

Supplemental information

An atlas of cells in the human tonsil

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Supplementary Figures

Supplementary Figure Legends (S1-S7)

Supplementary Figures (S1-S7)

Figure S1. A single-cell multiomic atlas of human tonsillar cells. Related to Figure 1. (A,G) UMAP projection of unintegrated (*left*) and harmony-based integrated single-cell datasets (*right*) splitted and colored by data modality [scRNA-seq and multiome (A), scATAC-seq and multiome (G) and external dataset (King *et al.* (A))] (B, H) Boxplot comparing local inverse Simpson's Index (LISI) across confounders of integrated and unintegrated scRNA-seq (B) and scATAC-seq (H) datasets. (C) UMAP projection of the King *et al.* dataset colored by the original publication. (D) UMAP projection of the tonsillar cells of the discovery cohort colored by the main 9 cellular compartments identified: Naive and Memory B cells (NBC_MBC), Germinal center B cells (GCBC), plasma cells (PC), CD4 T cells, cytotoxic (CD8 T cells, NK, ILC, double negative T cells (DN), myeloid cells (DC, macrophages, monocytes, granulocytes, mast cells), follicular dendritic cells (FDC), epithelial cells and plasmacytoid dendritic cells (PDC). (E) UMAP projection of tonsillar cells colored by cell cycle phase; *left*: S Score and *right*: G2M Score. (F) Pie chart representing the distribution of preB and preT cells across donors. For each donor, the number and percentage of cells (out of the total preB and of the total preT) is indicated. (M: male; F: female). (I) UMAP projection of the prediction score inferred for CITE-seq (*left*) and scATAC-seq (*right*) after the label and coordinate transfer.

Figure S2. CD4 T follicular and non-follicular cell fate decision in the human tonsil. Related to Figure 2. (A) Dotplot showing the average expression of markers for Naive, CM pre-non-Tfh and CM Pre Tfh. Dot size reflects the percentage of cells in a cluster expressing each gene and the color the average expression level. (B) UMAP projection colored by PRDM1 and BCL6 gene expression and TF-activity gen- (red) and region (green) based obtained with SCENIC+ (STAR methods). (C) Dotplot showing the average expression (color) and percentage of cells expressing (size) each of the top 20 markers that described the main CD4 T subpopulations identified. (D) Boxplots of BCL6 accessibility values considering the gene body and 2,000 bp upstream (*top*) and the predicted distal cis-regulatory region using Cicero as a potential enhancer (*bottom*). (E) Genomic snapshot of accessibility at BCL6 and distal enhancer locus across T cell clusters from King *et al.* (F) Violinplot of CD28 (*left*) and CD29 (*right*) protein expression across CD4 T cell subsets. (G) UMAPs projection colored by the estimated Nebulosa density expression (STAR Methods) for key interleukin and chemokine receptors. (H) Violinplot showing the effector Treg (eTreg, *top*) and circulating Tfr (cTfr, *bottom*) gene expression signatures from Wing JB, *et al.* computed using UCell R package for Treg subtypes.

Figure S3. Landscape of CD8 and innate lymphoid cells in the human tonsil. Related to Figure 3. (A) Heatmap showing the average expression of the top markers for CD8 T cells and innate lymphoid cells (ILC). (B) Violinplot showing the protein expression of seven representative markers for different subpopulations in CD8 T and ILC. (C) Violinplot showing the motif activity of TBX21 across the different subpopulations in CD8 T and ILC. (D-G) Immunofluorescence overlays of markers acquired by multiplex histology in one tissue section from a representative tonsil. Each image depicts the same field of view (FOV) of 665 x 665 μm , sequentially stained with the depicted fluorescence-labelled antibodies. Images contain 2048 x 2048 pixels and are generated using an inverted wide-field fluorescence microscope with a 20x objective, a lateral resolution of 325 nm and an axial resolution above 5 μm . The thin dotted line represents the B cell follicle and the thick dotted line represents the epithelial layer. (D) CD20 (cyan) depicts the B cell follicle, CD3 (yellow) marks the T cell zone and pancytokeratin (PCK; magenta) stains the epithelium. (E) The endothelial marker CD34 (green) and the myofibroblast marker smooth muscle actin (SMA; red) depict the vessels, and vimentin (blue) marks the fibroblast-dense areas. (F) Granzyme A (GzmA; magenta), Eomes (yellow) and CD8 (magenta) represent cytotoxic T cells. (G) CD49a (cyan), CD69 (magenta) and CD103 (yellow) mark tissue resident cells. (C-D) White outlined squares represent areas of interest, shown as enlargements and single channel images. RM CD8 T cells (CD103+CD69+CD49a+/-) are shown as white arrows and are preferentially localized within the epithelium and in the connective tissue septum lining the tonsillar crypts, but not within the B cell follicles and rarely deep within the T cell zone. (H) UMAP projection of CD4 T, CD8 T, NK, ILC and DN in the CITE-seq dataset. (I) Protein expression of CD3, CD4 and CD8 across major lymphocyte populations (CITE-seq dataset). (J) In silico gating of major lymphocyte populations based on CD4 and CD8 protein expression. (K) Distribution of gated groups across each major population. (L) FACS validation of DN T cells in tonsils.

Figure S4. B cell activation and GC dynamics. Related to Figure 4. (A) Dotplot showing marker expression for NBC and MBC subpopulations. (B) Dotplot showing marker expression per GCBC subpopulations. Dot size reflects the percentage of cells in a cluster expressing each gene and the color the average expression level. (C, D) UMAP projection of GCBC cells colored by cell cycle phase (C) or GCBC annotation (D) and splitted by cell cycle correction strategy. (E) Local inverse Simpson's Index (LISI) for both annotation [top, related with (D)] and cell cycle phase [bottom, related with (E)]. (F) UMAP projection of NFKB signature computed using *UCellR* package with seven genes (*NFKBIA*, *NFKBID*, *NFKB1*, *NFKB2*, *REL*, *RELA*, *RELB*) for NBC and MBC. (G) UMAP projection of *BATF* expression in NBC and MBC.

Figure S5. Plasma cell differentiation and cell identity regulation in human tonsils. Related to Figure 5. (A) PC BCR analysis. Top: CITE-seq/BCR cells projected in scRNA-seq UMAP coordinates. Cluster labels and coordinates are transferred via KNN classification and regression respectively. Bottom: Barplot representation of PC clonality. Blue, clonal expansion when ≥ 3 cells had identical CDR3 sequence; Green, two cells share identical sequence; Orange, cells with distinct CDR3 sequence. (B) Proliferative cells (PB committed, Transitional PB, PB) differentiation analysis. Top: UMAP projection of proliferative cells colored by cell cycle phase. Bottom: Barplot representation of PC-phenotypic markers expression across S and G2M cells from PB cluster (most representative proliferative cluster). (C) UMAPs projection of the expression of Ig and MBC genes (*BANK1*, *CELF2*, *TXNIP*). (D) Violin Plot of the Endoplasmic Reticulum (ER) signature across PC scRNA-seq clusters. Signature was obtained using the *UCellR* package with 70 genes obtained from the DAVID KEGG pathway analysis: Protein processing in endoplasmic reticulum. (E) Cell type proportions deconvoluted using SPOTlight (see Methods). (F) Spatially defined trajectories on H&E stained images from BCLL-10-T (left) and BCLL-12-T (right) patients and heatmap showing smoothed expression changes for specific genes across defined trajectories. BCLL-10-T trajectory starts in a LZ zone and includes an interfollicular zone. (G) Correlation plot showing co-localization of cell types on the Visium slides, plot for slide BCLL-10-T (top) and for all slides combined (bottom). (H) UMAP projection of the activity (AUCell score) of VDR and CREB3 TFs. (I) Barplot of *SIX5* expression in different subpopulations from peripheral blood scRNA-seq data from Hao Yuhan *et al.* (J) Boxplot of PC phenotypic markers, *SIX5* and *SIX5* predicted target in different populations from bone marrow scRNA-seq data from Hay Stuart B *et al.* Link <http://www.altanalyze.org/ICGS/HCA/Viewer.php>

Figure S6. Epithelial cells in the human tonsils. Related to Figure 6. (A) UMAP projection of epithelial tonsillar cells colored and numbered by scRNA-seq clusters. (B) Dotplot showing the average expression of the top markers that described the main clusters identified in the epithelial compartment. Dot size reflects the percentage of cells in a cluster expressing each gene and the color the average expression level. (C) Cell type proportions of populations of interest within each spot on slide BCLL-2-T along with an H&E image of the tissue slice. Cell type deconvolution was carried out with SPOTlight. We deconvoluted the spots using epithelial cell subpopulation annotations along with the general annotation of other cell types ensuring we captured the biological signal of all our cell types. (D) UMAP projection of follicular dendritic cells (FDC) and other cells from mesenchymal origin colored and numbered by scRNA-seq clusters. (E) Dotplot showing the average expression of the top markers that described the main clusters identified. (F) MAGIC-normalized gene expression of genes of interest on slide BCLL-10-T. (G) Dotplot showing the average expression of the top markers that described the main clusters identified in the DC (*left*) and aDC (*right*) compartment. Dot size reflects the percentage of cells in a cluster expressing each gene and the color the average expression level. (H) Boxplot showing the proliferation signatures (S.Score and G2M.Score) for DC1 precursor and DC1 mature cells. (I) Violinplot showing seven signatures computed using *UCellR* package for aDC subsets. (J) Stacked barplot showing the proportion of cells (*y-axis*) detected in each donor (*x-axis*) for each subpopulation. (K) Dotplot showing the average expression of three markers (*C1QA*, *MMP12*, *SELENOP*) across all defined cell types and states in the tonsil atlas.

Figure S7. Confirmation of presence, annotation and markers of tonsillar cell types using a validation cohort and Analysis of two MCL samples in the light of the tonsil atlas. Related to Figure 7. (A, C, E, G; I) UMAP projection of reference (left, discovery cohort) and query (right, validation cohort) for naive and memory B cells [NBC/MBC, (A)], germinal center B cells [GCBC, (C)], plasma cells [PC, (E)], CD8 T (G) and myeloid cells (I). (B, D, F, H, J) Heatmap showing scaled mean marker expression for NBC/MBC (B), GCBC (D), PC (F), CD8 T (H) and myeloid cells (J). Boxplots represent the annotation confidence for each cluster. (K) UMAP projection the expression of six genes encoded in chromosome Y for M102 (top) and M413 (bottom). (L) Distribution and UMAP projection of a chrY expression signature derived from the six genes shown in (A) for M102 (left) and M413 (right). Vertical dashed line shows the cutoff used to classify cells in chrY+/- . (M) UMAP projection of the annotated clusters for M413. (N) Dotplot showing the average expression (color) and percentage of expressing cells (size) of the top markers that described the main clusters identified in for M413. (O, P) Heatmaps showing the copy number alterations (CNA) inferred from transcriptomics data with infercnv for M102 (O) and M413 (P).

Figure S1

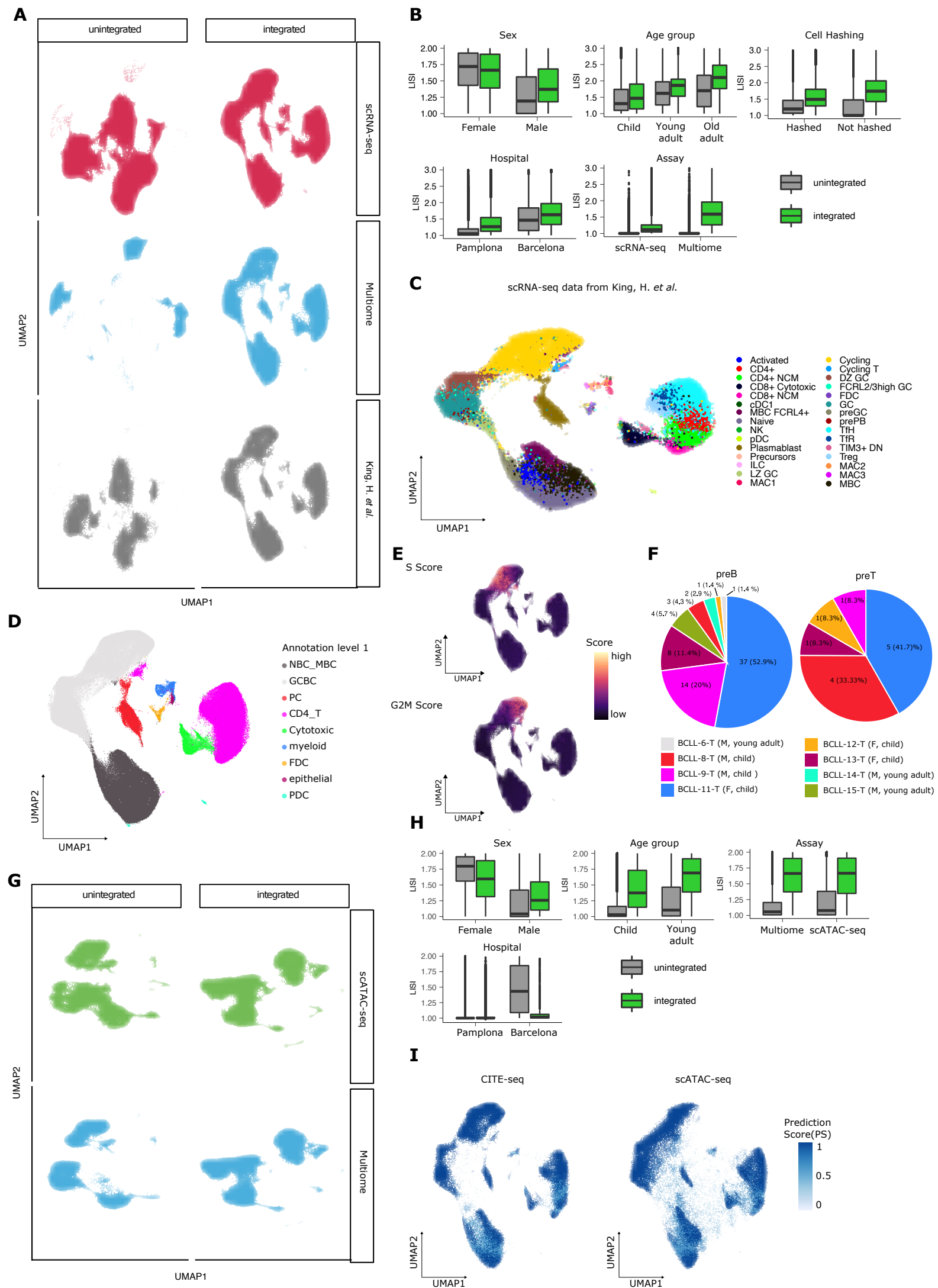


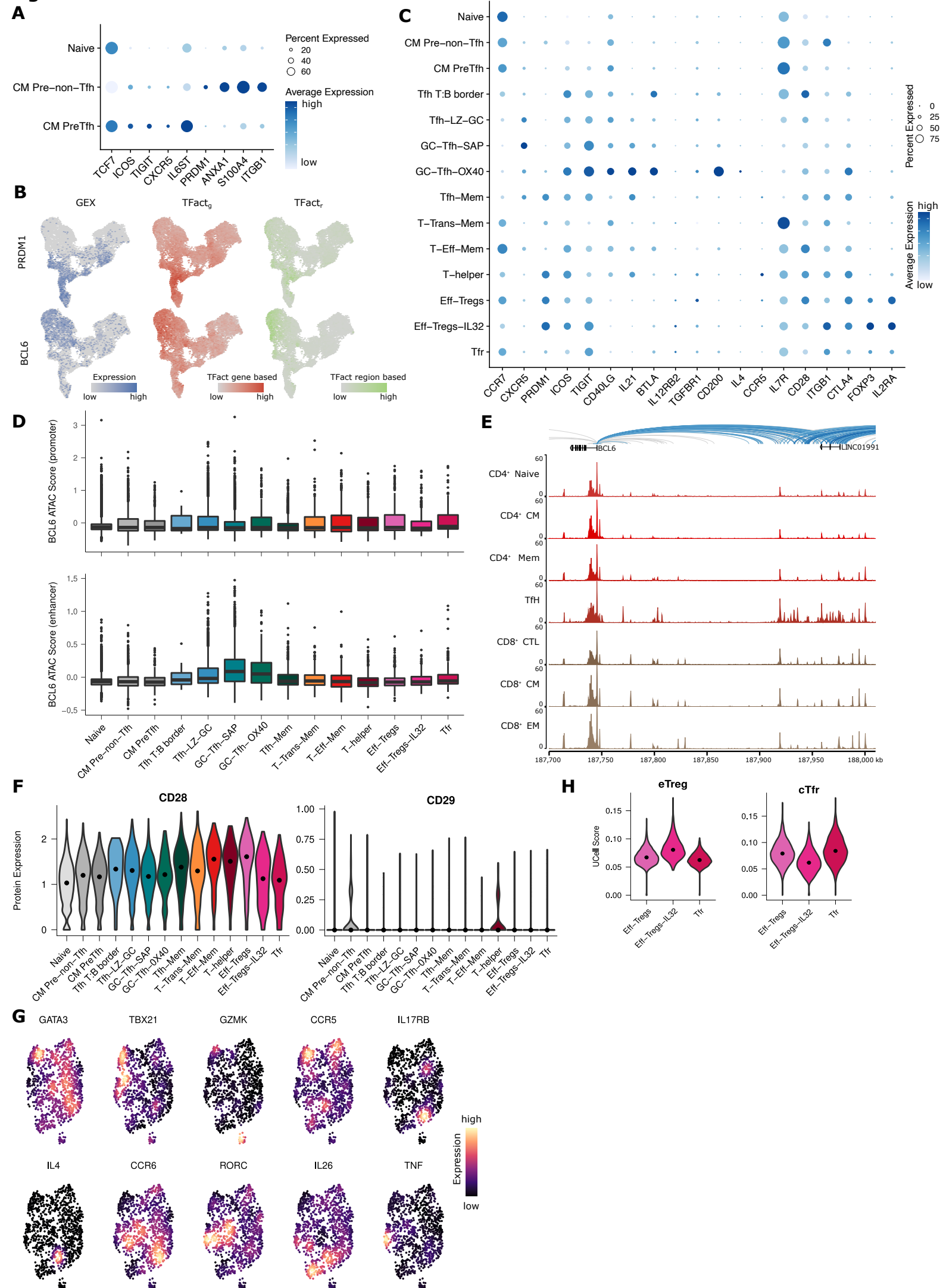
Figure S2

Figure S3

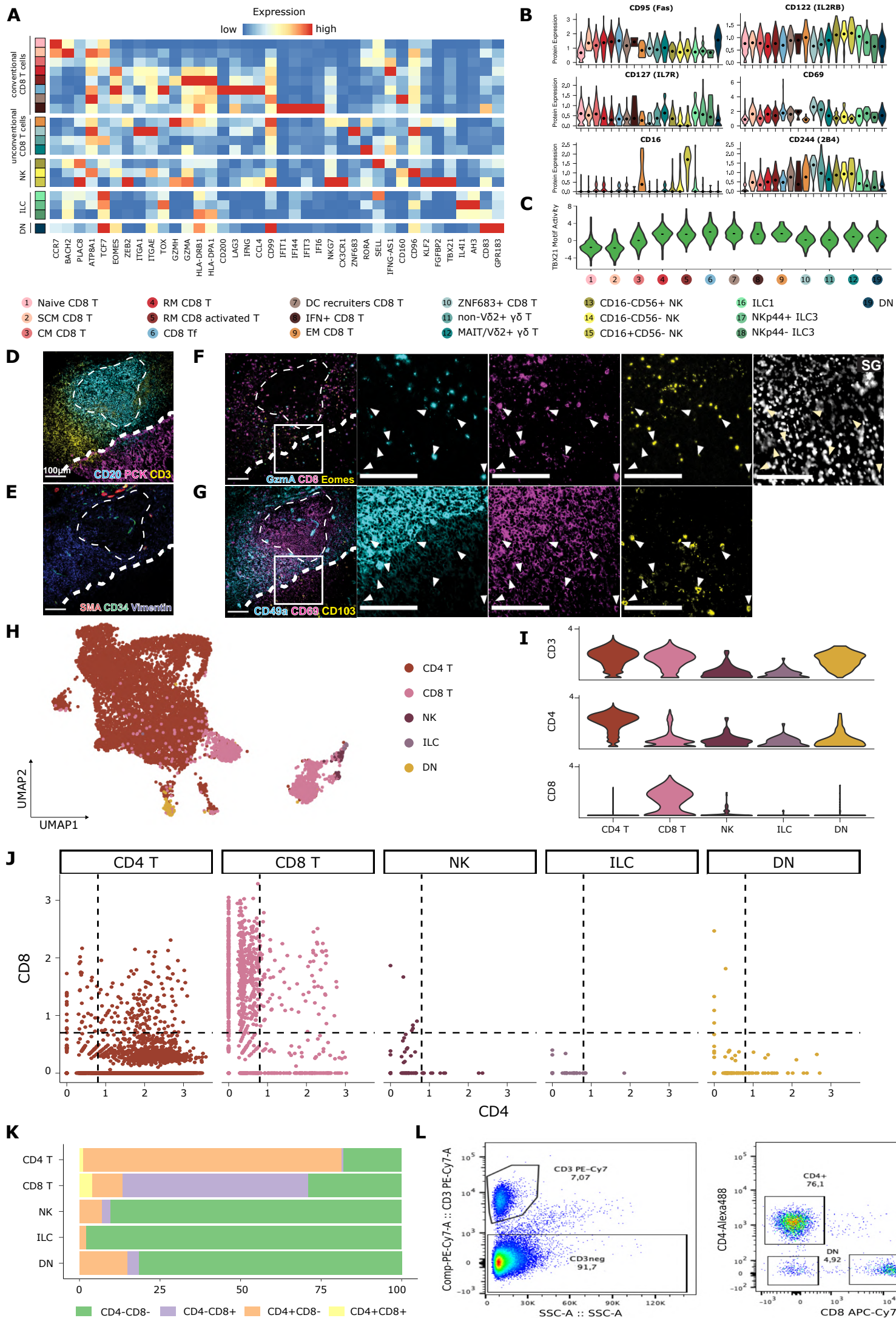


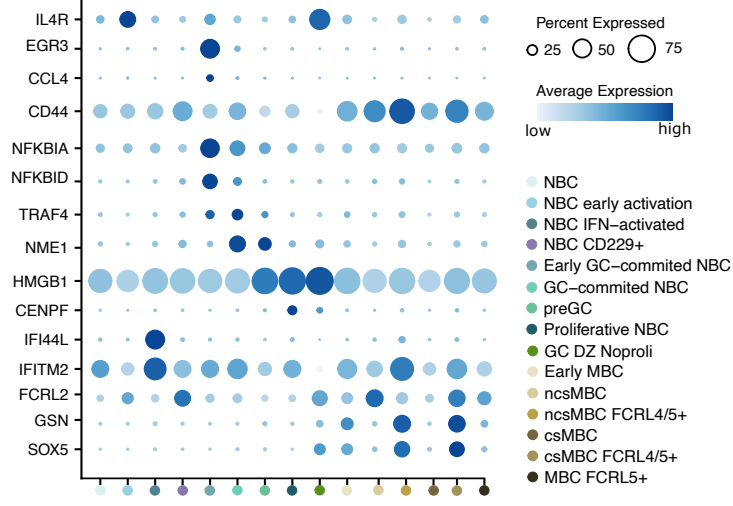
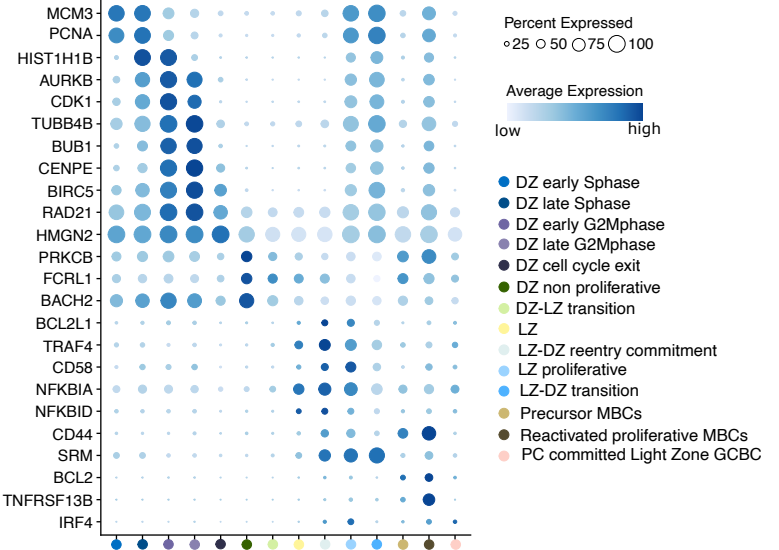
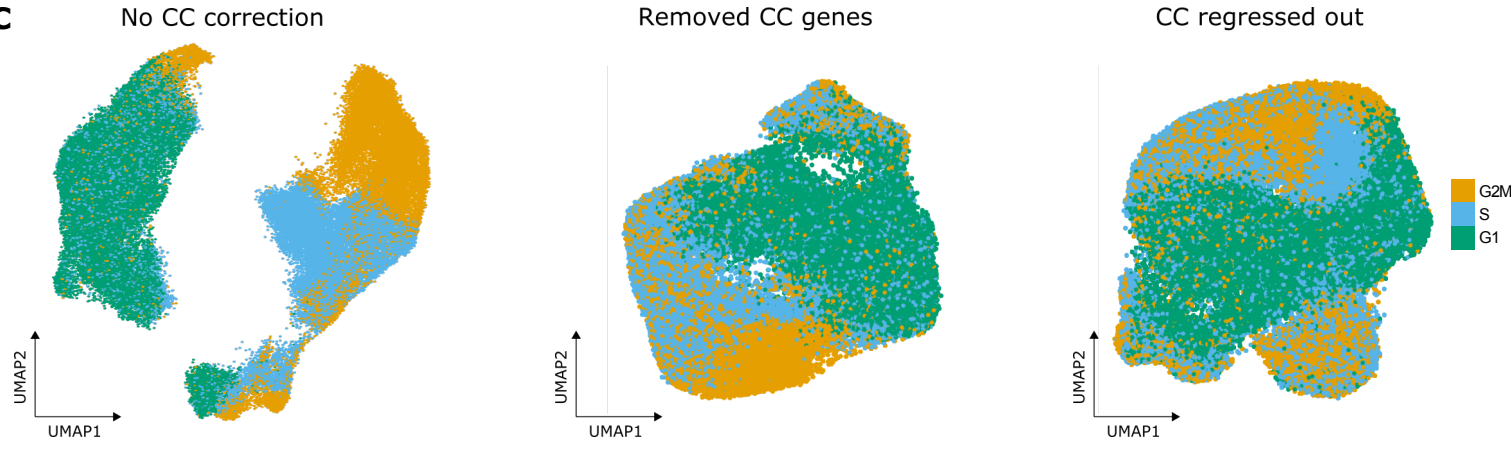
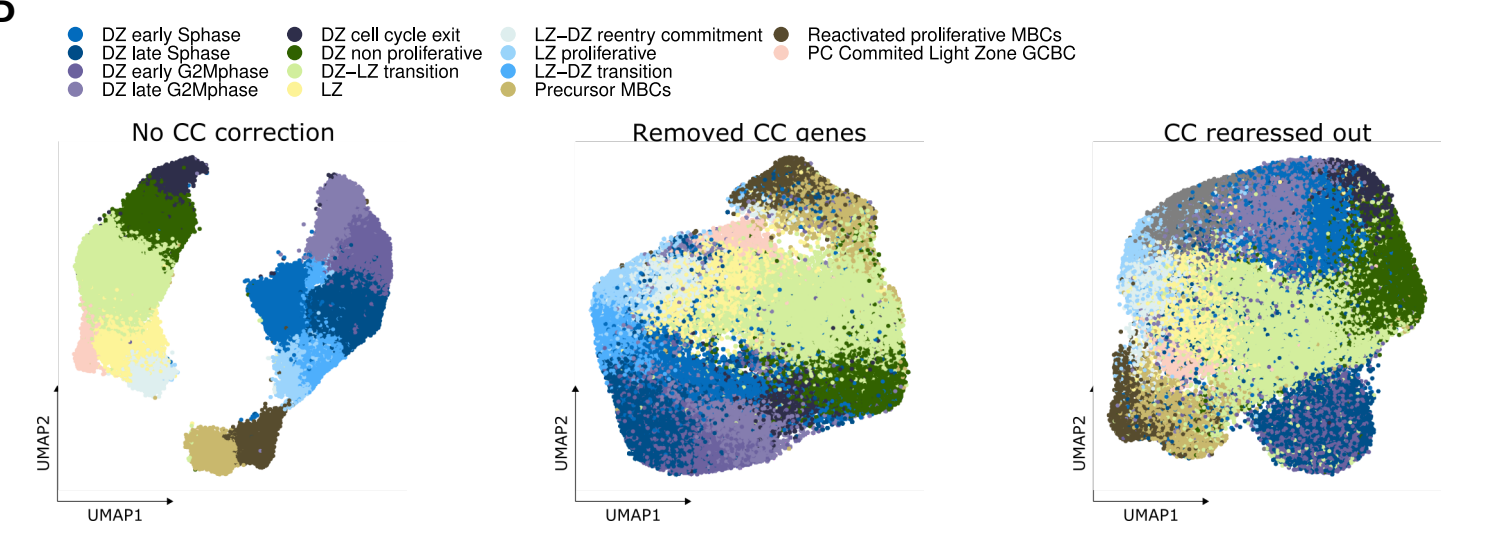
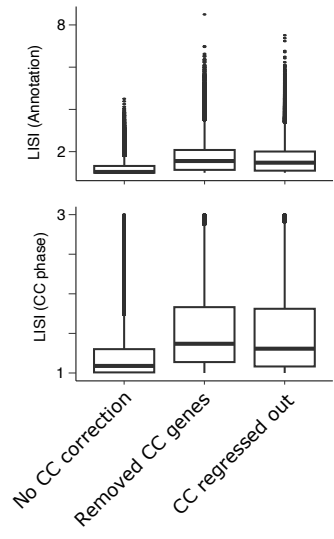
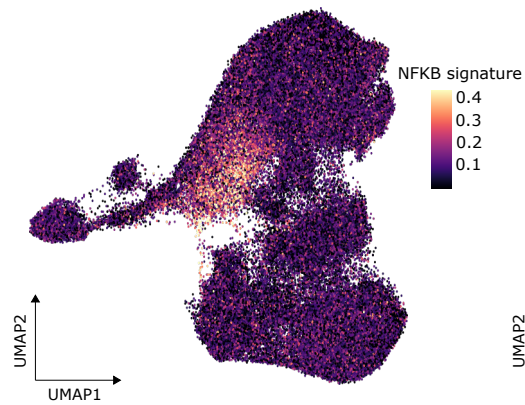
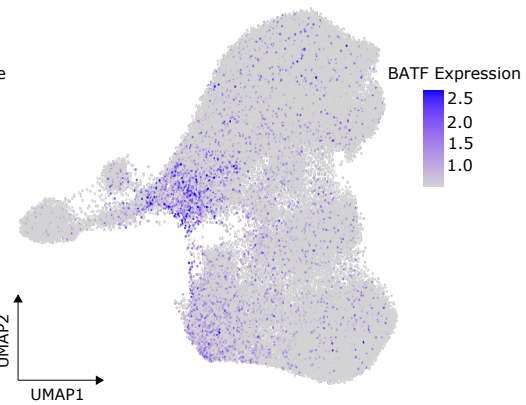
Figure S4**A****B****C****D****E****F****G**

Figure S5

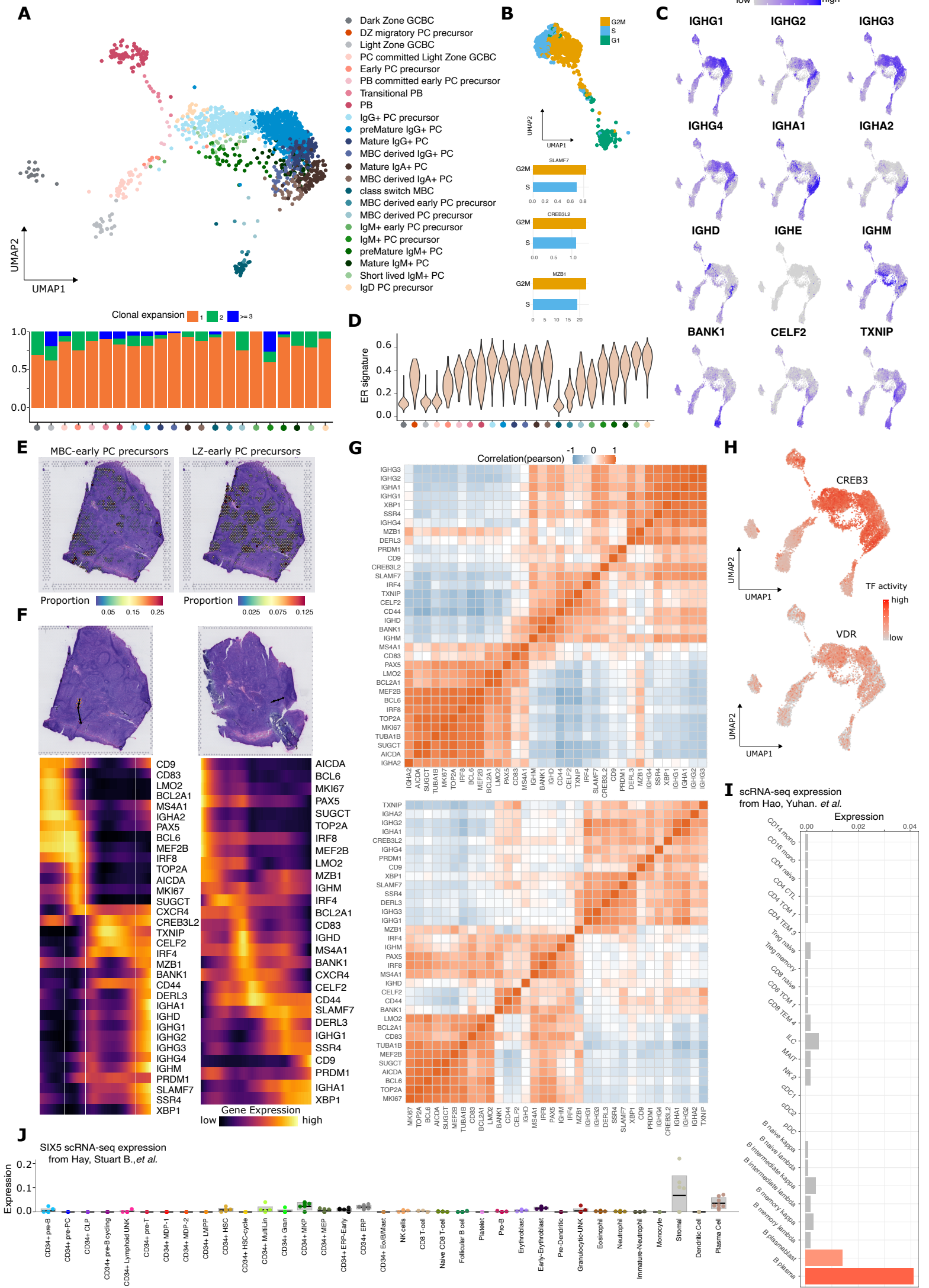


Figure S6

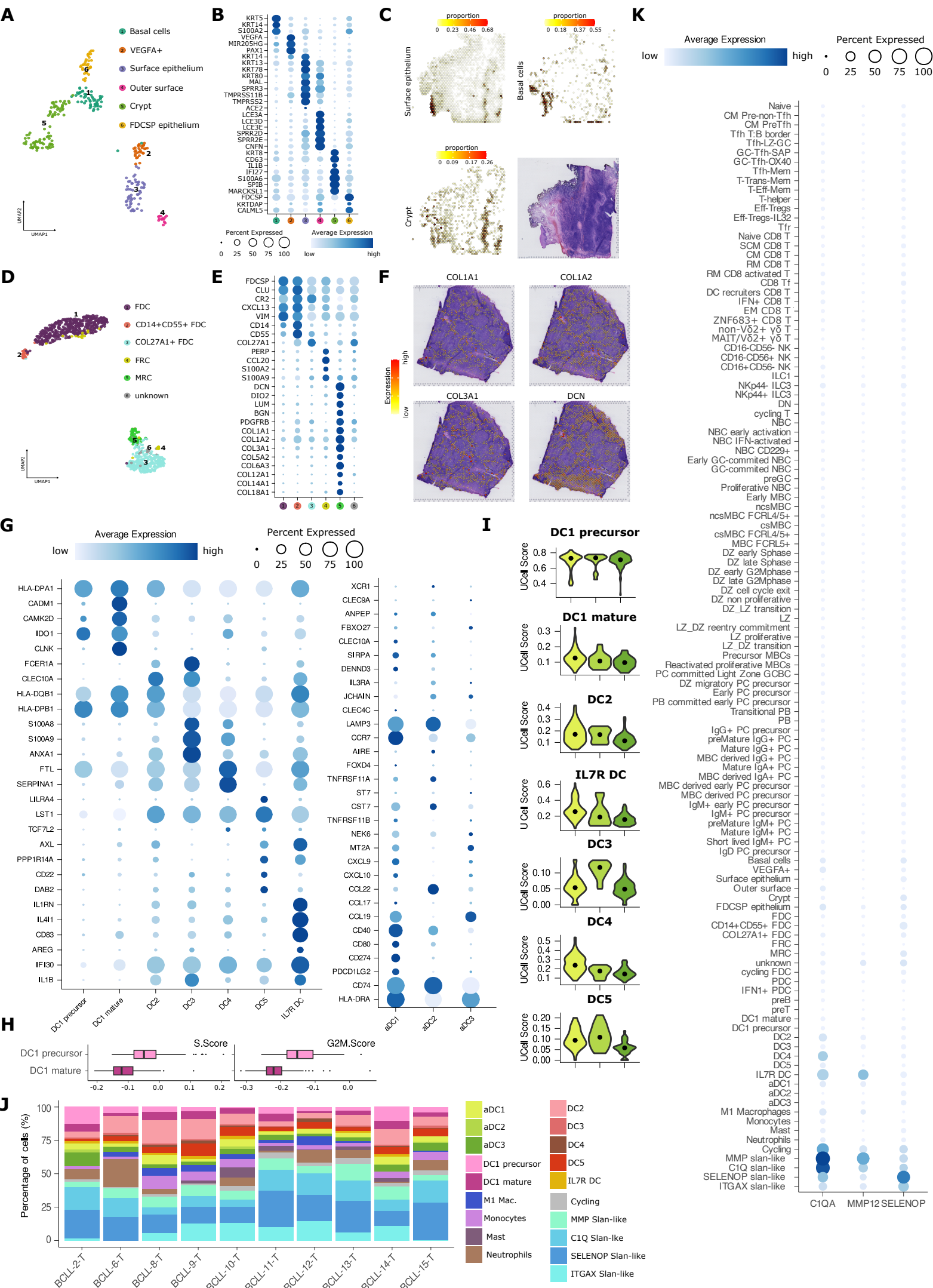
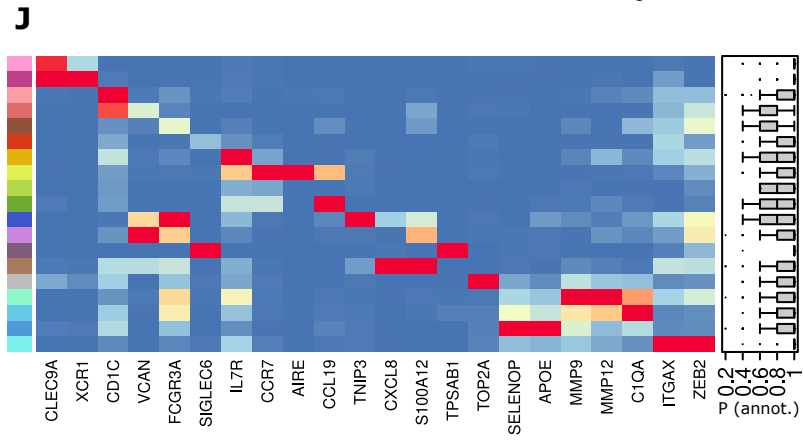
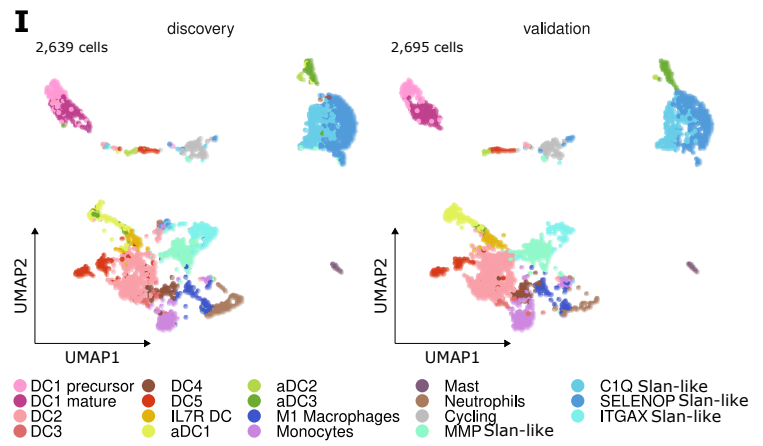
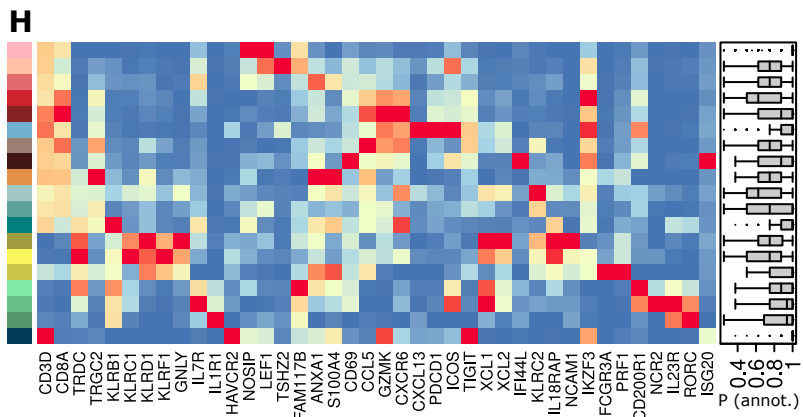
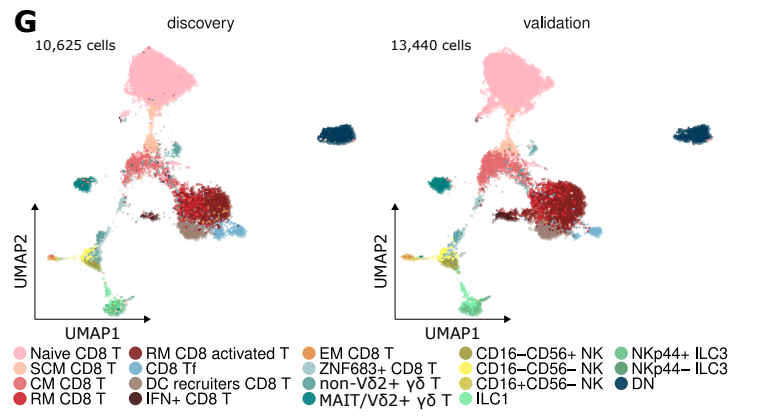
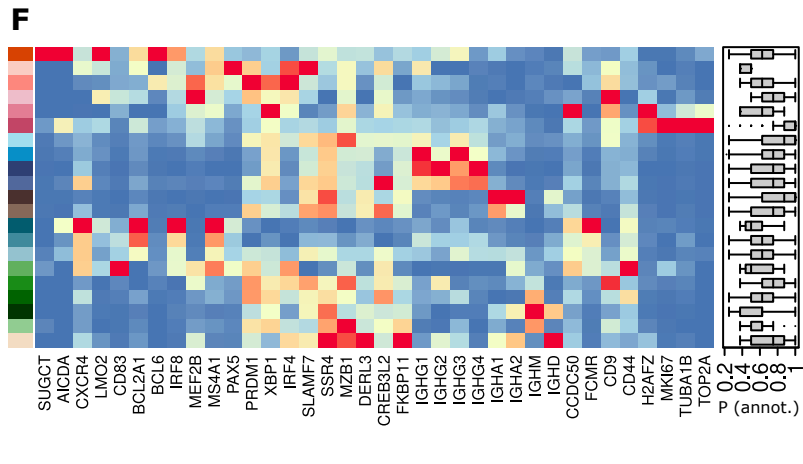
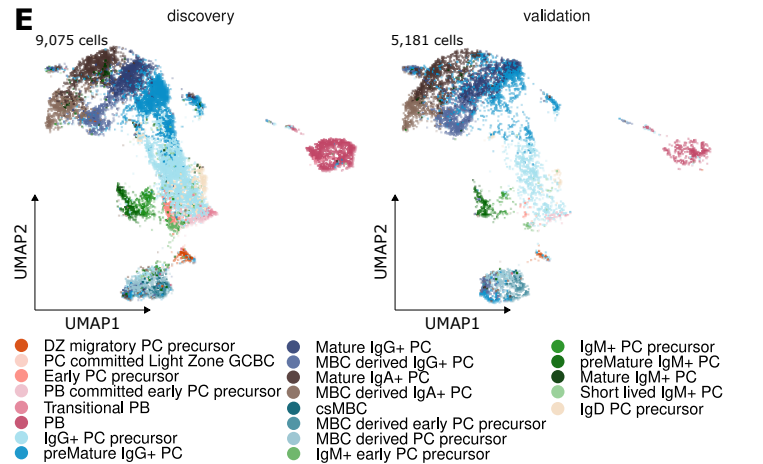
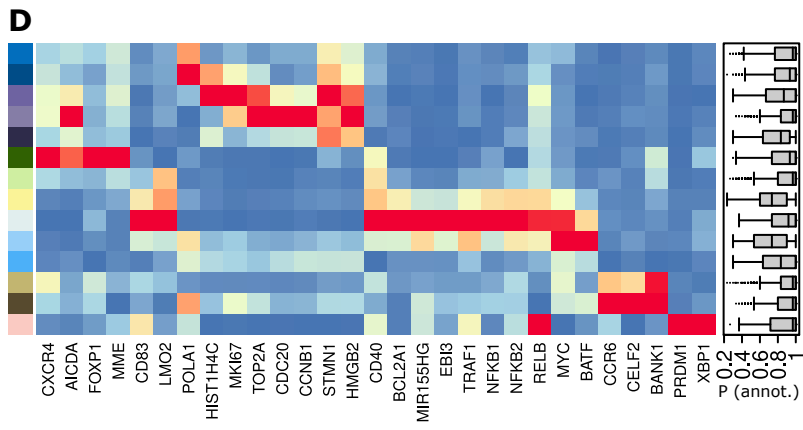
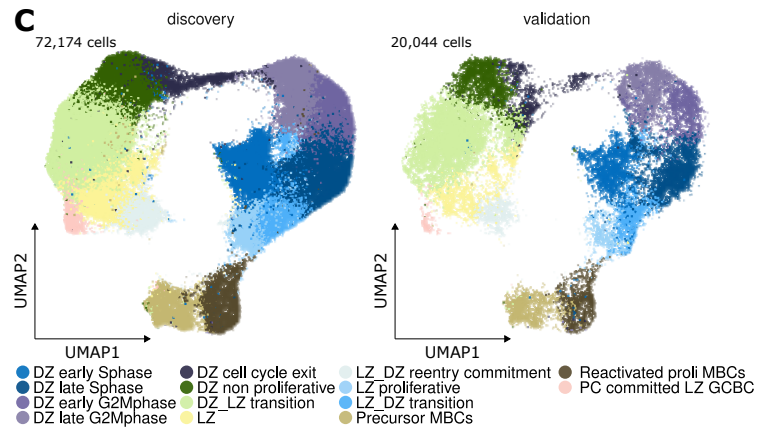
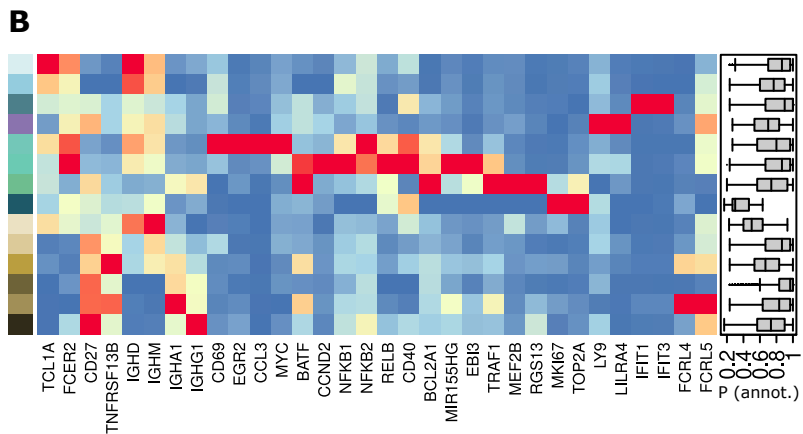
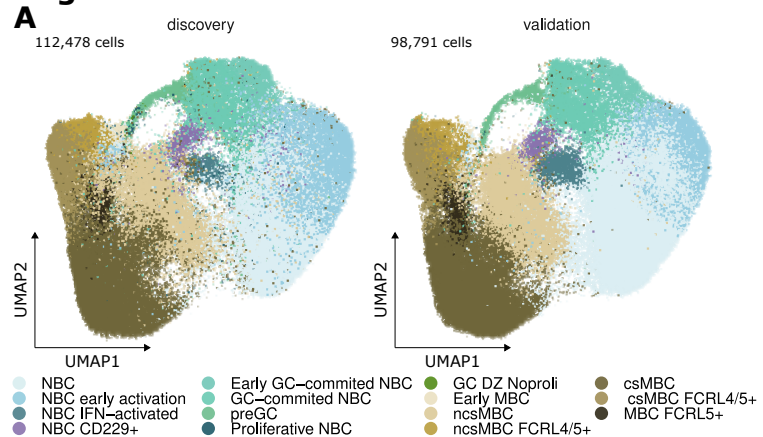
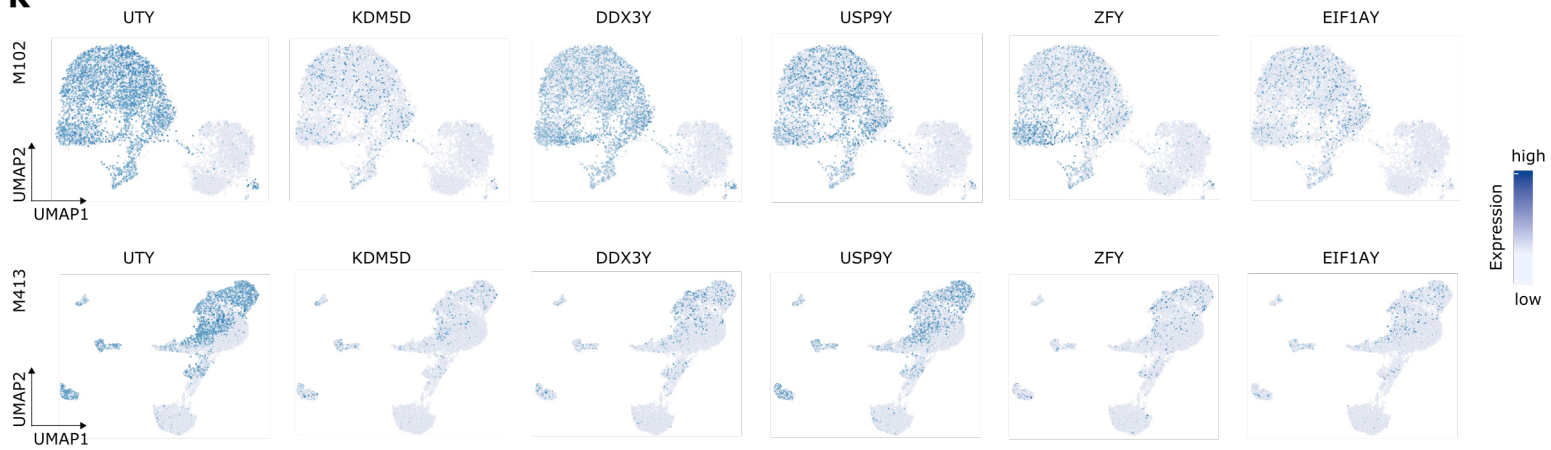
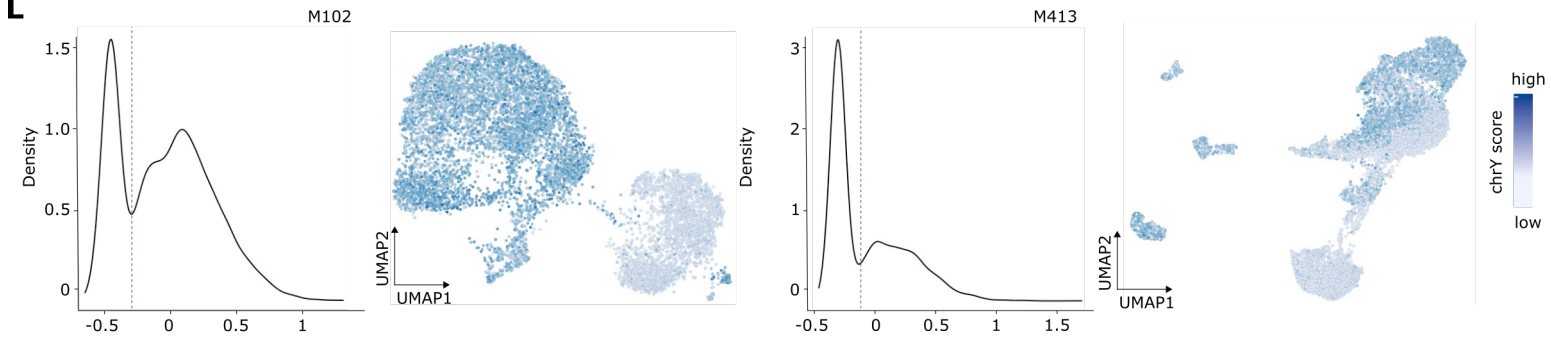
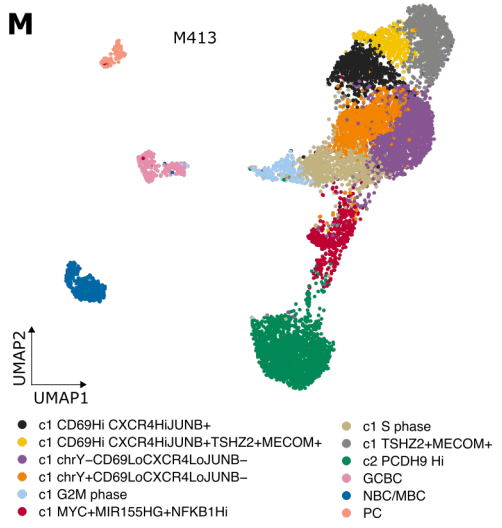
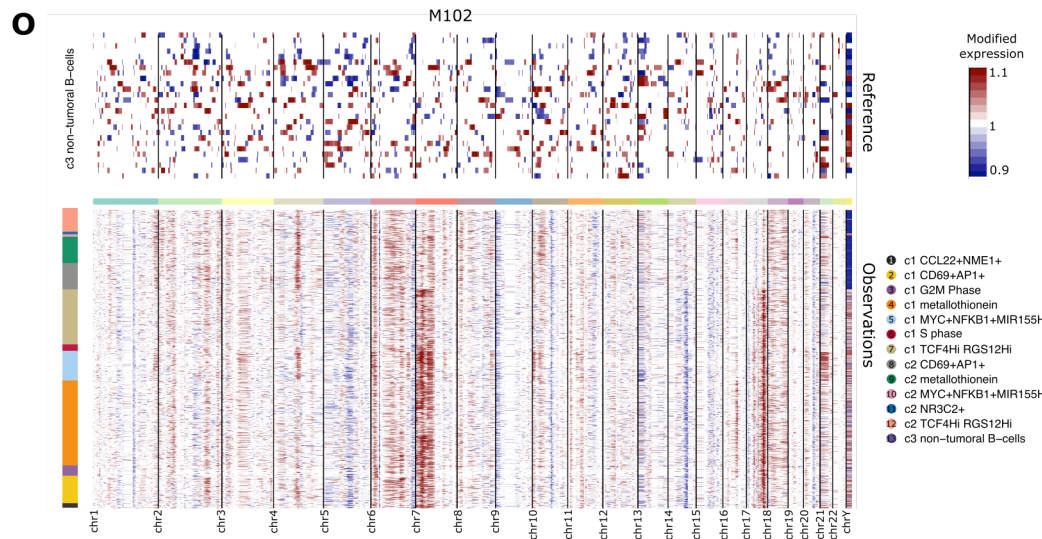
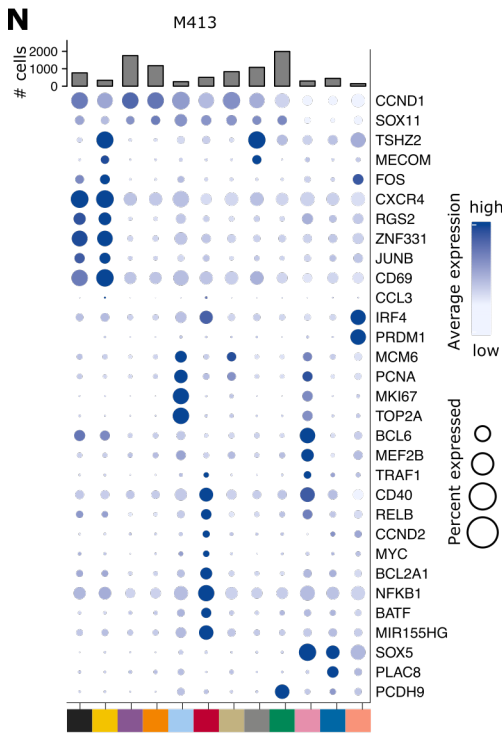


Figure S7



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