Supplementary Information

The surface charge both influences the penetration and safety of polystyrene nanoparticles despite the protein corona formation

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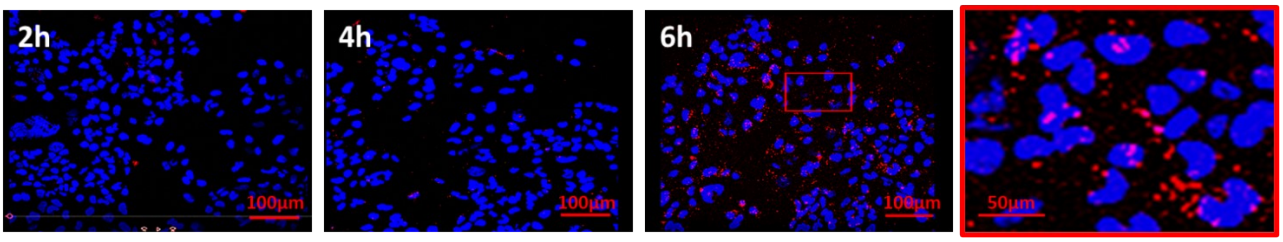
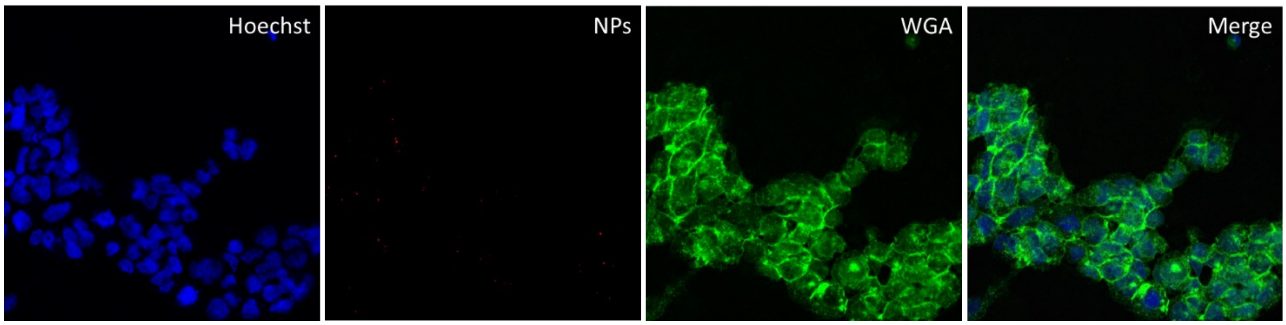
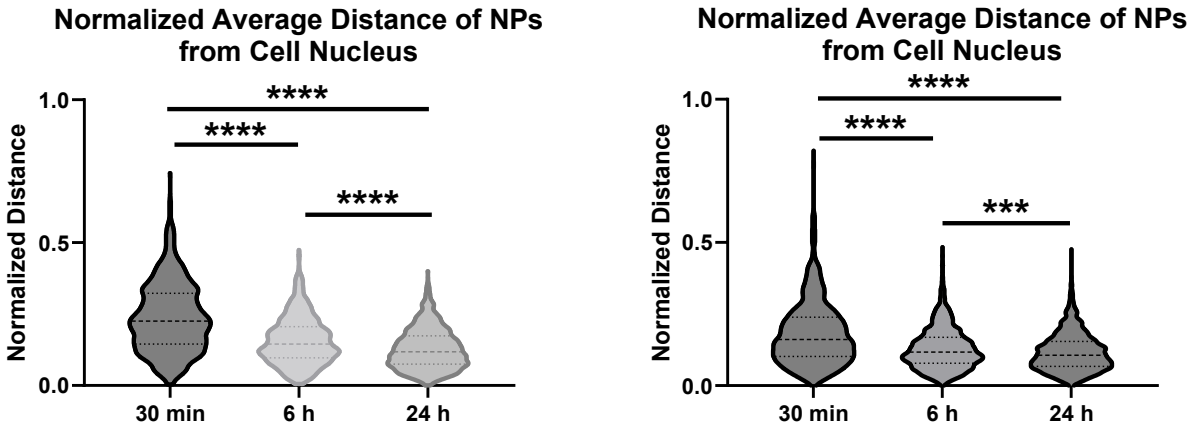
Supplementary Figures



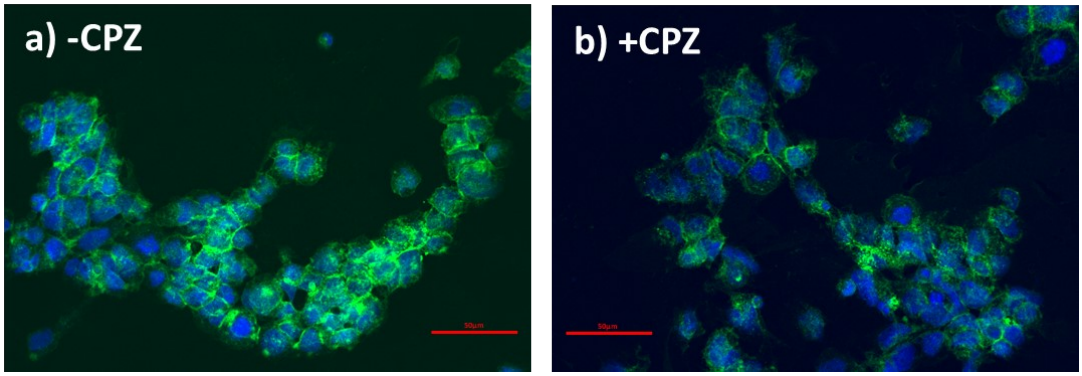
Supplementary Figure S1. Internalization pathway of negative PS-NPs over time. Screenshots of the time-lapse images of HEK 293 cells treated with 2.5 µg/ml negative PS-NPs (magnification 20X) at 2, 4, and 6 hours of incubation. In the red square on the right, a higher magnification shows the NPs internalized. Blue: Hoechst-stained nuclei; red: fluorescently labeled NPs. Scale bar: 100 µm.



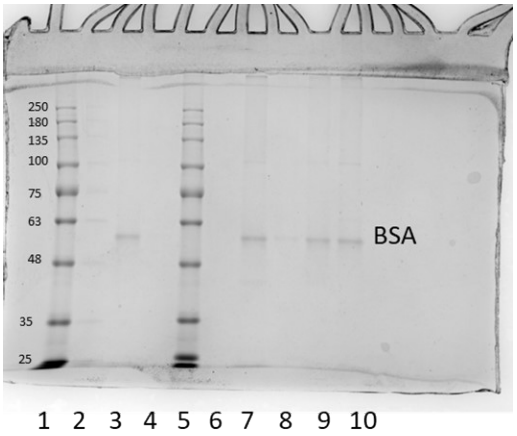
Supplementary Figure S2. Intracellular sub-localization of negative PS-NPs. Split channels of a confocal image of HEK 293 cells treated for 24 hours with negative PS-NPs and the merged image indicate the lack of distribution and internalization of this kind of PS-NPs in the cells.



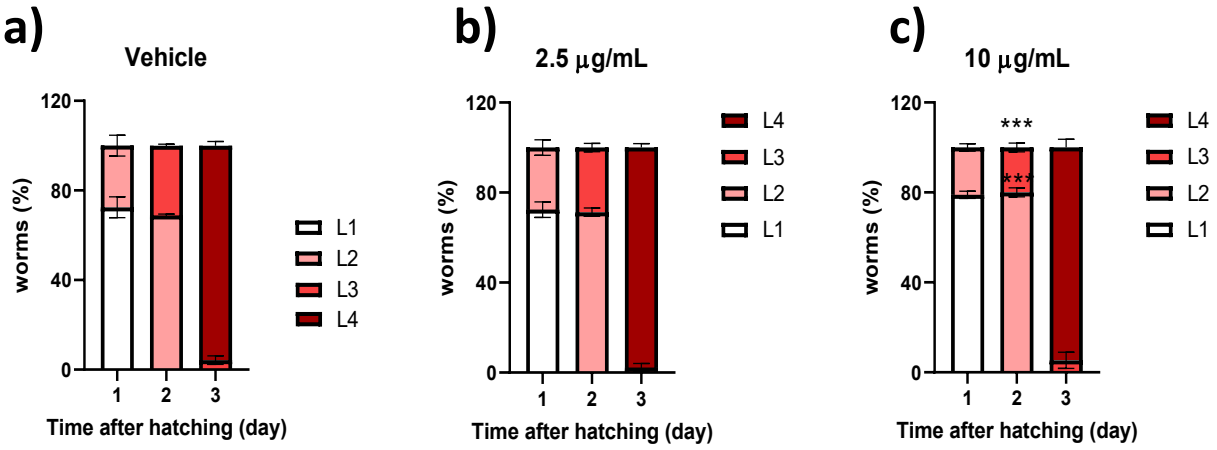
Supplementary Figure S3. Intracellular sub localization of positive and negative PS-NPs. Measure of the distance of positive (left) and negative (right) PS-NPs from the nuclei of the cells over time. ****p< 0.0001, ***p<0.001 according to one-way ANOVA and Tukey's *post hoc* test.



Supplementary Figure S4. Clathrin-mediated internalization pathway of negative PS-NPs Representative confocal images (magnification 60X) of HEK 293 cells treated for 2 hours with negative PS-NPs (**a**) or pre-treated with chlorpromazine for 30 minutes and then treated with negative PS-NPs for 2 hours (**b**). Blue: nuclei; red: PS-NPs; green: cell membranes.



Supplementary Figure S5. Formation of protein corona. SDS-PAGE of serum proteins absorbed on positive and negative PS-NPs. PS-NPs were incubated with 10% FBS for 1 hour at 37°C and collected by centrifugation as described in the Materials and Methods section. Proteins were separated by gel electrophoresis. Lane 1 and 5: markers. Lane 2: positive PS-NPs. Lane 3: FBS+ positive PS-NPs. Lanes 4 and 8: washing PBS. Lane 6: negative PS-NPs. Lane 7: FBS+ negative PS-NPs. Lane 9: FBS 10%. Lane 10: FBS 100%. A band with MW corresponding to BSA was observed in lanes 3 and 7 indicative of the corona formation



Supplementary Figure S6. Effect of positive PS-NPs on development of nematodes. Worms (100 worms/100 µl) were fed for 2 hours with 2.5 µg/ml or 10 µg/ml of positive PS-NPs suspended in 10 mM PBS, pH 7.4 in the absence of OP50 *E. coli*, then plated on NGM plates seeded with the bacteria. Control worms were fed only 10 mM PBS, pH 7.4 (Vehicle). Larval growth of the progeny generated by worms treated with Vehicle, 2.5 µg/ml, and 10 µg/ml positive PS-NPs was rated from 1 up to 3 days after eggs hatched by counting the worms at different larval stages (L1, L2, L3, L4). Data are percentages of the total worms ± SEM (N=10 worms/group).