

PB3483 Spatially-resolved, single-cell transcriptomics detects cell types and novel cell states in kidney diseases

Metzger Evelyn¹, Rosales Ivy², How Ira², Brousaides Nicole², Kim Sean¹, Murphy Sarah¹, Reeves Jason¹, Smith Rex², Colvin Robert²

¹NanoString Technologies, Inc., Seattle WA. ²Immunopathology Research Laboratory, Center for Transplantation Sciences, Massachusetts General Hospital, Boston, MA 02114

Abstract

Spatially-resolved, single-cell tissue atlases can provide mechanistic insights not appreciable by bulk RNAseq or spatially-agnostic scRNAseq. We have begun to harness the power of space in understanding mechanisms in kidney diseases, beginning with pathogenetically relevant transplant glomerulopathy in chronic antibody mediated rejection (CAMR), the major cause of late kidney graft dysfunction.

A necessary first step is to generate the spatial atlases themselves. To do this, gene expression was measured in FFPE biopsy sections from 7 CAMR cases, 4 T cell mediated rejection (TCMR) cases, and 3 cases with no evidence of rejection (NER) with 1000 gene probes using the CosMx™ Spatial Molecular Imager (SMI). Cells were detected by IF staining of B2M/CD298 and DAPI with the machinery in the AtoMx™ Spatial Informatics Platform. The analyzed dataset consisted of 295,937 cells and a total of 51,142,869 transcripts. Cell typing was accomplished by semi-supervised clustering with the Kidney Cell Atlas database.

With the disease-inclusive spatial atlas, we report on 3 tests related to kidney rejection and cell states:

1. We examined expressional differences of glomerular endothelial cells (GECs) within glomeruli between CAMR and controls. In the CAMR, GECs showed upregulation of genes related to repair and angiogenesis (e.g., *COTL1*; $\log_2FC=1.1$, $FDR=2.9e-5$); protective genes (e.g., *CD59*; $\log_2FC=0.96$, $FDR=9.2e-7$), and a downregulation of genes involved in VEGFR2 signaling (e.g., *EFNB2*; $\log_2FC=1.25$, $FDR=1.8e-31$).
2. We further interrogated the in-glomerulus CAMR GECs. We found two distinct classes of GECs with one class containing genes related to inflammation and angiogenesis and the other class previously identified in normal kidneys with genes related to cell adhesion and growth arrest.
3. We sought to understand the role of NK cells in CAMR. We found that NK cells tended to co-localize with GECs in CAMR ($p=0.08$). Moreover, differential expression showed evidence that CAMR-residing NK cells were activated relative to NER NK cells.

We found profound transcript changes in GECs affecting the VEGFA pathway, known to be required for normal GEC differentiation. NK cells were in proximity to glomerular endothelium and expressed increased activation transcripts in CAMR. Our data show that pathological phenotypes of cells may not be represented in normal cell atlases and these atlases can be improved with spatially-resolved, single cell approaches.

Background and Cohort Details

Chronic antibody mediated rejection (CAMR) is the predominant cause of late renal allograft dysfunction. A defining pathology is transplant glomerulopathy (Fig. 1), thought to be mediated by NK cells targeting endothelial cells.

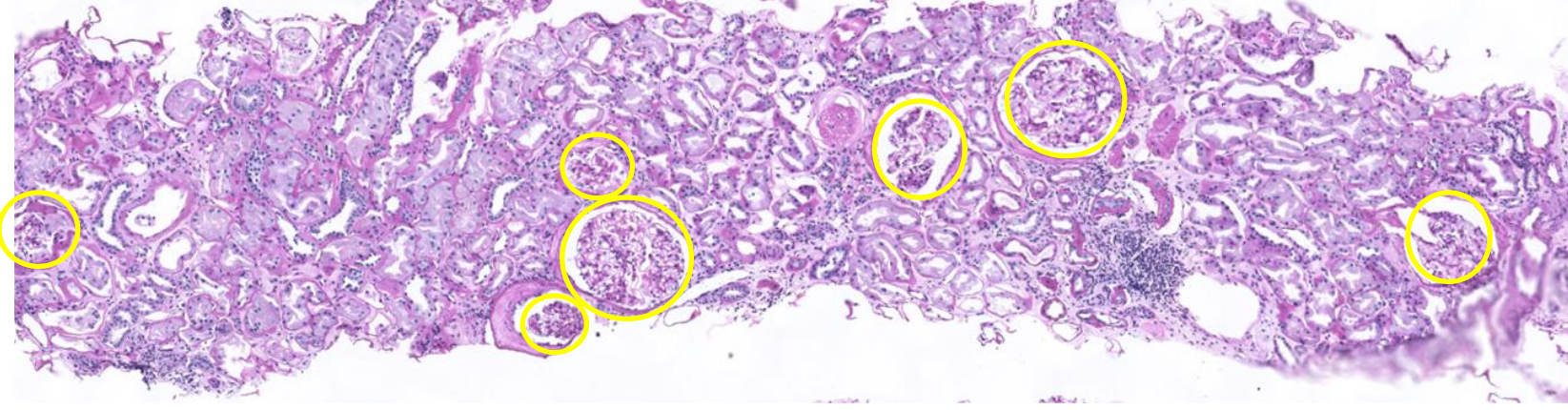


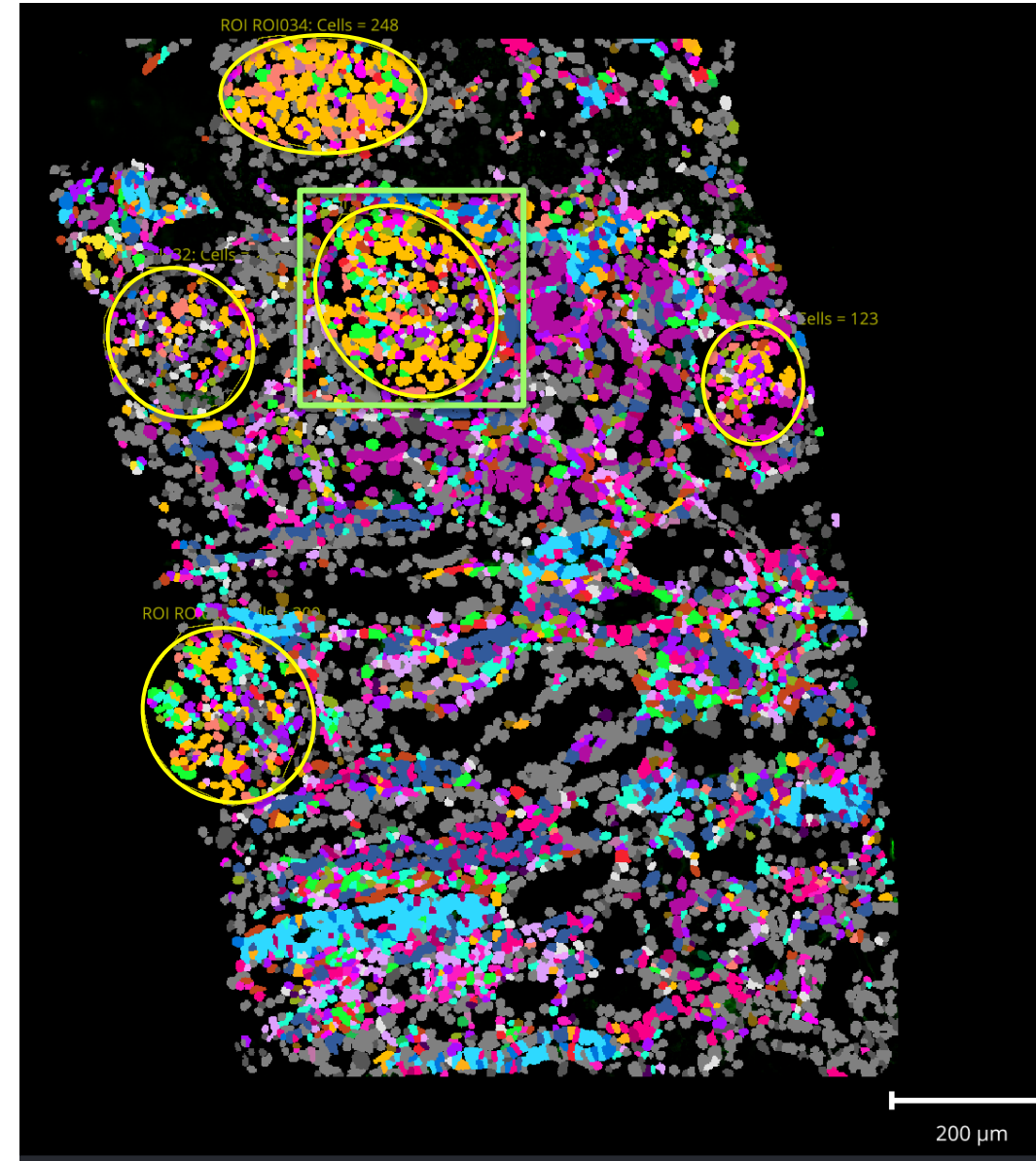
Fig 1. Glomeruli in a PAS-stained section of FFPE. The vast majority of glomerular endothelium cells (GECs) are found within these glomeruli (yellow circles).

Spatially localized transcripts at the cellular level were identified in FFPE renal biopsies with CAMR compared with controls with no evidence of rejection (NER), using the CosMx SMI with a 1,000 plex probe set. Analysis included cellular differential expression, cell proximity, and ligand-receptor localization.

Table 1. Cohort at a Glance.

Samples	NER: 3 CAMR: 7 TCMR only: 4
Plex	1000 RNA
FOVs	101
Total Transcripts	51,142,869
Total Cells	295,937

Fig 2. Representative section of core needle biopsy that was analyzed with the CosMx SMI. Shown here are cells for two FOVs. Five glomeruli (yellow outlines) containing a characteristic enrichment of GEC, Podocytes, and Mesangial cells are shown. Green box shows location of glomerulus shown in Fig. 4.



Disease-dependent Cell States

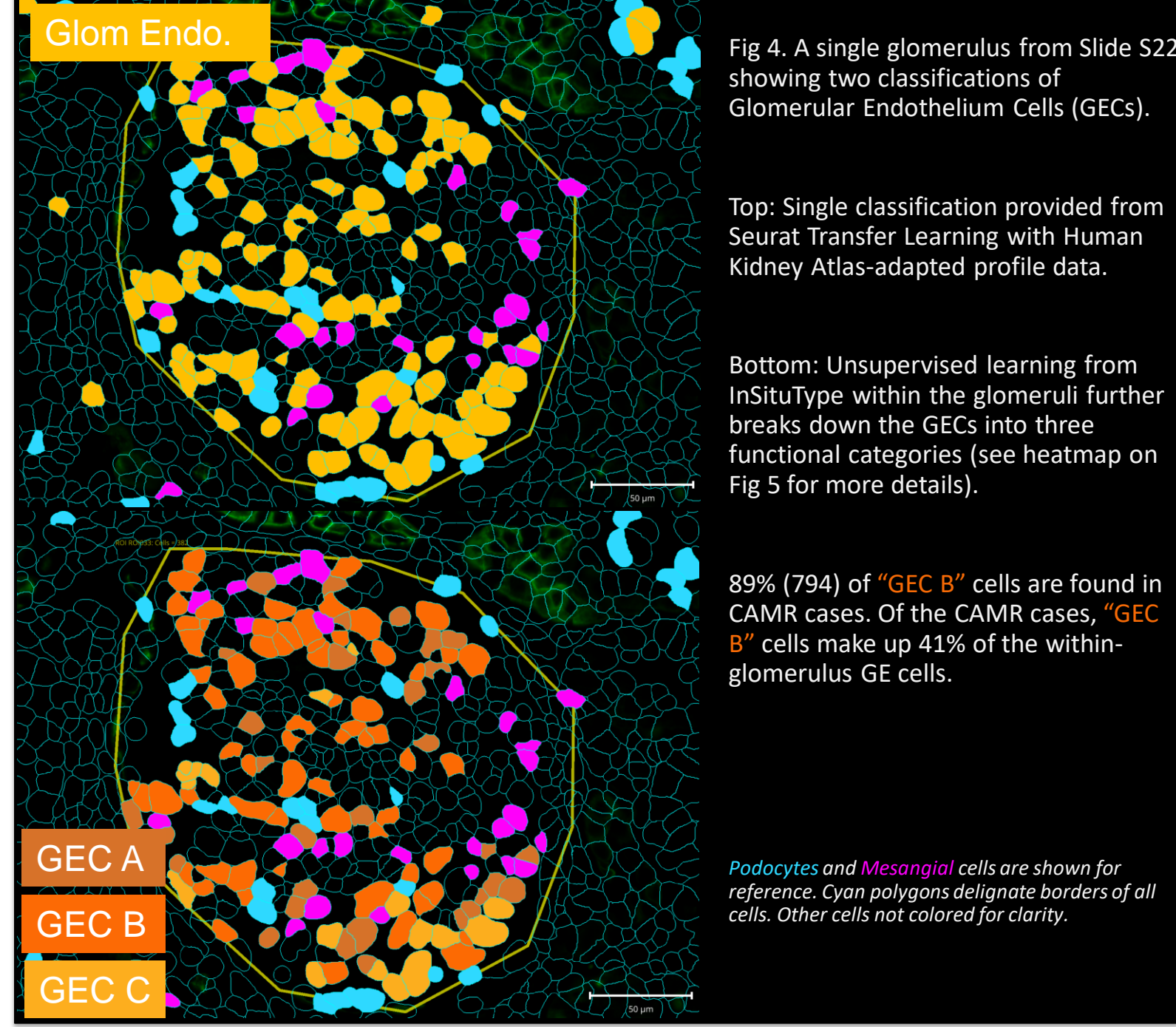


Fig 4. A single glomerulus from Slide S22 showing two classifications of Glomerular Endothelial Cells (GECs).

Top: Single classification provided from Seurat Transfer Learning with Human Kidney Atlas-adapted profile data.

Bottom: Unsupervised learning from In Situ type within the glomeruli further breaks down the GECs into three functional categories (see heatmap on Fig 5 for more details).

89% (794) of "GEC B" cells are found in CAMR cases. Of the CAMR cases, "GEC B" cells make up 41% of the within-glomerulus GE cells.

Podocytes and Mesangial cells are shown for reference. Cyan polygons delineate borders of all cells. Other cells not colored for clarity.

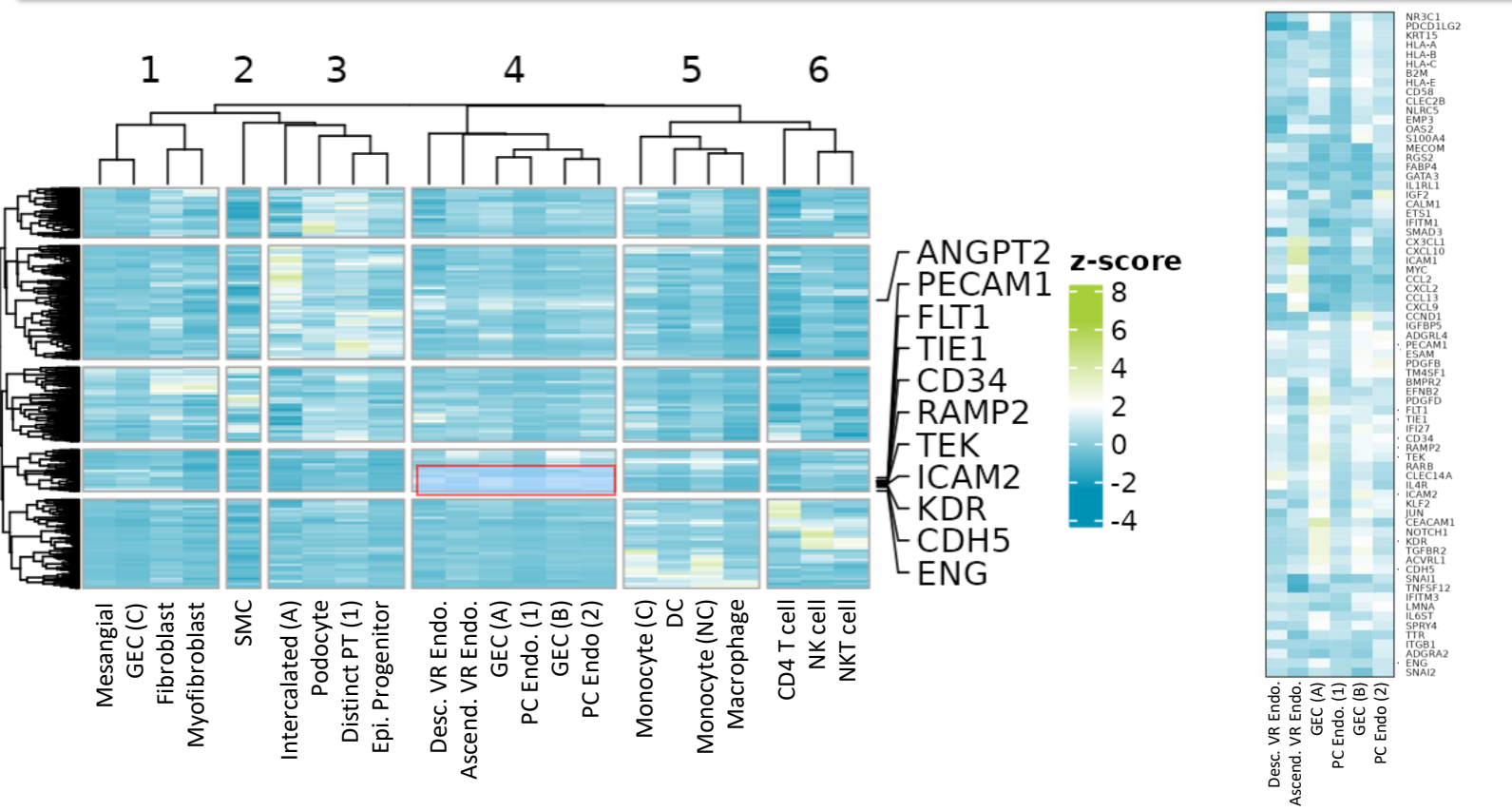


Fig 5. Disease-specific cell states identified when sub-clustering glomerular-resident GECs. Marker gene heatmap of cells (i.e., Z-scores of expression of nearly 1000 genes) is shown on the left. Right: zoom-in of genes present in the red box showing expression differences amongst endothelium cell types.

Cell Co-localization and Ligand-Receptor interactions of NK and GECs

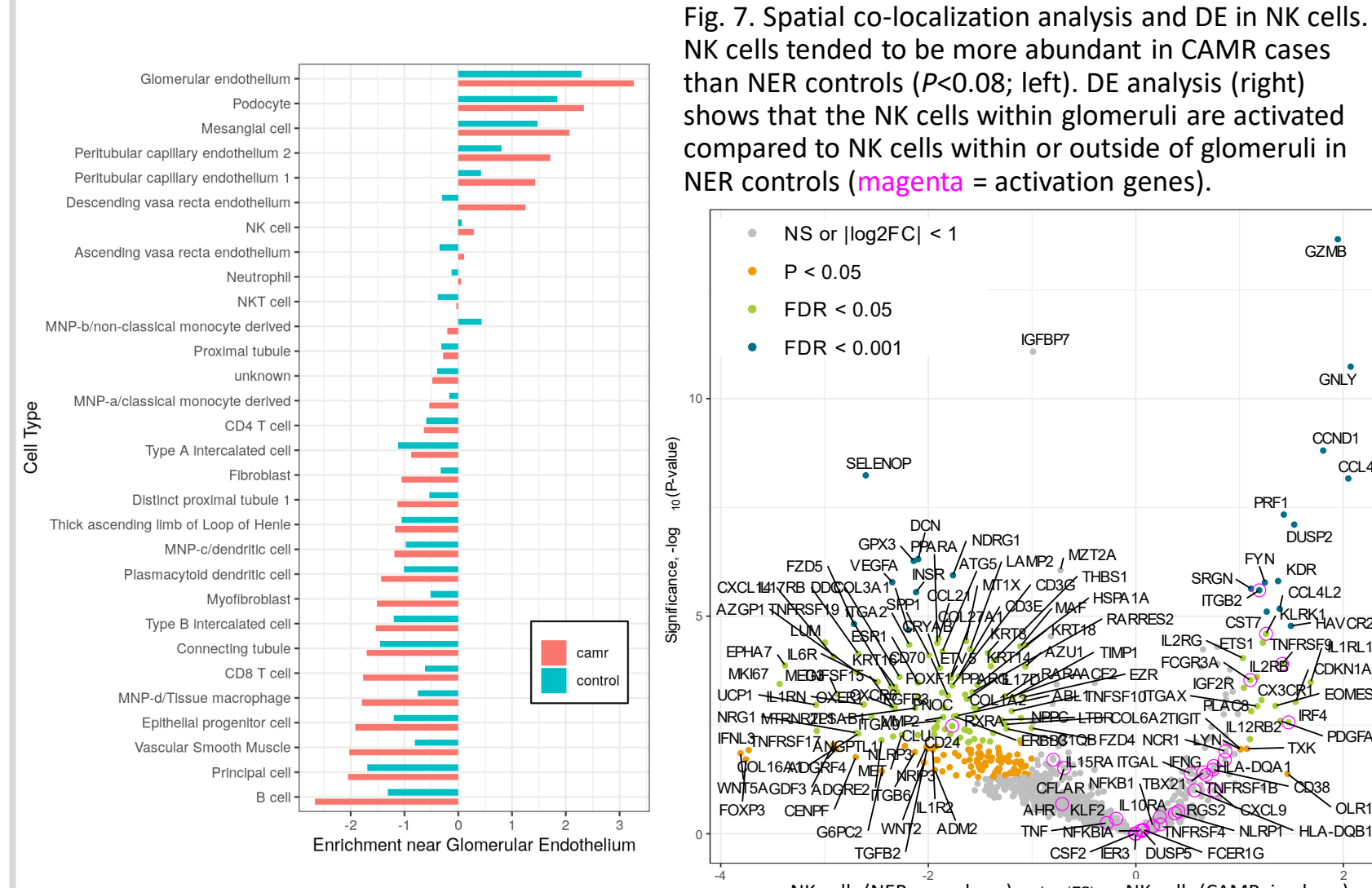


Fig. 7. Spatial co-localization analysis and DE in NK cells. NK cells tended to be more abundant in CAMR cases than NER controls ($P<0.08$; left). DE analysis (right) shows that the NK cells within glomeruli are activated compared to NK cells within or outside of glomeruli in NER controls (magenta = activation genes).

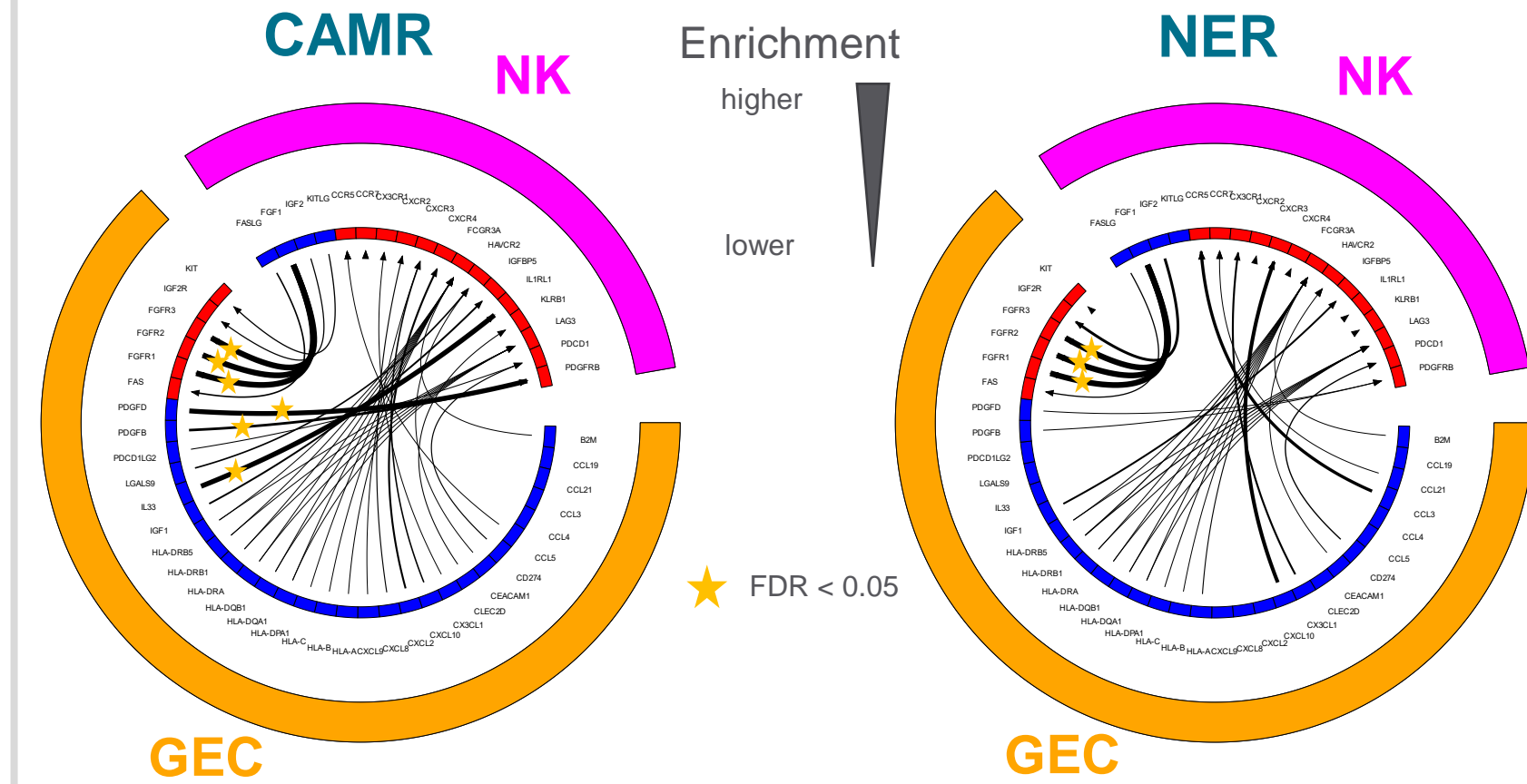


Fig 8. Ligand-Receptor (LR) hits for NK cells and GECs in CAMR (left) and NER controls (right). Outer ring shows a given cell type. Inner ring shows genes (blue = ligand; red = receptor). LR target expression in adjacent cells is used to calculate a co-expression enrichment score. Wider arcs show higher enrichment score of a LR pair. These enrichment scores are permuted to estimate a significance. Stars show significance below a False Discovery Rate of 5%.

CosMx SMI Profiling of FFPE Samples

Sequential flow of target-binding and fluorescently labelled reporter probes across FFPE tissues enable robust detection of RNA target fragments with <50 nucleotides of intact sequence required for target detection on standard histopathology slides.

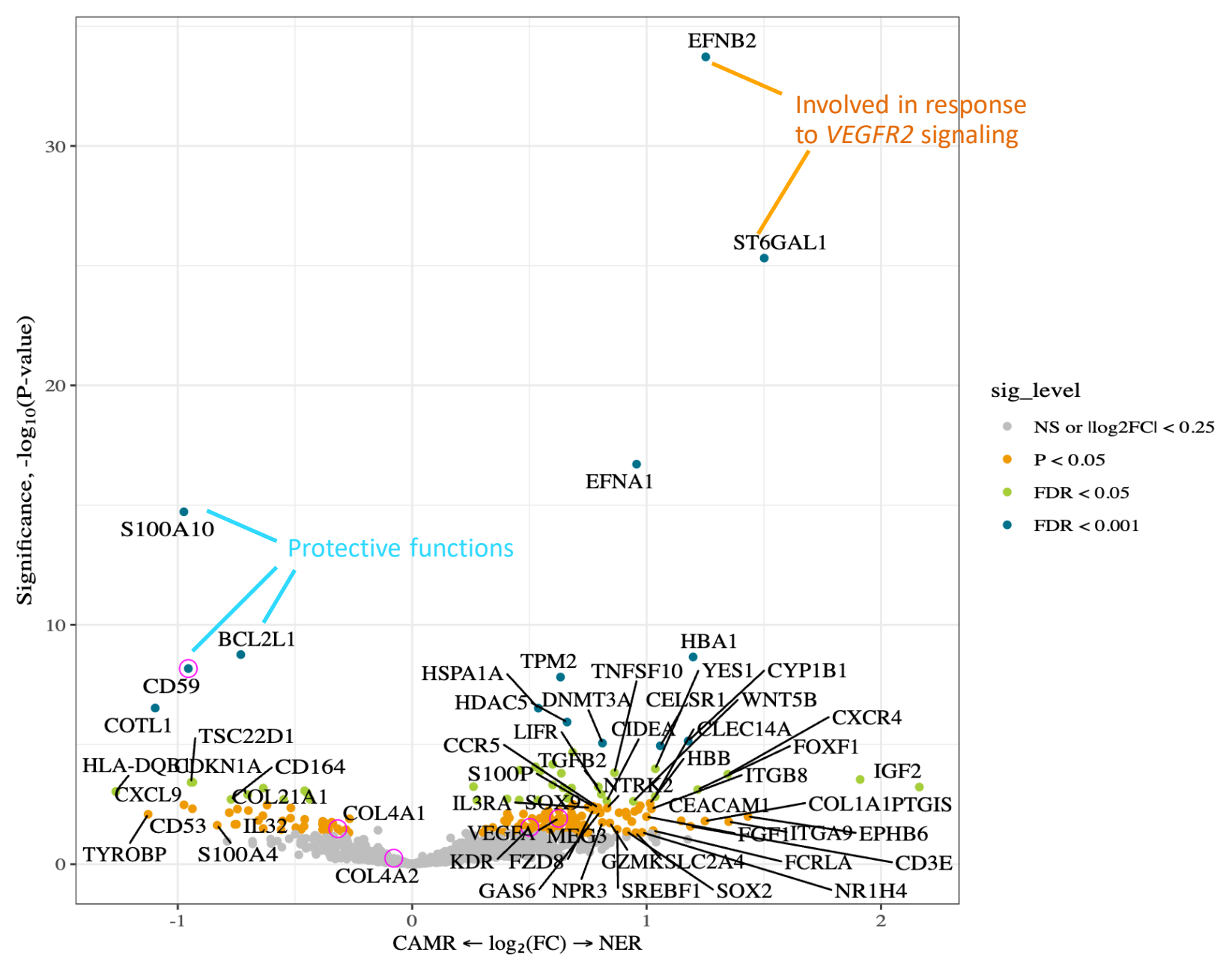
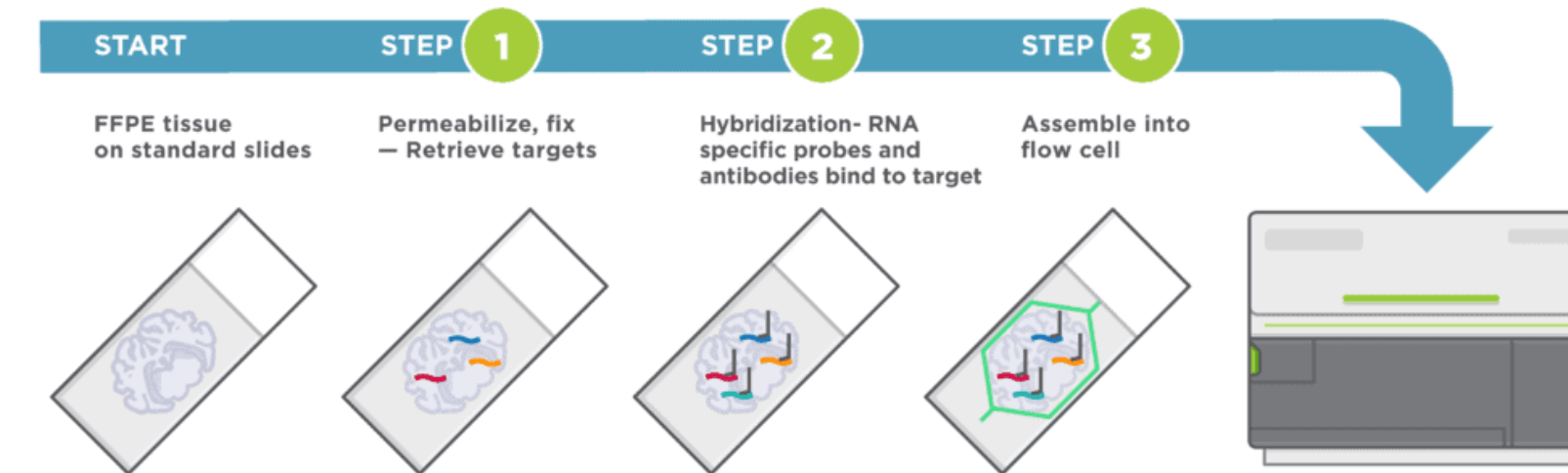
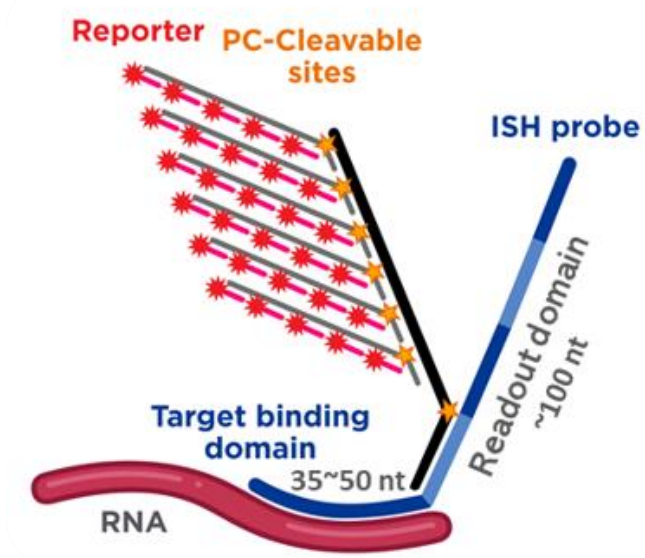


Fig 3. Expressional differences of glomerular endothelium cells (GECs) between CAMR and NER controls. CAMR GECs had increased transcripts for protective genes (BCL2L1, CD59, S100A10), endothelial repair/angiogenesis (COTL1, TSC22D1), and the target of donor specific antibody (HLA-DQ).

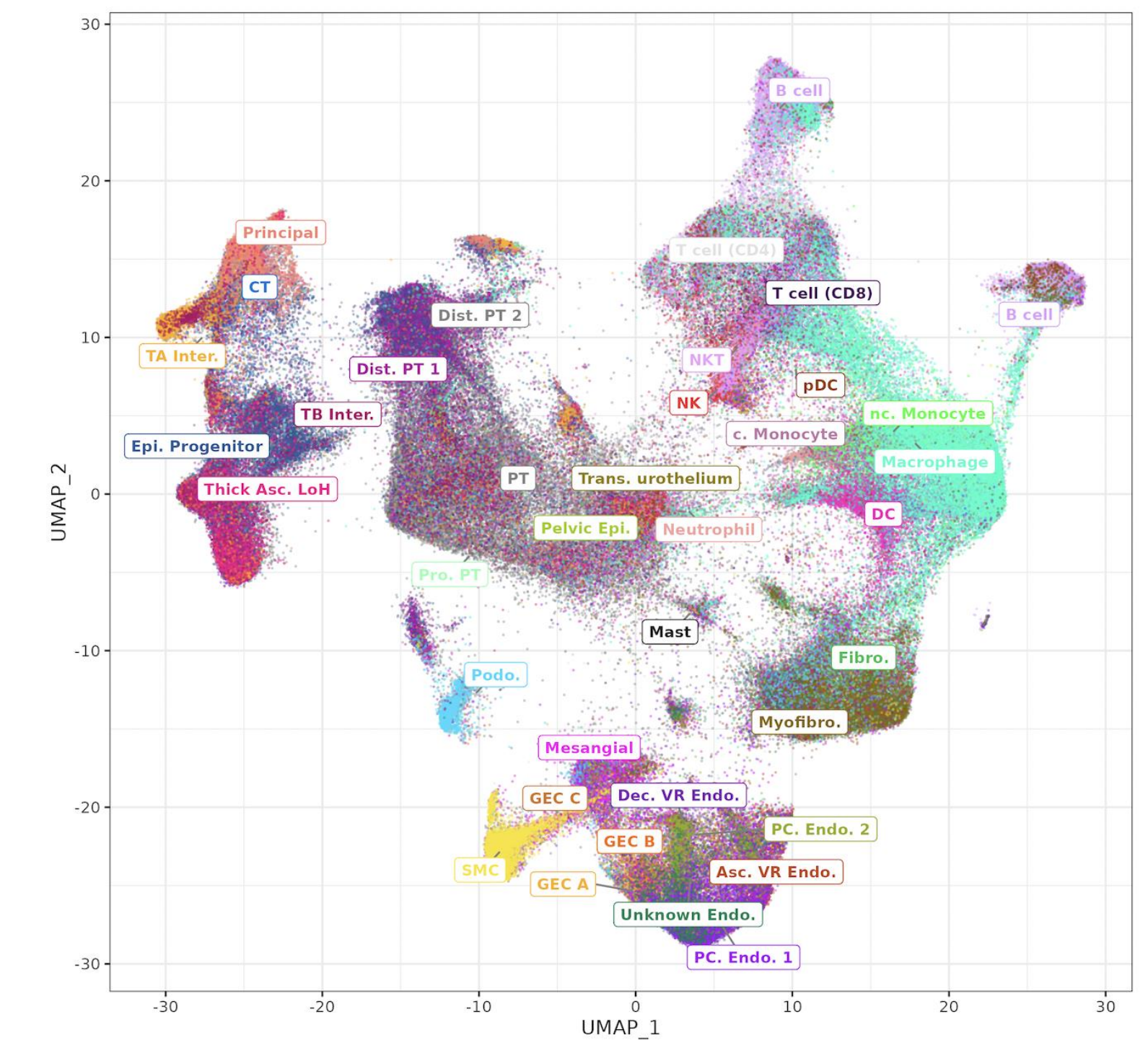


Fig 6. Projecting cells into 2D expression space. UMAP of 295,937 post-filtered cells in the study, colored by cell types.

Conclusions

CosMx SMI analyses quantify transcripts in individual cells along with their location in routine biopsy sections. This approach was applied to provide deeper insights into the pathogenesis of transplant glomerulopathy, a defining feature of chronic antibody mediated rejection (CAMR). The results revealed intraglomerular NK cell activation of genes related to cytotoxicity, Fc receptor and non-self recognition. Glomerular endothelial cells had increased transcripts related to protection, angiogenesis and MHC expression. Glomerular endothelial heterogeneity was revealed with two subsets, one restricted to CAMR glomeruli. Several NK-glomerular endothelial ligand-receptor pairs plausibly relevant to pathogenesis and a potential target of intervention were detected. Finally, our results suggest that incorporating 1) disease tissue and 2) spatial context in existing cell type atlases can provide a fuller understanding of cell states.

Scan here to download or learn more



The CosMx™ SMI and decoder probes are not offered and/or delivered to the following UPC member states* for use in these countries for the detection of RNA in a method used for the detection of a plurality of analytes in a cell or tissue sample without the consent of the President and Fellows of Harvard College (Harvard Corporation) as owner of the Unitary Patent EP 4 108 782 B1. The use for the detection of RNA is prohibited without the consent of the of the President and Fellows of Harvard College (Harvard Corporation).
*Austria, Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Portugal, Slovenia, Sweden

The CosMx™ SMI and decoder probes are not offered and/or delivered to the Federal Republic of Germany for use in the Federal Republic of Germany for the detection of cellular RNA, messenger RNA, microRNA, ribosomal RNA and any combinations thereof in a method used in fluorescence in situ hybridization for detecting a plurality of analytes in a sample without the consent of the President and Fellows of Harvard College (Harvard Corporation) as owner of the German part of EP 2 794 928 B1. The use for the detection of cellular RNA, messenger RNA, microRNA, ribosomal RNA and any combinations thereof is prohibited without the consent of the of the President and Fellows of Harvard College (Harvard Corporation).