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Effect of LncRNA UFC1 Downregulation on Alleviating Glucocorticoid Resistance in Chronic Obstructive Pulmonary Disease via the PI3K/AKT Pathway

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Received: 26 May 2026; Accepted: 27 August 2026; Published: 19 October 2026

ABSTRACT: Background: Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory airway disease frequently complicated by glucocorticoid (GC) resistance. This study investigated whether lncRNA UFC1 regulates cigarette smoke extract (CSE)-induced inflammation, apoptosis, and GC resistance through phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling. **Methods:** To simulate cellular damage and GC insensitivity *in vitro*, U937 cells were subjected to CSE stimulation. The influence of lncRNA UFC1 silencing on cell survival, inflammatory responses, apoptosis, and GC responsiveness was evaluated through a combination of Cell Counting Kit-8 (CCK-8), reverse transcription quantitative polymerase chain reaction (RT-qPCR), enzyme-linked immunosorbent assay (ELISA), Western blot, flow cytometry, and immunofluorescence assays. The PI3K activator 740Y-P was used for validation. *In vivo*, COPD rat models were induced by intermittent intratracheal lipopolysaccharide (LPS) instillation combined with chronic cigarette smoke exposure, and AAV-sh-UFC1 and dexamethasone (DEX) were used to evaluate lung injury, inflammation, and molecular expression. **Results:** CSE upregulated UFC1 and induced inflammation, apoptosis, and GC resistance in U937 cells. UFC1 knockdown improved cell viability ($p < 0.01$); reduced IL-6, IL-1 β , TNF- α , IL-8, GM-CSF, and MIP-3 α levels; decreased apoptosis ($p < 0.01$); and enhanced DEX sensitivity ($p < 0.01$), with increased GR α and HDAC2 and decreased GR β expression. Mechanistically, UFC1 downregulation inhibited PI3K/AKT activation (both $p < 0.001$), while 740Y-P partially reversed these effects (both $p < 0.05$). *In vivo* experiments confirmed that inhibiting Ufc1 attenuated lung injury and inflammation ($p < 0.01$ vs. COPD + AAV-NC), improved GC resistance indicators, and inhibited PI3K/AKT activation (both $p < 0.01$ vs. COPD + AAV-NC). **Conclusion:** Downregulation of lncRNA UFC1 alleviated CSE-induced inflammation, apoptosis, and GC resistance and COPD-related injury, potentially through PI3K/AKT inhibition, suggesting lncRNA UFC1 as a potential target for GC-resistant COPD.

KEYWORDS: Long non-coding RNA UFC1; pulmonary disease; chronic obstructive; glucocorticoid resistance; phosphatidylinositol 3-kinase/protein kinase b signaling pathway; inflammation; apoptosis

1 Introduction

Characterized by unrelenting inflammation of the airways and fixed airflow limitation, chronic obstructive pulmonary disease (COPD) is a globally prevalent respiratory condition that culminates in a steady deterioration of lung capacity. Globally, this condition remains a primary contributor to chronic morbidity and high mortality rates, posing a substantial public health challenge [1]. The pathogenesis of COPD is linked to multiple influences, with chronic tobacco consumption being recognized as a major risk factor [2]. Cigarette smoke contains numerous oxidative and pro-inflammatory components that can continuously

stimulate airway epithelial and immune cells, inducing the release of inflammatory mediators, cellular injury, and airway remodeling, which eventually drive the progression of the disease [3]. In addition to airway inflammation and remodeling, endothelial and mitochondrial dysfunction have also been implicated in the pathophysiology of COPD [4]. In addition to chronic inflammation, COPD patients often exhibit abnormal apoptosis, structural destruction of lung tissue, and reduced responsiveness to glucocorticoid (GC) treatment. These pathological changes collectively exacerbate the disease burden and limit clinical efficacy [5–7]. Although bronchodilators, inhaled corticosteroids, and combination therapies play important roles in COPD management, some patients—particularly those with smoking-related COPD—exhibit notable glucocorticoid resistance, which adversely affects disease control and prognosis [8]. Therefore, elucidating the molecular mechanisms of inflammatory damage and GC resistance in COPD and searching for new intervention targets are of significant theoretical and clinical importance.

Glucocorticoids are important therapeutic agents for managing airway inflammation, and their anti-inflammatory actions rely primarily on glucocorticoid receptor (GR)-mediated transcriptional regulation [9]. Under physiological conditions, GCs bind to cytoplasmic GR α and translocate into the nucleus, where they inhibit pro-inflammatory gene expression and promote anti-inflammatory responses [8]. However, in COPD—especially smoking-related COPD—the anti-inflammatory effects of GCs are often notably attenuated, manifesting as typical glucocorticoid resistance [10]. Studies suggest that the molecular mechanisms underlying glucocorticoid resistance are complex, involving reduced GR expression, an imbalance in isoform ratios (such as decreased GR α and increased GR β), post-translational modifications of the receptor (e.g., hyperphosphorylation and nitration), and impaired recruitment of co-repressors. For instance, a decrease in the expression or activity of histone deacetylase 2 (HDAC2) is considered a key mechanism of glucocorticoid resistance in COPD patients [11]. The involvement of HDAC2 in airway inflammation has also been demonstrated in asthma, where reduced HDAC2 expression was associated with enhanced inflammatory responses, while modulation of HDAC2 affected the production of multiple inflammatory cytokines [12]. Specifically, after entering the nucleus, the GR needs to recruit HDAC2 to deacetylate histones, which induces chromatin condensation and effectively silences the transcription of pro-inflammatory genes. The deficiency of HDAC2 leads to the persistent activation of inflammatory genes even in the presence of GC [13]. Therefore, exploring the molecular basis of GC resistance in COPD by focusing on HDAC2 and GR signaling abnormalities is of significance for identifying new strategies to reverse steroid resistance.

Recently, the respiratory medicine community has increasingly recognized the significance of long non-coding RNAs (lncRNAs), primarily due to their multifaceted involvement in transcriptional, epigenetic, and signaling pathways [14]. Growing evidence indicates that lncRNAs are extensively involved in regulating inflammation, oxidative stress, apoptosis, airway remodeling, and drug responses in COPD [15,16]. Certain lncRNAs can modulate the progression of COPD by acting as competitive endogenous RNAs, regulating protein stability, or influencing the activation of key signaling pathways [17–19]. The regulatory role of lncRNA UFC1 in governing neoplastic cell proliferation and apoptotic pathways has been well-documented [20]; with further research, its role in modulating tissue inflammation and immune homeostasis has gradually received attention [21]. Previous studies have reported that lncRNA UFC1 is upregulated in lung cancer and can promote lung cancer cell proliferation, migration, and tumor growth, suggesting that lncRNA UFC1 may be involved in the regulation of lung-related pathological processes [20]. However, systematic investigations into the role of lncRNA UFC1 in COPD remain limited, and its potential involvement in cigarette smoke-induced inflammatory injury and GC resistance has not yet been clearly reported.

As a well-established regulator of cellular survival, inflammation, and programmed cell death, the PI3K/AKT signaling axis exerts a pivotal influence on the pathogenesis and advancement of COPD [22]. Earlier research has demonstrated that cigarette smoke-related oxidative stress can activate PI3K/AKT signaling, impair HDAC2 function, and reduce corticosteroid sensitivity, thereby contributing to persistent pro-inflammatory responses [23]. Hyperactivation of the PI3K/AKT pathway not only amplifies the inflammatory cascade but also impairs the anti-inflammatory effects of GCs by affecting HDAC2 stability and GR signal transduction [24,25]. Therefore, modulating the PI3K/AKT signaling axis may offer a viable strategy for alleviating inflammation-driven injury and addressing steroid resistance in COPD. Furthermore, increasing research indicates that lncRNAs are involved in pathological developments through the regulation of PI3K/AKT signaling [26], yet whether a functional link exists between lncRNA UFC1 and the PI3K/AKT axis in COPD remains to be further elucidated.

Against this background, the present study utilized CSE-induced U937 cell models of inflammation/GC resistance and lipopolysaccharide (LPS)/cigarette smoke (CS)-exposed COPD rat models to systematically investigate the role of lncRNA UFC1 in COPD-related inflammatory injury, apoptosis, and glucocorticoid resistance. We hypothesized that lncRNA UFC1 contributes to glucocorticoid resistance through activation of the PI3K/AKT signaling pathway and dysregulation of HDAC2- and GR-related responses, whereas UFC1 knockdown may restore glucocorticoid sensitivity. Moreover, we investigated whether lncRNA UFC1 regulates the levels of HDAC2 and GR-associated factors via its interaction with the PI3K/AKT signaling axis. By integrating *in vitro* and *in vivo* models, this research aims to provide experimental observations that clarify the molecular basis of GC resistance in COPD. Furthermore, it seeks to offer a rationale for considering lncRNA UFC1 as a candidate therapeutic target for the condition.

2 Materials and Methods

2.1 Reagents and Treatments

Cigarette smoke extract (CSE) was generated by bubbling smoke from ignited unfiltered 1R6F cigarettes (University of Kentucky Center for Tobacco Reference Products, Lexington, KY, USA; tar, 8.58 mg/cigarette; nicotine, 0.721 mg/cigarette; carbon monoxide, 10.1 mg/cigarette under the ISO non-intense smoking regimen, according to the certificate of analysis) through 4 mL of PBS (0.01 M, pH 7.4) via a tube. This solution, designated as the 100% stock, was subsequently passed through a 0.22 µm membrane filter [27] and utilized in experimental procedures within 1 h of its preparation [28]. DEX (MedChemExpress, Monmouth Junction, NJ, USA; Cat# HY-14648) was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA; Cat# D2650) to prepare a stock solution. The stock solution was diluted with culture medium for *in vitro* experiments and with sterile PBS for *in vivo* administration to obtain the indicated working concentrations. The final DMSO concentration was kept at 0.1% in all treatment groups. The PI3K activator 740Y-P (MedChemExpress, Monmouth Junction, NJ, USA; Cat# HY-P0175) was dissolved in sterile double-distilled water with ultrasonic assistance to prepare a stock solution. The stock solution was diluted with culture medium for *in vitro* experiments and with sterile PBS for *in vivo* experiments. During the experiments, all stock solutions were diluted to predetermined working concentrations based on the experimental design. The si-UFC1 and si-NC used for cell transfection and the primers used for reverse transcription quantitative PCR (RT-qPCR) were synthesized by GeneChem Co., Ltd. (Shanghai, China). AAV-sh-UFC1 and AAV-NC used for animal experiments were constructed and packaged by GeneChem Co., Ltd. The specific siRNA sequences were as follows: si-UFC1, 5'-UUUAUUGAUGGGUUAUCUCCA-3'; si-NC, 5'-GAGAUAAACCAUCAUAAAAG-3'.

2.2 Cell Culture and Treatments

Short Tandem Repeat (STR) profiling was employed to authenticate the U937 human monocytic cell line, which was originally sourced from the American Type Culture Collection (ATCC; Manassas, VA, USA). RPMI-1640 medium (Gibco, Grand Island, NY, USA; Cat# 11875093) supplemented with 10% fetal bovine serum (FBS; Beyotime, Shanghai, China; Cat# C0226) and 1% penicillin-streptomycin (Beyotime, Shanghai, China; Cat# C0222) was utilized to culture the cells. Cultivation was conducted at 37°C in a 5% CO₂ humidified atmosphere. Additionally, the absence of mycoplasma was consistently verified through routine diagnostic checks to ensure culture purity.

For the induction of *in vitro* cellular injury, exponentially growing U937 cells were exposed to CSE at varying concentrations (0%, 0.125%, 0.25%, 0.5%, 1%, 2% and 5%) for 24 h [29]. For the purpose of identifying concentration-related response trends, the metabolic activity of the cells was monitored via the CCK-8 method. From these results, 5% CSE was selected as the working concentration for subsequent mechanistic assays because it produced a mild-to-moderate injury response while avoiding excessive cytotoxicity. Unless otherwise specified, the duration of CSE stimulation in all subsequent cell experiments was 24 h.

To evaluate the functional significance of lncRNA UFC1, the cells were partitioned into different experimental cohorts: Control, 5% CSE, CSE + si-NC, and CSE + si-UFC1. siRNA transfection was performed 48 h before CSE stimulation to ensure effective gene knockdown.

For the assessment of GC sensitivity, cells were divided into the Control, 5% CSE, CSE + DEX, CSE + si-NC, CSE + si-UFC1, and CSE + si-UFC1 + DEX groups. In this assay, LPS was used as a common terminal inflammatory stimulus in all groups and was therefore omitted from the group labels, which denote the different pretreatment conditions. The Control group received LPS stimulation without CSE, siRNA, or DEX pretreatment. For siRNA-transfected groups, CSE exposure was initiated at 48 h post-transfection. Before CSE exposure, cells were collected by centrifugation at 300× g for 5 min at room temperature using a centrifuge (Eppendorf, Hamburg, Germany), resuspended in fresh complete medium, and adjusted to the appropriate density of 2.5×10^5 cells/mL (5×10^5 cells in 2 mL per well) to avoid excessive cell accumulation. This medium replacement step also helped minimize residual vehicle- or transfection reagent-related effects. Cells were then exposed to 5% CSE as described above. After CSE exposure, cells were collected by centrifugation at 300× g for 5 min at room temperature, washed with PBS (0.01 M, pH 7.4), and resuspended in fresh complete medium. The DEX-treated groups were incubated with 1 μM DEX for 1 h, whereas the corresponding groups without DEX received an equivalent volume of vehicle. Subsequently, all groups were stimulated with 1 mg/L LPS (Gibco, Grand Island, NY, USA; Cat# 00-4976-03) for a 24-h duration. This protocol was established with reference to previous studies [29,30], in which CSE exposure was used to establish a COPD-related GC-insensitive cellular context, whereas LPS stimulation was applied to mimic acute inflammatory exacerbation. GC sensitivity was evaluated by assessing the inhibitory effect of DEX on LPS-induced IL-8 release.

In the PI3K signaling pathway intervention experiments, cells were categorized into the Control, 5% CSE, CSE + DEX, CSE + si-NC, CSE + si-UFC1, and CSE + si-UFC1 + 740Y-P groups. To activate the PI3K pathway, 740Y-P (20 μM) was introduced into the culture medium 1 h before CSE treatment or further pharmacological procedures.

2.3 Small Interfering RNA Transfection

U937 cells in the logarithmic growth phase were plated into 6-well plates at a density of 5×10^5 cells/well in 2 mL of complete medium before siRNA transfection. As per the supplier's guidelines, siRNA transfection was carried out with MCE® PolyFast Transfection Reagent (MedChemExpress, USA; Cat#

HY-K1014). At a final concentration of 50 nM, cells were transfected with either UFC1-targeted siRNA (si-UFC1) or a non-targeting negative control (si-NC). After 6 h of transfection, the transfection mixture was removed by centrifugation at 300× g for 5 min at room temperature, and cells were resuspended in fresh complete medium. To evaluate the knockdown efficiency, UFC1 mRNA abundance was assessed via RT-qPCR using cells collected exactly 48 h after the start of transfection. Following confirmation of successful transfection, subsequent CSE stimulation and functional assays were conducted.

2.4 Establishment of LPS/CS-Induced COPD Rat Model and Grouping

For the present study, a total of 36 male Sprague-Dawley rats aged 6–8 weeks within a weight range of 180–220 g were obtained from the Shanghai-based SLAC Laboratory Animal Co., Ltd. (Shanghai, China; production license No. SCXK [Hu] 2022-0004). Each rat was considered an independent experimental unit. The animals were housed under SPF conditions, where the ambient temperature and relative humidity were regulated at $22 \pm 2^\circ\text{C}$ and 50%–60%, respectively, alongside a 12-h light/dark photoperiod. The animals were allowed *ad libitum* access to a standard laboratory diet and water. To ensure environmental adjustment, all rats underwent a one-week acclimatization period before the commencement of any experiments [29]. The Experimental Animal Ethics Committee of the Affiliated Huai'an Hospital of Xuzhou Medical University approved all animal procedures (approval No. HEYLL2025115).

The COPD rat model was established by exposing the animals to a combination of LPS and CS. On days 1, 15, 30, and 45 of the experiment, rats were anesthetized with isoflurane and received an intratracheal instillation of LPS solution at 1 mg/kg in a volume of 200 μL . For the remaining days, the rats were placed in a smoking chamber for CS exposure twice daily, with each exposure session lasting 1 h and involving smoke from one cigarette, with an interval of >6 h between sessions [29]. Control rats inhaled fresh air.

Rats were randomly assigned to six groups ($n = 6$ per group) using a random-number table: Control, COPD, COPD + DEX, COPD + AAV-NC, COPD + AAV-sh-UFC1, and COPD + AAV-sh-UFC1 + DEX. No animals were excluded from the study unless they met the predefined humane endpoint criteria, including a body weight loss of $\geq 20\%$ from baseline, persistent severe respiratory distress, inability to eat or drink, or marked lethargy or recumbency; animal health was monitored daily. Before the start of CS exposure, gene intervention was performed via intranasal instillation of AAV-sh-UFC1 or AAV-NC (5×10^{11} v.g./mL; GeneChem, Shanghai) at 100 μL per rat [31]. The oligonucleotides used for construction of the sh-UFC1 expression cassette were as follows: Oligo 1, 5'-ACCTCGGAAGTCCCTGATCTGATTCATCAAGAGTGAATCAGATCAGGGACTTCCTT-3'; and Oligo 2, 5'-CAAAAAGGAAGTCCCTGATCTGATTCACTCTTGATGAATCAGATCAGGGACTTCCG-3'. The oligonucleotides used for construction of the non-targeting control shRNA (sh-NC) expression cassette were as follows: Oligo 1, 5'-ACCTCGTTTCGACAGTGCCATGATCTATCAAGAGTAGATCATGGCACTGTGCGAACTT-3'; and Oligo 2, 5'-CAAAAAGTTTCGACAGTGCCATGATCTACTCTTGATAGATCATGGCACTGTGCGAACG-3'. DEX (0.2 mg/kg/day) was administered via oral gavage in PBS at 5 mL/kg 1 h before daily CS exposure. The modeling and treatment period lasted for a total of 8 weeks [32]. Rats were randomly assigned to the experimental groups. Outcome assessment and data analysis were performed by investigators blinded to group allocation. After group allocation, the positions of the cages on the animal-room racks were systematically rotated once per week to minimize potential spatial differences in lighting, ventilation, and temperature. At the experimental endpoint, rats were euthanized by an overdose of sodium pentobarbital (150 mg/kg, intraperitoneally). Death was confirmed by the absence of spontaneous respiration, heartbeat, and corneal reflex, after which blood and lung tissues were collected for subsequent analyses.

2.5 Cell Viability/Proliferation Assay (CCK-8)

For the CCK-8 assay, non-transfected U937 cells were directly seeded into 96-well plates at a density of 5×10^3 cells per well for subsequent treatment. For siRNA-transfected groups, U937 cells were first transfected in 6-well plates as described above. At 48 h post-transfection, cells were collected by centrifugation, counted, and re-seeded into 96-well plates at a density of 5×10^3 cells/well for subsequent CSE and/or DEX treatment. Following the specified experimental treatments, cell viability/proliferation was measured using a CCK-8 assay (Beyotime, Shanghai, China; Cat# C0037). Each well received 10 μ L of CCK-8 reagent following the conclusion of the specified treatments. The plates were then maintained at 37°C for a further 1 h to allow for the reaction. A Multiskan FC microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) was employed to measure the optical density (OD) at 450 nm. Subsequently, cell viability/proliferation was determined in accordance with the manufacturer's protocol.

2.6 Reverse Transcription Quantitative PCR (RT-qPCR)

The expression levels of lncRNA UFC1 in human U937 cells and Ufc1 in rat lung tissues were assessed using RT-qPCR. Following extraction from lung specimens or cell lysates using TRIzol (Beyotime, China; R0016), total RNA was converted to cDNA templates utilizing a reverse transcription kit (Beyotime, D7168M). The subsequent amplification was performed on the CFX96 platform (Bio-Rad, Hercules, CA, USA) with SYBR Green qPCR Master Mix (Beyotime, D7601M). Normalization was carried out against GAPDH to determine relative expression via the $2^{-\Delta\Delta C_t}$ algorithm. Specific primer information is provided in Table 1.

Table 1: The primer sequences.

Species/Sample Source	Genes	Primer Sequences (5'–3')	
Human/U937 cells	UFC1	Forward	TCCAACCTGAGTGACATAGCGA
		Reverse	CTGACCTCCAACCTCCAACGAAT
Human/U937 cells	GAPDH	Forward	TCAAGAAGGTGGTGAAGCAGG
		Reverse	TCAAAGGTGGAGGAGTGGGT
Rat/lung tissues	Ufc1	Forward	CGTGTCTGAGATCCCGGTGC
		Reverse	CCAAACCACCGGTCCCTTC
Rat/lung tissues	Gapdh	Forward	ACAGCAACAGGGTGGTGGAC
		Reverse	TTTGAGGGTGCAGCGAACTT

2.7 Enzyme-Linked Immunosorbent Assay (ELISA)

For ELISA analysis, non-transfected U937 cells were directly seeded into 24-well plates at a density of 1.0×10^5 cells/well for subsequent treatment. For siRNA-transfected groups, U937 cells were first transfected as described above and then re-seeded into 24-well plates for subsequent treatments. Culture supernatants were collected and cleared of cellular debris via a 5-min centrifugation at 300× g. The secretion levels of various cytokines—namely IL-6 (Cat# PI330), IL-1 β (Cat# PI305), TNF- α (Cat# PT518), IL-8 (Cat# PI640), GM-CSF (Cat# PG355), and MIP-3 α (Cat# KOA0261)—were then measured using commercial ELISA assays (Beyotime, Shanghai, China) in accordance with the supplier's guidelines.

For the assessment of cytokine expression in rat serum, blood samples were gathered from all experimental groups and incubated at ambient temperature for 30 min to permit coagulation. Subsequently, the serum was isolated by centrifuging (3000× g, 15 min, 4°C). Subsequently, ELISA kits from Beyotime (Shanghai, China) were employed to quantify the concentrations of IL-6 (Cat# PI328), IL-1 β (Cat# PI303), and TNF- α (Cat# PT516).

Experimental steps were conducted strictly following the protocol provided by the supplier. Absorbance values were recorded at 450 nm using an iMark Microplate Absorbance Reader (Bio-Rad Laboratories, Hercules, CA, USA), and the levels of cytokines were determined by referring to the corresponding standard curves.

2.8 Flow Cytometric Analysis of Apoptosis

To assess apoptosis, 1×10^5 U937 cells per staining reaction were harvested and subjected to two rounds of PBS (0.01 M, pH 7.4) rinsing. Subsequently, the cells were taken up in binding buffer, strictly adhering to the protocol of the Annexin V-APC/PI Kit (Beyotime, Shanghai, China; Cat# AP107-100). Following staining with Annexin V-APC and PI, the cells were incubated for 15 min at room temperature in the dark; the cells were kept in the dark to prevent light exposure. Subsequent data collection was executed on a FACSCanto II flow cytometer (BD Biosciences, San Jose, CA, USA), and at least 10,000 gated events were acquired for each sample. The overall percentage of apoptosis was derived from the combined early and late apoptotic populations through analysis with FlowJo software version 10.8.1 (FlowJo LLC, Ashland, OR, USA).

2.9 Immunofluorescence Staining in Cells

For the visualization of protein localization, U937 cells were placed onto sterile coverslips previously modified with poly-L-lysine and incubated for 30 min at 37°C to allow the cells to settle and adhere to the coverslips before fixation. The cells were then fixed with 4% paraformaldehyde for 15 min and permeabilized with 0.1% Triton X-100 for 10 min. To minimize background interference, a 1-h blocking step with 5% BSA was implemented. Subsequently, specimens were probed overnight (4°C) with primary antibodies targeting GR α (Abcam, Cambridge, UK; Cat# ab3580; 1:200) or HDAC2 (Abcam, Cambridge, UK; Cat# ab124974; 1:200). On the second day, an Alexa Fluor 488-conjugated secondary antibody (Invitrogen, Carlsbad, CA, USA; Cat# A-11008; 1:500) was applied for 1 h in a light-protected environment, followed by a 10-min nuclear counterstain with DAPI. Fluorescence signals were captured via an Olympus IX73 microscope (Olympus Corporation, Hachioji, Tokyo, Japan), and ImageJ version 1.53t (National Institutes of Health, Bethesda, MD, USA) was utilized to assess expression levels across five random fields per slide.

2.10 Western Blot Analysis

Total protein was harvested from pulmonary tissues and cellular lysates via lysis in RIPA buffer (Abcam, UK; Cat# ab288006) containing a cocktail of protease and phosphatase inhibitors. The lung specimens used for protein extraction were collected from rats at the experimental endpoint. For lung specimens, thorough homogenization was performed before the lysis process. The extraction involved a 30-min incubation on ice, with intermittent vortexing every 10 min to ensure efficiency. After centrifuging for 15 min at $12,000 \times g$ (4°C), the harvested supernatants were assessed for protein content using a bicinchoninic acid (BCA) Protein Assay Kit (Beyotime, Shanghai, China; Cat# P0012). Subsequently, 30 μ g of total protein per lane loads were resolved via SDS-PAGE and transferred onto PVDF membranes by electro-blotting. After transfer, membranes used for the detection of phosphorylated proteins, including p-PI3K and p-AKT, were blocked with 5% BSA in Tris-buffered saline containing 0.1% Tween-20 (TBST), whereas membranes used for the detection of total or structural proteins were blocked with 5% non-fat milk in TBST for 1 h at room temperature. The membranes underwent overnight incubation with primary antibodies at 4°C. The next day, following triple rinsing with TBST, the membranes were treated with a goat anti-rabbit IgG H&L (HRP) secondary antibody (Abcam, Cambridge, UK; Cat# ab6721; 1:2000) for 60 min. Protein bands were

visualized using enhanced chemiluminescence (ECL; Thermo Fisher Scientific, Waltham, MA, USA; Cat# 32106) reagents, and band intensities were quantified using ImageJ software.

The primary antibodies used in this study were as follows: Cleaved caspase-3 (Abcam, UK; Cat# ab32042; 1:500), Caspase-3 (Abcam, Cambridge, UK; Cat# ab32351; 1:1000), Bax (Abcam, Cambridge, UK; Cat# ab32503; 1:1000), Bcl-2 (Abcam, Cambridge, UK; Cat# ab182858; 1:1000), PI3K (Abcam, Cambridge, UK; Cat# ab302958; 1:1000), PI3K p85 alpha (Abcam, Cambridge, UK; Cat# ab191606; 1:1000), AKT (Abcam, Cambridge, UK; Cat# ab8805; 1:1000), p-AKT at S473 (Abcam, Cambridge, UK; Cat# ab81283; 1:1000), HDAC2 (Abcam, Cambridge, UK; Cat# ab32117; 1:1000), GR α (Abcam, Cambridge, UK; Cat# ab183127; 1:2000), GR β (antibodies-online, Aachen, Germany; Cat# ABIN1714189; 1:1000), MKP-1 (Abcam, Cambridge, UK; Cat# ab61201; 1:1000) and β -actin (Abcam, UK; Cat# ab8227; 1:10000). Relative protein abundance was determined by dividing the target band density by the density of the internal control, with values subsequently normalized to the control group. The levels of phosphorylated proteins were determined by normalizing the phosphoprotein signals to their respective total protein counterparts. Similarly, Caspase-3 activation was quantified by calculating the relative abundance of Cleaved Caspase-3 compared to total Caspase-3.

2.11 Assessment of GC Responsiveness Based on DEX-Mediated Inhibition of IL-8 Secretion

To assess GC responsiveness, concentration-response curves were generated for the inhibitory effect of DEX on LPS-induced IL-8 secretion. For the CSE concentration-response experiment, U937 cells were plated in 24-well plates at a density of 1.0×10^5 cells/well and exposed to different concentrations of CSE (0%, 1%, 2%, and 5%) for 24 h. Following CSE exposure, the cells were collected, washed with PBS, and resuspended in fresh complete medium. The cells were then treated with a range of DEX concentrations (10^{-10} – 10^{-6} mol/L) for 1 h, followed by stimulation with 1 mg/L LPS for 24 h. To evaluate the effect of UFC1 knockdown on GC responsiveness, U937 cells were transfected with si-UFC1 or si-NC for 48 h, as described above, and subsequently exposed to 5% CSE for 24 h. After CSE exposure, the cells were collected, washed with PBS, and resuspended in fresh complete medium. The cells were then treated with the same range of DEX concentrations for 1 h, followed by stimulation with 1 mg/L LPS for 24 h. In both experiments, LPS was used as a common terminal inflammatory stimulus. IL-8 concentrations in the harvested culture supernatants were subsequently quantified via ELISA. Concentration-response curves for DEX-mediated inhibition of LPS-induced IL-8 secretion were constructed using GraphPad Prism software version 9.5.1 (GraphPad Software, Boston, MA, USA).

2.12 RNA Immunoprecipitation Assay (RIP)

RIP was performed using the Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore-Sigma, Burlington, MA, USA; Cat# 17-700) according to the manufacturer's instructions. Approximately 2×10^7 U937 cells were harvested, washed with ice-cold PBS, and lysed in RIP lysis buffer supplemented with protease and RNase inhibitors. A portion of each cell lysate was retained as the input control. The remaining lysates were incubated overnight at 4°C with protein A/G magnetic beads conjugated with a rabbit anti-PI3K p85 antibody (Cell Signaling Technology, Danvers, MA, USA; Cat# 4292) or normal rabbit IgG supplied with the RIP kit as the negative control. After extensive washing, the immunoprecipitated RNA-protein complexes were treated with proteinase K, and the associated RNA was purified under RNase-free conditions. The recovered RNA was reverse-transcribed, and UFC1 enrichment in the input, IgG, and PI3K immunoprecipitates was determined by qRT-PCR using the UFC1-specific primers described above.

2.13 Hematoxylin and Eosin Staining and Lung Injury Scoring

Following collection, rat lung specimens were immersed in 4% paraformaldehyde overnight for fixation and subsequently processed into paraffin blocks. Sections with a thickness of 4 μm were then generated. These sections underwent deparaffinization in xylene and were rehydrated through descending grades of ethanol (100%, 95%, 90%, 80%, and 70%, v/v), followed by a 1-min staining period with hematoxylin (Beyotime Biotechnology, Shanghai, China; Cat# C0107). A 3-s differentiation step was conducted using 1% acid alcohol, followed by counterstaining with eosin for 1 min. Lung injury was scored based on alveolar wall thickening, inflammatory cell infiltration, alveolar destruction, and hemorrhage/edema. Each parameter was graded from 0 to 4 according to lesion severity, and the average score was used to reflect the degree of lung injury [33]. To quantitatively assess the extent of lung parenchymal destruction caused by emphysema, the Destructive Index (DI) was analyzed [34]. Histopathological scoring and DI assessment were independently performed by two investigators who were blinded to the group allocation, and any discrepancies were resolved by consensus. Ten high-power fields (HPFs) were randomly selected per section to count normal and destroyed alveoli. Destroyed alveoli were defined as those exhibiting ruptured alveolar walls, significantly thinned septa, or fused alveolar cavities. The DI was determined according to the equation: $\text{DI} = (\text{number of damaged alveoli} / \text{total alveolar count})$.

2.14 Measurement of Airway Resistance

Baseline respiratory mechanics parameters were evaluated in each group using a pulmonary function testing system (FinePointe RC, DSI, St. Paul, MN, USA). Anesthesia was induced by administering 1% pentobarbital sodium (50 mg/kg) via intraperitoneal (i.p.) injection. Subsequently, a tracheotomy was performed, and a specialized tracheal cannula was inserted. Following tracheal cannulation, rats received pancuronium bromide (1 mg/kg, i.p.) to abolish spontaneous respiratory effort. Complete respiratory paralysis was confirmed by the absence of spontaneous respiratory movements. Subsequently, the cannula was attached to a mechanical ventilator, with the settings established at a tidal volume of 8 mL/kg and a breathing frequency of 120 breaths/min. After the respiratory baseline had stabilized for 5 min, the baseline lung resistance (Baseline RL) was continuously recorded for 3 min. Mean data were utilized to assess the influence of various interventions on pulmonary dysfunction and airway narrowing in the COPD rat model.

2.15 Determination of Cellular HDAC2 Activity and Lung Nuclear HDAC/HDAC2 Activities

HDAC2 activity in U937 cells and nuclear HDAC/HDAC2 activities in rat lung tissues were measured using commercial activity assay kits according to the manufacturers' instructions. For cellular assays, U937 cells were collected after the indicated treatments and washed twice with pre-cooled PBS (0.01 M, pH 7.4). The cells were then lysed on ice for 30 min, with gentle vortexing every 10 min. After lysis, the samples were centrifuged at $12,000\times g$ for 15 min at 4°C, and the supernatants were collected as total protein extracts. Protein concentrations were determined using the BCA method, and equal amounts of protein were used for HDAC2 activity detection. Cellular HDAC2 activity was measured using an HDAC2 Immunoprecipitation and Activity Assay Kit (Sigma-Aldrich, St. Louis, MO, USA; Cat# EPI012) following the supplier's protocol.

For animal experiments, rat lung tissues from each group were minced and homogenized on ice. Nuclear protein fractions were then isolated using a nuclear extraction kit (Thermo Fisher Scientific, USA; Cat# 78833). All extraction procedures were performed at 4°C to preserve enzymatic stability. After quantification by the BCA method, equal amounts of nuclear protein were used to determine nuclear HDAC activity and nuclear HDAC2 activity. Nuclear HDAC activity was measured using an HDAC Activity Assay Kit (Abcam, UK; Cat# ab156064), whereas nuclear HDAC2 activity was determined using

an HDAC2 Immunoprecipitation and Activity Assay Kit (Sigma-Aldrich, USA; Cat# EPI012). According to the manufacturer's specifications, the HDAC2 kit applies to human, mouse, and rat samples. For the respective assays, equal amounts of protein samples were incubated with the reaction substrate provided in the corresponding kits. For the HDAC2 activity assay, HDAC2 was first immunoprecipitated using the antibody supplied with the kit before incubation with the enzymatic substrate. After the respective enzymatic reactions, fluorescence intensity was recorded using a Synergy H1 multimode microplate reader (BioTek Instruments, Winooski, VT, USA), with excitation/emission wavelengths set at 355/460 nm for the HDAC activity assay and 380/500 nm for the HDAC2 activity assay. Background fluorescence from blank wells was subtracted, and the corrected fluorescence values were expressed as percentages relative to the control group, which was defined as 100%.

2.16 Statistical Analysis

All experimental results are expressed as the mean $\pm$ standard deviation (SD). Cell-based experiments were performed with three independent biological replicates ($n = 3$), whereas animal cohorts consisted of six rats per group ($n = 6$). An *a priori* sample size calculation for the animal experiments was performed using G*Power 3.1, with the significance level set at $\alpha = 0.05$ and the statistical power at $1 - \beta = 0.80$. The calculation indicated that six rats per group were sufficient to detect a large between-group effect. Statistical analyses were executed using GraphPad Prism 9.5.1 (GraphPad Software, Boston, MA, USA). Normality of the residuals was assessed using the Shapiro–Wilk test. For datasets analyzed using the unpaired Student's *t*-test or ordinary one-way ANOVA, homogeneity of variance was evaluated using the Brown–Forsythe test. All datasets satisfied the relevant assumptions for parametric analysis. Discrepancies between the two groups were determined via an unpaired two-tailed Student's *t*-test. For datasets involving more than two groups, one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test was implemented. For the DEX concentration–response experiment described in Section 2.11, the effects of DEX concentration and CSE exposure were analyzed using two-way repeated-measures ANOVA with the Geisser–Greenhouse correction, followed by Tukey's multiple-comparisons test. Exact *p* values are reported in the Results and figure legends where applicable, and the magnitudes of key experimental changes are additionally described using fold changes where appropriate. A value of $p < 0.05$ was considered to indicate statistical significance. Detailed statistical information for the relevant comparisons, including the corresponding *p*-value ranges, is provided in Supplementary Materials Table S1.

3 Results

3.1 Downregulation of lncRNA UFC1 Inhibits CSE-Induced Inflammatory Response and Apoptosis in U937 Cells

CSE is a commonly used stimulus for establishing *in vitro* COPD models [35]. To investigate the involvement of lncRNA UFC1 in CSE-mediated damage to U937 cells, we initially assessed how CSE affects cell survival. As demonstrated by the CCK-8 assay, CSE exposure led to a decline in U937 cell viability (Fig. 1A). RT-qPCR analysis showed that the expression level of UFC1 was markedly upregulated following CSE stimulation (Fig. 1B), suggesting its potential involvement in CSE-induced pathological processes. To further explore its function, we transfected cells with si-UFC1 to inhibit its expression and confirmed high knockdown efficiency by RT-qPCR (Fig. 1C). Follow-up functional analyses revealed that UFC1 silencing partially counteracted the reduction in cell survival induced by 5% CSE (Fig. 1D). IL-6, IL-1 β , and TNF- α are commonly used pro-inflammatory markers for evaluating inflammatory responses [36]. ELISA results indicated that CSE induced massive secretion of these factors, whereas UFC1 knockdown

markedly suppressed this pro-inflammatory effect (Fig. 1E–G). Furthermore, the upregulation of cleaved caspase-3/caspase-3 and Bax/Bcl-2 ratios suggested that CSE induced significant cell apoptosis (Fig. 1H,I), which was further confirmed by flow cytometry (Fig. 1J,K). Notably, treatment with si-UFC1 effectively reduced the apoptosis rate. Taken together, our results indicate that the CSE-induced inflammatory reactions and programmed cell death in U937 cells are effectively mitigated by the depletion of UFC1.

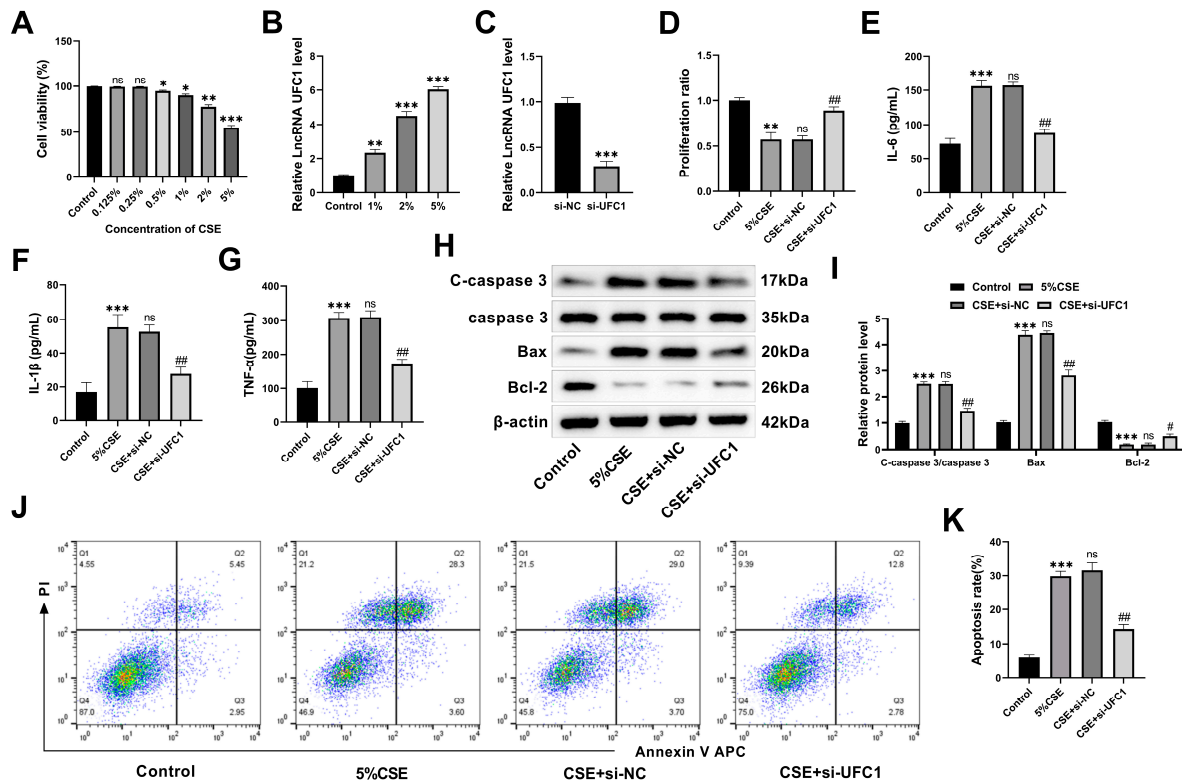


Figure 1: Downregulation of lncRNA UFC1 inhibits CSE-induced inflammatory response and apoptosis in U937 cells. (A) Evaluation of U937 cell viability via CCK-8 assay, illustrating a concentration-dependent decline upon CSE exposure. (B) UFC1 mRNA expression levels following CSE treatment as determined by RT-qPCR. (C) RT-qPCR validation of UFC1 silencing efficiency after si-UFC1 transfection. (D) CCK-8 assay results indicate that the CSE-mediated reduction in cell viability is attenuated by UFC1 knockdown. (E–G) ELISA quantification of pro-inflammatory cytokines, showing that UFC1 deficiency limits the CSE-triggered secretion of IL-6 (E), IL-1β (F), and TNF-α (G). (H,I) Immunoblotting results show that UFC1 knockdown prevents the CSE-induced accumulation of pro-apoptotic factors (cleaved caspase-3, bax) and simultaneously preserves the levels of anti-apoptotic bcl-2. (J,K) Representative flow cytometry plots (J) and corresponding quantification of apoptosis rates (K), demonstrating that UFC1 downregulation suppresses CSE-induced cell death. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control group or si-NC group; # $p < 0.05$, ## $p < 0.01$ vs. CSE + si-NC group; ns, no significance.

3.2 Downregulation of lncRNA UFC1 Alleviates CSE-Induced Glucocorticoid (GC) Resistance in LPS-Challenged U937 Cells

The inhibitory rate of DEX on LPS-induced IL-8 release is commonly utilized to evaluate GC sensitivity [29]. The results indicated that the suppressive capacity of DEX on LPS-induced IL-8 release was progressively diminished in response to rising CSE concentrations (Fig. 2A). Under 5% CSE exposure, DEX-mediated inhibition of LPS-induced IL-8 release was markedly attenuated across the tested concentration range, indicating reduced cellular responsiveness to GC (Fig. 2B,C). In contrast, UFC1 knockdown signifi-

cantly reduced the IC_{50} of DEX, as reflected by an increased $-\log(IC_{50})$ value, indicating restored cellular sensitivity to DEX. ELISA results further revealed that si-UFC1 enhanced the inhibitory effects of DEX on inflammatory mediators, including IL-8, GM-CSF, and MIP-3 α (Fig. 2D–F). The anti-inflammatory effects of GCs depend on GR α nuclear translocation and HDAC2-mediated transcriptional repression, whereas increased GR β can antagonize GR α -mediated signaling and contribute to reduced GC sensitivity [37–39]. Data from both Western blotting and immunofluorescence revealed that CSE exposure was associated with restricted nuclear entry of GR α and a decrease in HDAC2 protein levels, while Western blotting further showed increased GR β expression. Western blotting showed that CSE exposure markedly reduced MKP-1 protein expression, whereas DEX treatment partially restored its expression. UFC1 knockdown also increased MKP-1 levels, and this effect was further enhanced in the presence of DEX (Fig. 2G,H). Immunofluorescence analysis revealed that CSE exposure impaired the nuclear localization of GR α and reduced HDAC2 expression, whereas UFC1 knockdown promoted GR α nuclear localization and restored HDAC2 expression (Fig. 2I–L). These findings were further supported by Western blotting, which showed increased HDAC2 and GR α protein levels following UFC1 knockdown. In human U937 cells, UFC1 knockdown also attenuated the CSE-induced increase in GR β expression (Fig. 2M,N). Collectively, these results indicate that UFC1 downregulation may alleviate CSE-induced GC insensitivity by restoring MKP-1 and HDAC2 expression and enhancing GR α nuclear localization; in the human U937 cell model, this effect was also accompanied by reduced GR β expression. These findings suggest that the downregulation of UFC1 can mitigate CSE-induced GC resistance by improving GR α function and HDAC2 expression and suppressing GR β expression.

3.3 Downregulation of lncRNA UFC1 Inhibits the Activation of the PI3K/AKT Pathway

To clarify the regulatory role of UFC1 in the PI3K/AKT signaling axis, we first examined how UFC1 knockdown affected the phosphorylation status of these proteins. Our data demonstrated that the loss of UFC1 notably reduced the p-PI3K/PI3K and p-AKT/AKT ratios (Fig. 3A,B). While CSE exposure triggered the activation of the PI3K/AKT axis alongside a decrease in HDAC2 expression, DEX treatment attenuated these CSE-induced changes, as evidenced by reduced p-PI3K and p-AKT levels and partially restored HDAC2 expression in the CSE + DEX group. Similarly, the inhibition of UFC1 diminished PI3K and AKT phosphorylation and promoted a partial recovery of HDAC2 expression (Fig. 3C,D). The use of 740Y-P, a PI3K agonist, further supported this relationship. DEX consistently attenuated CSE-induced PI3K/AKT pathway activation and partially restored HDAC2 expression, whereas 740Y-P partially counteracted the effects of si-UFC1 by increasing PI3K and AKT phosphorylation and reversing the si-UFC1-mediated recovery of HDAC2 expression (Fig. 3E,F). Furthermore, RIP-qPCR analysis demonstrated significant enrichment of UFC1 in the PI3K immunoprecipitate compared with the IgG control, supporting an association between UFC1 and PI3K (Fig. 3G). These findings imply that UFC1 may contribute to HDAC2 downregulation by enhancing the activity of the PI3K/AKT pathway.

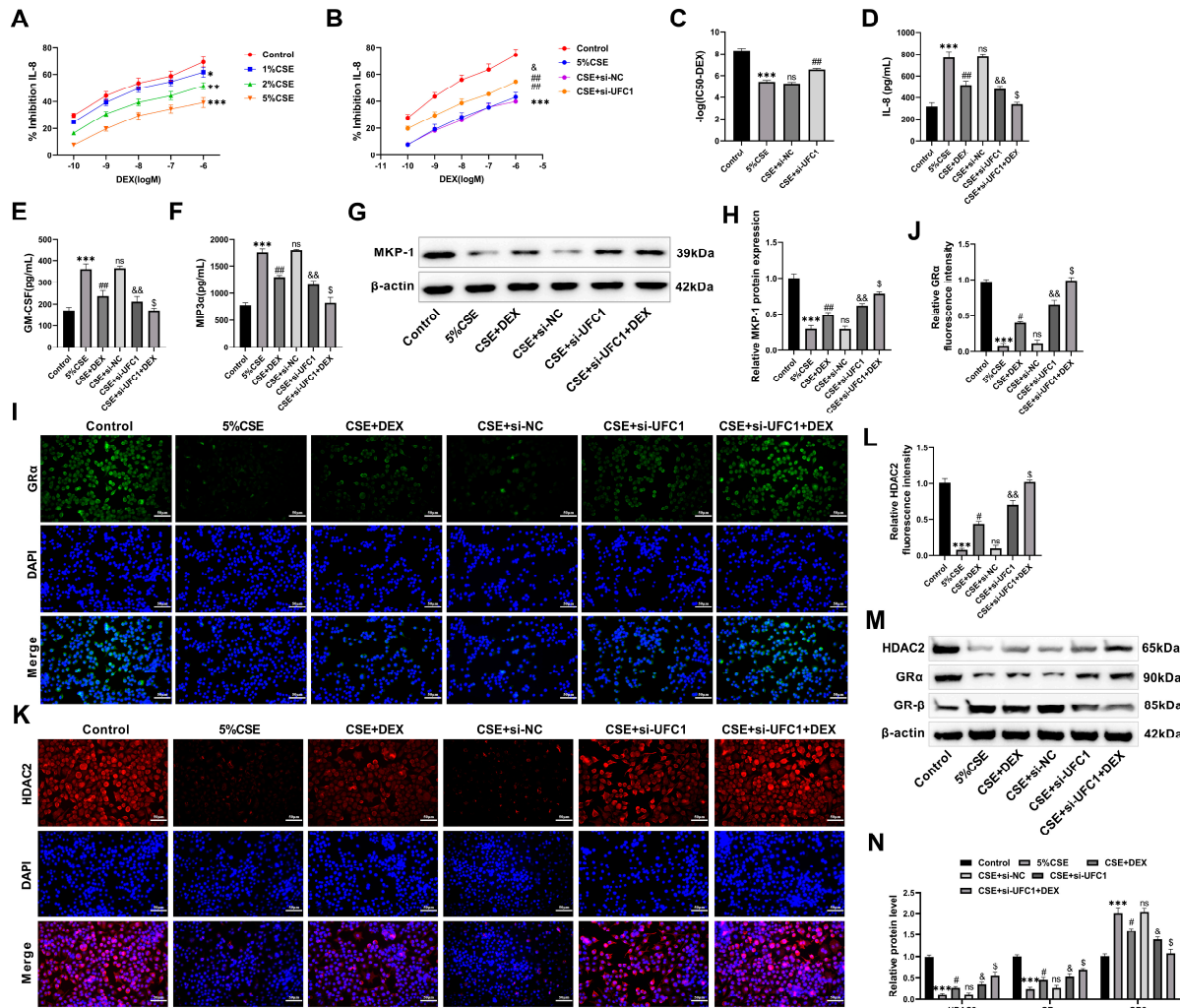


Figure 2: Downregulation of lncRNA UFC1 alleviates CSE-induced GC resistance in U937 cells. (A) A gradual loss of DEX-mediated suppression of IL-8 is observed as the dose of CSE increases. (B) Concentration-response profiles of DEX-mediated IL-8 inhibition across experimental cohorts. (C) Quantification shows that the IC₅₀ value of DEX is notably lowered upon UFC1 silencing, thereby alleviating CSE-induced GC insensitivity. (D–F) The inhibitory impact of DEX on the release of IL-8 (D), GM-CSF (E), and MIP-3α (F) is further augmented by UFC1 knockdown. (G,H) Representative western blot images (G) and densitometric analysis (H) showing that CSE exposure reduced MKP-1 protein expression, whereas UFC1 knockdown restored MKP-1 expression and further enhanced its induction by DEX. (I,J) Representative immunofluorescence micrographs (I) and statistical evaluation (J), showing that UFC1 deficiency promotes the nuclear entry of GRα, which CSE otherwise hindered. (K,L) Immunofluorescence assessment (K) and quantification (L) reveal that UFC1 silencing facilitates the recovery of HDAC2 expression following its downregulation by CSE. (M,N) Immunoblotting results (M) and densitometric analysis (N) indicate that the depletion of UFC1 enhances the protein levels of HDAC2 and GRα. **p* < 0.05, ***p* < 0.01, ****p* < 0.001 vs. control group; #*p* < 0.05, ##*p* < 0.01 vs. 5% CSE group; &*p* < 0.05, &&*p* < 0.01 vs. CSE + si-NC group; \$*p* < 0.05 vs. CSE + si-UFC1 group; ns, no significance.

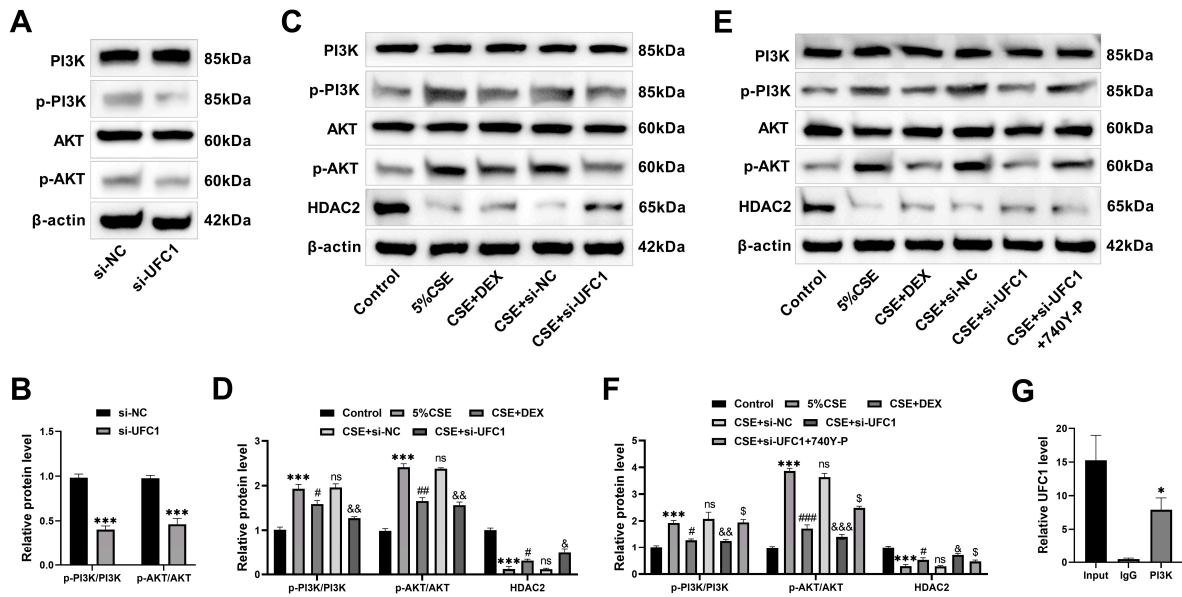


Figure 3: Downregulation of lncRNA UFC1 inhibits the activation of the PI3K/AKT signaling pathway. (A,B) Immunoblotting (A) and corresponding densitometric quantification (B) indicate that the silencing of UFC1 diminishes the phosphorylation of p-PI3K and p-AKT. (C,D) Western blot (C) and statistical analysis (D) reveal that UFC1 deficiency attenuates the CSE-mediated overactivation of PI3K/AKT signaling while helping to recover HDAC2 protein levels. (E,F) Western blot (E) and quantitative results (F) show that the PI3K agonist 740Y-P partly offsets the inhibitory influence of si-UFC1 on pathway protein phosphorylation, resulting in a secondary decline in HDAC2 expression. (G) RIP-qPCR analysis showed significant enrichment of UFC1 in the PI3K immunoprecipitate compared with the IgG control, supporting an association between UFC1 and PI3K. Input RNA was used as the positive control, and IgG immunoprecipitation served as the negative control. * $p < 0.05$ and *** $p < 0.001$ vs. control group or si-NC group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs. 5% CSE group; & $p < 0.05$, && $p < 0.01$, &&& $p < 0.001$ vs. CSE + si-NC group; \$ $p < 0.05$ vs. CSE + si-UFC1 group; ns, no significance.

3.4 Downregulation of lncRNA UFC1 Alleviates CSE-Induced GC Resistance by Inhibiting the PI3K/AKT Pathway

To investigate whether the PI3K/AKT axis mediates UFC1's effect on GC resistance, rescue experiments were conducted utilizing the PI3K agonist 740Y-P. Findings indicated that 740Y-P application attenuated the enhancement of cell survival induced by si-UFC1 (Fig. 4A) and partially offset its suppressive effect on programmed cell death (Fig. 4B–E). Furthermore, in the evaluation of GC responsiveness, 740Y-P treatment weakened the inhibitory effect of DEX on LPS-induced IL-8 secretion, thereby partially attenuating the improvement in GC responsiveness induced by UFC1 knockdown (Fig. 4F). Additionally, the secretion levels of IL-8, GM-CSF, and MIP-3 α were upregulated following 740Y-P administration (Fig. 4G–I). Furthermore, 740Y-P counteracted the enhanced GR α nuclear translocation and the restoration of HDAC2 expression and activity induced by si-UFC1 (Fig. 4J–N). Notably, the assay for HDAC2 enzymatic activity revealed a consistent regulatory trend (Fig. 4L). Activation of the PI3K/AKT signaling axis appears to be the primary mechanism through which UFC1 regulates GC resistance within U937 cells.

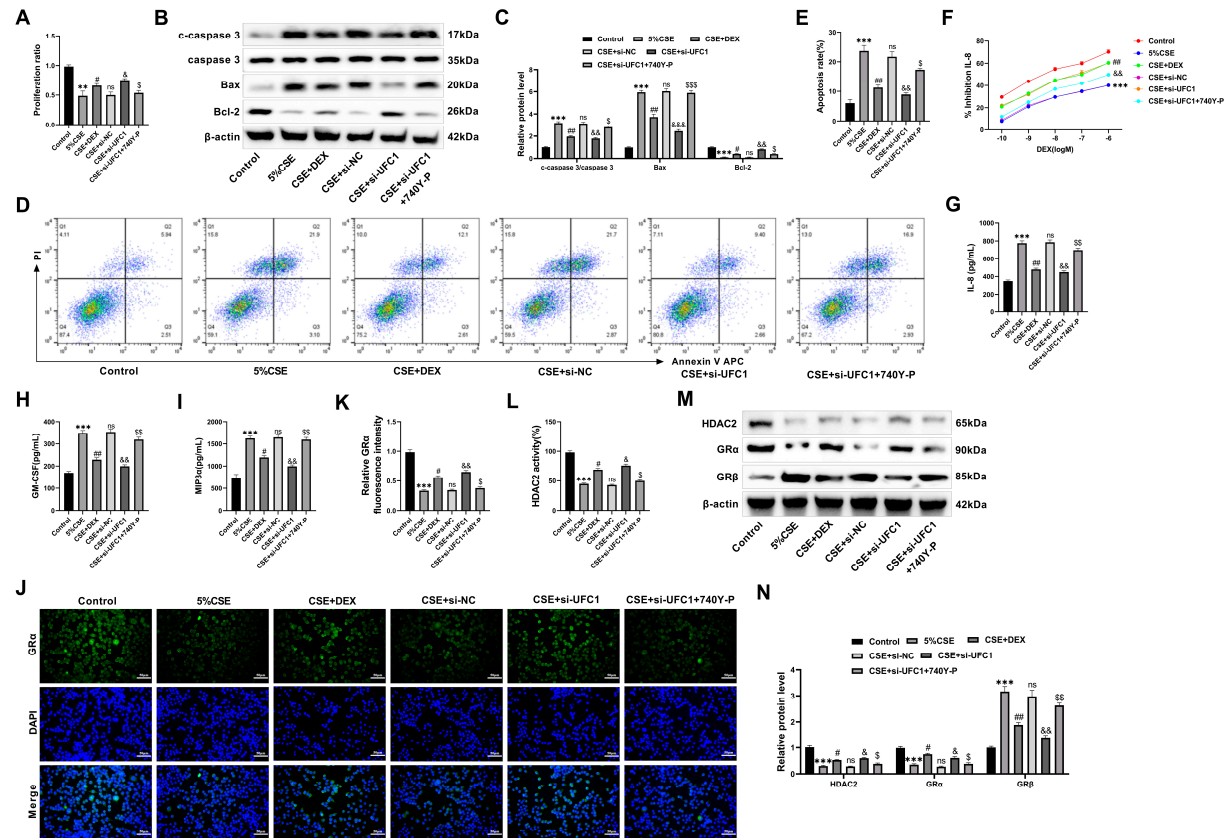


Figure 4: Downregulation of lncRNA UFC1 alleviates CSE-induced GC resistance in U937 cells by inhibiting the PI3K/AKT pathway. (A) CCK-8 assay results indicating that 740Y-P attenuates the enhancement of cell proliferation mediated by si-UFC1. (B,C) Immunoblotting (B) and subsequent densitometric assessment (C) regarding c-caspase 3, caspase-3, bax, and bcl-2, showing that 740Y-P counteracts the regulatory impact of si-UFC1 on apoptotic protein expression. (D,E) Flow cytometric evaluation (D) and statistical analysis of apoptosis rates (E) suggest that 740Y-P moderates the anti-apoptotic influence of si-UFC1. (F) Concentration-response curves for DEX-induced IL-8 suppression in U937 cells; 740Y-P leads to an upward shift in the IC₅₀ value, associated with decreased GC responsiveness. (G–I) ELISA quantification of supernatant IL-8 (G), GM-CSF (H), and MIP-3 α (I) levels, demonstrating that 740Y-P diminishes the anti-inflammatory action of si-UFC1, resulting in increased cytokine release. (J,K) Representative immunofluorescence images (J) and quantitative evaluation of fluorescence intensity (K) for GR α , illustrating that 740Y-P suppresses the facilitation of GR α nuclear entry by si-UFC1. (L) Measurement of HDAC2 enzymatic activity across cohorts reveals that 740Y-P reduces HDAC2 function. (M,N) Immunoblotting (M) and densitometry (N) of HDAC2, GR α , and GR β , indicating that 740Y-P offsets the si-UFC1-mediated upregulation of HDAC2 and GR α protein levels. ** p < 0.01, *** p < 0.001 vs. control group; # p < 0.05, ## p < 0.01 vs. 5% CSE group; & p < 0.05, && p < 0.01, &&& p < 0.001 vs. CSE + si-NC group; \$ p < 0.05, \$\$ p < 0.01, \$\$\$ p < 0.001 vs. CSE + si-UFC1 group; ns, no significance.

3.5 Inhibition of lncRNA UFC1 Alleviates Cigarette Smoke-Induced Lung Injury and Inflammation in COPD Rats

To examine the biological impact in an animal model, a COPD rat model was generated by LPS instillation and CS exposure. RT-qPCR results showed that UFC1 levels were notably higher in the lungs of the COPD cohort; however, this elevation was attenuated by the administration of AAV-sh-UFC1 (Fig. 5A,B). Histological analysis via H&E staining showed that the lung tissues in the COPD group exhibited significant

alveolar structural destruction, septal thickening, and inflammatory cell infiltration, accompanied by a markedly increased lung injury score (Fig. 5C,D). Downregulation of UFC1 significantly attenuated these pathological injuries.

Physiological monitoring demonstrated that airway resistance was increased in the COPD group, whereas AAV-sh-UFC1 treatment effectively decreased the resistance (Fig. 5E). Furthermore, AAV-sh-UFC1 treatment reduced the DI, which reflects the severity of emphysema, and decreased the serum levels of TNF- α , IL-1 β , and IL-6 (Fig. 5F–I). These *in vivo* findings further support that the inhibition of UFC1 can mitigate COPD-related lung pathological damage and inflammatory responses.

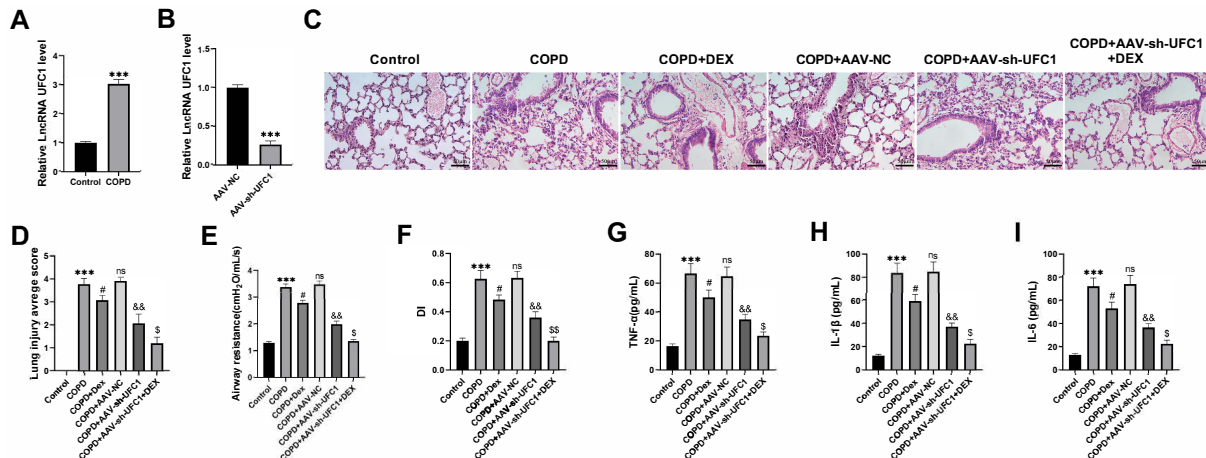


Figure 5: Targeting lncRNA UFC1 relieves pulmonary inflammation and injury in LPS/CS-induced COPD rats. (A) RT-qPCR analysis revealed a notable elevation of UFC1 expression in the pulmonary tissues of the COPD cohort. (B) RT-qPCR validation showing that AAV-sh-UFC1 administration leads to a reduction in UFC1 levels within lung tissues. (C,D) Histopathological observations via H&E staining (Scale bar = 100 μ m) (C) and statistical evaluation of lung injury scores (D) suggest that UFC1 deficiency attenuates pathological lung impairment in COPD rat models. (E) Measurement of respiratory resistance; the knockdown of UFC1 appears to mitigate the increase in airway resistance in LPS/CS-induced COPD rats. (F–I) Evaluation of the DI (F) and pro-inflammatory cytokine concentrations, including TNF- α (G), IL-1 β (H), and IL-6 (I), indicates that reduced UFC1 expression helps alleviate the severity of emphysema and systemic inflammatory responses in COPD rats. *** p < 0.001 vs. control group; # p < 0.05 vs. COPD group; && p < 0.01 vs. COPD + AAV-NC group; \$ p < 0.05, \$\$ p < 0.01 vs. COPD + AAV-sh-UFC1 group.

3.6 Downregulation of lncRNA UFC1 Alleviates Glucocorticoid Resistance in a COPD Rat Model by Inhibiting the PI3K/AKT Pathway

To further clarify the *in vivo* role of lncRNA UFC1 in regulating glucocorticoid responsiveness, PI3K/AKT signaling and GR-related factors were evaluated in rat lung tissues. Given that excessive PI3K/AKT activation is a central factor contributing to HDAC2 dysfunction, this pathway represents a vital link in the development of glucocorticoid resistance. Western blotting demonstrated that, relative to the Control group, the phosphorylation of PI3K and AKT was notably higher in the lung tissues of COPD rats, suggesting that this signaling axis was overactivated in the LPS/CS-induced COPD model (Fig. 6A,B). Treatment with DEX alone failed to significantly inhibit pathway activation, whereas downregulation of UFC1 markedly attenuated the phosphorylation status of both PI3K and AKT.

HDAC2 is a key co-repressor mediating the anti-inflammatory effects of glucocorticoids, and its reduced activity directly leads to the derepression of inflammatory gene transcription [40]. Enzymatic activity assays showed that nuclear HDAC activity in lung tissue extracts was markedly reduced in the COPD

group (Fig. 6C). Consistently, nuclear HDAC2 activity was also decreased in COPD rat lungs (Fig. 6D). In contrast, AAV-mediated silencing of UFC1 restored both nuclear HDAC activity and nuclear HDAC2 activity, with a more pronounced recovery observed in the group co-treated with DEX. Further analysis of GC receptor isoforms (GR α and GR β) revealed that COPD led to the downregulation of functional GR α protein levels and induced the expression of antagonistic GR β , thereby weakening the therapeutic efficacy of GCs (Fig. 6E,F). Silencing of UFC1 notably enhanced the protein levels of HDAC2 and GR α , while simultaneously attenuating the excessive rise of GR β . These *in vivo* findings confirm that the downregulation of UFC1 can alleviate glucocorticoid resistance in COPD rats by inhibiting the PI3K/AKT axis, upregulating HDAC2 expression and activity, and subsequently correcting the GR α /GR β imbalance.

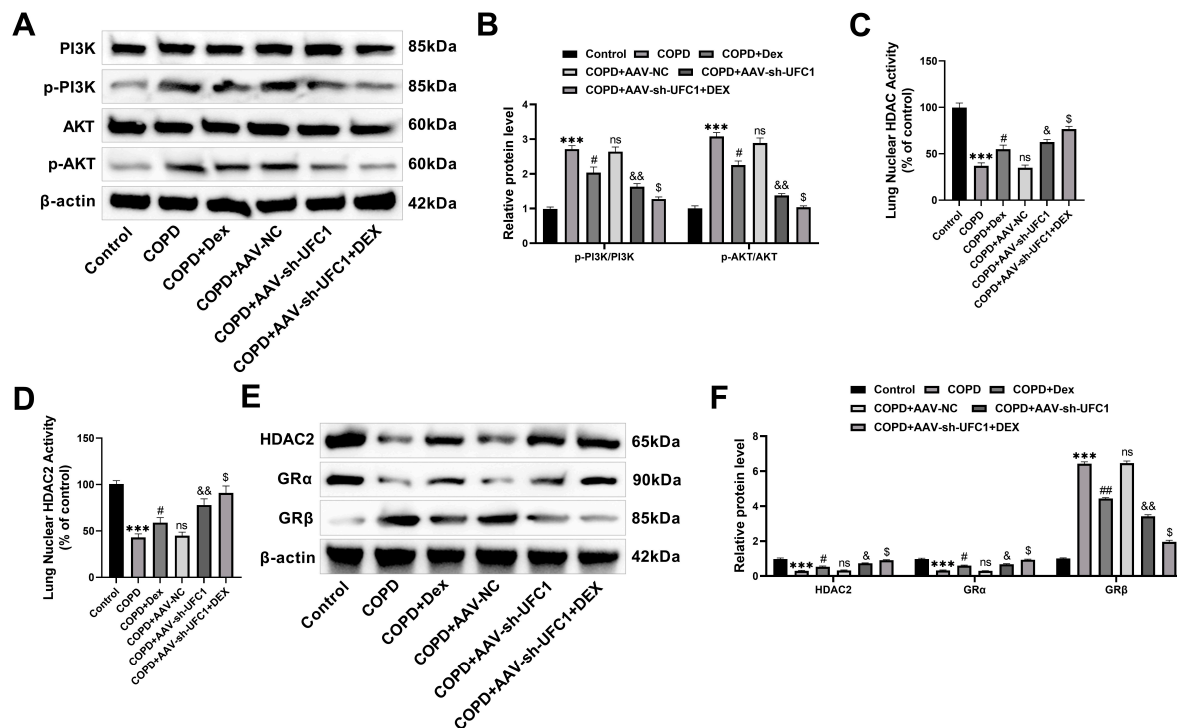


Figure 6: Downregulation of lncRNA UFC1 alleviates glucocorticoid resistance in a COPD rat model by inhibiting the PI3K/AKT pathway. (A,B) Western blot analysis (A) and densitometric evaluation (B) of PI3K, p-PI3K, AKT, and p-AKT in lung tissues, suggesting that UFC1 deficiency attenuates the phosphorylation status of PI3K and AKT in the COPD rat model. (C) Assessment of nuclear HDAC activity in lung tissue extracts, showing that UFC1 silencing facilitates the recovery of enzymatic function. (D) Nuclear HDAC2 activity assay showing that UFC1 knockdown promotes the recovery of nuclear HDAC2 activity, with a particularly notable effect observed in the cohort co-treated with DEX. (E,F) Immunoblotting (E) and densitometry (F) of HDAC2, GR α , and GR β expression, demonstrating that UFC1 downregulation enhances the protein levels of HDAC2 and GR α while moderating the excessive rise of GR β . *** p < 0.001 vs. control group; # p < 0.05, ## p < 0.01 vs. COPD group; & p < 0.05, && p < 0.01 vs. COPD + AAV-NC group; \$ p < 0.05 vs. COPD + AAV-sh-UFC1 group. ns, no significance.

4 Discussion

COPD is recognized as a long-term pulmonary ailment, primarily defined by enduring airway inflammation and chronic obstruction of the airways [41]. Although GCs serve as the cornerstone of clinical anti-inflammatory therapy, approximately 30% of COPD patients exhibit GC resistance, which represents a hurdle in effective treatment [42]. In our study, by establishing CSE-induced U937 cell models and

COPD rat models, we systematically demonstrated the pivotal role of lncRNA UFC1 in COPD-associated inflammatory responses and GC resistance. Our findings indicate that the downregulation of UFC1 can reverse GC resistance and alleviate lung injury by inhibiting the PI3K/AKT signaling pathway, restoring HDAC2 expression and activity, and correcting the GR α /GR β imbalance.

LncRNAs, acting as key epigenetic regulators, play pivotal roles in inflammatory lung diseases [43]. Our results demonstrate that UFC1 expression was increased in CSE-exposed U937 cells and in the lung tissues of LPS/CS-induced COPD rats. Furthermore, the downregulation of UFC1 markedly inhibited the secretion of pro-inflammatory cytokines and reduced cell apoptosis. Animal studies substantiated that the silencing of UFC1 helped to ameliorate pulmonary histopathology and lower both airway resistance and the DL. These results indicate that UFC1 acts as a key mediator of inflammatory responses in the COPD model primarily driven by chronic CS exposure with intermittent LPS challenge.

The pathogenesis of GC resistance is highly complex, with the decline in HDAC2 expression and activity playing an important role [44]. Mechanistically, HDAC2 facilitates the recruitment of GR to the promoters of inflammatory genes and deacetylates histones, thereby repressing the transcription of these genes [45]. We observed that CSE stimulation reduced HDAC2 activity in U937 cells and decreased nuclear HDAC/HDAC2 activities in rat lung tissues, accompanied by a reduced inhibitory effect of DEX on IL-8 secretion and an elevated IC₅₀-DEX value. Conversely, UFC1 downregulation enhanced DEX sensitivity, restored HDAC2 protein expression, and increased cellular HDAC2 activity as well as nuclear HDAC/HDAC2 activities in lung tissues. This suggests that UFC1-mediated GC resistance is closely associated with HDAC2 dysfunction. Furthermore, our findings revealed that UFC1 regulates GR α and GR β . GR α is the functional isoform that exerts anti-inflammatory effects, whereas GR β acts as a dominant-negative inhibitor [46]. Downregulation of UFC1 rectified the CSE-induced decrease in GR α and HDAC2 expression and enhanced GC responsiveness; in the human U937 cell model, it also attenuated the CSE-induced increase in GR β .

Activation of the PI3K/AKT pathway has been implicated in HDAC2 dysfunction, possibly by promoting HDAC2 phosphorylation and subsequent ubiquitin-proteasome-mediated degradation [47]. Importantly, rescue experiments using the PI3K activator 740Y-P revealed that activating the PI3K/AKT pathway partially counteracted the restorative effects of si-UFC1 on HDAC2 expression and the improvement of GC sensitivity. This evidence provides strong support for the pathological process in which UFC1 facilitates HDAC2 deficiency and GC resistance by sustaining the hyperactivation of the PI3K/AKT signaling axis.

However, this study has several limitations. The *in vitro* experiments were primarily conducted using U937 cells, which provide a reproducible model of monocyte/macrophage-associated inflammatory responses but cannot fully recapitulate the cellular heterogeneity and pathological microenvironment of COPD. Further validation in primary human bronchial epithelial cells, alveolar macrophages, and monocyte-derived macrophages is therefore required to determine whether the regulatory effects of UFC1 on the PI3K/AKT/HDAC2 signaling are broadly applicable. In addition, although the cigarette smoke-exposure conditions were standardized across experimental groups, serum or urinary cotinine, carboxyhemoglobin, and other exposure-related biomarkers were not measured. Consequently, individual variability in the actual systemic smoke exposure could not be quantitatively assessed. Future studies incorporating objective exposure biomarkers and primary human cell models will help strengthen the reproducibility and translational relevance of these findings. Moreover, the involvement of the PI3K/AKT pathway was mainly evaluated using the PI3K activator 740Y-P in rescue experiments, whereas complementary validation with a PI3K-specific inhibitor, such as LY294002, was not performed. Future studies combining primary human cell models, objective smoke-exposure biomarkers, and bidirectional pharmacological regulation of PI3K/AKT

signaling will further strengthen the mechanistic evidence, reproducibility, and translational relevance of these findings.

Overall, lncRNA UFC1 appears to be a significant regulatory factor in the onset and advancement of COPD. By modulating the PI3K/AKT signaling axis, it may contribute to the suppression of HDAC2 activity and a reduction in GR α expression, potentially resulting in an amplified inflammatory response and diminished glucocorticoid sensitivity. Although the present study identified the UFC1/PI3K/AKT/HDAC2 regulatory axis, whether UFC1 functions by directly binding to the PI3K protein or by acting as a competitive endogenous RNA (ceRNA) warrants further investigation.

5 Conclusion

The present investigation explored the functional significance and mechanistic basis of lncRNA UFC1 in the context of COPD-related inflammation and corticosteroid insensitivity. Our results confirmed that stimulation with CSE or cigarette smoke induced the aberrant overexpression of UFC1, whereas its inhibition significantly attenuated inflammatory injury and apoptosis in both U937 cells and COPD rats. A potential mechanism underlying these effects involves the following: the silencing of UFC1 attenuates PI3K/AKT signaling, which facilitates the recovery of HDAC2 expression and activity. This process further helps normalize the GR α -dependent glucocorticoid responsiveness, thereby potentially alleviating glucocorticoid insensitivity under COPD-related conditions.

These results broaden the current perspective on the involvement of lncRNAs in COPD development and suggest potential candidate targets for addressing clinical glucocorticoid insensitivity. Strategies focused on UFC1 or the associated PI3K/AKT signaling axis may offer a viable direction for the creation of supplemental treatments aimed at improving steroid responsiveness. Future research will focus on elucidating the direct protein interactants of UFC1 using advanced molecular biology techniques and evaluating its predictive value in clinical samples from patients with COPD.

Acknowledgement: None.

Funding Statement: This work was supported by the Natural Science Research Plan of the Huai'an Municipal Science and Technology Bureau (Grant No. HAB202419) and the General Category Medical Research Project of the Provincial Health Commission (Grant No. M2024003).

Author Contributions: The authors confirm their contributions to the paper as follows: conceptualization, Jingjing Wu, Jing Wang and Yufeng Wan; methodology, Jingjing Wu; formal analysis, Jingjing Wu and Yue Li; investigation, Jingjing Wu; data curation, Jingjing Wu and Yue Li; writing—original draft preparation, Jingjing Wu; writing—review and editing, Jingjing Wu, Yue Li, Jing Wang and Yufeng Wan. All authors reviewed and approved the final version of the manuscript.

Availability of Data and Materials: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval: This study was approved by the Affiliated Huai'an Hospital of Xuzhou Medical University Ethics Committee. (Approval number: HEYLL2025115).

Conflicts of Interest: The authors declare that they have no financial conflicts of interest.

Supplementary Materials: The supplementary material is available online at <https://www.techscience.com/doi/10.32604/biocell.2026.086220/s1>.

Abbreviations

Abbreviations	Full name
AAV	Adeno-associated virus
ANOVA	Analysis of variance
Bax	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma 2
BSA	Bovine serum albumin
CCK-8	Cell Counting Kit-8
COPD	Chronic obstructive pulmonary disease
CS	Cigarette smoke
CSE	Cigarette smoke extract
DEX	Dexamethasone
DI	Destructive Index
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal bovine serum
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GC	Glucocorticoid
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GR	Glucocorticoid receptor (α/β subtypes)
HDAC2	Histone deacetylase 2
H&E	Hematoxylin and eosin
IC ₅₀	Half maximal inhibitory concentration
IL	Interleukin (IL-6, IL-1 β , IL-8)
lncRNA UFC1	Long non-coding RNA UFC1
LPS	Lipopolysaccharide
MIP-3 α	Macrophage inflammatory protein-3 alpha
NC	Negative control
PBS	Phosphate-buffered saline
PI3K	Phosphoinositide 3-kinase
RT-qPCR	Quantitative real-time polymerase chain reaction
RL	Airway resistance
SD	standard deviation
siRNA	Small interfering RNA
SPF	Specific pathogen-free
STR	Short tandem repeat
TNF- α	Tumor necrosis factor-alpha

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