

COMBINING CANCER PATIENT SPATIAL TRANSCRIPTOMICS AND BULK RNA-SEQ DATA TO DRIVE INSIGHTS INTO NSCLC

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Translational research

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INTRODUCTION

Combining cancer patient spatial transcriptomics (ST) and bulk RNA-Seq allows for a more nuanced understanding of the gene expression profiles. While large-scale sequencing projects have revealed some NSCLC cancer drivers, they cannot fully address the complexity of the heterogeneous tissue composition present in patients. ST enables the measurement of gene expression in specific regions within a tissue sample, complementing and refining insights gained from bulk RNA-Seq.

METHODS

Collection of samples: Tumor tissues were globally collected using a standardized protocol, minimizing the ischemia time until freezing in liquid nitrogen. To ensure the quality of the samples, all tissues were Hematoxylin and Eosin stained and subjected to a pathological QC. Samples need to be invasive, have a tumor content of $\geq 30\%$ and Necrosis $\leq 30\%$. Normal tissues were processed in parallel and need to be free of tumor and representative regarding the tumor tissue to be included. Approximately 10 mg tissue were taken for nucleic acid extraction and protein lysate preparation each. To account for tumor heterogeneity, pathological QCs were performed on two sections, before and after taking the analysis material. The tissues stay frozen during the entire process.

RNA-Seq and Spatial Transcriptomics analyses were performed on one NSCLC patient, adenocarcinoma (ADC), stage IV. RNA-Seq differential expression was additionally done on 52 ADCs.

Nucleic Acid Extraction and Quality Assessment: Frozen tissue slices were mixed with beta-mercaptoethanol containing sample buffer and homogenized using the BeadBug system. RNA was extracted using the Qiagen AllPrep Universal Kit according to the manufacturer's instructions. RNA concentration was quantified using Qubit RNA BR assay respectively. RNA quality was assessed using Agilent High-Sensitivity RNA ScreenTape kit respectively. RNAs need to have a RIN ≥ 4 or a DV200 ≥ 60 to be selected for library preparation.

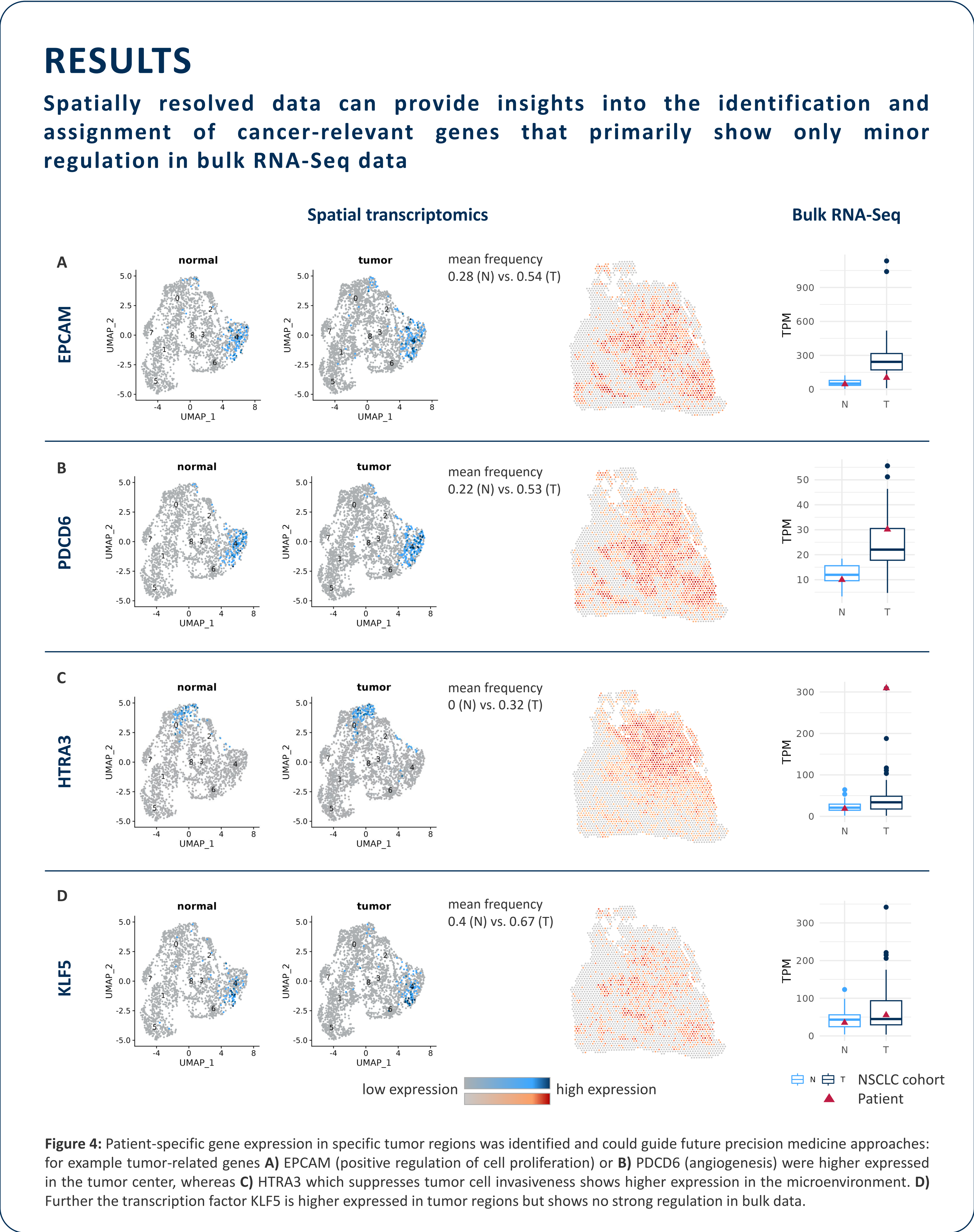
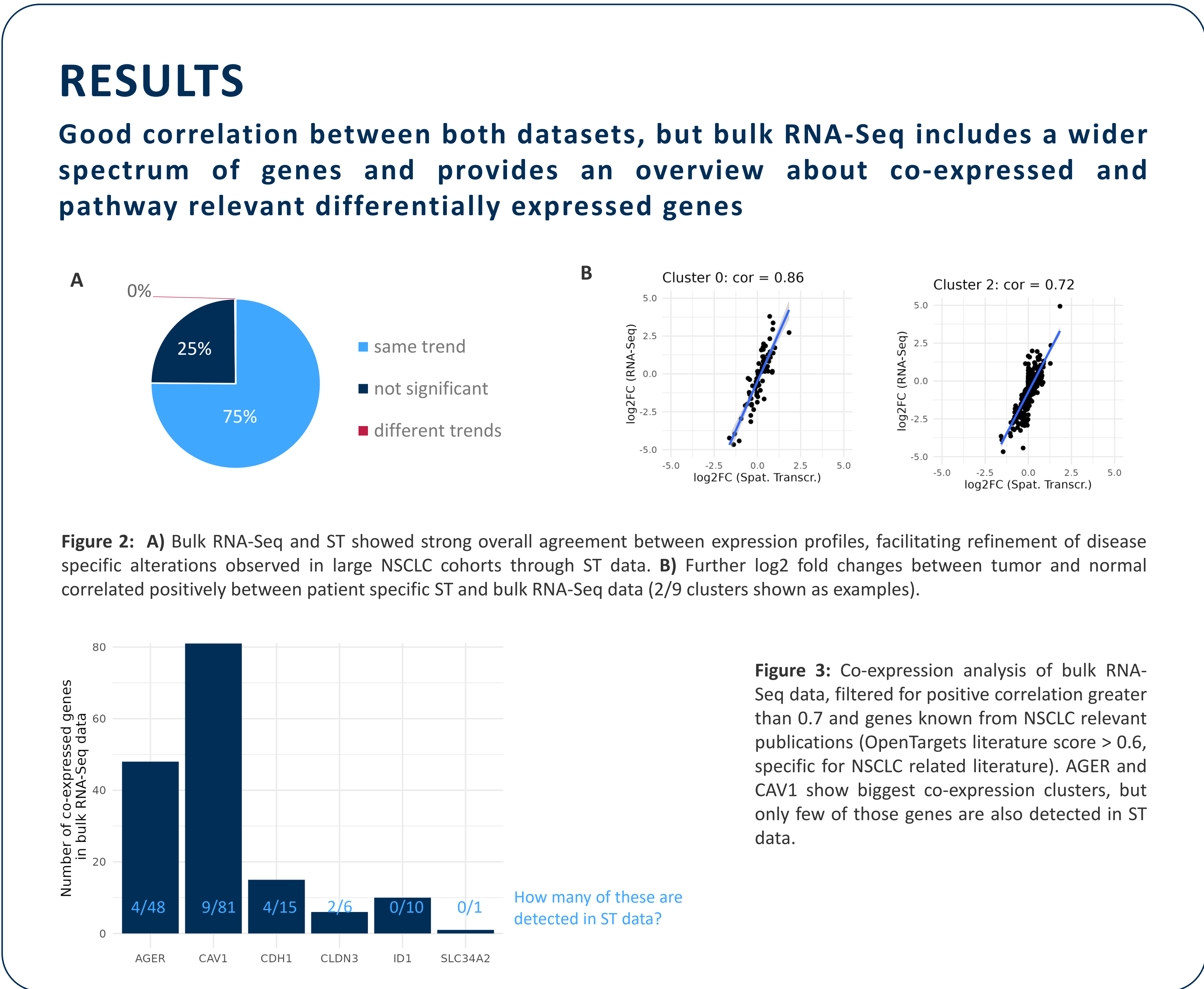
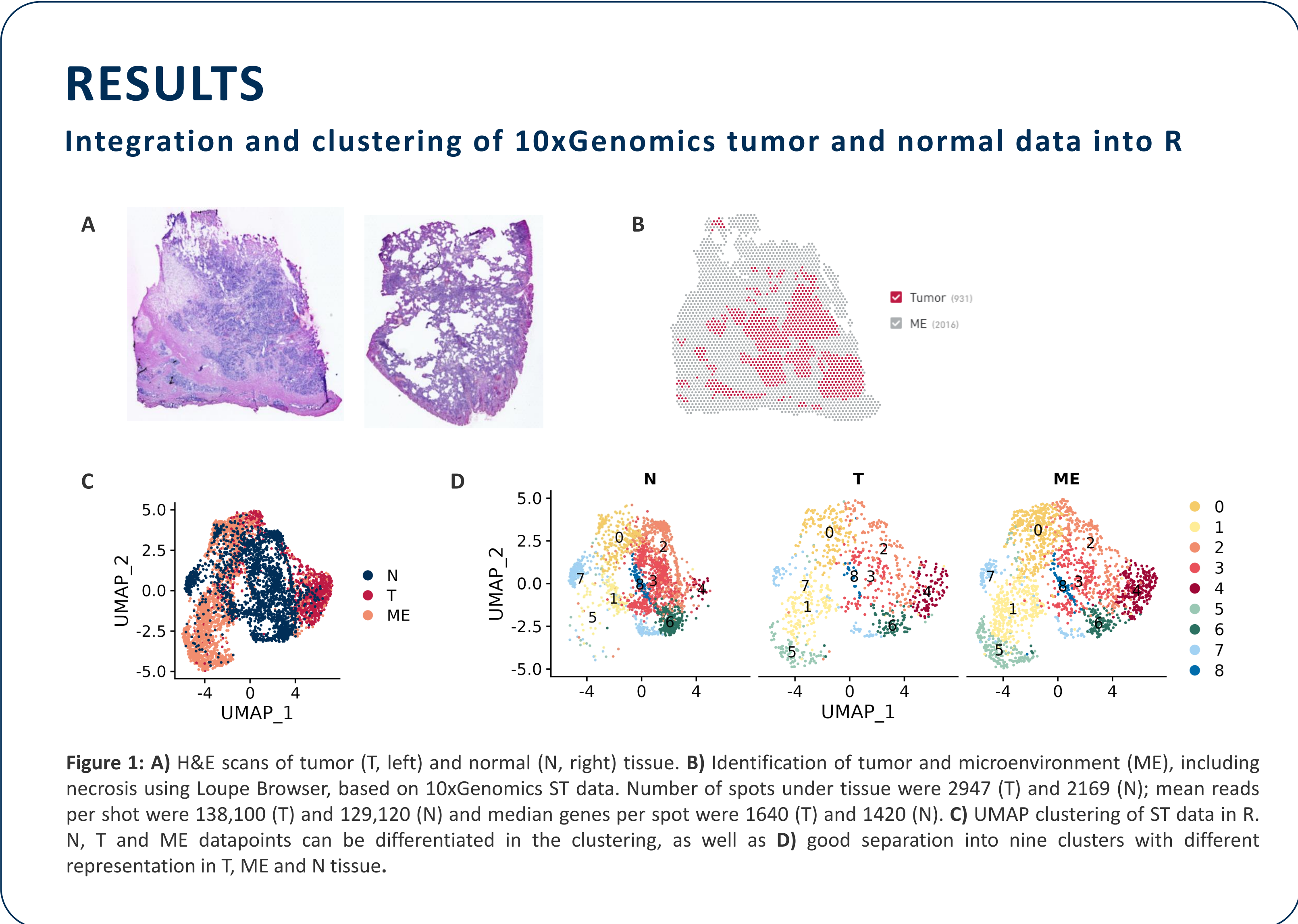
RNA-Seq: For whole transcriptome sequencing, RNA samples were depleted of the ribosomal RNA using the Ribo Zero Kit (Illumina) and library preparation was performed using the TruSeq Stranded Total RNA Kit (Qiagen). The library preparation kit was used according to manufacturer's instructions. Sequencing was performed on a NovaSeq6000 system (Illumina). Whole transcriptome sequencing datasets have ≥ 100 million total reads with less than 20% of ribosomal origin and ≥ 20 million reads mapping to mRNAs according to Ensembl reference. Ribosomal depletion was performed to remove nuclear rRNA and mt-rRNA. NGS data was aligned against Grch38 genome assembly.

10x Genomics Visium Spatial Gene Expression Manual Workflow for FFPE: One FFPE section was mounted on a Visium Spatial Gene Expression Slide containing spatially barcoded capture primers. The FFPE section was first H&E stained and scanned to visualize the histological tissue structure, followed by human whole transcriptome probe hybridization, targeting 17,943 human protein coding genes by 18,630 probe pairs. According to hybridization probes were ligated, followed by tissue permeabilization and probe release. Ligated probes were then captured via spatially barcoded primers and extended followed by Visium Spatial Gene Expression FFPE Library Preparation. The final library was sequenced at a concentration of 300 pM on a NovaSeq™6000 using a S1 v1.5 flowcell (Illumina) to reach a minimum sequencing depth of 25,000 read pairs per tissue covered spot by our sub-provider (IMGM Laboratories). Sequencing data containing the specific spatially sequencing barcodes were analyzed using the Space Ranger analysis pipeline (version 1.3.0) and the Loupe Browser (version 6.4.1) to assign the gene expression data to the corresponding histological positions in the tissue section.

Bioinformatical analyses: RNA-Seq differential and co-expression was based on normalized readcount data (TPM: transcripts per million). 10x Genomics data was integrated in R using Seurat package [1]. To remove artifacts, tumor and normal data were normalized separately, using SCTransform method. Both datasets were then combined for UMAP clustering [2] and differential expression analysis.

[1] Hao, Y. et al.: "Integrated analysis of multimodal single-cell data", Cell, 2021, 184(13); <https://doi.org/10.1016/j.cell.2021.04.048>, R package version 4.3.0.1

[2] Konopka, T.: "umap: Uniform Manifold Approximation and Projection", 2023, R package version 0.2.10.0



CONCLUSION

A more refined in situ understanding of gene expression profiles in the NSCLC microenvironment, combined with large cohort data will help guide therapeutic target selection. The combination not only refines target expression cell types (e.g. primary tumor or immune system) but also provide a strong indicator for target gene expression variability within patients' tumors for improved precision medicine approaches.