

# An *In Vitro* Coculture System for the Detection of Sensitization Following Aerosol Exposure

## Supplementary Data

**Tab. S1: Statistical information on analyses used in the paper including the statistical test, P values, F values, and degrees of freedom**

The exact n values, which represent the number of biological replicates, used to calculate the statistics is also presented in the table. If n varied among experiments, the conditions are indicated in brackets next to the n value. When n did not vary in the experiment, conditions are not specified in the table.

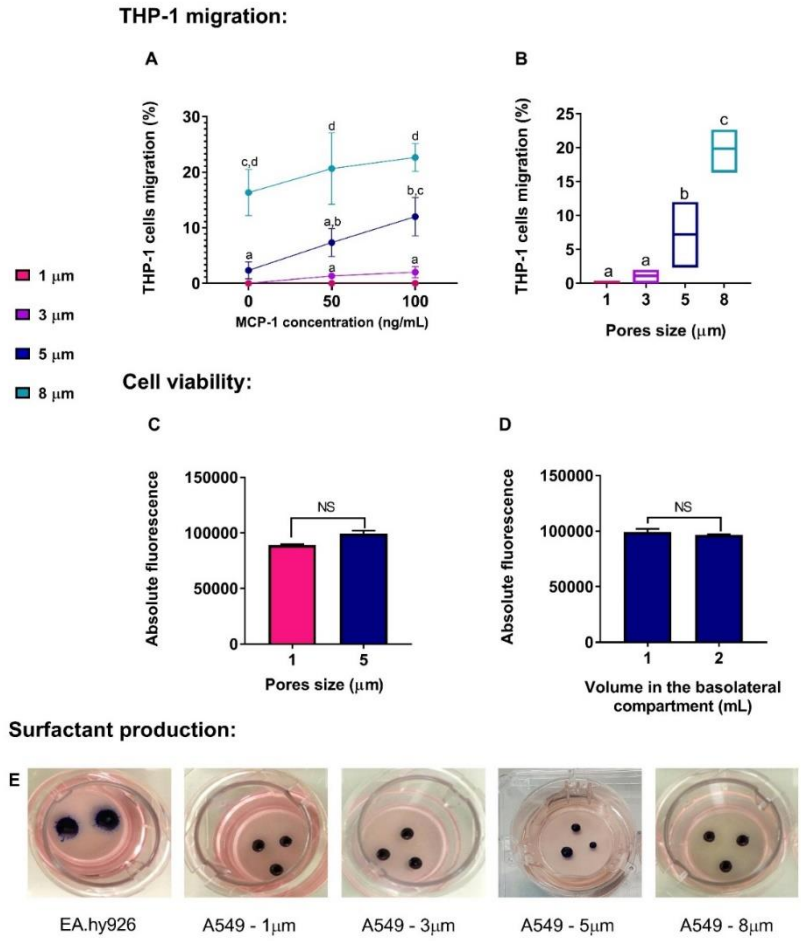
Experiments were done in at least 3 biological replicates. Unless otherwise specified, no technical replicates were included in the experimental design.

Related figures	Statistical tests	P values	F values	df	n values
S1A	2way ANOVA + Tukey post hoc test	0.002	8.475		3
S1B	2way ANOVA + Tukey post hoc test	< 0.001	103.4		3
S1C	Student's t-test	0.2212		4	3
S1D	Student's t-test	0.0888		4	3
S2E	2way ANOVA + Tukey post hoc test	0.513	0.6923		3
3B - CD54	Student's t-test	0.0003		5	4 (monoculture), 3 (coculture)
3B - CD86	Student's t-test	0.0076		5	4 (monoculture), 3 (coculture)
3C - CD54	Student's t-test	0.0003		5	4 (monoculture), 3 (coculture)
3C - CD86	Student's t-test	0.6209		5	4 (monoculture), 3 (coculture)
S3A - IL-6	Student's t-test	< 0,0001		13	7 (water), 6 (LPS)
S3A - MIP-3 $\alpha$	Student's t-test	0.004		7	4 (water), 5 (LPS)
S3A - RANTES	Student's t-test	< 0.0001		14	8
S3A - IL-1a	Student's t-test	0.0104		4	3
S3A - GM-CSF	Student's t-test	< 0,0001		13	7 (water), 8 (LPS)
S3A - IL-10	Student's t-test	0.0003		14	8
S3B - CIITA	Student's t-test	0.0026		6	4
S3B - MyD88	Student's t-test	0.0004		6	4
S3B - HLA-DMA	Student's t-test	0.0141		6	4
S3B - MAP2K1	Student's t-test	0.0193		6	4
S3B - G-CSF-R	Student's t-test	0.0019		6	4
S3B - CD80	Student's t-test	< 0.0001		6	4
4A1	ANOVA + Tukey post hoc test	< 0.001	10.4		3
4A2	ANOVA + Tukey post hoc test	0.001	8.52		3
4B1	Kruskal-Wallis test + Dunn's multiple comparisons test	0.01			4 (water, DMSO, Acr, MeSa), 3 (TMA, PA)
4B2	ANOVA + Tukey post hoc test	0.08	2.53		4 (water, DMSO, Acr, MeSa), 3 (TMA, PA)
5A	ANOVA + Tukey post hoc test	< 0.001	13.9		3
S5A	Kruskal-Wallis test + Dunn's multiple comparisons test	0.05			3
S5B	Kruskal-Wallis test + Dunn's multiple comparisons test	0.06			3
6A	ANOVA + Tukey post hoc test	< 0.001	20.2		3 (HDM), 4 (water, Bet v1)
S6A	ANOVA + Tukey post hoc test	0.8	0.226		3
S6B - IL-10	Kruskal-Wallis test + Dunn's multiple comparisons test	0.008			7 (water), 3 (Bet v1), 4 (HDM)
S6B - MCP-1	ANOVA + Tukey post hoc test	0.006	7.26		9 (water), 3 (Bet v1), 6 (HDM)
S6B - CCL20/MIP-3 $\alpha$	ANOVA + Tukey post hoc test	0.09	3.03		7 (water), 3 (Bet v1), 4 (HDM)
S6B - IL-6	Kruskal-Wallis test + Dunn's multiple comparisons test	< 0.001			7 (water), 3 (Bet v1), 4 (HDM)
S3C - IL1R1	ANOVA + Tukey post hoc test	0.009	9.82		4 (water), 3 (Bet v1, HDM)
S3C - MAP2K1	ANOVA + Tukey post hoc test	0.04	5.34		4 (water), 3 (Bet v1, HDM)
S3C - G-CSF-R	Kruskal-Wallis test + Dunn's multiple comparisons test	< 0.001			4 (water), 3 (Bet v1, HDM)
S3C - CIITA	Kruskal-Wallis test + Dunn's multiple comparisons test	0.02			4 (water), 3 (Bet v1, HDM)

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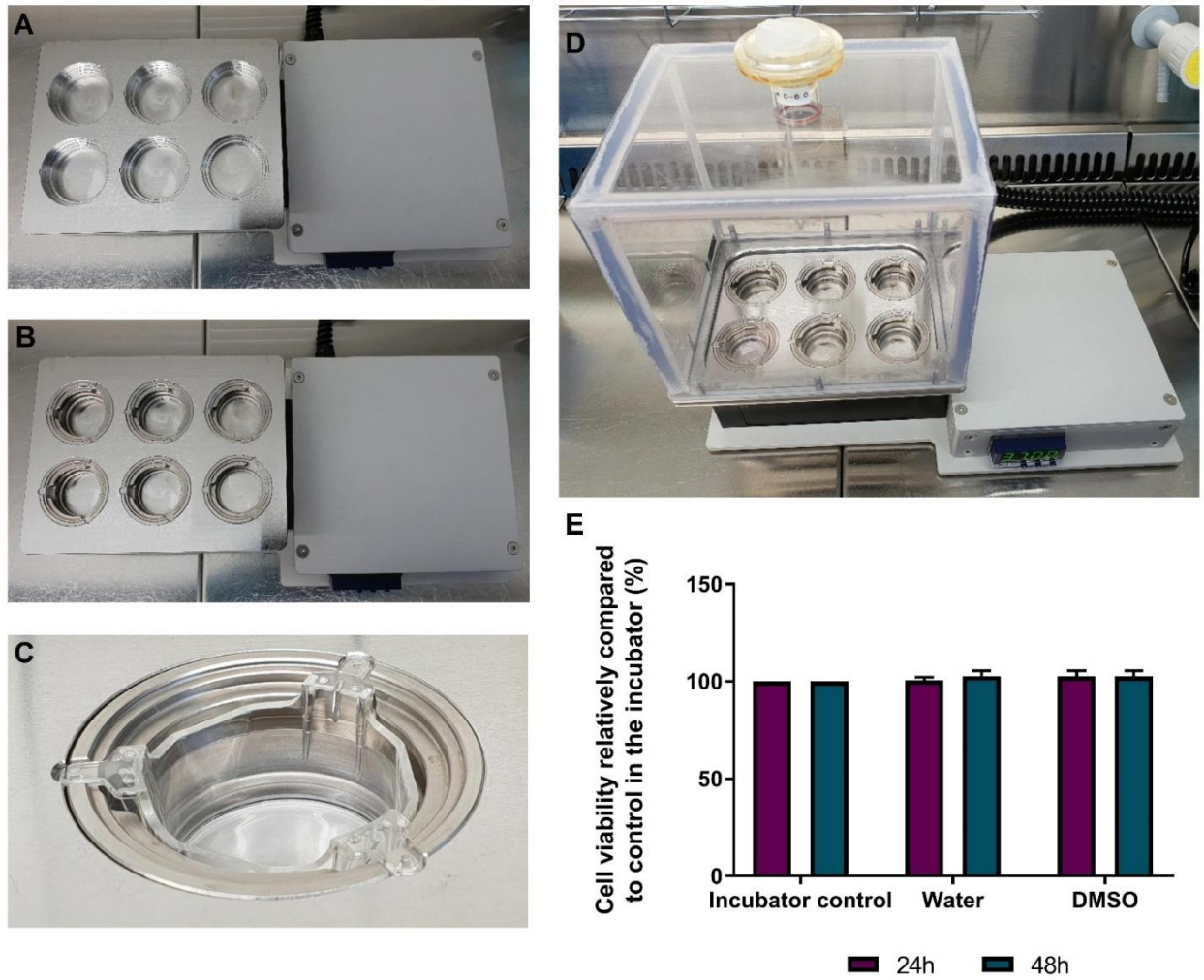
ALTEX 36(3), SUPPLEMENTARY DATA

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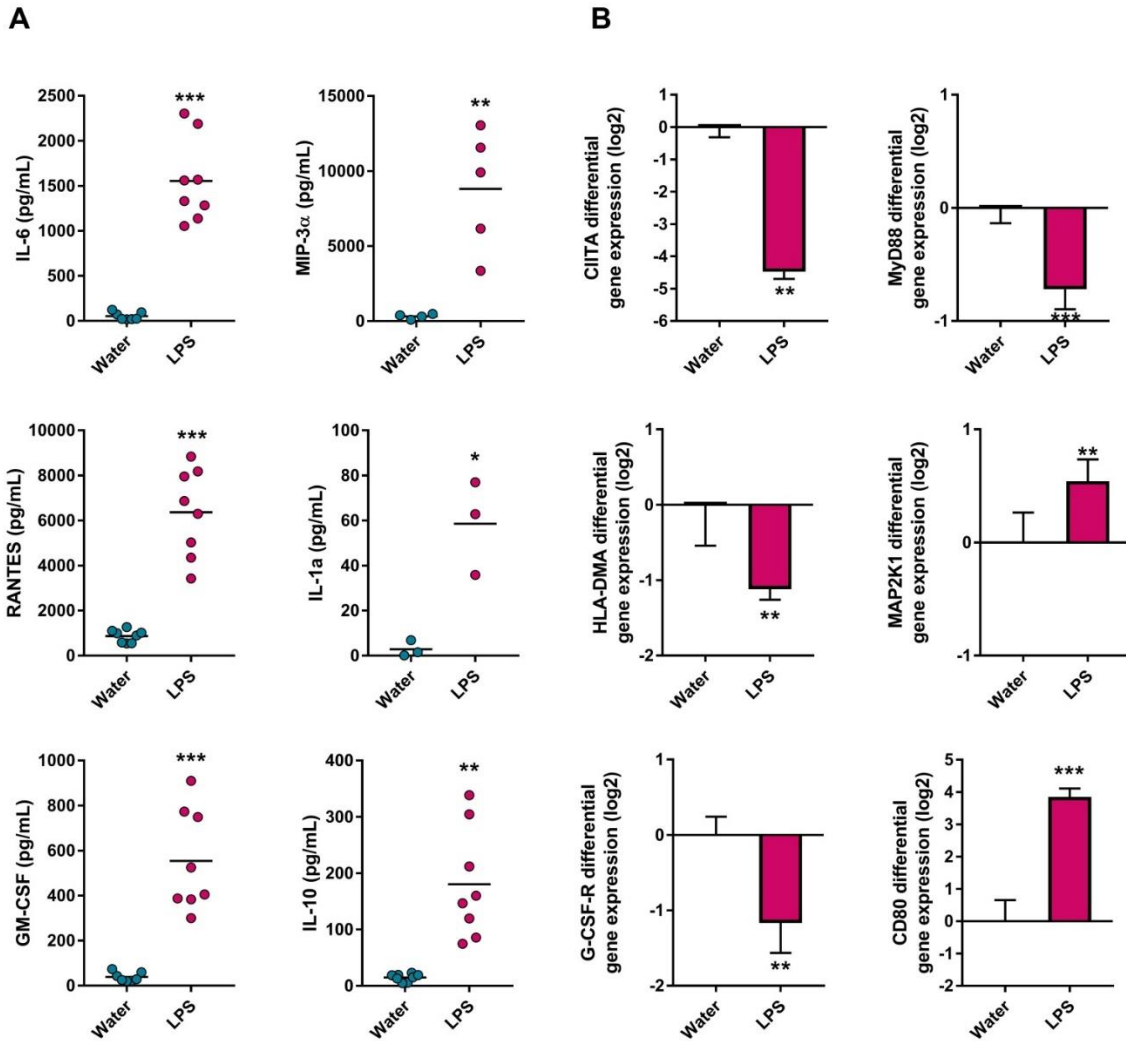


**Fig. S1: Determination of the most suitable pore size inserts allowing the migration of THP-1 cells**

(A) Percentage of THP-1 cells migrating through the membranes with different pore sizes after exposure to different concentrations of MCP-1 chemoattractant (mean  $\pm$  SEM, n=3, Groups that share the same letters are not significantly different ( $p > 0.05$ )). (B) Effect of the membrane pore sizes of the Transwell inserts on migration of THP-1 cells (mean  $\pm$  SEM, n=3, Groups that share the same letters are not significantly different ( $p > 0.05$ )). (C) Absolute fluorescence of converted Alamar Blue of A549 cells cultured at the ALI for 24 h on 1  $\mu$ m or 5  $\mu$ m pore size membranes (mean  $\pm$  SEM, n=3). (D) Absolute fluorescence of converted Alamar Blue of A549 cells cultured at the ALI for 24 h on 5  $\mu$ m pore size membranes with different volumes of medium (1 or 2 ml) in the basolateral compartment (mean  $\pm$  SEM, n=3). (E) Surfactant droplet test as described in (Schürch et al., 1978; Rothen-Rutishauser et al., 2008; Klein et al., 2013). DMP/O droplets were placed on the cell surface of the membrane to determine the surface tension. A large diameter of the droplet indicates a high surface tension (negative control: EA.hy926 cells), a small diameter of the droplet indicates a lower surface tension (A549 cells grown under ALI conditions on different pore sizes membranes).

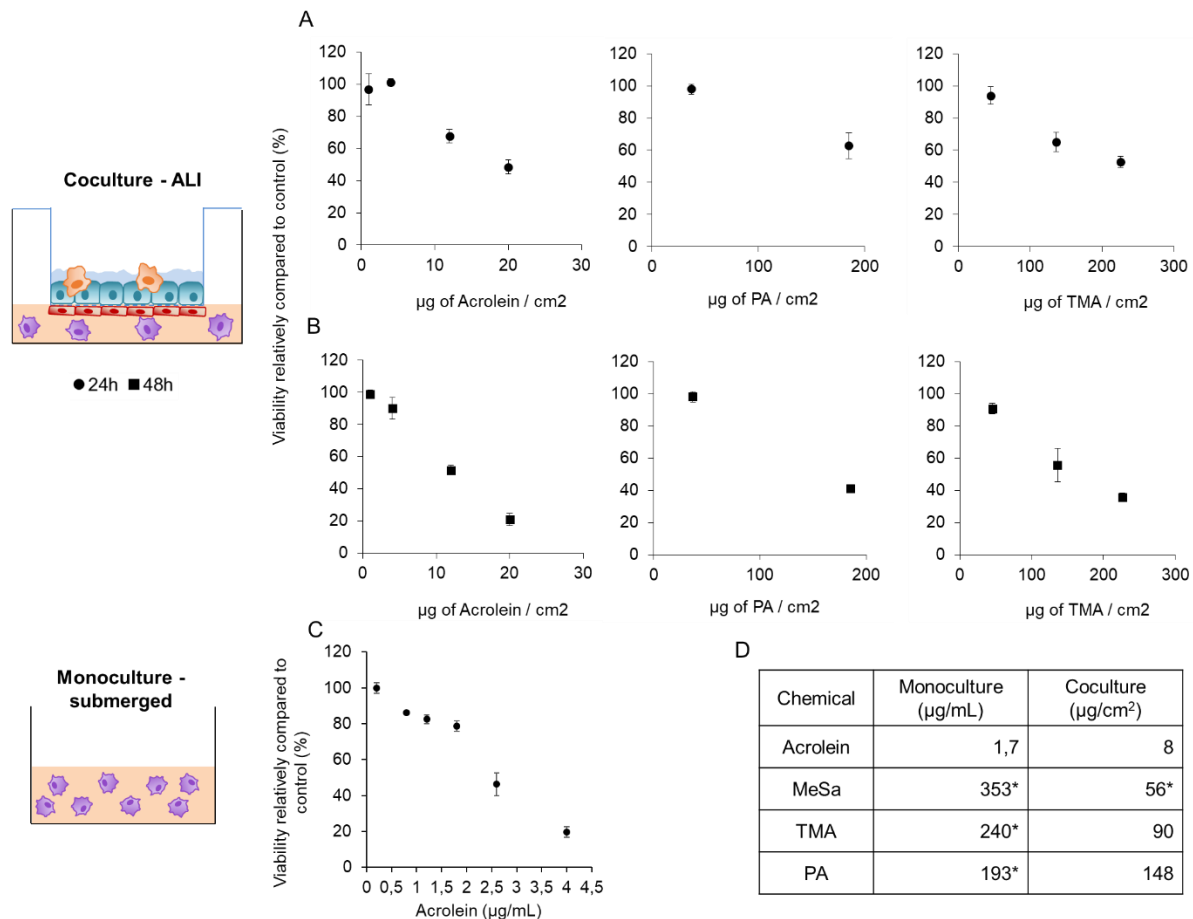


**Fig. S2: Exposure of coculture to vehicle controls at the ALI using the Vitrocell® cloud system has no influence on viability** (A) Picture of the exposure module in which (B) rings were placed to avoid direct exposure of the lower compartment. (C) Insert mounted into the ring and (D) overview of the complete modified Vitrocell® cloud system. (E) Viability of the coculture after exposure to water and DMSO vehicle controls after 24 h and 48 h as compared to unexposed inserts left in the incubator (mean ± SEM, n=3).



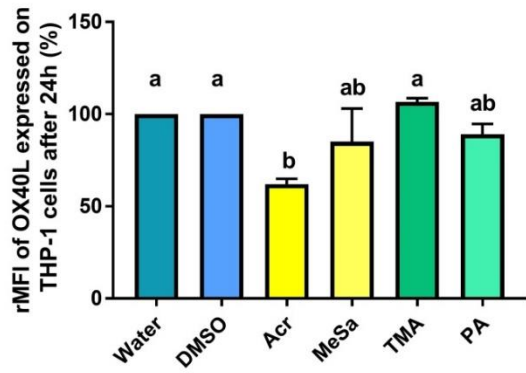
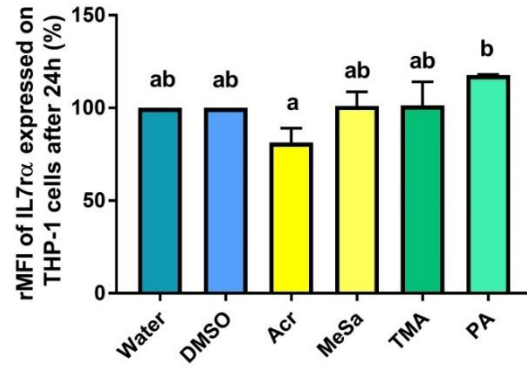
**Fig. S3: LPS stimulation induced release of cytokines and the regulation of genes involved in sensitization in THP-1 cells in the coculture 24 h after exposure**

(A) Concentration (pg/ml) of IL-6, MIP-3 $\alpha$  (CCL20), RANTES, IL-1 $\alpha$ , GM-CSF, and IL-10 cytokines released into medium 24 h after exposure to LPS or its vehicle control (water) at the ALI (Scatter plot and mean) and (B) expression of *CIITA*, *CD80*, *G-CSF-R*, *HLA-DMA*, *MAP2K1*, and *MyD88* genes 24 h after exposure to LPS or its vehicle control (water) at the ALI. Results are expressed on the log 2 scale for the differential gene expression (mean  $\pm$  SEM, n=4).

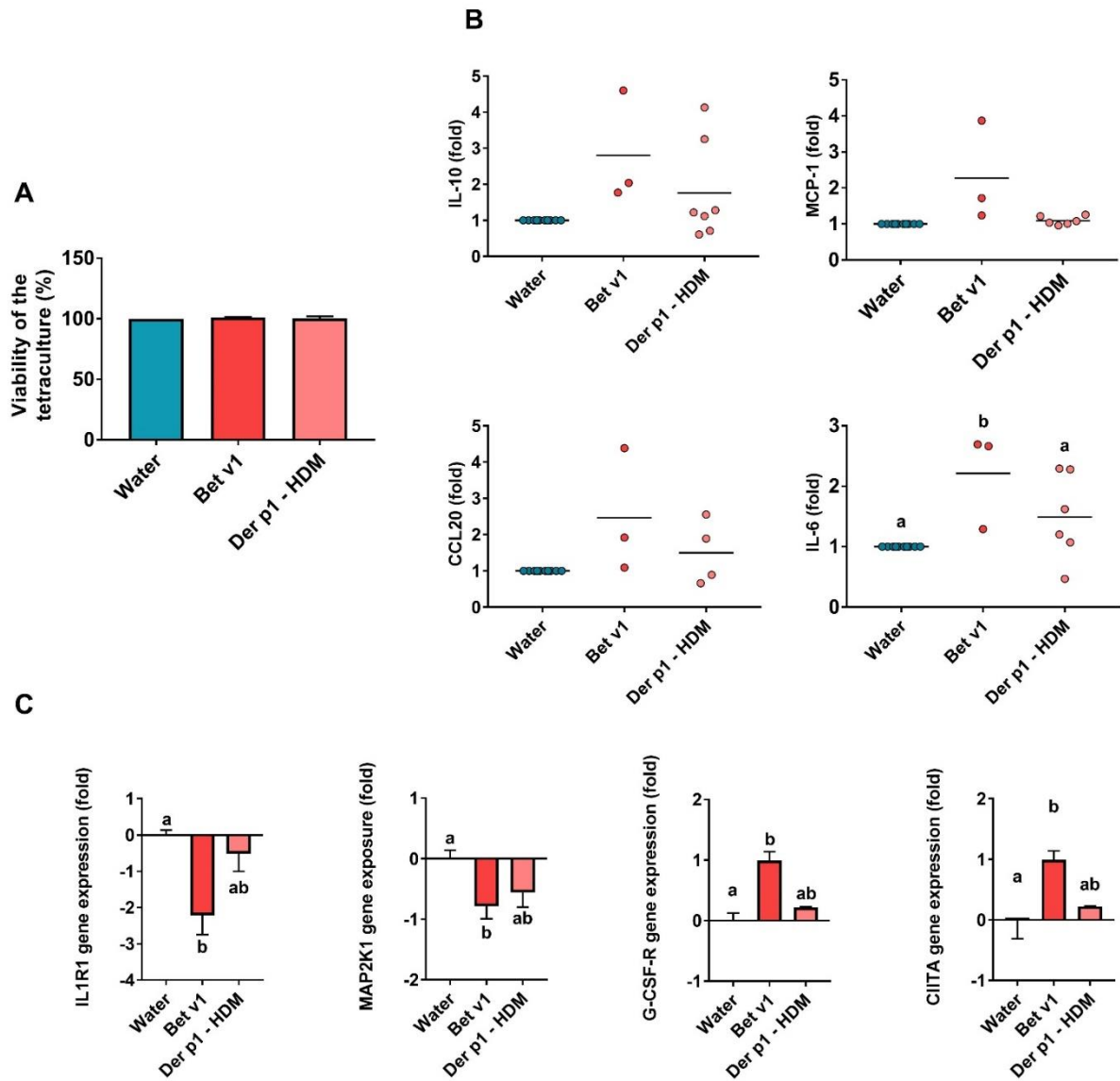


**Fig. S4: Viability assessment after chemical exposure**

(A) Relative viability of the complete coculture 24 h after exposure to chemical compounds (mean ± SEM). (B) Relative viability of the complete coculture 48 h after exposure to chemical compounds (mean ± SEM). (C) Viability of THP-1 cells in monoculture after 24 h exposure to acrolein (mean ± SEM). Table (D) summarizes calculated cell viability 75% (CV75) to which mono- and coculture were exposed for the following experiments, \* indicates when CV75 could not be reached; cells were then exposed to the chemicals at maximum solubility.

**A****B****Fig. S5: Additional THP-1 cell surface marker expression**

Relative MFI of (A) OX40L and (B) IL7ra expressed on THP-1 cells in the coculture 24 h after exposure to chemical sensitizers and irritants at the ALI (mean  $\pm$  SEM, n=3, Groups that share the same letters are not significantly different ( $p > 0.05$ )).



**Fig. S6: Additional tested markers to assess respiratory sensitization to proteins**

(A) Viability of the tissue after exposure to protein sensitizers (mean  $\pm$  SEM, n=3). (B) IL-10, MCP-1, CCL20 (MIP-3 $\alpha$ ), and IL-6 cytokines released in the medium (Scatter plot and mean) and (C) CIITA, G-CSF-R, MAP2K1, and IL1R1 gene expression after 24 h exposure to proteins. Results are expressed on the log<sub>2</sub> scale for the differential gene expression (mean  $\pm$  SEM, n=3-4, Groups that share the same letters are not significantly different (p > 0.05)).