

LPA1 receptor (LPAR1) localization in IPF tissue and functional profiling of PIPE-791, a highly selective, orally dosed LPAR1 antagonist for potential treatment of pulmonary fibrosis

James S Swaney, Daniel S Lorrain, Didier Bagnol, Kym I Lorrain, Timothy R Watkins, Michael M Poon, Jeffrey R Roppe, Thomas Schrader, Austin C Chen

Contineum Therapeutics, San Diego, CA. USA
<https://contineum-tx.com/>

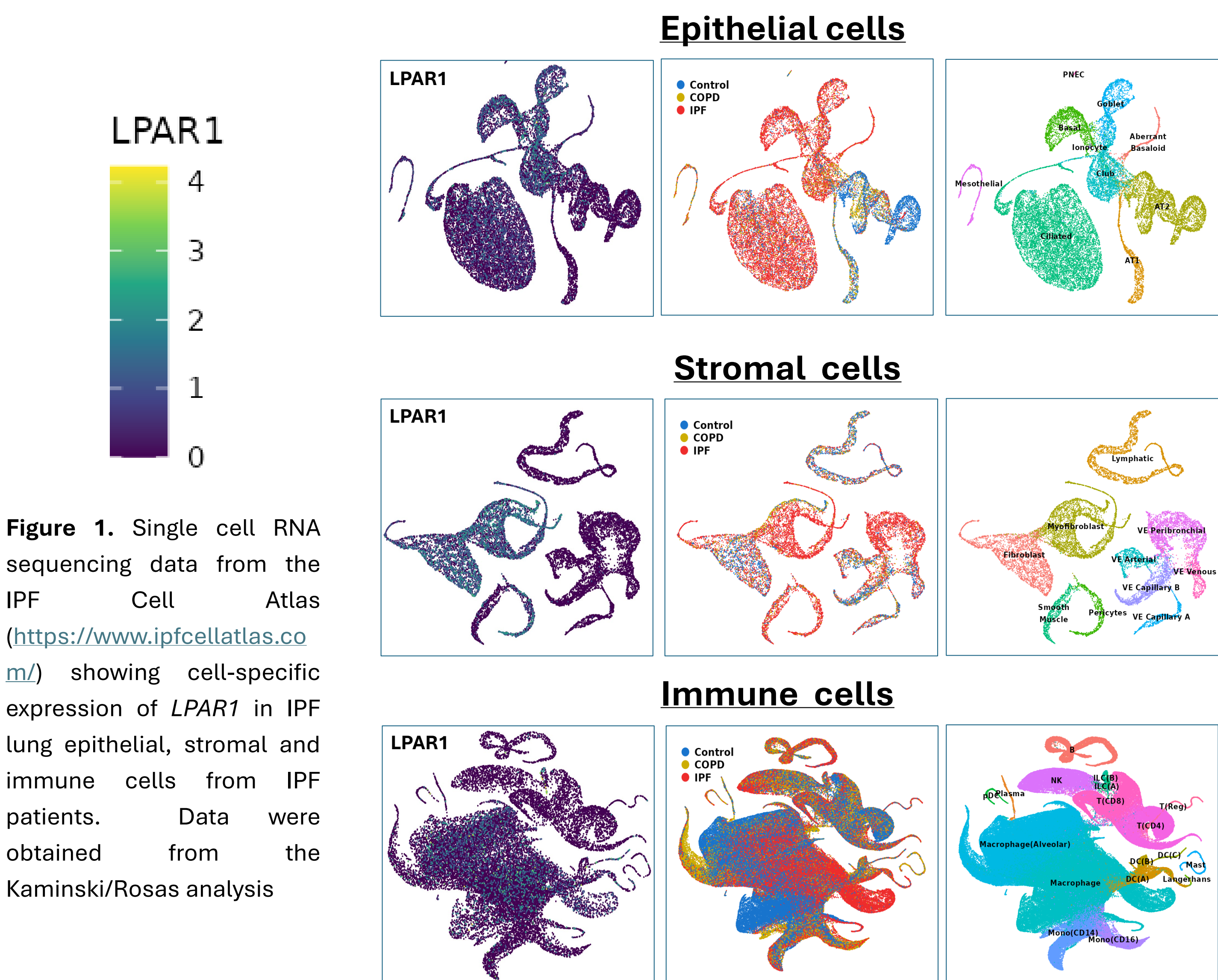
Background & Aims

Although the exact cellular pathobiology remains unknown, IPF is believed to result from interacting genetic and environmental risk factors in the setting of repeated alveolar epithelial injury. The LPA/LPA1 receptor axis is linked to lung injury and pulmonary fibrosis via cell-specific regulation of cell migration, survival, vascular leakage and fibrosis. PIPE-791, a potent, selective LPA1 receptor (LPAR1) antagonist has demonstrated efficacy in translational models of pulmonary fibrosis and is currently being investigated in a global Ph 2 IPF study. However, questions remain regarding cell-specific *LPAR1* expression in lung as it relates to the mechanism for LPAR1 antagonist efficacy in IPF. We characterized *LPAR1* localization and upregulation by specialized airway/alveolar cells in IPF and assessed PIPE-791 efficacy to block type I alveolar epithelial cells (AT1) apoptosis and fibrotic gene expression, induced by conditioned media from IPF tissue.

Methods

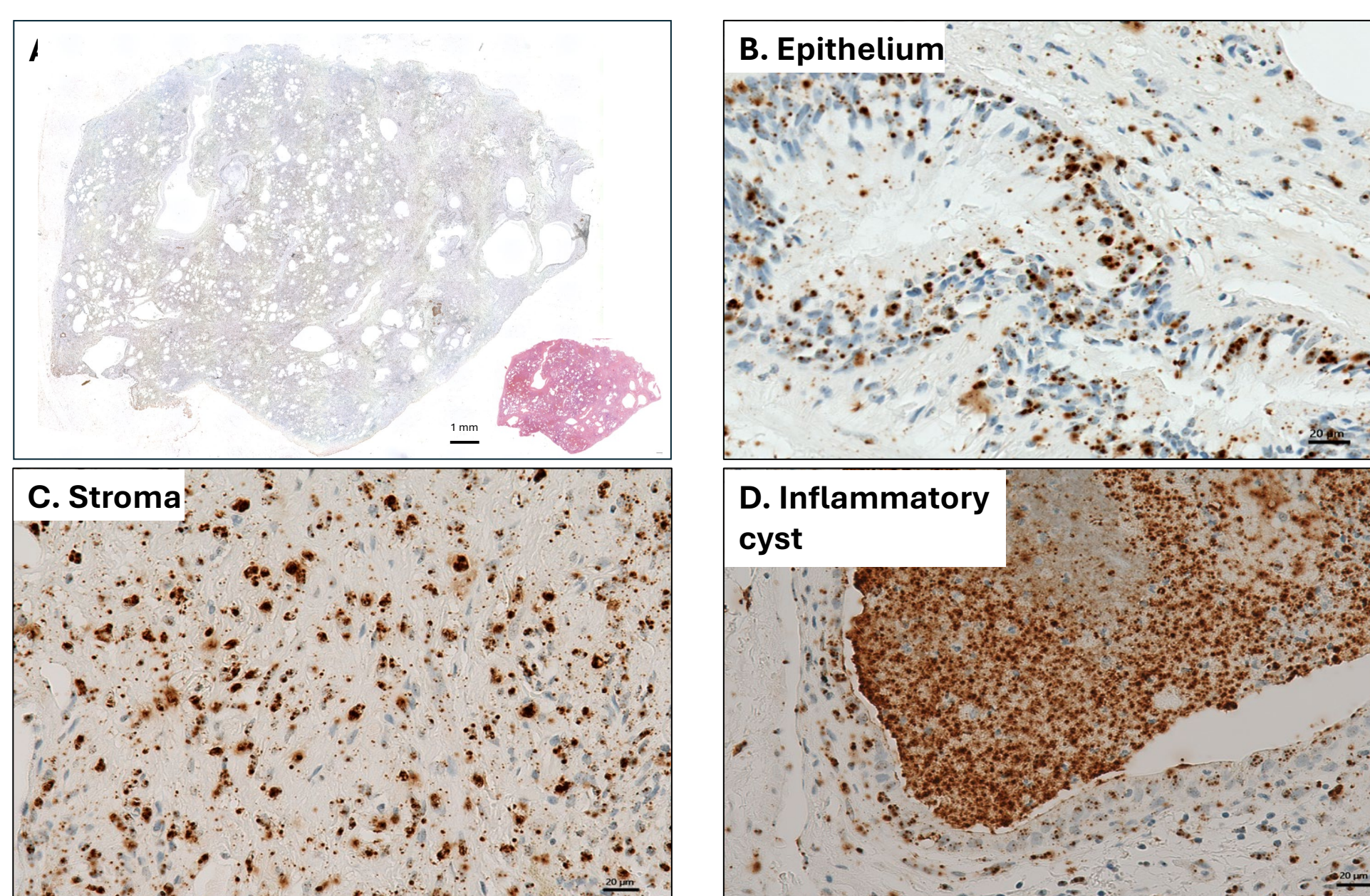
- ***In situ* hybridization:** Cell-specific expression of *LPAR1* was interrogated informatically using the IPF cell atlas and confirmed by *in situ* hybridization in IPF lung tissues, using RNAscope™ Multiplex Fluorescent V2 (ACD/Biotechne) or HCR™ Pro RNA-ISH (Molecular Instruments).
- ***Ex vivo* conditioned media studies.** Cryopreserved precision cut lung slices (PCLS; Anabios) were cultured in vehicle or 300 nM PIPE-791 for 6 days. Conditioned media was collected at study end and frozen (-80 °C). Cultured human AT1 cells were preincubated with vehicle or 300 nM PIPE-791 for 8 h, then 50% of culture media exchanged for conditioned media from IPF or healthy donor PCLS. In some cases, PIPE-791 was added to reach a 300 nM. After 1 day, cells were fixed and immunostained for podoplanin (Pdpn), cleaved caspase 3 (Asp175), and Hoechst. Apoptosis was measured as a percent cleaved caspase 3+/Pdpn+/Hoechst+ of total Pdpn+/Hoechst+. Apoptosis was measured as a percent cleaved caspase 3+/Pdpn+/Hoechst+ of total Pdpn+/Hoechst
- **mRNA expression:** The effects of PIPE-791 on *COL1A1*, *COL3A1*, *TIMP1* and *SERPINE1* mRNA expression were measured in IPF PCLS by quantitative RT-PCR.

LPAR1 expression data from the IPF cell atlas



LPAR1 expression in IPF tissue

Figure 2. In situ hybridization performed using HCR™ Pro RNA-ISH confirms high *LPAR1* expression in **A)** whole lung (inset; H&E), **B)** lung epithelium, **C)** fibrotic stroma and **D)** regions of alveolar inflammation, consistent with findings published on the IPF Cell Atlas. IPF donor tissue was obtained from a 55 year old African American male, smoker with BMI = 20.3 and anoxia as the cause of death.



Cellular LPAR1 expression in IPF tissue

Probe name	Cell type
COL1A1	Fibroblasts
CD68	Macrophages
AGER	AT1
SFTPC	AT2
MUC5B	Goblet>club cells>AT2 (secreted mucin)

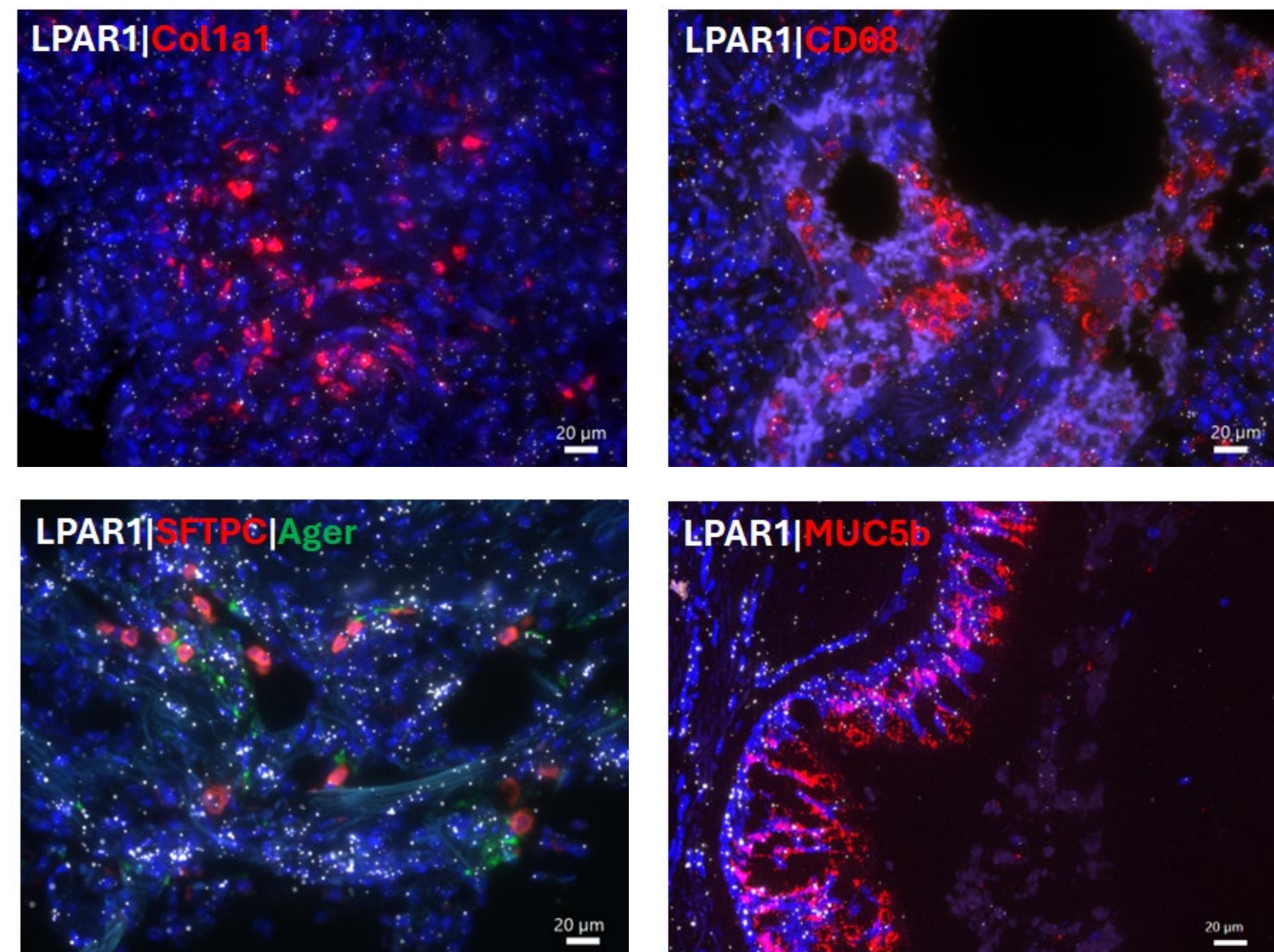


Figure 3. A) RNA probes and **B)** *In situ* hybridization was performed using RNAscope™ Multiplex Fluorescent V2 demonstrating LPAR1 expression by fibroblasts (COL1A1+), macrophages (CD68+) and AT1 (AGER)+ and AT2 (SFTPC+/MUC5B+) epithelial cell subtypes. Same IPF donor tissue as Figure 2.

PIPE-791 inhibits epithelial cell apoptosis

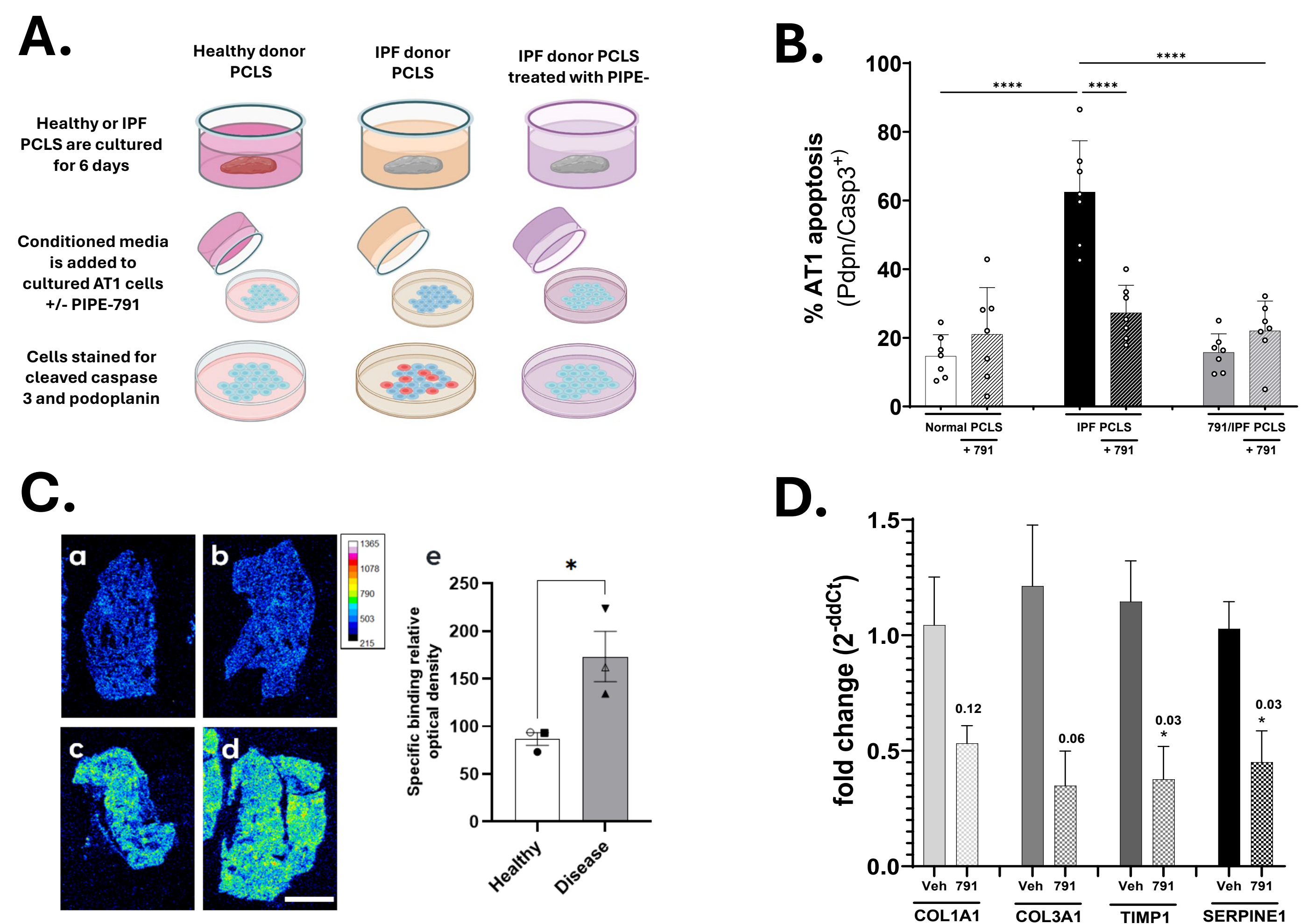


Figure 4. A) Experimental Design. Conditioned media (after 6 div) from healthy or IPF-donor precision cut lung slices was collected and applied to cultured AT1 cells. **B) IPF-donor conditioned media induced apoptosis is inhibited by PIPE-791; no effect of normal PCLS conditioned media.** IPF-donor conditioned media induces apoptosis, whereas normal PCLS conditioned media does not. Apoptosis was inhibited when PIPE-791 (300 nM) was added to AT1 cells prior to IPF conditioned media addition or when PIPE-791 (300 nM) was added to IPF-PCLS during media conditioning. Mass spec analysis indicates that PIPE-791 in the IPF-PCLS conditioned media was below limit of quantification (<1.05 nM, n=4). **C) LPAR1 binding in human lung sections:** Representative images illustrating the total binding of an LPAR1 radioligand ([3H]-PIPE497 (20nM)) on human lung sections from healthy donor (**a, b**) and patients (**c, d, e**). **e** Bar graph with individual donors showing an increased in LPA1 specific binding signal in IPF patients compared to healthy donors. Scale bar = 0.5 mm. **D) PIPE-791 treatment of PCLS reduces fibrosis-related transcripts** including *COL1A1*, *COL3A1*, *TIMP1*, and *SERPINE1*. **Donor:** 64yo, Caucasian female, IPF; symmetric bronchiectasis. Coronary artery disease. Hypertension 0-5yrs; Skin - squamous basal cell carcinoma on nose, removed in 2020.

Conclusions

LPAR1 localized primarily to airway epithelial cells, type II alveolar cells and alveolar macrophages, with significant expression in *COL1A1* positive fibroblasts and MUC5B expression epithelial cells within the fibrotic lung parenchyma. Consistent with *LPAR1* expression in epithelial cells and fibroblasts, PIPE-791 significantly reduced IPF condition media stimulated epithelial cell apoptosis and profibrotic factor expression by IPF tissue. Increase *LPAR1* expression was also confirmed by radioligand binding in IPF tissue. These data demonstrate that *LPAR1* is expressed by multiple cell lineages in IPF tissue and PIPE-791 may be therapeutic in IPF via effects on multiple epithelial, stromal and inflammatory cell types.