

Cell, Volume 182

Supplemental Information

Coordinated Cellular Neighborhoods

Orchestrate Antitumoral Immunity

at the Colorectal Cancer Invasive Front

Christian M. Schürch, Salil S. Bhate, Graham L. Barlow, Darci J. Phillips, Luca Noti, Inti Zlobec, Pauline Chu, Sarah Black, Janos Demeter, David R. McIlwain, Nikolay Samusik, Yury Goltsev, and Garry P. Nolan

Data S1. Screening and Validation of CODEX Antibodies, Related to Figure 2, Table S1 and STAR Methods.

Antibodies were conjugated to DNA oligonucleotides and tested individually along with cross-validation in standard IHC using the same, non-conjugated antibody clone. Clones, manufacturers, and staining specifications are listed for each antibody, and examples of IHC staining, CODEX staining (false gray color fluorescence images), and similar areas on independent H&E-stained sections are shown. Brightness and contrast adjusted. Scale bars, 100 μm .

Staining specifications

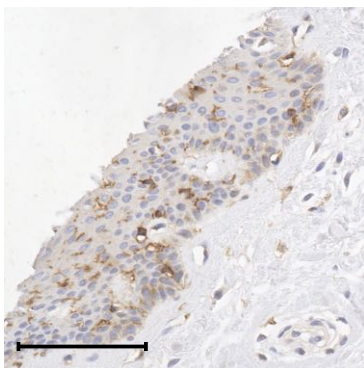
Antigen: CD1a
Clones: O10 + C1A/711

Company:
Novus Biologicals (NBP2-34698)

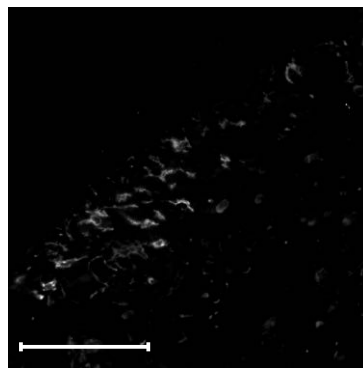
Tissue: Skin

Dilution:
IHC: 1:600
CODEX: 1:100

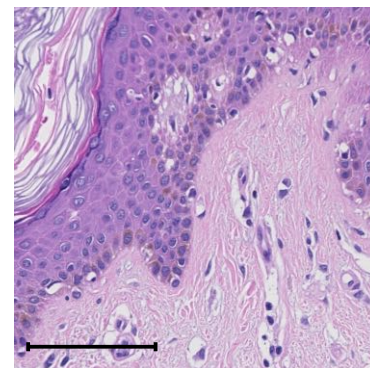
CODEX oligo: 43-Alexa647



CODEX



H&E



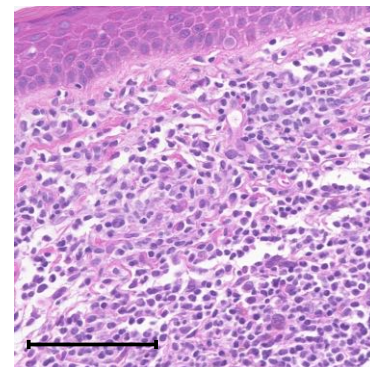
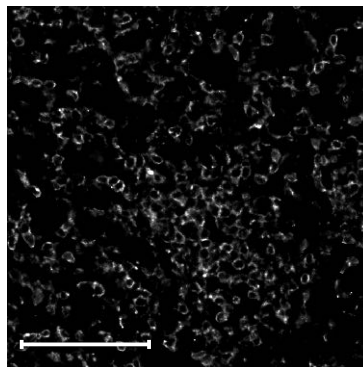
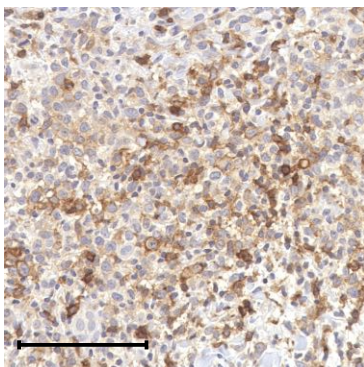
Antigen: CD2
Clone: RPA-2.10

Company:
BioLegend (300202)

Tissue: Skin T cell lymphoma

Dilution:
IHC: 1:5
CODEX: 1:25

CODEX oligo: 25-Alexa647



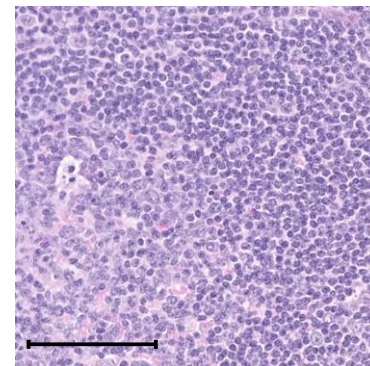
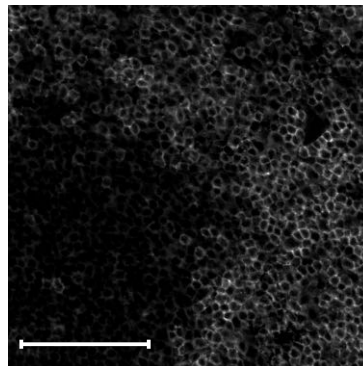
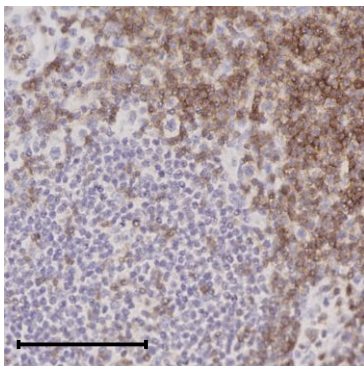
Antigen: CD3
Clone: D7A6E or MRQ-39

Companies:
Cell Signaling Technology (custom)
Cell Marque (custom)

Tissue: Tonsil

Dilution:
IHC: 1:100
CODEX: 1:100

CODEX oligo: 77-Alexa647



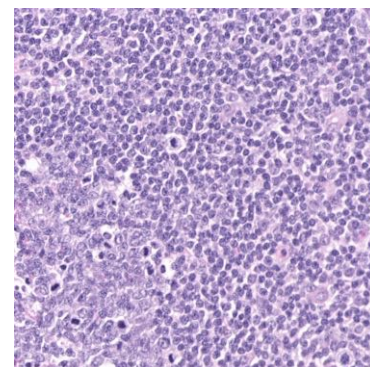
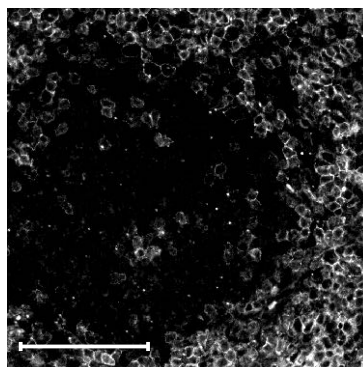
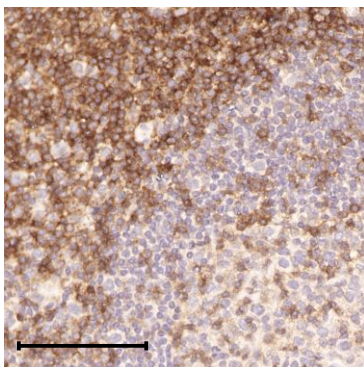
Antigen: CD4
Clone: EPR6855

Company:
Abcam (ab181724)

Tissue: Tonsil

Dilution:
IHC: 1:25
CODEX: 1:100

CODEX oligo: 20-ATTO550



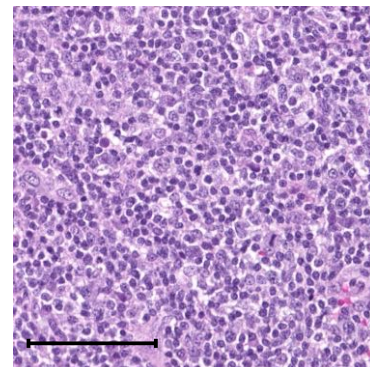
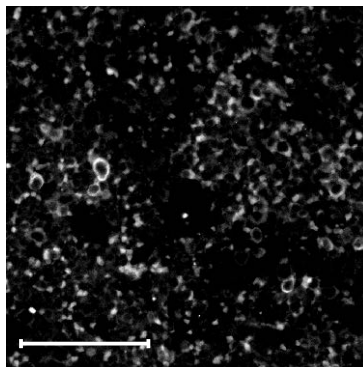
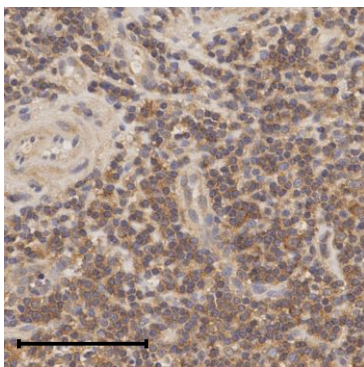
Antigen: CD5
Clone: UCHT2

Company:
BD Biosciences (555350)

Tissue: Tonsil

Dilution:
IHC: 1:5
CODEX: 1:25

CODEX oligo: 75-ATTO550



Staining specifications

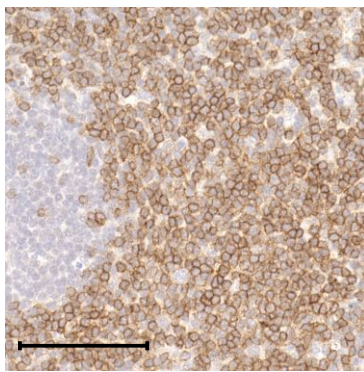
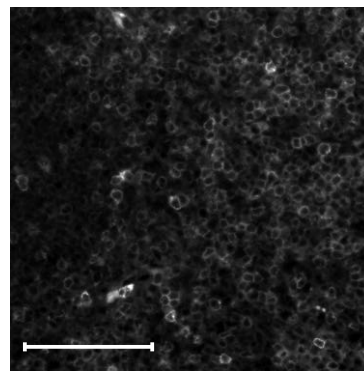
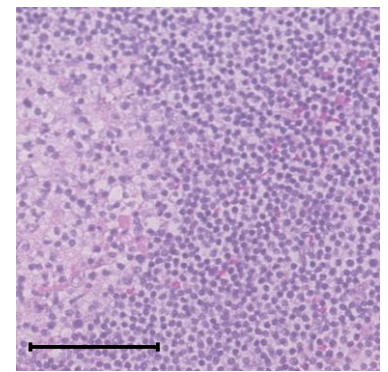
Antigen: CD7
Clone: MRQ-56

Company:
Cell Marque (custom)

Tissue: Tonsil

Dilution:
IHC: 1:50
CODEX: 1:100

CODEX oligo: 63-Alexa488

**CODEX****H&E**

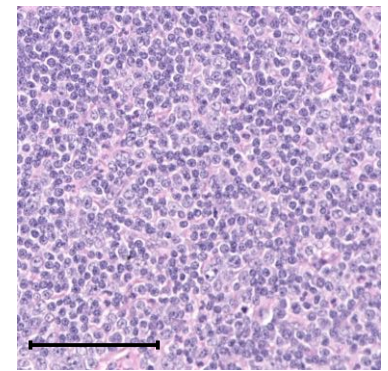
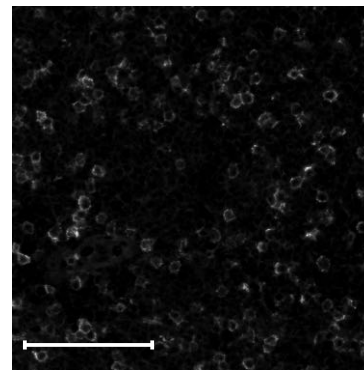
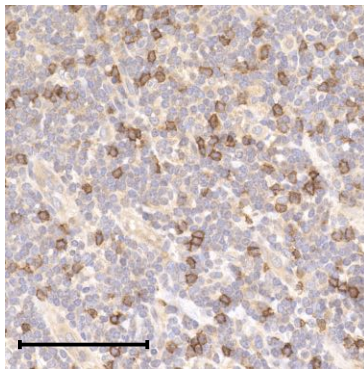
Antigen: CD8
Clone: C8/144B

Company:
Cell Marque (custom)
Santa Cruz Bio (sc-53212)

Tissue: Tonsil

Dilution:
IHC: 1:200
CODEX: 1:50

CODEX oligo: 8-Alexa488



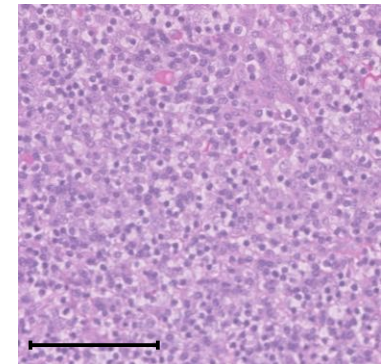
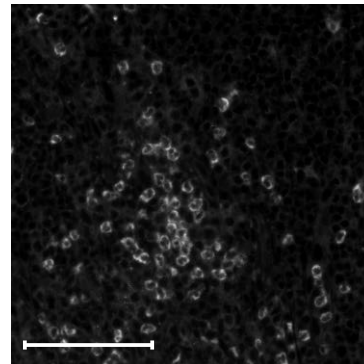
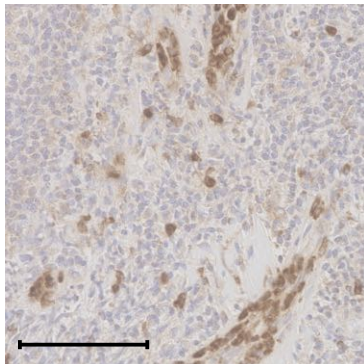
Antigen: CD11b
Clone: EPR1344

Company:
Abcam (ab216445)

Tissue: Tonsil

Dilution:
IHC: 1:100
CODEX: 1:50

CODEX oligo: 28-Alexa647



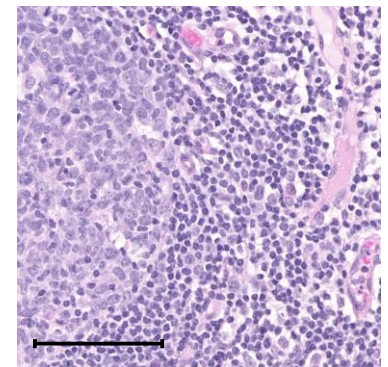
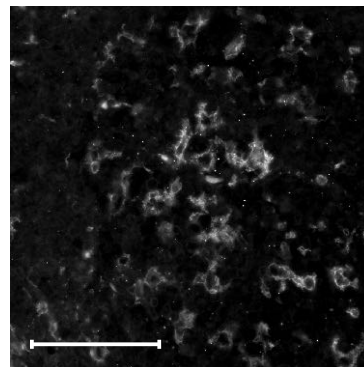
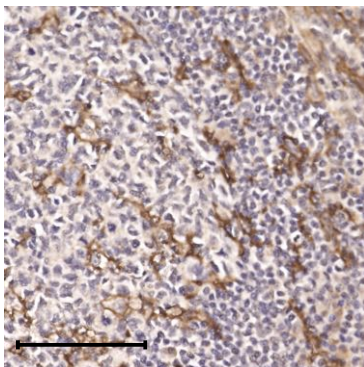
Antigen: CD11c
Clone: EP1347Y

Company:
Abcam (ab216655)

Tissue: Tonsil

Dilution:
IHC: 1:100
CODEX: 1:50

CODEX oligo: 49-ATTO550



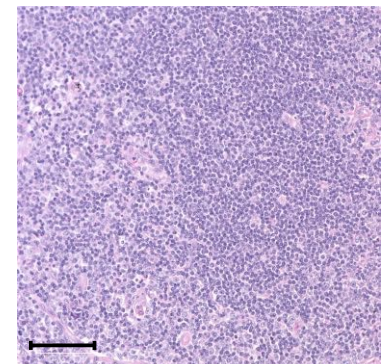
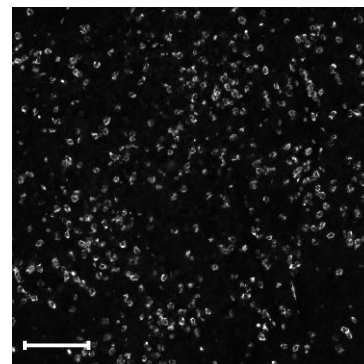
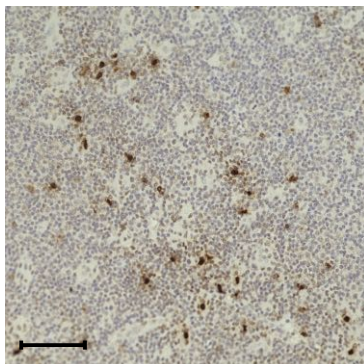
Antigen: CD15
Clone: MMA

Company:
BD Biosciences (559045)

Tissue: Tonsil

Dilution:
IHC: 1:100
CODEX: 1:200

CODEX oligo: 14-ATTO550

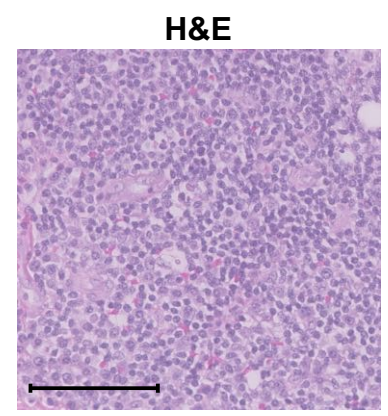
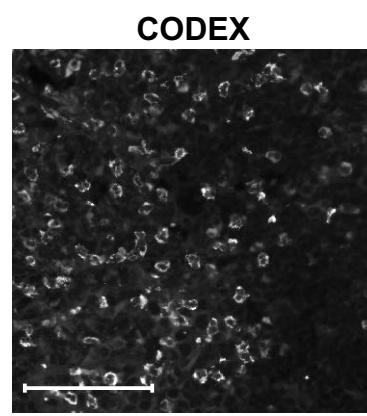
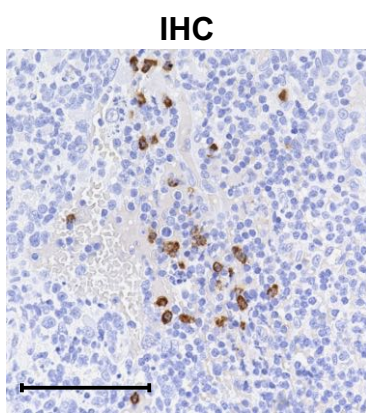


Staining specifications**Antigen: CD15**
Clone: HI98Company:
Biolegend (301902)

Tissue: Tonsil

Dilution:
IHC: 1:100
CODEX: 1:200

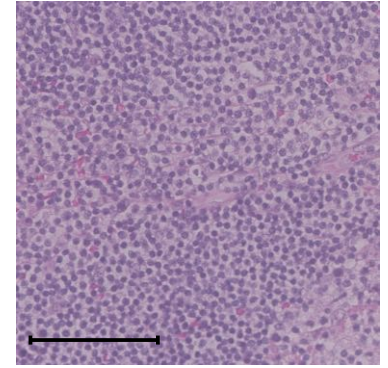
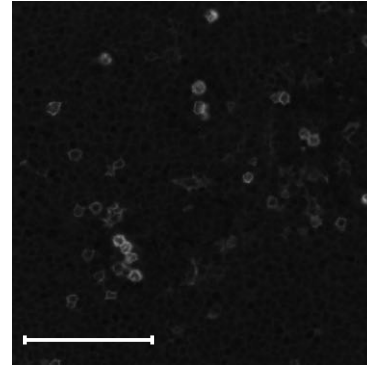
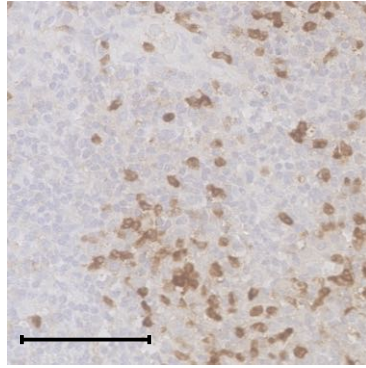
CODEX oligo: 15-Alexa488

**Antigen: CD16**
Clone: D1N9LCompany:
Cell Signaling Technology (custom)

Tissue: Tonsil

Dilution:
IHC: 1:100
CODEX: 1:100

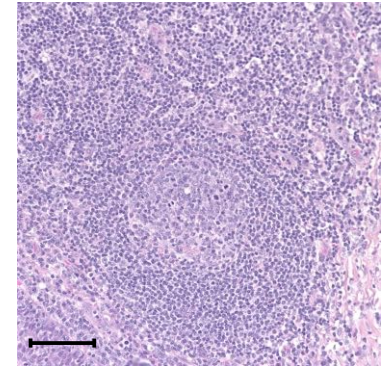
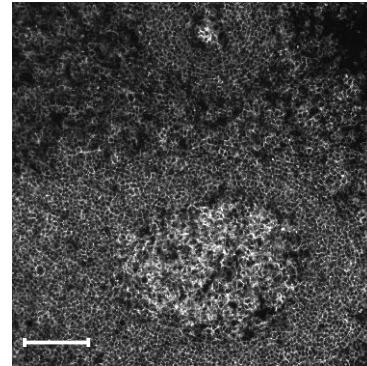
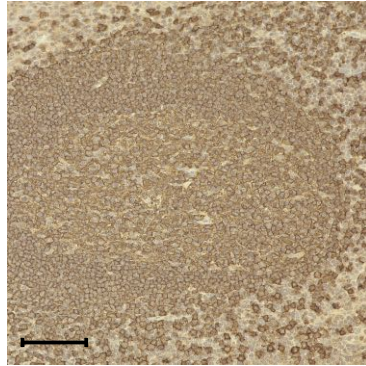
CODEX oligo: 26-Alexa647

**Antigen: CD20**
Clone: rIGEL/773Company:
Novus Biologicals (NBP2-54591)

Tissue: Tonsil

Dilution:
IHC: 1:100
CODEX: 1:200

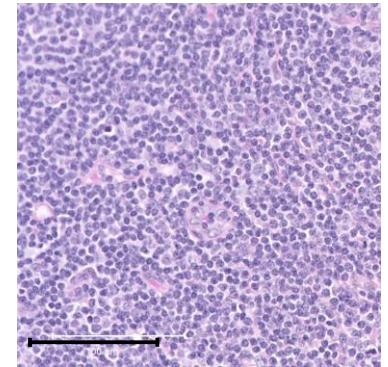
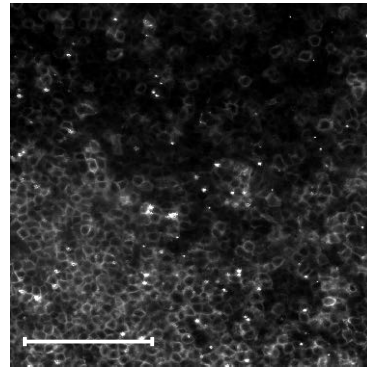
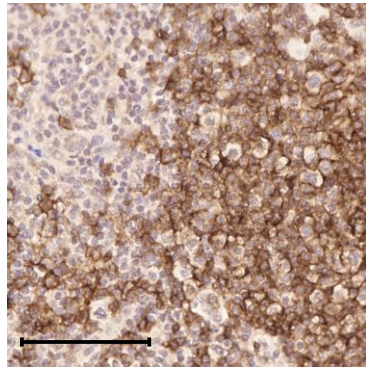
CODEX oligo: 48-ATTO550

**Antigen: CD20**
Clone: H1Company:
BD Biosciences (555677)

Tissue: Tonsil

Dilution:
IHC: 1:200
CODEX: 1:10

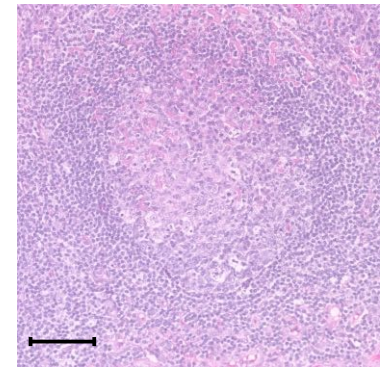
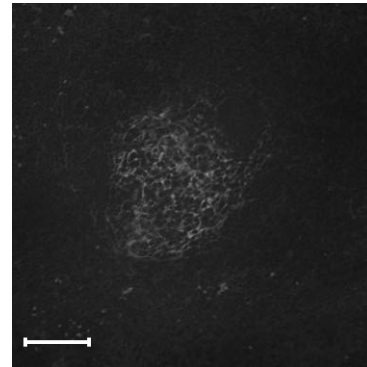
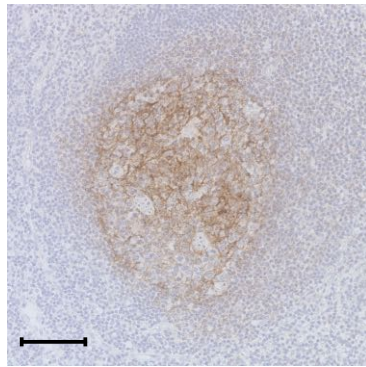
CODEX oligo: 48-ATTO550

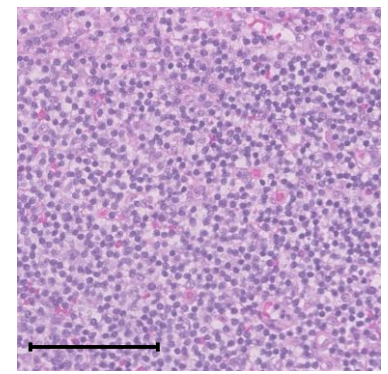
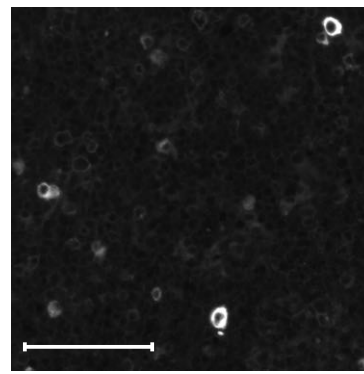
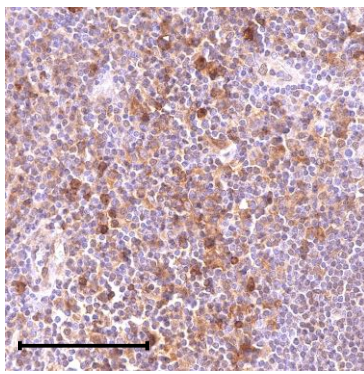
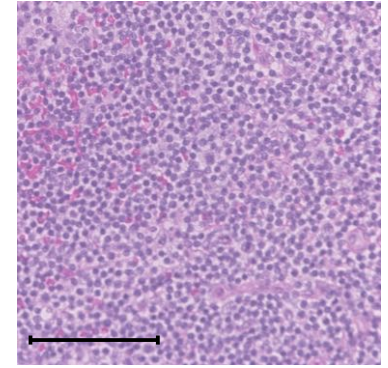
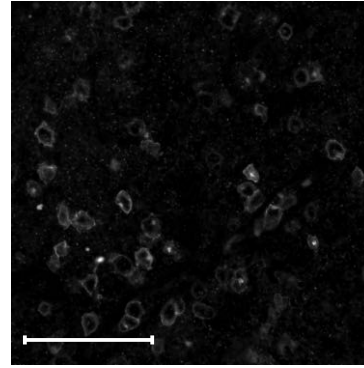
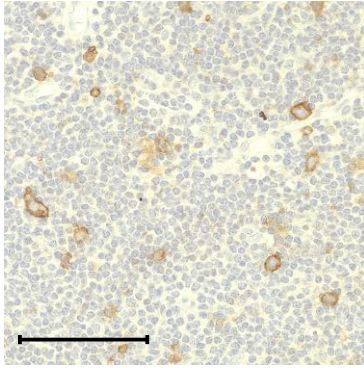
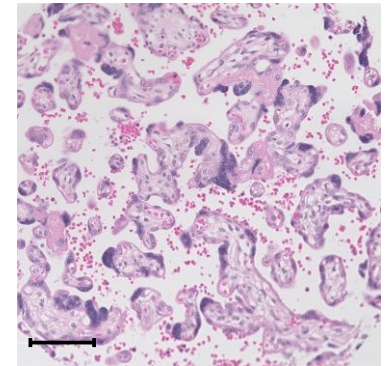
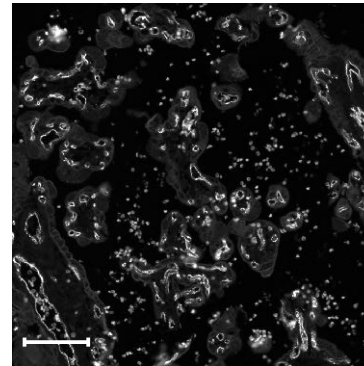
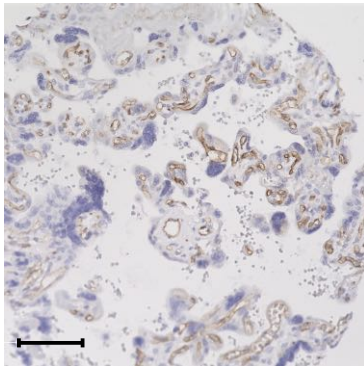
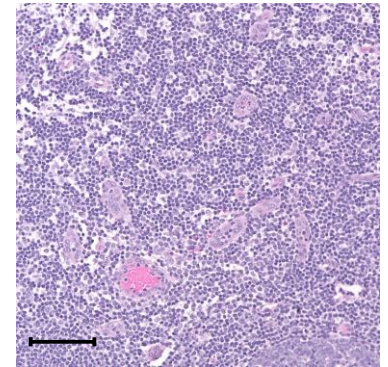
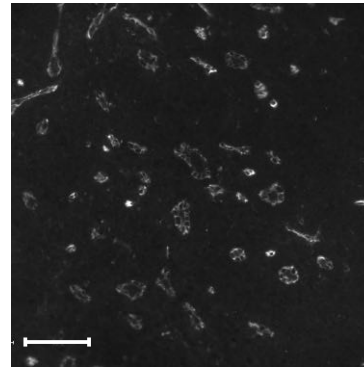
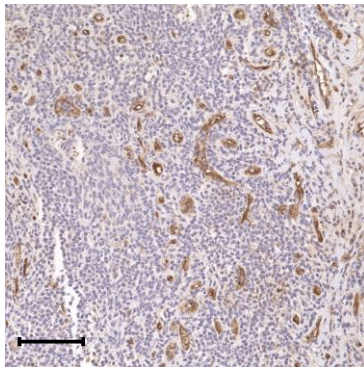
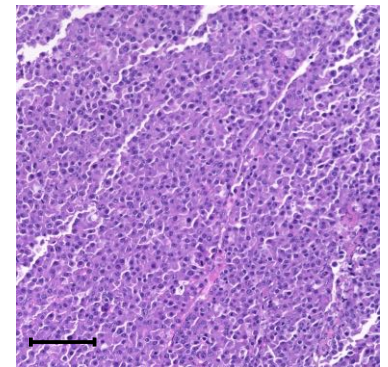
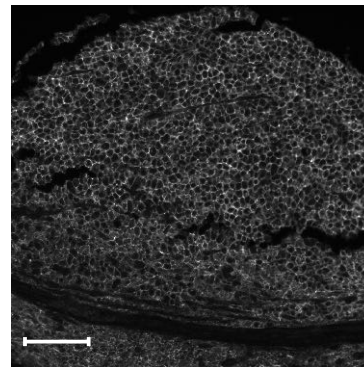
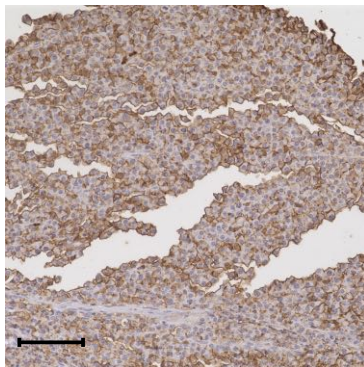
**Antigen: CD21**
Clone: Bu32Company:
Biolegend (354902)

Tissue: Tonsil

Dilution:
IHC: 1:100
CODEX: 1:50

CODEX oligo: 21-Alexa647



Staining specifications**Antigen: CD25**
Clone: 4C9**Company:**
Cell Marque (custom)**Tissue: Tonsil****Dilution:**
IHC: 1:
CODEX: 1:100**CODEX oligo: 24-ATTO550****Antigen: CD30**
Clone: Ber-H2**Company:**
Cell Marque (custom)**Tissue: Tonsil****Dilution:**
IHC: 1:100
CODEX: 1:25**CODEX oligo: 57-ATTO550****Antigen: CD31**
Clones:
C31.3 + C31.7 + C31.10**Company:**
Novus Biologicals (NBP2-47785)**Tissue: Placenta****Dilution:**
IHC: 1:100
CODEX: 1:200**CODEX oligo: 68-ATTO550****Antigen: CD34**
Clones:
QBEnd/10 + HPCA1/764**Company:**
Novus Biologicals (NBP2-47909)**Tissue: Tonsil****Dilution:**
IHC: 1:200
CODEX: 1:100**CODEX oligo: 38-ATTO550****Antigen: CD38**
Clone: EPR4106**Company:**
Abcam (ab176886)**Tissue: Plasmacytoma****Dilution:**
IHC: 1:200
CODEX: 1:100**CODEX oligo: 66-ATTO550**

Staining specifications

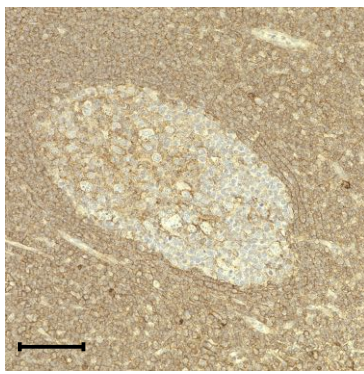
Antigen: CD44
Clone: IM-7

Company:
BD Biosciences (553131)

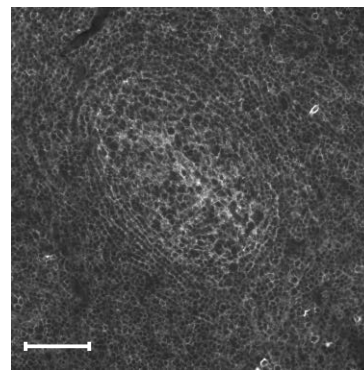
Tissue: Tonsil

Dilution:
IHC: 1:100
CODEX: 1:25

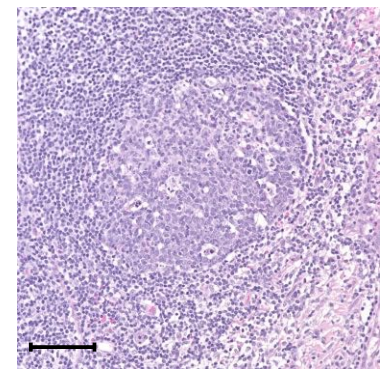
CODEX oligo: 44-Alexa488



CODEX



H&E



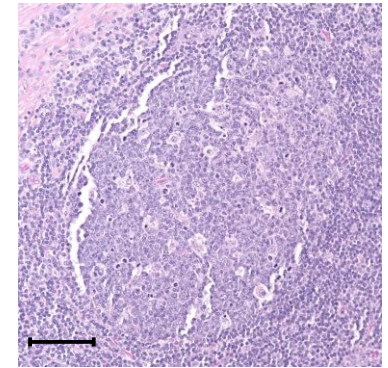
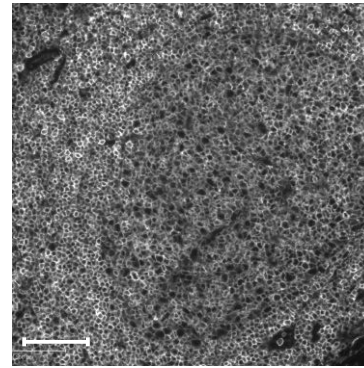
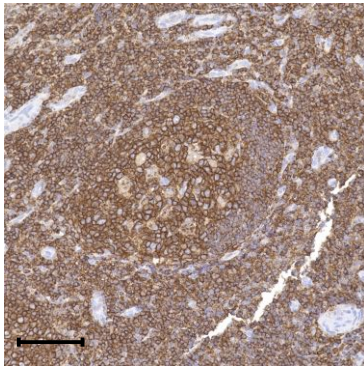
Antigen: CD45
Clones: 2B11 + PD7/26

Company:
Novus Biologicals (NBP2-34528)

Tissue: Tonsil

Dilution:
IHC: 1:400
CODEX: 1:400

CODEX oligo: 56-ATTO550



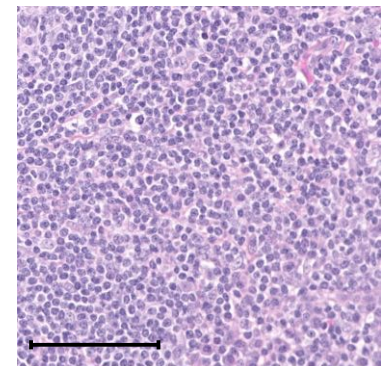
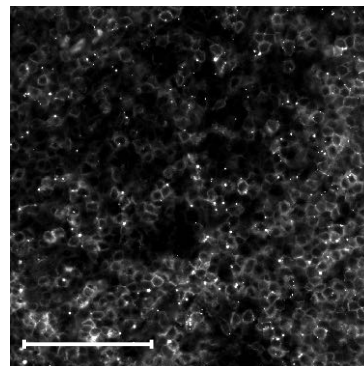
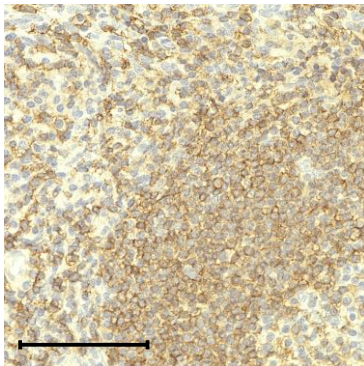
Antigen: CD45RA
Clone: HI100

Company:
BD Biosciences (555486)

Tissue: Tonsil

Dilution:
IHC: 1:50
CODEX: 1:50

CODEX oligo: 72-Alexa488



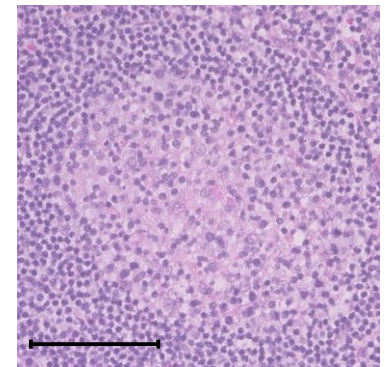
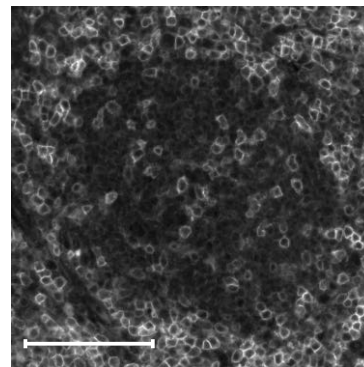
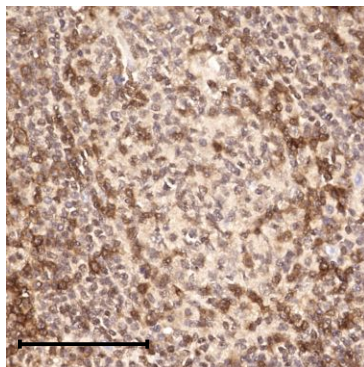
Antigen: CD45RO
Clone: UCH-L1

Company:
Santa Cruz Bio (sc-1183)

Tissue: Tonsil

Dilution:
IHC: 1:50
CODEX: 1:25

CODEX oligo: 2-ATTO550



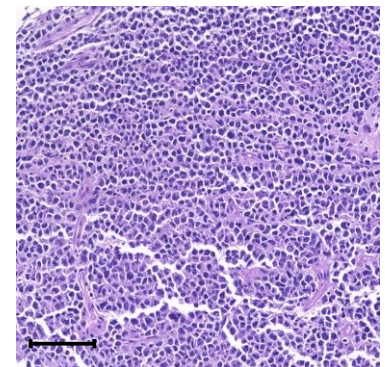
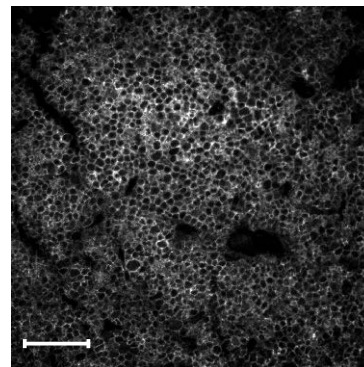
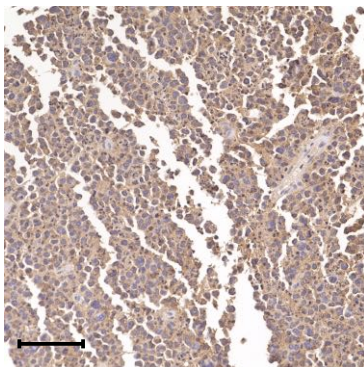
Antigen: CD56
Clone: MRQ-42

Company:
Cell Marque (custom)

Tissue: NK/T cell lymphoma

Dilution:
IHC: 1:200
CODEX: 1:100

CODEX oligo: 29-Alexa647



Staining specifications

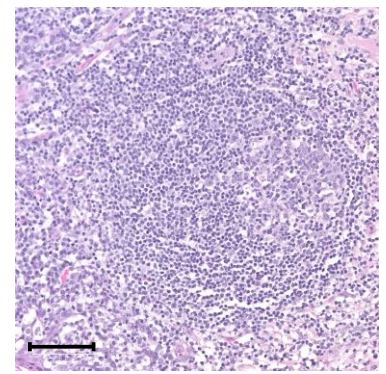
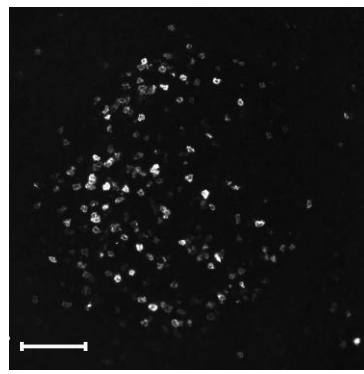
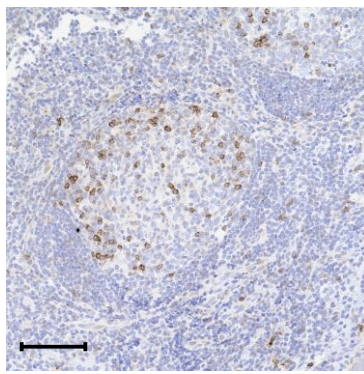
Antigen: CD57
Clone: HCD57

Company:
BioLegend (322325)

Tissue: Tonsil

Dilution:
IHC: 1:100
CODEX: 1:200

CODEX oligo: ST30-ATTO550



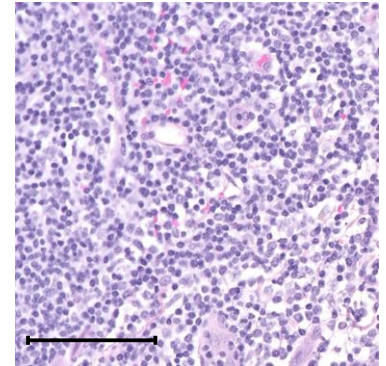
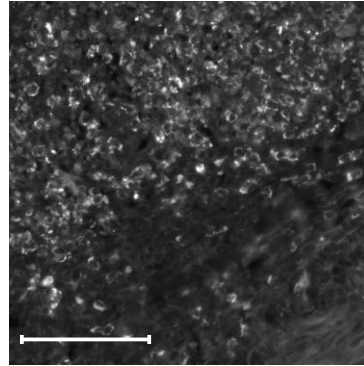
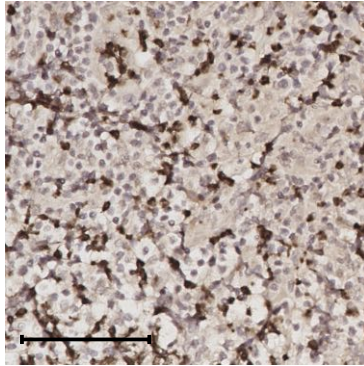
Antigen: CD66a
Clone: B1.1/CD66

Company:
BD Biosciences (551354)

Tissue: Tonsil

Dilution:
IHC: 1:200
CODEX: 1:200

CODEX oligo: 41-Alexa488



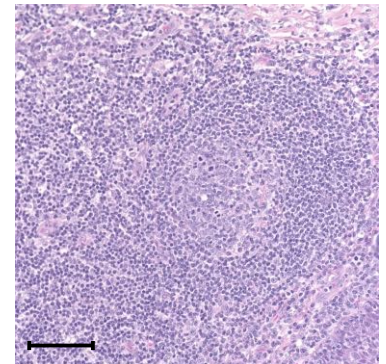
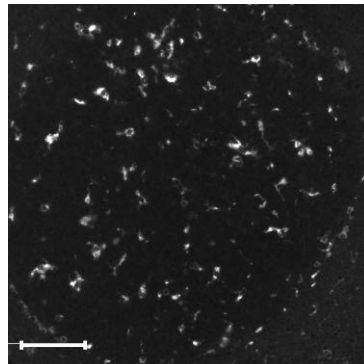
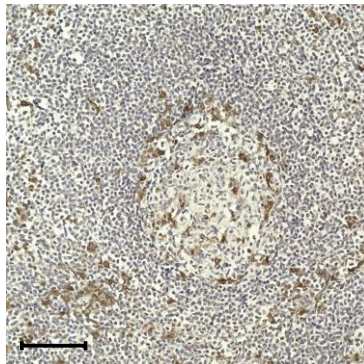
Antigen: CD68
Clone: D4B9C or KP-1

Companies:
Cell Signaling Technology (custom)
Biolegend (916104)

Tissue: Tonsil

Dilution:
IHC: 1:100
CODEX: 1:200

CODEX oligo: 70-Alexa647



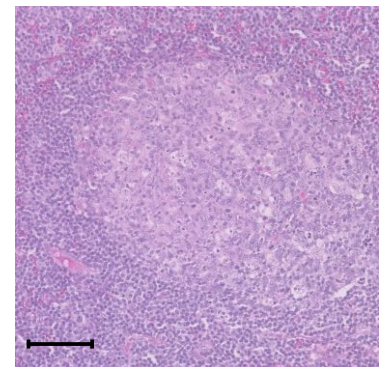
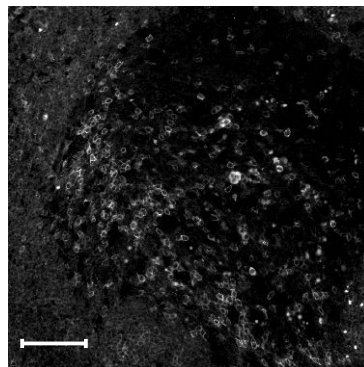
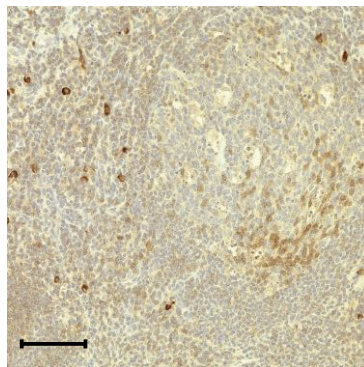
Antigen: CD69
Clone: polyclonal

Company:
Novus Biologicals (AF2359)

Tissue: Tonsil

Dilution:
IHC: 1:200
CODEX: 1:200

CODEX oligo: 36-ATTO550



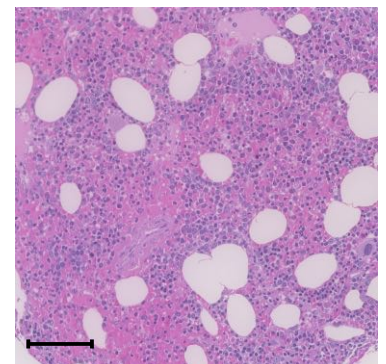
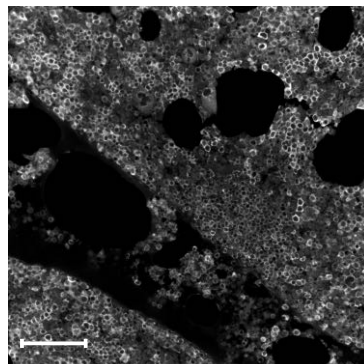
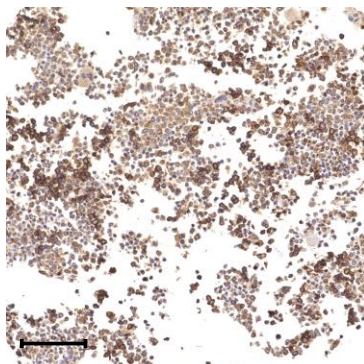
Antigen: CD71
Clone: MRQ-48

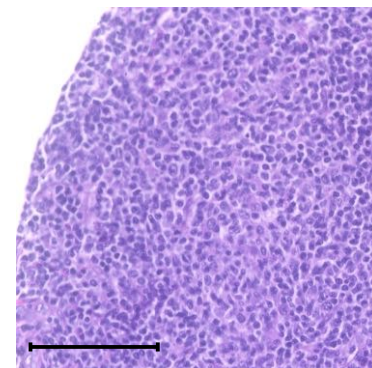
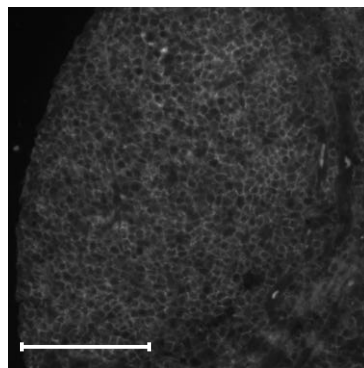
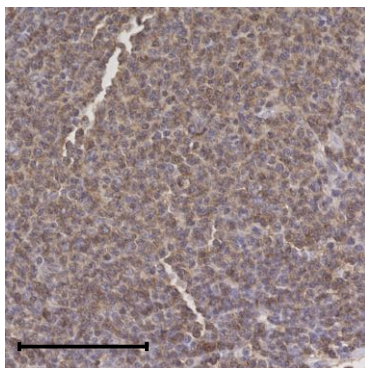
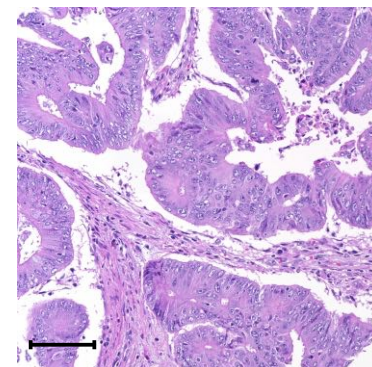
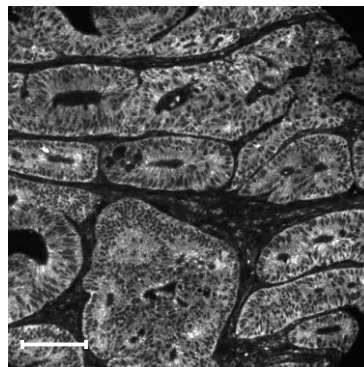
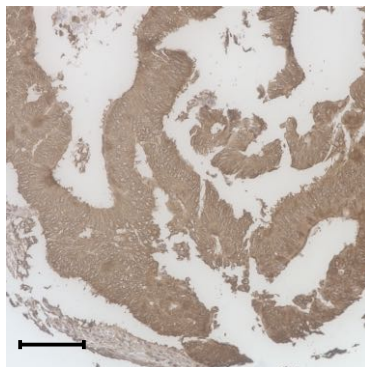
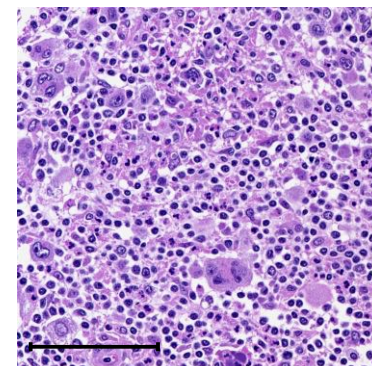
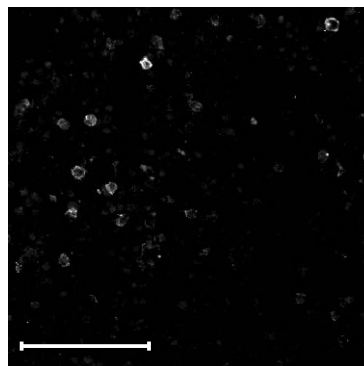
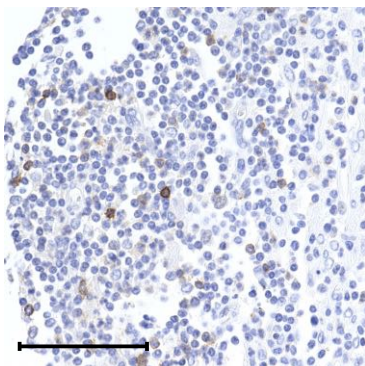
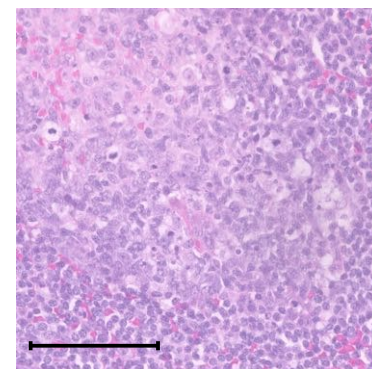
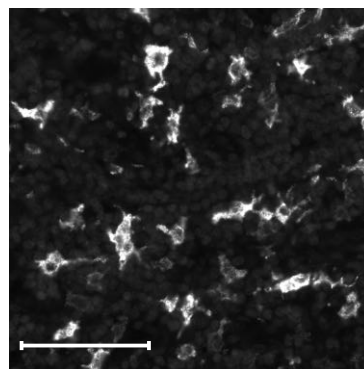
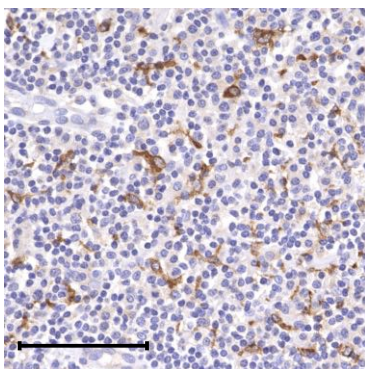
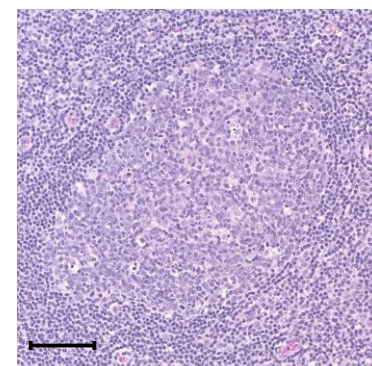
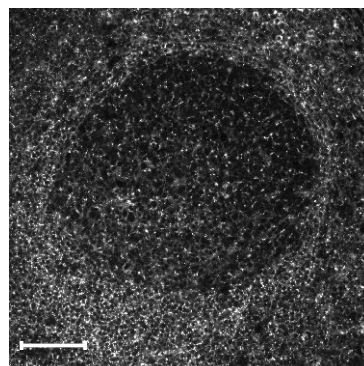
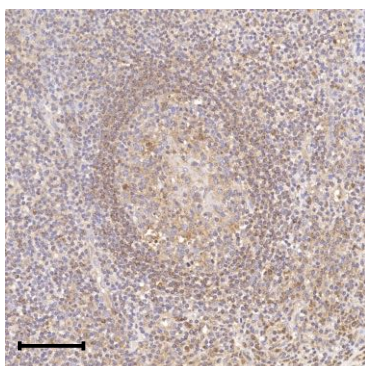
Company:
Cell Marque (custom)

Tissue: Myelolipoma

Dilution:
IHC: 1:400
CODEX: 1:100

CODEX oligo: 3-Alexa647



Staining specifications**Antigen: CD79a**
Clone: JBC117**Company:**
Cell Marque (custom)**Tissue: Follicular lymphoma****Dilution:**
IHC: 1:300
CODEX: 1:20**CODEX oligo: 46-Alexa488****Antigen: CD138 (Syndecan-1)**
Clone: B-A38**Company:**
Invitrogen (MA1-10091)**Tissue: Colorectal carcinoma****Dilution:**
IHC: 1:100
CODEX: 1:50**CODEX oligo: ST76-Alexa647****Antigen: CD162 (CLA)**
Clone: HECA-452**Company:**
BD Biosciences (555946)**Tissue: Classic Hodgkin lymphoma****Dilution:**
IHC: 1:600
CODEX: 1:200**CODEX oligo: 46-Alexa47****Antigen: CD163**
Clone: EDHu-1**Company:**
Novus Biologicals (NB110-48686)**Tissue: Tonsil****Dilution:**
IHC: 1:200
CODEX: 1:200**CODEX oligo: 45-Alexa647****Antigen: CD164**
Clone: N6B6**Company:**
BD Biosciences (551296)**Tissue: Tonsil****Dilution:**
IHC: 1:100
CODEX: 1:200**CODEX oligo: 69-Alexa488**

Staining specifications

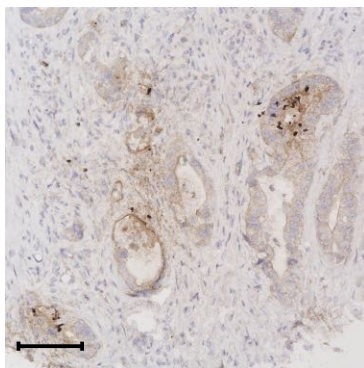
Antigen: CD194 (CCR4)
Clone: L291H4

Company:
BioLegend (359402)

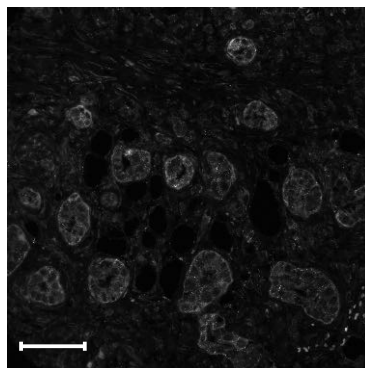
Tissue: Cholangiocarcinoma

Dilution:
IHC: 1:50
CODEX: 1:10

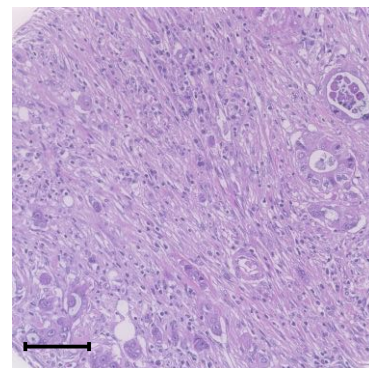
CODEX oligo: ST2-ATTO550



CODEX



H&E



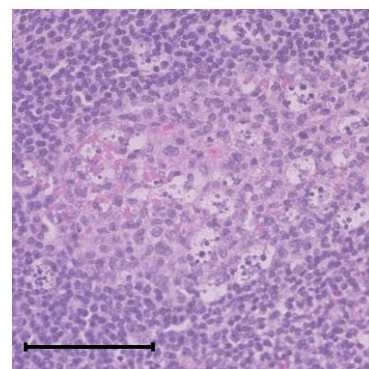
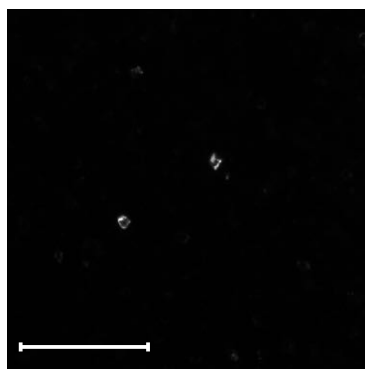
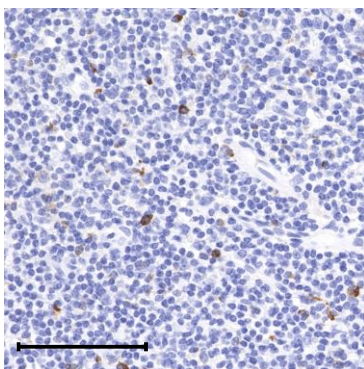
Antigen: CD223 (LAG-3)
Clone: D2G4O or 17B4

Companies:
Cell Signaling Technology (custom)
LSBio (LS-C18692-100)

Tissue: Tonsil

Dilution:
IHC: 1:300
CODEX: 1:20

CODEX oligo: 42-Alexa647



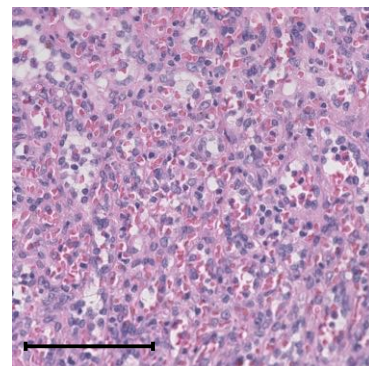
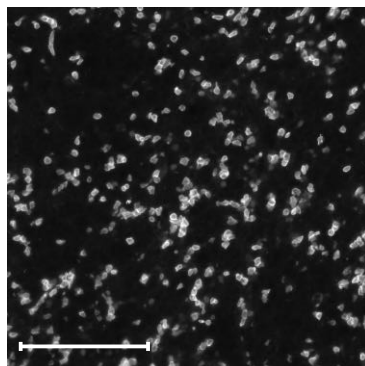
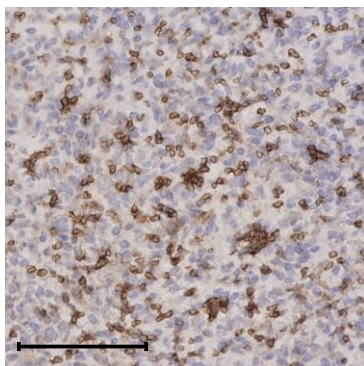
Antigen: CD235a
Clone: GA-R2

Company:
BD Biosciences (555569)

Tissue: Spleen

Dilution:
IHC: 1:400
CODEX: 1:200

CODEX oligo: 69-Alexa488



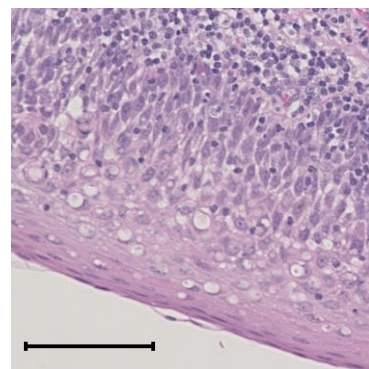
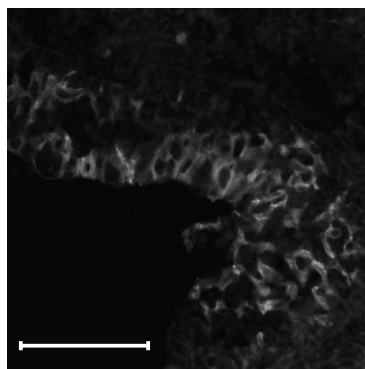
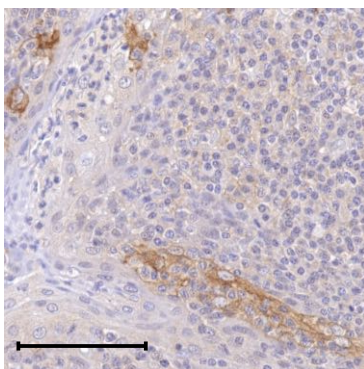
Antigen: CD274 (PD-L1)
Clone: E1L3N

Company:
Cell Signaling Technology (custom)

Tissue: Tonsil

Dilution:
IHC: 1:50
CODEX: 1:100

CODEX oligo: 11-ATTO550



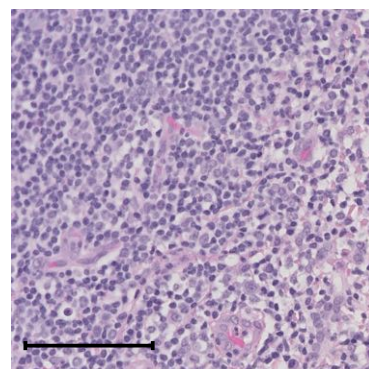
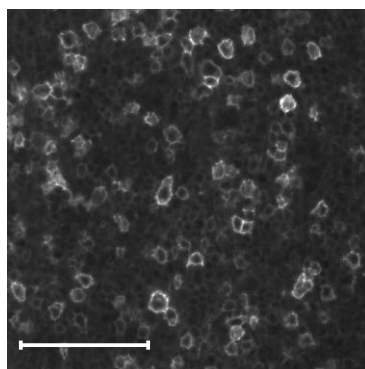
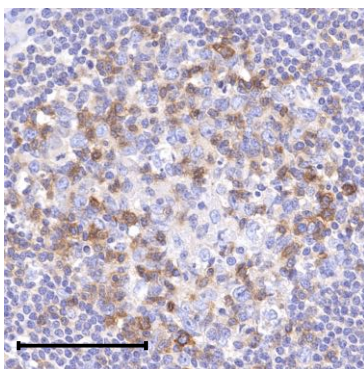
Antigen: CD278 (ICOS)
Clone: D1K2T

Company:
Cell Signaling Technology (custom)

Tissue: Tonsil

Dilution:
IHC: 1:200
CODEX: 1:20

CODEX oligo: 74-ATTO550



Staining specifications

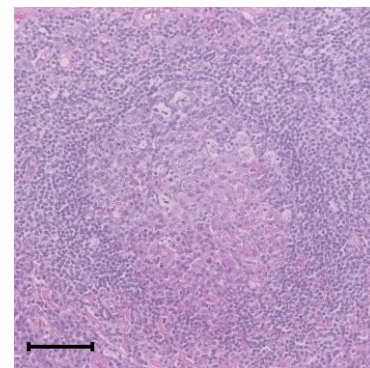
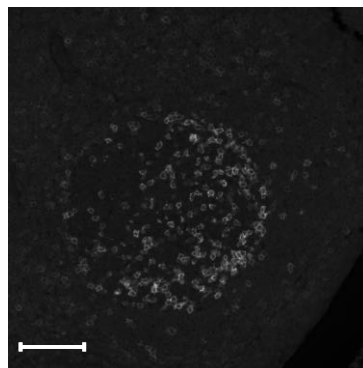
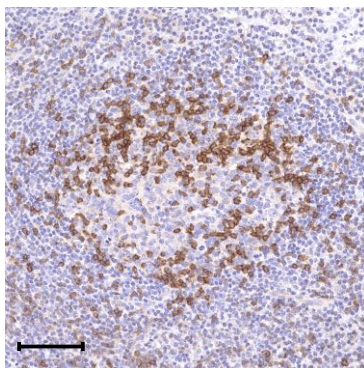
Antigen: CD279 (PD-1)
Clone: D1K2T

Company:
Cell Signaling Technology (custom)

Tissue: Tonsil

Dilution:
IHC: 1:200
CODEX: 1:25

CODEX oligo: 23-ATTO550



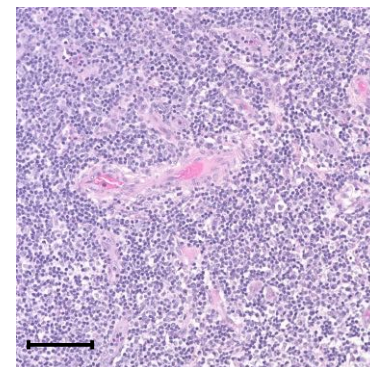
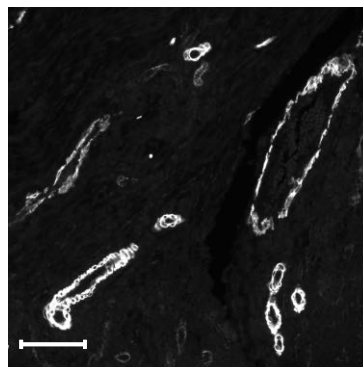
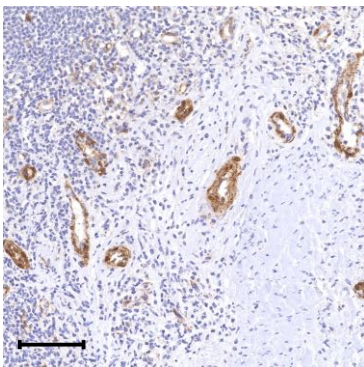
Antigen: α -SMA
Clone: polyclonal

Company:
Abcam (ab5694)

Tissue: Tonsil

Dilution:
IHC: 1:200
CODEX: 1:200

CODEX oligo: 69-Alexa488



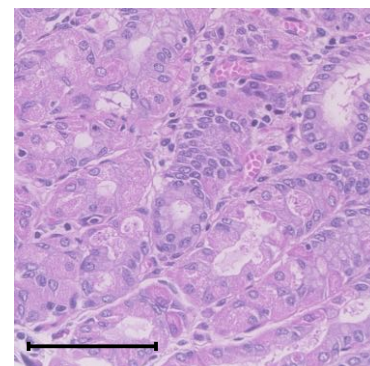
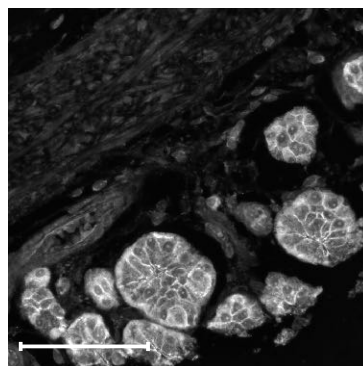
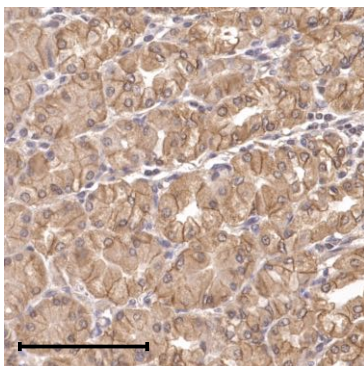
Antigen: β -catenin
Clones: polyclonal or 14

Company:
Novus Biologicals (AF1329)
Cell Marque (custom)

Tissue: Stomach

Dilution:
IHC: 1:100
CODEX: 1:25

CODEX oligo: 51-Alexa647



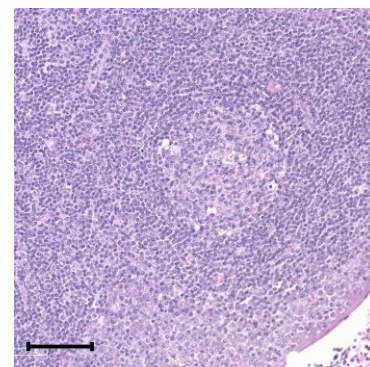
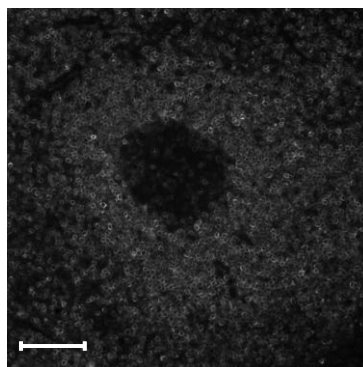
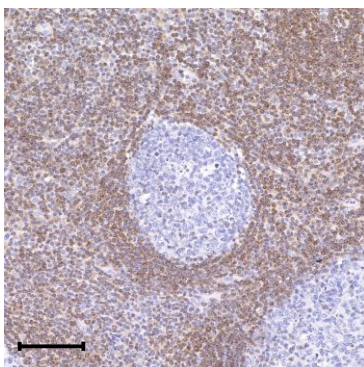
Antigen: BCL-2
Clone: 124

Company:
Cell Marque (custom)

Tissue: Tonsil

Dilution:
IHC: 1:200
CODEX: 1:25

CODEX oligo: 41-Alexa647



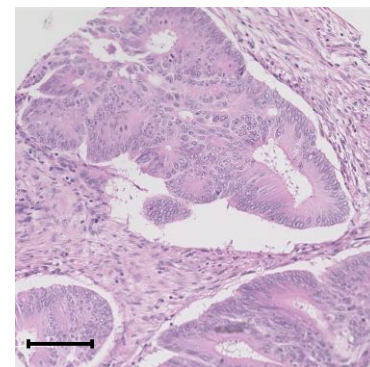
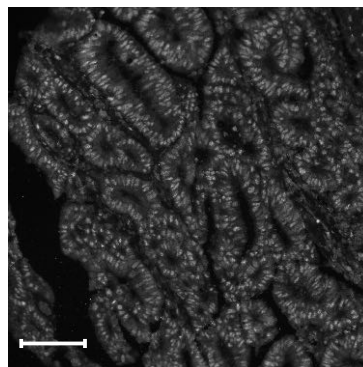
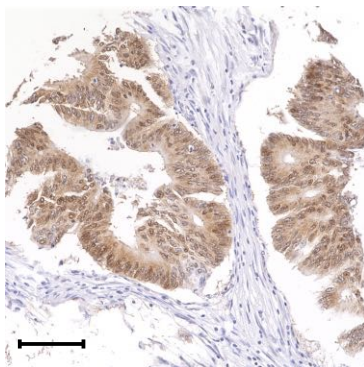
Antigen: CDX2
Clone: CDX2/1690

Company:
Novus Biologicals (NBP2-54472)

Tissue: Colorectal adenocarcinoma

Dilution:
IHC: 1:200
CODEX: 1:25

CODEX oligo: 53-Alexa647



Staining specifications

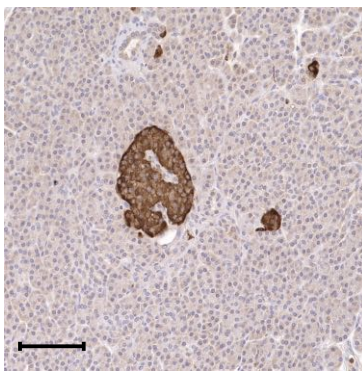
Antigen: Chromogranin A
Clones:
LK2H10 + PHE5 + CGA/414

Company:
Novus Biologicals (NBP2-34674)

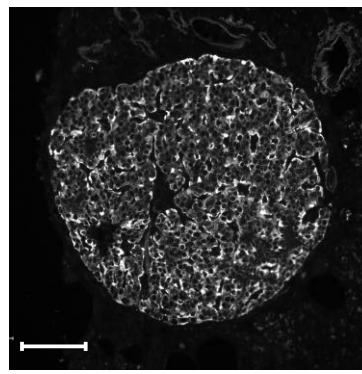
Tissue: Pancreas

Dilution:
IHC: 1:400
CODEX: 1:50

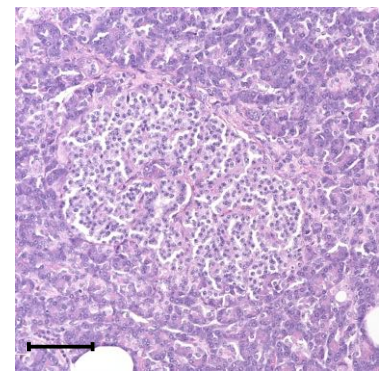
CODEX oligo: 43-Alexa488



CODEX



H&E



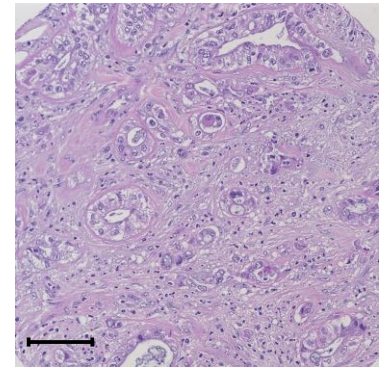
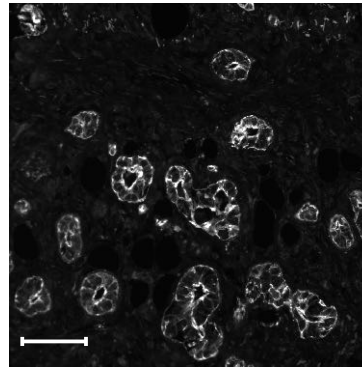
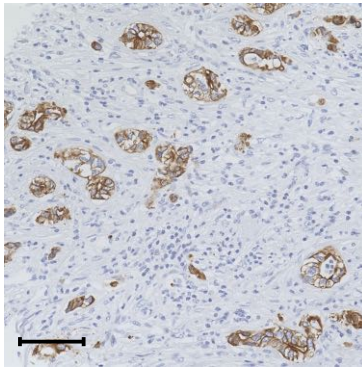
Antigen: CK7
Clone: OV-TL12/30

Company:
Novus Biologicals (NBP2-47940)

Tissue: Cholangiocarcinoma

Dilution:
IHC: 1:200
CODEX: 1:50

CODEX oligo: 3-Alexa488



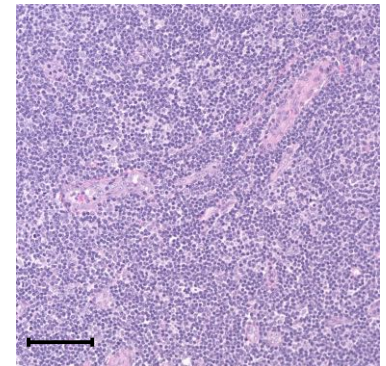
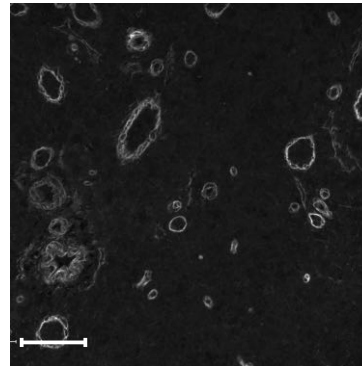
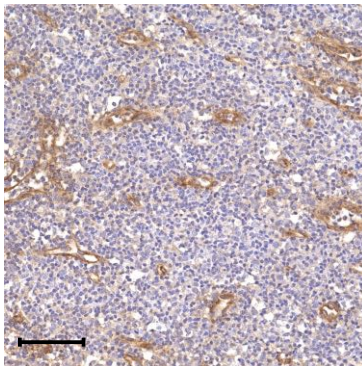
Antigen: Collagen IV
Clone: polyclonal

Company:
Abcam (ab6586)

Tissue: Tonsil

Dilution:
IHC: 1:200
CODEX: 1:200

CODEX oligo: 33-Alexa647



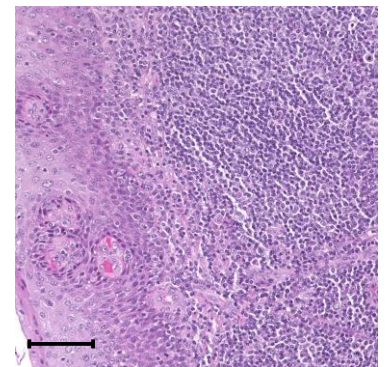
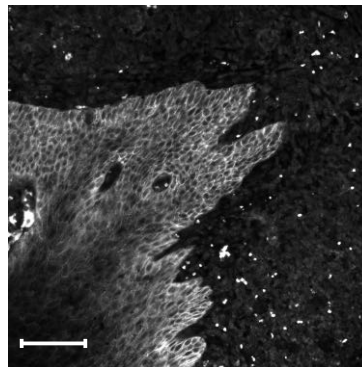
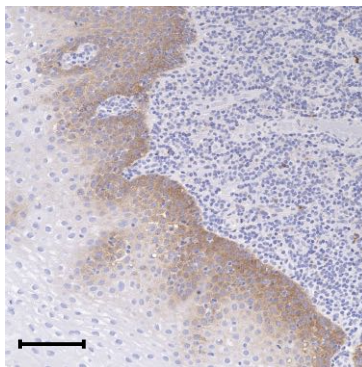
Antigen: EGFR
Clone: D38B1

Company:
Cell Signaling Technology (custom)

Tissue: Tonsil

Dilution:
IHC: 1:50
CODEX: 1:25

CODEX oligo: 58-Alexa488



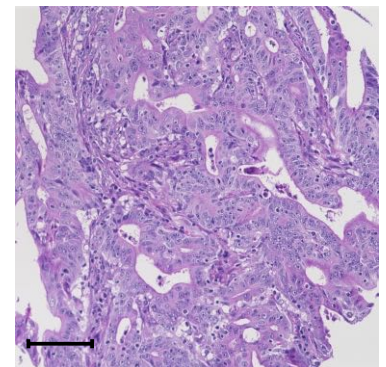
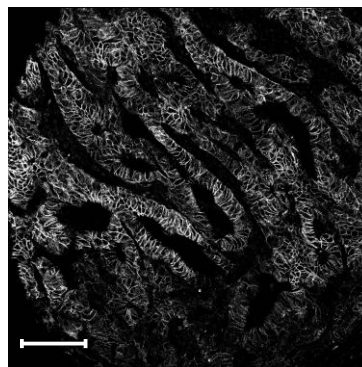
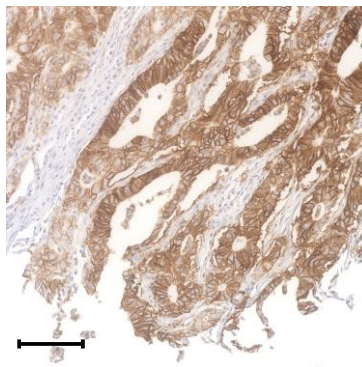
Antigen: EpCAM
Clone: Ber-EP4

Company:
Cell Marque (custom)

Tissue: Gastric adenocarcinoma

Dilution:
IHC: 1:100
CODEX: 1:25

CODEX oligo: 70-Alexa647



Staining specifications

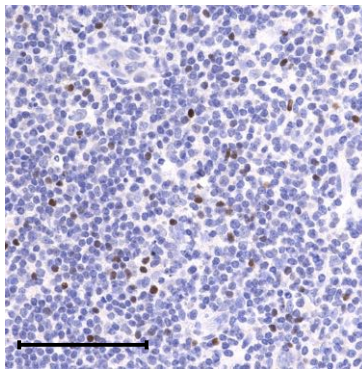
Antigen: FOXP3
Clone: 236A/E7

Company:
Invitrogen (14-4777-80)

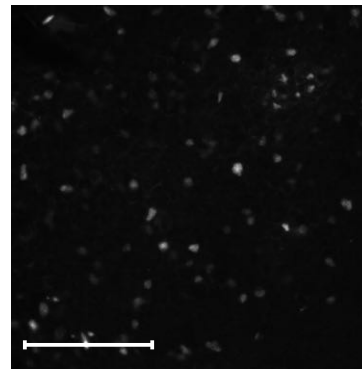
Tissue: Tonsil

Dilution:
IHC: 1:20
CODEX: 1:100

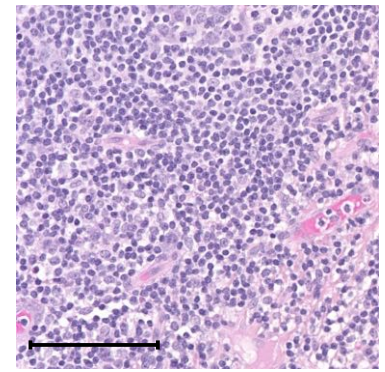
CODEX oligo: 61-Alexa647



CODEX



H&E



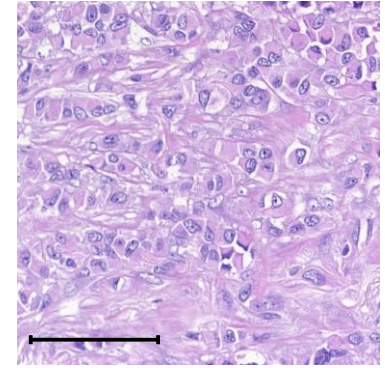
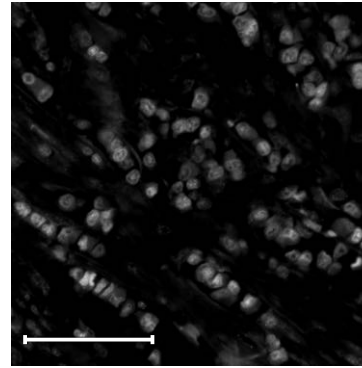
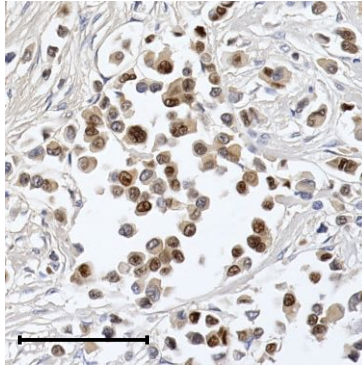
Antigen: GATA3
Clone: L50-823

Company:
Cell Marque (custom)

Tissue: Breast lobular carcinoma

Dilution:
IHC: 1:600
CODEX: 1:100

CODEX oligo: 60-Alexa647



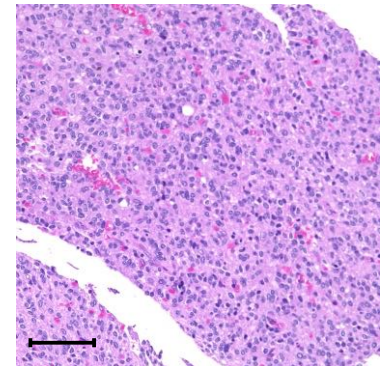
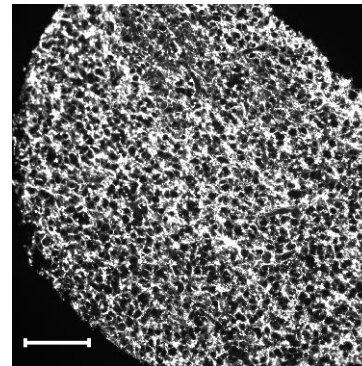
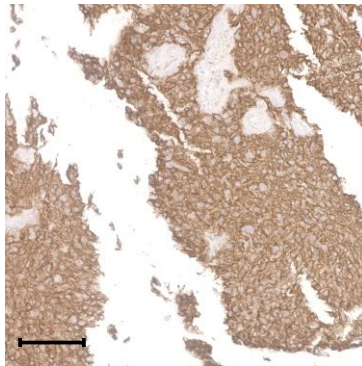
Antigen: GFAP
Clone: 2.2B10

Company:
Invitrogen (130300)

Tissue: Glioblastoma

Dilution:
IHC: 1:100
CODEX: 1:25

CODEX oligo: 46-Alexa488



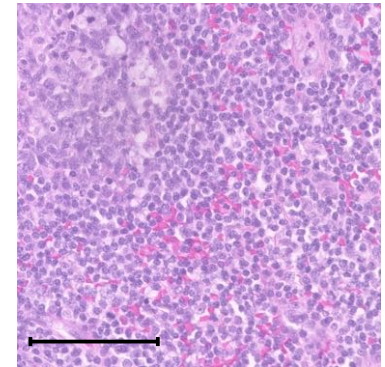
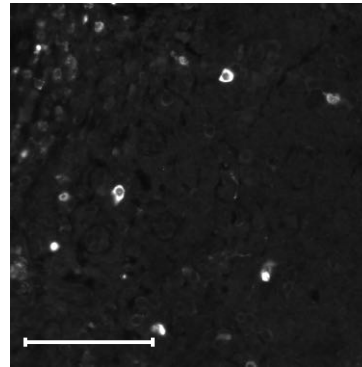
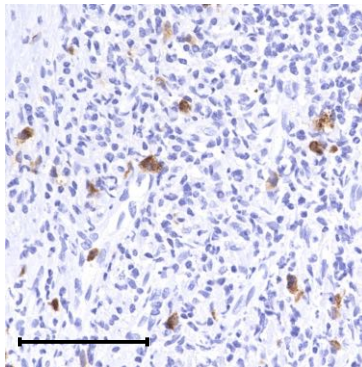
Antigen: Granzyme B
Clone: EPR20129-217

Company:
Abcam (ab219803)

Tissue: Tonsil

Dilution:
IHC: 1:200
CODEX: 1:100

CODEX oligo: 81-Alexa647



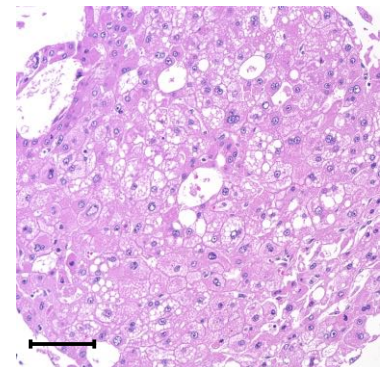
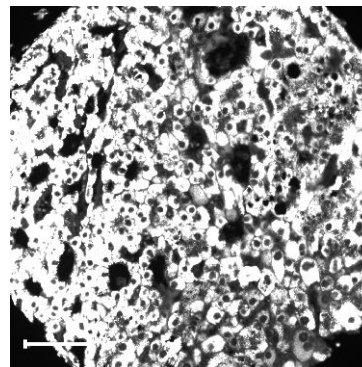
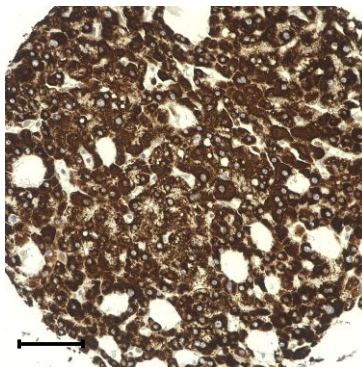
Antigen: Hep-Par 1
Clone: OCH1E5

Company:
Santa Cruz Bio (sc-58693)

Tissue: Hepatocellular carcinoma

Dilution:
IHC: 1:25
CODEX: 1:100

CODEX oligo: 28-Alexa488



Staining specifications

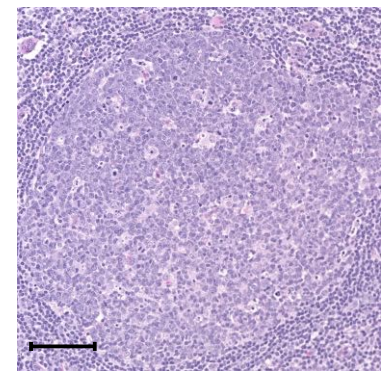
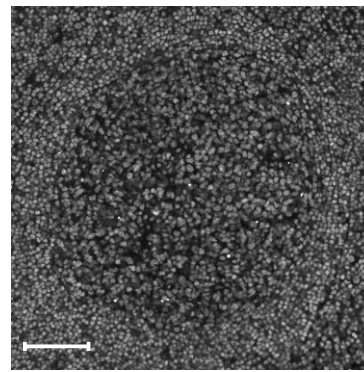
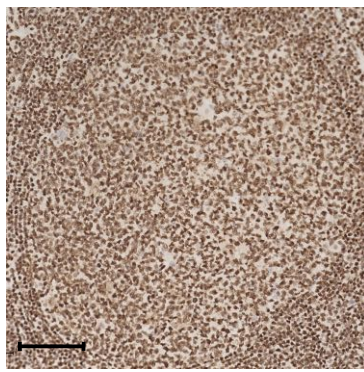
Antigen: Histone H3
Clone: D1H2

Company:
Cell Signaling Technology (custom)

Tissue: Tonsil

Dilution:
IHC: 1:100
CODEX: 1:50

CODEX oligo: 30-ATTO550



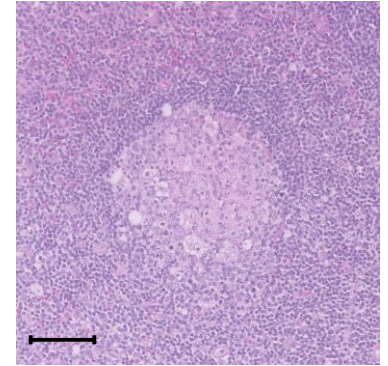
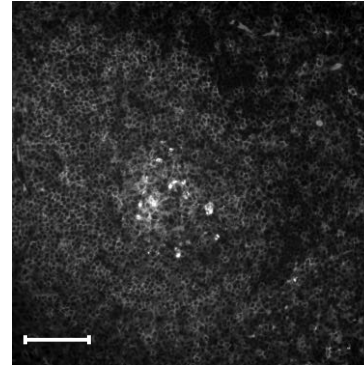
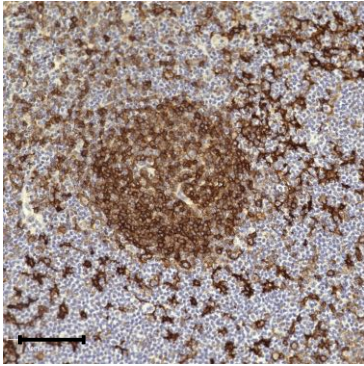
Antigen: HLA-DR
Clone: EPR3692

Company:
Abcam (ab215985)

Tissue: Tonsil

Dilution:
IHC: 1:100
CODEX: 1:50

CODEX oligo: 65-Alexa488



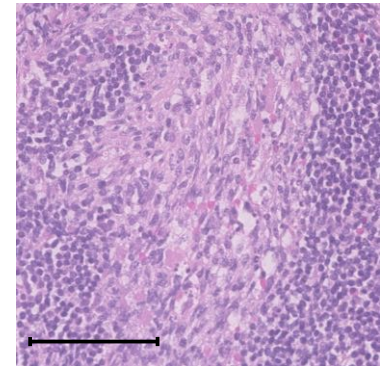
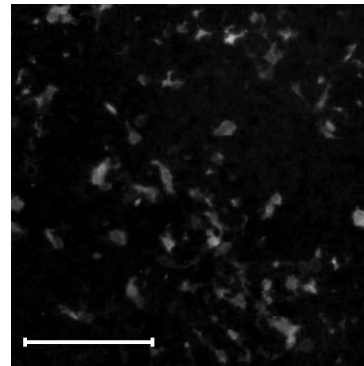
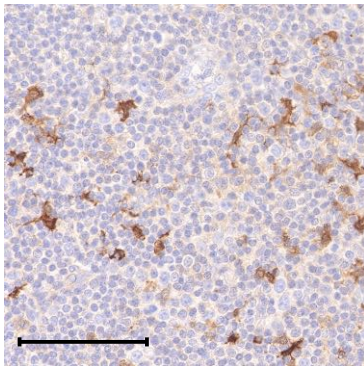
Antigen: IDO-1
Clone: D5J4E

Company:
Cell Signaling Technology (custom)

Tissue: Tonsil

Dilution:
IHC: 1:5000
CODEX: 1:20

CODEX oligo: 59-Alexa647



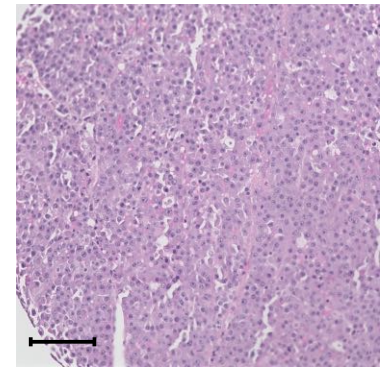
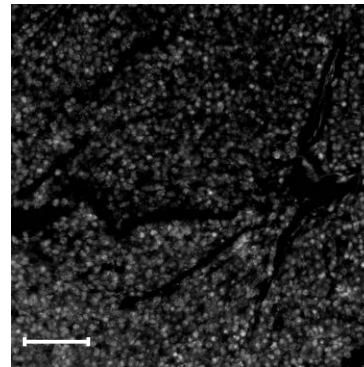
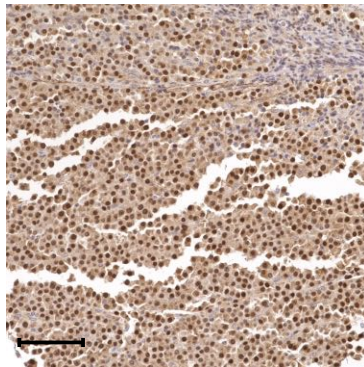
Antigen: IRF4
Clone: IRF4.3E4

Company:
Biolegend (646402)

Tissue: Plasmacytoma

Dilution:
IHC: 1:100
CODEX: 1:25

CODEX oligo: 51-Alexa647



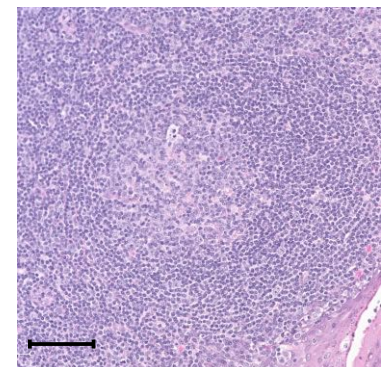
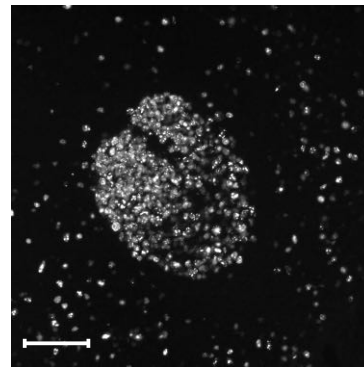
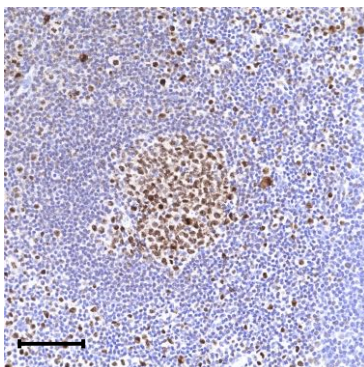
Antigen: Ki-67
Clone: B56

Company:
BD Biosciences (556003)

Tissue: Tonsil

Dilution:
IHC: 1:100
CODEX: 1:100

CODEX oligo: 6-Alexa647



Staining specifications

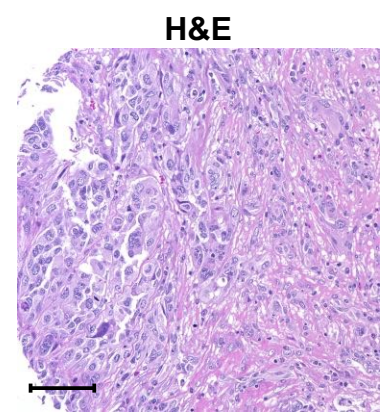
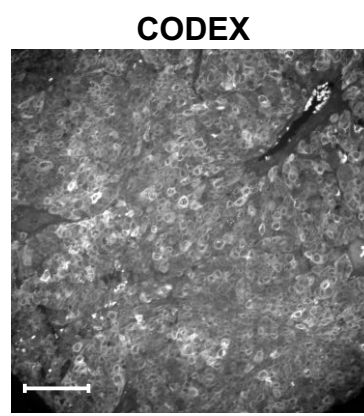
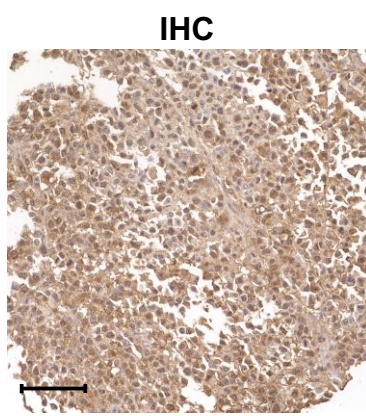
Antigen: Melan-A
Clones:
A103 + M2-7C10 + M2-9E3

Company:
Novus Biologicals (NBP2-34546)

Tissue: Malignant melanoma

Dilution:
IHC: 1:250
CODEX: 1:50

CODEX oligo: 44-ATTO550



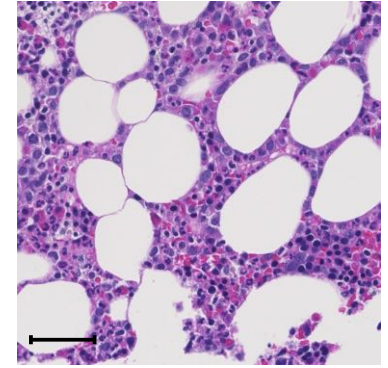
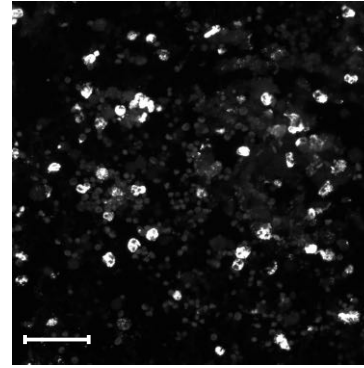
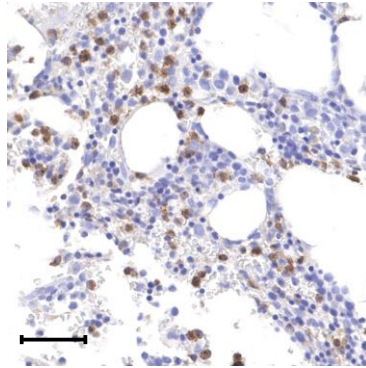
Antigen: MMP9
Clone: L51/82

Company:
Biolegend (819701)

Tissue: Bone marrow

Dilution:
IHC: 1:800
CODEX: 1:25

CODEX oligo: 65-Alexa488



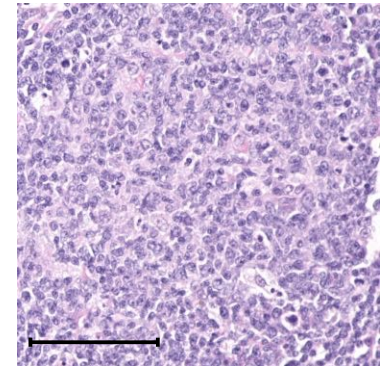
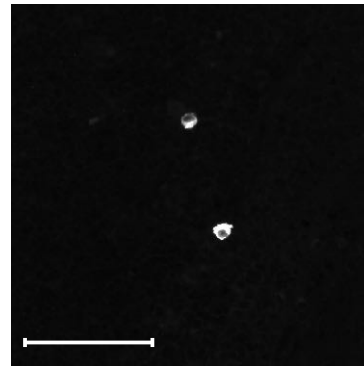
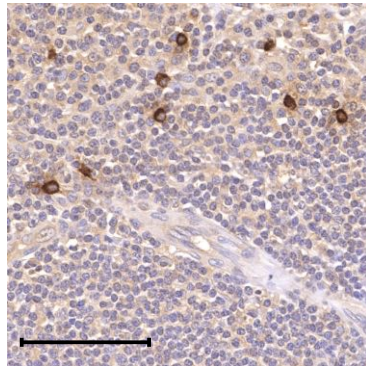
Antigen: MMP12
Clone: polyclonal

Company:
Abcam (ab137444)

Tissue: Tonsil

Dilution:
IHC: 1:200
CODEX: 1:25

CODEX oligo: 80-Alexa647



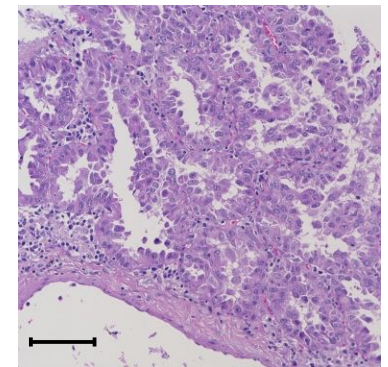
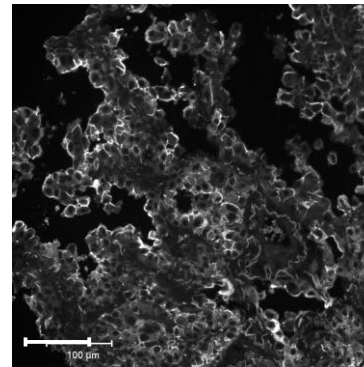
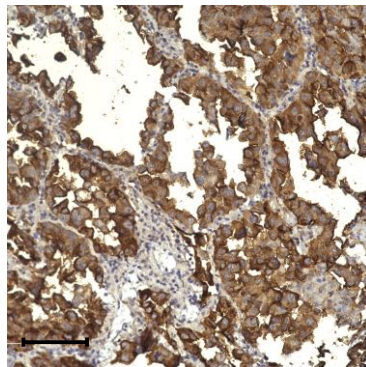
Antigen: Muc-1 (EMA)
Clone: 955

Company:
NSJ Bioreagents (V2372SAF)

Tissue: Lung adenocarcinoma

Dilution:
IHC: 1:200
CODEX: 1:100

CODEX oligo: 15-Alexa488



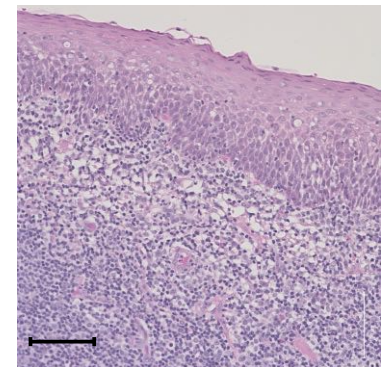
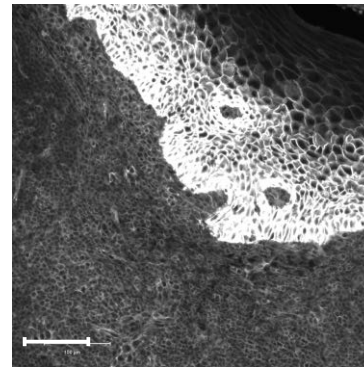
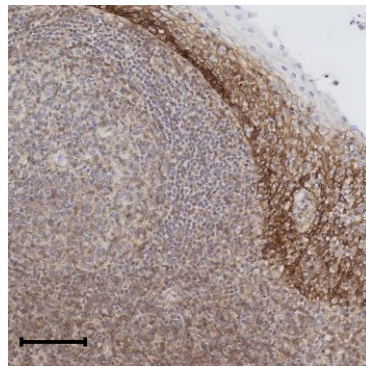
Antigen: Na-K-ATPase
Clone: EP1845Y

Company:
Abcam (ab167390)

Tissue: Tonsil

Dilution:
IHC: 1:100
CODEX: 1:100

CODEX oligo: 36-Alexa488



Staining specifications

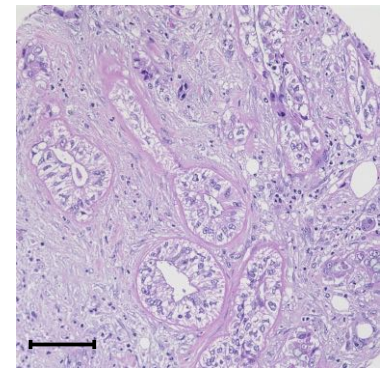
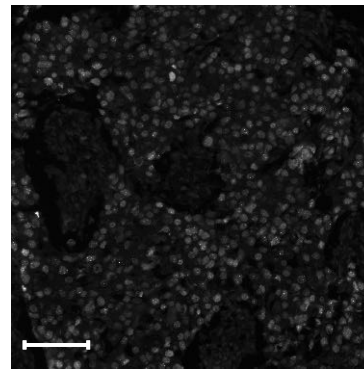
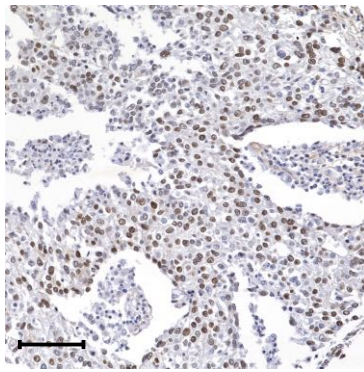
Antigen: p53
Clone: DO-7

Companies:
Cell Marque (custom)
Santa Cruz Bio (sc-47698)

Tissue: Breast cancer NST

Dilution:
IHC: 1:1000
CODEX: 1:25

CODEX oligo: 52-ATTO550



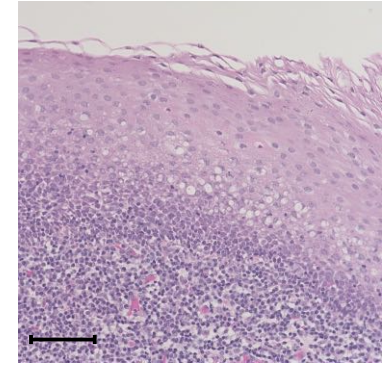
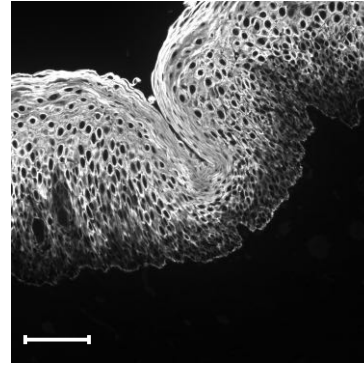
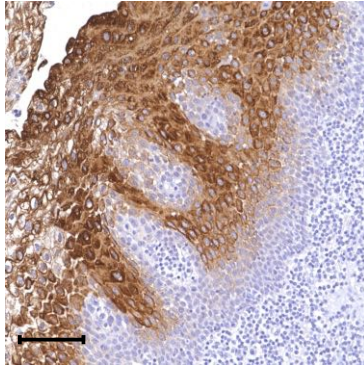
Antigen: Pan-Cytokeratin
Clones: AE-1 + AE-3 or C-11

Company:
Biolegend (914204 or 628602)

Tissue: Tonsil

Dilution:
IHC: 1:200
CODEX: 1:200

CODEX oligo: 67-ATTO550



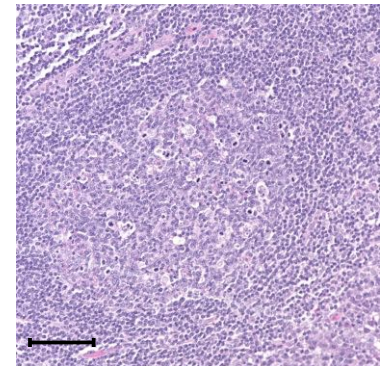
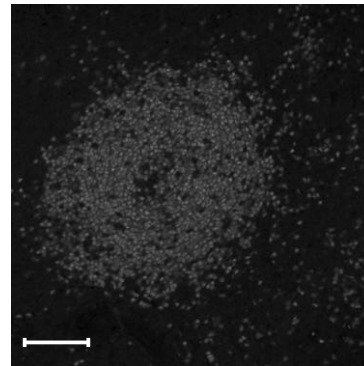
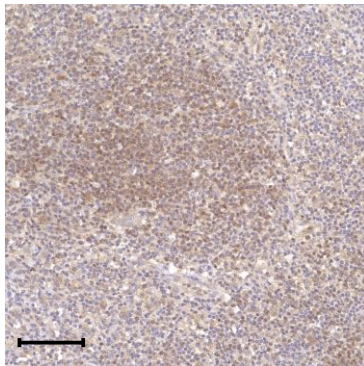
Antigen: PAX5
Clone: D7H5X

Company:
Cell Signaling Technology (custom)

Tissue: Tonsil

Dilution:
IHC: 1:200
CODEX: 1:25

CODEX oligo: 42-Alexa647



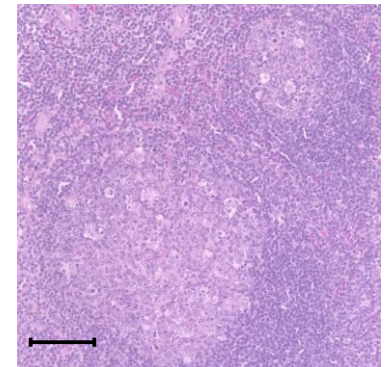
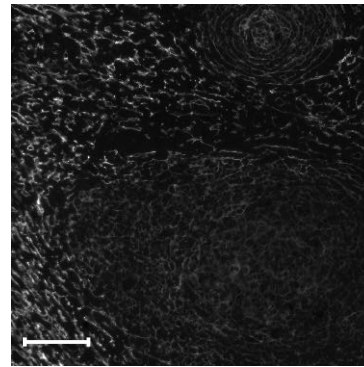
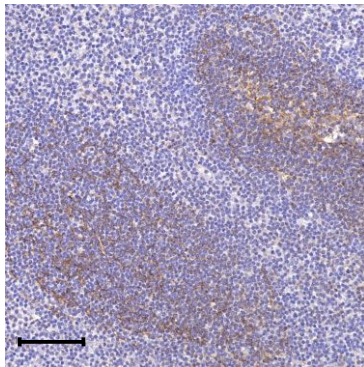
Antigen: Podoplanin
Clone: D2-40 or NC-08

Company:
Biolegend (916606 or 337002)

Tissue: Tonsil

Dilution:
IHC: 1:200
CODEX: 1:200

CODEX oligo: 32-Alexa647



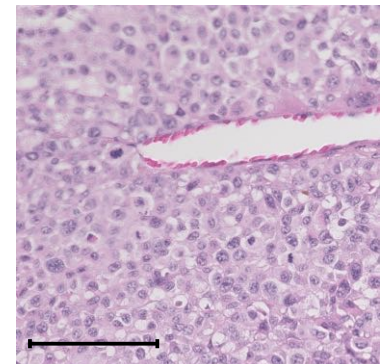
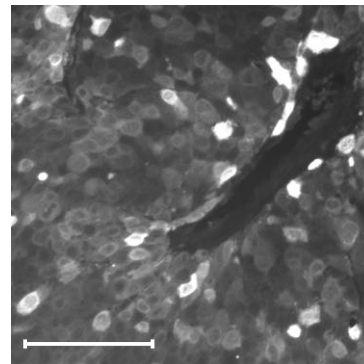
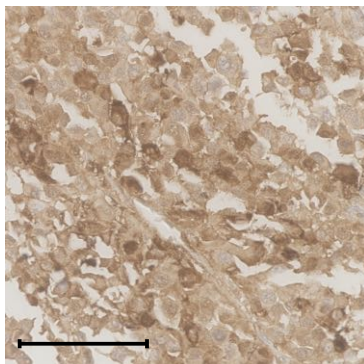
Antigen: S100A6
Clone: 7D11

Company:
Novus Biologicals (NB100-1765)

Tissue: Malignant melanoma

Dilution:
IHC: 1:100
CODEX: 1:20

CODEX oligo: 20-ATTO550



Staining specifications

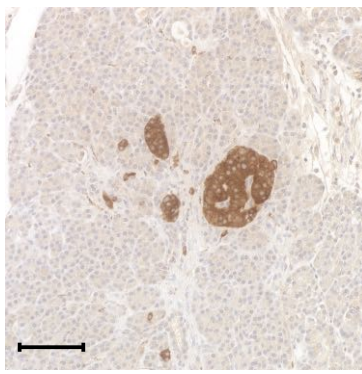
Antigen: Synaptophysin
Clone: 7H12

Company:
Novus Biologicals (NBP1-47483)

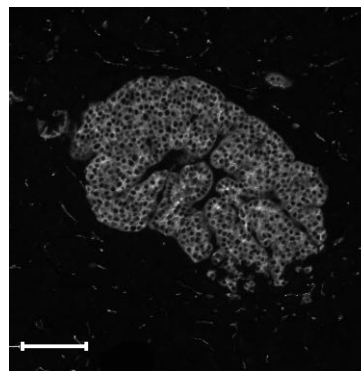
Tissue: Pancreas

Dilution:
IHC: 1:200
CODEX: 1:100

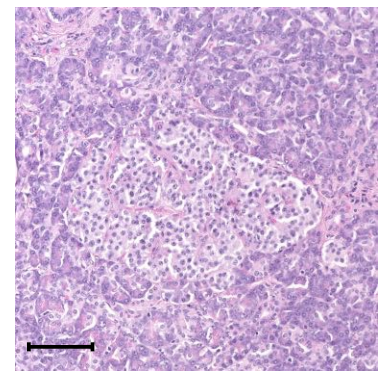
CODEX oligo: 26-ATTO550



CODEX



H&E



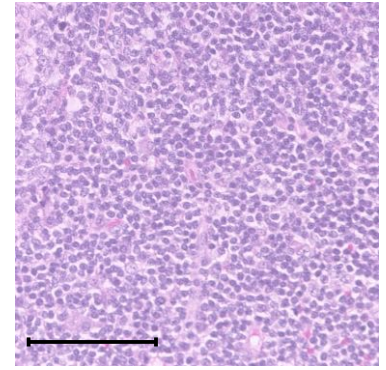
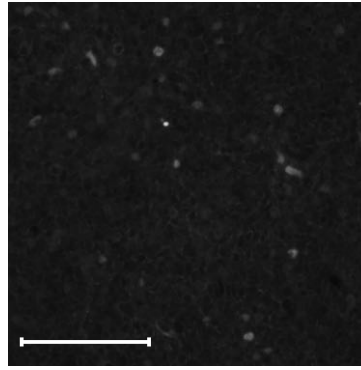
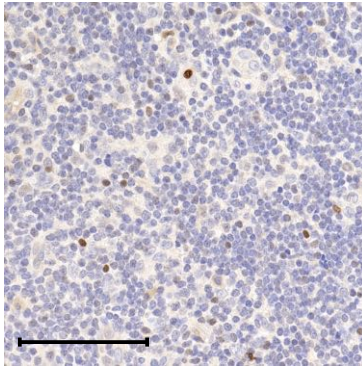
Antigen: T-bet
Clones: D6N8B

Company:
Cell Signaling Technology (custom)

Tissue: Tonsil

Dilution:
IHC: 1:100
CODEX: 1:100

CODEX oligo: 5-ATTO550



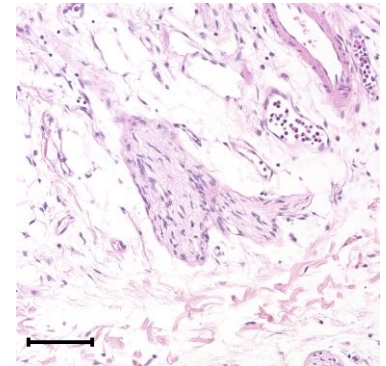
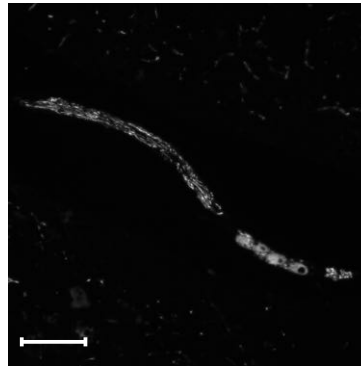
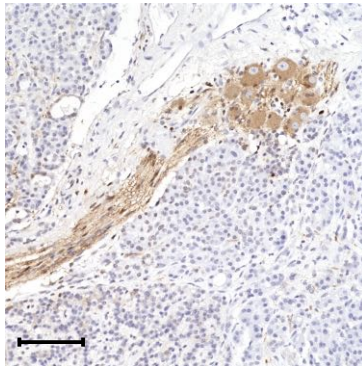
Antigen: Tubulin β 3
Clone: TUJ1

Company:
Biolegend (801201)

Tissue: Pancreas

Dilution:
IHC: 1:100
CODEX: 1:10

CODEX oligo: 80-Alexa647



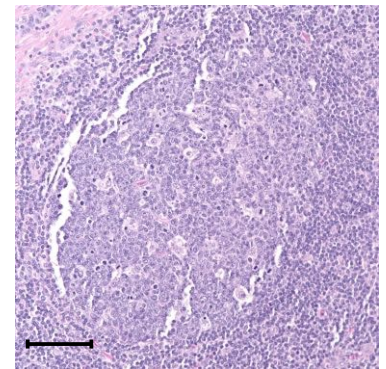
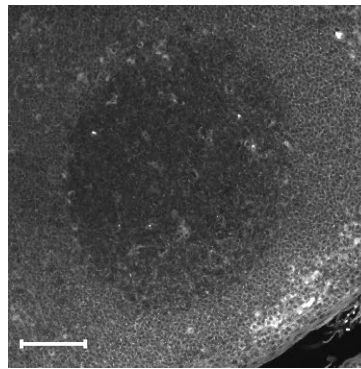
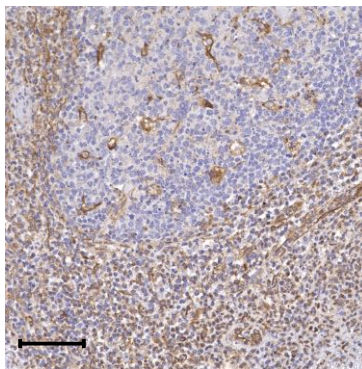
Antigen: Vimentin
Clone: RV202

Company:
BD Biosciences (550513)

Tissue: Tonsil

Dilution:
IHC: 1:300
CODEX: 1:200

CODEX oligo: 7-Alexa488



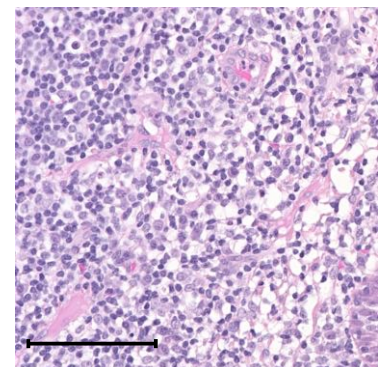
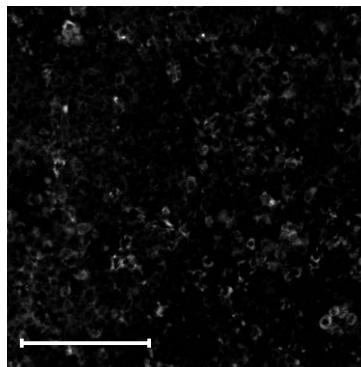
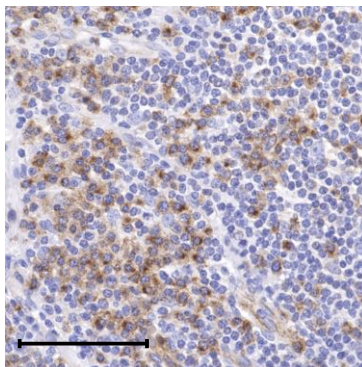
Antigen: VISTA
Clone: D1L2G

Company:
Cell Signaling Technology (custom)

Tissue: Tonsil

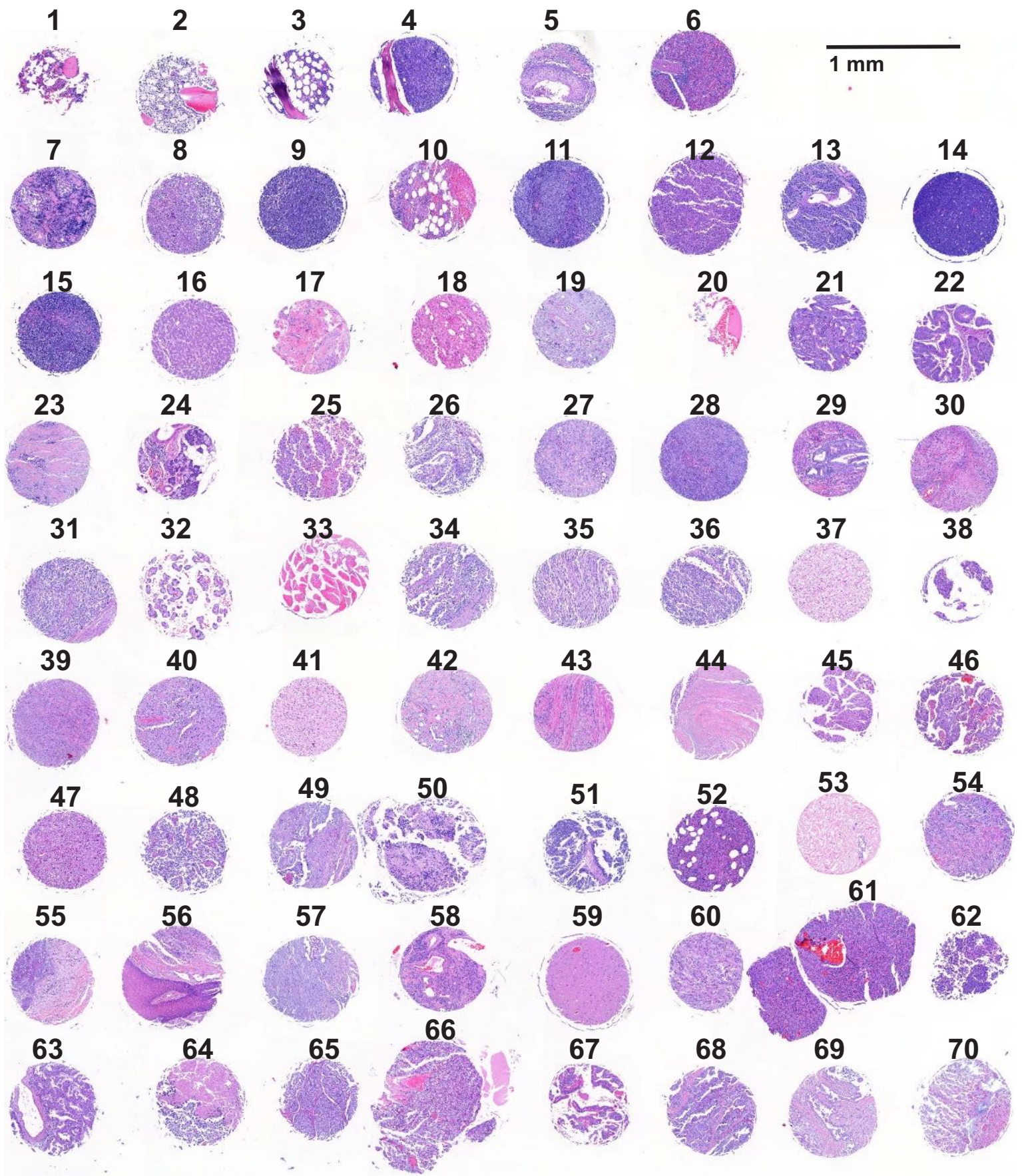
Dilution:
IHC: 1:200
CODEX: 1:50

CODEX oligo: 79-Alexa647

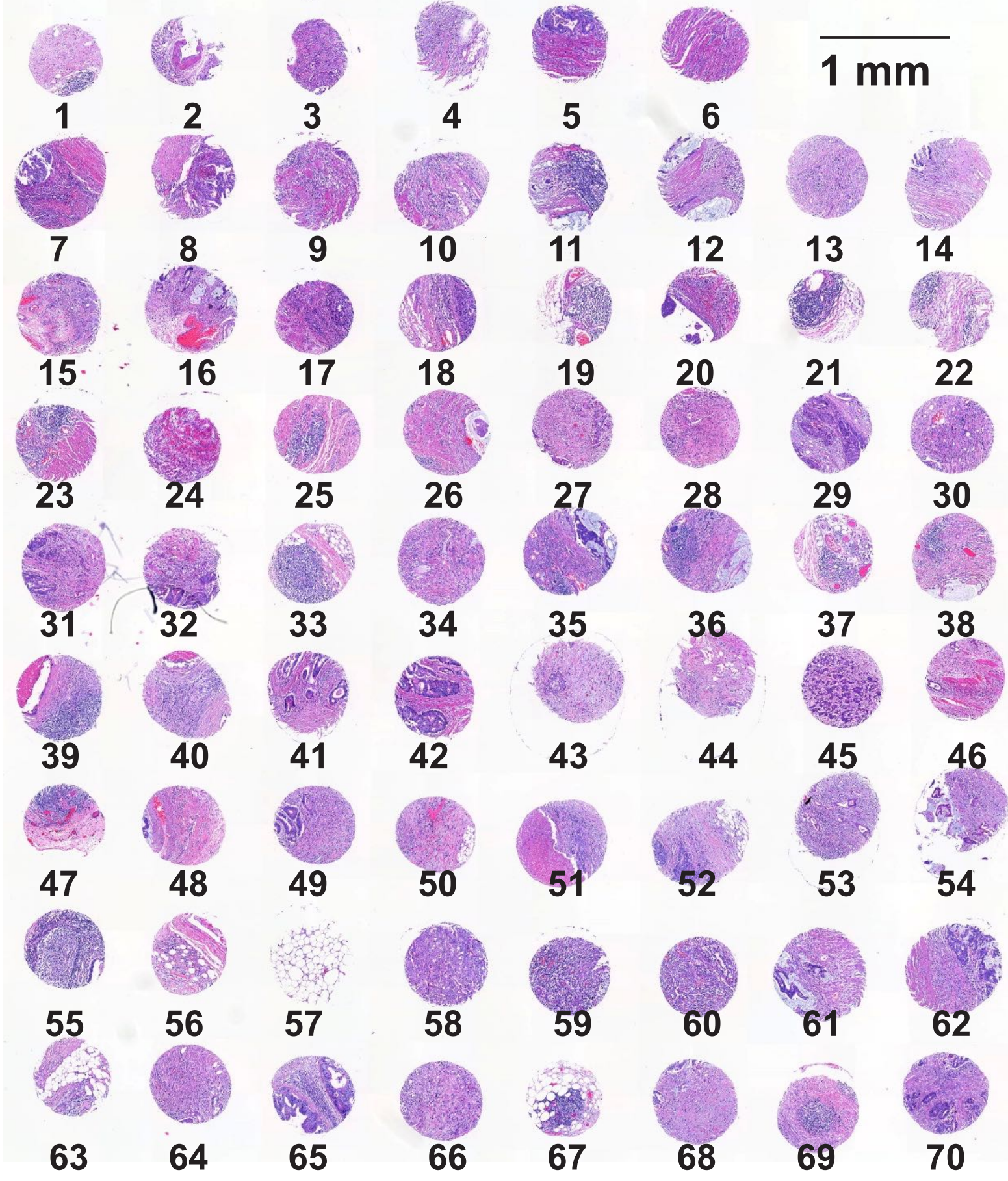


Data S2. Hematoxylin & Eosin (H&E) Stainings of TMAs and Corresponding Cellular Neighborhoods, Related to Figures 2-4 and Table S1. (A) Representative H&E-stained section of the multi-tumor TMA. For details of tissues and tumor types, see Table S1. (B-C) Representative H&E-stained sections of CRC TMAs A and B. Cores are arranged according to patient number (1-35), with two cores per patient per TMA (4 cores per patient in total). (B), TMA A and (C), TMA B. See Table S1 for detailed patients' characteristics. (A-C) Scale bars, 1 mm. (D-G) Cellular neighborhoods and corresponding H&E stainings for all cores of both CRC TMAs. Scale bars, 0.3 mm.

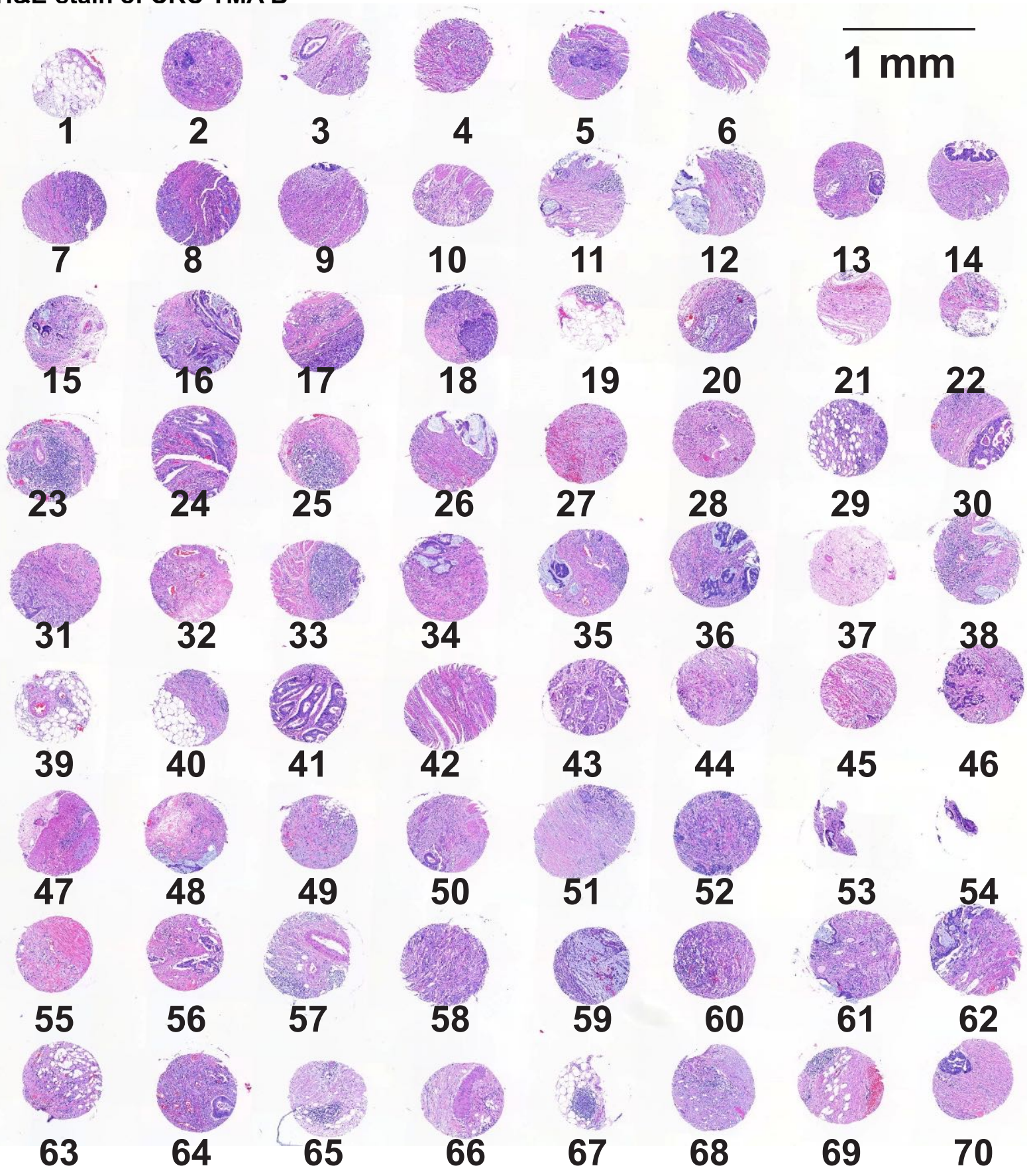
A H&E-stain of multi-tumor TMA



B H&E-stain of CRC TMA A

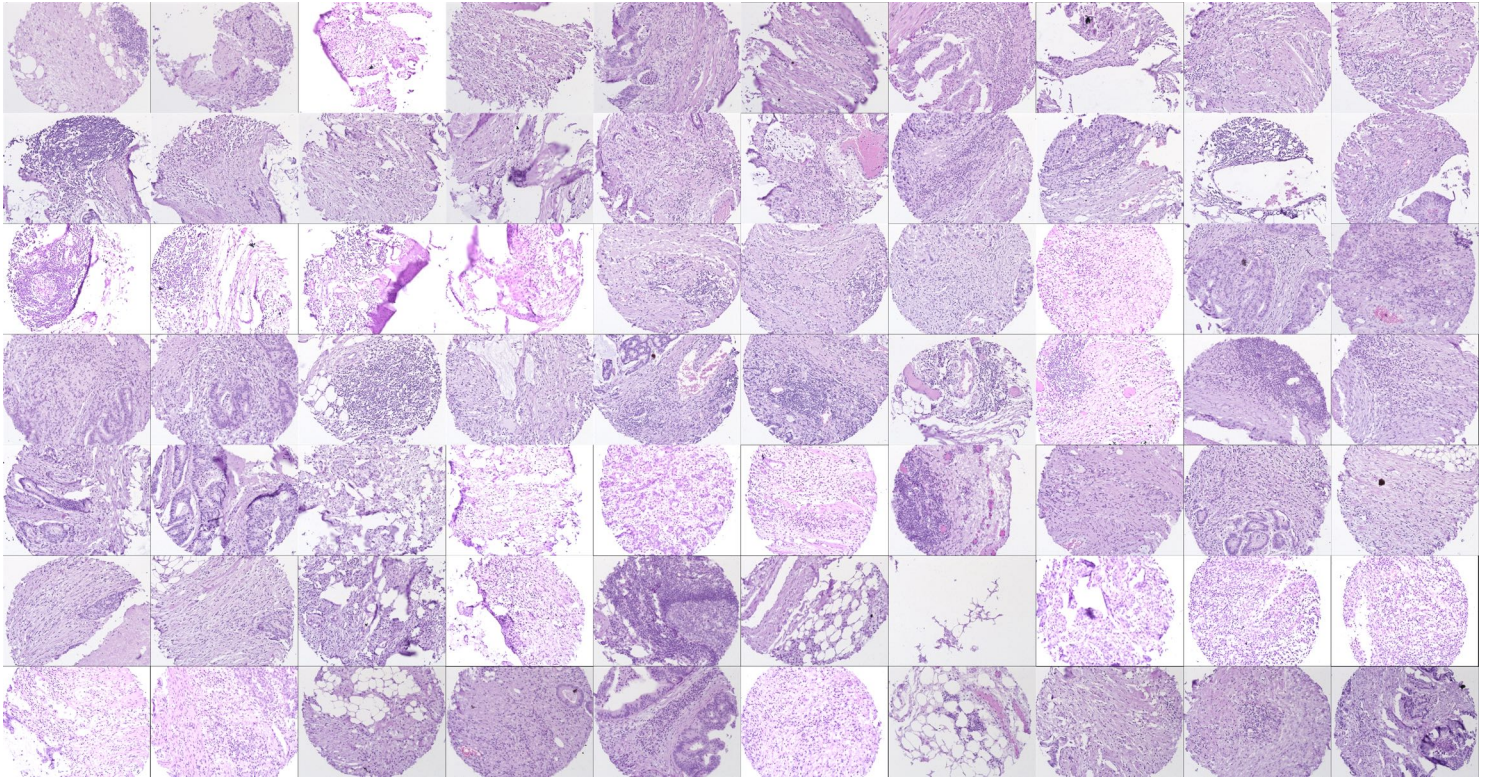


C H&E-stain of CRC TMA B



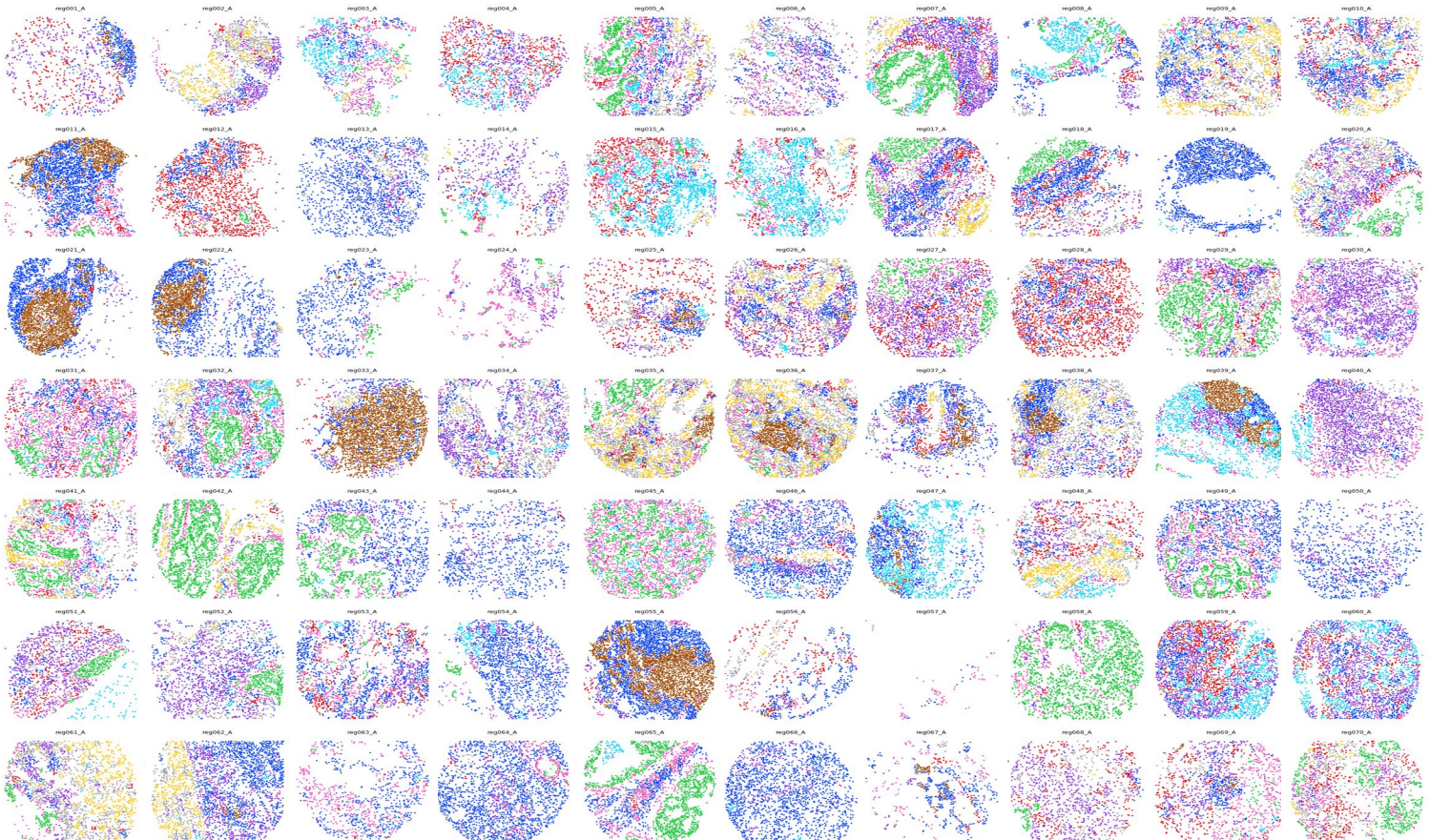
CRC TMA A

D



0.3 mm

E

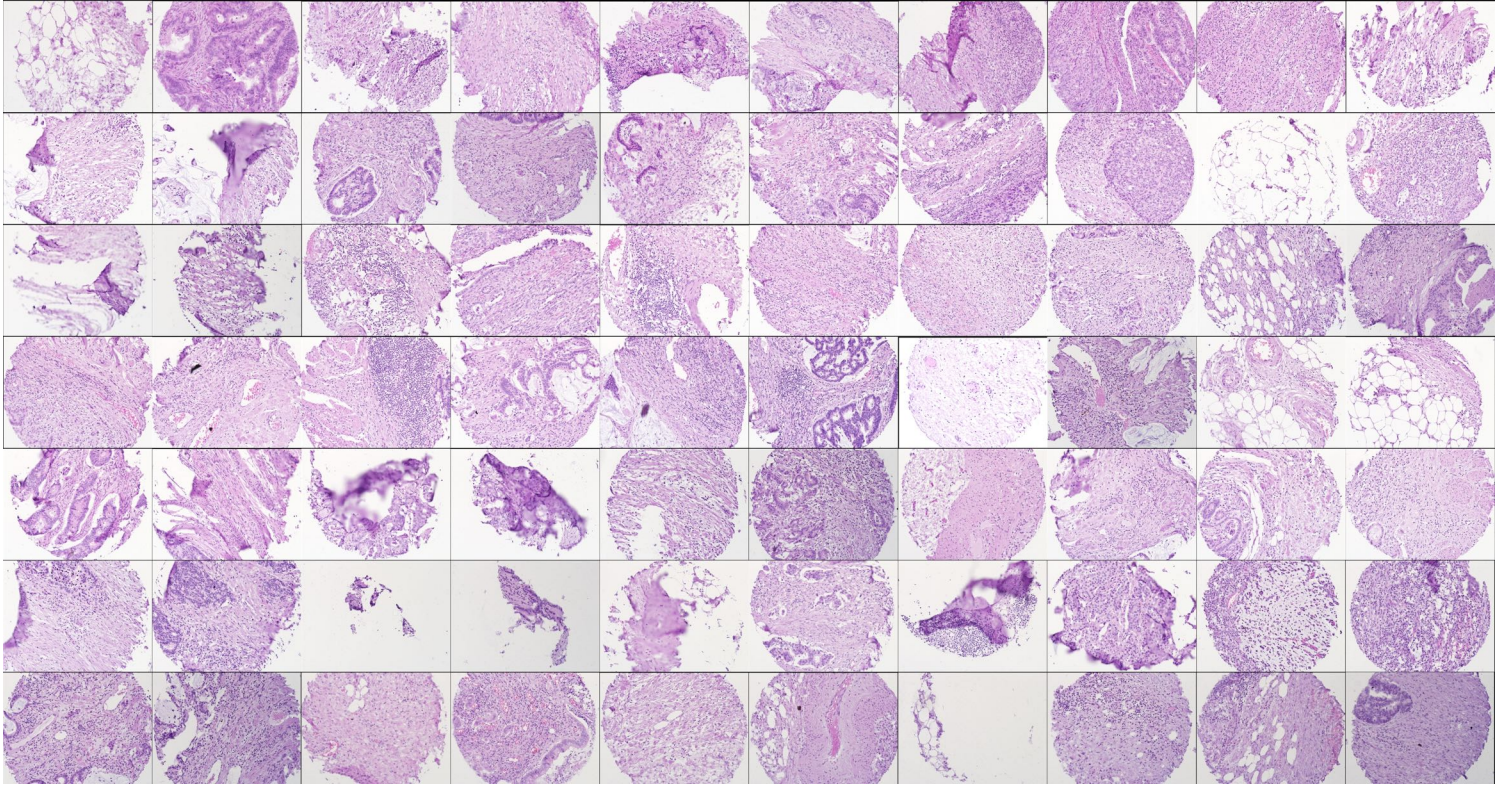


Neighborhood Legend

- 1 T cell enriched
- 3 immune-infiltrated stroma
- 5 follicle
- 7 vascular smooth muscle
- 2 bulk tumor
- 4 macrophage enriched
- 6 tumor boundary
- 8 smooth muscle
- 9 granulocyte enriched

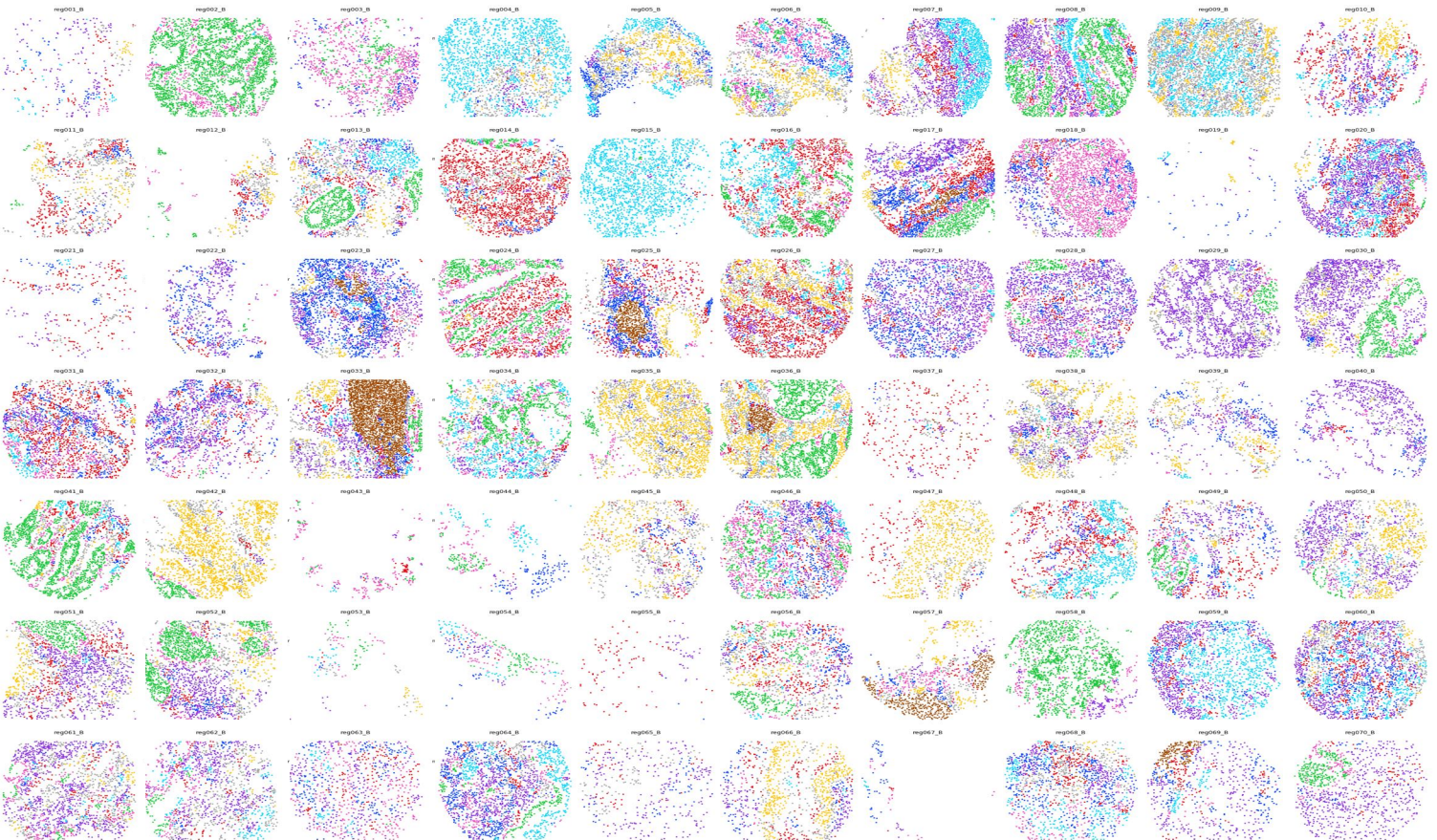
CRC TMA B

F



0.3 mm

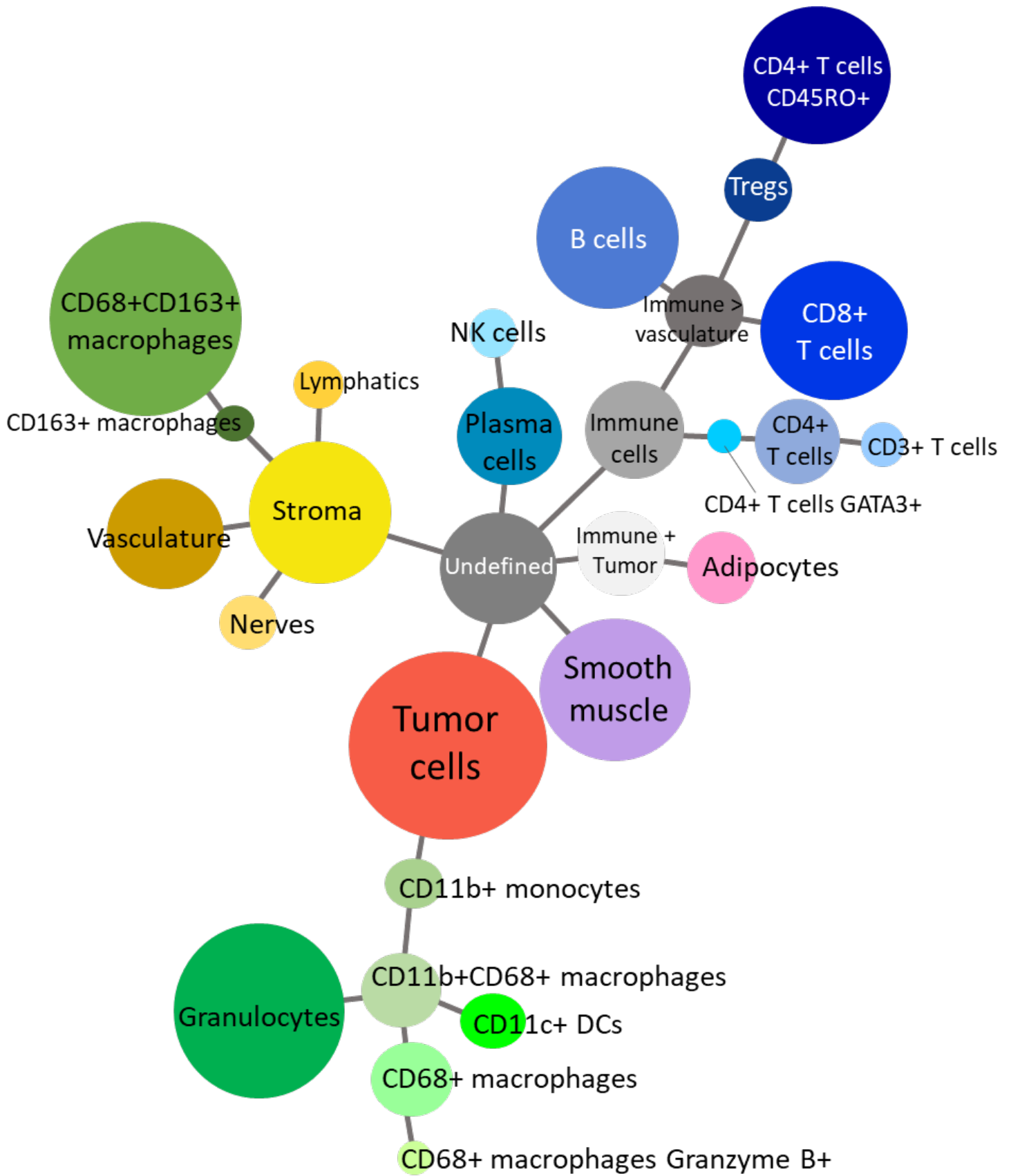
G



Neighborhood Legend

- 1 T cell enriched
- 3 immune-infiltrated stroma
- 5 follicle
- 7 vascular smooth muscle
- 2 bulk tumor
- 4 macrophage enriched
- 6 tumor boundary
- 8 smooth muscle
- 9 granulocyte enriched

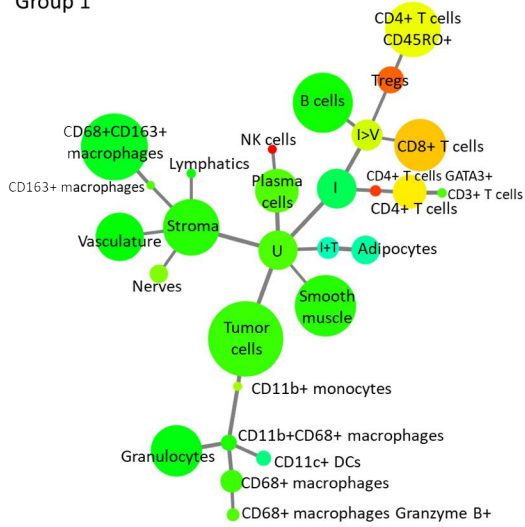
Data S3. Minimal Spanning Trees (MSTs) and Mean Marker Expression of 28 CRC Clusters from Unsupervised X-shift Clustering, Related to Figure 3 and STAR Methods. MSTs show the relationship between the clusters (edges and distances), their sizes, and their mean marker expression, for each patient group. MSTs were generated in Vortex for each marker analyzed. Group 1, CLR patients; group 2, DII patients.



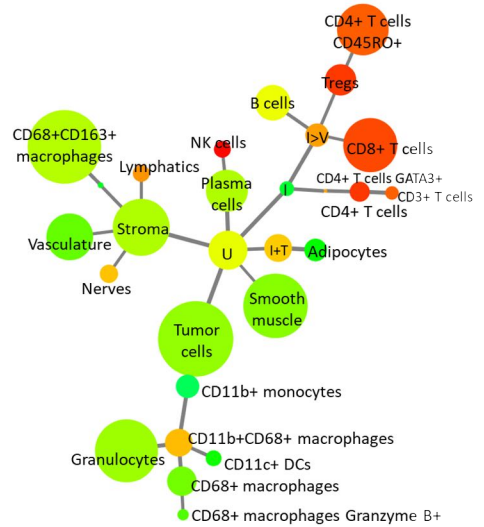
28 unique clusters

CD2

Group 1

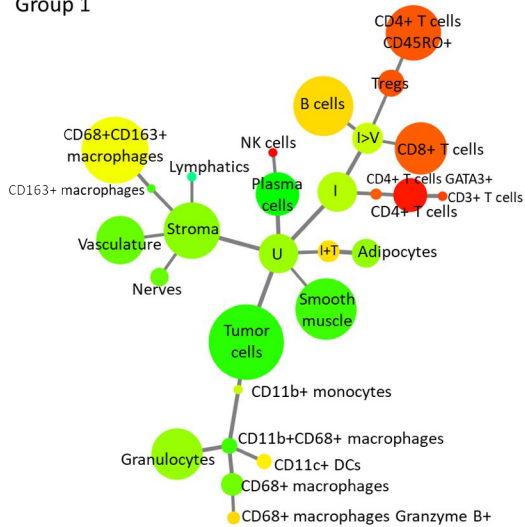


Group 2

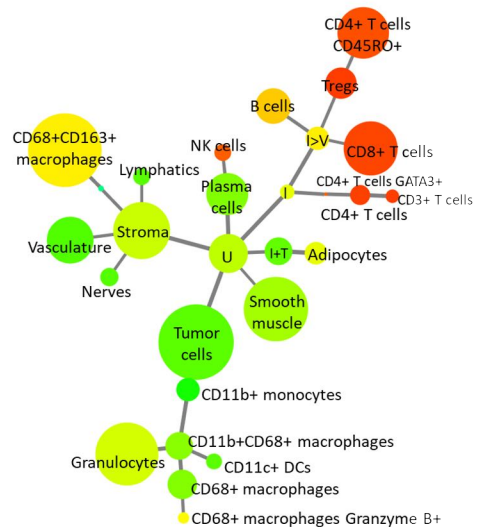


CD3

Group 1

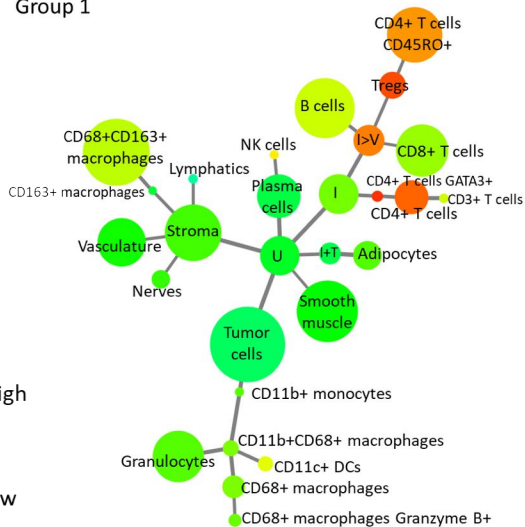


Group 2

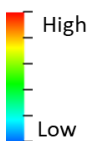
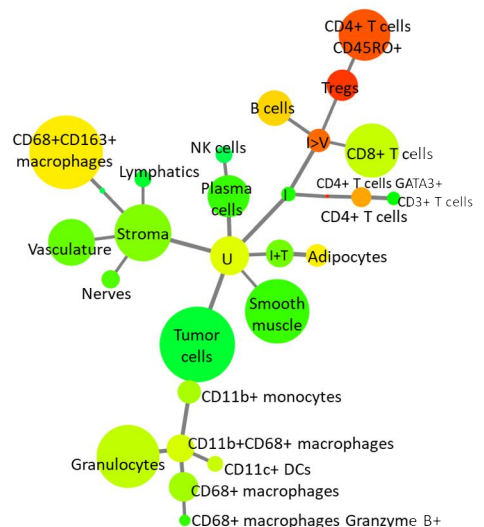


CD4

Group 1

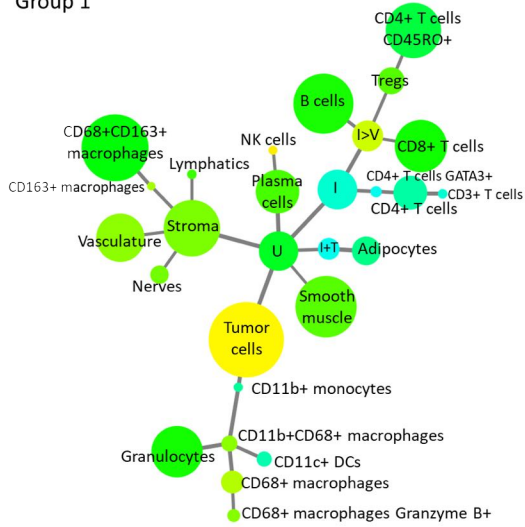


Group 2

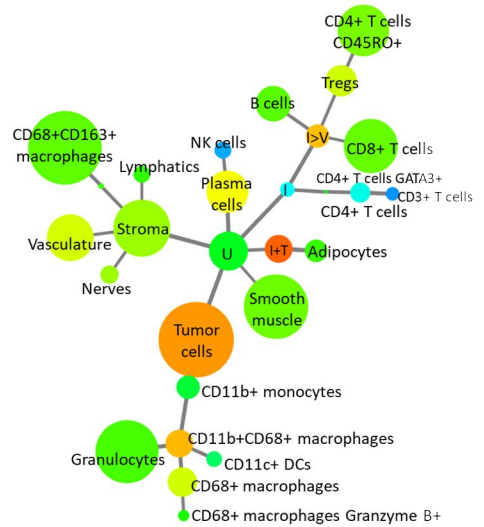


CD5

Group 1

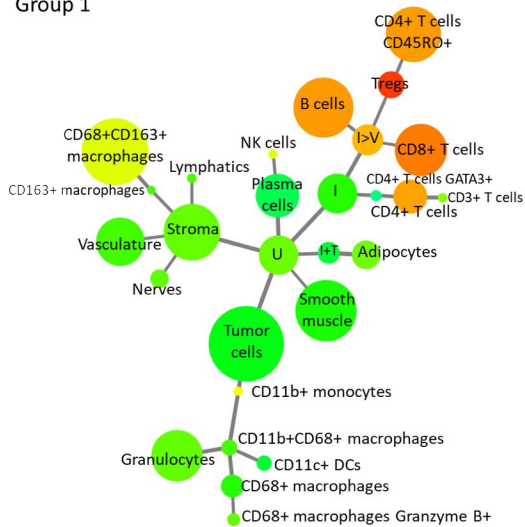


Group 2

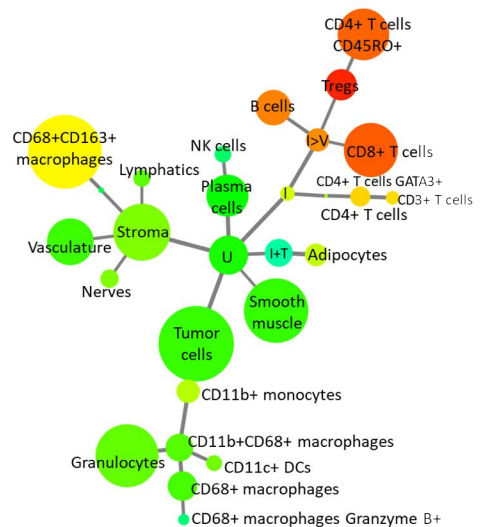


CD7

Group 1

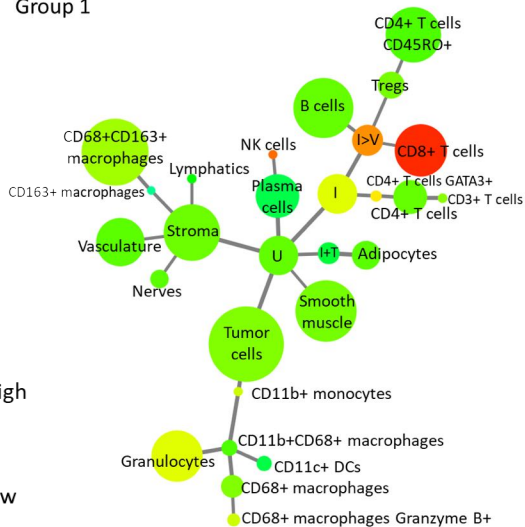


Group 2

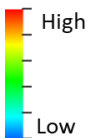
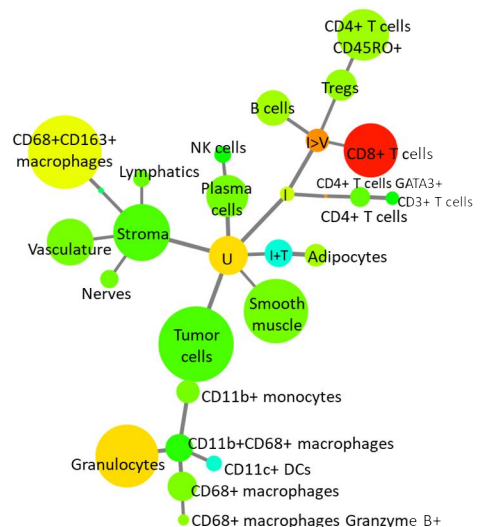


CD8

Group 1

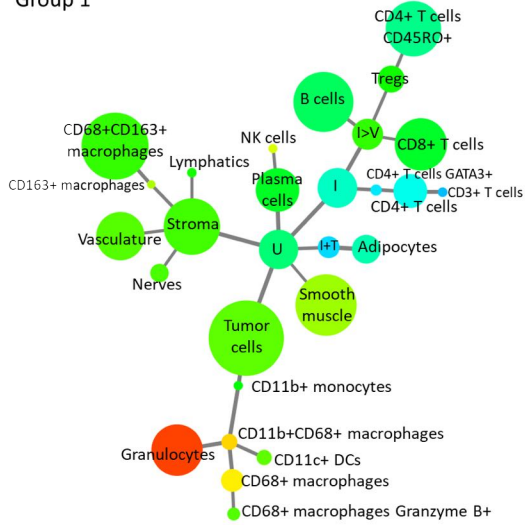


Group 2

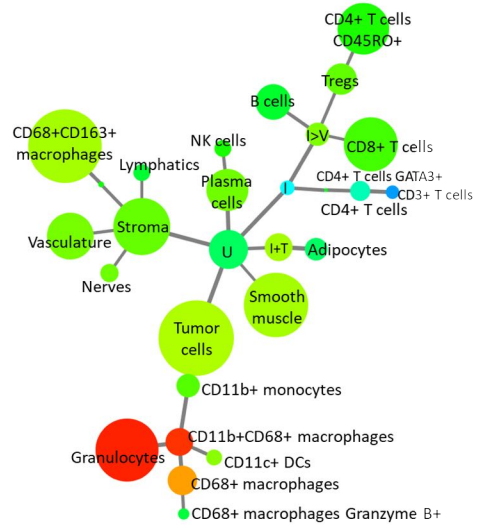


CD11b

Group 1

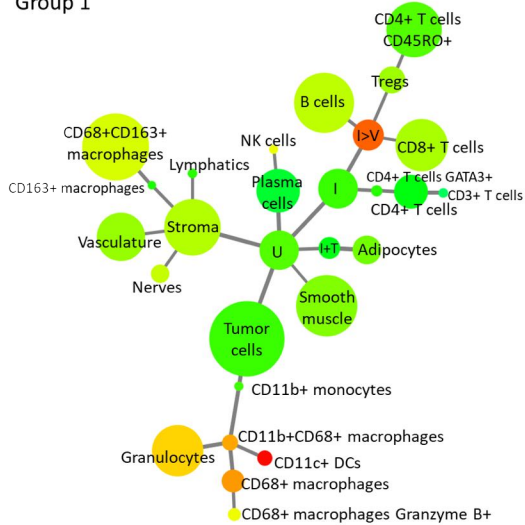


Group 2

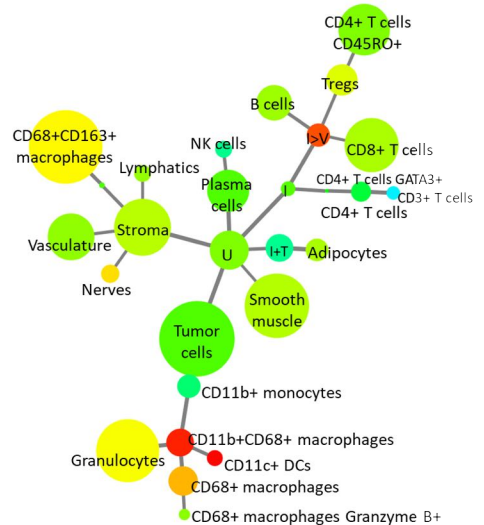


CD11c

Group 1

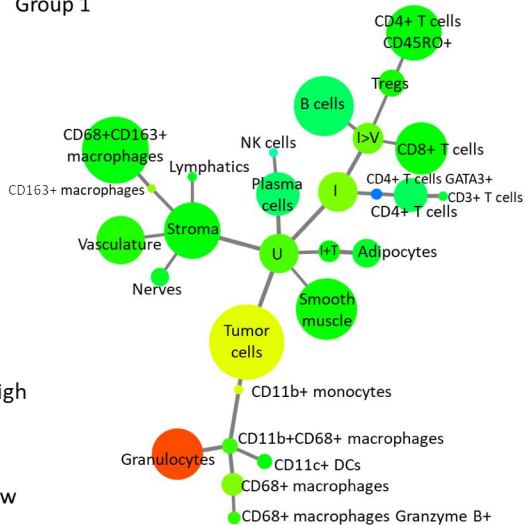


Group 2

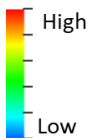
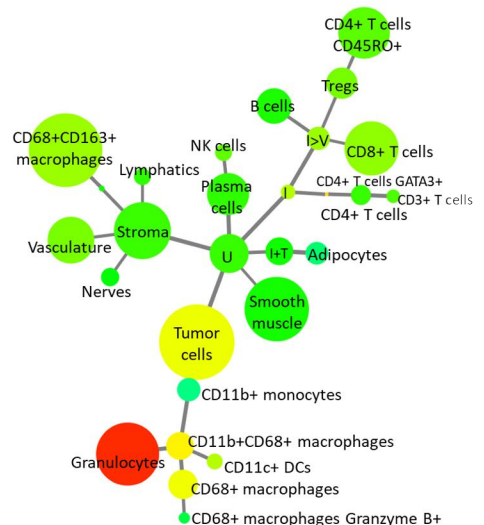


CD15

Group 1

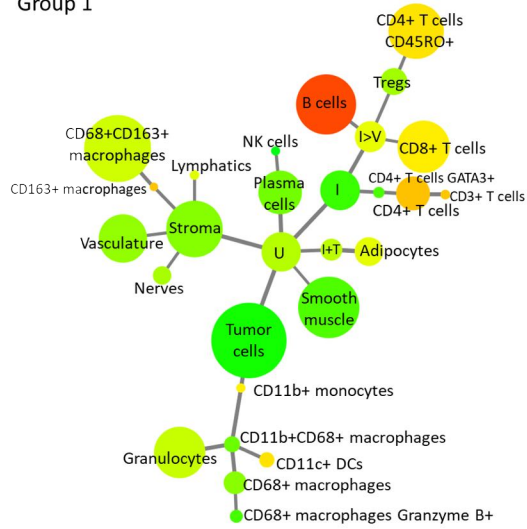


Group 2

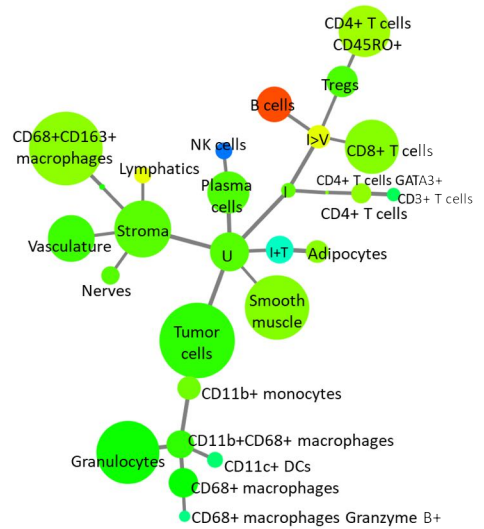


CD20

Group 1

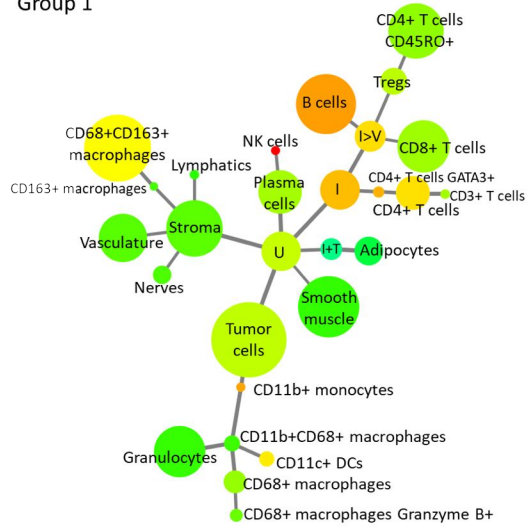


Group 2

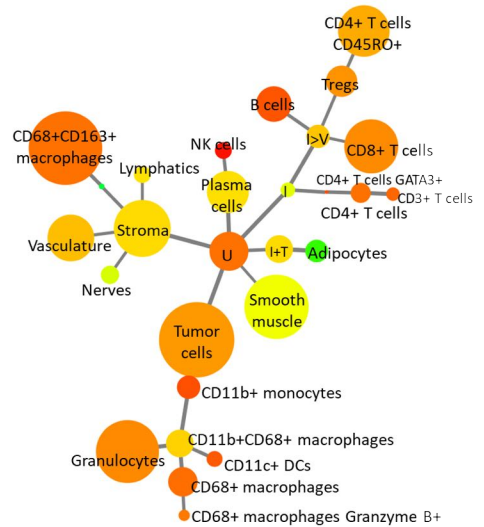


CD21

Group 1

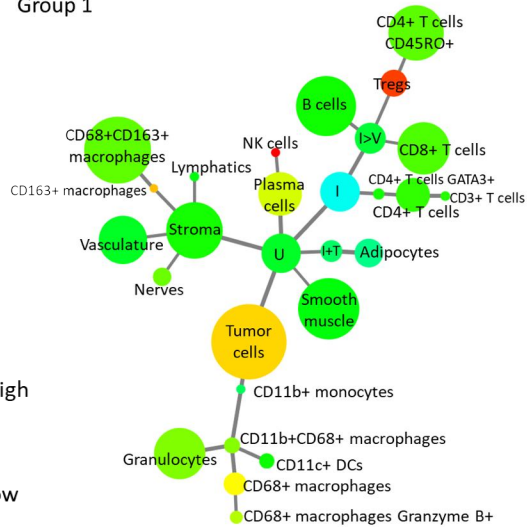


Group 2

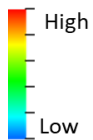
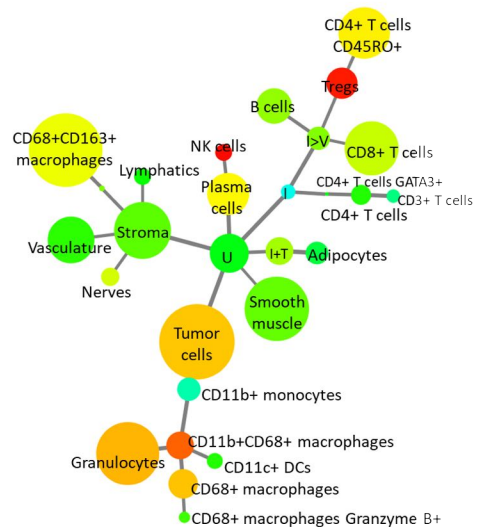


CD25

Group 1

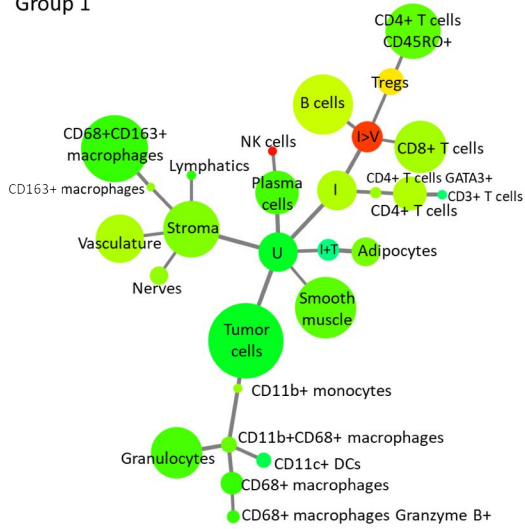


Group 2

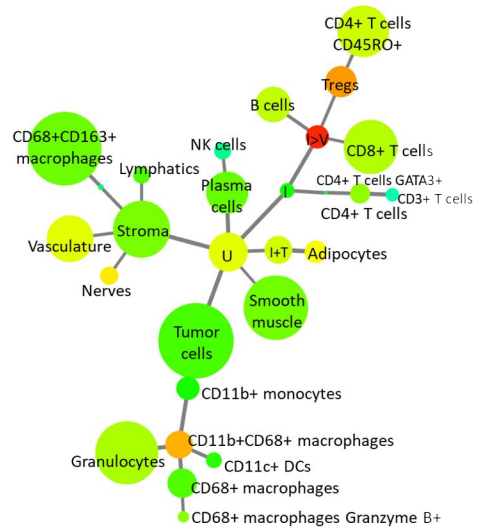


CD30

Group 1

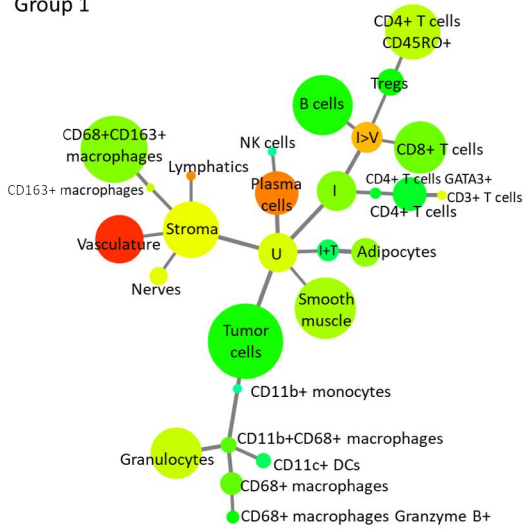


Group 2

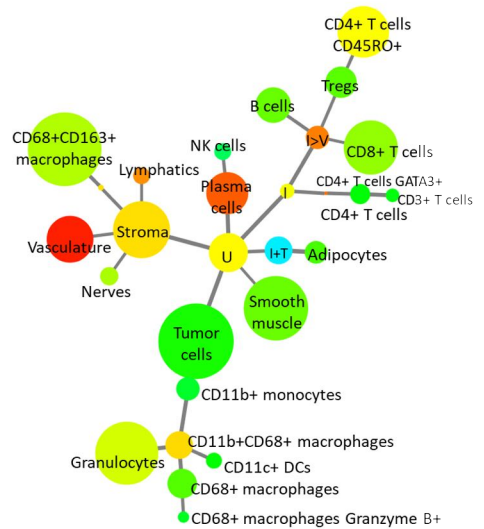


CD31

Group 1

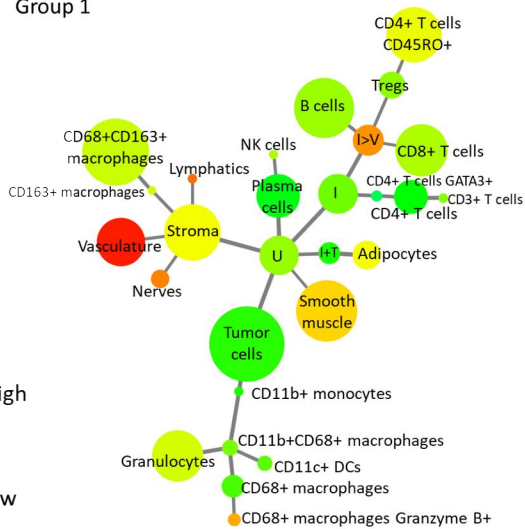


Group 2

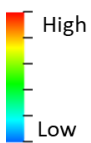
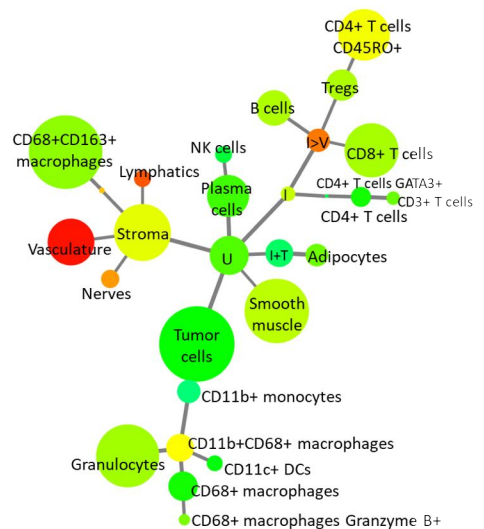


CD34

Group 1

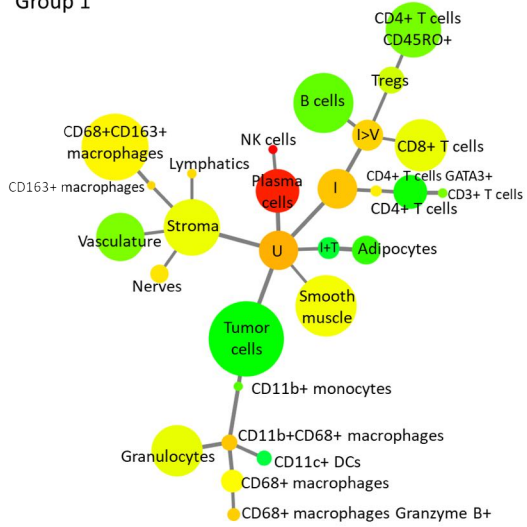


Group 2

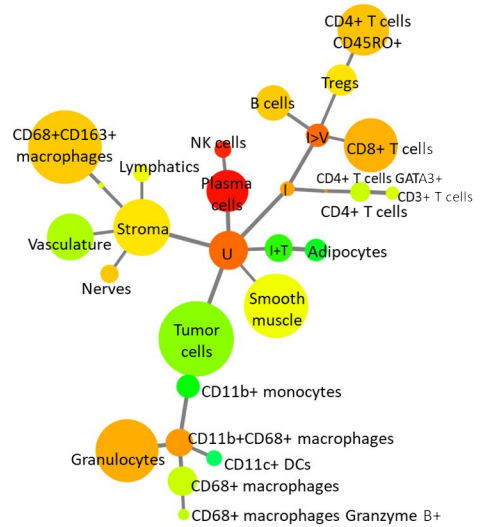


CD38

Group 1

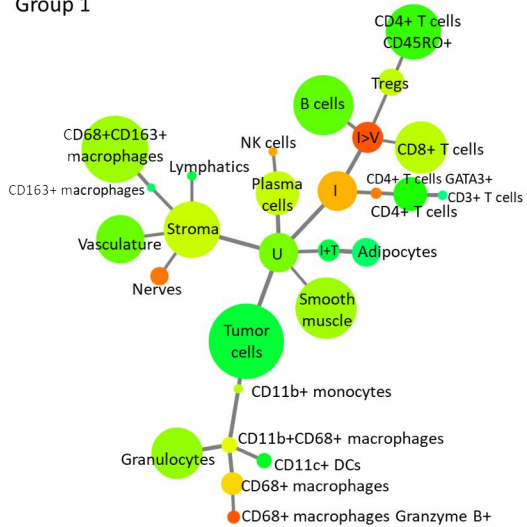


Group 2

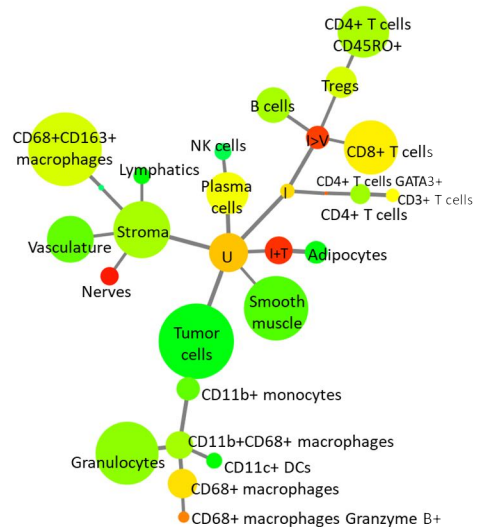


CD44

Group 1

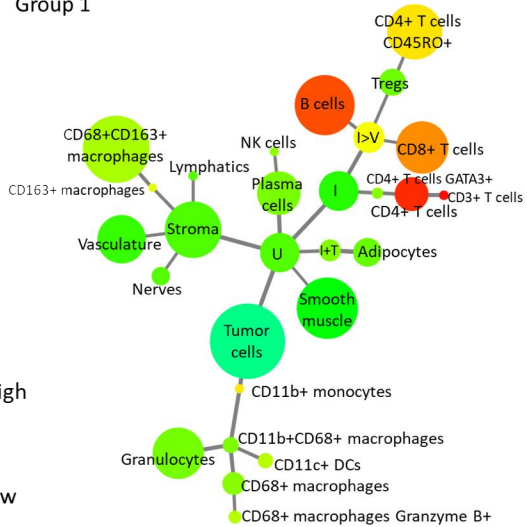


Group 2

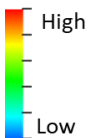
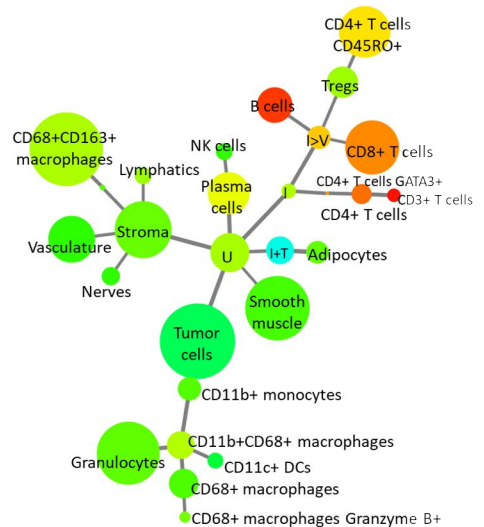


CD45

Group 1

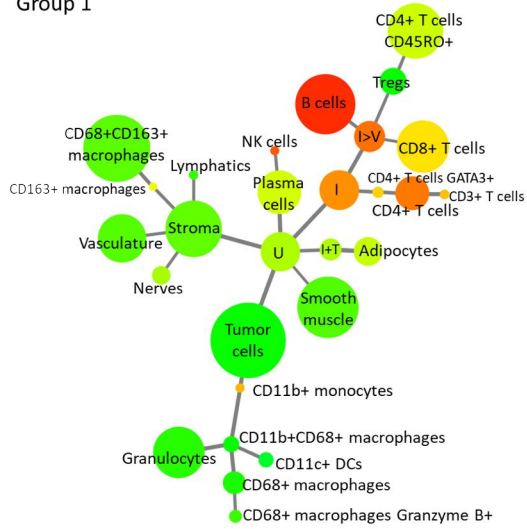


Group 2

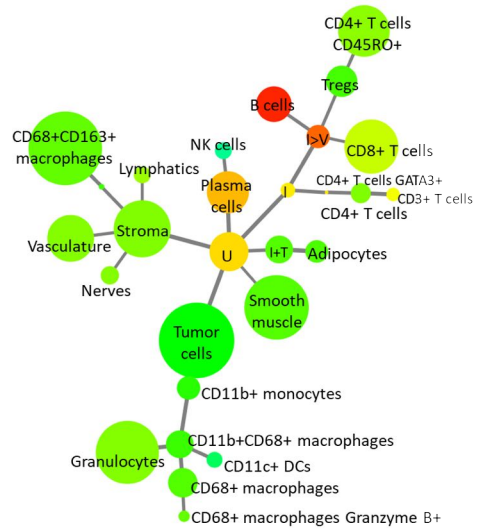


CD45RA

Group 1

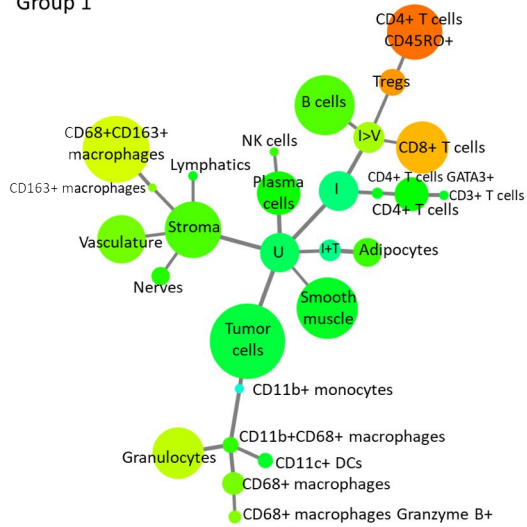


Group 2

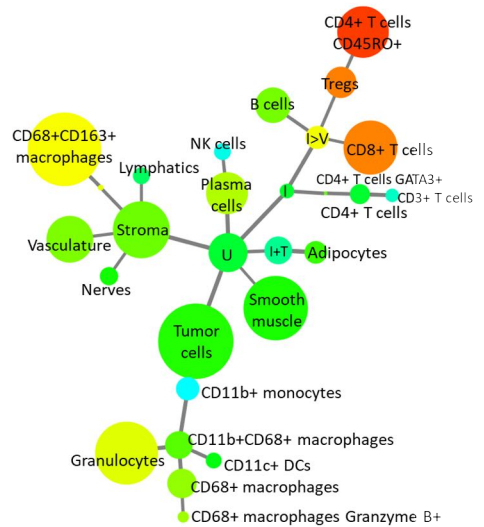


CD45RO

Group 1

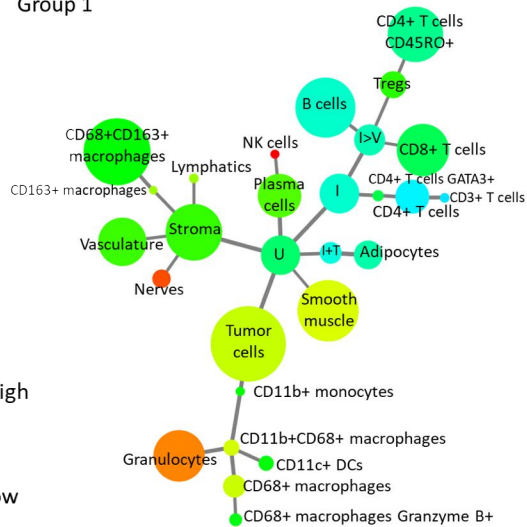


Group 2

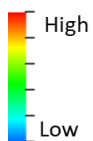
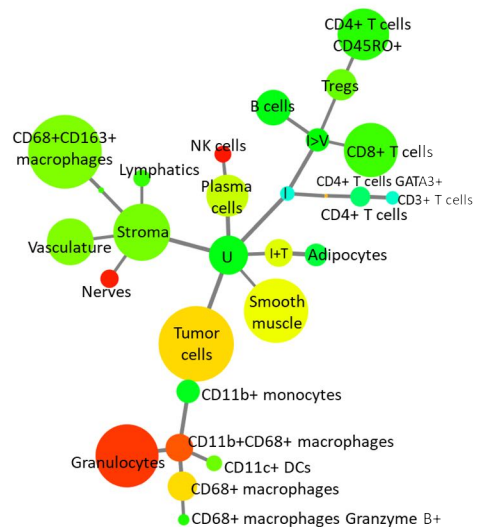


CD56

Group 1

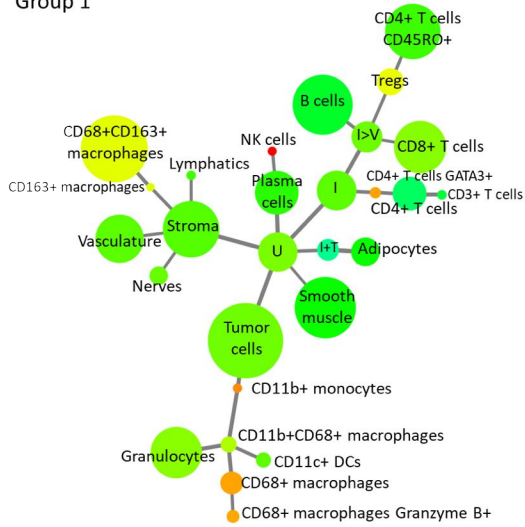


Group 2

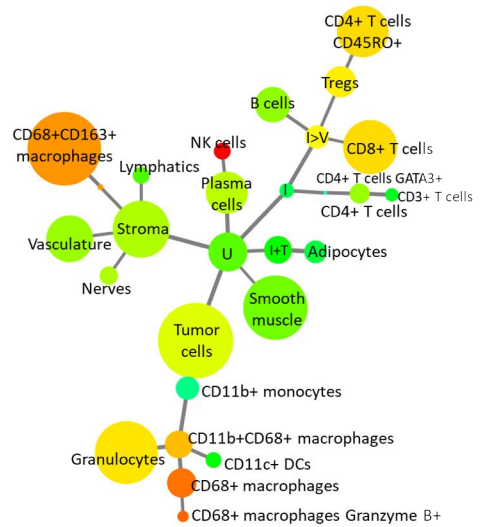


CD57

Group 1

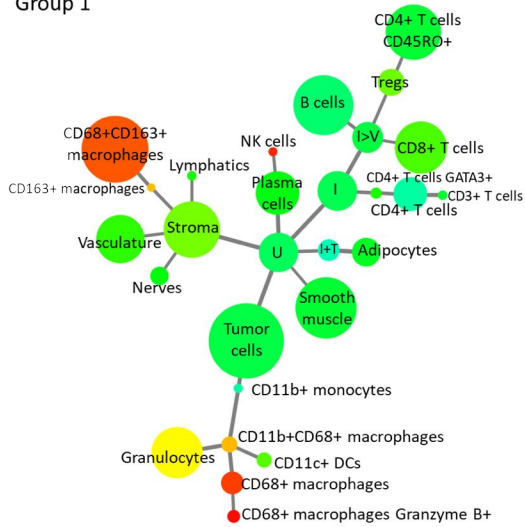


Group 2

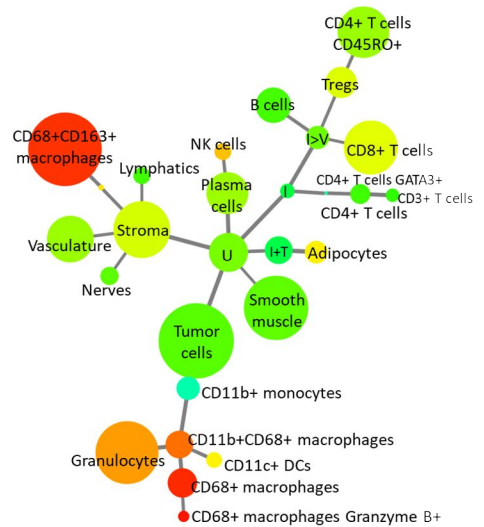


CD68

Group 1

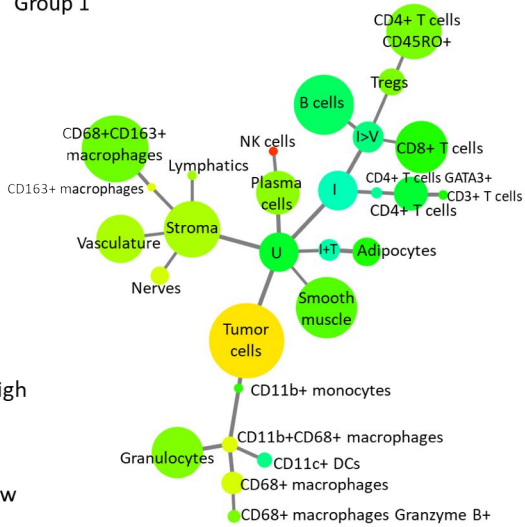


Group 2

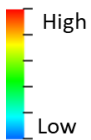
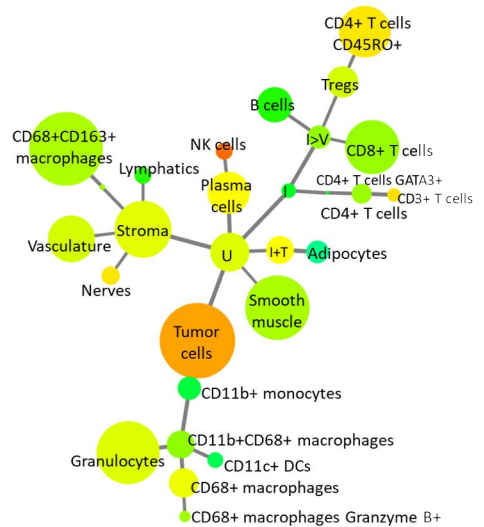


CD71

Group 1

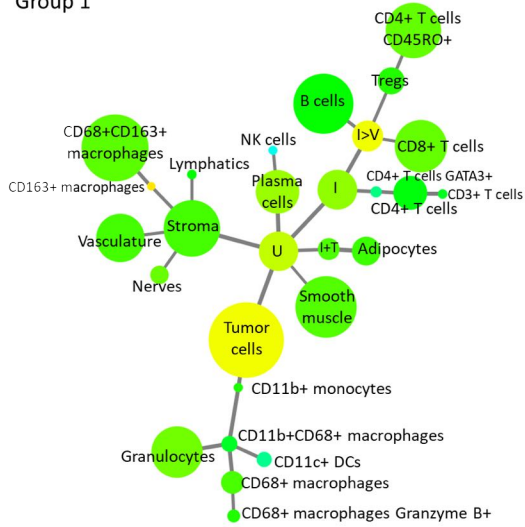


Group 2

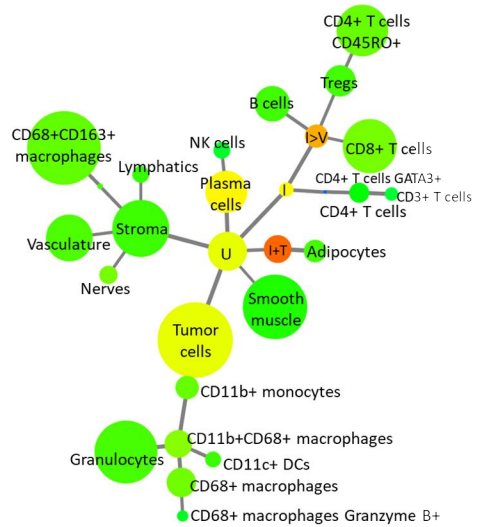


CD138

Group 1

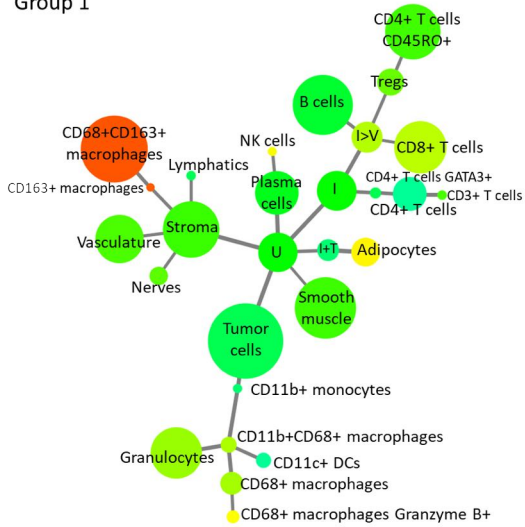


Group 2

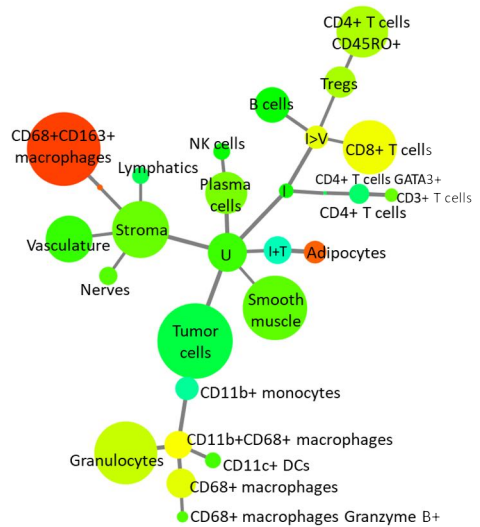


CD163

Group 1

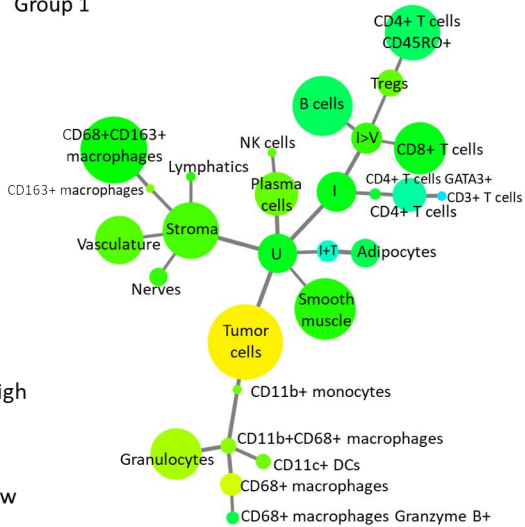


Group 2

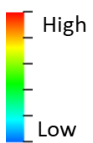
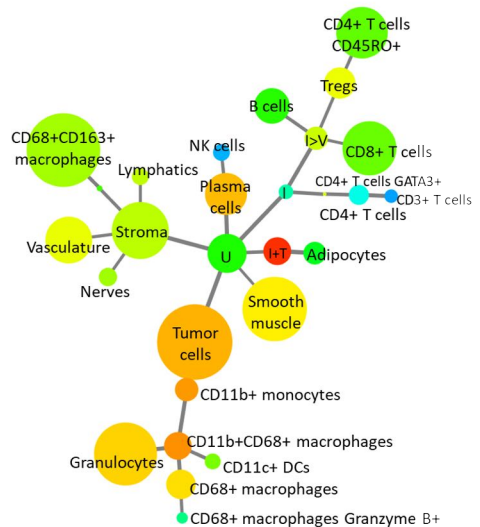


CD194

Group 1

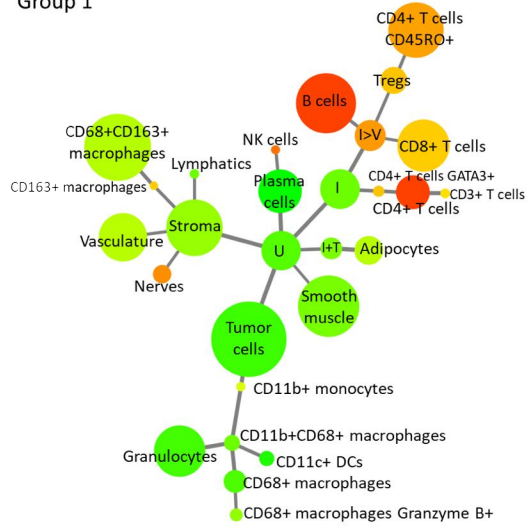


Group 2

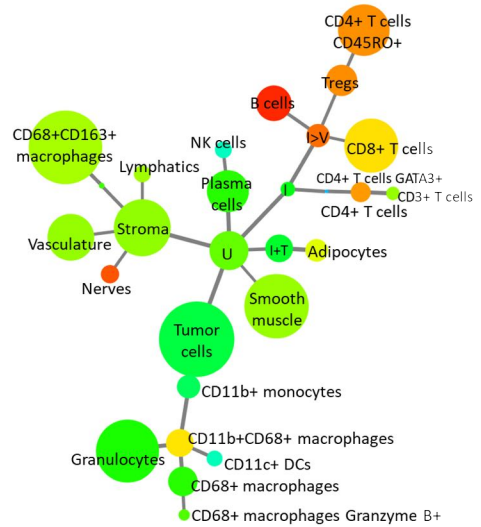


BCL-2

Group 1

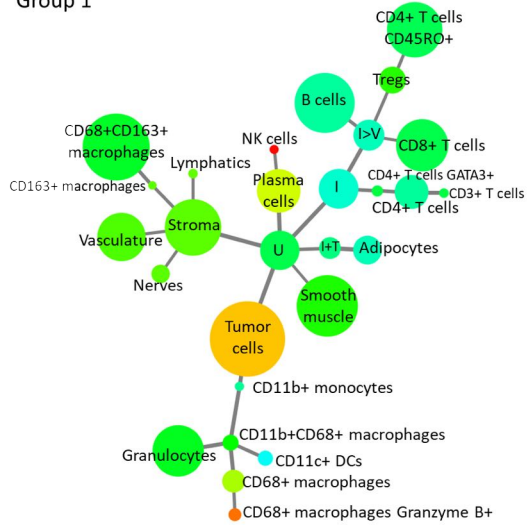


Group 2

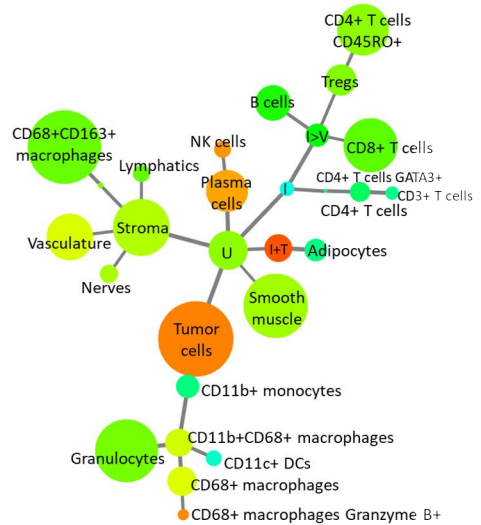


Beta-catenin

Group 1

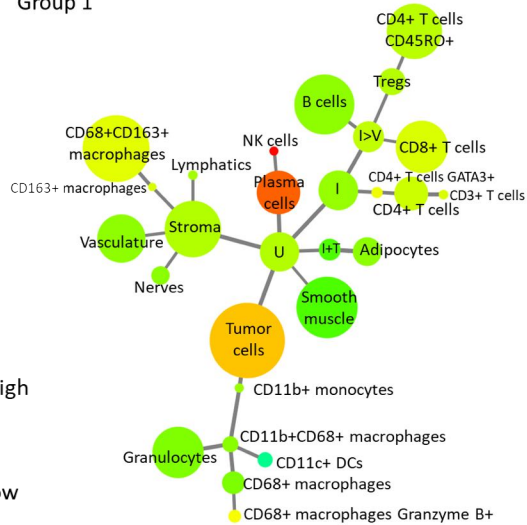


Group 2

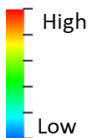
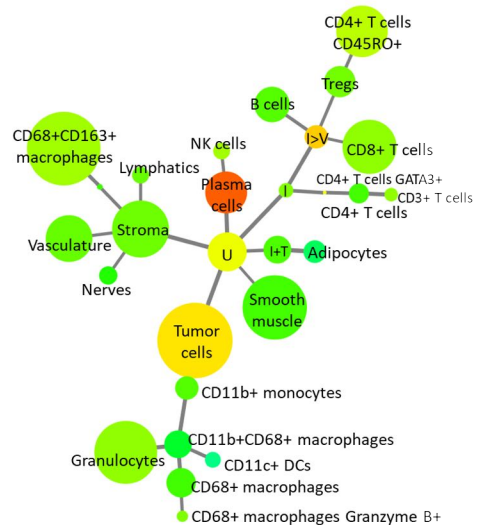


CDX2

Group 1

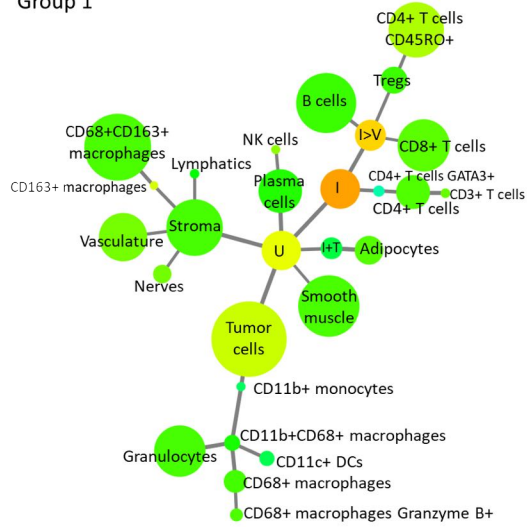


Group 2

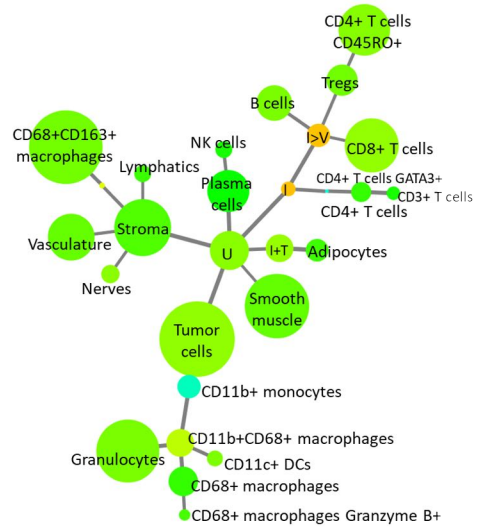


Chromogranin A

Group 1

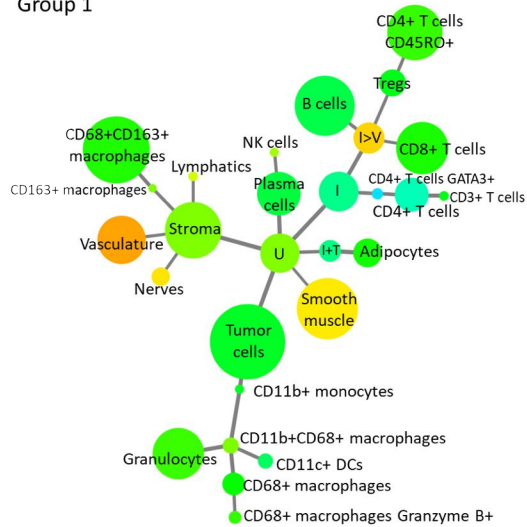


Group 2

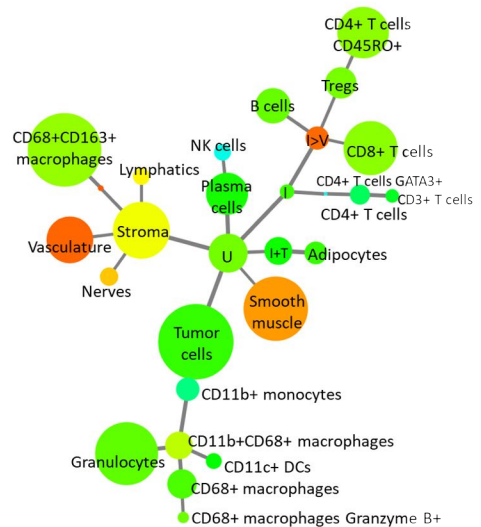


Collagen IV

Group 1

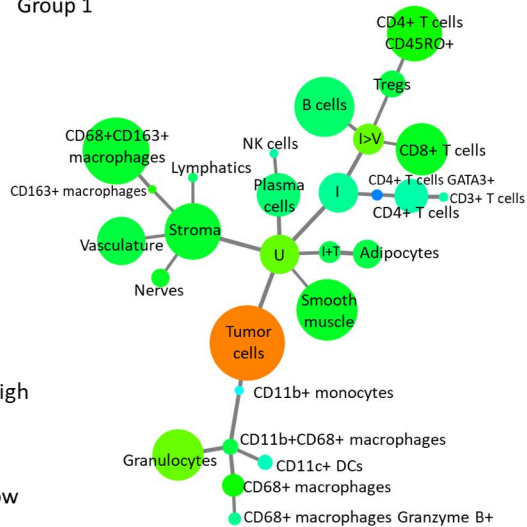


Group 2

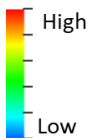
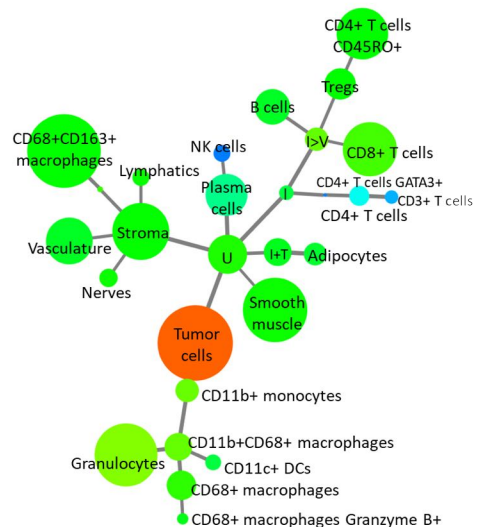


Cytokeratin

Group 1

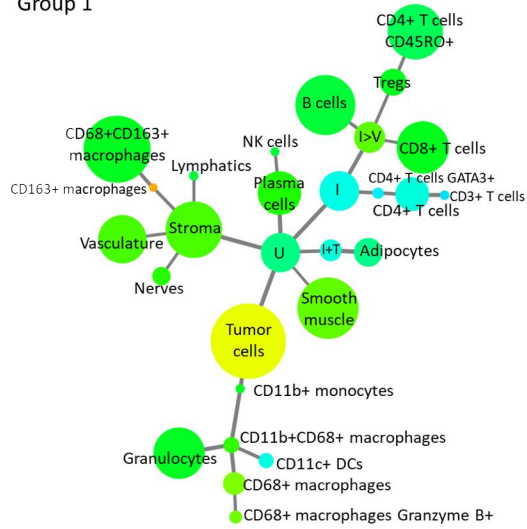


Group 2

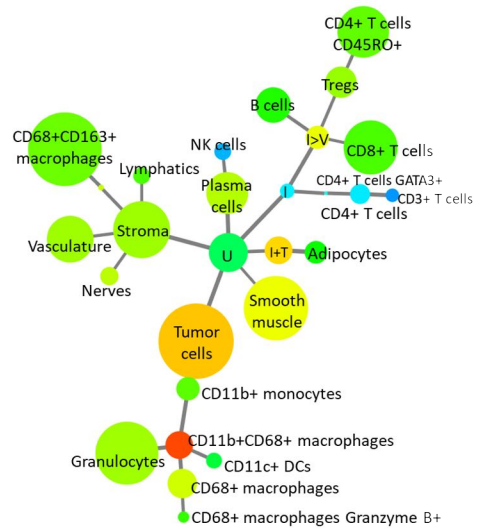


EGFR

Group 1

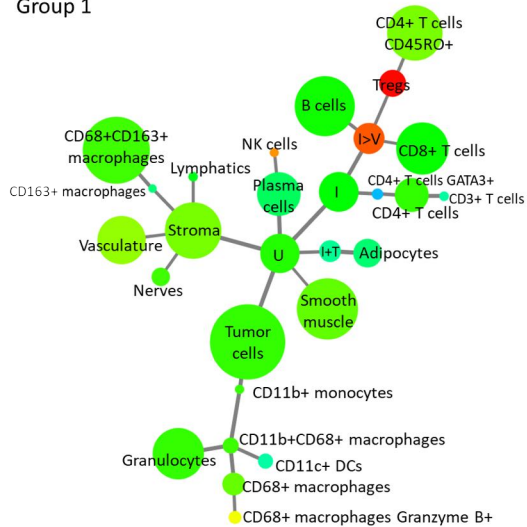


Group 2

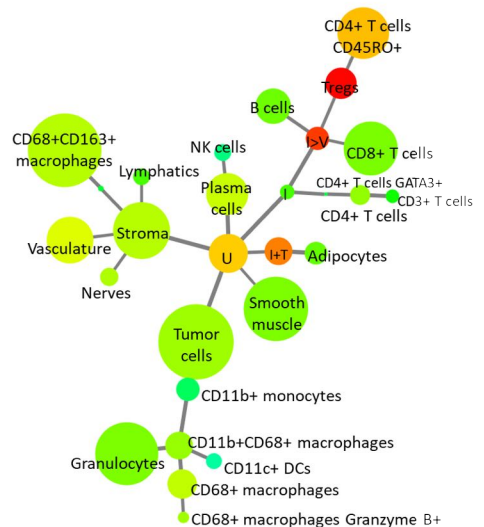


FoxP3

Group 1

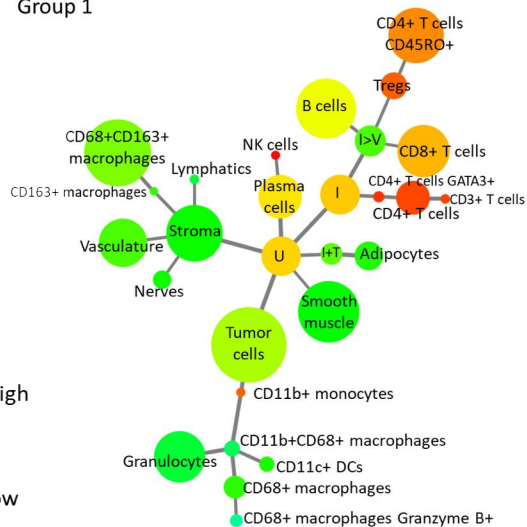


Group 2

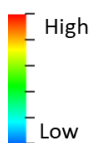
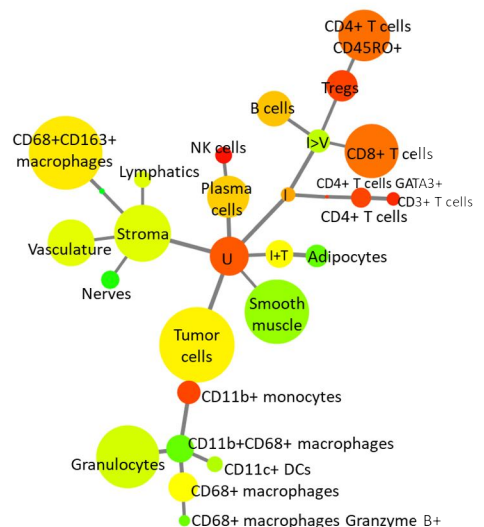


GATA3

Group 1

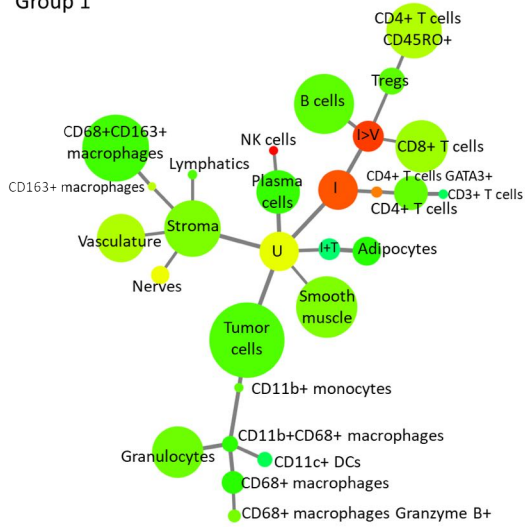


Group 2

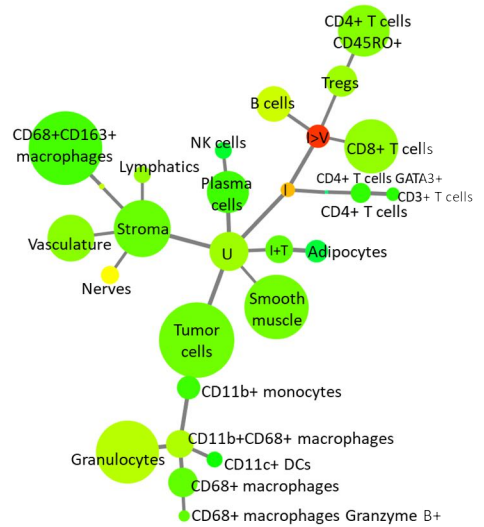


GFAP

Group 1

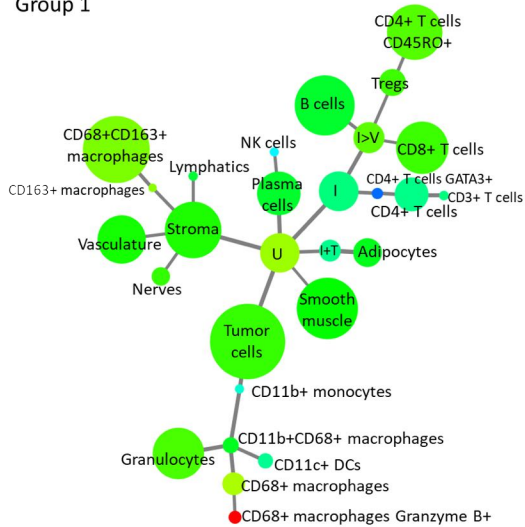


Group 2

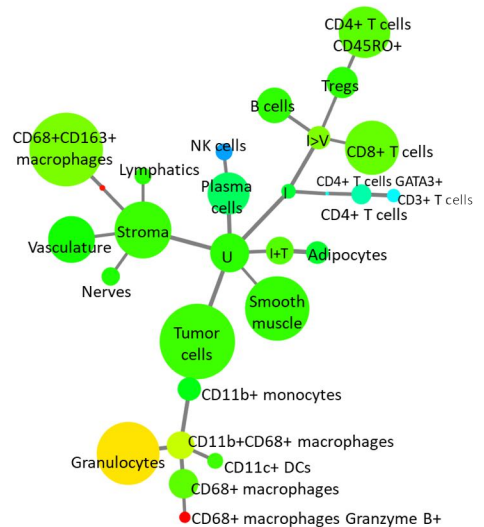


Granzyme B

Group 1

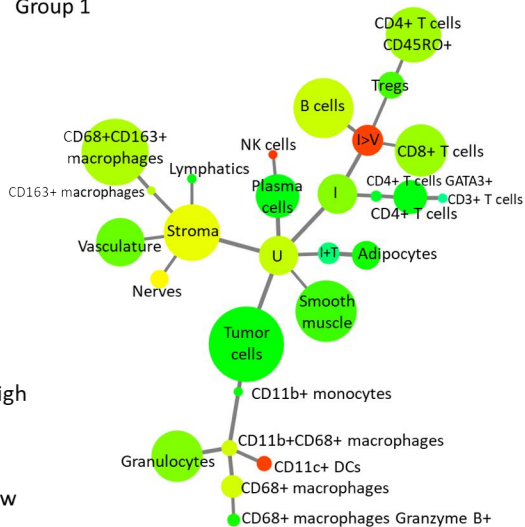


Group 2

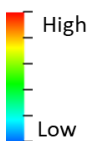
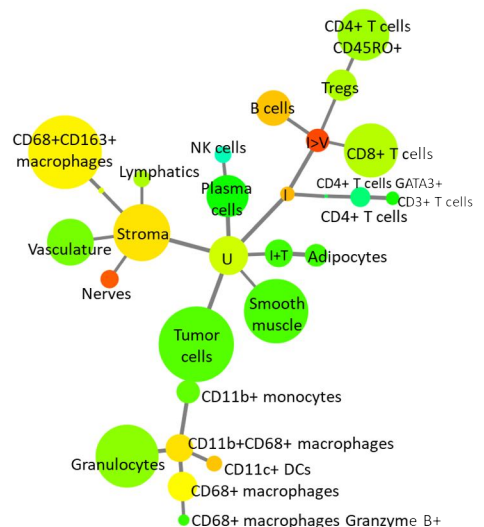


HLA-DR

Group 1

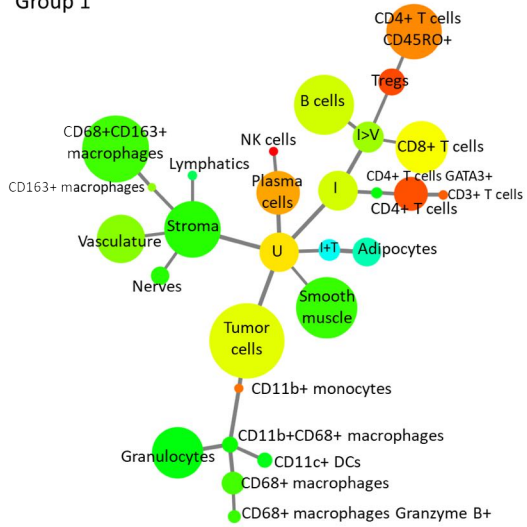


Group 2

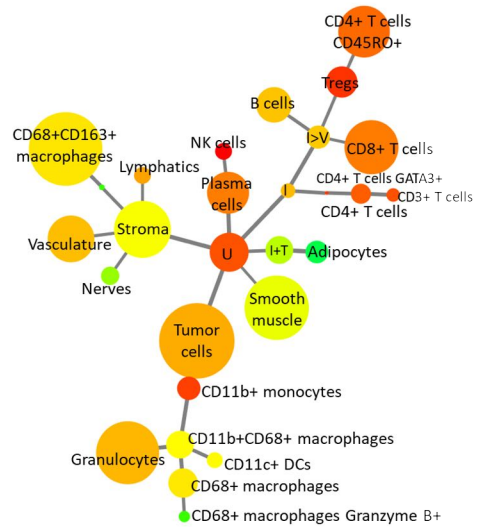


ICOS

Group 1

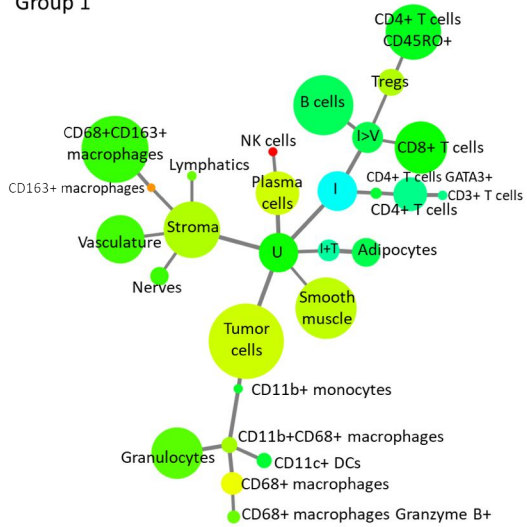


Group 2

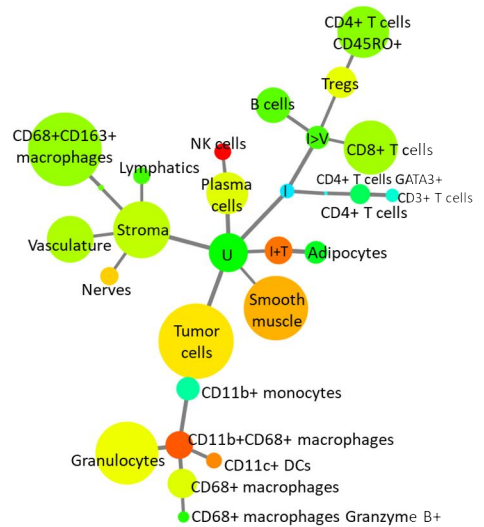


IDO-1

Group 1

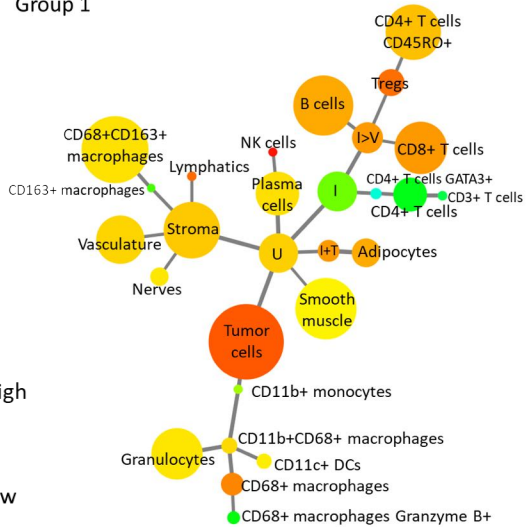


Group 2

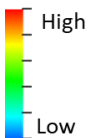
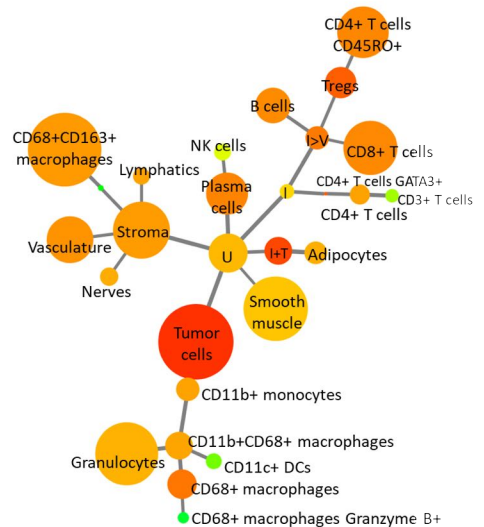


Ki-67

Group 1

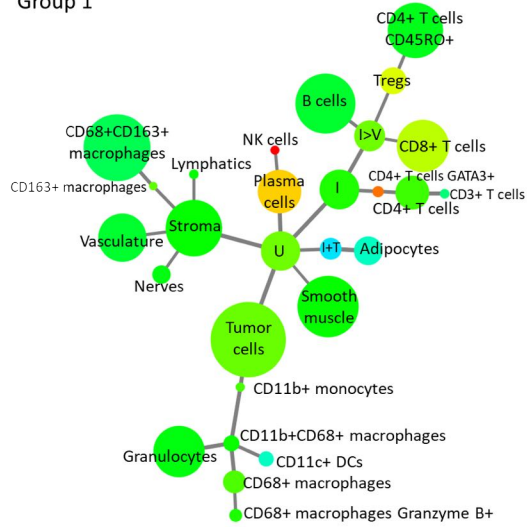


Group 2

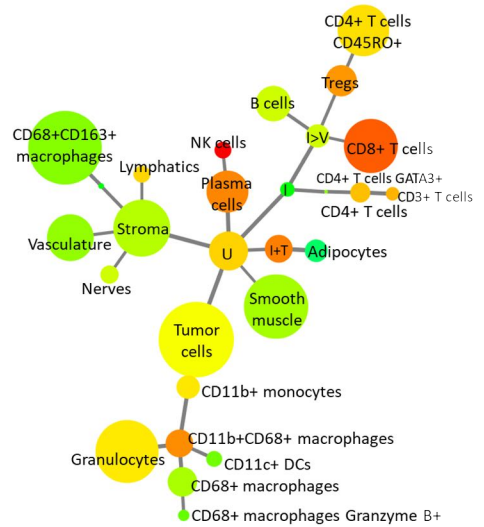


LAG-3

Group 1

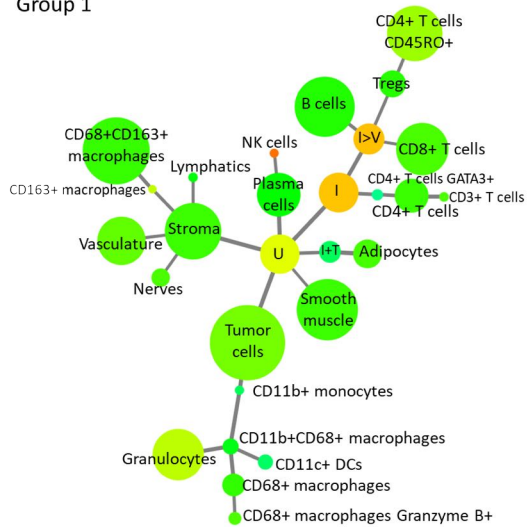


Group 2

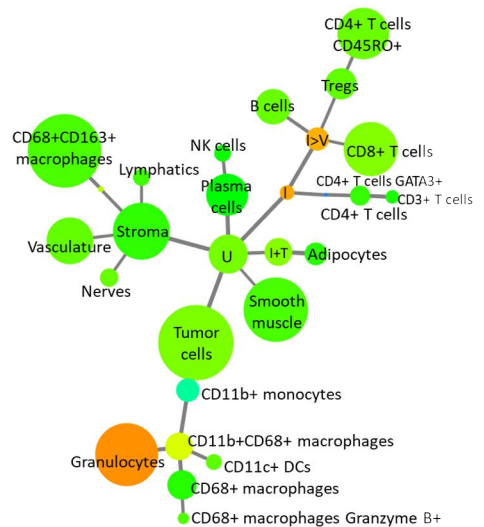


MMP-9

Group 1

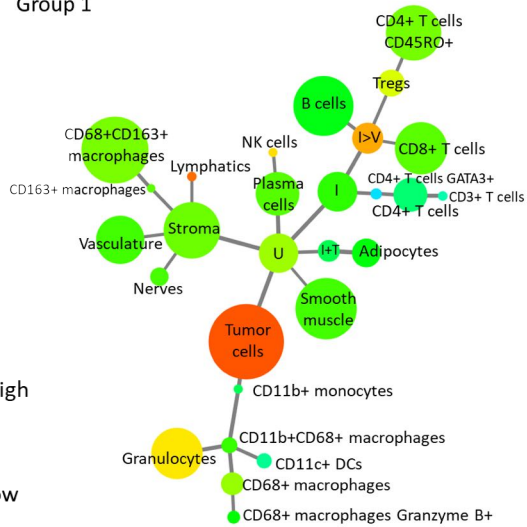


Group 2

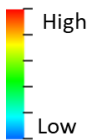
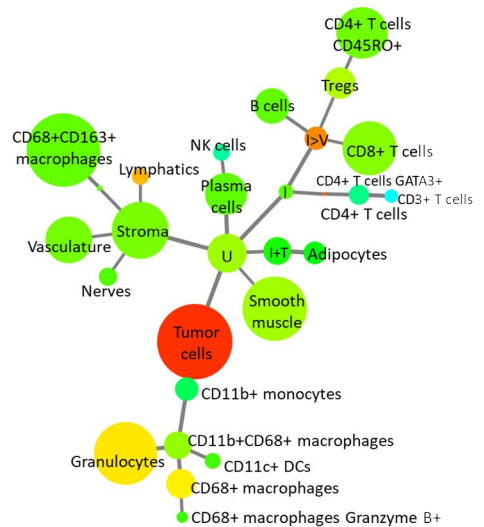


MUC-1

Group 1

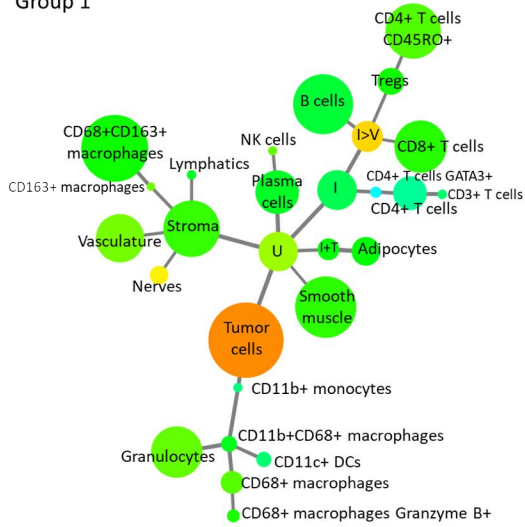


Group 2

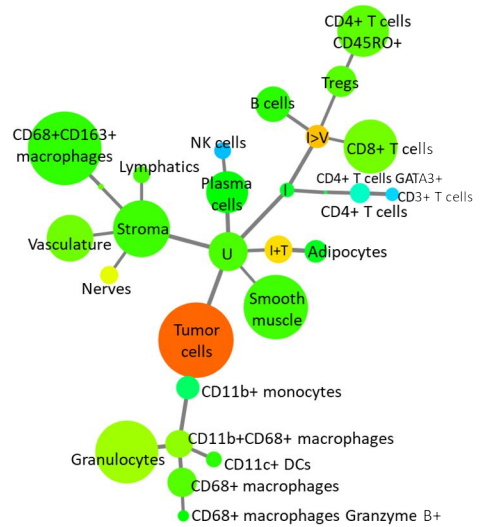


Na-K-ATPase

Group 1

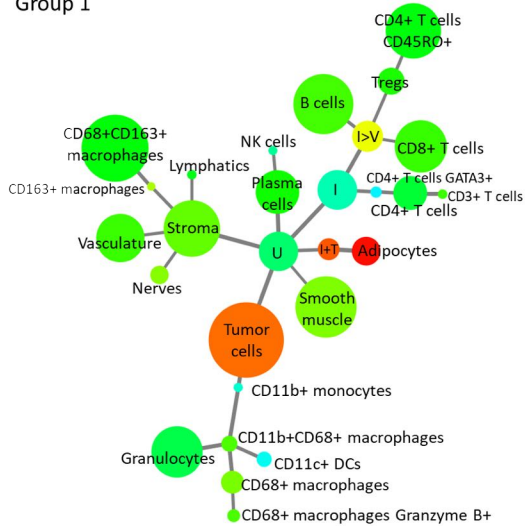


Group 2

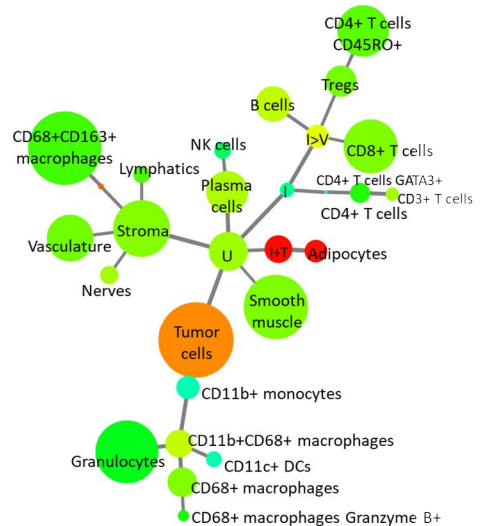


p53

Group 1

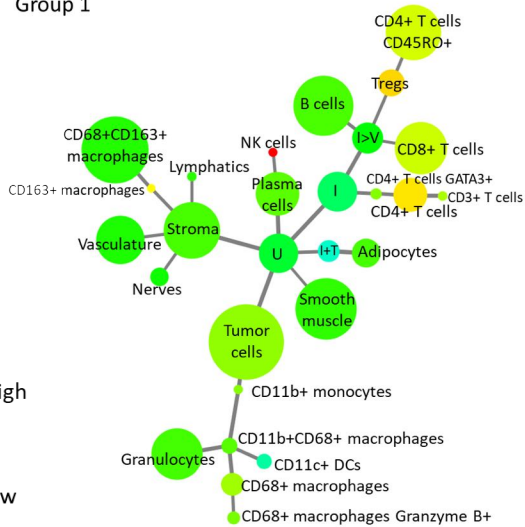


Group 2

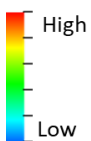
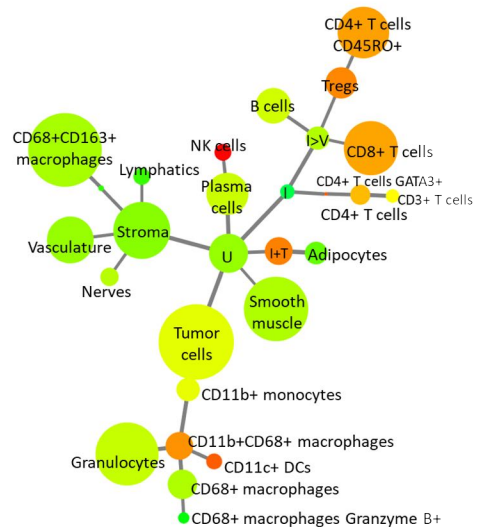


PD-1

Group 1

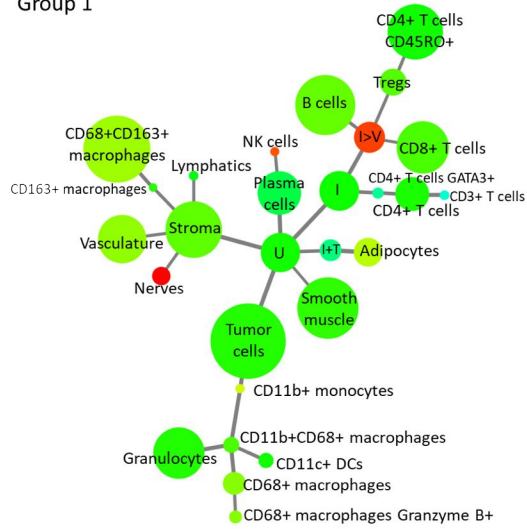


Group 2

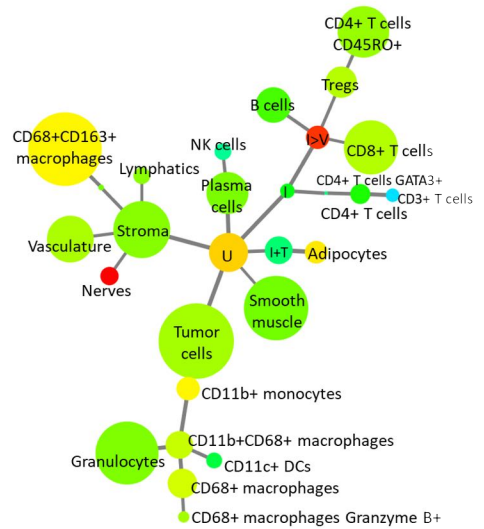


PD-L1

Group 1

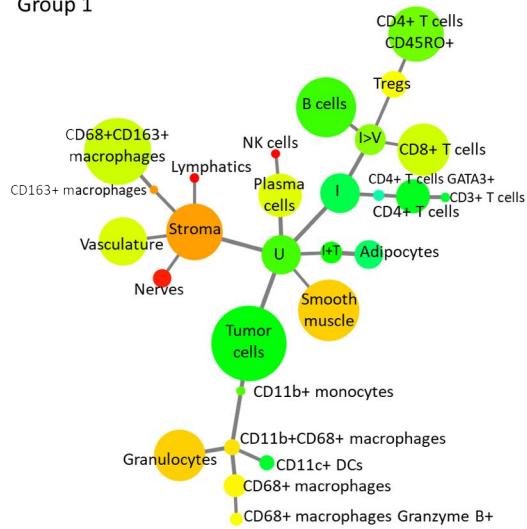


Group 2

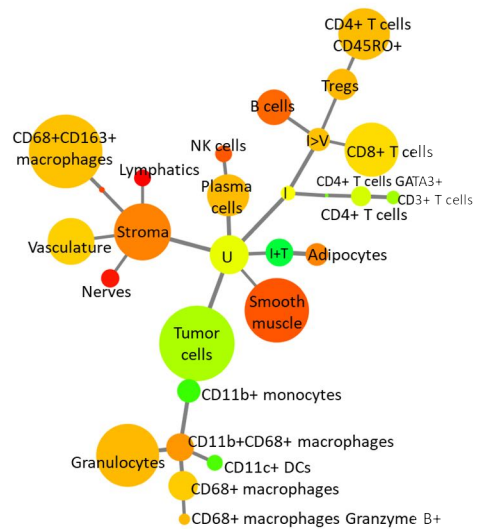


Podoplanin

Group 1

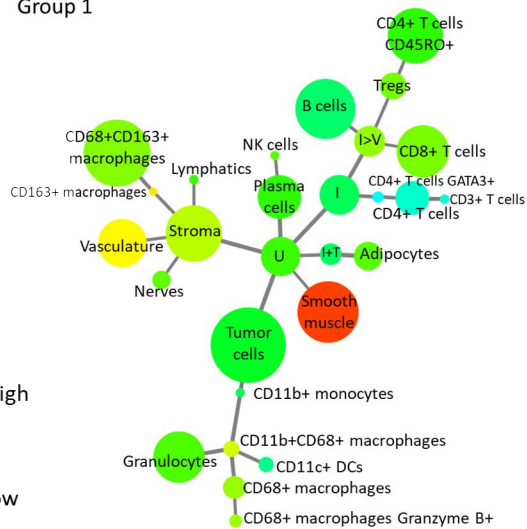


Group 2

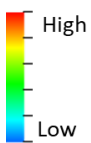
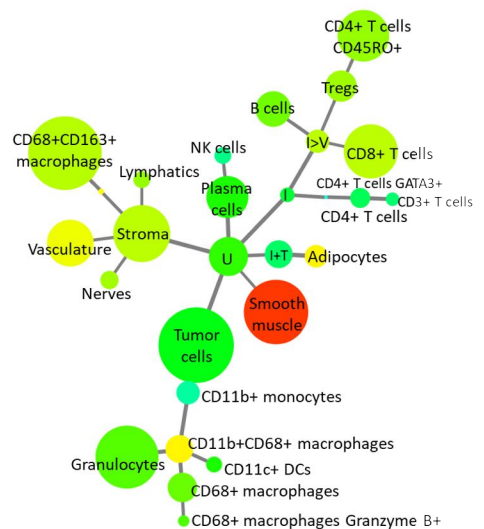


Smooth Muscle Actin (SMA)

Group 1

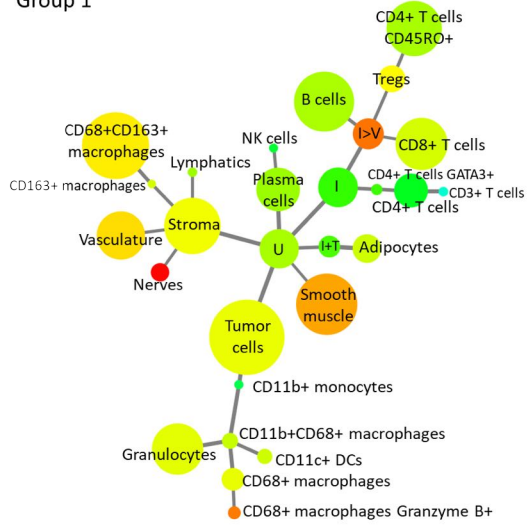


Group 2

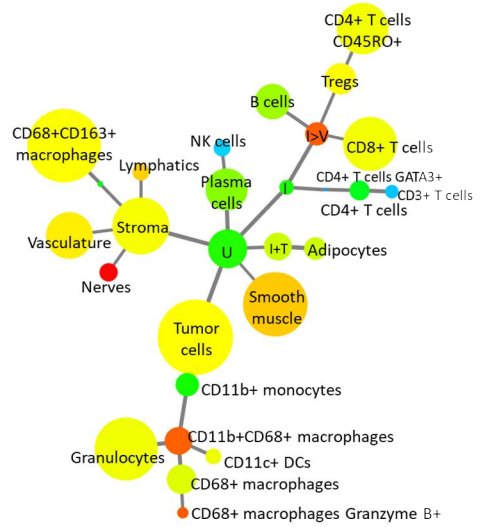


Synaptophysin

Group 1

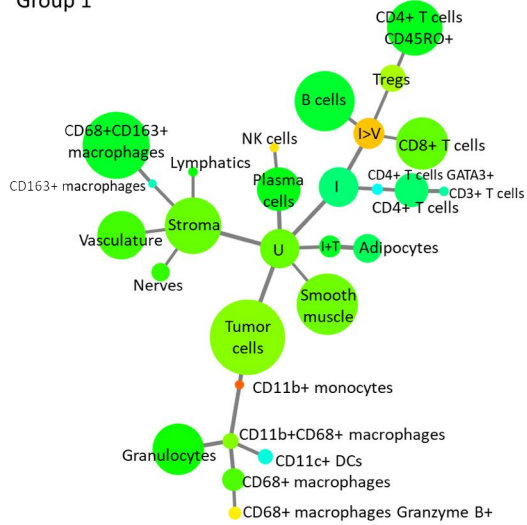


Group 2

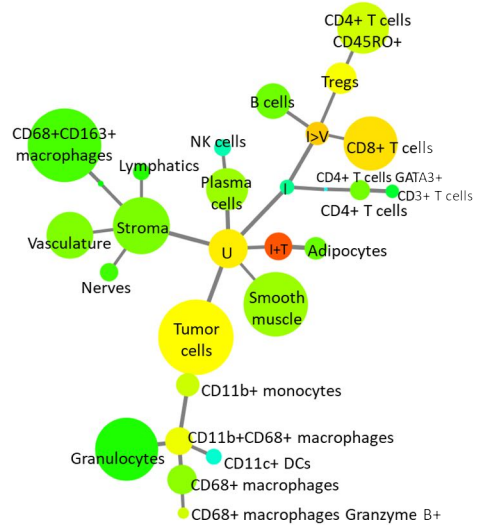


T-bet

Group 1

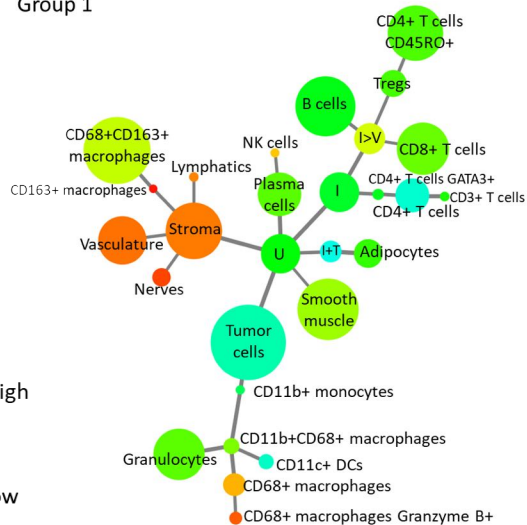


Group 2

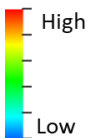
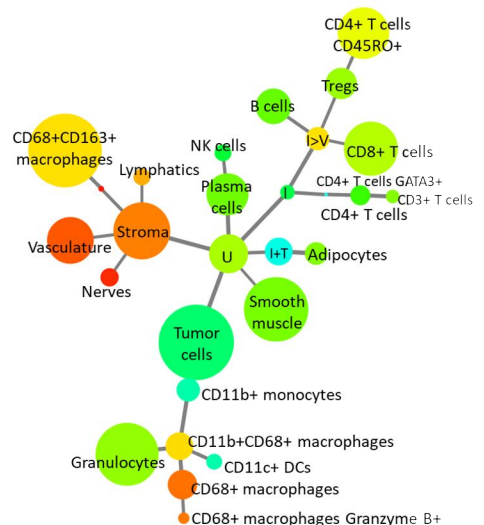


Vimentin

Group 1

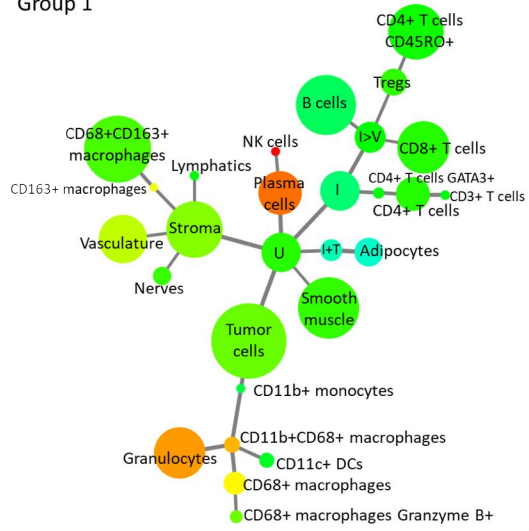


Group 2

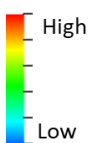
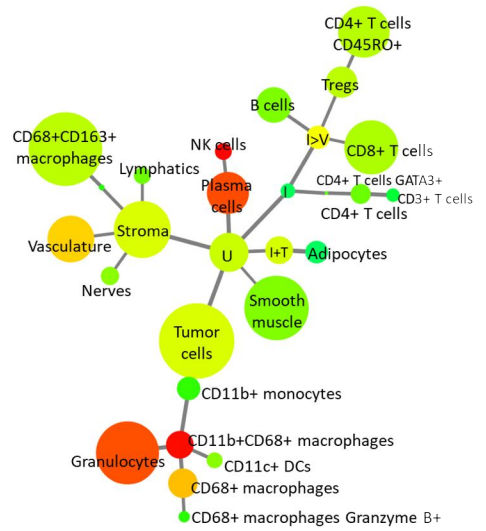


VISTA

Group 1

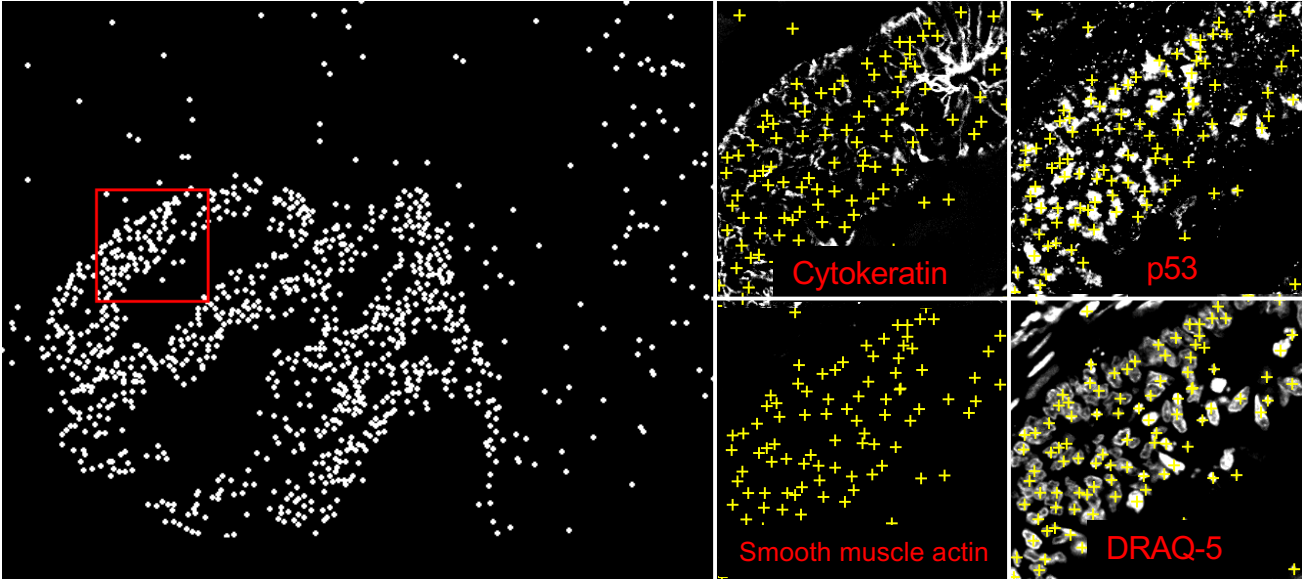


Group 2

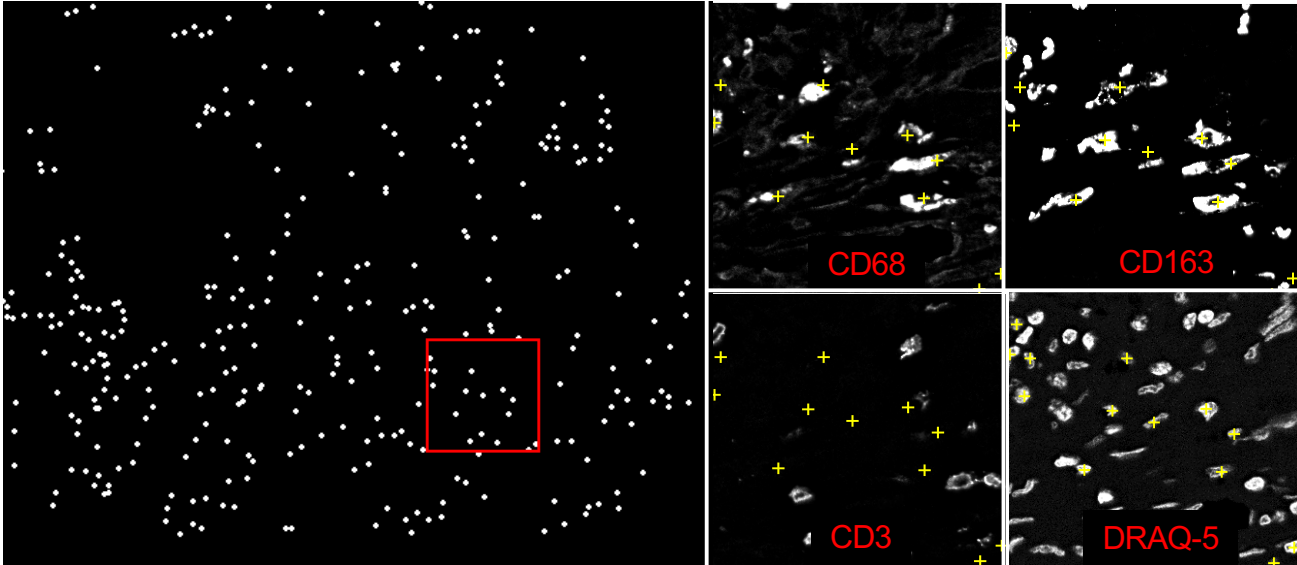


Data S4. Supervised Annotation of CRC Clusters Based on Marker Expression, Tissue Localization and Morphology, Related to Figure 3 and STAR Methods. After X-shift clustering, single cells from the 143 resulting initial clusters were overlaid on the raw data fluorescent images and on H&E stains of TMAs based on X/Y positions and visually verified based on marker expression profiles, morphology, and localization within the tissue. Similar clusters were manually merged, resulting in 28 final clusters. Here, 18 representative clusters are shown as yellow crosses based on X/Y coordinates of the cells contained in that cluster, overlaid on stitched montages of different TMA cores. For each of these 18 clusters, three examples of markers important for cluster identification (2 positive and 1 negative) and DRAQ5 nuclear stain (right panels) are shown as well as a global overview of cellular distribution of that cluster within a single TMA spot (yellow crosses on black background, left panel).

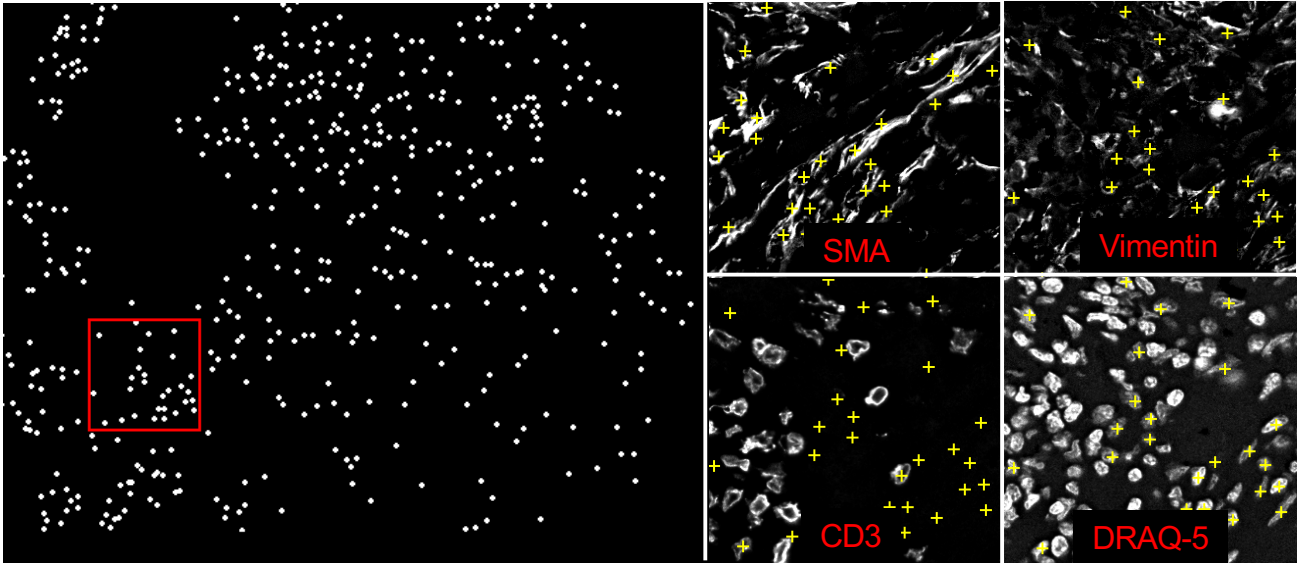
A. Tumor cell cluster



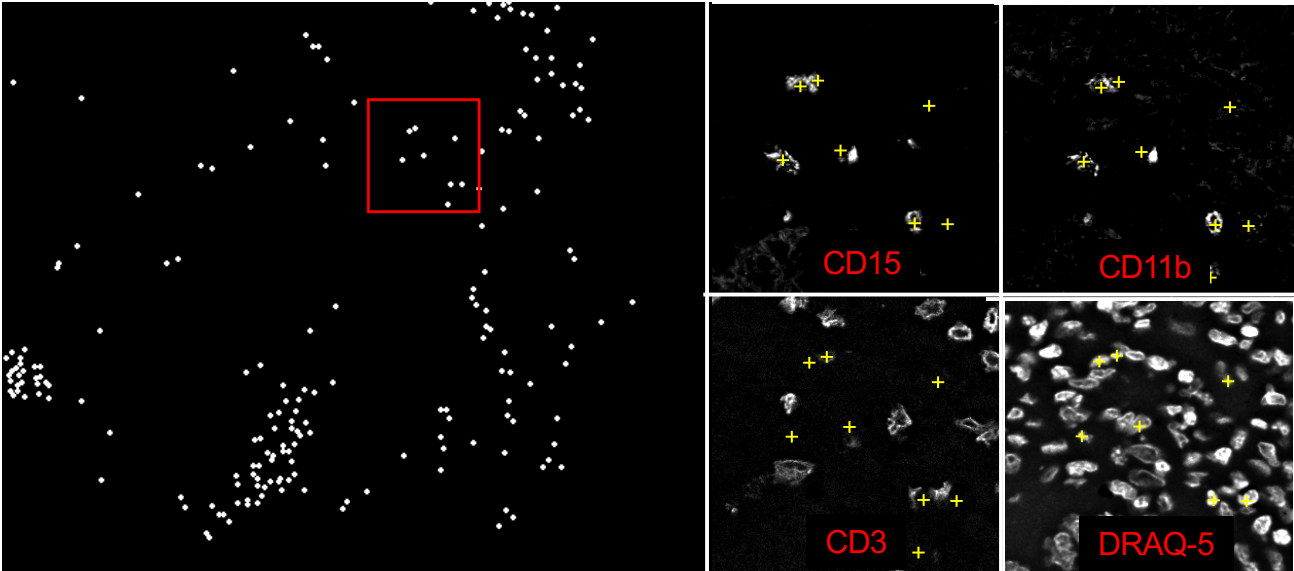
B. CD68+CD163+ macrophage cluster



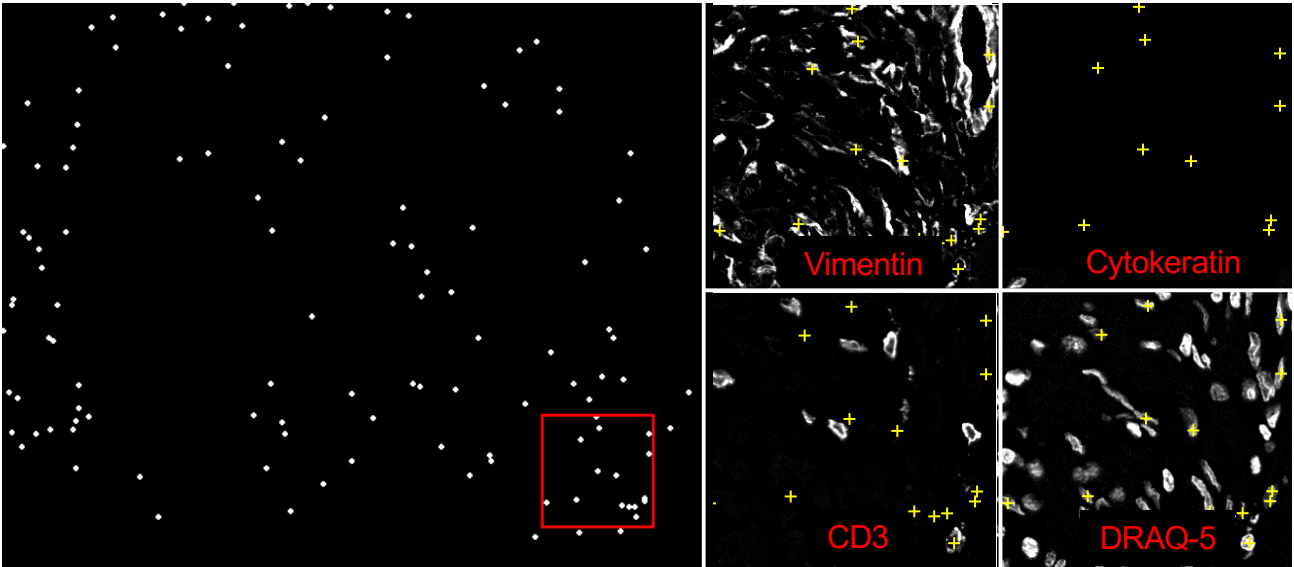
C. Smooth muscle cluster



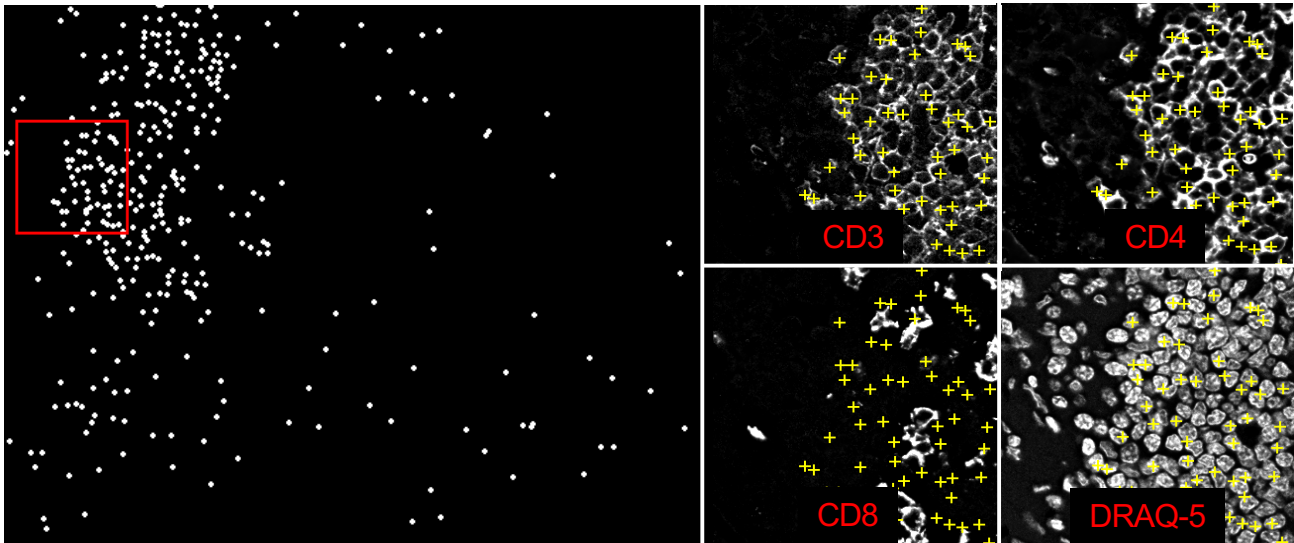
D. Granulocyte cluster



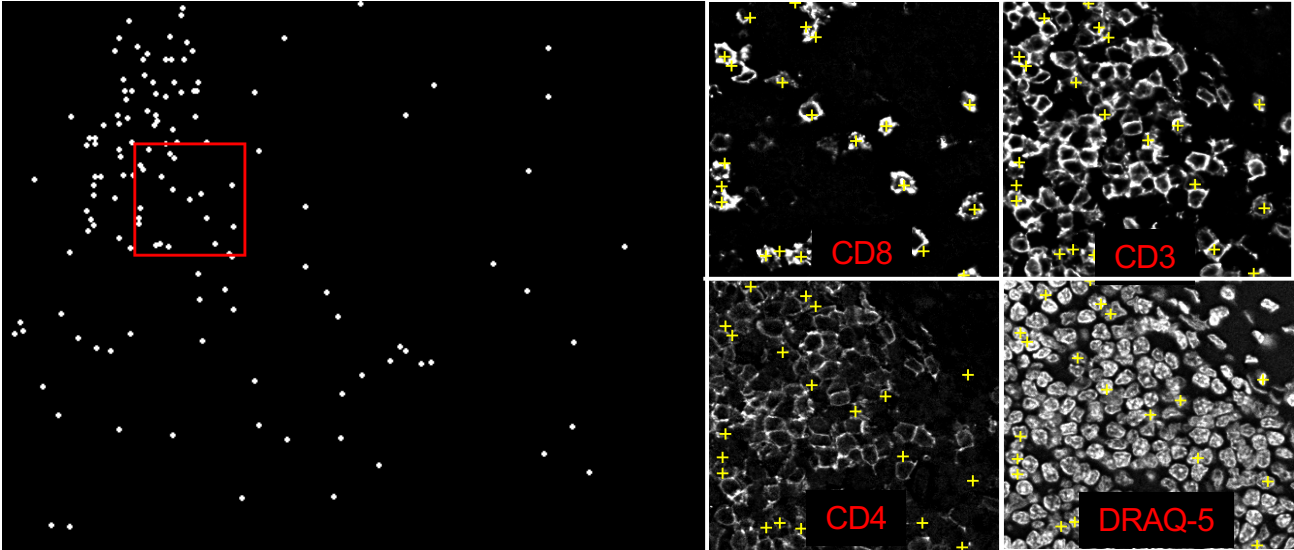
E. Stroma cluster



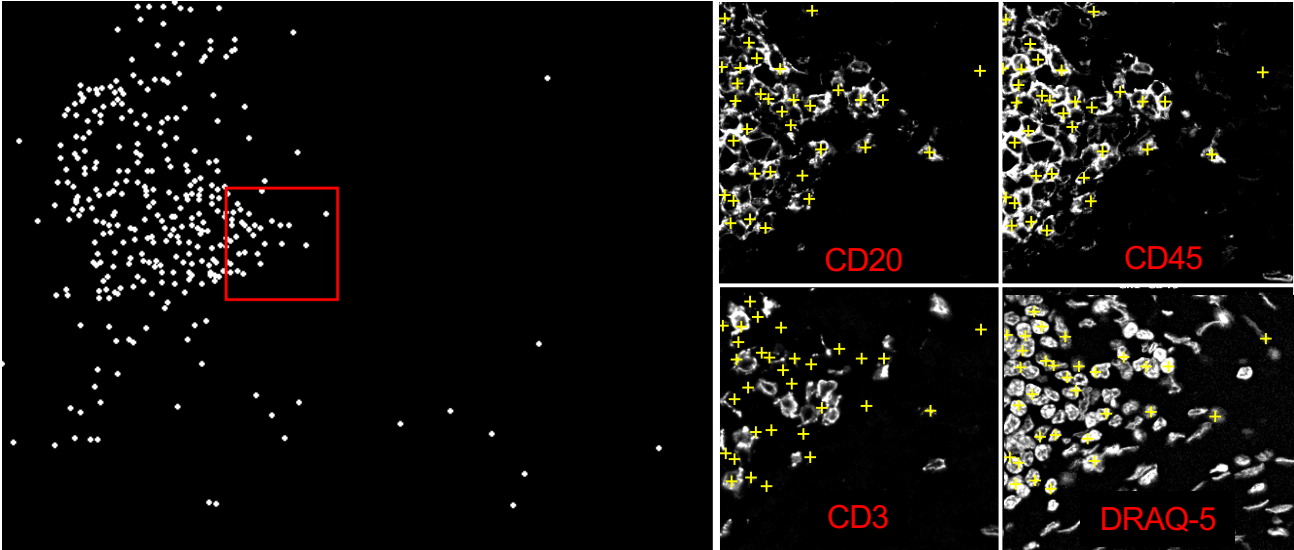
F. CD4+CD45RO+ T cell cluster



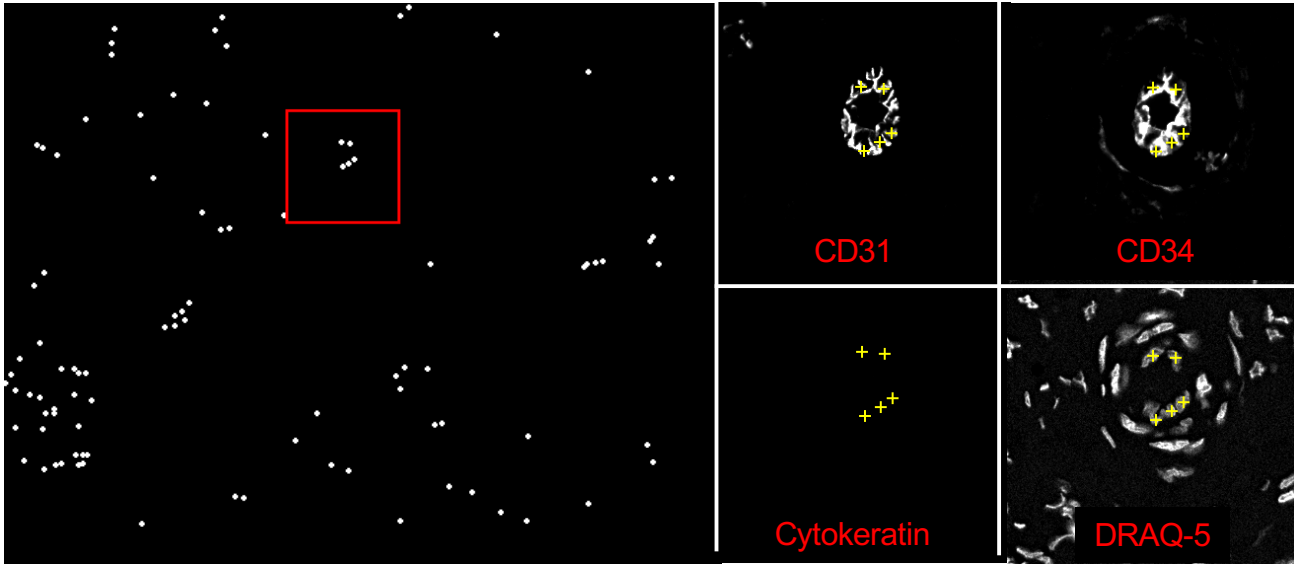
G. CD8+ T cell cluster



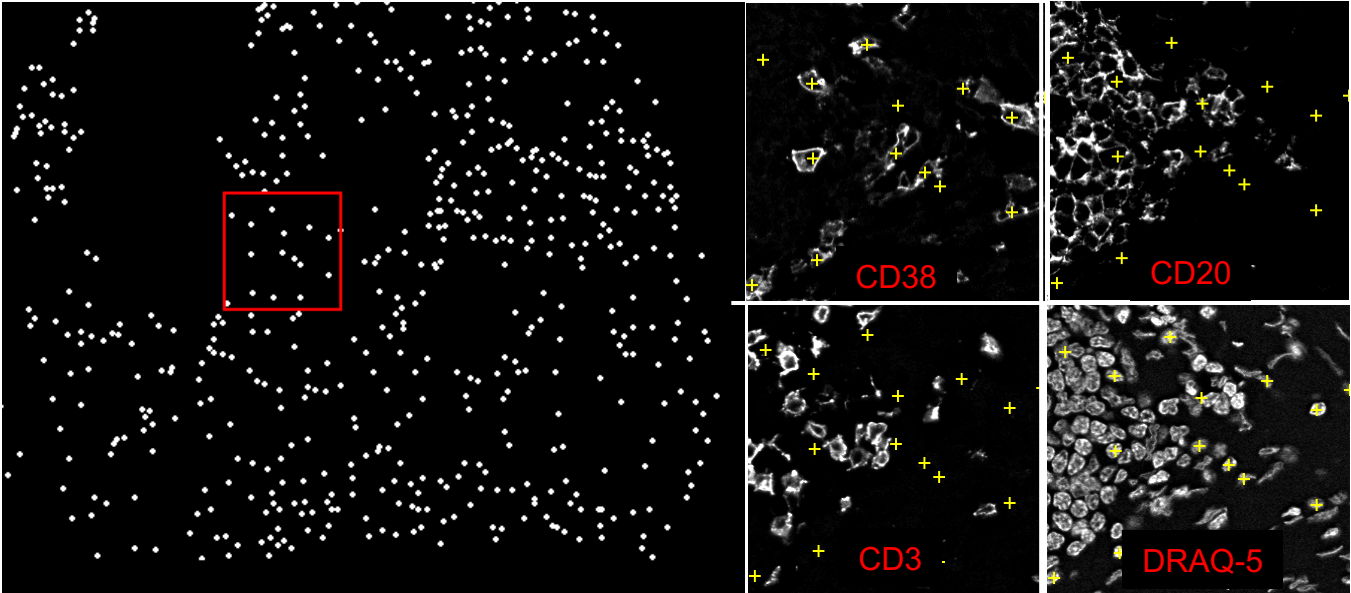
H. B cell cluster



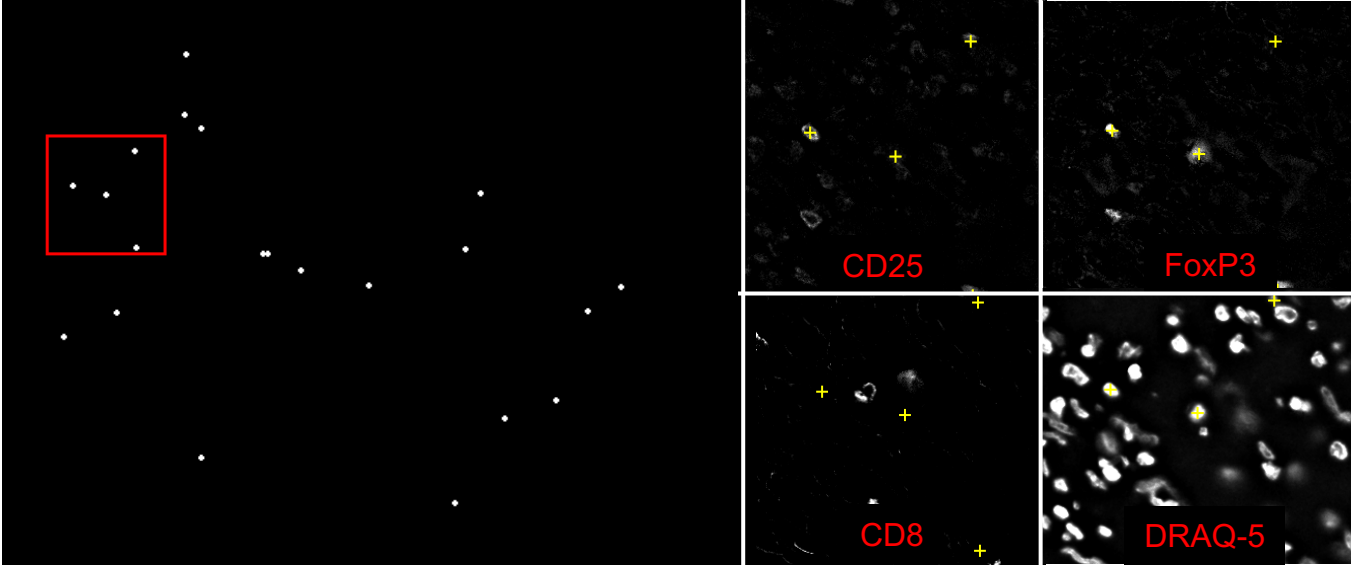
I. Vasculature cluster



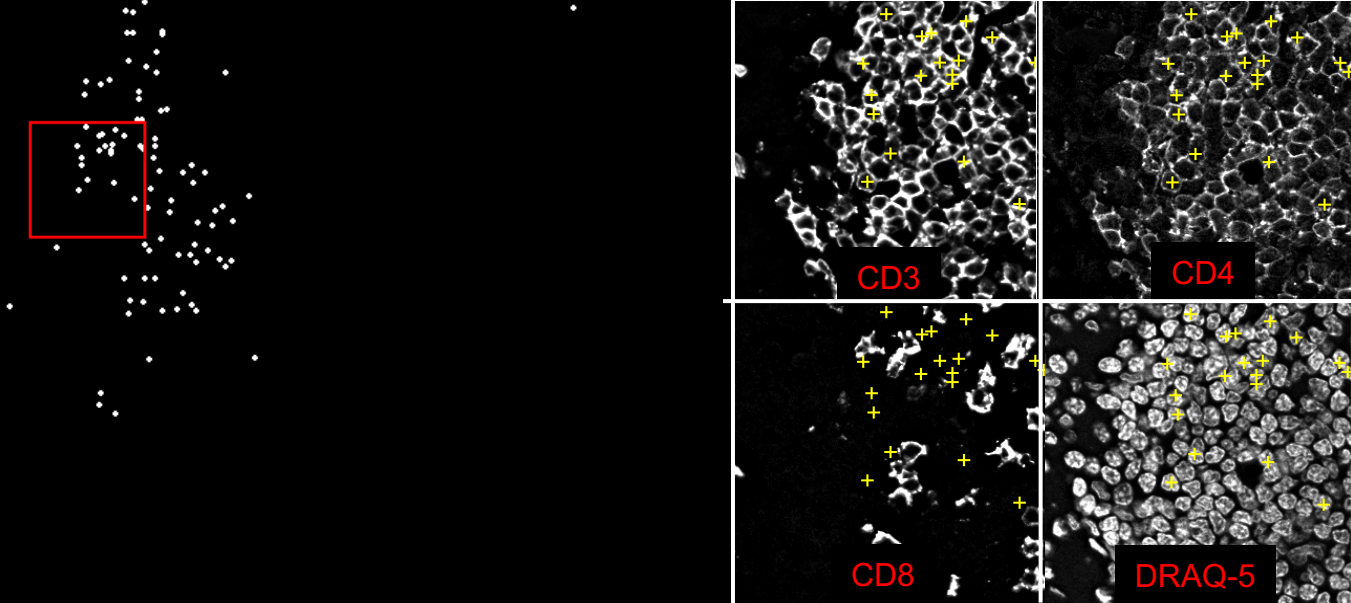
J. Plasma cell cluster



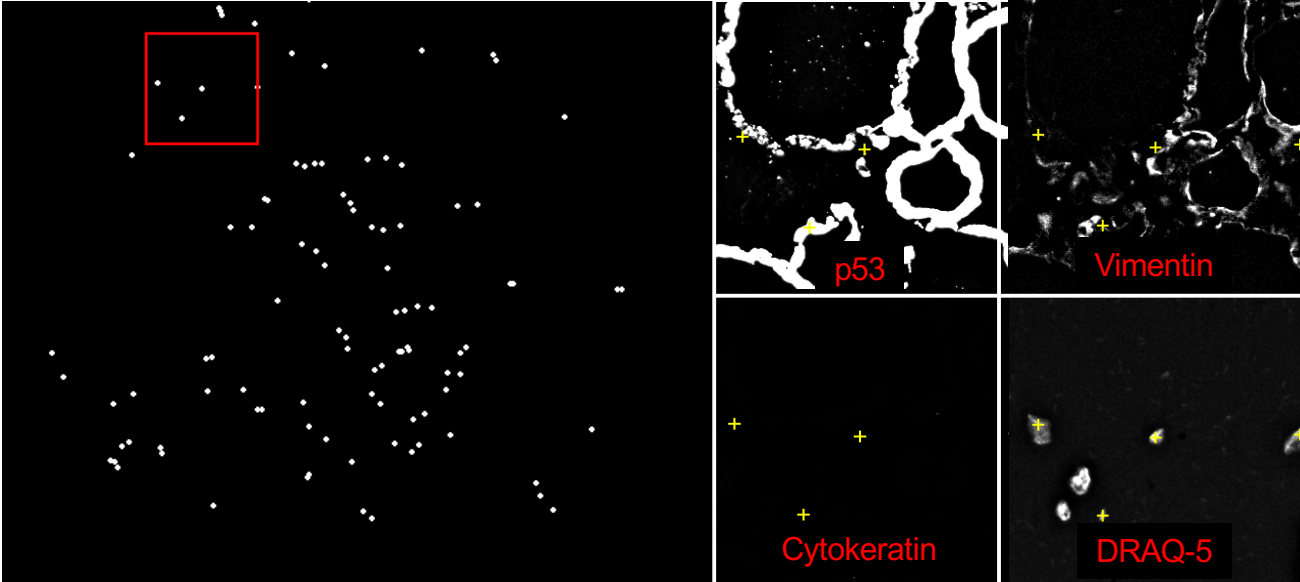
K. Treg cluster



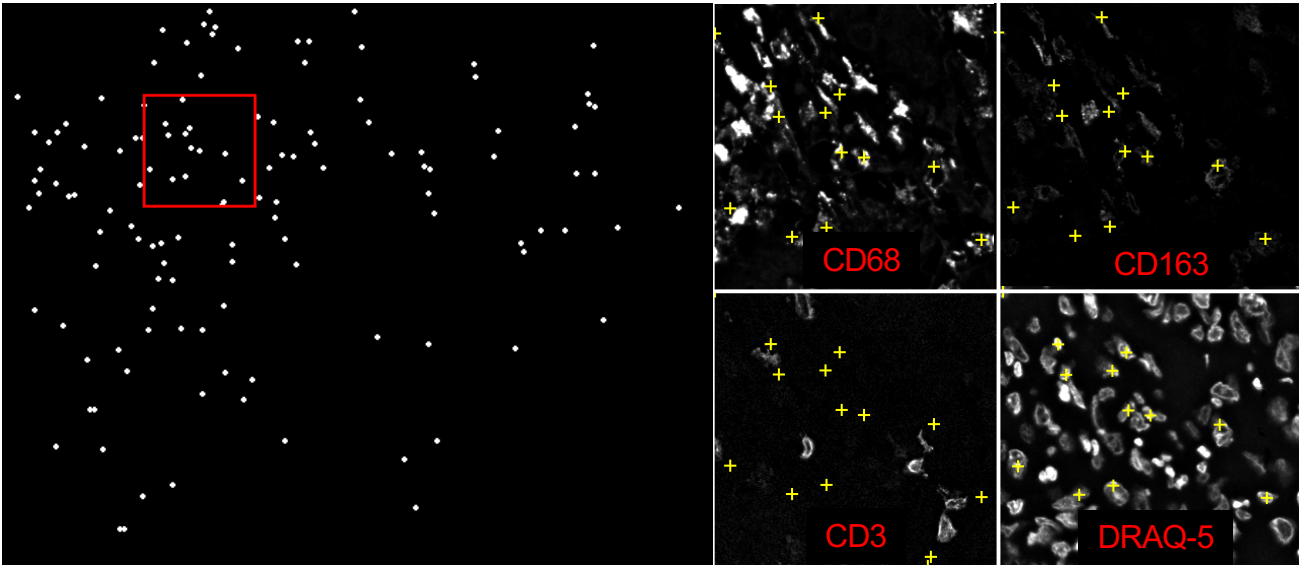
L. CD4+ T cell cluster



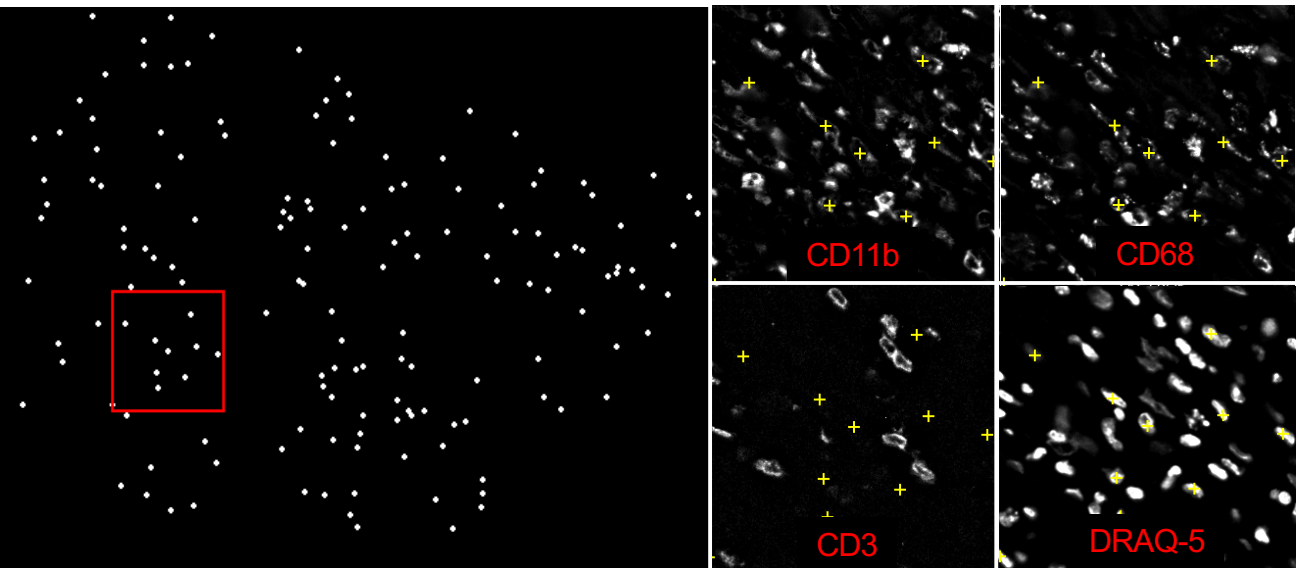
M. Adipocyte cluster



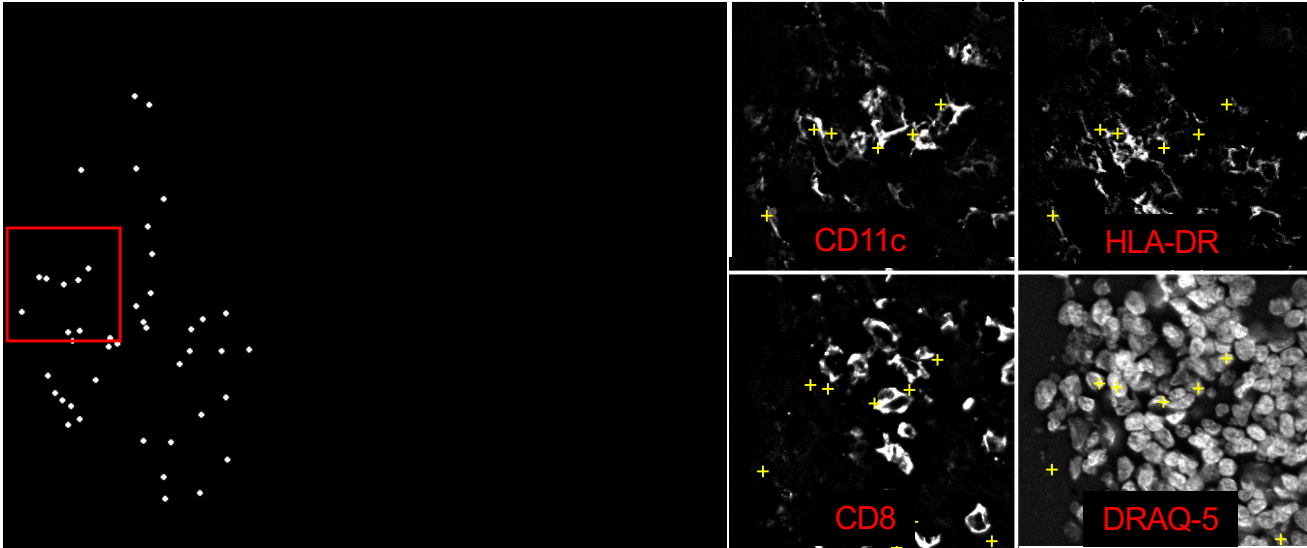
N. CD68+ macrophage cluster



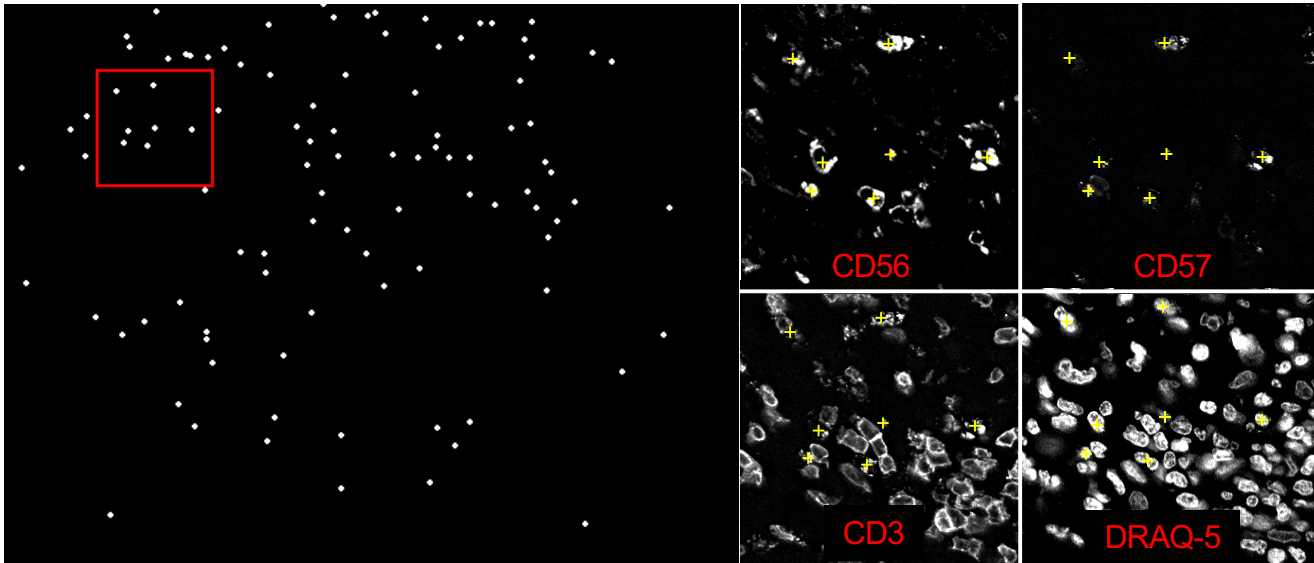
O. CD11b+CD68+ macrophage cluster



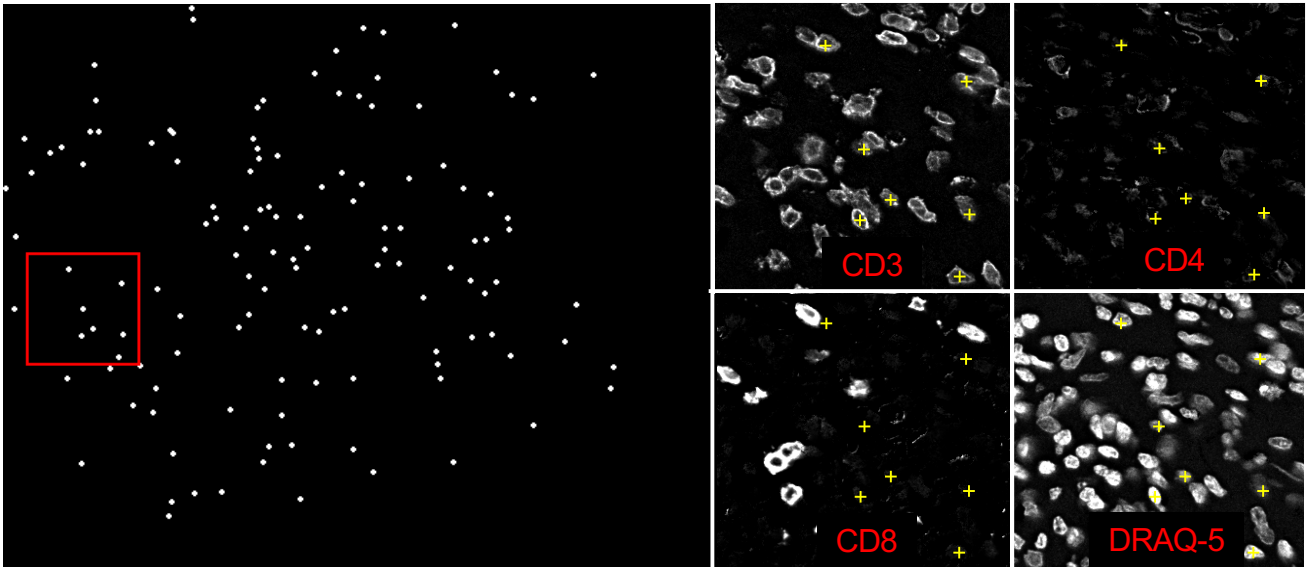
P. CD11c dendritic cell cluster



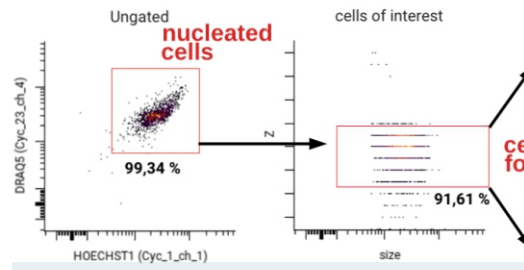
Q. NK cell cluster



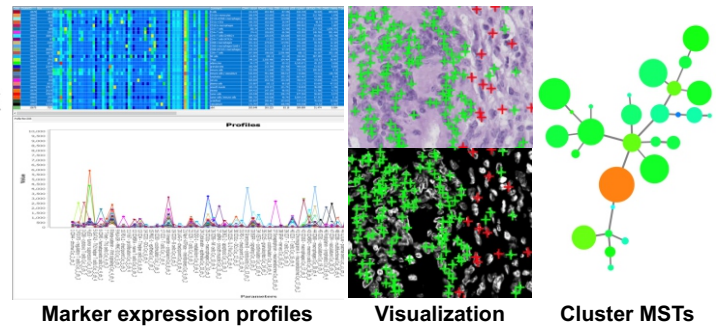
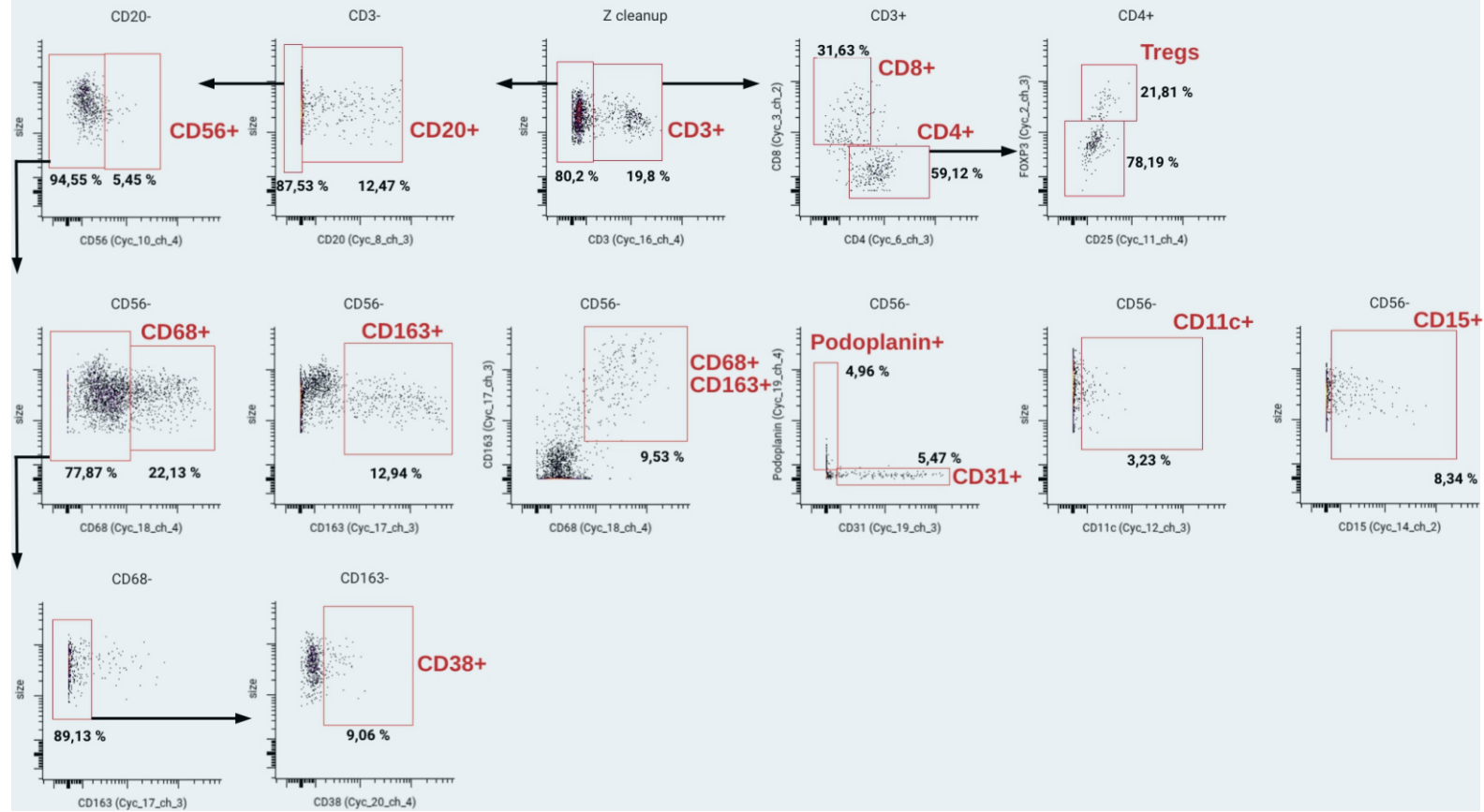
R. CD3+ T cell cluster

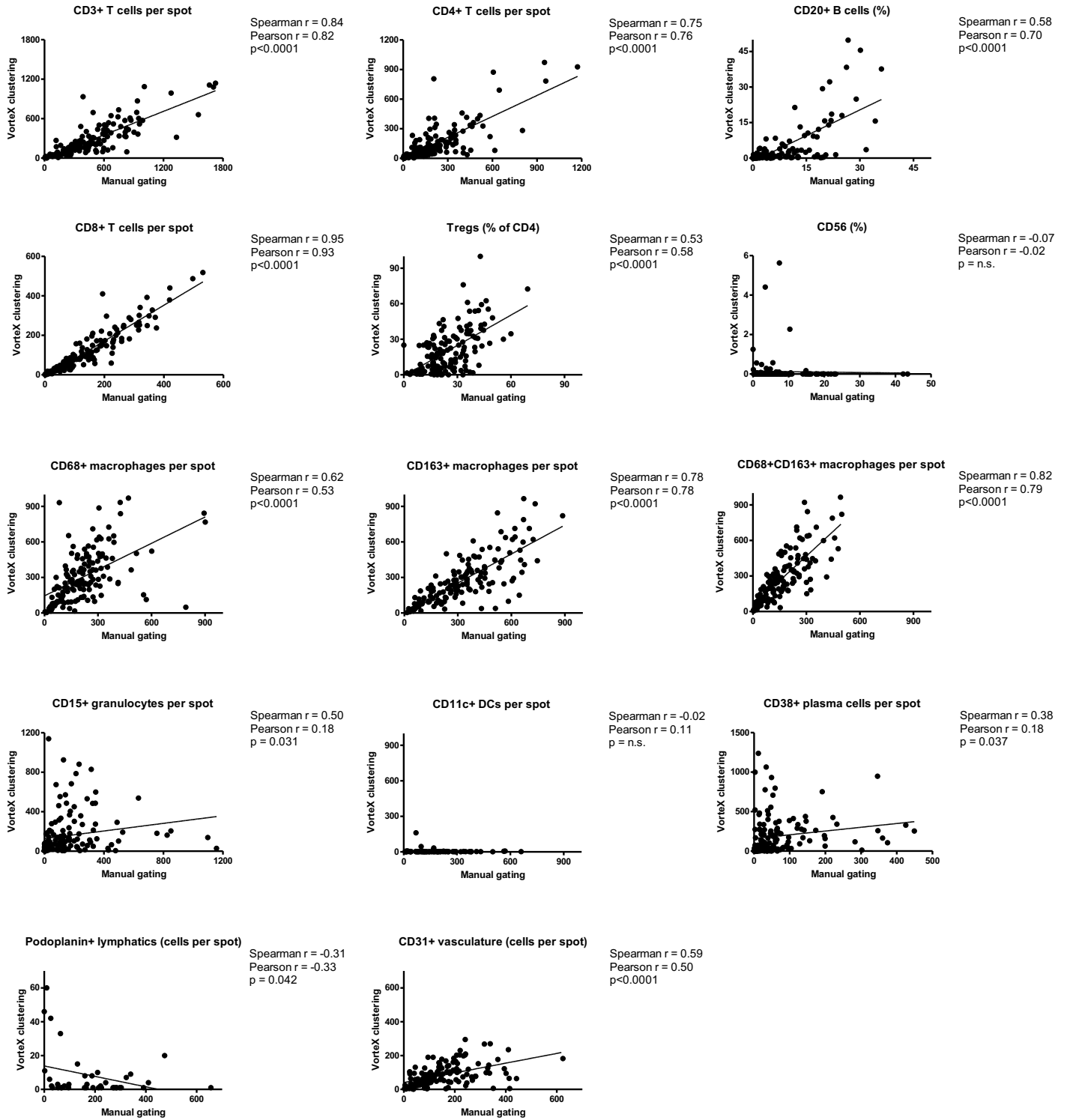


Data S5. Comparison of Unsupervised X-shift Clustering vs. Supervised Manual Gating of Cell Populations, Related to Figure 3 and STAR Methods. (A) Flow cytometry standard (FCS) files from segmented images were imported into CellEngine (www.cellengine.com). Gates were tailored individually for each file and cell population. (1) Cleanup gating: Nucleated cells were selected for by gating on cells positive for Hoechst (cycle 1) and DRAQ5 (cycle 23), and out-of-focus events were removed by gating on the focused Z planes. (2) FCS files were exported for X-shift unsupervised clustering in Vortex and (3) were further analyzed for major immune cell types in CellEngine. The gating strategy to identify T cells ($CD3^+$), cytotoxic T cells ($CD3^+CD8^+$), T helper cells ($CD3^+CD4^+$), Tregs ($CD3^+CD4^+CD25^+FOXP3^+$), B cells ($CD3^-CD20^+$), NK cells ($CD3^-CD20^-CD56^+$), $CD68^+$ macrophages ($CD3^-CD20^-CD56^-CD68^+$), $CD163^+$ macrophages ($CD3^-CD20^-CD56^-CD163^+$), $CD68^+CD163^+$ double-positive macrophages ($CD3^-CD20^-CD56^-CD68^+CD163^+$), lymphatics ($CD3^-CD20^-CD56^-Podoplanin^+$), vasculature ($CD3^-CD20^-CD56^-CD31^+$), dendritic cells ($CD3^-CD20^-CD56^-CD11c^+$), granulocytes ($CD3^-CD20^-CD56^-CD15^+$), and plasma cells ($CD3^-CD20^-CD56^-CD68^-CD163^-CD38^+$) is shown. (B) Identification and quantification of major immune cell populations in CODEX data is comparable between supervised manual gating and unsupervised X-shift clustering. Numbers of cells per TMA spot and relative cell frequencies for manual gating in CellEngine (left columns, blue background) vs. unsupervised clustering in Vortex (right columns, white background) of selected major cell subsets. (C) Correlation of major immune cell populations quantified by supervised manual gating and unsupervised X-shift clustering. Correlation diagrams based on individual TMA cores for each cell population.

A**1. Cleanup gating****2. Export FCS files for Vortex unsupervised clustering**

vs.

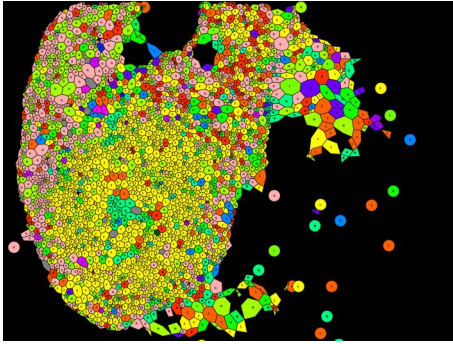
**3. Manual gating in CellEngine**

C

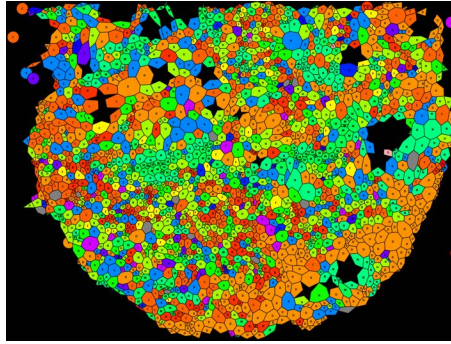
Data S6. Voronoi Diagrams and Distribution of the 28 CRC Clusters, and Distribution of the Eight Merged Immune Clusters, Related to Figure 3. (A) Five representative Voronoi diagrams of the 28 CRC clusters are shown for each patient group. (B) The frequencies of the 28 CRC clusters for all patients and for each group are listed. Significant differences are highlighted in bold (Mann-Whitney test). Data represent mean values from four biological replicates (TMA cores) per patient. (C) Distributions of the 28 clusters in all CRC patients and CLR and DII groups. (D) Distributions of the 28 clusters in each individual patient. (E) The frequencies of the eight merged immune clusters for all patients and for each group are listed. Significant differences are highlighted in bold (Mann-Whitney test). (F) Cell numbers (left panel) and distributions of the eight merged clusters (right panel) in each individual patient.

A**Group 1 (CLR)****Group 2 (diffuse)****Voronoi Map Legend**

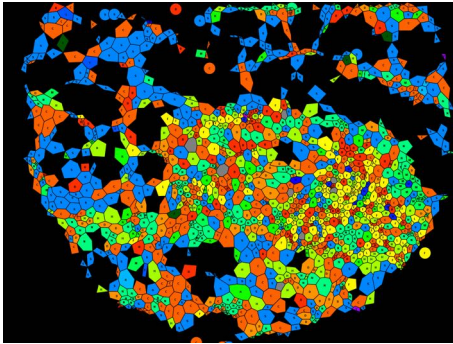
Patient 11



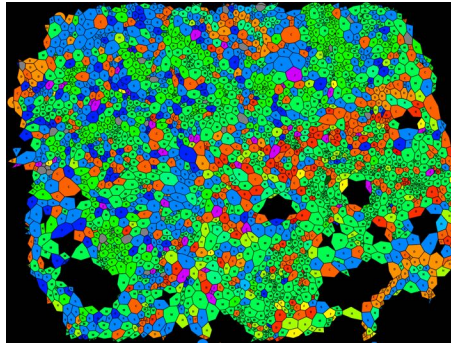
Patient 5



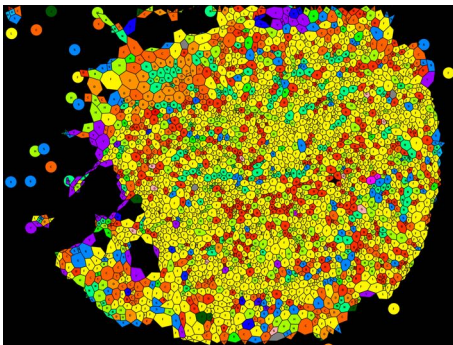
Patient 13



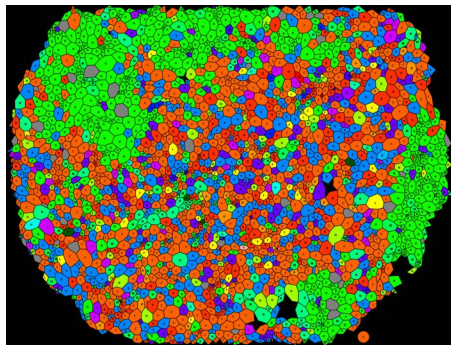
Patient 8



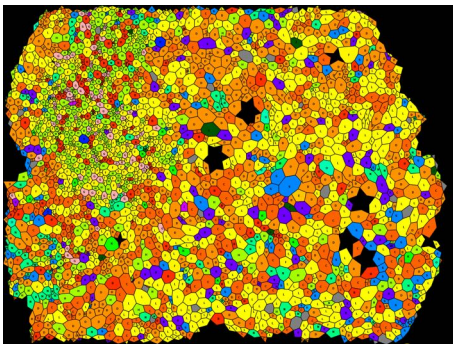
Patient 17



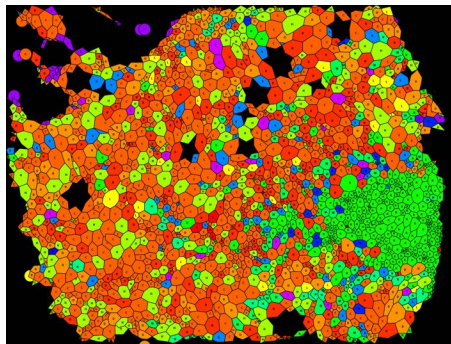
Patient 14



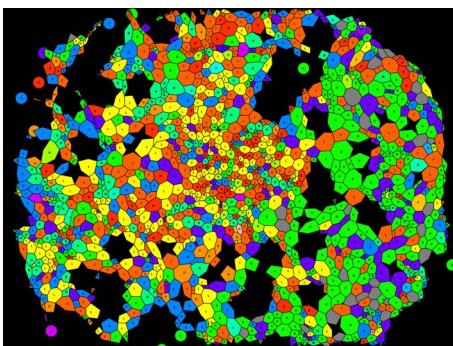
Patient 19



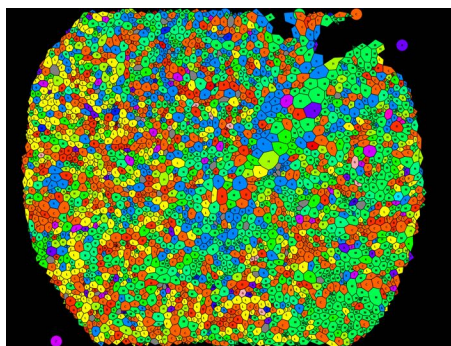
Patient 26



Patient 35



Patient 30

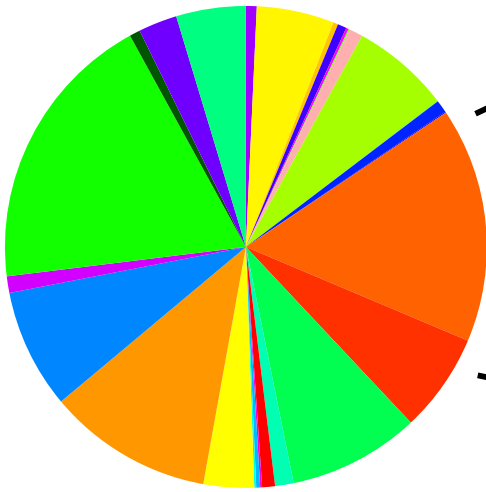


A	01-immune cells / vasculature
B	02-CD8+ T cells
C	03-CD68+CD163+ macrophages
D	04-smooth muscle
E	05-CD11b+ monocytes
F	06-B cells
G	07-CD4+ T cells
H	08-CD4+ T cells CD45RO+
I	09-CD3+ T cells
J	10-tumor cells / immune cells
K	11-tumor cells
L	12-plasma cells
M	13-granulocytes
N	14-vasculature
O	15-immune cells
P	16-NK cells
Q	17-CD4+ T cells GATA3+
R	18-nerves
S	19-stroma
T	20-CD163+ macrophages
U	21-CD68+ macrophages
V	22-CD68+ macrophages GzmB+
W	23-CD11b+CD68+ macrophages
X	24-undefined
Y	25-adipocytes
Z	26-Tregs
[27-CD11c+ DCs
\	28-lymphatics

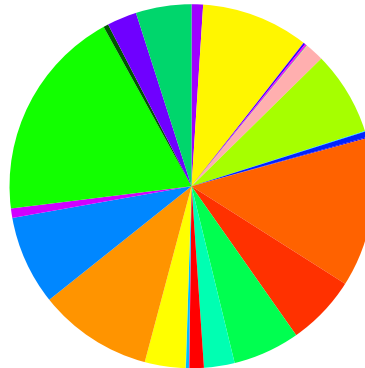
B	All CRC Patients (n=251,028 cells) Mean % ± SD	Group 1 (CLR) (n=107,966 cells) Mean % ± SD	Group 2 (Diffuse) (n=143,062 cells) Mean % ± SD	Mann-Whitney Test p-value
Tumor cells	19.3 ± 14.4%	19.3 ± 18.9%	19.3 ± 9.1%	0.3141
CD68+CD163+ macrophages	15.6 ± 7.1%	13.4 ± 6.4%	17.6 ± 7.2%	0.0831
Smooth muscle	10.2 ± 8.4%	9.5 ± 7.0%	10.9 ± 9.8%	0.9605
Granulocytes	8.5 ± 8.2%	5.8 ± 5.3%	11.0 ± 9.7%	0.0496
Stroma	8.0 ± 4.3%	7.9 ± 4.7%	8.0 ± 4.1%	0.9080
CD4+ T cells CD45RO+	7.0 ± 5.0%	7.5 ± 5.5%	6.6 ± 4.6%	0.7043
CD8+ T cells	7.0 ± 4.3%	6.0 ± 2.9%	7.9 ± 5.3%	0.6322
B cells	5.1 ± 7.5%	8.8 ± 9.4%	1.7 ± 2.3%	0.0009
Vasculature	4.5 ± 2.2%	4.8 ± 2.4%	4.2 ± 1.9%	0.4780
Plasma cells	3.3 ± 4.0%	3.8 ± 4.0%	2.9 ± 4.2%	0.2038
Undefined	2.9 ± 3.8%	2.9 ± 2.9%	3.0 ± 4.6%	0.2283
Immune cells (mixed)	1.5 ± 4.5%	3.0 ± 6.2%	0.1 ± 0.2%	0.0306
Tregs	1.1 ± 0.7%	0.9 ± 0.6%	1.4 ± 0.7%	0.0218
CD4+ T cells	1.0 ± 2.5%	1.8 ± 3.4%	0.3 ± 0.7%	0.0167
Immune cells / vasculature	1.0 ± 1.8%	1.5 ± 2.5%	0.5 ± 0.8%	0.1923
Adipocytes	0.8 ± 1.1%	1.2 ± 1.0%	0.5 ± 1.1%	0.0004
CD68+ macrophages	0.8 ± 1.0%	0.5 ± 0.5%	1.1 ± 1.3%	0.0333
Tumor cells / immune cells	0.6 ± 2.0%	0.5 ± 1.0%	0.8 ± 2.6%	0.0127
CD11b+CD68+ macrophages	0.5 ± 1.4%	0.2 ± 0.1%	0.9 ± 2.0%	0.0459
CD11b+ monocytes	0.3 ± 1.5%	0.0 ± 0.1%	0.6 ± 2.1%	0.4485
CD11c+ DCs	0.2 ± 0.4%	0.2 ± 0.2%	0.2 ± 0.5%	0.1326
Nerves	0.2 ± 0.3%	0.2 ± 0.3%	0.2 ± 0.3%	0.9605
NK cells	0.1 ± 0.4%	0.0 ± 0.1%	0.2 ± 0.5%	0.5323
Lymphatics	0.1 ± 0.3%	0.0 ± 0.1%	0.2 ± 0.4%	0.2452
CD3+ T cells	0.1 ± 0.2%	0.0 ± 0.1%	0.1 ± 0.3%	0.6111
CD68+ macrophages GzmB+	0.1 ± 0.1%	0.1 ± 0.1%	0.1 ± 0.1%	0.7965
CD4+ T cells GATA3+	0.0 ± 0.2%	0.1 ± 0.2%	0.0 ± 0.0%	0.3029
CD163+ macrophages	0.0 ± 0.0%	0.0 ± 0.1%	0.0 ± 0.0%	0.4905

C

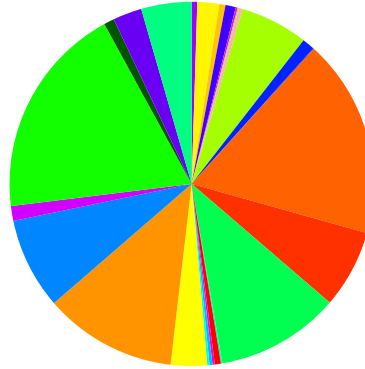
Distribution of 28 clusters in CRC (n=251,028)



Group 1 (CLR) (n=107,966)



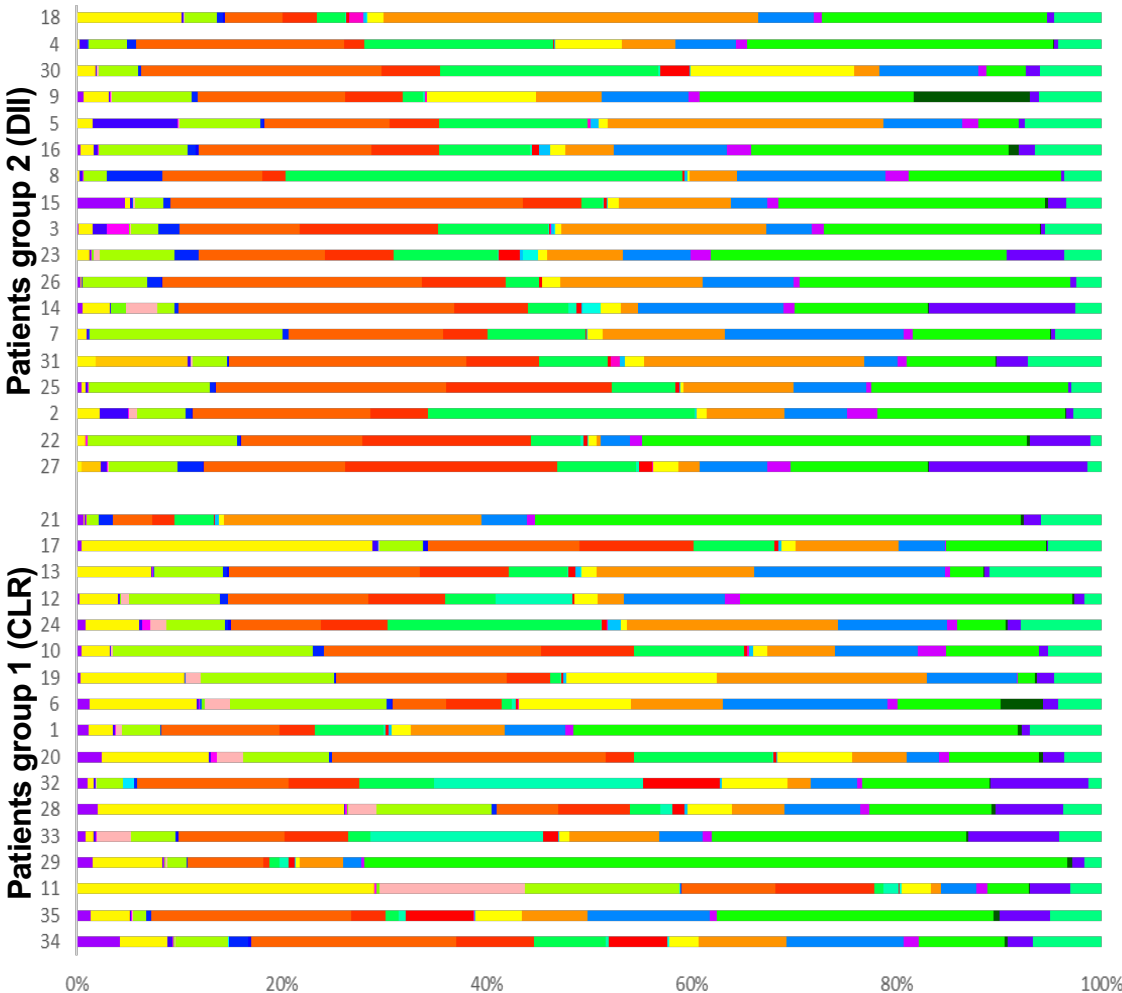
Group 2 (diffuse) (n=143,062)



- adipocytes
- B cells
- CD11b+ monocytes
- CD11b+CD68+ macrophages
- CD11c+ DCs
- CD163+ macrophages
- CD3+ T cells
- CD4+ T cells
- CD4+ T cells CD45RO+
- CD4+ T cells GATA3+
- CD68+ macrophages
- CD68+ macrophages GzmB+
- CD68+CD163+ macrophages
- CD8+ T cells
- granulocytes
- immune cells
- immune cells / vasculature
- lymphatics
- nerves
- NK cells
- plasma cells
- smooth muscle
- stroma
- Tregs
- tumor cells
- tumor cells / immune cells
- undefined
- vasculature

D

Distribution of 28 clusters in CRC, per patient

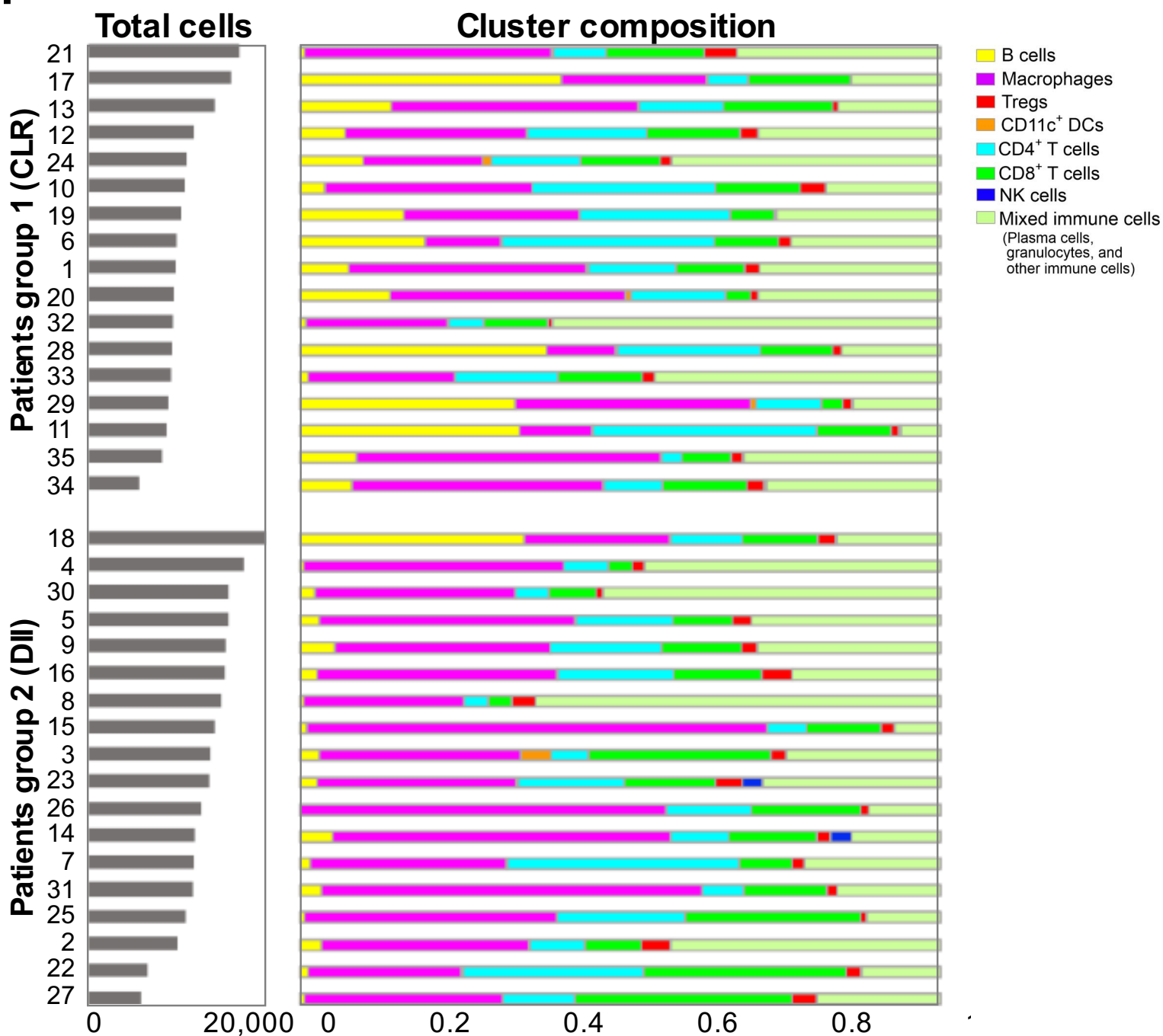


- adipocytes
- B cells
- CD11b+ monocytes
- CD11b+CD68+ macrophages
- CD11c+ DCs
- CD163+ macrophages
- CD3+ T cells
- CD4+ T cells
- CD4+ T cells CD45RO+
- CD4+ T cells GATA3+
- CD68+ macrophages
- CD68+ macrophages GzmB+
- CD68+CD163+ macrophages
- CD8+ T cells
- granulocytes
- immune cells
- immune cells / vasculature
- lymphatics
- nerves
- NK cells
- plasma cells
- smooth muscle
- stroma
- Tregs
- tumor cells
- tumor cells / immune cells
- undefined
- vasculature

M

	All CRC Patients (n=131,329 immune cells) Mean % ± SD	Group 1 (CLR) (n=56,343 immune cells) Mean % ± SD	Group 2 (Diffuse) (n=74,986 immune cells) Mean % ± SD	Mann-Whitney Test p-value
B cells	9.5 ± 12.2%	15.3 ± 13.5%	4.0 ± 7.9%	0.0008
Macrophages	33.5 ± 13.2%	28.4 ± 11.2%	38.4 ± 13.5%	0.0496
Tregs	2.2 ± 1.3%	1.8 ± 1.3%	2.6 ± 1.2%	0.0535
CD11c+ DCs	0.4 ± 0.8%	0.4 ± 0.4%	0.4 ± 1.1%	0.0512
CD4+ T cells	14.7 ± 9.0%	16.2 ± 9.5%	13.3 ± 8.6%	0.3641
CD8+ T cells	13.1 ± 7.3%	11.2 ± 3.9%	15.0 ± 9.2%	0.4187
NK cells	0.4 ± 1.0%	0.1 ± 0.1%	0.8 ± 1.4%	0.7197
Other immune cells	26.3 ± 14.4%	26.7 ± 13.3%	25.9 ± 15.7%	0.6322

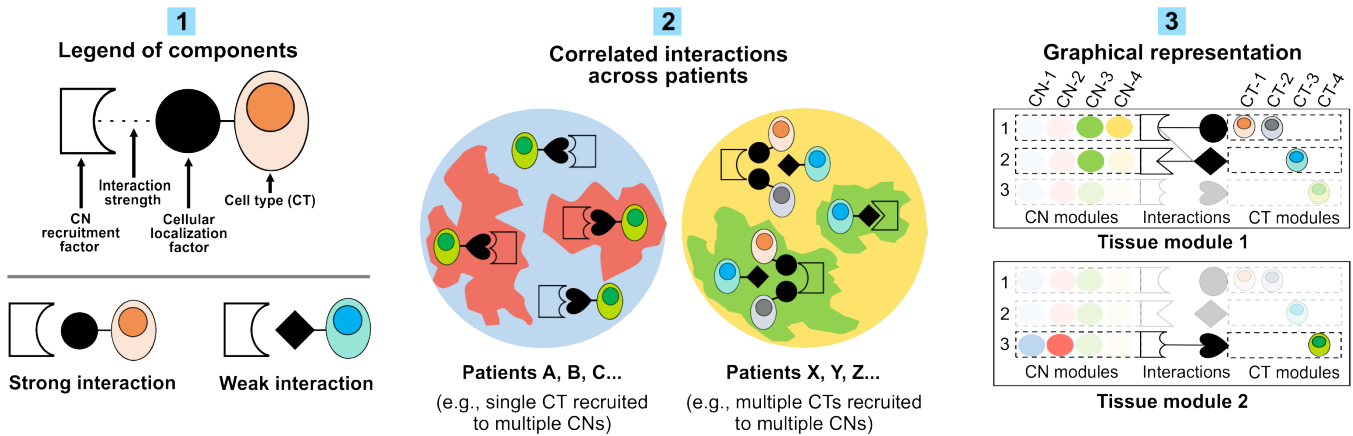
N



Data S7. Tensor Decomposition, Related to Figure 5.

We motivate our use of tensor methods for describing differences in the variation across patients' joint CN-CT compositions by discussing the limitations of traditional PCA for this purpose. One possibility for describing the differences, between patient groups, in variation across patients' joint CN-CT compositions, would have been to first perform PCA (by flattening each patient's 2D matrix to a 1D vector), and subsequently describe how the identified axes were different. However, this would have eliminated the information that CNs and CTs form two distinct but coupled views of the iTME. This coupling corresponds exactly to the fact that the underlying biological programs drive multiple distinct CTs to be found together in multiple distinct CNs. For example, multiple CTs might share combinations of cytokine receptors, and cytokine gradients might promote combinations of CNs.

An example which illustrates how underlying biology could give rise to the tensor decomposition output is depicted as a schematic in the **Figure** below: (1) The tissue is formed by the interaction of CN 'recruitment factors' (for example, cytokines) shared by multiple CNs to recruit cell types by interacting with cognate 'cellular localization factors' (for example, cytokine receptors) shared by multiple cell types (**Panel 1**, top aspect). The term factor should be viewed in a statistical sense and could represent more complicated programs than a single ligand or receptor. Different factors can interact to different extents (**Panel 1**, lower aspect). (2) Different interacting pairs of recruitment and localization factors are found together in the tissue, giving rise to the observed distribution of CNs and cell types (**Panel 2**). In the left region, the blue and red CNs share a recruitment factor (heart-shaped indentation), so share a common cell type (green) with a cognate localization factor (heart). In the right region, the orange and the gray cells share a localization factor (circle), so are found in multiple CNs. The green CN uses multiple recruitment factors, one shared with the yellow CN. Distinct interacting pairs of recruitment and localization factors co-occur across patients (red and blue found together, and yellow and green found together), each co-occurring collection of interacting pairs corresponding to a tissue module. These recruitment and localization factors are inferred from the tensor decomposition output, visualized as tissue modules comprised of CN modules and cell type (CT) modules, with interactions between them represented as edges (**Panel 3**). Note that there is a common collection of CT modules and CN modules that are present to different extents in each tissue module. The contribution of each CN module and CT module to each tissue module is represented by its shading (**Panel 3**). In tissue module 1 (top box), the CN module in the first row is interpreted as the recruitment factor with a circular indentation. This is because it contains yellow and green CNs, and there is a strong edge with the CT module containing the orange and grey cell types, and a weak edge with the CT module containing the blue cell type. The CN module with just the green CN (row 2) is interpreted as the recruitment factor with the square indentation. This is because that CN module does not contain any other CNs and has only one edge with one CT module containing the blue cell type. Since the red and green CNs are not found in the same patients, the CN module with the red and blue CNs and its cognate CT module with just the green cell type are faint in tissue module 1 and form tissue module 2. Note that the CN modules and the cell type modules are identified by their mutual dependence.



Schematic illustrating the interpretation of the tensor decomposition output. **(1)** Legend of components: A CN module corresponds to a cell recruitment program utilized by the CNs comprising that module, and a CT module corresponds to a cell type localization program utilized by the cell types comprising that module. Different pairs of recruitment programs and localization programs interact to different strengths. **(2)** Different pairs of interacting recruitment programs and localization programs co-occur to form the tissue through balanced interactions between recruitment and localization factors. These combinations yield similar combinations of CNs and cell types within them across patients. **(3)** Graphical representation of tissue modules corresponding to combinations of interacting pairs, indicated by edges, of CN modules (left column) and CT modules (right column). CN modules and CT modules are common across both tissue modules. In each tissue module, the transparency of each CN module and CT module corresponds to the weight of the maximum edge of which it is part, i.e. indicating its contribution to that tissue module.