

Generation of 3D Multicellular Primary Nasal Organoids and Cell-Line Alveolar Organoids

Tak Machiri^{1,2}, Tony Guo^{1,2,3}, Yidan Meng², Elia Kim^{1,2}, Emmanuel Osei^{1,3}, Min Hyung Ryu²

What are Primary Organoids?

- Organoids are **three-dimensional**, self-organizing cell cultures that model functional and structural features of their tissue of origin
- Unlike conventional cell lines, patient-derived organoids better **retain donor-specific characteristics**.
- Nasal epithelial organoids model the upper airway, including **basal, ciliated, and mucus-producing cells**, while alveolar organoids represent the **lung epithelium**.
- These models provide more **physiologically relevant** platforms for studying disease mechanisms and potential treatments, as they better recapitulate the **3D architecture** of the human body

Aims

- Establish **primary HNEC organoids** and **cell-line-derived triple co-culture** alveolar-like organoids
- Assess organoid formation**, growth, morphology, and reproducibility.
- Evaluate inflammatory and fibrotic responses following **TGF-β1** and **bleomycin** treatment by measuring **IL-8** and **procollagen I** production.
- Replace cell-line fibroblasts with primary fibroblasts to generate preliminary **primary-derived alveolar organoids** (PDAOs)

How to Generate Multicellular Organoids

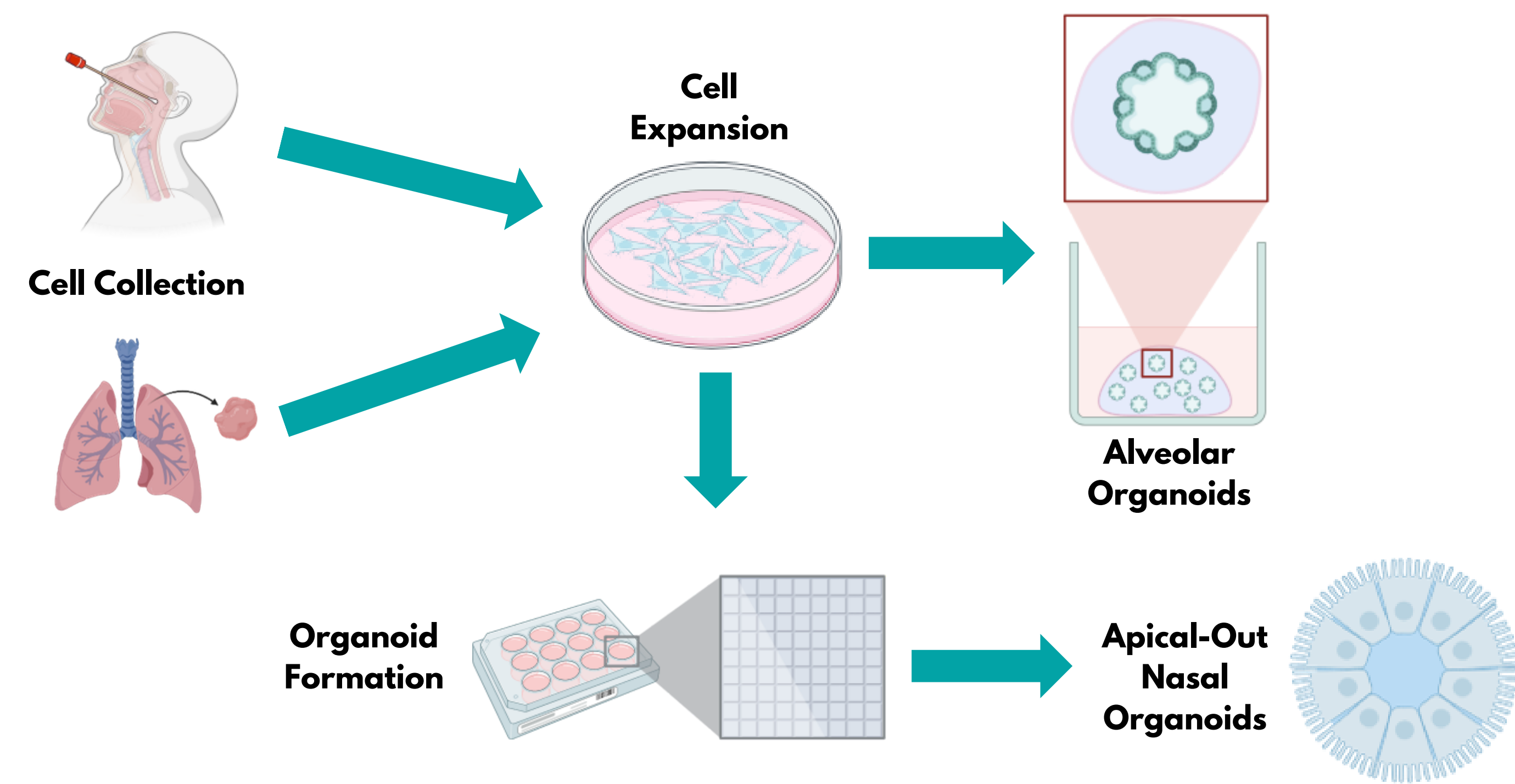


Figure 1. Primary nasal epithelial cells and alveolar cell populations are collected and expanded prior to three-dimensional culture. Expanded cells are then directed toward either alveolar organoid formation within an extracellular matrix or apical-out nasal organoid generation.

Effect of Profibrotic Stimuli

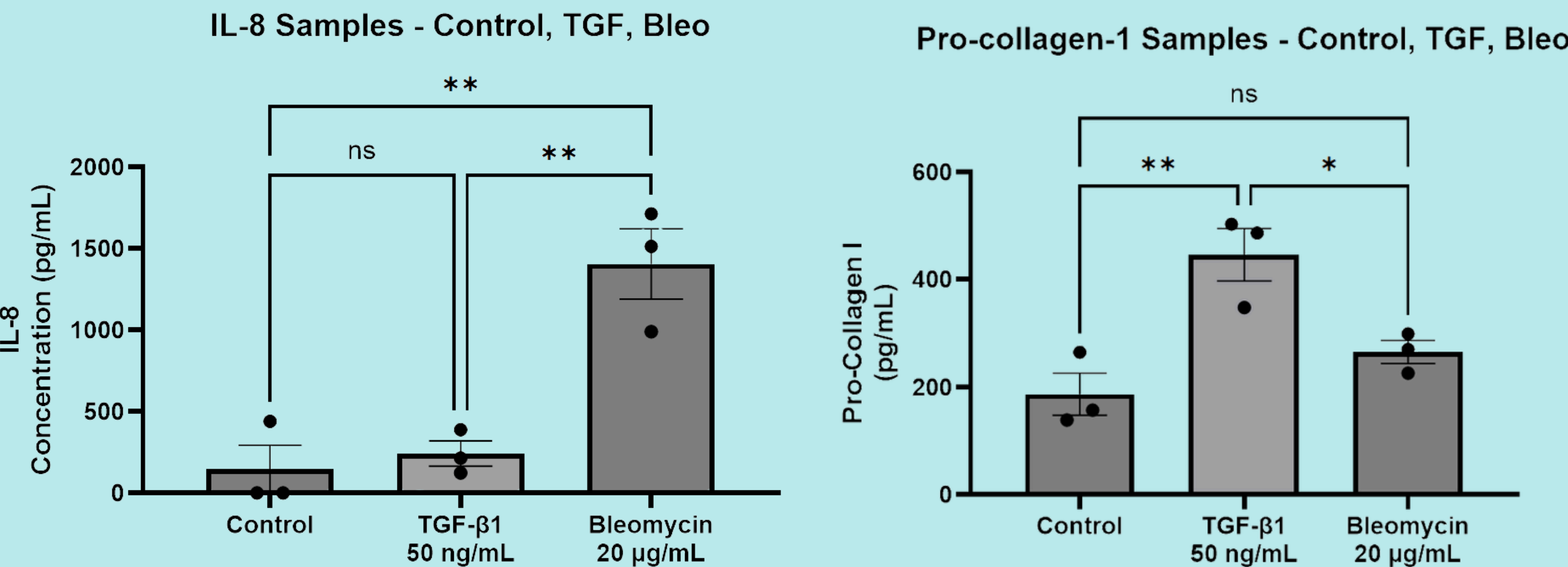


Figure 2. Effects of TGF-β1 and bleomycin on inflammatory and fibrotic marker release. IL-8 and pro-collagen I concentrations were measured in control samples and samples treated with TGF-β1 (50 ng/mL) or bleomycin (20 μg/mL). Bleomycin significantly increased IL-8 compared with both the control and TGF-β1 groups, whereas TGF-β1 significantly increased pro-collagen I compared with the control and bleomycin groups (n=3).

Organoid Characterization

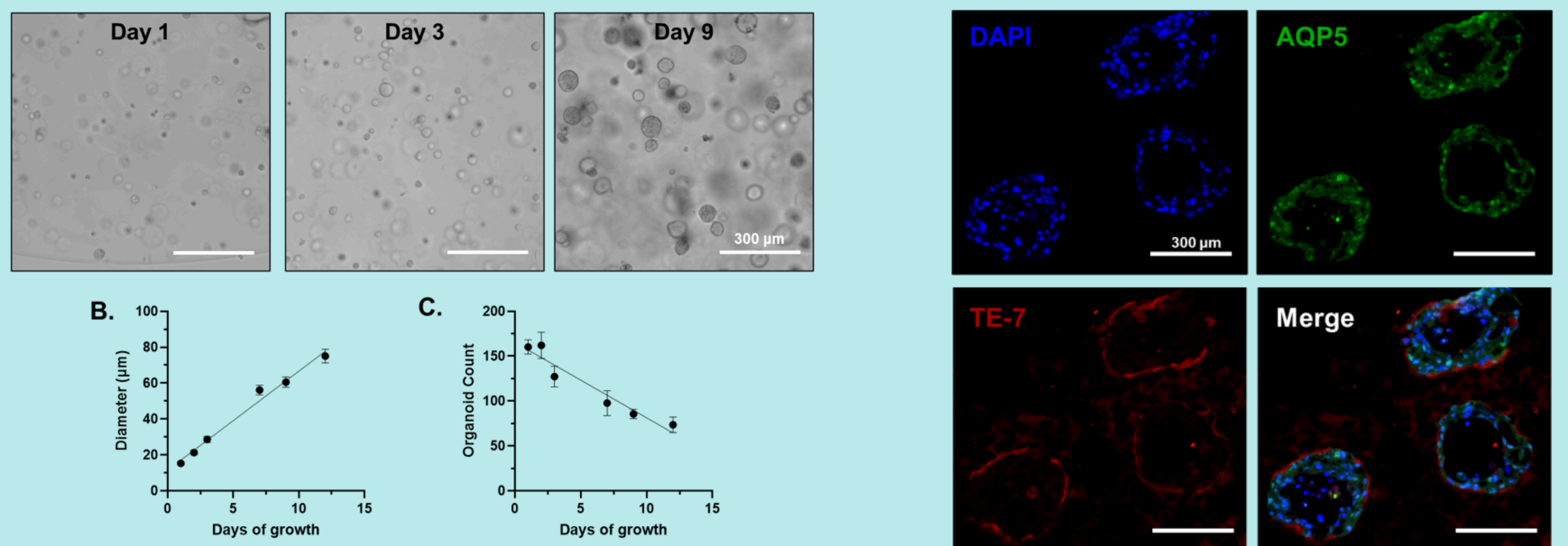


Figure 3. Growth and characterization of triple co-culture alveolar-like organoids. Brightfield images show progressive organoid growth from Day 1 to Day 9, with increasing diameter and decreasing organoid number over 14 days, consistent with growth and fusion. Immunofluorescence staining for DAPI, AQP5, and TE-7 confirms the presence of epithelial and fibroblast populations within the organoids.

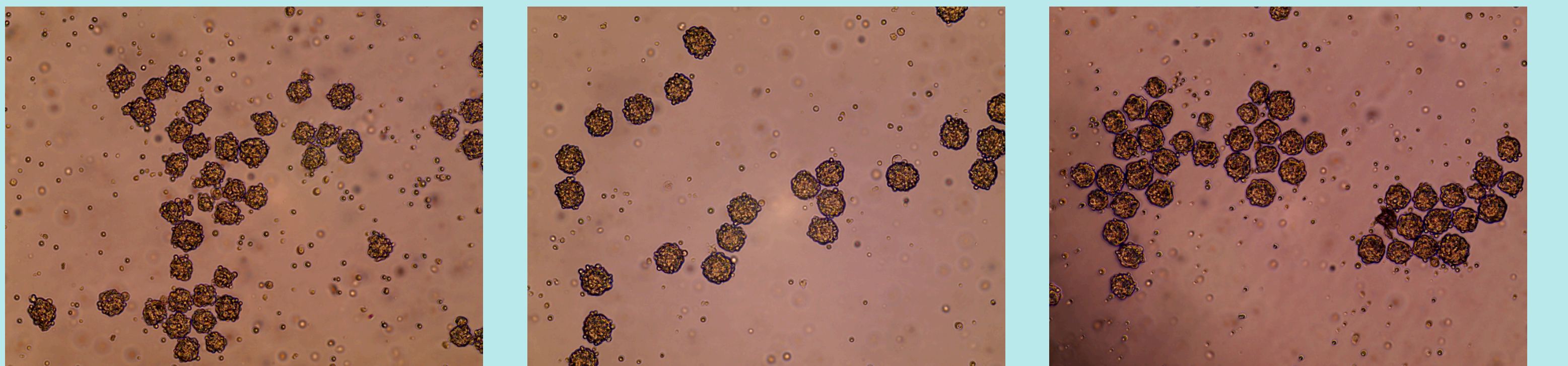
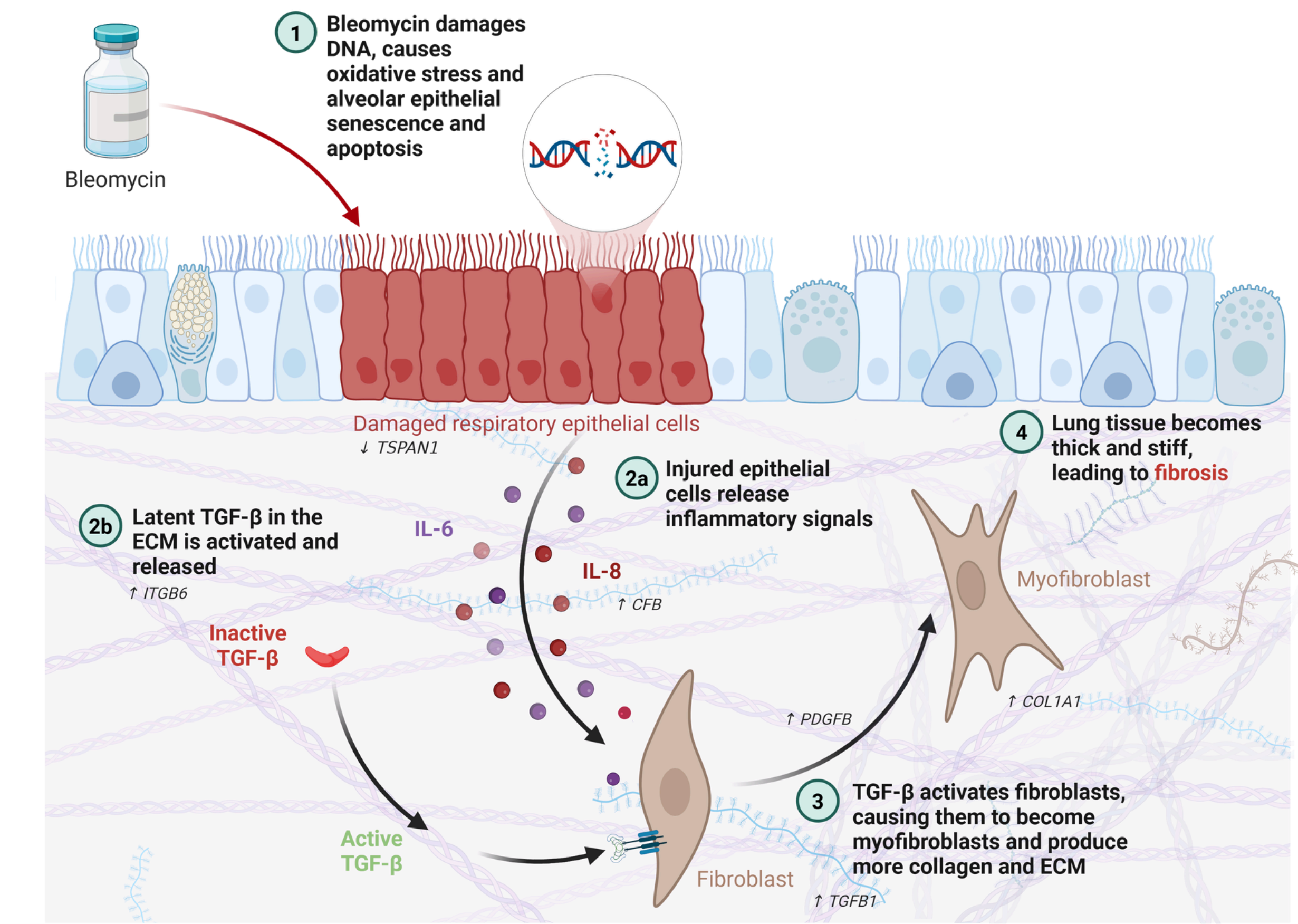


Figure 4. Donor 1 HNEC Organoids Figure 5. Donor 2 HNEC Organoids Figure 6. Donor 3 HNEC Organoids

Bleomycin Mechanism of Action



RNA Sequencing Data

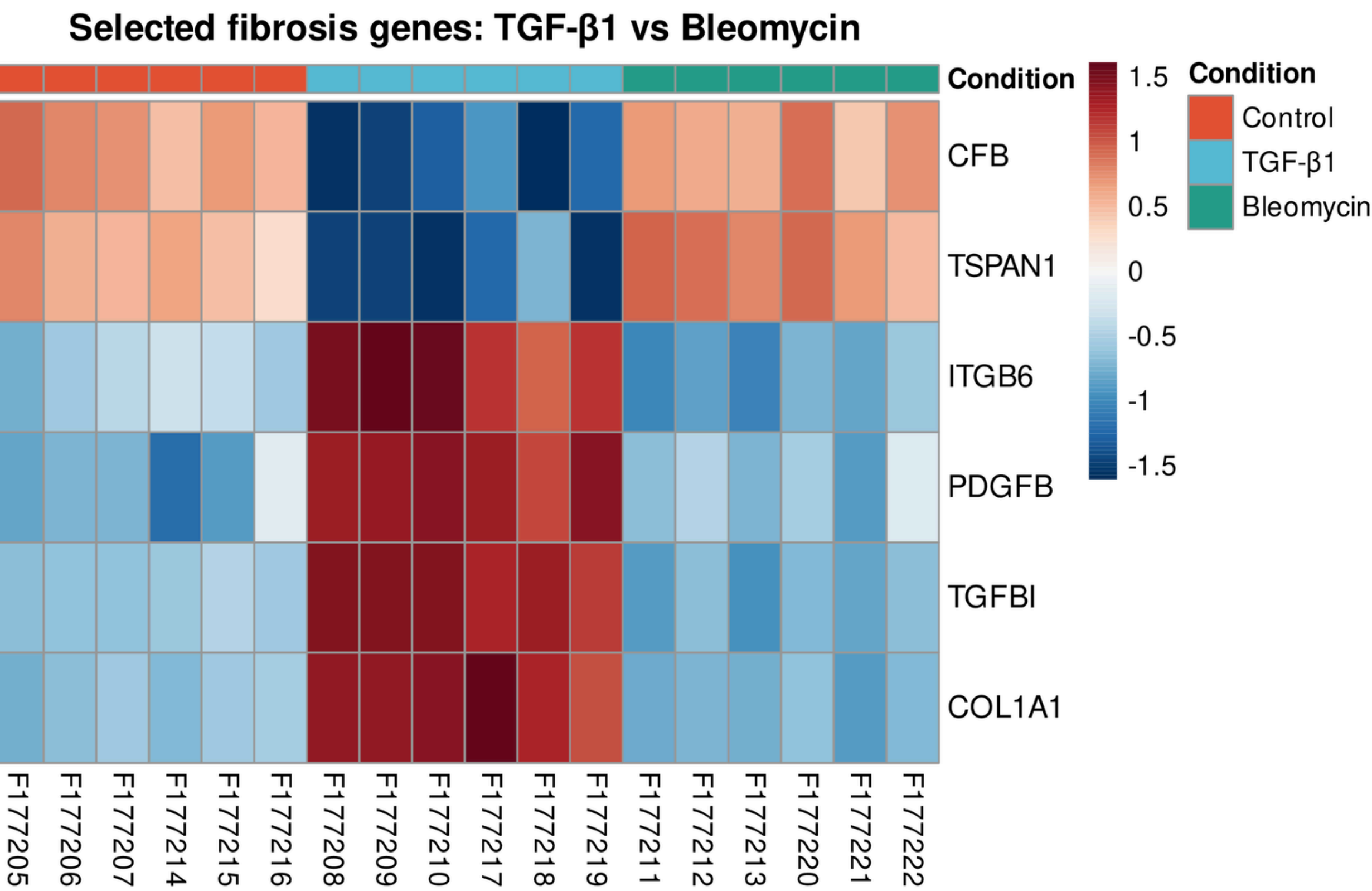


Figure 7. Selected fibrosis-associated gene expression following TGF-β1 and bleomycin treatment. TGF-β1 showed stronger expression of ITGB6, PDGFB, TGFBI, and COL1A1, consistent with a more profibrotic, matrix-remodelling response.

What's Next?

- Further optimize and characterize patient-derived HNEC and alveolar organoids using tissue-specific epithelial markers.
- Repeat** bleomycin and TGF-β1 treatments with **PDAOs**.
- Treat alveolar organoids with TGF-β1 and bleomycin, followed by **nintedanib**, to determine whether the drug can reduce or **reverse injury-associated changes** in organoid morphology, IL-8 release, and pro-collagen I production.

Key Sources & Acknowledgements

1.Mohammed SM, Al-Saedi HFS, Mohammed AQ, Amir AA, Radi UK, Sattar R, et al. Mechanisms of Bleomycin-induced Lung Fibrosis: A Review of Therapeutic Targets and Approaches. Cell Biochem Biophys. 2024 Sep 1;82(3):1845–70. doi:10.1007/s12013-024-01384-9
2.Ramezanpour M, et al. Biochim Biophys Acta Gen Subj. 2026;1870(6):130936 doi:10.1016/j.bbagen.2026.130936