Appendix 1: Methods

Collection of $PM_{2.5}$ and sample preparation

$PM_{2.5}$ samples were obtained from the rooftops of the platform at the Guangzhou Institute of Geochemistry, Chinese Academy of Sciences (100 m near the motorway). The sample was collected in a Tissuquartz (PALL, CA, USA) using high-volume air samplers (Thermo Fischer Scientific) at the flow rate of 1.05 m^3/min daily in August 2020. We placed small pieces of quartz fiber filter membrane (20×25 cm^2) loaded with $PM_{2.5}$ in 300 mL of ultrapure water. The particles were eluted from the quartz fiber filter membranes during a 30-min ultrasonic vibration process. After dehydrating the membrane, it oscillated for 30 min in an ultrasonic oscillator. After repeating the elution 3 times, all eluates were collected in a large vessel. The entire $PM_{2.5}$ solution was filtered through a sterile gauze, distributed into 50-mL tubes, and centrifuged for 60 min at 4 ℃ (12,000 rpm). We cryopreserved the bottom layer solution of the eluate containing $PM_{2.5}$ at –80 ℃. Freeze-dried powder of $PM_{2.5}$ was prepared by using a vacuum freeze dryer for 4 hours. For the cell experiment, the lyophilized $PM_{2.5}$ powder was accurately weighed, mixed into ultrapure water and autoclaved for 20 min, and then thawed in PBS before use. Ion chromatography and water-soluble total organic carbon were respectively analyzed by using inductively coupled plasma mass spectrometry (Thermo Finnegan), organic carbon/elemental carbon analyzer (Desert Research Institute) (date in *Table S1*).

Histopathology and IHC staining

The left lung tissues were harvested from five euthanized mice, fixed in 4% paraformaldehyde for 24 hours at room temperature, and embedded in paraffin for sectioning at 5-μm thickness. For scoring analysis, the sections were stained with HE. Using the Smith Lung Injury Scoring System, two pathologists blindly assessed 10 lung tissues for injury severity on HE sections. This study evaluated four pathological parameters: pulmonary edema; alveolar and interstitial inflammation; alveolar and interstitial hemorrhage; atelectasis and the formation of hyaline membranes. Based on the scores, the tissue lesions were scored as follows: 0 (no lesion); 1 (25%); 2 (>25–50%); 3 (>50–75%); and 4 (>75%). Thus, the lung injury total score is the sum of the four parameters.

The sections were deparaffinized and blocked with 3% bovine serum albumin (BSA) to perform the IHC staining. The sections were incubated overnight at 4 ℃ for 2 hours at room temperature with the primary antibodies of TLR4 (1:100, Abcam), anti-phospho-NF-κB (1:150, Abcam), NLRP3 (1:200, Abcam), caspase-1 (1:500, Bioss), IL-1β (1:100, Abcam), and gasdermin D (1:150, Abcam). After washing thrice with PBS, the sections were incubated at 37 ℃ for 2 hours with the goat anti-rabbit secondary antibody (Bioss; 1:5,000). Subsequently, the sections were stained by 3,3'-diaminobenzidine tetrahydrochloride (DAB) for 5 min at the room temperature. Finally, IHC images were randomly scanned by a light scanning microscope at ×200 magnification. We then ImageJ to quantify the high positive percentage contribution.

CCK-8 assay

The CCK-8 solution (Abcam, USA) was added to the culture medium and incubated for 2 hours after $PM_{2.5}$ or CC16 administration per the manufacturer's instructions to assess the cell viability. The viability of living cells in each group was tested using a microplate reader at a 450-nm optical density. The viability of living cells in each group was tested by using a microplate reader at 450-nm optical density.

Western blotting

The lung tissues and harvested cells were homogenized in RIPA lysis buffer (Solarbio, Beijing, China) containing protease, phosphatase, and phenylmethylsulfonyl fluoride (PMSF). The mixture was centrifuged at 12,000 rpm at 4 ℃ for 20 min. The supernatant was collected, and its protein content was measured with a bicinchoninic acid (BCA) protein assay kit (Solarbio). Then, 10 μg of protein was separated with SDS-PAGE gel (8%, 10%, and 12%) and then transferred onto a polyvinylidene fluoride (PVDF) membrane (Bio-Rad, Richmond, CA, USA). The blots with targeted molecules were cut during blotting before hybridization with antibodies. After washing, the blots were incubated at 4 ℃ overnight with the

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primary antibodies against TLR4 (CST, 1:1,000), NF-κB (CST; 1:1,000), polyclonal-anti-NF-κB (CST; 1:1,000), caspase-3 (CST; 1:1,000), NLRP3 (Abcam; 1:1,000), caspase-1 (CST; 1:1,000), IL-1β (CST; 1:1,000), gasdermin D (CST; 1:1,000), HMGB1 (CST; 1:1,000), ETS1 (CST; 1:1,000), and β-actin (CST; 1:3,000). Horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG) (CST; 1:5,000) or goat anti-mouse (CST; 1:5,000) was incubated as secondary antibodies for 2 hours at room temperature. Finally, the immunoreactive blots were visualized using the Enhanced Chemiluminescence Detection Kit (Beyotime Biotech; Shanghai, China) and quantified by Quality One software (Bio-Rad). We used ImageJ to quantify the Western blot strip gray value. To confirm the statistical conclusion, we conducted multiple repetitions of each Western blot band, exceeding three times, and performed a comparative analysis of the gray value.

PCR

The literature was consulted to identify the target protein gene subtype, and Guangzhou Aiji Company (Guangzhou, China) synthesized primers. BEAS-2B cells were subjected to pancreatic enzyme digestion and modeled with either $PM_{2.5}$ or CC16. Upon reaching 60–80% confluency, the reaction was halted by adding a culture medium. The collected cells were centrifuged at 1,000 g for 3 min and washed twice with PBS before undergoing another round of centrifugation at 1,000 g for 3 min. Total RNA was isolated from BEAS-2B cells under the TRIzol (Sigma) protocol. The RNA concentration was determined through an ELISA at 562 nm absorbance. Subsequently, the extracted RNA was promptly subjected to reverse transcription into cDNA utilizing the Thermo Scientific Revert Aid First Strand cDNA Synthesis Kit (Thermo, USA). The first step involves the generation of cDNA through reverse transcription, utilizing a 10 μL system. The reaction system (20) should be prepared on an ice box with the following components following the total RNA used, which should not exceed 500 ng. Real-time PCR was conducted using the QuantiFast SYBR Green PCR kit (Invitrogen) on the Roche Light Cycler 480II system. The primer list is as follows in *Table 1*.

Statistical analysis

Data are presented as the mean ± standard deviation. We performed the statistical analysis with SPSS 14.0 statistical software (SPSS Inc., Chicago, IL, USA). The Student's *t*-test was performed to analyze the difference between the two groups. One-way ANOVA followed by Bonferroni's post-hoc test was performed for comparisons among multiple experimental groups. $P<0.05$ was considered to indicate significant differences.

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Table S1 The MS/MS results in $PM_{2.5}$ or $PM_{2.5}$ with HP sterilization

Items	Elements (μg/mg) compositions		Trace elements (ng/mg)		Water soluble components (μg/mg)		Water soluble components (μg/mg)	
	$PM_{2.5}$	$PM_{2.5}$ with HP sterilization	$PM_{2.5}$	$PM_{2.5}$ with HP sterilization	$PM_{2.5}$	$PM_{2.5}$ with HP sterilization	$PM_{2.5}$	$PM_{2.5}$ with HP sterilization
TOC	341±74.25	364.6±39.66						
Ca	67.6±43.76	71.2±46.96						
K	33.4±14.4	34.6±15.33						
Na	32.60±16.27	61.4±33.17						
Zn	7.12±2.61	7.04±2.47						
Cu			2,410.8±1,780.33	2,104.6±1,553.22				
Ba			1,180.6±247.51	1,400.6±310.71				
Mn			1,108.4±358.83	1,110±404.58				
As			381.8±261.35	386.6±234.84				
Pb			353.4±263.46	244.2±177.41				
Na^+					19.64±13.5	48.36±50.73		
NH^{4+}					186±39.74	213.2±28.67		
K^+					32.2±13.33	32.2±14.14		
Mg^{2+}					6.76±4.38	8.02±4.49		
Ca^{2+}					76.2±44.68	76.2±49.30		
$SO4^{2-}$							774.2±151.61	857.4±186.15
NO^{3-}							98.4±25.46	114.2±31.33
NO^{2-}							2.68±1.875	2.34±1.63
Cl^-							7.58±9.84	6.62±3.37
Br^-							2.16±1.18	1.12±1.53

We examined untreated and high-pressure steam-sterilized $PM_{2.5}$ for ion chromatography, water-soluble total organic carbon, and metals, presenting the results as mean ± standard deviation. $PM_{2.5}$, particulate matter 2.5.

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Table S2 The expression of 10 genes that were originally upregulated in the $PM_{2.5}$ group decreased, while the expression of 21 genes that were originally downregulated in the $PM_{2.5}$ group increased after CC16 treatment

Gene name	Abbreviation	The expression in $PM_{2.5}$ group	The expression in $PM_{2.5}$ + CC16 group
Angiopoietin-like protein 4	ANGPTL4	Upregulation	Downregulation
Early growth response 1	EGR1	Upregulation	Downregulation
Intercellular cell adhesion molecule-1	ICAM1	Upregulation	Downregulation
Perilipin 2	PLIN2	Upregulation	Downregulation
Growth differentiation factor 15	GDF15	Upregulation	Downregulation
Carnitine palmitoyl transferase 1	CPT1A	Upregulation	Downregulation
Eukaryotic translation elongation factor 1 alpha 1	EEF1A1	Upregulation	Downregulation
Cyclin dependent kinase inhibitor 1A	CDKN1A	Upregulation	Downregulation
Ribosomal protein L29	RPL29	Upregulation	Downregulation
Guanine nucleotide binding protein beta polypeptide 2-like 1	GNB2L1	Upregulation	Downregulation
E-twenty-six-1	ETS1	Downregulation	Downregulation
Zinc finger and BTB domain containing 38	ZBTB38	Downregulation	Downregulation
Proteasome 26S subunit	PSMD2	Downregulation	Downregulation
Eucaryotic translation initiation factor 3	EIF3A	Downregulation	Downregulation
Collagen type VI alpha 2 chain	COL6A2	Downregulation	Upregulation
TAP binding protein	TAPBP	Downregulation	Upregulation
Collagen type VI alpha 1	COL6A1	Downregulation	Upregulation
Phospholipases D 3	PLD3	Downregulation	Upregulation
Chromosome 15 open reading frame 52	C15orf52	Downregulation	Downregulation
Latent transforming growth factor beta binding protein 4	LTBP4	Downregulation	Upregulation
Fusion	FUS	Downregulation	Downregulation
ATP binding cassette	–	Downregulation	Upregulation
Sub-family A member 2	ABCA2	Downregulation	Upregulation
CBF1 interacting corepressor	CIR	Downregulation	Upregulation
Nucleoporin 210kDa	NUP210	Downregulation	Upregulation
Notch homolog 3	NOTCH3	Downregulation	Upregulation
Complement 1	–	Downregulation	Upregulation
Complement component 1	–	Downregulation	Upregulation
Q subcomponent like protein 1	C1QL1	Downregulation	Upregulation
Tumor-associated calcium signal transducer 2	TACSTD2	Downregulation	Upregulation
Basal cell adhesion molecule	BCAM	Downregulation	Upregulation
Retinoic acid receptor responder 2	RARRES2	Downregulation	Upregulation
Sushi domain containing 2	SUSD2	Downregulation	Upregulation

Table S2 (*continued*)

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Table S2 (*continued*)

Gene name	Abbreviation	The expression in $PM_{2.5}$ group	The expression in $PM_{2.5}$ + CC16 group
Carboxypeptidase A	CPA	Downregulation	Upregulation
Interferon induced protein with tetratricopeptide repeats 2	IFIT2	Downregulation	Upregulation
Interleukin-7 receptor	IL7R	Downregulation	Upregulation
Kexin9 proprotein convertase subtilisin	PCSK9	Downregulation	Upregulation

$PM_{2.5}$, particulate matter 2.5; CC16, club cell secretory protein 16.

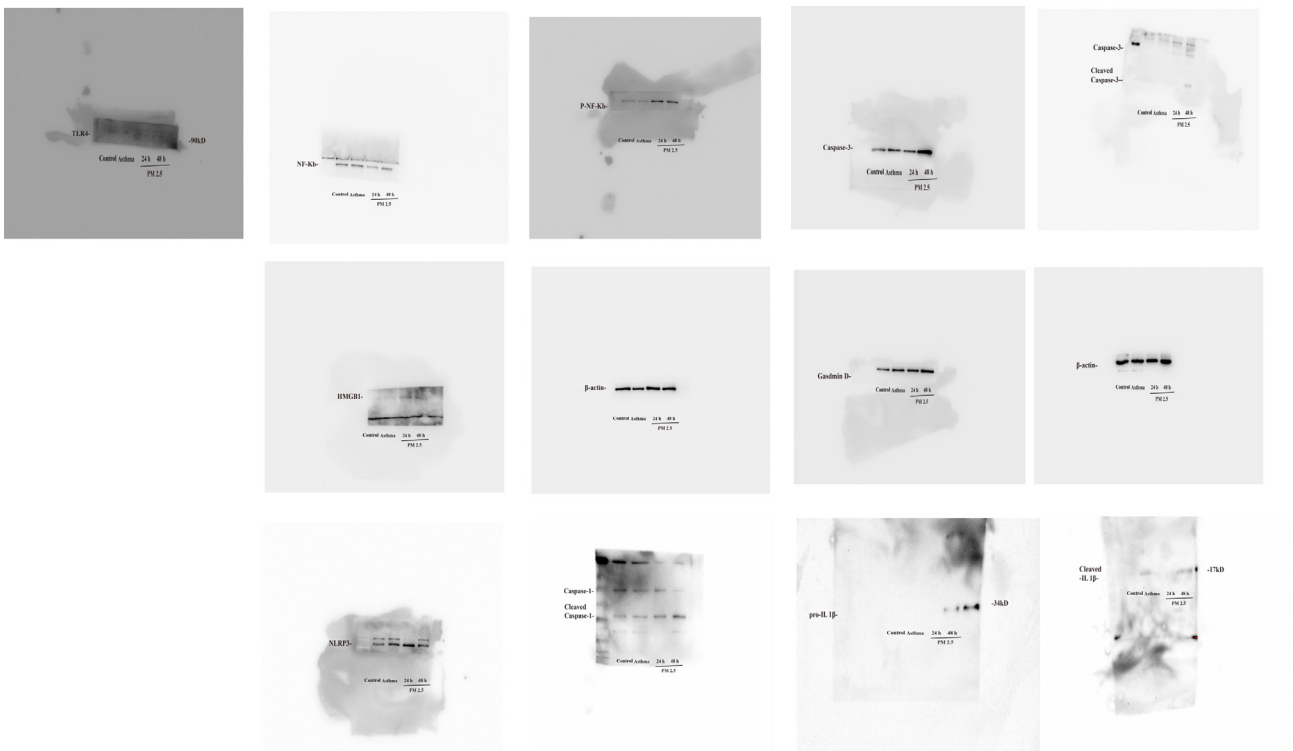

Figure S1 Western blot analysis of protein expression in various treatment groups. Lanes 1–4 represent control group, asthma group, $PM_{2.5}$ for 24 hours group, and $PM_{2.5}$ for 48 hours group, respectively. The blots were probed with anti-TLR4/NF-κB/p-NF-κB/cascade-3/cleaved cascade-3/HMGB1/gasdmin D/NLRP3/caspase-1/cleaved cascade-1/pro-IL-1β/cleaved IL-1β antibody (1:1,000 dilution), and β-actin was used as a loading control. Data are representative of three independent experiments.

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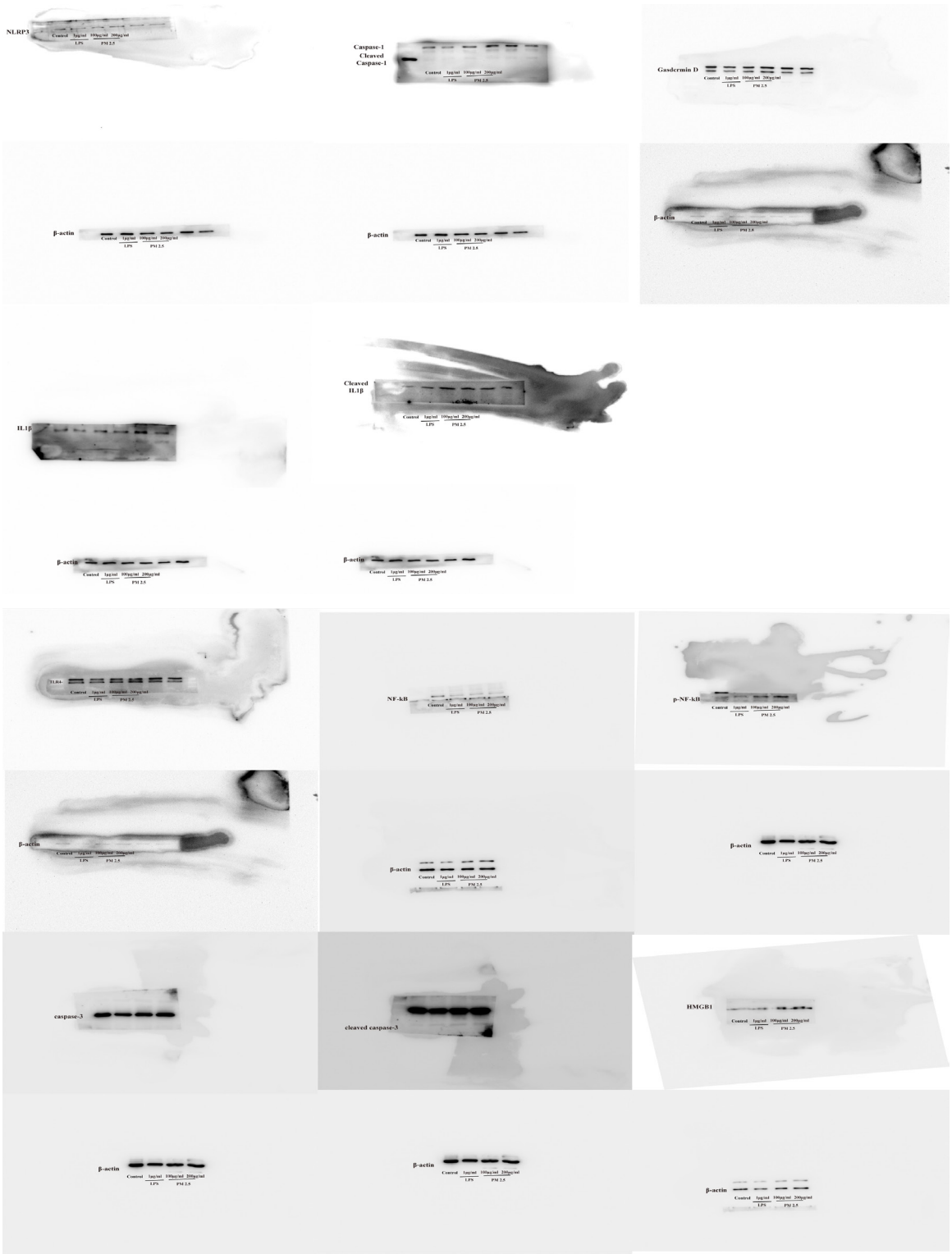

Figure S2 Western blot analysis of protein expression in various treatment groups. Lanes 1–4 represent control group, LPS (1 μg/mL) group, $PM_{2.5}$ (100 μg/mL) group, and $PM_{2.5}$ (200 μg/mL) group, respectively. The blots were probed with anti-NLRP3/caspase-1/cleaved cascade-1/gasdmin D/IL-1β/cleaved IL-1β/TLR4/NF-κB/p-NF-κB/cascade-3/cleaved cascade-3/HMGB1 antibody (1:1,000 dilution), and β-actin was used as a loading control. Data are representative of three independent experiments.

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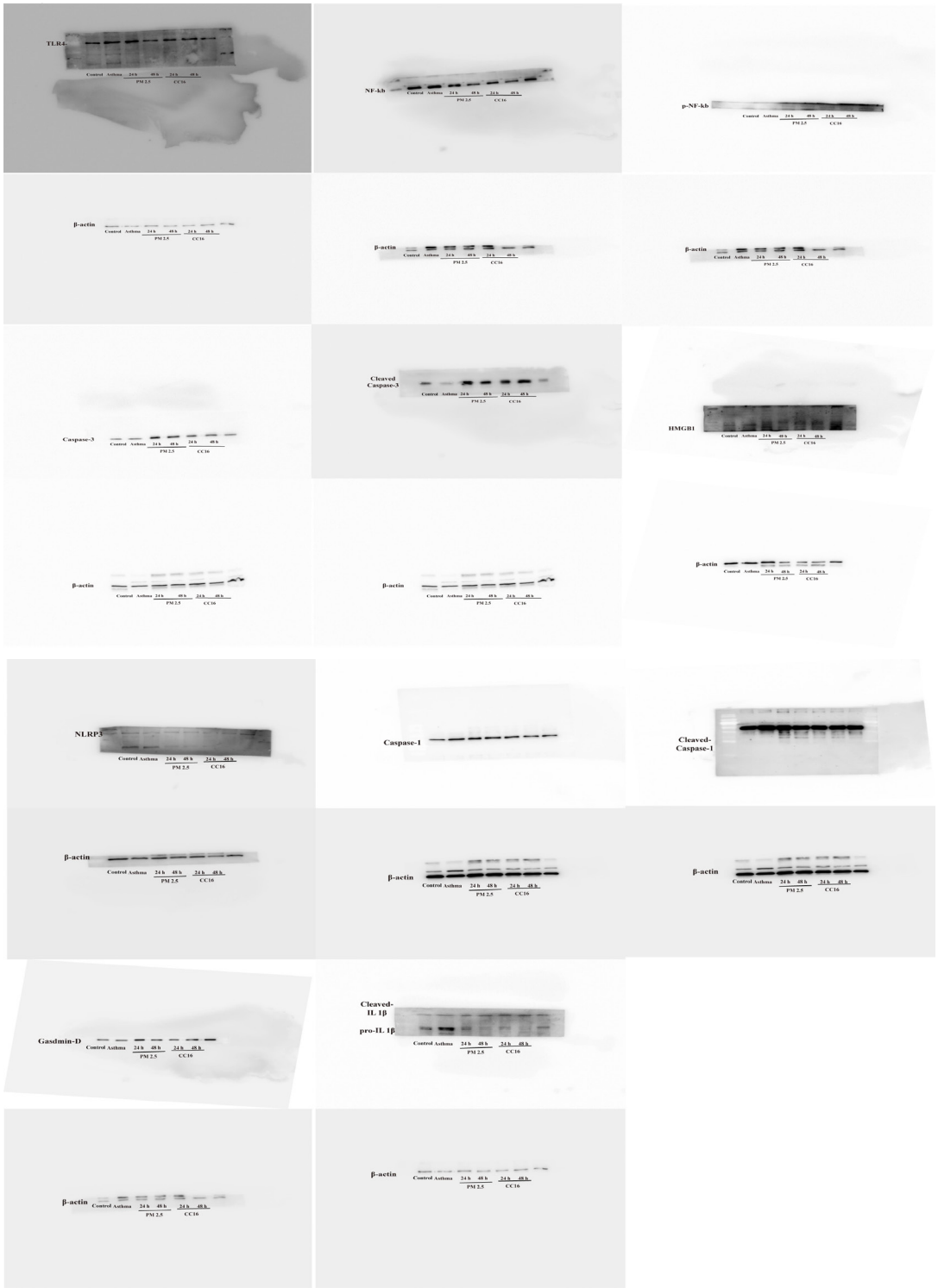

Figure S3 Western blot analysis of protein expression in various treatment groups. Lanes 1–6 represent control group, asthma group, $PM_{2.5}$ for 24 hours group, and $PM_{2.5}$ for 48 hours group, $PM_{2.5}$ + cc16 for 24 hours group, and $PM_{2.5}$ + cc16 for 48 hours group, respectively. The blots were probed with anti-TLR4/NF-κB/p-NF-κB/cascade-3/cleaved cascade-3/HMGB1/gasdmin D/NLRP3/caspase-1/cleaved cascade-1/pro-IL-1β/cleaved IL-1β antibody (1:1,000 dilution), and β-actin was used as a loading control. Data are representative of three independent experiments.

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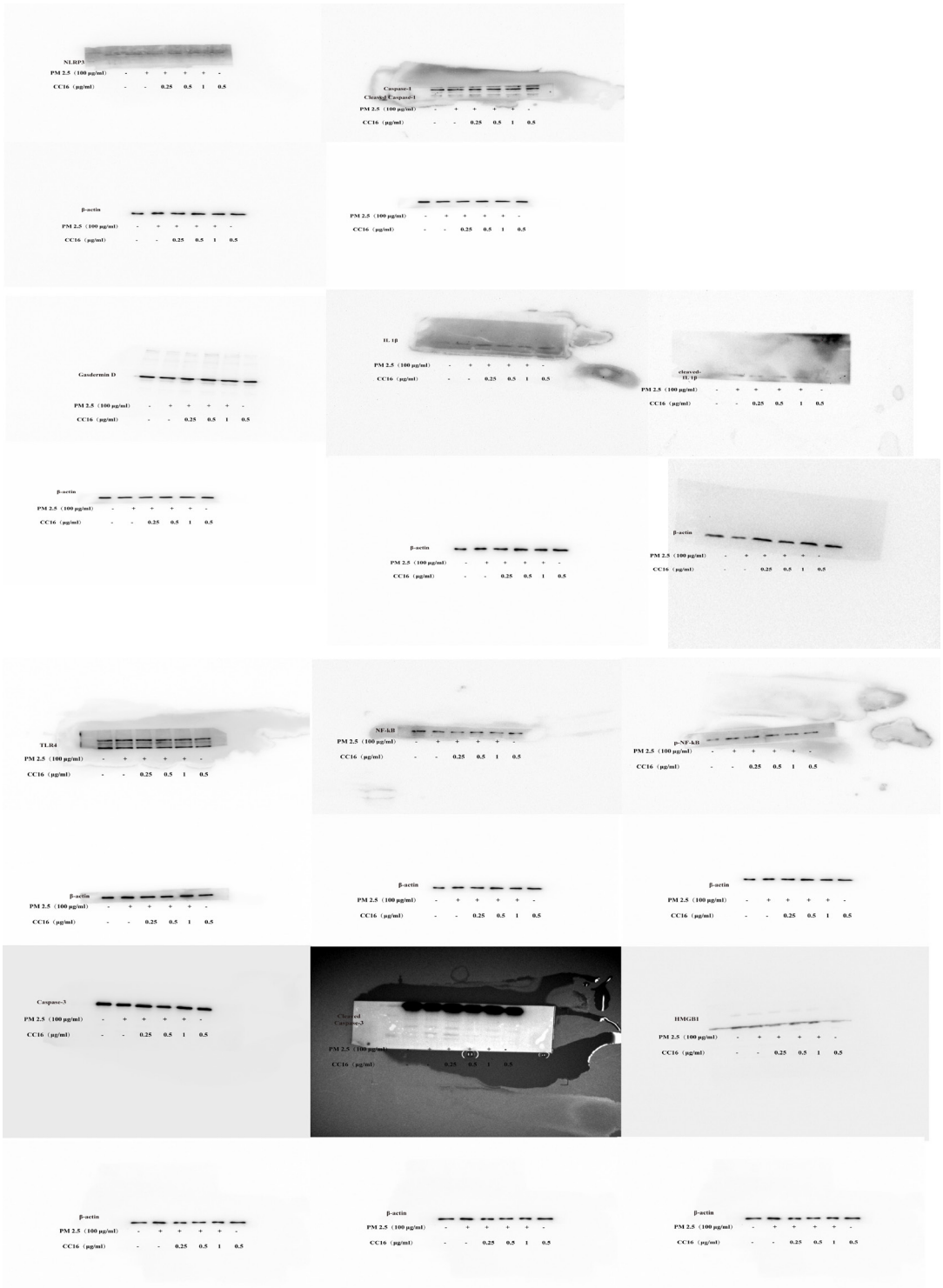

Figure S4 Western blot analysis of protein expression in various treatment groups. Lanes 1–6 represent control group, $PM_{2.5}$ (100 μg/mL) group, $PM_{2.5}$ (100 μg/mL) + cc16 (0.25 μg/mL) group, $PM_{2.5}$ (100 μg/mL) + cc16 (0.5 μg/mL) group, $PM_{2.5}$ (100 μg/mL) + cc16 (1 μg/mL) group, and cc16 (0.5 μg/mL) group, respectively. The blots were probed with anti-NLRP3/caspase-1/cleaved cascade-1/gasdmin D/IL-1β/cleaved IL 1β/TLR4/NF-κB/P-NF-κB/cascade-3/cleaved cascade-3/HMGB1 antibody (1:1,000 dilution), and β-actin was used as a loading control. Data are representative of three independent experiments.

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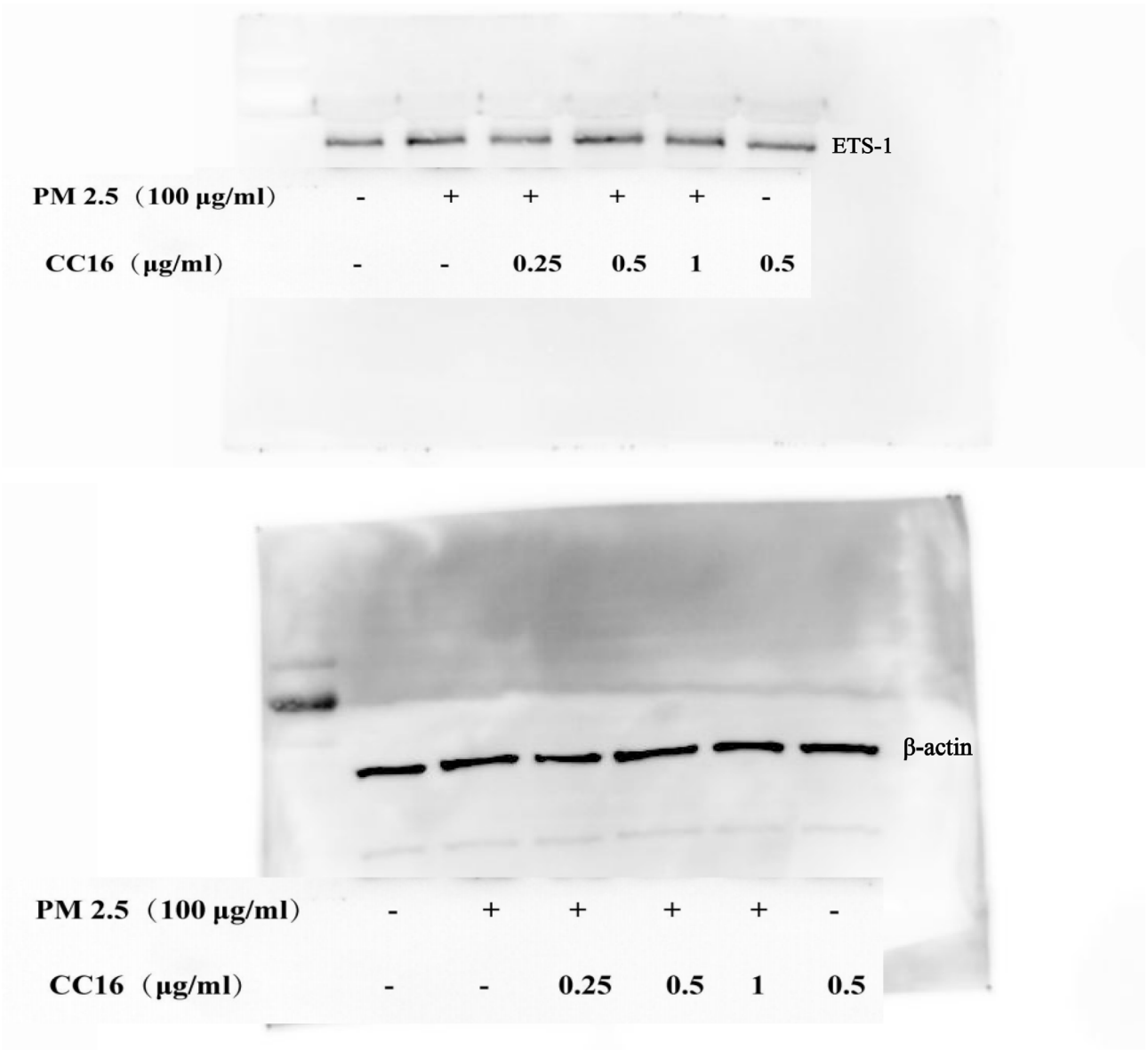

Figure S5 Western blot analysis of protein expression in various treatment groups. Lanes 1–6 represent control group, $PM_{2.5}$ (100 μg/mL) group, $PM_{2.5}$ (100 μg/mL) + cc16 (0.25 μg/mL) group, $PM_{2.5}$ (100 μg/mL) + cc16 (0.5 μg/mL) group, $PM_{2.5}$ (100 μg/mL) + cc16 (1 μg/mL) group, and cc16 (0.5 μg/mL) group, respectively. The blots were probed with anti-ETS-1, and β-actin was used as a loading control. Data are representative of three independent experiments.

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