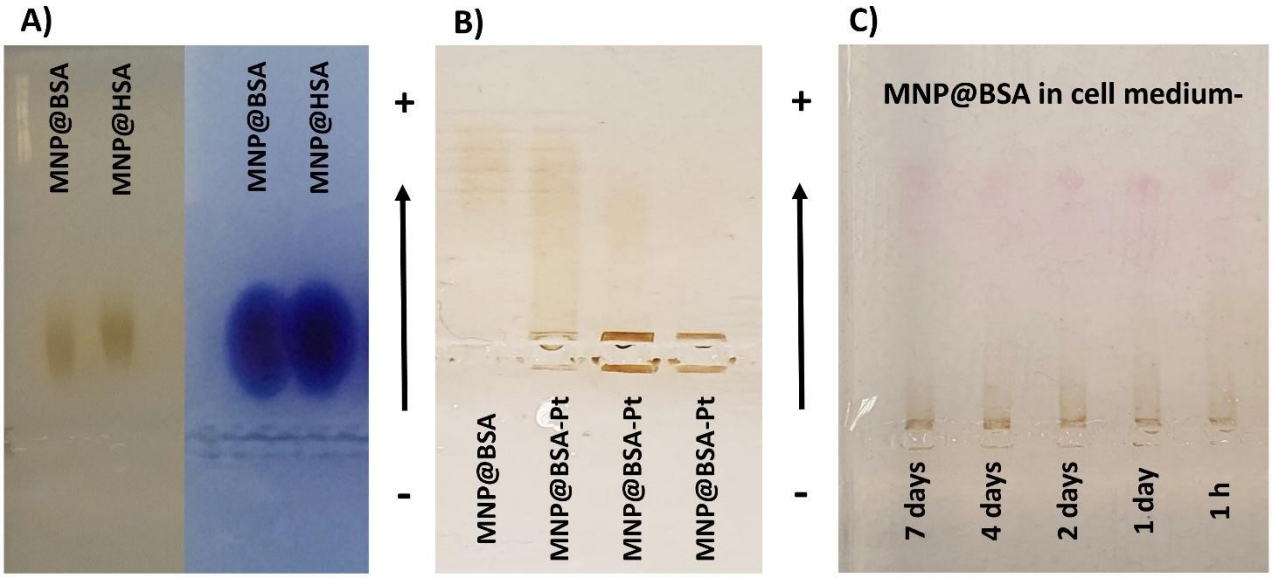

Figure S2. Gel electrophoresis images. A) Migration pattern of MNP@BSA and MNP@HSA before (left) and after (right) staining with Coomassie Blue. B) Migration pattern of MNP@BSA-Pt loaded with increasing amounts of cisPt (i.e., either 75, 150, or 300 µL of a 20 mM solution in DMSO). C) Migration pattern of MNP@BSA dispersed in 10% FBS cell culture medium.

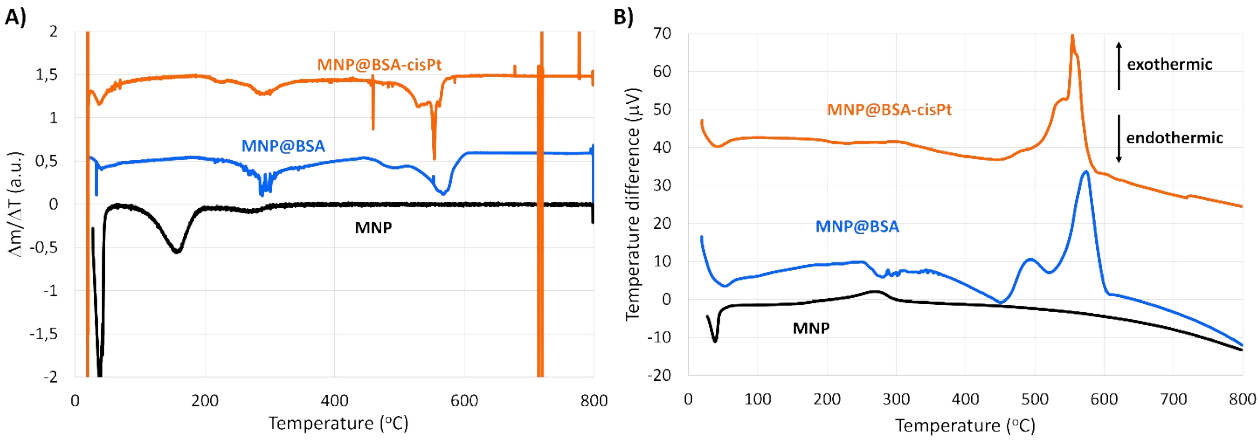

Figure S3. TGA measures of the nanoparticles, either MNP, MNP@BSA or MNP@BSA-cisPt: A) first derivative curves of the measures reported in the main manuscript; B) Differential Thermal Analysis (DTA) of the three types of nanoparticles.

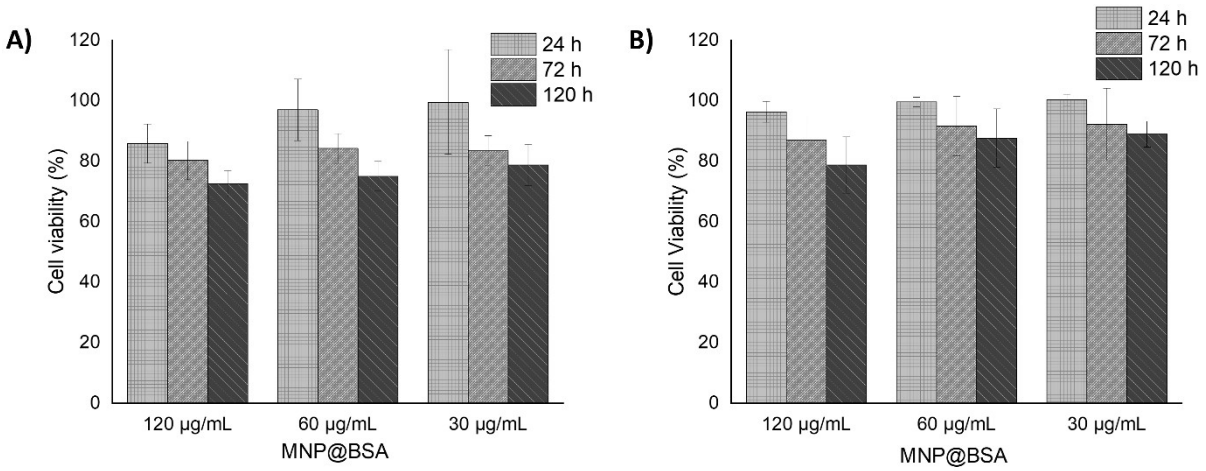

Figure S4. MTT viability assay performed upon incubation of either A) MCF-7 or B) MDA-MB-231 cells with the MNP@BSA for 24, 72, and 120 h at three different concentrations (30, 60, and 120 µg/mL, referring to the inorganic domain, corresponding to about 290, 580, and 1160 µg/mL of BSA).

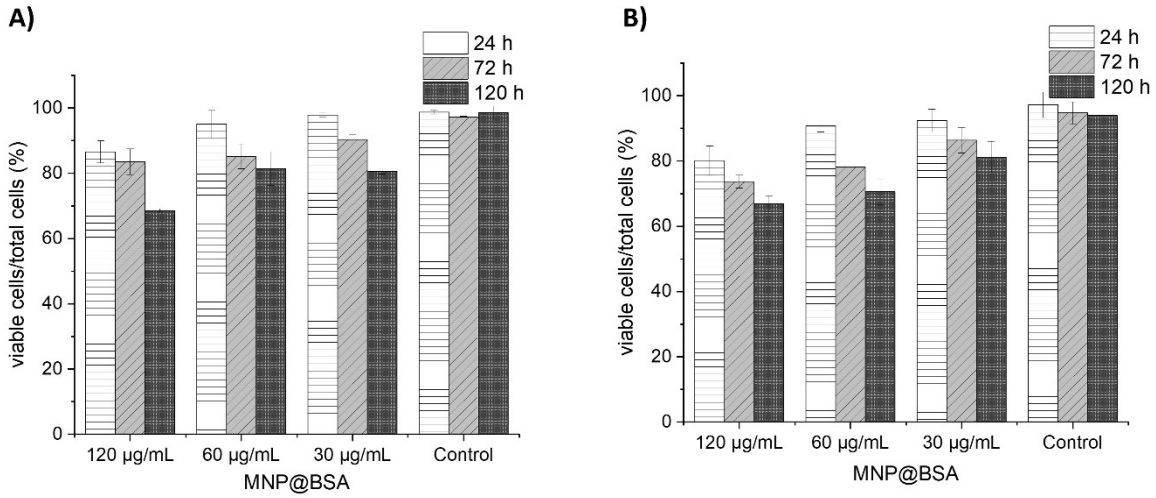

Figure S5. Trypan Blue viability assay performed upon incubation of either A) MCF-7 or B) MDA-MB-231 cells with the MNP@BSA for 24, 72, and 120 h at three different concentrations (30, 60, and 120 µg/mL, referring to the inorganic domain, corresponding to about 290, 580, and 1160 µg/mL of BSA).

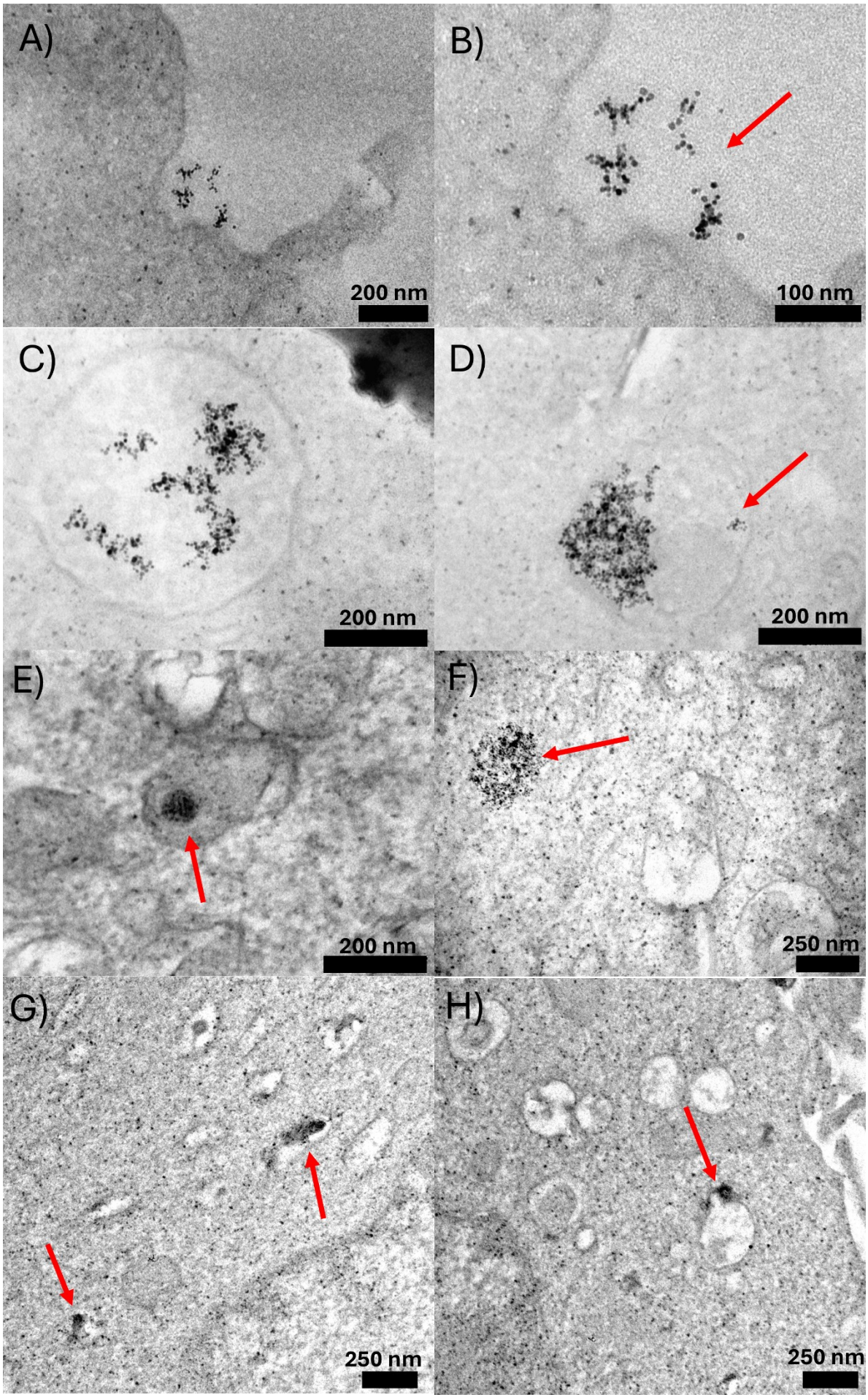
A)
200 nm
B)
100 nm
C)
200 nm
D)
200 nm
E)
200 nm
F)
250 nm
G)
250 nm
H)
250 nm

Figure S6. TEM images of the MCF-7 cells incubated with the MNP@BSA. Panels A-B) correspond to the cells incubated with the nanoparticles for 1 h. Panels C-D) correspond to the cells incubated with the MNP@BSA for 24 h and processed for ultrastructural analysis. Panels E-F) and G-H) correspond to the cells treated with the MNP@BSA for 24 h and then kept under incubation in fresh medium for additional 6 and 14 days, respectively. The red arrows indicate either the nanoparticles close to the cellular membrane in B), the endosomes-localized nanoparticles in C-F) and the degraded nanoparticles in G-H).

Functionalization with Rhodamine 101

To study the internalization of the nanoparticles into the cells, the MNP@BSA were labeled with the organic fluorophore Rhodamine 101 (Rhod101) using EDC-SulfoNHS chemistry. In detail, 1 mL of each of the following solutions was mixed: 10 μM MNP@BSA, 200 μM Rhod101, 50 mM EDC, and 10 mM SulfoNHS. The volume of the mixture was brought to 10 mL and left stirring overnight at 4 °C. To remove the excess of unbound rhodamine, centrifugal filters and size exclusion chromatography were used. Then, the samples were loaded onto an agarose gel for characterization, and the fluorescence spectra were analyzed.

The migration of the nanoparticles is delayed compared to that of the starting sample and a weak fluorescent signal is visible under UV light. It is worth mentioning that the UV lamp excitation at 365 nm excites the fluorophore at the left tail of the excitation curve, not at the peak. Indeed, under proper excitation at 555 nm, the fluorescence curve of the nanoparticles is clearly detectable (Figure S6B) and slightly red-shifted compared to that of free Rhodamine (Figure S6B).

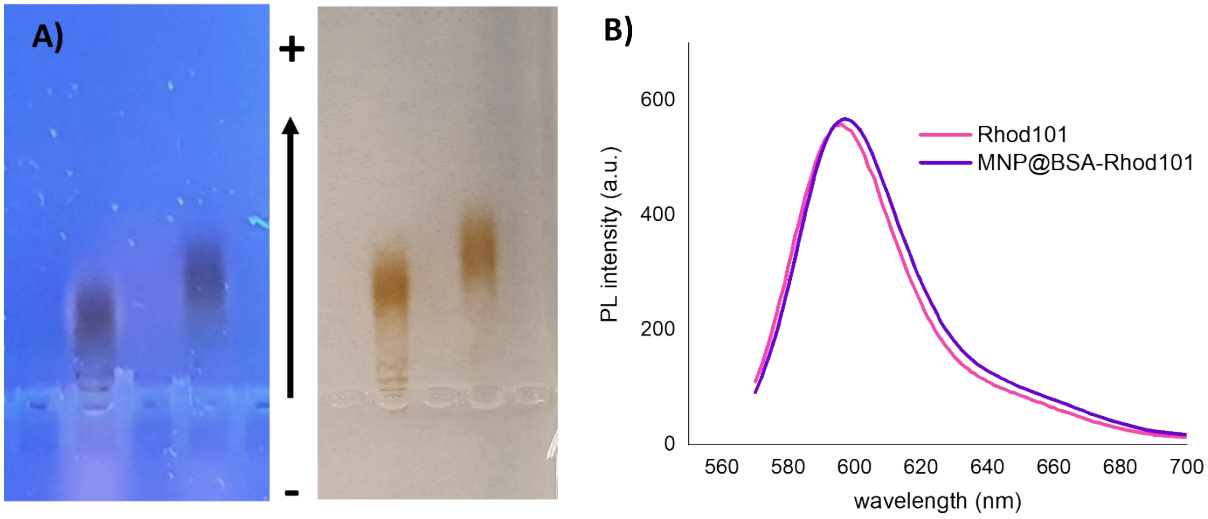

Figure S7. A) Agarose gel electrophoresis of MNP@BSA-Rhod101 and MNP@BSA: the left panel shows the fluorescence emission of the nanoparticles conjugated to the fluorophore under UV light irradiation (at 365 nm), while the right panel shows the same gel under ambient light. B) Photoluminescence spectra of the MNP@BSA-Rhod101 and of the free fluorophore (excitation at 555 nm).

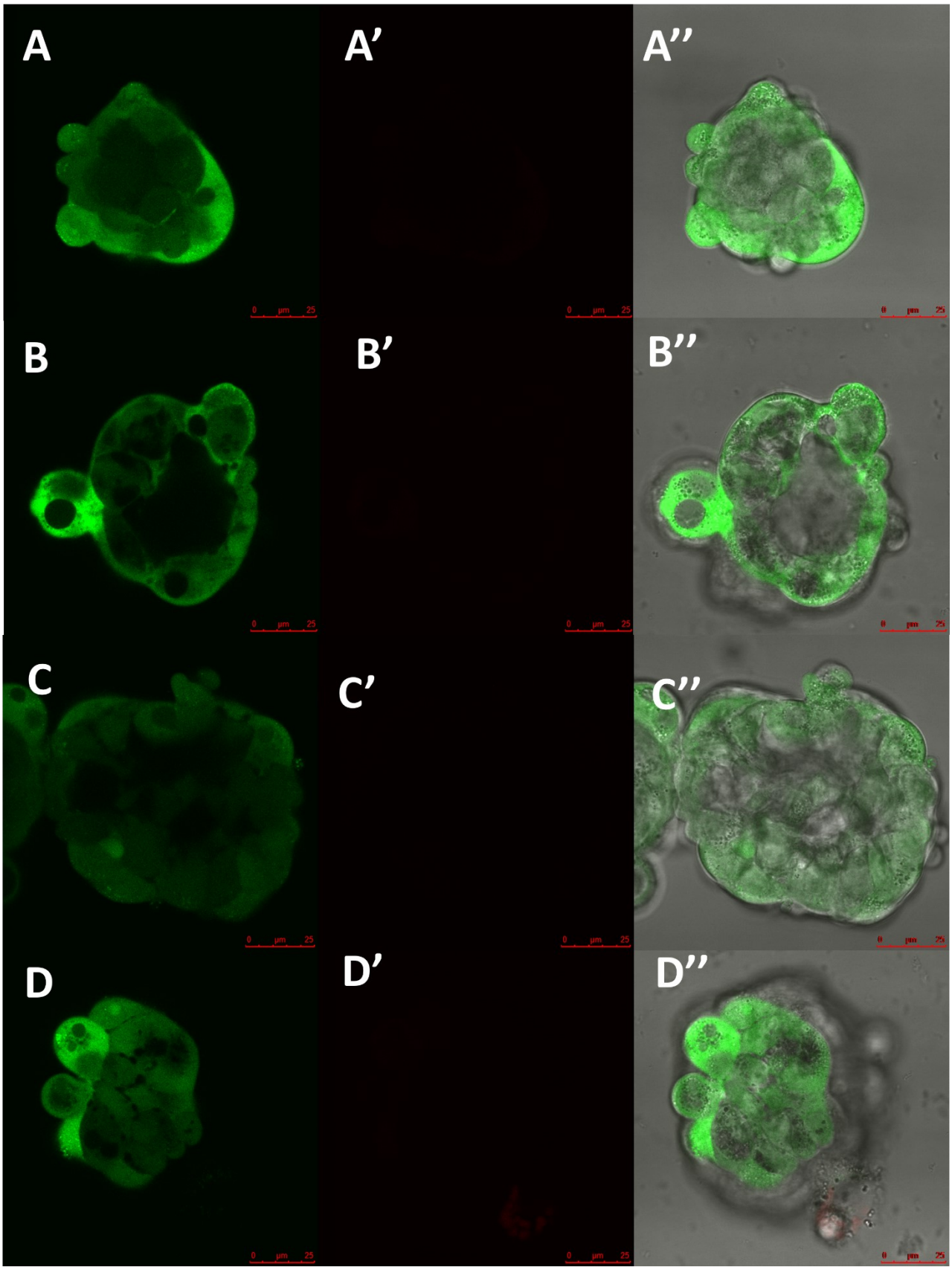

Figure S8. Images of the mammospheres upon L&D assay performed after 48 and 72 h incubation with MNP@BSA.

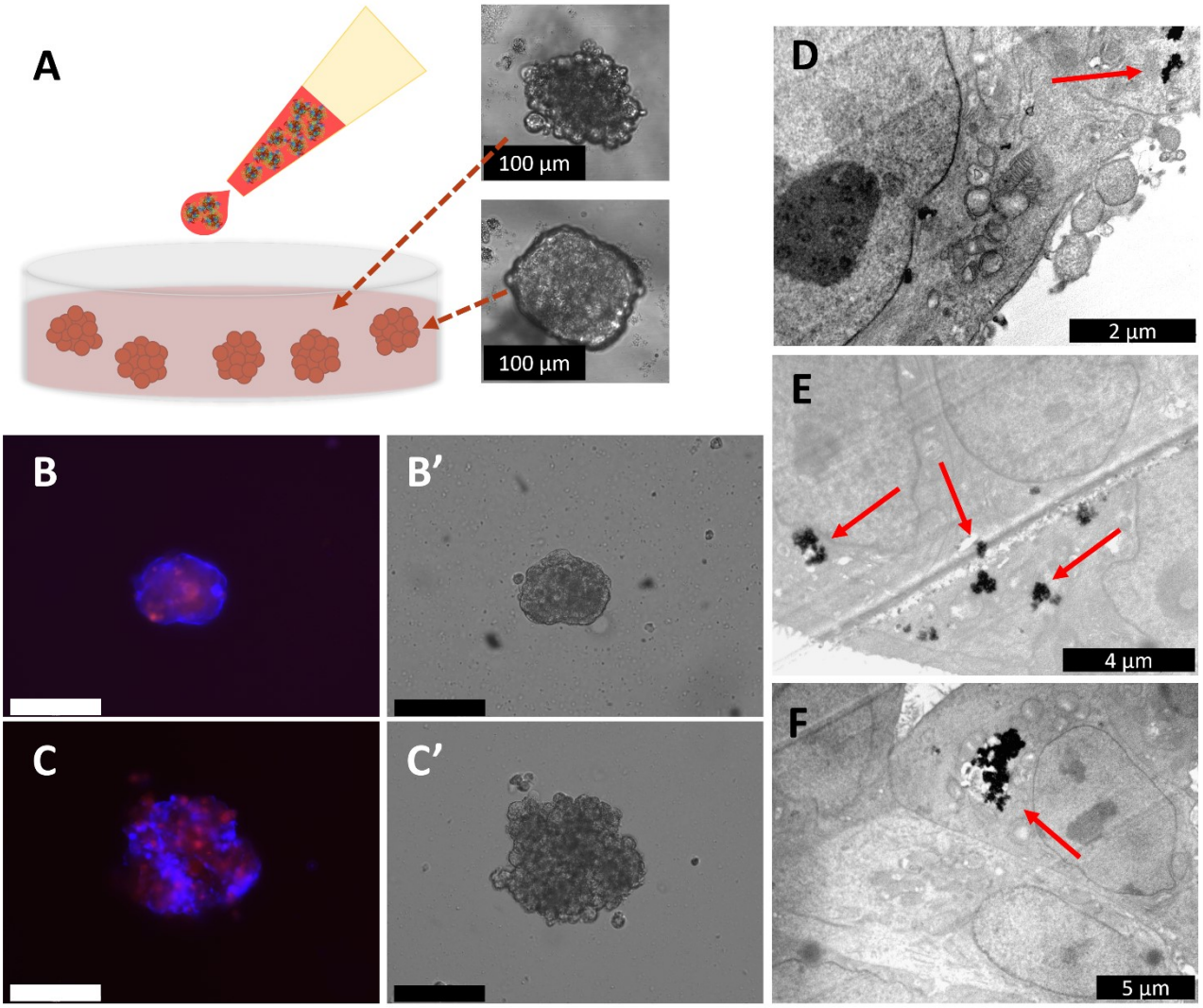

Figure S9. A) The sketch depicts the agarose-embedded 3D tumor spheroids and the two optical images of the side inset show the typical structural of the spheroids upon 12 days growth. B-B') and C-C') are the fluorescent and the bright field images of the spheroids incubated with the Rhodamine labelled MNP@BSA for 6 and 24 h, respectively. The cell nuclei were stained with DAPI. Scale bar corresponds to 150 µm. D-E-F) are the TEM images of the spheroids incubated with MNP@BSA for 24 h, recovered from the hydrogel and processed for ultrastructural analysis. The red arrows indicate the endosomes-localized nanoparticles.

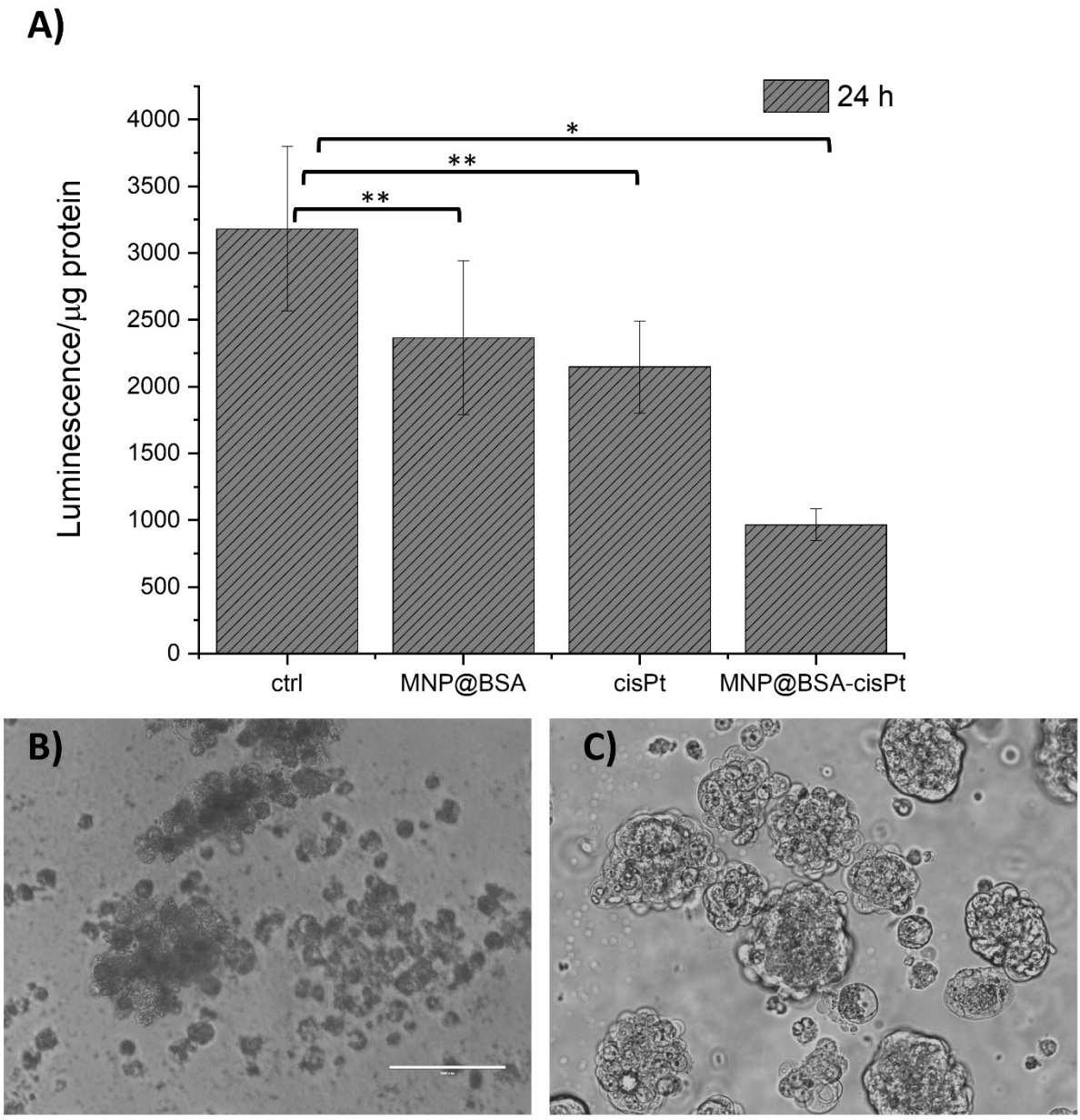

Figure S10. A) Cell Titer Glo assay performed with mammospheres incubated with MNP@BSA-cisPt, MNP@BSA and free cisPt for 24 h (statistical analysis was performed by t-test:* is $p<0.01$; *is $p<0.05$). B-C) Optical images of the mammospheres upon incubation with MNP@BSA-cisPt and MNP@BSA, respectively.

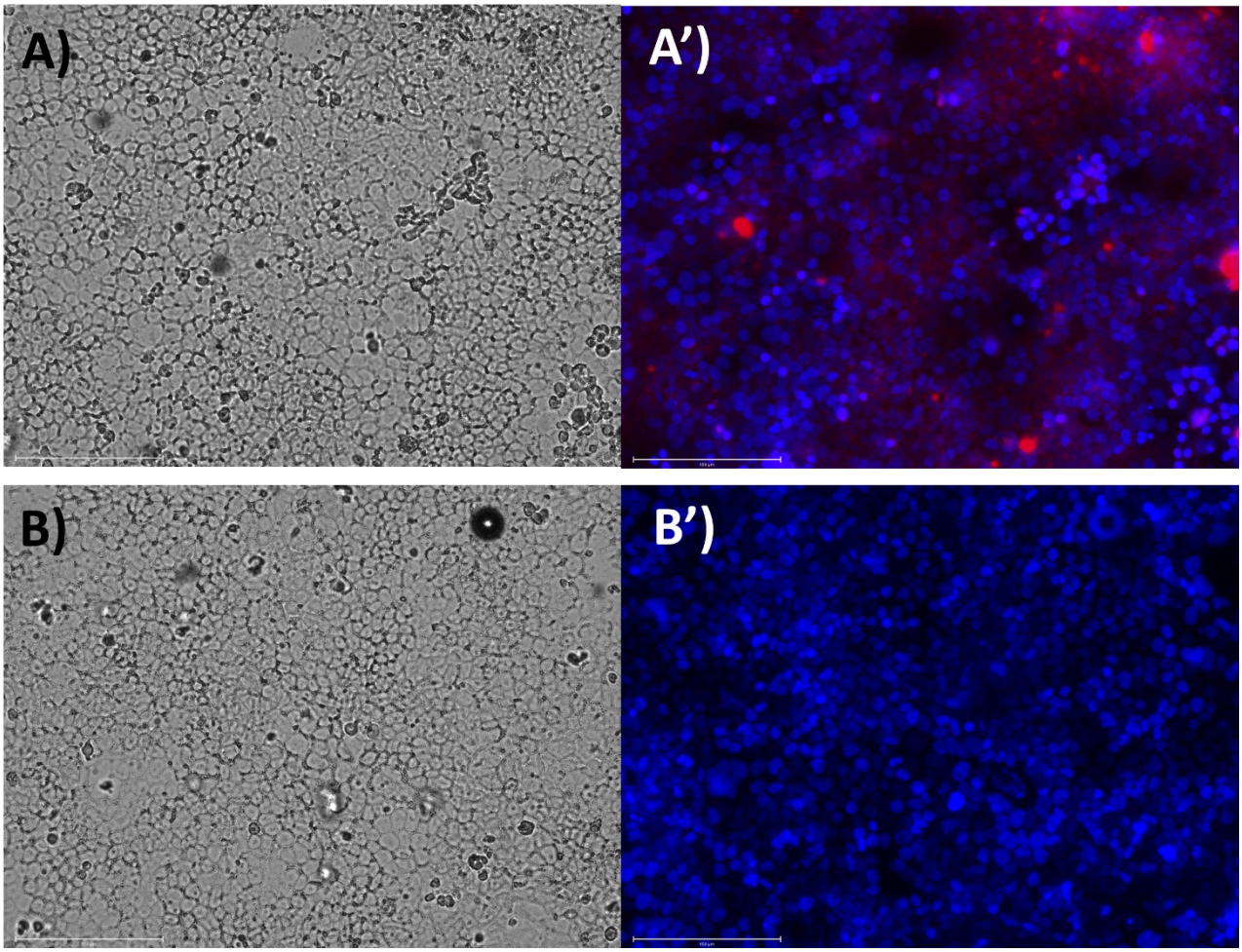

Figure S11. Images of the Caco2 cell monolayer grown on the transwell membrane. A-A') correspond to the cells incubated with Rhodamine-labelled MNP@BSA. B-B') correspond to the control cell sample. The cell nuclei were stained with DAPI.