



Dissecting myeloid and T cells
using spatial transcriptome

interaction niches in the TME

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07/18/2023

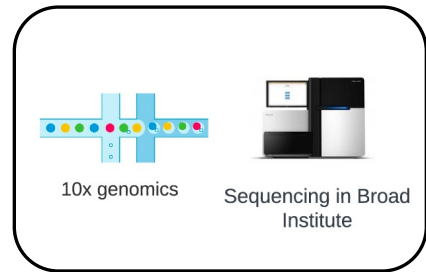
Overview of Immunitas Integrated Computational Pipeline & Workflow for Novel Drug Target Identification

In-house sample prep
by Immunology Group

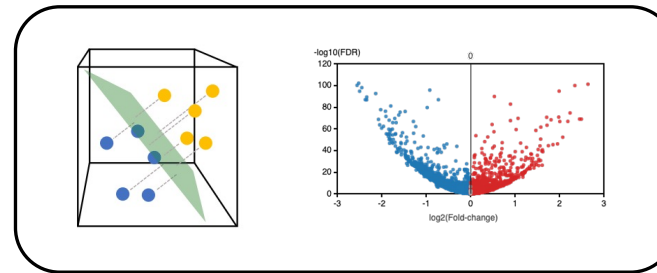
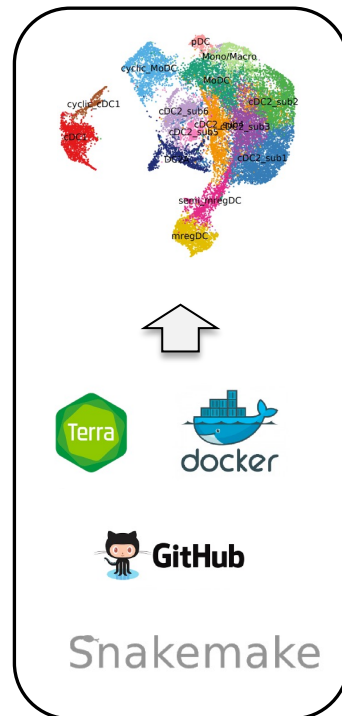
Single-cell data
processing pipeline

Supervised ML identifies genes in myeloid
cells correlated with immune response

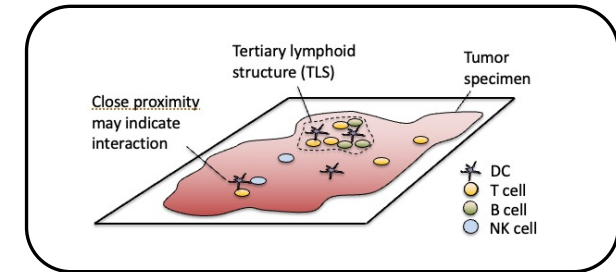
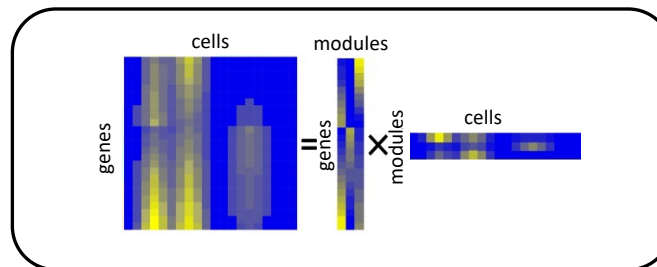
Target validation with spatial
transcriptomics



Curation of publicly available
datasets



Unsupervised ML identifies gene co-
expression modules in myeloid cells



Target validation by cross-
referencing with external data

	Toxicology	CRISPR KO	...	Novelty
Gene 1	✓	✓		✓
Gene 2	✓	✗		✓
...				
Gene N	✗	✓		✗

Location, Location, Location

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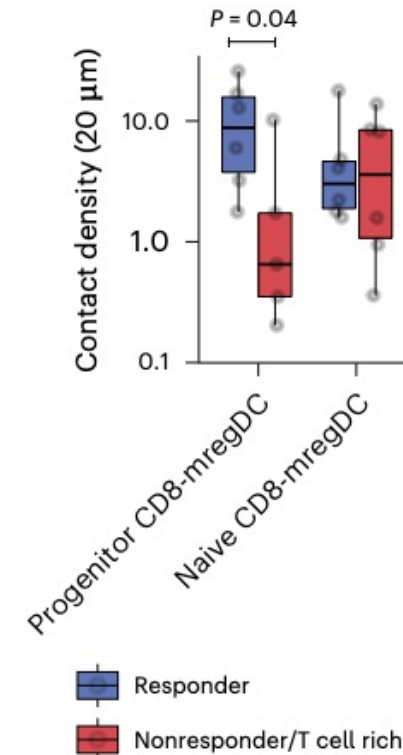
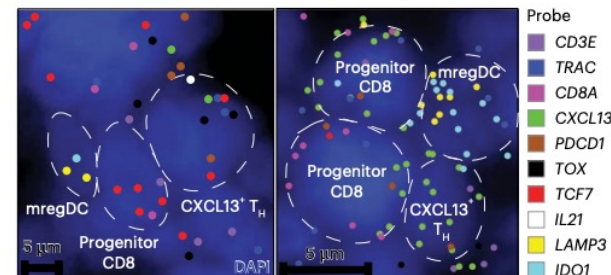
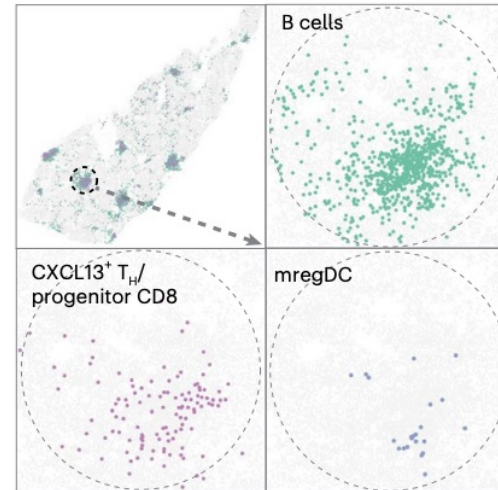
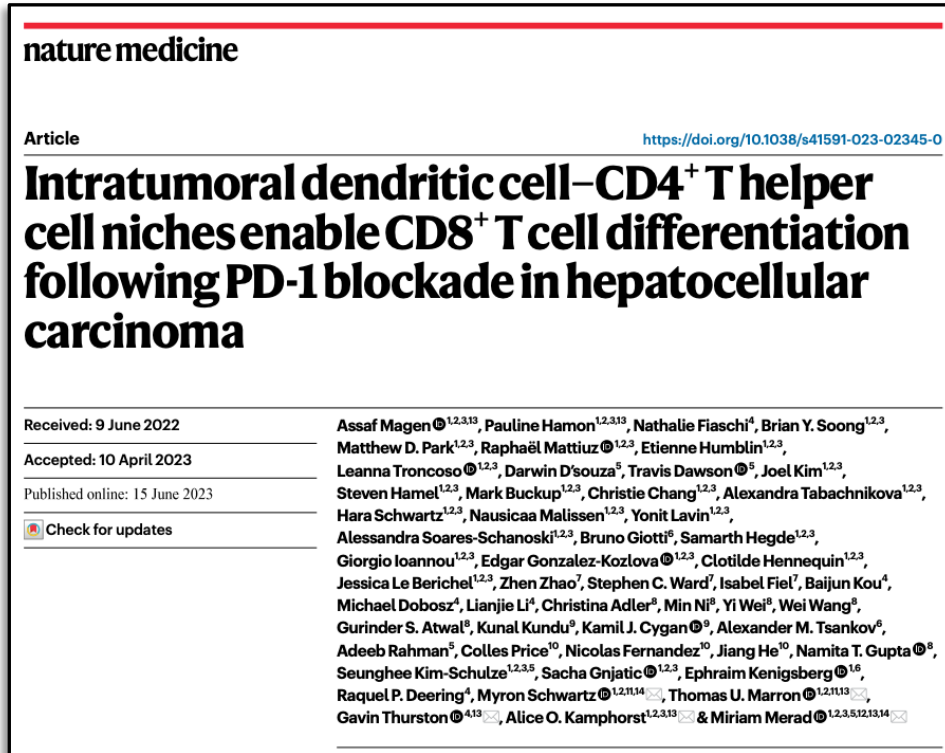
Technology Feature | [Published: 06 January 2021](#)

Method of the Year: spatially resolved transcriptomics

[Vivien Marx](#) 

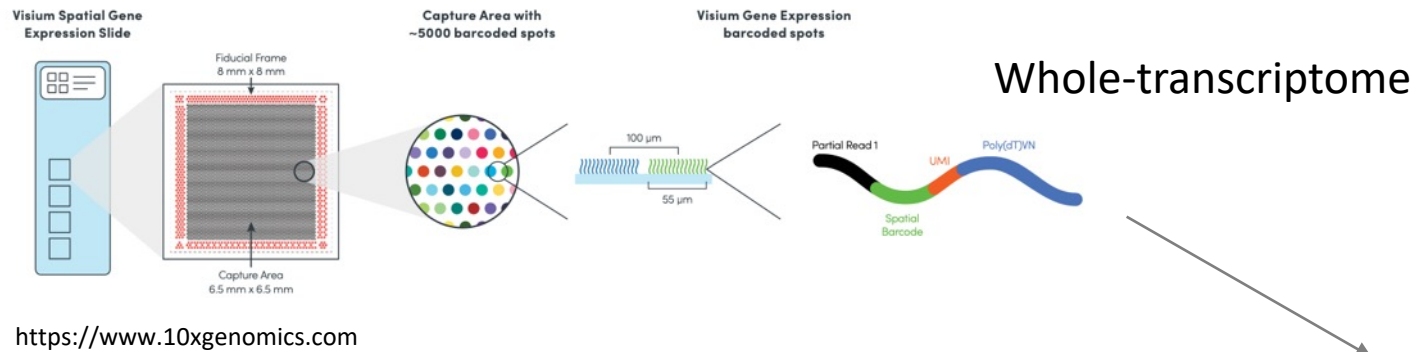
[Nature Methods](#) **18**, 9–14 (2021) | [Cite this article](#)

Spatial transcriptomics enables exploration of cellular niches in the tissue microenvironment



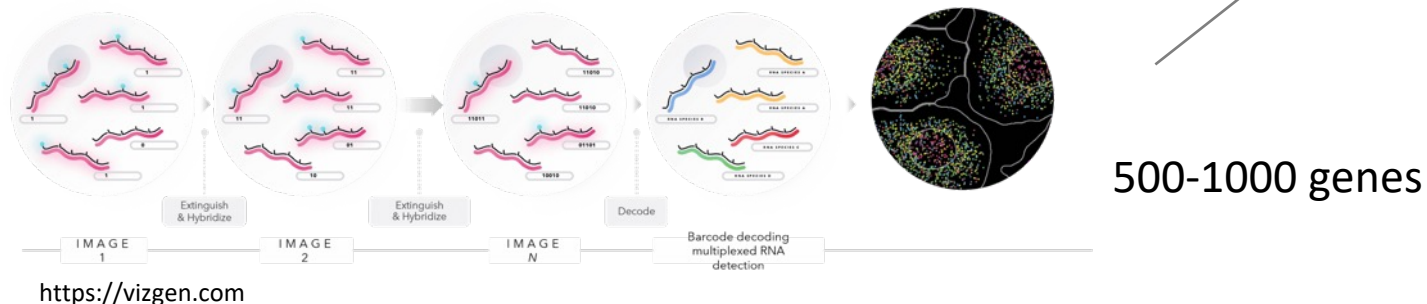
Spatial transcriptomics: spatially resolved gene expression

Sequencing-based: Spatial Transcriptomics, Visium



	Cell/Spot 1	Cell/Spot 2	...	Cell/Spot N
Gene 1	3	0	...	11
Gene 2	5	8	...	0
⋮	⋮	⋮	⋮	⋮
Gene M	1	0	...	4

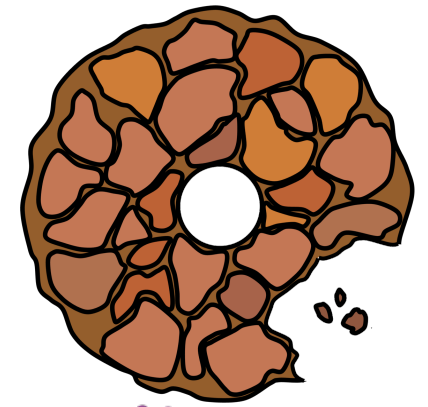
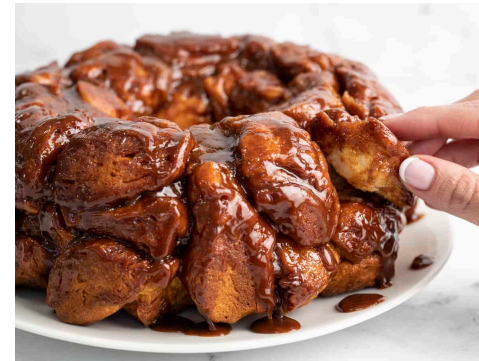
Fluorescent *in situ* hybridization(FISH): MERSCOPE, Xenium, Nanostring COSMX



	Cell/Spot 1	Cell/Spot 2	...	Cell/Spot N
x	36	0	...	91
y	50	17	...	37

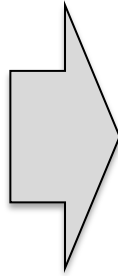
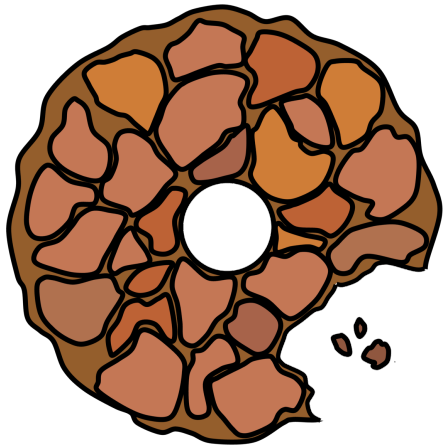
Exploring cellular niches in tumors with Monkeybread

- Monkeybread is a Python package developed at Immunitas that facilitates exploration of cellular organization in spatial transcriptomics data
- Available at GitHub:
<https://github.com/immunitastx/monkeybread>
- Developed by Dillon Scott and Matt Bernstein

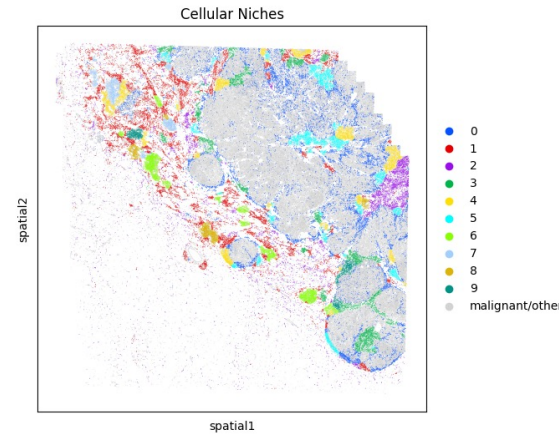


Exploring cellular niches in tumors with Monkeybread

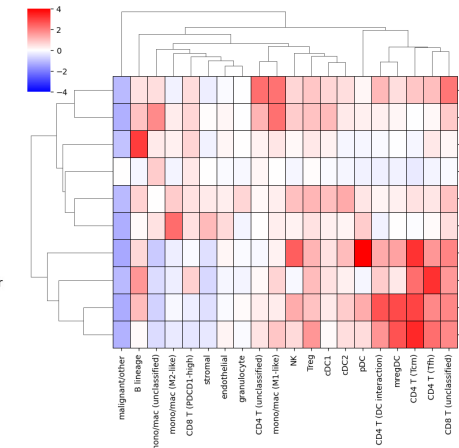
Monkeybread



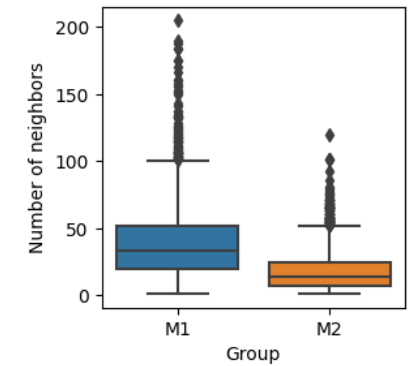
Identify cellular niches



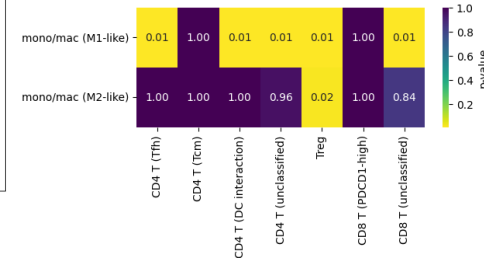
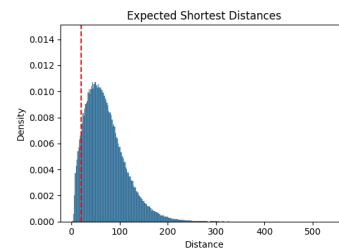
Explore cell type enrichment in niches



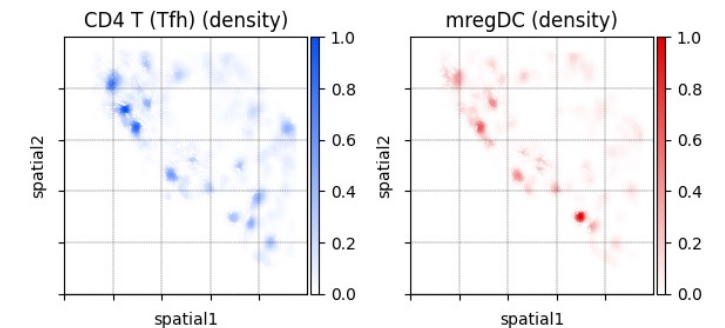
Compare neighborhood composition between cell types



Statistical tests for cell co-localization

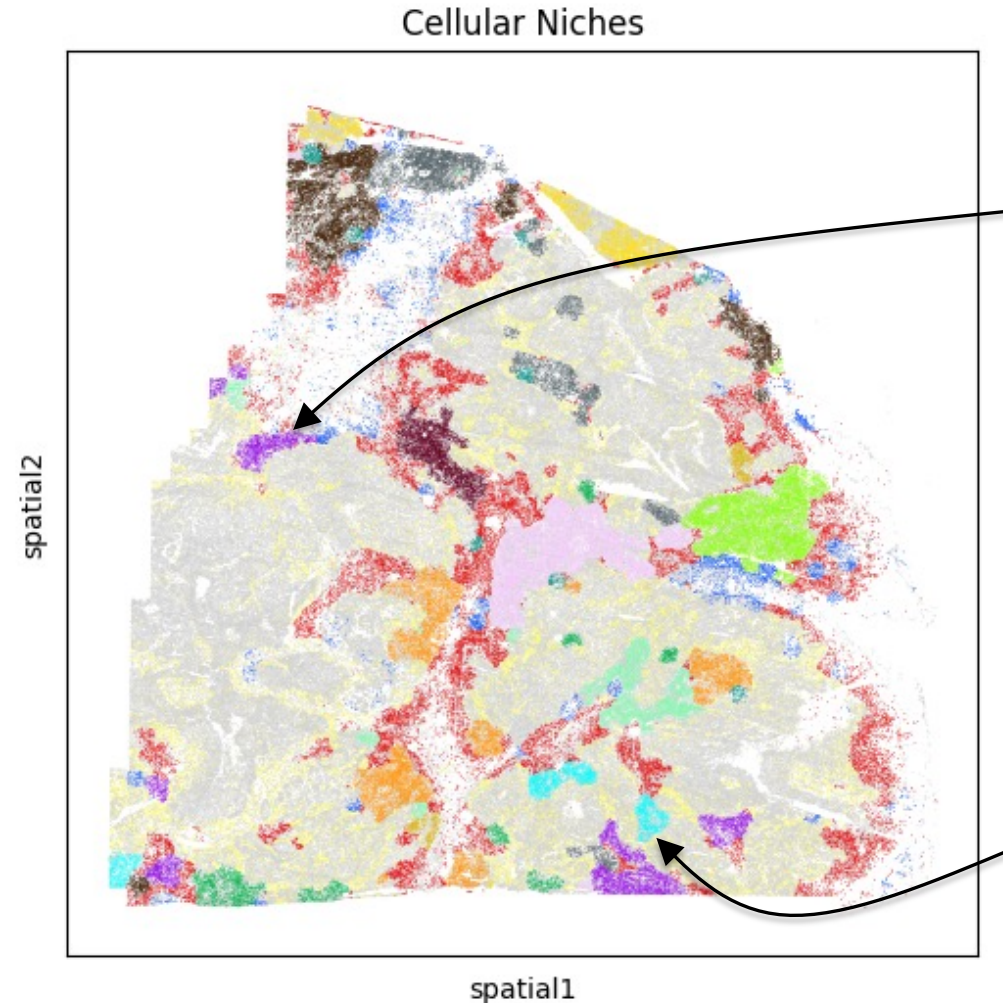


Compare spatial distribution between cell types

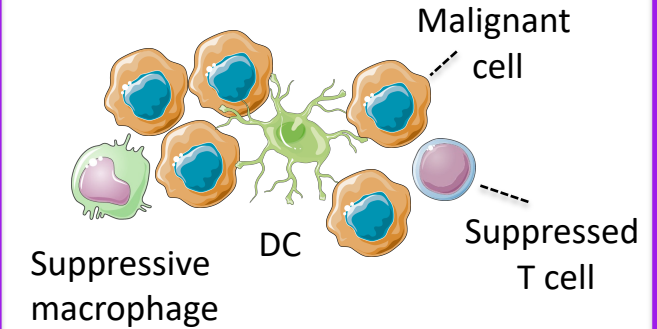


Comparing Immune cells located in anti vs. pro-tumor cellular niches

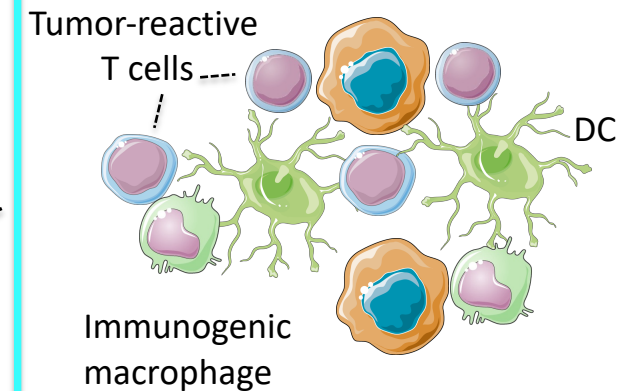
- Identify niches of co-localizing cell types and phenotypes in the TME
- Compare gene expression between cells in suppressive vs. immunogenic niches



Suppressive niche



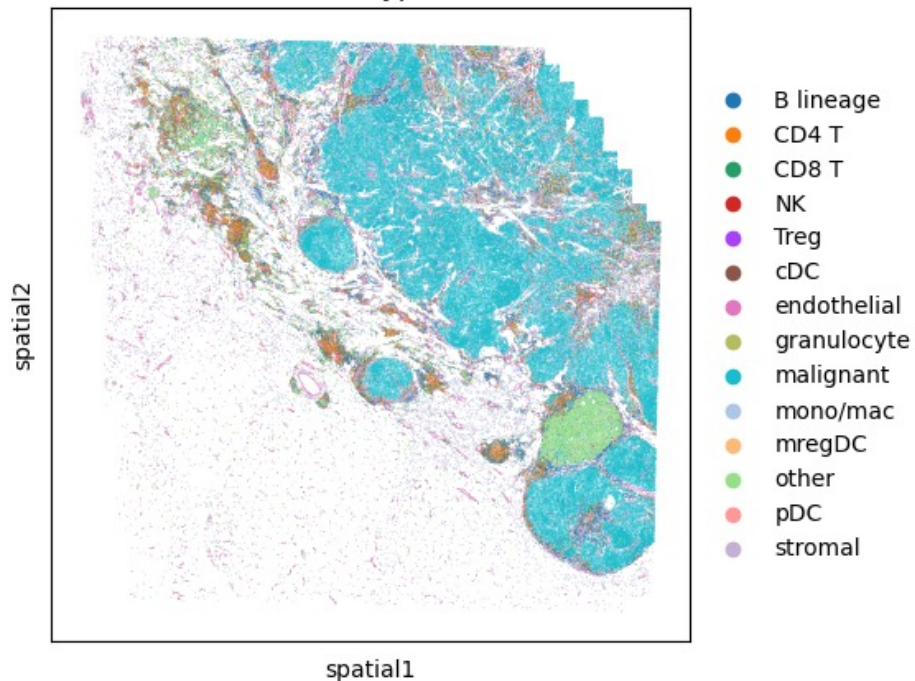
Immunogenic niche



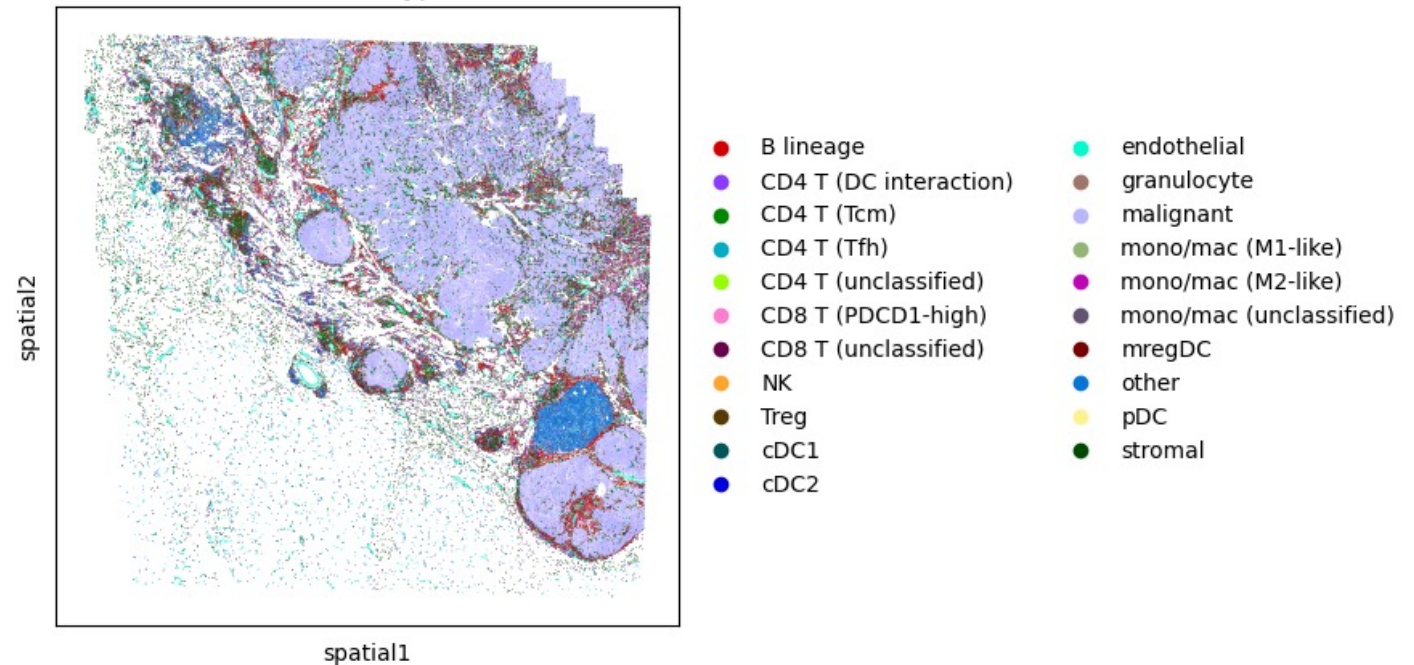
Case study: Human melanoma sample

- Retrieved and annotated a melanoma dataset from Vizgen's public immuno-oncology data release
 - <https://vizgen.com/human-ffpe-immunooncology-release-roadmap/>

Cell Type



Cell Subtype



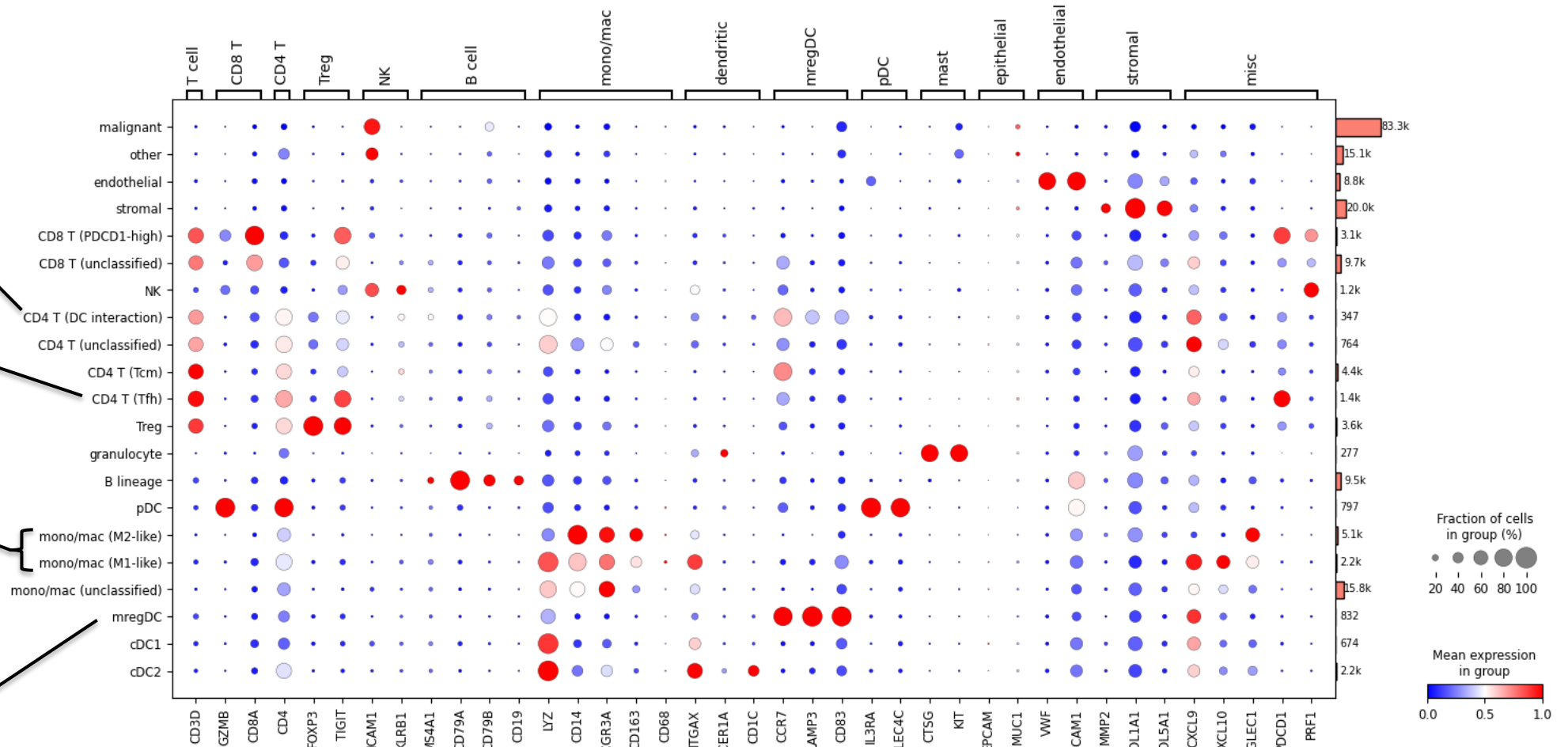
Fine-grained cell type annotations

T cells enriched for DC markers may be T cells interacting with DCs

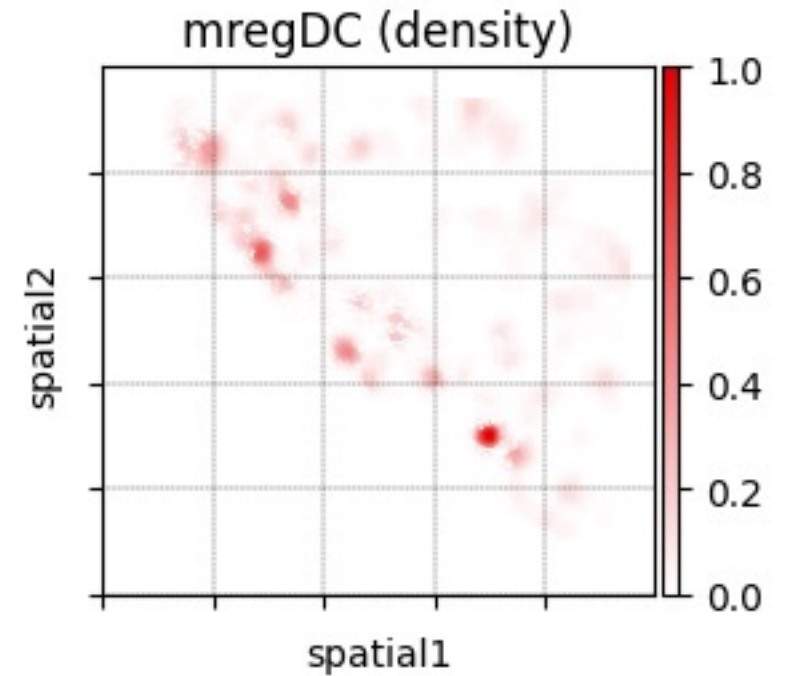
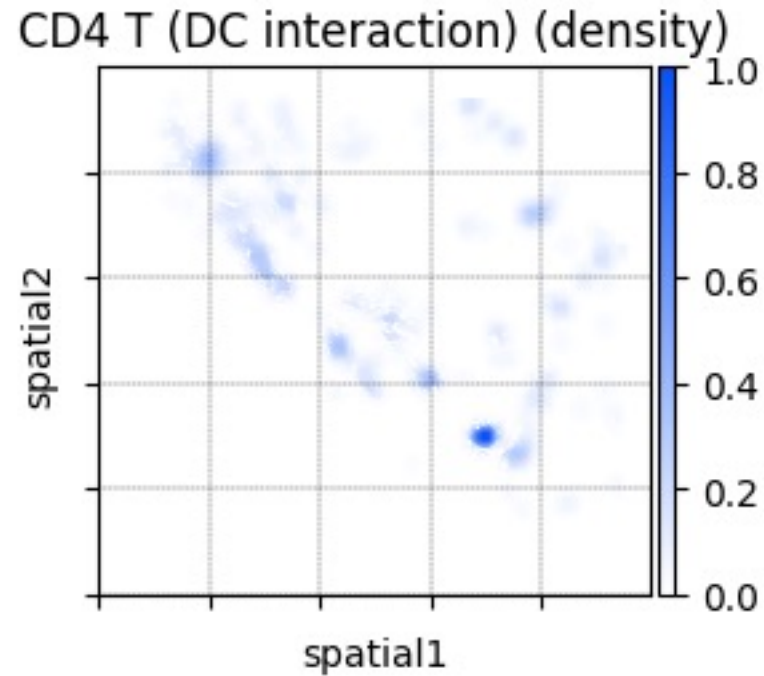
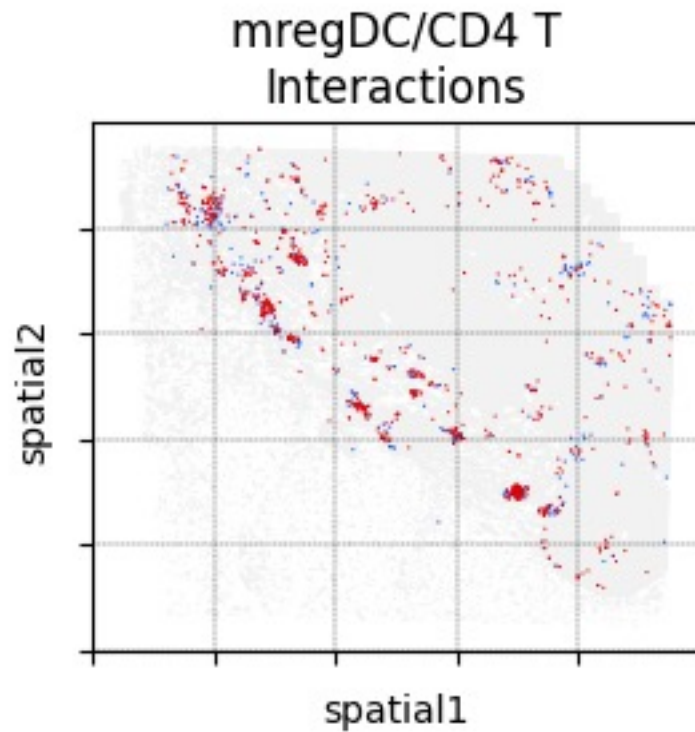
Tfh cells are high for PD-1

Suppressive and immunogenic macrophages

mature DCs enriched in immunoregulatory modules

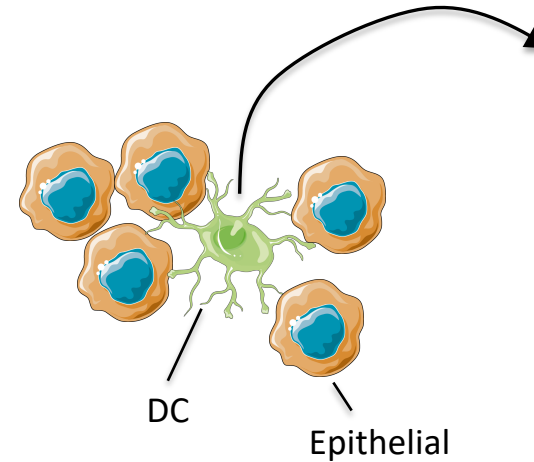


DC-marker-expressing CD4 T cells co-localize with mregDCs



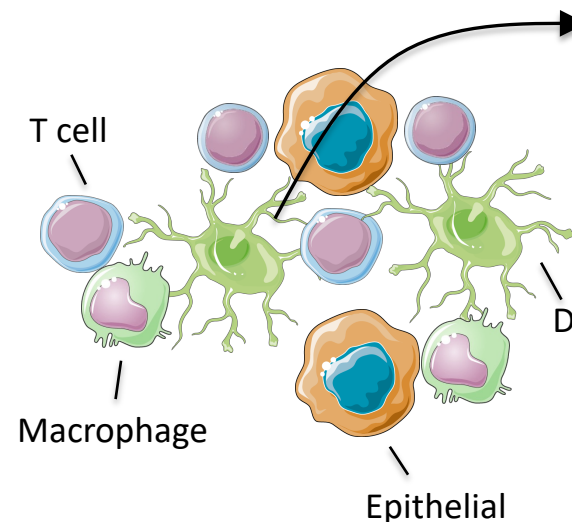
Computing neighborhood profiles

1. For each cell, determine the frequency of cell types in its neighborhood
2. Assign each cell a “profile” of neighbor-frequencies
3. Normalize cellular frequencies to account for very common cell types (e.g., malignant cells)
4. Clusters of neighborhood profiles define cellular niches



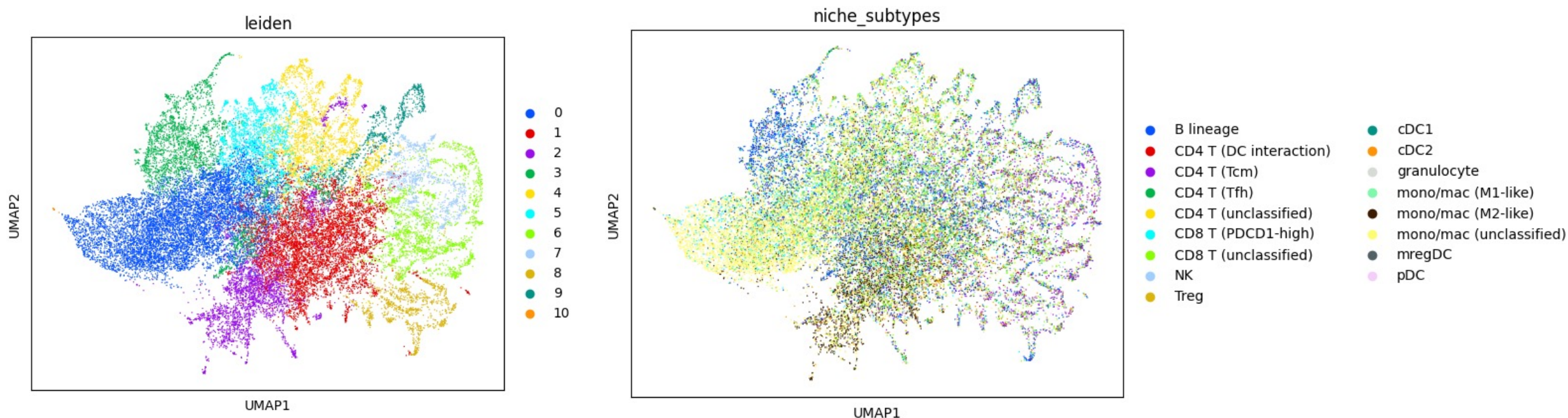
Neighborhood profile

Cell type	Frequency in neighborhood
Epithelial	5
CD8 T	0
DC	1
...	...
Macrophage	0



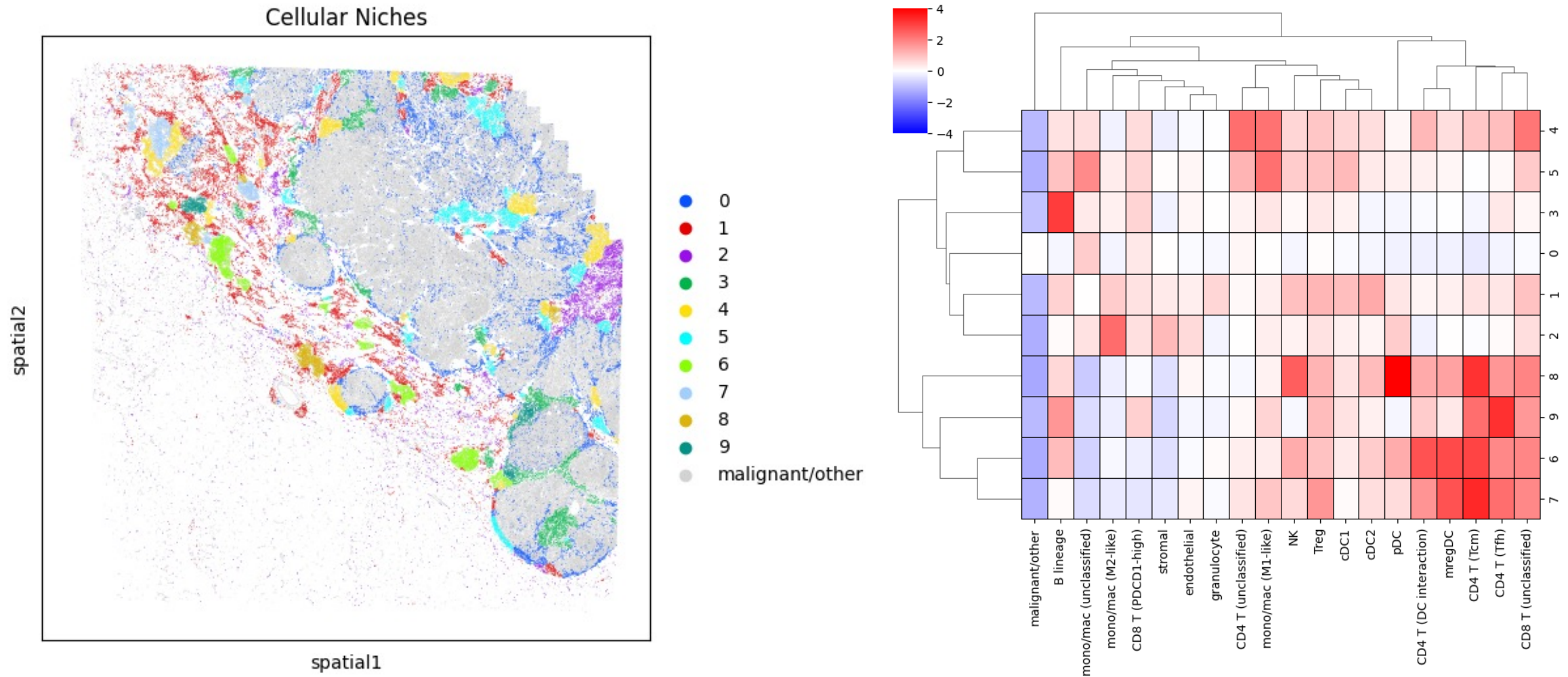
Cell type	Frequency in neighborhood
Epithelial	2
CD8 T	4
DC	2
...	...
Macrophage	2

Define cellular niches as clusters of neighborhood profiles



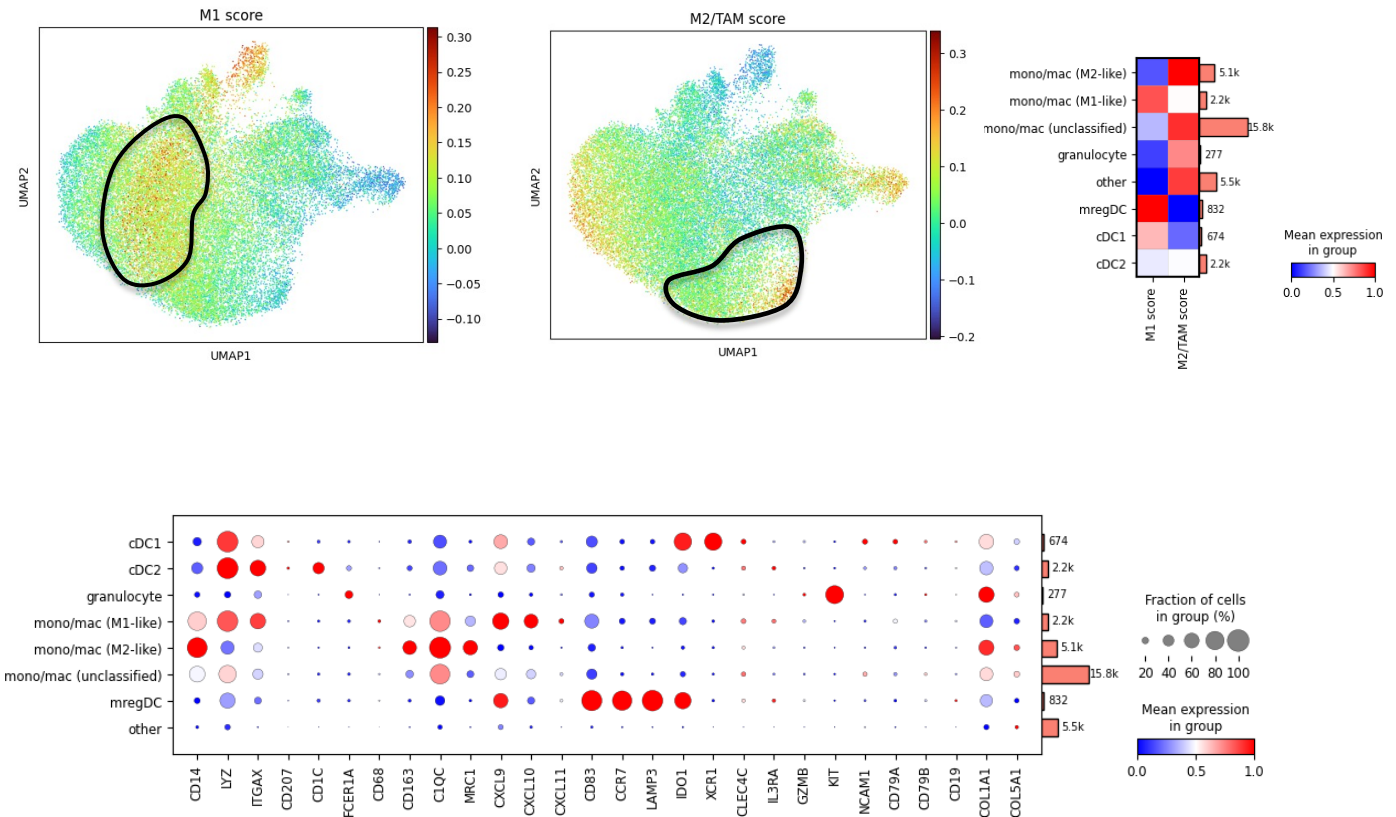
A cellular niche: a spatial region containing a similar composition of the different cell types

Niche analysis characterizes heterogeneity of tumor microenvironment

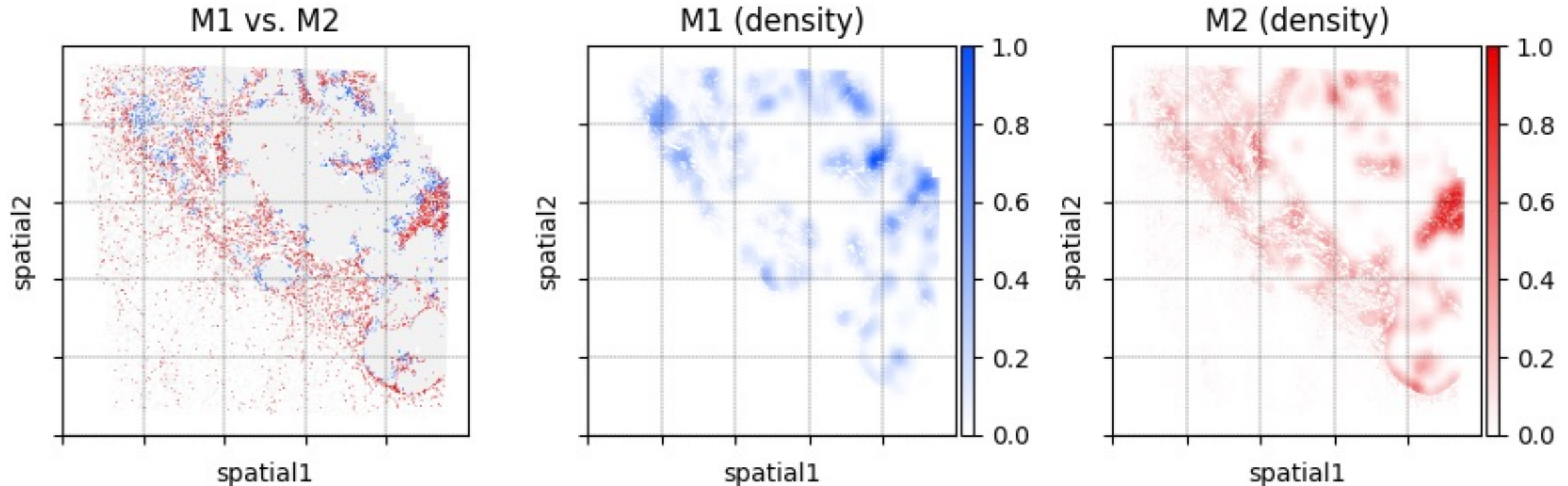


Vignette: Immunogenic vs. suppressive macrophages

- Examination of myeloid cells revealed a population enriched for an M2 signature (suppressive) and M1 signature (immunogenic)
 - Signatures derived from in-house in vitro experiments

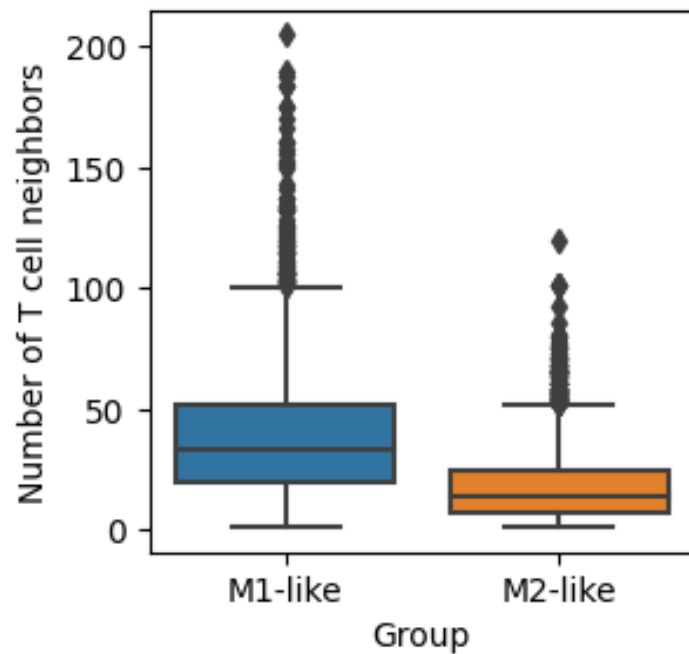


Macrophages with an immunogenic phenotype are in different regions from those with a suppressive phenotype

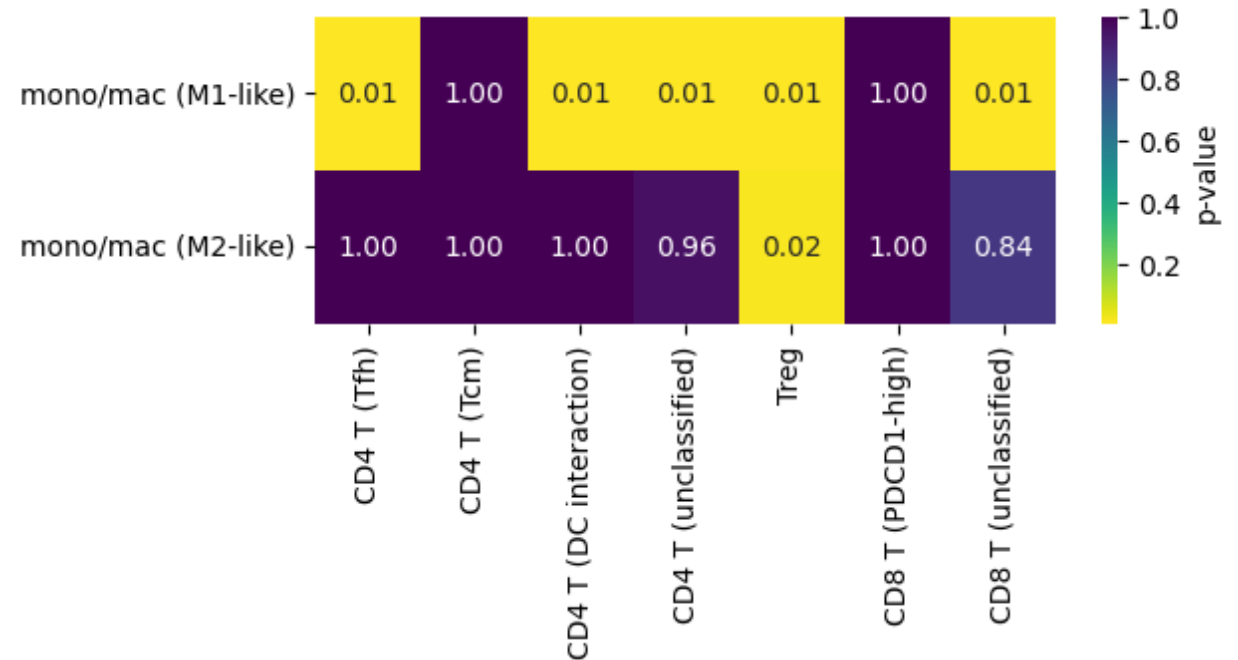


Immunogenic macrophages co-locate with T cells whereas suppressive macrophages co-locate with Tregs

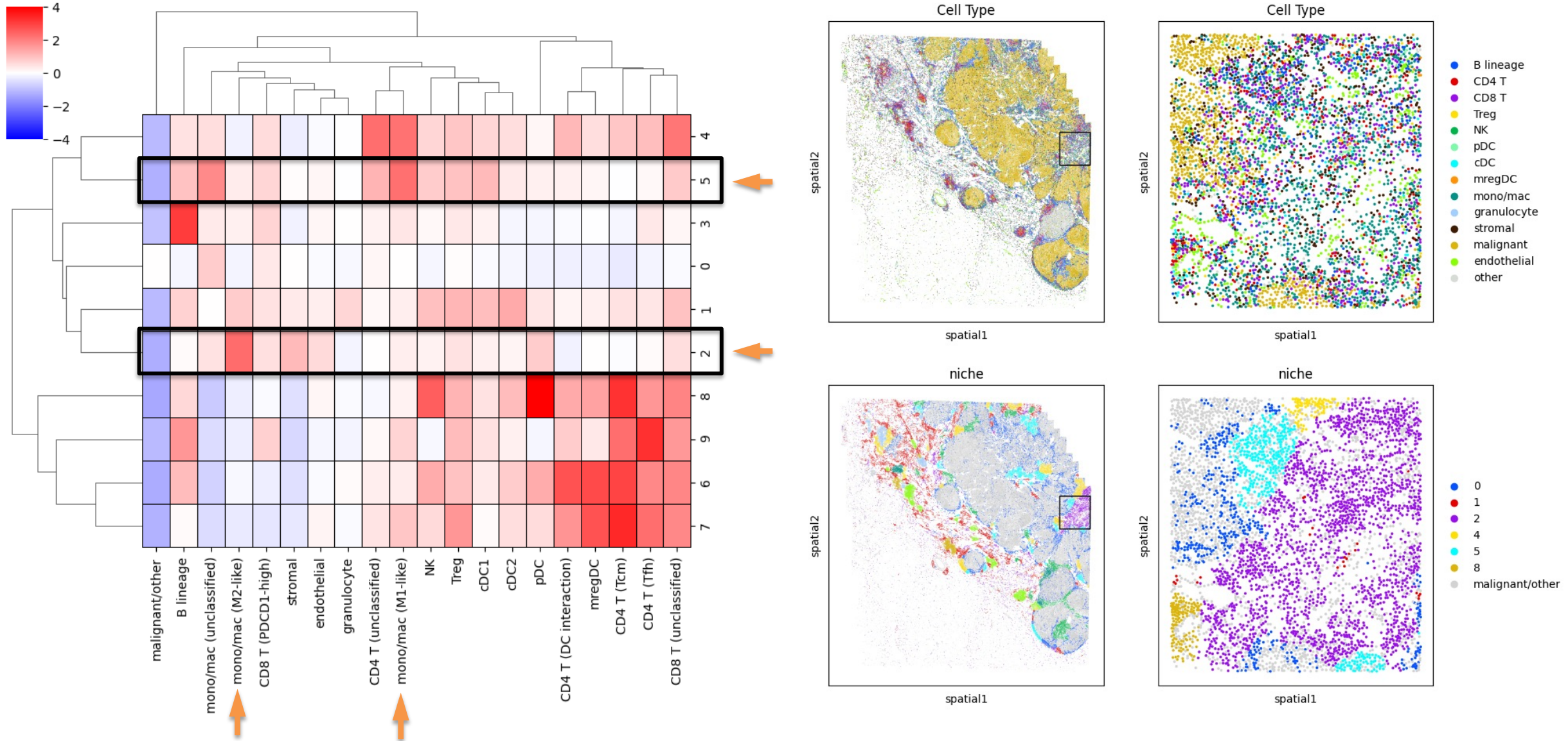
Compare number of T cells in vicinity of macrophages of each phenotype



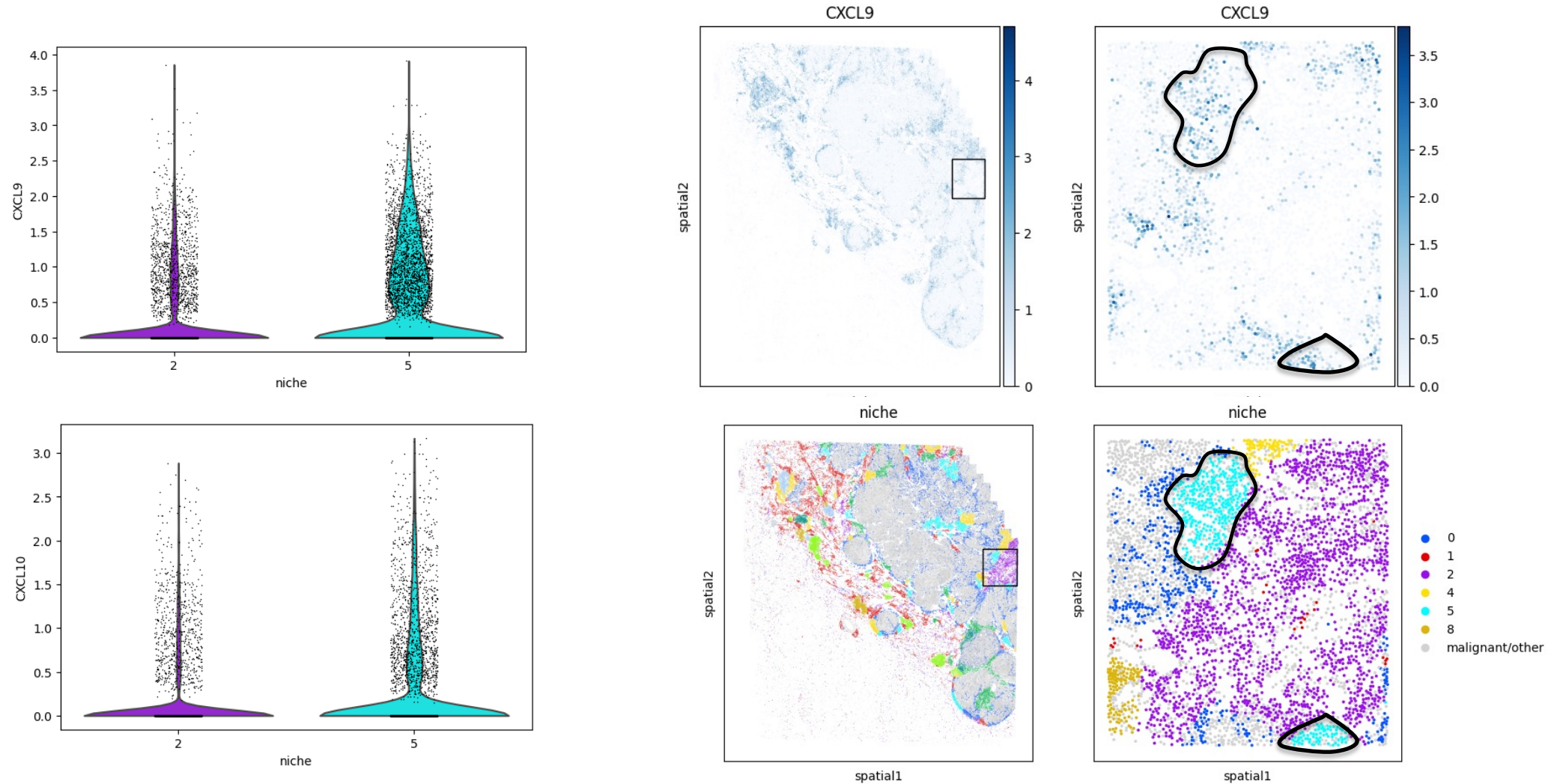
Permutation test examining distance of macrophages to their nearest T cell neighbor



Niche 2 and 5 are enriched for suppressive vs. immunogenic macrophages

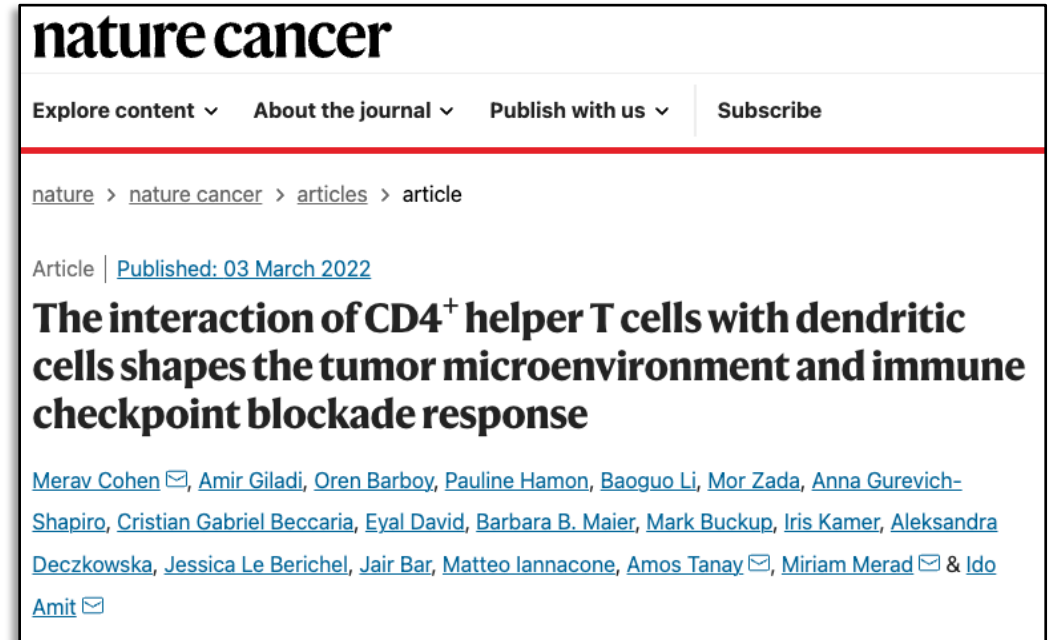


Niche 2 and 5 differ in expression of interferon response genes



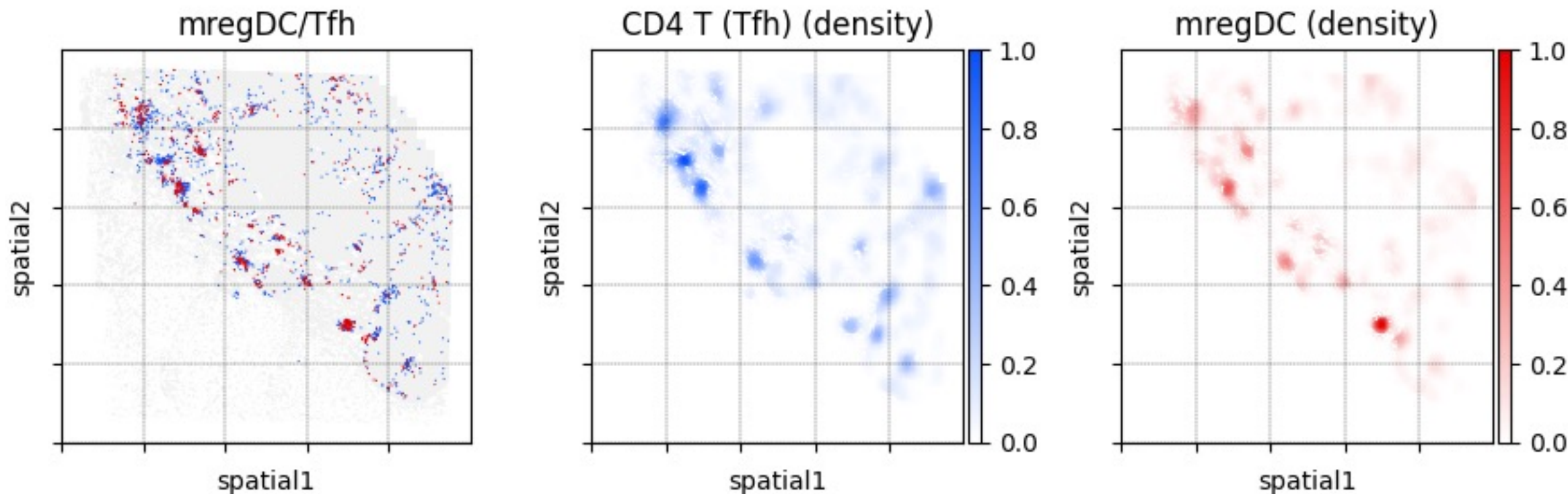
Vignette: Spatial distribution of DC-T cell interaction

- mregDCs tend to co-locate with CD4 T cells (especially PD-1-high Tfh cells)
- We identify a niche enriched for mregDCs and CD4 T cells

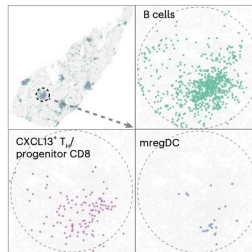
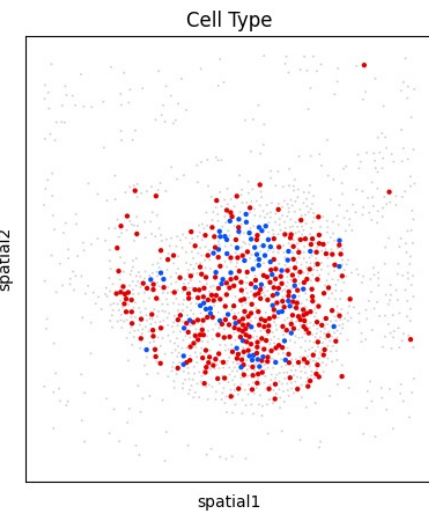
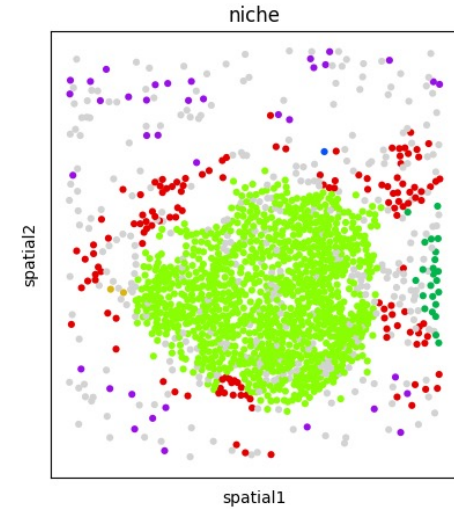
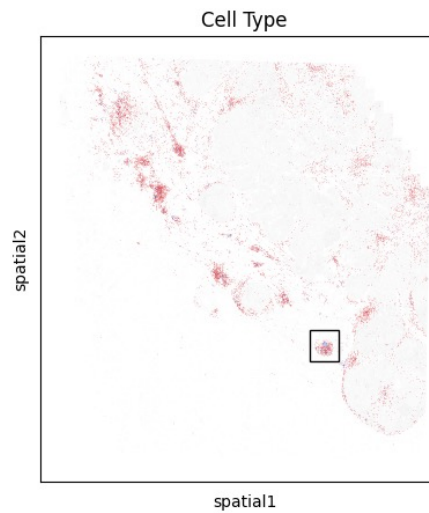
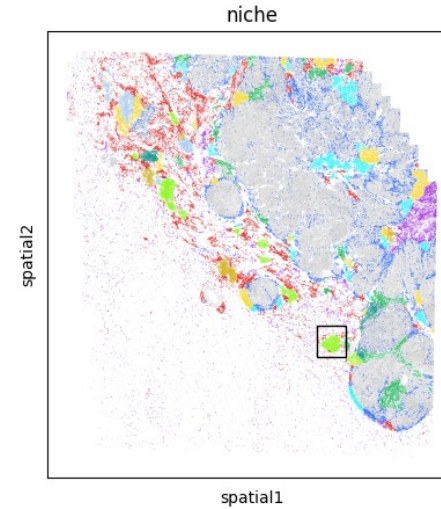
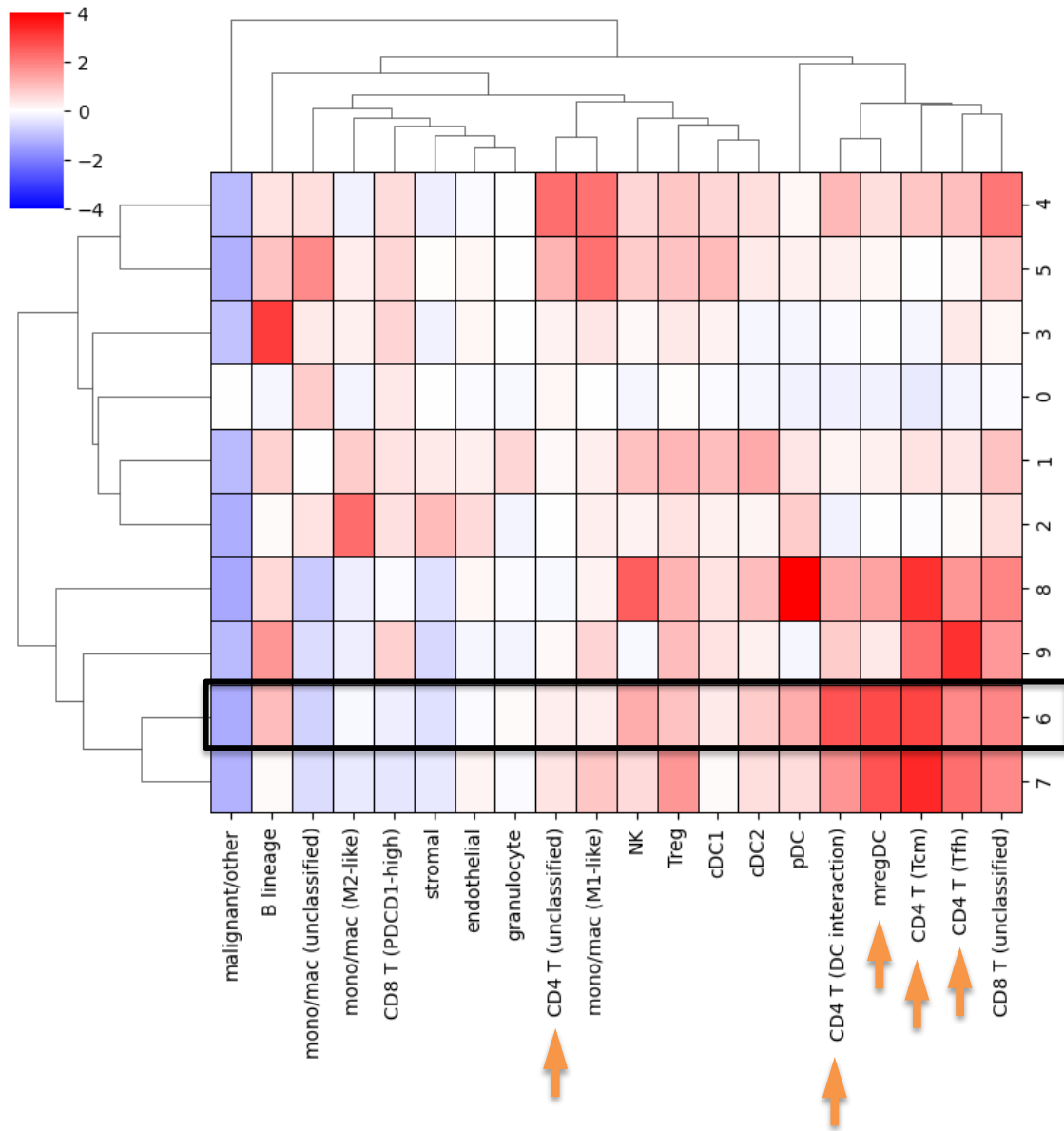


PIC-seq

mregDCs and PD-1-high Tfh cells co-localize

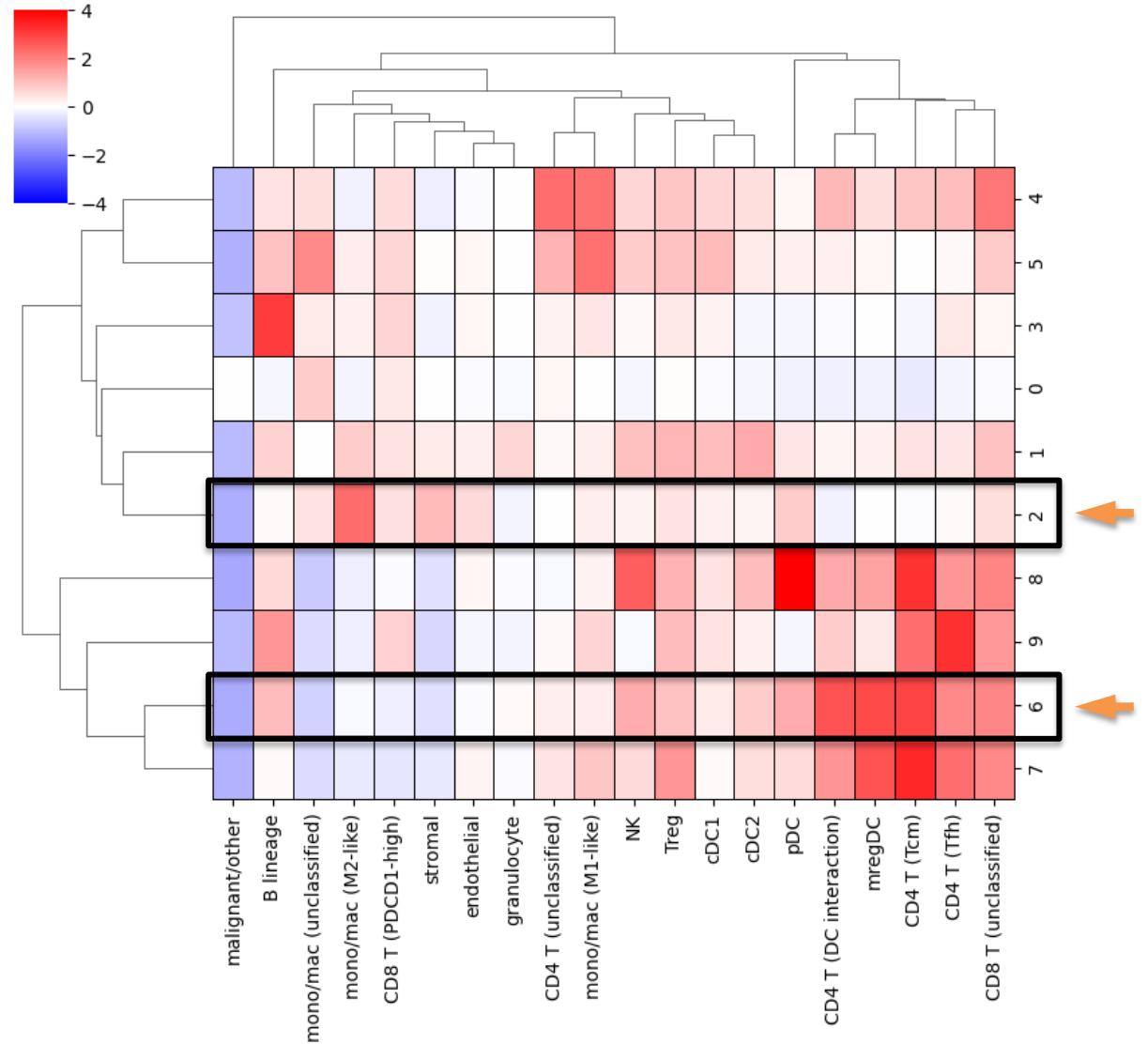


Niche 6 is particularly enriched for mregDCs and CD4 T cells



Vignette: Comparing T cells between immunogenic vs. suppressive niches

- Compare T cells located in Niches 2 and 6
 - Excluding Tregs
- Perform differential expression analysis between these two groups of T cells



Differentially expressed genes higher in Niche 6 indicate T cell activation

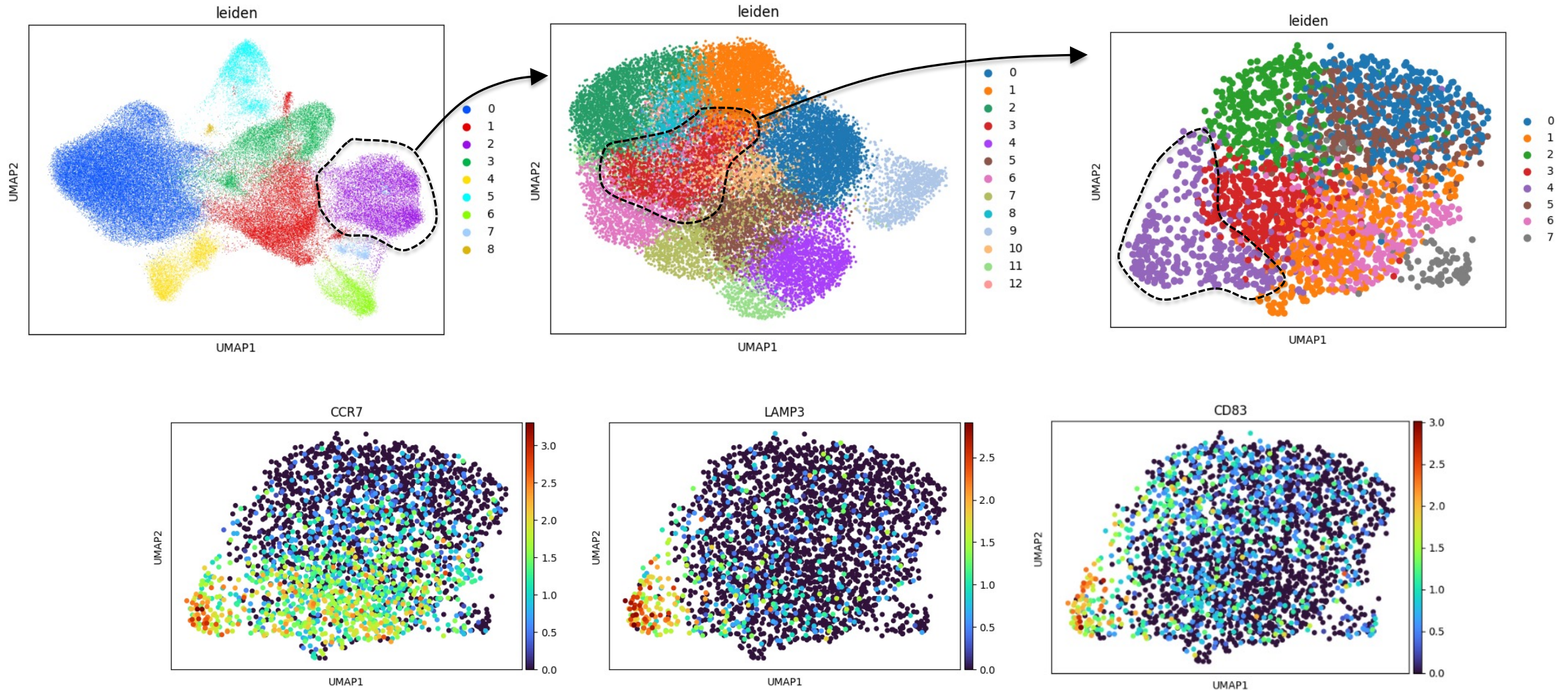
- 92 DE genes (adj. $p < 0.05$) in Niche 6 T cells are enriched for interferon response and T cell activation as evidenced by Gene Set Enrichment Analysis
- Enriched GO terms (adj. $p < 0.1$) include:
 - Activation of immune response
 - Alpha beta T cell activation
 - Antigen receptor mediated signaling

Summary

- Spatial transcriptomics is a powerful tool for exploring cellular organization within the tissue microenvironment
- Monkeybread is a Python package that implements a suite of tools to facilitate such analyses
- We applied Monkeybread to a melanoma sample and identified cellular niches that may be sites of both immune activation and suppression

Extra slides

A subcluster of cells in the T cell cluster over-express mregDC markers



Spatial data analysis resources

- <https://lmweber.org/OSTA-book/spatialexperiment.html>
- https://github.com/crazyhottommy/awesome_spatial_omics



