PMI RESEARCH & DEVELOPMENT

ASSESSING THE EFFECTS OF REPEATED CIGARETTE SMOKE-EXPOSURE USING HUMAN ORGANOTYPIC SYSTEMS REPRODUCING THE RESPIRATORY TRACT IN VITRO

Carole Mathis

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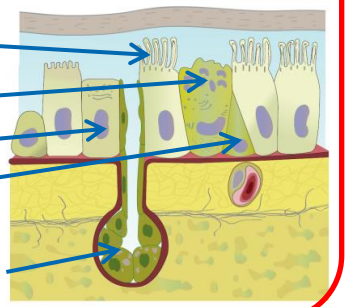
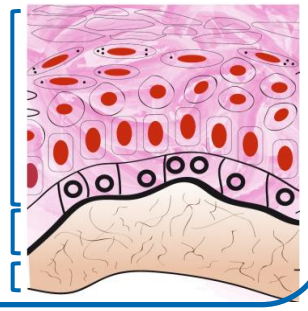
Recapitulation of In Vivo Biology by Organotypic Systems

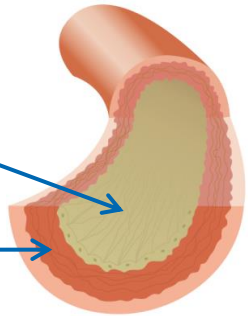
Nasal epithelium

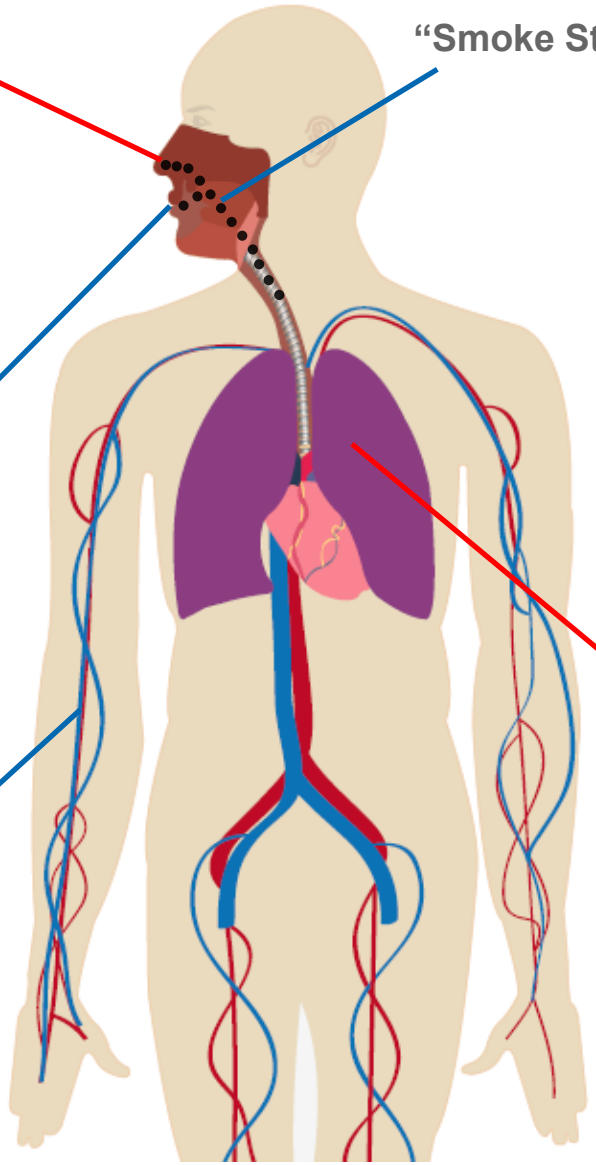


Buccal and Gingival Mucosa

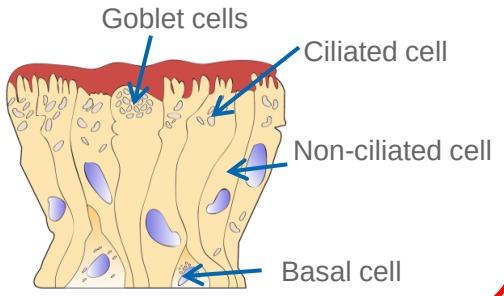


Blood vessel





Bronchial Epithelium



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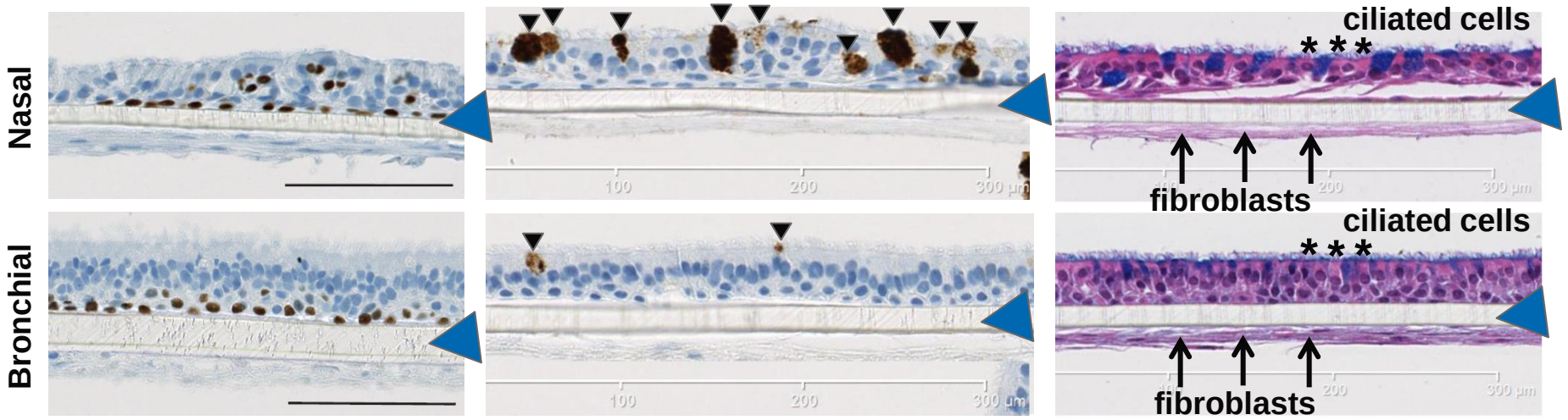
Human Organotypic Cultures of Primary Bronchial and Nasal Epithelial Cells

COMPARISON AT THE MORPHOLOGICAL LEVEL

(ANALYSIS DONE ON UNTREATED TISSUES)



p63⁺ basal cells

Muc5AC⁺ mucus secreting cells

Hematoxylin/Eosin

(POROUS MEMBRANE)

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Whole cigarette smoke/aerosol exposure system (Vitrocell®)

Smoking Machine

Humidification System

Vitrocell 24/48

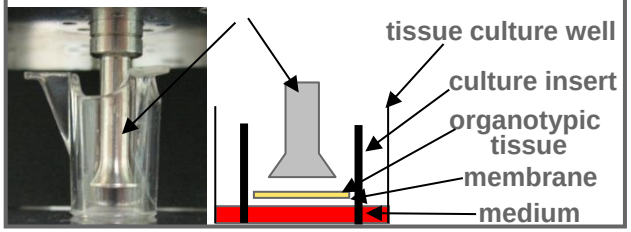
Climatic chamber
Dilution system
(8 dilutions @ 6 replicates)

Microbalance

Exposure Chamber

37°C
24 well plate

Position of the aerosol inlet above the AIR-100 tissue insert



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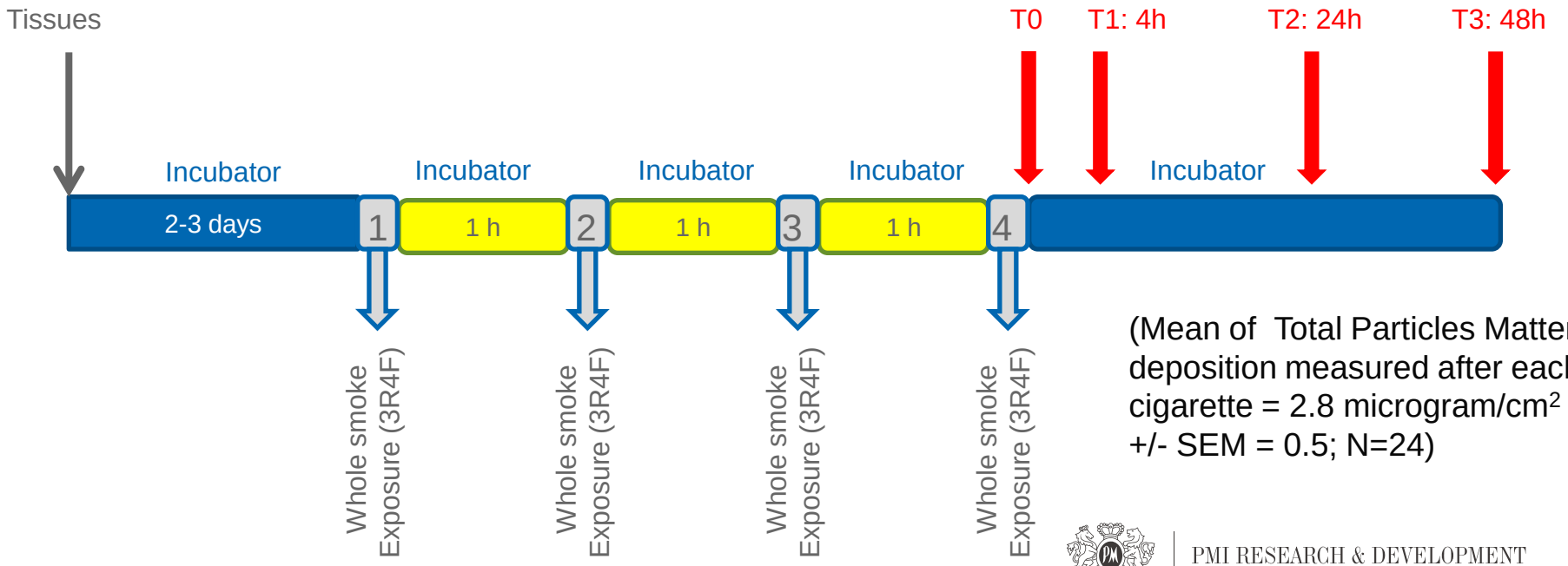
Whole Smoke Repeated Exposure of Organotypic Cultures of Human Primary Bronchial and Nasal Epithelial Cells

Experimental Design

HUMAN TISSUES	CONDITIONS	ENDPOINTS	POST-EXPOSURE TIME 0h	4h	24h	48h
BRONCHIAL MUCILAIR™	SHAM	Cytotoxicity (LDH assay)			X	X
		Membrane Integrity (TEER)			X	X
NASAL MUCILAIR™	CS 10%	Gene Expression Profiles (Affymetrix)	X	X	X	X
	CS 16%	MicroRNA Expression Profiles (GeneChip)	X	X	X	X
		Histology (Alcian blue, H&E)			X	
		Immunohistology (p63, Ki67, B-Tubulin, Muc5AC)			X	



(Mean of Total Particles Matter deposition measured after each cigarette = 2.8 microgram/cm^2 +/- SEM = 0.5; N=24)

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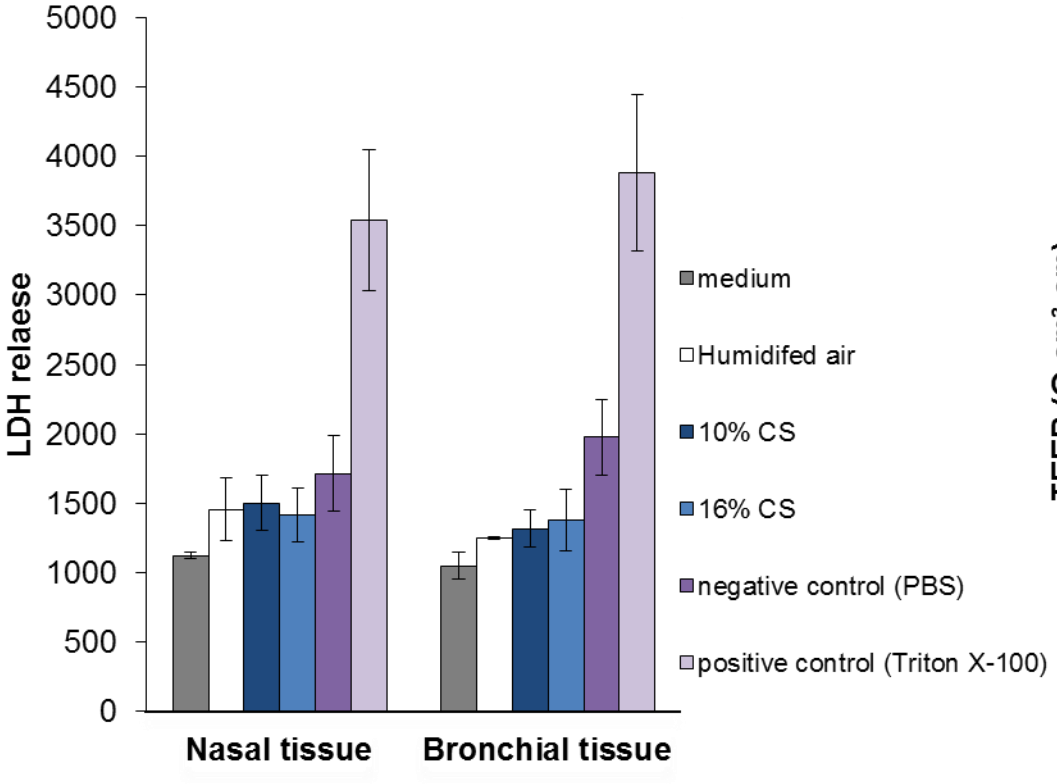
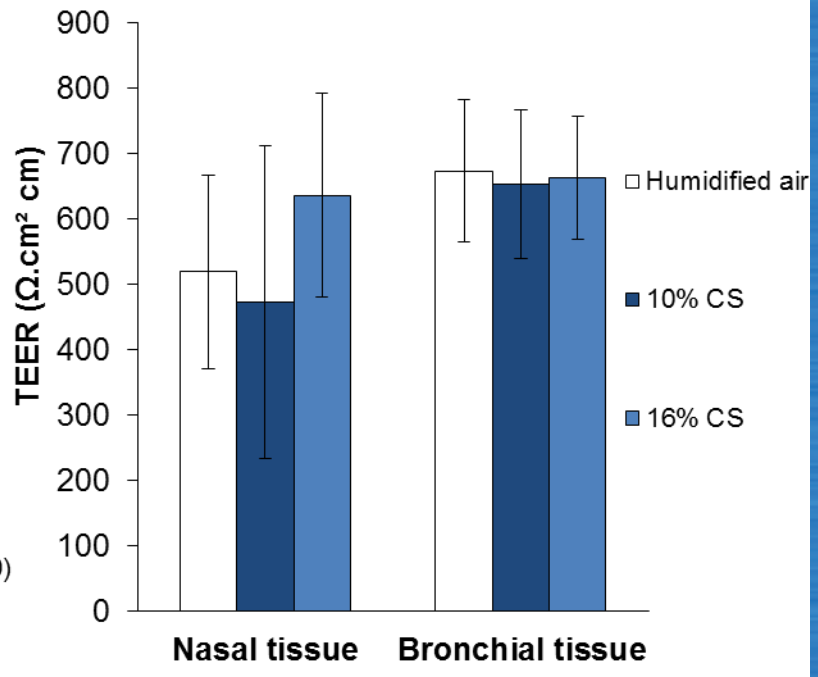
Whole Smoke Repeated Exposure of Organotypic Cultures of Human Primary Bronchial and Nasal Epithelial Cells

The two doses of CS applied on both nasal and bronchial tissue cultures do not induce high toxicity or impair tissue integrity allowing the capture of systems biology endpoints.





24h after exposure

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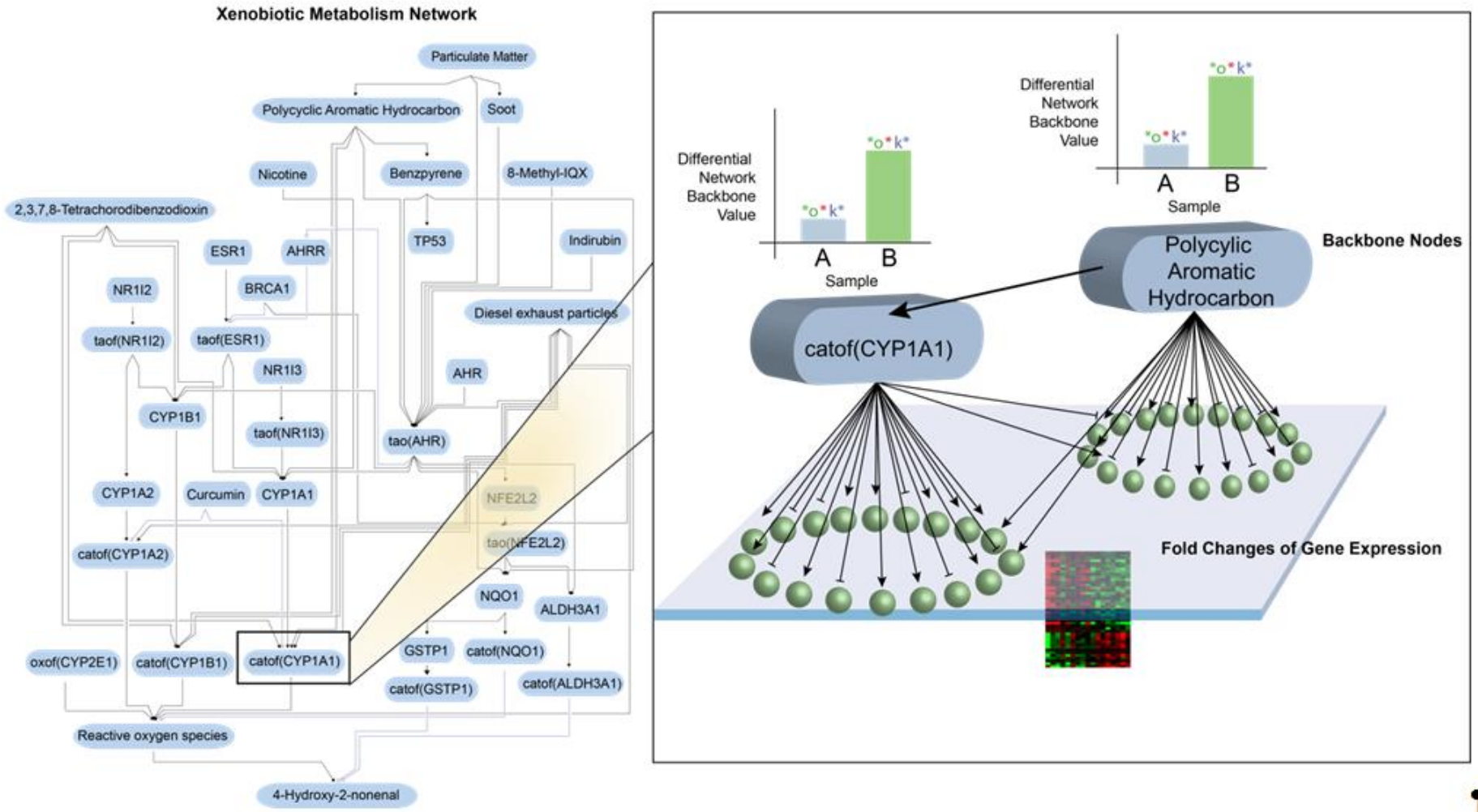
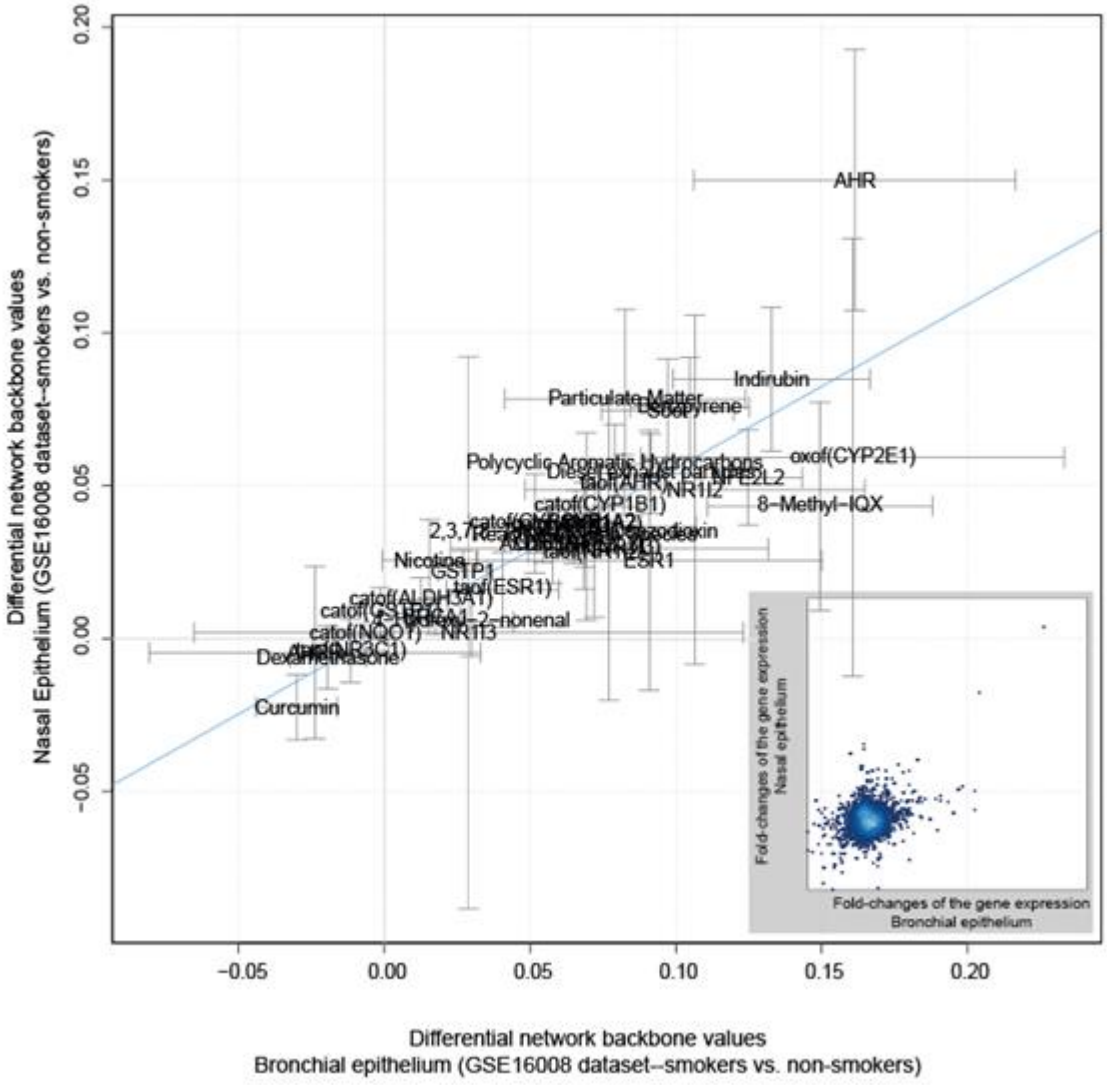
Xenobiotic Metabolism Network Model and Biological Perturbation Assessment



(Ref. Iskandar A. R. et al. BioMed Research International - In press)

PMI RESEARCH & DEVELOPMENT

Nasal as a Surrogate for Bronchus – IN VIVO



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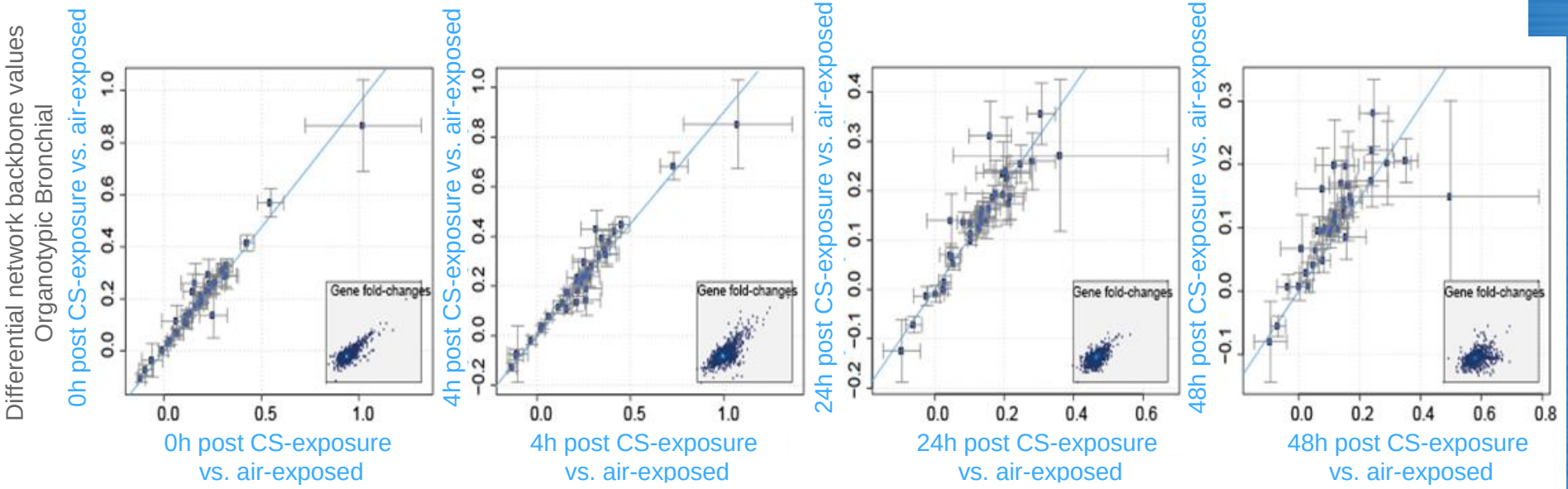
Nasal as a Surrogate for Bronchus – IN VITRO



(Ref. Iskandar A. R. et al. BioMed Research International - In press)

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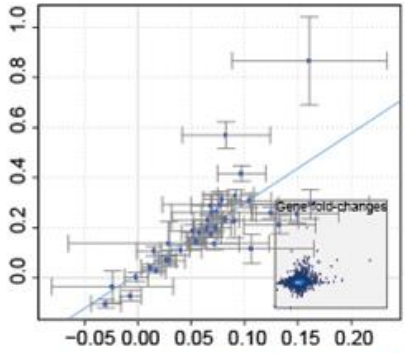
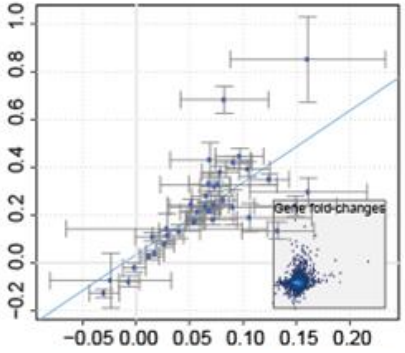
In Vivo/In Vitro Comparison in the Xenobiotic Metabolism Network Model

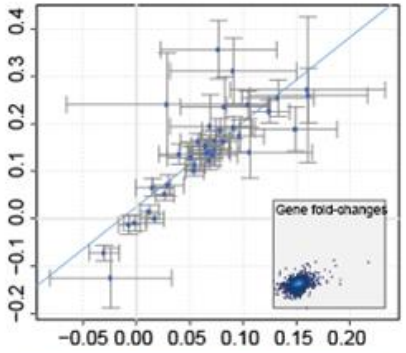
Differential network backbone values
Bronchial Epithelium (Dataset GSE16008
Smokers vs. non smokers



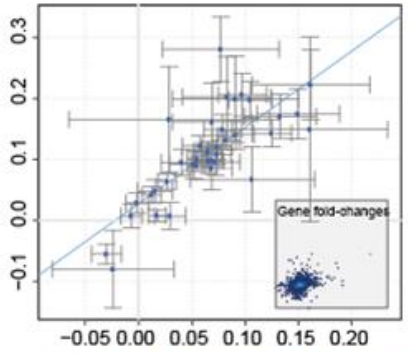
0h post CS-exposure
vs. air-exposed



4h post CS-exposure
vs. air-exposed



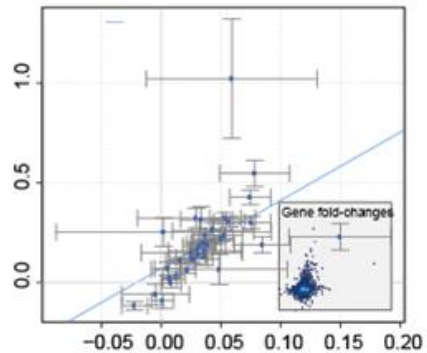
24h post CS-exposure
vs. air-exposed



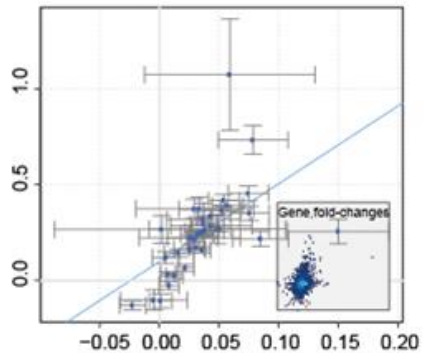
48h post CS-exposure
vs. air-exposed

Differential network backbone values – Organotypic bronchial

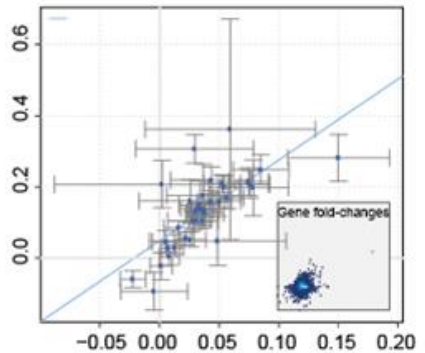
Differential network backbone values
Nasal Epithelium (Dataset GSE16008
Smokers vs. non smokers



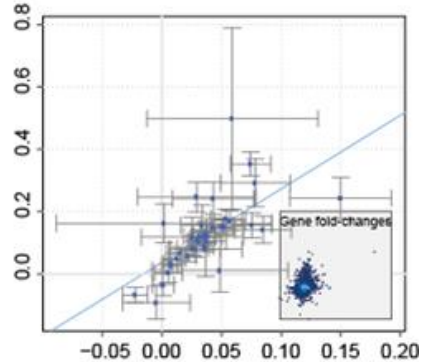
0h post CS-exposure
vs. air-exposed



4h post CS-exposure
vs. air-exposed



24h post CS-exposure
vs. air-exposed



48h post CS-exposure
vs. air-exposed

(Ref. Iskandar A. R. et al. BioMed Research International - In press)

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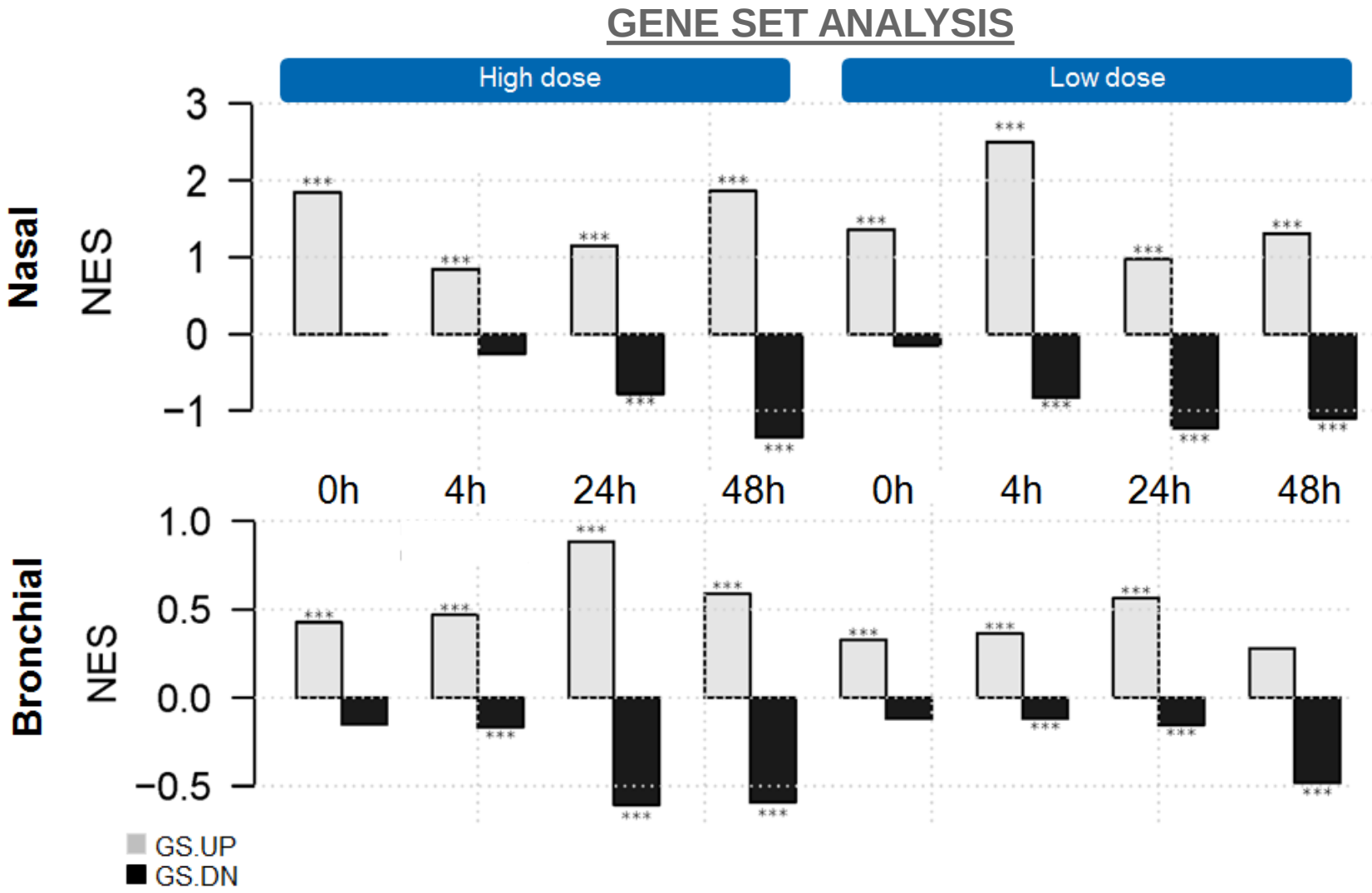
In Vivo/In Vitro Comparison



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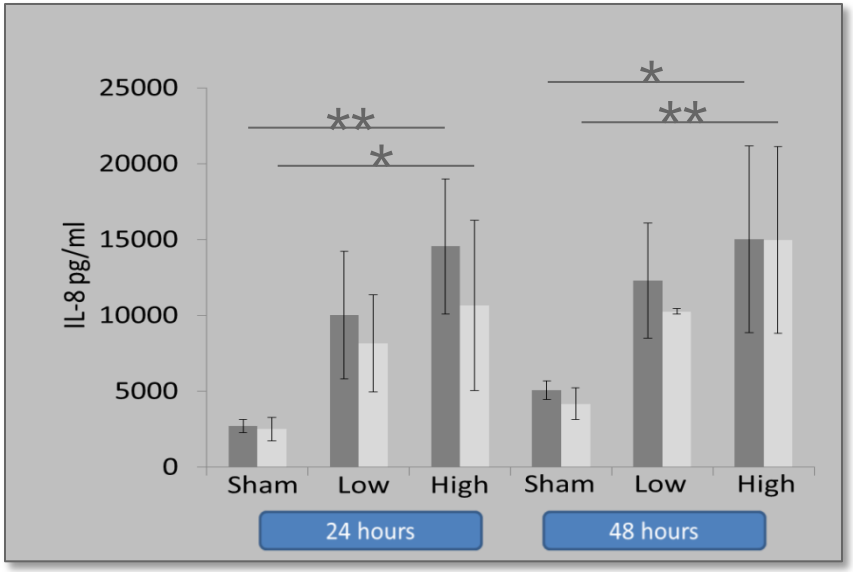
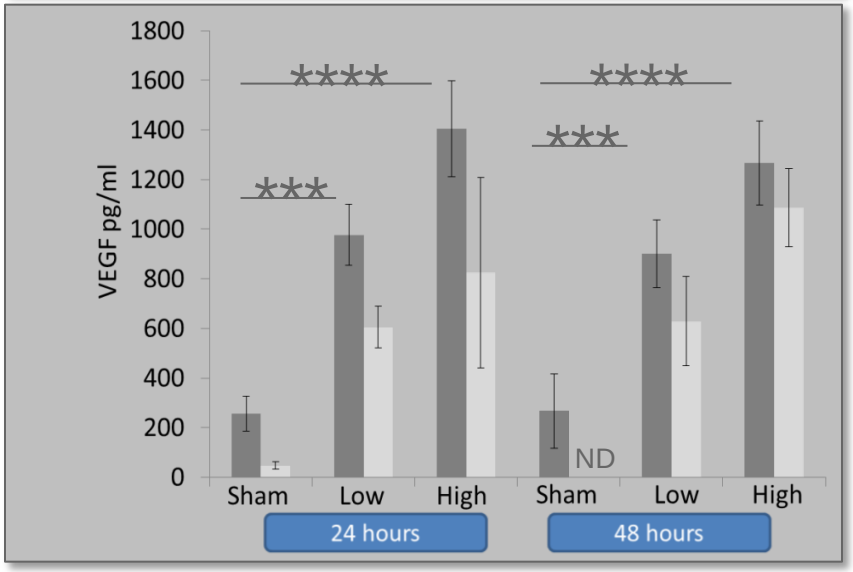
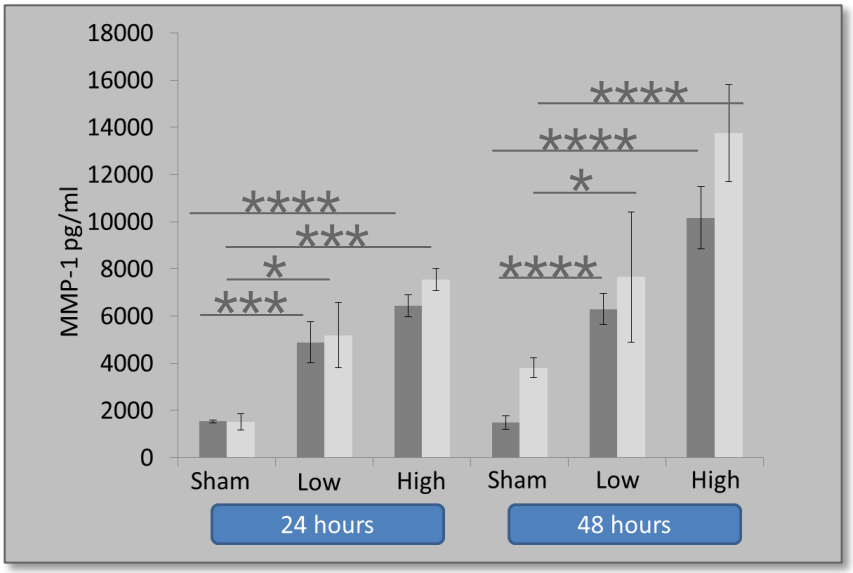
Top 10 Canonical Pathways Perturbed Over Post-Exposure Time and Over Dose in both Nasal and Bronchial Exposed Tissues

Dose- and Time-dependent Release of Pro-inflammatory Markers in both CS-exposed Bronchial and Nasal Tissues







BRONCHIAL

NASAL

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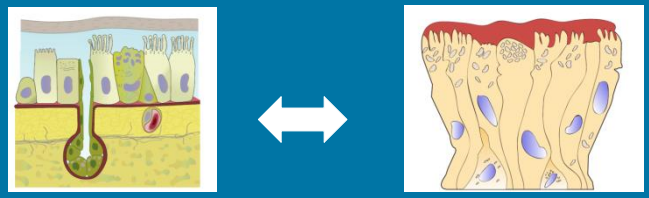
CONCLUSIONS

CS DOSE- AND TIME-DEPENDENCY EFFECTS

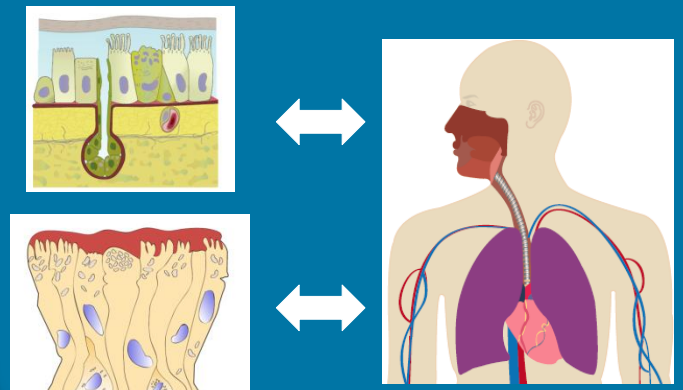
- Activation of AP-1 pathway or of the xenobiotic metabolism at early post-exposure time points (0h and 4h).
- Similar dose- and time-dependent regulation of the release of pro-inflammatory markers (VEGF, IL-8 and MMP-1).

- Up-regulation of genes involved in cell cycle only in nasal tissue culture 48h after exposure
  ➡ recovery process specific to the nasal epithelium?

NASAL AS A SURROGATE FOR BRONCHUS



COMPARISON IN VIVO / IN VITRO



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