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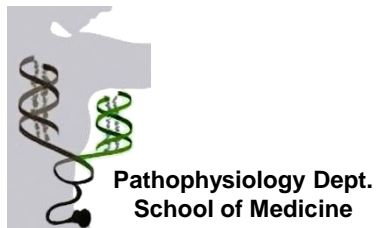


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Η ΧΩΡΙΚΗ ΠΟΛΥΠΑΡΑΓΟΝΙΚΗ ΑΝΑΛΥΣΗ ΦΛΕΓΜΟΝΩΔΩΝ  
ΔΙΗΘΗΜΑΤΩΝ ΑΠΟ ΒΙΟΨΙΕΣ ΧΕΙΛΟΥΣ ΑΣΘΕΝΩΝ ΜΕ  
ΣΥΝΔΡΟΜΟ SJÖGREN ΑΠΟΚΑΛΥΠΤΕΙ ΑΝΑΒΑΘΜΙΣΜΕΝΟ  
ΡΟΛΟ ΣΤΑ DOUBLE NEGATIVE (IgD-,CD27-) Β ΚΥΤΤΑΡΑ

*Χατζής Λ., Patrice H., Γουλές Α., Καψογεώργου Ε., Scuille V., Jacques-Olivier P., Τζιούφας Α.*



Crete 2022

# 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

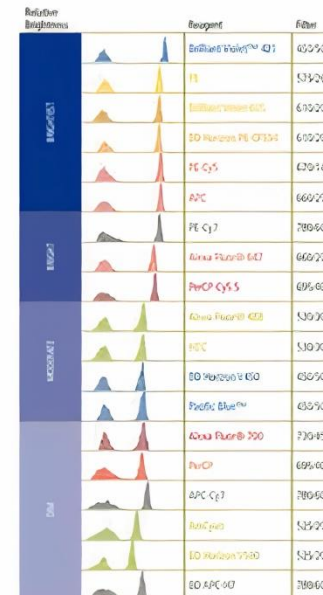
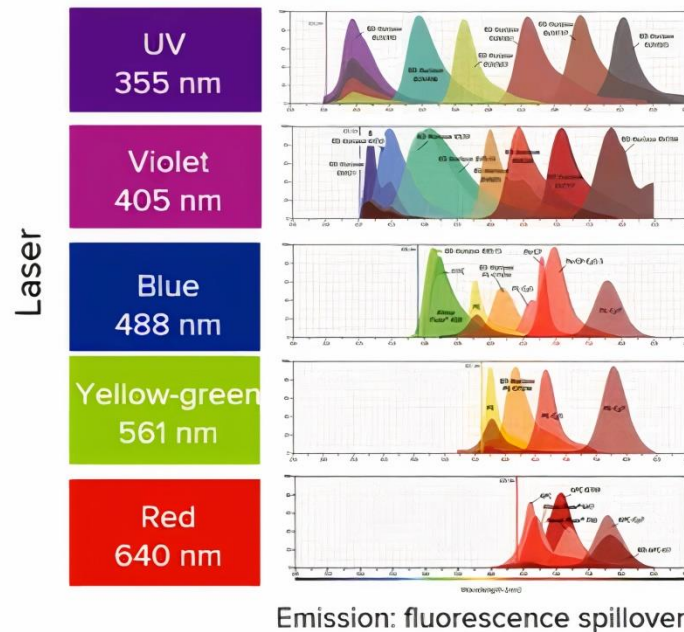
# Methods

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

# 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



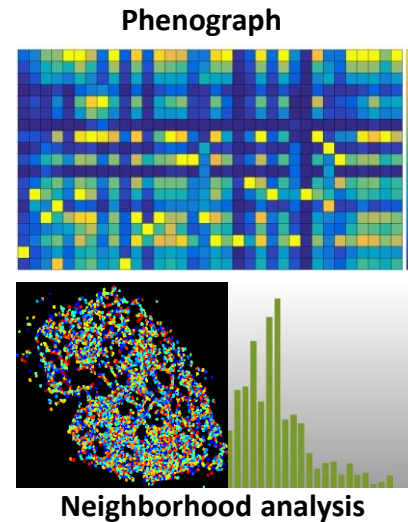
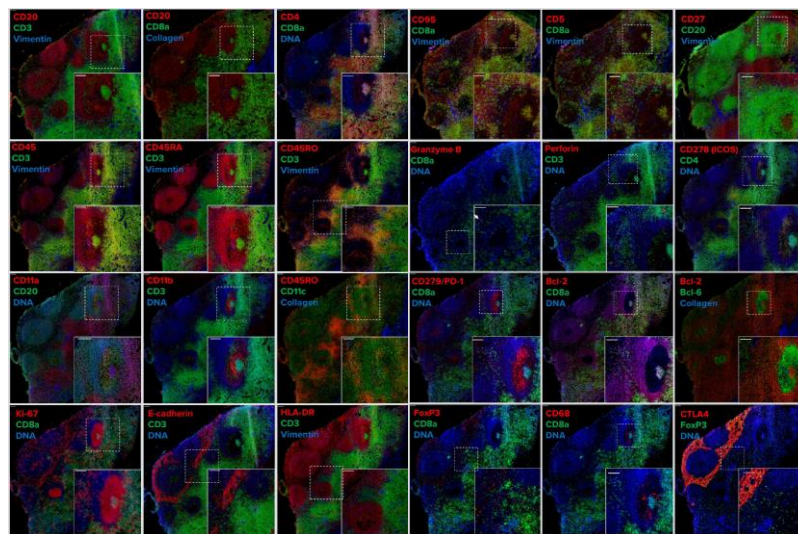
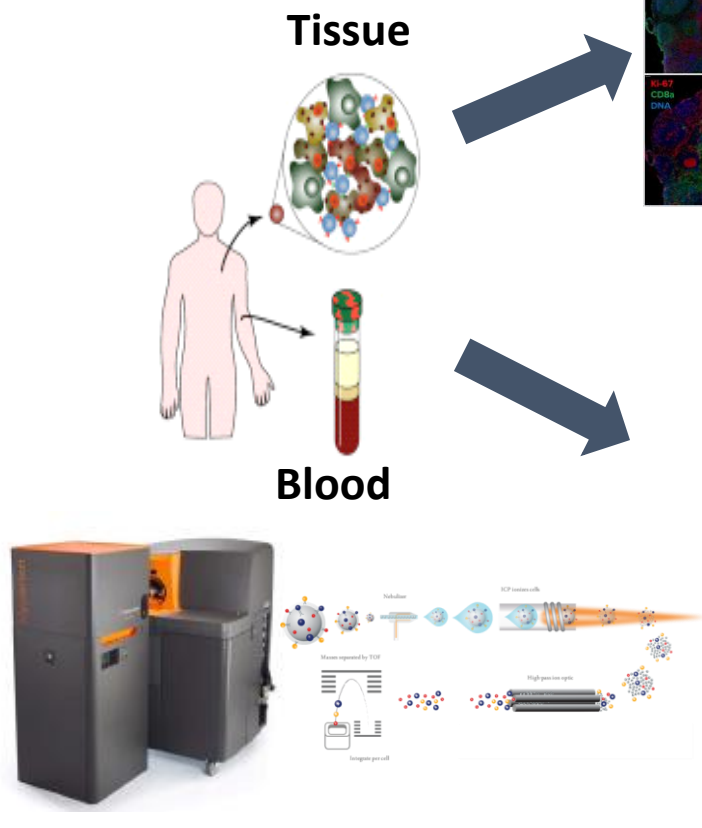


Plateforme de cytométrie en flux et de masse



HYPE Research in Immunology and Oncology

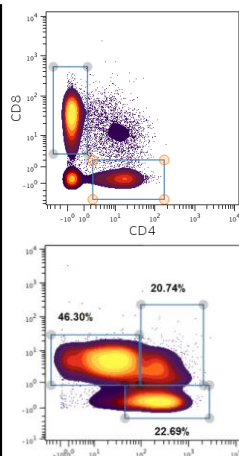
Imaging mass cytometry (IMC) is a new multiparametric and quantitative technique for phenotypic and functional analysis of cells and tissue sections. It can measure up to 50 parameters simultaneously in tissues at a spatial resolution of 1  $\mu\text{m}$ .



Panel

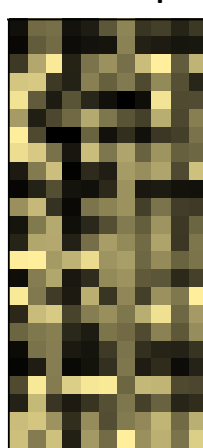
- CD45
- CD196 CCR6
- CD113
- CD113
- CD4
- CD8a
- CD11c
- CD16
- CD45RO
- CD45RA
- CD161
- CD194 CCR4
- CD25
- CD27
- CD57
- CD183 CXCR3
- CD185 CXCR5
- CD28
- CD38
- CD56 NCAM
- TCR $\beta$
- CD294
- CD197 CCR7
- CD14
- CD3
- CD20
- CD68
- HLA-DR
- IgD
- CD127
- CD11d
- CD24
- CD11b
- CD163
- IgM
- CD21
- CD5

Dot Plot

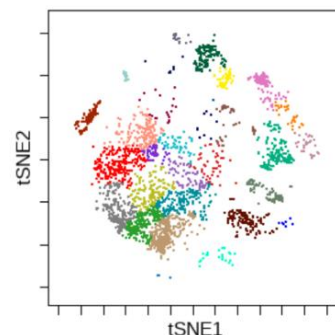


Supervised analysis

Heatmap

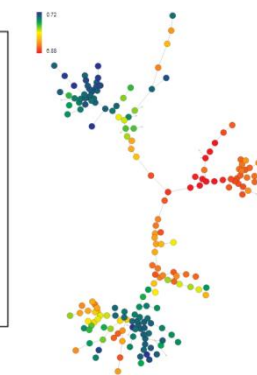


tSNE



Unsupervised analysis

SPADE

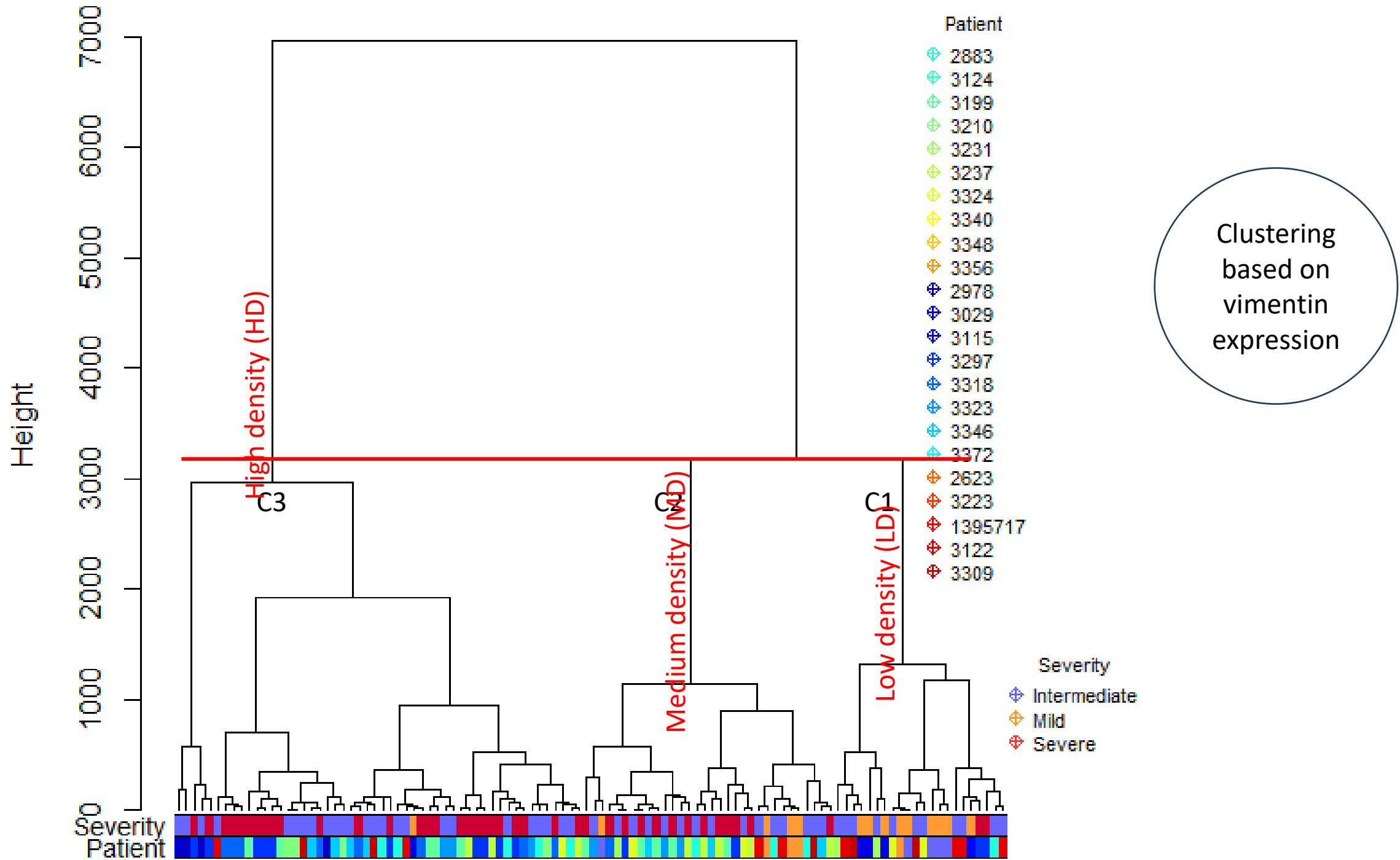


## Clinical characteristics of patients analyzed by IMC

	Age	Gender (F/M)	anti-Ro60 <sup>+</sup> / anti-Ro60 <sup>-</sup>	anti-SSB <sup>+</sup> / anti-SSB <sup>-</sup>	ESSDAI	ESSPRI
<b>SCs</b>	57.8± 9.2	5/0	0/5	0/5	NA	NA
<b>Mild</b>	52.4± 3.6	6/1	5/2	4/3	4.0±2.7	4.3±1.5
<b>Intermediate</b>	38.1± 10.2	6/1	7/0	5/2	11.1±8.7	4.0±1.7
<b>TLS</b>	52.5± 6.6	3/1	3/1	2/2	13.2±10.3	4.7±0.9
<b>Severe</b>	57.6± 6.6	0/3	3/0	3/0	20.0±6.2	3.6±1.4
<b>Lymphoma</b>	55.5± 7.8	0/2	2/0	2/0	24.0±8.5	4.1±1.6

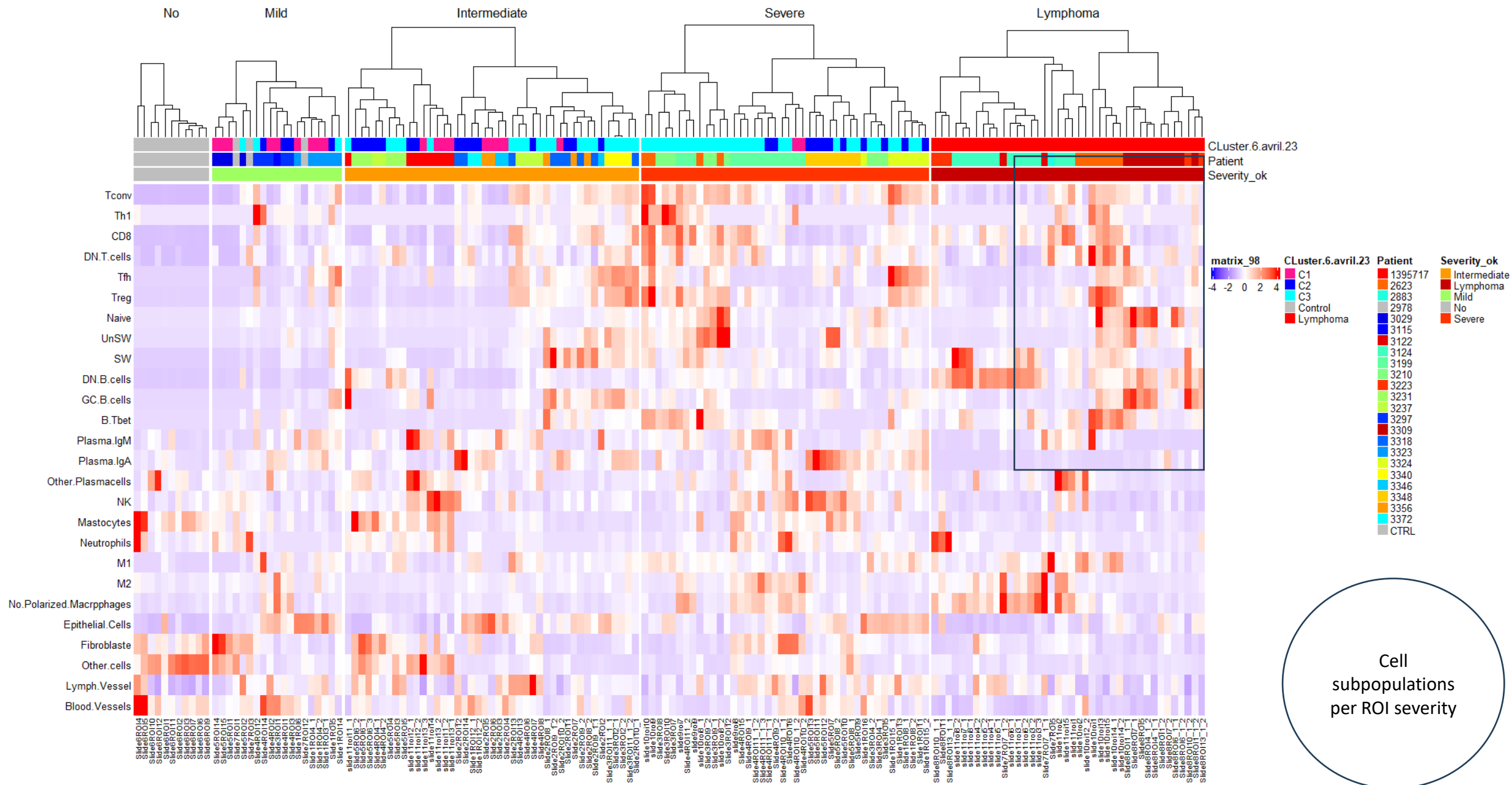
## IMC panel

Antigen	Cell type/function	Antigen	Cell type/function
CD204	Macrophages	Collagen Type I	Fibroblasts
CD34	Endothelial cells	CD3	T cells
CD163	Macrophages	CD27	T cells/B cells
Pan-Keratin	Epithelial cells	HLA DR	APCs
TSLP	Lymphoma	pS6	Activation
CD31	Endothelial cells	IgA	Plasma cells
Ki-67	Proliferation	Vimentin	Structure
IgD	B cells/Plasma cells	CD38	B cells/Plasma cells
IgM	B cells	Tbet	Transcription factor
FoxP3	Tregs	Bcl6	Transcription factor
CD4	T cells	Cleaved Caspase 3	Apoptose
cKit	Mastocytes/Epithelial cells	Podoplanin	Lymph vessels
CD68	Macrophages	FLT3R	Lymphoma
CD20	B cells	FLT3L	Lymphoma
CD8a	T cells	CXCL13	Chemokine
CD138	Plasma cells	CXCR3	Chemokine receptor
MPO	Neutrophils	CD127 (IL-7Ra)	T cells
CD56	NK cells		





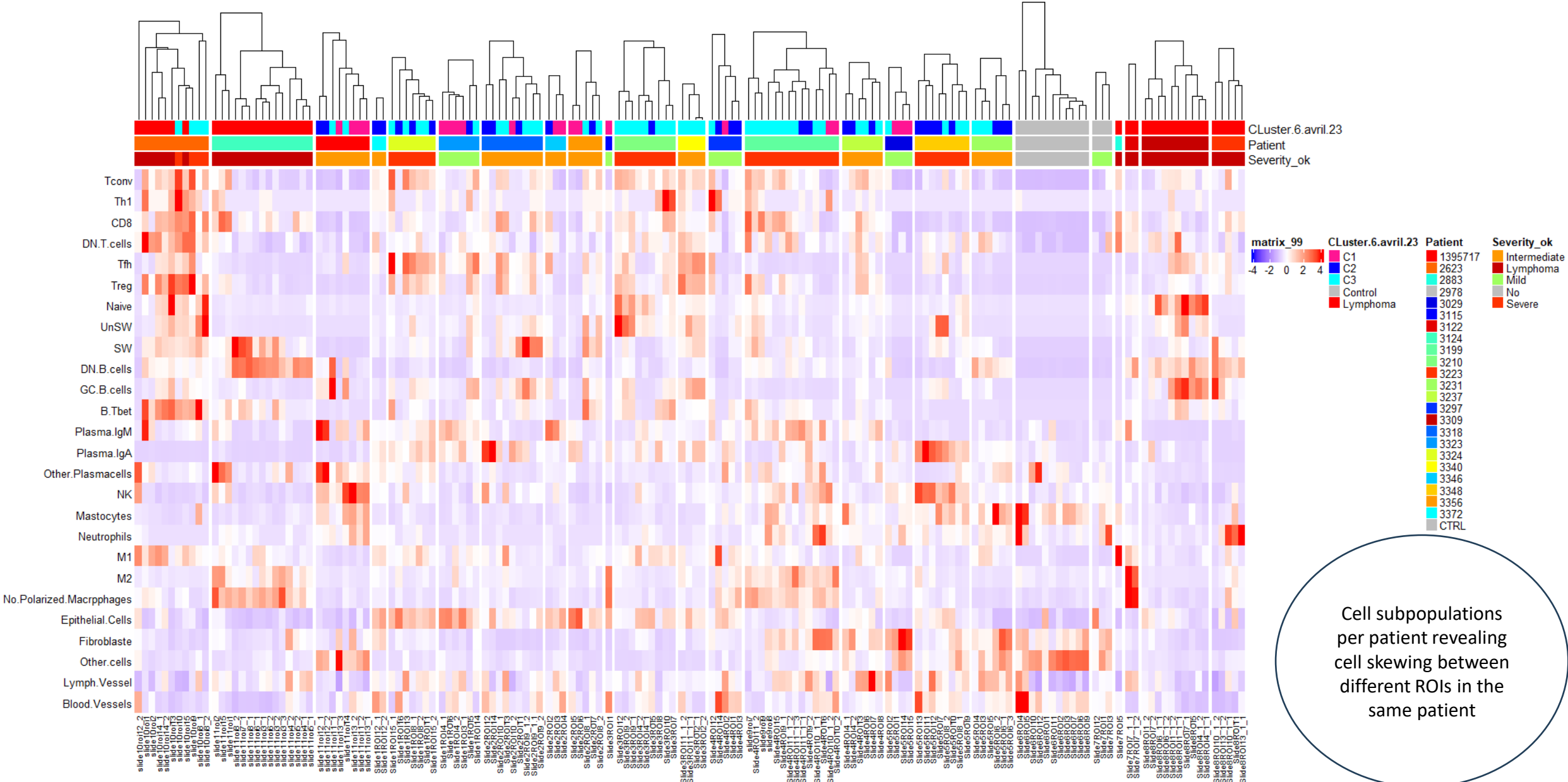
# Heatmap representing the supervised analysis per severity





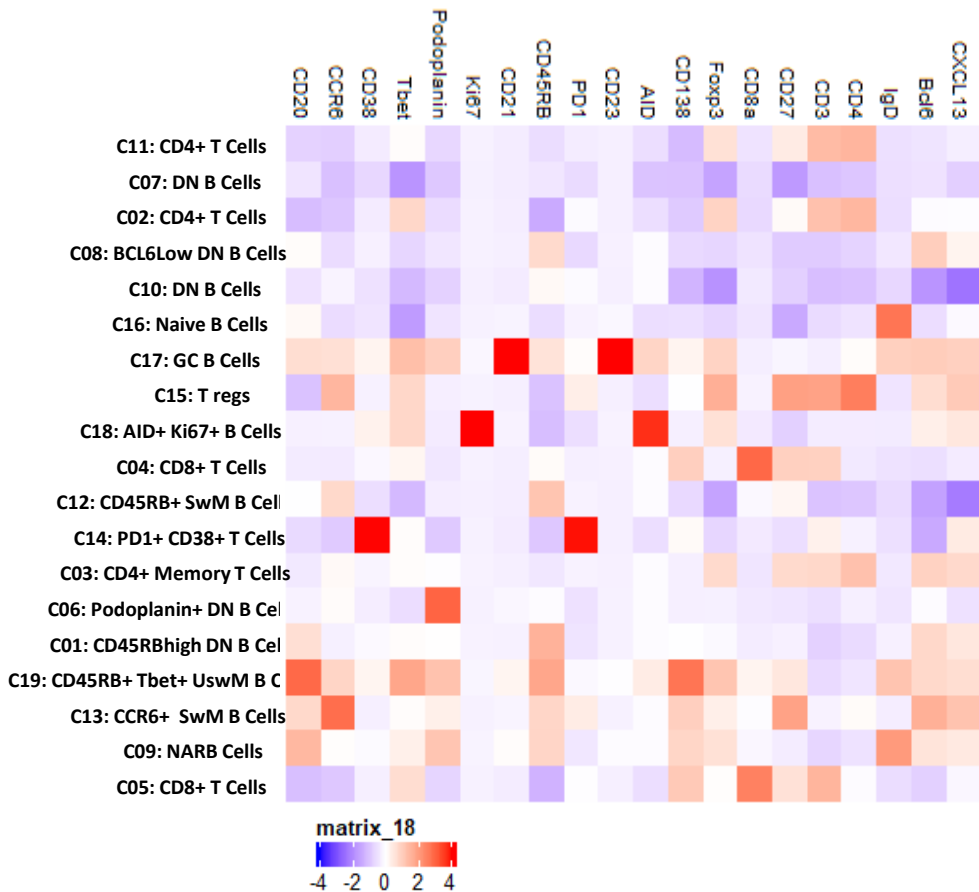
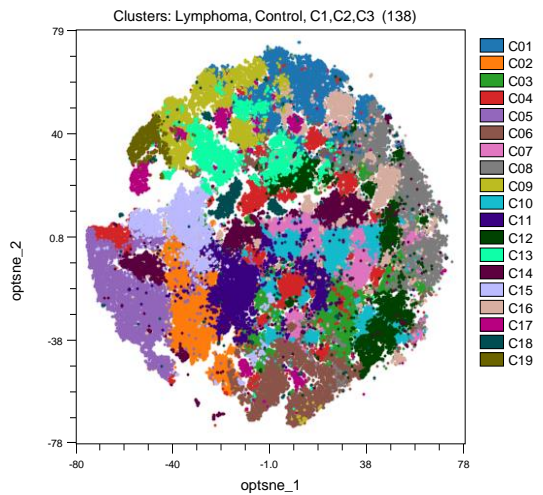
# Heatmap representing the supervised analysis per patient

2623 3124 1395717 3372 3324 3323 3318 3346 3356 3115 3210 3340 3297 3199 3237 3029 3348 3231 CTRL 2978 2883 3309 3223



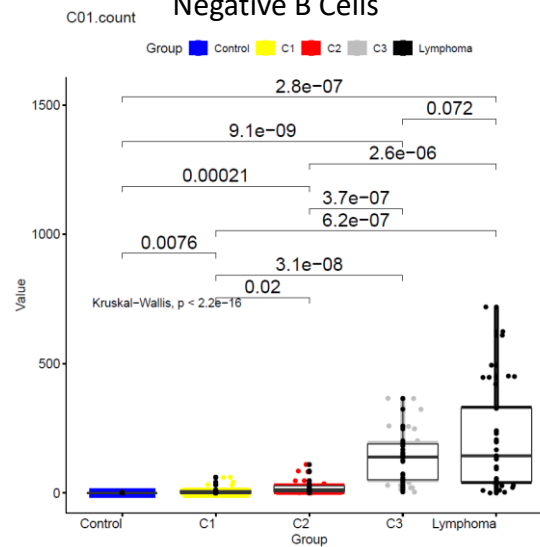
Cell subpopulations per patient revealing cell skewing between different ROIs in the same patient

b)

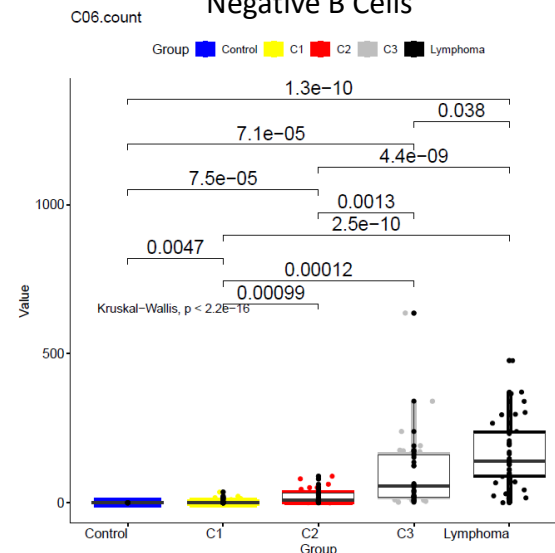


c)

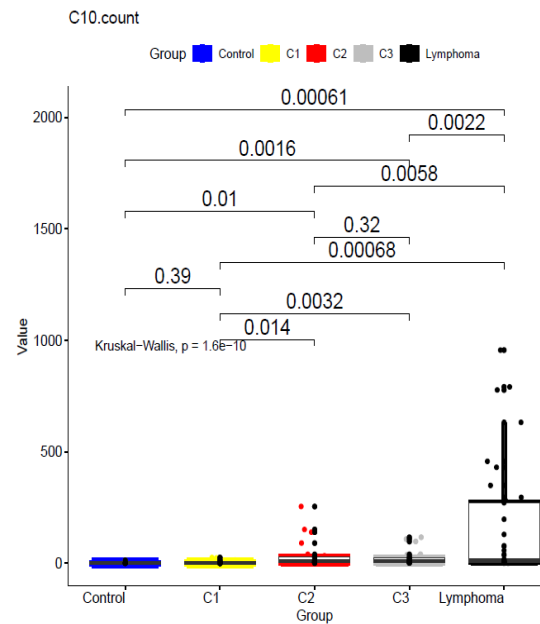
### CD45RBhigh Double Negative B Cells



### Podoplanin+ Double Negative B Cells



### Double Negative B cells



### CD45RB+ Switched Memory B cells

