



Cannabichromeorcin targets cathepsin L to alleviate oxidative stress–Driven airway inflammation in severe asthma

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ABSTRACT

Background: Severe asthma (SA) is characterized by corticosteroid insensitivity, persistent airway inflammation, and excessive oxidative stress, underscoring an urgent unmet therapeutic need. Meroterpenoids derived from *Rhododendron capitatum* Maxim have demonstrated potent anti-inflammatory properties; however, their therapeutic potential in SA has not been elucidated.

Methods: A meroterpenoid-rich fraction was isolated from *R. capitatum* Maxim, and cannabichromeorcin (CBCO) was identified as its most abundant constituent. The therapeutic effects of CBCO were evaluated in murine models of SA induced by toluene diisocyanate or by combined house dust mite and lipopolysaccharide exposure. Transcriptomic profiling, TargetNet-based target prediction, molecular docking, and molecular dynamics simulations were employed to identify pharmacological targets. Clinical relevance was examined using transcriptomic datasets from the *Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes* project (U-BIOPRED) and validated in an independent clinical cohort.

Results: Pretreatment with CBCO ameliorated airway inflammation and oxidative stress in murine models of SA. An integrative approach identified cathepsin L (CTSL) as a redox-sensitive pharmacological target of CBCO. Pretreatment with CBCO inhibited CTSL, thereby reducing the formation of neutrophil extracellular traps containing MMP8, and attenuating the activation of the unfolded protein response (UPR). Pharmacological inhibition of CTSL, MMP8, or the UPR recapitulated the protective effects of CBCO. In vitro, CBCO preserved airway epithelial barrier integrity, reduced reactive oxygen species accumulation, and suppressed alarmin release. Clinically, transcriptomic analyses from the U-BIOPRED cohort revealed elevated MMP8 and DDIT3 expression in sputum and blood samples from patients with SA. In our independent validation cohort, serum MMP8 and DDIT3 levels were increased in SA. Receiver operating characteristic analyses confirmed serum MMP8 and DDIT3 as predictive biomarkers for SA.

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Conclusion: CBCO conferred protection against the development of SA by targeting CTSL to suppress NET formation, UPR activation, and downstream oxidative stress. MMP8 and DDIT3 emerged as promising biomarkers for predicting disease severity.

1. Introduction

Asthma affects more than 300 million individuals worldwide, with severe asthma (SA) representing approximately 5–10% of cases but contributing disproportionately to morbidity, mortality, and healthcare expenditures [1,2]. This phenotype is characterized by a poor response to corticosteroid therapy, frequent exacerbations, and an accelerated decline in lung function [3]. Among the inflammatory phenotypes of SA, the eosinophilic subtype has been extensively targeted by various biologic agents, resulting in substantial clinical improvements [4]. In contrast, the neutrophilic and mixed-granulocytic phenotypes—primarily associated with interleukin-17 (IL-17), tumor necrosis factor- α (TNF- α), and oxidative stress—still lack effective targeted therapies [5]. Increasing evidence indicates that these mixed inflammatory patterns reflect a redox-driven pathological process in SA, characterized by excessive oxidative stress, mitochondrial dysfunction, and steroid resistance [6,7].

Our group previously established and characterized a murine model of toluene diisocyanate (TDI)-induced asthma that reproduces the hallmark features of SA, including steroid resistance, airway hyper-responsiveness (AHR), mixed-granulocytic airway inflammation, airway remodeling, and mitochondrial dysfunction within the airways [7–11]. Using this model, we and others have demonstrated the pivotal role of neutrophil extracellular traps (NETs) in amplifying airway inflammation and promoting steroid resistance [12]. NETs are web-like structures composed of extracellular DNA (eDNA), histones, and granular proteins such as NE, myeloperoxidase (MPO), and matrix metalloproteinases (MMPs), which, upon release, damage the airway epithelium and perpetuate inflammation [13]. Although several NET components have been investigated, the specific contribution of matrix metalloproteinase-8 (MMP8) to the pathogenesis of SA remains poorly defined, representing a potential target for therapeutic intervention.

The search for novel agents capable of modulating oxidative stress, NETosis, and neutrophilic inflammation prompted our investigation of natural products. We previously identified the *Rhododendron* genus as a rich source of structurally diverse and biologically active meroterpenoids [14]. Among these, *Rhododendron capitatum* Maxim, an evergreen shrub traditionally used in Tibetan medicine to treat inflammation, gastric disorders, and cough [15,16], has emerged as a particularly promising candidate. Nevertheless, its active constituents and their potential therapeutic effects in SA remain largely unexplored.

To systematically identify the molecular target of the active fraction derived from *R. capitatum* Maxim, we employed an integrative approach that combined transcriptomic analysis with computational prediction. This strategy enabled us to move beyond conventional anti-inflammatory screening and directly identify the key molecular target underlying its bioactivity. Cathepsin L (CTSL)—a lysosomal protease involved in extracellular matrix degradation, epithelial barrier disruption, and immune cell activation [17,18]—was identified as the sole overlapping target between the predicted targets of the active compound and the differentially expressed genes in our asthma model. Although dysregulated CTSL activity has been associated with several chronic airway diseases [19,20], its role in SA and its potential linkage to oxidative stress and NET formation have not yet been elucidated.

Therefore, this study aimed to isolate the active meroterpenoid constituents from *R. capitatum* Maxim, identify the principal bioactive compound, and elucidate its mechanistic role in mitigating the development of SA. Particular emphasis was placed on the CTSL–MMP8/UPR axis, its potential association with oxidative stress, and the translational relevance of these findings.

2. Methods

2.1. Extraction and isolation procedure of *Rhododendron capitatum* Maxim

The aerial parts (mainly stems and leaves, 20.0 kg) of *Rhododendron capitatum* Maxim were extracted three times by heating under reflux with 70% aqueous EtOH. The resulting extract was then partitioned five times with EtOAc. The EtOAc soluble fraction (2.7 kg) was eluted with dichloromethane (100%) and MeOH (100%) and eluted by silica gel column chromatography (200–300 μ m) to give three final fractions Fr. A, Fr. B and Fr. C. The Fr. A (424.8 g) was separated by MCI gel column chromatography (MCI gel CHP 20P, 75–150 μ m; Mitsubishi Chemical, Japan) using a gradient elution of MeOH/H₂O (60:40 to 100:0, v/v) to afford four subfractions (Fr. a–Fr. d). Fr. c (148.3 g) was further chromatographed on an MCI column with a MeOH/H₂O gradient (70:30 to 100:0, v/v) to yield six fractions (Fr. 1–Fr. 6). Fr. 3 (3.0 g) was fractionated on a Sephadex LH-20 gel column (25–100 μ m) using 100% MeOH as the mobile phase at a flow rate of 2.0 mL/min. A total of 100 fractions (5.0 mL each) were collected and combined based on thin layer chromatography (TLC) profiles to give two subfractions, Fr. 3.1 and Fr. 3.2 (2.1 g). Fr. 3.2 was purified by semi-preparative HPLC (Shimadzu LC-20AT system; column: Zorbax SB-C18, 250 mm $\times$ 9.4 mm i.d., 5 μ m) with MeOH–H₂O (90:10, containing 0.05% HCOOH) as the eluent at a flow rate of 3.0 mL/min. At a retention time (t_R) of 20.0 min, compound 1 (Cannabichromeol, 67.0 mg) [21] was obtained. Fr. 4 (23.7 g) was separated and purified by silica gel column chromatography using a gradient elution system of PE/EtOAc with volume ratios of 200:1, 150:1, 100:1, 50:1, 40:1, 30:1, 20:1, 10:1, 5:1, and 1:1 (v/v) sequentially. Based on the elution profile, Fr. 4.1–Fr. 4.3 were obtained. Fr. 4.1 (0.4 g) was separated into three subfractions (Fr. 4.1.1–4.1.3) on preparative thin-layer chromatography (PTLC) using a gradient of PE/EtOAc (5:1, v/v). Subfraction. Fr. 4.1.3 (78.6 mg) was further purified by semi-preparative HPLC (MeOH–H₂O, 88:12, v/v, containing 0.05% formic acid) to yield compound 2 (Rubiginosins G, 28.0 mg, t_R = 15.6 min) [22]. Fr. 4.2 (2.0 g) was first separated by gel column chromatography (eluted with 100% methanol) to yield two fractions: Fr. 4.2.1 (0.5 g) and Fr. 4.2.2 (1.5 g). Fr. 4.2.1 was purified by semi-preparative HPLC (MeOH–H₂O, 85:15, v/v, containing 0.05% HCOOH, flow rate 3.0 mL/min) to afford compound 3 (Nyingchinoid F, 32.0 mg, t_R = 21.6 min) [23] and compound 4 (Ranhuduanine B, 7.3 mg, t_R = 16.0 min) [21]. Fr. 4.2.2 was purified by semi-preparative HPLC (MeCN–H₂O, 78:22, v/v, containing 0.05% HCOOH, flow rate 3.0 mL/min) to yield compound 5 [24] (Grifolin, 7.0 mg, t_R = 16.7 min). Fr. 4.3 was subsequently refined by a gel column chromatography using MeOH/H₂O (100:0, v/v) to afford Fr. 4.3.1–Fr. 4.3.2 (2.7 g). Compounds 6 (Ranhuduanine D, 7.3 mg, t_R = 22.5 min) [21] were afforded using semi-preparative HPLC (MeCN–H₂O, 70:30, v/v, containing 0.05% HCOOH, flow rate 3.0 mL/min). Relevant chemical data are provided in Figs. S1–S12. ¹H and ¹³C NMR spectra were recorded on Bruker AVANCE III 400 MHz and 600 MHz spectrometers (Bruker, Germany) using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in parts per million (ppm). Rotary evaporation was performed using an EYELAN-1001D-W instrument (Tokyo Rikakikai, Japan).

2.2. Clinical trial

Participants were recruited from the respiratory clinic of Nanfang Hospital and Dalian municipal friendship hospital. The hospital's Ethics

Committee granted approval for this study under approval number NFEC-2023-259 and LL-2025-033-02. Before being enrolled in the study, each participant signed the informed consent. In total, 172 individuals were progressively included in the trial after receiving an asthma diagnosis in accordance with the Global Initiative for Asthma (GINA) 2024 guidelines. Respiratory tract infections and respiratory disease flare-ups within 4 weeks of the initial research visit were both grounds for exclusion. Severe asthma patients are classified according to GINA guidelines, who belong to 4–5 GINA steps. As control, 68 healthy individuals without a history of lung conditions were included with normal spirometry results. Standardized processes were used to induce and process sputum as described in our previous study [12]. Informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.

2.3. Animal

6 weeks-old male Balb/c mice were gained from Southern Medical University. All mice were kept in an specific pathogen-free (SPF) laboratory with a 12-h dark/light cycle at a temperature of $23 \pm 2^\circ\text{C}$ and humidity range of 40–70%. Mice were continuously given irradiated food and sterilized water. All mice were randomly housed in separate cages, and interventions and analyses were conducted at the same time point. The experimental protocols were approved by the Animal Subjects Committee of Nanfang Hospital and followed the Principles of Laboratory Animal Care.

2.4. Establishment of the TDI-induced mice asthma model

110 mice were averagely randomized to 11 groups by simple randomization. A mixture of 2 vol of acetone and 3 vol of olive oil was used as the vehicle to dissolve TDI for cutaneous sensitization, and 1 vol of acetone and 4 vol of olive oil was utilized for airway challenge. The procedure of establishing animal models was described in our previous reporting [7]. CBCO (1, 2, 4 or 6 mg/kg, intratracheally), TDI + Z-Phe-Tyr-CHO (Z-FY-CHO, 5 mg/kg, intratracheally), MMP8 inhibitor-1 (20 mg/kg, intratracheally), ATF4-IN-1 (5 mg/kg, intratracheally), Dexamethasone (1 mg/kg, intratracheally) and Tunicamycin (1 mg/kg, intratracheally) dissolved in DMSO and saline were given to the mice immediately before each sensitization and airway challenge. The dosages for in vivo and in vitro experiments were determined with reference to previous studies and adjusted according to differences in administration routes to ensure safety, and relatively low doses were selected to avoid excessive pharmacological effects [12,25–31]. Sham mice were given the same amount of vehicle.

2.5. Establishment of the HDM/LPS-induced mice asthma model

60 mice were averagely randomized to 5 groups. Mice were sensitized and challenged with 20 μg HDM i.n. on day 0, day 7, days 14–18 and days 21–25. LPS (10 μg /mice) was treated intratracheally on day 21. The mice in control group were sensitized and challenged by inhaling saline. CBCO (2 mg/kg, intratracheally), TDI + Z-FY-CHO (5 mg/kg, intratracheally) ATF4-IN-1 (5 mg/kg, intratracheally) and MMP8 inhibitor-1 (20 mg/kg, intratracheally) dissolved in DMSO and saline were given to the mice immediately before each sensitization and airway challenge.

2.6. Sample size calculation

Sample size was determined based on previous similar studies [7] and power analysis to detect significant differences in airway inflammation. The final sample size will be larger than the number required for calculation to meet different experimental needs.

2.7. Blinding

Group allocation was concealed during the allocation process and the conduct of the experiment. Investigators responsible for outcome assessment and data analysis were blinded to the group assignments to minimize bias.

2.8. Preparation of TDI-human serum albumin (HSA) conjugates

In accordance with our reported procedure [32], TDI-human serum albumin (HSA) conjugates were prepared.

2.9. Cell culture and treatment

Primary human airway epithelial cells (HAECs) were obtained from ATCC. They were expanded in PneumaCult™-Ex Plus Medium. Once reaching 85% convergence in tissue culture flasks (NEST Science Co.Ltd, Shanghai, China), the cells were passaged and seeded into suitable culture plates at a density of 1×10^5 cells/cm². The dispersed cells were then passaged at the same plating density into further T25 flasks or seeded on apical chambers of 12 mm Transwell (0.4 μm Pore Polyester Membrane Inserts, Costar). Within the inserts, cells were incubated at 37°C until 100% confluence was reached (2–4 days). Upon reaching confluence, the medium for expansion was removed from the inserts and washed with D-PBS. Then the PneumaCult™-ALI Maintenance Medium was added to the basal chamber only, leaving the apical chamber empty, to create an air-liquid interface (ALI) for the cells. The PneumaCult-ALI protocol for Maintenance Phase was then followed for 28 days. Cells within five passages were used for our experiments. Cells were harvested for protein extraction and supernatant in the basal chamber was collected 24 h post exposure. A test for mycoplasma contamination should be performed prior to each cell respassaging and sample harvesting to ensure the absence of contamination.

2.10. Assessment of airway hyperresponsiveness (AHR)

Mice was endotracheal intubated and placed in a chamber to measure airway responsiveness to methacholine on day 25. To achieve this, lung resistance (RL) was gauged in response to escalating dosages (3.125, 6.25, 12.5 and 25 mg/mL) of methacholine through ultrasonic nebulization on anesthetized and mechanically ventilated mice. After every nebulization process, measurements of RL were taken at 5 min intervals until a plateau phase was attained. The results were presented as a percentage of the RL value at baseline for each methacholine concentration.

2.11. Analysis of serum

After resting at room temperature for an hour, the blood samples were centrifuged (3000 g, 20 min), and the supernatant fluid was collected and kept at -80°C . Following the manufacturer's instructions, we evaluated total serum IgE and MMP8 via ELISA kits.

2.12. Analysis of bronchoalveolar lavage (BAL) fluids

The number of cells in BAL fluid was calculated via cell counter (Count-star, Shanghai, China). Then, the BAL fluid was centrifuged (1000 g, 10 min). According to the manufacturer's recommendations, the supernatant was frozen (-80°C) for subsequent Luminex multiplex assay (IL-1 β , IL-4, IL-5, IL-17A, IL-13, TNF- α). To classify different cells, cytosin samples were made from cell precipitation after centrifugation and stained using hematoxylin and eosin (H&E). A total of 200 cells from each sample were counted to determine the number of macrophages, eosinophils, neutrophils, and lymphocytes.

2.13. Pulmonary histopathological examination

The left lungs of the mice were separated, fixed with 4% paraformaldehyde, then dehydrated, and paraffin embedded. Lung tissue sections (4 μ m) were cut and stained with periodic acid-Schiff (PAS), H&E, Masson's trichrome and Immunofluorescence. Briefly, two criteria were scored to document the pulmonary inflammation: peribronchial inflammation and perivascular inflammation. A value of 0 was adjudged when no inflammation was detectable, a value of 1 for occasional cuffing with inflammatory cells, a value of 2 for most bronchi or vessels surrounded by thin layer (one to ten cells) of inflammatory cells and a value of 3 when most bronchi or vessels were surrounded by a thick layer (more than ten cells) of inflammatory cells [33]. As 8–10 tissue sections per mouse were scored, inflammation scores could be expressed as a mean value and could be compared between groups. Sections were assigned a random code to blind the examiner to the identity of each specimen.

2.14. Quantitative PCR (qPCR)

In accordance with the manufacturer's instructions, total RNA was extracted with the use of the Total RNA Extraction Reagent. Using 2 μ g of RNA in 20 μ l reaction buffer of HiScript® IV All-in-one RT SuperMix Perfect for qPCR kits and random primers, the first strand of complementary DNA was generated by sequentially heating the mixture to 37 °C for 15 min and 85 °C for 5 s. Real-time PCR was performed using a ChamQ Universal SYBR qPCR Master Mix. All primer sequences were listed in Table S1.

2.15. Detection of cell survival rate

Cell Counting Kit-8 (Beyotime, China) was utilized for the detection of cell survival rate in accordance with the manufacturer's protocol.

2.16. Detection of cell permeability

Cell permeability was detected by FITC-labeled Dextran (Beyotime, Shanghai, China) according to manufacturer's protocols.

2.17. Analysis of datasets from Gene Expression Omnibus

The datasets used in this study are sourced from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>), with the downloaded data in MINiML format. The detailed processing procedure can be found in the method description on the dataset selection page. Statistical analysis was conducted using R software, version v4.0.3. Results were considered statistically significant when the p-value was less than 0.05.

2.18. Detection of eDNA

The BALF and serum of mice were collected for measurement of eDNA, using Quant-iT PicoGreen DNA reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions.

2.19. Measurement of oxidative stress levels

Total antioxidant capacity (TAC), malonaldehyde (MDA), and superoxide dismutase (SOD) activity were assayed using the TAC assay kit, MDA assay kit, and SOD assay kit, respectively, according to the manufacturer's instructions.

2.20. Molecular docking

The crystal structure of CTSL was obtained from the RCSB Protein Data Bank (PDB ID: 7Z3T). The docking grid box was defined according

to the reported active sites S1, S2, S3, S1', S2', and S3' [34]. The compound CBCO, which exists as a neutral molecule under physiological conditions, was retrieved from the PubChem database in the form of a SMILES string. Twenty three-dimensional conformations of CBCO were generated using RDKit and subsequently energy-minimized with OpenBabel.

Molecular docking of CTSL with each CBCO conformation was performed using AutoDock Vina, generating 20 docking poses per ligand conformation. The resulting docking complexes were ranked based on their binding affinity scores. The optimal binding pose was selected considering both the docking score and detailed interaction analyses, including hydrogen bonding, van der Waals forces, and other non-covalent forces.

2.21. Molecular dynamics simulation

To further investigate the dynamic stability and interaction behavior of the CTSL-CBCO complex, all-atom molecular dynamics (MD) simulations were conducted using the GROMACS package (version 2022.6) with the AMBER14SB force field [35]. The RESP charges and ligand topology files were generated using Multiwfn in combination with ORCA [36,37], followed by topology conversion with sobtop, while the protein topology was prepared with GROMACS. The complex was solvated in a dodecahedron periodic box containing TIP3P water molecules, and the system was neutralized by adding appropriate counterions.

Energy minimization was performed using the conjugate gradient algorithm until the maximum force on any atom was reduced below 100.0 k mol⁻¹ nm⁻¹. The minimized system was equilibrated under position restraints for 5 ns to allow proper relaxation of solvent molecules and ions. Subsequently, a 50 ns unrestrained production MD simulation was carried out under isothermal-isobaric (NPT) ensemble conditions at 298.15 K and 1 bar, maintained using the V-rescale thermostat and Parrinello-Rahman barostat, respectively.

Trajectory data from the production phase were analyzed to calculate the root-mean-square deviation (RMSD) and binding free energy using gmx_MMPBSA, in order to evaluate the conformational stability and binding affinity of the CTSL-CBCO complex throughout the simulation. The dynamic behavior of the last 5 ns of the production trajectory was visualized and converted into a video using PyMOL.

2.22. Detection of reactive oxygen species (ROS)

ROS level in HAECs were measured by DCFH-DA fluorescent probe in accordance with the manufacturer's protocol (Reactive Oxygen Species Assay Kit, Solarbio).

2.23. Detection of autophagy

Autophagy level in HAECs were measured by monodansylcadaverine (MDC) in accordance with the manufacturer's protocol (Autophagy Staining Assay Kit with MDC, Beyotime).

2.24. Transcriptomics

Total RNA was extracted from lung tissue using the accurate cracking mixture reagent (Singleron, China). RNA integrity was assessed with the Agilent Bioanalyzer 2100. Sequencing libraries were prepared using the Illumina TruSeq kit and sequenced on the Illumina platform. Differentially expressed genes were identified using DESeq2 with adjusted p < 0.05 as the cutoff. Data was stored in BioStudies database (S-BSST2238).

2.25. Cellular thermal shift assay (CETSA)

Cells were treated with the indicated compounds or vehicle,

harvested, and heated at defined temperatures for 3 min. After cooling, cells were lysed by freeze–thaw cycles, and soluble proteins were collected by centrifugation. Target protein levels were analyzed by immunoblotting to assess changes in thermal stability.

2.26. Statistical analysis

Statistical analyses were performed using SPSS 26.0 software. Continuous variables were expressed as mean $\pm$ standard error of the mean (SEM). Normality of data distribution was assessed using the Shapiro–Wilk test. For comparisons among multiple groups, one-way analysis of variance (ANOVA) was applied, followed by Bonferroni post hoc correction for multiple comparisons. When data did not meet the assumptions of normality, nonparametric tests were applied as appropriate. Correlation analyses were performed using Spearman's rank correlation due to the non-normal distribution of most clinical

parameters. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of candidate biomarkers, and the area under the curve (AUC) with 95% confidence intervals (CIs) was calculated. The optimal cutoff value was determined using the Youden index. Two-way repeated-measures ANOVA with Bonferroni's post hoc test was used to analyze airway hyper-responsiveness curves. All statistical tests were two-sided, and a P value < 0.05 was considered statistically significant.

3. Results

3.1. Meroterpenoid fraction analysis of *R. capitatum maxim*

The aerial parts of *R. capitatum* Maxim (20.0 kg) were extracted with 70% aqueous ethanol, and the extract was partitioned with EtOAc to afford an EtOAc-soluble fraction (2.7 kg). This fraction was subjected to

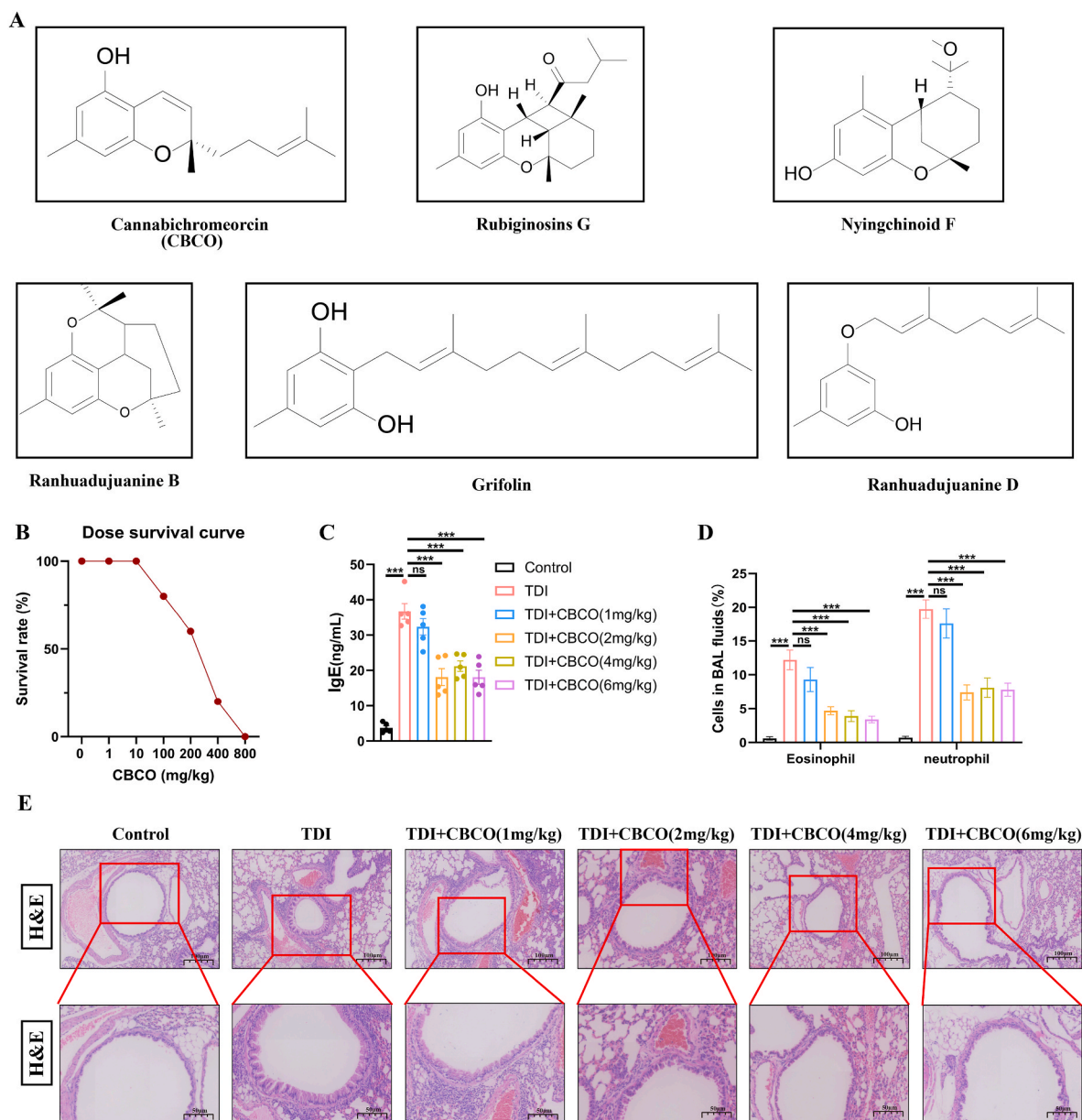


Fig. 1. Meroterpenoid fraction analysis of *Rhododendron capitatum* Maxim.

(A) Molecular structure of different Meroterpenoid fractions from *Rhododendron capitatum* Maxim. (B) Dose survival curve of different dosage of CBCO ($n = 10$). (C) Measurement of IgE in serum by ELISA ($n = 5$). (D) Total and differential inflammatory cells in BALF ($n = 5$). (E) Representative H&E stained lung sections of different groups ($n = 5$). * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$, ns > 0.05 .

successive chromatographic separations, including silica gel, MCI gel, and Sephadex LH-20 column chromatography, followed by semi-preparative HPLC, to yield six meroterpenoids (Fig. 1A). From Fr. 3.2, compound 1 (Cannabichromeorcin, 67.0 mg) was obtained. Fraction 4 was further separated to afford compound 2 (Rubiginosins G, 28.0 mg) from Fr. 4.1.3, compound 3 (Nyingchinoid F, 32.0 mg) and compound 4 (Ranhuaduanine B, 7.3 mg) from Fr. 4.2.1, compound 5 (Grifolin, 7.0 mg) from Fr. 4.2.2, and compound 6 (Ranhuaduanine D, 7.3 mg)

from Fr. 4.3.2. As Cannabichromeorcin (CBCO) represented the most abundant meroterpenoid and was reported here for the first time in *Rhododendron capitatum* Maxim, it was selected for further structural and bioactivity investigations.

To determine the minimum effective dose of CBCO, we performed a dose–response survival analysis. Our findings indicated that intra-tracheal administration of CBCO at 10 mg/kg resulted in no mortality among mice within 14 days (Fig. 1B). We then established a steroid-

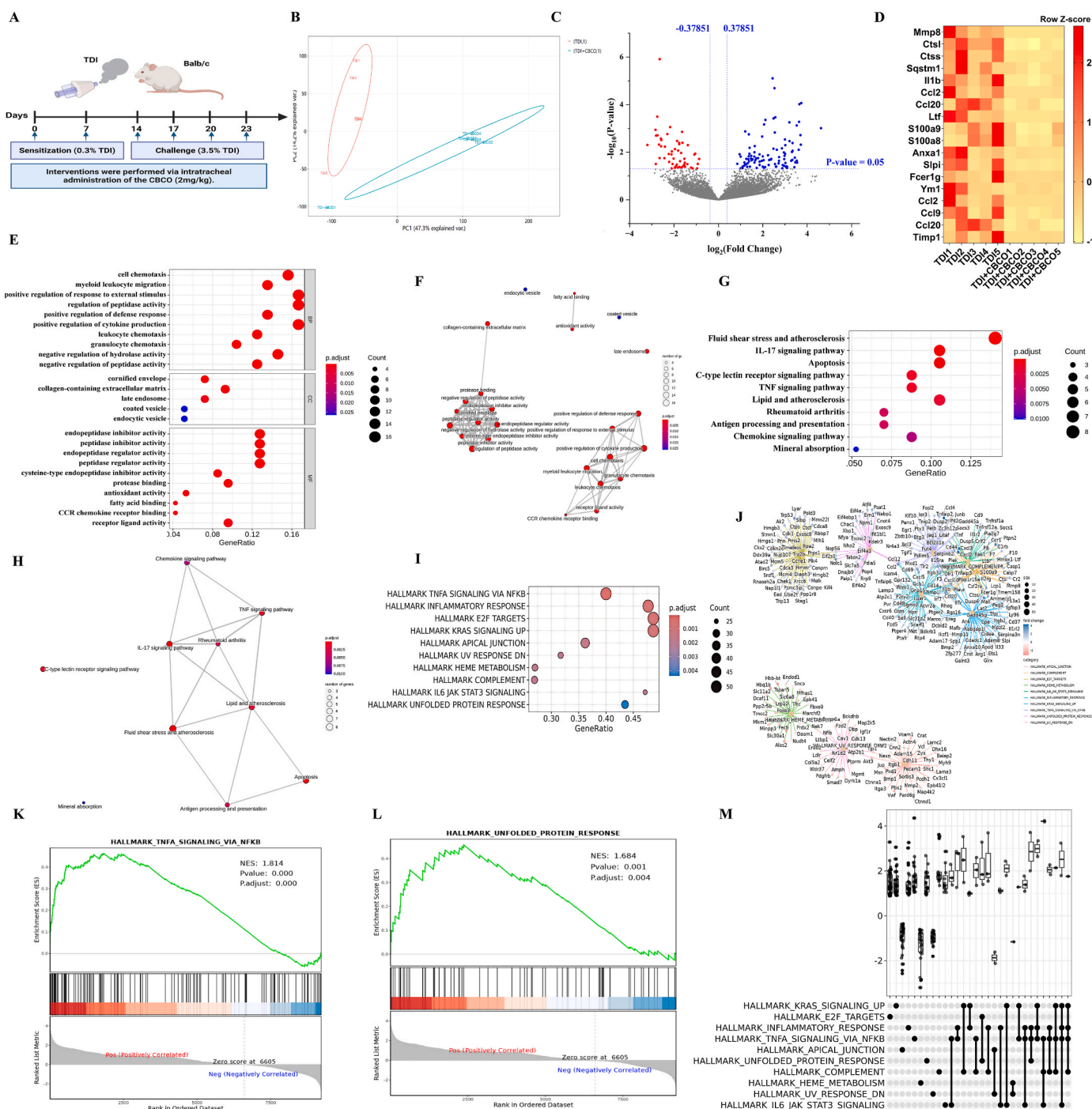


Fig. 2. Transcriptomics analysis of CBCO-treated TDI asthma group and TDI asthma group.

(A) Schematic diagram representation of experimental plan. (B) Principal component analysis (PCA) of transcriptomics. (C) Volcano analysis of transcriptomics (TDI vs TDI + CBCO). (D) Heat map analysis of inflammatory differential genes. (E) GO pathway enrichment of the differential genes of transcriptomics. (F) Network analysis of GO pathway enrichment. (G) Kegg pathway enrichment of the differential genes of transcriptomics. (H) Network analysis of Kegg pathway enrichment. (I) GSEA pathway enrichment of transcriptomics. (J) Network analysis of GSEA pathway enrichment. (K) GSEA pathway enrichment analysis of TNFA signaling via NF- κ B pathway. (L) GSEA pathway enrichment analysis of Unfolded protein response pathway. (M) Multiple hallmark pathways analysis of GSEA pathway.

insensitive asthma model by TDI, as evidenced by previous research [12, 38] and our validation (Fig. S13). As shown in Fig. 1C–E, TDI-exposed mice were treated with varying doses of CBCO (1, 2, 4, and 6 mg/kg). Administration of 1 mg/kg CBCO failed to reduce the TDI-induced elevations in immunoglobulin E (IgE), neutrophil and eosinophil counts, and airway inflammation. In contrast, treatment with higher doses of CBCO (2–6 mg/kg) significantly alleviated these pathological changes. Therefore, a dose of 2 mg/kg was selected for subsequent experiments in TDI-exposed mice.

3.2. Transcriptomics analysis of CBCO-treated TDI asthma group and TDI asthma group

RNA sequencing (RNA-seq) analysis revealed that CBCO treatment markedly reversed the transcriptomic alterations induced by TDI exposure (Fig. 2A–C). As shown in Fig. 2D and Table S2, heatmap analysis demonstrated that CBCO regulated oxidative stress-related genes (*Hmox1*, *Gclc*, *Gclm*, *Txnrd1*, *Akr1b8*, *Ptgr1*), and significantly suppressed the expression of neutrophilic inflammation-related genes (*Mmp8*, *Il1b*, *Ctss*, *Ltf*, *S100a8*, *S100a9*, *Anxa1*, and *Slpi*) [39–45], as well as eosinophilic inflammation-related genes (*Fcer1g*, *Ym1*, *Ccl2*, *Ccl9*, *Ccl20*, and *Timp1*) [46–51]. Gene Ontology analysis indicated significant enrichment in pathways related to antioxidant activity, leukocyte chemotaxis, and granulocyte chemotaxis (Fig. 2E and F). Moreover, gene set enrichment analysis and Kyoto Encyclopedia of Genes and Genomes

pathway analysis revealed that differentially expressed genes (DEGs) were predominantly enriched in inflammatory response pathways, including the unfolded protein response (UPR), TNF- α , IL-17, IL-6, and NF- κ B signaling pathways (Fig. 2G–J). Specifically, gene set enrichment analysis showed significant enrichment of the UPR pathway (NES = 1.684, adjusted p = 0.004) and TNF- α signaling via NF- κ B (NES = 1.814, adjusted p < 0.001) in the TDI group compared with the TDI + CBCO group, indicating that CBCO effectively inhibited these inflammatory pathways (Fig. 2K and L). Multiple hallmark pathways—including TNF- α signaling via NF- κ B, IL-6/JAK/STAT3 signaling, inflammatory response, and UPR—were significantly enriched in the disease group relative to the treatment group, with substantial gene overlap, suggesting coordinated activation of inflammatory and stress-related signaling under disease conditions (Fig. 2M). Collectively, these findings provide a robust foundation for subsequent mechanistic studies and underscore the potential of CBCO as a candidate anti-inflammatory therapy for SA.

3.3. Computational prediction and target validation of CBCO

To elucidate the potential molecular targets of CBCO, we performed computational target prediction using TargetNet (<http://targetnet.scbdd.com/>) based on its SMILES sequence (CC1=CC(=C2C=CC(OC2=C1)(C)CCC=C(C)C)O). By computational drug-target prediction, we identified a total of 580 predicted targets enriched in airway

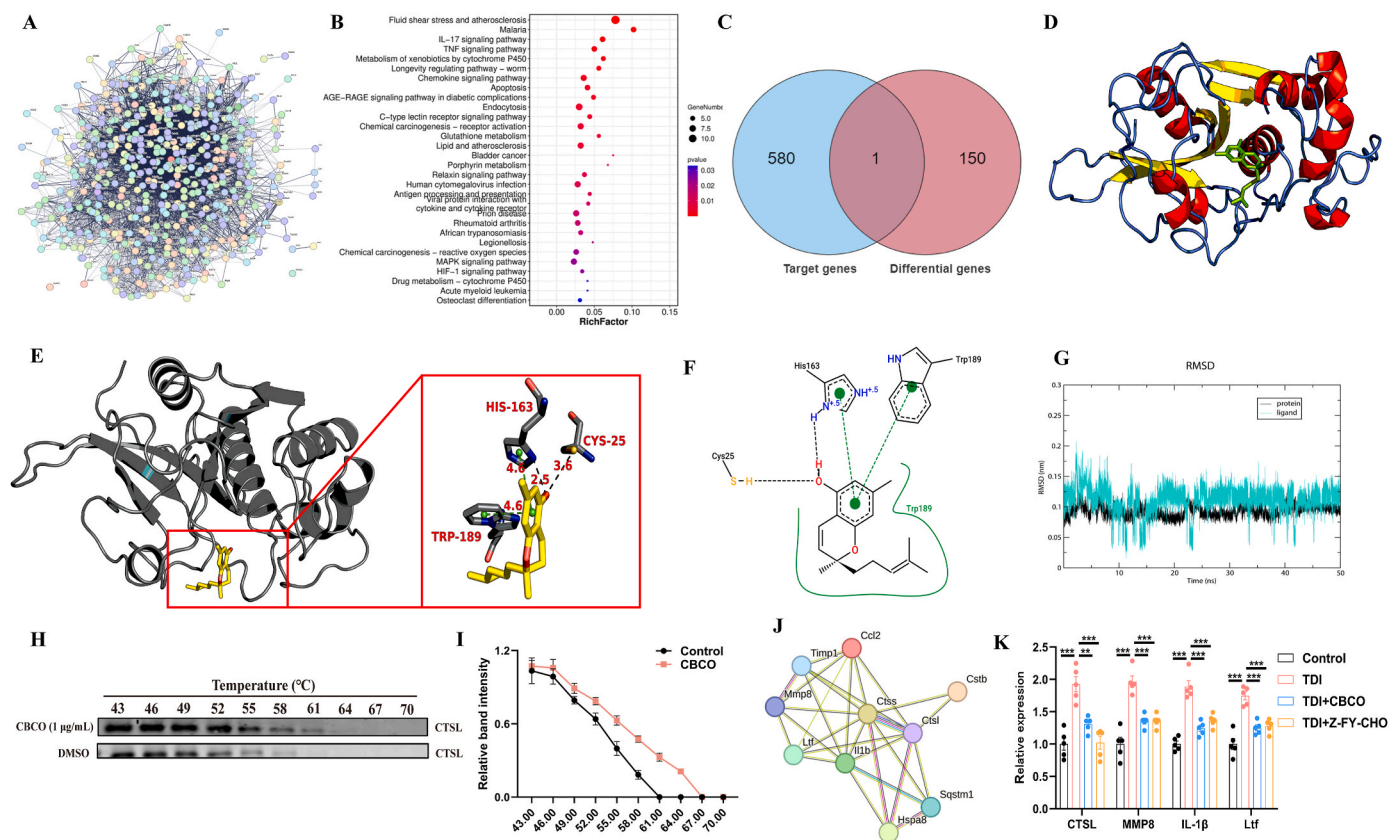


Fig. 3. Computational prediction and target validation of CBCO.

(A) String analysis of target genes of CBCO predicted by Target-Net. (B) Kegg pathway enrichment of target genes of CBCO predicted by Target-Net. (C) Venn diagram analysis of target genes of CBCO predicted by Target-Net and differential genes of transcriptomics. (D) Schematic diagram of CBCO-CTSL molecular docking. (E) 3D schematic diagram of CBCO-CTSL molecular dynamics simulation (Green dashed line: π - π interaction; Black dashed line: hydrogen bond; Grey cartoon: CTSL; Yellow sticks: CBCO; Grey sticks: Amino acids that interact with CTSL and CBCO). (F) 2D schematic diagram of CBCO-CTSL molecular dynamics simulation (Green dashed line: π - π interaction; Black dashed line: hydrogen bond; Green solid line: hydrophobic effect). (G) The RMSD plot over time of CBCO and CTSL, displays the changes in the spatial structure of CBCO and CTSL over 100ns, highlighting any deviations from its original conformation. (H–I) CETSA analysis of the effect of CBCO on HAECs. (J) String analysis of CTSL and differential genes of transcriptomics. (K) qPCR analysis of the mRNA expression levels of CTSL, MMP8, IL-1 β , Ltf. * P < 0.05, ** P < 0.005, *** P < 0.0005, ns > 0.05.

inflammation-related pathways, including the IL-17 signaling pathway, TNF signaling pathway, apoptosis, and AGE-RAGE signaling pathway (Fig. 3A and B). Venn diagram analysis comparing the 580 predicted targets with 150 DEGs of transcriptomic data (TDI + CBCO vs TDI) identified CTSL as the sole overlapping target (Fig. 3C). Furthermore,

RNA-seq analysis revealed that CTSL was upregulated in severe asthma models induced by TDI or HDM/LPS compared to control group (Fig. S14). CTSL was strongly related to oxidative stress-related genes, neutrophilic inflammation-related genes, as well as eosinophilic inflammation-related genes.

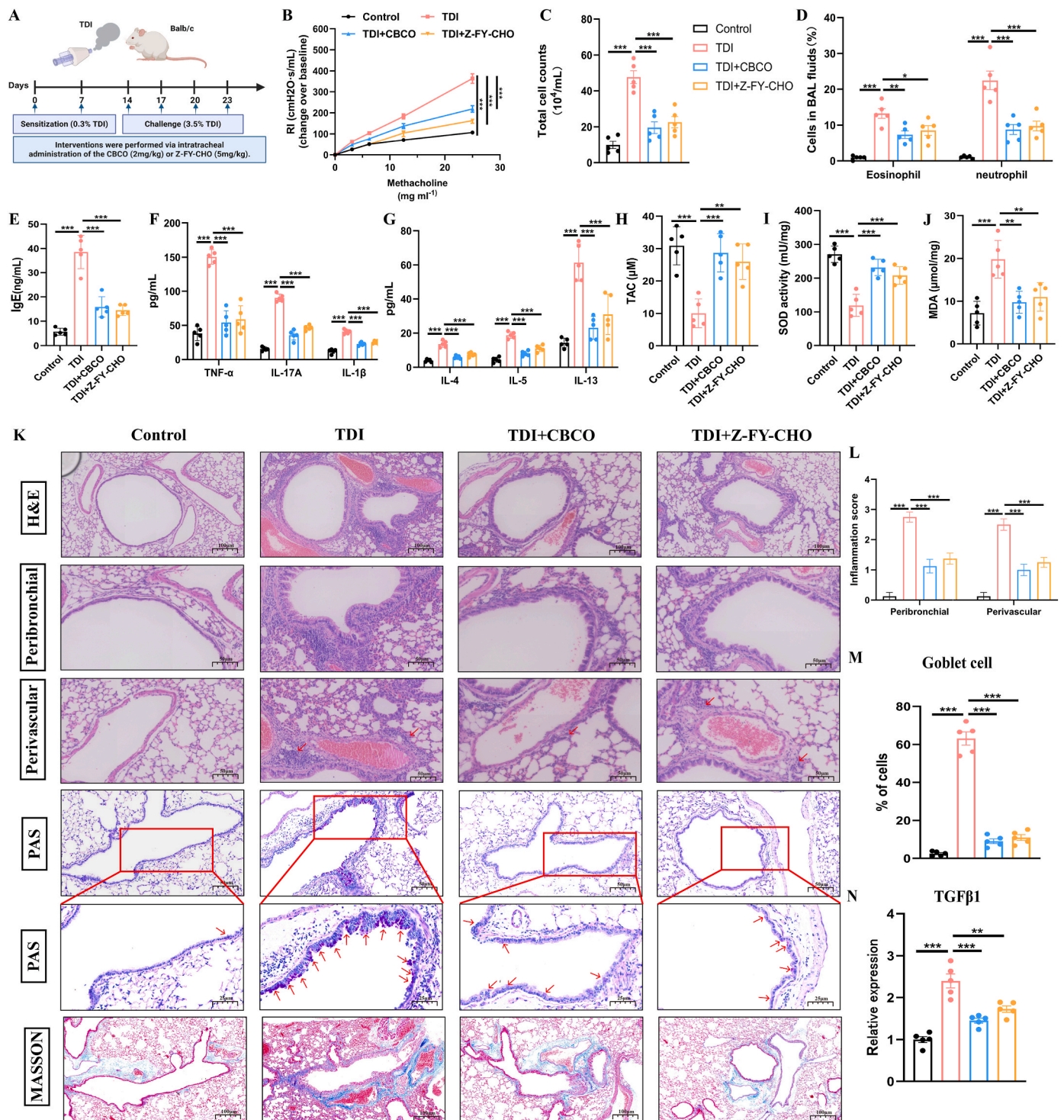


Fig. 4. CBCO or pharmacological inhibition of CTSL attenuates the development of TDI-induced asthma and reduces NETs formation.

(A) Schematic diagram representation of experimental plan. (B) Airway hyperresponsiveness was measured by lung resistance (RL). Results were shown as percentage over baseline value. (C–D) Total and differential inflammatory cells in BALF. Percentages of different inflammatory cells were calculated by counting a total of 200 cells in H&E stained cytospin samples. (E) Measurement of serum IgE by ELISA. (F–G) Measurement of IL-1 β , IL-4, IL-5, IL-13, IL-17A, TNF- α in BALF by ELISA. (H) Measurement of TAC in BALF. (I–J) Measurement of SOD, MDA in lung tissues. (K) Representative H&E stained lung sections of different groups, and periodic acid-Schiff base (PAS) stained sections and Masson's trichrome stained sections of different groups. (L–M) Semi-quantitative analysis of airway inflammation and goblet cell metaplasia. (N) qPCR analysis of the mRNA expression level of TGF β 1. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$, ns > 0.05 , $n = 5-8$.

Molecular docking analysis revealed that CBCO conformation 9 exhibited the most favorable binding interaction with CTSL, with a docking score of -6.745 kcal/mol. CBCO formed hydrogen bonds with Cys25 and His163 at the S1 site, a π - π interaction with His163, and additional π - π stacking and hydrophobic interactions with Trp189 at the S1' site, indicating high binding affinity (Fig. 3D; Table S3). Molecular dynamics simulations further confirmed the stability of the CTSL–CBCO complex (Fig. 3E–G; Table S4). The RMSD values remained stable below 0.2 nm, and the binding free energy calculated using *gmx_MMPBSA* was -26.03 kcal/mol. Visualization of the final 5 ns trajectory in PyMOL demonstrated a consistently stable CTSL–CBCO complex throughout the simulation (Video 1). CETSA analysis showed that CBCO treatment increased the thermal stability of the target protein in primary human airway epithelial cells (HAECs), as evidenced by a rightward shift in the thermal denaturation curve and an elevation of the apparent melting temperature (Fig. 3H–I).

Supplementary data related to this article can be found online at <http://doi.org/10.1016/j.redox.2026.104232>

STRING (<https://string-db.org/>) protein–protein interaction network analysis of CTSL and transcriptomic DEGs revealed interactions between CTSL and *MMP8*, *IL1B*, *LTF*, *CTSS*, and *HSPA8* (Fig. 3J). In vivo validation showed that treatment with either CBCO or the CTSL inhibitor Z-Phe-Tyr-CHO (Z-FY-CHO; 5 mg/kg, intratracheally) significantly reduced the expression of *Ltf*, *Ctsl*, *Mmp8*, and *Il1b* in the lung tissues of TDI-exposed mice (Fig. 3K). Collectively, CETSA, molecular docking, molecular dynamics simulations, and functional inhibition studies confirm CTSL as a molecular target engaged by CBCO, suggesting that CBCO inhibits airway inflammation (*MMP8*, *IL1B*, and *Ltf*) [39,40,42] through CTSL-mediated pathways.

3.4. CBCO or pharmacological inhibition of CTSL attenuates the development of TDI-induced asthma and reduces NETs formation

To further validate these findings, we administered intratracheal injections of either CBCO or the CTSL inhibitor Z-FY-CHO to TDI-induced asthmatic mice, as illustrated in Fig. 4A. TDI exposure markedly increased AHR, serum IgE, proinflammatory cytokines (TNF- α , IL-1 β , IL-4, IL-5, IL-13, and IL-17A), as well as total cell counts, eosinophils, and neutrophils in bronchoalveolar lavage fluid (BALF) (Fig. 4B–G). Moreover, levels of NETs components—including MPO, extracellular DNA, MMP8, and neutrophil elastase (NE)—were markedly elevated in both serum and BALF samples from TDI-exposed mice, accompanied by significant elevated levels of citrullinated histone H3 (CitH3) combined with MPO around airways (Fig. S15). Pretreatment with either CBCO or Z-FY-CHO markedly attenuated these changes above.

Pretreatment with CBCO or Z-FY-CHO significantly enhanced total antioxidant capacity (TAC) and superoxide dismutase (SOD) activity while reducing malondialdehyde (MDA) levels (Fig. 4H–J). Histopathological and semi-quantitative analyses revealed that TDI exposure induced characteristic features of airway inflammation, including peribronchial and perivascular inflammatory cell infiltration, mucus hypersecretion, and collagen deposition, as demonstrated by H&E, PAS, and Masson's trichrome staining, respectively (Fig. 4K–N). Notably, pretreatment with either CBCO or Z-FY-CHO markedly attenuated these pathological changes. Collectively, these results support that CBCO exerts a protective effect against TDI-induced airway hyperresponsiveness, inflammatory cell infiltration, and NET formation, while restoring antioxidant defenses and ameliorating histopathological damage, likely through modulation of key regulatory nodes.

3.5. Inhibition of MMP8 attenuates the development of TDI-induced asthma

Among these CTSL-associated molecules, MMP8 exhibited potential interaction with CTSL and its consistent reduction following both CBCO treatment and CTSL inhibition in serum, BALF, and lung tissues,

suggesting that MMP8 may represent a downstream effector associated with CTSL-mediated inflammatory responses and NET formation in TDI-induced asthma. To further evaluate the role of MMP8 in TDI-induced asthma, we treated TDI-exposed mice with MMP8 inhibitor-1 (Fig. 5A). As expected, TDI exposure markedly increased AHR, serum IgE levels, proinflammatory cytokines (TNF- α , IL-1 β , IL-4, IL-5, IL-13, and IL-17A), and inflammatory cell infiltration (Fig. 5B–G). Treatment with MMP8 inhibitor-1 significantly enhanced TAC and SOD activity while decreasing MDA levels (Fig. 5H–J).

Histopathological examination revealed characteristic asthmatic lesions, including peribronchial and perivascular inflammatory cell infiltration, mucus hypersecretion, and collagen deposition, as demonstrated by H&E, PAS, and Masson's trichrome staining (Fig. 5K–N). Notably, inhibition of MMP8 substantially attenuated these inflammatory and structural abnormalities, resulting in decreased cytokine release and reduced airway remodeling.

In vitro, cell viability detection under recombinant human MMP8 stimulation with different gradients suggested that 2 μ g/mL might be the lowest concentration that caused significant cell deaths on primary human airway epithelial cells (HAECs) (Fig. 5O). Treatment with recombinant human MMP8 protein (2 μ g/mL) not only increased oxidative stress, autophagy, and the expression of epithelial alarmins (IL-33 and thymic stromal lymphopoietin [TSLP]) but also caused epithelial disruption, leading to structural cell damage and disorganization (Fig. 5P–V).

Collectively, these findings support a central role for MMP8 in TDI-induced airway inflammation, oxidative stress, and tissue remodeling, and indicate that pharmacological inhibition of MMP8 exerts a protective effect against asthmatic pathology by targeting this key regulatory node.

3.6. CBCO attenuates the development of TDI-induced asthma by inhibiting unfolded protein response

Given that oxidative stress functions as a key upstream driver of proteolytic and inflammatory cascades—disrupting cellular homeostasis and activating adaptive signaling pathways—and that our transcriptomic analysis identified activation of the UPR with a potential association with airway inflammation, we next examined the interplay among oxidative stress, the UPR, CTSL, and MMP8. Transcriptomic profiling demonstrated that CBCO inhibited the expression of the UPR-related transcription factors *Atf4* and *Ddit3* (Fig. 6A). Correlation analysis further revealed a strong positive association between UPR-related transcription factors (*Atf4* and *Ddit3*) and airway inflammation-associated genes (*S100a9*, *S100a8*, *Anxa1*, *Slpi*, *Il1b*, *Mmp8*, and *Ctsl*) (Fig. 6B; Table S5). Quantitative analysis confirmed that treatment with CBCO or inhibition of CTSL or MMP8 significantly downregulated *Atf4* and *Ddit3* expression (Fig. 6C).

These findings highlight a strong interconnection between the UPR and the CTSL–MMP8 axis. Given the lack of specific pharmacological inhibitors targeting DDIT3, we further investigated whether inhibition of its upstream regulator ATF4 could help validate the involvement of this pathway in airway inflammation (Fig. 6D). Pharmacological inhibition of ATF4 using ATF4-IN-1 markedly reduced AHR, serum IgE levels, proinflammatory cytokines (TNF- α , IL-1 β , IL-4, IL-5, IL-13, and IL-17A), oxidative stress, and inflammatory cell infiltration (Fig. 6E–M). Consistently, histopathological examination demonstrated that ATF4-IN-1 treatment attenuated peribronchial and perivascular inflammatory cell infiltration, mucus hypersecretion, collagen deposition and the levels of NETs components in BALF and serum, CitH3 and MPO combination in lung tissues (Fig. 6N–Q, Fig. S16).

To determine whether UPR activation is functionally required for the protective effects conferred by MMP8 or CTSL inhibition in TDI-induced asthma, rescue experiments were performed by pharmacologically reactivating UPR signaling with tunicamycin under conditions of MMP8 or CTSL suppression (Fig. S17). Notably, forced activation of UPR

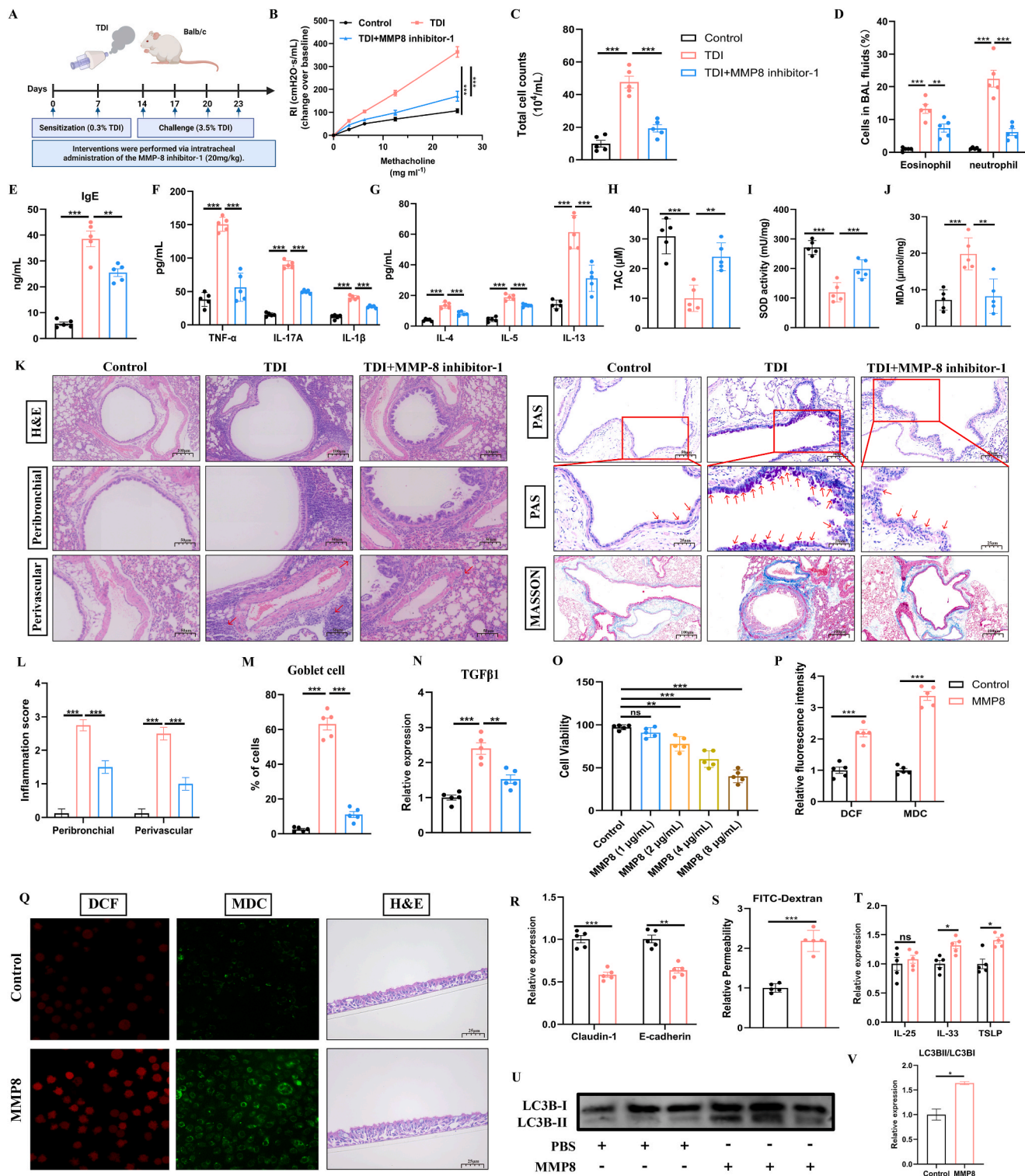


Fig. 5. Inhibition of MMP8 attenuates the development of TDI-induced asthma.

(A) Schematic diagram representation of experimental plan. (B) Airway hyperresponsiveness was measured by lung resistance (RL). Results were shown as percentage over baseline value. (C–D) Total and differential inflammatory cells in BALF. Percentages of different inflammatory cells were calculated by counting a total of 200 cells in H&E stained cytopsin samples. (E) Measurement of serum IgE by ELISA. (F–G) Measurement of IL-1 β , IL-4, IL-5, IL-13, IL-17A, TNF- α in BALF by ELISA. (H) Measurement of TAC in BALF. (I–J) Measurement of SOD, MDA in lung tissues. (K) Representative H&E stained lung sections of different groups, and periodic acid-Schiff base (PAS) stained sections and Masson's trichrome stained sections of different groups. (L–M) Semi-quantitative analysis of airway inflammation and goblet cell metaplasia. (N) qPCR analysis of the mRNA expression level of TGF β 1. (O) Detection of cytotoxicity by CCK8 of different groups. (P) Relative fluorescence intensity of DCF stained and MDC stained HAECs. (Q) Representative DCF stained, MDC stained and H&E stained HAECs of different groups. (R) qPCR analysis of the mRNA expression levels of E-cadherin and Occludin of the ALI-cultured-HAECs. (S) Relative permeability of ALI-cultured-HAECs detected by FITC-Dextran. (T) qPCR analysis of the mRNA expression levels of TSLP, IL-25, IL-33 of the ALI-cultured-HAECs. (U–V) Western blot analysis of expression levels of LC3B. *P < 0.05, **P < 0.005, ***P < 0.0005, ns > 0.05, n = 5–8.

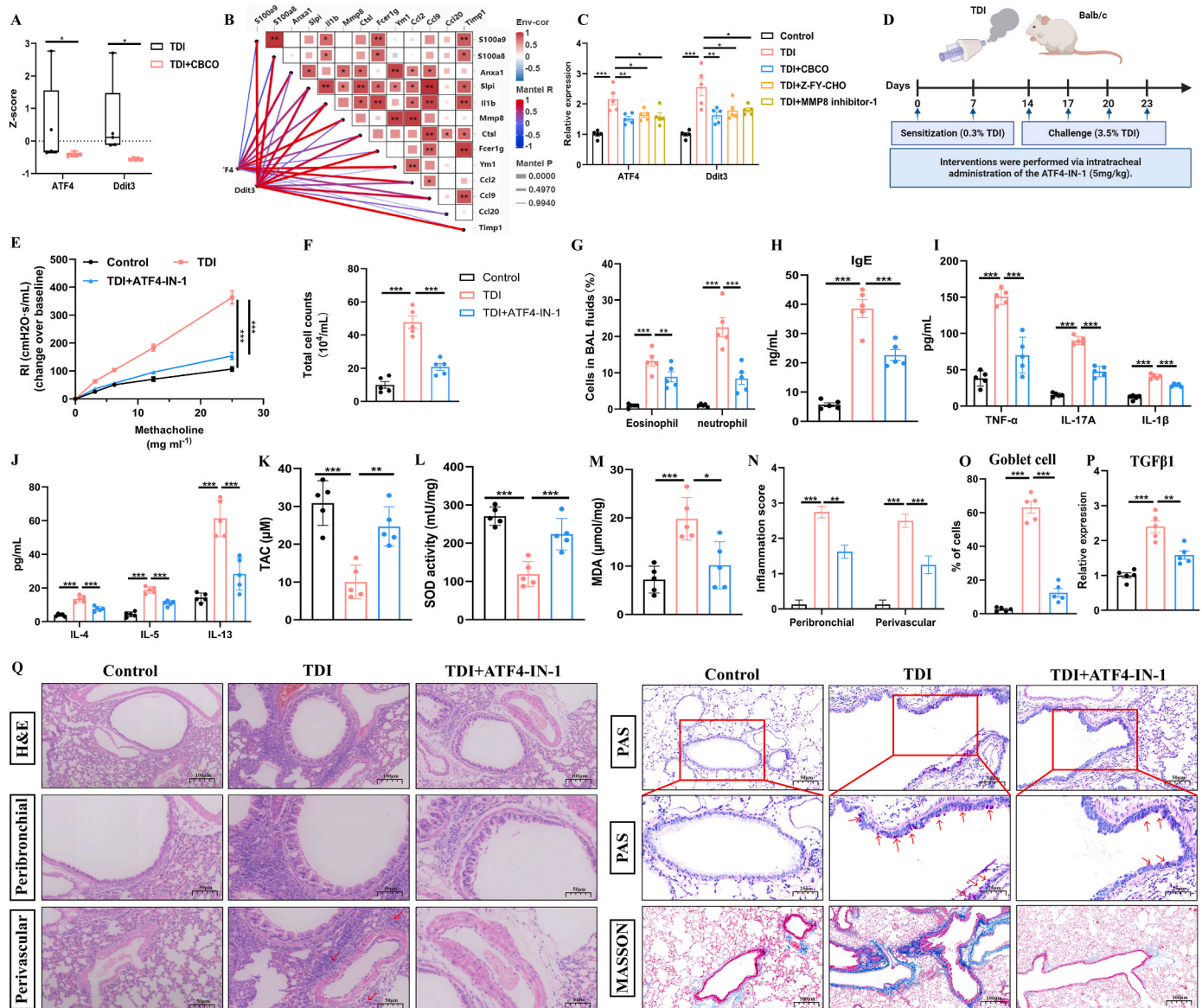


Fig. 6. CBCO attenuates the development of TDI-induced asthma by inhibiting unfolded protein response. (A) Z-score of ATF4 and Ddit3 of transcriptomics. (B) Heat map of correlation analysis between unfolded protein response-related transcription factors (ATF4, Ddit3) and inflammation-related genes (S100a9, S100a8, Anxa1, Slpi, IL-1 β , MMP8, CTSL). (C) qPCR analysis of the mRNA expression levels of ATF4 and Ddit3. (D) Schematic diagram representation of experimental plan. (E) Airway hyperresponsiveness was measured by lung resistance (RL). Results were shown as percentage over baseline value. (F–G) Total and differential inflammatory cells in BALF. Percentages of different inflammatory cells were calculated by counting a total of 200 cells in H&E stained cytospin samples. (H) Measurement of serum IgE by ELISA. (I–J) Measurement of IL-1 β , IL-4, IL-5, IL-13, IL-17A, TNF- α in BALF by ELISA. (K) Measurement of TAC in BALF. (L–M) Measurement of SOD, MDA in lung tissues. (N–O) Semi-quantitative analysis of airway inflammation and goblet cell metaplasia. (P) qPCR analysis of the mRNA expression level of TGF β 1. (Q) Representative H&E stained lung sections of different groups, and PAS stained sections and Masson's trichrome stained sections of different groups. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$, ns > 0.05 , $n = 5-8$.

signaling partially attenuated the protective effects conferred by MMP8 or CTSL inhibition. Although UPR reactivation robustly induced canonical UPR markers, including *Atf4* and *Ddit3*, it failed to fully restore AHR, airway inflammation and oxidative stress to levels observed in the TDI group. Furthermore, UPR reactivation increased the levels of MMP8 in serum, while exhibited no effect on the expression of CTSL.

Given that no specific agonist for MMP8 is available and recombinant protein is prone to degradation in the in vivo environment, we performed in vitro experiments using recombinant human MMP8 to stimulate primary human airway epithelial cells (Fig. S18). We found that MMP8 promoted airway epithelial barrier damage and the expression of UPR-related genes, including ATF4 and DDIT3, but had no significant effect on CTSL expression. Notably, targeted inhibition of

ATF4 markedly reversed MMP8-induced changes above, whereas inhibition of CTSL had no such effect.

Collectively, these results demonstrate that pharmacological inhibition of UPR attenuated the development of TDI-induced asthma. Rescue experiments suggest that UPR and MMP8 signaling function as parallel interacting pathways in TDI-induced airway inflammation, whereas CTSL acts upstream of both and coordinately regulates their activation.

3.7. CBCO alleviates human primary airway epithelial cells damage induced by TDI-HSA

We first assessed the cytotoxicity of the tested compounds and found that CBCO (1 μ g/mL), the CTSL inhibitor Z-FY-CHO (10 μ M), and ATF4-

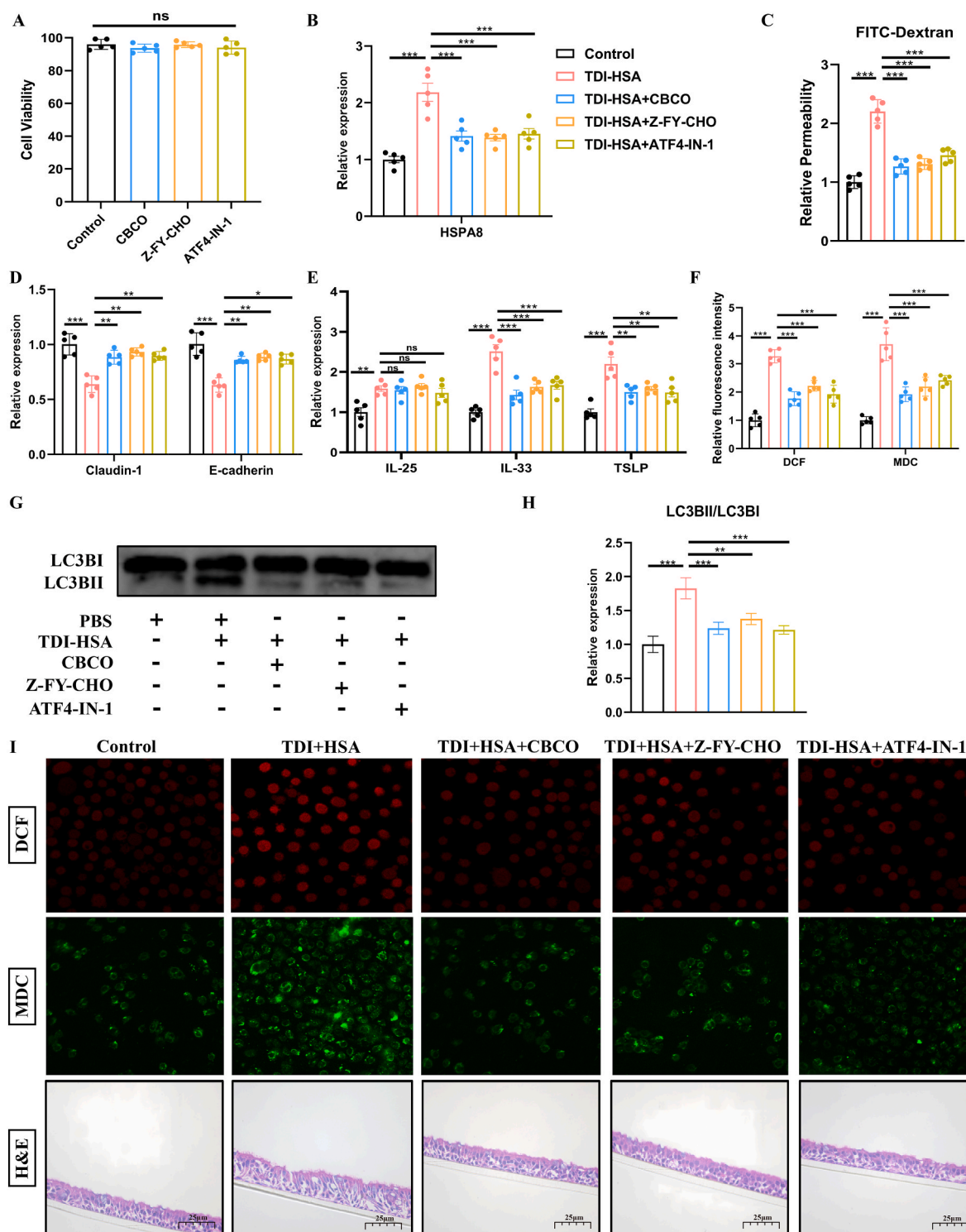


Fig. 7. CBCO alleviates human primary airway epithelial cells damage induced by TDI-HSA.

(A) Detection of cytotoxicity by CCK8 of different groups. (B) qPCR analysis of the mRNA expression levels of HSPA8 of the HAECs. (C) Relative permeability of ALI-cultured-HAECs detected by FITC-Dextran. (D) qPCR analysis of the mRNA expression levels of E-cadherin and Occludin of the ALI-cultured-HAECs. (E) qPCR analysis of the mRNA expression levels of TSLP, IL-25, IL-33 of the ALI-cultured-HAECs. (F) Relative fluorescence intensity of DCF stained and MDC stained HAECs. (G–H) Western blot analysis of expression levels of LC3B-II and LC3B-I. (I) Representative DCF stained, MDC stained and H&E stained HAECs of different groups. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$, ns > 0.05 , $n = 5$.

IN-1 (1 μM) had no significant effect on the viability of HAECs (Fig. 7A). In vitro exposure of monolayer HAECs to TDI-HSA induced pronounced autophagy and oxidative stress, accompanied by elevated mRNA expression of *HSPA8*, and protein expression of LC3B-II/LC3B-I ratio (Fig. 7B–F–I). Furthermore, when HAECs were cultured under air-liquid interface conditions, co-exposure to TDI-HSA caused marked epithelial disruption, reduced expression of barrier-associated proteins (ZO-1, occludin, and E-cadherin), and increased levels of epithelial alarmins (IL-33 and TSLP) and cellular permeability (Fig. 7C–E, I).

Pretreatment with CBCO, Z-FY-CHO, or ATF4-IN-1 effectively mitigated TDI-HSA-induced autophagy, oxidative stress, and alarmin release, thereby preserving airway epithelial structure and barrier integrity. Collectively, these findings underscore the protective effects of CBCO, the CTSL inhibitor Z-FY-CHO, and ATF4-IN-1 in maintaining epithelial homeostasis under TDI-induced injury.

3.8. CBCO attenuates the development of HDM/LPS-induced asthma

To validate our findings beyond the TDI-induced asthma model, we further examined the effects of CBCO and inhibition of CTSL, MMP8, or ATF4 in a house dust mite/lipopolysaccharide (HDM/LPS)-induced SA model (Fig. 8A). Consistent with observations in the TDI model, HDM/LPS-exposed mice displayed marked AHR, elevated serum IgE levels, increased proinflammatory cytokines (TNF- α , IL-1 β , IL-4, IL-5, IL-13, MMP8, and IL-17A), enhanced oxidative stress, and inflammatory cell infiltration (Fig. 8B–L). Histopathological evaluation revealed classical asthmatic features, including peribronchial and perivascular inflammatory cell accumulation, as shown by H&E staining (Fig. 8M).

Pretreatment with CBCO or pharmacological inhibition of CTSL, MMP8, or ATF4 markedly attenuated these pathological changes, leading to reduced airway inflammation. Collectively, these preclinical

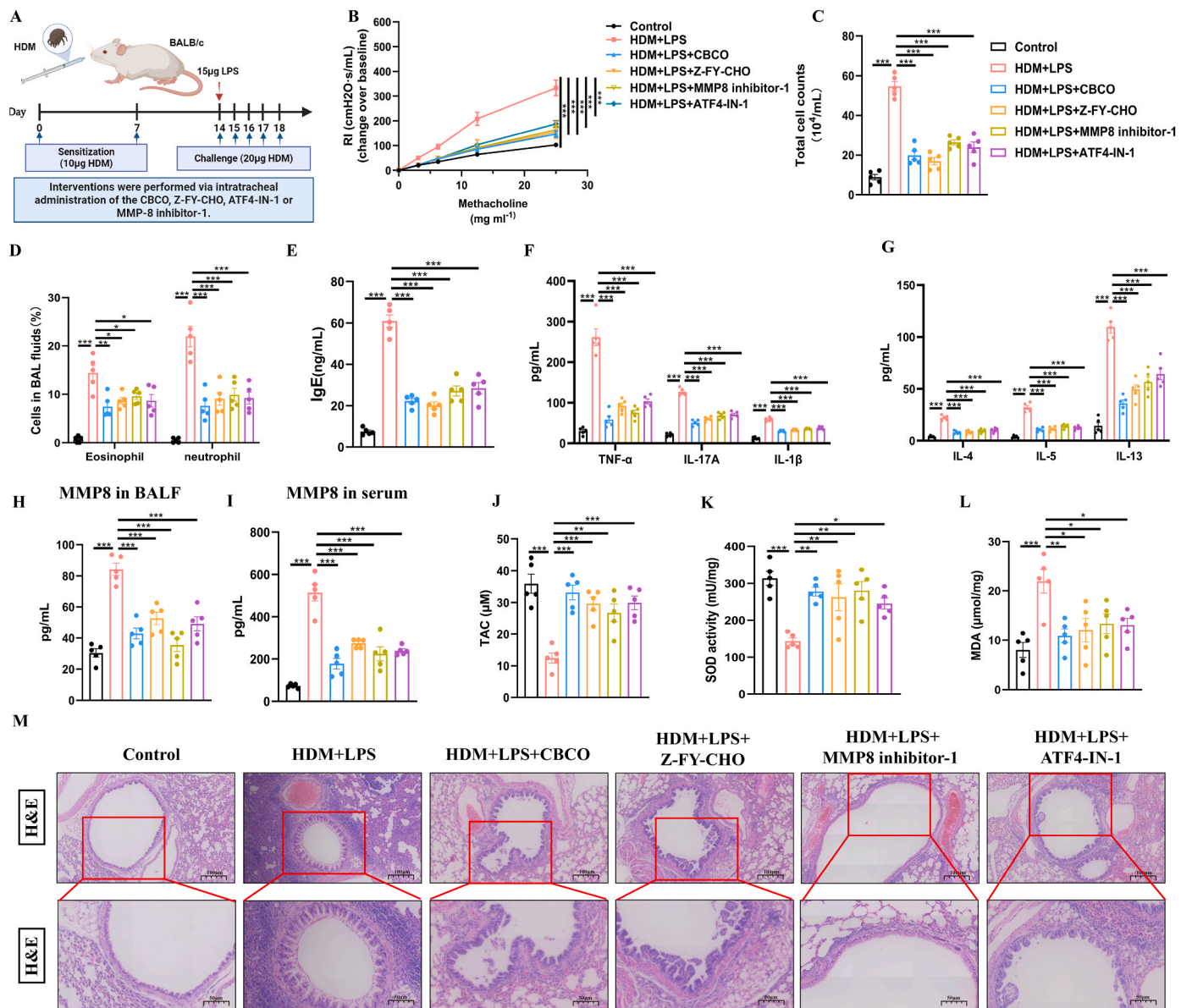


Fig. 8. CBCO attenuates the development of HDM/LPS-induced asthma.

(A) Schematic diagram representation of experimental plan. (B) Airway hyperresponsiveness was measured by RL. Results were shown as percentage over baseline value. (C–D) Inflammatory cells in BALF. Percentages of different inflammatory cells were calculated by counting a total of 200 cells in H&E stained cytoplasmic samples. (E) Measurement of serum IgE by ELISA. (F–G) Measurement of IL-1 β , IL-4, IL-5, IL-13, IL-17A, TNF- α in BALF by ELISA. (H) Measurement of MMP8 in BALF. (I) Measurement of MMP8 in serum. (J) Measurement of TAC in BALF. (K–L) Measurement of SOD, MDA in lung tissues. (M) Representative H&E stained lung sections of different groups. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$, ns > 0.05 , $n = 5–8$.

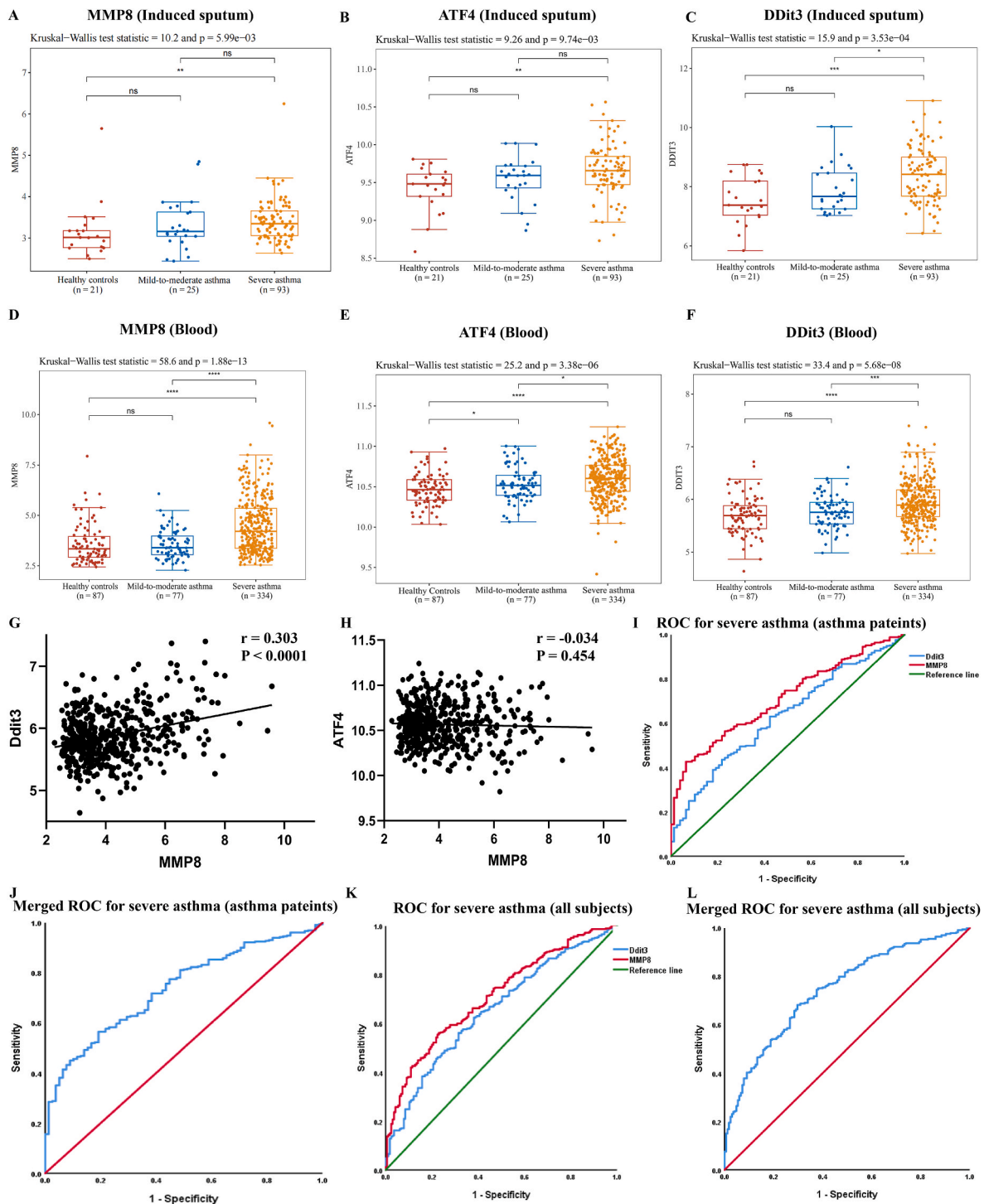


Fig. 9. Identification of MMP8 and DDit3 as potential clinical biomarkers in SA using the Unbiased BIOMarkers in Prediction of REspiratory Disease outcomes Project.

(A–C) Expression levels of MMP8, ATF4 and Ddit3 in the blood samples of transcriptomics. (D–F) Expression levels of MMP8, ATF4 and Ddit3 in the induced sputum samples of transcriptomics. (G) Correlation analysis between MMP8 and Ddit3 in all subjects. (H) Correlation analysis between MMP8 and Ddit3 in all subjects. (I) Correlation analysis between MMP8 and Ddit3 in all subjects. (J) ROC curve for MMP8 and Ddit3 of blood samples in predicting the diagnosis of severe asthma in asthma patients. (K) ROC curve for MMP8 combined with Ddit3 of blood samples in predicting the diagnosis of severe asthma in asthma patients. (L) ROC curve for MMP8 and Ddit3 of blood samples in predicting the diagnosis of severe asthma in all subjects. (M) ROC curve for MMP8 combined with Ddit3 of blood samples in predicting the diagnosis of severe asthma in all subjects. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$, ns > 0.05 .

findings suggest that CTSL, MMP8 and the UPR act as critical effectors linking oxidative stress with airway inflammation in SA. To evaluate their potential clinical relevance, we next investigated their expression in well-characterized asthma cohorts.

3.9. Identification of MMP8 and DDIT3 as potential clinical biomarkers in SA using the unbiased BIOMarkers in prediction of REspiratory disease outcomes project

Building on our experimental evidence that MMP8 upregulation and UPR activation play critical roles in airway inflammation in SA models, we next investigated whether these molecular signatures are also evident in human SA. Transcriptomic analysis of induced sputum samples ($n = 139$) from the *Unbiased BIOMarkers in Prediction of REspiratory Disease Outcomes* (U-BIOPRED) project (GSE76262) revealed significantly elevated expression of *MMP8*, *ATF4*, and *DDIT3* in patients with SA compared with healthy controls (HCs) (Fig. 9A–C). Similarly, transcriptomic profiling of peripheral blood samples ($n = 498$) from the U-BIOPRED cohort (GSE69683) demonstrated increased expression of *MMP8*, *ATF4*, and *DDIT3* in SA patients compared with HCs and mild-to-moderate asthma (MMA) patients (Fig. 9D–F).

Correlation analysis showed a significant positive relationship between *MMP8* and *Ddit3* expression ($r = 0.303$, $p < 0.0001$), whereas *MMP8* expression was not significantly correlated with *ATF4* ($p = 0.454$). As illustrated in Fig. 9G–L and Table S6, both blood *MMP8* and *DDIT3* individually exhibited significant diagnostic potential for SA, while their combined analysis provided improved predictive accuracy (area under the curve [AUC] = 0.744 in all subjects; AUC = 0.737 among asthma patients, both $p < 0.001$).

Collectively, transcriptomic analyses from the U-BIOPRED cohorts revealed upregulated *MMP8* and *DDIT3* expression in SA, with both genes demonstrating moderate diagnostic accuracy, suggesting their potential utility as biomarkers of disease severity. To further validate these findings, we conducted clinical confirmation in our independent patient cohort.

3.10. Clinical validation of MMP8 and DDIT3 as biomarkers in SA

To further substantiate these findings, we measured serum *MMP8* levels in our clinical cohort and examined their associations with lung function parameters and inflammatory cell counts in patients with SA. A total of 240 participants were enrolled, comprising 68 HCs, 92 individuals with MMA, and 80 individuals with SA (Fig. 10A). The demographic and clinical characteristics of the participants are summarized in Table 1. No significant differences were observed among the three groups in age ($p = 0.127$), sex distribution ($p = 0.150$), or body mass index ($p = 0.890$).

In contrast, significant differences were identified across all pulmonary function indices. Compared with the HC group, both asthma groups exhibited markedly reduced lung function. The forced vital capacity (FVC, % predicted), forced expiratory volume in 1 s (FEV₁, % predicted), FEV₁/FVC ratio, peak expiratory flow (PEF, %), and maximal expiratory flow rates (MEF_{75/25}, MEF₇₅, MEF₅₀, and MEF₂₅) were all significantly lower in asthma patients (all $p < 0.001$) than in HCs. Post hoc analyses revealed a stepwise decline in FVC, % predicted, FEV₁ % predicted, FEV₁/FVC, MEF₇₅, MEF₅₀, and MEF₂₅ values across the groups (HCs > MMA > SA). For PEF and MEF_{75/25}, both asthma groups showed significantly lower values than HCs, but there was no significant difference between the MMA and SA groups.

Hematological analyses also revealed several significant differences among the groups. White blood cell count was significantly higher in the SA group than in the HCs and MMA groups ($p < 0.001$). Red blood cell count and hemoglobin levels were also elevated in the SA and MMA groups compared with the HCs group ($p = 0.014$ and $p = 0.001$, respectively). Neutrophil count and percentage were significantly higher in the SA group than in the HCs and MMA groups ($p < 0.001$ for

both). Likewise, eosinophil count and percentage were markedly increased in the MMA and SA groups relative to HCs (both $p < 0.001$). Platelet counts did not differ significantly among groups ($p = 0.097$).

Consistent with the U-BIOPRED cohort findings, serum *MMP8* and *DDIT3* levels were elevated in the SA group compared with the HCs and MMA groups (Fig. 10B). Notably, the MMA group exhibited higher levels of *MMP8* or *DDIT3* than the HCs group. Correlation analysis demonstrated that serum *MMP8* and *DDIT3* were negatively correlated with lung function across all participants (Fig. 10C; Table 2). Furthermore, serum *MMP8* was significantly positively correlated with peripheral white blood cell count ($p < 0.001$, $r = 0.305$; Fig. 10D), blood neutrophil counts ($p < 0.001$, $r = 0.329$; Fig. 10E), and serum *Ddit3* levels ($p = 0.008$, $r = 0.328$; Fig. 10G) but negatively correlated with maximal expiratory flow at 25% of FVC (MEF₂₅) ($p = 0.003$, $r = -0.384$; Fig. 10F) in asthma patients. Similarly, serum *Ddit3* was negatively correlated with MEF₂₅ ($p = 0.004$, $r = -0.366$; Fig. 10H).

As shown in Fig. 10I–L and Table 3, both serum *MMP8* and *DDIT3* individually exhibited significant diagnostic potential for SA, and their combined analysis yielded superior predictive accuracy (AUC = 0.840 in all subjects; AUC = 0.773 among asthma patients, both $p < 0.001$). Collectively, evidence from in vivo and in vitro experiments, together with clinical data analyses, indicates that *MMP8* and *DDIT3* serve as potential biomarkers of SA.

4. Discussion

In this study, we uncovered a previously unrecognized redox-regulatory mechanism underlying airway inflammation, wherein CTSL-driven NETs formation and UPR activation amplified oxidative stress, exacerbating epithelial injury and inflammation. We further identified CBCO—an abundant meroterpenoid isolated from *R. capitatum* Maxim—as a potent modulator of this redox axis. CBCO suppressed NETs- and UPR-associated ROS generation, restored redox homeostasis, and attenuated downstream inflammatory signaling by inhibiting CTSL activity. Through the integration of phytochemistry, multi-omics, computational biology, and translational clinical validation, we delineated a previously unappreciated pathogenic pathway centered on oxidative stress-driven airway inflammation and proposed a novel phytochemical strategy for its modulation. Collectively, our findings position CBCO as a promising lead compound with potential for targeting oxidative stress and inflammatory cascades in SA.

We successfully isolated six meroterpenoids from the aerial parts of *R. capitatum* Maxim using a series of chromatographic techniques, with CBCO identified as the most abundant constituent—reported here for the first time in this species. Guided by its yield and structural novelty, CBCO was selected for further evaluation to determine its safety profile and therapeutic potential in neutrophilic airway inflammation. Our findings demonstrated that intratracheal administration of CBCO at a dose of 10 mg/kg was well tolerated, with no mortality observed over 14 days, confirming its suitability for in vivo studies. Notably, pretreatment with CBCO conferred a protective effect against TDI-induced airway inflammation at a concentration of 2 mg/kg, but not at 1 mg/kg, suggesting that 2 mg/kg represents the minimal effective dose for this protective effect, as evidenced by reduced IgE levels, diminished inflammatory cell infiltration, and improved histopathological outcomes.

A pivotal finding of this study was the integrative identification of CTSL as a molecular target engaged by CBCO, achieved through the combined use of computational prediction, CETSA analysis and transcriptomic analysis. Cathepsins, particularly CTSL, are lysosomal proteases that mediate extracellular matrix degradation, epithelial barrier disruption, and immune cell activation [17,18]. Although dysregulated CTSL activity has been implicated in chronic airway disease [19,20], its precise contribution to SA has remained unclear. Through our integrative analytical approach, CTSL was identified as the sole overlapping target between the predicted CBCO targets and differentially expressed genes. Molecular docking and molecular dynamics simulations further

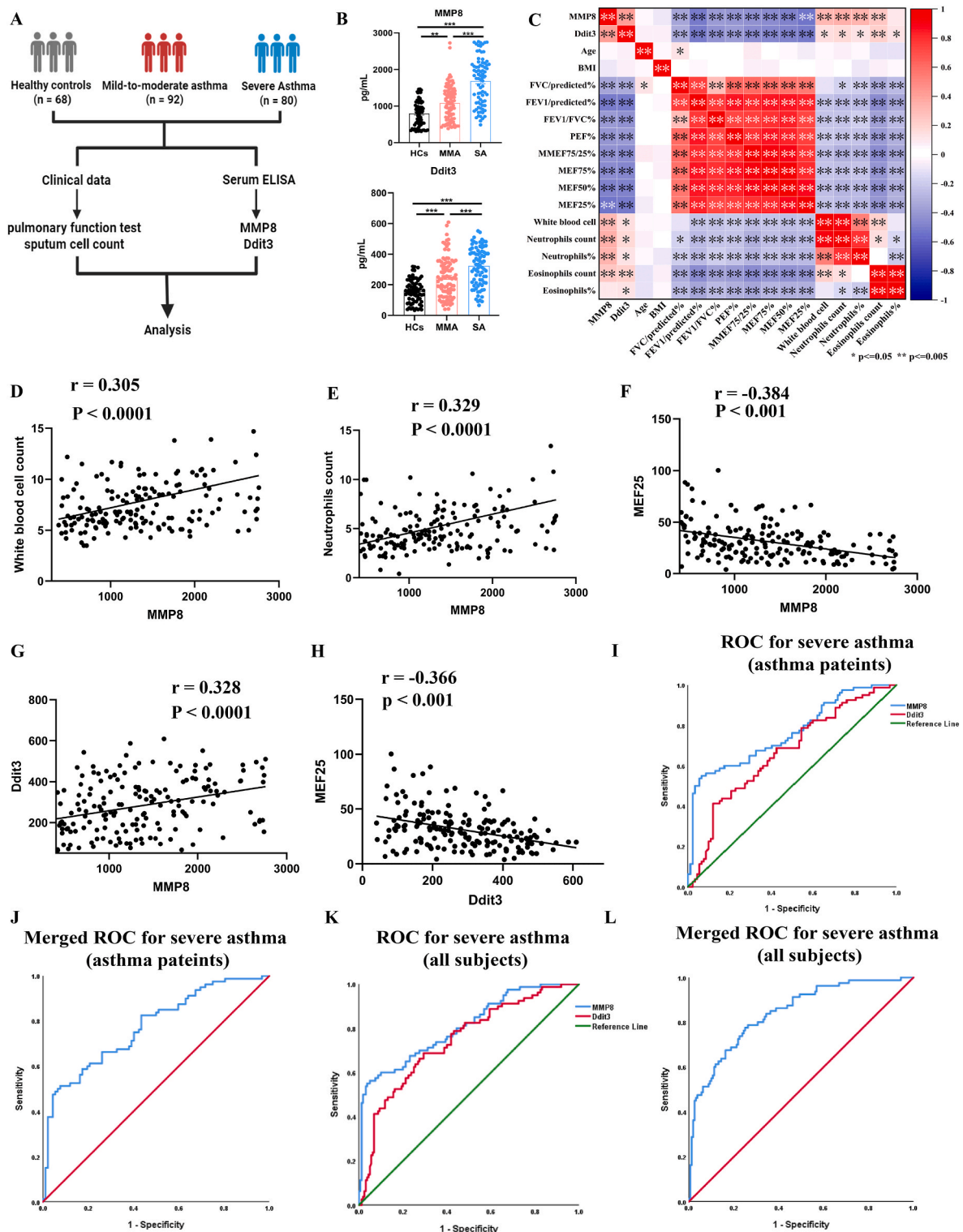


Fig. 10. Clinical validation of MMP8 and Ddit3 as biomarkers in SA.

(A) Schematic diagram representation of clinical validation plan. (B) Expression levels of MMP8 and Ddit3 in different groups of serum. (C) Correlation analysis between serum MMP8, Ddit3, and clinical features in all subjects. (D) Correlation analysis between serum MMP8 and white blood cell count in asthma patients. (E) Correlation analysis between serum MMP8 and neutrophil counts in asthma patients. (F) Correlation analysis between serum MMP8 and MEF25 in asthma patients. (G) Correlation analysis between serum MMP8 and serum Ddit3 in asthma patients. (H) Correlation analysis between serum Ddit3 and MEF25 in asthma patients. (I) ROC curve for serum MMP8 and Ddit3 in predicting the diagnosis of severe asthma in asthma patients. (J) ROC curve for MMP8 combined with Ddit3 in predicting the diagnosis of severe asthma in asthma patients. (K) ROC curve for serum MMP8 and Ddit3 in predicting the diagnosis of severe asthma in all subjects. (L) ROC curve for MMP8 combined with Ddit3 in predicting the diagnosis of severe asthma in all subjects. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$, ns > 0.05 .

Table 1
Characteristics of healthy subjects and patients with asthma.

	Healthy controls (HCs)	Mild to moderate asthma (MMA)	Severe asthma (SA)	Overall test P- value	Significant paired comparison Results (p ≤ 0.05) ^{&}
N	68	92	80		
Age, years	53.6 ± 13.3	49.5 ± 15.0	51.6 ± 17.0	P = 0.127	
Gender, M/F	28/40	46/46	38/42	P = 0.150	
BMI, kg/m ²	24.2 ± 3.9	24.4 ± 3.4	23.4 ± 3.9	P = 0.890	
FVC/predicted, %	105.9 ± 10.5	94.0 ± 18.3	84.5 ± 12.8	P < 0.001	HCs > MMA > SA
FEV1/predicted, %	99.5 (9.5)	74.1 (22.3)	56.4 (22.8)	P < 0.001	HCs > MMA > SA
FEV1/FVC, %	90.5 (13.9)	74.7 (21.0)	60.3 (18.8)	P < 0.001	HCs > MMA > SA
PEF, %	91.8 (10.0)	60.1 (23.1)	49.8 (24.4)	P < 0.001	HCs > MMA > SA
MEF75/25, %	86.8 (9.8)	44.8 (24.2)	30.5 (22.8)	P < 0.001	HCs > MMA > SA
MEF75, %	87.6 (10.4)	49.9 (22.2)	31.6 (24.3)	P < 0.001	HCs > MMA > SA
MEF50, %	91.9 (12.3)	38.5 (22.3)	21.6 (16.5)	P < 0.001	HCs > MMA > SA
MEF25, %	70.8 (16.0)	35.4 (19.7)	19.4 (17.2)	P < 0.001	HCs > MMA > SA
White blood cell (10 ⁹ /L)	6.1 (2.5)	6.5 (2.8)	8.0 (4.3)	P < 0.001	SA > MMA, HCs
Red blood cell (10 ¹² /L)	4.4 (1.1)	4.6 (1.0)	4.7 (0.8)	P = 0.014	SA, MMA > HCs
Hemoglobin (g/L)	131.5 (17.5)	137.0 (15.8)	141.0 (21.8)	P = 0.001	SA, MMA > HCs
Platelet (10 ⁹ /L)	241.9 ± 64.7	247.5 ± 58.7	262.1 ± 100.5	P = 0.097	
Neutrophils count (10 ⁹ /L)	3.2 (1.9)	4.2 (2.2)	5.2 (3.6)	P < 0.001	SA > MMA > HCs
Neutrophils, %	55.9 ± 13.4	61.9 ± 10.5	67.2 ± 13.8	P < 0.001	SA > MMA > HCs
Eosinophils count (10 ⁹ /L)	0.09 (0.09)	0.22 (0.21)	0.30 (0.61)	P < 0.001	SA, MMA > HCs
Eosinophils, %	1.5 (1.2)	4.0 (3.5)	3.6 (6.8)	P < 0.001	SA, MMA > HCs
Sputum cell count, %					
Neutrophils, %	27.5 (31.8)	48.0 (32.8)	62.5 (31.8)	P < 0.001	SA > MMA > HCs
Eosinophils, %	0.0 (0.0)	4.0 (12.0)	6.0 (17.0)	P < 0.001	SA > MMA > HCs
Macrophages, %	72.5 (31.5)	41.0 (30.8)	23.5 (26.8)	P < 0.001	HCs > MMA > SA
Leukomonocyte, %	0.0 (0.0)	0.0 (0.0)	1.0 (1.0)	P < 0.001	SA > HCs, MMA

BMI, body mass index; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; PEF, peak expiratory flow; MEF, maximal expiratory flow; Data are reported in mean ± standard deviation or median (Eterquartile range).

Table 2
Correlation analysis between serum MMP8 and clinical features.

V1	V2	Correlation	p_value
Age	MMP8	0.043	0.506
	Ddit3	−0.058	0.368
BMI	MMP8	−0.093	0.152
	Ddit3	0.064	0.326
FVC/predicted, %	MMP8	−0.351**	<0.001
	Ddit3	−0.332**	<0.001
FEV1/predicted, %	MMP8	−0.507**	<0.001
	Ddit3	−0.520**	<0.001
FEV1/FVC, %	MMP8	−0.382**	<0.001
	Ddit3	−0.434**	<0.001
PEF, %	MMP8	−0.415**	<0.001
	Ddit3	−0.394**	<0.001
MEF75/25, %	MMP8	−0.438**	<0.001
	Ddit3	−0.446**	<0.001
MEF75, %	MMP8	−0.459**	<0.001
	Ddit3	−0.465**	<0.001
MEF50, %	MMP8	−0.484**	<0.001
	Ddit3	−0.481**	<0.001
MEF25, %	MMP8	−0.521**	<0.001
	Ddit3	−0.514**	<0.001
White blood cell	MMP8	0.315**	<0.001
	Ddit3	0.155*	0.016
Neutrophils count	MMP8	0.354**	<0.001
	Ddit3	0.160*	0.013
Neutrophils %	MMP8	0.323**	<0.001
	Ddit3	0.138*	0.033
Eosinophils count	MMP8	0.245**	<0.001
	Ddit3	0.202**	0.002
Eosinophils %	MMP8	0.117	0.070
	Ddit3	0.127*	0.049

BMI, body mass index; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; PEF, peak expiratory flow; MEF, maximal expiratory flow.

supported a stable binding interaction between CBCO and CTSL, while in vivo validation demonstrated that CBCO administration significantly suppressed CTSL expression in lung tissue. This inhibition was

associated with pronounced reductions in UPR signaling, NET formation, and MMP8 levels, accompanied by decreased ROS accumulation and restoration of redox homeostasis. Collectively, these results establish CTSL as a redox-sensitive upstream regulator of the NET–MMP8 axis and UPR, extending current understanding by linking proteolytic stress to oxidative signaling pathways that sustain neutrophilic inflammation and steroid resistance in SA [12].

NETs are increasingly recognized as critical amplifiers of neutrophilic inflammation in SA [52,53]. Although NE and MPO have received substantial attention, the contribution of NET-associated MMP8 has been largely overlooked. Our findings clearly demonstrated that MMP8 is abundantly released during NETosis and plays a pivotal role in mediating AHR, inflammatory cytokine production, and structural remodeling. This process was accompanied by excessive ROS generation, indicating that MMP8-driven inflammation is tightly linked to redox dysregulation. Pharmacological inhibition of MMP8 conferred a protective effect against asthma pathology and oxidative stress, supporting its role as a key pathogenic mediator.

By modulating CTSL-associated signaling and suppressing NETs formation, CBCO effectively downregulated MMP8 expression and restored redox balance, thereby revealing a novel and therapeutically targetable CTSL–MMP8 axis in SA. Collectively, our data position MMP8 not as a passive bystander but as a redox-sensitive effector molecule within NETs that drives airway hyperresponsiveness, tissue remodeling, and persistent inflammation.

In this study, UPR activation was identified as a central mechanism linking protease-driven signaling to airway inflammation. Transcriptomic analysis revealed strong correlations between UPR-associated transcription factors (*Atf4* and *Ddit3*) and inflammatory genes, indicating transcriptional coupling between endoplasmic reticulum stress, oxidative stress, and inflammation. Notably, UPR activation is inherently redox-sensitive, and excessive ROS production amplifies maladaptive UPR signaling, thereby promoting chronic inflammation [54]. Pharmacological inhibition of ATF4 with ATF4-IN-1 conferred a protective effect against airway hyperresponsiveness, inflammatory cytokine production, immune cell infiltration, and tissue remodeling in

Table 3
Predictive values of serum MMP8 and Ddit3 for predicting severe asthma patients.

Group	Test variables	AUC	Cutoff value	Sensitivity	Specificity	PPV	NPV	Youden index	P-value
All subjects	MMP8 (pg/mL)	0.806 (95% CI 0.75-0.87)	1677.85	0.56	0.95	0.85	0.81	0.51	$P < 0.001$
	Ddit3 (pg/mL)	0.743 (95% CI 0.68-0.81)	252.79	0.69	0.61	0.54	0.82	0.39	$P < 0.001$
	Merged	0.840 (95% CI 0.79-0.89)	N.A.	0.79	0.74	0.60	0.87	0.53	$P < 0.001$
Asthma	MMP8 (pg/mL)	0.762 (95% CI 0.69-0.84)	1740.50	0.54	0.95	0.90	0.70	0.48	$P < 0.001$
	Ddit3 (pg/mL)	0.665 (95% CI 0.58-0.75)	375.83	0.41	0.88	0.75	0.63	0.29	$P < 0.001$
	Merged	0.773 (95% CI 0.70-0.84)	N.A.	0.51	0.93	0.85	0.69	0.44	$P < 0.001$

AUC, area under the curve; PPV, positive predictive values; NPV, negative predictive values; N.A., Not applicable.

SA models.

Pretreatment with CBCO or inhibition of the CTSL–MMP8 axis significantly decreased oxidative stress and downregulated *ATF4/DDIT3* expression. Importantly, suppression of *ATF4* also reduced *MMP8* expression and NET formation, suggesting crosstalk between the UPR and NET pathways. These findings indicate that UPR activation and NETs formation represent two parallel downstream branches of CTSL signaling, each contributing to oxidative injury—a concept consistent with previous reports [55–57]. This notion is further supported by rescue experiments showing that pharmacological reactivation of UPR only partially reversed the protective effects of MMP8 or CTSL inhibition and increased MMP8 levels without affecting CTSL expression, indicating that UPR is not strictly downstream of MMP8 but can modulate its activity. Moreover, recombinant MMP8 directly induced UPR-related gene expression, while *ATF4* inhibition—but not CTSL inhibition—effectively reversed these effects, suggesting that CTSL acts upstream to coordinately regulate both MMP8 and UPR signaling. Collectively, our results suggest that oxidative stress, UPR, and NET activity form a self-reinforcing regulatory network rather than a strict linear signaling cascade, converging on a CTSL–MMP8/UPR regulatory axis that amplifies airway inflammation during the development of SA and represents a key target for protective intervention to limit disease progression.

Equally important are the epithelial-protective effects of CBCO. In SA, the airway epithelial barrier is profoundly compromised, facilitating allergen penetration and perpetuating chronic inflammation [58]. Our findings demonstrated that CBCO, as well as inhibition of CTSL or *ATF4*, mitigated TDI–HSA-induced autophagy dysregulation, suppressed the release of epithelial alarmins (IL-33 and TSLP), and restored the expression of tight junction proteins, thereby maintaining epithelial integrity and homeostasis. Because oxidative stress is a principal driver of epithelial barrier disruption [59], the capacity of CBCO to attenuate ROS generation provides a mechanistic basis for its barrier-protective effects.

Conversely, exposure to recombinant MMP8 protein directly induced epithelial barrier dysfunction, elevated alarmin production, and increased oxidative stress, further supporting a causal relationship between NETs-derived proteases and redox imbalance. These observations offer a coherent mechanistic explanation for how MMP8—a neutrophil-derived protease—can simultaneously drive both neutrophilic and eosinophilic inflammatory phenotypes in SA.

Asthma is a highly heterogeneous disease, and SA encompasses multiple distinct inflammatory phenotypes. Although recent advances in type 2 biologics have substantially improved the management of eosinophilic SA, effective targeted therapies remain unavailable for the more complex mixed-granulocytic and neutrophilic subtypes [60]. According to the global initiative for Asthma guidelines and the U-BIOPRED consensus [2,61], the diagnosis of SA is primarily defined by airway hyperresponsiveness, symptom control, and treatment response rather than by the underlying inflammatory profile.

To account for this heterogeneity, we employed two steroid-insensitive murine models with differing inflammatory characteristics and triggers for validation. The consistent protective efficacy of CBCO across both the TDI-induced and HDM/LPS-induced SA models underscores the robustness and translational relevance of our findings.

These models represent complementary etiologies of SA: the TDI model simulates chemically induced, mixed-granulocytic, steroid-insensitive asthma [62], whereas the HDM/LPS model recapitulates allergen- and endotoxin-driven mixed-granulocytic inflammation [63]. The consistent protective effects of CBCO across both models indicate that it confers protection against severe asthma through a shared mechanism involving inhibition of the CTSL–MMP8/UPR regulatory axis, accompanied by attenuation of oxidative stress, mucus hypersecretion, and collagen deposition, thereby modulating the multifaceted, redox-driven pathology that characterizes the disease.

Beyond mechanistic insights, this study provides substantial translational relevance by identifying *MMP8* and *DDIT3* as robust biomarkers of SA. Analysis of the U-BIOPRED dataset revealed that *MMP8* and *DDIT3* expression levels were significantly elevated in both sputum and blood samples from patients with SA compared with HCs and those with MMA. Receiver operating characteristic curve analyses confirmed their predictive value, findings that were further validated in our independent clinical cohort. Importantly, serum MMP8 and DDIT3 levels correlated positively with peripheral neutrophil counts and inversely with lung function, underscoring their clinical relevance. These results extend previous observations linking NETs- and UPR-related biomarkers to asthma severity and position *MMP8* as a reliable surrogate marker of neutrophilic inflammation.

Notwithstanding these compelling findings, several limitations warrant acknowledgment. First, although no overt toxicity was observed, the comprehensive pharmacokinetic and toxicological profiles of CBCO have yet to be fully characterized—an essential step toward clinical translation. In addition, while our current data support target engagement of CTSL, they do not yet definitively establish direct pharmacological binding or selectivity, and further biophysical validation will be required. Second, the clinical validation of MMP8 and DDIT3 as biomarkers was conducted in a relatively modest cohort. While our data support their potential utility in disease discrimination and severity stratification, the lack of longitudinal sampling and the absence of a CBCO-treated patient cohort preclude conclusions regarding their value in monitoring treatment response or predicting therapeutic efficacy. Larger, multicenter studies with longitudinal designs will be required for further validation. Third, although our mechanistic investigations were supported by in vivo models and primary human airway epithelial cells, the current in vivo design primarily reflects a preventive rather than a therapeutic paradigm, as interventions were administered prior to sensitization/challenge. In addition, human tissue-relevant experimental systems, such as airway organoids or precision-cut lung slices, could provide complementary mechanistic insights and better recapitulate the complex multicellular microenvironment of the human airway. Incorporating these advanced ex vivo models and delayed-treatment designs in future studies would further strengthen the translational relevance of the proposed CTSL–MMP8/UPR regulatory axis.

In conclusion, this study identifies CBCO as a promising phytotherapeutic candidate for the prevention or modulation of SA-associated inflammation, exerting protective effects through modulation of CTSL-associated redox signaling and its downstream MMP8 and UPR. Our findings highlight CTSL as a key regulator of NETs formation and UPR activation, establish *MMP8* and *DDIT3* as biomarkers, and provide a robust scientific foundation for the development of phytochemical-

based interventions to treat SA. By bridging oxidative stress biology with translational validation, this work offers a compelling rationale for advancing CBCO through preclinical development and underscores the potential value of targeting redox-driven airway inflammation in SA.

Authors consent for publication

All authors give consent for publication.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

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CRediT authorship contribution statement

Junwen Huang: Conceptualization, Writing – original draft, Writing – review & editing. **Ying Chen:** Formal analysis, Writing – original draft, Writing – review & editing. **Yi Deng:** Formal analysis, Writing – original draft, Writing – review & editing. **Dapeng Hu:** Investigation, Writing – original draft, Writing – review & editing. **Yaixin Chen:** Software, Writing – original draft, Writing – review & editing. **Yuemao Li:** Methodology, Writing – review & editing. **Cancan Xie:** Investigation. **Keke Fan:** Validation. **Bang Zhu:** Validation. **Zhaoqian Gong:** Validation. **Jie Yang:** Investigation. **Shuyu Huang:** Funding acquisition. **Xiaoqian Peng:** Investigation. **Xianru Peng:** Formal analysis. **Bing Huang:** Funding acquisition. **Wenqu Zhao:** Funding acquisition, Writing – original draft, Writing – review & editing. **Qingfeng Du:** Formal analysis, Writing – original draft, Writing – review & editing. **Haijin Zhao:** Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.redox.2026.104232>.

Data availability

Data will be made available on request.

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