Short term Pm2.5 exposure caused a robust lung inflammation, vascular remodeling, and exacerbated transition from left ventricular failure to right ventricular hypertrophy

Short title: Pm2.5 and heart failure

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Supplementary Material and Methods

Animals and experimental design: To determine the effect of mild short-term PM2.5 exposure (described below) on the progression of transverse aortic constriction (TAC)-induced congestive heart failure (HF), Balb/c background male mice 4-5 weeks of age were subjected to TAC created with a 27G needle. Two weeks after TAC, LV ejection fraction (EF) of these mice was determined and the mice were divided into different experimental groups to assure similar initial LV dysfunction. After the division of the groups, mice were either treated with the local polluted PM2.5 air for 10 hours each day, 7 days each week for total 3 weeks (from April 30th to May 21, 2017) in the research facility at the Beijing Zhongguancun District (the local Campus of the University of Chinese Academy of Sciences; N39°57′39.83″E116°20′10.97″). The daily average PM2.5 concentrations of Beijing and the local monitoring station during the study period are obtained from http://www.pm25.com and http://www.zhb.gov.cn. The PM2.5 concentrations in the research facility were also recorded.

During the entire exposure stage, the mice were fed commercial mouse chow and distilled water ad libitum, and were housed under controlled temperatures (22 ± 2 °C) and relative humidity (40–60%) conditions, with a 12 hour light/dark cycle. Samples were collected 5 weeks after TAC. LV hypertrophy, cardiac function and pulmonary congestion were assessed. All mouse studies were approved by the Institutional Animal Care and Use Committee at TongJi University.

Echocardiography and evaluation of LV hemodynamics: Echocardiographic images were obtained with a Visualsonics Vevo 2100 system as previously described [1].

Sample collection: Mouse heart and lung samples for protein and RNA analysis were flash frozen in liquid nitrogen, weighed and then transferred into a -80 °C freezer. Samples for histological analysis

were fixed in formaldehyde at room temperature and embedded in paraffin blocks.

Western blotting: Western blot analysis was processed as previously described[1]. Protein was extracted from the lung using complete RIPA buffer (10Mm Tris-HCl pH7.4, 150Mm NaCl,1%NP40, 0.1% sodium dodecyl sulfate(SDS) and 1× protease inhibitor cocktail(Roche). Equal amounts of soluble protein were separated on 10% polyacrylamide gels, transferred onto Polyvinylidene difluoride (PVDF) membranes followed by routine Western blot analysis. Antibody against Vcam1 and Icam1 were purchased from R&D Systems, Gapdh was from Santa Cruz Biotechnology, Inc..

Histological staining: Sections of 5 μm thickness were sliced. Immunostaining was conducted to identify leukocytes ($CD45^+$cells) and macrophages ($Mac2^+$cells) in mouse lung. Immunostaining of Vcam1 and Icam1 was used to detect the expression levels of lung adhesion molecules. Immunostaining of α-smooth muscle actin and CD31 co-staining was used to detect the degree of vascular muscularization. Antibody against CD45, Mac2, Vcam1 , Icam1, α-smooth muscle actin and CD31 were from R&D Systems, Cedarlane laboratories, Santa Cruz Biotechnology, Inc. or Millipore. 4', 6'-diamidino-2-phenylindole (DAPI) was from Southern Biotech. Antibody staining was visualized by using a secondary Alexa Fluor 549-conjugated antibody or FITC-conjugated antibody (Jackson ImmunoResearch Laboratories, Inc. and Abcam). The staining of the lung was quantified from 5 random fields per slide. Lung fibrosis was identified using Masson's Trichrome Stain Kit from Sigma-Aldrich. Relative tissue fibrosis was calculated by dividing the area of red staining by the total measured section area using digitized images. All slides were examined using a microscope; IX83 Olympus or LSM710 ZEISS. Samples from 5-6 mice were analyzed per group.

Quantitative real-time PCR: Quantitative PCR was processed as described before[1]. Total RNA of mouse lung was extracted using Trizol reagent (Invitrogen), and 2μg of total RNA was used for each reverse transcription reaction using a PrimeScript RT Reagent Kit (TaKaRa) followed by quantitative PCR using KAPA SYBR FAST Universal Kit (Kapa biosystems). Primer pairs used in this study are listed in Table S1. The relative amount of each gene in each sample was estimated by the ΔΔCT method. Results were normalized to 18S rRNA level.

Statistics: A normality test (Shapiro-Wilk) provided by SigmaPlot was used to determine whether data were normally distributed. If data were normally distributed, the data were presented as mean ± SEM. A Student's t-test was used to test for differences between 2 groups. A two-way ANOVA followed by a Bonferroni correction post-hoc test was used to test for differences among more than 2 groups. If mouse physiological data were not normally distributed or the sample size in one of the experimental groups was less than 10, a non-parametric test (Mann-Whitney or Kruskal-Wallis) followed by a Bonferroni post hoc correction was performed. All pairwise p-values are two-sided. The null hypothesis was rejected at $P < 0.05$[1].

Reference

1. Wang H, Xu X, Fassett J, Kwak D, Liu X, Hu X, Falls TJ, Bell JC, Li H, Bitterman P, Bache RJ, Chen Y (2014) Double-stranded rna-dependent protein kinase deficiency protects the heart from systolic overload-induced congestive heart failure. *Circulation* 129:1397-1406. doi:10.1161/CIRCULATIONAHA.113.002209.

Table S1. Primers used in quantitative real-time PCR

Mouse Gene		
IL-1β	sense	5’-TCC TGT GTA ATG AAA GAC GGC-3’
	antisense	5’-ACT CCA CTT TGC TCT TGA CTT C-3’
TNF-α	sense	5’-CTT CTG TCT ACTGAA CTT CGG G-3’
	antisense	5’-CAG GCT TGT CAC TCG AAT TTT G-3’
MCP-1	sense	5’-GCA TCC ACG TGT TGG CTC A-3’
	antisense	5’-CTC CAG CCT ACT CAT TGG GAT CA-3’
IL-10	sense	5’-GAT GCC CCA GGC AGA GAA-3’
	antisense	5’-CAC CCA GGG AAT TCA AAT GC-3’
Vcam1	sense	5’-ACA AAG GCA GAG TAC ACA GAC-3’
	antisense	5’-CAC AGG ATT TTG GGA GTT GG-3’
Icam1	sense	5’-GAG AAG TTG GAC AGA ACC CTG-3’
	antisense	5’-GTT ACT TGG TCC CCT TCT GAG-3’
TGF-β	sense	5’-CCT GAG TGG CTG TCT TTT GA-3’
	antisense	5’-CGT GGA GTT TGT TAT CTT TGC TG-3’
18S	sense	5’-TCG AGG CCC TGT AAT TGG AA-3’
	antisense	5’-CCC TCC AAT GGA TCC TCG TT-3’

Table S2 Anatomic date for mice

Parameters	Sham-Filtered Air	Sham-PM2.5	TAC-Filtered Air	TAC-PM2.5
Number of mice	10	13	13	13
Bodyweight(g)	25.4±0.582	24.8±0.622	22.6±0.681*	21±0.554*†
Left ventricular (LV) weight (mg)	81.9±2.13	84.9±3.33	121±3.85*	124±2.17*
Left atria (LA) weight (mg)	3.91±0.265	4.06±0.161	17.3±4.07*	36.5±5.67*†
Lung mass (mg)	172±15.1	151±2.34	306±30.6*	410±20.8*†
Right ventricular (RV) weight (mg)	24.2±0.953	25.0±1.01	25.3±1.09	27.9±1*†
Ratio of LV weight to body weight (mg/g)	3.23±0.087	3.42±0.091	5.41±0.22*	5.92±0.131*†
Ratio of LA weight to body weight (mg/g)	0.154±0.009	0.164±0.004	0.822±0.22*	1.8±0.305*†
Ratio of lung weight to body weight (mg/g)	6.73±0.499	6.15±0.207	13.9±1.61*	19.8±1.4*†
Ratio of RV weight to body weight (mg/g)	0.952±0.031	1.01±0.03	1.14±0.07*	1.34±0.07*†
Tibial length(mm)	17.6±0.177	17.5±0.124	17.3±0.132	17.2±0.12*
Ratio of LV weight to tibial length(mg/mm)	4.65±0.103	4.84±0.191	7.0±0.19*	7.2±0.129*
Ratio of LA weight to tibial length(mg/mm)	0.223±0.017	0.231±0.009	1.0±0.236*	2.12±0.33*†
Ratio of Lung weight to tibial length(mg/mm)	9.75±0.811	8.63±0.18	17.7±1.75*	23.9±1.29*†
Ratio of RV weight to tibial length(mg/mm)	1.37±0.057	1.43±0.053	1.46±0.06	1.62±0.061*†

*p<0.05 as compared with corresponding control conditions; † p<0.05 as compared with control group mice under the TAC condition.

Supplemental Results

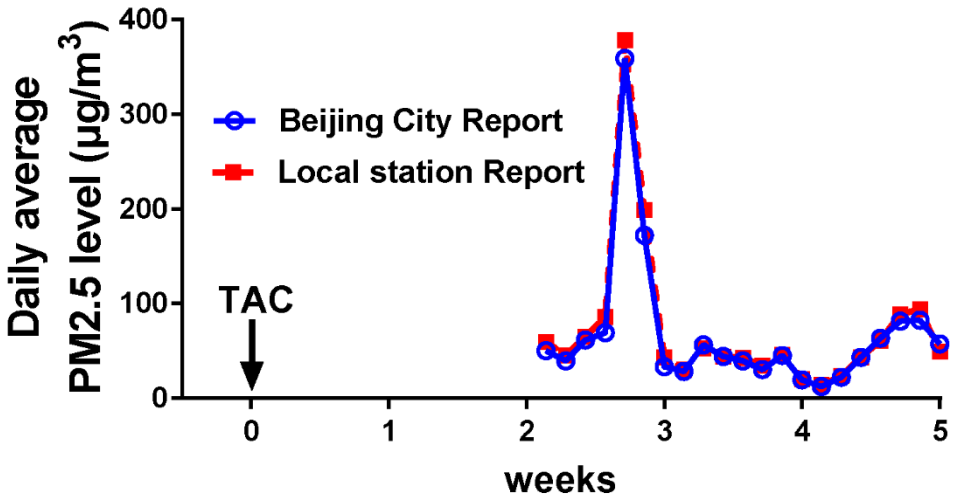

Fig. S1 Daily PM2.5 concentrations and mice survival during the exposure period. The ambient mean daily PM2.5 concentrations in Beijing city and Wanliu monitoring station were also recorded during the exposed period.

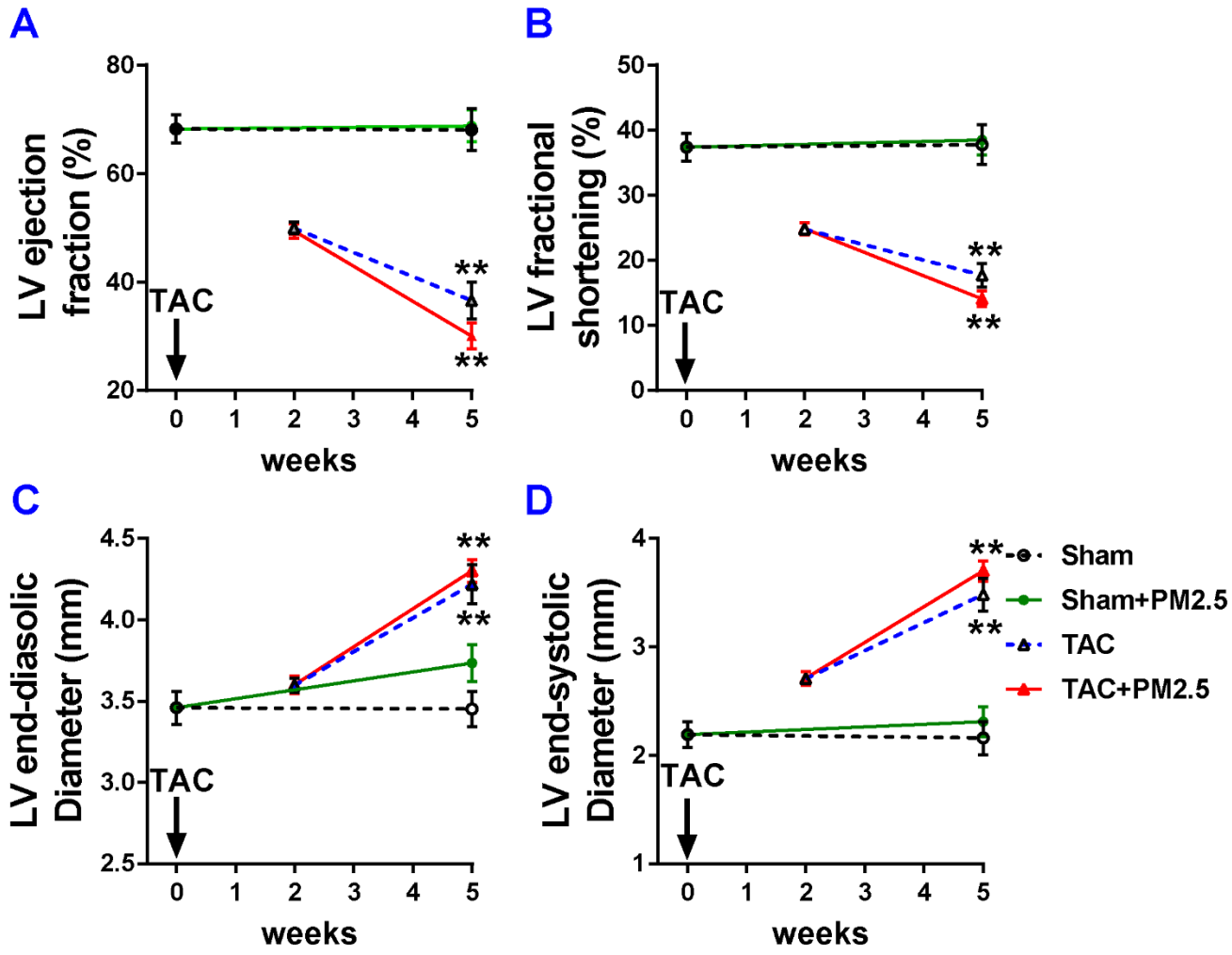

Fig. S2 The LV function before and after PM2.5 exposure. Data were collected from sham-operated and TAC mice before and after exposed to Filtered Air or PM2.5. Echocardiographic measurements of left ventricle (LV) ejection fraction (EF), LV fractional shortening (FS), LV end-systolic diameter (LVESD), LV end-diastolic diameter(LVEDD) (A-D) .*$P < 0.05$ as compared with corresponding sham group.**$P < 0.01$ as compared with corresponding sham group.

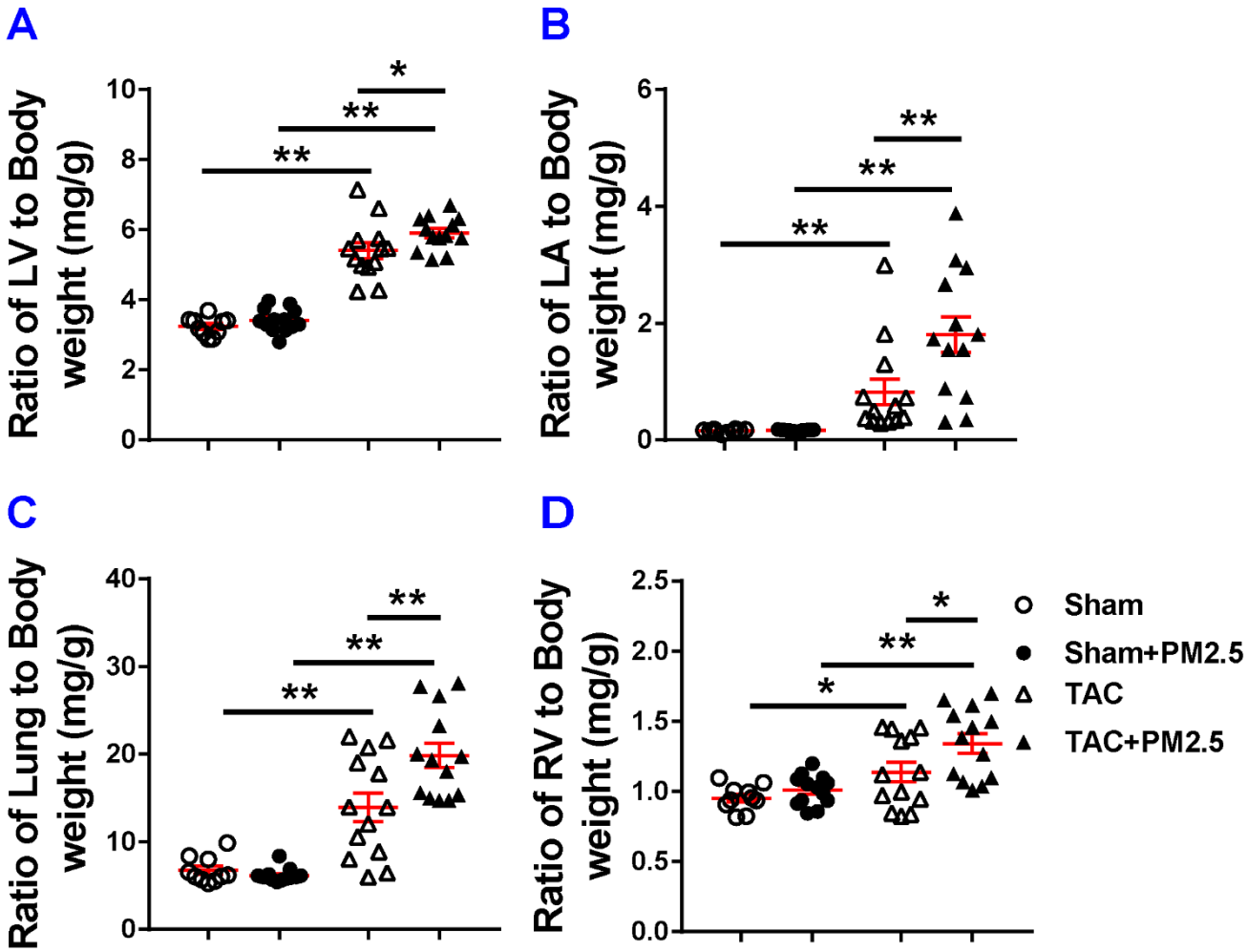

Fig. S3 Moderate PM2.5 exposure did not affect LV hypertrophy in mice with existing LV dysfunction but enhanced the increase of the ratio of lung weight to body weight. Data were collected from sham-operated and TAC mice exposed to Filtered Air or PM2.5.The ratios of LV, LA, lung and RV (right ventricle) weight to body weight of the mice are shown (A-D). *$P < 0.05$ between corresponding groups.**$P < 0.01$ between corresponding groups.

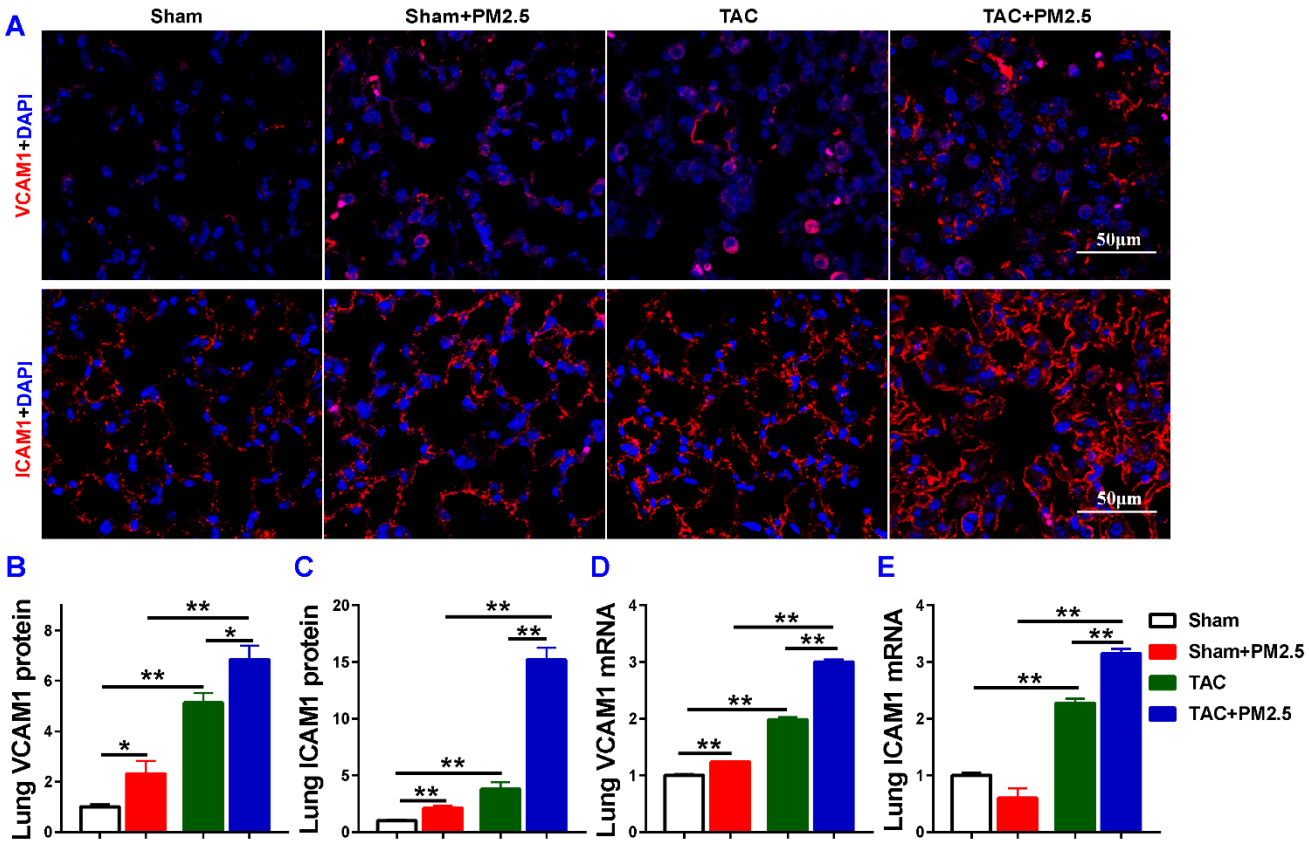

Fig. S4 PM2.5 exposure increased lung VCMA-1 and ICAM-1 expression in mice. Data were collected from sham-operated and TAC mice exposed to filtered air (FA) or PM2.5.Representative images of adhesion molecules vascular cell adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM-1) immunostaining (red) of the lung (A).The quantitative data of VCAM-1 and ICAM-1 protein and mRNA levels in lung tissues(B-E). *$P < 0.05$ between corresponding groups.**$P < 0.01$ between corresponding groups. n=3 per group.

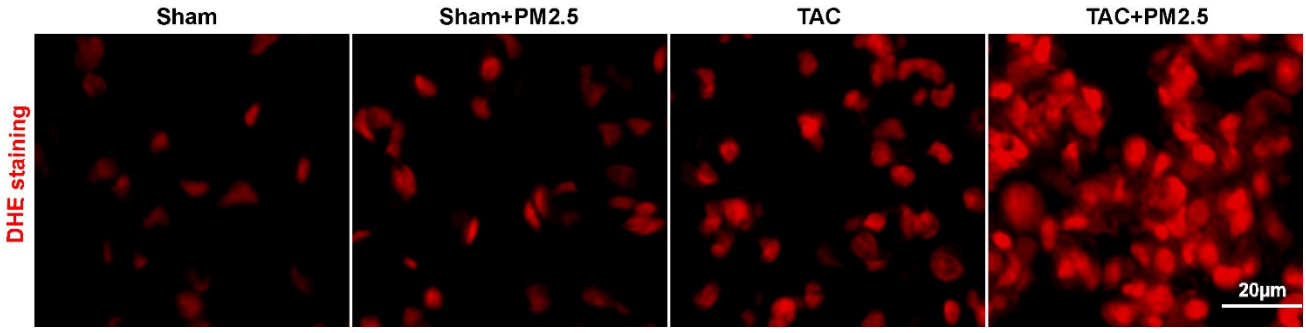

Fig. S5 The DHE staining of lung. Dihydroethidium (DHE) staining of representative frozen lung sections from corresponding sham and TAC mice with or without PM2.5 exposure. Scale bar = 20μm.