

Capturing the complex molecular composition of human lungs by spatial multi-omic analysis and integration

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Background

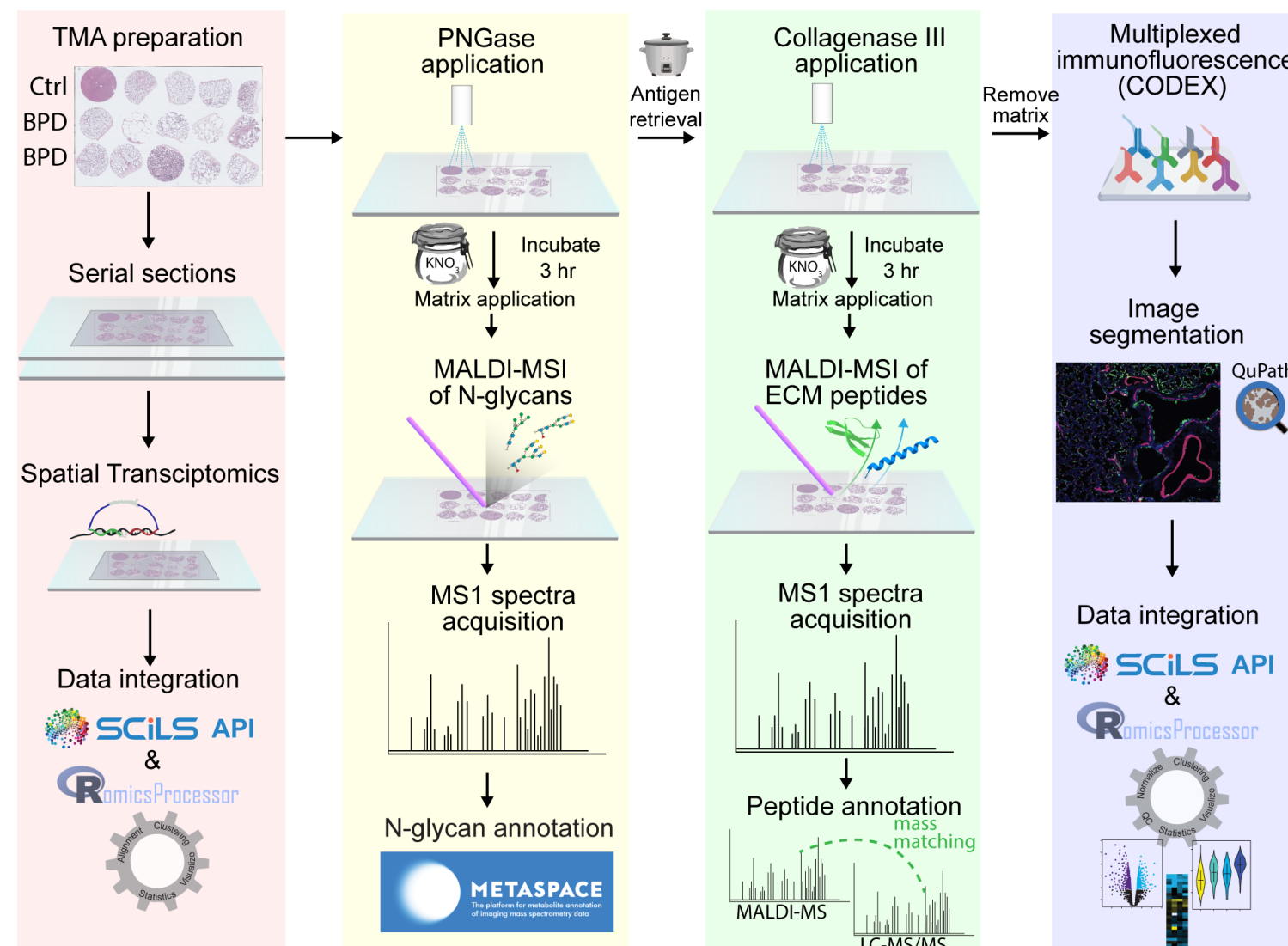
The lung is a highly heterogeneous organ composed of various functional regions, which are difficult to resolve with bulk-omics techniques. This complicates the investigation of fatal lung diseases like bronchopulmonary dysplasia (BPD). Therefore, many spatially resolved omics assays have been applied to understand this complexity, such as spatial transcriptomics (ST), multiplexed immunofluorescence (MxIF), and matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI). However, in multimodal studies, connections between the data from separate analyses are generally inferred. A combined approach on the same sample provides a comprehensive biological picture of functional regions in heterogeneous tissues.

Overview

Tissue microarrays (TMAs) were prepared from 14 formalin-fixed paraffin-embedded human lung samples. The TMAs were sectioned serially at 5 µm thicknesses onto Xenium and indium tin oxide (ITO)-coated glass slides.

The imaging workflow was as follows:

- 1) Spatial transcriptomics – Xenium
- 2) Spatial N-glycomics – MALDI-MSI
- 3) Spatial extracellular matrixomics (ECM-omics) – MALDI-MSI
 - Bulk ECM-omics – LC-MS/MS (on adjacent tissue sections)
- 4) Multiplexed Immunofluorescence – Co-detection by indexing (CODEX)



MALDI-MS analysis:

- MALDI-MSI performed on a Bruker timsTOF fleX in positive ion mode 800-3,000 m/z, mass resolution 40k @ 1000 m/z, and step size 20 µm

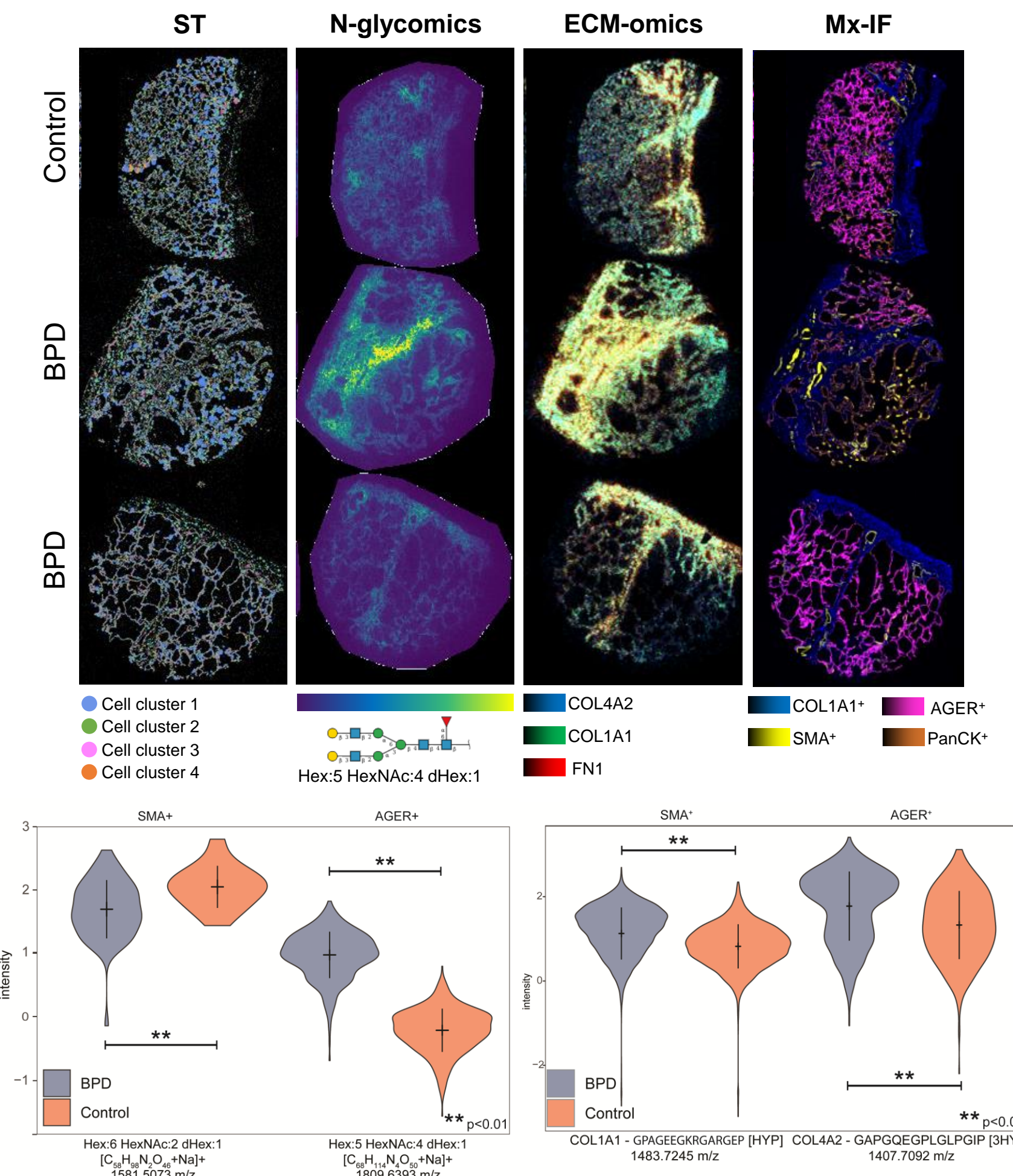
LC-MS/MS:

- Peptides were separated with 50 µm diameter, C18 column at 100 nL/min using a 30 min gradient from 8% to 35% acetonitrile with 0.1% formic acid.
- Analysis was performed on a Bruker timsTOF SCP operated in DDA-PASEF mode. For DDA-PASEF acquisition, one MS1 scan was followed by 8 PASEF MS/MS scans per acquisition cycle.

Multimodal analysis reveals molecular signatures within lung functional regions

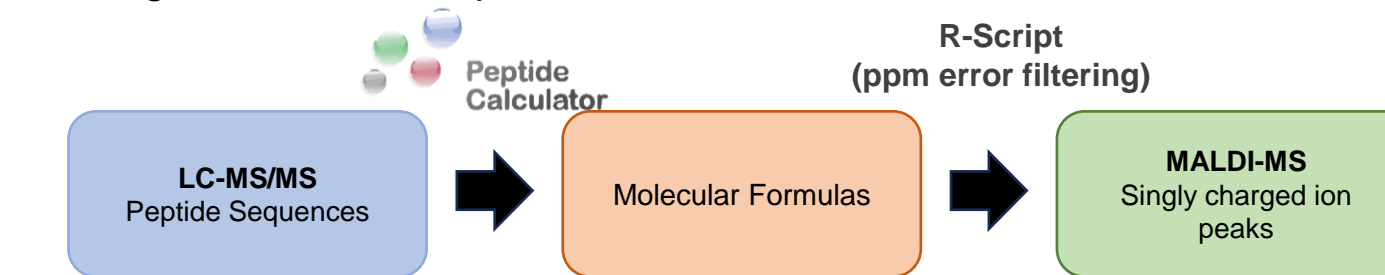
Since all datasets were collected from serial sections of the same sample, images were easily co-registered and visualized in Bruker's SCiLS software. This combined analysis permitted the localization of specific N-glycans and ECM peptides within distinct cellular regions.

The MxIF images were segmented based on distinct IF markers into cell populations and functional tissue units, and changes in the N-glycan and ECM composition were assessed using our SCiLS API to RomicsProcessor pipeline designed for the analysis of multimodal MSI datasets (**MP 449**). In the BPD tissues, smooth muscle cells (SMA⁺) displayed higher abundances within the MxIF images. These SMA⁺ regions also displayed increases in COL4A2 and decreases in high mannose glycans (i.e., Hex:6 HexNAc:2 dHex:1). In alveolar type 1 cells (AGER⁺), we detected an increase COL1A1 and COL1A2 peptides and specific N-glycans including Hex:5HexNAc:4 dHex:1 in the BPD tissues.



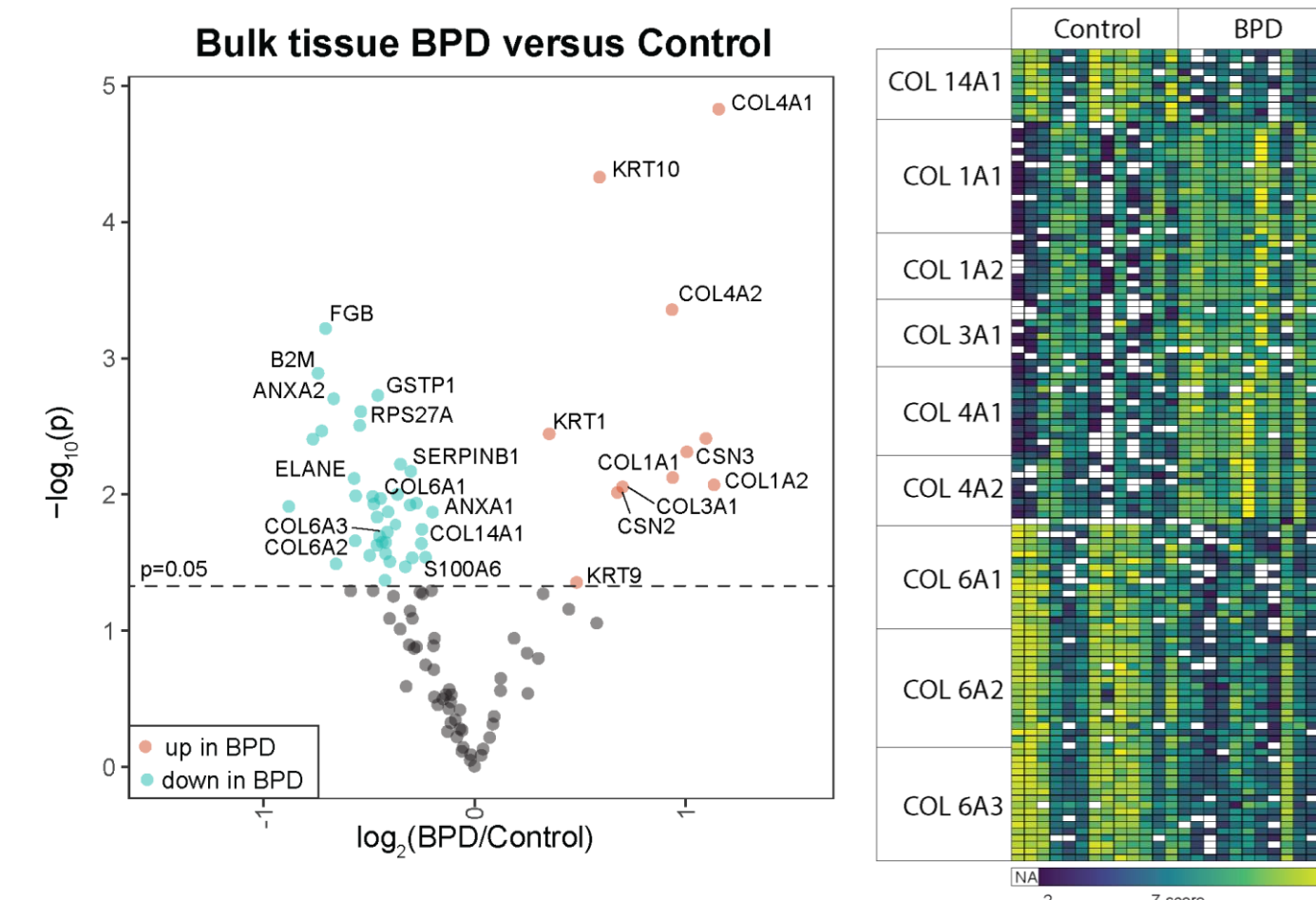
Library building through bulk proteomics

Bulk ECM-omics data was also used to create a peptide database for annotation of MALDI-MS ion peaks. First, identified ECM peptides (Fragpipe; unspecified enzyme) were converted to molecular formulas using the peptide calculator API. Then, the MALDI-MSI peak list is cross-referenced to the computed m/z values for [H]⁺, [Na]⁺, and [K]⁺ ions using a custom R-script.



Proteomic changes detected through bulk Collagenase III digestion

The use of Collagenase III for a parallel bulk digestion of the ECM proteins provided a detailed view of the ECM composition between the healthy and BPD human lung tissue. These results suggest large scale remodeling of the ECM proteins, notably, COL1, COL3, and COL4 displayed a significant increase in BPD tissues, whereas COL6 and several ECM-associated proteins decreased in the BPD tissues.



Conclusions and future work

- Uniting multimodal datasets permitted the identification of proteomic and N-glycomics changes within cellular niches. For example:
 - We identified changes in abundance in COL4 peptides using bulk proteomics and verified alterations in the spatial abundance of COL4A2 in the AGER⁺ alveolar cells.
- Uniting this datasets also allowed investigation of the molecular changes associated with an increase in specific cell markers
 - We noted an increase in SMA⁺ cells in the BPD tissues and a correlated increase of COL1A1
- Next, we will integrate ST images through segmentation of specific cell-states.