Supplementary Information

Aerosol Delivery of Functionalized Gold Nanoparticles Target and Activate Dendritic Cells in a 3D Lung Cellular Model

Kleanthis Fytianos,[1] Savvina Chortarea,[1] Laura Rodriguez-Lorenzo,[1] Fabian Blank,[3] Christophe von Garnier,[3] Alke Petri-Fink,[1,2] Barbara Rothen-Rutishauser*[1]

[1] Adolphe Merkle Institute, University of Fribourg, Fribourg, Switzerland
[2] Department of Chemistry, University of Fribourg, Fribourg,
[3]Switzerland Respiratory Medicine, Inselspital, University of Bern,
Bern, Switzerland
Corresponding author:
Barbara Rothen-Rutishauser
Adolphe Merkle Institute
Chemin des Verdiers 4
CH-1700 Fribourg
Switzerland
Phone: +41 26 300 9502
Email: barbara.rothen@unifr.ch

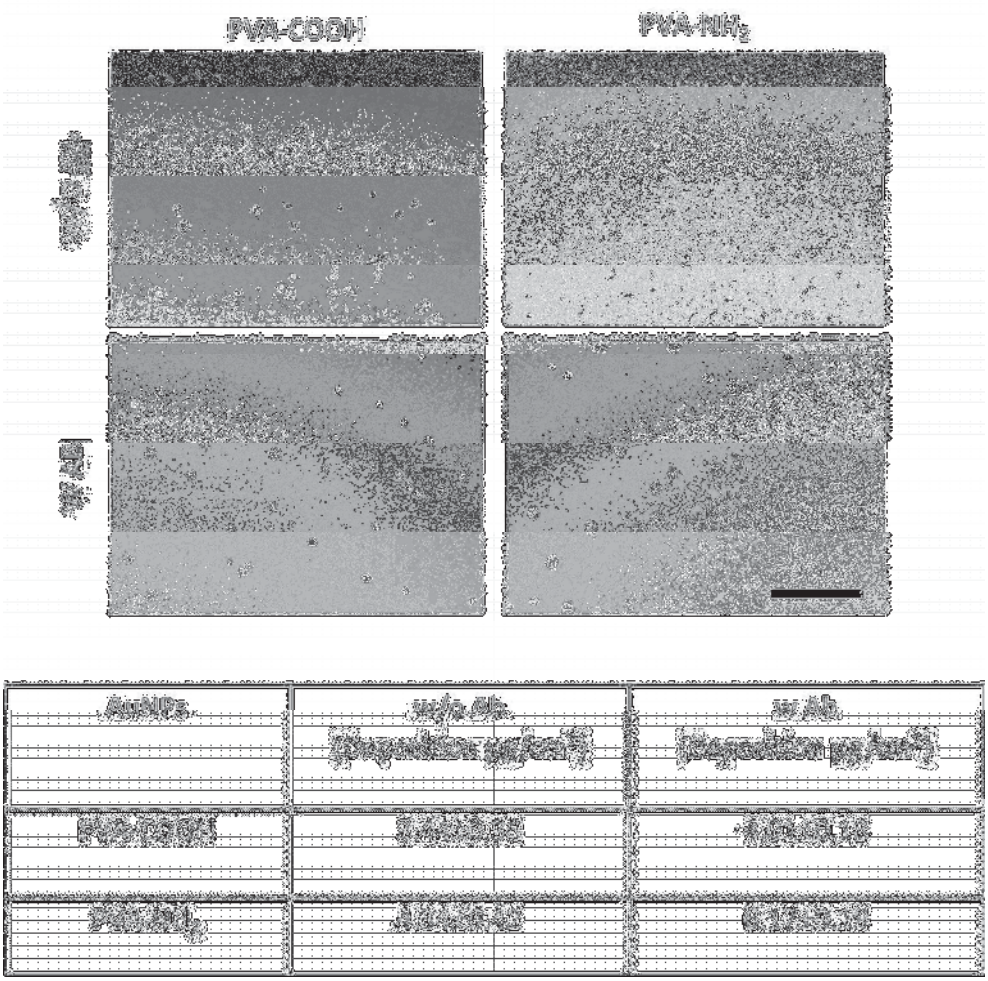

Figure S1: TEM images and QCM values of the aerosolized AuNPs. TEM images of the AuNPs deposited onto TEM grids after aerosolization. TEM grids were placed inside the exposure chamber of the ALICE and left to dry after the aerosolization process. For the QCM values (expressed in μg/cm^2), three different aerosolization steps were performed and the results are expressed as mean±SD (n=3).

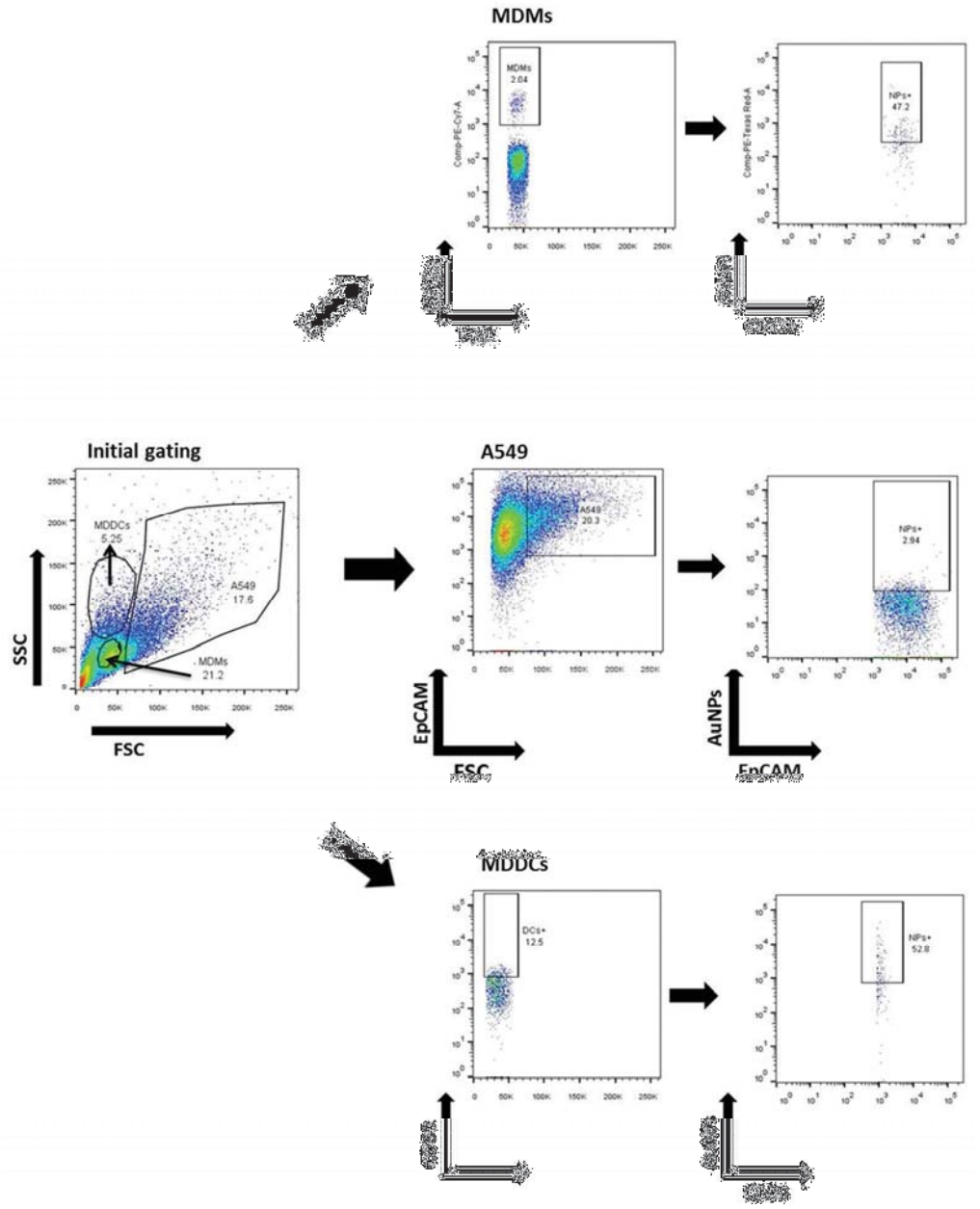

Figure S2: Cellular uptake gating strategy. Representative dot plots show the gating strategy that was applied for the cellular uptake of AuNPs in the 3CCCM, for all the three different cell populations sperately (A: A549 cells; B: MDDCs; C: MDMs). First, the cell population was immunophenotypically identified by specific surface marker expression. Then, the MFI of ATTO590 of this population was calculated. $MFI_{ATTO590}$ is compared with the $MFI_{ATTO590}$ of the negative control (cells exposed to aerosolized H_2O). FSC: forward scattering; SSC: side scattering; CD11a: MDMs marker, cluster of differentiation 11a, conjugated to PE-Cy7 fluorochrome; EpCAM: epithelial cells marker, epithelial cell adhesion molecule, conjugated to APC fluorochrome; CD80: dendritic cells marker, cluster of differentiation 80, conjugated to FITC fluorochrome; AuNPs: AuNPs conjugated with ATTO590 fluorochrome.

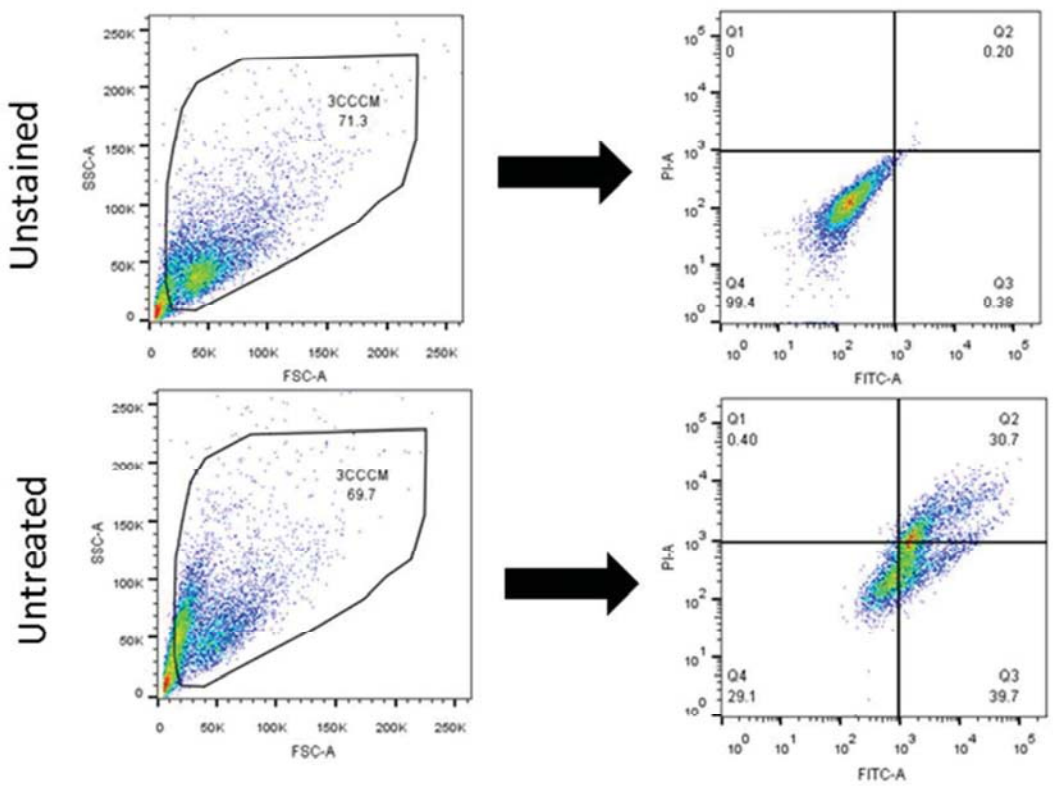

Figure S3: Annexin V/PI gating strategy. Representative dot plots and gating strategy for measuring healthy (quadrant Q4), early (quadrant Q3) and late apoptotic (quadrant Q2) as well as dead cells (quadrant Q1) of the 3CCCM. Cells exposed to aerosolized H_2O were used as negative control. Cells exposed to 2 mM camptothecin for 24 h as control for apoptosis and cells exposed to –80 ^{o}C / 30 min as positive control for cell death. FSC: forward scattering; SSC: side scattering; FITC: annexin-V FITC expression; PI: propidium iodide expression.

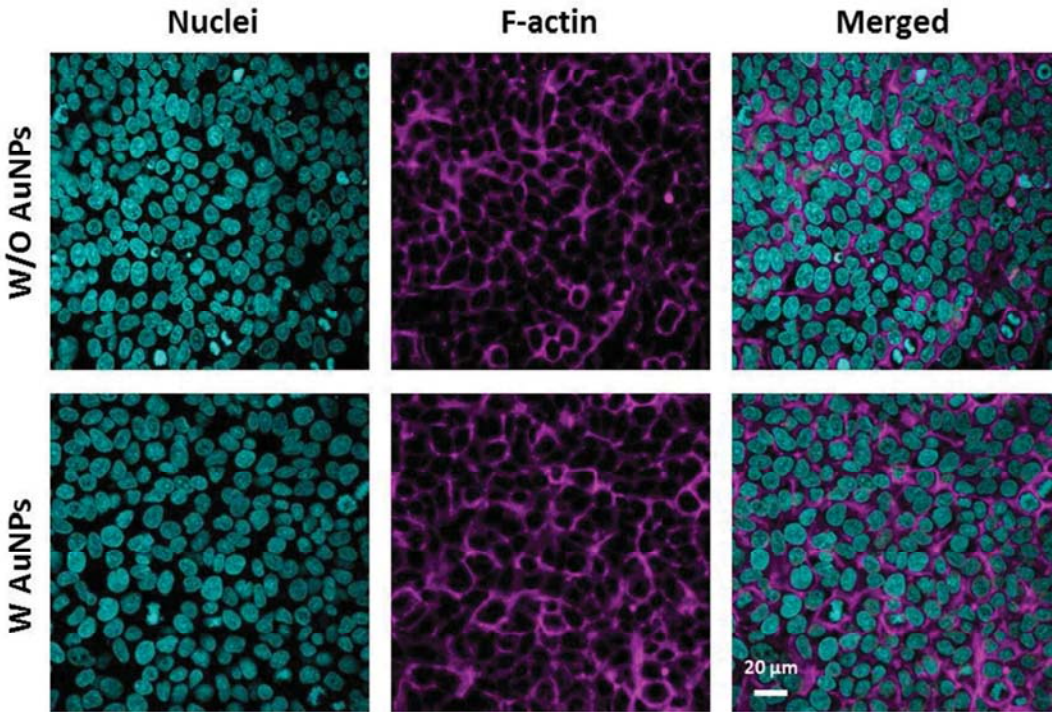

Figure S4. LSM images. In order to ensure epithelial monolayer integrity and tightness, LSM images were obtained. Representative images are shown and no gaps were observed. Furthermore, in the presence of PVA-NH_2-AuNPs (lower panel) there is no effect on the epithelial layer morphology. Samples were fixed with 3% PFA and stained with Phalloidin-Alexa488 (purple) plus DAPI (light blue), washed and mounted in glycergel. Samples were examined microscopically under a Zeiss LSM710.

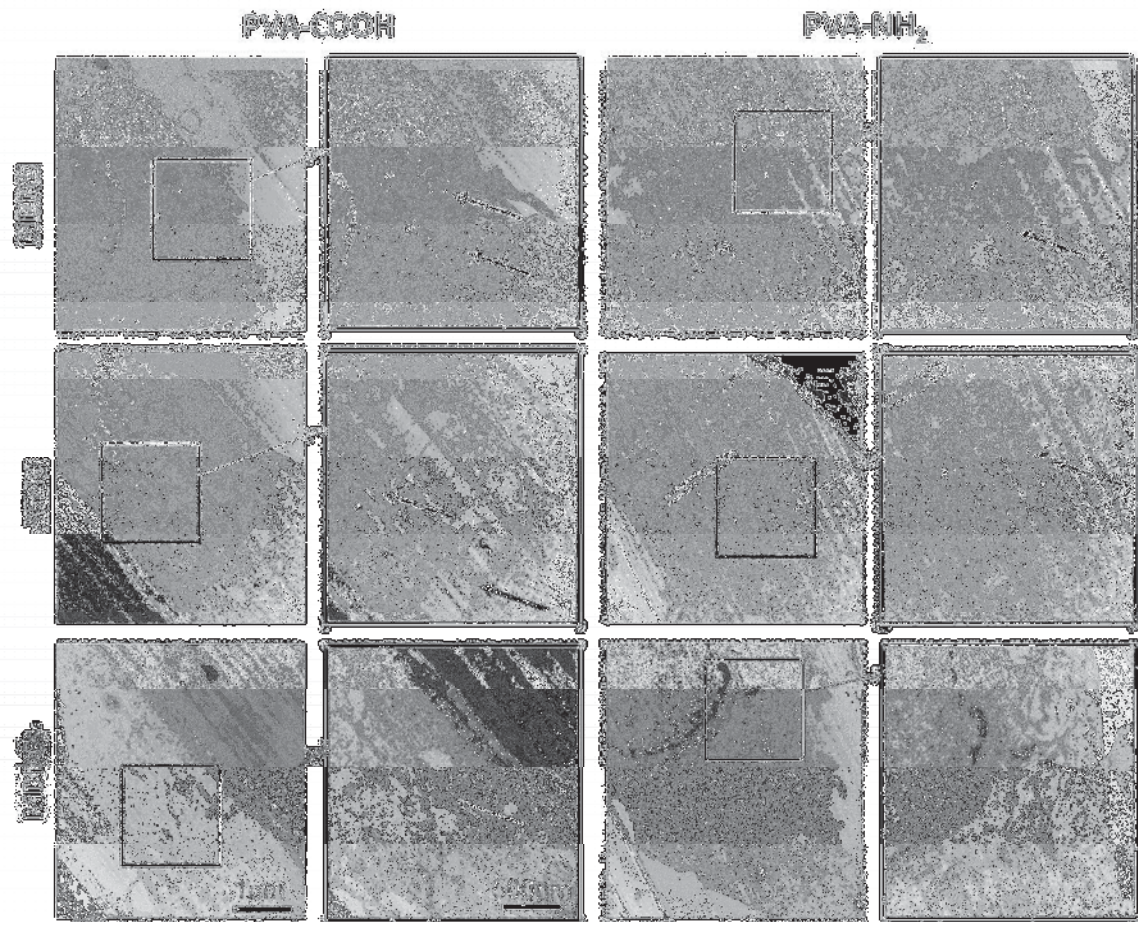

Figure S5. TEM images. TEM images of internalized AuNPs without DC-SIGN Abs in MDMs, A549 (apical side of the 3D co-cultures) and MDDCs (basolateral side of the 3D co-cultures). (Left panel: PVA-COOH AuNPs. Right panel: PVA-NH_2-AuNPs. AuNPs were found inside cellular vesicles, 24 h after exposure using the ALICE system. The boxes (overview) and the zoomed images show the intracellular particles (arrows) (samples without lead citrate and uranyl acetate staining).