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PII: S0006-291X(16)32198-2

DOI: [10.1016/j.bbrc.2016.12.138](https://doi.org/10.1016/j.bbrc.2016.12.138)

Reference: YBBRC 37012

To appear in: *Biochemical and Biophysical Research Communications*

Received Date: 9 December 2016

Accepted Date: 21 December 2016

Please cite this article as: L. Wang, J. Li, J. Zhang, Q. He, X. Weng, M. Guan, C. Qiu, Inhibition of TRPC3 downregulates airway hyperresponsiveness, remodeling of OVA-sensitized mouse, *Biochemical and Biophysical Research Communications* (2017), doi: 10.1016/j.bbrc.2016.12.138.

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Inhibition of TRPC3 Downregulates Airway Hyperresponsiveness, Remodeling of OVA-sensitized mouse

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Abstract

Airway hyperresponsiveness (AHR), airway remodeling and inflammation are the fundamental pathological alterations that occur in asthma. Transient receptor potential canonical 3 (TRPC3) has been implicated in diverse functions of airway smooth muscle cells (ASMCs) in asthma. However, the underlying mechanisms remain incompletely understood. We investigated the mRNA and protein expression of TRPC3 in ASMCs from normal and OVA-sensitized mouse. And the effects of inhibition or knockdown of TRPC3 with Ethyl-1- (4- (2,3,3-trichloroacrylamide) phenyl) -5 - (trifluoromethyl) -1H -pyrazole -4-carboxylate (Pyr3) and lentiviral shRNA on OVA-sensitized mouse AHR, airway remodeling, circulating inflammatory cytokines, cell proliferation and migration. We found that TRPC3 mRNA and protein expression levels were significantly increased in ASMCs from OVA-sensitized mouse. Inhibiting TRPC3 with continuous subcutaneous administration of Pyr3 decreased

enhanced pause (Penh) of OVA-sensitized mouse. Meanwhile, both Pyr3 and lentiviral shRNA treatment of ASMCs in OVA-sensitized mouse significantly decreased their proliferation and migration. These results suggest that TRPC3 plays a critical role in asthma and represents a promising new target for asthma treatment.

Key words TRPC3; ASMCs; AHR; proliferation; migration; asthma

1. Introduction

Asthma prevalence increased markedly worldwide during the second half of the 20th century, especially in developing countries as they became more westernized or communities became urbanized [1]. Inhaled glucocorticoids are the first-line treatment for asthma [2]. Unfortunately, 5-10% patients remain symptomatic despite high-dose glucocorticoids treatments. Moreover, according to community-based studies, adherence to such therapy can be as low as 20% [3], resulting in poor asthma control. These concerns have prompted the search for alternative therapies in patients whose asthma are not well controlled by glucocorticoids alone. We attempt to develop novel and effective intervention targeting and methods in the treatment of asthma, for which there has not yet been a breakthrough.

Intracellular calcium ($[Ca^{2+}]_i$) participates in various diseases and regulates a variety of cellular processes, including proliferation, contraction, migration and phenotypic transformation. Canonical transient receptor potential channel (TRPC)-mediated $[Ca^{2+}]_i$ entry has been implicated in the control of smooth muscle cell proliferation and demonstrated to represent a pivotal mechanism underlying in-stent restenosis [4], but the role of TRPC in the pathogenesis of asthma is not yet fully understood. Multiple TRPC molecules are expressed in rodent and human ASMCs, among which TRPC3 [5,6] acts as an important molecular component of the native nonselective cation channels (NSCCs) [7] although it displays moderate selectivity for Ca^{2+} over monovalent cations. A single glutamate residue (E630) was recognized as the key determinant of ion selectivity [8]. Neutralization of this negative charge completely disrupts Ca^{2+} permeation. Hofmann et al. [9] suggested that TRPC3 preferentially associates with TRPC6 and TRPC7 to form heterotetrameric channel complexes. Previous studies found that the expression of

TRPC3 significantly increased in ASMCs of patients with asthma and of mouse model of allergic asthma [10,11]. In this study, we investigated the roles of TRPC3 in AHR, airway remodeling, circulating inflammatory cytokines and specific cellular functions in OVA-sensitized mice after knockdown of TRPC3 with lentivirus-mediated shRNA or with a selective TRPC3 antagonist.

2. Materials and Methods

2.1 Preparation of chronic OVA-sensitized/-challenged mouse model

BALB/c female mice (6 wks old, n=10) were sensitized by i.p. injection of 20 µg grade V ovalbumin (OVA, Sigma-Aldrich, St. Louis, MO, USA) mixed in 50 µl of 36 mg/ml Imject ®Alum (Thermo Fisher Scientific, Boston, MA, USA) on days 0 and 7. Mice were challenged with 5% aerosolized OVA for 30 min per day, three days per week, from day 14 to day 53. Normal control mice (n=10) were treated with the equivalent volume of 0.9% saline solution alone. At 24 h after the last aerosolized exposure to the OVA or saline, mice were subjected to a determination of Penh to acetyl-β-methyl-choline chloride (methacholine, mACh, Sigma-Aldrich, St. Louis, MO, USA) and then sacrificed by cervical dislocation. All procedures related to animal use were approved by the Committee on the Use of Live Animals for Teaching and Research, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences.

2.2 Subcutaneous pump implantation

Ethyl-1-(4-(2,3,3-trichloroacrylamide)phenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylate (Pyr3, 0.1 mg/kg/d, Sigma-Aldrich, St. Louis, MO, USA) was administered through osmotic pumps (Alzet, Cupertino, CA, USA) that delivered a continuous dosage of the treatment for 7 days before the mice were sacrificed. The Pyr3 dose was chosen according to previous data [4]. Micro-osmotic pumps were implanted under the dorsal skin of the mice by first anesthetizing each mouse with 3% pentobarbital sodium salt (25mg/kg, Merck, Kenilworth, NJ, USA). Following anesthesia, the skin was cleaned with 75% alcohol, a small incision was made in the dorsal skin between the scapulae, and a small pocket was formed by spreading the subcutaneous connective tissues apart. The osmotic pump was inserted into the pocket.

The incision was closed with wound sutures. Mice in the Sham group were subjected to the same procedure but given 0.9% saline instead of Pyr3.

2.3 Non-invasive measurement of Penh

Penh was measured 24 h after the last challenge by recording respiratory pressure curves using barometric unrestrained whole-body plethysmography (Buxco electronics, St. Paul, MN, USA) in response to inhale increasing doses of mACh (0-100 mg/ml) in mice. mACh was aerosolized for 2 min, and readings were recorded and averaged for 5 min after each nebulization. At the end of the measurement, mice were sacrificed by cervical dislocation, and their lungs were excised for the histopathology studies.

2.4 Measurement of IL-13 and TNF α in Plasma

The mice plasma were stored at -80°C before analysis. The concentrations of IL-13 and TNF α in plasma were measured by ELISA kits (Neobioscience Biotechnology Co, Shenzhen, China) according to the manufacturer's instructions.

2.5 Histopathology

The middle lobe of right lung was harvested for histopathological examination. Tissue was fixed in 4% formaldehyde overnight before paraffin embedding, and 5- μ m slides were prepared with a vibratome from tissue sections for staining. Slides were stained with hematoxylin and eosin (H&E). Morphometry was performed by individuals blinded to the experimental design. The parameters bronchial basement membrane perimeter (Pbm), the area of airway wall (WAt) were measured by digital image analysis (Image J, 1.43u; Wayne Rasband, NIH, USA). The ratios of WAt to Pbm (WAt/Pbm) were used to evaluate the thickness of the airway wall. A minimum of 10 fields for each group were randomly examined for the morphometric analyses in cross-sections which were defined by a ratio of minimal to maximal internal diameter of less than 0.5. The tissue analysis was blinded to the observers .

2.6 Primary culture of ASMCs from mice

Primary ASMCs from normal or OVA-sensitized mice were isolated following a protocol that was used in our laboratory based on previously reported data [12]. Briefly, the trachea and lungs were completely detached and the extraneous

membranes and connective tissues were removed. The residual tissues were cut into 1 mm×3 mm blocks that were tiled on culture flask and immersed in Dulbecco's modified eagle medium (DMEM, Hyclone, Logan, Utah, USA) supplied with 20% FBS (Gibco, Grand Island, NY, USA), 4 mM glutamine and 1% penicillin - streptomycin (Gibco, Grand Island, NY, USA). The cultures were incubated at 37°C, 5% CO₂ until reaching approximately 80% confluence. After purification, cells at passages 3-8 were used for the experiments described below. The cells were identified as ASMCs by morphology and immunofluorescence staining.

2.7 Real-time polymerase chain reaction (qPCR)

Total RNA was extracted according to the RNAiso Plus (Takara Bio, Dalian, China) protocol. The first-strand cDNAs were synthesized from RNAs using the ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo, Osaka, Japan). The resultant cDNA templates were amplified with oligonucleotide primers as follow: 5'-GCGAGCAAGAACTGCGAGAT-3' and 3'-TGCACCACCTCGTACTTATGG-5' for mouse *Trpc3* (NM_019510) and 5'-CTGGCACCACACCTTCTACAATG-3' and 3'-AATGTCACGCACGATTTCCCGC-5' for mouse β -actin (NM_007393.5). The relative mRNA levels were evaluated using the comparative threshold cycle (dCt) method and normalized to the β -actin. Real-time PCR assays were carried out using ABI TaqMan Universal Master Mix (Toyobo, Osaka, Japan) and all the samples were measured in triplicate.

2.8 Western blotting analysis

Expression of TRPC3 protein was analyzed by western blotting. Protein was electrophoresed (20 μ g/lane) through 8% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Membranes were incubated with the primary antibody (rabbit anti-TRPC3, 1: 1000 dilution, Alomone Labs, Jerusalem, Israel) overnight at 4°C after blocking with 5% non-fat milk in Tris-buffered saline and 0.05% Tween20, followed by incubation with secondary goat anti-rabbit IgG (1: 4000 dilution, Abcam, Cambridge, UK) conjugated with horseradish peroxidase 2 h at room temperature. Enzyme-linked chemiluminescence (ECL Plus, Beyotime, Shanghai, China) was used to detected the

reaction. Data were normalized to the β -actin (Sigma-Aldrich, St. Louis, MO, USA) in the same lane. The experiment had been repeated three times.

2.9 Generation of lentiviral shRNA TRPC3 knockdown ASMCs

Lentivirus packaged TRPC3 shRNA (TRPC3 KD) and scrambled, nonsilencing shRNA (NS) were purchased from GenePharma (GenePharma, Suzhou, China). ASMCs were seeded (2×10^5 cells/well) in 6-well plates, transfected with lentivirus (multiplicity of infection, MOI=100) encoding TRPC3 or NS shRNA at 37°C for 48 h, and then treated with puromycin (1 μ g/ml). ASMCs were passaged after cell confluence achieved at approximately 100%. Western blotting analysis was used to confirm the effectiveness of the TRPC3 knockdown.

2.10 Cell proliferation assays

Cells were seeded in 96-well plates at a density of 4000 cells per well. After incubation with Pyr3 at increasing concentrations (0 μ M, 3 μ M, 5 μ M and 10 μ M) overnight at 37°C, Cell proliferation was analyzed at 24, 48, 72 h by determining the concentration of formazan in cell supernatants with Cell Counting Kit-8 (CCK-8 kit, Dojindo, Kumamoto, Japan). After incubation for 2 h at 37°C, the absorbance of each well was measured at 450 nm with a microplate reader (Thermo Scientific, Rockford, IL, USA). Pyr3 was dissolved in dimethylsulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA) and diluted with PBS (Hyclone, Logan, Utah, USA). Pyr3 0 μ M was added the equivalent DMSO as other concentrations. The experiment had been repeated three times.

2.11 Cell migration assays

Cells (5×10^4 cells per transwell) resuspended in 200 μ l serum-free DMEM were cultured onto the upper surfaces of transwell inserts (8 μ m pores, Corning, Lowell, MA, USA). Transwells were placed into wells of 24-well plates containing 600 μ l DMEM supplemented with 5% FBS. Cells were incubated at 37°C, 5% CO₂ incubator for 6 h before fixing in precooled methanol and stained with 0.5% crystal violet (Sigma-Aldrich, St. Louis, MO, USA). Migrated cells were counted under high power fields (HPFs, $\times 20$) and 10 HPFs were counted to determine the average cell number migrated per HPF.

2.12 Data analysis

All data are represented as the mean $\pm$ SEM. Statistical analyses (two-sample t test, one-way ANOVA, and two-way ANOVA) were performed with GraphPad Prism 5 (GraphPad, La Jolla, CA, USA). Differences were considered significant $P < 0.05$.

3. Results

3.1 *Penh, airway remodeling and the gene expression and protein of TRPC3 are increased in OVA-sensitized mice*

In the OVA-sensitized group (abbreviated as Asthma group), mice demonstrated tachypnea, irregular respiratory rhythm, sneeze, irritability, tremor, and cyanosis of lips and reduced activity. Mice of asthma group and normal group were generally good, eating as usual. There was no significant difference in body weight. The Penh of the asthma group was significantly higher than that of the normal group (Fig. 1C). For the histology study, in addition to the apparent increase in inflammatory cells in the lung mucosal and submucosal areas, the thickness of airway walls was considerably greater in the OVA-sensitized mice than in the normal group (Fig. 1A and B). Meanwhile, ASMCs manifested consistent staining of α -smooth muscle actin (α -SMA) which was specific protein for the smooth muscle in the immunohistological assay and immunofluorescent staining, indicating that these were ASMCs (data not shown). Real-time PCR analysis revealed that mRNA expression of TRPC3 in OVA-sensitized ASMCs was significantly higher than in the normal group (Fig.1D). Western blotting demonstrated an important increase in the protein expression of TRPC3 compared with the normal group (Fig.1E).

3.2 *In vivo antagonism of TRPC3 by Pyr3 suppresses the OVA-sensitized mice Penh, airway remodeling and circulating IL-13*

Our previous experiments have demonstrated increased TRPC3 expression at both the mRNA and protein levels. Pyr3 can selectively antagonize TRPC3 by binding to the extracellular side of the receptor [13], we therefore used this small molecule antagonist to clarify the role of TRPC3 in AHR of OVA-sensitized mice in vivo. Our results showed that compared with the Sham group, the increased mACh-induced Penh in OVA-sensitized mice was significantly decreased by continuous subcutaneous

administration of Pyr3 via the Alzet pump. The Penh of the Pyr3 group was decreased, showing responses similar to the normal group (Fig. 2A). Pyr3 also reduced the airway wall thickness compared with the asthma group, as demonstrated by H&E staining (Fig. 2C). Meanwhile, the circulating levels of IL-13 decreased after Pyr3 administration (Fig. 2B), TNF α level was not affected by the administration of Pyr3 (data not shown).

3.3 In vitro antagonism of TRPC3 by Pyr3 decreases OVA-sensitized mice ASMC proliferation and migration

Based on the aforementioned observed activity, the TRPC3 antagonist Pyr3 was used to test the role of TRPC3 in cellular functions of ASMCs. Treatment with Pyr3 decreased ASMC proliferation at 24 h, 48 h and 72 h (Fig. 3A). Similarly, Pyr3 at 3 μ M, 5 μ M and 10 μ M significantly decreased ASMC migration (Fig. 3B and C).

3.4 In vitro knockdown of TRPC3 by lentiviral shRNA decreased OVA-sensitized ASMCs proliferation and migration

We further investigated the role of TRPC3 in ASMCs using lentiviral shRNA. Western blotting analysis confirmed the knockdown effect of TRPC3, and lentivirus No. 2593 was chosen as the most effective (Fig. 4A) and used in the following experiments. TRPC3 shRNA significantly decreased the ASMC proliferation at 48 h (Fig. 4B). Similarly, ASMC migration was significantly decreased by TRPC3 shRNA (Fig. 4C).

4. Discussion

The present study mainly examines the effects of TRPC3 inhibition on Penh and airway remodeling. We found that Pyr3 decreased Penh, airway wall thickness and the circulating IL-13 level of OVA-sensitized mouse *in vivo*. Moreover, *in vitro* proliferation and migration of OVA-sensitized mouse ASMC was significantly attenuated by Pyr3 or TRPC3 shRNA treatment. Given a plethora of previous studies demonstrating that TRPC3 contributes to $[Ca^{2+}]$ and the resting membrane potential (V_m) in normal ASMCs along with V_m agonist-induced $[Ca^{2+}]_i$ entry and membrane depolarization in OVA-sensitized ASMCs [10], the present study further suggests that TRPC3 plays an important role in regulating Penh and specific cellular functions of

ASMCs.

TRPC channels are termed “classical” or “canonical” because they are structurally the closest to the originating family member found in *Drosophila* [14]. The coupling of IP3R1 and membrane-resident TRPC3 has been reported to be directly involved in human cerebellar artery myocyte vasoconstriction [15]. TRPC3 has been described as a determinant factor of phenotype switching in vascular smooth muscle cell (VSMC) and induces cerebrovascular remodeling during hypertension via the transactivation of EGF receptor signals [16]. Pyr3 is a potent and selective antagonist of TRPC3 [17]. Remodeling of airway smooth in asthma shares similarities with the remodeling of cardiomyocytes and VSMCs that occurs in cardiac hypertrophy, restenosis and hypertension [18]. However, compared with the relatively detailed research of TRPC3 in cardiomyocytes and VSMCs [19], the potential roles of TRPC3 in asthmatic ASMCs remain incompletely understood. Previous studies have revealed that TRPC3 expression and function are increased in ASMCs derived from mice sensitized with OVA or stimulated with inflammatory factors or from patients with asthma and TRPC3 mediates OVA-sensitized membrane depolarization in ASMCs [20]. In line with the role of Pyr3 in VSMCs preventing in-stent restenosis in human arteries [4], our data show that TRPC3 antagonism by Pyr3 in ASMCs significantly decreases OVA-sensitized mouse airway remodeling and Penh.

In our study, *in vivo* continuous subcutaneous administration of Pyr3 also decreased the circulating IL-13 level in OVA-sensitized mouse. To our knowledge, this is the first report that continuous subcutaneous administration of Pyr3 could attenuate systemic IL-13 *in vivo*. Thomas and colleagues reported that siRNA knockdown of TRPC3 decreased agonist-induced Ca^{2+} concentration and store-operated Ca^{2+} entry (SOCE) [7]. Meanwhile, inhibition of TRPC3 with siRNA or Pyr3 decreased TNF α -stimulated brain-derived neurotrophic factor secretion in hASMCs. On the other hand, inflammatory cytokines are important asthma mediators such as TNF α increases TRPC3 expression in hASMCs and $[\text{Ca}^{2+}]_i$, thus increasing airway contraction.

In our results, the TRPC3 antagonized by Pyr3 and the TRPC3 knockdown by

TRPC3 shRNA both demonstrated inhibitory effects on the proliferation and migration of ASMCs from OVA-sensitized mouse. Though the mechanisms of Pyr3 function and TRPC3 shRNA might be different, as a whole-cell patch clamp study indicated that Pyr3 functioned directly and extracellularly, whereas the shRNA functions through knockdown of the TRPC3 mRNA, both proved the importance of a functional role for TRPC3 in the proliferation and migration of ASMCs from OVA-sensitized mouse. The pathophysiological airway remodeling is a phenotypic effect of the change of ASMCs from a quiescent to a migratory and proliferative/hypertrophic phenotype [21]. The proliferation, migration, synthesis and secretion functions of ASMCs increased in the airway remodeling group [22]. Increased airway smooth muscle mass, which is at least partially caused by hyperplasia, is one of the characteristic changes of airway remodeling and impaired lung function. Exposure of ASMCs to mitogenic stimuli such as platelet-derived growth factor results in changing from a hypoproliferative to a proliferative phenotype, as demonstrated by our and other laboratories [23,24]. Our previous work has shown that NF- κ B signaling is essential for phenotype switching in ASMCs and that inhibition of NF- κ B alleviates ASMC accumulation both in vivo and in vitro. The current study suggests that TRPC3 contributes to proliferation and migration signaling in asthma and also represents a promising target for the treatment of airway remodeling in asthma.

The functional roles of TRPC3 in asthma were further proved in the OVA-sensitized mouse, in which continuous subcutaneous delivery of Pyr3 for 7 days significantly rescued the Penh and the airway remodeling. Moreover, the circulating level of the inflammatory cytokine IL-13 was suppressed by Pyr3 compared to the OVA-sensitized group. The essentials of our results are supported by another independent study by Song, T. et al. In their study, treatment of the OVA-sensitized mouse with TRPC3 shRNA and Pyr3 intranasally administered also abolished the allergen-induced AHR as well as airway remodeling [25]. The inflammatory cytokines in the bronchoalveolar lavage fluid remained unaffected by those treatments. Compared to the effect of TRPC3 blockage by Pyr3 on the circulating IL-13 level in

our study, the difference might be due in part to the route of drug delivery, the treatment duration and the difference between the levels of inflammatory cytokines in bronchoalveolar lavage fluid and plasma.

In conclusion, we have found that in vivo antagonism of TRPC3 by Pyr3 resulted in decreased OVA-sensitized mouse Penh and decreased circulation of inflammatory cytokines and that in vitro antagonism of TRPC3 by Pyr3 and knockdown of TRPC3 by shRNA resulted in decreased proliferation and migration of ASMCs from OVA-sensitized mouse. We therefore suggest that TRPC3 might be a promising therapeutic target in asthma and that Pyr3 is an encouraging candidate drug, although more detailed investigations are required.

Acknowledgments

This work was supported by the NSFC (81270074), the Natural Science Foundation of Guangdong (2012CB518200, 2016A030310087), the Project of Shenzhen Basic Research Plan (JCYJ20140417113430710, JCYJ20130402092657770, JCYJ2016042216583605), the China postdoctoral science foundation (2016M592602).

Conflict of interest

The authors declare that they have no conflict of interest.

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Figure Legends

Figure 1. Identification of chronic OVA-sensitized mice model and the mRNA and protein expression of TRPC3

(A) The photomicrographs are representative of the right-middle lobes of each group using hematoxylin and eosin (H&E) staining. Scale bar, 20 μ m. (B) Penh in OVA-sensitized group (abbreviated as Asthma group) and normal group. *** $P < 0.001$ compared with the normal group ($n=10$). (C) The thickness of airway wall was increased in the OVA-sensitized group. ** $P < 0.01$ compared with the normal group. (D) Relative mRNA level of TRPC3 in ASMCs of the OVA-sensitized group and the normal group. ** $P < 0.01$ compared with the normal group ($n=10$). (E) The protein expression of TRPC3 in the OVA-sensitized group and the normal group.

Figure 2. In vivo pharmacological inhibition of TRPC3 by Pyr3 decreased Penh, airway remodeling and circulating IL-13

(A) Penh to the mACh was measured in the OVA-sensitized group (Control, $n=6$), OVA-sensitized mice with implantation of a pump containing Pyr3 (Pyr3, $n=6$) and OVA-sensitized mice with a sham operation providing 0.9% saline instead of Pyr3 (Sham, $n=4$). *** $P < 0.001$ compared with control group. (B) ELISA of circulating IL-13 levels of each group. * $P < 0.05$ compared with the control group. (C) Photomicrographs representative of the airways of each group using H&E staining.

Scale bar, 20 μm . Bar graph showing the relative airway thickness of each group. $*P < 0.05$ compared with the control group.

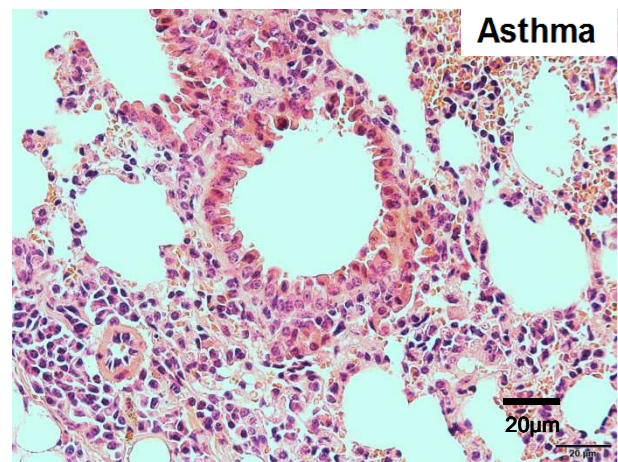
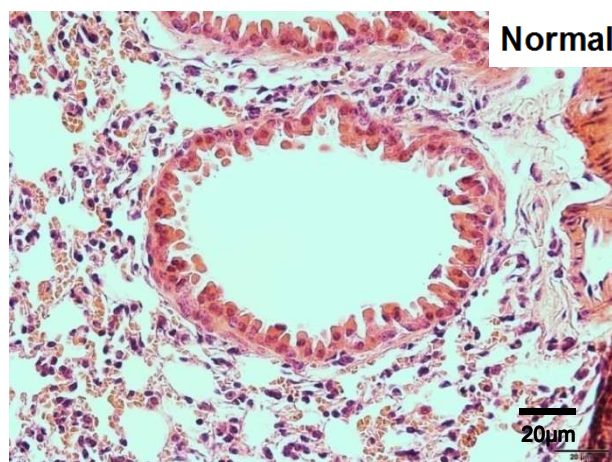
Figure 3. In vitro inhibition of TRPC3 by Pyr3 blocked OVA-sensitized mouse ASMC proliferation, migration

Effect of Pyr3 on ASMC proliferation (A) and migration (B), a, b, c were represented as comparing with the 0 μM group, 3 μM group and 5 μM group respectively. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with the 0 μM group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with the 3 μM group, $^{\&\&\&}P < 0.001$ compared with the 5 μM group. (C) The photomicrographs are representative of cell migration. Scale bar, 50 μm .

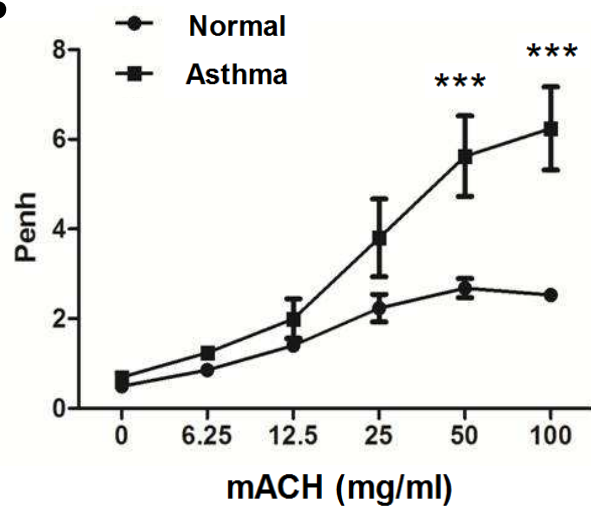
Figure 4. In vitro lentiviral TRPC3 shRNAs blocked the OVA-sensitized mouse ASMC proliferation and migration

(A) TRPC3 expression was detected in ASMCs. 1681, 2241, 2387 and 2593 represent ASMCs transfected with different TRPC3 shRNA. (B) TRPC3 shRNA decreases ASMC proliferation. Cell proliferation was assessed at 24 h, 48 h, and 72 h after seeding in 96-well plates. $***P < 0.001$ compared with the NS group. (C) TRPC3 shRNA reduces ASMC migration. Scale bar, 50 μm . $**P < 0.01$ compared TRPC3 KD group with NS group.

A

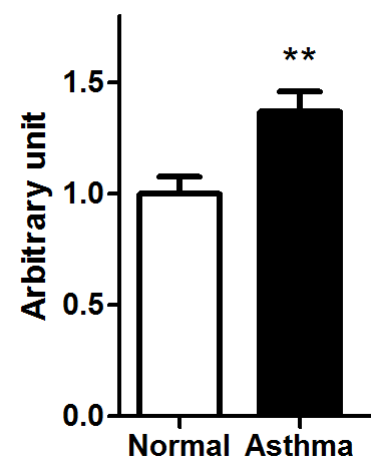


B



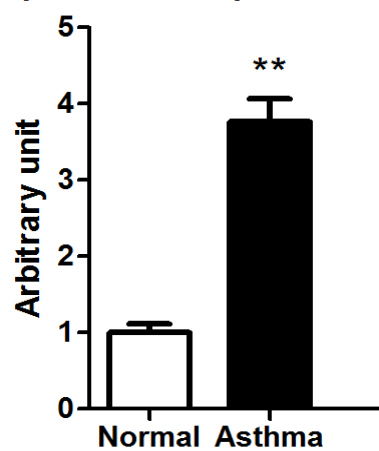
C

Airway wall thickness



D

Trpc3 mRNA expression



E

TPPC3 protein expression

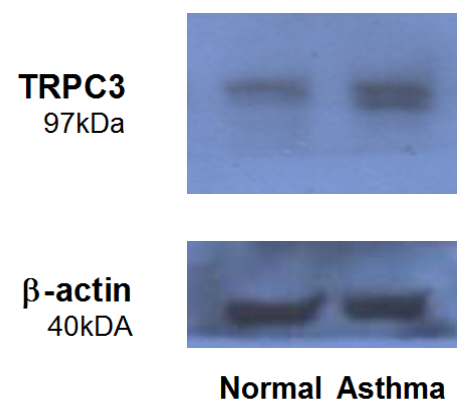


Fig 1.

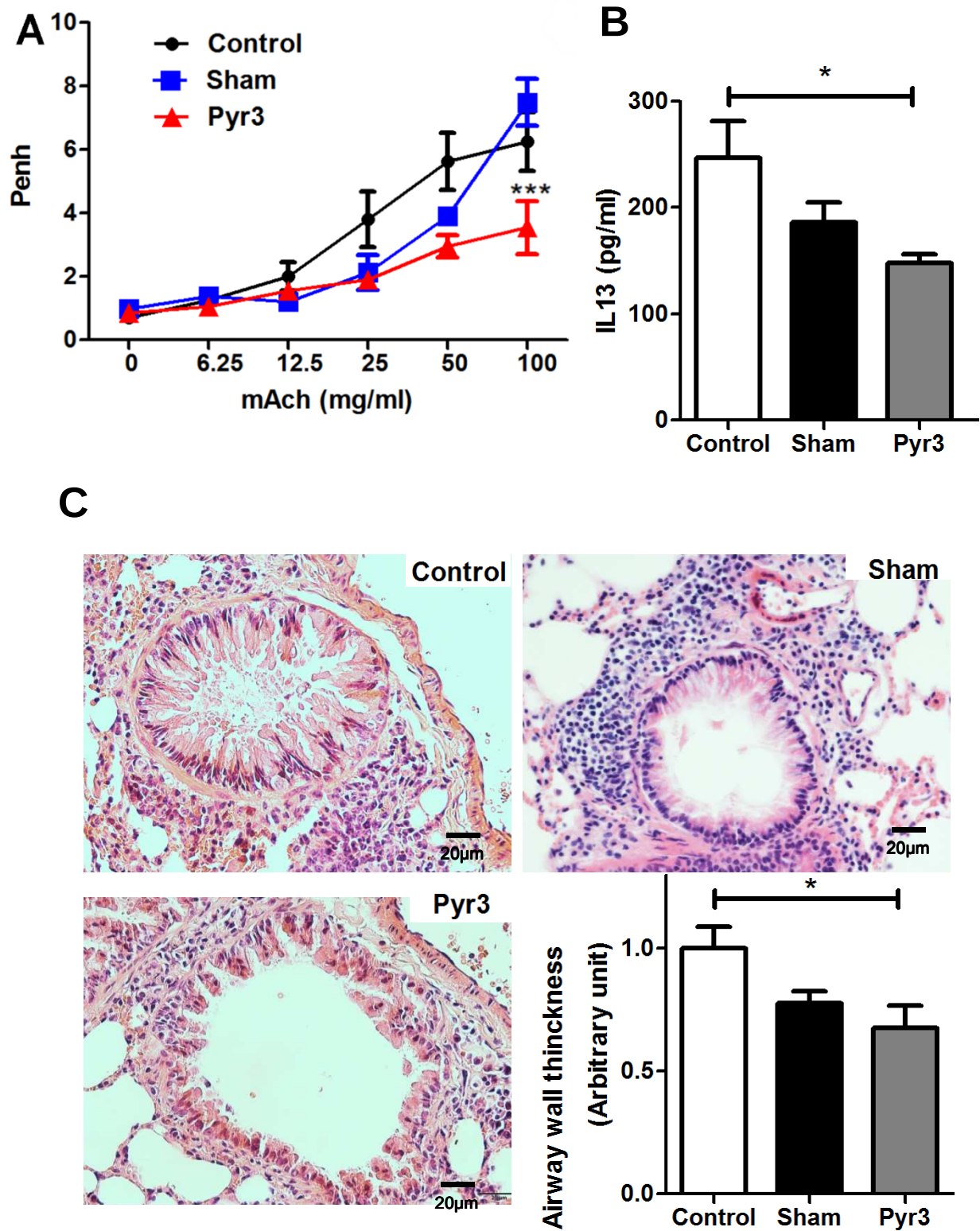
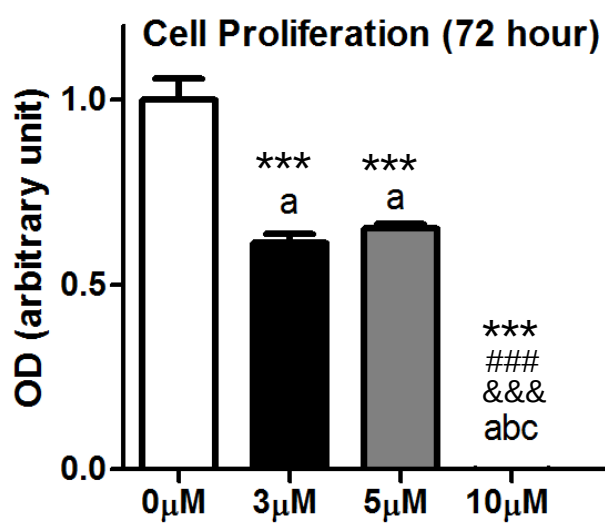
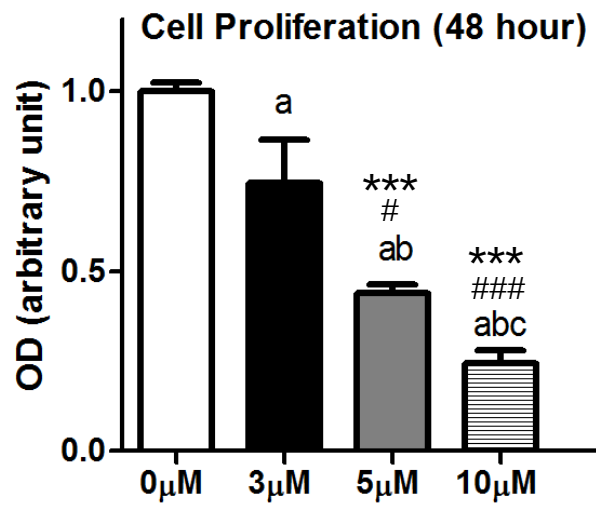
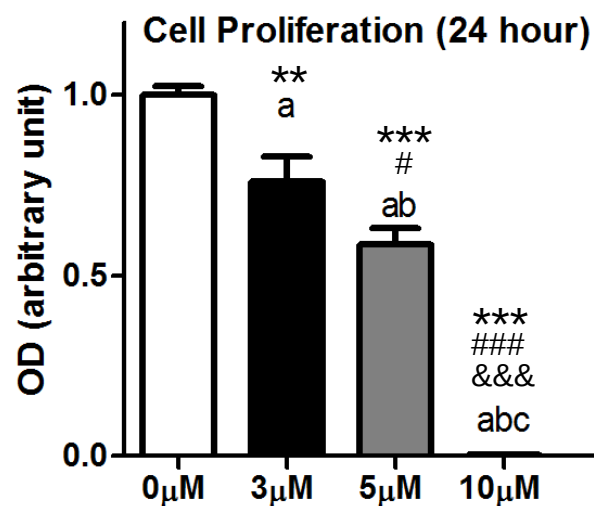
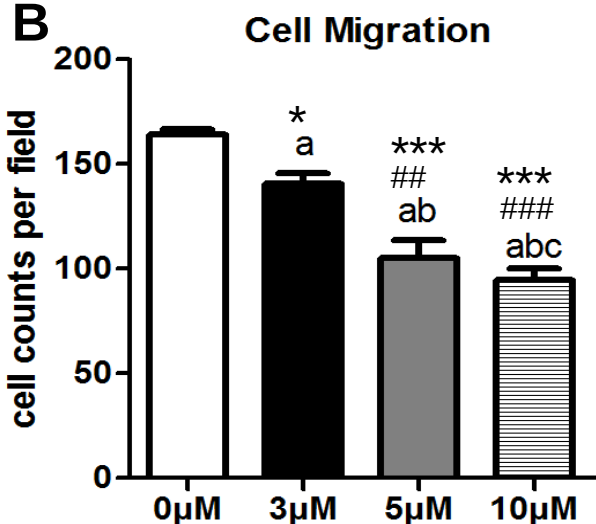


Fig 2.

A



B



C

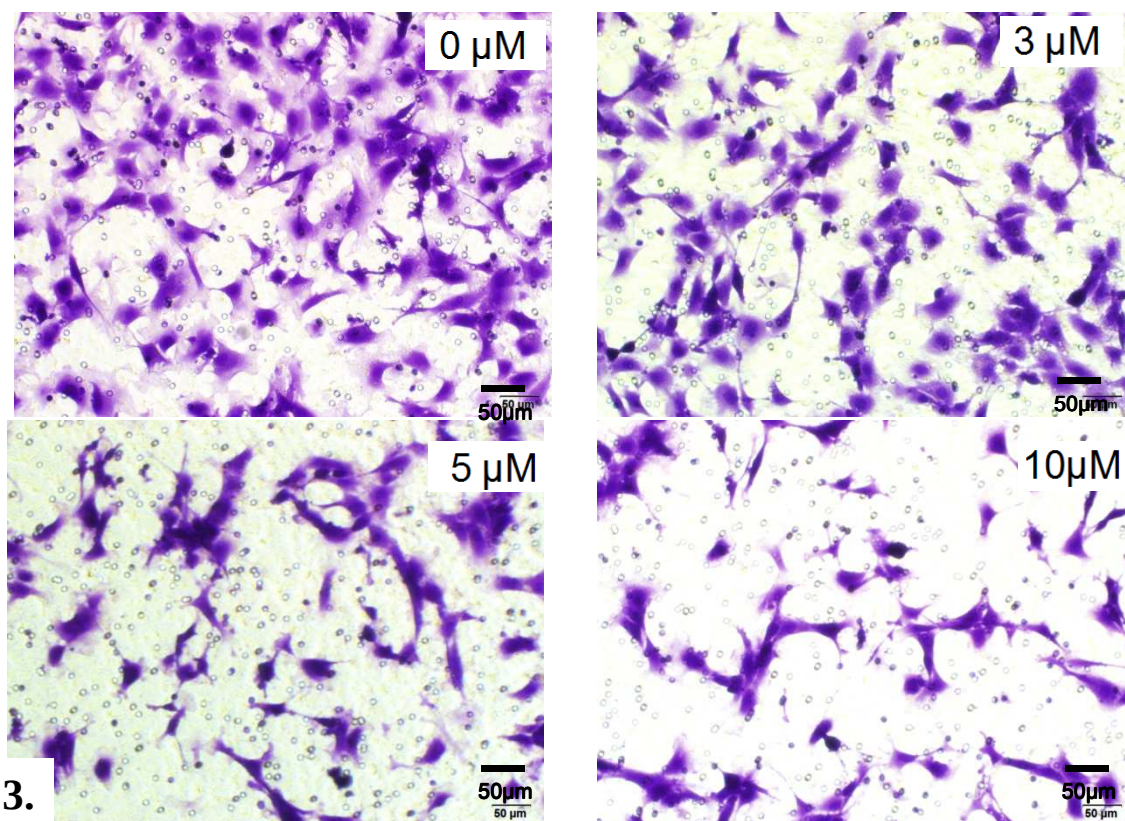
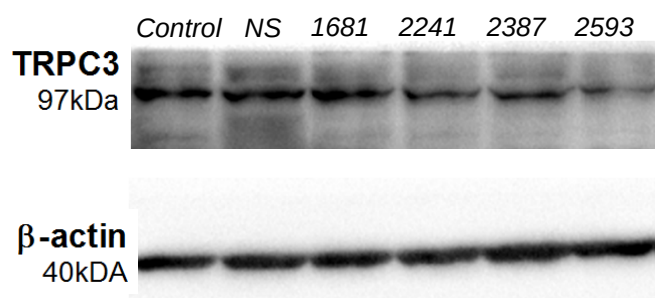
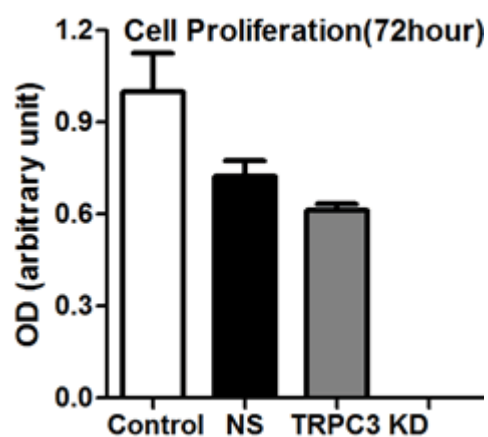
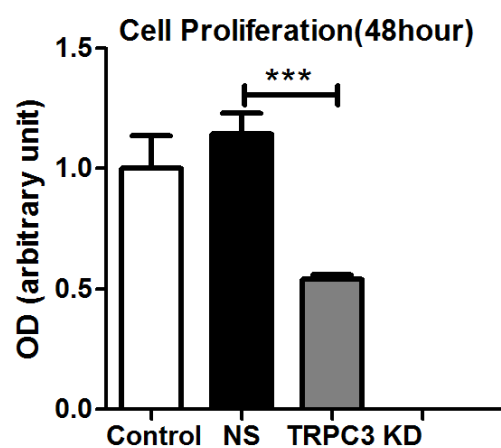
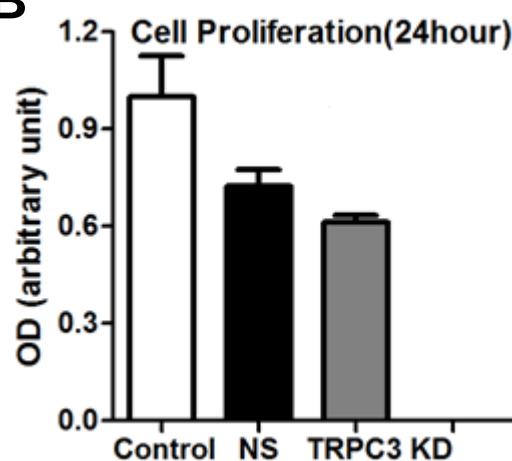
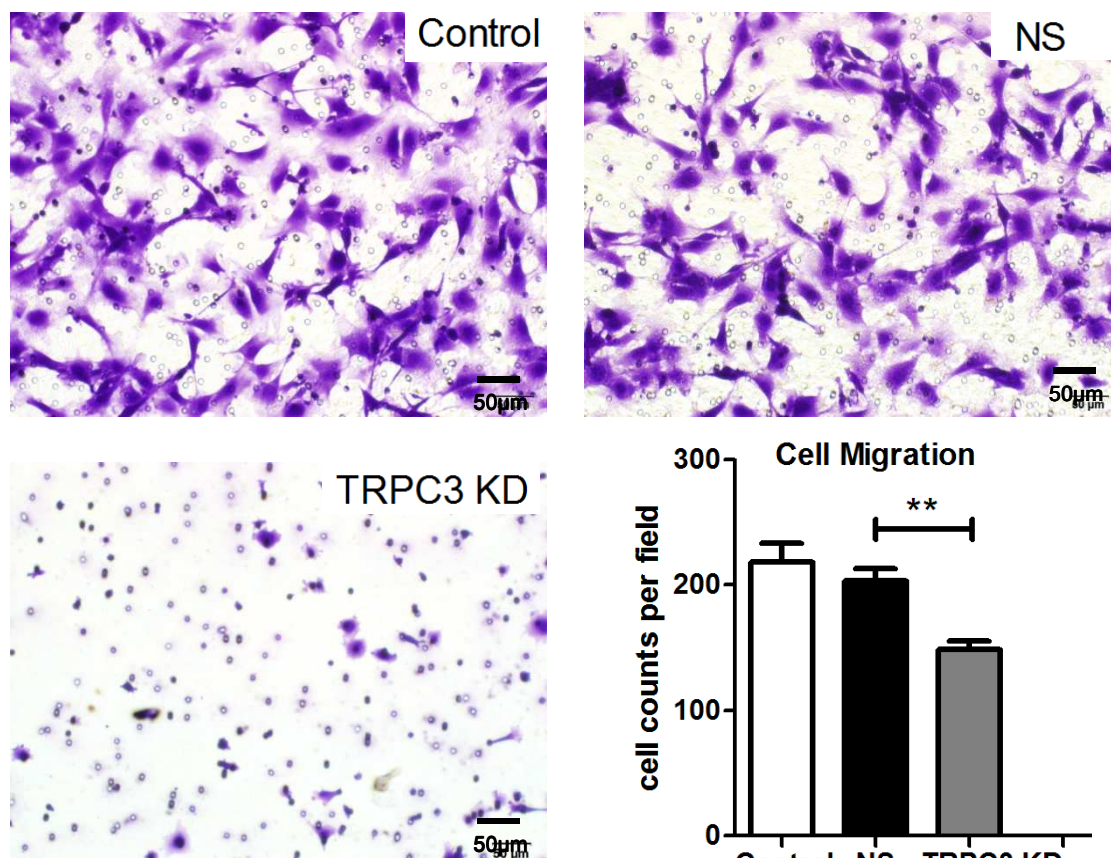


Fig 3.

A**B****C****Fig 4.**

1. Penh, airway remodeling and the gene expression and protein of TRPC3 are increased in OVA-sensitized mice
2. Inhibition of TRPC3 suppresses the OVA-sensitized mice Penh and airway remodeling.
3. Inhibition of TRPC3 decreases OVA-sensitized mice ASMC proliferation and migration.