



National and  
Kapodistrian  
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Χωρική πολυπαραγοντική ανάλυση φλεγμονωδών διηθημάτων  
από βιοψίες χείλους ασθενών με σύνδρομο Sjögren

Χατζής Λ., Patrice H., Γουλές Α., Καψογεώργου Ε., Scuiller V., Pers J.O., Τζιούφας Α.

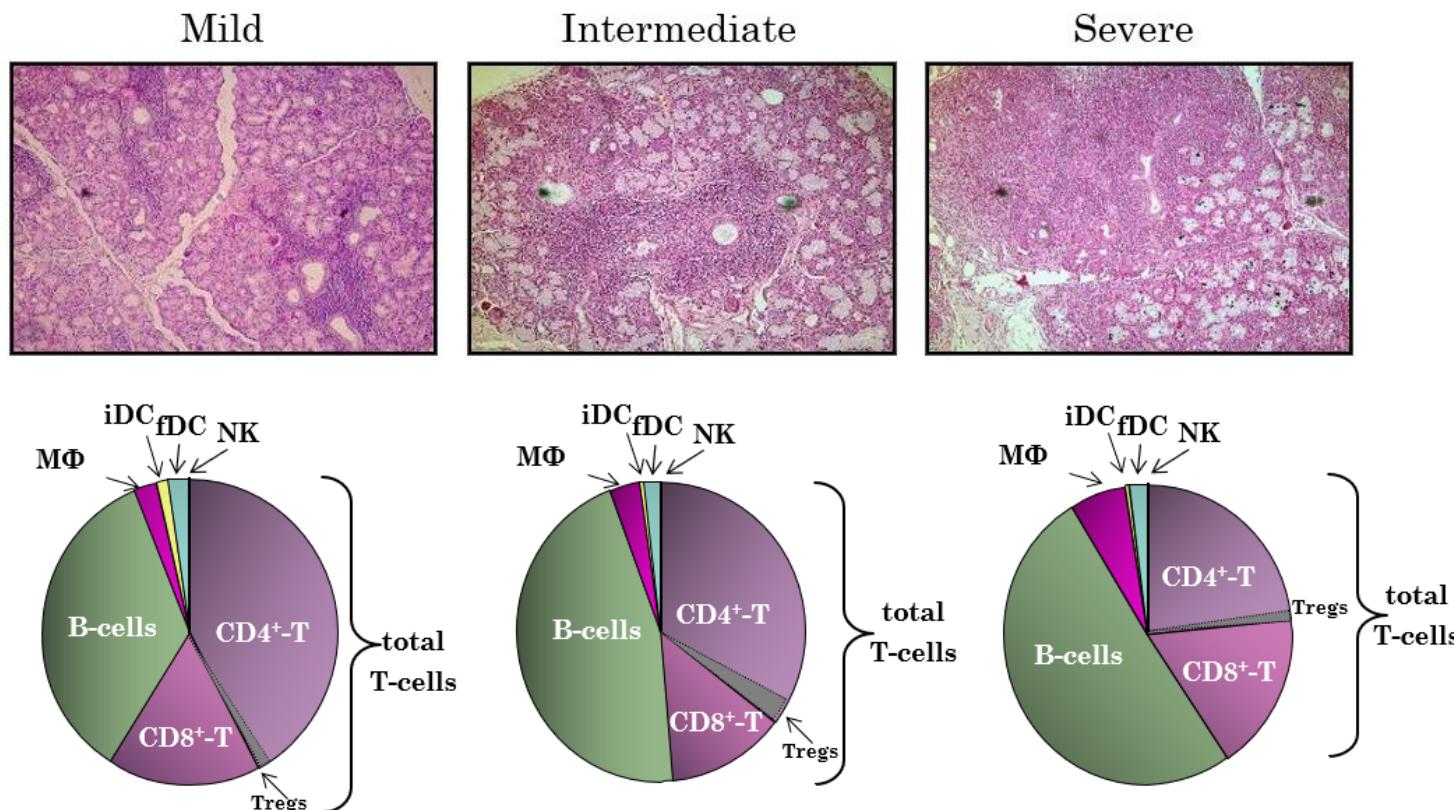
# Our Target



## Hyperion project

To redefine the glandular architecture and the inflammatory infiltrate of Sjogren Syndrome patients, discover markers associated with the inflammatory burden, uncover cell to cell interactions elucidating potential pathogenetic roads towards both autoimmune inflammation and lymphomagenesis

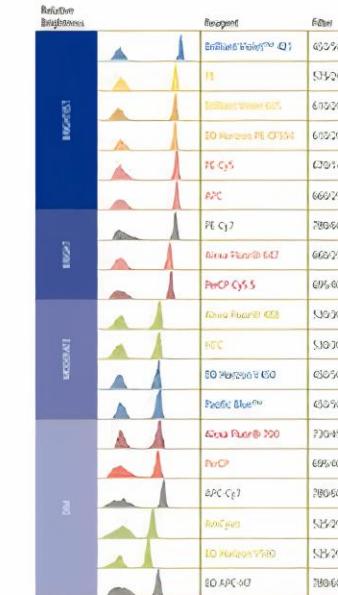
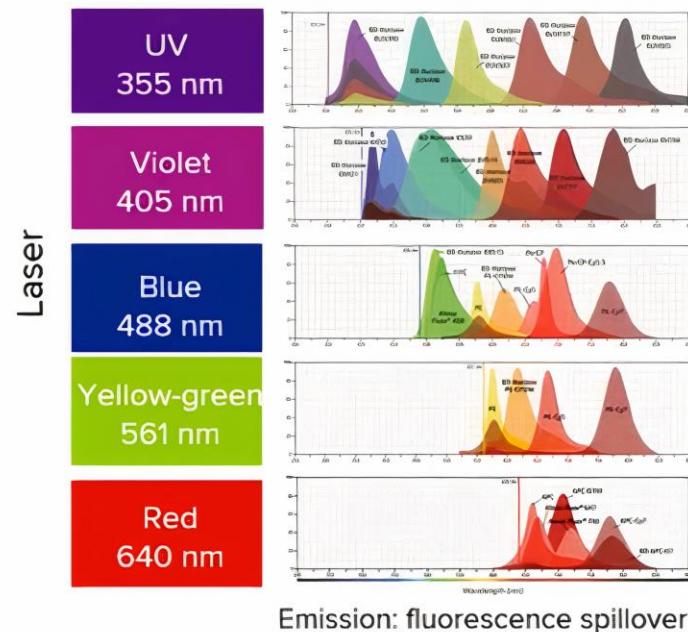
# Background



# Antibody-mediated multiparameter protein detection

- Fluorochrome-conjugated antibodies are widely used but have limited utility for high-parameter studies. These limitations contribute complexities into experimental design and interpretation

## Fluorescence spillover | Variable staining intensities | Background signal



Staining intensities and background



## The highly-multiplexed tissue imaging: HYPERION® technologies



### Capture

Hyperion tissue imager

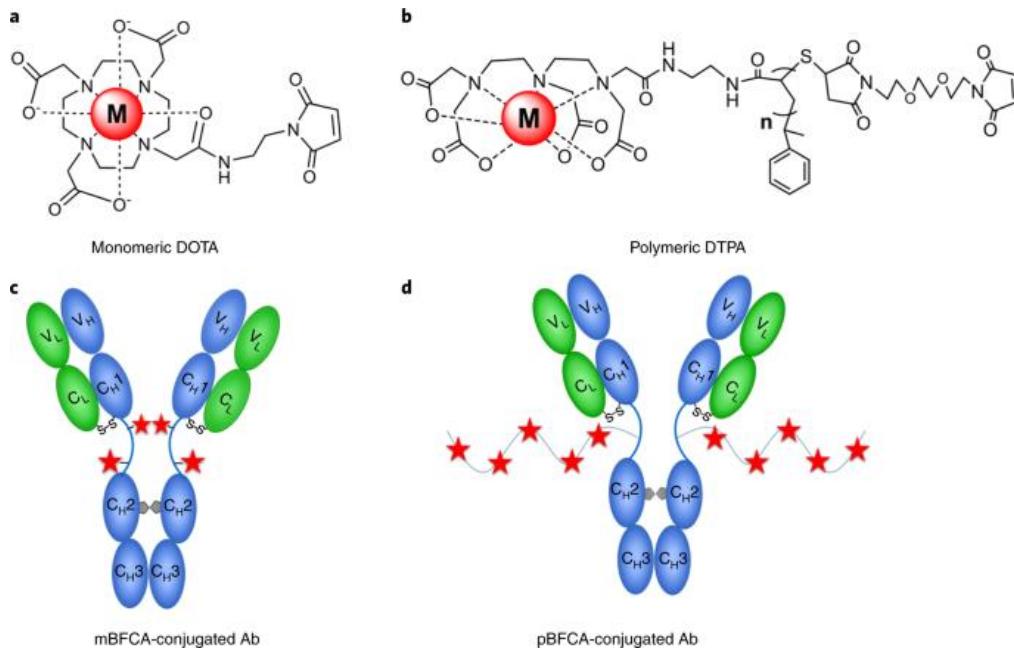
### Detection

CyTOF technology

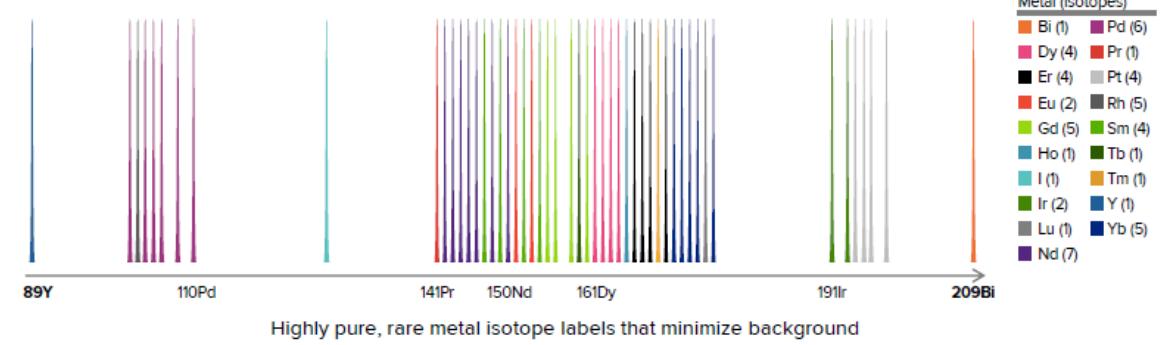
- ▶ Up to **40 proteins** simultaneous detection
- ▶ Stain samples with **all antibodies simultaneously**
- ▶ **Spatial** analysis of protein markers on a single tissue section
- ▶ **FFPE or fresh frozen** tissue

## Labelling

# HYPERION® technology overview



- ▶ Each Antibody in the panel is conjugated to a **metal isotope** with a **unique atomic mass**
- ▶ One single **staining step** with the antibody panel

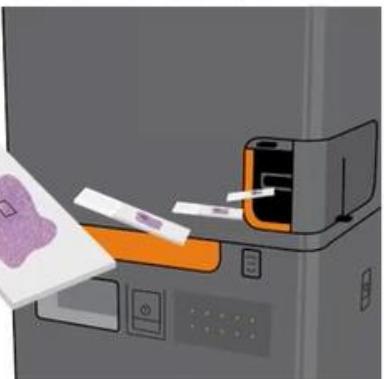




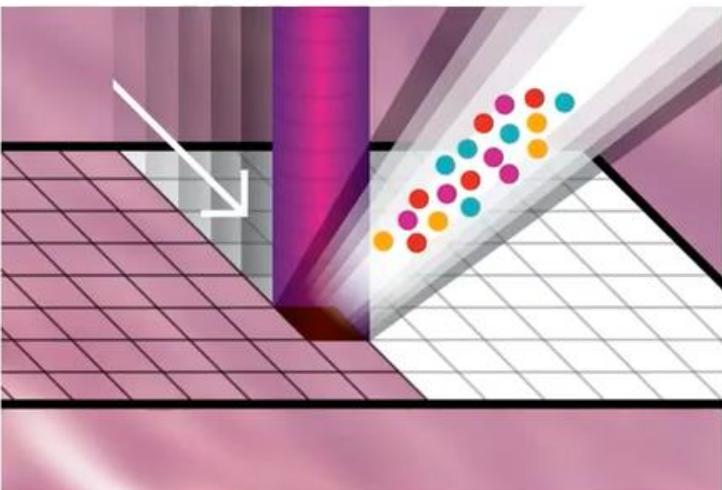
## Instrument

# HYPERION® technology overview

Load the sample into the Hyperion Imaging System



Precise laser imaging of the region of interest



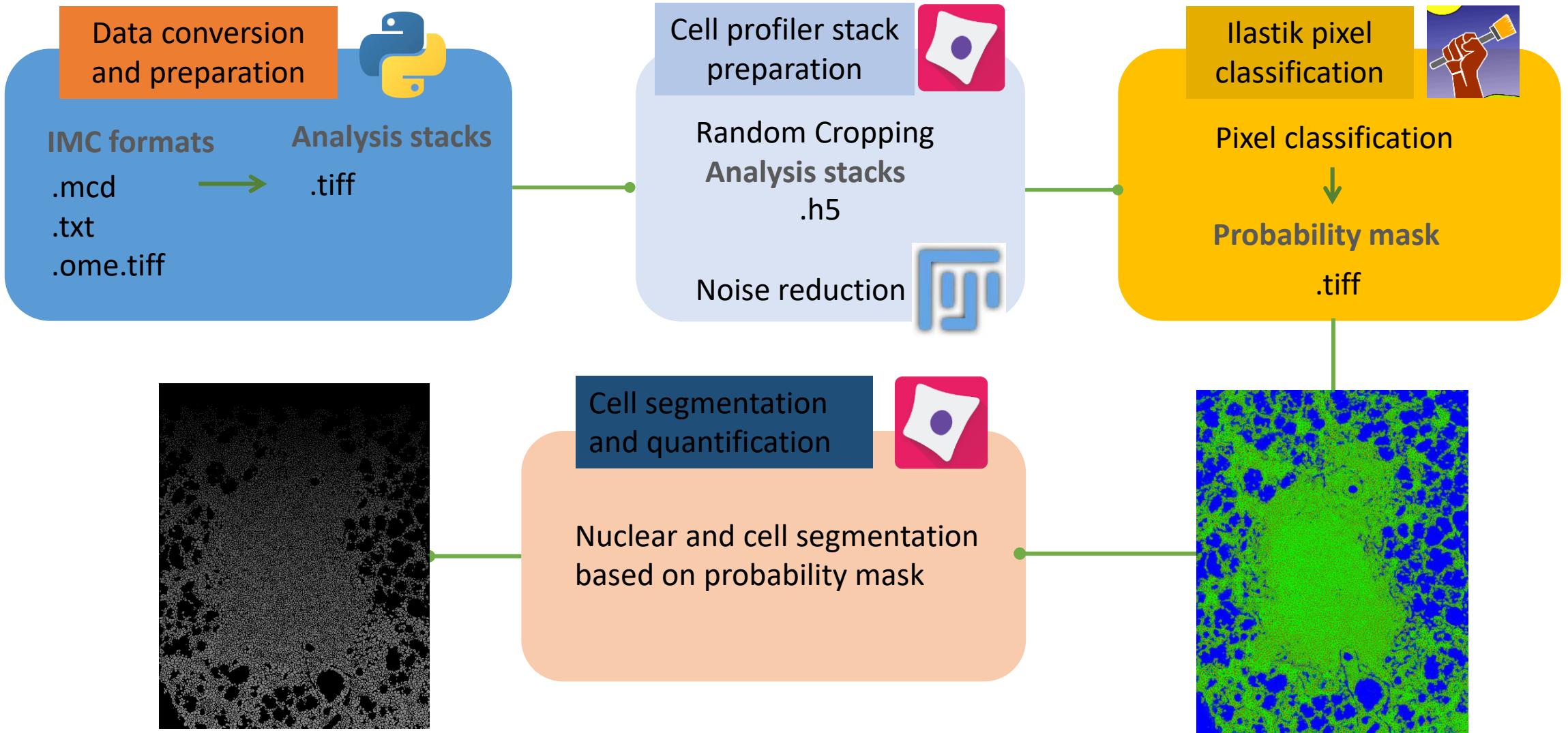
Laser beam focuses on  $1 \mu\text{m}^2$  spots in the selected region

Collects metal-tagged proteins

Sends for CyTOF analysis

- ▶ Laser beam focuses on  $1 \mu\text{m}^2$  spots in the selected region
- ▶ Collects **metal-tagged proteins**
- ▶ Sends for **CyTOF analysis**

# Data pre-processing – Pipeline



# Analysis

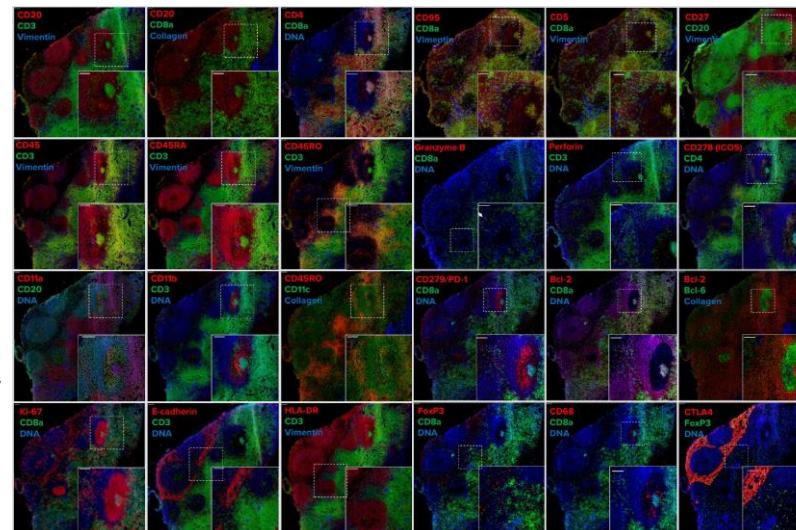
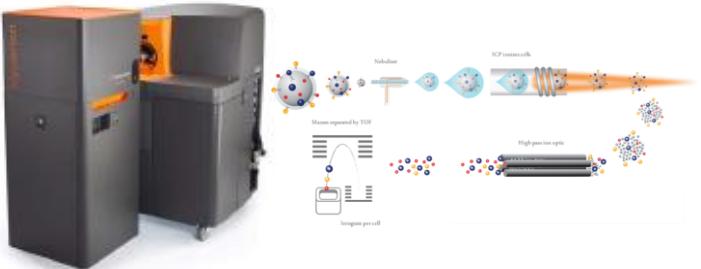
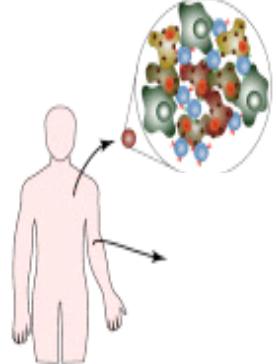


Plateforme de cytométrie en flux et de masse

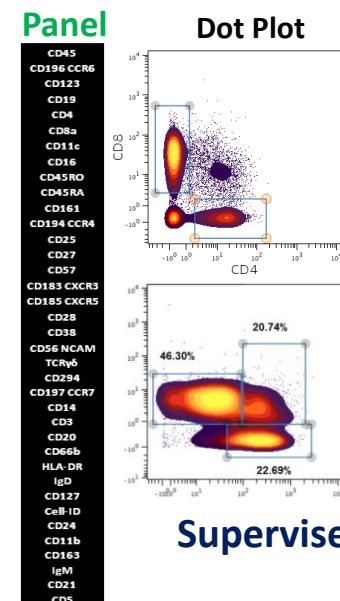
# HYPERION

Imaging mass cytometry (IMC) is a new multiparametric and quantitative technique for phenotypic and functional analysis of cells and tissue sections. It can measure up to 40 parameters simultaneously in tissues at a spatial resolution of 1  $\mu\text{m}$ .

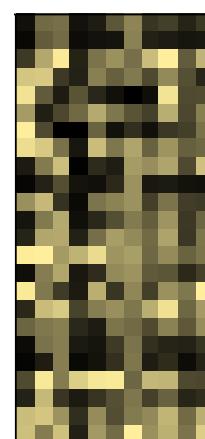
# Tissue



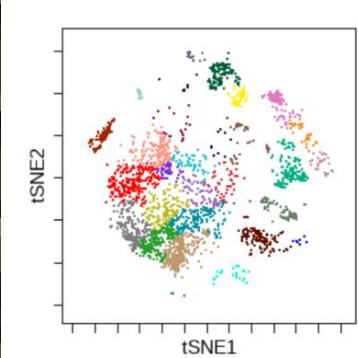
## Supervised analysis



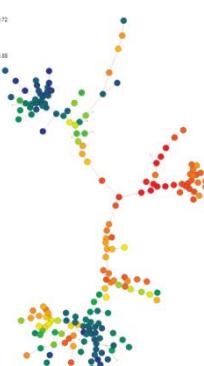
## Supervised analysis      Unsupervised analysis



tSNE



## Unsupervised analysis



# Methods

1

5 sicca controls



FS:0-1, Anti – Ro/SSA (-),  
Anti-La/SSB (-)

2

5 Mild infiltrates



FS:1-1.79, TS:1

3

5 Intermediate  
infiltrates



FS:1.8-3.5, TS:2

4

5 Severe infiltrates



FS: 3.6-11, TS: 3-4

5

4 patients with SS  
associated lymphoma

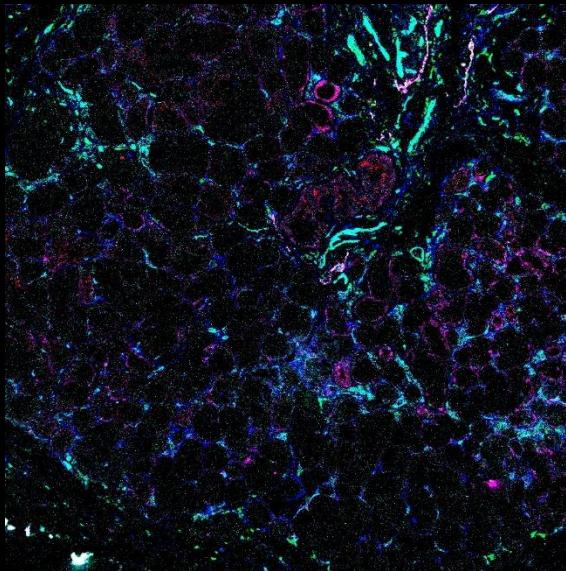


MALT Lymphoma diagnosis

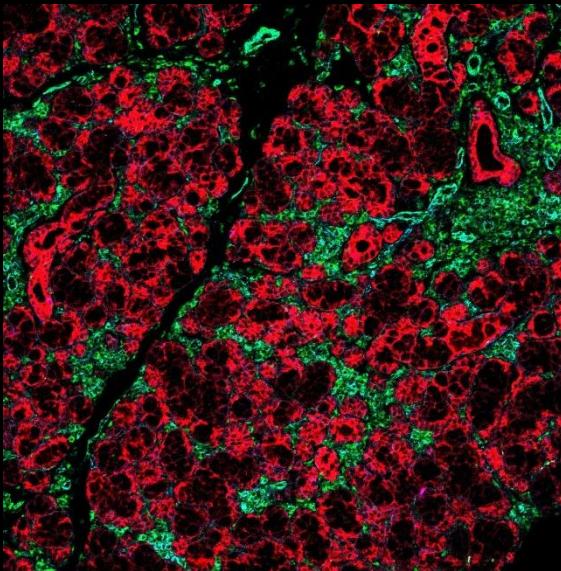
## 2 Antibody Panels

General Panel	Properties	General Panel	Properties	>>>>	B cell panel	Properties
CD38	Plasma cells, Immature/ transitional b cells, effector T cells	Bcl6	GC B cells and T follicular helper cells		CD45RB	Memory B cells, Cd38 positive B cells outside of GM
CD204	M2 macrophage	CD20	B lymphocytes		Vimentin	Mesenchymal cells
Vimentin	Mesenchymal cells	CD8a	Cytotoxic T cells		Tbet	Th1 T cells
CD14	<b>Macrophages</b> , dendritic cells, neutrophils	CD138	Plasma cells		Pan-Keratin	Epithelial cells
Tbet	Th1 T cells	MPO	Neutrophils		Ki-67	Proliferation
CD34	Hematopoietic progenitor cells, stem cells	Flt3Ligand	T cells		IgD	Mantle zone B cells
CD163	M2 macrophages	CD56	NK cells		FoxP3	T regulatory cells
Pan-Keratin	Epithelial cells	CD127	Memory and Effector T cells not on Treg, Precursors B cells		CD4	T helper cells
CD11b	Monocytes, B1 B cells, natural killer (NK) cells, and dendritic cells, cDC2	Collagen	Collagen type I		CD68	M1 Macrophages
TSLP	Lymphoma, Epithelial cells	CD3	T cells		Bcl6	GC B cells and T follicular helper cells
CD31	Endothelial cells in blood vessels, platelets	CD27	Memory T cells (except an effector memory population), B cells (not effector), Plasmablasts (High) NK cells		CD20	B lymphocytes
Ki-67	Proliferation	Caspase-3	Cell apoptosis		CD8a	Cytotoxic T cells
IgD	Mantle zone B cells, naive B cells	Podoplanin	Follicular DCs, Lymphatic endothelial cells		CD138	Plasma cells
IgM	Mantle zone B cells, naive B cells	HLA-DR	Dendritic cells, macrophages, B cells, stimulated epithelial cells		MPO	Neutrophils
FoxP3	T regulatory cells	pS6	Akt/PI3K/mTOR pathway related proliferation		PD-1	T follicular helper cells
Cd4	T helper cells	Flt-3	Plasmacytoid DCs, B progenitor cells		Collagen	Collagen type I
cKit	Cancer, Stem cells	CXCL13	Follicular dendritic cells		CCR6	Bmem
CD68	M1 Macrophages	CXC3	Activated Th1 cells, CD8 effector cells, Nk cells, proinflammatory B cells		CD3	T cells
IgA	Plasma cells and germinal center immunoblasts				CD27	Memory B cells (except an effector memory population), T cells (not effector), NK cells
AID	Somatic hypermutation and immunoglobulin class switch recombination				Podoplanin	Follicular DCs, Lymphatic endothelial cells
					SMA	Smooth muscle antibodies
					CD38	Plasma cells, Immature/transitional B cells, effector T cells
					AID	Somatic hypermutation and immunoglobulin class switch recombination

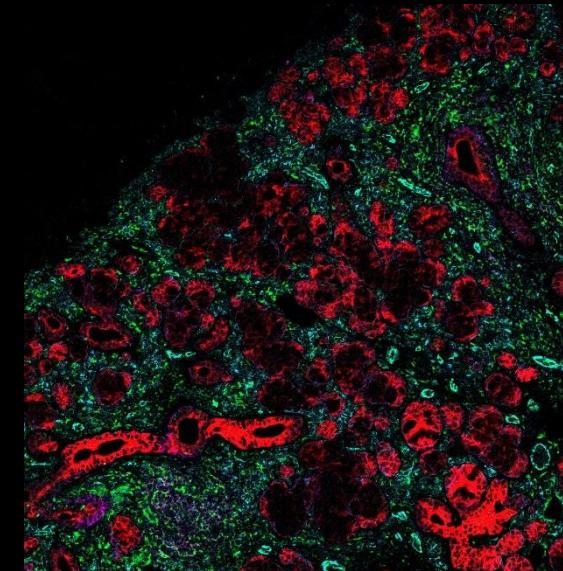
**SC**



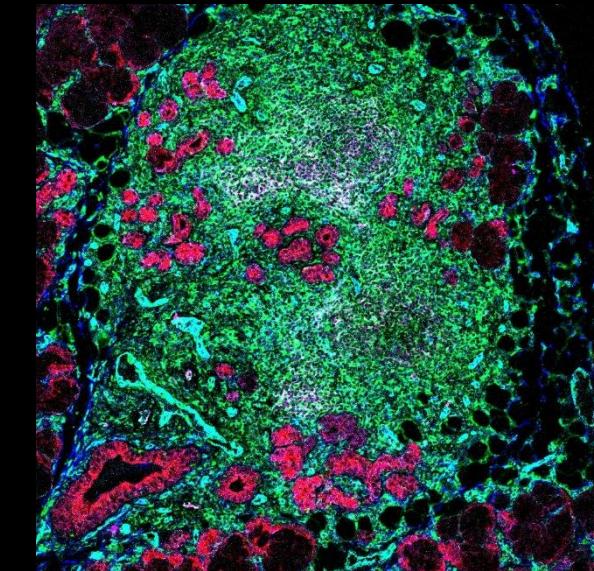
**Mild**



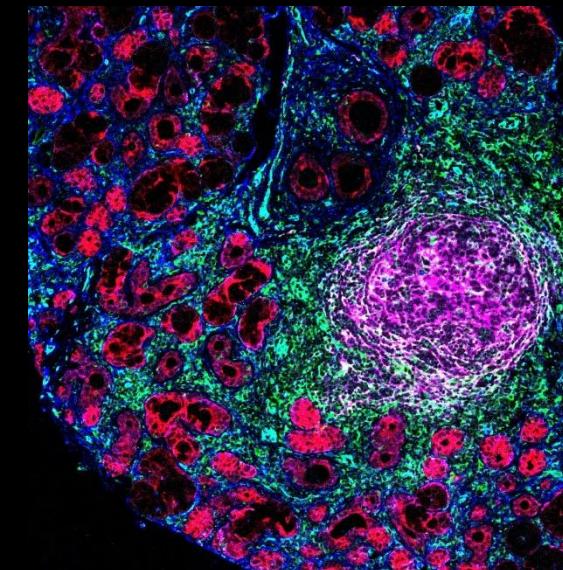
**Intermediate**



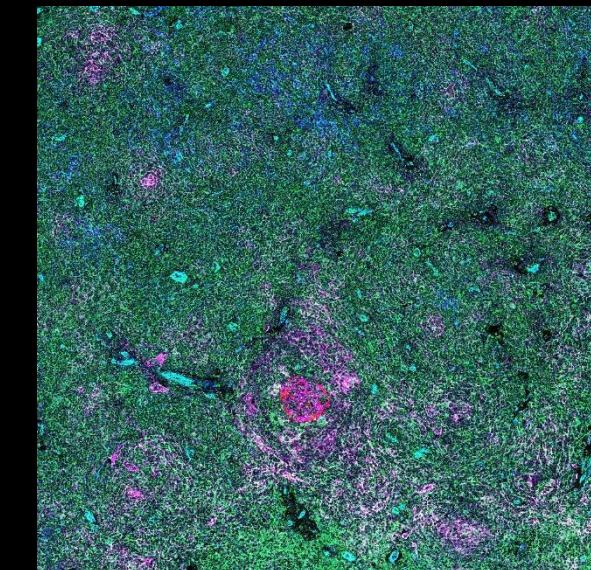
**Severe**



**TLS**



**Lymphoma**



**PanKeratin**

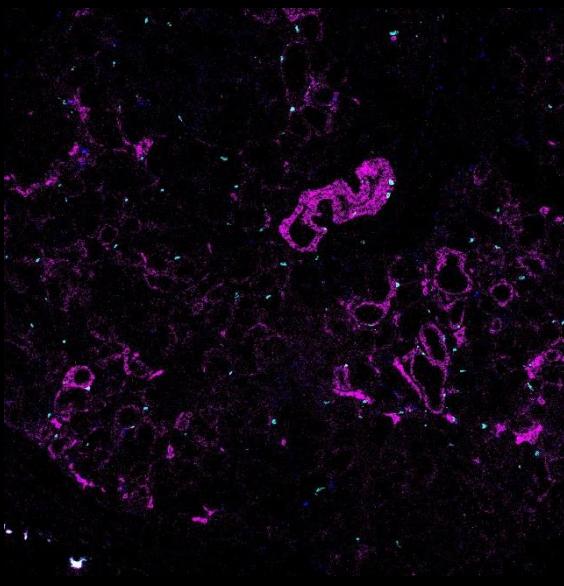
**Vimentin**

**CD34**

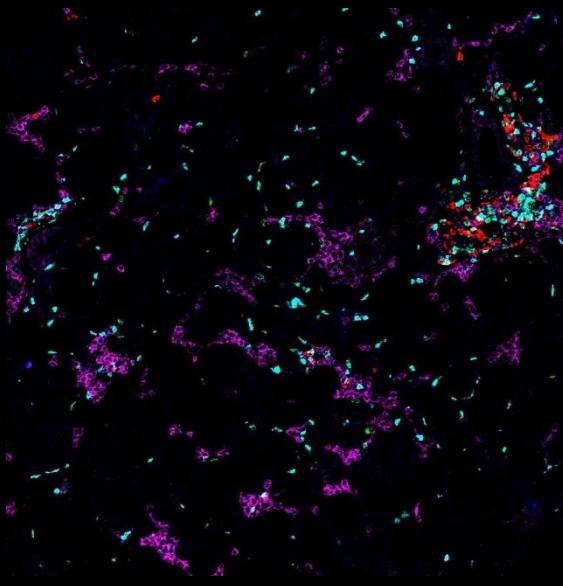
**CD31**

**Podoplanin**

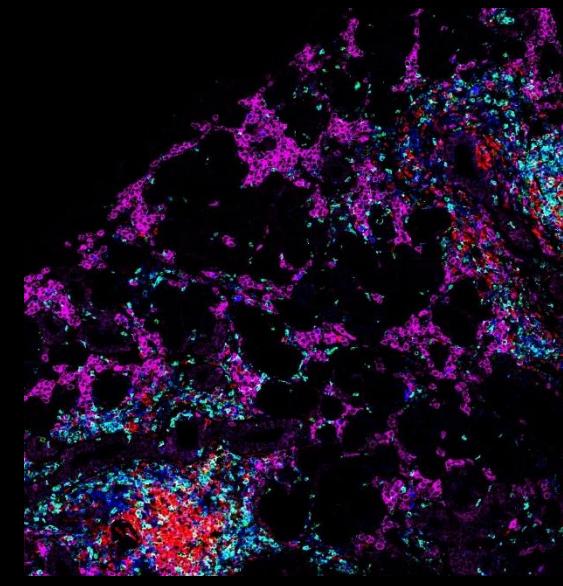
**SC**



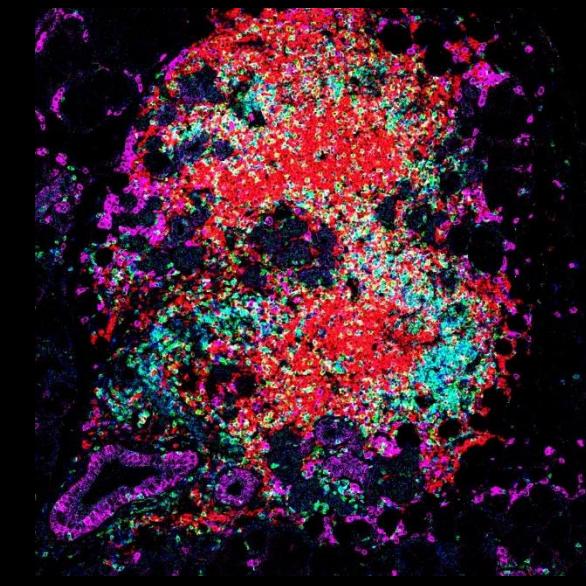
**Mild**



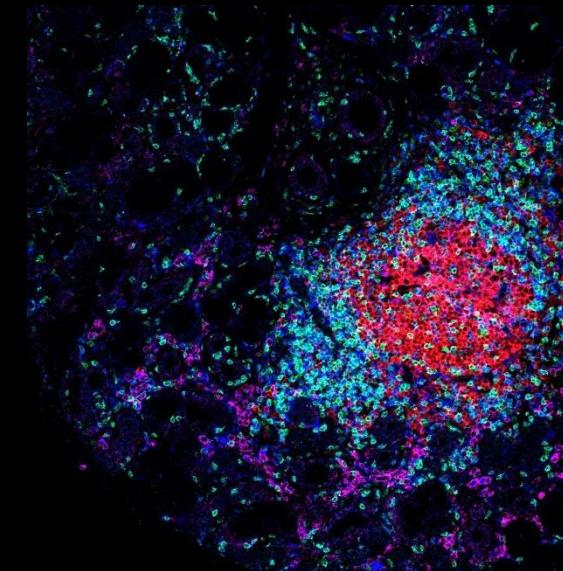
**Intermediate**



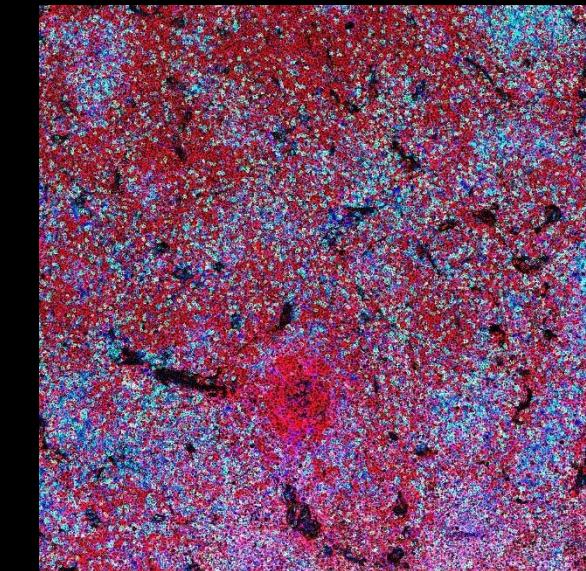
**Severe**



**TLS**



**Lymphoma**



**CD20**

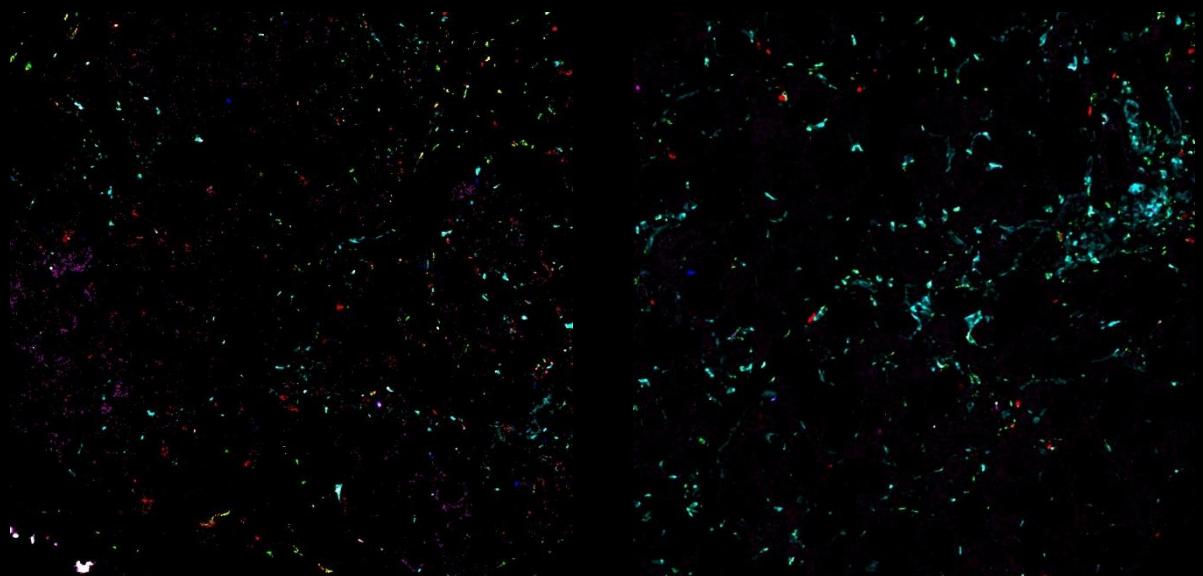
**CD3**

**CD4**

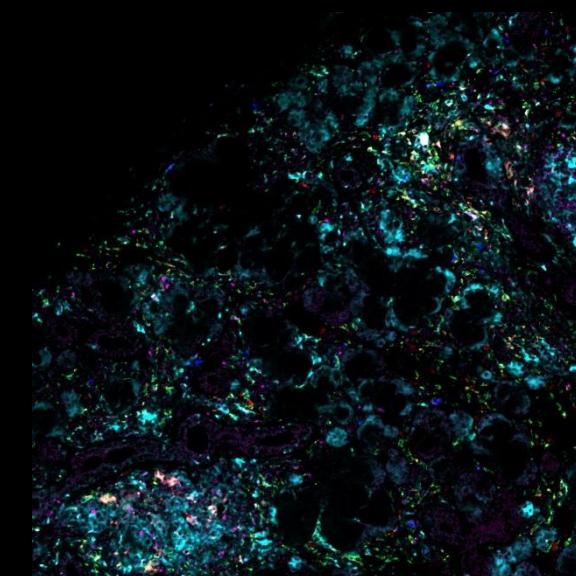
**CD8**

**CD138**

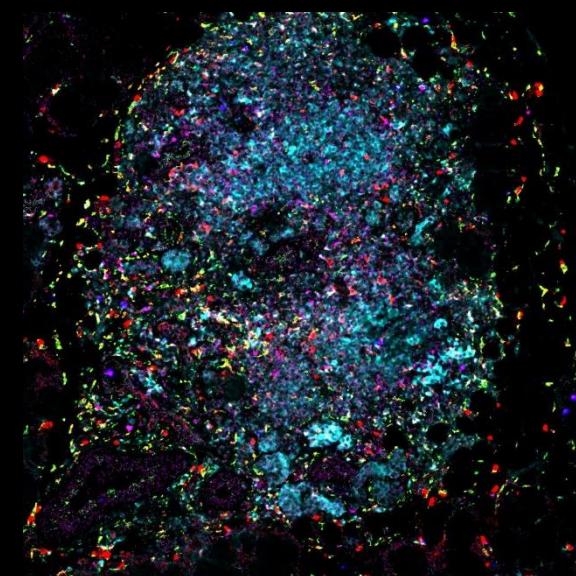
**SC**



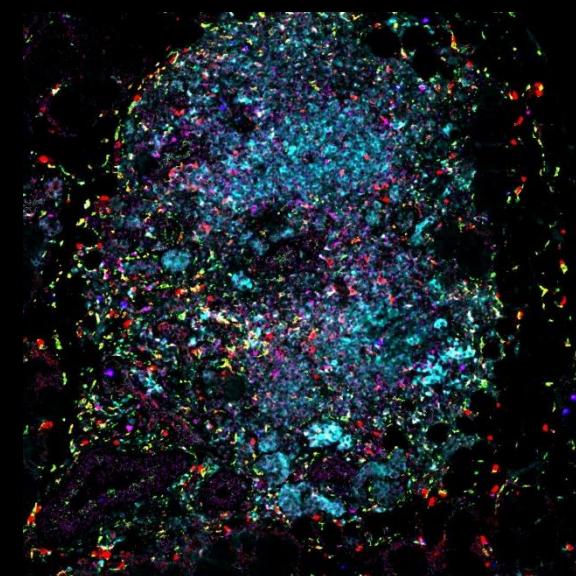
**Mild**



**Intermediate**

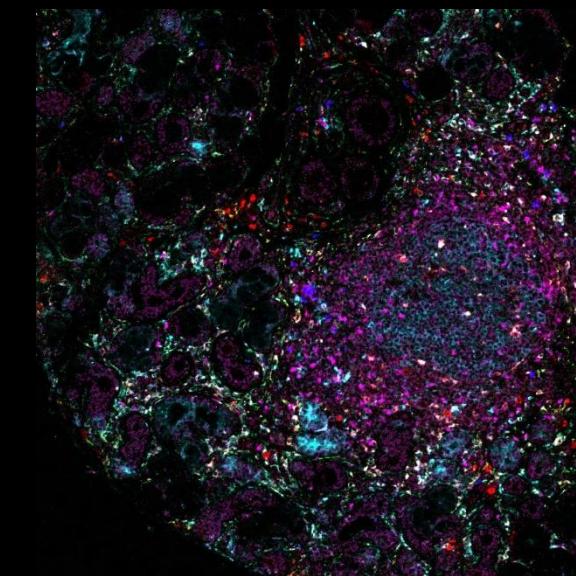


**Severe**

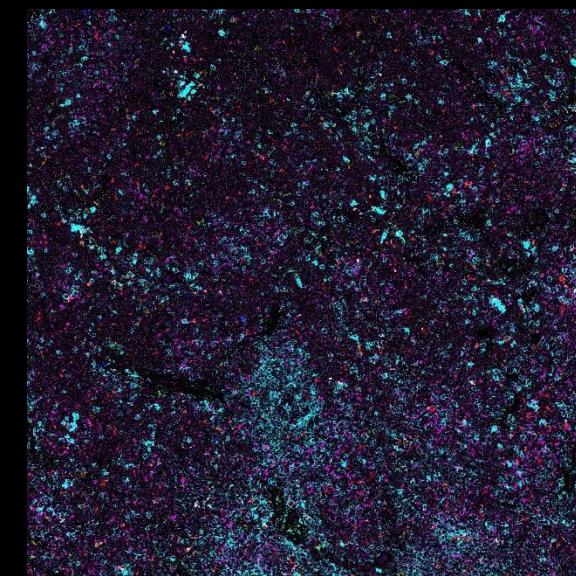


**CD68**  
**CD163**  
**MPO**  
**HLA-DR**  
**FoxP3**

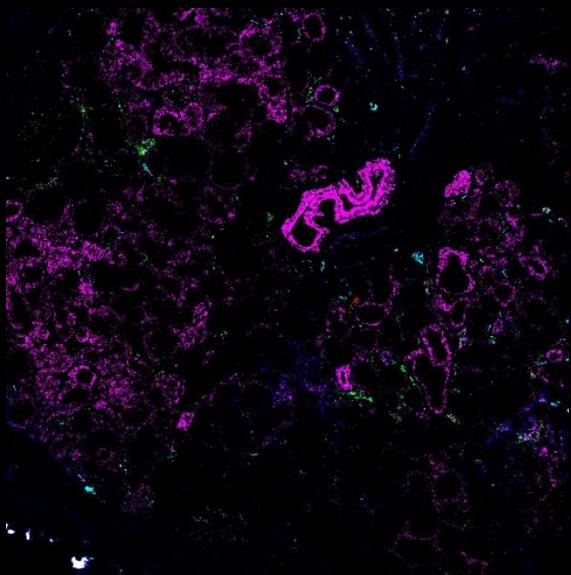
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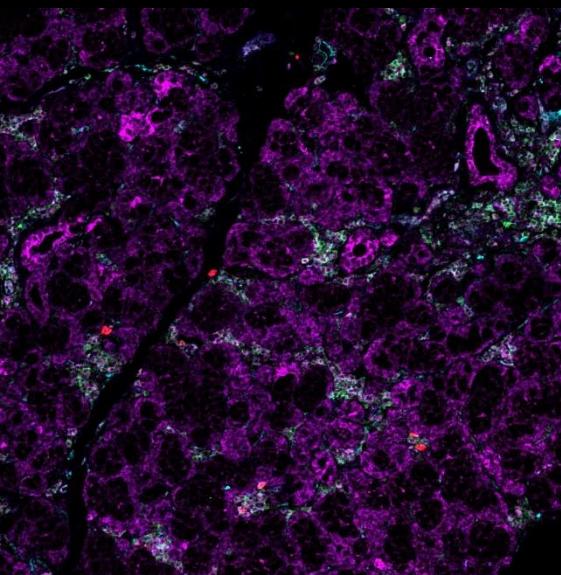
**Lymphoma**



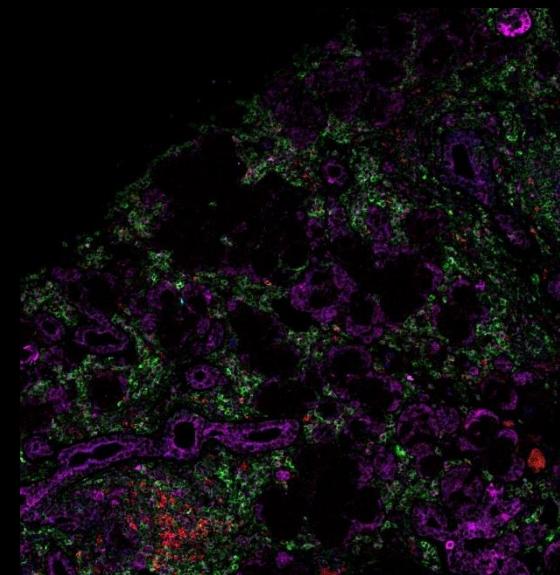
**SC**



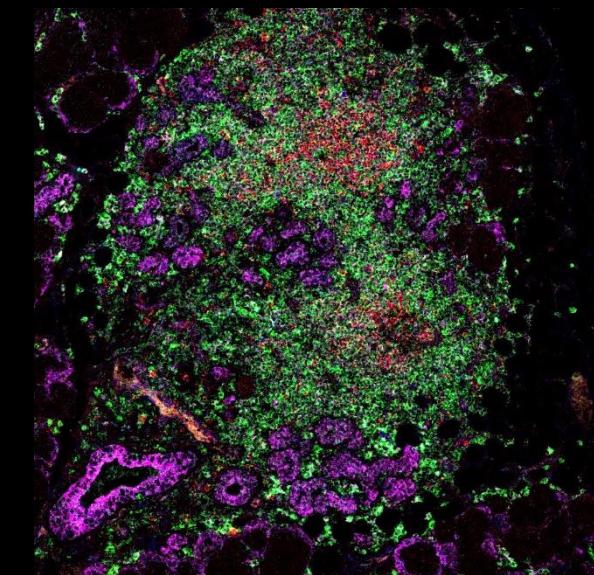
**Mild**



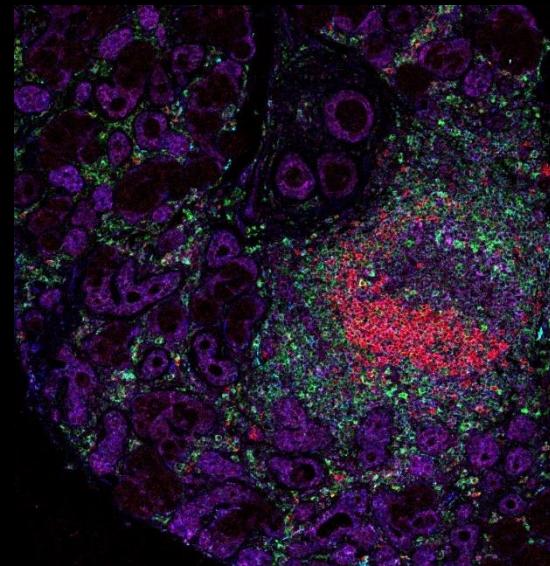
**Intermediate**



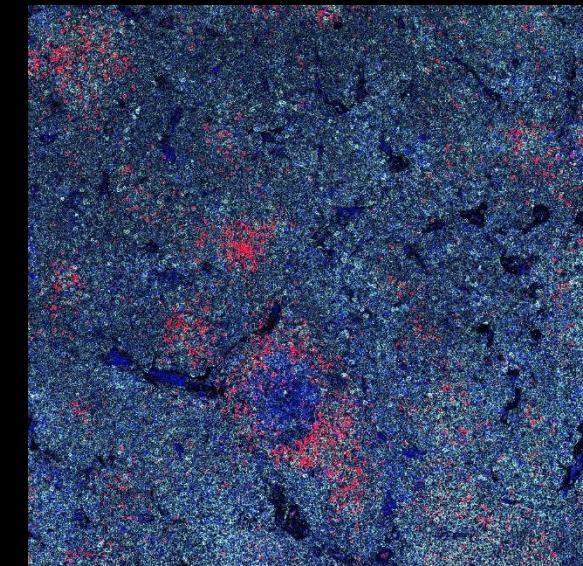
**Severe**



**TLS**



**Lymphoma**



**IgD**

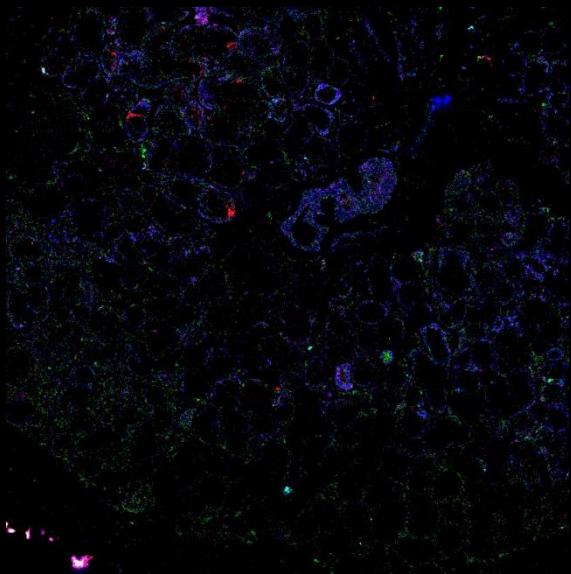
**CD27**

**Tbet**

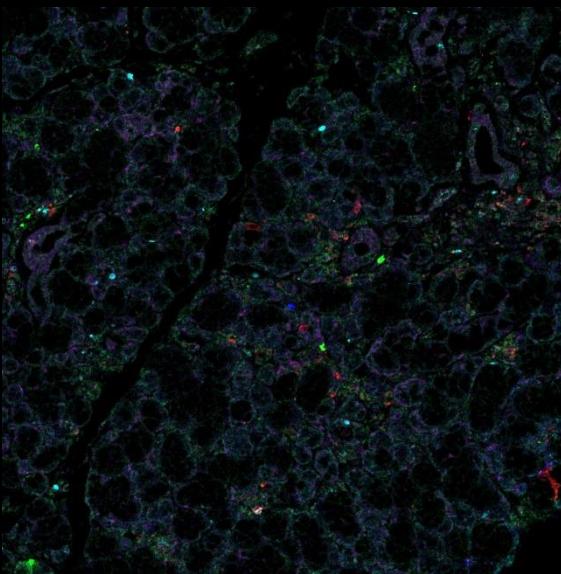
**CD56**

**CD127**

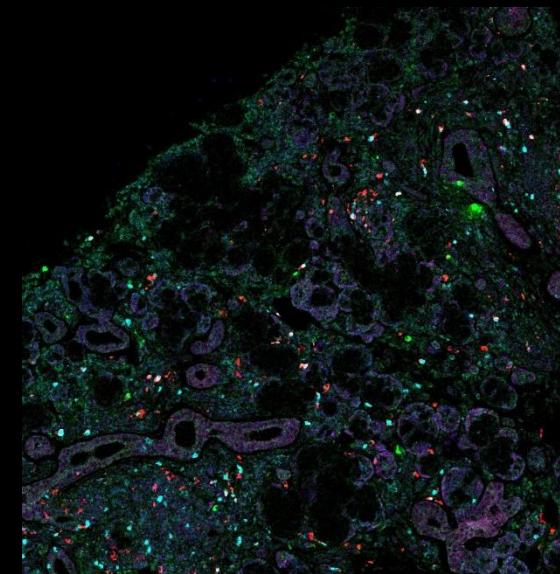
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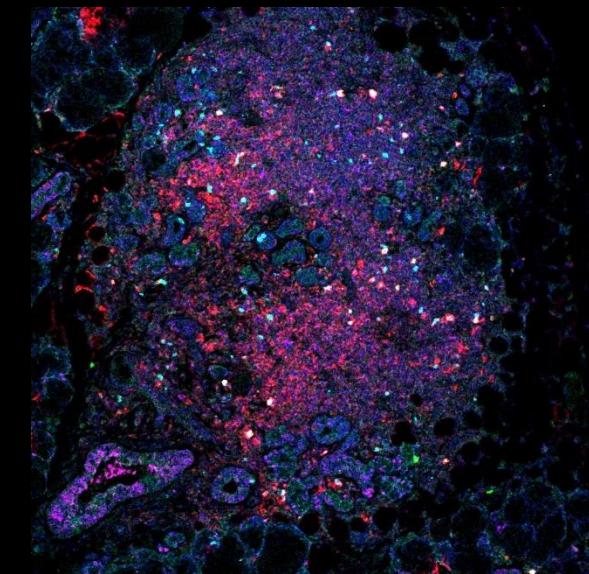
**Mild**



**Intermediate**

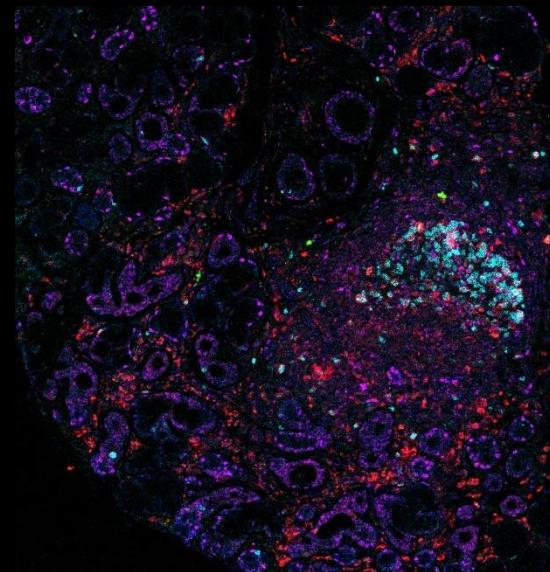


**Severe**

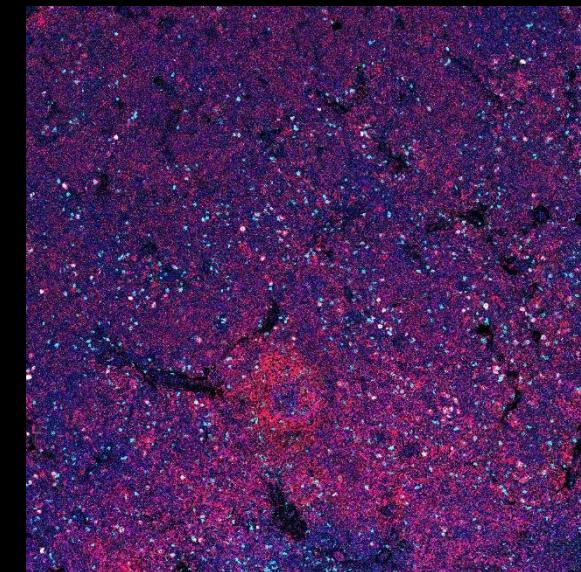


**pS6**  
**CXCL13**  
**TSLP**  
**Ki67**  
**Bcl6**

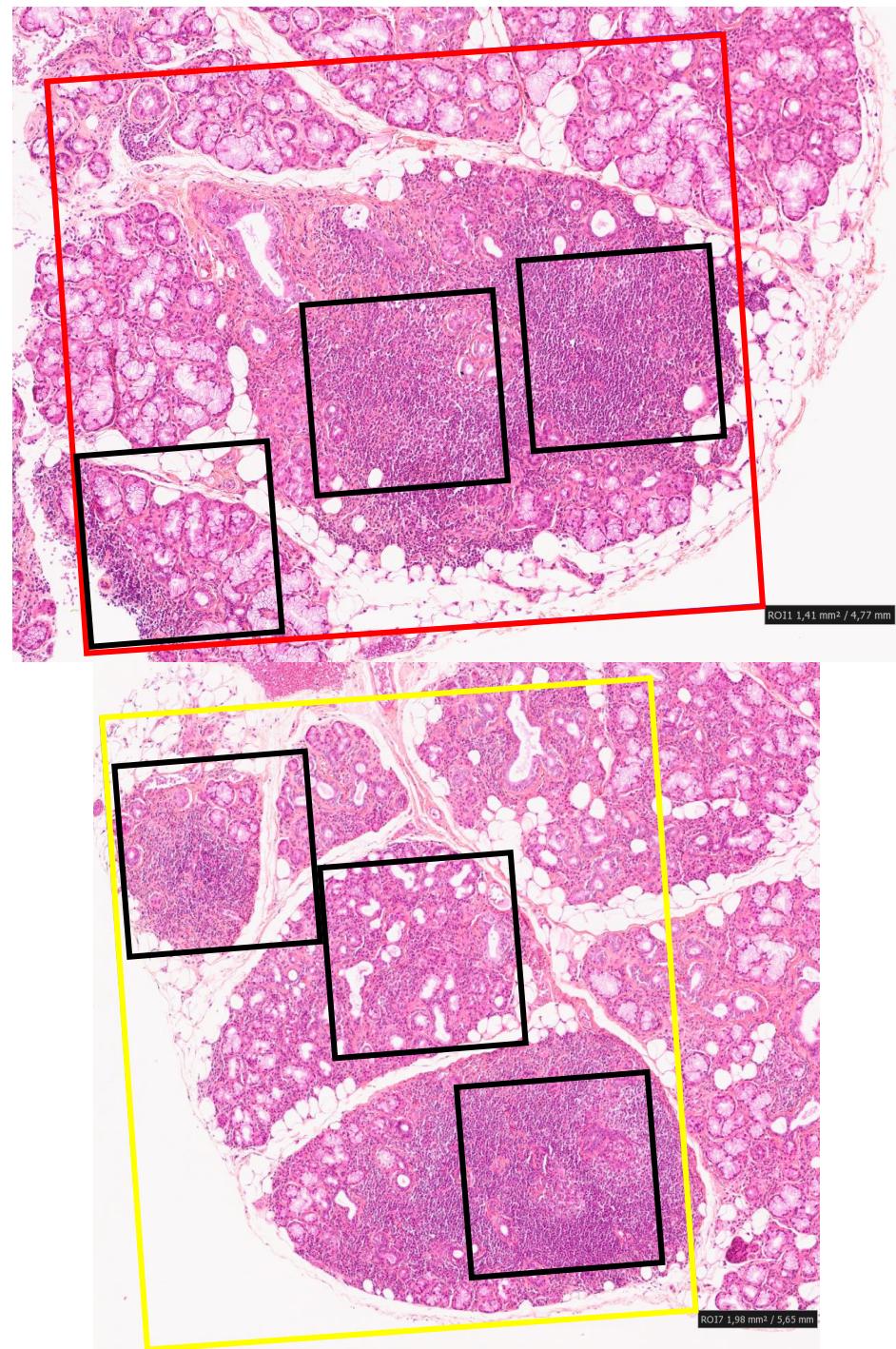
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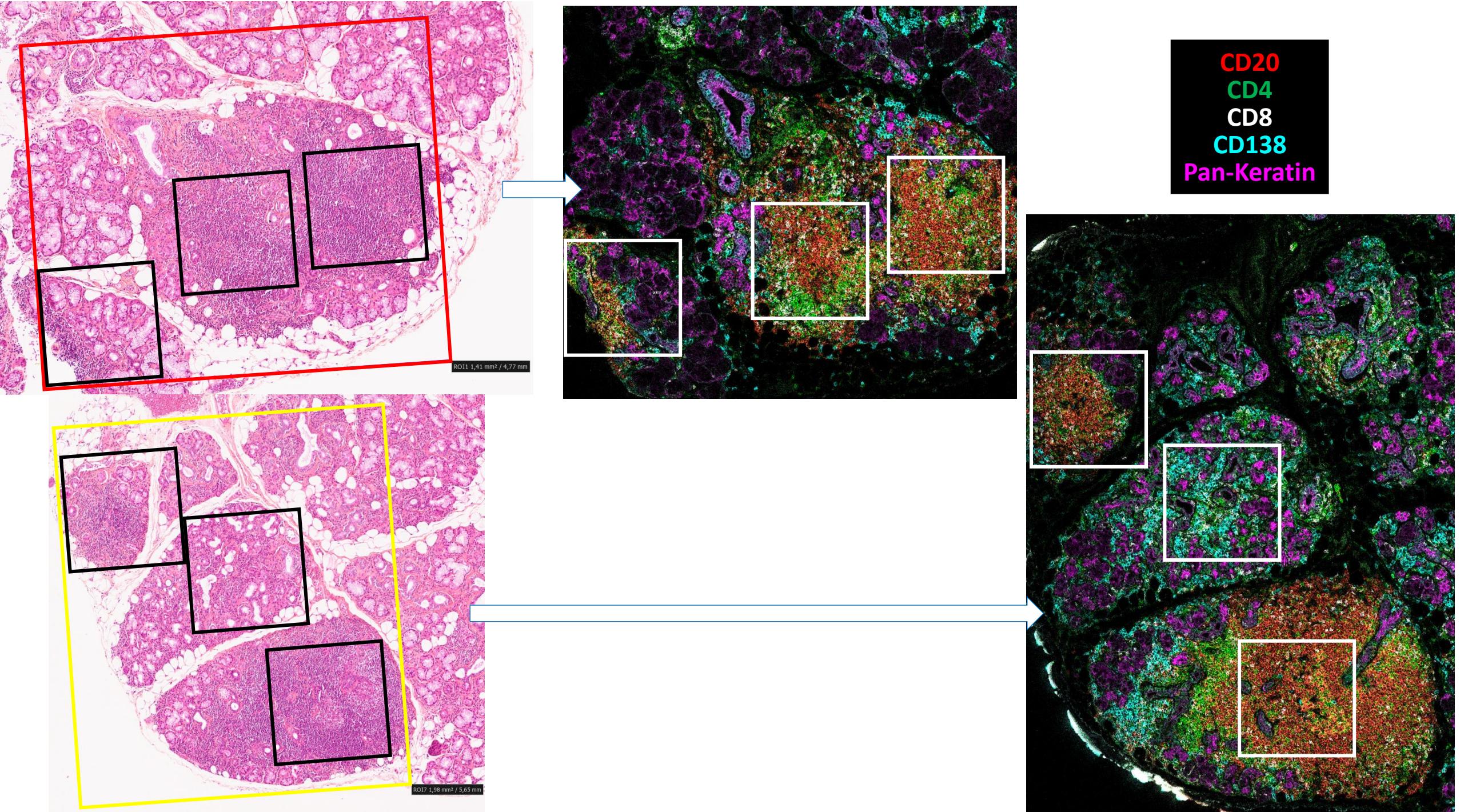


**Lymphoma**



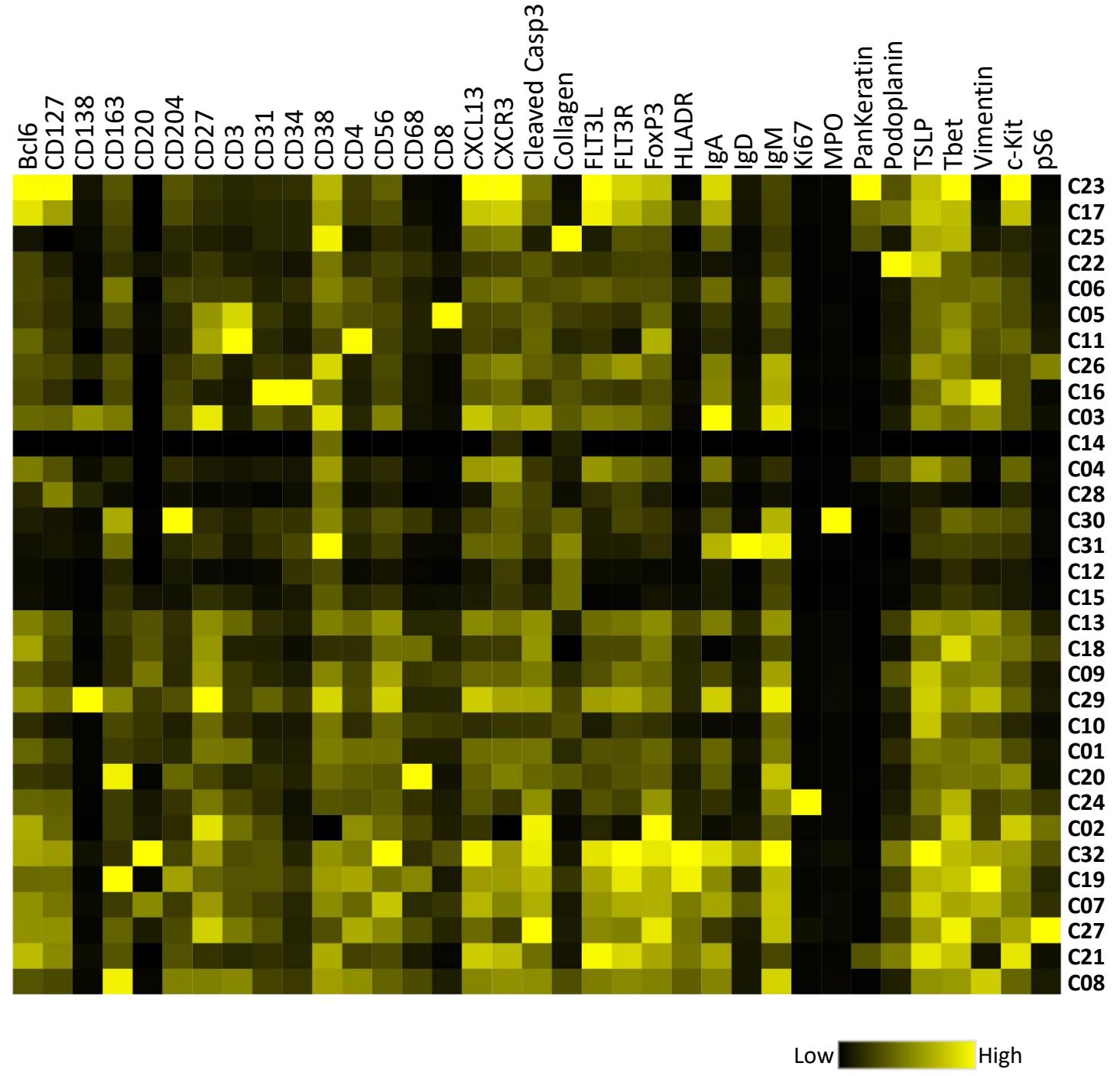
pSS patient  
Focus score >2  
Severe infiltrates



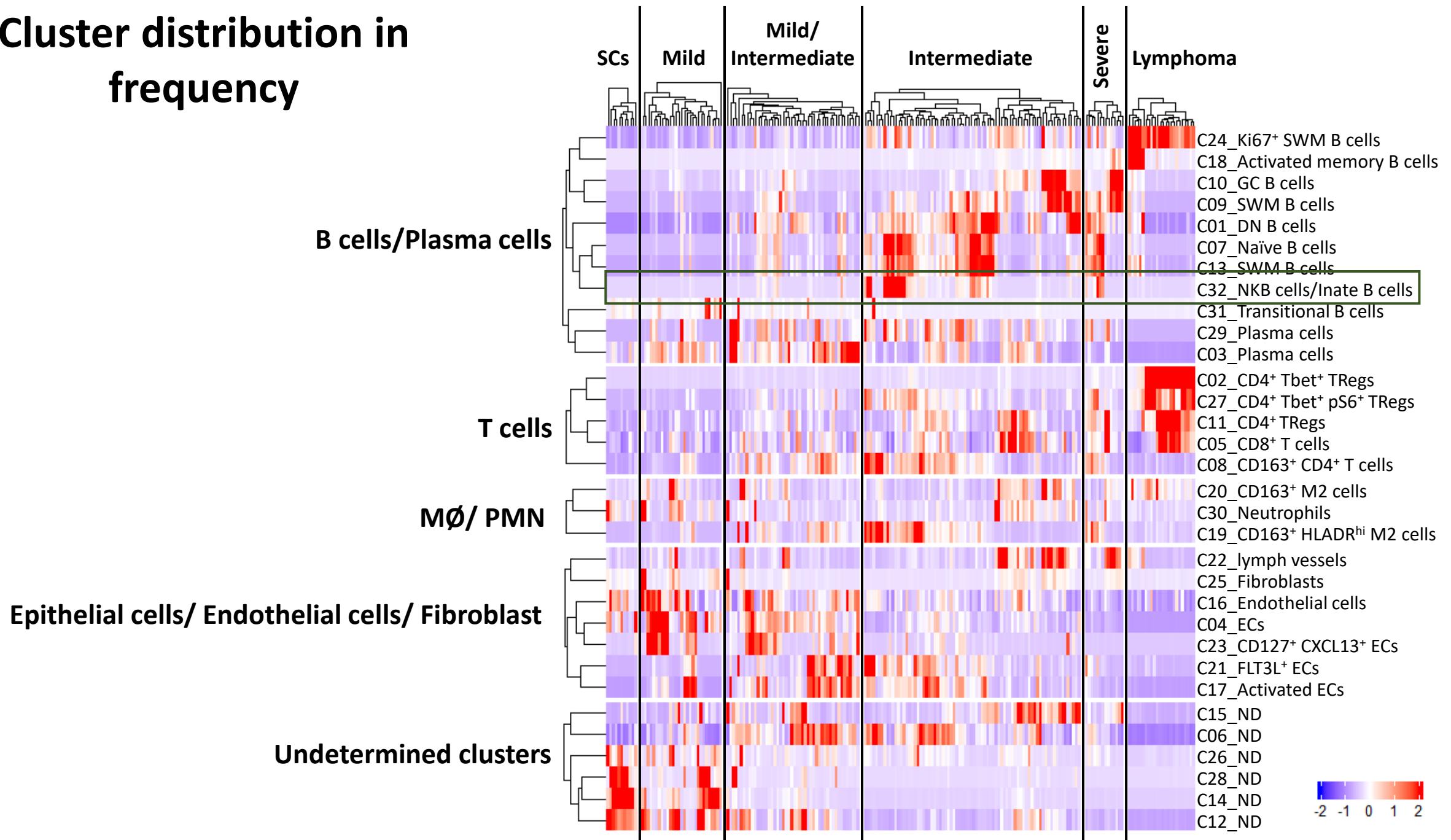


# Unsupervised analysis

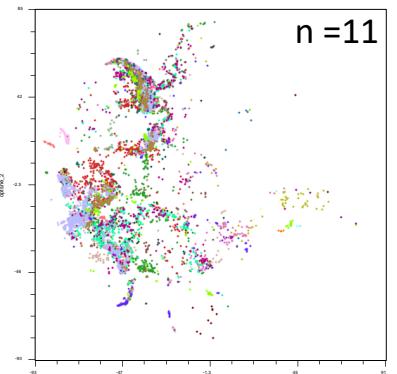
ALL ROI



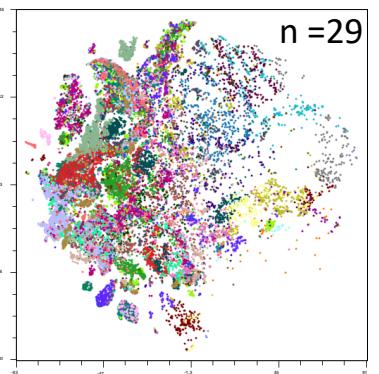
# Cluster distribution in frequency



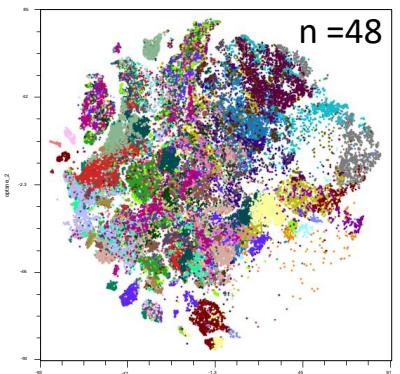
SCs



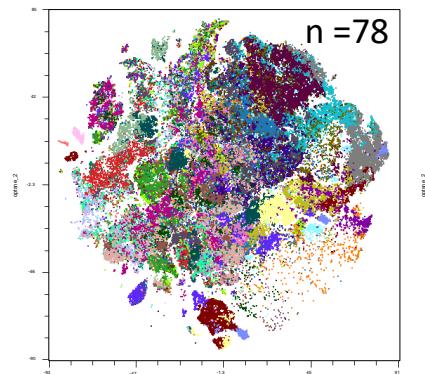
Mild



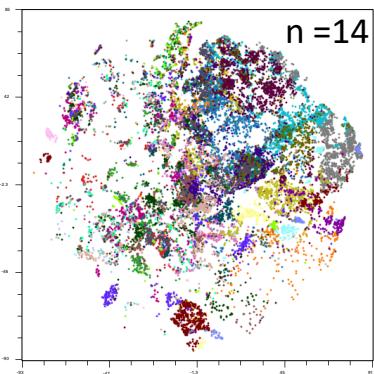
## Mild/Intermediate



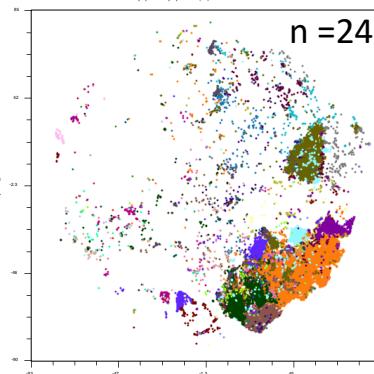
## Intermediate



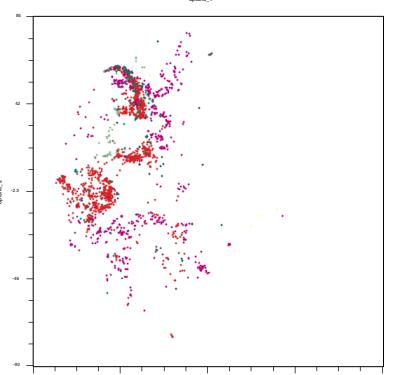
## Severe



## Lymphoma

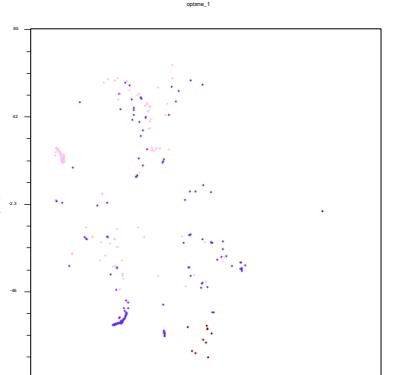


Global clusters



The figure is a scatter plot with the x-axis labeled 'k' ranging from 1 to 10 and the y-axis labeled 'silhouette width' ranging from -0.5 to 1.0. There are five data series representing different clustering methods: K-Means (black dots), K-Medoids (red dots), Agglomerative (green dots), DBSCAN (blue dots), and Spectral (cyan dots). The K-Means and K-Medoids series show a general upward trend as k increases, with K-Medoids reaching a higher silhouette width (~0.75) than K-Means (~0.65) at k=10. The Agglomerative series shows a more scattered pattern with a peak around k=5. The DBSCAN series has a single data point at k=4 with a silhouette width of approximately 0.4. The Spectral series shows a sharp increase starting from k=5, reaching a silhouette width of approximately 0.85 at k=10.

M $\emptyset$ /PMN



The figure is a scatter plot titled "optone\_1". The x-axis ranges from -10 to 10, and the y-axis ranges from -10 to 10. The data points are represented by small dots and are colored according to their density or value. The colors range from dark purple (low values) to bright yellow (high values). The pattern is highly complex and non-linear, featuring numerous overlapping and branching clusters. A prominent central cluster is located around (0, 0) with a dense core of yellow and orange points. Other significant clusters are visible along the axes and in the lower-right quadrant, characterized by large, irregular shapes of varying colors.

A scatter plot showing the expression levels of two genes, optine\_1 (x-axis) and optine\_2 (y-axis). The x-axis ranges from 0 to 10, and the y-axis ranges from 0 to 10. The data points are colored according to their expression levels, forming several distinct clusters. A color scale bar on the right indicates intensity from low (blue) to high (red).

The plot shows a complex pattern of gene expression. There are several clusters of high-expression points (red/orange) and many more clusters of lower-expression points (blue/purple). The overall distribution is somewhat circular or radial, with higher expression levels generally occurring at higher values for both genes.

A scatter plot showing the relationship between two variables, optane\_1 (Y-axis) and optane\_2 (X-axis). The Y-axis ranges from 0 to 10, and the X-axis ranges from 0 to 10. The data points are colored according to their density, with darker shades representing higher concentrations. Several distinct clusters of points are visible, primarily concentrated in the lower-left, upper-left, and upper-right regions of the plot. A small cluster of red points is located near the bottom center.

A horizontal bar chart consisting of seven colored squares arranged from left to right. The colors are: dark teal (C25), red (C04), dark teal (C17), light green (C23), yellow (C21), magenta (C16), and dark grey (C22). Below the bars is a thick black horizontal line.

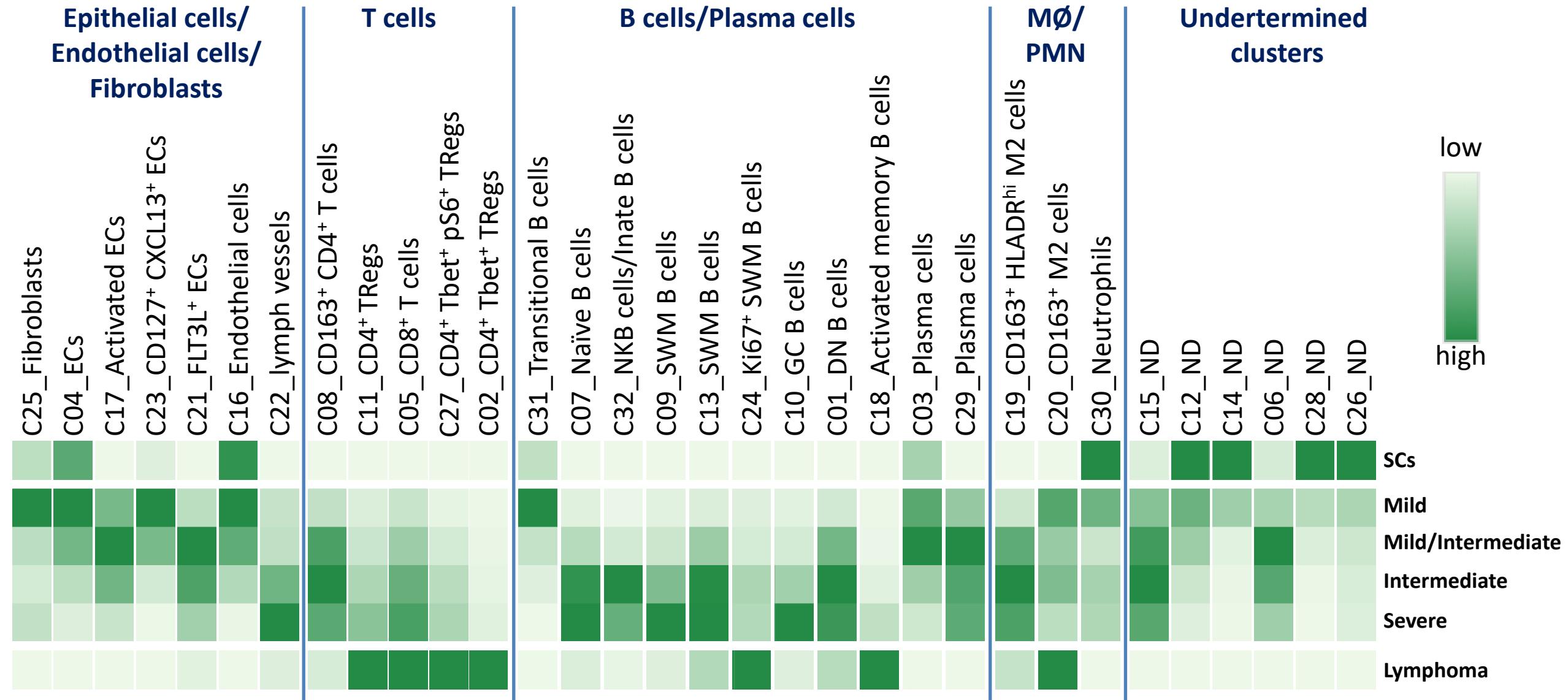
A horizontal color bar consisting of twelve colored squares arranged from left to right. The colors are: red (C31), grey (C07), blue (C32), cyan (C09), dark purple (C13), light blue (C24), dark purple (C10), blue (C01), dark olive green (C18), green (C03), and brown (C29). Above the color bar, the labels C31 through C29 are written vertically above each corresponding square. To the right of the color bar, the text "optique\_1" is written.

optane\_1

C19    C20    C30

A horizontal row of six colored squares, each labeled with a two-letter code above it. From left to right, the colors are brown (C15), teal (C12), light blue (C14), magenta (C06), olive green (C28), and lime green (C26). The labels are positioned above the squares.

# Cluster distribution within the different type of infiltrates





# Acknowledgements

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Athens

- Athanasios G. Tzioufas
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- Andreas Goules
- Michael Voulgarelis
- Chiara Baldini
- Salvatore De Vita
- Ourania Argyropoulou
- Ioanna Stergiou
- Vasilis Pezoulas



Pathophysiology Dept.  
School of Medicine

