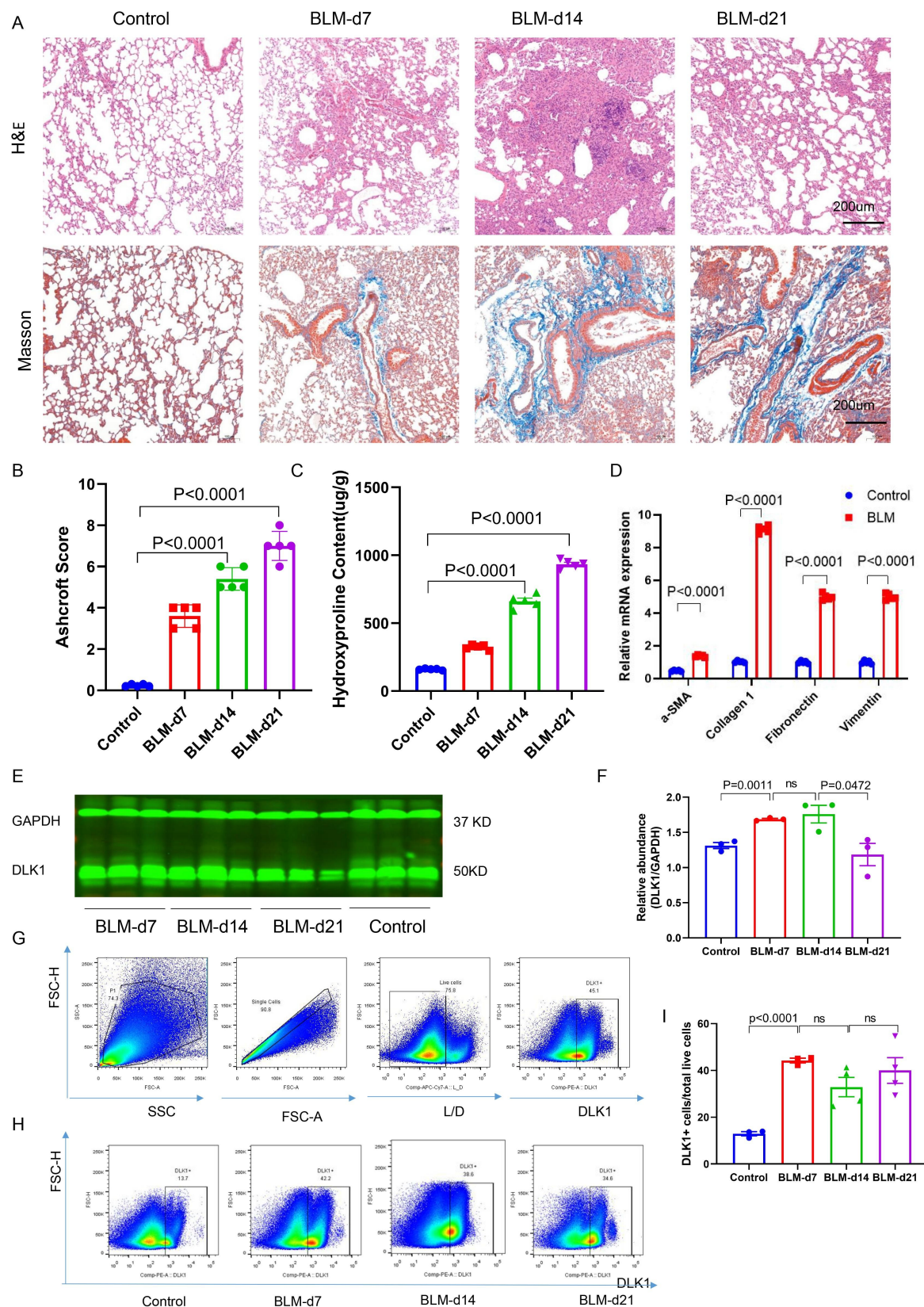
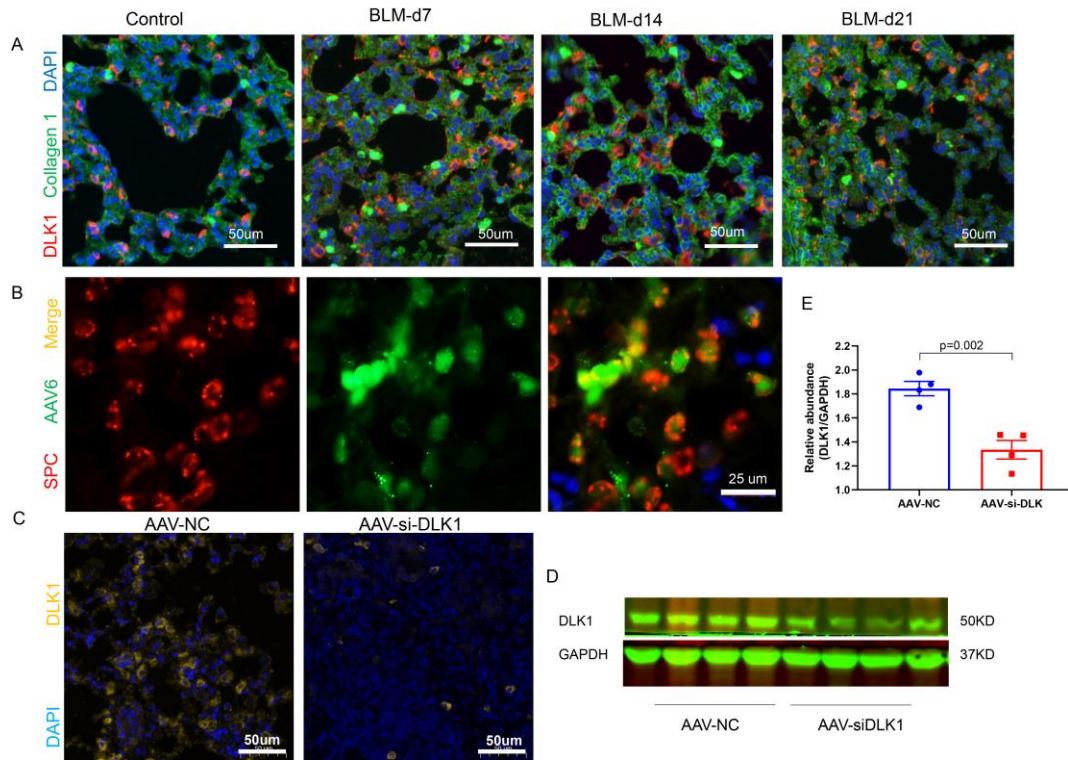


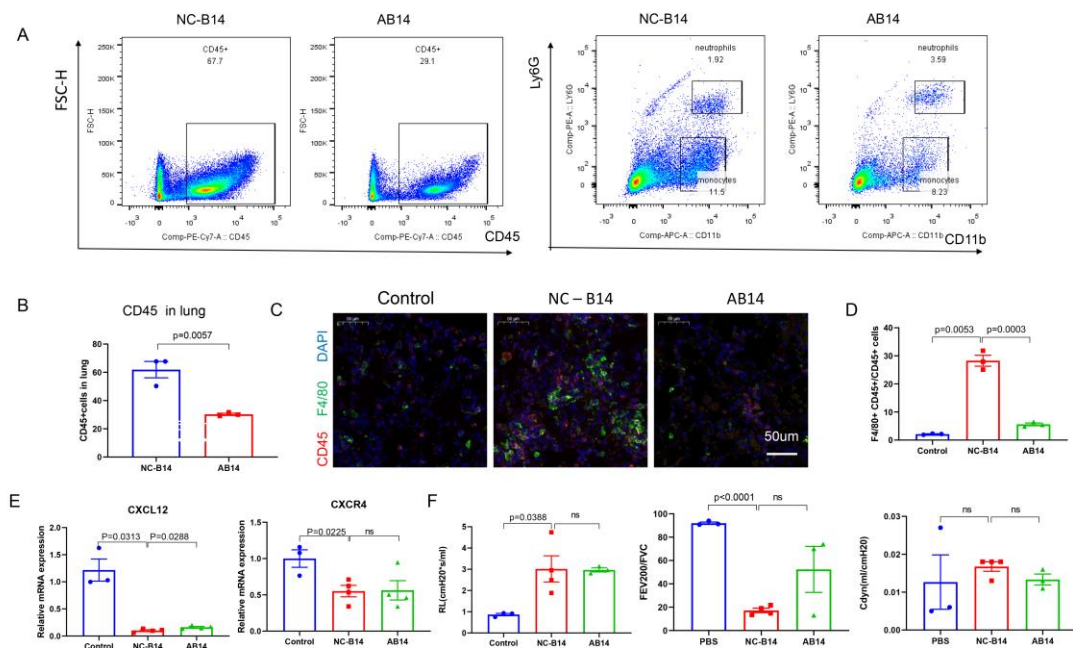
Supplementary Figures



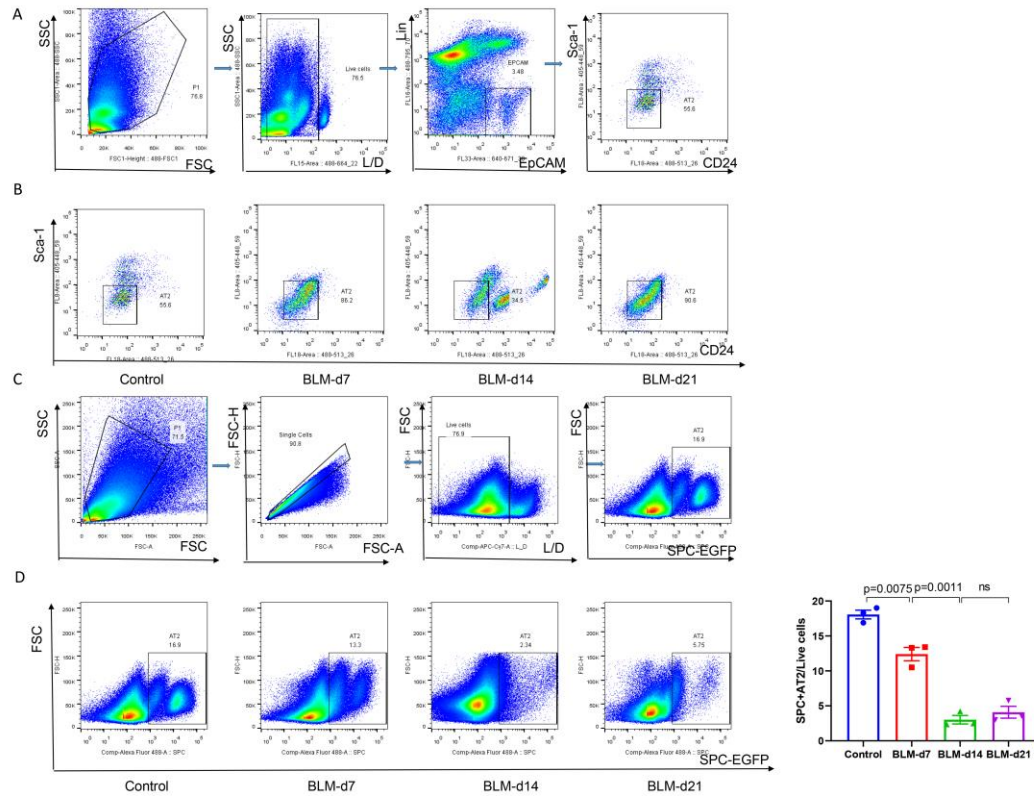
Sup Figure 1. Increased expression of DLK1 in pulmonary fibrosis. (A) pathological changes by H&E and Masson in the control lung and 7,14,21days following BLM induction, the scale bar 200µm. (B) Ashcroft score of indicated time point.(C)Hydroxyproline assay.(D) PCR analysis of fibrotic genes.(E, F) Western blot analysis of DLK1 in control lungs and BLM-treated fibrotic lungs at indicated times. (G, H, I) FCM analysis showed the gating strategy and the proportion of DLK1 during BLM-induced fibrosis.



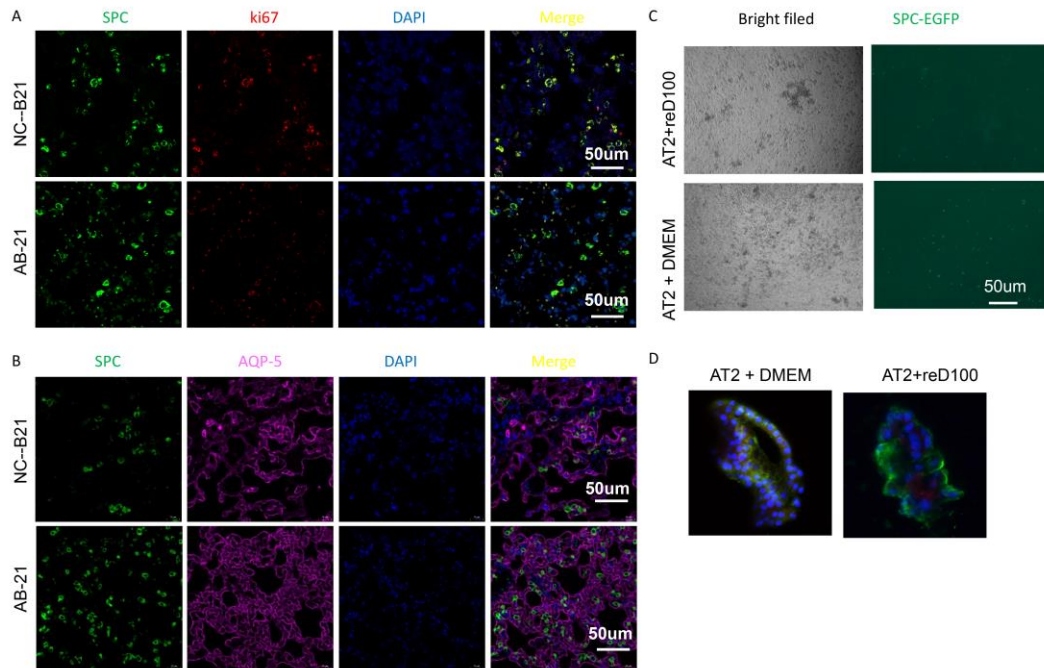
Sup Figure 2. DLK1 was predominantly expressed in AT2 cells during pulmonary fibrosis. (A) Co-immunostaining of DLK1 (red), Collagen 1 (green), and DAPI (blue) in control and BLM-induced mice, showing that DLK1 was hardly expressed in mesenchymal cells, the scale bar 50um. (B) immunostaining of AAV6 in SPC-EGFP mice showed the precise targeting of AAV6 on AT2 cells. (C) immunostaining of DLK1 in AAV-NC or AAV-siDLK1 treated mice demonstrated the efficiency of AAV-mediated knockdown, the scale bar 50um. (D, E) Western blot analysis of DLK1 testified the eligibility of AAV-siDLK1.



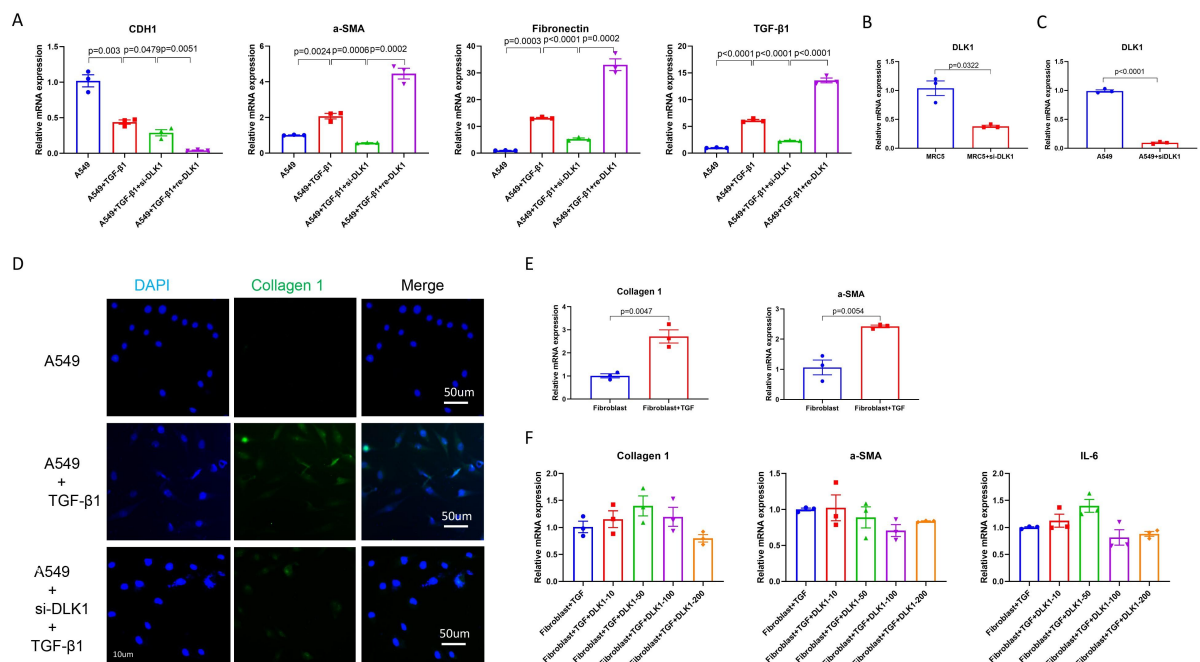
Sup Figure 3. AAV6-mediated alveolar epithelial knockdown of DLK1 mitigated the inflammatory infiltration. (A, B) FCM analysis of CD45⁺ cells and neutrophils in the lung of AAV-NC and AAV-siDLK1 treated mice. (C, D) Representative fluorescence images of CD45 (red), F4/80 (green), and DAPI (blue) in control, AAV-NC, and AAV-siDLK1 treated BLM mice, the scale bar 50um. (E) Relative mRNA expression of fibroblast recruitment-associated genes, including CXCL12 and CXCR4 in the indicated mice. (F) Non-invasive lung function measurement by WBP analysis . Data are presented as mean±SEM.



Sup Figure 4. Less alveolar injury in the lung of DLK1 knockdown mice during fibrosis. (A, B) Gating strategy of Lin⁺Epcam⁺ CD24⁻ Sca-1⁻ AT2 cells and their dynamic changes on day 7,14,21. (C, D) Gating strategy and of SPC⁺AT2 cells their dynamic changes during fibrosis using SPC-EGFP mice.



Sup Figure 5. Knockdown of DLK1 promoted AT2 proliferation and differentiation. (A) Representative fluorescence images of SPC (green), Ki67 (red), and DAPI (blue) on day 21 in AAV-NC and AAV-siDLK1 treated BLM mice, the scale bar 50um. (B) Representative fluorescence images of SPC (green), AQP-5 (pink), and DAPI (blue) on day 21 in the indicated group. (C) Bright and fluorescence field of fresh AT2 clones cultured in control medium or 100ng/ml DLK1. (D) Representative fluorescence images of 3D organoids. SPC-EGFP labeled AT2 cells were supplied with the control medium or 100ng/ml recombinant DLK1 for 14 days and stained with DAPI (blue), the scale bar 50um.



Sup Figure 6. Re-DLK1 promoted TGF-β induced fibrosis and si-DLK1 rescued it in vitro. (A)

Relative mRNA expression of fibronectin, α -SMA, TGF- β 1 in A549 cells treated with si-DLK1 or 50ng/ml re-DLK1 followed by TGF- β stimulation for 48h. (B, C) The efficiency of si-DLK1 in MRC5 and A549 cells. (D) Representative fluorescence images of collagen 1 (green) and DAPI (blue) in A549 cells treated with or without si-DLK1 followed by TGF- β induction for 48h, the scale bar 50um. (E) Relative mRNA expression of collagen1, α -SMA in primary lung fibroblasts treated with TGF- β (n=3). (F). Relative mRNA expression of collagen1, α -SMA and IL-6 in primary lung fibroblasts treated with 0,10,25,50,100,200ng/ml re-DLK1 6h earlier followed by TGF- β treatment for 48h.