

Combination Xenium 5K and Hyperion

Immunity and cancer team
Julien Faget



ircm
Institut de Recherche
en Cancérologie de Montpellier

Inserm

La science pour la santé
From science to health



ICM
Institut régional du Cancer
Montpellier | Val d'Aurelle



UNIVERSITÉ DE
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Workflow

Xenium

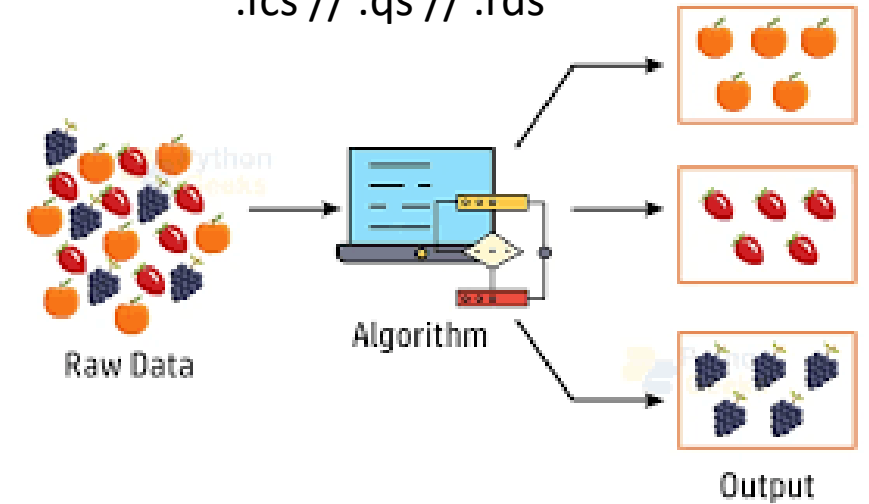
(Tissue segmentation + RNA expression (5K))



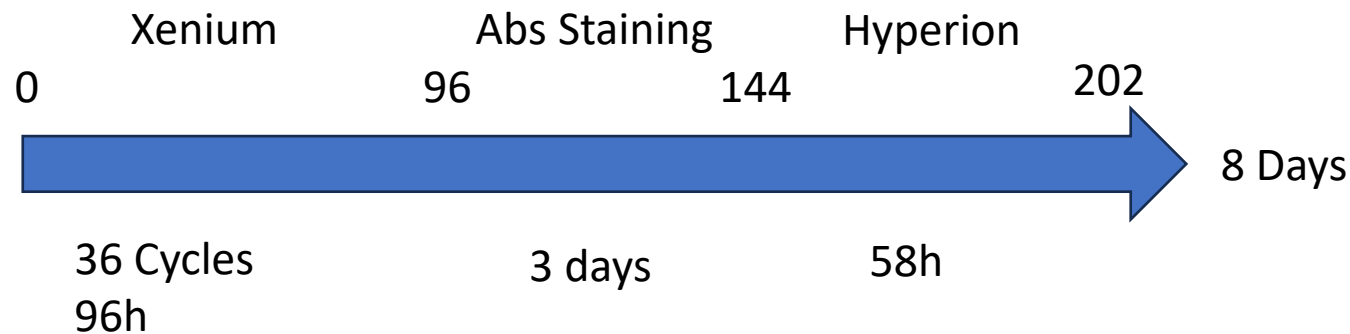
Hyperion XTi
Antibody panel (n=43)



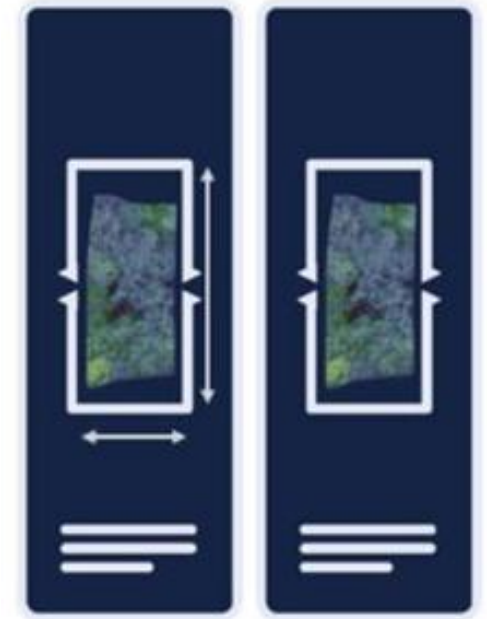
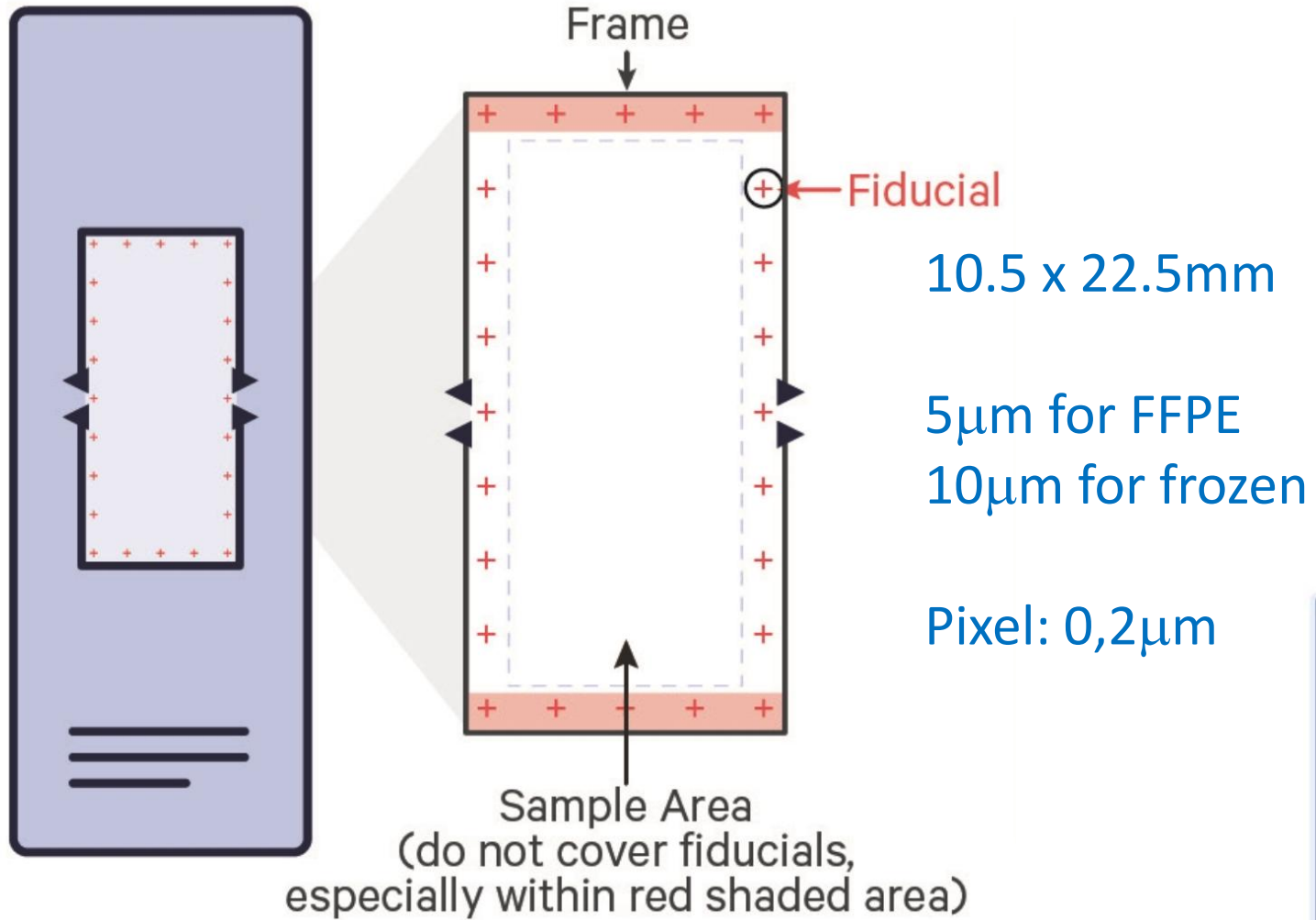
Clustering
.fcs // .qs // .rds



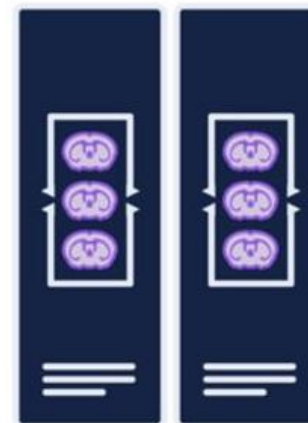
Visualization (Xenium explorer)



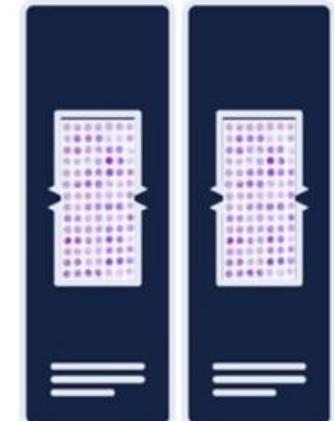
Preparing a Xenium slide



Two 10.5 x 22.5mm
sections



Six 6 x 10mm mouse brain
sections



196 x 1mm biopsies in
TMAs

FFPE

Ag retrieval EDTA pH9

Resolution 1µm

Panel IMC (Hyperion)

Channel	Marker
Y89	CD15
In113	Iba1
In115	pNAD
Pr141	p4EBP1
Nd142	CD20
Nd143	Vimentin
Nd144	CD36
Nd145	4EBP1
Nd146	MPC1
Sm147	CD163
Nd148	PanCK
Sm149	S6
Nd150	SDHA
Eu151	CD31
Sm152	CD3
Eu153	CD66b
Sm154	GlutSynthase
Gd155	pS6
Gd156	CD4
Gd157	CD68
Gd158	ECadherin
Tb159	PHGDH
Gd160	ATP5A
Dy161	TCRgd
Dy162	CD8a
Dy163	CD45
Dy164	LDHB
Ho165	PD1

Channel	Marker
Er166	GPT2
Er167	GrB
Er168	Ki67
Tm169	PKM2
Er170	Zeb1
Yb171	CD27
Yb172	Casp3
Yb173	MCT1
Yb174	MCT4
Lu175	STING
Yb176	CPT1a
Ir191	DNA1
Ir193	DNA2
Pt195	HLADR
Pt196	CD45RA
Pt198	CD45RO
Bi209	aSMA



Stromal and functional markers
Y. Glasson
P. Dupre

Methabolic effector molecules
L. LeCam team
M. Lacroix / P. Dupre

Analyzed Area with both modalities

➤ 28 ROIs – 4 tissus :

- C2103096 → 5 ROIs
- C2109288BB → 3 ROIs
- ND23-4710-1A → 1 ROI
- 19H08921 → 19 ROIs

➤ ROI de 2,9 à 10,8mm²

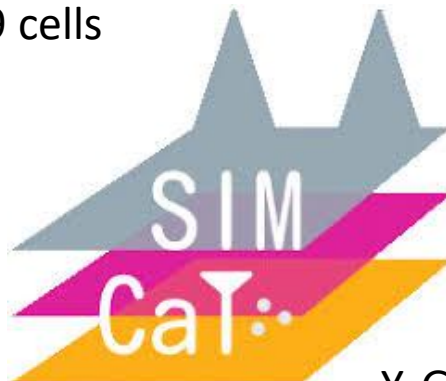
➤ Alignement Score de 57,71% à 78,49%

➤ Panel IMC → 43 marqueurs

➤ Panel Xenium → 5001, Xenium Human 5K Pan Tissue & Pathways Panel

151 mm²

928819 cells

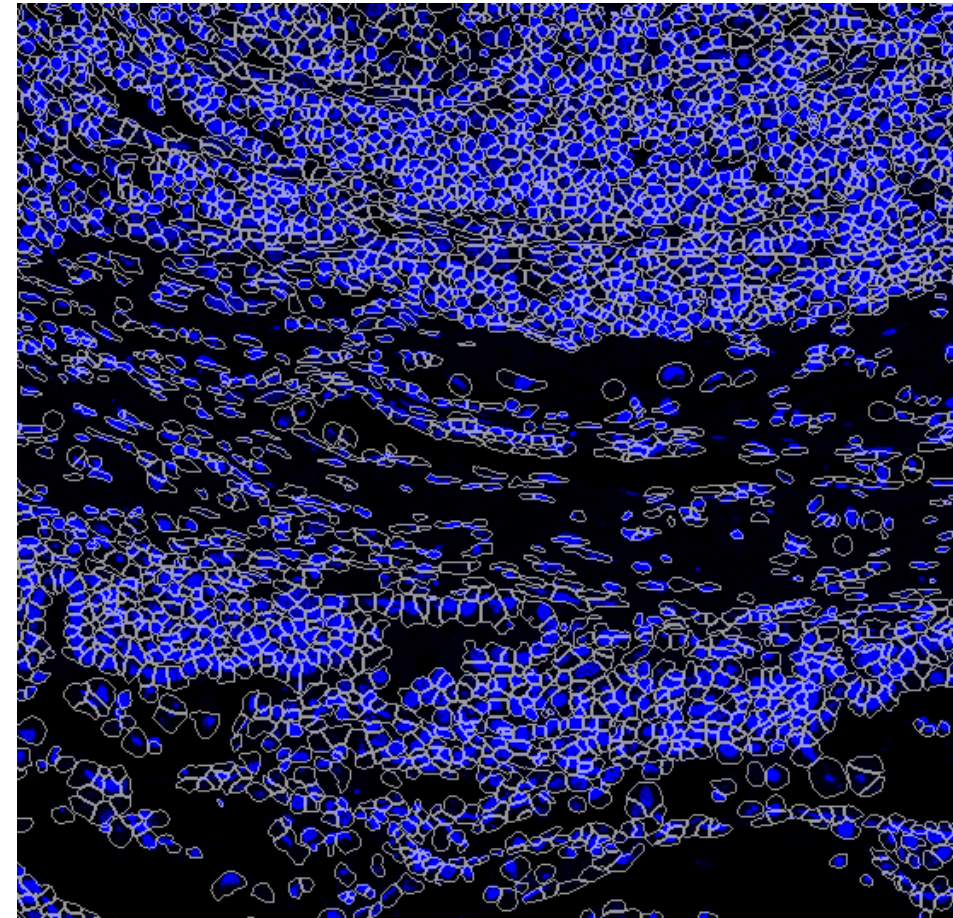


Y. Glasson

Samples	Area (mm ²)	Score (%)	Nb Cells
C2103096-ROI1	6,8816	76,26	20346
C2103096-ROI2	6,534	75,96	23404
C2103096-ROI3	9,703575	78,32	28787
C2103096-ROI4	10,855758	74,03	28543
C2103096-ROI5	8,312512	78,49	21746
C2109288BB-ROI1	5,020712	74,4	16746
C2109288BB-ROI2	4,684362	73,1	22397
C2109288BB-ROI3	6,040608	78,32	23831
ND23-4710-1A-ROI1	3,963012	77,52	12356
19H08921-ROI1	5,57382	74,22	54302
19H08921-ROI2	5,295168	71,69	40272
19H08921-ROI3	5,07584	77,98	34958
19H08921-ROI4	4,793984	75,7	36731
19H08921-ROI5	4,283902	74,13	42472
19H08921-ROI6	5,384886	71,38	45857
19H08921-ROI7	5,646057	72,18	50103
19H08921-ROI8	5,443004	74,43	47107
19H08921-ROI9	4,499746	74,46	44131
19H08921-ROI10	2,90394	66,42	13067
19H08921-ROI11	4,455897	69,19	35878
19H08921-ROI12	4,703391	71,8	50444
19H08921-ROI13	4,536738	74,12	42074
19H08921-ROI14	4,68864	72,83	30920
19H08921-ROI15	3,473964	73,83	20813
19H08921-ROI16	3,751251	74,85	32681
19H08921-ROI17	4,995523	73,4	41613
19H08921-ROI18	5,39609	75,36	42413
19H08921-Sain-ROI19	4,95504	57,71	24827

Results from Fusionomics

Overlap:
Hyperion (Nucleus) morphology (Xenium)

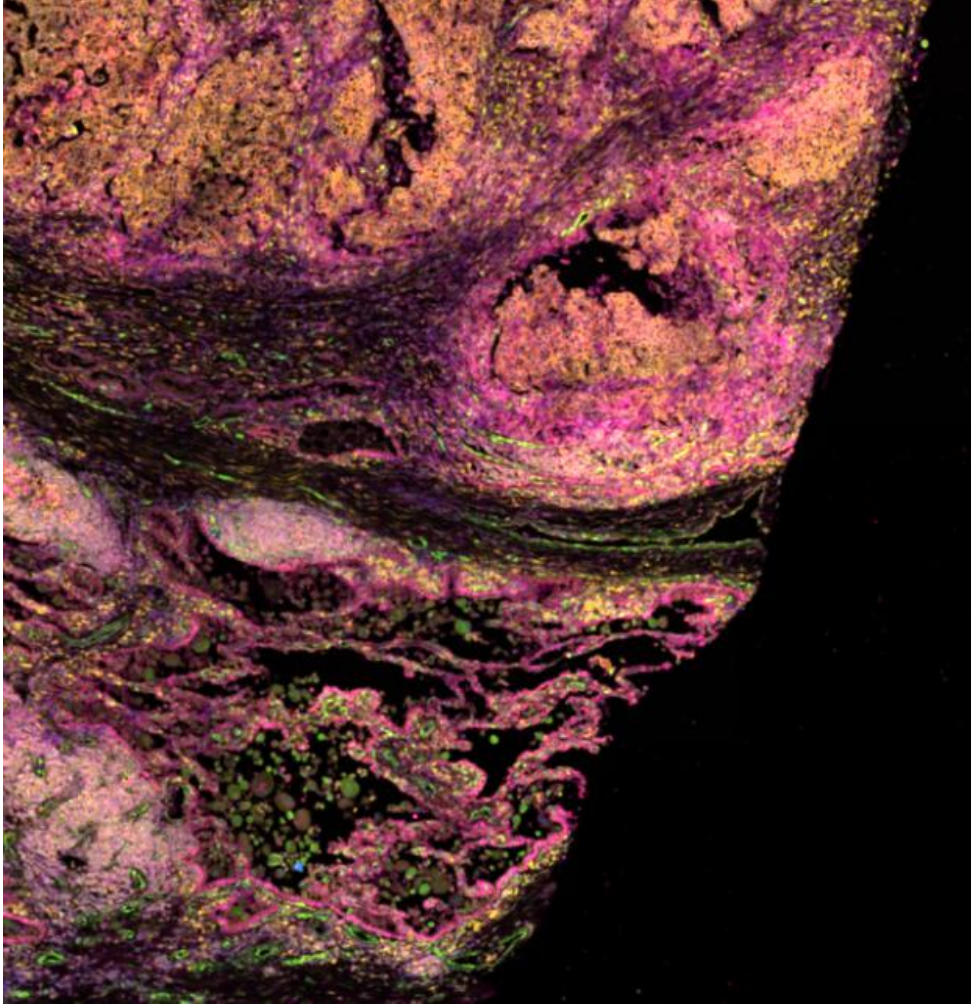


Hyperion nucleus

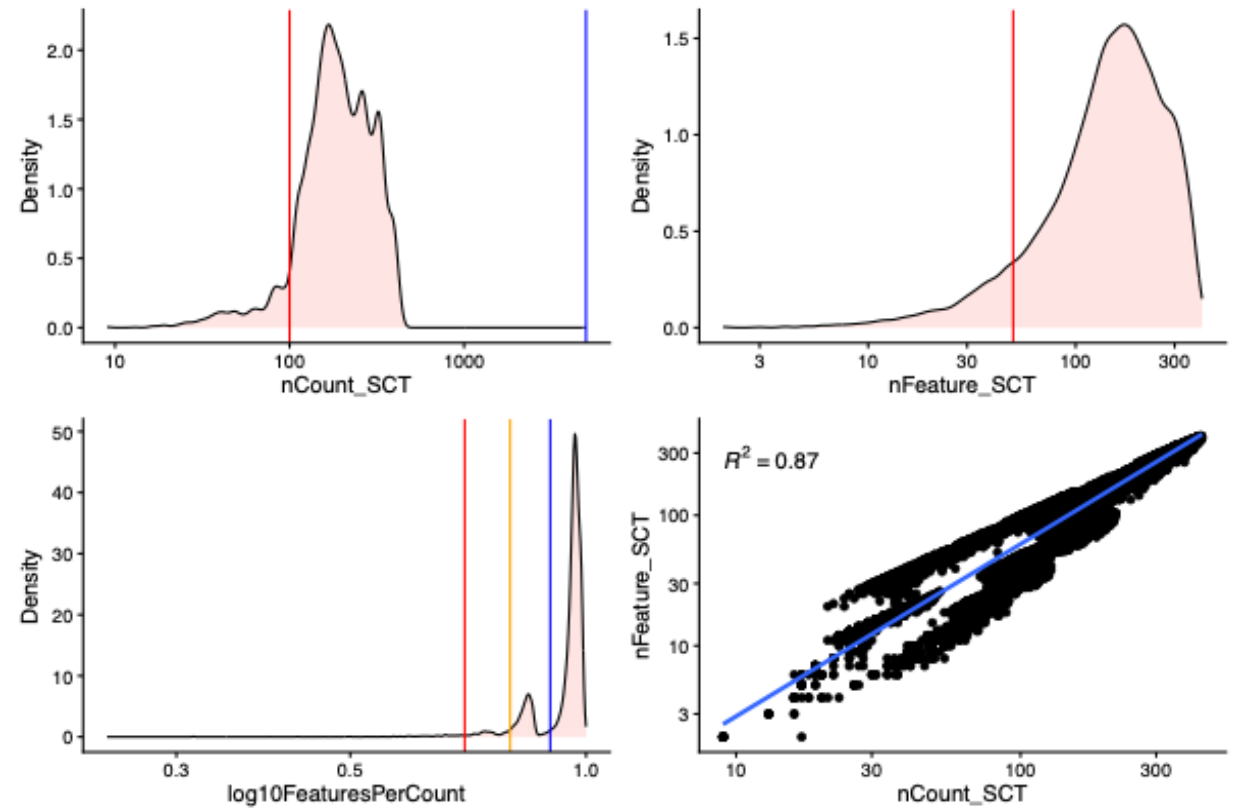
Xenium morphology

- ⇒ DAPI
- ⇒ ATP1A1/CD45/E-Cadherin
- ⇒ 18s
- ⇒ Alpha-SMA / Vimentine

Data complexity

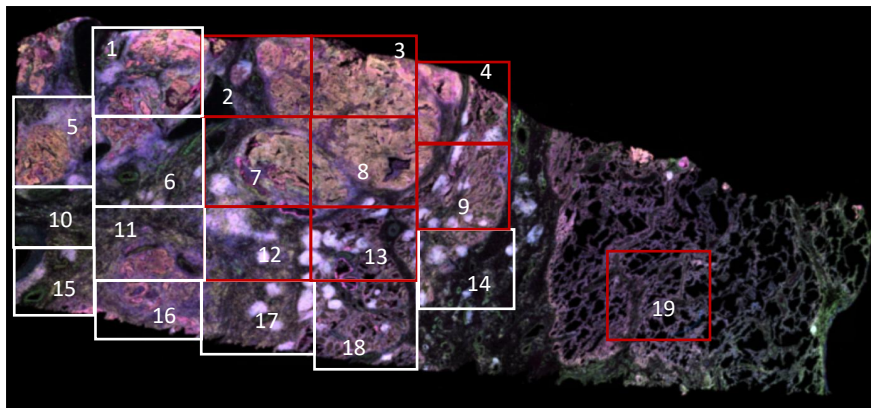


Xenium (RNA)

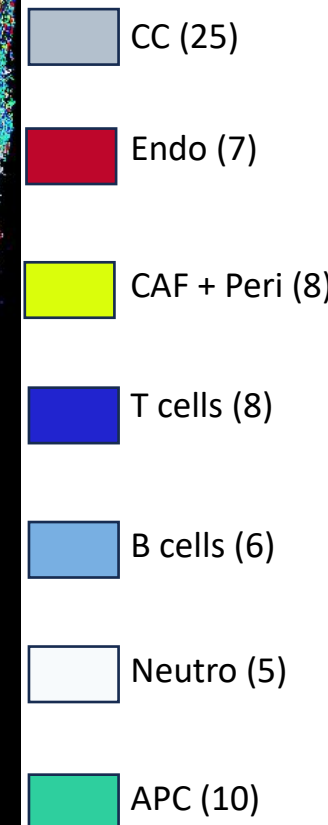
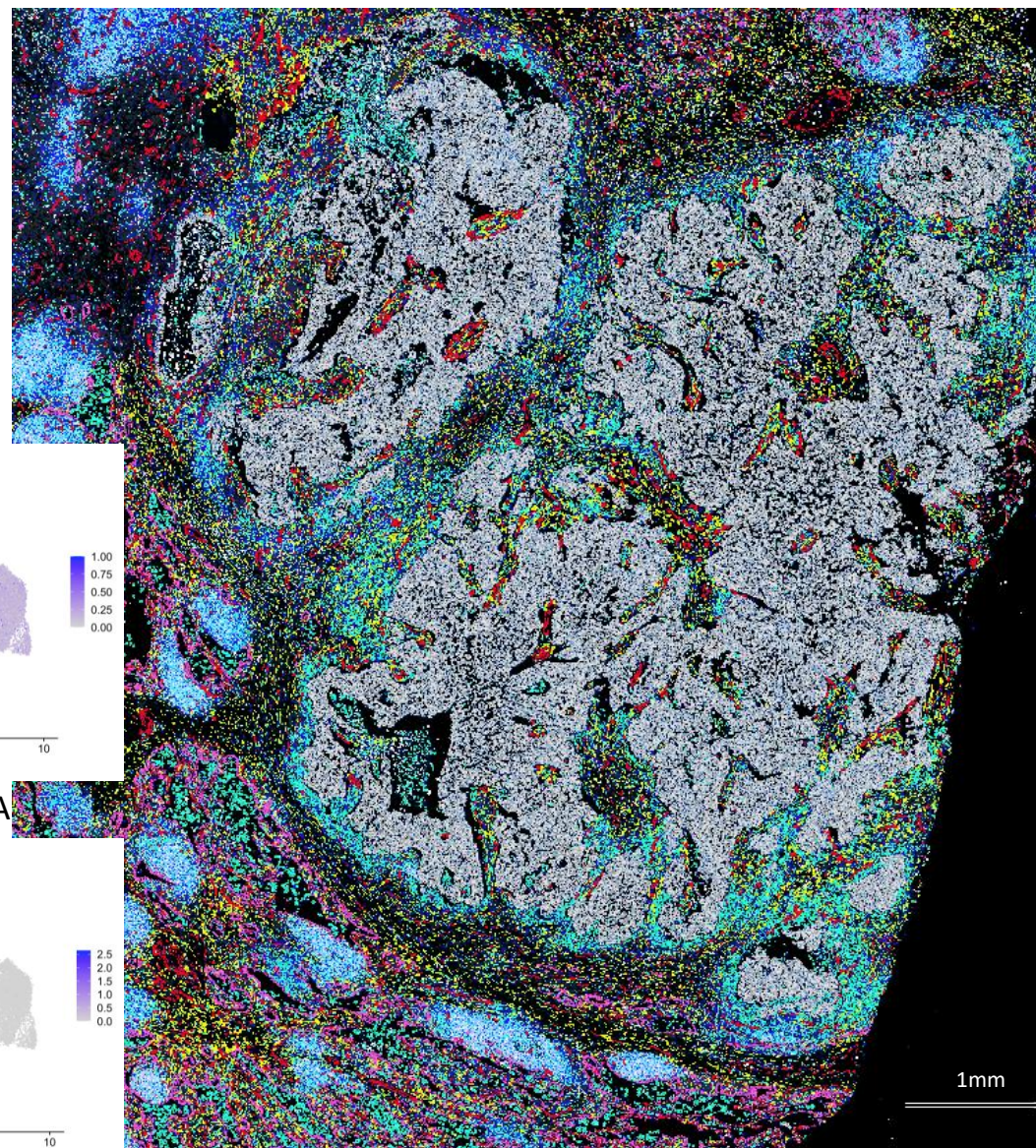


About 1-5 RNA copy / gene / cell

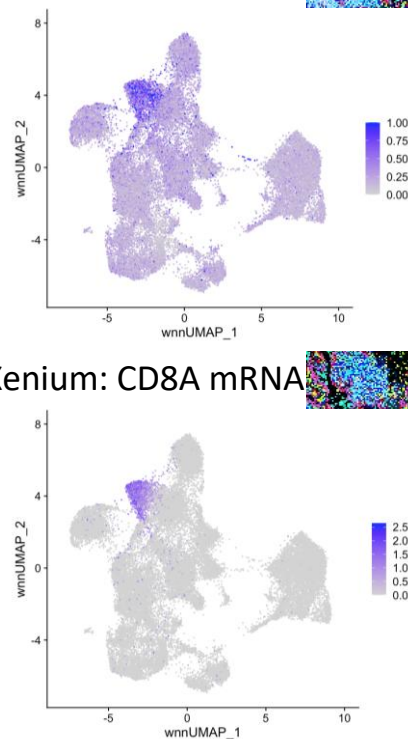
Integration of multiple ROIs



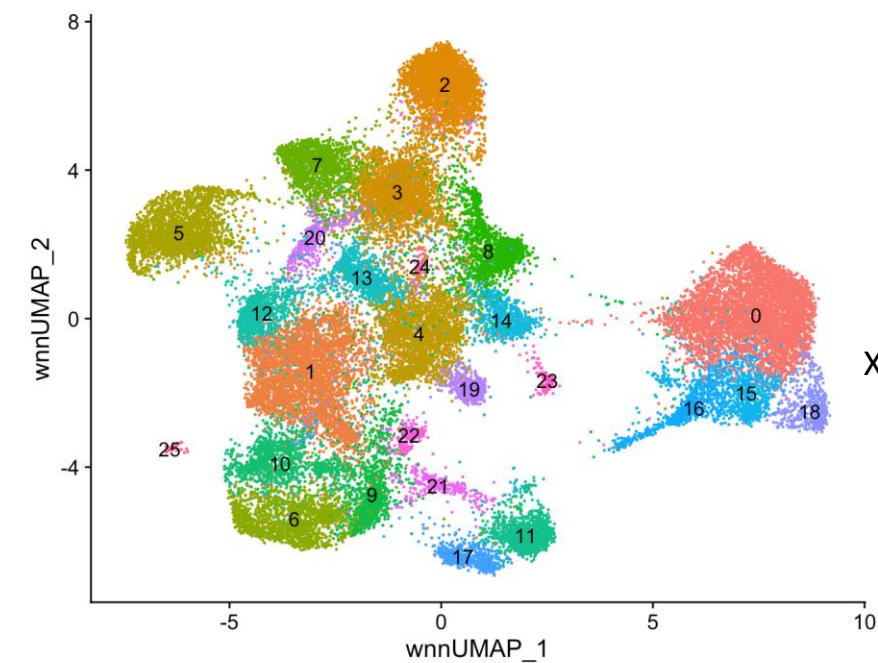
Hyperion : CD8AProtein



Xenium: CD8A mRNA

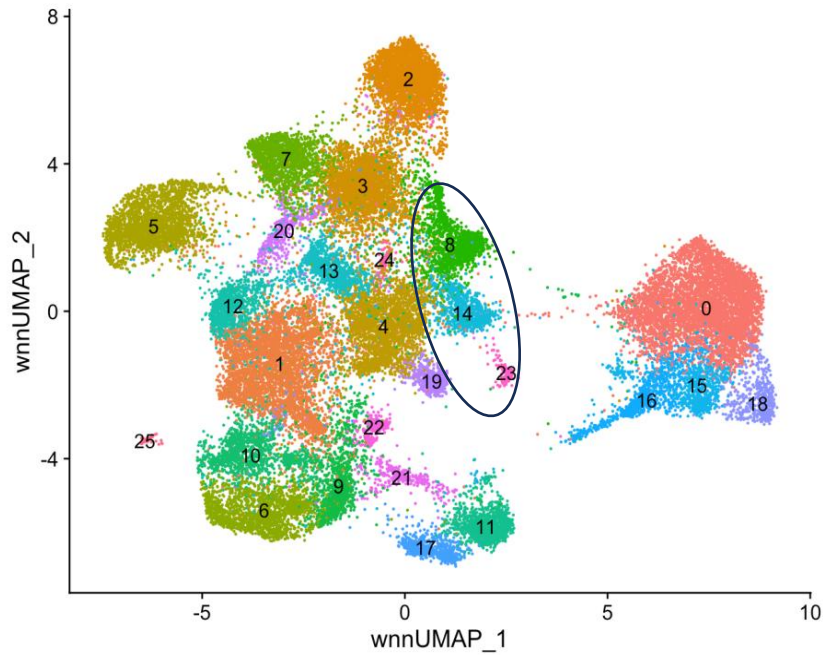


+ healthy lung cells (6) = 75 cell populations

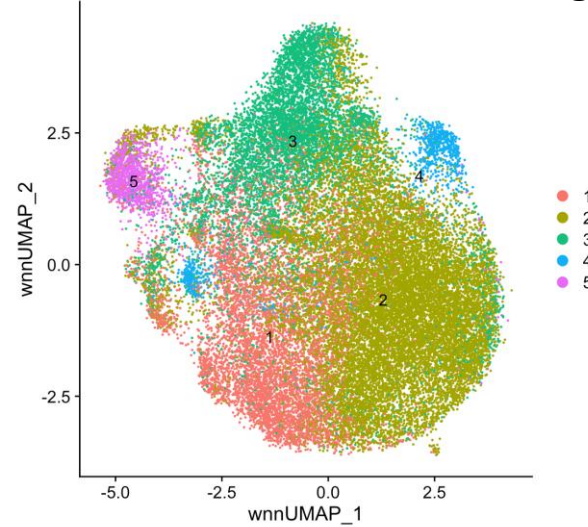


Identification of rare cell populations

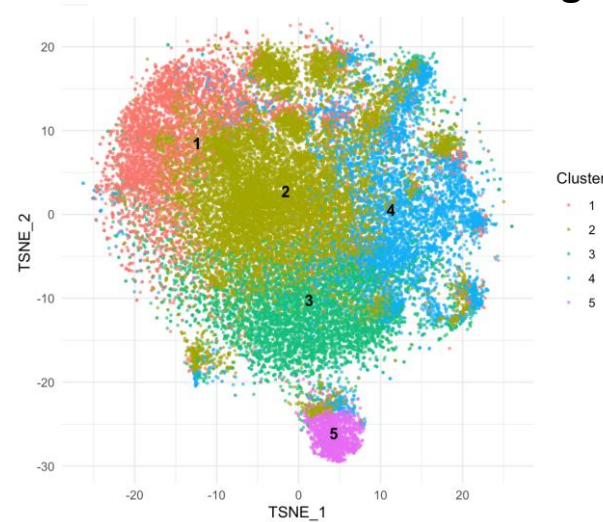
Focus on neutrophils



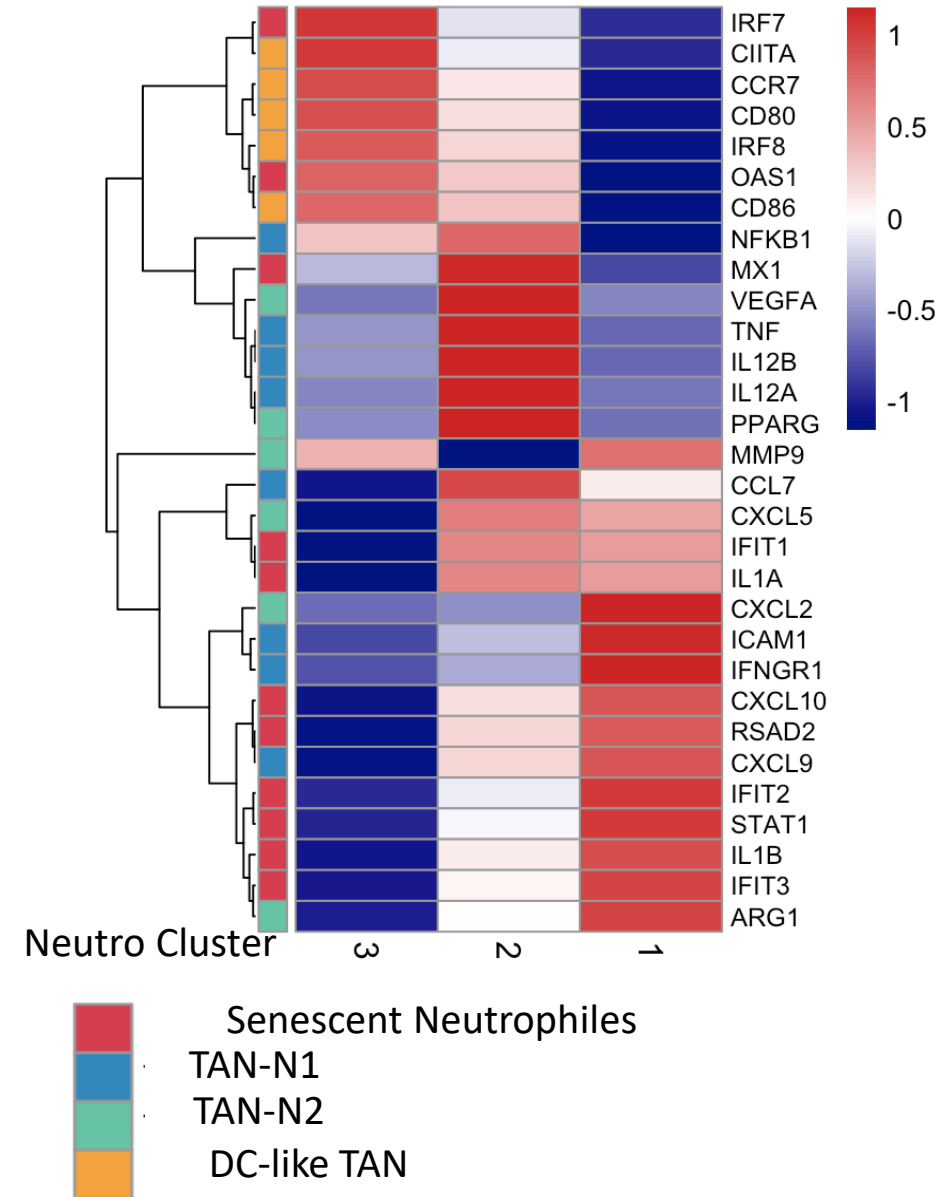
UMAP K-mean clustering



T-SNE K-mean clustering

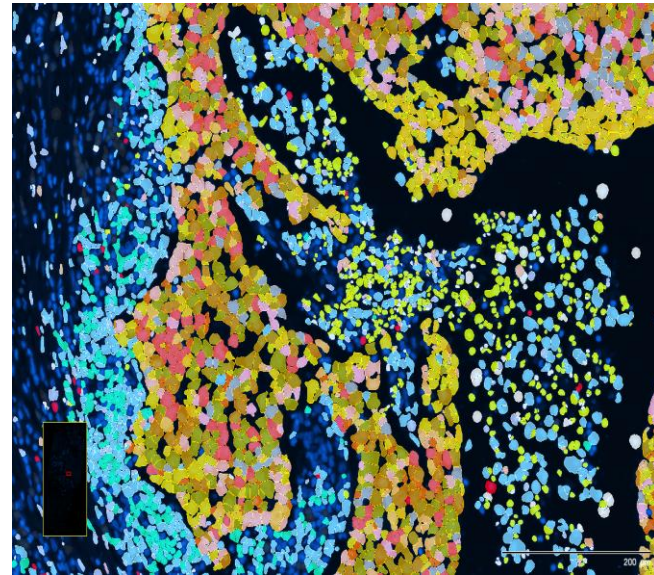


Three main Neutrophil clusters

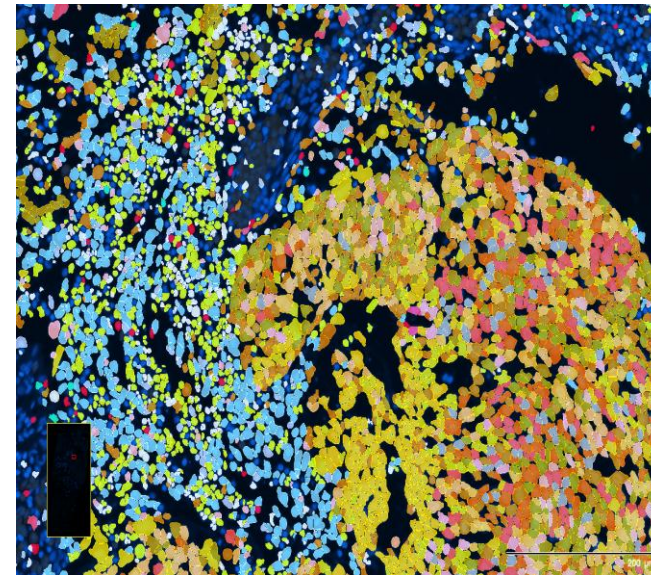


Localizing neutrophil clusters on tumor section

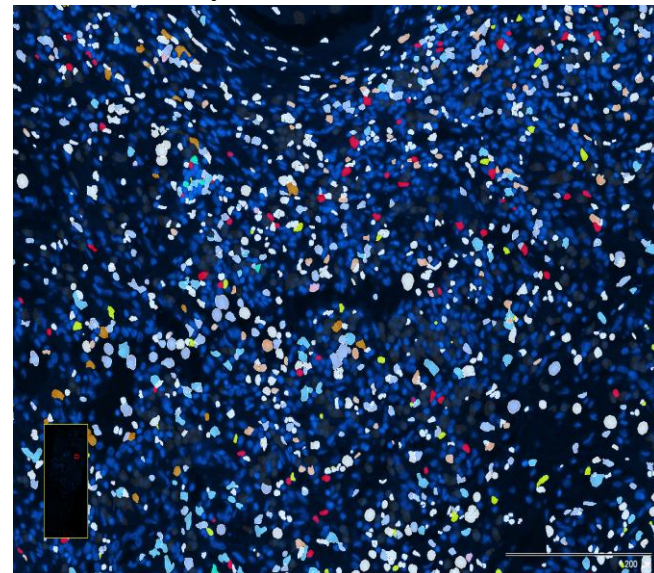
D: Tumor islet #1



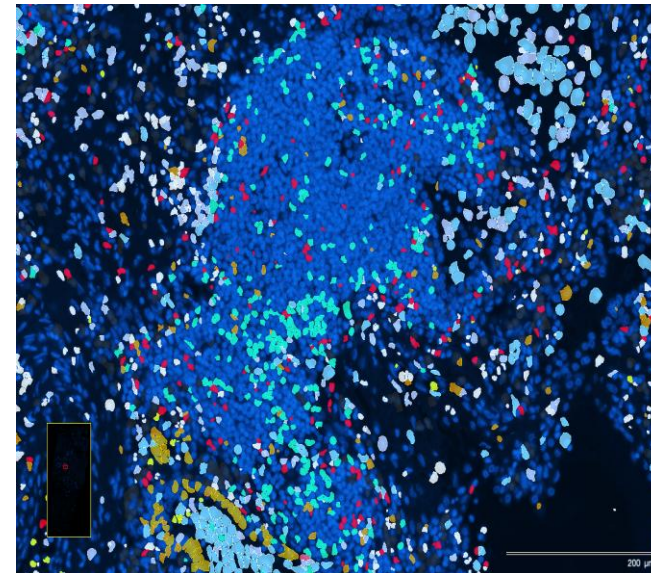
A: Tumor Islet #2



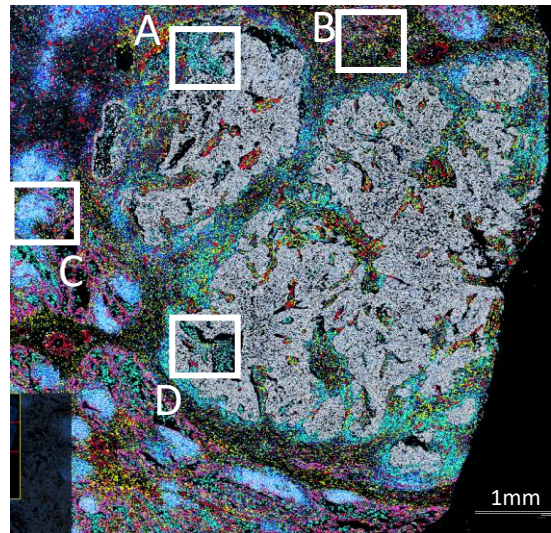
B: Parenchyma



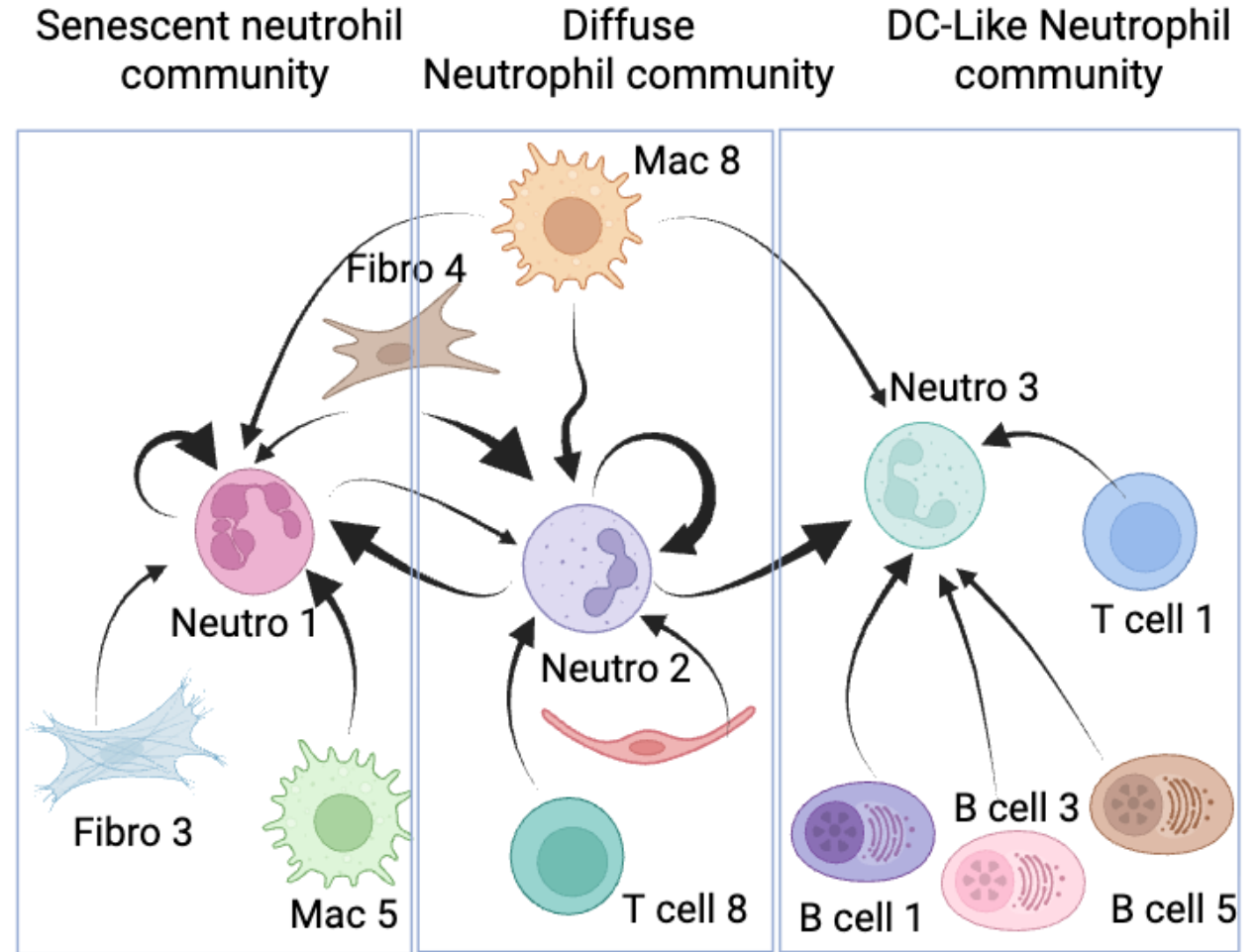
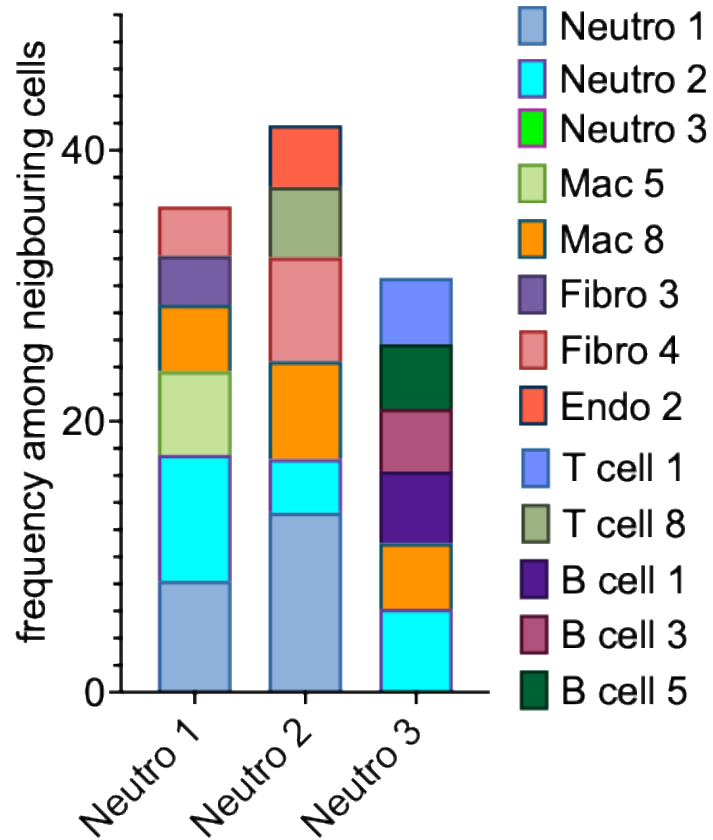
C: TLS



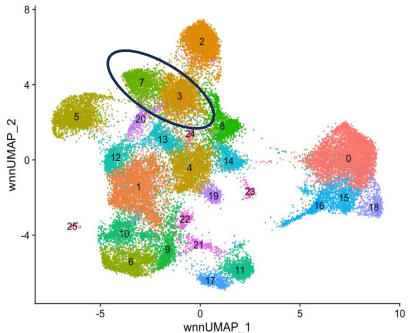
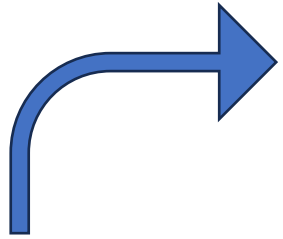
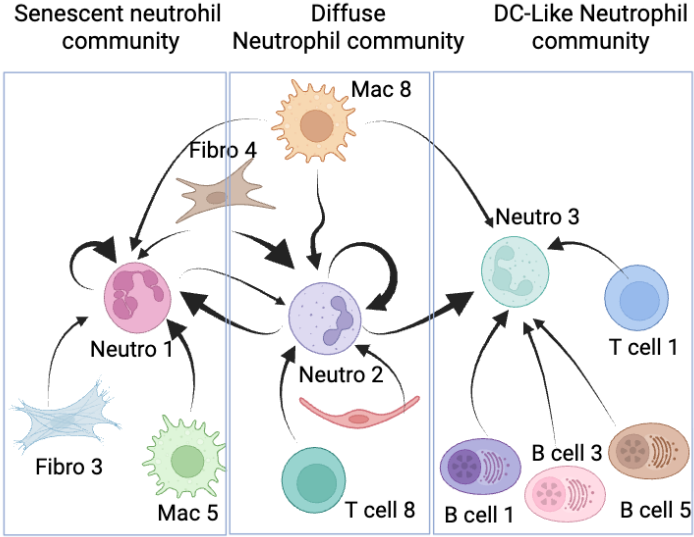
- Neutro C1
- Neutro C2
- Neutro C3
- DC (APC-C6+C7)
- Macro (APC C1-5+8-10)
- CC (C1-25)
- Nucleus



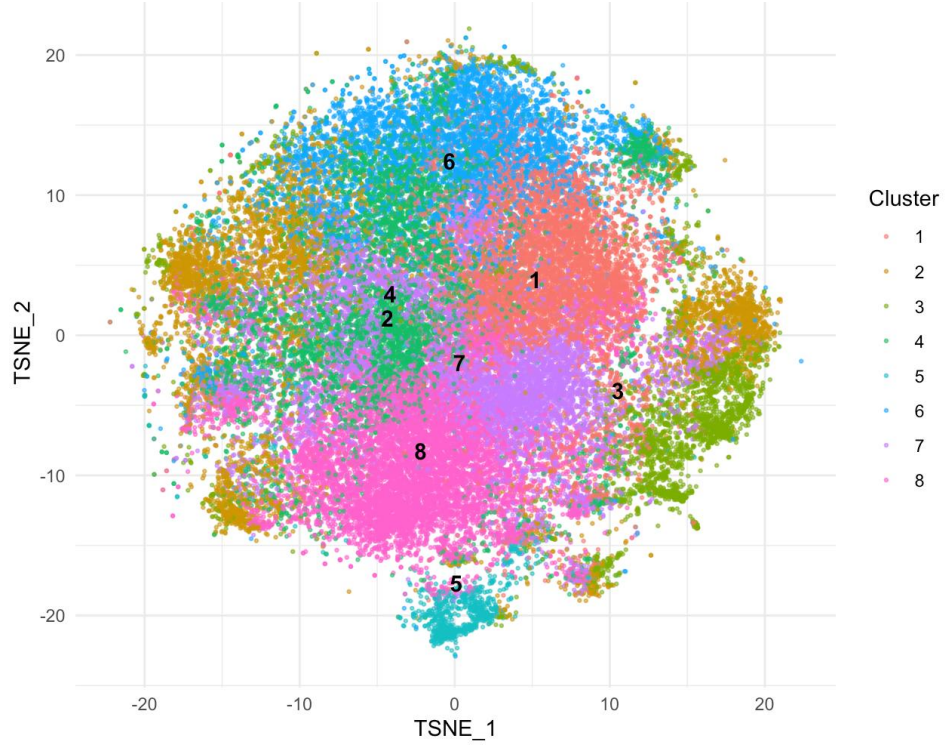
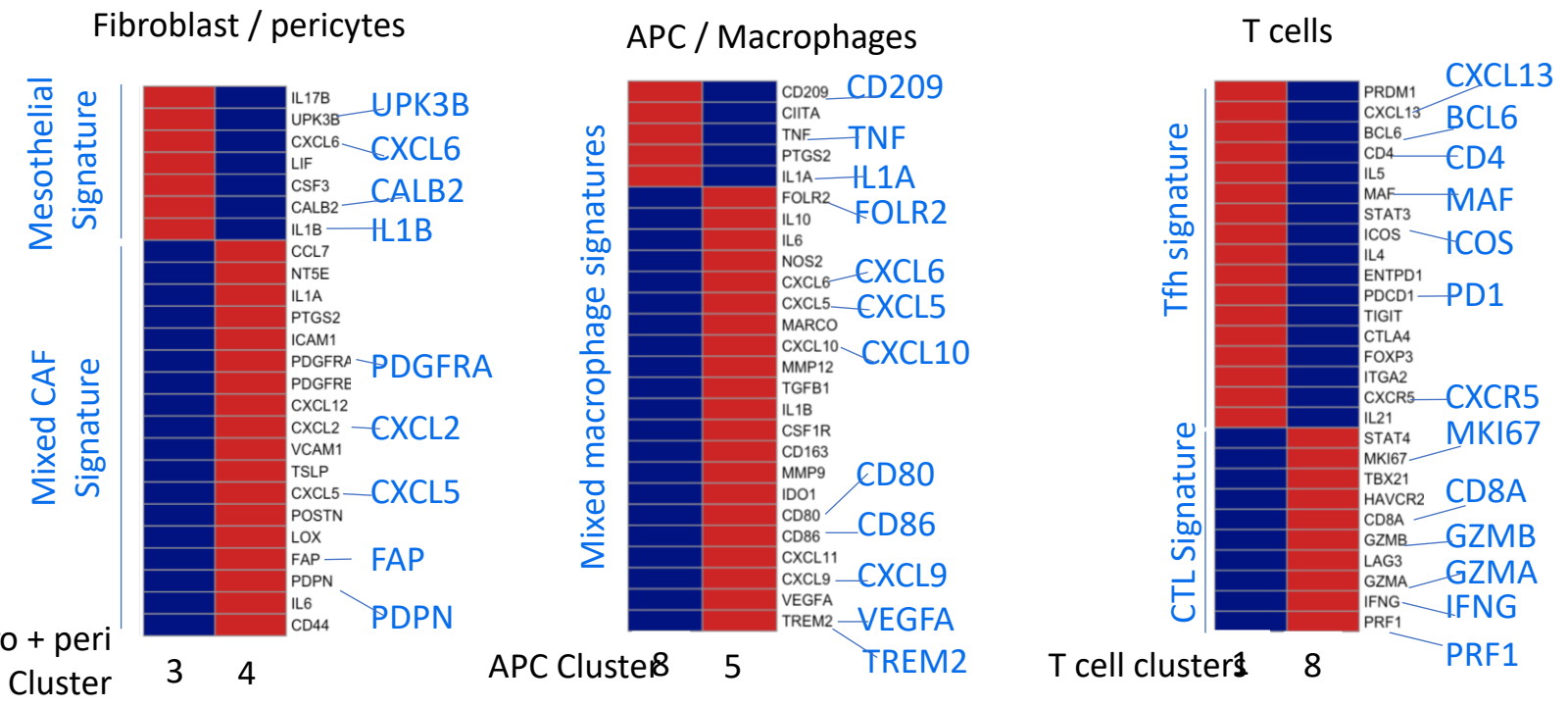
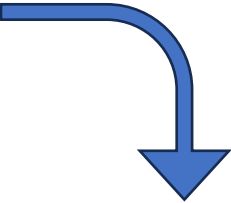
Neutrophil neighborhood



Hard to re-identify T cells sub-clusters

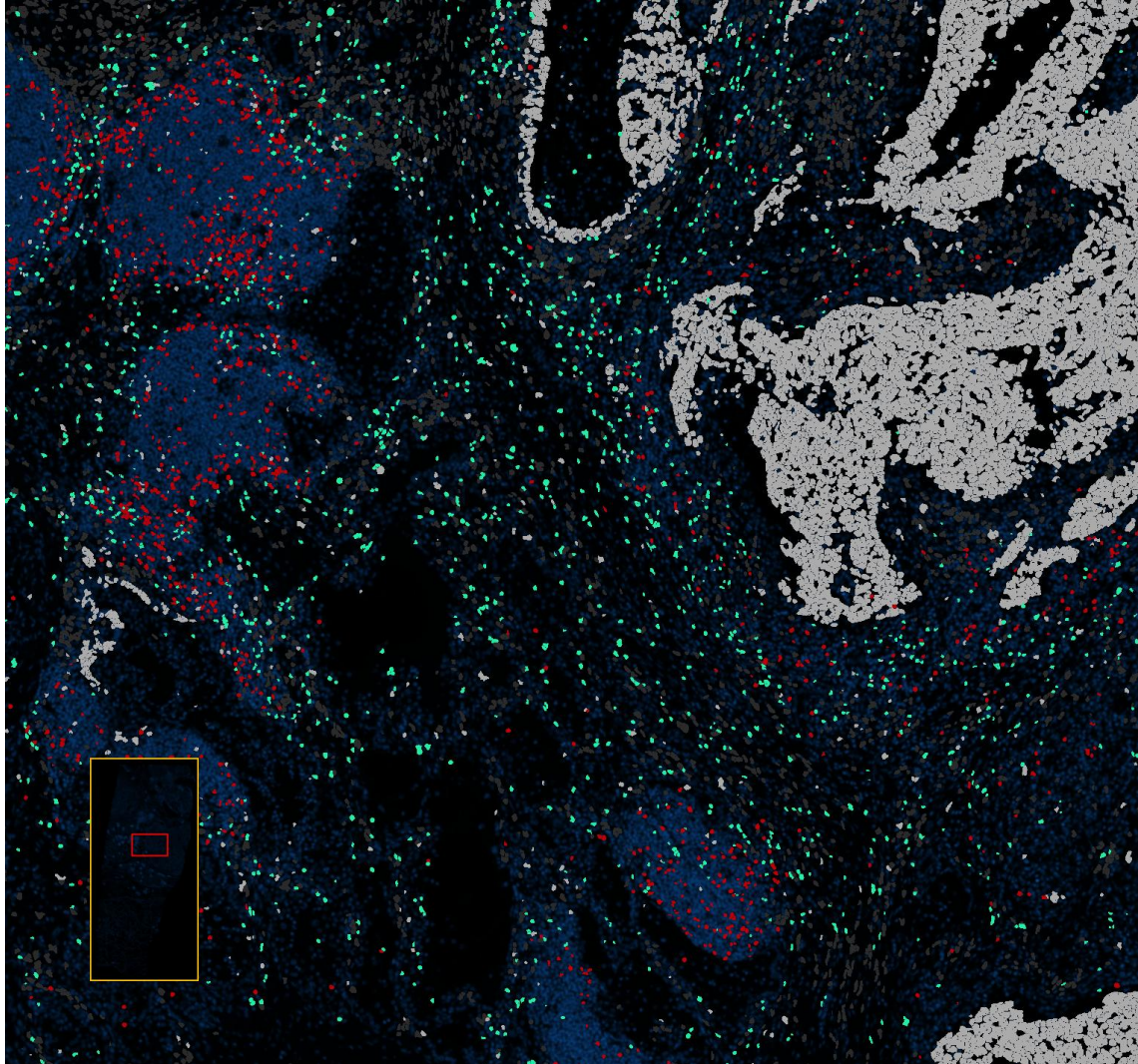


T cells sub-clustering

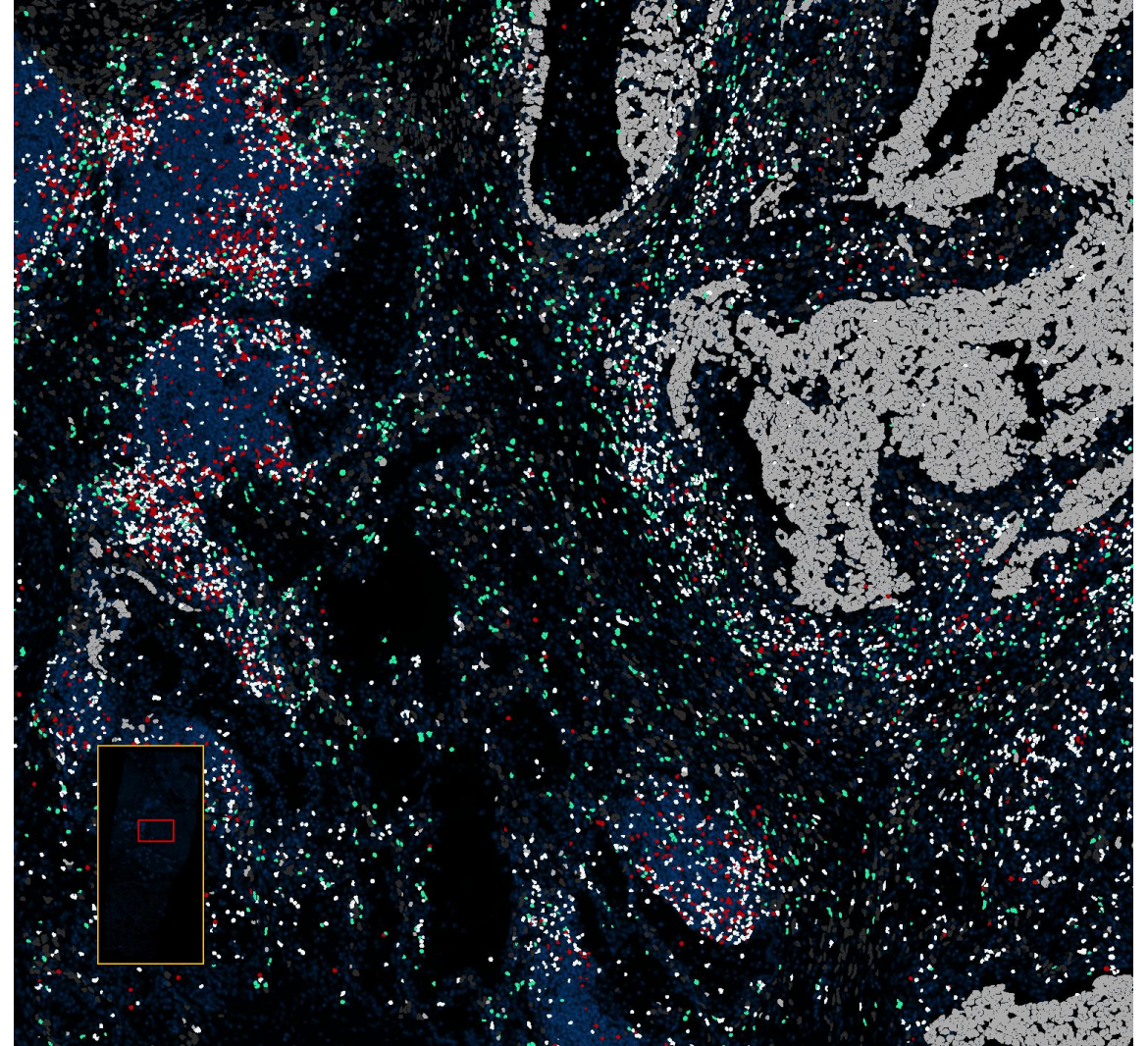


T cell clusters show specific localization

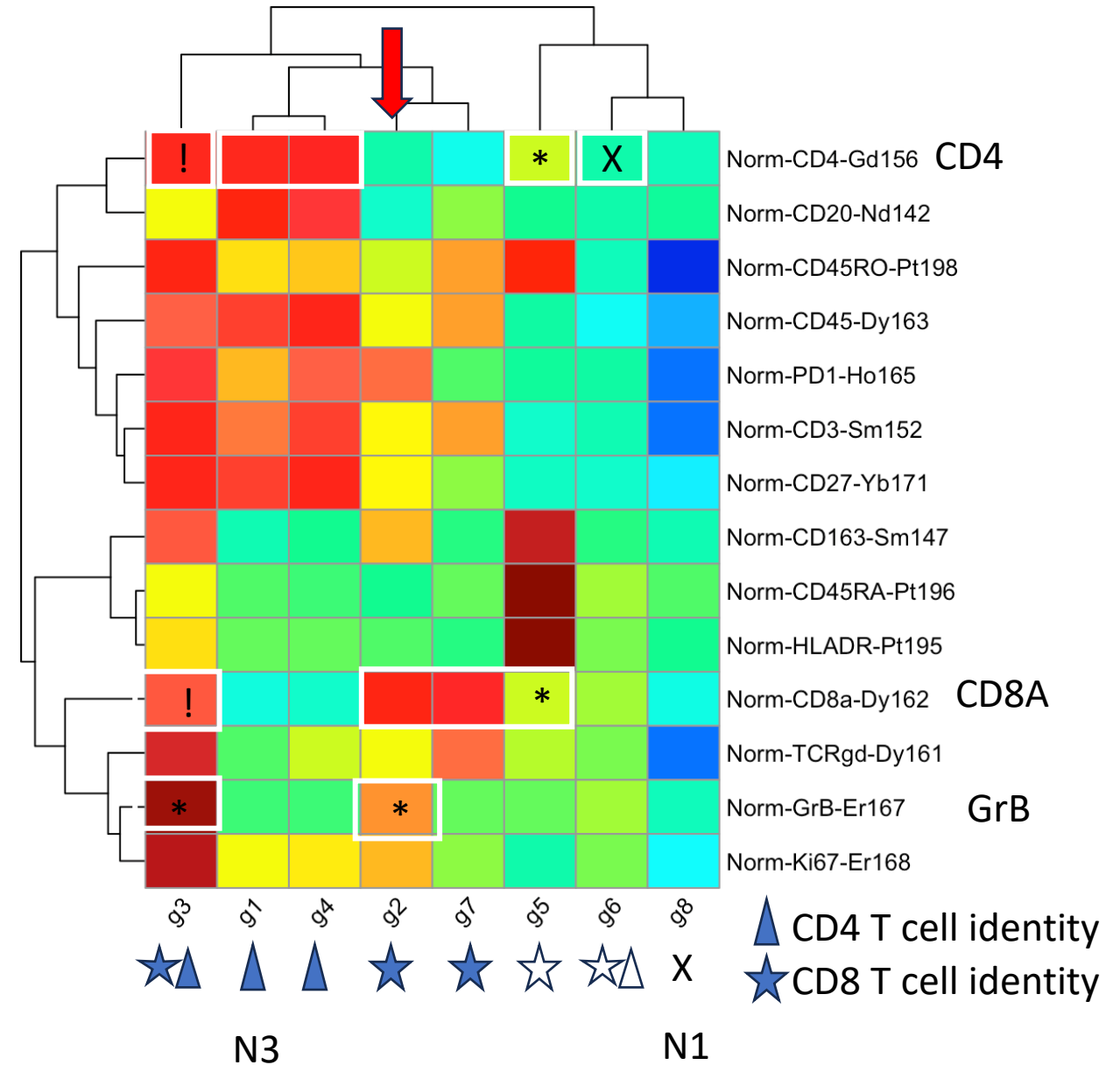
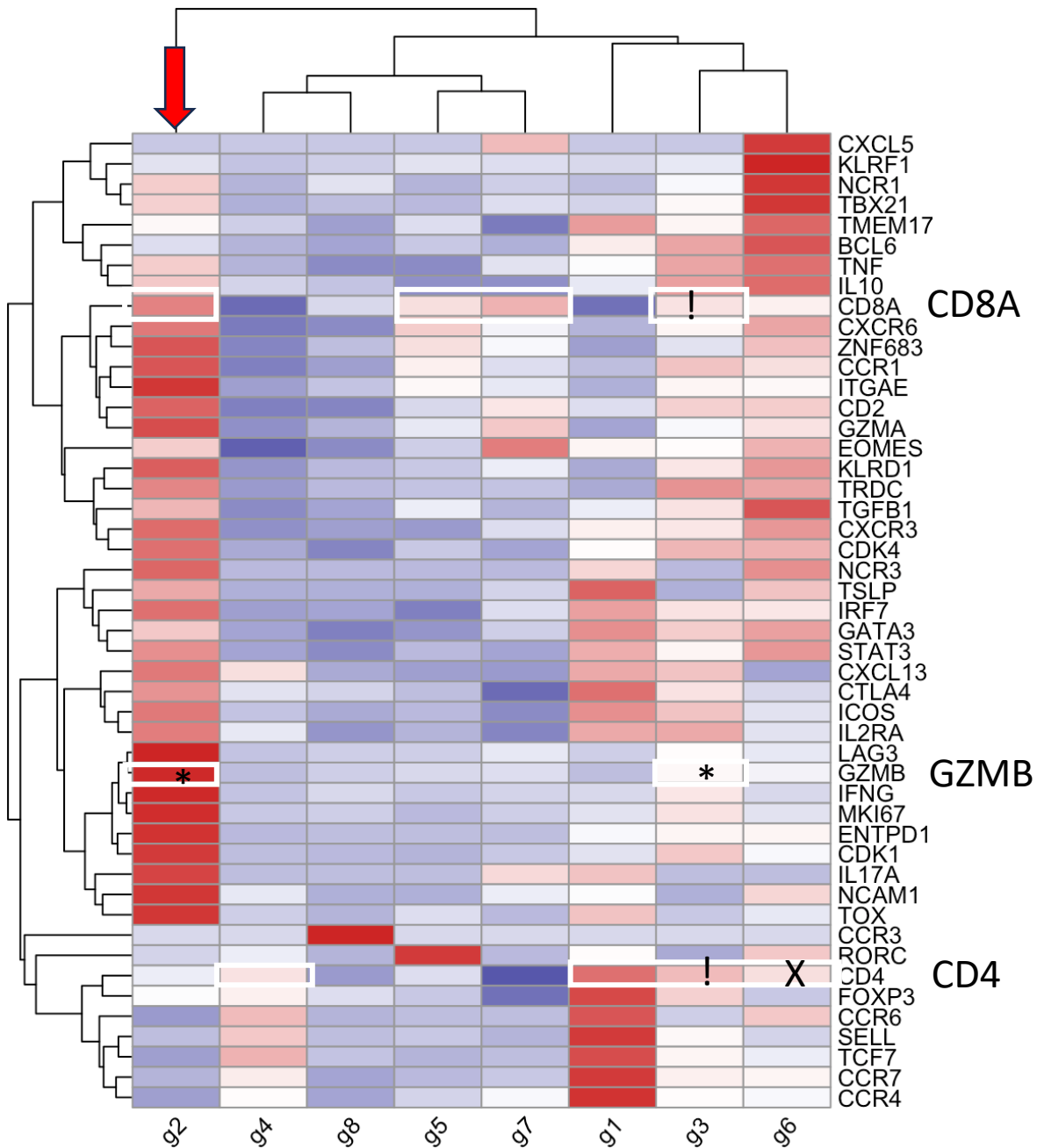
T Cells interacting with Neutro **C2** or **C3**



All T Cells



Hard to re-identify T cells sub-clusters



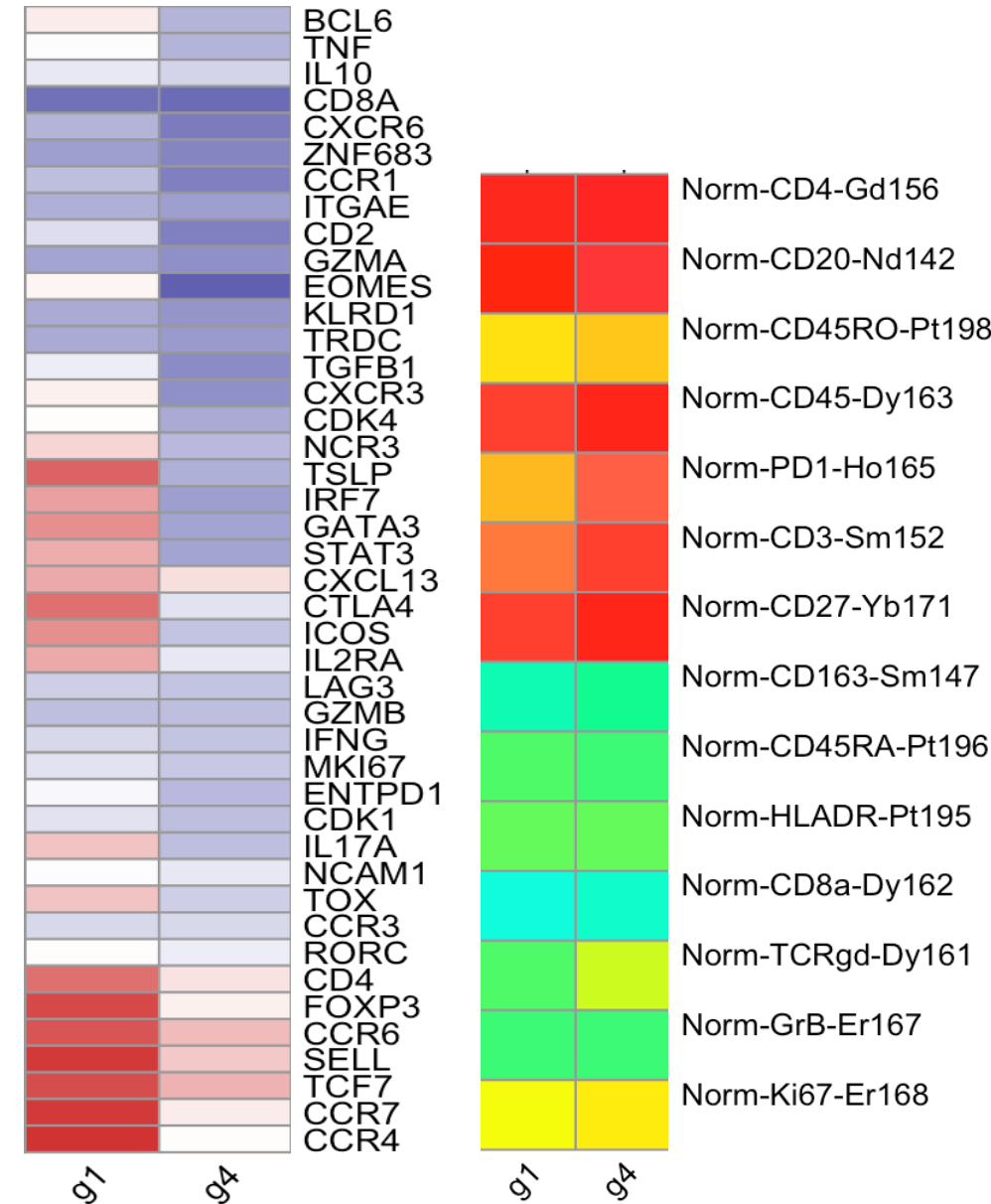
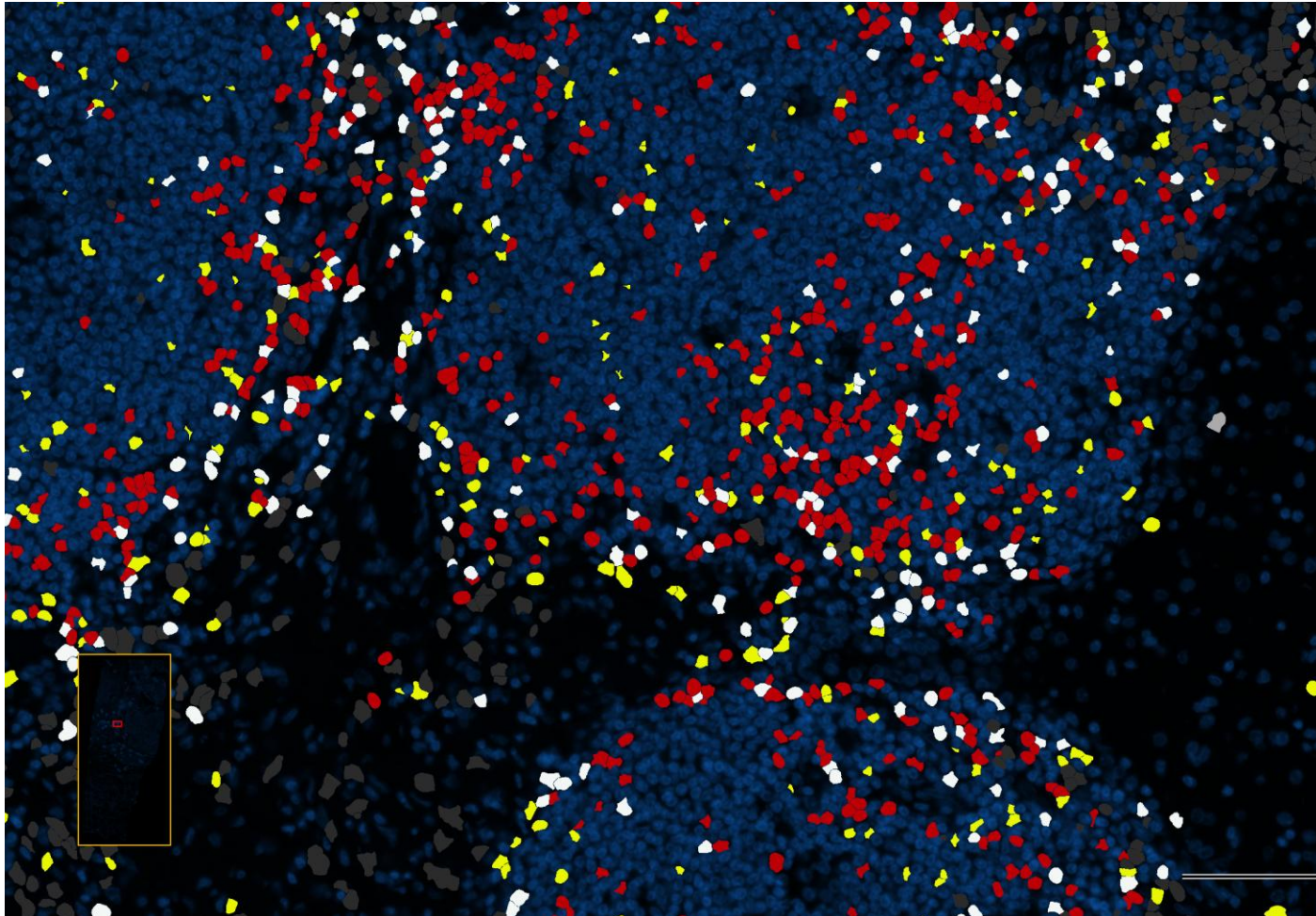
integrating proteomics + transcriptomics + spatial

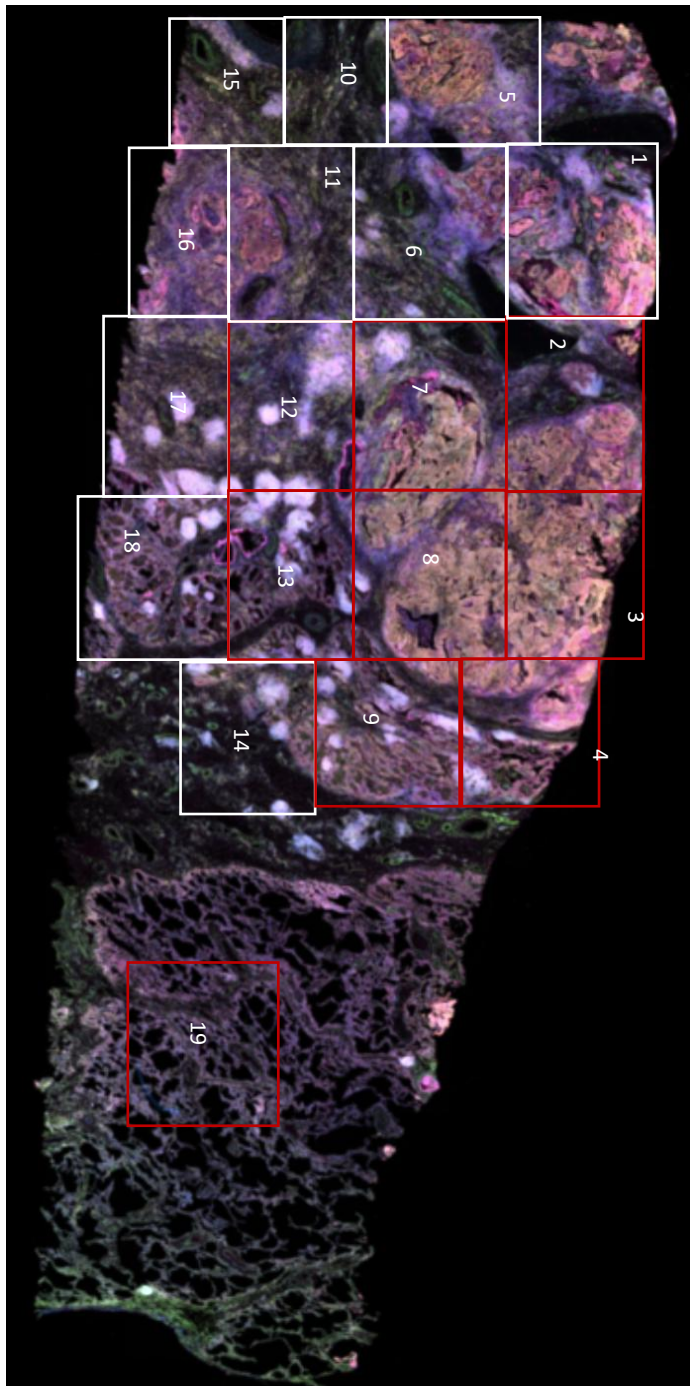
DC-Like Neutrophils interacts with Tfr cells in TLS

Neutrophils C3

interacting T Cell C1

not interacting T cell C4





Most of the Abs validated by SIM-Cat are working after long Xenium assay



Co-integrating Abs and RNA in a single dataset helps identifying cell clusters



Spatial distribution is critical for proper cell types annotation



Cell clustering contains spatial information



Enable functional exploration



Integrating multiple ROIs necessitate huge informatic power