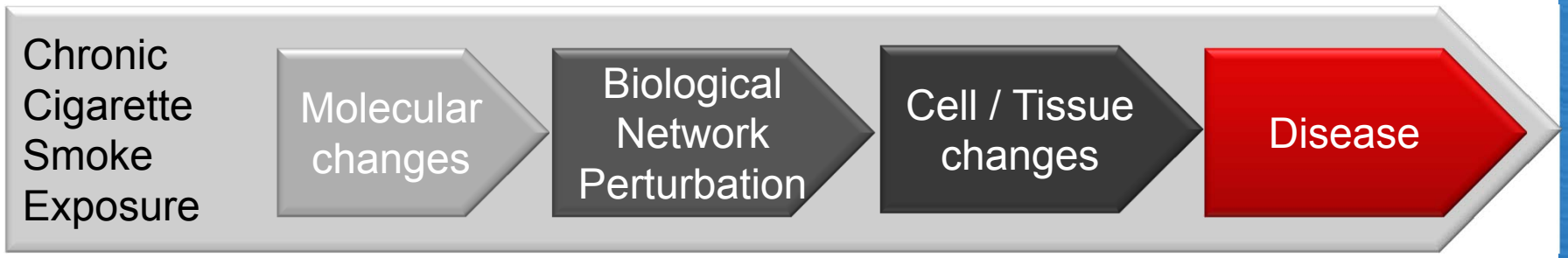
Animal Model for CVD and Inflammation – pMRTP Switching Study

Understanding Tobacco and Tobacco Harm Reduction: A Path Forward

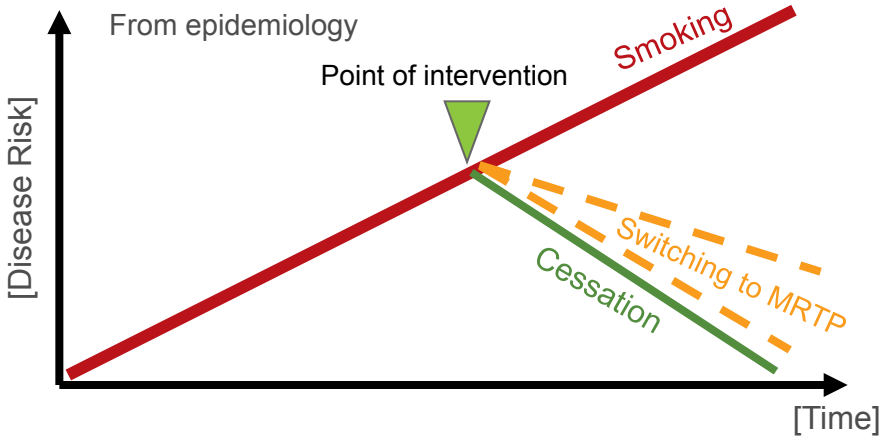
Julia Hoeng

July 2nd 2013

How does Biology Respond to MRTP Aerosols?

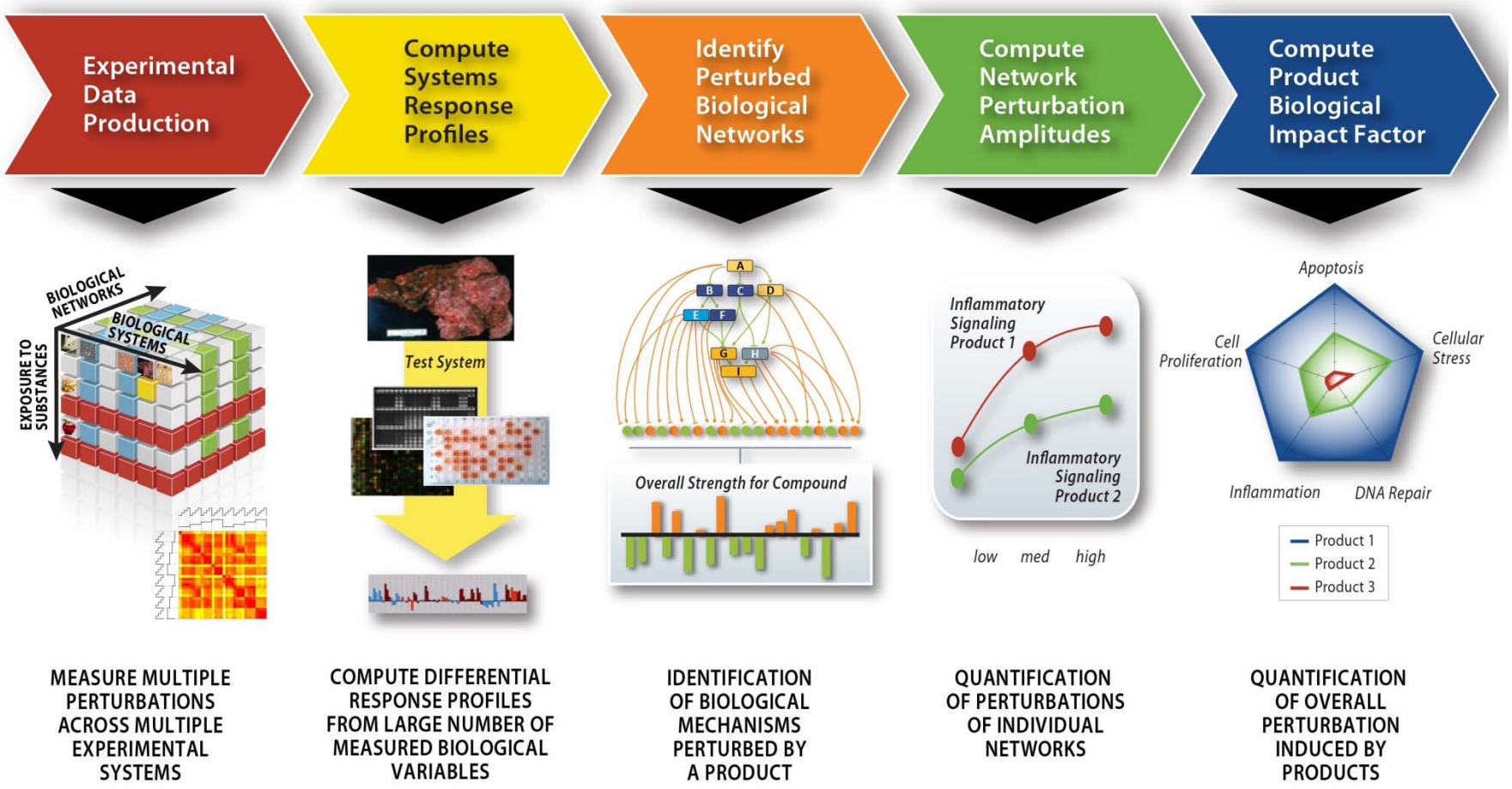


What is the Biological Impact of an MRTP Aerosol compared to Cigarette Smoke?



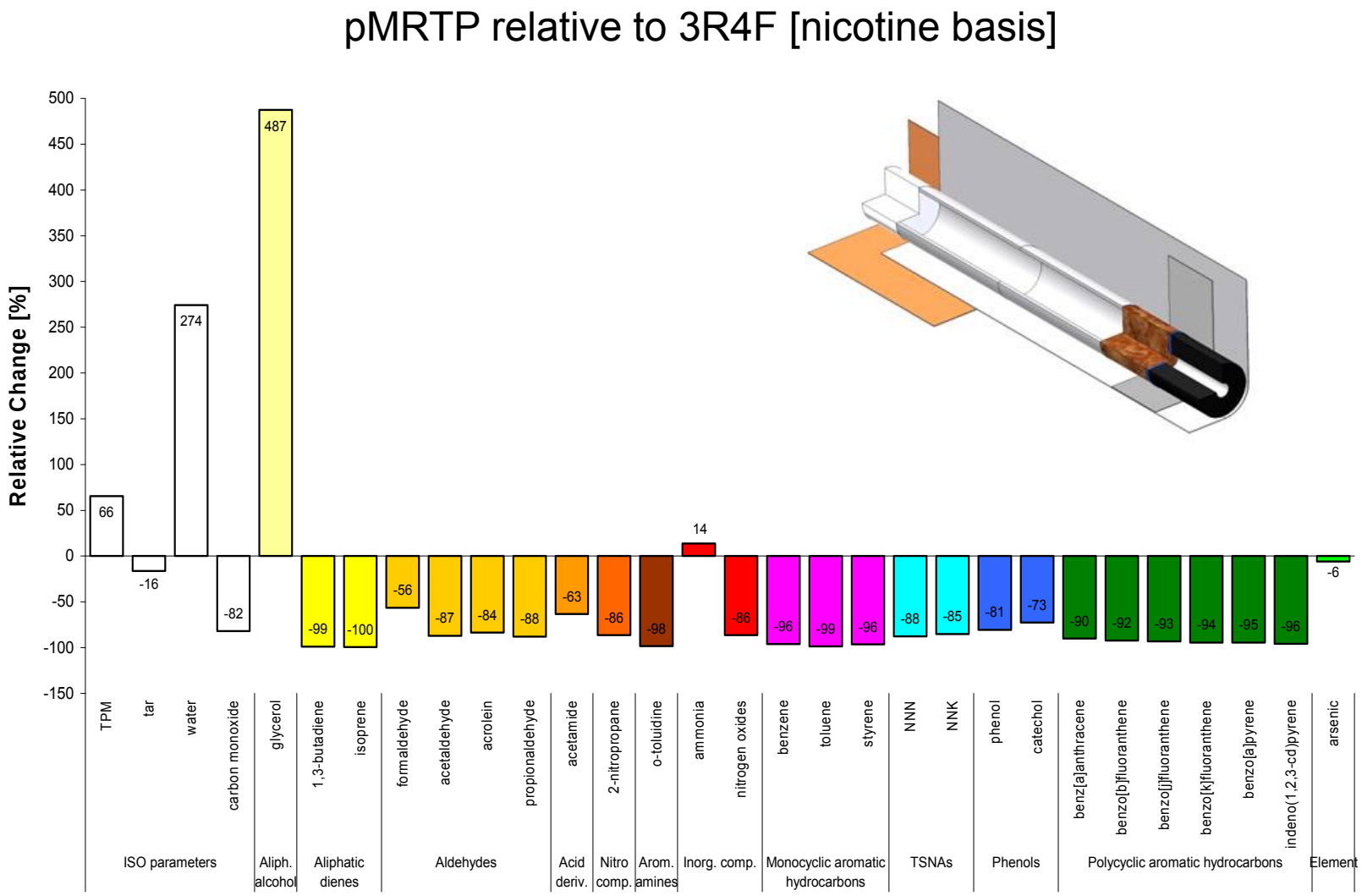
- Compare switching to pMRTP with continued smoking and benchmark against smoking cessation.
- Assess how close switching to pMRTP is to smoking cessation

Quantitative Mechanism-Based Systems Impact Assessment



Hoeng J, Deehan R, Pratt D, Martin F, Sewer A, Thomson TM, Drubin DA, Waters CA, de Graaf D, and Peitsch MC.
A network-based approach to quantifying the impact of biologically active substances. *Drug Discov Today* 17: 413-418, 2012.

Smoke Chemistry is Indicative of Reduced Exposure Potential in pMRTP Compared to Reference Cigarette (3R4F)



Inhalation Study Design

Study Design

The aim of this study was to evaluate the potential reversibility of COPD- and CVD-related endpoints upon switching from conventional cigarette exposure to a pMRTP exposure. The study was performed to identify CVD- and COPD-related biology perturbed by smoke exposure, taking advantage of the experimental and computational tools and methods developed in-house to enable the Systems Biology-based assessment of pMRTP.

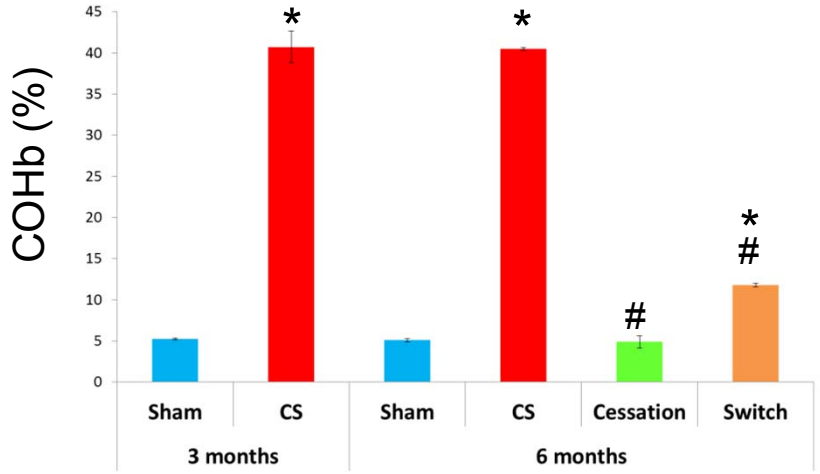
Systems Toxicology Endpoints

Endpoint	Assay	Assay Description	Number of Mice per Group (Total Number of Mice*)
Test atmosphere characterization		Smoke generation and test atmosphere characterization	-
Markers of exposure	Nicotine metabolites	Trans-3'-Hydroxycotinine (3'HOCOT), nornicotine (NNIC), norcotinine (NCOT), cotinine (COT), and nicotine-N'-oxide (NN'O) were quantified by HPLC in urine	
	COHb	Carboxyhemoglobin measurement	8 (48)
In life observation	Body weight	Measure of body weight	All
CVD	Plaque size	Planimetry of aortic arch	16 (96)
	Lipoproteins	Lipoproteins concentrations in plasma and in the plaque measured by high-performance liquid chromatography	8 (144)
	Lipidomics	Mass-spectrometry based lipidomics (outsourced to Zora Biosciences, Finland)	8 (64)
COPD	BALF	- Cells counts in BALF (FACS analysis) - Mediator analysis in BALF outsourced to Rules Based Medicine (USA)	8 (32)
	Lung histology and morphometry	Histopathological and morphometrical analyses of lung tissues	20 (80)
	Lung function	Evaluation of respiratory mechanics	8 (32)
All	Transcriptomics	- mRNA levels measured using Affymetrix GeneTitan microarrays - Computational analysis	8 (112)
	Statistics	Sample number and Statistical Analysis	-

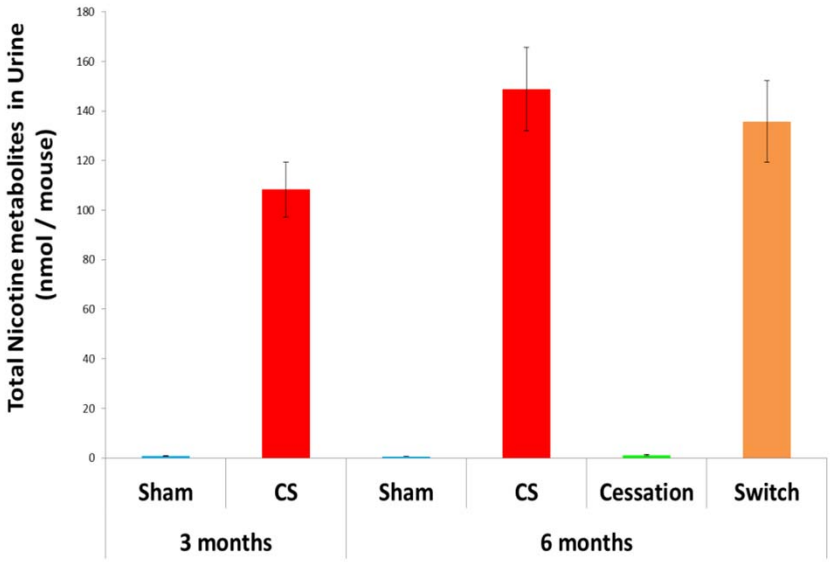
* Whenever possible, mice were used to measure multiple endpoints

Reduced Exposure For Similar Nicotine Delivery, Illustrated by COHb Levels in Blood and Nicotine Metabolites in Urine

Reduced COHb in blood



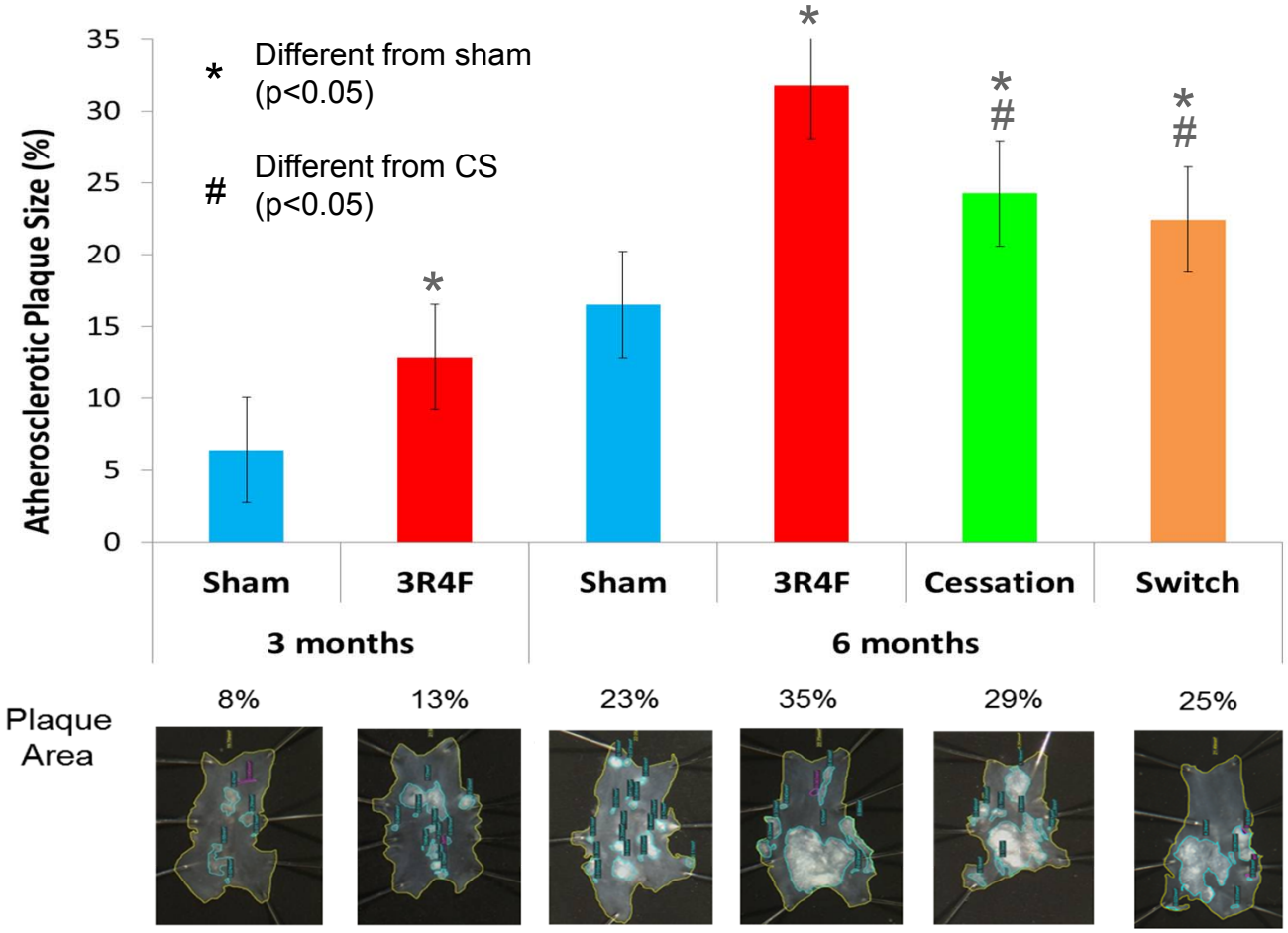
Similar nicotine metabolites content in urine



* Different from sham ($p<0.05$)

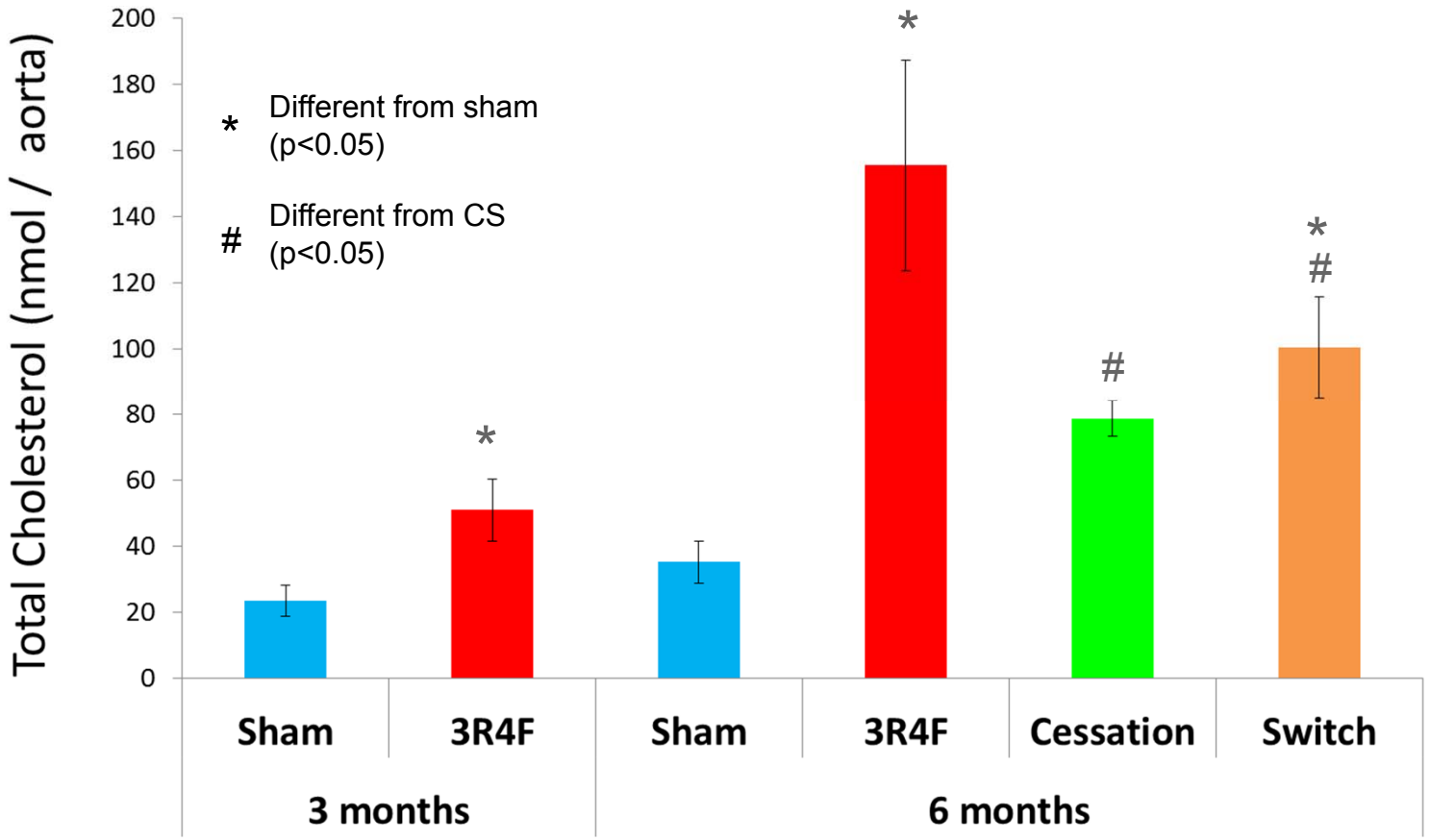
\# Different from CS ($p<0.05$)

CVD - Reduced Atherosclerotic Plaque Burden



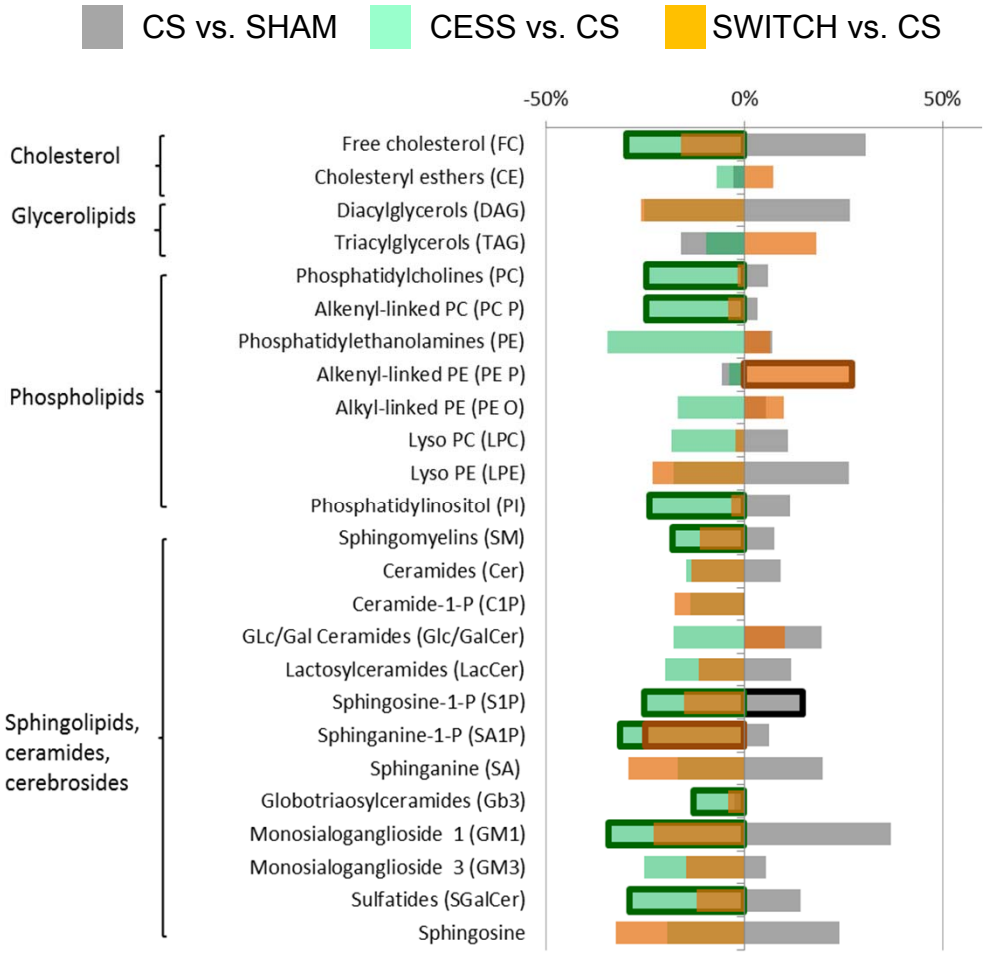
Data (except for pMRTP) published in Lietz M, Berges A, Lebrun S, Meurrens K, Steffen Y, Stolle K, Schueller J, Boué S, Vuillaume G, Vanscheeuwijck P, Moehring M, Schlage W, De Leon H, Hoeng J, Peitsch M: Cigarette-smoke-induced atherogenic lipid profiles in plasma and vascular tissue of apolipoprotein E-deficient mice are attenuated by smoking cessation. Atherosclerosis 2013, In press.

CVD - Reduced Total Cholesterol in Aorta



Data (except for pMRTP) published in Lietz M, Berges A, Lebrun S, Meurrens K, Steffen Y, Stolle K, Schueller J, Boué S, Vuillaume G, Vanscheeuwijck P, Moehring M, Schlage W, De Leon H, Hoeng J, Peitsch M: Cigarette-smoke-induced atherogenic lipid profiles in plasma and vascular tissue of apolipoprotein E-deficient mice are attenuated by smoking cessation. Atherosclerosis 2013, In press.

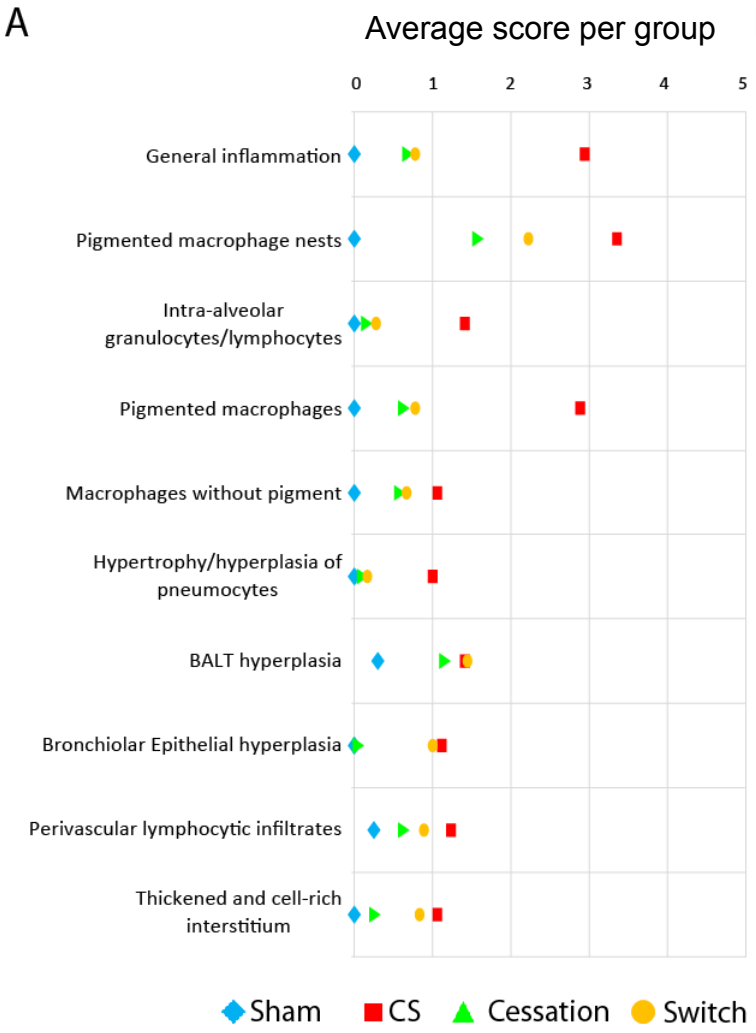
Reduced Lipid Concentrations in Serum

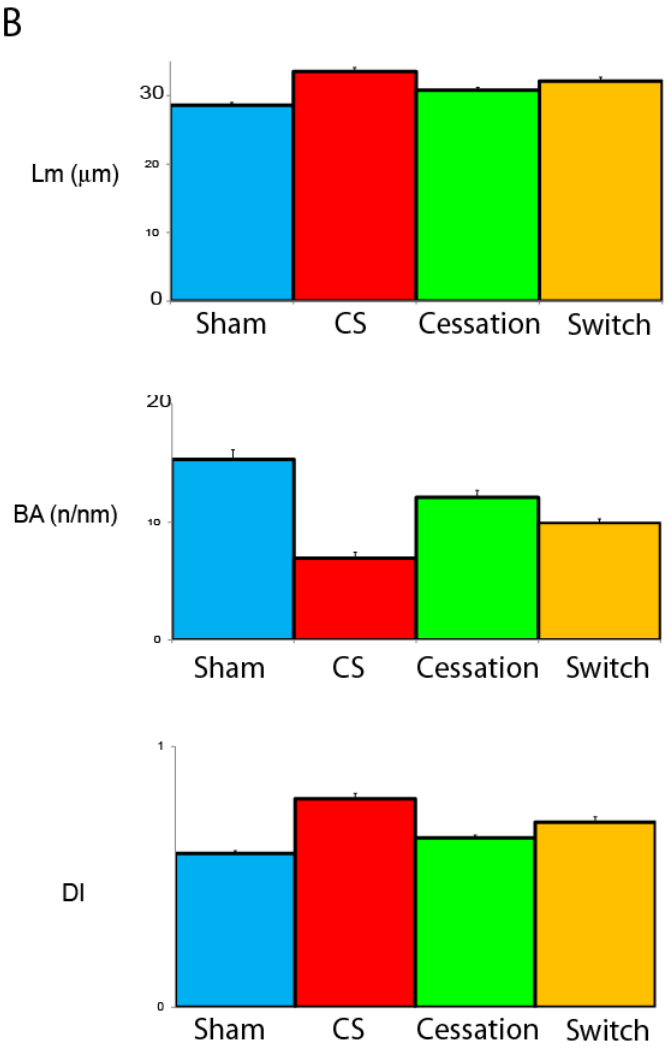


Switching to pMRTP from CS (3R4F) resulted in CESS level concentrations of cholesteryl esters, free cholesterol, ceramides and lactosylceramides.

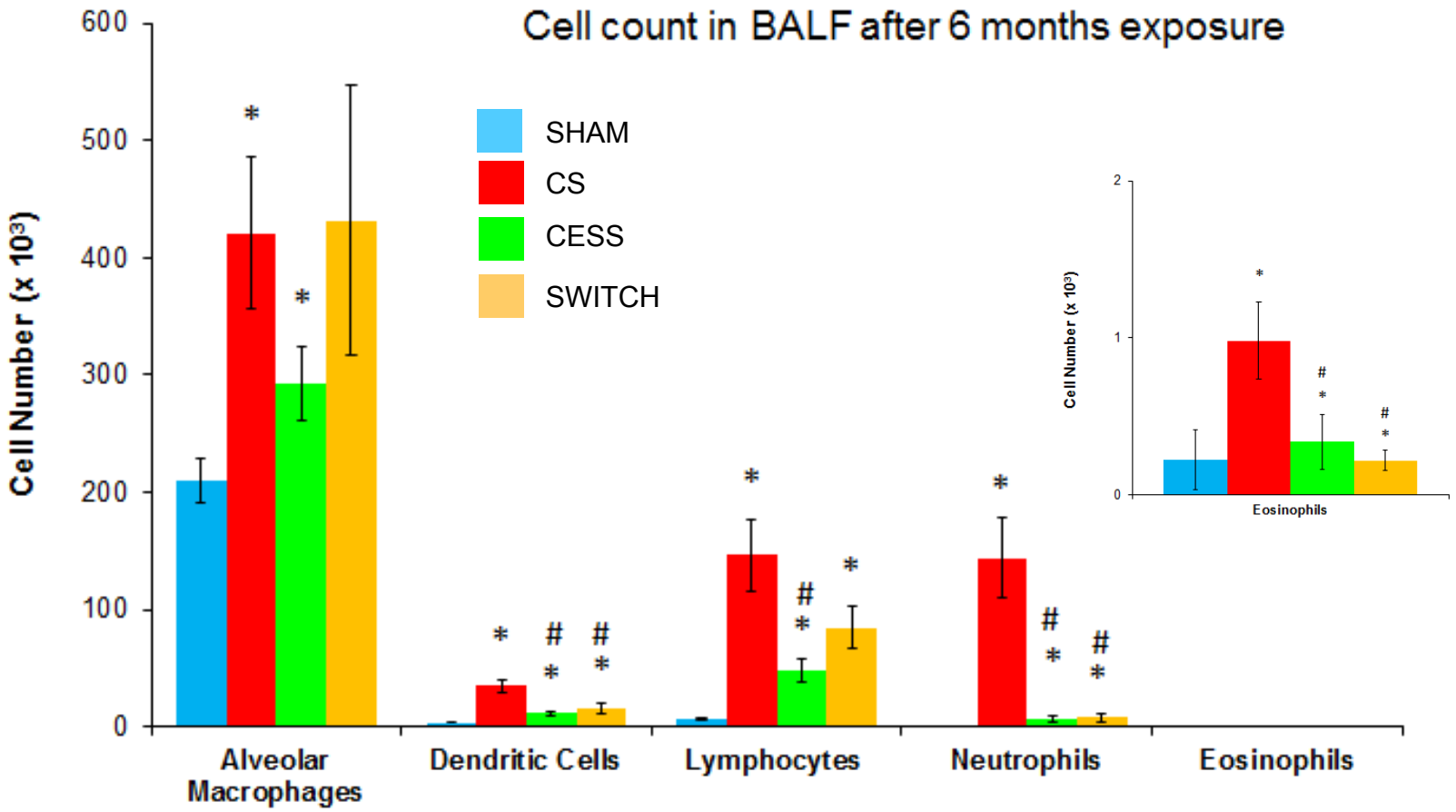
Data (except for pMRTP) published in Lietz M, Berges A, Lebrun S, Meurrens K, Steffen Y, Stolle K, Schueller J, Boué S, Vuillaume G, Vanscheeuwijck P, Moehring M, Schlage W, De Leon H, Hoeng J, Peitsch M: Cigarette-smoke-induced atherogenic lipid profiles in plasma and vascular tissue of apolipoprotein E-deficient mice are attenuated by smoking cessation. Atherosclerosis 2013, In press.

Reduced Histopathology Evidence of Lung Emphysema and Inflammation





Reduced Pulmonary Inflammation Evidenced in BALF Free Lung Cells



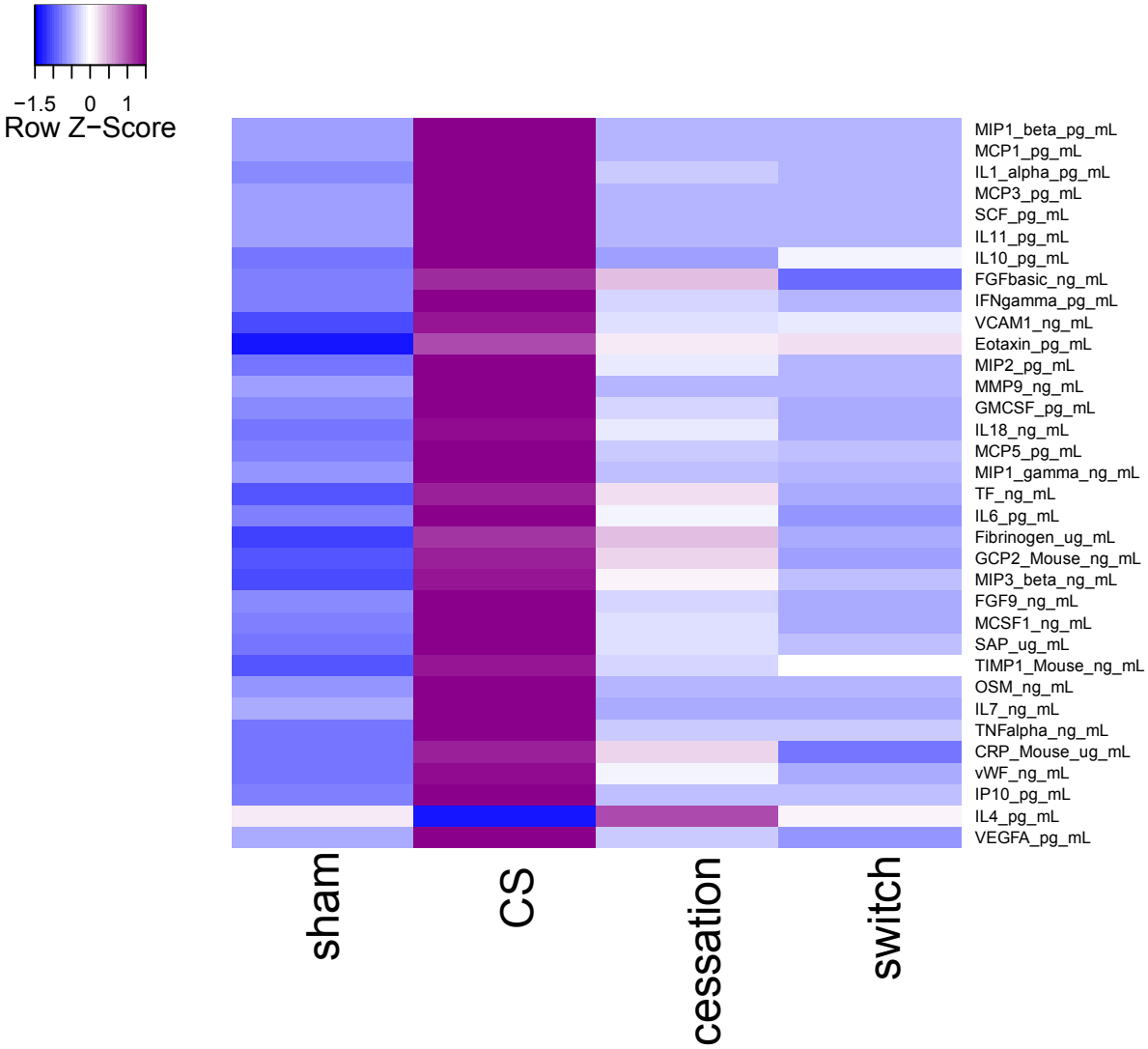
Wilcoxon rank-sum test

* Different from sham ($p<0.05$)

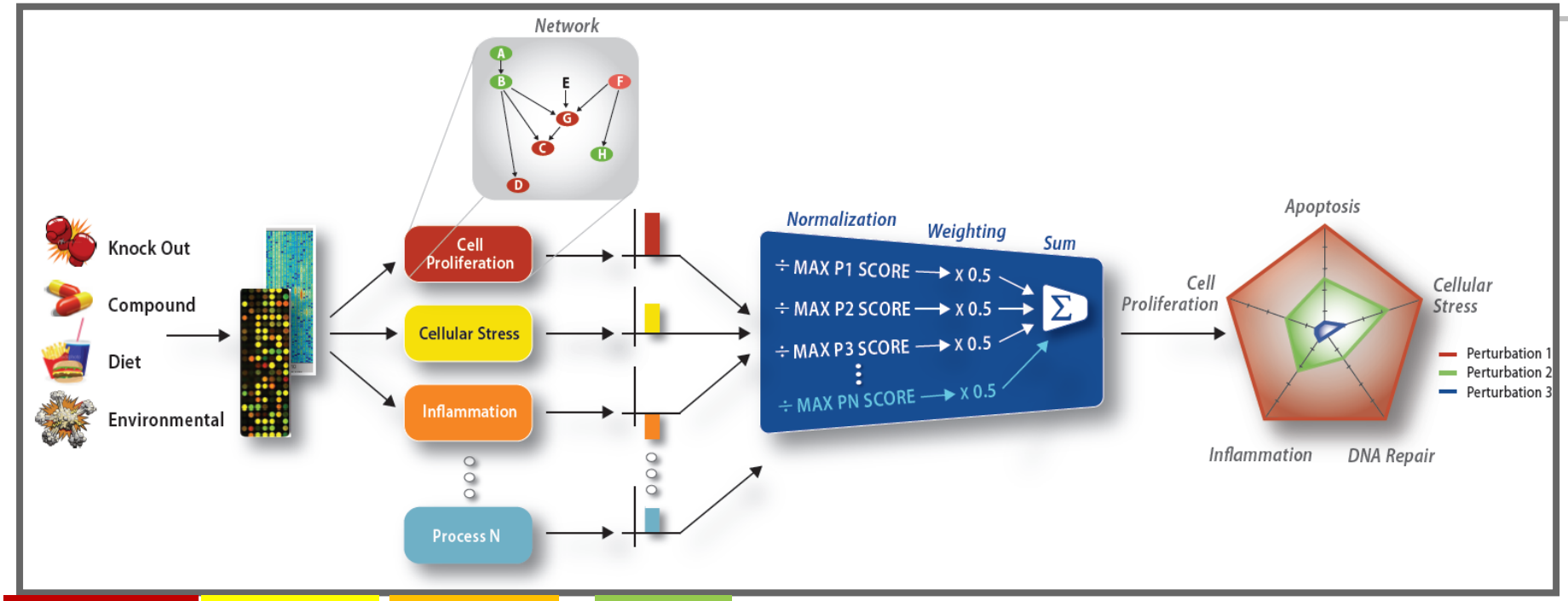
\# Different from CS ($p<0.05$)

PMI RESEARCH & DEVELOPMENT

Reversal of Inflammation Markers in the BALF (Proteomics at the 6-month time point)



From an Exposure to an Assessment of Impact



Exposure and data Systems Biology production

Systems Response Profile

Biological Network Model

NPA Scores

Score Aggregation

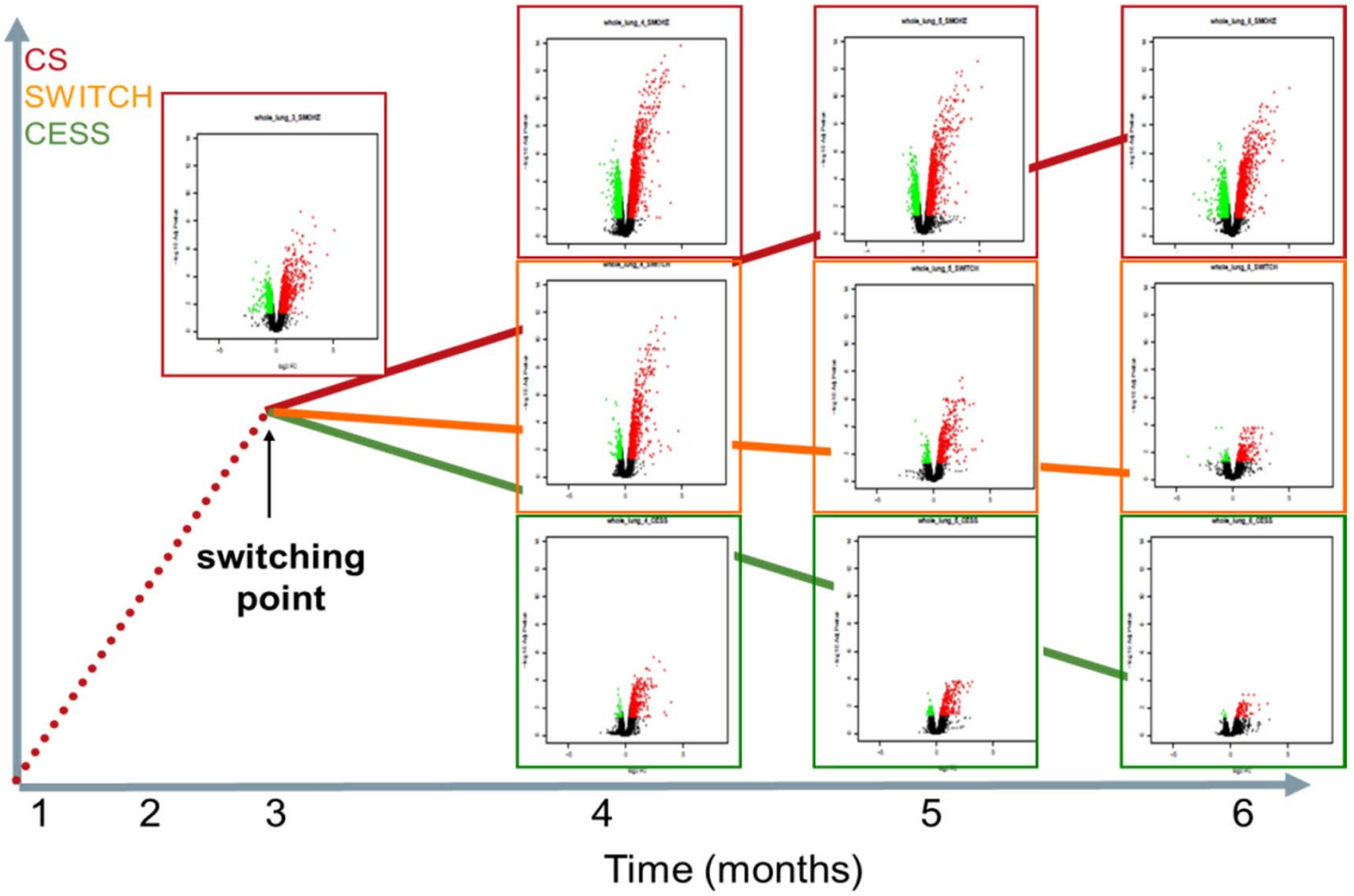
Biological Impact Factor (BIF)

Biological Networks describe the mechanism

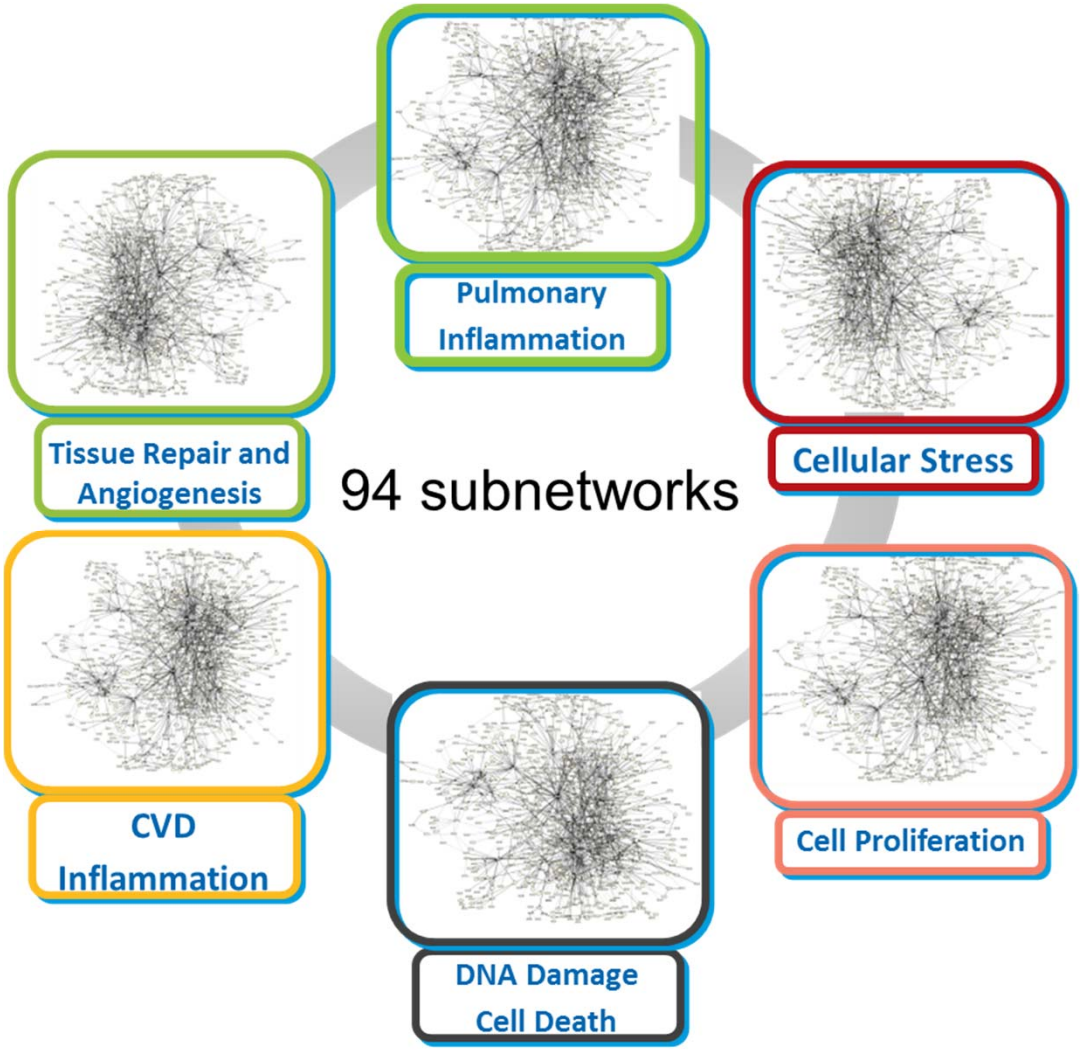
The NPA algorithms uses the data in the context of a defined biological network model

NPA scores can be aggregated to produce a Biological Impact Factor (BIF).

Reduced Overall Gene Expression Perturbation in the Lung



Biological Networks describing Disease Mechanisms



Park JS et al. **Construction of a Computable Network Model of Tissue Repair and Angiogenesis in the Lung.** Clinical Toxicology. 2013;S12.

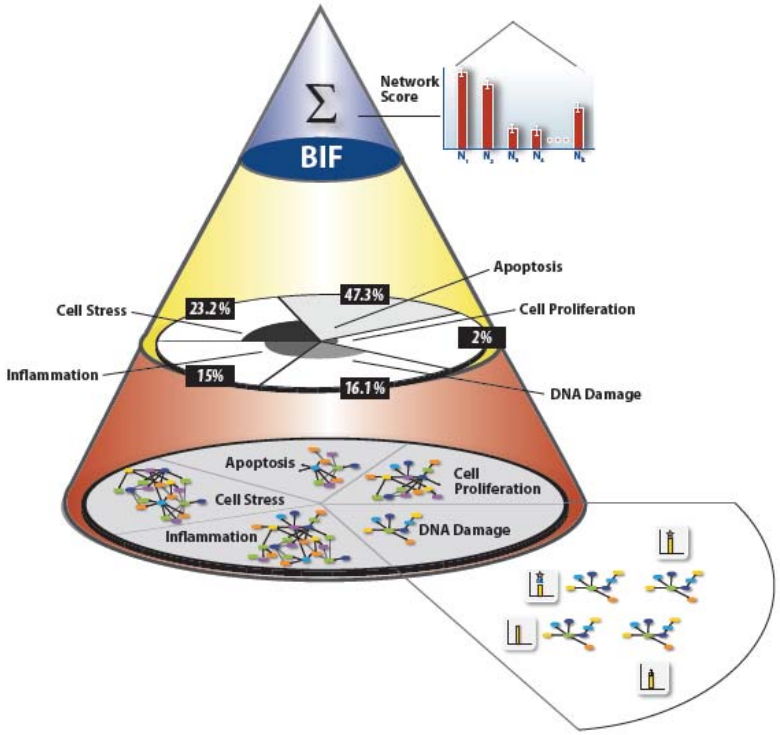
Schlage WK et al. **A computable cellular stress network model for non-diseased pulmonary and cardiovascular tissue.** BMC Syst Biol. 2011;5:168.

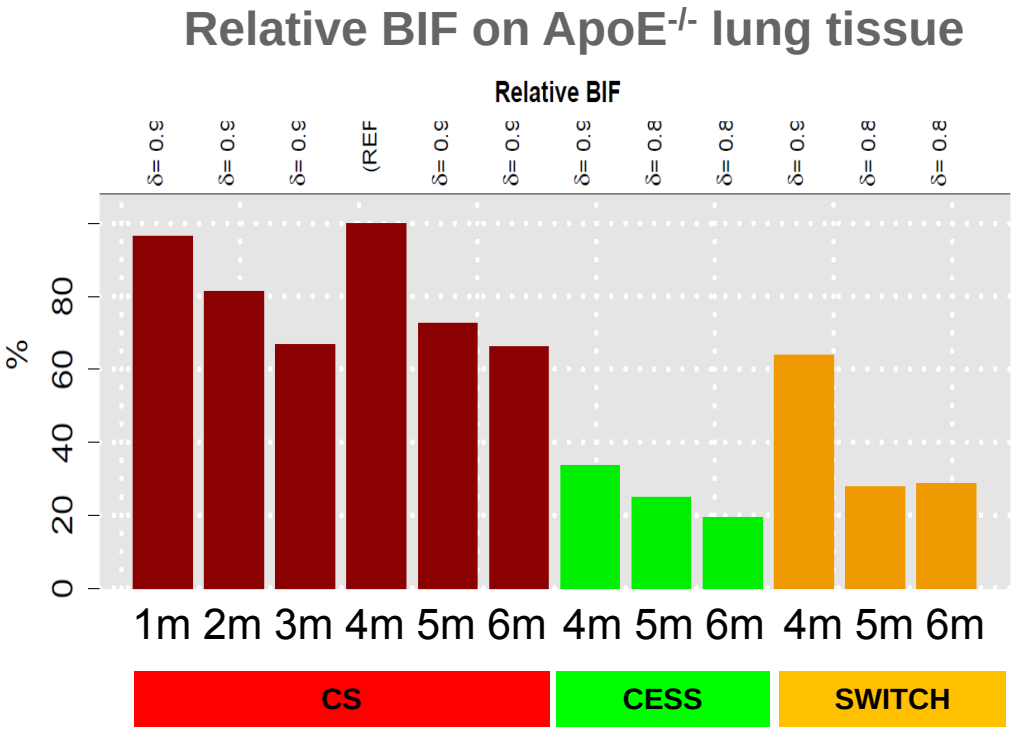
Westra JW et al. **Construction of a computable cell proliferation network focused on non-diseased lung cells.** BMC Syst Biol. 2011;5:105.

Westra JW et al. **A Modular Cell-Type Focused Inflammatory Process Network Model for Non-diseased Pulmonary Tissue.** Bioinformatics and biology insights. 2013;7:1-26.

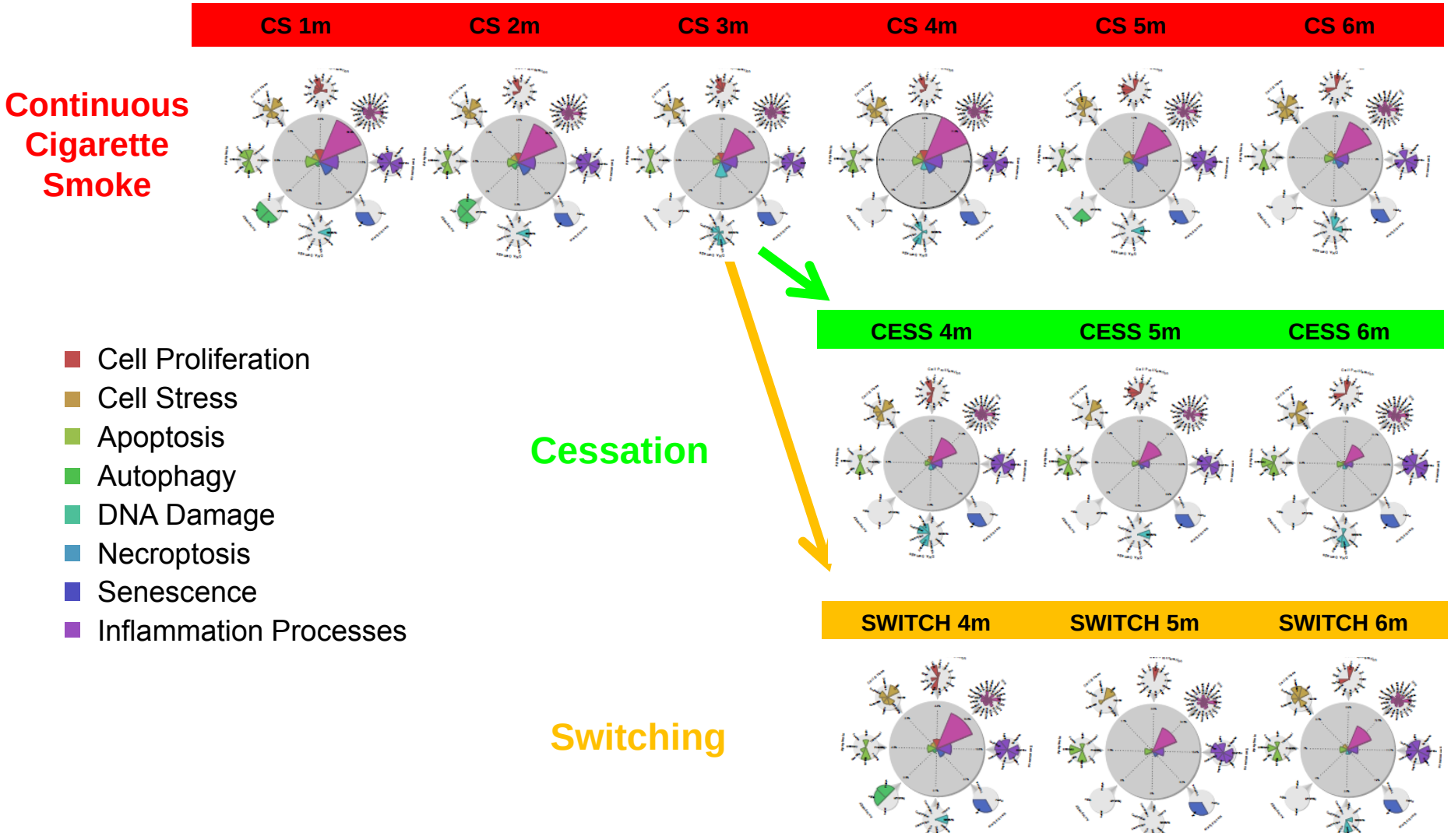
Gebel S et al. **Construction of a computable network model for DNA damage, autophagy, cell death, and senescence.** Bioinformatics and biology insights. 2013, 7:97-117.

The Biological Impact Factor (BIF)





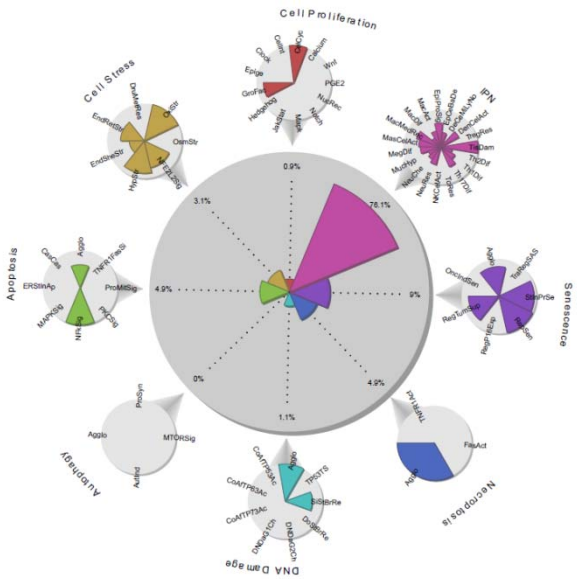
Network Perturbations and Biological Impact in the Lung (Gene Expression)



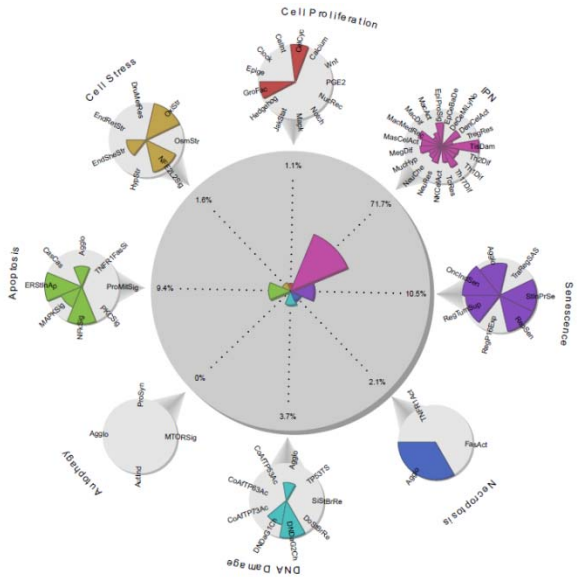
Source: PMI

Network Perturbations and Biological Impact in the Lung at the Final Time Point

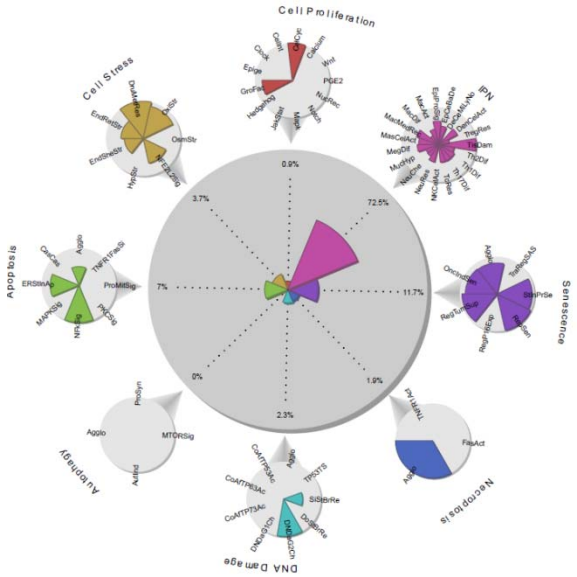
CS 6m



CESS 6m

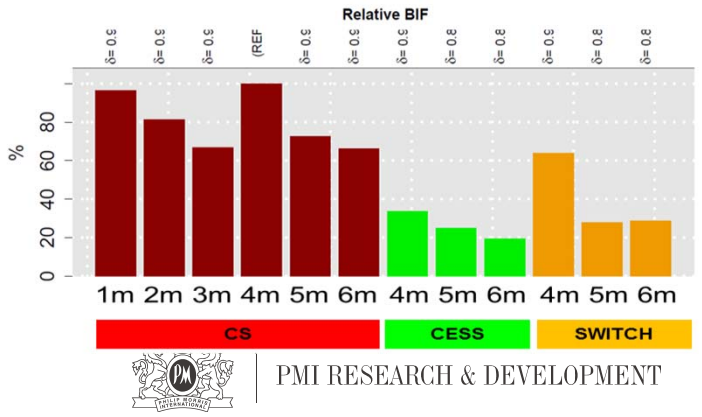


SWITCH 6m



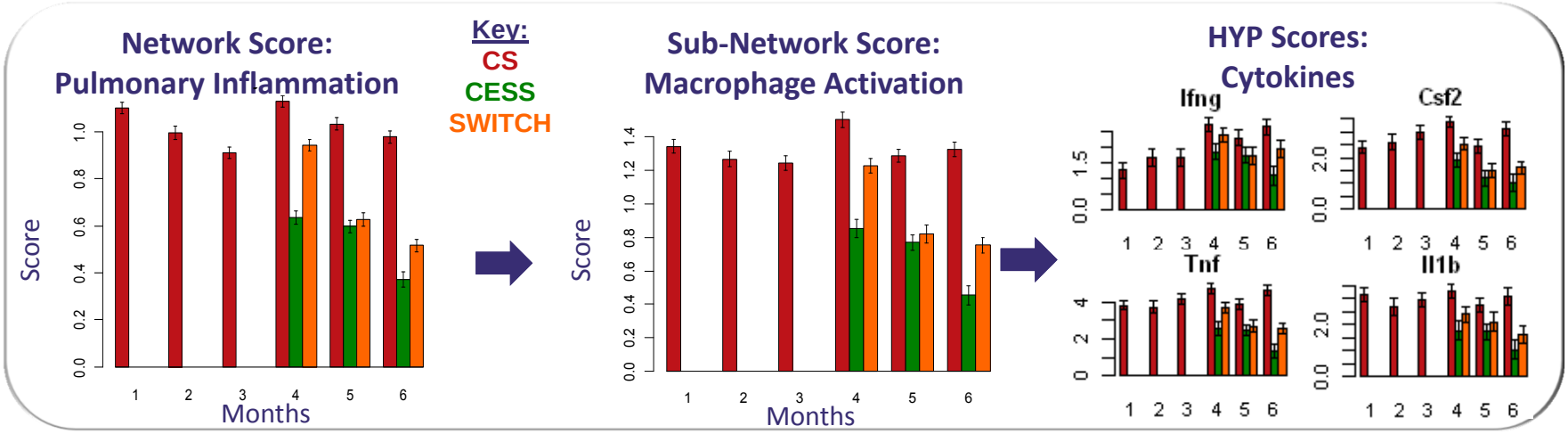
- Cell Proliferation
- Cell Stress
- Inflammation Processes
- Apoptosis
- Autophagy
- DNA Damage
- Necroptosis
- Senescence

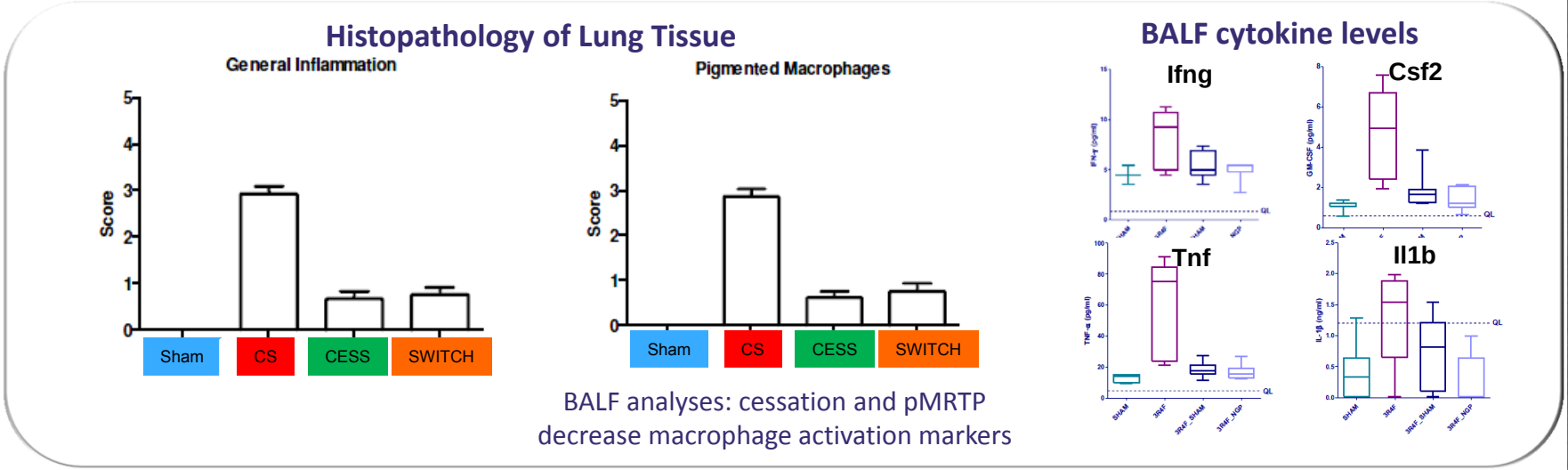
Relative BIF on ApoE$^{-/-}$ lung tissue



PMI RESEARCH & DEVELOPMENT

Inflammatory Network Perturbations determined by Gene Expression Analysis of Lung Tissue substantiate the Inflammatory Biomarkers measured in Broncheoalveolar Lavage Fluid (BALF)

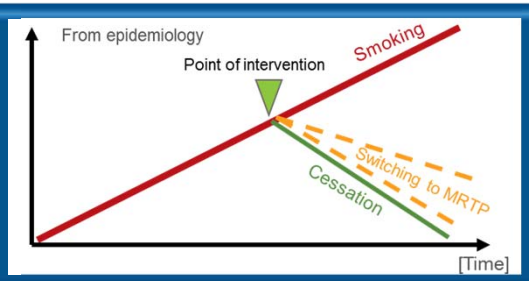




BALF analyses: cessation and pMRTP decrease macrophage activation markers

Summary of Results

Plaque burden ~ CVD risk
Atherogenic lipids ~ CVD risk
Lung emphysema ~ COPD risk
Inflammation ~ x diseases risk



- These data highlight the potential of heating (as opposed to burning) tobacco to reduce cigarette smoke exposure-related disease risks.
- The results obtained from a comprehensive list of endpoints in this study suggest that the systems toxicology is powerful approach for the simultaneous investigation of lung and cardiovascular disease mechanisms in vivo.
- This approach could have applications in the development of a systems biology-based assessment to compare the biological impact of Modified Risk Tobacco Products (as defined by the US FDA) with conventional cigarettes and smoking cessation as a benchmark.