National and Kapodistrian University of Athens

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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.*

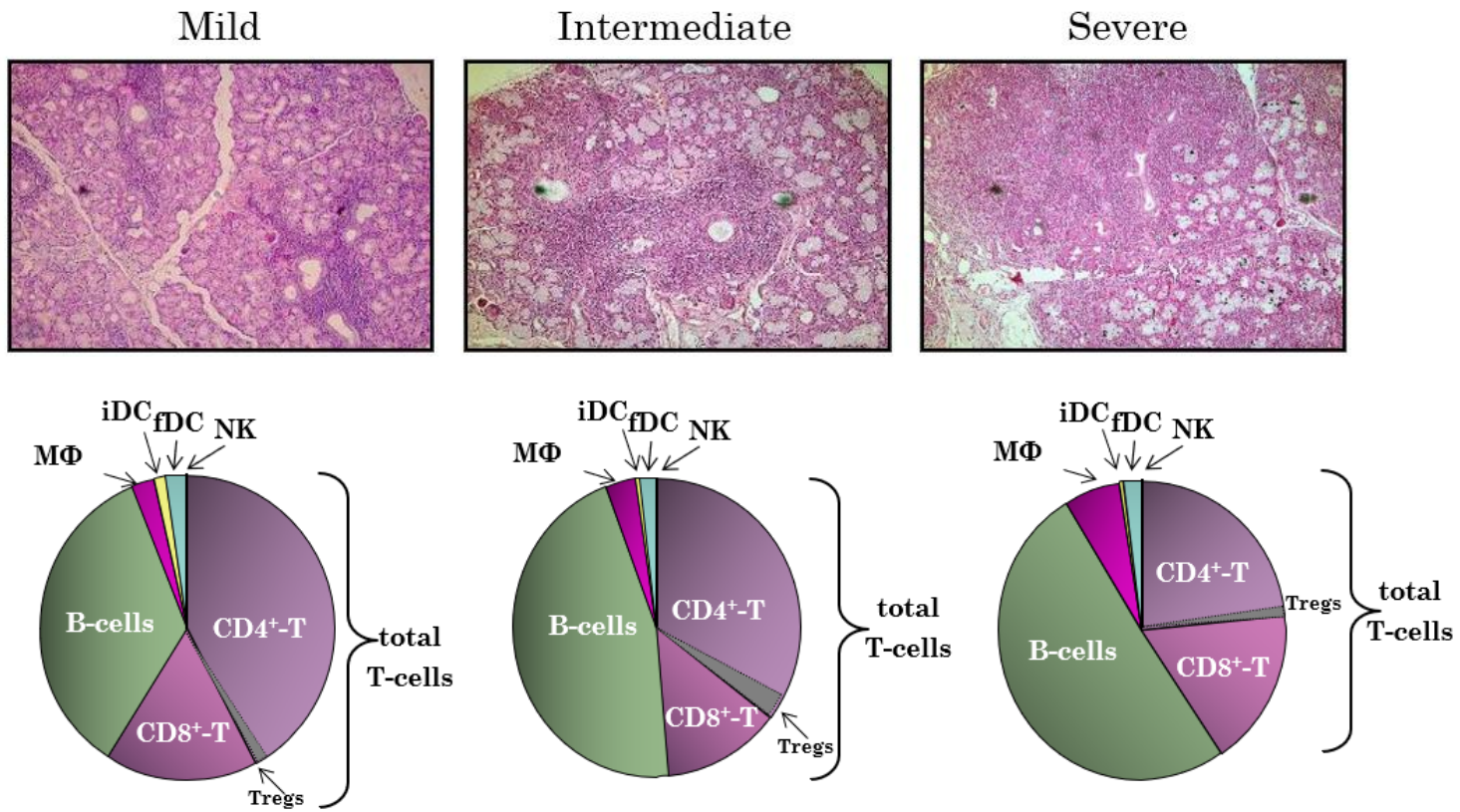
Pathophysiology Dept.
School of Medicine

# 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

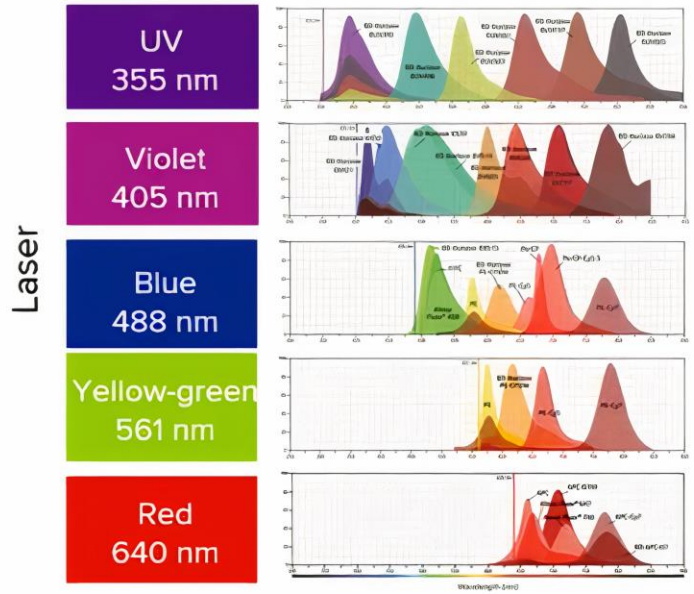



*Christodoulou et al., J Autoim., 2009*

# Antibody-mediated multiparameter protein detection

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

**Fluorescence spillover | Variable staining intensities | Background signal**




Emission: fluorescence spillover

Staining intensities and background

# HYPERION® (Fluidigm)

# The highly-multiplexed tissue imaging:

# HYPERION ® technologies


**Capture**

Hyperion tissue imager

**Detection**

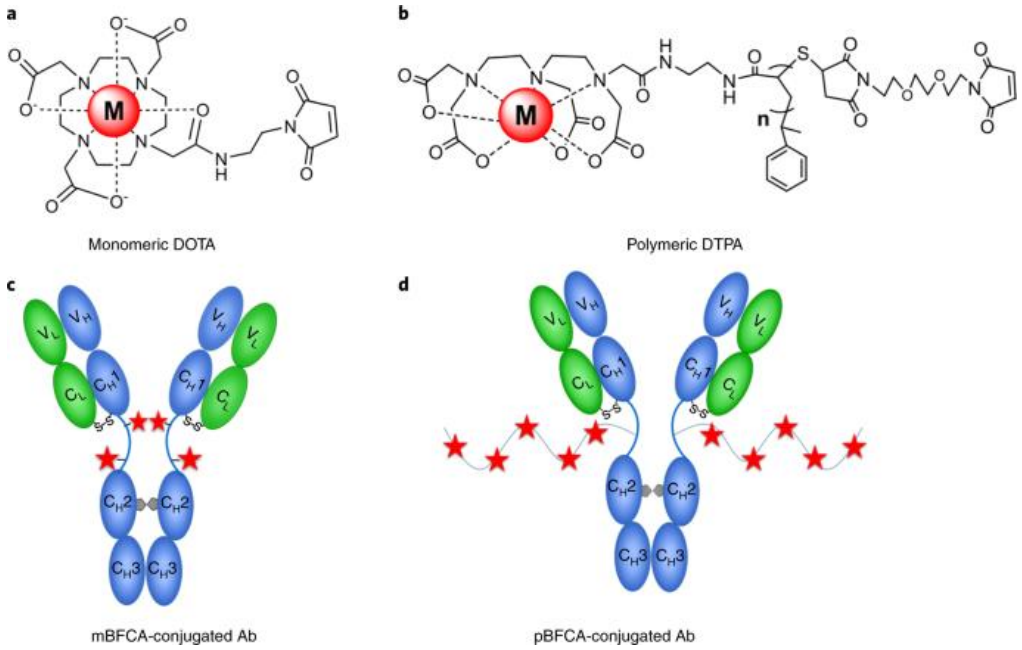
CyTOF technology

Labelling → Instrument → Analysis

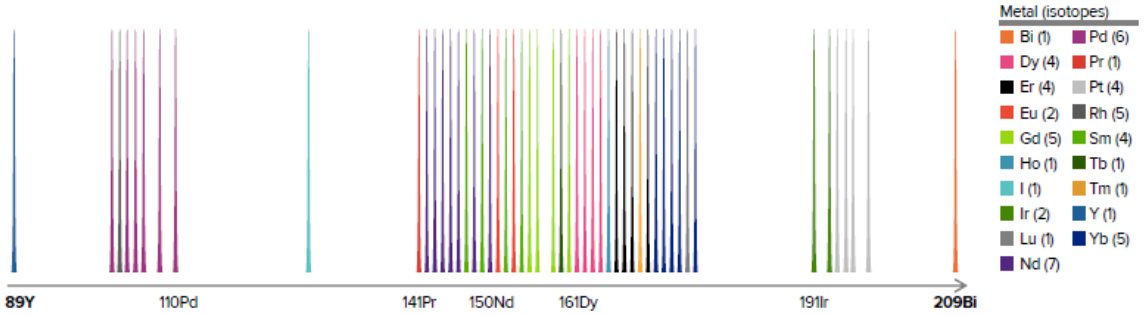
- Up to **40 proteins** simultaneous detection
- Stain samples with **all antibodies simultaneously**
- **Spatial** analysis of protein markers on a single tissue section
- **FFPE** or **fresh frozen** tissue

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




Highly pure, rare metal isotope labels that minimize background



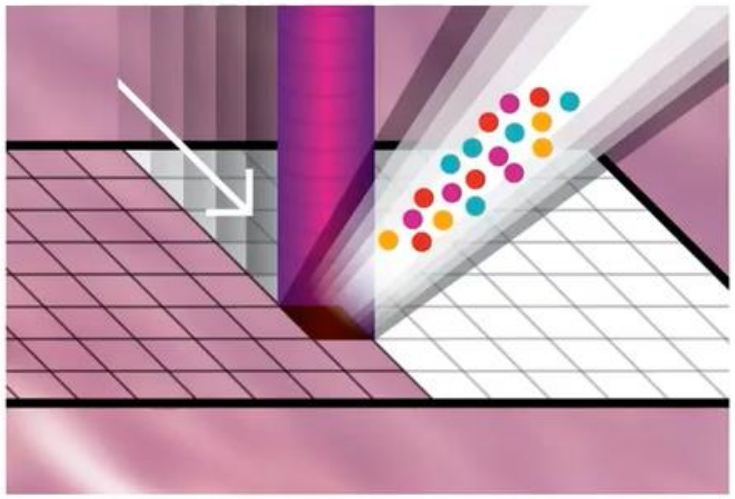
*Metal-isotope-tagged monoclonal antibodies for high-dimensional mass cytometry - Nature Protocols – Garry P. Nolan*

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 µm² spots in the selected region

Collects metal-tagged proteins

Sends for CyTOF analysis

- Laser beam focuses on **1 µm2** spots in the selected region
- Collects **metal-tagged proteins**
- Sends for **CyTOF analysis**

# Data pre-processing – Pipeline

Data conversion and preparation

IMC formats

.mcd
.txt
.ome.tiff

→ Analysis stacks

.tiff

Cell profiler stack preparation

Random Cropping

Analysis stacks

.h5

Noise reduction

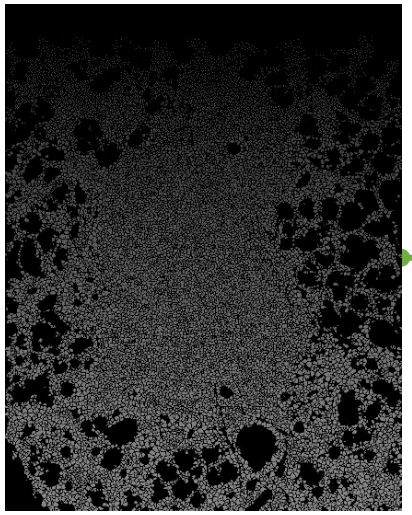
Ilastik pixel classification

Pixel classification

↓

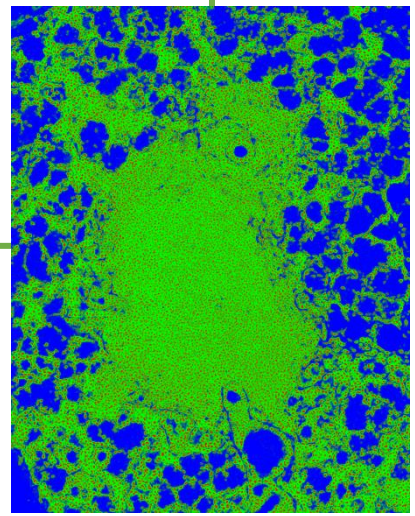
Probability mask

.tiff



Cell segmentation and quantification

Nuclear and cell segmentation based on probability mask



Analysis

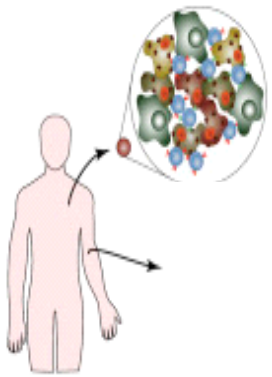
Plateforme de cytométrie en flux et de masse

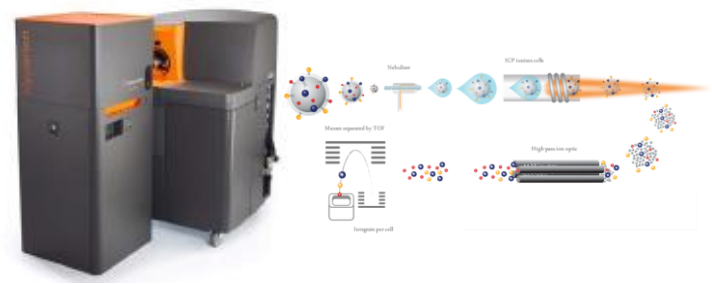
HYPERION

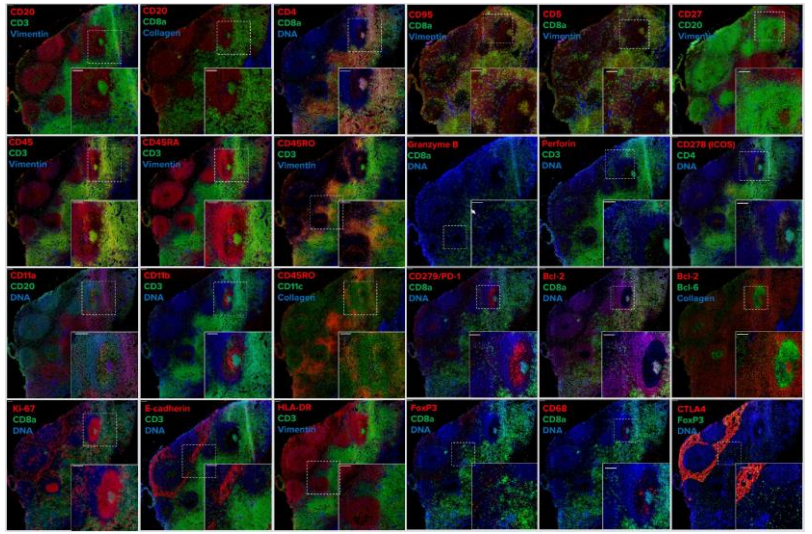
HYPE Research in Immunology and ONcology

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 µm.

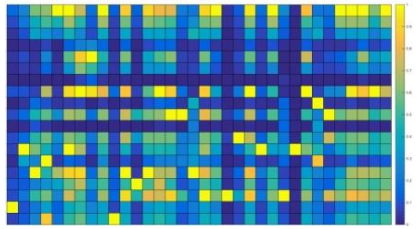
## Tissue

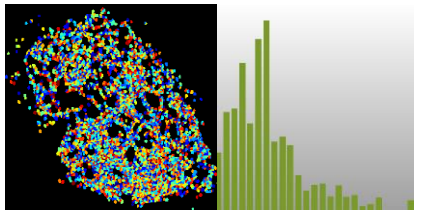






**Phenograph**



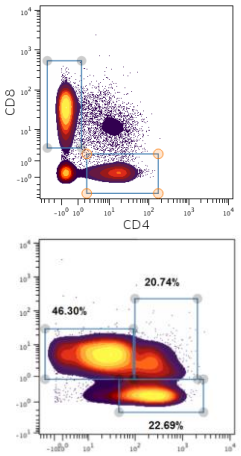


**Neighborhood analysis**

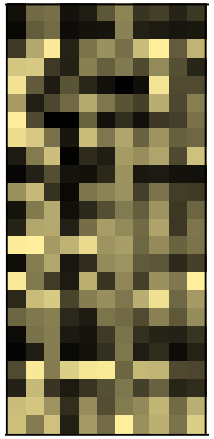
**Panel**

CD45
CD196 CCR6
CD123
CD19
CD4
CD8a
CD11c
CD16
CD45RO
CD45RA
CD161
CD194 CCR4
CD25
CD27
CD57
CD183 CXCR3
CD185 CXCR5
CD28
CD38
CD56 NCAM
TCRγδ
CD294
CD197 CCR7
CD14
CD3
CD20
CD66b
HLA-DR
IgD
CD127
Cell-ID
CD24
CD11b
CD163
IgM
CD21
CD5

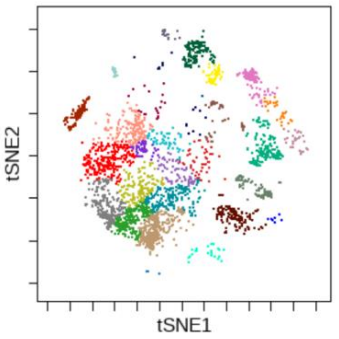
**Dot Plot**



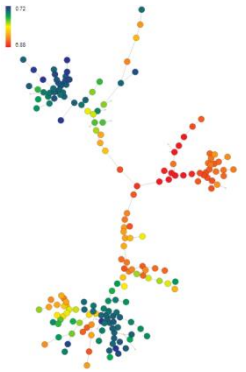

**Heatmap**



**tSNE**




**SPADE**



**Supervised analysis**

**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

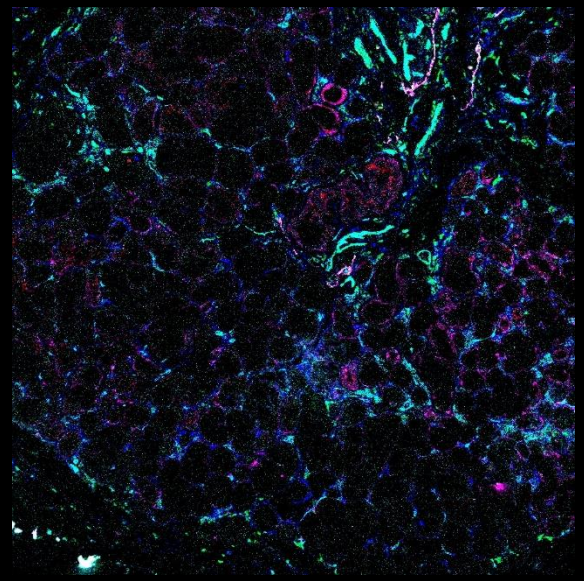
| General Panel | Properties |
|---|---|
| CD38 | Plasma cells, Immature/ transitional b cells, effector T cells |
| CD204 | M2 macrophage |
| Vimentin | Mesenchymal cells |
| CD14 | **Macrophages**, dendritic cells, neutrophils |
| Tbet | Th1 T cells |
| CD34 | Hematopoietic progenitor cells, stem cells |
| CD163 | M2 macrophages |
| Pan-Keratin | Epithelial cells |
| CD11b | Monocytes, B1 B cells, natural killer (NK) cells, and dendritic cells, cDC2 |
| TSLP | Lymphoma, Epithelial cells |
| CD31 | Endothelial cells in blood vessels, platelels |
| Ki-67 | Proliferation |
| IgD | Mantle zone B cells, naive B cells |
| IgM | Mantle zone B cells, naive B cells |
| FoxP3 | T regulatory cells |
| Cd4 | T helper cells |
| cKit | Cancer, Stem cells |
| CD68 | M1 Macrophages |
| IgA | Plasma cells and germinal center immunoblasts |
| AID | Somatic hypermutation and immunoglobulin class switch recombination |

| General Panel | Properties |
|---|---|
| Bcl6 | GC B cells and T follicular helper cells |
| CD20 | B lymphocytes |
| CD8a | Cytotoxic T cells |
| CD138 | Plasma cells |
| MPO | Neutrophils |
| Flt3Ligand | T cells |
| CD56 | NK cells |
| CD127 | Memory and Effector T cells not on Treg, Precursos B cells |
| Collagen | Collagen type I |
| CD3 | T cells |
| CD27 | Memory T cells (except an effector memory population), B cells (not effector), Plasmablasts (High) NK cells |
| Caspase-3 | Cell apoptosis |
| Podoplanin | Follicular DCs, Lymphatic endothelial cells |
| HLA-DR | Dendritic cells, macrophages, B cells, stimulated epithelial cells |
| pS6 | Akt/PI3K/mTOR pathway related proliferation |
| Flt-3 | Plasmacytoid DCs, B progenitor cells |
| CXCL13 | Follicular dendritic cells |
| CXC3 | Activated Th1 cells, CD8 effector cells, Nk cells, proinflmmatory B cells |

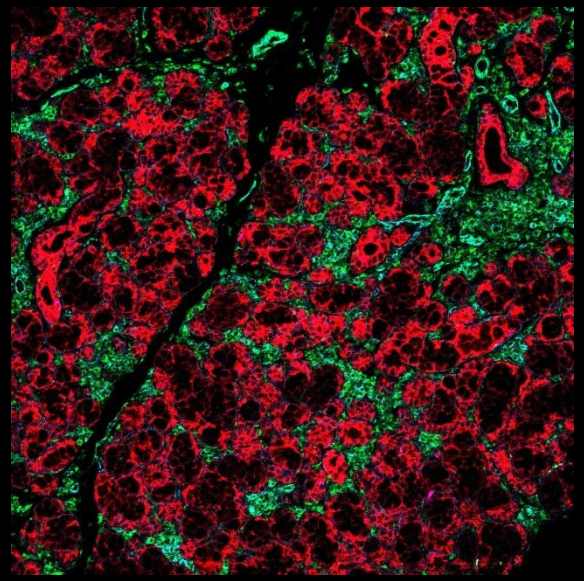
>>>>>>

| B cell panel | Properties |
|---|---|
| CD45RB | Memory B cells, Cd38 positive B cells outside of GM |
| Vimentin | Mesenchymal cells |
| Tbet | Th1 T cells |
| Pan-Keratin | Epithelial cells |
| Ki-67 | Proliferation |
| IgD | Mantle zone B cells |
| FoxP3 | T regulatory cells |
| CD4 | T helper cells |
| CD68 | M1 Macrophages |
| Bcl6 | GC B cells and T follicular helper cells |
| CD20 | B lymphocytes |
| CD8a | Cytotoxic T cells |
| CD138 | Plasma cells |
| MPO | Neutrophils |
| PD-1 | T follicular helper cells |
| Collagen | Collagen type I |
| CCR6 | Bmem |
| CD3 | T cells |
| CD27 | Memory B cells (except an effector memory population), T cells (not effector), NK cells |
| 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 |

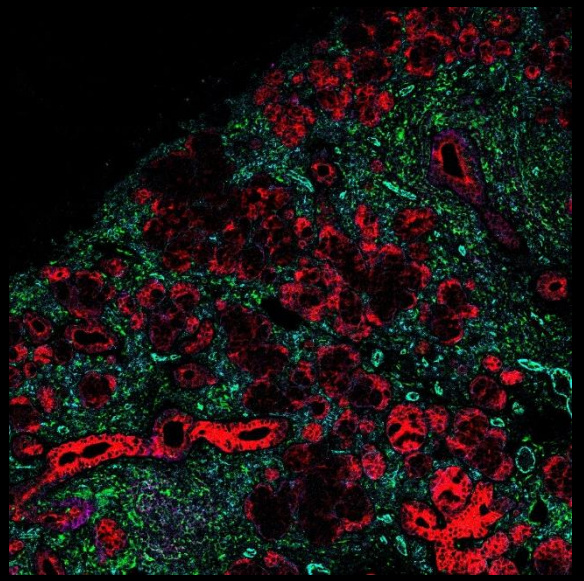
SC



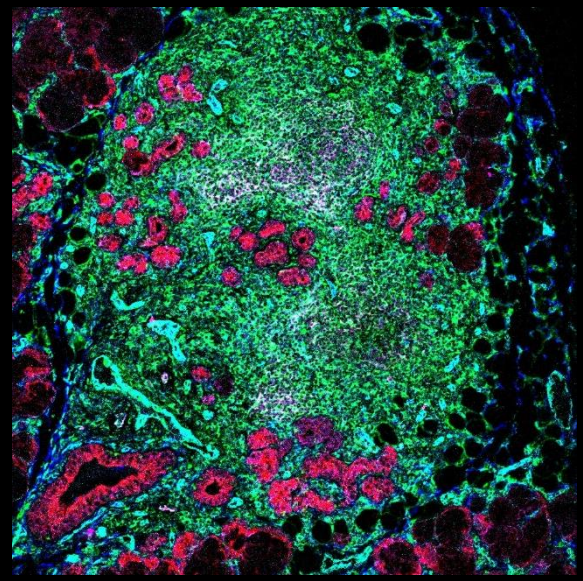
Mild



Intermediate



Severe



PanKeratin

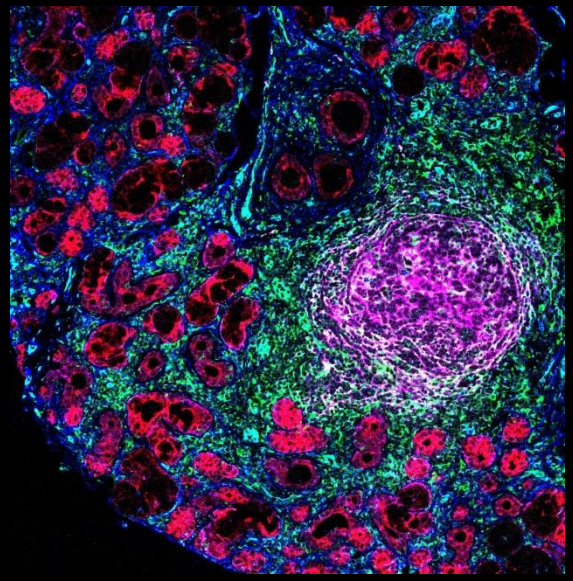
Vimentin

CD34

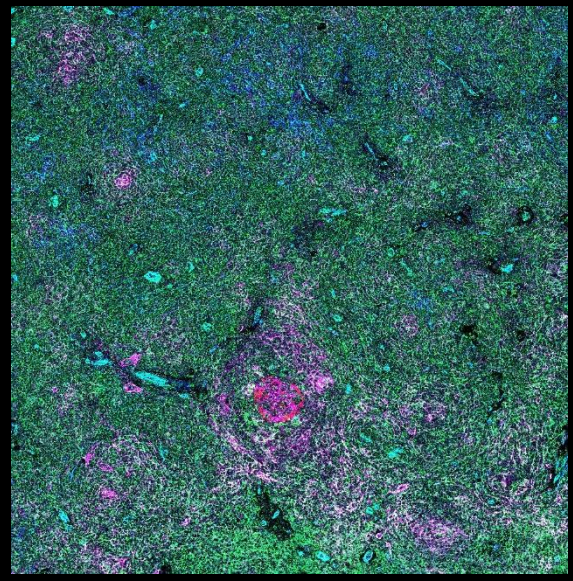
CD31

Podoplanin

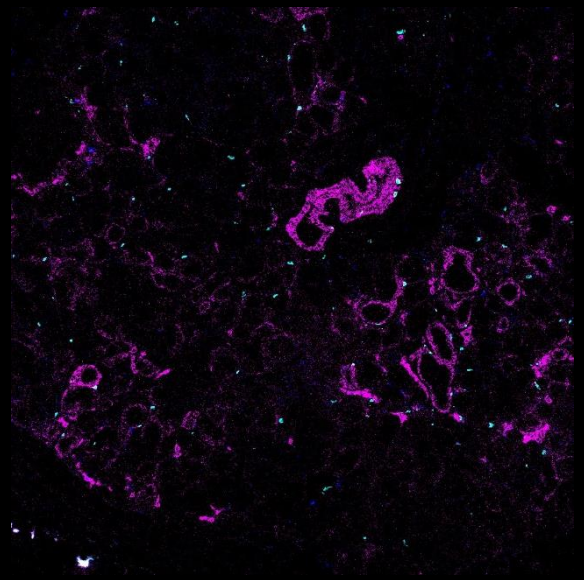
TLS



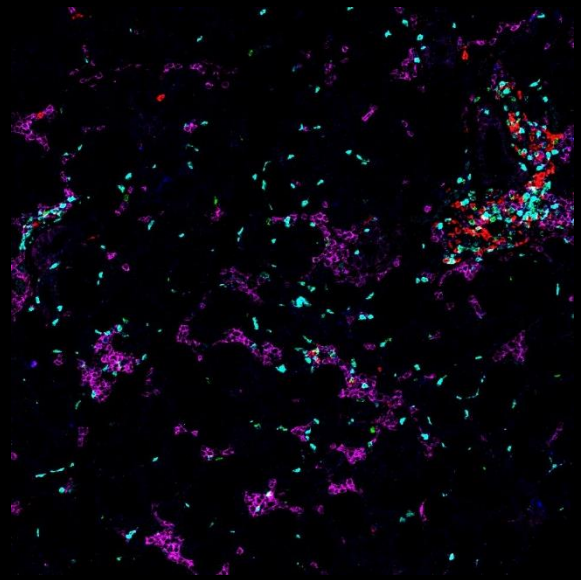
Lymphoma



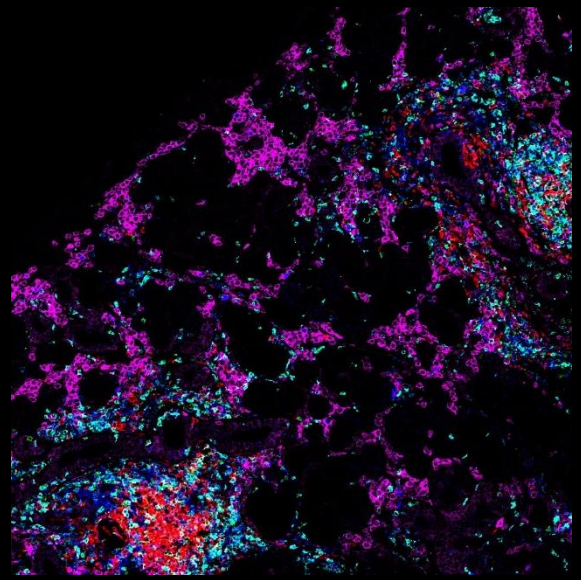
SC



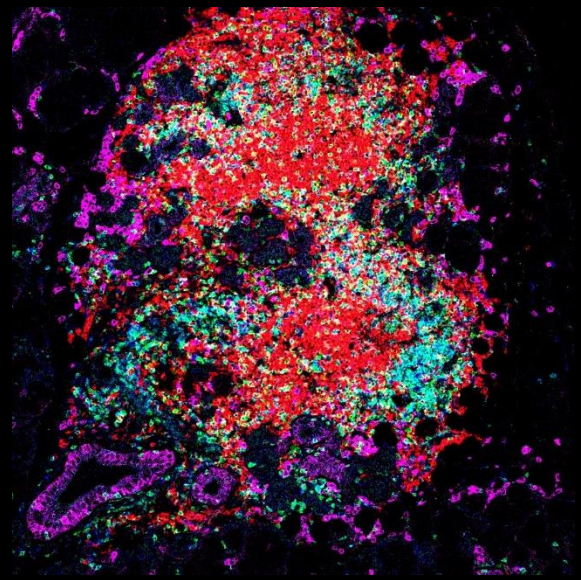
Mild



Intermediate



Severe



CD20

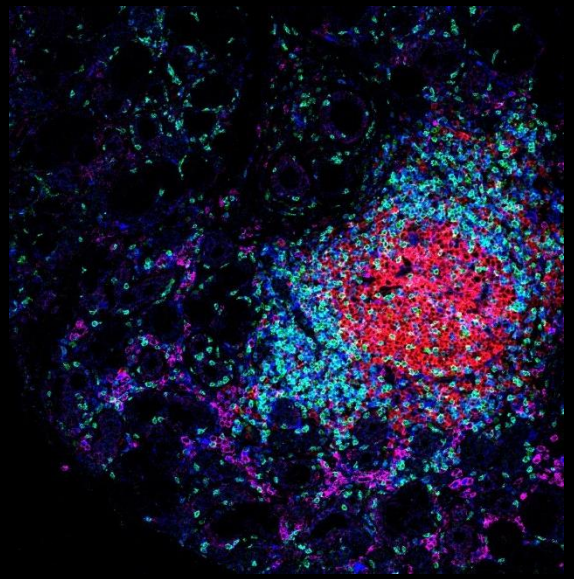
CD3

CD4

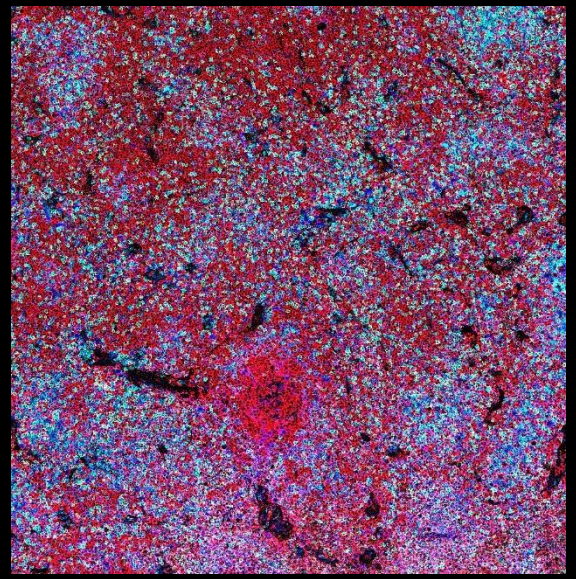
CD8

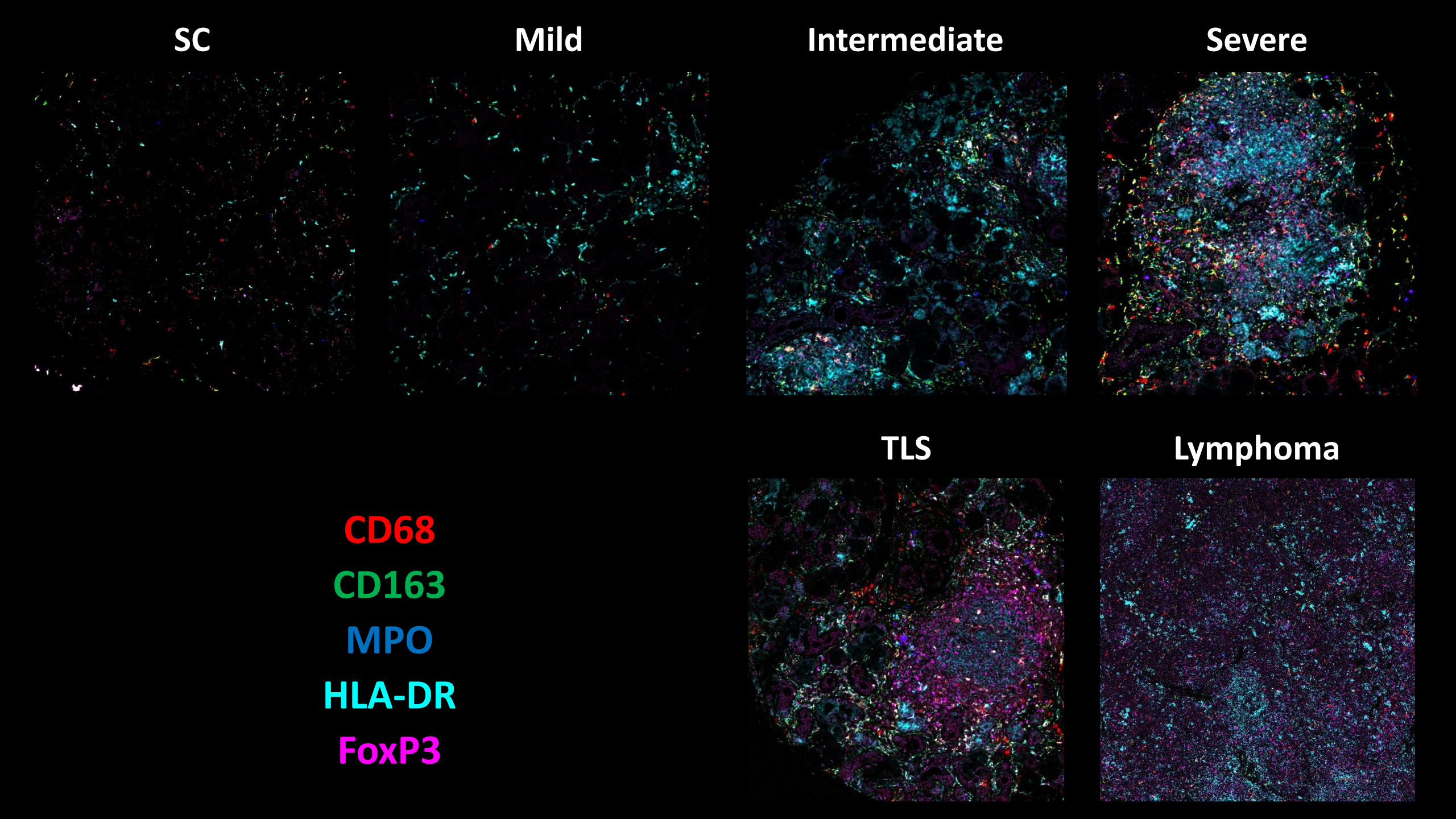
CD138

TLS

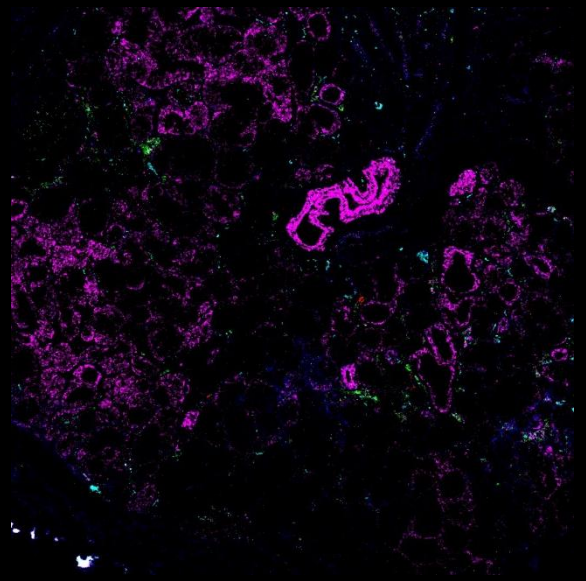


Lymphoma

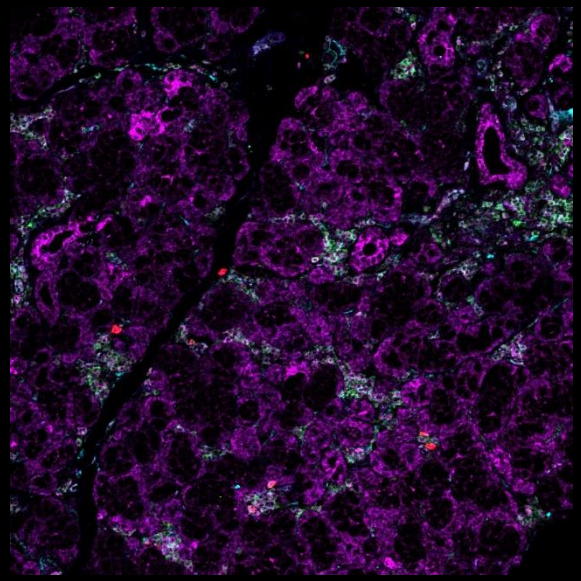



SC
Mild
Intermediate
Severe
CD68
CD163
MPO
HLA-DR
FoxP3
TLS
Lymphoma

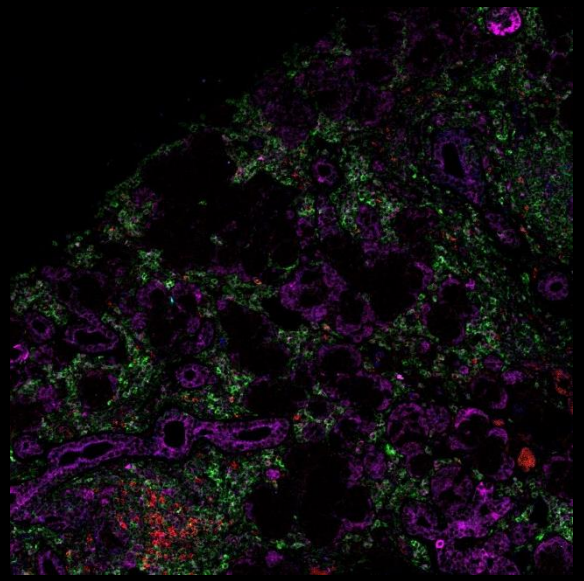
SC



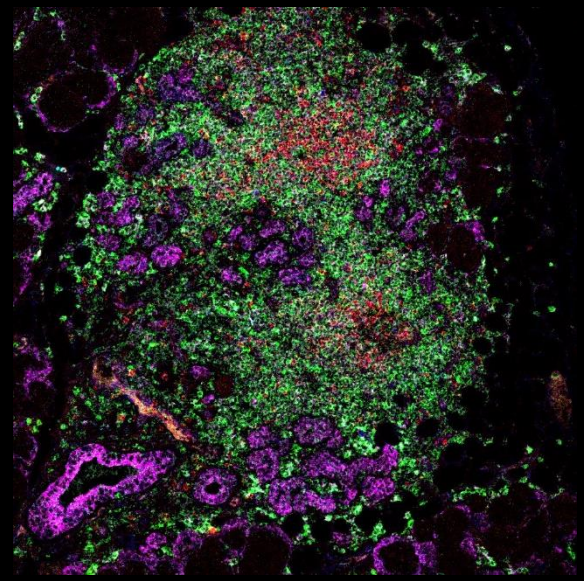
Mild



Intermediate



Severe



IgD

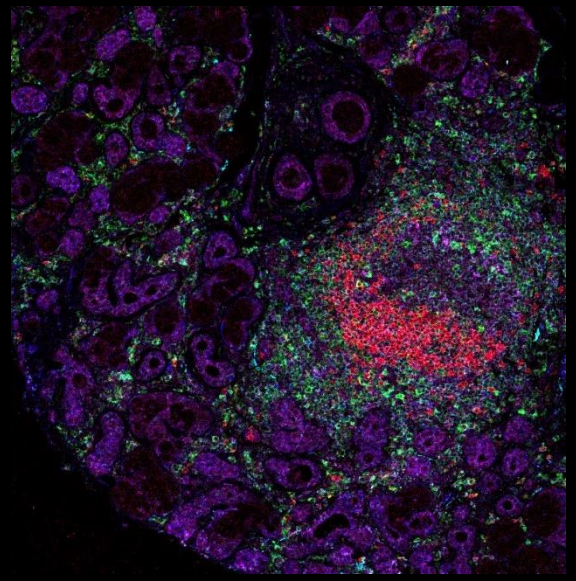
CD27

Tbet

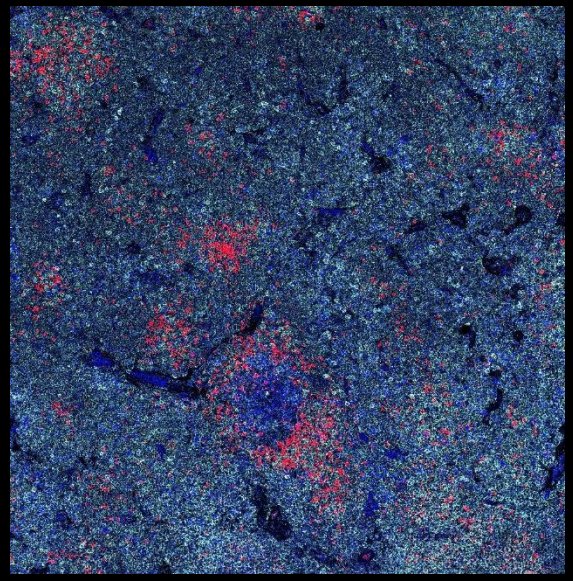
CD56

CD127

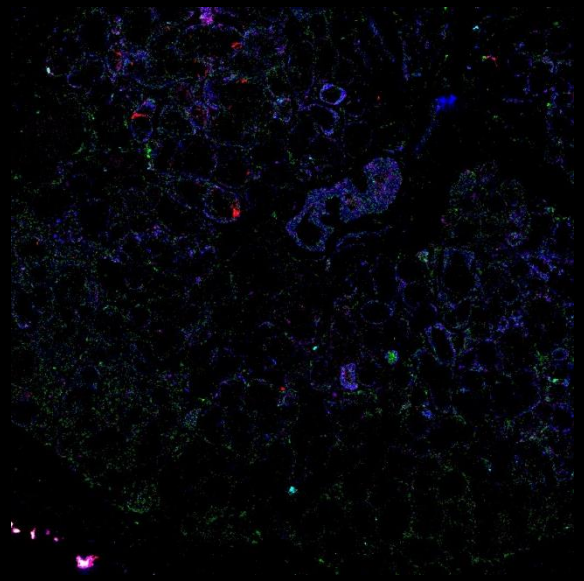
TLS



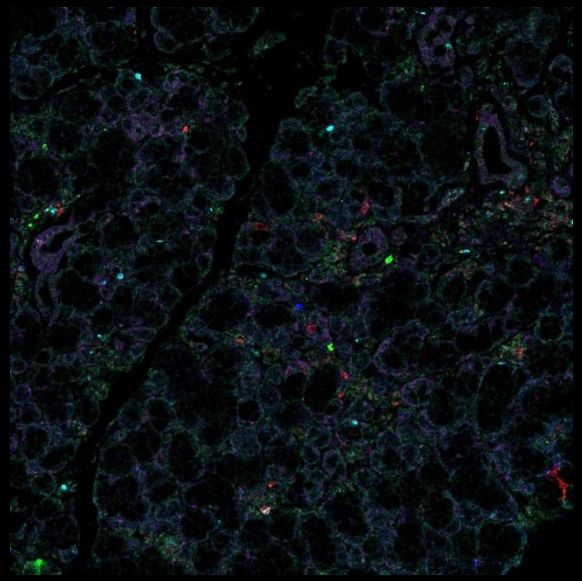
Lymphoma



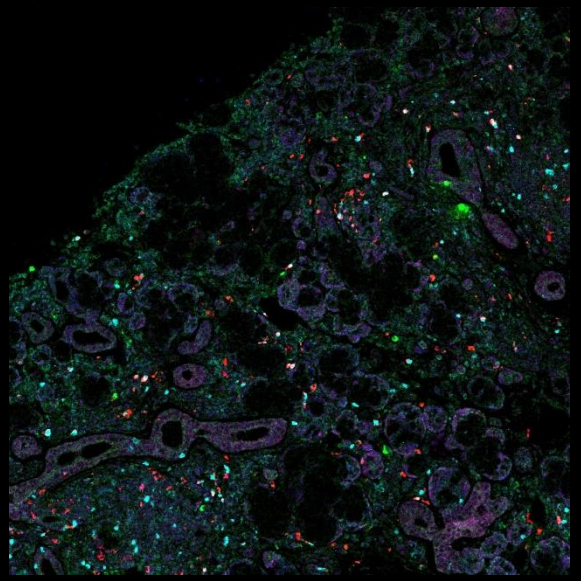
SC



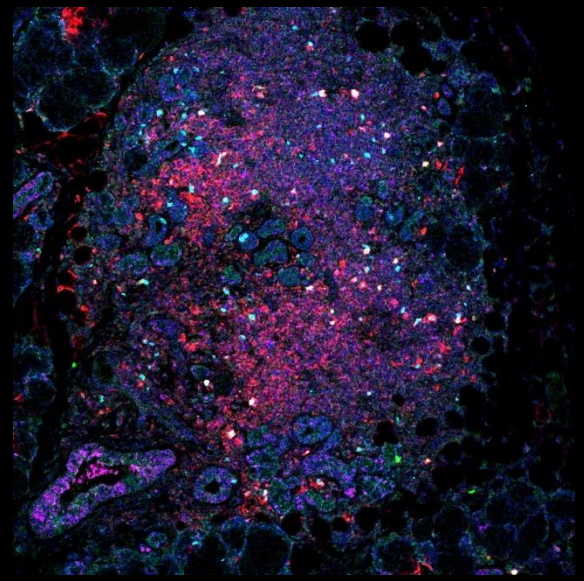
Mild



Intermediate



Severe



pS6

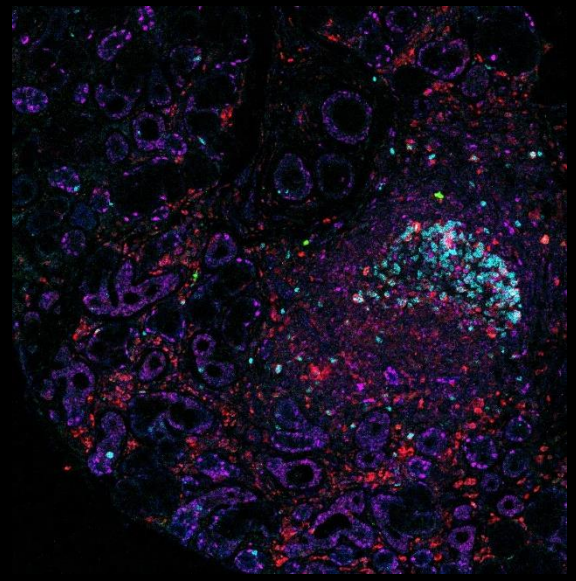
CXCL13

TSLP

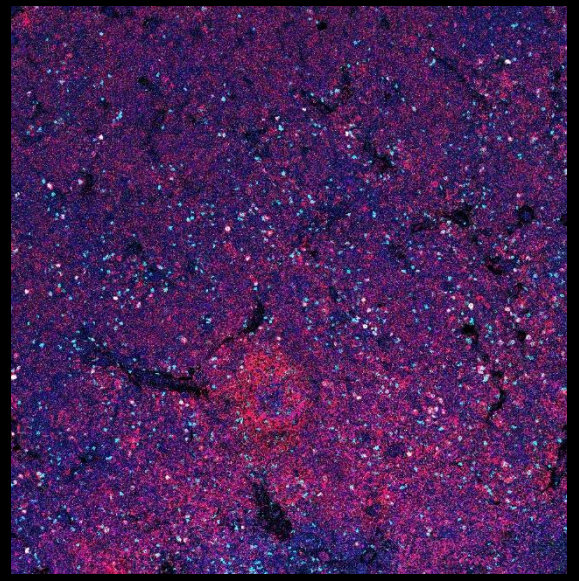
Ki67

Bcl6

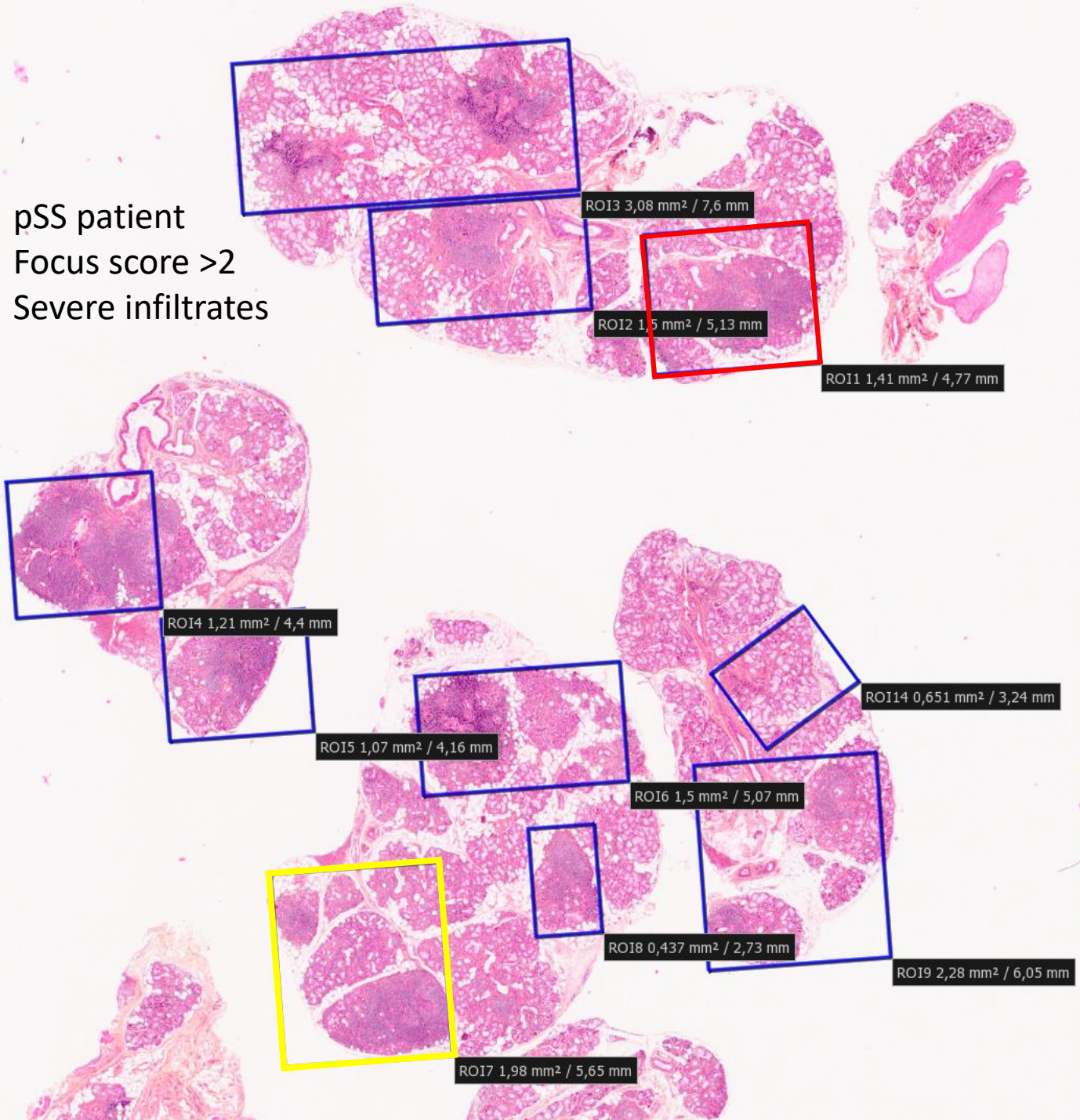
TLS

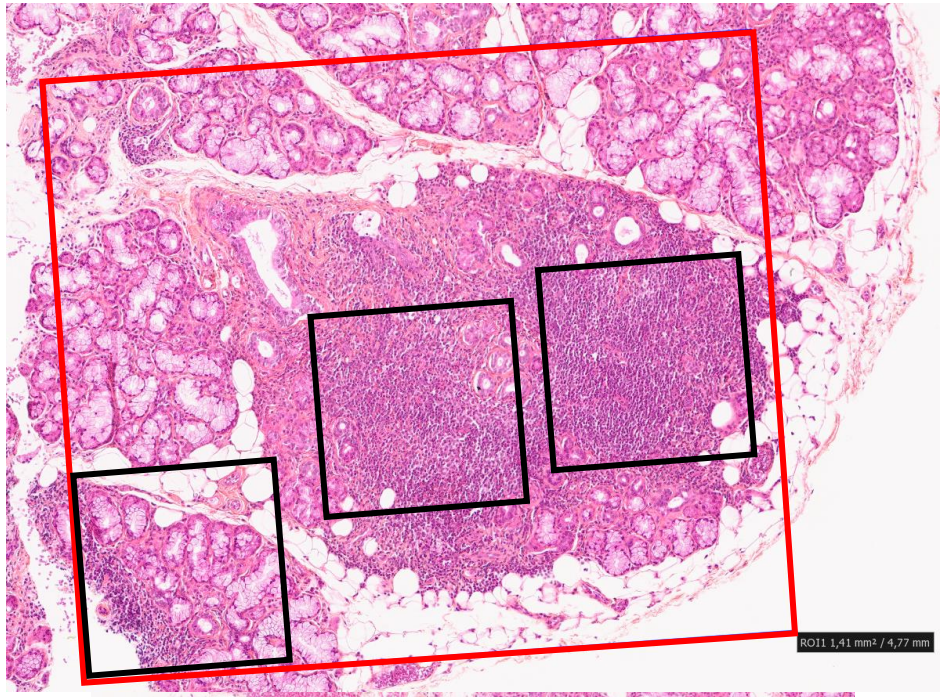


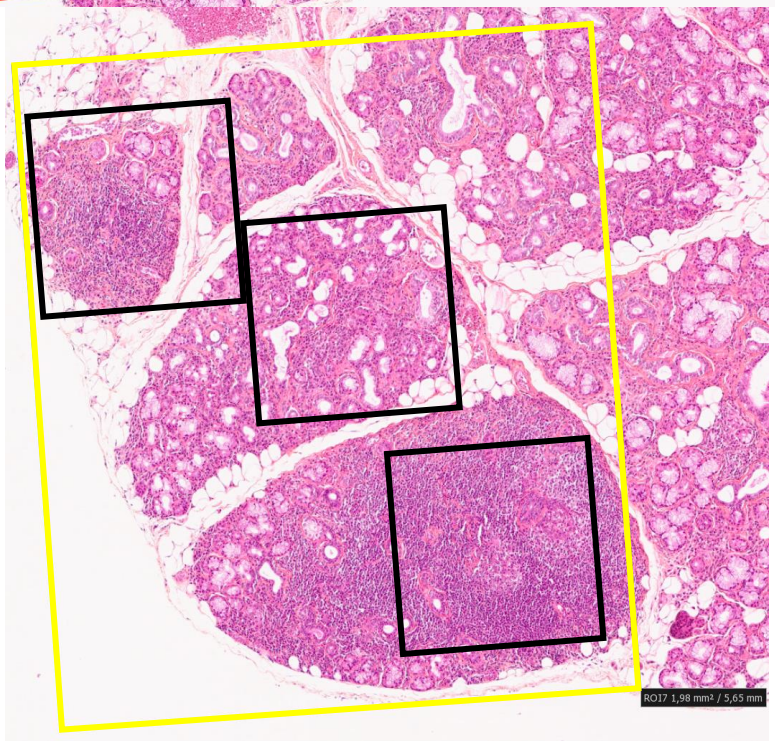
Lymphoma

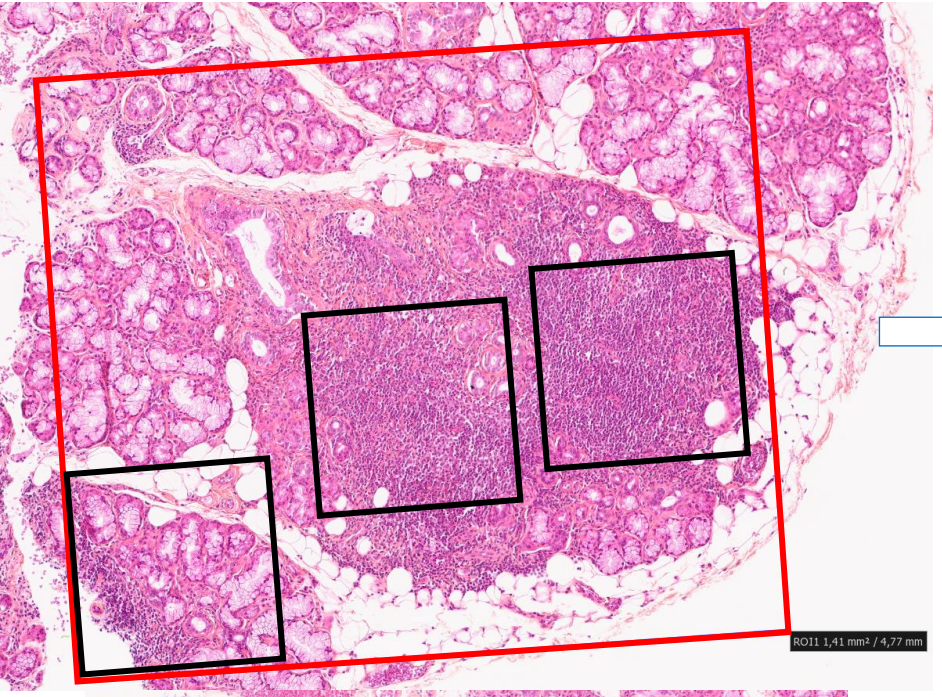


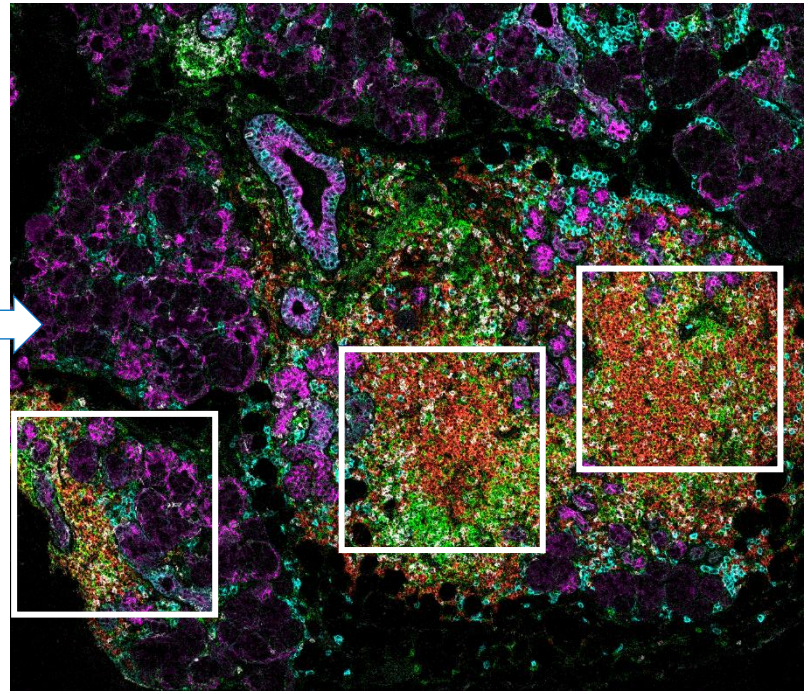
pSS patient
Focus score >2
Severe infiltrates



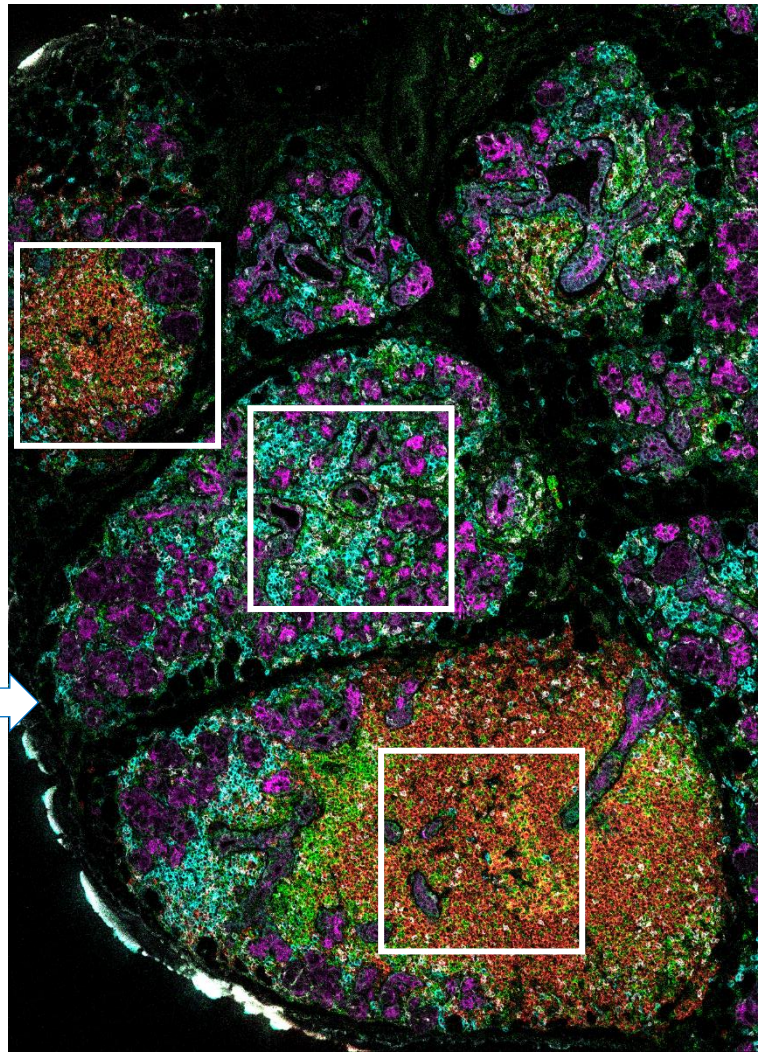



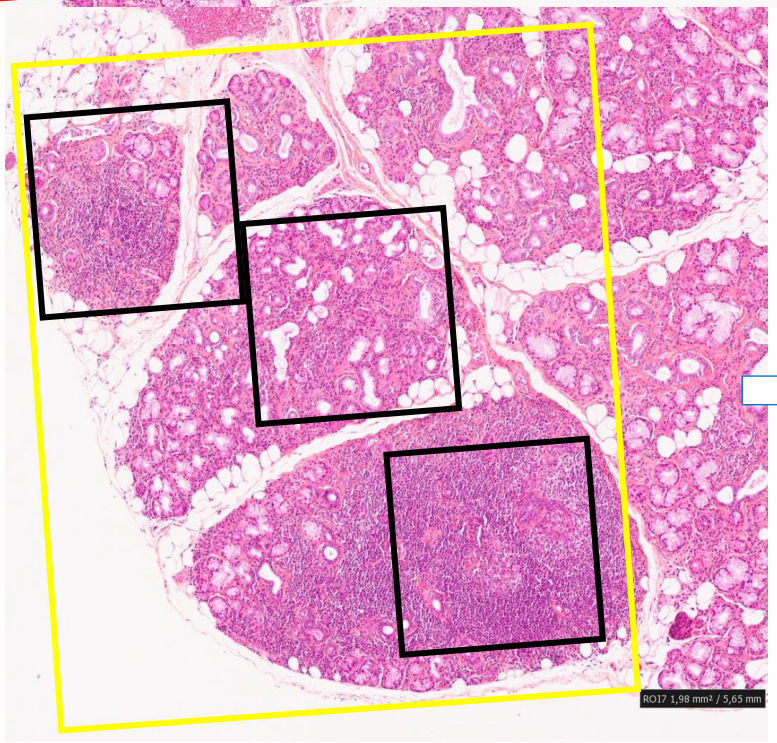





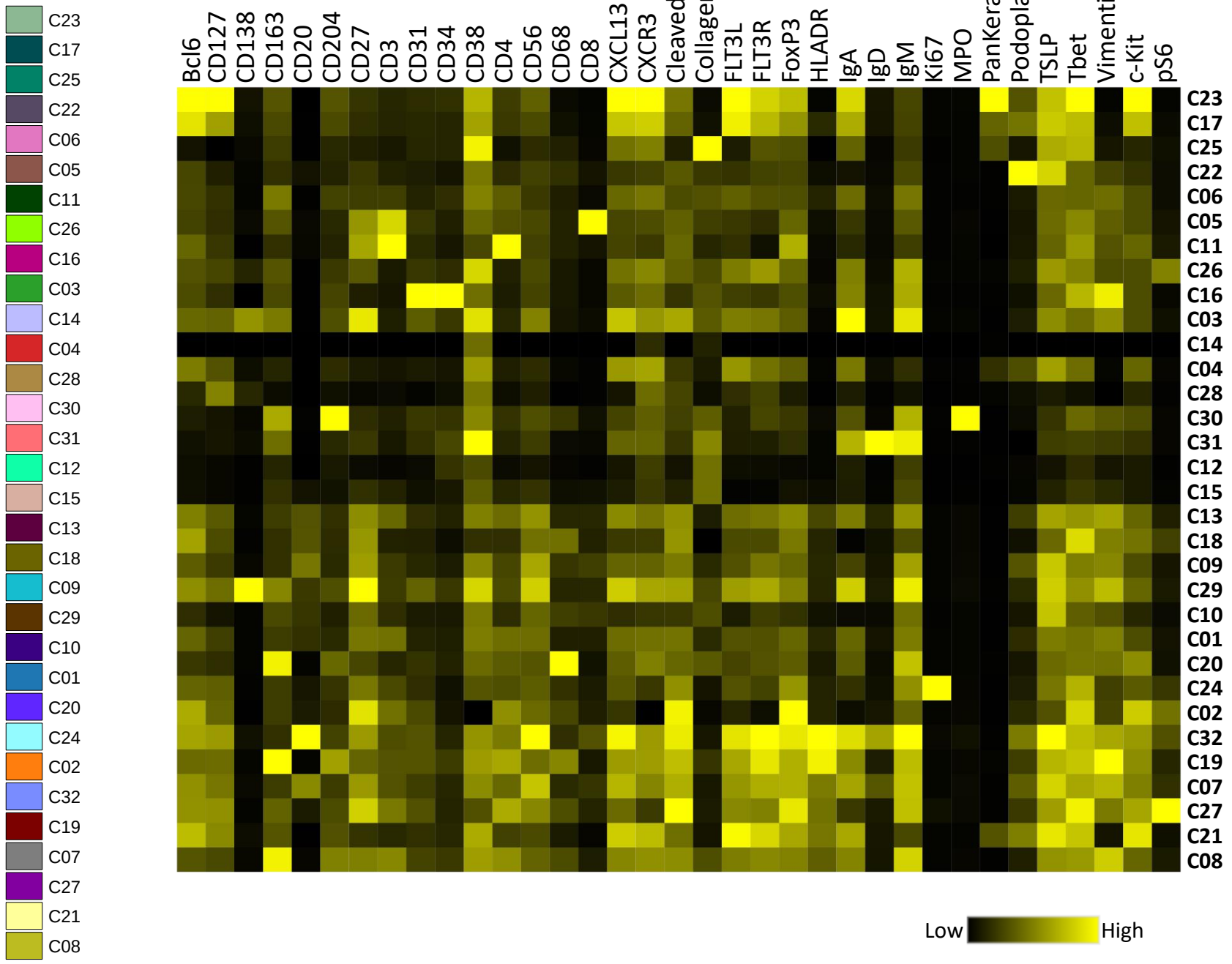
CD20
CD4
CD8
CD138
Pan-Keratin



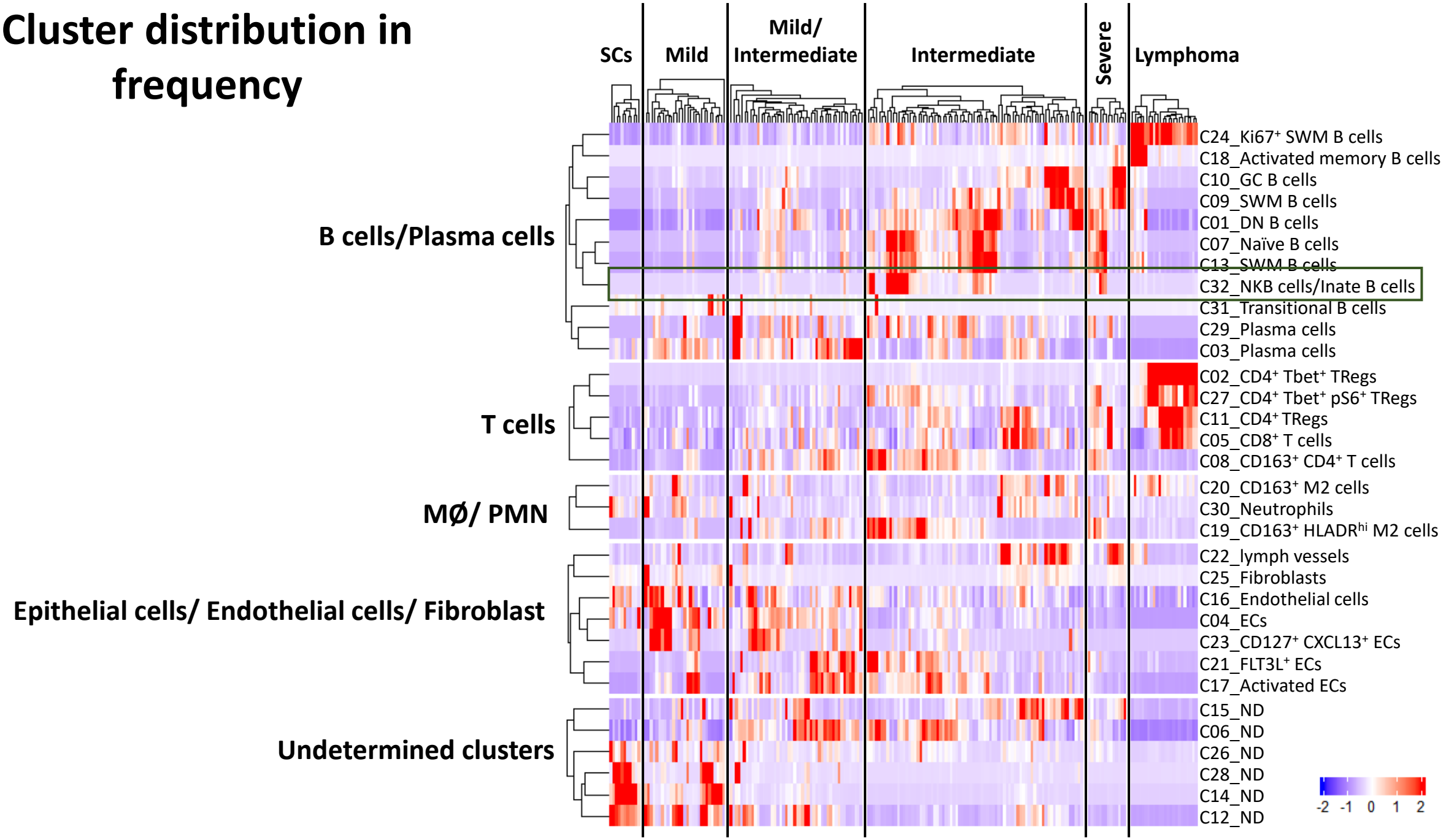


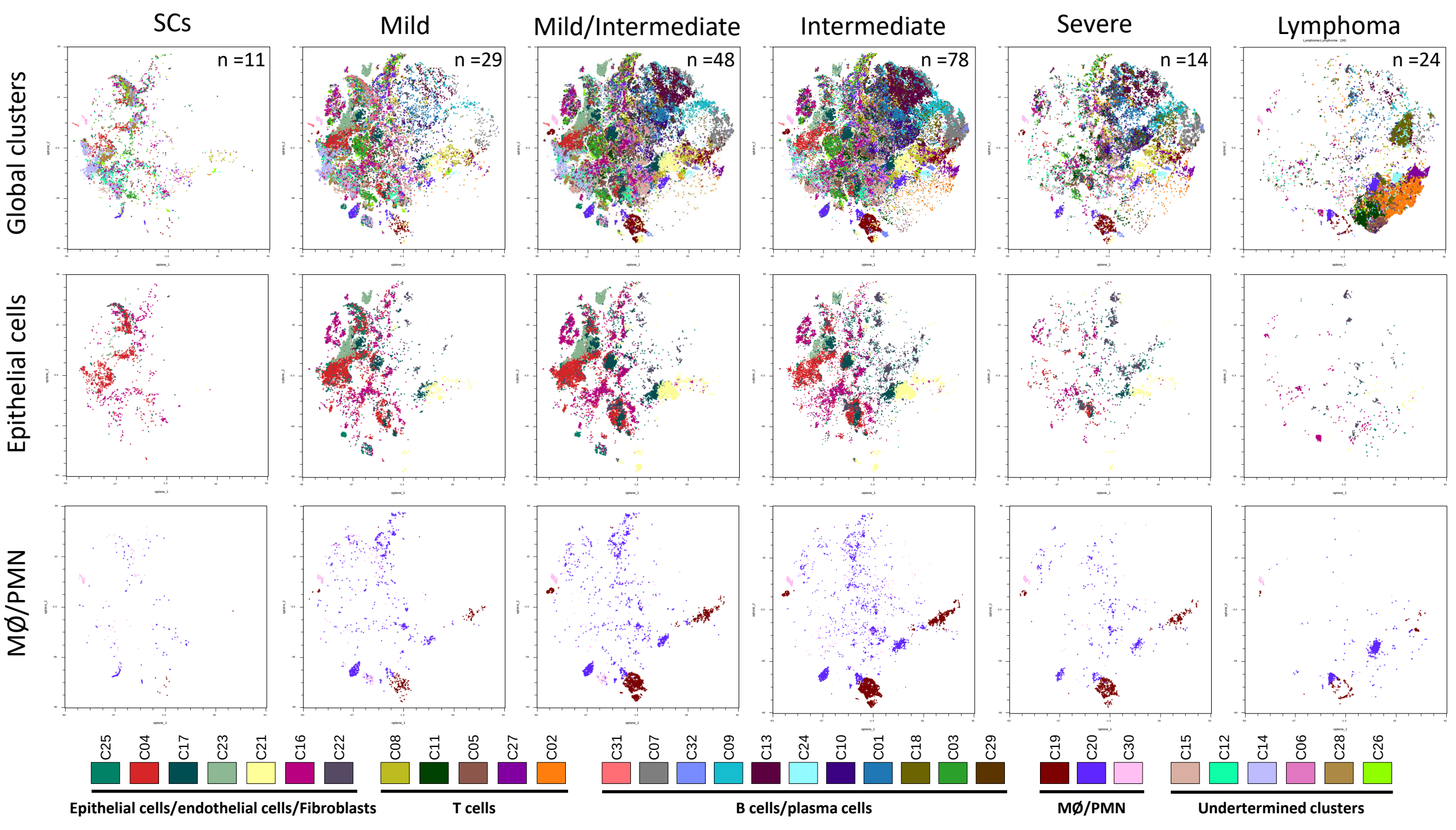
# Unsupervised analysis

ALL ROI

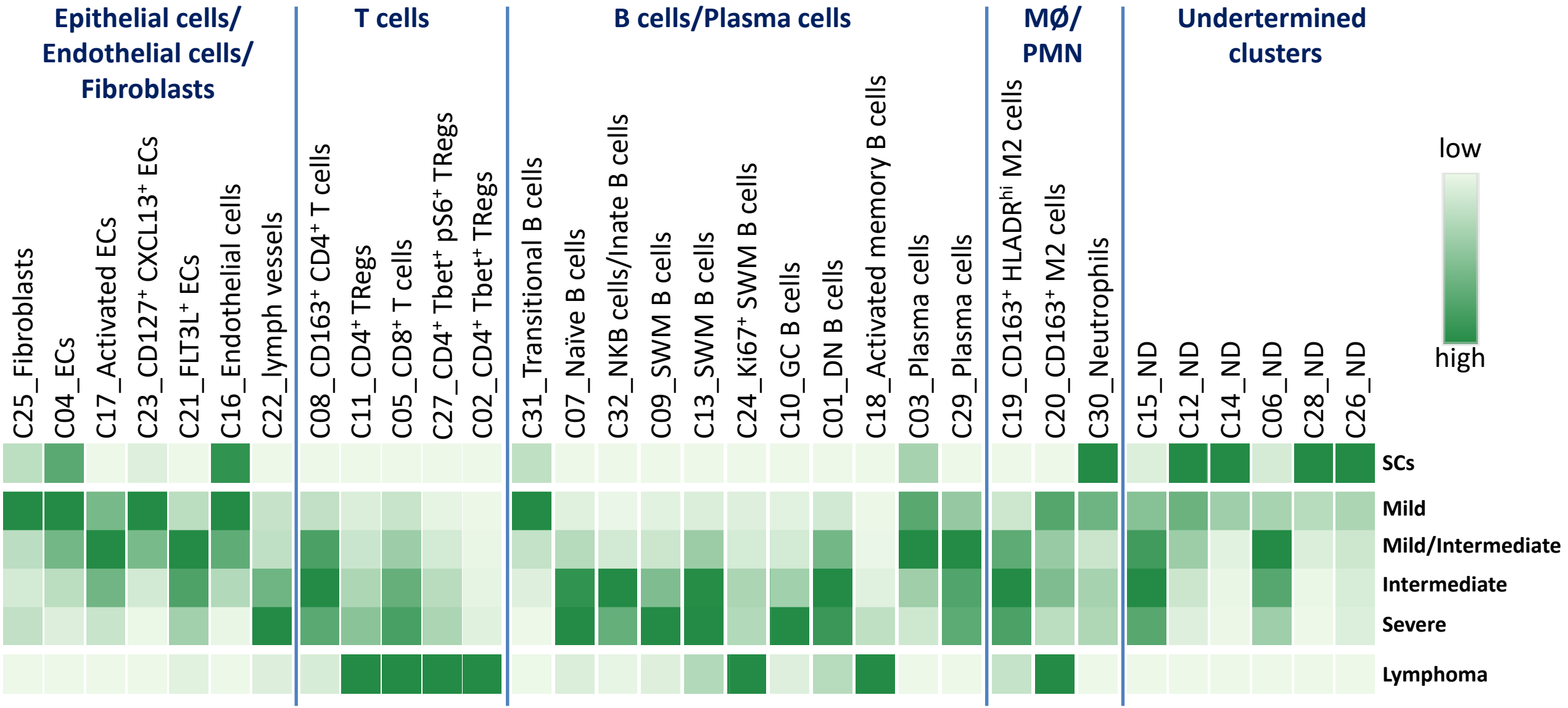


# Cluster distribution in frequency




SCs
Mild
Mild/Intermediate
Intermediate
Severe
Lymphoma
Global clusters
Epithelial cells
MØ/PMN
n =11
n =29
n =48
n =78
n =14
n =24
C25 C04 C17 C23 C21 C16 C22
C08 C11 C05 C27 C02
C31 C07 C32 C09 C13 C24 C10 C01 C18 C03 C29
C19 C20 C30
C15 C12 C14 C06 C28 C26
Epithelial cells/endothelial cells/Fibroblasts
T cells
B cells/plasma cells
MØ/PMN
Undertermined clusters

# Cluster distribution within the different type of infiltrates



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