

Supplementary Information

Time and Space Resolved Uptake Study of Silica Nanoparticles by Human Cells

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	50nm			100nm			300nm		
Dispersant	Water	PBS	cMEM	Water	PBS	cMEM	Water	PBS	cMEM
Size									
(hydrodynamic diameter, nm)	49	66	*****	94	87	*****	326	298	*****
Polydispersity Index	0.02	0.234	*****	0.052	0.073	*****	0.018	0.076	*****
Zeta Potential (mV)	----	-19.6	-8.23	----	-24.7	-9.51	----	-30.8	1.25
Mob (μmcm/Vs)	----	-1.49	-0.64	----	-1.88	-0.74	----	-2.37	0.09
Conductivity (mS/cm)	----	17.4	15.2	----	17.3	15.4	----	11.6	17.7

Table S1. Nanoparticle Characterisation: Size (diameter), size distribution and zeta potential of fluorescent SiO₂ nanoparticle dispersions in water, phosphate buffer and complete cell culture medium (cMEM), determined by dynamic light scattering using a photon correlation spectrophotometer at 25°C.

*** The size and PDI in complete cell culture medium have been studied more extensively at 37°C and the data are shown in Figure S1 F-G as a function of time. ---Zeta potential in water could not be determined due to the dispersion conductivity being too low (< 0.1 ms/cm).

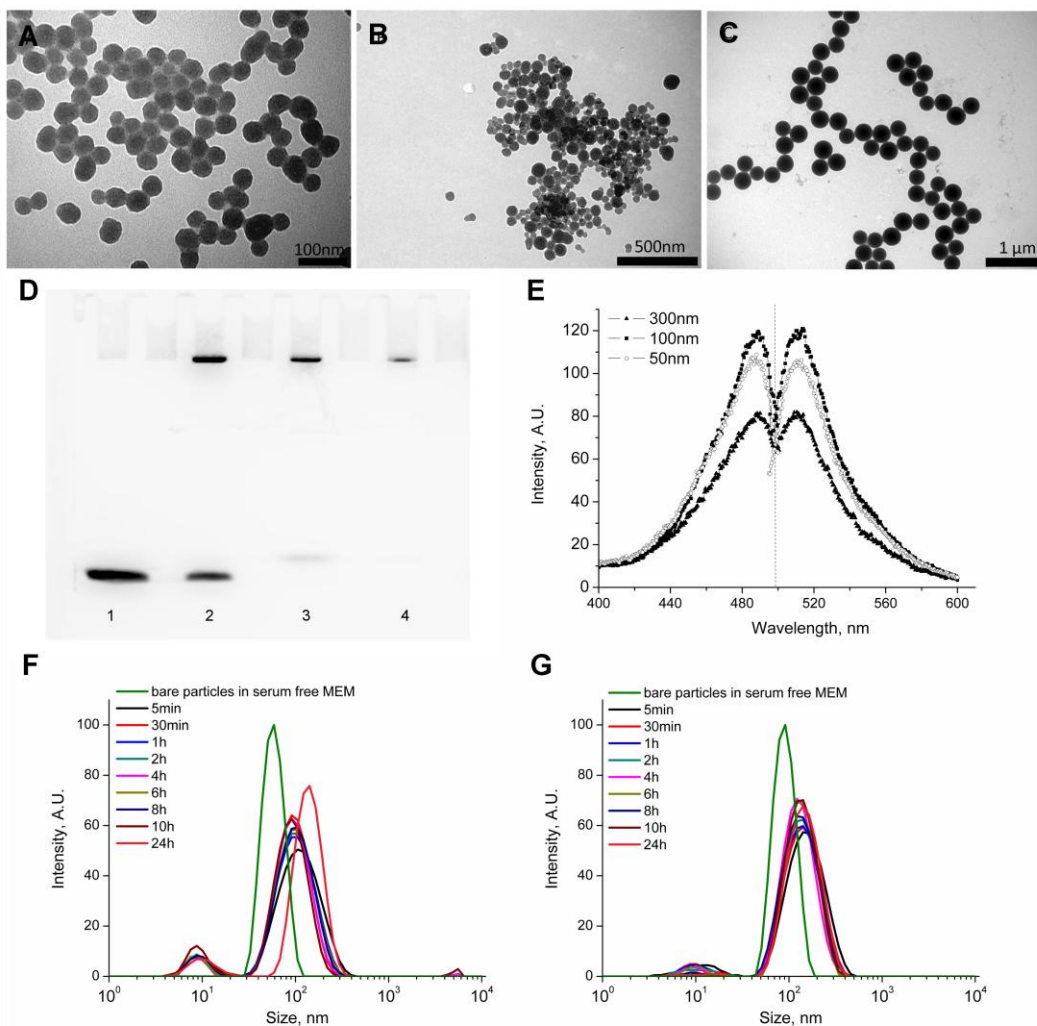


Figure S1. Nanoparticle Characterisation: EM images of the 50 (A), 100 (B) and 300 (C) nm SiO₂ nanoparticles. (D): Fluorescence image of an SDS PAGE gel of SiO₂ nanoparticle, compared with the YG dye and fluorescently-labelled polystyrene nanoparticles as a reference. The upper region of the gel shows the fluorescence bands from the nanoparticles which are too large to enter the gel. The fluorescence bands in the lower region of the gel show the presence of a fluorescence molecule comparable to YG dye alone (in lane 1) which is the labile dye eluting from the nanoparticles. Lane 1: Free YG dye from Polysciences, Lane 2: Polysciences 50nm FITC labelled polystyrene nanoparticles, as a reference. Lane 3: 50nm green labelled Kisker SiO₂ nanoparticles, Lane 4: 100nm green labelled Kisker SiO₂ nanoparticles. (E): Fluorescence spectra (excitation and emission) of the 50, 100 and 300 nm SiO₂ nanoparticles at 100 μ g/ml in PBS. (F): Comparison of the size distribution curves obtained by DLS for 100 μ g/ml 50nm SiO₂ nanoparticles in complete medium cMEM at 37°C for multiple

incubation times showing the full protein corona. **(G)**: As above for the 100nm SiO₂ nanoparticles.

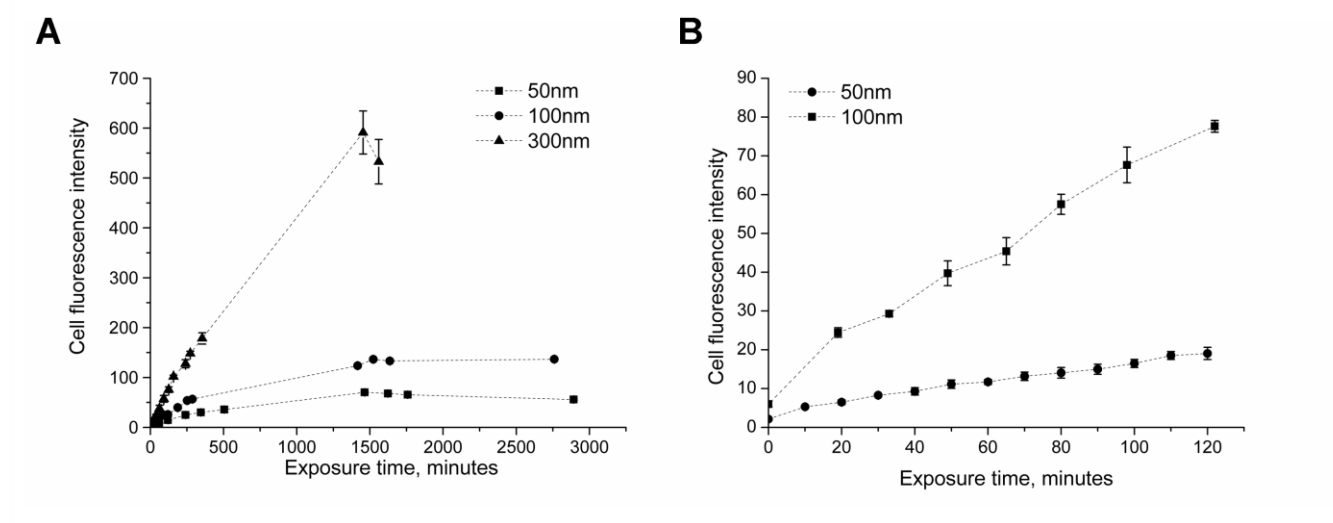


Figure S2(A): Time profile of uptake of 100 µg/ml of 50, 100 and 300 nm SiO₂ nanoparticles, as obtained by flow cytometry, without data normalisation. **(B)**: Two hour time profile of accumulation of 50nm SiO₂ nanoparticles presented at 100µg/ml to A549 cells, without data normalisation.

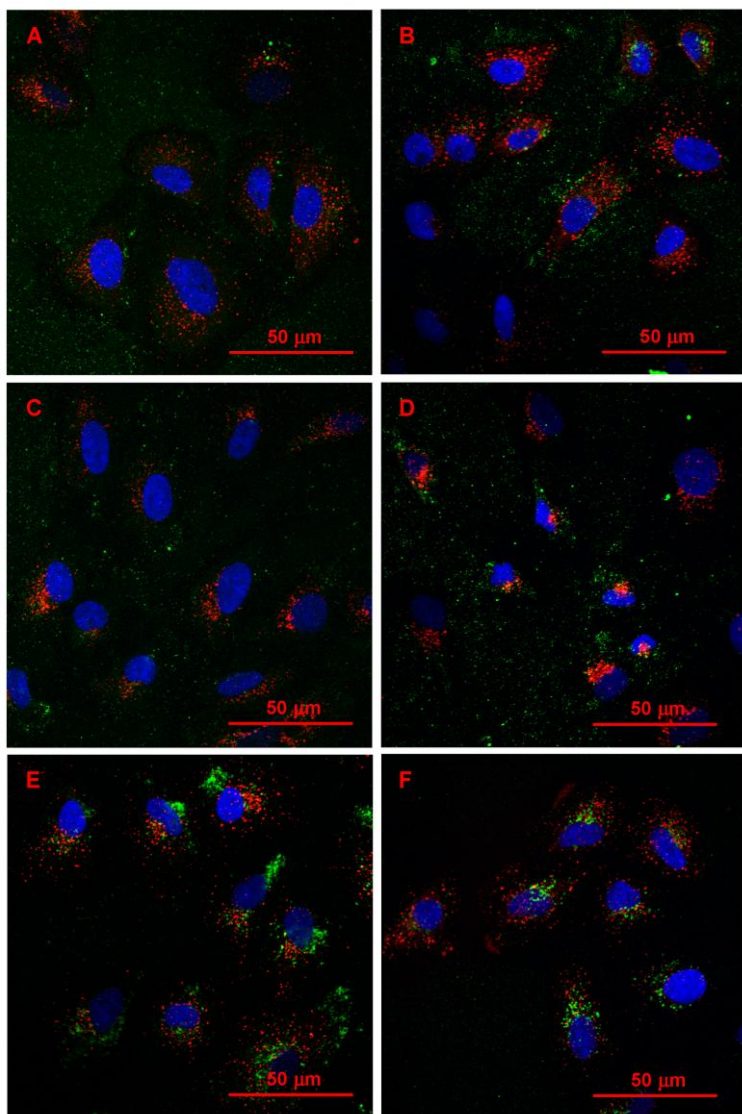


Figure S3: Confocal images of A549 cells after exposure for 2h to 100 µg/ml 50 or 100 nm green SiO₂ particles (A, C and B, D respectively). **(A-B):** Red - staining of early endosomes with EEA1 antibody. **(C-D):** Red - staining of lysosomes with LAMP1 antibody. (For both cases: secondary Alexa-647 antibody). Blue: DAPI stained nuclei. **(E-F):** Confocal images of A549 cells after exposure for 24h to 100µg/ml 50 or 100 nm green SiO₂ nanoparticles (E and F respectively). Red: staining of early endosomes with EEA1 antibody (secondary Alexa-647 antibody). Blue: DAPI stained nuclei.

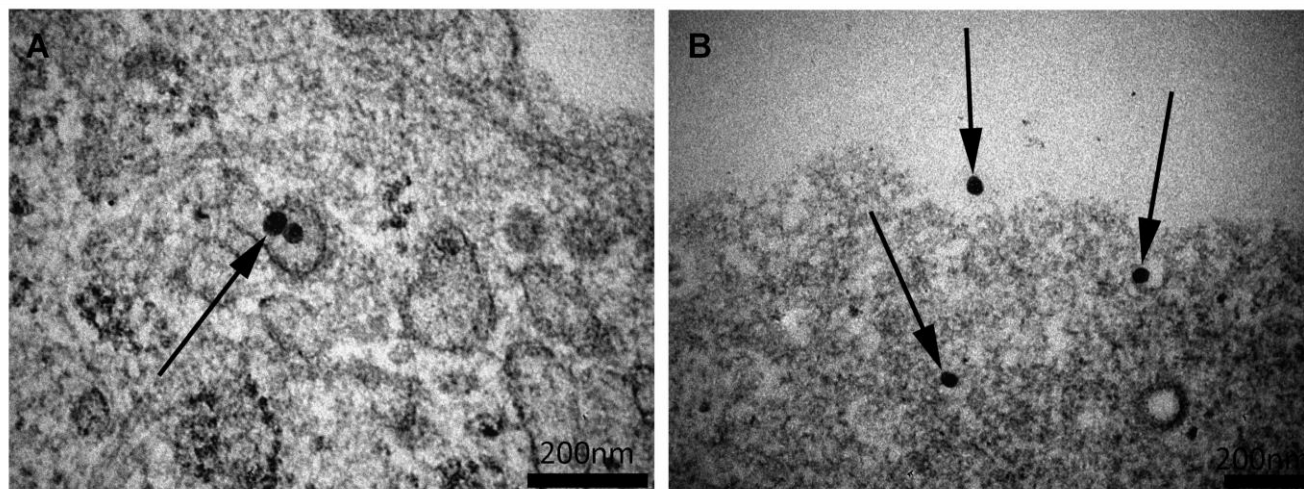


Figure S4: EM images of A549 cells exposed to 100 $\mu\text{g/ml}$ 50 nm green SiO_2 particles for 10 (A) and 20 (B) minutes, showing the early stages of uptake. Arrows indicate some of the nanoparticles.

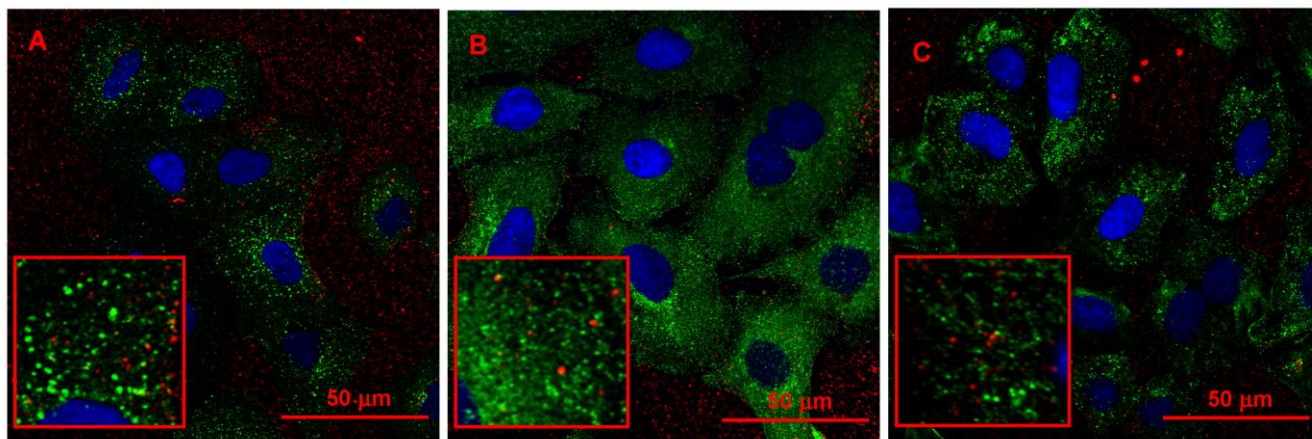


Figure S5: Confocal microscopy images of A549 cells after exposure for 30 minutes to 100µg/ml 50 nm red SiO₂ nanoparticles. Green: immunostaining of (A) EEA1; (B) clathrin heavy chain and (C) caveolin1 (secondary Alexa-488 antibody). Blue: DAPI stained nuclei. In the lower squares: enlarged view of a detail of the same images.

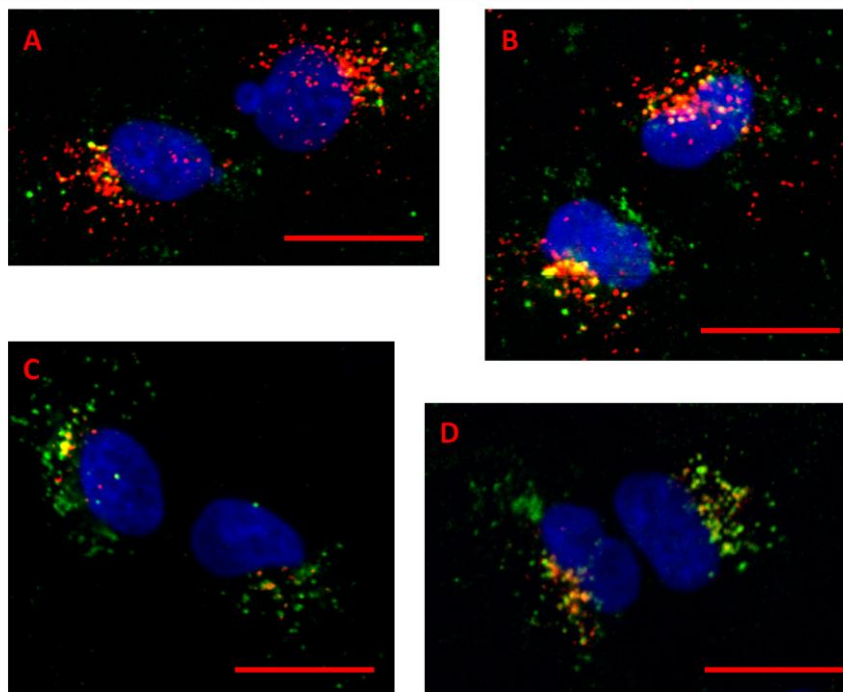


Figure S6: Confocal microscopy images of A549 cells at the final stage of cell division, showing the splitting of the nanoparticle load (green) between the two daughter cells. Cells were exposed for 24 hours to 100 μ g/ml 50 nm (A-C) or 100 nm (D) green SiO₂ nanoparticles. Red: immunostaining of LAMP1 (secondary Alexa-647 antibody). Blue: DAPI stained nuclei. Scale bar: 20 μ m.

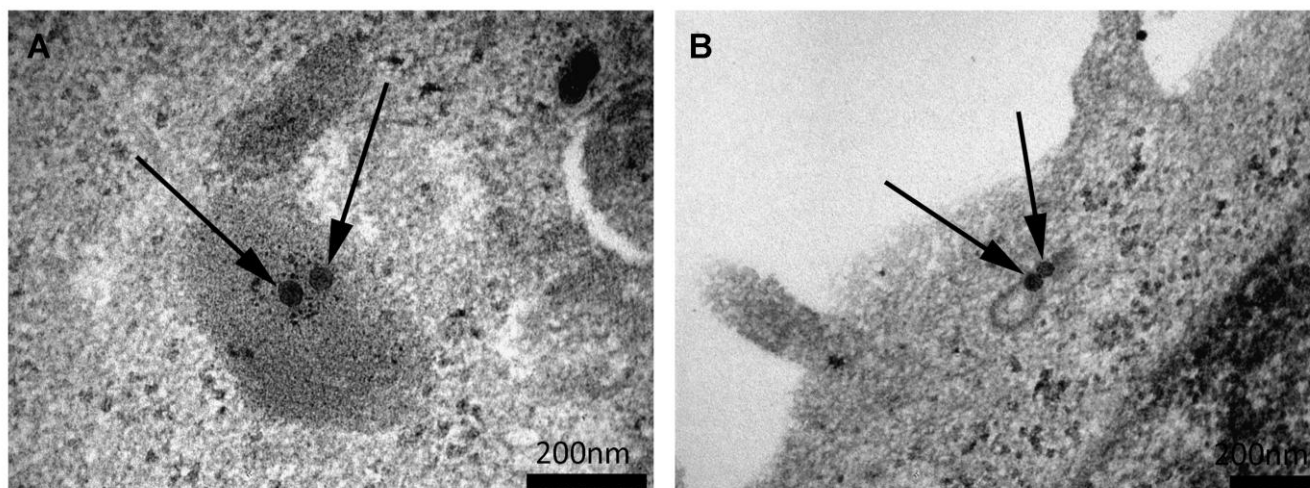


Figure S7: EM images of A549 cells exposed for 4h to 100 $\mu\text{g/ml}$ 50 nm green SiO_2 particles in the presence of 5mg/ml NaN_3 (**A**) or at 4 $^{\circ}\text{C}$ (**B**). Arrows indicate the location of nanoparticles in the cells.

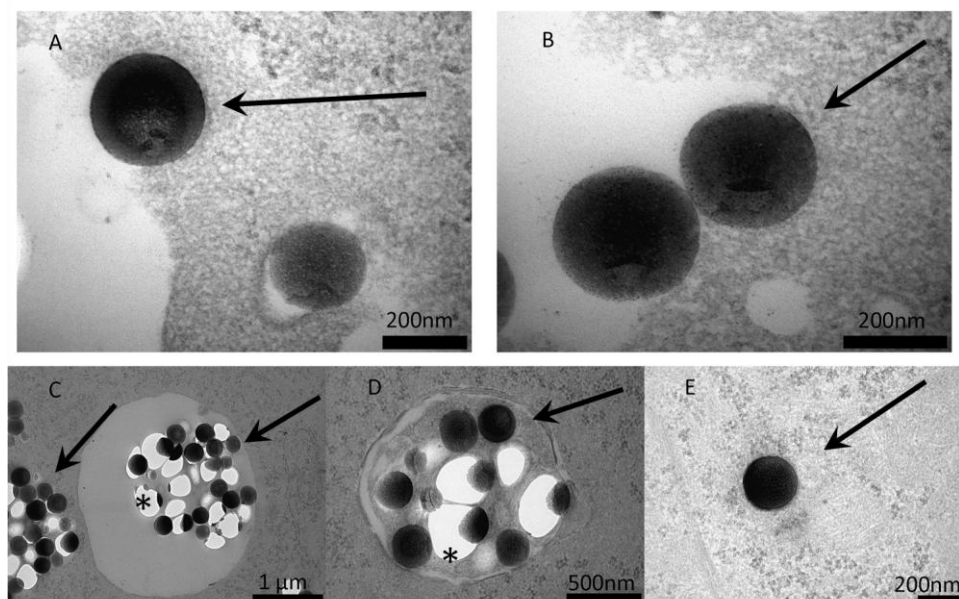


Figure S8: EM images of A549 cells exposed for 4h and 24h to 100 μ g/ml 300 nm green SiO₂ particles. **(A-B):** early events of uptake; **(C):** nanoparticles in an organelle, which might be a micropinosome; **(D):** nanoparticles in a lysosome; **(E):** a nanoparticle in the cytoplasm (no clear evidence of a vesicle surrounding it). Arrows indicate some of the nanoparticles in the cells. The holes (indicated by the symbol * in panels C and D) are a result of the 300nm particles being much larger than the 80 nm microtome slices, and likely represent areas where particles remained behind when the slice was taken.

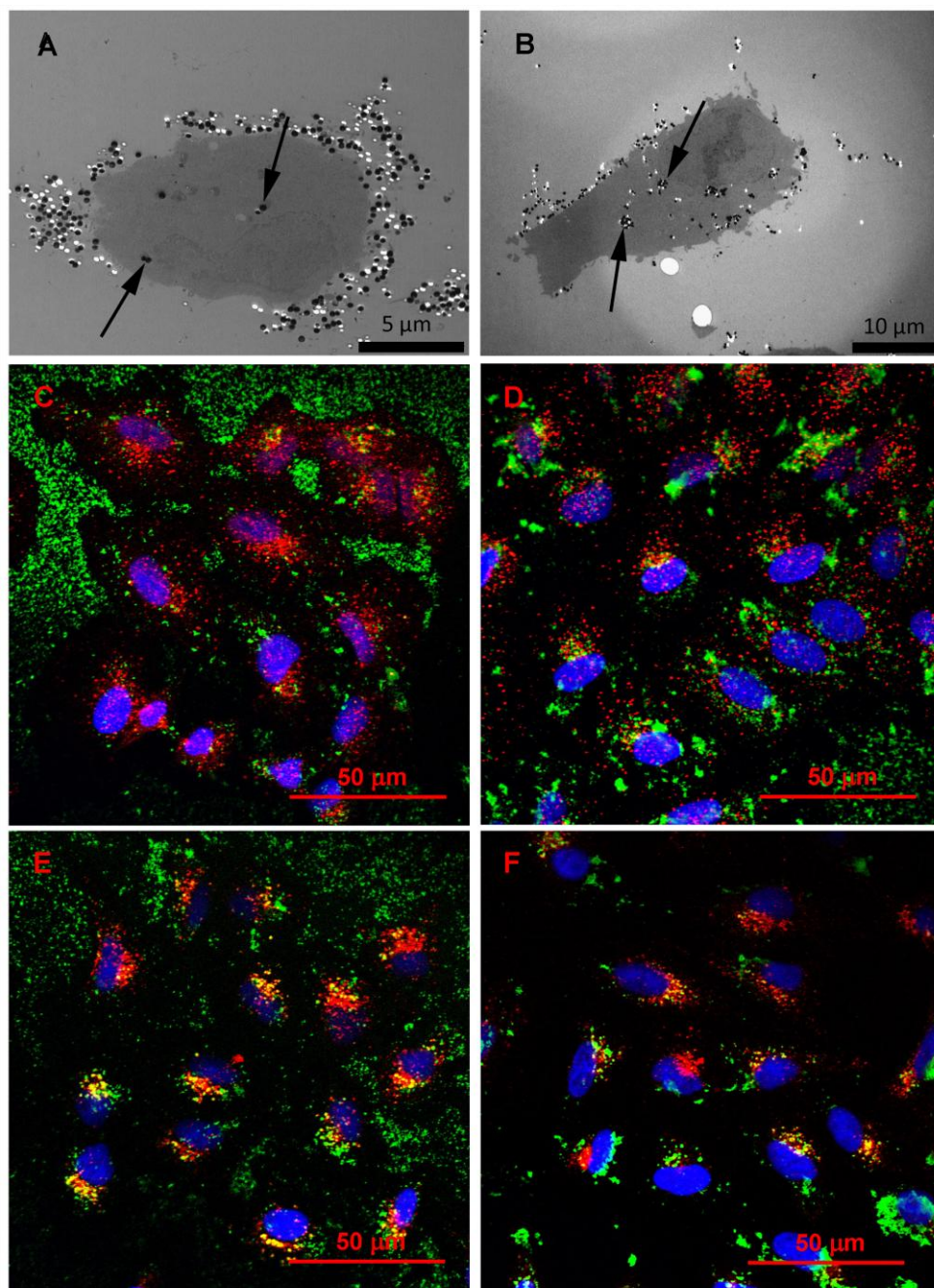


Figure S9: EM images of A549 cells exposed for 4h (**A**) and 24h (**B**) to 100μg/ml 300 nm green SiO₂ particles. Arrows indicate nanoparticles in the cells. (**C-F**): Confocal microscopy images of A549 cells after exposure to 100 μg/ml 300 nm green SiO₂ nanoparticles. C and D: 4h exposure; red: immunostaining of early endosomes with EEA1 antibody. E and F: 4h and 24h exposure; red: immunostaining of lysosomes with LAMP1 antibody (in both cases: secondary Alexa-647 antibody). Blue: DAPI stained nuclei.