



A tri-culture alveolar organoid platform for human-relevant mechanistic studies of fibrotic lung disease

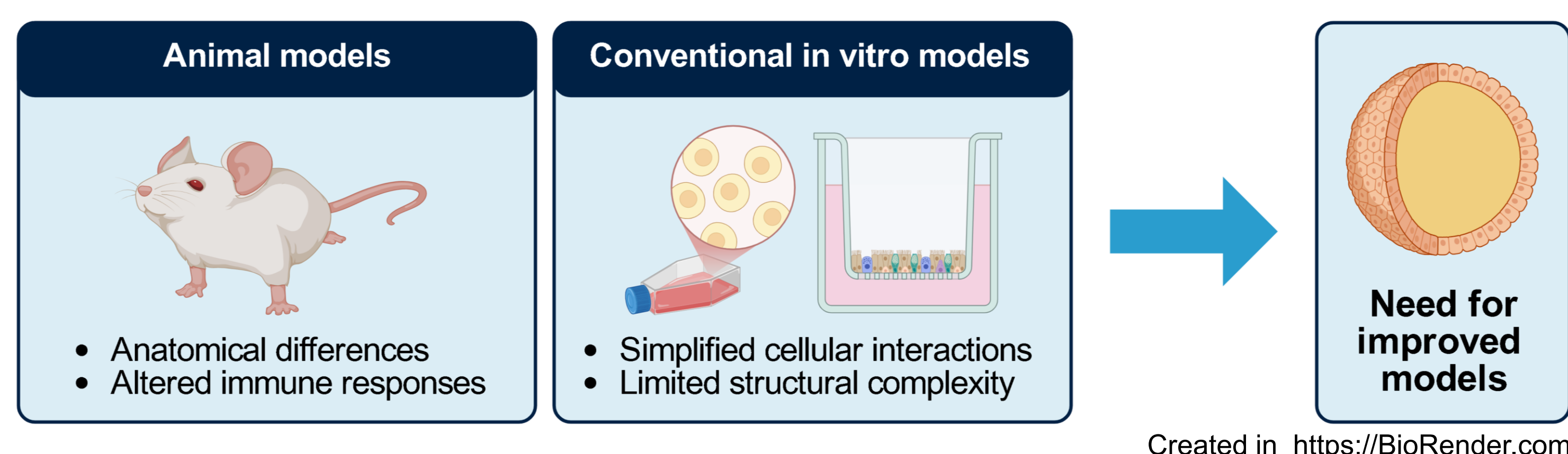
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Human 3D tri-culture alveolar-like organoids are a promising experimental model for studying fibrosis-associated cellular interactions.

Why Model the Lung in 3D?

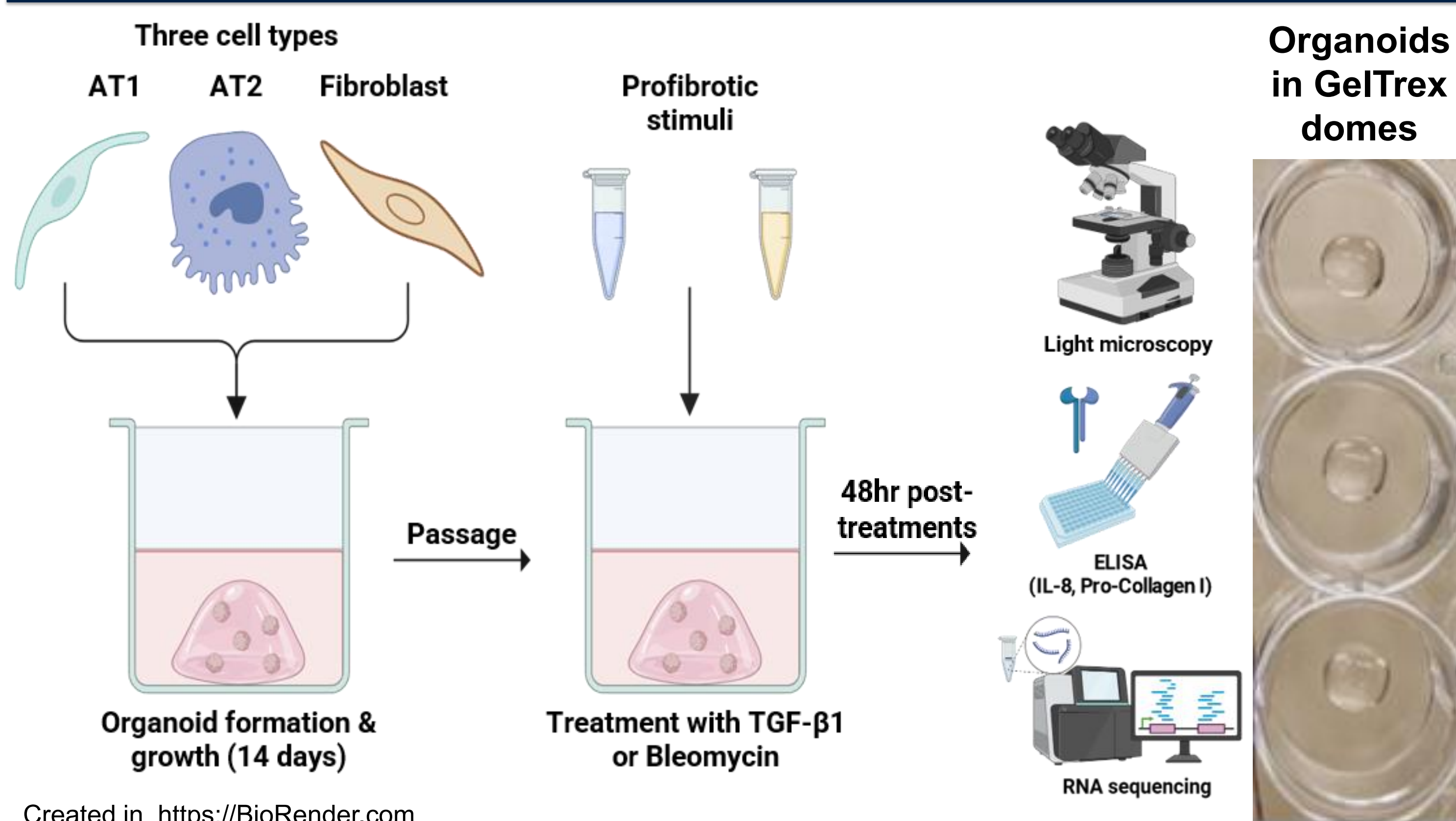
- Fibrotic interstitial lung diseases (fILD) involve **repeated epithelial injury and abnormal repair**, leading to progressive lung scarring¹
- Epithelial-fibroblast interactions drive injury and repair but are **difficult to study in conventional 2D cultures**
- 3D organoid models** better capture tissue architecture, multicellular interactions, and the surrounding matrix²



Objectives

- Develop and characterize a **defined tri-culture alveolar-like organoid model**
- Assess the model's ability to capture distinct fibrosis-associated cellular responses

Methods



Results

Tri-culture organoids form and progressively grow over 14 days

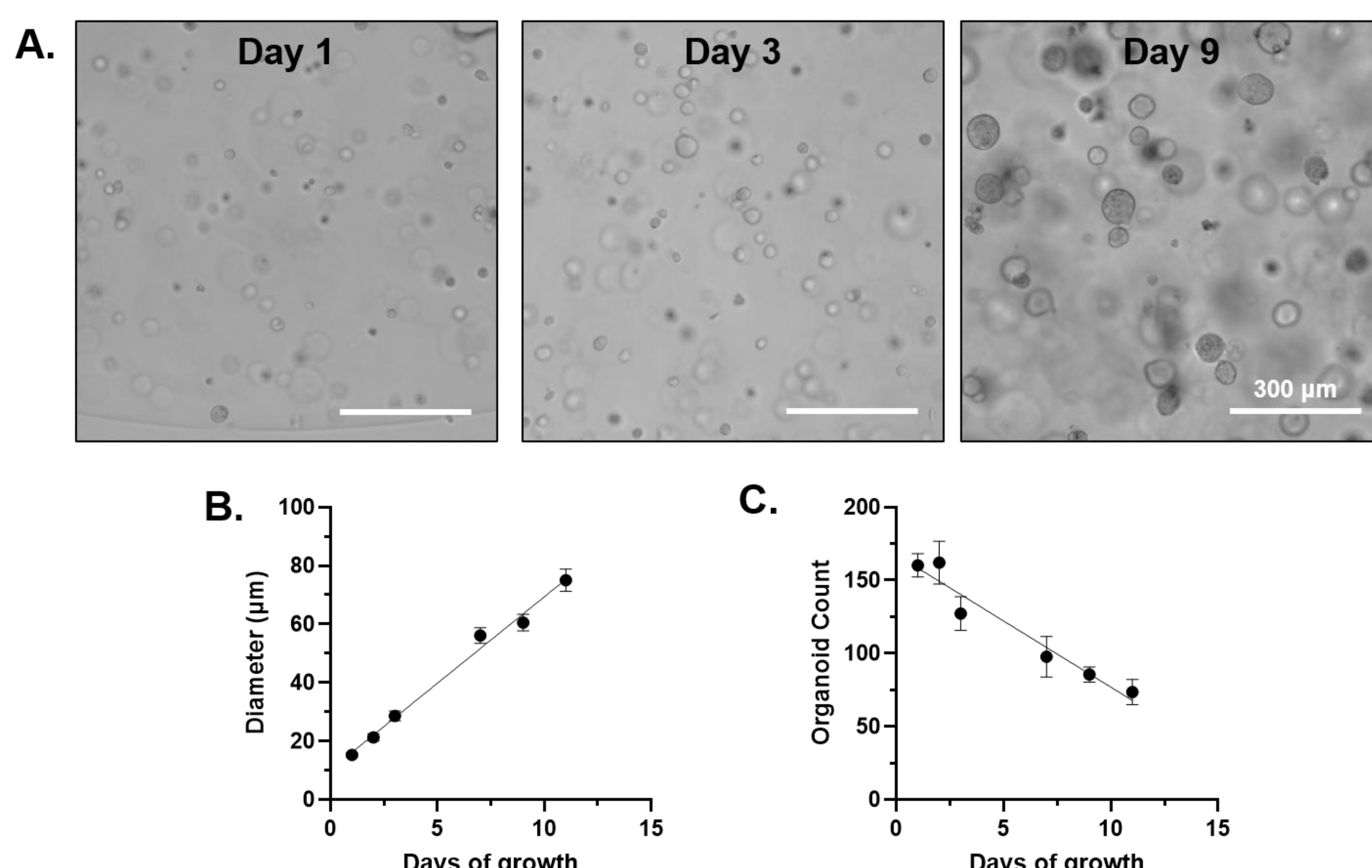


Figure 1. Growth of tri-culture alveolar-like organoids over time. Representative brightfield images show organoid formation at days 1, 3, and 9. Organoids progressively increased in diameter while organoid counts decreased over 14 days of culture.

Results

Organoids retain distinct epithelial and stromal compartments

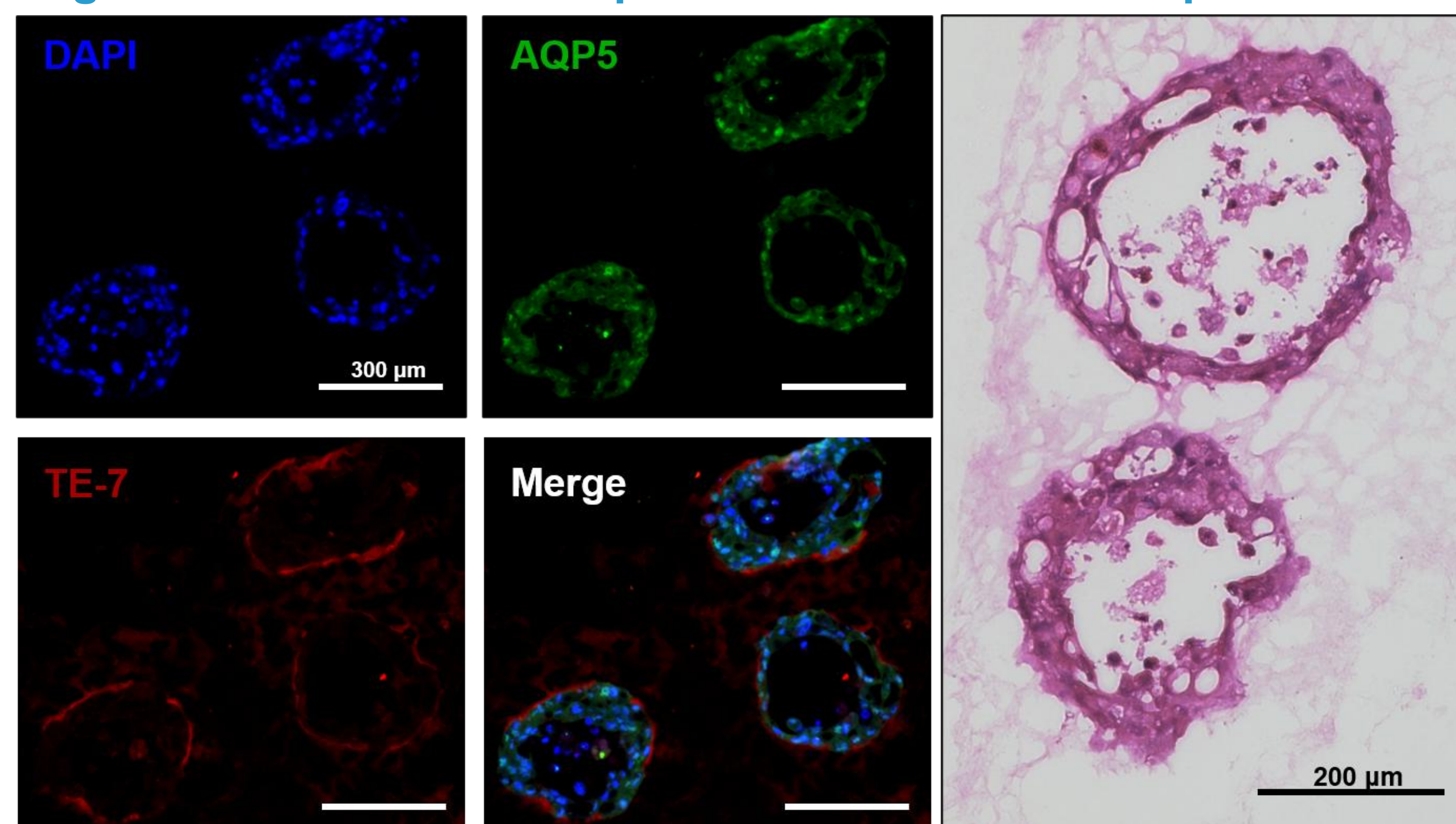


Figure 2. Cellular composition and morphology of tri-culture alveolar-like organoids. AQP5 and TE-7 staining identified epithelial and fibroblast populations, while H&E staining showed hollow organoid structures.

Profibrotic stimuli induce inflammatory and matrix-associated responses.

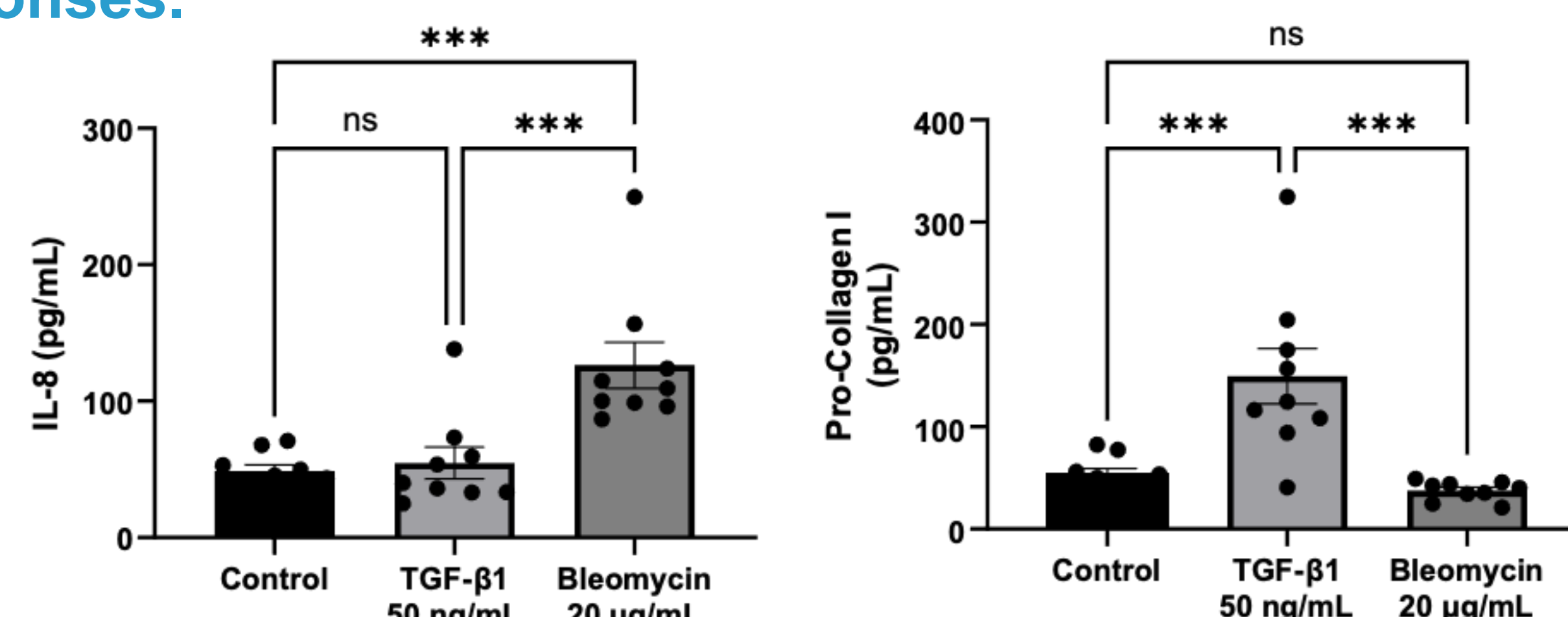


Figure 3. TGF-β1 and bleomycin induce distinct responses in tri-culture alveolar-like organoids. Bleomycin increased IL-8 secretion, whereas TGF-β1 increased pro-collagen I secretion.

Treatments induce distinct fibrosis-associated gene expression

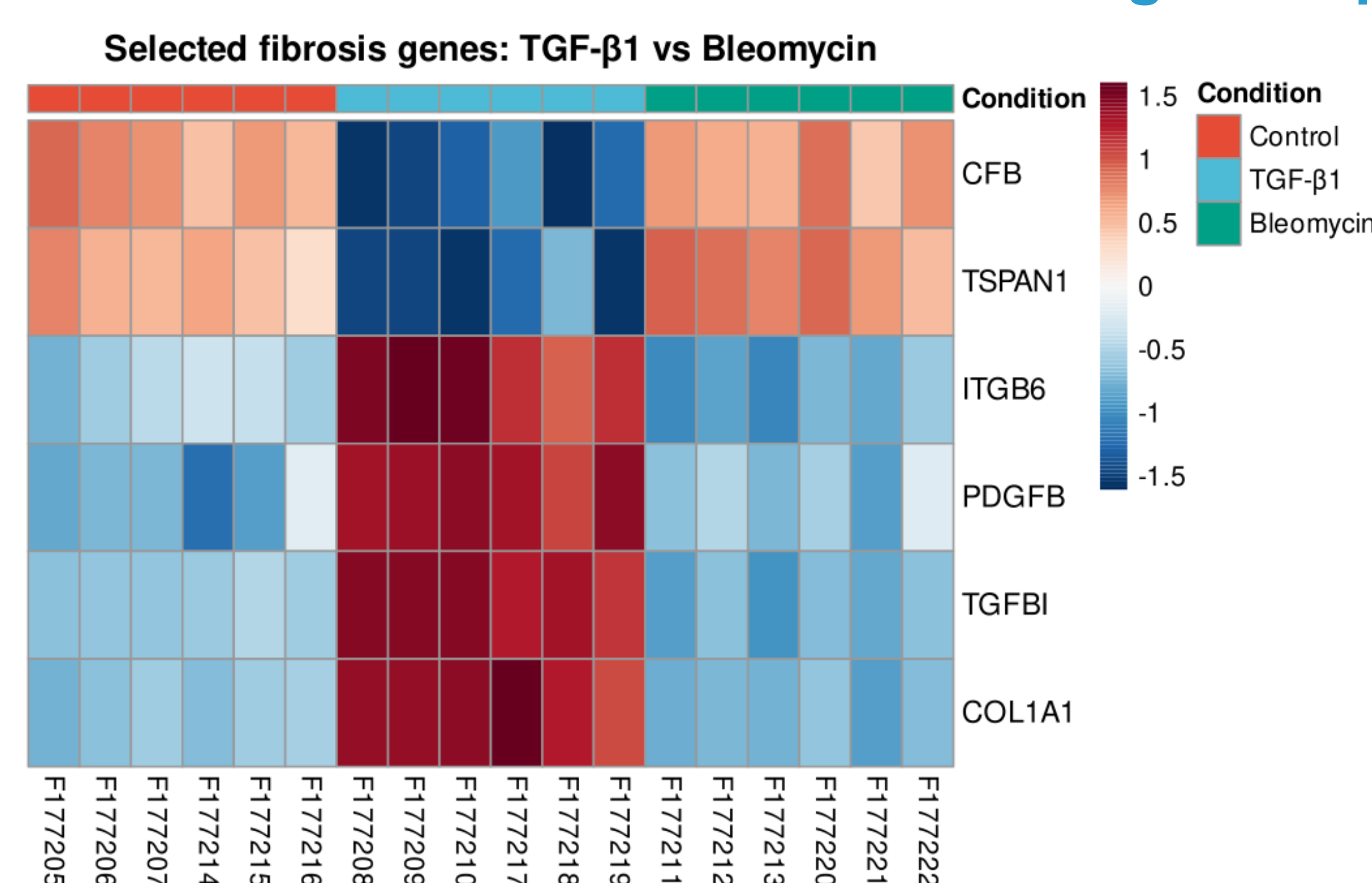


Figure 4. Fibrosis-associated gene expression following TGF-β1 and bleomycin treatment. TGF-β1 showed higher expression of *ITGB6*, *PDGFB*, *TGFBI*, and *COL1A1*, reflecting a matrix-remodelling response.

Limitations

- Established cell lines do not capture donor-specific differences
- The model lacks immune and endothelial cells, and 48-hour treatments represent acute responses

Next Steps

- Incorporate primary cells and additional cell types to increase biological complexity
- Expand the model's applications by exploring environmental exposures and fibrosis treatment drugs

References:

- Chambers RC, Mercer PF. Mechanisms of alveolar epithelial injury, repair, and fibrosis. *Ann Am Thorac Soc* 2015;12 Suppl 1:S16-20. <https://doi.org/10.1513/AnnalsATS.201410-448MG>.
- Kong J, Wen S, Cao W, Yue P, Xu X, Zhang Y, et al. Lung organoids, useful tools for investigating epithelial repair after lung injury. *Stem Cell Res Ther* 2021;12:95. <https://doi.org/10.1186/s13287-021-02172-5>.
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