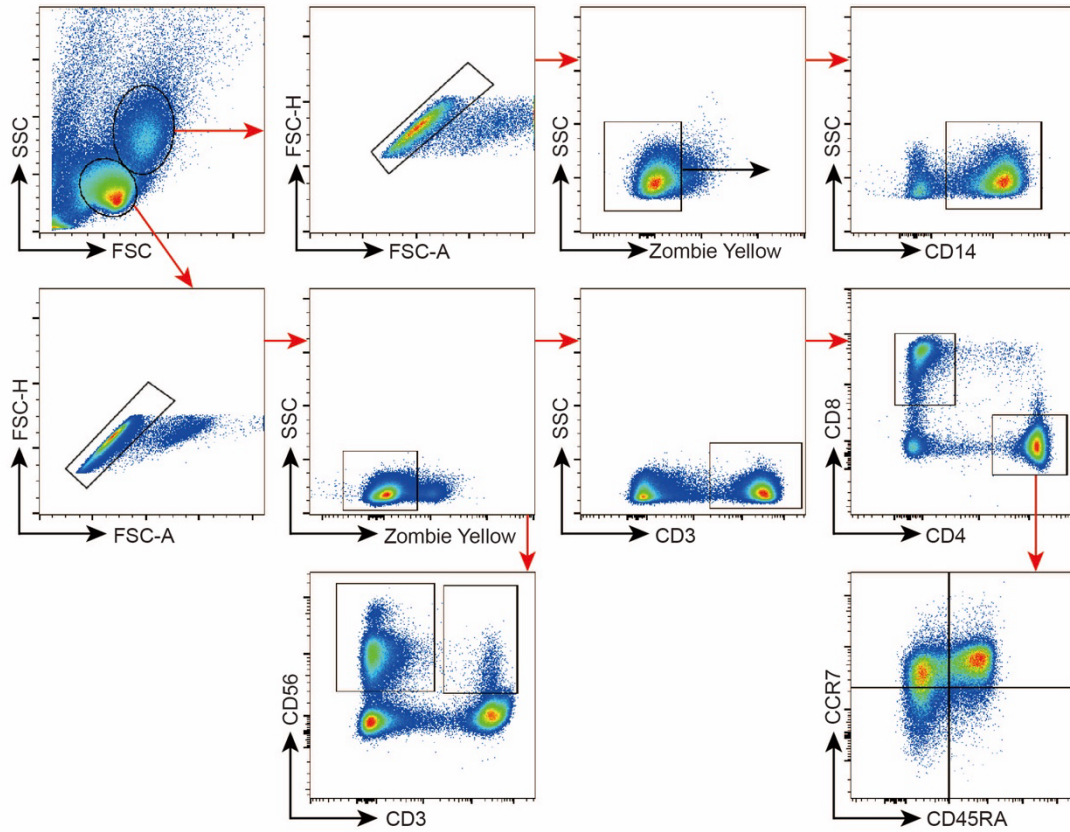


Supplementary Material

Supplementary Figure 1: Flow cytometry analysis of PBMCs from CTD-ILD

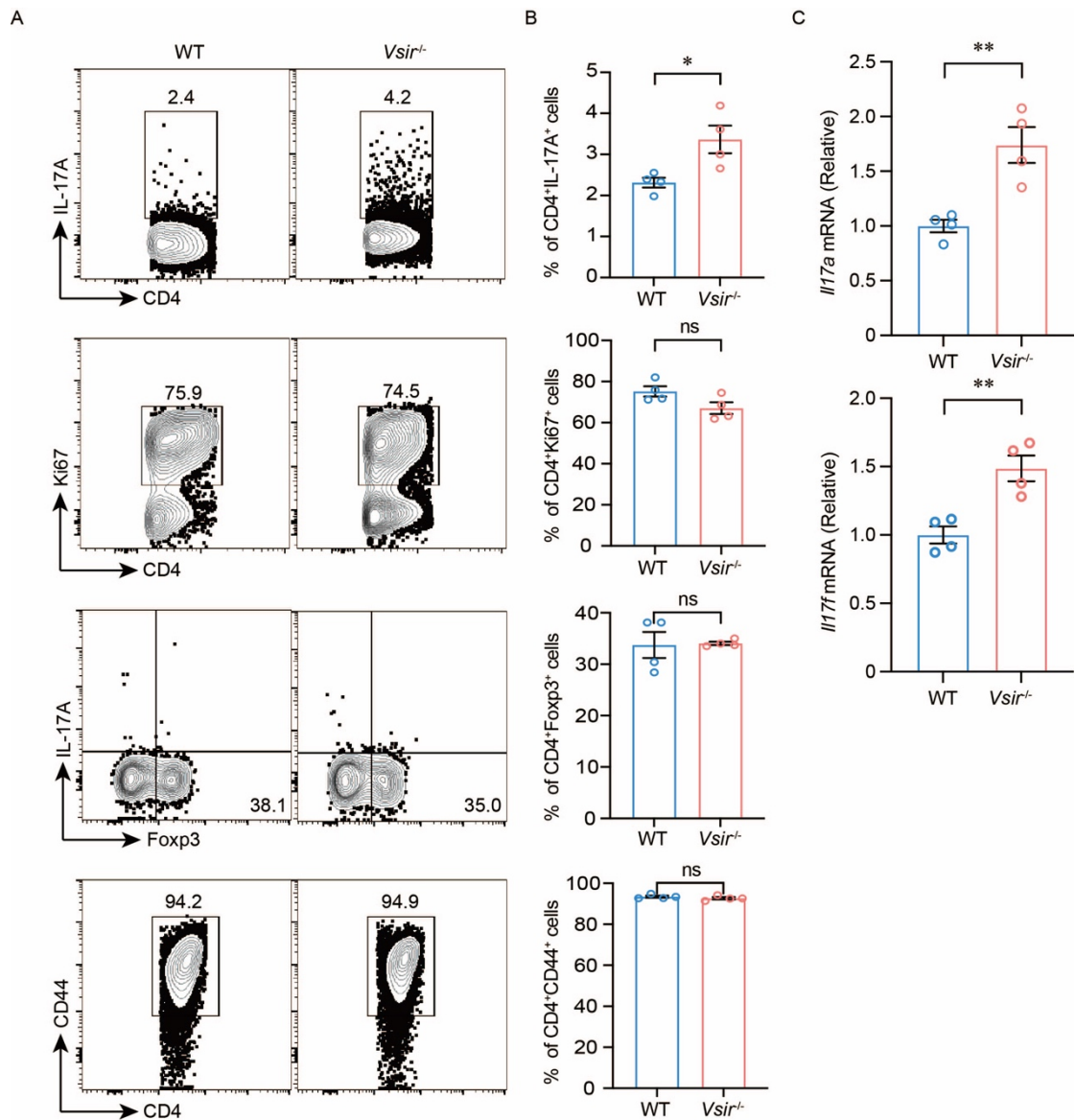
patients and HCs.

A



(A) Gating strategies of different types of immune cells.

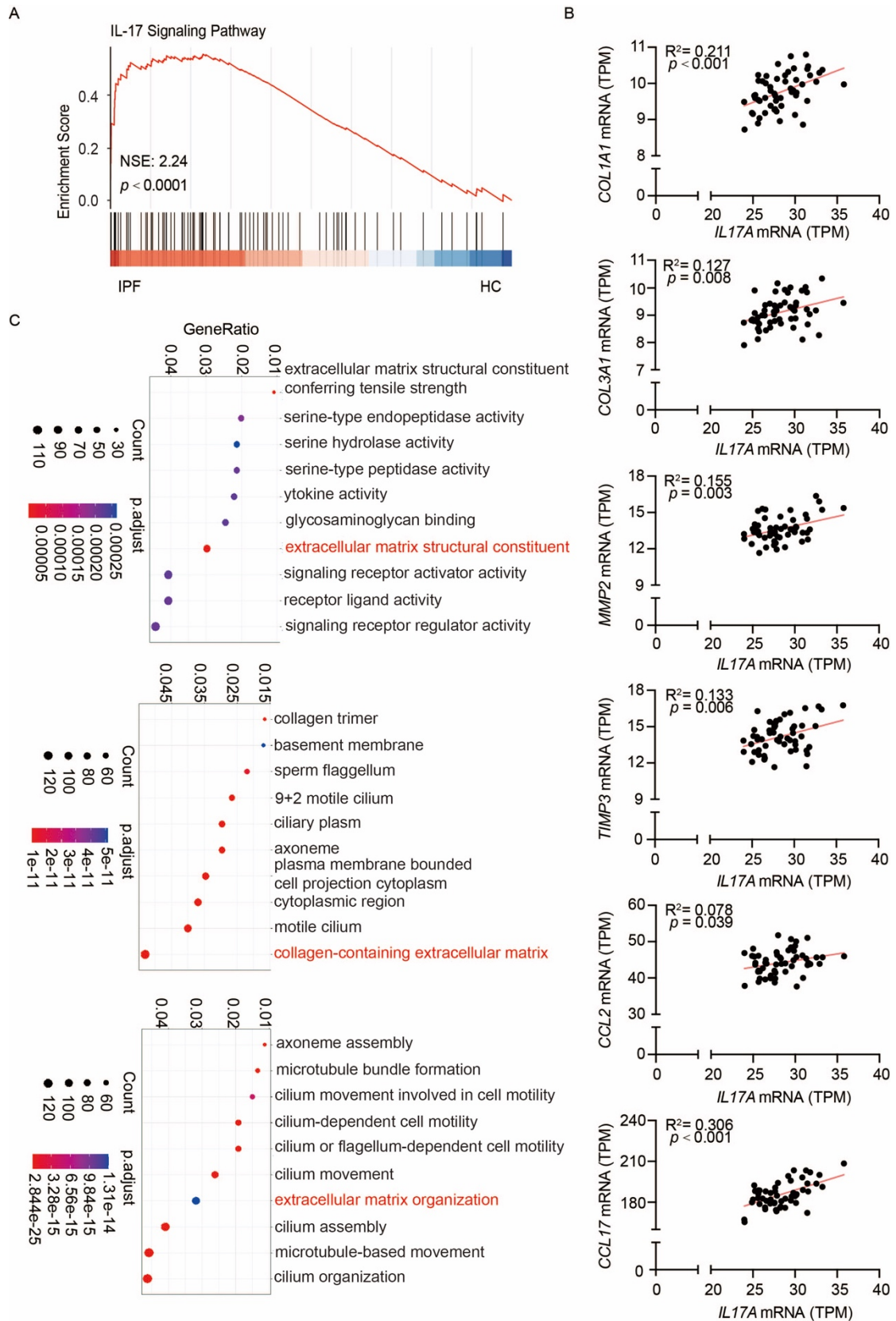
Supplementary Figure 2: Down-regulation of VISTA promotes Th17 differentiation in vitro



(A and B) Spleen cell suspensions were prepared and stimulated with anti-CD3 antibody, anti-CD28 antibody, TGF- β , IL-6, IL-1 β , IL-23, anti-IFN- γ and anti-IL4 for 4 days (n = 4 in WT group, n = 4 in *Vsir*^{-/-} group). Representative gating images were shown (A). The expression levels of IL-17A, Ki-67, Foxp3 and CD44 were compared in WT group and *Vsir*^{-/-} group (B). (C) mRNA levels of *Il17a* and *Il17f* were measured by real-time PCR. Data are mean \pm SEM. * p < 0.05, ** p < 0.01 by Student's t test.

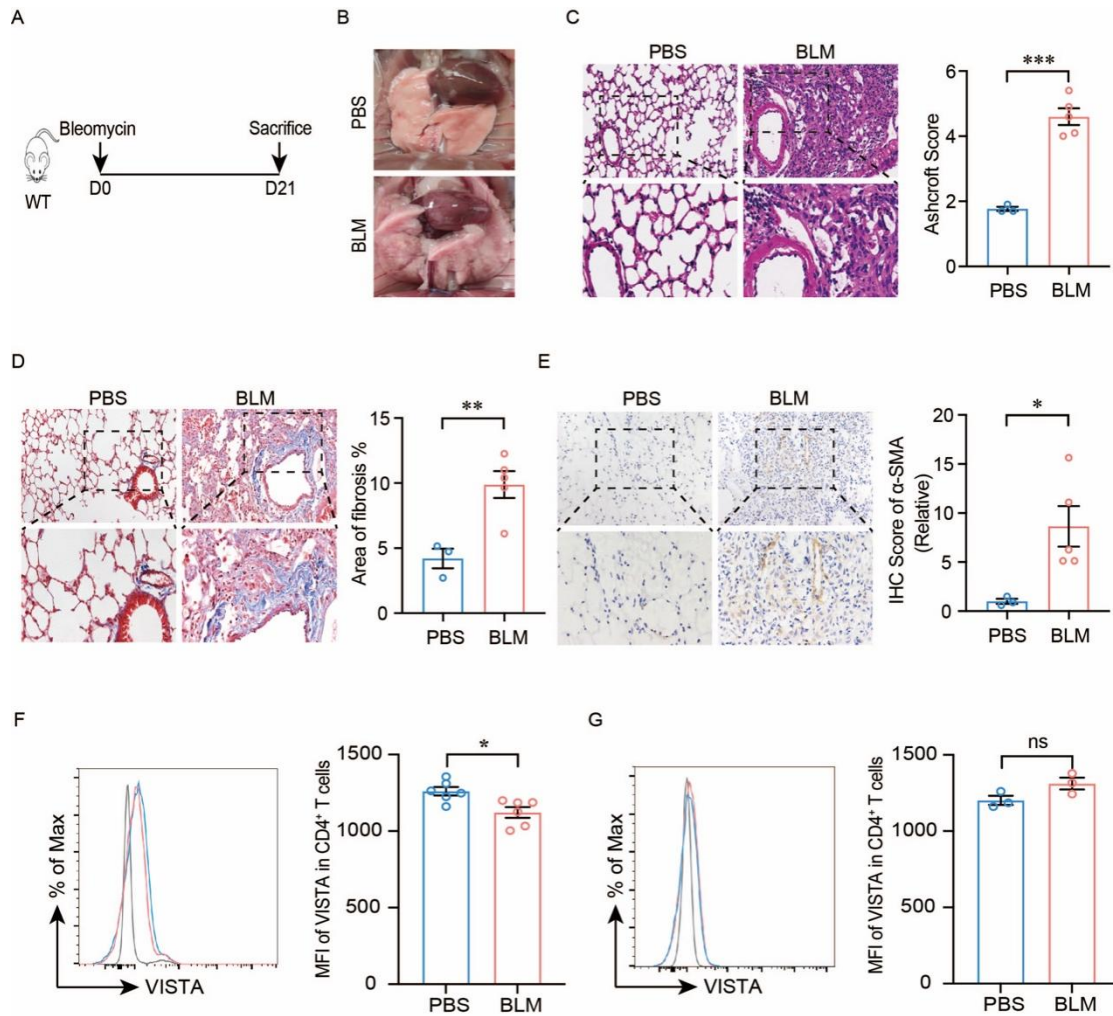
Supplementary Figure 3: IL-17 signaling pathway is involved in the pathogenesis

of PF



(A) IL-17 signaling pathway through GSEA (GSE150910). (B) The relationship between *IL17A* and *COL1A1*, *COL3A1*, *MMP2*, *TIMP3*, *CCL2* and *CCL17* (GSE93606). (C) GO analysis of DEGs from IPF and HCs (GSE150910). Data are mean \pm SEM. Correlation was calculated by Pearson's correlation coefficient.

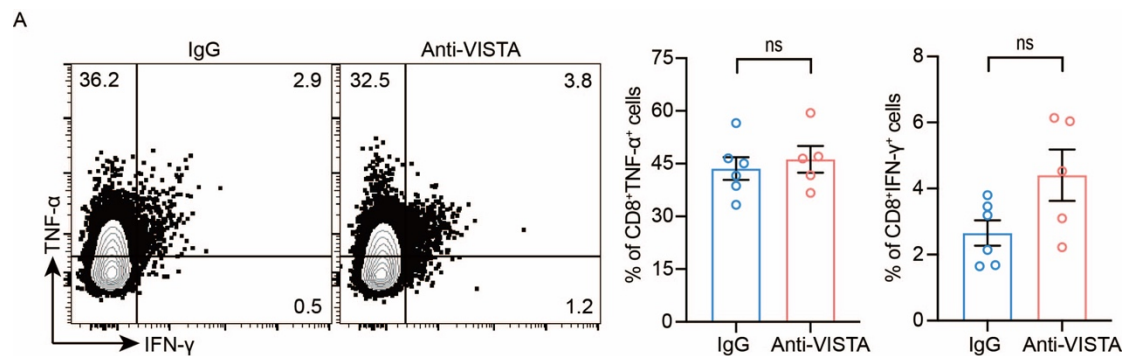
Supplementary Figure 4: BLM induced lung fibrosis.



(A) Schematic graph of murine model. WT mice were divided into two groups, and received single intratracheal injection of BLM or PBS. Mice were sacrificed at day 21 (n = 3 in PBS group, n = 5 in BLM group). (B) Images of lungs from mice in PBS group (upper) or BLM group (lower). (C) H&E staining of lung sections and Ashcroft score of each mouse in two groups. (D) Masson's trichrome staining of lung sections and the area of fibrosis. (E) Immunohistochemistry staining of lung sections and relative score of α -SMA. (F) Spleen cells from PBS group and BLM group were isolated, and VISTA expression was measured by flow cytometry (n = 6 in PBS group, n = 6 in BLM group). (G) CD4 T cells from spleens of WT mice were stimulated with PBS or BLM (15 μ g/ml)

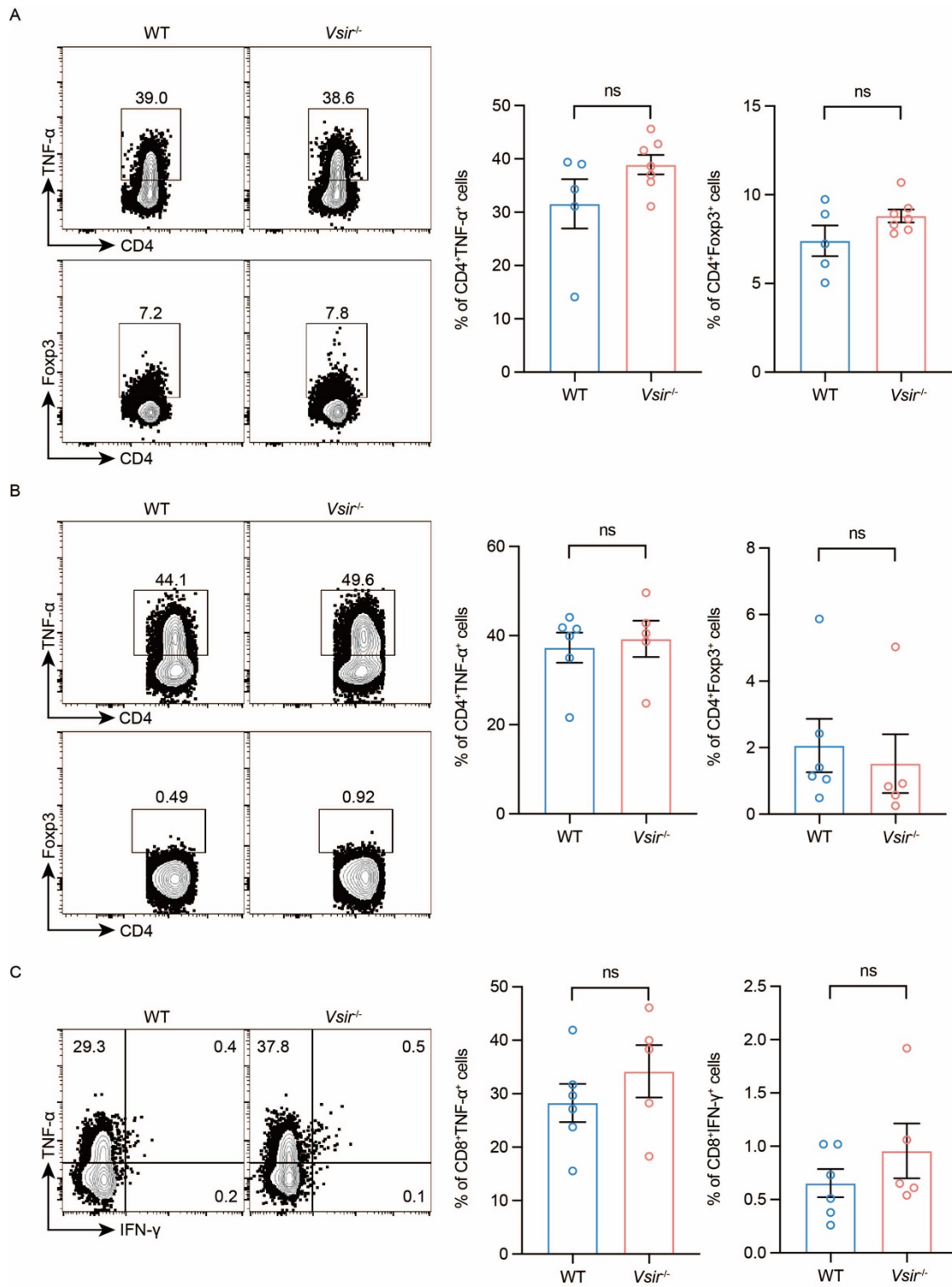
for 2 days. VISTA expression was measured by flow cytometry (n = 3 in PBS group, n = 3 in BLM group). Original magnification: ×20 (upper), ×40 (lower). Data are mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by Student's t test.

Supplementary Figure 5: Anti-VISTA antibody do not influence cytokines production in CD8 T cells



(A) Single cell suspensions of draining lymph nodes from mice were prepared and subjected to flow cytometry analysis ($n = 6$ in isotype antibody group, $n = 5$ in anti-VISTA antibody group). Representative gating strategies for cytokines expression in CD8 T cells and cytokines TNF- α and IFN- γ expression levels in CD8 T cells. Data are mean \pm SEM. Data were analyzed by Student's t test.

Supplementary Figure 6: Flow cytometry analysis of single cells from draining lymph nodes and lung tissues.



(A) Gating strategies of draining lymph nodes and TNF- α and Fcpx3 expression levels in CD4 T cells ($n = 5$ in WT group, $n = 7$ in $Vsir^{-/-}$ group). (B) Gating strategies

of CD4 T cells from lung tissues and TNF- α and Foxp3 expression levels in CD4 T cells (n = 6 in WT group, n = 5 in *Vsir^{-/-}* group). (C) Gating strategies of CD8 T cells from lung tissues and TNF- α and IFN- γ expression levels in CD8 T cells (n = 6 in WT group, n = 5 in *Vsir^{-/-}* group). Data are mean \pm SEM. Data were analyzed by Student's t test.

Supplementary Table 1: Baseline characteristics of patients

Parameters	CTD-ILD (n=18)	HC (n=9)
Age, years (mean \pm SD)	52.11 \pm 14.41	26.56 \pm 2.30
Gender		
Male (%)	5 (27.78%)	4 (44.44%)
Female (%)	13 (72.22%)	5 (55.56%)
Pulmonary function		
FVC, % predicted	61.38 \pm 9.87	NA
TLC, % predicted	64.63 \pm 13.39	NA
DLCO, % predicted	49.63 \pm 18.74	NA

CTD-ILD, connective-tissue disease-related interstitial disease; HC, health control; FVC, forced vital capacity; TLC, total lung capacity; DLCO, the diffusing capacity of the lung for carbon monoxide.

Supplementary Table 2: Primers used for real time PCR

Mouse	Forward Primer (5'-3')	Reverse Primer (5'-3')
genes		
<i>Cxcl1</i>	CTGGGATTCACCTCAAGAACATC	CAGGGTCAAGGCAAGCCTC
<i>Il6</i>	CCCAATTTCCAATGCTCTCC	CGCACTAGGTTTGCCGAGTA
<i>Il17a</i>	TCAGCGTGTCCAAACACTGAG	CGCCAAGGGAGTTAAAGACTT
<i>Il17f</i>	TGCTACTGTTGATGTTGGGAC	AATGCCCTGGTTTTGGTTGAA
<i>Col3a1</i>	AACCTGGTTTCTTCTCACCCCTC	ACTCATAGGACTGACCAAGGTGG
<i>Mmp2</i>	ATCGAGACCATGCGGAAGC	ATCCACGGTTTCAGGGTCC
<i>Coll1a1</i>	TAAGGGTCCCAATGGTGAGA	GGGTCCCTCGACTCCTACAT
<i>Tgfb1</i>	GACATTCGGGAAGCAG	CCACTCAGGCGTATCAG
<i>Gapdh</i>	AACCTGGTTTCTTCTCACCCCTC	AACCTGGTTTCTTCTCACCCCTC