Effects of Particulate Matter\textsubscript{10} on RNA Expression in Lung Tissue of a Murine Model

Running title: Inhalation of PM\textsubscript{10} in murine model

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Abstract

**Background:** Particulate matter (PM10; airborne particles <10 μm) has been demonstrated to induce airway and lung diseases. We aimed to investigate the effects of PM10 on RNA expression in lung using a murine model.

**Methods:** Female BALB/c mice were treated with PM10, ovalbumin (OVA), or both (OVA/PM10). PM10 was intranasally administered four times over 2 weeks, and OVA was intraperitoneally injected and then intranasally administered. After 2 days from the last challenges, mice were sacrificed. Full RNA sequencing using lung homogenates was conducted.

**Results:** PM10 did not induce cell proliferation in bronchoalveolar fluid, and it also did not lead to airway hyper-responsiveness, but it did cause airway inflammation and lung fibrosis. Levels of IL-1β, TNF-α, and TGF-β in lung homogenates were significantly higher in the PM10-treated group, compared to the control group. The PM10 model showed increased RNA expression of Rn45a, Snord22, Atp6v0c-ps2, Snora28, Snord15b, Snora70, and Mmp12. Generally, genes associated with RNA splicing, DNA repair, the inflammatory response, the immune response, cell death, and apoptotic processes were highly expressed in the PM10-treated group. OVA/PM10 treatment did not produce greater effects than OVA alone. However, the OVA/PM10-treated group did show increased RNA expression of Clca1, Snord22, Retnla, Prg2, Tff2, Atp6v0c-ps2, and Fegbp compared to the control group. These genes are associated with RNA splicing, DNA repair, the inflammatory response, and the immune response.

**Conclusions:** Inhalation of PM10 changed RNA expression in extensive range, and it also induced increase inflammatory cytokines, cellular inflammation, and fibrosis, in murine model.
Introduction

Air pollution is an important problem worldwide, and it certainly has negative effects on general health\textsuperscript{1-3}. Particulate matter\textsubscript{10} (<10 μm) is one of the major components of air pollution. It includes high levels of elements such as silicon, barium, aluminum, zinc, copper, and lead\textsuperscript{4,5}. PM\textsubscript{10} enters the airway through the nose and mouth, and as a result it can potentially cause injury to the respiratory tract, including the trachea, bronchus, alveoli, and even lung parenchyma. Studies have also indicated that chronic and intensive inhalation of PM\textsubscript{10} can induce and enhance airway and lung diseases. For example, epidemiologic data have shown that asthma can be developed and aggravated by ambient pollutants like PM\textsubscript{10}\textsuperscript{6-8}, and chronic obstructive pulmonary disease (COPD) is also sensitive to PM\textsubscript{10} exposure\textsuperscript{9-12}.

Some indications of the mechanisms underlying these effects have been found\textsuperscript{13}. For example innate and adaptive immune responses in the airway and lung can be altered by extrinsic irritants in general\textsuperscript{14}, and PM\textsubscript{10} exposure can alter mechanical and immunological barriers in airway disease\textsuperscript{15}. At the molecular level, evidence indicates that IL-1β, IL-6, NLRP3, and CCL-20 may be key mediators of the effects of PM\textsubscript{10} on airway and lung tissue\textsuperscript{16-18}. However, PM\textsubscript{10} particles are extremely small and consist of variable elements. We therefore hypothesized that PM\textsubscript{10} can alter RNA expression \textit{in extensive range}, potentially leading to visible inflammation and other side effects. Elucidating the patterns of RNA expression changes in response to PM\textsubscript{10} in a murine model may be helpful for predicting its effects on human health.
Materials and methods

Animal model designs

Female BALB/c mice, between 5 and 6 weeks old (Orient, Daejeon, Korea), were maintained at conventional animal facilities under pathogen-free conditions, and 5 mice were assigned in each group. To establish the PM10-induced murine model (PM10 model), PM10 (ERMCZ-120® certified reference material; Sigma-Aldrich, St Louis, MO, USA; 100 μg [PM100] or 200 μg [PM200]) suspended in 20 μL normal saline was intranasally administered four times over 2 weeks. To establish the OVA-induced asthma murine model (OVA model), mice were sensitized with 20 μg OVA (Sigma-Aldrich, St Louis, MO, USA) suspended in 1% aluminum hydroxide (Resorptar, Indergen, New York, NY, USA) by intraperitoneal injection on days 1 and 14. On days 21, 22, and 23, the OVA-sensitized mice were challenged intranasally with 30 μL of OVA (1 mg/mL) in saline solution. An OVA/PM10-treated model was established by the above two treatments simultaneously. All mice were sacrificed 2 days after their last treatment (supplementary figure 1). All experimental procedures of mice studies were approved by the Institutional Animal Care and Use Committee, Animal Research Ethics Board of Yonsei University (Seoul, Korea) (IACUC approval number, 2020-0087) and were performed in accordance with the Committee’s guidelines and regulations for animal care.

Measurement of airway hyper-responsiveness

Airway hyper-responsiveness (AHR) to inhaled aerosolized methacholine (MCh; Sigma-Aldrich, St Louis, MO, USA) was measured using a forced oscillation technique (FlexiVent; SCIREQ, Montreal, QC, Canada) on the sacrifice day, as described in a previous study19-21. Aerosolized phosphate-buffered saline (PBS) or methacholine at varying concentrations (3.125 mg/mL, 6.25 mg/mL, 12.5 mg/mL, 25.0 mg/mL, or 50.0 mg/mL), was administered to mice for 10 s via a nebulizer connected to a ventilator. Then, AHR was assessed by
measurements of airway resistance.

**Inflammatory cell counting in bronchoalveolar lavage fluid**

To collect bronchoalveolar lavage fluid (BALF), lung lavage was performed, using 1 mL of Hank’s balanced salt solution (HBSS) through a tracheal tube. The recovered BALF was centrifuged and resuspended in 300 µL HBSS. Total cell numbers were determined using a hemocytometer and trypan blue staining. BALF cells were centrifuged by cytocentrifugation (Cytospin 3; ThermoFisher Scientific, Waltham, MA, USA) and were pelleted to cytospin slides. The slides were stained with hematoxylin and eosin (H&E Hemacolor®, Merck, Darmstadt, Germany) and a differential count of inflammatory cells was performed (200 cells per slide).

**Histological analysis**

The lung that was not used for BALF collection was fixed in 4% formalin and embedded in paraffin. Lung sections were cut into 3–4-µm-thick slices and stained with H&E, periodic acid-Schiff (PAS), and Masson trichrome (M&T) for histological analysis. The slides were observed under a light microscope (×200 magnification). Fibrosis area was measured by estimating the color-pixel count over the pre-set threshold color on MT-stained slides at 200× magnification using MetaMorph program (Molecular Devices, Sunnyvale, CA).

**Lung homogenate**

After collecting BALF, remaining lung tissue was resected and homogenized using a tissue homogenizer (Biospec Products, Bartlesville, OK, USA) in lysis buffer and protease inhibitor solution (Sigma-Aldrich, St Louis, MO, USA). After incubation and centrifugation, supernatants were harvested and passed through a 0.45-micron filter (Gelman Science, Ann
Arbor, MI, USA). The final preparations were stored at -20°C for cytokine analysis as described previously\textsuperscript{19}.

**Analysis of cytokines**

Concentrations of interleukin (IL)-1β, TNF-α, IL-13, and TGF-β in lung homogenates were assessed by enzyme-linked immunosorbent assay (R&D Systems, San Diego, USA) according to the manufacturer’s instructions. All samples were assessed in duplicate.

**Full RNA sequencing**

Total RNA was extracted from lung tissue using Trizol reagent (Invitrogen). The isolated mRNAs were used for cDNA synthesis. Libraries were prepared using the NEBNext Ultra II Directional RNA Seq Kit (NEW ENGLAND BioLabs, Inc., UK). Indexing was performed using the Illumina indexes 1–12. The enrichment step was carried out using PCR. Subsequently, libraries were checked using the Agilent 2100 bioanalyzer (Agilent Technologies, Amstelveen, The Netherlands), to evaluate the mean fragment size. Quantification was performed using the library quantification kit with an ND 2000 Spectrophotometer (Thermo Inc., DE, USA) and StepOne Real Time PCR System (Life Technologies, Inc., USA). High-throughput sequencing was performed as paired end 100 sequencing using NovaSeq 6000 (Illumina, Inc., USA).

Quality control of raw sequencing data was performed using FastQC (Simon, 2010). The results of fast QC are presented in supplementary figure 2. Adapter and low-quality reads (<Q20) were removed using FASTX\_Trimmer (Hannon Lab, 2014) and BBMap (Bushnell, 2014). Then, the trimmed reads were mapped to the reference genome using TopHat.\textsuperscript{22} Gene expression levels were estimated by calculating fragments per kb per million reads (FPKM) using Cufflinks.\textsuperscript{23} The FPKM values were normalized based on a quantile normalization method using EdgeR within R (R development Core Team, 2016). Data mining and graphic
visualization including define upregulated or downregulated gene expression were performed using ExDEGA (E-Biogen, Inc., Korea).

**Statistical analysis**

All results are expressed as the mean ± standard error. The AHR data were analyzed using repeated-measure analysis of variance (ANOVA), followed by a post-hoc Bonferroni test. One-way ANOVA was performed to assess the significance of differences in BALF cell count, cytokine levels, and quantitative fibrosis among groups. All statistical analyses were performed with IBM SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). *P*-values < 0.05 were considered statistically significant.
Results

Comparison of weight changes, AHR, and BALF between control and PM$_{10}$-treated groups
All mice increased in weight over the course of the experiment. There was a non-significant trend for the PM$_{10}$-treated group (PM100 and PM200) to gain less weight (Fig. 1A). AHR obtained by methacholine challenge showed no significant changes among the three groups (Fig. 1B). BALF cell counts were also not significantly different among groups (Fig. 1C).

Comparison of pathologic findings between control and PM$_{10}$-treated groups
Compared to the control group, the PM$_{10}$-treated group (PM100 and PM200) showed cellular infiltration in the airway and lung parenchyme. Airway wall thickness, goblet cell hyperplasia, and inflammatory cellular proliferation were observed predominantly in the PM$_{10}$-treated group. In addition, fibrosis in lung parenchyme and peribronchial tissues were also predominant in the PM$_{10}$-treated group, compared to the control group (Fig. 2).

Comparison of cytokine levels in lung homogenates and quantitative fibrosis between the control and PM$_{10}$-treated groups
The levels of IL-1$\beta$, TNF-$\alpha$, and TGF $\beta$ in lung homogenates were higher in the PM$_{10}$-treated group than in the control group, but statistical significance was observed only for the PM100 group (Fig. 3A–C). As evidence by the results of the fibrosis-area analysis, PM$_{10}$ induced significant lung fibrosis (Fig. 3D).

Comparison of RNA expression between the control and PM$_{10}$-treated groups
The PM$_{10}$ model showed increased RNA expression of Rn45a, Snord22, Atp6v0c-ps2, Snora28, Snord15b, Snora70, and Mmp12 compared to control group (Fig. 4A). Generally, genes associated with RNA splicing, DNA repair, inflammatory response, immune response, cell death, and the apoptotic process were highly expressed in the PM$_{10}$ model compared to control group (Fig. 4B).
Comparison of weight changes, airway hyper-responsiveness, and bronchoalveolar lavage fluid cell count between the control, OVA, and OVA/PM$_{10}$-treated groups

All mice increased in weight over the course of the experiment. Among all the groups, the final weight of the control group was heaviest (Fig. 5A). AHR obtained by methacholine challenge in both OVA-treated groups (OVA and OVA/PM$_{10}$) was predominant compared to the control group. However, it was not significantly different between the OVA and OVA/PM$_{10}$-treated groups (Fig. 5B). Total cell, macrophage, and eosinophil counts in BALF were highly elevated in all OVA-treated groups compared with the control group. However, they were not significantly different between the OVA and OVA/PM$_{10}$-treated groups (Fig. 5C).

Comparison of pathologic findings between control, OVA, and OVA/PM$_{10}$-treated group

All OVA-treated group showed prominent inflammatory cell proliferation and fibrosis in airway, peribronchial tissue, and lung parenchyma, compared to control group. However, treatment of OVA/PM$_{10}$ did not have additive effect to OVA alone (Fig 6).

Comparison of cytokine levels in lung homogenates and quantitative fibrosis between in control, OVA, and OVA/PM$_{10}$-treated group

The levels of IL-1$\beta$, TNF-$\alpha$, IL-13, and TGF-$\beta$ in lung homogenates were increased in the OVA-treated group. However, the effects of OVA/PM$_{10}$ treatment were not greater than those of OVA alone (Fig. 7A-D). Both OVA and OVA/PM$_{10}$ treatment induced significant lung fibrosis as evident in fibrosis-are analysis, however, OVA/PM$_{10}$ treatment were not greater than those of OVA alone (Fig. 7E).

Comparison of RNA expression between the control and OVA/PM$_{10}$-treated groups

The OVA/PM$_{10}$-treated model showed increased RNA expression of Clea1, Snord22, Retnl, Prg2, Tff2, Atp6v0c-ps2, and Fcgbp compared to the control (Fig. 8A). Overall, this model
showed increased RNA expression of genes associated with RNA splicing, DNA repair, inflammatory response, and immune response compared to control group (Fig. 8B).

Discussion

This study confirmed that PM$_{10}$ can alter immune and inflammatory processes of the lung at the gene, protein, and cellular levels, using a murine model. In a substantial advance on previous work, we showed that exposure to PM$_{10}$ can extensively alter RNA expression in lung homogenates. PM$_{10}$ induced increased RNA expression associated with RNA splicing, DNA repair, cell death, apoptotic processes, the inflammatory response, and the immune response. The above processes are associated with the cell cycle, cell viability, and cellular proliferation. Potential consequences of such widely altered RNA expression profiles include necrosis, malignancy, and other diseases. Referring to the results of our RNA expression analysis, we can potentially predict various clinical effects of PM$_{10}$, and conduct further studies concerning mechanisms underlying these effects.

Inhalation of PM$_{10}$ induced proliferation of inflammatory cells and fibrosis in peri-bronchial and lung tissue. We speculated that abundant helper T cell type I (Th1) type inflammatory cytokines increased in lung homogenates might led to these changes. Some previous studies have showed similar results: Th1 type inflammatory cytokines increased in PM$_{10}$ treated model.$^{24,25}$ Other studies also showed PM$_{10}$ is associated with inflammation$^{26}$ or fibrosis$^{27}$ of lung. Based on this study and previous in vitro and in vivo studies, PM$_{10}$ is definitely toxic material to airway and lung parenchyme. Many human studies also support that PM$_{10}$ has negative effects on lung and airway diseases.$^{28}$

It is notable that we observed extremely high expression of Rn45s (8,058-fold change), Snord22 (676-fold change), and Atp6v0c-ps2 (196-fold change) in the PM$_{10}$ treated group,
compared to the control group. Rn45s is known to be associated with RNA toxicity, but its function has not been fully elucidated. Snord22 is small nucleolar RNA. Atp6v0c-ps2 is associated with ATPase, H+ transporting, and lysosomal V0 subunit C. This plays a central role in H(+) transport across cellular membranes. In addition, Snora28, Snord15b, Snora70, Mmp12, Rprl3, Bcl, Snora17, AA467197, Snora26, Ccl17, Rpph1, and Clec4d were also highly expressed in the PM10-treated group compared to the control group. These genes are associated with small nucleolar RNA, brain cytoplasmic RNA, or specific chemokines. In the OVA/PM10-treated group, the genes Cleca1 (Chloride channel accessory 1), Snord 22 (small nucleolar RNA), Retnla (resistin like alpha), Prg2 (proteoglycan 2, bone marrow), Tff2 (trefoil factor 2), Atp6v0c-ps2 (ATPase, H+ transporting, lysosomal V0 subunit C, pseudogene 2), Fcgbp (Fc fragment of IgG binding protein), Muc5ac, Iltn1, Ngp (neutrophilic granule protein), Fxvd4 (FXYD domain-containing ion transport regulator 4), Mzb1 (marginal zone B and B1 cell-specific protein 1), Mmp12 (matric metallopeptidase 12), Camp (cathelicidin antimicrobial peptide), and Tff1 were upregulated compared to control group.

Some genes were extremely suppressed in the PM10-treated group, compared to the control group: Chil4, Krt13, Krtiap (associated with keratinocyte differentiation), Sprr2a3 (small proline-rich protein 2A3), Krt4, Tnnc2 (troponin C2, fast), Acta1 (actin, alpha 1, skeletal muscle), Mt4, Serpinb3c (serine peptidase inhibitor, clade B, member 3C), Lgals7 (lectin, galactose binding, soluble 7), Crtcl (cysteine-rich C-terminal 1), Serpinb12 (serine peptidase inhibitor, clade B, member 12), Tnnt3 (troponin T3, skeletal, fast), Mylpf (myosin light chain, phosphorylatable, fast skeletal muscle), and Lce3a (late cornified envelope 3A). In the OVA/PM10-treated group, the genes Krt6b, serpinb12 (serine peptidase inhibitor, clade B, member 12), Crtcl (cysteine-rich C-terminal 1), Mylpf (myosin light chain, phosphorylatable, fast skeletal muscle), Lce3b (late cornified envelope 3B), Defb4 (defensin beta 4), Lce3a (late cornified envelope 3A), Lgals7 (lectin, galactose binding, soluble 7), Serpinb3c (serine
peptidase inhibitor, clade B, member 3C), Mt4, Krt4, Acta1 (actin, alpha1, skeletal muscle), Tnnc2 (troponin C2, fast), Krtdap (keratinocyte differentiation associated protein), and Krt13 were substantially downregulated compared to the control group.

PM$_{10}$ altered RNA expression in extensive range. It also increased production of inflammatory cytokines. Inflammation and fibrosis were also induced. However, its effects were only slightly greater than those of OVA. We used an acute OVA model with intraperitoneal OVA sensitization and intranasal OVA challenge. This model also showed extensive changes of RNA expression and abundant inflammation. Because of the magnitude of the changes caused by OVA, additional effects of PM$_{10}$ were not well revealed. In clinics, severe asthma often leads to hide the clinical effects of other underlying disease, like stable COPD. However, Gold et al. showed that PM mediates and augments allergic sensitization and cellular proliferation using a murine model, and Clifford et al. showed that PM$_{10}$ exposure exacerbates various response to respiratory viral infection, e.g. increased inflammation and impaired lung function. Then, we are not sure whether additive or synergic effects of PM$_{10}$ in mild or chronic asthma model. In order to further clarify whether PM$_{10}$ has additive or synergic effects on an allergy model, a further-modified OVA model which does not hide the effects of PM$_{10}$, is needed.

PM$_{10}$ is a major air pollutant, and thus ends up in the human respiratory system where it can facilitate and aggravate allergic sensitization and airway inflammation. This also alters defense mechanisms, including innate immunity in the lungs. Thus, respiratory diseases can be developed and aggravated by exposure to PM$_{10}$. However, studies elucidating the effects of PM$_{10}$ using murine models are rare, and changes of RNA expression induced by PM$_{10}$ have not been well studied. This study used standardized PM$_{10}$ in a murine model, and showed extensive RNA expression changes. Our results can be used to inform future work using PM$_{10}$-treated murine models, including further investigation of mechanisms underlying the
damaging effects of PM$_{10}$ on the airway and lung. Finally, this study will be helpful to search for therapeutic agents in PM$_{10}$-exposed human airway and lung diseases.

**Conclusion**

We showed that inhalation of PM$_{10}$ changed RNA expression in extensive range in a murine model. PM$_{10}$ also induced increased production of inflammatory cytokines, cellular proliferation, and fibrosis. In an acute-OVA model, additional effects of PM$_{10}$ were not observed. Our findings suggest PM$_{10}$ can affect various airway and lung diseases.

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**TRD**

**Disclosure of Interests:**

The authors report no conflicts of interest.

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Figure Legends

Figure 1. Weight change did not significantly differ among groups (A). Airway hyperresponsiveness (AHR) obtained by methacholine challenge showed no significant difference among groups (B). There was no significant difference of BALF cell count among groups (C).

OVA, ovalbumin; BALF, bronchoalveolar lavage fluid
Figure 2. Pathologic findings revealed that PM$_{10}$ treatment led to airway inflammation and lung fibrosis (H&E, PAS, and M&T; all × 200 magnification).

H&E, hematoxylin and eosin; PAS, periodic acid-Schiff; M&T, Masson trichrome
Figure 3. IL-1β (A), TNF-α (B), and TGF-β (C) levels in lung homogenates were significantly higher in the PM100-treated group compared to the control group. Quantitative fibrosis was significant and severe in the PM10-treated group compared to the control group (D).

* $P < 0.05$
Figure 4. Top genes showing the most extreme difference between the control and PM_{10}-treated groups (A). RNA expression of genes associated with RNA splicing, DNA repair, the inflammatory response, the immune response, cell death, and apoptotic process were increased in the PM_{10}-treated group compared to the control group (B).

| Gene       | Fold change (PM_{10} vs. control) | Normalized data (log2) Control | Normalized data (log2) PM_{10} |
|------------|----------------------------------|--------------------------------|--------------------------------|
| Rnk10a     | 8068.366                        | 0.048                          | 13.023                         |
| Snora22    | 875.879                         | 0.048                          | 9.446                          |
| Abf6v0c-p2  | 194.598                         | 0.048                          | 7.853                          |
| Snora28    | 84.314                          | 0.040                          | 6.437                          |
| Snora156   | 76.070                          | 0.039                          | 6.326                          |
| Snora70    | 70.963                          | 0.038                          | 6.187                          |
| Mmp12      | 56.102                          | 1.246                          | 7.056                          |
| Rps3       | 51.280                          | 0.036                          | 5.717                          |
| Bcl1       | 42.404                          | 0.024                          | 5.440                          |
| Snora17    | 31.237                          | 0.031                          | 4.967                          |
| Aa467197    | 27.230                          | 0.818                          | 5.586                          |
| Snora26    | 26.536                          | 0.030                          | 4.760                          |
| Ccld17     | 19.580                          | 3.338                          | 7.630                          |
| Ryh1       | 19.420                          | 1.865                          | 6.144                          |
| Clec4d     | 18.464                          | 1.815                          | 6.021                          |
| Loco3a     | 0.004                           | 8.049                          | 0.000                          |
| Myc81      | 0.004                           | 9.441                          | 1.341                          |
| Trm3       | 0.003                           | 9.594                          | 1.204                          |
| Sexpni12    | 0.003                           | 8.326                          | 0.000                          |
| Cccl1      | 0.003                           | 8.528                          | 0.000                          |
| Lgat3T     | 0.003                           | 8.740                          | 0.162                          |
| Sexpni3c    | 0.002                           | 0.134                          | 0.000                          |
| Mm (metallothionein 4) | 0.002                  | 9.223                          | 0.000                          |
| Actb       | 0.001                           | 9.954                          | 0.000                          |
| Trm2       | 0.001                           | 9.988                          | 0.000                          |
| Klf4 (keratin 4) | 0.001                      | 10.223                         | 0.246                          |
| Svp2a3     | 0.001                           | 10.174                         | 0.000                          |
| Kdrlap     | 0.001                           | 10.468                         | 0.000                          |
| Klf13 (keratin 15) | 0.000                      | 11.586                         | 0.500                          |
| Ccl4 (cellulose-like 4) | 0.000                      | 11.664                         | 0.581                          |
Figure 5. Weight change was not significantly different among groups (A). Airway hyperresponsiveness (AHR) obtained by methacholine challenge were increased in the OVA and/or PM$_{10}$-treated group (B). BALF cell counts revealed significantly increased total, macrophage, and eosinophil counts in the OVA or OVA/PM$_{10}$-treated group compared to the control group (C).

* $P < 0.05$ between it and others
Figure 6. Pathologic findings revealed that OVA or OVA/PM$_{10}$ treatment led to airway inflammation and lung fibrosis (H&E, PAS, and M&T; all × 200 magnification).

H&E, hematoxylin and eosin; PAS, periodic acid-Schiff; M&T, Masson trichrome
Figure 7. IL-1β (A), TNF-α (B), IL-13 (C), and TGF-β (D) levels in lung homogenates were increased in the OVA or OVA/PM10-treated group. Quantitative fibrosis was significant and severe in the OVA or OVA/PM10-treated group compared to the control group (D).
Figure 8. Top genes showing the most extreme difference between the control and OVA/PM$_{10}$-treated groups (A). RNA expression of genes associated with RNA splicing, DNA repair, the inflammatory response, and the immune response were increased in the PM$_{10}$-treated group compared to the control group (B).

* $P < 0.05$

| Genes       | Fold change (OVA/PM$_{10}$ vs. control) | Normalized data (log2) Control | Normalized data (log2) OVA/PM$_{10}$ |
|-------------|----------------------------------------|--------------------------------|--------------------------------------|
| Ccna1       | 3.235.352                              | 2.454                          | 0.804                                |
| Snored22    | 2.037.589                              | 7.718                          | 0.046                                |
| Petlia      | 1.491.163                              | 15.198                         | 7.978                                |
| Ptg2        | 0.718.841                              | 7.593                          | 1.073                                |
| IF11        | 20.658.258                             | 8.905                          | 2.763                                |
| Atp6v0c-1s2 | 67.243.243                             | 6.122                          | 0.048                                |
| Fgfbp       | 59.644.243                             | 6.875                          | 0.977                                |
| Muc5ac (mucin SAC) | 58.342.243 | 5.978                          | 0.111                                |
| Fth1 (anticlock 1) | 48.569.243 | 5.978                          | 0.142                                |
| Ngf         | 45.142.243                             | 7.188                          | 1.973                                |
| Fagy4       | 41.619.243                             | 5.638                          | 0.212                                |
| Mb21        | 47.767.243                             | 8.722                          | 3.305                                |
| Mmp12       | 38.894.243                             | 6.528                          | 1.246                                |
| Ccng        | 37.851.243                             | 6.822                          | 1.587                                |
| Tef1 (effol factor 1) | 38.465.243 | 5.093                          | 0.083                                |
| Krt6b (keratin 6B) | 0.004.243   | 8.045                          | 0.126                                |
| Serpemb12   | 0.004.243                             | 8.323                          | 0.376                                |
| Ccnd1       | 0.004.243                             | 8.578                          | 0.565                                |
| Mypf        | 0.004.243                             | 9.441                          | 1.463                                |
| Lce3b       | 0.004.243                             | 7.697                          | 0.006                                |
| Cgyfb       | 0.004.243                             | 8.042                          | 0.006                                |
| Lce3a       | 0.004.243                             | 8.043                          | 0.006                                |
| Lggb7       | 0.003.243                             | 8.742                          | 0.393                                |
| Serpemb3c   | 0.002.243                             | 9.134                          | 0.006                                |
| Vt4 (metathionine-4) | 0.002.243 | 9.233                          | 0.006                                |
| Krt4 (keratin 4) | 0.001.243   | 10.342                         | 0.046                                |
| Acta3       | 0.001.243                             | 9.954                          | 0.174                                |
| Trusc2      | 0.001.243                             | 9.988                          | 0.006                                |
| Krt19a       | 0.001.243                             | 10.465                         | 0.006                                |
| Krt13 (keratin 13) | 0.000.243   | 11.348                         | 0.159                                |
**Supplementary figure 1.** Animal model design protocol

![Diagram showing animal model design protocol]

**Supplementary figure 2.** Fast QC data in control (A), PM$_{10}$-treated (B), and OVA/PM$_{10}$-treated group (C)

![Graphs showing fast QC data for different groups]