

Tito Panciera¹, Francesca Zanconato¹, Michelangelo Cordenonsi¹, Mattia Forcato¹, Giada Vanni¹, Silvio Bicciato¹, Julian Preciado², Dan McGuire², Saskia Ilcisin², Katrina van Raay², Megan Vandenberg², Margaret Hoang², Michael Patrick², Shanshan He², Joseph Beechem² and Stefano Piccolo¹

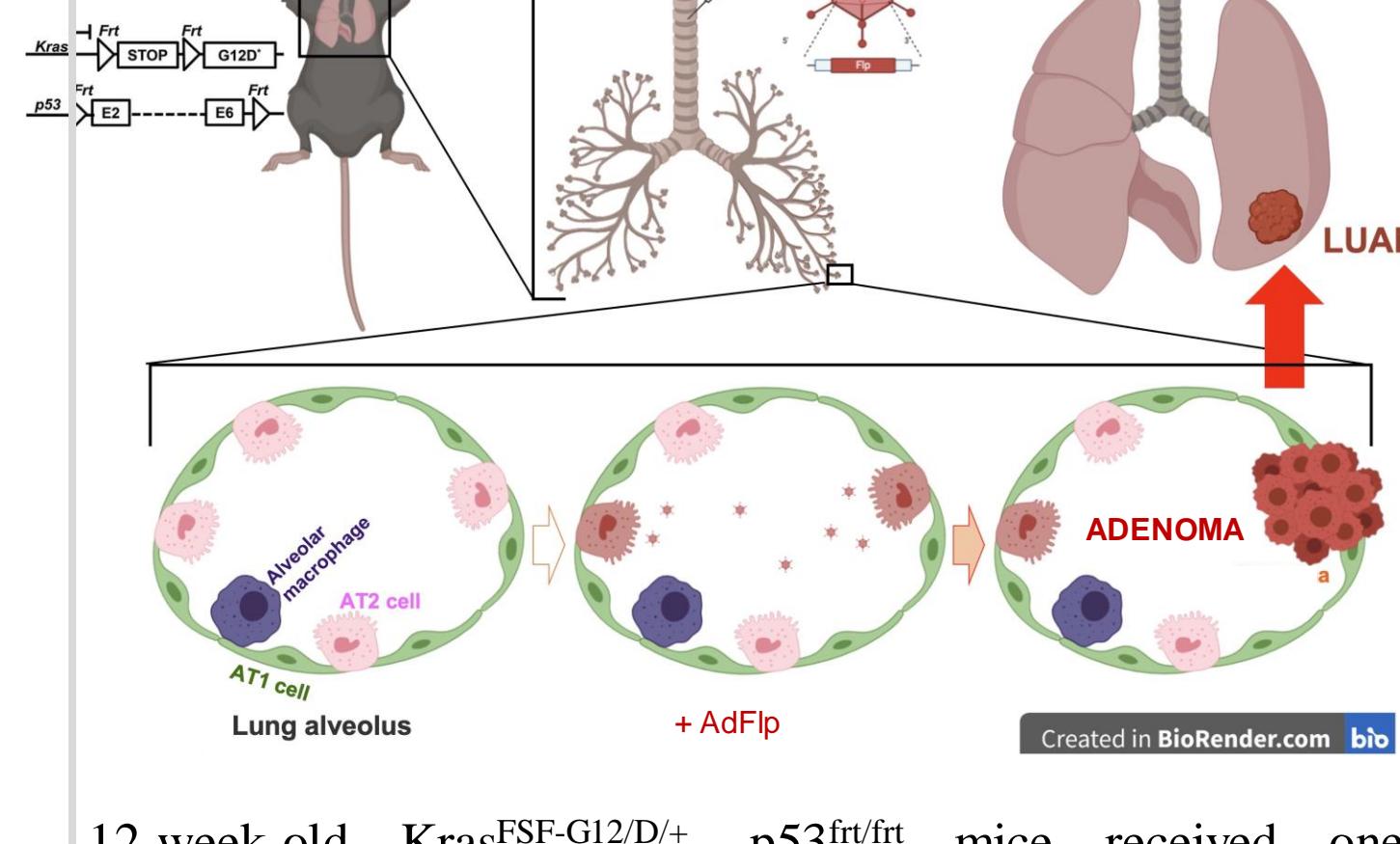
¹Department of Molecular Medicine (DMM), University of Padua School of Medicine, Via Ugo Bassi 58/B, 35131 Padua, Italy.
²NanoString[®] Technologies, Seattle WA.

Abstract

Despite the ever-expanding weaponry of molecularly targeted and immunotherapy approaches, lung adenocarcinoma continues to stand as the leading cause of cancer-related mortality. One of the most frequently occurring mutations in lung cancer are KRAS mutations. For many years, these mutations were considered untreatable. However, the recent development of chemical inhibitors that specifically target oncogenic variants of Ras, particularly the commonly mutated KRAS-G12D isoform, represents a significant breakthrough in targeted therapeutics. An appealing aspect of KRAS mutation targeted drugs is their ability to alert the immune system and enhance its ability to attack cancer cells. Nevertheless, the tissue-level mechanisms underlying the cell-autonomous and non-cell-autonomous effects of KRas-G12D inhibitors are poorly understood. Additionally, the effectiveness of KRas-G12D inhibitors in lung cancer models remains unknown. To address these gaps in knowledge, we aimed to investigate tumor regression and the body's ability to combat established tumors. Specifically, we analyzed the spatial interactions between cancer cells and the surrounding tissue microenvironment during the process of tumor eradication mediated through KRas-G12D inhibitors.

Methods

Induction of lung adenocarcinoma (LUAD) in mice



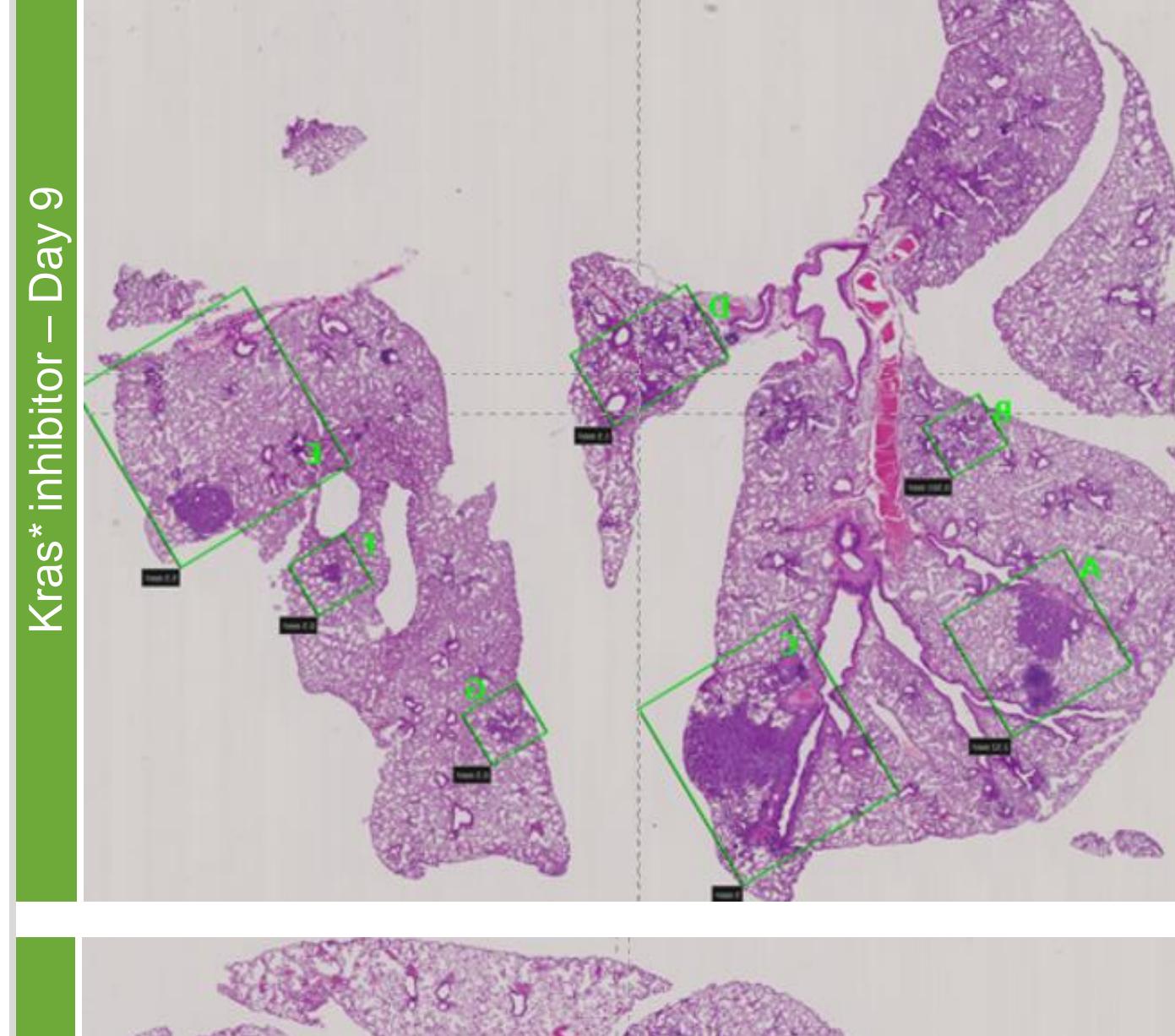
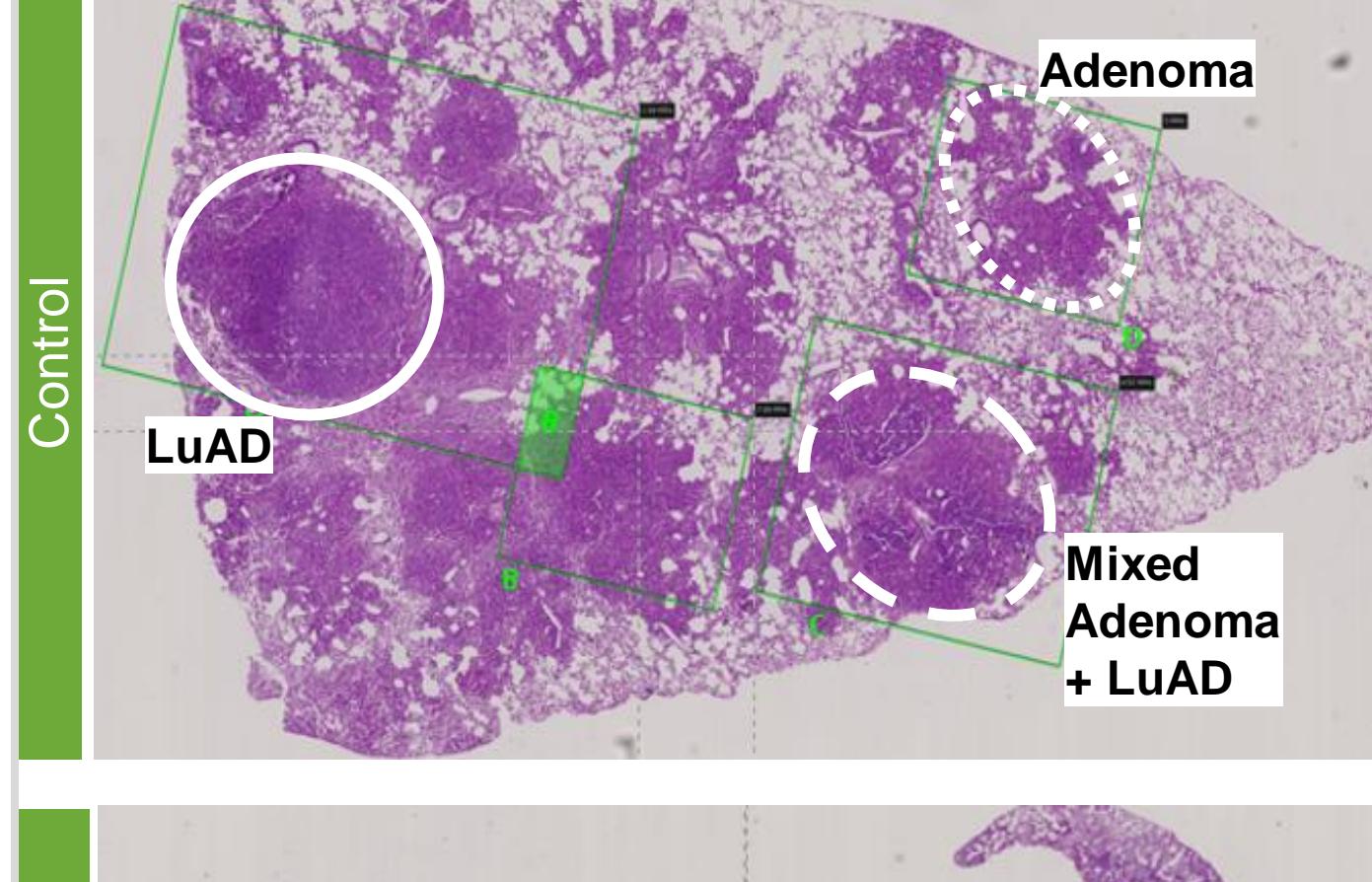
12-week-old Kras^{FSF-G12D/+}, p53^{fl/fl} mice received one intratracheal instillation of adenoviral particles serotype 5, an established tool to target epithelial cells of the lung encoding Flp recombinase^{1,2,3}. Activation of mutant Kras^{G12D} expression and KO of p53 trigger the formation of multifocal adenomas from AT2 cells in 2-3 weeks and of LuADs in 2 months.

Pharmacological treatment and tissue processing

Starting two months after AdFlp instillation, mice received daily injections of a pharmacological inhibitor of mutant Kras. Control mice were euthanized at Day 0 (n=6), and treated mice were sacrificed after 9 or 15 days of treatment (n=5). Lungs were perfused with PBS, fixed in 4% PFA for 24 hours and room temperature and paraffin embedded. 2 consecutive 4µm-thick sections were used for H&E staining and CosMx SMI 1,000-plex RNA assay.

Sample overview

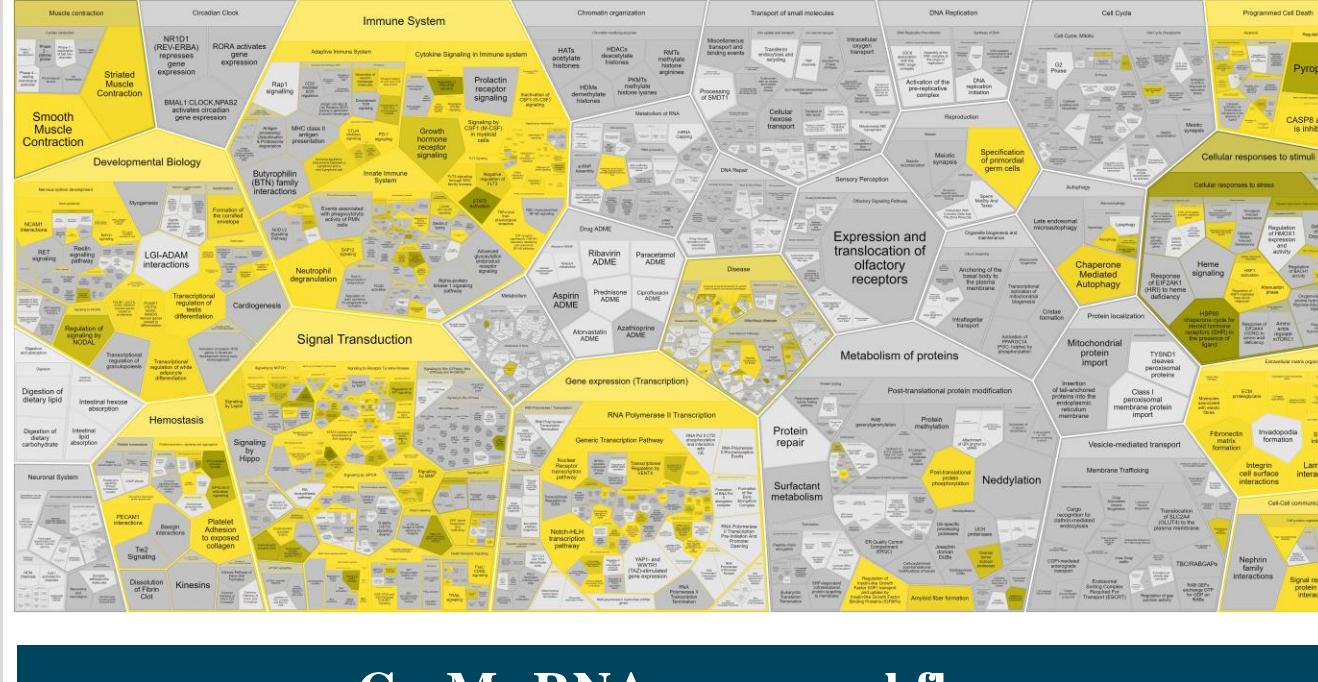
Prior to Kras inhibitor treatment, mice displayed both LuAD and lung adenomas. Lesion size and histological grade progressively decrease during pharmacological treatment with Kras inhibitor.



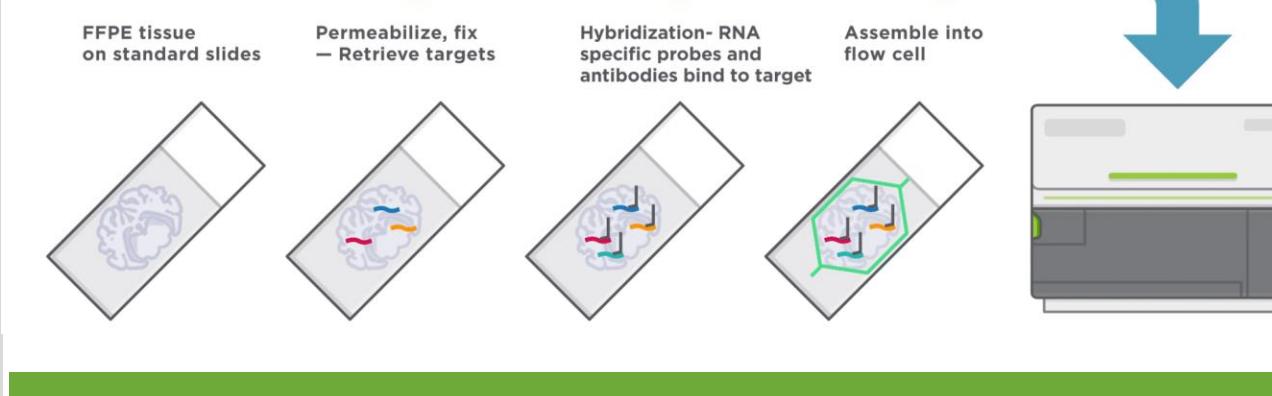
Kras^{*} inhibitor – Day 15

Panel provide broad coverage of biological pathways

CosMx SMI 1,000-plex Mouse Universal Cell Characterization panel is the highest-plex RNA panel with wide coverage of key marker genes in immunology, signaling transduction and cell response to stimulation during oncology process

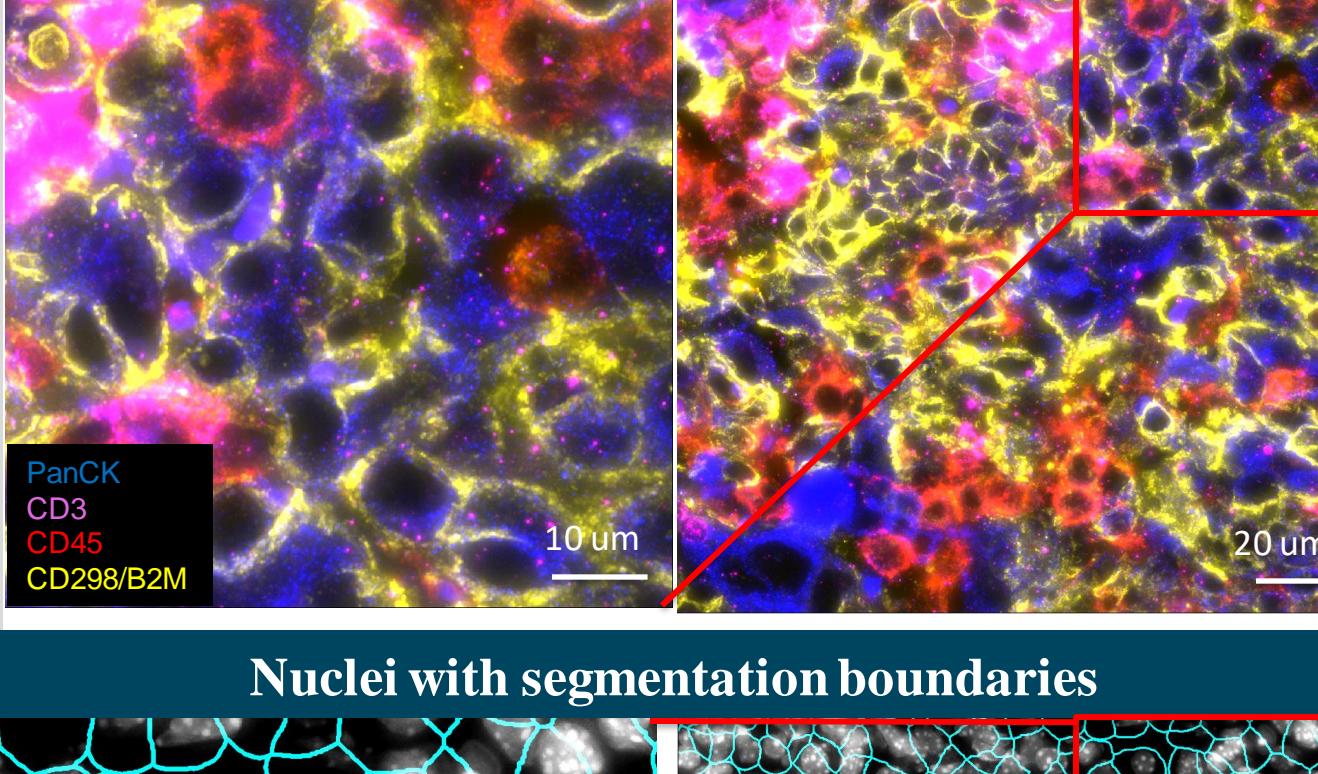
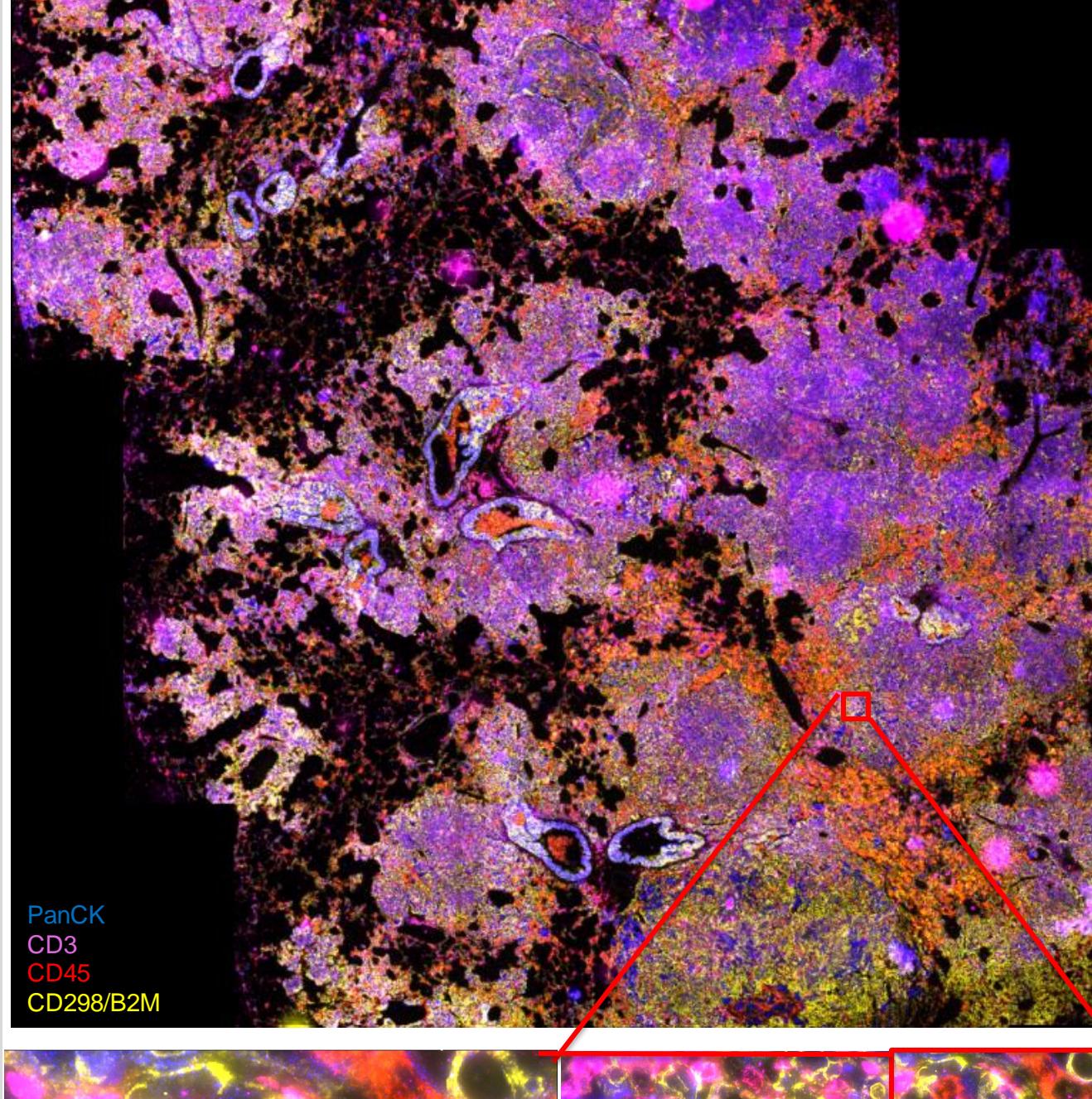


CosMx RNA assay workflow

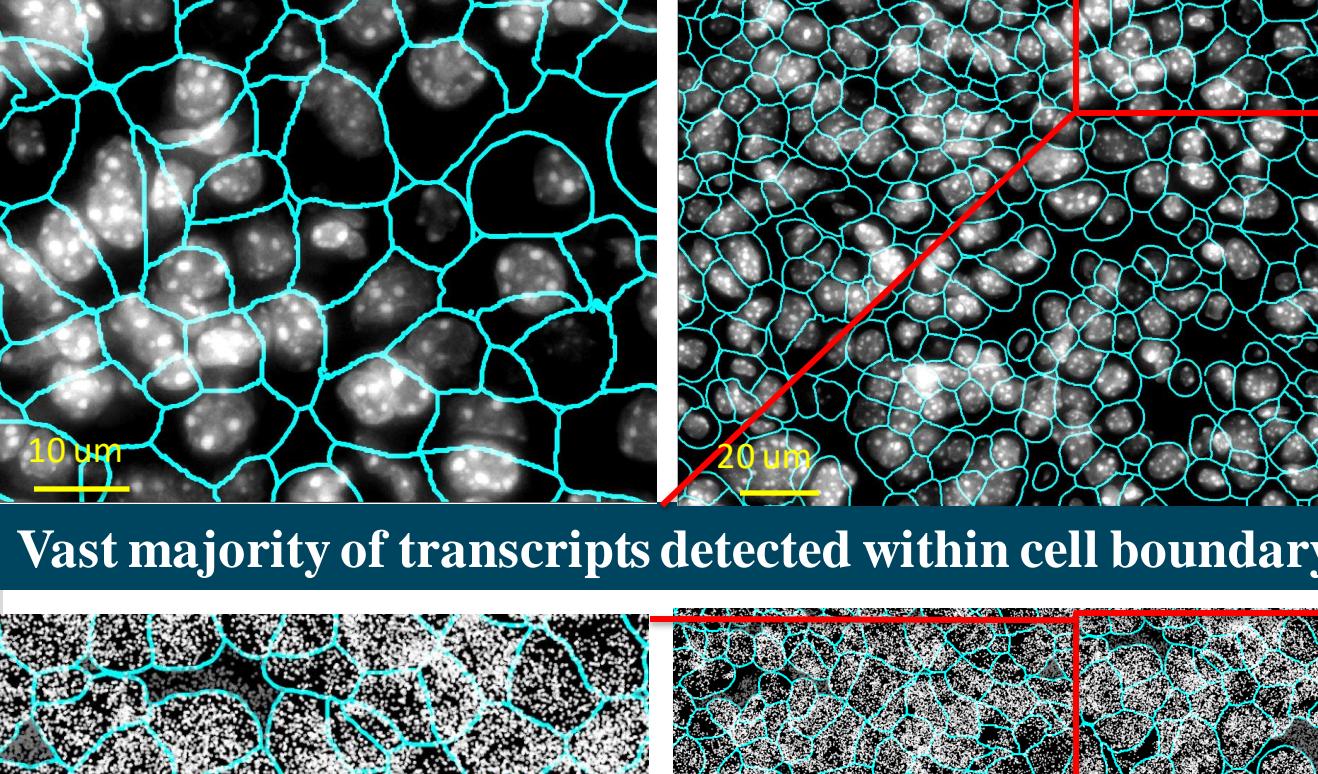


Results

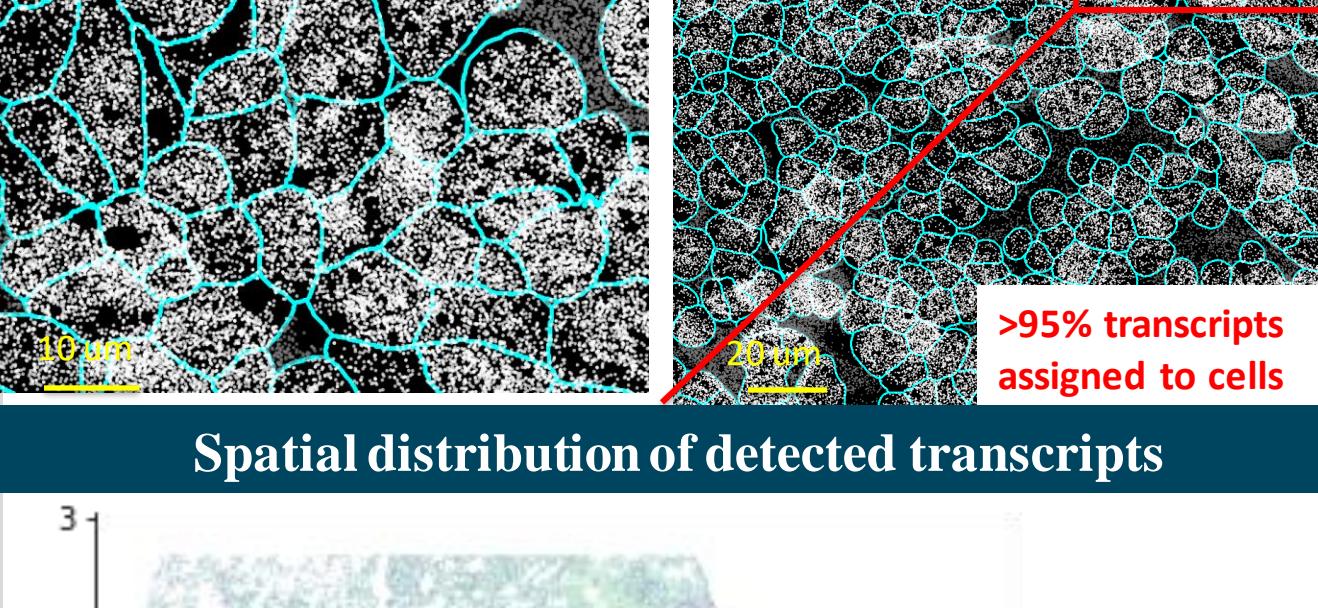
Morphological staining aids in segmentation accuracy



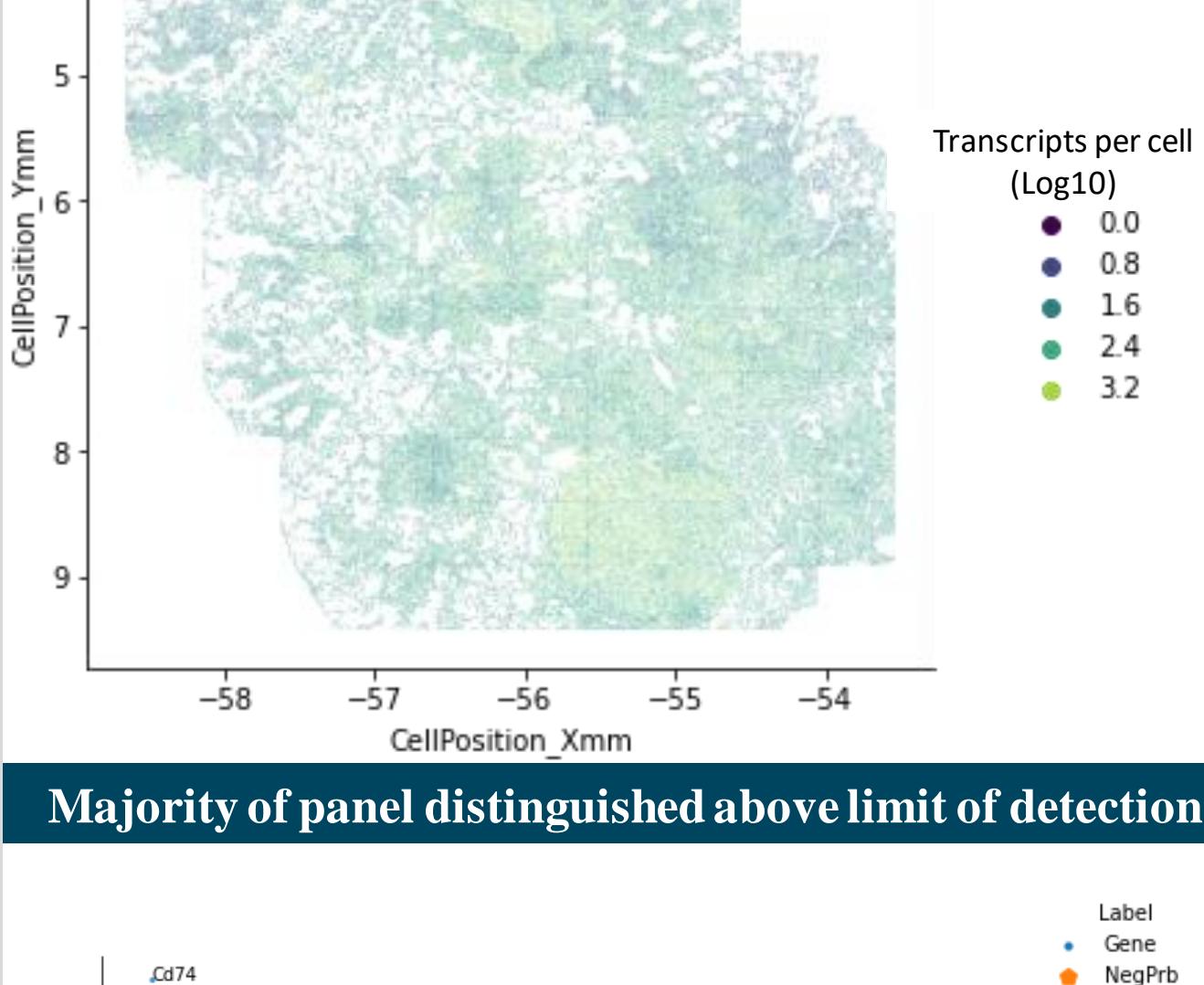
Nuclei with segmentation boundaries



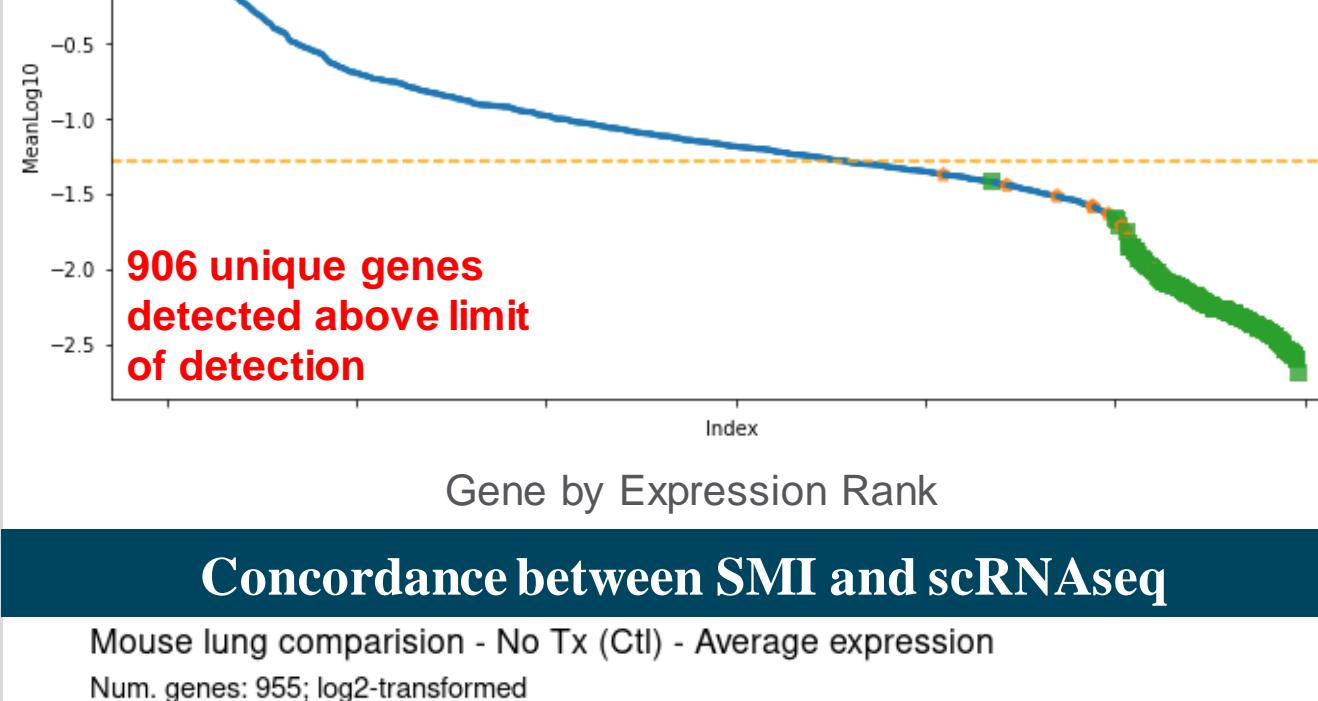
Vast majority of transcripts detected within cell boundary



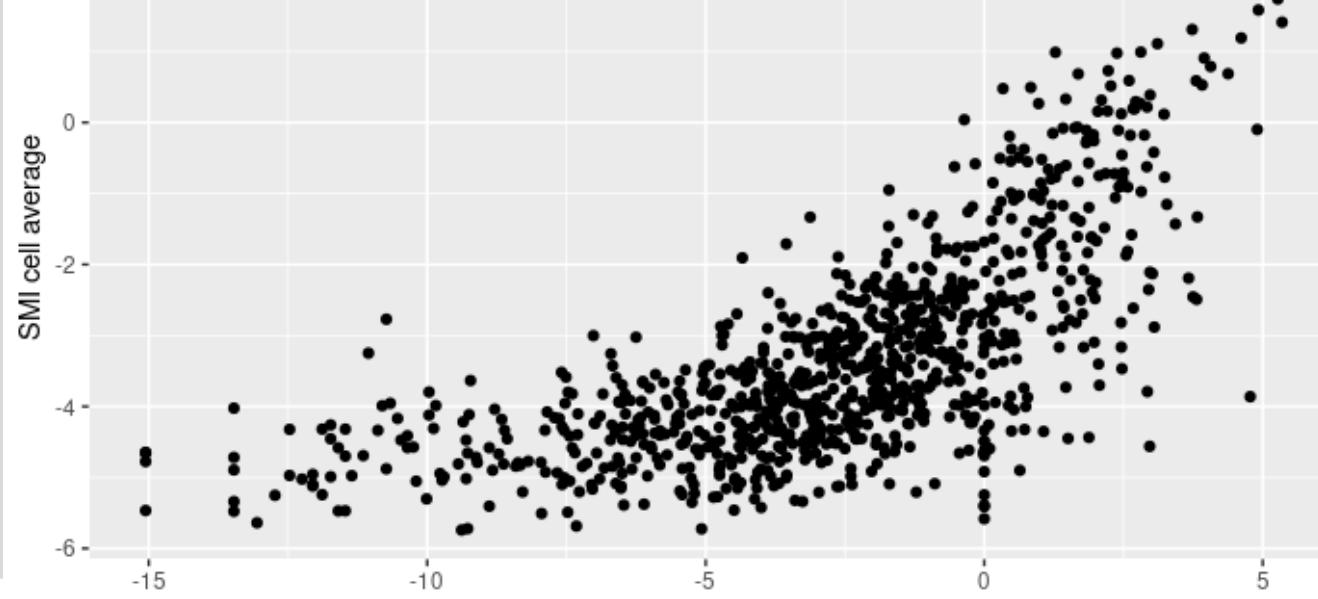
Spatial distribution of detected transcripts



Majority of panel distinguished above limit of detection



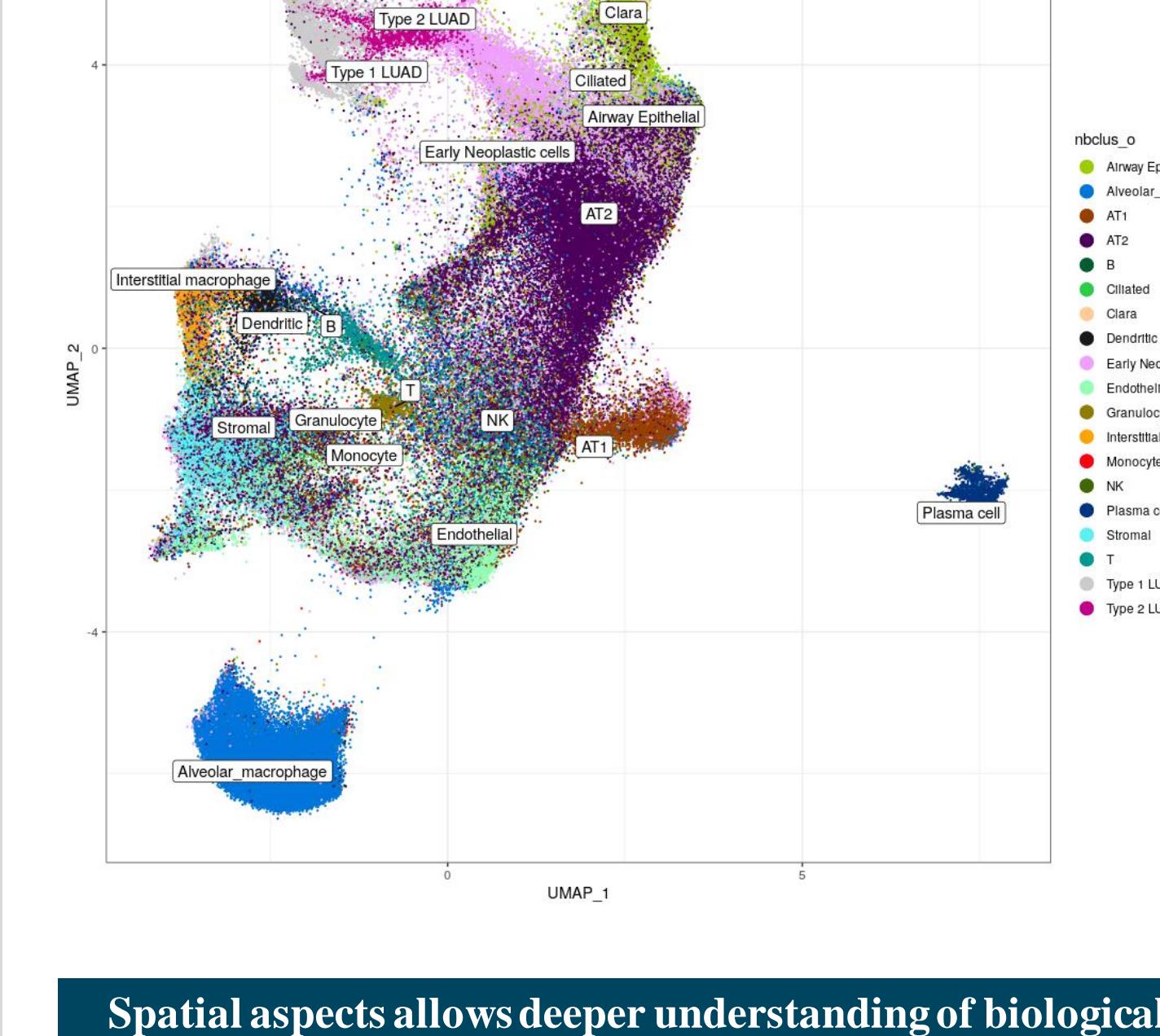
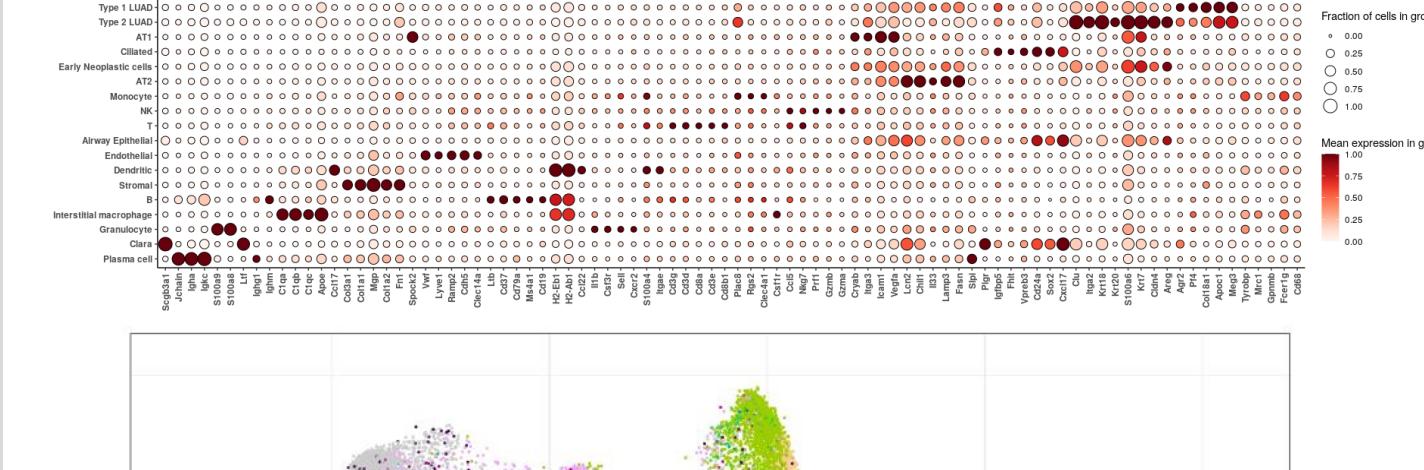
Concordance between SMI and scRNAseq



CosMx SMI results match "gold standard"

Cell-typing using CosMx technology recovered all major cell types identified by single-cell RNA-seq performed on the same samples using droplet-based technology (10X). These cell types were unambiguously mapped on the slides, for example:

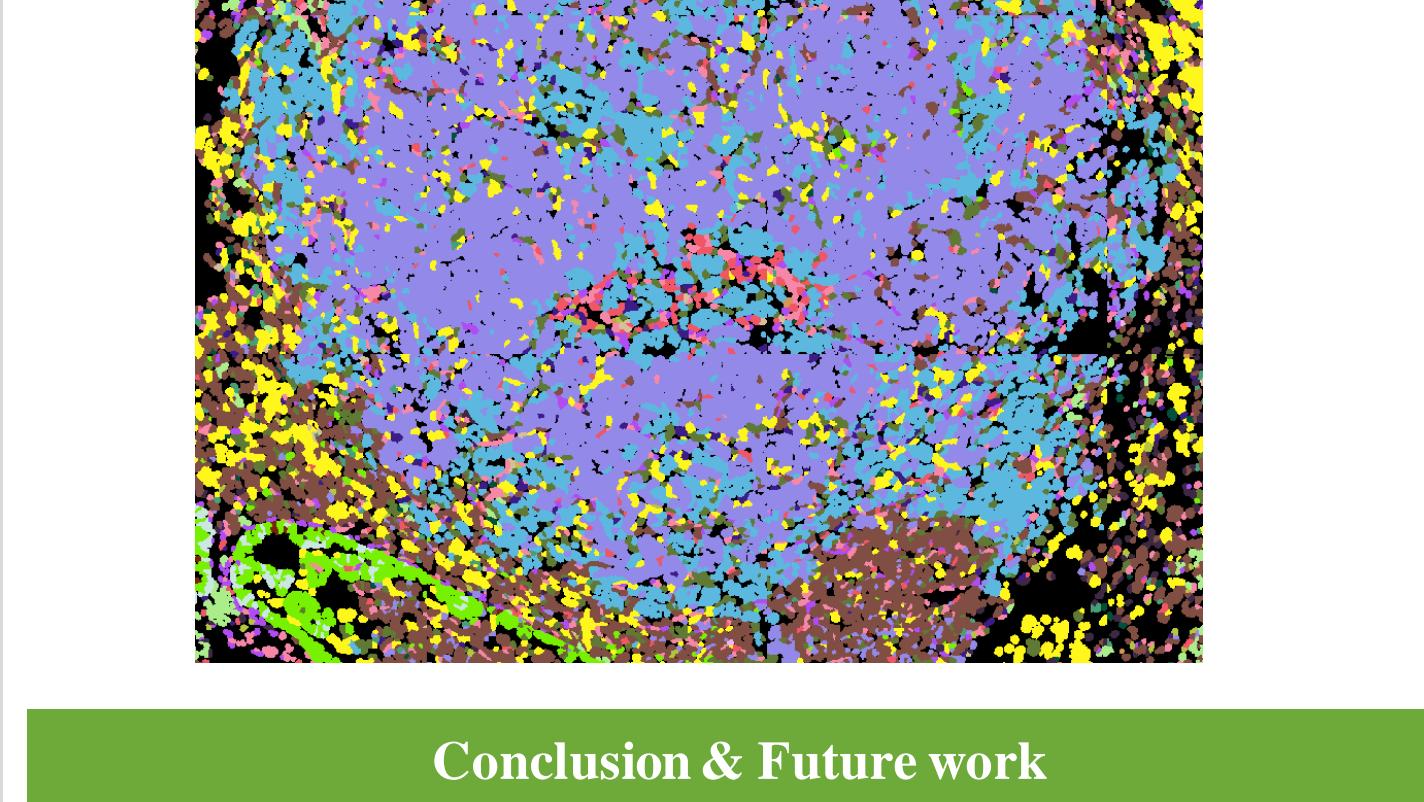
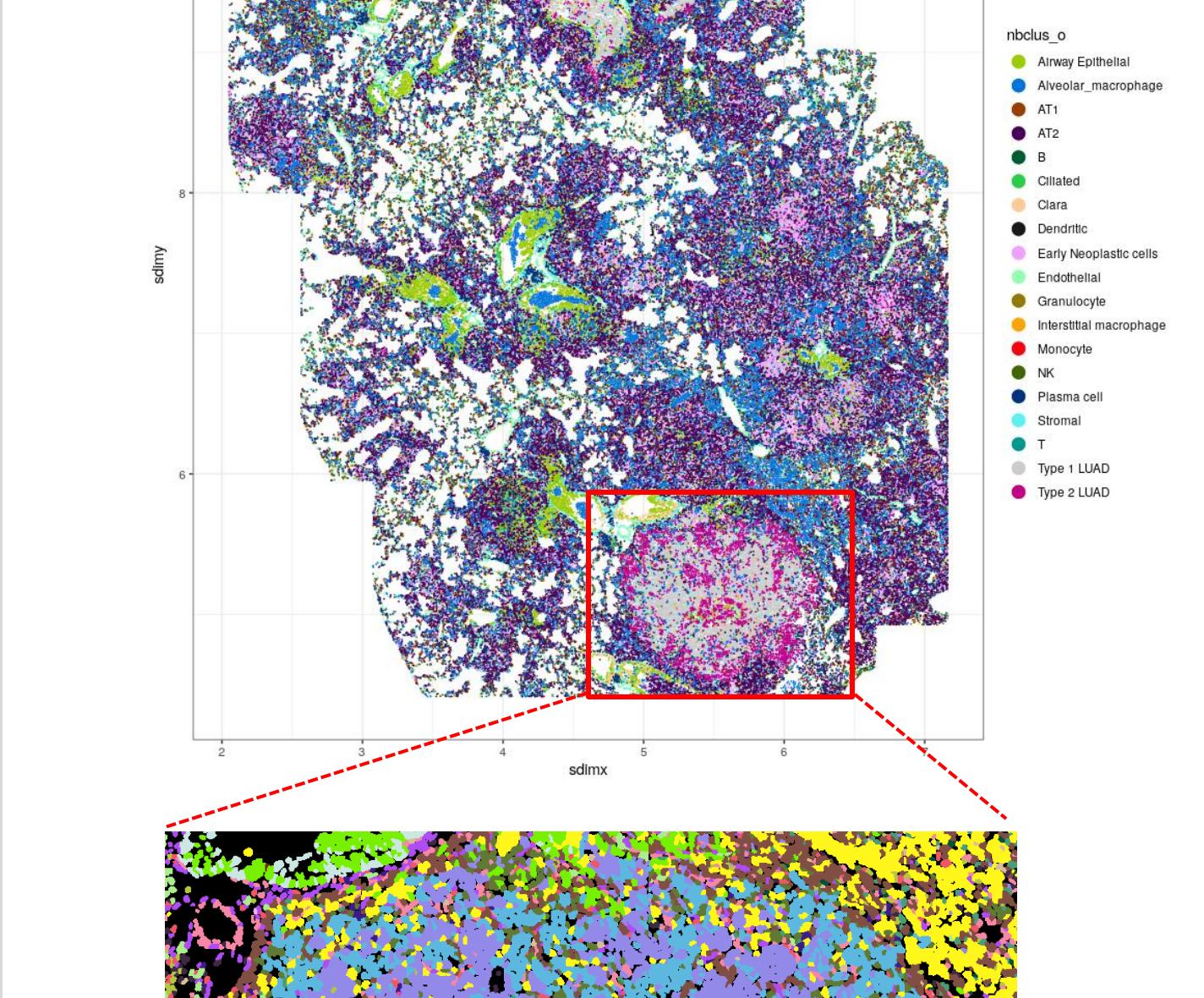
- normal lung alveoli were found to contain AT1, AT2, and endothelial cells and alveolar macrophages, as expected for this type of tissue.
- Tumor masses identified as LUAD by an expert pathologist were in fact composed mainly by epithelial cells expressing a highly malignant lung tumor signature (LUAD cells)
- Smaller tumor masses, identified as adenomas by the pathologist, were composed by cells (Early Neoplastic cells) with molecular phenotypes intermediate between that of LUAD and AT2 cells. This can be visualized on the t-SNE graph, as Early Neoplastic cells form "a bridge" between AT2 and LUAD cells.



Spatial aspects allows deeper understanding of biological context compared to scRNAseq

CosMx SMI allows in-depth characterization and functional analysis of cellular states, potentially surpassing standard single cell analyses, especially for rare cell types within specific niches. scRNA-seq revealed two distinct LUAD cell types (Type1 and Type2) with unique transcriptional programs, which we successfully extracted from our CosMx dataset and mapped onto the histological section.

CosMx SMI adds a new layer of tissue-level information on cell types and cell states when compared to standard scRNA approaches. Remarkably, Type2 LUAD cells were found mainly on the border of the tumor masses, facing stromal areas. On the contrary, Type1 LUAD cells were found inside the tumor mass, surrounded by Type2 LUAD cells, or as cells protruding inside the airways, where they are surrounded by airway epithelial cells.



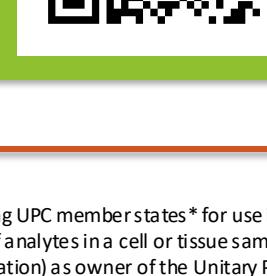
Conclusion & Future work

Spatial transcriptomics allowed to define the cell types identified by scRNA-seq as histological structures, paving the way to the functional characterization of their different spatial distribution in the tissue.

We now aim at understanding how the three types of neoplastic cells interact with each other and with TME cells. For this, we will take advantage of the fact that the CosMx SMI 1,000-plex Mouse Universal Cell Characterization panel contains a wide array of ligand-receptor pairs, allowing to map cell-cell interactions while taking in the account their reciprocal position in the tissue.

We also aim at understanding how inhibition of oncogenic KRAS activity affects the lung tumors. This includes Ras-controlled cell-autonomous and non-cell autonomous effects.

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The CosMx[®] Cell and Tissue Profiling kit is not cleared or approved by the U.S. Food and Drug Administration (FDA) or any other regulatory authority for use in humans. The CosMx[®] Cell and Tissue Profiling kit is intended for research use only. The CosMx[®] Cell and Tissue Profiling kit is a multiplexed, quantitative, fluorescence-based assay for the detection of cellular RNA, messenger RNA, microRNA, ribosomal RNA and any combinations thereof in a method used in fluorescence in situ hybridization for detecting a plurality of analytes in a sample without the consent of the President and Fellows of Harvard College (Harvard Corporation) as owner of the United States Patent EP 4 108 782 B1. The use for the detection of RNA is prohibited without the consent of the President and Fellows of Harvard College (Harvard Corporation).

¹ Austria, Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Portugal, Slovenia, Sweden, Switzerland

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