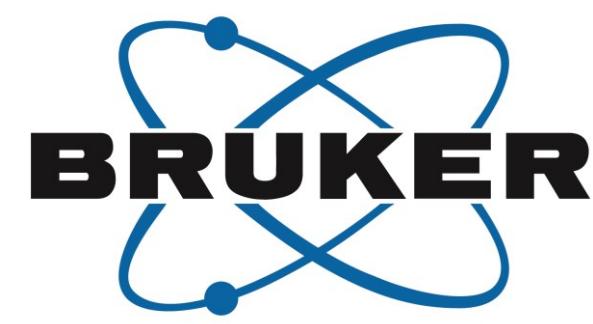


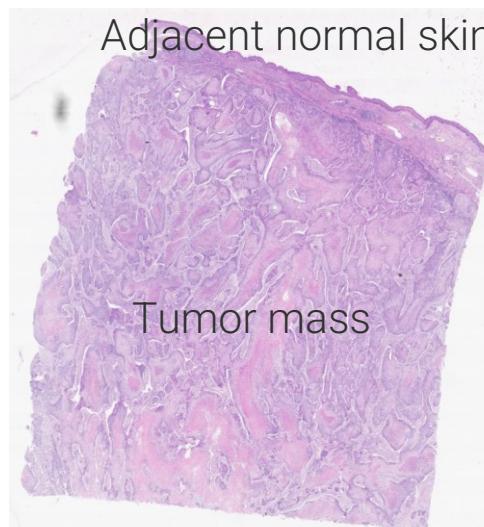
# High-resolution and AI-enabled single-cell spatial transcriptomics and histopathology integrated to reveal tumor differentiation and immune exclusion in skin squamous cell carcinoma



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## Introduction

The CosMx® Whole Transcriptome assay is the world's first technology capable of measuring spatially-resolved single-cell whole transcriptomes with single-molecule resolution. This new class of data gives us the opportunity to discover new biology in each new sample we assay.



In this spirit, we profiled an archived FFPE squamous cell carcinoma, then sought to uncover whatever biological insights this sample held.

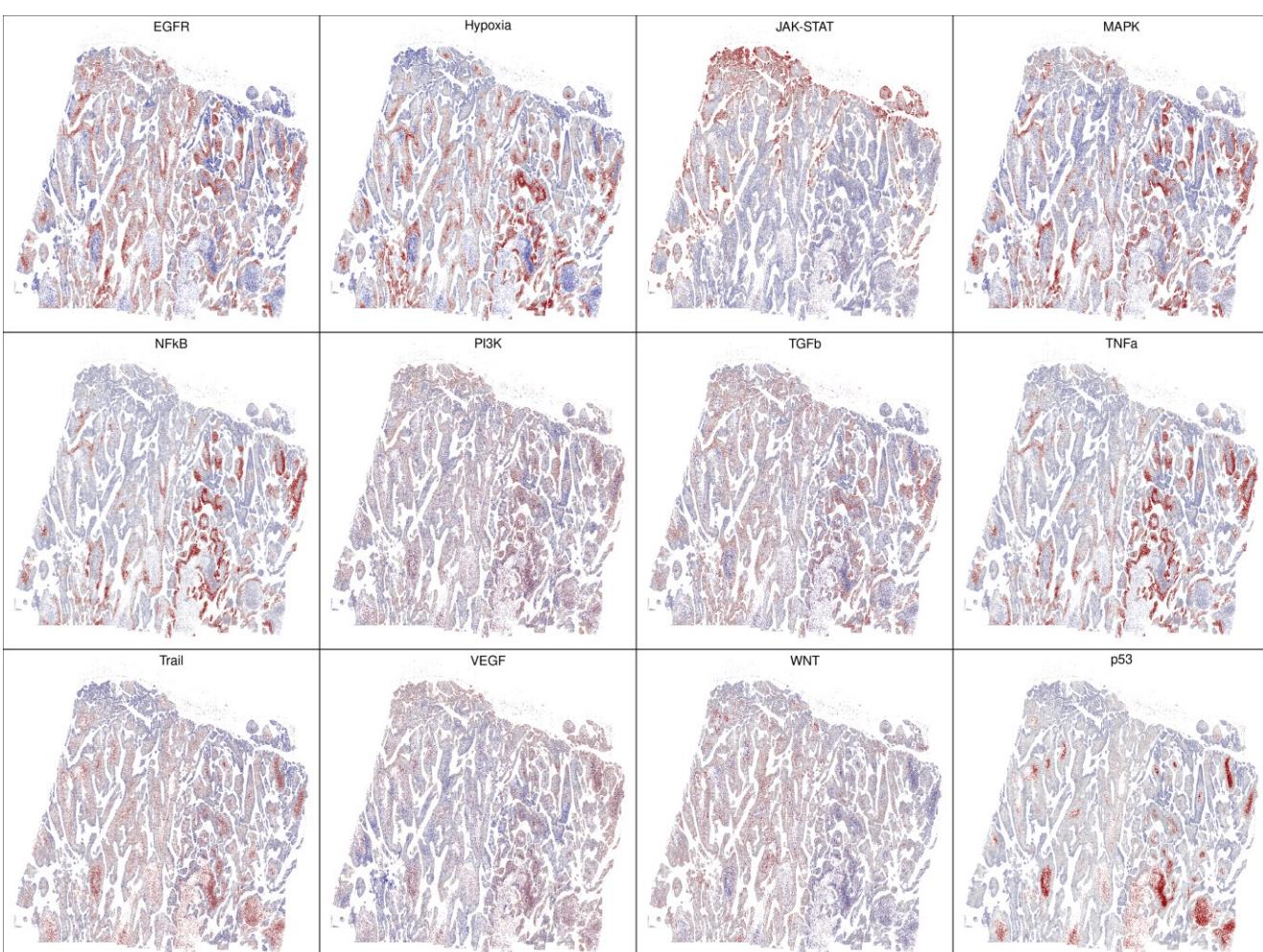
## Technical Performance

Key summary statistics of technical performance are shown below

Genes	18,935
Cells	555,754
Cells passing QC (>2^8 counts):	96%
Transcripts/cell: 0.1 quantile	483
Transcripts/cell: 0.25 quantile	1,041
Transcripts/cell: 0.5 quantile	2,249
Transcripts/cell: 0.75 quantile	4,191
Transcripts/cell: 0.9 quantile	6,198
Transcripts/cell: mean	2,894.10
Mean background / cell / plex	0.045
Unique genes / cell: mean	1,440.70

## Scoring the Hallmarks of Cancer

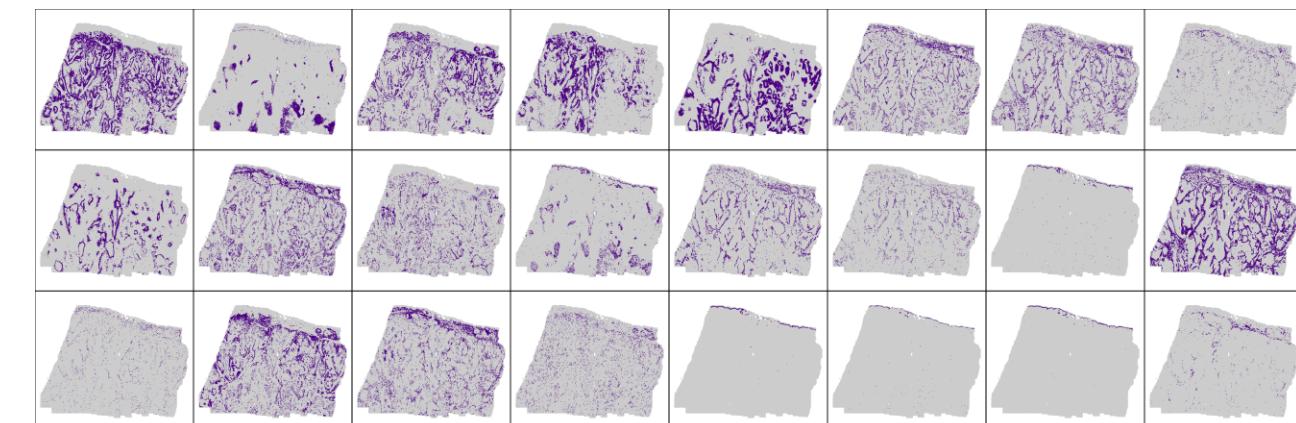
The PROGENy database reports 500-gene scores for the hallmarks of cancer. The WTX assay allows us to score them in their entirety:



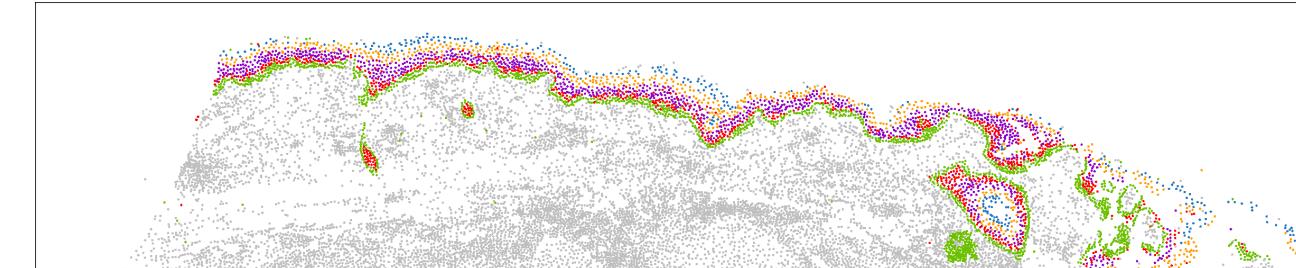
PROGENy scores for 12 canonical pathways. Only cancer cells are shown.

## Cell Type Landscape

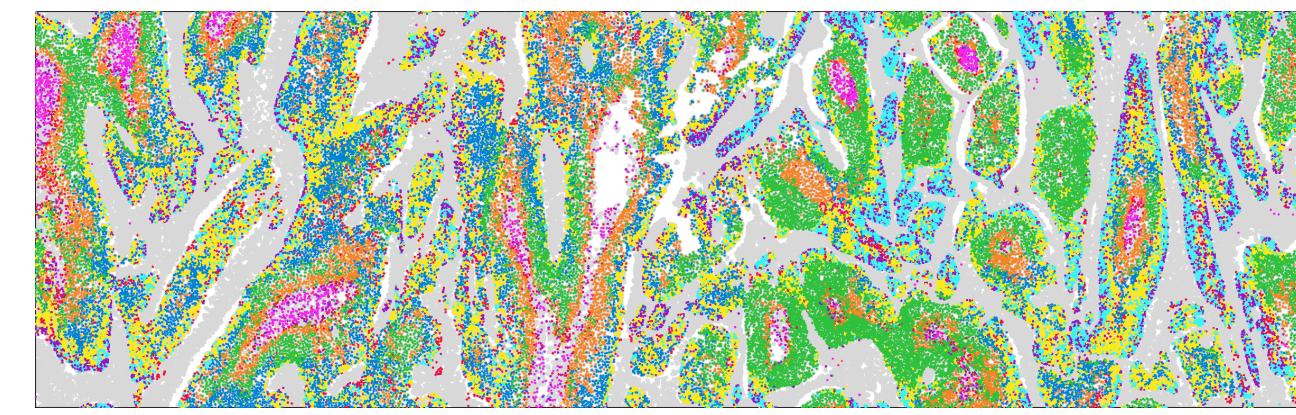
We used InSituType to finely cluster the cells:



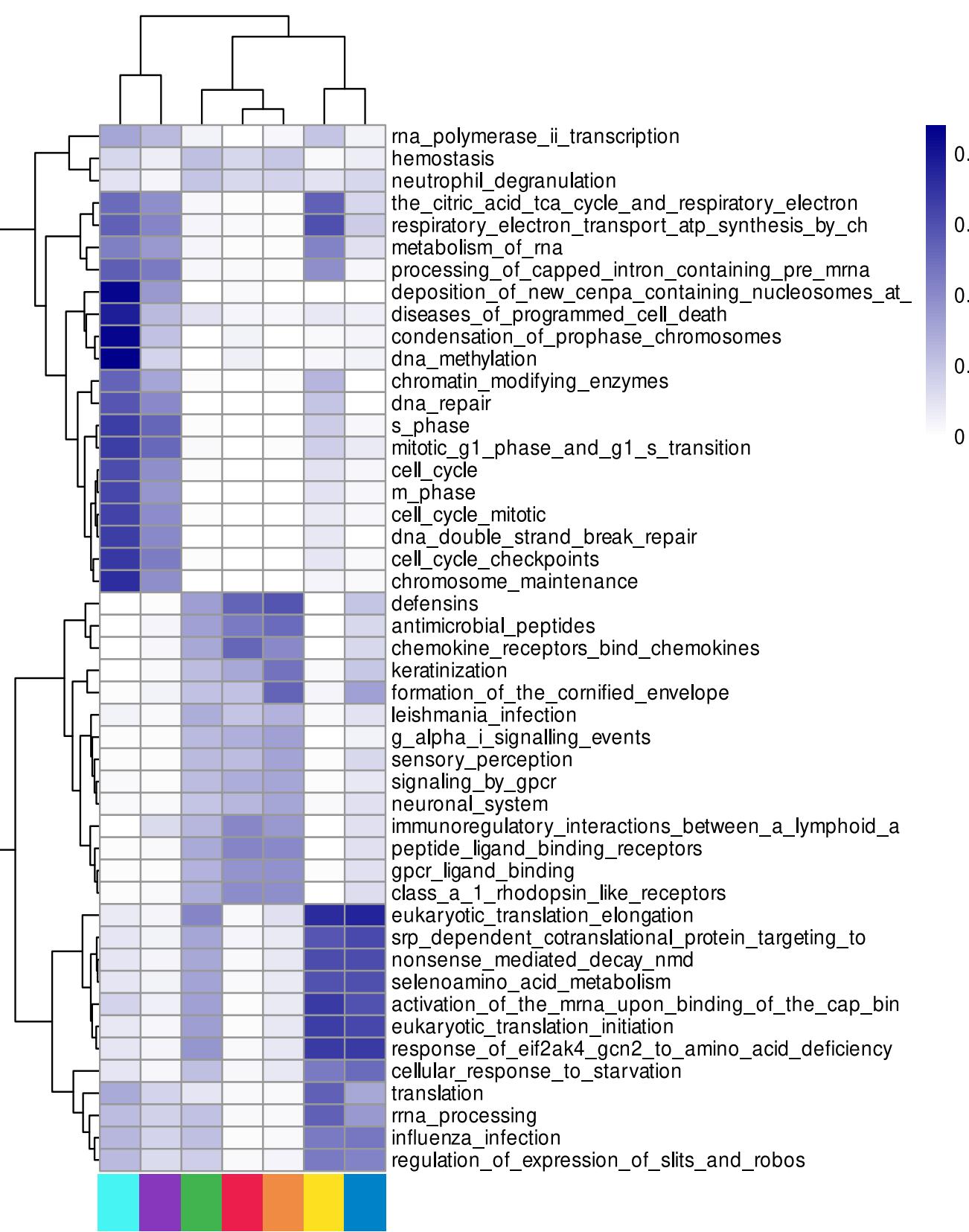
This produced accurate and nuanced subtyping of healthy skin:



And discovered 8 tumor subclusters with distinct spatial patterns:

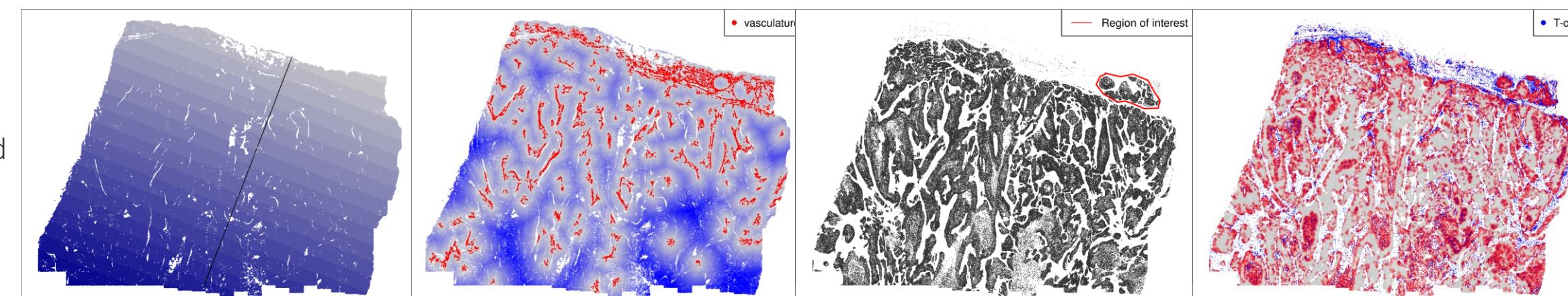


These subclusters are enriched for distinct REACTOME pathways:



## Hypothesis-Driven Analysis: Differential Expression

### Hypothesis-driven variables describing spatial context of tumor cells

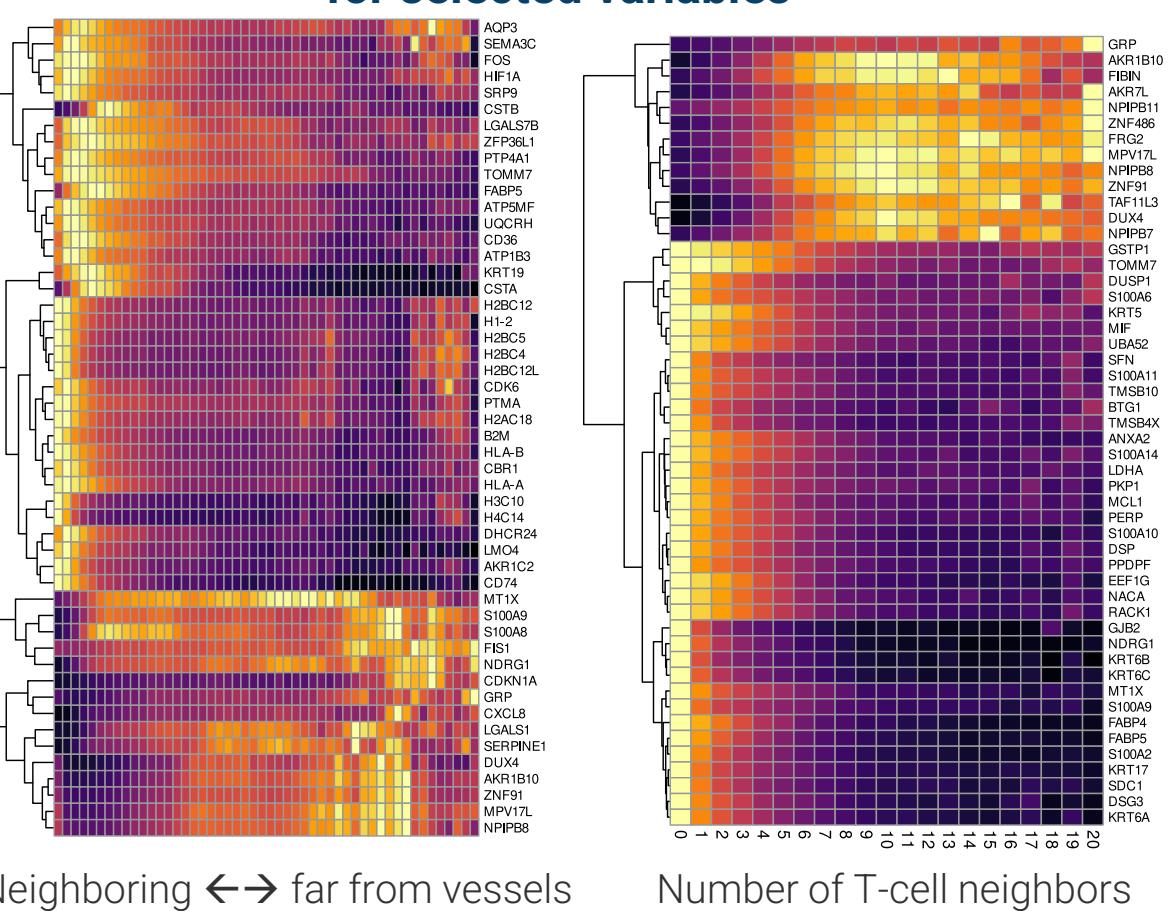


Distance from surface      Distance from vasculature

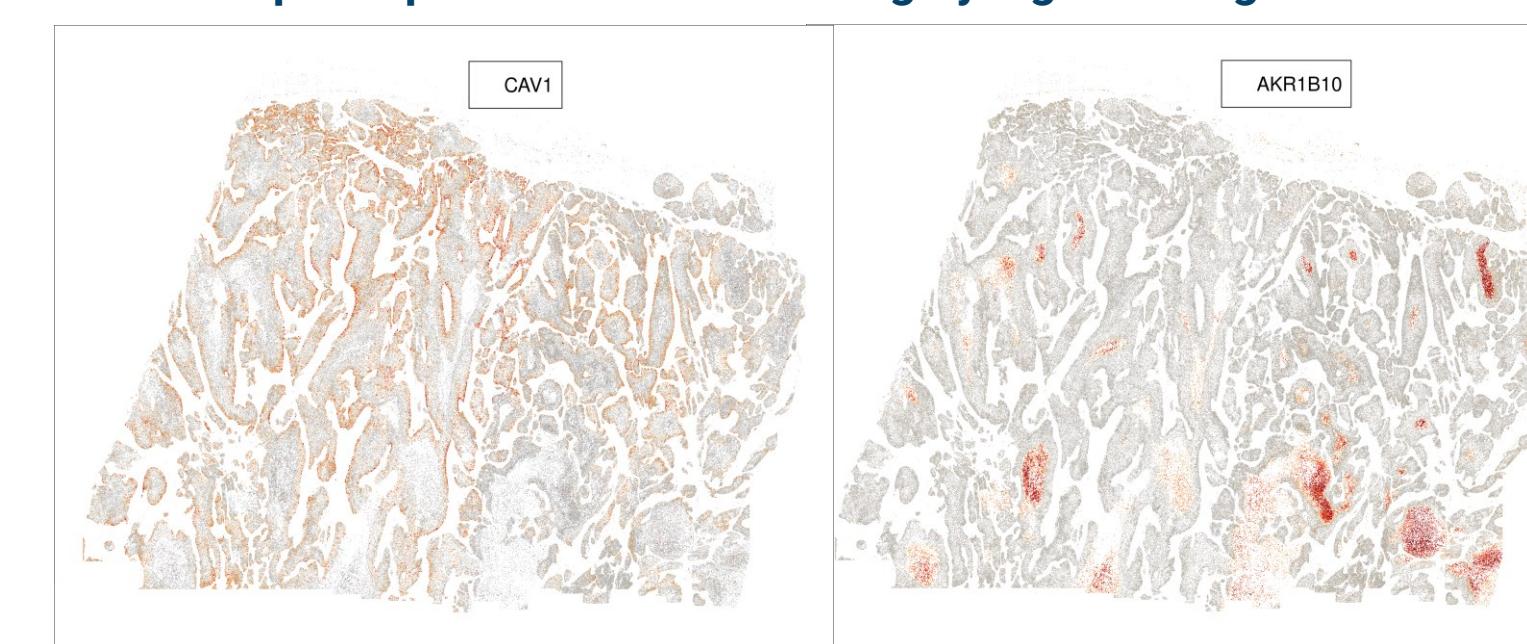
Region of interest (tumor inside epidermis)

Number of T-cell neighbors

### Mean expression of 50 most significant genes for selected variables



### Spatial patterns of selected highly significant genes



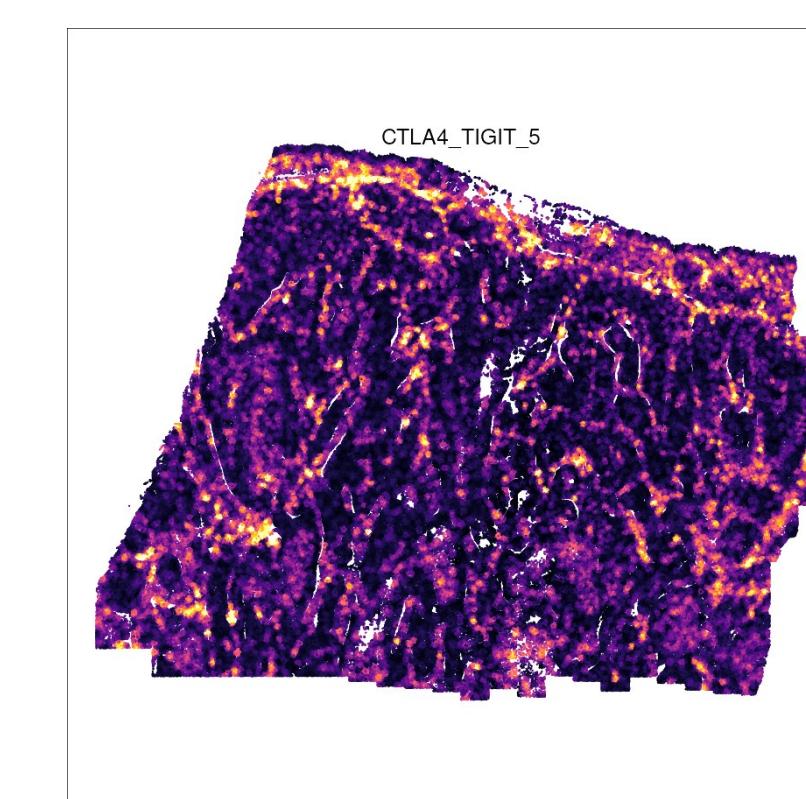
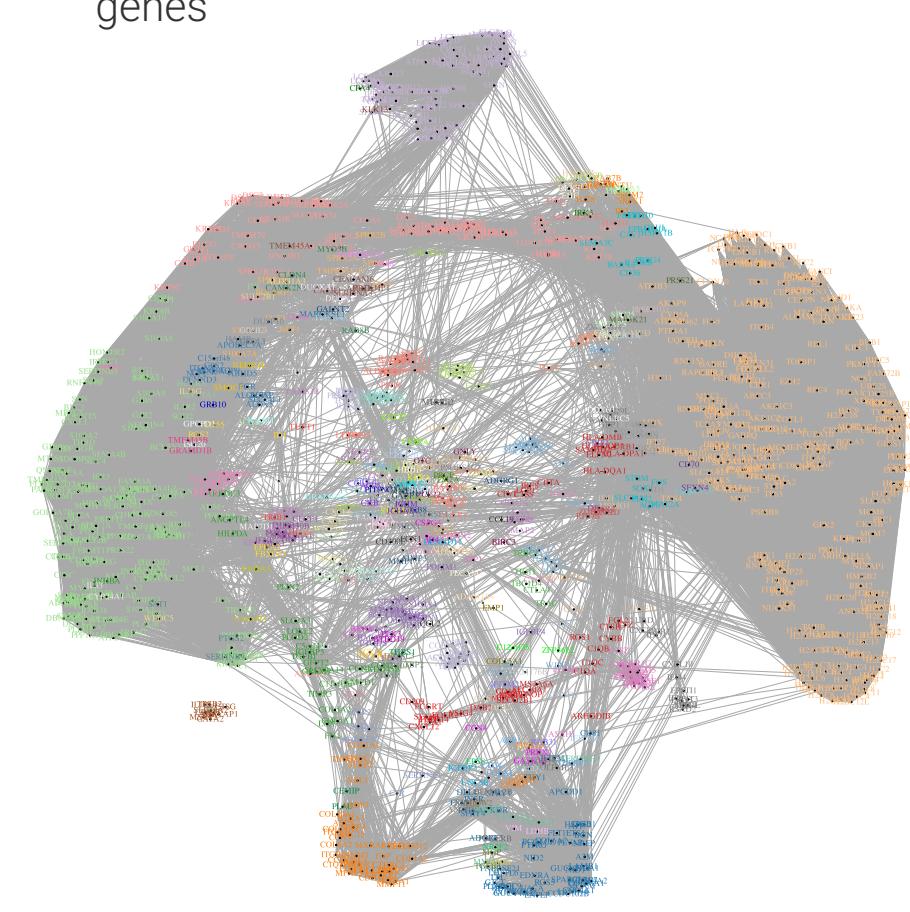
## Unbiased, Data-Driven Search for Trends with InSituCor

The InSituCor algorithm looks for sets of spatially correlated genes. These gene sets often reveal interesting biology.

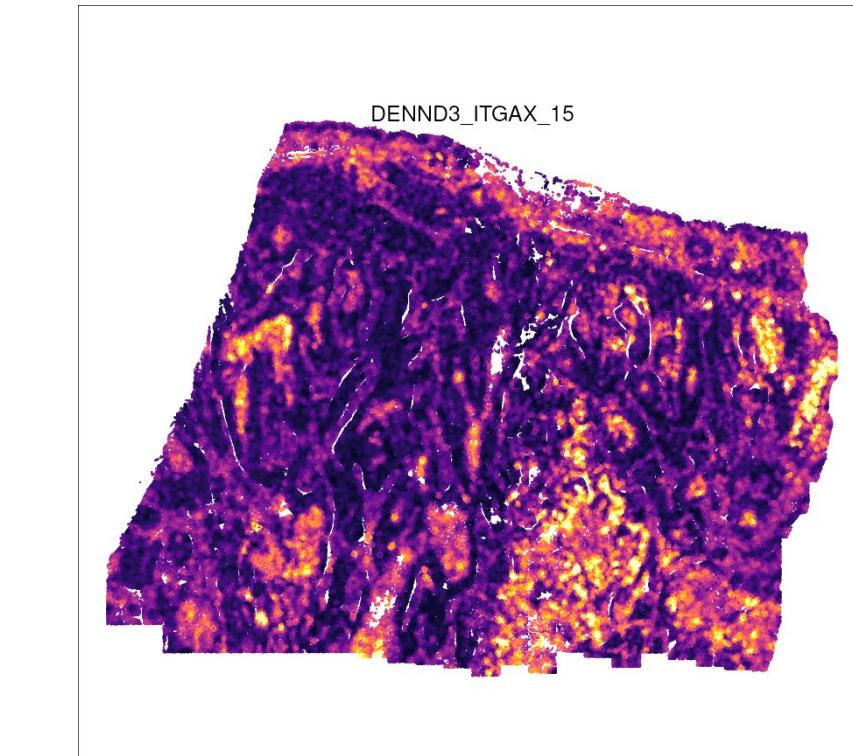
**T-cell markers:** TRAC, TRBC1  
**T-cell exhaustion genes:** CTLA4, TIGIT  
**T-cell metabolism:** TBC1D4

Right: selected gene modules discovered by InSituCor

Below: connectome of spatially correlated genes



**Innate immunity:** ITGAX, FGR, CSF3R, AQP9  
**Lipid-handling macrophages:** ABHD5, ALOX5  
**Immune sensing:** APOBEC3A, C15orf48  
**Immune trafficking/adhesion:** DENND3, SEC14L1



## References

- Danaher, Patrick, et al. "InSituType: likelihood-based cell typing for single cell spatial transcriptomics." *BioRxiv* (2022): 2022-10.
- Danaher, Patrick, et al. "InSituCor: a toolkit for discovering non-trivial spatial correlations in spatial transcriptomics." *bioRxiv* (2023): 2023-09. (In Press)
- Schubert, Michael, et al. "Perturbation-response genes reveal signaling footprints in cancer gene expression." *Nature communications* 9.1 (2018): 20.

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