Supporting Information

Particulate Matter Exposure History Affects Antioxidant Defense Response of Mouse Lung to Haze Episodes

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A total of 13 pages, 6 tables, 3 figures

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Detailed structure and parameters of the filtration unit

The filtration unit, composed of a HEPA filter (39cm×27cm×2 cm, IKIN HPF35M1120, China), pneumatic sensing system and a DustMate environmental monitor (Turnkey Instruments Ltd., U.K.), was installed between the air inlet and exposure area. When the filtration unit was off, the HEPA filter was beneath the air inlet, but when the filtration was switched on it rose and blocked the air inlet. The on/off state of the filtration unit was controlled either manually or by a pneumatic sensing system according to signals generated by the $PM_{2.5}$ concentration coming from a DustMate (Turnkey Instruments Ltd., U.K.). In the first chamber, the filtration unit was switched off, such that ambient air was provided during the entire pre-exposure phase (whole-air group). In the second chamber, the filtration unit was controlled automatically and was switched on only when the $PM_{2.5}$ concentration exceeded 75 $\mu g/m^3$ (Chinese 24-h Guideline for $PM_{2.5}$). This ensured that mice in the second chamber were exposed only to ambient air with a low level of $PM_{2.5}$ pollution (low-PM group). In the third chamber, the filtration unit was switched on during the entire pre-exposure phase, such that the mice were exposed only to filtered, very clean air (filtered-air group). The HEPA filter had a particle removal capacity of at least 99.97% for particles with a diameter of 0.3 μm, thus including dust, smoke, pollen, pet dander and other allergens. A test run showed that no obvious differences of $PM_{2.5}$ concentrations were found in different positions of the same chamber. The $PM_{2.5}$ concentration differences between the unfiltered-air in the chamber and the ambient air were no more than 5 $\mu g/m^3$.

Prevention of infectious diseases

(1) Three mice per cage was kept in order to keep a suitable feeding density to decrease the incidence of infections.

(2) Drinking water and bedding materials were replaced per 2 ~ 3 days.

(3) The cages, chambers and shed were all disinfected once a week with 1:5000 potassium permanganate solution.

(4) Infected people were forbidden to contact with exposed mice.

$PM_{2.5}$ sampling and preparation

The Chinese city of Nanjing covers an area of ~6600 km^2 and is located in a north subtropical monsoon climate zone. It has an annual mean temperature of 16 ℃ and a mean annual precipitation of 1106 mm. The prevailing wind normally blows from the southeast in summer and from the northwest in winter. Automobiles, electrical power production, electronics, petrochemical, and steel are its five main industries. $PM_{2.5}$ samples were collected from the Xianlin Campus of Nanjing University, which is located in the northern suburbs of Nanjing.

Each filter used in sample collection was conditioned for 48 h in a desiccator at 25 ℃ and 40% relative humidity before and after sampling. The filters were weighed using a microbalance (Mettler-Toledo, Greifensee,

Switzerland). Samples were collected throughout the day (from 8 am in the morning to 8 am of the next morning) from December 9, 2017 to January 20, 2018.

Analyses of trace metals (TM), polycyclic aromatic hydrocarbons (PAH) and persistent free radicals (PFR)

TM analyses. Metal elements in $PM_{2.5}$ were digested using HF and reverse aqua regia (a mixture of HCl and HNO3 in a 1:3 ratio) successively. The As, Cd, Co, Cr, Cu, Ni, Pb and V concentrations in the extracts were determined using inductively coupled plasma mass spectrometry (Elan 9000, PerkinElmer). Elemental concentrations are expressed as the volume-related concentrations (normalized by the volume of air sampled through the filters). Quality control was ensured by analyzing certified reference material SRM 1648a (urban particulate matter). Recovery was within ±10% of the certified values for the studied elements. More details can be found in our previous studies.[1,2]

PAH analysis. After sampling, the filters were stored in a closed, dark chamber at −20 ℃ and PAH were measured within 30 days after filter extraction in a Soxhlet extraction apparatus. The PAH concentrations were determined using a Thermo Trace GC ultra gas chromatograph in connection with a mass spectrometer detector (electron ionization mode and quadrupole analyzer) fitted with a fused silica capillary (30m×0.25mm ID×0.25 μm df) working in simple ion monitoring mode according to the protocol provided by Thermo Scientific. Helium served as the carrier gas, supplied at a constant flow rate of 1 ml/min and the transfer line was heated to 280 ℃. The column temperature was programmed as follows: 40 ℃ (held 2 min), increasing at a rate of 10 ℃/min until 320 ℃ (held 5.5 min). The quantifications were performed using an internal standard method. The limits of detection were determined to be in the range of < 0.00007 to < 0.00026 $\mu g/m^3$. Recovery efficiencies for the 16 kinds of PAHs ranged from 0.802 to 0.911, with an average values of 0.867.

PFR analysis. Two pieces (10 mm×10 mm) were cut from the sample filters and then further cut into small pieces before they were placed in a glass centrifuge tube. The filter pieces were directly analyzed using an electron paramagnetic resonance (EPR) spectrometer (EMX-10/12, Bruck, Germany). The instrument and operating parameters were as follows: center field, 3484 G; microwave frequency, 9.77 GHz; microwave power, 0.63 mW; modulation frequency, 100 kHz; modulation amplitude, 1.0 G; sweep width, 200 G. Radical quantification was conducted by comparing the signal intensity of the C standard in the EPR spectrum. The results were expressed as the radical number of spins in the PM samples ($spins/m^3$).

Biochemical tests for the levels of ROS and NO, as well as the activity of Sod and Cat

The measurements of ROS. ROS were assayed with the method of 2′, 7′-dichloro-dihydrofluorescein diacetate (DCFH-DA) detection, which is the most commonly used and sensitive method for the detection of ROS in the cell.

Specifically, the harvested fresh lung tissues were soaked in a 9× volume of PBS and stored at 4℃ before completing the batch of sampling. Then all of the samples were prepared into single cell suspensions one-off finished by high throughput single cell suspension equipment (SM-12/24/64, Jingxin Institute, China). The single cell suspensions were incubated with 10 μM DCFH-DA for 30 min at 37℃, then centrifuged at 1000 g for 10 min to collect the fluorescence labeled cells. After washed three times with PBS, the labeled single cell were suspended again with a 9× volume of PBS, and the fluorescence intensity were immediately measured using an automatic microplate reader (Multiskan FC, Thermo Fisher Scientific. U.S.A.) with maximum excitation and emission spectra of 495 and 529 nm, respectively. Each batch of the ROS detection was finished on the same day of sampling.

The measurements of NO. The production of nitric oxide (NO) were determined with the method of nitrate reductase, which indirectly evaluated the level of NO through measuring the contents of NO metabolite such as nitrate and nitrite. According to the instructions for the kit provided by manufacturer (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), 5 ml tissue homogenate (10%) was incubated with 0.4 ml mixed reagent containing reagent 1 and reagent 2 (1:1) at 37℃ for 60 min. After adding 0.2 ml reagent 3 and 0.1 ml regent 4, the mixed sample were rested for 40 min at room temperature, then centrifuged at 500 ~ 4000 r/min for 10 min. Another 0.6 ml chromogenic agent composed of reagent 5, reagent 6 and reagent 7 (2.5:1:1) were mixed with 0.8 ml supernatant and absorbance was determined with spectrophotometer (DR3900, HACH, U.S.A.) at 550 nm wavelengths and 50 mm optical path.

The measurement of Sod activity. The total Sod were determined using a Total Superoxide Dismutase Assay Kit (Nanjing Jiancheng Bioengineering Institute, China). According to the instructions for the kit provided by manufacturer (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), 50 μl fresh tissue homogenate (0.5%) was incubated with a mixed reagents contained 0.1 ml reagent 1, 0.1 ml regent 2, 0.1 ml regent 3 and regent 4 at 37℃ for 40 min, then added 2 ml chromogenic agent composed of reagent 5, reagent 6 and glacial acetic acid (3:3:2:1). Absorbance was determined with spectrophotometer (DR3900, HACH, U.S.A) at 550 nm wavelengths and 1cm optical path. Protein concentrations were determined using the Bradford reagent (CW biotech).

The measurement of Cat activity. The Cat activities in lung tissues were determined using a Catalase Assay Kit (Nanjing Jiancheng Bioengineering Institute, China). According to the instructions provided by manufacturer (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), 50 μl fresh tissue homogenate (0.5%) was incubated with a mixed reagents contained 1ml reagent 1, 0.1 ml regent 2 at 37℃ for 1 min, then added 1ml reagent 5 and 0.1 ml reagent 6. Absorbance was determined with spectrophotometer (DR3900, HACH, U.S.A.) at 405 nm wavelengths and 5 mm optical path. Protein concentrations were determined using Bradford reagent (CW biotech).

Table S1. Trace metal (TM) concentration in $PM_{2.5}$ samples during pre-exposure and haze-exposure and limits values of standards

(ng/m^3)

TM	Pre-exposure		Haze-exposure		Limit of NAAQS	Limit of WHO air
	Mean	Range	Mean	Range	(GB3095-2012)	quality guidelines
Cu	169.21	34.47-383.87	288.15	90.70-447.9	/	/
As	7.291	1.078-21.69	14.77	13.78-16.66	6	6.6
Cd	5.664	1.509-15.28	6.073	6.055-6.087	5	5
Co	5.152	2.364-8.953	8.418	7.226-10.70	/	/
Cr	63.10	38.82-108.5	83.41	79.93-86.21	/	/
Ni	19.05	7.796-36.13	29.02	26.79-33.10	/	25
Pb	69.55	22.32-170.7	110.85	86.78-109.7	500	500
V	7.693	1.002-24.93	15.59	13.48-15.03	/	1000

Table S2. The concentrations of polycyclic aromatic hydrocarbons (PAHs) in $PM_{2.5}$ samples during pre-exposure and haze-exposure

(ng/m^3)

PAHs	Pre-exposure		Haze-exposure		Detection limit
	Mean	Range	Mean	Range	
NAP	ND	ND	ND	ND	0.26
ACPY	0.153	ND-10.11	0.67	0.407-0.92	0.13
ACP	ND	ND	ND	ND	0.13
FLU	ND	ND	ND	ND	0.13
PHE	0.29	ND-0.66	1.68	ND -2.63	0.14
ANT	ND	ND	ND	ND	0.10
FLUA	0.28	ND-3.10	1.38	ND-2.63	0.14
PYR	0.11	ND-0.94	0.47	0.21-0.73	0.10
BaA	1.40	0.44-3.99	2.69	1.64-3.10	0.12
CHR	0.96	0.20-3.09	1.58	1.20-1.80	0.10
BbF	2.38	ND -0.77	0.54	0.29-0.87	0.12
BkF	0.48	0.21-0.79	0.54	0.37-0.77	0.12
BaP	0.13	ND-0.32	0.21	0.11-0.22	0.12
InP	0.93	0.44-3.99	1.49	1.05-2.19	0.07
DahA	0.48	ND-1.71	1.36	1.14-1.47	0.13
BghiP	0.42	ND-1.57	1.17	0.87-1.42	0.13
ΣLMW-PAHs(1)	1.04	0.21-4.76	3.99	0.78-2.81	/
ΣMMW-PAHs(2)	2.48	1.07-9.67	4.75	4.62-6.20	/
ΣHMW-PAHs(3)	4.80	0.81-5.64	5.31	3.48-4.93	/
Σ16 PAHs	8.32	3.47-20.39	14.05	8.37-15.80	/

(1) Low molecular weight (LMW)- PAHs, included naphthalene (NAP), acenaphthylene (ACPY), acenaphthene (ACP), fluorine (FLU), phenanthrene (PHE), and anthracene (AMT).
(2) Medium molecular weight (MMW)-PAHs were fluoranthene (FLUA), pyrene (PYR), benzo[a]anthracene (BaA), and chrysene (CHR).
(3) The high molecular weight (HMW)-PAHs were the group of dibenzo [a,h] anthracene (DahA), benzo [b] fluoranthene (BbF), benzo [k] fluoranthene (BkF), benzo (a) pyrene (BaP), indeno [1,2,3,-cd] pyrene (INP), benzo [ghi] p erylene (Bghip).

Table S3. Sequences of the primers used in the quantitative reverse transcription polymerase chain reaction.

mRNA	Accession no.	Nucleotide sequence (5'→3')	Size (bp)
Sod1	NM_011434.1	F: ATGTGACTGCTGGAAAGGACG	200
		R: CGCAATCCCAATCACTCCAC	
Sod2	NM_013671.3	F: TCCCAGACCTGCCTTACGACT	244
		R: CCCTTAGGGCTCAGGTTTGTC	
Sod3	NM_011435.3	F: TTGTTCTACGGCTTGCTACTGG	198
		R: AGCATCCACCTCCCTTCGTC	
Cat	NM_009804.2	F: TTCTTGTTCAGTGACCGAGGGA	131
		R: CCCTGGTCGGTCTTGTAATGG	
Hsp60	NM_001356512.1	F: GTGGTGCAGTGTTTGGAGAAGA	232
		R: GAAAGTTTAGCAAGTCGCTCGTT	
Hsp70	NM_010478.2	F: CCGACAAGGAGGAGTTCGTG	249
		R: ACAGTAATCGGTGCCCAAGC	
Hsp90	NM_008302.3	F: AGGCTATCCCATCACCCTCTATT	117
		R: GGCTTCTCCTCATCCTCCTTATC	
Pmsb4		F: CTCGGCCAGATGGTGATT	
		R: ACGGGCATCTCGGTAGTA	
Pmsd8		F: CTGGCCCGTGACATACTGGA	
		R: TTCCGGCTTCTGCTGCTG	
P62	NM_001290769.1	F: CCTGTGGTGGGAACTCGCTAT	299
		R: TTGGGATCTTCTGGTGGAGCA	
Beclin1	NM_019584.3	F: GCAGCAGTTCAAAGAAGAGGTG	122
		R: TTTTGATGGAATAGGAGCCGC	
Nrf2	NM_010902.3	F: CTGGCTGATACTACCGCTGTTC	208
		R: AGGTGGGATTTGAGTCTAAGGAG	
P38	NM_001168508.1	F: GTGCCCGAACGATACCAGAAC	241
		R: TGAATTCCTCCAGTGACCTTGC	
P21	NM_009429.3	F: CAGCCATGACGAGCTGTTCT	168
		R: CTTTCGGTACCTTCGCCCTC	

Table S4. The levels of reactive oxygen species (ROS) and nitric oxide (NO) in the three groups of mice on pre-exposure termination (PE) and haze exposure termination (HE) , as well as first day (FA-1), third day (FA-3) and seventh day (FA-7) during the filtered-air phase

Index	Groups	Timepoins				
		PE	HE	FA-1	FA-3	FA-7
ROS	Whole-air group	1.000±0.058	1.575±0.044	1.037±0.162	0.764±0.158	1.001±0.093
	Low-PM group	0.994±0.071	2.885±0.045	1.892±0.064	1.283±0.051	0.896±0.088
	Filtered-air group	0.466±0.179	2.738±0.048	2.914±0.051	2.039±0.077	1.274±0.081
NO	Whole-air group	1.400±0.105	1.333±0.121	1.098±0.122	1.144±0.167	1.077±0.225
	Low-PM group	1.146±0.116	1.472±0.270	2.235±0.212	1.707±0.181	1.309±0.159
	Filtered-air group	1.074±0.077	0.985±0.145	1.239±0.054	2.234±0.172	2.788±0.327

Table S5 Relative mRNA expression levels of genes encoding the antioxidant enzymes superoxide dismutase 1–3 and catalase (*Sod1*, *Sod2*, *Sod3* and *Cat*), chaperones (*Hsp60*, *Hsp70* and *Hsp90*) and proteolytic systems (*Pmsb4*, *Pmsd8*, *P62* and *Beclin1*) in the three groups of mice on pre-exposure termination (PE) and haze exposure termination (HE), as well as first day (FA-1), third day (FA-3) and seventh day (FA-7) during the filtered-air phase

Index	Groups	Timepoins				
		PE	HE	FA-1	FA-3	FA-7
Sod1	Whole-air group	1.000±0.101	1.209±0.243	1.145±0.206	1.250±0.161	1.031±0.235
	Low-PM group	1.868±0.184	5.124±0.538	7.989±0.748	3.744±0.177	1.584±0.205
	Filtered-air group	2.550±0.157	5.154±0.638	1.990±0.485	4.227±0.380	10.094±0.789
Sod2	Whole-air group	1.000±0.215	5.019±0.783	1.470±0.532	2.377±0.668	1.773±0.338
	Low-PM group	4.545±0.372	8.675±0.572	11.532±1.139	4.629±0.629	3.621±0.263
	Filtered-air group	6.453±0.422	14.807±1.213	4.182±1.048	13.565±1.179	23.021±1.089
Sod3	Whole-air group	1.000±0.117	3.840±0.142	1.065±0.194	1.369±0.231	0.996±0.158
	Low-PM group	1.616±0.225	4.782±0.579	8.517±0.301	3.172±0.497	1.524±0.179
	Filtered-air group	4.825±0.181	9.892±0.807	1.204±0.479	5.326±0.699	14.418±1.188
Cat	Whole-air group	1.000±0.160	1.370±0.233	1.599±0.302	2.494±0.340	2.103±0.368
	Low-PM group	4.439±0.268	4.130±0.656	15.426±0.433	21.320±1.129	3.599±0.256
	Filtered-air group	5.709±0.494	16.054±1.476	3.774±0.585	20.946±0.917	34.729±1.468
Hsp60	Whole-air group	1.001±0.136	0.982±0.180	0.980±0.205	0.936±0.209	0.882±0.167
	Low-PM group	1.030±0.130	2.343±0.251	3.441±0.514	1.304±0.138	1.070±0.144
	Filtered-air group	1.237±0.063	1.599±0.211	1.005±0.112	1.222±0.272	1.572±0.308
Hsp70	Whole-air group	1.002±0.208	1.519±0.280	1.079±0.156	1.160±0.275	1.196±0.260
	Low-PM group	1.164±0.144	1.102±0.234	1.117±0.284	1.601±0.301	2.979±0.129
	Filtered-air group	2.114±0.193	1.975±0.342	1.297±0.140	1.654±0.390	5.084±0.494
Hsp90	Whole-air group	1.000±0.152	5.549±0.284	0.931±0.339	1.389±0.369	1.116±0.374
	Low-PM group	1.467±0.232	5.827±0.764	8.026±0.349	6.038±0.714	1.496±0.259
	Filtered-air group	3.303±0.267	4.947±0.725	2.285±0.416	4.919±0.987	11.042±0.737
Pmsb4	Whole-air group	1.000±0.216	0.897±0.223	1.471±0.130	2.302±0.229	1.436±0.131
	Low-PM group	4.311±0.149	3.957±0.837	11.184±1.310	9.550±0.684	3.200±0.233
	Filtered-air group	3.539±0.231	11.186±1.206	2.513±0.443	10.860±1.018	24.700±1.697
Pmsd8	Whole-air group	1.003±0.153	0.589±0.254	1.108±0.235	1.714±0.204	1.318±0.201
	Low-PM group	4.533±0.293	4.348±0.632	16.187±0.300	7.381±0.803	2.720±0.318
	Filtered-air group	5.221±0.237	8.299±0.863	1.771±0.381	10.111±0.991	20.377±1.293
P62	Whole-air group	1.000±0.162	1.968±0.354	1.071±0.188	1.353±0.257	1.242±0.180
	Low-PM group	2.353±0.173	4.617±0.492	6.143±0.699	7.418±0.712	2.162±0.116
	Filtered-air group	2.439±0.155	5.373±0.750	1.705±0.344	9.703±1.085	17.623±1.275
Beclin1	Whole-air group	1.000±0.182	2.939±0.316	0.971±0.254	1.367±0.436	0.932±0.347
	Low-PM group	1.780±0.165	4.571±0.438	7.467±0.608	5.444±0.558	1.360±0.315
	Filtered-air group	3.025±0.183	4.053±0.258	1.212±0.119	5.225±0.829	11.247±0.925

Table S6. Relative levels of mRNA expression of *P38*, *Nrf2*, and *P21* in the three groups of mice on pre-exposure termination (PE) and haze exposure termination (HE)

Groups	*P38*		*Nrf2*		*P21*	
	PE	HE	PE	HE	PE	HE
Whole-air group	1.000±0.113	1.542±0.197	1.000±0.192	1.024±0.227	1.000±0.047	0.903±0.068
Low-PM group	1.747±0.145	2.900±0.363	0.982±0.027	2.421±0.290	0.538±0.035	0.468±0.031
Filtered-air group	4.058±0.215	8.125±1.105	1.565±0.247	2.962±0.219	0.278±0.011	1.456±0.076

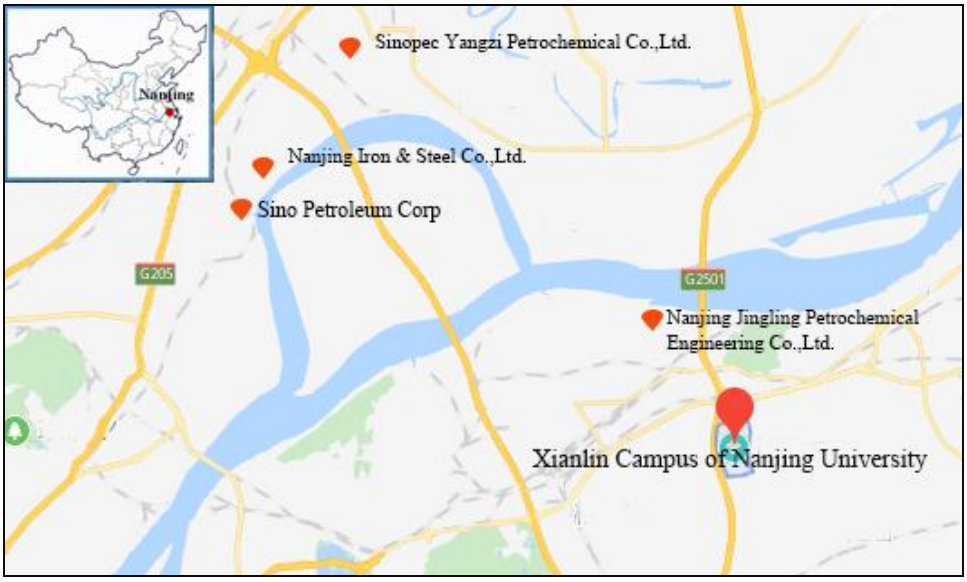


Figure S1. Study area and the nearby industrial point sources of air pollutants

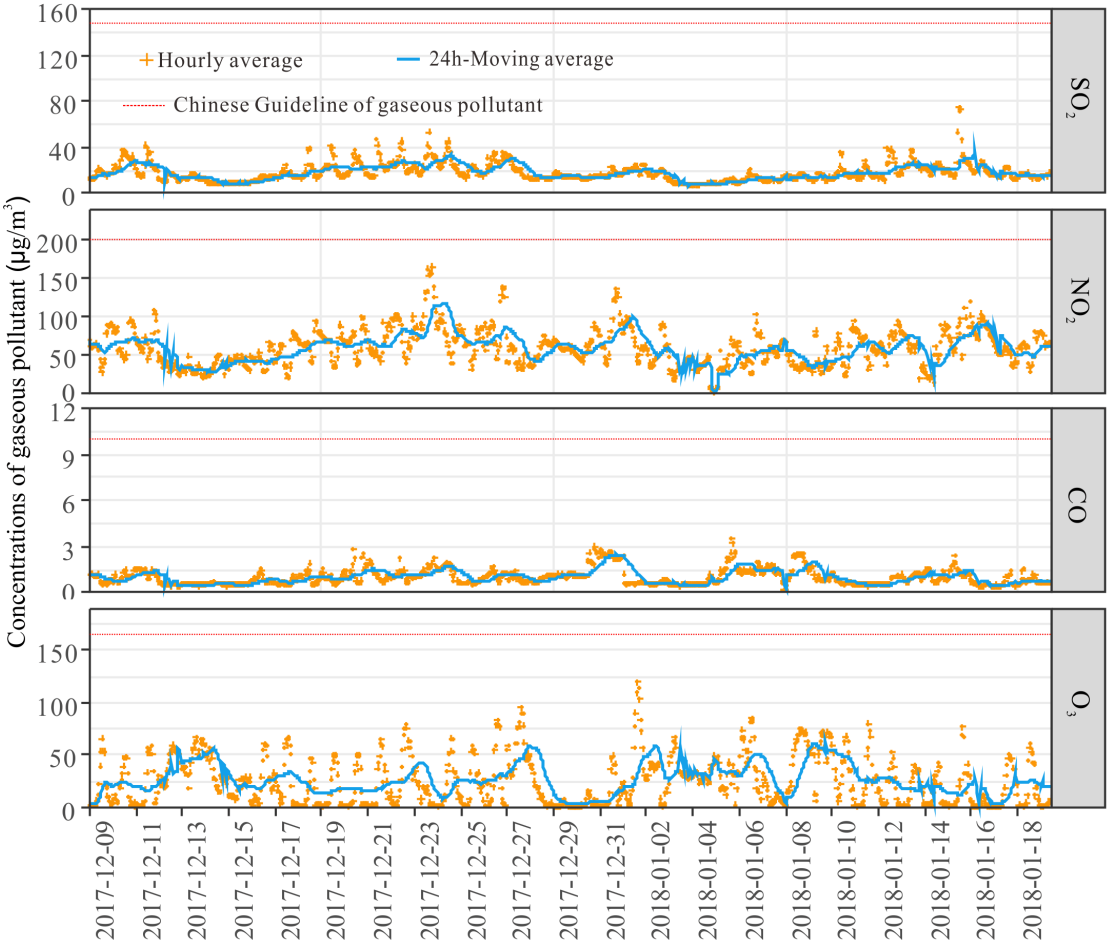


Figure S2. Concentrations of SO_2, NO_2, CO and O_3 in the ambient air during the pre-exposure and haze exposure phases.

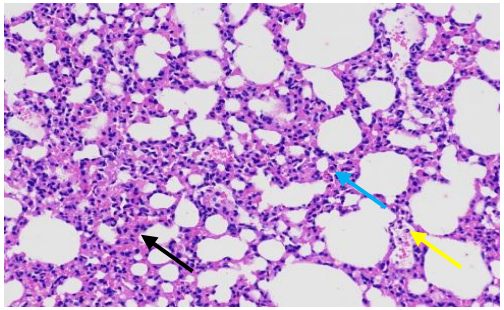
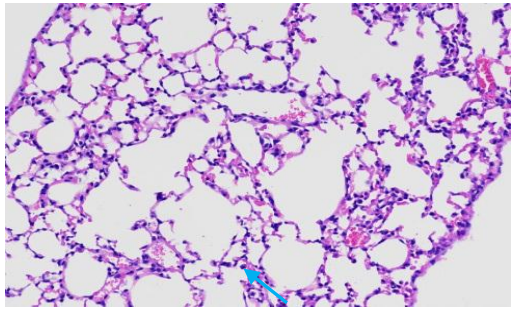
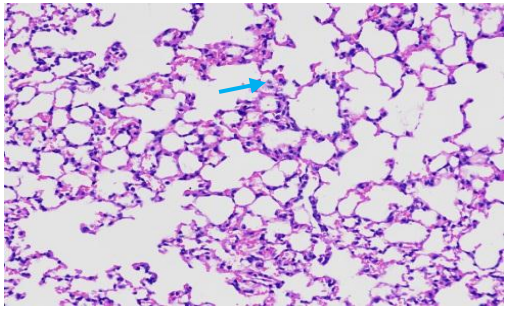
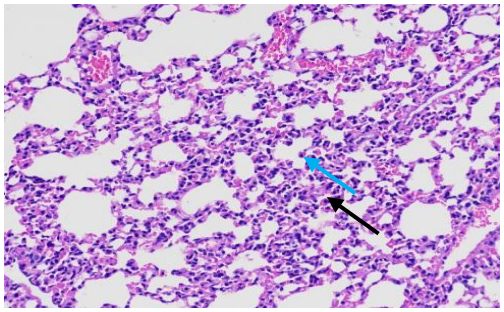
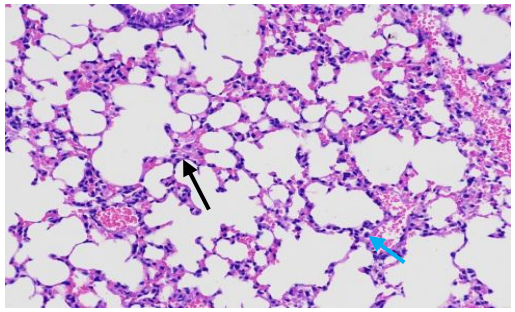
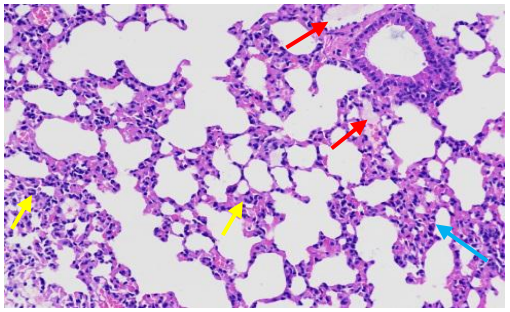
Time	Whole-air group	Low-PM group	Filtered-air group
PE			
HE			

Figure S3. The morphological changes in the lungs of the three groups of mice on the termination of pre-exposure termination (PE) and haze exposure termination (HE). Hematoxylin-eosin staining, ×200. Black arrows label thickened alveolar walls, narrowed alveolar spaces. Blue arrows and yellow arrows label infiltrates of macrophages and neutrophils, respectively. Red arrows label pink effusion in alveolar space.

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