Use of EpiAlveolar™ Lung Model to Predict Fibrotic Potential of Multi-Walled Carbon Nanotubes

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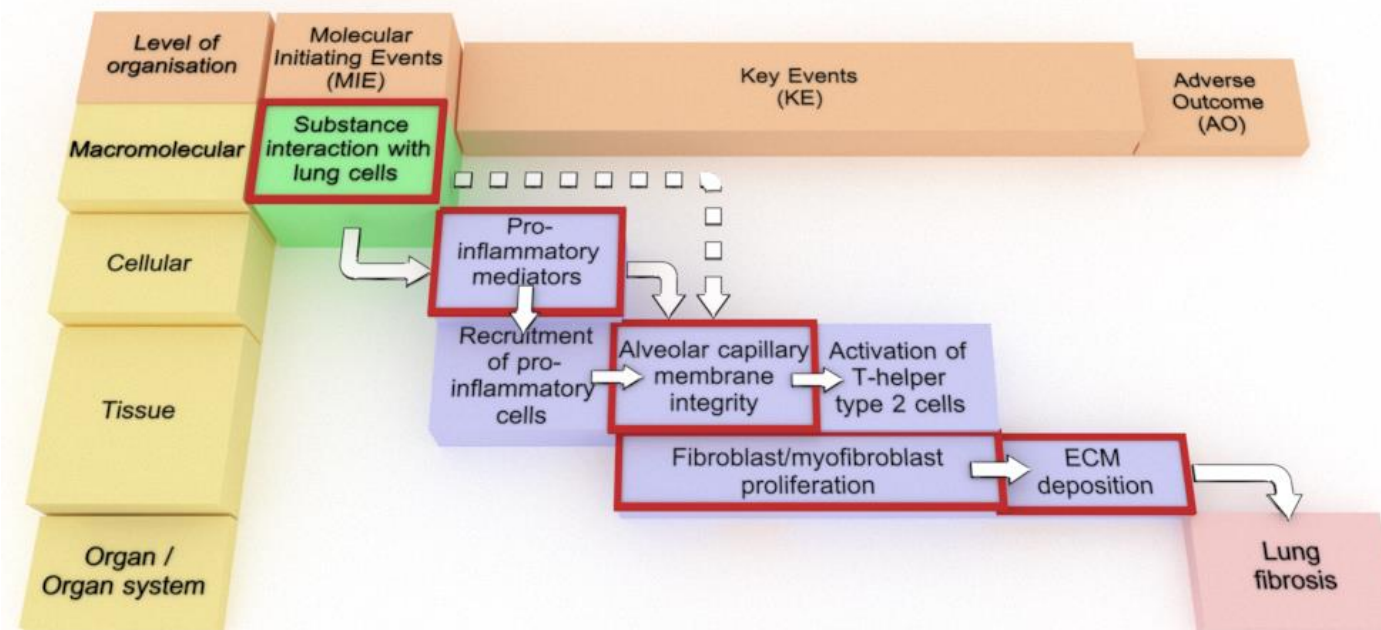
*Corresponding Author:

Prof. Barbara Rothen-Rutishauser

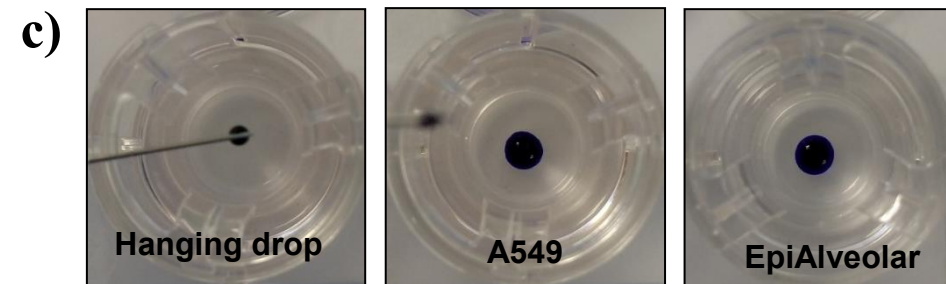Adolphe Merkle Institute, Université de Fribourg, Chemin des Verdiers 4
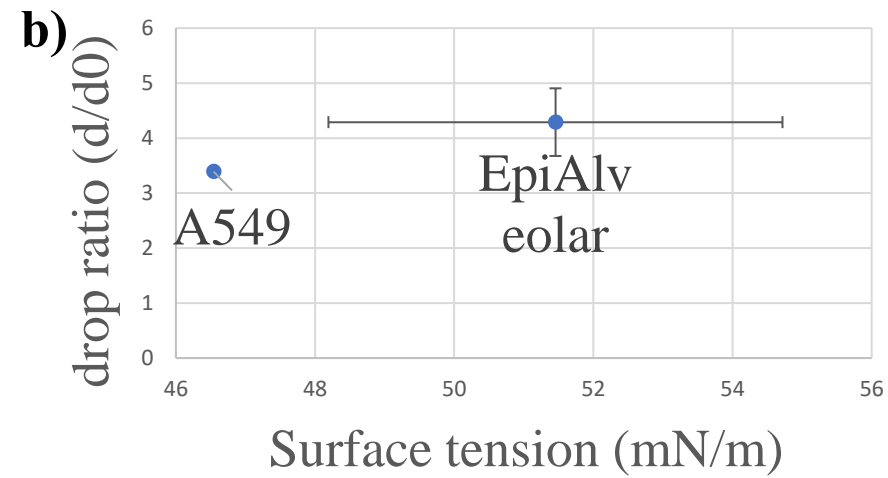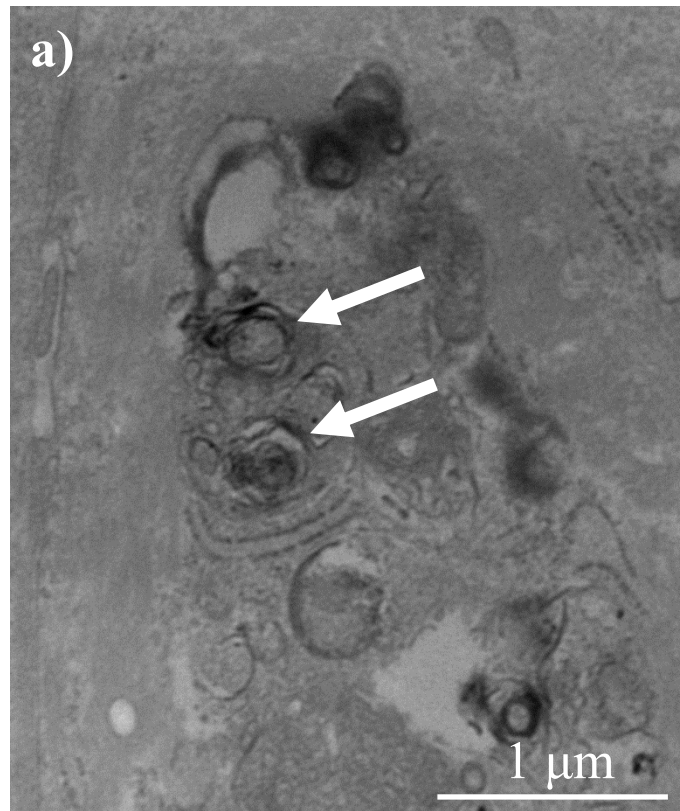
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Supplementary Figure 1
Figure 1: Schematic depicting the adverse outcome pathway (AOP) for pulmonary fibrosis.

Supplementary Figure 2
EpiAlveolar characterization: a) Representative Transmission Electron Microscopy micrograph showing a lamellar body (white arrow) present in ATII cells. Data obtained in Laboratory 2. And surface tension investigation, b) the graph showing droplet diameter vs. surface tension, data presented as mean±standard deviation for EpiAlveolar (n=3), A549 single value (n=1) and c) droplet diameter before (hanging drop) and after placement on the cell culture surface. B). Data obtained in Laboratory 1.
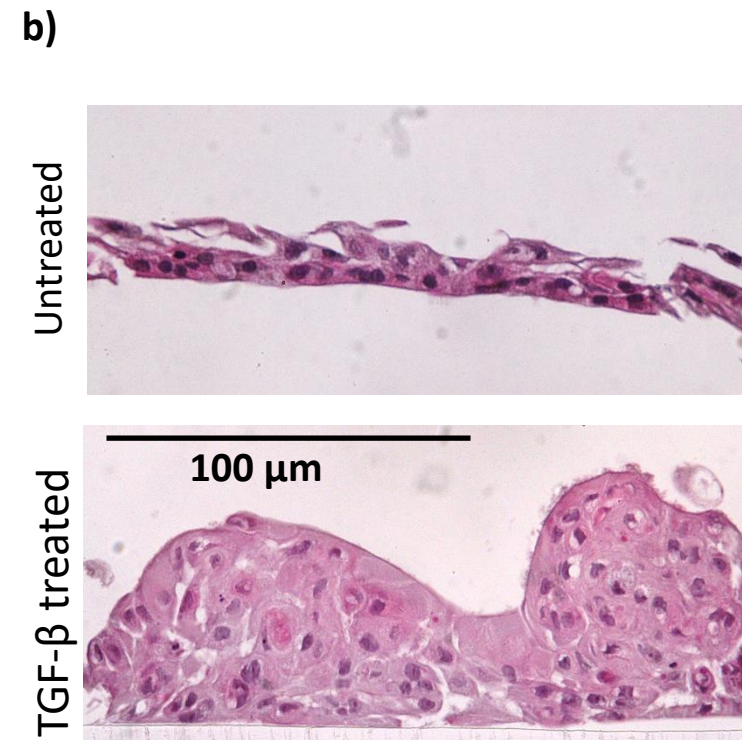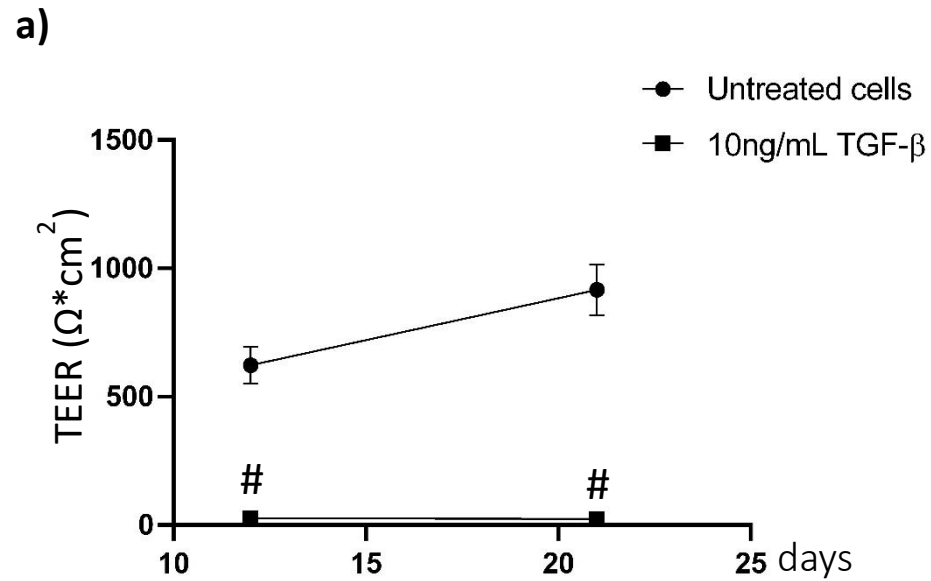
Supplementary Figure 3

EpiAlveolar response upon TGF-β treatment: a) Transepithelial electrical resistance, data presented as mean±standard deviation, # marks statistically significant decrease (compared to untreated cells) b) Hematoxylin and eosin staining showing the thickness of the untreated tissues (upper), and nodules formation with tissue contraction upon exposure to TGF-β (lower). Scale bar is 100 μm. Data obtained in Laboratory 3.
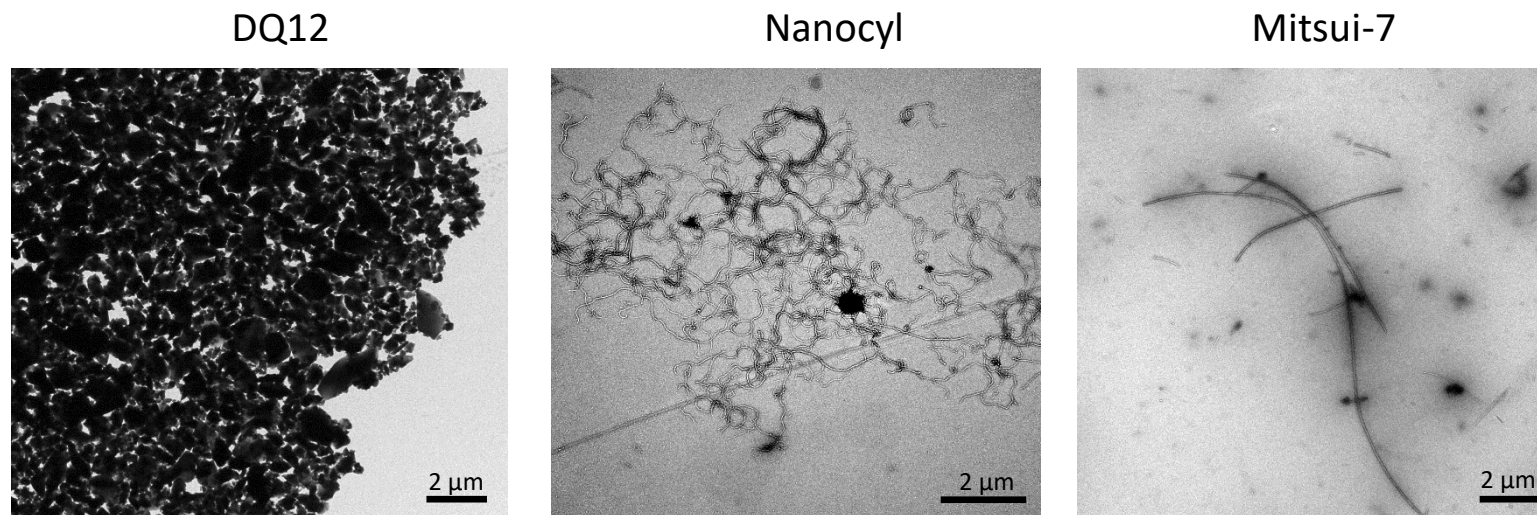
Supplementary Figure 4

Representative TEM images of suspended particles prior nebulization. The stock solution of DQ12 (left), Nanocyl (middle) and Mitsui-7 (right) suspended particles was pipetted onto TEM grid. Scale bar is 2 μm. Data obtained in Laboratory 2.

Material	Daily deposition (μg/cm²)$^{\Psi}$	Weekly deposition (μg/cm²)$^{\theta}$	Total deposition after 21 days (μg/cm²) $^{\theta}$	Dispersant
DQ12	0.18 ± 0.04	0.91 ± 0.22	2.72 ± 0.66	Ultrapure water
Nanocyl	1.02 ± 0.10	5.08 ± 0.52	15.24 ± 1.56	0.1 % BSA
Mitsui-7	0.90 ± 0.31	4.50 ± 1.56	13.50 ± 4.67	0.1 % BSA
	1.98 ± 0.42	9.88 ± 2.10	29.63 ± 6.30	

$^{\Psi}$ deposition measured by QCM

$^{\theta}$ deposition calculated based on daily deposition and number of days

Supplementary Figure 5

Particle deposition during the experiment presented in (μg/cm²), data are presented as mean ± standard deviation. Data obtained in Laboratory 2.
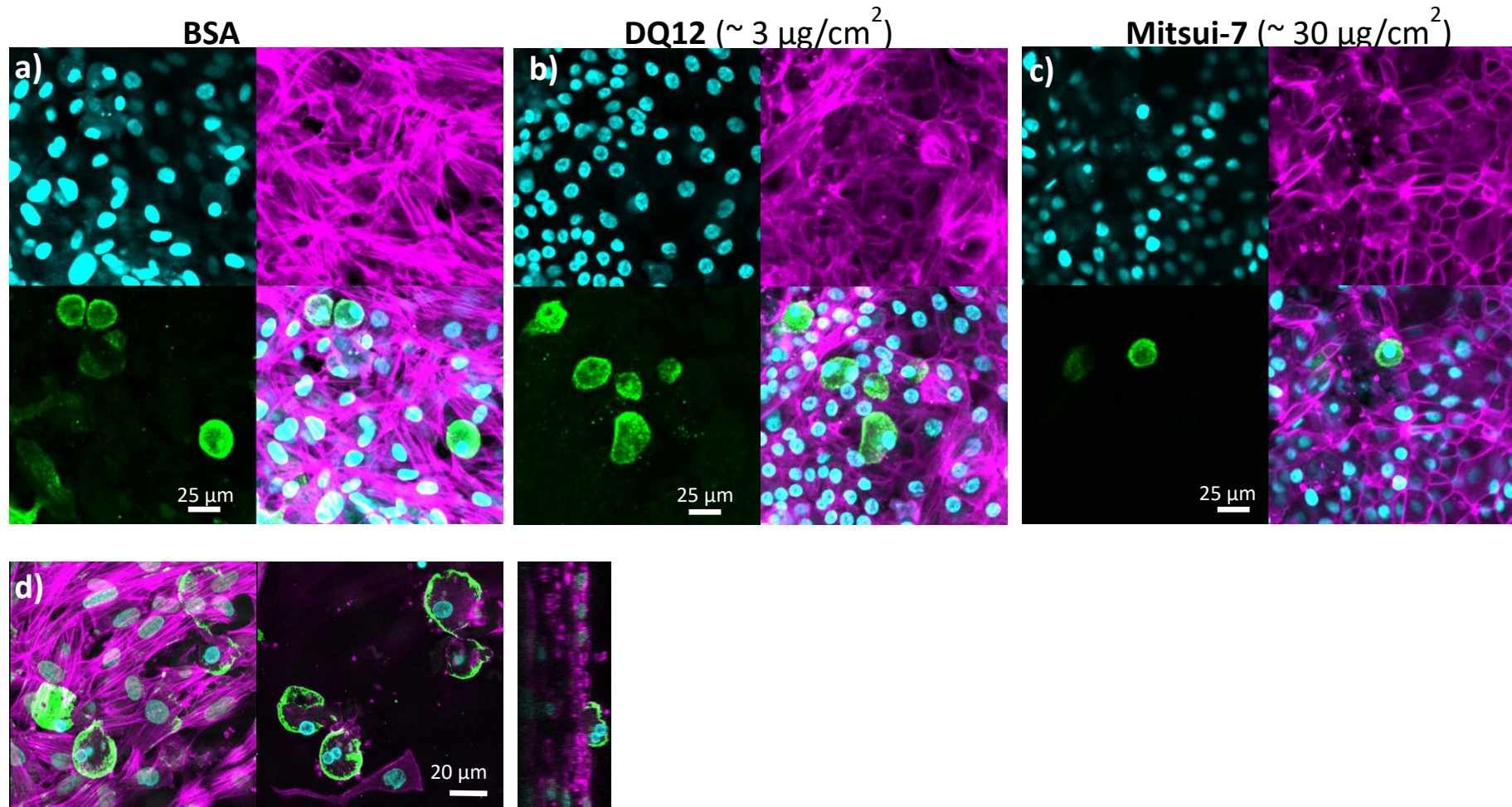
Supplementary Figure 6

Representative LSM images of (+) MDMs tissues showing the presence of MDMs within the (a) BSA, (b) DQ12, and Mitsui-7 (higher dose) treated tissues at D21. (d) shows that MDMs are located on the top of apical side of the tissue. Magenta represents cytoskeleton, cyan cell nuclei, and green represents macrophages (CD206). Data obtained in Laboratory 2.
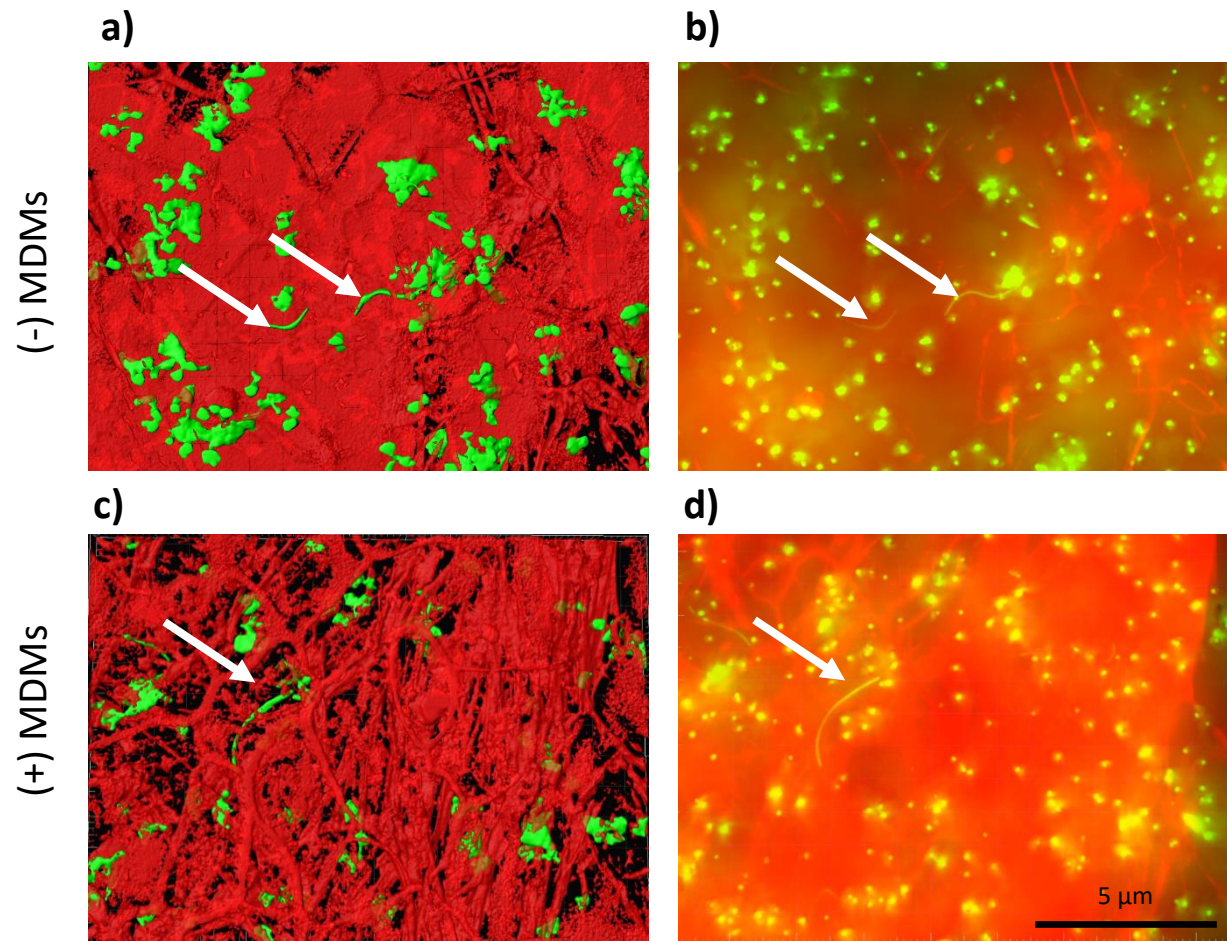
Supplementary Figure 7

Fluorescence-enhanced dark-field in vitro imaging of Mitsui-7 in EpiAlveolar tissue. Representative images of the (-) MDMs (a, b), and (+) MDMs (c, d) model are presented. In order to visualize the spatial location of MWCNTs within the tissue both 3D rendered (a, c) and as xy projections (b, d) are presented. Red color represents F-actin cytoskeleton, while green color represents Mitsui-7. In addition, as the cells were growing on PET membrane inserts a strong scattering of the insert pores resulted in unspecific signals (round-shaped green objects), and white arrows point to the fiber structures derived from Mitsui-7. Scale bar is 5 μm. Data obtained in Laboratory 2.
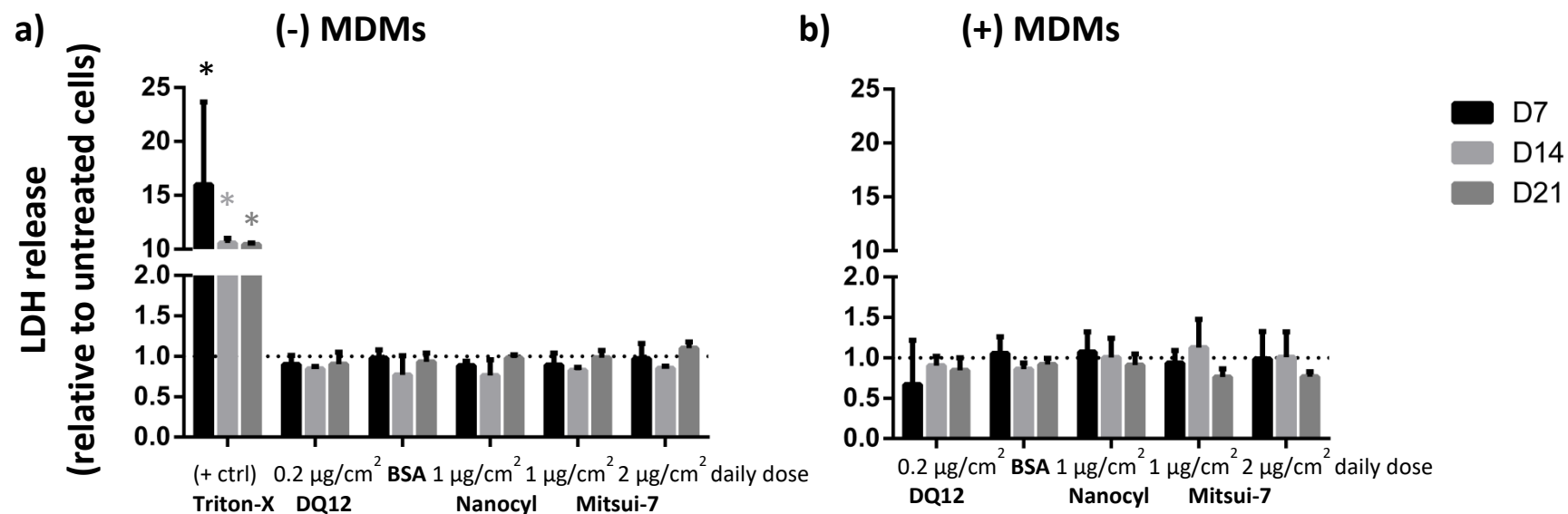
Supplementary Figure 8
LDH release from co-culture model (a) (-) MDMs and (b) (+) MDMs upon exposure to DQ12, Nanocyl and Mitsui-7 MWCNTs at D7, D14 and D21. Triton-X (0.2%) was used as positive control. Data are presented relative to untreated cells (dash line), mean ± standard deviation (SD), n=3. Data marked as (*) are considered statistically significant ($p < 0.05$) increased compared to untreated cells. Data obtained in Laboratory 2.
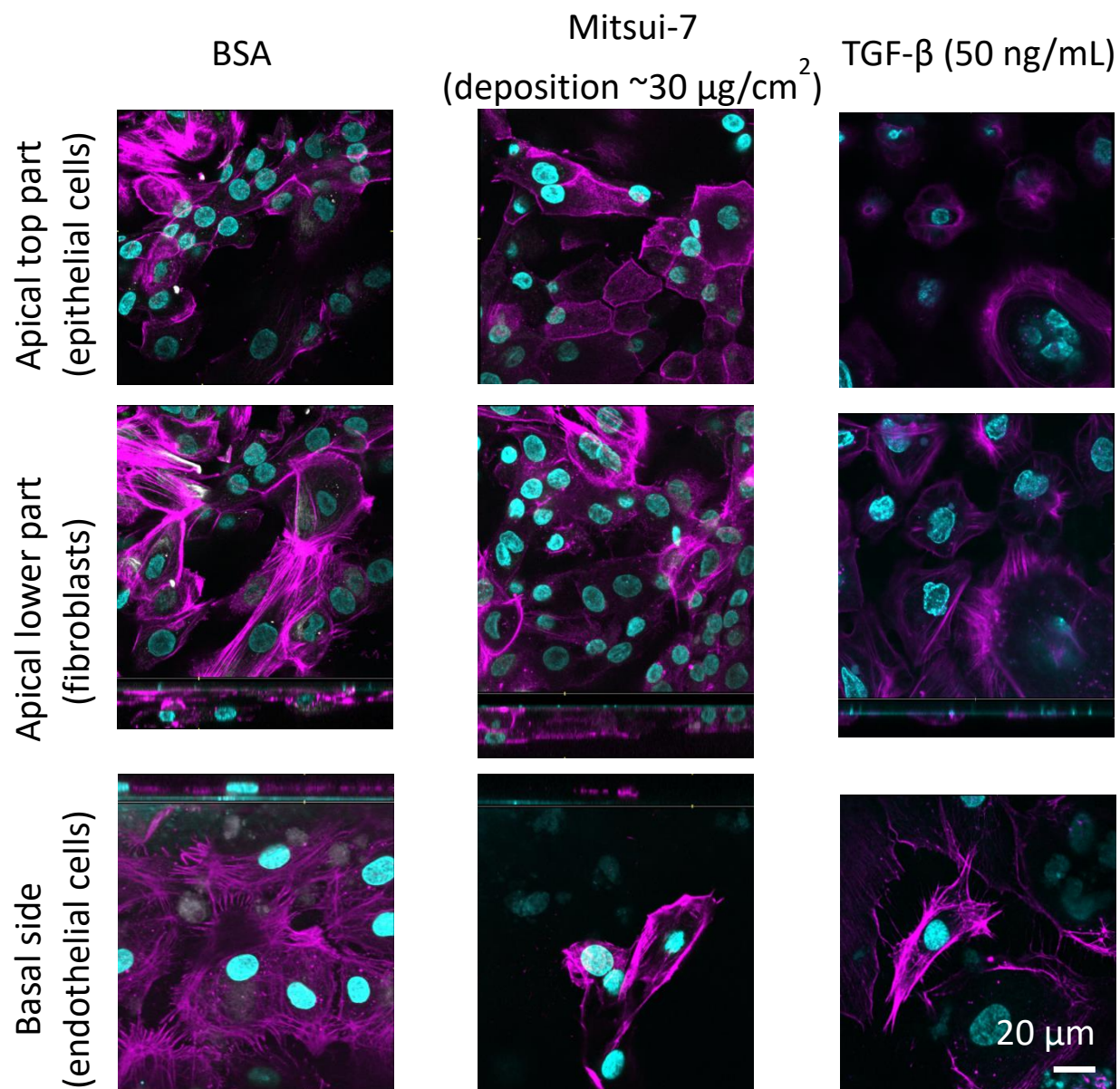
Supplementary Figure 9

Representative confocal laser scanning microscopy images of (-) MDMs model at D21 exposed to BSA, Mitsui-7 MWCNTs and 50 ng/mL TGF-β. Magenta represents the F-actin cytoskeleton, cyan stains cell nuclei. Scale bar is 20 μm. Data obtained in Laboratory 2.
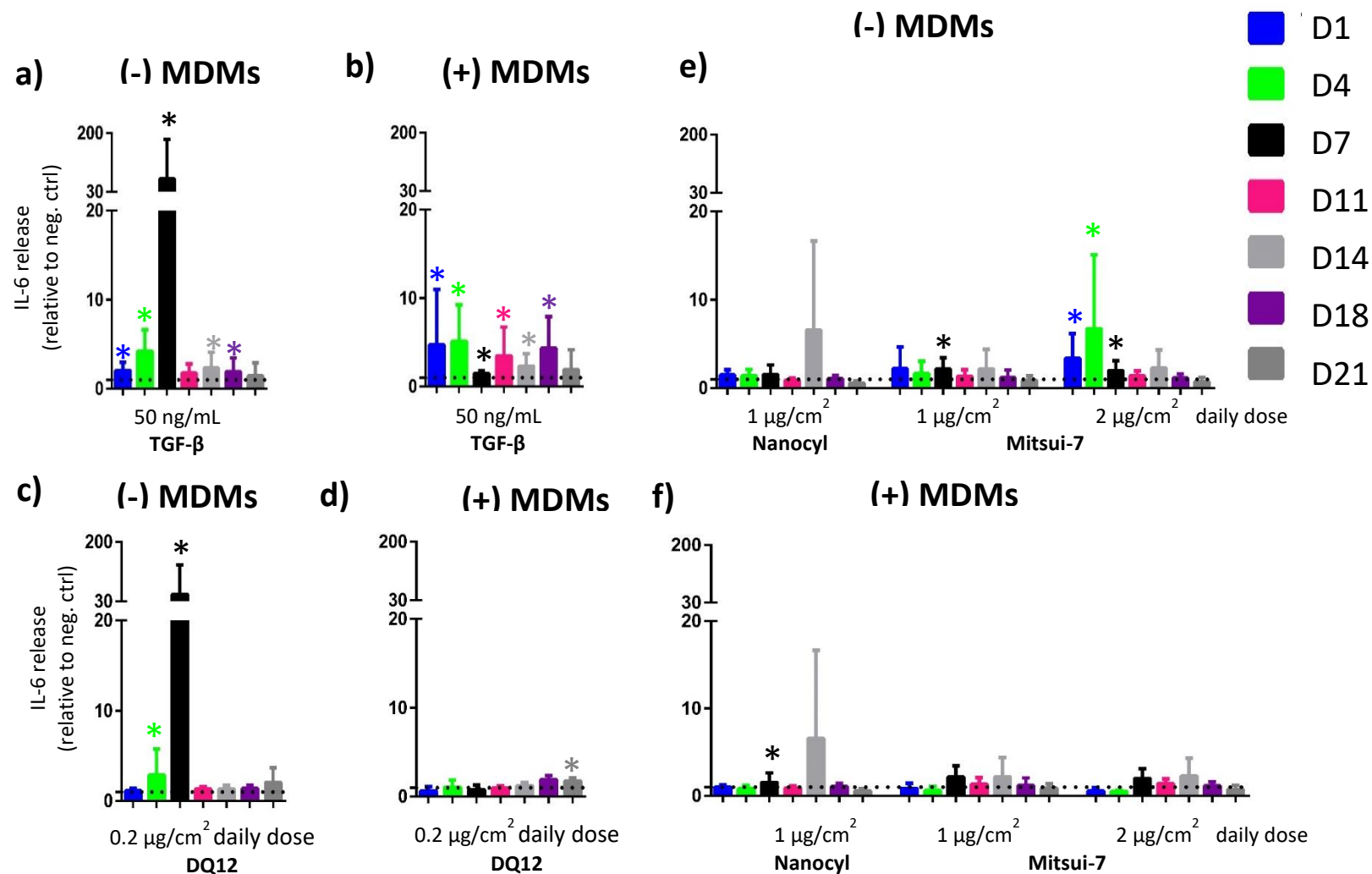
Supplementary Figure 10

Interleukin 6 release as a marker of (pro-)inflammatory response from tissues without macrophages (a, c, e) and with macrophages (b, d, f). Data are presented as relative to negative control (dash line); untreated cells served as negative control for positive control-treated cells (a, b) and DQ12-treated tissues (c, d), while BSA-treated cells served as negative control for MWCNTs-treated cells (e, f). Data are presented as mean±standard deviation (SD) (n=3). Data marked as (*) were considered statistically significant (p<0.05) increased compared to negative control. Data obtained in Laboratory 2.

Supplementary Figure 11

Interleukin 8 release as a marker of (pro-)inflammatory response from tissues without macrophages (a, c, e) and with macrophages (b, d, f). Data are presented as relative to negative control (dash line); untreated cells served as negative control for positive control-treated cells (a, b) and DQ12-treated tissues (c, d), while BSA-treated cells served as negative control for MWCNTs-treated cells (e, f). Data are presented as mean±standard deviation (SD) (n=3). Data marked as (*) were considered statistically significant ($p < 0.05$) increased compared to negative control. Data obtained in Laboratory 2.

Supplementary Figure 12

Fibronectin release as a marker of (pro-)fibrotic response from tissues without macrophages (a, c, e) and with macrophages (b, d, f). Data are presented as relative to negative control (dash line); untreated cells served as negative control for positive control-treated cells (a, b) and DQ12-treated tissues (c, d), while BSA-treated cells served as negative control for MWCNTs-treated cells (e, f). Data are presented as mean±standard deviation (SD) (n=3). Data marked as (*) were considered statistically significant (p<0.05) increased compared to negative control. Data obtained in Laboratory 2.
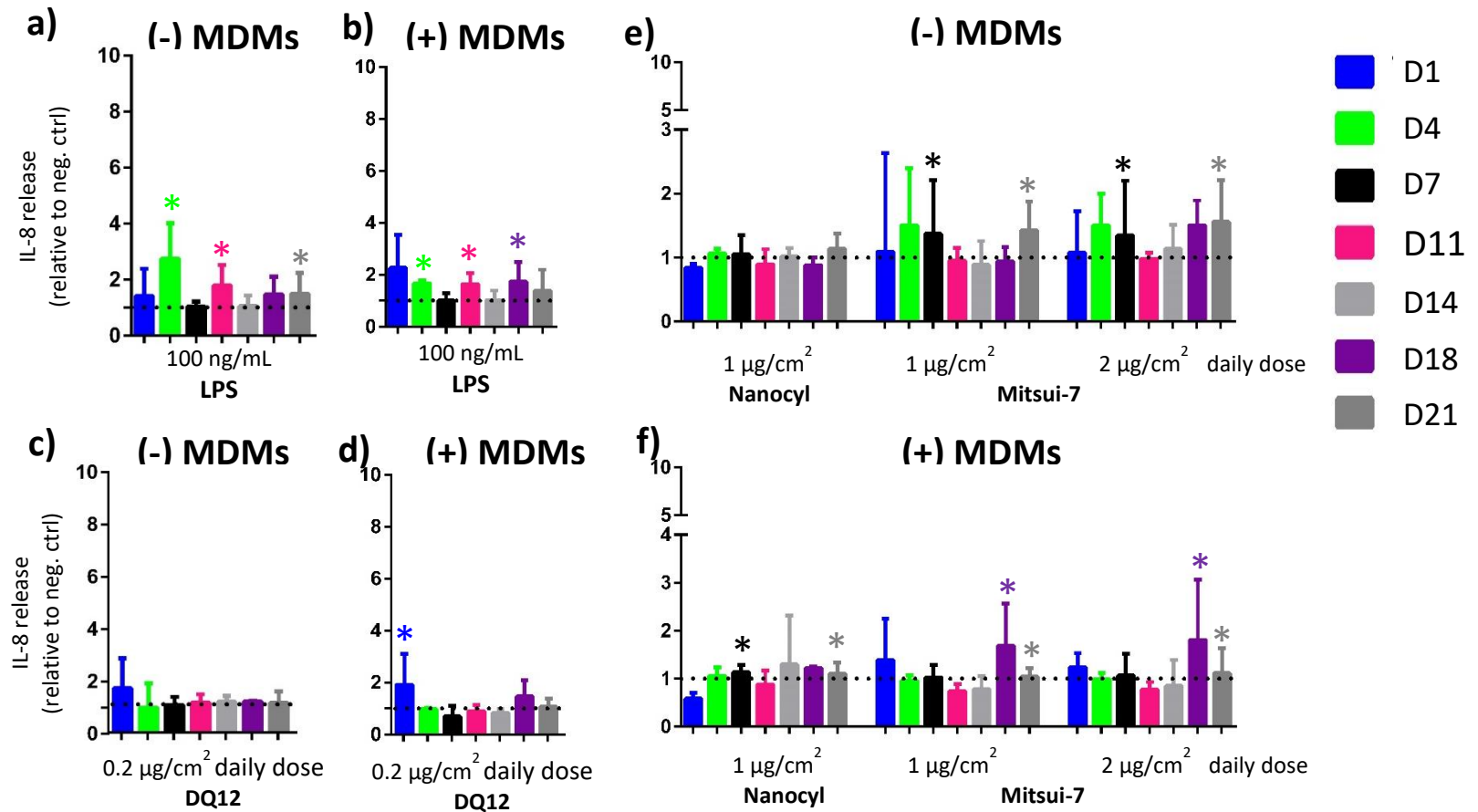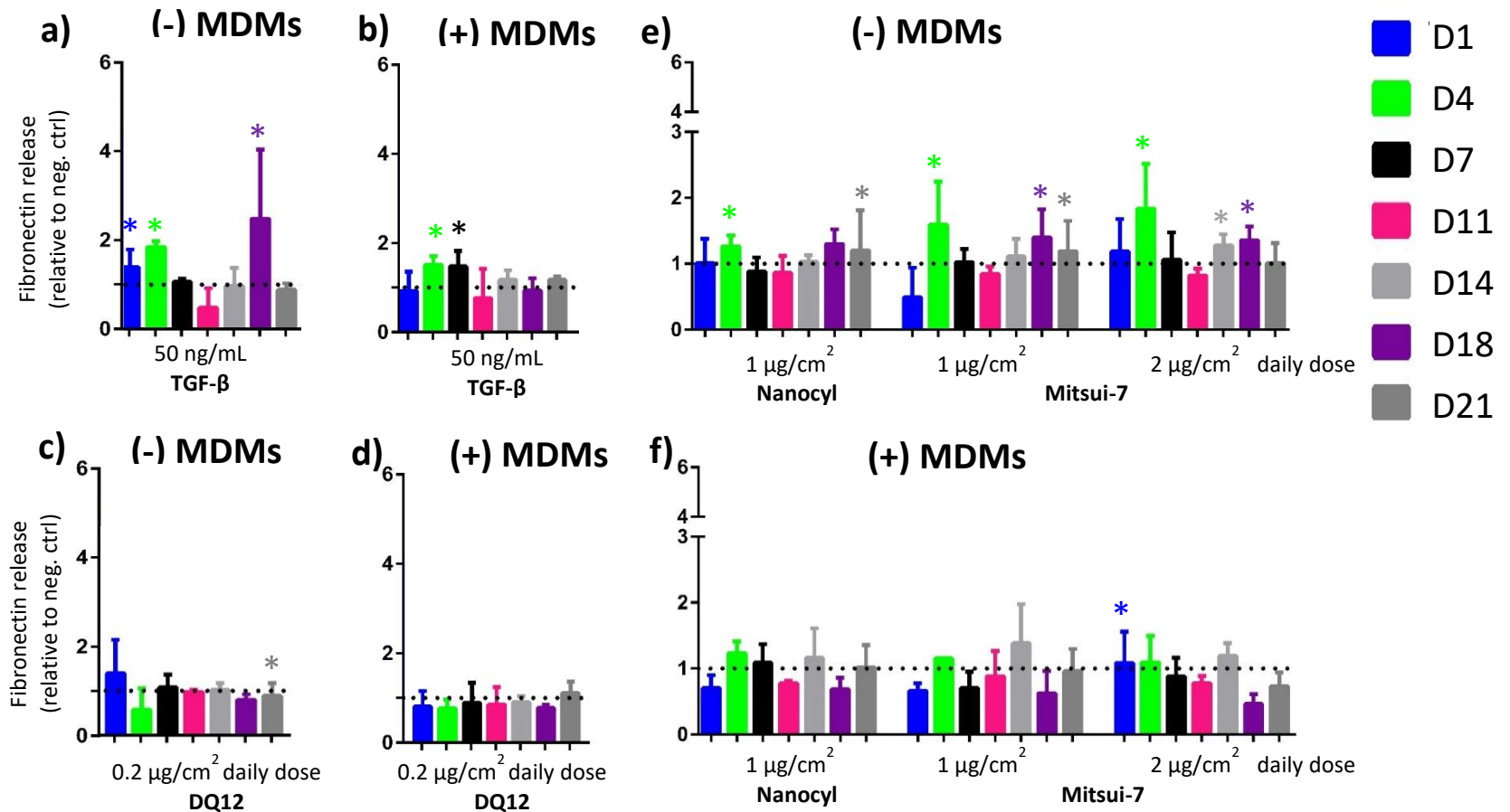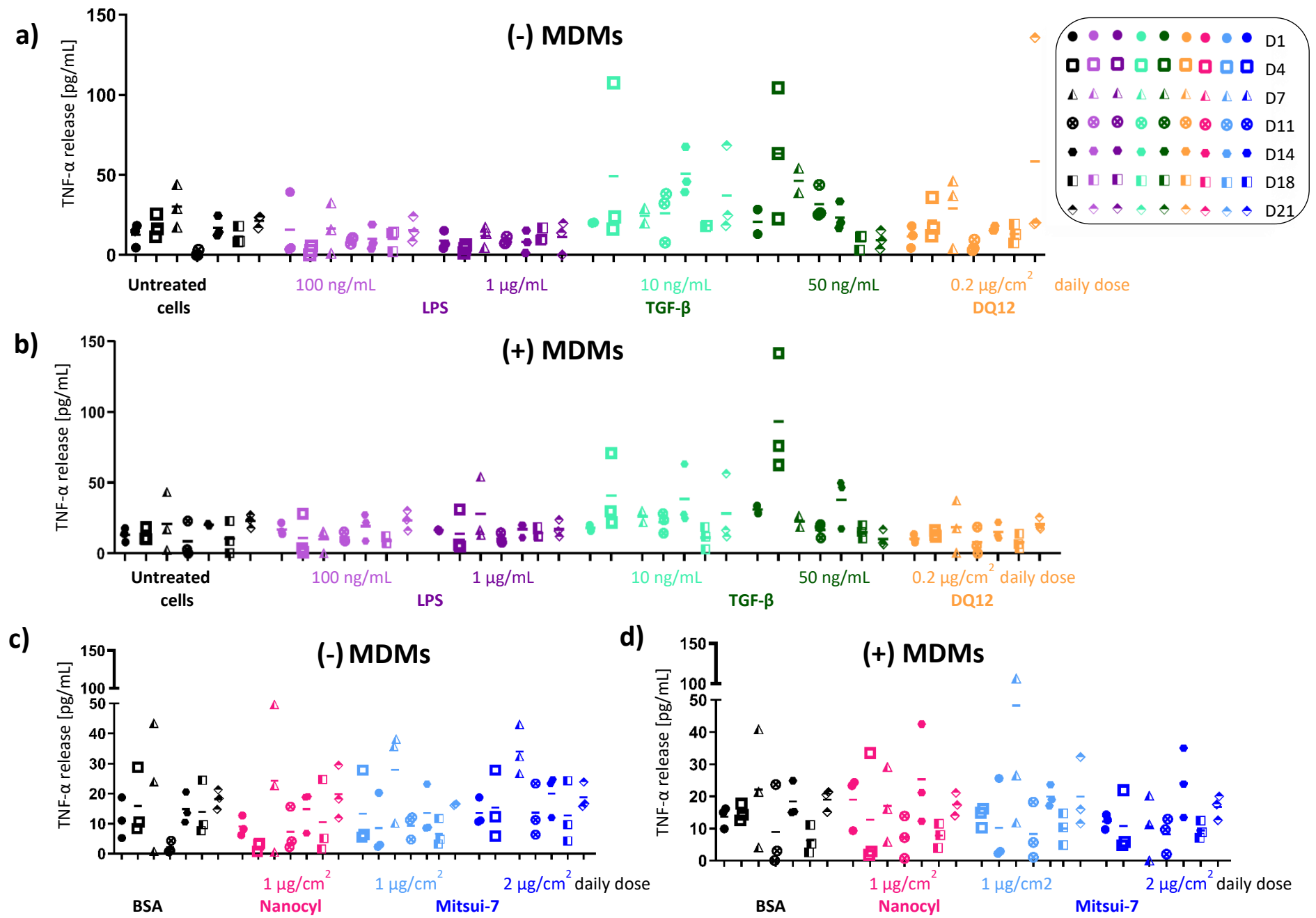
Supplementary Figure 13

Tumor necrosis factor α release from tissues without macrophages (a, c) and with macrophages (b, d). Data are presented as single values (n=3). Data obtained in Laboratory 2.
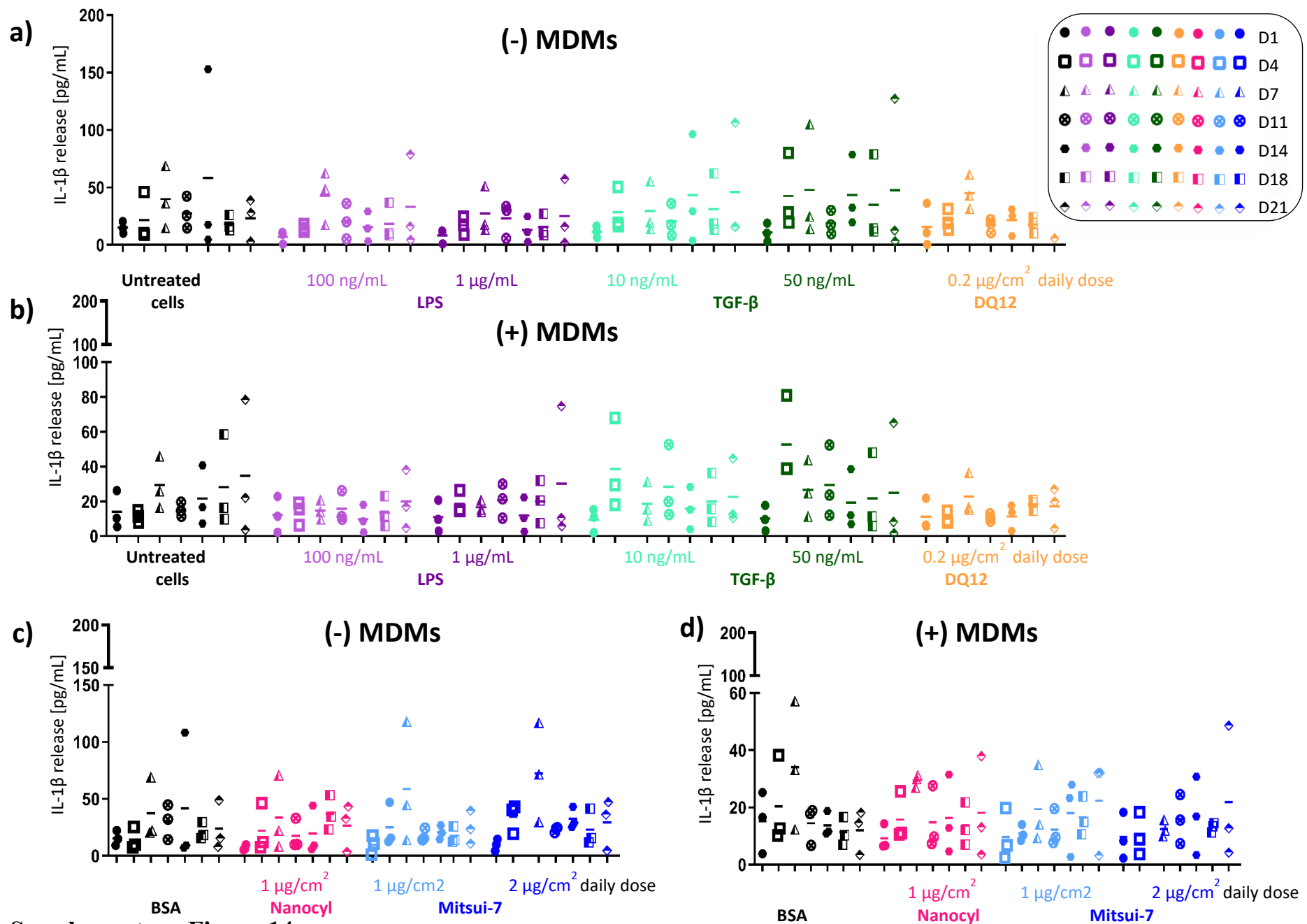
Supplementary Figure 14

Interleukin 1β release from tissues without macrophages (a, c) and with macrophages (b, d). Data are presented as single values (n=3). Data obtained in Laboratory 2.
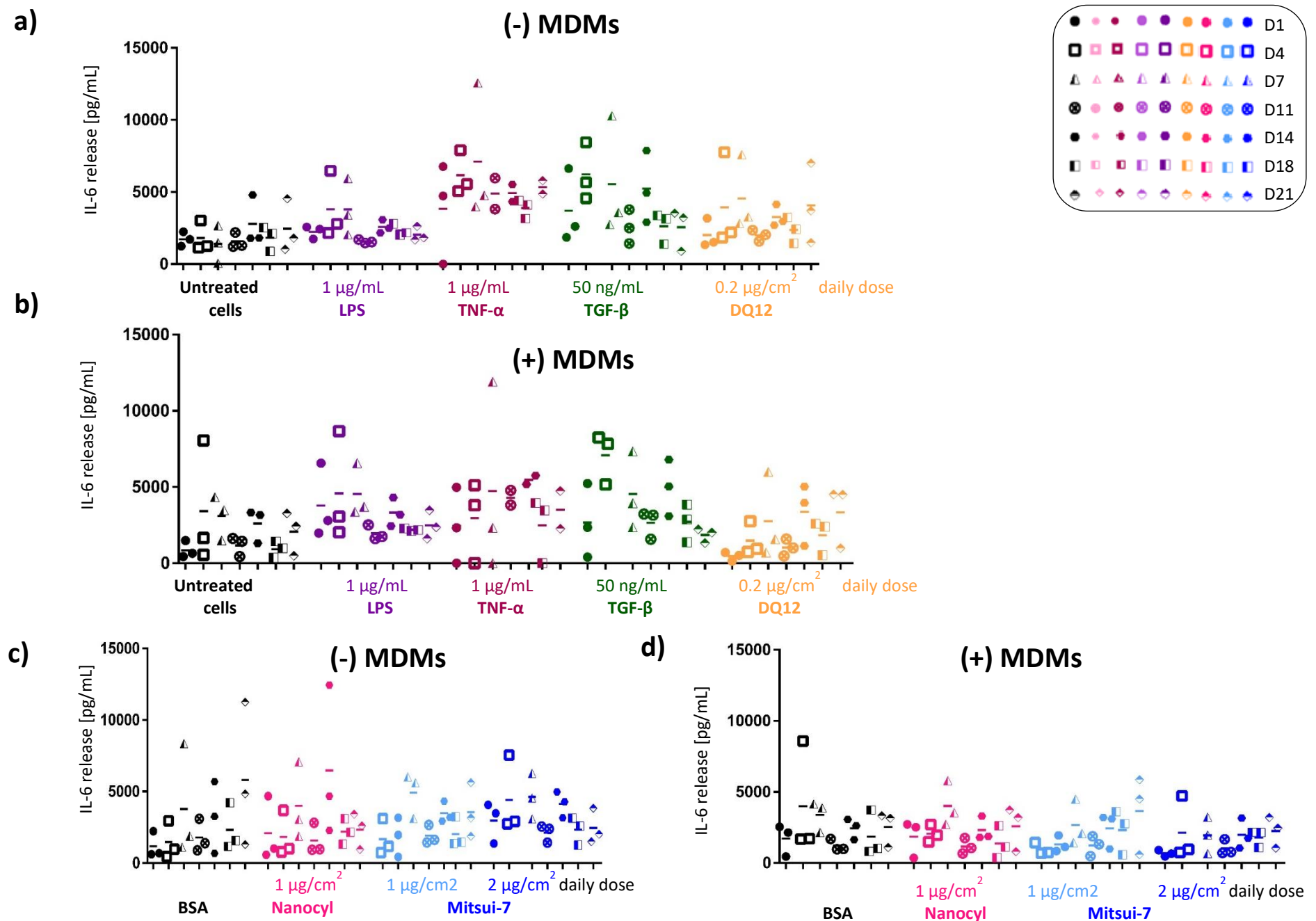
Supplementary Figure 15
Interleukin 6 release from tissues without macrophages (a, c) and with macrophages (b, d). Data are presented as single values (n=3). Data obtained in Laboratory 2.
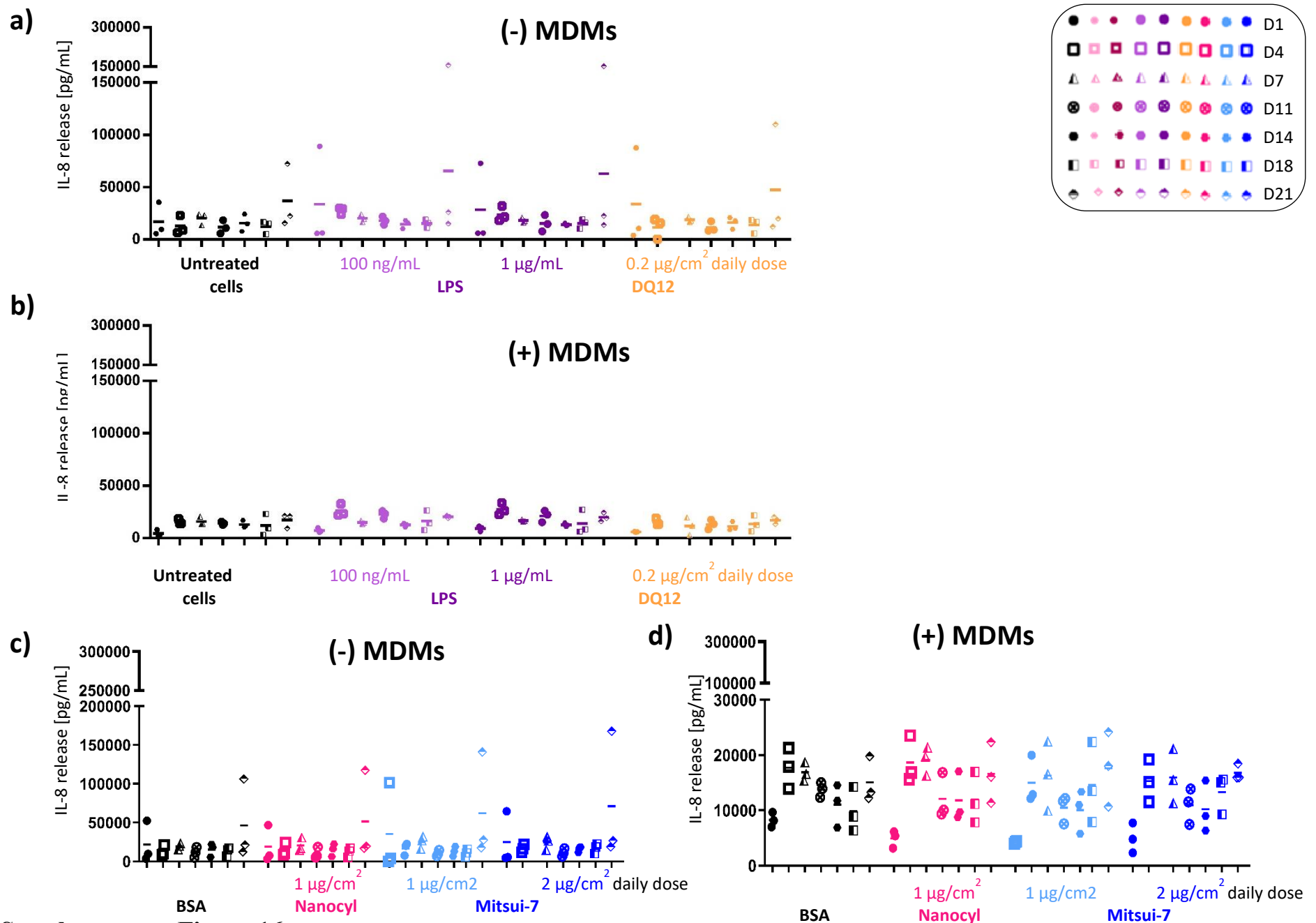
Supplementary Figure 16
Interleukin 8 release from tissues without macrophages (a, c) and with macrophages (b, d). Data are presented as single values (n=3). Data obtained in Laboratory 2.
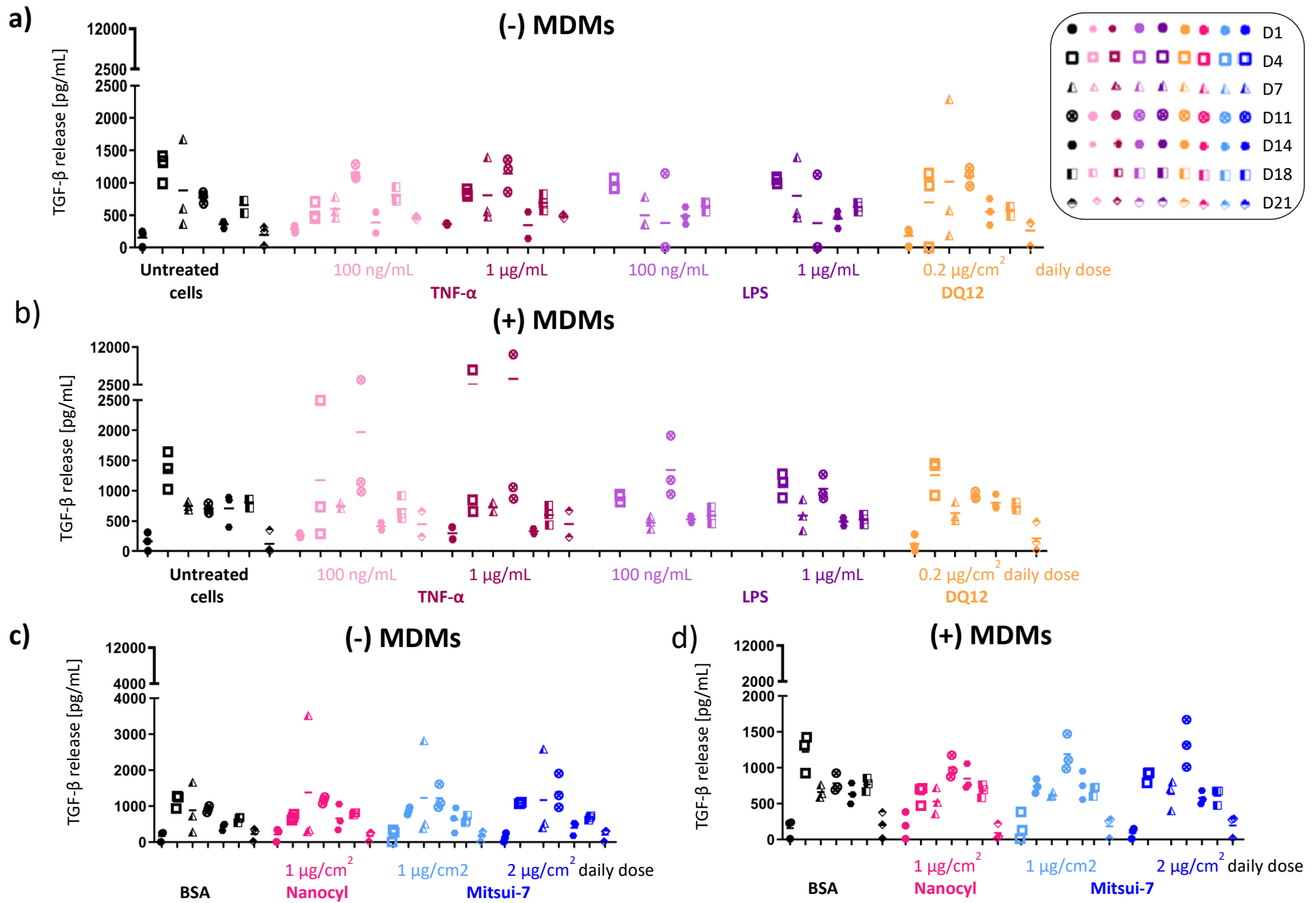
Supplementary Figure 17

Transforming growth factor β release from tissues without macrophages (a, c) and with macrophages (b, d). Data are presented as single values (n=3). Data obtained in Laboratory 2.
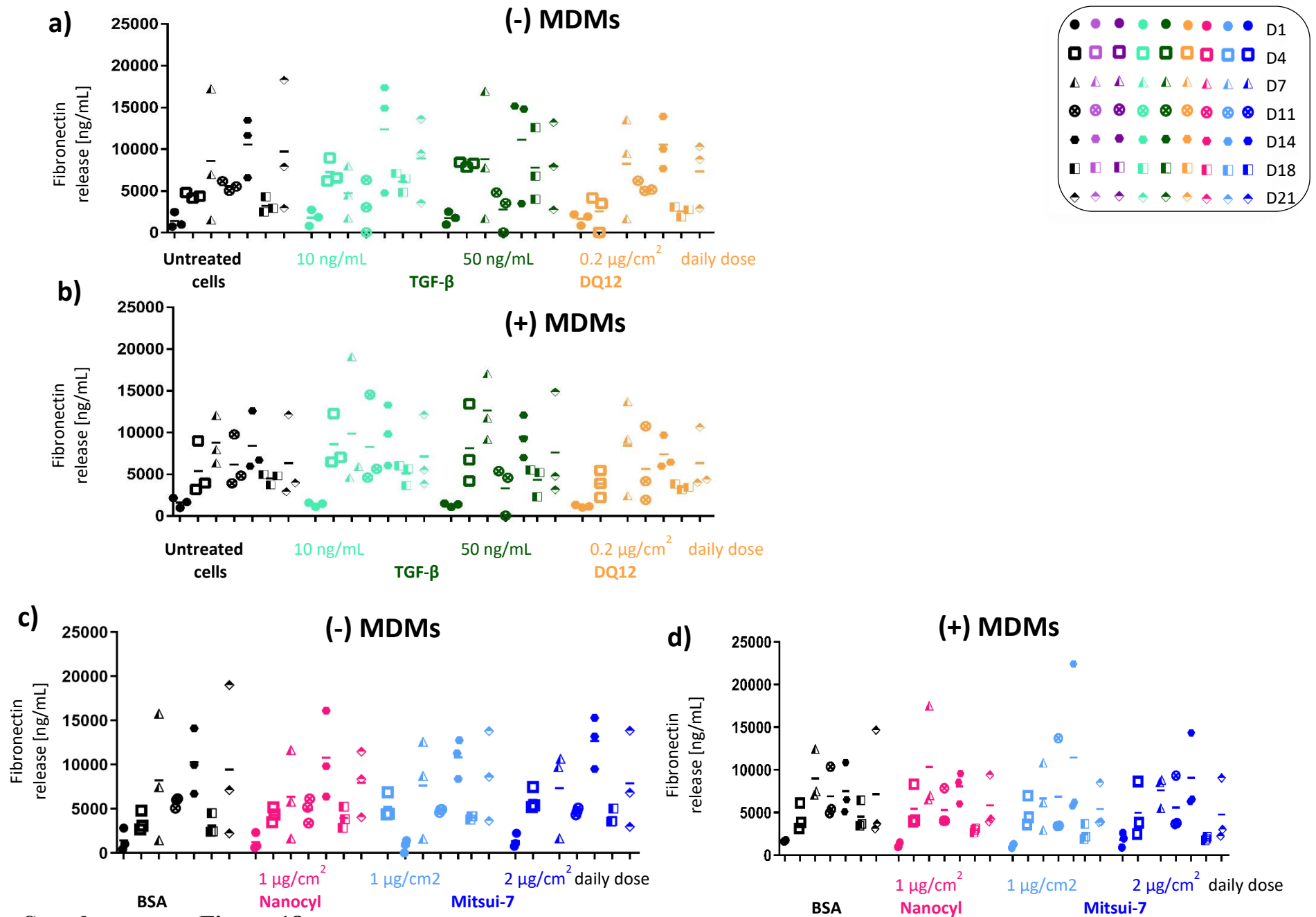
Supplementary Figure 18

Fibronectin release from tissues without macrophages (a, c) and with macrophages (b, d). Data are presented as single values (n=3). Data obtained in Laboratory 2.
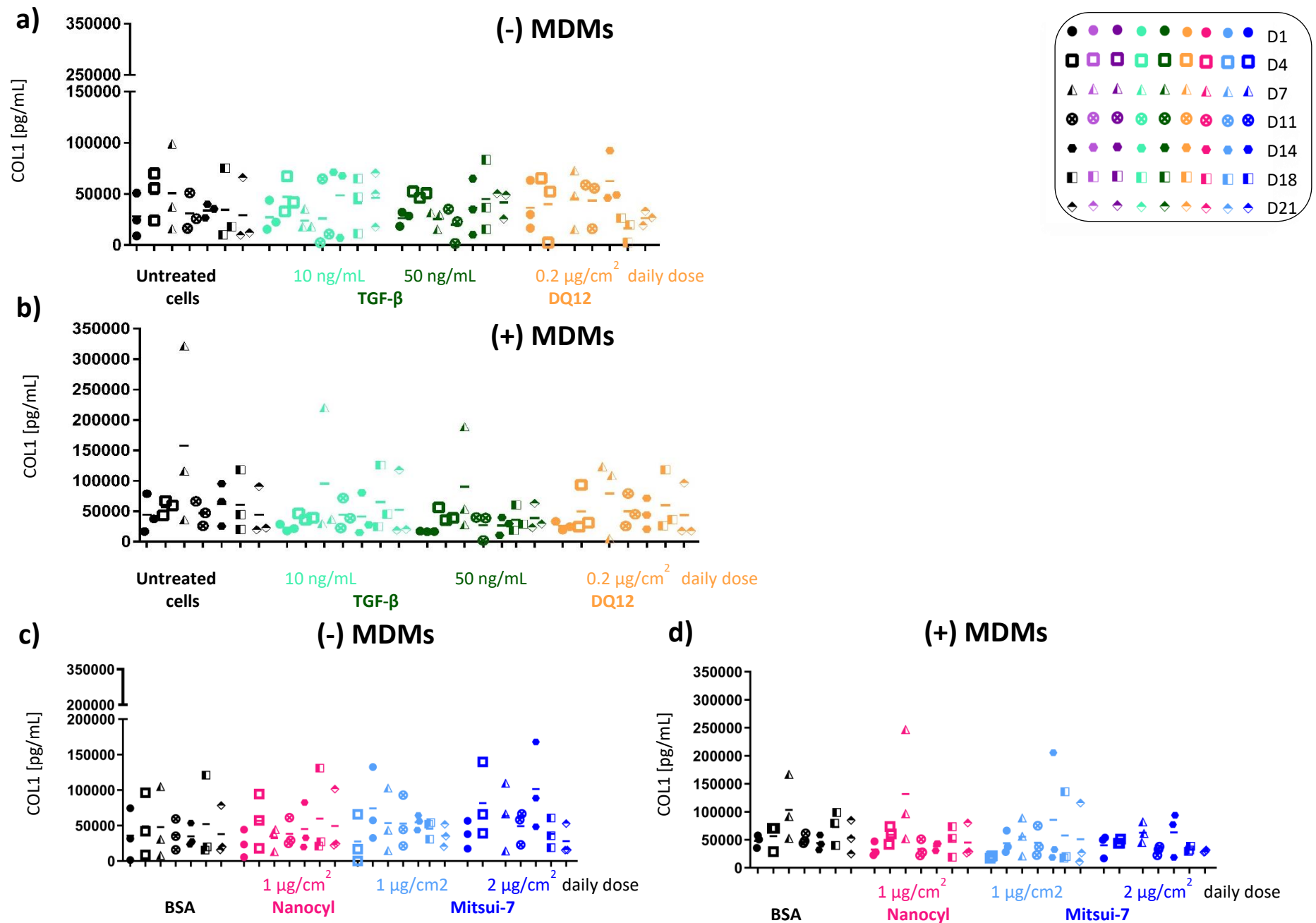
Supplementary Figure 19
Collagen type 1 release from tissues without macrophages (a, c) and with macrophages (b, d). Data are presented as single values (n=3). Data obtained in Laboratory 2.
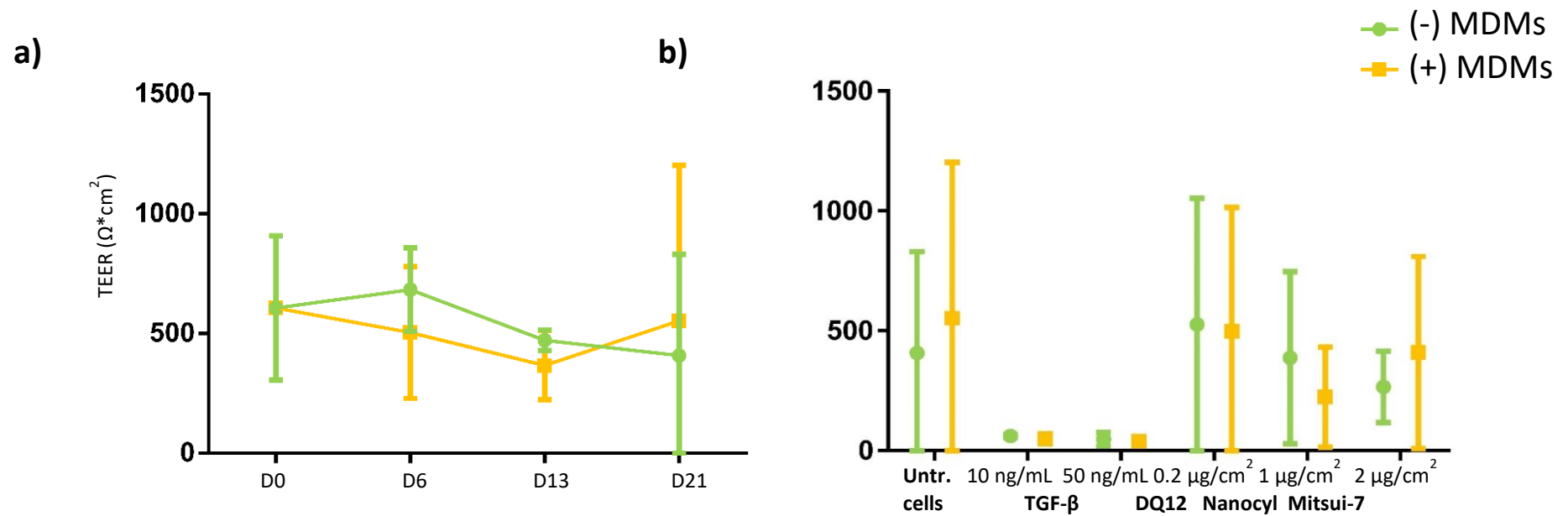
Supplementary Figure 20

Transepithelial electrical resistance measurement as a marker of cell layer continuity of tissues with (+) and without (-) macrophages. Data are presented as mean±standart deviation (SD) (n=3). Data obtained in Laboratory 2.
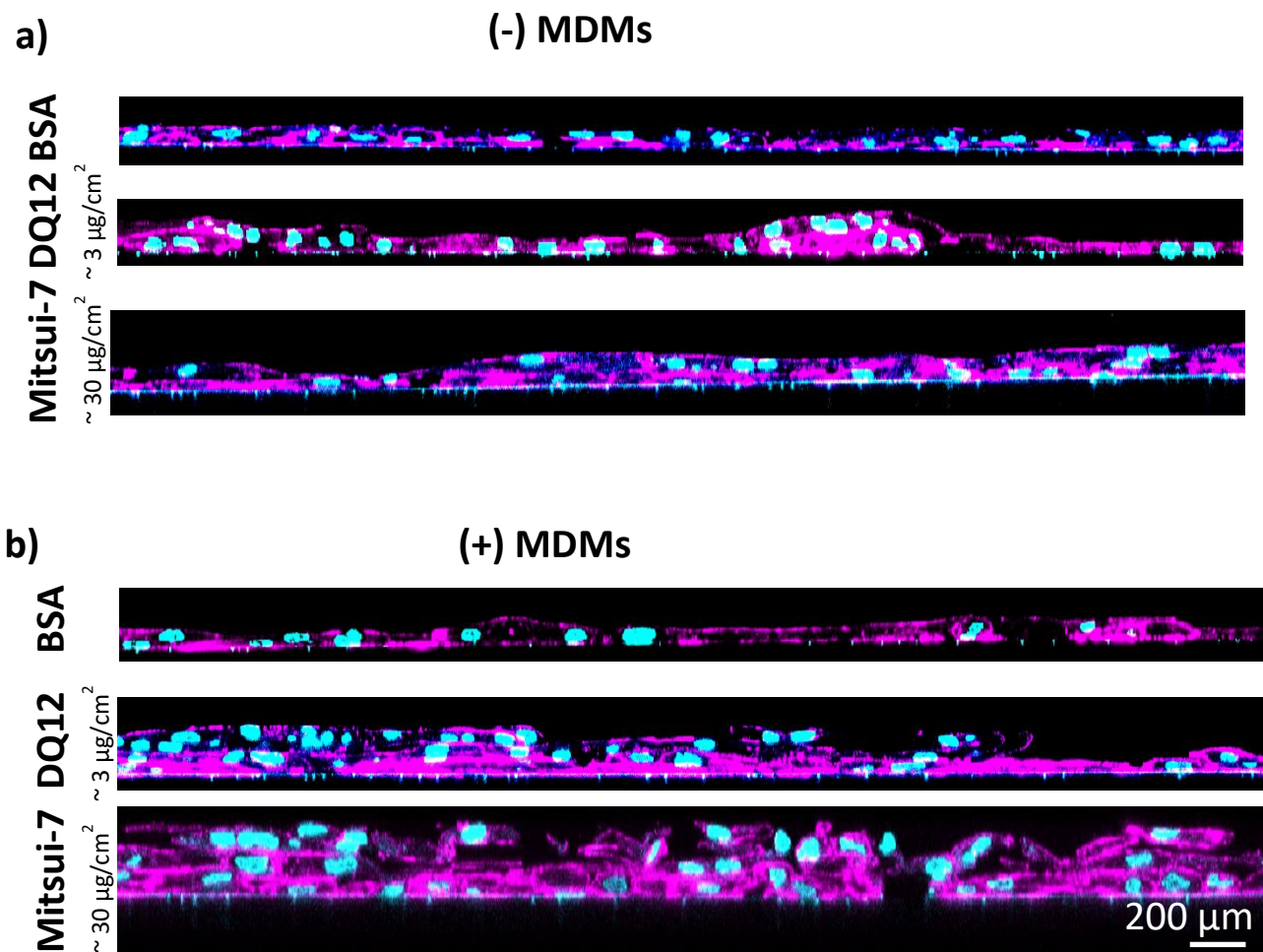
Supplementary Figure 21

Confocal laser scanning microscopy images of cell layer thickness of tissues (a) (-) MDMs and (b) (+) MDMs after 21 days exposures to BSA, DQ12, and Mitsui-7 MWCNTs. Scale bar is 200 µm. Magenta represents the F-actin cytoskeleton, cyan stains cell nuclei. Data obtained in Laboratory 2.
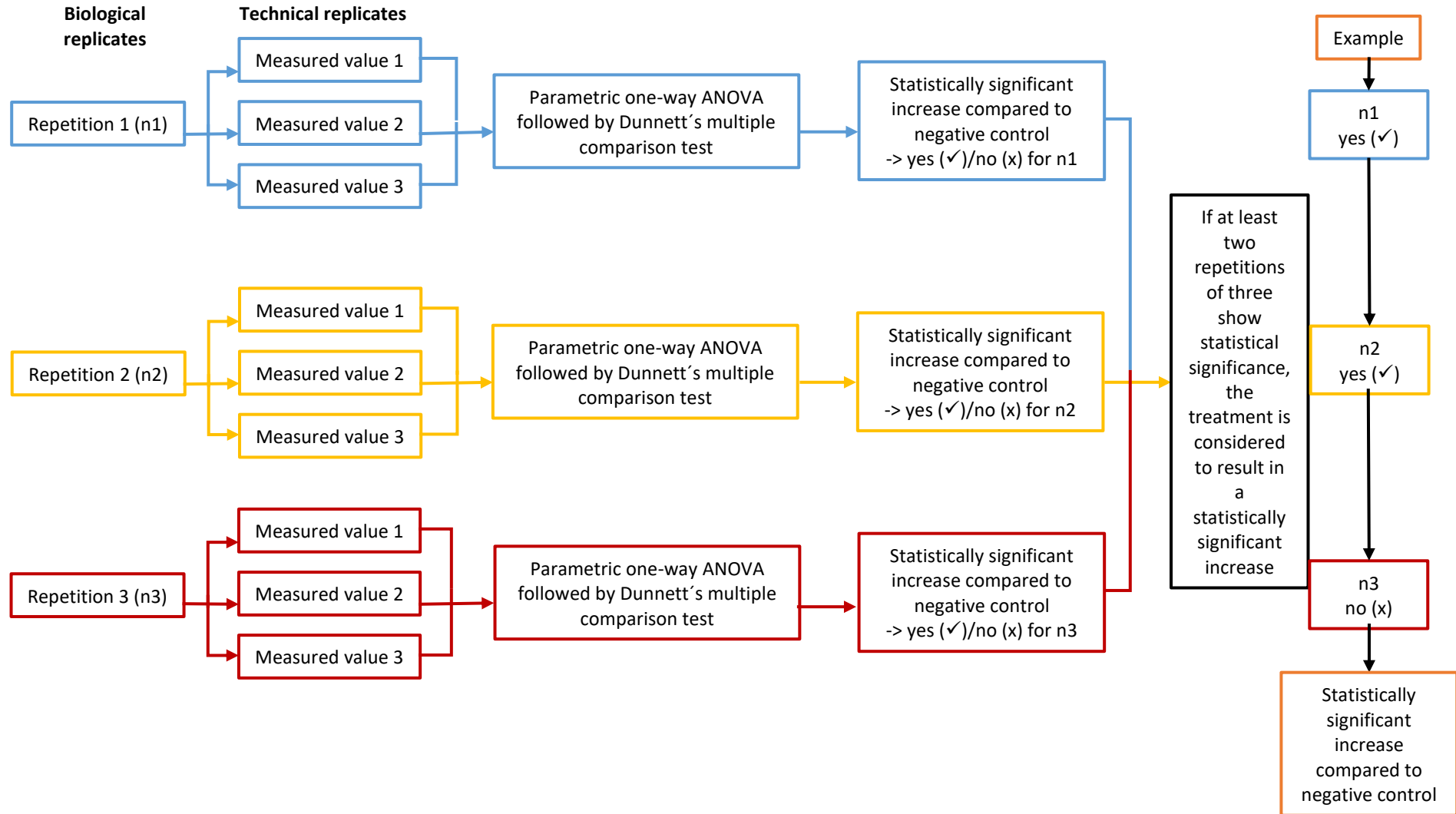
Supplementary Figure 22

Flowchart depicting statistical analysis performed for protein release.

TNF-α

	D1			D4			D7			D11			D14			D18			D21			MDMs
	n1	n2	n3	n1	n2	n3	n1	n2	n3	n1	n2	n3	n1	n2	n3	n1	n2	n3	n1	n2	n3	
LPS [100 ng/mL]	x	✓	x	x	x	x	x	x	x	✓	✓	✓	x	✓	x	✓	x	x	x	x	x	−
	✓	✓	x	x	x	✓	x	x	x	✓	✓	x	x	✓	x	✓	✓	x	✓	x	x	+
LPS [1 µg/mL]	x	x	x	x	x	x	x	x	x	✓	✓	✓	x	x	x	✓	x	x	x	x	x	−
	✓	✓	x	x	x	✓	x	x	x	✓	✓	✓	x	x	x	✓	✓	x	x	x	x	+
TGF-β [10 ng/mL]	x	x	x	x	✓	x	x	x	x	✓	✓	✓	✓	✓	✓	✓	✓	x	x	x	x	−
	x	✓	✓	✓	✓	✓	x	✓	x	✓	✓	x	x	✓	✓	✓	x	x	x	✓	x	+
TGF-β [50 ng/mL]	x	x	✓	x	✓	✓	x	✓	x	✓	✓	✓	✓	✓	x	✓	x	x	x	x	x	−
	x	✓	✓	✓	✓	✓	x	x	x	✓	✓	x	✓	✓	x	✓	✓	x	x	x	x	+
DQ12 (0.2 µg/cm²/day)	✓	✓	x	x	✓	✓	x	✓	x	✓	✓	✓	✓	x	x	x	✓	x	x	x	x	−
	x	x	x	x	✓	x	x	x	x	x	✓	x	x	x	x	x	x	x	x	x	x	+
Nanocyl (1 µg/cm²/day)	x	x	x	x	x	x	x	x	✓	x	x	✓	x	✓	x	✓	x	x	x	x	✓	−
	x	✓	✓	x	x	✓	x	x	x	✓	✓	x	x	✓	x	✓	x	x	x	x	x	+
Mitsui-7 (1 µg/cm²/day)	x	✓	x	x	✓	x	x	x	x	✓	✓	✓	x	✓	x	✓	x	x	x	x	x	−
	x	x	x	x	x	✓	x	x	x	x	✓	x	x	x	x	✓	x	✓	x	x	✓	+
Mitsui-7 (2 µg/cm²/day)	✓	✓	x	x	✓	x	x	x	x	✓	✓	✓	x	✓	✓	✓	x	x	x	x	✓	−
	x	x	x	x	x	✓	x	x	x	✓	✓	x	x	✓	x	✓	✓	x	✓	x	x	+

X – not statistically significant (compared to negative control)

✓ - statistically significant (compared to negative control)

☐ - statistical significance

(-) macrophages

(+) macrophages

Supplementary table 1

Summary of statistical analysis of the obtained data of Tumor necrosis factor α release from both tissues (with and without macrophages), x marks no statistically significant increase (p>0.05), ✓ marks statistically significant increase (p<0.05) compared to negative control. Untreated cells served as negative control for positive controls and DQ12 treated samples, while BSA-treated samples served as negative control for MWCNTs-treated cells. Data obtained in Laboratory 2.

IL-1β

	D1			D4			D7			D11			D14			D18			D21			MDMs
	n1	n2	n3	n1	n2	n3	n1	n2	n3	n1	n2	n3	n1	n2	n3	n1	n2	n3	n1	n2	n3	
LPS [100 ng/mL]	x	x	x	x	x	x	x	x	x	x	x	x	✓	x	x	x	x	x	✓	x	x	-
	x	x	x	✓	✓	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	+
LPS [1 µg/mL]	x	x	x	x	x	x	x	x	x	x	x	x	✓	x	x	x	x	x	✓	x	x	-
	x	x	x	✓	✓	x	x	x	x	✓	x	x	x	x	x	x	x	x	x	x	x	+
TGF-β [10 ng/mL]	x	x	x	x	x	x	✓	x	x	x	x	x	✓	x	x	x	x	x	✓	✓	x	-
	x	x	x	✓	x	✓	✓	x	x	✓	x	x	x	x	x	x	x	x	x	✓	x	+
TGF-β [50 ng/mL]	x	x	x	x	x	✓	✓	x	x	x	x	x	x	✓	x	✓	x	x	✓	x	x	-
	x	x	x	✓	x	✓	✓	x	x	✓	x	x	x	x	x	x	x	x	x	✓	x	+
DQ12 (0.2 µg/cm²/day)	✓	x	x	x	✓	x	✓	x	x	x	✓	x	✓	✓	x	x	✓	✓	x	x	x	-
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	✓	x	x	x	+
Nanocyl (1 µg/cm²/day)	x	x	x	x	x	✓	x	x	x	x	x	x	x	x	x	x	✓	✓	✓	x	x	-
	x	x	✓	x	x	x	x	✓	x	✓	x	x	✓	x	x	✓	x	x	✓	x	x	+
Mitsui-7 (1 µg/cm²/day)	x	x	x	x	✓	x	x	✓	x	x	✓	x	✓	✓	x	x	x	x	✓	x	x	-
	x	x	x	x	x	x	x	x	x	x	x	x	✓	x	✓	✓	x	✓	✓	x	✓	+
Mitsui-7 (2 µg/cm²/day)	x	x	x	x	✓	✓	✓	✓	x	x	✓	x	✓	✓	x	x	x	✓	✓	x	x	-
	x	x	x	x	x	x	x	x	x	x	x	x	✓	x	✓	x	x	✓	✓	x	x	+

X – not statistically significant (compared to negative control)

✓ - statistically significant (compared to negative control)

▢ - statistical significance

(-) macrophages

(+) macrophages

Supplementary table 2

Summary of statistical analysis of the obtained data of Interleukin 1β release from both tissues (with and without macrophages), x marks no statistically significant increase (p>0.05), ✓ marks statistically significant increase (p<0.05) compared to negative control. Untreated cells served as negative control for positive controls and DQ12 treated samples, while BSA-treated samples served as negative control for MWCNTs-treated cells. Data obtained in Laboratory 2.

IL-6

Treatment	D1 n1	D1 n2	D1 n3	D4 n1	D4 n2	D4 n3	D7 n1	D7 n2	D7 n3	D11 n1	D11 n2	D11 n3	D14 n1	D14 n2	D14 n3	D18 n1	D18 n2	D18 n3	D21 n1	D21 n2	D21 n3	MDMs
LPS [1 µg/mL] (−)	x	✓	x	✓	✓	x	✓	x	✓	✓	x	x	✓	x	x	x	✓	x	x	✓	x	−
LPS [1 µg/mL] (+)	✓	✓	✓	x	✓	x	x	✓	x	x	✓	x	x	✓	x	x	✓	✓	x	✓	x	+
TNF-α [1 µg/mL] (−)	x	✓	✓	✓	✓	✓	✓	✓	✓	x	✓	✓	x	✓	x	x	✓	✓	x	✓	x	−
TNF-α [1 µg/mL] (+)	x	✓	✓	x	✓	✓	x	✓	✓	x	✓	✓	x	✓	✓	x	✓	✓	x	✓	x	+
TGF-β [50 ng/mL] (−)	x	✓	✓	✓	✓	✓	✓	✓	✓	x	✓	x	✓	✓	x	x	✓	✓	x	✓	x	−
TGF-β [50 ng/mL] (+)	x	✓	✓	x	✓	✓	x	✓	✓	x	✓	✓	x	✓	✓	x	✓	✓	x	✓	x	+
DQ12 (0.2 µg/cm²/day) (−)	x	x	x	✓	✓	x	✓	✓	✓	✓	x	x	✓	x	x	x	x	✓	x	✓	x	−
DQ12 (0.2 µg/cm²/day) (+)	x	x	✓	x	x	x	x	x	✓	x	x	x	✓	x	x	x	x	✓	x	✓	✓	+
Nanocyl (1 µg/cm²/day) (−)	x	x	✓	x	x	x	✓	x	x	x	x	x	✓	x	x	x	x	x	x	x	x	−
Nanocyl (1 µg/cm²/day) (+)	x	x	x	x	x	x	x	✓	✓	x	x	x	x	x	x	x	x	x	x	x	x	+
Mitsui-7 (1 µg/cm²/day) (−)	x	✓	x	x	✓	x	✓	✓	x	✓	x	x	✓	x	x	✓	x	x	x	✓	x	−
Mitsui-7 (1 µg/cm²/day) (+)	✓	x	x	x	x	x	x	x	x	x	x	✓	x	✓	x	x	x	✓	x	x	✓	+
Mitsui-7 (2 µg/cm²/day) (−)	✓	✓	✓	✓	✓	x	✓	✓	x	✓	x	x	✓	x	x	x	x	x	x	x	x	−
Mitsui-7 (2 µg/cm²/day) (+)	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	✓	x	x	x	x	+

X – not statistically significant (compared to negative control)

✓ – statistically significant (compared to negative control)

▢ – statistical significance

(−) macrophages

(+) macrophages

Supplementary table 3

Summary of statistical analysis of the obtained data of Tumor necrosis factor α release from both tissues (with and without macrophages), x marks no statistically significant increase ($p > 0.05$), ✓ marks statistically significant increase ($p < 0.05$) compared to negative control. Untreated cells served as negative control for positive controls and DQ12 treated samples, while BSA-treated samples served as negative control for MWCNTs-treated cells. Data obtained in Laboratory 2.

Supplementary table 4

Summary of statistical analysis of the obtained data of Interleukin 8 release from both tissues (with and without macrophages), x marks no statistically significant increase (p>0.05), ✓ marks statistically significant increase (p<0.05) compared to negative control. Untreated cells served as negative control for positive controls and DQ12 treated samples, while BSA-treated samples served as negative control for MWCNTs-treated cells. Data obtained in Laboratory 2.

TGF-β

Treatment	D1 n1	D1 n2	D1 n3	D4 n1	D4 n2	D4 n3	D7 n1	D7 n2	D7 n3	D11 n1	D11 n2	D11 n3	D14 n1	D14 n2	D14 n3	D18 n1	D18 n2	D18 n3	D21 n1	D21 n2	D21 n3	MDMs
LPS [100 ng/mL]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	✓	x	x	x	x	x	x	−
LPS [100 ng/mL]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	✓	x	+
LPS [1 µg/mL]	x	x	x	x	x	x	x	✓	x	x	x	x	x	x	✓	x	x	x	x	x	x	−
LPS [1 µg/mL]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	✓	x	x	✓	x	x	+
TNF-α [100 ng/mL]	x	x	x	x	x	x	x	✓	x	x	x	✓	x	x	✓	✓	✓	x	x	x	✓	−
TNF-α [100 ng/mL]	x	x	✓	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	✓	x	+
TNF-α [1 µg/mL]	x	✓	x	x	x	x	x	✓	x	x	x	x	x	x	✓	✓	x	x	x	x	✓	−
TNF-α [1 µg/mL]	x	x	✓	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	✓	✓	+
DQ12 (0.2 µg/cm²/day)	x	x	x	x	x	x	✓	x	x	✓	x	✓	✓	x	✓	x	x	x	x	x	x	−
DQ12 (0.2 µg/cm²/day)	x	x	x	x	x	x	x	x	x	✓	x	✓	x	x	x	✓	x	x	✓	✓	x	+
Nanocyl (1 µg/cm²/day)	x	x	x	x	x	x	✓	✓	x	x	x	✓	✓	x	x	✓	✓	✓	x	x	x	−
Nanocyl (1 µg/cm²/day)	x	x	✓	x	x	x	x	x	x	x	✓	x	x	✓	x	✓	x	x	✓	✓	x	+
Mitsui-7 (1 µg/cm²/day)	x	x	x	x	x	x	✓	✓	x	x	✓	x	✓	x	✓	x	x	x	x	x	x	−
Mitsui-7 (1 µg/cm²/day)	x	x	✓	x	x	x	x	x	x	x	✓	x	x	x	x	x	x	x	✓	x	x	+
Mitsui-7 (2 µg/cm²/day)	x	x	x	x	x	x	✓	✓	x	x	✓	x	✓	x	x	x	x	x	x	x	x	−
Mitsui-7 (2 µg/cm²/day)	x	x	x	x	x	x	x	✓	x	x	✓	✓	x	x	x	x	x	x	x	x	x	+

X – not statistically significant (compared to negative control)
✓ – statistically significant (compared to negative control)
☐ – statistical significance

(−) macrophages
(+) macrophages

Supplementary table 5

Summary of statistical analysis of the obtained data of Transforming growth factor β release from both tissues (with and without macrophages), x marks no statistically significant increase (p>0.05), ✓ marks statistically significant increase (p<0.05) compared to negative control. Untreated cells served as negative control for positive controls and DQ12 treated samples, while BSA-treated samples served as negative control for MWCNTs-treated cells. Data obtained in Laboratory 2.

Columns span days D1, D4, D7, D11, D14, D18, D21 (each with sub-columns n1, n2, n3), plus a final MDMs column.

Rows: TGF-β [10 ng/mL], TGF-β [50 ng/mL], DQ12 (0.2 μg/cm²/day), Nanocyl (1 μg/cm²/day), Mitsui-7 (1 μg/cm²/day), Mitsui-7 (2 μg/cm²/day). Each treatment has two rows corresponding to MDMs (-) and (+).

Legend:
- X – not statistically significant (compared to negative control)
- ✓ – statistically significant (compared to negative control)
- [red box] – statistical significance
- (-) macrophages (green)
- (+) macrophages (orange/yellow)

Supplementary table 6

Summary of statistical analysis of the obtained data of Fibronectin release from both tissues (with and without macrophages), x marks no statistically significant increase (p>0.05), ✓ marks statistically significant increase (p<0.05) compared to negative control. Untreated cells served as negative control for positive controls and DQ12 treated samples, while BSA-treated samples served as negative control for MWCNTs-treated cells. Data obtained in Laboratory 2.

COL1

Treatment	MDMs	D1 n1	D1 n2	D1 n3	D4 n1	D4 n2	D4 n3	D7 n1	D7 n2	D7 n3	D11 n1	D11 n2	D11 n3	D14 n1	D14 n2	D14 n3	D18 n1	D18 n2	D18 n3	D21 n1	D21 n2	D21 n3
TGF-β [10 ng/mL]	−	✓	x	x	✓	x	x	✓	x	x	✓	x	x	✓	x	✓	x	x	x	✓	✓	x
TGF-β [10 ng/mL]	+	x	✓	x	x	x	x	x	x	x	x	x	✓	x	x	✓	x	x	x	x	x	✓
TGF-β [50 ng/mL]	−	✓	x	x	✓	x	x	x	x	x	✓	x	x	✓	x	x	x	✓	x	✓	✓	x
TGF-β [50 ng/mL]	+	x	x	x	x	x	x	x	✓	x	x	✓	x	x	x	x	x	x	x	x	✓	x
DQ12 (0.2 µg/cm²/day)	−	✓	✓	x	✓	x	x	x	✓	x	x	✓	x	✓	✓	✓	x	✓	x	✓	✓	x
DQ12 (0.2 µg/cm²/day)	+	x	✓	x	x	x	✓	x	x	x	x	x	✓	x	x	✓	✓	✓	x	x	x	✓
Nanocyl (1 µg/cm²/day)	−	x	x	x	✓	✓	x	✓	✓	x	✓	x	x	x	x	✓	x	x	✓	x	✓	✓
Nanocyl (1 µg/cm²/day)	+	x	x	x	x	x	x	✓	✓	x	x	x	x	x	x	✓	x	x	x	x	x	x
Mitsui-7 (1 µg/cm²/day)	−	x	✓	x	✓	✓	x	✓	✓	x	✓	✓	x	✓	✓	x	x	✓	x	x	✓	x
Mitsui-7 (1 µg/cm²/day)	+	x	x	x	x	x	x	✓	x	x	✓	x	x	✓	x	x	✓	x	✓	x	x	✓
Mitsui-7 (2 µg/cm²/day)	−	x	✓	x	✓	✓	x	✓	✓	x	x	✓	x	✓	✓	✓	x	x	x	x	✓	x
Mitsui-7 (2 µg/cm²/day)	+	x	x	x	✓	x	x	x	✓	x	x	x	x	✓	x	✓	x	x	x	x	✓	x

Legend:

X – not statistically significant (compared to negative control)
✓ - statistically significant (compared to negative control)
▯ - statistical significance

(−) macrophages (green)
(+) macrophages (orange)

Supplementary table 7
Summary of statistical analysis of the obtained data of Collagen type 1 release from both tissues (with and without macrophages), x marks no statistically significant increase (p>0.05), ✓ marks statistically significant increase (p<0.05) compared to negative control. Untreated cells served as negative control for positive controls and DQ12 treated samples, while BSA-treated samples served as negative control for MWCNTs-treated cells. Data obtained in Laboratory 2.