Supplementary data

Materials and Methods

Cell motility assay

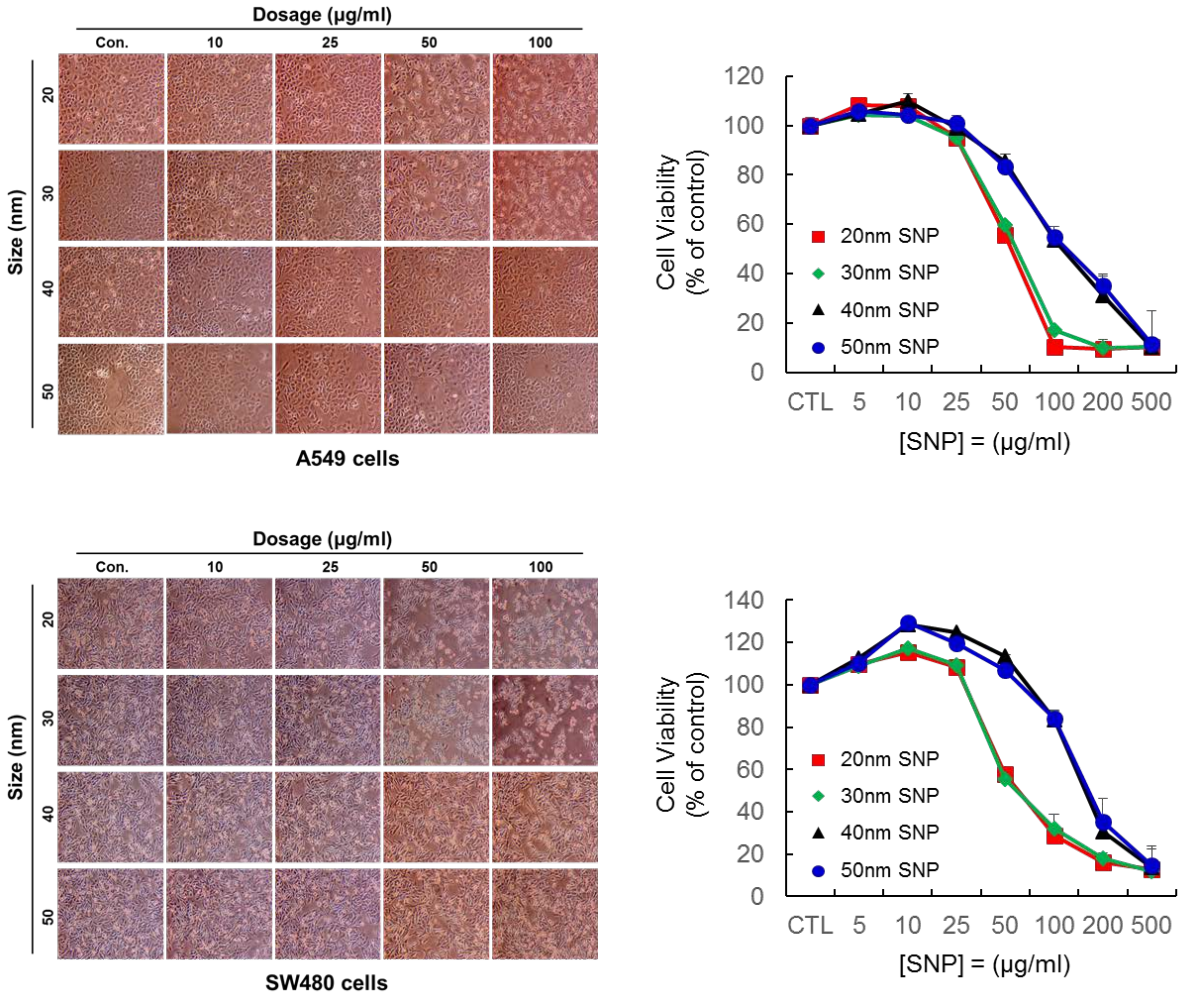
We examined a scratch 'wound-healing' assay to identify the effect of SNPs on cell motility. A549, SW480, and HepG2 cells were seeded in 12-well plates (1×10^5 cells/well) and wounded by scratching with sterile plastic 200 μl micropipette tips. After washing cells using phosphate-buffered saline (PBS), cells were cultured in media containing 20, 30, 40, or 50 nm SiNPs at different concentrations (10, 25, 50, 100, or 200 μg/ml) for 24 h. The distances of migrating cells were imaged with a microscope.

Determination of intracellular ROS levels

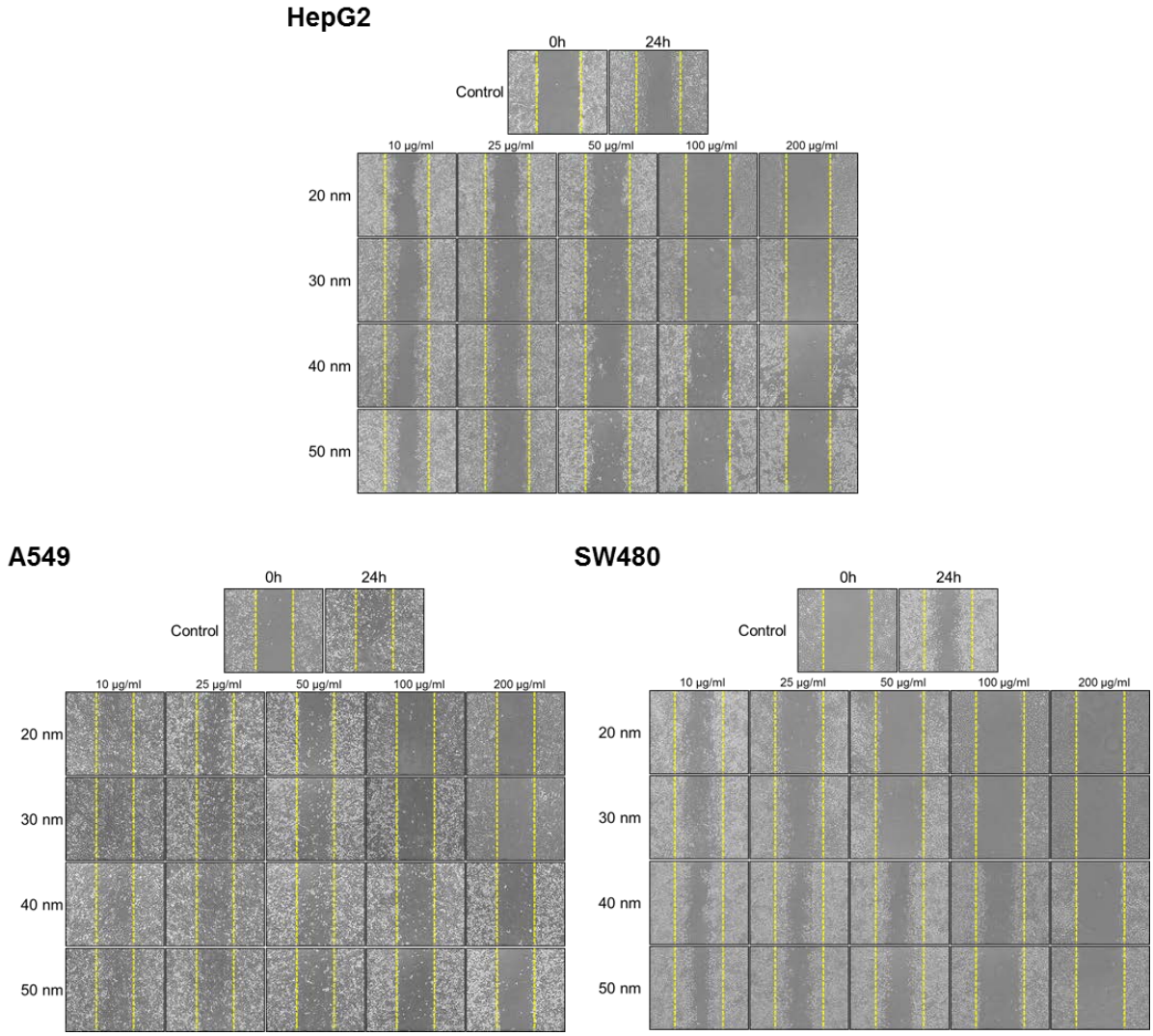
The HepG2 cells were seeded (1×10^4 cells/well) in a 96-well dark plate and incubated for 24 h at 37°C. The cells were treated SNPs with various sizes, respectively, and stained by H2DCFDA (Molecular probes) solution for 30 min at room temperature. Then, it was added general oxidative stress indicator, H2DCFDA (Molecular probes) solution for 30 min. After the solution was discarded carefully, the well were added PBS buffer and immediately DCF fluorescence signals were detected by flow cytometer (Ex/Em = 485/535). Data analysis was carried out using WinMDI2.9 software.

Genotoxicity test

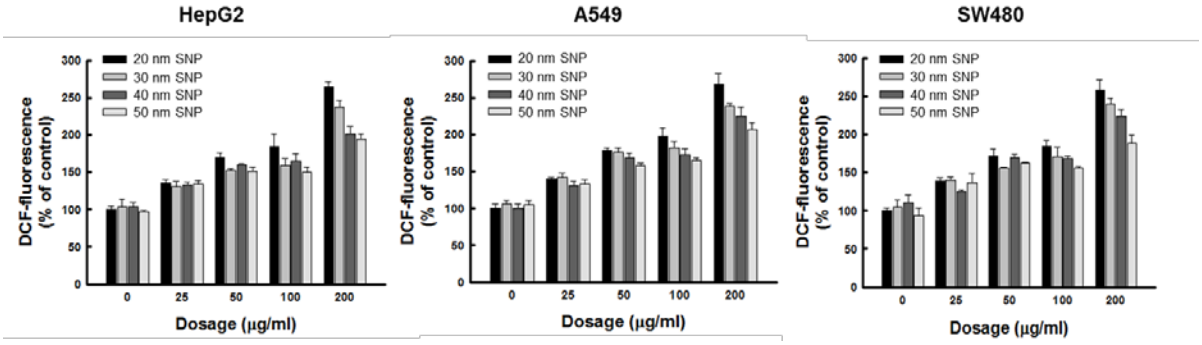
In order to evaluate nanoparticle-mediated genotoxicity, we identified micronuclei generation in the cells treated with SNPs. The cells were seeded in 96-well plates (1×10^4 cells/well) and treated with 20, 30, 40, or 50 nm sizes of SNPs with 5, 10, 25, 50, or 100 μg/ml concentrations for 24 h. The micronuclei assay was performed using a BD™ Gentest Micronucleus Assay Kit following the standard protocol provide by the manufacturer. Cell images were obtained via florescence microscopy and analyzed using the Metamorph software.



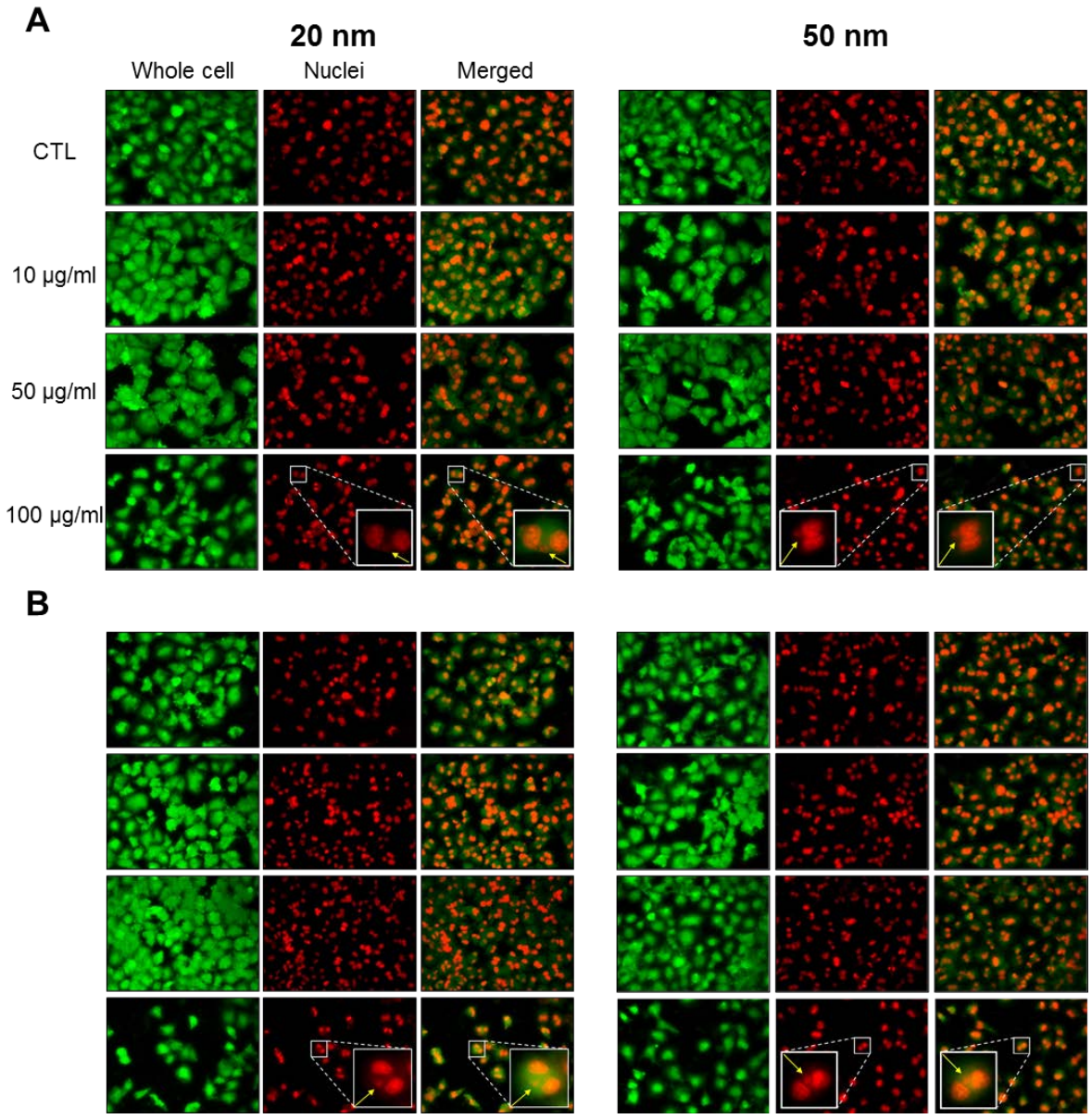
Supplementary Figure S1 Cytotoxicity of SNP with the different sizes. Assessment of A549 and SW480 cell viability following treatment with different sizes of SNPs. The cells were treated with the indicated concentrations of the SNPs for 24 h in low serum-containing condition and analyzed using WST-1 assay. Morphologies of these cells following treatment with SNPs for 24 h were showed using an optical microscopy.



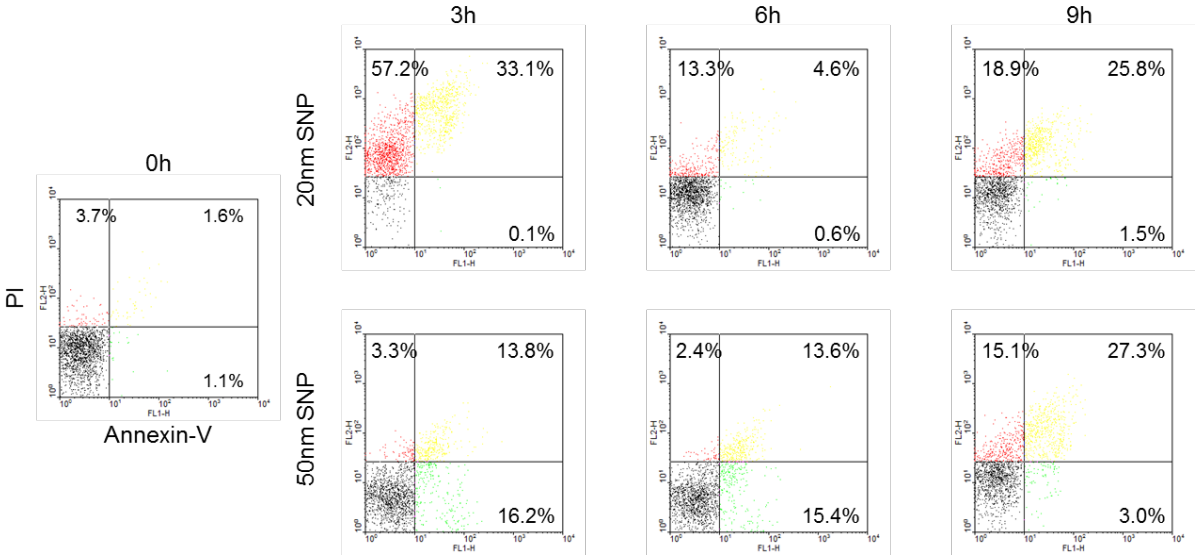
Supplementary Figure S2 Size-dependent effect of the SNPs on cell motility. Scratch wound healing assay of HepG2, A549, and SW480 cells treated with the indicated concentrations of the SNPs for 24 h in low serum-containing condition.



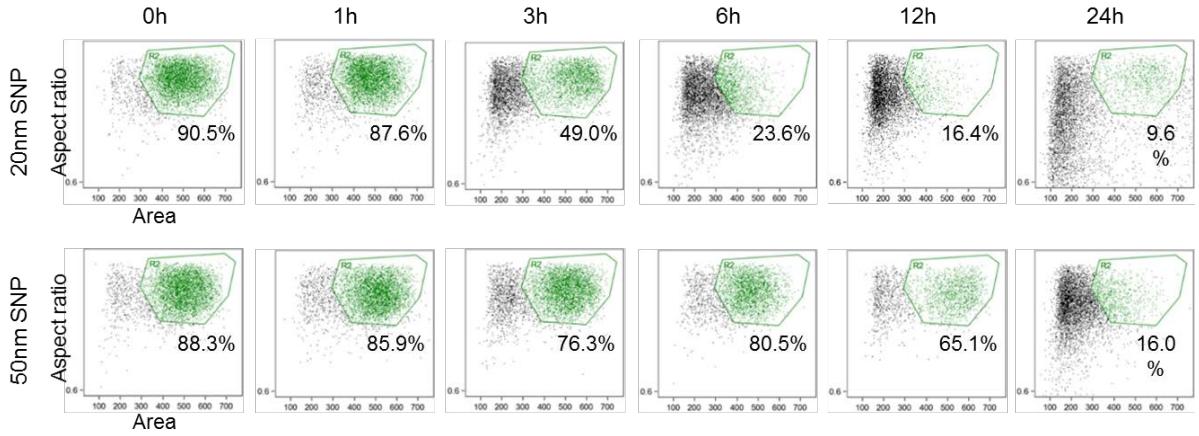
Supplementary Figure S3 Determination of intracellular ROS levels. Relative ROS level in HepG2, A549, and SW480 cells treated with the indicated sizes and concentration of SNPs for 24 h was examined by the oxidative stress indicator (H2DCFDA).



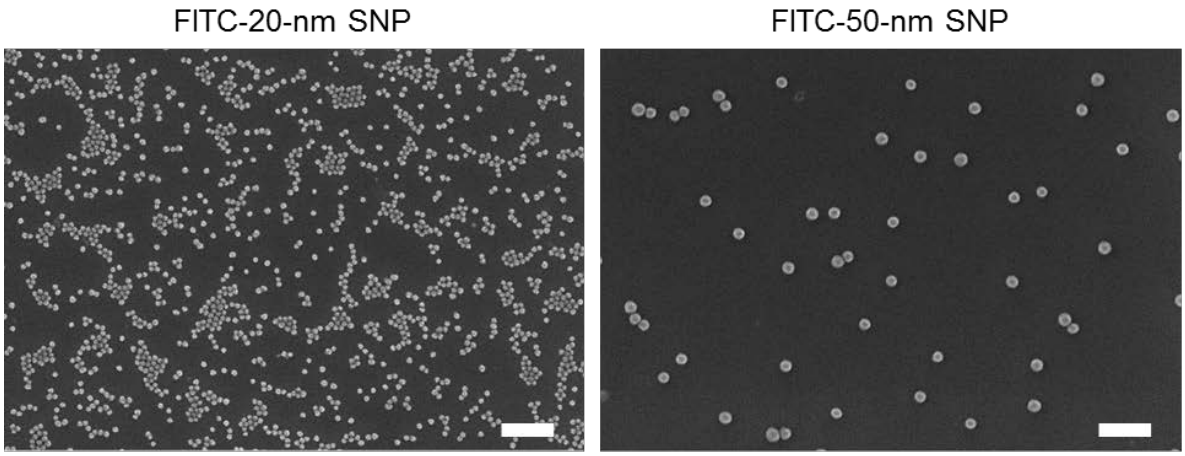
Supplementary Figure S4 Induction of micronuclei in cells following SNPs treatment. Representative image of binucleated cells in the (**A**) HepG2 and (**B**) A549-treated the indicated sizes and concentrations of the SNPs for 24 h. Yellow arrow indicates the micronucleus formed among the binucleated cells.



Supplementary Figure S5 Time-lapse induction of apoptosis and necrosis depending on SNP size. Flow cytometric analysis of apoptosis and necrosis of HepG2 cells treated with 20-nm SNP (75.2 μg/mL) and 50-nm SNPs (175.2 μg/mL) in the indicated exposure time (early apoptosis (green): annexin-V(+)/PI (-), late apoptosis (yellow): annexin-V(+)/PI (+), and necrosis (red): annexin-V(-)/PI (+)).

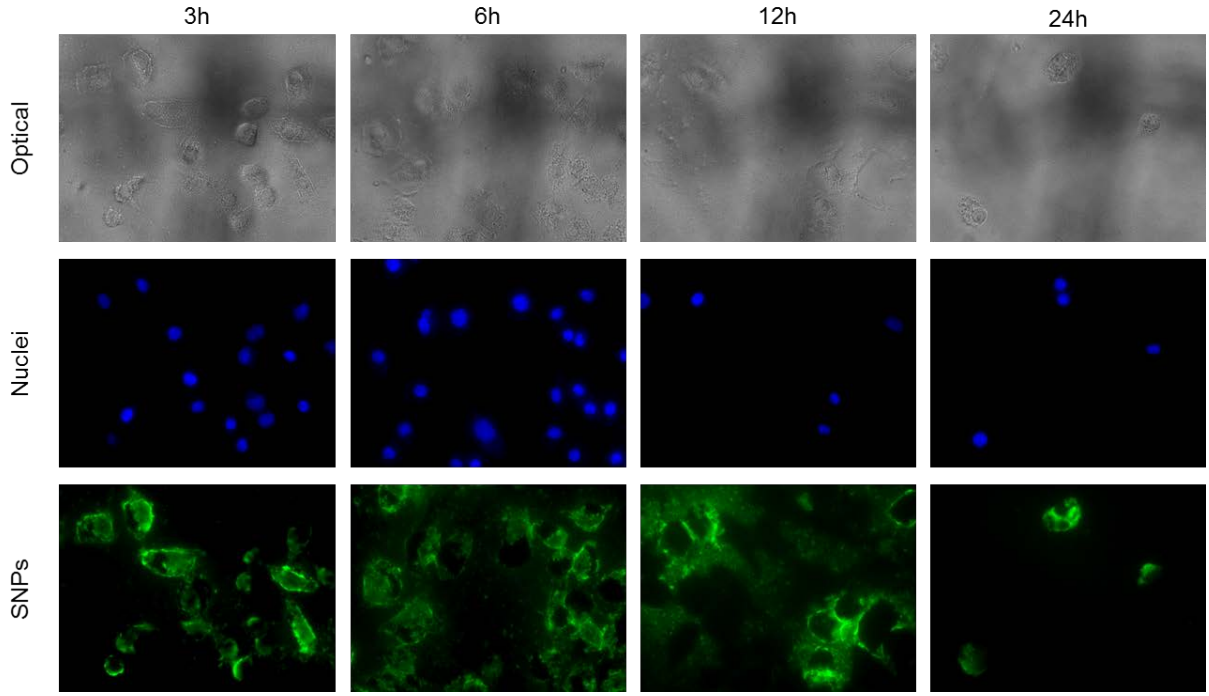


Supplementary Figure S6 Gating on bivariate plot of aspect ratio versus area of HepG2 cells treated with the 20- and 50-nm SNPs. Normal single cells were separated from destroyed cells, doublets or debris using this scatter plot. R2 region defines a normal cell population with an aspect ratio close to 1. Each percentage represents the ratio of cells gated in the R2 region among the total number of counted cells.

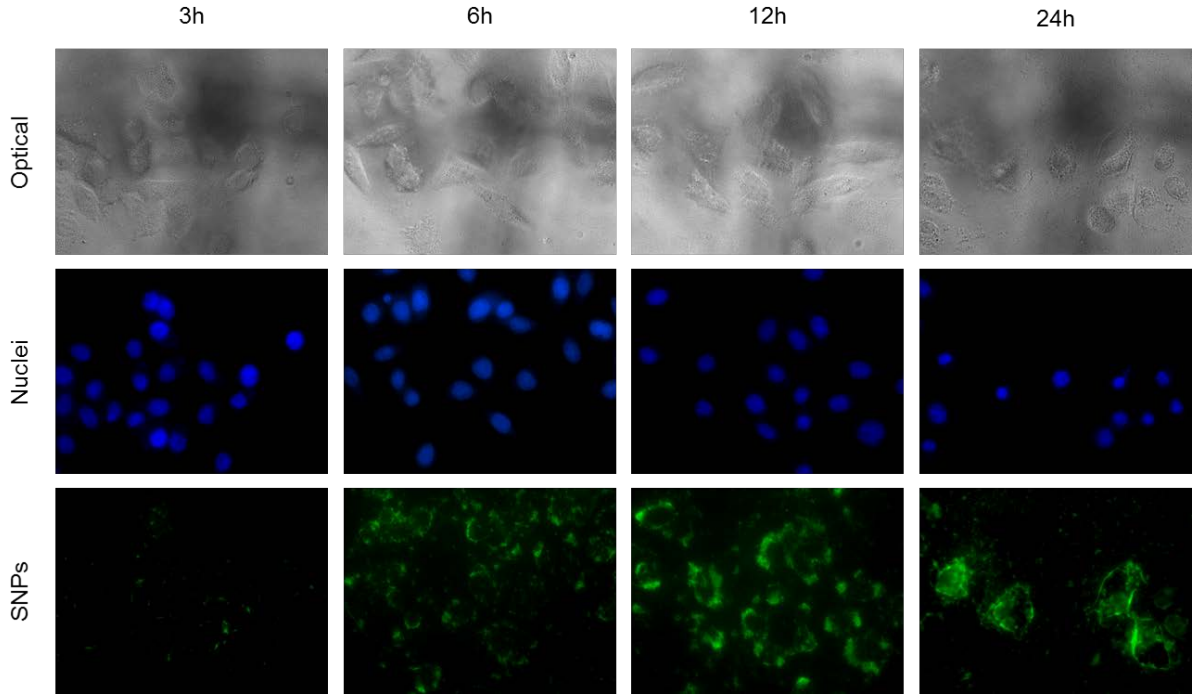


Nominal size	SEM size (nm)		DLS size (nm)	
(nm)	Mean	S.D	Mean ± S.D	PDI
FITC-20-nm SNP	22.5	1.8	21.7 ± 0.77	0.19
FITC-50-nm SNP	52.4	5.6	58.6 ± 0.25	0.07

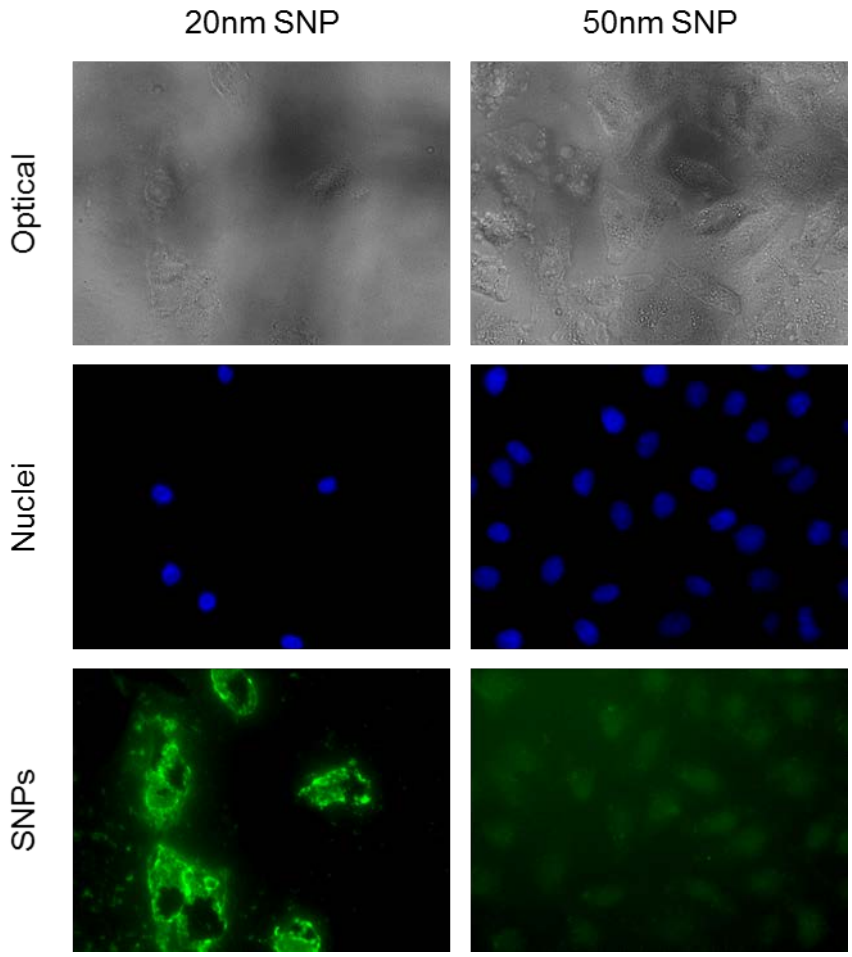
Supplementary Figure S7 Preparation and characteristics of FITC-labeled SNPs. SEM images and size distribution analysis results of FITC-20- and 50-nm SNPs (scale bar = 200 nm). The SEM images show that all SNPs are monodispersed in size and spherical shape. Analysis of DLS data from aqueous suspensions of SNPs ([FITC-SNPs] = 1 mg/ml). No significant difference of size and distribution was found compared to the non-labeled SNPs.



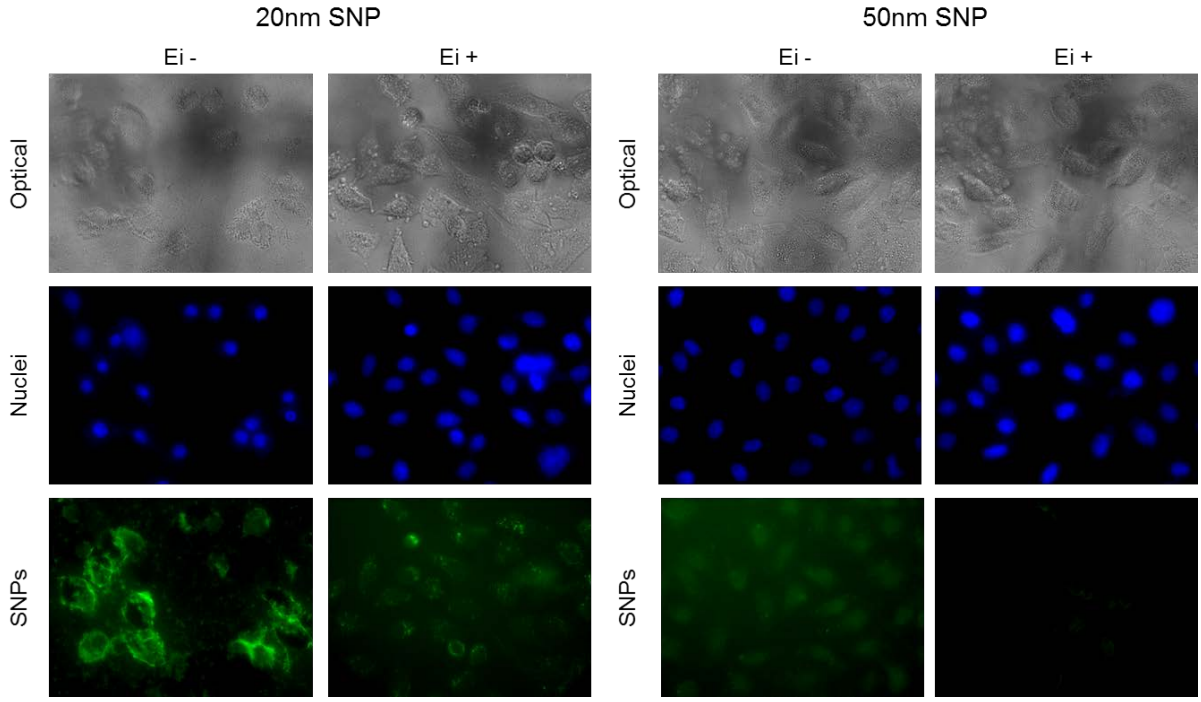
Supplementary Figure S8 Time-lapse imaging of the cellular uptake of the 20-nm SNP in HepG2 cells over 24 h. FITC-labeled 20-nm SNP was prepared, and it was treated into the HepG2 cells during the indicated time intervals. After fixation, the cells treated with DAPI solution for nuclei staining. Its cellular uptake was observed by fluorescent microscopy.



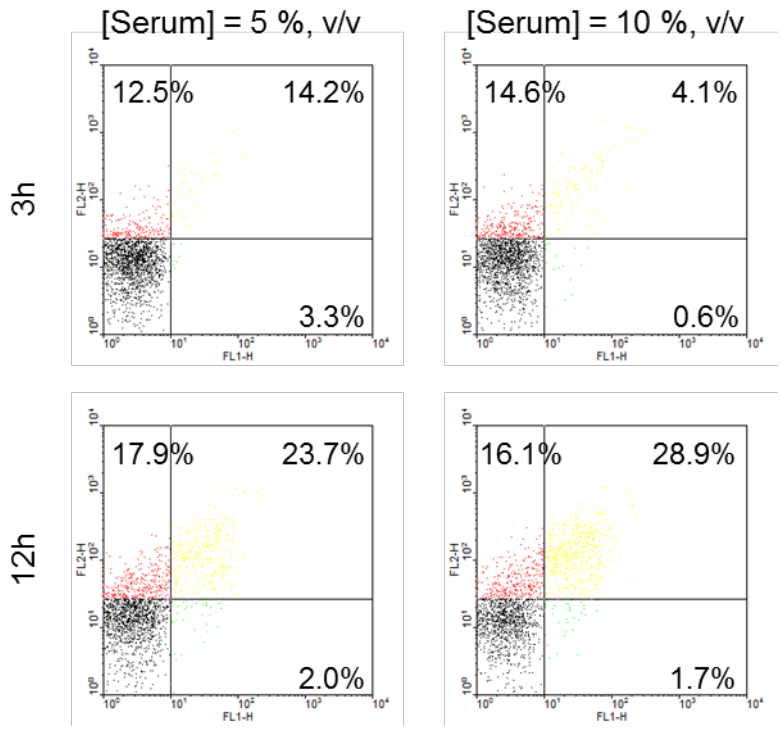
Supplementary Figure S9 Time-lapse imaging of the cellular uptake of the 50-nm SNP in HepG2 cells over 24 h. FITC-labeled 50-nm SNP was prepared, and it was treated into the HepG2 cells during the indicated time intervals. After fixation, the cells treated with DAPI solution for nuclei staining. Its cellular uptake was observed by fluorescent microscopy.



Supplementary Figure S10 Cellular uptake of 20- and 50-nm SNPs in the presence of endocytosis inhibitors. After treatment of the endocytosis inhibitors, the uptake of 20- and 50-nm SNPs were observed by fluorescent microscopy, respectively.



Supplementary Figure S11 Effect of serum proteins on cellular uptake of 20- and 50-nm SNPs. At the 5% serum-containing condition, cellular uptake of 20- and 50-nm SNPs into the HepG2 cells with/without endocytosis inhibitors were observed by fluorescent microscopy.



Supplementary Figure S12 Effect of serum proteins on cytotoxic mechanisms of 20-nm SNP. Flow cytometric analysis of apoptosis and necrosis of HepG2 cells treated with 20-nm SNP (75.2 μg/mL) in the indicated serum concentration and exposure time (early apoptosis (green): annexin-V(+)/PI (-), late apoptosis (yellow): annexin-V(+)/PI (+), and necrosis (red): annexin-V(-)/PI (+)).