

Electronic Supplementary Information

Transport across cell-membrane dictates nanoparticle fate and toxicity: a new paradigm in nanotoxicology

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Figure S1. Quantification of internalized endo and shot NPs through ICP elemental analysis after 24 h incubation in bEnd.3 cells. Au NPs (a), Ag NPs (b) and Fe₃O₄ NPs (c). Data were expressed as NP number/cell. Error bars indicate the standard deviation.

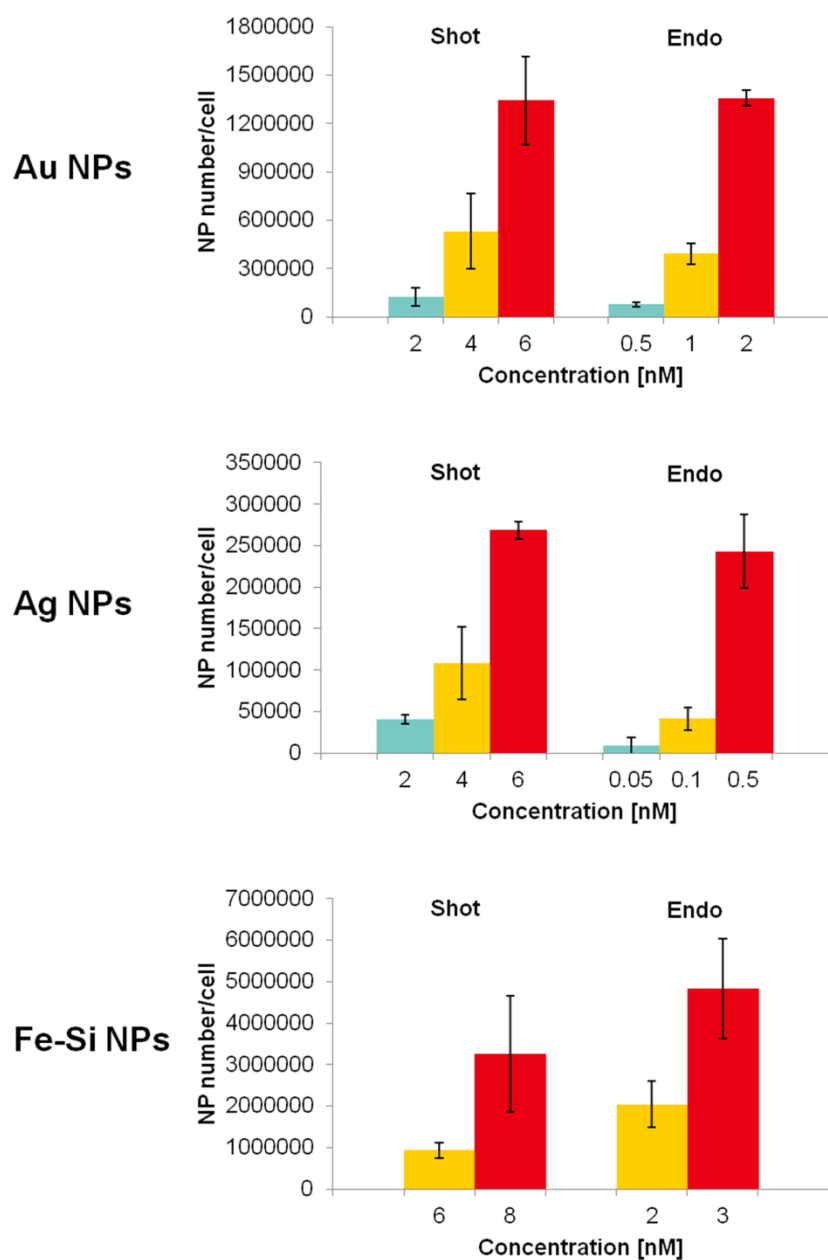


Figure S2. Quantification of internalized shot and endo 44 PS NPs, at the concentrations of 4.2×10^{13} and 2.1×10^{11} NPs/ml, respectively, in bEnd.3 cells through spectrofluorometer analysis. Data were expressed as NP number per cell. Error bars indicate the standard deviation.

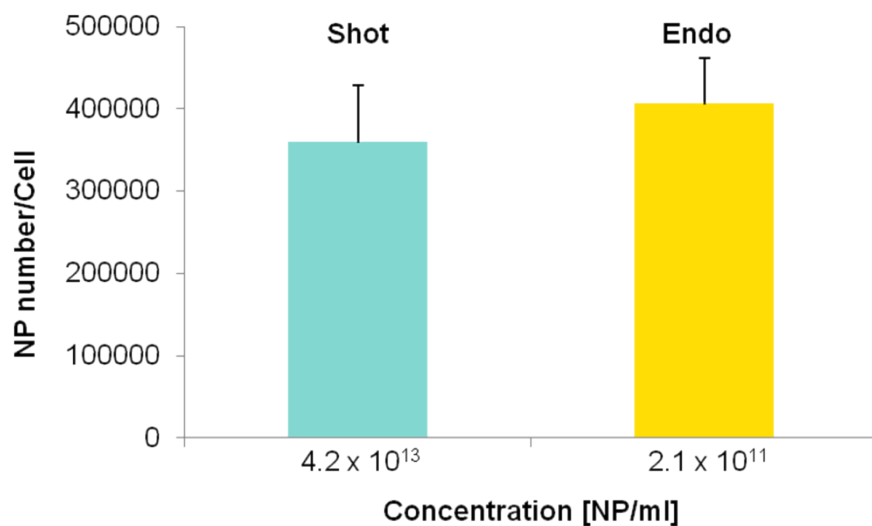


Figure S3. *In vitro* cytotoxicity by Alamar blue assay of shot and endo 44 PS NPs incubated 24 h with bEnd.3 cells at the concentration of 4×10^5 NPs/cell. Data were reported as percentage of cell viability normalized to control cells. Error bars indicate the standard deviation. No statistically significant differences were found between NPs' treatments and controls.

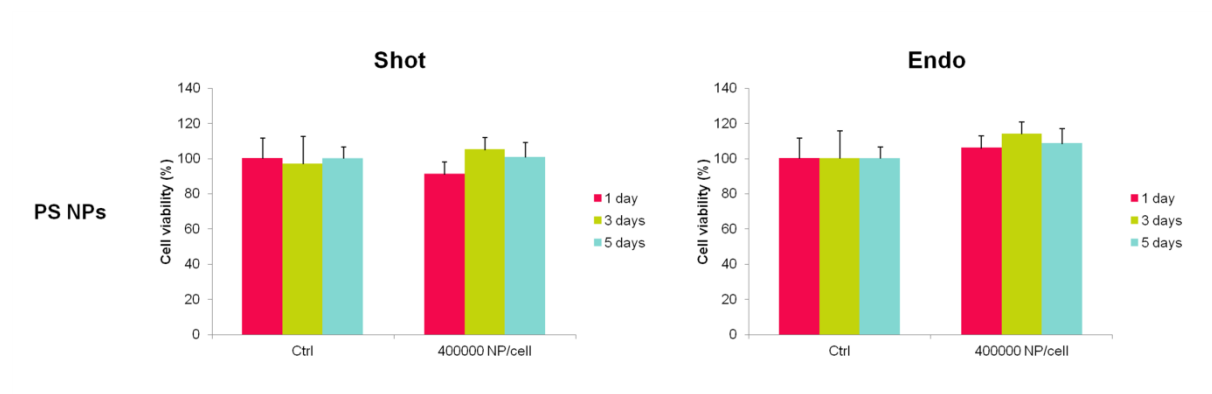


Figure S4. Quantification of internalized shot and endo 44 PS NPs in HeLa cells through spectrofluorometer. HeLa cells were exposed to 1.5×10^{13} PS NPs/ml for shooting and 3.0×10^{11} PS NPs/ml for endocytosis. Data were expressed as NP number per cell. Error bars indicate the standard deviation.

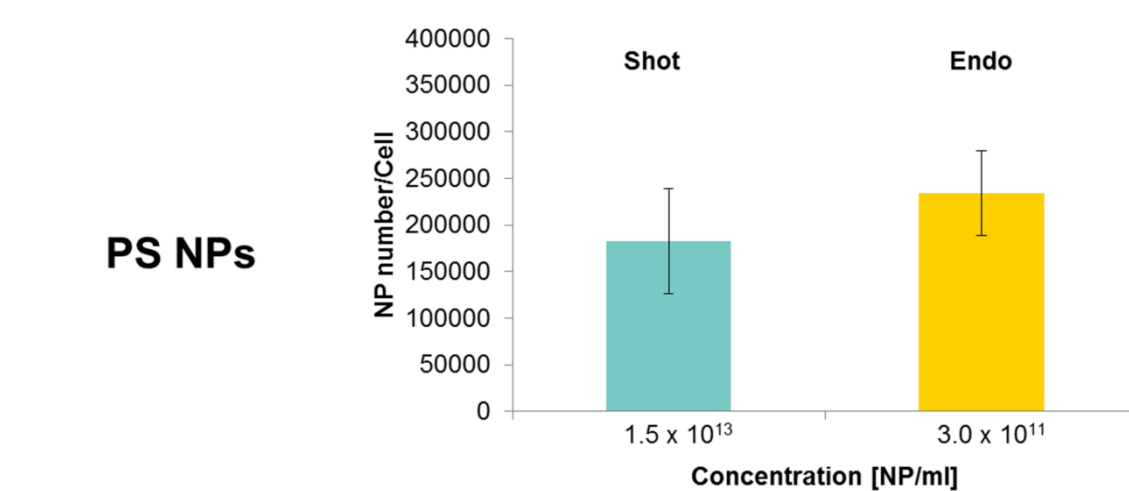


Figure S5. Quantification of internalized shot and endo Au, Ag and Fe₃O₄ NPs in HeLa cells through ICP analysis. For Au NPs, HeLa cells were exposed to 6 nM for shooting and 0.5 nM for endocytosis. For Ag NPs, HeLa cells were exposed to 4 nM for shooting and 0.6 nM for endocytosis. For Fe₃O₄ NPs, HeLa cells were exposed to 6 nM for shooting and 0.1 nM for endocytosis. Data were expressed as NP number per cell. Error bars indicate the standard deviation.

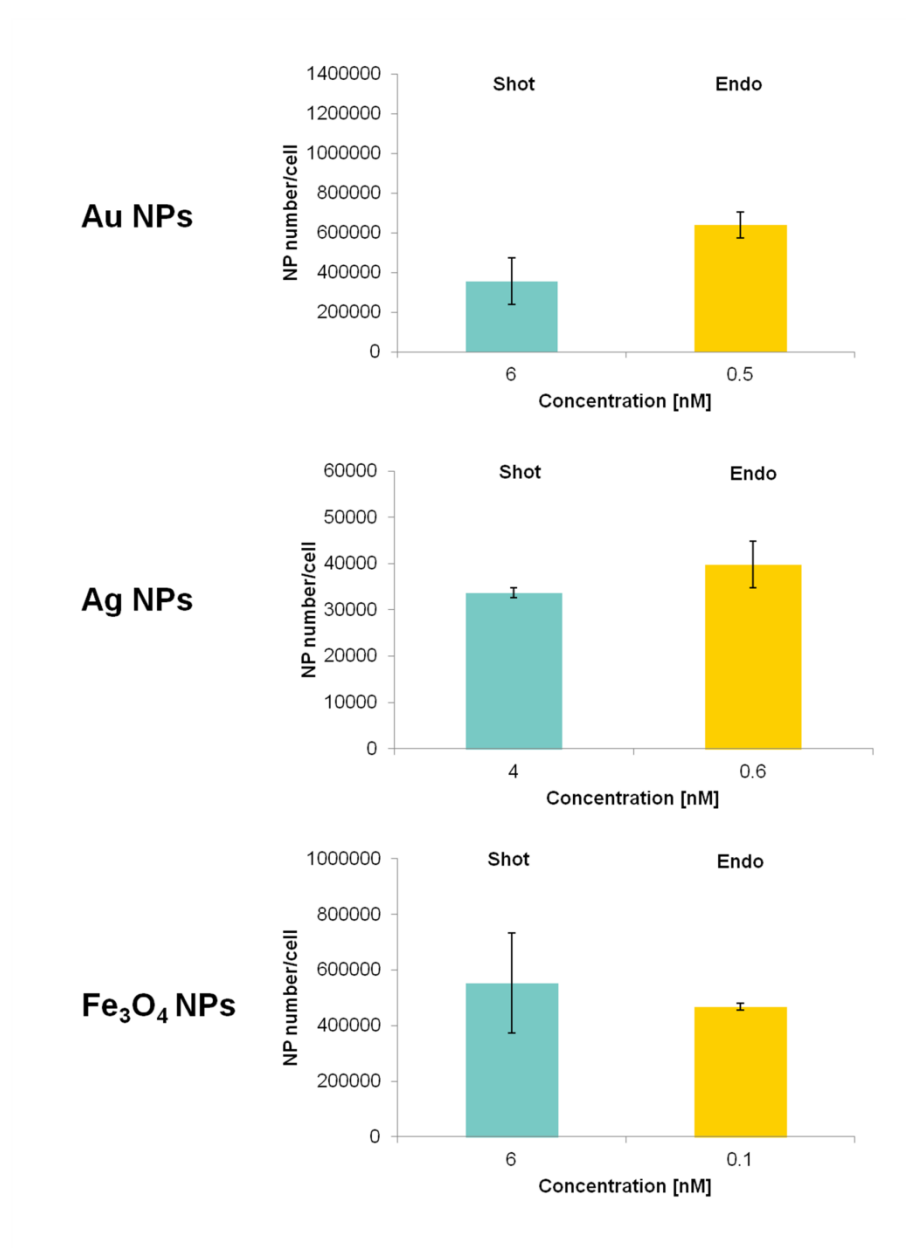


Figure S6. *In vitro* cytotoxicity by Alamar blue assay of shot and endo 44 PS NPs incubated 24 h with HeLa cells at the concentration of 4×10^5 NPs/cell. Data were reported as percentage of cell viability normalized to control cells. Error bars indicate the standard deviation. * $p < 0.05$ compared to control (n= 9).

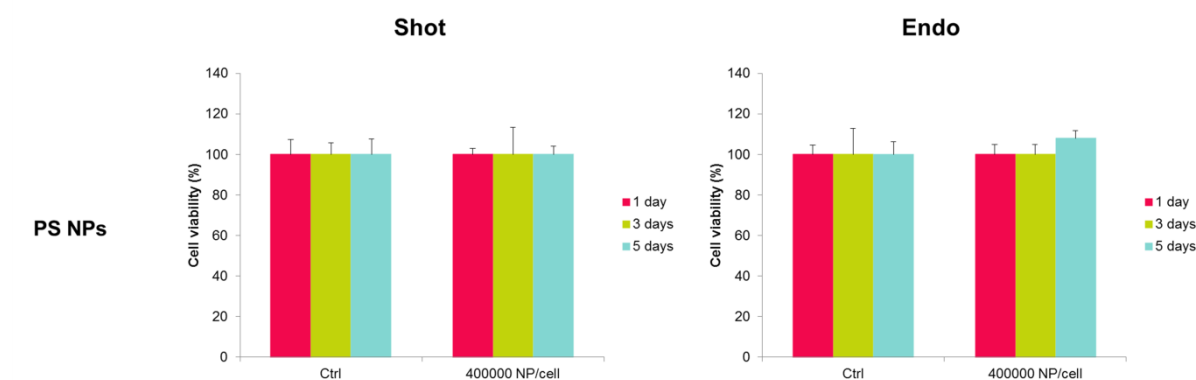


Figure S7. Effects of NP shooting (a) and endocytosis (b) on the level of ROS in bEnd.3 cell line, probed by the DCFH-DA assay. Cells were treated with 4×10^5 44 PS NPs/cell. For the experiment, red fluorescent 44 PS NPs were used in order to avoid interferences with green fluorescent DCF. The ROS level of nanoparticle-treated cells was expressed relative to non-treated control cells (Ctrl -). As a positive control (Ctrl +), cells were incubated with 100 μ M H₂O₂. Error bars indicate the standard deviation. No statistically significant differences were found between NPs' treatments and negative control.

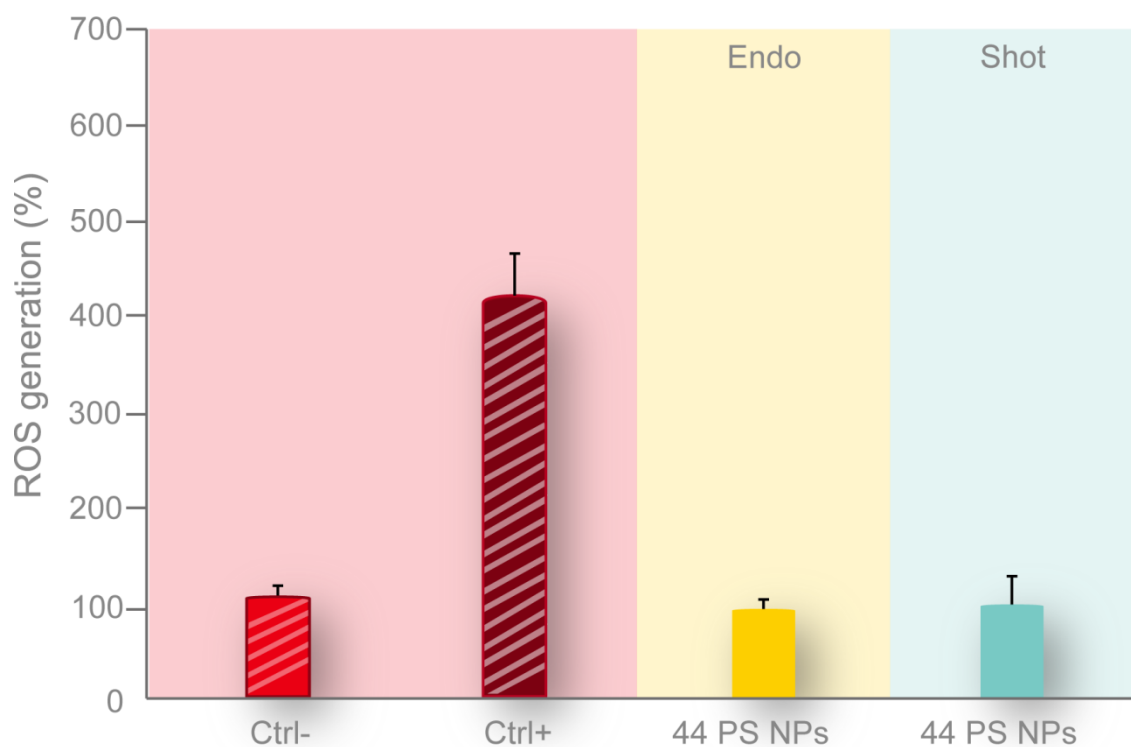


Figure S8. Metallothioneins' expression in HeLa cells. Images represent cells non treated with Ag NPs (control endo).

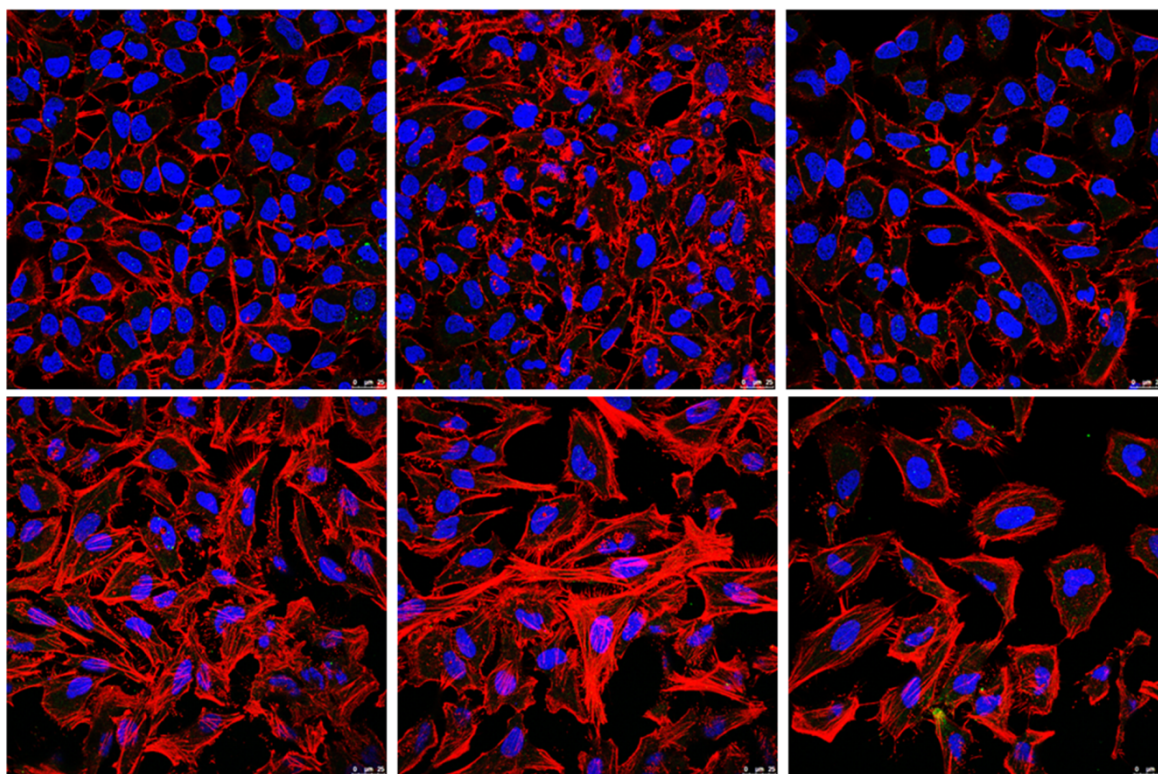


Figure S9. Metallothioneins' expression in HeLa cells. Images represent cells treated with endo Ag NPs (4×10^4 NPs/cell) upon 3h incubation.

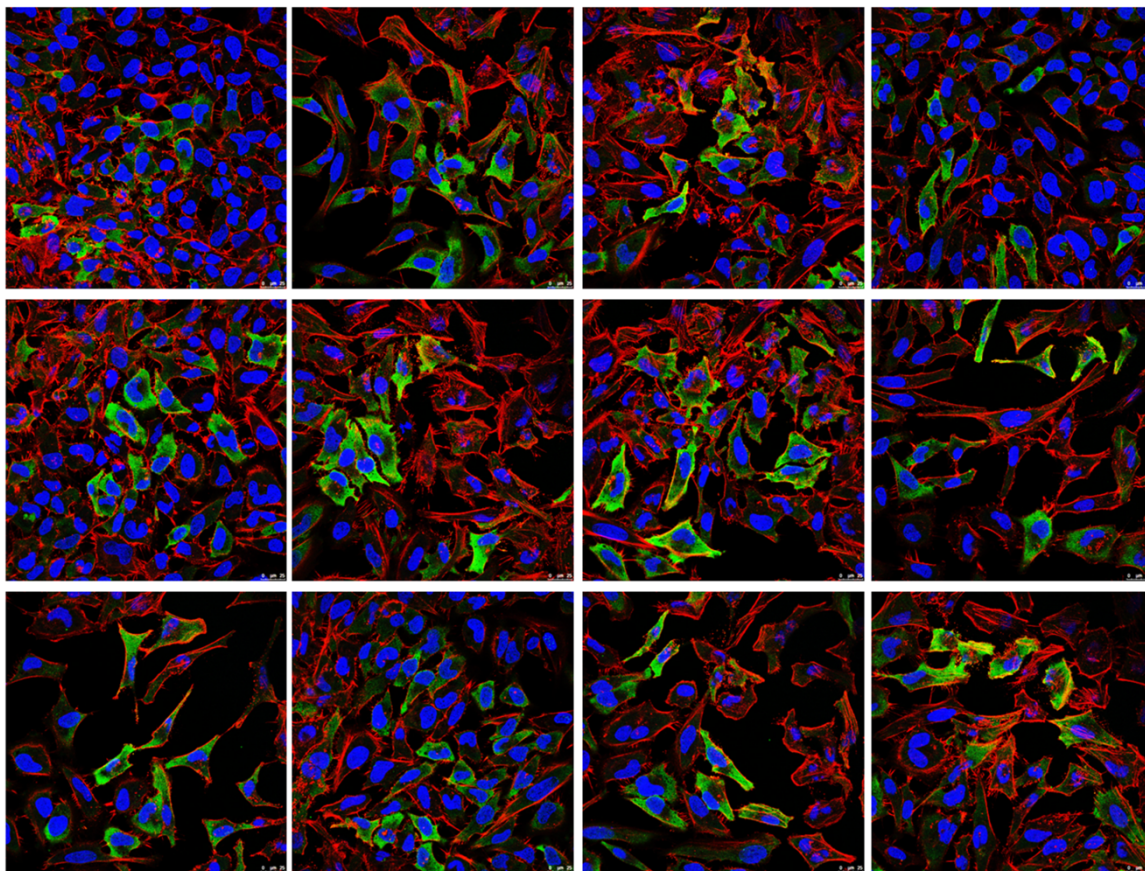


Figure S10. Metallothioneins' expression in HeLa cells. Images represent cells treated with endo Ag NPs (4×10^4 NPs/cell) upon 24h incubation.

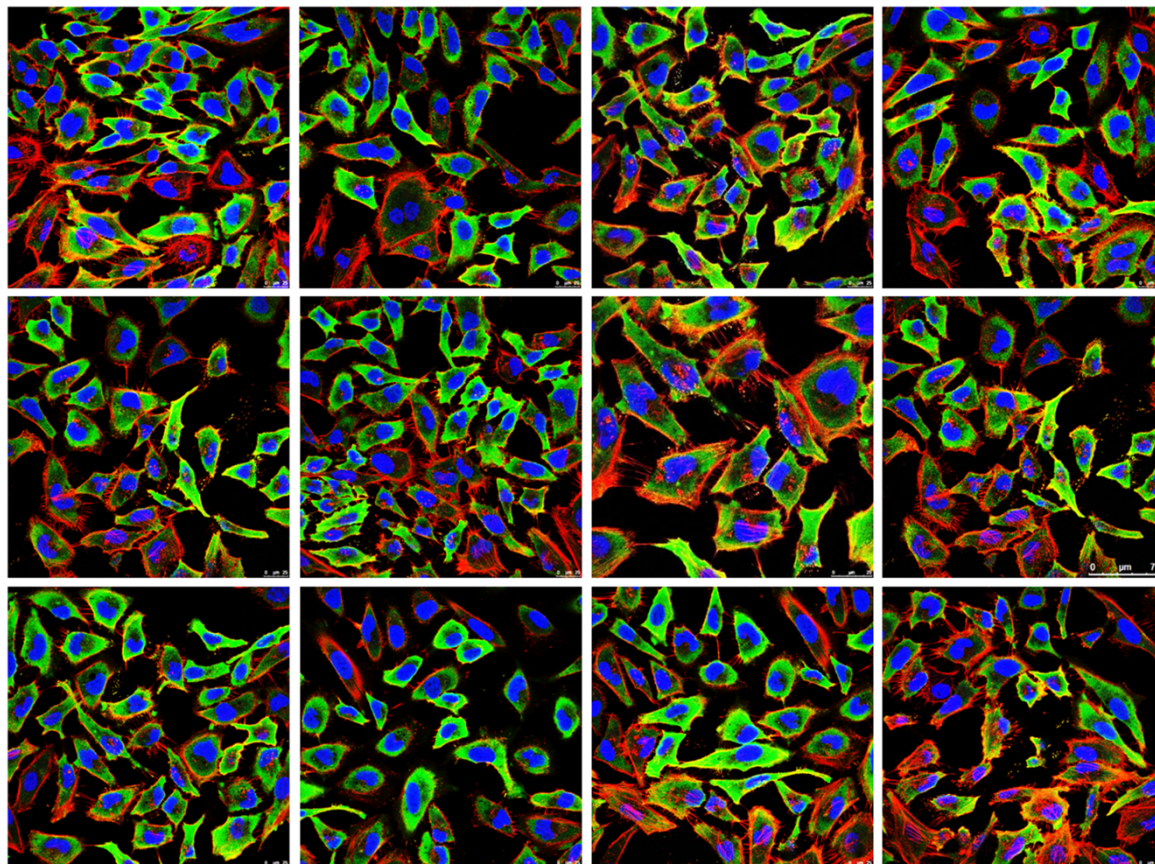


Figure S11. Metallothioneins' expression in HeLa cells. Images represent cells treated with gene gun method without Ag NPs (control shot).

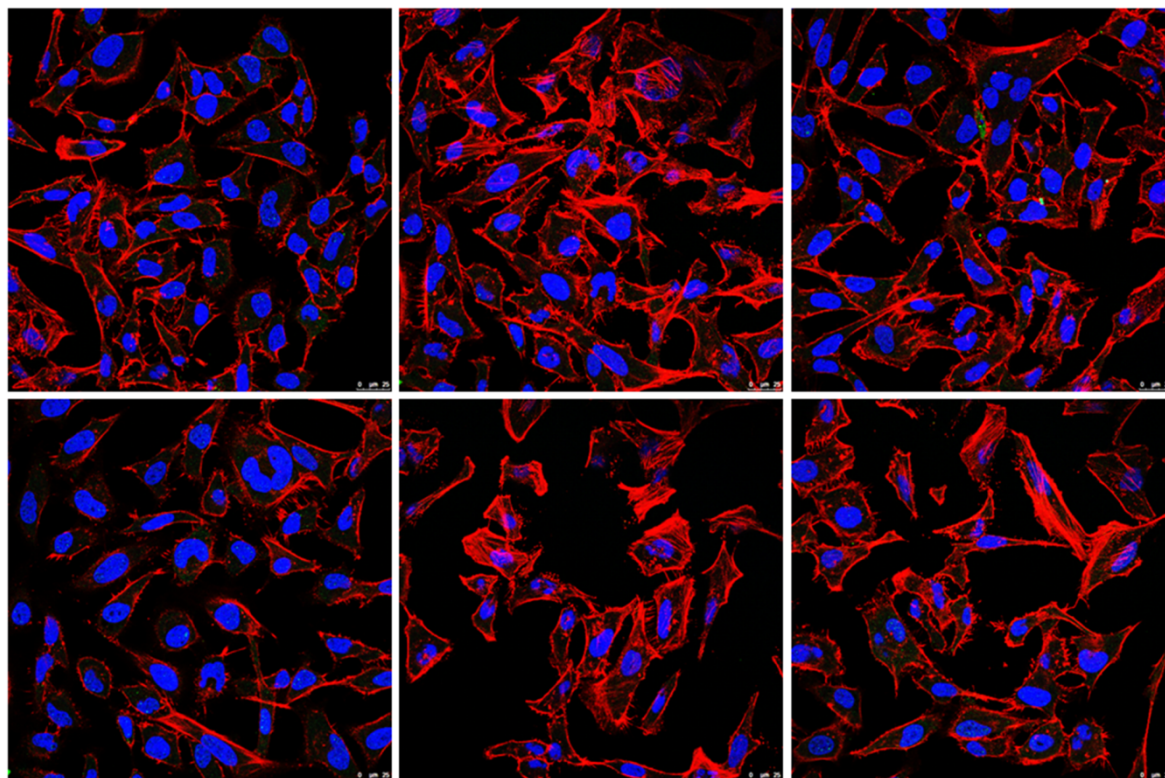


Figure S12. Metallothioneins' expression in HeLa cells. Images represent cells treated with shot Ag NPs (4×10^4 NPs/cell) after 24 hours.

