



Lung single cell transcriptomics to guide the development for AOP anchored-cell based assays in response to nanoparticle exposure

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Defense mechanisms for inhaled particles:

- **airway epithelium: mucus mucociliary clearance**

mechanism found in marine invertebrates >500 Mio. years ago

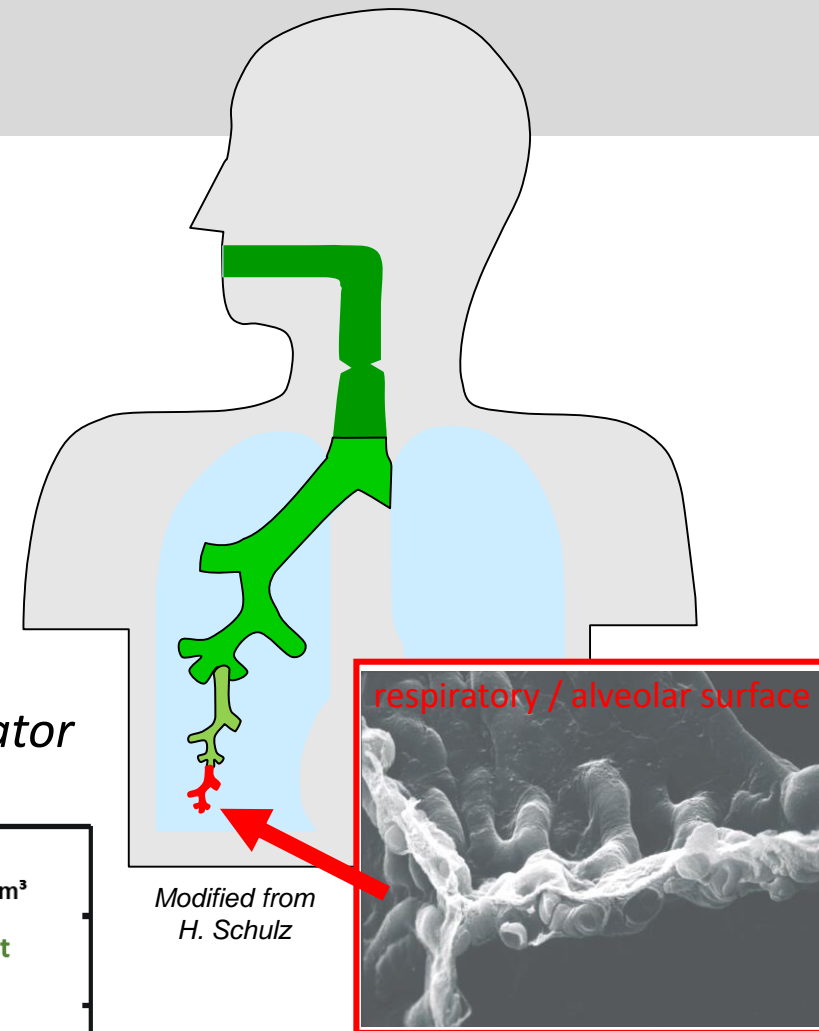
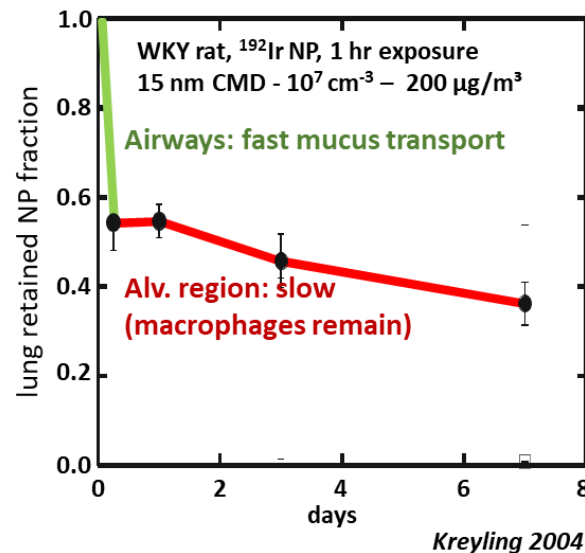
→ very fast (min.) & effective removal of PM from the lung

- **respiratory / alveolar epithelium: particle phagocytosis**

→ fast (hrs.) phagocytic removal of PM from respiratory surface, but slow removal from lung tissue (*clearance of AMs*)

slow removal of particle laden macrophages via the mucociliary escalator

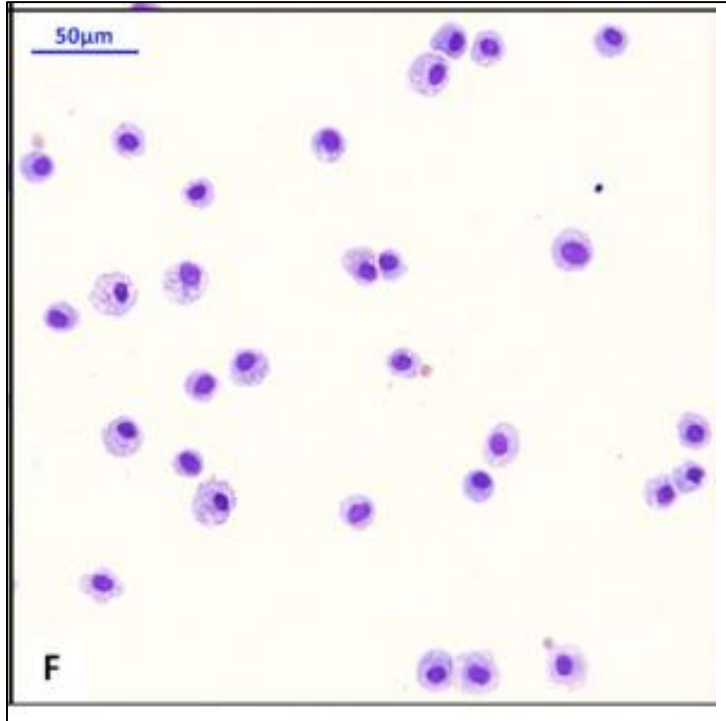
*Paradox for effective alveolar clearance:
Long half-life and low turn-over rates of
tissue resident alveolar macrophages
(mouse: 1-2 years; human: several years)*



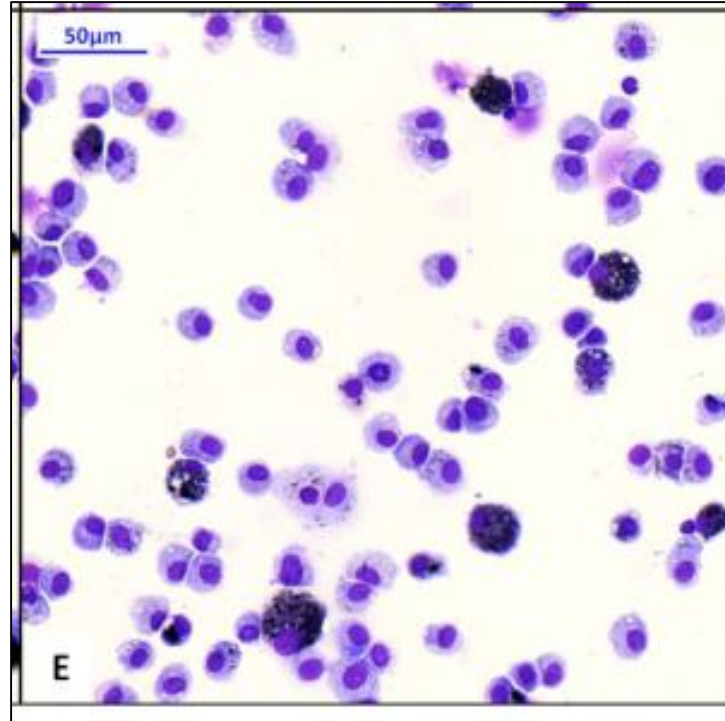
Slow removal of alveolar deposited NPs from the lungs

Mouse cytopins show presence of CNP-laden alveolar macrophages (AM) at day 90 after exposure

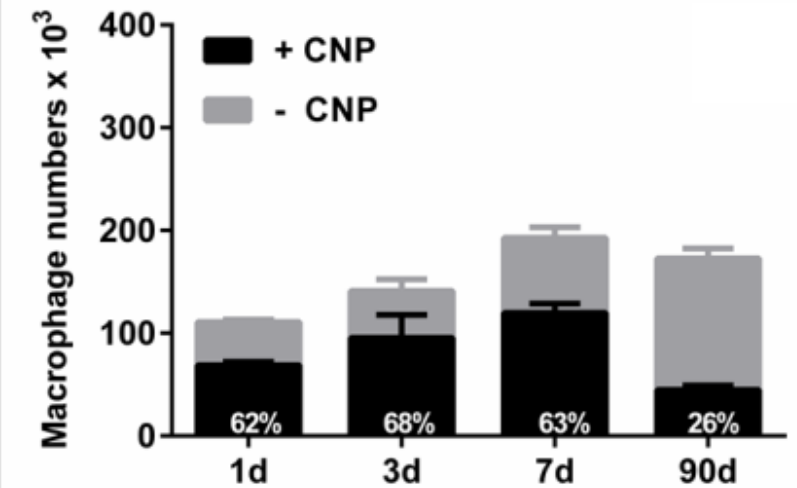
control



day 90 (50ug CNP)

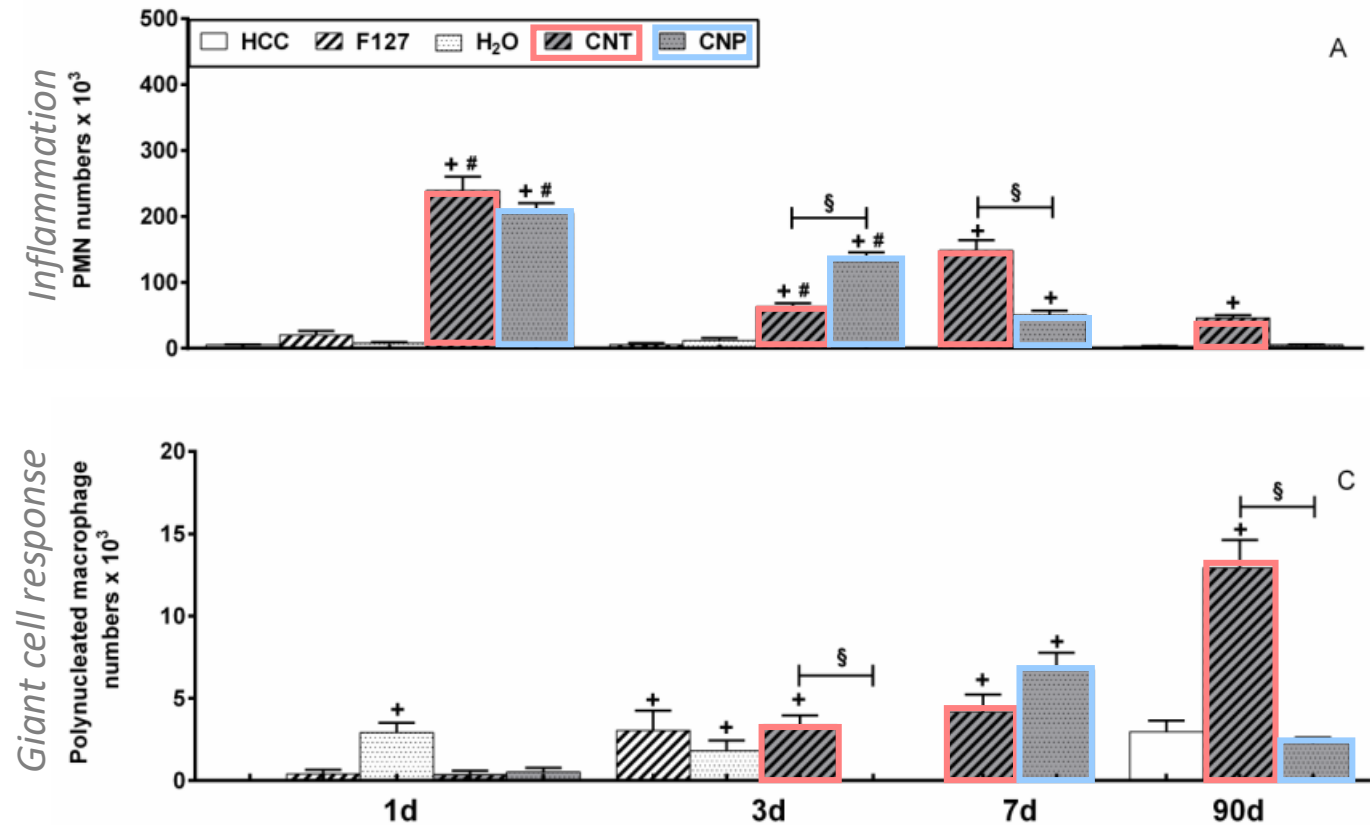
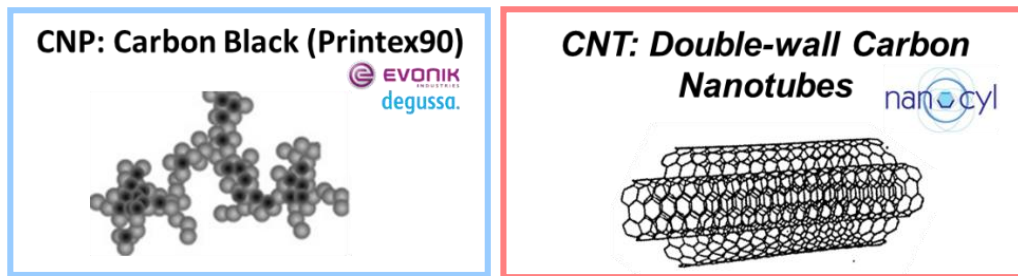
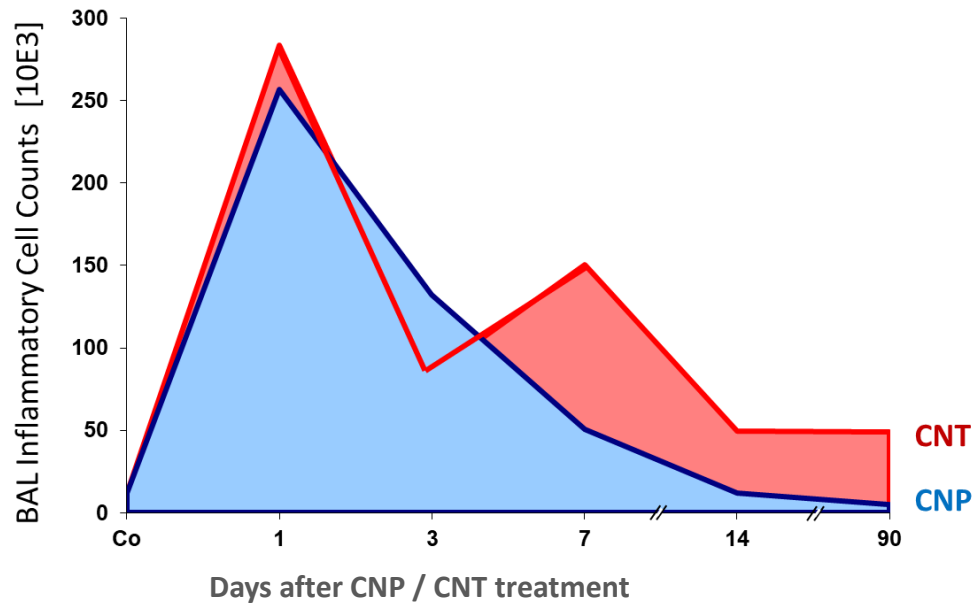


Persistent incorporation of CNPs in AM determined by light microscopic analysis of respective BAL cytopin images



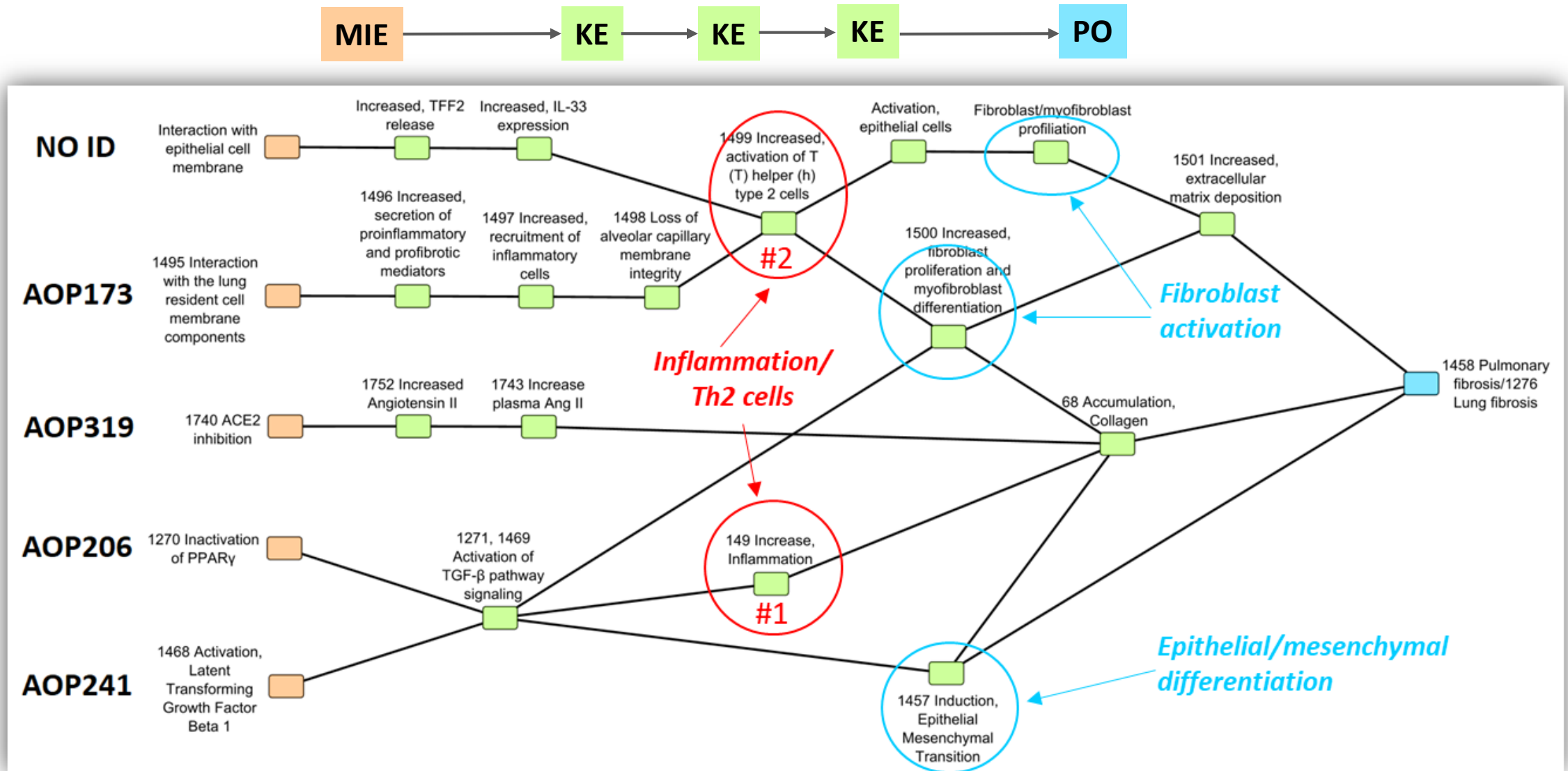
Dependent of shape, NPs of equal composition (carbon) and dose may cause acute or chronic inflammation

Lung inflammation time course of mice exposed to equal doses (mass and surface area) of spherical CNP or fiber-shaped CNTs



AOP anchored-cell based assays to predict nanomaterial specific toxicity

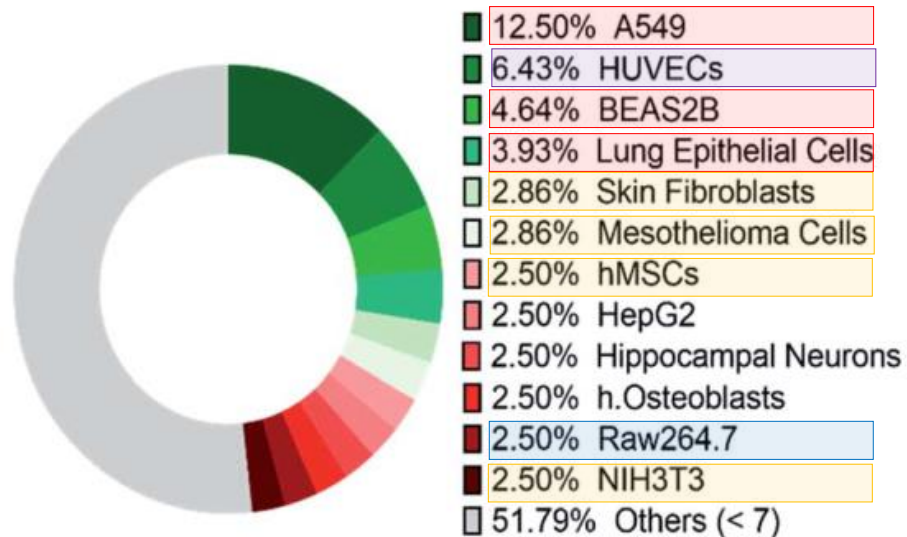
Pulmonary fibrosis based AOPs



Are we using the appropriate cells & cell types?

Overview of cell types and cell toxic test applied for CNT toxicity studies

Type of cells



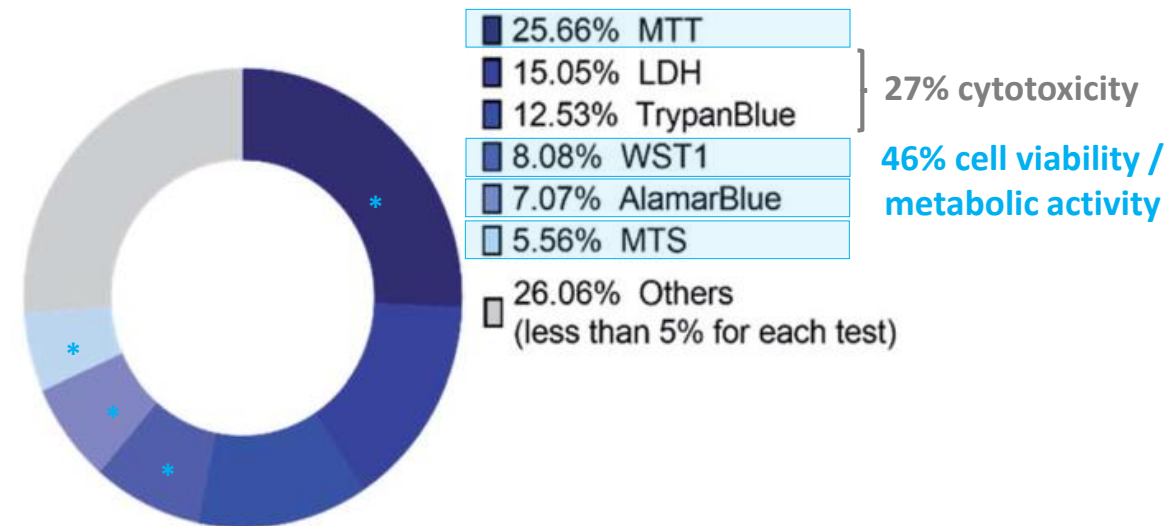
20% lung epithelium

6% endothelium

11% mesothelium + fibroblast

3% phagocytes

Cytotoxicity assay



Single cell RNA sequencing to gain new insights in cell-particle interactions in the lung

human lung:

85 molecular cell types

33 'tissue' cells types:

15 epithelial cell types

airway, alveolar,...

9 endothelial cell types

artery/vein, capillary, bronchiolar, lymphatic, ...

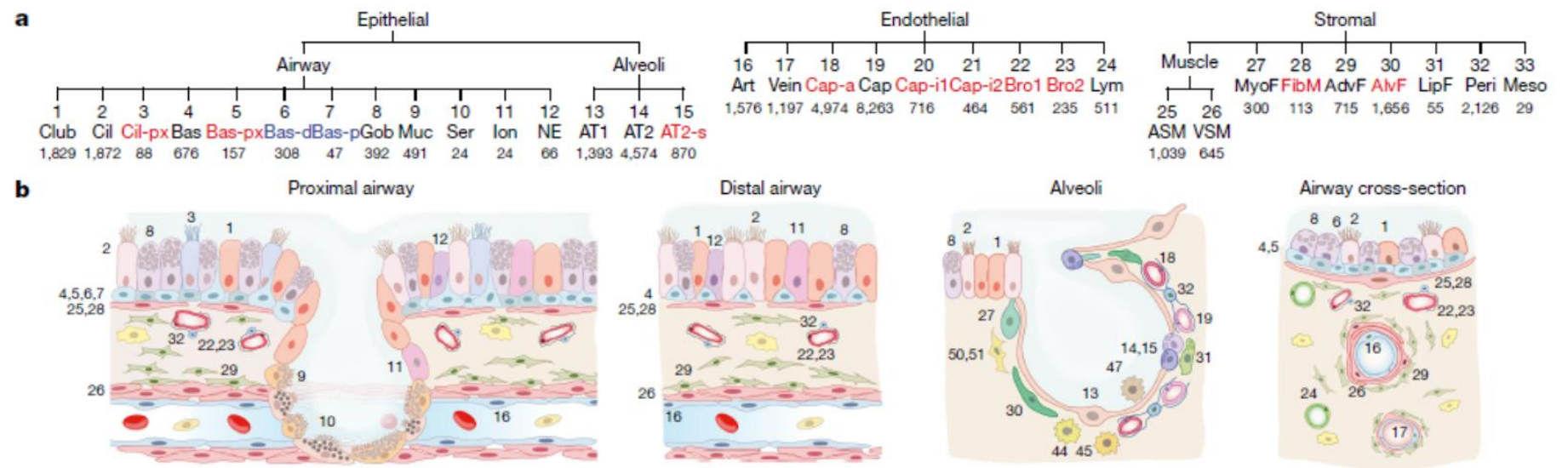
9 stromal cell types:

muscle, fibroblasts, mesothelial, ...

Article

A molecular cell atlas of the human lung from single-cell RNA sequencing

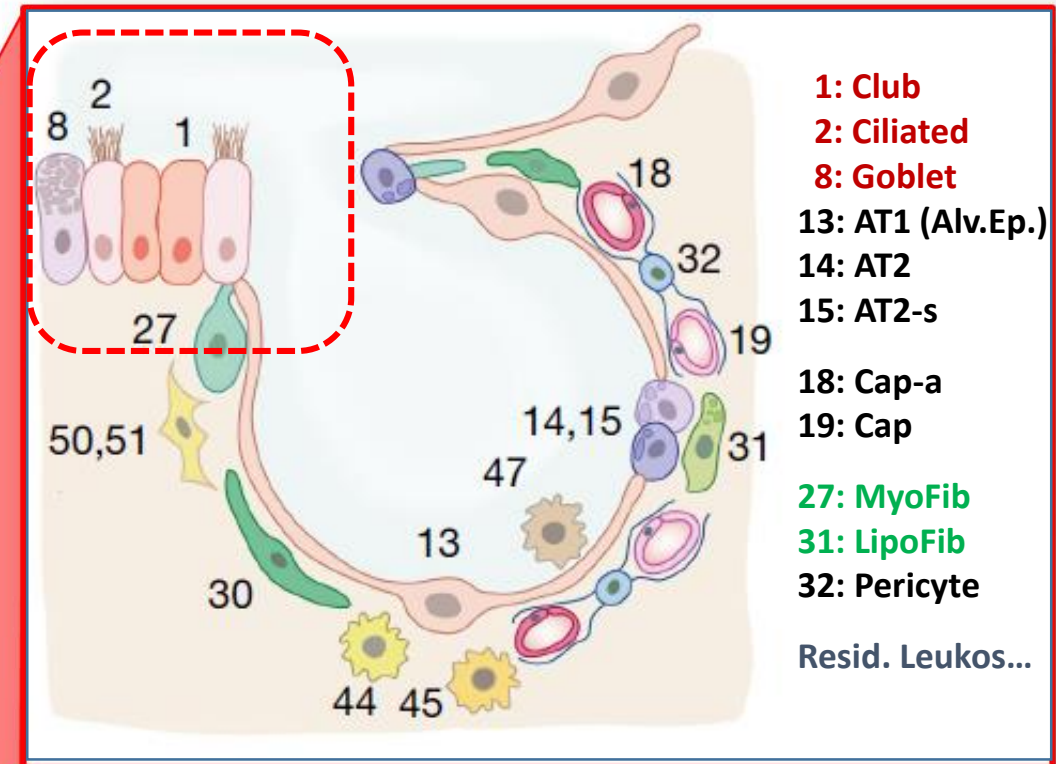
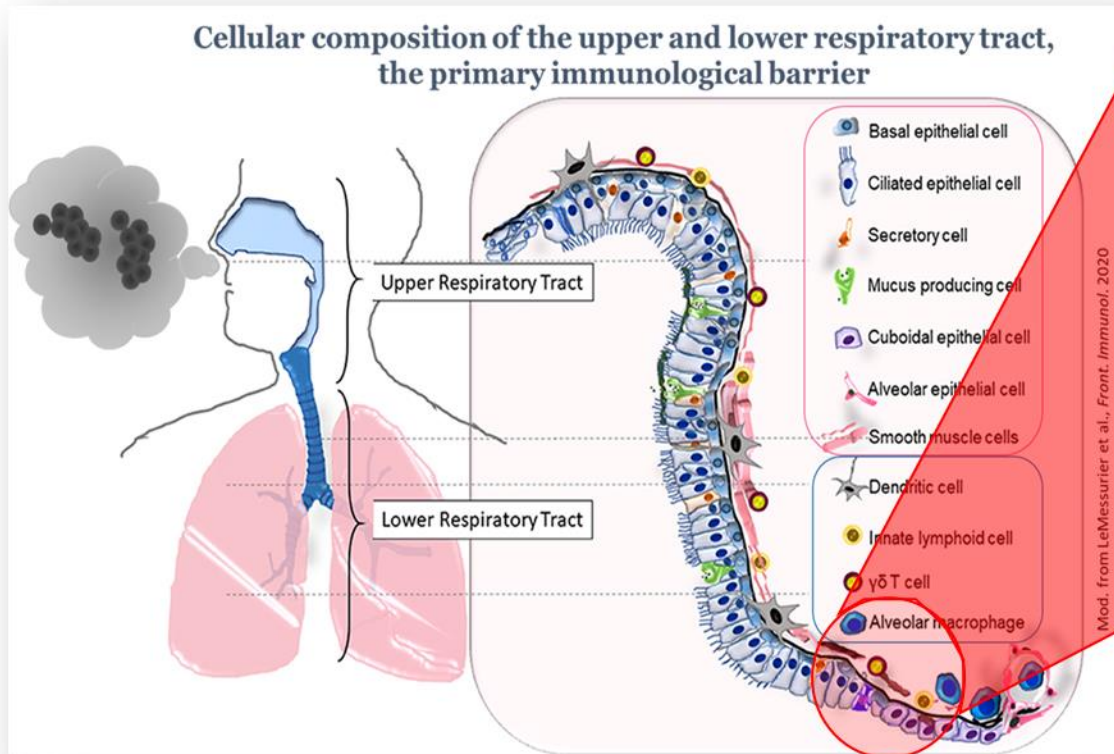
Travaglini et al., Nature, 2020



black, canonical types; blue, proliferating or differentiating subpopulations; red, novel populations; number of cells shown below cluster name

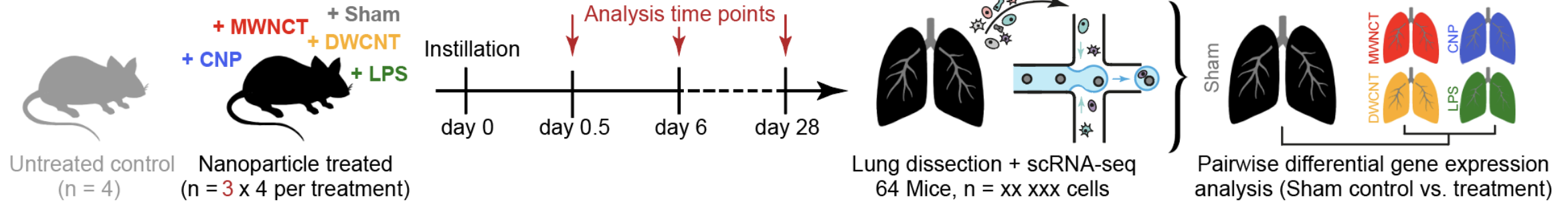
Deposition hotspots of inhaled NPs

Deposition hot spots for inhaled particles



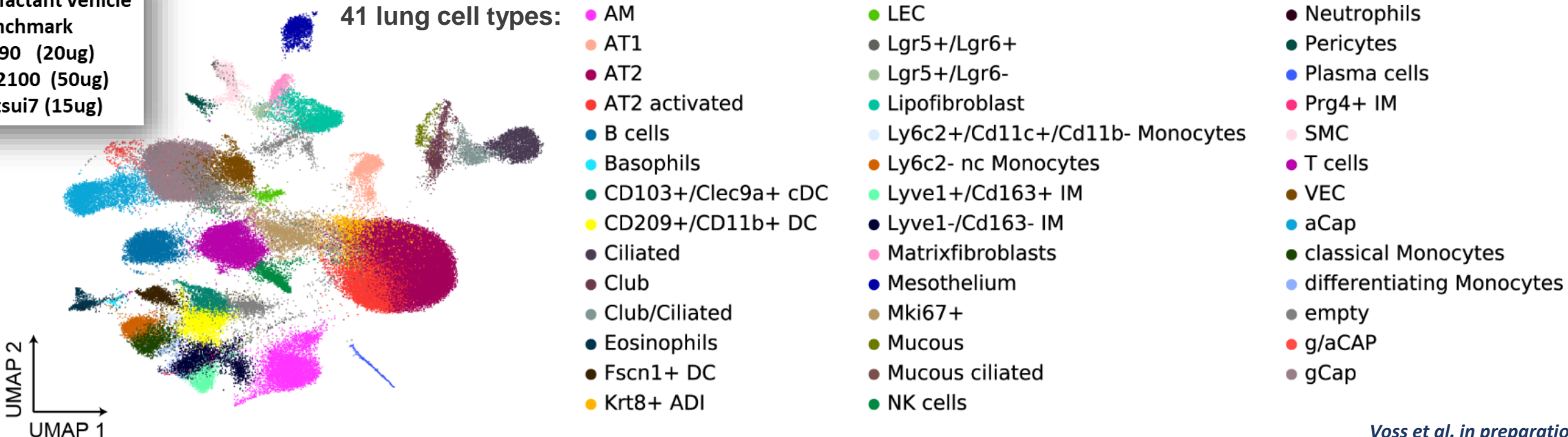
Single cell RNA sequencing to gain new insights in material specific cell-particle interactions in the lung

a



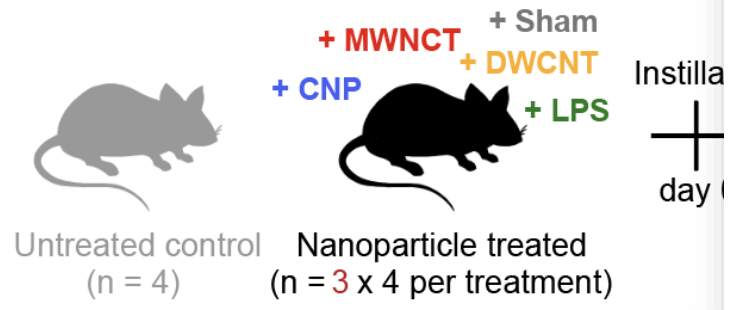
Exposure / NMs:

| | |
|-------|--------------------|
| Sham | surfactant vehicle |
| LPS | benchmark |
| CNP | Ptx90 (20ug) |
| DWCNT | NC2100 (50ug) |
| MWCNT | Mitsui7 (15ug) |



Single cell RNA sequencing to gain new insights in material specific cell-particle interactions

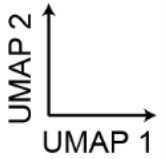
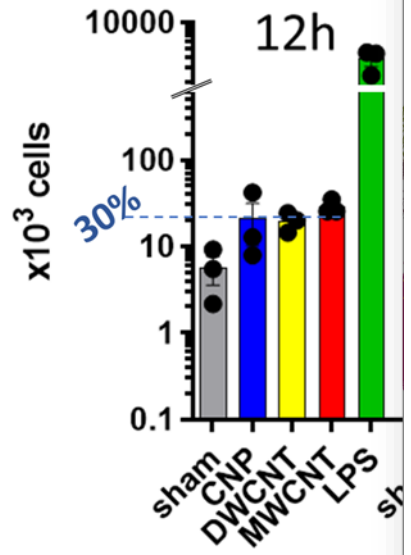
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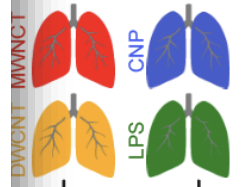
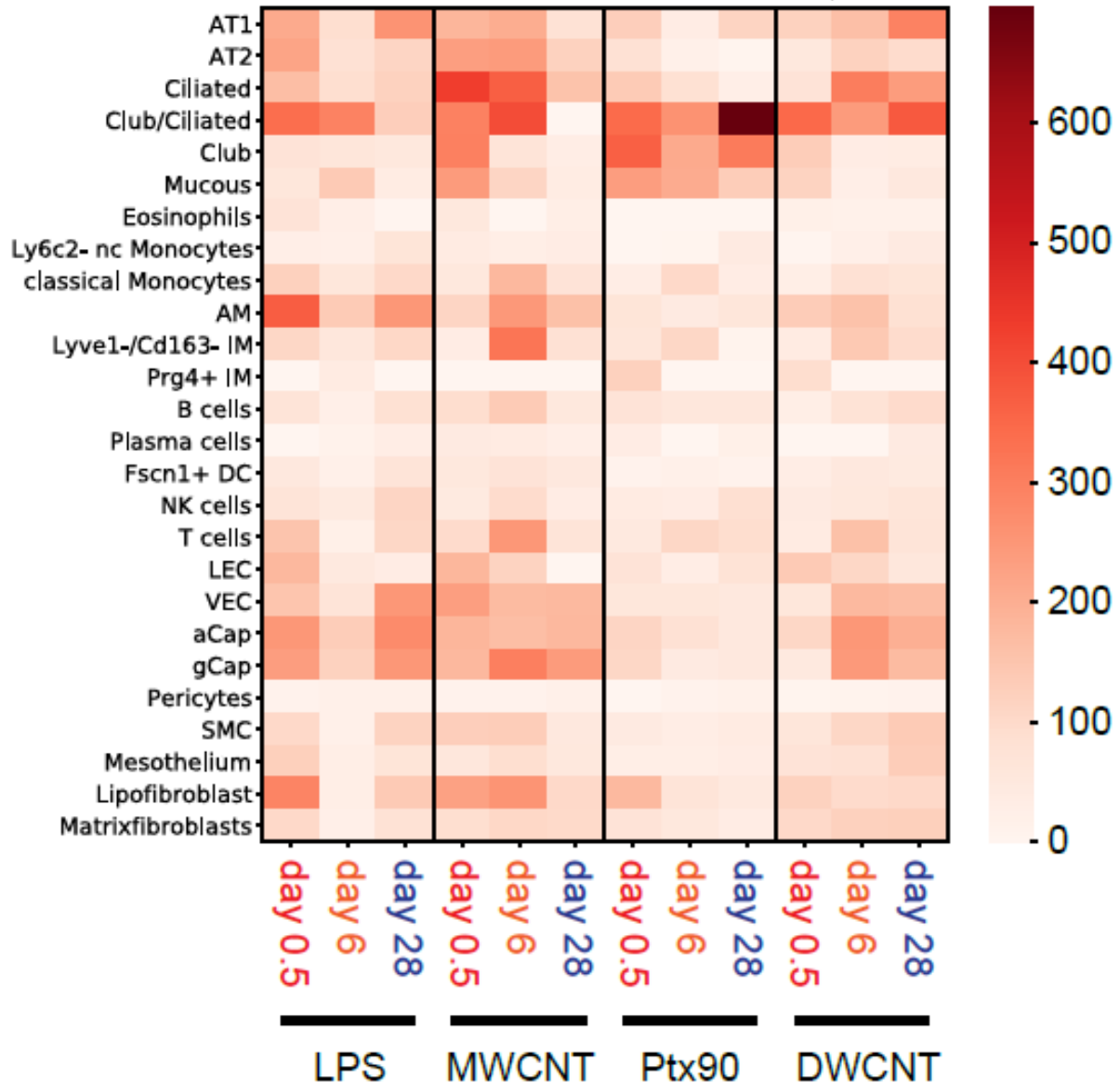
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| Sham | surfactant vehicle |
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BAL Neutrophils



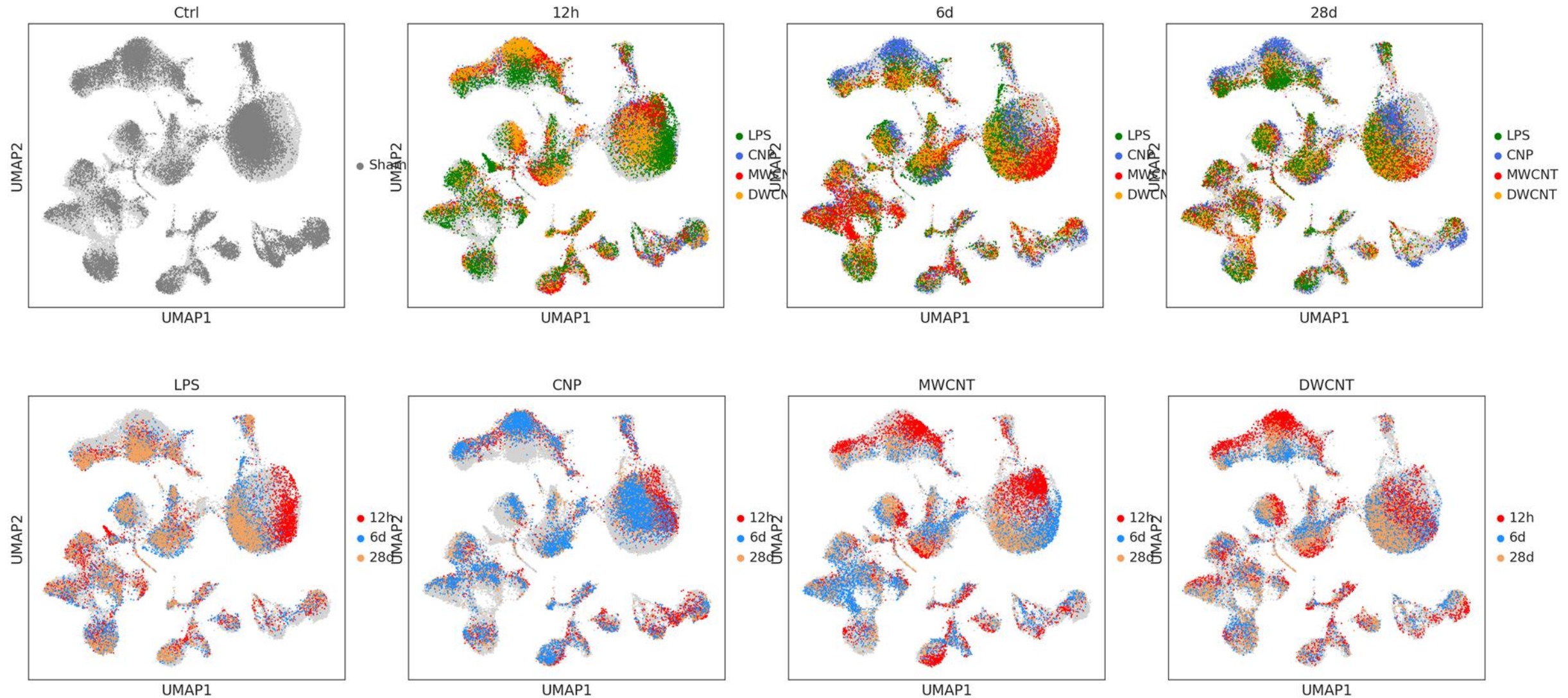
of upregulated genes with logFC > 0.5



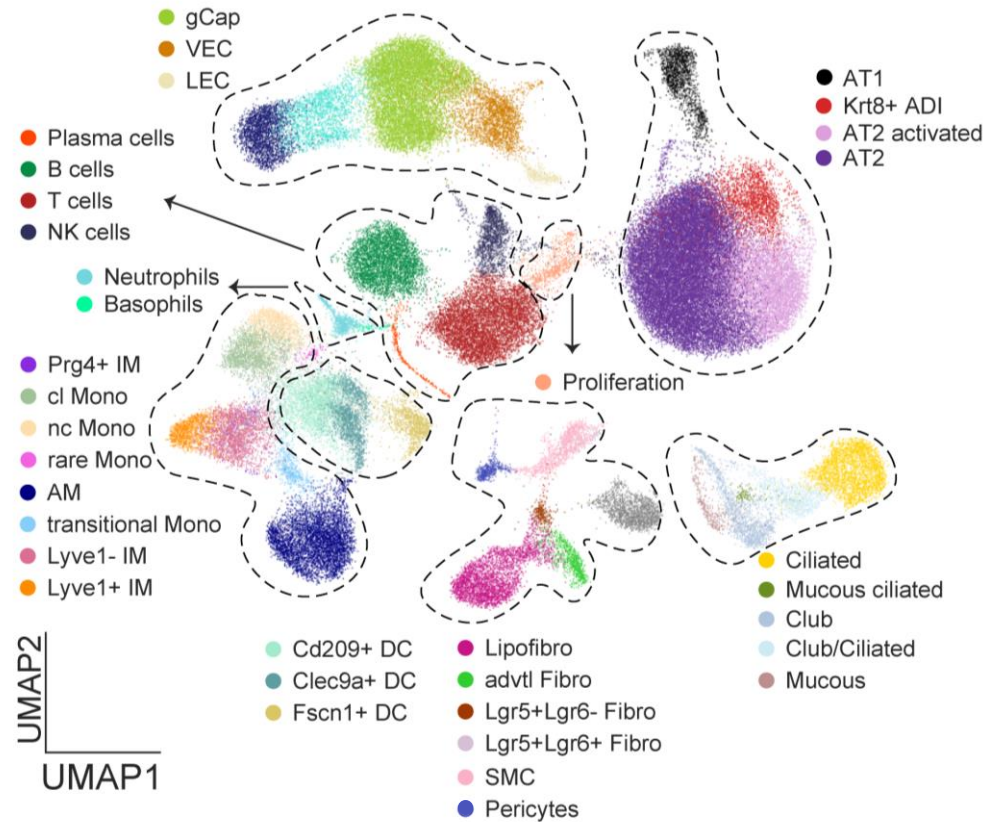
al gene expression
control vs. treatment)

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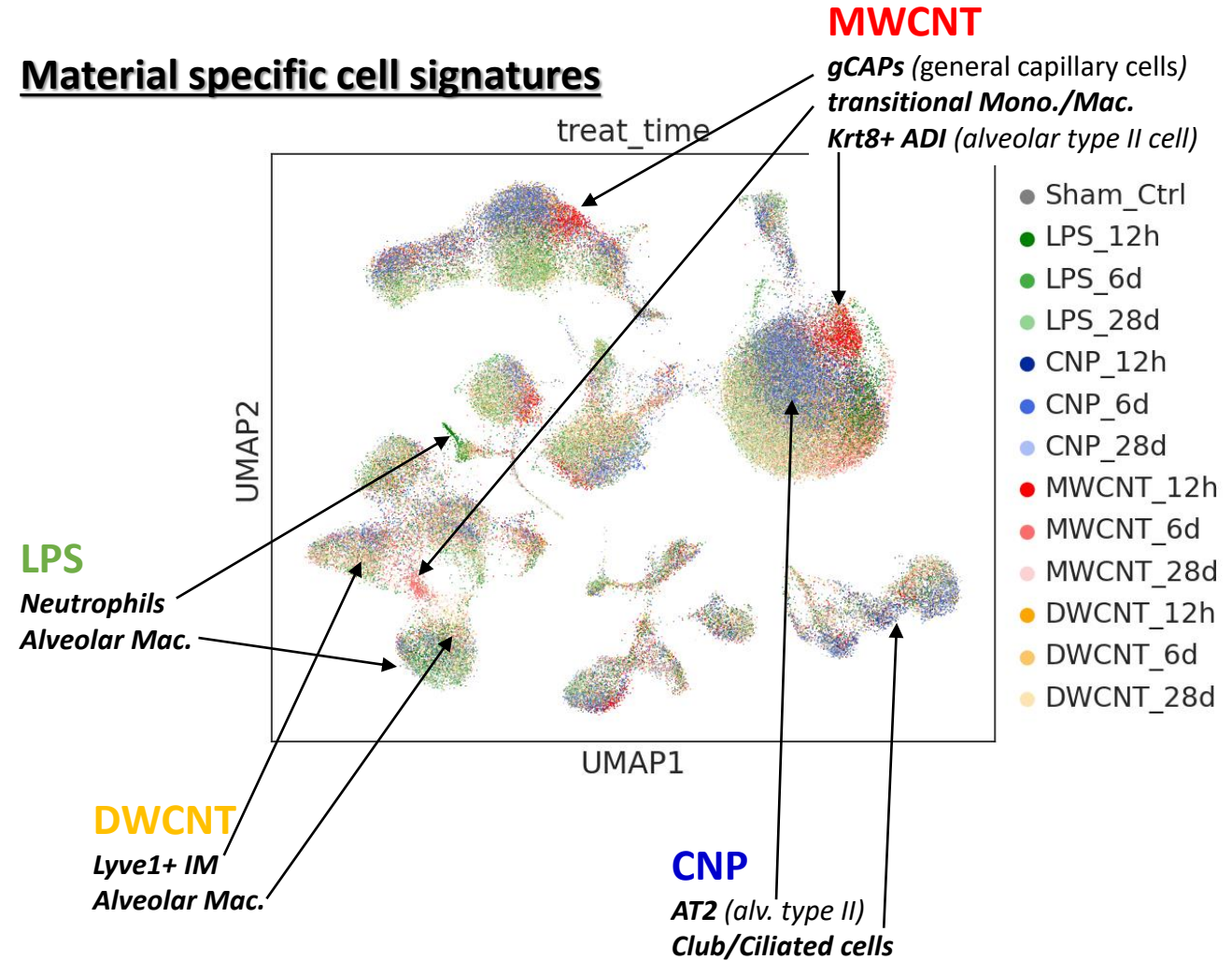
Single cell RNA sequencing of NM exposed mouse lungs reveals material-specific cellular perturbation pattern



Single cell RNA sequencing of NM exposed mouse lungs reveals material-specific cellular perturbation pattern

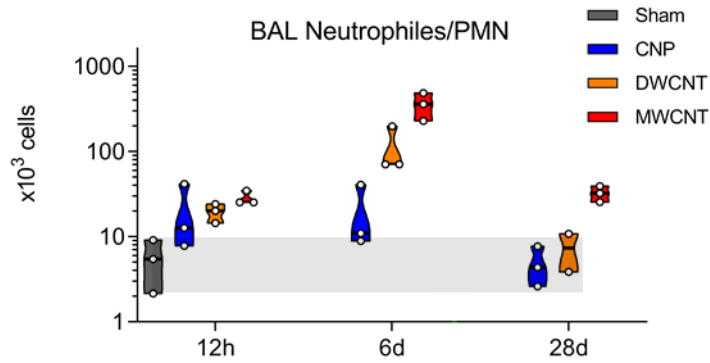


Material specific cell signatures



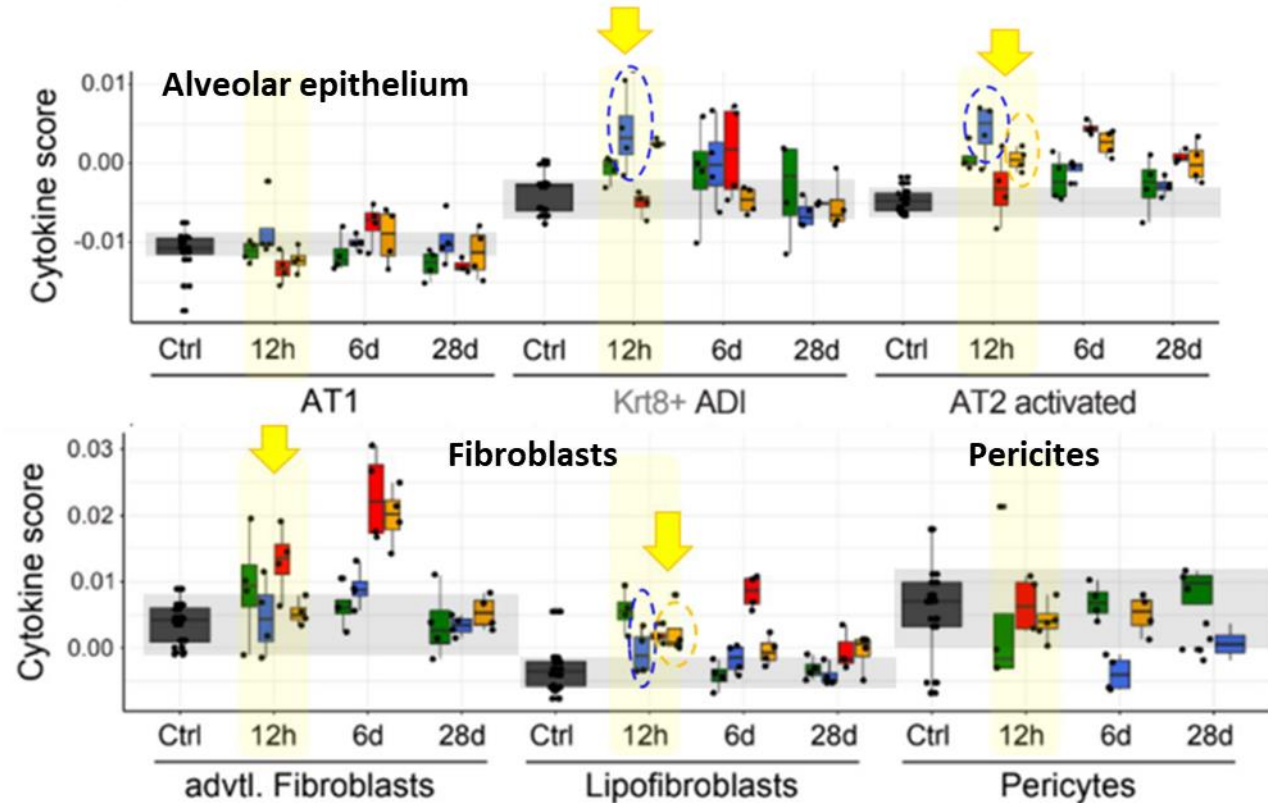
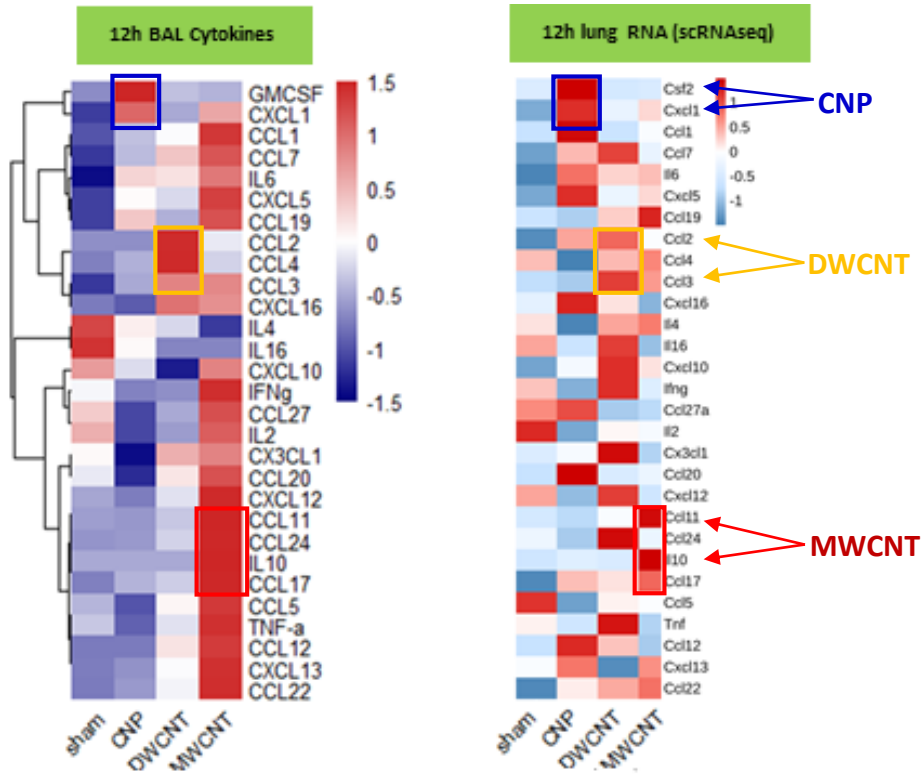
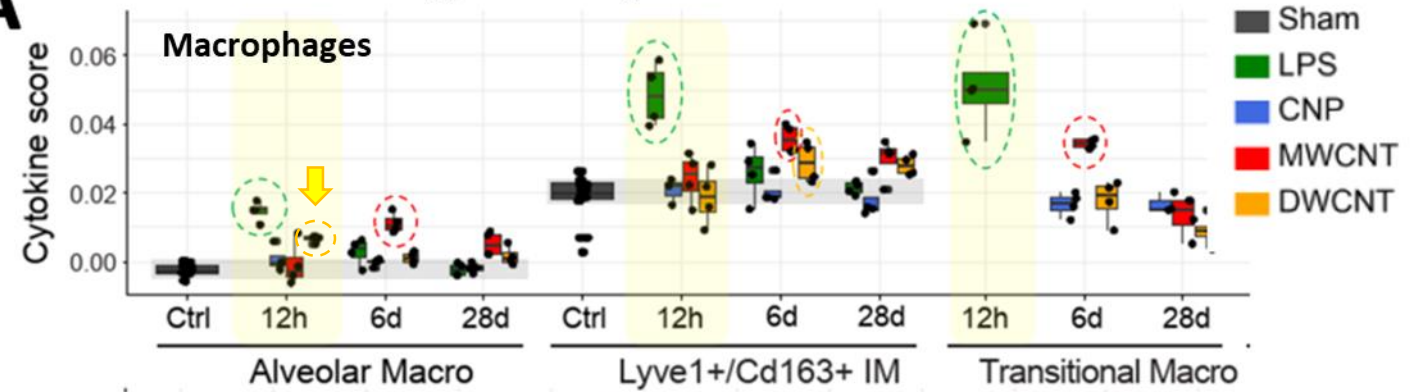
Material specific initiation of the inflammatory response

Equal level of acute lung neutrophilia



A

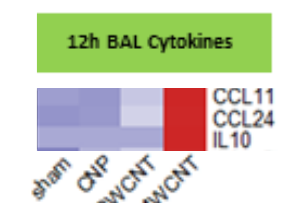
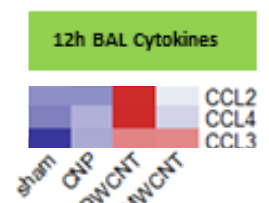
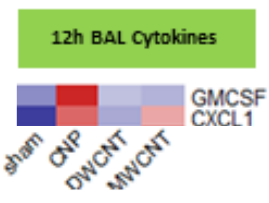
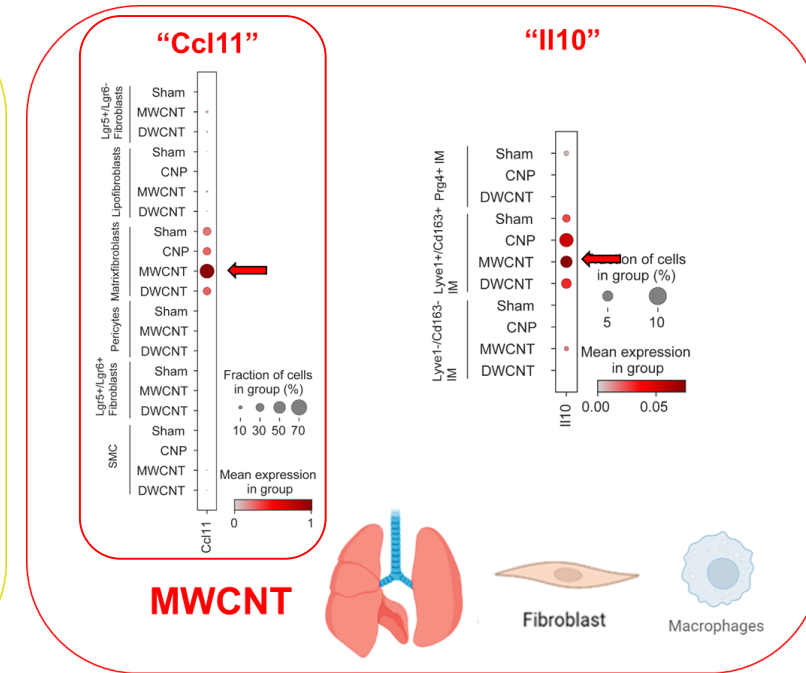
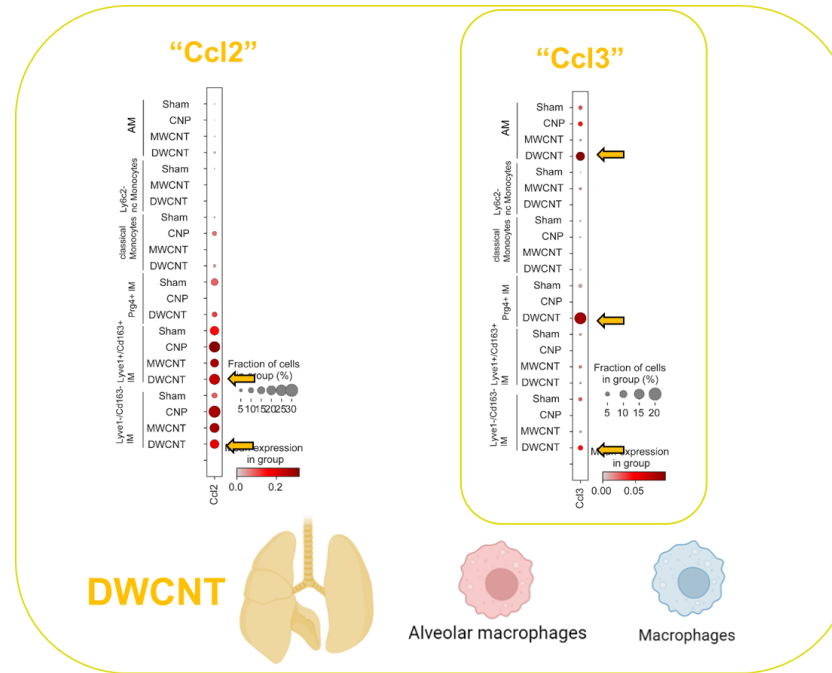
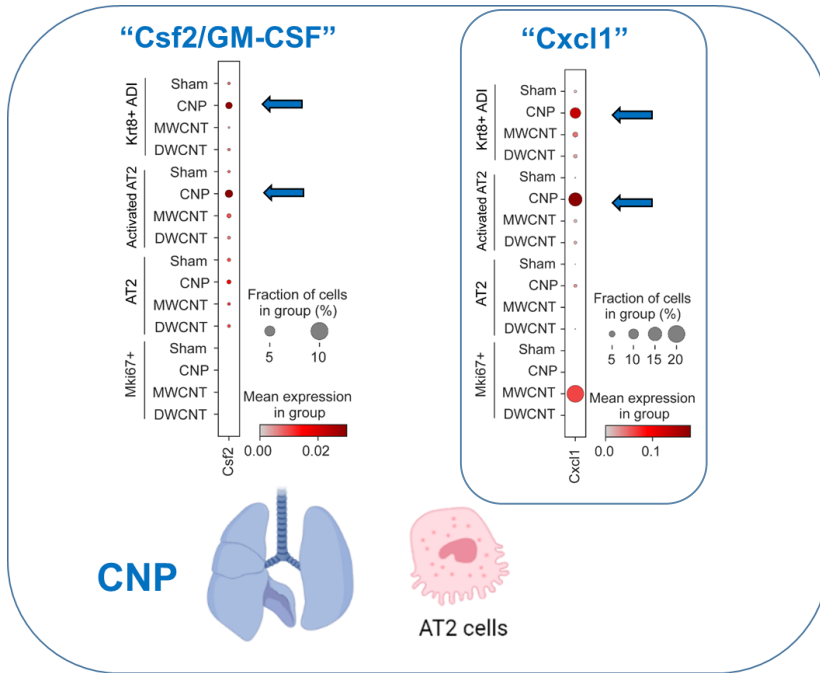
single cell cytokine scores



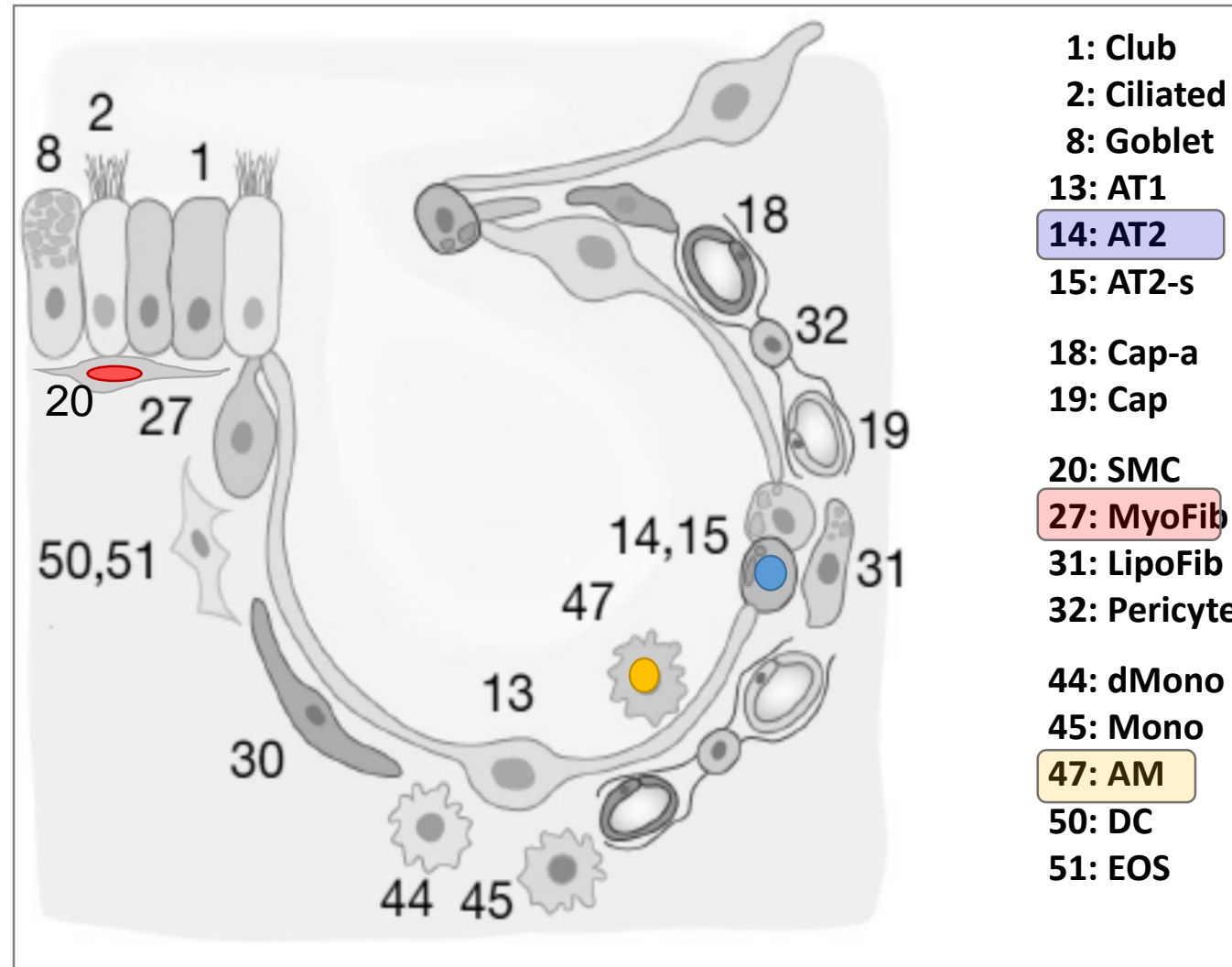
Material specific initiation of the inflammatory response

single gene level

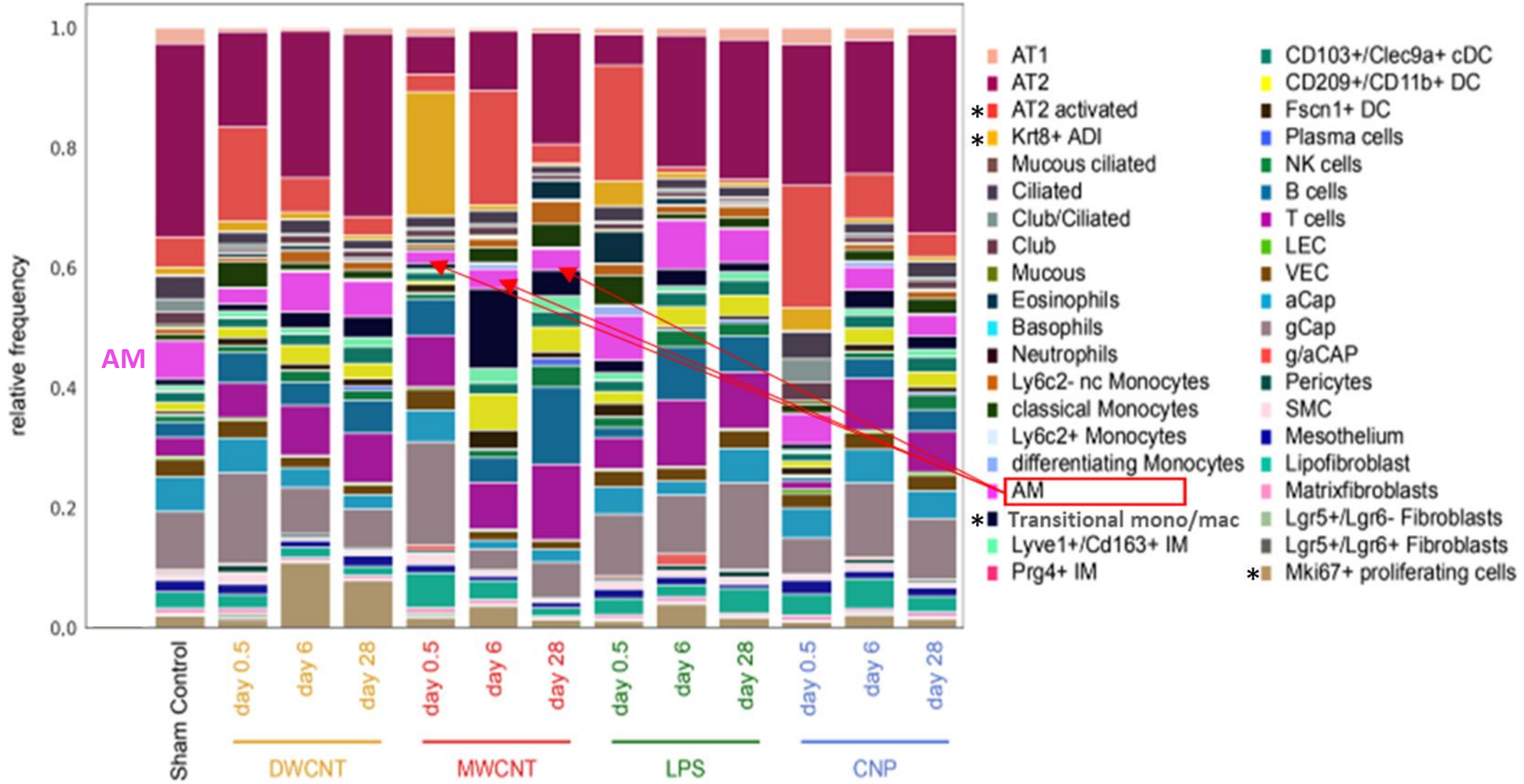
Dot blots for selected **granulocyte chemoattractant genes** with the top expressing cell types (12h)



Material specific initiation of the inflammatory response *transcriptional level only!*

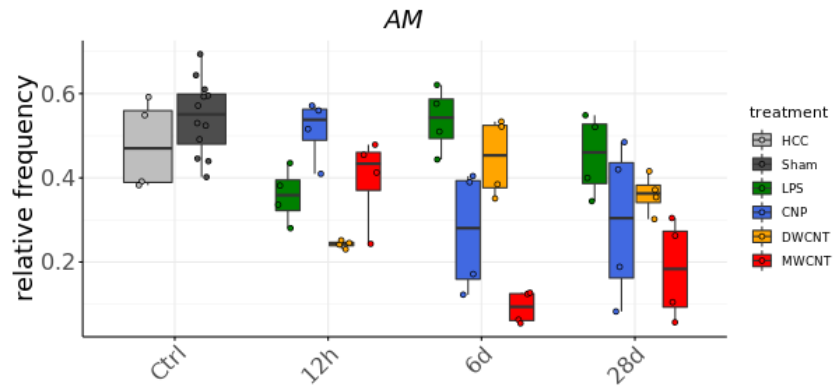


scRNAseq reveals material specific depletion of cell types and expansion of specific cell states



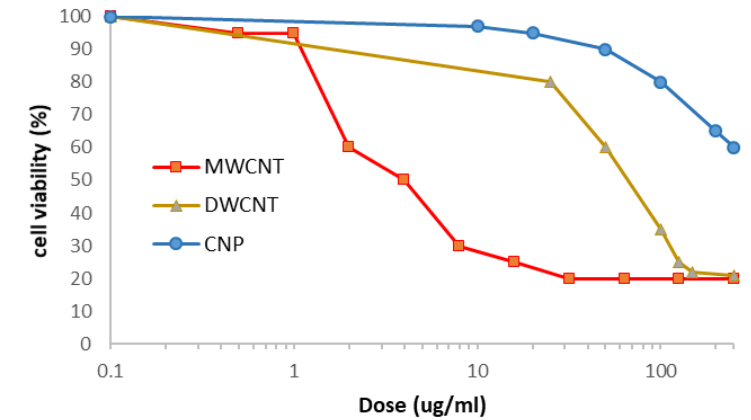
scRNAseq reveals material specific depletion of cell types and expansion of specific cell states

scRNAseq Cell frequency



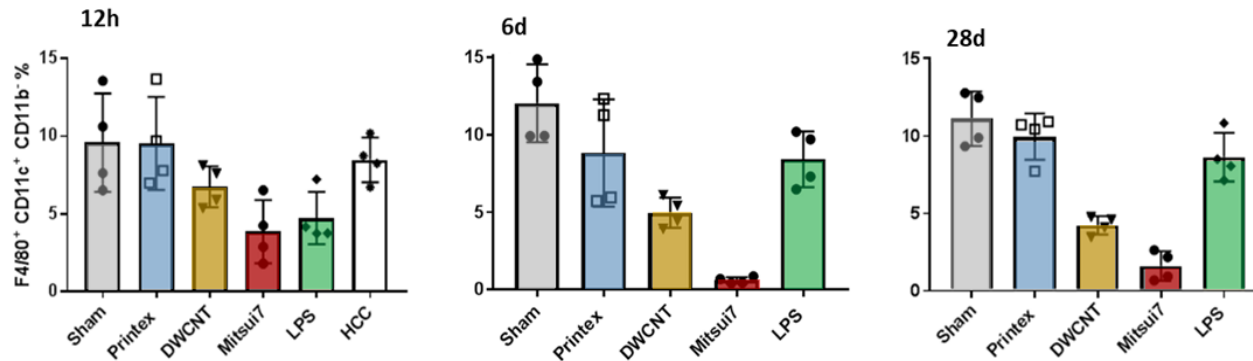
in vitro studies using MH-S cells

in vitro Macrophage Viability (MH-S, 24h)

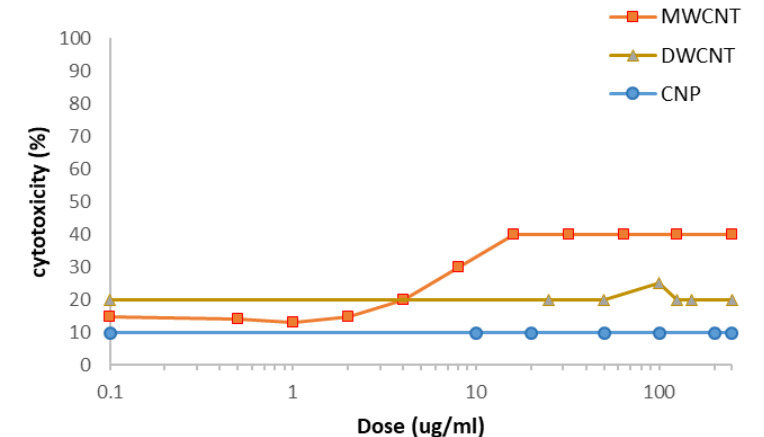


In Vivo FACS analysis of lungs from NM exposed mice

Lung FACS Percentage of alveolar macrophages 12h, 6d and 28d after exposure

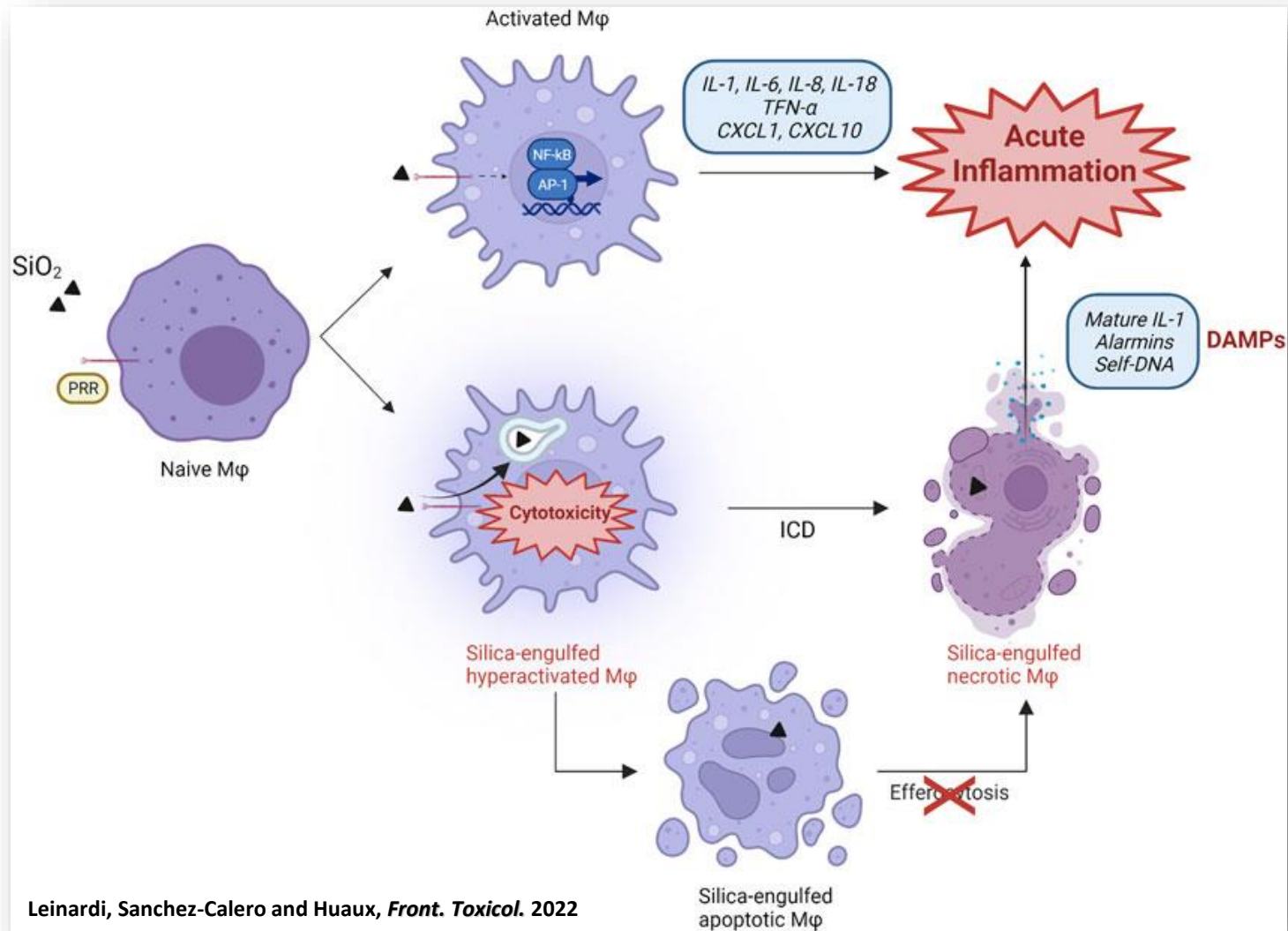


in vitro Macrophage LDH release (MH-S, 24h)



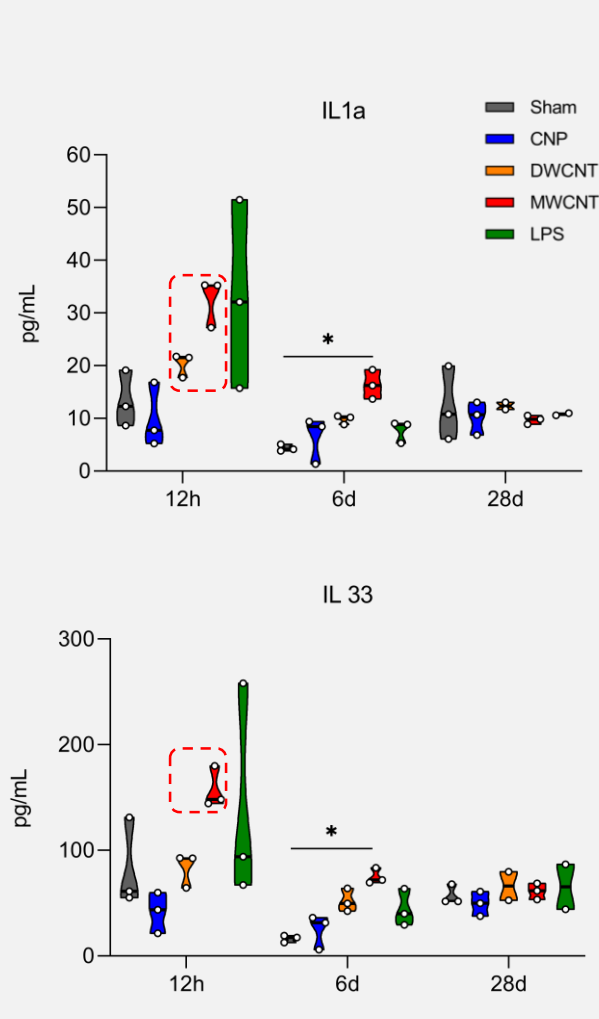
Nanoparticle-Mediated Immunogenic Cell Death (ICD) of Macrophages as Trigger of Lung Inflammation

Proposed scenario in silica-induced lung acute inflammation

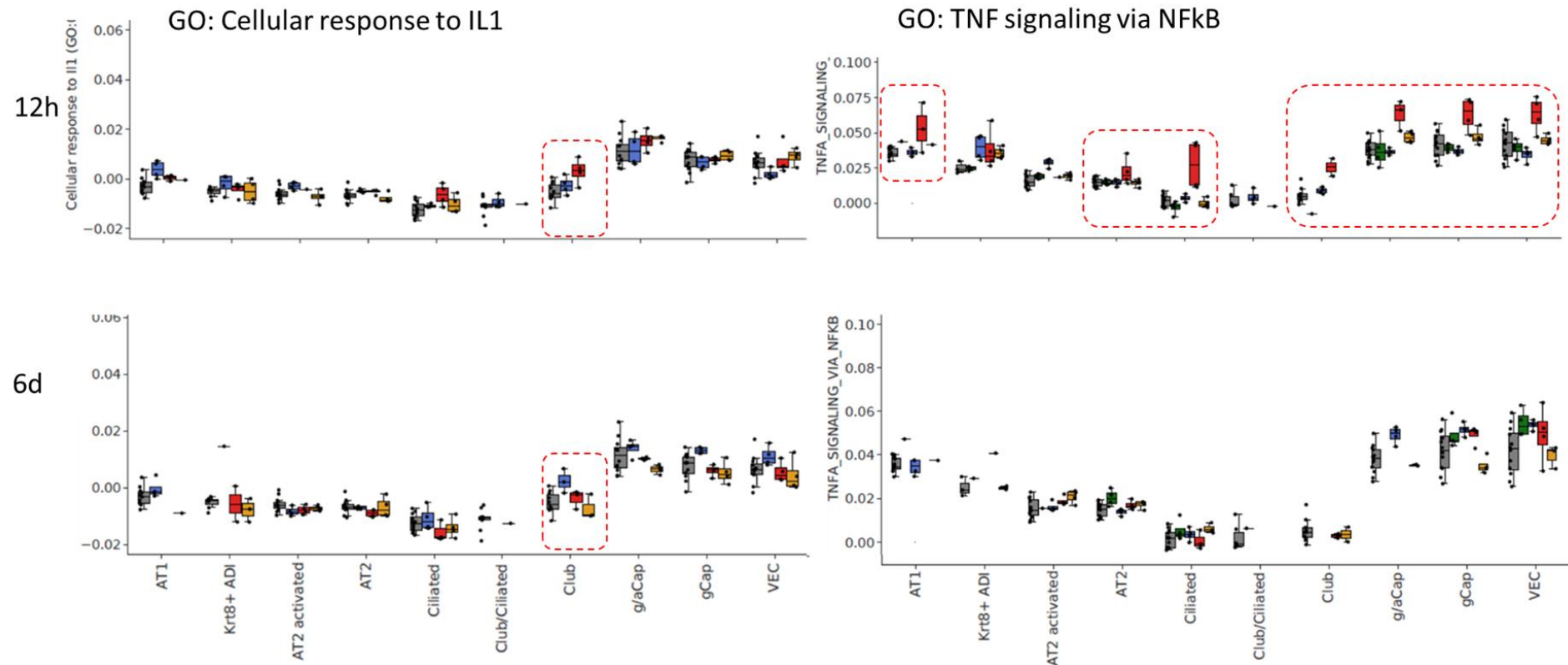


MWCNT specific depletion of alveolar macrophages might trigger the release of DAMPs and alarmins

BAL alarmin levels

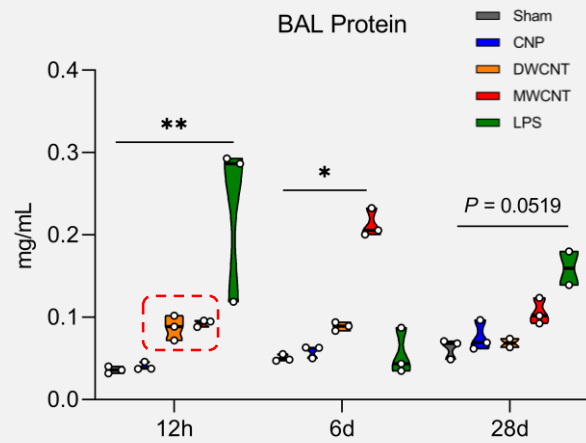


Signaling in epithelial cells in response to alarmins

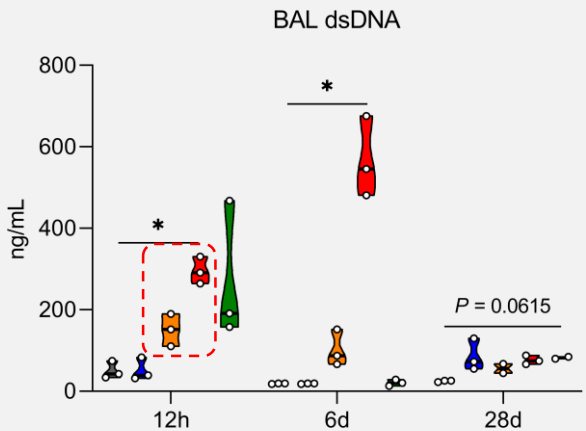


MWCNT specific depletion of alveolar macrophages might trigger the release of DAMPs and alarmins

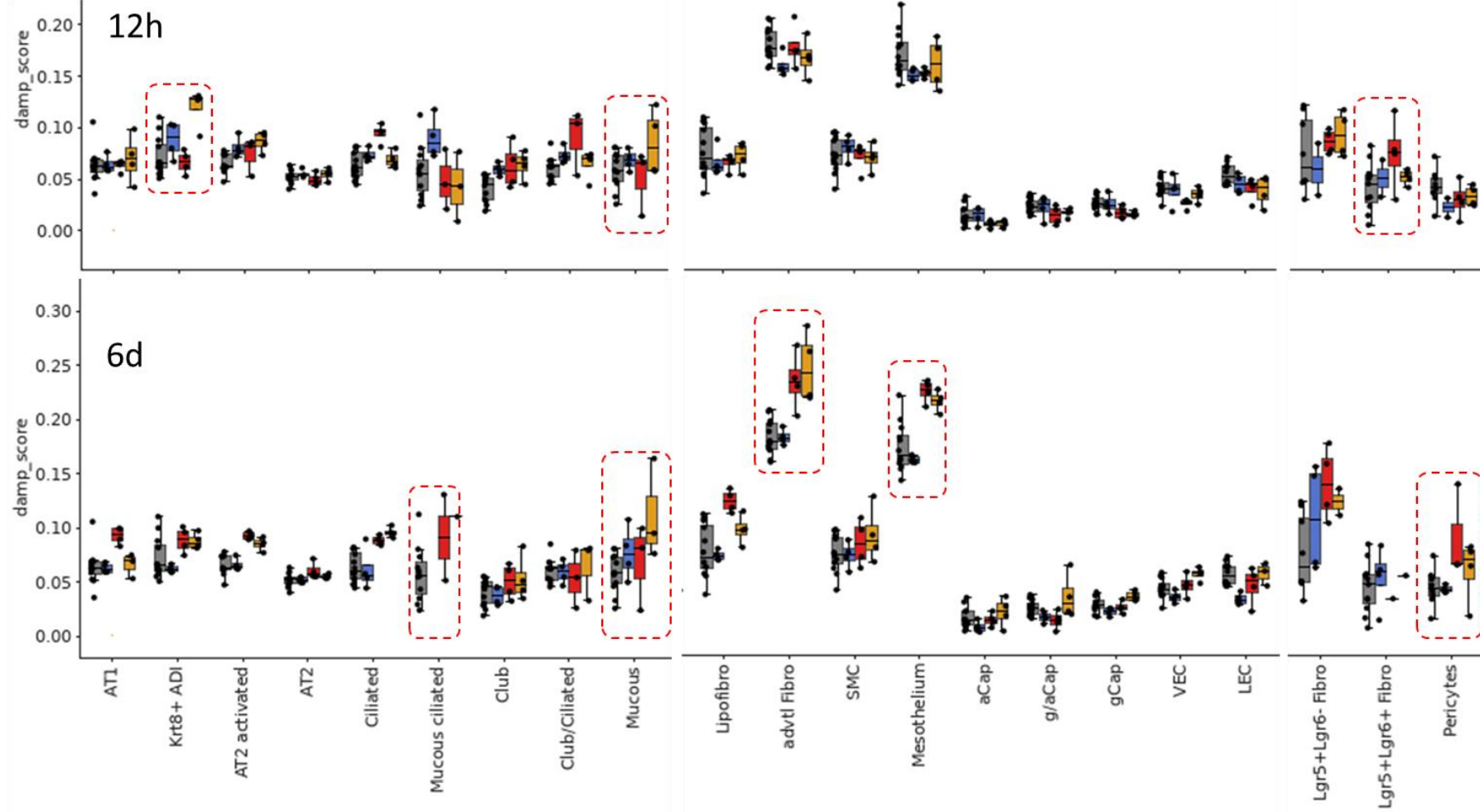
Blood-air barrier damage



BAL DAMP levels

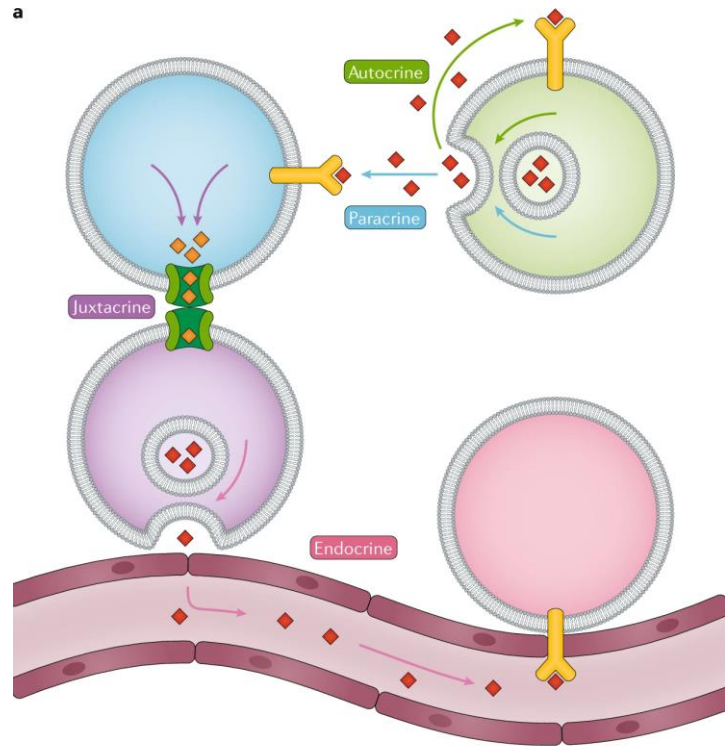


Signaling in related to DAMP sensing



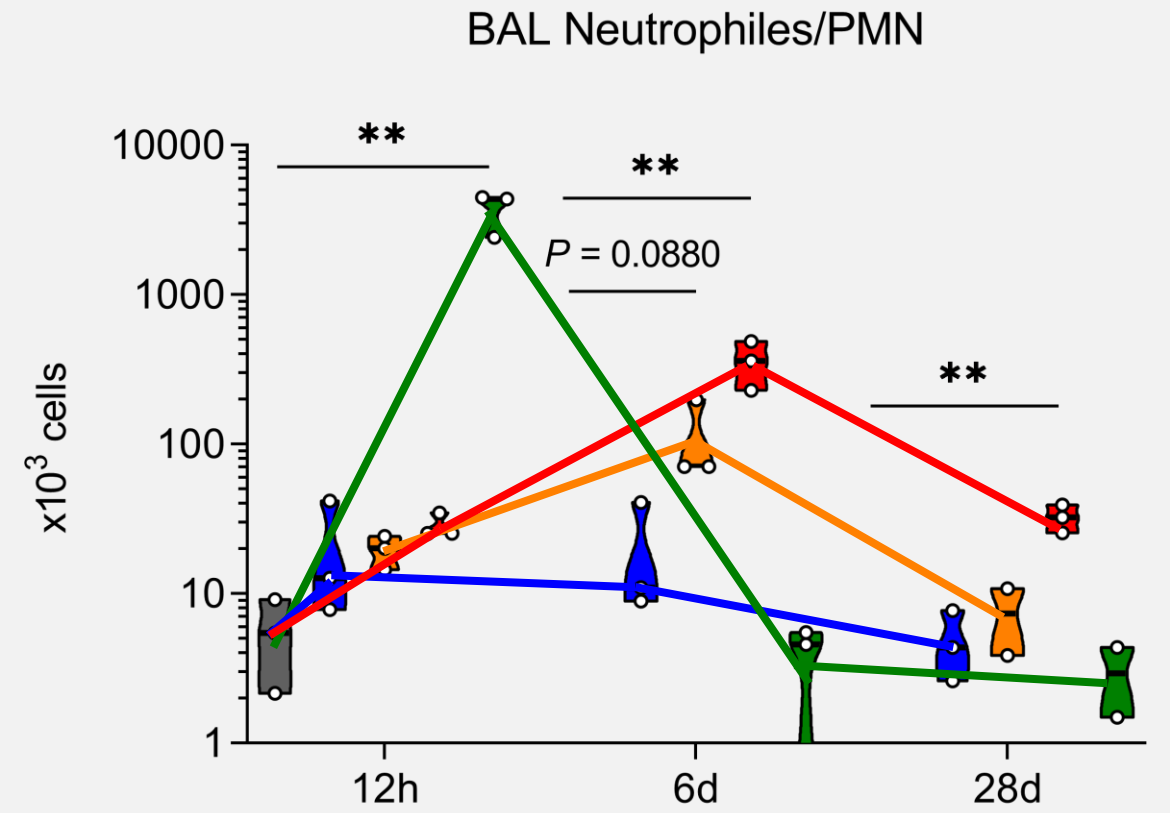
Deciphering NM triggered cell – cell interactions using scRNAseq

Modeling intercellular communication by linking ligands to target genes (e.g. NicheNet analysis)



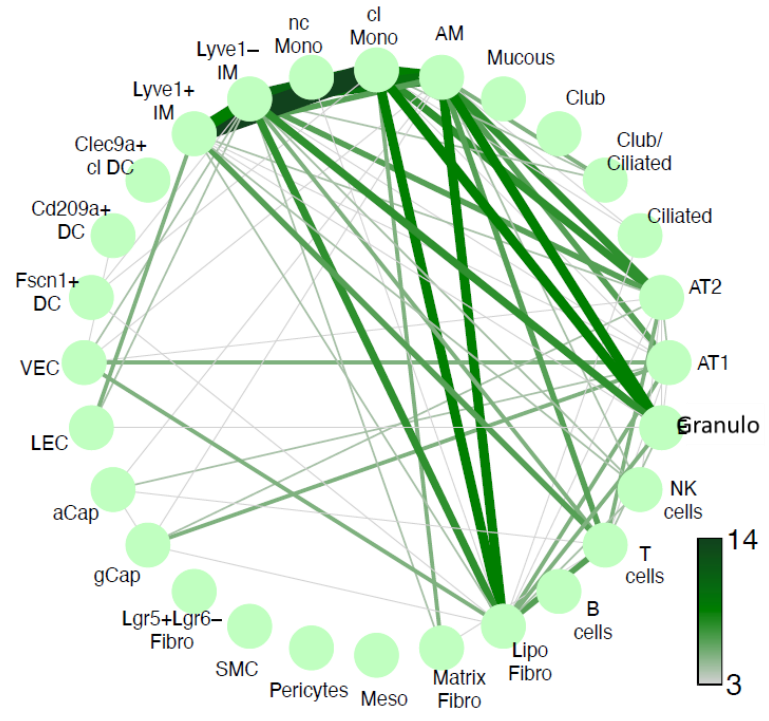
Armingol et al., Deciphering cell–cell interactions and communication from gene expression, *Nat Rev Genet.* 2021

Course of lung NM triggered inflammation

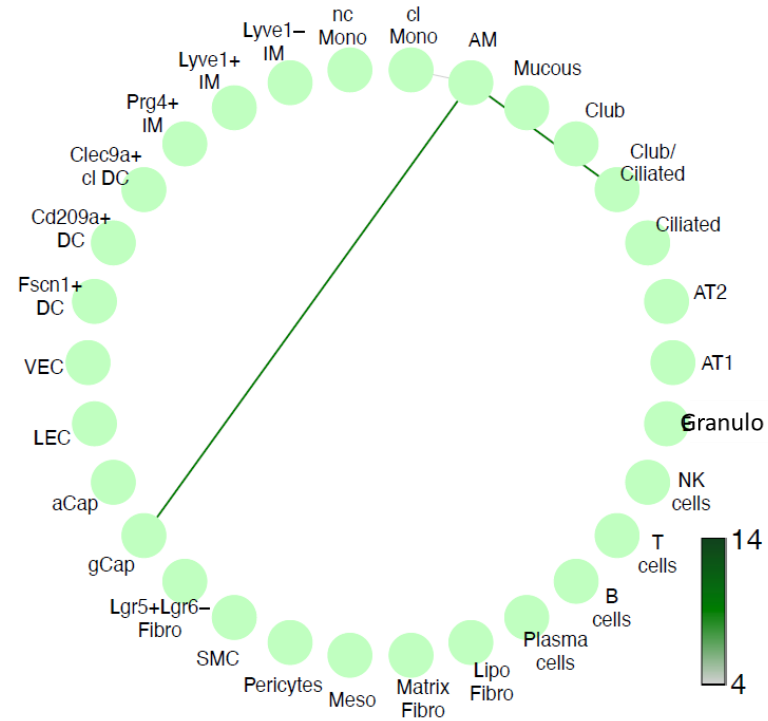


Deciphering NM triggered cell – cell interactions using scRNAseq

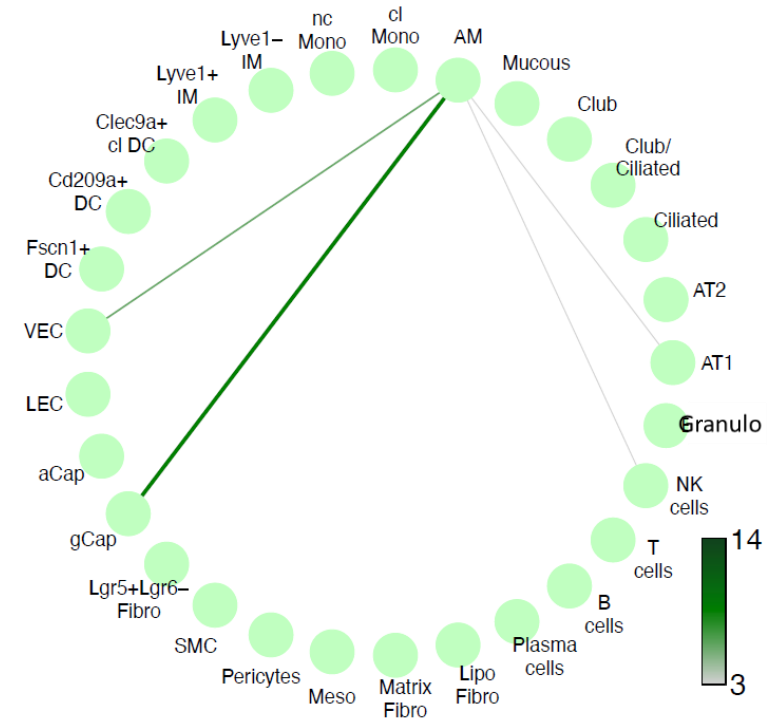
LPS 12h (logFC 05)



LPS 6d (logFC 05)

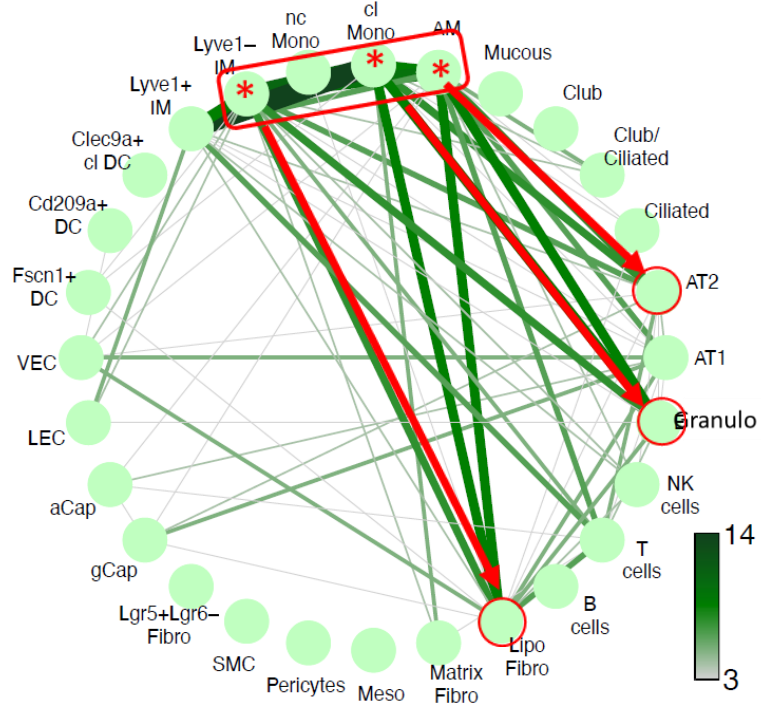


LPS 28d (logFC 05)



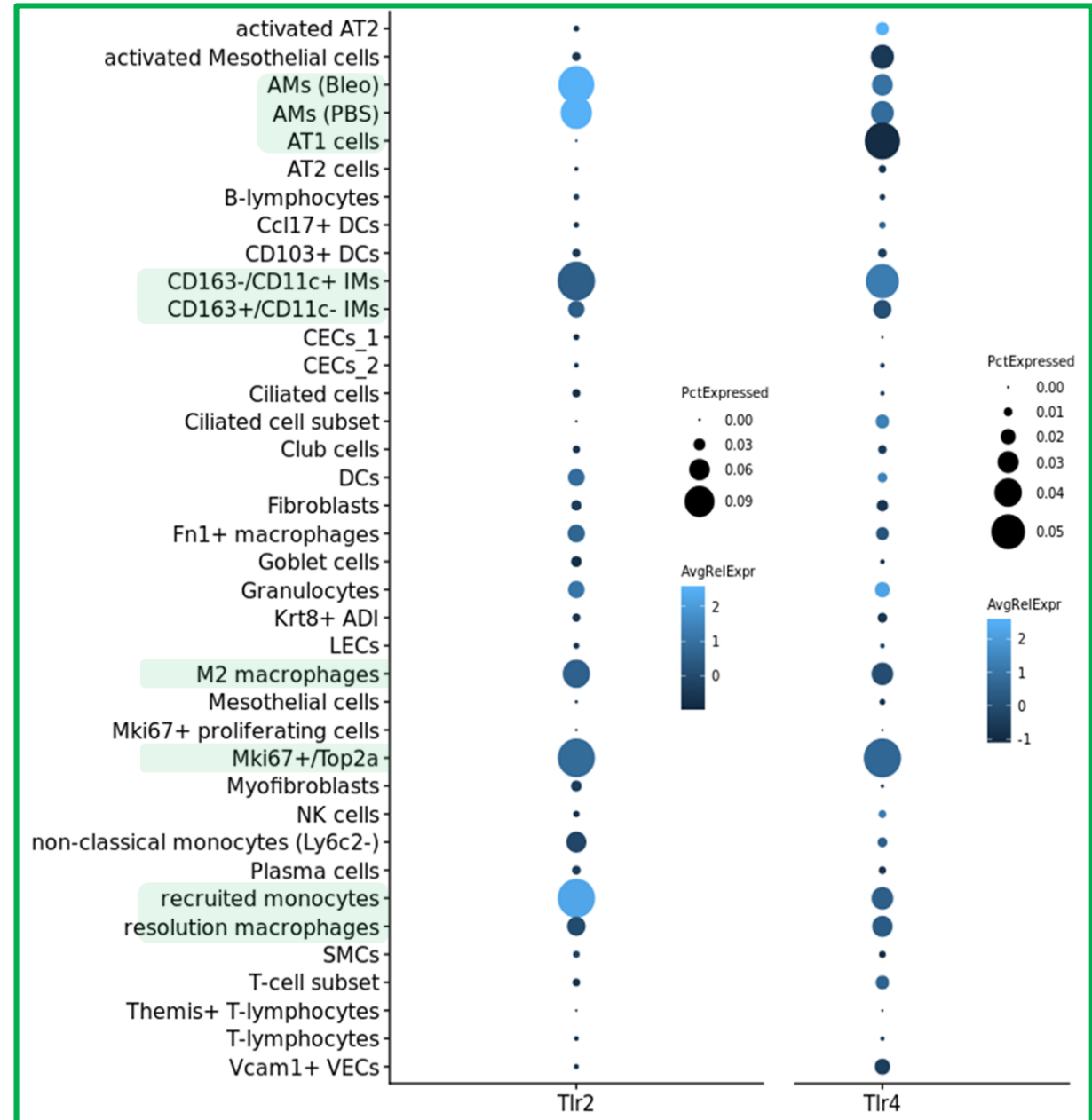
Deciphering NM triggered cell – cell interactions using scRNAseq

LPS 12h (logFC 05)



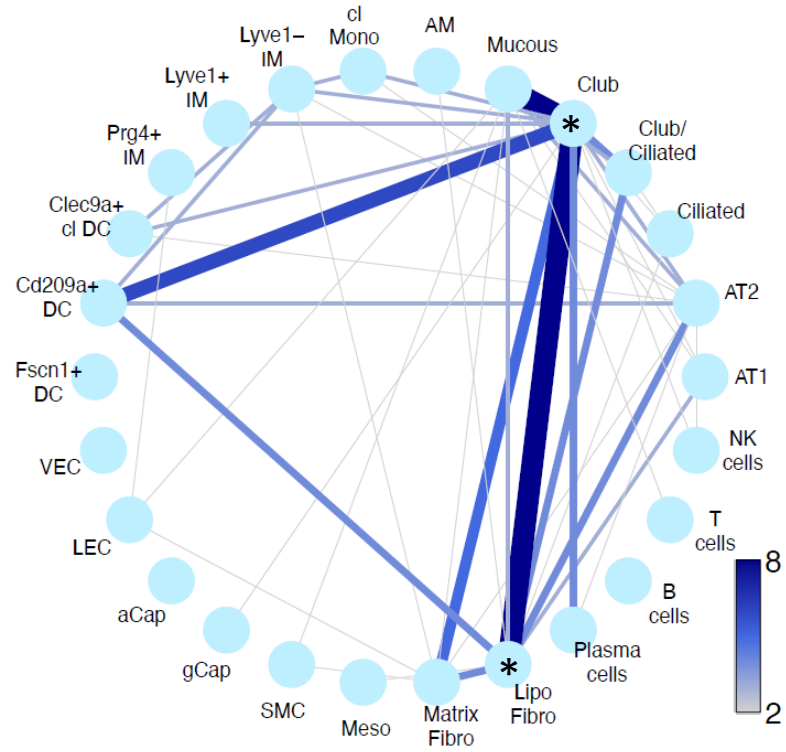
LPS triggers crosstalk of

AM / IM / Mono
to
LipoFibrobl, AT2,
Granulocytes

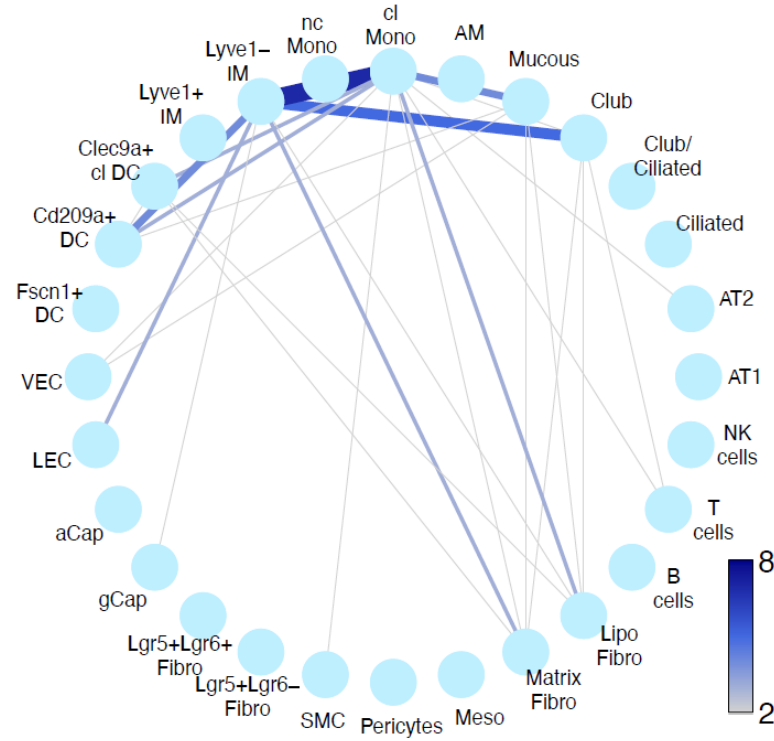


Deciphering NM triggered cell – cell interactions using scRNAseq

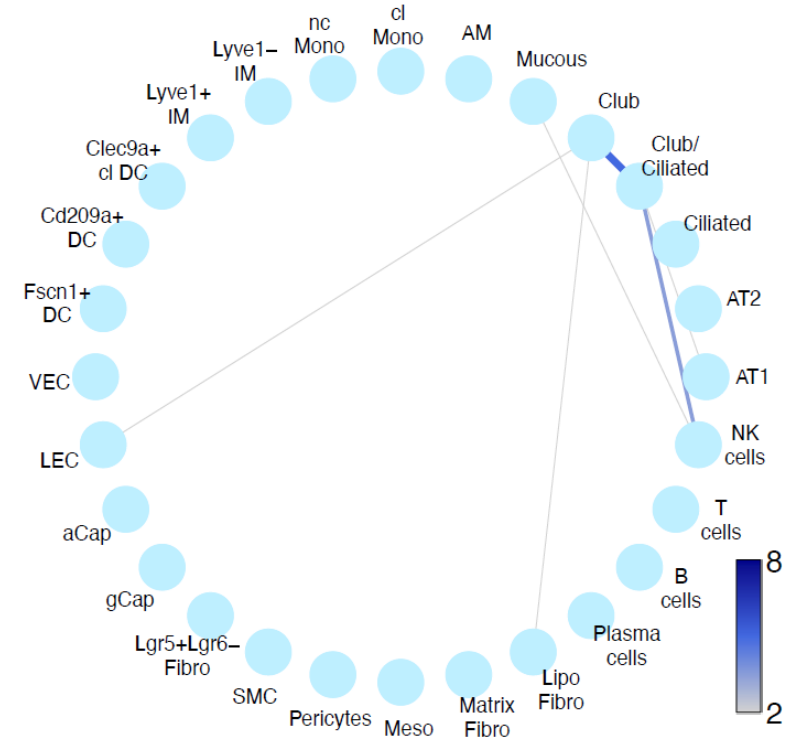
CNP 12h (logFC 05)



CNP 6d (logFC 05)



CNP 28d (logFC 05)



Club: *Cxcl5, Ltf, Serpine2, Icam1, C3*

LipoF: *Timp1, Thbs1, C3, Icam1*

Club

Cxcl5



LipoFibroblast

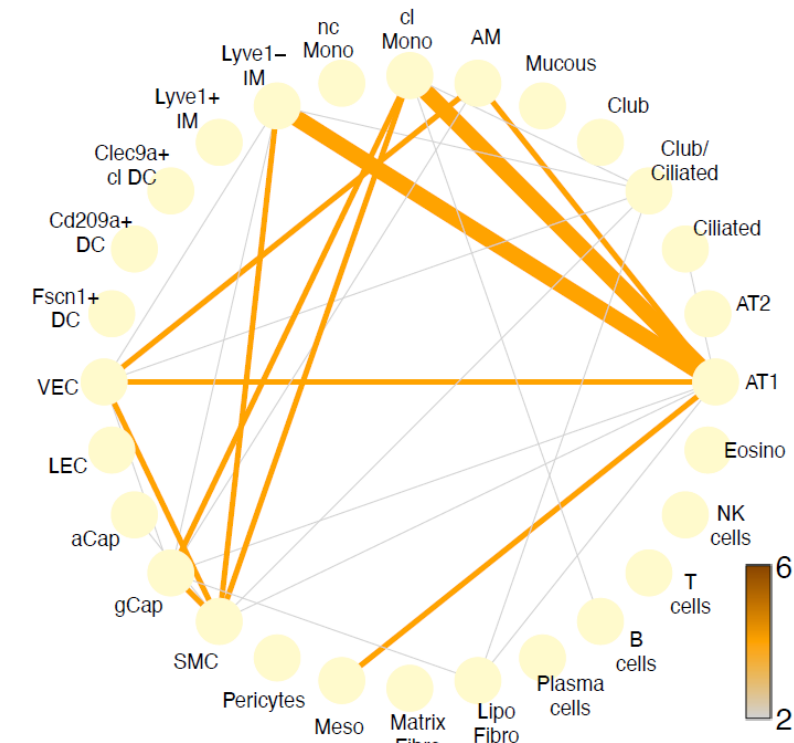
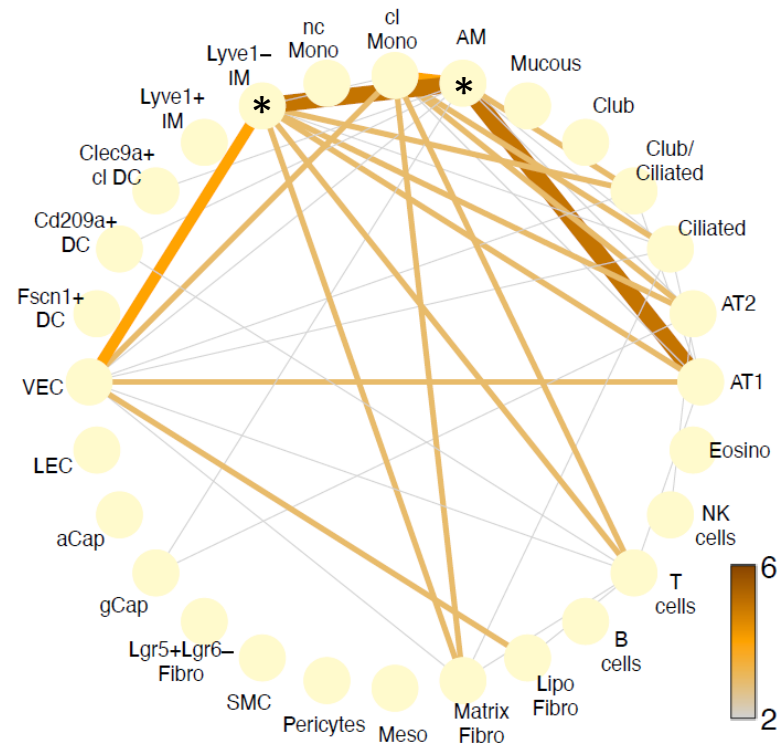
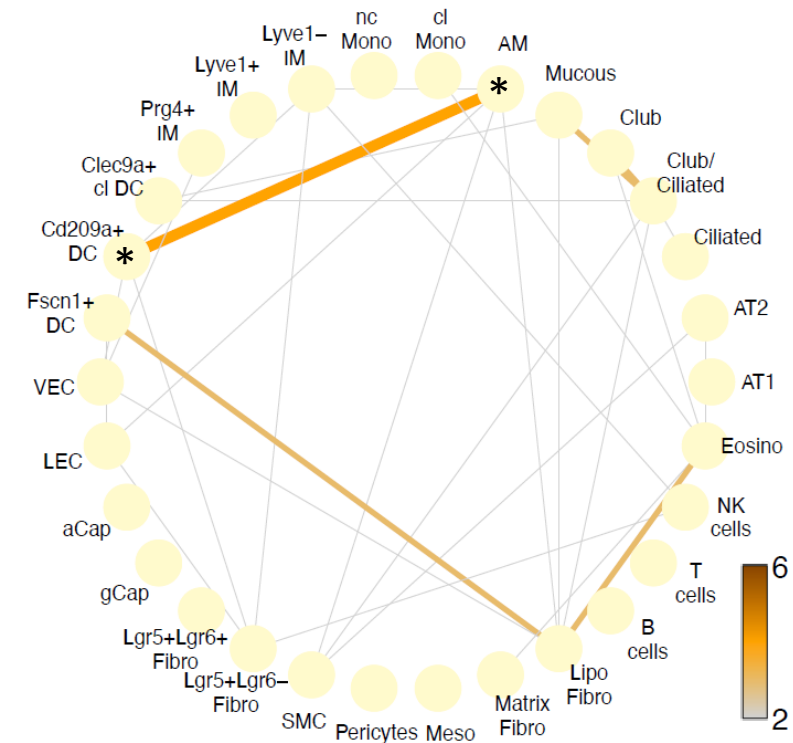
Lpar1, S1pr3 (GPCR signaling)

Deciphering NM triggered cell – cell interactions using scRNAseq

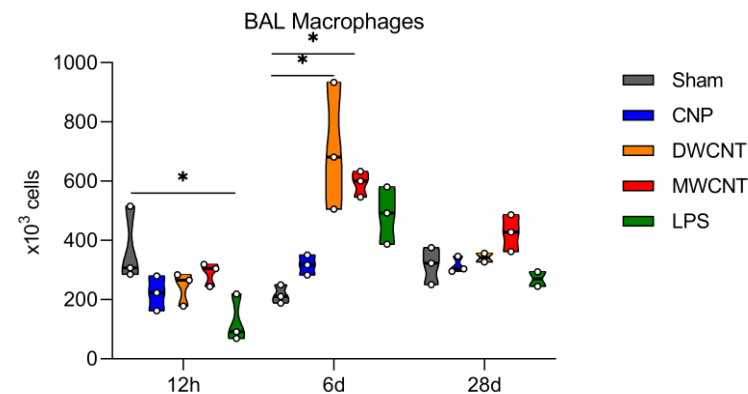
DWCNT 12h (logFC 05)

DWCNT 6d (logFC 05)

DWCNT 28d (logFC 05)

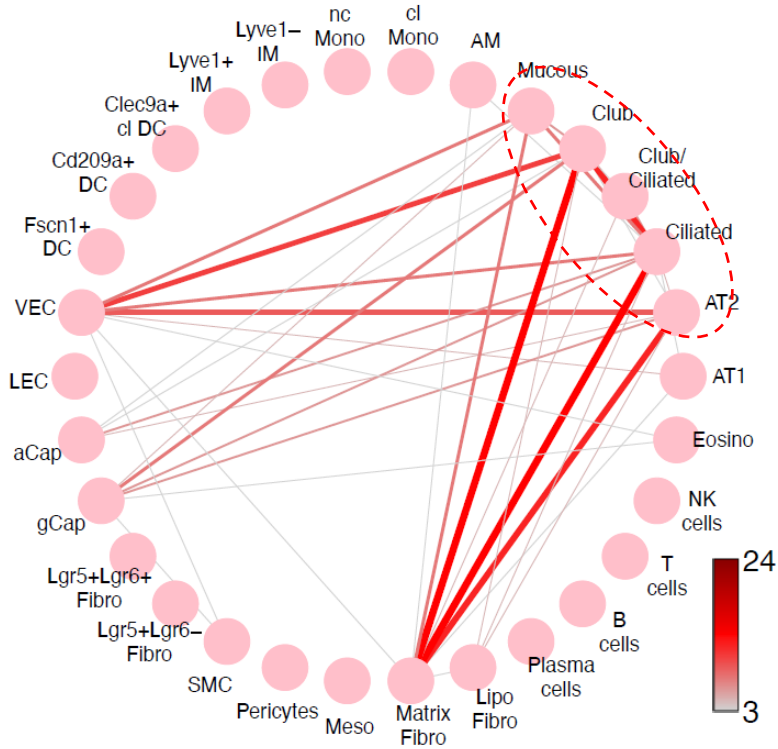


alveolar Macrophages
 ⇕
 monocyte-derived DC
 (CD209+ DC)

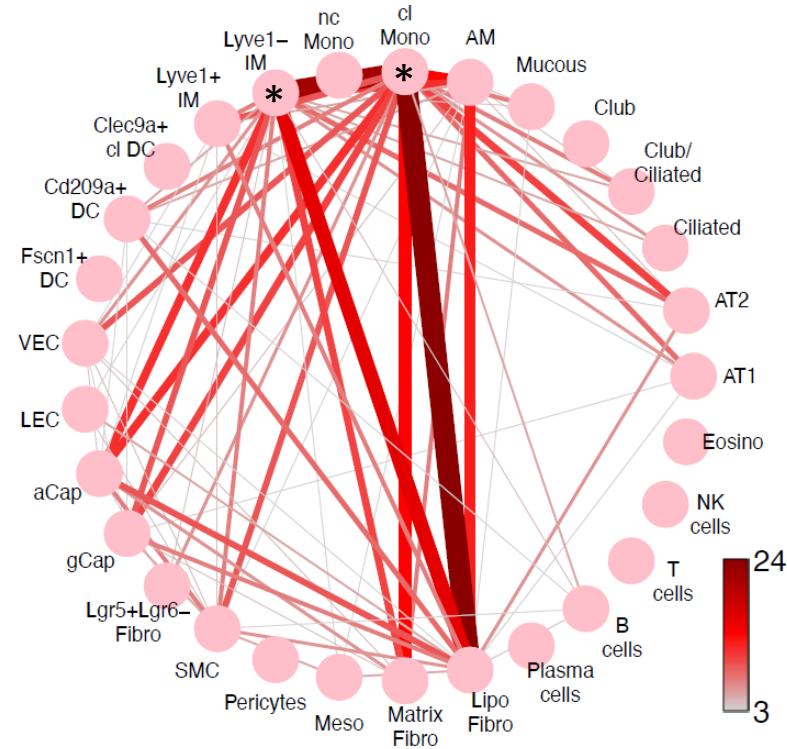


Deciphering NM triggered cell – cell interactions using scRNAseq

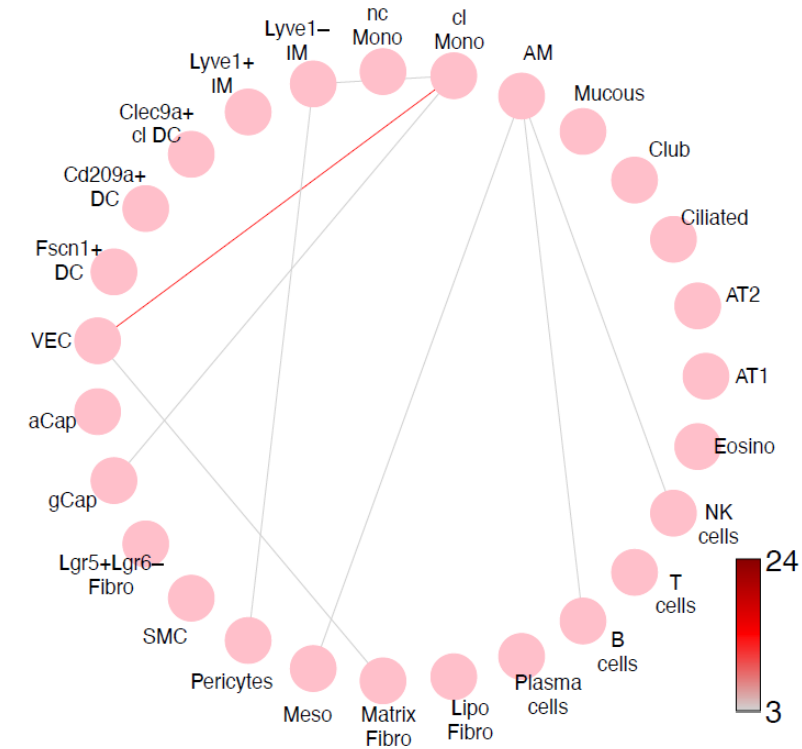
MWCNT 12h (logFC 05)



MWCNT 6d (logFC 05)

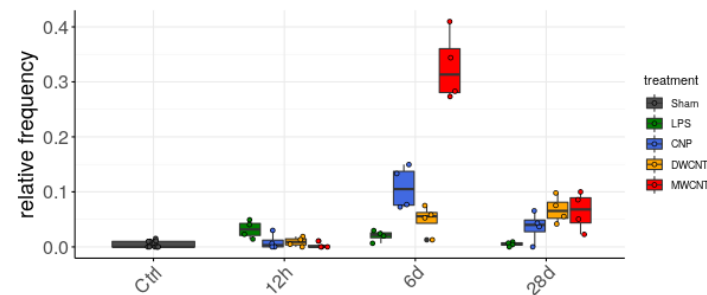


MWCNT 28d (logFC 05)

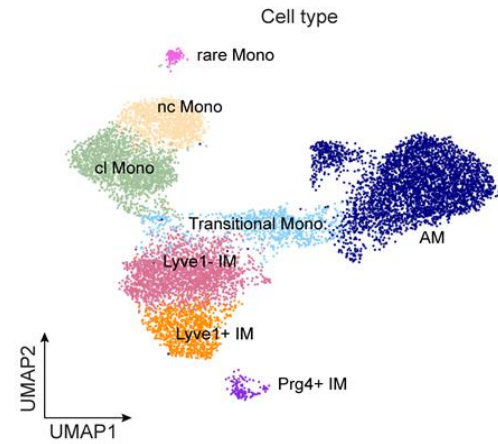


(bronchiolar) epithelial cells
 ⇕
 vascular endothelial cells
 + matrix fibroblasts

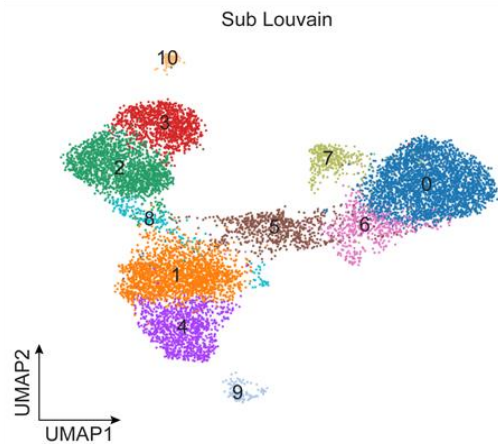
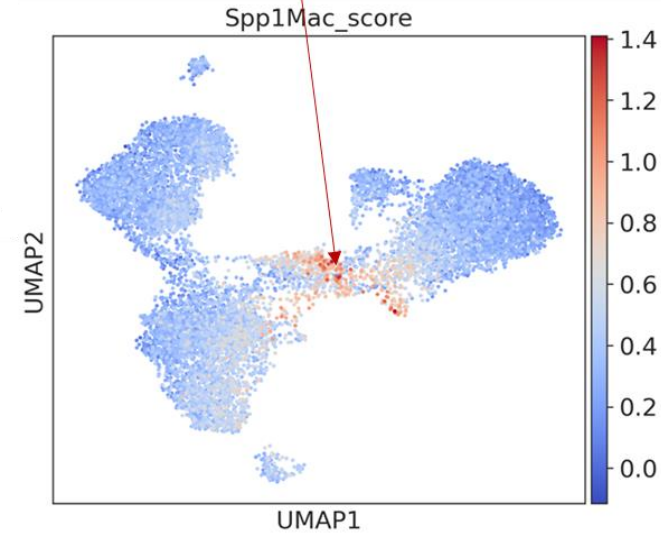
transitional Mono



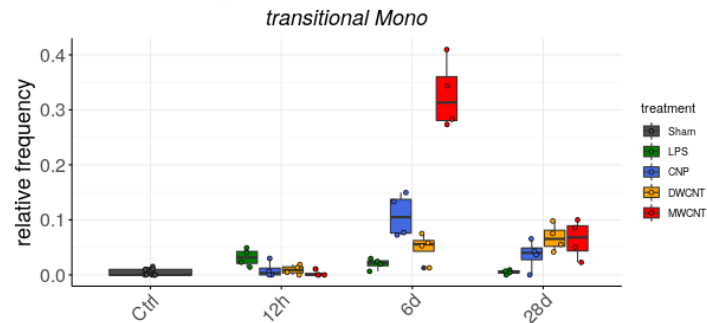
Deciphering NM triggered cell – cell interactions using scRNAseq



'SPP1+ Mac' in IPF lungs
(Wendisch et al., *Cell*. 2021)
Profibrotic macrophage state

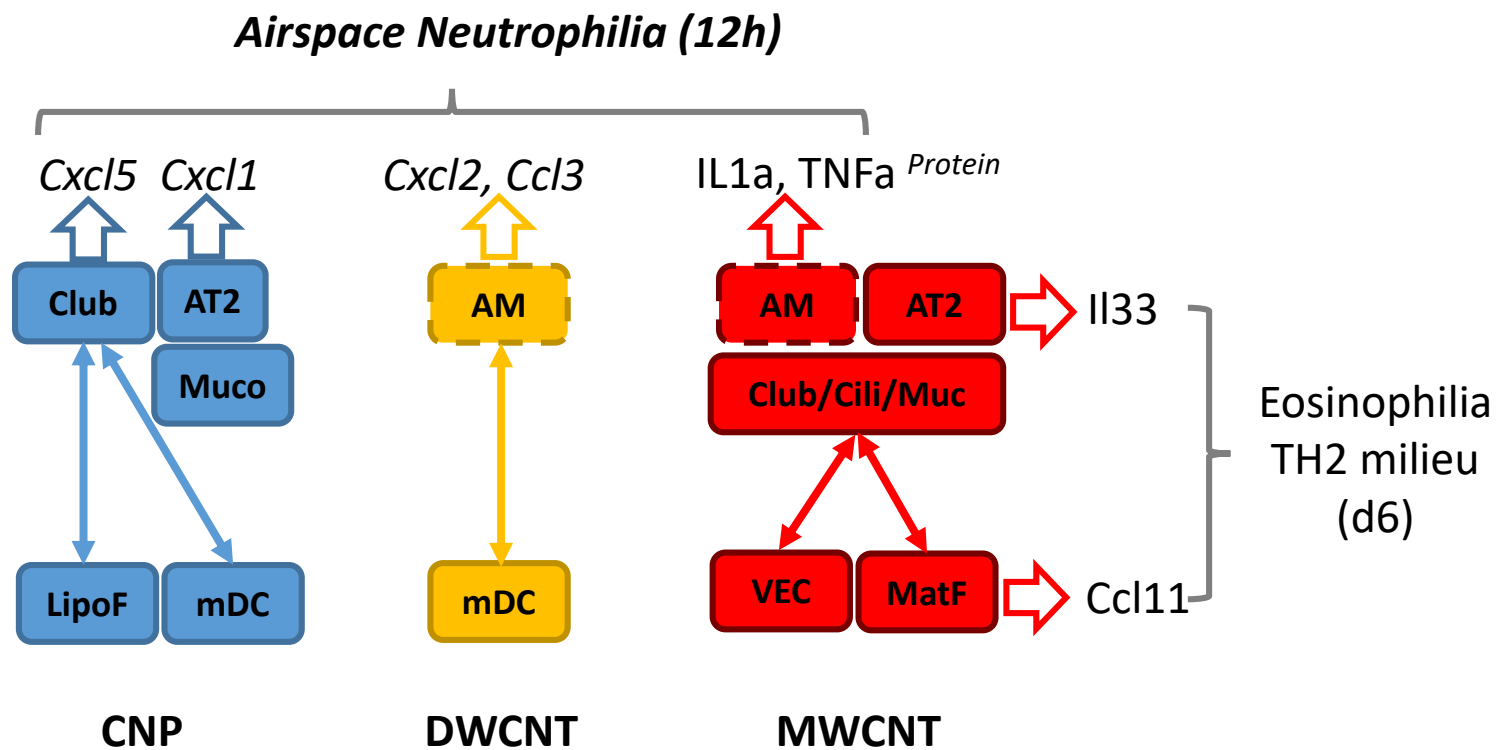


(bronchiolar) epithelial cells
↕
vascular endothelial cells
+ matrix fibroblasts



Summary of early (12h) cell-cell communication events after NM inhalation

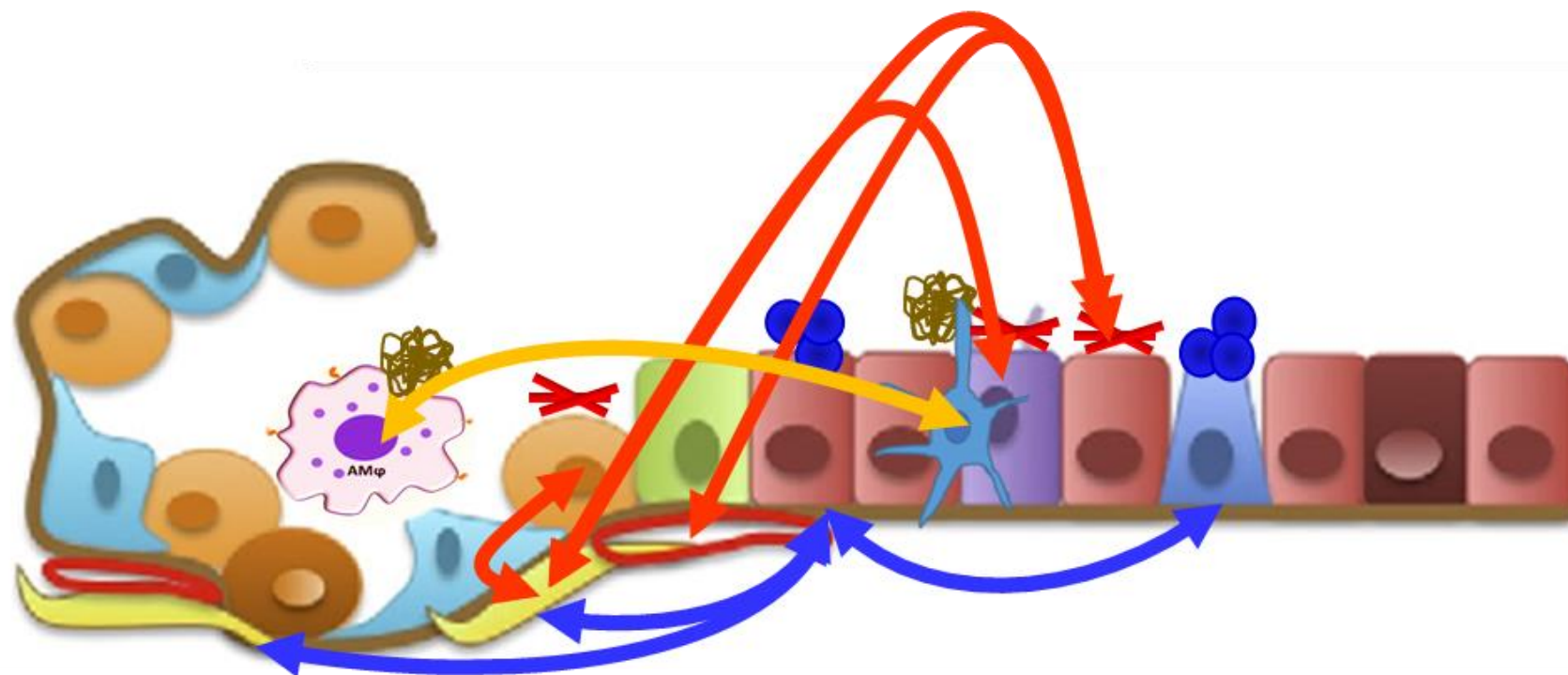
To be considered for the development for AOP anchored-cell based assays



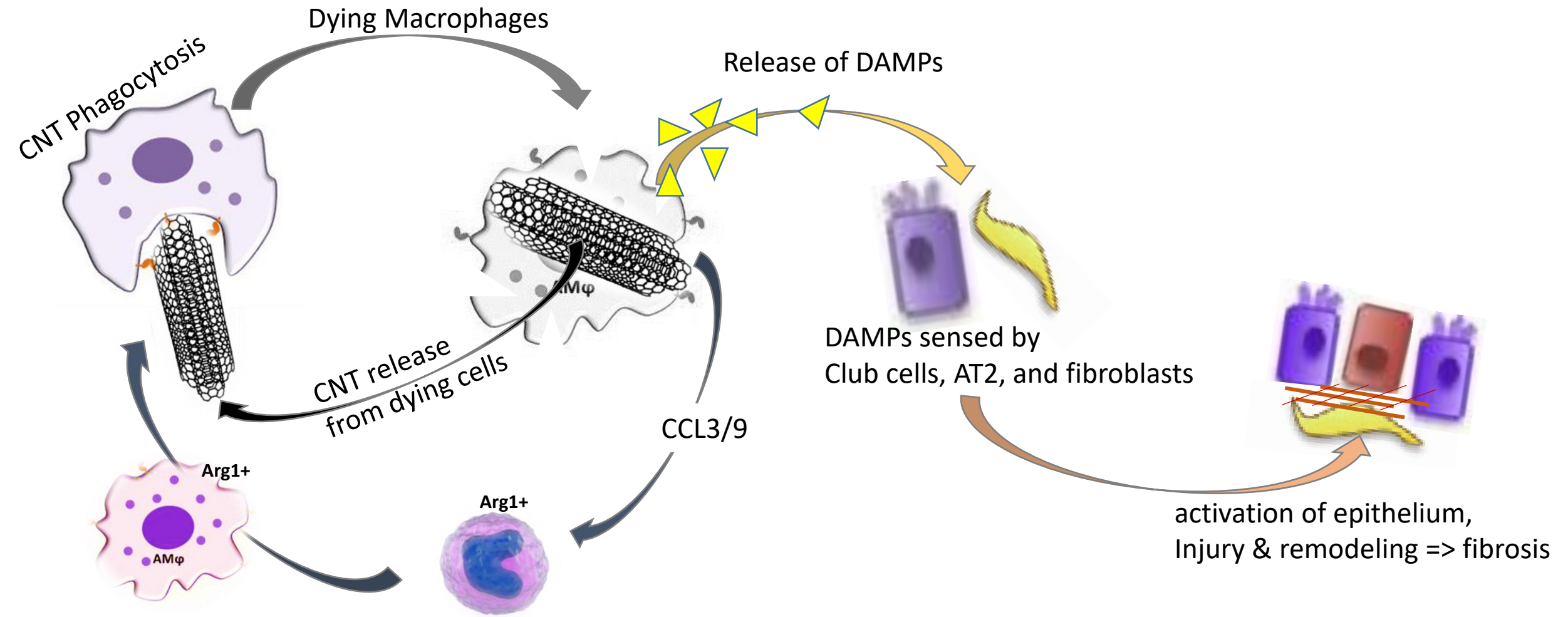
Summary of early (12h) cell-cell communication events after NM inhalation

To be considered for the development for AOP anchored-cell based assays

Cell Interaction in the Bronchio-Alveolar Duct Junction



Hypothesis for MΦ centered Mode of Action for CNTs



Recruitment and Differentiation of Monocytes into AMs and eventual giant cell / granuloma formation

Summary

- **Deposition hotspots** in the **bronchiolar-alveolar duct** region leads to high local (acute) doses.
- **Tissue resident macrophages (AMs)** seem for spherical particles (CNP) **not involved** in the initiation of lung inflammation. Particle laden macrophages are not necessarily inflammatory activated.
- Biopersistent materials with high **cytotoxicity to phagocytes** (MWCNTs) cause long term **AM depletion** and their replenishment with '*profibrotic macrophages*' causes chronic lung injury.
- **Immunogenic phagocyte death** and subsequent **release of alarmins & DAMPs** needs consideration for cell based assays.
- **MWCNT specific cellular response pattern** suggest a complex interplay of epithelial, endothelial and mesenchymal cells for the **fibrosis AOP**

acknowledgements

HELMHOLTZ

RESEARCH FOR
GRAND CHALLENGES

DZL Deutsches Zentrum für
Lungenforschung

my group @iLBD



Carola Voss
Markus Rehberg
Qiongliang Liu



Carolina B. Lopez
Qiaoxia Zhou
Lianyong Han



Anna Fuchs
David Kutschke
Verena Häfner



Herbert Schiller
Meshal Ansari

“THE scExperts”

@iLBD



Schmid Otmar
Lin Yang

“The Aerosol
Experts”

@iLBD



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nanoPASS

Bridging the gaps in nanosafety for animal-free prediction of adverse outcomes



HARMLESS

Advanced High Aspect Ratio and Multicomponent materials:
towards comprehensive intelLigent tEsting and Safe by design Strategies



SmartNanoTox

Smart Tools for Gauging Nano Hazards