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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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Table of Contents

Table S1. Detailed information and Reproducibility of each sample in scRNA-seq analysis.

Table S2. Primer lists for qRT-PCR.

Table S3. The analysis of representative components in organic or water-soluble components of PM_{2.5}.

Table S4. Top 10 conserved markers of 14 cell clusters used for cell type annotations in scRNA-seq dataset.

Table S5. Summary data (mean, SD, SEM and sample size n) for plots.

Table S6. *P*-values and power (1- β) values for results.

Table S7. Corresponding cell number and proportions of 12 cell types from data of Figure 4D.

Table S8. Corresponding cell number and proportions of cell sub-clusters of fibroblasts and monocyte-derived cells from data of Figure 7C.

Table S9. Corresponding Z-scores of for representative enriched pathways of neutrophils, monocyte-derived cells and fibroblasts from data of Figure 8D.

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 ($\%/\mu\text{m}^2$ total area) examined by ORO staining (n = 4 per group). Data was analyzed using Student's *t*-test. The data are expressed as mean \pm 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 H₂O-BS and NMN-BS groups (n = 10 per group). **(B)** Average daily food intake monitored every 3 days and calculated in H₂O-BS and NMN-BS groups (n = 10 per group). **(C)** Average daily water consumption recorded every 3 days and calculated in H₂O-BS and NMN-BS groups (n = 10 per group). Data was analyzed using Student's *t*-test. The data are expressed as mean \pm 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 H₂O-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 H₂O-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 \pm 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-1 β content measured in lung tissue from mice in Con-H₂O, Exp-H₂O, 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-H₂O, Exp-H₂O, 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-H₂O, Exp-H₂O, Con-NMN, and Exp-NMN mice. (H) The tail moment of comet assay (DNA damage index) conducted on peripheral blood in Con-H₂O, Exp-H₂O, 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-H₂O, Exp-H₂O, 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 \pm 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-H₂O mice (Exp-NMN vs Exp-H₂O). 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-H₂O and Exp-H₂O 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-H₂O 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-H₂O, Exp-H₂O, Con-NMN and Exp-NMN groups. (A) Immunosuppressive activity of BM-derived MDSCs derived from Con-H₂O, Exp-H₂O, 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**. *** $P < 0.001$. ## $P < 0.01$ compared with the Exp-H₂O mice (Exp-NMN vs Exp-H₂O). 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

Supplementary tables

Table S1. Detailed information and Reproducibility of each sample in scRNA-seq analysis.

Sample ID	Sample Contents			Estimated cell counts in raw data	nUMIs in raw data	Estimated cell counts after quality control	nUMIs after quality control
	Air-filtered	PM exposure	NMN treatment				
Con-H ₂ O-1*	√	-	×	9471	31053	8394	18608
Con-H ₂ O-2*	√	-	×	9417	31053	8321	18388
Con-H ₂ O-3*	√	-	×	11332	31053	10039	17926
Exp-H ₂ O-1*	-	√	×	9209	31053	8140	17624
Exp-H ₂ O-2*	-	√	×	10802	31053	9503	18987
Exp-H ₂ O-3*	-	√	×	10832	31053	9003	18782
Exp-NMN-1	-	√	√	7760	31053	6570	18034
Exp-NMN-2	-	√	√	9062	31053	7597	17035
Exp-NMN-3	-	√	√	8265	31053	7054	17585
Con-H ₂ O-Aggr*	data pooled from 3 samples in Con-H ₂ O group by using Cellranger aggr			30220	31053	26745	20160
Exp-H ₂ O-Aggr*	data pooled from 3 samples in Exp-H ₂ O group by using Cellranger aggr			30843	31053	26532	20367
Exp-NMN-Aggr	data pooled from 3 samples in control group by using Cellranger aggr			25087	31053	21495	19638

*The data has been reported previously¹.

Table S2. Primer lists for qRT-PCR.

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
IL1 β	ATGGGCAACCACTTACCTATTT	GTTCTAGAGAGTGCTGCCTAATG
IL17a	CAAACATGAGTCCAGGGAGAG	GCTGAGCTTTGAGGGATGAT
TGF β 1	GGTGGTATACTGAGACACCTTG	CCCAAGGAAAGGTAGGTGATAG
Acta2	CCATCATGCGTCTGGACTT	GGCAGTAGTCACGAAGGAATAG
Col1a1	GCTTGAAGACCTATGTGGGTATAA	GGTGGAGAAAGGAGCAGAAA
p65	GGTGCATCCCTGTGTTGATA	CGTGGAGGAAGACACTTGATAG
S100a9	GCAAGAAGATGGCCAACAAAG	GGTGTCCCTCCTTCCTAGAGTA
β -actin	GAGGTATCCTGACCCTGAAGTA	CACACGCAGCTCATTGTAGA

Table S3. The analysis of representative components in organic or water-soluble components of PM_{2.5}.

Organic components			
PAHs (ng/m³)	Abbreviation	TEF^a	Week 1-16
Benzo[a]anthracene	BaA	0.1	4.36
Benzo[a]pyrene	BaP	1	8.43
Dibenzo[a,h]anthracene	DBahA	1	2.66
∑16PAHs			87.58
nitro-PAHs (ng/m³)	Abbreviation	TEF^a	Week 1-16
1-Nitropyrene	1-NPyr	0.1	0.68
6-Nitrochrysene	6-NC	10	0.57
∑nito-PAHs			18.49
PCDD/F (pg/m³)	Abbreviation	I-TEF^b	Week 1-16
2,3,7,8-Tetrachlorodibenzofuran	2,3,7,8-TCDF	0.1	0.21
2,3,7,8 -Tetrachlorodibenzo-p-dioxin	2,3,7,8-TCDD	1	0.04
1,2,3,7,8-Pentachlorodibenzo-p-dioxin	1,2,3,7,8-PeCDD	0.5	0.06
∑PCDF			4.29
∑PCDD			0.95
∑PCDF+PCDD			5.24
PCB (pg/m³)	Abbreviation	TEF^c	Week 1-16
3,3',4,4'-Tetrachlorobiphenyl	PCB 77	0.0001	0.22
2,3',4,4',5-Pentachlorobiphenyl	PCB 118	0.00003	0.16
3,3',4,4',5-Pentachlorobiphenyl	PCB 126	0.1	0.22
2,2',4,4',5,5'-Hexachlorobiphenyl	PCB 153		0.18
∑ PCB			3.08
Water-soluble components			
Metal element (ng/m³)			Week 1-16

Cr	0.12
Mn	1.25
As	1.18
Cd	0.08
Pb	0.25
Σ Metal	1050.80
Anion (ng/m³)	Week 1-16
SO ₄ ²⁻	2344.53
NO ₃ ⁻	2095.91
Σ Anion	4939.55

Note: TEF^a, Toxic Equivalency Factor; **I-TEF^b**, International Toxicity Equivalency Factor for PCDD/F; TEF^c, 2005 World Health Organization re-evaluation of human and mammalian toxic equivalency factors (TEFWHO-05) for PCB.

*The analysis results of representative component in PM_{2.5} was from the detailed information of component analysis has been reported previously¹.

Table S4. Top 10 conserved markers of 14 cell clusters used for cell type annotations in scRNA-seq dataset.

Seurat cluster	annotation	Top 10 markers identified by using FindAllMarkers function										Con-H ₂ O	Exp-H ₂ O	Exp-NMN
0	Alveolar macrophages	Lpl	Chil3	Plet1	Ear2	Abcg1	Mrc1	Ctsd	Ear1	Atp6v0d2	Cd9	9724	7249	6022
1	Neutrophils	S100a9	S100a8	Retnlg	Il1b	Csf3r	Slc7a11	Hdc	G0s2	Mmp9	Ifitm1	5104	9867	5011
2	B cells 1	Cd79a	Igkc	Ebf1	Ighm	Ly6d	Cd79b	Ms4a1	Ighd	Cd74	H2-Eb1	5257	3531	4541
3	T cells 2	Trbc2	Ccl5	Il7r	Ms4a4b	Cd3d	Cd3g	Trbc1	Thy1	Cd8b1	Bcl11b	2557	1608	1999
4	Monocyte-derived cells	Apoe	S100a4	Plac8	Ifitm3	Ms4a6c	Cst3	Csflr	Ccr2	Crip1	Ace	1666	1944	1930
5	Dividing DCs	Stmn1	Top2a	Mki67	Tubb5	Pclaf	Birc5	Tuba1b	Ube2c	Rrm2	Cenpf	888	518	681
6	NK cells	Gzma	Ccl5	AW112010	Nkg7	Il2rb	Prfl	Klra4	Serpinb9	Klra8	Gzmb	800	457	684
7	Fibroblasts	Dcn	Mgp	Igfbp5	Col1a2	Col3a1	Sparc	Clec3b	Col1a1	Serping1	Igfbp4	331	771	236
8	B cells 2	Ms4a1	Ebf1	Ly6d	Cd79b	Pax5	Bank1	Ighd	Ralgps2	Cd79a	Iglc2	164	227	159
9	Club cells	Alas2	Snca	Hbb-bt	Bpgm	Fam46c	Hba-a2	Ube2l6	Hba-a1	Ube2o	Fech	25	178	70
10	DCs	Ccl22	Ccl17	Fscn1	Cxcl16	Cacnb3	Tspan3	Basp1	Tbc1d4	Traf1	Ccr7	85	74	73
11	T cells 2	Cd3d	Cd3g	Trbc2	Il7r	Tcf7	Ms4a4b	Ear1	Fabp1	Krt79	Lpl	99	52	58
12	AT2	Reg3g	Lypd2	Sftpd	Tff2	Krt8	Wfdc2	Ces1d	Sftpb	Sftpa1	Aldh1a1	33	50	24
13	AT1	Resp18	Calca	Ascl1	Nnat	Nov	Meis2	Ptn	Pcsk1	Piezo2	Krt8	12	6	7

*Note: Dividing DC, dividing dendritic cells; DC, dendritic cells; AT1, type 1 alveolar epithelial cells; AT2, type 2 alveolar epithelial cells.

Table S5. Summary data (mean, SD, SEM and sample size n) for plots.

Figure 2								
NAD ⁺ (pg/mg protein)	Con				Exp			
	mean	SD	SEM	n	mean	SD	SEM	n
Lung	2715.130	272.529	157.3	3	1554.009	43.772	25.3	3
Liver	3800.026	743.768	429.4	3	2383.558	476.122	274.9	3
Spleen	2138.599	431.343	249.0	3	1132.438	190.839	110.2	3
Bone marrow	2309.985	249.667	144.1	3	1928.191	332.904	192.2	3
ATP (pmol/mg protein)	Con				Exp			
	mean	SD	SEM	n	mean	SD	SEM	n
Lung	44.793	5.346	3.087	3	29.990	2.139	1.235	3
Liver	243.544	23.281	13.440	3	140.564	18.163	10.490	3
Spleen	33.480	5.111	2.951	3	19.797	4.034	2.329	3
Bone marrow	2095.484	450.039	259.800	3	878.646	83.664	48.300	3
NAD ⁺ (pg/mg protein)	H ₂ O-BS				NMN-BS			
	mean	SD	SEM	n	mean	SD	SEM	n
Lung	2785.899	26.435	15.3	3	6767.567	895.232	516.9	3
Liver	2786.401	484.497	279.7	3	5094.216	361.409	208.7	3
Spleen	1745.420	178.327	103.0	3	2561.583	787.746	454.8	3
Bone marrow	1404.772	269.474	279.7	3	3173.774	529.316	208.7	3
Plasma	271.157	43.469	25.1	3	427.359	33.275	19.2	3
ATP (pmol/mg protein)	H ₂ O-BS				NMN-BS			
	mean	SD	SEM	n	mean	SD	SEM	n
Lung	65.137	13.089	7.557	3	128.661	21.442	12.380	3
Liver	286.113	29.790	17.200	3	940.885	46.540	26.870	3
Spleen	34.917	5.059	2.921	3	42.143	8.843	5.106	3
Bone marrow	2366.033	392.652	226.700	3	6693.834	475.500	274.500	3

Figure 3								
ALI scores	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	0.227	0.0461	0.0206	5	0.187	0.0148	0.00660	5
Exp	0.354	0.0423	0.0189	5	0.251	0.0160	0.00714	5
TP (g/L)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	0.486	0.116	0.0517	5	0.460	0.105	0.0467	5
Exp	1.142	0.227	0.102	5	0.686	0.203	0.0907	5
LDH (U/L)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	323.8	32.229	14.41	5	325.4	30.689	13.72	5
Exp	879.6	104.054	46.53	5	585.4	70.436	31.50	5
Total cell number (x10 ⁵)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	3.820	0.920	0.0287	5	3.634	1.221	0.0292	5
Exp	7.650	1.386	0.0477	5	6.222	1.011	0.0354	5
Ashcroft scores	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	0.32	0.0447	0.0316	5	0.32	0.0447	0.0490	5
Exp	0.96	0.1673	0.0748	5	0.40	0.0707	0.0316	5
Collagen content (%)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	9.944	0.663	0.297	5	10.918	0.995	0.445	5

Exp	14.679	1.154	0.516	5	10.728	0.700	0.313	5
Hyp concentration (pg/mg protein)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	0.574	0.143	0.0823	3	0.590	0.022	0.0126	3
Exp	0.814	0.040	0.0229	3	0.618	0.020	0.0117	3

Figure 6

Blood MDSCs (% Viable cells)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	18.457	1.186	0.530	5	18.899	3.314	1.382	5
Exp	50.631	6.713	3.002	5	38.070	6.115	2.735	5
BM MDSCs (% Viable cells)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	56.946	1.887	0.844	5	57.928	5.724	2.560	5
Exp	81.370	5.367	2.400	5	57.362	2.452	1.096	5
Lung MDSCs (% Viable cells)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	10.244	1.721	0.770	5	10.461	3.139	1.404	5
Exp	20.142	5.933	2.653	5	12.176	2.955	1.322	5
Spleen MDSCs (% Viable cells)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	1.862	0.666	0.279	5	1.752	0.437	0.196	5
Exp	3.081	0.980	0.438	5	1.688	0.524	0.234	5
Plasma TNF- α (pg/mL)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	8.773	0.267	0.154	3	8.062	1.540	0.889	3
Exp	25.052	0.905	0.523	3	11.884	2.155	1.244	3
Plasma IL-1 β (pg/mL)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	19.563	1.126	0.650	3	19.149	1.199	0.692	3
Exp	25.310	1.673	0.907	3	19.609	0.484	0.280	3
Plasma IL-17A (pg/mL)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	3.662	0.963	0.556	3	3.682	0.945	0.546	3
Exp	32.515	6.195	3.577	3	14.106	4.960	2.863	3

Figure 7

Hepatic TG (μ mol/mg protein)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	0.350	0.0394	0.0197	4	0.370	0.0041	0.0021	4
Exp	0.536	0.0711	0.0356	4	0.382	0.0781	0.0390	4
Plasma TG (mmol/L)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	0.527	0.0541	0.0270	4	0.495	0.1357	0.0679	4
Exp	0.773	0.0877	0.0439	4	0.576	0.1228	0.0614	4

Figure S1

Oil red O staining (%)	H ₂ O-BS				NMN-BS			
	mean	SD	SEM	n	mean	SD	SEM	n
	0.300	0.152	0.0760	4	1.351	0.135	0.0677	4

Figure S2

Body Weight (g)	H ₂ O-BS				NMN-BS			
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	mean	SD	SEM	n	mean	SD	SEM	n
Week 1	22.35	1.517	0.480	10	21.99	0.937	0.296	10
Week 2	22.94	1.438	0.455	10	22.26	1.051	0.332	10
Week 3	24.52	1.175	0.372	10	24.30	1.735	0.549	10
Week 4	26.02	1.380	0.436	10	24.60	2.177	0.689	10
Week 5	27.05	1.317	0.416	10	25.96	2.248	0.711	10
Week 6	27.11	1.314	0.416	10	26.48	1.922	0.608	10
Week 7	27.26	1.573	0.497	10	27.00	1.892	0.598	10
Week 8	29.28	1.512	0.478	10	27.90	2.085	0.660	10
Week 9	29.65	1.410	0.446	10	27.95	2.312	0.731	10
Week 10	30.98	1.440	0.455	10	29.24	2.162	0.684	10
Week 11	30.83	1.443	0.456	10	29.36	2.045	0.647	10
Week 12	30.96	1.605	0.508	10	29.45	2.082	0.659	10
Week 13	31.53	1.500	0.475	10	29.64	1.659	0.525	10
Week 14	31.56	1.565	0.495	10	30.21	2.042	0.646	10
Week 15	31.57	2.111	0.668	10	30.38	1.579	0.499	10
Week 16	32.73	1.814	0.574	10	31.18	1.826	0.577	10
Week 17	32.60	1.729	0.547	10	31.23	2.199	0.695	10
Week 18	33.30	1.548	0.489	10	31.27	1.639	0.518	10
Average daily water consumption (ml per mouse/day)	H ₂ O-BS				NMN-BS			
	mean	SD	SEM	n	mean	SD	SEM	n
Week 1	4.220	1.575	1.114	10	4.427	0.221	0.156	10
Week 2	4.929	1.515	1.071	10	5.000	1.355	0.958	10
Week 3	4.190	0.539	0.381	10	4.375	0.295	0.208	10
Week 4	4.143	0.471	0.333	10	3.938	0.029	0.021	10
Week 5	5.024	0.572	0.405	10	4.833	0.471	0.333	10
Week 6	4.024	0.101	0.071	10	4.083	0.059	0.042	10
Week 7	3.786	0.168	0.119	10	4.125	0.059	0.042	10
Week 8	4.333	0.471	0.333	10	4.625	0.295	0.208	10
Week 9	5.500	0.236	0.167	10	5.361	0.668	0.472	10
Week 10	3.208	0.413	0.292	10	3.722	0.236	0.167	10
Week 11	4.167	0.000	0.000	10	3.750	0.196	0.139	10
Week 12	3.292	0.530	0.375	10	3.611	0.786	0.556	10
Week 13	3.274	0.084	0.060	10	3.420	0.270	0.191	10
Week 14	4.060	0.859	0.607	10	4.156	0.722	0.510	10
Week 15	4.286	0.842	0.595	10	3.802	0.663	0.469	10
Week 16	3.244	0.463	0.327	10	3.993	0.638	0.451	10
Week 17	4.167	0.000	0.000	10	3.611	0.786	0.556	10
Week 18	3.292	0.530	0.375	10	3.420	0.270	0.191	10
Average daily food intake (g per mouse/day)	H ₂ O-BS				NMN-BS			
	mean	SD	SEM	n	mean	SD	SEM	n
Week 1	3.112	0.057	0.040	10	3.169	0.127	0.090	10
Week 2	3.443	0.061	0.043	10	3.515	0.027	0.019	10
Week 3	3.179	0.091	0.064	10	3.148	0.027	0.019	10
Week 4	3.310	0.088	0.062	10	3.188	0.177	0.125	10
Week 5	3.400	0.168	0.119	10	3.242	0.006	0.004	10
Week 6	3.521	0.172	0.121	10	3.219	0.032	0.023	10
Week 7	3.345	0.111	0.079	10	3.481	0.121	0.085	10
Week 8	3.202	0.280	0.198	10	3.354	0.083	0.058	10
Week 9	4.992	1.261	0.892	10	5.178	1.131	0.800	10
Week 10	4.575	0.106	0.075	10	4.844	1.241	0.878	10

Week 11	4.408	0.083	0.058	10	4.750	0.526	0.372	10
Week 12	4.525	0.035	0.025	10	4.422	0.157	0.111	10
Week 13	4.333	0.165	0.117	10	5.283	0.369	0.261	10
Week 14	3.789	0.416	0.294	10	3.865	0.663	0.469	10
Week 15	3.933	0.775	0.548	10	3.231	1.152	0.815	10
Week 16	3.786	0.296	0.210	10	3.208	0.141	0.100	10
Week 17	4.596	0.006	0.004	10	4.142	0.035	0.025	10
Week 18	4.542	0.165	0.117	10	4.325	0.236	0.167	10

Figure S4								
qPCR in liver tissue (relative fold change)	H ₂ O-BS				NMN-BS			
	mean	SD	SEM	n	mean	SD	SEM	n
Cpt1c	0.936	0.102	0.059	3	1.408	0.179	0.103	3
Acsbg1	1.003	0.083	0.048	3	1.363	0.335	0.194	3
Adipoq	1.007	0.145	0.084	3	2.011	0.133	0.077	3
Slc27a1	0.994	0.167	0.059	3	2.114	0.476	0.275	3
B3gnt5	1.004	0.102	0.059	3	2.426	0.505	0.292	3
Fasted glucose (mg/L)	H ₂ O-BS				NMN-BS			
	mean	SD	SEM	n	mean	SD	SEM	n
	4.86	0.385	0.172	5	5.04	0.321	0.144	5
Hepatic TG (μ mol/mg protein)	H ₂ O-BS				NMN-BS			
	mean	SD	SEM	n	mean	SD	SEM	n
	0.180	0.0084	0.0042	4	0.140	0.0133	0.0067	4
IPGTT % (relative to initial)	H ₂ O-BS				NMN-BS			
	mean	SD	SEM	n	mean	SD	SEM	n
0 min	100.000	0.000	0.000	5	100.000	0.000	0.000	5
15 min	418.500	71.602	32.020	5	328.717	56.580	25.300	5
30 min	417.500	96.420	43.120	5	339.412	44.301	19.810	5
60 min	347.500	107.979	48.290	5	275.195	35.425	15.840	5
90 min	279.500	114.009	50.990	5	231.988	24.493	10.950	5
120 min	247.000	98.050	43.850	5	194.798	27.583	12.340	5
IPGTT AUC	H ₂ O-BS				NMN-BS			
	mean	SD	SEM	n	mean	SD	SEM	n
	38936	4056.0	1814.0	5	27962	1385.00	619.00	5
ITT % (relative to initial)	H ₂ O-BS				NMN-BS			
	mean	SD	SEM	n	mean	SD	SEM	n
0 min	99.763	12.966	7.486	3	101.277	14.766	8.525	3
15 min	55.560	0.000	0.000	3	48.610	7.368	5.210	3
30 min	44.800	0.000	0.000	3	40.510	4.369	2.522	3
60 min	38.230	1.039	0.600	3	35.880	3.612	2.086	3
90 min	46.590	0.000	0.000	3	40.795	3.684	2.605	3
ITT AUC	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
	4435	99.7	57.6	3	4089	180.7	104.3	3

Figure S5								
ALB (g/L)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	0.314	0.064	0.0287	5	0.318	0.065	0.0292	5
Exp	0.469	0.107	0.0477	5	0.394	0.079	0.0354	5
IL-1 β (pg/mg protein)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	855.387	137.845	79.58	3	659.457	189.251	109.30	3

Exp	3108.186	248.241	143.30	3	1153.880	380.308	219.60	3
II1b (relative fold change)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	1.029	0.102	0.059	3	1.121	0.379	0.219	3
Exp	2.221	0.859	0.496	3	1.263	0.093	0.046	4
S100a9 (relative fold change)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	0.937	0.117	0.067	3	0.990	0.014	0.008	3
Exp	2.105	0.725	0.419	3	1.065	0.131	0.076	3
p65 (relative fold change)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	1.104	0.176	0.102	3	1.172	0.115	0.007	3
Exp	1.271	0.252	0.145	3	1.040	0.070	0.040	3
Pulmonary MDA (μ mol/g protein)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	110.169	21.093	12.180	3	99.210	14.086	8.132	3
Exp	162.714	15.599	9.006	3	117.328	6.075	3.507	3
MDA (μ mol/L)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	73.594	0.478	0.276	3	47.717	28.804	15.530	3
Exp	159.109	40.088	23.140	3	60.072	9.889	5.709	3
Tail moment	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	0.113	0.038	0.017	4	0.084	0.026	0.013	4
Exp	0.244	0.097	0.049	4	0.081	0.014	0.007	4
Acta2 (relative fold change)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	1.080	0.118	0.068	3	1.086	0.168	0.097	3
Exp	3.090	0.590	0.341	3	1.807	0.120	0.069	3
Tgfb1 (relative fold change)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	0.904	0.074	0.043	3	1.139	0.212	0.122	3
Exp	1.949	0.162	0.093	3	1.131	0.320	0.185	3
II17a (relative fold change)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	1.066	0.154	0.090	3	1.031	0.189	0.085	3
Exp	2.479	0.990	0.571	3	1.006	0.036	0.021	3
IL-17A (pg/mg protein)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	508.634	72.260	41.720	3	467.485	58.371	33.700	3
Exp	798.078	87.802	50.690	3	566.584	29.922	17.280	3

Figure S8								
Functional assay (proliferation rate %)	H ₂ O				NMN			
	mean	SD	SEM	n	mean	SD	SEM	n
Con	72.760	1.963	0.0287	3	72.387	0.415	0.0292	3
Exp	66.290	0.360	0.0477	3	72.320	1.405	0.0354	3

Table S6. *P*-values and power (1- β) values for results.

Figure 2			
NAD ⁺ (pg/mg protein)	Con vs Exp		
	<i>P</i> -value	summary	power (1- β)
Lung	0.0019	**	0.9993
Liver	0.0499	*	0.5574
Spleen	0.0209	*	0.7868
Bone marrow	0.1872	ns	
ATP (pmol/mg protein)	Con vs Exp		
	<i>P</i> -value	summary	power (1- β)
Lung	0.0112	*	0.9067
Liver	0.0038	**	0.9916
Spleen	0.0220	*	0.7755
Bone marrow	0.0100	**	0.9228
NAD ⁺ (pg/mg protein)	H ₂ O-BS vs NMN-BS		
	<i>P</i> -value	summary	power (1- β)
Lung	0.0164	*	0.9997
Liver	0.0027	**	0.9972
Spleen	0.1550	ns	
Bone marrow	0.0067	**	0.9641
Plasma	0.0078	**	0.9510
ATP (pmol/mg protein)	H ₂ O-BS vs NMN-BS		
	<i>P</i> -value	summary	power (1- β)
Lung	0.0119	*	0.8981
Liver	<0.0001	***	1.0000
Spleen	0.2866	ns	
Bone marrow	0.0003	***	1.0000

Figure 3			
Examinations	<i>P</i> -value	summary	power (1- β)
ALI scores	<0.0001	***	1.0000
TP (g/L)	<0.0001	****	0.9999
LDH (U/L)	<0.0001	****	1.0000
Total cell number (x10 ⁵)	0.0240	*	0.9985
Ashcroft scores	<0.0001	****	1.0000
Collagen (%)	<0.0001	****	1.0000
Hyp content (pg/mg protein)	0.0150	*	0.8371
post hoc tests			
ALI scores	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	<0.0001	****	
Con-H ₂ O vs. Con-NMN	0.2703	ns	
Con-H ₂ O vs. Exp-NMN	0.6545	ns	
Exp-H ₂ O vs. Con-NMN	<0.0001	****	
Exp-H ₂ O vs. Exp-NMN	0.0009	***	
Con-NMN vs. Exp-NMN	0.0340	*	
TP (g/L)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	<0.0001	****	
Con-H ₂ O vs. Con-NMN	0.9951	ns	
Con-H ₂ O vs. Exp-NMN	0.2879	ns	
Exp-H ₂ O vs. Con-NMN	<0.0001	****	
Exp-H ₂ O vs. Exp-NMN	0.0033	**	
Con-NMN vs. Exp-NMN	0.1997	ns	
LDH (U/L)	Adjusted <i>P</i>	summary	

Con-H ₂ O vs. Exp-H ₂ O	<0.0001	****	
Con-H ₂ O vs. Con-NMN	>0.9999	ns	
Con-H ₂ O vs. Exp-NMN	<0.0001	****	
Exp-H ₂ O vs. Con-NMN	<0.0001	****	
Exp-H ₂ O vs. Exp-NMN	<0.0001	****	
Con-NMN vs. Exp-NMN	<0.0001	****	
total cell number (x10 ⁵)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.0356	*	
Con-H ₂ O vs. Con-NMN	0.9998	ns	
Con-H ₂ O vs. Exp-NMN	0.4268	ns	
Exp-H ₂ O vs. Con-NMN	0.0415	*	
Exp-H ₂ O vs. Exp-NMN	0.4744	ns	
Con-NMN vs. Exp-NMN	0.47	ns	
Ashcroft scores	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	<0.0001	****	
Con-H ₂ O vs. Con-NMN	0.9918	ns	
Con-H ₂ O vs. Exp-NMN	0.509	ns	
Exp-H ₂ O vs. Con-NMN	<0.0001	****	
Exp-H ₂ O vs. Exp-NMN	<0.0001	****	
Con-NMN vs. Exp-NMN	0.3571	ns	
Collagen (%)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	<0.0001	****	
Con-H ₂ O vs. Con-NMN	0.3516	ns	
Con-H ₂ O vs. Exp-NMN	0.5314	ns	
Exp-H ₂ O vs. Con-NMN	<0.0001	****	
Exp-H ₂ O vs. Exp-NMN	<0.0001	****	
Con-NMN vs. Exp-NMN	0.9868	ns	
Hyp (pg/mg protein)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.0192	*	
Con-H ₂ O vs. Con-NMN	0.9936	ns	
Con-H ₂ O vs. Exp-NMN	0.8942	ns	
Exp-H ₂ O vs. Con-NMN	0.0272	*	
Exp-H ₂ O vs. Exp-NMN	0.0505	ns	
Con-NMN vs. Exp-NMN	0.9691	ns	

Figure 6

Examinations	<i>P</i> -value	summary	power (1-β)
Blood MDSCs (% Viable cells)	<0.0001	****	1.0000
BM MDSCs (% Viable cells)	<0.0001	****	1.0000
Lung MDSCs (% Viable cells)	0.0021	**	0.9582
Spleen MDSCs (% Viable cells)	0.0158	*	0.8015
TNF-α (pg/mL)	0.0011	**	1.0000
IL-1β (pg/mL)	0.0006	***	0.9988
IL-17A (pg/mL)	0.0008	***	1.0000
post hoc tests			
Blood MDSCs (% Viable cells)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	<0.0001	****	
Con-H ₂ O vs. Con-NMN	0.9989	ns	
Con-H ₂ O vs. Exp-NMN	<0.0001	****	

Exp-H ₂ O vs. Con-NMN	<0.0001	****	
Exp-H ₂ O vs. Exp-NMN	0.0044	**	
Con-NMN vs. Exp-NMN	<0.0001	****	
BM MDSCs (% Viable cells)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	<0.0001	****	
Con-H ₂ O vs. Con-NMN	0.9823	ns	
Con-H ₂ O vs. Exp-NMN	0.9986	ns	
Exp-H ₂ O vs. Con-NMN	<0.0001	****	
Exp-H ₂ O vs. Exp-NMN	<0.0001	****	
Con-NMN vs. Exp-NMN	0.9965	ns	
Lung MDSCs (% Viable cells)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.0037	**	
Con-H ₂ O vs. Con-NMN	0.9997	ns	
Con-H ₂ O vs. Exp-NMN	0.8483	ns	
Exp-H ₂ O vs. Con-NMN	0.0045	**	
Exp-H ₂ O vs. Exp-NMN	0.0194	*	
Con-NMN vs. Exp-NMN	0.8877	ns	
Spleen MDSCs (% Viable cells)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.0542	ns	
Con-H ₂ O vs. Con-NMN	0.994	ns	
Con-H ₂ O vs. Exp-NMN	0.9772	ns	
Exp-H ₂ O vs. Con-NMN	0.0332	*	
Exp-H ₂ O vs. Exp-NMN	0.0247	*	
Con-NMN vs. Exp-NMN	0.9988	ns	
TNF- α (pg/mL)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	<0.0001	****	
Con-H ₂ O vs. Con-NMN	0.923	ns	
Con-H ₂ O vs. Exp-NMN	0.1002	ns	
Exp-H ₂ O vs. Con-NMN	<0.0001	****	
Exp-H ₂ O vs. Exp-NMN	<0.0001	****	
Con-NMN vs. Exp-NMN	0.0418	*	
IL-1 β (pg/mL)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.0017	**	
Con-H ₂ O vs. Con-NMN	0.9729	ns	
Con-H ₂ O vs. Exp-NMN	>0.9999	ns	
Exp-H ₂ O vs. Con-NMN	0.0011	**	
Exp-H ₂ O vs. Exp-NMN	0.0018	**	
Con-NMN vs. Exp-NMN	0.9636	ns	
IL-17A (pg/mL)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.0001	***	
Con-H ₂ O vs. Con-NMN	>0.9999	ns	
Con-H ₂ O vs. Exp-NMN	0.0517	ns	
Exp-H ₂ O vs. Con-NMN	0.0001	***	
Exp-H ₂ O vs. Exp-NMN	0.0023	**	
Con-NMN vs. Exp-NMN	0.0522	ns	

Figure 7			
Examinations	<i>P</i> -value	summary	power (1- β)
Hepatic TG (μ mol/mg protein)	0.0020	**	0.9719
Plasma TG (mmol/L)	0.0120	**	0.8450

Figure S1			
Examination	Con vs Exp		
	<i>P</i> -value	summary	power (1- β)
Oil red O staining (%)	<0.0001	***	1.0000

Figure S2			
Body Weight (g)	H ₂ O-BS vs NMN-BS		
	<i>P</i> -value	summary	
Week 1	>0.9999	ns	
Week 2	0.9998	ns	
Week 3	>0.9999	ns	
Week 4	0.7143	ns	
Week 5	0.9563	ns	
Week 6	>0.9999	ns	
Week 7	>0.9999	ns	
Week 8	0.7555	ns	
Week 9	0.4072	ns	
Week 10	0.3674	ns	
Week 11	0.6602	ns	
Week 12	0.6156	ns	
Week 13	0.239	ns	
Week 14	0.7848	ns	
Week 15	0.9093	ns	
Week 16	0.5705	ns	
Week 17	0.7655	ns	
Week 18	0.1513	ns	
Average daily water consumption (ml per mouse/day)	H ₂ O-BS vs NMN-BS		
	<i>P</i> -value	summary	
Week 1	>0.9999	ns	
Week 2	>0.9999	ns	
Week 3	>0.9999	ns	
Week 4	>0.9999	ns	
Week 5	>0.9999	ns	
Week 6	>0.9999	ns	
Week 7	>0.9999	ns	
Week 8	>0.9999	ns	
Week 9	>0.9999	ns	
Week 10	>0.9999	ns	
Week 11	>0.9999	ns	
Week 12	>0.9999	ns	
Week 13	>0.9999	ns	
Week 14	>0.9999	ns	
Week 15	>0.9999	ns	
Week 16	0.9922	ns	
Week 17	0.9998	ns	
Week 18	>0.9999	ns	
Average daily food intake (g per mouse/day)	H ₂ O-BS vs NMN-BS		
	<i>P</i> -value	summary	
Week 1	>0.9999	ns	
Week 2	>0.9999	ns	

Week 3	>0.9999	ns	
Week 4	>0.9999	ns	
Week 5	>0.9999	ns	
Week 6	>0.9999	ns	
Week 7	>0.9999	ns	
Week 8	>0.9999	ns	
Week 9	>0.9999	ns	
Week 10	>0.9999	ns	
Week 11	>0.9999	ns	
Week 12	>0.9999	ns	
Week 13	0.5997	ns	
Week 14	>0.9999	ns	
Week 15	0.9364	ns	
Week 16	0.9898	ns	
Week 17	0.9994	ns	
Week 18	>0.9999	ns	

Figure S4			
qPCR in liver tissue (relative fold change)	H ₂ O-BS vs NMN-BS		
	<i>P</i> -value	summary	power (1- β)
Cpt1c	0.0165	*	0.8392
Acsbg1	0.1453	ns	
Adipoq	0.0009	***	1.0000
Slc27a1	0.1000	ns	0.8002
B3gnt5	0.0088	**	0.9387
Metabolic alterations	H ₂ O-BS vs NMN-BS		
	<i>P</i> -value	summary	power (1- β)
Fasted glucose (mg/L)	0.4450	ns	
Hepatic TG (μ mol/mg protein)	0.0022	**	0.9875
IPGTT AUC (% relative to initial)	0.0004	***	0.9986
ITT AUC (% relative to initial)	0.0440	*	0.5927

Figure S5			
Examinations	<i>P</i> -value	summary	power (1- β)
ALB (g/L)	0.0240	*	0.7465
IL-1 β (pg/mg protein)	0.0108	*	1.0000
Il1b (relative fold change)	0.0334	*	0.7016
S100a9 (relative fold change)	0.0418	*	0.8434
p65 (relative fold change)	0.4277	ns	
Pulmonary MDA (μ mol/g protein)	0.0042	**	0.9594
MDA (μ mol/L)	0.0025	**	0.9819
Tail moment	0.0022	**	0.8042
Acta2 (relative fold change)	0.0002	***	1.0000
Tgfb1 (relative fold change)	0.0015	**	0.9927
Il17a (relative fold change)	0.0189	*	0.8893
IL-17A (pg/mg protein)	0.0012	**	0.9955
post hoc tests			
ALB (g/L)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.0356	*	
Con-H ₂ O vs. Con-NMN	0.9998	ns	

Con-H ₂ O vs. Exp-NMN	0.4268	ns	
Exp-H ₂ O vs. Con-NMN	0.0415	*	
Exp-H ₂ O vs. Exp-NMN	0.4744	ns	
Con-NMN vs. Exp-NMN	0.47	ns	
IL-1 β (pg/mg protein)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	<0.0001	****	
Con-H ₂ O vs. Con-NMN	0.7856	ns	
Con-H ₂ O vs. Exp-NMN	0.5163	ns	
Exp-H ₂ O vs. Con-NMN	<0.0001	****	
Exp-H ₂ O vs. Exp-NMN	<0.0001	****	
Con-NMN vs. Exp-NMN	0.1609	ns	
Il1b (relative fold change)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.0409	*	
Con-H ₂ O vs. Con-NMN	0.994	ns	
Con-H ₂ O vs. Exp-NMN	0.9007	ns	
Exp-H ₂ O vs. Con-NMN	0.0597	ns	
Exp-H ₂ O vs. Exp-NMN	0.0816	ns	
Con-NMN vs. Exp-NMN	0.9745	ns	
S100a9 (relative fold change)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.0208	*	
Con-H ₂ O vs. Con-NMN	0.9979	ns	
Con-H ₂ O vs. Exp-NMN	0.9732	ns	
Exp-H ₂ O vs. Con-NMN	0.0265	*	
Exp-H ₂ O vs. Exp-NMN	0.0372	*	
Con-NMN vs. Exp-NMN	0.9943	ns	
p65 (relative fold change)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.6294	ns	
Con-H ₂ O vs. Con-NMN	0.9569	ns	
Con-H ₂ O vs. Exp-NMN	0.9645	ns	
Exp-H ₂ O vs. Con-NMN	0.8842	ns	
Exp-H ₂ O vs. Exp-NMN	0.387	ns	
Con-NMN vs. Exp-NMN	0.7719	ns	
Pulmonary MDA (μ mol/g protein)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.0122	*	
Con-H ₂ O vs. Con-NMN	0.8136	ns	
Con-H ₂ O vs. Exp-NMN	0.9362	ns	
Exp-H ₂ O vs. Con-NMN	0.004	**	
Exp-H ₂ O vs. Exp-NMN	0.0265	*	
Con-NMN vs. Exp-NMN	0.5007	ns	
MDA (μ mol/L)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.0134	*	
Con-H ₂ O vs. Con-NMN	0.6106	ns	
Con-H ₂ O vs. Exp-NMN	0.9099	ns	
Exp-H ₂ O vs. Con-NMN	0.0028	**	
Exp-H ₂ O vs. Exp-NMN	0.0058	**	
Con-NMN vs. Exp-NMN	0.9289	ns	
Tail moment	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.0067	**	
Con-H ₂ O vs. Con-NMN	0.9996	ns	
Con-H ₂ O vs. Exp-NMN	0.9975	ns	
Exp-H ₂ O vs. Con-NMN	0.0056	**	
Exp-H ₂ O vs. Exp-NMN	0.0048	**	

Con-NMN vs. Exp-NMN	0.9998	ns	
Acta2 (relative fold change)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.0003	***	
Con-H ₂ O vs. Con-NMN	>0.9999	ns	
Con-H ₂ O vs. Exp-NMN	0.0883	ns	
Exp-H ₂ O vs. Con-NMN	0.0003	***	
Exp-H ₂ O vs. Exp-NMN	0.005	**	
Con-NMN vs. Exp-NMN	0.0913	ns	
Tgfb1 (relative fold change)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.0014	**	
Con-H ₂ O vs. Con-NMN	0.5555	ns	
Con-H ₂ O vs. Exp-NMN	0.582	ns	
Exp-H ₂ O vs. Con-NMN	0.0068	**	
Exp-H ₂ O vs. Exp-NMN	0.0064	**	
Con-NMN vs. Exp-NMN	>0.9999	ns	
Il17a (relative fold change)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.0382	*	
Con-H ₂ O vs. Con-NMN	0.9998	ns	
Con-H ₂ O vs. Exp-NMN	0.9988	ns	
Exp-H ₂ O vs. Con-NMN	0.0339	*	
Exp-H ₂ O vs. Exp-NMN	0.0312	*	
Con-NMN vs. Exp-NMN	>0.9999	ns	
IL-17A (pg/mg protein)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.0029	**	
Con-H ₂ O vs. Con-NMN	0.8667	ns	
Con-H ₂ O vs. Exp-NMN	0.7098	ns	
Exp-H ₂ O vs. Con-NMN	0.0012	**	
Exp-H ₂ O vs. Exp-NMN	0.0109	*	
Con-NMN vs. Exp-NMN	0.3197	ns	

Figure S8			
Examination	<i>P</i> -value	summary	power (1-β)
Functional assay (proliferation rate %)	0.0005	***	0.9992
post hoc tests			
Functional assay (proliferation rate %)	Adjusted <i>P</i>	summary	
Con-H ₂ O vs. Exp-H ₂ O	0.0009	***	
Con-H ₂ O vs. Con-NMN	0.9816	ns	
Con-H ₂ O vs. Exp-NMN	0.9706	ns	
Exp-H ₂ O vs. Con-NMN	0.0014	**	
Exp-H ₂ O vs. Exp-NMN	0.0015	**	
Con-NMN vs. Exp-NMN	0.9999	ns	

Table S7. Corresponding cell number and proportions of 12 cell types from data of Figure 4D.

Cell cluster	Cell number			Cell proportion (%)		
	Con-H ₂ O	Exp- H ₂ O	Exp-NMN	Con- H ₂ O	Exp- H ₂ O	Exp-NMN
Alveolar macrophages	9724	7249	6022	36.36%	27.32%	28.02%
Neutrophils	5104	9867	5011	19.08%	37.19%	23.31%
B cells	5421	3758	4700	20.27%	14.16%	21.87%
T cells	2656	1660	2057	9.93%	6.26%	9.57%
Monocyte-derived cells	1666	1944	1930	6.23%	7.33%	8.98%
Dividing DCs	888	518	681	3.32%	1.95%	3.17%
NK cells	800	457	684	2.99%	1.72%	3.18%
Fibroblasts	331	771	236	1.24%	2.91%	1.10%
Club cells	25	178	70	0.09%	0.67%	0.33%
DCs	85	74	73	0.32%	0.28%	0.34%
AT2	33	50	24	0.12%	0.19%	0.11%
AT1	12	6	7	0.04%	0.02%	0.03%

*Note: Dividing DC, dividing dendritic cells; DC, dendritic cells; AT1, type 1 alveolar epithelial cells; AT2, type 2 alveolar epithelial cells.

Table S8. Corresponding cell number and proportions of cell sub-clusters of fibroblasts and monocyte-derived cells from data of Figure 7C.

Cell cluster	Cell number			Cell proportion (%)		
	Con-H ₂ O	Exp- H ₂ O	Exp-NMN	Con- H ₂ O	Exp- H ₂ O	Exp-NMN
Monocyte-derived cells						
Monocyte-derived cells 0	518	440	664	31.09%	22.63%	34.40%
Monocyte-derived cells 1	372	513	640	22.33%	26.39%	33.16%
Monocyte-derived cells 2	152	295	97	9.12%	15.17%	5.03%
Monocyte-derived cells 3	148	221	127	8.88%	11.37%	6.58%
Monocyte-derived cells 4	186	147	125	11.16%	7.56%	6.48%
Monocyte-derived cells 5	97	110	67	5.82%	5.66%	3.47%
Monocyte-derived cells 6	96	87	74	5.76%	4.48%	3.83%
Monocyte-derived cells 7	53	52	89	3.18%	2.67%	4.61%
Monocyte-derived cells 8	26	25	42	1.56%	1.29%	2.18%
Monocyte-derived cells 9	18	54	5	1.08%	2.78%	0.26%
Fibroblasts						
Fibroblasts 0	71	136	102	21.45%	17.64%	43.22%
Fibroblasts 1	95	162	24	28.70%	21.01%	10.17%
Fibroblasts 2	2	224	1	0.60%	29.05%	0.42%
Fibroblasts 3	53	45	26	16.01%	5.84%	11.02%
Fibroblasts 4	33	43	37	9.97%	5.58%	15.68%
Fibroblasts 5	35	33	15	10.57%	4.28%	6.36%
Fibroblasts 6	14	58	9	4.23%	7.52%	3.81%
Fibroblasts 7	22	36	7	6.65%	4.67%	2.97%
Fibroblasts 8	6	34	15	1.81%	4.41%	6.36%

Table S9. Corresponding Z-scores of for representative enriched pathways of neutrophils, monocyte-derived cells and fibroblasts from data of Figure 8D.

Ingenuity Canonical Pathways	Z-scores		
	Neutrophils	Monocytes	Fibroblasts
LXR/RXR Activation	1.000	2.236	2.449
IL-17 Signaling	/	-2.236	-1.342
Pulmonary Fibrosis Idiopathic Signaling Pathway	/	-2.236	-2.236
Aryl Hydrocarbon Receptor Signaling	-1.000	/	/
Tumor Microenvironment Pathway	0.000	-1.342	-2.000
ID1 Signaling Pathway	0.000	-1.342	0.816

Supplementary figures

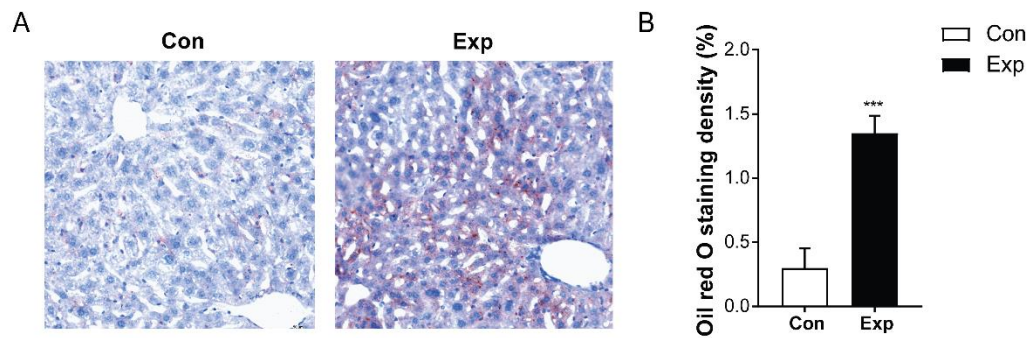


Figure. S1 Effect of PM exposure on lipid accumulation in mouse liver tissue. (A) Representative images of oil red O (ORO)-stained liver sections from air-filtered control group (Con) and PM-exposed group (Exp) following 16-week PM exposure (n = 4 per group). Magnifications: 200X. Scale bar = 50 μm . (B) Hepatic lipid content (%) was calculated and expressed as the ratio of labeled red areas to total area of liver section ($\%/\mu\text{m}^2$ total area) examined by ORO staining (n = 4 per group). Data was analyzed using Student's *t*-test. The data are expressed as mean \pm 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.

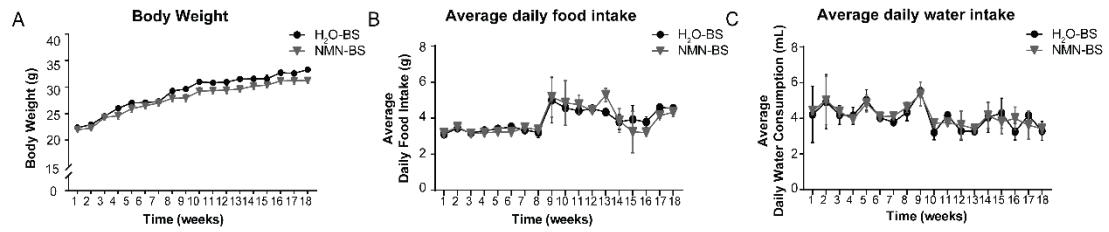


Figure S2 Body weights, average daily food intake and average daily water intake of NMN-treated mice without being exposed to PM. (A) Weekly body weights monitored in mice from H₂O-BS and NMN-BS groups (n = 10 per group). (B) Average daily food intake monitored every 3 days and calculated in H₂O-BS and NMN-BS groups (n = 10 per group). (C) Average daily water consumption recorded every 3 days and calculated in H₂O-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.

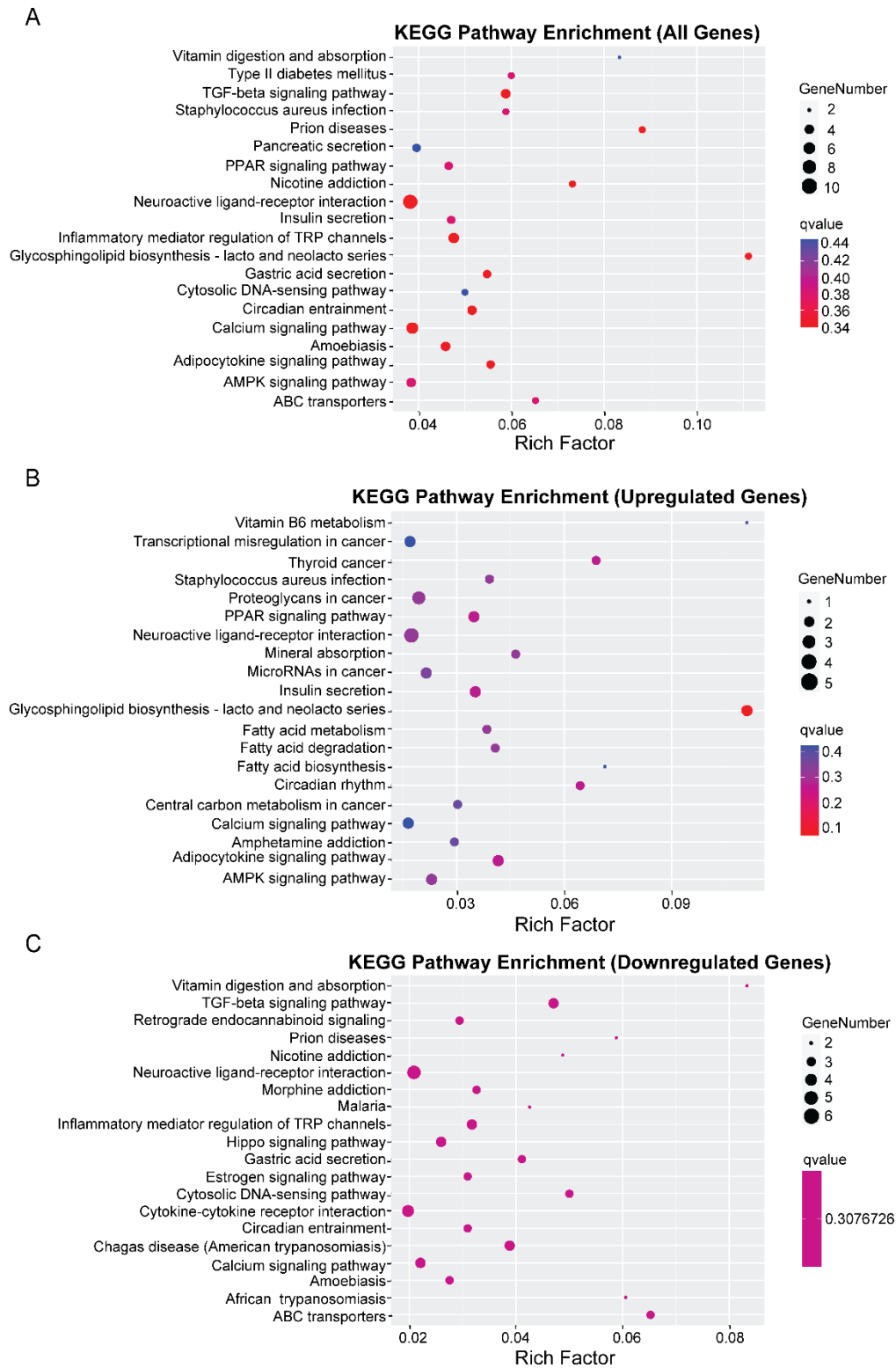


Figure S3 Annotations of enriched pathways in mouse liver tissue of H₂O-BS and NMN-BS groups. Pathway enrichment analysis based on all DEGs (A), upregulated DEGs (B) and downregulated DEGs (C), by using KEGG pathway enrichment programs. DEGs: differentially expressed genes; KEGG: Kyoto Encyclopedia of Genes and Genomes. The pathway analysis in this figure is listed in **Excel Table S3**.

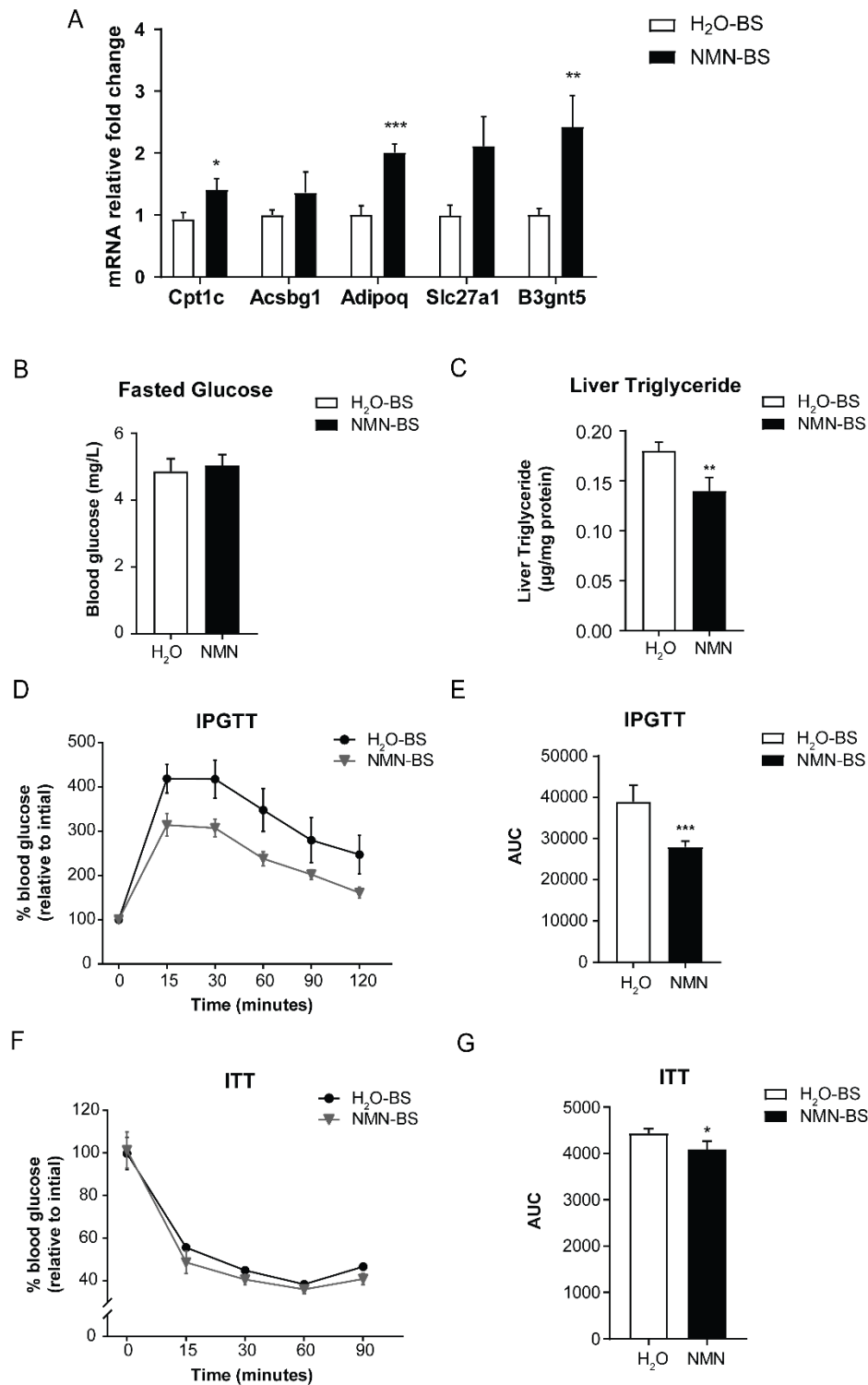


Figure S4 Metabolic alterations in mice following 18-week oral NMN supplementation. (A) The relative mRNA expression levels of Cpt1c, Acsbg1, Adipoq, Slc27a1 and B3gnt5 in mouse liver tissue of H₂O-BS and NMN-BS groups (n = 3 per group). (B) Fasted glucose levels measured in mouse tail vein blood (n = 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 \pm 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.

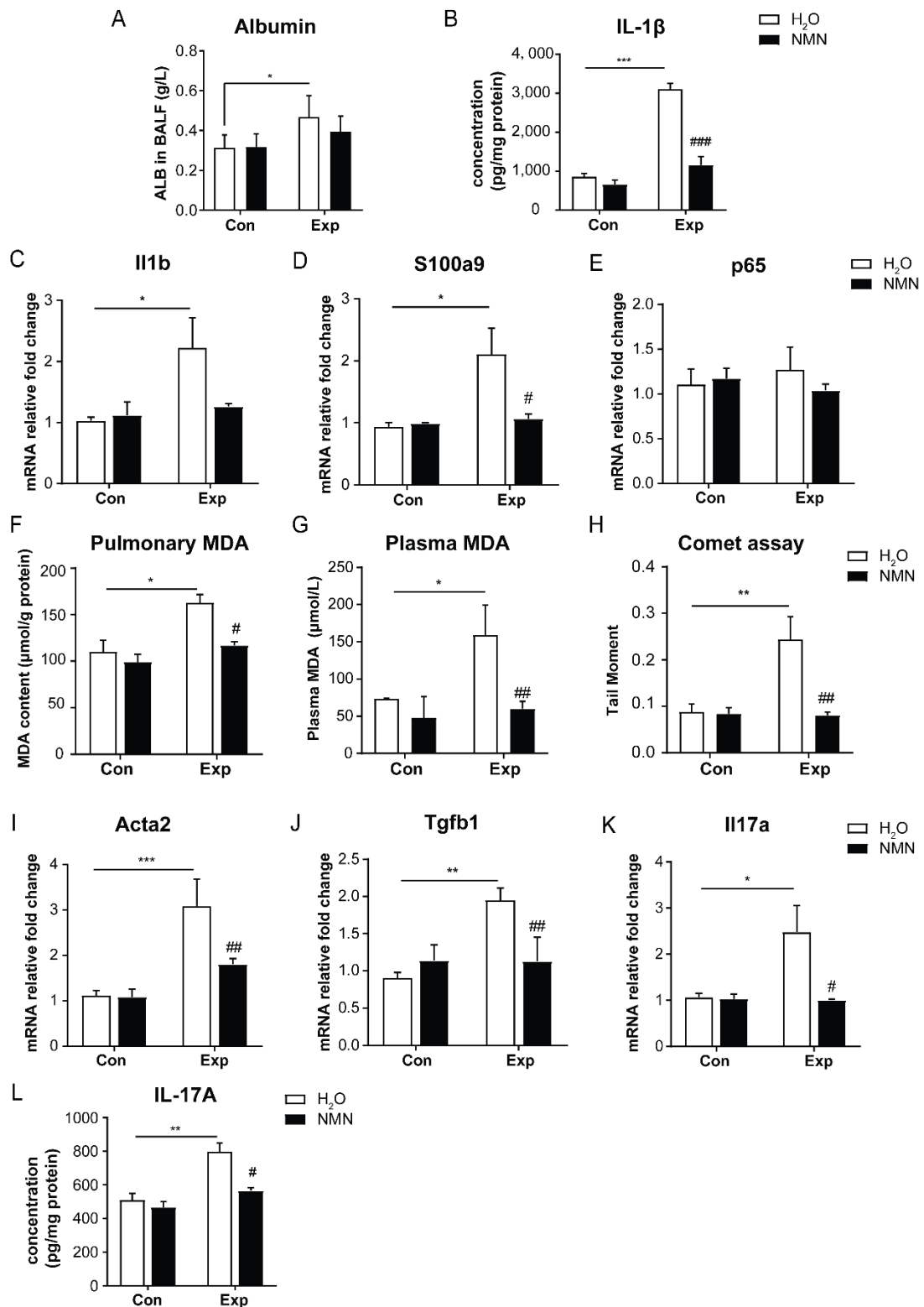


Figure S5 Effects of NMN supplementation on oxidative stress, chronic inflammation and pro-fibrotic changes induced by PM exposure. (A) The albumin (ALB) contents examined in the bronchoalveolar lavage fluid (BALF) (n = 5 per group). (B) IL-1 β content measured in lung tissue from mice in Con-H₂O, Exp-H₂O, 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-H₂O, Exp-H₂O, 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-H₂O, Exp-H₂O, Con-NMN, and Exp-NMN mice. (H) The tail moment of comet assay (DNA damage index) conducted on peripheral blood in Con-H₂O, Exp-H₂O, 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-H₂O, Exp-H₂O, 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-H₂O mice (Exp-NMN vs Exp-H₂O). *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.

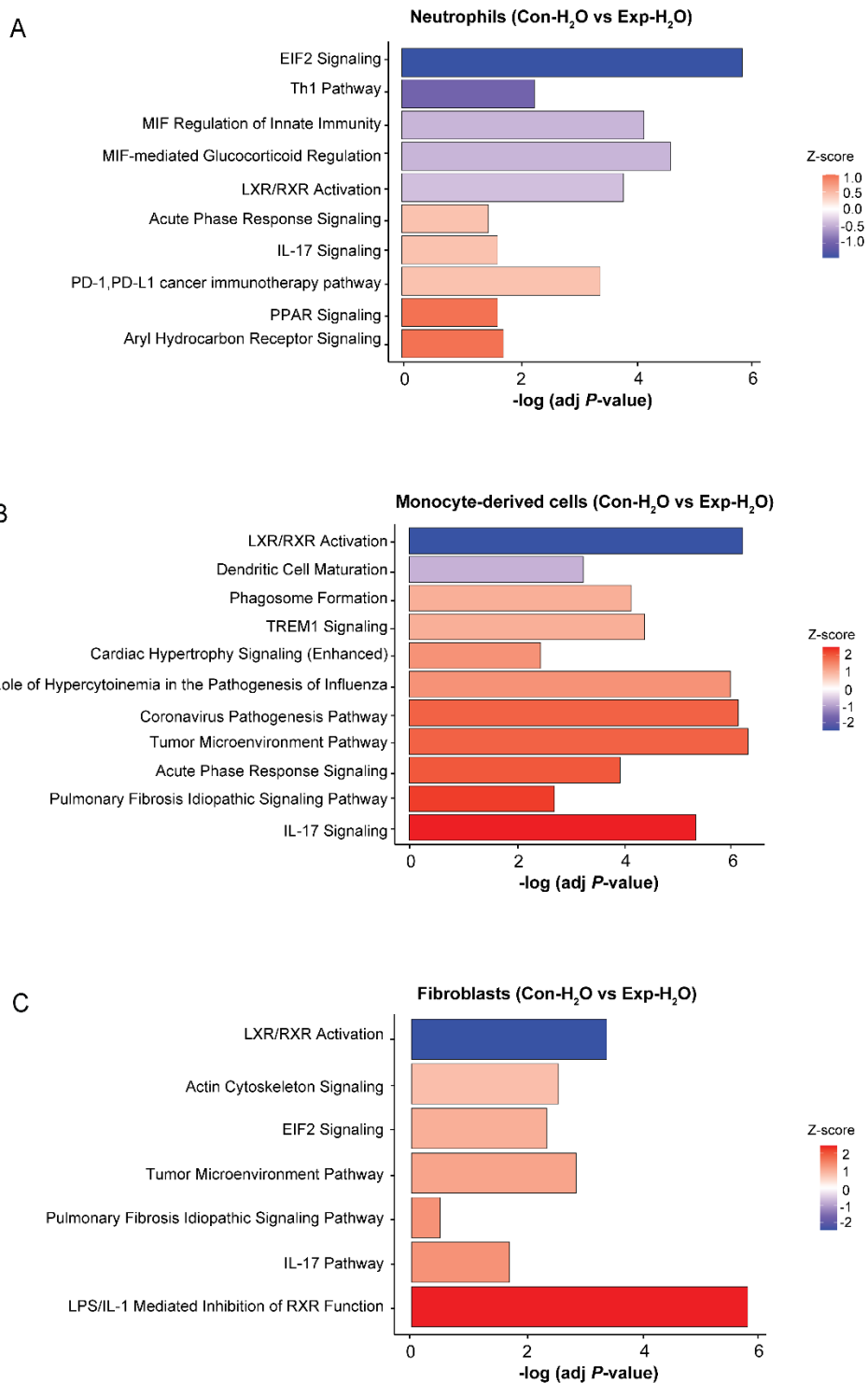


Figure S6 Canonical pathway analysis based on the “PM Exposure DEGs” in neutrophils, monocyte-derived cells, and fibroblasts. (A-C) Enrichment analysis was carried out by canonical pathway analysis on DEGs ($\log_2|FC|>0.25$, $P<0.05$) identified in neutrophils (A), monocyte-derived cells (B), and fibroblasts (C) between Con-H₂O and Exp-H₂O 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**.

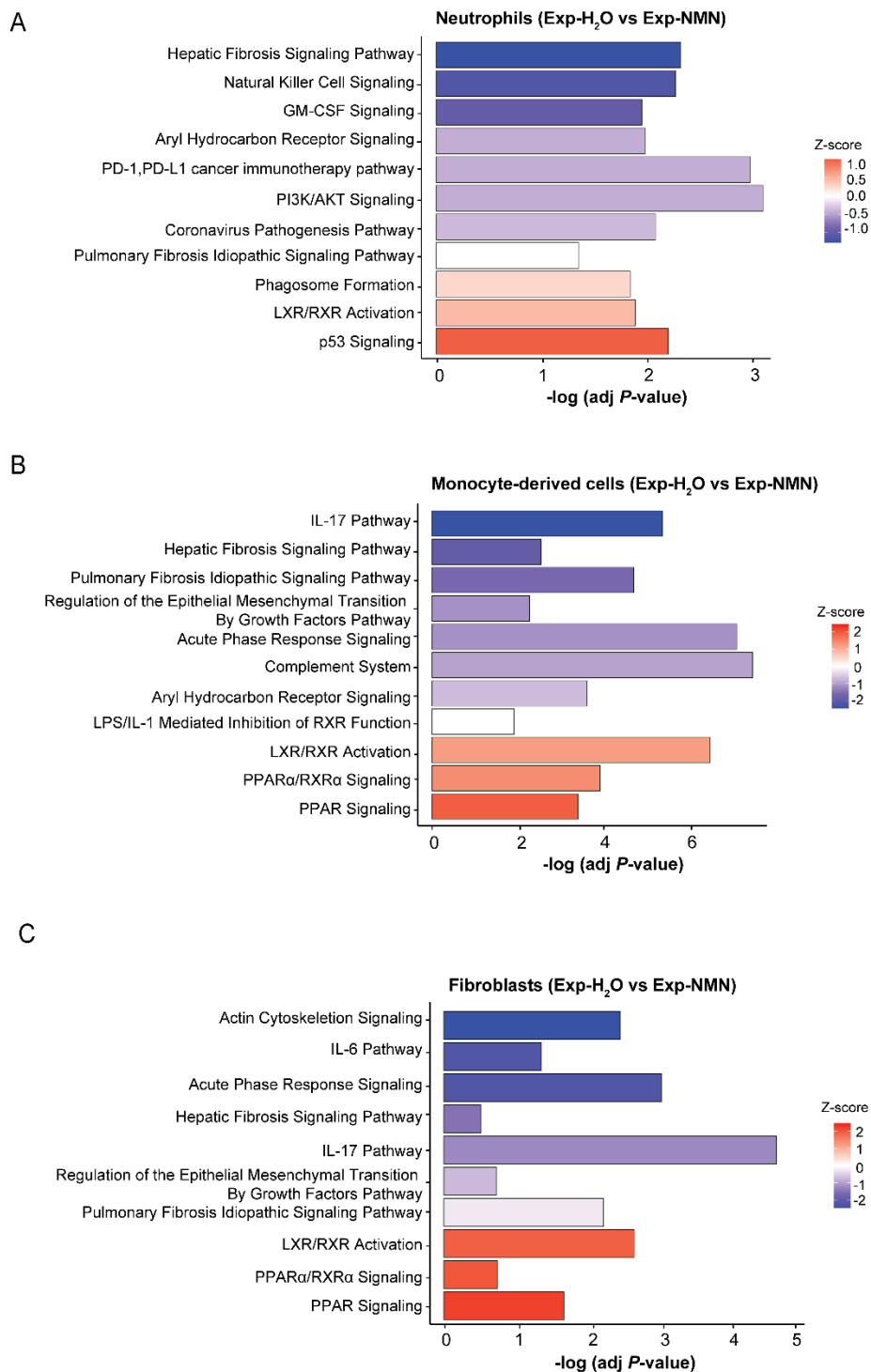


Figure S7 Canonical pathway analysis based on the “NMN Treatment DEGs” in neutrophils, monocyte-derived cells, and fibroblasts. (A-C) Enrichment analysis was carried out by canonical pathway analysis on DEGs ($\log_2|FC| > 0.25$, $P < 0.05$) identified in neutrophils (A), monocyte-derived cells (B), and fibroblasts (C) between Exp-H₂O 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**.

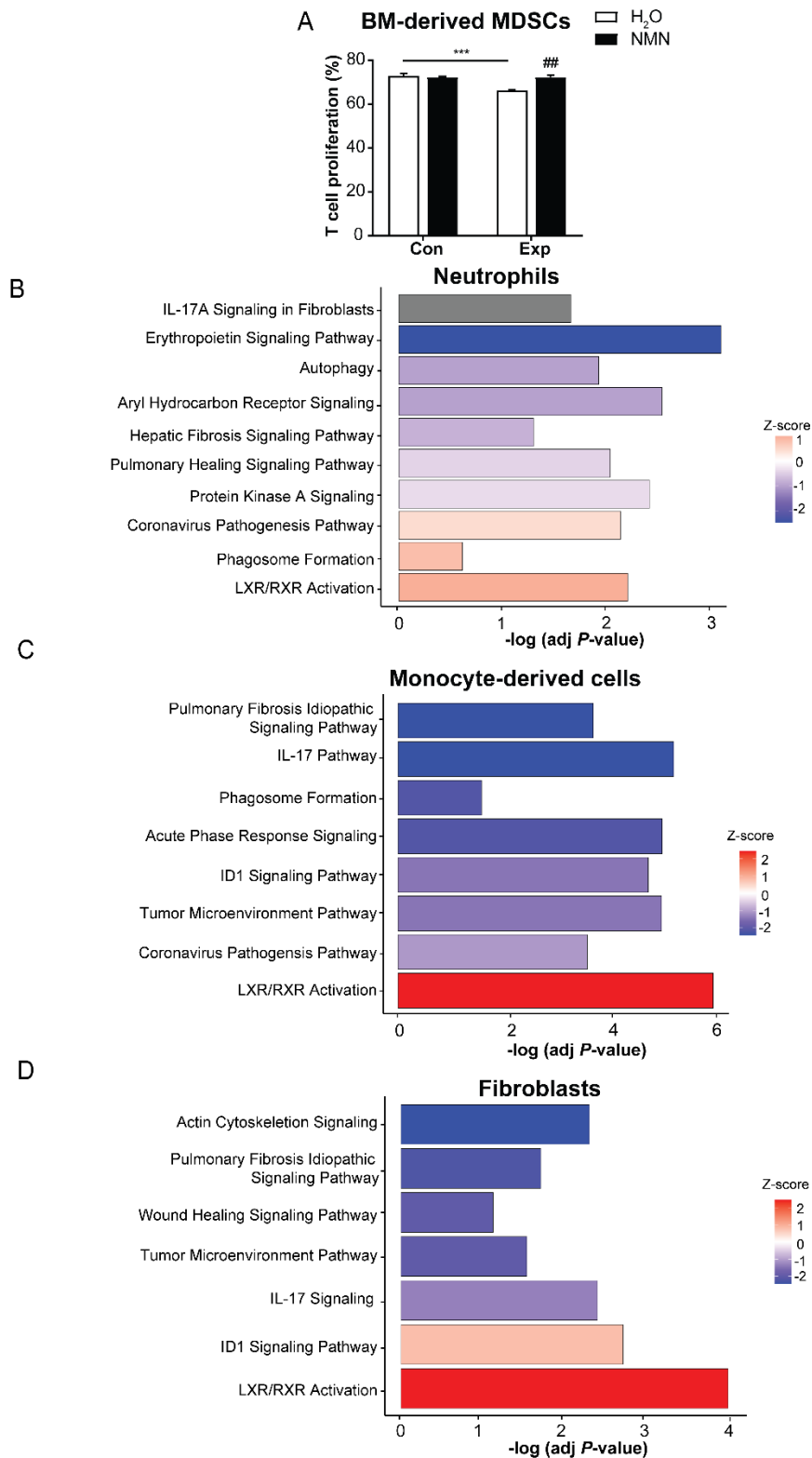


Figure S8 Effects of NMN supplementation on immune functions in mouse lung tissue of Con-H₂O, Exp-H₂O, Con-NMN and Exp-NMN groups. (A) Immunosuppressive activity of BM-derived MDSCs derived from Con-H₂O, Exp-H₂O, Con-NMN, and Exp-NMN mice indicated by T cell proliferation (%) after coculturing at the 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 \pm SD. The summary data (mean, SD and SEM values) of bar graph in this figure is shown in **Table S5**. *** $P < 0.001$. ## $P < 0.01$ compared with the Exp-H₂O mice (Exp-NMN vs Exp-H₂O). 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**.

Reference:

1. Zhang R, Chen S, Chen L, Ye L, Jiang Y, Peng H, Guo Z, Li M, Jiang X, Guo P, Yu D, Zhang R, Niu Y, Zhuang Y, Aschner M, Zheng Y, Li D, Chen W. 2022. Single-cell transcriptomics reveals immune dysregulation mediated by IL-17A in initiation of chronic lung injuries upon real-ambient particulate matter exposure. *Part Fibre Toxicol* 19 (1):42. PMID: 35739565, 10.1186/s12989-022-00483-w.