# Role of LncRNA H19/miR-93-5p/Orai1 axis in the regulation of human bronchial smooth muscle cell functions and airway remodeling in murine models of asthma

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## Abstract

# Background

Type 2 cytokine Interleukin (IL)-13 regulates airway remodeling in asthma by acting on store-operated $Ca^{2+}$ entry (SOCE) in airway smooth muscle cells. The underlying mechanisms of this regulating effect of IL-13 are not fully understood.

# Methods

Bioinformatic analysis identified interactions of microRNA 93-5p with Orai1, the pore-forming molecule of SOCE, and long non-coding RNA H19 respectively. We investigated the role of H19/miR-93-5p/Orai1 axis in the regulation of proliferation and migration of *in vitro* cultured human bronchial smooth muscle cells (hBSMCs) induced by IL-13. Functional relevance of H19 in airway inflammation and airway remodeling was investigated in acute and chronic murine models of asthma.

# Results

IL-13 dose-dependently increased the expression of H19 and Orai1 and decreased the expression of miR-93-5p in hBSMCs; H19 siRNA reversed IL-13-induced miR-93-5p and Orai1 expression, and the proliferation and migration of hBSMCs. IL-13-promoted expression of H19 and Orai1 was reduced by miR-93-5p mimic and enhanced by miR-93-5p inhibitor. IL-13-promoted hBSMCs proliferation was enhanced by miR-93-5p inhibitor but not changed by miR-93-5p mimic; whereas IL-13-promoted hBSMCs migration was enhanced by miR-93-5p inhibitor and reduced by miR-93-5p mimic. MiR-93-5p mimic enhanced the inhibiting effect of H19 siRNA on IL-13-induced Orai1 mRNA expression, whereas miR-93-5p inhibitor reversed the inhibiting effects of H19 siRNA on IL-13-induced H19 and Orai1 mRNA and protein expression. The inhibiting effect of H19 siRNA on IL-13-induced hBSMCs proliferation and migration was reversed by miR-93-5p inhibitor but not changed by miR-935p mimic. In the lungs of both asthma mice models, the expression of H19 and Orai1 was higher than in control mice. In acute asthma mice, H19 siRNA reduced Orai1 expression, inflammatory cell infiltration and goblet cell hyperplasia in the lungs, and IL-13 levels in the bronchoalveolar lavage fluid. In chronic asthma mice, H19 siRNA reduced Orai1 expression, inflammatory cell infiltration, goblet cell hyperplasia, collagen deposition and smooth muscle mass in the lungs, as well as IL-13 levels in the BALF.

# Conclusion

IL-13 increases the proliferation and migration of airway smooth muscle cells by acting on H19/miR-93-5p/Orai1 axis, which also regulates airway inflammation and airway remodeling in murine models of asthma.

# 1. Background

Asthma is a heterogeneous disease characterized by variable respiratory symptoms and airflow limitation[1]. The pathogenesis of asthma is not fully understood; however, type 2 immunity has been accounted for most allergic asthma[2]. IL-13 is an important cytokine of type 2 inflammation and can be produced by T helper 2 (Th2) cells and group 2 innate lymphoid cells (ILC2)[2].

Airway remodeling is another important feature of asthma and contributes to persistent airflow limitation and decreased reversibility in response to bronchodilators. Airway remodeling has been found to be presented in early childhood with asthma, suggesting that it may be dissociated with airway inflammation[1]. Pathologically, airway remodeling is characterized by changes in structural cells, including epithelial damage, cilial dysfunction, goblet cell hyperplasia, thickened lamina reticularis and reticular basement membrane, increased subepithelial collagen deposition and angiogenesis, airway smooth muscle hypertrophy and hyperplasia[1]. Airway smooth muscle cells (ASMCs) hypertrophy and hyperplasia represent the most important components of airway remodeling and ASM mass is the best predictor for airflow limitation in asthma[1].

Type 2 cytokines IL-4 and IL-13 plays a pivotal role in airway remodeling[2] and airway hyperreactivity (AHR)[3]. IL-13 has been established as an indispensable cytokine in the development of airway remodeling and contributes to steroid-resistance in pediatric asthma patients[4]. IL-13 acts on different cell types to induce airway remodeling, such as airway epithelial cell[5] and fibroblasts[6]. More importantly, IL-13 also acts on ASMCs via IL-4 receptor α (IL-4Rα) to participate in AHR[7].

We have previously demonstrated that IL-13 induces ASMCs proliferation by promoting $Ca^{2+}$ release and store-operated $Ca^{2+}$ entry (SOCE)[8]. Orai1 is the pore-forming subunit of store-operated $Ca^{2+}$ (SOC) channels and mediates $Ca^{2+}$ influx after interaction with stromal interacting molecule 1 (STIM1). STIM1 senses the $Ca^{2+}$ level of endoplasmic reticulum (ER) $Ca^{2+}$ store and translocate to ER membrane underneath plasma membrane upon $Ca^{2+}$ store depletion, where they interact with Orai1 and activate SOCE[9]. Orai1 was shown to be upregulated in proliferating ASMCs and knock-down of Orai1 attenuated SOCE and ASMC proliferation[10]. Orai1 protein levels are greatly upregulated in ASMCs isolated from asthmatic mice and contribute to ASMCs proliferation and migration[11]. Caveolae also regulates ASMC SOCE via enhance the expression of Orai1[12]. All these results suggesting a critical role of Orai1 in the functional regulation of ASMCs in airway inflammation of asthma. However, the mechanisms underlying the regulating effects of type 2 cytokine IL-13 on Orai1 in ASMCs are still not fully elucidated.

Long non-coding RNA (lncRNA) is a type of non-coding RNAs that are at least 200 nucleotides[13]. Three major functional principles are assigned to lncRNAs: RNA-based function, regulatory element in the gene body and transcription-based function[14]. LncRNAs have been shown to participate in the pathogenesis of airway inflammation and airway remodeling and may act as biomarkers for phenotypes of asthma and glucocorticoids sensitivity in asthma[15]. A few lncRNAs have been proved to be involved in the airway remodeling by acting on ASMCs, such as growth arrest specific-5 (GAS5), plasmacytoma variant

translocation (PVT1), TCF7, TUG1, Brain cytoplasmic RNA 1 (BCYRN1), LINC00882, metastasis-associated lung adenocarcinoma transcript 1(MALAT1) and COPDA1[15, 16]. Almost all of these lncRNAs promoted proliferation of ASMCs, except PVT1, which was shown to inhibit the growth of ASMCs[15, 16].

LncRNA H19 plays a critical role in diverse biological and pathophysiological processes including tumor metastasis and progression, hypoxia, metabolism, oxidative stress and inflammation[17]. Role of H19 in airway inflammation and ASMCs is still not elucidated. It is reported that H19 promotes pulmonary smooth muscle cell proliferation by sponging microRNA (miRNA) let-7b[17]. Since LncRNA/miRNA/mRNA axis is the major mechanism of lncRNA in regulating biological processes[18], we performed a bioinformatic analysis to identify miRNAs that interact with both H19 and Orai1. Has-miR-93-5p was identified as a candidate miRNA. A previous study has demonstrated that miR-93-5p participates in the gene expression in asthma[19]. In the present study, we investigated the possible roles of H19/miR-93-5p/Orai1 axis in the regulation of ASMCs proliferation and migration stimulated by IL-13; in addition, the role of H19 in regulating airway inflammation and airway remodeling was also determined with murine asthma models.

# 2. Materials And Methods

## 2.1 *In vitro* culture of hBSMCs

Segmental bronchi were obtained from lung cancer patients undergoing pulmonary lobectomy or pneumonectomy in Zhongnan Hospital of Wuhan University. Informed consents were acquired from all patients and the study was approved by Medical Ethics Committee of Zhongnan Hospital (approval number: 2019044). Bronchial smooth muscle was dissected out and cut into small pieces, which were then placed into 25 $cm^2$ culture flasks and cultured in RPMI-1640 medium containing L-glutamine (2 mM), penicillin (50 U/mL) and streptomycin (50 mg/mL) and supplemented with 10% fetal bovine serum (FBS). After reaching ~ 80% confluence, cells were passaged by tyrosination with 0.05% trypsin-EDTA and then grown in high glucose DMEM/F12 medium (Gibco-BRL, Carlsbad, CA, USA) supplemented with 20% FBS (Gibco-BRL). Typical hBSMCs exhibited a hill-and-valley pattern when reaching confluence. The purity of hBSMCs was confirmed by immunocytochemistry with mouse anti-human α-smooth muscle actin (SMA) antibody, and 95% cells were positively stained with anti-α-SMA (Fig. S1). hBSMCs before passage 5 were used for the experiments. In our experiments, hBSMCs were seeded in 6-well plates at a density of $5\times10^4$ cells/ml and cultured in DMEM/F12 with 10% FBS for 24 h. At ~ 70% confluence, cells were treated accordingly to the purpose of the experiments.

## 2.2 Transfection of hBSMCs

To knockdown the expression of lncRNA H19, hBSMCs were transfected with 50 nM of siRNA against H19 or scrambled siRNA as negative control (Qijing, Wuhan, China) diluted in riboFECTTMCP reagent (Ribobio, Guangzhou, China). Sequence of H19 siRNA were as follows: sense 5’-CCAACAUCAAAGACACCAUTT-3’, antisense 5’-AUGGUGUCUUUGAUGUUGGTT-3’. The expression of miR-

93-5p was up- or down- regulated with miR-93-5p mimic or inhibitor (Ribobio, Guangzhou, China) which was transfected into hBSMCs with riboFECTTMCP reagent. The mimic or inhibitor binds to miR-93-5p and regulates its function. For transfection, hBSMCs were inoculated into 24-well plates with a density of $1\times10^4$ cells/well. Transfection was performed when cells grew to 50 ~ 60% confluence. The transfection complex was prepared and added into penicillin-streptomycin free DMEM/F12 supplemented with 10% FBS with a final volume of 500 μl. Control cells were treated only with the same amount of PBS without transfection and IL-13. The medium containing transfection complex was removed after 6 h, and the cells were cultured in DMEM/F12 in the presence of IL-13 for additional 24–72 h. The silencing efficiency of siRNA was examined by qRT-PCR.

# 2.3 Quantitative Real-time PCR (qRT-PCR)

Total RNA from lung tissues or hBSMCs was extracted via TRIzol Reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. For Orai1 and H19, RNA was reverse transcribed into cDNA using a ReverTra Ace qPCR RT kit (Toyobo, Tokyo, Japan). cDNA was then amplified with SYBR Premix Ex Taq™ II (RR820A, Takara, Japan) by the following three steps: 1 cycle at 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s, and a melting curve collected at 95°C for 5 s and 60°C for 1 min. For miR-93-5p detection, reverse transcription and qRT-PCR was performed using Bulge-LoopTM miRNA qRT-PCR Starter Kit (R11067.2, RiboBio Co., Guangzhou, China) and Bulge-LoopTM hsa-miR-93-5p qRT-PCR Primer Set (R10031.7, RiboBio Co., Guangzhou, China). The amplification parameters were: 1 cycle at 95°C for 10 min, 40 cycles of 95°C 2 s, 60°C 20 s, and 70°C 10 s. The cycle threshold (CT) values of the target genes were normalized to U6 for miR-93-5p and β-actin for H19 or mRNA respectively. The reaction was performed using the CFX96 Real-Time system (Bio-Rad, USA), and the data were analyzed using the $2^{-\Delta\Delta CT}$ method.

# 2.4 Western-Blot

Lung tissues or hBSMCs were lysed in RIPA buffer containing phosphatase and protease inhibitors (ST505, Beyotime, China) on ice for 30 min and centrifuged at 12,000 rpm for 20 min at 4°C. Protein concentrations were detected using a BCA protein assay kit (Beyotime, China), and equal amounts of protein (40 μg each lane) were subjected to SDS-PAGE and then transferred to Poly (vinylidene fluoride) (PVDF) membranes (Millipore, USA). Membranes were incubated by primary antibody of Orai1 (rabbit anti human, 1:4000, Abcam, USA) and β-actin (goat anti rabbit, 1:5000, Sigma, St. Louis, MO, USA) for 18 h, followed by horseradish peroxidase (HRP)-conjugated secondary antibodies for 2 h at room temperature. enhanced chemiluminescence (ECL) was used to detect the immunoreactive bands, and blots densitometry was analyzed by ImageJ software.

# 2.5 CCK-8 assay

Cell counting kit-8 (CCK-8) assay (Dojindo, Shanghai, China) was used to determine the proliferation rates of hBSMCs. hBSMCs in 96-wells plates at a concentration of $1\times10^4$ cells/well were treated with PBS as control group or transfected with siRNA against H19 or miR-93-5p mimic or inhibitor respectively. After 6 h, medium containing transfection complex was removed. Cells were then synchronized in FBS-free

medium for 24 h, and then incubated in 1% FBS-containing medium in the presence of 10 μg/ml IL-13 for 24 h. 10 μl CCK-8 reagent was added into each well and incubated for 2–4 h and the optical density (OD) values of absorbance at 450 nm were measured.

## 2.6 Scratch wound healing assay

A scratch wound healing assay was conducted to determine the migration rates of hBSMCs. hBSMCs were cultured in 6-well plates in DMEM/F12 supplemented with 10% FBS until the cells reaching 60% confluency. Cells were then transfected with siRNA against H19 or miR-93-5p mimic or inhibitor in the same fashion as in CCK-8 assay. After removing transfection complex and incubated for 24 h in 10% FBS- containing medium, linear wound tracks were generated with sterile 1 ml pipettes and IL-13 (10 μg/ml) was added into each well. Non-adherent cells were removed by rinsing twice with sterile PBS before adding IL-13. DMEM/F12 with 1% FBS was used in this assay. The wound distance at two random wound gap locations was measured 24 h after scratching.

## 2.7 Dual luciferase reporter assay

The targets of H19 and miR-93-5p were searched via StarBase and TargetScan online. The sequences of H19 and Orai1 3'UTR containing miR-93-5p binding sites were cloned into the downstream of pmirGLO luciferase reporter vector to form the wild-type (WT) constructs WT-H19 and Orai1 3'UTR-WT, respectively. The corresponding mutant (MUT) constructs MUT-H19 and Orai1 3'UTR-MUT were generated by mutating the miR-599 binding sites. For dual-luciferase reporter assay, 293T cells were co-transfected with these constructs and miR-599 mimic or miR-NC via Lipofectamine™ 2000. After 48 h, cells were harvested and lysed for luciferase activity analysis with a dual-luciferase assay system.

## 2.8 Murine models of asthma

Female BALB/c mice (6–8 weeks) were obtained from the Animal Experiment Center of Wuhan University and housed under pathogen-free conditions at a 12 h:12 h light: dark cycle. This study was approved by Animal Ethics Committee of Zhongnan Hospital (Approval No. 20200708). House dust mites (HDM) extract (Stallergenes Greer, London, UK) resolved in PBS was intranasally instilled into mice. In the 2-week acute asthma mice model, 100 μg HDM in 25 μL PBS was introduced to each on day 1–5, whereases the control mice were introduced only with equal volume of PBS (Fig. S3A). To inhibition of H19 expression, acute asthma mice were treated with 50 μl lentivirus ($1\times10^8$ TU/ml) carrying H19-specific siRNA or scramble siRNA and green fluorescence protein (GFP) gene (Genechem Co., LTD, Shanghai, China) respectively by intranasal instillation on day 11 and 13. Asthma mice were challenged with 100 μg HDM in 25 μL PBS on day 12 and 14, and the control mice were treated with equal volume of PBS.

In the 8-week chronic asthma model, mice were sensitized with 10 μg HDM in 35 μL PBS for 5 consecutive days in the first week. From 2nd -8th week, mice were challenged with the same amount of HDM in PBS for 5 consecutive days per week. Control mice for chronic model were only treated with PBS in a same pattern of HDM-treated asthma mice. To inhibit the expression of H19 in the lungs, same amount of lentivirus carrying H19-specific siRNA or scramble siRNA and GFP gene was introduced into

chronic asthma mice by intranasal instillation on the previous day before the first HDM challenge every week from week 2 to week 8. Evaluation of the endpoint metrics was performed 24 h after the last instillation (Fig. S3B).

## 2.9 Bronchoalveolar lavage fluid (BALF) collection and enzyme linked immunosorbent assay (ELISA)

24 h after the last challenge, mice were sacrificed by cervical dislocation. The indwelling needle was inserted from thyroid cartilage along the tracheal and fixed. 0.5 ml PBS was infused into the lungs and were aspirated in and out for 3–5 times, the BALF was then collected. The process was repeated for another 2 times to collect enough BALF. IL-13 level in BALF was tested by ELISA kit (eBioscience, CA, USA) according to the manufacturer's protocol.

## 2.10 Immunohistochemical analysis

The left lung from each mouse was fixed in 10% neutral formalin for 24 h, embedded in paraffin and sectioned at 4 mm for hematoxylin-eosin (HE) staining, periodic acid-Schiff (PAS) staining (Solarbio Technology, Beijing, China), Masson staining and α-smooth muscle actin (α-SMA; 1:100, Abcam, USA) immunostaining to observe the pathological changes of lung tissues in mice.

### 2.11 Statistical analysis

All data were presented as mean ± standard deviation (SD) from at least three independent assays. Student's *t*-test was used to compare the difference between two groups and one-way ANOVA was used to compare difference among three or more groups. All statistic were performed with GraphPad Prism 8 software (La Jolla, CA, USA). $P < 0.05$ was regards as statistically significant.

# 3. Results

## 3.1 MiR-93-5p interacts with lncRNA H19 and Orai1

Bioinformatics predictions using TargetScan and ENCORI indicated that H19 and Orai1 sequence contains miR-93-5p binding sites (Fig. S2). To determine the direct interactions between miR-93-5p and the Orai1 3'UTR and between miR-93-5p and H19, we mutated the miR-93-5p binding site in Orai1 and H19 to generate Luc-Orai1-MUT and Luc-H19-MUT respectively. Functional miR-93-5p mimic, functional plasmid-mediated Orai1 overexpression construct (Luc-Orai1-WT) and functional plasmid-mediated H19 overexpression (Luc-H19-WT) construct were generated, and dual-luciferase reporter assay was performed in 293T cells (Fig. 1A, B). The results showed that mutant Orai1 reversed the luciferase activity when compared to WT Orai1 in the presence of sufficient functional miR-93-5p mimic (Fig. 1D), confirming the direct interaction between miR-93-5p and Orai1. Mutant H19 reversed also the luciferase activity relative to that of WT H19 when there was sufficient functional miR-93-5p mimic, indicating that miR-93-5p directly targets H19 (Fig. 1C).

## 3.2 Effects of IL-13 on the expression of H19, miR-93-5p and Orai1 in hBSMCs.

We have previously shown that IL-13 promoted ASMCs proliferation by enhancing SOCE. Here we explored the effects of IL-13 on H19 and miR-93-5p expression in *in vitro* cultured hBSMCs. RT-PCR showed that IL-13 increased lncRNA H19 expression in hBSMCs, with a maximal effect at concentration of 1000 μg/ml (Fig. 2A). IL-13 dose-dependently deceased the expression of miR-93-5p in hBSMCs (Fig. 2B). Similar to our previous results, IL-13 treatment increased also Orai1 mRNA and protein expression in hBSMCs, with a maximal effect at concentration of 1000 μg/ml (Fig. 2C, D).

## 3.3 Effects of H19 knock-down on the expression of miR-93-5p and Orai1 in hBSMCs.

We then inhibited H19 expression by siRNA and investigated expression changes of miR-93-5p and Orai1 in hBSMcs. qRT-PCR showed that H19-specific siRNA but not control scramble siRNA significantly decreased H19 expression in hBSMCs (Fig. 3A), suggesting the efficiency and specificity of this siRNA sequence in inhibiting H19 expression. Transfection of this H19-specific siRNA significantly decreased 10 μg/ml of IL-13-induced H19 expression (Fig. 3B) and reversed the inhibition effect of IL-13 on the miR-93-5p expression (Fig. 3C). H19 expression inhibition with RNAi also reduced Orai1 mRNA and protein expression induced by IL-13 in hBSMCs (Fig. 3D, E). These results indicated that H19 have an inhibitory effect on IL-13-induced miR-93-5p expression and promoting effect on IL-13-induced Orai1 expression in hBSMCs.

## 3.4 Effects of H19 siRNA on the proliferation and migration of hBSMCs.

HBSMCs transfected with or without H19-specific siRNA were cultured in DMEM with 1% FBS in the presence 10 μg/ml of IL-13. Both at 24h and 48h, IL-13 increased the proliferation rates of hBSMCs significantly when compared to control cells without IL-13 stimulation and H19 siRNA transfection; this effect of IL-13 was inhibited by H19 siRNA (Fig. 3F). Similarly, IL-13 also significantly increased the migration rate of BSMCs, which was also inhibited by H19 siRNA, as indicated with mean width changes in wound repair tests (Fig. 3G). These results suggested that H19 have a promoting effect on IL-13-promoted proliferation and migration of human BSMCs.

## 3.5 Role of miR-93-5p in IL-13-induced H19 and Orai1 expression

To explore the role of miR-93-5p in IL-13-induced H19 and Orai1 expression, miR-93-5p-specific mimic or inhibitor was transfected to hBSMCs in the presence of 10 μg/ml IL-13. H19 expression increased by IL-13 was inhibited by miR-93-5p mimic and enhanced by miR-93-5p inhibitor (Fig. 4A). Both the mRNA and protein expression of Orai1 were inhibited by miR-93-5p mimic and enhanced by miR-93-5p inhibitor

(Fig. 4B, C). These results indicated that miR-93-5p and H19 have a reciprocal inhibiting effect in each other, whereas miR-93-5p has an inhibitory effect on Orai1 expression.

## 3.6 Role of miR-93-5p in IL-13-induced hBSMCs proliferation and migration

In hBSMCs cultured in 1% FBS-containing DMEM and stimulated with 10 μg/ml IL-13, miR-93-5p mimic did not have effect on proliferation rate at both 24 h and 48 h; by contrast, miR-93-5p inhibitor enhanced the proliferation rate at both 24 h and 48 h (Fig. 4D). miR-93-5p mimic decreased whereas miR-93-5p inhibitor enhanced hBSMCs migration rate, as indicated with wound repair test (Fig. 4E). These results indicated that miR-93-5p plays an inhibiting effect on IL-13-induced hBSMCs proliferation and migration.

## 3.7 Mutual effects of H19 and miR-93-5p on Orai1 expression

We further determined the effects of miR-93-5p mimic and inhibitor on IL-13-induced Orai1 in hBSMCs with or without co-transfection of H19-specific siRNA. As expected, miR-93-5p mimic enhanced, whereas miR-93-5p inhibitor partly reversed H19 expression inhibited by H19-speific siRNA (Fig. 5A). Similarly, miR-93-5p mimic enhanced, whereas miR-93-5p inhibitor partly reversed Orai1 mRNA expression inhibited by H19-speific siRNA (Fig. 5B); miR-93-5p mimic had no effect on Orai1 protein expression inhibited by H19 siRNA, whereas miR-93-5p inhibitor significantly promoted Orai1 protein expression even in hBSMCs transfected with H19 siRNA (Fig. 5C). These results indicated that the inhibitory effect of H19 siRNA on Orai1 expression was overwhelmed by miR-93-5p inhibition in IL-13-treated hBSMCs.

## 3.8 Mutual effects of H19 and miR-93-5p on the proliferation and migration of hBSMCs.

We then assessed the effects of co-transfection of H19 siRNA and miR-93-5p mimic or inhibitor on the proliferation and migration rates of human BSMCs. miR-93-5p mimic inhibited, whereas miR-93-5p inhibitor promoted the proliferation of hBSMCs co-transfected with H19 siRNA at 24 h; miR-93-5p mimic had no effect, whereas the inhibitor dramatically increased the proliferation of hBSMCs co-transfected with H19 siRNA at 48 h (Fig. 5D). The inhibitory effect of H19 siRNA on hBSMCs migration was overwhelmed by miR-93-5p inhibitor but not changed by the mimic (Fig. 5E).

## 3.9 Role H19 in Orai1 expression and airway inflammation in a murine model of acute asthma

To learn more about the regulating effect of lncRNA H19 on Orai1 expression in the scenario of asthma, HDM-sensitized acute asthma mice were transfected with lentivirus carrying H19-spcific siRNA (Fig. S3A). Transfection of H19-specific siRNA but not scrambled siRNA significantly inhibited the expression of H19 in the lungs of asthma mice, indicating the efficacy and specificity of H19-speific siRNA (Fig. 6A). In the lungs of acute asthma mice, both the mRNA and the protein expression of H19 were significantly higher than in control mice (Fig. 6B). H19 siRNA transfection significantly inhibited both the mRNA and protein

expression of Orai1 in the lungs of asthma mice; by contrast, scramble siRNA had no effect on the Orai1 expression in the lungs of acute asthma mice (Fig. 6C).

More inflammatory cell infiltration was observed in peri-bronchial and perivascular area in the lungs of acute asthma mice when compared to that in control mice (Fig. 6Di, ii). H19 siRNA, but not scrambled siRNA significantly reduced inflammatory cell infiltration in the lungs of asthma mice (Fig. 6Diii, iv). Inflammatory score confirmed these results, as indicated in Fig. 6E. The concentration of IL-13 in the BALF from asthma mice was significantly higher than that in control mice, and H19 siRNA, but not Scrambled siRNA significantly inhibited IL-13 secretion in the BALF of acute asthma mice (Fig. 6F). These results indicate that H19 inhibition with Specific siRNA attenuated Orai1 expression and also airway inflammation in acute asthma mice.

3.10 Role of H19 in Orai1 expression and airway inflammation, airway remodeling in a murine model of chronic asthma

In the lungs of HDM-induced chronic asthma mice, the H19 expression was higher than that in control mice; transfection of H19-specic siRNA, but not scrambled siRNA with lentivirus significantly reduced the expression of H19 in the lungs of chronic asthma mice (Fig. 7A). Similar to that in acute asthma mice, H19 inhibition with siRNA inhibited Orai1 mRNA and protein expression in the lungs of chronic asthma mice; scrambled siRNA transfection did not affect the both the mRNA and protein expression of Orai1 (Fig. 7B, C).

Inflammatory cell infiltration was more frequently observed in lungs of chronic asthma mice than in control mice; H19 siRNA but not scramble siRNA reduced inflammatory cell infiltration in the lungs of chronic asthma mice (Fig. 7D), as quantified with inflammatory score (Fig. 7E). PAS staining showed that goblet cell hyperplasia was more prominent in asthma mice than in control mice; H19 siRNA but not scrambled siRNA inhibited goblet cell hyperplasia in bronchial mucosa (Fig. 7G), as quantified with the ratio of $PAS^+$ area to perimeter of bronchi (Fig. 7H).

To elucidate the role of H19 in airway remodeling, lung tissue from chronic asthma mice were stained with Masson trichrome and immune-stained with anti-α-SMA antibody. Masson staining showed higher collagen deposition beneath bronchial mucosa in chronic asthma mice than in control mice; H19 siRNA but not scramble siRNA inhibited bronchial collagen deposition in chronic asthma mice (Fig. 7I), as quantified by ratio of area of positive Masson staining to area of peri-bronchial mucus (Fig. 7J). Anti-α-SMA antibody showed larger smooth muscle area in beneath bronchial mucus in chronic asthma mice than in control mice; and H19 siRNA but not scrambled siRNA reduced smooth muscle area in the lungs of chronic asthma mice (Fig. 7K); using ratio of area of positively stained with α-SMA to that of perimeter of bronchi confirmed these results (Fig. 7L).

Similar to that in acute asthma mice, IL-13 levels in BALF of chronic asthma mice were significantly higher than in control mice; and H19 siRNA but not scramble siRNA reduced IL-13 levels in chronic

asthma mice (Fig. 7F). Taken together, these data indicate that H19 may promote airway inflammation and airway remodeling by enhancing Orai1 expression in the lung in chronic asthma mice.

# 4. Discussion

The dual luciferase reporter assay confirmed the interactions of miR-93-5p with H19 and Orai1, suggesting the existence of a possible functional H19/miR-93-5p/Orai1 axis in ASMCs. We found that IL-13 increased the expression of H19 and Orai1 and decreased the expression of miR-93-5p in hBSMCs, and this effect of IL-13 was inhibited by H19 knockdown. MiR-93-5p inhibited Orai1 expression, as demonstrated by miR-93-5p mimic and inhibitor. Moreover, the promoting effects of IL-13 on hBSMCs proliferation and migration were attenuated by H19 siRNA and miR-93-5p mimic but aggravated by miR-93-5p inhibitor. In murine asthma models, the expression of H19 and Orai1, as well as the airway inflammation and remodeling in the lungs of asthmatic mice, were higher than in control mice; H19 inhibition with siRNA attenuated both the expression of H19 and Orai1 mRNA in the lungs of asthmatic mice, and also the airway inflammation and airway remodeling.

Type 2 cytokine IL-13 can be produced by Th2 as well as ILC2 cells[2]. IL-13 is required to the development of asthma[20] and induces different biological effects in airway inflammatory and structural cells[21]. More importantly, IL-13 is central to the pathogenesis of airway remodeling of asthma by acting on airway epithelial cells[22, 23], fibroblasts[6], ASMCs[21] and induces goblet cell metaplasia, mucus hypersecretion, ASMCs proliferation and migration, fibroblasts proliferation, airway hyperreactivity and elevated IgE levels[21], all features of airway remodeling. We have previously shown that IL-13 upregulated Orai1 and STIM1 expression and SOCE in cultured ASMCs[8], consistent with the results in the current study that IL-13 dose-dependently increased the mRNA and protein expression of Orai1 in hBSMCs.

For the first time, we demonstrated that IL-13 increased the expression of lncRNA H19 and decreased the expression of miR-93-5p in hBSMCs. Other lncRNAs have been demonstrated to play a role in IL-13-associated responses, such as lncRNA BANCR in eosinophilic esophagitis[24], multiple lncRNAs in mycobacterium tuberculosis-infected macrophages[25]. In allergic rhinitis, IL-13 decreased lncRNA Linc00632 expression in nasal epithelial cells and Linc00632 inhibited IL-13-induced GM-CSF, eotaxin and MUAC5AC production via targeting miR-498[26]. On the other hand, lncRNAs also regulate the expression of IL-13. For example, lncRNA GATA3-AS1 is essential to IL-13 expression in peripheral blood mononuclear cells[27]; depletion of the Th2-locus control region (LCR) lncRNA by siRNA demonstrates that it is necessary for the expression of genes encoding Th2 cytokines IL-4, IL-5 and IL-13[28]. Consistently, in the present study, we found that H19 inhibition with siRNA decreased IL-13 levels in BALF from asthma mice, also suggesting a possible regulating effect of lncRNA on IL-13 expression.

IL-13 has also been demonstrated to regulate the expression of miRNAs in airway inflammation. In nasal epithelial cells, IL-13 promoted the expression of miR-498 that was inhibited by lncRNA Linc00632[26], and also dose-dependently decreased the expression of miR-15a-5p[29]. In human bronchial epithelial

cells, IL-13-induced MUC5AC secretion was inhibited by miR-330 mimic[30]. Using miRNA microarray analysis, it was found that 79 miRNAs were higher, and 138 miRNAs, including miR-93-5p, were lower in airway epithelial cells from asthma patients than in healthy control subjects[31]. Moreover, IL-13 stimulation altered the expression patterns of miRNAs in *in vitro* cultured human bronchial epithelial cells, similar to that in asthma[31]. We also observed that IL-13 decreased the expression of miR-93-5p in cultured hBSMCs. Similar to our results, IL-13 also downregulated the expression of miR-133a[32] and miR-140-3p[33] in BSMCs, and thus up-regulation of RhoA protein, a possible key regulator of bronchial hyperreactivity. These data suggest that microRNAs, including miR-93-5p, may be actively involved in type 2 inflammation and airway remodeling in asthma. Further studies are needed to confirm the expression changes of miR-93-5p in ASMCs from asthma patients.

On the other hand, there is evidence showing that IL-13 is also subjective to the regulation by microRNAs. MiR-let-7 was shown to decrease IL-13 levels in cultured T cells and in the lungs of a murine asthma model[34]. Another study found that in the plasma of asthma children, levels of miR-146a and miR-106b were positively correlated with IL-13 levels[35]; miR-155 was also reported to regulate IL-13 signaling[36]. MiR-143 suppressed IL-13-related inflammation by targeting of IL-13Rα1 in epidermal keratinocytes[37]. In allergic asthma models, miR-126 and miR-145 were correlated with the secretion of IL-13[38]. Our study also found that LncRNA H19 regulated the IL-13 levels in BALF of asthma mice. The role of miR-93-5p in IL-13 secretion in asthma mice should be evaluated in future studies.

The interaction between H19 and miR-93-5p was not studied previously, even that H19 has been reported to interact with multiple miRNAs to regulate diverse biological processes. In smooth muscle cells, H19 is shown to interact with miR-Let-7b and upregulate the expression angiotensin II type 1 receptor ($AT_1R$) to promote pulmonary artery smooth muscle cell proliferation[17], interact with miR-21 to regulate the proliferation and migration of ASMCs[39], and interact with miR-599 to promote vascular smooth muscle cell proliferation[40]. In skeletal muscle, the only tissue with persistent H19 expression after birth, H19 was found to interact with miR-675-3p and miR-675-5p to promote skeletal muscle differentiation and regeneration[41]. Together with our results, all of these data support the conclusion that H19 has an important regulating role in diverse function that is mediated by miRNAs.

We also showed that miR-93-5p regulated the expression of Orai1, the pore-forming molecule of SOC channels in hBSMCs. In addition to miR-93-5p, a few other microRNAs have also been reported to regulate the expression of Orai1 and thus different biological activities. MiR-93 was reported to downregulate Orai1 expression and then suppressed transforming growth factor-β1-mediated epithelial mesenchymal transformation and fibrogenesis[42]. MiR-519 downregulated Orai1 expression and thus inhibited cancer cell growth[43, 44]. In $CD4^+$ T cells, overexpression of miR-10a-5p decreased Orai1 expression and SOCE, and thus the proliferation of this kind of cells[45]. Deletion of Dicer, the enzyme responsible for processing precursor miRNAs to mature miRNAs, inhibited Orai1 expression and SOCE in $CD4^+$ T cells, indicating the importance of microRNAs to the expression and function of Orai1[46]. Consistent with our results, these data suggested that miRNAs are required for the proper function of Orai1 and may also SOCE.

Functionally, H19 has been proven to participate in fibrosis diseases such as pulmonary fibrosis by regulating the miR-196a/COL1A1[47], in renal fibrosis[48] and in cholestatic liver fibrosis[49]. H19 also plays a pivotal role in diverse inflammations. In rheumatoid arthritis, H19 promoted the inflammation by degradation of miR-103a[50]; in ankylosing spondylitis, H19 interacts with miR-675-5p and miR-22-5p to increase IL-17A/IL-23 release[51]; H19 also regulates ageing and age-related diseases by interplay with inflammation[52]. H19 also contributes to the genesis and metastasis of different type of cancers by re-expression in cancer tissues, e. g., hepatocellular carcinoma[53], thyroid cancer cells[54], non-small cell lung cancer[55], etc. Other functional relevance of H19 includes regulation of hypoxia-induced tissue injury[56] and oxidative distress. The role of H19 in the regulation of intracellular $Ca^{2+}$ signaling is not clear. Our results demonstrated a role of H19 in the regulation of Orai1 expression and thus proliferation and migration of hBSMCs. Our results are contradictory to another study which showed that H19 inhibited platelet-derived growth factor (PDGF)-induced proliferation and migration of ASMCs[39]. H19 was also found to be decreased in ASMCs from patients with mild asthma when compared to healthy controls[57]. Therefore, further studies are warranted to clarify the role of H19 in the functional regulation of ASMCs and also the source of the controversy.

Our study had limitations. Firstly, role of H19 in the functional regulation of hBSMCs was investigated only with H19 siRNA but not with H19 overexpression; secondly, the role of H19 in the airway inflammation and airway remodeling need to be confirmed in H19 knock-out mice; finally, the activity of SOCE was not determined with $Ca^{2+}$ fluorescence measurements and/or electrophysiological measurement with whole-cell patch-clamp technique, due to the technical unavailability. Thus, further studies are warranted to investigate the effects and functional relevance of H19-miR-93-5p axis on SOCE in ASMCs and may also other cell types that contribute to the pathogenesis of asthma.

# 5. Conclusions

Our results demonstrated that IL-13 increases the proliferation and migration of airway smooth muscle cells by promoting the expression of H19 and Orai1 and decreasing the expression of miR-93-5p. H19 upregulates the expression of Orai1 via inhibition of miR-93-5p in hBSMCs. H19 aggregates airway inflammation and airway remodeling in murine models of asthma possibly by inhibition of the expression of Orai1. Targeting H19/miR-93-5p/Orai1 axis may represent a novel strategy for the treatment of airway remodeling in asthma.

# Abbreviations

α-SMA: α-smooth muscle actin; ASMCs: Airway smooth muscle cells; BALF: Bronchoalveolar lavage fluid; DMEM: Dulbecco's modified eagle medium; FBS: fetal bovine serum; GFP: Green fluorescent protein; hBSMCs: human bronchial smooth muscle cells; HDM: House dust mites; PBS: Phosphate buffered solution; qRT-PCR: Quantitative Real-time PCR; IL-13: Interleukin-13; ILC2: Group 2 innate lymphoid cells; lncRNA: long non-coding RNAs; miRNA: microRNA; Orai1: =CRACM1, Calcium Release-Activated Calcium

Modulator 1; siRNA: small interfering RNA; STIM1: Stromal interacting molecule 1: Th2: T helper 2 cells; WT: Wild-type;

# Declarations


### Acknowledgement

Not applicable


### Authors' contributions

YDG designed the study, analyzed the data and wrote the manuscript; LLX, QQW, YMW, SJH, and WJX contributed to the experiments, LLX and QQW also contributed to the data analysis and the writing.


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Availability of data and materials

All data generated or analysed during this this study are included in this manuscript (and also the supplementary information files).

### Declarations

### Ethics approval and consent to participate

This manuscript contains samples collected from human subjects that have been approved by the Medical Ethics Committee of Zhongnan Hospital (approval number: 2019044). The animal experiments were approved by Animal Ethics Committee of Zhongnan Hospital (Approval number: 20200708).

### Consent for publication

The publication of this manuscript has been approved by administration of Zhongnan Hospital of Wuhan University.

### Competing interests

The authors declared that they have no competing interests.


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# References

1. Papi A, Brightling C, Pedersen SE, Reddel HK. Asthma. Lancet. 2018; 391 (10122):783–800.

2. Akdis CA, Arkwright PD, Bruggen MC, Busse W, Gadina M, Guttman-Yassky E, Kabashima K, Mitamura Y, Vian L, Wu J, Palomares O. Type 2 immunity in the skin and lungs. Allergy. 2020; 75 (7):1582–1605.

3. Manson ML, Safholm J, James A, Johnsson AK, Bergman P, Al-Ameri M, Orre AC, Karrman-Mardh C, Dahlen SE, Adner M. IL-13 and IL-4, but not IL-5 nor IL-17A, induce hyperresponsiveness in isolated human small airways. J Allergy Clin Immunol. 2020; 145 (3):808–817 e802.

4. Saglani S, Lui S, Ullmann N, Campbell GA, Sherburn RT, Mathie SA, Denney L, Bossley CJ, Oates T, Walker SA, et al. IL-33 promotes airway remodeling in pediatric patients with severe steroid-resistant asthma. J Allergy Clin Immunol. 2013; 132 (3):676–685 e613.

5. Wang X, Xu C, Ji J, Cai Y, Shu Y, Chao Y, Wu X, Zou C, Wu X, Tang L. IL-4/IL-13 upregulates Sonic hedgehog expression to induce allergic airway epithelial remodeling. Am J Physiol Lung Cell Mol Physiol. 2020; 318 (5):L888-L899.

6. Doucet C, Brouty-Boye D, Pottin-Clemenceau C, Canonica GW, Jasmin C, Azzarone B. Interleukin (IL) 4 and IL-13 act on human lung fibroblasts. Implication in asthma. J Clin Invest. 1998; 101 (10):2129–2139.

7. McKnight CG, Potter C, Finkelman FD. IL-4Ralpha expression by airway epithelium and smooth muscle accounts for nearly all airway hyperresponsiveness in murine allergic airway disease. Mucosal Immunol. 2020; 13 (2):283–292.

8. Gao YD, Zou JJ, Zheng JW, Shang M, Chen X, Geng S, Yang J. Promoting effects of IL-13 on Ca2 + release and store-operated Ca2 + entry in airway smooth muscle cells. Pulm Pharmacol Ther. 2010; 23 (3):182–189.

9. Prakriya M, Lewis RS. Store-Operated Calcium Channels. Physiol Rev. 2015; 95 (4):1383–1436.

10. Zou JJ, Gao YD, Geng S, Yang J. Role of STIM1/Orai1-mediated store-operated Ca(2)(+) entry in airway smooth muscle cell proliferation. J Appl Physiol (1985). 2011; 110 (5):1256–1263.

11. Spinelli AM, Gonzalez-Cobos JC, Zhang X, Motiani RK, Rowan S, Zhang W, Garrett J, Vincent PA, Matrougui K, Singer HA, Trebak M. Airway smooth muscle STIM1 and Orai1 are upregulated in asthmatic mice and mediate PDGF-activated SOCE, CRAC currents, proliferation, and migration. Pflugers Arch. 2012; 464 (5):481–492.

12. Sathish V, Abcejo AJ, Thompson MA, Sieck GC, Prakash YS, Pabelick CM. Caveolin-1 regulation of store-operated Ca(2+) influx in human airway smooth muscle. Eur Respir J. 2012; 40 (2):470–478.

13. Atianand MK, Caffrey DR, Fitzgerald KA. Immunobiology of Long Noncoding RNAs. Annu Rev Immunol. 2017; 35:177–198.

14. Ali T, Grote P. Beyond the RNA-dependent function of LncRNA genes. Elife. 2020; 9.

15. Zhu X, Wei Y, Dong J. Long Noncoding RNAs in the Regulation of Asthma: Current Research and Clinical Implications. Front Pharmacol. 2020; 11:532849.

16. Zheng M, Hong W, Gao M, Yi E, Zhang J, Hao B, Liang C, Li X, Li C, Ye X, et al. Long Noncoding RNA COPDA1 Promotes Airway Smooth Muscle Cell Proliferation in Chronic Obstructive Pulmonary Disease. Am J Respir Cell Mol Biol. 2019; 61 (5):584–596.

17. Su H, Xu X, Yan C, Shi Y, Hu Y, Dong L, Ying S, Ying K, Zhang R. LncRNA H19 promotes the proliferation of pulmonary artery smooth muscle cells through AT1R via sponging let-7b in monocrotaline-induced pulmonary arterial hypertension. Respir Res. 2018; 19 (1):254.

18. Lopez-Urrutia E, Bustamante Montes LP, Ladron de Guevara Cervantes D, Perez-Plasencia C, Campos-Parra AD. Crosstalk Between Long Non-coding RNAs, Micro-RNAs and mRNAs: Deciphering Molecular Mechanisms of Master Regulators in Cancer. Front Oncol. 2019; 9:669.

19. Mao Z, Shi Y, Cao Q, Chen Y, Sun Y, Liu Z, Zhang Q, Huang M. Transcriptional regulation on the gene expression signature in combined allergic rhinitis and asthma syndrome. Epigenomics. 2018; 10 (2):119–131.

20. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, Donaldson DD. Interleukin-13: central mediator of allergic asthma. Science. 1998; 282 (5397):2258–2261.

21. Ingram JL, Kraft M. IL-13 in asthma and allergic disease: asthma phenotypes and targeted therapies. J Allergy Clin Immunol. 2012; 130 (4):829–842; quiz 843 – 824.

22. Sugita K, Steer CA, Martinez-Gonzalez I, Altunbulakli C, Morita H, Castro-Giner F, Kubo T, Wawrzyniak P, Ruckert B, Sudo K, et al. Type 2 innate lymphoid cells disrupt bronchial epithelial barrier integrity by targeting tight junctions through IL-13 in asthmatic patients. J Allergy Clin Immunol. 2018; 141 (1):300–310 e311.

23. Yang SJ, Allahverdian S, Saunders ADR, Liu E, Dorscheid DR. IL-13 signaling through IL-13 receptor alpha2 mediates airway epithelial wound repair. FASEB J. 2019; 33 (3):3746–3757.

24. Sherrill JD, Kiran KC, Blanchard C, Stucke EM, Kemme KA, Collins MH, Abonia JP, Putnam PE, Mukkada VA, Kaul A, et al. Analysis and expansion of the eosinophilic esophagitis transcriptome by RNA sequencing. Genes Immun. 2014; 15 (6):361–369.

25. Roy S, Schmeier S, Kaczkowski B, Arner E, Alam T, Ozturk M, Tamgue O, Parihar SP, Kawaji H, Itoh M, et al. Transcriptional landscape of Mycobacterium tuberculosis infection in macrophages. Sci Rep. 2018;

8 (1):6758.

26. Yue L, Yin X, Hao F, Dong J, Ren X, Xu O, Shan C. Long Noncoding RNA Linc00632 Inhibits Interleukin-13-Induced Inflammatory Cytokine and Mucus Production in Nasal Epithelial Cells. J Innate Immun. 2020; 12 (1):116–128.

27. Gibbons HR, Shaginurova G, Kim LC, Chapman N, Spurlock CF, 3rd, Aune TM. Divergent lncRNA GATA3-AS1 Regulates GATA3 Transcription in T-Helper 2 Cells. Front Immunol. 2018; 9:2512.

28. Spurlock CF, 3rd, Tossberg JT, Guo Y, Collier SP, Crooke PS, 3rd, Aune TM. Expression and functions of long noncoding RNAs during human T helper cell differentiation. Nat Commun. 2015; 6:6932.

29. Wang L, Lv Q, Song X, Jiang K, Zhang J. ADRB2 suppresses IL-13-induced allergic rhinitis inflammatory cytokine regulated by miR-15a-5p. Hum Cell. 2019; 32 (3):306–315.

30. Su Y, Wang J, Zou J, Han W, Li S. miR-330 regulates interleukin-13-induced MUC5AC secretion by targeting Munc18b in human bronchial epithelial cells. Int J Clin Exp Pathol. 2018; 11 (7):3463–3470.

31. Solberg OD, Ostrin EJ, Love MI, Peng JC, Bhakta NR, Hou L, Nguyen C, Solon M, Nguyen C, Barczak AJ, et al. Airway epithelial miRNA expression is altered in asthma. Am J Respir Crit Care Med. 2012; 186 (10):965–974.

32. Chiba Y, Tanabe M, Goto K, Sakai H, Misawa M. Down-regulation of miR-133a contributes to up-regulation of Rhoa in bronchial smooth muscle cells. Am J Respir Crit Care Med. 2009; 180 (8):713–719.

33. Chiba Y, Ando Y, Kato Y, Hanazaki M, Sakai H. Down-regulation of miR-140-3p is a cause of the interlukin-13-induced up-regulation of RhoA protein in bronchial smooth muscle cells. Small GTPases. 2021:1–6.

34. Kumar M, Ahmad T, Sharma A, Mabalirajan U, Kulshreshtha A, Agrawal A, Ghosh B. Let-7 microRNA-mediated regulation of IL-13 and allergic airway inflammation. J Allergy Clin Immunol. 2011; 128 (5):1077–1085 e1071-1010.

35. Elnady HG, Sherif LS, Kholoussi NM, Ali Azzam M, Foda AR, Helwa I, Sabry RN, Eissa E, Fahmy RF. Aberrant Expression of Immune-related MicroRNAs in Pediatric Patients with Asthma. Int J Mol Cell Med. 2020; 9 (4):246–255.

36. Biethahn K, Orinska Z, Vigorito E, Goyeneche-Patino DA, Mirghomizadeh F, Foger N, Bulfone-Paus S. miRNA-155 controls mast cell activation by regulating the PI3Kgamma pathway and anaphylaxis in a mouse model. Allergy. 2014; 69 (6):752–762.

37. Zeng YP, Nguyen GH, Jin HZ. MicroRNA-143 inhibits IL-13-induced dysregulation of the epidermal barrier-related proteins in skin keratinocytes via targeting to IL-13Ralpha1. Mol Cell Biochem. 2016; 416 (1–2):63–70.

38. Foster PS, Maltby S, Rosenberg HF, Tay HL, Hogan SP, Collison AM, Yang M, Kaiko GE, Hansbro PM, Kumar RK, Mattes J. Modeling TH 2 responses and airway inflammation to understand fundamental mechanisms regulating the pathogenesis of asthma. Immunol Rev. 2017; 278 (1):20–40.

39. Yu H, Qi N, Zhou Q. LncRNA H19 Inhibits Proliferation and Migration of Airway Smooth Muscle Cells Induced by PDGF-BB Through miR-21/PTEN/Akt Axis. J Asthma Allergy. 2021; 14:71–80.

40. Lu G, Chu Y, Tian P. Knockdown of H19 Attenuates Ox-LDL-induced Vascular Smooth Muscle Cell Proliferation, Migration, and Invasion by Regulating miR-599/PAPPA Axis. J Cardiovasc Pharmacol. 2021; 77 (3):386–396.

41. Dey BK, Pfeifer K, Dutta A. The H19 long noncoding RNA gives rise to microRNAs miR-675-3p and miR-675-5p to promote skeletal muscle differentiation and regeneration. Genes Dev. 2014; 28 (5):491–501.

42. Ma J, Zhang L, Hao J, Li N, Tang J, Hao L. Up-regulation of microRNA-93 inhibits TGF-beta1-induced EMT and renal fibrogenesis by down-regulation of Orai1. J Pharmacol Sci. 2018; 136 (4):218–227.

43. Abdelmohsen K, Srikantan S, Tominaga K, Kang MJ, Yaniv Y, Martindale JL, Yang X, Park SS, Becker KG, Subramanian M, et al. Growth inhibition by miR-519 via multiple p21-inducing pathways. Mol Cell Biol. 2012; 32 (13):2530–2548.

44. Deng W, Wang J, Zhang J, Cai J, Bai Z, Zhang Z. Orai1, a Direct Target of microRNA-519, Promotes Progression of Colorectal Cancer via Akt/GSK3beta Signaling Pathway. Dig Dis Sci. 2016; 61 (6):1553–1560.

45. Zhang S, Al-Maghout T, Cao H, Pelzl L, Salker MS, Veldhoen M, Cheng A, Lang F, Singh Y. Gut Bacterial Metabolite Urolithin A (UA) Mitigates Ca(2+) Entry in T Cells by Regulating miR-10a-5p. Front Immunol. 2019; 10:1737.

46. Zhang S, Al-Maghout T, Zhou Y, Bissinger R, Abousaab A, Salker MS, Pelzl L, Cobb BS, Cheng A, Singh Y, Lang F. Role of Dicer Enzyme in the Regulation of Store Operated Calcium Entry (SOCE) in CD4 + T Cells. Cell Physiol Biochem. 2016; 39 (4):1360–1368.

47. Lu Q, Guo Z, Xie W, Jin W, Zhu D, Chen S, Ren T. The lncRNA H19 Mediates Pulmonary Fibrosis by Regulating the miR-196a/COL1A1 Axis. Inflammation. 2018; 41 (3):896–903.

48. Shi S, Song L, Yu H, Feng S, He J, Liu Y, He Y. Knockdown of LncRNA-H19 Ameliorates Kidney Fibrosis in Diabetic Mice by Suppressing miR-29a-Mediated EndMT. Front Pharmacol. 2020; 11:586895.

49. Xiao Y, Liu R, Li X, Gurley EC, Hylemon PB, Lu Y, Zhou H, Cai W. Long Noncoding RNA H19 Contributes to Cholangiocyte Proliferation and Cholestatic Liver Fibrosis in Biliary Atresia. Hepatology. 2019; 70 (5):1658–1673.

50. Miao C, Bai L, Yang Y, Huang J. Dysregulation of lncRNAs in Rheumatoid Arthritis: Biomarkers, Pathogenesis and Potential Therapeutic Targets. Front Pharmacol. 2021; 12:652751.

51. Zhang X, Ji S, Cai G, Pan Z, Han R, Yuan Y, Xu S, Yang J, Hu X, Chen M, et al. H19 Increases IL-17A/IL-23 Releases via Regulating VDR by Interacting with miR675-5p/miR22-5p in Ankylosing Spondylitis. Mol Ther Nucleic Acids. 2020; 19:393–404.

52. Wang B, Suen CW, Ma H, Wang Y, Kong L, Qin D, Lee YWW, Li G. The Roles of H19 in Regulating Inflammation and Aging. Front Immunol. 2020; 11:579687.

53. Gamaev L, Mizrahi L, Friehmann T, Rosenberg N, Pappo O, Olam D, Zeira E, Bahar Halpern K, Caruso S, Zucman-Rossi J, et al. The pro-oncogenic effect of the lncRNA H19 in the development of chronic inflammation-mediated hepatocellular carcinoma. Oncogene. 2021; 40 (1):127–139.

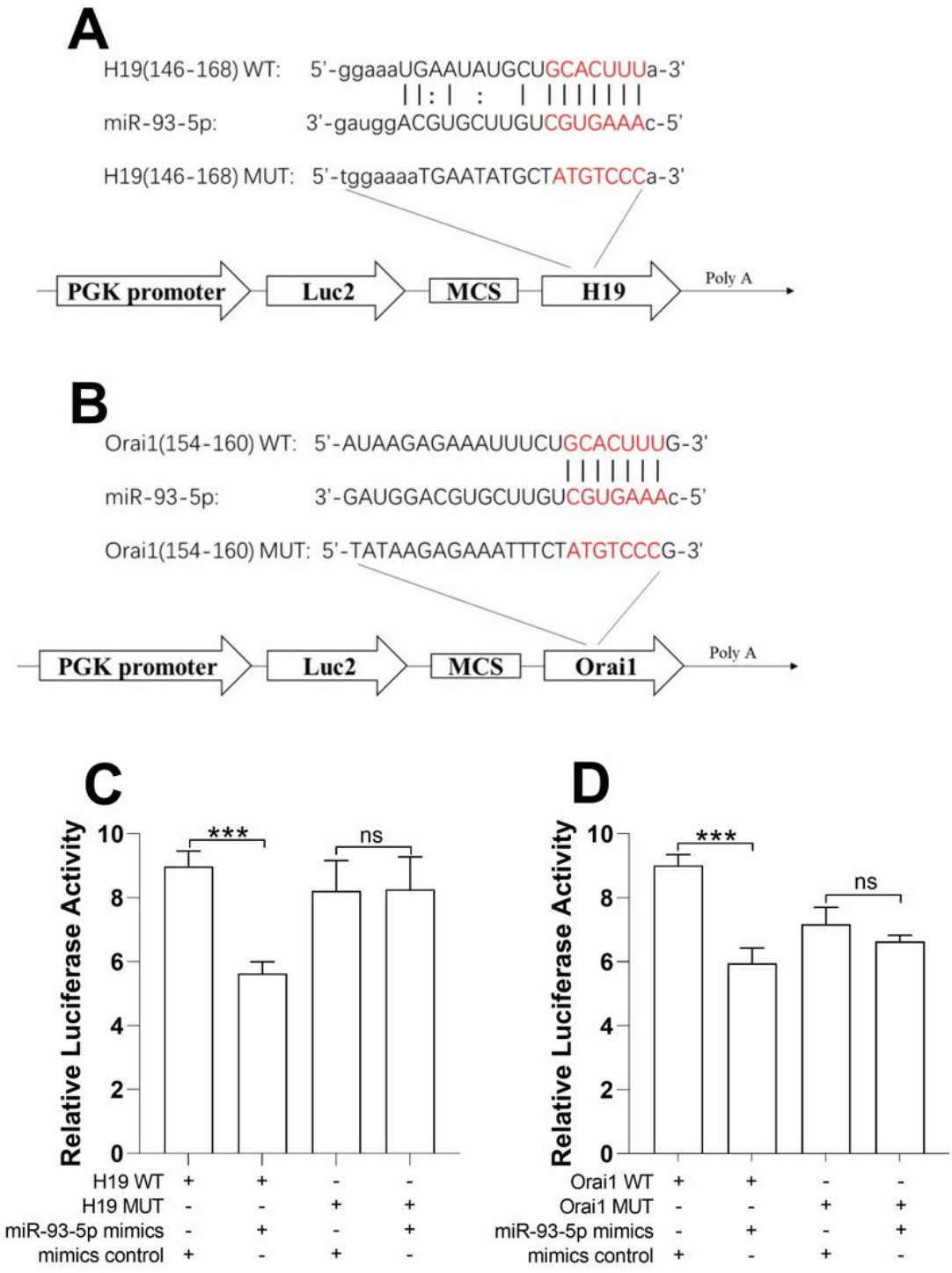
54. Lan X, Sun W, Dong W, Wang Z, Zhang T, He L, Zhang H. Downregulation of long noncoding RNA H19 contributes to the proliferation and migration of papillary thyroid carcinoma. Gene. 2018; 646:98–105.

55. Chen C, Liu WR, Zhang B, Zhang LM, Li CG, Liu C, Zhang H, Huo YS, Ma YC, Tian PF, et al. LncRNA H19 downregulation confers erlotinib resistance through upregulation of PKM2 and phosphorylation of AKT in EGFR-mutant lung cancers. Cancer Lett. 2020; 486:58–70.

56. Yuan Y, Li X, Chu Y, Ye G, Yang L, Dong Z. Long Non-coding RNA H19 Augments Hypoxia/Reoxygenation-Induced Renal Tubular Epithelial Cell Apoptosis and Injury by the miR-130a/BCL2L11 Pathway. Front Physiol. 2021; 12:632398.

57. Austin PJ, Tsitsiou E, Boardman C, Jones SW, Lindsay MA, Adcock IM, Chung KF, Perry MM. Transcriptional profiling identifies the long noncoding RNA plasmacytoma variant translocation (PVT1) as a novel regulator of the asthmatic phenotype in human airway smooth muscle. J Allergy Clin Immunol. 2017; 139 (3):780–789.

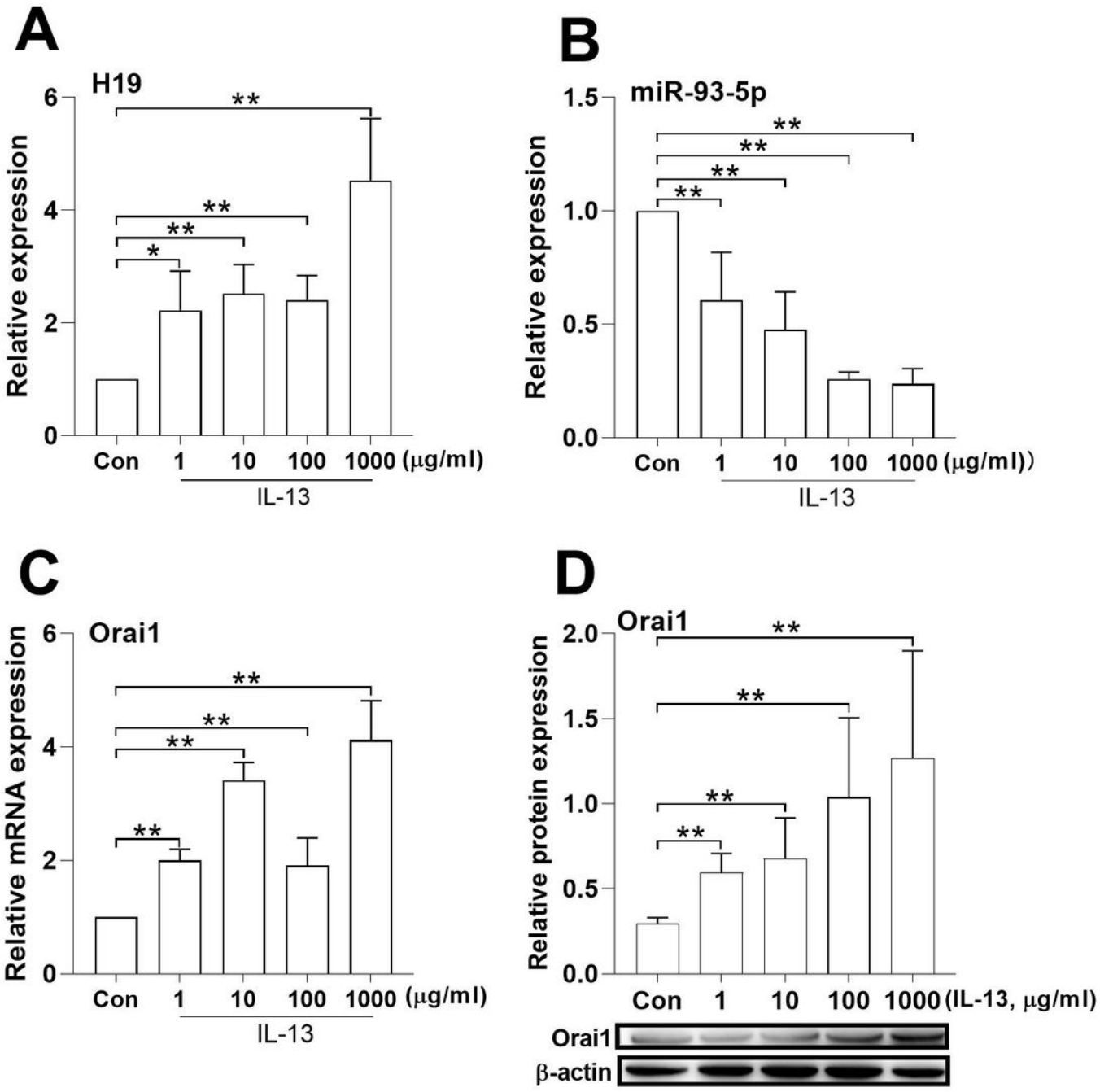
# Figures




## Figure 1

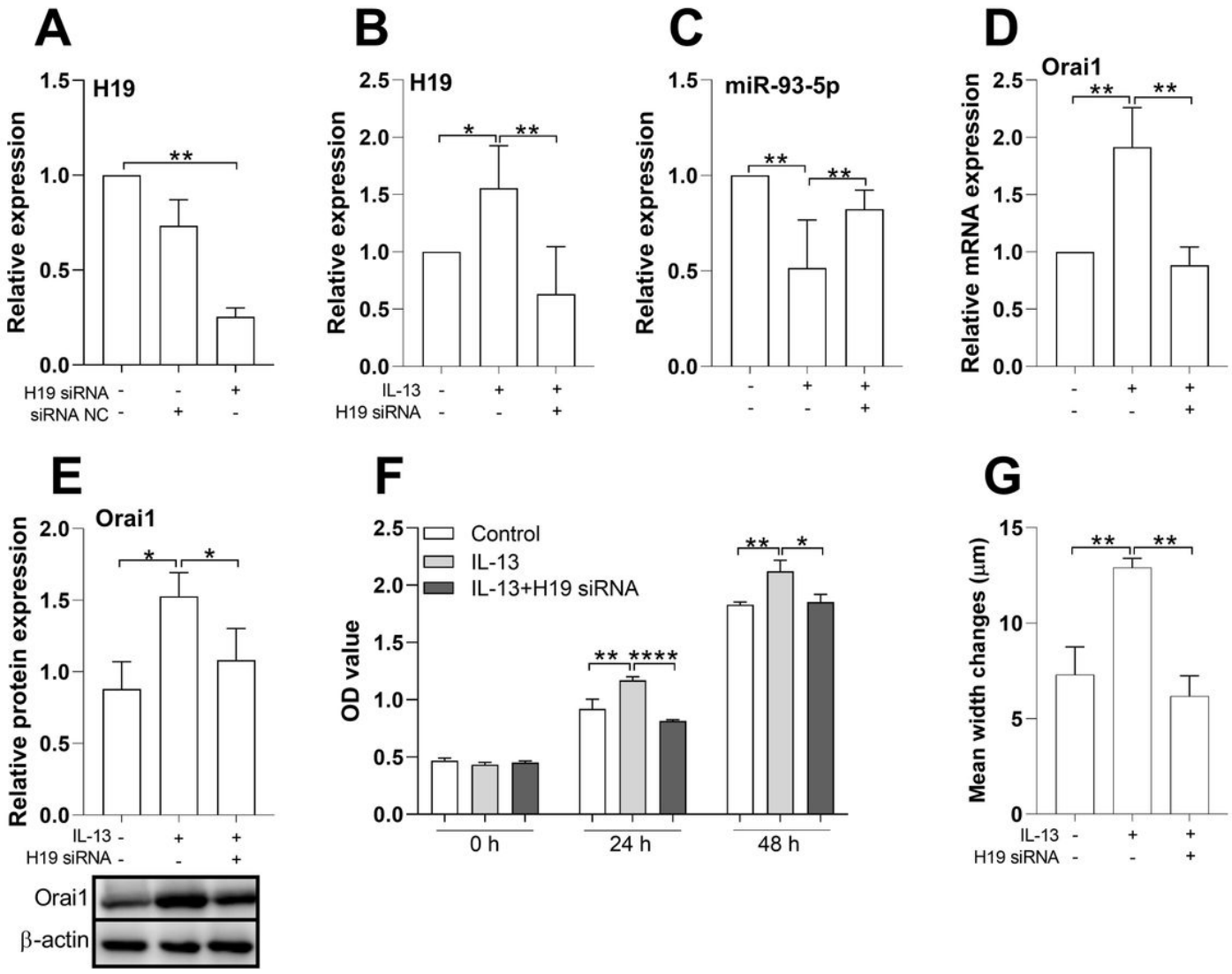
miR-93-5p interacts with H19 and Orai1 respectively. A. Diagram shows the structure of H19 luciferase in PGK reporter. The predicted binding sites for sponging miR-93-5p and the corresponding mutation sites in H19 are shown in red. B. Diagram shows the structure of Orai1 luciferase in PGK reporter. The predicted binding sites for sponging miR-93-5p and the corresponding mutation sites in Orai1 are shown in red. C-D.

Luciferase reporter gene assay was used to determine the interaction between miRNA-93-5p with H19 (C) and Orai1 (D). ***P<0.001. ns, not significant.



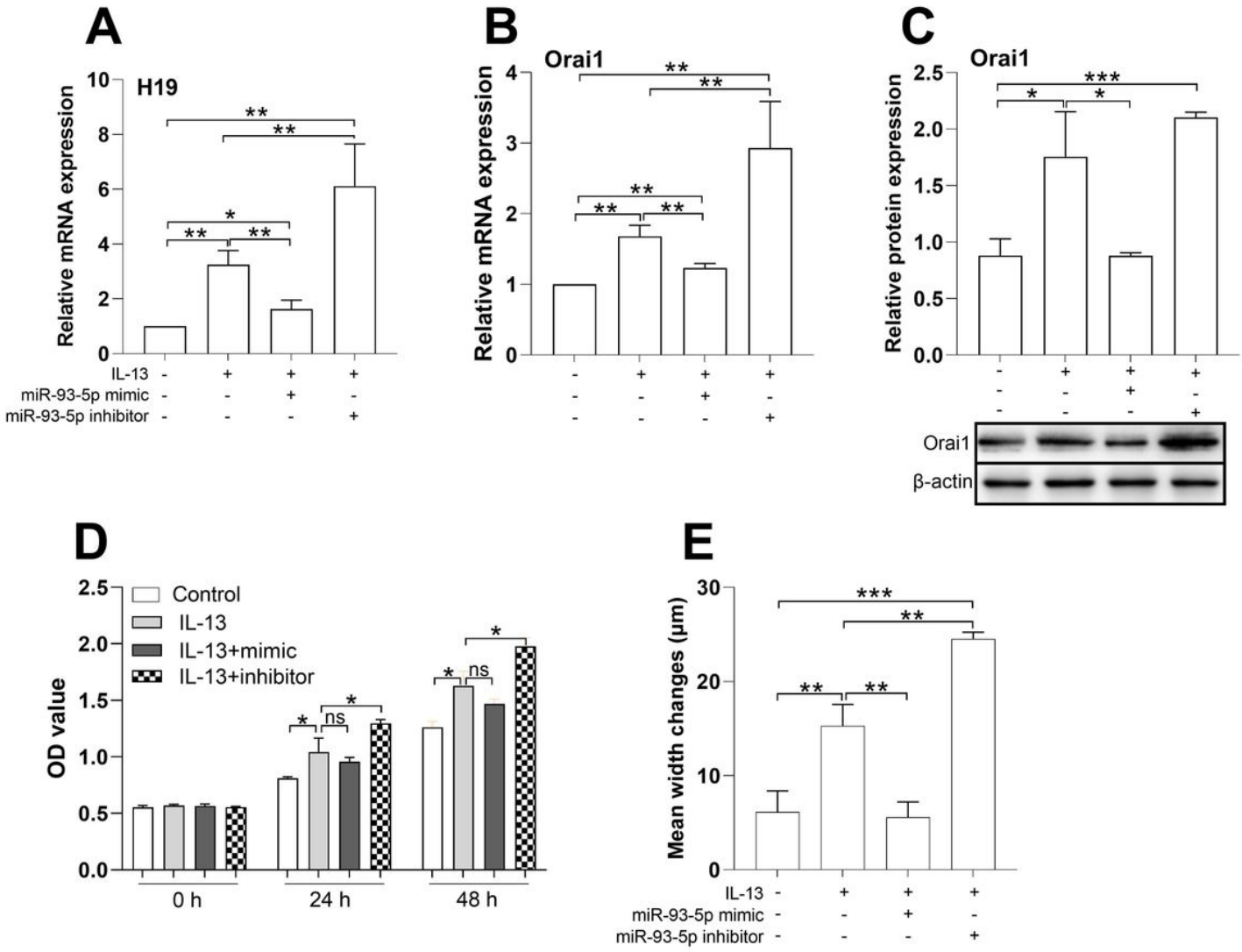

## Figure 2

Effects of IL-13 on the expression of H19, miR-93-5p and Orai1 in hBSMCs. In vitro cultured human bronchial smooth muscle cells (hBSMCs) were stimulated with different concentrations of IL-13 for 24 h and the relative expression of lncRNA H19 (A), miR-93-5p (B) and Orai1 mRNA (C) was assayed with RT-qPCR, the protein expression of Orai1 (D) was determined with western blotting. β-actin was used as internal control for protein expression. Con: control hBSMCs without IL-13 treatment; *p<0.05, **p<0.01.



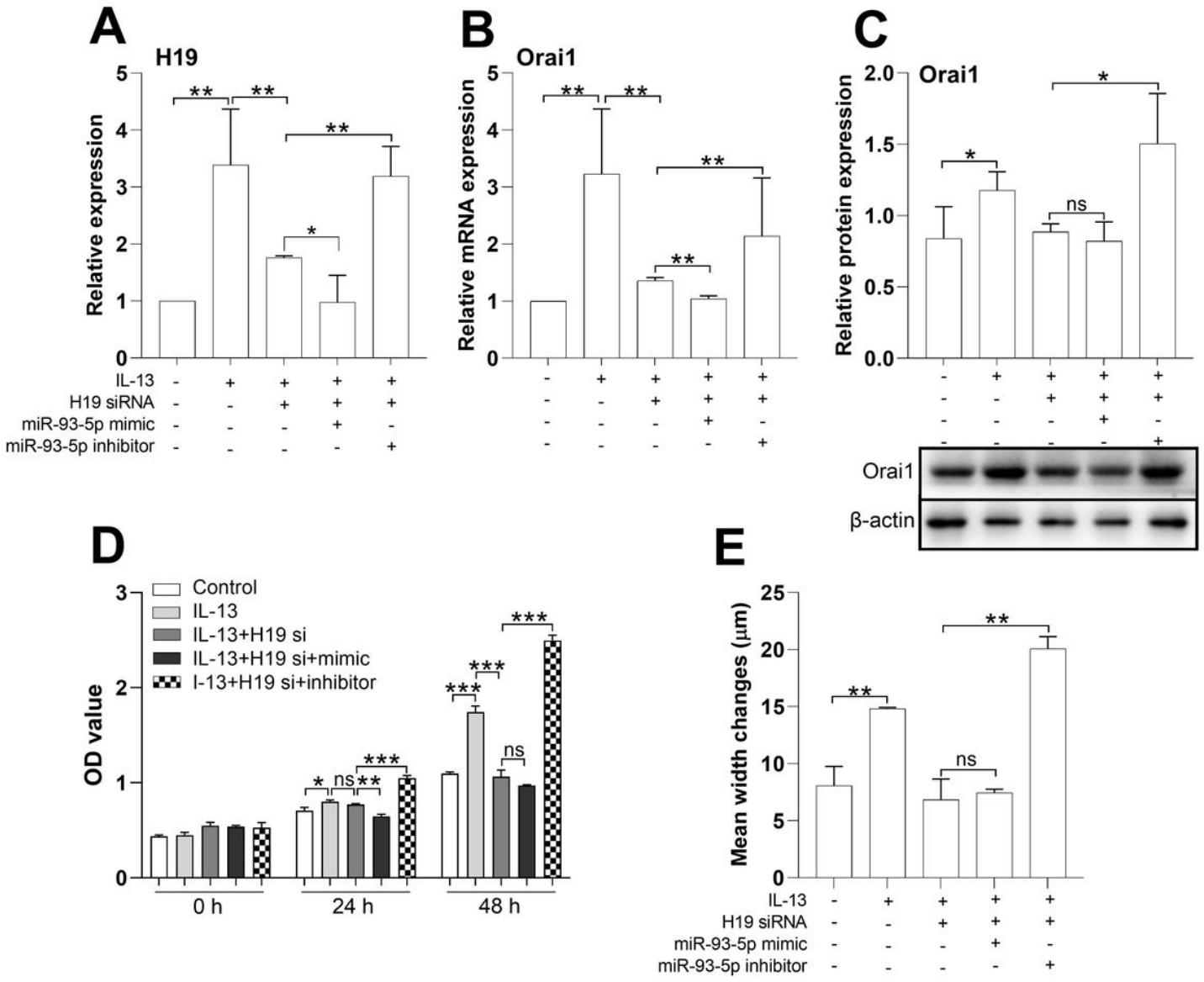

## Figure 3

Effects of H19 knock-down with siRNA on the expression of H19, miR-93-5p and Orai1 and hBSMCs proliferation and migration. A. In vitro cultured hBSMCs were treated with H19-specific siRNA (H19 siRNA) or negative control siRNA (siRNA NC), the expression of H19 was assessed with RT-qPCR. B-D. In vitro cultured hBSMCs were treated as indicated, the mRNA expression of H19, miR-93-5p and Orai1 were assessed with RT-qPCR. E. The protein expression of Orai1 in hBSMCs was determined with Western blotting, β-actin was used as internal control. F. The effect of H19-specific siRNA on IL-13-induced hBSMCs proliferation rates after 24 h and 48 h was assayed with CCK-8 methods. G. The effect of H-19-specific siRNA on IL-13-induced hBSMCs migration rates was investigated with wound healing test. *p<0.05; **P<0.01.



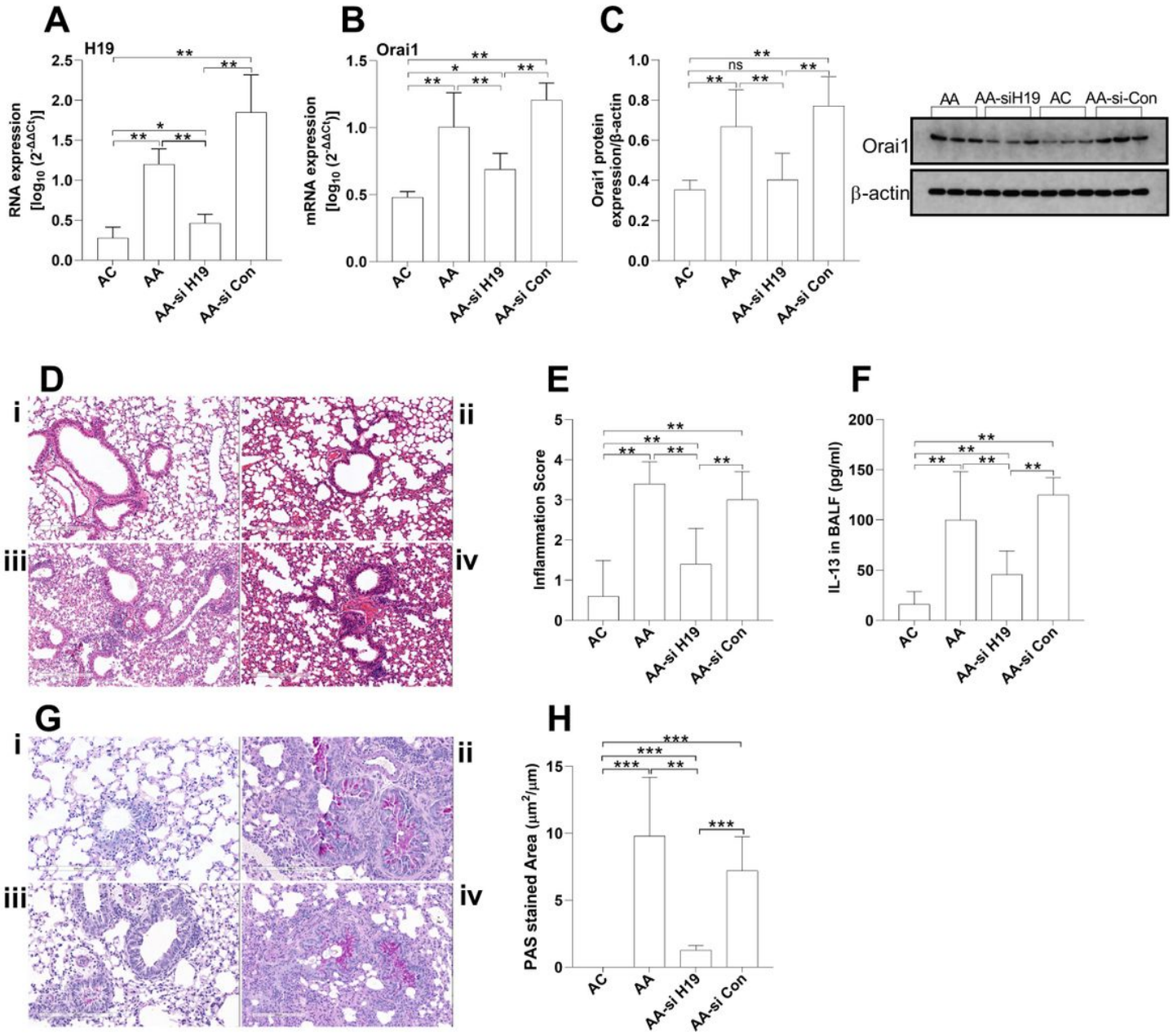

## Figure 4

Effects of miR-93-5p mimic and inhibitor on the expression of H19 and Orai1, and the proliferation and migration rates of hBSMCs. In vitro cultured hBSMCs were transinfected with miR-93-5p mimic and inhibitor respectively under stimulation with IL-13 (10 μg/ml). A-B. Relative mRNA expression of H19 (A) and Orai1 (B) compared to control (without IL-13 and transfection). C. Relative protein expression of Orai1 under different treatments, β-actin was used as internal control. D. Proliferation rates of hBSMCs after 24 h and 48 h with different treatments as indicated. E. Migration rates of hBSMCs under different treatments as indicated. *P<0.05; **P<0.01; ***P<0.001. ns, not significant.




## Figure 5

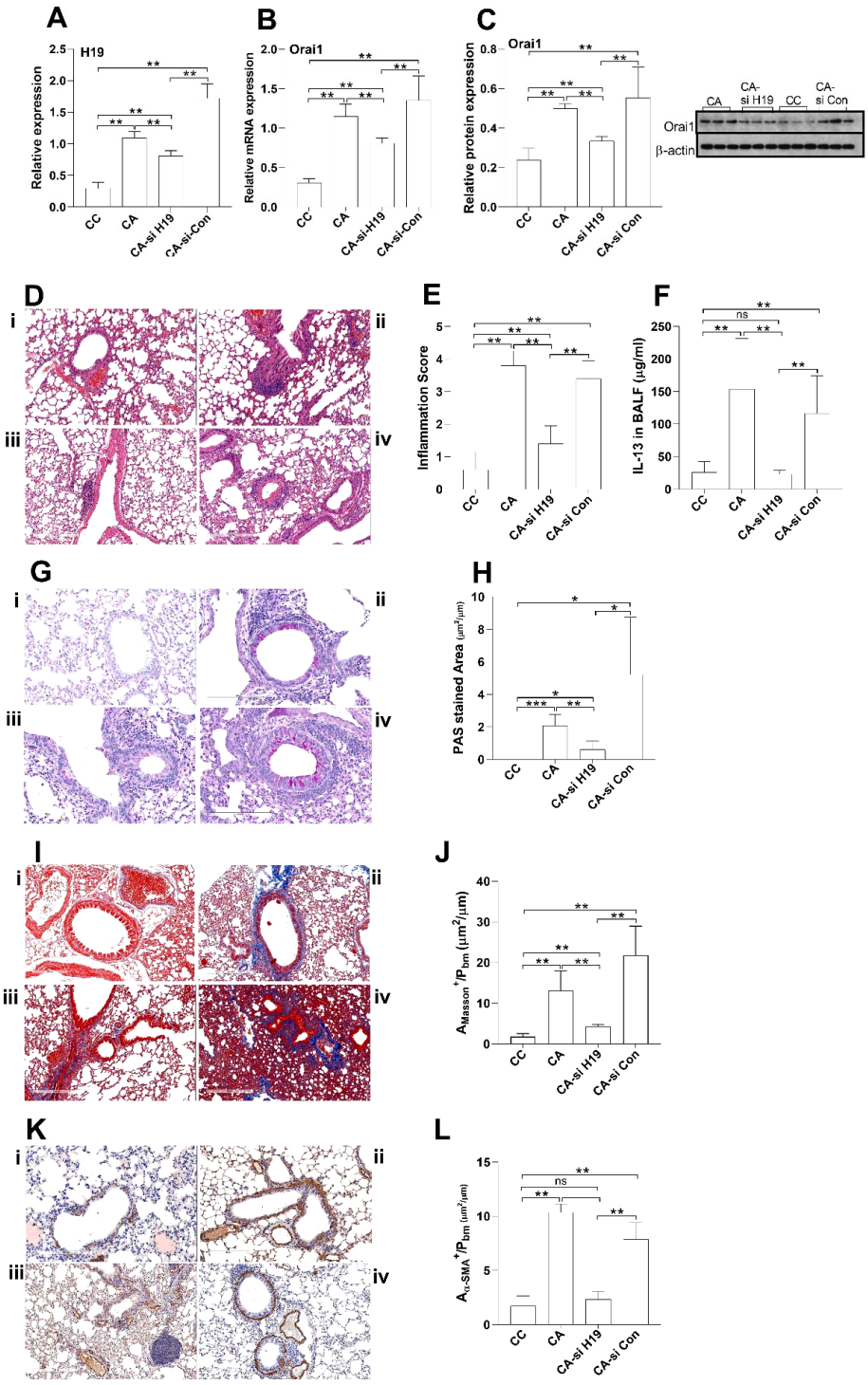
miR-93-5p mediated the effects of H19 on Orai1 expression, and the proliferation and migration rates of hBSMCs. In vitro cultured hBSMCs were transinfected with H19-specific siRNA with or without miR-93-5p mimic or inhibitor respectively under stimulation with IL-13 (10 μg/ml). A-B. Relative mRNA expression of H19 (A) and Orai1 (B) compared to control (without IL-13 and transfection). C. Relative protein expression of Orai1 under different treatments, β-actin was used as internal control. D. Proliferation rates of hBSMCs after 24 h and 48 h with different treatments as indicated. E. Migration rates of hBSMCs under different treatments as indicated. *P<0.05; **P<0.01; ***P<0.001. ns, not significant.




## Figure 6

The effects of H19 inhibition on Orai1 expression and airway inflammation in acute asthma murine model. Acute asthma mice (AA) were intranasal administrated with lentivirus-carrying H19-specific siRNA (AA-si H19) or control scramble siRNA (AA-si-Con). The expression of H19 (A) and mRNA expression of Orai1 (B) in the lungs was assessed with RT-qPCR. C. The protein expression of Orai1 in the lungs. Inserted are typical western blotting assays of Orai1 and internal control protein β-actin. D. Representative H&E staining of lung sections to show inflammatory cell infiltration in the lungs (200×). E. Inflammation scores (n=3 in each group). F. IL-13 concentration in BALF (n=3 in each group). G. Representative PAS staining of lung sections to show mucus hypersecretion. H. Ratio of PAS+ area to perimeter of bronchi to

quantify mucus hypersecretion. *p<0.05; **p<0.01; ***p<0.001; ns, no significance; i. control (AC) mice; ii. AA mice; iii. AA-si H19 mice; iv. AA-si-Con mice.




## Figure 7

The impact of H19 inhibition on Orai1 expression, airway inflammation and airway remodeling in chronic asthma murine model. Chronic asthma mice (CA) were intranasal administrated with lentivirus carrying H19-specific siRNA (CA-si H19) or control scramble siRNA (CA-si-Con). The expression of H19 (A) and

mRNA expression of Orai1 (B) in the lungs was assessed with RT-qPCR. C. The protein expression of Orai1 in the lungs. Inserted are typical western blotting assays of Orai1 and internal control protein β-actin. D. Representative H&E staining of lung sections to show inflammatory cell infiltration in the lungs (200×). E. Inflammation scores (n=3 in each group). F. Representative PAS staining to show goblet cell hyperplasia in bronchial mucus. G. Ratio of PAS+ area to perimeters of bronchi to quantify mucus hypersecretion. H. Representative Masson staining of lung sections to show collagen deposition in mouse lungs (200×). I. Ratio of Masson+ area to perimeters of bronchi to quantify collagen deposition. J. Representative α-SMA immunostaining to show hyperplasia and hypertrophy of ASM in the lungs of mice (200×). K. Ratio of α-SMA+ area to perimeter of bronchi to quantify hypertrophy of ASM. L. IL-13 concentration in BALF (n=3 in each group). *p<0.05; **p<0.01; ns, no significance. i: control (CC) mice; ii: CA mice; iii: CA-si H19 mice; iv: CA-si Con mice.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FIgureS3.docx
- FigureS2.jpg
- figureS1.jpg