Methods

The mice were exposed to smoke from 3RF (750 micrograms/liter of total particulate matter – TPM), aerosol from pMRTP or filtered air for 6 hours per day, 5 days per week, up to 7 months. Both tobacco products used had the same nicotine concentration in smoke and aerosol - 3.4-micrograms/liter. After 2 months of exposure to 3RF smoke, switching and cessation groups were exposed to pMRTP aerosol or filtered air, respectively. Right lung samples from month 1, 3, 5, and 7 were analysed.

All procedures were approved by the Animal Ethics Committee of the University of Neuchâtel, by the Ethics Committee on Animal Experimentation for the State of Neuchâtel, and by the Veterinary Services of the State of Neuchâtel. Animal experiments were performed according to Directive 2010/63/EU and Swiss Cantonal regulations on animal welfare.

ITRAQ: 50 μg of extracted proteins from the 5 respective treatment groups were reduced, alkylated and tryptic digested in parallel followed by labeling using ITRAQ tags and samples were pooled. Finally, the tagged peptides were analysed by LC-MS/MS to be identified and quantified.

2D-PAGE: 150 μg of protein was loaded and separated on 11 cm strip, 3-10 NL, then on 13 cm 12% SDS-PAGE and finally stained with Sypro Ruby SanoSpot software (SlixiLab) was used for the detection of differentially expressed proteins by comparison to the control sample (Sham). Tryptic digested samples were analysed using MALDI TOF/TOF-MS and proteins were identified using Mascot search engine against the Uniprot Mouse database.

RPPA: Spots were measured in 3 replicates on a screen format and the images were measured using the Zephotainer.

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