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Supplemental Material

Assessing the Effects of Nicotinamide Mononucleotide Supplementation on Pulmonary Inflammation in Male Mice Subchronically Exposed to Ambient Particulate Matter

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Figure S1. Effect of PM exposure on lipid accumulation in mouse liver tissue. (**A**) Representative images of oil red O (ORO)-stained liver sections from air-filtered control group (Con) and PM-exposed group (Exp) following 16-week PM exposure (n = 4 per group). Magnifications: 200X. Scale bar = 50 μm. (**B**) Hepatic lipid content (%) was calculated and expressed as the ratio of labeled red areas to total area of liver section (%/μm^2 total area) examined by ORO staining (n = 4 per group). Data was analyzed using Student's *t*-test. The data are expressed as mean ± SD. The mean, SD and SEM values for data are shown in **Table S5**. ***P<0.001 compared with the corresponding control mice. *P* values for all tests are reported in **Table S6**. Note: PM, particulate matter; Con, air-filtered control group; Exp, PM exposure group; SD, standard deviation; SEM, standard error of mean.

Figure S2. Body weights, average daily food intake and average daily water intake of NMN-treated mice without being exposed to PM. (**A**) Weekly body weights monitored in mice from H_2O-BS and NMN-BS groups (n = 10 per group). (**B**) Average daily food intake monitored every 3 days and calculated in H_2O-BS and NMN-BS groups (n = 10 per group). (**C**) Average daily water consumption recorded every 3 days and calculated in H_2O-BS and NMN-BS groups(n = 10 per group). Data was analyzed using Student's *t*-test. The data are expressed as mean ± SD. The mean, SD and SEM values for data are shown in **Table S5**. *P* values for all tests are reported in **Table S6**. Note: SD, standard deviation; SEM, standard error of mean.

Figure S3. Annotations of enriched pathways in mouse liver tissue of H_2O-BS and NMN-BS groups. Pathway enrichment analysis based on all DEGs (**A**), upregulated DEGs (**B**) and downregulated DEGs (**C**), by using KEGG pathway enrichment programs. DEGs: differentially expressed genes; KEGG: Kyoto Encyclopedia of Genes and Genomes. The pathway analysis in this figure is listed in **Excel Table S3**.

Figure S4. Metabolic alterations in mice following 18-week oral NMN supplementation. (**A**) The relative mRNA expression levels of Cpt1c, Acsbg1, Adipoq, Slc27a1 and B3gnt5 in mouse liver tissue of H_2O-BS and NMN-BS groups (n = 3 per group). (**B**) Fasted glucose levels measured in mouse tail vein blood (n = 5 per group). (**C**) The TG levels examined in mouse liver tissue (n = 4 per group). (**D**) Relative blood glucose levels measured at the time points of 15, 30, 60, 90 and 120 min after mice were intraperitoneally injected with 1 g/kg 20% glucose solution (n = 5 per group). (**E**) AUCs calculated based on the curves shown at (**D**). (**F**) Relative blood glucose levels measured at the time points of 15, 30, 60, and 90 min after 0.5 U/kg insulin administration(n = 3 per group). (**G**) AUCs calculated based on the curves shown in (**F**). Data was analyzed using Student's *t*-test or Wilcoxon rank sum test where appropriate. The data are expressed as mean ± SD. The mean, SD and SEM values for data are shown in **Table S5**. *P<0.05; **P<0.01; ***P<0.001 compared with the control mice. *P* values for all tests are reported in **Table S6**. Note: TG, triglyceride; IPGTT, intraperitoneal glucose tolerance testing; ITT, insulin tolerance testing; AUCs, area under curves; SD, standard deviation; SEM, standard error of mean.

Figure S5. Effects of NMN supplementation on oxidative stress, chronic inflammation and pro-fibrotic changes induced by PM exposure. (**A**) The albumin (ALB) contents examined in the bronchoalveolar lavage fluid (BALF) (n = 5 per group). (**B**) IL-1β content measured in lung tissue from mice in Con-H_2O, Exp-H_2O, Con-NMN and Exp-NMN groups (n = 3 per group). (**C-E**) The relative mRNA expression levels of Il1b (**C**), S100a9 (**D**) and p65 (**E**) in lung tissue of Con-H_2O, Exp-H_2O, Con-NMN and Exp-NMN mice (n = 3 per group). (**F-G**) MDA content examined in lung tissue (n = 3 per group) (**F**) and plasma (n = 3 per group) (**G**) of Con-H_2O, Exp-H_2O, Con-NMN, and Exp-NMN mice. (**H**) The tail moment of comet assay (DNA damage index) conducted on peripheral blood in Con-H_2O, Exp-H_2O, Con-NMN, and Exp-NMN groups (n = 4 per group). (**I-K**) The relative mRNA expression levels of Acta2 (**I**), Tgfb1 (**J**) and Il17a (**K**) in lung tissue of Con-H_2O, Exp-H_2O, Con-NMN and Exp-NMN mice (n = 3 per group). (**L**) IL-17A content measured in lung tissue from mice in 4 groups at the end of PM exposure (n = 3 per group). Data presented in this figure was analyzed using one-way ANOVA followed by Tukey's multiple comparison post hoc test or Kruskal-Wallis test followed by Dunn's multiple comparisons test where appropriate. The results were presented as mean ± SD. The summary data (mean, SD and SEM values) of bar graphs in this figure is shown in **Table S5**. *P<0.05; **P<0.01; ***P<0.001. $^{\#}P$<0.05; $^{\#\#}P$<0.01; $^{\#\#\#}P$<0.001 compared with the Exp-H_2O mice (Exp-NMN vs Exp-H_2O). P values for all tests are reported in **Table S6**. Note: PM, particulate matter; NMN, nicotinamide mononucleotide; ALB, albumin; BALF, bronchoalveolar lavage fluid; MDA, malondialdehyde; ANOVA, analysis of variance; SD, standard deviation; SEM, standard error of mean.

Figure S6. Canonical pathway analysis based on the "PM Exposure DEGs" in neutrophils, monocyte-derived cells, and fibroblasts. (**A-C**) Enrichment analysis was carried out by canonical pathway analysis on DEGs (log_2|FC|>0.25, P<0.05) identified in neutrophils (**A**), monocyte-derived cells (**B**), and fibroblasts (**C**) between Con-H_2O and Exp-H_2O groups. Data was analyzed by using IPA software. Note: DEGs, differentially expressed genes; IPA, Ingenuity Pathway Analysis. The detailed information of enriched pathways is listed in **Excel Table S12**.

Figure S7. Canonical pathway analysis based on the "NMN Treatment DEGs" in neutrophils, monocyte-derived cells, and fibroblasts. (**A-C**) Enrichment analysis was carried out by canonical pathway analysis on DEGs (log_2|FC|>0.25, P<0.05) identified in neutrophils (**A**), monocyte-derived cells (**B**), and fibroblasts (**C**) between Exp-H_2O and Exp-NMN groups. Data was analyzed by using IPA software. Note: DEGs, differentially expressed genes; IPA, Ingenuity Pathway Analysis. The detailed information of enriched pathways is listed in **Excel Table S13**.

Figure S8. Effects of NMN supplementation on immune functions in mouse lung tissue of Con-H_2O, Exp-H_2O, Con-NMN and Exp-NMN groups. (**A**) Immunosuppressive activity of BM-derived MDSCs derived from Con-H_2O, Exp-H_2O, Con-NMN, and Exp-NMN mice indicated by T cell proliferation (%)after coculturing at the ratio of 1:1. The experiment was conducted at 3 duplicates. (**B-D**) Enrichment analysis was carried out by canonical pathway analysis on "rescue" DEGs identified in neutrophils (**B**), monocyte-derived cells (**C**), and fibroblasts (**D**). Data presented in (**A**) was analyzed using one-way ANOVA followed by Tukey's multiple comparison post hoc test. The result in bar graph was presented as mean ± SD. The summary data (mean, SD and SEM values) of bar graph in this figure is shown in **Table S5**. ***P<0.001. $^{\#\#}P$<0.01compared with the Exp-H_2O mice (Exp-NMN vs Exp-H_2O). P values for all tests are reported in **Table S6**. Note: NMN, nicotinamide mononucleotide; BM, bone marrow; DEGs, differentially expressed genes; IPA, Ingenuity Pathway Analysis; ANOVA, analysis of variance; SD, standard deviation; SEM, standard error of mean. The detailed information of enriched pathways is listed in **Excel Table S14**.

Additional File- Excel Document

References