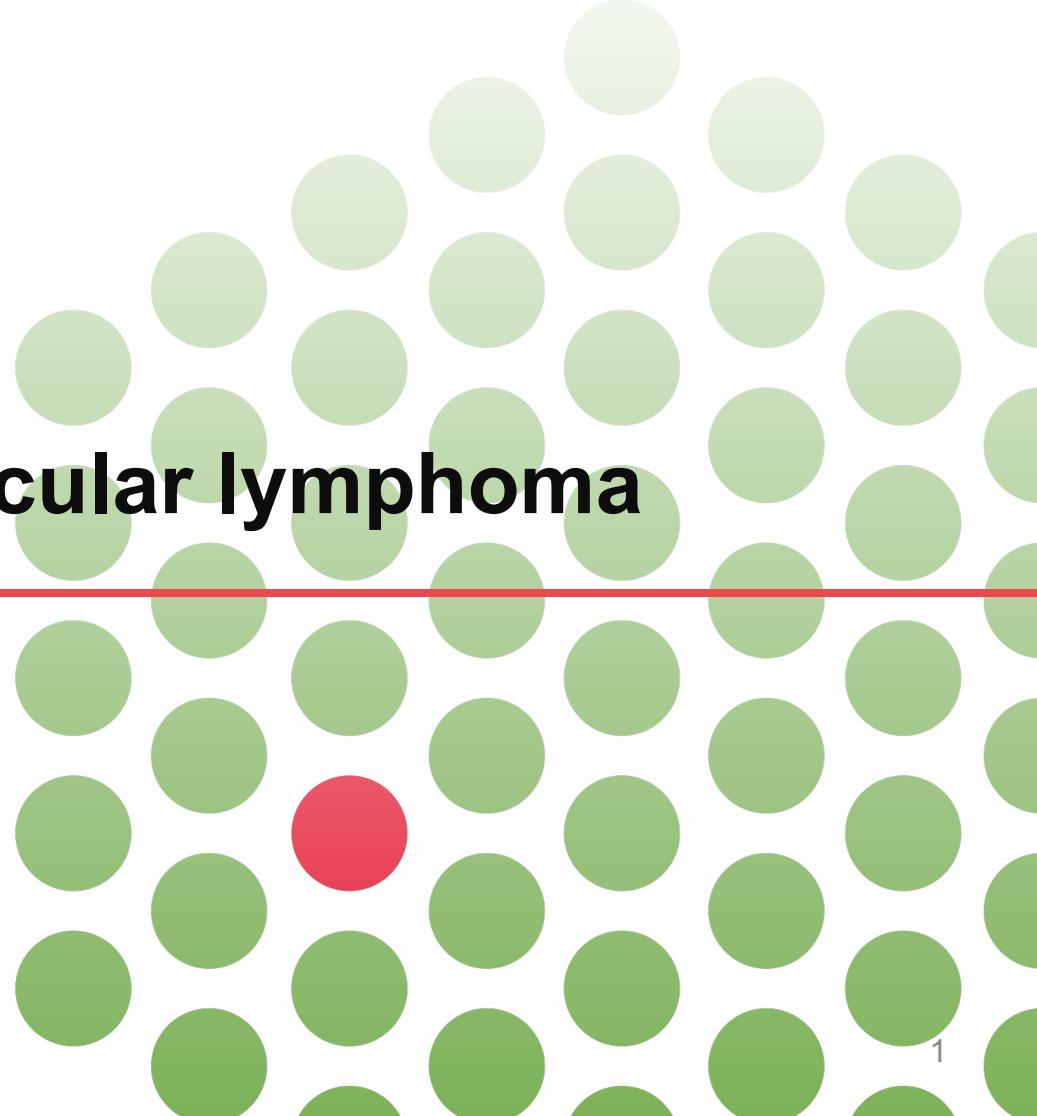


Epigenetic changes underlying histologic transformation of follicular lymphoma

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Saka / Zaugg Groups
Apr. 10, 2024



Follicular Lymphoma (FL)

- Histologically resembles B cell follicles in the lymph node
- 20-25% of B cell lymphomas
- Slow, indolent behavior
- Advanced stages incurable

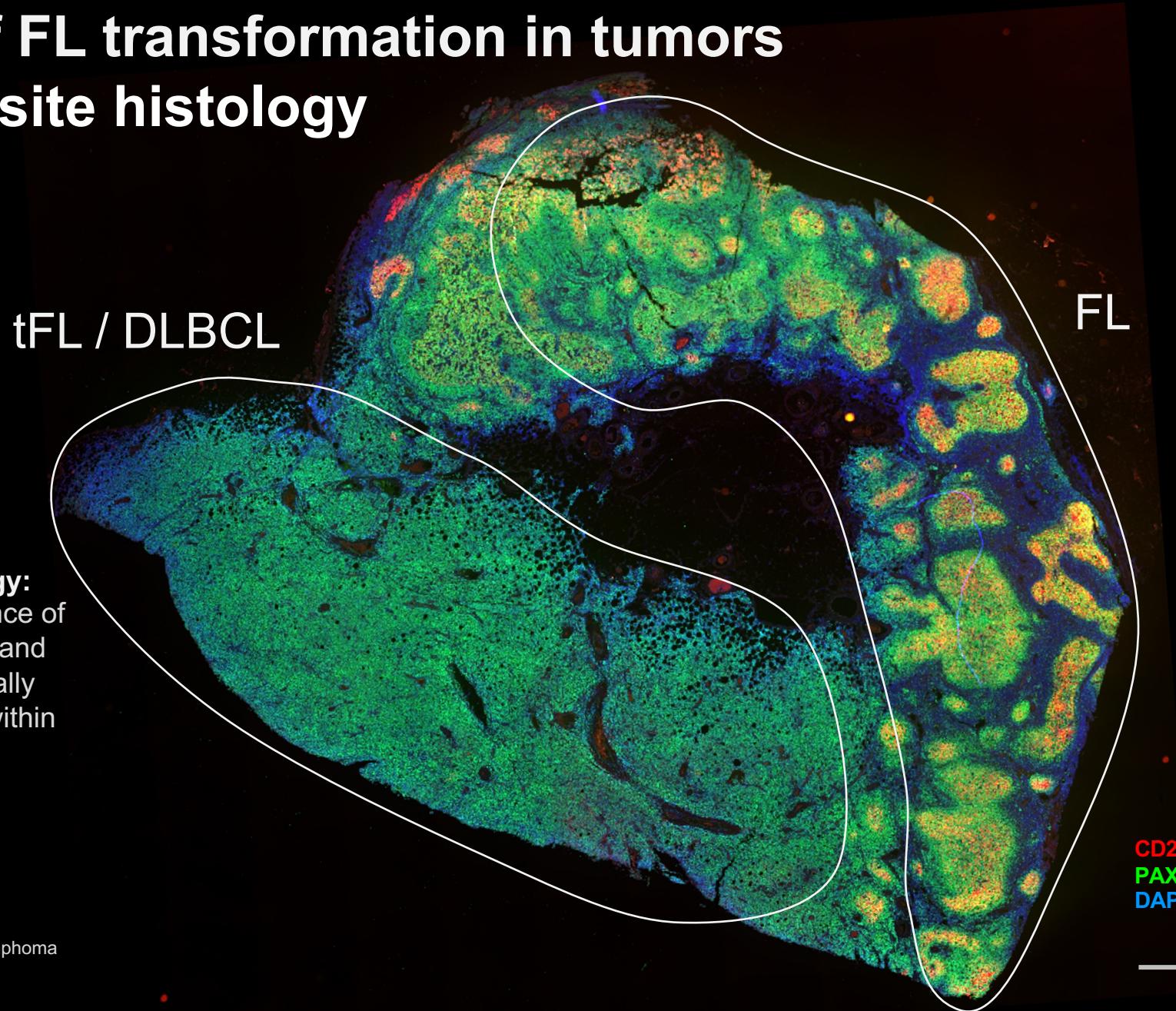
FL Transformation

- 
- Occurs in 10-60% of FL cases
 - Leads to poor clinical outcome
 - Mechanism is not understood

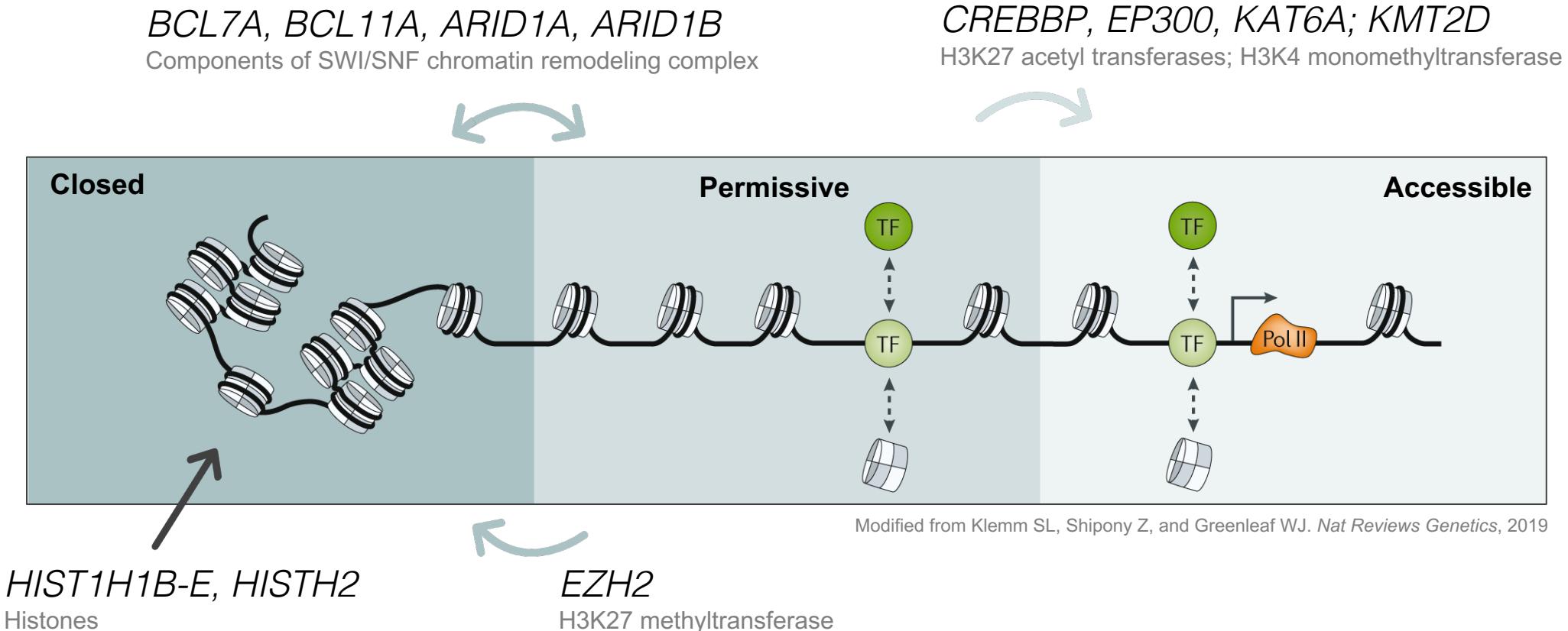
Diffuse Large B Cell Lymphoma (DLBCL)

- Follicular organization ablated
- 30-35% of B cell lymphomas
- Aggressive behavior
- 20-40% of patients relapse or do not respond to standard treatment

Evidence of FL transformation in tumors with composite histology



90% of FL cases have mutations in epigenetic machinery genes



Hypothesis

Cell-Intrinsic Factors

Genetic mutations in epigenetic machinery



Altered chromatin permissibility



Changes in transcription factor motif accessibility

Single cell profiling methods

Cell-Extrinsic Factors

Cell-cell signaling in tumor microenvironment



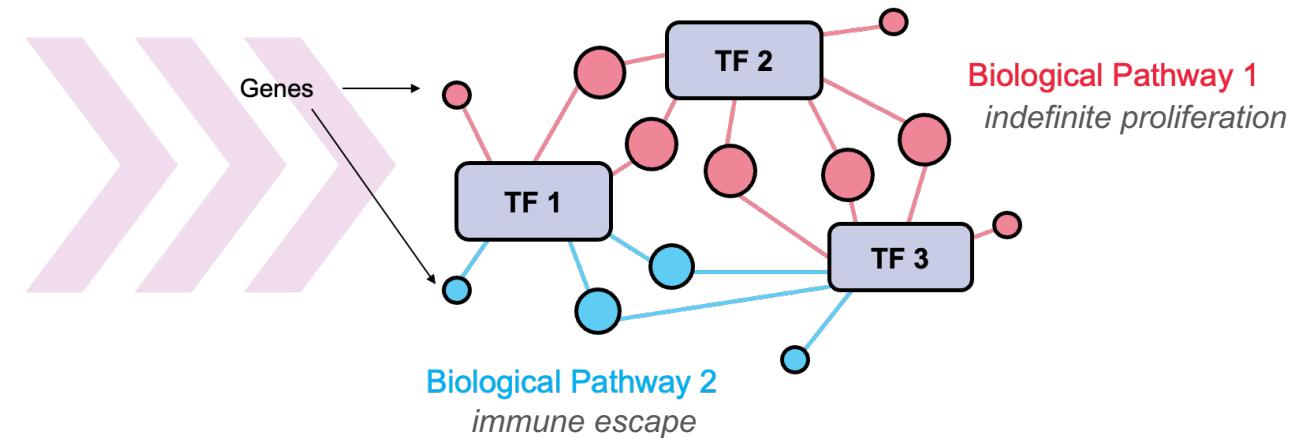
Signal cascade through surface receptors



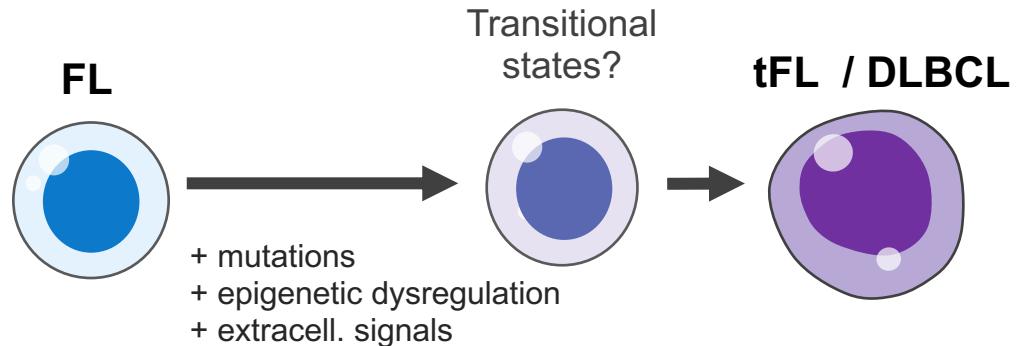
Activation of downstream transcription factors

Spatially resolved techniques

Upregulation regulatory networks promoting transformation



Project Aims



- Resolve cellular states underlying tumor cell remodeling along the FL – tFL axis
- Link regulatory networks to cell-cell interactions within compositionally distinct tumor niches
- Predict perturbable pathways which may (1) interfere with transformation or (2) promote anti-tumor activity of TILs
 - Molecular level: intracellular kinases, transcription factors
 - Cellular level: cell-surface receptors, secreted signaling factors

Experimental Approach

Sample Description

ID	Age	Sex	Diagnosis	Histological Composition
1	51	F	DLBCL	FL 2 (90%); tFL - GCB (10%)
2	68	F	DLBCL	FL 3A (75%); tFL - GCB (25%)
3	63	F	DLBCL	FL 3B; tFL - GCB (unknown distribution)
4	57	M	DLBCL	FL 3A (40%); tFL - GCB (60%)
5	70?	M	DLBCL	FL 3A (80%); tFL - nonGCB (20%)

All patients were therapy naive at the time of sampling.
FFPE tissue available for 4/5 samples.

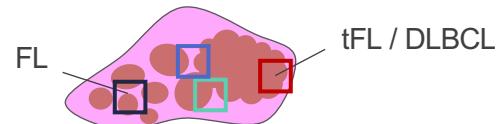


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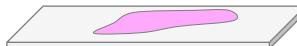
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Universitätsklinikum
Düsseldorf

Patient-matched FFPE tissue



Regions of interest annotated by hematopathologist.

CODEX



- Single cell resolution
- 60-plex protein
- Cell-specific markers
- **Quantify cellular compositional differences in TME**

10X Visium



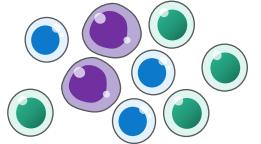
- 10 - 20 cells / array spot
- Whole transcriptome
- **Identify FL and tFL signatures**

10X Xenium



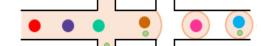
- Single cell resolution
- ~ 400-plex RNA
- **Anchor single cell data**
- **Resolve cellular states and subclones in space**

Bio-banked lymphocytes



CD3⁺ and CD19⁺ lymphocytes sorted by FACS.

10X Multiome

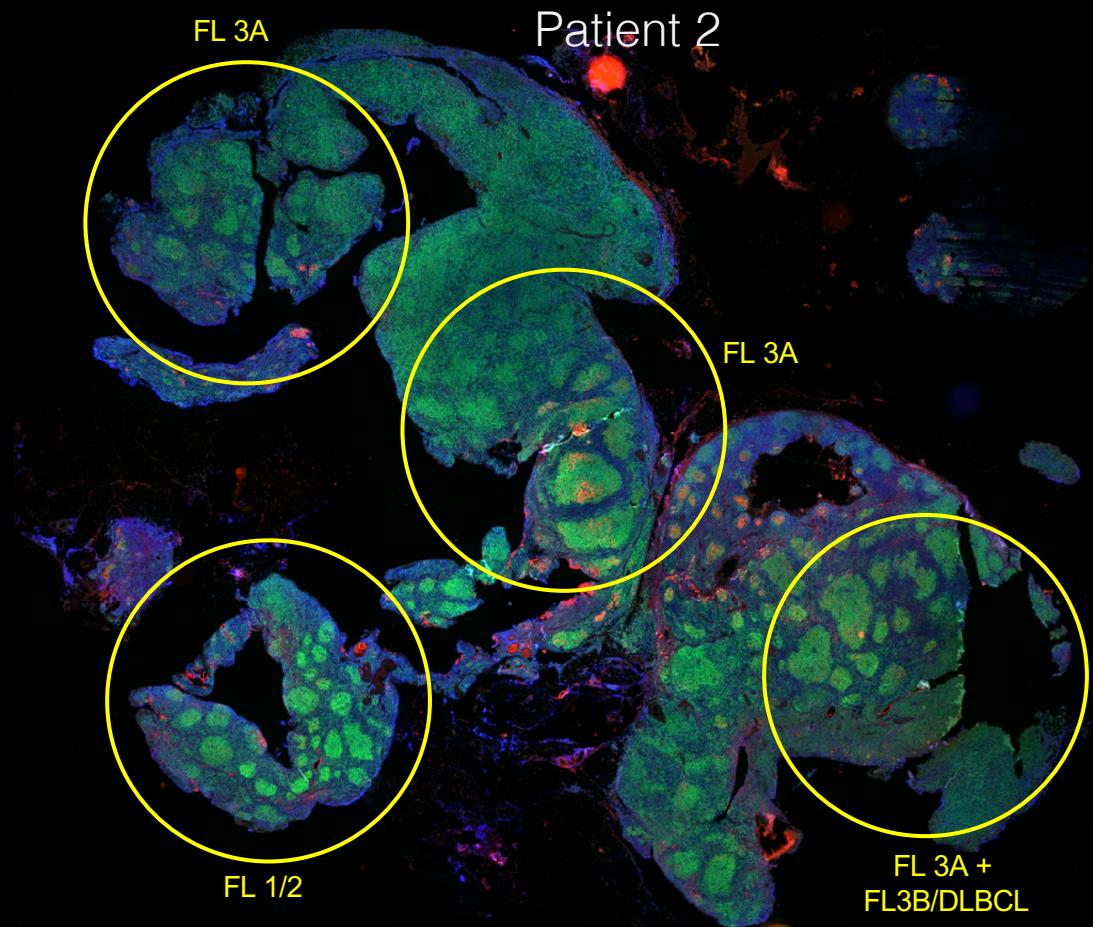
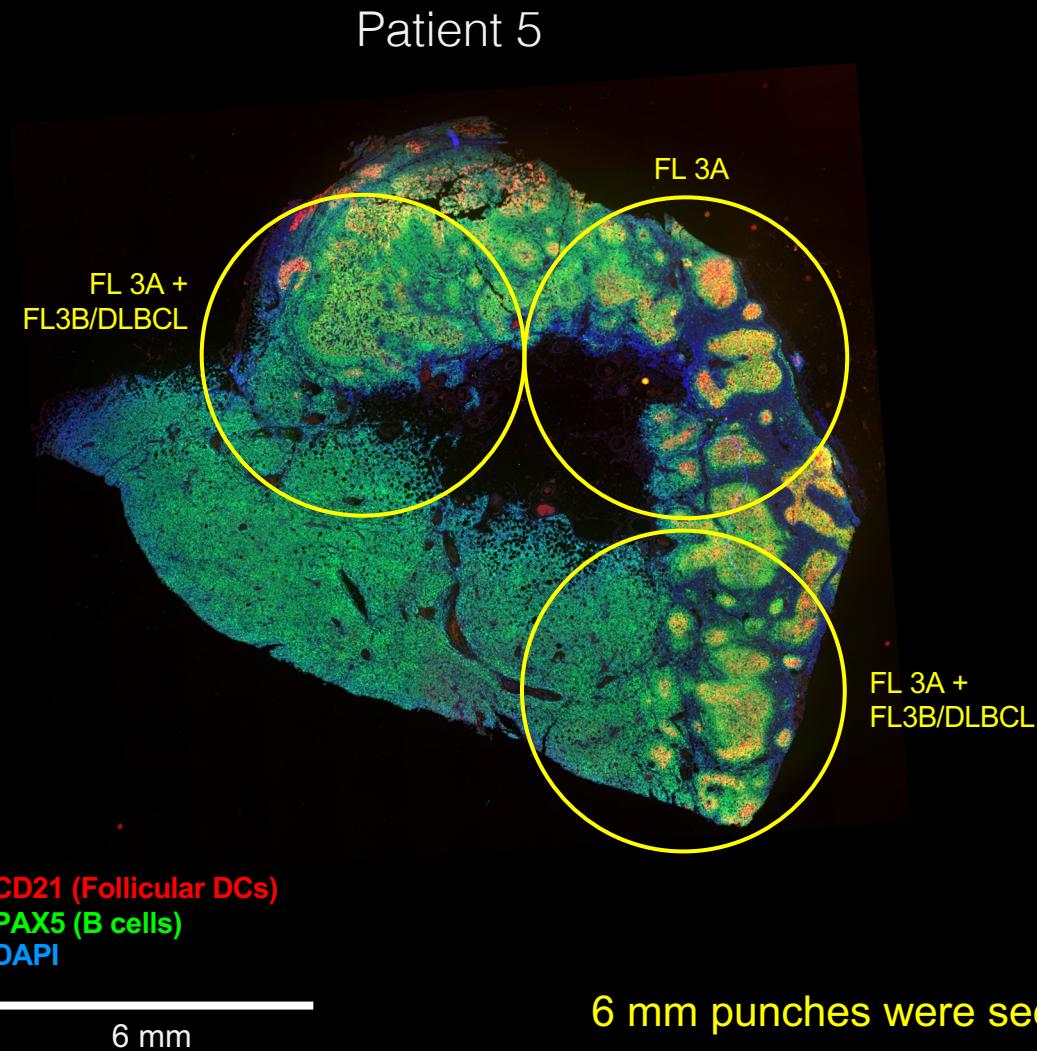


- Single cell
- Joint RNA+ATAC-seq
- Only lymphocytes
- **Reconstruct gene regulatory networks**



Valeriy Pak
EMBL

Selecting regions of interest for downstream spatial analysis

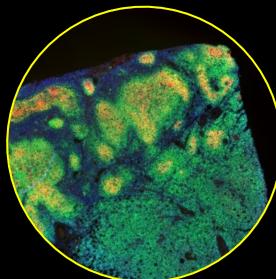


6 mm punches were sectioned onto Visium Capture Areas

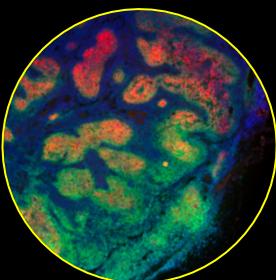
Patient 5

IF for Pathology

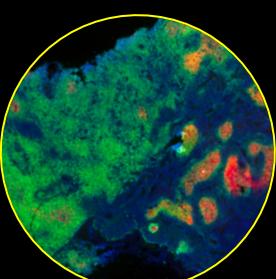
FL3A + DLBCL



FL1/2 + FL3A



FL1/2 + Diffuse FL3A



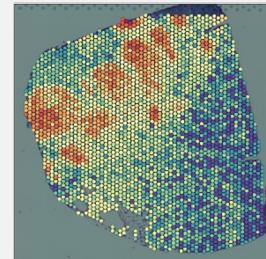
+ 15 - 20 μ m



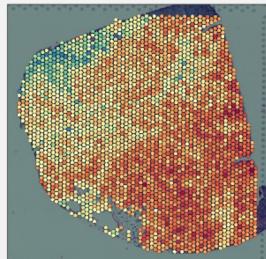
CD21 (FDCs)
PAX5 (B cells)
DAPI

Gene Expression

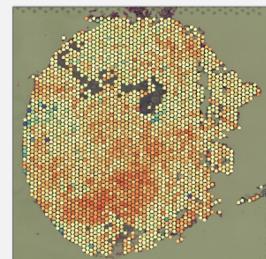
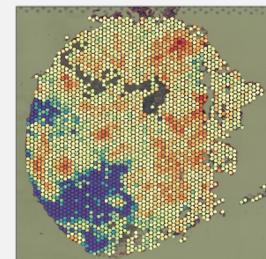
CR2 (CD21)



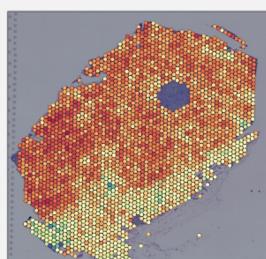
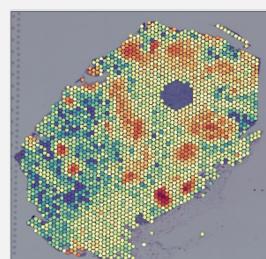
MS4A1



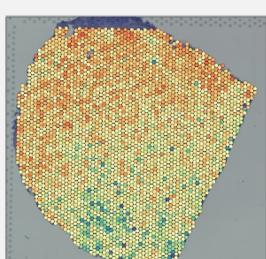
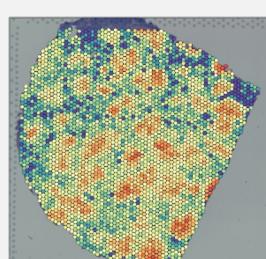
n = 29009



n = 2553



n = 2542



n = 3392

Integration of 4 ROIs from Patient 5



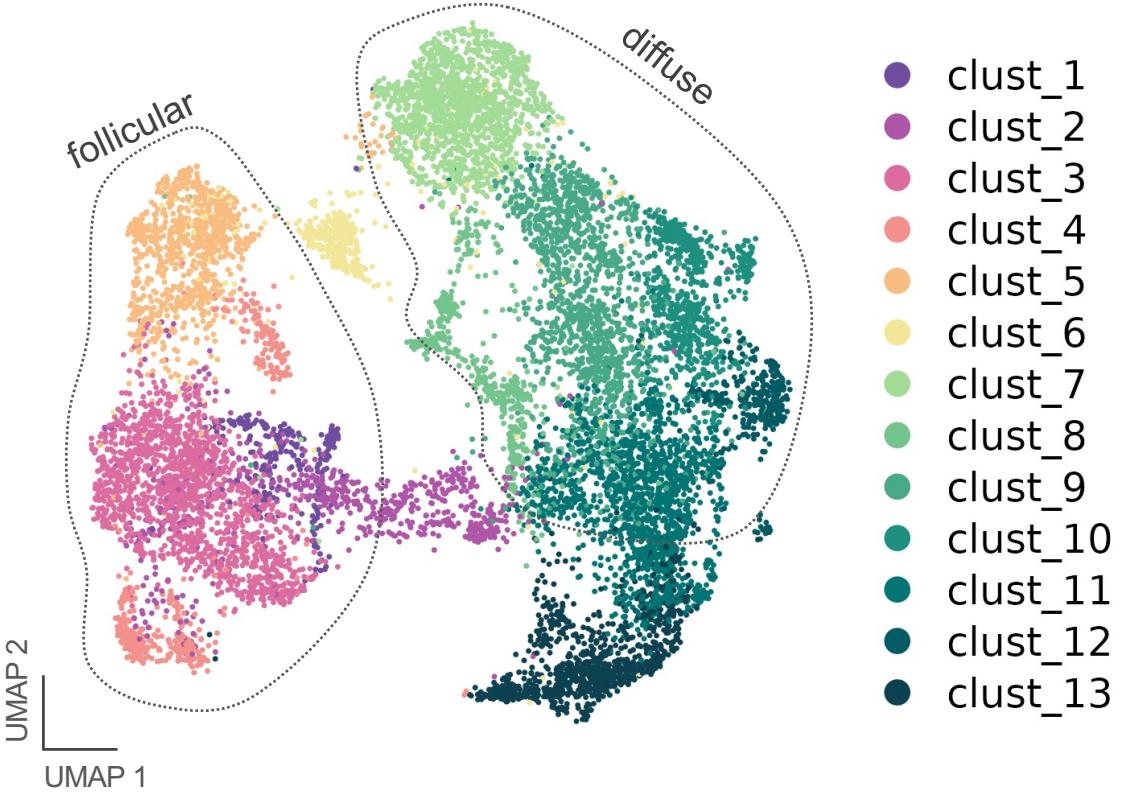
UMAP 2

UMAP 1

n (total spots) = 11,396

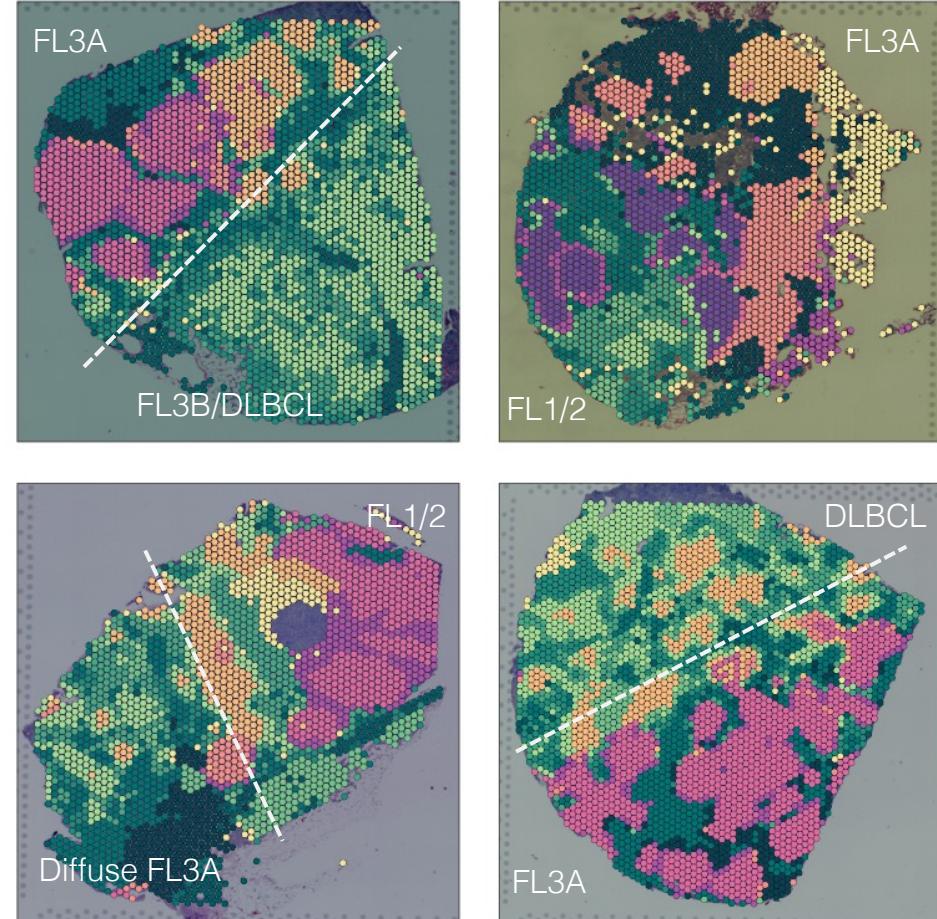
- clust_1
- clust_2
- clust_3
- clust_4
- clust_5
- clust_6
- clust_7
- clust_8
- clust_9
- clust_10
- clust_11
- clust_12
- clust_13

Spot-level clusters separate according to follicular and diffuse zones



Can integrate with single cell data to:

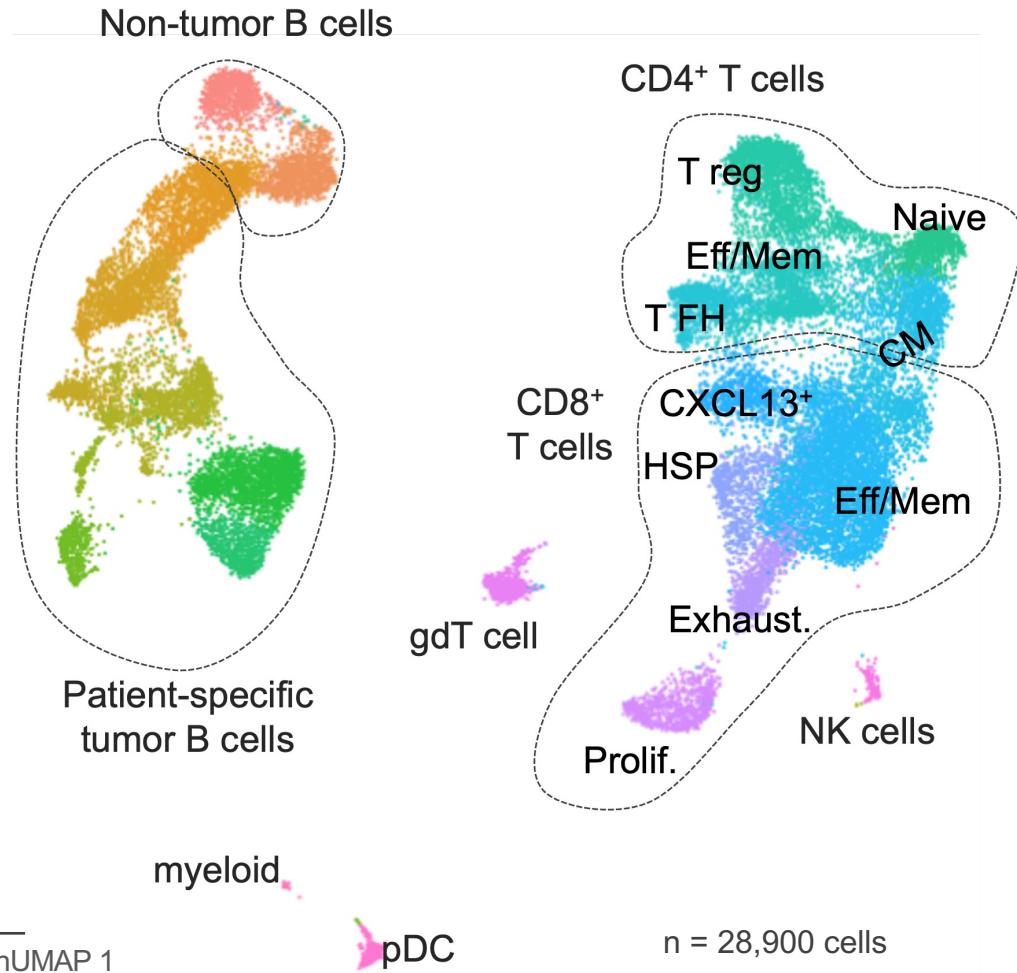
1. Deconvolute cellular composition of spots
2. Annotate malignant B cells as follicular or diffuse



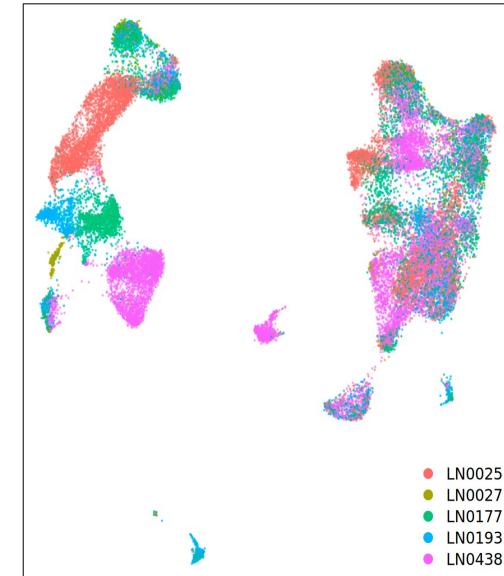
Do FL and DLBCL cells resolve in single cell data?

Intratumoral heterogeneity is reflected in patient-specific clusters of malignant B cells

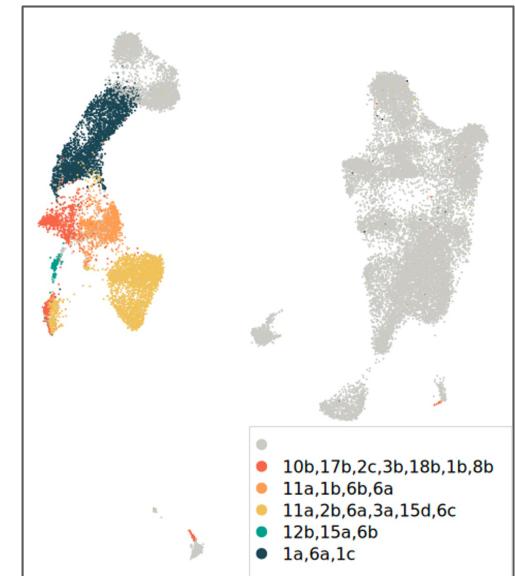
Joint Embedding (RNA+ATAC)



Patient ID

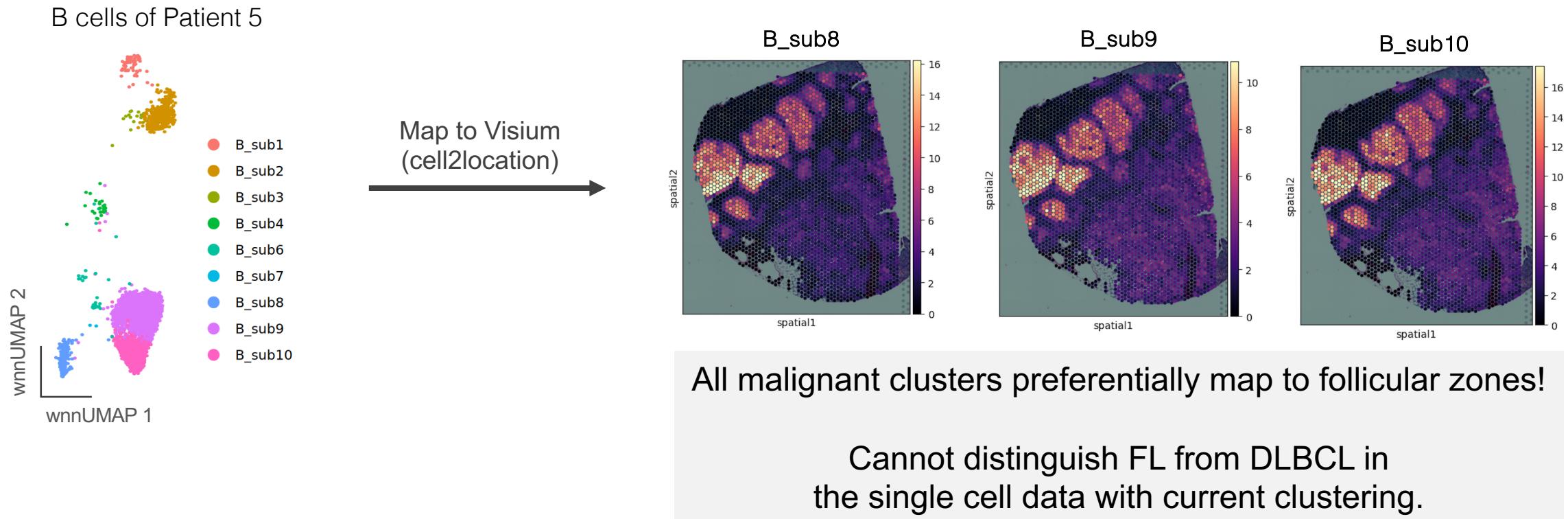


CNV Genotype (Numbat)

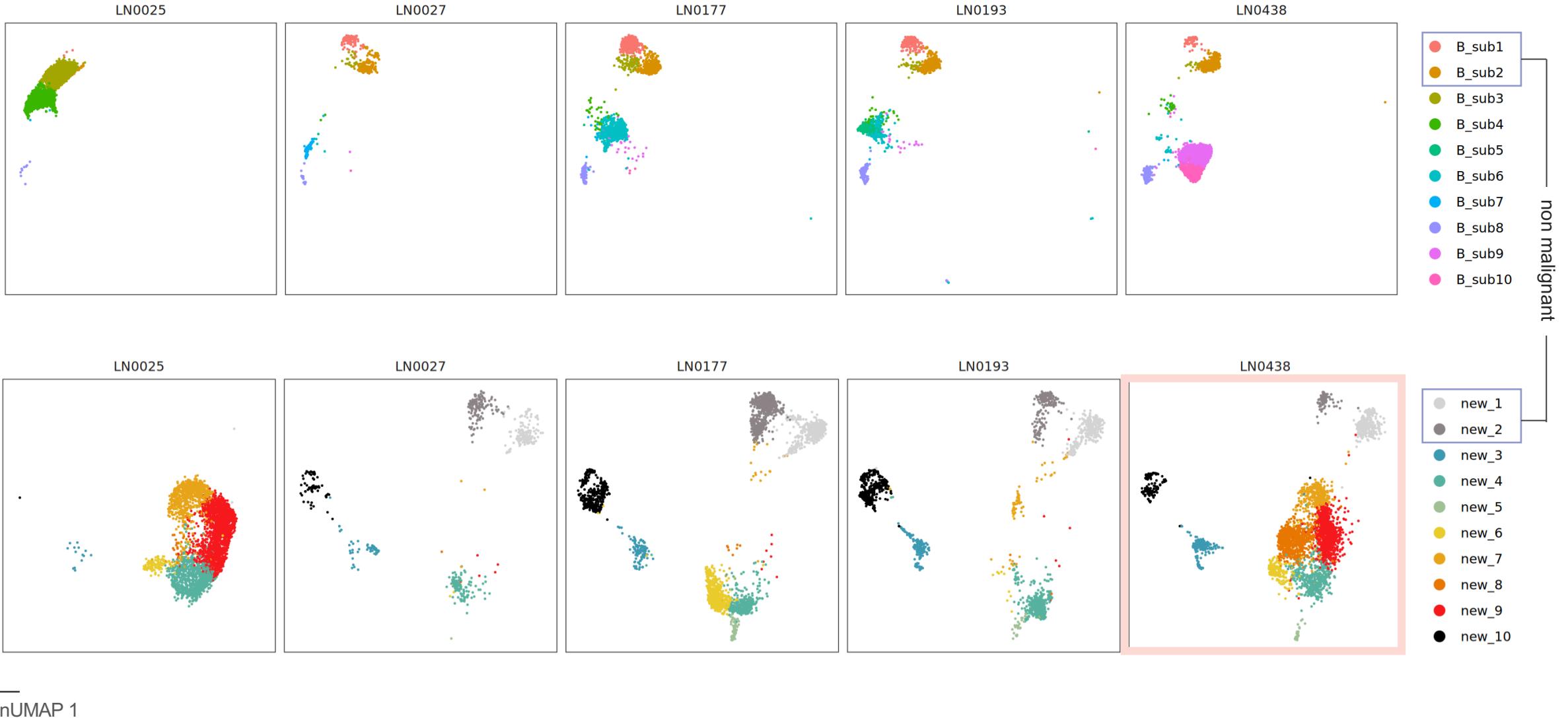


Can we annotate the tumor B cell clusters based on how they map in space?

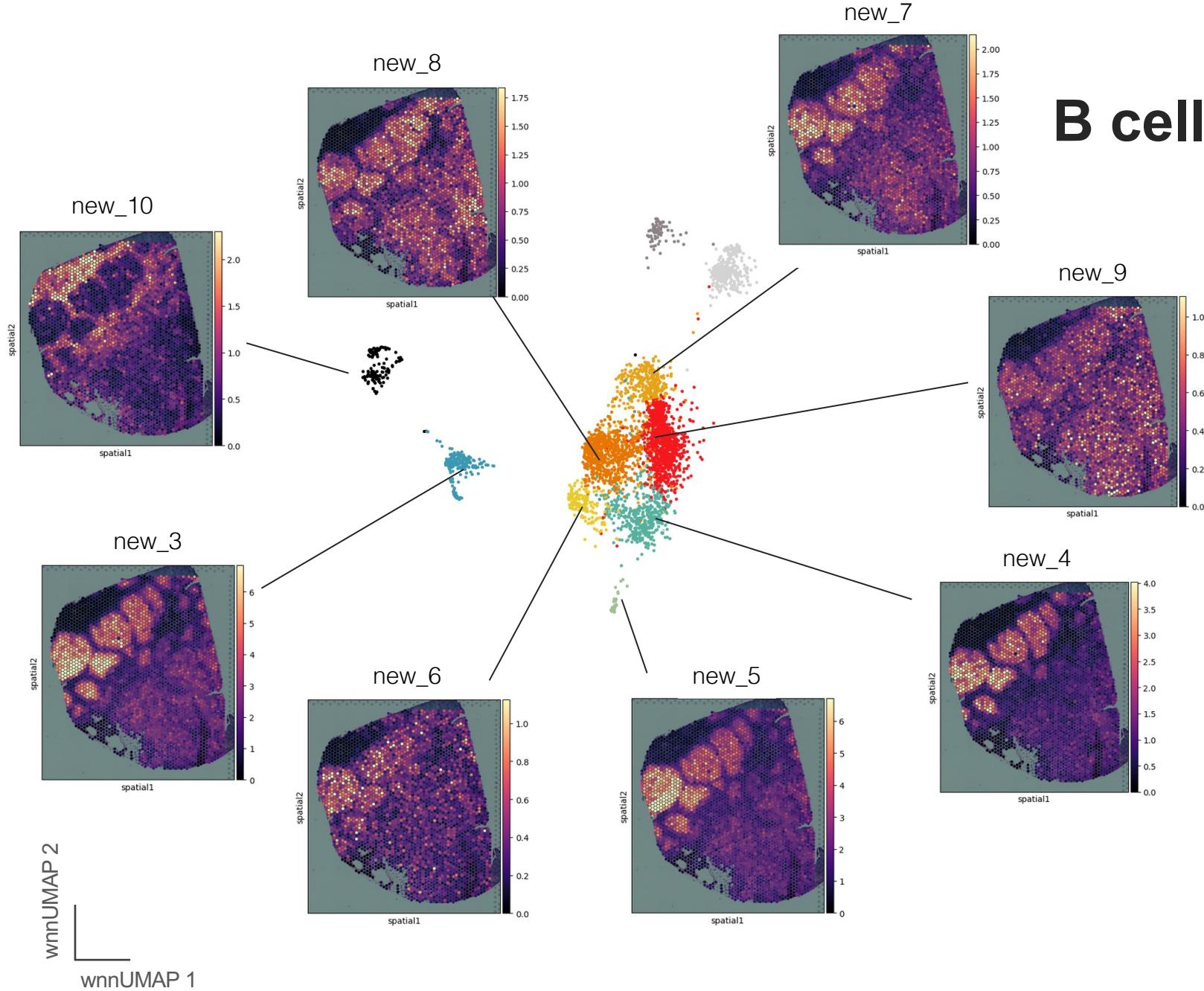
Can we annotate the tumor B cell clusters based on how they map in space?



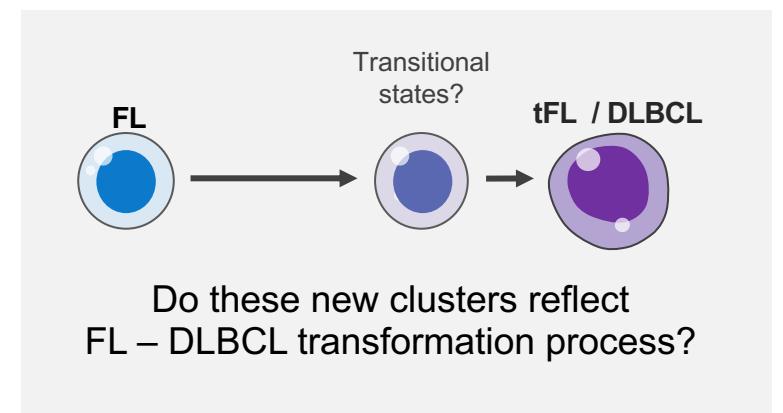
Reclustering of tumor B cells



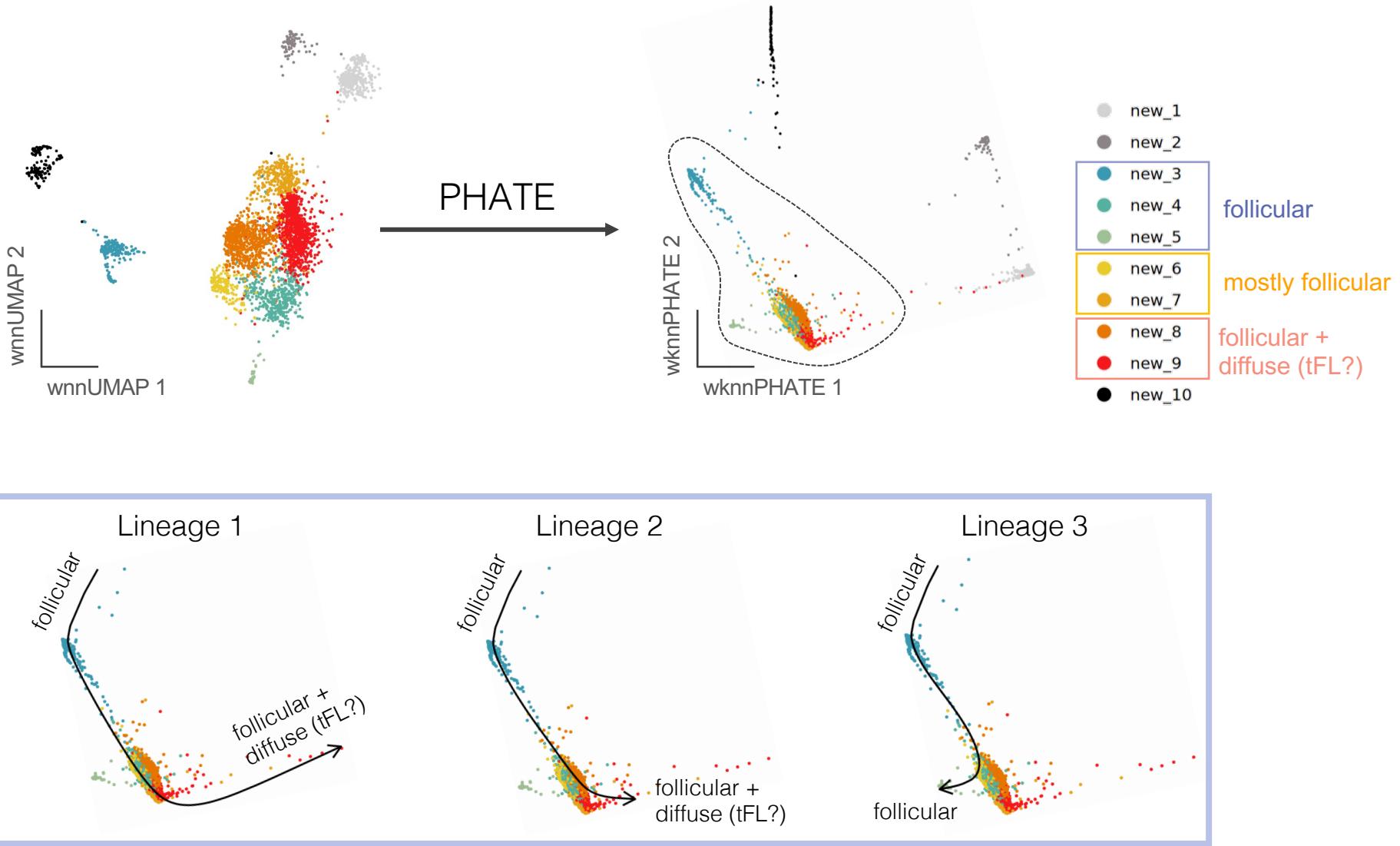
Spatial mapping of B cell clusters for Patient 5



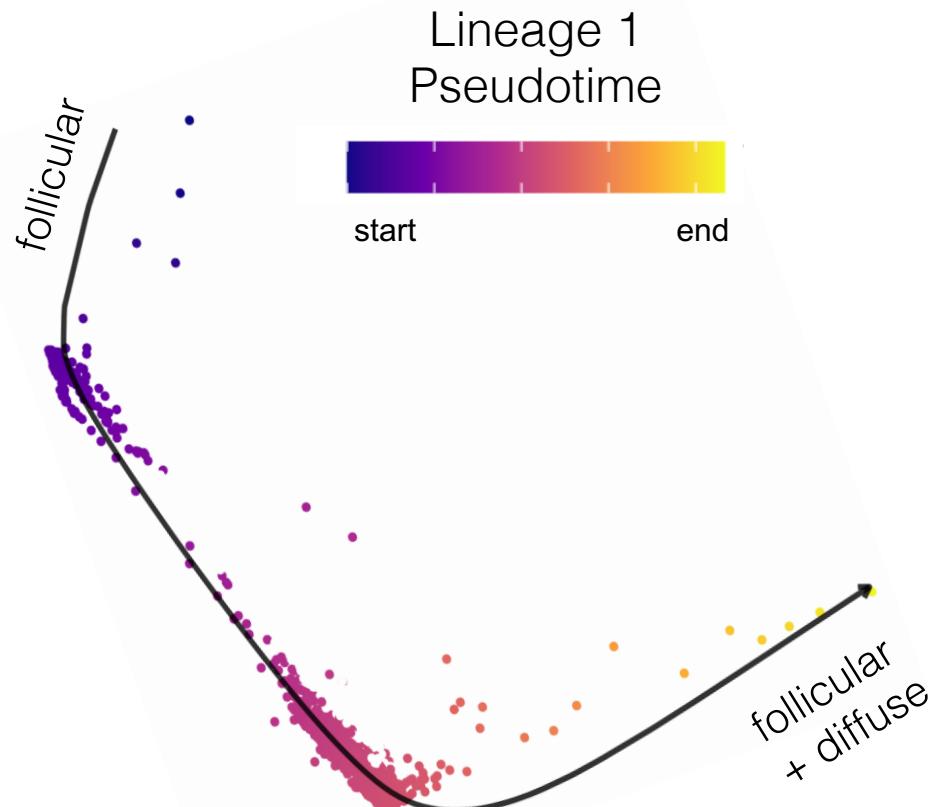
new_1	non-malignant
new_2	
new_3	follicular
new_4	
new_5	
new_6	mostly follicular
new_7	
new_8	follicular + diffuse (tFL?)
new_9	
new_10	plasma cell



Resolving transitional cell states and lineages with PHATE

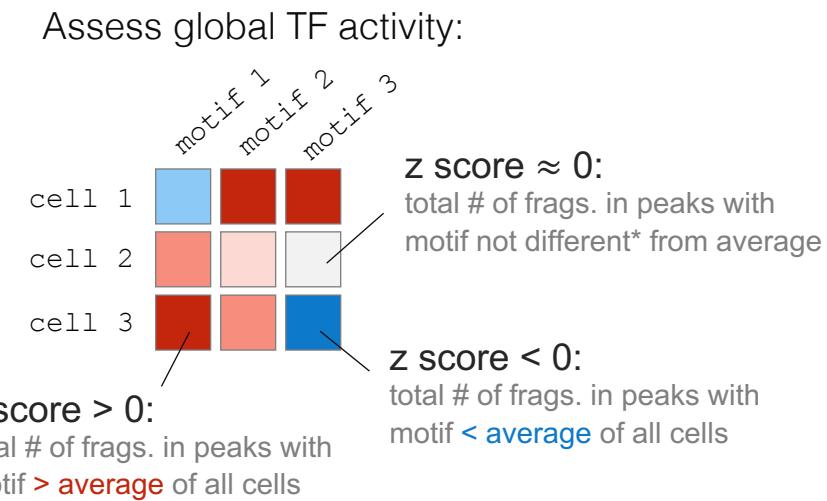
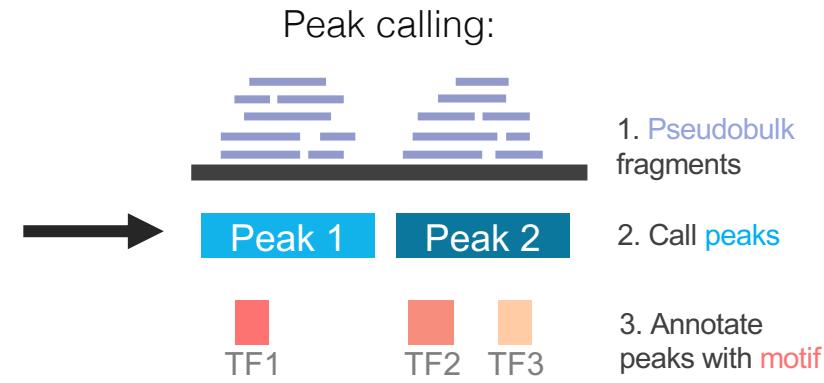
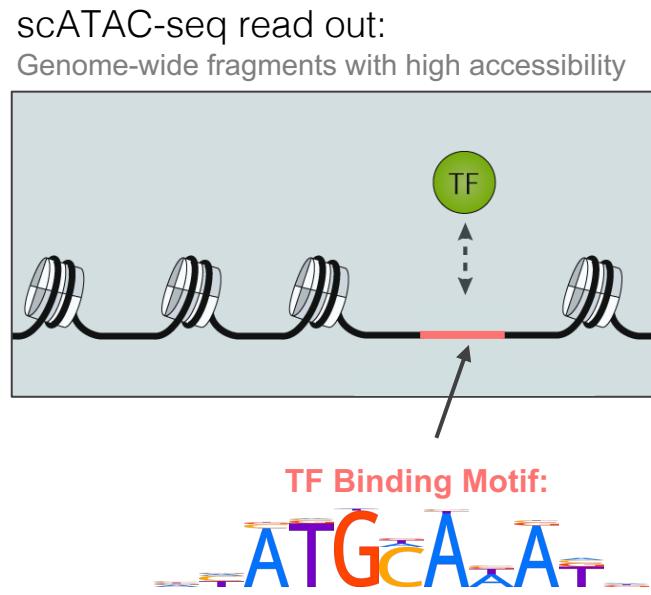


What changes occur in the epigenetic landscape during histologic transformation towards diffuse phenotype?

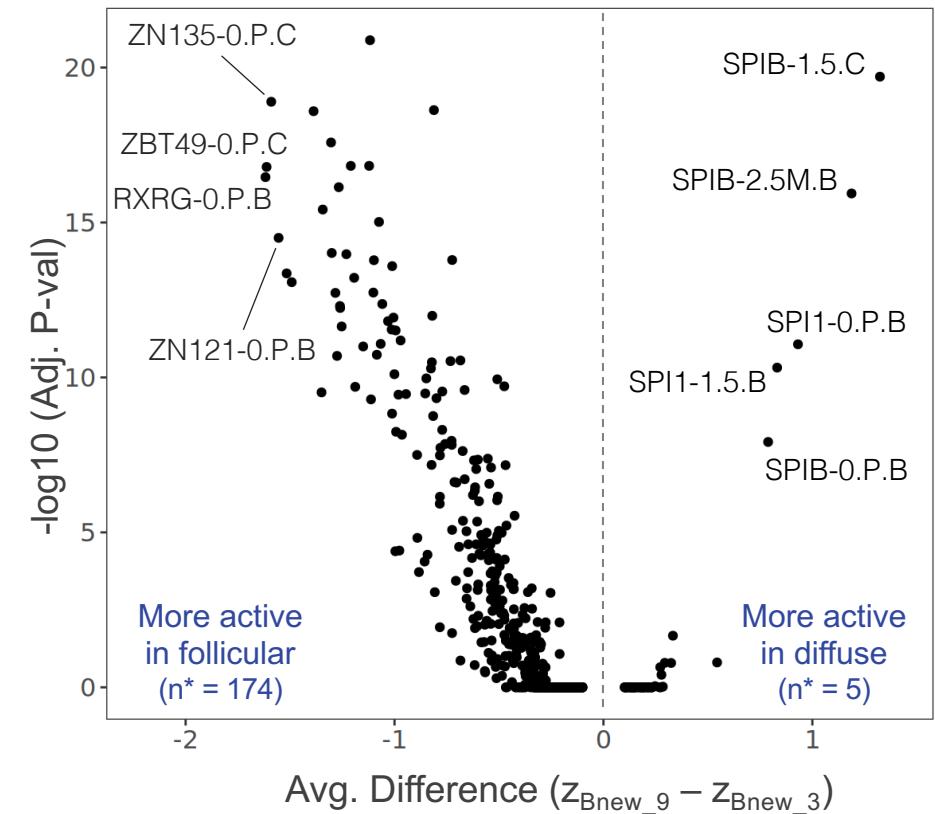
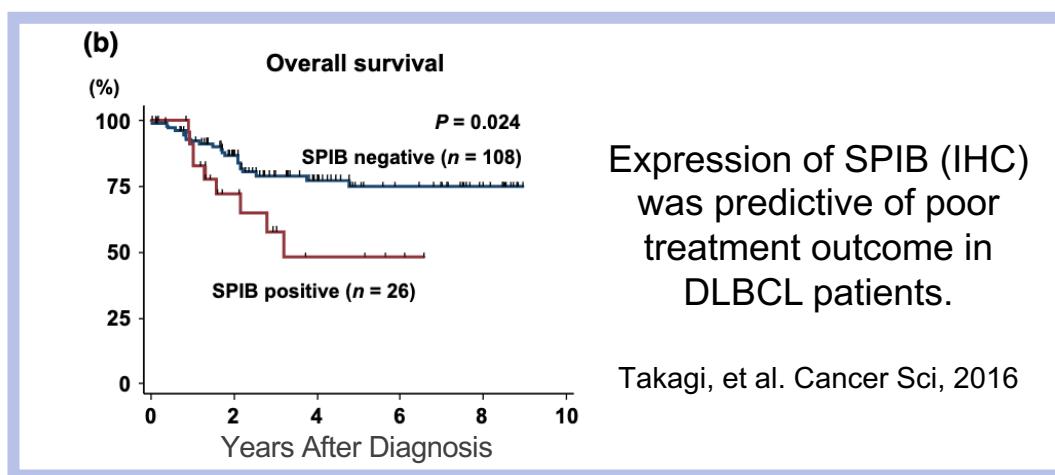
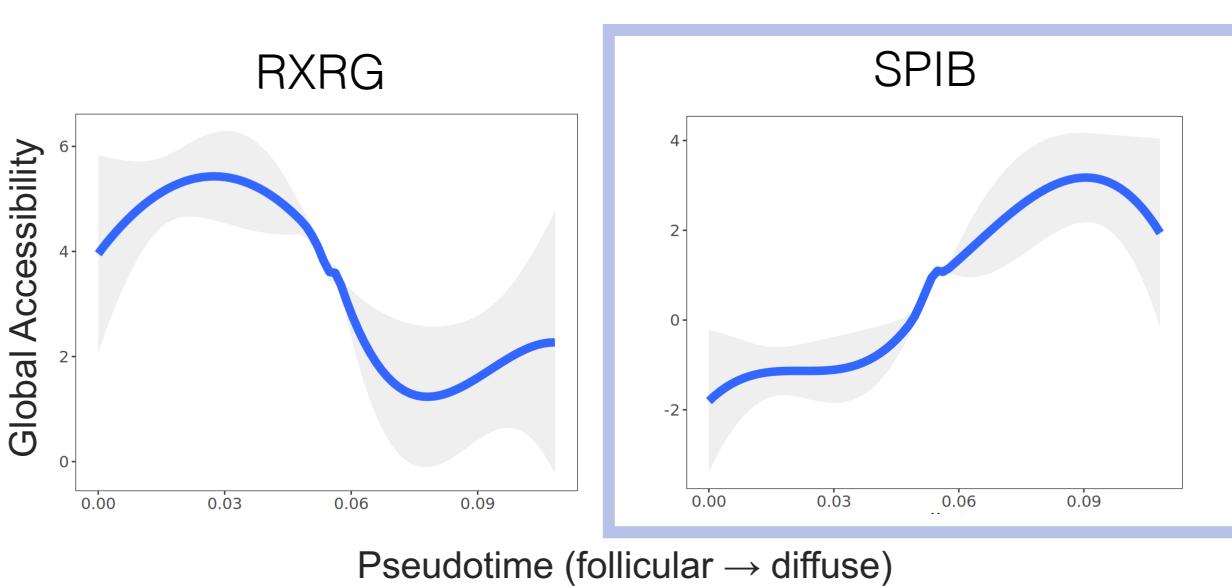


1. Are there global changes in transcription factor (TF) motif accessibility?
2. Which enhancers become progressively more/less accessible?
3. Which genes are regulated by the enhancers linked to the transformation process?

Inferring global TF activity with scATAC-seq

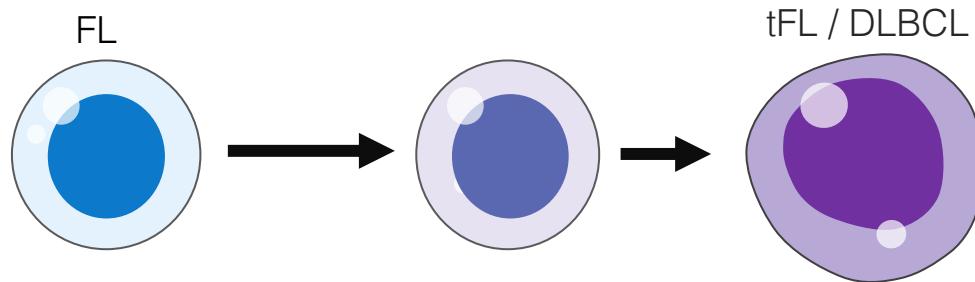


tFL B cells in Lineage 1 show a significant increase in SPIB and SPI1 TF activity



* n of sig. differentially active TFs determined by adj. p-val < 0.01

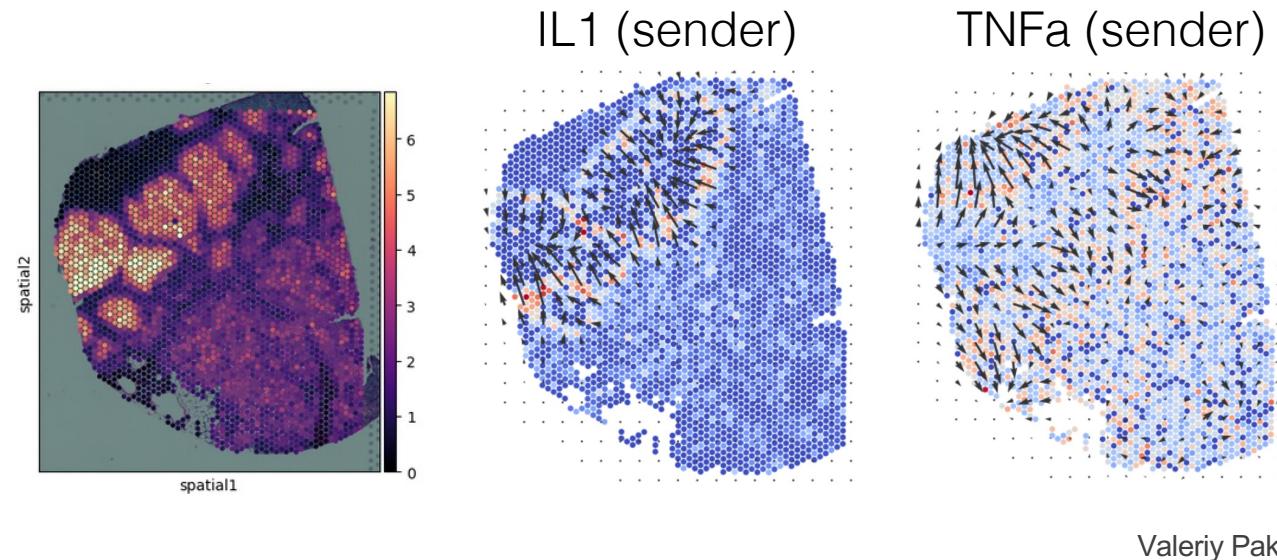
Conclusions



- Several malignant B cell subpopulations are resolved through joint single cell RNA and ATAC-seq profiling, reflecting inter- and intra-tumoral heterogeneity
- Malignant B cell subpopulations map to either follicular or follicular + diffuse zones
 - Those mapping to both follicular + diffuse zones may represent tFL cells
- Changes in TF activity and target gene expression along the FL-tFL axis can be inferred through pseudotime analysis
 - tFL cells display sig. increased activity of SPIB and SPI1 compared to cells restricted to the follicle

Next steps

- Characterize locus-specific enhancers which gain/lose accessibility along pseudotime
- Correlate enhancer accessibility changes to expression of target genes; reconstruct gene regulatory networks (GRNs)
- Anchor GRNs to spatial data and correlate to cell-cell signaling patterns



Cell-Intrinsic Factors

Genetic mutations in epigenetic machinery



Altered chromatin permissibility



Changes in transcription factor motif accessibility

Single cell profiling methods

Cell-Extrinsic Factors

Cell-cell signaling in tumor microenvironment



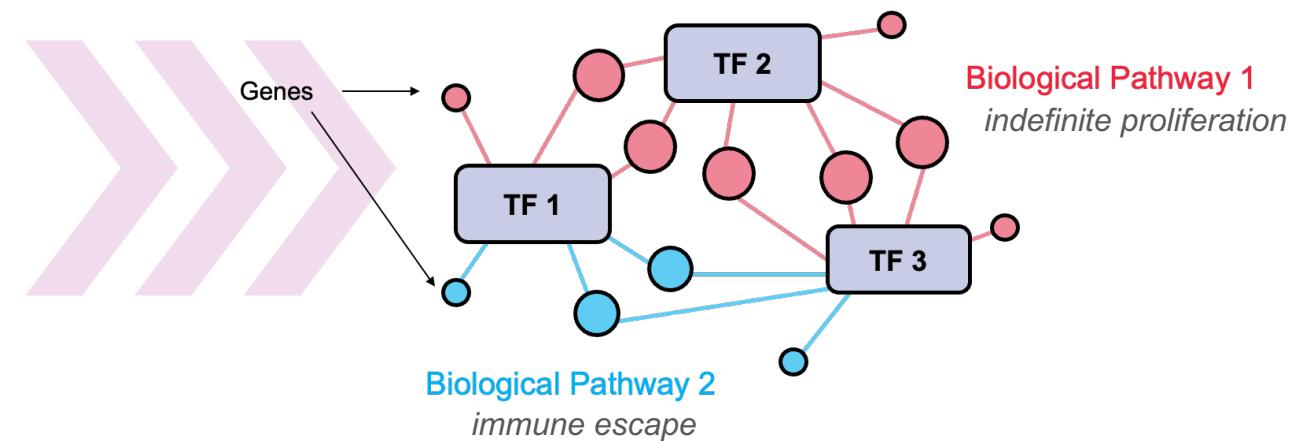
Signal cascade through surface receptors



Activation of downstream transcription factors

Spatially resolved techniques

Upregulation regulatory networks promoting transformation



Acknowledgements



Sascha Dietrich
Universitätsklinikum
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Felix Czernilofsky
Universitätsklinikum
Heidelberg



Alexander Brobeil
Universitätsklinikum
Heidelberg

Sinem Saka, EMBL

Valeriy Pak

Yulia Ermakova
Davide Floris
Jennifer Heck
Berkan Kanmaz
Sora Matsumoto
Pablo Mejias
Maria Puschhof
Robert Reinhardt
Jorge Trojanowski
Wouter-Michiel Vierdag
Yuchen Xiang
Jennifer Zilke

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Felix Czernilofsky

Christina Schniederjohann

Peter-Martin Bruch

Harald Vöhringer
Donnacha Fitzgerald
Tobias Roider

Alexander Brobeil,

**Institute of Pathology at
Uniklinik. Heidelberg**

Carolin Kerber

Fabio Tabone

**Judith Zaugg, Structural
and Comp. Bio. Unit**

Christian Arnold
Guido Barzaghi
Gwen Bauersachs
Josephine Brysting
Annie Claringbould
Charles Dussiau
Dirk Hart
Daniel Heid
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