Supplementary Materials for

Nanoparticle dynamics in macrophages investigated with a laboratory soft x-ray microscope

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This PDF file includes:

Figs. S1 to S4

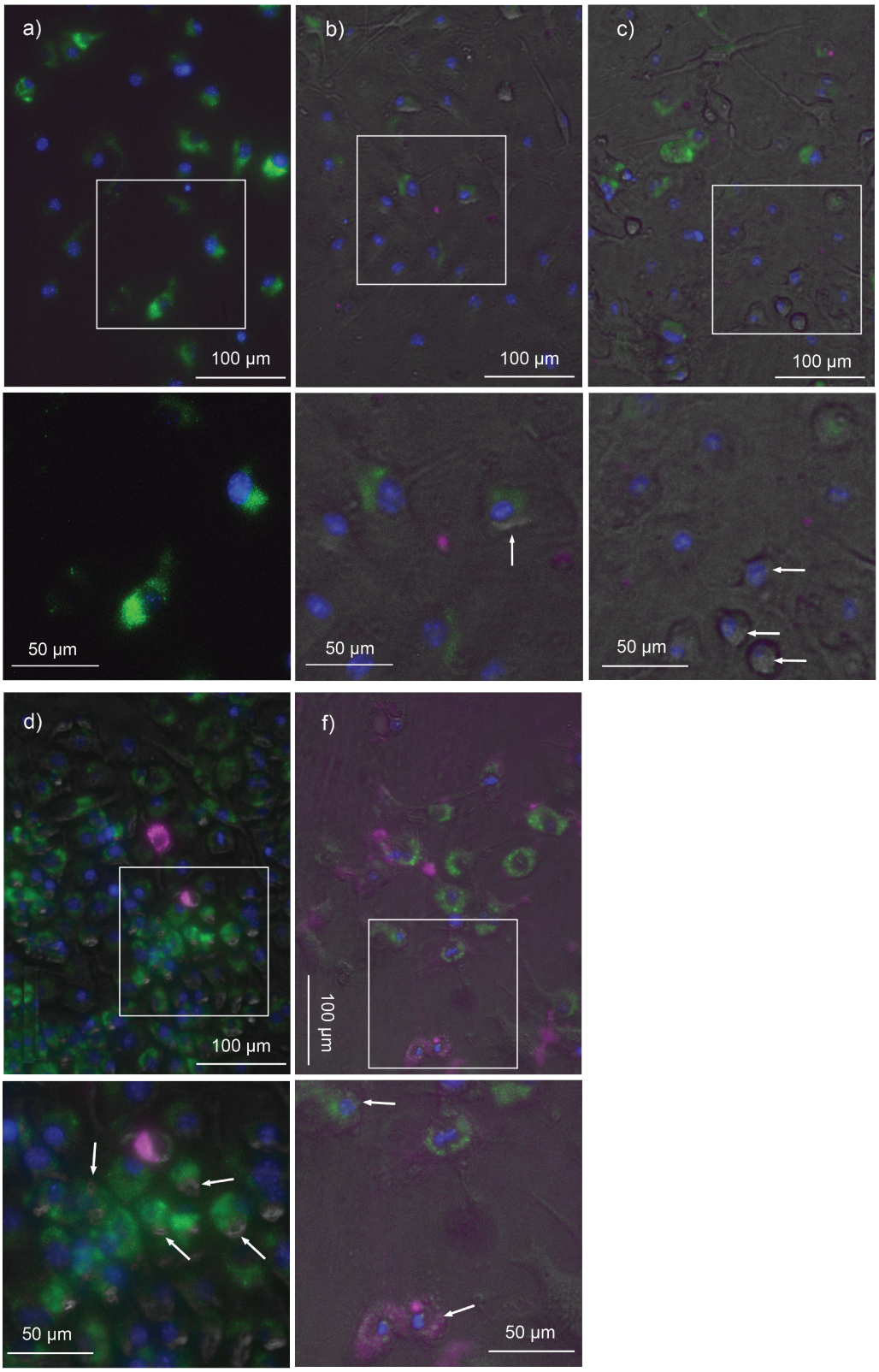

Fig. S1. Live fluorescence microscopy images of macrophages exposed to MoO_2-SiO_2 NPs at five different time points. Acidic compartments (lysosomes), nuclei, and MoO_2-SiO_2 NPs are visualized in green, blue, and magenta, respectively. **a)** Unexposed (control) cells show the normal lysosomal load in the absence of MoO_2-SiO_2 NP exposure. **b)** Macrophages exposed to MoO_2-SiO_2 NPs for 1 hour. At this time point, the NPs begin to be internalized by the macrophages, as shown by red color in the image (see magnified image of in **b**. Additionally, the white structure (overlapping green and magenta, indicated by an arrow) shows that MoO_2-SiO_2 NPs are co-localized with the lysosomes. **c)** Macrophages exposed to MoO_2-SiO_2 NPs for 2 hours. More MoO_2-SiO_2 NPs are found inside the lysosomes, and these structures are also marked by arrows in the image. **d)** Macrophages exposed to MoO_2-SiO_2 NPs for 4 hours. The white structures become more prominent at this time point, with ring-like structures also observed. **e)** Macrophages exposed to MoO_2-SiO_2 NPs for 24 hours.

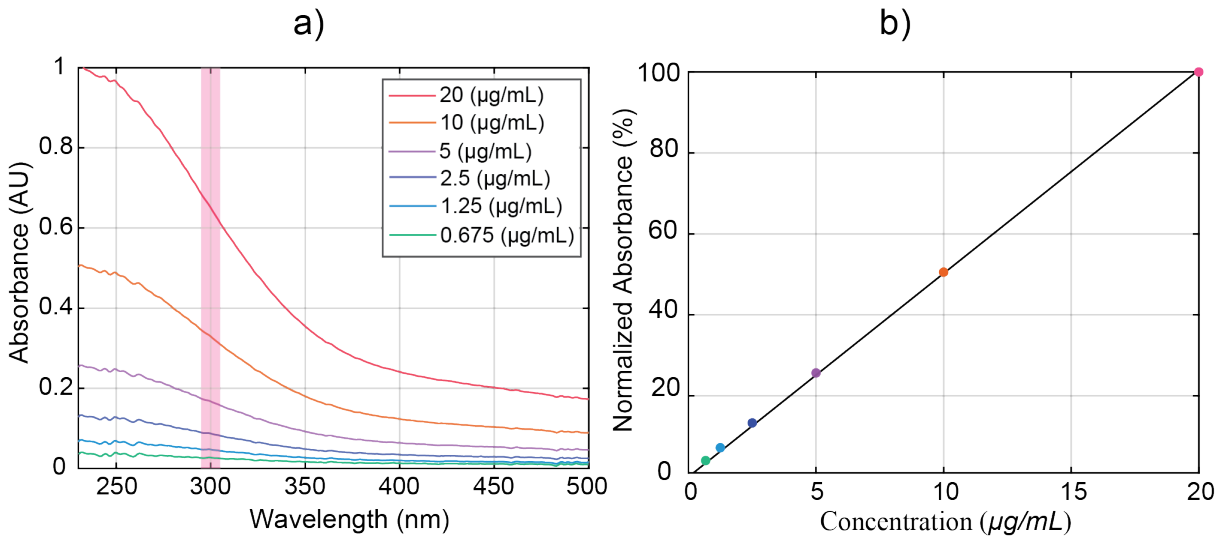

Fig. S2. a) Ultraviolet-visible (UV-Vis) spectra of six different concentrations of MoO_2-SiO_2 in DI water. **b)** Normalized absorbance values of the UV-Vis spectra for six different concentrations of MoO_2-SiO_2 at a wavelength range of 290-310 nm. The experiments were conducted at 37℃

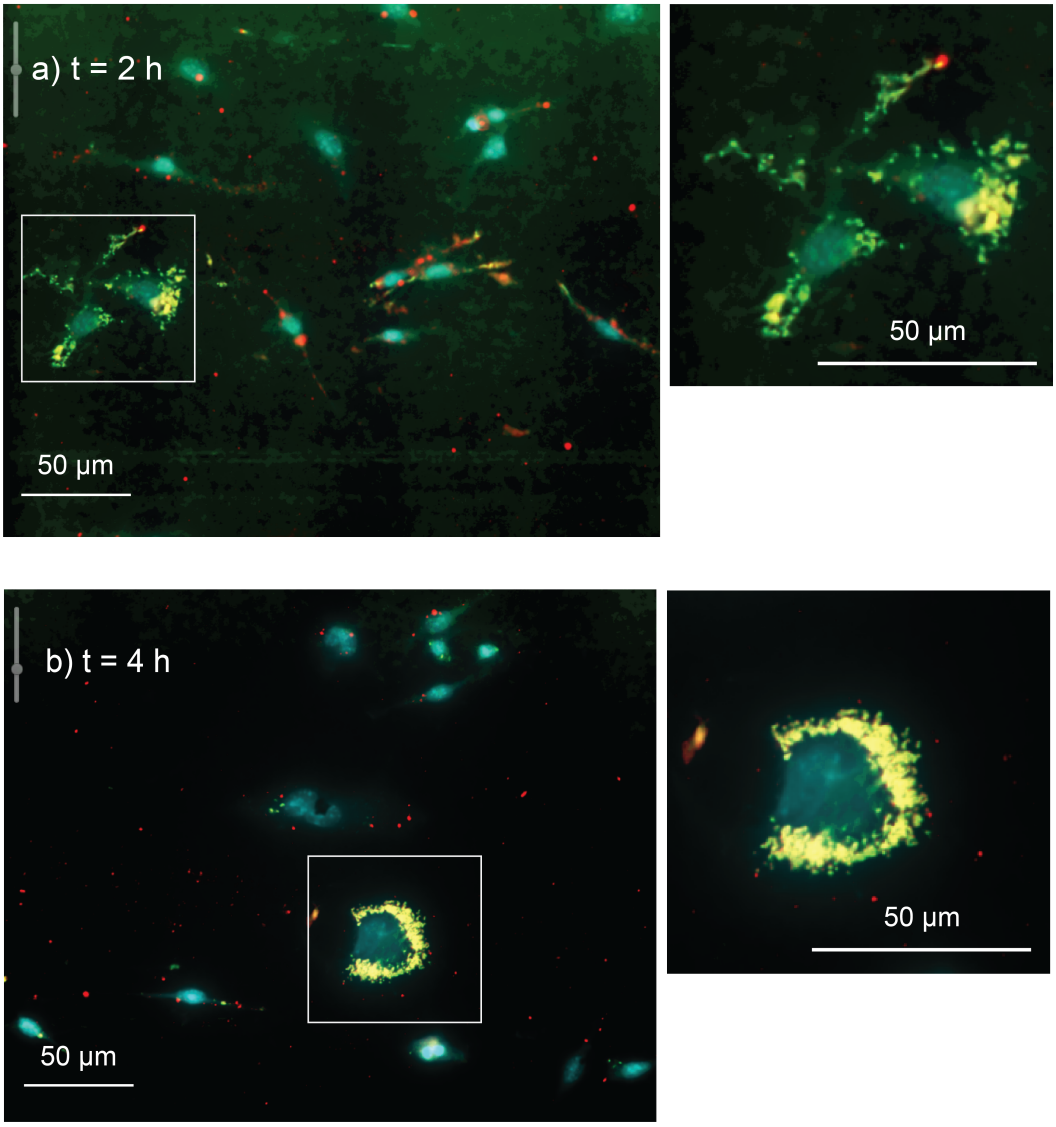

Fig. S3. Confocal microscopy images of macrophages exposed to MoO_2-SiO_2 nanoparticles (NPs) at different time points. The cells were stained with LC3B antibody to demonstrated autophagy in the samples. The LC3B antibody, nuclei, and MoO_2-SiO_2 NPs are visualized in yellow, blue, and red, respectively. Macrophages exposed to MoO_2-SiO_2 NPs for 2 hours and 4 hours are shown in **a)** and **b)**, respectively. The presence of LC3B antibody, indicated by yellow structures in both **a)** and **b)**, suggests that autophagy is occurring in the cells during these time points. However, this autophagy activity is observed only in some cells, indicating that phagocytosis is likely the primary pathway for NP uptake.

Figure S4 shows confocal microscopy images of macrophages undergoing autophagy. The samples were prepared by seeding murine liver macrophages (Sigma-Aldrich, Stockholm, Sweden) in chamber slides (Millipore, MA, USA) and allowing them to attach to the surface for 24 hours. Following this, MoO_2-SiO_2 NPs were added to the cells at a concentration of 100 μg/mL, and the cells were incubated in the presence of the NPs for for 2 hours and 4 hours. At the end of the incubation period, the medium contain NPs was removed, and the slides were washed three times with PBS. The cells were then fixed with 4% paraformaldehyde (PFA) for 15 minutes, followed by permeabilization with 0.1% Triton X-100 for 30 minutes. After permeabilization, the cells were blocked with 3% BSA and incubated with Alexa Fluor 488-conjugated LC3B antibody (Millipore, MA, USA) for 2 hours. After the incubation, unbound antibody was removed by washing the slides three times with PBS. Finally, the slides were mounted using ProLong™anti-fade mounting medium (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) before imaging.

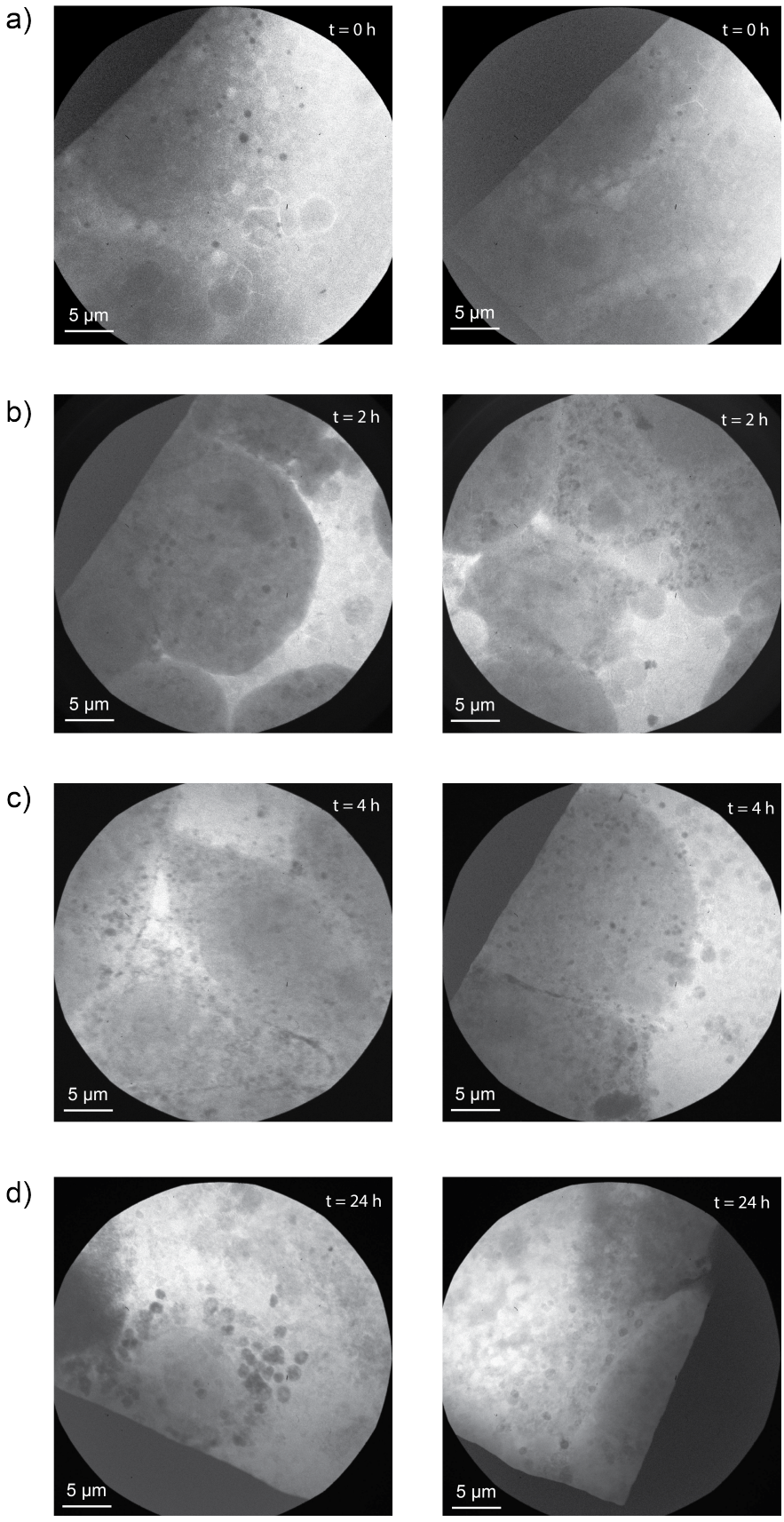

Fig. S4. X-ray micrographs of macrophages exposed to MoO_2-SiO_2 NPs at four different time points. **a)** Control cells (cells were not exposed to MoO_2-SiO_2 NPs). **b-d)** Macrophages were exposed to NPs for 2, 4, and 24 hours, respectively. The images show the progression of NP uptake over time, with clear evidence of NP distribution within the macrophages. Figure 2d (right) illustrates that some cells appear to have burst after 24 hours of exposure.