

Systems toxicology analysis of cardiovascular and respiratory endpoints from ApoE^{-/-} mice showed similar effects when switching to a candidate Modified Risk Tobacco Product, THS2.2, or ceasing smoking

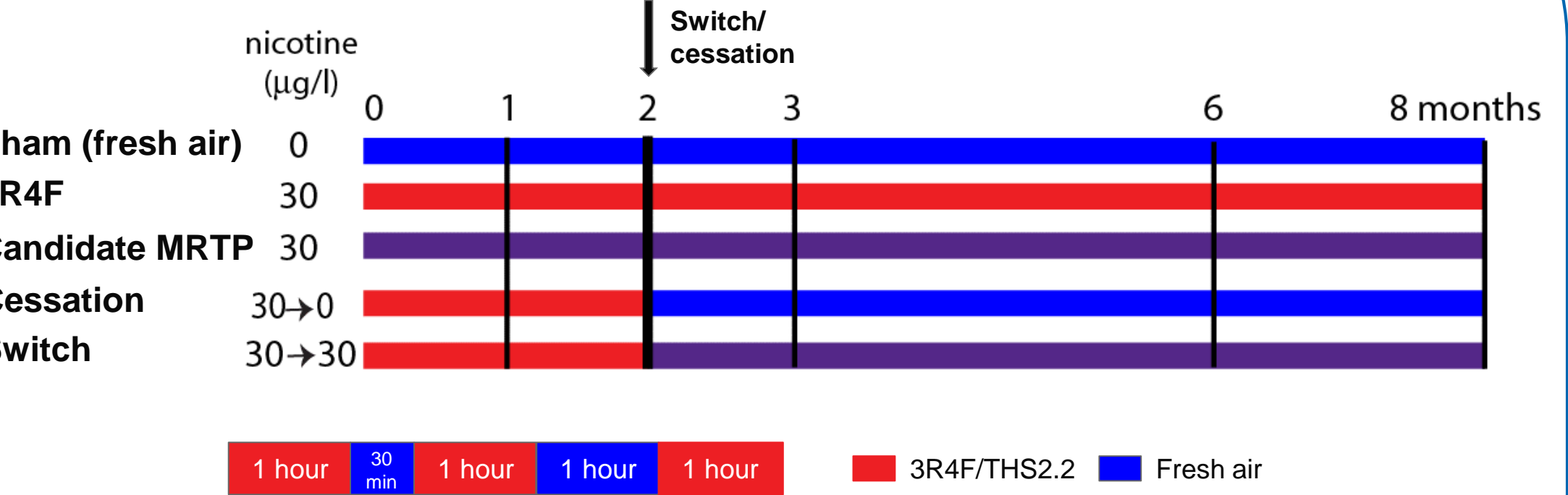
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Background

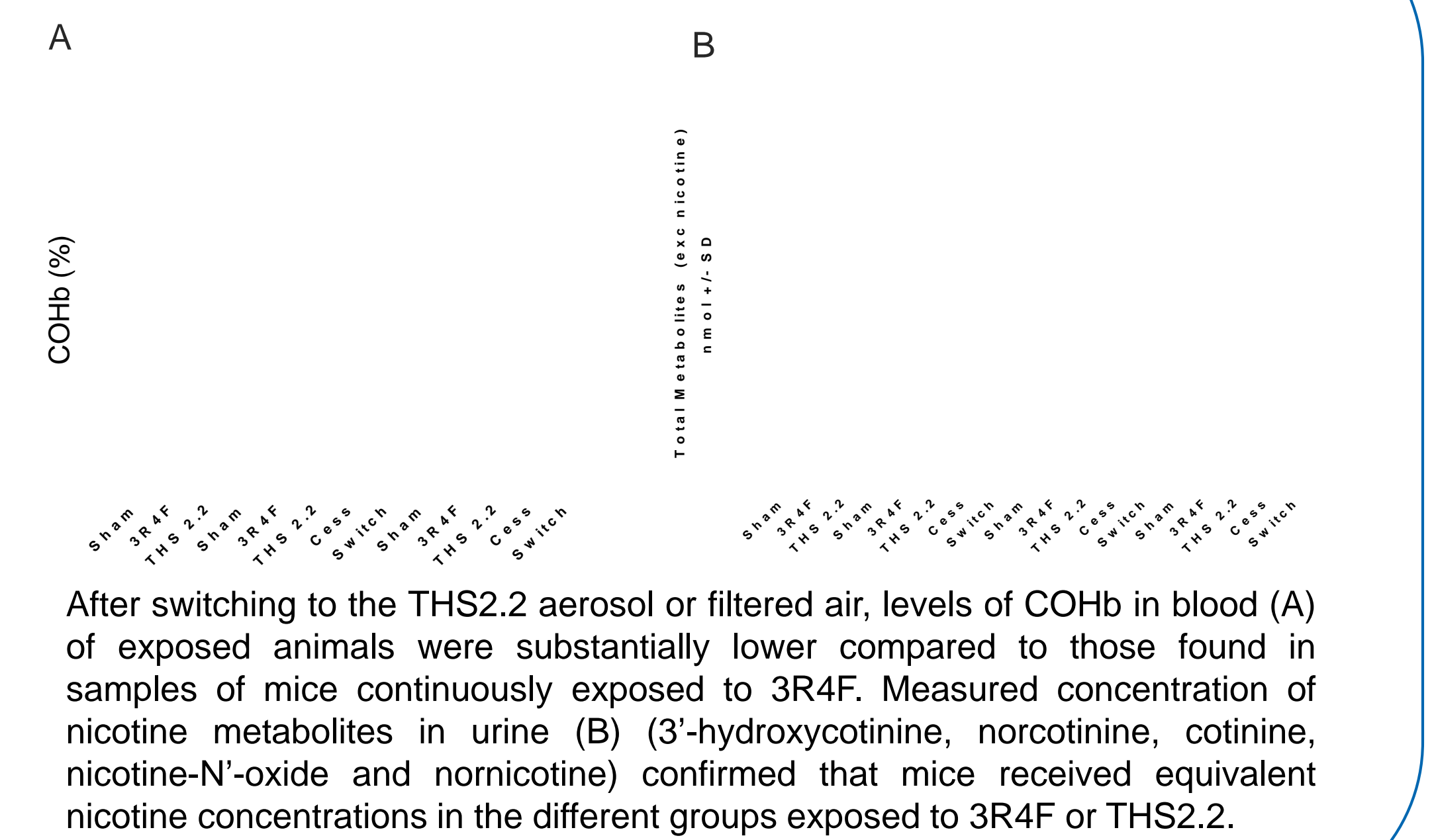
Smoking causes many serious diseases including cardiovascular disease (CVD) and chronic obstructive pulmonary disease (COPD). Suitable animal models play an important role in understanding of smoke-induced pathogenesis. This study examined the development of hallmarks of both COPD and CVD in ApoE^{-/-} mice exposed to either cigarette smoke (CS) or to an aerosol from a candidate modified risk tobacco product (MRTP), the tobacco heating system (THS2.2) over an 8-month period. In addition to chronic exposure regimes, a comparison of exposure cessation or switching to THS2.2 after 2 months of exposure to CS was performed using a battery of assays (physiological, morphological and molecular). Biological interpretation of various endpoints enables to understand the biological effects of cessation/switching as compared to continuous smoking.

Study design and endpoints

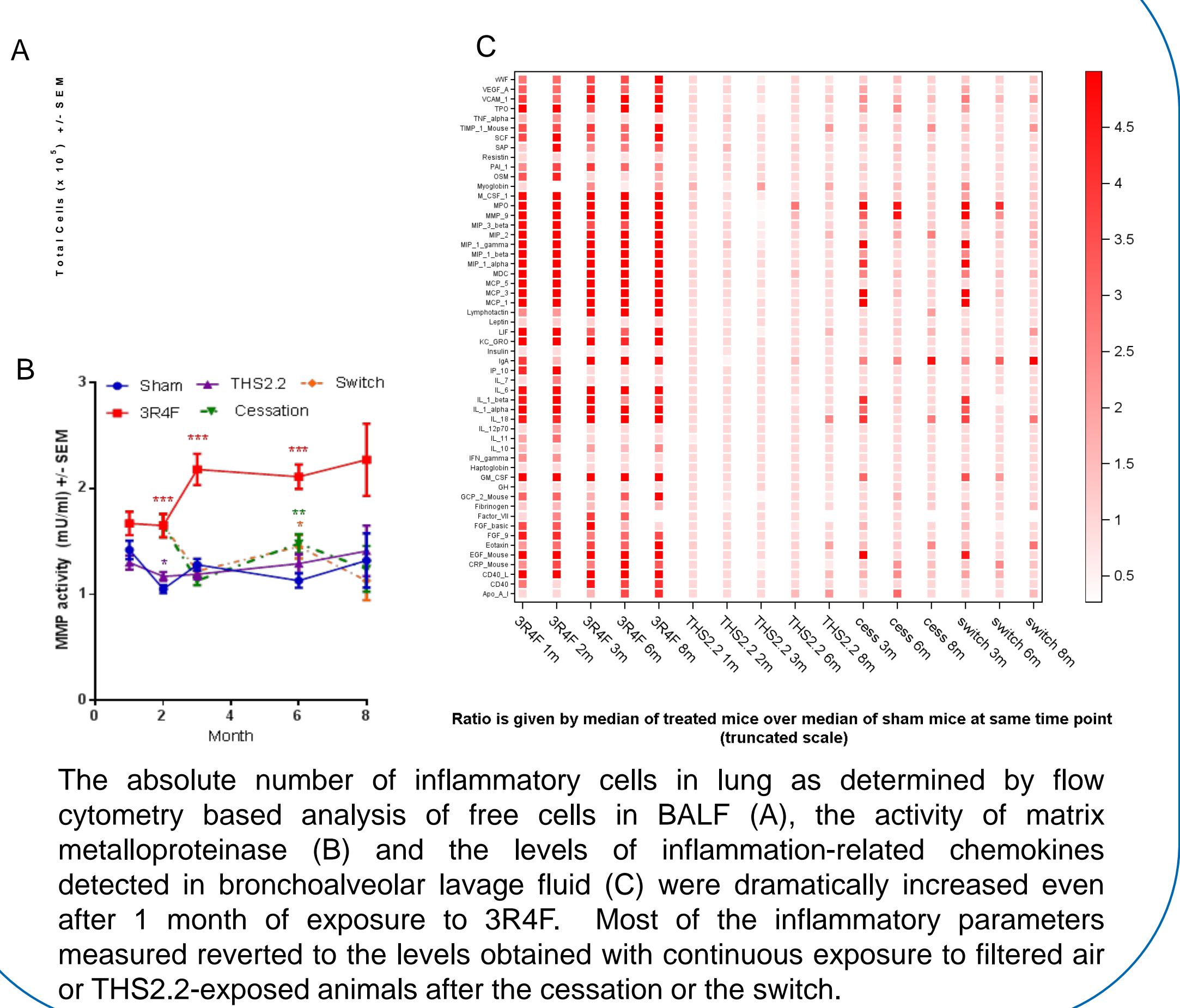


Female ApoE^{-/-} mice were exposed to 3R4F (600 mg/m³ TPM), THS2.2 (matched to the nicotine in 3R4F – 30 µg/l) or filtered air for 3 hours per day, 5 days per week, for up to 8 months. After 2 months of exposure to 3R4F, switching and cessation groups were exposed to aerosol from THS2.2 or filtered air, respectively. Animals were observed on a daily basis, body weight progression was monitored twice per week, exposure parameters (carboxyhemoglobin (COHb) in blood and nicotine metabolites in urine) were measured 3 times during the study. Dissections were performed after 1, 2, 3, 6, and 8 months of exposure. At each time point animals were allocated for the following endpoints: bronchoalveolar lavage fluid (BALF), identification of infiltrated inflammatory cells in lungs and multi-analyte (cytokines/chemokines) profiling; histopathological evaluation and morphometry of lungs; lung function; plaque surface determination and an extensive molecular high throughput analysis (transcriptomics, proteomics and lipidomics).

Exposure biomarkers



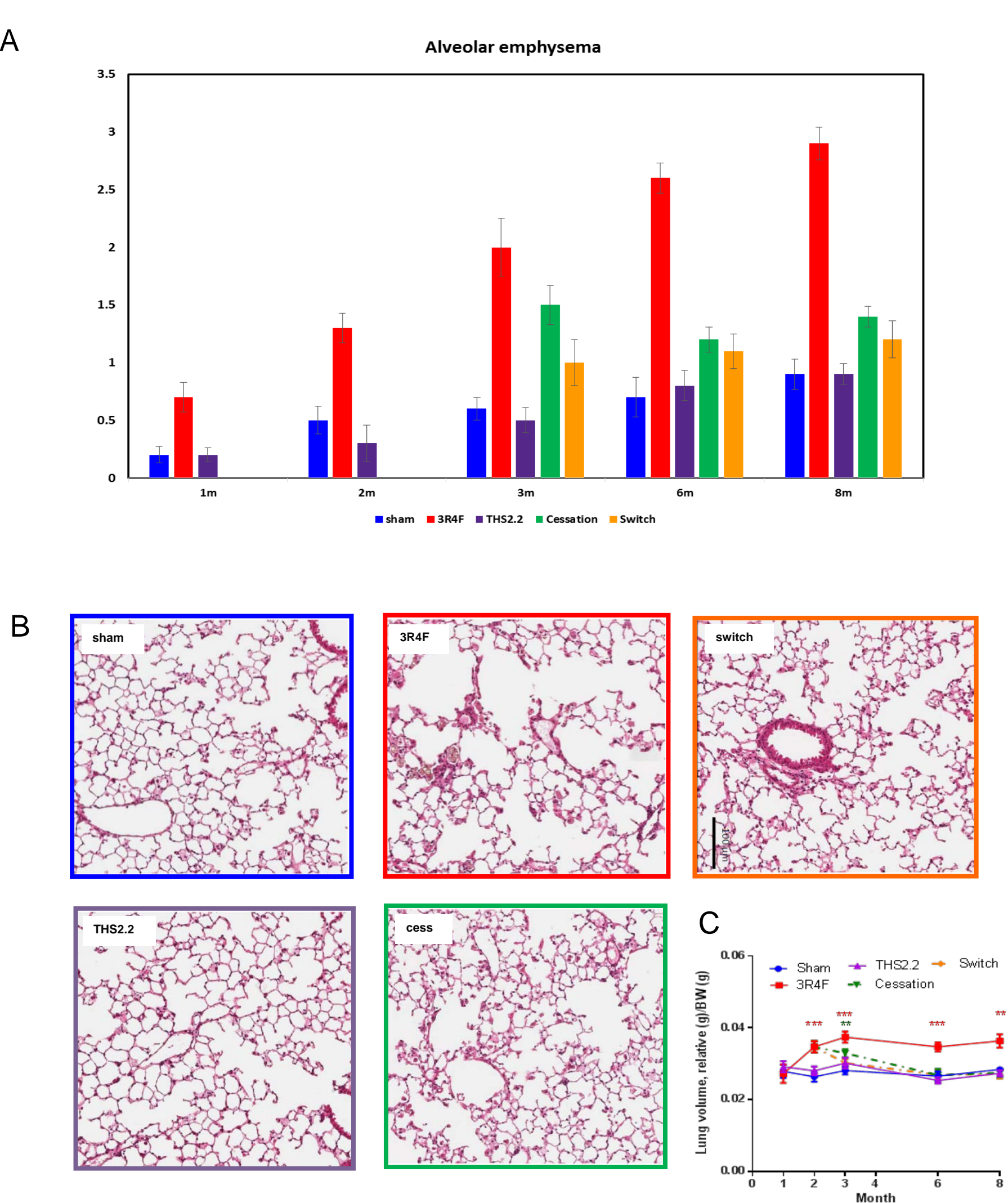
Lung inflammation



- The exposure to 3R4F cigarette smoke resulted in significant levels of pulmonary inflammation, decline in pulmonary function, and histopathological changes. These phenotypic changes were coherent with the molecular data.
- Chronic exposure to an aerosol from the THS2.2 resulted in very little difference in all measured parameters related to COPD and CVD when compared to the filtered air-exposed animals.
- The biological response to switching to a THS2.2 aerosol or filtered air following 2 months of 3R4F cigarette smoke exposure were very similar between the two conditions across the spectrum of endpoints assessed, and showed a generally positive effect.

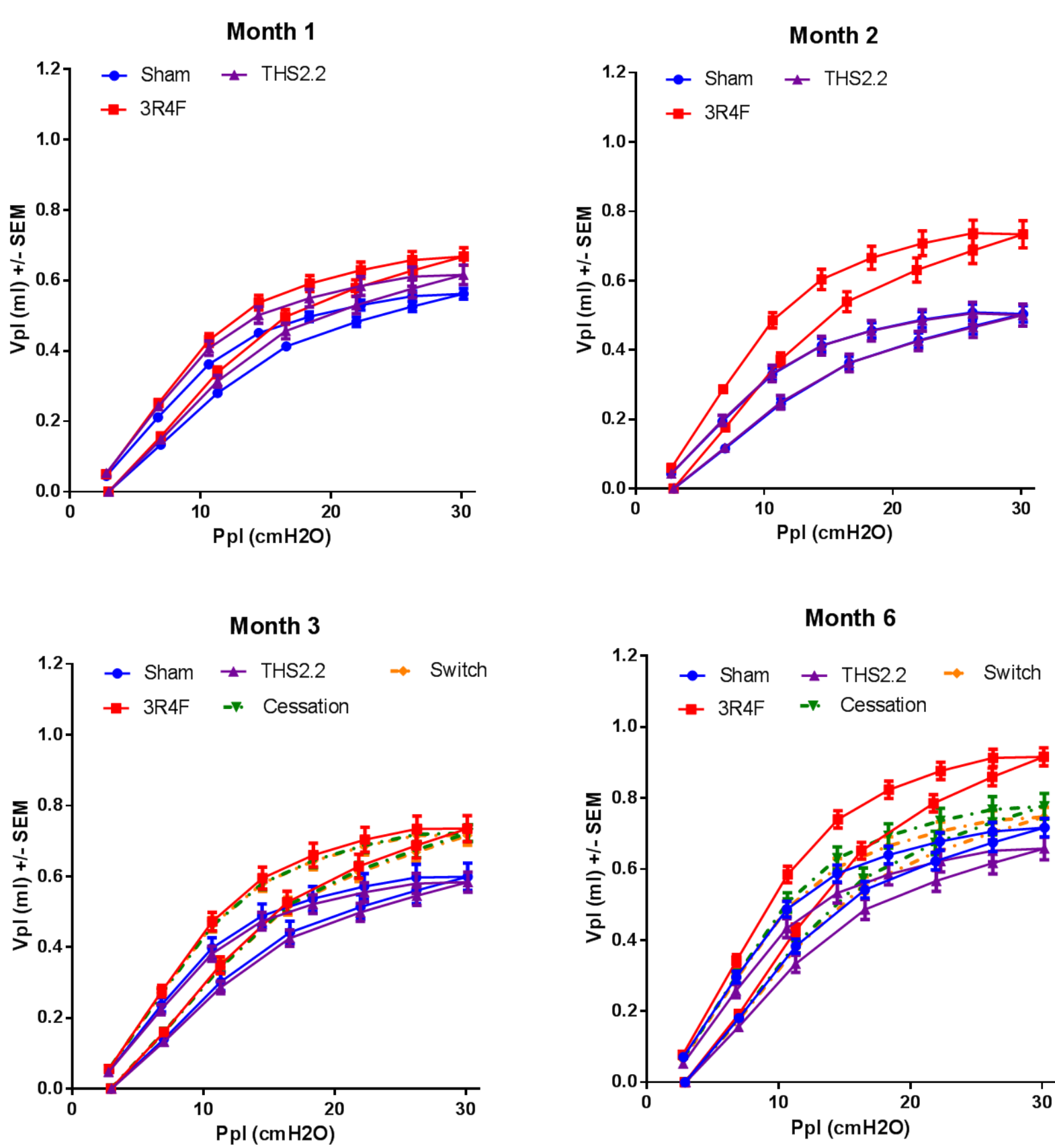
Conclusions

Lung volume and histopathological evaluation



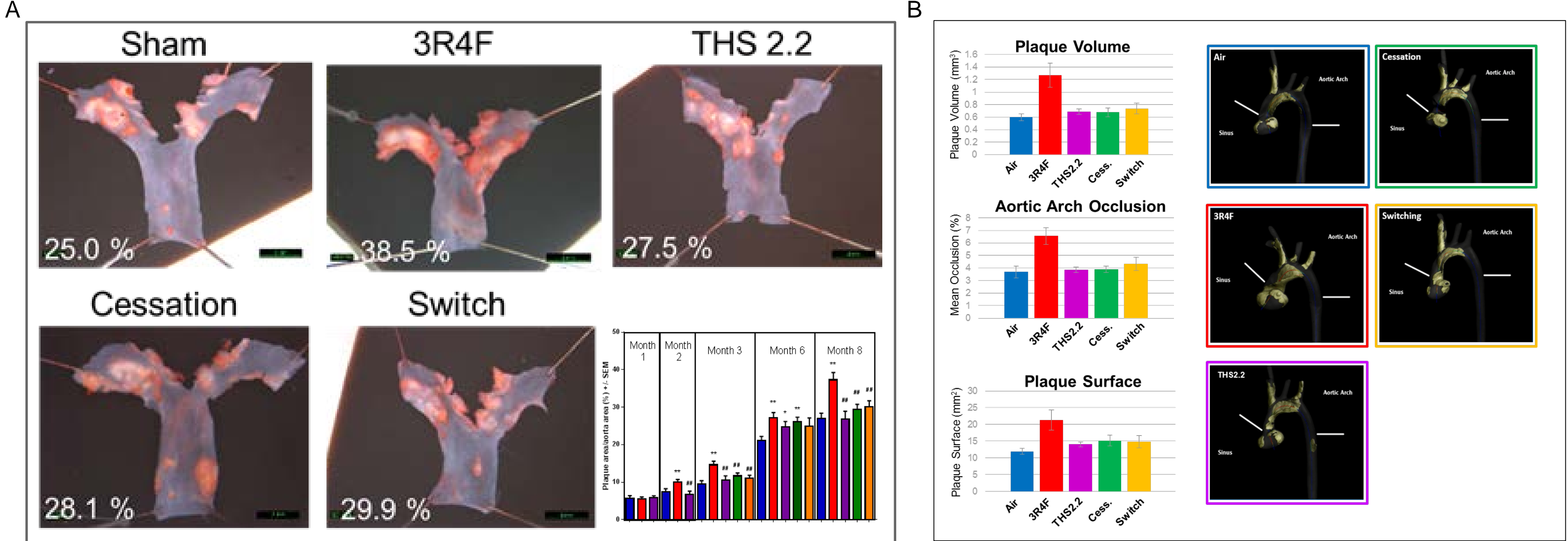
Panel A – Scoring of alveolar emphysema; panel B – Representative haematoxylin and eosine stained lung sections; panel C – Lung volume. The level of emphysema was evaluated by the scoring of severity and by morphometrical measurements performed in a blinded manner by an external pathologist. After the switch or cessation, the evaluation revealed a certain level of reduction of severity compared to the emphysema seen in lungs from continuously exposed animals. However, even 6 months after the switch some tissue degradation was still evident (A and B). Those changes are well reflected in the liquid displacement based lung volume measurement (C).

Pulmonary function: pressure-volume (P-V) loops



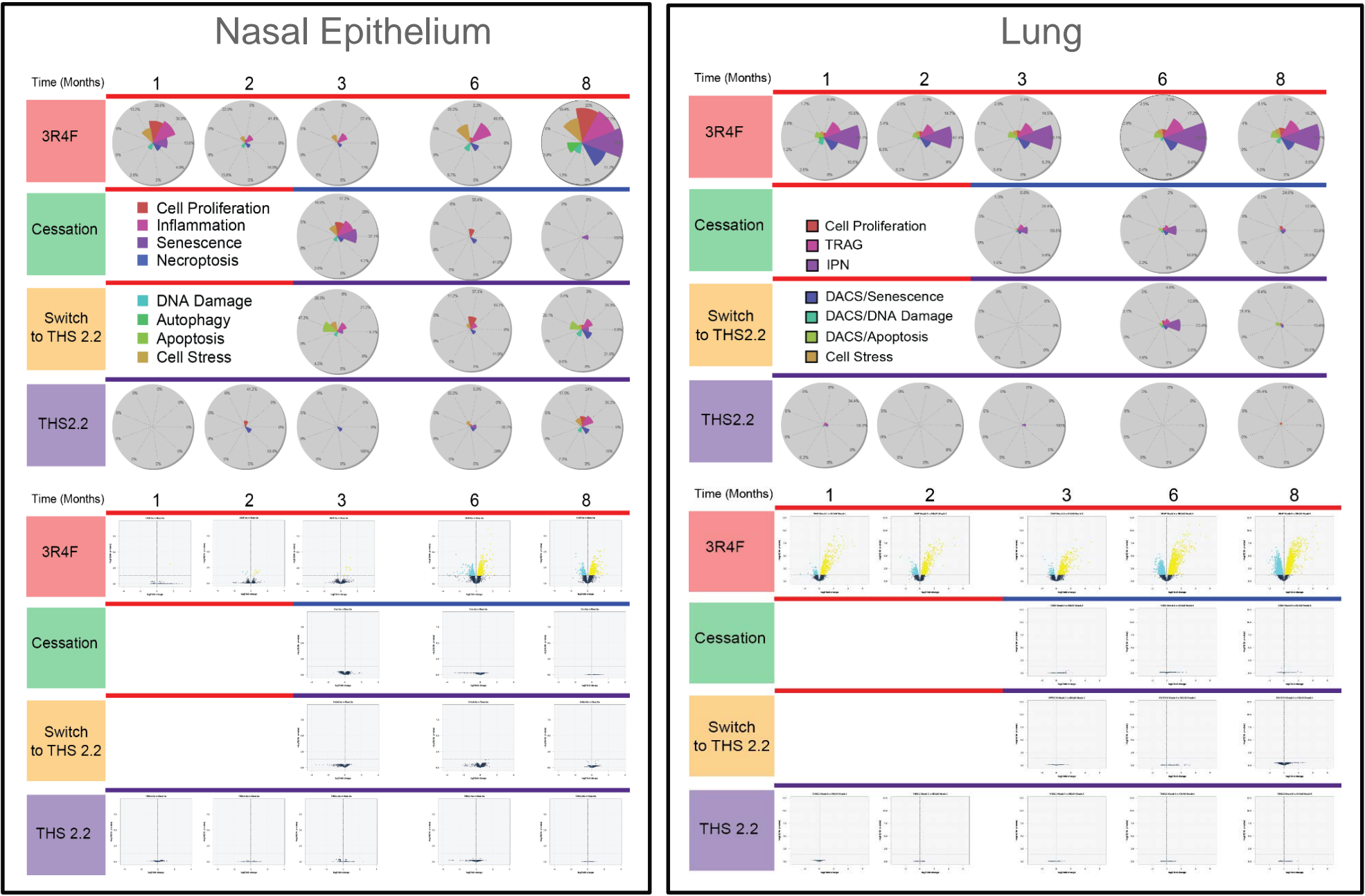
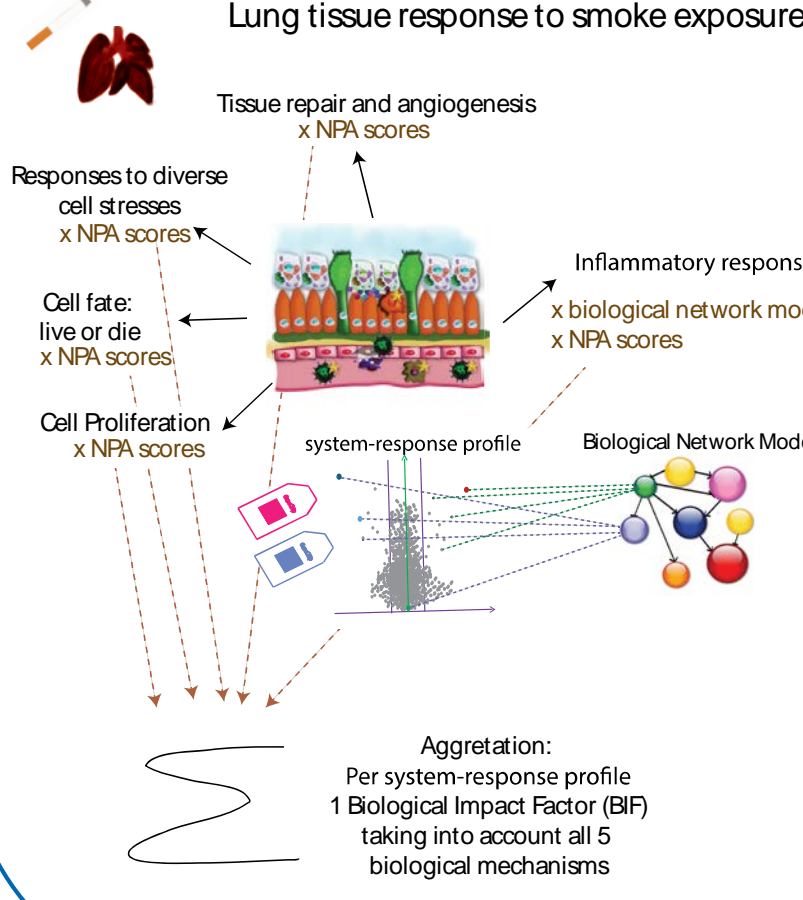
Exposure of mice to 3R4F cigarette smoke resulted in a leftward shift of the P-V loops as compared to the results obtained with filtered air exposed animals. Measurements from THS2.2 and filtered air exposed animals revealed very similar values during the course of the study. After the switch to the aerosol from THS2.2 or cessation, P-V loops shifted right, but did not reach values obtained from animals continuously exposed to filtered air or THS2.2.

Plaque area measurements



Exposure to 3R4F resulted in increased plaque formation in aortic arch of ApoE^{-/-} mice compared with sham exposure. These changes were alleviated by smoking cessation or switching to THS2.2 within one month, and nearly reached control levels at the 8-month time point. Panel A – oil red O stained plaque, two-dimensional quantification, percentages indicate the surface of aorta under plaque; panel B – µCT scanned aorta after the staining, 3D reconstruction showing position and the thickness of the plaque (collaboration with an external partner).

Gene expression profiles from lung and nasal epithelium



For each gene, the gene expression change, calculated as the log2 fold change, is plotted on the x-axis and the statistical significance, proportional to the negative log10-adjusted P-value, is plotted on the y-axis. Yellow and blue dots highlight genes that are statistically significantly up- or down-regulated, respectively, compared with the sham group at each respective time point. Star plots illustrate the decomposition of the overall transcriptional changes into its eight mechanistic components (from cell proliferation to inflammation, indicated by colours) for each treatment group.



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