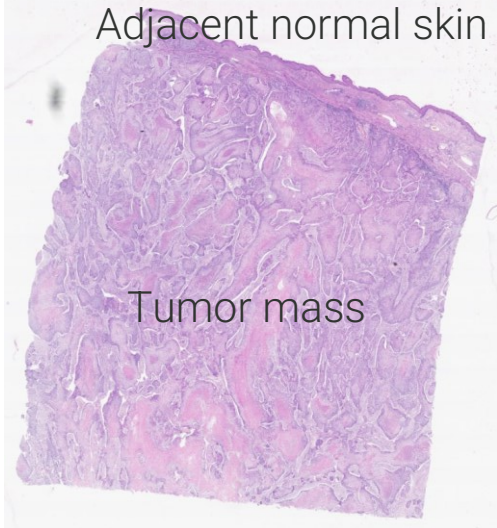


High-resolution and AI-enabled single-cell spatial transcriptomics and histopathology integrated to reveal tumor differentiation and immune exclusion in skin squamous cell carcinoma

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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

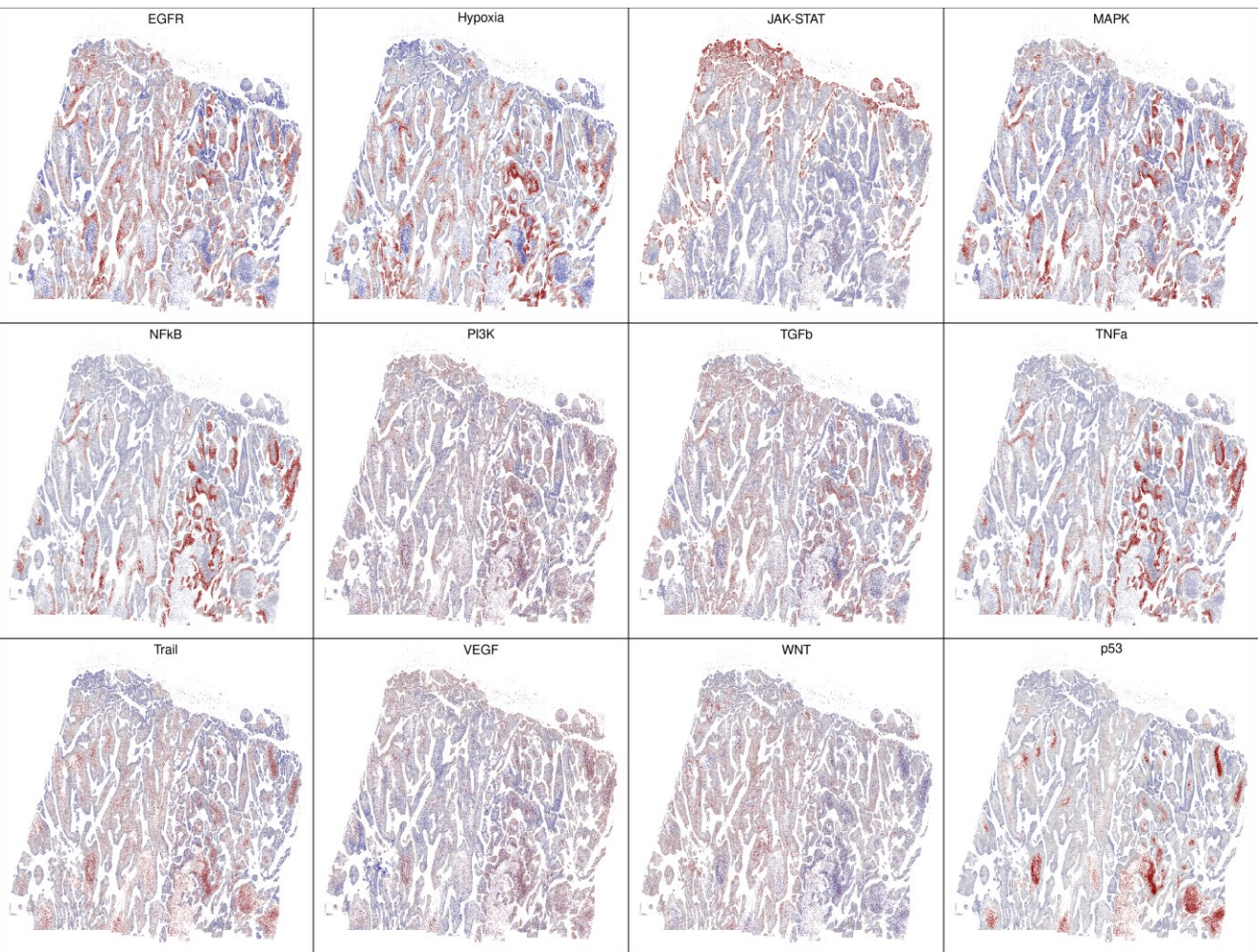
Key summary statistics of technical performance are shown below

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:

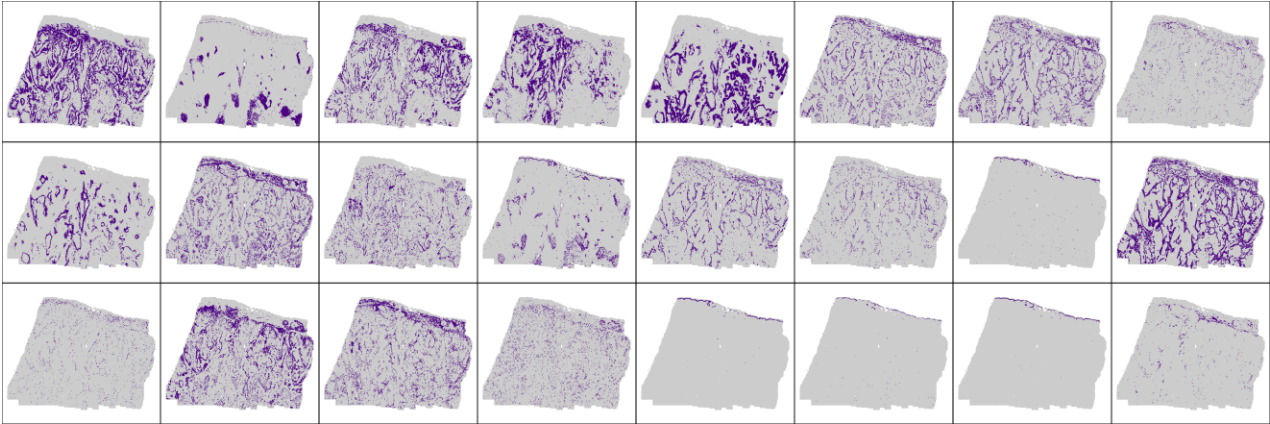
Perturbation-response genes reveal signaling footprints in cancer gene expression
Michael Schubert, Bertram Klingner, Martina Klonemann, Anja Sieber, Florian Uhlitz, Sascha Sauer, Matthew J. Gammitt, Nils Bläutig, & Julio Saez-Rodriguez ES
Nature Communications 9 Article number: 20 (2018) | [cite this article](#)



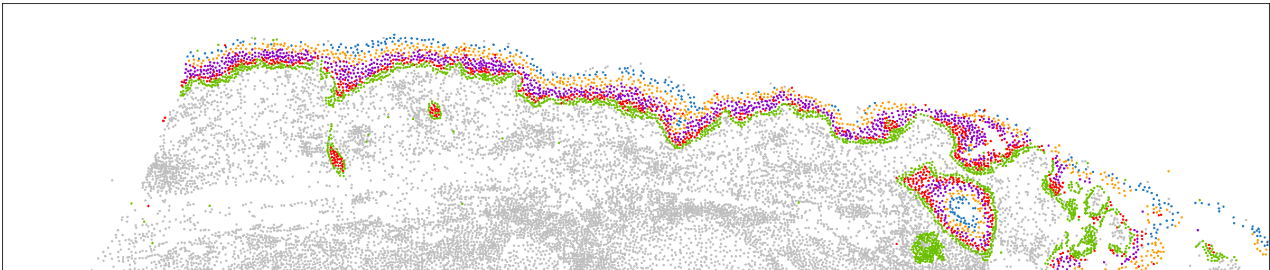
PROGENy scores for 12 canonical pathways. Only cancer cells are shown.

Cell Type Landscape

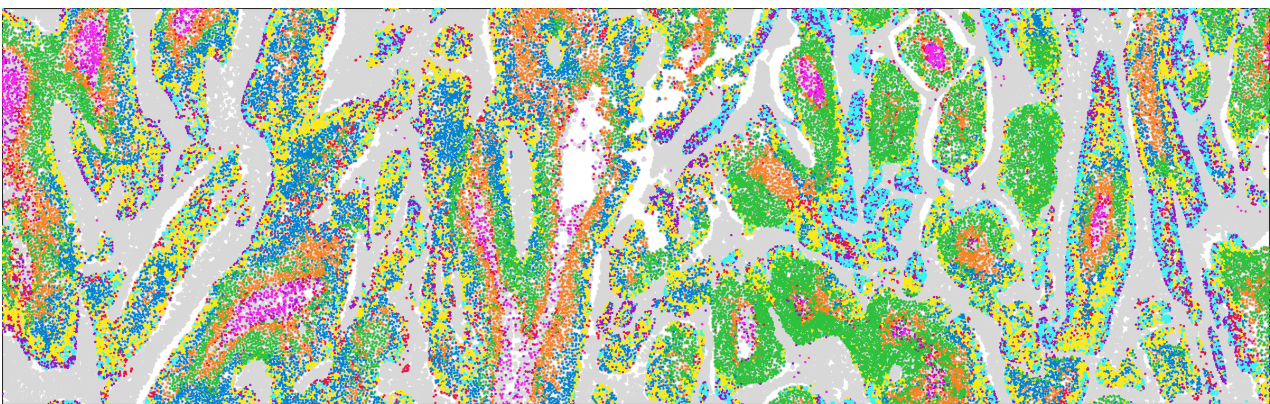
We used InSituType to finely cluster the cells:



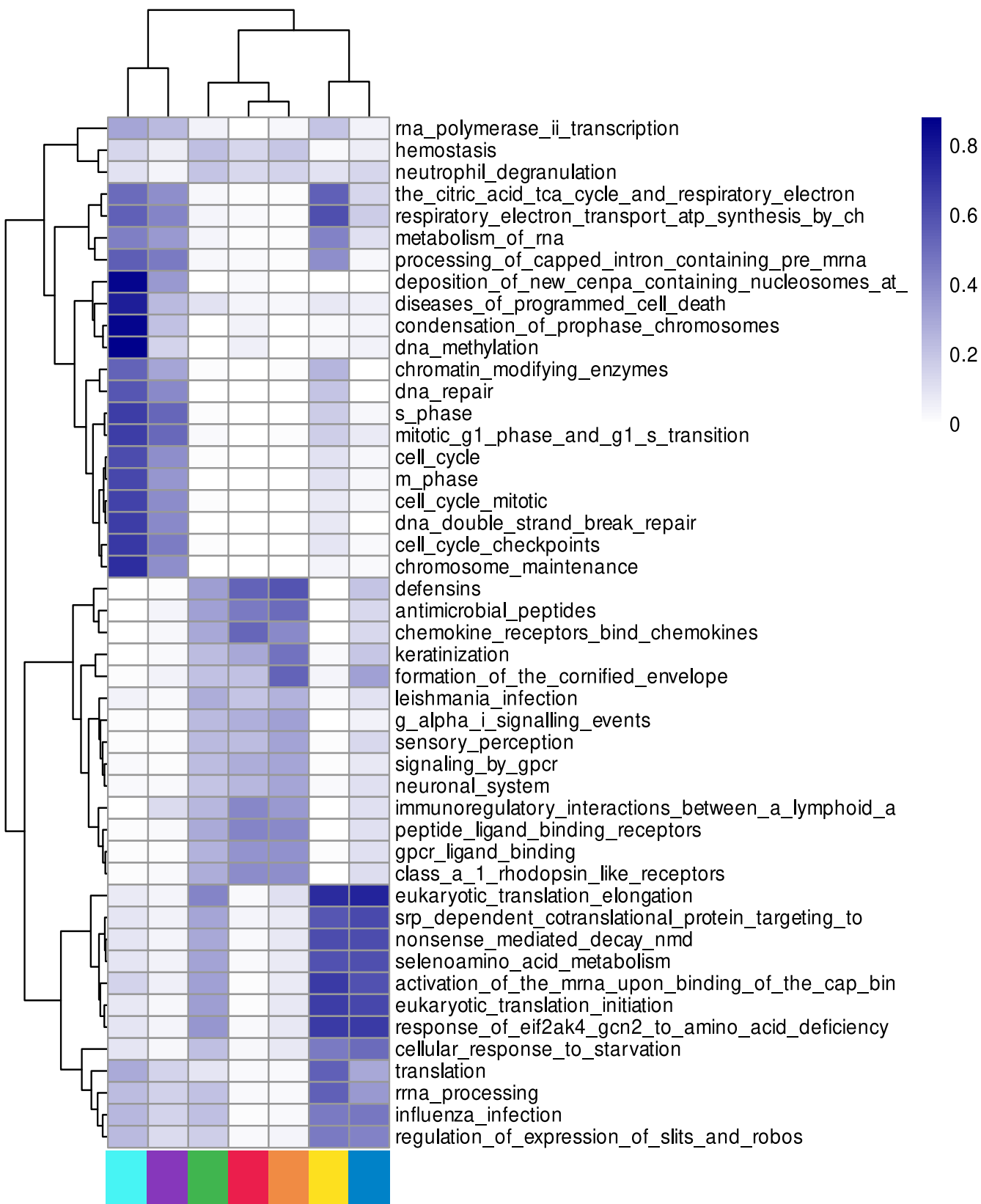
This produced accurate and nuanced subtyping of healthy skin:



And discovered 8 tumor subclusters with distinct spatial patterns:



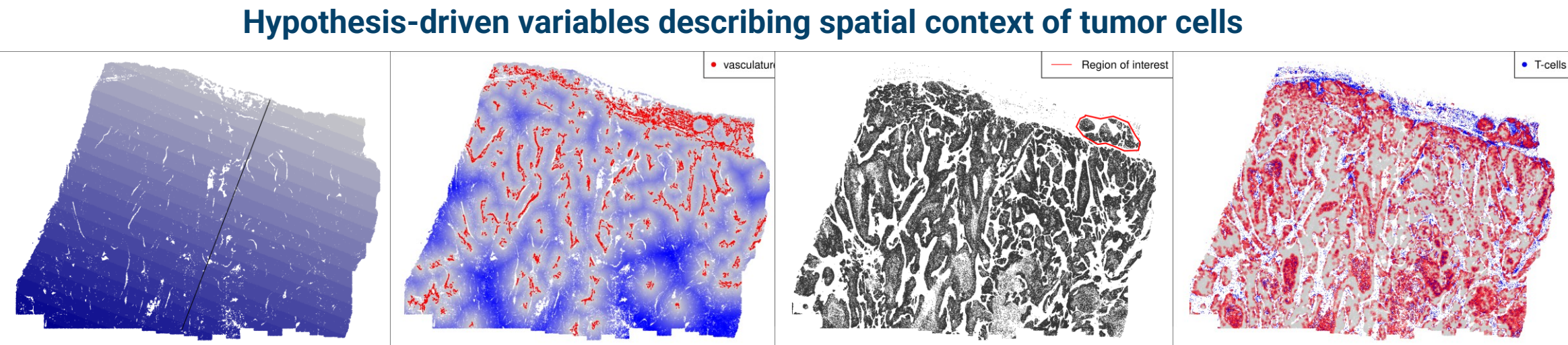
These subclusters are enriched for distinct REACTOME pathways:



REACTOME pathway enrichment scores in tumor cell subclusters

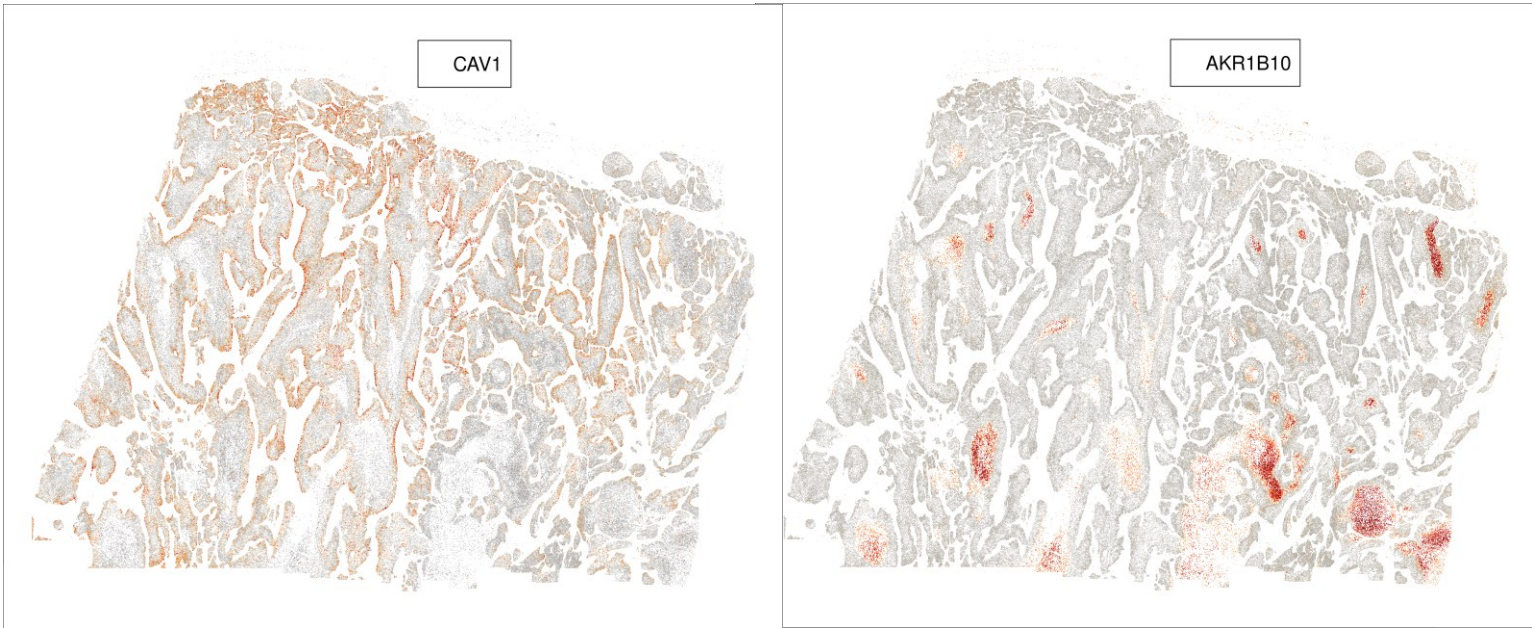
Hypothesis-Driven Analysis: Differential Expression

Next we asked, "how do tumor cells change behavior in response to their environment?" To this end, we defined variables measuring the spatial context of tumor cells, then predicted gene expression from these variables.



Our variables returned between 609 and 3108 significant genes (BH p -value < 0.05). Here we show the spatial trends for the top genes for some of these variables.

Spatial patterns of selected highly significant genes

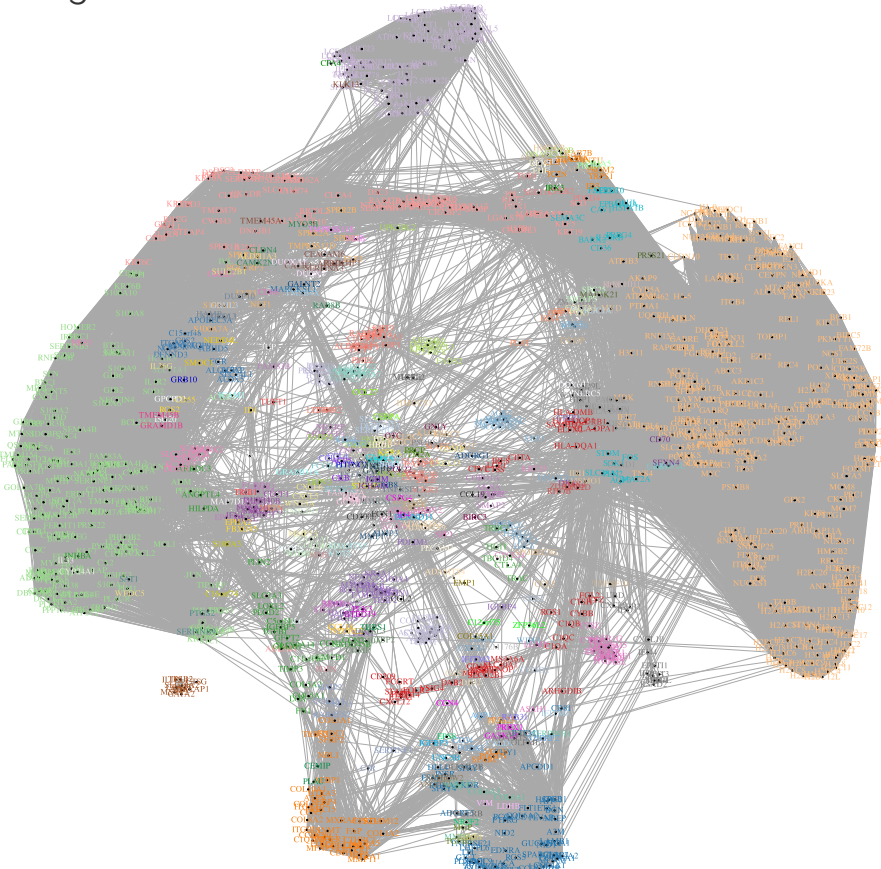


Unbiased, Data-Driven Search for Trends with InSituCor

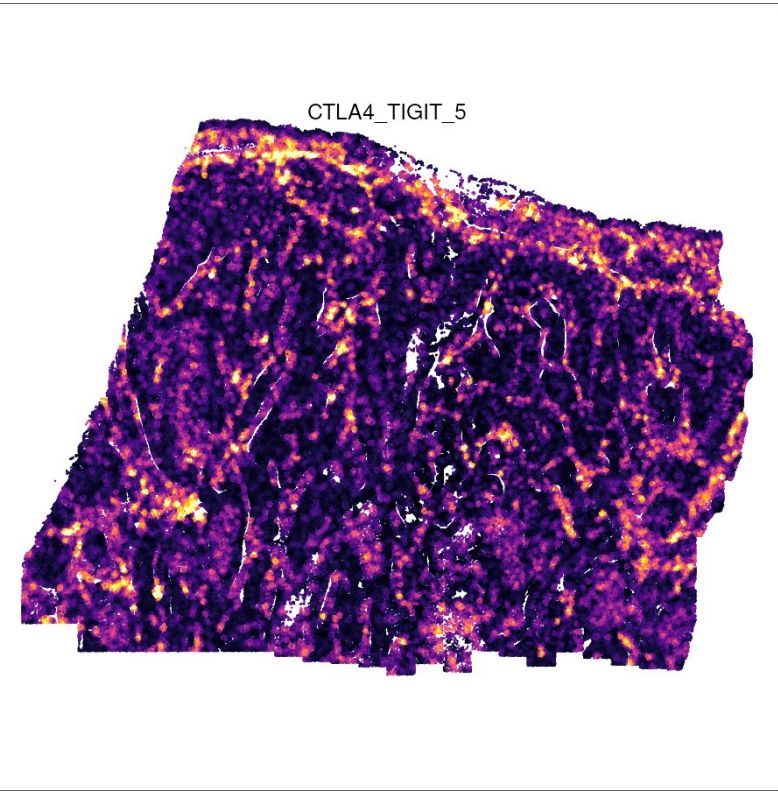
The InSituCor algorithm looks for sets of spatially correlated genes. These gene sets often reveal interesting biology.

Right: selected gene modules discovered by InSituCor

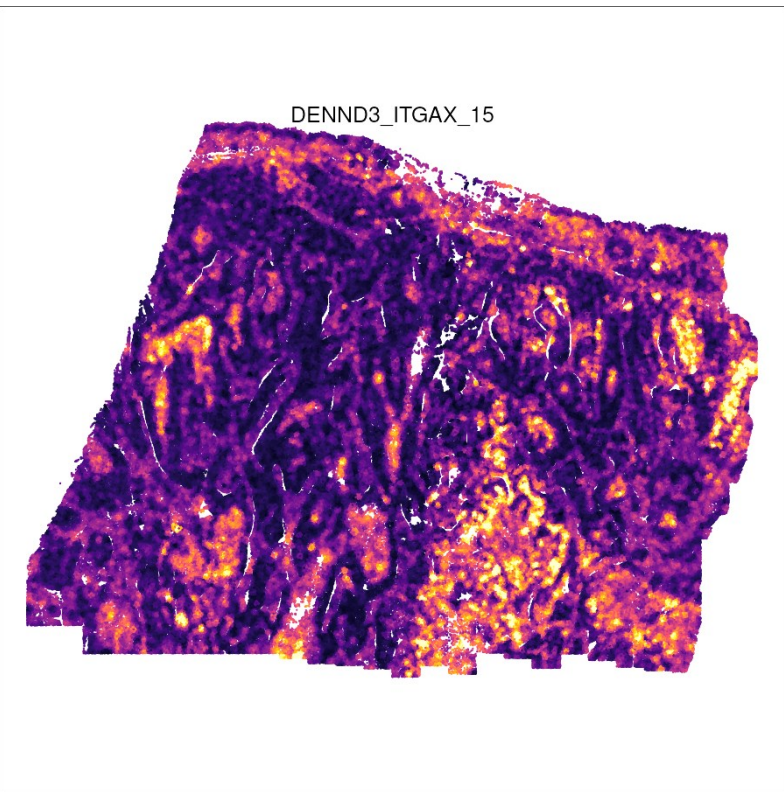
Below: connectome of spatially correlated genes



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



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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