SUPPLEMENTAL INFORMATION

Coordinated cellular neighborhoods orchestrate antitumoral immunity at the colorectal cancer invasive front

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Screening and validation of CODEX antibodies. 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. Scale bars, 100 μm.

Figure S2. Validation and titration of CODEX antibody panel. FFPE tonsil tissue was stained with a cocktail of 55 different DNA-conjugated antibodies (**Table S4**), and a multi-cycle experiment was performed followed by H&E staining. Images of identical tissue regions at the interface of a follicle (top left in each image) and epithelium (bottom right in each image) are depicted in false gray color for each antibody; H&E staining is also shown.

Figure S3. CODEX antibody validation. An FFPE tonsil section was stained with a 55-marker CODEX panel (**Table S4**), and a multi-cycle reaction was performed. Top left panel: Overview in five-color overlay image with Hoechst (blue; nuclei), CD31 (yellow; vasculature), CD3 (red; T cells), CD20 (green; B cells), and pan-cytokeratin (CK, white; epithelium). Inset: H&E staining. Regions 1-4 are indicated by white rectangles. Region 1: Six-color overlay and single-marker images of a follicle with CD57 (red), ICOS (also known as CD278, green), PD-1 (also known as CD279, blue), VISTA (cyan), LAG-3 (also known as CD223, white), and Ki-67 (magenta). Region 2: Six-color overlay and single-marker images of an inflamed

epithelial-lymphoid parenchyma interface with CD15 (blue), CD68 (red), CD163 (cyan), CD56 (white), PD-L1 (also known as CD274, green), and EGFR (magenta). Region 3: Six-color overlay and single-marker images of a follicle with CD4 (red), CD8 (green), CD25 (yellow), CD45RA (blue), CD45RO (cyan), and FOXP3 (magenta). A $CD4^{+}CD25^{hi}FOXP3^{+}CD45RO^{+}$ regulatory T cell is indicated by the white arrow. Region 4: Six-color and two-color overlay images of an epithelial region with Pdpn (green), CD34 (yellow), EMA (also known as MUC-1, white), CD45 (blue), vimentin (cyan), and SMA (magenta).

Figure S4. Multi-tumor tissue microarray (TMA). Representative H&E-stained section. For details of tissues and tumor types, see **Table S5**. Scale bar, 1 mm.

Figure S5. Intact tissue and DNA-antibody-antigen complexes during CODEX multicycle experiment. (A) FFPE tonsil tissue was stained with a cocktail of nine different DNA-conjugated antibodies. Antibodies were repeatedly rendered visible using complementary fluorescent oligonucleotides in 33 cycles with blank cycles to measure autofluorescence (no fluorescent oligonucleotides added) at the beginning, between each rendering cycle, and at the end. The microscope light exposure times were kept constant for each antibody in each cycle. Hoechst nuclear stain was used as a reference. **(B)** Example images of nuclear marker Ki-67-Alexa488 and membrane markers CD20-ATTO550 and CD3-Alexa647 in cycles 4, 10, and 20 are shown. Images are representative of cycles and nuclear and membrane markers that are not shown. **(C)** Comparison of fluorescence intensity profiles from cycles 4 and 20, as measured by ImageJ software on the yellow lines in panel B.

Figure S6. Marker signal strength, autofluorescence, and tissue integrity during CODEX multicycle experiment. Data are from the FFPE tonsil tissue stained with nine DNA-conjugated antibodies as described in **Figure S5**. Cells were segmented using the CODEX toolkit and clustered using X-shift (VorteX). **(A)** Mean marker expression for CD45 (ATTO550), CD20 (ATTO550), and CD3 (Alexa647) on lymphocytes (combined $CD20^{+}$ cells and $CD3^{+}$ cells). **(B)** Mean marker expression for Na-K-ATPase (Alexa488) and pan-cytokeratin (ATTO550) on epithelial cells (pan-$cytokeratin^{+}$ cells). **(C)** Mean marker expression for Ki-67 (Alexa488) and CD45 (ATTO550) on proliferating cells ($Ki\text{-}67^{+}$ cells). **(D)** Mean marker expression for HLA-DR (Alexa488) and CD45 (ATTO550) on antigen-presenting cells ($HLA\text{-}DR^{+}$ cells). For lymphocytes, epithelial cells, and proliferating cells, 1500 cells were sampled; for antigen presenting cells, >250 cells were sampled. **(E)** Mean autofluorescence levels on all cells combined measured in each channel in blank cycles (no fluorescent DNA probes added). **(F)** Mean expression of the nuclear marker Hoechst per cell in cycle 20 vs. cycle 1. **(G)** Representative image of H&E staining performed after cycle 33.

Figure S7. Colorectal cancer (CRC) TMAs. Representative H&E-stained section for each CRC TMA. Cores are arranged according to patient number (1-35), with two cores per patient per TMA (4 cores per patient in total). **(A)**, TMA A and **(B)**, TMA B. See Table S1 for detailed patients' characteristics. Scale bar, 1 mm.

Figure S8. CRC CODEX antibody panel. Each marker of the CRC CODEX panel (**Table S4**) is depicted individually for one representative TMA spot (spot 36 of TMA 1; patient 18). CD30 and MMP12 were not detectable in any spots in either TMA and were therefore omitted. H&E and Hoechst nuclear stainings are shown for morphological reference.

Figure S9. Comparison of unsupervised X-shift clustering vs. supervised manual gating of cell populations. 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.

Figure S10. Supervised annotation of CRC clusters. After X-shift clustering, single cells from the 143 resulting 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 clusters. For each cluster, yellow crosses based on X/Y coordinates of the cells contained in that cluster were overlaid on stitched montages of all TMA cores. For each cluster, 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).

Figure S11. Minimal spanning trees (MSTs) and mean maker expression of 28 CRC clusters from unsupervised X-shift clustering. MSTs show the relationship between the clusters (edges and distances), their sizes, and their mean marker expression. MSTs were generated in VorteX for each marker analyzed.

Figure S12. Voronoi diagrams and distribution of 28 CRC clusters. (A) Five representative Voronoi diagrams are shown for each patient group. **(B)** The frequencies of clusters for all CRC patients and for each group. Significant differences are

highlighted in bold (Mann-Whitney test). Data represent mean values from four biological replicates (TMA cores) per patient.

Figure S13. 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.

Figure S14. 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. Data from **Figure S13**.

Figure S15. Percentages and distributions of eight merged clusters. (A) Distributions of eight merged clusters in all CRC patients and CLR and DII groups. **(B)** Cell numbers (left panel) and distributions of the eight merged clusters (right panel) in each individual patient.

Figure S16. Pairwise cell -cell contacts and CNs. (A) PCA correlating combinations of cell-type abundances in CLR vs. DII patients. Cell-type loading in principal component 1 is shown. **(B)** Heatmap of likelihood ratios of direct cell-to-cell interactions for 14 selected clusters is shown for clusters with at least 100 unique interacting cells. Gray boxes indicate less than 100 unique interacting cells; these data were omitted. Pooled data from all TMA cores are shown. **(C)** Frequencies of each CN in each patient are shown. Frequencies are z scored by column to highlight major differences between CLR patients (blue) and DII patients (orange). **(D)** The contacts between CN 1 (T cell-enriched) and CN 4 (macrophage-enriched) were computed (see Methods) and are displayed as "CN mixing" by patient group. For each patient, the mean mixing score of four TMA cores is shown (*$p<0.05$, Student's *t*-test).

Figure S17. Neighborhoods and corresponding H&E stainings. H&E-stained images **(A, C)** and the 9 identified CNs **(B, D)** are shown for all cores of TMA A (A-B) and TMA B (C-D).

Figure S18. Neighborhood analysis independently identifies comparable cluster sets in each patient group. Both CLR and DII patient groups were clustered separately. CNs were annotated manually. CN-0 corresponds to the imaging artifacts cluster; this cluster was omitted from the analysis shown in **Figure 4C**. Neighborhoods that did not have matching counterparts in the analysis of the combined groups are labeled "not defined".

Figure S19. Elbow points for Tucker tensor decomposition. Tensor decomposition loss for choices of rank in patient space, CN space, and cell-type space used for selection of decomposition rank in each patient group. Blue lines, one tissue module; red lines, two tissue modules. The elbow point was found at CN-6 and cell-type modules (red line).

Figure S20. Gating strategy for analysis of checkpoint molecule expression on T cells and macrophage populations. **(A)** Representative dot plots from CellEngine are shown for each marker and population. **(B)** Heatmap of marker-positive cell populations per patient. **(C)** Frequencies of marker-positive cell populations per patient. Data are mean values from four biological replicates (TMA cores) per patient (*p<0.05, **p<0.01, Student's *t*-test).

Figure S21. Heatmap of estimated differential enrichment coefficients for cell types not shown in Figure 6. Asterisks indicate CNs and cell types with a regression p-value <0.05 (not adjusted for multiple tests). A positive coefficient (red) indicates that the corresponding cell type is more enriched in DII patients than in CLR patients in the given CN.

Figure S22. Feature importance for classification model. Bar plot of absolute coefficient z-scores for CN-specific cell type frequencies, estimated from a model classifying patient groups using iterative resampling, as described in **Figure 6F**. The five CN-specific cell type frequencies with a coefficient importance of 0.3 or higher (left to red dotted line) were considered for assessment with respect to survival in DII patients.

SUPPLEMENTAL TABLES

Table S1: CRC patient characteristics and cell types per patient (see separate .xlsx file online)
Table S2: Antibodies, clones, manufacturers and corresponding CODEX oligonucleotides
Table S3: Sequences of CODEX oligonucleotides
Table S4: CODEX multi-cycle panels
Table S5: Tissue composition of the multi-tumor TMA
Table S6: Key resources

SCRIPTS

Folder containing 12 scripts for computational CODEX data analysis (see separate .zip file online)

Figure S1a

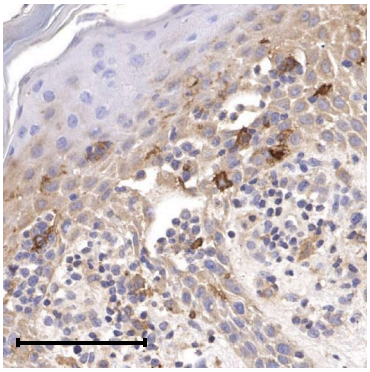
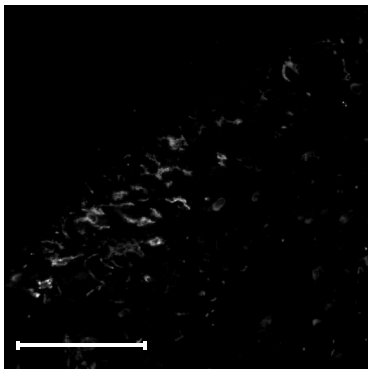
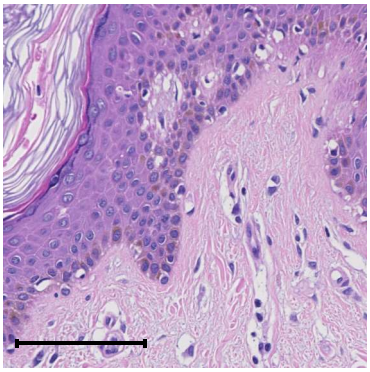
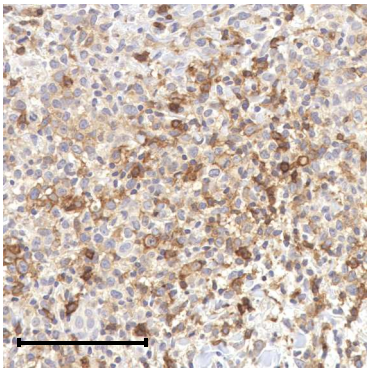
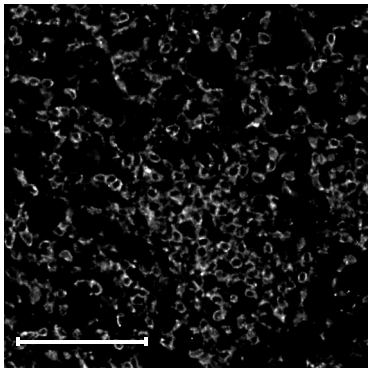
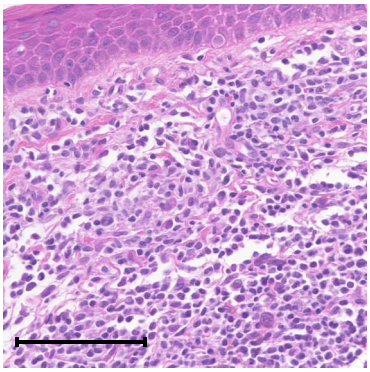
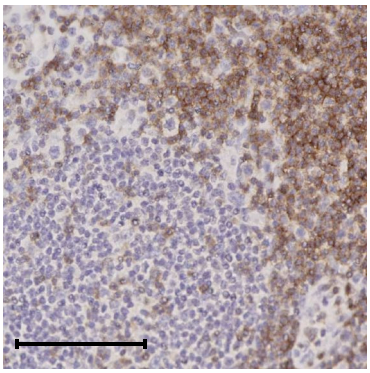
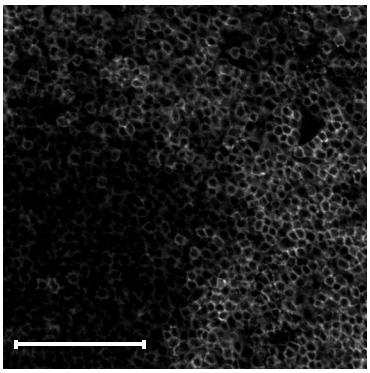
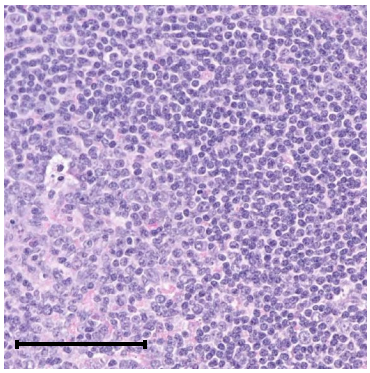
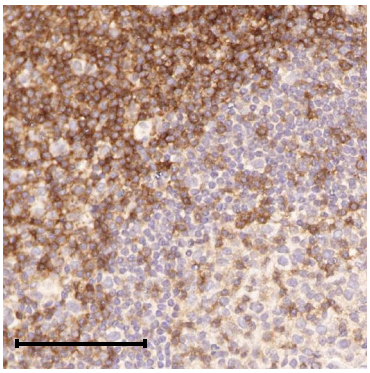
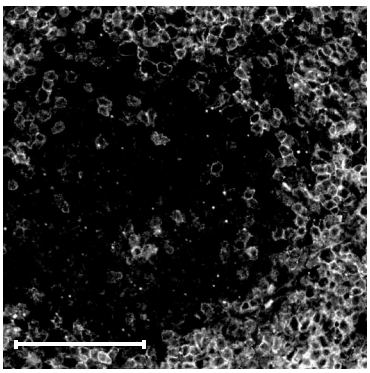
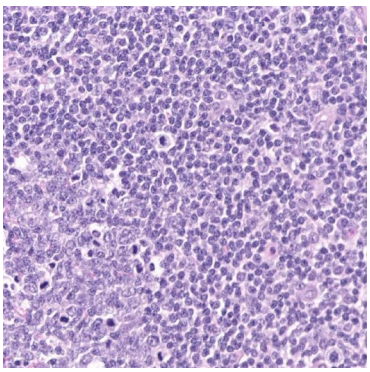
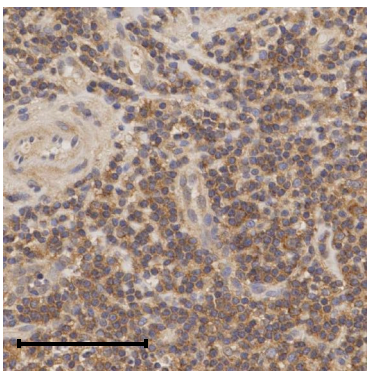
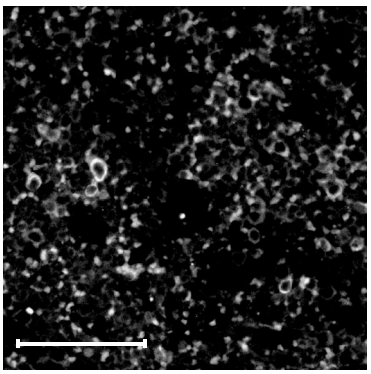
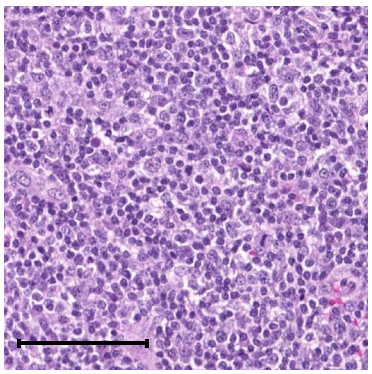
Staining specifications	IHC	CODEX	H&E
Antigen: CD1a **Clones: O10 + C1A/711** Company: Novus Biologicals (NBP2-34698) Tissue: Skin Dilution: IHC: 1:600 CODEX: 1:100 CODEX oligo: 43-Alexa647			
Antigen: CD2 **Clone: RPA-2.10** Company: BioLegend (300202) Tissue: Skin T cell lymphoma Dilution: IHC: 1:5 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			

Figure S1b

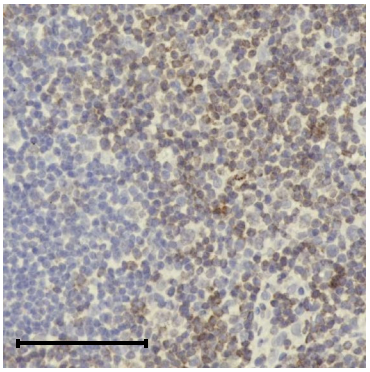
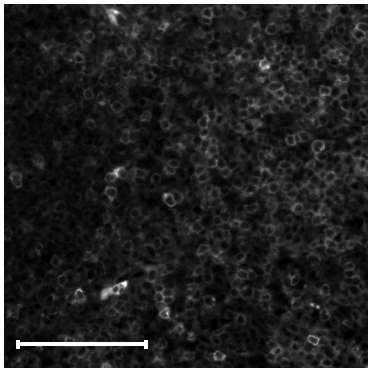
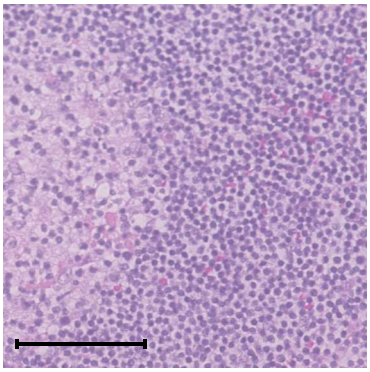
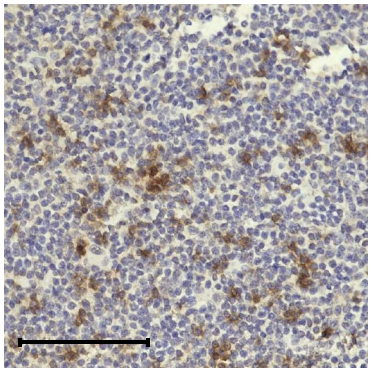
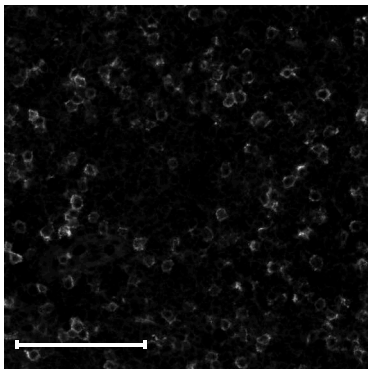
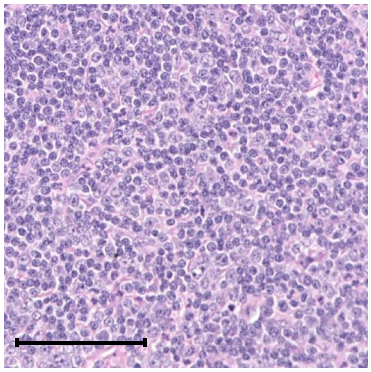
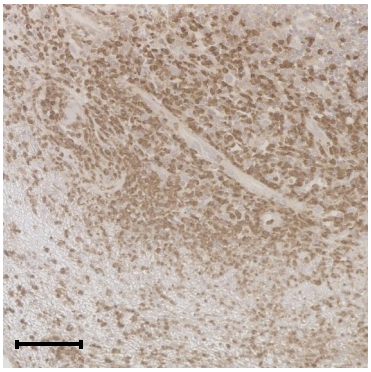
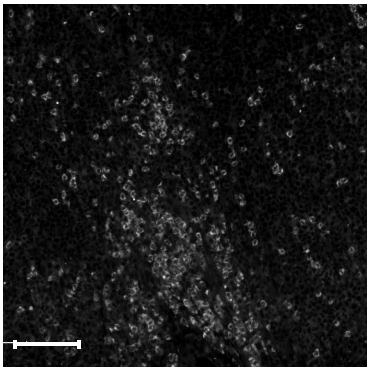
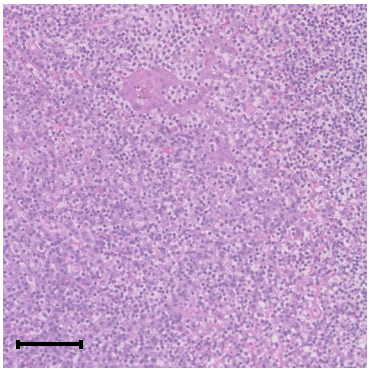
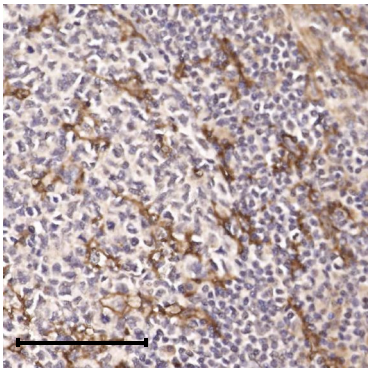
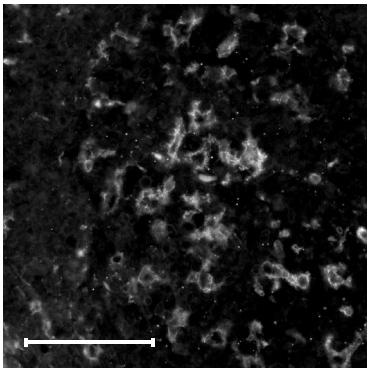
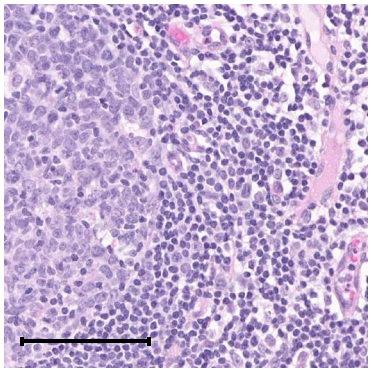
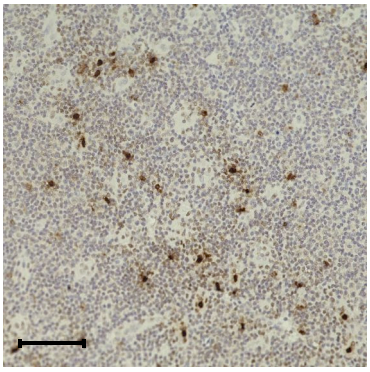
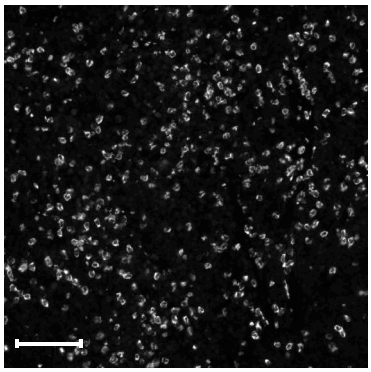
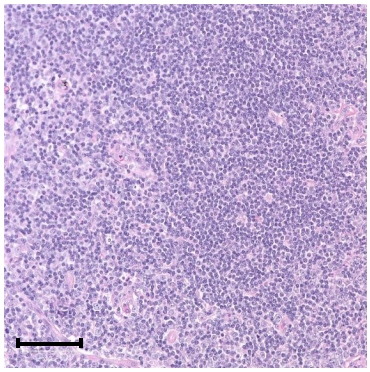
Staining specifications	IHC	CODEX	H&E
Antigen: CD7 **Clone: MRQ-56** **Company:** **Cell Marque (custom)** **Tissue: Tonsil** **Dilution:** **IHC: 1:100** **CODEX: 1:100** **CODEX oligo: 63-Alexa488**			
Antigen: CD8 **Clone: C8/144B** **Company:** **Cell Marque (custom)** **Santa Cruz Bio (sc-53212)** **Tissue: Tonsil** **Dilution:** **IHC: 1:100** **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**			

Figure S1c

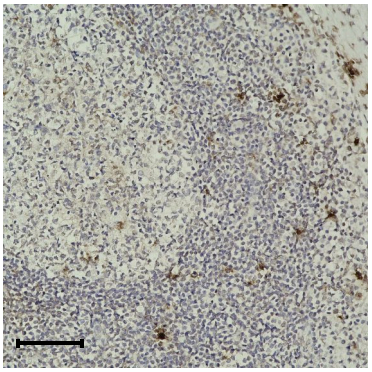
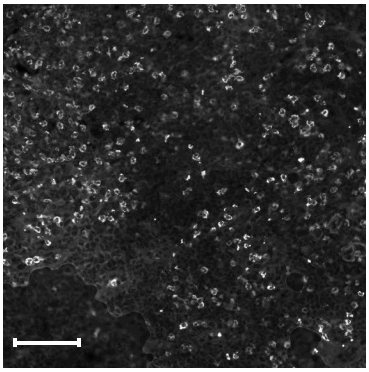
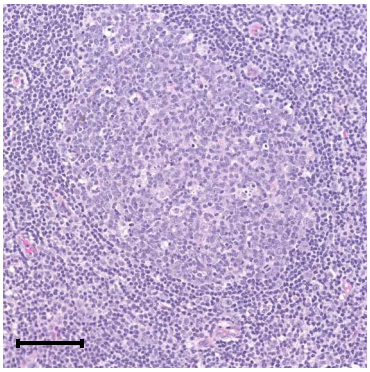
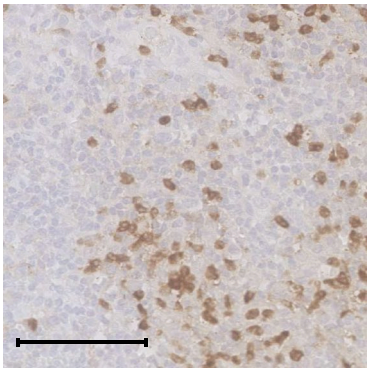
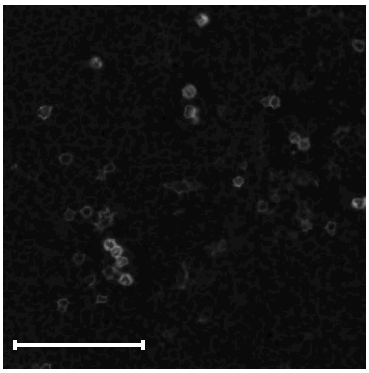
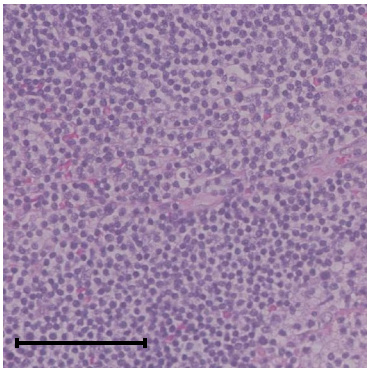
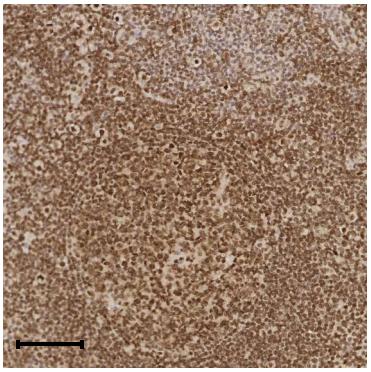
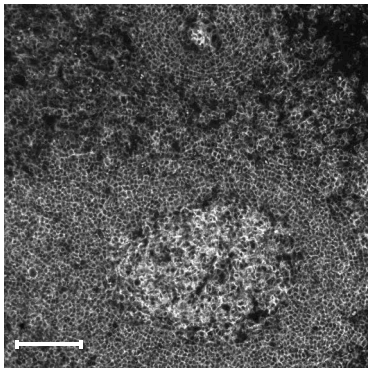
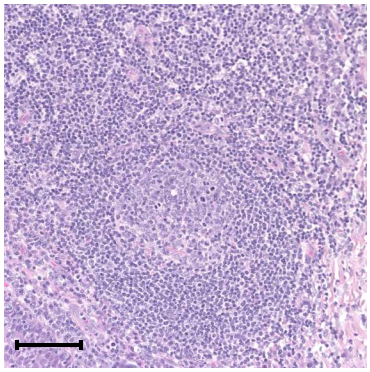
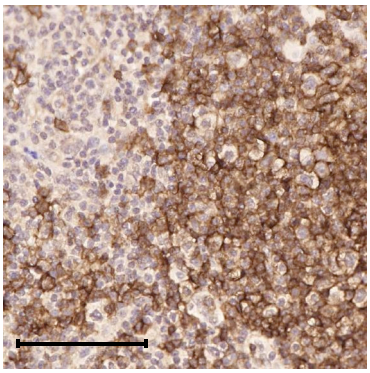
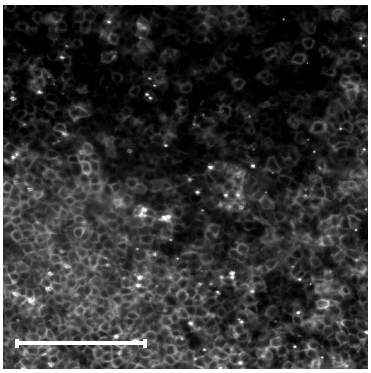
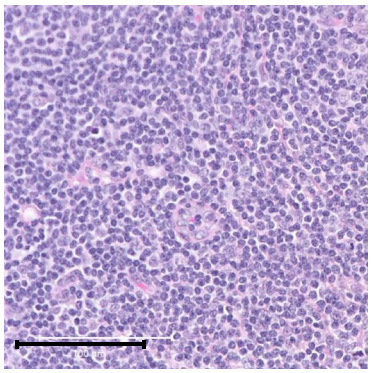
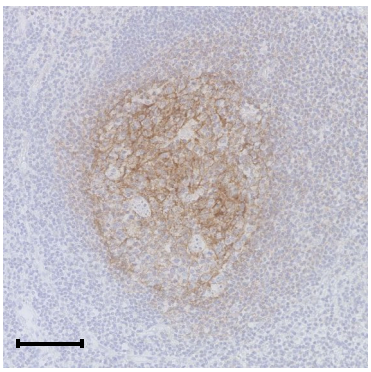
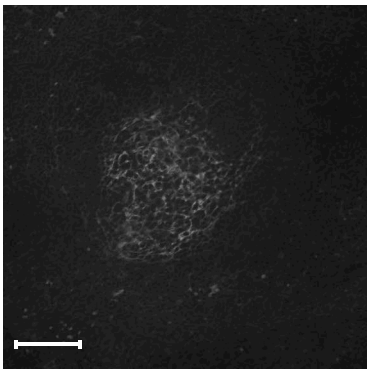
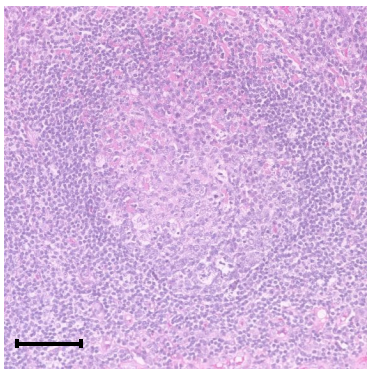
Staining specifications	IHC	CODEX	H&E
Antigen: CD15 **Clone: HI98** **Company:** **Biolegend (301902)** **Tissue: Tonsil** **Dilution:** **IHC: 1:100** **CODEX: 1:200** **CODEX oligo: 15-Alexa488**			
Antigen: CD16 **Clone: D1N9L** **Company:** **Cell Signaling Technology (custom)** **Tissue: Tonsil** **Dilution:** **IHC: 1:100** **CODEX: 1:100** **CODEX oligo: 26-Alexa647**			
Antigen: CD20 **Clone: rIGEL/773** **Company:** **Novus Biologicals (NBP2-54591)** **Tissue: Tonsil** **Dilution:** **IHC: 1:100** **CODEX: 1:200** **CODEX oligo: 48-ATTO550**			
Antigen: CD20 **Clone: H1** **Company:** **BD Biosciences (555677)** **Tissue: Tonsil** **Dilution:** **IHC: 1:200** **CODEX: 1:10** **CODEX oligo: 48-ATTO550**			
Antigen: CD21 **Clone: Bu32** **Company:** **Biolegend (354902)** **Tissue: Tonsil** **Dilution:** **IHC: 1:100** **CODEX: 1:50** **CODEX oligo: 21-Alexa647**			

Figure S1d

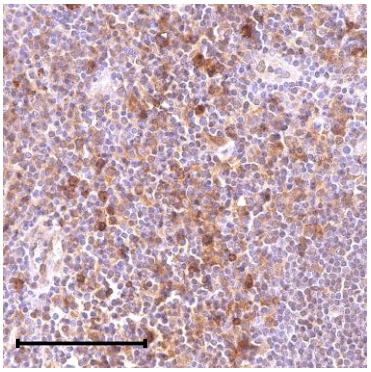
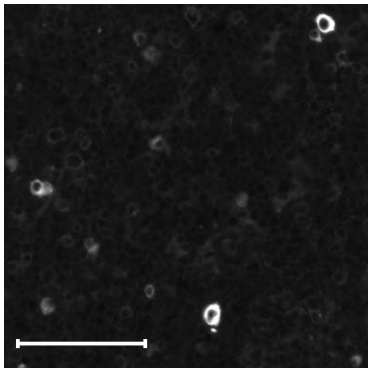
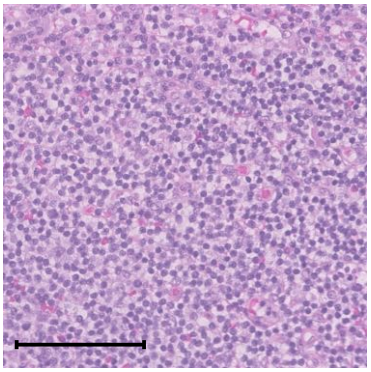
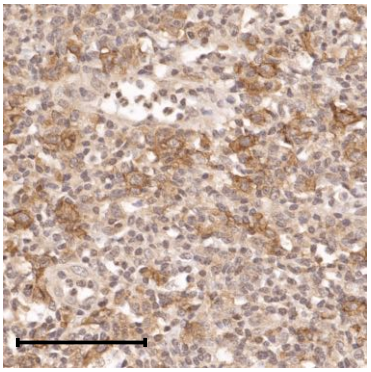
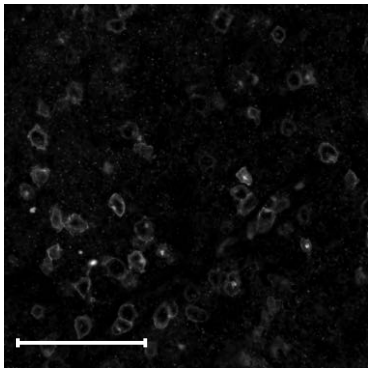
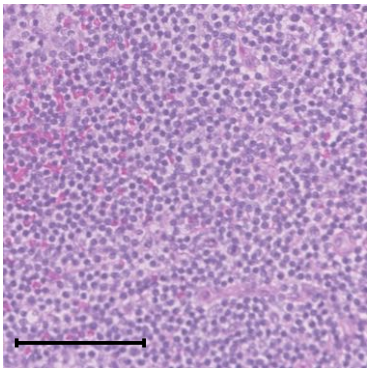
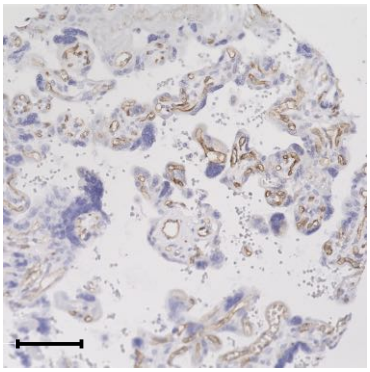
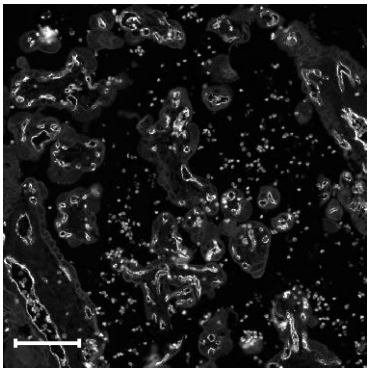
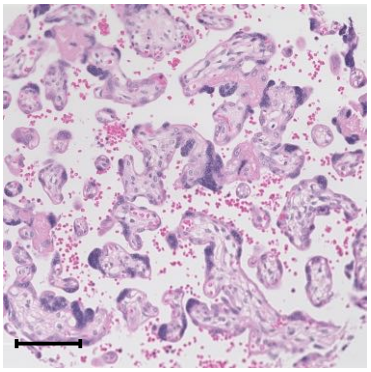
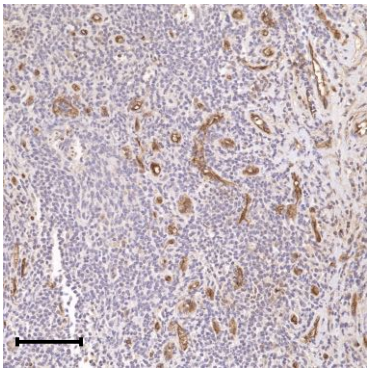
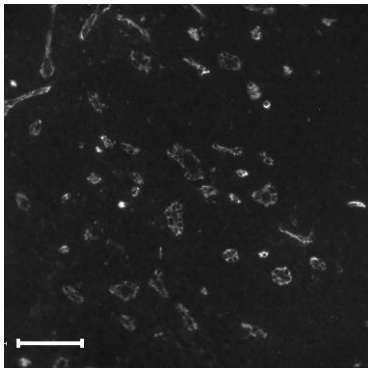
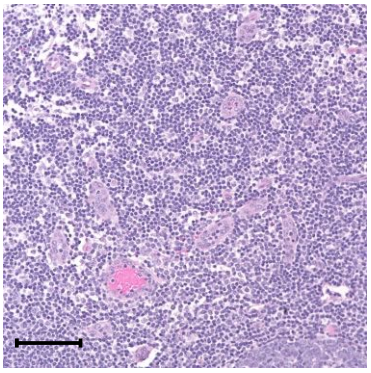
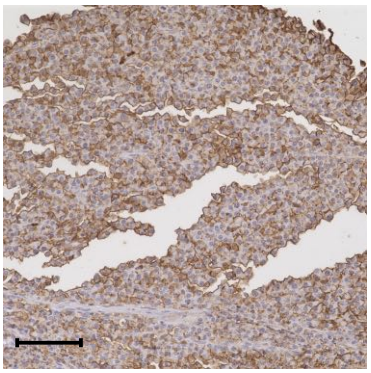
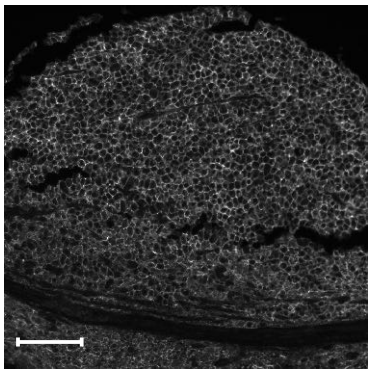
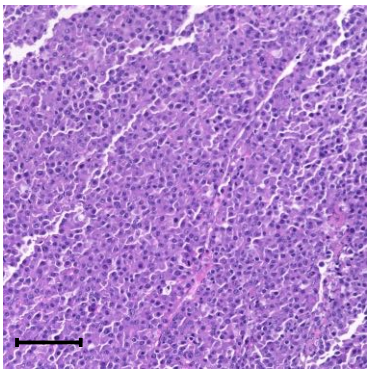
Staining specifications	IHC	CODEX	H&E
Antigen: CD25 **Clone: 4C9** **Company:** **Cell Marque (custom)** **Tissue: Tonsil** **Dilution:** **IHC: 1:** **CODEX: 1:100** **CODEX oligo: 24-ATTO550**			
Antigen: CD30 **Clone: Ber-H2** **Company:** **Cell Marque (custom)** **Tissue: Tonsil** **Dilution:** **IHC: 1:200** **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**			

Figure S1e

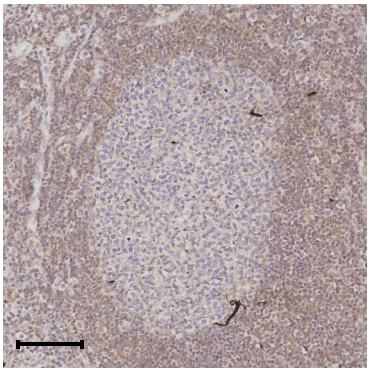
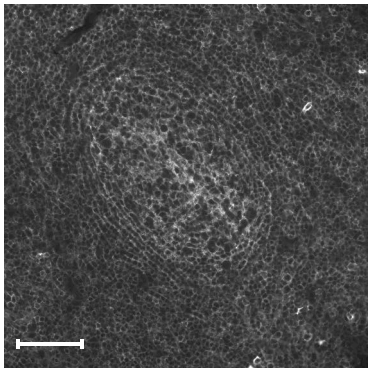
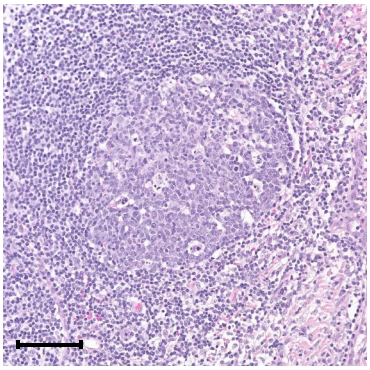
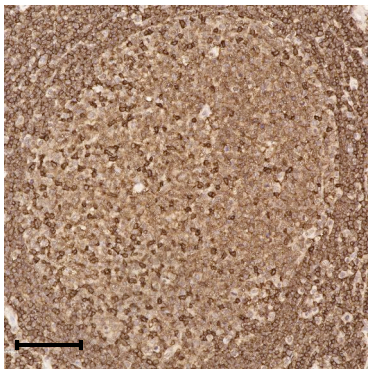
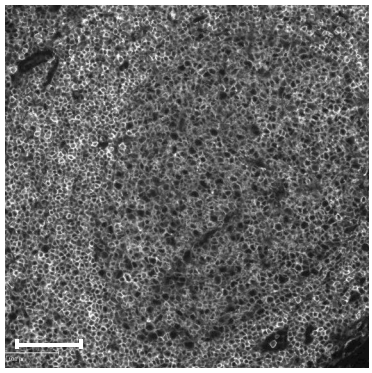
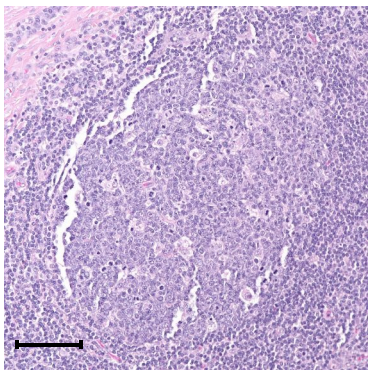
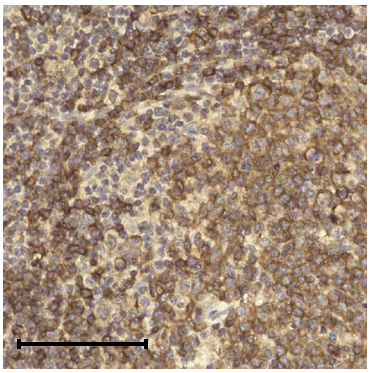
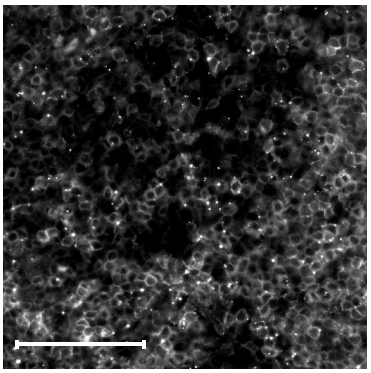
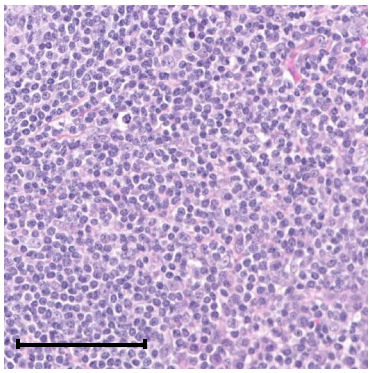
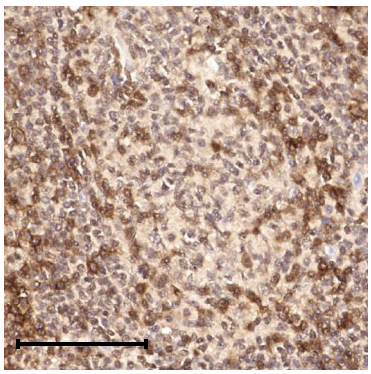
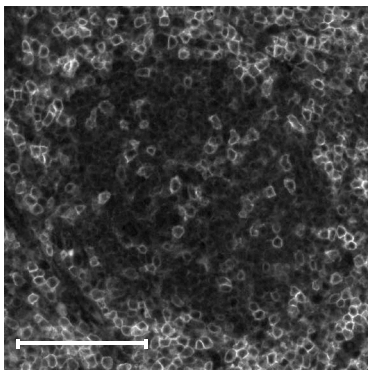
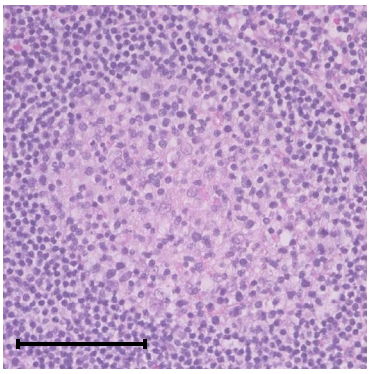
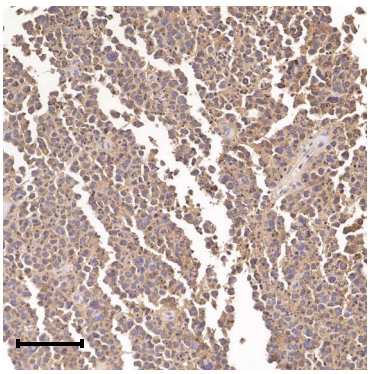
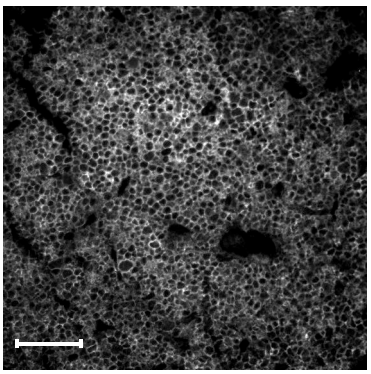
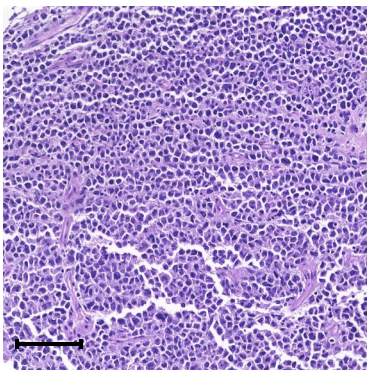
Staining specifications	IHC	CODEX	H&E
Antigen: CD44 **Clone: IM-7** **Company:** **BD Biosciences (553131)** **Tissue: Tonsil** **Dilution:** **IHC: 1:25** **CODEX: 1:25** **CODEX oligo: 44-Alexa488**			
Antigen: CD45 **Clones: 2B11 + PD7/26** **Company:** **Novus Biologicals (NBP2-34528)** **Dilution:** **IHC: 1:200** **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**			

Figure S1f

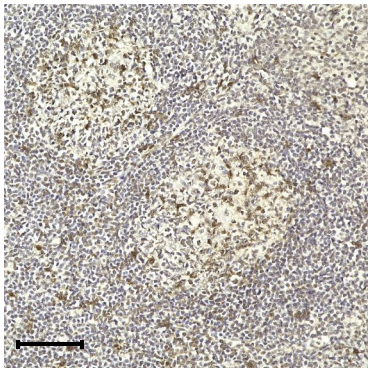
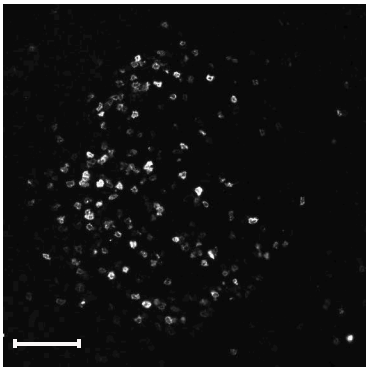
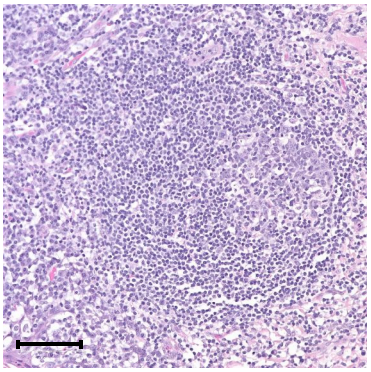
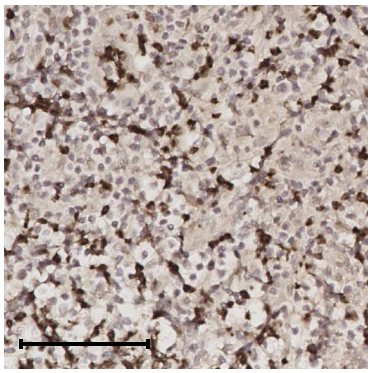
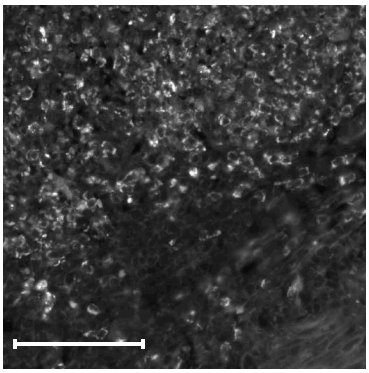
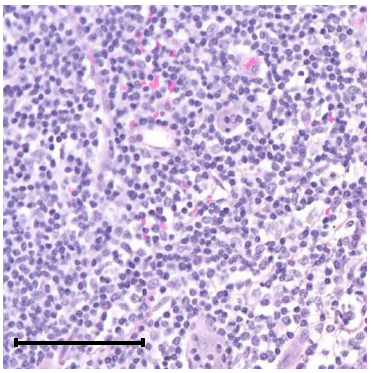
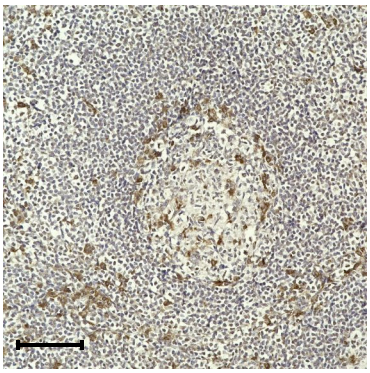
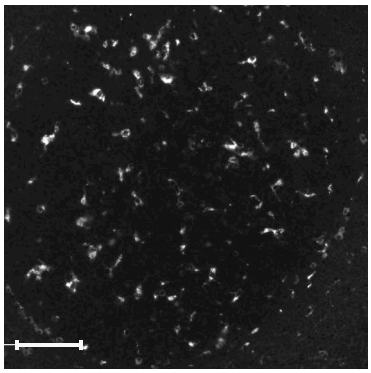
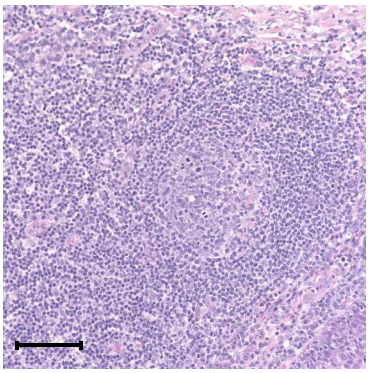
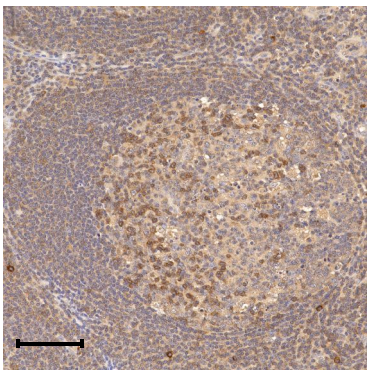
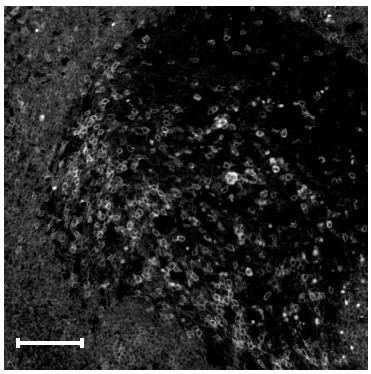
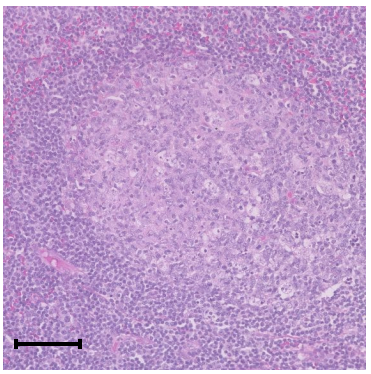
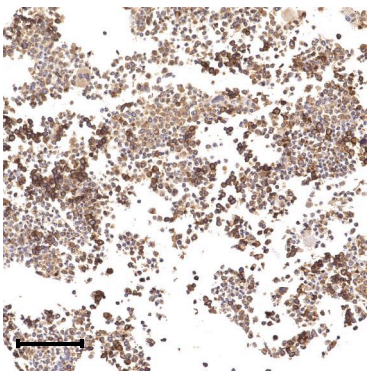
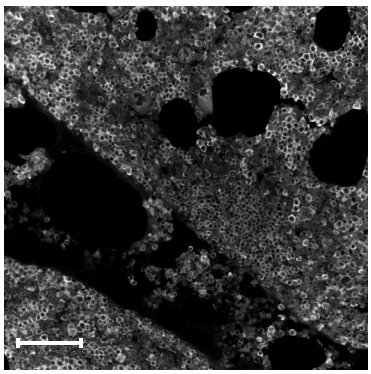
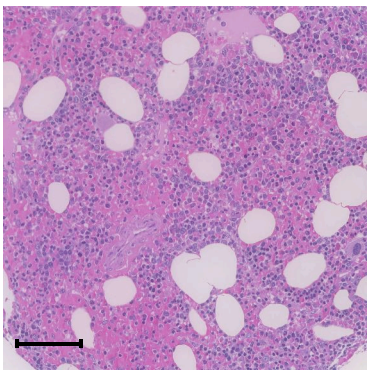
Staining specifications	IHC	CODEX	H&E
Antigen: CD57 **Clone: HCD57** **Company:** **BioLegend (322325)** **Tissue: Tonsil** **Dilution:** **IHC: 1:100** **CODEX: 1:200** **CODEX oligo: ST30-ATTO550**			
Antigen: CD66a **Clone: B1.1/CD66** **Company:** **BD Biosciences (551354)** **Tissue: Tonsil** **Dilution:** **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: 1:100** **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**			

Figure S1g

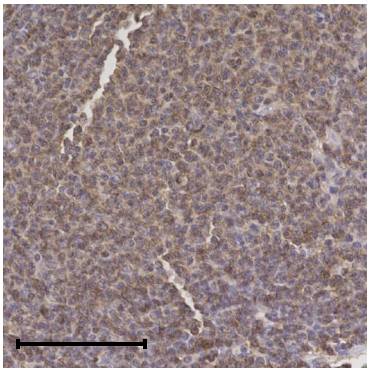
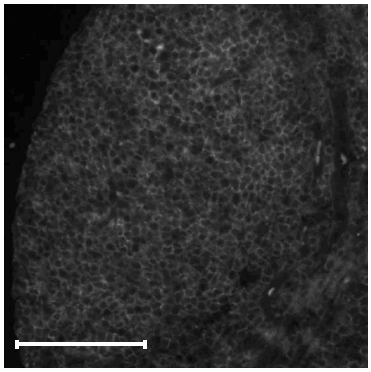
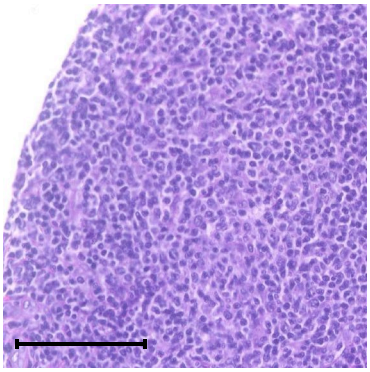
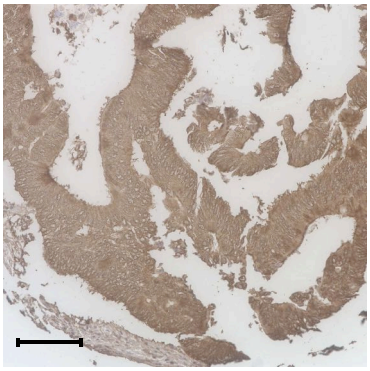
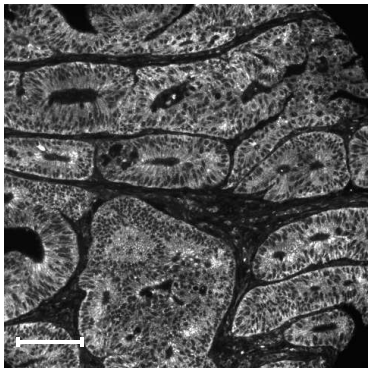
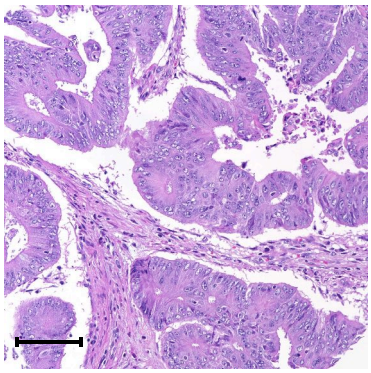
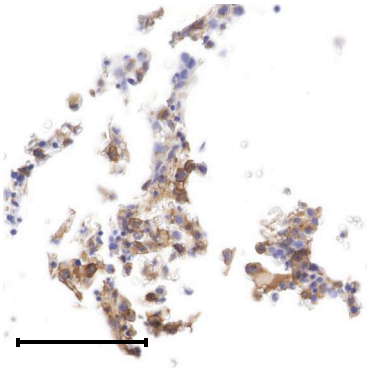
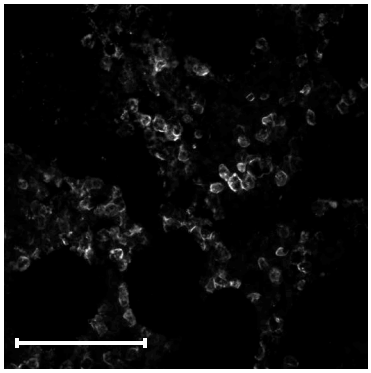
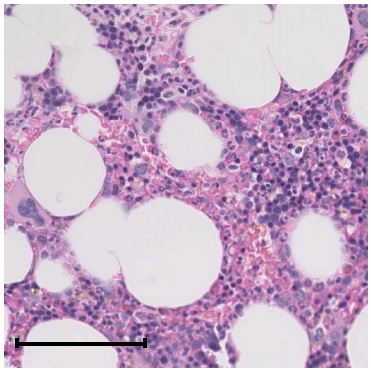
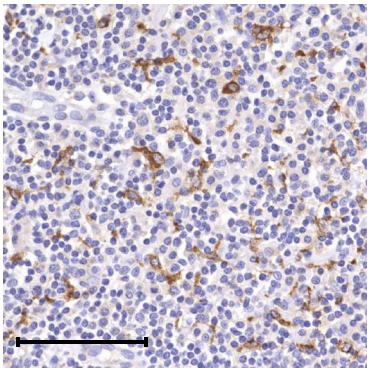
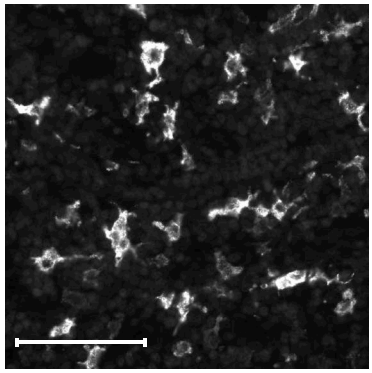
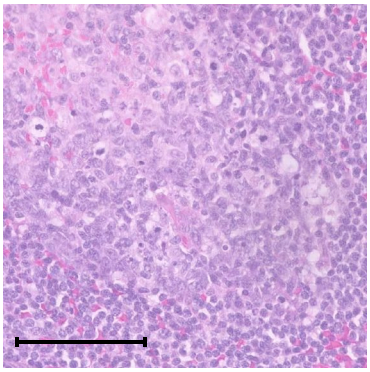
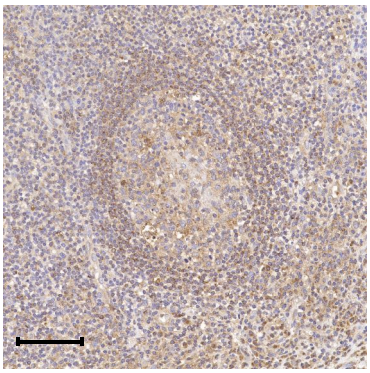
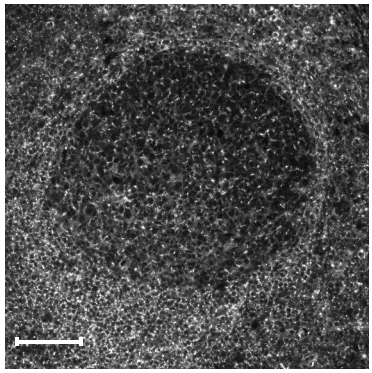
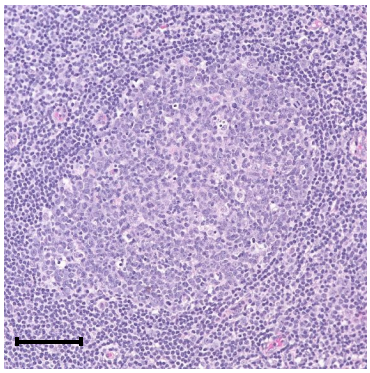
Staining specifications	IHC	CODEX	H&E
Antigen: CD79a **Clone: JBC117** **Company:** **Cell Marque (custom)** **Tissue: Follicular lymphoma** **Dilution:** **IHC: 1:300** **CODEX: 1:20** **CODEX oligo: 46-Alexa488**			
Antigen: CD138 (Syndecan-1) **Clone: B-A38** **Company:** **Invitrogen (MA1-10091)** **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: Bone marrow** **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**			

Figure S1h

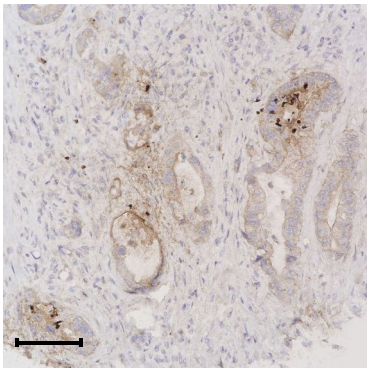
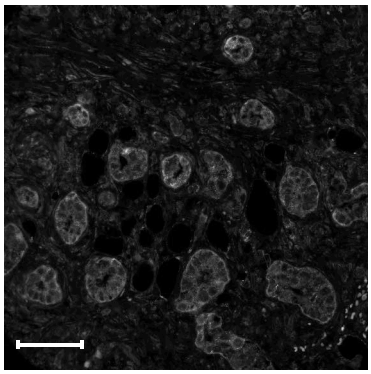
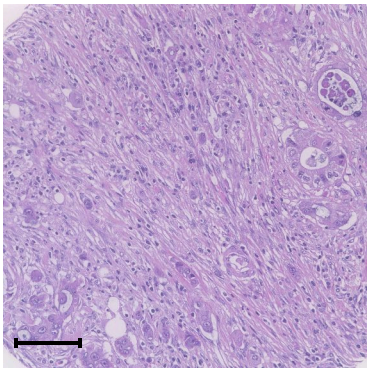
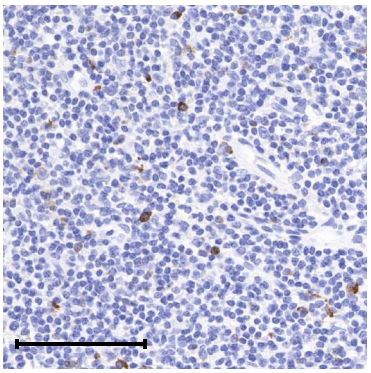
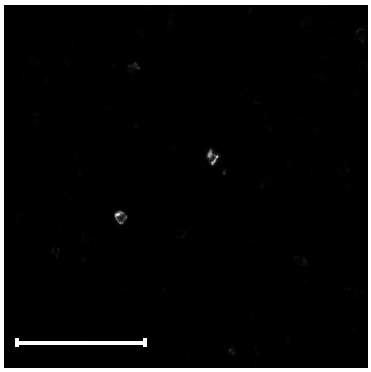
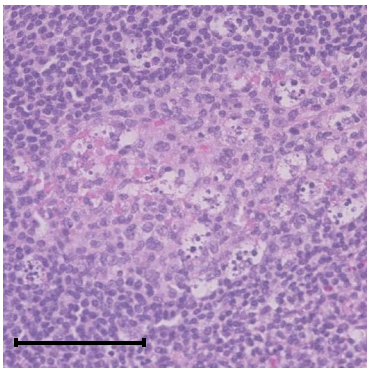
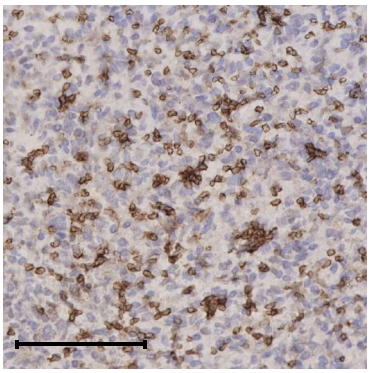
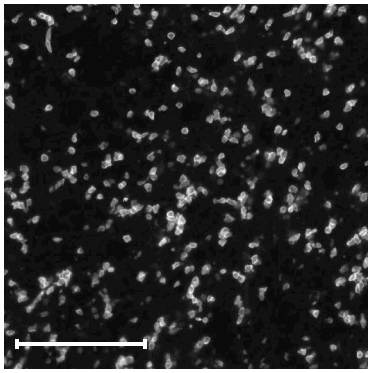
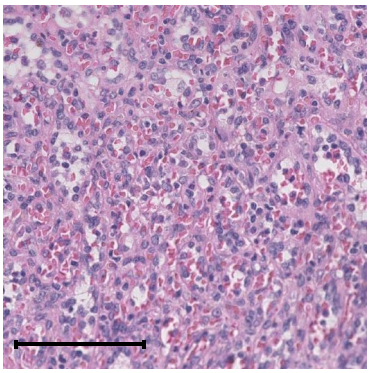
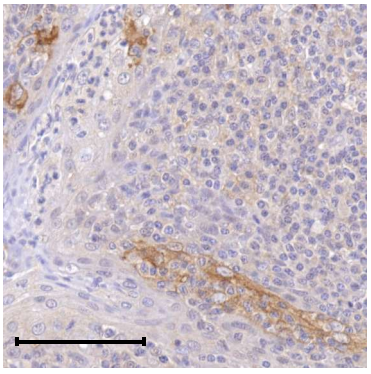
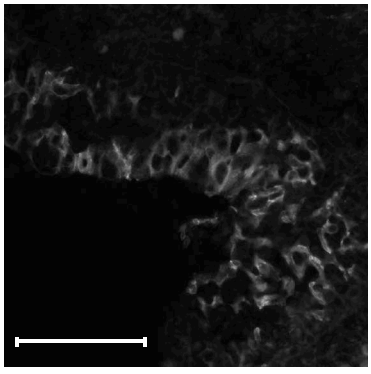
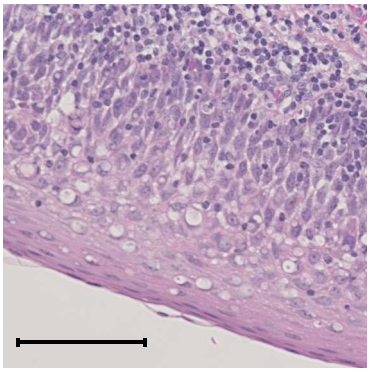
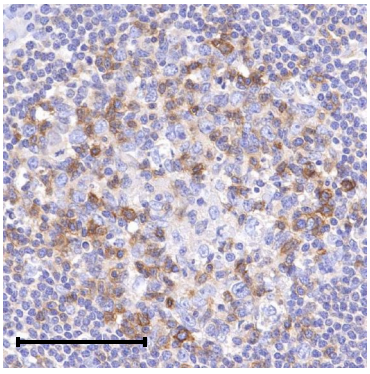
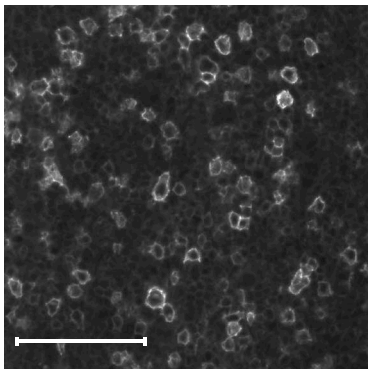
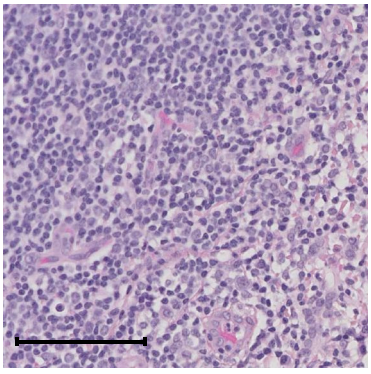
Staining specifications	IHC	CODEX	H&E
Antigen: CD194 (CCR4) **Clone: L291H4** Company: BioLegend (359402) Tissue: Cholangiocarcinoma Dilution: IHC: 1:50 CODEX: 1:10 CODEX oligo: ST2-ATTO550			
Antigen: CD223 (LAG-3) **Clone: D2G4O or 17B4** Companies: Cell Signaling Technology (custom) LSBio (LS-C18692-100) 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			

Figure S1i

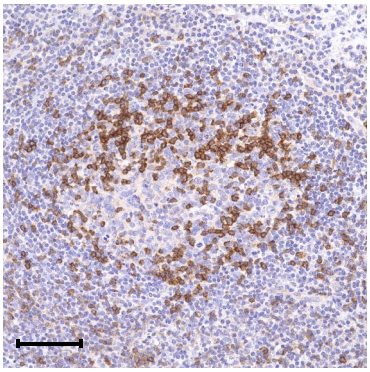
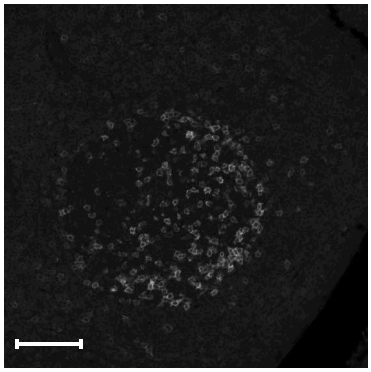
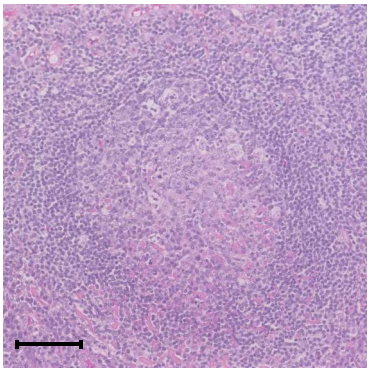
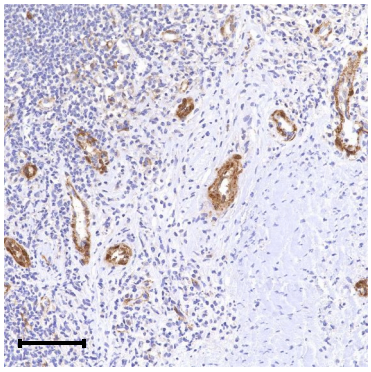
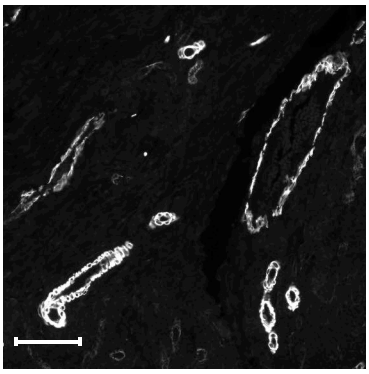
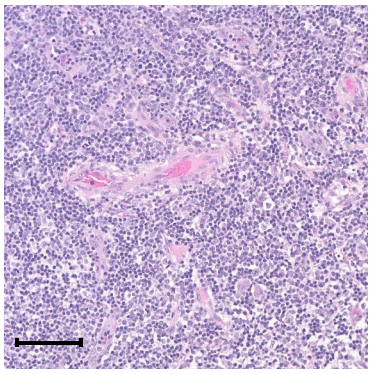
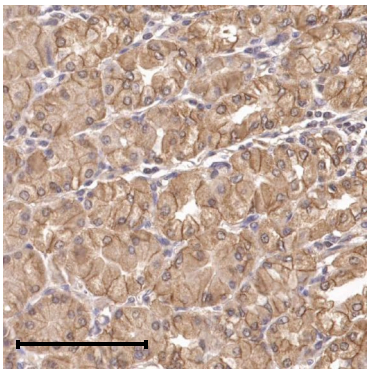
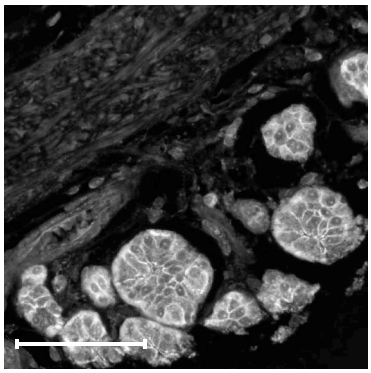
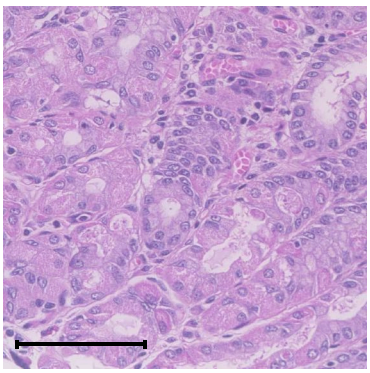
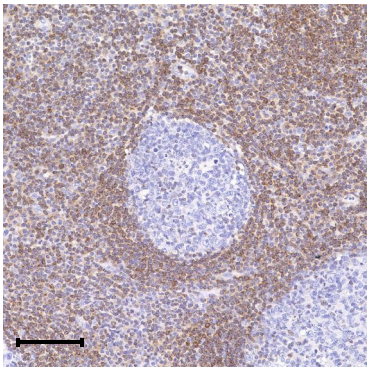
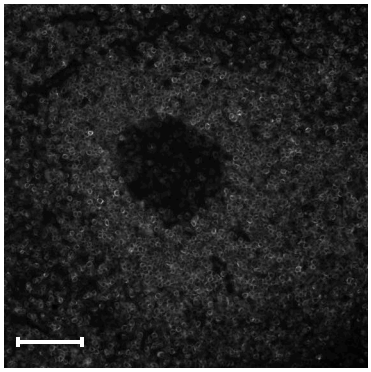
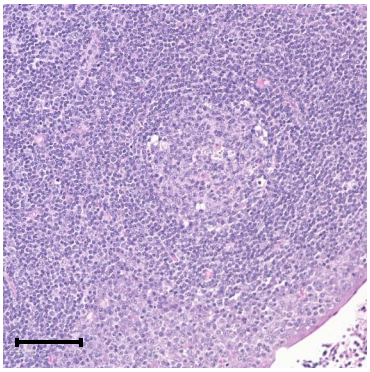
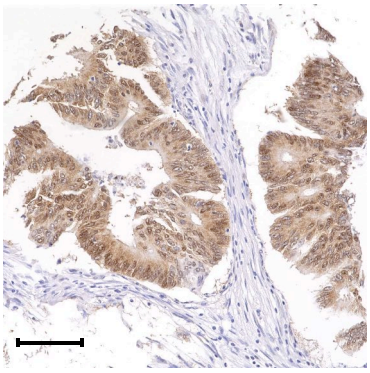
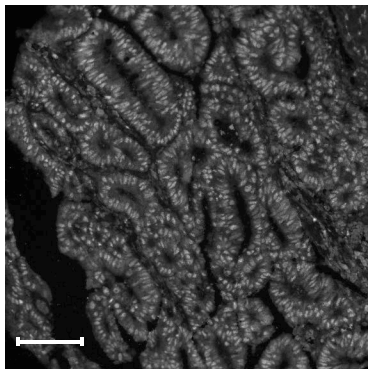
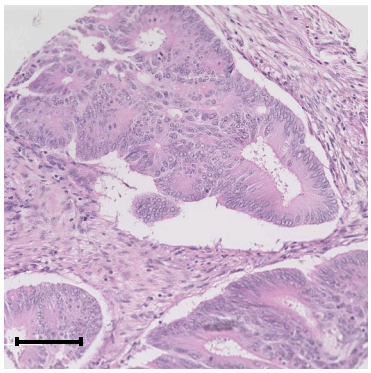
Staining specifications	IHC	CODEX	H&E
Antigen: CD279 (PD-1) **Clone: D1K2T** Company: Cell Signaling Technology (custom) Tissue: Tonsil Dilution: IHC: 1:200 CODEX: 1:25 CODEX oligo: 23-ATTO550			
Antigen: α-SMA **Clone: polyclonal** Company: Abcam (ab5694) Tissue: Tonsil Dilution: IHC: 1:200 CODEX: 1:200 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			

Figure S1j

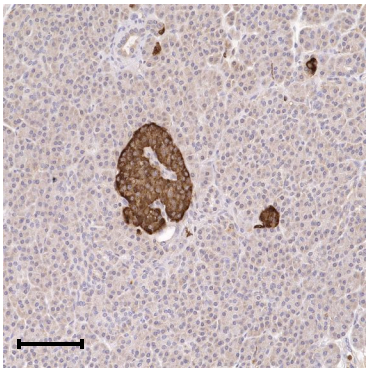
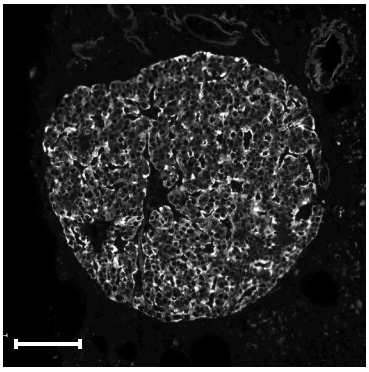
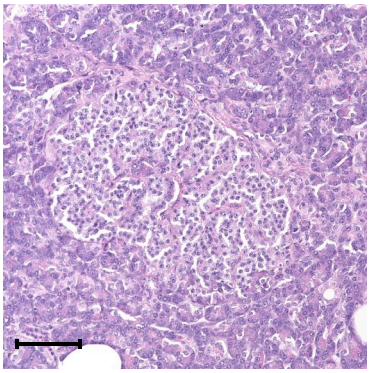
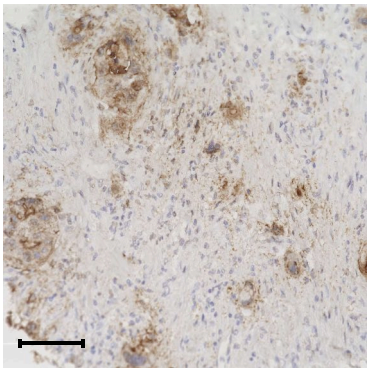
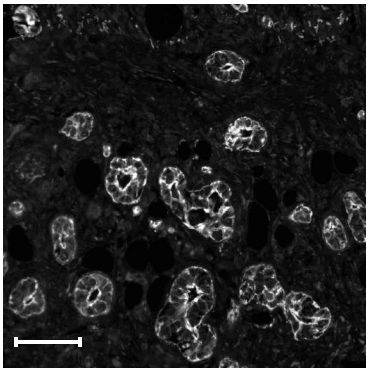
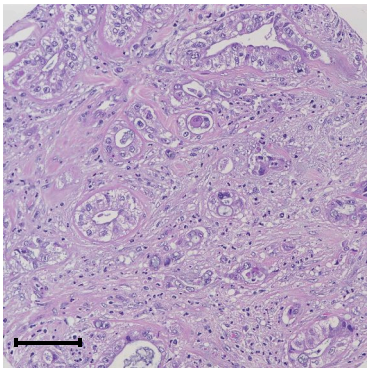
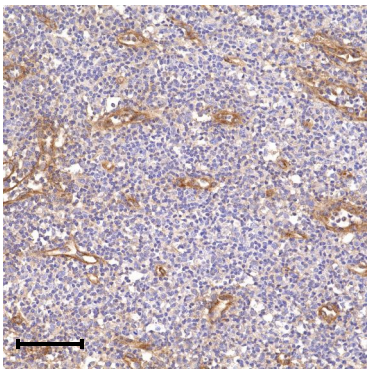
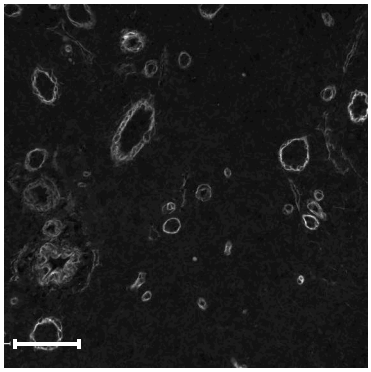
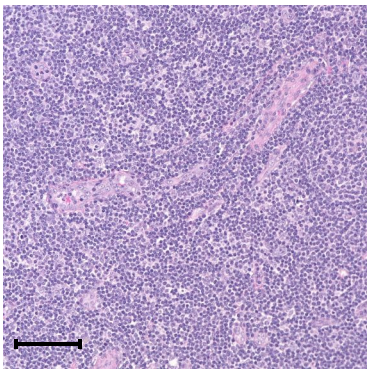
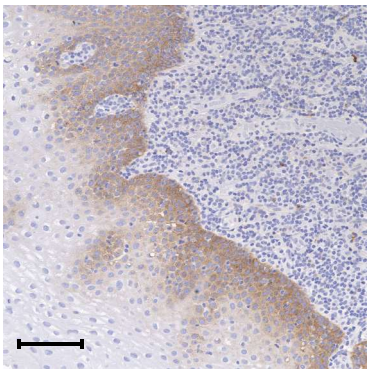
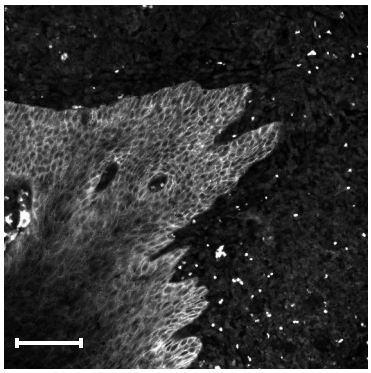
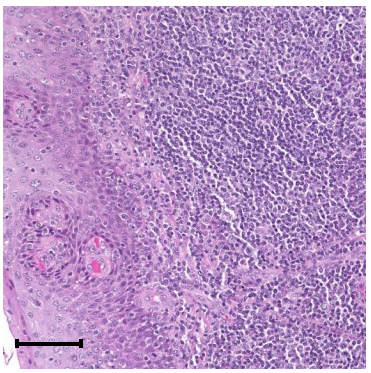
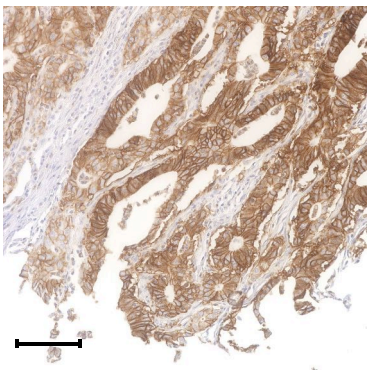
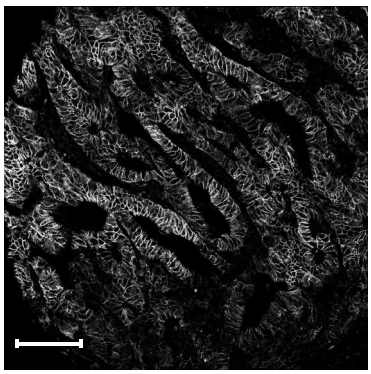
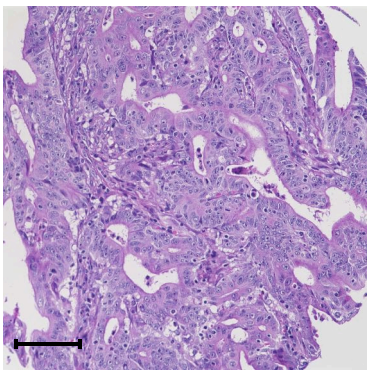
Staining specifications	IHC	CODEX	H&E
Antigen: Chromogranin A **Clones:** **LK2H10 + PHE5 + CGA/414** **Company:** **Novus Biologicals (NBP2-34674)** **Tissue: Pancreas** **Dilution:** **IHC: 1:400** **CODEX: 1:50** **CODEX oligo: 43-Alexa488**			
Antigen: CK7 **Clone: OV-TL12/30** **Company:** **Novus Biologicals (NBP2-47940)** **Tissue: Cholangiocarcinoma** **Dilution:** **IHC: 1:100** **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**			

Figure S1k

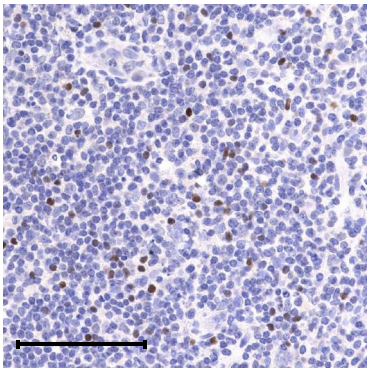
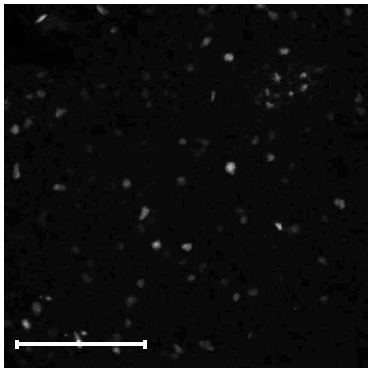
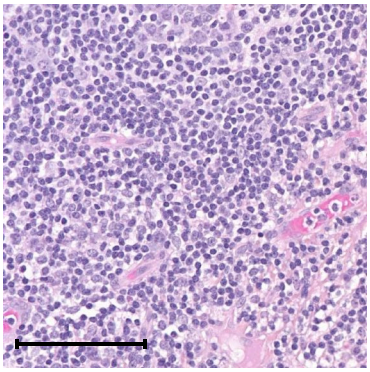
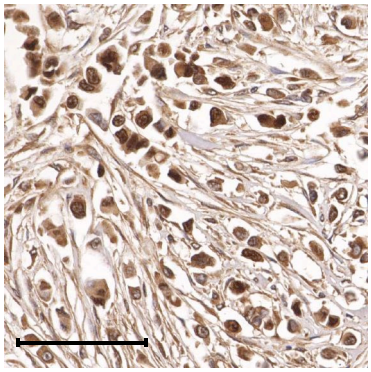
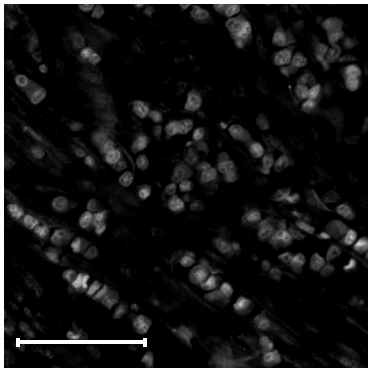
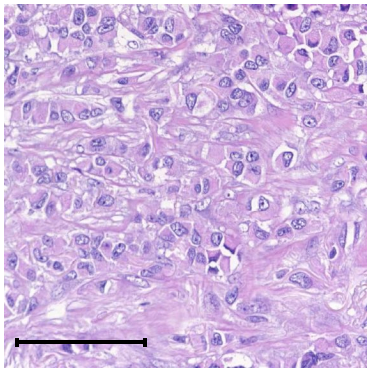
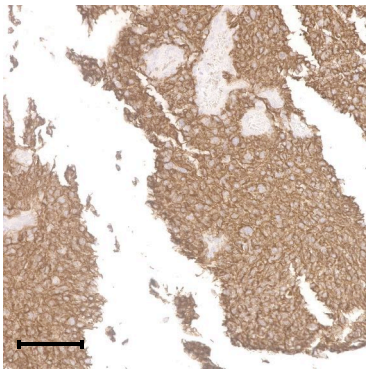
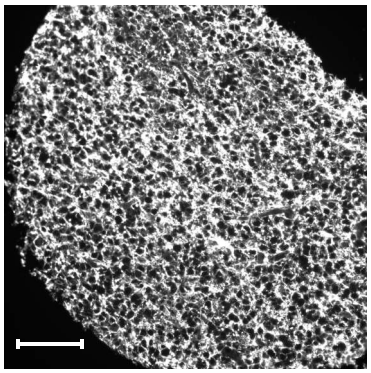
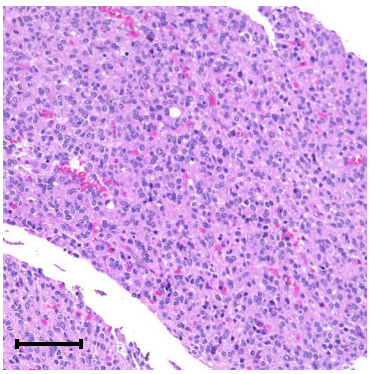
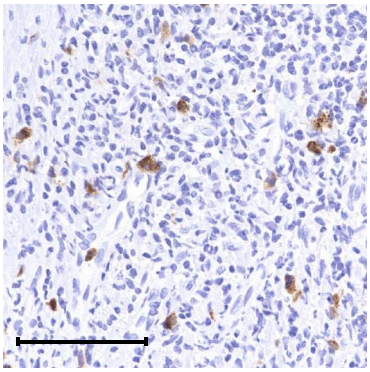
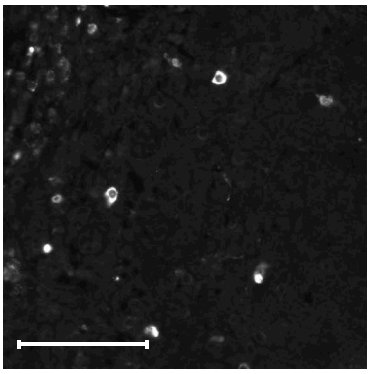
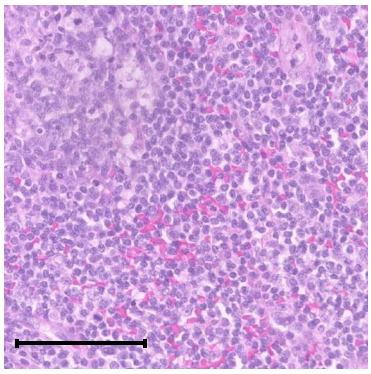
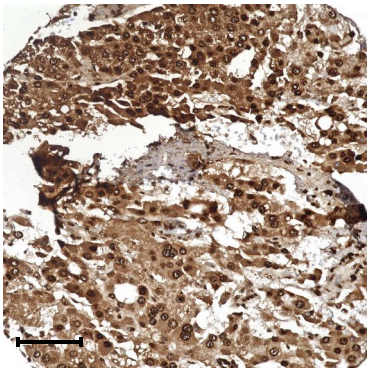
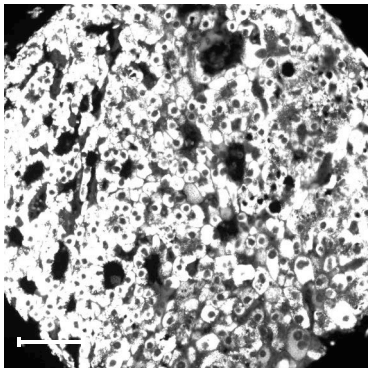
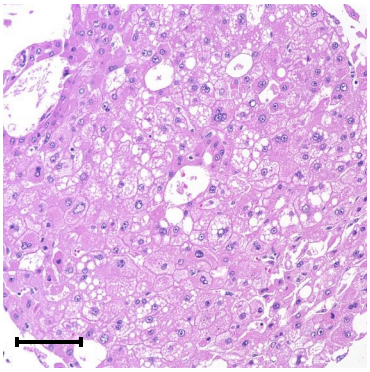
Staining specifications	IHC	CODEX	H&E
Antigen: FOXP3 **Clone: 236A/E7** **Company:** **Invitrogen (14-4777-80)** **Tissue: Tonsil** **Dilution:** **IHC: 1:20** **CODEX: 1:100** **CODEX oligo: 61-Alexa647**			
Antigen: GATA3 **Clone: L50-823** **Company:** **Cell Marque (custom)** **Tissue: Breast lobular carcinoma** **Dilution:** **IHC: 1:300** **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**			

Figure S1I

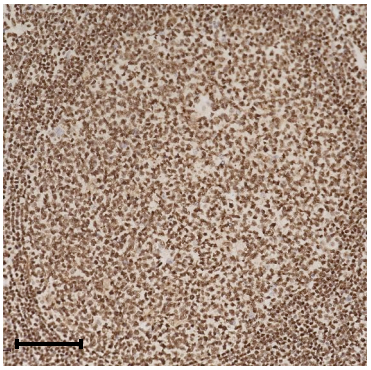
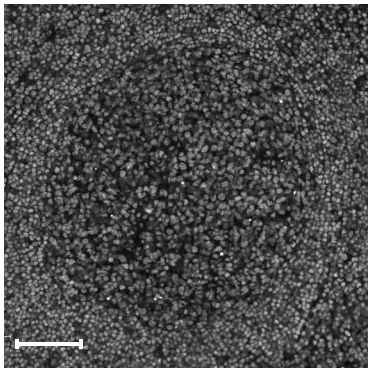
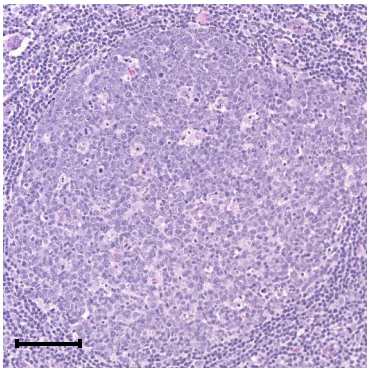
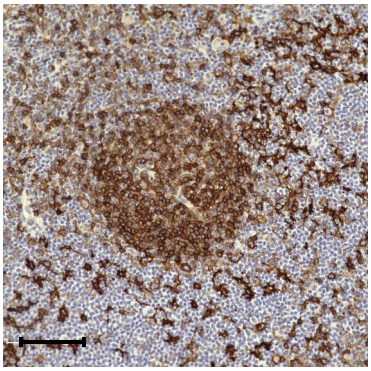
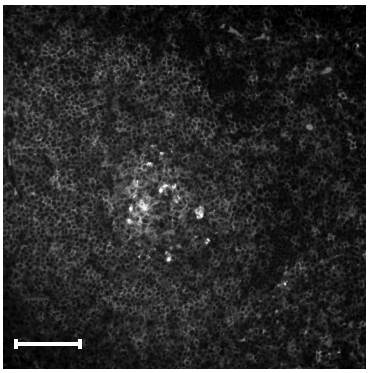
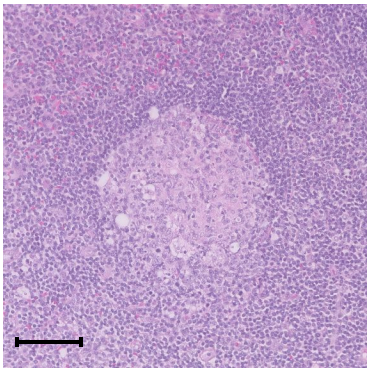
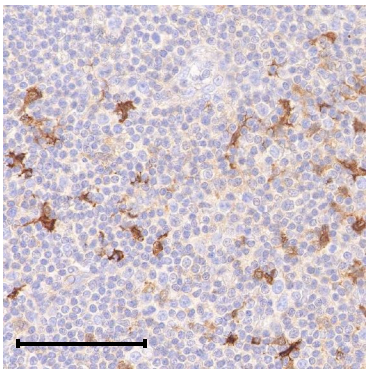
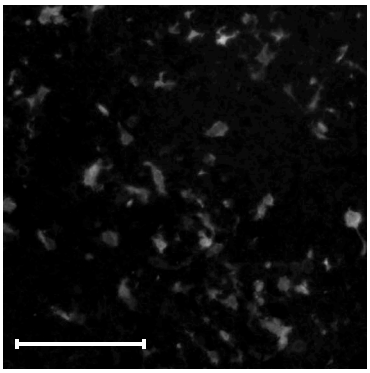
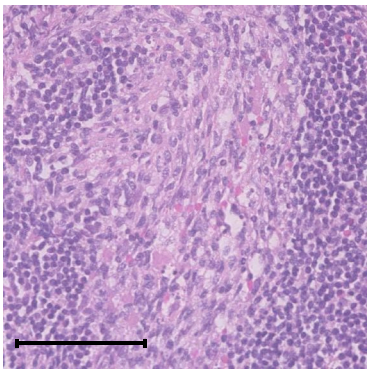
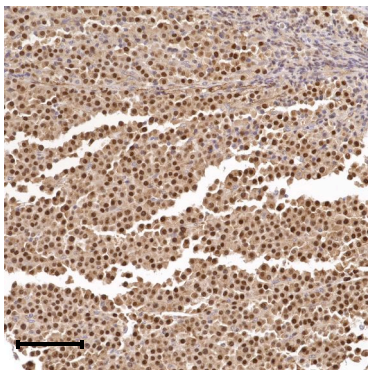
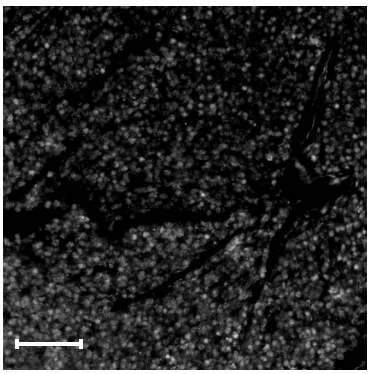
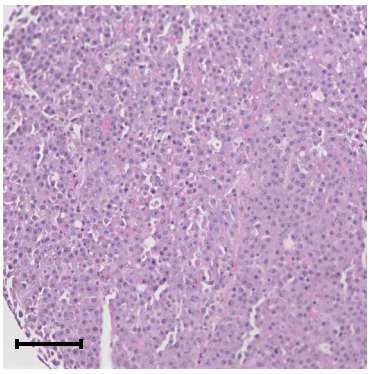
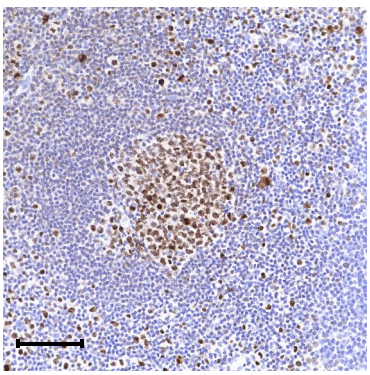
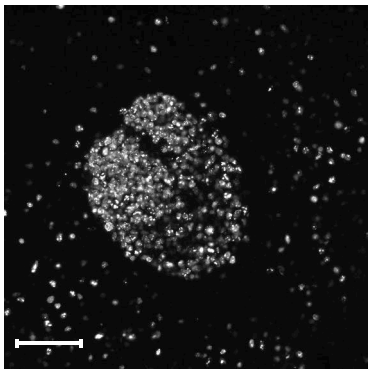
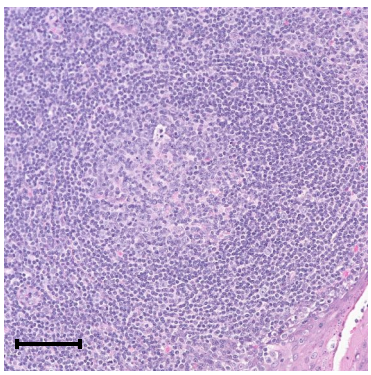
Staining specifications	IHC	CODEX	H&E
Antigen: Histone H3 **Clone: D1H2** **Company:** **Cell Signaling Technology (custom)** **Tissue: Tonsil** **Dilution:** **IHC: 1:100** **CODEX: 1:50** **CODEX oligo: 30-ATTO550**			
Antigen: HLA-DR **Clone: EPR3692** **Company:** **Abcam (ab215985)** **Tissue: Tonsil** **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**			

Figure S1m

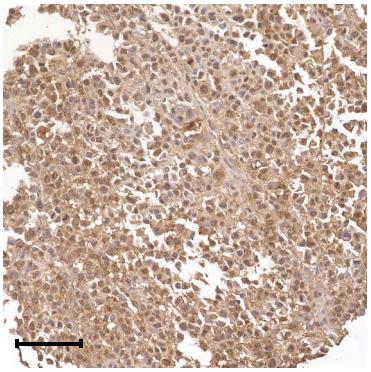
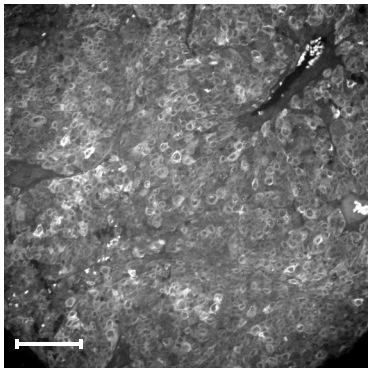
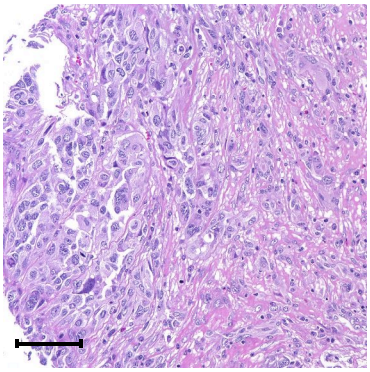
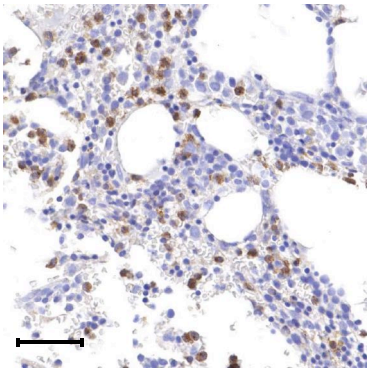
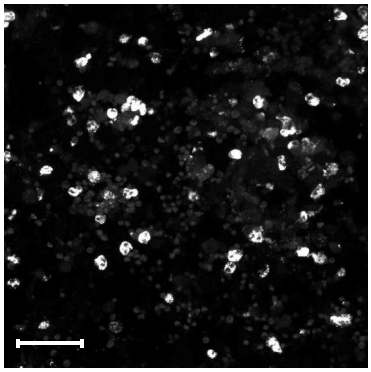
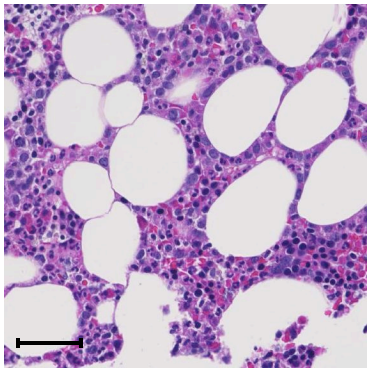
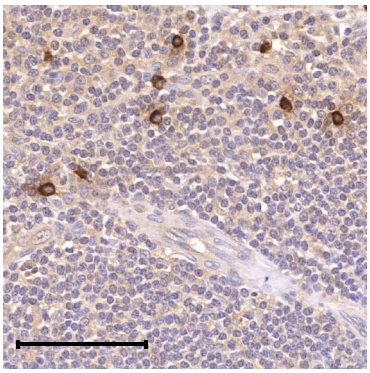
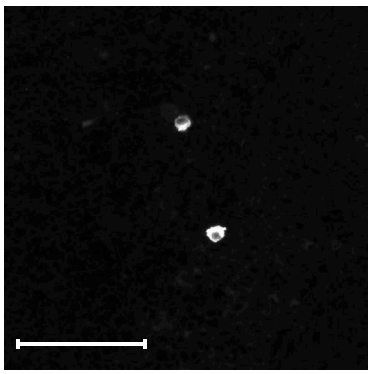
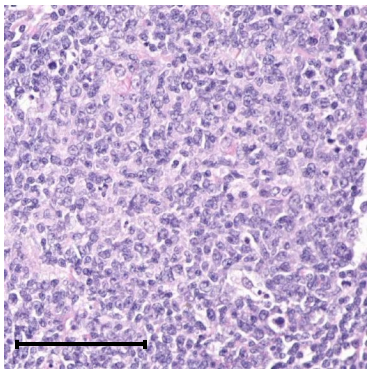
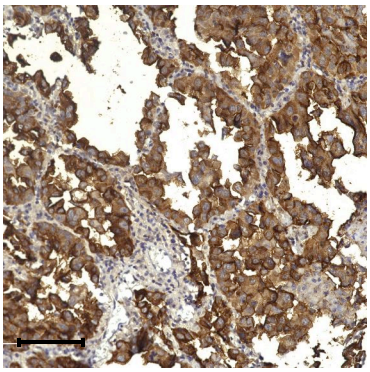
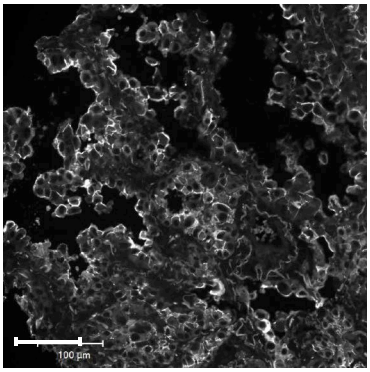
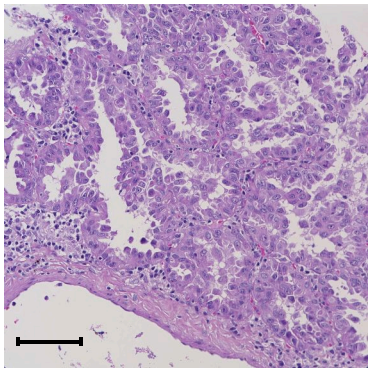
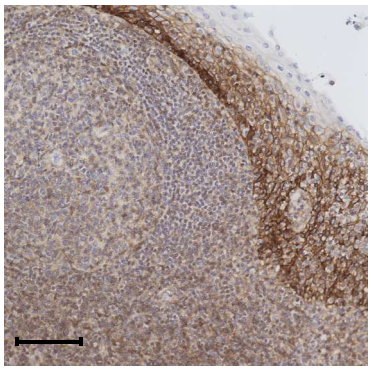
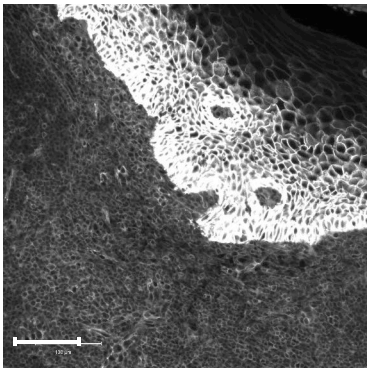
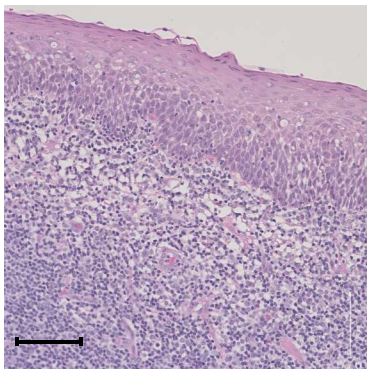
Staining specifications	IHC	CODEX	H&E
Antigen: Melan-A **Clones:** **A103 + M2-7C10 + M2-9E3** **Company:** **Novus Biologicals (NBP2-34546)** **Tissue: Malignant melanoma** **Dilution:** **IHC: 1:250** **CODEX: 1:50** **CODEX oligo: 44-ATTO550**			
Antigen: MMP9 **Clone: L51/82** **Company:** **Biolegend (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**			

Figure S1n

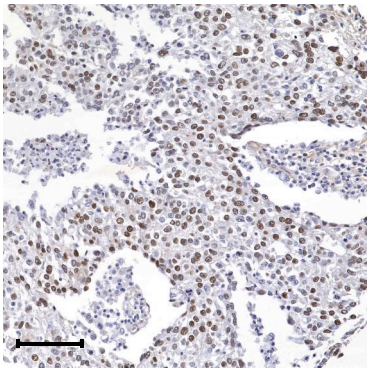
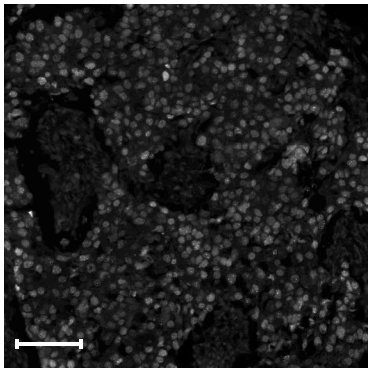
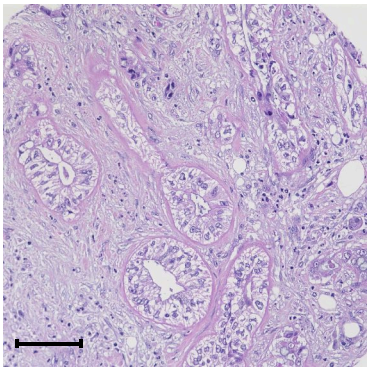
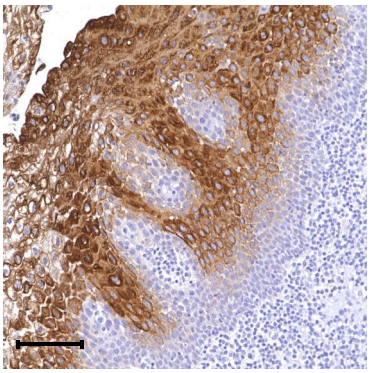
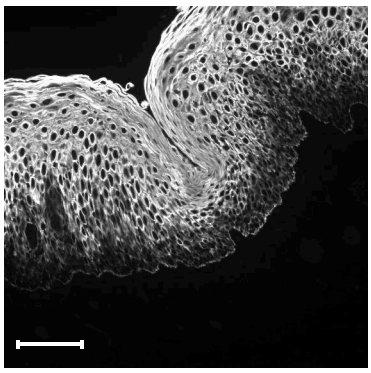
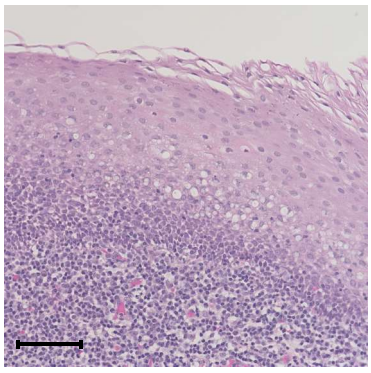
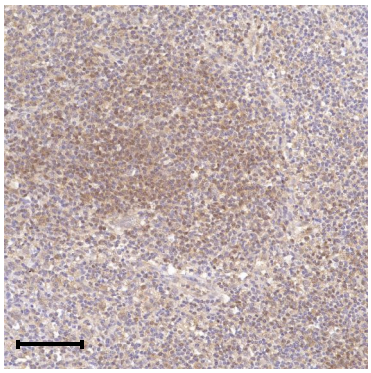
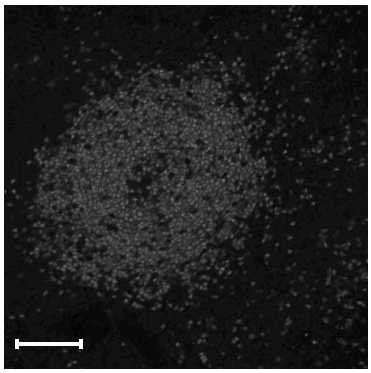
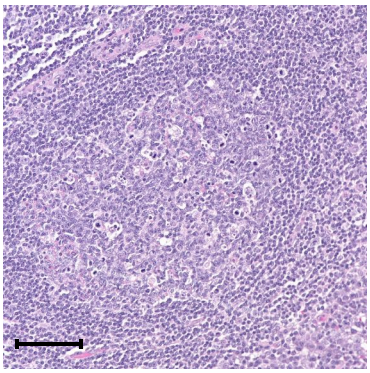
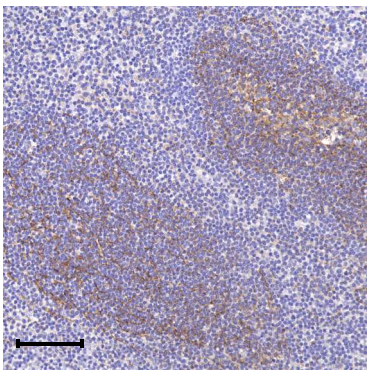
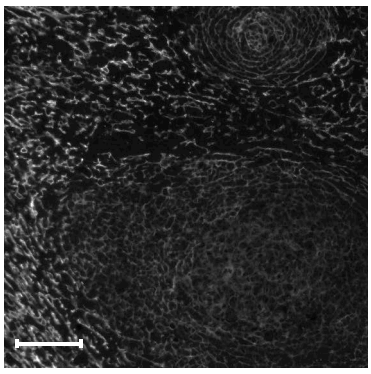
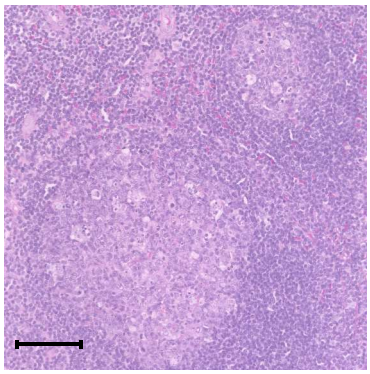
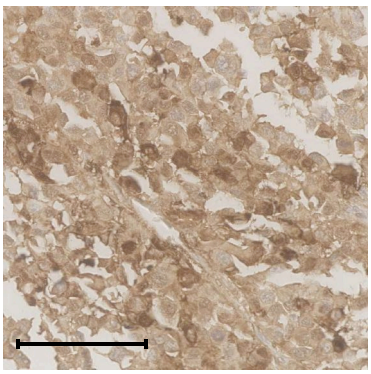
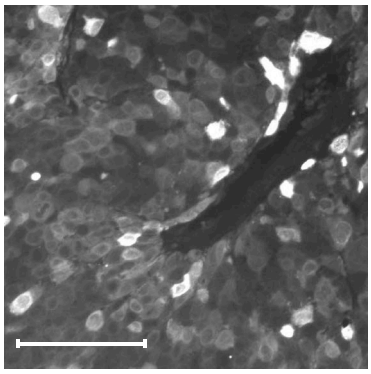
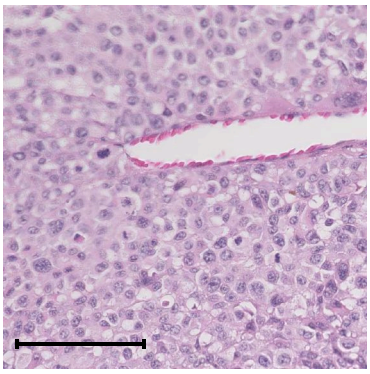
Staining specifications	IHC	CODEX	H&E
Antigen: p53 **Clone: DO-7** **Companies:** **Cell Marque (custom)** **Santa Cruz Bio (sc-47698)** **Tissue: Breast cancer NST** **Dilution:** **IHC: 1:1000** **CODEX: 1:25** **CODEX oligo: 52-ATTO550**			
Antigen: Pan-Cytokeratin **Clones: AE-1 + AE-3 or C-11** **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**			

Figure S1o

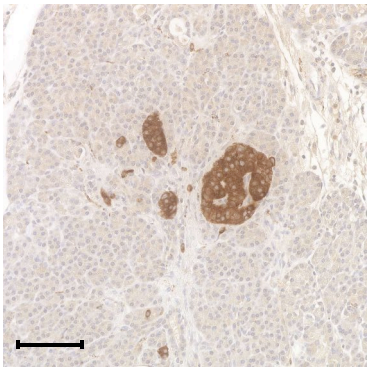
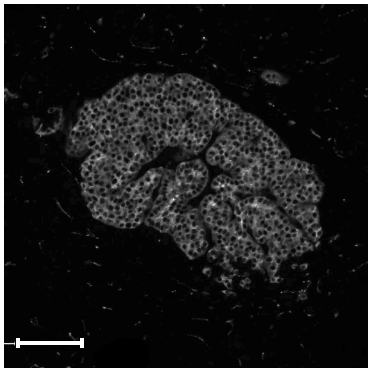
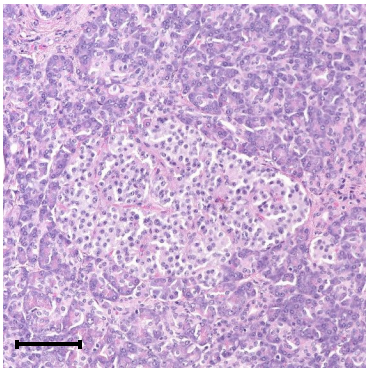
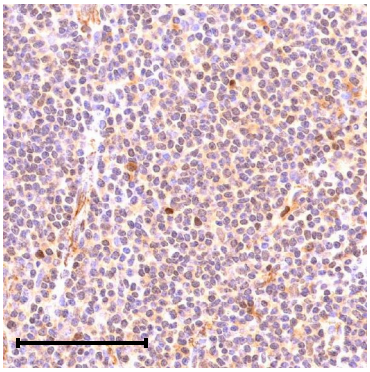
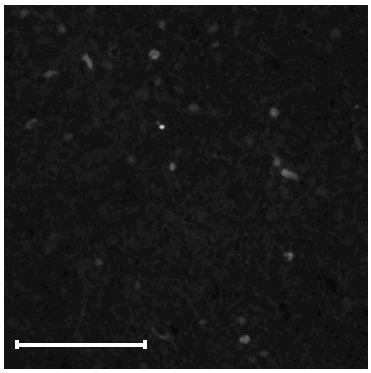
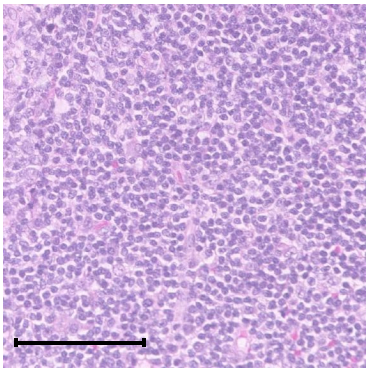
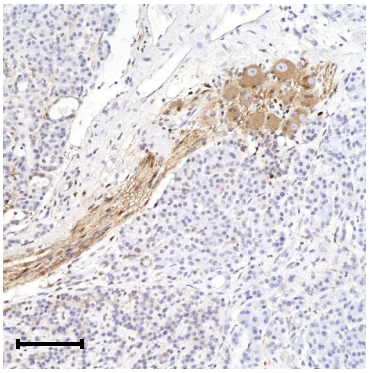
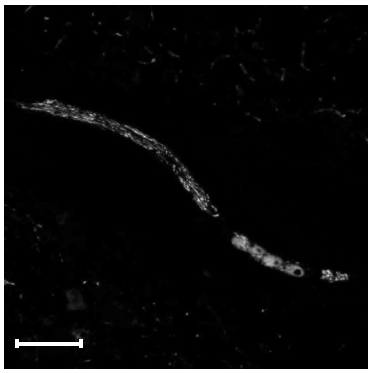
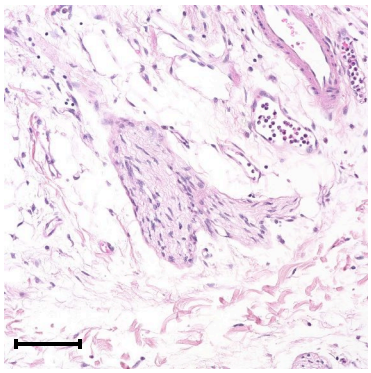
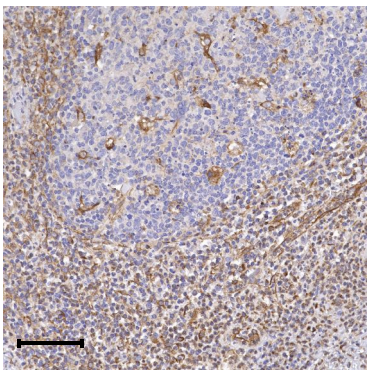
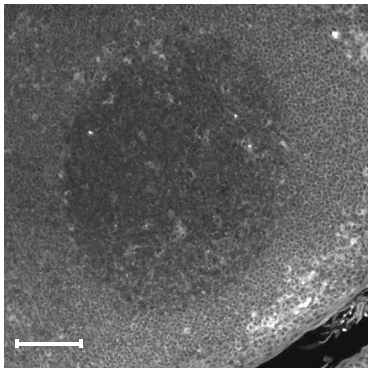
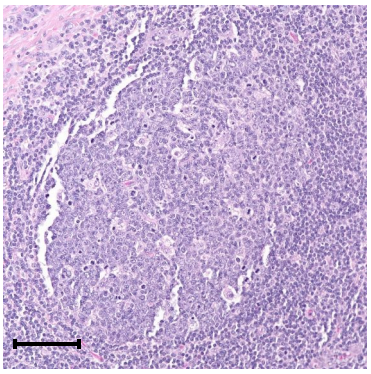
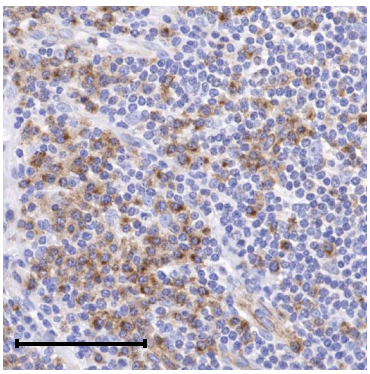
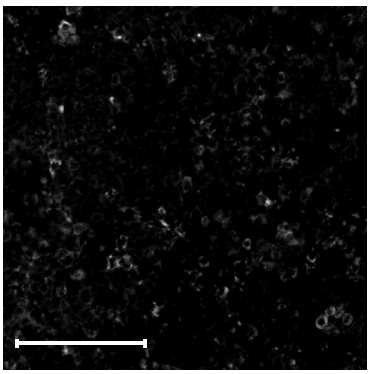
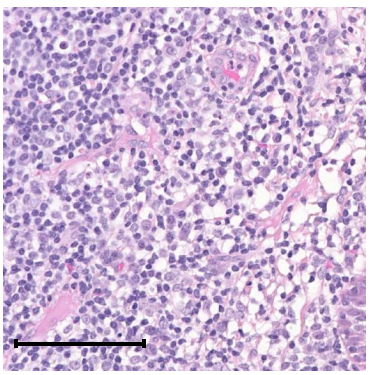
Staining specifications	IHC	CODEX	H&E
Antigen: Synaptophysin **Clone: 7H12** **Company:** **Novus Biologicals (NBP1-47483)** **Tissue: Pancreas** **Dilution:** **IHC: 1:200** **CODEX: 1:100** **CODEX oligo: 26-ATTO550**			
Antigen: T-bet **Clones: D6N8B** **Company:** **Cell Signaling Technology (custom)** **Tissue: Tonsil** **Dilution:** **IHC: 1:25** **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**			

Figure S2

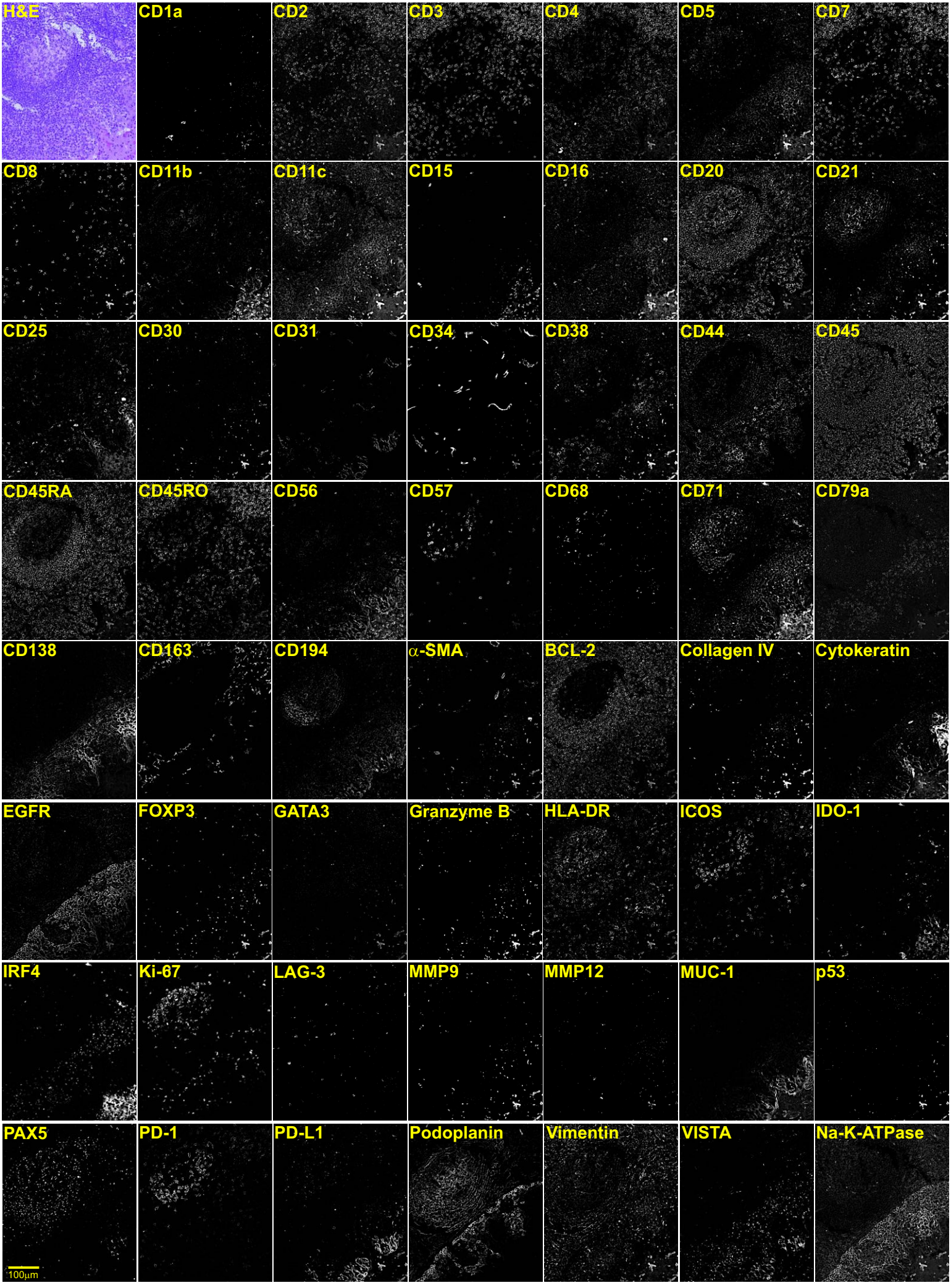

H&E
CD1a
CD2
CD3
CD4
CD5
CD7
CD8
CD11b
CD11c
CD15
CD16
CD20
CD21
CD25
CD30
CD31
CD34
CD38
CD44
CD45
CD45RA
CD45RO
CD56
CD57
CD68
CD71
CD79a
CD138
CD163
CD194
α-SMA
BCL-2
Collagen IV
Cytokeratin
EGFR
FOXP3
GATA3
Granzyme B
HLA-DR
ICOS
IDO-1
IRF4
Ki-67
LAG-3
MMP9
MMP12
MUC-1
p53
PAX5
PD-1
PD-L1
Podoplanin
Vimentin
VISTA
Na-K-ATPase
100μm

Figure S3

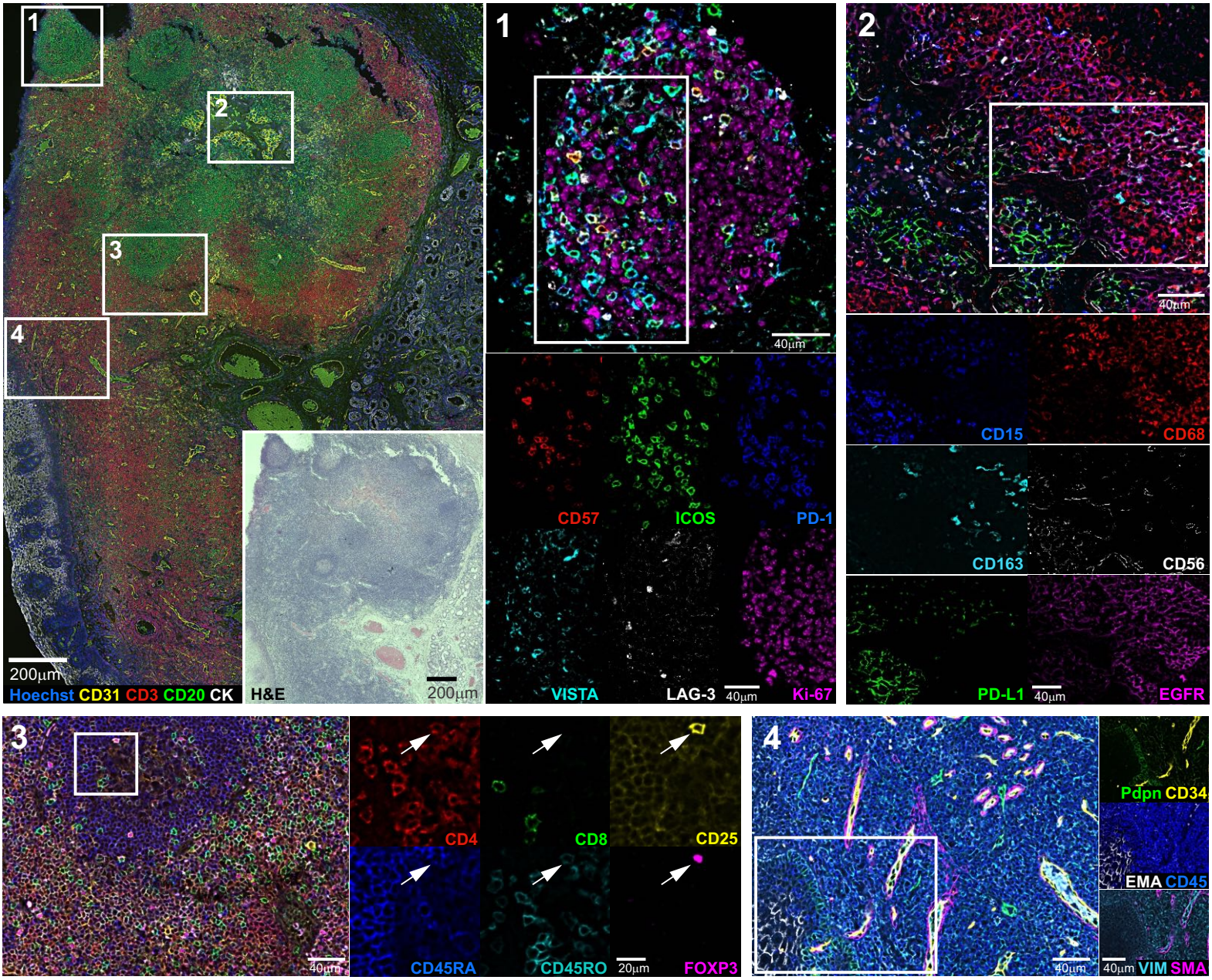
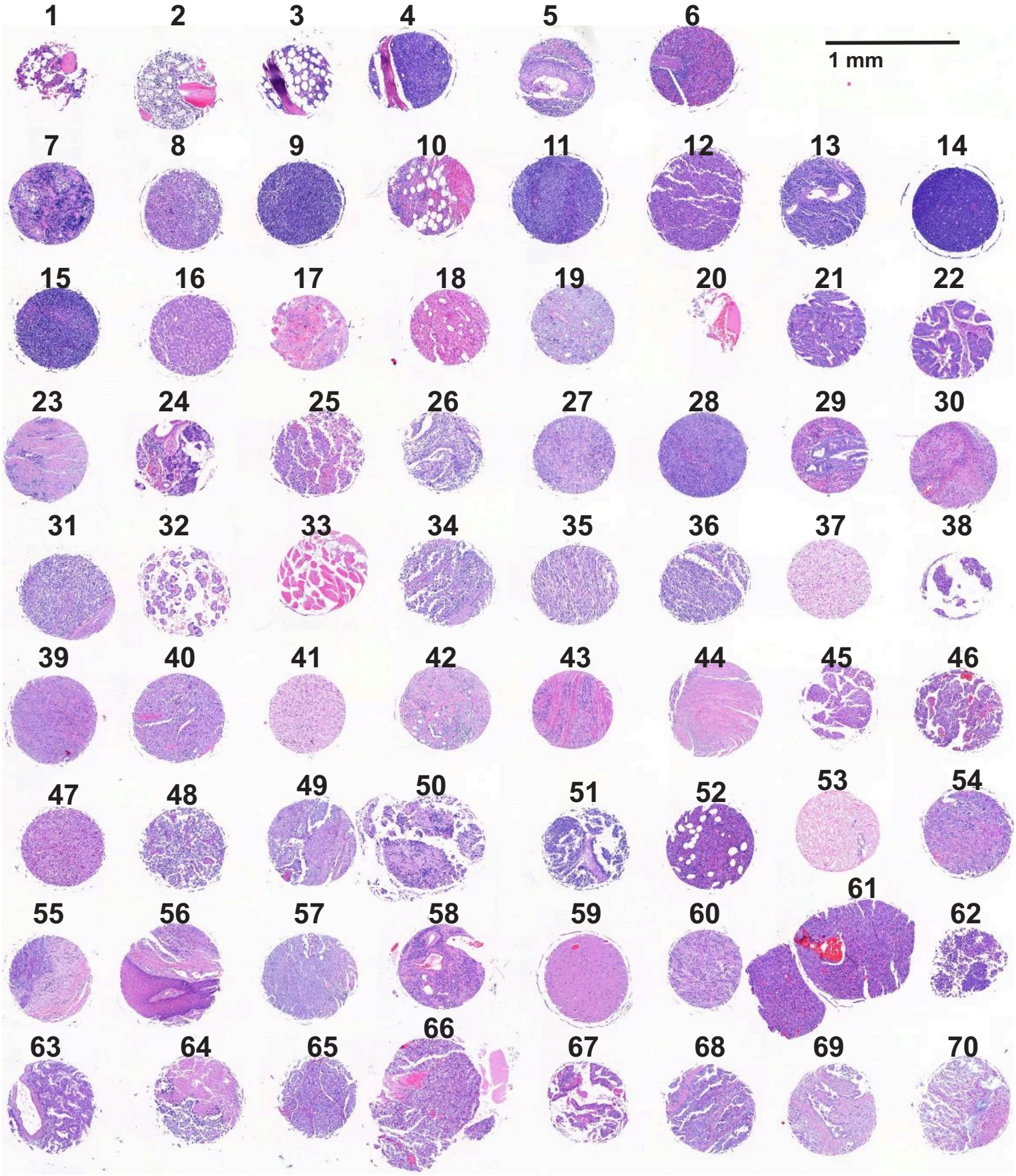

1
2
3
4
200μm
Hoechst CD31 CD3 CD20 CK
H&E
200μm
1
40μm
CD57
ICOS
PD-1
VISTA
LAG-3
40μm
Ki-67
2
40μm
CD15
CD68
CD163
CD56
PD-L1
40μm
EGFR
3
40μm
CD4
CD8
CD25
CD45RA
CD45RO
20μm
FOXP3
4
40μm
Pdpn CD34
EMA CD45
40μm
VIM SMA

Figure S4


1
2
3
4
5
6
1 mm
7
8
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10
11
12
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14
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16
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69
70

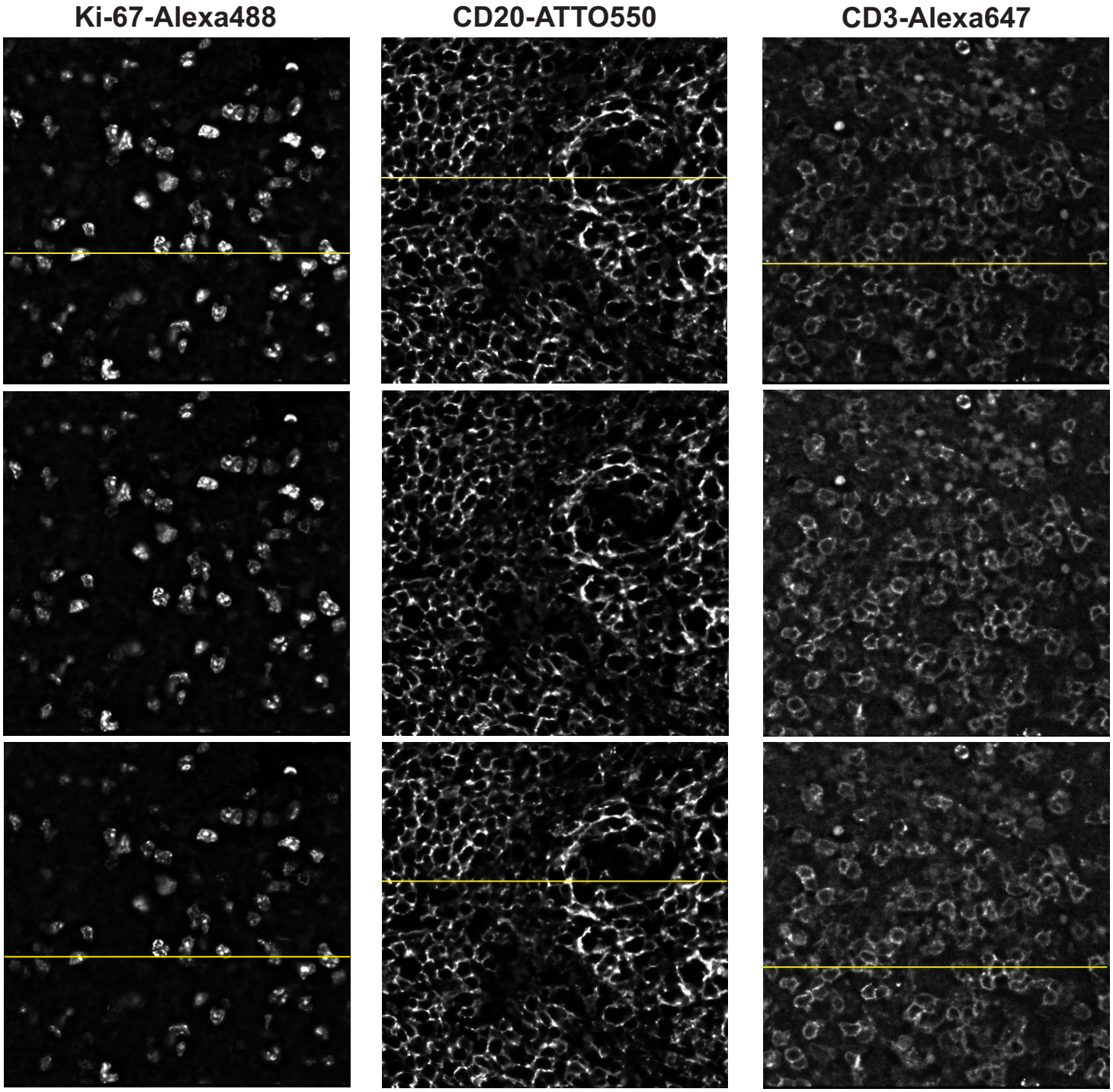
Figure S5

A

Cycles	Channel 1	Alexa488	ATTO550	Alexa647
1, 11, 21, 31, 32, 33	HOECHST	-	-	-
2, 5, 8, 12, 15, 18, 22, 25, 28	HOECHST	HLA-DR	CD45	FOXP3
3, 6, 9, 13, 16, 19, 23, 26, 29	HOECHST	Na-K-ATPase	Pan-Cytokeratin	p53
4, 7, 10, 14, 17, 20, 24, 27, 30	HOECHST	Ki-67	CD20	CD3

repeat

B



C

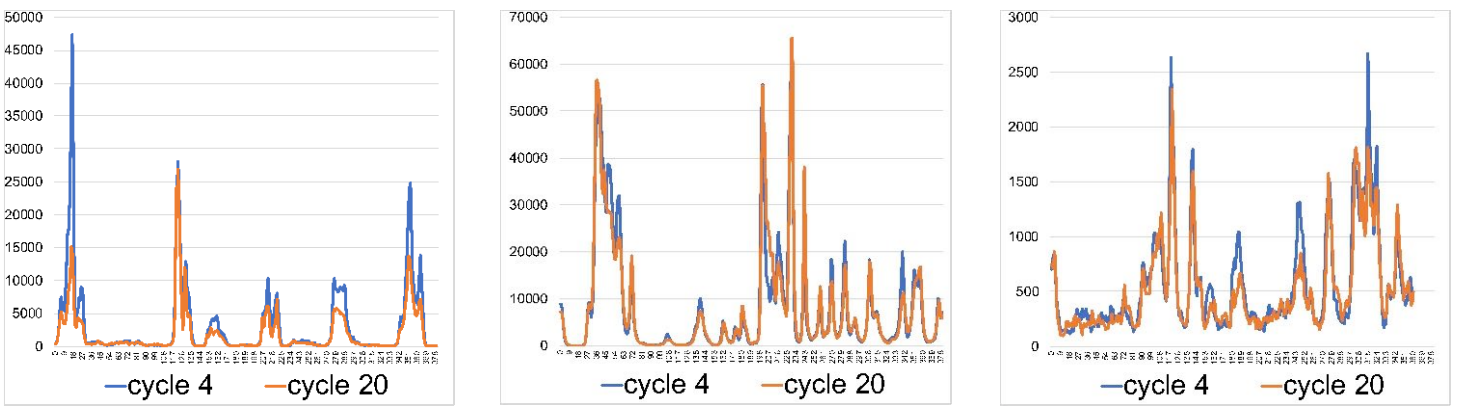


Figure S6

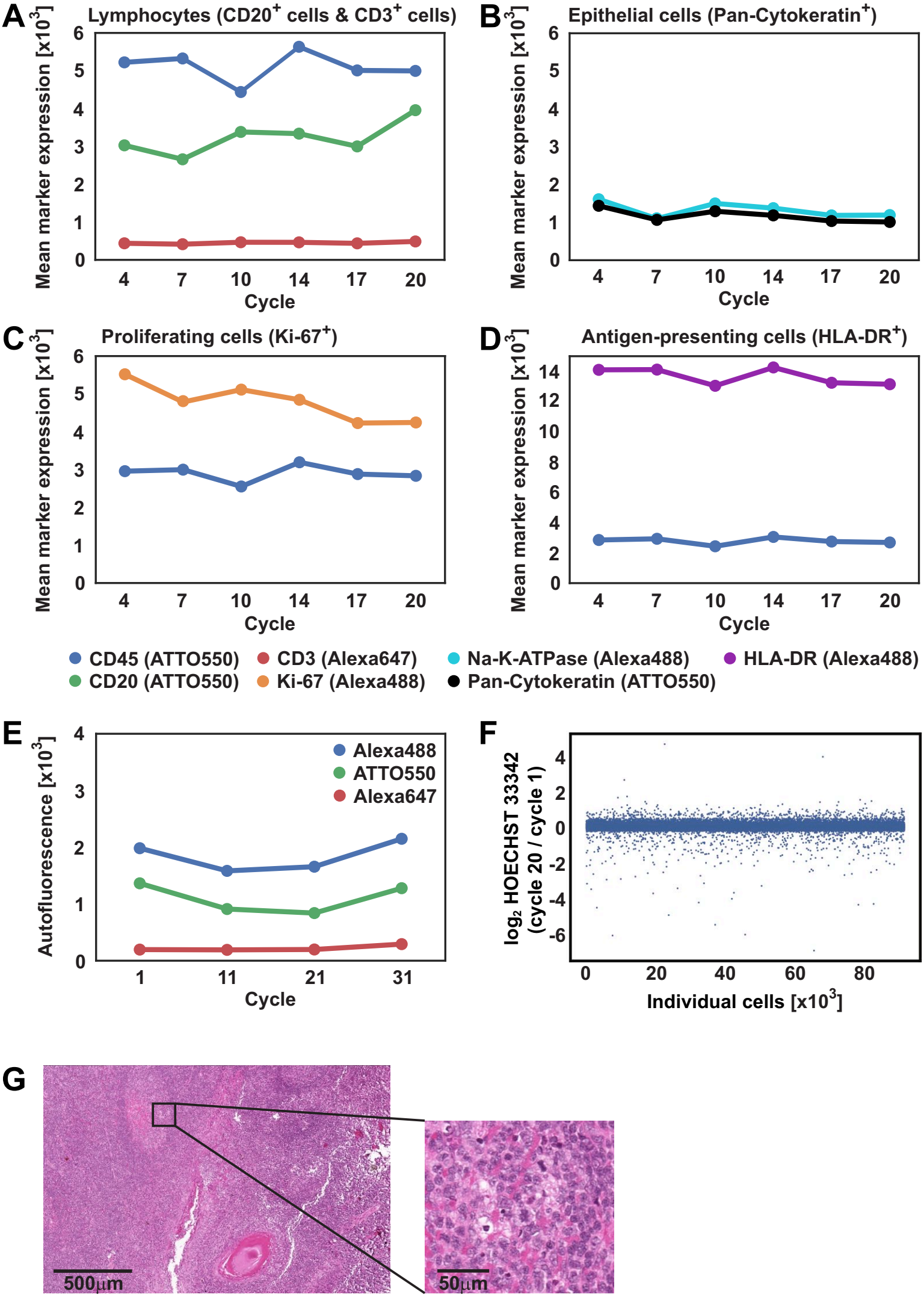
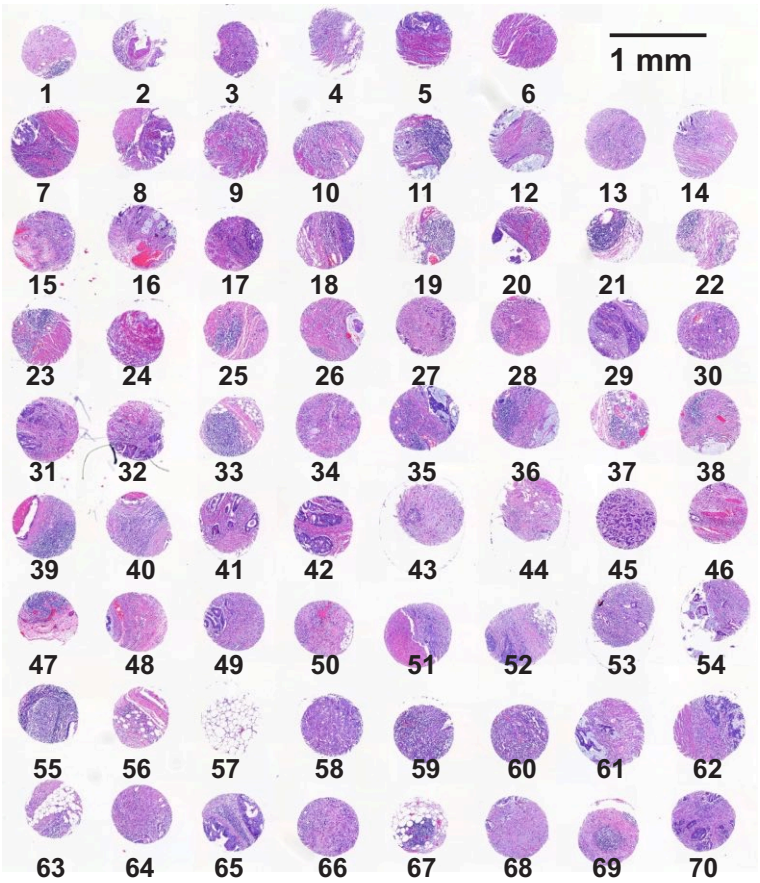

A
Lymphocytes (CD20+ cells & CD3+ cells)
Mean marker expression [x10^3]
Cycle
B
Epithelial cells (Pan-Cytokeratin+)
C
Proliferating cells (Ki-67+)
D
Antigen-presenting cells (HLA-DR+)
CD45 (ATTO550)
CD20 (ATTO550)
CD3 (Alexa647)
Ki-67 (Alexa488)
Na-K-ATPase (Alexa488)
Pan-Cytokeratin (ATTO550)
HLA-DR (Alexa488)
E
Autofluorescence [x10^3]
Alexa488
ATTO550
Alexa647
F
log2 HOECHST 33342 (cycle 20 / cycle 1)
Individual cells [x10^3]
G
500µm
50µm

Figure S7


A
1 mm
1 2 3 4 5 6
7 8 9 10 11 12 13 14
15 16 17 18 19 20 21 22
23 24 25 26 27 28 29 30
31 32 33 34 35 36 37 38
39 40 41 42 43 44 45 46
47 48 49 50 51 52 53 54
55 56 57 58 59 60 61 62
63 64 65 66 67 68 69 70

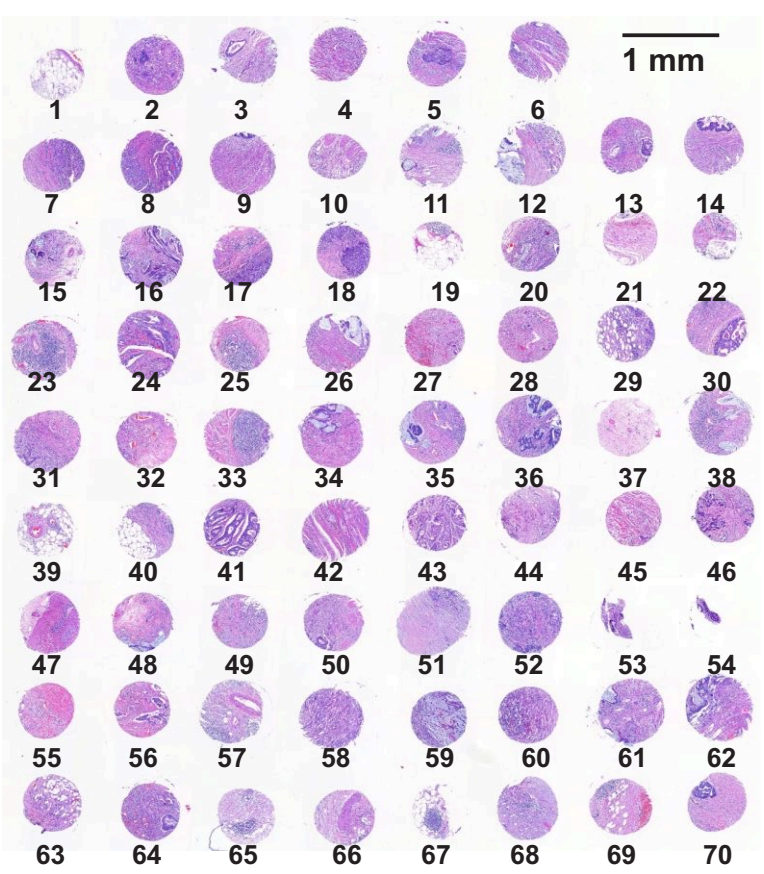

B
1 mm
1 2 3 4 5 6
7 8 9 10 11 12 13 14
15 16 17 18 19 20 21 22
23 24 25 26 27 28 29 30
31 32 33 34 35 36 37 38
39 40 41 42 43 44 45 46
47 48 49 50 51 52 53 54
55 56 57 58 59 60 61 62
63 64 65 66 67 68 69 70

Figure S8

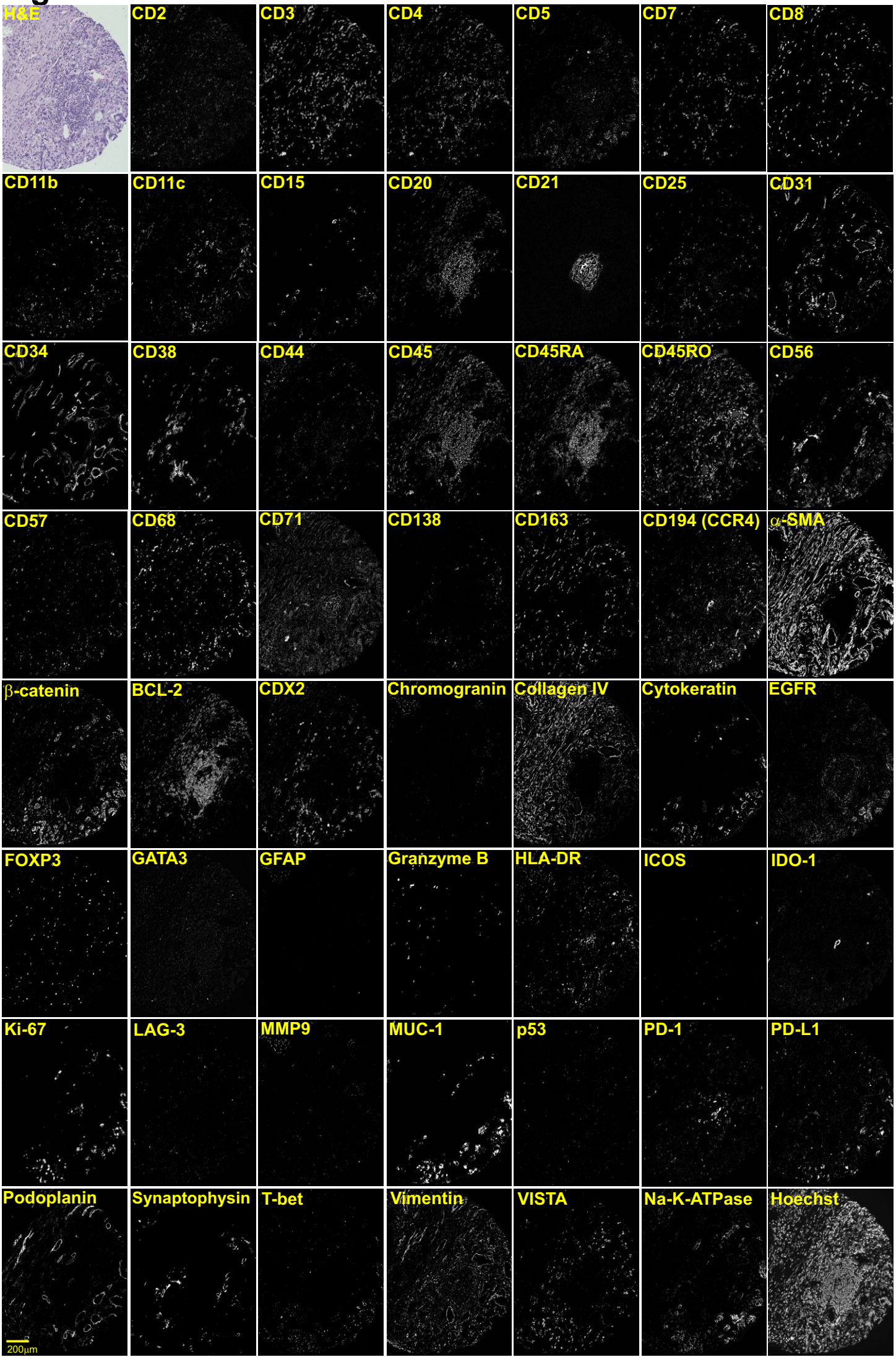

H&E
CD2
CD3
CD4
CD5
CD7
CD8
CD11b
CD11c
CD15
CD20
CD21
CD25
CD31
CD34
CD38
CD44
CD45
CD45RA
CD45RO
CD56
CD57
CD68
CD71
CD138
CD163
CD194 (CCR4)
α-SMA
β-catenin
BCL-2
CDX2
Chromogranin
Collagen IV
Cytokeratin
EGFR
FOXP3
GATA3
GFAP
Granzyme B
HLA-DR
ICOS
IDO-1
Ki-67
LAG-3
MMP9
MUC-1
p53
PD-1
PD-L1
Podoplanin
Synaptophysin
T-bet
Vimentin
VISTA
Na-K-ATPase
Hoechst
200μm

Figure S9

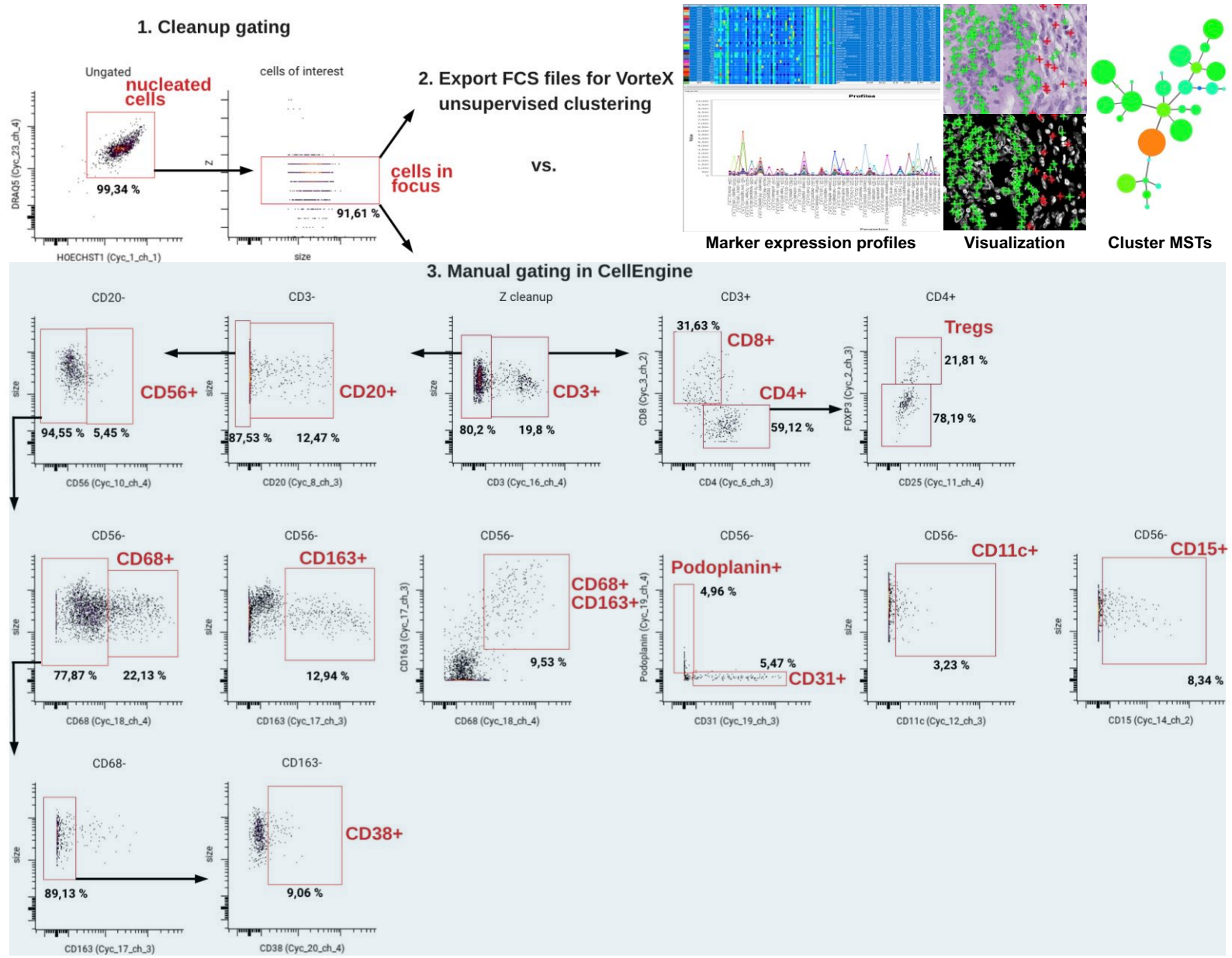
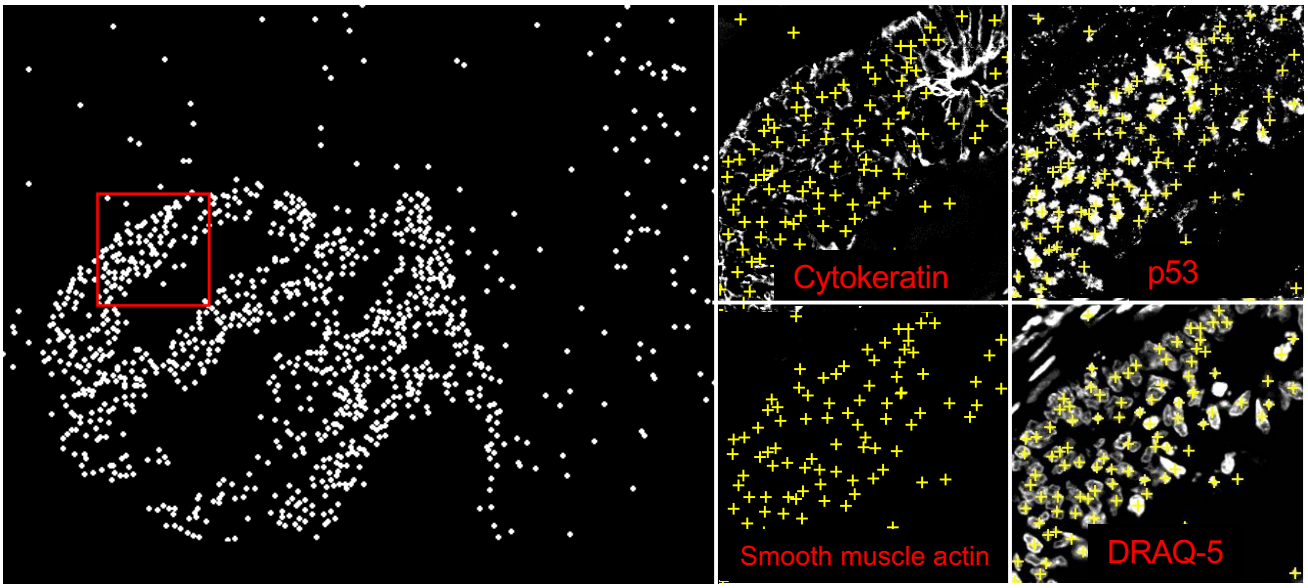
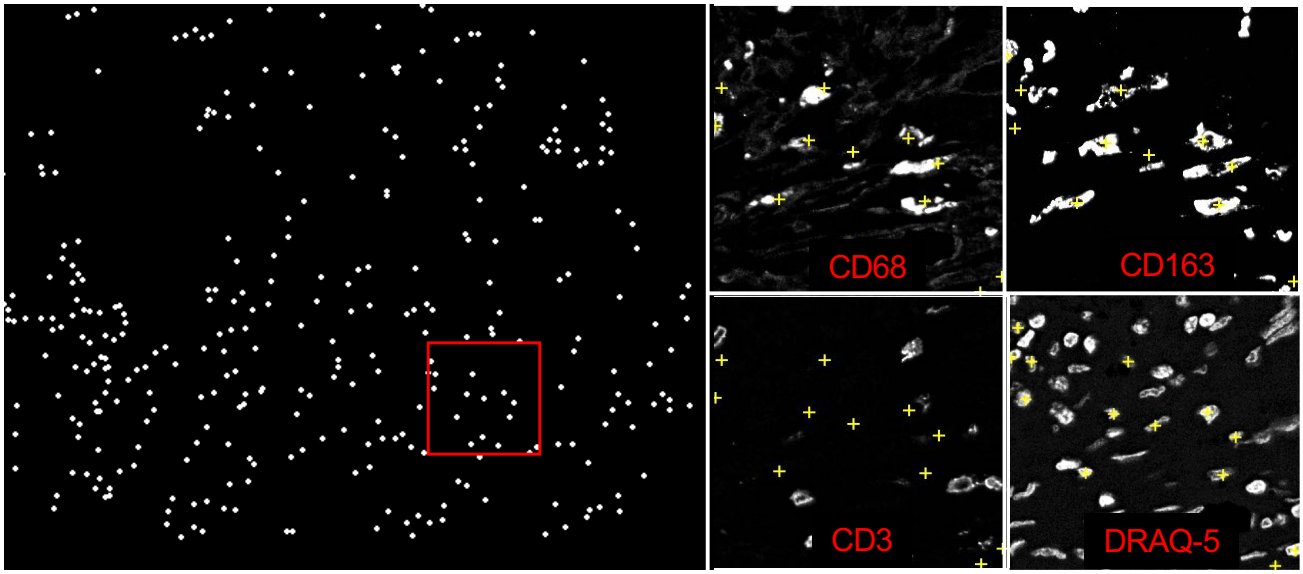

1. Cleanup gating
Ungated
nucleated cells
cells of interest
99,34 %
91,61 %
DRAQ5 (Cyc_23_ch_4)
HOECHST1 (Cyc_1_ch_1)
Z
size
cells in focus
2. Export FCS files for VorteX unsupervised clustering
vs.
Marker expression profiles
Visualization
Cluster MSTs
3. Manual gating in CellEngine
CD20-
CD56+
94,55 %
5,45 %
CD56 (Cyc_10_ch_4)
CD3-
CD20+
87,53 %
12,47 %
CD20 (Cyc_8_ch_3)
Z cleanup
CD3+
80,2 %
19,8 %
CD3 (Cyc_16_ch_4)
CD3+
CD8+
31,63 %
CD4+
59,12 %
CD8 (Cyc_3_ch_2)
CD4 (Cyc_6_ch_3)
CD4+
Tregs
21,81 %
78,19 %
FOXP3 (Cyc_2_ch_3)
CD25 (Cyc_11_ch_4)
CD56-
CD68+
77,87 %
22,13 %
CD68 (Cyc_18_ch_4)
CD56-
CD163+
12,94 %
CD163 (Cyc_17_ch_3)
CD56-
CD68+
CD163+
9,53 %
CD163 (Cyc_17_ch_3)
CD68 (Cyc_18_ch_4)
CD56-
Podoplanin+
4,96 %
5,47 %
CD31+
Podoplanin (Cyc_19_ch_4)
CD31 (Cyc_19_ch_3)
CD56-
CD11c+
3,23 %
CD11c (Cyc_12_ch_3)
CD56-
CD15+
8,34 %
CD15 (Cyc_14_ch_2)
CD68-
89,13 %
CD163 (Cyc_17_ch_3)
CD163-
CD38+
9,06 %
CD38 (Cyc_20_ch_4)

Figure S10

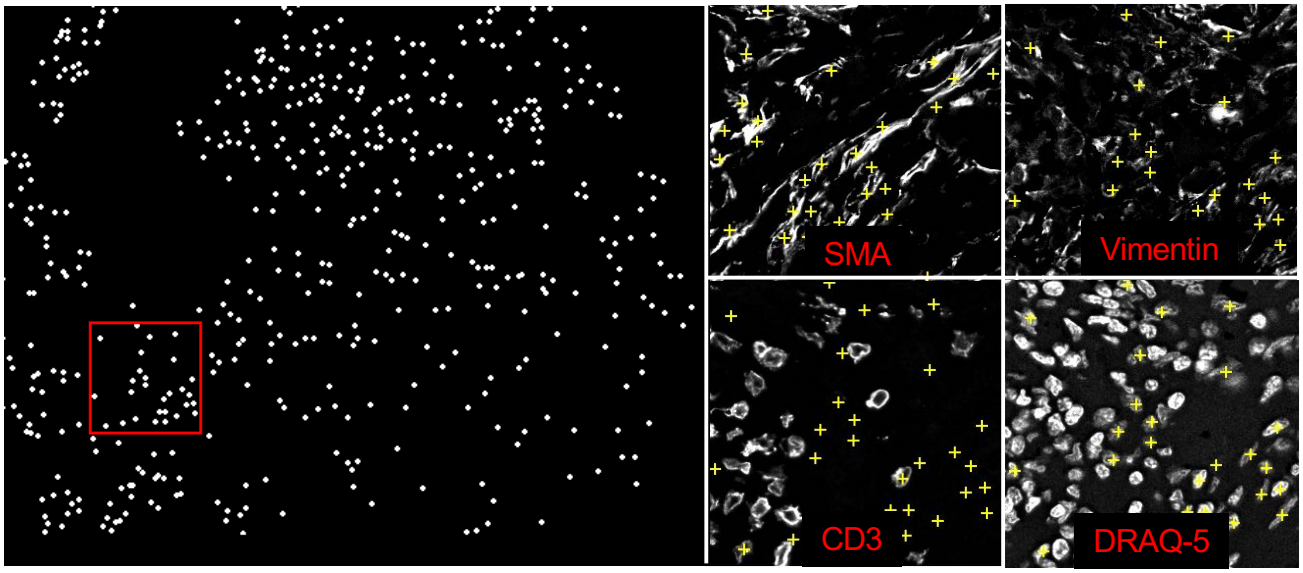
A. Tumor cell cluster



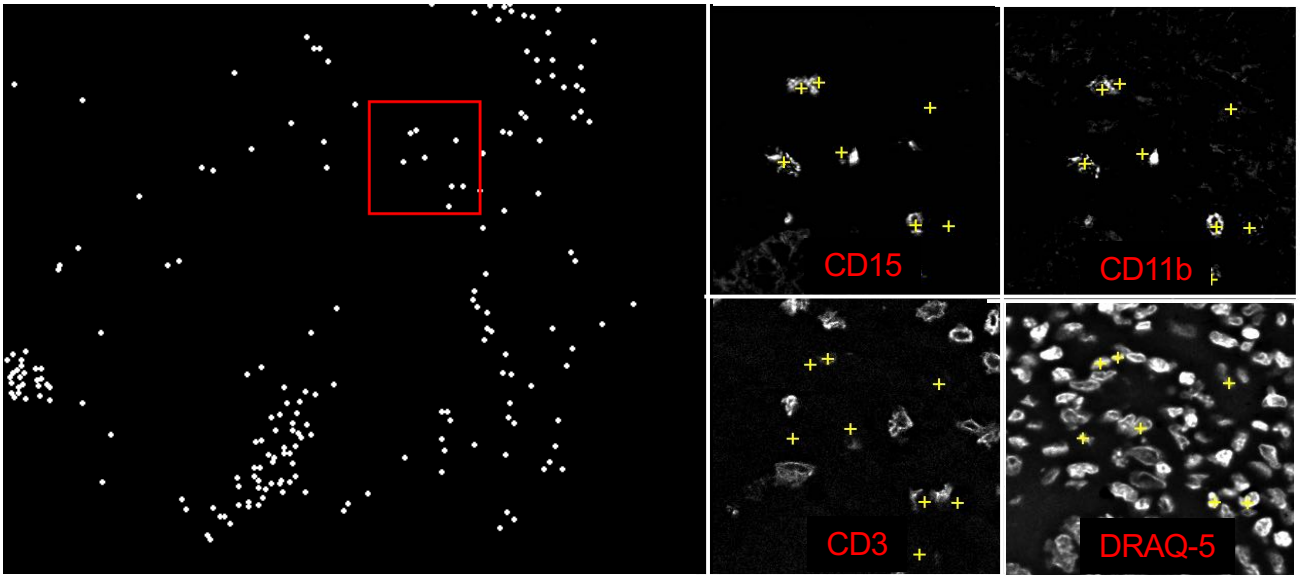
B. CD68+CD163+ macrophage cluster



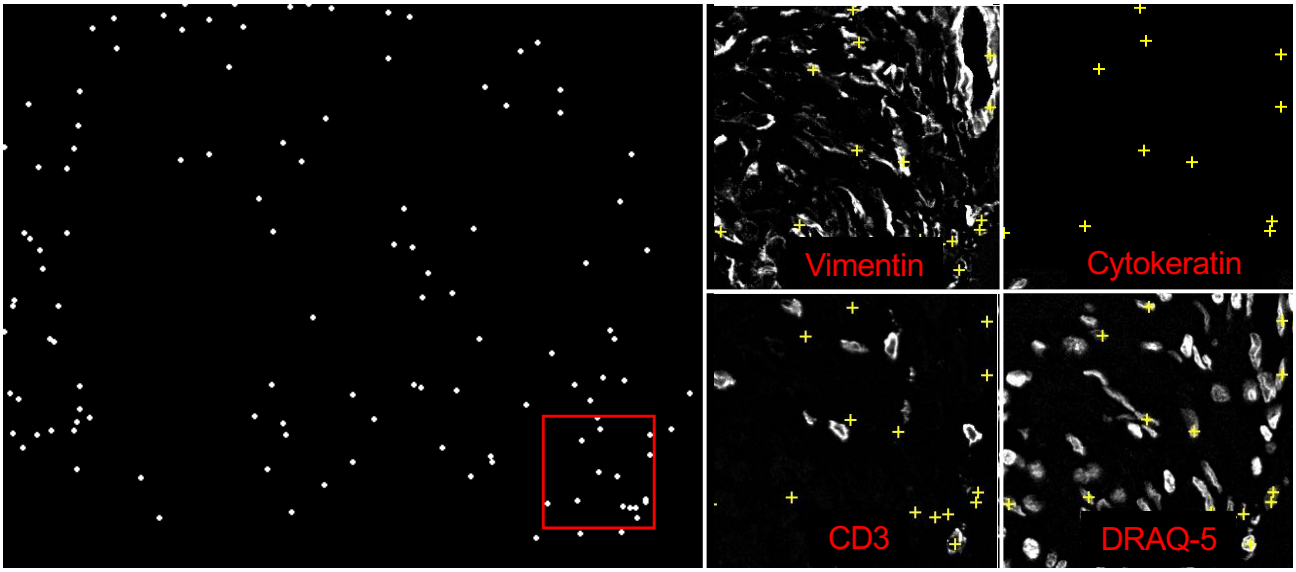
C. Smooth muscle cluster



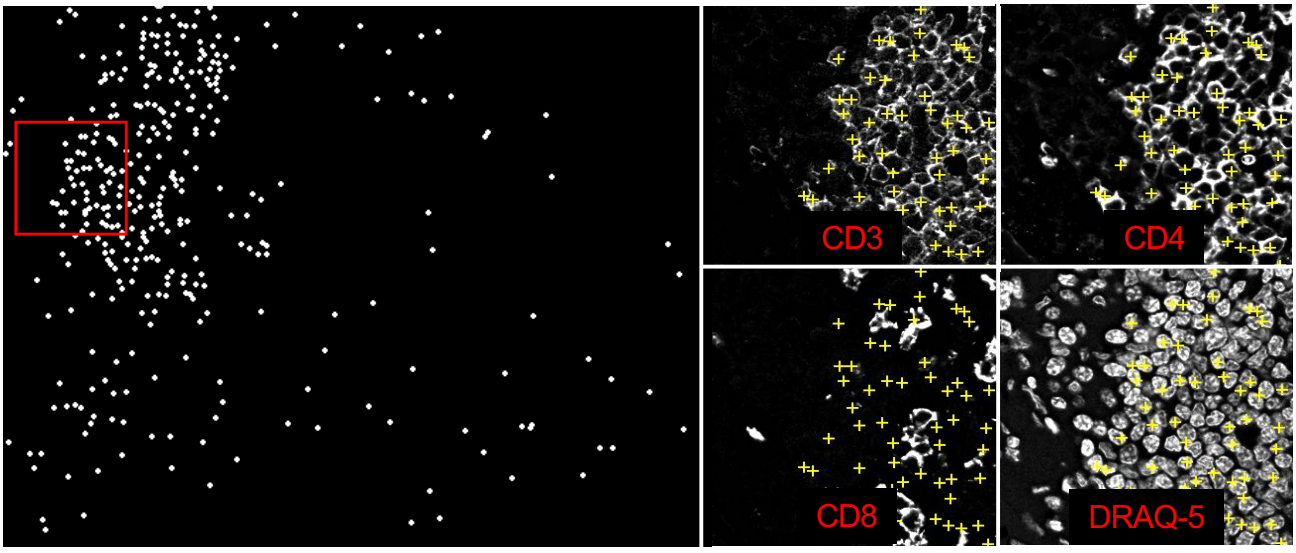
D. Granulocyte cluster



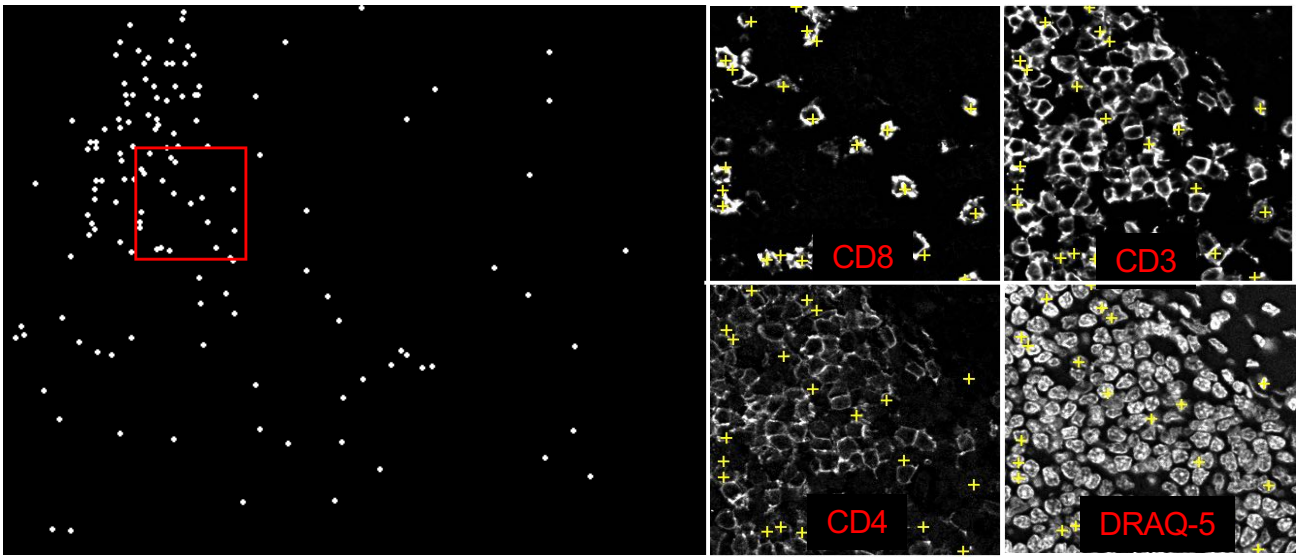
E. Stroma cluster



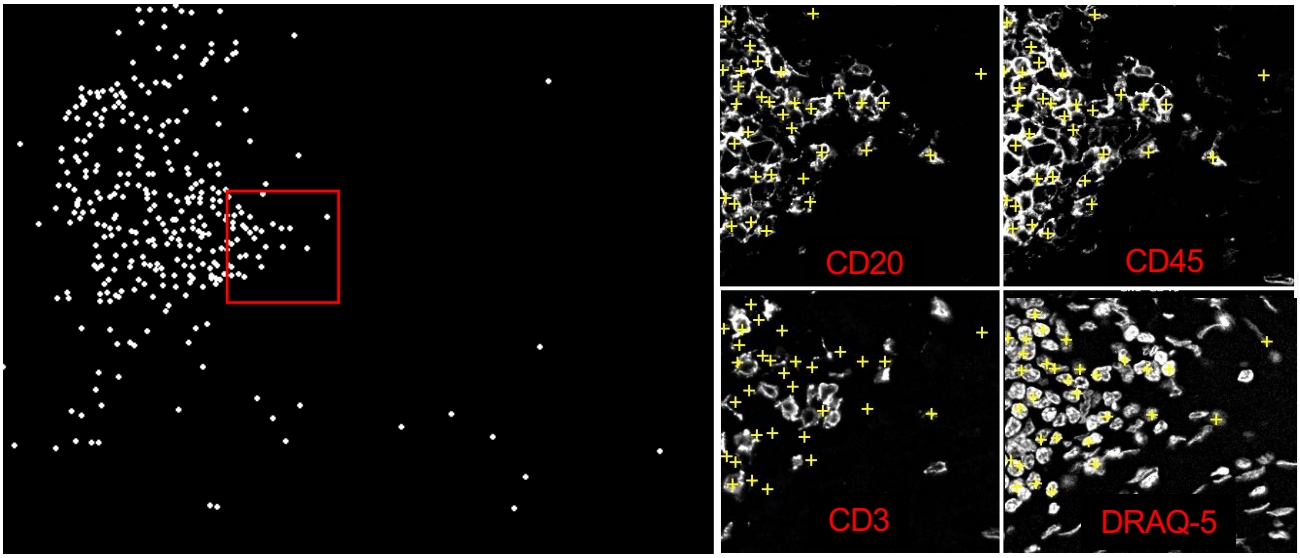
F. CD4+CD45RO+ T cell cluster



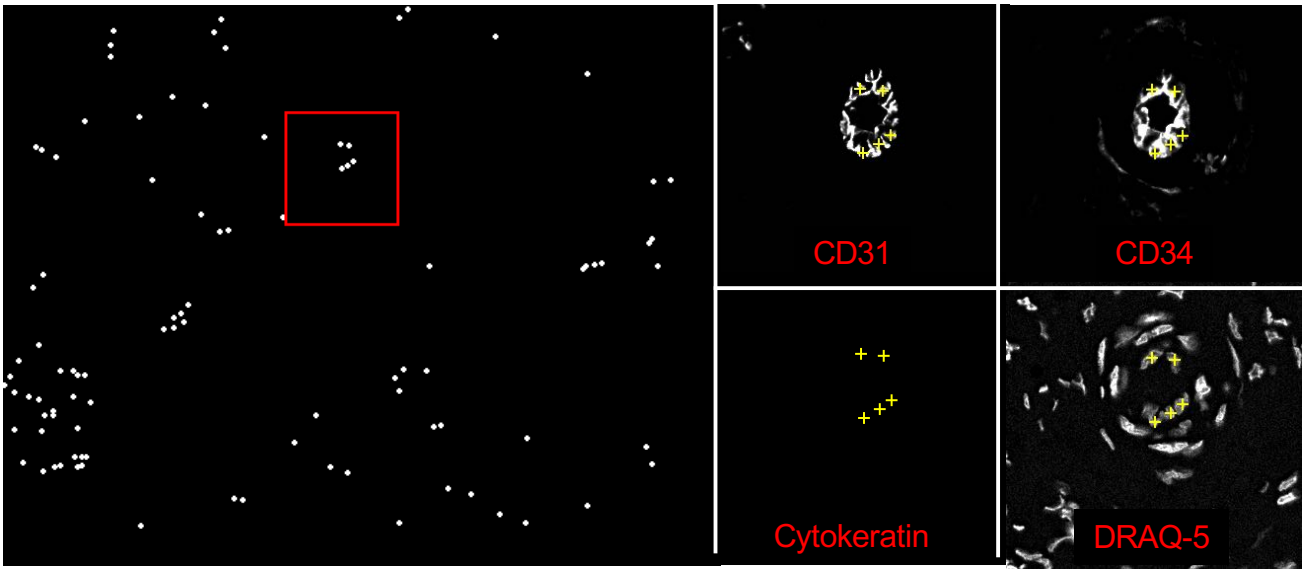
G. CD8+ T cell cluster



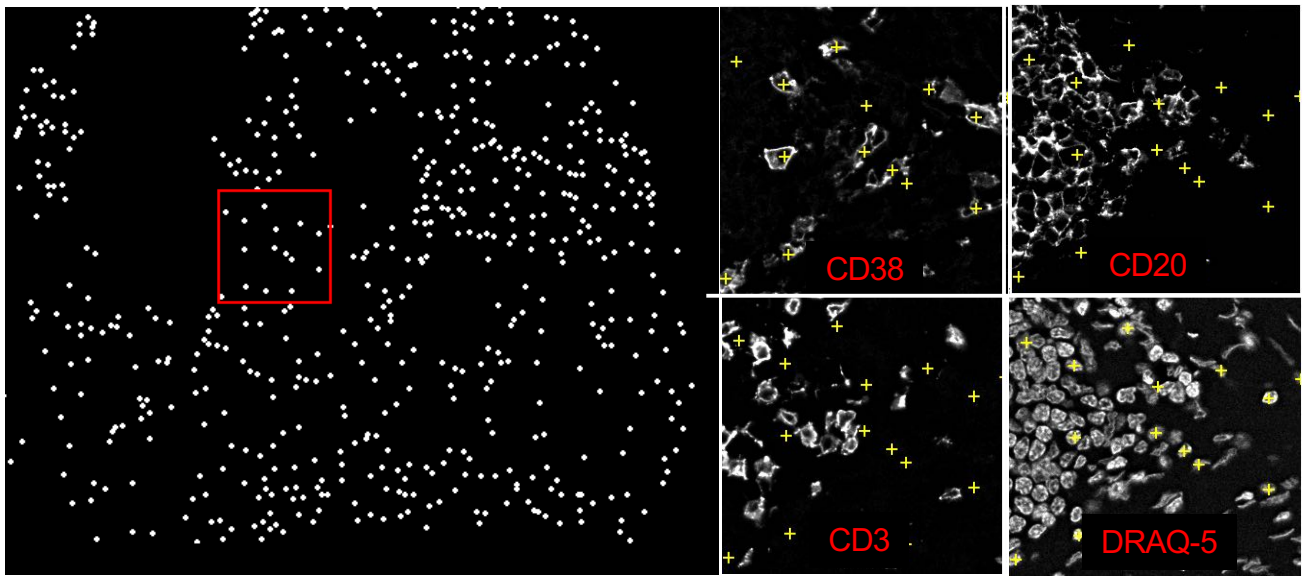
H. B cell cluster



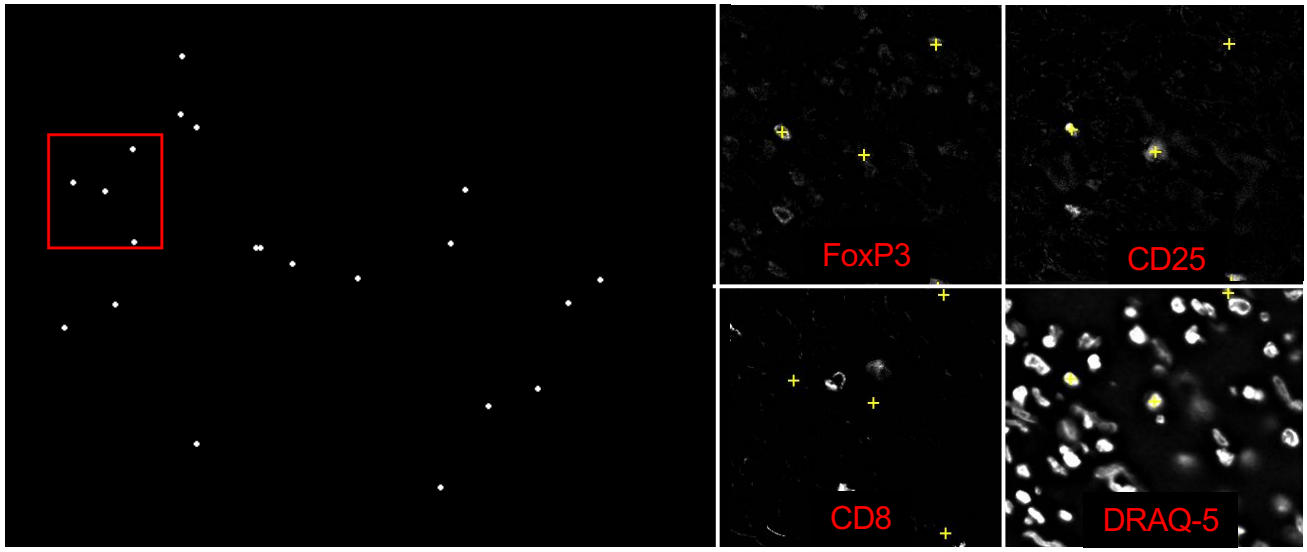
I. Vasculature cluster



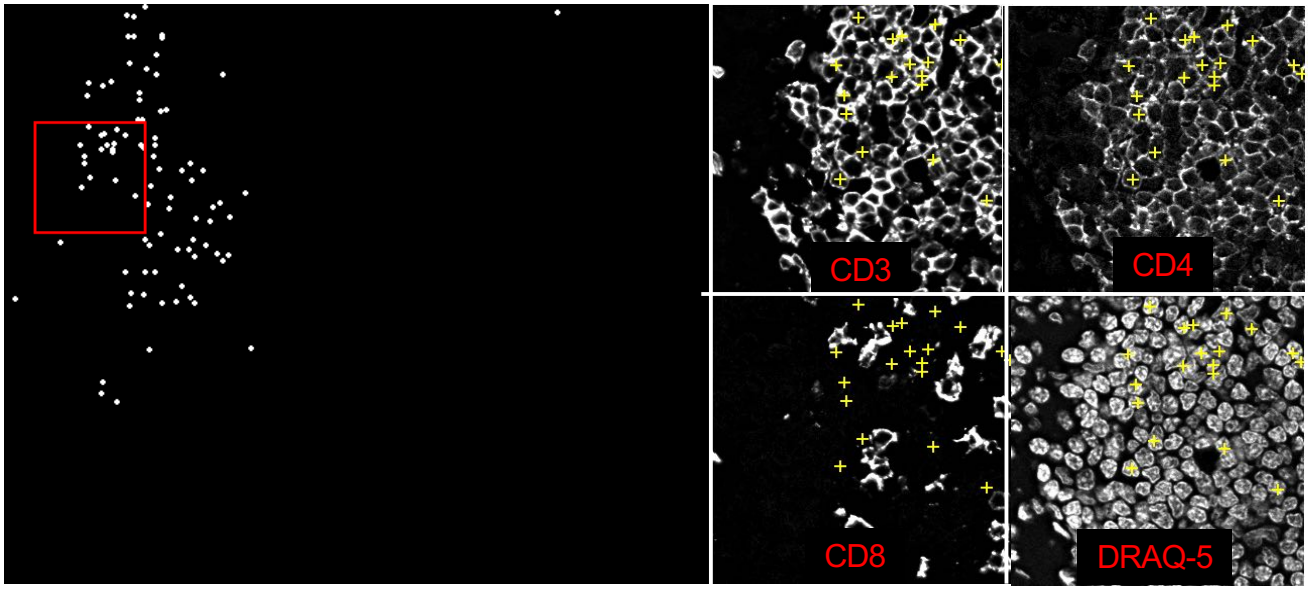
J. Plasma cell cluster



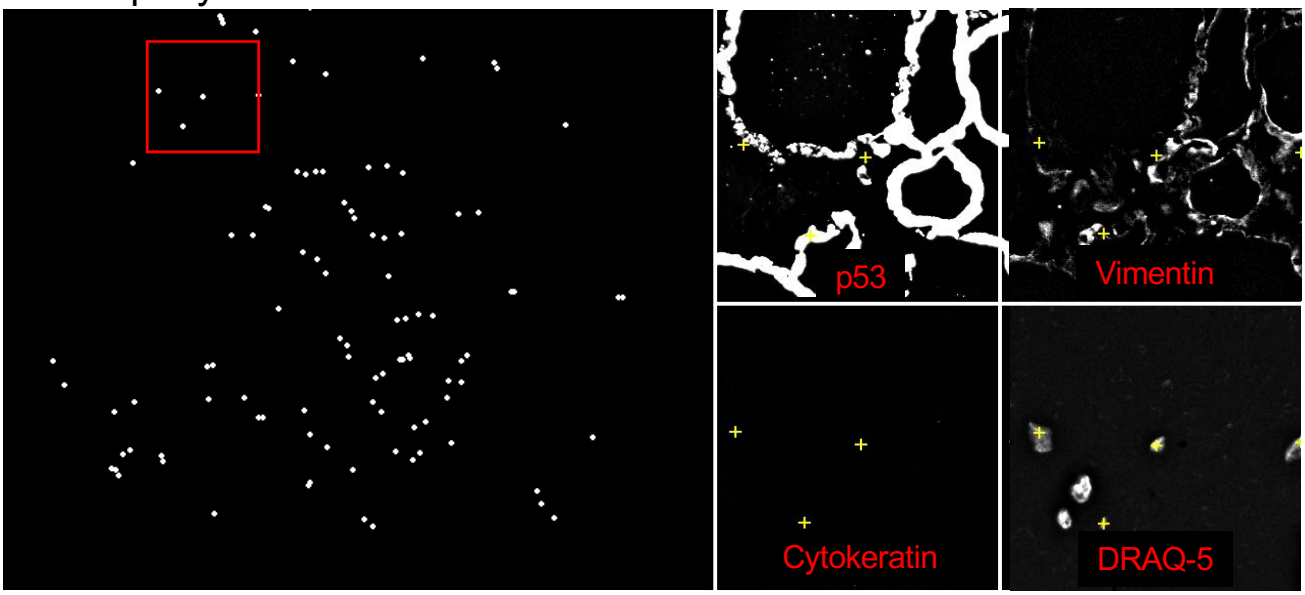
K. Treg cluster



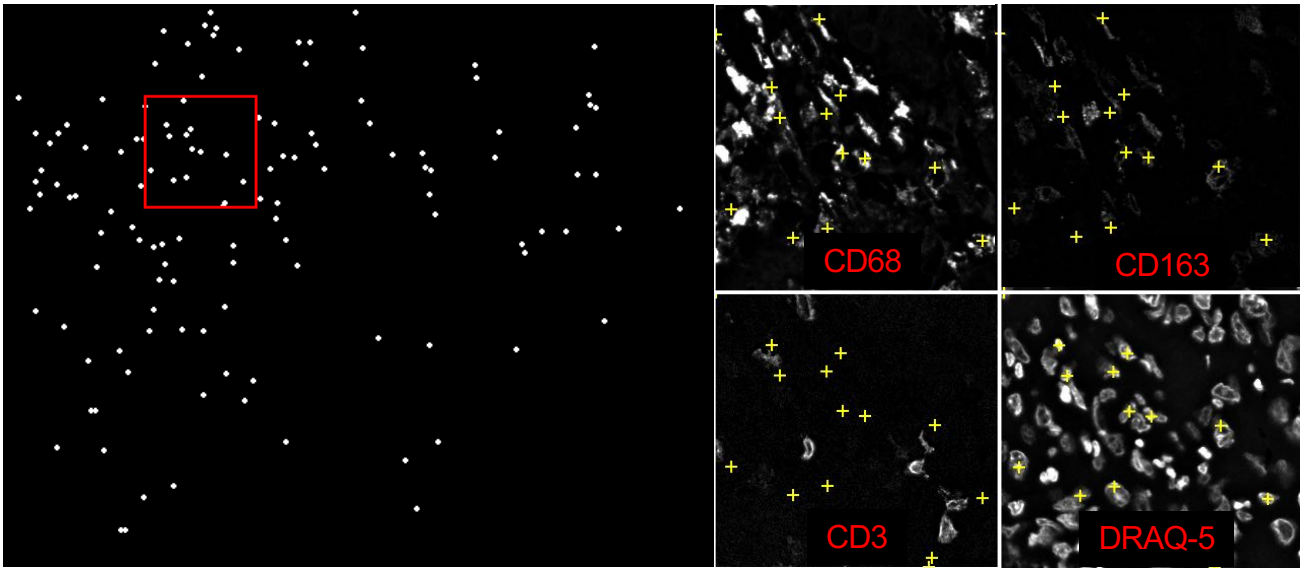
L. CD4+ T cell cluster



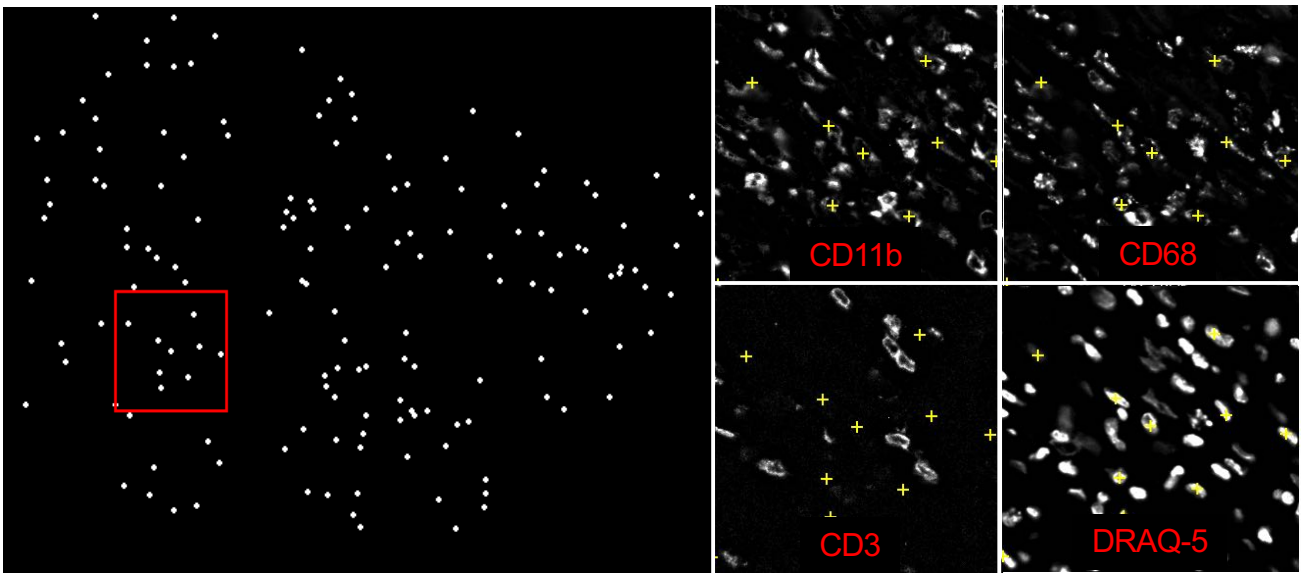
M. Adipocyte cluster



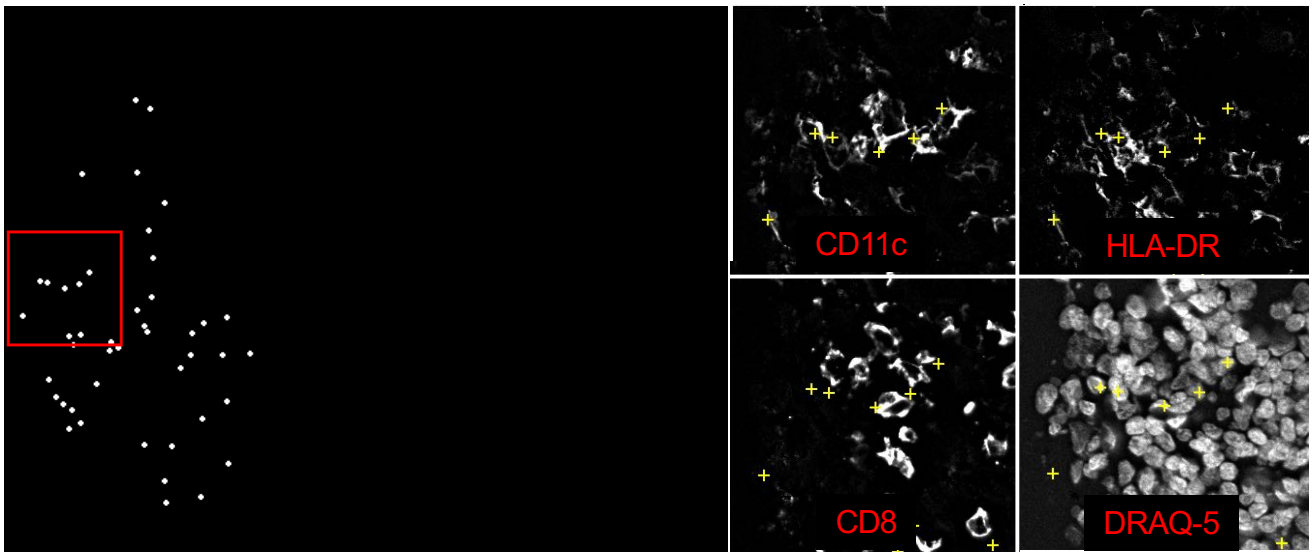
N. CD68+ macrophage cluster



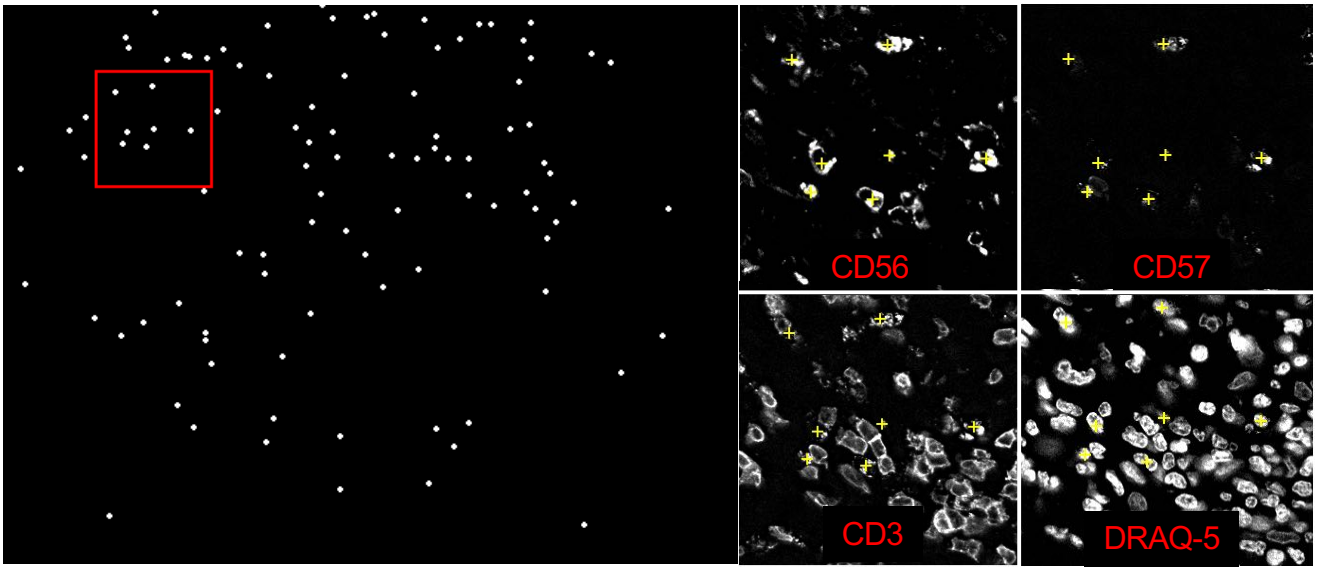
O. CD11b+CD68+ macrophage cluster



P. CD11c dendritic cell cluster



Q. NK cell cluster



R. CD3+ T cell cluster

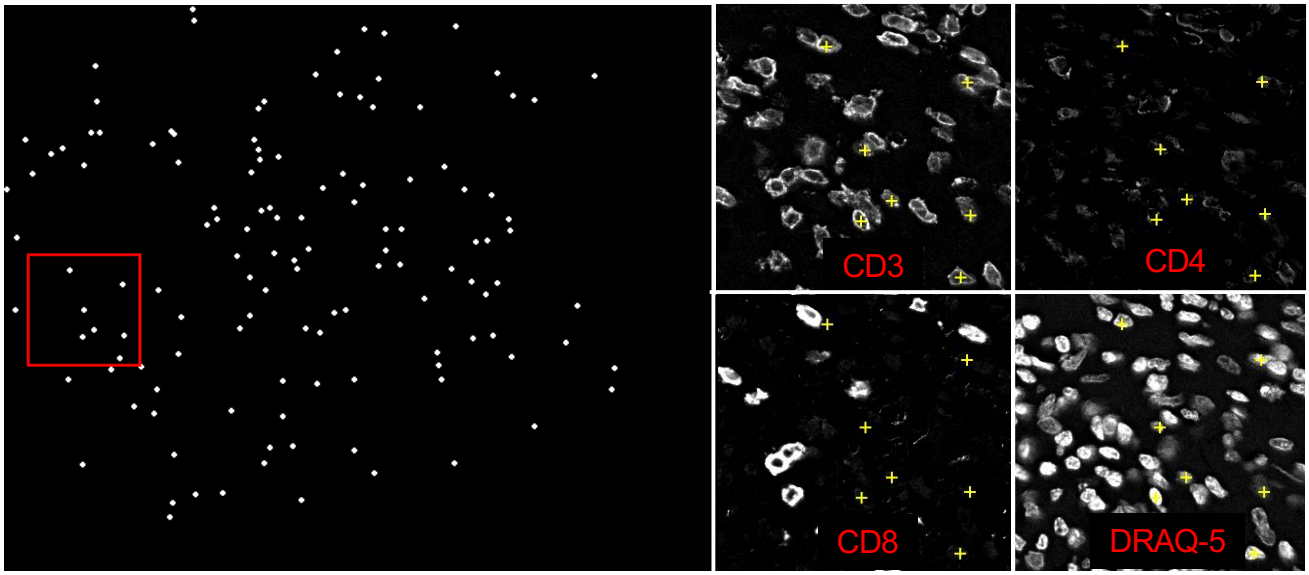
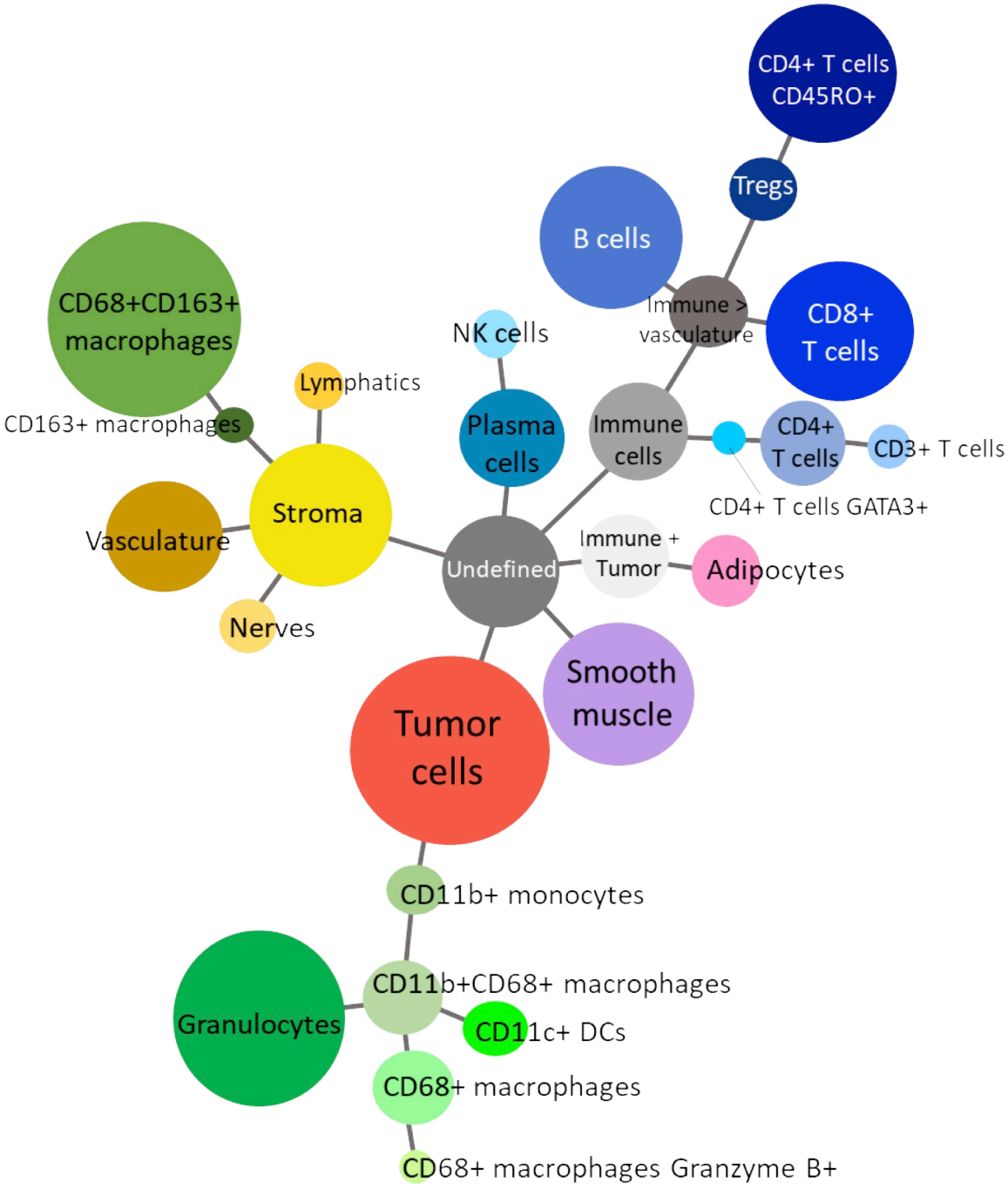


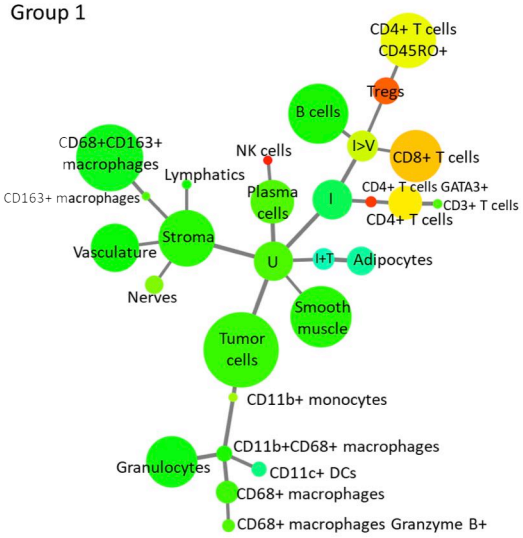
Figure S11



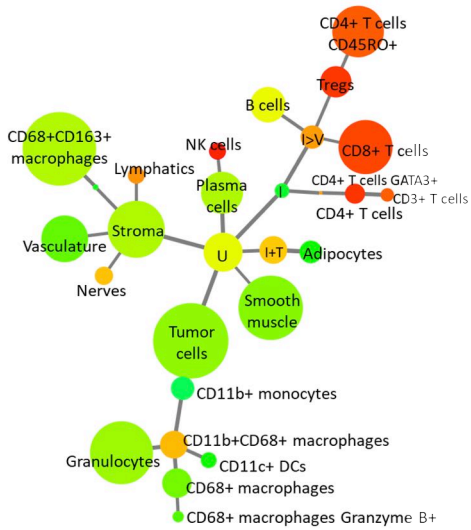
28 unique clusters

CD2

Group 1

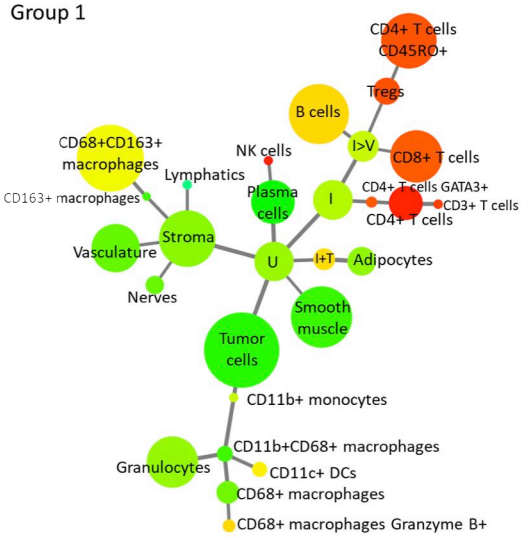


Group 2



CD3

Group 1



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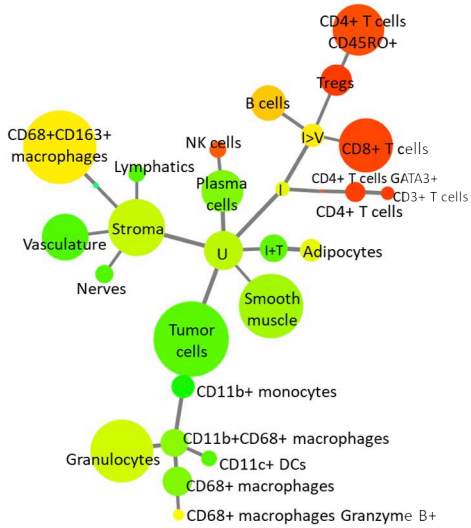
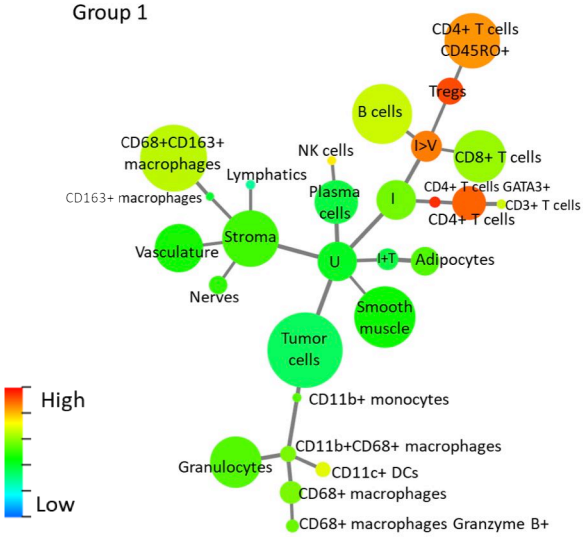
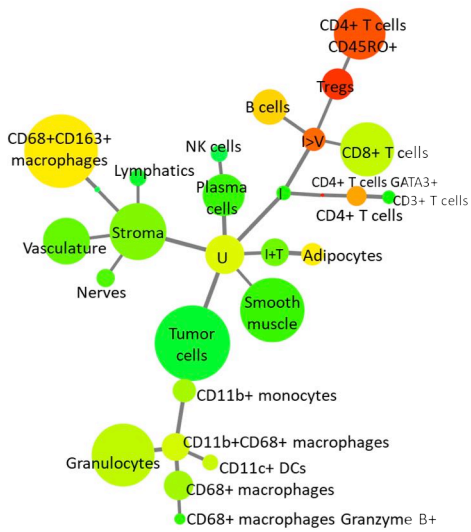


CD4

Group 1

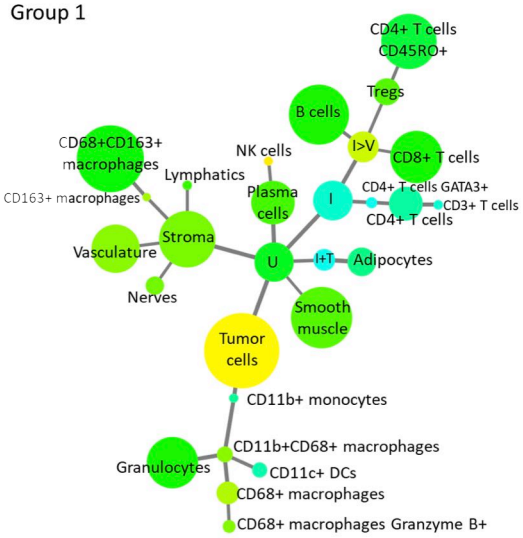


Group 2

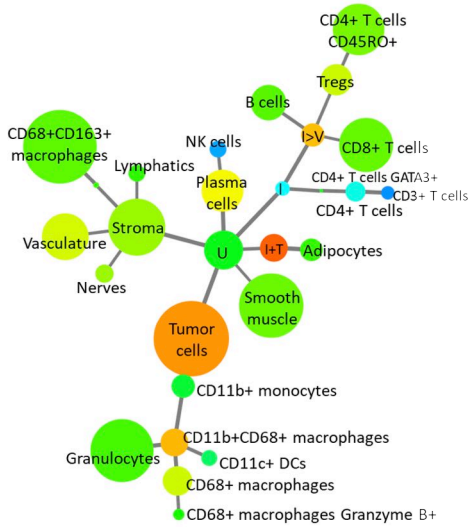


CD5

Group 1

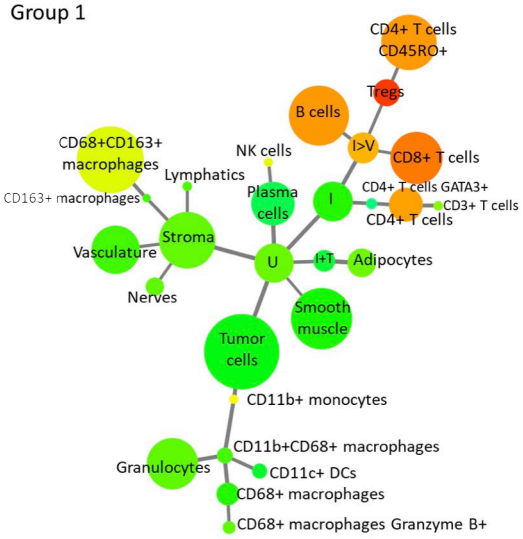


Group 2



CD7

Group 1



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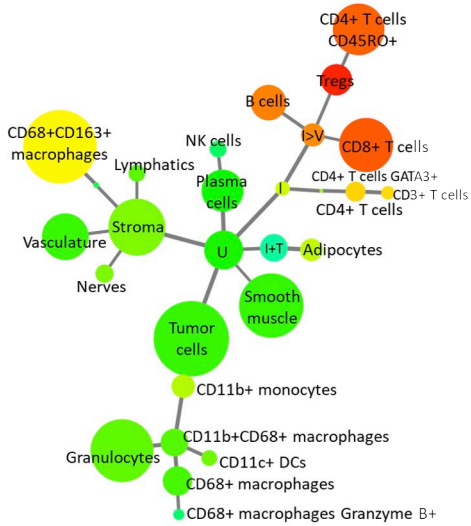
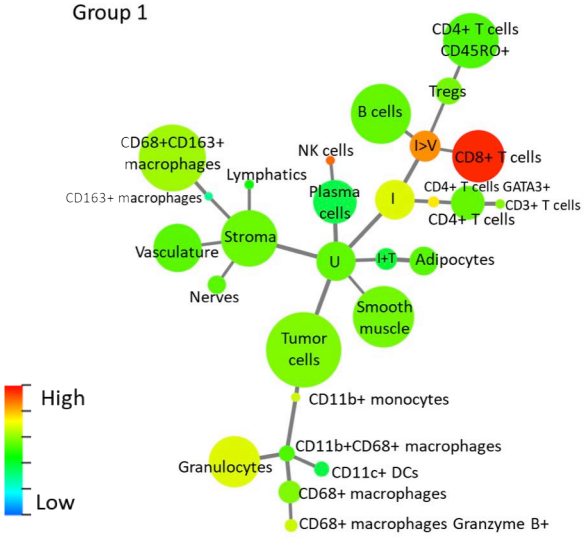
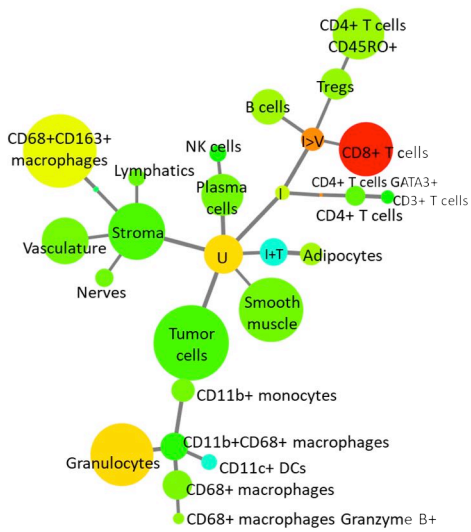


CD8

Group 1

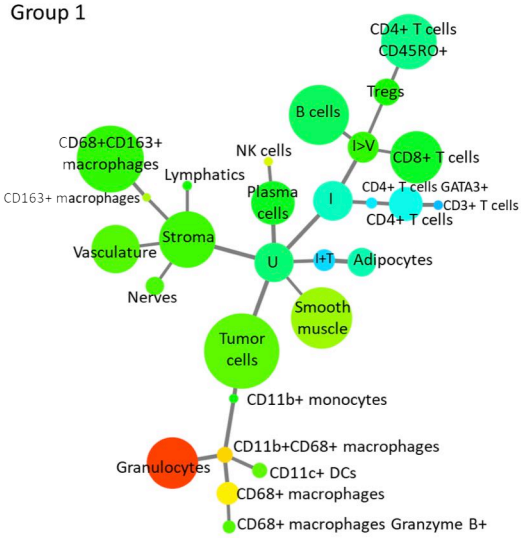


Group 2

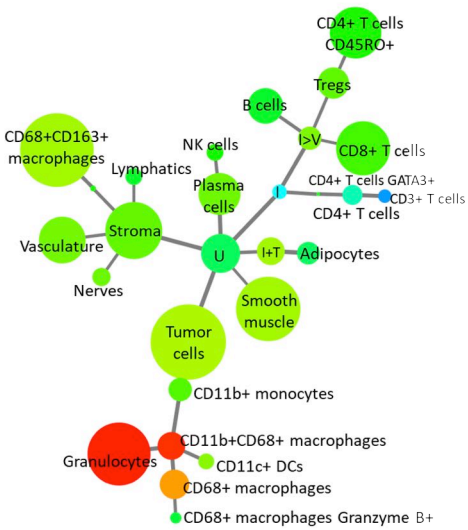


CD11b

Group 1

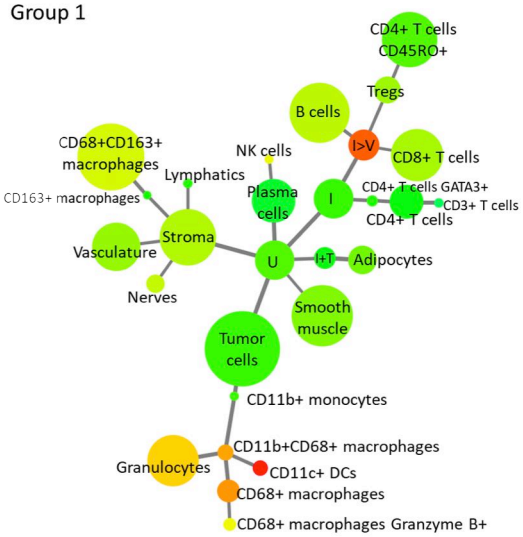


Group 2



CD11c

Group 1



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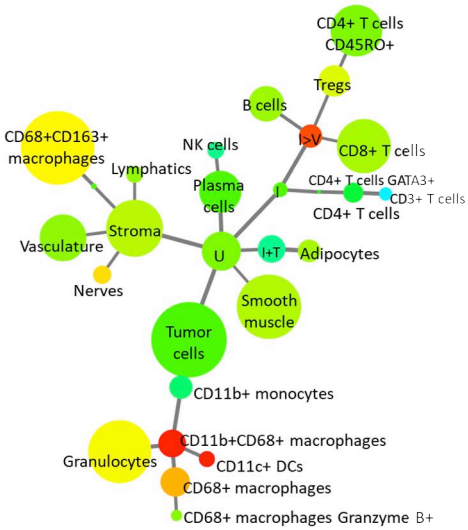
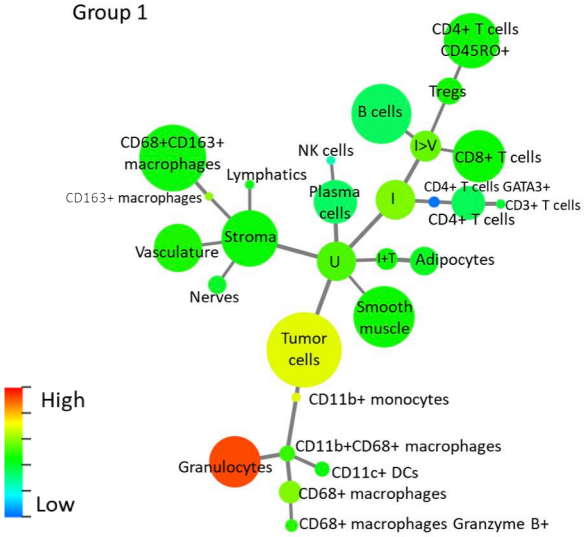


CD15

Group 1



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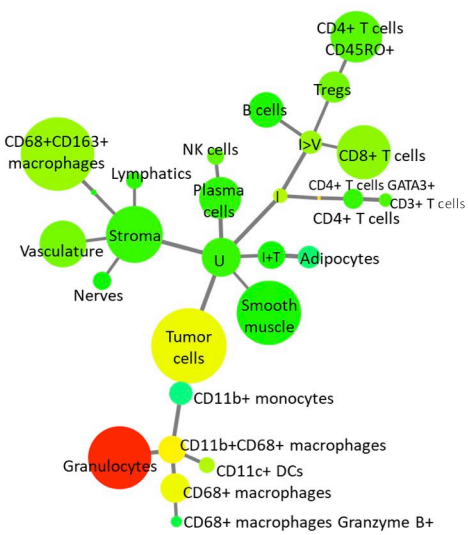
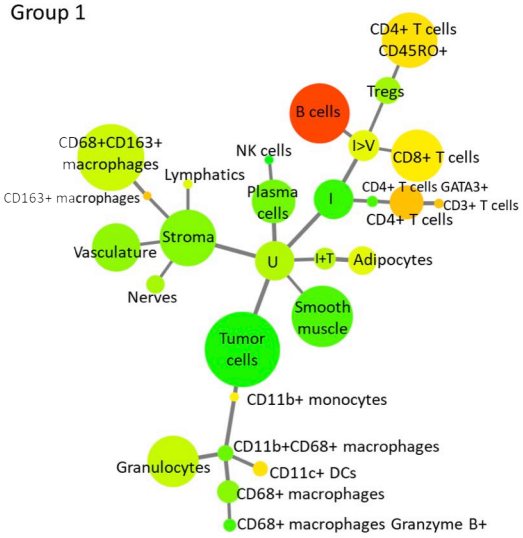


CD20

Group 1



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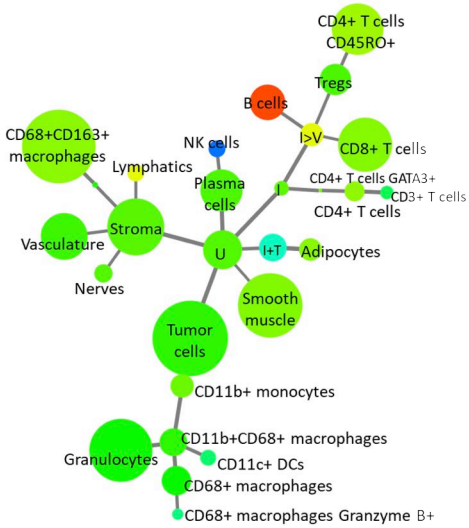
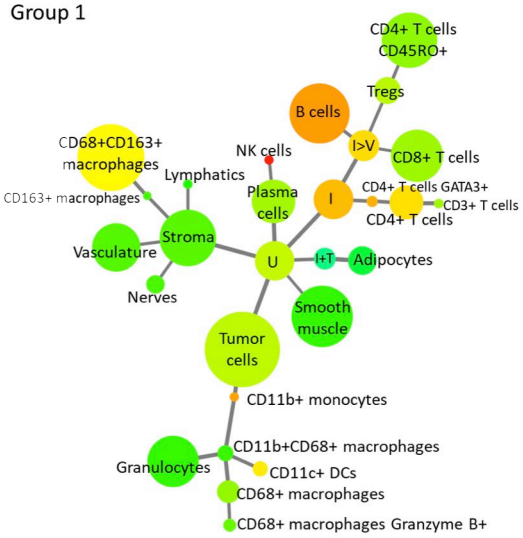


CD21

Group 1



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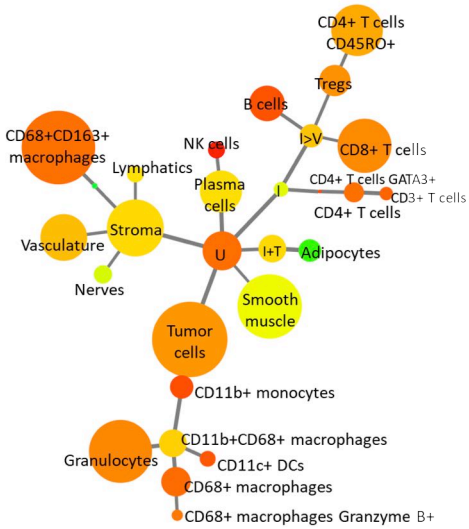
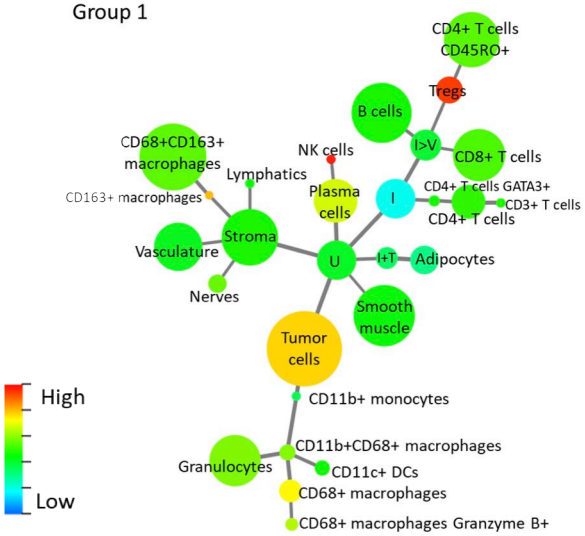
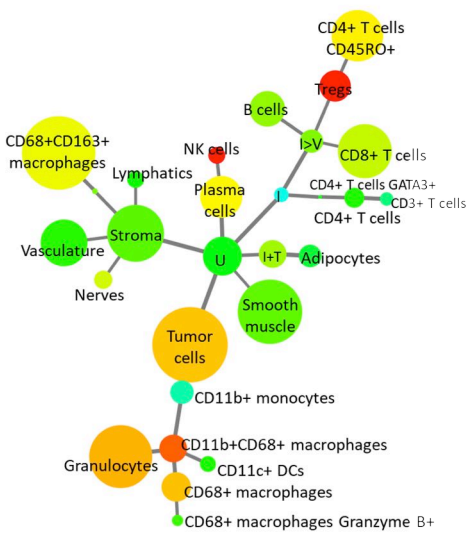


CD25

Group 1

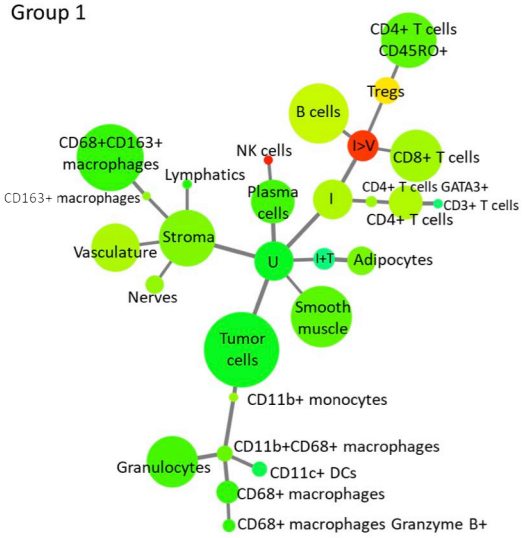


Group 2



CD30

Group 1



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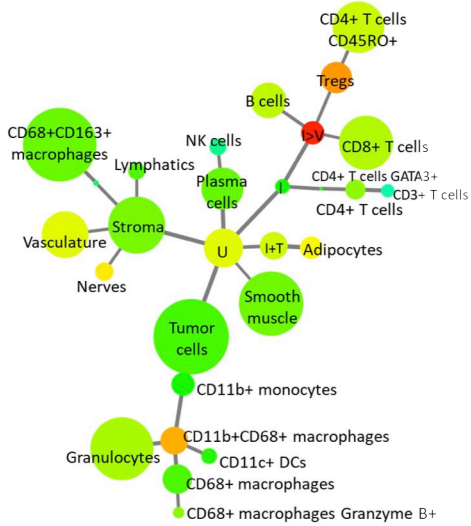
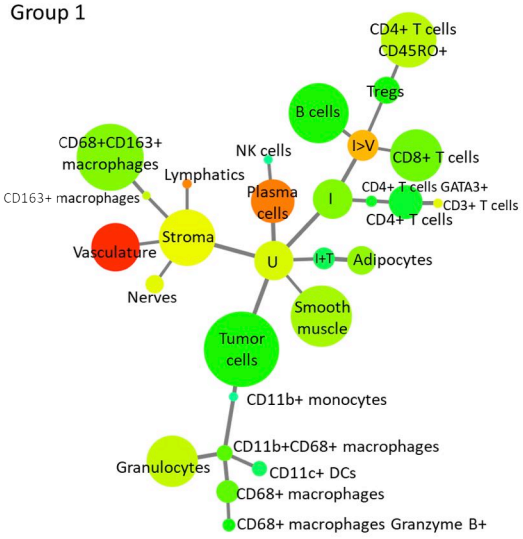


CD31

Group 1



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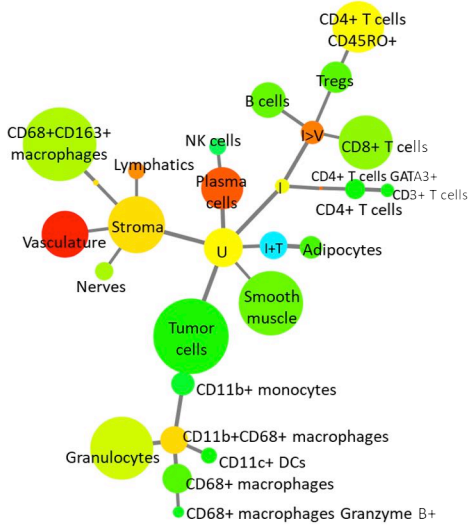
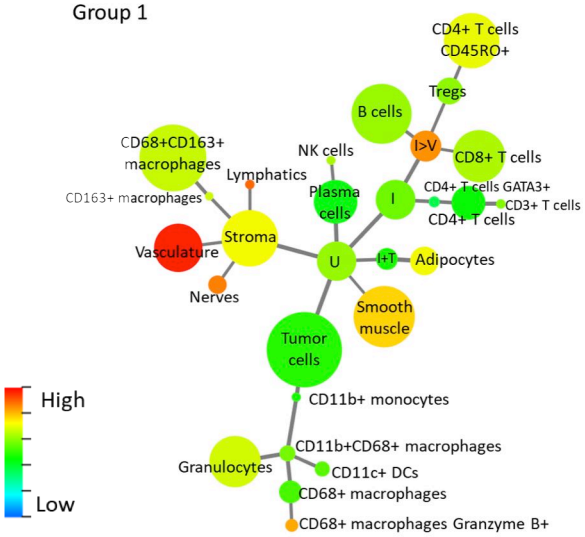


CD34

Group 1



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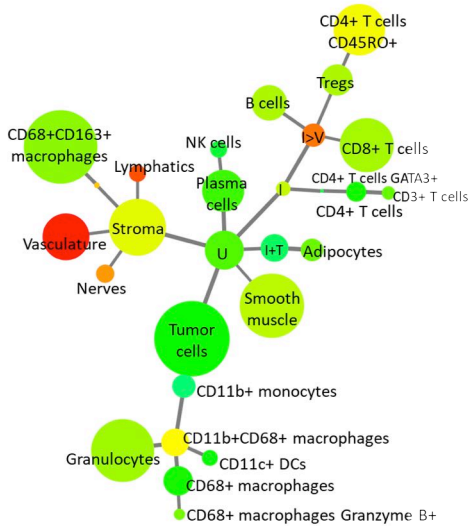
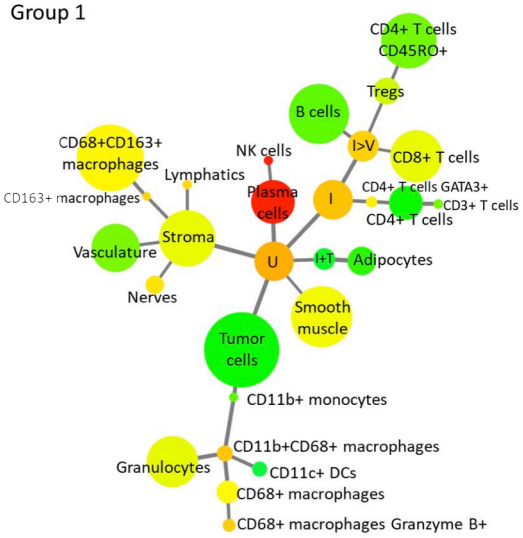


CD38

Group 1



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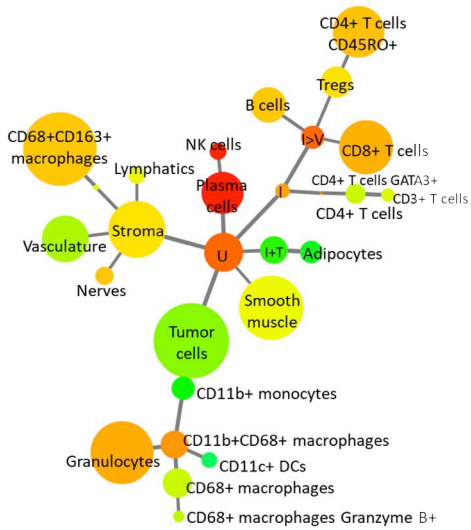
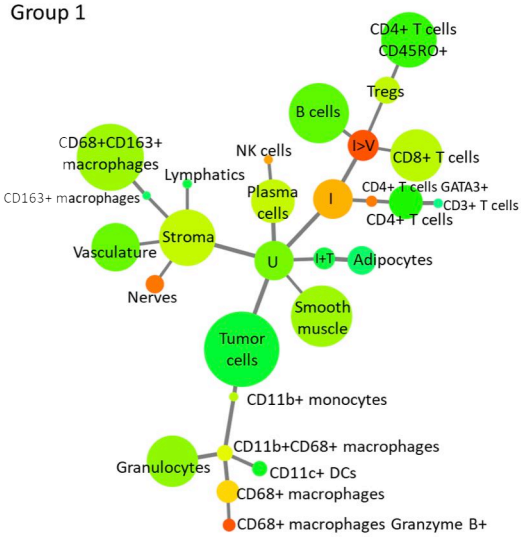
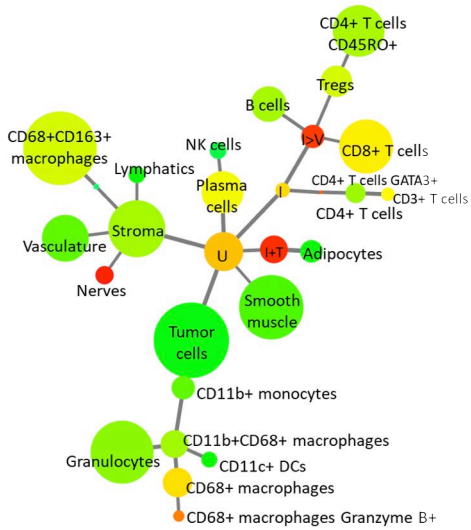


CD44

Group 1

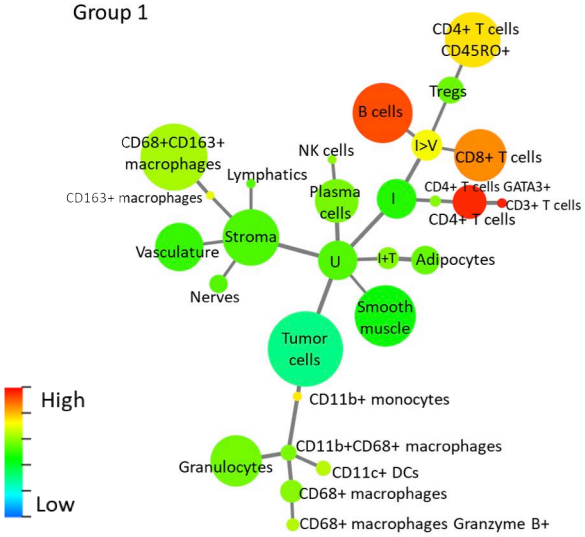


Group 2

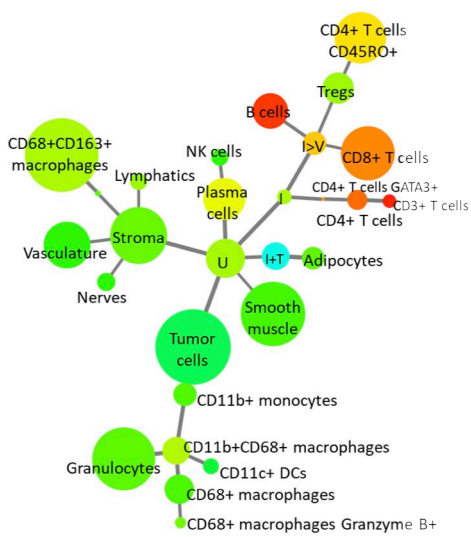


CD45

Group 1

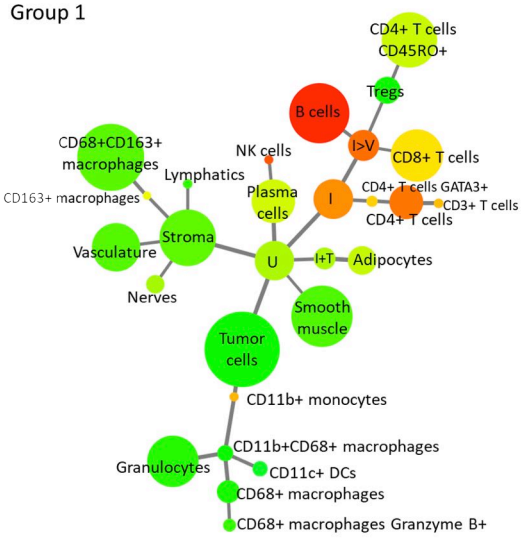


Group 2

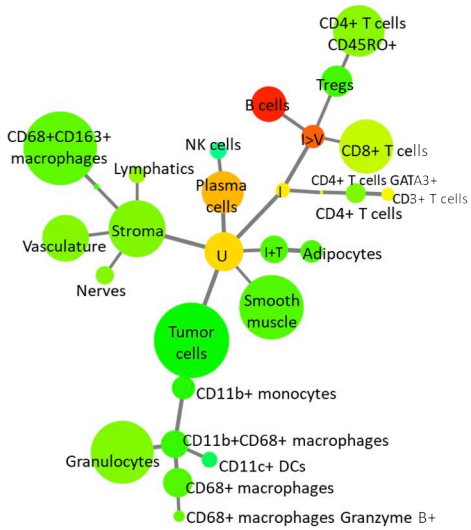


CD45RA

Group 1

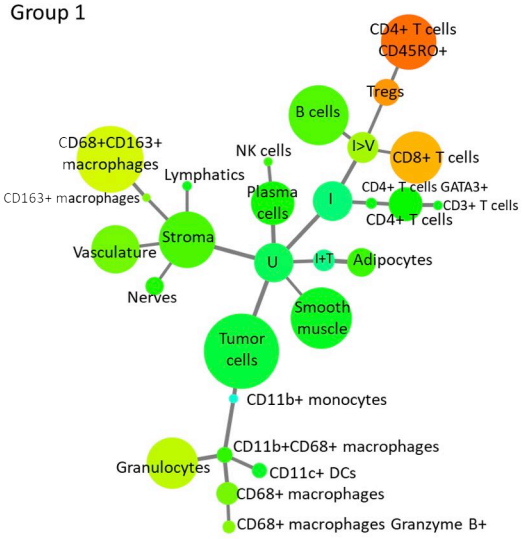


Group 2

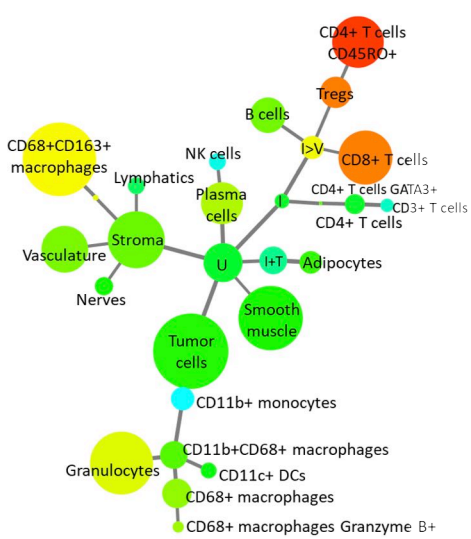


CD45RO

Group 1

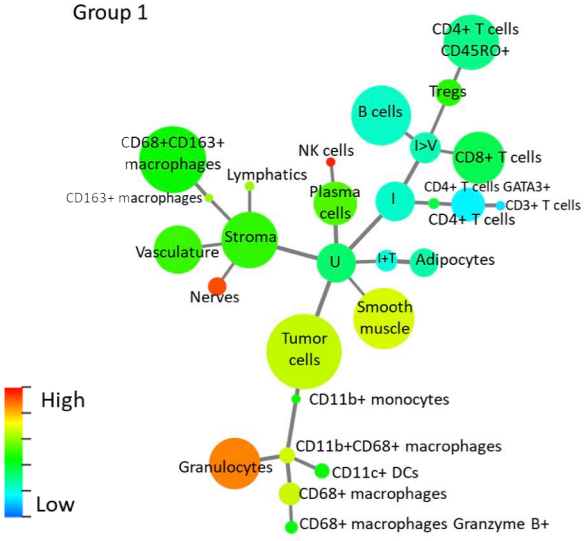


Group 2

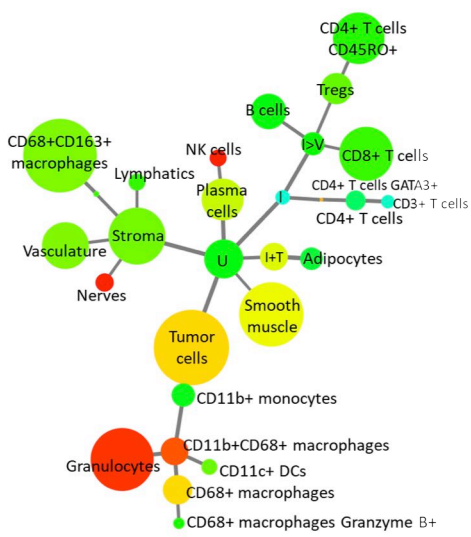


CD56

Group 1

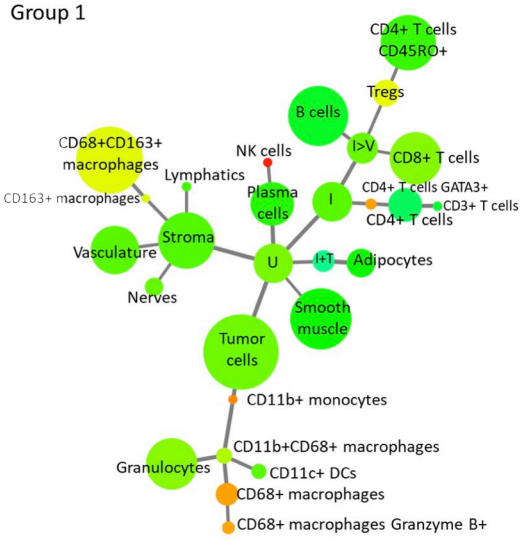


Group 2

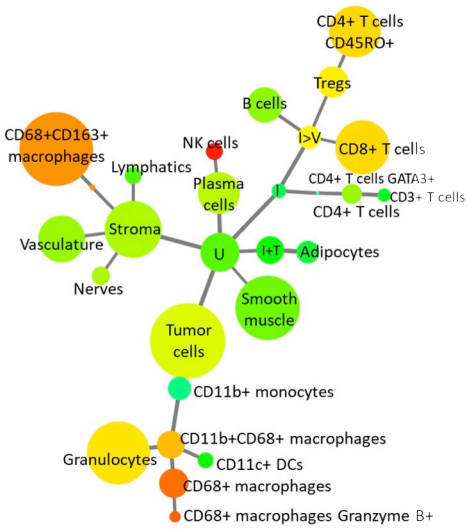


CD57

Group 1

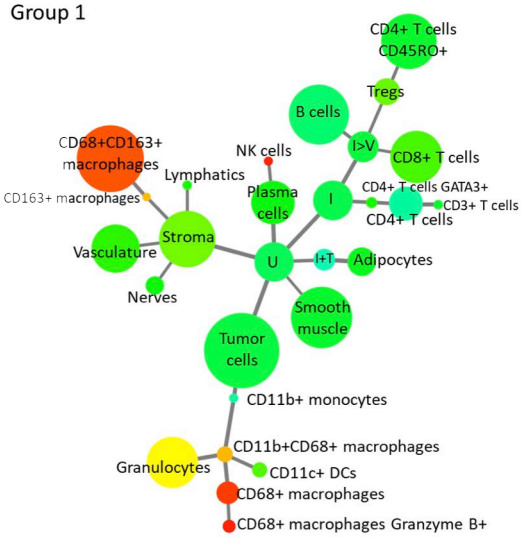


Group 2

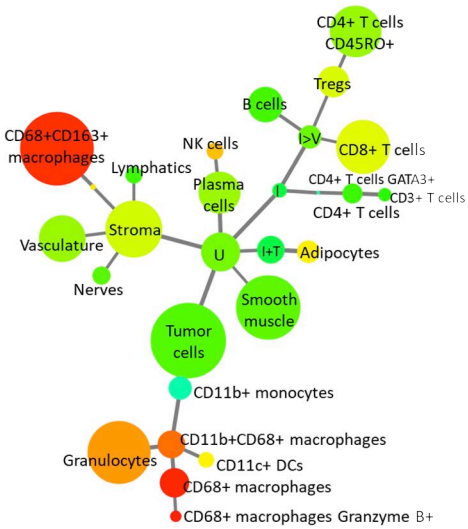


CD68

Group 1

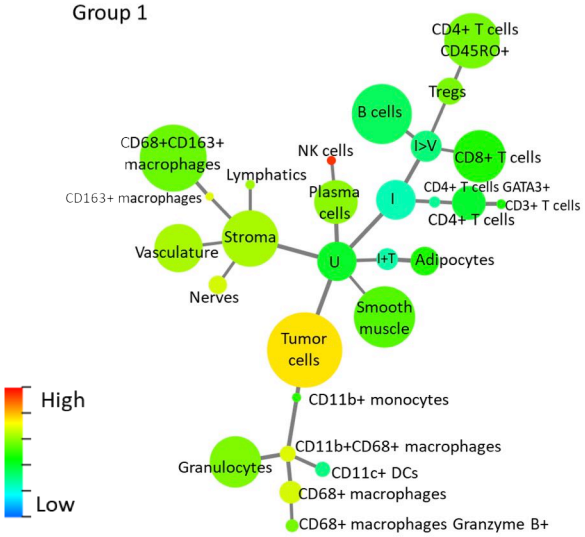


Group 2

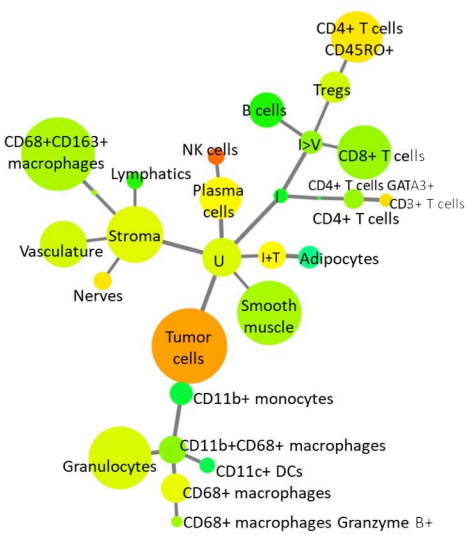


CD71

Group 1

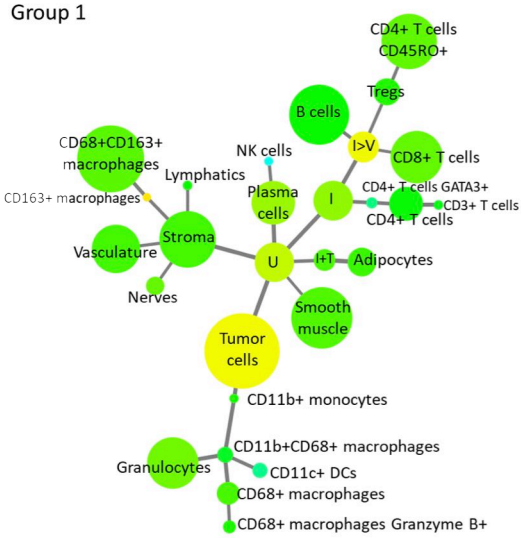


Group 2

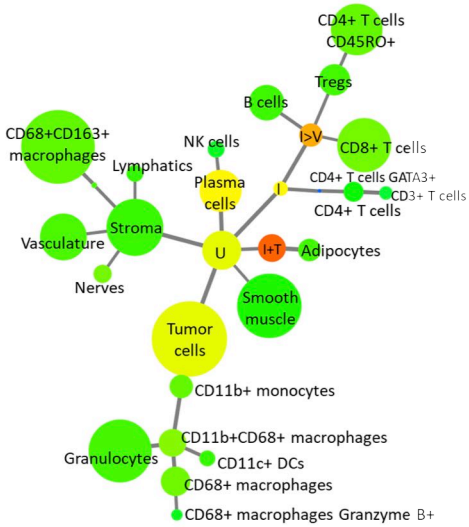


CD138

Group 1

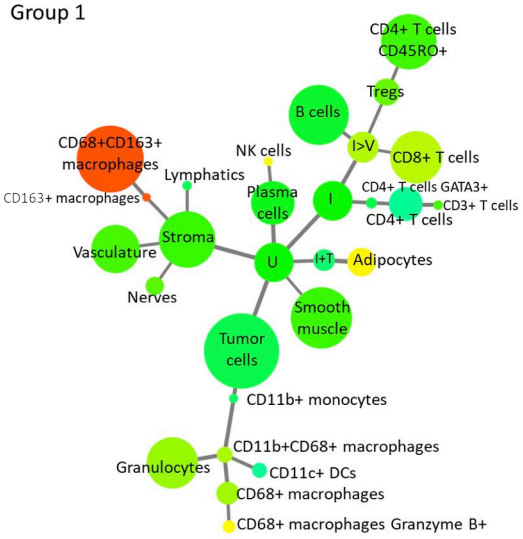


Group 2

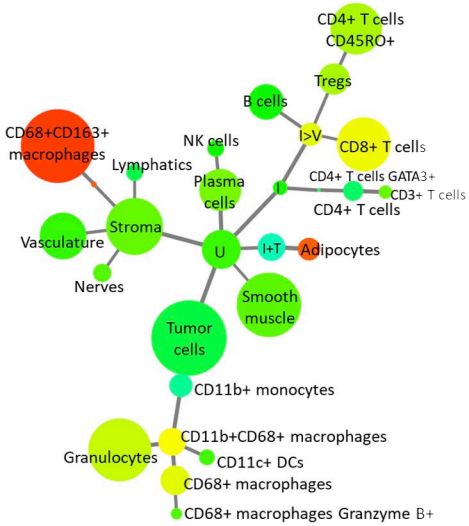


CD163

Group 1

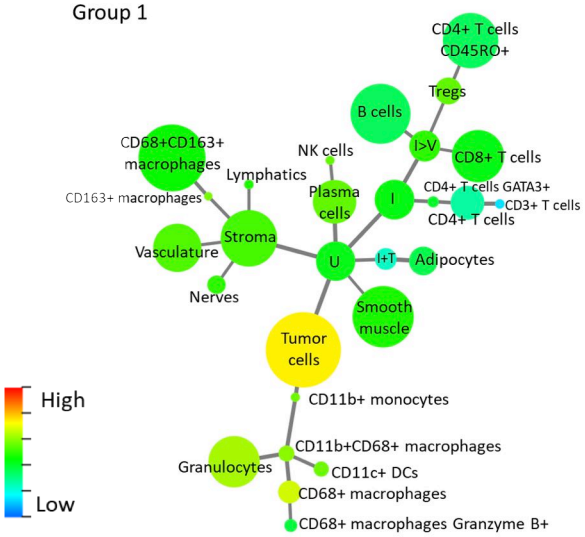


Group 2

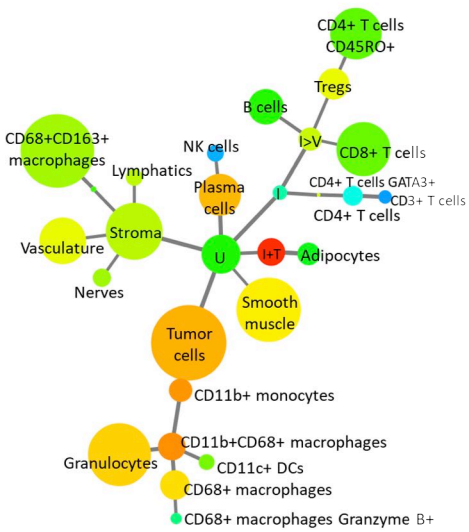


CD194

Group 1

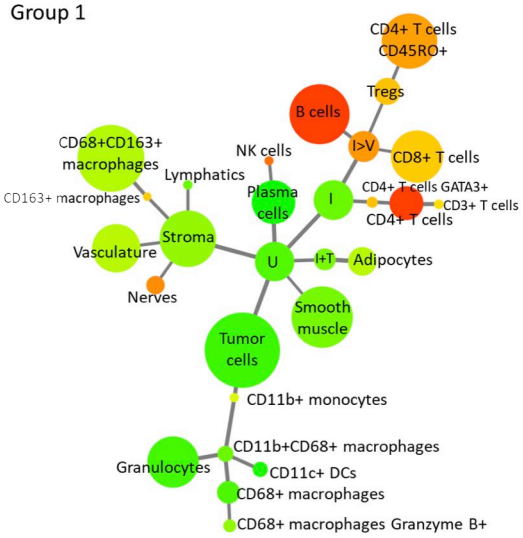


Group 2

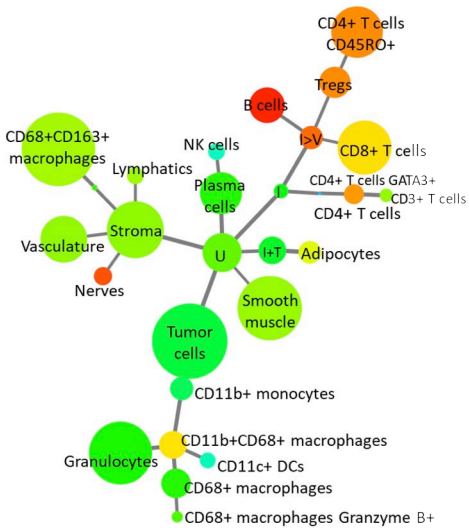


BCL-2

Group 1

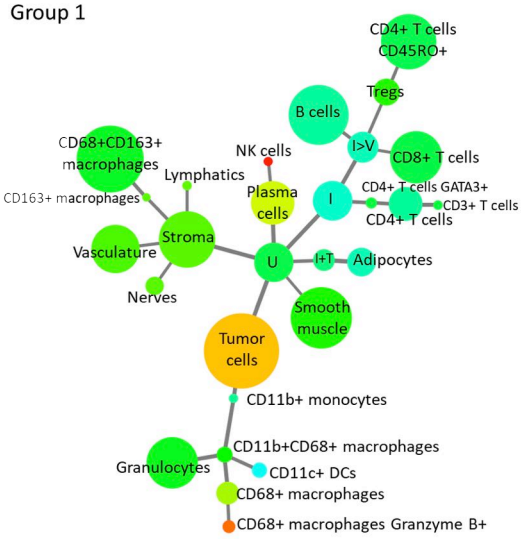


Group 2



Beta-catenin

Group 1



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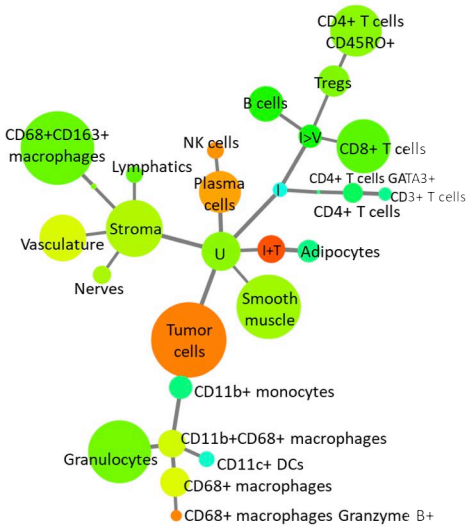
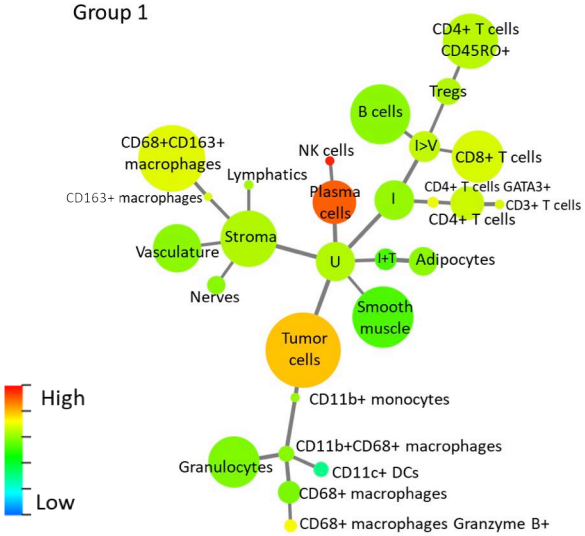
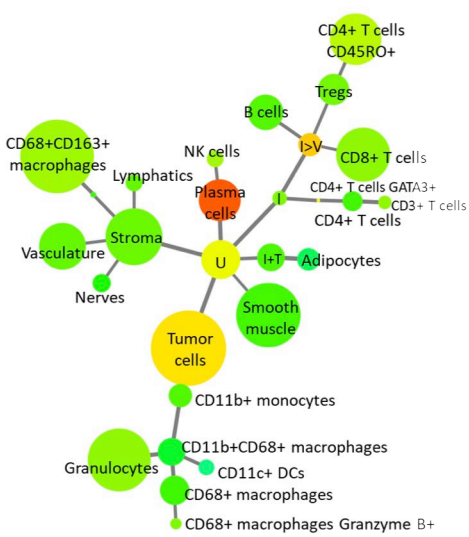


CDX2

Group 1

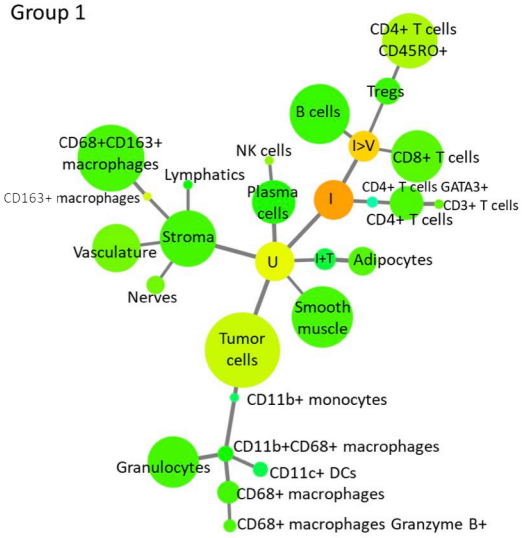


Group 2



Chromogranin A

Group 1



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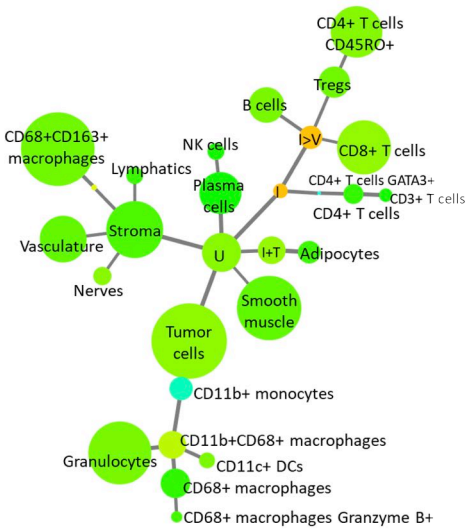
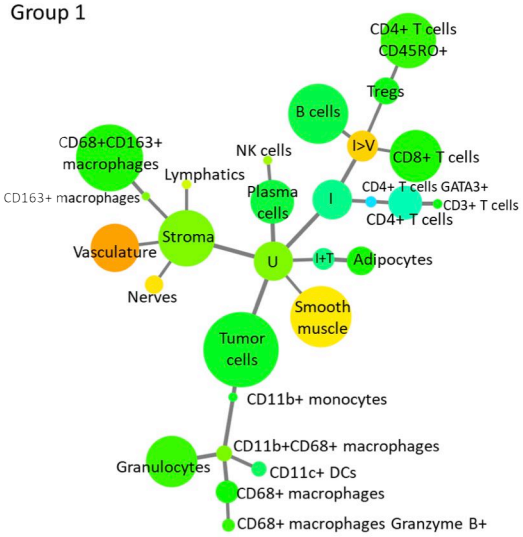


Collagen IV

Group 1



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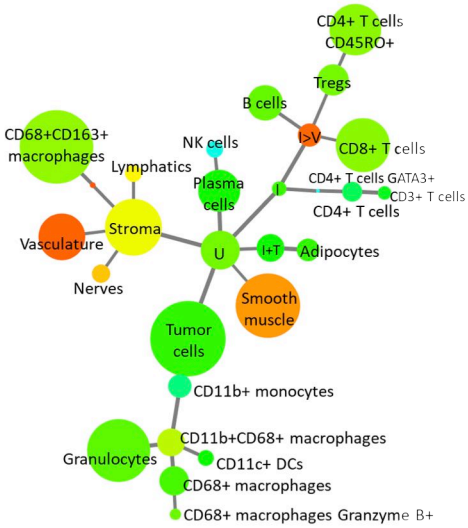
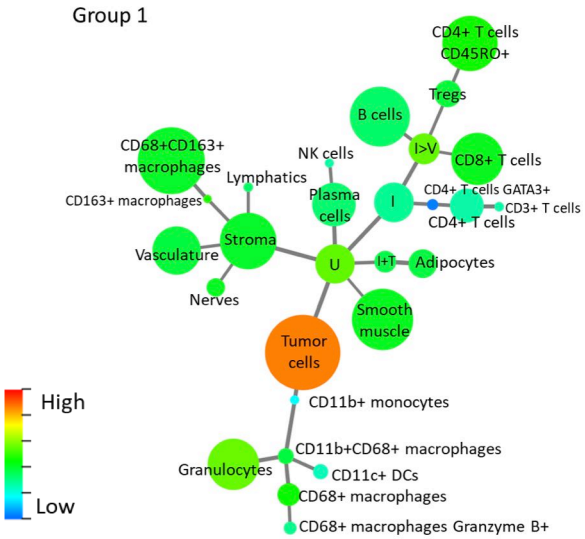


Cytokeratin

Group 1



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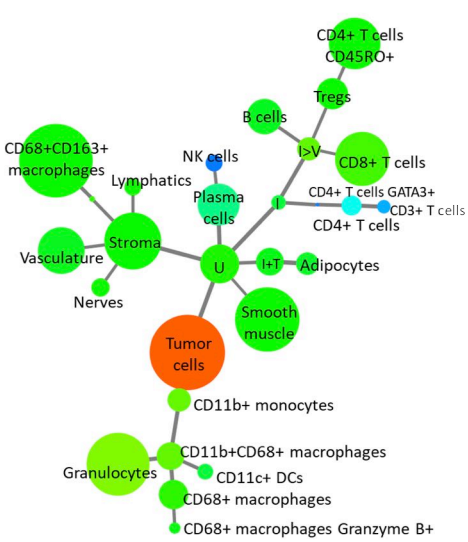
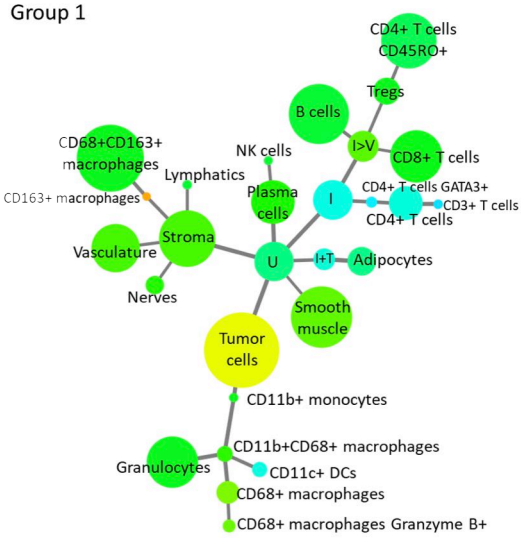


EGFR

Group 1



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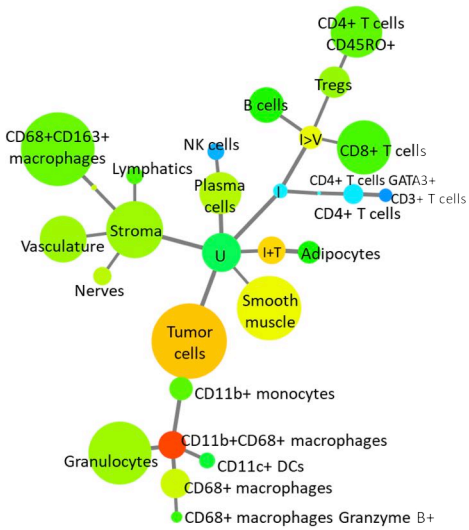
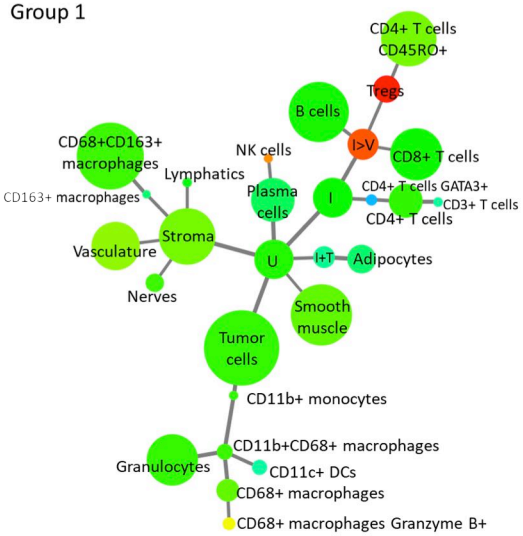


FoxP3

Group 1



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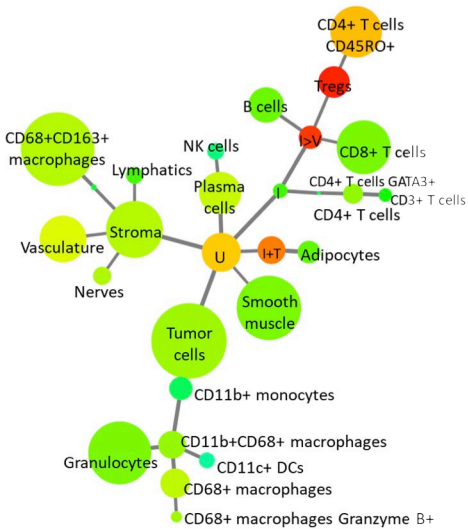
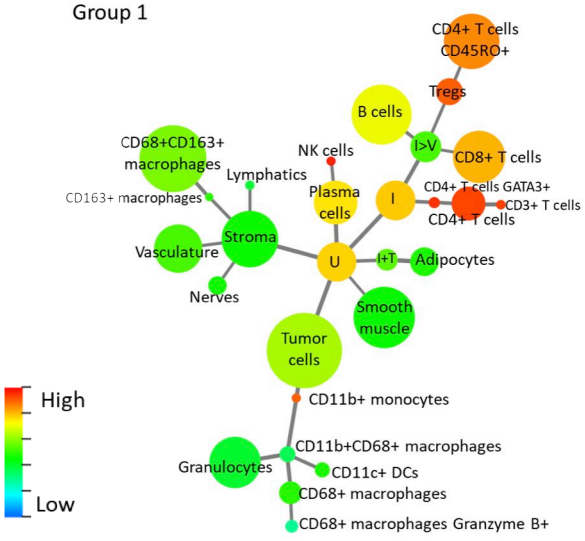


GATA3

Group 1



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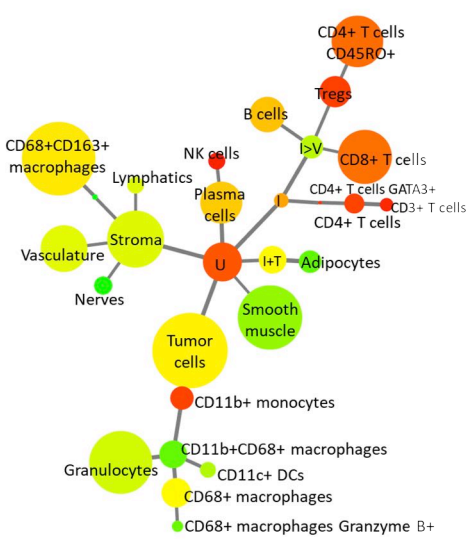
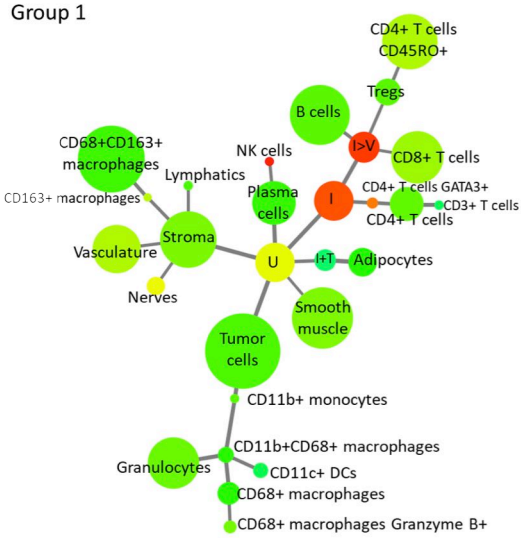
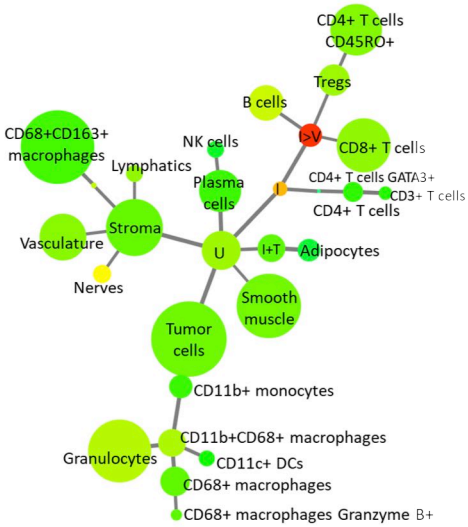


GFAP

Group 1

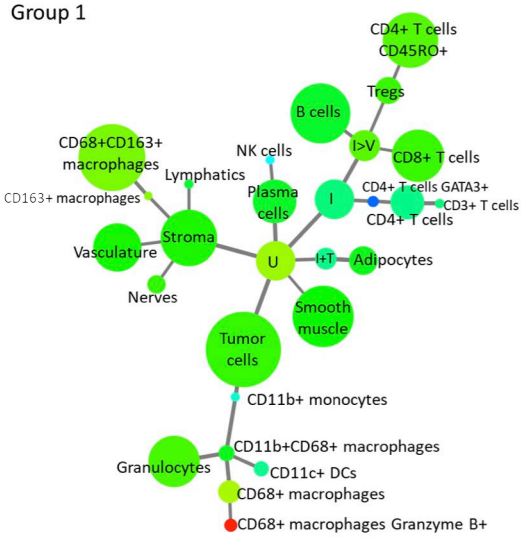


Group 2



Granzyme B

Group 1



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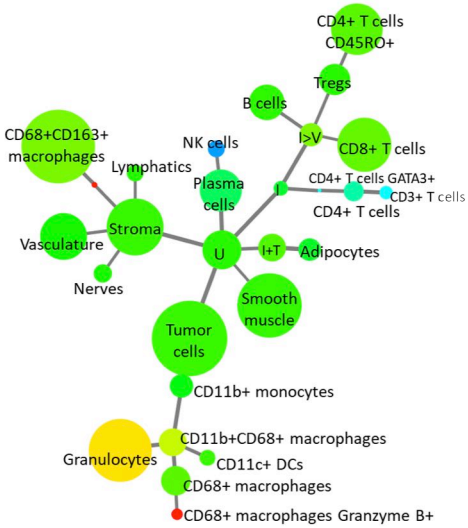
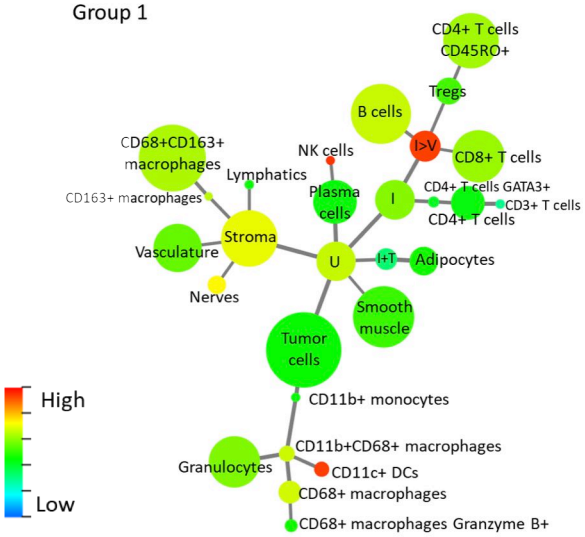


HLA-DR

Group 1



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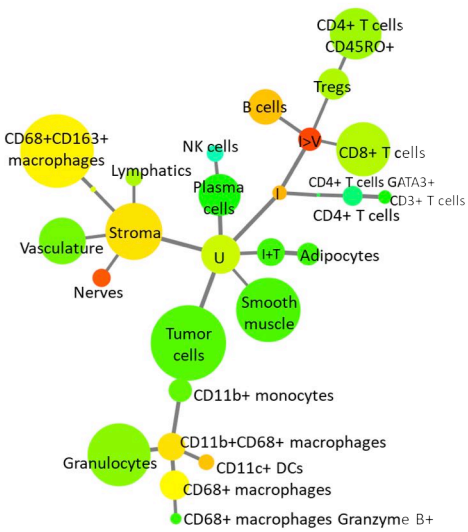
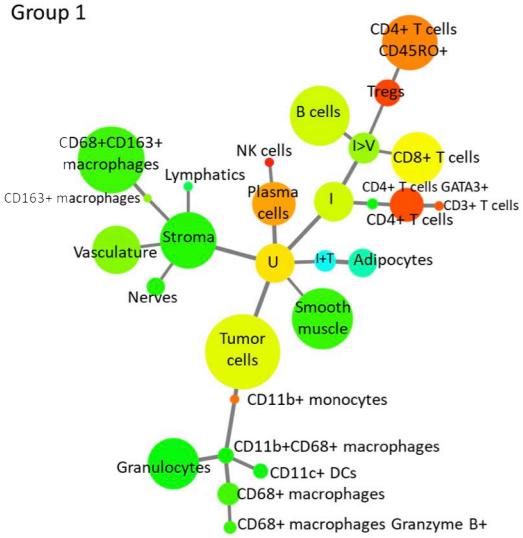


ICOS

Group 1



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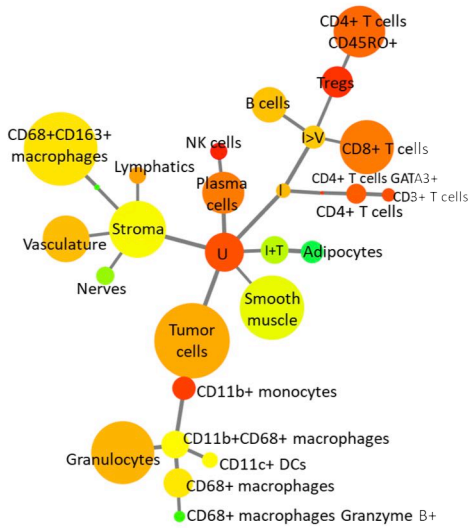
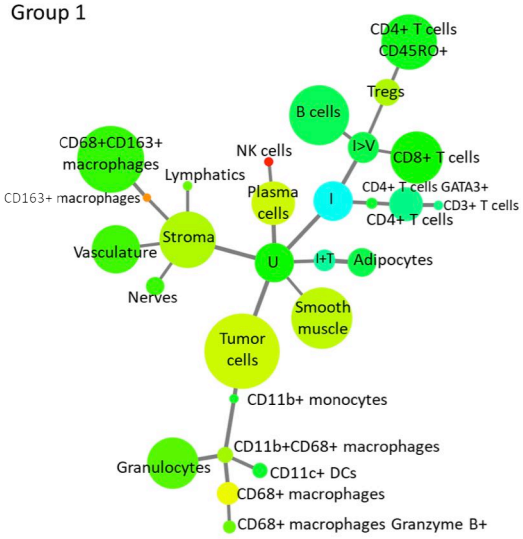


IDO-1

Group 1



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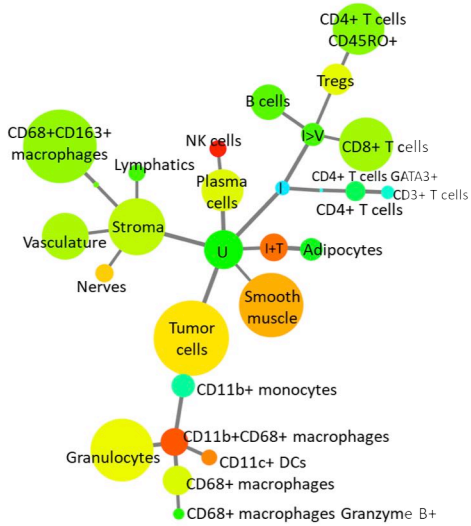
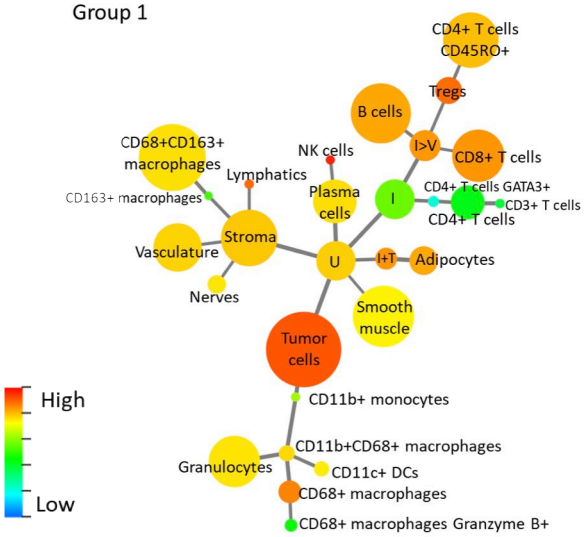


Ki-67

Group 1



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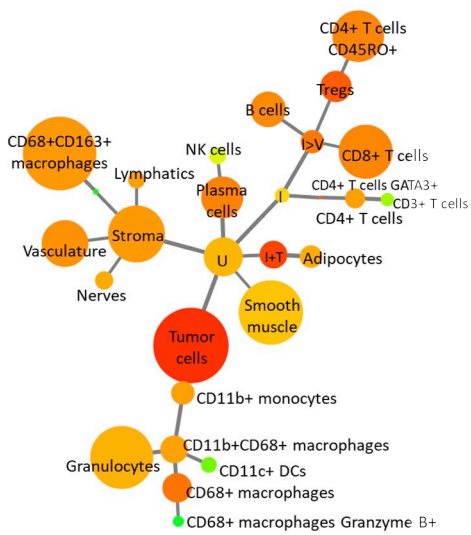
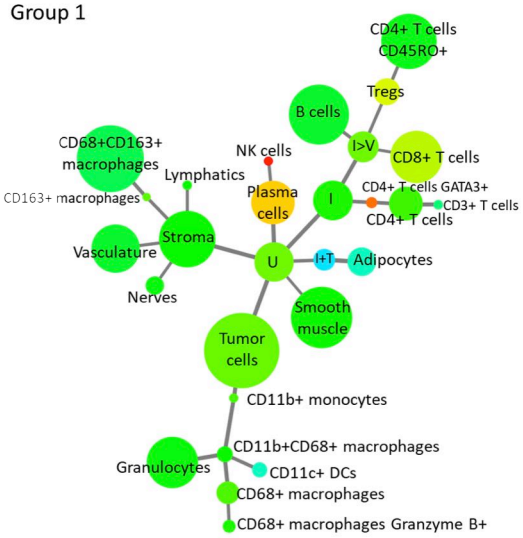
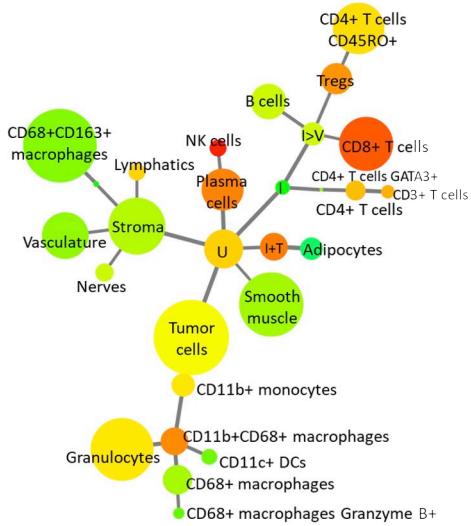


LAG-3

Group 1

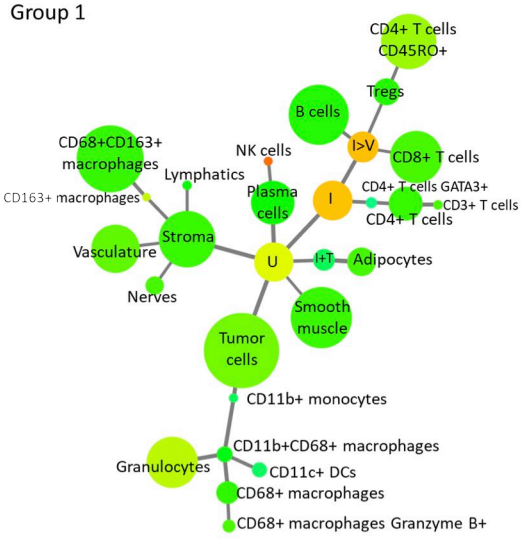


Group 2

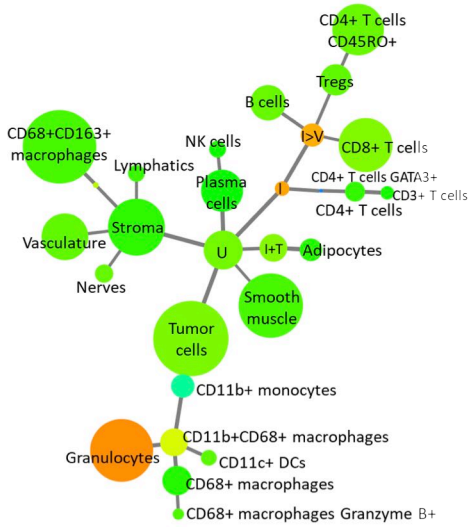


MMP-9

Group 1

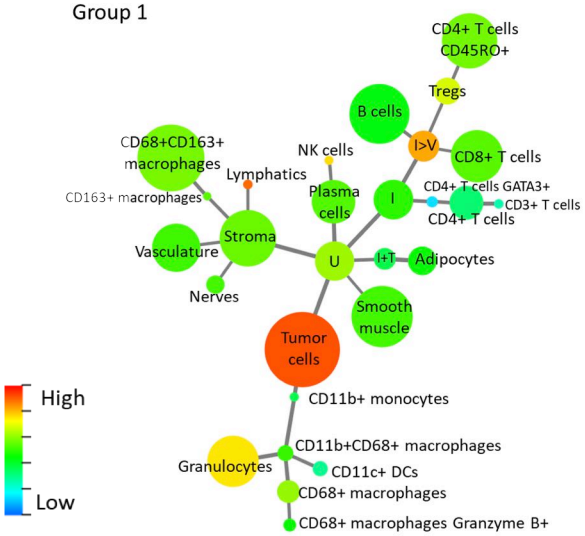


Group 2

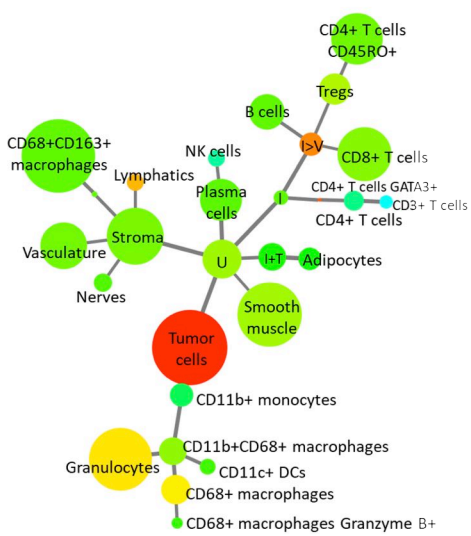


MUC-1

Group 1

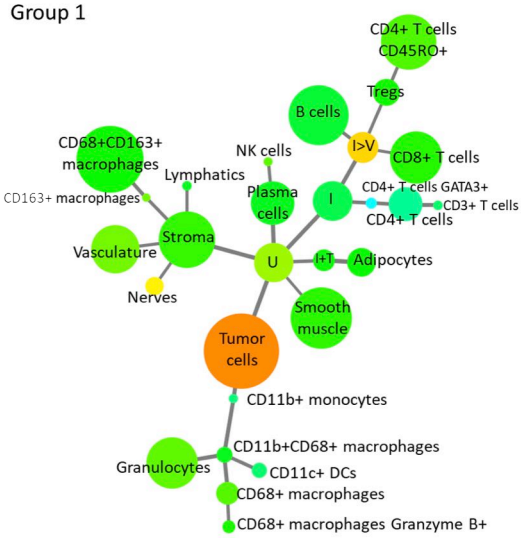


Group 2



Na-K-ATPase

Group 1



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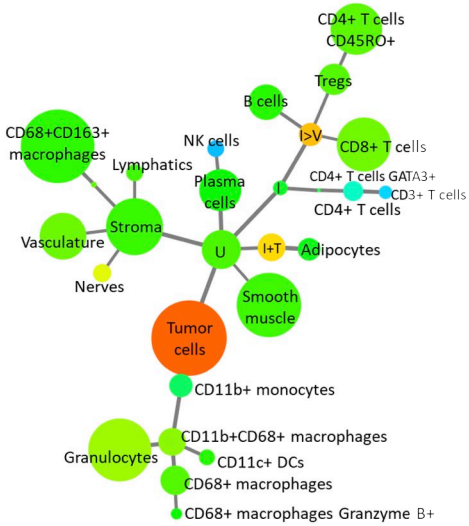
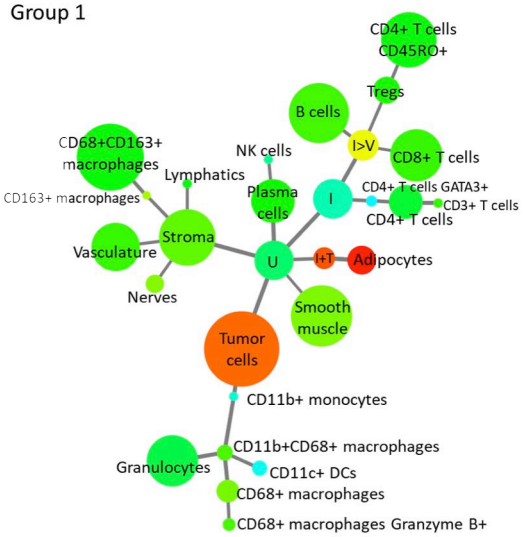
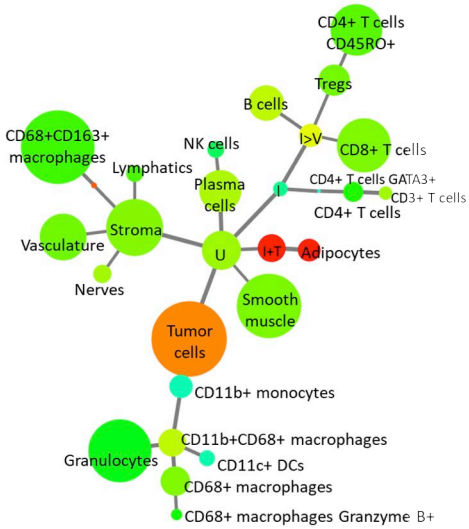


p53

Group 1

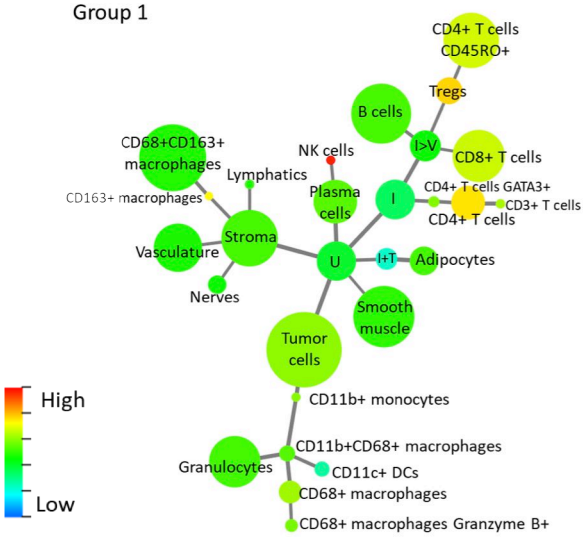


Group 2

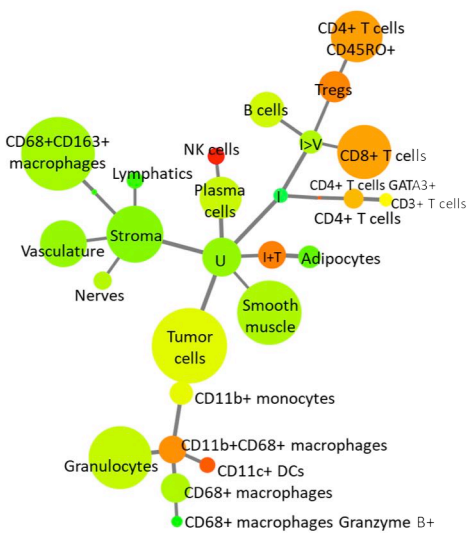


PD-1

Group 1

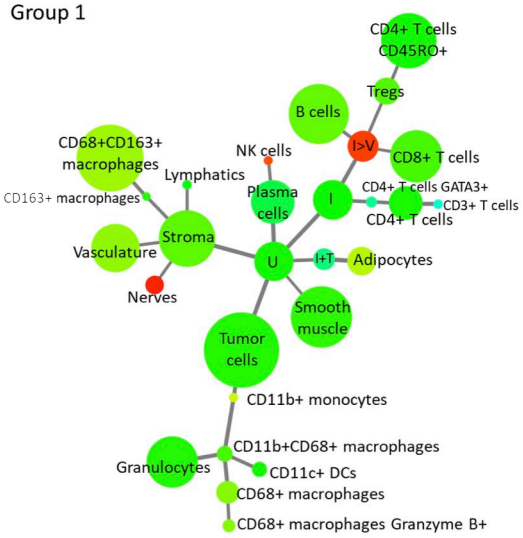


Group 2

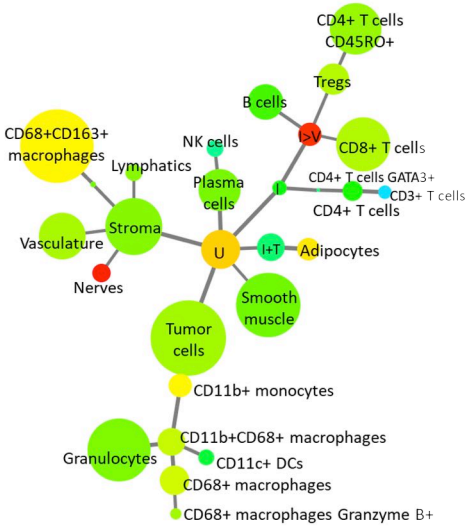


PD-L1

Group 1

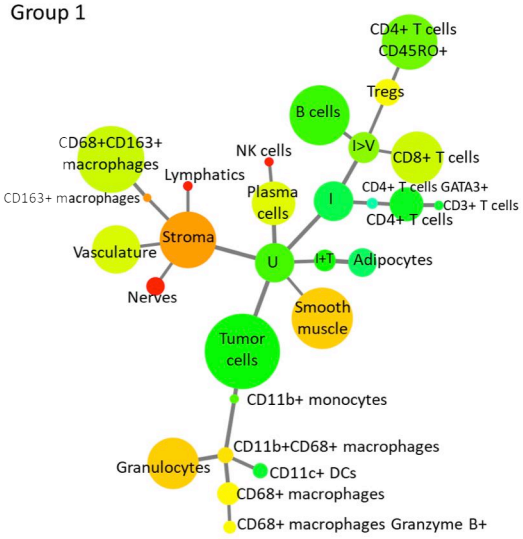


Group 2

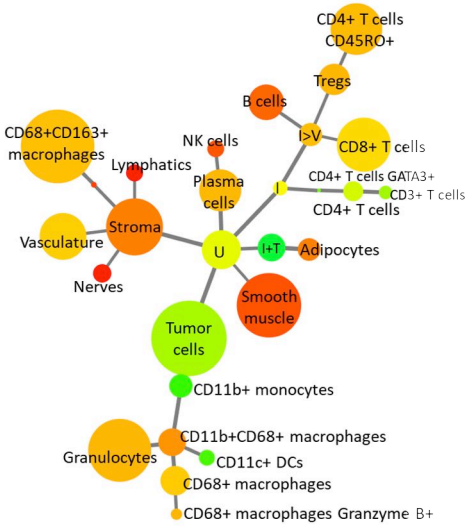


Podoplanin

Group 1

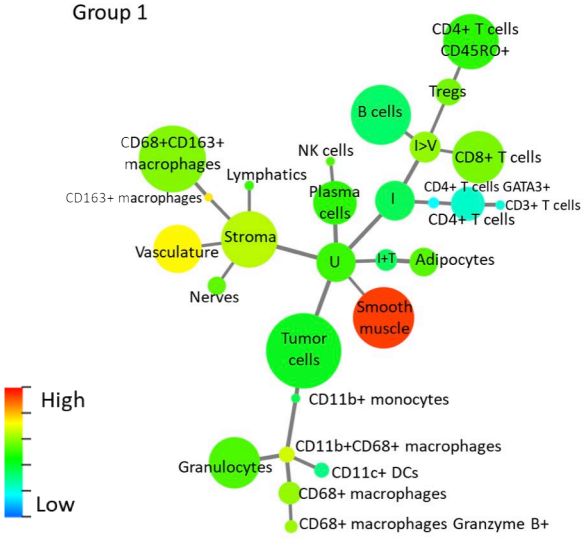


Group 2

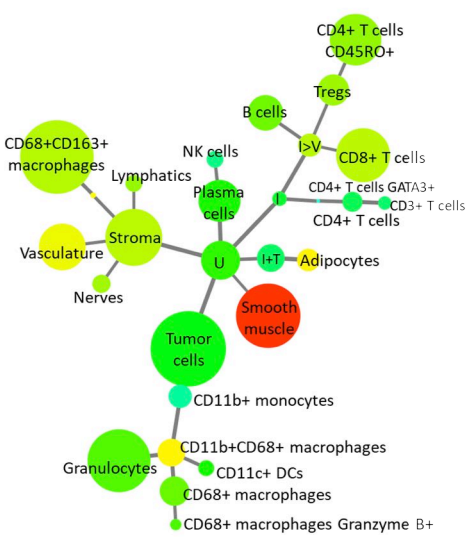


Smooth Muscle Actin (SMA)

Group 1

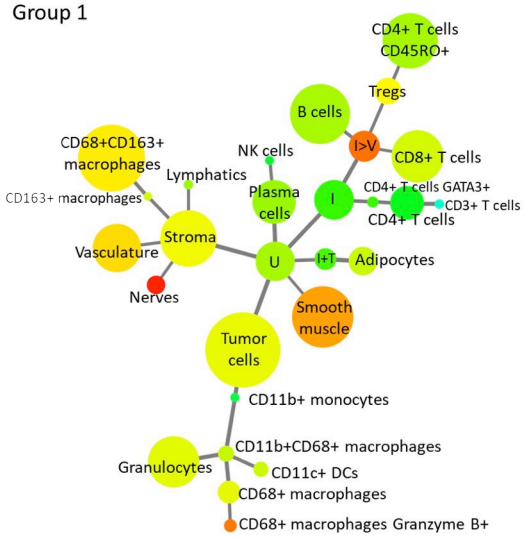


Group 2

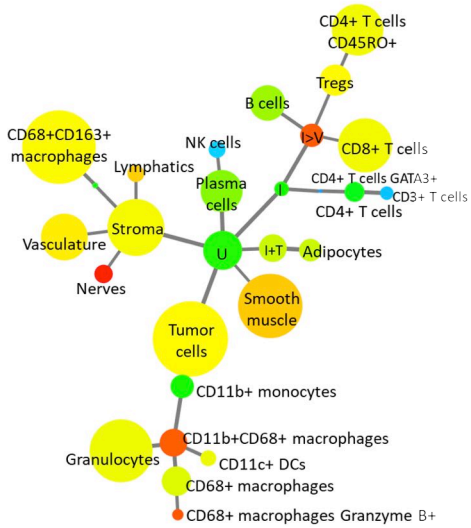


Synaptophysin

Group 1

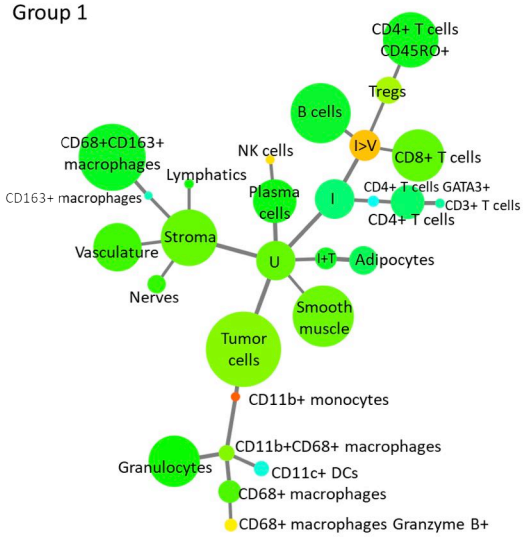


Group 2

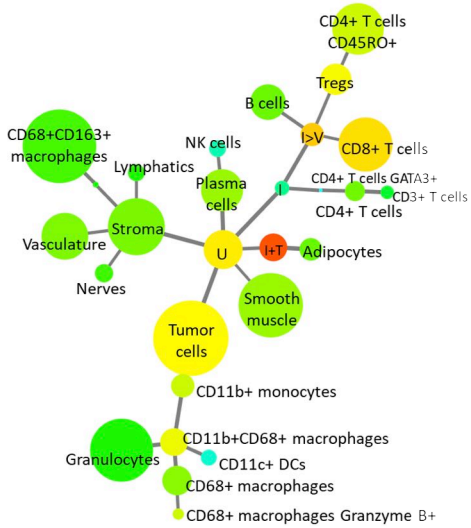


T-bet

Group 1

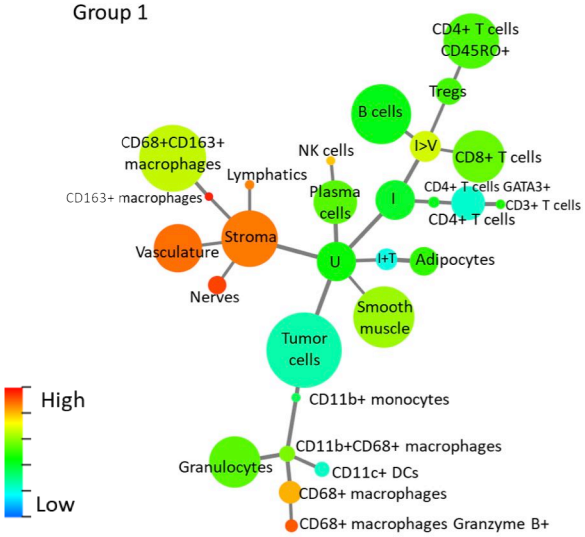


Group 2

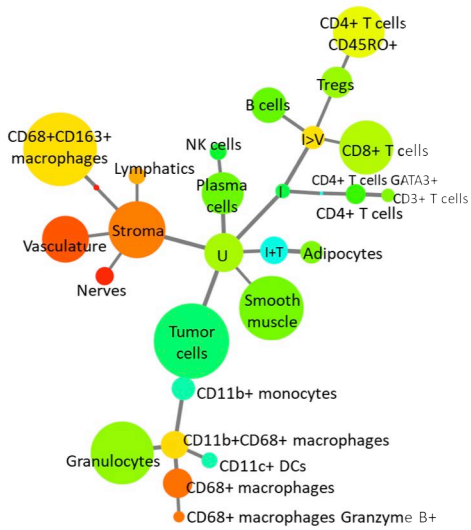


Vimentin

Group 1

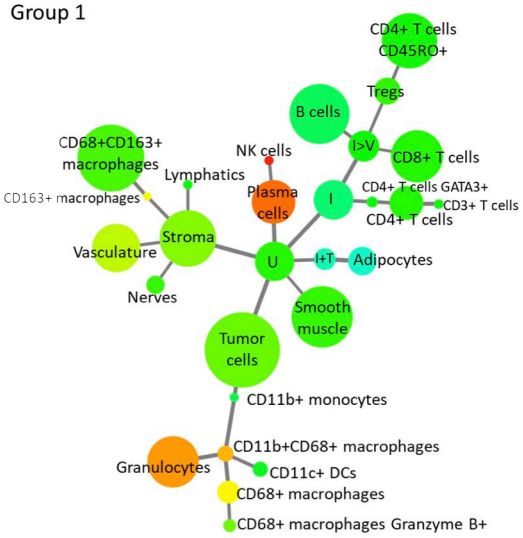


Group 2

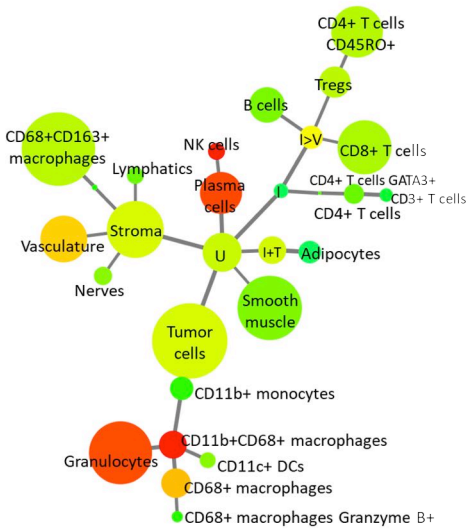


VISTA

Group 1



Group 2



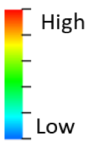
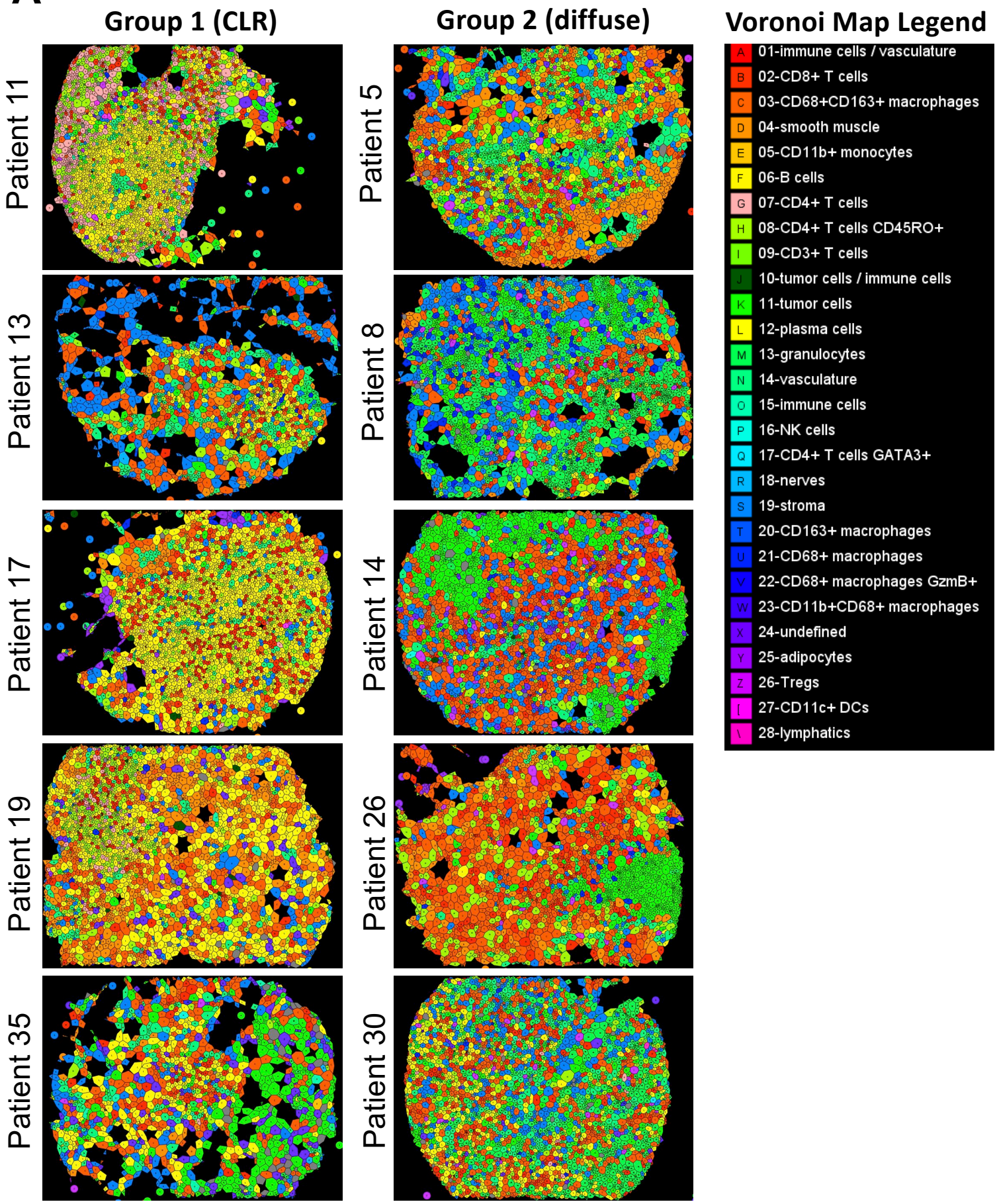


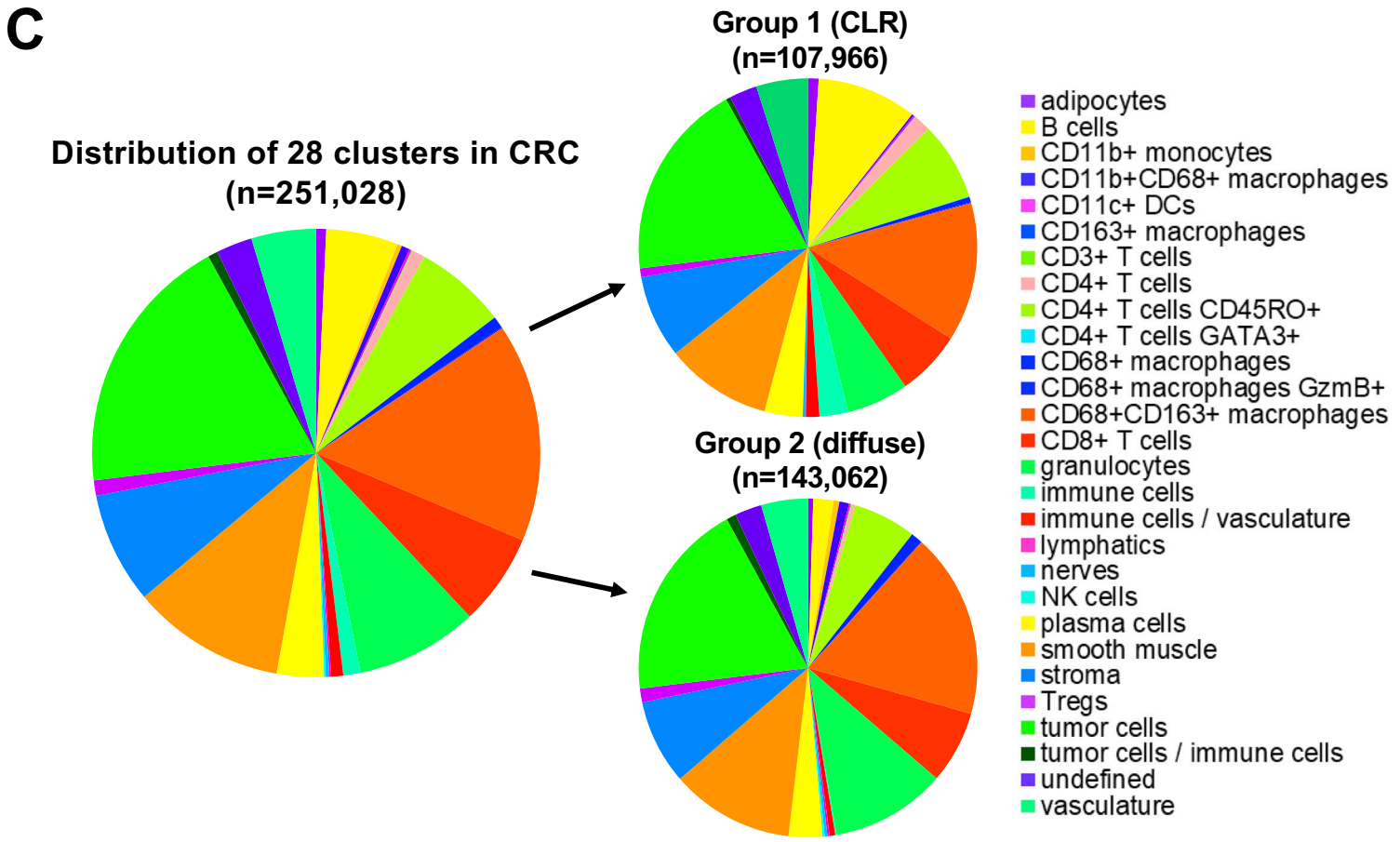
Figure S12

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**
Immmune 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

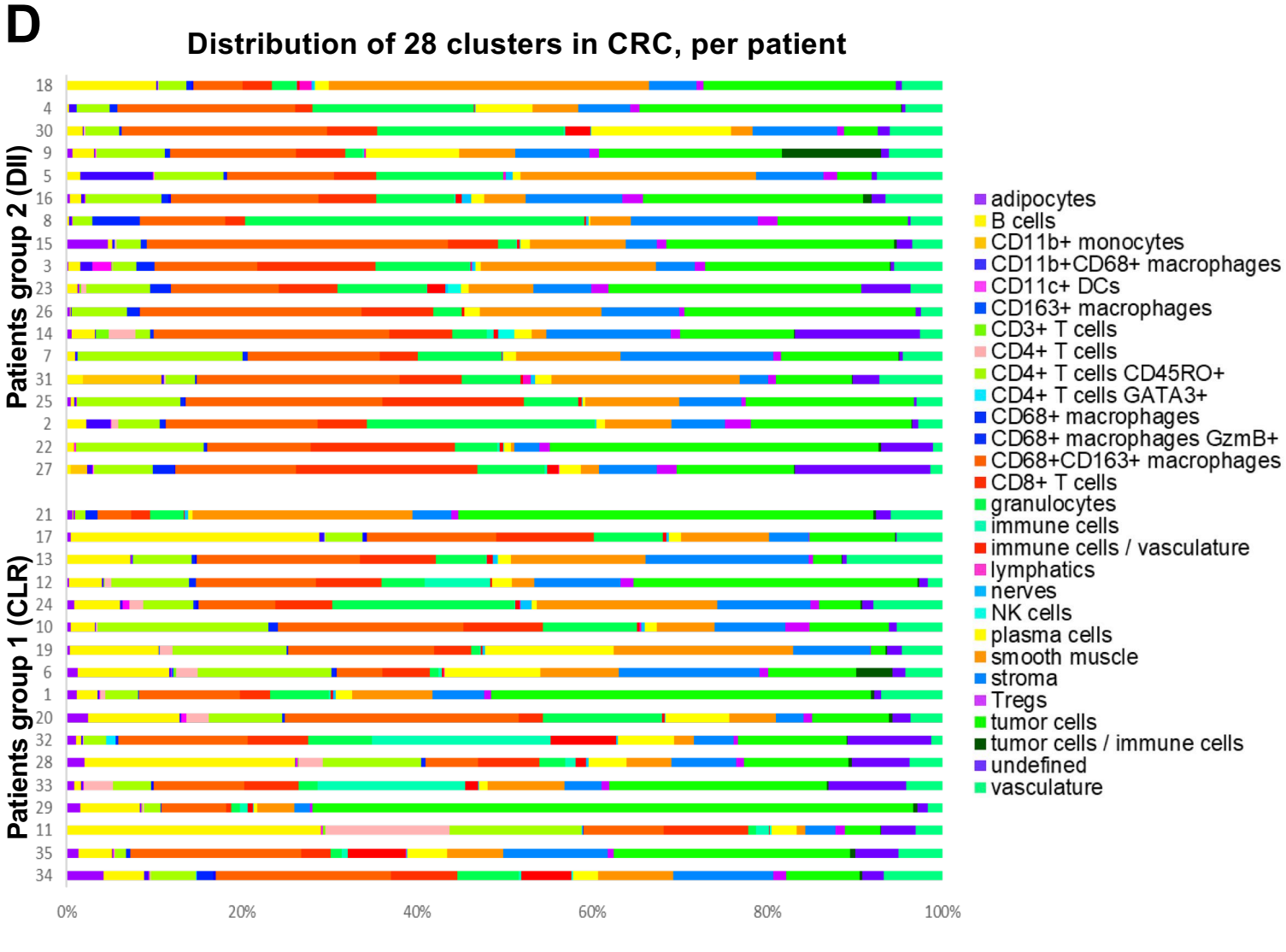

D
Distribution of 28 clusters in CRC, per patient
Patients group 2 (DII)
18
4
30
9
5
16
8
15
3
23
26
14
7
31
25
2
22
27
Patients group 1 (CLR)
21
17
13
12
24
10
19
6
1
20
32
28
33
29
11
35
34
0%
20%
40%
60%
80%
100%
adipocytes
B cells
CD11b+ monocytes
CD11b+CD68+ macrophages
CD11c+ DCs
CD163+ macrophages
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

Figure S13

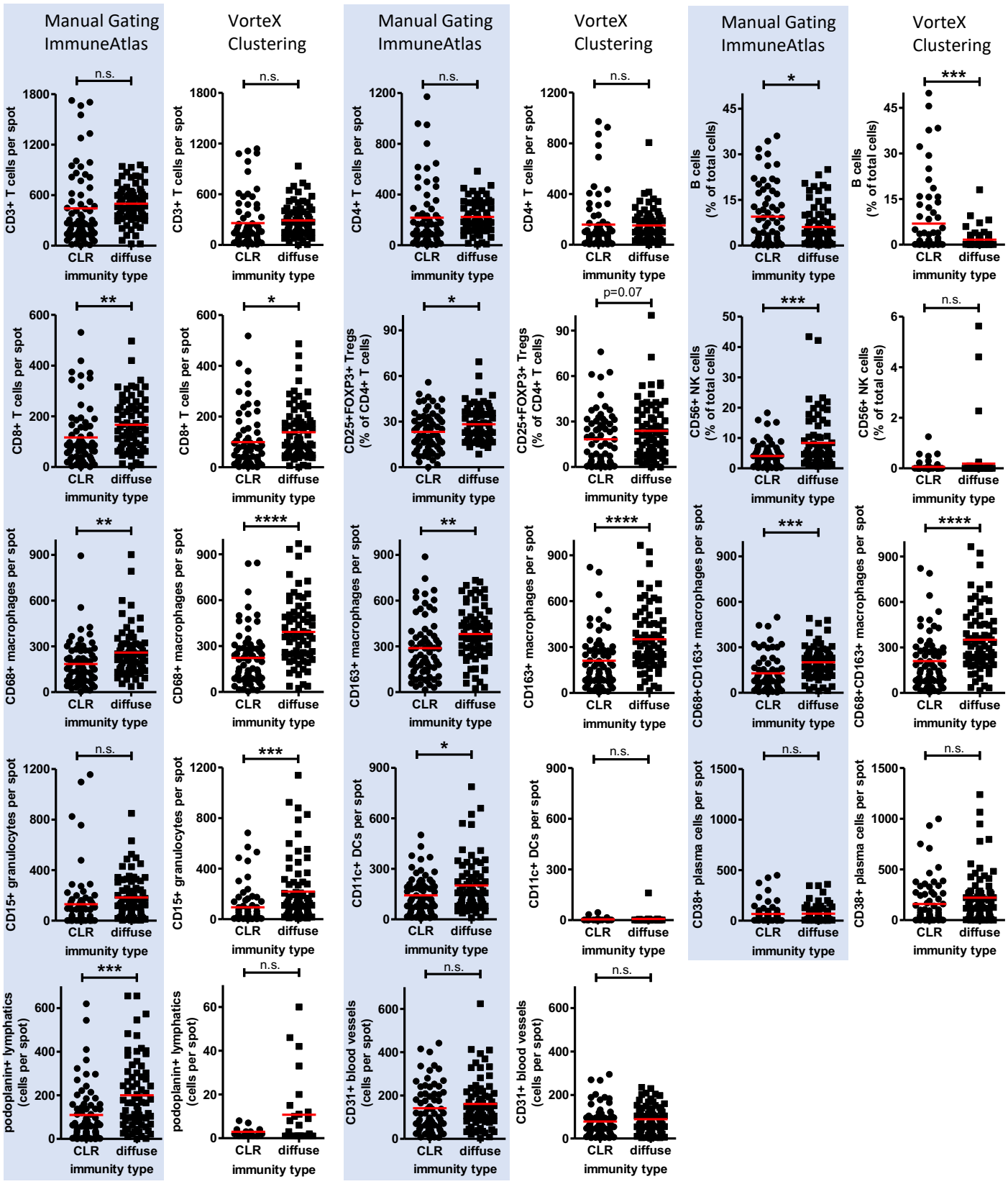
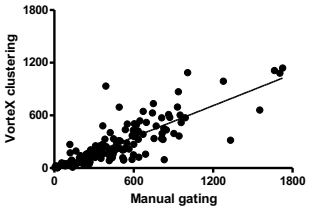

Manual Gating ImmuneAtlas
VorteX Clustering
Manual Gating ImmuneAtlas
VorteX Clustering
Manual Gating ImmuneAtlas
VorteX Clustering
CD3+ T cells per spot
CD8+ T cells per spot
CD68+ macrophages per spot
CD15+ granulocytes per spot
podoplanin+ lymphatics (cells per spot)
CD4+ T cells per spot
CD25+FOXP3+ Tregs (% of CD4+ T cells)
CD163+ macrophages per spot
CD11c+ DCs per spot
CD31+ blood vessels (cells per spot)
B cells (% of total cells)
CD56+ NK cells (% of total cells)
CD68+CD163+ macrophages per spot
CD38+ plasma cells per spot
CLR
diffuse
immunity type
n.s.
p=0.07

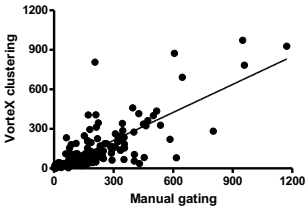
Figure S14

CD3+ T cells per spot



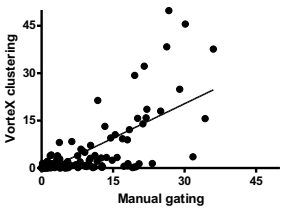
Spearman r = 0.84
Pearson r = 0.82
p<0.0001

CD4+ T cells per spot



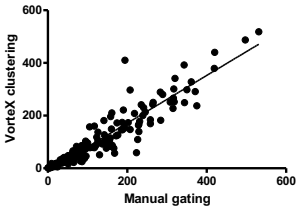
Spearman r = 0.75
Pearson r = 0.76
p<0.0001

CD20+ B cells (%)



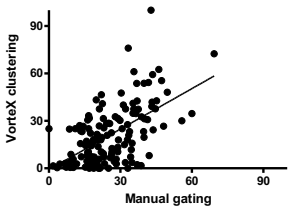
Spearman r = 0.58
Pearson r = 0.70
p<0.0001

CD8+ T cells per spot



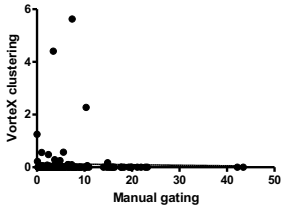
Spearman r = 0.95
Pearson r = 0.93
p<0.0001

Tregs (% of CD4)



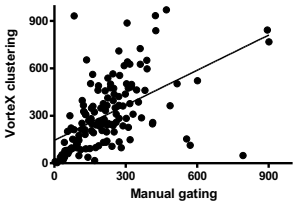
Spearman r = 0.53
Pearson r = 0.58
p<0.0001

CD56 (%)



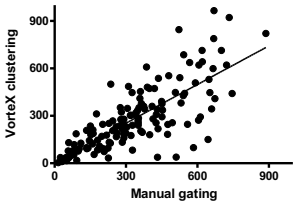
Spearman r = -0.07
Pearson r = -0.02
p = n.s.

CD68+ macrophages per spot



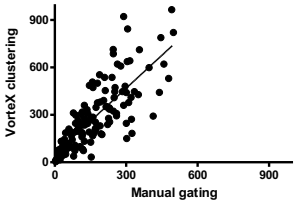
Spearman r = 0.62
Pearson r = 0.53
p<0.0001

CD163+ macrophages per spot



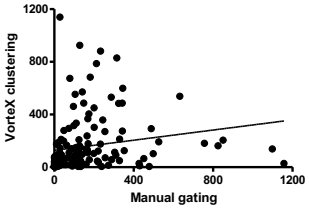
Spearman r = 0.78
Pearson r = 0.78
p<0.0001

CD68+CD163+ macrophages per spot



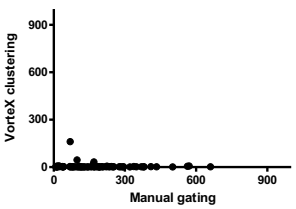
Spearman r = 0.82
Pearson r = 0.79
p<0.0001

CD15+ granulocytes per spot



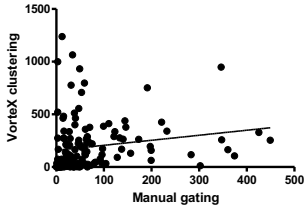
Spearman r = 0.50
Pearson r = 0.18
p = 0.031

CD11c+ DCs per spot



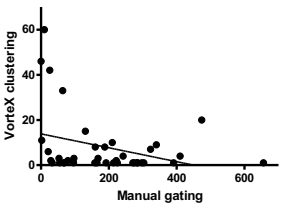
Spearman r = -0.02
Pearson r = 0.11
p = n.s.

CD38+ plasma cells per spot



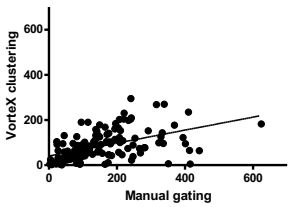
Spearman r = 0.38
Pearson r = 0.18
p = 0.037

Podoplanin+ lymphatics (cells per spot)



Spearman r = -0.31
Pearson r = -0.33
p = 0.042

CD31+ vasculature (cells per spot)



Spearman r = 0.59
Pearson r = 0.50
p<0.0001

Supplemental Figure 15

A

	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

B

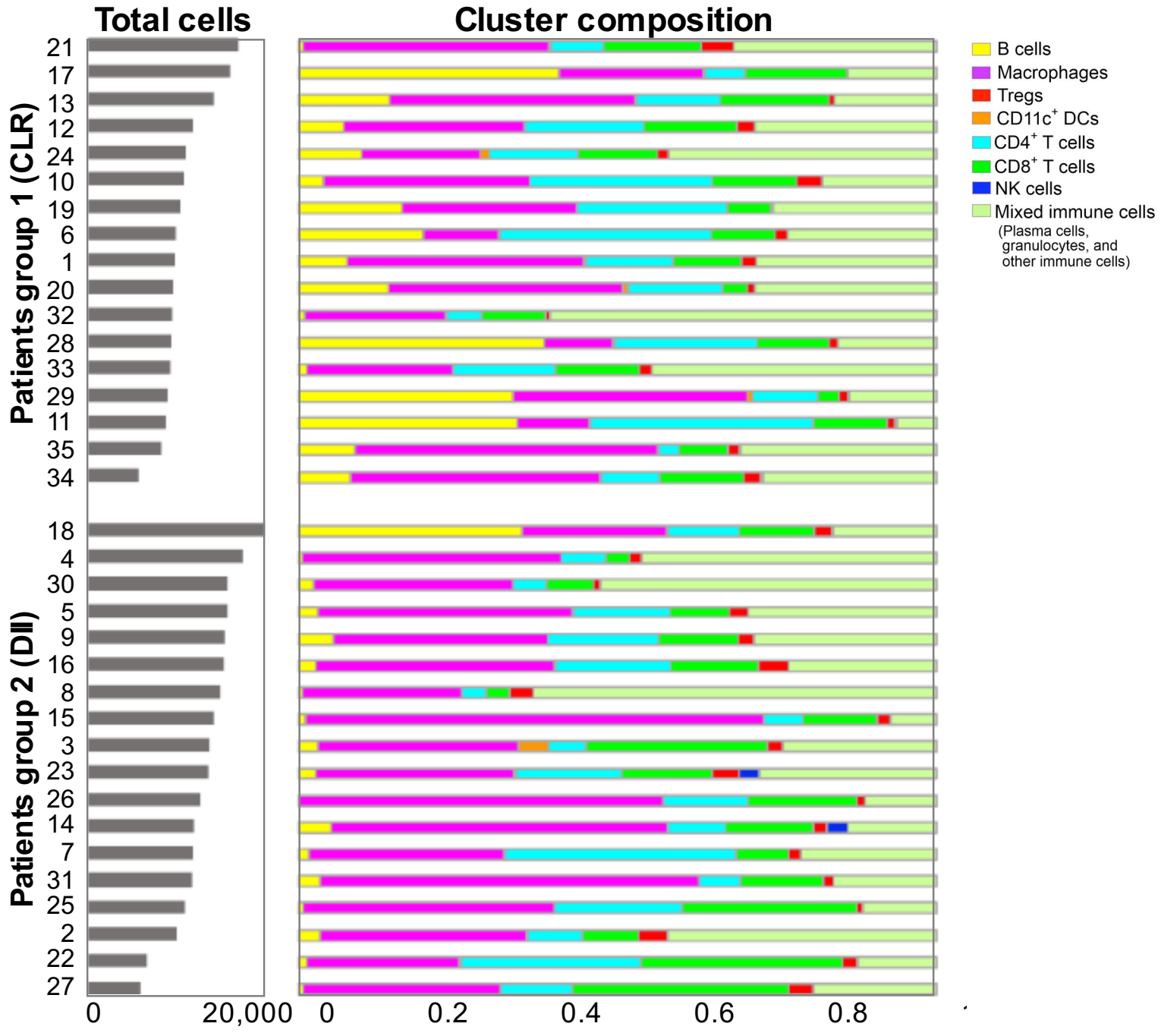


Figure S16

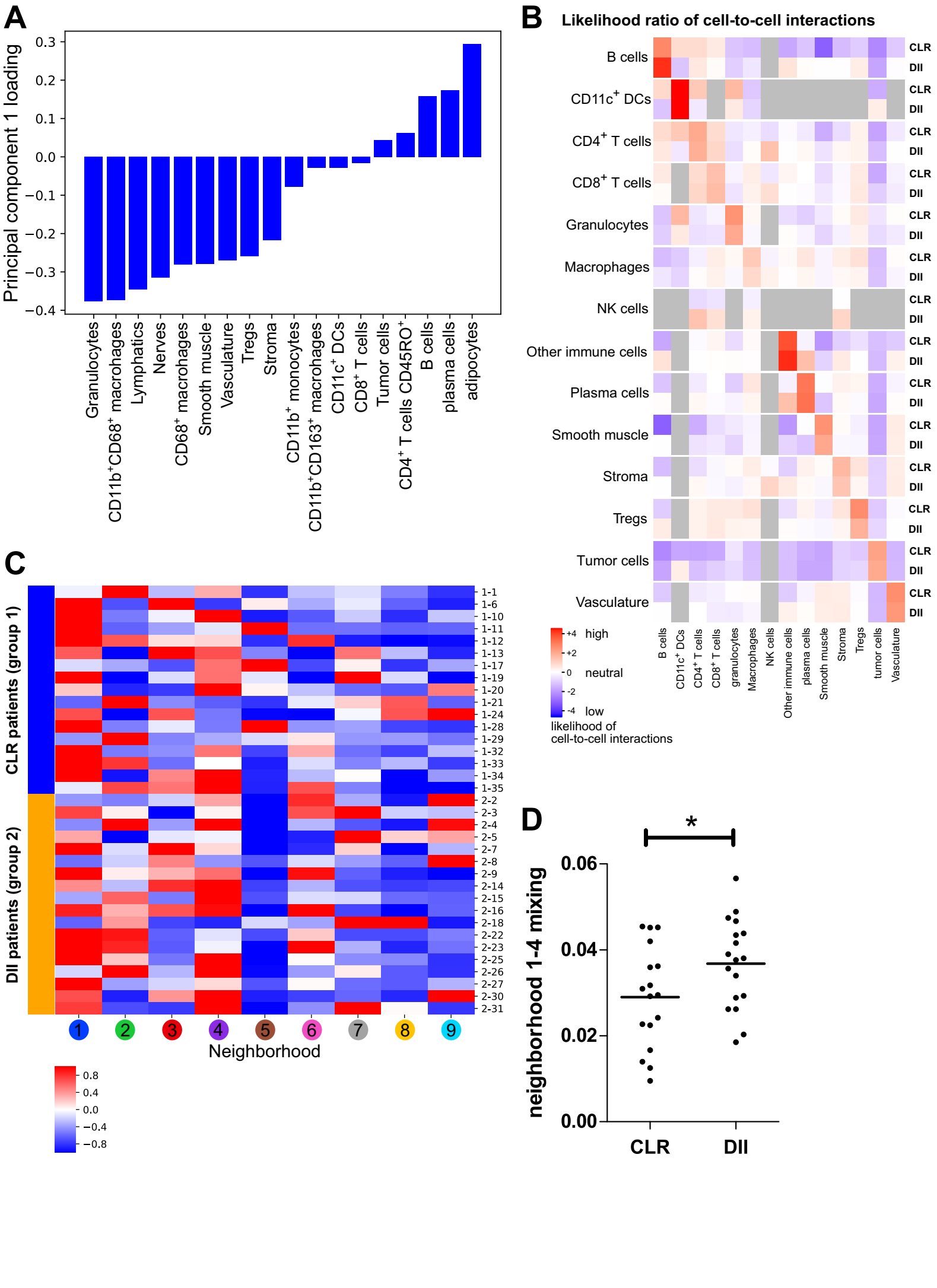
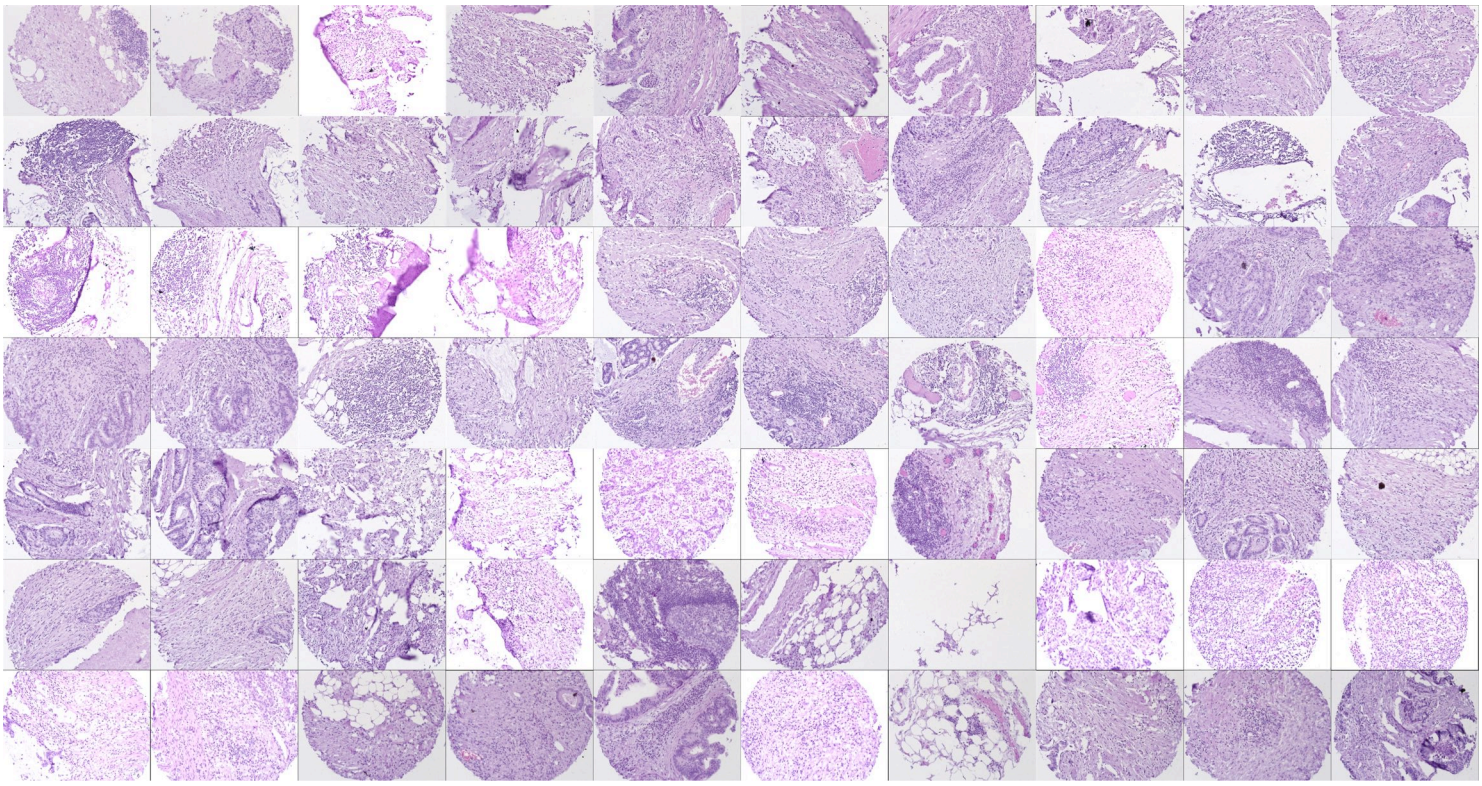
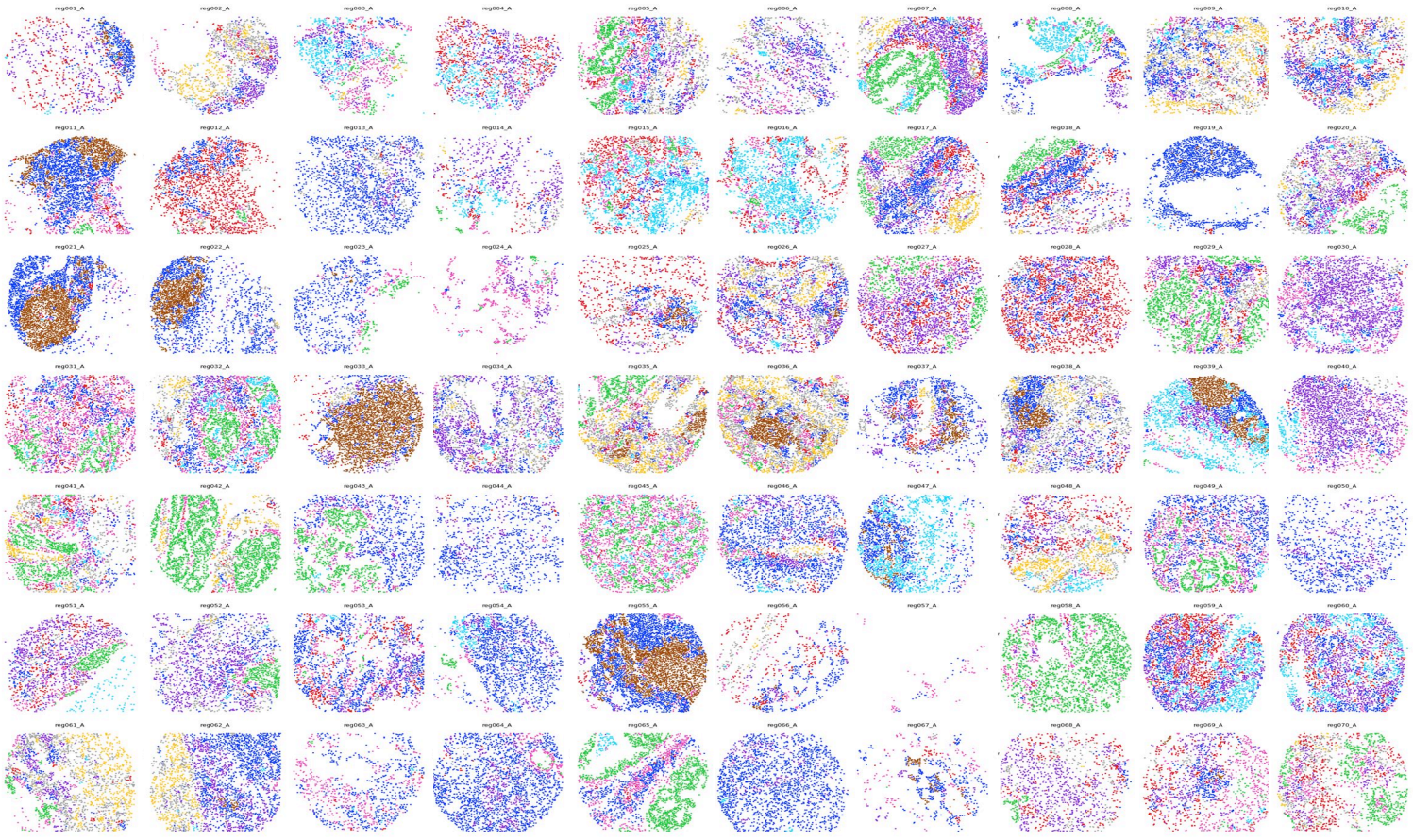

A
Principal component 1 loading
0.3
0.2
0.1
0.0
−0.1
−0.2
−0.3
−0.4
Granulocytes
CD11b+CD68+ macrophages
Lymphatics
Nerves
CD68+ macrophages
Smooth muscle
Vasculature
Tregs
Stroma
CD11b+ monocytes
CD11b+CD163+ macrophages
CD11c+ DCs
CD8+ T cells
Tumor cells
CD4+ T cells CD45RO+
B cells
plasma cells
adipocytes
B
Likelihood ratio of cell-to-cell interactions
B cells
CD11c+ DCs
CD4+ T cells
CD8+ T cells
Granulocytes
Macrophages
NK cells
Other immune cells
Plasma cells
Smooth muscle
Stroma
Tregs
Tumor cells
Vasculature
CLR
DII
+4 high
+2
0 neutral
-2
-4 low
likelihood of
cell-to-cell interactions
C
CLR patients (group 1)
DII patients (group 2)
1-1
1-6
1-10
1-11
1-12
1-13
1-17
1-19
1-20
1-21
1-24
1-28
1-29
1-32
1-33
1-34
1-35
2-2
2-3
2-4
2-5
2-7
2-8
2-9
2-14
2-15
2-16
2-18
2-22
2-23
2-25
2-26
2-27
2-30
2-31
1
2
3
4
5
6
7
8
9
Neighborhood
0.8
0.4
0.0
−0.4
−0.8
D
neighborhood 1-4 mixing
0.06
0.04
0.02
0.00
*
CLR
DII

Figure S17

A



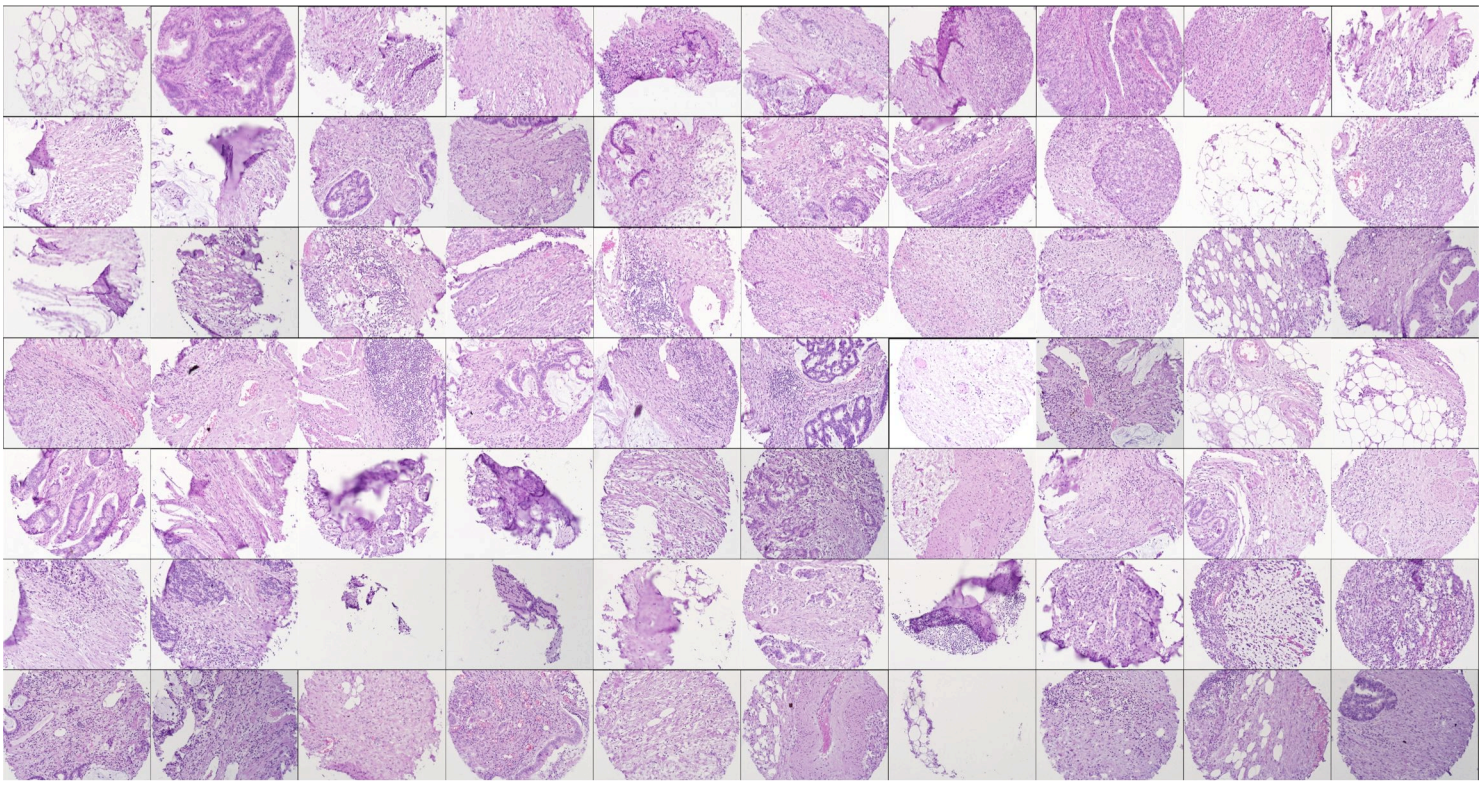
B



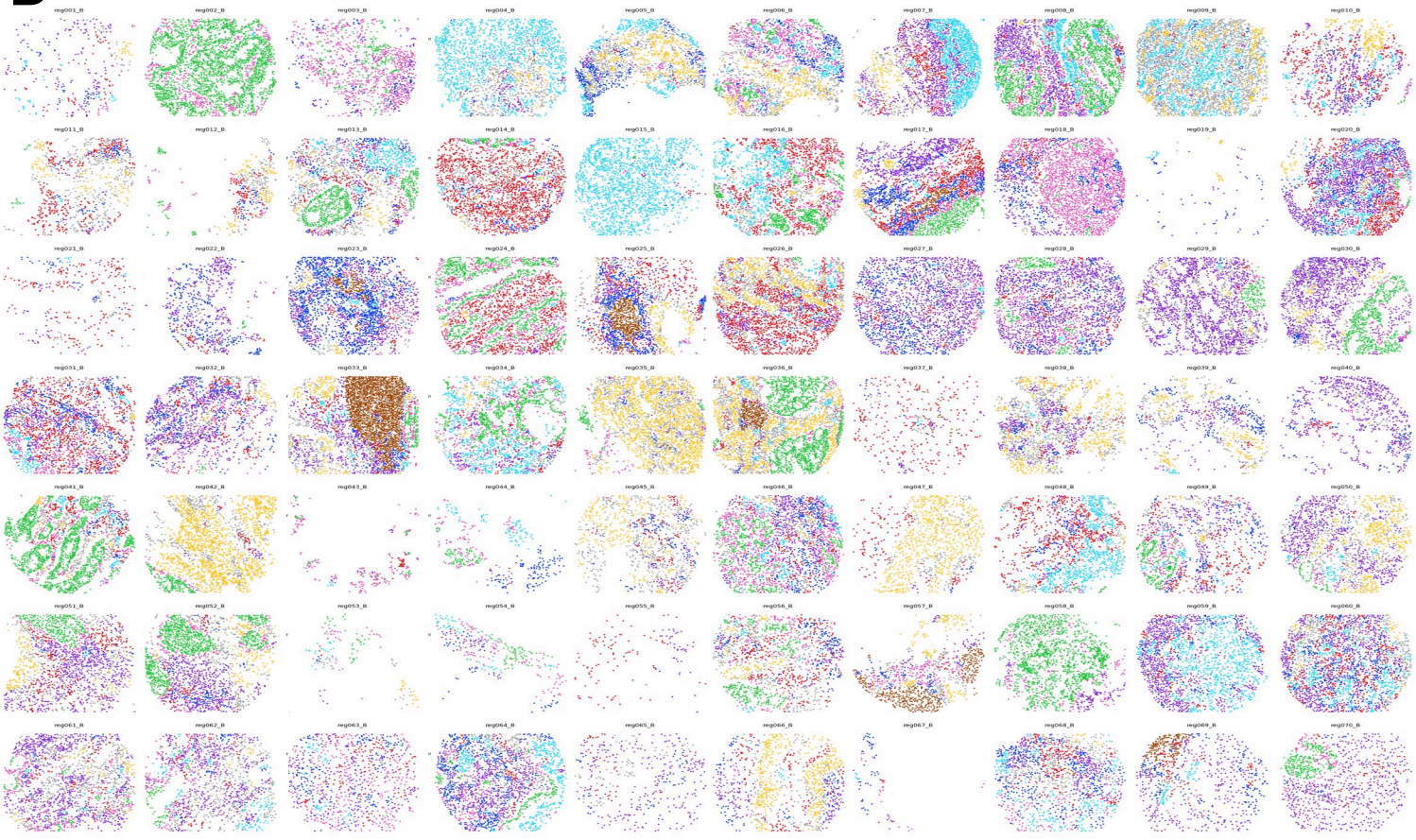
Neighborhood Legend

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

C



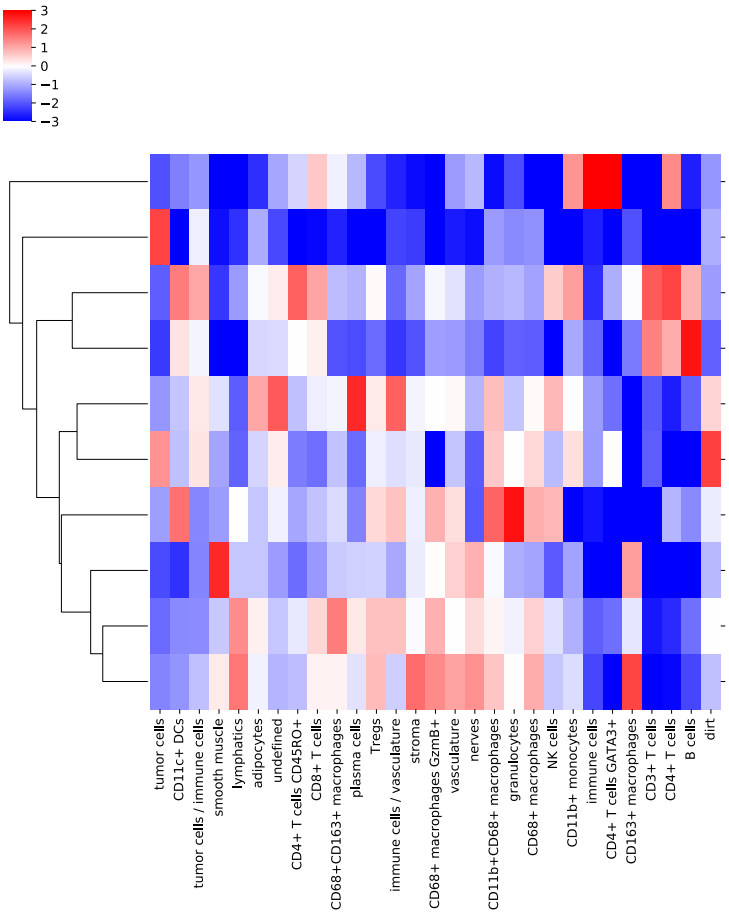
D



Neighborhood Legend

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

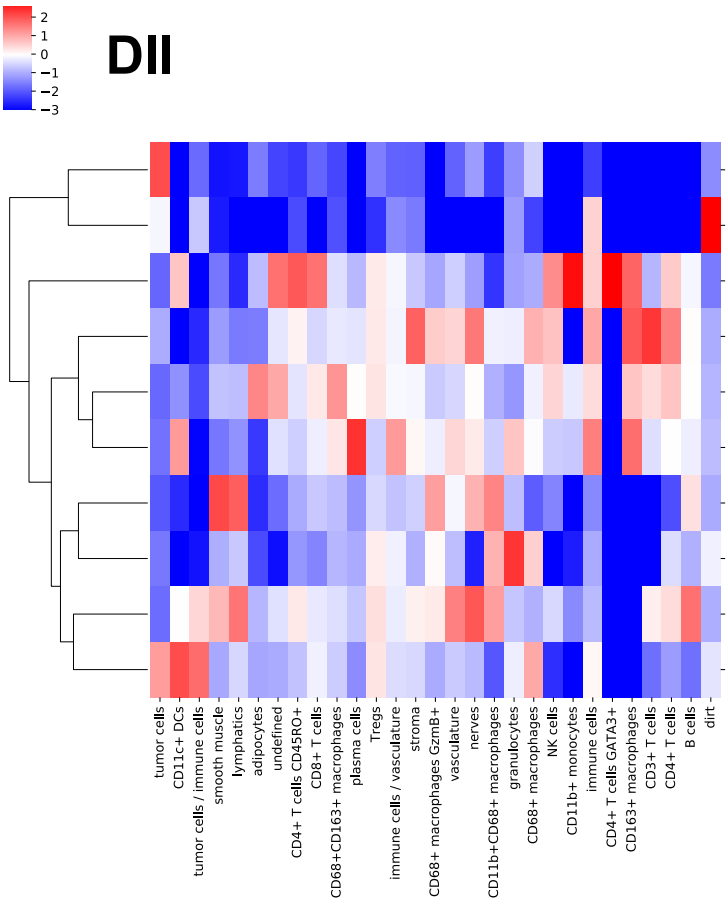
Figure S18



cellular neighborhood most similar to:

- not defined (CN-X)
- main tumor (CN-2)
- T cell enriched (CN-1)
- follicle (CN-5)
- tumor boundary (CN-6)
- artifacts (CN-0, not shown in Figure 4B)
- granulocyte enriched (CN-9)
- smooth muscle (CN-8)
- macrophage enriched (CN-4)
- immune-infiltrated stroma / vascularized smooth muscle (CN-3 / CN-7)

DII

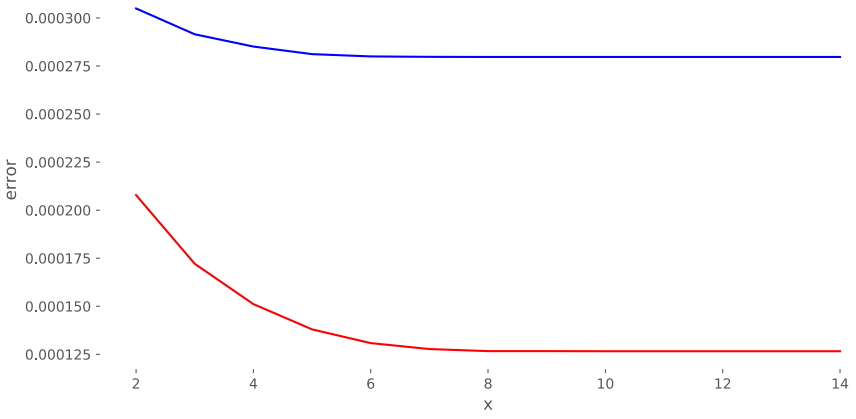


cellular neighborhood most similar to:

- main tumor (CN-2)
- artifacts (CN-0, not shown in Figure 4B)
- T cell enriched (CN-1)
- immune-infiltrated stroma (CN-3)
- macrophage enriched (CN-4)
- not defined (CN-X)
- smooth muscle (CN-8)
- granulocyte enriched (CN-9)
- vascularized smooth muscle (CN-7)
- tumor boundary (CN-6)

Figure S19

CLR



DII

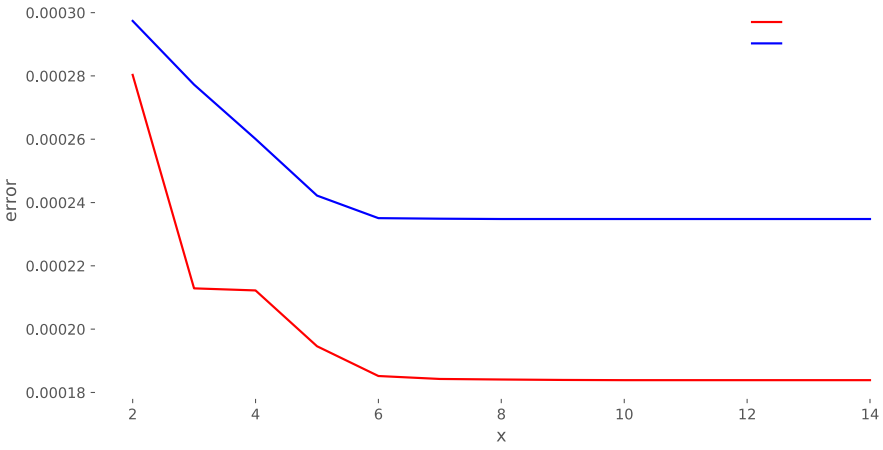
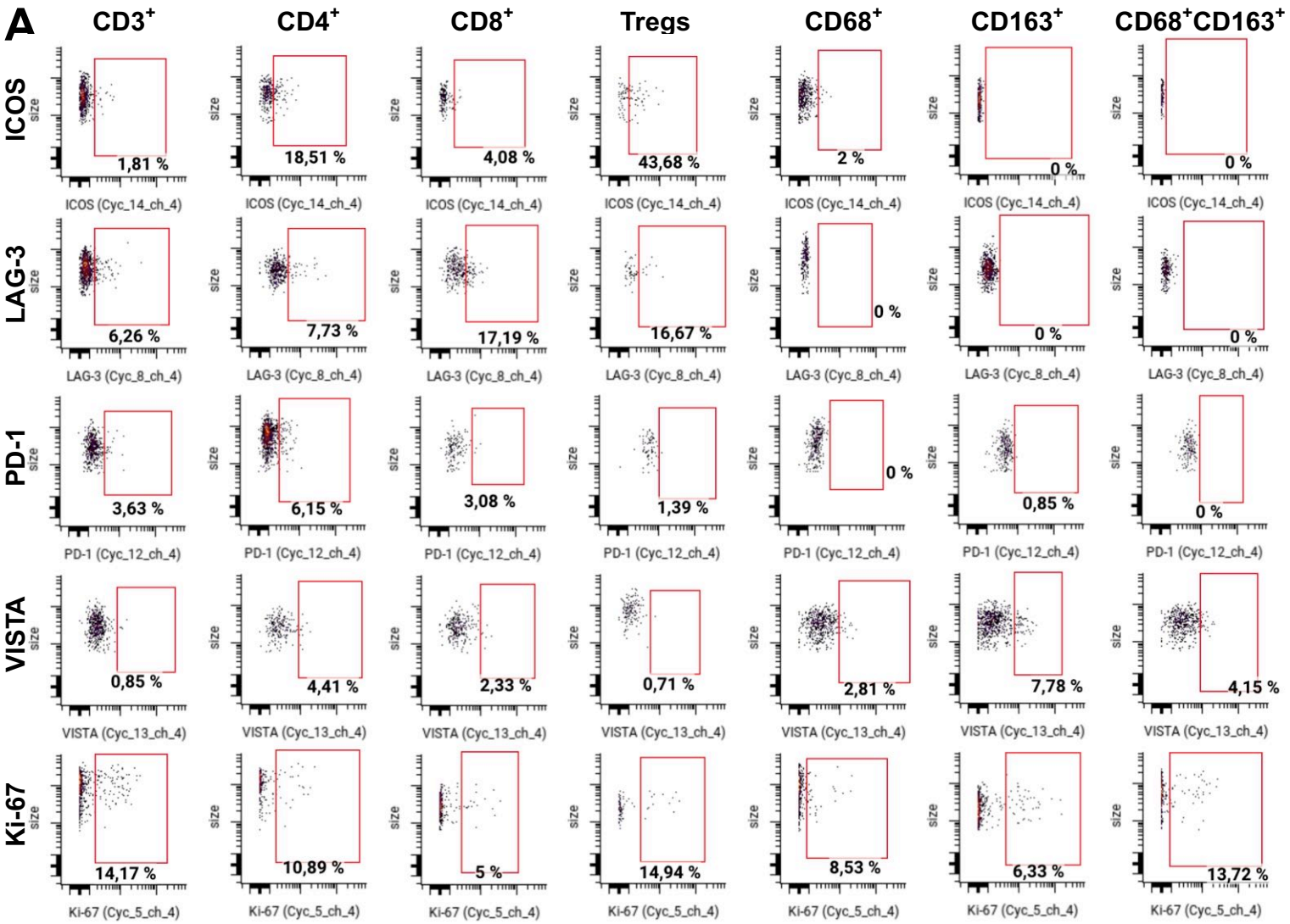
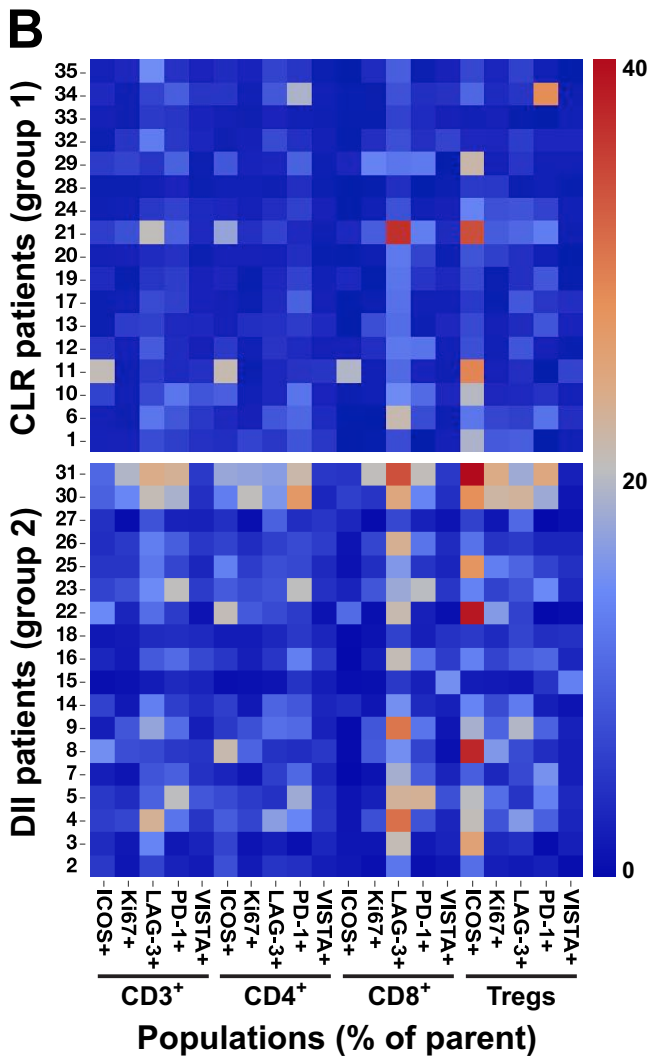


Figure S20


A
CD3+
CD4+
CD8+
Tregs
CD68+
CD163+
CD68+CD163+
ICOS
LAG-3
PD-1
VISTA
Ki-67
1,81 %
18,51 %
4,08 %
43,68 %
2 %
0 %
0 %
6,26 %
7,73 %
17,19 %
16,67 %
0 %
0 %
0 %
3,63 %
6,15 %
3,08 %
1,39 %
0 %
0,85 %
0 %
0,85 %
4,41 %
2,33 %
0,71 %
2,81 %
7,78 %
4,15 %
14,17 %
10,89 %
5 %
14,94 %
8,53 %
6,33 %
13,72 %


B
CLR patients (group 1)
DII patients (group 2)
40
20
0
ICOS+
Ki67+
LAG-3+
PD-1+
VISTA+
CD3+
CD4+
CD8+
Tregs
Populations (% of parent)

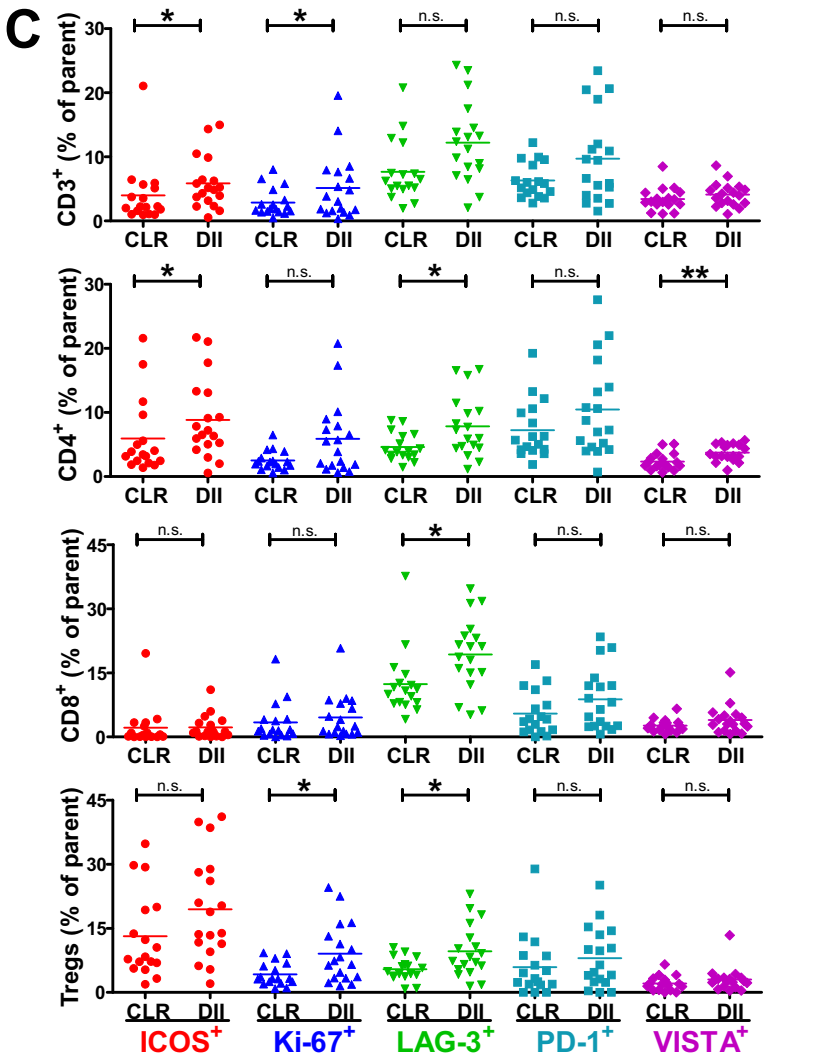
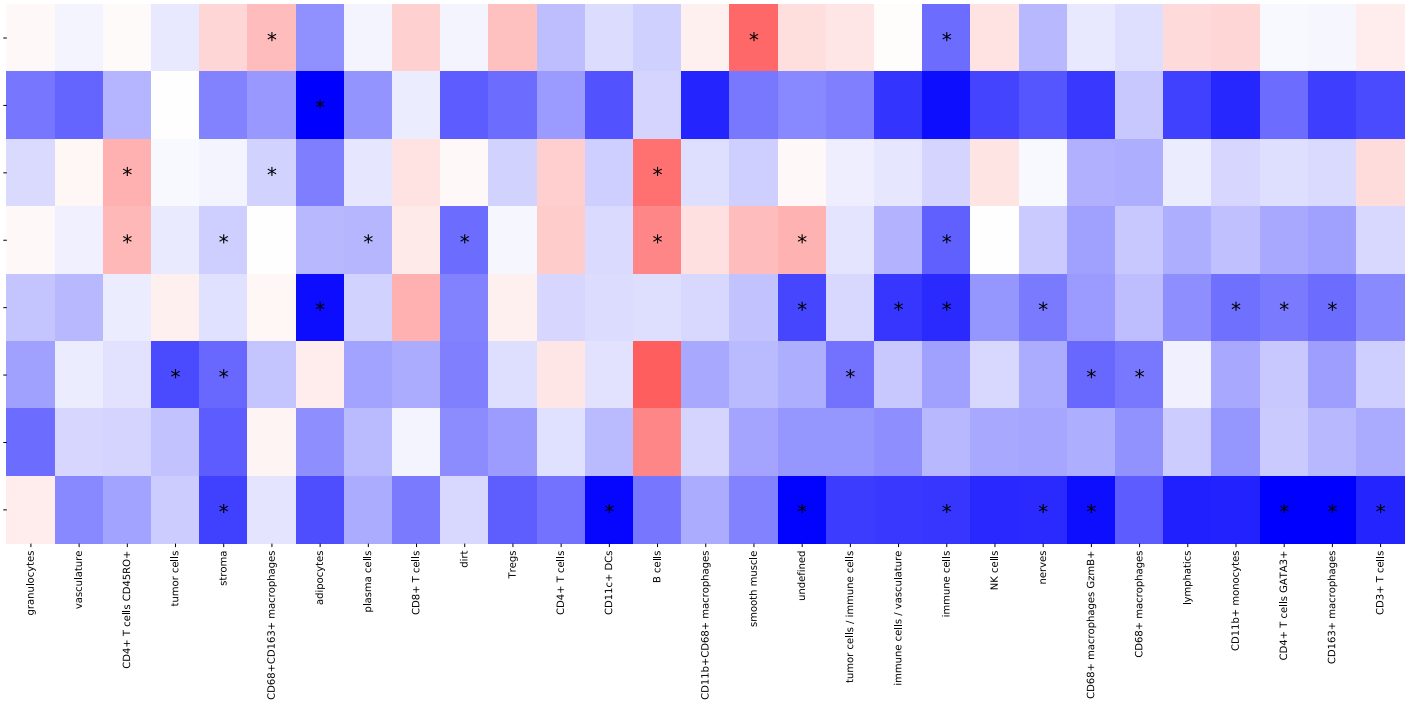

C
CD3+ (% of parent)
CD4+ (% of parent)
CD8+ (% of parent)
Tregs (% of parent)
CLR
DII
n.s.
ICOS+
Ki-67+
LAG-3+
PD-1+
VISTA+

Figure S21


0
2
3
4
6
7
8
9
granulocytes
vasculature
CD4+ T cells CD45RO+
tumor cells
stroma
CD68+CD163+ macrophages
adipocytes
plasma cells
CD8+ T cells
dirt
Tregs
CD4+ T cells
CD11c+ DCs
B cells
CD11b+CD68+ macrophages
smooth muscle
undefined
tumor cells / immune cells
immune cells / vasculature
immune cells
NK cells
nerves
CD68+ macrophages GzmB+
CD68+ macrophages
lymphatics
CD11b+ monocytes
CD4+ T cells GATA3+
CD163+ macrophages
CD3+ T cells

Supplemental Figure 22

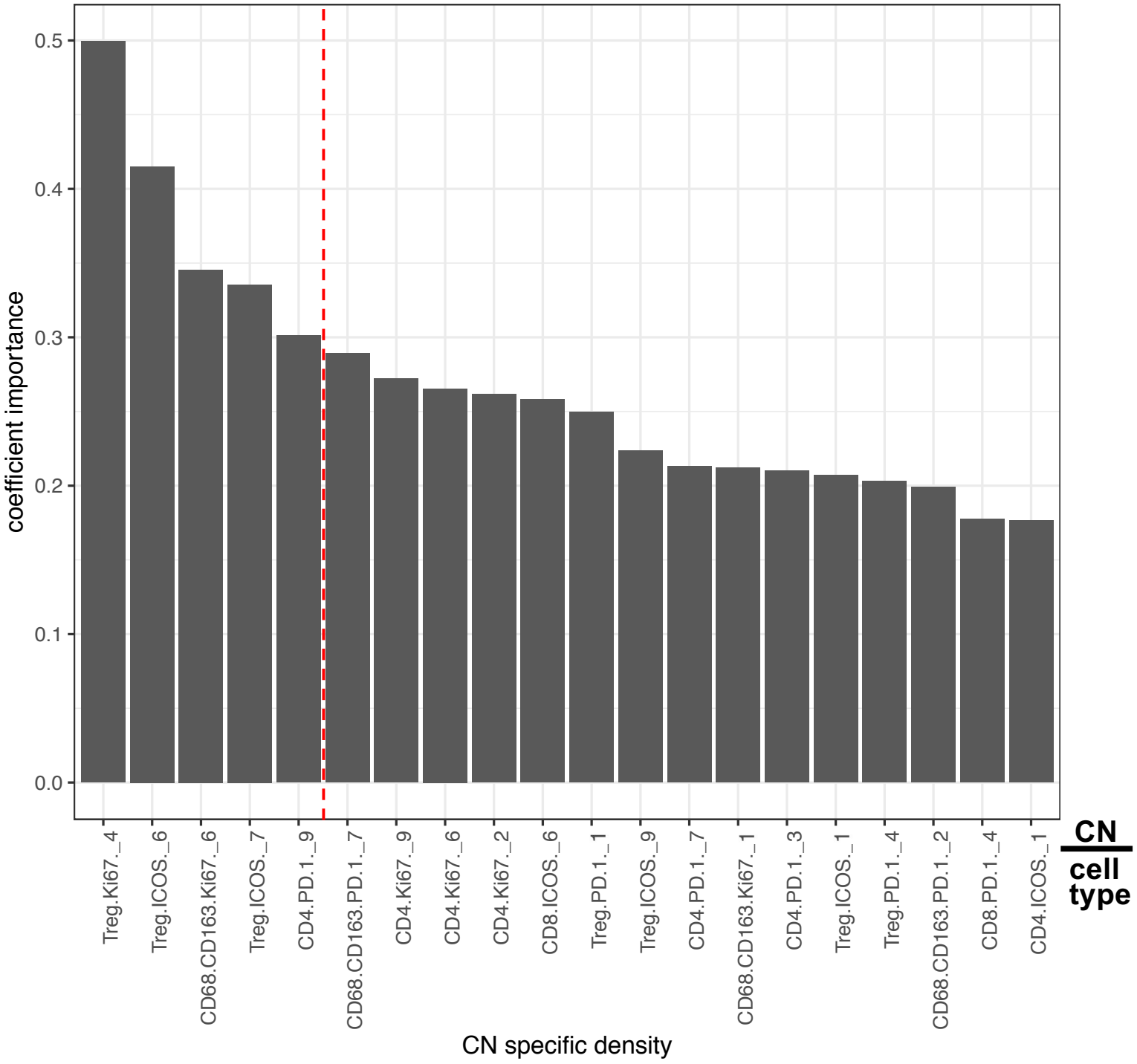


TABLE S2

Antigen	Clone(s)	Manufacturer	Catalog No.	CODEX oligo	Working dilution	Exposure time
α-SMA	polyclonal	Abcam	ab5694	69	1:200	1/4s
β-catenin	polyclonal	Novus Biologicals	AF1329	51	1:25	1/2s
BCL-2	124	Cell Marque	custom	41	1:50	1/2s
CD11b	EPR1344	Abcam	ab216445	28	1:25	1/2s
CD11c	EP1347Y	Abcam	ab216655	49	1:50	1/2s
CD134 (OX40)	Ber-ACT35	Biolegend	350002	71	1:100	1/2s
CD138	B-A38	Thermo Fisher Scientific	MA1-10091	76	1:100	1/8.5s
CD15	MMA	BD Biosciences	559045	14	1:200	1/8.5s
CD163	EDHu-1	Novus Biologicals	NB110-40686	45	1:50	1/2s
CD194 (CCR4)	L291H4	Biolegend	359402	55	1:25	1/2s
CD2	RPA-2.10	Biolegend	300202	25	1:25	1/2s
CD20	rIGEL/773	Novus Biologicals	NBP2-54591	48	1:200	1/4s
CD21	Bu32	Biolegend	354902	21	1:50	1/2s
CD223 (LAG3)	D2G4O	Cell Signaling Technology	custom	42	1:25	1/2s
CD25	4C9	Cell Marque	custom	24	1:100	1/2s
CD274 (PD-L1)	E1L3N	Cell Signaling Technology	custom	11	1:50	1/2s
CD278 (ICOS)	D1K2T	Cell Signaling Technology	custom	74	1:100	1/4s
CD279 (PD-1)	D4W2J	Cell Signaling Technology	custom	23	1:50	1/2s
CD3	MRQ-39	Cell Marque	custom	77	1:100	1/2s
CD30	BerH2	Cell Marque	custom	57	1:25	1/2s
CD31	C31.3 + C31.7 + C31.10	Novus Biologicals	NBP2-47785	68	1:200	1/5s
CD34	QBEnd/10	Novus Biologicals	NBP2-34713	38	1:100	1/2s
CD38	EPR4106	Abcam	ab176886	66	1:100	1/2s
CD4	EPR6855	Abcam	ab181724	20	1:100	1/2s
CD44	IM-7	Biolegend	103002	44	1:25	1/2s
CD45	2B11 + PD7/26	Novus Biologicals	NBP2-34528	56	1:100	1/4s
CD45RA	HI100	BD Biosciences	555486	72	1:50	1/2s
CD45RO	UCH-L1	Santa Cruz Biotechnology	sc-1183	2	1:25	1/2s
CD5	UCHT2	BD Biosciences	555350	75	1:25	1/2s
CD56	MRQ-42	Cell Marque	custom	29	1:50	1/2s
CD57	HCD57	Biolegend	322325	30	1:200	1/2s
CD68	KP-1	Biolegend	916104	70	1:200	1/3s

CD68	D4B9C	Cell Signaling Technology	custom	5	1:100	1/2s
CD7	MRQ-56	Cell Marque	custom	63	1:100	1/4s
CD71	MRQ-48	Cell Marque	custom	3	1:100	1/2s
CD79a	JBC117	Cell Marque	custom	46	1:25	1/2s
CD8	C8/144B	Cell Marque	custom	8	1:50	1/2s
CDX2	CDX2/1690	Novus Biologicals	NBP2-54472	53	1:25	1/2s
Chromogranin A	LK2H10 + PHE5 + CGA/414	Novus	NBP2-34674	43	1:400	1/4s
Collagen IV	polyclonal	Abcam	ab6586	33	1:100	1/4s
Cytokeratin 7	OV-TL12/30	Novus	NBP2-47940	3	1:200	1/4s
EGFR	D38B1	Cell Signaling Technology	custom	58	1:25	1/2s
EpCAM	BerEp4	Cell Marque	custom	70	1:25	1/2s
FoxP3	236A/E7	Invitrogen	14-4777-80	61	1:100	1/2s
GATA3	L50-823	Cell Marque	custom	60	1:100	1/2s
GFAP	2.2B10	Thermo Fisher Scientific	13-0300	46	1:25	1/2s
Granzyme B	EPR20129-217	Abcam	ab219803	81	1:200	1/4s
Hep-Par-1	OCH1E5	Santa Cruz Biotechnology	sc-58693	28	1:100	1/2s
HLA-DR	EPR3692	Abcam	ab215985	65	1:100	1/4s
IDO-1	D5J4E	Cell Signaling Technology	custom	59	1:25	1/2s
IRF4	IRF4.3E4	Biolegend	646402	51	1:25	1/2s
Ki-67	B56	BD Biosciences	556003	6	1:100	1/8.5s
Melan-A	A103 + M2-7C10 + M2-9E3	Novus Biologicals	NBP2-34546	44	1:50	1/2s
MMP12	polyclonal	Abcam	ab137444	80	1:200	1/4s
MMP9	L51/82	Biolegend	819701	62	1:400	1/4s
MUC-1 (EMA)	955	NSJ Bioreagents	V2372SAF	15	1:100	1/4s
Na-K-ATPase	EP1845Y	Abcam	ab167390	36	1:100	1/4s
p53	D07	Cell Marque	custom	52	1:25	1/2s
Pan-Cytokeratin	C11	Biolegend	628602	67	1:200	1/8.5s
PAX5	D7H5X	Cell Signaling Technology	custom	42	1:25	1/2s
Podoplanin	D2-40	Biolegend	916606	32	1:200	1/4s
Synaptophysin	7H12	Novus	NBP1-47483	26	1:100	1/4s
T-bet	D6N8B	Cell Signaling Technology	custom	5	1:100	1/2s
Vimentin	RV202	BD Biosciences	550513	7	1:200	1/2s
VISTA	D1L2G	Cell Signaling Technology	custom	79	1:50 / 1:100	1/2s

TABLE S3

Oligo No.	Antibody oligo sequence (5'-3')	Fluorescent oligonucleotide sequences (5'-3')			Blocking component 4 (BC4) oligos (5'-3')
2	/mal/ATGGTTTAGGACTAC	/5Alex488N/GTAGTCCTAAACCAT	/5ATTO550N/GTAGTCCTAAACCAT		GTAGTCCTAAACCAT
3	/mal/TACTCCTCGCCG	/5Alex488N/CGGCGAGGAGTA	/5ATTO550N/CGGCGAGGAGTA	/5Alex647N/CGGCGAGGAGTA	CGGCGAGGAGTA
5	/mal/TCTCCCATTAGTCGG	/5Alex488N/CCGACTAATGGGAGA	/5ATTO550N/CCGACTAATGGGAGA		CCGACTAATGGGAGA
6	/mal/TGGATGTGTTACGAT	/5Alex488N/ATCGTAACACATCCA	/5ATTO550N/ATCGTAACACATCCA	/5Alex647N/ATCGTAACACATCCA	ATCGTAACACATCCA
7	/mal/CGCTAAGATATTCTAAG	/5Alex488N/CTTAGAATATCTTAGCG	/5ATTO550N/CTTAGAATATCTTAGCG		CTTAGAATATCTTAGCG
8	/mal/CGCAGATGAATATTC	/5Alex488N/GAATATTCATCTGCG	/5ATTO550N/GAATATTCATCTGCG	/5Alex647N/GAATATTCATCTGCG	GAATATTCATCTGCG
11	/mal/GGGTTATACACTCGT	/5Alex488N/ACGAGTGTATAACCC	/5ATTO550N/ACGAGTGTATAACCC		ACGAGTGTATAACCC
14	/mal/AGATTCAGTCTCTCG	/5Alex488N/CGAGAGACTGAATCT	/5ATTO550N/CGAGAGACTGAATCT		CGAGAGACTGAATCT
15	/mal/CTGTAATAGGCACTA	/5Alex488N/TAGTGCCTATTACAG	/5ATTO550N/TAGTGCCTATTACAG	/5Alex647N/TAGTGCCTATTACAG	TAGTGCCTATTACAG
20	/mal/ATGTAGGATGGTCTC		/5ATTO550N/GAGACCATCCTACAT	/5Alex647N/GAGACCATCCTACAT	GAGACCATCCTACAT
21	/mal/CGTGCCGTTTCAC	/5Alex488N/GTGAAACGGCACG	/5ATTO550N/GTGAAACGGCACG	/5Alex647N/GTGAAACGGCACG	GTGAAACGGCACG
23	/mal/GGTTTCCTCAGACAC	/5Alex488N/GTGTCTGAGGAAACC	/5ATTO550N/GTGTCTGAGGAAACC	/5Alex647N/GTGTCTGAGGAAACC	GTGTCTGAGGAAACC
24	/mal/ATAAGGGCTCATTGT	/5Alex488N/ACAATGAGCCCTTAT	/5ATTO550N/ACAATGAGCCCTTAT	/5Alex647N/ACAATGAGCCCTTAT	ACAATGAGCCCTTAT
25	/mal/GCACGCCCTTTTA		/5ATTO550N/TAAAAGGGCGTGC	/5Alex647N/TAAAAGGGCGTGC	TAAAAGGGCGTGC
26	/mal/CACTTGTCTAACCAA	/5Alex488N/TTGGTTAGACAAGTG	/5ATTO550N/TTGGTTAGACAAGTG	/5Alex647N/TTGGTTAGACAAGTG	TTGGTTAGACAAGTG
28	/mal/TGGTCCACTAACGTA	/5Alex488N/TACGTTAGTGGACCA	/5ATTO550N/TACGTTAGTGGACCA	/5Alex647N/TACGTTAGTGGACCA	TACGTTAGTGGACCA
29	/mal/ATAGGGCATTTGAAG	/5Alex488N/CTTCAAATGCCCTAT	/5ATTO550N/CTTCAAATGCCCTAT	/5Alex647N/CTTCAAATGCCCTAT	CTTCAAATGCCCTAT
30	/mal/CACATGAGCGAATCA		/5ATTO550N/TGATTCGCTCATGTG	/5Alex647N/TGATTCGCTCATGTG	TGATTCGCTCATGTG
32	/mal/TACCAAATCCTGATG	/5Alex488N/CATCAGGATTTGGTA	/5ATTO550N/CATCAGGATTTGGTA	/5Alex647N/CATCAGGATTTGGTA	CATCAGGATTTGGTA
33	/mal/TTATCATGAGGAGCG	/5Alex488N/CGCTCCTCATGATAA		/5Alex647N/CGCTCCTCATGATAA	CGCTCCTCATGATAA
36	/mal/ACCTACACAATGCTA	/5Alex488N/TAGCATTGTGTAGGT	/5ATTO550N/TAGCATTGTGTAGGT		TAGCATTGTGTAGGT
38	/mal/GCGTCTACTTATAAG	/5Alex488N/CTTATAAGTAGACGC	/5ATTO550N/CTTATAAGTAGACGC		CTTATAAGTAGACGC
41	/mal/TGTATGAGTAGTAATCT	/5Alex488N/AGATTACTACTCATACA	/5ATTO550N/AGATTACTACTCATACA	/5Alex647N/AGATTACTACTCATACA	AGATTACTACTCATACA
42	/mal/TCTAAGTCAGAGAGC		/5ATTO550N/GCTCTCTGACTTAGA	/5Alex647N/GCTCTCTGACTTAGA	GCTCTCTGACTTAGA
43	/mal/GACATTATCCGTGAT	/5Alex488N/ATCACGGATAATGTC	/5ATTO550N/ATCACGGATAATGTC	/5Alex647N/ATCACGGATAATGTC	ATCACGGATAATGTC
44	/mal/TCACTACTATTAGTACT	/5Alex488N/AGTACTAATAGTAGTGA	/5ATTO550N/AGTACTAATAGTAGTGA		AGTACTAATAGTAGTGA
45	/mal/GCCAGAATGCCA		/5ATTO550N/TGGCATTCTGGC	/5Alex647N/TGGCATTCTGGC	TGGCATTCTGGC
46	/mal/GACTCGAACCTGAG	/5Alex488N/CTCAGGTTCGAGTC		/5Alex647N/CTCAGGTTCGAGTC	CTCAGGTTCGAGTC
48	/mal/GCACGGCAAAGTG	/5Alex488N/CACTTTGCCGTGC	/5ATTO550N/CACTTTGCCGTGC	/5Alex647N/CACTTTGCCGTGC	CACTTTGCCGTGC
49	/mal/ATAACGCCTCGTATC	/5Alex488N/GATACGAGGCGTTAT	/5ATTO550N/GATACGAGGCGTTAT		GATACGAGGCGTTAT
51	/mal/CGTGCGGGAAAAT	/5Alex488N/ATTTTCCCGCACG		/5Alex647N/ATTTTCCCGCACG	ATTTTCCCGCACG
52	/mal/ACCAGGTGATTGCAT		/5ATTO550N/ATGCAATCACCTGGT	/5Alex647N/ATGCAATCACCTGGT	ATGCAATCACCTGGT
53	/mal/TATCCCGTAAACTTC		/5ATTO550N/GAAGTTTACGGGATA	/5Alex647N/GAAGTTTACGGGATA	GAAGTTTACGGGATA
55	/mal/AGGTCAACTCGCAC	/5Alex488N/GTGCGAGTTGACCT	/5ATTO550N/GTGCGAGTTGACCT		GTGCGAGTTGACCT
56	/mal/GGTCACATGGTCGTT	/5Alex488N/AACGACCATGTGACC	/5ATTO550N/AACGACCATGTGACC	/5Alex647N/AACGACCATGTGACC	AACGACCATGTGACC
57	/mal/GCGGATTTCGTATTT	/5Alex488N/AAATACGAAATCCGC	/5ATTO550N/AAATACGAAATCCGC	/5Alex647N/AAATACGAAATCCGC	AAATACGAAATCCGC
58	/mal/CGTCAGTACTTTCAG	/5Alex488N/CTGAAAGTACTGACG	/5ATTO550N/CTGAAAGTACTGACG		CTGAAAGTACTGACG
59	/mal/GCTTATTATGGACTTC		/5ATTO550N/GAAGTCCATAATAAGC	/5Alex647N/GAAGTCCATAATAAGC	GAAGTCCATAATAAGC
60	/mal/ACAAAACTGCTGTCG		/5ATTO550N/CGACAGCAGTTTTGT	/5Alex647N/CGACAGCAGTTTTGT	CGACAGCAGTTTTGT
61	/mal/TCTTATTCCCGAATA		/5ATTO550N/TATTCGGGAATAAGA	/5Alex647N/TATTCGGGAATAAGA	TATTCGGGAATAAGA
62	/mal/TAGGGGAACAGGTTG	/5Alex488N/CAACCTGTTCCCCTA	/5ATTO550N/CAACCTGTTCCCCTA		CAACCTGTTCCCCTA
63	/mal/AATTAGCTTAAGAGAGT	/5Alex488N/ACTCTCTTAAGCTAATT	/5ATTO550N/ACTCTCTTAAGCTAATT		ACTCTCTTAAGCTAATT
65	/mal/GATAAATATTTTACAGAGT	/5Alex488N/ACTCTGTAAAATATTTAT(	/5ATTO550N/ACTCTGTAAAATATTTATC		ACTCTGTAAAATATTTATC
66	/mal/TACGTGCTTTGGTT	/5Alex488N/AACCAAAGCACGTA	/5ATTO550N/AACCAAAGCACGTA	/5Alex647N/AACCAAAGCACGTA	AACCAAAGCACGTA
67	/mal/GACGACGAAGGC	/5Alex488N/GCCTTCGTCGTC	/5ATTO550N/GCCTTCGTCGTC	/5Alex647N/GCCTTCGTCGTC	GCCTTCGTCGTC
68	/mal/CTTCTTGTGGAACC	/5Alex488N/GGTTCCACAAGAAG	/5ATTO550N/GGTTCCACAAGAAG		GGTTCCACAAGAAG
69	/mal/CCCGGCAGTT	/5Alex488N/AACTGCCGGG		/5Alex647N/AACTGCCGGG	AACTGCCGGG
70	/mal/AACCAAACTGACCG	/5Alex488N/CGGTCAGTTTGGTT		/5Alex647N/CGGTCAGTTTGGTT	CGGTCAGTTTGGTT
71	/mal/TCACCCCCAGC		/5ATTO550N/GCTGGGGGTGA	/5Alex647N/GCTGGGGGTGA	GCTGGGGGTGA
72	/mal/AACGCGACGGAT	/5Alex488N/ATCCGTCGCGTT	/5ATTO550N/ATCCGTCGCGTT	/5Alex647N/ATCCGTCGCGTT	ATCCGTCGCGTT
74	/mal/ATTTGCTTCGACGA		/5ATTO550N/TCGTCGAAGCAAAT	/5Alex647N/TCGTCGAAGCAAAT	TCGTCGAAGCAAAT
75	/mal/CGCTTGGGTGTTTA	/5Alex488N/TAAACACCCAAGCG	/5ATTO550N/TAAACACCCAAGCG		TAAACACCCAAGCG
76	/mal/CTGTCGGTCGTCA		/5ATTO550N/TGACGACCGACAG	/5Alex647N/TGACGACCGACAG	TGACGACCGACAG
77	/mal/ATTTCAACAAATATTGTT		/5ATTO550N/AACAATATTTGTTGAAA	/5Alex647N/AACAATATTTGTTGAAAT	AACAATATTTGTTGAAAT
79	/mal/GCCGGAAGTGGT		/5ATTO550N/ACCACTTCCGGC	/5Alex647N/ACCACTTCCGGC	ACCACTTCCGGC
80	/mal/CGGGGCACCA		/5ATTO550N/TGGTGCCCCG	/5Alex647N/TGGTGCCCCG	TGGTGCCCCG
81	/mal/CAAGGAACTACCGA	/5Alex488N/TCGGTAGTTCCTTG	/5ATTO550N/TCGGTAGTTCCTTG	/5Alex647N/TCGGTAGTTCCTTG	TCGGTAGTTCCTTG

TABLE S4

CRC panel

Cycle	Channel 1	Exposure Time	Alexa488	Oligo	Clone	Ab dilution	Exposure Time	ATTO550	Oligo	Clone	Ab dilution	Exposure Time	Alexa647	Oligo	Clone	Ab dilution	Exposure Time
1	Hoechst 33342	1/175s	blank				1/2s	blank				1/2s	blank				1/2s
2	Hoechst 33342	1/175s	CD44	44	IM-7	1:25	1/2s	FoxP3	61	236A/E7	1:100	1/2s	CDX2	53	CDX2/1690	1:25	1/2s
3	Hoechst 33342	1/175s	CD8	8	C8/144B	1:50	1/2s	p53	52	D07	1:25	1/2s	GATA3	60	L50-823	1:100	1/2s
4	Hoechst 33342	1/175s	CD45	56	2B11+PD7/26	1:100	1/4s	T-bet	5	D6N8B	1:100	1/2s	β-catenin	51	polyclonal	1:25	1/2s
5	Hoechst 33342	1/175s	HLA-DR	65	EPR3692	1:100	1/4s	CD274 (PD-L1)	11	E1L3N	1:50	1/2s	Ki-67	6	B56	1:100	1/8.5s
6	Hoechst 33342	1/175s	CD45RA	72	HI100	1:50	1/2s	CD4	20	EPR6855	1:100	1/2s	CD21	21	Bu32	1:50	1/2s
7	Hoechst 33342	1/175s	MUC-1 (EMA)	15	955	1:100	1/4s	CD30	57	BerH2	1:25	1/2s	CD2	25	RPA-2.10	1:25	1/2s
8	Hoechst 33342	1/175s	Vimentin	7	RV202	1:200	1/2s	CD20	48	rIGEL/773	1:200	1/4s	CD223 (LAG3)	42	D2G4O	1:25	1/2s
9	Hoechst 33342	1/175s	Na-K-ATPase	36	EP1845Y	1:100	1/4s	CD5	75	UCHT2	1:25	1/2s	IDO-1	59	D5J4E	1:25	1/2s
10	Hoechst 33342	1/175s	Pan-Cytokeratin	67	C11	1:200	1/8.5s	CD11b	28	EPR1344	1:25	1/2s	CD56	29	MRQ-42	1:50	1/2s
11	Hoechst 33342	1/175s	α-SMA	69	polyclonal	1:200	1/4s	BCL-2	41	124	1:50	1/2s	CD25	24	4C9	1:100	1/2s
12	Hoechst 33342	1/175s	Collagen IV	33	polyclonal	1:100	1/4s	CD11c	49	EP1347Y	1:50	1/2s	CD279 (PD-1)	23	D4W2J	1:50	1/2s
13	Hoechst 33342	1/175s	Granzyme B	81	EPR20129-217	1:200	1/8.5s	EGFR	58	D38B1	1:25	1/2s	VISTA	79	D1L2G	1:50	1/2s
14	Hoechst 33342	1/175s	CD15	14	MMA	1:200	1/8.5s	CD194 (CCR4)	55	L291H4	1:25	1/2s	CD278 (ICOS)	74	D1K2T	1:100	1/4s
15	Hoechst 33342	1/175s	MMP9	62	L51/82	1:400	1/4s	Synaptophysin	26	7H12	1:100	1/4s	CD71	3	MRQ-48	1:100	1/2s
16	Hoechst 33342	1/175s	GFAP	46	2.2B10	1:25	1/2s	CD7	63	MRQ-56	1:100	1/4s	CD3	77	MRQ-39	1:100	1/2s
17	Hoechst 33342	1/175s	Chromogranin A	43	LK2H10+PHE5+CGA/414	1:400	1/4s	CD163	45	EDHu-1	1:50	1/4s	CD57	30	HCD57	1:200	1/2s
18	Hoechst 33342	1/175s	blank				1/600s	CD45RO	2	UCH-L1	1:25	1/2s	CD68	70	KP-1	1:200	1/3s
19	Hoechst 33342	1/175s	blank				1/600s	CD31	68	C31.3+C31.7+C31.10	1:200	1/5s	Podoplanin	32	D2-40	1:200	1/4s
20	Hoechst 33342	1/175s	blank				1/600s	CD34	38	QBEnd/10	1:100	1/2s	CD38	66	EPR4106	1:100	1/2s
21	Hoechst 33342	1/175s	blank				1/600s	CD138	76	B-A38	1:100	1/8.5s	MMP12	80	polyclonal	1:200	1/4s
22	Hoechst 33342	1/175s	blank				1/8.5s	blank				1/8.5s	blank				1/8.5s
23	Hoechst 33342	1/175s	blank				1/600s	blank				1/600s	DRAQ5			1:100	1/8.5s
24	H&E (brightfield)																

Multi-tumor TMA panel

Cycle	Channel 1	Exposure Time	Alexa488	Oligo	Clone	Ab dilution	Exposure Time	ATTO550	Oligo	Clone	Ab dilution	Exposure Time	Alexa647	Oligo	Clone	Ab dilution	Exposure Time
1	Hoechst 33342	1/175s	blank				1/2s	blank				1/2s	blank				1/2s
2	Hoechst 33342	1/175s	CD79a	46	JBC117	1:25	1/2s	FoxP3	61	236A/E7	1:100	1/2s	CDX2	53	CDX2/1690	1:25	1/2s
3	Hoechst 33342	1/175s	CD8	8	C8/144B	1:50	1/2s	p53	52	D07	1:25	1/2s	GATA3	60	L50-823	1:100	1/2s
4	Hoechst 33342	1/175s	CD21	21	Bu32	1:50	1/2s	CD274 (PD-L1)	11	E1L3N	1:50	1/2s	Ki-67	6	B56	1:100	1/8.5s
5	Hoechst 33342	1/175s	CD45	56	2B11+PD7/26	1:100	1/4s	CD30	57	BerH2	1:25	1/2s	PAX5	42	D7H5X	1:25	1/2s
6	Hoechst 33342	1/175s	HLA-DR	65	EPR3692	1:100	1/4s	CD5	75	UCHT2	1:50	1/2s	CD2	25	RPA-2.10	1:25	1/2s
7	Hoechst 33342	1/175s	CD45RA	72	HI100	1:50	1/2s	CD4	20	EPR6855	1:100	1/2s	CD279 (PD-1)	23	D4W2J	1:50	1/2s
8	Hoechst 33342	1/175s	MUC-1 (EMA)	15	955	1:100	1/4s	BCL-2	41	124	1:50	1/2s	CD56	29	MRQ-42	1:100	1/2s
9	Hoechst 33342	1/175s	Cytokeratin 7	3	OV-TL12/30	1:200	1/4s	CD25	24	4C9	1:100	1/2s	VISTA	79	D1L2G	1:100	1/2s
10	Hoechst 33342	1/175s	Hep-Par-1	28	OCH1E5	1:100	1/2s	CD11c	49	EP1347Y	1:50	1/2s	IRF4	51	IRF4.3E4	1:25	1/2s
11	Hoechst 33342	1/175s	CD20	48	rIGEL/773	1:400	1/8.5s	EGFR	58	D38B1	1:25	1/2s	IDO-1	59	D5J4E	1:25	1/2s
12	Hoechst 33342	1/175s	Granzyme B	81	EPR20129-217	1:200	1/4s	Melan-A	44	A103 + M2-7C10 + M2	1:50	1/2s	-				
13	Hoechst 33342	1/175s	Vimentin	7	RV202	1:200	1/2s	Synaptophysin	26	7H12	1:100	1/4s	-				
14	Hoechst 33342	1/175s	Na-K-ATPase	36	EP1845Y	1:100	1/4s	CD194 (CCR4)	55	L291H4	1:25	1/2s	CD57	30	HCD57	1:200	1/2s
15	Hoechst 33342	1/175s	Pan-Cytokeratin	67	C11	1:200	1/8.5s	CD68	5	D4B9C	1:100	1/2s	CD3	77	MRQ-39	1:100	1/2s
16	Hoechst 33342	1/175s	α-SMA	69	polyclonal	1:200	1/4s	CD34	38	QBEnd/10	1:50	1/2s	EpCAM	70	BerEp4	1:25	1/2s
17	Hoechst 33342	1/175s	Collagen IV	33	polyclonal	1:200	1/4s	CD45RO	2	UCH-L1	1:25	1/2s	Podoplanin	32	D2-40	1:200	1/4s
18	Hoechst 33342	1/175s	CD15	14	MMA	1:200	1/8.5s	CD7	63	MRQ-56	1:25	1/2s	CD163	45	EDHu-1	1:50	1/2s
19	Hoechst 33342	1/175s	Chromogranin A	43	LK2H10+PHE5+CGA/414	1:400	1/4s	CD31	68	C31.3+C31.7+C31.10	1:200	1/5s	-				
20	Hoechst 33342	1/175s	MMP9	62	L51/82	1:400	1/4s	CD138	76	B-A38	1:100	1/8.5s	CD38	66	EPR4106	1:25	1/2s
21	Hoechst 33342	1/175s	blank				1/2s	blank				1/2s	blank				1/2s
22	Hoechst 33342	1/175s	blank				1/600s	Hyaluronan-BP-bi	Streptavidin-PE		1:500 each	1/4s	DRAQ5			1:100	1/8.5s
23	H&E (brightfield)																

Tonsil panel

Cycle	Channel 1	Exposure Time	Alexa488	Oligo	Clone	Ab dilution	Exposure Time	ATTO550	Oligo	Clone	Ab dilution	Exposure Time	Alexa647	Oligo	Clone	Ab dilution	Exposure Time
1	Hoechst 33342	1/175s	blank				1/2s	blank				1/2s	blank				1/2s
2	Hoechst 33342	1/175s	CD79a	46	JBC117	1:25	1/2s	FoxP3	61	236A/E7	1:100	1/4	GATA3	60	L50-823	1:100	1/2s
3	Hoechst 33342	1/175s	CD8	8	C8/144B	1:50	1/2s	p53	52	D07	1:50	1/2s	PAX5	42	D7H5X	1:25	1/2s
4	Hoechst 33342	1/175s	CD21	21	Bu32	1:50	1/2s	CD274 (PD-L1)	11	E1L3N	1:50	1/2s	Ki-67	6	B56	1:100	1/8.5s
5	Hoechst 33342	1/175s	CD45	56	2B11+PD7/263	1:100	1/4s	CD30	57	BerH2	1:25	1/2s	CD2	25	RPA-2.10	1:25	1/2s
6	Hoechst 33342	1/175s	HLA-DR	65	EPR3692	1:100	1/4s	CD5	75	UCHT2	1:50	1/2s	CD279 (PD-1)	23	D4W2J	1:50	1/2s
7	Hoechst 33342	1/175s	CD45RA	72	HI100	1:50	1/2s	CD4	20	EPR6855	1:50	1/2s	CD56	29	MRQ-42	1:50	1/2s
8	Hoechst 33342	1/175s	MUC-1 (EMA)	15	955	1:100	1/4s	BCL-2	41	124	1:50	1/2s	CD223 (LAG3)	53	17B4	1:25	1/2s
9	Hoechst 33342	1/175s	CD71	3	MRQ-48	1:100	1/8.5s	CD25	24	4C9	1:100	1/2s	VISTA	79	D1L2G	1:50	1/2s
10	Hoechst 33342	1/175s	CD11c	49	EP1347Y	1:100	1/4s	EGFR	58	D38B1	1:25	1/2s	CD1a	43	O10 + CA1/7	1:25	1/2s
11	Hoechst 33342	1/175s	Na-K-ATPase	36	EP1845Y	1:100	1/4s	CD44	44	IM-7	1:25	1/2s	IDO-1	59	D5J4E	1:25	1/2s
12	Hoechst 33342	1/175s	CD38	66	EPR4106	1:100	1/2s	CD16	26	D1N9L	1:50	1/2s	IRF4	51	IRF4.3E4	1:25	1/2s
13	Hoechst 33342	1/175s	CD20	48	rIGEL/773	1:200	1/8.5s	CD7	63	MRQ-56	1:50	1/2s	CD3	77	MRQ-39	1:100	1/2s
14	Hoechst 33342	1/175s	Granzyme B	81	EPR20129-217	1:200	1/4s	CD194 (CCR4)	55	L291H4	1:50	1/2s	CD57	30	HCD57	1:100	1/2s
15	Hoechst 33342	1/175s	Vimentin	7	RV202	1:200	1/2s	CD68	5	D4B9C	1:100	1/8.5s	CD268 (ICOS)	74	D1K2T	1:100	1/2s
16	Hoechst 33342	1/175s	Pan-Cytokeratin	67	C11	1:200	1/8.5s	CD34	38	QBEnd/10	1:100	1/2s	CD163	45	EDHu-1	1:50	1/2s
17	Hoechst 33342	1/175s	SMA	69	polyclonal	1:200	1/8.5s	CD45RO	2	UCH-L1	1:25	1/2s	CD11b	28	EPR1344	1:25	1/2s
18	Hoechst 33342	1/175s	Collagen IV	33	polyclonal	1:200	1/4s	CD31	68	C31.3+C31.7+C31.10	1:200	1/5s	-				
19	Hoechst 33342	1/175s	CD15	14	MMA	1:200	1/4s	CD138	76	B-A38	1:100	1/8.5s	-				
20	Hoechst 33342	1/175s	MMP9	62	L51/82	1:400	1/4s	MMP12	80	polyclonal	1:200	1/4s	Podoplanin	32	D2-40	1:100	1/2s
21	Hoechst 33342	1/175s	blank				1/2s	blank				1/2s	blank				1/2s
22	Hoechst 33342	1/175s	blank				1/600s	blank				1/600s	DRAQ5			1:100	1/8.5s
23	H&E (brightfield)																

Table S5: Tissue composition of the multi-tumor TMA

#	tissue	normal / neoplasia	diagnosis
1	bone marrow	normal	Normal bone marrow (patient with diffuse large B cell lymphoma)
2	bone marrow	normal	Normal bone marrow (pediatric patient post chemotherapy for neuroblastoma)
3	bone marrow	cancer	Acute myeloid leukemia (80% blasts)
4	bone marrow	cancer	B lymphoblastic leukemia (B-ALL)
5	tonsil	normal	Normal tonsil - follicular hyperplasia
6	spleen	normal	Normal spleen (patient with mesenterial infarction)
7	lymph node	normal	Normal lymph node (iliacal)
8	lymph node	cancer	Classic Hodgkin's lymphoma - nodular sclerosis
9	lymph node	cancer	Chronic lymphocytic leukemia / small lymphocytic lymphoma (CLL / SLL)
10	lymph node	cancer	Diffuse large B cell lymphoma (DLBCL), NOS
11	lymph node	cancer	Follicular lymphoma (FL), low-grade (G 1-2)
12	lymph node	cancer	Plasmacytoma / Plasma cell myeloma
13	nasopharyngeal	cancer	Extranodal NK/T cell lymphoma, nasal type
14	lymph node	cancer	T lymphoblastic lymphoma (T-LBL)
15	thymus	cancer	Thymoma type AB with predominant type B component
16	liver	normal	Normal liver
17	appendix	normal	Normal appendix
18	liver	cancer	Hepatocellular carcinoma, G3
19	biliary system	cancer	Cholangiocellular carcinoma, G3
20	stomach	normal	Normal stomach (sleeve gastrectomy for obesity)
21	stomach	cancer	Gastric Adenocarcinoma, intestinal type Laurén, G3
22	colon	cancer	Colonic Adenocarcinoma, G2
23	pancreas	cancer	Neuroendocrine tumor, G1, insulinoma
24	salivary gland	normal	Normal seromucous salivary gland (submandibular)
25	pancreas	cancer	Anaplastic pancreatic carcinoma with osteoclast-like giant cells
26	breast	cancer	Breast invasive carcinoma, no special type (NST)
27	breast	cancer	Breast invasive carcinoma, lobular
28	ovary	cancer	Serous high-grade ovarian carcinoma
29	uterus	cancer	Endometrioid endometrial carcinoma
30	cervix	cancer	Cervical squamous cell carcinoma
31	uterus	cancer	Leiomyosarcoma
32	placenta	normal	Normal placenta
33	muscle	normal	Normal muscle
34	muscle	cancer	Alveolar rhabdomyosarcoma
35	muscle	cancer	Embryonal rhabdomyosarcoma
36	kidney	cancer	Ewing sarcoma / PNET
37	nerve	non-malignant tumor	Neurofibroma
38	soft tissue	cancer	Monophasic synovial sarcoma
39	tendon	non-malignant tumor	Giant cell tumor of tendon sheath, localized
40	bone	cancer	Telangiectatic osteosarcoma, G3
41	nerve	cancer	Malignant peripheral nerve sheath tumor (MPNST)
42	skin	cancer	Angiosarcoma
43	prostate	cancer	Prostate acinar adenocarcinoma
44	kidney	cancer	Clear cell renal cell carcinoma, G4
45	kidney	cancer	Papillary urothelial carcinoma, minimally invasive, of the renal pelvis
46	adrenal gland	cancer	Pheochromocytoma
47	adrenal gland	cancer	Adrenocortical carcinoma
48	testis	cancer	Seminoma
49	testis	cancer	Embryonal carcinoma
50	adrenal gland	cancer	Neuroblastoma
51	kidney	cancer	Wilms tumor / Nephroblastoma
52	adrenal gland	non-malignant tumor	Myelolipoma
53	skin	normal	"Normal skin" (chronic inflammation)
54	skin	cancer	Malignant melanoma, nodular
55	skin	cancer	Basal cell carcinoma
56	skin	cancer	Kaposi's sarcoma
57	skin	cancer	Dermatofibrosarcoma protuberans (DFSP)
58	skin	non-malignant tumor	Glomangioma
59	brain	normal	Normal brain
60	meninges	non-malignant tumor	Meningioma
61	brain	cancer	Glioblastoma multiforme, IDH-mutant
62	pituitary	non-malignant tumor	Pituitary adenoma
63	lung	cancer	Lung Adenocarcinoma
64	lung	cancer	Lung small cell carcinoma
65	lung	cancer	Lung carcinoid, typical
66	parathyroid	non-malignant tumor	Parathyroid adenoma
67	thyroid	cancer	Papillary thyroid carcinoma, cribriform-morular variant
68	thyroid	cancer	Medullary thyroid carcinoma, lymph node metastasis
69	pleura	cancer	Malignant pleural mesothelioma
70	musculoscelettal	cancer	Chordoma, sacral

TABLE S6. KEY RESOURCES

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies and proteins		
Purified antibodies, see **Table S2**	various	various
Mouse IgG	Sigma	I5381
Rat IgG	Sigma	I4131
Biotinylated VG1 hyaluronan-detection reagent	Bollyky Lab, Stanford U	(Clark et al., 2011)
Oligonucleotides		
CODEX oligonucleotides, see **Table S3**	TriLink Biotechnologies and Integrated DNA Technologies	N/A
Biological Samples		
FFPE tissue blocks	Institute of Pathology, University of Bern	N/A
Chemicals and Reagents		
Streptavidin-PE	Biolegend	405203
PBS	Thermo Fisher Scientific	14190-250
NaCl	Thermo Fisher Scientific	S271-10
Na_2HPO_4	Sigma	S7907
$NaH_2PO_4 \cdot 7\ H_2O$	Sigma	S9390
$MgCl_2 \cdot 6\ H_2O$	Sigma	M2670
NaN_3	Sigma	S8032
EDTA	Sigma	93302
TCEP	Sigma	C4706
NaOH	Sigma	S8263
BS3	Thermo Fisher Scientific	21580
DMSO	Thermo Fisher Scientific	D128-4
DMSO	Sigma	472301
DMSO ampoules	Sigma	D2650
Paraformaldehyde ampoules, 16%	Thermo Fisher Scientific	50-980-487
BSA	Sigma	A3059
Tris 1 M, pH 8.0	Teknova	T1080
Candor PBS antibody stabilizer solution	Thermo Fisher Scientific	NC0436689
Salmon sperm DNA, sheared	Thermo Fisher Scientific	AM9680
Triton™ X-100	Sigma	T8787
Ethanol, 100%	Sigma	E7023
Acetone, 100%	Thermo Fisher Scientific	A929-4
Methanol, 100%	Thermo Fisher Scientific	A412-4
Trizma® HCl	Sigma	T3253
Trizma® Base	Sigma	T1503
Drierite indicating desiccant	Thermo Fisher Scientific	07-578-3A
Bondic polyacrylamide gel	Amazon	B018IBEHQU
Dako target retrieval solution, pH 9.0	Agilent	S236784-2
TBS IHC wash buffer with Tween® 20	Cell Marque	935B-09
Antibody diluent	Agilent	S080981-2
Protein block, serum-free	Agilent	X090930-2
Dual endogenous enzyme-blocking reagent	Agilent	S200380-2
Liquid DAB+ substrate chromogen system	Agilent	K346711-2
EnVision+ HRP Mouse	Agilent	K400011-2
EnVision+ HRP Rabbit	Agilent	K400211-2
Hematoxylin, ready-to-use	Agilent	S330930-2
Eosin Y solution	Sigma	HT110116
Cytoseal XYL	Thermo Fisher Scientific	8312-4
Sally Hansen Nail Polish, clear	Amazon	B00CMFMYEG
Critical Commercial Instruments, Consumables, Kits and Assays		
LTS filter tips, 10 µl	Rainin	30389225
LTS filter tips, 200 µl	Rainin	30389239
LTS filter tips, 1000 µl	Rainin	30389212
Amicon™ Ultra Centrifugal Filters, 50kDa	Thermo Fisher Scientific	UFC505096
Nalgene™ Rapid Flow 500 ml filter, 0.2 µm	Thermo Fisher Scientific	09-740-28C
Glass coverslips, 22x22 mm, # 1 1/2	Electron Microscopy Sciences	72204-01
Frosted microscope slides	Thermo Fisher Scientific	12-550-343

Glass coverslip storage box	Qintay	CS-22
22x22 mm coverslip mounting gaskets	Qintay	TMG-22
Wheaton™ Coverslip glass jars	Thermo Fisher Scientific	02-912-637
Dumont #5/45 cover slip forceps	Fine Science Tools	11251-33
ST4020 small linear stainer	Leica	14050946425
Labconco™ Fast-Freeze™ Flasks, Complete Assembly, 900ml	Thermo Fisher Scientific	10-269-63
FreeZone® 4.5 Plus Cascade Benchtop Freeze Dry System	Labconco	7386030
8-strip tubes, 0.2 ml	E&K Scientific	280008
8-strip caps, flat top	E&K Scientific	491008
8-strip caps, dome top	E&K Scientific	491018
CODEX acrylic plates	Bayview Plastic Solutions	custom made
BZ-X710 fluorescence microscope	Keyence	N/A
Hoechst 33342	Thermo Fisher Scientific	62249
DRAQ5	Cell Signaling Technology	4084L
Vectabond™	Vector Labs	SP-1800
Vacuum Desiccators, 23L	Thermo Fisher Scientific	08-648-112
Corning™ black 96-well plates	Thermo Fisher Scientific	07-200-762
Axygen aluminum sealing film	VWR Scientific	47734-817
TMA Grand Master	3DHistech	N/A
Pannoramic P250 digital slide scanner	3DHistech	N/A
CODEX System	Akoya Biosciences	N/A
Deposited Data		
Software and Algorithms		
BZ-X viewer	Keyence	N/A
CODEX driver	Akoya Biosciences	N/A
CODEX Toolkit, version 1.3.5	https://github.com /nolanlab/CODEX	(Goltsev et al., 2018)
Microvolution software for deconvolution	www.microvolution.com	N/A
ImageJ (Fiji version 2.0.0)	http://imagej.net	N/A
VorteX (X-shift clustering algorithm)	https://github.com /nolanlab/VORTEX	(Samusik et al., 2016)
CellEngine	www.cellengine.com	(Bjornson-Hooper et al., 2019)
R, version 3.4.3	www.r-project.org	N/A
R studio desktop, version 1.1.423	www.rstudio.com	N/A
Neighborhood analysis notebooks	This paper	N/A
Tensorly Python package	http://tensorly.org/	(Kossaifi et al., 2019)
Statsmodel Python package	https://www.statsmodels.org/	(Seabold and Perktold, 2010)
Scikit learn Python package	https://scikit-learn.org/	(Pedregosa et al., 2011)
Survival R package	https://cran.r-project.org/web/packages/survival/index.html	(Therneau, 2015)
Glmnet R package	https://cran.r-project.org/web/packages/glmnet/index.html	(Friedman et al., 2010)
Visreg R package	https://cran.r-project.org/web/packages/visreg/index.html	(Breheny and Burchett, 2013)
Deldir R package	https://cran.r-project.org/web/packages/deldir/index.html	N/A
ComplexHeatmap R package	https://bioconductor.org/packages/release/bioc/html/ComplexHeatmap.html	(Gu et al., 2016)
The Human Protein Atlas	www.proteinatlas.org	N/A
Pathology Outlines	www.pathologyoutlines.com	N/A