Supporting Information

Applicability of avidin protein coated mesoporous silica nanoparticles as drug carriers in the lung

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Synthesis of MSN-SH_{in}-$NH_{2,out}$. Core-shell functionalized MSNs were synthesized using a similar method, as previously reported.[1] In brief, a mixture of tetraethyl orthosilicate (TEOS, 1.63 g, 7.82 mmol), mercaptopropyl triethoxysilane (MPTES, 112 mg, 0.48 mmol) and triethanolamine (TEA, 14.3 g, 95.6 mmol) was heated under static conditions at 90 °C for 20 min in a polypropylene reactor. Then, a solution of cetyltrimethylammonium chloride (CTAC, 2.41 mL, 1.83 mmol, 25 wt% in H_2O) and ammonium fluoride (NH_4F, 100 mg, 2.70 mmol) in H_2O (21.7 g, 1.21 mmol) was preheated to 60 °C, and added to the TEOS solution rapidly. The reaction mixture was stirred vigorously (700 rpm) for 20 min while cooling down to room temperature. Subsequently, TEOS (138.2 mg, 0.922 mmol) was added in four equal increments every three minutes. After another 30 min of stirring at room temperature, TEOS (19.3 mg, 92.5 µmol) and aminopropyl triethoxysilane (APTES, 20.5 mg, 92.5 µmol) were added to the reaction. The resulting mixture was then allowed to stir at room temperature overnight. After addition of ethanol (100 mL), the MSNs were collected by centrifugation (19,000 rpm, 43,146 rcf, for 20 min) and re-dispersed in absolute ethanol. The template extraction was performed by heating the MSN suspension under reflux (90 °C, oil bath temperature) for 45 min in an ethanolic solution (100 mL) containing ammonium nitrate (NH_4NO_3, 2 g), followed by 45 min heating under reflux in a solution of concentrated hydrochloric acid (HCl, 10 mL) and absolute ethanol (90 mL). The mesoporous silica nanoparticles were collected by centrifugation and washed with absolute ethanol after each extraction step.

Heptapeptide functionalisation (MSN-HP).[2] Bio-PLLMWSR (HP-biotin, 90.1 %, 5.0 mg, 4.0 µmol) was dissolved in 100 µL DMSO. The solution was diluted by addition of 400 µL H_2O. Then, EDC (0.8 mg, 5.2 µmol) was added, and the reaction mixture was stirred for 5 min at room temperature. Subsequently, sulfoNHS (1 mg, 5.0 µmol) was added, and the reaction mixture was stirred for another 5 min at room temperature. This mixture was added to a suspension containing 50 mg of MSN-$NH_{2\,OUT}$ in a total volume of 8 mL (EtOH:H_2O 1:1). The resulting mixture was then allowed to stir at room temperature overnight. The MSNs were thoroughly washed by EtOH and H_2O (3 times) and finally collected by centrifugation (19,000 rpm, 43,146 rcf, 20 min). The HP-biotin functionalized MSNs were stored as colloidal suspension in absolute ethanol.

Avidin coating (MSN-AVI). 1 mg of MSN-HP (in 500 µL HBSS buffer) was added to 500 µL HBSS buffer containing 1 mg of avidin. The solution was mixed by 5 sec of vortexing and allowed to react for 30 min under static conditions at room temperature. The resulting suspension was then centrifuged (5000 rpm, 2200 rcf, 4 min, 15 °C) and washed three times with HBSS buffer. The particles were finally re-dispersed in HBSS buffer and used *in vitro* and *in vivo* studies.

For Atto633 labeling, 1 mg MSNs in 1 mL ethanol were reacted with 1 uL Atto633Mal for 12 h. Afterwards the particles were washed three times with ethanol and resuspended in 1 mL HBSS buffer.

Characterisation of MSN-SH_{in}-$NH_{2,out}$ and the stepwise addition of Avidin.

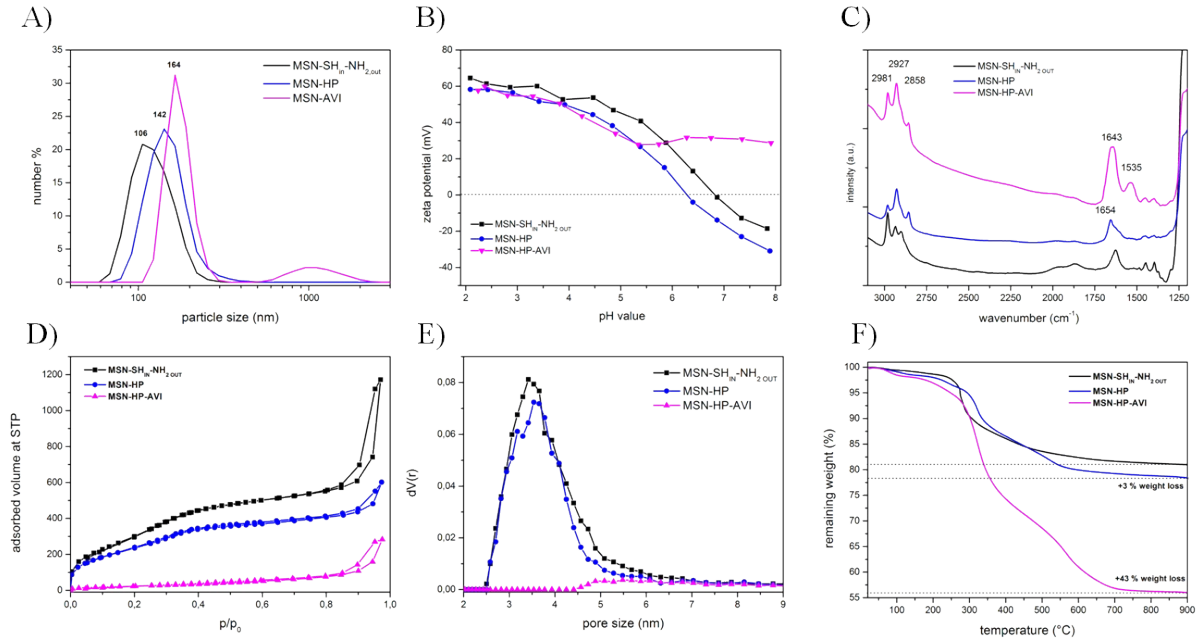

Figure S1. Characterisation of MSN-SH_{in}-$NH_{2,out}$, MSN-HP and MSN-AVI particles A) DLS measurements of functionalised MSNs in ethanol. B) Zeta potential of the different functionalisation stages. C) A stepwise attachment of the peptide linker followed by the addition of avidin on the outer periphery can be visualised by infrared spectroscopy. D) Nitrogen sorption isotherms show mesoporous pore structure and huge surface areas for MSNs. E) After addition of avidin the pores are sealed and no pore size distribution is visible. F) Thermogravimetric analysis (TGA) data show increasing amount of organic residues in different functionalisation steps.

Non-functionalised MSNs (nonMSN). Non-MSN were synthesized using a similar method, as previously reported.[1] In brief, a mixture of tetraethyl orthosilicate (TEOS, 1.92 g, 9.22 mmol) and triethanolamine (TEA, 14.3 g, 95.6 mmol) was heated under static conditions at 90 °C for 20 min in a polypropylene reactor. Then, a solution of cetyltrimethylammonium chloride (CTAC, 2.41 mL, 1.83 mmol, 25 wt% in H_2O) and ammonium fluoride (NH_4F, 100 mg, 2.70 mmol) in H_2O (21.7 g, 1.21 mmol) was preheated to 60 °C, and added to the TEOS solution rapidly. The reaction mixture was stirred vigorously (700 rpm) for 20 min while cooling down to room temperature. Subsequently, the resulting mixture was then allowed to stir at room temperature overnight. After addition of ethanol (100 mL), the MSNs were collected by centrifugation (19,000 rpm, 43,146 rcf, for 20 min) and re-dispersed in absolute ethanol. The template extraction was performed by heating the MSN suspension under reflux (90 °C, oil bath temperature) for 45 min in an ethanolic solution (100 mL) containing ammonium nitrate (NH_4NO_3, 2 g), followed by 45 min heating under reflux in a solution of concentrated hydrochloric acid (HCl, 10 mL) and absolute ethanol (90 mL). The non-functionalized mesoporous silica nanoparticles were collected by centrifugation and washed with absolute ethanol after each extraction step.

Characterisation of nonMSN.

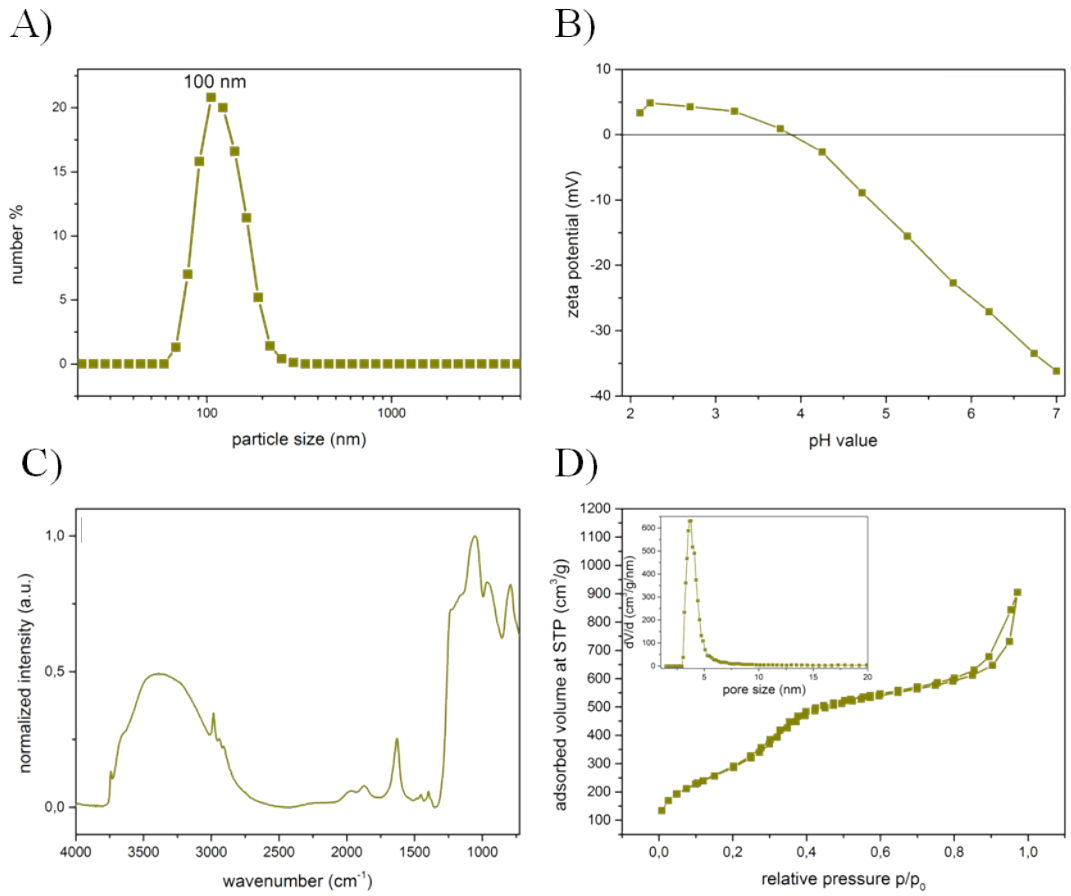

Figure S2. A) DLS measurements of non-functionalized MSNs (nonMSN) in ethanol show a particle size of about 100 nm. B) Zeta potential of nonMSN with an isoelectric point of 4. C) IR spectrum. D) Nitrogen sorption isotherms show mesoporous pore structure (pore size 3.7 nm) and huge surface areas (1050 m^2/g) for nonMSNs.

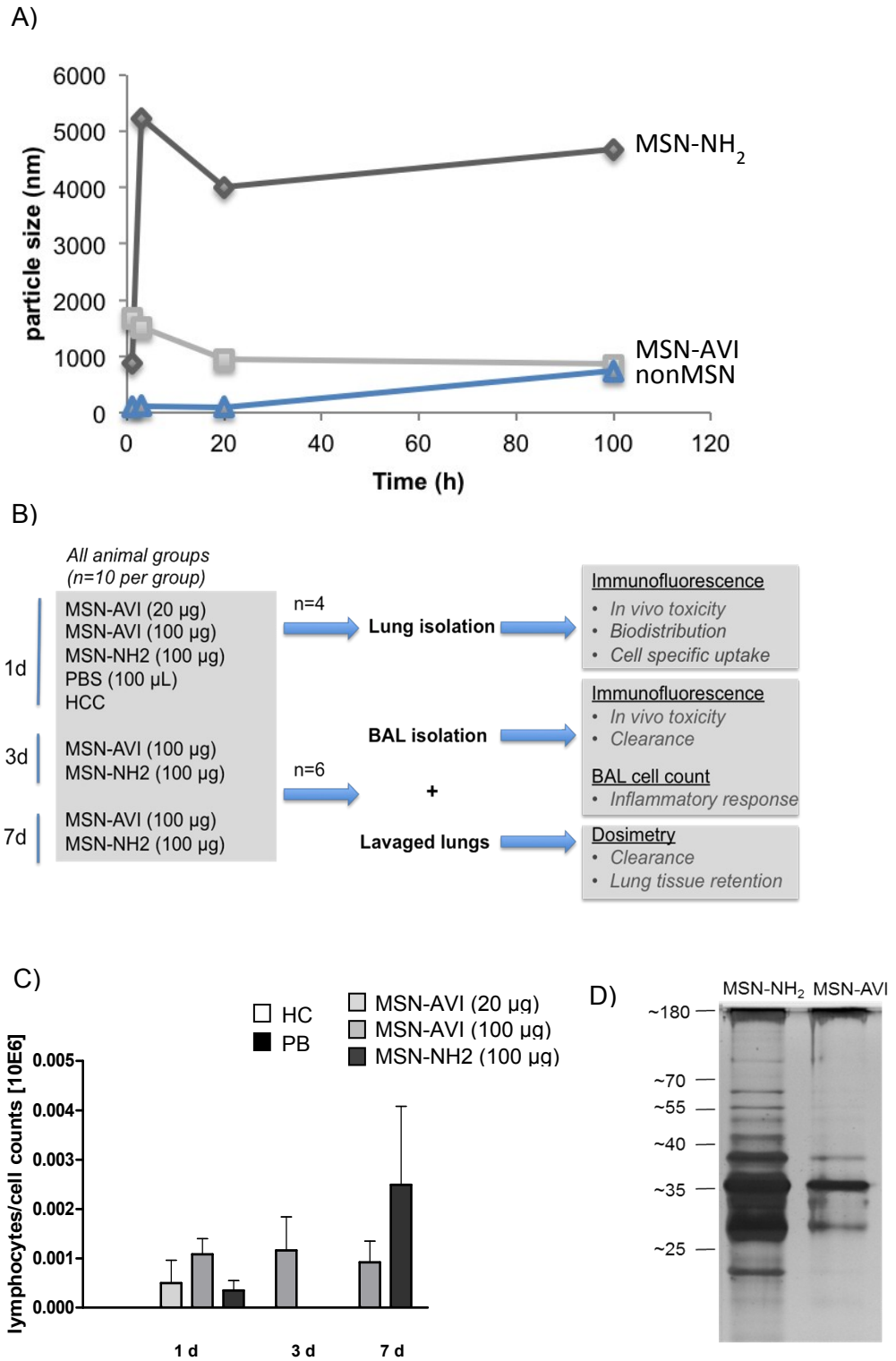

Figure S3. A) DLS measurements of MSN-NH_2, nonMSN and MSN-AVI suspensions over time. Data show that MSN-NH_2 particles suspended in cell culture buffer with 10% FCS, form larger agglomerates compared to MSN-AVI and nonMSN in time. B) Experimental set-up of animal work, C) Lymphocyte cell count in bronchoalveolar lavage (BAL). Lymphocyte counts are overall close to the detection limit (1% of total BAL cells) and no significant changes ($p<0.05$) have been observed between MSN treated and control mice (n=6). D) Protein corona formation on MSN-AVI and MSN-NH_2: Native gel analysis of MSN-NH_2 and MSN-AVI incubated with mouse BALF fluid.

Table S1. The inflammatory effects detected by BAL cell differentiation, 24 h after instillation of 100 µg of MSN-NH2 or nonMSN, compared to 100 µL PBS (vehicle control) and untreated HCC (home cage control) .

	N (mice)	Total cell count x 10^6	Macrophage cell count x 10^3	Multinucleated macrophages cell count x 10^3	PMN cell count x 10^3 (%)	Lymphocyte cell count x 10^3 (%)
[a] **HCC**	2	0.36 ± 0.00	356.2 ± 4.4	0.0 ± 0.0	0.0 ± 0.0 (0)	0.0 ± 0.0
[b] **PBS**	6	0.29 ± 0.03	290.6 ± 29.5	0.0 ± 0.0	3.2 ± 1.6 (0)	0.0 ± 0.0
MSN-NH2	6	0.33 ± 0.03	135.0 ± 15.1	0.2 ± 0.1	191.5 ± 13.9 (59)	0.4 ± 0.2
nonMSN	6	0.35 ± 0.03	308.7 ± 31.4	0.9 ± 0.4	37.33 ± 3.0 (11)	0.8 ± 0.2

[a] HCC = home cage control animals (non-treated mice)
[b] PBS = 100 µL PBS instilled mice (vehicle/sham control)

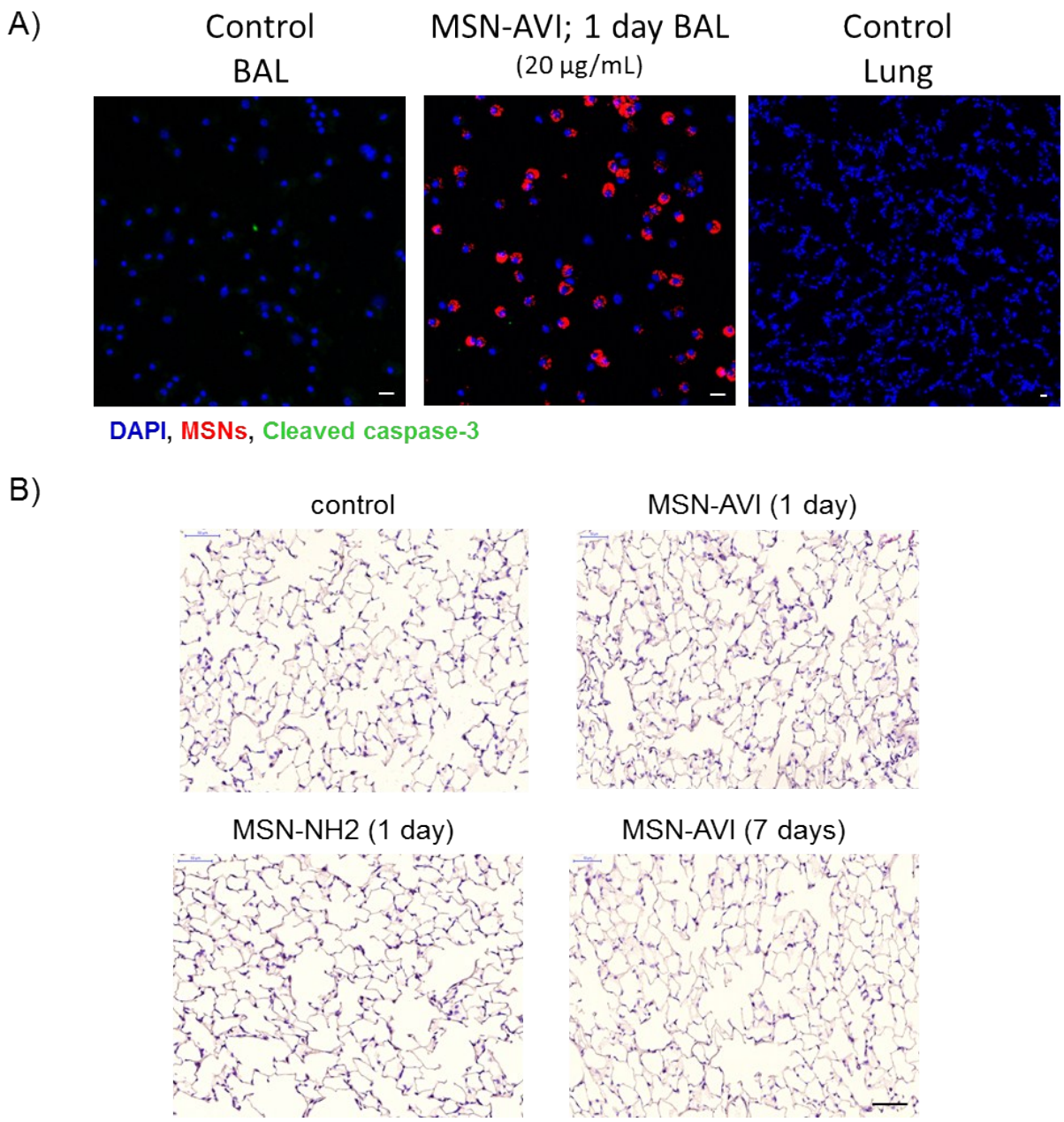

Figure S4. A) Toxicity of BAL recovered cells of PBS control mice (left); MSN-AVI in Balb/c mice exposed to 20 µg for 1 day in BAL (middle) and lung tissue of PBS control mice (right). Cell nuclei are shown in blue (DAPI), ATTO 633 labeled MSNs are shown in red. Scale bar is 20 µm. B) Hematoxylin and eosin staining of lung sections from mice exposed to MSN-AVI or MSN-NH_2 particles for 1 or 7 days. Scale bar is 50 µm.

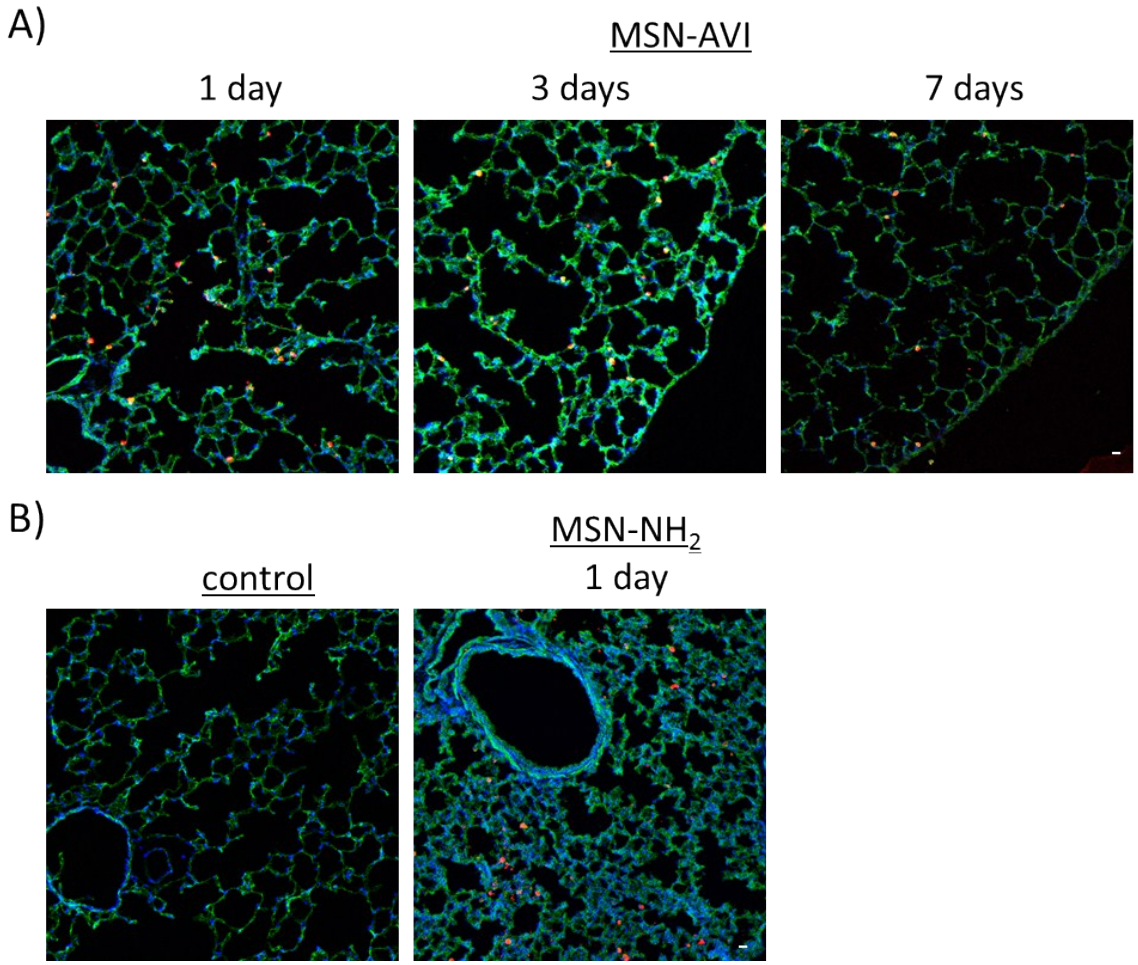

Figure S5. Nanoparticle distribution in lung cryo-slices of Balb/c mice exposed to 100 µg MSN-AVI after 1, 3, and 7 days. B) PBS control and nanoparticle distribution in lung cryo-slices after 1 day exposure to 100 µg MSN-NH2. Cell nuclei are shown in blue (DAPI), ATTO 633 labeled MSNs are shown in red, cell actin staining (phalloidin) is shown in green. Images are representative for n=4 animals. Scale bar is 20 µm.

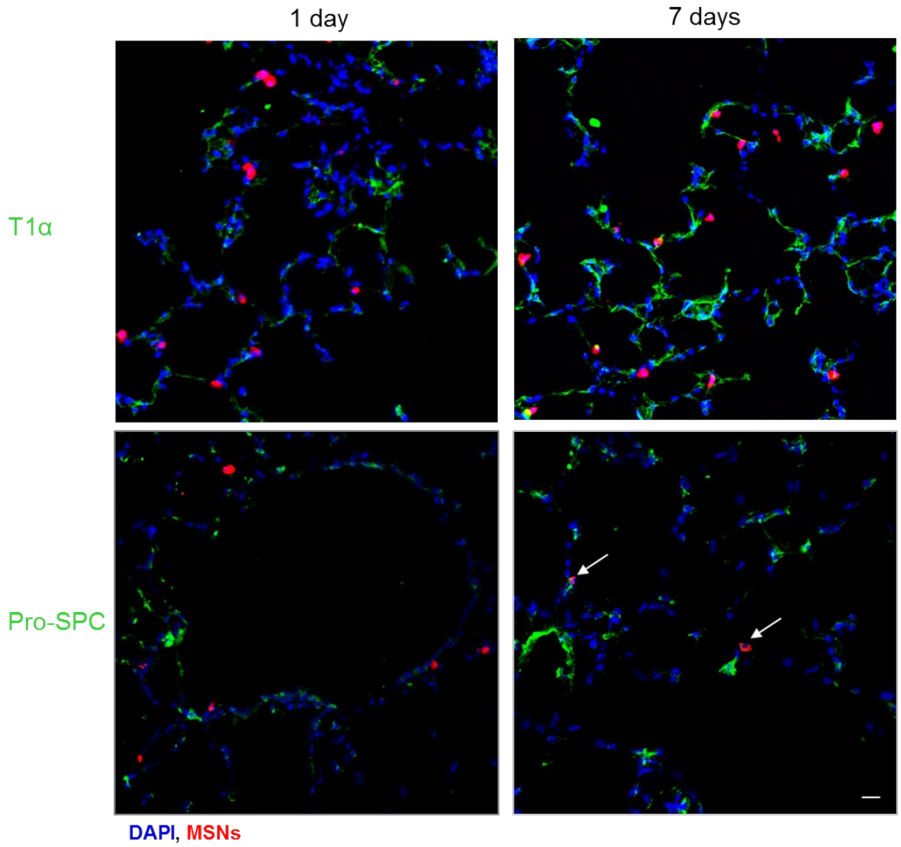

Figure S6. Confocal microscopy images (20x objective) of cryo-slices of Balb/c mice exposed to MSN-AVI for 1 or 7 days, co-stained with alveolar epithelial cell type 1 marker (T1α, green, top) or with alveolar epithelial cell type 2 marker (pro-SPC, green, bottom). Cell nuclei are shown in blue (DAPI) and ATTO 633 labeled MSNs are shown in red. Scale bar is 20 µm.

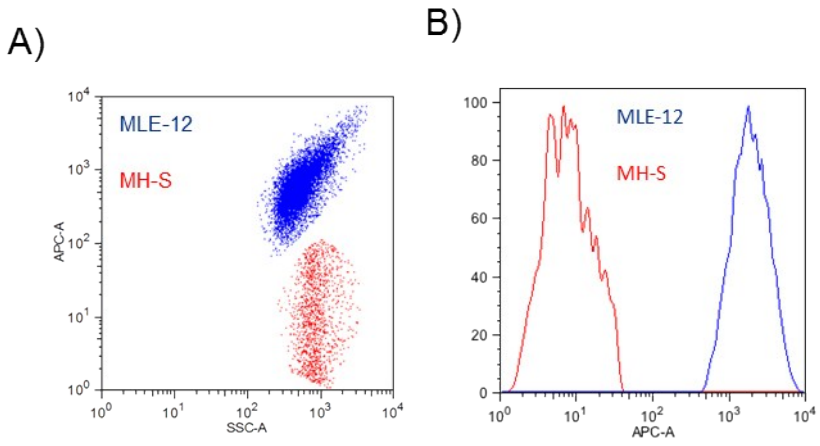

Figure S7. DiD selective labeling of MLE-12 cells in a MLE-12/ MH-S co-culture set-up. A) dot plot of SSC against APC-A channel, B) histogram of co-culture in APC-A channel.

(1) Cauda, V.; Schlossbauer, A.; Kecht, J.; Zürner, A.; Bein, T. *J. Am. Chem. Soc.* **2009**, *131*, 11361.
(2) van Rijt, S. H.; Bölükbas, D. A.; Argyo, C.; Datz, S.; Lindner, M.; Eickelberg, O.; Königshoff, M.; Bein, T.; Meiners, S. *ACS Nano* **2015**, *9*, 2377.