Comparative assessment of lung inflammation, pulmonary function and emphysema caused by the aerosol from potential Reduced Risk Products and cigarette smoke in mouse models of COPD.

11 July, 2016 – COPD-2016 - Brisbane

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Philip Morris International is the sole source of funding and sponsor of this project.

PMI's Goal for Harm Reduction

Offering adult smokers satisfying products that reduce risk

- Smoking is addictive and causes a number of serious diseases
- Worldwide it is estimated that more than one billion people will continue to smoke in the foreseeable future*

- Successful harm reduction requires that current adult smokers be offered a range of Reduced Risk Products (RRPs) so that consumer acceptance can be best fulfilled

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*The Tobacco Atlas 3rd Edition. American Cancer Society, 2009
Figure adapted from Clive Bates presentation to E-Cigarette Summit (19 Nov 2013)

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Reduced-Risk Products ("RRPs") is the term the company uses to refer to products with the potential to reduce individual risk and population harm in comparison to smoking cigarettes. PMI's RRPs are **in various stages of development and commercialization**, and we are conducting **extensive and rigorous scientific studies** to determine whether we can support claims for such products of reduced exposure to harmful and potentially harmful constituents in smoke, and ultimately claims of reduced disease risk, when **compared to smoking cigarettes**.

Before making any such claims, we will **rigorously evaluate the full set of data** from the relevant scientific studies to determine whether they substantiate reduced exposure or risk. Any such claims **may also be subject to government review and authorization** as is the case in the US today.

Aim and scope of the presentation

- Assessment of the effects of cigarette smoke and a RRP (THS2.2), using PMI's Heat-not-Burn technology, in 2 animal models of COPD:
  - ApoE$^{-/-}$ mouse (C57Bl6 background), typically used as model for cardiovascular disease
  - A/J mouse, used as model for lung cancer
- Both animal models are responsive to cigarette mainstream smoke and develop different pathologies, among which aspects of COPD such as *lung inflammation*, changed *pulmonary function*, *emphysema**
- Other endpoints, such as general (chronic) toxicity, atherosclerosis, lung tumor development determined in these studies will not be reported here

*Lo Sasso G, Schlage WK, Boué S, Veljkovic E, Peitsch MC, Hoeng J. (2016) The Apoe(-/-) mouse model: a suitable model to study cardiovascular and respiratory diseases in the context of cigarette smoke exposure and harm reduction. J Transl Med. 2016 14(1):146.Epub. Review. **PMID 27207171**.

*Stinn W, Buettner A, Weiler H, Friedrichs B, Luetjen S, van Overveld F, Meurrens K, Janssens K, Gebel S, Stabbert R, Haussmann HJ. (2013) Lung inflammatory effects, tumorigenesis, and emphysema development in a long-term inhalation study with cigarette mainstream smoke in mice.
Toxicol Sci. 131(2):596-611. **PMID: 23104432**

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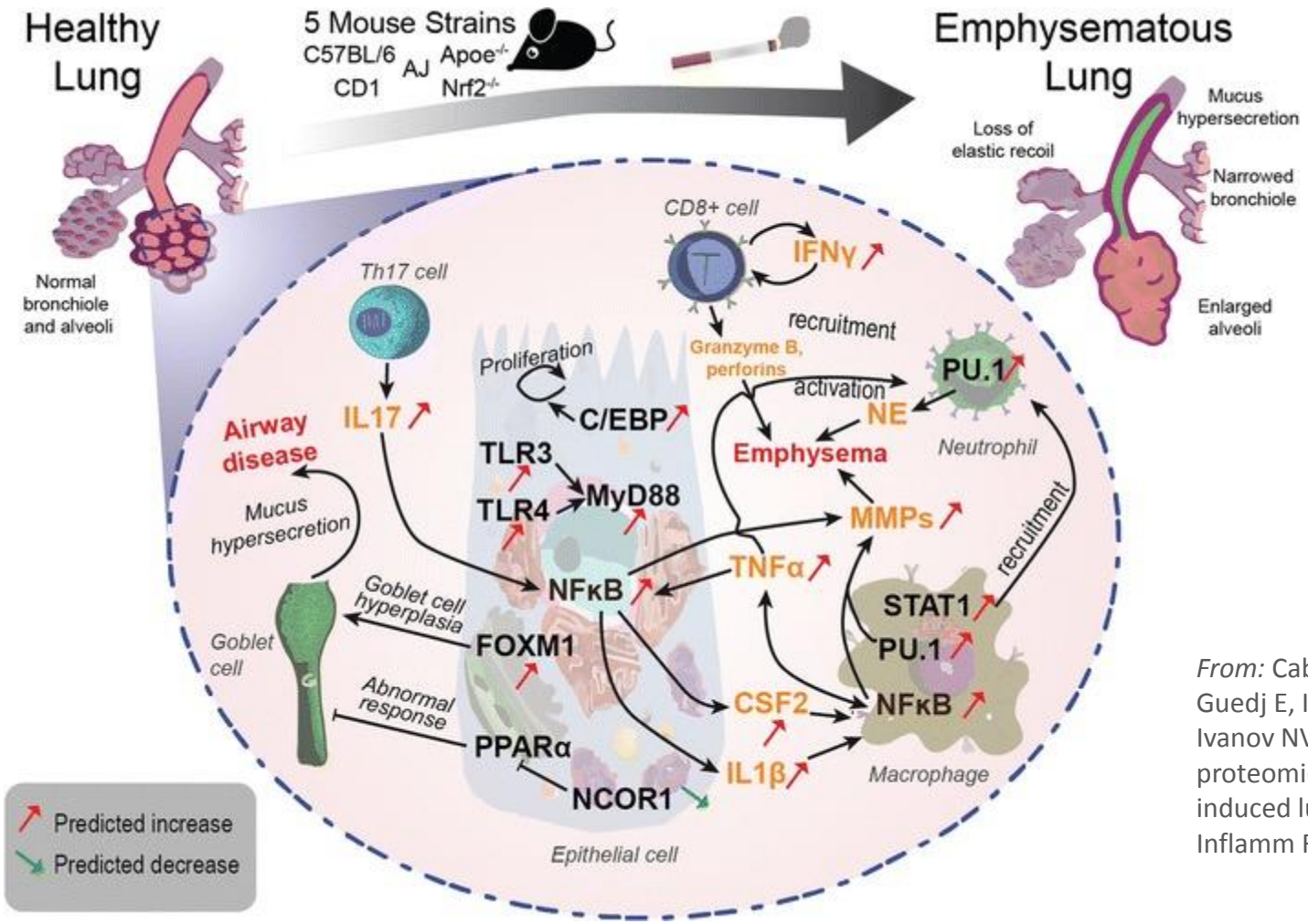
Common disease mechanisms in different mouse models, relevance to human situation



- Possible interrelationships and roles for the identified common mechanisms (HYPs) in five mouse models of emphysema in a framework of classical human COPD mechanisms.
  - transcription factors (*black font*)
  - inflammatory mediators (*orange font*) classical pathways of human COPD pathogenesis (*black arrows*) as depicted,

From: Cabanski M, Fields B, Boue S, Boukharov N, DeLeon H, Dror N, Geertz M, Guedj E, Iskandar A, Kogel U, Merg C, Peck MJ, Poussin C, Schlage WK, Talikka M, Ivanov NV, Hoeng J, Peitsch MC. (**2015**): Transcriptional profiling and targeted proteomics reveals common molecular changes associated with cigarette smoke-induced lung emphysema development in five susceptible mouse strains. Inflamm Res.64(7):471-86. ***PMID: 25962837***

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Methods -Cigarette smoke and aerosol from a RRP

Assessment of smoke/aerosol – Health Canada Intense smoke protocol

Conventional cigarettes:
Smoke from University of Kentucky Standard Reference Cigarette 3R4F

Potentially Reduced-Risk product:
Aerosol generated by Tobacco Heating System, commercialized as *iQOS* (also designated as THS 2.2)

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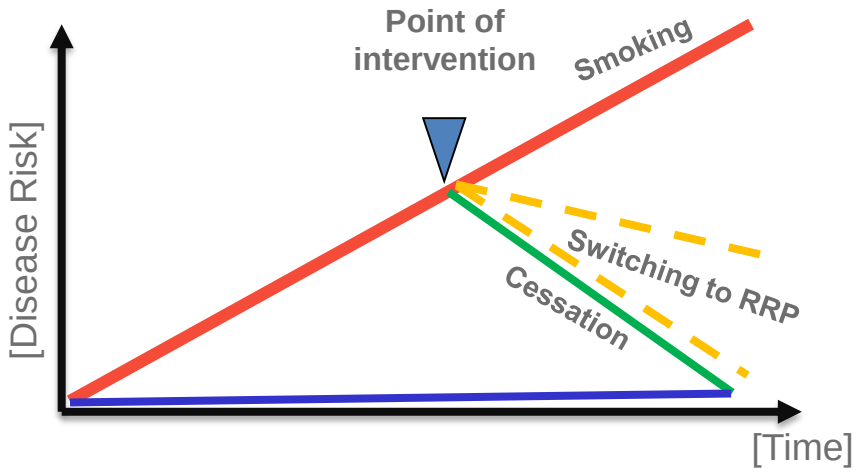
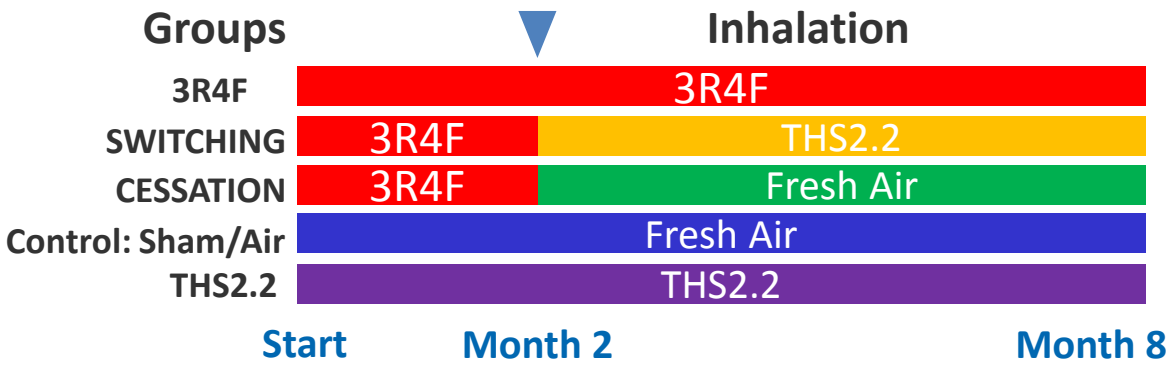
ApoE$^{-/-}$ mouse switching study
Study design

- Comparative assessment of effects of THS2.2 and 3R4F
- Switching design upon initiation of disease:
  - to assess reversibility (switch to fresh air, i.e. cessation) and
  - To quantify how similar switching to THS2.2 is to cessation





Dissection time points: Month 1, 2, 3, 6 and 8

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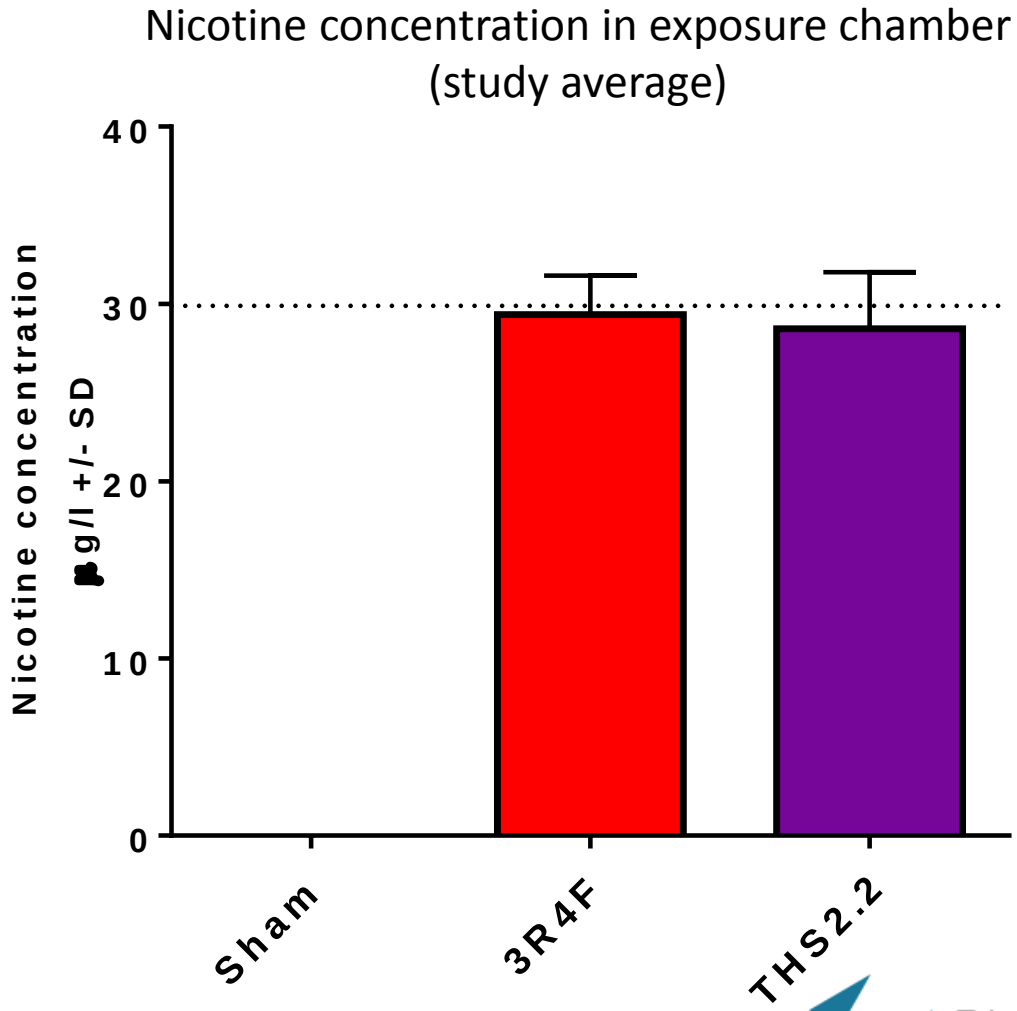
ApoE$^{-/-}$ mouse switching study
Methods - Exposure regime

1 hour	30 min	1 hour	1 hour	1 hour

Fresh air

THS2.2 or 3R4F

- Animals were exposed 3 hours per day (3 x 1 hour interrupted exposure periods), 5 days per week
- Nicotine was measured during every exposure period (3 samples per chamber per day)
- Aerosol delivery (nicotine) was within +/- 10% of the targeted **29.9 µg/l** nicotine concentration



*29.9 µg/l nic corresponds to 6.5 mg/kg, daily dose- or the nicotine amount from approx. 32 cig/day for a 60 kg human, based on body surface comparison, Guidance document Heq dose, FDA

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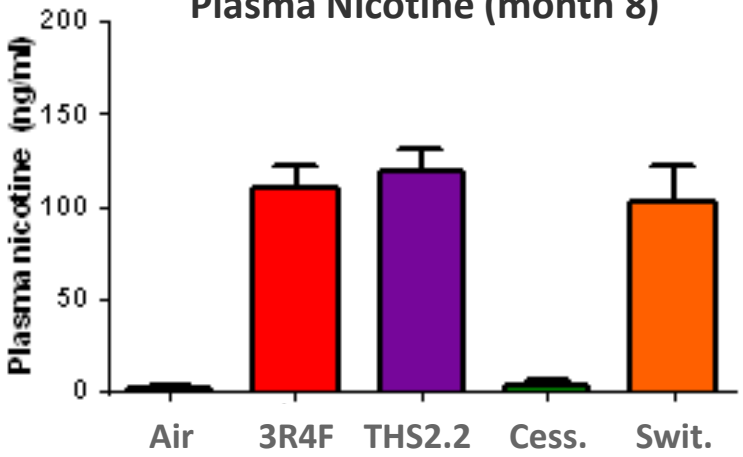
ApoE$^{-/-}$ mouse switching study
Aerosol uptake (biomarkers of exposure)

Plasma Nicotine (month 8)



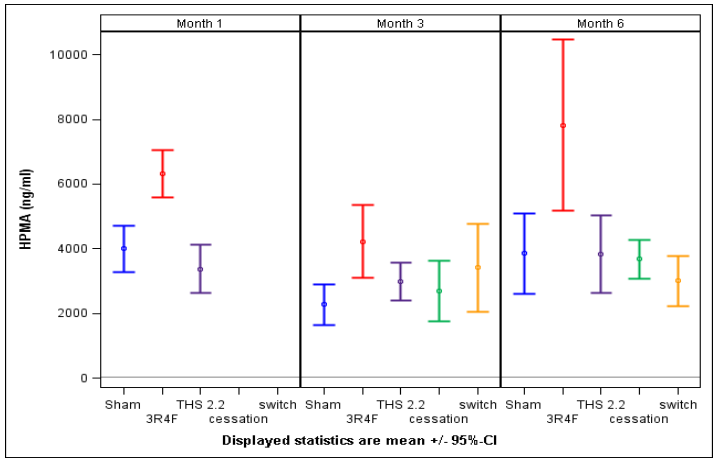
Means ± SEM

Groups

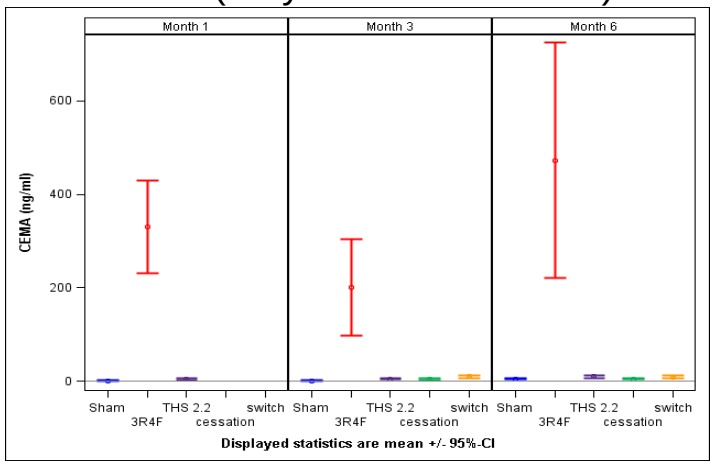
- 3R4F
- Cessation
- Switching
- THS2.2
- Sham / Air

Urinary Metabolites (months 3, 6, 8)

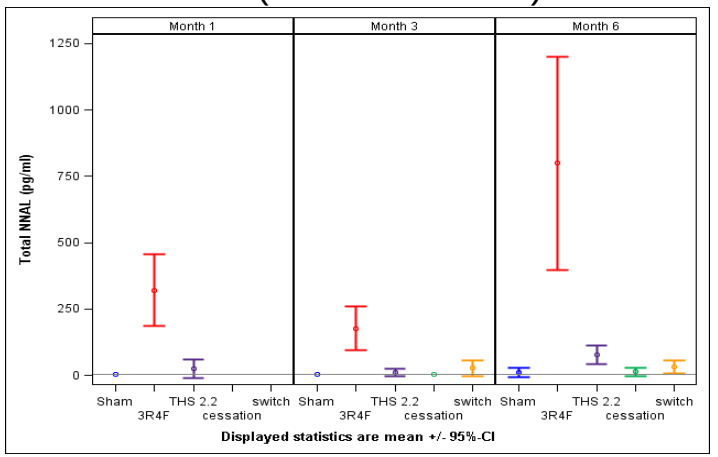
HPMA (Acrolein metabolite)



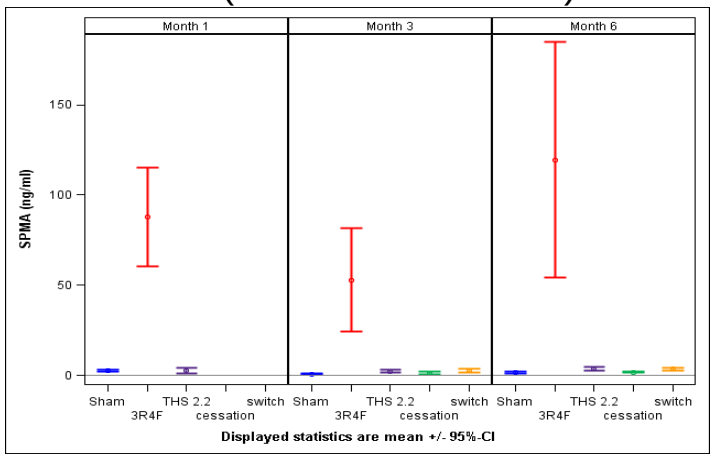
CEMA (Acrylonitrile metabolite)



NNAL (NNK metabolite)



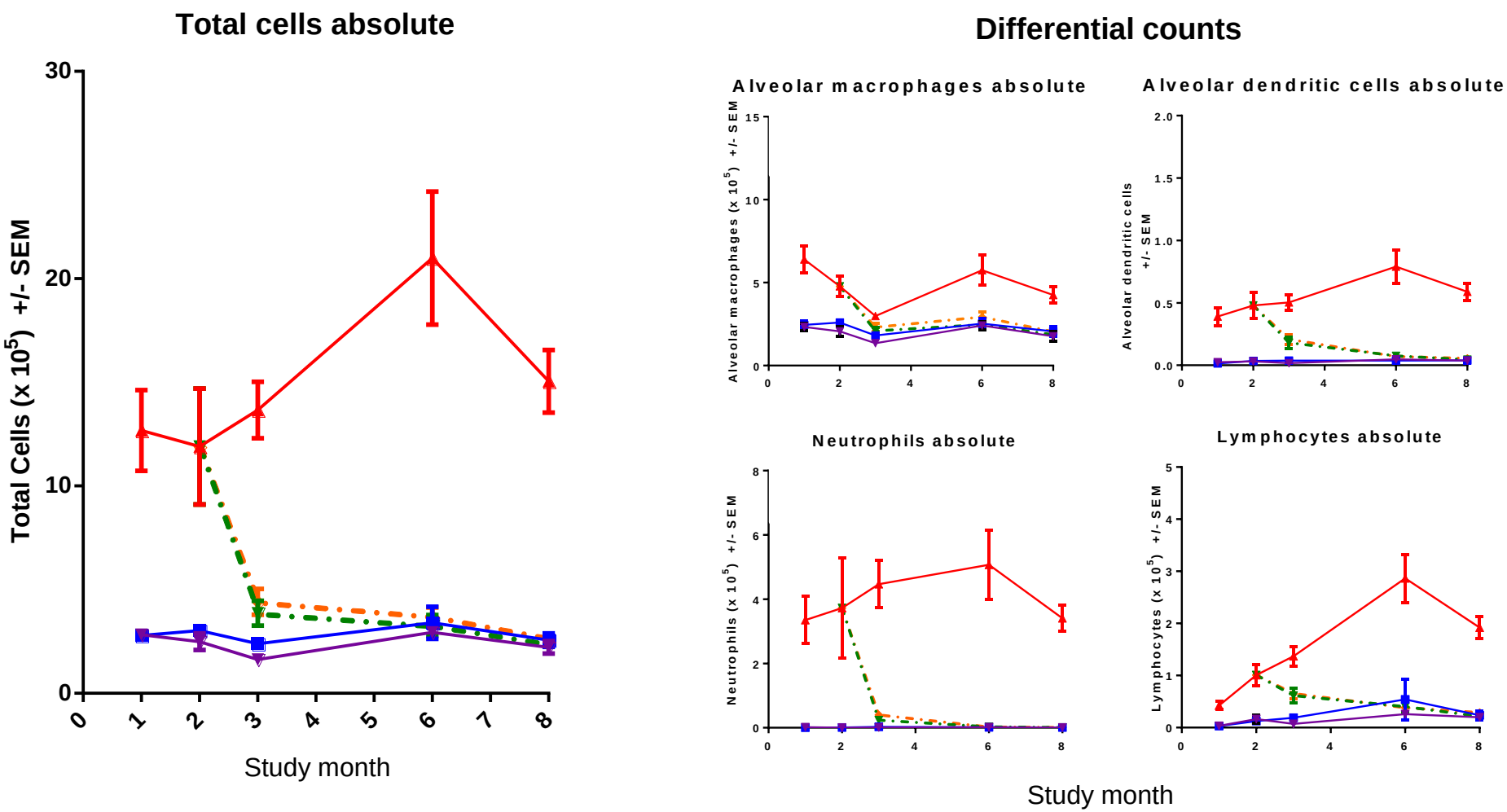
SPMA (Benzene metabolite)



Means ± SEM

ApoE$^{-/-}$ mouse switching study
Result summary: Disease mechanisms - Lung inflammation

Free lung cells in Broncho-alveolar lavage fluid (BALF)



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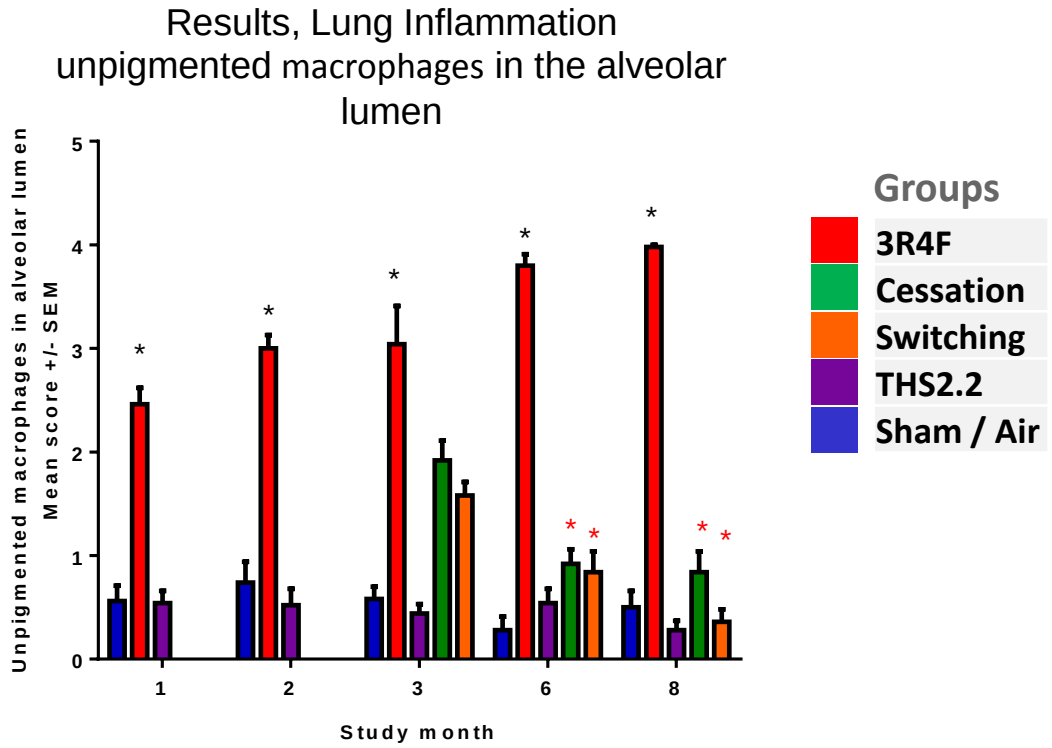
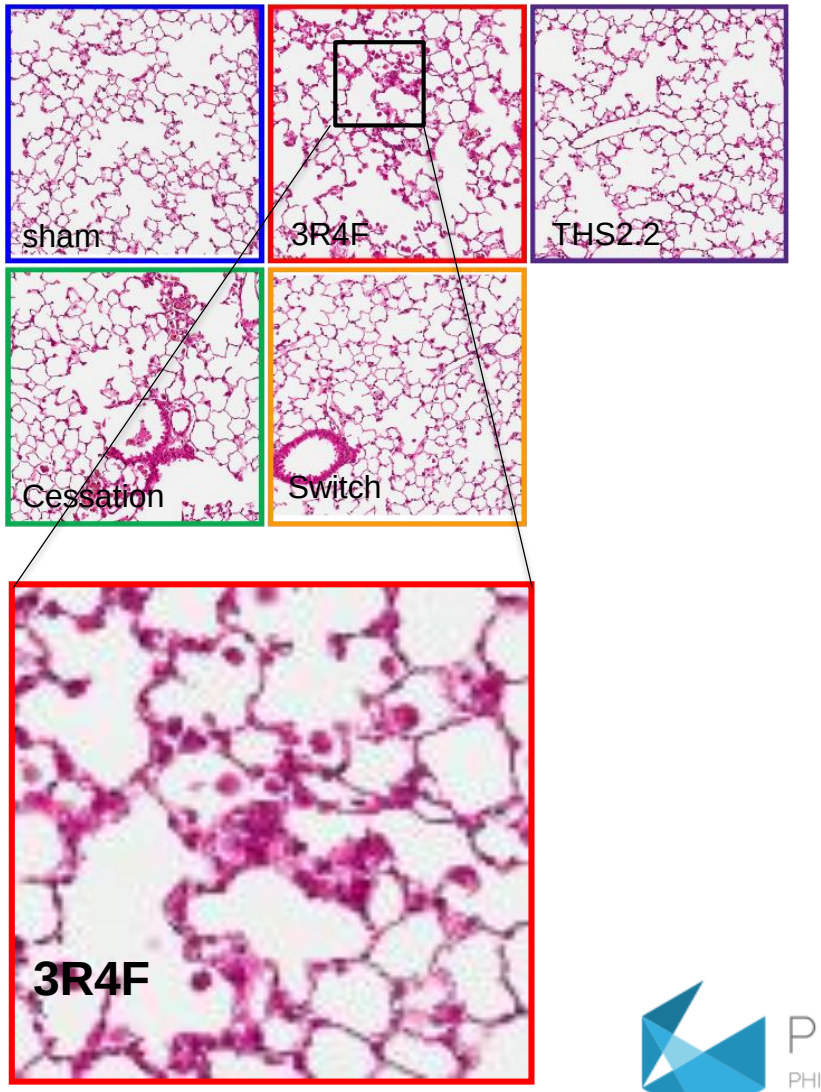
ApoE$^{-/-}$ mouse switching study
Result summary: Histopathology of the lung – Pulmonary inflammation



*: Statistically significant compared to sham
*: Statistically significant compared to 3R4F:CONT at month 2



- Decrease in mean scores after switching to fresh air or THS2.2 (statistically significant from month 6)
- No statistically significant difference between Cessation group and THS2.2-Switch group at month 3

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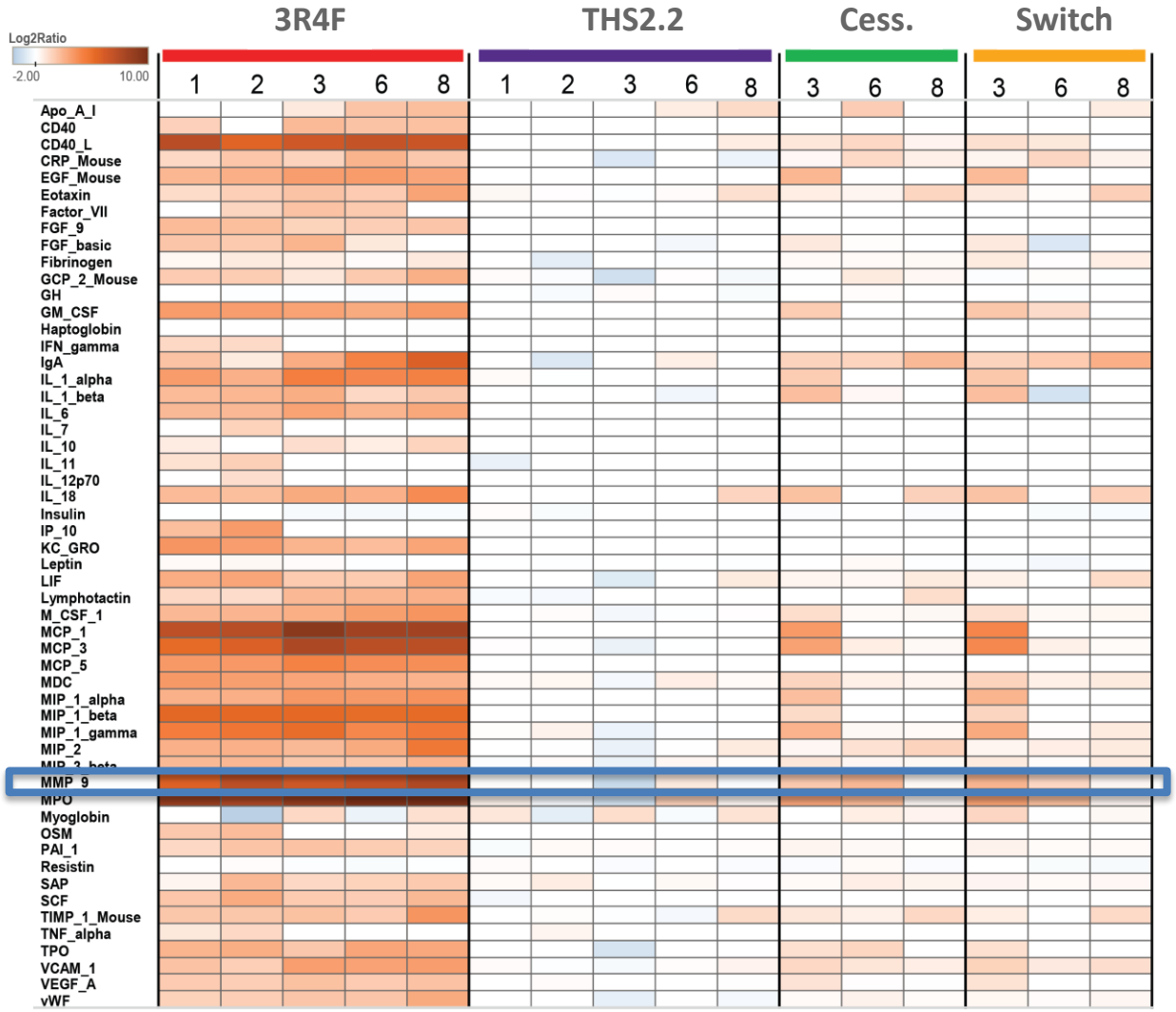
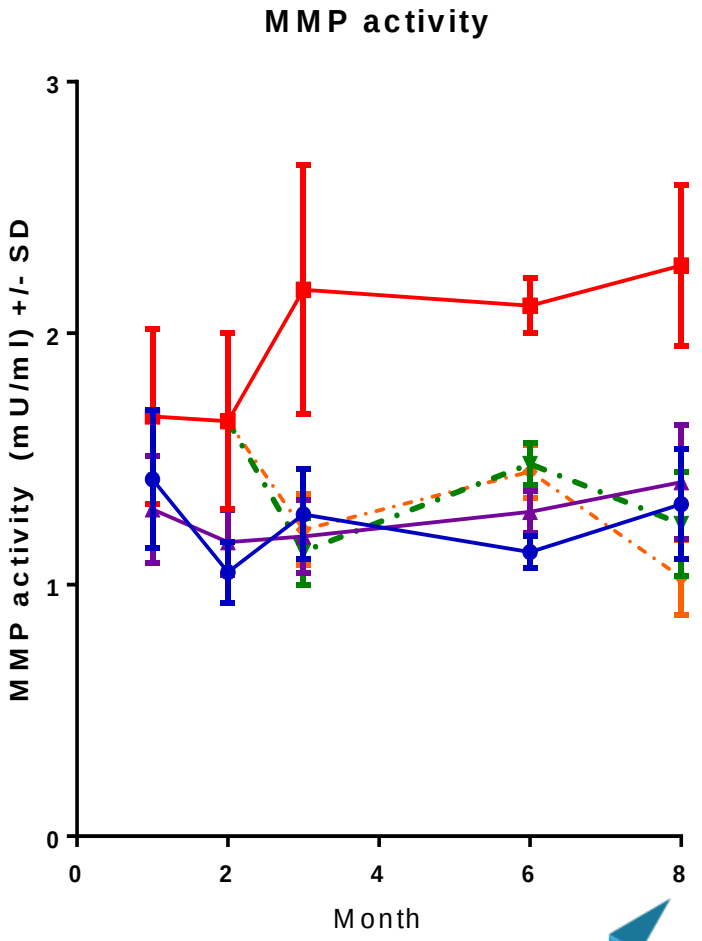
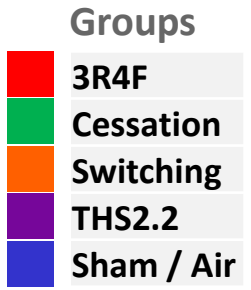
ApoE$^{-/-}$ mouse switching study
Result summary: Disease mechanisms - Lung inflammation

Multiple analyte profiling in Broncho-alveolar Lavage Fluid







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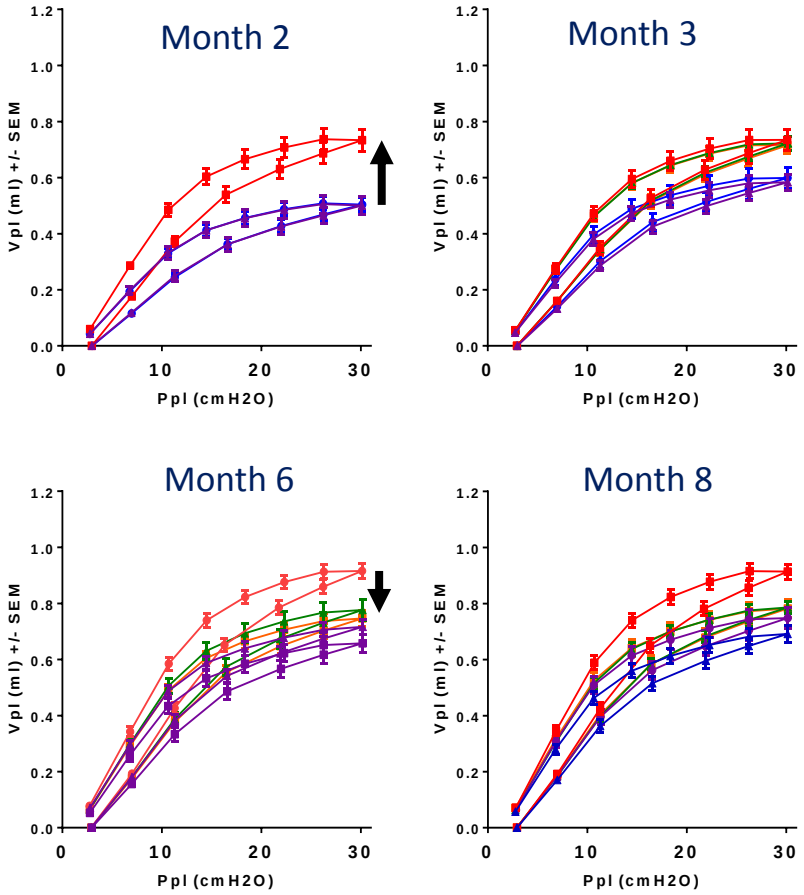
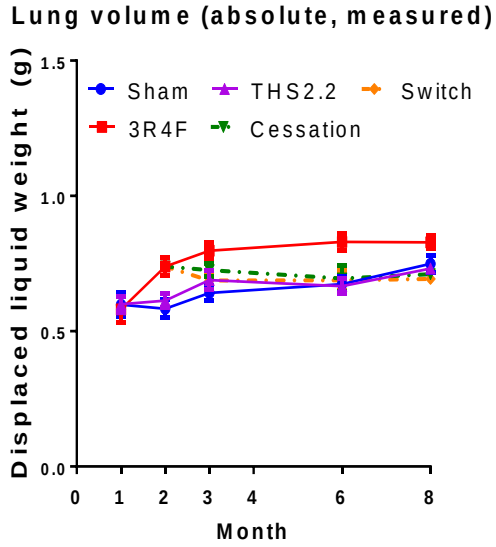
ApoE$^{-/-}$ mouse switching study
Result summary: Disease endpoints- Lung function and lung volume

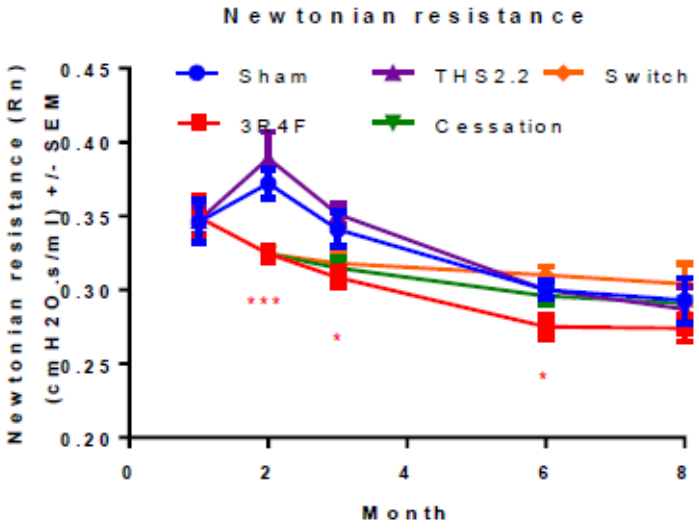
Lung function: Pressure Volume Loops (PVsP) (FlexiVent (Scireq))



Groups

- 3R4F
- Cessation
- Switching
- THS2.2
- Sham / Air





Means ± SEM

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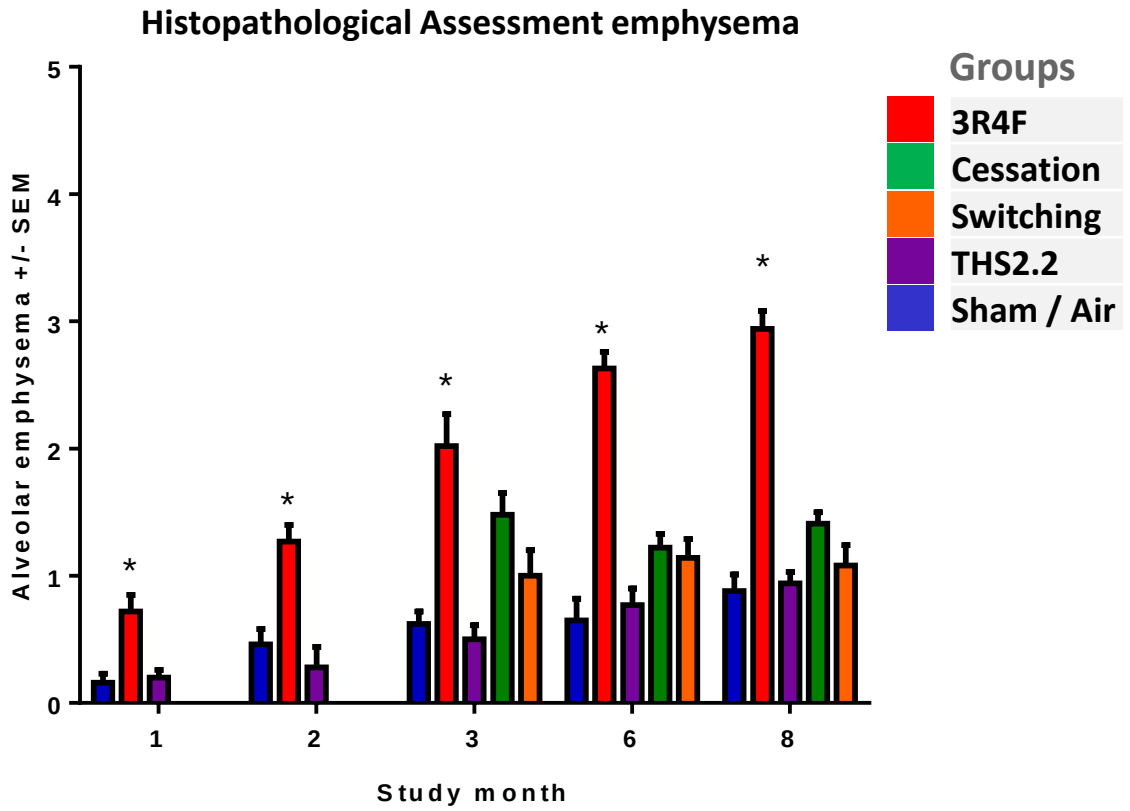
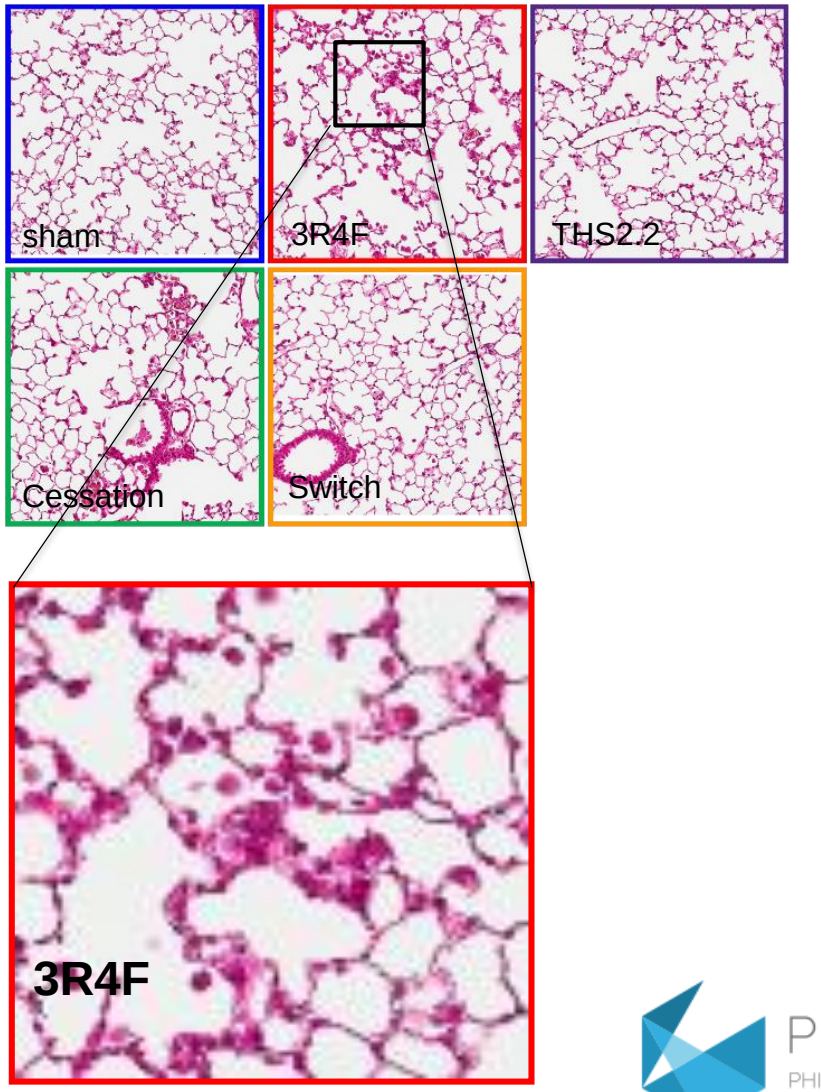
Switching Study in an Animal Model of Disease
Result summary: Tissue changes - Histopathology



*: Statistically significant compared to sham
*: Statistically significant compared to 3R4F:CONT at month 2



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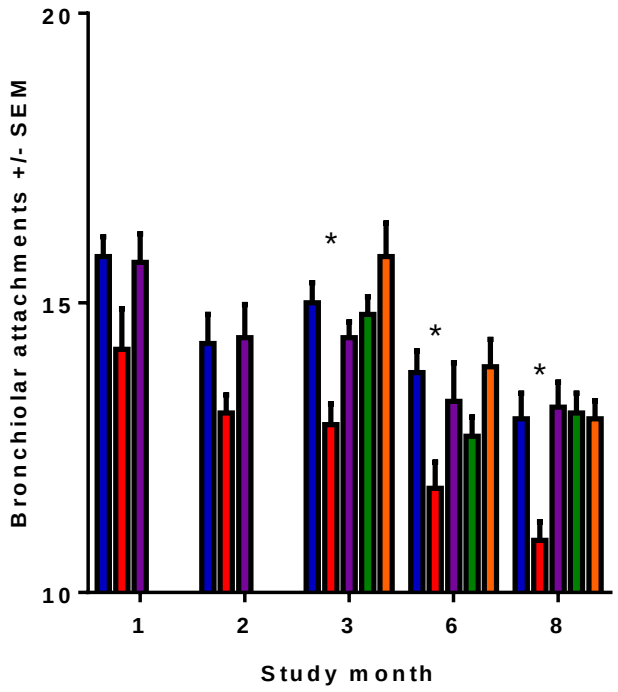
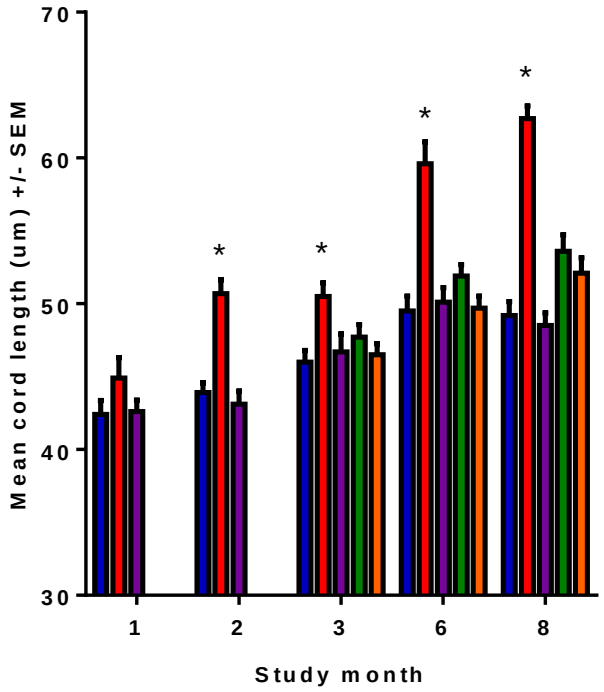
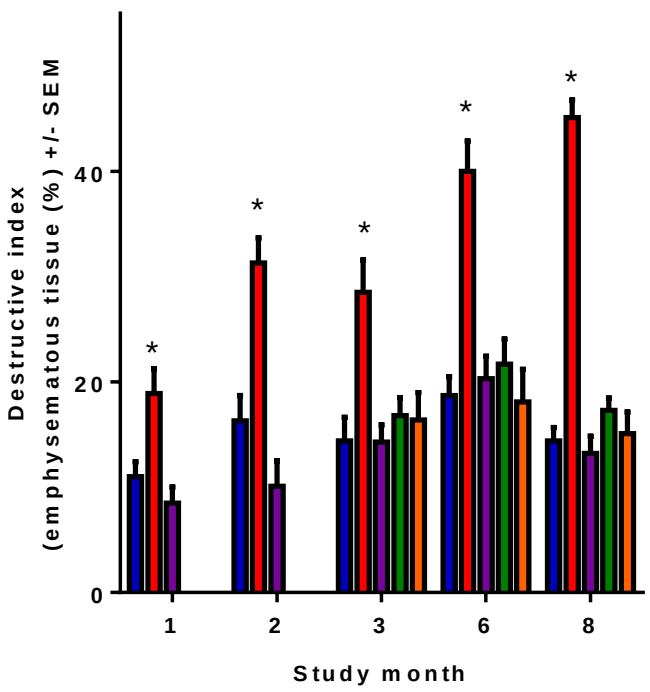
ApoE$^{-/-}$ mouse switching study
Result Summary: Lung tissue changes - Morphometry



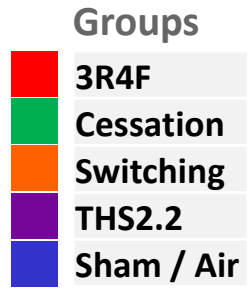
Bronchiolar attachments
- Fewer Bronchiolar attachments in 3R4F-exposed group



Mean chord length (MCL)
- Mean linear intercept length
- Increased MCL in 3R4F-exposed group



Destructive index (DI)
- Index of parenchymal destruction
- Increased DI in 3R4F-exposed group



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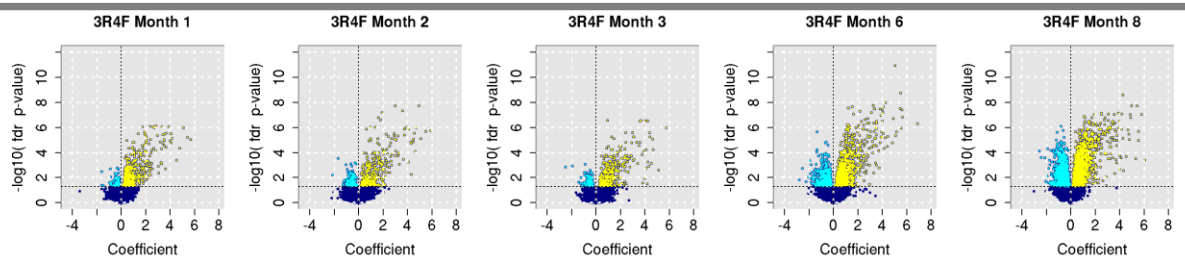
ApoE$^{-/-}$ mouse switching study
Result Summary: Systems response profile: differential gene expression - Lung

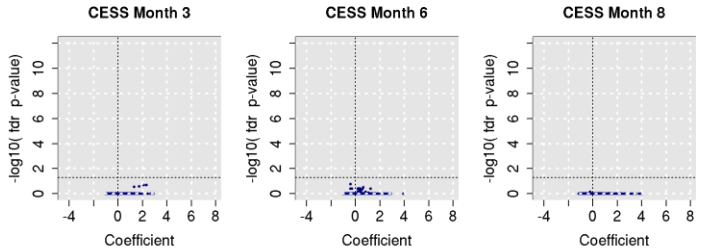
3R4F

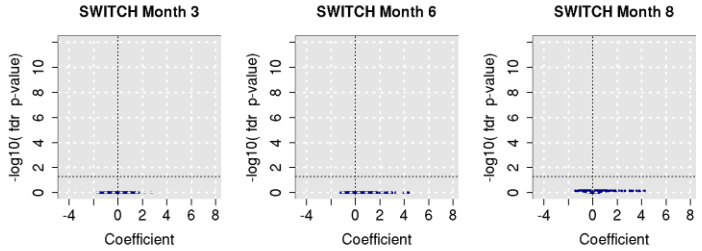
Cessation

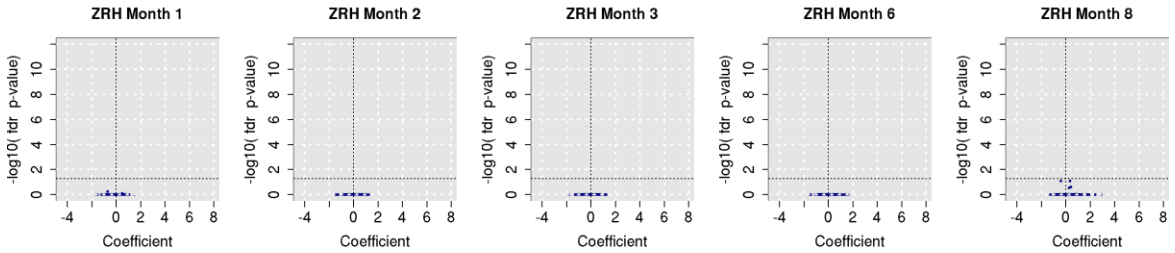
Switch

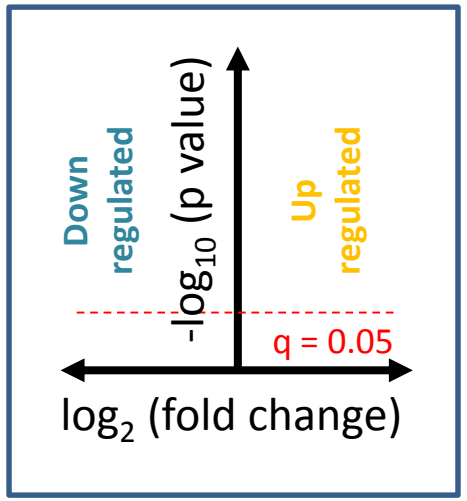
THS2.2











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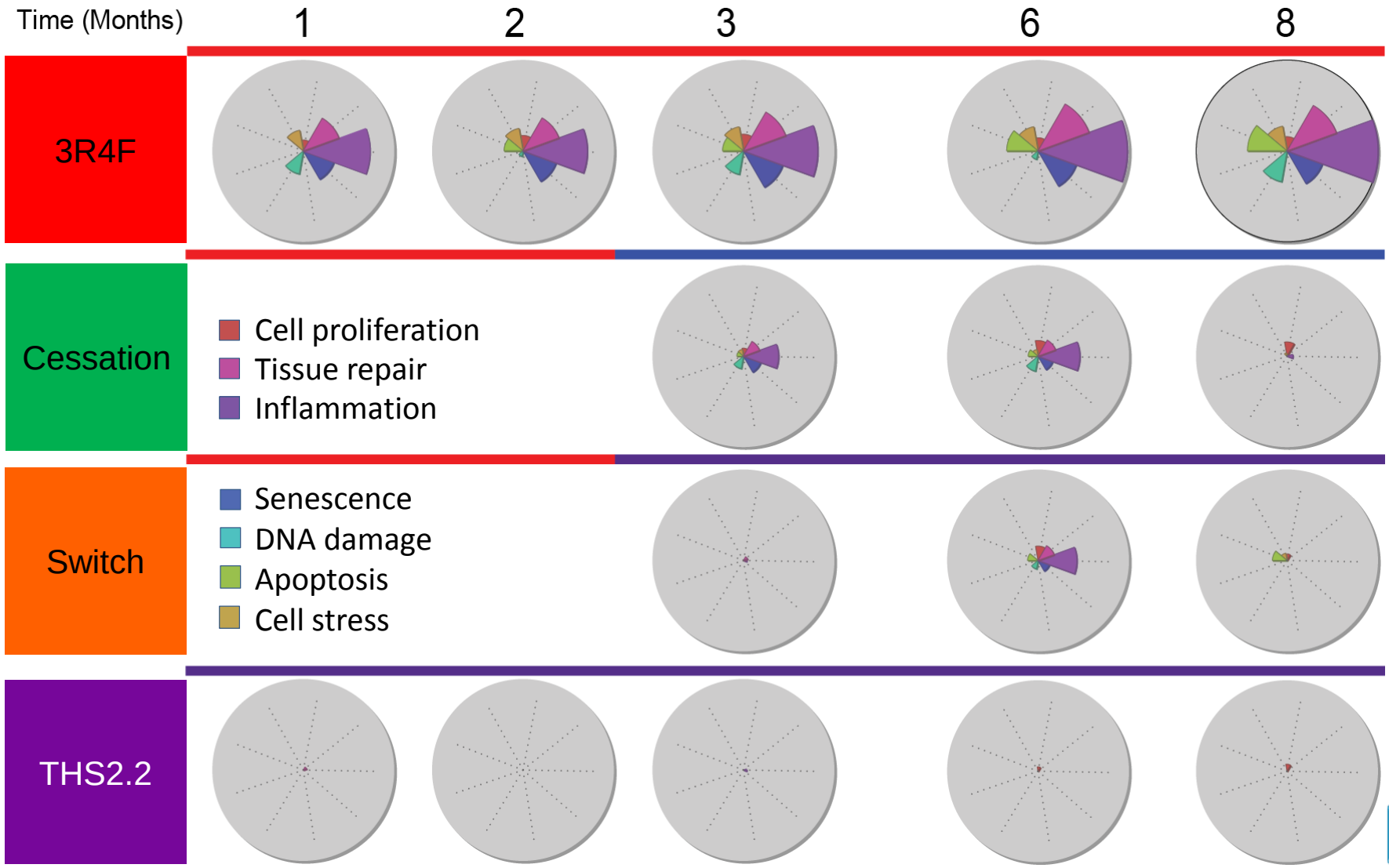
ApoE$^{-/-}$ mouse switching study
Result Summary: Disease mechanisms - Network perturbations - Lung



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Switching Study in an Animal Model of Disease
Summary and Conclusions

- The ApoE$^{-/-}$ mouse model is suitable for studying smoke-related aspects of COPD
- Continuous exposure to smoke from 3R4F causes lung inflammation, lung function and emphysematous changes as of one month of treatment
- Continuous exposure to aerosol from THS2.2 for up to 8 months does not increase inflammation and emphysema in comparison to Sham group
- Switching from cigarette smoke exposure after 2 months to fresh air (Sham) exposure reverses the onset of disease as measured in apical, functional, and molecular endpoints
- Switching from cigarette smoke exposure to THS2.2 aerosol exposure reverses the onset of disease in a similar manner as cessation

Phillips B, Veljkovic E, Boué S, Schlage WK, Vuillaume G, Martin F, Titz B, Leroy P, Buettner A, Elamin A, Oviedo A, Cabanski M, De León H, Guedj E, Schneider T, Talikka M, Ivanov NV, Vanscheeuwijck P, Peitsch MC, Hoeng J. (2016). An 8-Month Systems Toxicology Inhalation/Cessation Study in Apoe-/- Mice to Investigate Cardiovascular and Respiratory Exposure Effects of a Candidate Modified Risk Tobacco Product, THS 2.2, Compared With Conventional CigarettesToxicol Sci. 149(2):411-32. ***PMID 26609137*.**

Titz, B., Boue, S., Phillips, B., Talikka, M., Vihervaara, T., Schneider, T., Nury, C., Elamin, A., Guedj, E., Peck, M.J., Schlage WK, Cabanski M, Leroy P, Vuillaume G, Martin F, Ivanov NV, Veljkovic E, Ekroos K, Laaksonen R, Vanscheeuwijck P, Peitsch MC, Hoeng J.(2016). Effects of Cigarette Smoke, Cessation, and Switching to Two Heat-Not-Burn Tobacco Products on Lung Lipid Metabolism in C57BL/6 and Apoe-/- Mice-An Integrative Systems Toxicology Analysis. Toxicol Sci *149*, 441-457. ***PMID 26582801*.**

Lo Sasso, G., Titz, B., Nury, C., Boue, S., Phillips, B., Belcastro, V., Schneider, T., Dijon, S., Baumer, K., Peric, D, Dulize R, Elamin A, Guedj E, Buettner A, Leroy P, Kleinhans S, Vuillaume G, Veljkovic E, Ivanov NV, Martin F, Vanscheeuwijck P, Peitsch MC, Hoeng J. (2016). Effects of cigarette smoke, cessation and switching to a candidate modified risk tobacco product on the liver in Apoe−/− mice – a systems toxicology analysis. Inhal Toxicol. 28(5): 226-4. ***PMID 27027324*.**

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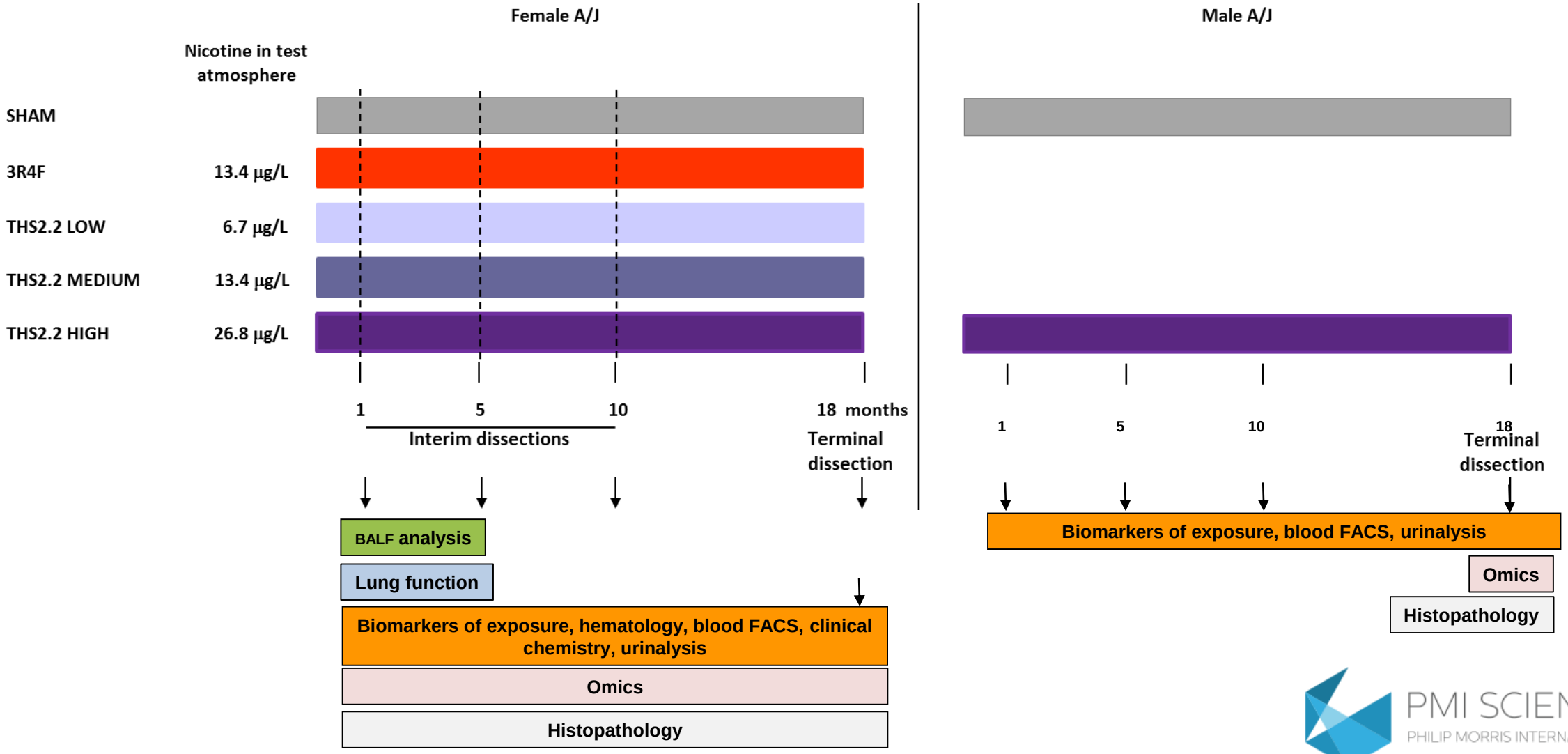
A/J mouse study
Study design (OECD TG 453 - Chronic toxicity)



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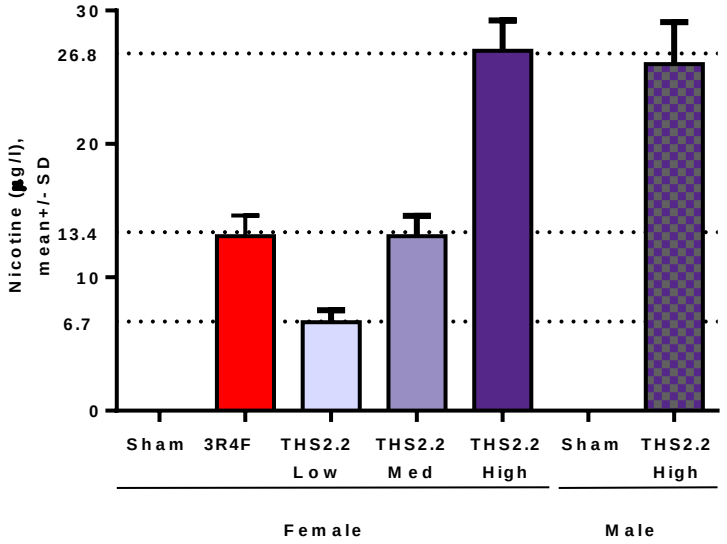
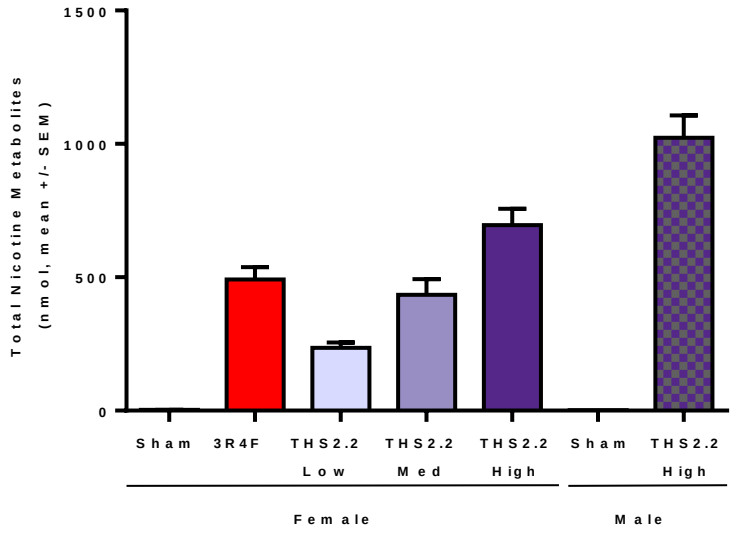
A/J mouse study
Methods - Exposure and aerosol uptake

- Animals were exposed 6 hours per day, 5 days per week
- Nicotine was measured 3 samples per chamber per day)
- Aerosol delivery (nicotine) was within +/- 10% of the targeted nicotine concentration
- Aerosol uptake was in line with test atmosphere nicotine concentration

Nicotine concentration in exposure chamber (study average)



Nicotine metabolites in urine



*26.8 µg/l nic (high dose) corresponds to 11.6 mg/kg, daily dose- or the nicotine amount from approx. 56 cig/day for a 60 kg human, based on body surface comparison, Guidance document Heq dose, FDA

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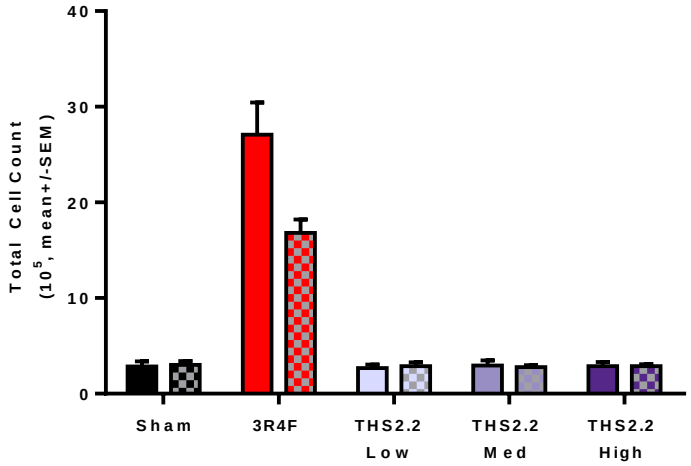
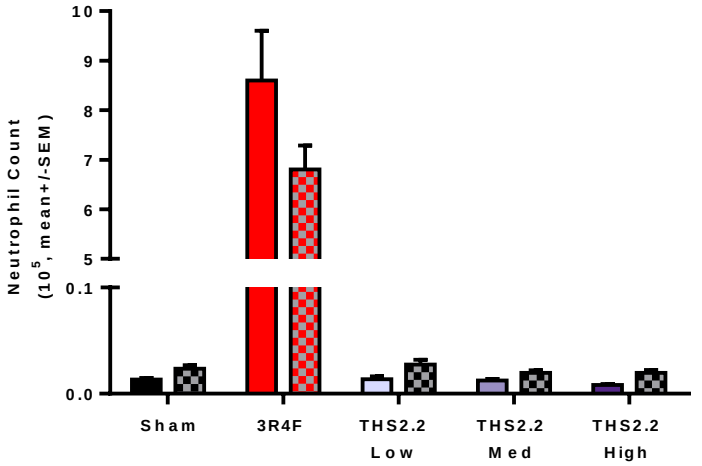
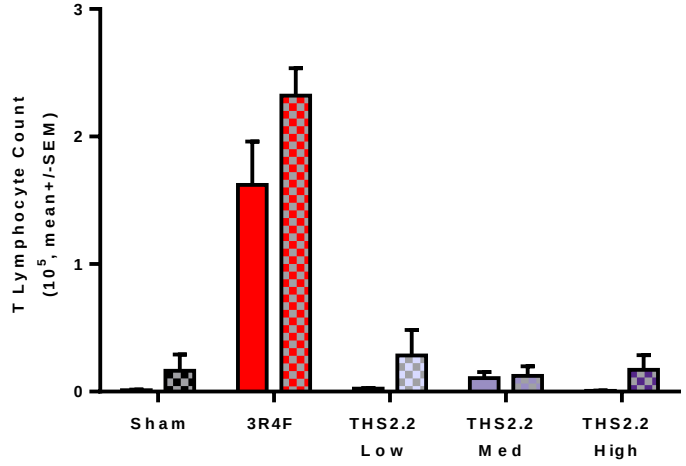
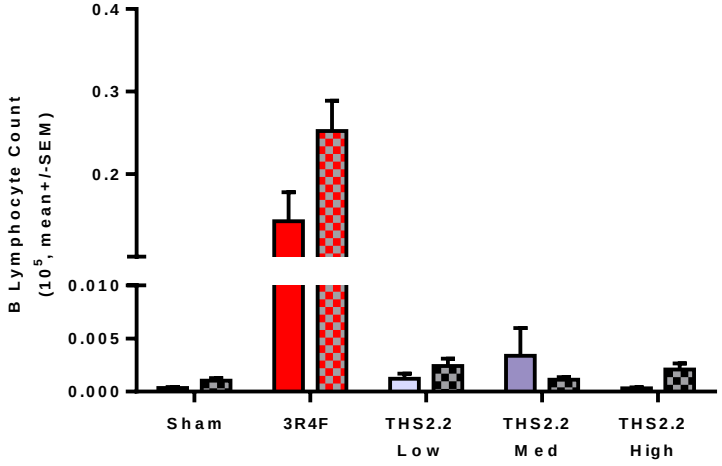
A/J mouse study
Result summary: Inflammation - Free lung cell analysis in BALF

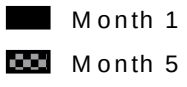
- 3R4F exposure-related increases in neutrophil, alveolar macrophage, dendritic cell, and lymphocyte count
- No obvious increase in the immune cell counts in lungs (BALF) of THS2.2 aerosol-exposed mice











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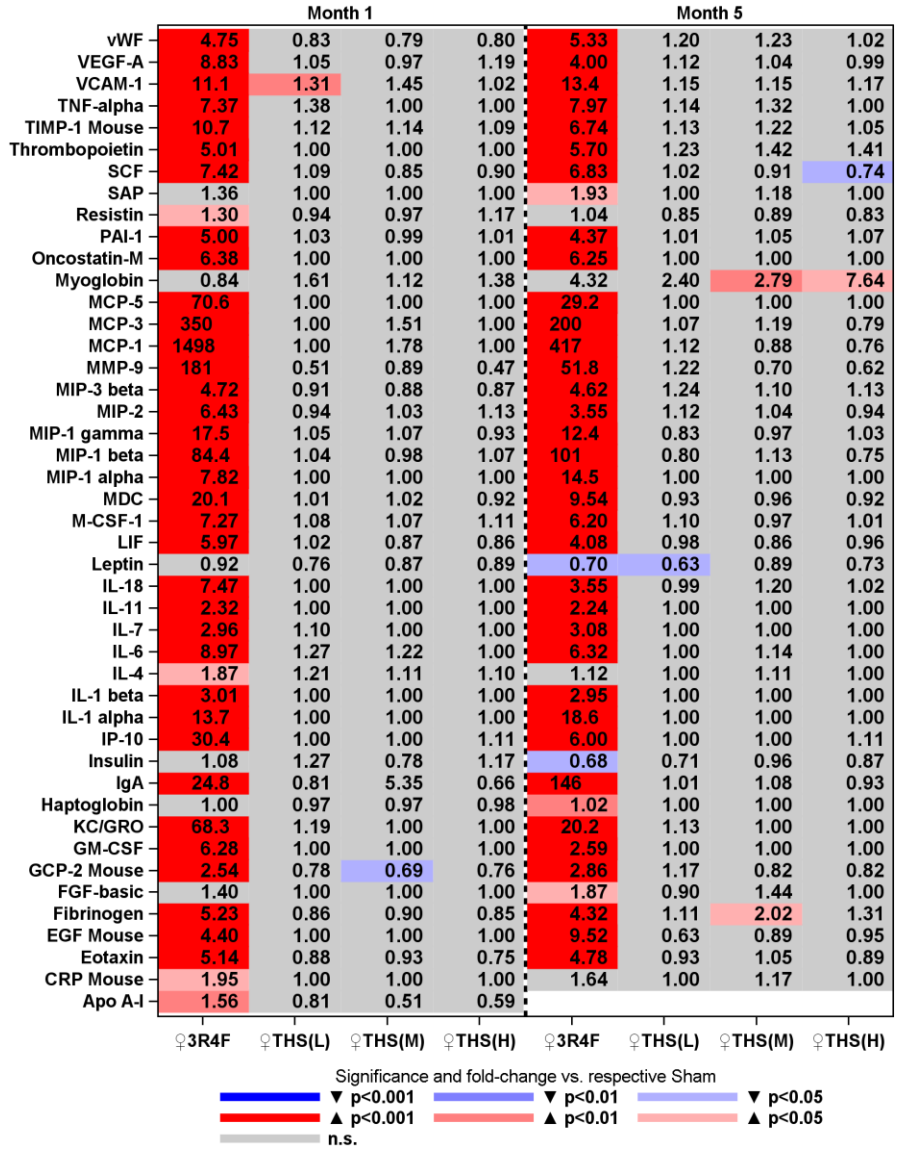
A/J mouse study
Result summary: Lung inflammation – BALF cytokines/chemokines

- Minimal up-regulation of key inflammatory factors in THS2.2 aerosol-exposed mice
- 3R4F exposure-related increases in
  - Levels of inflammatory cytokines, e.g. IL-6, TNF-α, IL1-β
  - Levels of chemotactic factors, e.g. MCP1, KC
  - Growth factors, e.g. EGF, VEGF



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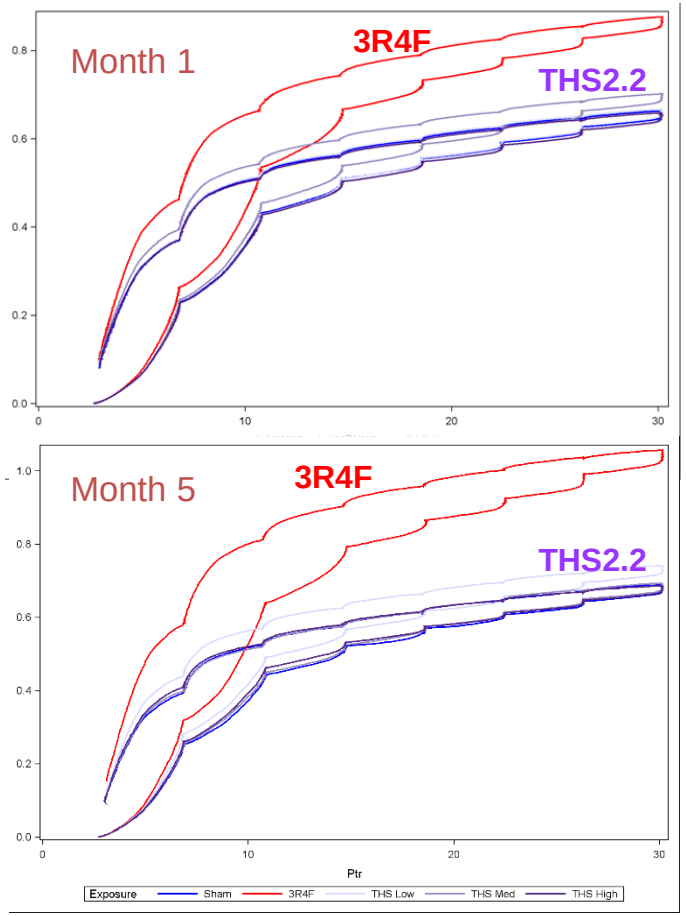
A/J mouse study
Result summary: Lung function

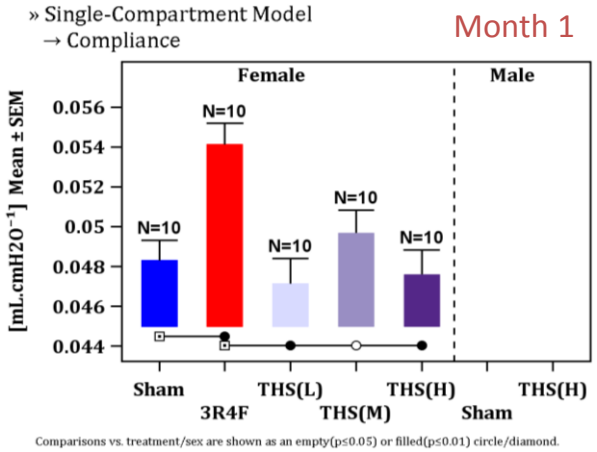
No obvious changes in lung function in THS2.2 aerosol-exposed mice

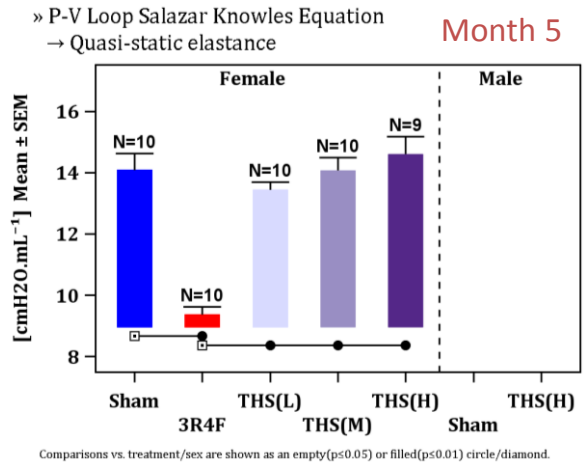
3R4F exposure-related changes

- Leftward & upward shift of the P-V loops for both the inflation and deflation phases
- Increased lung volumes at specified pressure; greater ease with which the lungs may be extended at a specified pressure
- Lower pressure at specified volume of air in the lung

Lung function Flexivent - Pressure-Volume loops







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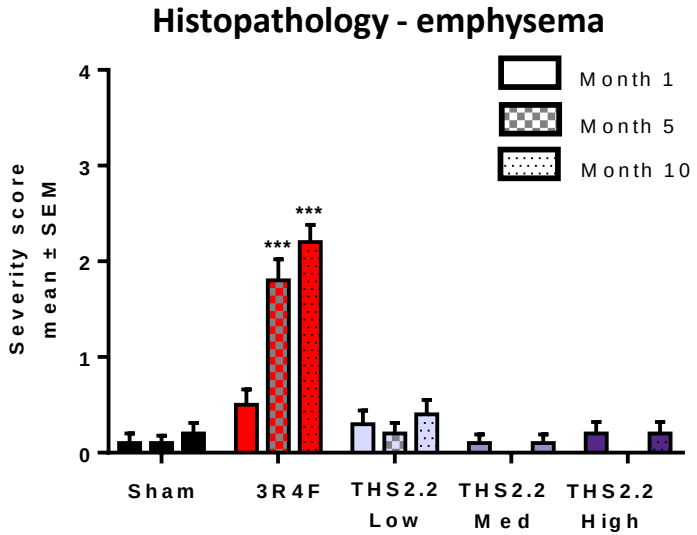
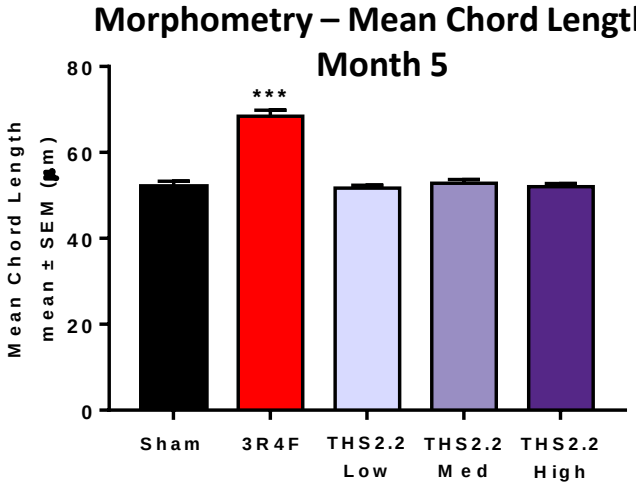
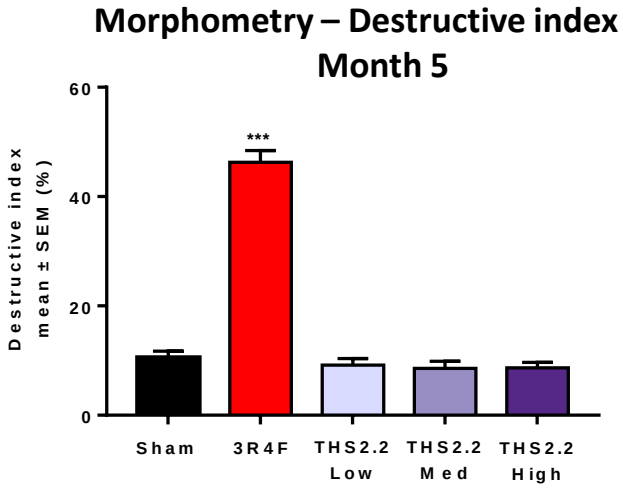
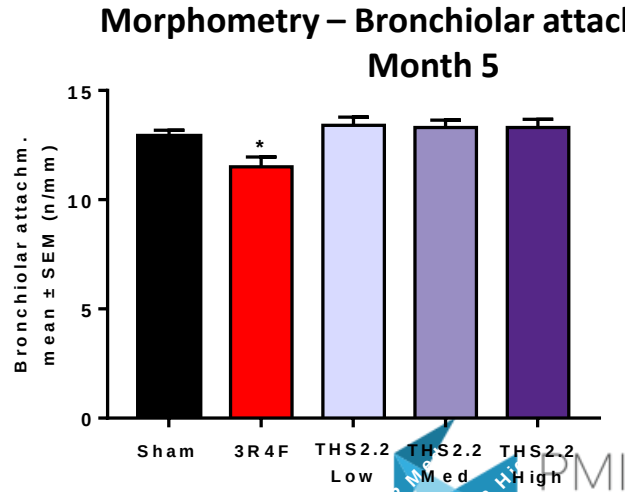
A/J mouse study
Result summary: Lung tissue changes - Histopathology and morphometry

- Histopathological assessment and morphometric analysis shows consistent emphysema in 3R4F-exposed A/J mice but in THS2.2-exposed animals.









* p<0.05; **p<0.01; ***p<0.001 differences relative to sham

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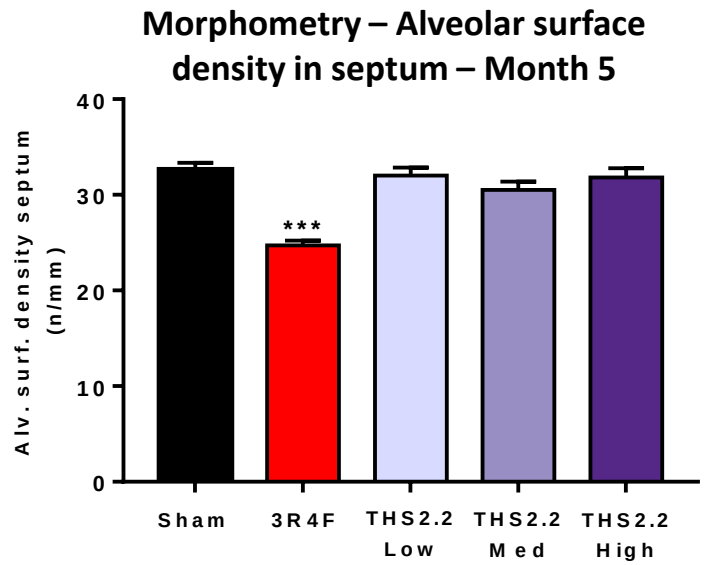
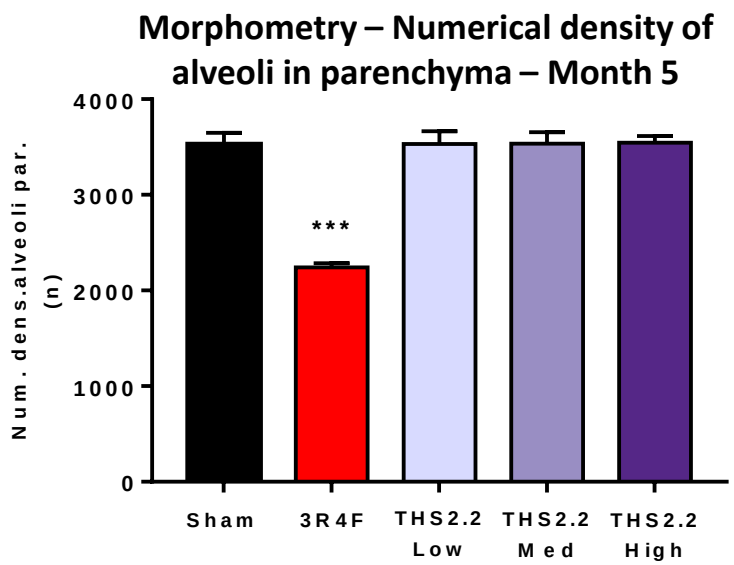
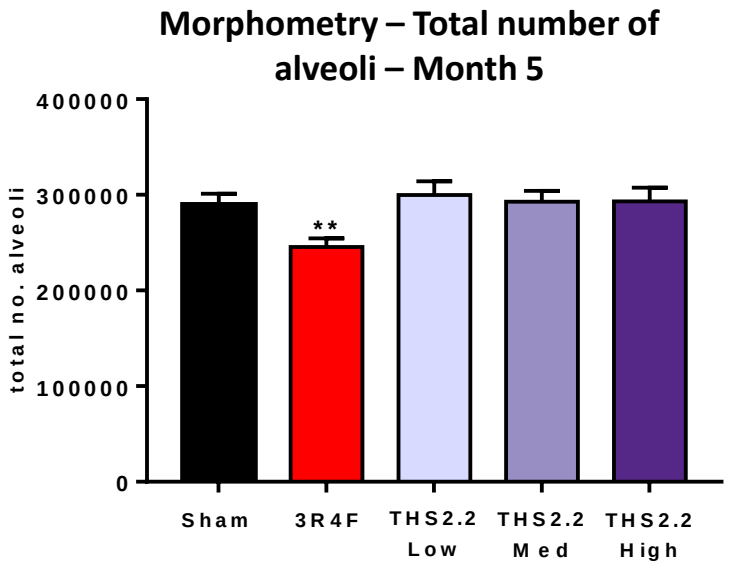
A/J mouse study
Result summary: Lung tissue changes -Morphometry

- Supplemental analysis of lung volume-independent state-of-the-art morphometric parameters confirm the emphysematous changes in 3R4F-exposed but in the THS2.2-exposed mice







* p<0.05; **p<0.01; ***p<0.001 differences relative to sham

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A/J mouse study
Summary and Conclusions

- The A/J mouse model is suitable for studying smoke-related aspects of COPD
- After 1 month of exposure to cigarette smoke, lung inflammation is clearly present and changes in lung function are obvious – lung emphysema is present at the 5 months time point
- Exposure to aerosol for THS2.2 doesn't cause any changes in lung inflammation, lung function and emphysema

Stinn W, Buettner A, Weiler H, Friedrichs B, Luetjen S, van Overveld F, Meurrens K, Janssens K, Gebel S, Stabbert R, Haussmann HJ. (2013) Lung inflammatory effects, tumorigenesis, and emphysema development in a long-term inhalation study with cigarette mainstream smoke in mice.
Toxicol Sci. 131(2):596-611. **PMID: 23104432**

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Comparison mouse models

- Both independent mouse studies have shown that:
  - Lung inflammation and changes in pulmonary function are induced after 1 month of exposure to cigarette smoke
  - Lung emphysema is caused by cigarette smoke and significant after 2 months (or 5 months A/J mice) of exposure to cigarette smoke
  - Aerosol from THS2.2, a RRP, causes only minimal changes in lung inflammation, but no changes in lung function and pulmonary emphysema.

THANK YOU

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