



Dissecting myeloid and T cells using spatial transcriptome

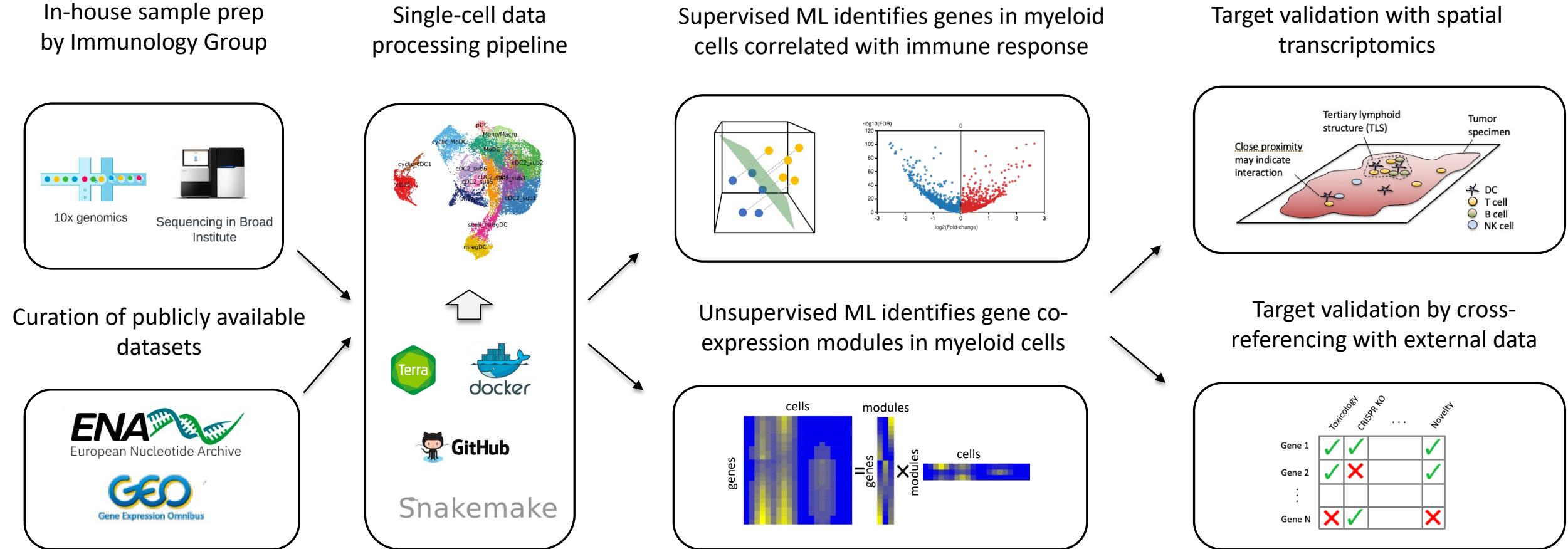
interaction niches in the TME

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Director of Computational biology, Immunitas

07/18/2023

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



Location, Location, Location

nature methods

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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

nature medicine

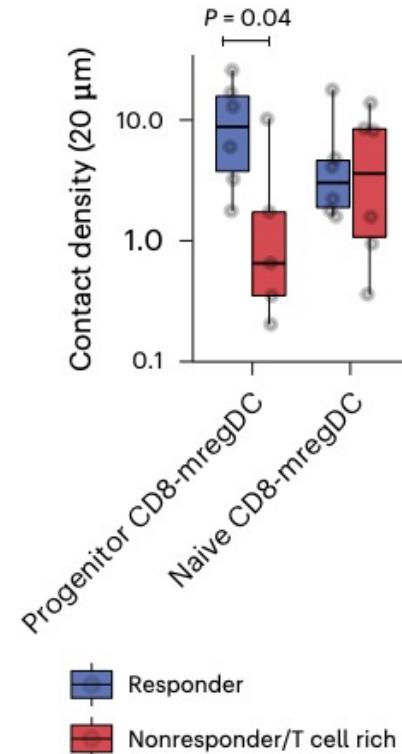
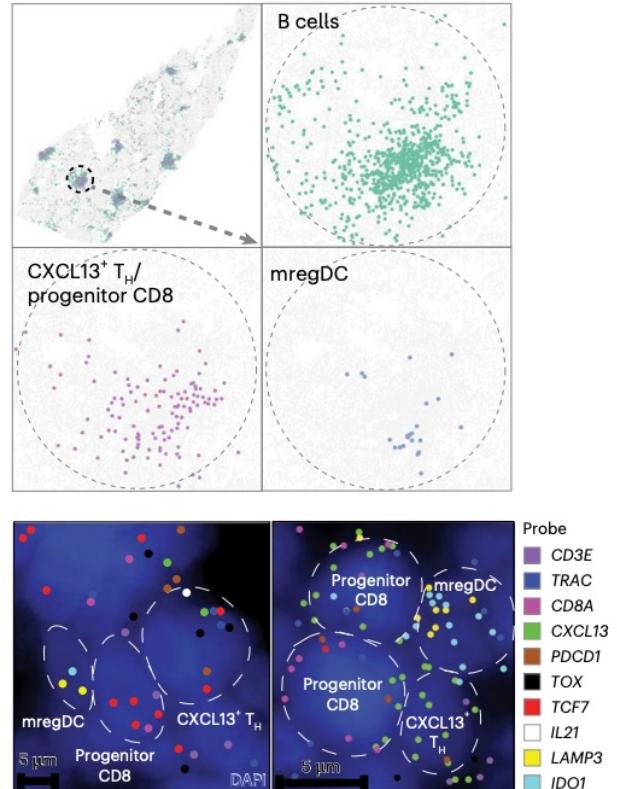
Article <https://doi.org/10.1038/s41591-023-02345-0>

Intratumoral dendritic cell–CD4⁺ T helper cell niches enable CD8⁺ T cell differentiation following PD-1 blockade in hepatocellular carcinoma

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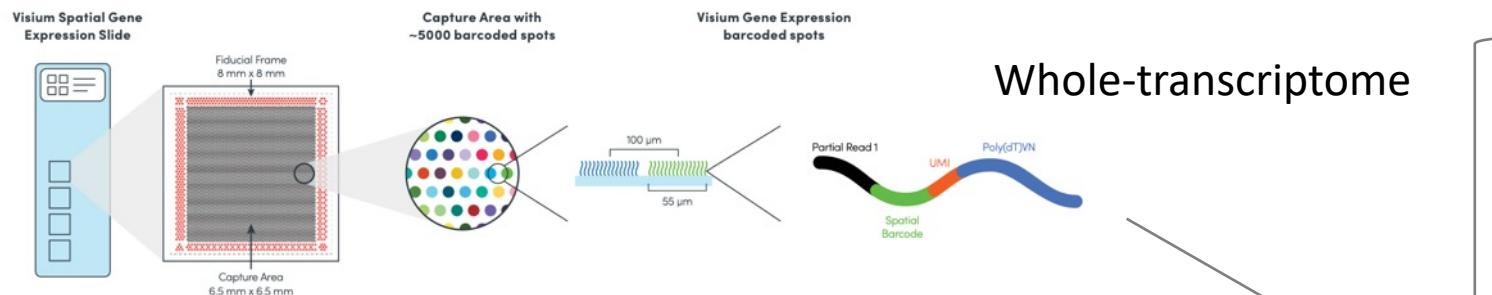
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Spatial transcriptomics: spatially resolved gene expression

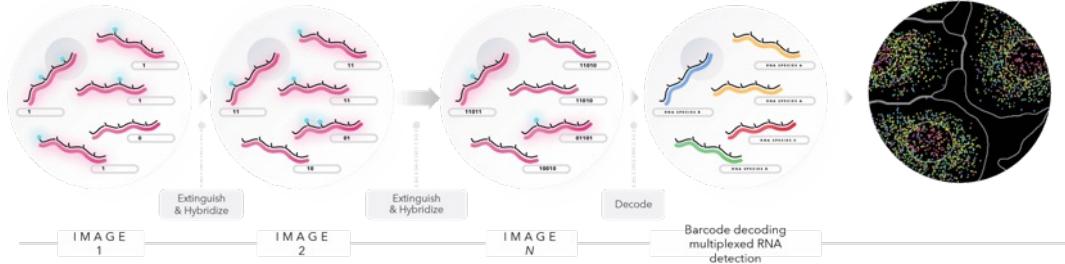
Sequencing-based: Spatial Transcriptomics, Visium



<https://www.10xgenomics.com>

	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



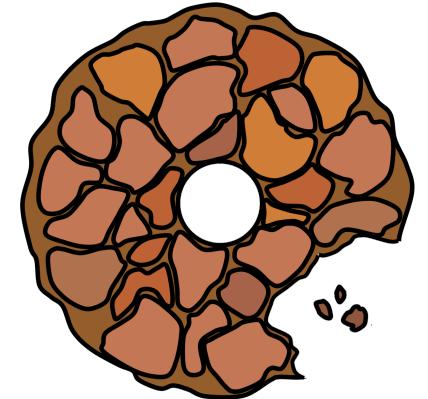
<https://vizgen.com>

500-1000 genes

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

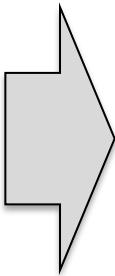
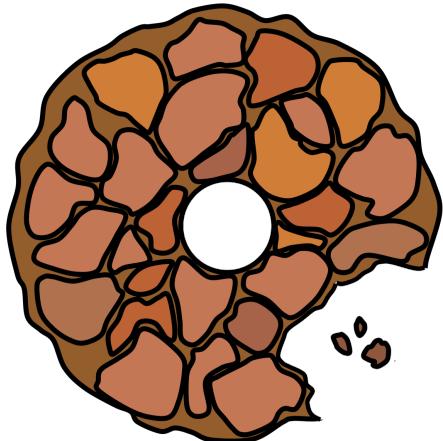
Exploring cellular niches in tumors with Monkeybread

- 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

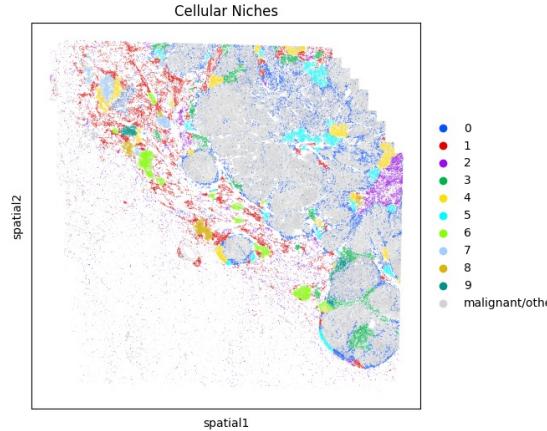


Exploring cellular niches in tumors with Monkeybread

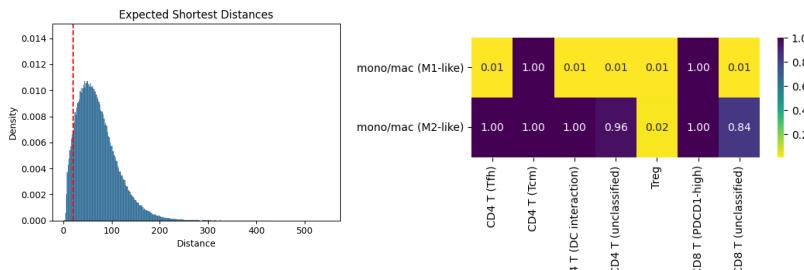
Monkeybread



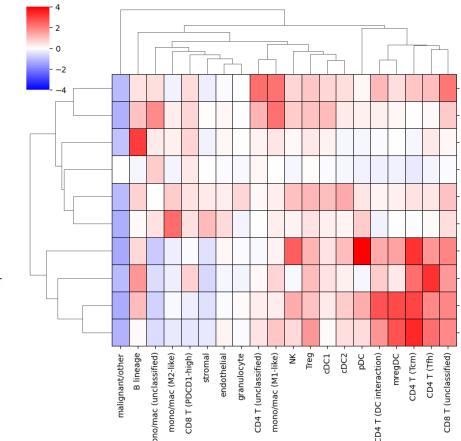
Identify cellular niches



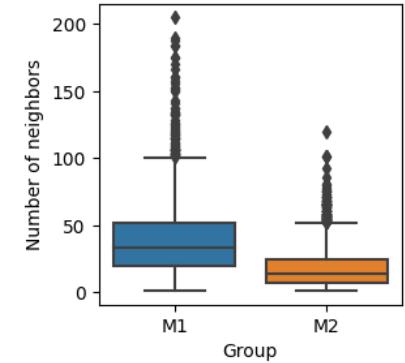
Statistical tests for cell co-localization



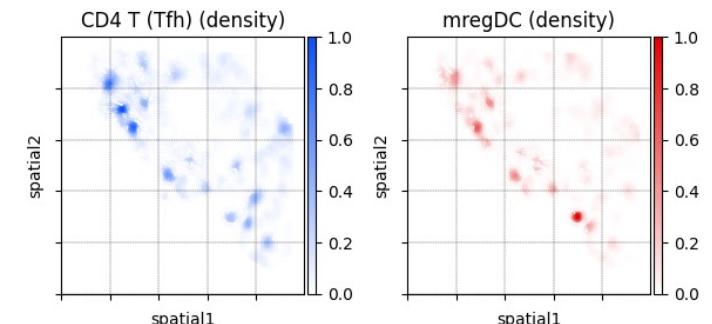
Explore cell type enrichment in niches



Compare neighborhood composition between cell types

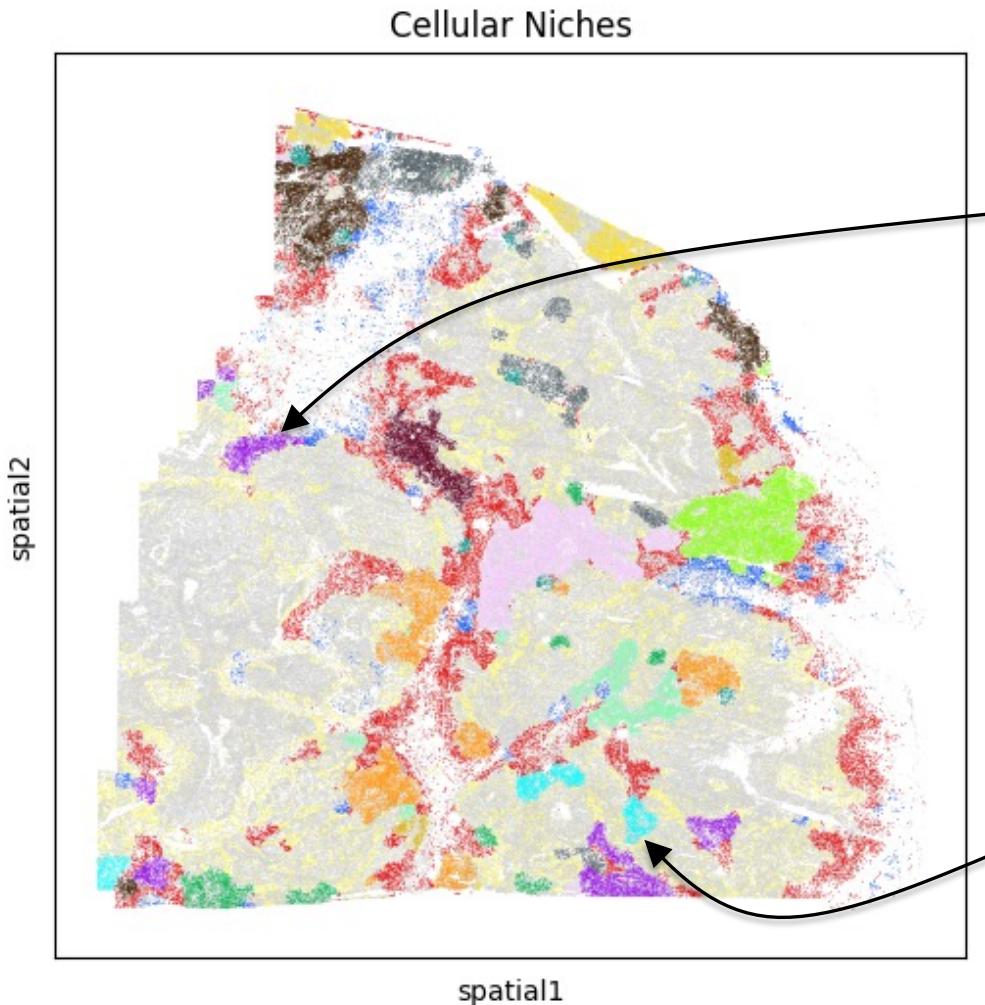


Compare spatial distribution between cell types

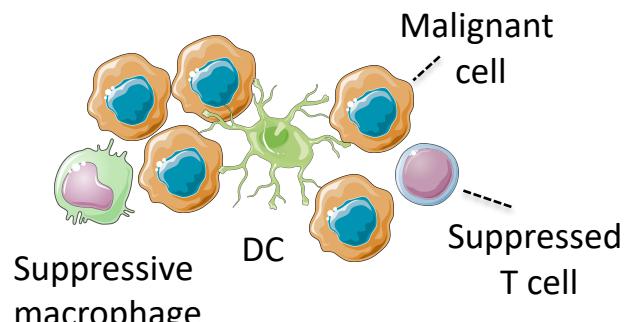


Comparing Immune cells located in anti vs. pro-tumor cellular niches

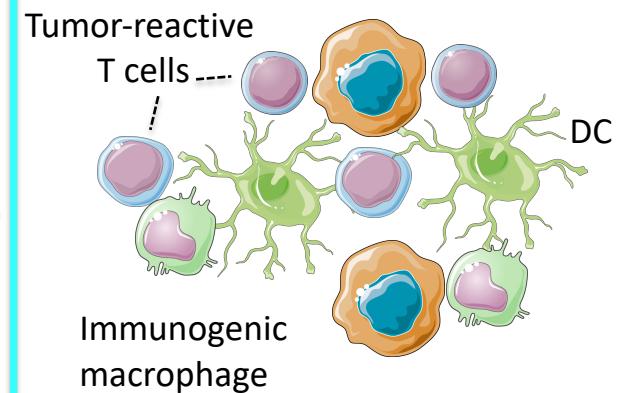
- Identify niches of co-localizing cell types and phenotypes in the TME
- Compare gene expression between cells in suppressive vs. immunogenic niches



Suppressive niche

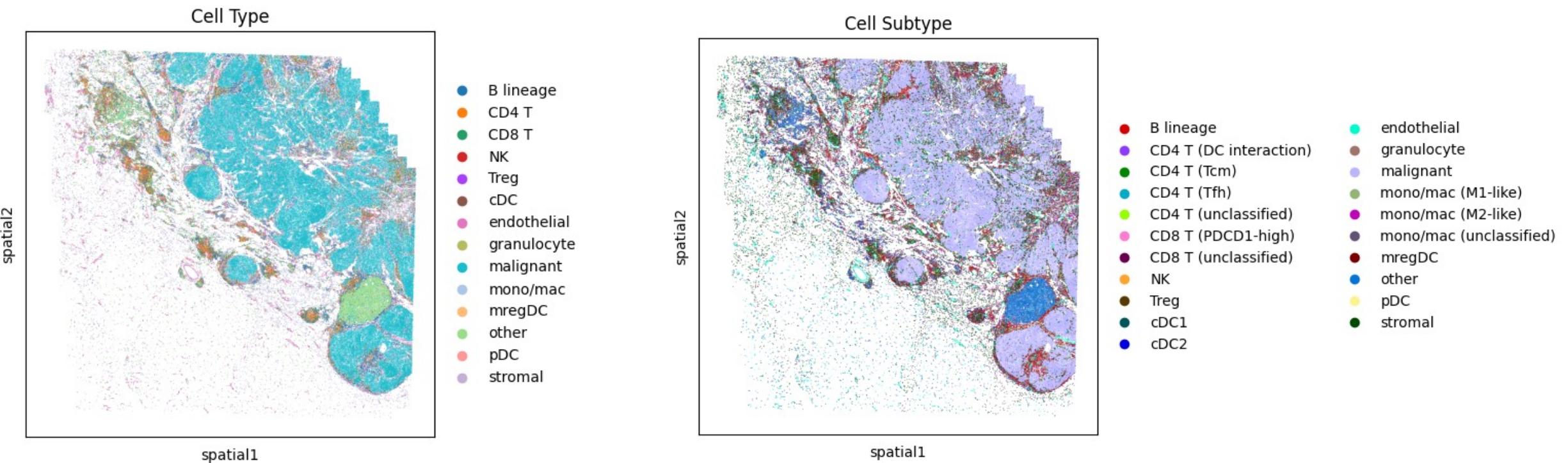


Immunogenic niche



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/>



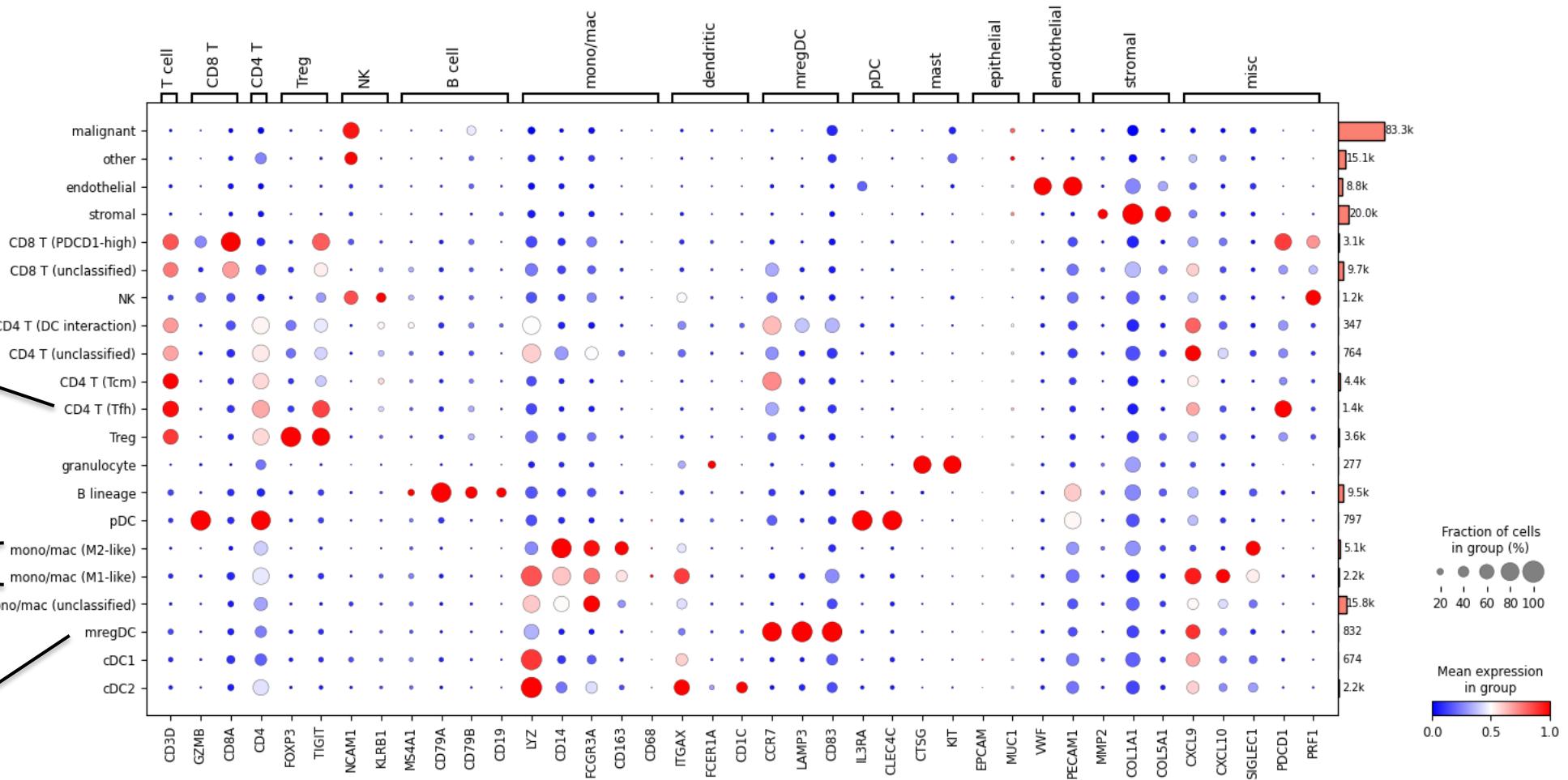
Fine-grained cell type annotations

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

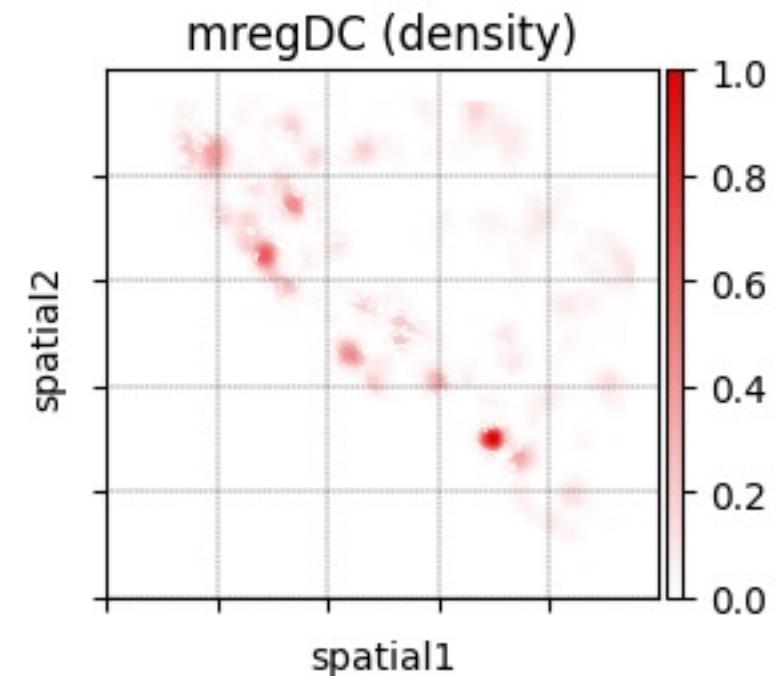
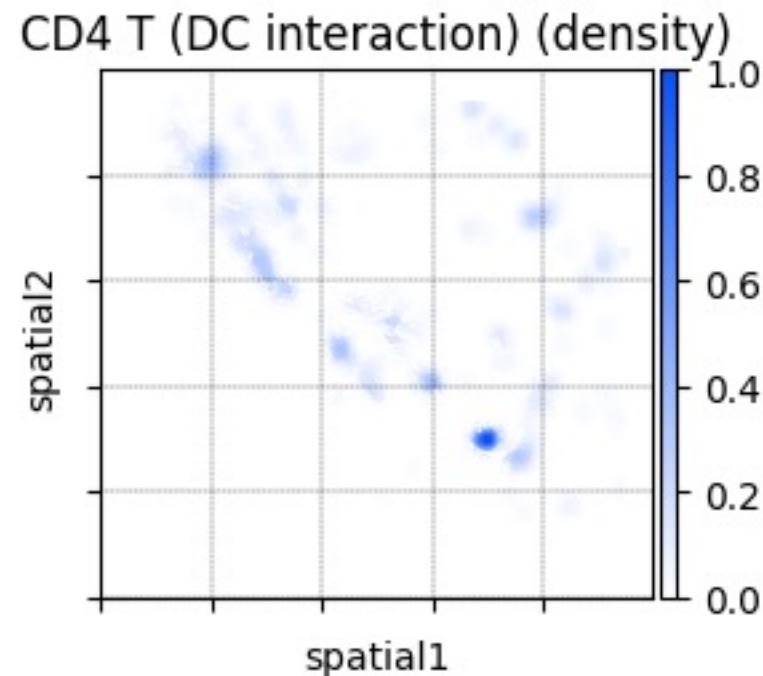
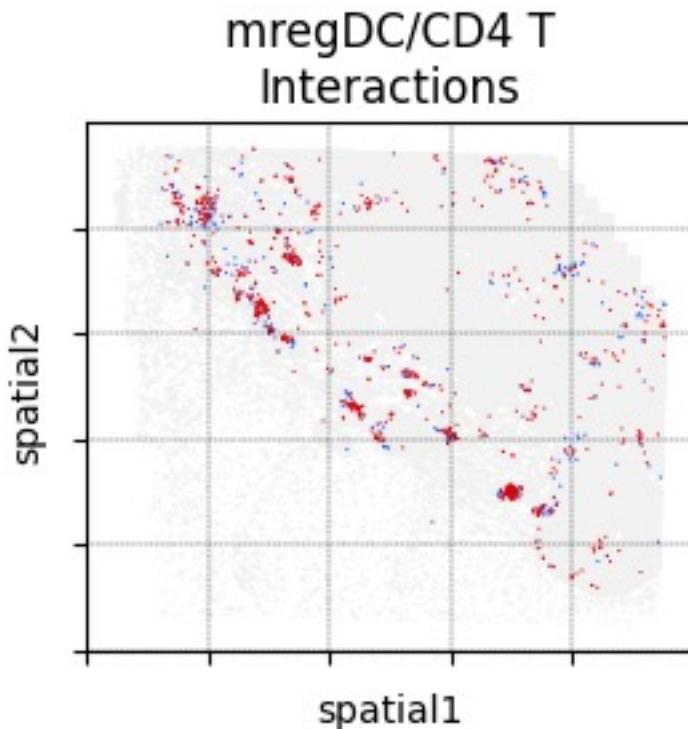
Tfh cells are high for PD-1

Suppressive and immunogenic macrophages

mature DCs enriched in immunoregulatory modules

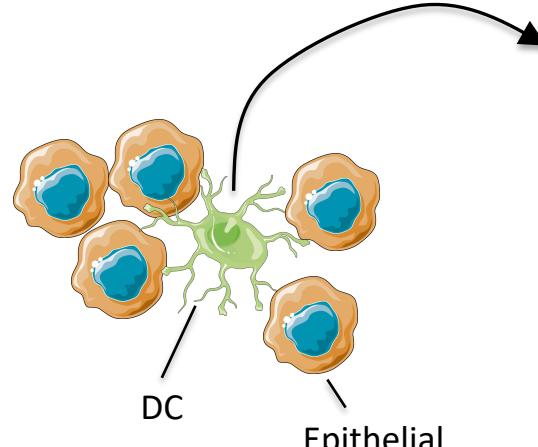


DC-marker-expressing CD4 T cells co-localize with mregDCs

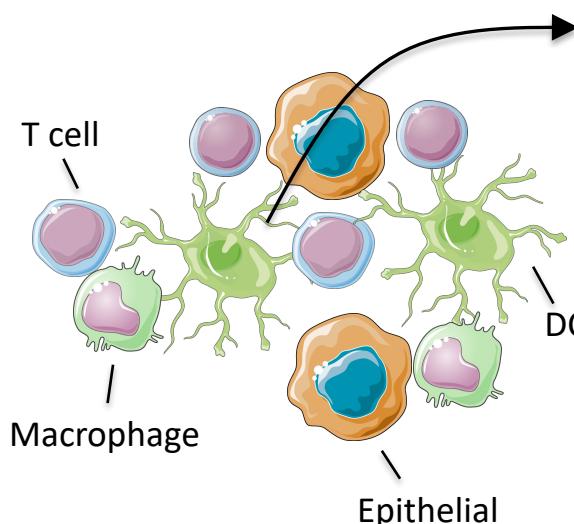


Computing neighborhood profiles

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

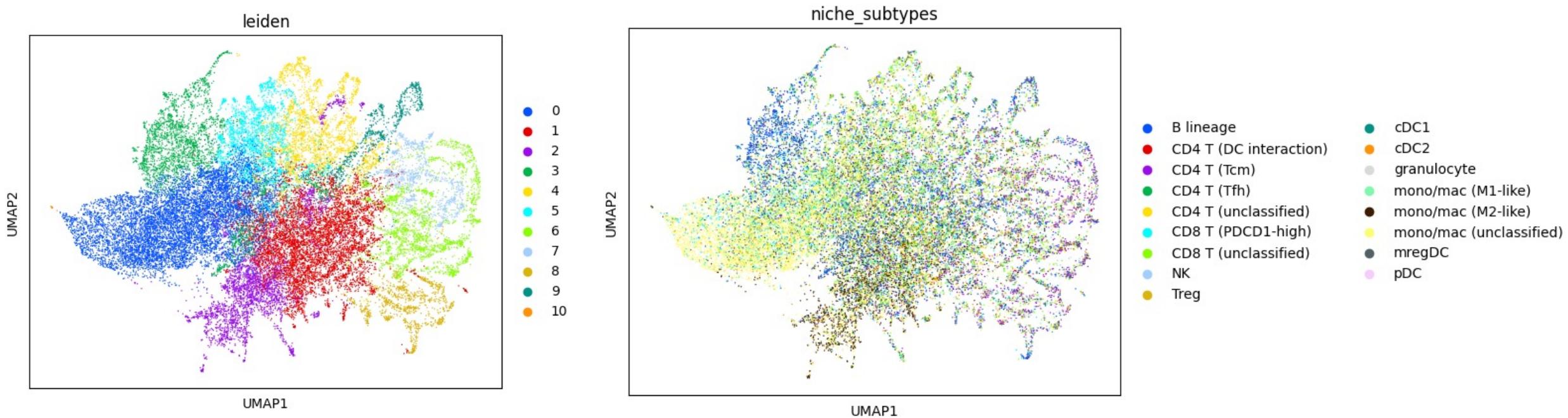


Neighborhood profile	
Cell type	Frequency in neighborhood
Epithelial	5
CD8 T	0
DC	1
...	...
Macrophage	0



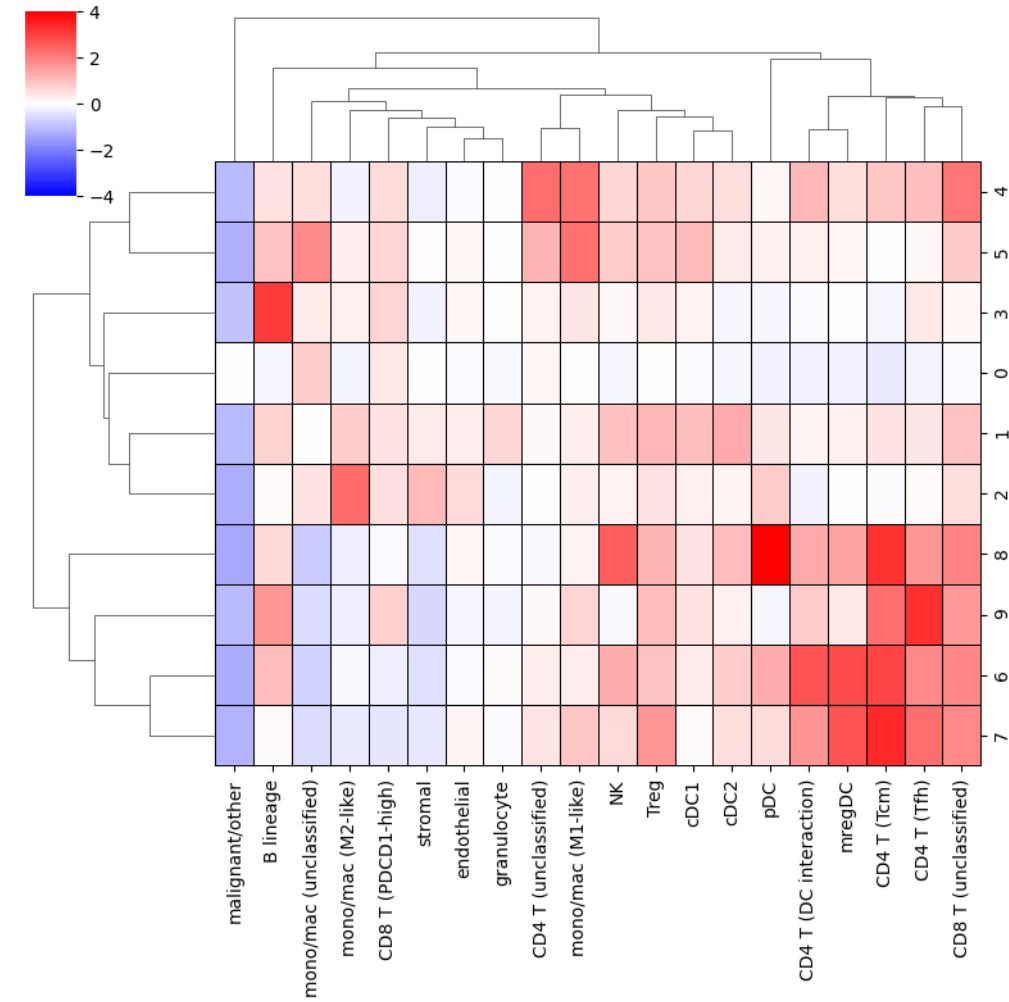
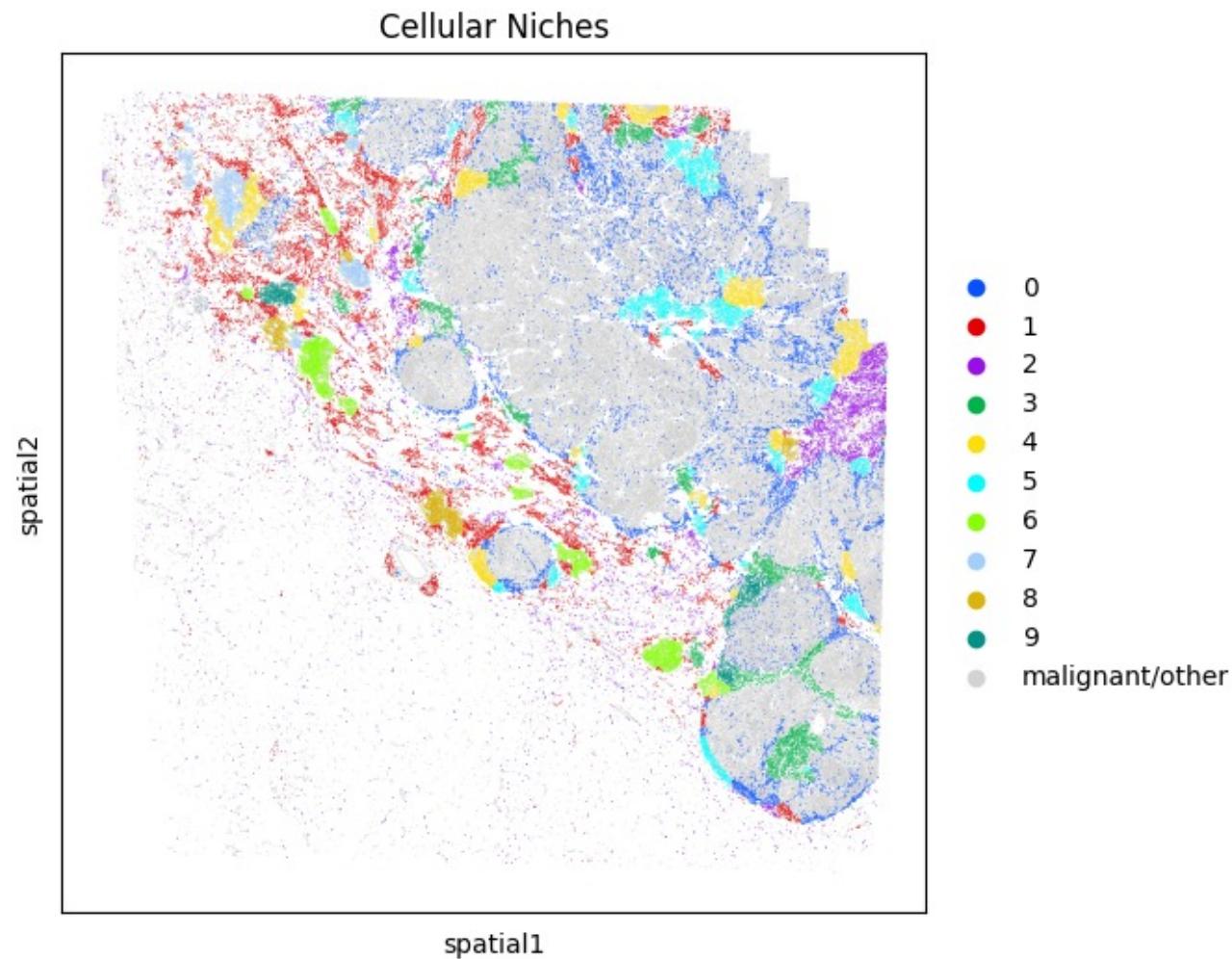
Neighborhood profile	
Cell type	Frequency in neighborhood
Epithelial	2
CD8 T	4
DC	2
...	...
Macrophage	2

Define cellular niches as clusters of neighborhood profiles



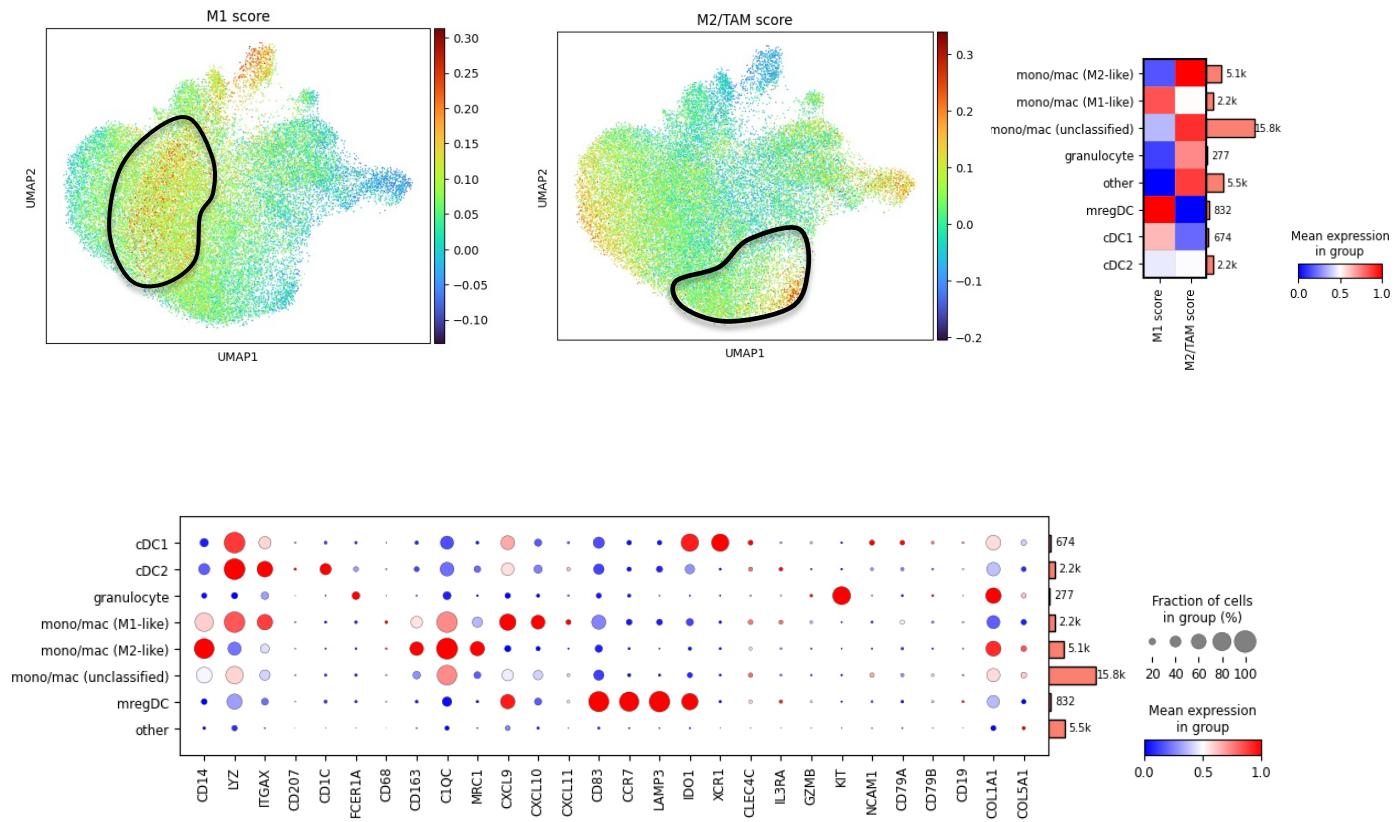
A cellular niche: a spatial region containing a similar composition of the different cell types

Niche analysis characterizes heterogeneity of tumor microenvironment

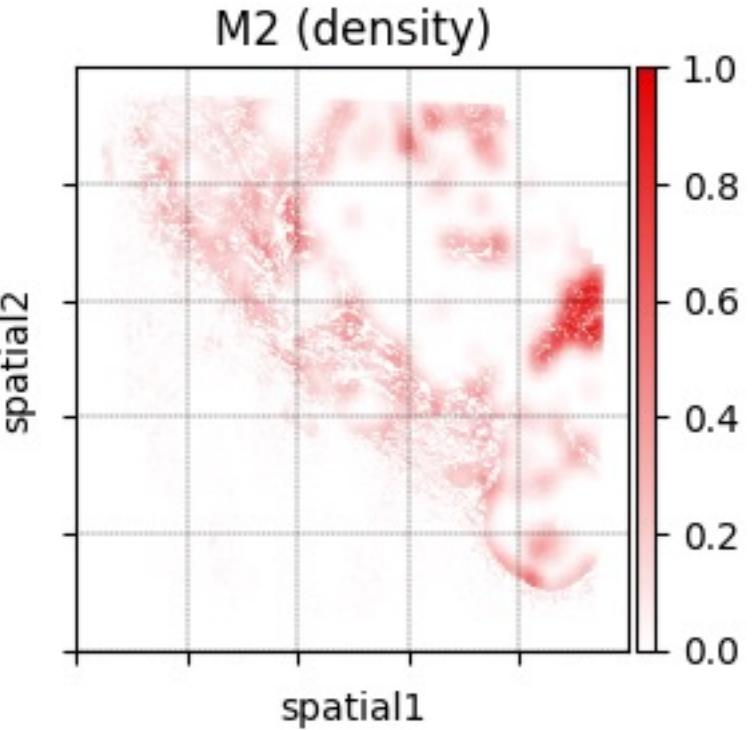
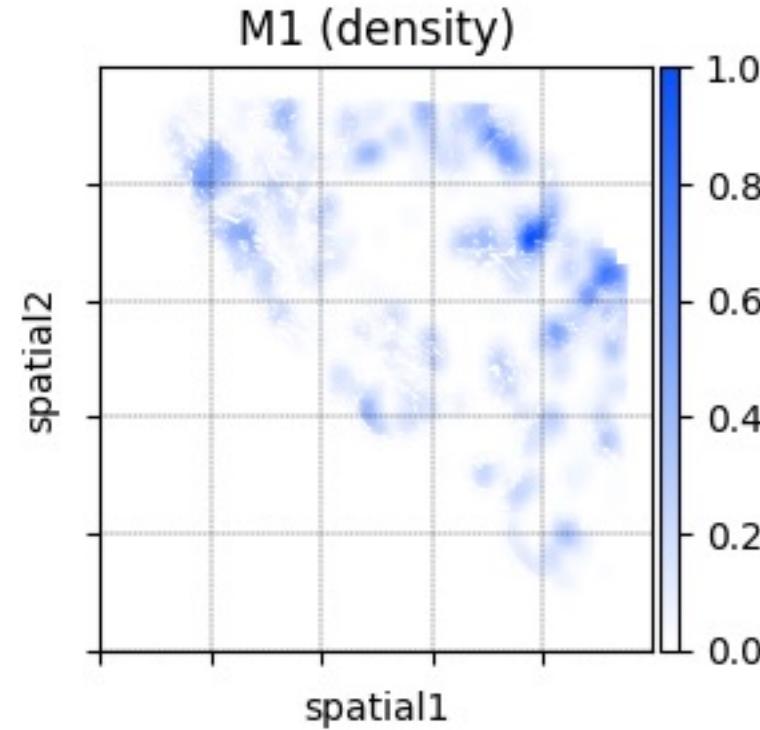
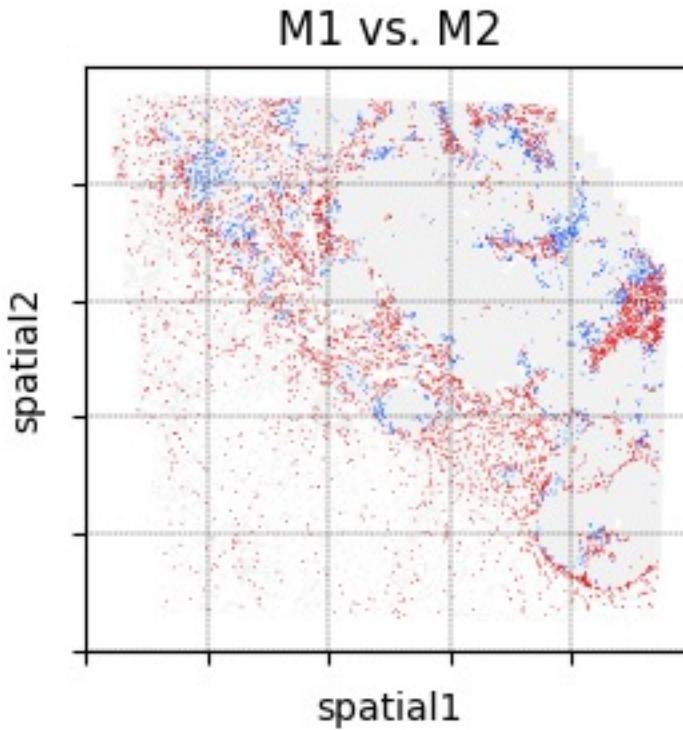


Vignette: Immunogenic vs. suppressive macrophages

- 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

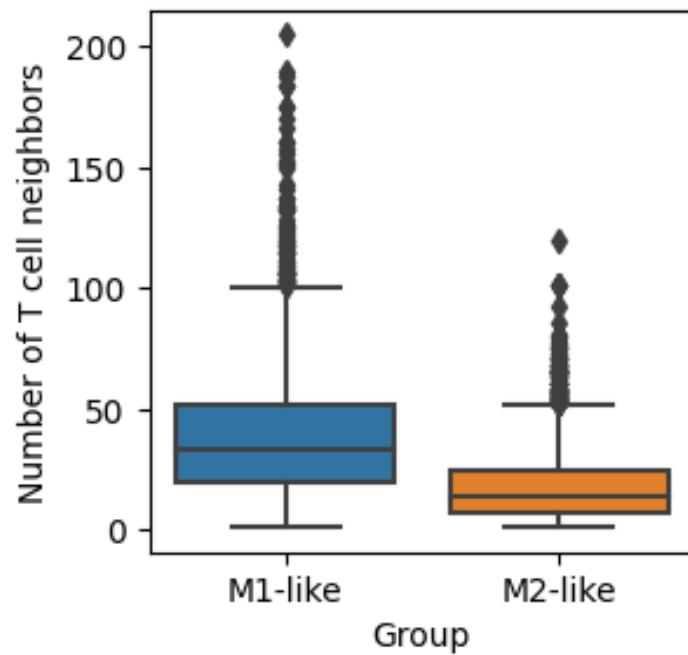


Macrophages with an immunogenic phenotype are in different regions from those with a suppressive phenotype

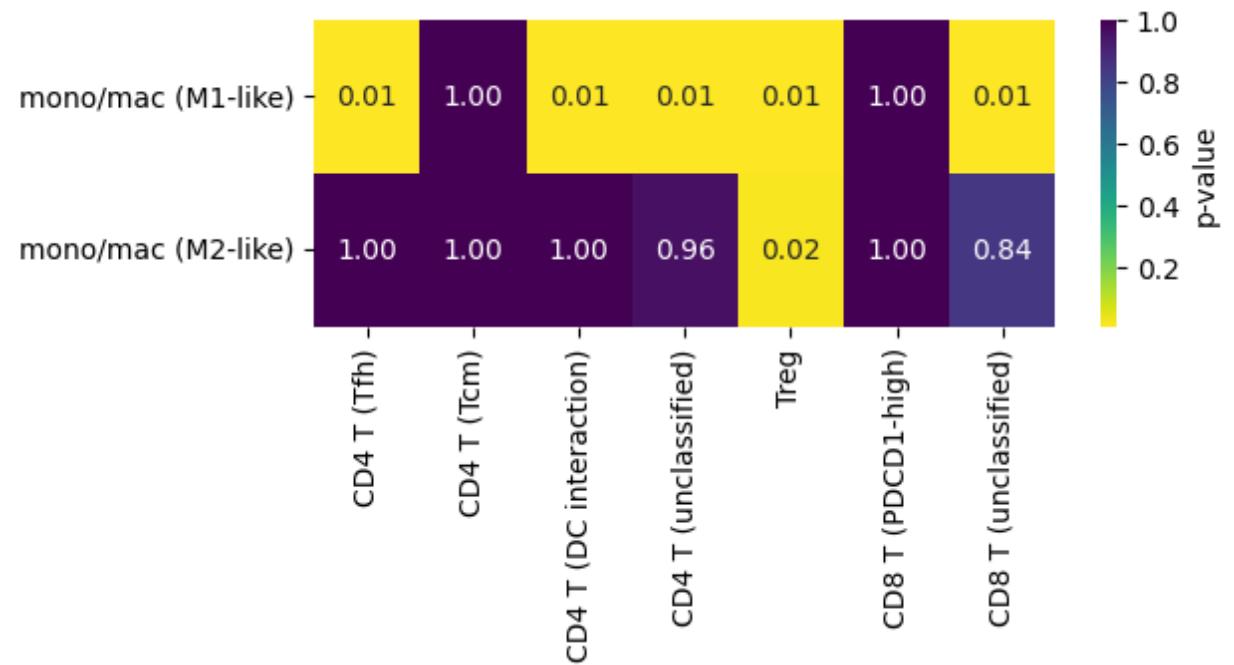


Immunogenic macrophages co-locate with T cells whereas suppressive macrophages co-locate with Tregs

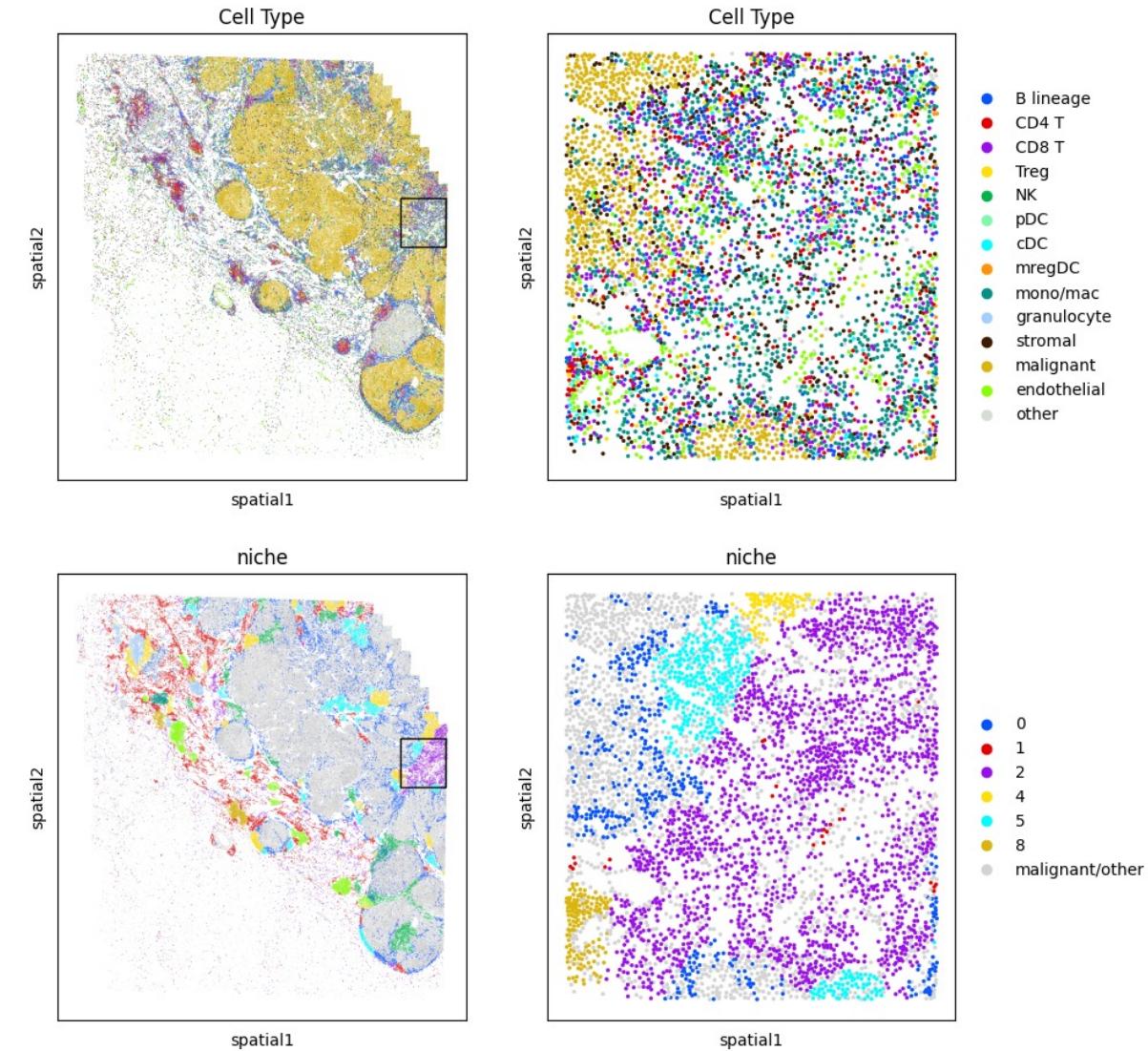
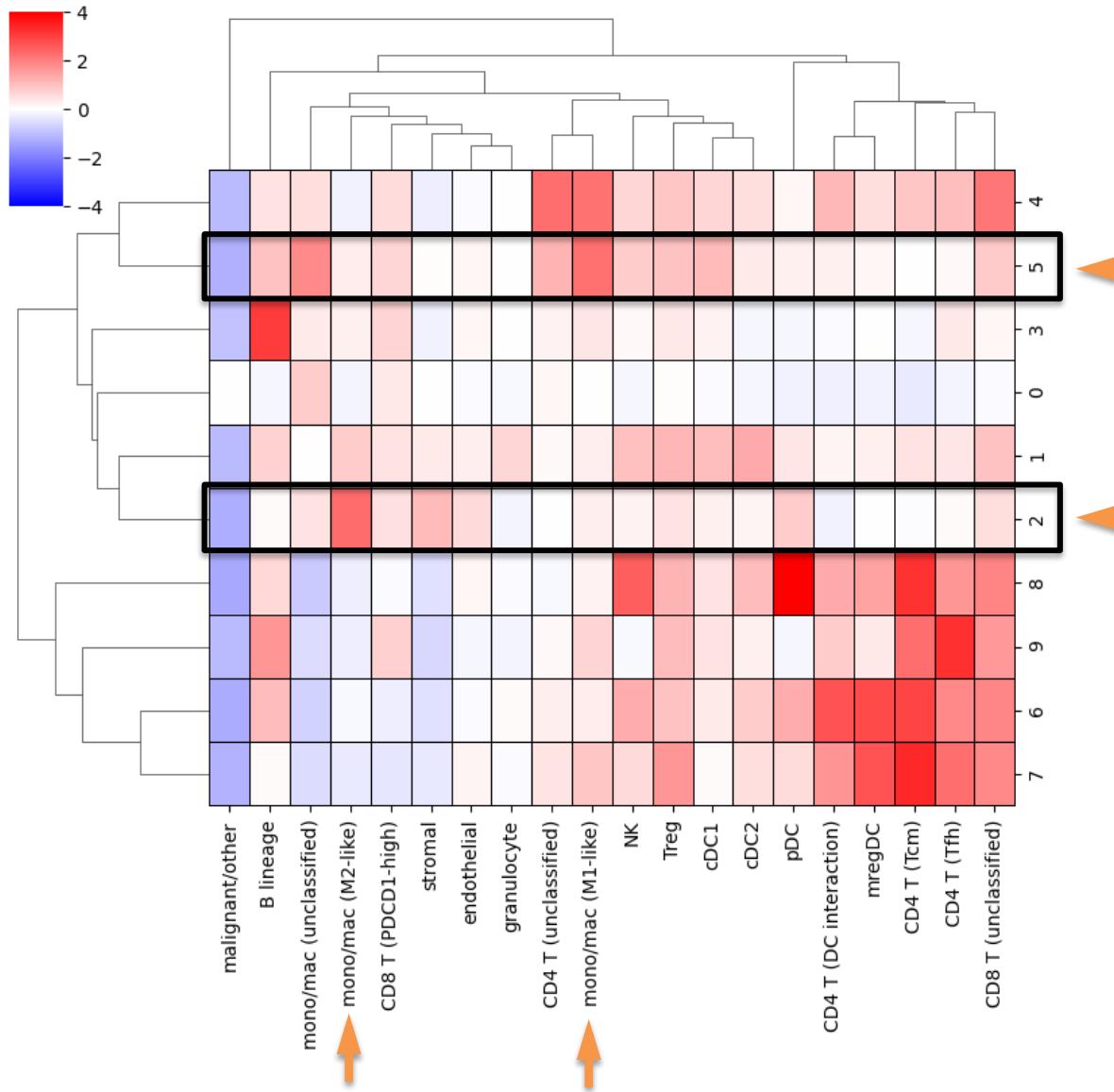
Compare number of T cells in vicinity of macrophages of each phenotype



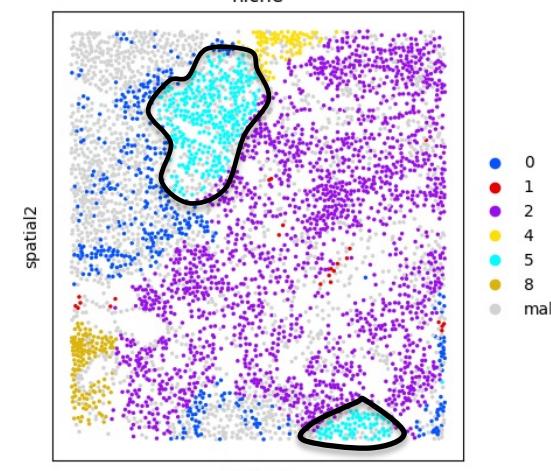
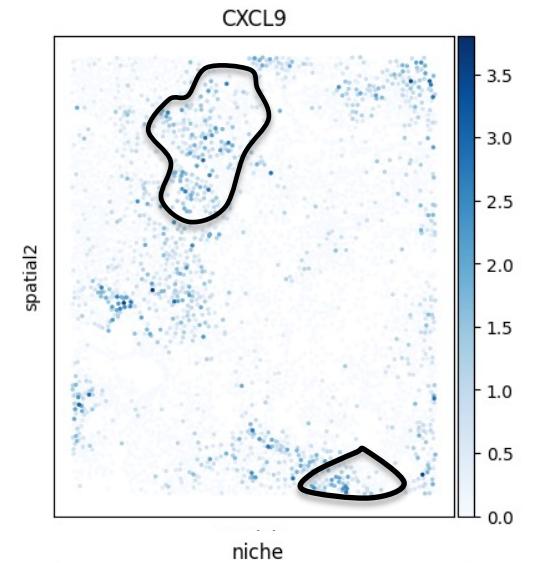
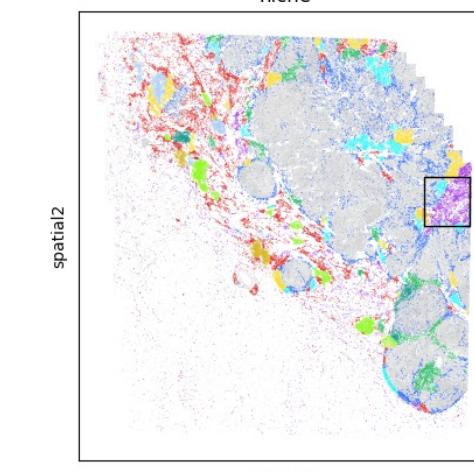
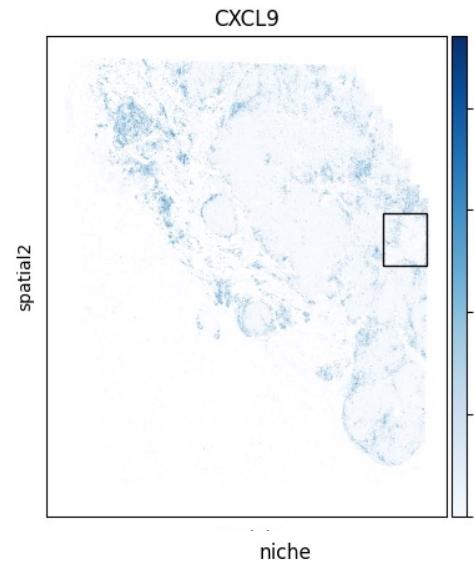
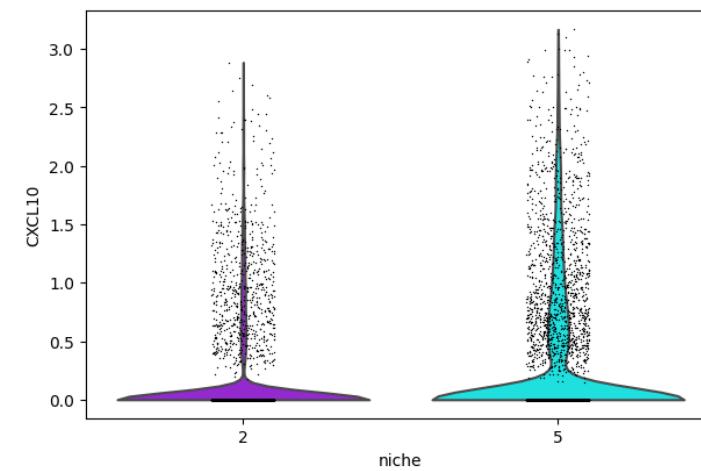
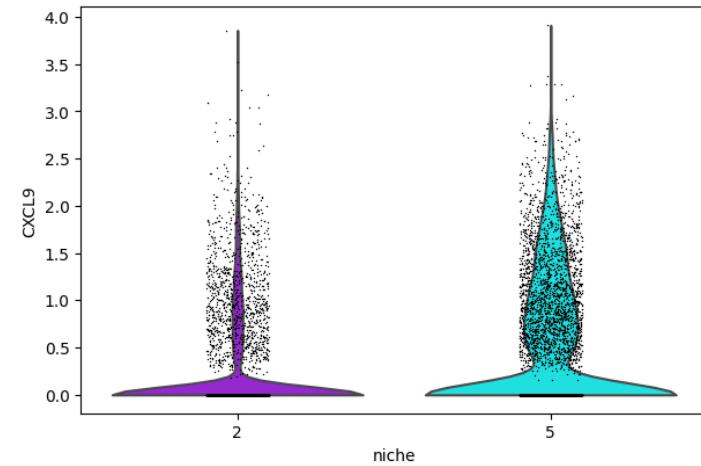
Permutation test examining distance of macrophages to their nearest T cell neighbor



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

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Article | Published: 03 March 2022

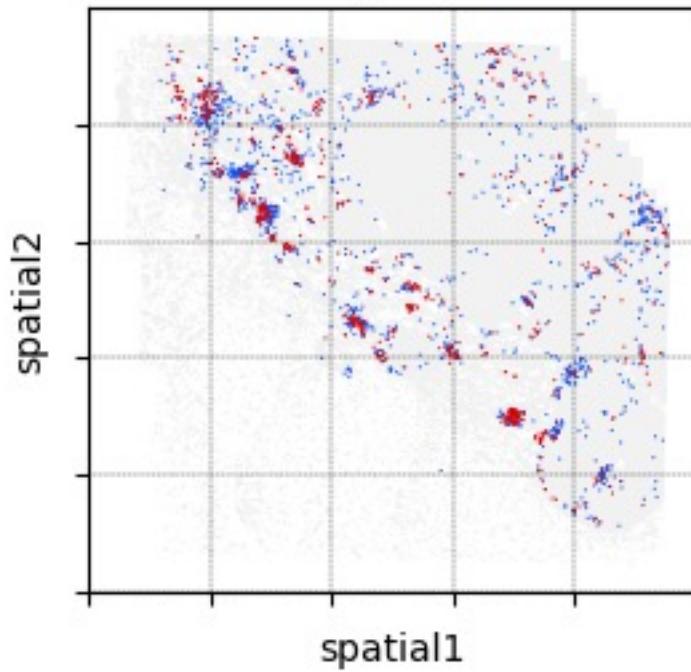
The interaction of CD4⁺ helper T cells with dendritic cells shapes the tumor microenvironment and immune checkpoint blockade response

Merav Cohen , Amir Giladi, Oren Barboy, Pauline Hamon, Baoguo Li, Mor Zada, Anna Gurevich-Shapiro, Cristian Gabriel Beccaria, Eyal David, Barbara B. Maier, Mark Buckup, Iris Kamer, Aleksandra Deczkowska, Jessica Le Berichel, Jair Bar, Matteo Iannacone, Amos Tanay , Miriam Merad  & Ido Amit 

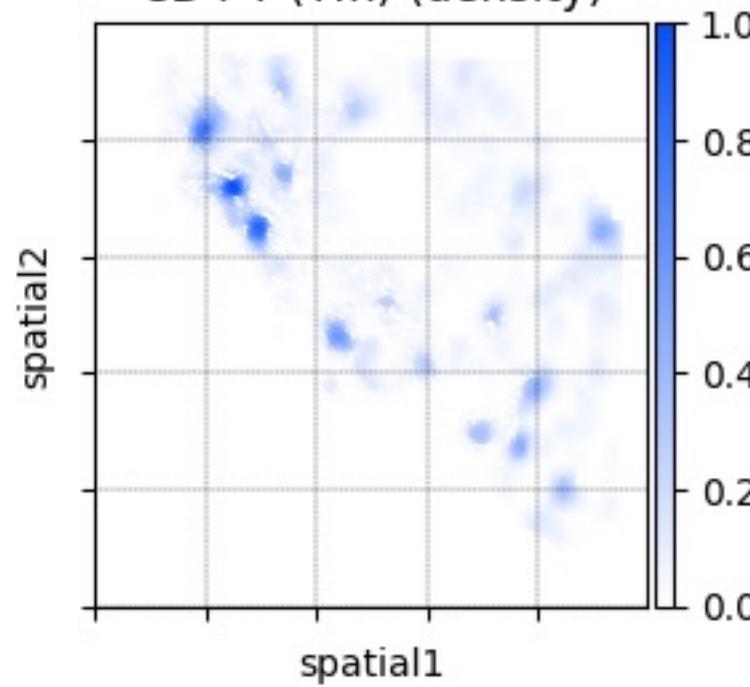
PIC-seq

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

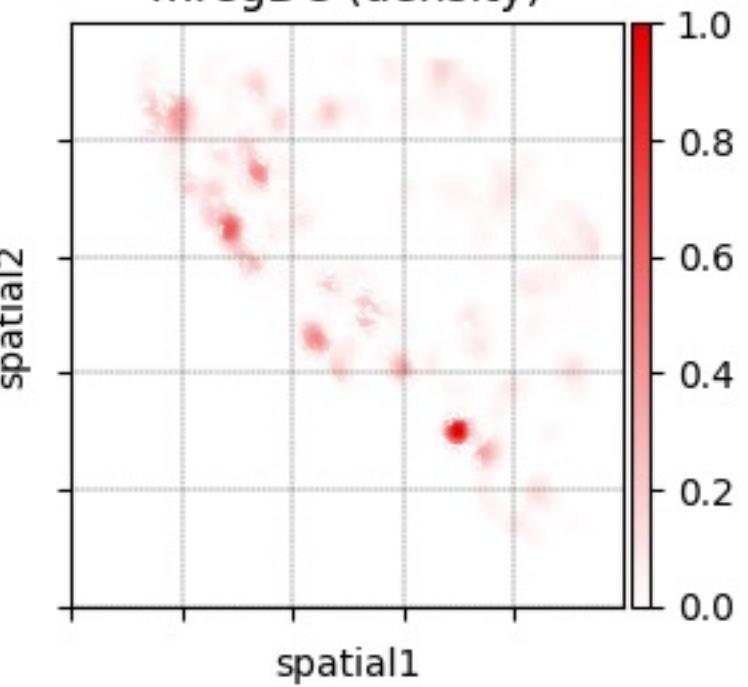
mregDC/Tfh



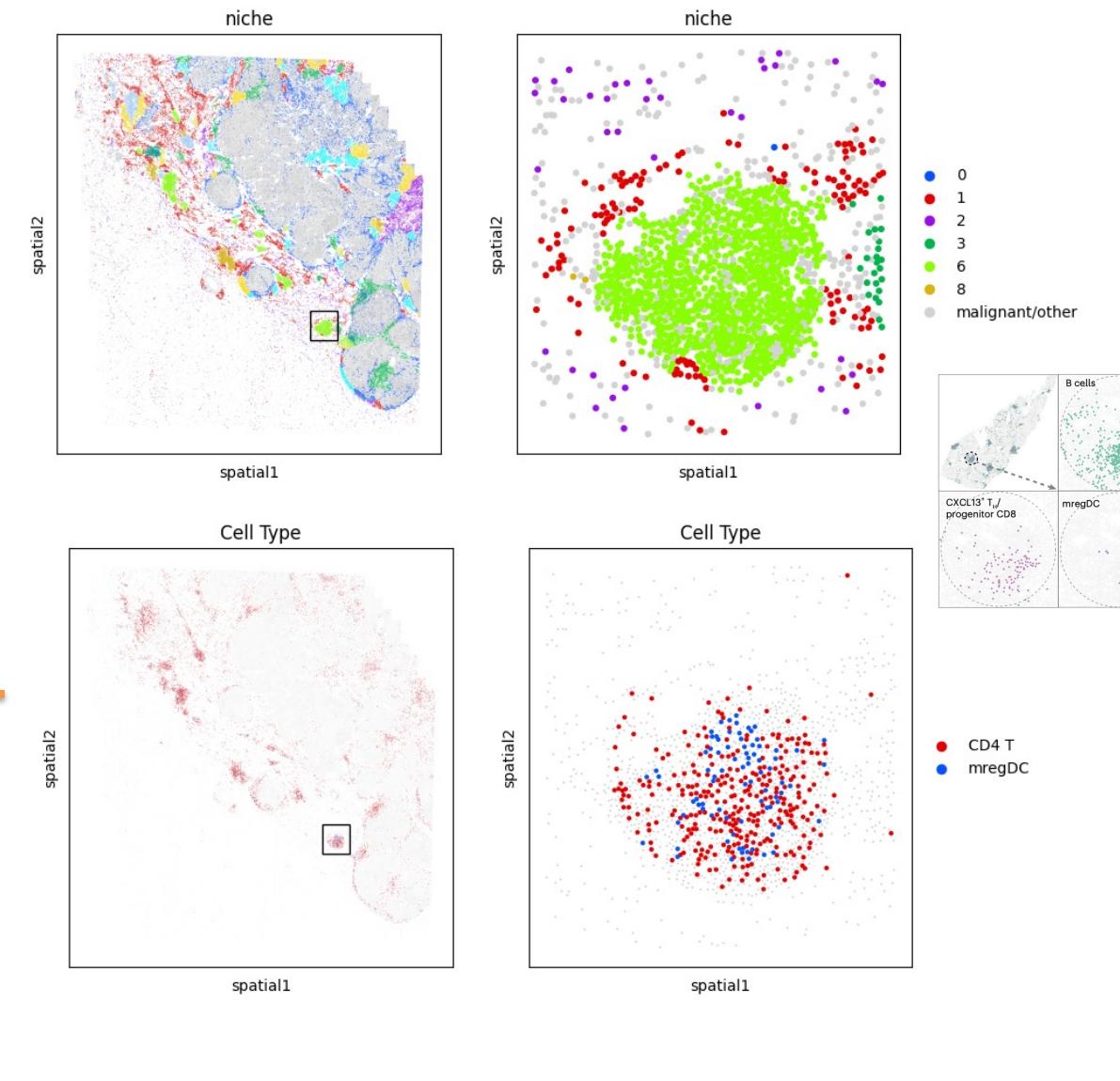
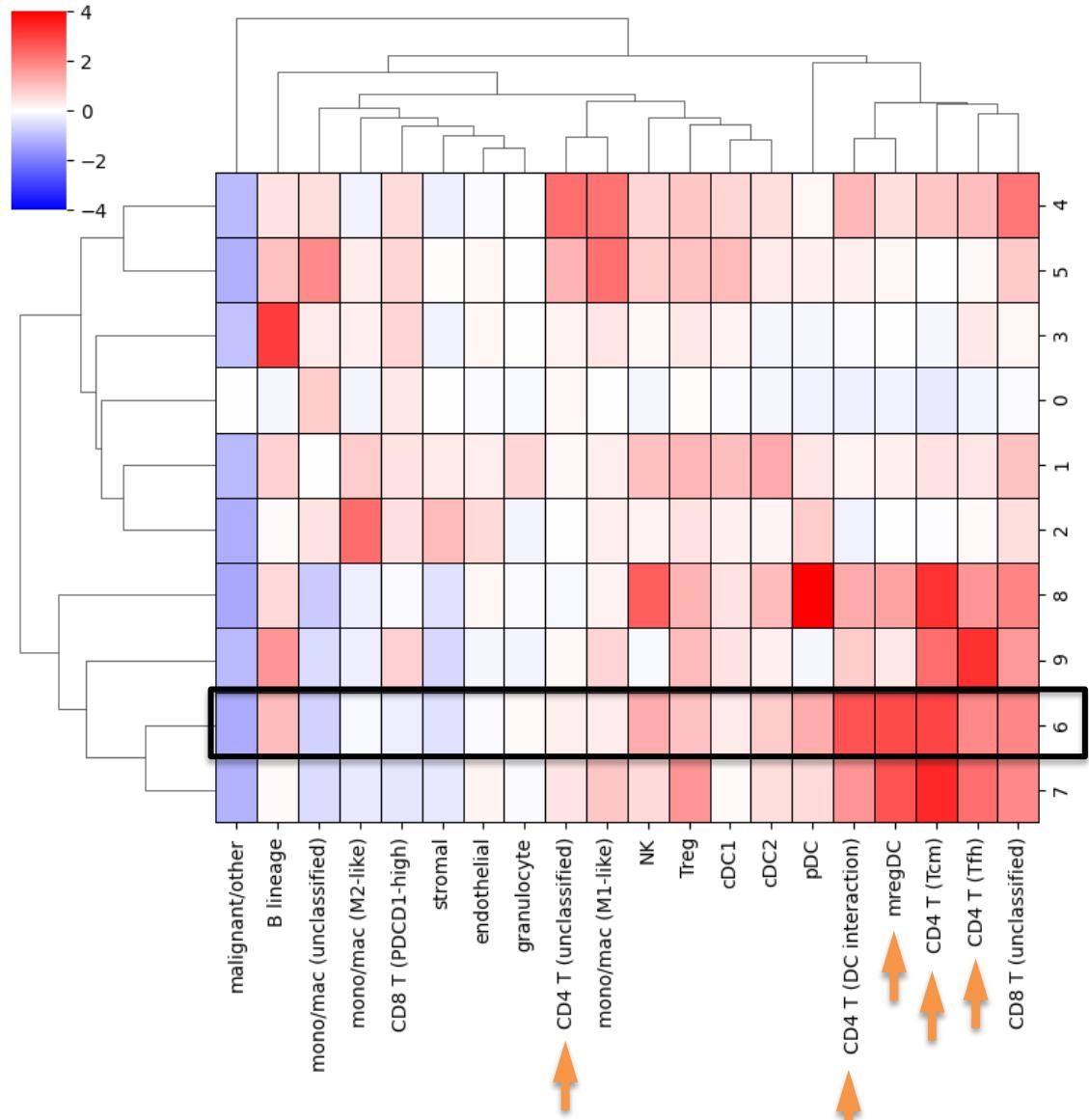
CD4 T (Tfh) (density)



mregDC (density)

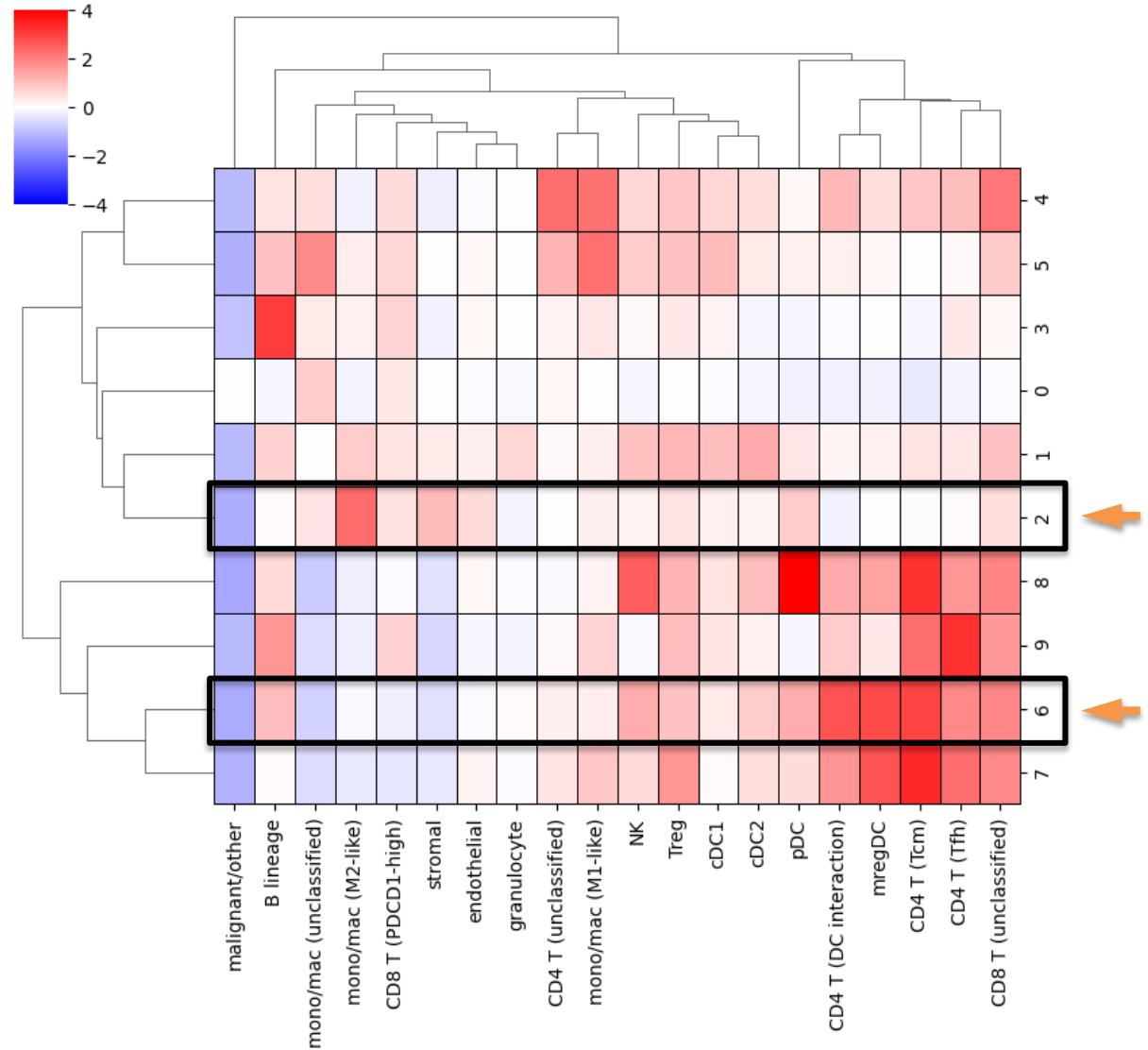


Niche 6 is particularly enriched for mregDCs and CD4 T cells



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

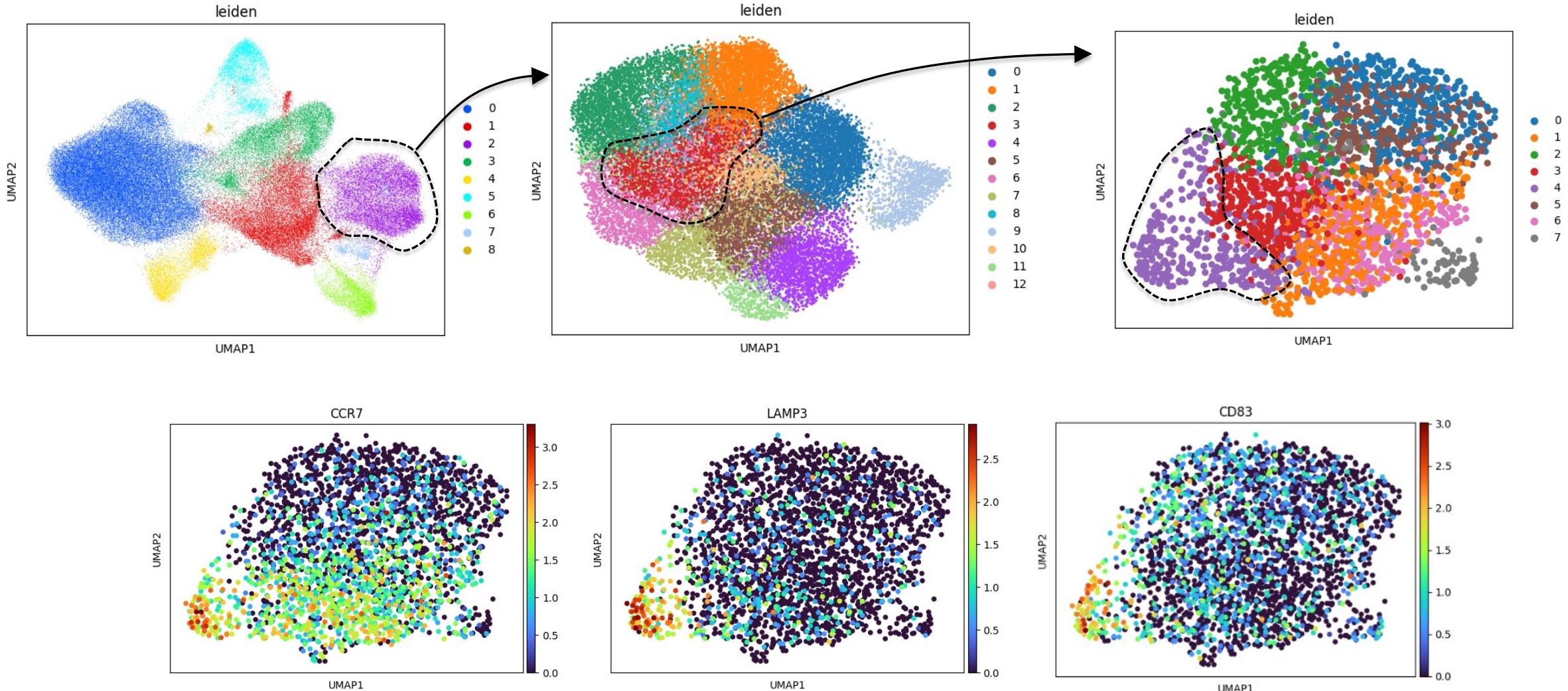
- 92 DE genes (adj. p < 0.05) in Niche 6 T cells are enriched for interferon response and T cell activation as evidenced by Gene Set Enrichment Analysis
- Enriched GO terms (adj. p < 0.1) include:
 - Activation of immune response
 - Alpha beta T cell activation
 - Antigen receptor mediated signaling

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](https://github.com/crazyhottommy/awesome_spatial_omics)





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