

# Decoding cancer hallmarks through single-cell whole transcriptome imaging in invasive ductal carcinoma

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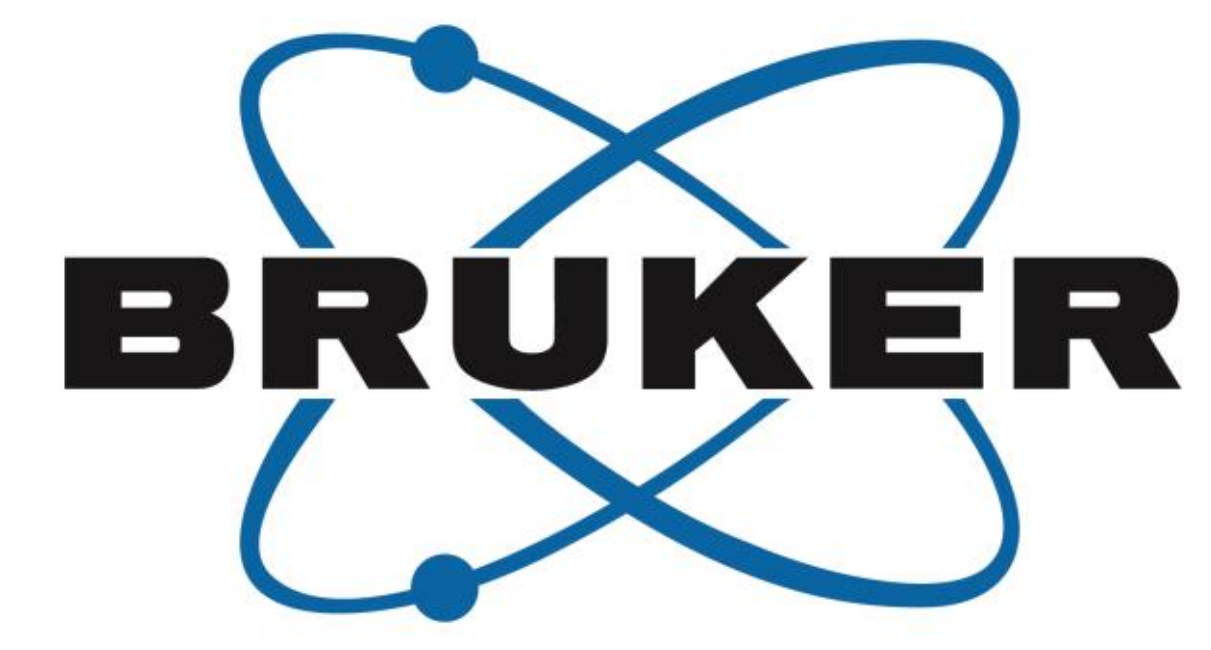
Bruker Spatial Biology, Seattle WA



BSB @ AACR



Preprint



## Introduction

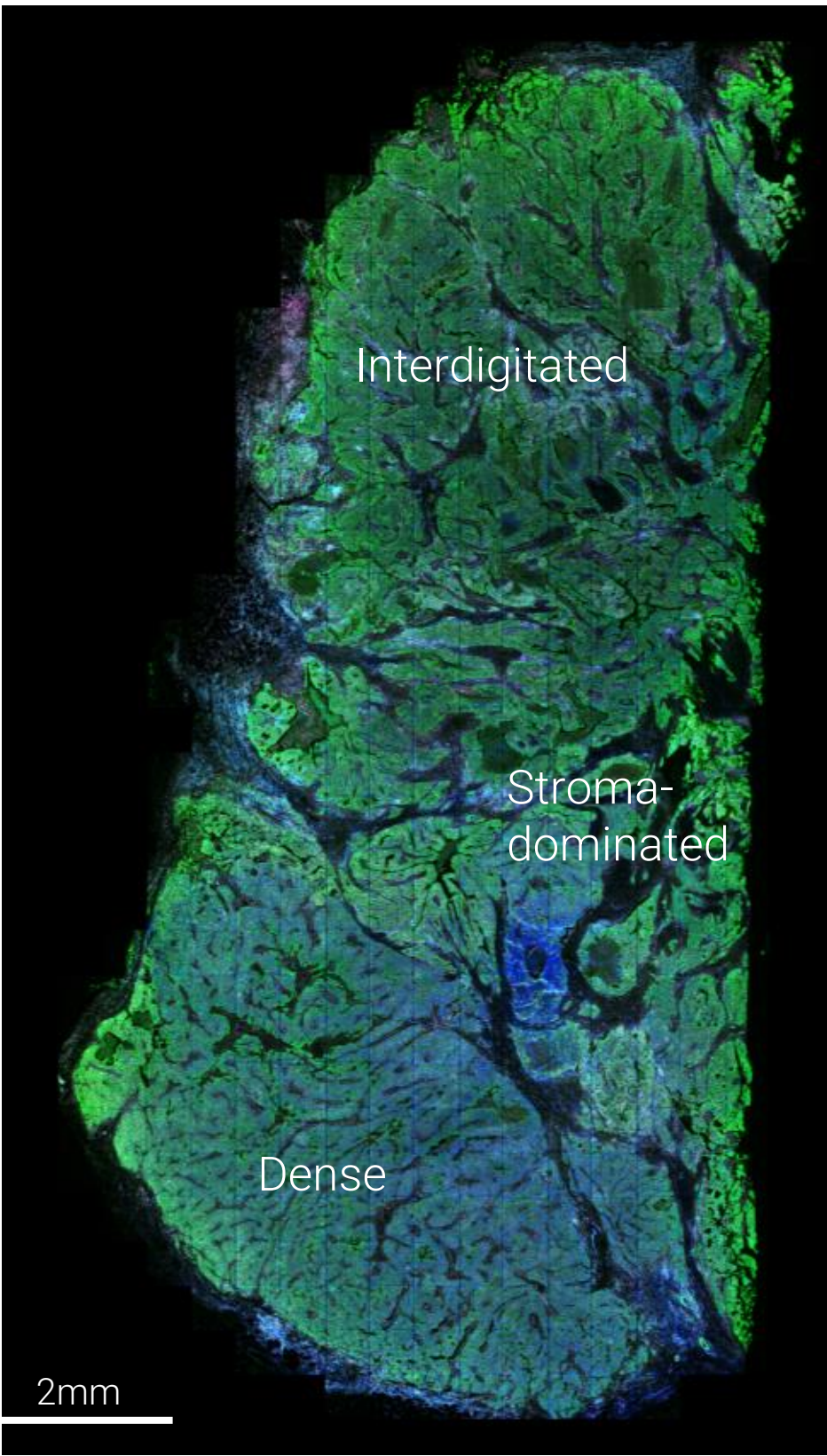
We performed the world's first transcriptome-scale spatial imaging of a breast tumor.

Through analyses focused on the hallmarks of cancer, we assessed what could be learned about one patient's disease.

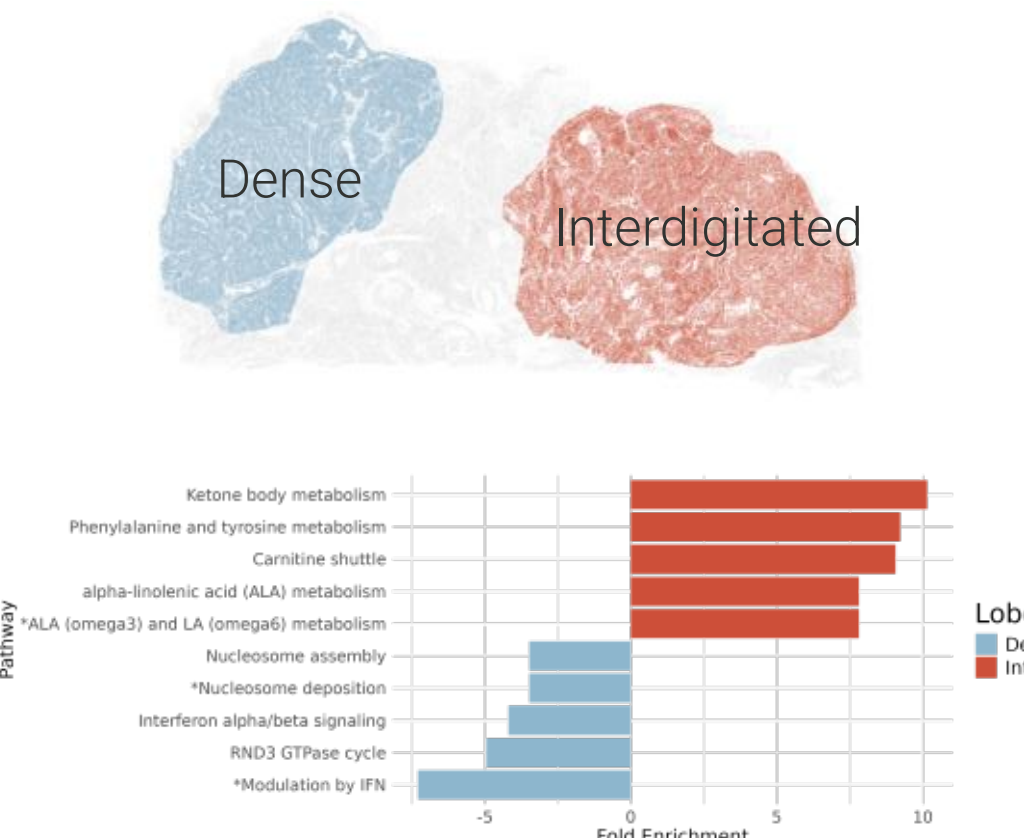
We measured >600000 cells, 18935 genes, 3 morphological regions, 9 spatial domains, 37 cell types, 1692 pathways, 64 proteins.

## Transcriptomics yield a nuanced map of the tumor landscape

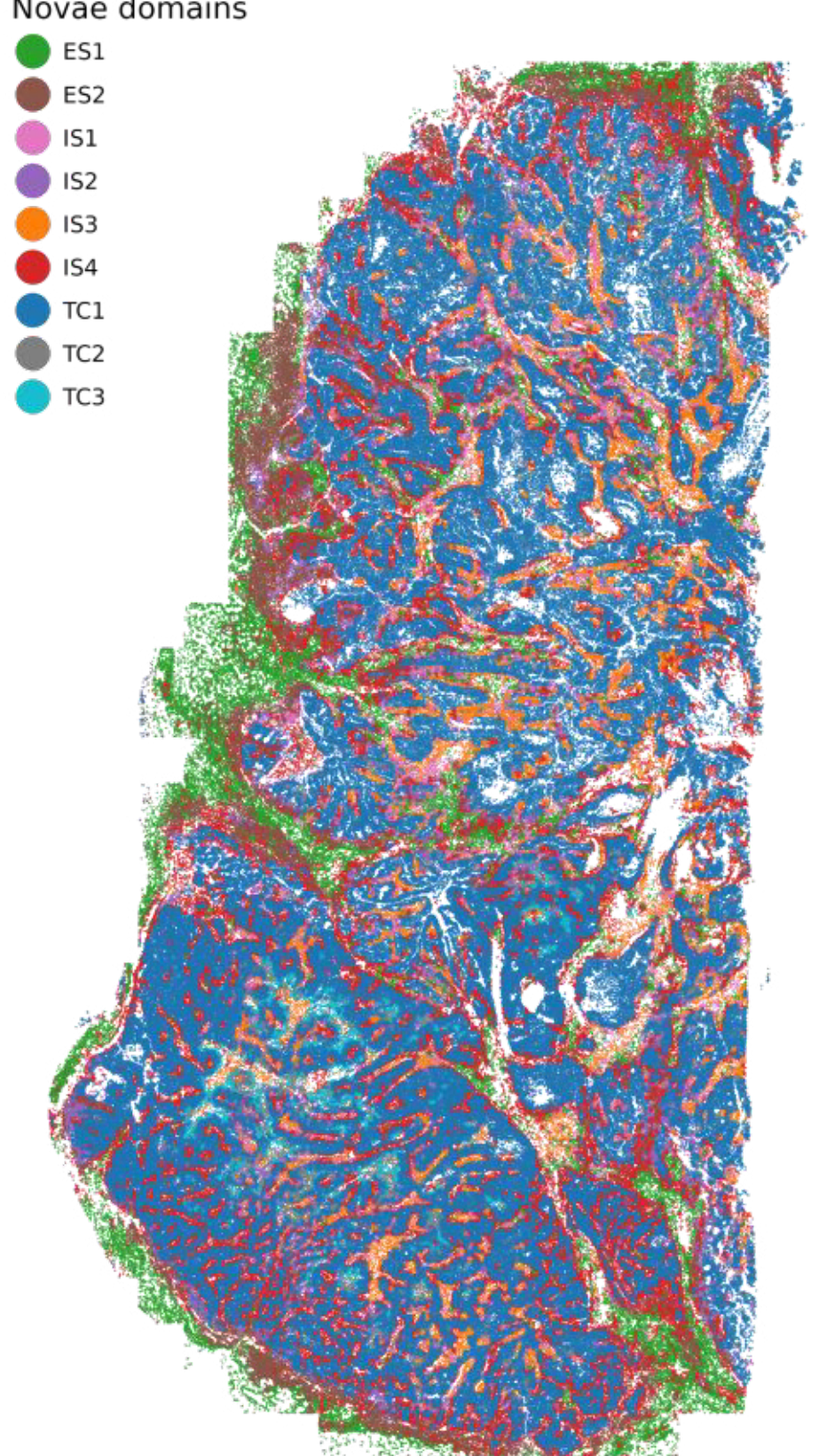
Pathology view: gross morphological structures



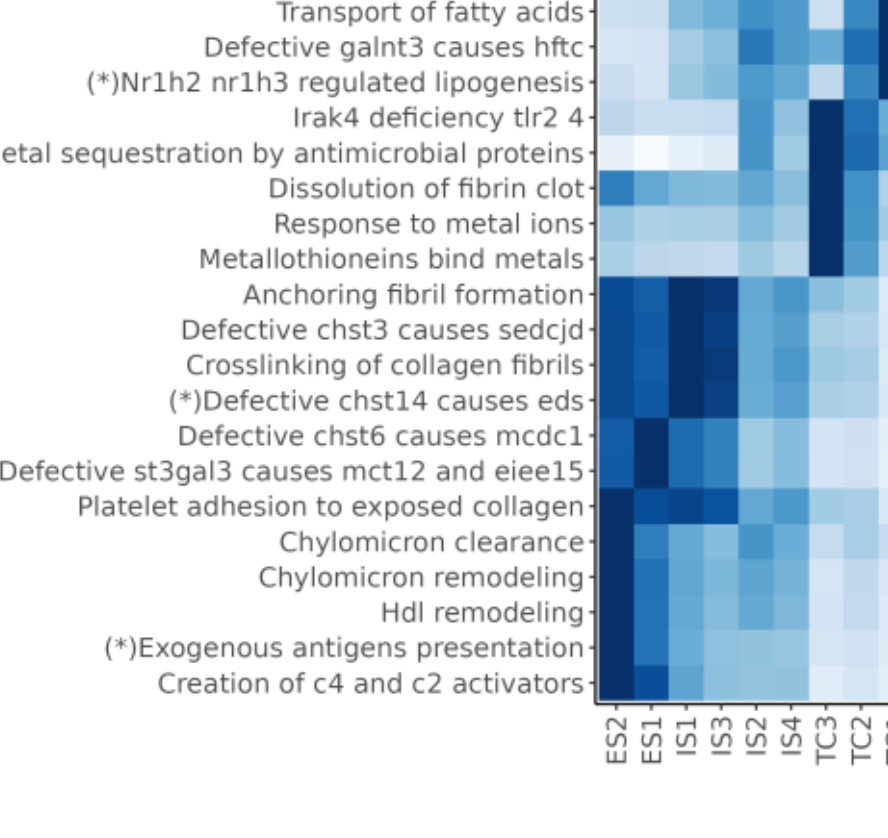
Differential pathways between morphological regions



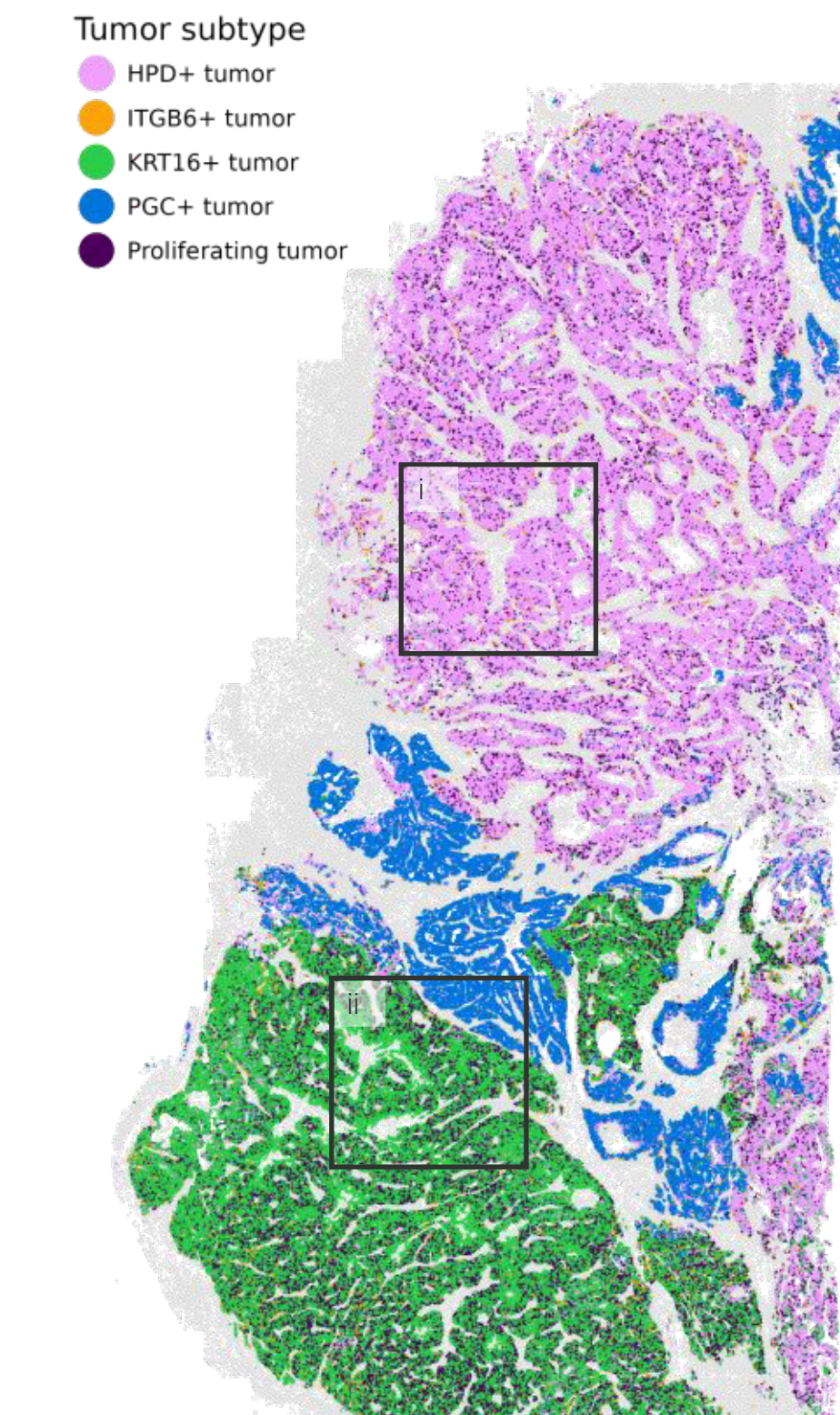
Transcriptomic domains: nuanced view of microenvironment



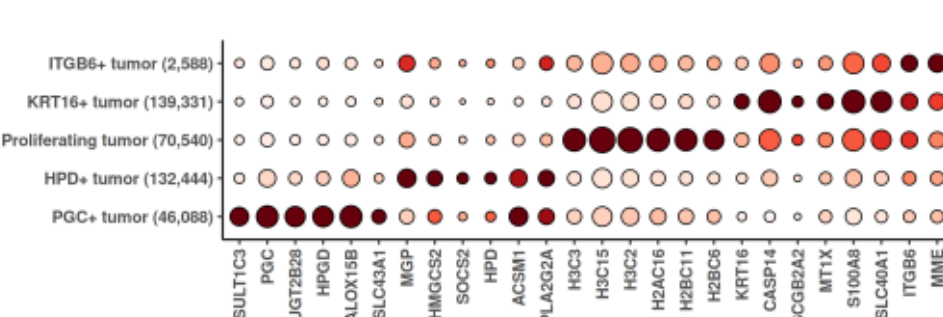
Domain function seen through REACTOME pathway scores



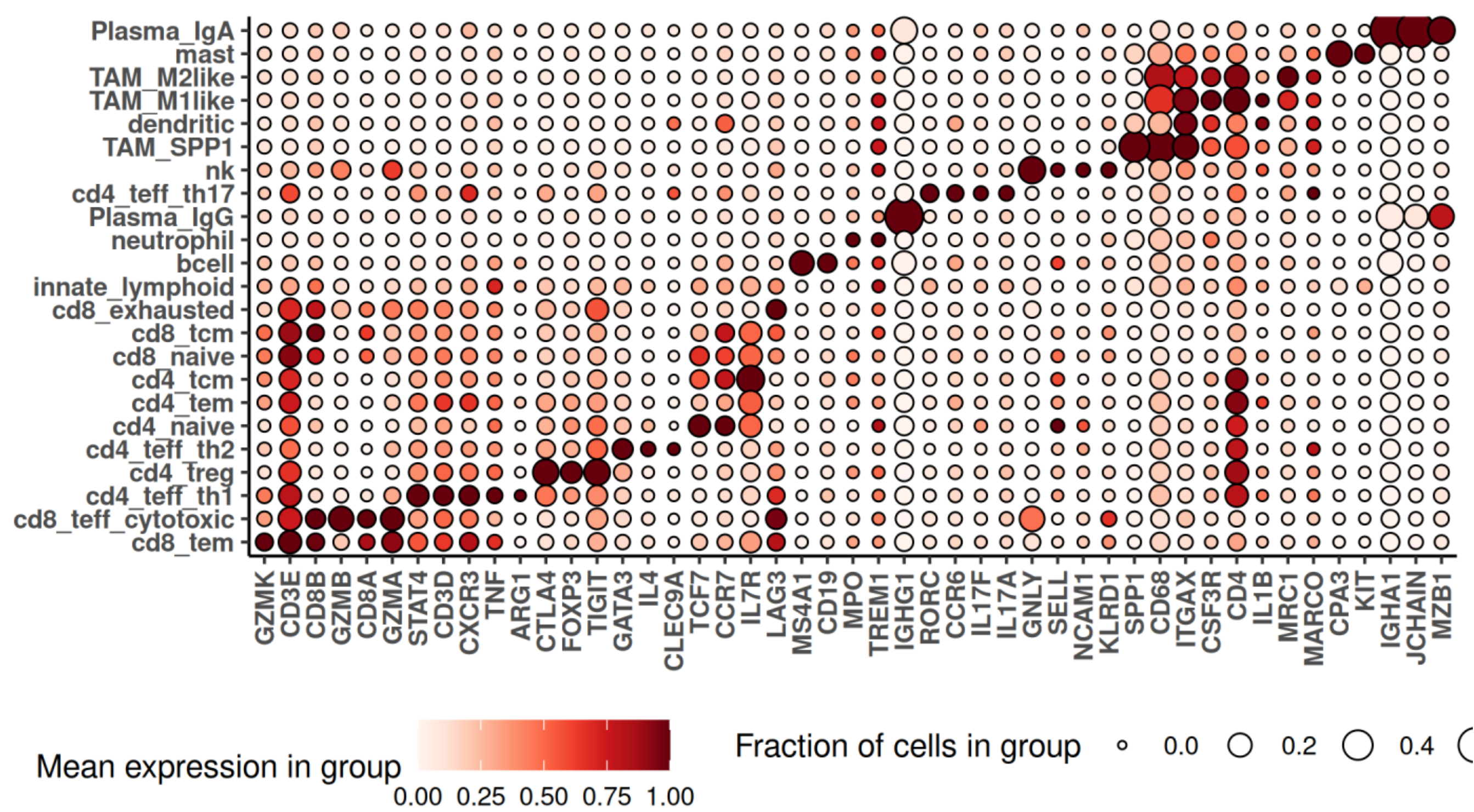
Subclusters of cancer cells: detailed phenotypes (lineages?) of clonal outgrowth



Marker genes of cancer subtypes



## Deep immune cell typing



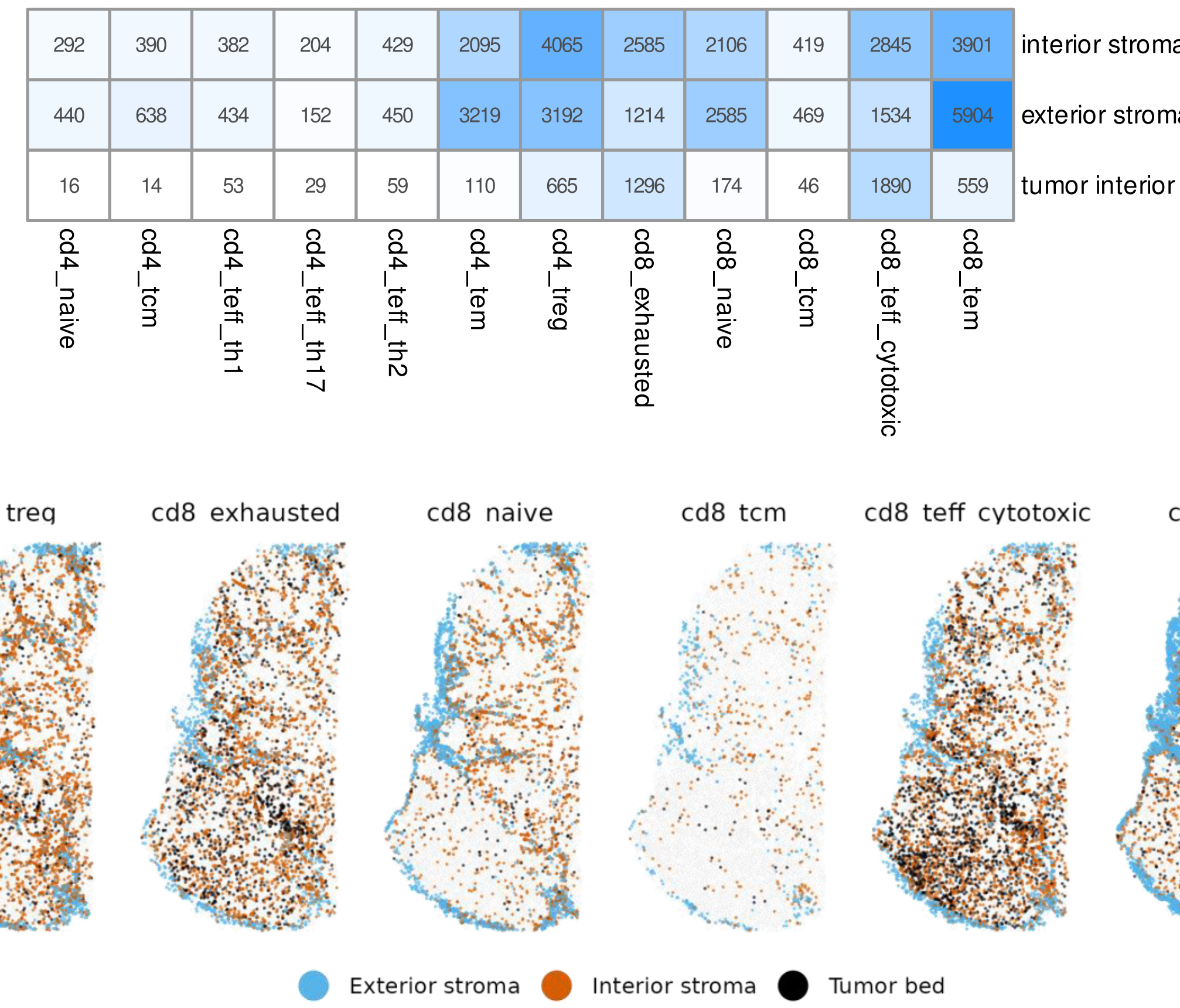
We used the HierAtype algorithm to classify immune cells based on canonical marker genes. We classified 23 immune cell populations, including 12 T-cell subsets.

## Mechanisms of immune escape

T cells have infiltrated the tumor bed.

Upper panel: T-cell subtype abundance by spatial context.

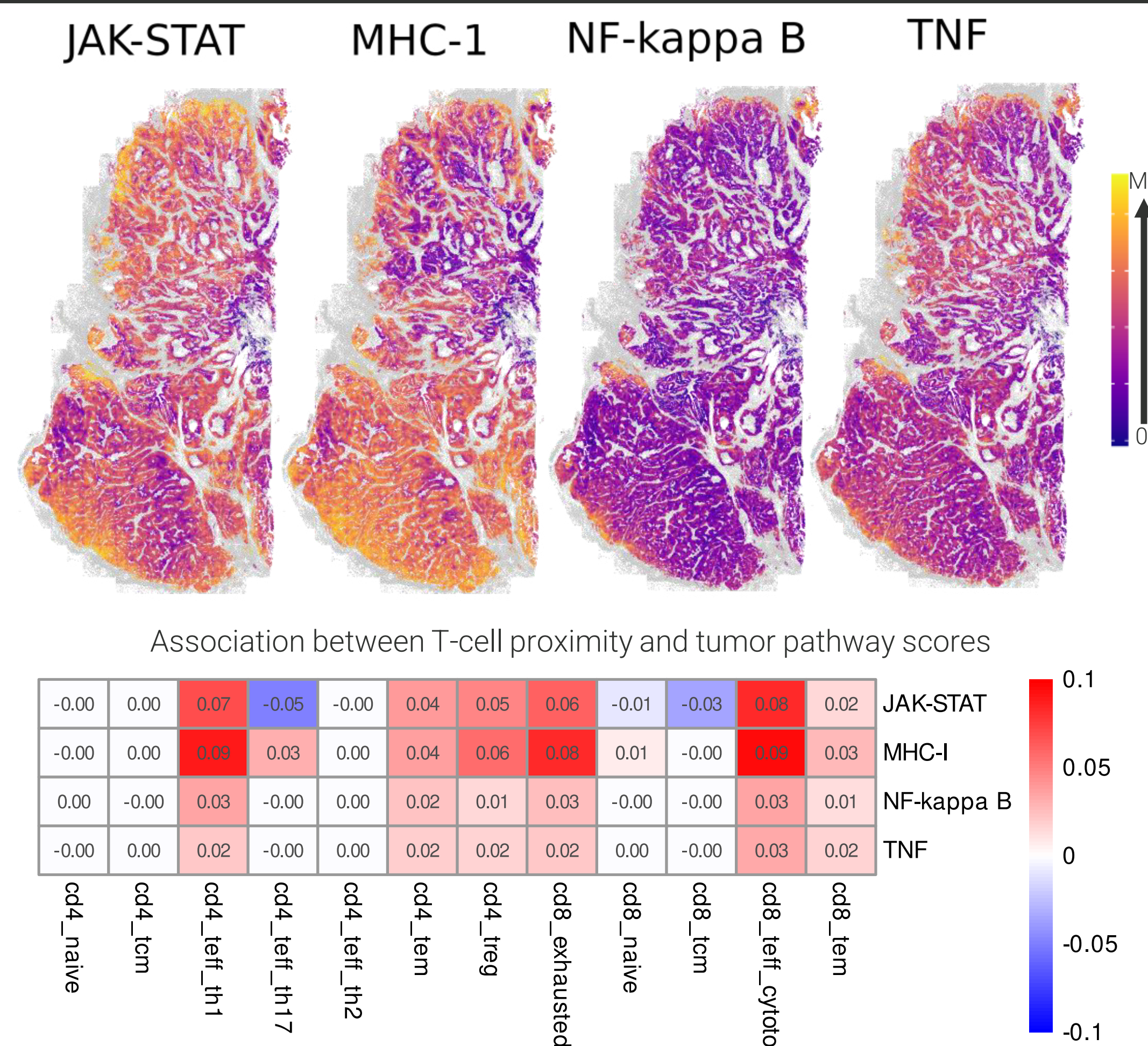
Lower panel: Points show T cell locations; color denotes spatial context.



Cancer-intrinsic pathway activity confirms T-cell signaling is having an effect

Upper panel: Pathway scores in tumor cells show response to T-cell signaling in areas of dense T cells.

Lower panel: tumor immune response pathways are enriched with proximity to effector T cells.



Cytotoxic CD8 T cells inside tumor bed lose effector function and up-regulate exhaustion genes

Right: Differential expression analysis of cytotoxic CD8 T cells vs. spatial context.

Up-regulated in tumor bed:

The checkpoint and exhaustion genes PDCD1, ID2 and SARDH

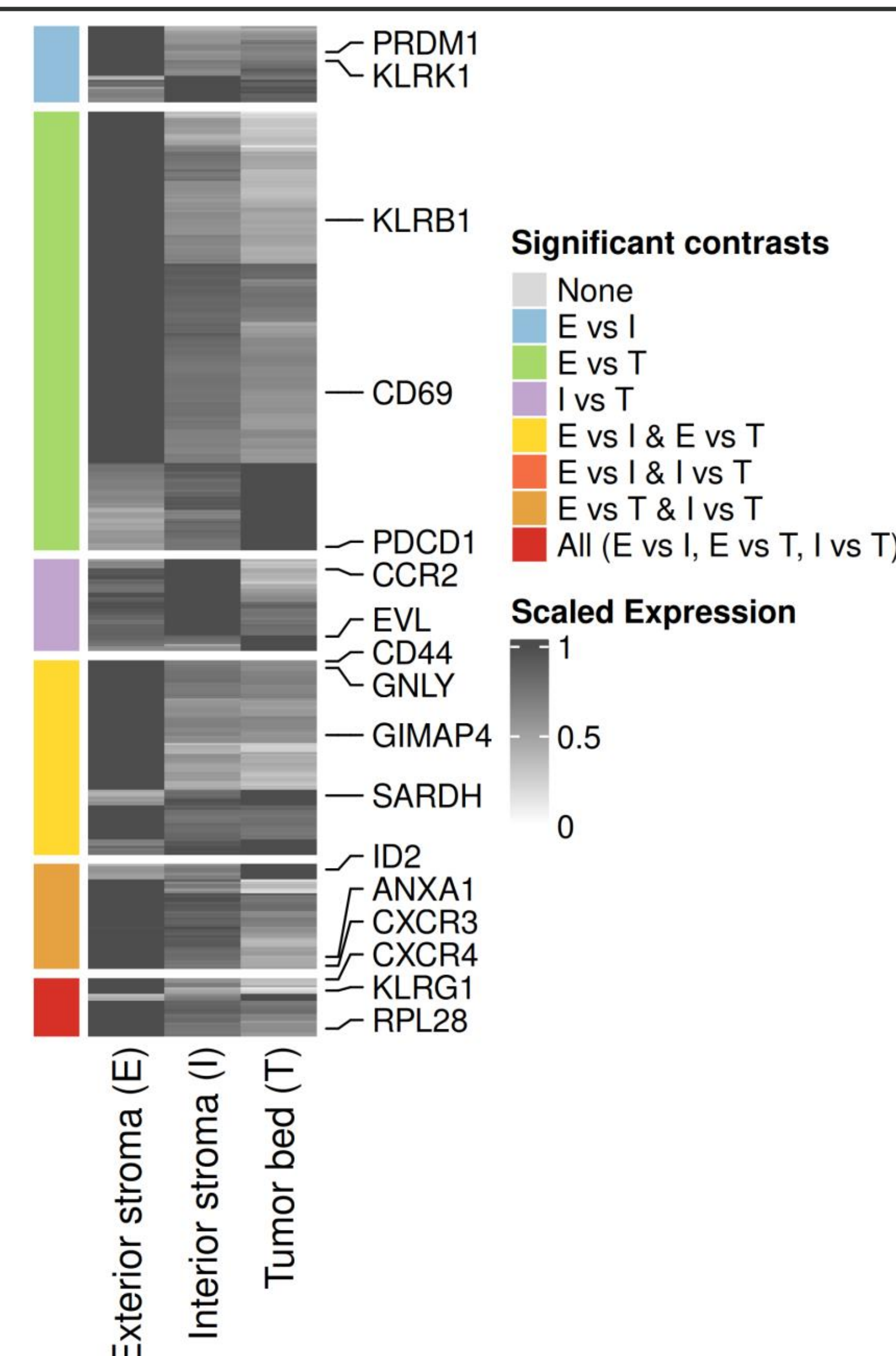
Down-regulated in tumor bed:

Effector genes: KLRK1, KLRG1, GNLY and PRDM1

Homing and adhesion: CD44

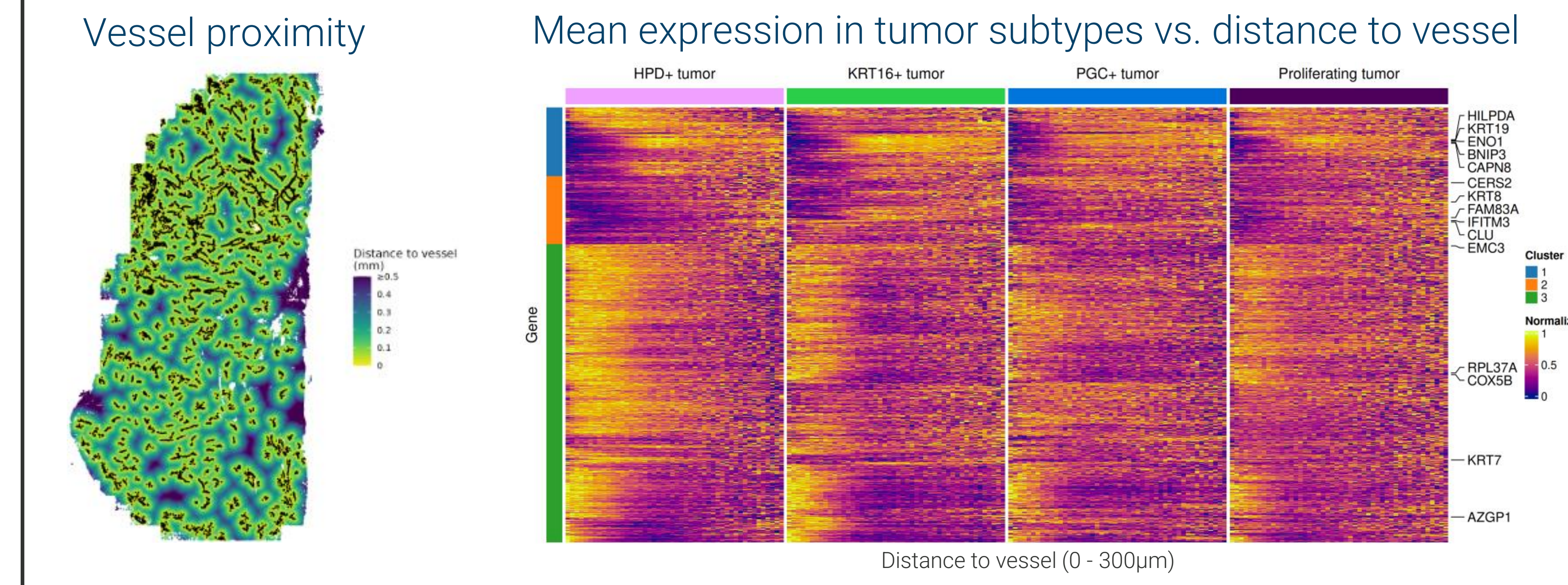
Chemotaxis: CXCR4 and SELPLG

GIMAP4: potential new mechanism of T-cell inhibition

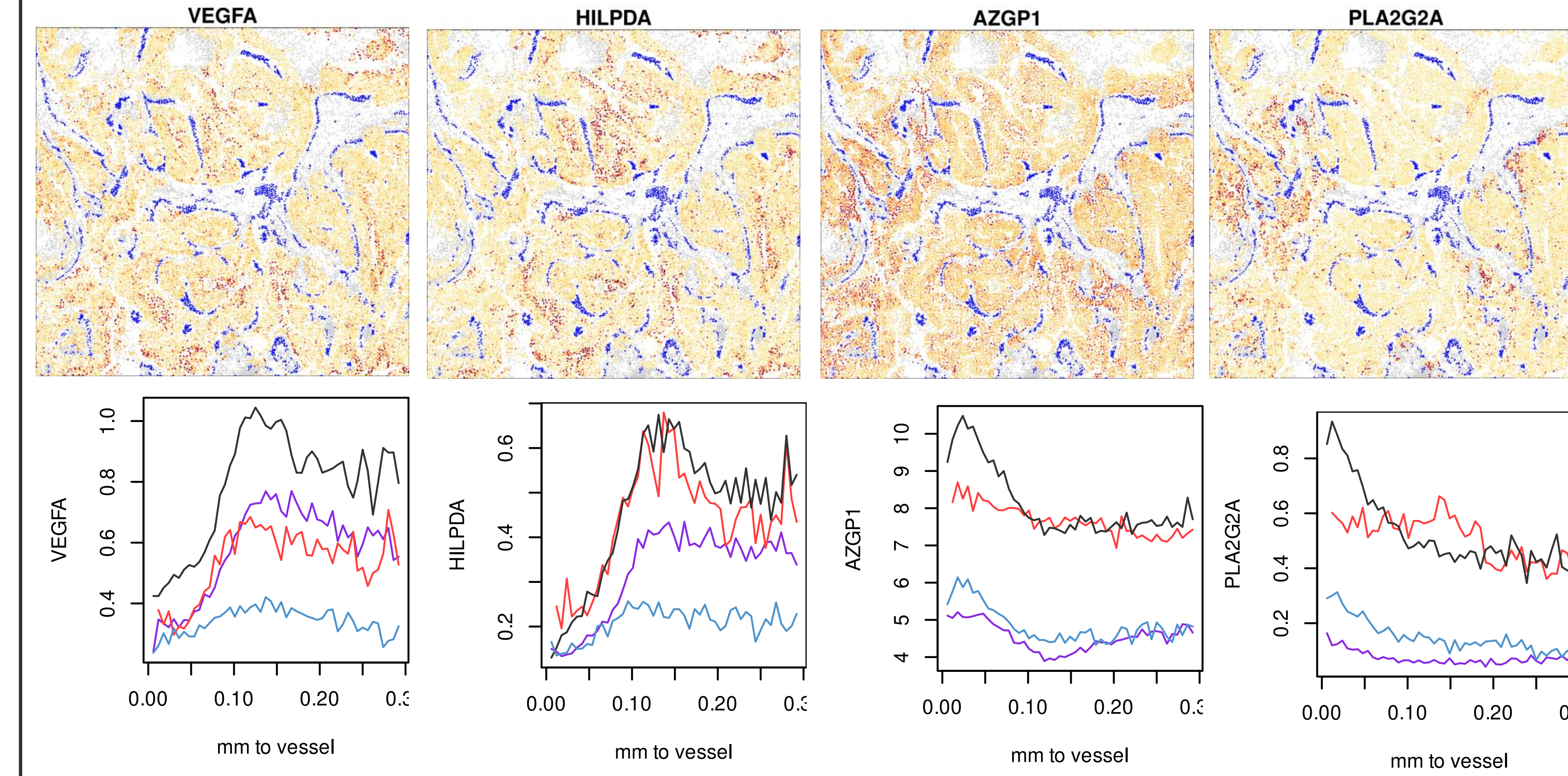


## Cancer cell response to vasculature proximity

Differential expression analysis of cancer cells vs. distance to blood vessels



Detailed results for notable genes



## Impact of cancer metabolism on surrounding cells

Differential expression analysis vs. neighborhood glycolysis: For each of the 32 non-cancer cell types, we ran differential expression analysis against the intensity of the glycolysis signature in nearby cells. This entailed 310,823 hypothesis tests.

Notable results in high-glycolysis regions:

- Fibroblasts increased ECM remodeling and tumor-supporting activities
- M1 TAM's became more inflammatory and metabolically active
- Endothelial cells showed angiogenic and stress-response signatures.

