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Supplemental Material

Pathological Cardiopulmonary Evaluation of Rats Chronically Exposed to Traffic-Related Air Pollution

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Figure S1. Animal exposure protocol. At the age of 28 days, 24 rats (12 male and 12 female) were randomly divided into two groups and exposed to traffic-related air pollution (TRAP) or filtered air (FA) continuously 24 hours a day, 7 days a week, for a total of 14 months in an exposure facility adjacent to a heavily trafficked tunnel in Northern California. Air was collected from the eastbound exit of the tunnel bores continuously without any modification for the TRAP group, while ambient air was drawn from outside near the facility and passed through filters to remove particles, activated charcoal to remove organic compounds and BaO to remove NO_2 before being delivered to the FA exposure chambers. Concentrations of $PM_{2.5}$ in $\mu g/m^3$ were determined in the 14 months of exposure and calculated using a gravimetric method by weighing the PM samplers pre- and post-exposure. Note: FA, filtered air; TRAP, traffic-related air pollution; PND, postnatal day.

Figure S2. Carbon percent total $PM_{2.5}$ mass concentrations and mass concentration of $PM_{2.5}$ Thermal optical reflectance determined elemental and organic carbon (EC/OC) analysis results for FA and TRAP. ***Left Axis*:** Carbon mass concentration of $PM_{2.5}$ in $\mu g/m^3$ ***Right Axis:*** Total concentration average percentage of $PM_{2.5}$; error bars ± SEM (Filtered Air: FA, traffic-related air pollution: TRAP) Note: EC, elemental carbon, OC, organic carbon, OM, organic matter. N=10. Two-way analysis of variance (ANOVA) followed by Sidak's multiple comparisons test was used for statistical analysis. Mean ± SEM. The exact mean and SEM values for data presented in Figure S1 can be found in Table S12.

Figure S3. Expression of fibrosis-related genes were examined in the lung tissues by qRT-PCR Fold change of fibrosis-related genes in lungs from both female and male rats exposed to FA or TRAP. Note: FA, filtered air; TRAP, traffic-related air pollution; F, female; M, male. Mean ± SEM; n=6; Two-way analysis of variance (ANOVA) followed by Sidak's multiple comparisons test was used for statistical analysis. *P<0.05 compared to FA group in the same sex animals. The exact mean and SEM values for data presented in Figure S1 can be found in Table S13.

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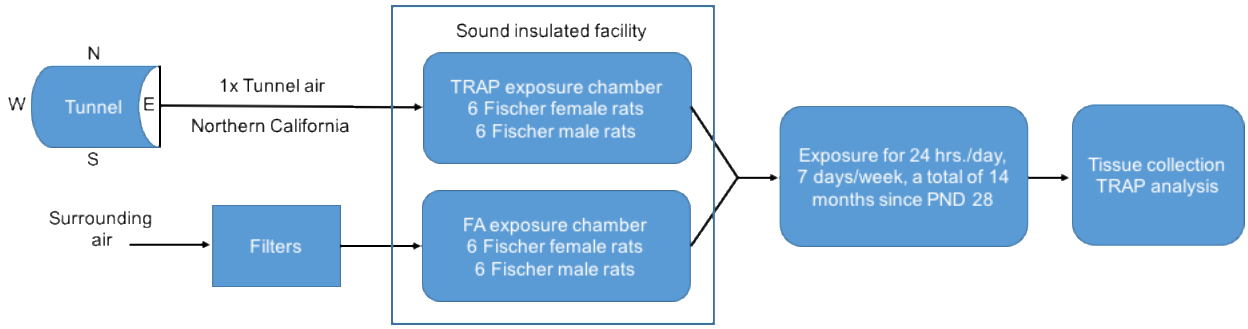
Table S12. Carbon percent total $PM_{2.5}$ mass concentrations and mass concentration of $PM_{2.5}$. Mean and SEM values are the ones presented in Figure S2.

Table S13. Expression of fibrosis-related genes were examined in the lung tissues by qRT-PCR. Mean and SEM values are the ones presented in Figure S3.



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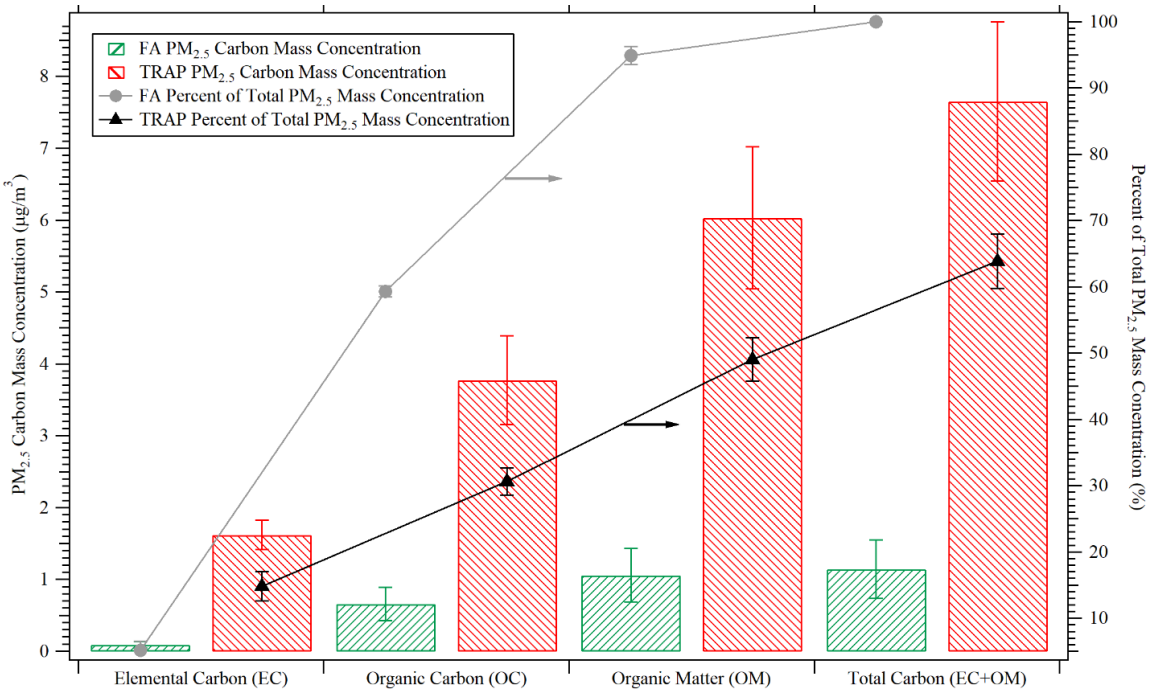

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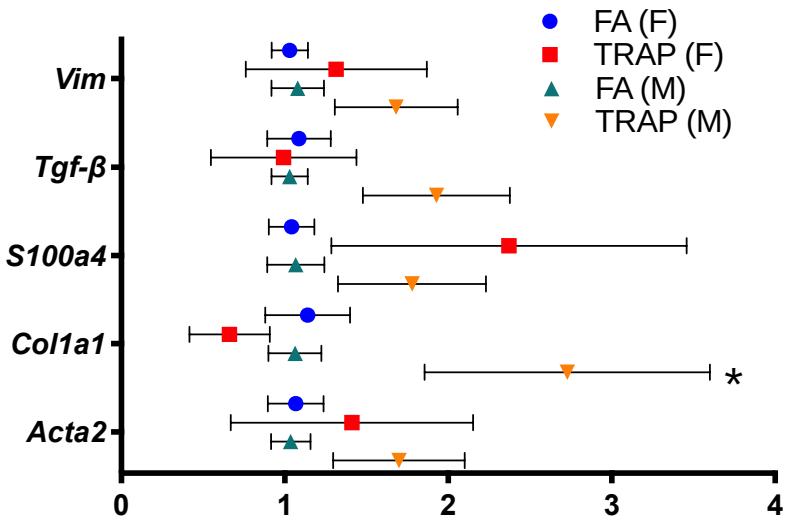

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Table S1. Elemental analysis including detection limit (DL) in the lung tissues after exposure. Mean and SEM values in Figure 4 are also presented in this table.

	FA (F) (µg/l)	TRAP (F) (µg/l)	FA (M) (µg/l)	TRAP (M) (µg/l)	DL (µg/l)
7 Li	UD	UD	UD	UD	0.43
9 Be	UD	UD	UD	UD	0.33
11 B	413.39 ± 128.65	364.58 ± 137.67	263.58 ± 74.37	286.49 ± 67.41	0.92
12 C	1.51e^8 ± 2.33e^7	1.98e^8 ± 8.82e^6	1.89e^8 ± 9.03e^6	1.79e^8 ± 1.03e^7	0.36
14 N	1.93e^9 ± 2.87e^8	1.77e^9 ± 1.56e^8	1.30e^9 ± 8.93e^7	1.53e^9 ± 9.17e^7	$1.4e^7$
23 Na	1.08e^6 ± 1.63e^5	1.25e^6 ± 1.05e^5	1.12e^6 ± 3.68e^4	1.14e^6 ± 5.79e^4	6.40
24 Mg	8.34e^4 ± 9.88e^3	1.04e^5 ± 4.59e^3	9.10e^4 ± 2.90e^3	9.45e^4 ± 2.34e^3	4.50
27 Al	3.9e^3 ± 8.9e^2	2.9e^3 ± 3.8e^2	2.0e^3 ± 1.9e^2	2.3e^3 ± 4.4e^2	28.50
28 Si	UD	UD	UD	UD	0.0
31 P	1.36e^6 ± 1.56e^5	1.75e^6 ± 3.36e^4	1.62e^6 ± 3.95e^4	1.62^6 ± 6.14e^5	2.2
34 S	4.88e^5 ± 6.52e^4	6.88e^5 ± 2.61e^4	5.89e^5 ± 3.06e^4	6.16e^5 ± 0.30e^4	0.0
35 Cl	1.10e^6 ± 1.32e^5	1.07e^6 ± 6.02e^4	1.12e^6 ± 8.07e^4	1.20e^6 ± 1.14e^5	0.0
39 K	9.35e^5 ± 1.32e^5	1.08e^6 ± 6.96e^4	1.01e^6 ± 4.34e^4	1.05e^6 ± 5.46e^4	2.7
43 Ca	1.29e^4 ± 3.55e^3	1.23e^4 ± 3.39e^3	1.73e^4 ± 4.38e^3	1.54e^4 ± 3.26e^3	18
45 Sc	10.10 ± 2.08	20.27 ± 4.98	11.06 ± 2.83	9.08 ± 2.17	0.11
47 Ti	141.66 ± 41.23	116.25 ± 25.56	234.96 ± 67.57	295.51 ± 58.86	0.24
51 V	9.60 ± 1.42	7.15 ± 1.56	7.05 ± 2.01	5.83 ± 1.03	0.04
52 Cr	55.45 ± 22.37	269.35 ± 116.42	26.10 ± 8.06	21.81 ± 5.95	0.30
55 Mn	167.35 ± 23.97	190.78 ± 11.12	213.36 ± 18.15	193.71 ± 3.41	0.11
56 Fe	6.43e^4 ± 1.02e^3	6.43e^4 ± 1.02e^3	6.43e^4 ± 1.02e^3	6.43e^4 ± 1.02e^3	6.6
59 Co	18.31 ± 3.05	16.14 ± 1.83	21.83 ± 6.56	16.11 ± 1.87	0.02
60 Ni	7.03 ± 1.67	84.38 ± 30.61	12.84 ± 4.80	41.49 ± 31.88	0.01
63 Cu	1.00e^3 ± 1.46e^2	1.12e^3 ± 15.40	1.09e^3 ± 32.04	1.11e^3 ± 47.89	0.09
66 Zn	1.14e^4 ± 1.58e^3	1.37e^4 ± 3.72e^2	1.24e^4 ± 4.12e^2	1.22e^4 ± 2.35e^2	2.00
69 Ga	14.01 ± 4.66	6.74 ± 1.00	8.00 ± 2.16	13.17 ± 1.96	0.02
72 Ge	UD	UD	UD	UD	0.32
75 As	728.36 ± 180.83	770.72 ± 166.12	440.18 ± 80.57	485.26 ± 71.50	0.03
78 Se	UD	UD	UD	UD	0.32
79 Br	3.08e^3 ± 2.41e^2	3.79e^3 ± 1.95e^2	3.41e^3 ± 3.46e^2	3.65e^3 ± 3.31e^2	0.82
85 Rb	2.84e^3 ± 3.91e^2	3.39e^3 ± 9.38e^1	3.25e^3 ± 7.67e^1	3.25e^3 ± 1.26e^2	0.03

88 Sr	76.24 ± 13.15	69.13 ± 10.31	71.27 ± 11.17	48.47 ± 4.84	0.30
89 Y	UD	UD	UD	UD	0.01
90 Zr	7.23 ± 1.81	5.53 ± 1.99	4.86 ± 1.41	8.75 ± 2.04	0.06
93 Nb	UD	UD	UD	UD	0.01
95 Mo	98.95 ± 18.66	118.37 ± 12.32	141.51 ± 6.83	139.71 ± 7.66	0.08
107 Ag	UD	UD	UD	UD	0.00
111 Cd	UD	UD	UD	UD	0.02
115 In	1.64e^3 ± 6.81e^2	2.06e^3 ± 3.60e^2	3.79e^3 ± 1.25e^3	4.06e^3 ± 1.08e^3	0.01
118 Sn	UD	UD	UD	UD	0.12
121 Sb	16.34 ± 6.32	15.80 ± 3.52	17.98 ± 1.56	17.96 ± 3.80	0.01
127 I	106.96 ± 23.44	116.18 ± 18.41	106.69 ± 14.87	125.43 ± 11.37	0.18
133 Cs	13.31 ± 2.62	9.79 ± 1.91	16.13 ± 2.05	12.87 ± 1.49	0.01
137 Ba	19.03 ± 5.16	34.46 ± 9.82	25.26 ± 6.21	34.73 ± 13.27	0.10
139 La	UD	UD	UD	UD	0.01
140 Ce	2.24 ± 0.83	1.70 ± 0.58	1.46 ± 0.42	1.71 ± 0.39	0.01
141 Pr	UD	UD	UD	UD	0.01
146 Nd	UD	UD	UD	UD	0.01
147 Sm	UD	UD	UD	UD	0.01
153 Eu	UD	UD	UD	UD	0.01
157 Gd	UD	UD	UD	UD	0.01
159 Tb	2.13 ± 0.33	2.53 ± 0.58	1.47 ± 0.22	1.93 ± 0.42	0.01
163 Dy	UD	UD	UD	UD	0.01
165 Ho	UD	UD	UD	UD	0.01
166 Er	UD	UD	UD	UD	0.01
169 Tm	UD	UD	UD	UD	0.01
172 Yb	UD	UD	UD	UD	0.01
175 Lu	2.81 ± 0.94	1.32 ± 0.24	2.08 ± 0.36	1.65 ± 0.36	0.01
178 Hf	7.27 ± 2.12	3.27 ± 1.08	4.93 ± 1.66	7.07 ± 2.41	0.38
181 Ta	UD	UD	UD	UD	0.01

182 W	UD	UD	UD	UD	0.01
185 Re	UD	UD	UD	UD	0.01
208 Pb	4.26 ± 1.38	2.00 ± 0.45	2.89 ± 0.74	1.42 ± 0.33	0.01
209 Bi	UD	UD	UD	UD	0.01
232 Th	4.69 ± 1.67	1.44 ± 0.39	1.56 ± 0.42	1.93 ± 0.38	0.13
238 U	UD	UD	UD	UD	0.01

DL: detection limit; UD: undetected; FA, filtered air; TRAP, traffic-related air pollution; F, female; M, male

Table S2. List of genes analyzed, and their forward and reverse primers.

Gene	Forward Sequence (5'-3')	Reverse Sequence (3'-5')
Sod2	GGACAAACCTGAGCCCTAAG	CAAAAGACCCAAAGTCACGC
Gpx	AATCAGTTCGGACATCAGGAG	GAAGGTAAAGAGCGGGTGAG
Sod1	TGTGTCCATTGAAGATCGTGTG	CTTCCAGCATTTCCAGTCTTTG
Gsr	TGTGAAAATCTACTCGACCGC	ATCTCATCGCAGCCAATCC
G6pd	ACATCCGCAAACAGAGTGAG	CTGTTGAGGTGCTTGTAGGAG
Ho-1	CTTTCAGAAGGGTCAGGTGTC	TGCTTGTTTCGCTCTATCTCC
Nrf2	GCTATTTTCCATTCCCGAGTTAC	ATTGCTGTCCATCTCTGTCAG
Nos2	GGAGCAGGTTGAGGATTACTTC	TCAGAGTCTTGTGCCTTTGG
TNF-α	CTTCTCATTCCTGCTCGTGG	TGATCTGAGTGTGAGGGTCTG
Il-6	AAGCCAGAGTCATTCAGAGC	GTCCTTAGCCACTCCTTCTG
IFN-γ	CCAAGGCACACTCATTGAAAG	TCTGGCTCTCAAGTATTTTCGTG
Mcp-1	GGTCTCTGTCACGCTTCTG	TTCTCCAGCCGACTCATTG
TGF-β	CCTGAGTGGCTGTCTTTTGA	CGTGGAGTACATTATCTTTGCTG
IL-1β	TGCAGGCTTCGAGATGAAC	GGGATTTTGTCGTTGCTTGTC
Nlrp3	GACCTCAACAGACGCTACAC	AGTTCATGCTCCCTTTCCTG
Tlr4	CATGACATCCCTTATTCAACCAAG	GCCATGCCTTGTCTTCAATTG
Tlr9	GCTGTCCTATAACCTCATCGTC	ACCCACATCAAGCACTCG
Acta2	GTGAAGAGGAAGACAGCACAG	ATTCCAACCATCACTCCCTG
Col1a1	AGCATGTCTGGTTTGGAGAG	GTGATAGGTGATGTTCTGGGAG
S100a4	TCCACCTTCCACAAATACTCAG	CAGCTTCGTCTGTCCTTCTC
Vim	CATTGAGATCGCCACCTACAG	AGGAGTGTTCTTTTGGAGTGG
Myh6	CTTCTCCACCTATGCTTCTGC	CAGCTTGTTCAGATTTTCCCG
Myh7	ACAACCCCTACGATTATGCG	CGCCTGTCAGCTTGTAAATG
Atp2a2	TTTGGGCAGGATGAGGATG	TTGTGGGAAGGTTCAACTCG
Adcy5	ATGAAGAGGATGGGCTTTGAG	AAGAGGAACTTTCGGACGTG
Ercc1	GACTCCTGATTATGTGCTGGG	CCGTTCATGGATGTAGTCTGG
Igf1r	AACCCCGAGTATTTCAGCG	GCCACTCCTTCATAGACCATC
Hprt1	GGTGAAAAGGACCTCTCGAAG	GCTTTTCCACTTTCGCTGATG
Act-b	CACTTTCTACAATGAGCTGCG	CTGGATGGCTACGTACATGG

Table S3. Average daily $PM_{2.5}$ and TSP concentrations in FA and TRAP group. Mean and SEM values are the ones presented in Figure 1A.

Day	FA $PM_{2.5}$ ($\mu g/m^3$)	FA TSP ($\mu g/m^3$)	TRAP $PM_{2.5}$ ($\mu g/m^3$)	TRAP TSP ($\mu g/m^3$)
Sunday	0.66 ± 0.37	0.89 ± 0.35	6.95 ± 0.94	11.49 ± 0.85
Monday	0.37 ± 0.22	0.54 ± 0.18	5.08 ± 0.27	9.00 ± 0.66
Tuesday	0.56 ± 0.24	0.64 ± 0.19	7.57 ± 1.01	12.26 ± 2.33
Wednesday	0.32 ± 0.14	0.50 ± 0.22	7.55 ± 1.17	12.34 ± 1.94
Thursday	0.26 ± 0.09	1.73 ± 0.74	9.87 ± 2.58	14.90 ± 2.63
Friday	0.42 ± 0.17	1.11 ± 0.55	8.21 ± 1.87	13.19 ± 2.54
Saturday	0.34 ± 0.09	1.30 ± 0.75	8.07 ± 1.46	12.38 ± 1.57

FA, filtered air; TRAP, traffic-related air pollution; PM, particulate matter; TSP, total suspended particle

Table S4. 24-hour average concentrations of crustal elements and traffic related metals. Mean and SEM values are the ones presented in Figure 1B.

Element	FA $PM_{2.5}$ Mass Fraction	TRAP $PM_{2.5}$ Mass Fraction	FA $PM_{2.5}$ Concentration ($\mu g/m^3$)	TRAP $PM_{2.5}$ Concentration ($\mu g/m^3$)
CRUSTAL ELEMENTS (soil derived minerals/clay forming minerals)				
Na	5.72E-3 ± 3.06E-3	2.48E-2 ± 8.25E-03	5.88E-3 ± 4.05E-3	2.36E-1 ± 7.03E-2
Mg	1.14E-2 ± 7.86E-3	4.23E-3 ± 1.13E-03	1.25E-3 ± 4.66E-4	4.26E-2 ± 1.14E-2
Al	5.80E-3 ± 3.18E-3	3.93E-3 ± 8.81E-4	1.07E-3 ± 2.48E-3	3.87E-2 ± 7.21E-3
Si	6.67E-3 ± 3.30E-3	7.19E-3 ± 2.26E-3	1.58E-3 ± 5.00E-4	6.72E-2 ± 1.52E-2
P	4.36E-5 ± 3.35E-5	3.43E-4 ± 8.95E-5	4.81E-5 ± 3.84E-5	3.39E-3 ± 6.04E-4
S	2.46E-2 ± 2.74E-3	2.95E-2 ± 3.75E-3	2.55E-2 ± 8.81E-3	3.25E-1 ± 3.28E-2
Cl	2.90E-3 ± 2.04E-3	8.34E-3 ± 6.68E-3	3.46E-3 ± 3.33E-3	8.10E-2 ± 6.49E-2
K	6.34E-3 ± 1.70E-3	6.99E-3 ± 1.05E-3	6.56E-3 ± 2.54E-3	7.92E-2 ± 1.25E-2
Ca	2.17E-3 ± 1.48E-3	4.36E-3 ± 7.88E-4	6.96E-4 ± 2.55E-4	4.34E-2 ± 4.48E-3
Fe	1.22E-2 ± 7.92E-3	1.71E-2 ± 3.64E-3	2.83E-3 ± 7.41E-4	1.73E-1 ± 1.95E-2
Ba	9.60E-3 ± 6.49E-3	2.29E-3 ± 5.44E-4	1.46E-3 ± 4.66E-4	2.30E-2 ± 3.09E-3
TRAFFIC RELATED METALS (brake wear/tire wear/asphalt)				
Ti	6.36E-4 ± 3.98E-4	1.12E-3 ± 2.37E-4	1.96E-4 ± 5.67E-5	1.13E-2 ± 1.26E-3
Cr	5.64E-4 ± 3.84E-4	6.89E-5 ± 1.43E-5	6.29E-5 ± 1.71E-5	6.83E-4 ± 6.75E-5
Mn	5.83E-4 ± 3.17E-4	2.09E-4 ± 4.11E-5	1.13E-4 ± 4.84E-5	2.12E-3 ± 2.28E-4
Cu	9.44E-4 ± 4.57E-4	1.33E-3 ± 2.57E-4	1.84E-4 ± 4.46E-5	1.36E-2 ± 1.28E-3
Zn	1.38E-3 ± 7.23E-3	9.85E-4 ± 2.86E-4	3.24E-4 ± 1.10E-4	9.53E-3 ± 1.54E-3
Br	4.42E-4 ± 3.93E-4	2.57E-4 ± 3.18E-5	1.12E-4 ± 4.68E-5	2.80E-3 ± 2.87E-4
Sr	9.92E-5 ± 5.22E-5	2.59E-4 ± 1.25E-4	7.42E-5 ± 4.41E-5	2.90E-3 ± 1.63E-3
Zr	6.02E-4 ± 2.45E-4	5.49E-4 ± 1.38E-4	3.72E-4 ± 1.53E-4	5.27E-3 ± 7.13E-4
Cd	3.21E-4 ± 2.02E-4	4.03E-5 ± 1.94E-5	7.24E-5 ± 3.61E-5	5.76E-4 ± 2.71E-4
Sn	1.39E-3 ± 8.25E-4	2.50E-4 ± 4.73E-5	3.35E-4 ± 1.25E-4	2.70E-3 ± 3.83E-4
Sb	1.15E-3 ± 9.16E-4	1.70E-4 ± 7.43E-5	1.94E-4 ± 1.11E-4	1.56E-3 ± 4.79E-4
Ce	6.52E-4 ± 4.45E-4	1.05E-4 ± 5.80E-5	2.01E-4 ± 1.27E-4	7.92E-4 ± 3.64E-4
Pb	1.72E-4 ± 1.03E-4	7.92E-5 ± 2.01E-5	8.55E-5 ± 3.78E-5	7.38E-4 ± 1.43E-4

FA, filtered air; TRAP, traffic-related air pollution; PM, particulate matter

Table S5. Comparison of body weight and normalized tissue weights in both female and male rats exposed to FA or TRAP. Mean and SEM values are the ones presented in Figure 2.

	FA (F)	TRAP (F)	FA (M)	TRAP (M)
Body weight (g)	258.67 ± 5.44	273.33 ± 5.89	463.50 ± 13.00	501.00 ± 12.30
Lung: % body weight	0.36 ± 0.02	0.36 ± 0.02	0.29 ± 0.01	0.30 ± 0.01
Heart: % body weight	0.29 ± 0.01	0.30 ± 0.01	0.26 ± 0.01	0.25 ± 0.01
Spleen: % body weight	0.18 ± 0.01	0.17 ± 0.01	0.20 ± 0.01	0.23 ± 0.02

FA, filtered air; TRAP, traffic-related air pollution; F, female; M, male

Table S6. Macroscopic view of black nodules in the lungs after chronic exposure. Mean and SEM values are the ones presented in Figure 3.

	FA (F)	TRAP (F)	FA (M)	TRAP (M)
area of nodules/lung (%)	0.03 ± 0.01	0.52 ± 0.04	0.02 ± 0.01	0.48 ± 0.11

FA, filtered air; TRAP, traffic-related air pollution; F, female; M, male

Table S7. Expression of oxidative stress and inflammation-related genes were examined in the lung tissues by qRT-PCR. Mean and SEM values are the ones presented in Figure 5. All gene expressions were normalized by expression of housekeeping gene *beta-actin* and FA group was used as a reference group when calculating the ΔΔCt values. N=6. Two-way analysis of variance (ANOVA) followed by Sidak's multiple comparisons test was used for statistical analysis. *P<0.05 compared to FA group in the same sex animals.

Gene	Full Name	FA (F)	TRAP (F)	P	FA (M)	TRAP (M)	P
G6pd	Glucose 6-phosphate dehydrogenase	1.06 ± 0.14	1.05 ± 0.12	0.96	1.04 ± 0.12	1.12 ± 0.08	0.63
Gpx1	Glutathione peroxidase	1.04 ± 0.12	0.88 ± 0.1	0.32	1.04 ± 0.13	1.16 ± 0.08	0.46
Gsr	Glutathione reductase	1.01 ± 0.07	0.99 ± 0.07	0.79	1.03 ± 0.12	1.14 ± 0.08	0.49
Ho-1	Heme oxygenase I	1.04 ± 0.12	0.94 ± 0.04	0.43	1.05 ± 0.25	1.59 ± 0.19	0.04*
Nos2	Nitric oxide synthases 2	1.02 ± 0.08	1.04 ± 0.11	0.93	1.0 ± 0.14	1.07 ± 0.14	0.73
Nrf2	Nuclear factor erythroid 2-related factor 2	1.02 ± 0.08	0.82 ± 0.04	0.04*	1.0 ± 0.08	1.05 ± 0.09	0.69
Sod1	Superoxide dismutase 1	1.12 ± 0.2	0.84 ± 0.14	0.28	0.96 ± 0.31	1.09 ± 0.2	0.70
Sod2	Superoxide dismutase 2	1.04 ± 0.12	0.70 ± 0.08	0.04*	1.10 ± 0.22	1.09 ± 0.13	0.97
Ifn-γ	Interferon gamma	1.07 ± 0.16	1.21 ± 0.12	0.48	1.0 ± 0.20	0.74 ± 0.15	0.33
Il-1β	Interleukin 1 beta	1.18 ± 0.37	2.06 ± 0.6	0.26	1.0 ± 0.14	0.68 ± 0.17	0.71
Il-6	Interleukin 6	1.15 ± 0.31	1.58 ± 0.30	0.34	1.0 ± 0.20	0.69 ± 0.08	0.18
Mcp-1	Monocyte chemoattractant protein-1	1.16 ± 0.28	0.87 ± 0.09	0.35	1.05 ± 0.15	0.93 ± 0.09	0.97
Nlrp3	NLR family pyrin domain containing 3	1.02 ± 0.07	0.99 ± 0.08	0.83	1.25 ± 0.38	1.23 ± 0.25	0.99
Tlr4	Toll-like receptor 4	1.01 ± 0.07	0.97 ± 0.07	0.66	1.02 ± 0.09	0.94 ± 0.10	0.99
Tlr9	Toll-like receptor 9	1.01 ± 0.07	1.30 ± 0.11	0.06	1.15 ± 0.29	1.65 ± 0.29	0.33
Tnf-α	Tumor necrosis factor alpha	1.03 ± 0.1	0.97 ± 0.12	0.73	1.0 ± 0.07	1.07 ± 0.16	0.71

FA, filtered air; TRAP, traffic-related air pollution; F, female; M, male

Table S8. Expression of genes mediating oxidative stress, inflammation, fibrosis, and aging in female and male hearts of rats exposed to FA or TRAP. Mean and SEM values are the ones presented in Figure 6. All gene expressions were normalized by expression of housekeeping gene *Hprt* and FA group was used as a reference group when calculating the ΔΔCt values. N=6. Two-way analysis of variance (ANOVA) followed by Sidak's multiple comparisons test was used for statistical analysis. *P<0.05 compared to FA group in the same sex animals.

Gene	Full Name	FA (F)	TRAP (F)	P	FA (M)	TRAP (M)	P
G6pd	Glucose 6-phosphate dehydrogenase	1.00 ± 0.22	1.56 ± 0.26	0.12	1.00 ± 0.20	1.03 ± 0.16	0.91
Gpx1	Glutathione peroxidase	1.00 ± 0.19	0.75 ± 0.18	0.36	1.04 ± 0.14	1.41 ± 0.19	0.14
Gsr	Glutathione reductase	1.00 ± 0.08	1.25 ± 0.05	0.02*	1.01 ± 0.07	1.14 ± 0.05	0.18
Ho-1	Heme oxygenase I	1.00 ± 0.25	0.97 ± 0.17	0.40	1.03 ± 0.12	1.57 ± 0.15	0.02*
Nos2	Nitric oxide synthases 2	1.00 ± 0.31	1.32 ± 0.26	0.52	1.07 ± 0.17	1.02 ± 0.21	0.86
Nrf2	Nuclear factor erythroid 2-related factor 2	1.00 ± 0.11	1.33 ± 0.12	0.07	1.02 ± 0.09	1.17 ± 0.09	0.27
Sod1	Superoxide dismutase 1	1.00 ± 0.10	0.89 ± 0.07	0.24	1.09 ± 0.20	1.15 ± 0.24	0.84
Sod2	Superoxide dismutase 2	1.00 ± 0.09	0.94 ± 0.03	0.28	1.01 ± 0.06	1.04 ± 0.10	0.76
Ifn-γ	Interferon gamma	1.00 ± 0.14	2.13 ± 0.28	0.01*	1.02 ± 0.08	1.62 ± 0.24	0.04*
Il-1β	Interleukin 1 beta	1.00 ± 0.34	0.86 ± 0.14	0.71	1.05 ± 0.15	1.50 ± 0.36	0.28
Il-6	Interleukin 6	1.00 ± 0.21	2.26 ± 0.33	0.01*	0.93 ± 0.21	1.42 ± 0.09	0.06
Mcp-1	Monocyte chemoattractant protein-1	1.00 ± 0.11	1.55 ± 0.28	0.09	1.08 ± 0.18	1.93 ± 0.44	0.08
Nlrp3	NLR family pyrin domain containing 3	1.00 ± 0.26	2.33 ± 0.51	0.04*	1.06 ± 0.16	1.88 ± 0.26	0.04*
Tlr4	Toll-like receptor 4	1.00 ± 0.21	1.80 ± 0.21	0.02*	1.01 ± 0.05	1.33 ± 0.12	0.03*
Tlr9	Toll-like receptor 9	1.00 ± 0.31	1.38 ± 0.22	0.33	1.10 ± 0.21	1.55 ± 0.25	0.20
Tnf-α	Tumor necrosis factor alpha	1.00 ± 0.17	1.79 ± 0.23	<0.01*	1.02 ± 0.10	1.52 ± 0.25	0.09
Actα2	Smooth muscle aortic alpha-actin	1.00 ± 0.24	1.99 ± 0.21	<0.01*	1.00 ± 0.14	1.31 ± 0.33	0.63
Col1α1	Collagen, type I, alpha 1	1.00 ± 0.20	1.92 ± 0.32	0.03*	1.08 ± 0.17	1.01 ± 0.15	0.99
S100a4	S100 calcium-binding protein a4	1.00 ± 0.19	1.85 ± 0.32	0.04*	1.02 ± 0.08	1.19 ± 0.19	0.94
Tgf-β	Transforming growth factor-beta	1.00 ± 0.09	1.68 ± 0.22	0.01*	1.03 ± 0.11	1.18 ± 0.10	0.31
Vim	Type III intermediate filament protein	1.00 ± 0.16	1.57 ± 0.21	0.06	1.04 ± 0.12	1.11 ± 0.06	0.99
Adcy5	Adenylate cyclase 5	1.00 ± 0.12	0.99 ± 0.08	0.95	1.01 ± 0.05	1.33 ± 0.17	0.09
Atp2a2	Sarcoplasmic/endoplasmic reticulum calcium	1.00 ± 0.04	1.14 ± 0.10	0.20	1.00 ± 0.04	1.14 ± 0.10	0.24
Ercc1	Excision repair cross-complementation group 1	1.08 ± 0.19	1.63 ± 0.16	<0.01*	1.08 ± 0.15	1.01 ± 0.11	0.74
Igf1r	Insulin-like growth factor-1	1.00 ± 0.15	1.46 ± 0.13	0.04*	1.05 ± 0.12	1.17 ± 0.09	0.44
Myh6	Myosin heavy chain, alpha isoform	1.00 ± 0.12	1.10 ± 0.06	0.38	1.01 ± 0.06	1.38 ± 0.17	0.03*
Myh7	Myosin heavy chain, beta isoform	1.04 ± 0.10	0.76 ± 0.03	0.06	1.01 ± 0.07	1.00 ± 0.08	0.86

FA, filtered air; TRAP, traffic-related air pollution; F, female; M, male

Table S9. Gömöri trichrome staining in hearts from rats exposed to FA or TRAP. Mean and SEM values are the ones presented in Figure 7.

	FA (F)	TRAP (F)	FA (M)	TRAP (M)
Collagen positive area (%)	8.26 ± 0.59	14.27 ± 1.36	10.59 ± 0.81	9.66 ± 0.62

FA, filtered air; TRAP, traffic-related air pollution; F, female; M, male

Table S10. Cytokines/chemokines were tested in the plasma of rats exposed to FA or TRAP by ELISA. Concentration of cytokines in TRAP group were normalized by that in FA group to calculate the fold difference. Mean and SEM values are the ones presented in Figure 8A & 8B.

	FA (F)	TRAP (F)	FA (M)	TRAP (M)
IL-1α	1.00 ± 0.03	1.33 ± 0.17	1.00 ± 0.17	0.76 ± 0.11
IL-1β	1.00 ± 0.03	0.92 ± 0.04	1.00 ± 0.02	1.23 ± 0.10
IL-2	1.00 ± 0.05	0.92 ± 0.04	1.00 ± 0.09	0.93 ± 0.02
IL-4	1.00 ± 0.04	0.95 ± 0.09	1.00 ± 0.06	1.38 ± 0.32
IL-6	1.00 ± 0.05	1.08 ± 0.10	1.00 ± 0.05	1.27 ± 0.23
IL-10	1.00 ± 0.04	0.94 ± 0.08	1.00 ± 0.05	0.88 ± 0.03
IL-12	1.00 ± 0.03	0.96 ± 0.03	1.00 ± 0.06	0.94 ± 0.08
IL-13	1.00 ± 0.02	0.96 ± 0.03	1.00 ± 0.05	1.11 ± 0.05
IFN-γ	1.00 ± 0.13	1.25 ± 0.05	1.00 ± 0.04	1.16 ± 0.15
TNFα	1.00 ± 0.04	1.13 ± 0.05	1.00 ± 0.12	0.85 ± 0.07
GM-CSF	1.00 ± 0.03	1.03 ± 0.04	1.00 ± 0.06	1.05 ± 0.22
Rantes	1.00 ± 0.01	0.97 ± 0.02	1.00 ± 0.12	0.81 ± 0.25

FA, filtered air; TRAP, traffic-related air pollution; F, female; M, male

Table S11. Macrophage and T cell markers were detected in the spleen of rats exposed to FA or TRAP by flow cytometry. Mean and SEM values are the ones presented in Figure 8C & 8D & 8E.

	FA (F)	TRAP (F)	FA (M)	TRAP (M)
M1/total macrophages (%)	12.77 ± 1.37	11.34 ± 0.79	13.60 ± 0.78	16.11 ± 0.65
M2/total macrophages (%)	5.395 ± 0.54	6.20 ± 0.49	10.24 ± 1.88	5.28 ± 0.66
Activated/total T cells (%)	71.74 ± 3.97	69.62 ± 3.19	69.65 ± 1.96	69.12 ± 1.60

FA, filtered air; TRAP, traffic-related air pollution; F, female; M, male

Table S12. Carbon percent total $PM_{2.5}$ mass concentrations and mass concentration of $PM_{2.5}$. Mean and SEM values are the ones presented in Figure S2.

	FA		TRAP	
	$PM_{2.5}$ ($\mu g/m^3$)	**% of total $PM_{2.5}$**	**$PM_{2.5}$ ($\mu g/m^3$)**	**% of total $PM_{2.5}$**
EC	0.09 ± 0.04	5.13 ± 1.36	1.62 ± 0.20	14.84 ± 2.17
OC	0.66 ± 0.23	59.29 ± 0.85	3.77 ± 0.62	30.64 ± 2.06
OM	1.06 ± 0.37	94.87 ± 1.36	6.03 ± 0.99	49.02 ± 3.29
EC+OC	0.75 ± 0.27	64.42 ± 0.51	5.39 ± 0.75	45.47 ± 3.11
EC+OM	1.14 ± 0.41	100 ± 0.38	7.65 ± 1.11	63.86 ± 4.08

EC, elemental carbon; OC, organic carbon; OM, organic matter; FA, filtered air; TRAP, traffic-related air pollution; F, female; M, male

Table S13. Expression of fibrosis-related genes were examined in the lung tissues by qRT-PCR. Mean and SEM values are the ones presented in Figure S3.

Gene	Full Name	FA (F)	TRAP (F)	FA (M)	TRAP (M)
Actα2	Smooth muscle aortic alpha-actin	1.07 ± 0.17	1.41 ± 0.74	1.03 ± 0.12	1.70 ± 0.40
Col1α1	Collagen, type I, alpha 1	1.14 ± 0.26	0.67 ± 0.25	1.06 ± 0.16	2.72 ± 0.87
S100a4	S100 calcium-binding protein a4	1.04 ± 0.14	2.37 ± 1.10	1.07 ± 0.17	1.78 ± 0.45
Tgf-β	Transforming growth factor-beta	1.09 ± 0.20	1.00 ± 0.45	1.03 ± 0.11	1.92 ± 0.45
Vim	Type III intermediate filament protein	1.03 ± 0.11	1.32 ± 0.56	1.08 ± 0.16	1.68 ± 0.38

FA, filtered air; TRAP, traffic-related air pollution; F, female; M, male