HHS Public Access

Author manuscript

J Respir Biol Transl Med. Author manuscript; available in PMC 2024 June 01.

Published in final edited form as:

J Respir Biol Transl Med. 2024 June ; 1(2): . doi:10.35534/jrbtm.2024.10006.

Arrestin beta 1 Regulates Alveolar Progenitor Renewal and Lung Fibrosis

Guanling Huang[1,2], **Yan Geng**[1,3], **Vrishika Kulur**[1], **Ningshan Liu**[1], **Xue Liu**[1], **Forough Taghavifar**[1], **Jiurong Liang**[1], **Paul W. Noble**[1,*], **Dianhua Jiang**[1,4,*]

[1]Division of Pulmonary, Women's Guild Lung Institute, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA;

[2]Current Address: GH, Sanofi, 500 Kendall Street, Cambridge, MA 02142, USA

[3]Current Address: YG, Jiangnan University, 1800 Lihu Avenue, Wuxi, Jiangsu, 214122, China

[4]Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA

Abstract

The molecular mechanisms that regulate progressive pulmonary fibrosis remain poorly understood. Type 2 alveolar epithelial cells (AEC2s) function as adult stem cells in the lung. We previously showed that there is a loss of AEC2s and a failure of AEC2 renewal in the lungs of idiopathic pulmonary fibrosis (IPF) patients. We also reported that beta-arrestins are the key regulators of fibroblast invasion, and beta-arrestin 1 and 2 deficient mice exhibit decreased mortality, decreased matrix deposition, and increased lung function in bleomycin-induced lung fibrosis. However, the role of beta-arrestins in AEC2 regeneration is unclear. In this study, we investigated the role and mechanism of Arrestin beta 1 (ARRB1) in AEC2 renewal and in lung fibrosis. We used conventional deletion as well as cell type-specific deletion of *ARRB1* in mice and found that *Arrb1* deficiency in fibroblasts protects mice from lung fibrosis, and the knockout mice exhibit enhanced AEC2 regeneration in vivo, suggesting a role of fibroblast-derived ARRB1 in AEC2 renewal. We further found that *Arrb1*-deficient fibroblasts promotes AEC2 renewal in 3D organoid assays. Mechanistically, we found that CCL7 is among the top downregulated cytokines in *Arrb1* deficient fibroblasts and CCL7 inhibits AEC2 regeneration in 3D organoid experiments. Therefore, fibroblast ARRB1 mediates AEC2 renewal, possibly by releasing chemokine CCL7, leading to fibrosis in the lung.

*Correspondence authors. paul.noble@cshs.org (P.W.N.); dianhua.jiang@cshs.org (D.J.).

Author Contributions

J.L., P.W.N., and D.J. conceived the study. G.H., Y.G. designed, performed experiments, and analyzed data. V.K., F.T., and N.L. performed experiments. X.L. performed data analysis and interpretion. G.H., J.L., P.W.N., and D.J. wrote the paper.

Ethics Statement

The mouse studies were approved by the Institutional Animal Care and Use Committee at Cedars-Sinai Medical Center (IACUC008529).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Keywords

Lung; Fibrosis; IPF; Arrb1; AEC2; Alveolar stem cell; CCL7; Stem cell

1. Introduction

The molecular mechanisms that regulate progressive tissue fibrosis remain poorly understood. Although the causes of pulmonary fibrosis remain largely unknown, it is believed that progressive pulmonary fibrosis is an epithelial-fibroblastic disorder that results from numerous microinjuries to the alveolar epithelia that lead to excessive fibroblast activation, deposition of extracellular matrix, and eventually loss of normal lung architecture [1]. Lung fibrosis is regulated by a variety of processes including TGFbeta signaling [2,3], metabolism [4–6], non-coding RNAs [7–9], epigenetic changes [10], as well as aging [11].

In the lung, type 2 alveolar epithelial cells (AEC2s) function as adult stem cells [12]. Our previous work showed that there is a loss of AEC2s [13,14] and a failure of AEC2 renewal in the lungs of idiopathic pulmonary fibrosis (IPF) patients [13]. We and others have showed that AEC2 renewal is regulated by several pathways [13,15–19] and metabolic reprogramming [6,20]. Furthermore, AEC2 stem cell activity is also regulated by the AEC2 niche [18,21–23], including basal-like cells [24], fibroblasts [18,25,26], endothelial cells [20,27,28], macrophages [17,29], and T cells [30], through various mechanisms such as extracellular vesicle trafficking [18], cytokine release [18,24], or cell–cell interactions.

Beta-arrestins are classically known to participate in agonist-mediated G-protein-coupled receptor (GPCR) signaling through receptor desensitization and internalization [31–33], playing a wide range of roles in development, cancer, asthma [34,35], and tissue fibrosis [36]. We previously reported that beta-arrestin 1 and 2 deficient mice exhibit decreased mortality, decreased matrix deposition, and increased lung function in a model of bleomycin-induced lung fibrosis [37], and serve as the key regulators of fibroblast invasion [37]. However, the role of beta-arrestins in AEC2 regeneration is unclear.

In this study, we investigated the role of Arrestin beta 1 (ARRB1) [38] in AEC2 renewal and in lung fibrosis. We investigated the role of ARRB1 in regulating AEC2 regeneration using conventional deletion as well as cell type-specific deletion of ARRB1 in mice with a lung fibrosis mouse model. We found that targeted deletion of ARRB1 in fibroblasts promoted AEC2 regeneration and ameliorated lung fibrosis.

2. Materials and Methods

2.1. Mice

Arrb1$^{-/-}$ mice (Strain #: 011131, full name B6.129X1(Cg)-*Arrb1*^{tm1Jse}/J) [39] and wild type (WT) C57BL/6J were purchased from The Jackson Laboratory. *Arrb1*$^{-/-}$ mice were crossbred onto C57BL/6J background for more than five generations. *Arrb1*$^{flox/flox}$ mice [37] were from R. Lefkowitz of Duke University. Col1a2-Cre mice were from Dr. P. Angel of German Cancer Research Center (Das Deutsche Krebsforschungszentrum, DKFZ) [40]. SPC-Cre mice were described previously [41]. All mice were housed in a pathogen-

free facility at Cedars-Sinai Medical Center. All animal experiments were approved by the Institutional Animal Care and Use Committee at Cedars-Sinai Medical Center (IACUC008529).

2.2. Bleomycin-induce Lung Injury and Fibrosis in Mice

Bleomycin-induce lung injury and fibrosis in age matched 8- to 12-week mice was described previously [13]. Under anesthesia, the trachea was surgically exposed. 1.25 U/kg bleomycin (Hospira, Lake Forest, IL, USA) in 25 mL PBS was instilled into the mouse trachea with a 25-G needle inserted between the cartilaginous rings of the trachea. On day 0, day 7, and day 14, mice were sacrificed and lung tissues were collected for experiments.

2.3. Mouse Lung Tissue Digestion

Mouse lung tissue digestion was described previously [6,12,13,42]. In brief, mouse lungs were first digested with 4 U/mL Elastase in DMEM/F12 for 15 min. Then tissues were chopped into small pieces, and digested further with 100 mg/mL DNase I in DMEM/F12 for 15 min. Digestion was terminated with DMEM/F12 medium. Single cells were filtered through 100 mm filter, spined down, and resuspend with 700 mL $HBSS^+$ buffer for downstream experiments.

2.4. Flow Cytometry

Flow cytometrical analysis was described previously [6,13]. Mouse lung single cell suspension was stained with fixable viability dye, EPCAM, CD45, CD31, CD34, $PDGFRa^+$, Sca-1 and CD24. AEC2s were gated as live $EPCAM^+CD45^-CD31^-CD34^-Sca\text{-}1^-CD24^-$ cells, $PDGFRa^+$ fibroblasts were gated as live $EPCAM^-CD45^-CD31^-CD34^-$ $PDGFRa^+$ cells. Primary antibodies Percp-Cy5.5-EPCAM (clone G8.8, catalog# 118220), APC-Cy7-CD45 (clone 30-F11, catalog# 103116), APC-Cy7-CD31 (clone MEC13.3, catalog# 102534), APC-Cy7-CD34 (clone HM34, catalog# 128622), FITC-CD24 (clone M1/69, catalog# 101806), PE-Cy7-Sca-1 (clone D7, catalog# 108114) and APC-$PDGFRa^+$ (clone APA5, catalog# 135908) were from BioLegend (San Diego, CA, USA). Fixable viability dye (catalog# 65-0865-14) was from Thermo Fisher (Waltham, MA, USA). Stained cells were sorted on Aria III (BD Immunocytometry Systems; Franklin Lakes, NJ, USA) or analyzed on Fortessa (BD Immunocytometry Systems) and the data were analyzed with FlowJo software.

2.5. Cell Cycle Analysis

Surface marker-stained single cell suspension was fixed with Foxp3/Transcription Factor Staining Buffer Set (catalog# 00-5523-00, Thermo Fisher) for 1 h, then stained with conjugated APC-Ki-67 antibody (clone 16A8, catalog 652406, BioLegend) for another hour, then cells were washed, resuspended, and analyzed on Fortessa and the data were analyzed with FlowJo software.

2.6. RNA Isolation and Analysis

RNA isolation and analysis was described previously [6,24]. Briefly, 500 mL Trizol reagent (catalog# 15596026, Thermo Fisher) was added to freshly isolated $PDGFRa^+$ fibroblasts,

and incubated at room temperature for 5 min. 100 mL chloroform solution was added and incubated for 3 min. 250 mL isopropanol was then added and incubated for another 10 min. The mixture was then centrifuged for 10 min at 13,500 rpm, and supernatant was discarded, pellets were washed with 500 mL of 75% ethanol, followed by centrifuging for 5 min at 7500 *g* at 4 °C. After removal of the supernatant, RNA was air dried for 10 min, resuspend in 30 mL RNase-free water. Total RNAs were sent for RNA-Seq and reverse-transcribed into cDNA for real-time PCR.

2.7. Immunofluorescence

Mouse lung tissues were fixed with 10% formalin solution and embedded in paraffin. The tissues were sectioned, and the slices were blocked with 5% goat serum, stained with primary antibodies against pro-SPC (catalog# ab170699, Abcam; Boston, MA, USA) overnight at 4 °C. On the next day, the samples were washed with PBS+0.01% Tween 20 buffer and stained with secondary antibody. Results were visualized with Zeiss LSM 780 confocal microscope (Carl Zeiss AG, Oberkochen, Germany).

2.8. Hydroxyproline Assay

Hydroxyproline assay to determine collagen contents in mouse lungs was described previously [6,24]. In brief, lung tissues were heat-dried, minced, and hydrolyzed overnight. The other day, pH was adjusted and samples were diluted with PBS. Then, samples were incubated with Chloramine T solution, perchloric acid and P-DMAB solution. Finally, samples were read under 557 nm wavelength. The ability of the assay to completely hydrolyze and recover hydroxyproline from collagen was confirmed using samples containing known amounts of purified collagen.

2.9. 3D Organoid Cultures of AEC2s

AEC2 organoid assays were described previously [6,12,13,24,42]. Col1a2-Cre;*Arrb1*$^{fl/fl}$ and *Arrb1*$^{fl/fl}$ mice were treated with 1.25 U/kg bleomycin. 14 days after treatment, 1×10^5 fresh isolated lung PDGFRa$^+$ fibroblasts were mixed with 3000 flow-sorted WT AEC2 cells, resuspended in 100 μL matrigel/medium (1:1) mixture (catalog# 356252, Corning Inc.; Corning, NY, USA) and plated into 24-well transwell insert, and cultured for another 14 days. For colony-formation assay with CCL7, WT AEC2 cells were pre-treated with 10 ng/mL recombinant CCL7 protein (catalog# 250–08, Peprotech; Cranbury, NJ, USA) at 37 °C for 30 min, then mixed with mlg2908 fibroblasts. 400 μL medium was added to the lower chamber of the insert with a half medium change every other day. In the treatment group, 10 ng/mL CCL7 was also added to lower chamber medium. 14 days after culturing, colonies were counted and colony-formation efficiency was calculated.

2.10. Statistics

Results were shown as mean ± SD, statistical difference was calculated with Prism 8 (GraphPad Software, San Diego, CA, USA). Student's two-tailed *t* test was used for two-group comparisons. Results were considered statistically significant at $p < 0.05$.

2.11. Data and Material Availability

The data used in this paper can be accessed via GSE47460 [43] and GSE48455 [44]. Raw data of RNA-seq is available upon request. Further information and requests for resources and reagents should be directed to D. Jiang (Dianhua.Jiang@cshs.org).

3. Results

3.1. Arrb1 Deficiency in Fibroblasts Protects Mice from Lung Fibrosis

Our previous data showed that mice with conventional deletion of *Arrb1* were protected from bleomycin-induced injury and fibrosis [37]. To study the cell type responsible for this protective role, we analyzed *Arrb1* expression in different cell types in the lung. Results showed that *Arrb1* transcripts were highly expressed in lung fibroblasts, especially PDGFRa^{+} fibroblasts (Figure 1A). We further generated a mouse strain with lung fibroblast-specific deficiency of *Arrb1*. When challenged with bleomycin, mice with lung fibroblast-specific deficiency of *Arrb1* showed to a decrease in hydroxyproline content in the lungs compared to littermate control mice (Figure 1B), and reduced lung fibrosis was confirmed with Trichrome staining (Figure 1C). In contrast, deletion of Arrb1 in SPC^{+} AEC2 compartment did not affect fibrosis when challenged with bleomycin (Figure 1D). These data demonstrate a detrimental role of ARRB1 in fibroblasts in bleomycin-induced lung fibrosis.

3.2. Enhanced AEC2 Renewal in Mouse Lungs with Arrb1 Deficiency in Fibroblasts In Vivo

The mounting evidence indicates that the stem cell niche plays a crucial role in governing the regeneration of stem cells [45]. We recently showed that injured fibroblasts were less supportive role of AEC2 renewal [18]. We thus investigated the potential impact of *Arrb1* deletion in fibroblasts on the regeneration of AEC2. We found that mouse lungs with fibroblast-specific *Arrb1* deficiency have a higher percentage (Figure 2A) and number (Figure 2B) of AEC2 cells after bleomycin treatment. In contrast, deletion of *Arrb1* in SPC^{+} AEC2 compartment did not affect the percentage and number of AEC2 cells after bleomycin treatment (Figure 2C,D). These data indicated that deficiency of *Arrb1* in fibroblasts may promote AEC2 regeneration after injury. The results were further confirmed with immunofluorescence staining of pro-SPC (Figure 2E). These data showed that *Arrb1* deficiency in fibroblasts leads to enhanced recovery of AEC2 cells after lung injury.

3.3. Arrb1-deficient Fibroblasts Promotes AEC2 Proliferation and Renewal

AEC2 cells are adult stem cells in the lung, and regenerate the alveolar epithelial cells after injury [12,13]. To further demonstrate that ARRB1 affects AEC2 regeneration, we first assessed AEC2 proliferation in *Arrb1*$^{-/-}$ total knockout mice after bleomycin injury. The results showed that 7 days after bleomycin, there are more Ki-67^{+} AEC2 cells in *Arrb1*$^{-/-}$ mice compared to control mice (Figure 3A). However, the increase of Ki-67^{+} AEC2 cells was not observed in the lungs of mice with *Arrb1* deletion specifically in AEC2s (SPC-Cre;*Arrb1*$^{fl/fl}$ mice) (Figure 3B), indicating that ARRB1 promotes AEC2 proliferation via fibroblasts rather than AEC2s themselves. Indeed, when we deleted Arrb1 in Col1a2^{+}

fibroblasts, an increase of Ki-67^{+} AEC2 cells was observed (Figure 3C). To further validate that *Arrb1*$^{-/-}$ fibroblasts promote AEC2 proliferation, WT AEC2 cells were cocultured with *Arrb1*-deficient PDGFRa^{+} fibroblasts. Results showed that *Arrb1* deficiency in fibroblasts significantly increased colony-formation ability of AEC2 cells (Figure 3D), confirming that ARRB1 from fibroblasts affects AEC2 cell proliferation and renewal.

3.4. ARRB1 Promotes CCL7 Expression in Fibroblasts

Next, we sought to determine how *Arrb1*-deficent fibroblasts promote AEC2 proliferation. We isolated PDGFRa^{+} fibroblasts from *Arrb1*$^{+/+}$ and *Arrb1*$^{-/-}$ mice, and performed RNA sequencing (RNA-Seq). Results showed that differentially expressed genes were enriched in cytokine-cytokine receptor interactions and chemokine signaling pathways (Figure 4A). Cytokines and chemokines analysis showed that *Ccl7* was among the top differential expressed genes in *Arrb1* deficiency (Figure 4B). RPKM results also confirmed that *Ccl7* was downregulated in *Arrb1*-deficient fibroblasts (Figure 4C). We confirmed these results by RT-PCR analysis, showing that Ccl7 expression was decreased in *Arrb1*-deficient fibroblasts (Figure 4D). Thus, these data demonstrated that *Arrb1* deficiency downregulates *Ccl7* expression in fibroblasts.

3.5. CCL7 Inhibits AEC2 Renewal In Vitro

Our data show that mice with *Arrb1* deficiency in fibroblasts were protected from bleomycin-induced lung fibrosis, *Arrb1*-deficient fibroblasts promoted AEC2 proliferation, and *Arrb1* deficiency in fibroblasts led to decreased Ccl7 expression. CCL7 (C–C Motif Chemokine Ligand 7, aka Monocyte Chemoattractant Protein 3, MCP3) is a member of the C–C subfamily of chemokines [46], which plays a role in macrophage recruitment during inflammation [47] and in tumor metastasis [46]. *Ccl7* is among several upregulated inflammatory genes identified during early injury [44].

Next, we determined whether CCL7 directly affects AEC2 renewal. Analysis of rat lung injury data [44] showed that Ccl7 expression was increased after bleomycin treatment (Figure 5A). Importantly, CCL7 expression was also found to be increased in lungs of IPF patients compared to healthy lungs (Figure 5B). Furthermore, CCL7 expression is correlated with reduced lung function shown as the negative correlation between CCL7 expression and the percentage of predicted diffusion capacity for carbon monoxide (DLCO) (Figure 5C). In addition, 3D organoid experiments showed that adding CCL7 in organoid assays significantly decreased AEC2 renewal capacity (Figure 5D), confirming that CCL7 inhibits AEC2 regeneration.

In summary, these results demonstrate that fibroblast ARRB1 mediates AEC2 regeneration, possibly by releasing chemokine CCL7, which inhibits AEC2 regeneration, leading to fibrosis in the lung.

4. Discussion

AEC2s are adult stem cells in the lung [12]. We and others have demonstrated a loss of AEC2s and a failure of AEC2 renewal in IPF lungs [13,14]. We previously reported that beta-arrestins are the key regulators of fibroblast invasion, and beta-arrestin 1 and 2

deficient mice exhibit decreased lung fibrosis [37]. However, the role of beta-arrestins in AEC2 regeneration is unclear. In this study, we investigated the role of ARRB1 in AEC2 renewal and in lung fibrosis and found that *Arrb1* deficiency in fibroblasts enhanced AEC2 regeneration and protected mice from lung fibrosis.

AEC2 stem cell regenerative capacity is regulated by the AEC2 stem cell niche [18,21–23]. We recently showed that basal-like cells may produce WNT ligands, mediating AEC2 renewal [24]. Several studies have showed that lipofibroblasts and PDGFRa$^+$ fibroblasts regulate alveolar progenitor cell activities [18,25,26]. Other cell types such as endothelial cells [20,27,28], macrophages [17,29], and T cells [30] regulate alveolar progenitor cell activities. The current study is consistent with these studies and makes the novel observation that fibroblast ARRB1 is crucial for alveolar progenitor cell regeneration. Beta-Arrestins are known to act as a scaffold to regulate GPCR signaling through receptor desensitization and internalization [31–33]. Further studies are needed to determine how ARRB1 interacts with specific GPCRs in fibroblasts for this effector function.

Chemokine CCL7 (MCP3) is a member of the C–C subfamily [46]. It has been shown to play a role in macrophage recruitment during inflammation [47]. Both lung macrophages and myofibroblasts secrete CCL7 into the blood [48]. We found that CCL7 is increased in IPF and negatively correlated with lung function, consistent with a previous report showing an increase in CCL7 in fibroblasts from patients with usual interstitial pneumonia [49], in serum of patients with systemic sclerosis [50], and a recent preprint showing a significant increase of CCL7 in IPF plasma compared to that of matched controls [48]. We showed that CCL7 is also increased after bleomycin injury in mice, consistent with a previous report that CCL7 and CCL2 (MCP1) are increased in the BALF of Sftpc BRICHOS mutant mice [51]. CCL7 may also be a feature of aging [52]. Inhibition of CCL7 receptor CCR2 enhanced aged muscle regeneration and functional recovery after skeletal muscle injury [52].

CCL7 is known for its role in macrophage recruitment, mediating immune response [46]. It is reported that CCL7 promotes activation of the TGF-beta signaling pathways leading to increased type I collagen production [53]. We previously reported that beta-arrestins are key regulators of fibroblast invasion [37]. It is unclear if CCL7 has a role in fibroblast invasion. In this study, we showed that fibroblast-derived CCL7 participates in the AEC2 stem cell niche and inhibits AEC2 renewal. We previously showed that CXC chemokines such as CXCL1 [18] and cytokines such as IL-6 promote AEC2 renewal [13,18]. CCL7 binds CCR2 [54], CCR1, and CCR3, which are typical GPCRs. Future studies are needed to determine which and how CCL7 receptor participates in the AEC2 niche. At the cellular level, CCL7 may bind to CCR2 on macrophages, the latter may negatively regulate the AEC2 niche. Macrophages can influence lung stem cells in a pneumonectomy model in mice [29]. Since CCR2 expression level on AEC2 cells is notably scant, it is unlikely that CCL7 inhibits AEC2 regeneration through CCL7–CCR2 interactions on AEC2s. Future studies are warranted to determine if the effect of CCL7 on AEC2 renewal is direct or indirect.

In conclusion, we demonstrate that fibroblast ARRB1 impedes AEC2 regeneration, possibly by releasing chemokine CCL7, leading to fibrosis in the lung.

Acknowledgments

The authors thank the members of our laboratory for support and helpful discussion during the study. We thank Ning Yu, and Jo Suda from Cedars-Sinai Medical Center Flow Core for help with FACS sorting.

Funding

This work was supported in part by National Institutes of Health grants P01 HL108793, R01 AG078655, and R01 HL122068.

References

1. Noble PW, Barkauskas CE, Jiang D. Pulmonary fibrosis: patterns and perpetrators. J. Clin. Invest 2012, 122, 2756–2762. [PubMed: 22850886]
2. Massague J, Sheppard D. TGF-beta signaling in health and disease. Cell 2023, 186, 4007–4037. [PubMed: 37714133]
3. Taleb SJ, Ye Q, Baoyinna B, Dedad M, Pisini D, Parinandi NL, et al. Molecular Regulation of Transforming Growth Factor-beta1-induced Thioredoxin-interacting Protein Ubiquitination and Proteasomal Degradation in Lung Fibroblasts: Implication in Pulmonary Fibrosis. J. Respir. Biol. Transl. Med 2024, 1, 10002. [PubMed: 38529321]
4. Xie N, Tan Z, Banerjee S, Cui H, Ge J, Liu RM, et al. Glycolytic Reprogramming in Myofibroblast Differentiation and Lung Fibrosis. Am. J. Respir. Crit. Care. Med 2015, 192, 1462–1474. [PubMed: 26284610]
5. Bueno M, Lai YC, Romero Y, Brands J, St Croix CM, Kamga C, et al. PINK1 deficiency impairs mitochondrial homeostasis and promotes lung fibrosis. J. Clin. Invest 2015, 125, 521–538. [PubMed: 25562319]
6. Liang J, Huang G, Liu X, Taghavifar F, Liu N, Wang Y, et al. The ZIP8/SIRT1 axis regulates alveolar progenitor cell renewal in aging and idiopathic pulmonary fibrosis. J. Clin. Invest 2022, 132, e157338. [PubMed: 35389887]
7. Xie T, Liang J, Geng Y, Liu N, Kurkciyan A, Kulur V, et al. MicroRNA-29c Prevents Pulmonary Fibrosis by Regulating Epithelial Cell Renewal and Apoptosis. Am. J. Respir. Cell. Mol. Biol 2017, 57, 721–732. [PubMed: 28799781]
8. Ge L, Habiel DM, Hansbro PM, Kim RY, Gharib SA, Edelman JD, et al. miR-323a-3p regulates lung fibrosis by targeting multiple profibrotic pathways. JCI Insight 2016, 1, e90301. [PubMed: 27942594]
9. Liu G, Friggeri A, Yang Y, Milosevic J, Ding Q, Thannickal VJ, et al. miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. J. Exp. Med 2010, 207, 1589–1597. [PubMed: 20643828]
10. Huan C, Yang T, Liang J, Xie T, Cheng L, Liu N, et al. Methylation-mediated BMPER expression in fibroblast activation in vitro and lung fibrosis in mice in vivo. Sci. Rep 2015, 5, 14910. [PubMed: 26442443]
11. Liang J, Huang G, Liu X, Liu N, Taghavifar F, Dai K, et al. Reciprocal interactions between alveolar progenitor dysfunction and aging promote lung fibrosis. Elife 2023, 12, e85415. [PubMed: 37314162]
12. Barkauskas CE, Cronce MJ, Rackley CR, Bowie EJ, Keene DR, Stripp BR, et al. Type 2 alveolar cells are stem cells in adult lung. J. Clin. Invest 2013, 123, 3025–3036. [PubMed: 23921127]
13. Liang J, Zhang Y, Xie T, Liu N, Chen H, Geng Y, et al. Hyaluronan and TLR4 promote surfactant-protein-C-positive alveolar progenitor cell renewal and prevent severe pulmonary fibrosis in mice. Nat. Med 2016, 22, 1285–1293. [PubMed: 27694932]
14. Morse C, Tabib T, Sembrat J, Buschur KL, Bittar HT, Valenzi E, et al. Proliferating SPP1/MERTK-expressing macrophages in idiopathic pulmonary fibrosis. Eur. Respir. J 2019, 54, 1802441. [PubMed: 31221805]
15. Chen Q, Suresh Kumar V, Finn J, Jiang D, Liang J, Zhao YY, et al. CD44(high) alveolar type II cells show stem cell properties during steady-state alveolar homeostasis. Am. J. Physiol. Lung Cell. Mol. Physiol 2017, 313, L41–l51. [PubMed: 28473330]

16. Glisinski KM, Schlobohm AJ, Paramore SV, Birukova A, Moseley MA, Foster MW, et al. Interleukin-13 disrupts type 2 pneumocyte stem cell activity. JCI Insight 2020, 5, e131232. [PubMed: 31941839]
17. Choi J, Park JE, Tsagkogeorga G, Yanagita M, Koo BK, Han N, et al. Inflammatory Signals Induce AT2 Cell-Derived Damage-Associated Transient Progenitors that Mediate Alveolar Regeneration. Cell Stem Cell 2020, 27, 366–382. [PubMed: 32750316]
18. Xie T, Kulur V, Liu N, Deng N, Wang Y, Rowan SC, et al. Mesenchymal growth hormone receptor deficiency leads to failure of alveolar progenitor cell function and severe pulmonary fibrosis. Sci. Adv 2021, 7, eabg6005. [PubMed: 34108218]
19. Liu X, Zhang X, Yao C, Liang J, Noble PW, Jiang D. Transcriptomics Analysis Identifies the Decline in the AT2 Stem Cell Niches in Aged Human Lungs. Am. J. Respir. Cell. Mol. Biol 2024, 70, DOI: 10.1165/rcmb.2023-0363OC.
20. Chen Q, Rehman J, Chan M, Fu P, Dudek SM, Natarajan V, et al. Angiocrine Sphingosine-1-Phosphate Activation of S1PR2-YAP Signaling Axis in Alveolar Type II Cells Is Essential for Lung Repair. Cell Rep. 2020, 31, 107828. [PubMed: 32610129]
21. Donne ML, Lechner AJ, Rock JR. Evidence for lung epithelial stem cell niches. BMC Dev. Biol 2015, 15, 32. [PubMed: 26376663]
22. Basil MC, Katzen J, Engler AE, Guo M, Herriges MJ, Kathiriya JJ, et al. The Cellular and Physiological Basis for Lung Repair and Regeneration: Past, Present, and Future. Cell Stem Cell 2020, 26, 482–502. [PubMed: 32243808]
23. Juul NH, Stockman CA, Desai TJ. Niche Cells and Signals that Regulate Lung Alveolar Stem Cells In Vivo. Cold Spring Harb. Perspect. Biol 2020, 12, a035717. [PubMed: 32179507]
24. Huang G, Liang J, Huang K, Liu X, Taghavifar F, Yao C, et al. Basal Cell-derived WNT7A Promotes Fibrogenesis at the Fibrotic Niche in Idiopathic Pulmonary Fibrosis. Am. J. Respir. Cell. Mol. Biol 2023, 68, 302–313. [PubMed: 36318668]
25. Zysman M, Baptista BR, Essari LA, Taghizadeh S, Thibault de Menonville C, Giffard C, et al. Targeting p16(INK4a) Promotes Lipofibroblasts and Alveolar Regeneration after Early-Life Injury. Am. J. Respir. Crit. Care. Med 2020, 202, 1088–1104. [PubMed: 32628504]
26. Gao F, Li C, Danopoulos S, Al Alam D, Peinado N, Webster S, et al. Hedgehog-responsive PDGFRa(+) fibroblasts maintain a unique pool of alveolar epithelial progenitor cells during alveologenesis. Cell Rep. 2022, 39, 110608. [PubMed: 35385750]
27. Rafii S, Cao Z, Lis R, Siempos II, Chavez D, Shido K, et al. Platelet-derived SDF-1 primes the pulmonary capillary vascular niche to drive lung alveolar regeneration. Nat. Cell. Biol 2015, 17, 123–136. [PubMed: 25621952]
28. Li K, Wu Q, Sun X, Geng Y, Leng D, Li H, et al. Tsp1 promotes alveolar stem cell proliferation and its down-regulation relates to lung inflammation in intralobar pulmonary sequestration. Oncotarget 2017, 8, 64867–64877. [PubMed: 29029397]
29. Lechner AJ, Driver IH, Lee J, Conroy CM, Nagle A, Locksley RM, et al. Recruited Monocytes and Type 2 Immunity Promote Lung Regeneration following Pneumonectomy. Cell Stem Cell 2017, 21, 120–134. [PubMed: 28506464]
30. Kaiser KA, Loffredo LF, Santos-Alexis KL, Ringham OR, Arpaia N. Regulation of the alveolar regenerative niche by amphiregulin-producing regulatory T cells. J. Exp. Med 2023, 220, e20221462. [PubMed: 36534084]
31. Luttrell LM, Lefkowitz RJ. The role of beta-arrestins in the termination and transduction of G-protein-coupled receptor signals. J. Cell Sci 2002, 115, 455–465. [PubMed: 11861753]
32. Ma L, Pei G. Beta-arrestin signaling and regulation of transcription. J. Cell Sci 2007, 120, 213–218. [PubMed: 17215450]
33. Pydi SP, Barella LF, Zhu L, Meister J, Rossi M, Wess J. beta-Arrestins as Important Regulators of Glucose and Energy Homeostasis. Annu. Rev. Physiol 2022, 84, 17–40. [PubMed: 34705480]
34. Walker JK, Fong AM, Lawson BL, Savov JD, Patel DD, Schwartz DA, et al. Beta-arrestin-2 regulates the development of allergic asthma. J. Clin. Invest 2003, 112, 566–574. [PubMed: 12925697]

35. Hollingsworth JW, Theriot BS, Li Z, Lawson BL, Sunday M, Schwartz DA, et al. Both hematopoietic-derived and non-hematopoietic-derived beta-arrestin-2 regulates murine allergic airway disease. Am. J. Respir. Cell. Mol. Biol 2010, 43, 269–275. [PubMed: 19805483]
36. Jiang D, Xie T, Liang J, Noble PW. beta-Arrestins in the immune system. Prog. Mol. Biol. Transl. Sci 2013, 118, 359–393. [PubMed: 23764061]
37. Lovgren AK, Kovacs JJ, Xie T, Potts EN, Li Y, Foster WM, et al. beta-arrestin deficiency protects against pulmonary fibrosis in mice and prevents fibroblast invasion of extracellular matrix. Sci. Transl. Med 2011, 3, 74ra23.
38. Parruti G, Peracchia F, Sallese M, Ambrosini G, Masini M, Rotilio D, et al. Molecular analysis of human beta-arrestin-1: cloning, tissue distribution, and regulation of expression. Identification of two isoforms generated by alternative splicing. J. Biol. Chem 1993, 268, 9753–9761. [PubMed: 8486659]
39. Conner DA, Mathier MA, Mortensen RM, Christe M, Vatner SF, Seidman CE, et al. beta-Arrestin1 knockout mice appear normal but demonstrate altered cardiac responses to beta-adrenergic stimulation. Circ. Res 1997, 81, 1021–1026. [PubMed: 9400383]
40. Florin L, Alter H, Grone HJ, Szabowski A, Schutz G, Angel P. Cre recombinase-mediated gene targeting of mesenchymal cells. Genesis 2004, 38, 139–144. [PubMed: 15048811]
41. Eblaghie MC, Reedy M, Oliver T, Mishina Y, Hogan BL. Evidence that autocrine signaling through Bmpr1a regulates the proliferation, survival and morphogenetic behavior of distal lung epithelial cells. Dev. Biol 2006, 291, 67–82. [PubMed: 16414041]
42. Liang J, Liu N, Liu X, Mena JM, Xie T, Geng Y, et al. Mitogen-activated Protein Kinase-activated Protein Kinase 2 Inhibition Attenuates Fibroblast Invasion and Severe Lung Fibrosis. Am. J. Respir. Cell. Mol. Biol 2019, 60, 41–48. [PubMed: 30130411]
43. Kusko RL, Brothers JF 2nd, Tedrow J, Pandit K, Huleihel L, Perdomo C, et al. Integrated Genomics Reveals Convergent Transcriptomic Networks Underlying Chronic Obstructive Pulmonary Disease and Idiopathic Pulmonary Fibrosis. Am. J. Respir. Crit. Care. Med 2016, 194, 948–960. [PubMed: 27104832]
44. Bauer Y, Tedrow J, de Bernard S, Birker-Robaczewska M, Gibson KF, Guardela BJ, et al. A novel genomic signature with translational significance for human idiopathic pulmonary fibrosis. Am. J. Respir. Cell. Mol. Biol 2015, 52, 217–231. [PubMed: 25029475]
45. Wu H, Tang N. Stem cells in pulmonary alveolar regeneration. Development 2021, 148, dev193458. [PubMed: 33461972]
46. Opdenakker G, Froyen G, Fiten P, Proost P, Van Damme J. Human monocyte chemotactic protein-3 (MCP-3): molecular cloning of the cDNA and comparison with other chemokines. Biochem. Biophys. Res. Commun 1993, 191, 535–542. [PubMed: 8461011]
47. Mercer PF, Williams AE, Scotton CJ, Jose RJ, Sulikowski M, Moffatt JD, et al. Proteinase-activated receptor-1, CCL2, and CCL7 regulate acute neutrophilic lung inflammation. Am. J. Respir. Cell. Mol. Biol 2014, 50, 144–157. [PubMed: 23972264]
48. Unterman A, Zhao AY, Neumark N, Schupp JC, Ahangari F, Cosme C Jr, et al. Single-cell profiling reveals immune aberrations in progressive idiopathic pulmonary fibrosis. medRxiv. 2023, doi: 10.1101/2023.1104.1129.23289296.
49. Choi ES, Jakubzick C, Carpenter KJ, Kunkel SL, Evanoff H, Martinez FJ, et al. Enhanced monocyte chemoattractant protein-3/CC chemokine ligand-7 in usual interstitial pneumonia. Am. J. Respir. Crit. Care. Med 2004, 170, 508–515. [PubMed: 15191918]
50. Yanaba K, Komura K, Kodera M, Matsushita T, Hasegawa M, Takehara K, et al. Serum levels of monocyte chemotactic protein-3/CCL7 are raised in patients with systemic sclerosis: association with extent of skin sclerosis and severity of pulmonary fibrosis. Ann. Rheum. Dis 2006, 65, 124–126. [PubMed: 16344498]
51. Katzen J, Wagner BD, Venosa A, Kopp M, Tomer Y, Russo SJ, et al. An SFTPC BRICHOS mutant links epithelial ER stress and spontaneous lung fibrosis. JCI Insight 2019, 4, e126125. [PubMed: 30721158]
52. Blanc RS, Kallenbach JG, Bachman JF, Mitchell A, Paris ND, Chakkalakal JV. Inhibition of inflammatory CCR2 signaling promotes aged muscle regeneration and strength recovery after injury. Nat. Commun 2020, 11, 4167. [PubMed: 32820177]

53. Ong VH, Carulli MT, Xu S, Khan K, Lindahl G, Abraham DJ, et al. Cross-talk between MCP-3 and TGFbeta promotes fibroblast collagen biosynthesis. Exp. Cell Res 2009, 315, 151–161. [PubMed: 19038247]

54. Szymczak WA, Deepe GS Jr. The CCL7-CCL2-CCR2 axis regulates IL-4 production in lungs and fungal immunity. J. Immunol 2009, 183, 1964–1974. [PubMed: 19587014]

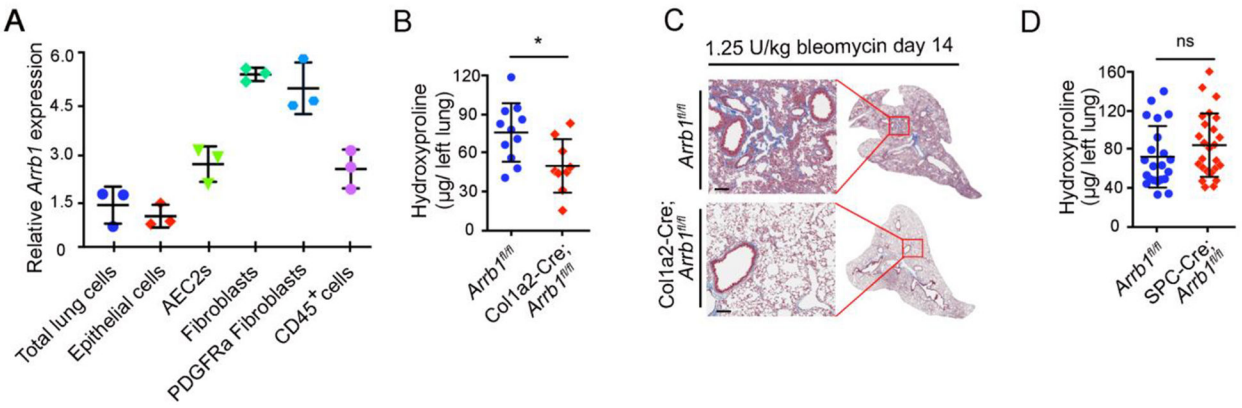

Figure 1.

Arrb1 deficiency in fibroblasts protects mice from lung fibrosis. (**A**) Expression of *Arrb1* in different cell types from mouse lungs was determined. Cells were isolated via FACS, and total RNAs were extracted. *Arrb1* expression was determined with RT-PCR ($n = 3$). (**B**) Hydroxyproline assays of mouse left lungs 14 days after 1.25 U/kg bleomycin treatment ($Arrb1^{fl/fl}$ $n = 11$, Col1a2-Cre; $Arrb1^{fl/fl}$ $n = 9$, $^{*}p < 0.05$). (**C**) Trichrome staining of mouse lungs 14 days after 1.25 U/kg bleomycin injury. (**D**) Hydroxyproline assays of mouse left lungs 14 days after 1.25 U/kg bleomycin treatment ($Arrb1^{fl/fl}$ $n = 22$, SPC-Cre; $Arrb1^{fl/fl}$ $n = 25$, $^{*}p < 0.05$).

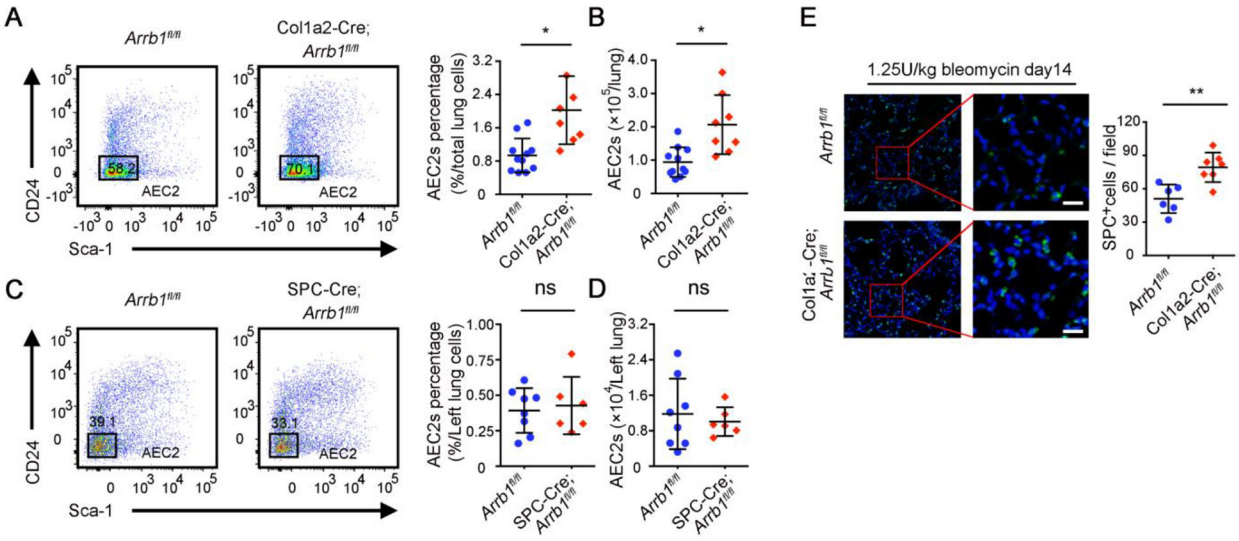

Figure 2.

Arrb1 deficiency in fibroblasts leads to increased AEC2 recovery in injured mouse lung. (**A**–**D**) 14 days after 1.25 U/kg bleomycin treatment, AEC2 cells were analyzed as live $CD45^{-}CD31^{-}CD34^{-}Epcam^{+}CD24^{-}Sca\text{-}1^{-}$ cells with flow cytometry. Percentage (**A**, $Arrb1^{fl/fl}$ $n = 11$, Col1a2-Cre; $Arrb1^{fl/fl}$ $n = 7$; $*p < 0.05$) and total number (**B**, $Arrb1^{fl/fl}$ $n = 11$, Col1a2-Cre; $Arrb1^{fl/fl}$ $n = 8$; $*p < 0.05$) of AEC2 cells in Col1a2-Cre;$Arrb1^{fl/fl}$ mouse lungs as well as percentage (**C**, $Arrb1^{fl/fl}$ $n = 8$, SPC-Cre;$Arrb1^{fl/fl}$ $n = 6$; ns, not significant) and total number (**D**, $Arrb1^{fl/fl}$ $n = 8$, SPC-Cre; $Arrb1^{fl/fl}$ $n = 6$; ns, not significant) of AEC2 cells in SPC-Cre;$Arrb1^{fl/fl}$ mouse lungs were calculated according to flow results. (**E**) Immunofluorescence staining of SPC^{+} cells from mouse lungs 14 days after 1.25 U/kg bleomycin treatment ($Arrb1^{fl/fl}$ $n = 7$, Col1a2-Cre; $Arrb1^{fl/fl}$ $n = 8$; $**p < 0.01$).

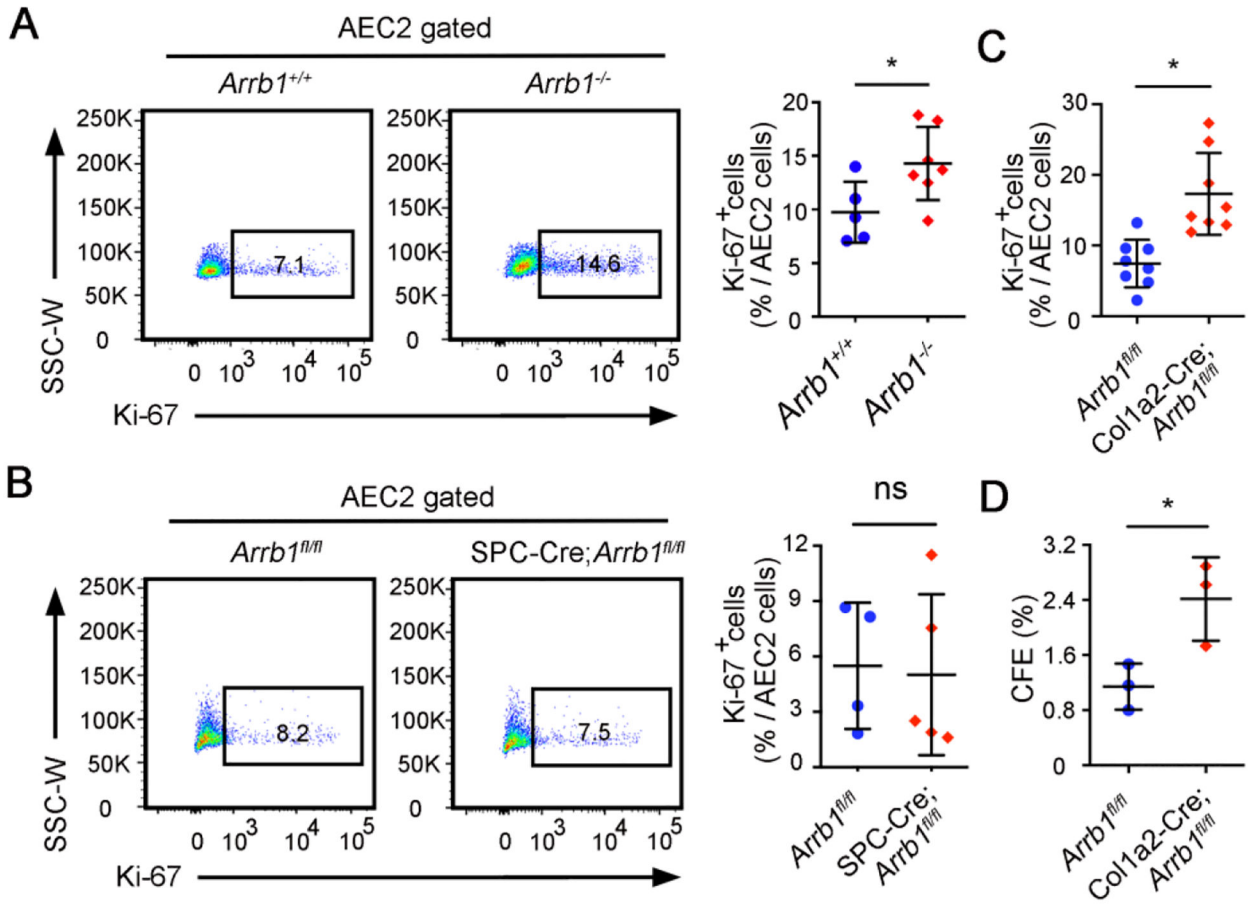

Figure 3.

Arrb1-deficient fibroblasts promote AEC2 proliferation and renewal. (**A**–**C**) All mice were subjected to 1.25 U/kg bleomycin and mouse lungs were harvested 14 days after injury. AEC2 cells were gated as live $CD45^{-}CD31^{-}CD34^{-}Epcam^{+}CD24^{-}Sca\text{-}1^{-}$, percentage of $Ki\text{-}67^{+}$ cells were calculated. $Ki\text{-}67^{+}$ AEC2 cells were analyzed by FACS in $Arrb1^{+/+}$ and $Arrb1^{-/-}$ mice (**A**, $Arrb1^{+/+}$ $n = 5$, $Arrb1^{-/-}$ $n = 7$; $^{*}p < 0.05$), $Arrb1^{fl/fl}$ and SPC-Cre;$Arrb1^{fl/fl}$ mice (**B**, $Arrb1^{fl/fl}$ $n = 7$, SPC-Cre;$Arrb1^{fl/fl}$ $n = 6$; ns, not significant), $Arrb1^{fl/fl}$ and Col1a2-Cre; $Arrb1^{fl/fl}$ mice (**C**, $n = 8$; $^{*}p < 0.05$). (**D**) AEC2 cells from wild type C57BL/6 mice were cocultured with freshly isolated $PDGFRa^{+}$ fibroblasts ($CD45^{-}CD31^{-}CD34^{-}EPCAM^{-}PDGFRa^{+}$) from $Arrb1^{fl/fl}$ and Col1a2-Cre;$Arrb1^{fl/fl}$ mice lungs 7 days after 1.25 U/kg bleomycin treatment. 14 days after culture, colony-formation efficiency (CFE) was calculated ($n = 3$, $^{*}p < 0.05$).

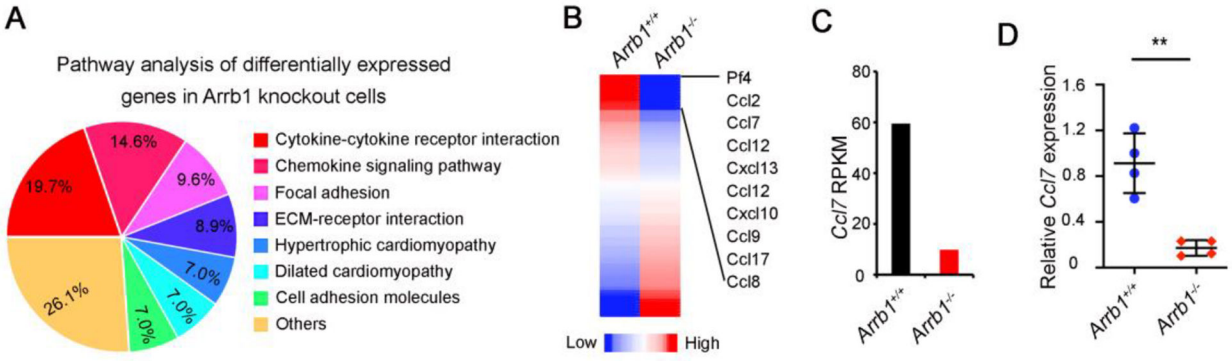

Figure 4.

Arrb1 promotes Ccl7 expression in fibroblasts. (**A**) *Arrb1*$^{+/+}$ and *Arrb1*$^{-/-}$ mice were subjected to 1.25 U/kg bleomycin injury, PDGFRa^{+} fibroblasts were isolated with FACS 7 days after treatment. RNA-seq was performed with total RNAs isolated from these cells. Pathway analysis was conducted with Ingenuity Pathway Analysis (IPA) on the differentially expressed genes (DEGs). (**B**) Differentially expressed cytokines and chemokines were extracted. C–C chemokine genes are indicated. (**C**) RPKM of *Ccl7* in PDGFRa^{+} fibroblasts from *Arrb1*$^{+/+}$ and *Arrb1*$^{-/-}$ lungs in RNA-Seq analysis. (**D**) *Ccl7* expression in PDGFRa^{+} fibroblasts was analyzed with RT-PCR ($n = 4$; $^{**}p < 0.01$).

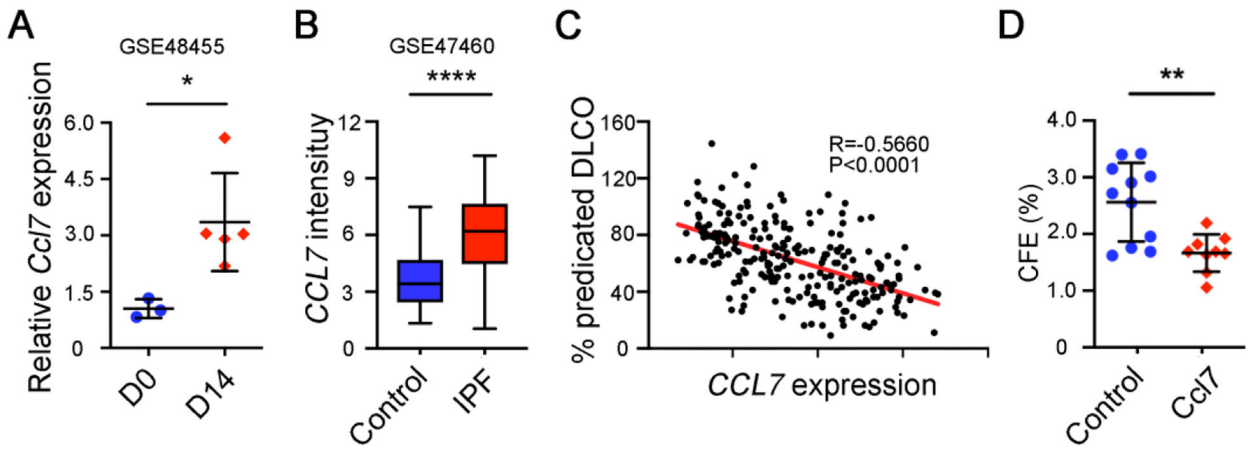

Figure 5.

CCL7 inhibits AEC2 renewal. (**A**) *Ccl7* expression on day 0 and day 14 rat lungs after bleomycin treatment in GSE48455 dataset was analyzed (Day 0 $n = 3$, Day 14 $n = 5$, $^{*}p < 0.05$). (**B**) *CCL7* expression in IPF patients and healthy controls in GSE47460 dataset was analyzed (Control $n = 108$, IPF $n = 160$, $^{****}p < 0.0001$). (**C**) Pearson correlation of *CCL7* expression and the percentage of predicted diffusion capacity for carbon monoxide (DLCO) in GSE47460 dataset was determined ($n = 242$, $R = -0.5660$, $p < 0.0001$). (**D**) Colony formation efficiency of AEC2 from wild type C57BL/6 mice treated with 10 ng/mL CCL7 (Control $n = 11$, CCL7 $n = 9$; $^{**}p < 0.01$).