Supplementary Figure S1. Single-Cell Transcriptomic Profiling Reveals Fibrosis-Associated Gene Expression Shifts in Lung Epithelial Subpopulations.

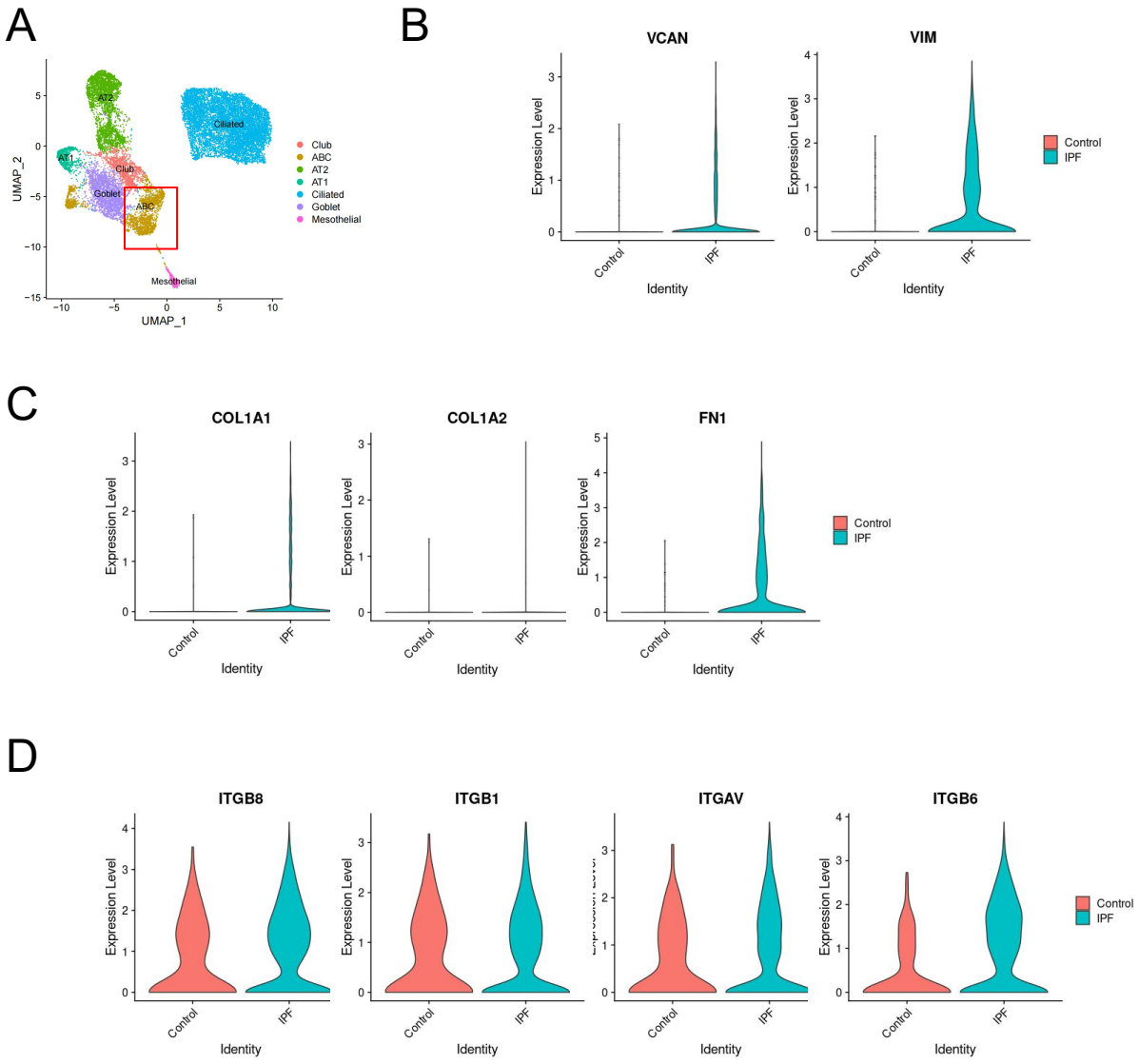

Figure S1. Fibrotic remodeling drives distinct gene expression signatures across lung epithelial subtypes. Data from GSE141939.A. Uniform Manifold Approximation and Projection (UMAP) of epithelial subclusters in human lung tissues. Color-coded clusters denote major subtypes: alveolar type 1 (AT1) and type 2 (AT2) cells, airway basal stem cells (BASC), club, ciliated, goblet, ionocyte, and tuft cells. The pathogenic subpopulation under investigation is highlighted (red box).B-D. Violin plots comparing expression of fibrosis-linked genes between control (red) and IPF (teal) samples:B.Extracellular matrix regulators: VCAN (versican) and VIM (vimentin).C. Collagen biosynthesis genes: COL1A1, COL1A2, and FN1 (fibronectin 1).D.Integrin family members: ITGB1, ITGB3, ITGB6, and ITGAV.

Supplementary Figure S2. RCN3 Co-Localizes with KRT5+ in Human IPF Tissues and fibrotic RNA expression levels in KRT5+ cells.

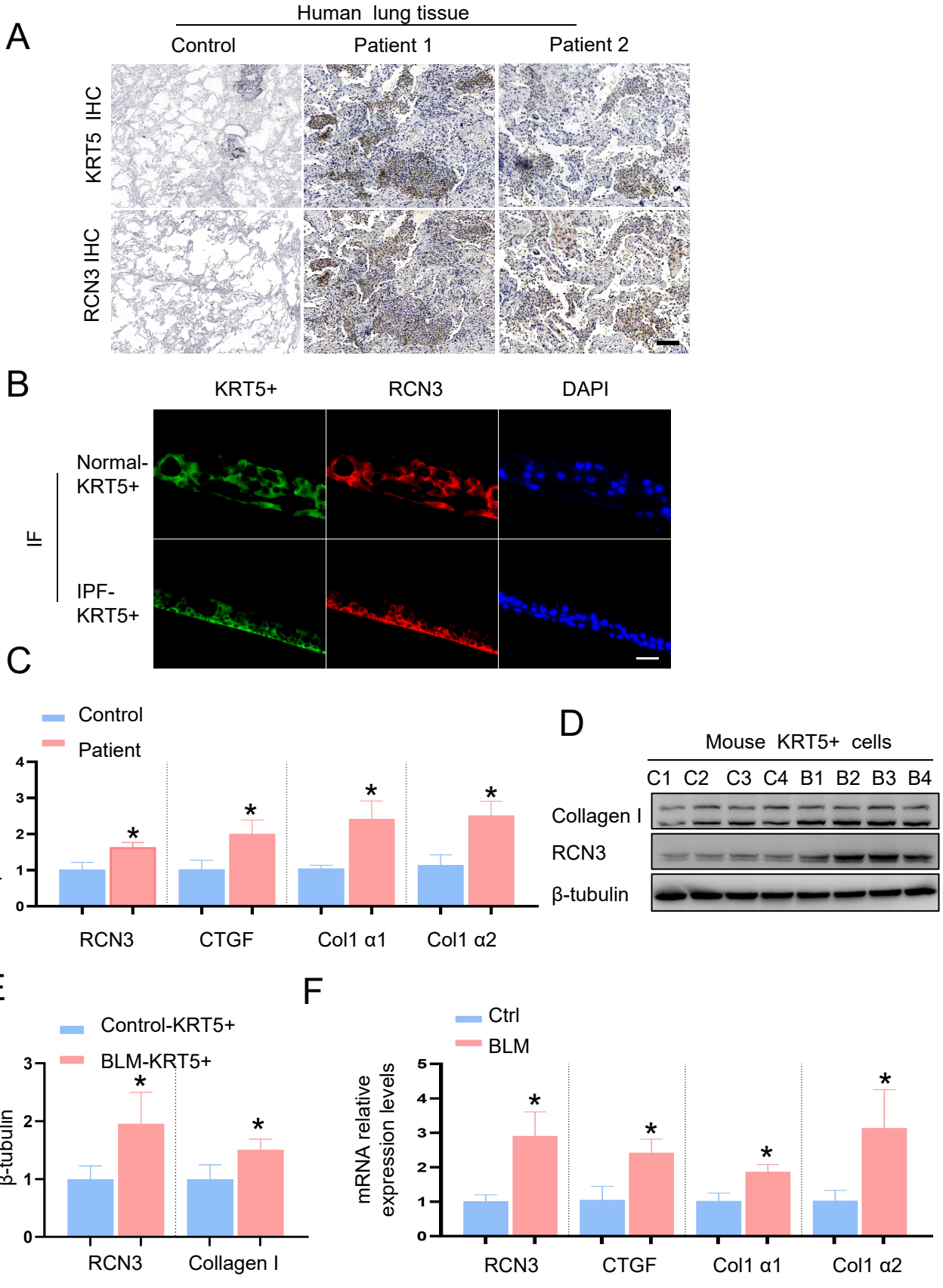

Figure S2.RCN3 colocalizes with pathogenic KRT5^{+}basal cells and drives fibrotic gene signatures in IPF.

A. RCN3-KRT5^{+}spatial association in human IPF. Immunohistochemistry of lung sections from healthy controls and IPF patients shows epithelial restructuring: KRT5^{+} basal cells (top) expand abnormally in fibrotic regions, with RCN3 (bottom) exhibiting parallel upregulation in diseased epithelia. Scale bars: 400 μm.B.Subcellular co-distribution in isolated epithelia. Immunofluorescence confirms RCN3 (red)-KRT5^{+} (green) colocalization in patient-derived cells, showing enhanced nuclear/cytoplasmic RCN3 accumulation specifically in IPF-KRT5^{+} populations vs. normal controls. Nuclei counterstained with DAPI (blue). Scale bars: 400 μm.C.qRT-PCR demonstrates significant upregulation of RCN3, CTGF, collagen I chains (Col1α1/Col1α2) in KRT5 cells drived from bleomycin-injured (BLM) lung tissues versus saline controls (* $P<0.05$; n=3).D-E. Western blots of murine KRT5^{+} lysates reveal elevated RCN3 and collagen I in BLM-treated groups versus controls. β-tubulin validates equal loading.F. Disease-specific reprogramming in human IPF-KRT5^{+} cells. Patient-derived KRT5^{+} basal cells exhibit amplified RCN3 and profibrotic effector transcripts (CTGF, Col1α1/Col1α2) compared to non-fibrotic controls (* $P<0.05$; n=3).All qRT-PCR data normalized to GAPDH genes and presented as mean ± SD. Statistical significance determined by unpaired t-test (*$P<0.05$).

Supplementary Figure S3. Modulation of cell proliferation by RCN3 and pharmacological Inhibition of Wnt/β-Catenin Signaling .

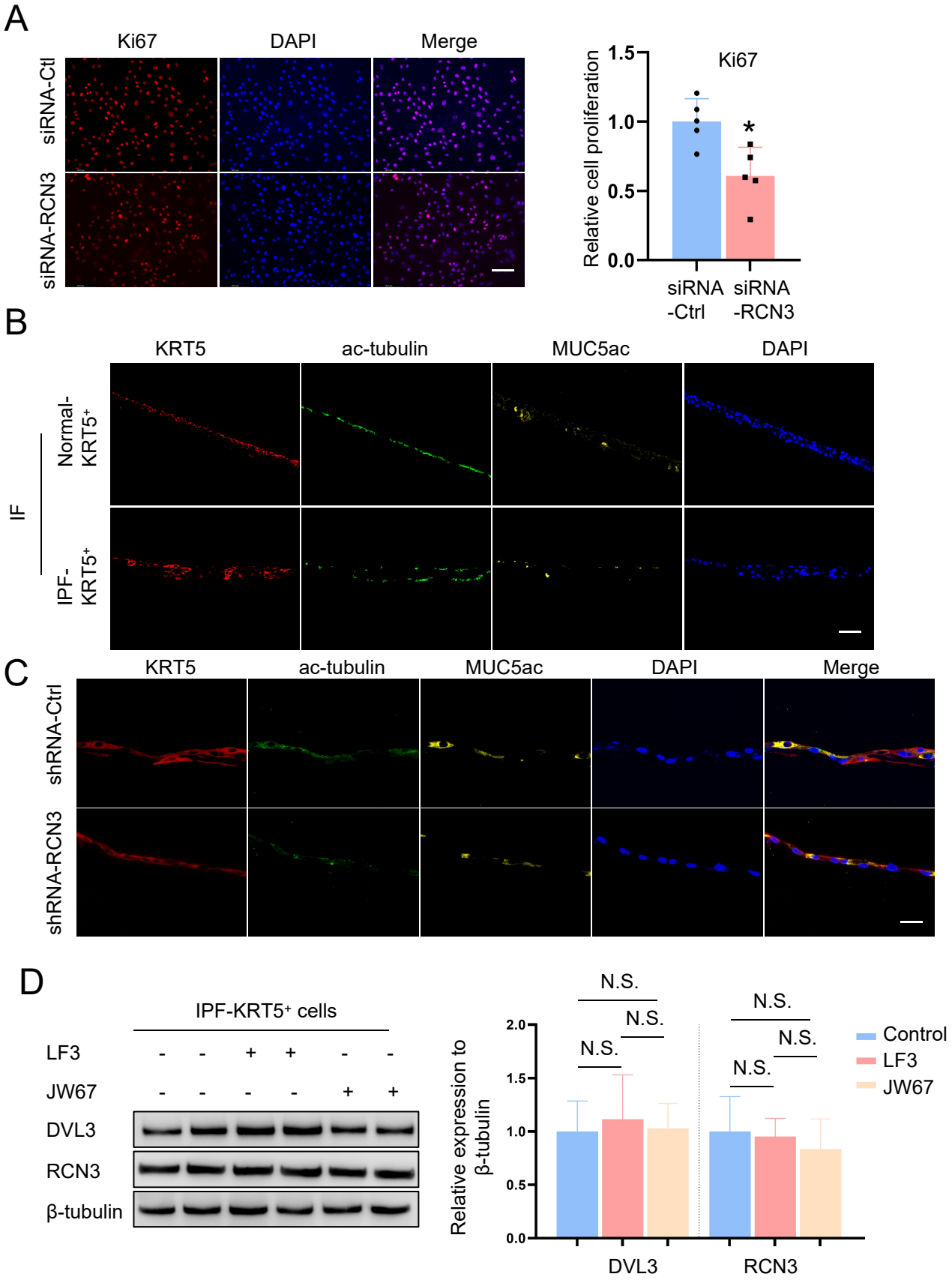

Figure S3. RCN3 regulated cell proliferation and mediated signaling operates independently of β-catenin degradation.

A.Immunofluorescence analysis of Ki67 in siRNA-transfected IPF-KRT5$^+$ cells. Cells treated with RCN3-targeting siRNA (siRNA-RCN3) or control siRNA (siRNA-Ctrl) are shown, co-stained with DAPI (blue) to mark nuclei. Merged images depict proliferative activity within the KRT5$^+$ population. Scale bar: 400 μm.Proliferation was quantified by Ki67 assay. siRNA-RCN3 treatment significantly reduced proliferative activity in IPF-KRT5$^+$ cells compared to the siRNA-Ctrl group (*$p < 0.05$, unpaired t-test). Data are mean ± SD.B.Immunofluorescence analysis of differentiation markers in KRT5$^+$ cells under ALI culture. Shown are KRT5 (basal, red), α-tubulin (ciliated, green), and MUC5AC (goblet, yellow), with DAPI (blue) marking nuclei. Scale bar: 400 μm. C.Immunofluorescence of patient-derived IPF-KRT5$^+$ cells transduced with control (shRNA-CT) or RCN3-targeting shRNA (shRNA-RCN3). Markers: KRT5 (basal cells, red), acetylated α-tubulin (ciliated cells, green), MUC5AC (goblet cells, yellow), DAPI (nuclei, blue). Merged images demonstrate altered epithelial composition upon RCN3 depletion. Scale bars: 400 μm.D. Western blot analysis of DVL3 and RCN3 protein levels in IPF-KRT5$^+$ cells treated with β-catenin inhibitors LF3 or JW67 vs. untreated controls. β-tubulin confirms equal loading.Quantification of DVL3 and RCN3 expression normalized to β-tubulin (mean ± SD, n = 3 biological replicates). N.S. = not significant (one-way ANOVA with Tukey post-hoc, $P > 0.05$).