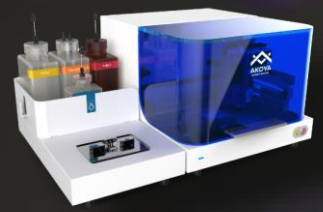
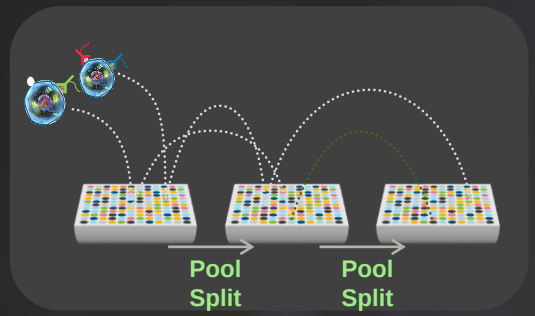
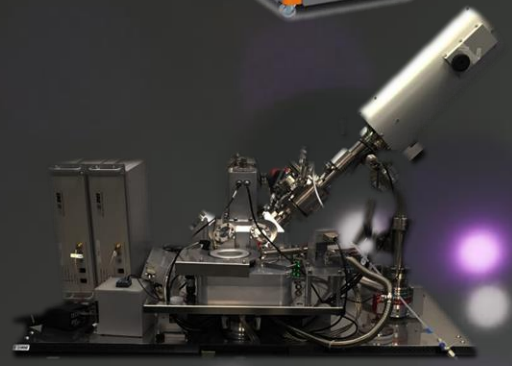
How cancer rearranges the rules for immune tissue schematics: New Class of Targets for Therapy

Architecture as defined by organization of niches and INTER-CELLULAR relationships







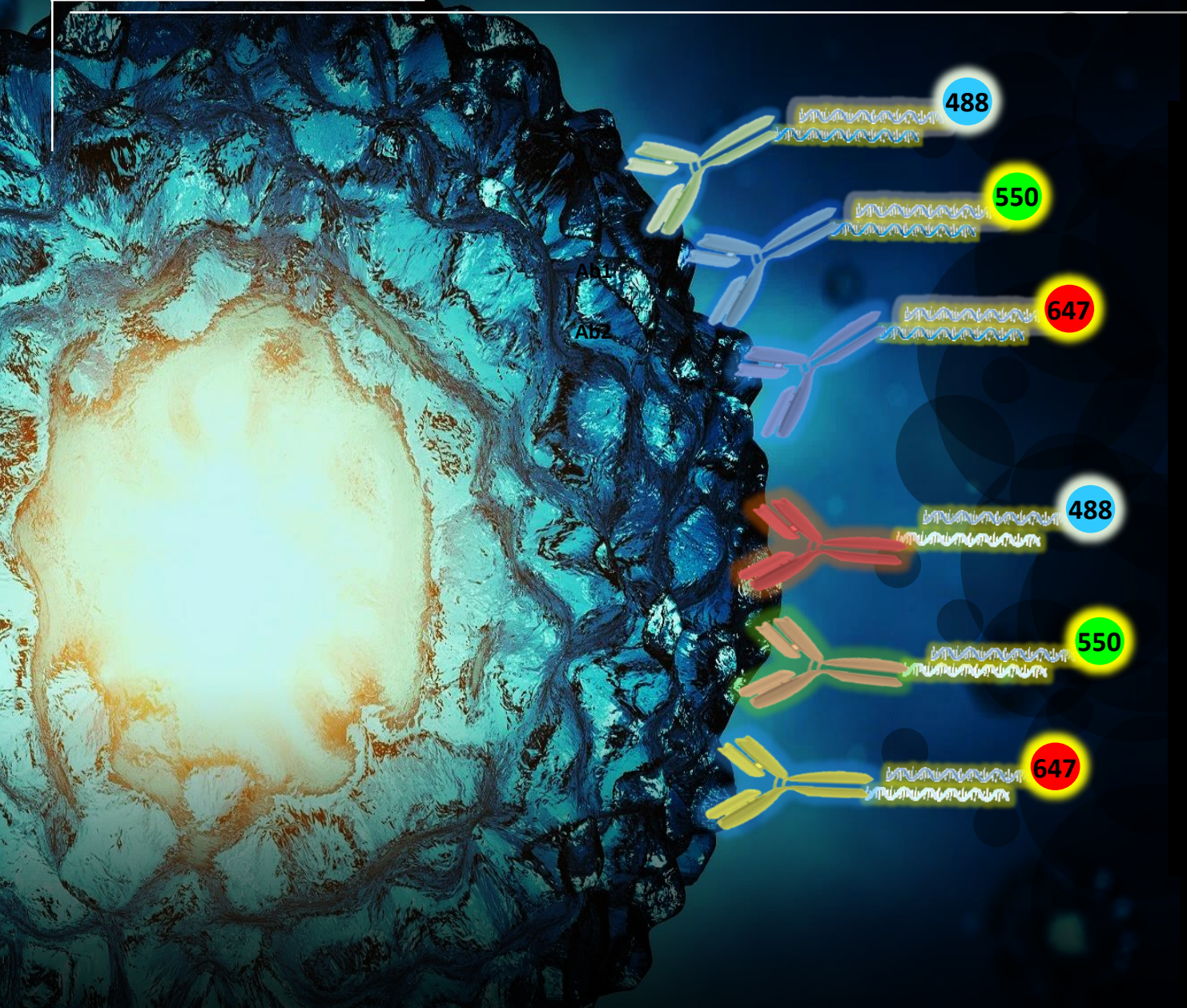
Commercial conflicts:
- Akoya Bio
- IonPath
- Scale Bio

Garry P. Nolan, Ph.D.
Rachford and Carlota A. Harris Professor
Dept. of Pathology
Stanford University

Parker Institute for Cancer Immunotherapy

CODEX2 • Multiplexing by Reannealing

Cell, August 2018



Equity holder - potential conflict

CODEX FFPE • Validated DNA-Barcoded Antibodies

CD / immune markers		
CD1a	CD38	CD103
CD2	CD39	CD117 (c-kit)
CD3	CD44	CD134 (OX-40)
CD4	CD45	CD138
CD5	CD45RA	CD162
CD7	CD45RO	CD163
CD8	CD47	CD164
CD11b	CD56	CD194 (CCR4)
CD11c	CD57	CD206
CD15	CD62L	CD223 (LAG-3)
CD16	CD66a	CD235a
CD20	CD68	CD271
CD21	CD69	CD274 (PD-L1)
CD25	CD70	CD278 (ICOS)
CD30	CD71	CD279 (PD-1)
CD31	CD73	CD366 (TIM-3)
CD34	CD74	
CD36	CD79a	

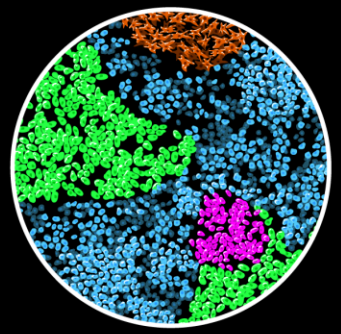
Tissue-Specific and other markers				
AIF-1	dsDNA	IRF4 (MUM-1)	p16	Synaptophysin
alpha-SMA	EGFR	Kappa	p63	T-bet
Annexin A1	EpCAM	Ki-67	Nucleolin	TCR-a/b
Arginase-1	FoxP3	Lambda	p53	TCR-g/d
beta-catenin	GATA3	Lamin A/C	Pancreatic amylase	TIA-1
BCL-2	GFAP	Lamin B1	Pancreatic lipase	TMEM119
beta-tubulin 3	Glucagon	Mast cell tryptase	Pancreatic PP	TTF-1
Cathepsin D	Granzyme B	Melan-A	PAX5	Vimentin
CCR6	Hep-Par-1	MHC-1	PNAd	VISTA
CDX2	Histone pH2A.X	MMP9	Podoplanin	
Chromogranin A	Histone H3	MMP12	Pro-insulin	
CK 7	Hist H3K9ac	MPO	pSTAT1	
Collagen IV	Hist H3K27me3	MUC-1	pSTAT3	
COX-2	Hist H3pS28	MUC-2	pSTAT5	
CX3CR1	HLA-DR	MUC-5AC	S100A6	
CXCL13	IDH-1 R132H	MYC	S100A9	
CXCR3	IDO-1	Na-K-ATPase	Somatostatin	
Cytokeratin (Pan)	Insulin	Nestin	SOX11	

TOTAL
:
133

TISSUE SCHEMATICS • Strategy for Representing Assembly

with Salil Bhate, Nolan lab

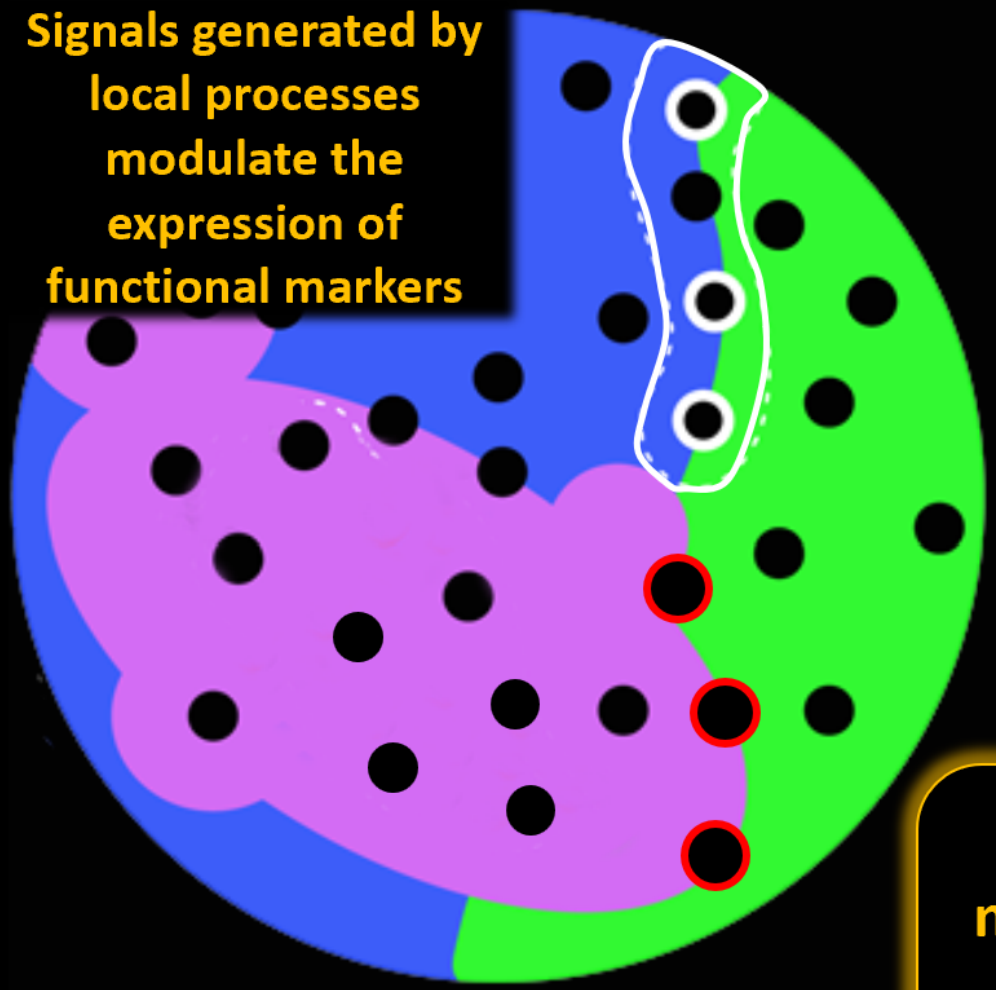
Tissue with CNs assembled into motifs

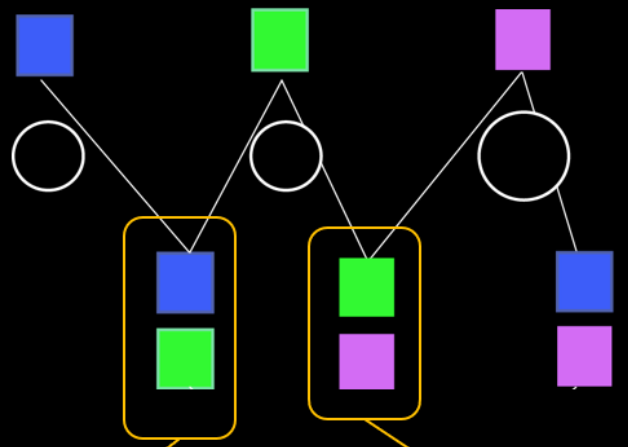


Identify Tissue Motifs

CELLS AT THE INTERFACE • DRIVING INTERACTIVE BIOLOGY

Signals generated by local processes modulate the expression of functional markers



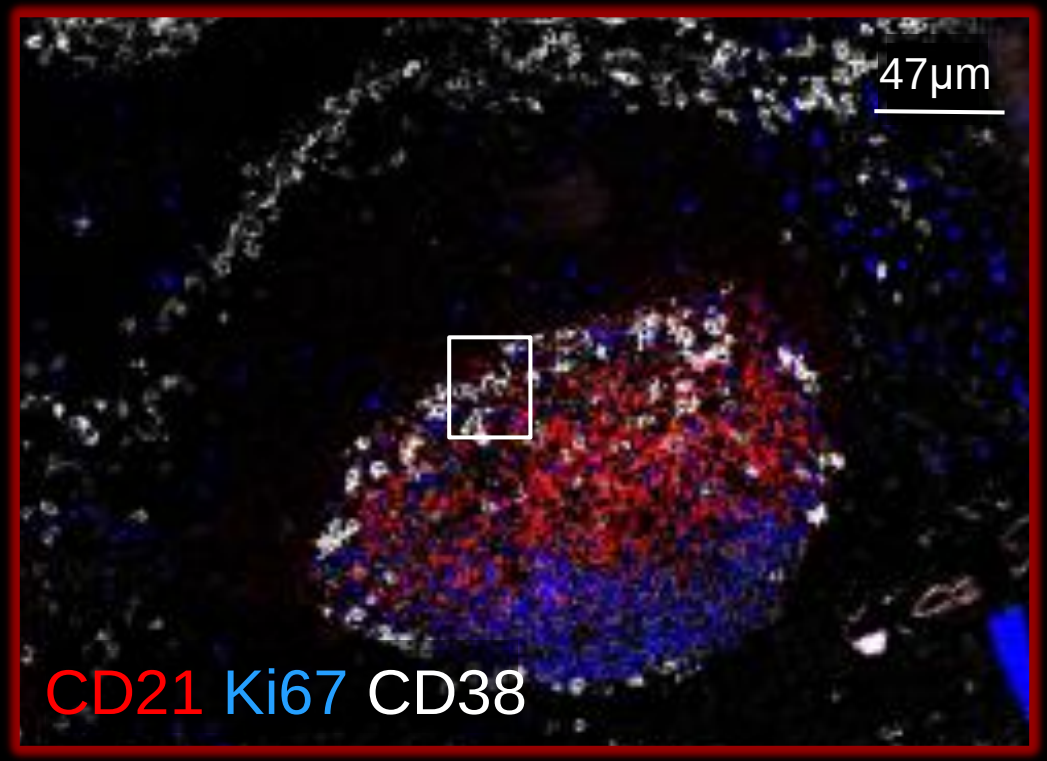


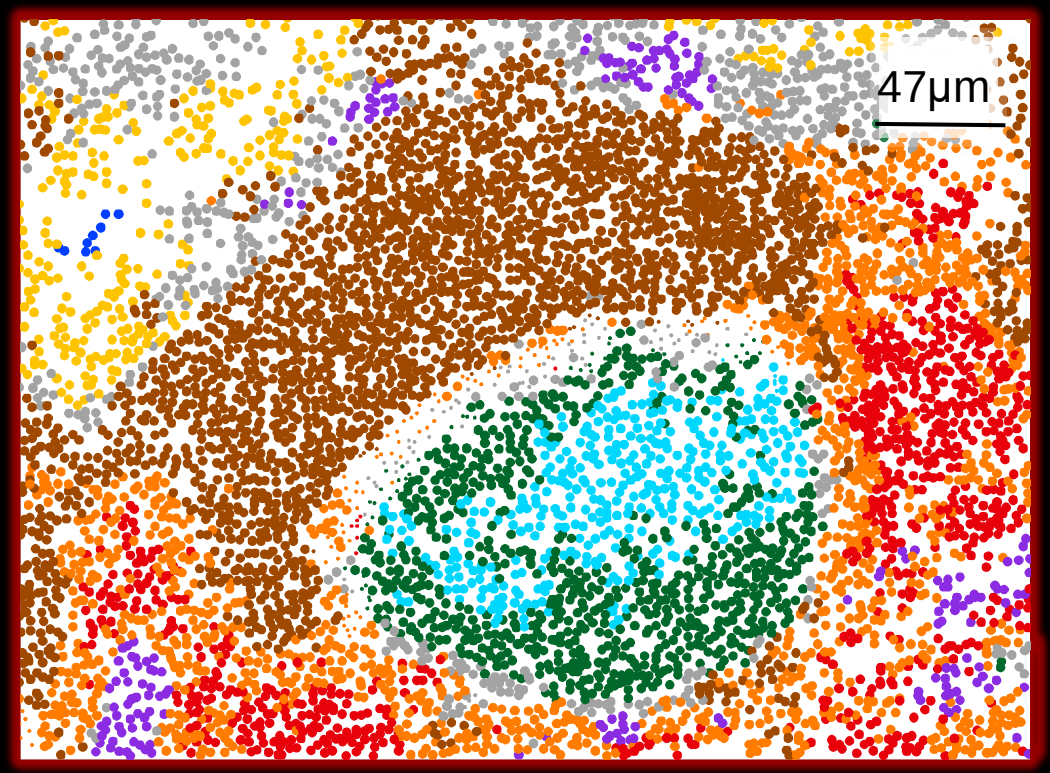
Increase in WHITE marker expression in SC at the green / blue interface

Increase in RED marker expression in SC at the green / purple interface

SPATIAL CONTEXT: OUTER ZONE EXHIBITS DYNAMIC CD38 EXPRESSION

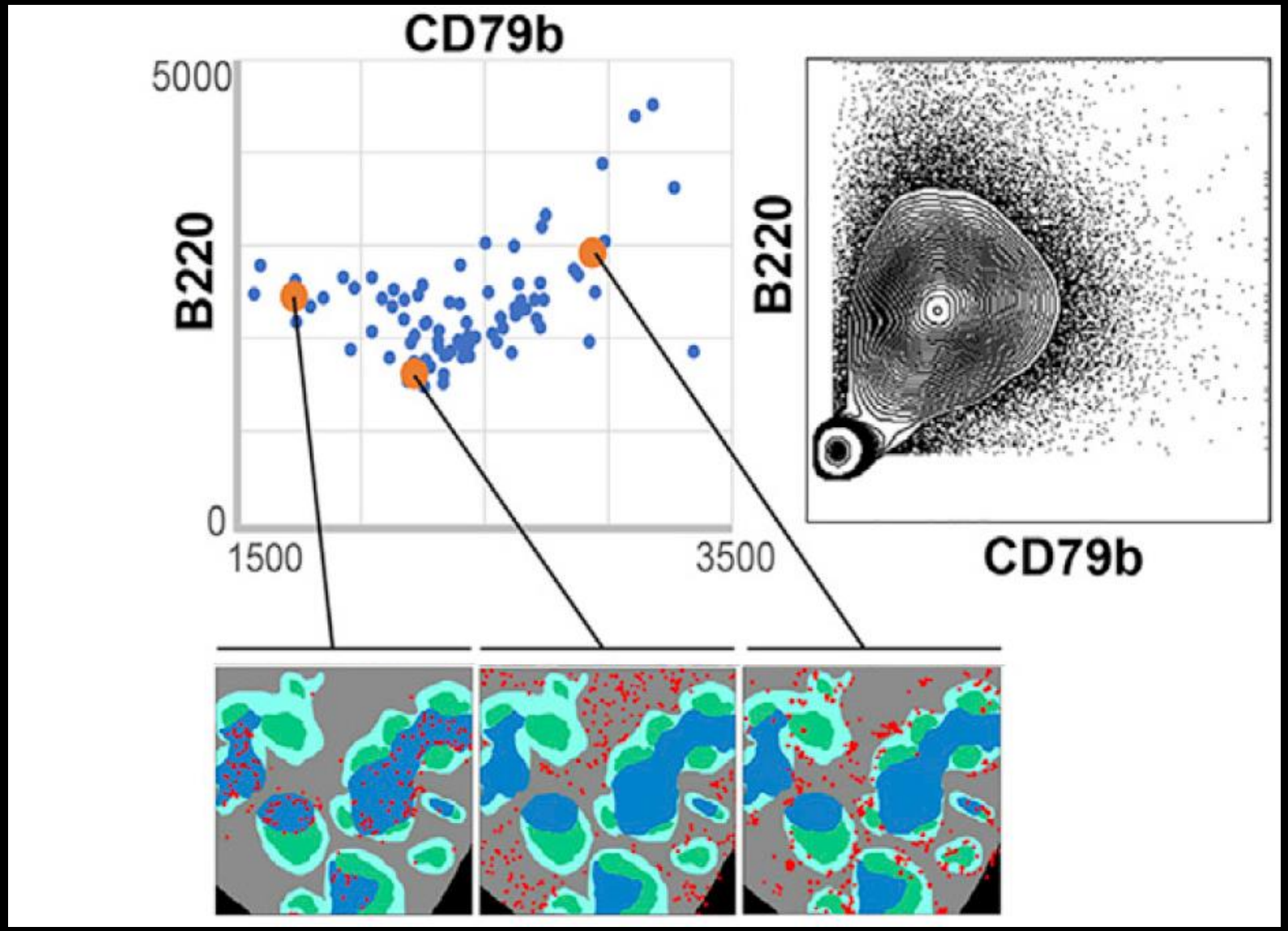
Follicle **WITH** CD38 Expression in Outer Zone





Minor changes in expression levels determines your neighbors

CD79b, immunoglobulin-associated beta



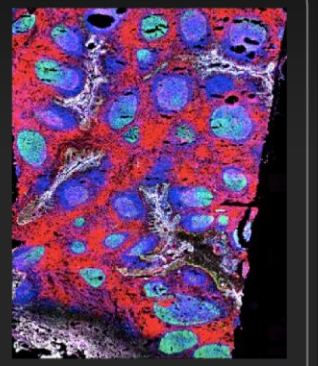
Most algorithms DO NOT APPRECIATE how minor changes in expression levels influence localization.

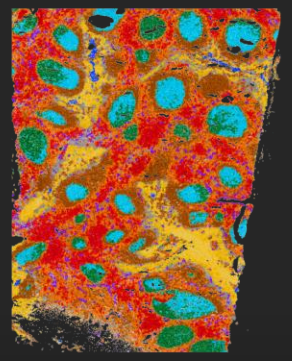
What you "think" and say determines your neighbors.

This data be used in AI algorithms to better classify cell types. Make sure your algorithms use this subtle information.

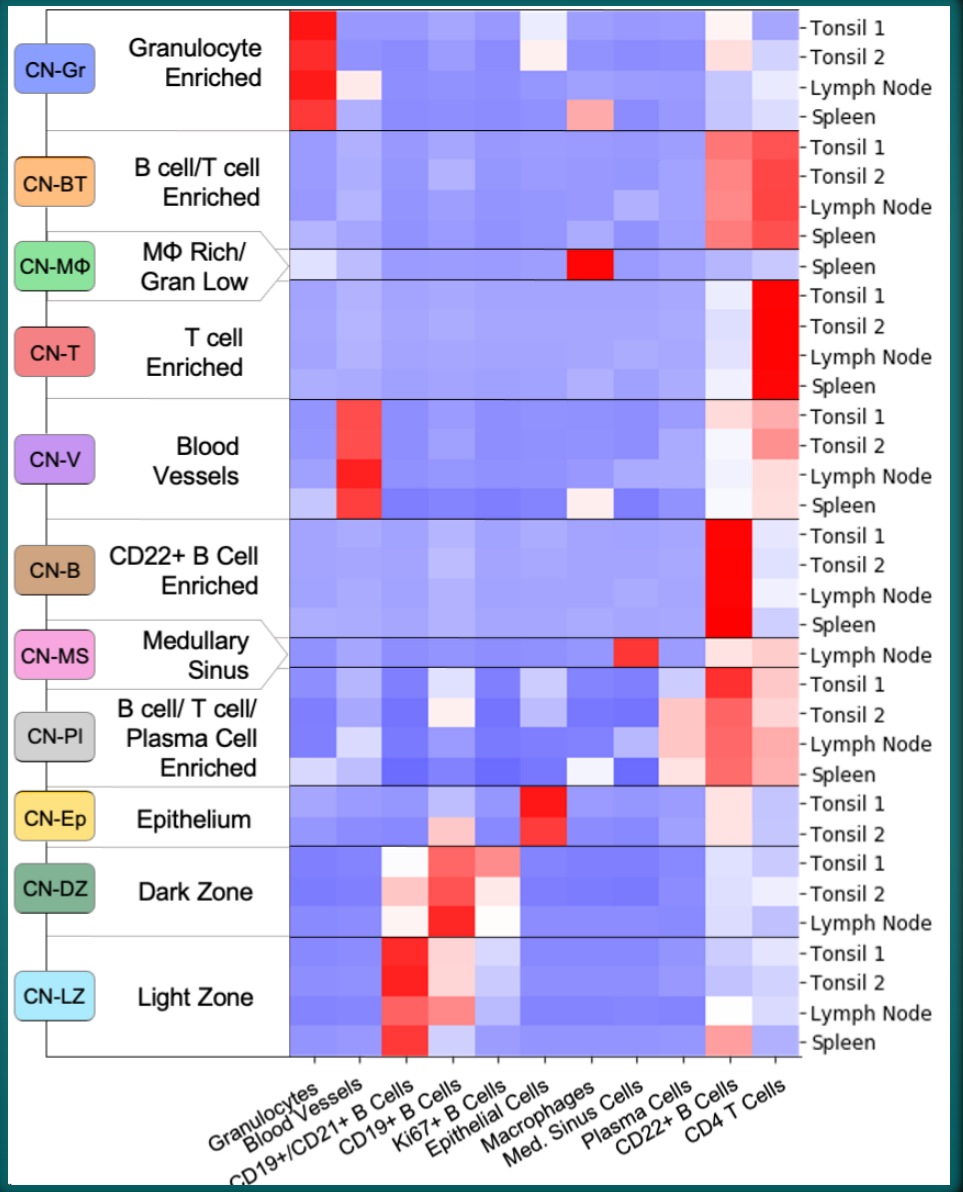
Goltsev Y, Samusik N, Kennedy-Darling J, Bhate S, Hale M, Vazquez G, Black S, **Nolan, GP**. *Cell* 2018

LYMPH NODES ARE SIMILAR•...BUT NOT ALL THE SAME





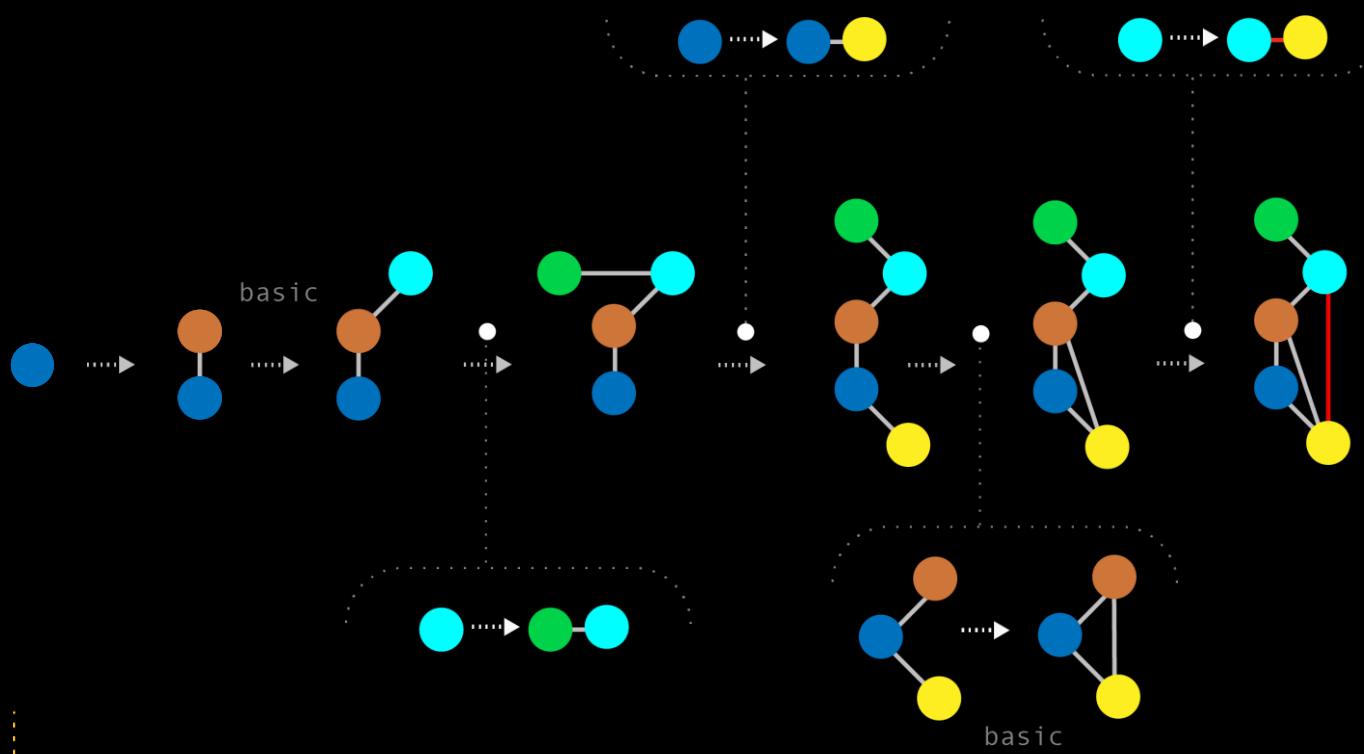
TONSIL 1



3 TYPES OF HLT

TISSUE SCHEMATICS • Strategy for Representing Assembly

with Salil Bhate, Nolan lab



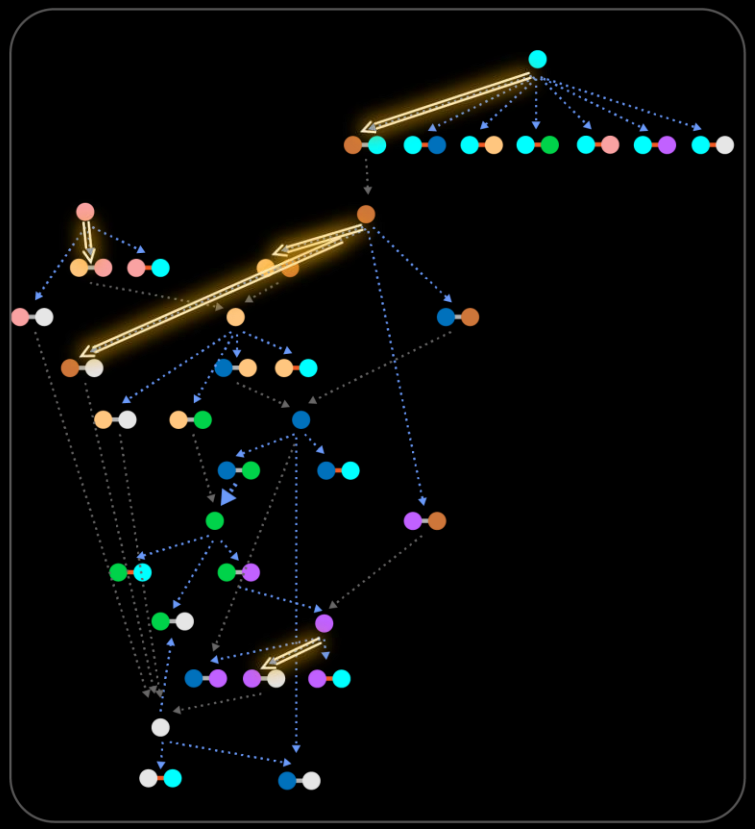
INTERPRETATION:

Rest of motif required for instance of blue CN to form constraints on 3D architecture (only have 2D images)

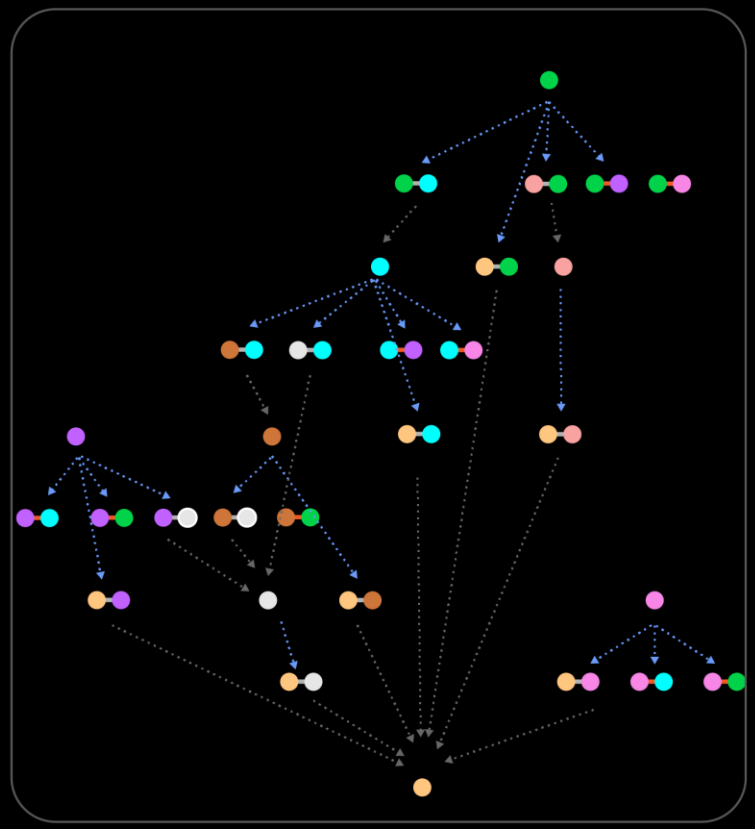
TISSUE SCHEMATICS • Strategy for Representing Assembly

with Salil Bhate, Nolan lab

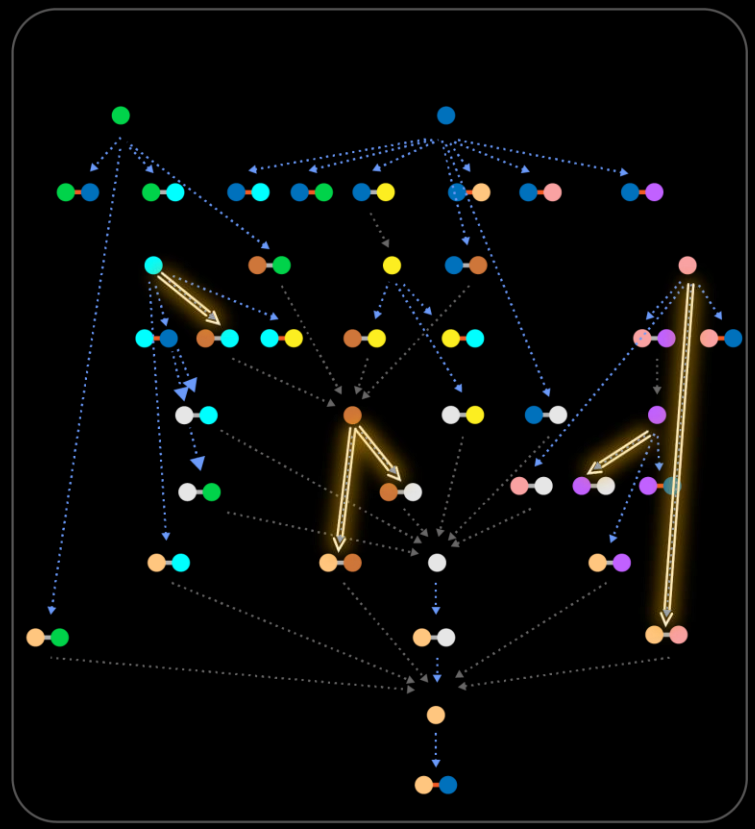
CN-Gr
CN-BT
CN-MΦ
CN-T
CN-V
CN-B
CN-MS
CN-Pl
CN-Ep
CN-DZ
CN-LZ



SPLEEN



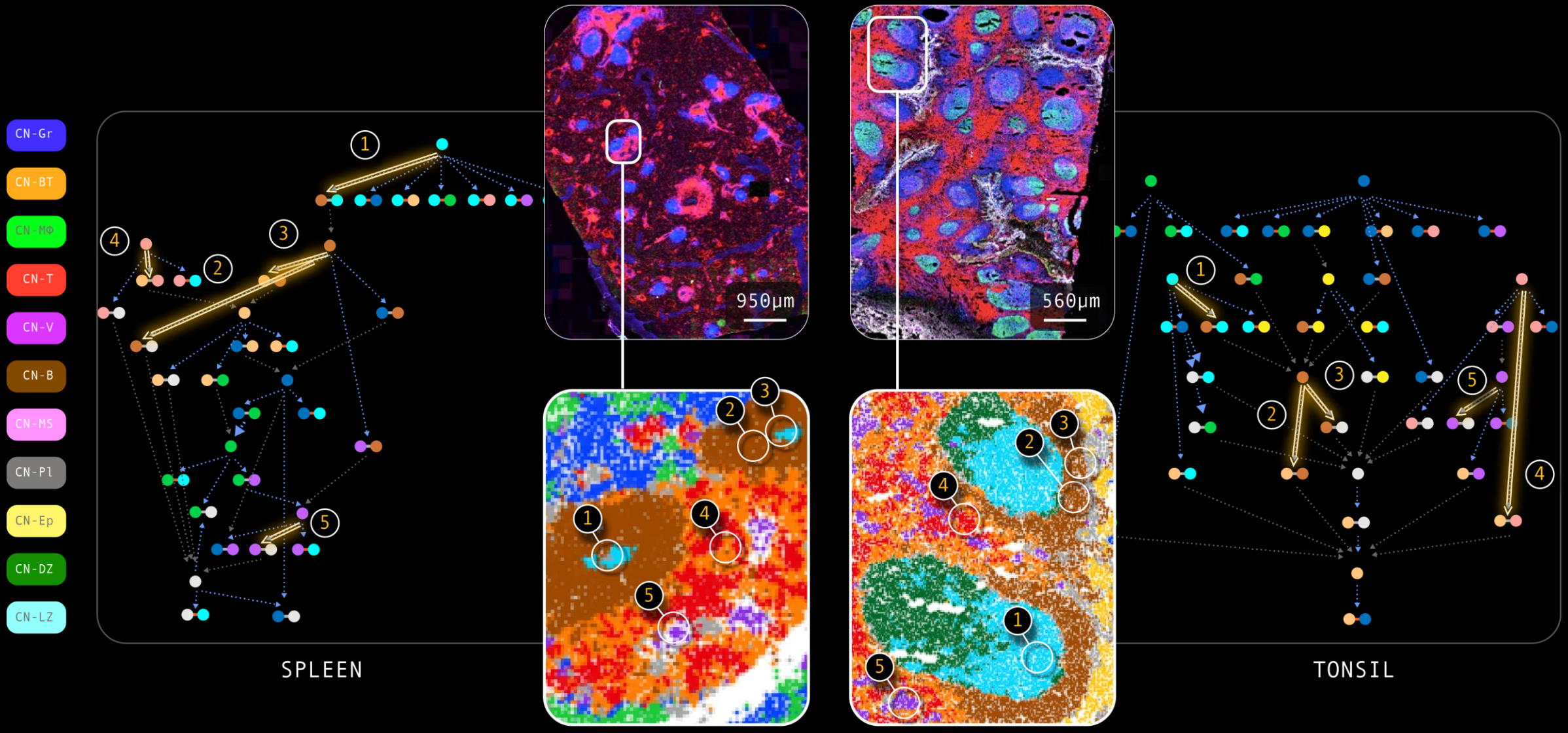
LYMPH NODE



TONSIL

TISSUE SCHEMATICS • Strategy for Representing Assembly

with Salil Bhate, Nolan lab



CASE STUDY • CRC Tumor Immune Microenvironment

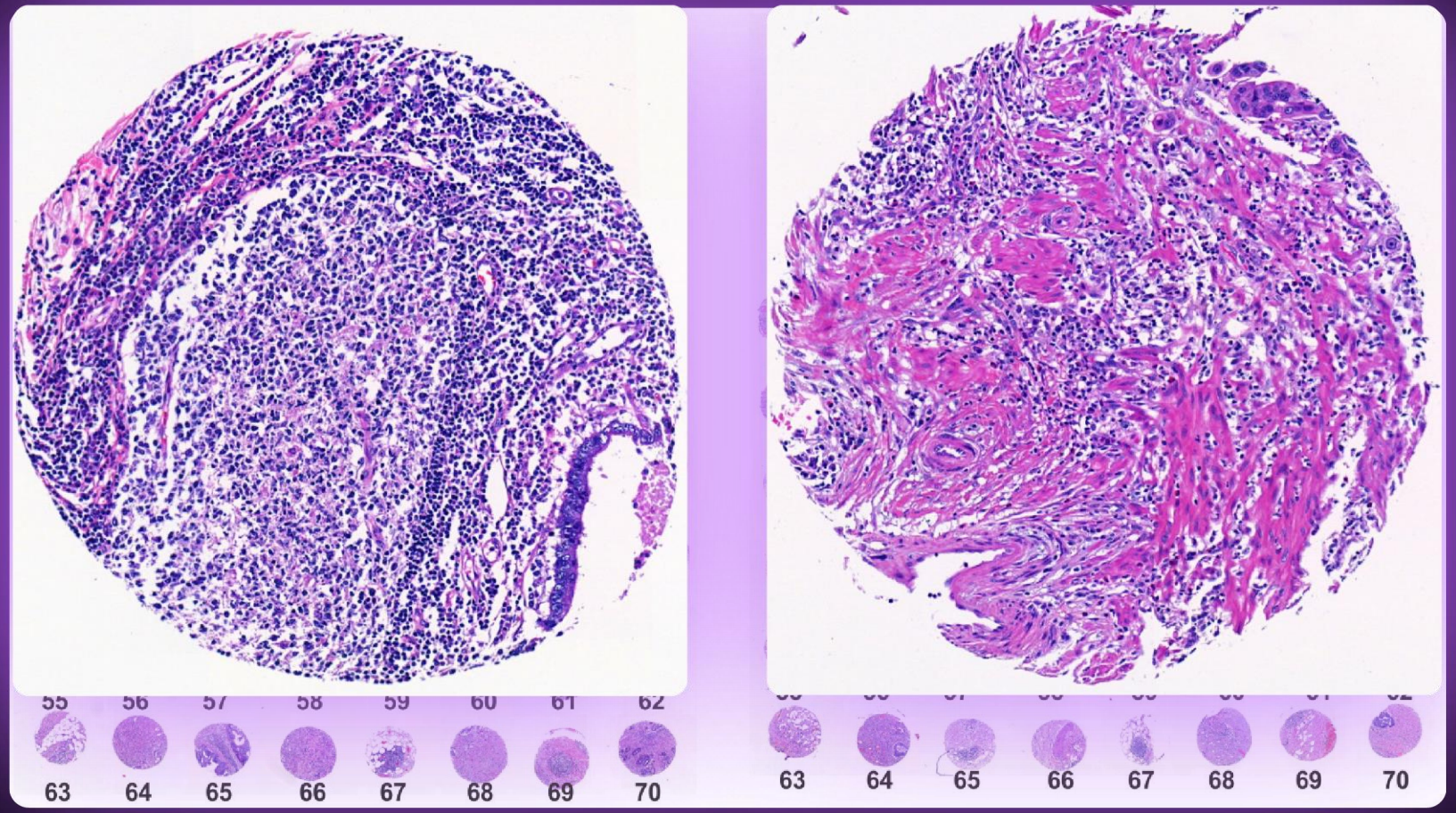
Christian Schuerch & Darci Phillips

750 CRC patients, all stages fully clinically annotated

250 stage III / IV cases

17 cases CLR only

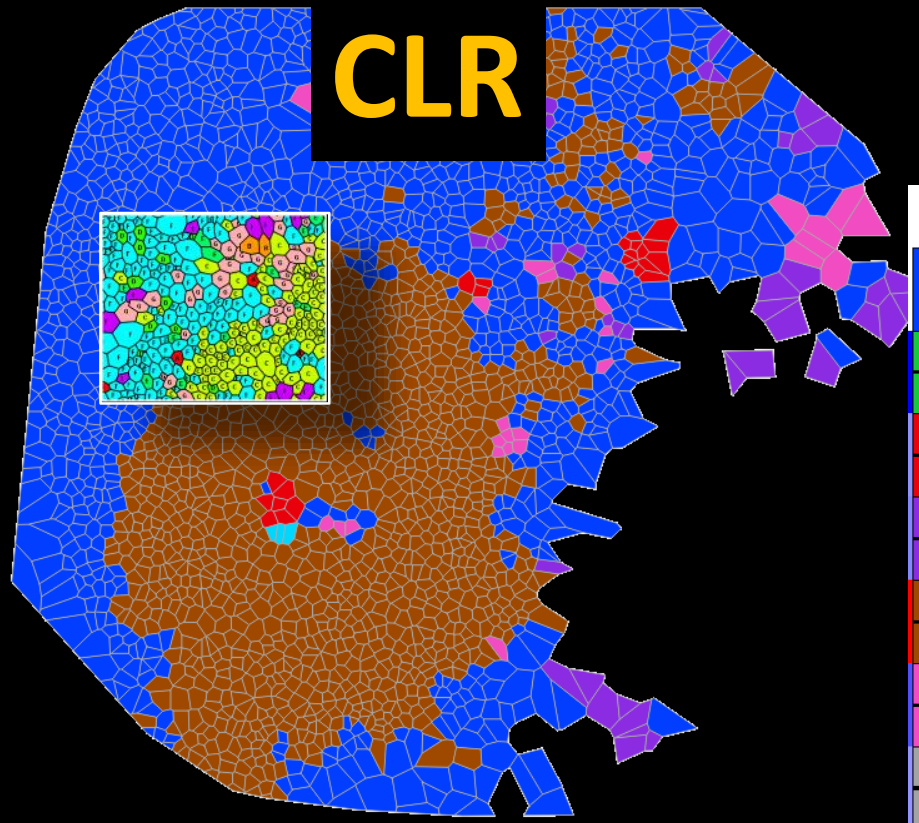
18 cases diffuse only

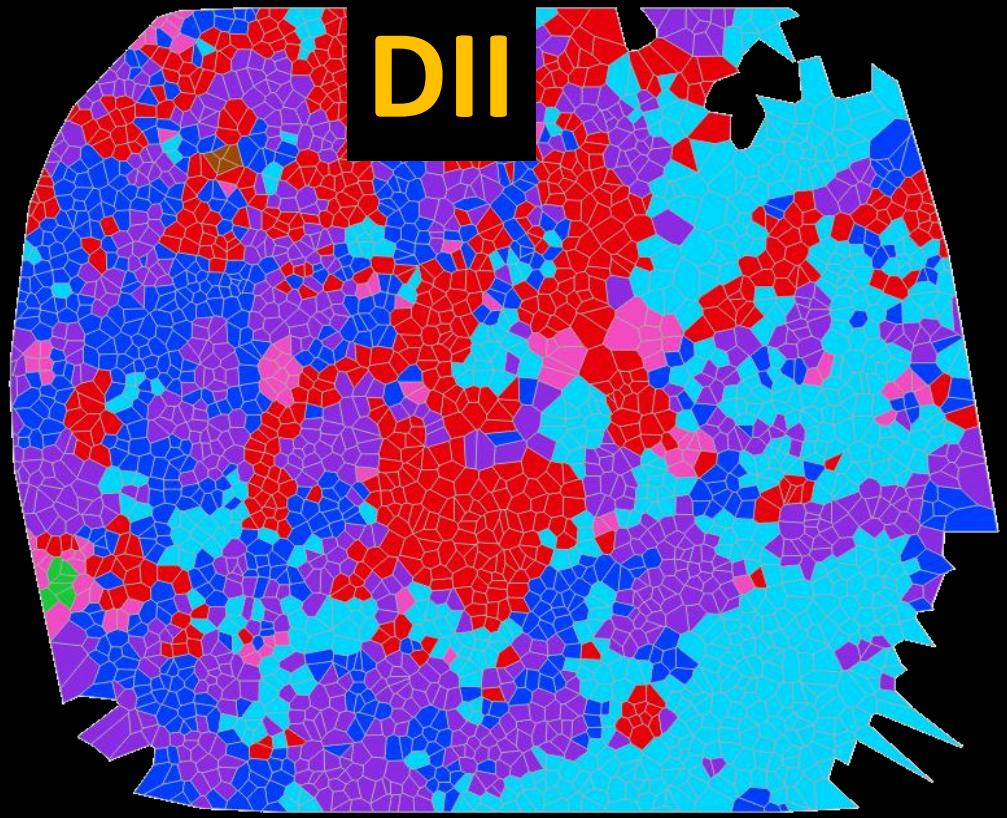


CRC • In DII, Immune Modules do no Aggregate/Coalesce

There are no significant differences in “types” of neighborhoods

But … The neighborhoods are smaller and more fragmented

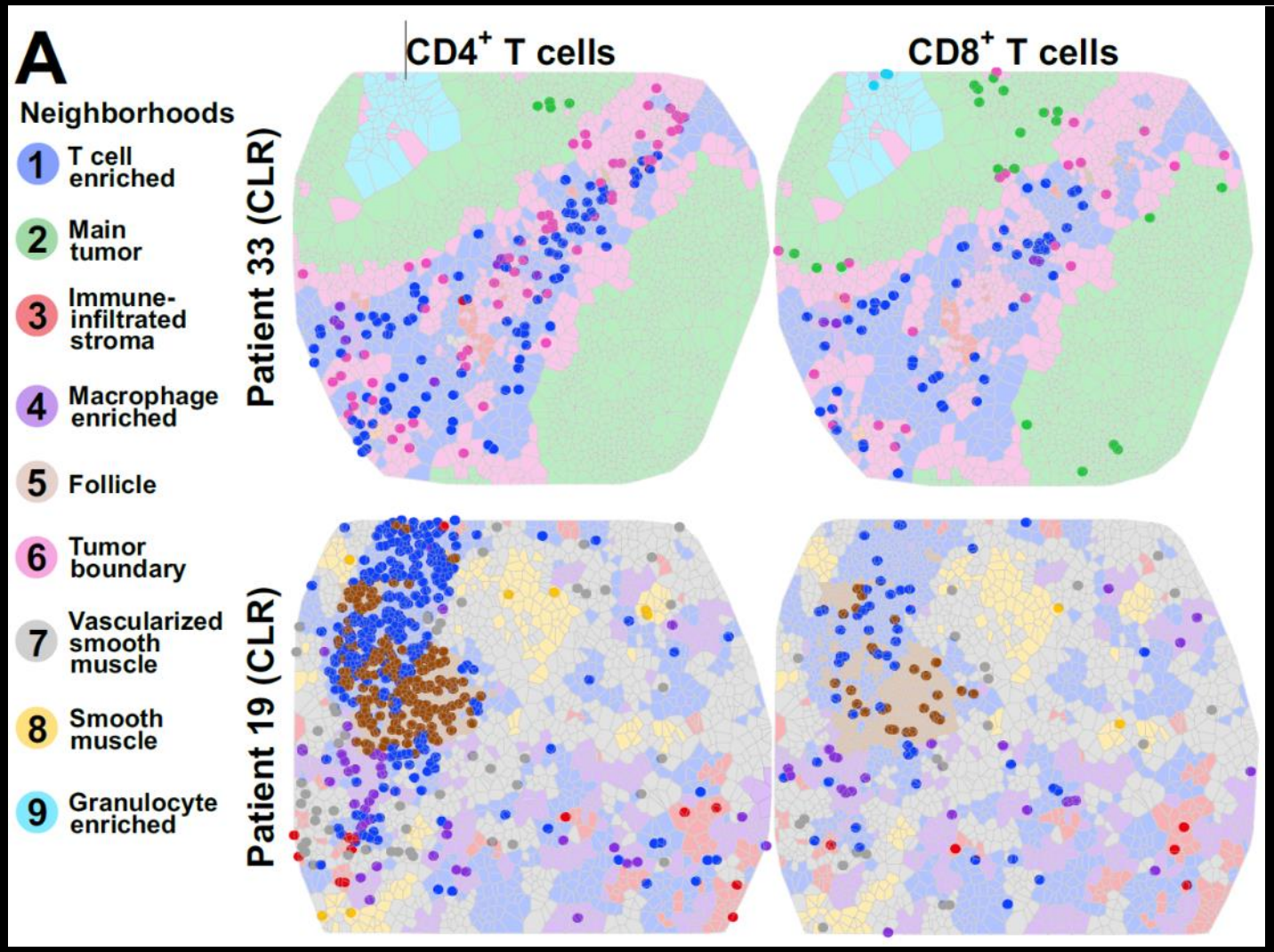


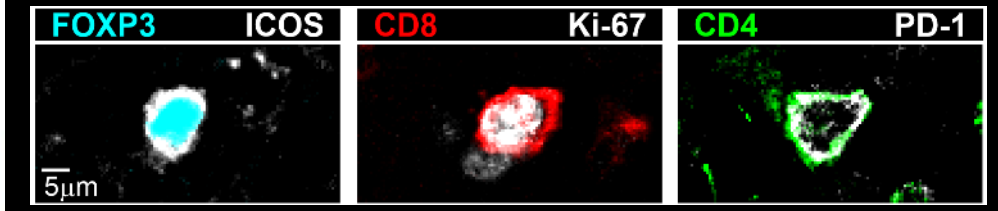


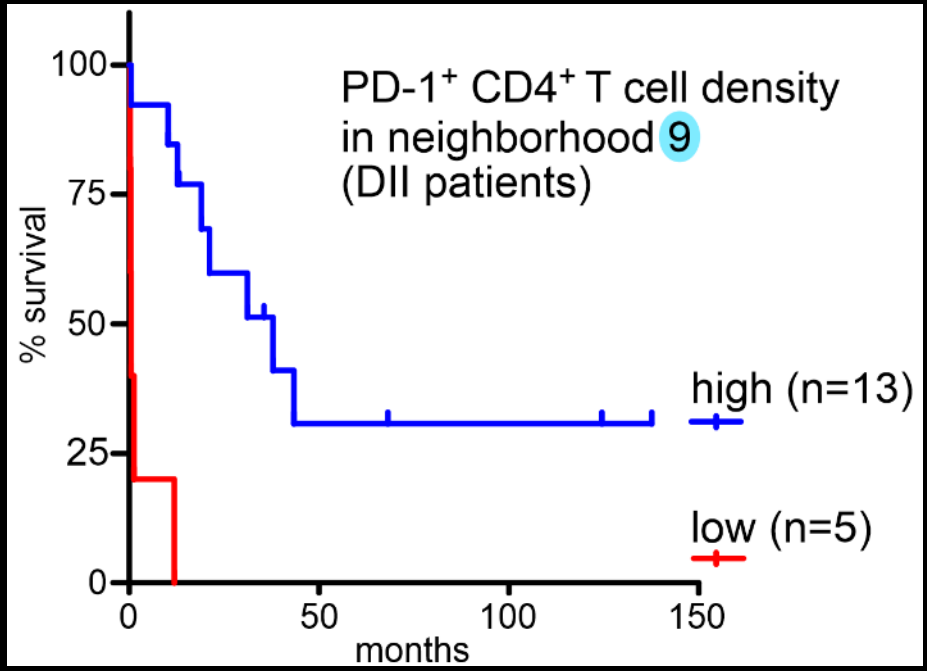
Neighborhood	
1	T cell enriched
2	Main tumor
3	Immune-infiltrated stroma
4	Macrophage enriched
5	Follicle
6	Tumor boundary
7	Vascularized smooth muscle
8	Smooth muscle
9	Granulocyte enriched

A Tregs	B CD11c$^+$ DCs	C B cells	D CD8$^+$ T cells	E Other immune cells
F CD4$^+$ T cells	G Tumor & stromal cells	H NK cells	I Macrophages	

Cell Type Prognostic Value in Neighborhoods

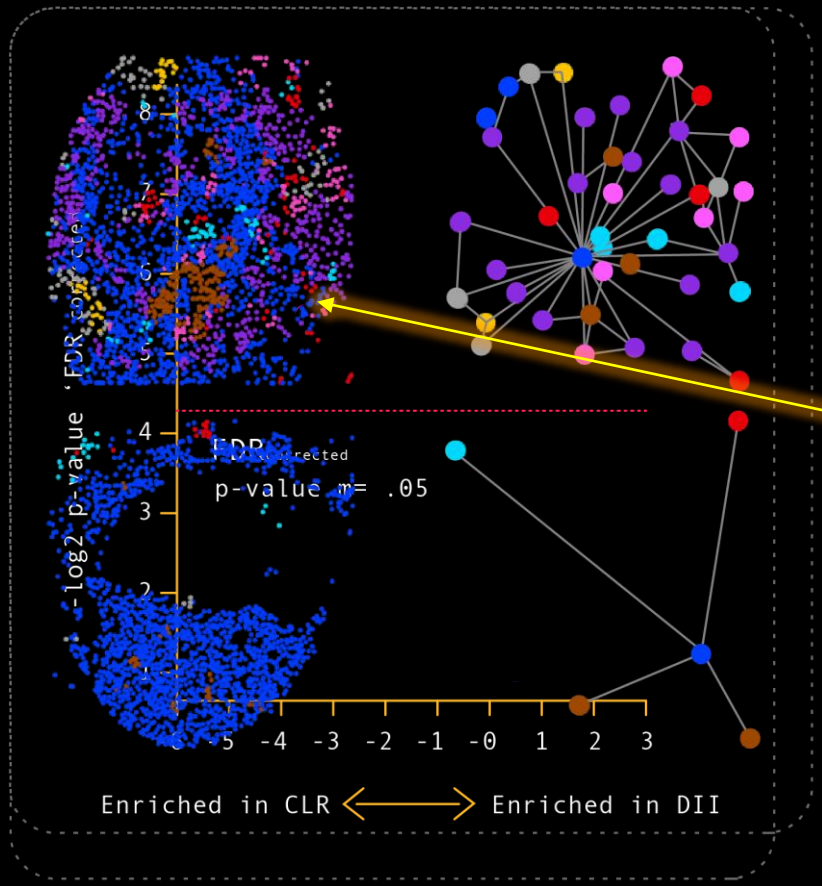


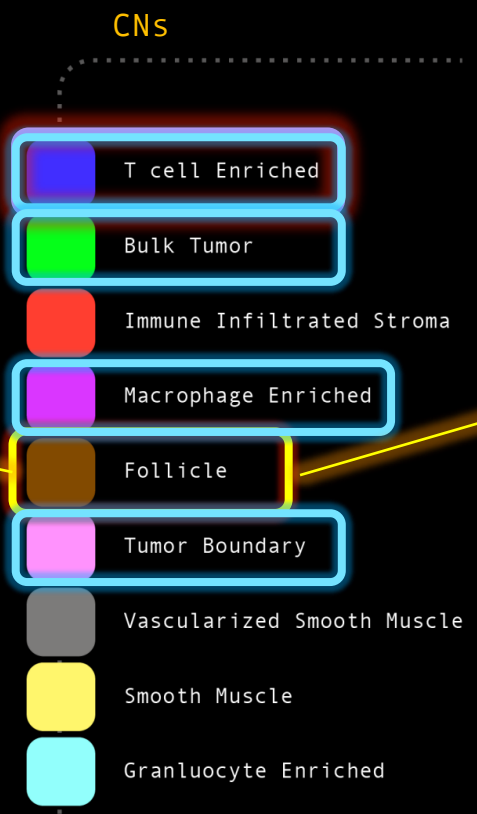


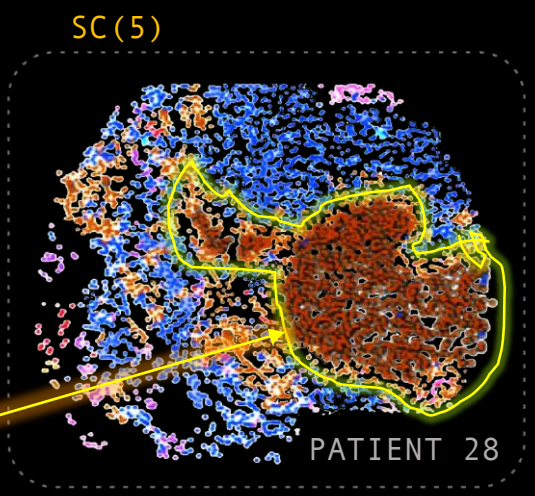


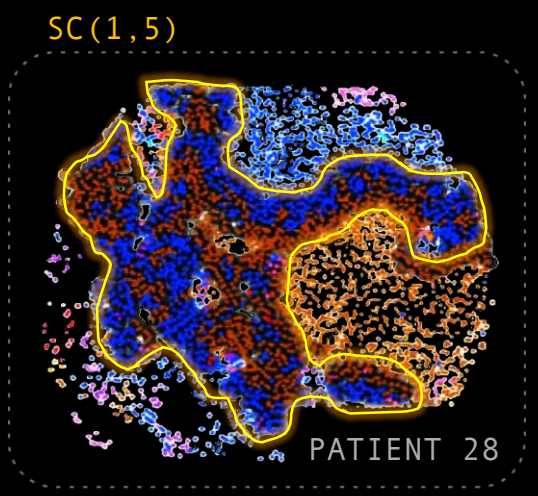
NEIGHBORHOOD ADJACENCIES ARE REFLECTIVE OF DISEASE STATE

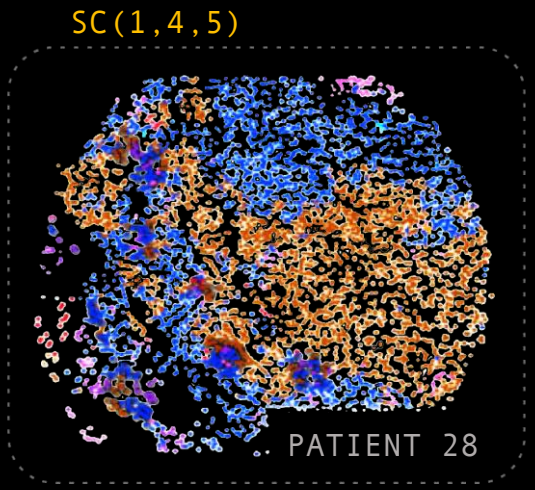
Salil Bhate & Graham Barlow

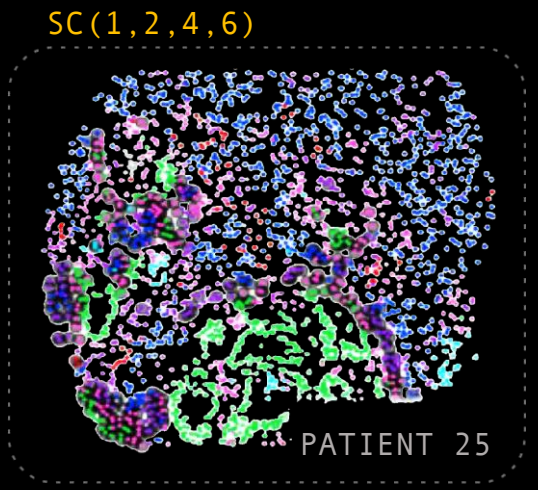




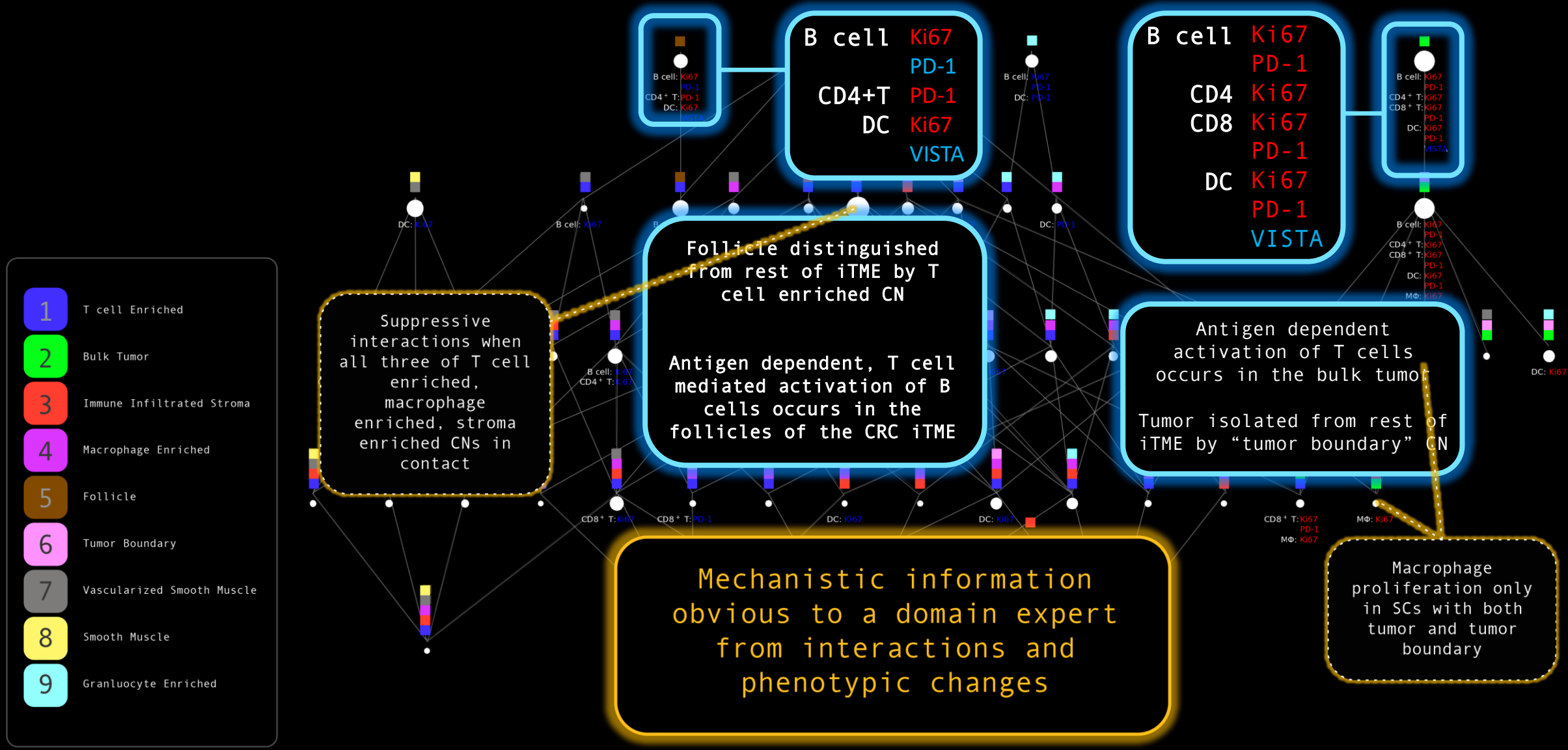




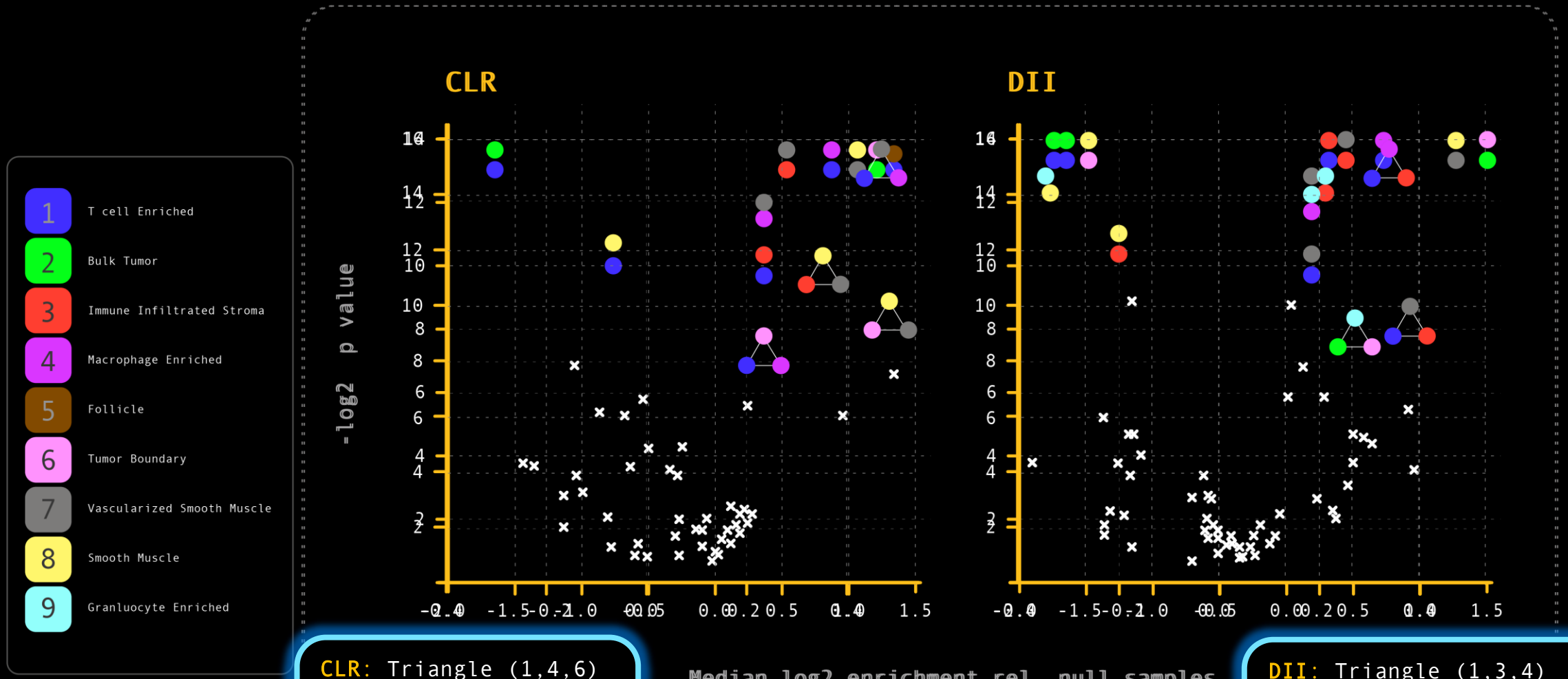




Neighborhoods, SCs and mechanism



CNs w/ certain SCs predict disease state



CLR: Triangle (1,4,6)

- T cell enriched,
- macrophage enriched,
- tumor boundary

DII: Triangle (1,3,4)

- T cell enriched,
- immune infiltrated stroma
- macrophage enriched

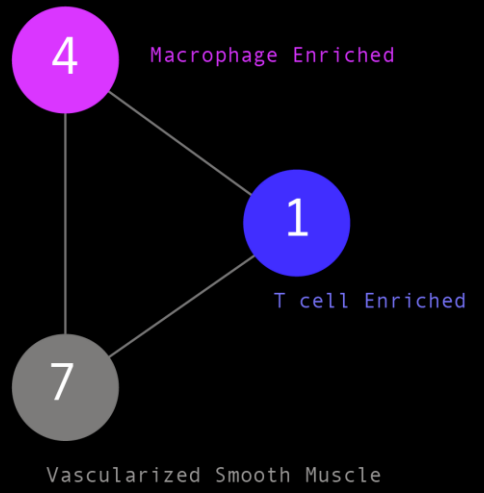
DII PATIENTS • Tumor Appropriates Higher-Order Tissue Motif

Bhate*, Barlow* et al., accepted

CNs

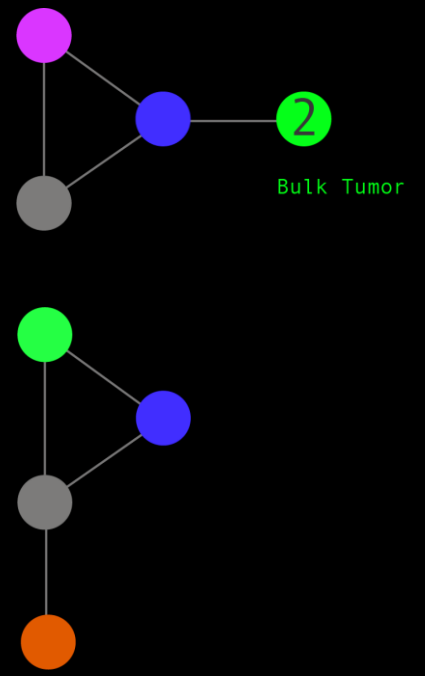
- T cell Enriched
- Bulk Tumor
- Immune Infiltrated Stroma
- Macrophage Enriched
- Follicle
- Tumor Boundary
- Vascularized Smooth Muscle
- Smooth Muscle
- Granluocyte Enriched

T cell & Macrophage Enriched, Vasculature Triangle



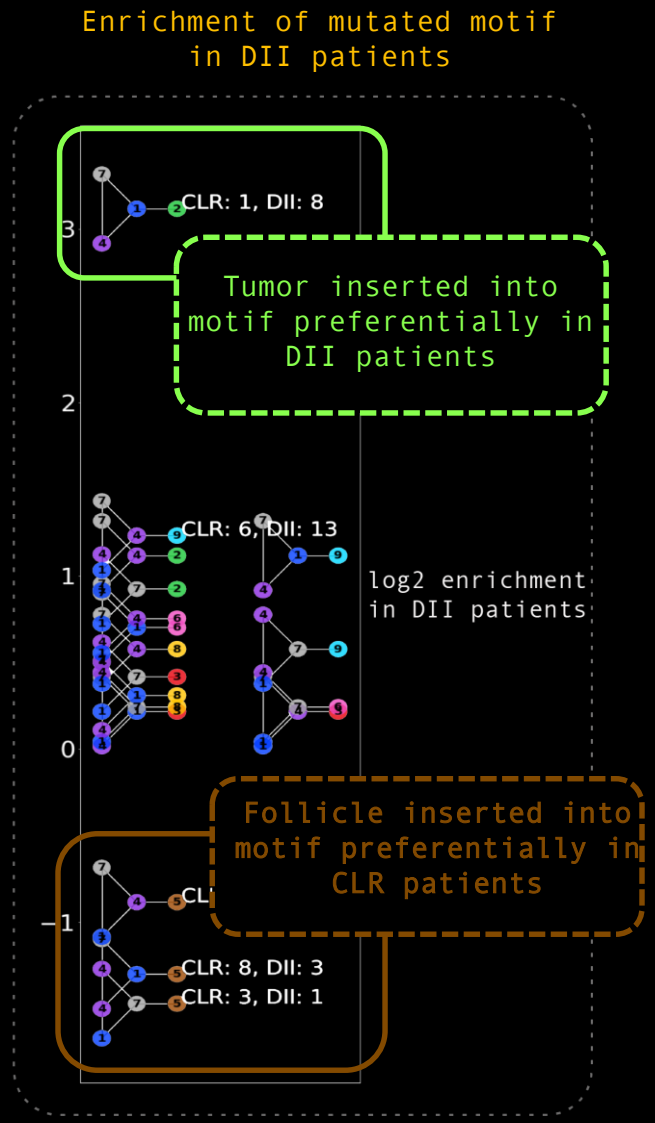
PRESENT IN ALL PATIENTS

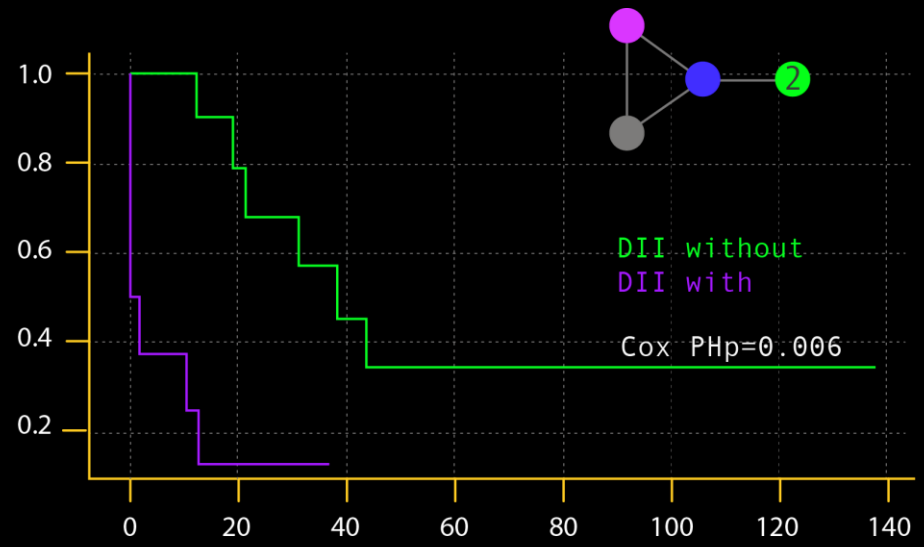
Mutations by Insertions to Triangle



Cell Type Prognostic Value in Neighborhoods

Salil Bhate & Graham Barlow

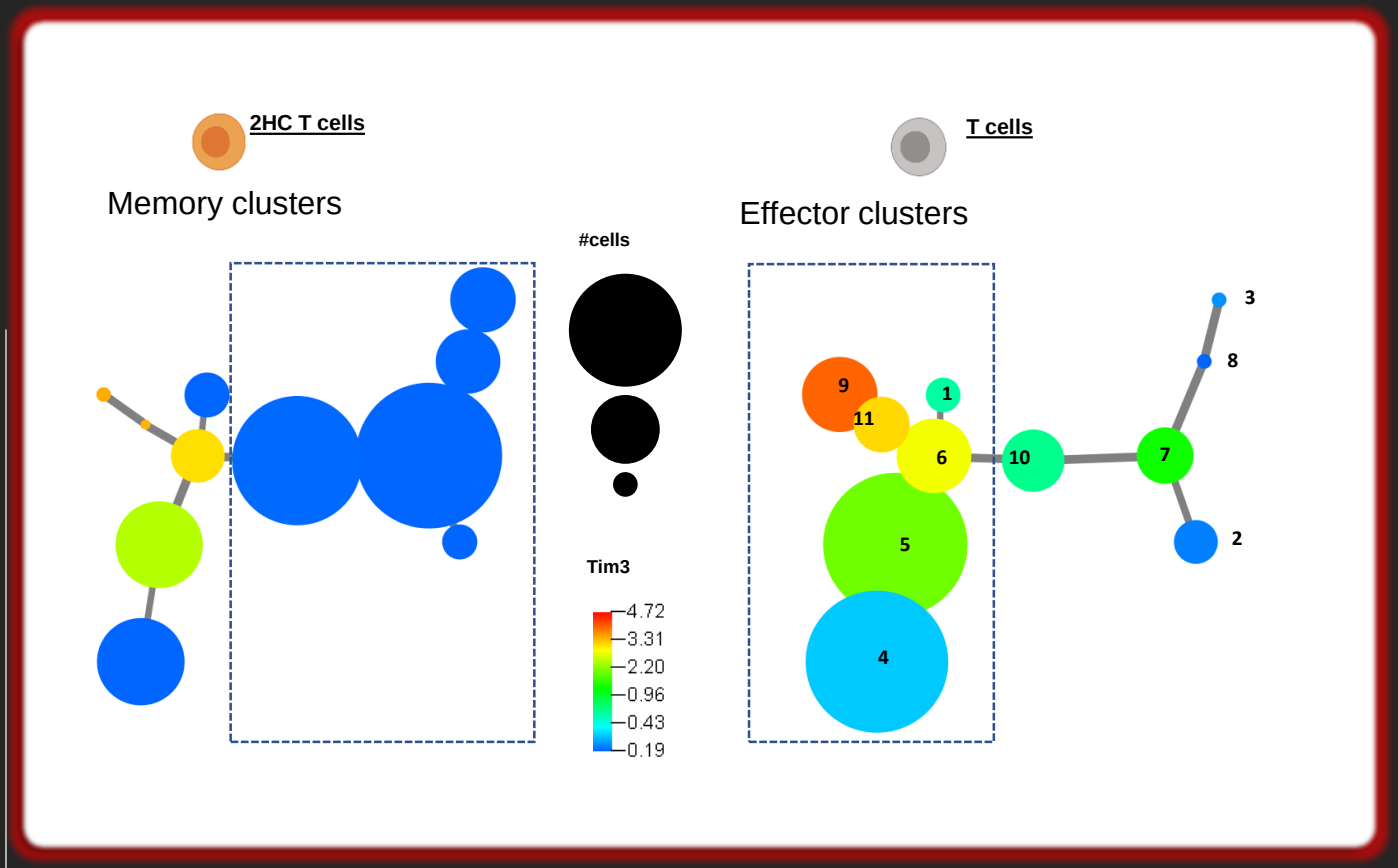


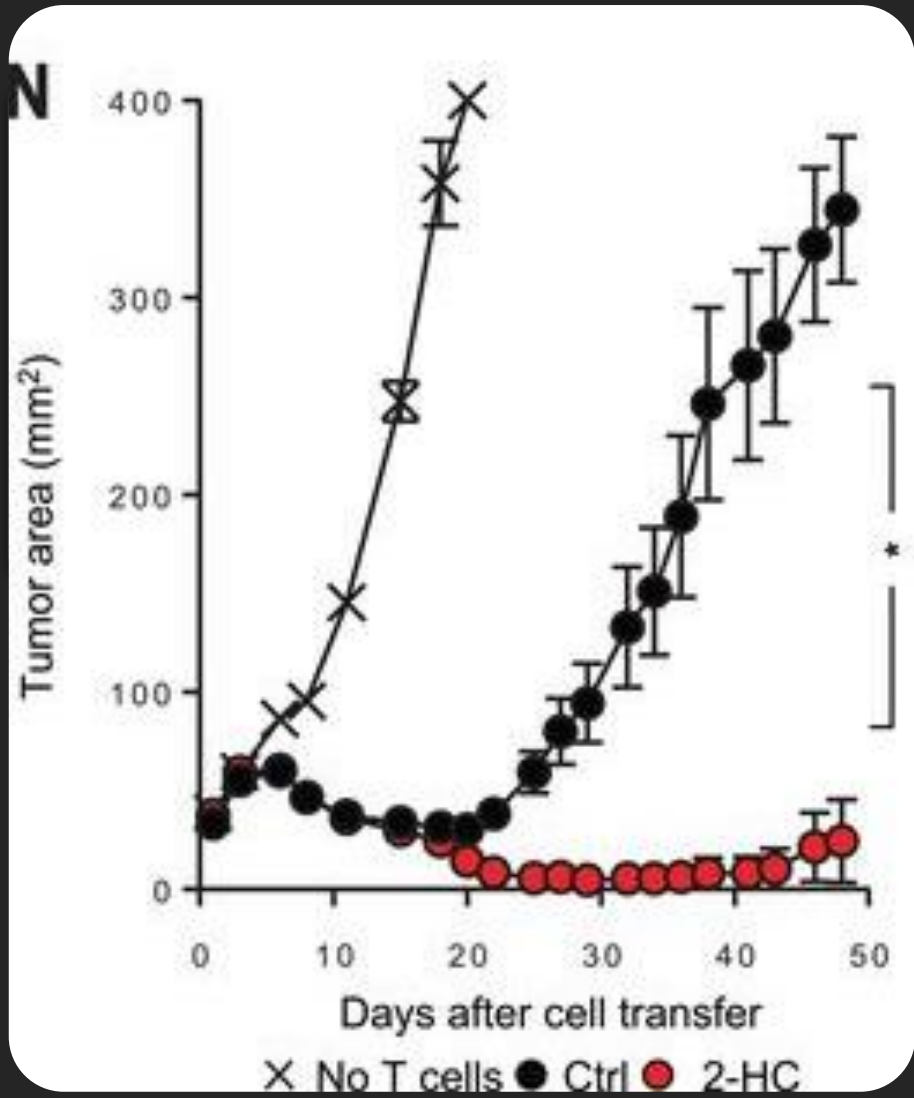


VARIABLE	COEF	LOWER 95%	UPPER 95%	P-VALUE
PD-1+, CD4+, T cell frequency in cn9	-0.55	-0.93	-0.18	0.004
Has cn2-Triangle (1,4,7) Motif	2.09	0.64	3.55	0.005

JUMP

Making “Super” CD8+ T Cells by Controlling Metabolism

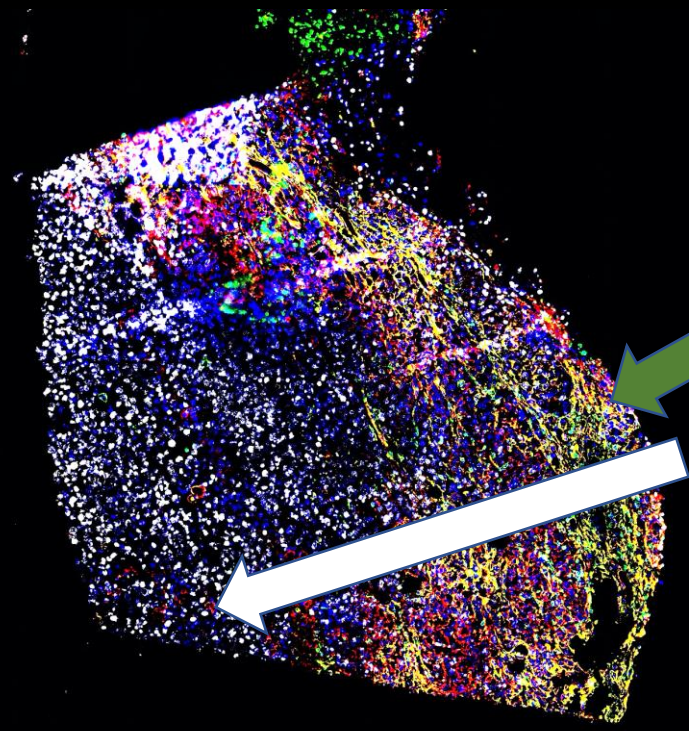




(Vodnala, *Science*, 2019)

CODEX Imaging of Tumors (day 3 Post-treatment)

Canonically Activated T cells



Hoechst -- Tumor

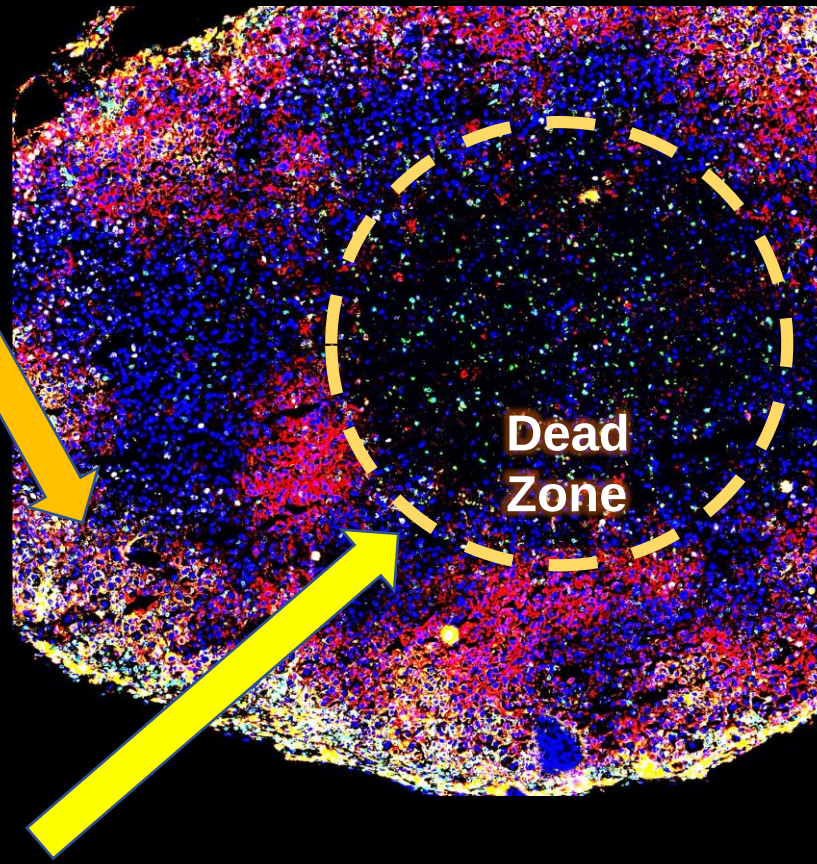
PDL1 -- Inflamed tumor

H2Kb -- Inflamed tumor

CD45 -- Immune cells

Ki67 -- Dividing cells

Metabolically Transformed T cells



How did this happen??

Inflammatory, Anti-Tumor Programs

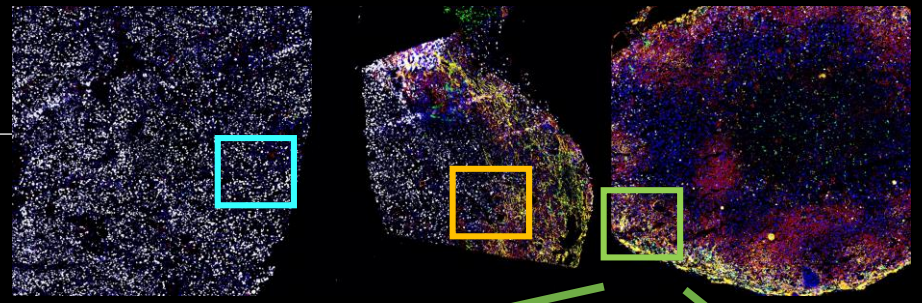
Hoechst - Nuclear

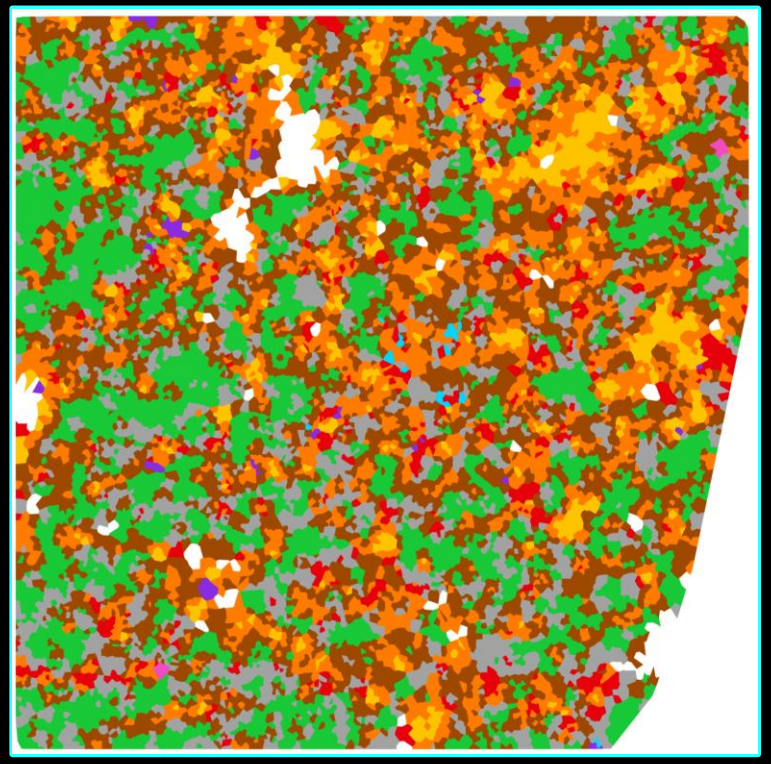
CD45 = Immune

Ki67 = Proliferation

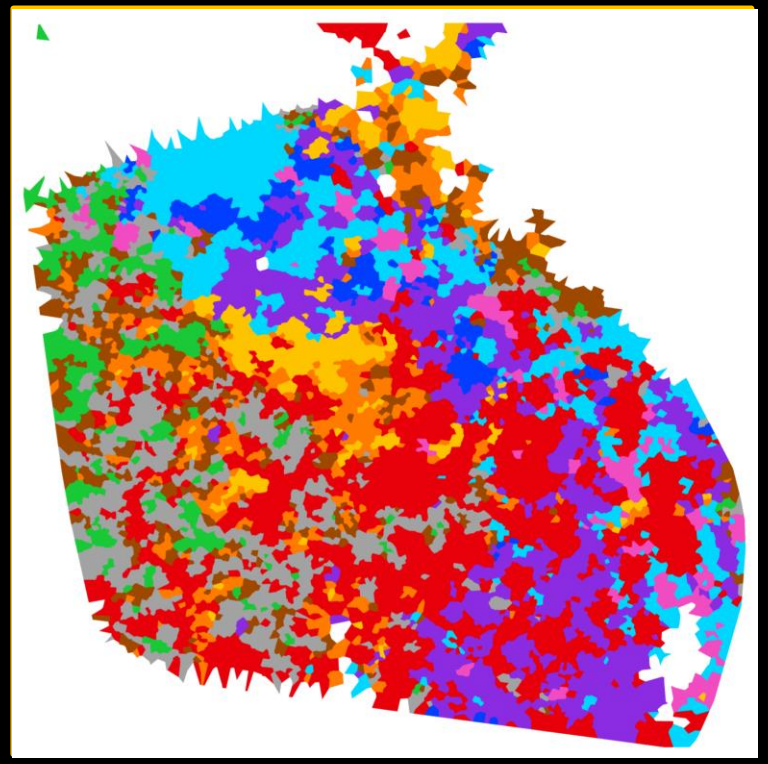
H2Kb = Antigen Presentation

PDL1 = Immune inhibitory

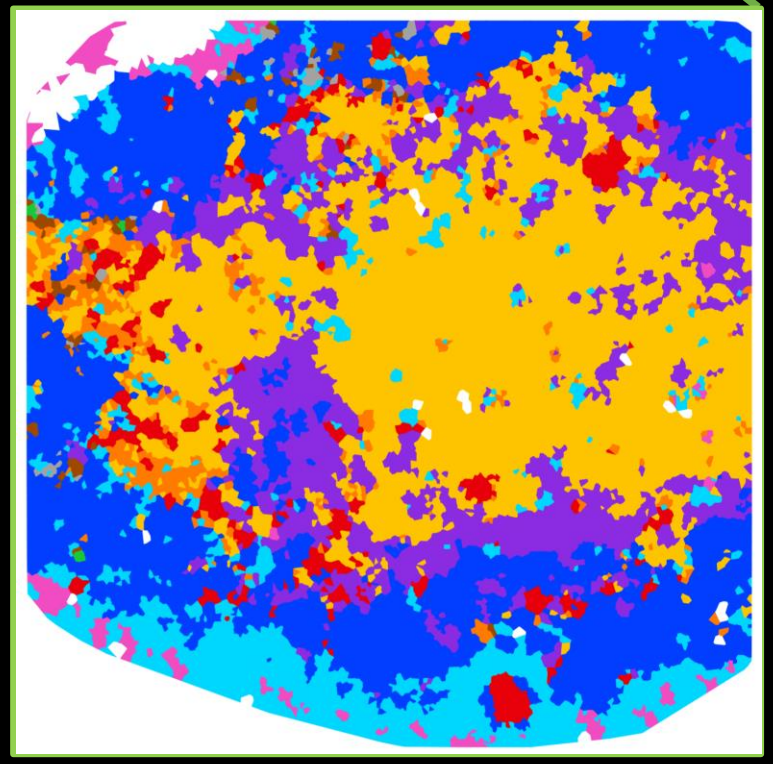




No T cells



Canonical Activated T cells



Metabolically Transformed T cells

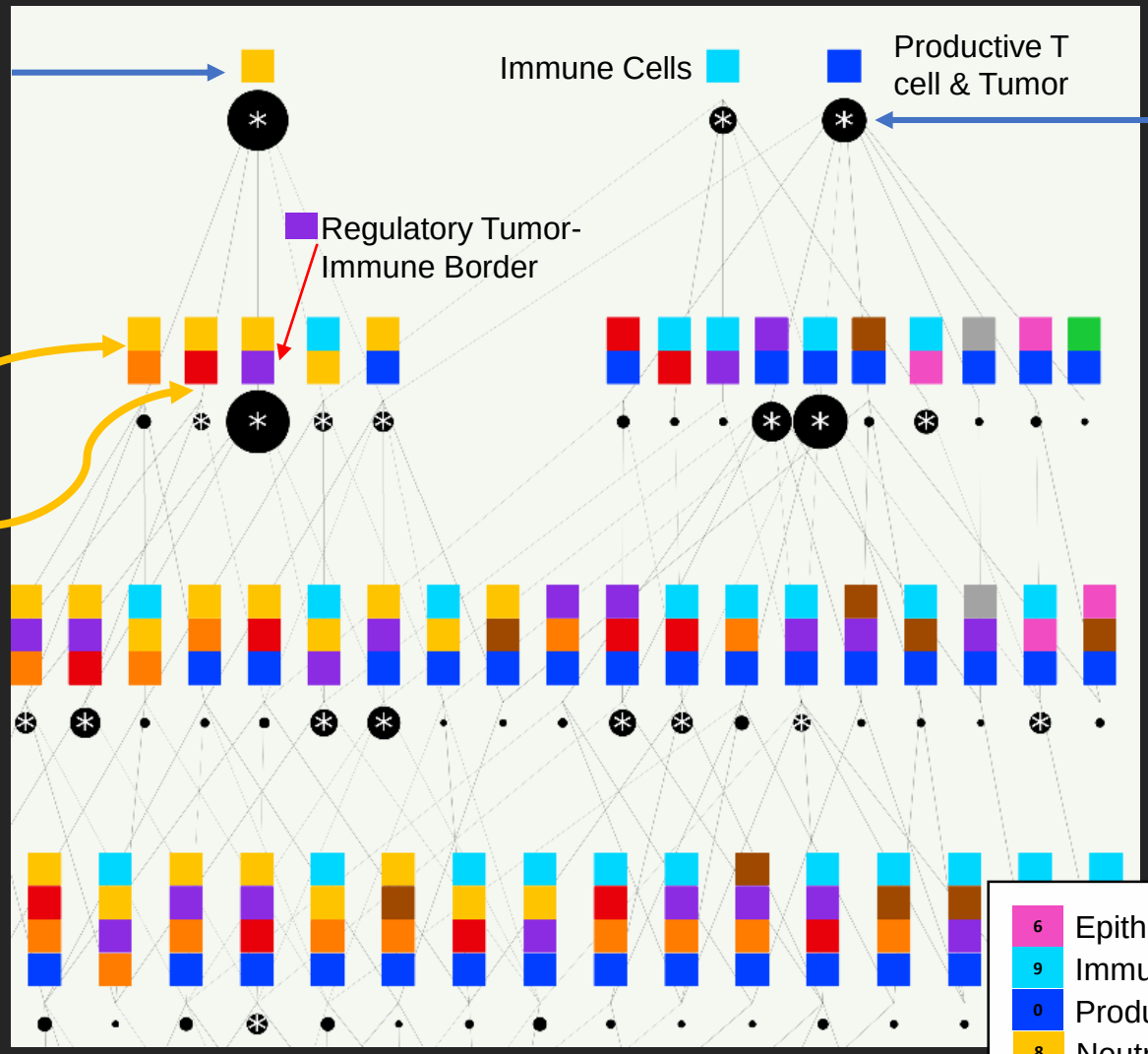
Spatial Context Map Analysis: Inter-neighborhood interactions

Neutrophil/Tumor Neighborhood

Neutrophil/Tumor found with:

Tumor

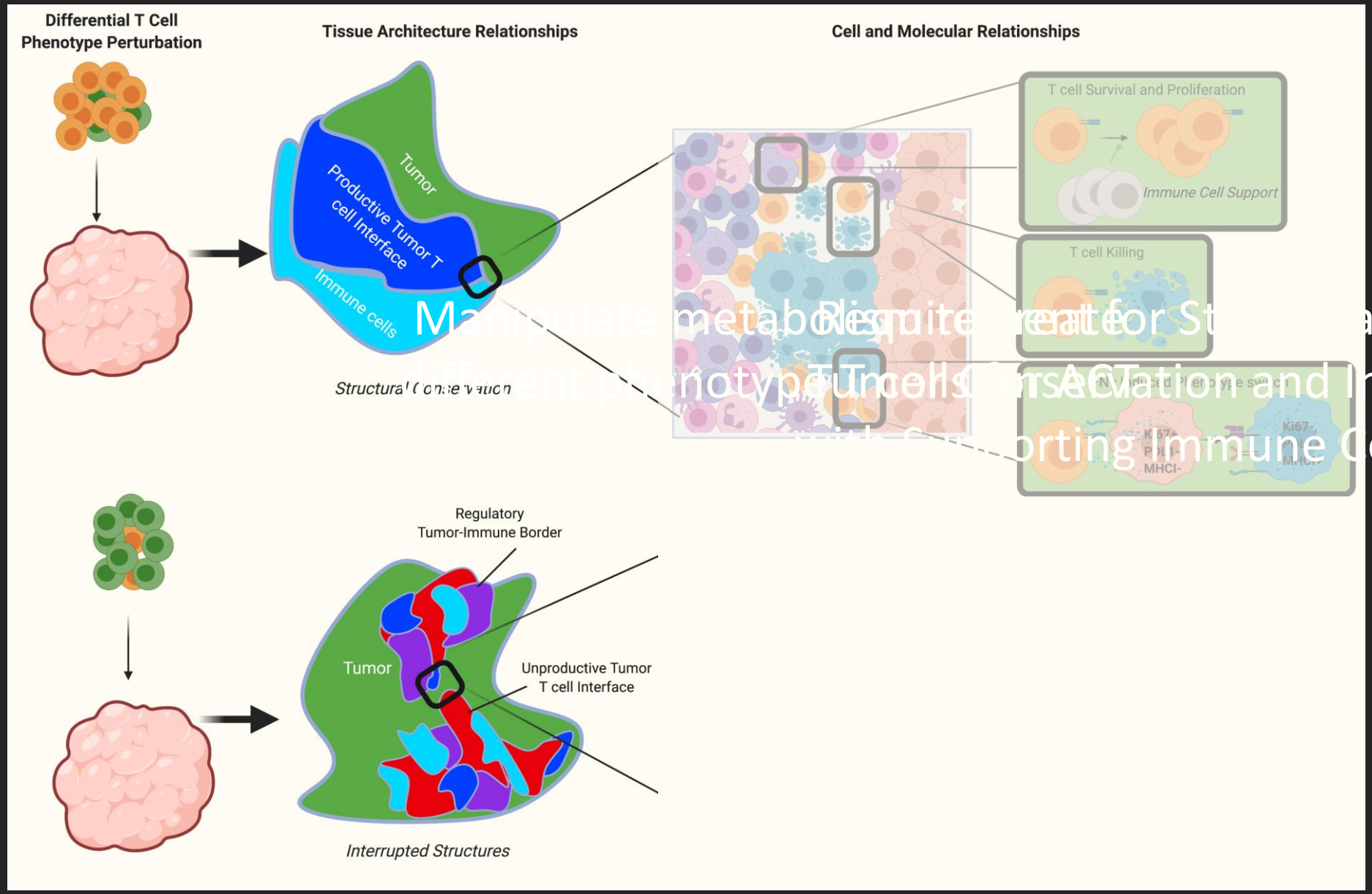
Unproductive T cell & Tumor



Relative Frequency

6 Epithelial
9 Immune Cells
0 Productive T cell & Tumor
8 Neutrophils & Tumor
2 Ki67+ Tumor
1 Tumor Stem Cell
5 Proliferating Tumor Edge
7 Mixed Tumor Phenotype
3 Unproductive T cell & Tumor
4 Regulatory Tumor-Immune Border

Critical Intercellular and Molecular Relationships



Manipulate metabolic requirements for Structural T cell-different phenotypes to Influence Activation and Interface with Supporting Immune Cell Zone

Tissue schematics can teach us about rules of interaction

The boundary space can inform "where the action is"

An expert observer can infer mechanistic consequences of these interaction zones

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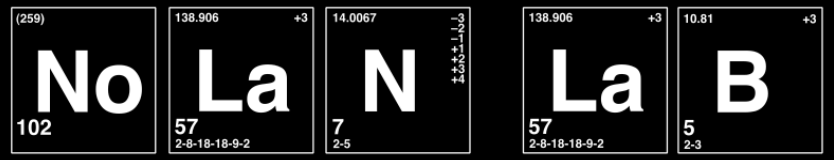
By stopping or enabling their development these are new therapeutic targets (small molecule/Abs)

SC-RNASeq data can be 'transferred' to CODEX to infer mechanism and suggest therapeutics

ACKNOWLEDGEMENTS •



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