CD8 T-cell dynamics in biopsies from patients with melanoma treated with combination immune checkpoint inhibitors

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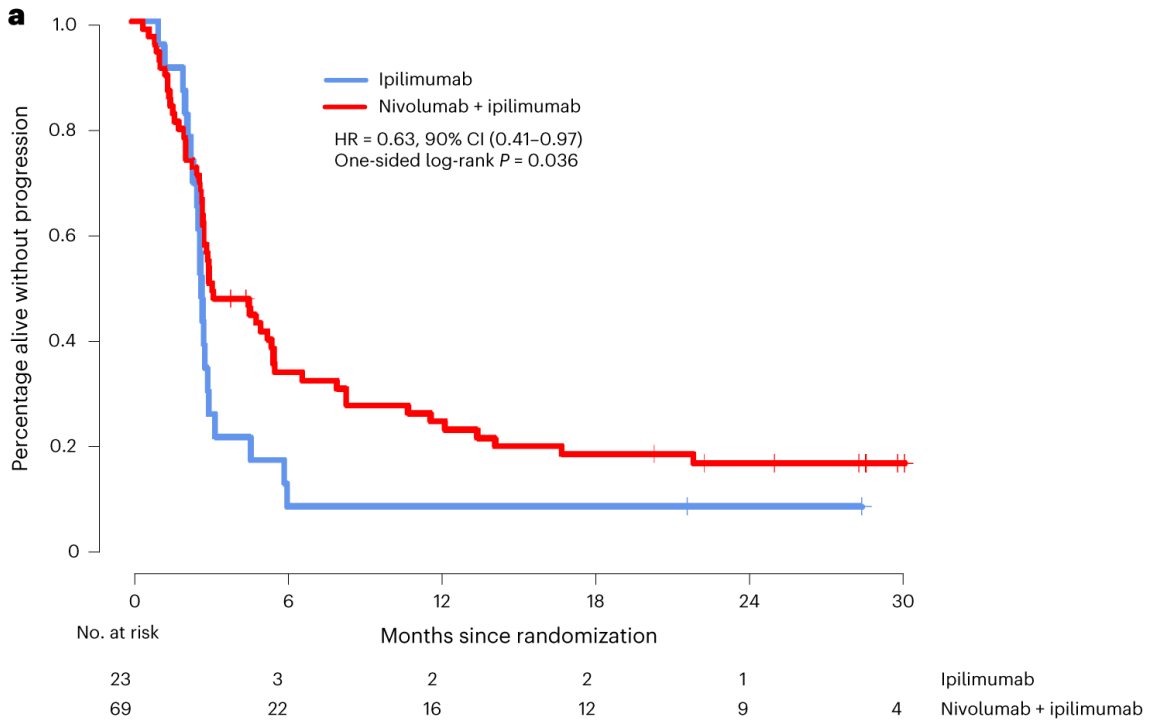
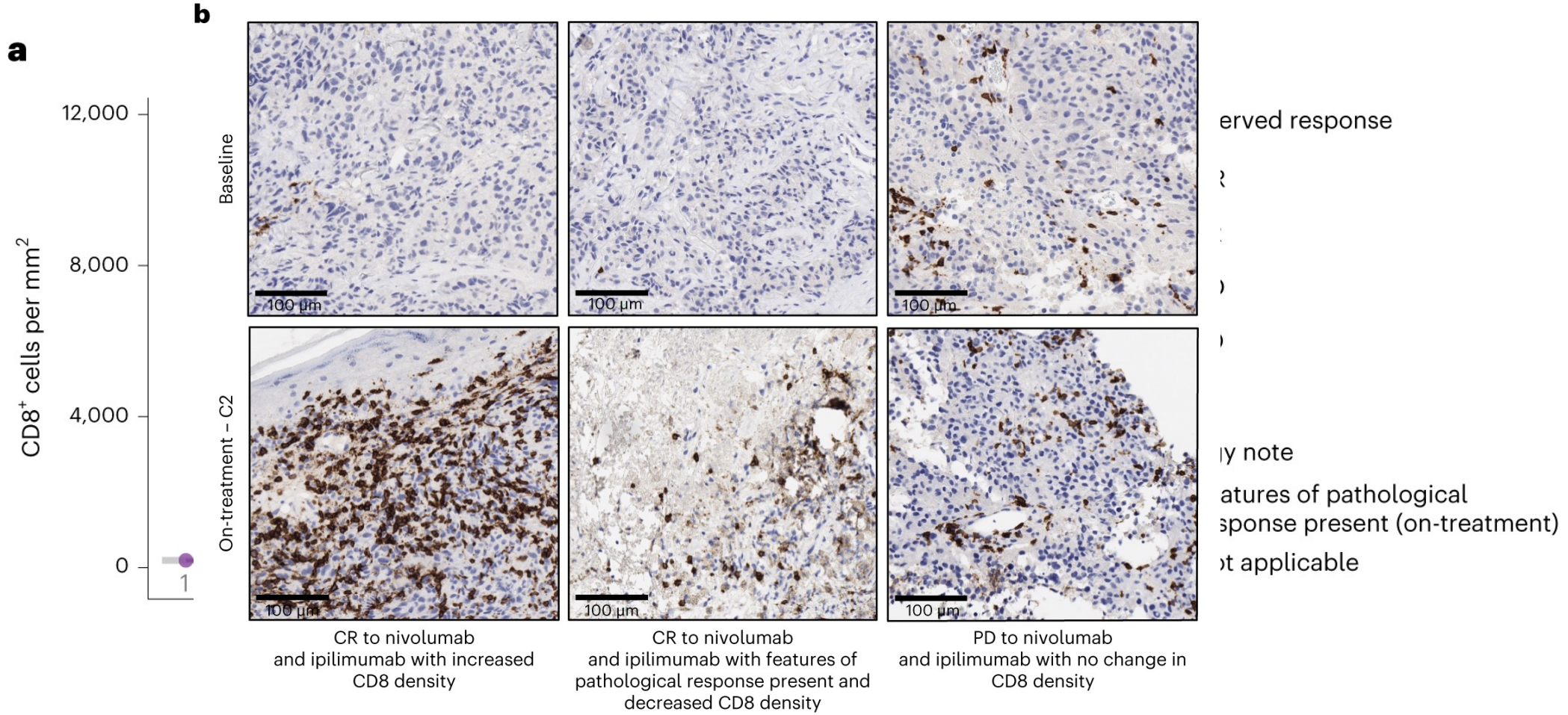
Patients with melanoma refractory to anti-PD-1 have improved outcomes following treatment with combined ipilimumab + nivolumab



- SWOG S1616 (N=94) was a 3:1 randomized phase II clinical trial comparing ipilimumab with or without nivolumab

VanderWalde et al. *Nat Med.* 2023

Biopsies from patients with melanoma responding to combination depict patterns of immune-mediated pathologic response



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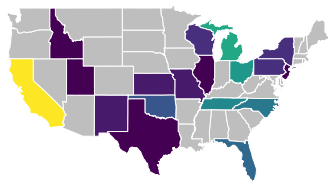
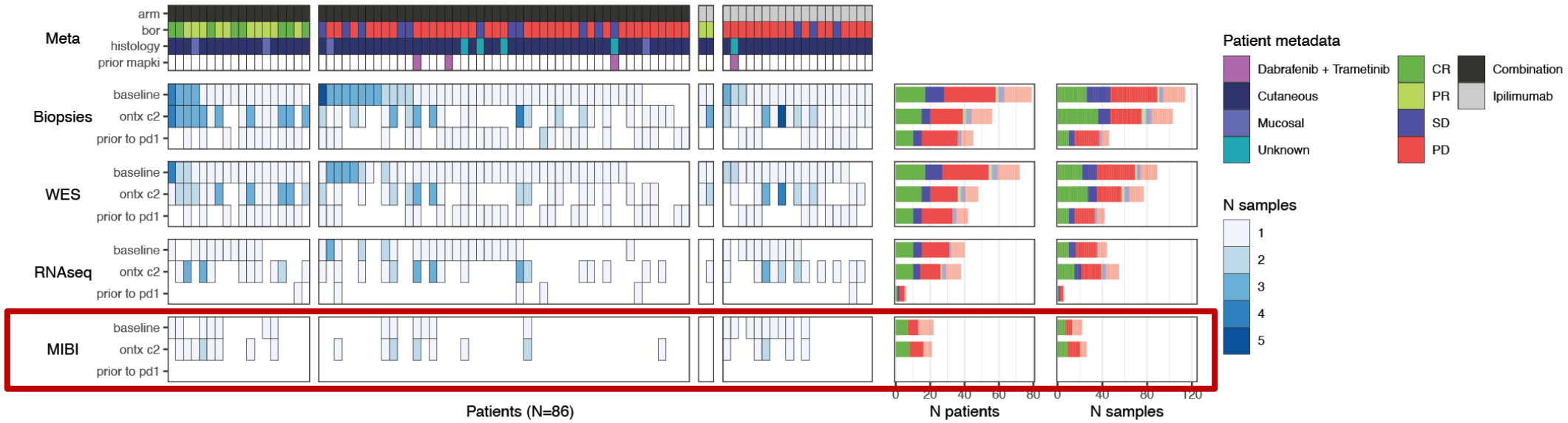
How do immune cells coordinate with CD8 T cells to mediate clinical response to combination therapy?

Baseline and early on-therapy biopsies were collected from patients enrolled in S1616 for integrated translational analyses





- Baseline biopsies collected **following** prior anti-PD1/PD-L1
- On-therapy biopsies collected after one cycle of therapy (Days 28-35)

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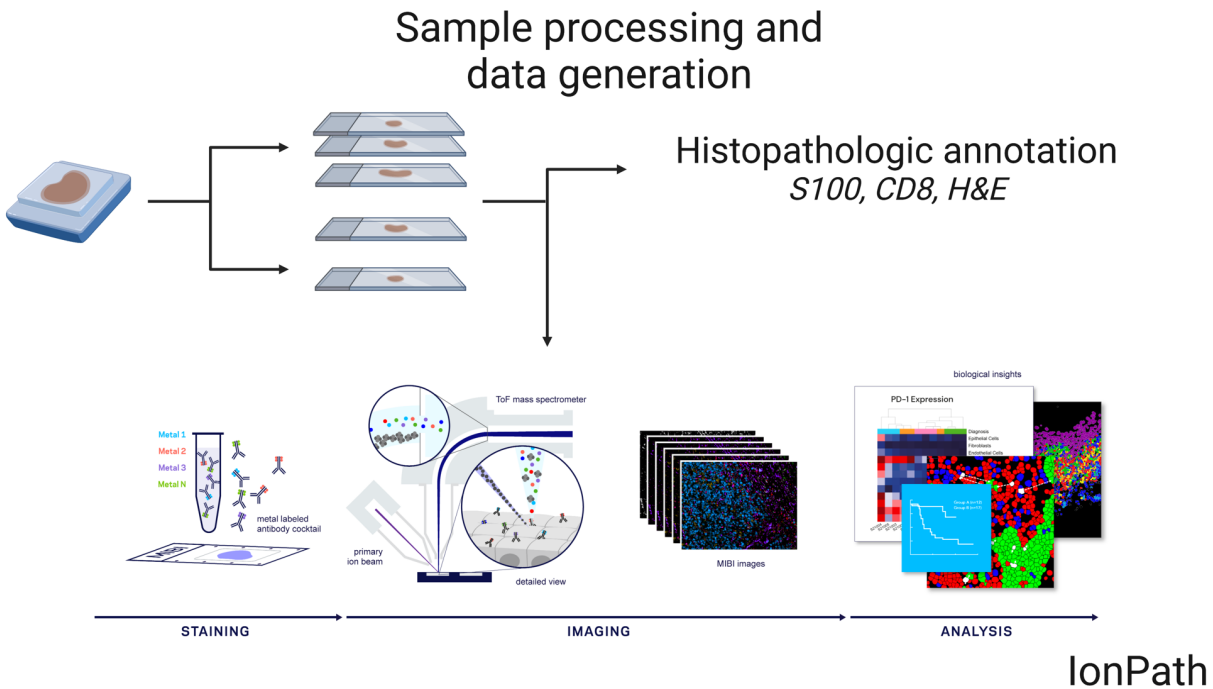
Over 200 ROIs were queried by MIBI across baseline and on-therapy biopsies

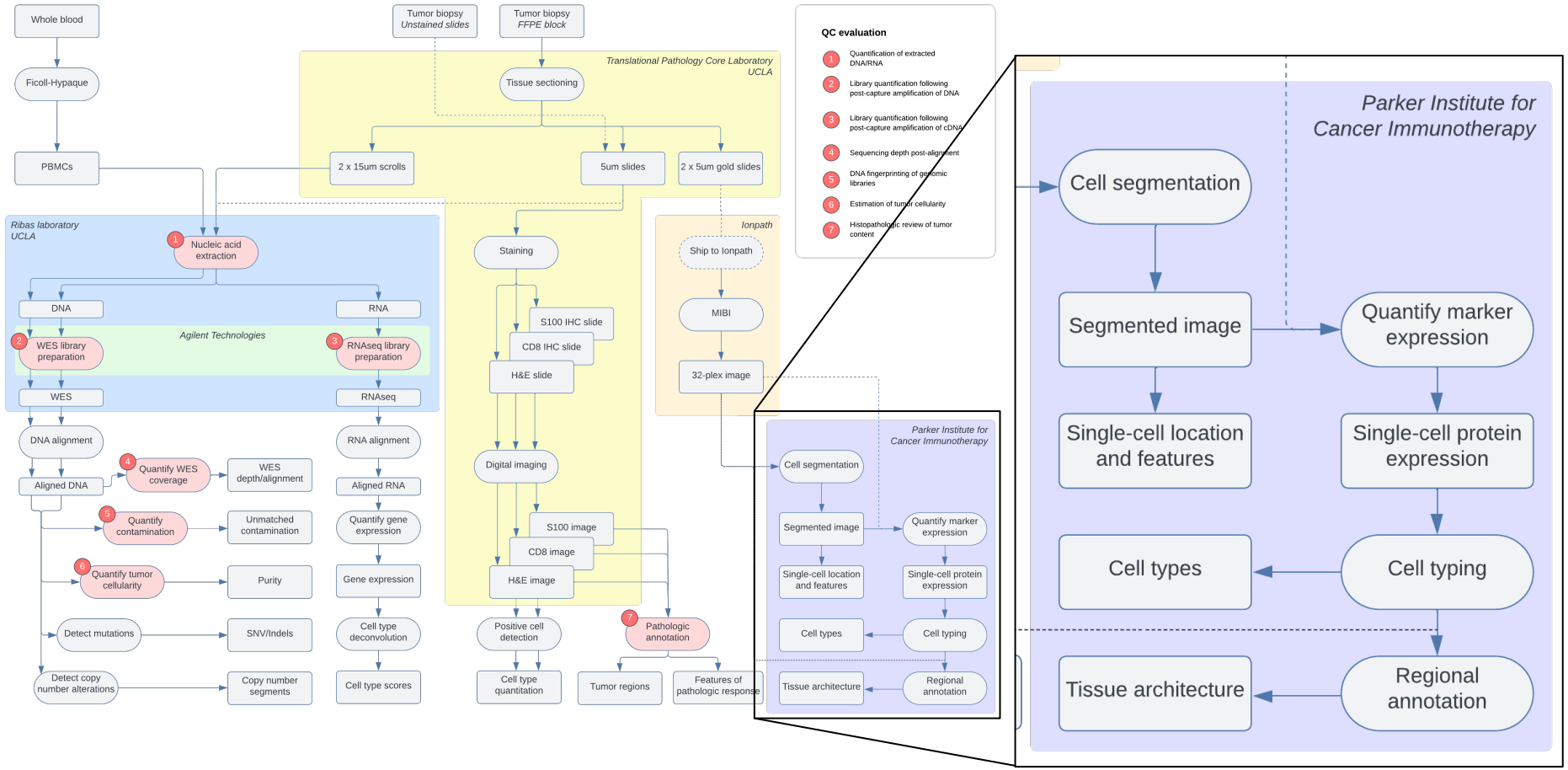


		N Patients			
Treatment Arm	**Response**	**Screening**	**OnTx**	**Paired**	**Total**
Combination	CR/PR	7	8	5	10
Combination	PD	6	9	6	9
Ipilimumab	PD	8	5	5	8

		N Biopsies (N ROIs)	
Treatment Arm	**Response**	**Screening**	**OnTx**
Combination	CR/PR	7 (30)	8 (26)
Combination	PD	6 (34)	13 (64)
Ipilimumab	PD	8 (46)	6 (20)

- 800x800um ROIs were selected to span entire biopsies
- Sequential sections were assessed by H&E or IHC (S100 or CD8)

Pipeline modifications automate cellular and regional analyses across biopsies



- Hierarchical cell typing assignment of 14 cell types
  - Nuclear markers > whole cell markers
- Cell segmentation improved by typing of pixels and segment expansion
- Regional annotation of tissue architecture
  - Vasculature (CD31+ endothelial vessels)
  - Tumor regions (SOX10+ melanma cells)
  - Lymphoid aggregates (CD20+ B cell areas)

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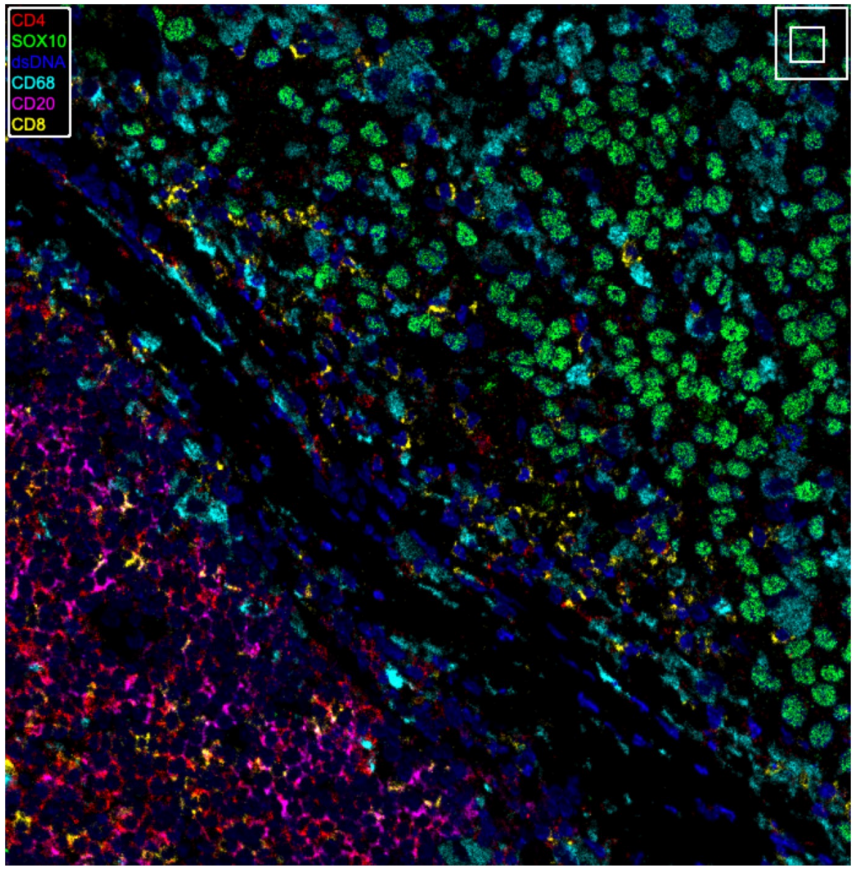
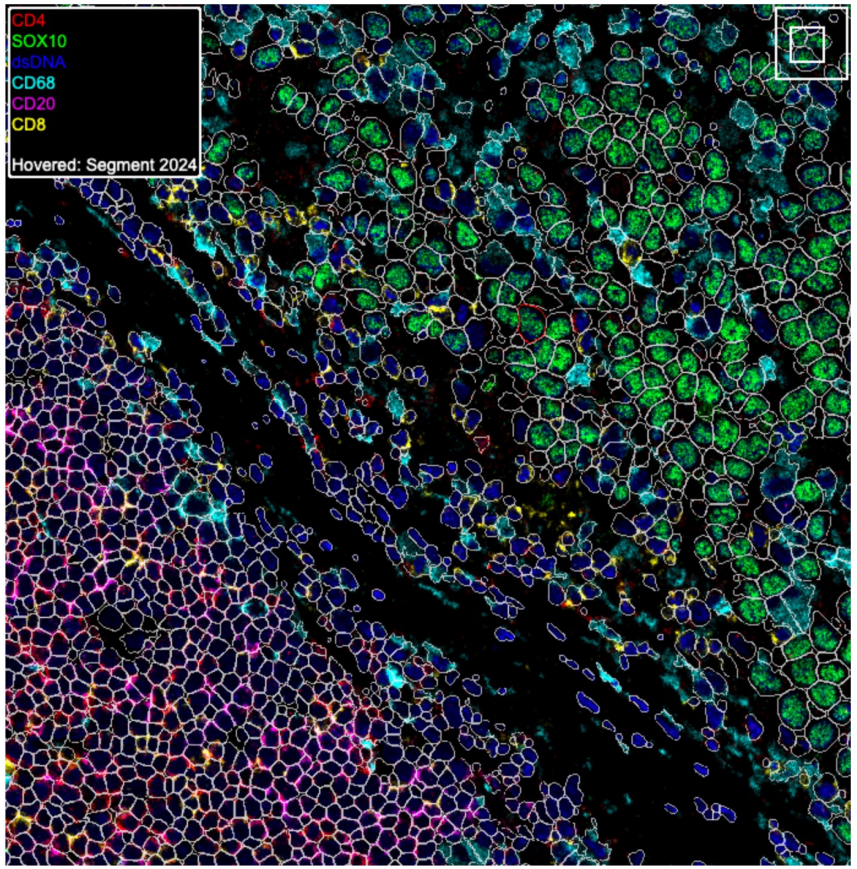
Sandra Santulli-Marotto

Cell type annotation of pixels and segments improves capturing of larger and amorphous cell types





- Mesmer algorithm, Angelo/Van Valen labs
- Nuclear channels: dsDNA
- Whole-cell channels: HLA class 1, CD45, CD68, CD14, CD11c, PD-1, PD-L1, HLA-DR, beta-tubulin

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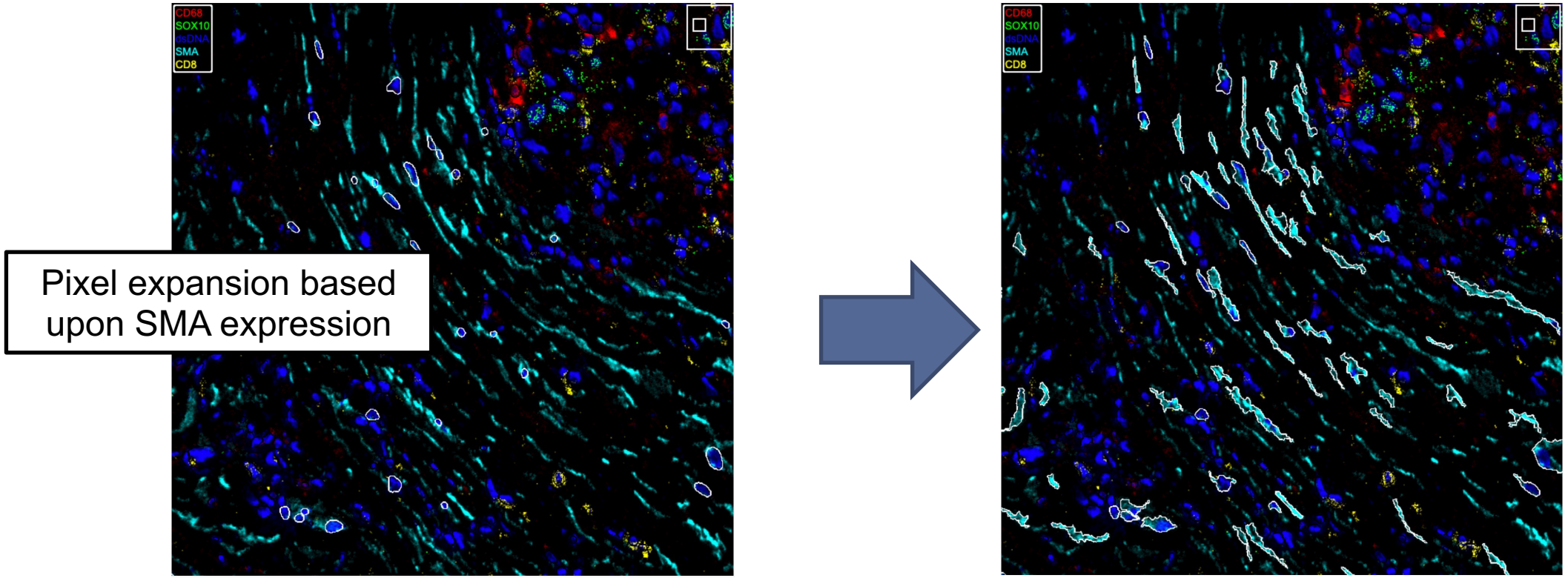
Zaid Bustami
Rob Schiemann
Marshall Thompson
Jessica Maxey
Ben Kamphaus

Cell type annotation of pixels and segments improves capturing of larger and amorphous cell types



	NKT cell	Treg	T cell	B cell	CD8 T cell	CD4 T cell	Neutrophil	Immune	Plasma cell	Dendritic	Monocyte	Myeloid	Endothelial	NK cell	Tumor	Macrophage	Fibroblast
Increase in segment area	0.94	1.4	4.5	5.5	7.6	7.6	9	12	13	20	20	32	36	37	39	46	98

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Zaid Bustami
Rob Schiemann
Marshall Thompson
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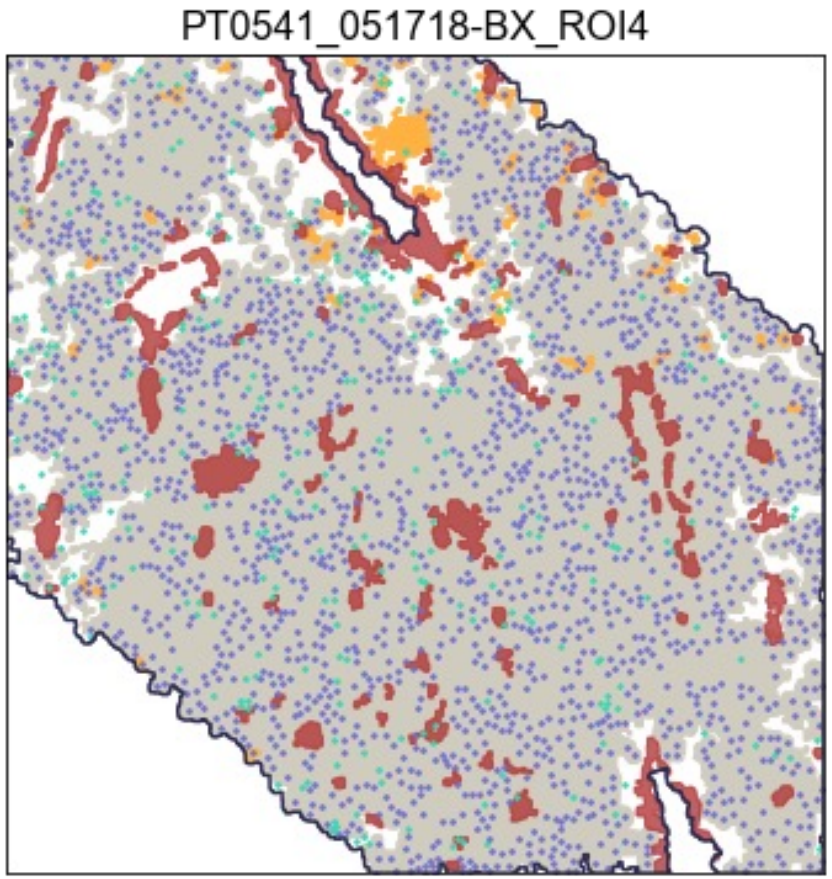
Reconstruction of the tissue architecture enables comparison of cells across regions

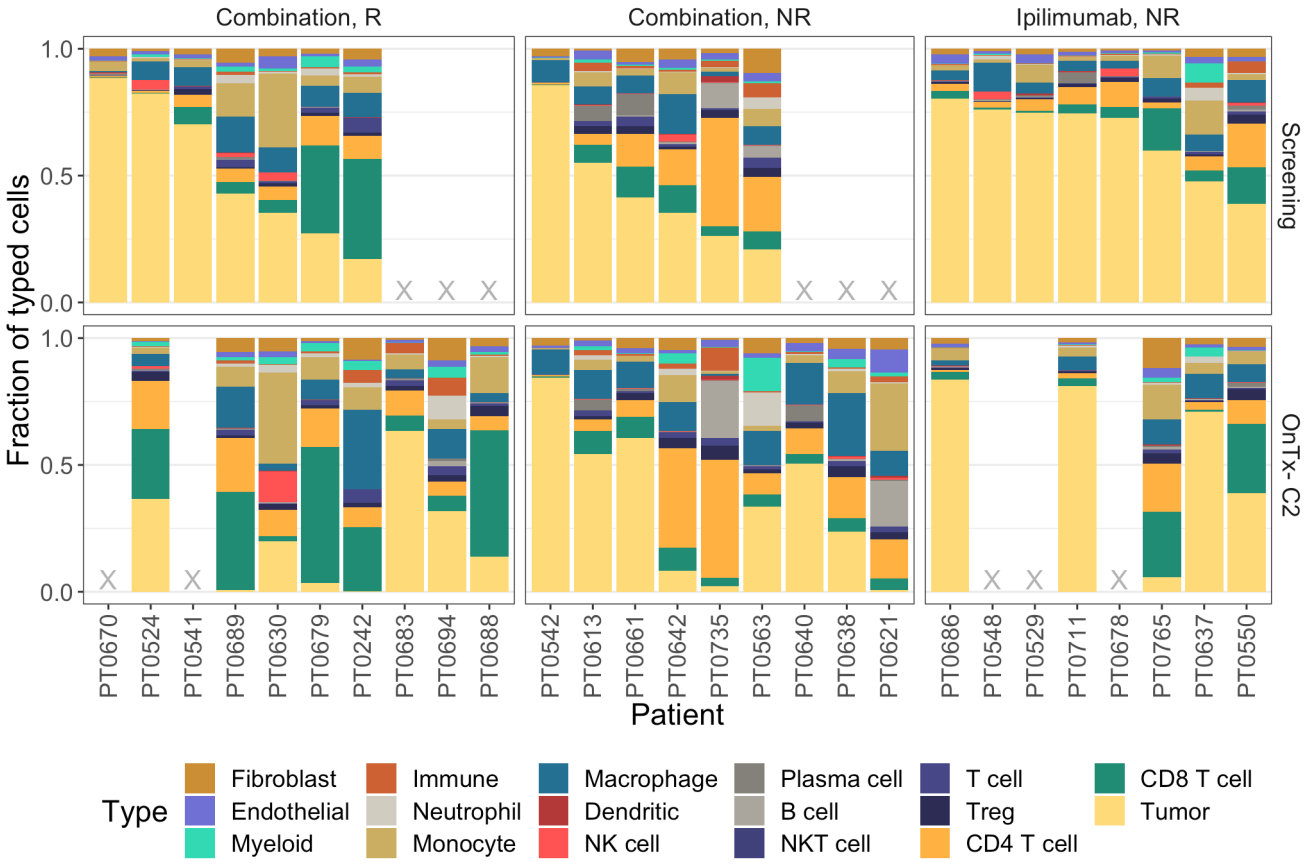


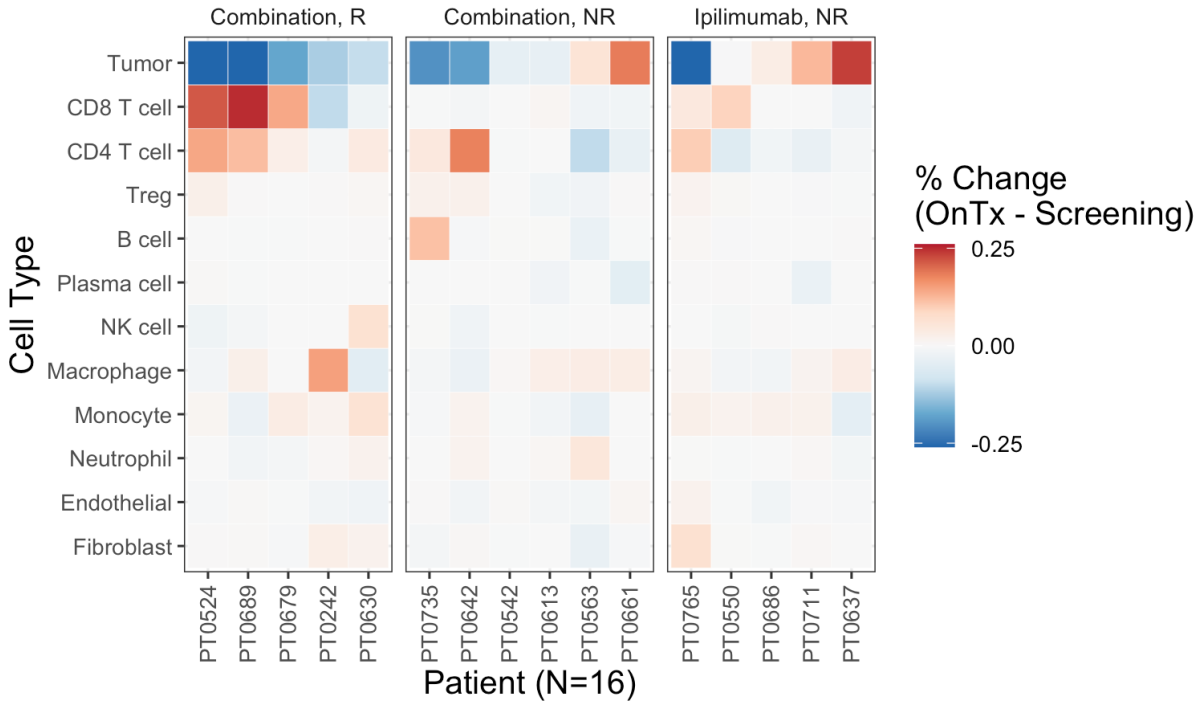
Lymphoid aggregate: CD20+ area, B cells

Blood vessel: CD31+ area, Endothelial cells

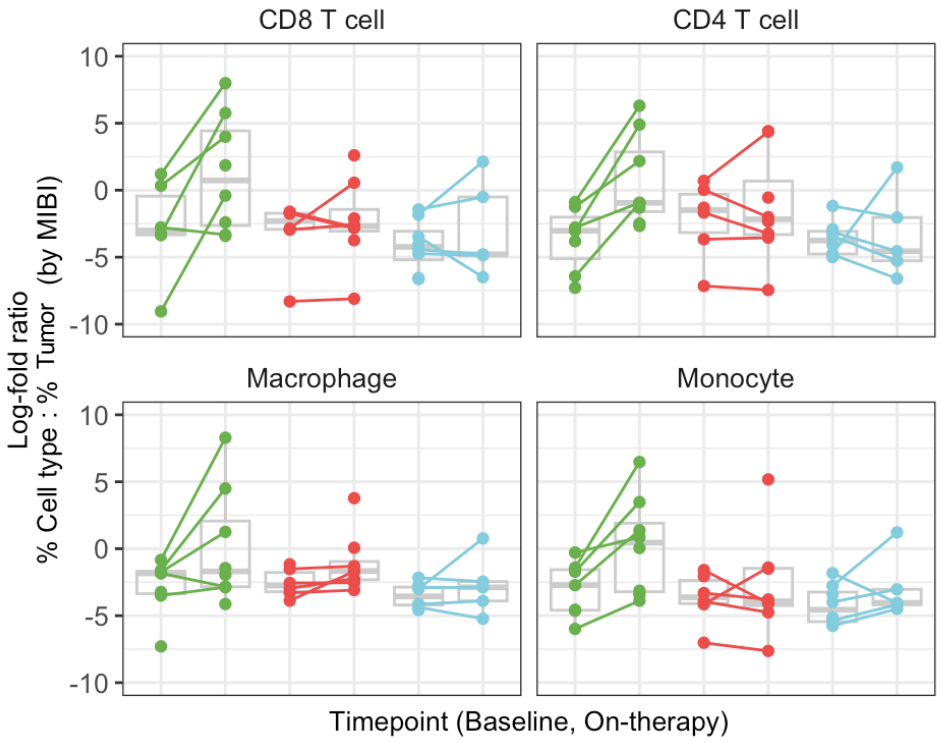
Tumor region: SOX10+ area, Melanoma cells

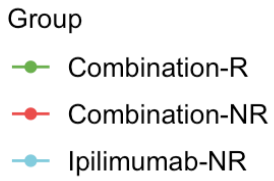
Biopsies responding to combination have increased CD8 T cells and decreased melanoma cells

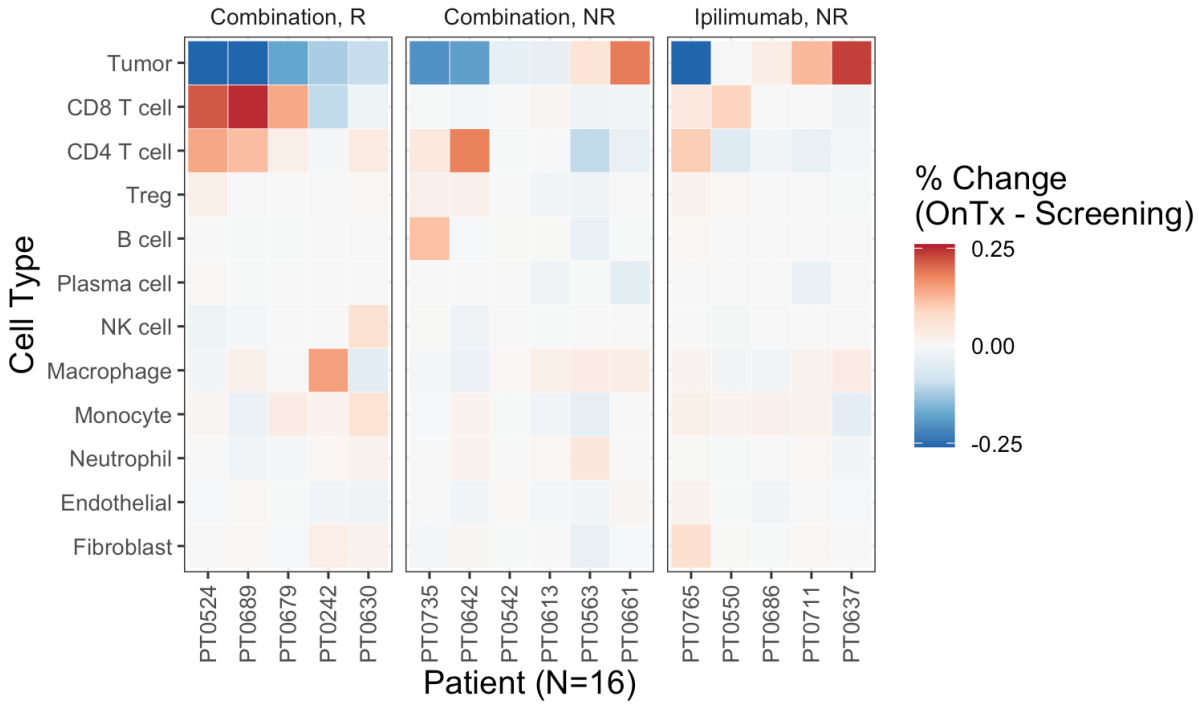




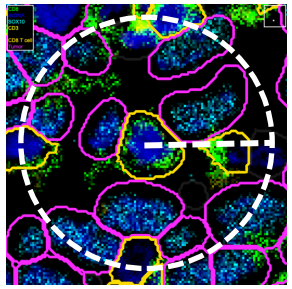
Biopsies responding to combination have increased monocytes, macrophages, and T cells and decreased melanoma cells



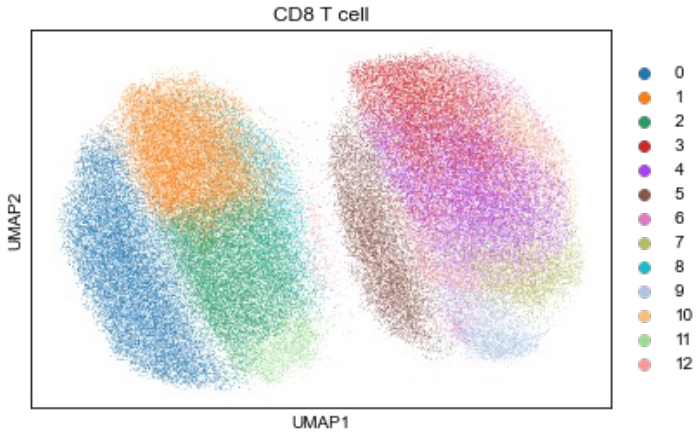


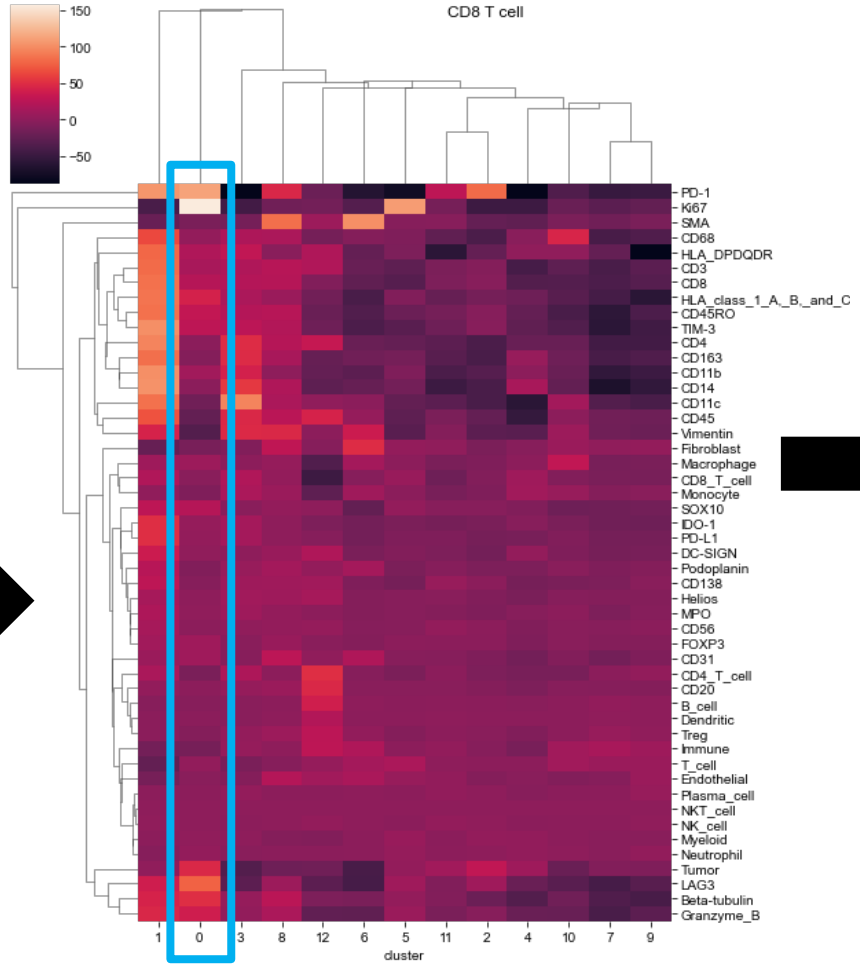


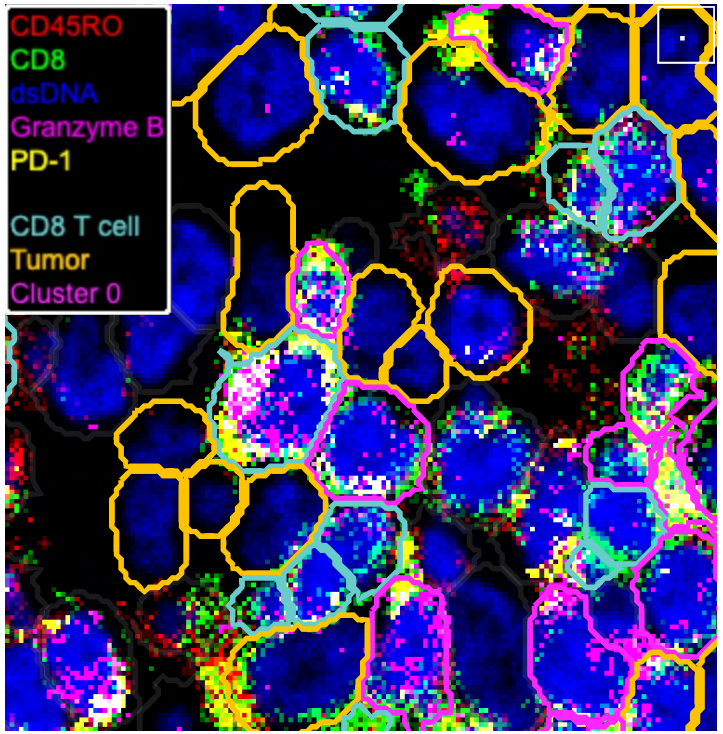
Unsupervised clustering identifies tumor-reactive CD8 T cell populations



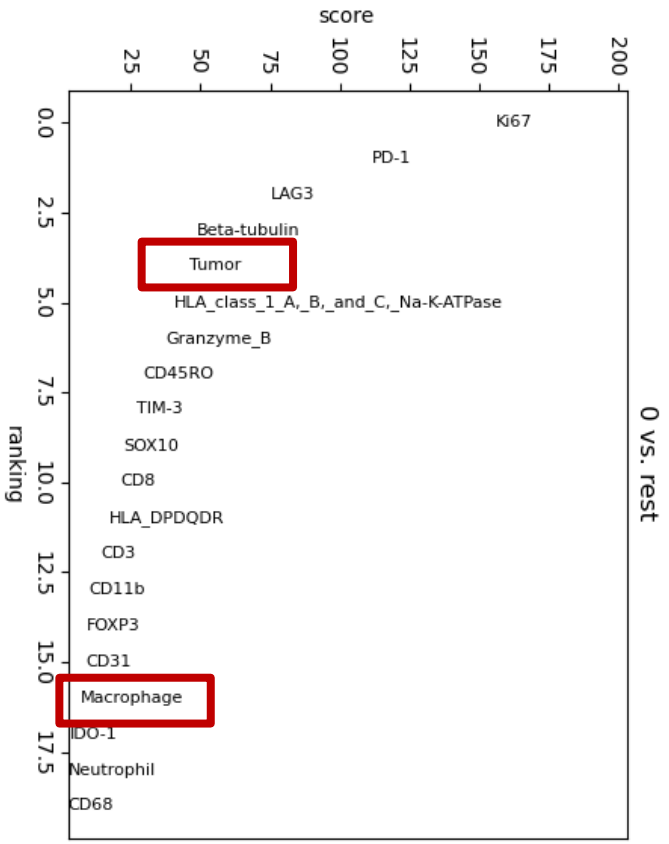
Features for Leiden clustering
- Expression of each marker
- % of each cell type within 30um

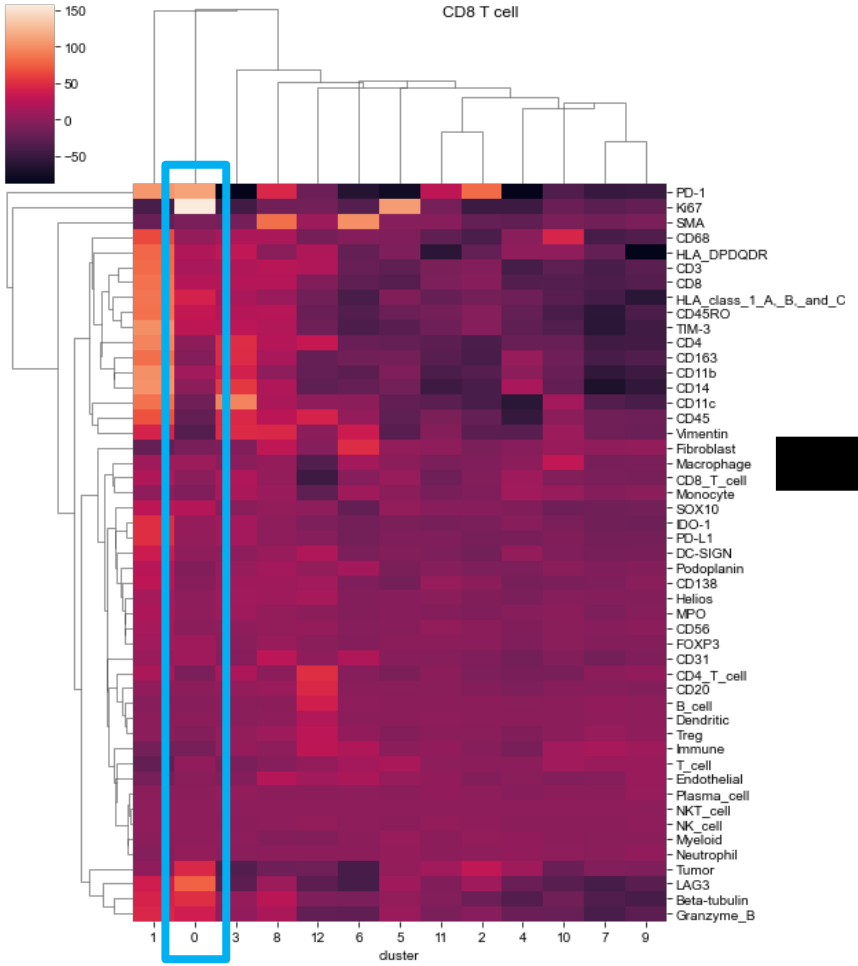


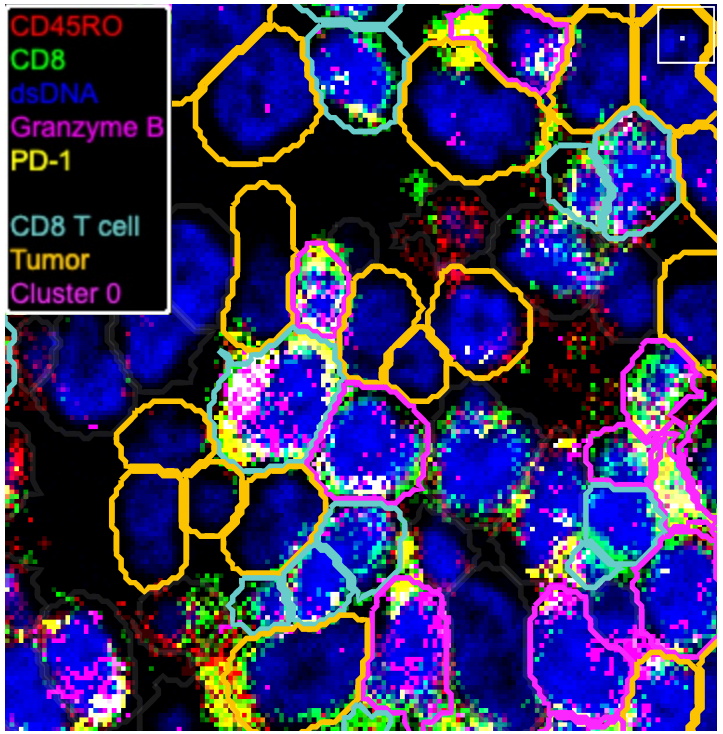




Hypothesis: CD8 T cells with shared location features also share phenotypic characteristics







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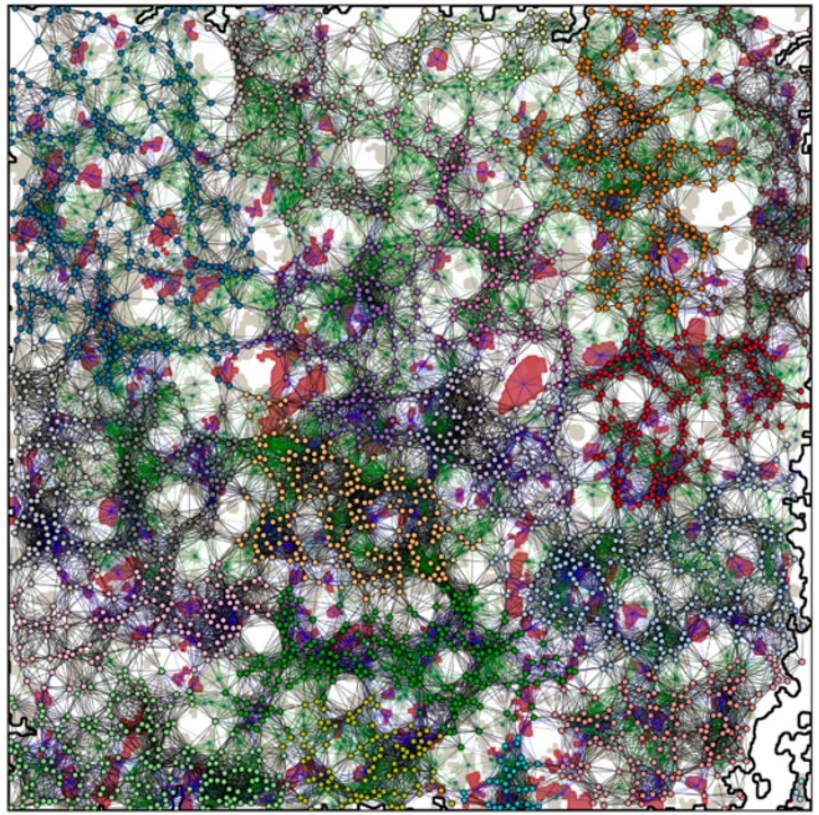
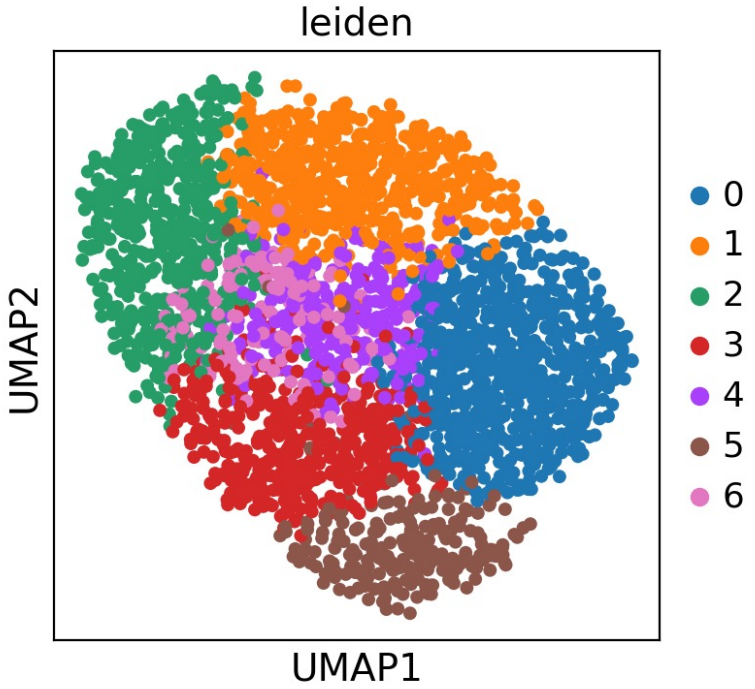
Analysis strategy: Analyze expression and communities of spatially defined groups of CD8 T cells

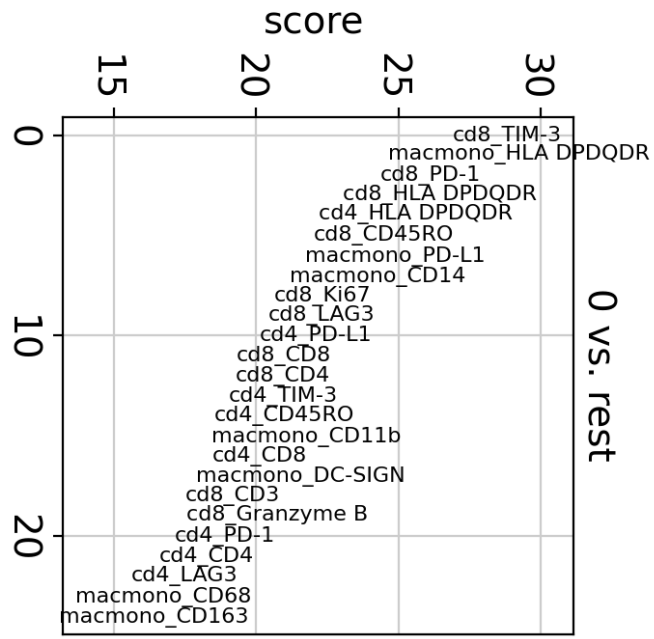
Group CD8 T cells into spatially distinct **partitions**



Cluster partitions based upon expression and location

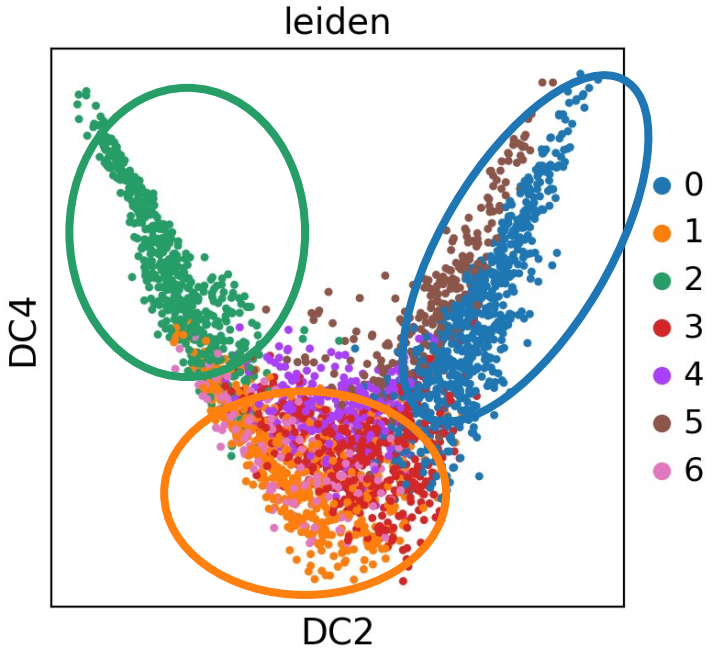


Evaluate Leiden features per cluster

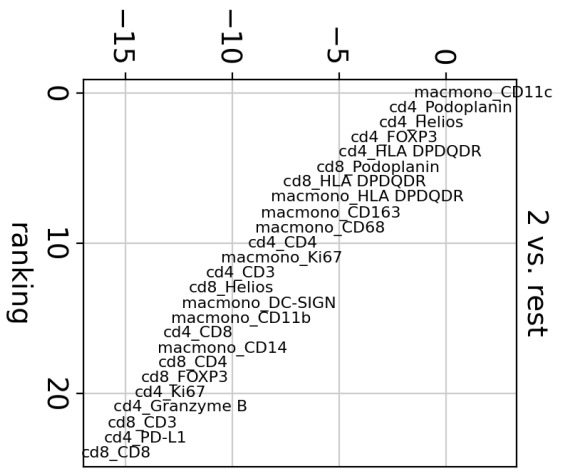


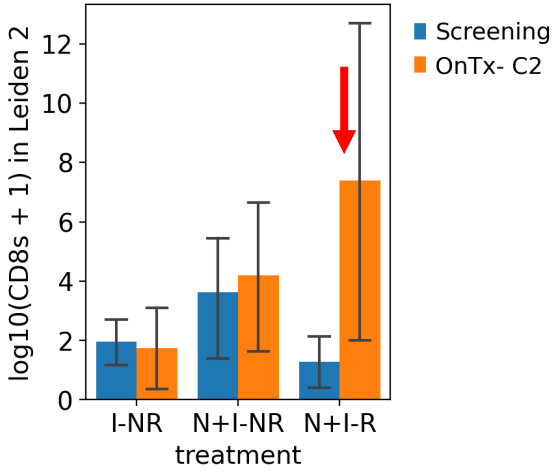
Responding biopsies have increased percentages of effector CD8 T cells and an influx of nonactivated CD8 T cells

DC = Diffusion components (Pseudotime analysis)

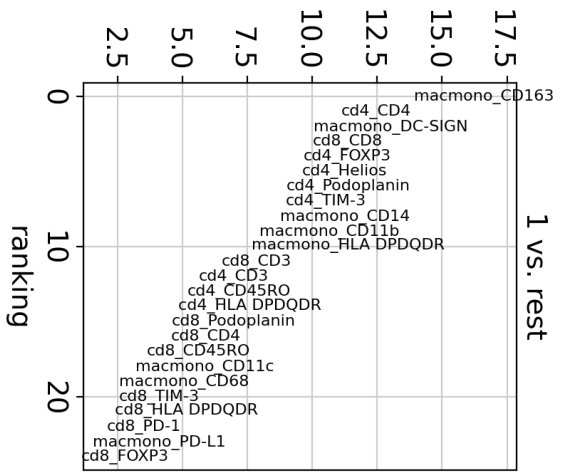


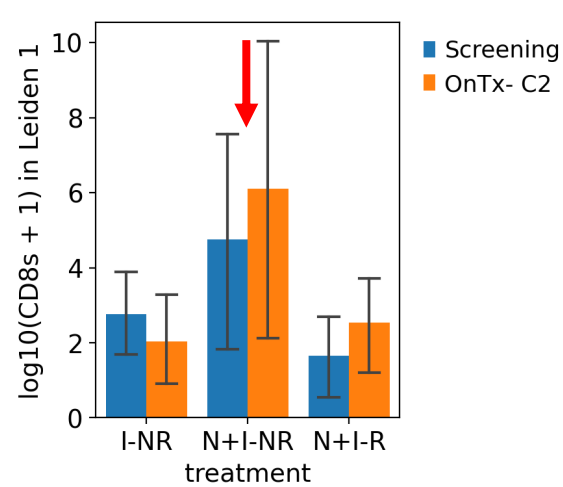
Leiden 2: Near blood vessels, no activation



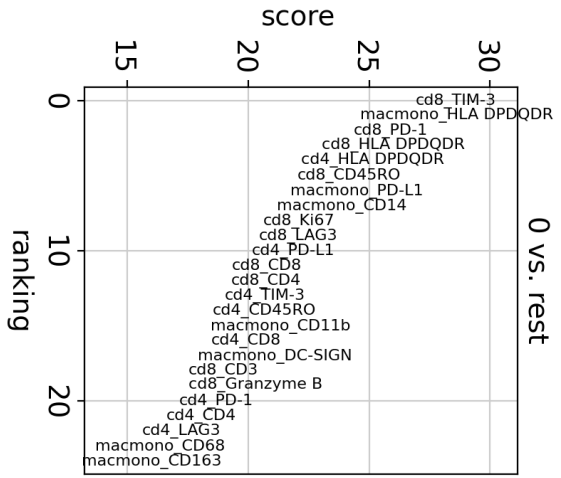


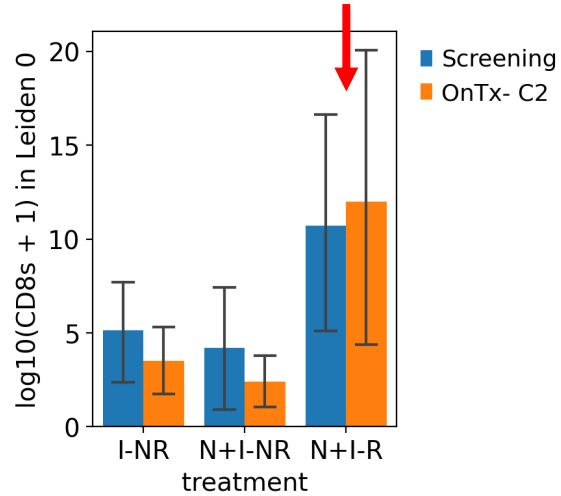
Leiden 1: M2, Treg, Tex





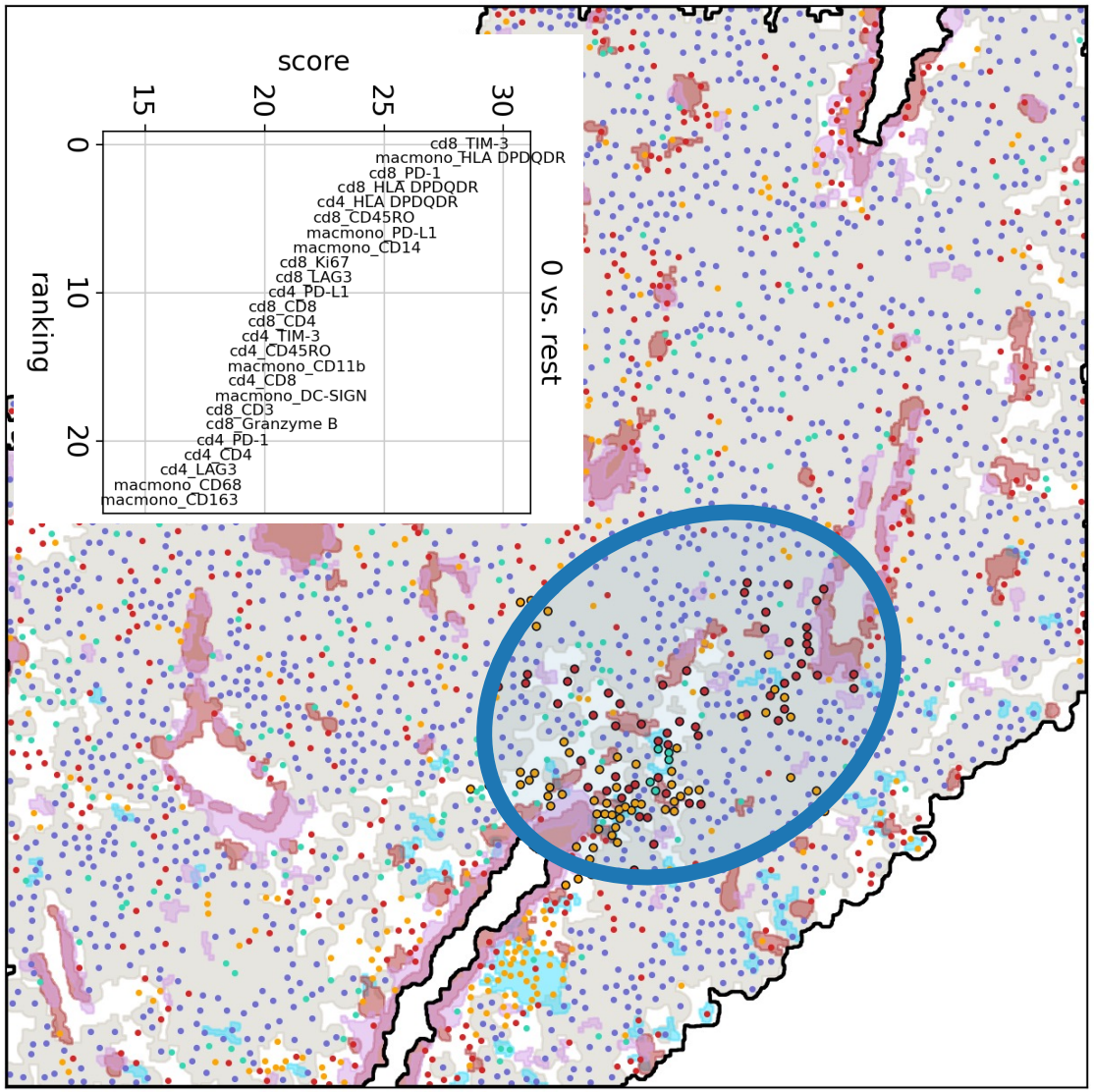
Leiden 0: M1, Th1, Teff



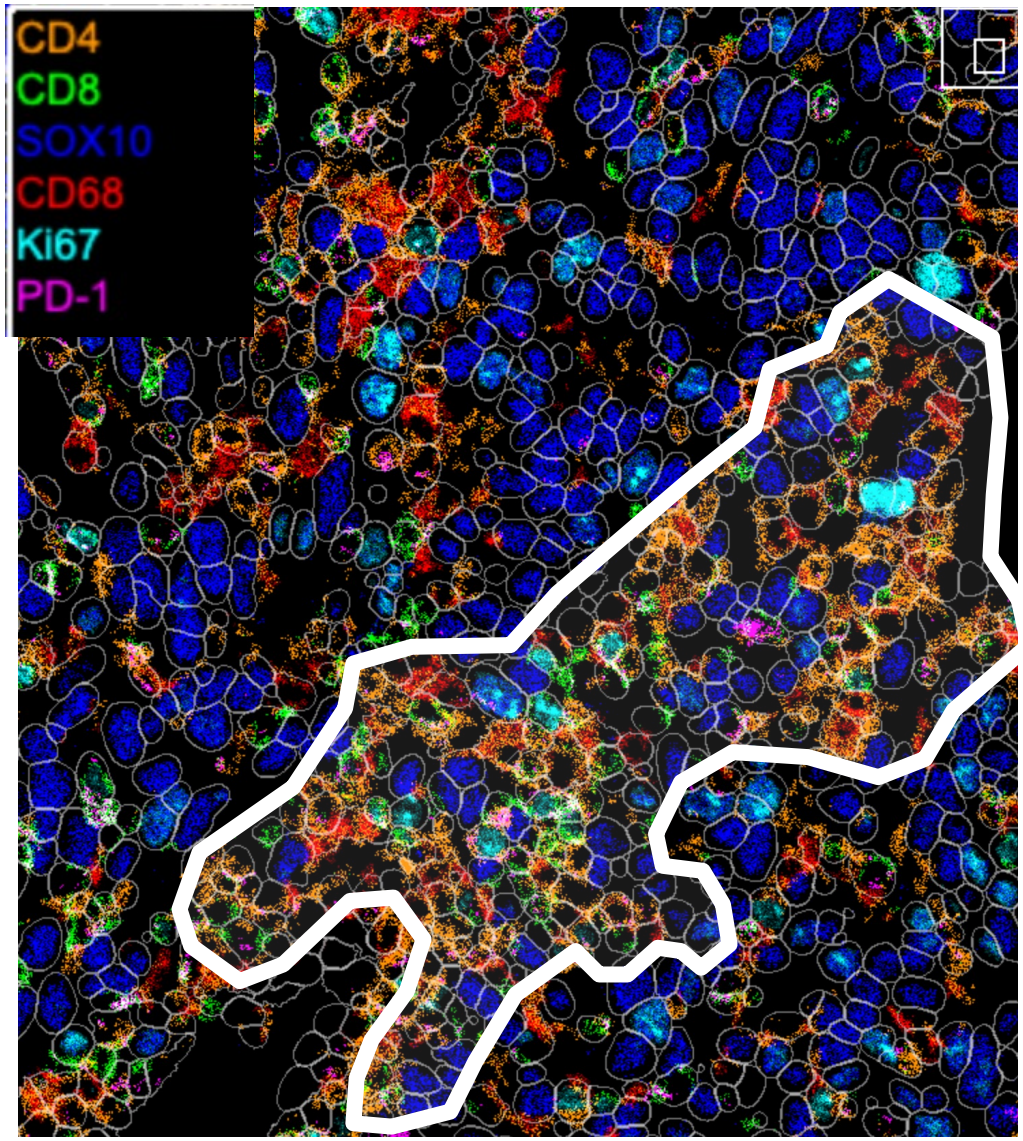


Daniel Chen

Leiden 0: exhausted, effector CD8 T-cells colocalize with M1 and Th1 cells



SOX10
CD20
CD31
SMA
dsDNA
dsDNA outline
CD8 T cell
Tumor
CD4 T cell
Treg
Monocyte
Macrophage



Summary

- Higher plexity and more cell types improves analysis of (sub)cellular and regional features
- Patients who respond to combination therapy demonstrate increased CD8 T-cell influx
  - Higher exhausted, effector CD8 T-cell phenotypes, colocalized with Th1 and M1 macrophages
  - More nonactivated CD8 T cells nearby blood vessels, on-therapy
- CD8 T cells present in biopsies from patients who do not respond to combination therapy colocalize with M2 and Treg populations
- Quantitated cell dynamics correlate with qualitative annotation/observation by dermatopathologists

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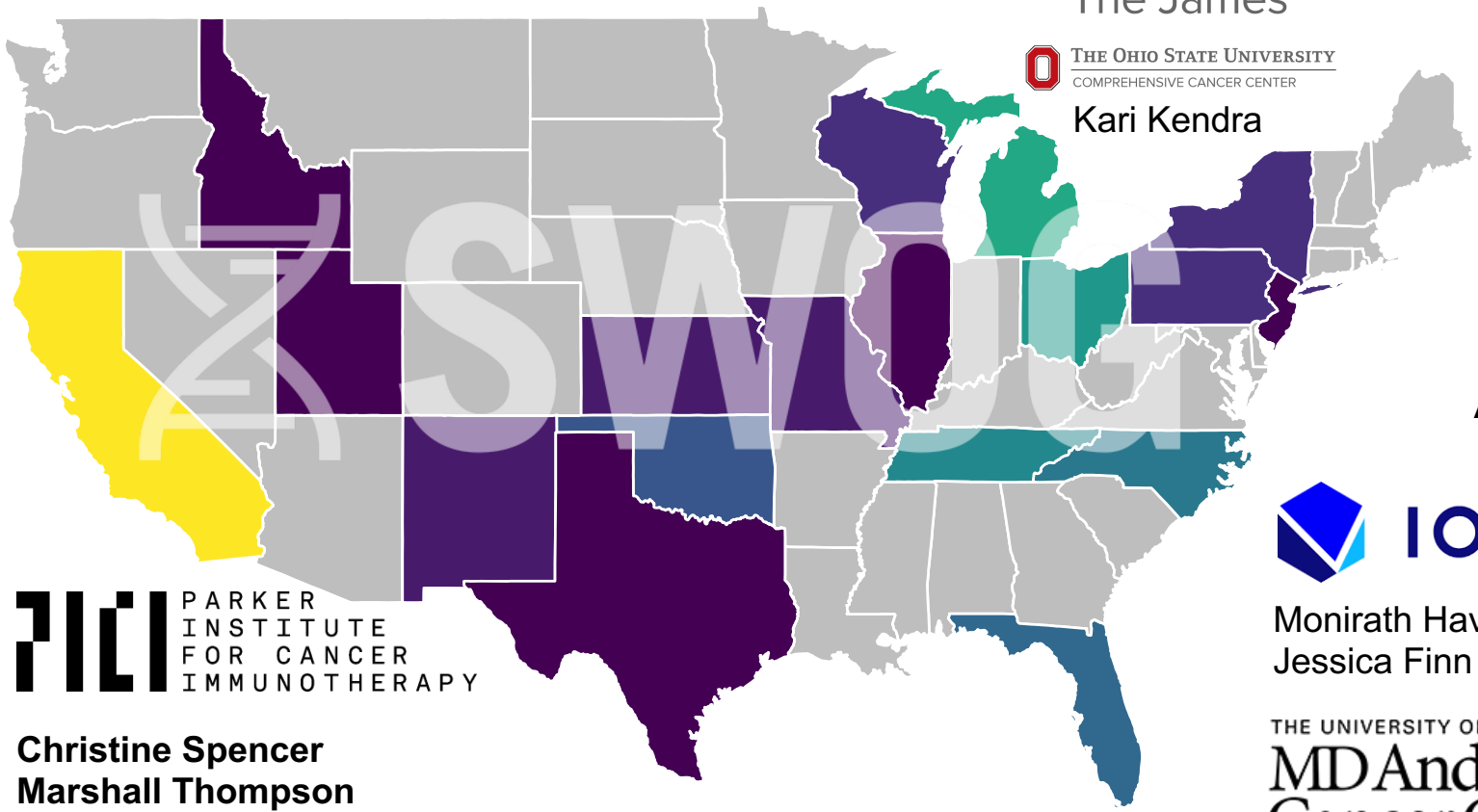

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