STIM1/Orai1-mediated Ca 2+ influx contributes to the ASM phenotype modulation and ASM-related ECM deposition in asthma

Hangqi Ni
Department of Respiratory and Critical Care Medicine, First Affiliated Hospital, Zhejiang University School of Medicine, Zhejiang University

Ting Li
Department of Respiratory and Critical Care Medicine, First Affiliated Hospital, Zhejiang University School of Medicine, Zhejiang University

Junjun Chen
Department of Respiratory and Critical Care Medicine, First Affiliated Hospital, Zhejiang University School of Medicine, Zhejiang University

Yuying Wei
Department of Respiratory and Critical Care Medicine, First Affiliated Hospital, Zhejiang University School of Medicine, Zhejiang University

Mengling Xia
Department of Respiratory and Critical Care Medicine, First Affiliated Hospital, Zhejiang University School of Medicine, Zhejiang University

Qing Wang (wqingss@zju.edu.cn)
Department of Respiratory and Critical Care Medicine, First Affiliated Hospital, Zhejiang University School of Medicine, Zhejiang University

Research Article

Keywords: airway smooth muscle, STIM1, SOCE, Orai1, asthma

Posted Date: January 17th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-3863076/v1

Additional Declarations: No competing interests reported.

Abstract

Background: Phenotype modulation of airway smooth muscle cells (ASMC), defined as a more proliferative/synthetic type switched from contractile cells, plays an important role in airway remodeling of asthma. STIM1 and Orai1, the key aspects mediating store-operated Ca^{2+} entry (SOCE), have been shown to promote ASMC proliferation and migration. In this study, we explored the role of STIM1/Orai1-mediated SOCE in ASMC phenotype transition, and further investigated their involvement in the extracellular matrix (ECM) deposition in asthma.

Methods: The ASMCs from C57BL/6 mice were prepared and incubated with PDGF-BB to induced the phenotype switching. SKF-96365, an inhibitor of STIM1/Orai1, was used to detect the effect of SOCE in the ASMC phenotype transition and ASMC-related ECM doposition. Cell counting kit-8 assay, immunocytochemistry staining, enzyme-linked immunosorbent assay, and western blot assay were employed to detect the ASMC's proliferation and the expressions of contractile proteins, inflammatory cytokines as well as exacellular matrix. Moreover, we prepared the asthmatic mice model with SKF-96365 intranasal or intratracheal instillation and western blot assay were employed to determine the effect of SOCE repression in ECM deposition in vivo.

Results: We prepared the "proliferative/synthetic" type ASMCs with PDGF-BB treatment. and detected the increased expressions of STIM1 and Orai1 in phenotype switched ASMCs, accompanied by an enhance of SOCE. SKF-96365 could obviously block the activation of SOCE in ASMC. Meanwhile, the addition of SKF-96365 in phenotype switched ASMCs could significantly attenuate their increased proliferation ability, inflammatory cytokines secretion, and decreased contractile proteins contents induced by PDGF-BB. Moreover, we detected that PDGF-BB-induced "proliferative/synthetic" ASMCs can produce more ECM components, including collagen I, elastin and fibronectin, and metalloproteinases (MMPs) such as MMP2 and MMP9, which could be inhibited by the STIM1/Orai1 blocker SKF-96365. In vivo experiments also showed the similar results that SKF-96365 reduced the ECM deposition and MMPs production in the asthmatic mice model.

Conclusion: These observations demonstrated the prominent role of STIM1/Orai1-mediated SOCE in the phenotype modulation of ASMCs and their influence in the ASMC-induced excessive and altered ECM deposition. Therefore, our results indicated that STIM1/Orai1-mediated SOCE may take part in the airway remodeling of asthma.

1. Background

Airway remodeling has been reported to be an important feature of asthma that contributes to the clinical manifestations of the disease. In recent years, much attention has been focused on the potential role of airway smooth muscle (ASM) in the progress of airway remodeling[1]. The diverse functional and structural alterations of ASM were defined as phenotype modulation, which refers to the ability to switch from a contractile phenotype to a "proliferative/synthetic" phenotype. These cells have an increased

proliferative capacity, a diminished abundance of contractile apparatus, and a marked abundance of protein and lipid synthesis, which might cause the deterioration of airway inflammation, the narrowing of airway wall and airway remodeling[2]. However, the mechanisms underlying ASMC phenotype modulation and its effect on airway remodeling have not been fully understood.

The airways of asthmatic patients have increased amounts and altered composition of extracellular matrix (ECM) deposition, which is another hallmark characteristic of asthma[3]. Aberrant ECM deposition causes airway rigidity and narrowing, and the altered ECM protein profile, which could be regulated by matrix metalloproteinases (MMPs), may represent specific asthma endotypes and intimately correlate with asthma severity[3, 4]. The pulmonary cells, including inflammatory cells, fibroblasts, and airway epithelial cells may produce a lot of matrix proteins to accelerate ECM deposition[3, 5]. However, little information is available regarding the potential role of ASMC, especially the effect of phenotype switched cells, on exaggerated ECM deposition.

In smooth muscle, Ca^{2+} influx through both receptor-operated calcium (ROC) channels and store-operated calcium (SOC) channels is believed to provide the Ca^{2+} signals required for long-term signals, which is essential for ASMC contractility, growth, and migration[6]. The relative contribution of SOC entry (SOCE) to the control of Ca^{2+} was believed to be particularly prominent in ASM[7]. Extensive studies had defined the key aspects of SOCE including a Ca^{2+} sensor for store depletion (STIM1) and a Ca^{2+} entry pore (Orai1)[6]. Spinelli's study showed that STIM1 and Orai1 were upregulated in the ASM of asthmatic mice and mediated PDGF-BB-activated ASM proliferation and migration[8, 9]. Several other studies using ASMCs from humans or rats also showed similar results that STIM1 or STIM1/Orai1-mediated SOCE was involved in ASMC proliferation and migration[10, 11]. However, whether STIM1/Orai1-mediated SOCE was further involved in the ASMC phenotype modulation, as well as the altered ECM protein profile was still uncertain. In the current study, we explored the involvement of STIM1/Orai1 in the process of phenotype modulation of ASMC and their effect on ECM deposition.

In the present study, we prepared the "proliferative/synthetic" ASMCs with PDGF-BB treatment, and found upregulated expressions of STIM1, Orai1 and enhanced SOCE in phenotype switched ASMCs. SKF-96365, an inhibitor of STIM1/Orai1, was detected to block PDGF-BB-induced increased proliferation ability, inflammatory cytokines secretion, and reduced contractile protein profile in ASMCs, indicating the involvement of STIM1/Orai1-mediated SOCE in phenotype modulation of ASMCs. Moreover, we observed the upregulated ECM protein and MMPs in phenotype switched ASMCs, and SKF-96365 could significantly ameliorate this effect. In conclusion, our results demonstrated STIM1/Orai1-mediated SOCE was involved in the phenotype switching of ASMCs and ASMC-related ECM deposition.

2. Methods

2.1 Animals

Male C57BL/6 mice (6 weeks of age) were obtained from Zhejiang Experiment Animal Center (Hangzhou, Zhejiang, China) and housed under specific pathogen free conditions. This study was approved by the Animal Experimental Ethical Inspection of the First Affiliated Hospital, Zhejiang University School of Medicine. All mice were anesthetized with isoflurane before intranasal or intratracheal instillation and were euthanized by cervical dislocation with 10% chloral hydrate anesthesia .

2.2 Isolation and culture of ASM cell

ASMCs were obtained as previously described[12, 13]. Briefly, the mice tracheae were excised and digested with 0.2% type IV collagenase (Sigma, C5138) and 0.05% elastase (Sigma, E7885) at 37°C for 45 min. The cell suspension was filtered and centrifuged (500g, 5 min), and the pellets were resuspended and cultured with 1:1 DMEM/Ham's F12 (Biological Industries, sh00016) containing 20% FBS (Gibco, 10099141). ASMCs were identified by the typical "hill and valley" growth pattern. For the experiments, the ASMCs at passages 3–6 were used.

2.3 Cell proliferation assay

Cell proliferation was detected using the cell counting kit-8 assay according to the manufacturer's instruction (Dojindo, CK04). The optical density (OD) value was measured by a microplate reader (Molecular Devices) at a wavelength of 450 nm.

2.4 Immunocytochemistry

ASMCs were stimulated with PDGF-BB (Invitrogen, CR40) or PDGF-BB combined with SKF-96365 (selleck, S7999) for 24h. The cells were fixed, permeabilized and blocked. After that, ASMCs were immunolabeled with anti-proliferating cell nuclear antigen (PCNA) mAb (Abcam, ab18197, 1:500), and probed by biotin-conjugated secondary antibodies (Beyotime, A0297, 1:100), followed by incubation with a SABC-HRP kit (Beyotime, P0603) to enhance the immunoreactivity. Finally, ASMCs were visualized using a DAB horseradish peroxidase color development kit (Beyotime, P0203) with a microscope (Olympus IX71, ×10 objective, cellSensStandard). The percentage of $PCNA^+$ ASMCs was calculated after 500 cells were randomly counted.

2.5 Western blot assay

After treatment, whole cells lysates from ASMCs or lung homogenates were prepared and equal amounts of proteins (20 μg/lane for cells lysates, 40 μg/ml for lung homogenates) were electrophoresed by 10% SDS-PAGE before being transferred onto PVDF membranes. The membranes were blocked and probed at 4°C overnight with primary antibodies. After incubation with HRP-conjugated secondary antibodies at room temperature for 1h, the membranes were washed, and the separated protein bands were visualized by an electrochemiluminescence reagent (Fdbio, #FD8030). The data were expressed as relative values to control values.

The primary antibodies of STIM1 (PA1-46217, 1:500), Orai1 (PA5-109270, 1:500) and α-smooth muscle actin (α-SMA) (A5228, RRID AB_1087373, 1:1000) were purchased from Invitrogen. The anti-sm22α

(ab14106, 1:1000) and anti-fibronectin (ab2415, 1:1000) were purchased from Abcam. The primary antibodies of MMP9 (db1869, 1:500), MMP2 (db5134, 1:500) were purchased from Diag Biotechnology and GAPDH (60004-1-Ig, 1:10000), collagen-I (66761-1-Ig, 1:1000), smooth muscle-myosin heavy chain (sm-MHC) (21404-1-AP, 1:1000) were purchased from proteintech. The HRP-conjugated anti-mouse IgG (FDR007, 1:10000) and anti-rabbit IgG (FDR006, 1:10000) were obtained from FDbio.

2.6 Enzyme-linked immunosorbent assay

The expression levels of inflammatory cytokines in the cell supernatants were detected by the ELISA kits according to the manufacturer's protocol. The ELISA kits of IL-1β (432604), IL-6 (431304) were obtained from Biolegend and CXCL1 (70-EK296/2) was from Multi Sciences Biotech.

2.7 Intracellular Ca^{2+} measurement

To detect intracellular calcium concentration, ASMCs were incubated with 5μM Fluo-4 AM (Invitrogen, A14201) according to the manufacture's protocol. Fluorescence was examined using excitation at 494 nm and emission at 506 nm and detected with either a fluorescence inverted microscope (Olympus IX71, ×10 objective, filter for FITC) or a microplate reader (Molecular Devices). ASMCs were first incubated in zero Ca^{2+} Tyrode solution. After that, 10 μM cyclopiazonic acid (CPA, Sigma,C1530) was added to empty Ca^{2+} stores[14]. Finally, ASMCs were exposed to 2 mM Ca^{2+} to evaluate the extracellular Ca^{2+} influx by SOCE. CellSensStandard was used to capture and analyze the image.

2.8 Ovalbumin sensitization and challenge

All mice were sensitized using 100 μg ovalbumin (OVA, Sigma, A5503) in 0.1 ml alum i.p. on days 0 and 12. The experimental group was challenged with aerosolized 5% OVA for 30 min daily between days 18 and 23 and three times per week for the prolonged exposure from day 26 to 34. Mice were euthanized on day 35 represented asthma model with the characteristic of airway remodeling[12, 13]. Control group was subjected to the same protocol but received PBS instead of OVA in the challenge phase.

2.9 Treatment of model mice

The SKF96365 (200μg/mouse) was dissolved in 20ul PBS and intranasal or intratracheal instilled in the asthmatic mice model three times perweek day from day 18 to 34, 1hour before nebulization. Mice were sacrificed on day 35, and the lung homogenates were obtained for the western blot analysis and lung collagen measurement.

2.10 Measurement of collagen

Lung homogenates from mice were collected and analyzed for total lung collagen content using the Sircol Collagen Assay Kit (Biocolor, S1000) according to the manufacturer's instructions.

2.11 Statistical analysis

Statistical analysis was performed using Prism 9.00 software (GraphPad Software Inc.). All experiments were carried out in at least triplicates and all quantitative data were presented as mean ± SD. For multiple

comparisons, one-way ANOVA was employed and Tukey's HSD test was used for the post-hoc analysis. For analysis between two groups, an unpaired student's t-test was utilized. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1 PDGF-BB induces phenotype switching of ASMCs

Previous studies have revealed the role of PDGF-BB in the phenotype modulation of ASMC. Various concentrations of PDGF-BB were used to activate ASMC switching from contractile type to a more proliferative/synthetic phenotype[10, 11]. In this study, we applied PDGF-BB to prepare the proliferative/synthetic ASMC. As shown in Fig. 1A, PDGF-BB treatment for 24h accelerated cells proliferation in a dose-dependent manner. Thus, we selected the concentrations of 20 and 50 ng/ml for the following experiments.

The "proliferative/synthetic" ASMCs were assessed by the proliferation ability, inflammatory cytokines secretion and the profile of contractile protein expression. PCNA immunocytochemistry analysis revealed the increased expression of PCNA protein after PDGF-BB treatment (Fig. 1B,1C), indicating an enhanced proliferation ability of ASMCs. The contractile proteins, including α-SMA, sm22α and sm-MHC, were decreased in PDGF-BB-treated ASMCs, which was shown by western blot results (Fig. 1D ~ 1G), suggesting the decreased abundance of contractile apparatus in ASMC. For the inflammatory cytokines' secretion, the results of ELISA exhibited that the secretion of IL-1β, IL-6 and CXCL1 were up-regulated in ASMCs stimulated by PDGF-BB (Fig. 1I ~ 1K). These results confirmed that PDGF-BB induced the phenotype transition of ASMCs from a contractile to a more proliferative, synthetic and secretive type.

3.2 STIM1/Orai1 expressions and SOCE are upregulated in "proliferative/ synthetic" ASMCs

To determine whether STIM1/Orai1 were involved during ASMCs phenotype switching, we evaluated the expressions of STIM1 and Orai1 in PDGF-BB-activated ASMCs at the protein level. According to the results of western blot analysis (Fig. 2A,2B), STIM1 and Orai1 were obviously upregulated in the PDGF-BB-activated ASMCs compared to the control group.

To investigate the role of STIM1/Orai1-mediated SOCE in phenotype switched ASMCs, we used the inhibitor of sarcoplasmic reticulum Ca^{2+} ATPase (SERCA), CPA (10μM) to empty the Ca^{2+} store in the absence of external Ca^{2+} and then detect the Ca^{2+} influx right after the addition of Ca^{2+}. We found that SOCE was significantly increased after 20 and 50ng/ml PDGF-BB treatment as compared with the control group (Fig. 2C ~ 2E), which was in line with the increased expressions of STIM1/Orai1. Therefore, our results revealed the possible involvement of STIM1/Orai1-mediated SOCE during phenotype modulation of ASMC.

3.3 SOCE inhibitor SKF-96365 attenuates PDGF-BB-induced ASMC phenotype modulation

Since the possible involvement of STIM1/Orai1 in the ASMC phenotype switching, we used SKF-96365, an inhibitor of STIM1/Orai1-mediated SOCE, to further explore their effect during this process. Intracellular calcium analysis showed that STIM1/Orai1-mediated SOCE was obviously blocked by SKF-96365 in PDGF-BB-treated ASMCs (Fig. 3).

Then we investigated the effect of SKF-96365 in the phenotype switched ASMCs. As indicated in Fig. 4A, the proliferation rate of ASMCs treated with SKF-96365 was significantly lower than that of the group stimulated with PDGF-BB only. The expression of PCNA protein was also repressed with the inhibitor (Fig. 4B,4C), which was consistent with the result of the CCK-8 assay. For the contents of contractile proteins, the addition of SKF-96365 obviously increased the expressions of α-SMA, sm22 , and sm-MHC both in the PDGF 20ng/ml and 50ng/ml treatment groups (Fig. 4D ~ 4G). For the inflammatory cytokines' secretion, the synthesis of IL-1β, IL-6 and CXCL1 was also inhibited by SKF 96365 treatment (Fig. 4I ~ 4K). In conclusion, SKF-96365 obviously attenuated PDGF-BB-induced phenotype modulation of ASMC, with reduced proliferation rates, cytokines' secretions and upregulated contractile protein contents. These results indicated the prominent role of STIM1/Orai1-mediated SOCE in the phenotype transition of ASMC.

3.4 SOCE-induced ASMC phenotype switching facilitates ECM deposition

Since SOCE-induced "proliferative/synthetic" ASMC could synthesis and secret more cytokines, we further explored its role in the synthesis of excessive ECM. According to the results of western blot staining, the phenotype switched ASMC produced increased levels of collagen I and fibronectin, which could be inhibited by SKF-96365 treatment. Moreover, the expressions of matrix metalloproteinases, including MMP2 and MMP9, were also elevated in PDGF-BB-activated ASMCs and their contents were obviously attenuated by SKF-96365 (Fig. 5). Our results demonstrated that the "proliferative/synthetic" type ASMC might have a role in the production of excessive and altered ECM deposition, and STIM1/Orai1-mediated SOCE was involved in this process.

In order to further confirm the effect of SKF-96365 on ECM deposition, in vivo experiments were performed by administering SKF-96365 in an asthmatic mice model. Our previous study has demonstrated that prolonged OVA exposure could induce the asthmatic mice model with the characteristics of airway remodeling including ASM remodeling and ECM deposition[12, 13]. We prepared the prolonged OVA challegend mice model, and some of them were intranasal or intratracheal instilled with SKF-96365. Control group were challegened with PBS. The results of western blot analysis showed that prolonged OVA challegen induced the elevated expressions of collagen-I and fibronectin, and both intranasal and intratracheal exposure to SKF-96365 could reduce their expressions obviously (Fig. 6A-C). The analysis of the total lung collagen content also revealed the similar results that SKF-96365

abrogated the increased levels of lung collagen deposition induced by OVA exposure (Fig. 6F). The increased levels of MMP2 and MMP9 induced by OVA challenge were also significantly attenuated by SKF-96365 instillation (Fig. 6A,D,E). In conclusion, the in vivo experiments also detected the inhibitory effect of SKF-96365 on the expression of ECM and MMPs, which were consistent with the results of cell experiments.

4. Discussion

STIM1/Orai1, as the molecular components of the SOCE pathway in smooth muscle cells, has been demonstrated to contribute to the phenotypic regulation of vascular smooth muscle and vascular remodeling[15, 16]. Potier et al prepared the “synthetic type” aorta smooth muscle cells and showed upregulated STIM1 and Orai1 expressions in these cells. Knockdown of STIM1/Orai1 inhibited the proliferation and migration of these cells[17]. After that, More studies showed the involvement of that STIM1/Orai1 in the proliferation of vascular smooth muscle cells(VSMC) as well as the neointimal formation, indicating the promising role of STIM1/Orai1 in the phenotype modulation of VSMC[18, 19]. In our study, we detected similar effects of STIM1/Orai1 in ASMC. Our results showed that the expressions of STIM1/Orai1 and their induced SOCE were enhanced in “proliferative/ synthetic” type ASMC and the inhibition of STIM1/Orai1 obviously attenuated the proliferation of ASMC. Moreover, we further showed that the blockage of STIM1/Orai1 could attenuate the reduced contractile apparatus expressions induced by PDGF treatment in ASMCs. Although the effect of STIM1/Orai1-mediated SOCE in the contraction of ASMC has been fully elucidated, little literature was focused on the effect of SOCE in the expression of contractile protein profile. Imoto K et al’s study regarding pulmonary hypertesion showed an inverse correlation between the expression of -SMA and STIM1 in fibroblasts[20]. In our study, the blockage of SOCE obviously upregulated the expressions of multiple contractile proteins including -SMA, sm-MHC and sm22 , indicating the prominent role of STIM1/Orai1-mediated SOCE in ASMC switching from contractile type to a more proliferative type.

Besides the proliferation and increased ASM mass in the asthmatic airway, ASM plays a pivotal role in airway inflammation, the characteristic of asthma as well[21]. Our results showed that the phenotypic switched ASMCs could express several pro-inflammatory cytokines, including IL-1β, IL-6, CXCL1. IL-1β and IL-6 are not only well-recognized inducers of airway inflammation, including neutrophilic lung inflammation and Th17 immunological inflammation, but also contribute to airway remodeling, such as collagen repair and mucus hypersecretion[22–24]. Mouse CXCL1 corresponds to human IL-8 that could recruit an abundance of inflammatory cells to drive airway inflammation[25]. Moreover, these cytokines might be the inducer to promote lots of pro-inflammatory and pro-fibrotic cytokines secretion including IL-33, TSLP, CCL5 and so on[26], which could further accelerate the airway remodeling of asthma. In our study, we observed that the production of these inflammatory cytokines in phenotype switched ASMCs could partly mediated by STIM1/Orai1-related SOCE. In conclusion, our work provides evidence that SOCE was involved in the ASMC-induced pro-inflammatory cytokines’ secretion.

Besides, the present study further showed the involvement of SOCE in ASM-related ECM deposition. Although there were several reports regarding cardiac fibrosis or heart failure showed the increased SOCE contributed to the collagen-rich ECM deposition in heart[27–29], few studies investigated its effect in the ECM deposition in asthma. Our study demonstrated that STIM1/Orai1-activated SOCE could accelerate the ECM and MMPs production from ASMCs both in vitro and in vivo, suggesting the possible involvement of Ca^{2+} influx of ASMC in the excessive and altered ECM deposition.

The interplay between ASM mass and ECM has been described by many studies. Previous studies of airways from asthmatic patients showed altered ECM proteins profile with enhanced collagen I and fibronectin[3]. This altered profile of ECM proteins has been shown to play a role in the phenotype plasticity of asthmatic ASMCs[3, 30, 31]. Extensive studies have demonstrated that the exposure of ASM to fibronectin and collagen I altered the proliferation, survival and inflammatory mediators' release from ASMCs[32]. However, most of the studies have focused on the effect of the ECM components on the function of ASMCs. In our study, we showed that the phenotype switched ASMCs could synthesis and secrete more collagen I and fibronectin which may further aggravate the ECM deposition. In addition, increased expression of MMP2 and MMP9 from synthetic ASMC were also detected, which may take part in the matrix turnover[32–34]. Given the degrative ability of MMPs and the specific type of ECM produced by ASMC, we believed an intimate interdependence between ASM mass and ECM deposition, which combined to make the deterioration of airway remodeling.

5. Conclusions

In summary, we demonstrated that STIM1/Orai1-mediated SOCE may play a role in the PDGF-BB-induced phenotype modulation of ASMCs, which presented as more proliferation, synthesis cells with less contractile protein contents. Moreover, the phenotype switched ASM could produce excessive ECM and matrix metalloproteinase, indicating the important role in the ECM deposition. Therefore, our findings provide a more comprehensive insight into the involvement of STIM1/Orai1-induced SOCE not only in the phenotype transition of ASMC, but also in the aggravated ECM deposition.

Abbreviations

ASM airway smooth muscle

ASMC airway smooth muscle cells

α-SMA α-smooth muscle actin

CPA cyclopiazonic acid

ECM extracellular matrix

MMP metalloproteinase

OD optical density

OVA ovalbumin

PCNA proliferating cell nuclear antigen

ROC receptor-operated calcium

SERCA sarcoplasmic reticulum Ca^{2+} ATPase

sm-MHC smooth muscle-myosin heavy chain

SOC store-operated calcium

SOCE store-operated Ca^{2+} entry

VSMC vescular smooth muscle cells

Declarations

Acknowledgements

We thank Dr. Guohua Lu for his excellent technical support and valuable contributions to the interpretation of this study.

Availability of data and materials The data that support the findings of this study are available in figshare at http://doi.org/10.6084/m9.figshare.24649200

Conflict-of-Interest Statement The authors declare no conflicts of interest.

Funding: This research was funded by the National Natural Science Foundation of China (81870018)

Author's Contribution: Wang Q designed and supervised the study. Ni H, Li T and Wei Y conducted the experiments and collected the data. Chen J and Xia M analyzed and interpreted the data. Ni H and Wei Y drafted the manuscript. All the authors made revisions of the article and final approval of the version submitted. All the authors were agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated.

References

1. Camoretti-Mercado B, Lockey RF (2021) Airway smooth muscle pathophysiology in asthma. J Allergy Clin Immunol 147(6):1983–1995
2. Wright DB, Trian T, Siddiqui S, Pascoe CD, Johnson JR, Dekkers BG et al (2013) Phenotype modulation of airway smooth muscle in asthma. Pulm Pharmacol Ther 26(1):42–49

3. Hough KP, Curtiss ML, Blain TJ, Liu RM, Trevor J, Deshane JS et al (2020) Airway remodeling in asthma. Front Med (Lausanne) 7:191
4. Bajbouj K, Ramakrishnan RK, Hamid Q (2021) Role of matrix metalloproteinases in angiogenesis and its implications in asthma. J Immunol Res 2021:6645072
5. Michalik M, Wójcik-Pszczoła K, Paw M, Wnuk D, Koczurkiewicz P, Sanak M et al (2018) Fibroblast-to-myofibroblast transition in bronchial asthma. Cell Mol Life Sci 75(21):3943–3961
6. Liu B, Peel SE, Fox J, Hall IP (2010) Reverse mode Na+/Ca2 + exchange mediated by STIM1 contributes to Ca2 + influx in airway smooth muscle following agonist stimulation. Respir Res 11(1):168
7. Chen J, Sanderson MJ (2017) Store-operated calcium entry is required for sustained contraction and Ca(2+) oscillations of airway smooth muscle. J Physiol 595(10):3203–3218
8. Spinelli AM, González-Cobos JC, Zhang X, Motiani RK, Rowan S, Zhang W et al (2012) Airway smooth muscle STIM1 and Orai1 are upregulated in asthmatic mice and mediate PDGF-activated SOCE, CRAC currents, proliferation, and migration. Pflugers Arch 464(5):481–492
9. Spinelli AM, Trebak M (2016) Orai channel-mediated Ca2 + signals in vascular and airway smooth muscle. Am J Physiol Cell Physiol 310(6):C402–413
10. Suganuma N, Ito S, Aso H, Kondo M, Sato M, Sokabe M et al (2012) STIM1 regulates platelet-derived growth factor-induced migration and Ca2 + influx in human airway smooth muscle cells. PLoS ONE 7(9):e45056
11. Zou JJ, Gao YD, Geng S, Yang J (2011) Role of STIM1/Orai1-mediated store-operated Ca2 + entry in airway smooth muscle cell proliferation. J Appl Physiol (1985) 110(5):1256–1263
12. Wang Q, Li H, Yao Y, Lu G, Wang Y, Xia D et al (2016) HB-EGF-promoted airway smooth muscle cells and their progenitor migration contribute to airway smooth muscle remodeling in asthmatic mouse. J Immunol 196(5):2361–2367
13. Wang Q, Li H, Yao Y, Xia D, Zhou J (2010) The overexpression of heparin -binding epidermal growth factor is responsible for Th17-induced airway remodeling in an experimental asthma model. J Immunol 185(2):834–841
14. Sathish V, Abcejo AJ, Thompson MA, Sieck GC, Prakash YS, Pabelick CM (2012) Caveolin-1 regulation of store-operated Ca(2+) influx in human airway smooth muscle. Eur Respir J 40(2):470–478
15. Avila-Medina J, Mayoral-Gonzalez I, Dominguez-Rodriguez A, Gallardo-Castillo I, Ribas J, Ordoñez A et al (2018) The complex role of store operated calcium entry pathways and related proteins in the function of cardiac, skeletal and vascular smooth muscle cells. Front Physiol 9:257
16. Johnson MT, Gudlur A, Zhang X, Xin P, Emrich SM, Yoast RE et al (2020) L-type Ca(2+) channel blockers promote vascular remodeling through activation of STIM proteins. Proc Natl Acad Sci U S A 117(29):17369–17380
17. Potier M, Gonzalez JC, Motiani RK, Abdullaev IF, Bisaillon JM, Singer HA et al (2009) Evidence for STIM1- and Orai1-dependent store-operated calcium influx through ICRAC in vascular smooth

muscle cells: role in proliferation and migration. FASEB J 23(8):2425–2437

18. Huang Z, Li P, Wu L, Zhang D, Du B, Liang C et al (2020) Hsa_circ_0029589 knockdown inhibits the proliferation, migration and invasion of vascular smooth muscle cells via regulating miR-214-3p and STIM1. Life Sci 259:118251
19. Liu B, Zhang B, Roos CM, Zeng W, Zhang H, Guo R (2020) Upregulation of Orai1 and increased calcium entry contribute to angiotensin II-induced human coronary smooth muscle cell proliferation: Running title: Angiotensin II-induced human coronary smooth muscle cells proliferation. Peptides 133:170386
20. Imoto K, Okada M, Yamawaki H (2018) Characterization of fibroblasts from hypertrophied right ventricle of pulmonary hypertensive rats. Pflugers Arch 470:1405–1417
21. Doeing DC, Solway J (2013) Airway smooth muscle in the pathophysiology and treatment of asthma. J Appl Physiol (1985) 114(7):834–843
22. Rincon M, Irvin CG (2012) Role of IL-6 in asthma and other inflammatory pulmonary diseases. Int J Biol Sci 8(9):1281–1290
23. Xue Y, Zhou Y, Bao W, Fu Q, Hao H, Han L et al (2021) STAT3 and IL-6 Contribute to Corticosteroid Resistance in an OVA and Ozone-induced Asthma Model with Neutrophil Infiltration. Front Mol Biosci 8:717962
24. Osei ET, Brandsma CA, Timens W, Heijink IH, Hackett TL (2020) Current perspectives on the role of interleukin-1 signalling in the pathogenesis of asthma and COPD. Eur Respir J 55(2):1900563
25. Chenuet P, Fauconnier L, Madouri F, Marchiol T, Rouxel N, Ledru A et al (2017) Neutralization of either IL-17A or IL-17F is sufficient to inhibit house dust mite induced allergic asthma in mice. Clin Sci (Lond) 131(20):2533–2548
26. Mahmutovic Persson I, Menzel M, Ramu S, Cerps S, Akbarshahi H, Uller L (2018) IL-1beta mediates lung neutrophilia and IL-33 expression in a mouse model of viral-induced asthma exacerbation. Respir Res 19(1):16
27. Zhang B, Jiang J, Yue Z, Liu S, Ma Y, Yu N et al (2016) Store-Operated Ca(2+) Entry (SOCE) contributes to angiotensin II-induced cardiac fibrosis in cardiac fibroblasts. J Pharmacol Sci 132:171–180
28. Ross GR, Bajwa T Jr, Edwards S, Emelyanova L, Rizvi F, Holmuhamedov EL et al (2017) Enhanced store-operated Ca2 + influx and ORAI1 expression in ventricular fibroblasts from human failing heart. Biol Open 6(3):326–332
29. Chen PH, Chung CC, Lin YF, Kao YH, Chen YJ (2021) Lithium reduces migration and collagen synthesis activity in human cardiac fibroblasts by inhibiting Store-Operated Ca(2+) Entry. Int J Mol Sci 22(2):842
30. Polio SR, Stasiak SE, Jamieson RR, Balestrini JL, Krishnan R, Parameswaran H (2019) Extracellular matrix stiffness regulates human airway smooth muscle contraction by altering the cell-cell coupling. Sci Rep 9(1):9564

31. Liu G, Cooley MA, Nair PM, Donovan C, Hsu AC, Jarnicki AG et al (2017) Airway remodelling and inflammation in asthma are dependent on the extracellular matrix protein fibulin-1c. J Pathol 243(4):510–523
32. Burgess JK (2009) The role of the extracellular matrix and specific growth factors in the regulation of inflammation and remodelling in asthma. Pharmacol Ther 122(1):19–29
33. Vieira CP, de Oliveira LP, Da Silva MB, Majolli Andre D, Tavares EBG, Pimentel ER et al (2020) Role of metalloproteinases and TNF-α in obesity-associated asthma in mice. Life Sci 259:118191
34. Freeman MR, Sathish V, Manlove L, Wang S, Britt RD Jr, Thompson MA et al (2017) Brain-derived neurotrophic factor and airway fibrosis in asthma. Am J Physiol Lung Cell Mol Physiol 313(2):L360–L370

Figures

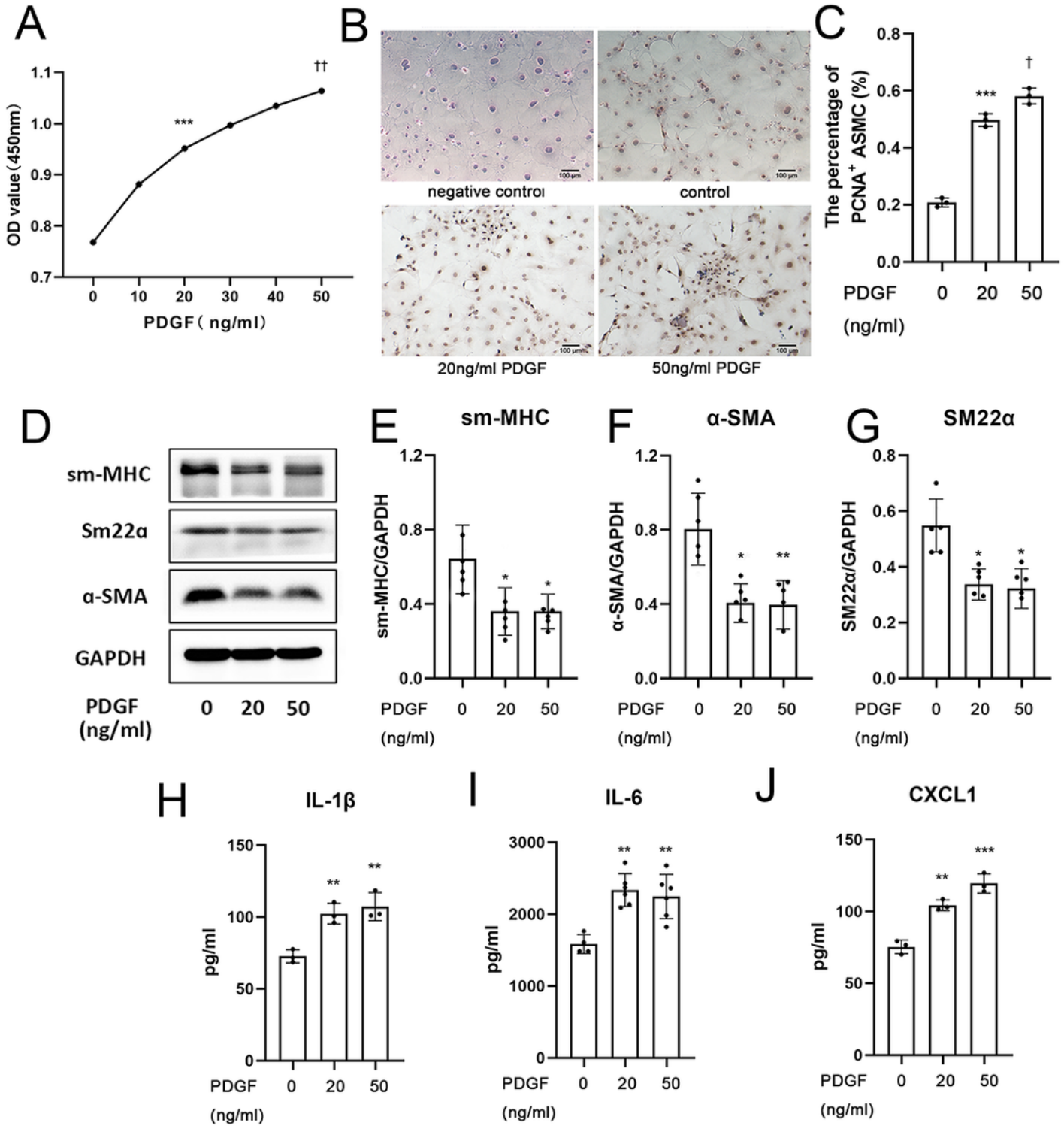

Figure 1

PDGF-BB induces phenotype switching of ASMCs

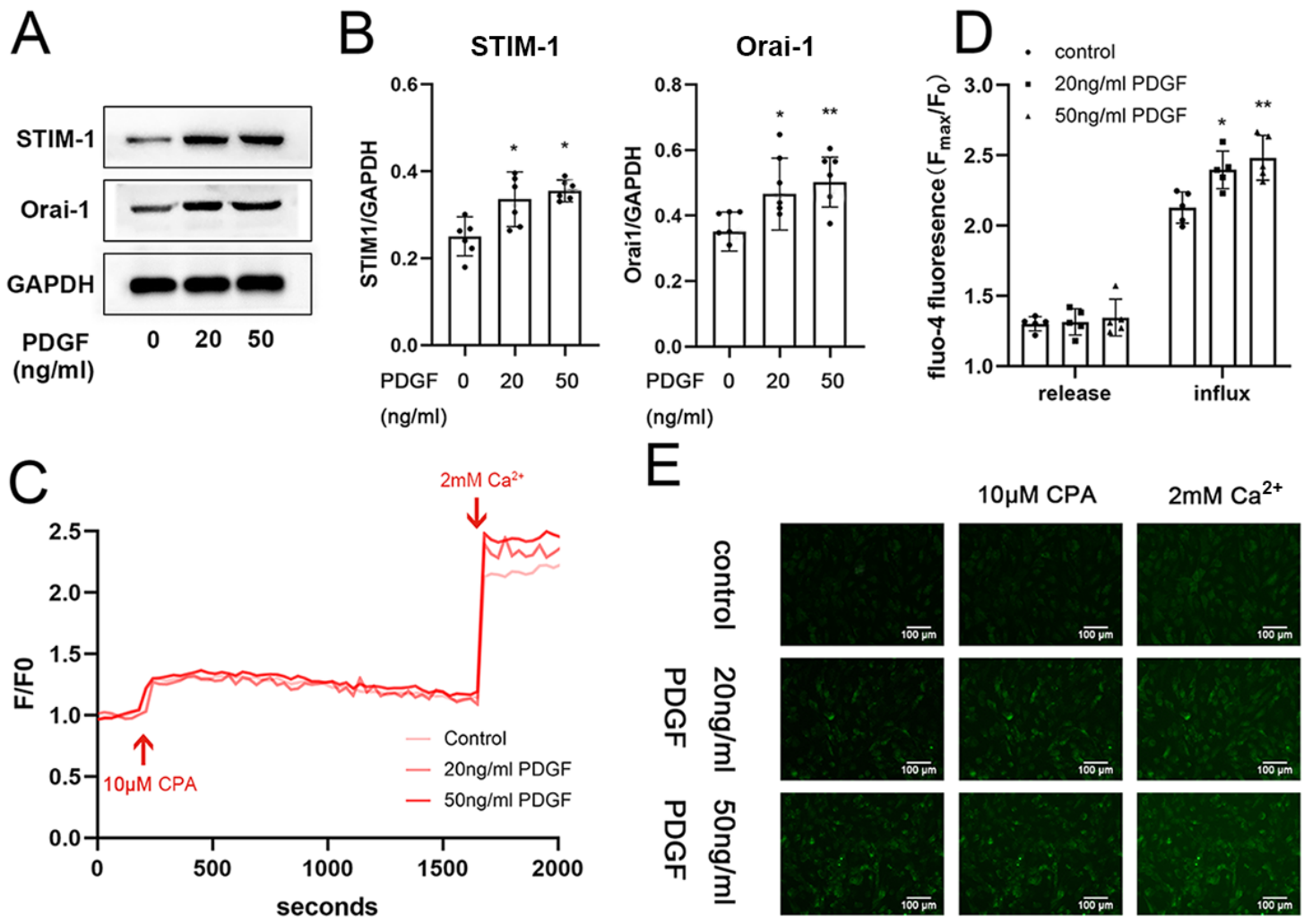

Figure 2

STIM1/Orai1 expressions and SOCE are upregulated in “proliferative/ synthetic” ASMCs

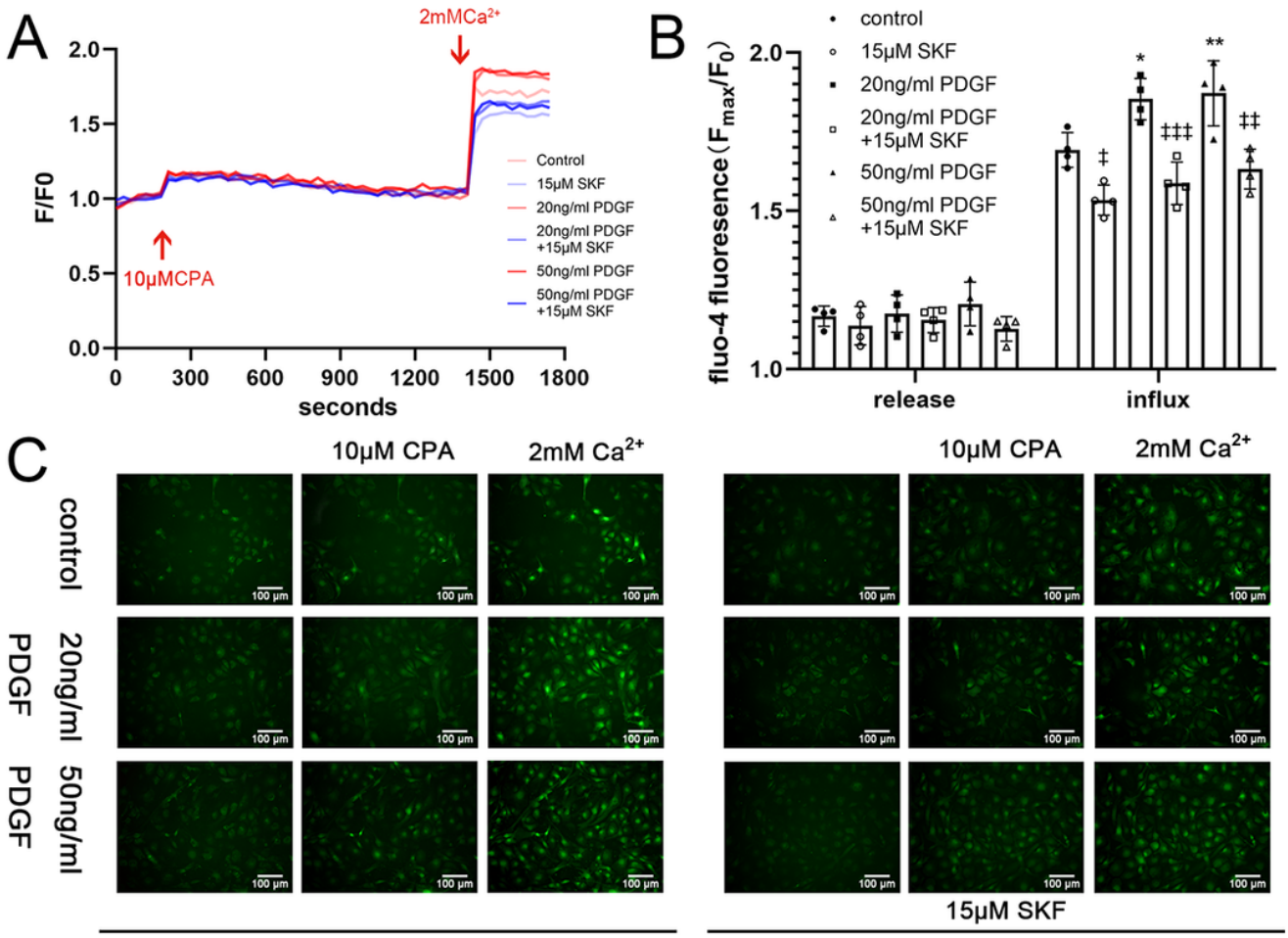

Figure 3

SKF-96365 ameliorates enhanced SOCE induced by PDGF-BB

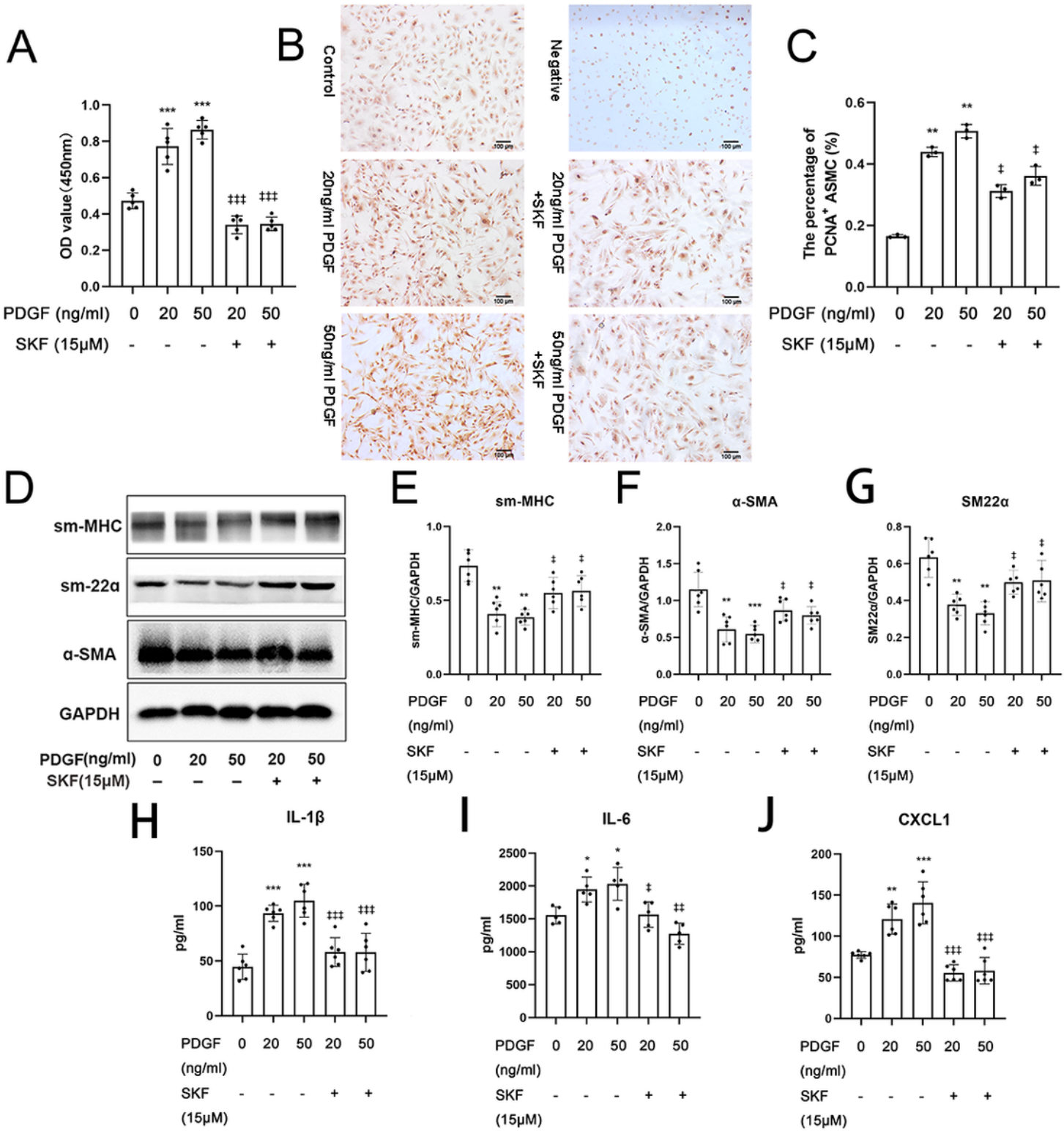

Figure 4

SKF-96365 attenuates PDGF-BB-induced ASMC phenotype modulation

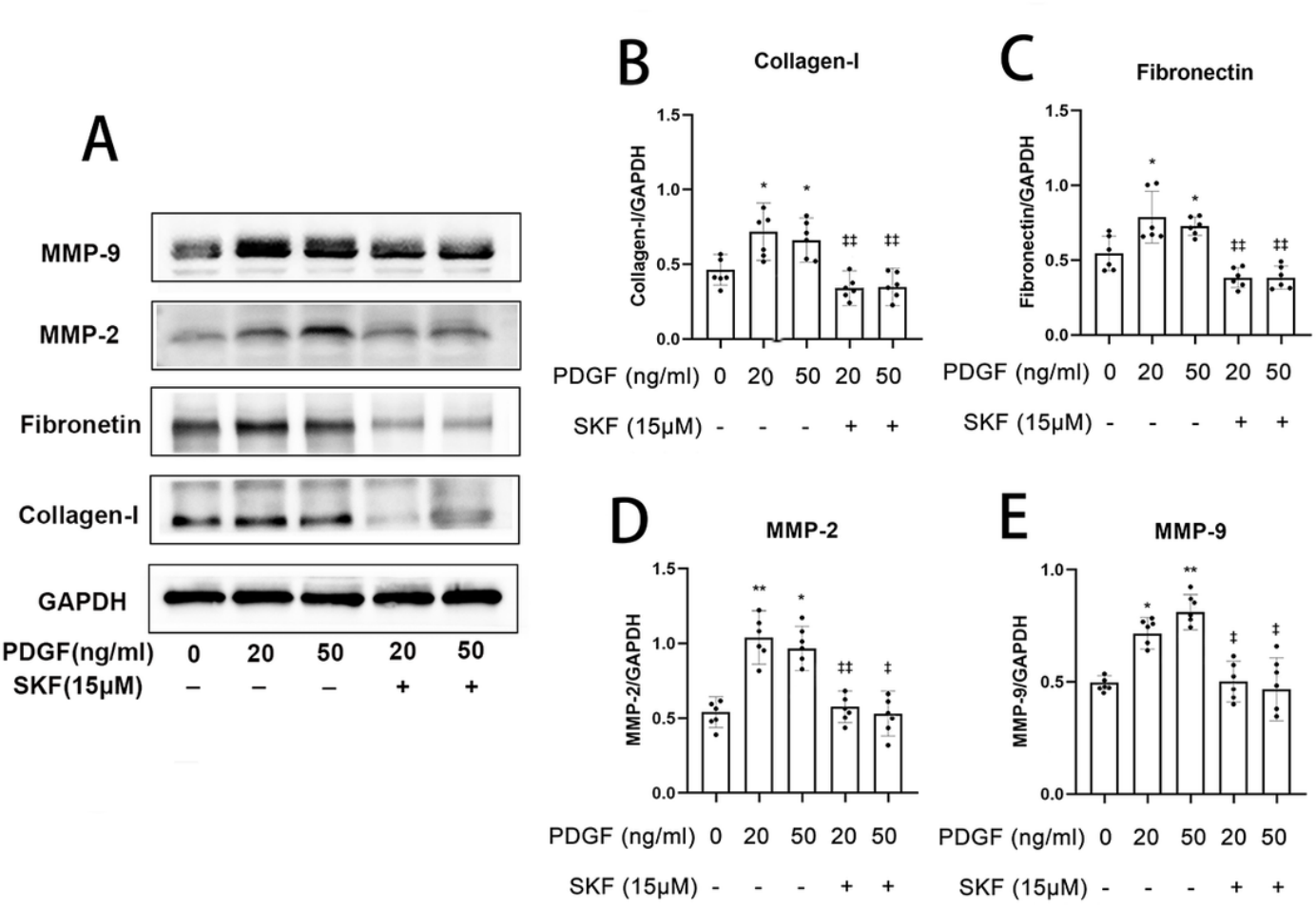

Figure 5

SOCE-induced ASMC phenotype switching facilitates ECM deposition

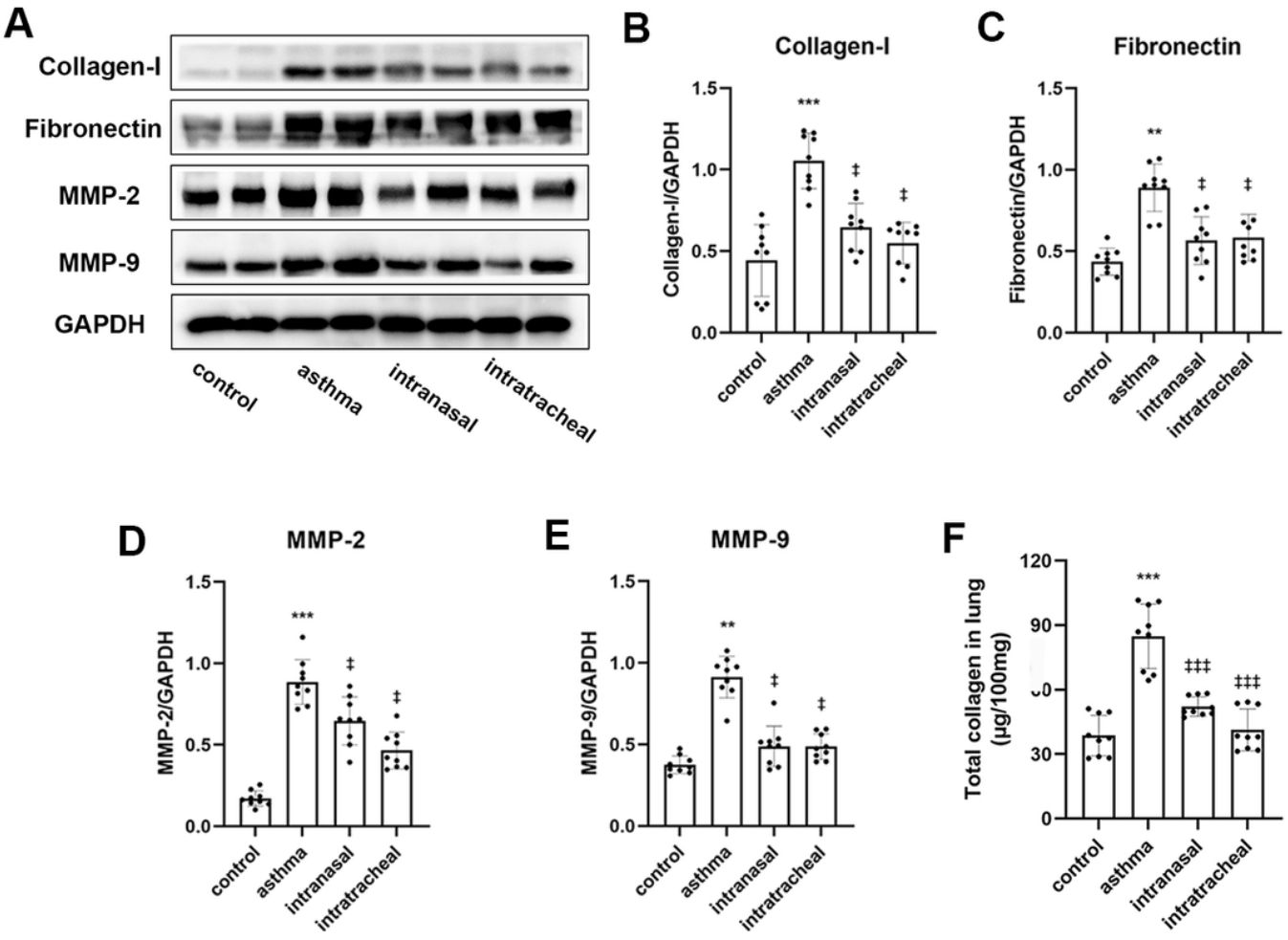

Figure 6

SKF-96365 attenuates prolonged OVA-induced ECM deposition in asthmatic mice