Supporting Information

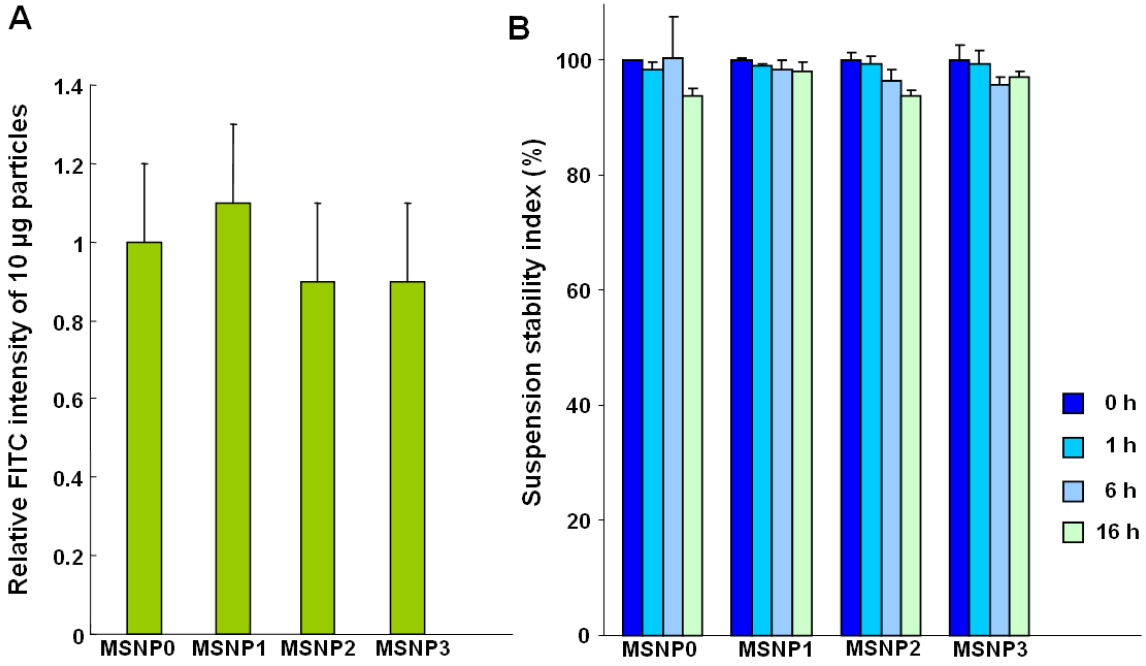

Figure S1. (A) Fluorescent labeling efficiency of the different particle types were determined by microplate reader. The FITC-labeled MSNPs were washed and suspended in water at 100 µg/mL. 100 µL each particle suspensions were loaded into a 96-well plate and the fluorescent intensity was detected at excitation and emission wavelength of 488/525 nm with a microplate reader (M5e, Molecular Device). The fluorescent intensities of MSNP1-MSNP3 were expressed as a unit of the fluorescence intensity of MSNP0, which was regarded as 1.0. The result indicated that there was no significant difference ($p>0.05$) in the fluorescent labeling efficiency among different MSNPs. (B) The suspension stability index of the each particle types was measured at 20 µg/mL (same concentration as in the cellular experiment) in complete RPMI cell culture medium. The FITC-labeled spheres and rods were respectively suspended in 2 mL phenol red free complete RPMI medium containing 10% FBS at 20 µg/mL and sonicated for 10 s before

the experiment. The fluorescence intensities of 10 μL in each of the particle suspensions were measured by a microplate reader (SpectroMax M5e, Molecular Devices Corp., CA) using excitation and emission weigh lengths of at 488 and 525 nm, respectively. This value was designated F_0. A kinetic analysis of suspension stability was performed by monitoring the fluorescence in each particle solutions for different lengths of time. The test tubes, covered by aluminum foil, were placed into tissue culture incubator without agitation for different lengths of time. At indicated time points, 10 μL particle suspension was withdrawn at $1/10^{th}$ of the liquid height from below the meniscuses to measure the fluorescence (F_t) at that time point. The particle sedimentation index (%) was calculated as: $(F_t/F_0)\times 100\%$.

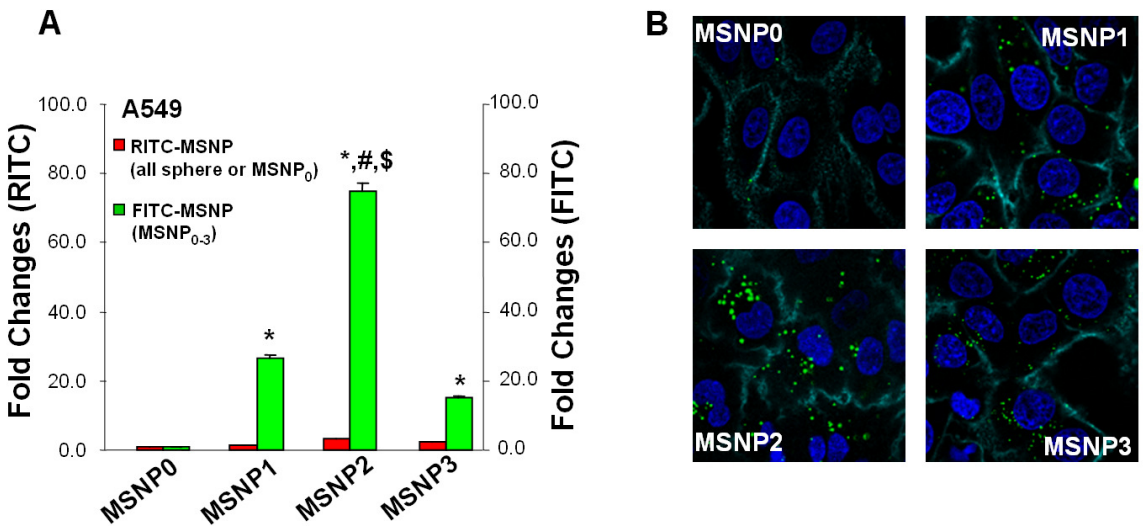

Figure S2. Cellular uptake of FITC-labeled MSNP in A549 lung cancer cells. (A) A549 cells were treated with 20 µg/mL FITC-labeled particles in complete RPMI for 6 h. The fold increase in mean fluorescence intensity (MFI) compared to spherical FITC-labeled MSNP (MSNP0) was used for making comparisons. RITC-labeled nanosphere uptake was used as an internal control for comparing to each FITC-labeled particle type. The RITC-labeled particles were introduced 1 h prior to the PBS washing and introduction of the FITC-labeled particles followed by additional PBS washing. *, $p<0.05$, compared with spherical FITC-labeled MSNP0; #, $p<0.05$, compared with FITC-labeled MSNP1; $, $p<0.05$, compared with FITC-labeled MSNP3. (B) A549 cells were seeded into 8-well chamber slides before addition of the FITC-labeled particles in complete RPMI at 20 µg/mL for 6 h. After fixation and permeabilization, cells were stained with 5 µg/mL wheat germ agglutinin 633 and Hoechst dye, following by visualization under a confocal 1P/FCS inverted microscope.

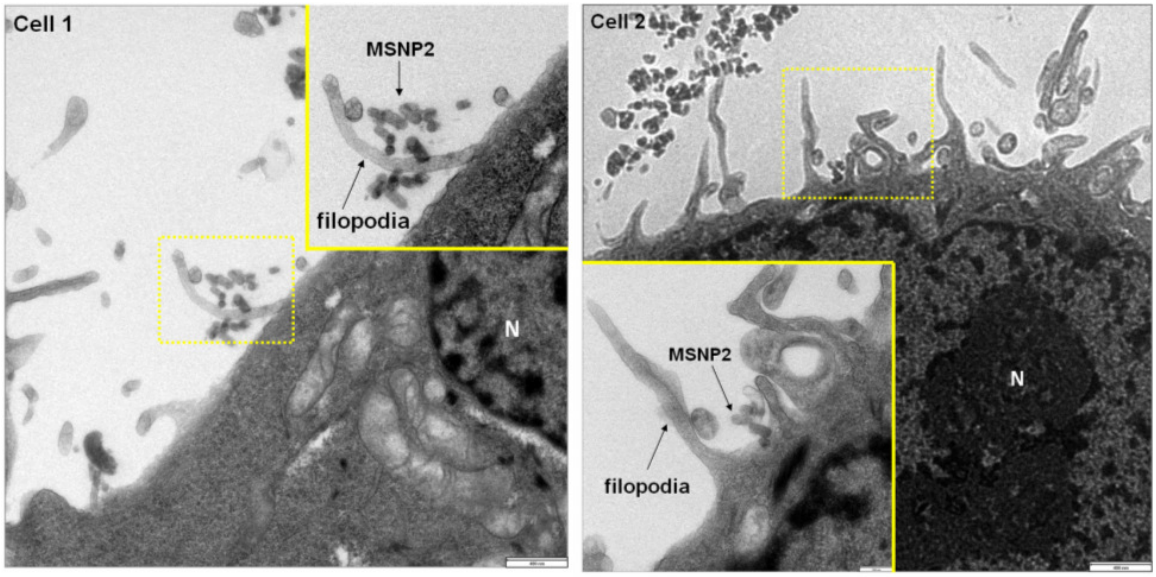

Figure S3. Additional ultrastructure analysis using TEM to elucidate MSNP uptake by macropinocytosis in HeLa cells. HeLa cells were exposed to 20 μg/mL MSNP2 for 3 h. The arrows in the figures point out filopodia and MSNP2 nanoparticles, respectively. "N" denotes nuclear.

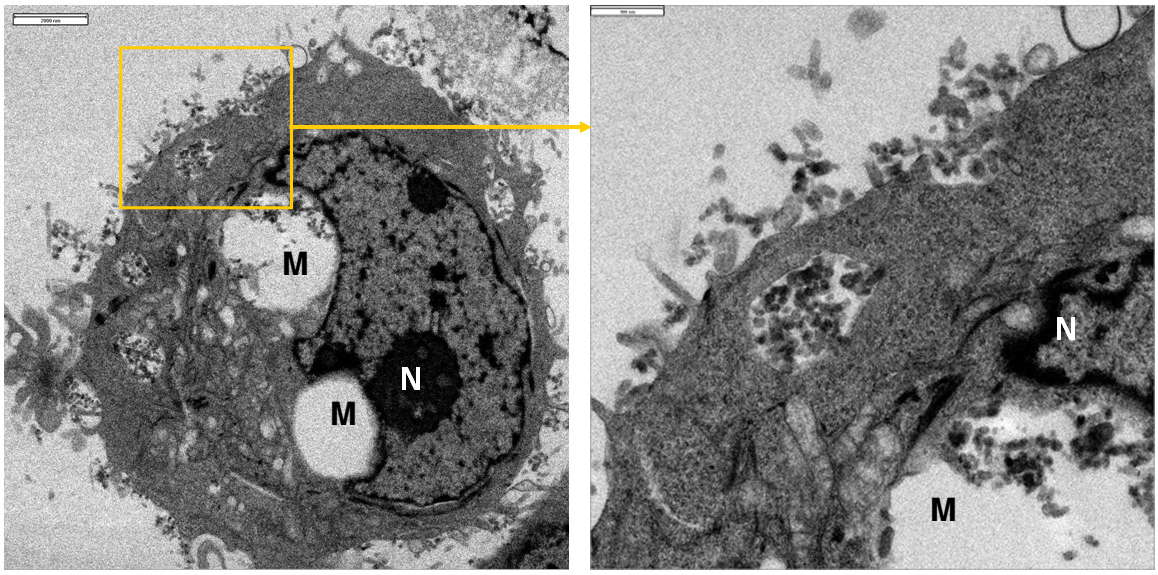

Figure S4. Electron tomography analysis and 3D image reconstruction of the TEM images generated during HeLa cell incubation with 20 μg/mL MSNP2 for 3 h. TEM grids were used for the tomography analysis on an FEI Tecnai F20 microscope operated at 200 kV. Images were captured by tilting the specimen from -70˚ to 70˚ with 1˚ steps. Tilt series images were captured with a TIETZ F415MP 16 megapixel CCD camera at a magnification of 26,600× and then aligned using the Etomo program in the IMOD package. The aligned tilt series were further processed using the reconstruction features of the FEI software, Inspect3d's SIRT, to improve the contrast. Videos of the aligned tilt series were generated using both Inspect3d and Windows movie maker. These data confirm MSNP2 uptake by macropinocytosis and actually yield resolution of the particles pores inside the cell. The accompanying videos are available online as V1 and V2. M: macropinosome. N: nuclear.

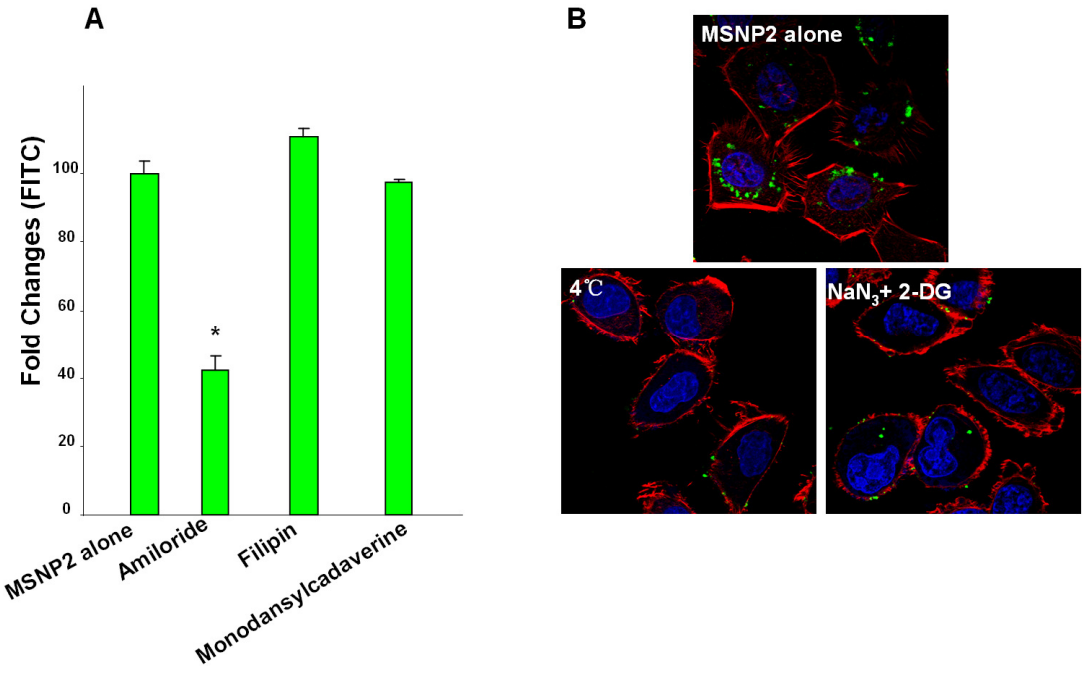

Figure S5. (A) Flow cytometry data showing inhibition of FITC-labeled MSNP2 uptake in the presence of amiloride (which interferes in macropinocytosis by inhibiting Na^+/H^+ exchange), filipin (which blocks caveolae-mediated endocytosis through cholesterol sequestration), or monodansylcadaverine (which inhibits formation of clathrin-coated pits). The fold-increase in mean fluorescence intensity (MFI) compared to the treatment using FITC-labeled MSNP2 alone was used for comparison. The cells were pre-cultured in RPMI 1640 medium containing amiloride (75 μM), filipin (5 μM), or monodansylcadaverine (50 μM) for 3 h. At this time point, the medium was replaced by fresh RPMI containing 20 μg/mL FITC-labeled MSNP2 and one of the chemical inhibitors (amiloride, filipin or monodansylcadaverine) and cells incubated for a further 6 h time period. Cells were washed with PBS and then processed for flow cytometry analysis as described in Fig. 2A. *, $p<0.05$, compared with FITC-labeled MSNP2 alone. (B) Confocal images corresponding to the data in Fig. 3D. This experiment was carried out at the same time in Fig. 3D and the image of MSNP2 alone is reshown together with

the images of cells kept at 4 °C and ATP depletion condition using NaN_3 (15 mM) plus 2-DG (50 mM) as described in Material and Methods section.

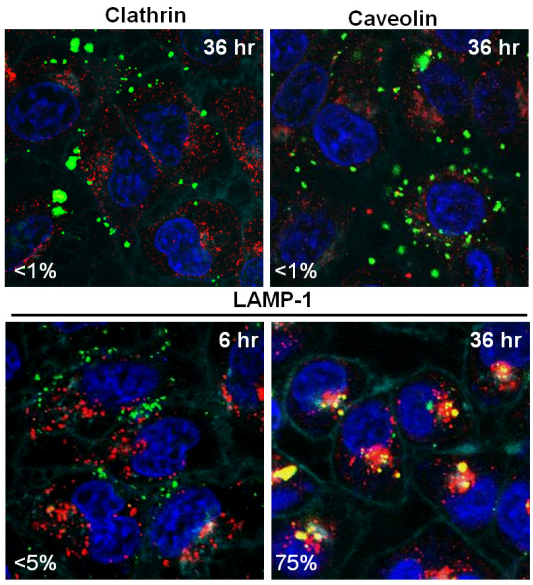

Figure S6. Confocal microscopy to study the subcellular localization of FITC-labeled MSNP2 in HeLa cells. HeLa cells were treated with 20 µg/mL FITC-labeled MSNP for the indicated time periods. After fixation and permeabilization, cells were stained with anti-clathrin (Santa Cruz, Biotechnology), anti-caveolin-1 (BD BioSciences), and anti-LAMP-1 (Abcam) antibodies, and visualized with Alexa 594-conjugated secondary antibody. The colocalization ratio, as determined by Imaging J software, indicates <1% colocalization of the green-labeled nanoparticles with the red-labeled clathrin and caveolae at 36 h. The colocalization ratios with the LAMP-1 positive compartment were estimated to be <5% and 75%, respectively, at the 6 h and 36 h time points.

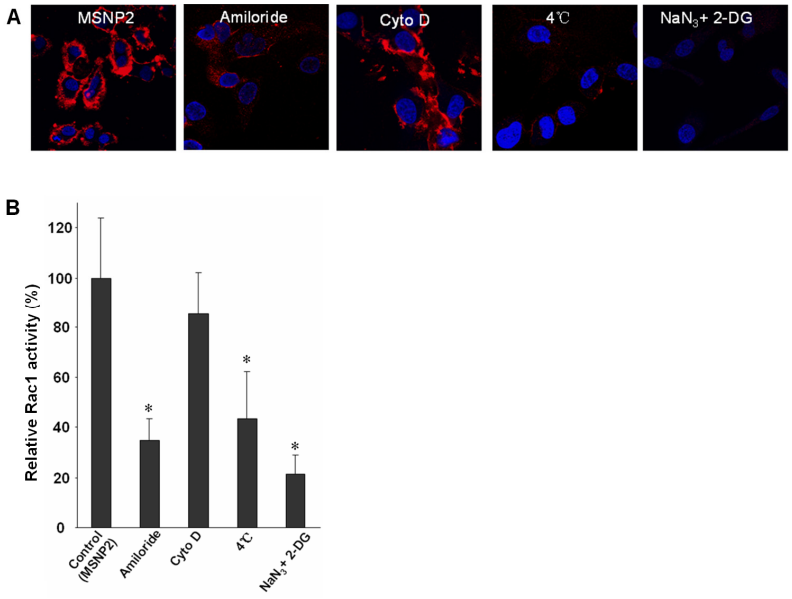

Figure S7. (A) Confocal microscopy to show the effects of the chemical inhibitors and low temperature on Rac1 activation in HeLa cells. The cells were pre-cultured for 3 h in serum free RPMI 1640 medium containing amiloride (75 μM), Cyto D (2.5 μg/mL), or 15 mM NaN_3/50 mM 2-DG. Alternatively, cells were placed at 4 °C for 3 h. Subsequently, the media were exchanged with fresh complete RPMI 1640 that included 20 μg/mL FITC-labeled MSNP2 as well as one of the chemical inhibitors (amiloride, Cyto D or NaN_3/50 mM) for a further 6 h. The 4 °C culture was maintained at this temperature. To remove free particles, the cells were gentally washed by PBS three times. After fixation and permeabilization, cells were stained with a primary antibody recognizing GTP-Rac1 and an Alexa 594-labeled secondary antibody. Nuclei were stained with Hoechst dye. (B) The fluorescence intensity of GTP-Rac1 was analyzed by Image J software. The fluorescence intensity of GTP-Rac1 in cells incubated with MSNP2 was regarded as 100% as baseline for comparison to cells treated with MSNP2 in the presence of inhibitors. *, $p<0.05$, demotes a significant decrease compared with the treatment using MSNP2 alone.

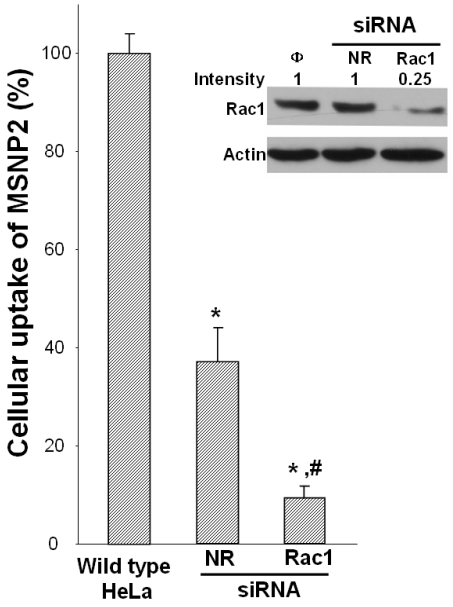

Figure S8. Abundance of cellular uptake of FITC-labeled MSNP2 in wild type HeLa cells *vs* cells in which Rac1 was knocked down by siRNA treatment. HeLa cells were plated at 2×10^5 cells per well and cultured for 12 h to allow cell attachment. Gene knockdown was performed by adding 150 ng/μL Rac1 siRNA (Santa Cruz Biotechnology, CA) or non-relevant (NR) siRNA (that targets at GFP protein) to 100 μL serum free RPMI medium. This was followed by adding Lipofectamine 2000 at a concentration of 1 mg/mL to 100 μL serum free RPMI medium and mixing the two solutions and 30 min incubation at room temperature. HeLa cells were cultured in the combined solution for 4 h. The medium was replaced with fresh RPMI containing 10% FBS 4 h after incubation and cultured for a further 3 days. The protein knockdown was determined by immunoblotting and compared to the relative abundance of a household protein, actin. Cells treated with Rac1 or NR siRNA were subsequently exposed to FITC-labeled MSNP2 at 20 μg/mL for 6 h. After PBS washing, all the cells were trypsinized and washed 3 times in PBS. Cells were analyzed in a SCAN flow cytometer

to assess FITC fluorescence. Uptake of FITC-labeled particles in wild type HeLa cells was regarded as reference value (100%) and the comparisons were made for cells treated with Rac1 or NR siRNA. *, $p<0.05$, compared to wild type HeLa; #, $p<0.05$, compared to NR siRNA treated HeLa cells.

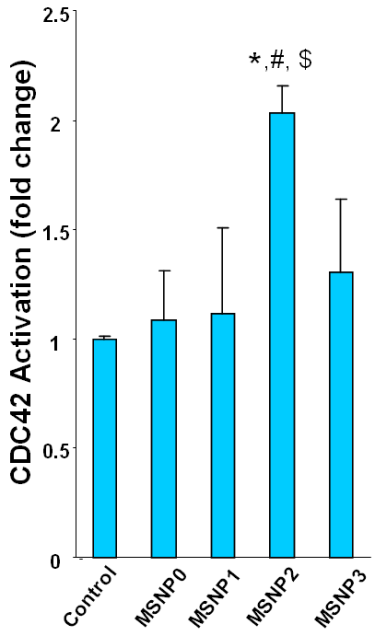

Figure S9. To study CDC42 activation, a G-LISA activation kit (Cytoskeleton, Inc.) was used according to the manufacture's instruction. Briefly, HeLa cells were serum-starved for 4 h before introduction of the particles that were dispersed in RPMI containing 10% FBS. Cells were incubated with the various particles for 30 min in complete RPMI at a concentration of 20 μg/mL. Serum starved cells that were re-exposed to complete RPMI for 30 min was used as control and was chosen as reference. After various treatments, the cells were washed in cold PBS and the pellets lysed in an ice-cold cell lysis buffer (Cytoskeleton, Inc.). The protein content of the supernatants was determined by the Bradford method. Diluted cell lysates were added into CDC42-GTP affinity plate (Cytoskeleton, Inc.). After incubation and thorough washing, the bound CDC42-GTP was detected through antibody recognition reaction. HRP detection reagent was added into each well for 10 min at 37 °C. After incubation, stop buffer was added and the OD value was measured by a mircoplate reader at 490 nm. *, $p<0.05$ compared to control; #,

$p<0.05$ compared to MSNP1; \$, $p<0.05$ compared to MSNP3. In a similar assay to determine RhoA activation was observed no effect on the activation of this small GTPase in response to any MSNP type (not shown).

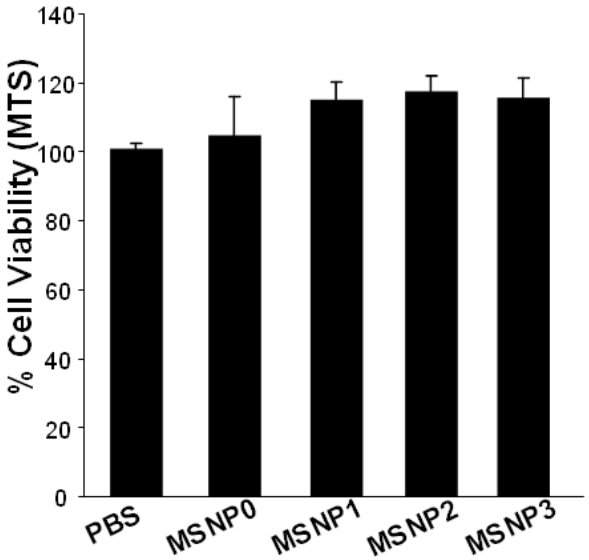

Figure S10. Cell viability assessment using a MTS assay. 1×10^4 HeLa cells in 100 μL completed RPMI were plated onto 96-multi-well plates (Costar; Corning, NY). After incubation with various empty particles at doses of 200 μg/mL for 36 h, HeLa cells were incubated with the MTS reagent for 2 h and the absorbance measured at 490 nm. Cells receiving treatment with 5 μL PBS alone served as control. The data show that all MSNPs were devoid of toxicity.