



Around the Block: Neighborhoods in Kidney Health and Disease

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Indiana University School of Medicine

7-12-24

Disclosures

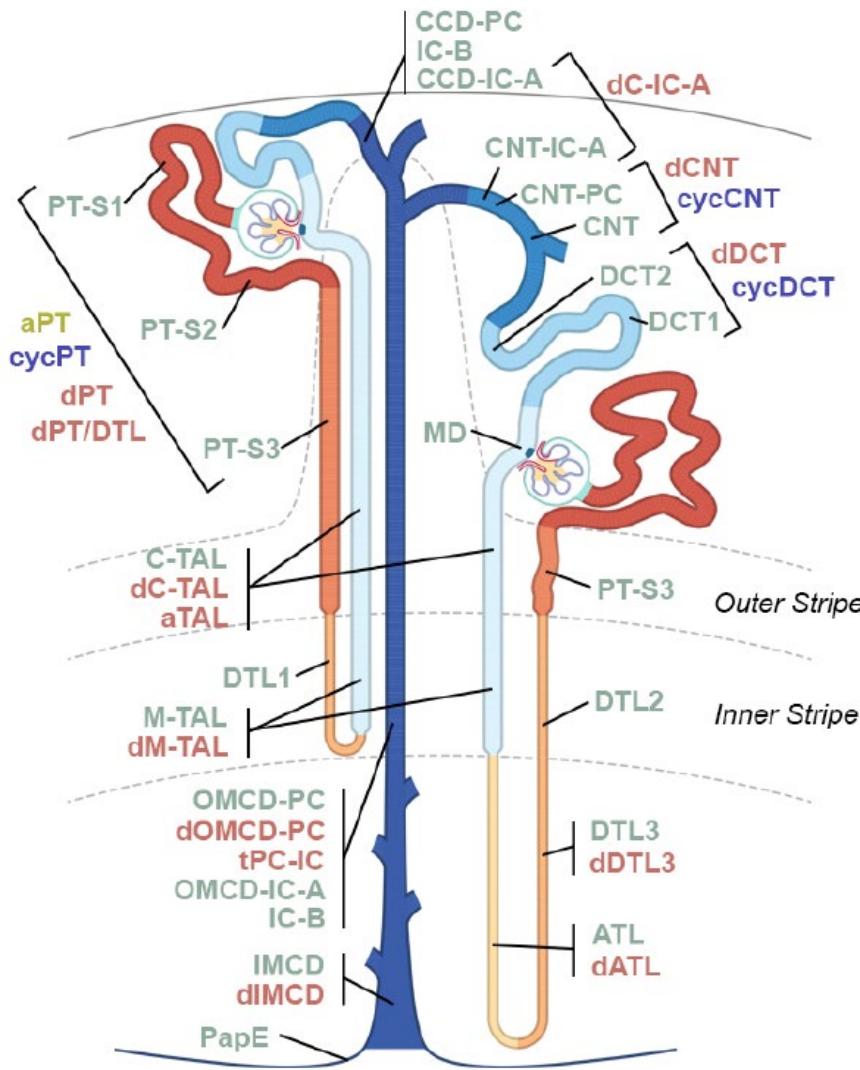
- NIH funding

Contents

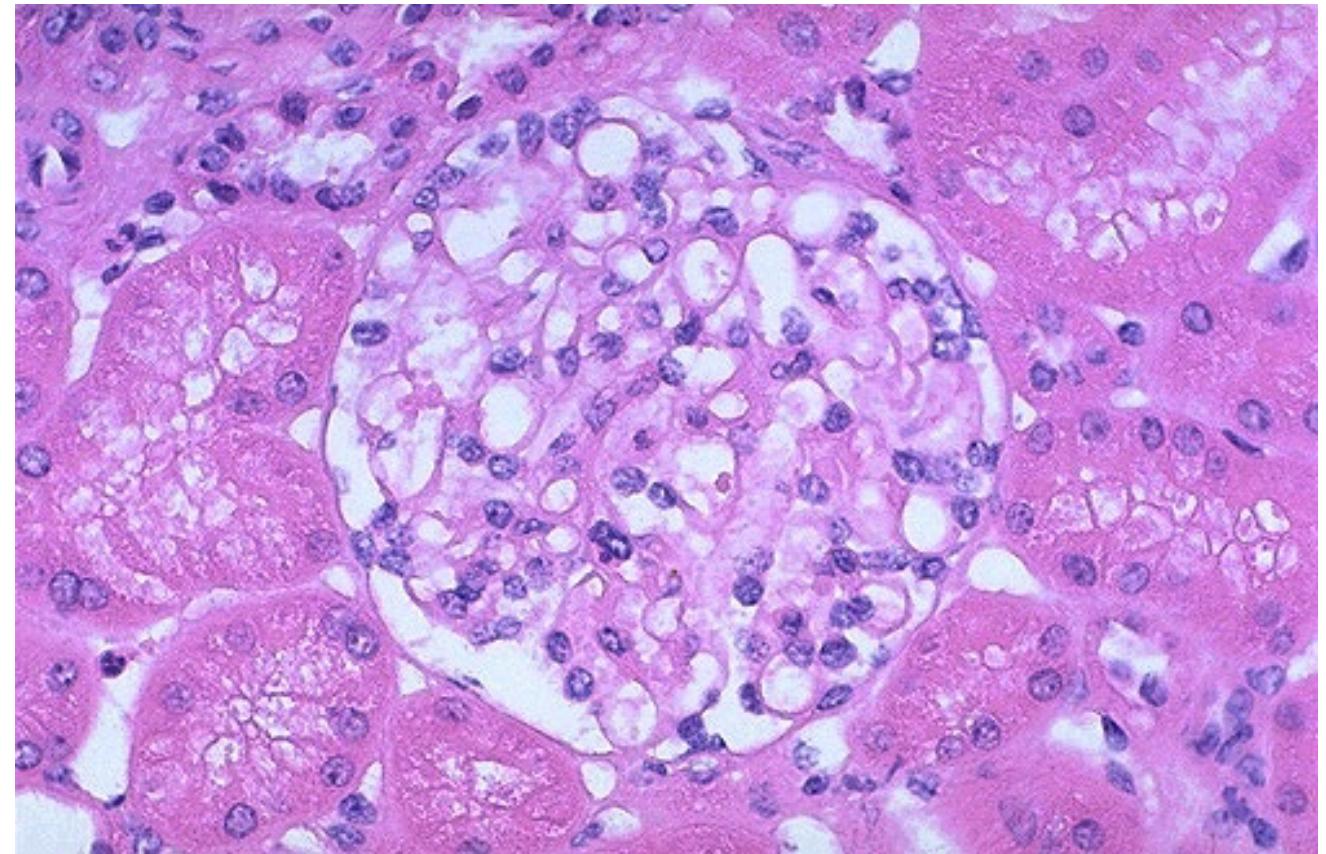
1. Data Sources
2. QC considerations
3. Analysis approaches
4. Histologic Annotation with AI

The Kidney

Nephron



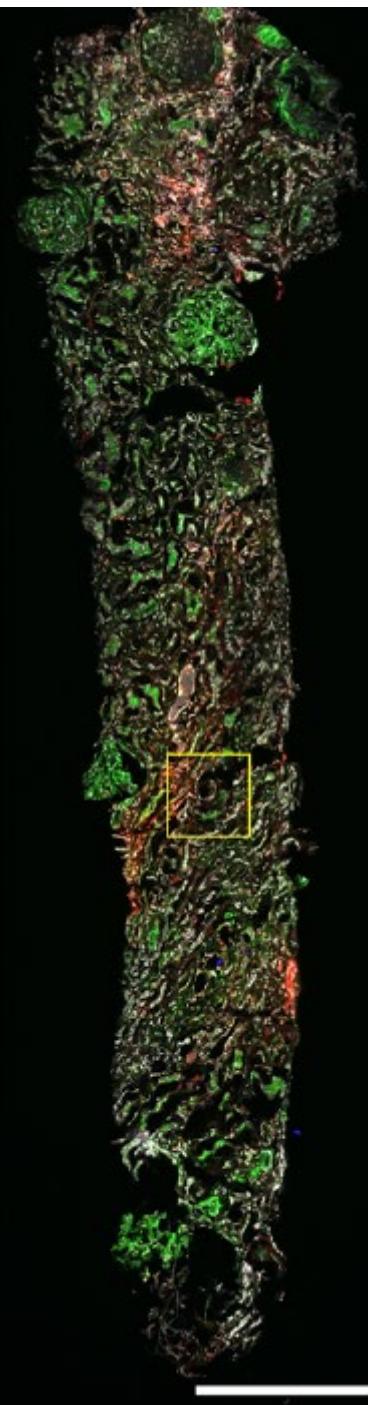
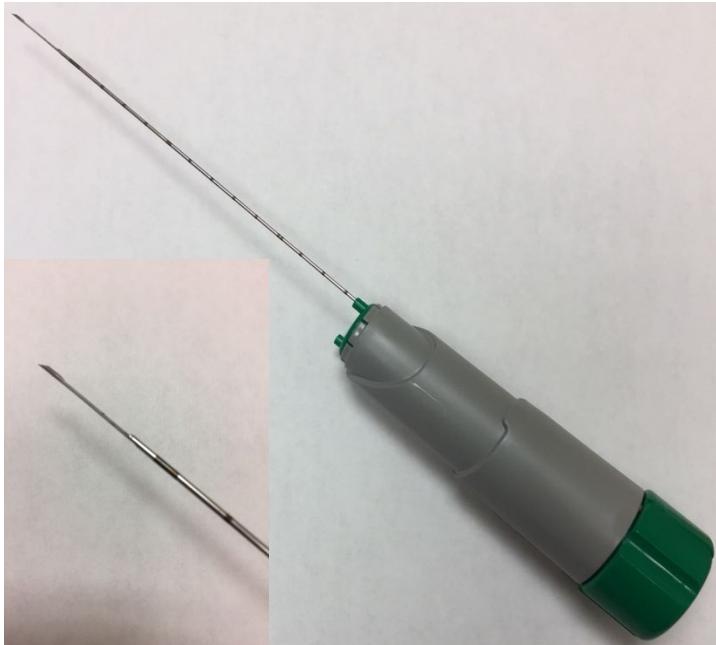
- Interface between blood and urine
- 1.25 million nephrons
- Over 100 cell types
- Mosaic like structure challenging for ST



<https://webpath.med.utah.edu/RENAHTML/RENAL101.html>

The Renal Biopsy

- Performed when a clinical diagnosis is in question or to determine therapy
- KPMP – prospective cohort of altruistic kidney tissue donation in early CKD or AKI





www.kpmp.org



TRANSCRIPTOMICS

- Single Cell RNA-seq
- Single Nucleus RNA-seq
- Regional Transcriptomics
- Spatial Transcriptomics
- Slide-seq Spatial Transcriptomics

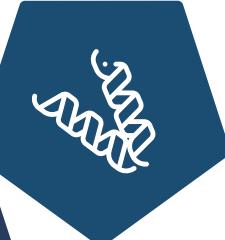
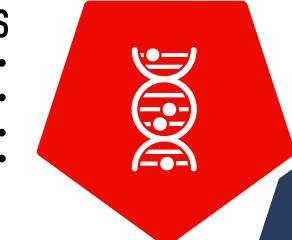
EPIGENETICS

- DNA Methyl-seq •
- microRNA-seq •
- CUT & RUN •
- ATAC-seq •

PROTEOMICS

PROTEOMICS

- Regional Proteomics
- Spatial Proteomics



INTEGRATED FUNCTIONAL KIDNEY MAP

IMAGING

- 3D Tissue Imaging •
- and Cytometry
- mlFISH •
- CODEX •
- Imaging Mass Cytometry •
- DART-FISH •



METABOLOMICS

- Spatial Metabolomics
- Spatial Lipidomics
- Spatial N-glycomics

Altruistically donated biopsy specimens in kidney disease

HuBMAP

The Human BioMolecular Atlas Program

HuBMAP

About ▾ Data ▾ News & Events ▾ Member Services ▾ Data Portal

What is HuBMAP?

The Human BioMolecular Atlas Program is working to catalyze the development of a framework for mapping the human body at single cell resolution.

[Find Out More](#)



1039 1013

Datasets



Samples

Explore our Data

86

Donors

21

Organs

Goal to map healthy cells in the human body across multiple organs.

KPMP data atlas

- <https://atlas.kpmp.org/>

Go forward one page (Alt+Right Arrow)
Right-click or pull down to show history as [Dashboard \(Home\)](#) [Explorer](#) [Repository](#) [Spatial Viewer](#) Help ▾

KIDNEY PRECISION MEDICINE PROJECT

Kidney Tissue Atlas

The Kidney Tissue Atlas is a set of interactive tools built to promote retrieval, exploration, discovery, and analysis of the KPMP data by the greater research community.
[Learn more about our data types and methodologies](#)

Explorer
Search for markers or cell types of interest and view summary data visualizations across the various KPMP 'omics' technologies.

[Go to Explorer](#)

Repository
Download raw gene data generated from KPMP participant biopsies and reference tissue samples.

[Go to Repository](#)

Spatial Viewer
View and interact with spatial data from various KPMP

Atlas Data Summary

IMPORTANT: Please follow this [citation guideline](#) when presenting or publishing data from the Kidney Tissue Atlas.

 **PARTICIPANTS**

281	70	19	125
CKD	AKI	DM-R	REFERENCE

Participants by -omics type
A subset of the raw data from the Data Repository has been analyzed and made available for interactive mining in Explorer and Spatial Viewer.

OMICS TYPE	REFERENCE	CKD	AKI	DM-R	ALL
Explorer					

HuBMAP Data Repository

<https://portal.hubmapconsortium.org>

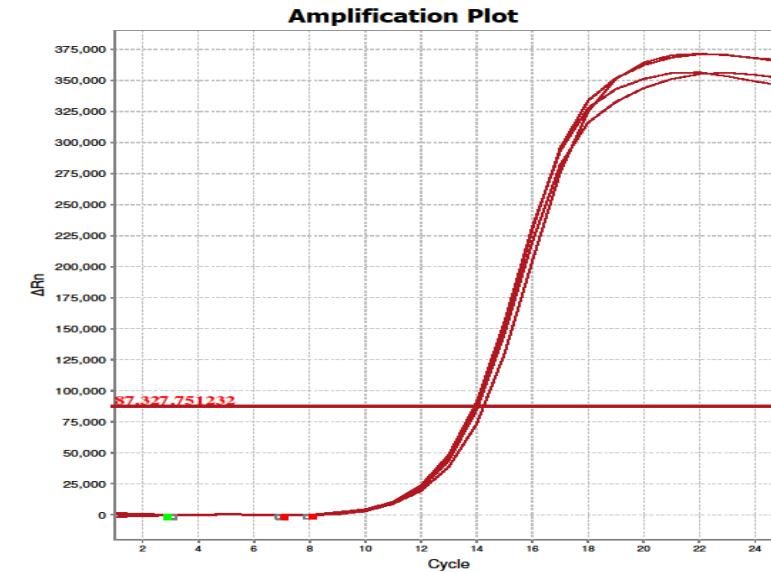
The screenshot shows the HuBMAP Data Portal interface. At the top, there is a navigation bar with links for 'HuBMAP', 'Data', and 'Resources'. Below the navigation bar, the main title 'Datasets' is displayed next to a three-line icon. A 'Getting Started' box is present, containing a welcome message and a 'Begin The Dataset Search Tutorial' button. The search bar includes a 'Search' input field and a 'Metadata' dropdown. Below the search bar, several filters are applied: 'Dataset Type: Slideseq', 'Dataset Type: Slideseq [Salmon]', 'Organ: Kidney (Right)', 'Organ: Kidney (Left)', and 'Analyte Class: RNA'. On the left side, a sidebar displays 'Dataset Metadata' with filters for 'Dataset Type' (RNAseq: 248, Slide-seq: 84), 'Organ' (Kidney (Left): 46, Kidney (Right): 38), 'Analyte Class' (RNA: 84), and 'Sample Category' (section: 46, block: 38). The main content area shows a table of dataset results. The columns are: HuBMAP ID, Group, Data Types, Organ, Status, and Last Modified. The table lists five entries:

HuBMAP ID	Group	Data Types	Organ	Status	Last Modified
HBM232.MBNR.586	Broad Institute RTI	Slideseq [Salmon]	Kidney (Right)	Published	2023-11-07 00:34:27
HBM986.KFWG.239	Broad Institute RTI	Slideseq [Salmon]	Kidney (Right)	Published	2023-11-07 00:32:26
HBM532.KKRC.477	Broad Institute RTI	Slideseq [Salmon]	Kidney (Right)	Published	2023-11-07 00:30:25
HBM846.KVCF.674	Broad Institute RTI	Slideseq [Salmon]	Kidney (Right)	Published	2023-11-07 00:28:25
HBM766.NZWP.682	Broad Institute RTI	Slideseq [Salmon]	Kidney (Right)	Published	2023-11-07 00:26:24

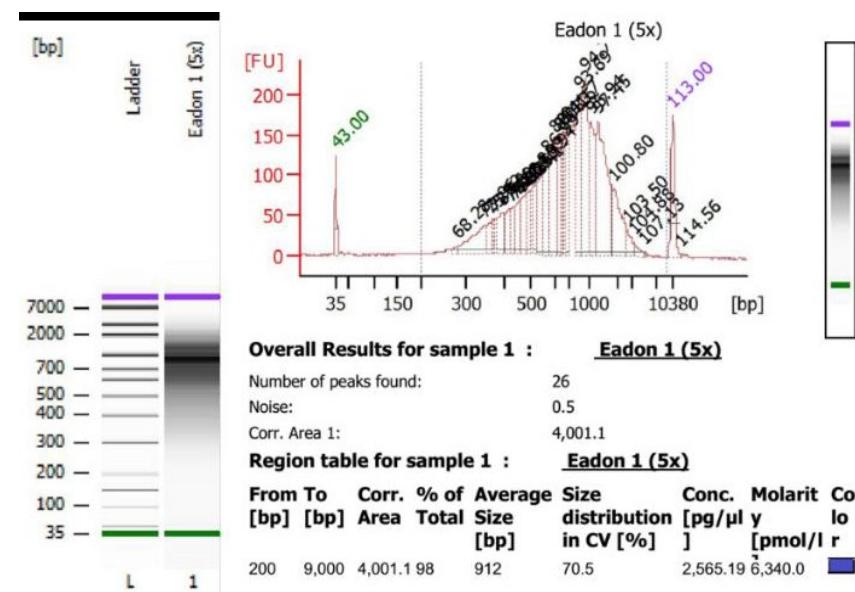
Quality Assurance Considerations

Quality Control Metrics

- QC metrics:
 - Bulk RNA quality (minimum RIN or DV200 by bioanalyzer)
 - Tissue affixment without folds or overlap
 - Microscope image visual inspection
 - Post-permeabilization RNA quantity and quality
 - cDNA quality
 - Sequencing Q30
 - Transcript mapping under spots/cells and to exons
 - Expected differential expression of marker genes



RNA assessment after permeabilization and isolation using rtPCR



cDNA quality assessed after amplification

Sequencing Output Metrics

3,007

Number of Spots Under Tissue

71,192

Mean Reads per Spot

2,970

Median Genes per Spot

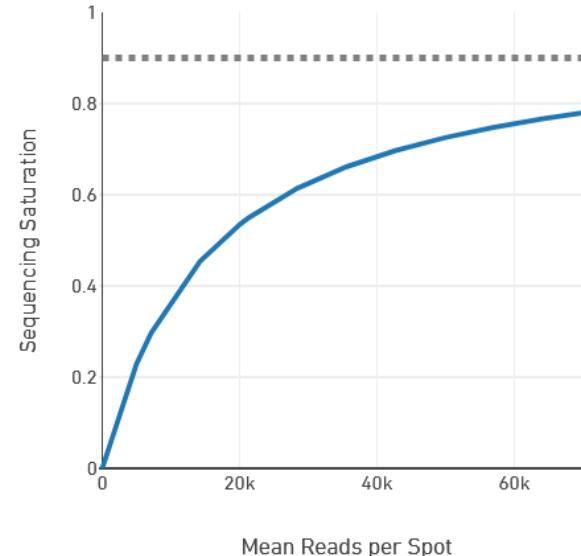
Sequencing ?

Number of Reads	214,074,559
Valid Barcodes	97.2%
Valid UMIs	99.9%
Sequencing Saturation	78.2%
Q30 Bases in Barcode	96.4%
Q30 Bases in RNA Read	93.2%
Q30 Bases in UMI	96.2%

Mapping ?

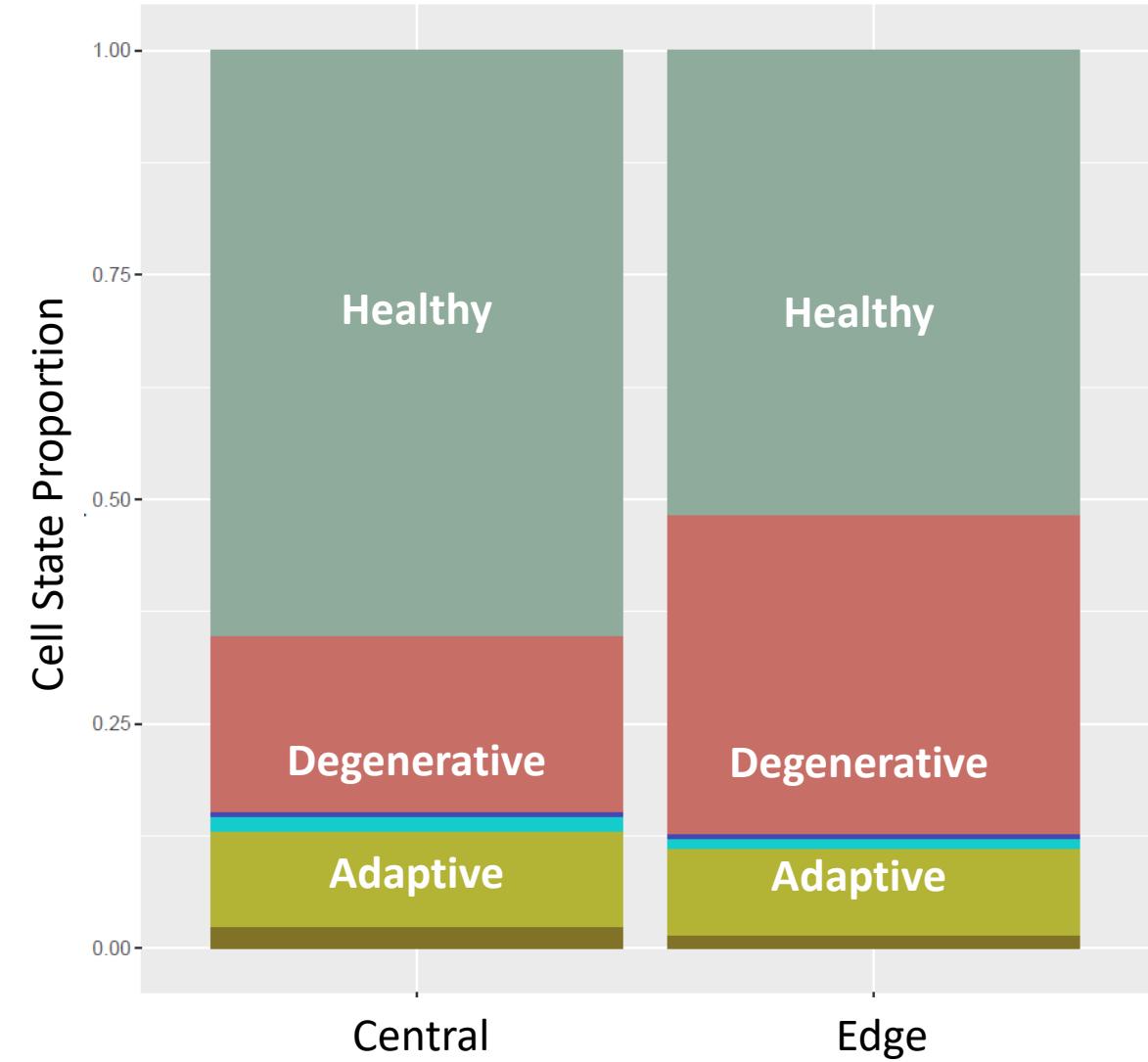
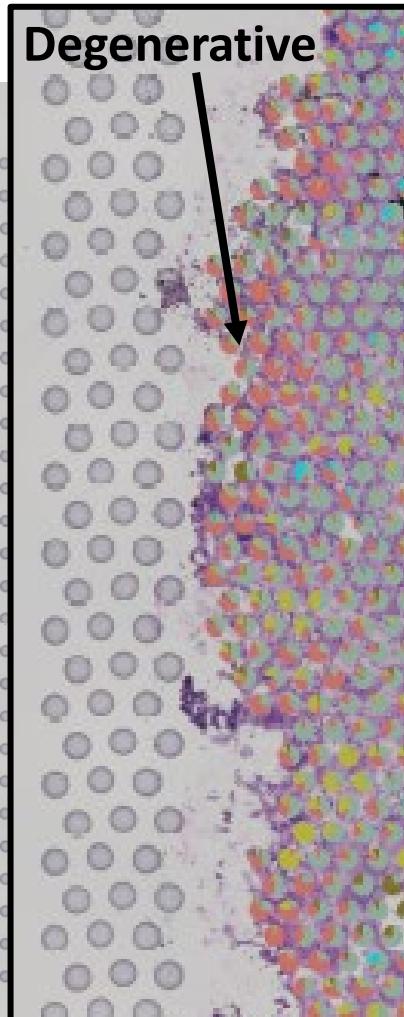
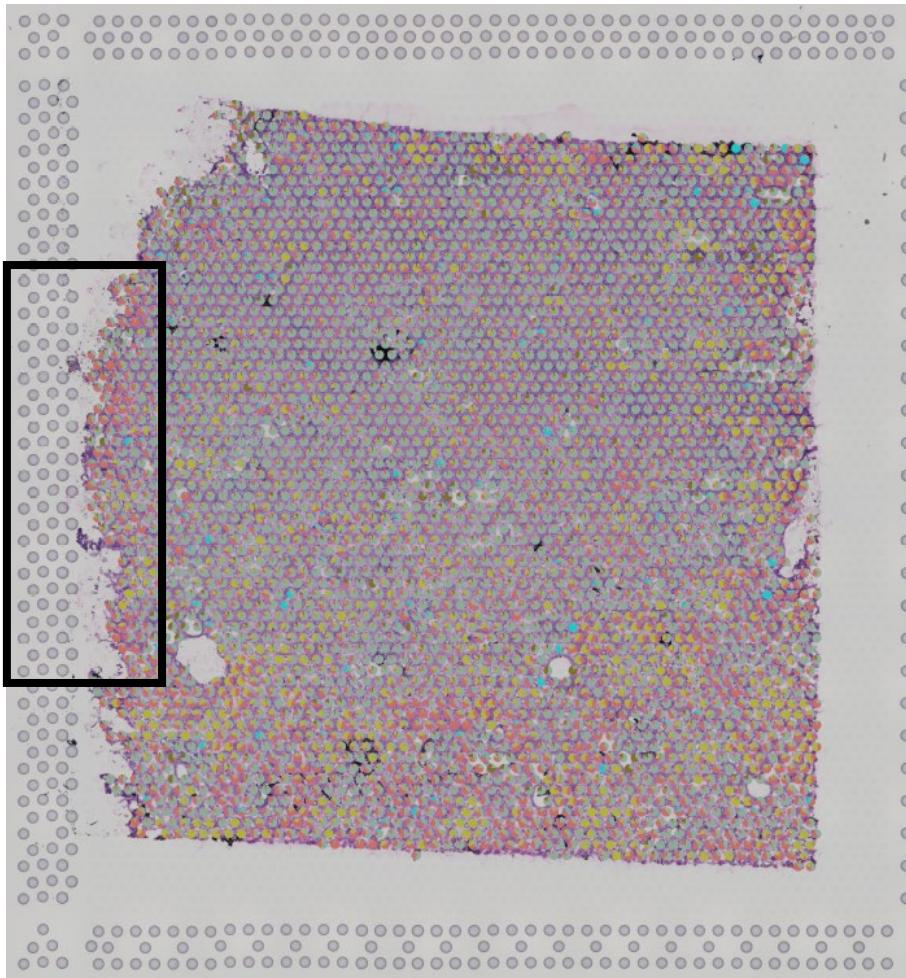
Reads Mapped to Genome	92.6%
Reads Mapped Confidently to Genome	87.9%
Reads Mapped Confidently to Intergenic Regions	4.9%
Reads Mapped Confidently to Intronic Regions	2.5%
Reads Mapped Confidently to Exonic Regions	80.5%
Reads Mapped Confidently to Transcriptome	75.9%
Reads Mapped Antisense to Gene	0.8%
Fraction Reads in Spots Under Tissue	87.2%
Mean Reads per Spot	71,192
Median Genes per Spot	2,970
Total Genes Detected	21,721
Median UMI Counts per Spot	8,349

Sequencing Saturation ?

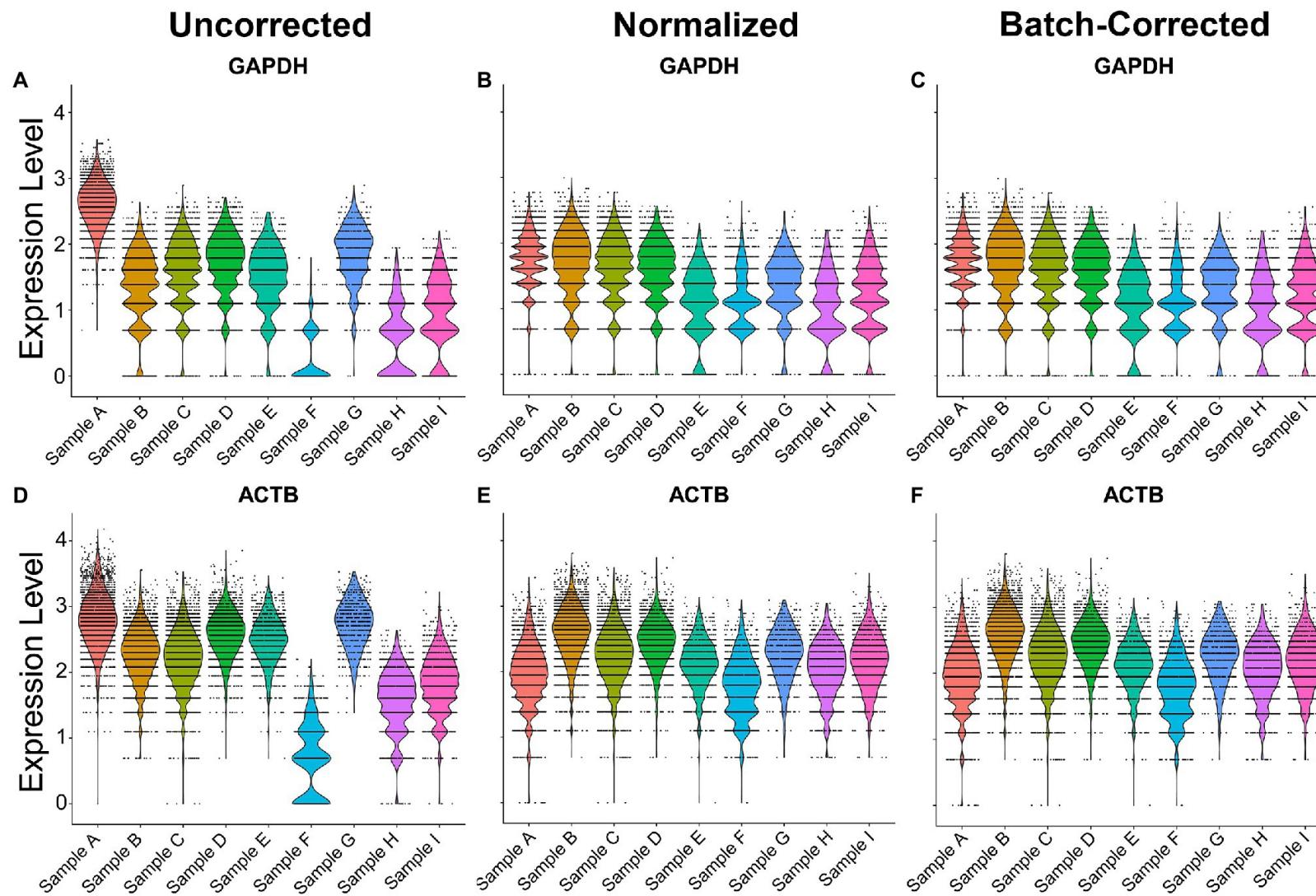


Artifactual Cell State Distribution

- We remove the outer border of cells or spots



Normalization and Batch Correction



- Normalized *GAPDH* expression with SCTransform
- Normalization and batch correction were performed by adding a batch as a variable in SCTransform.

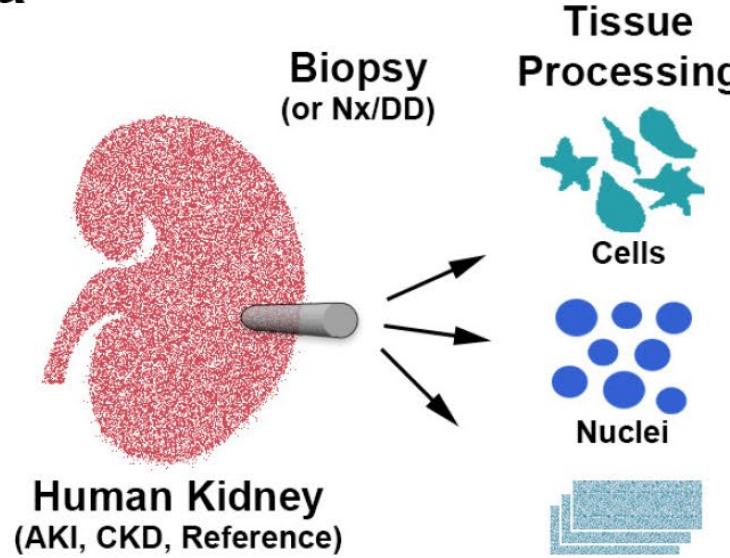
Analysis Approaches

- Label Transfer
- Deconvolution
- Neighborhood Analysis
- Spatial Variable Gene Analysis
- Segmentation

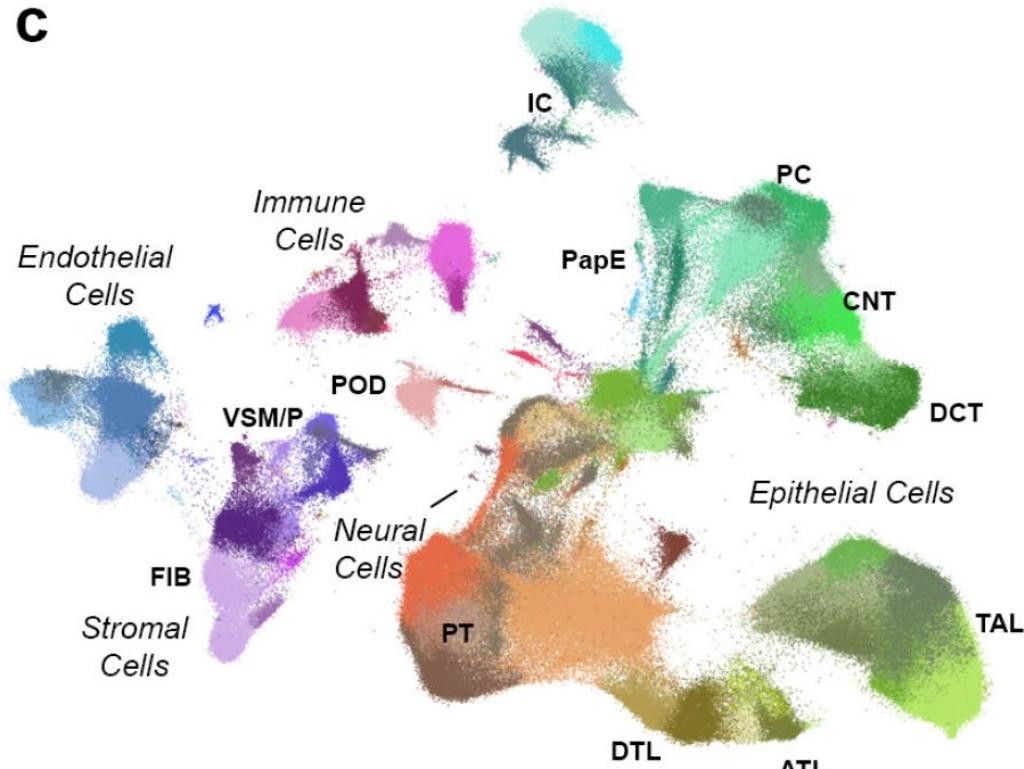
Kidney Cell Atlas Overview: Technologies

100 cell types defined, 6 cell states, 122 samples

a

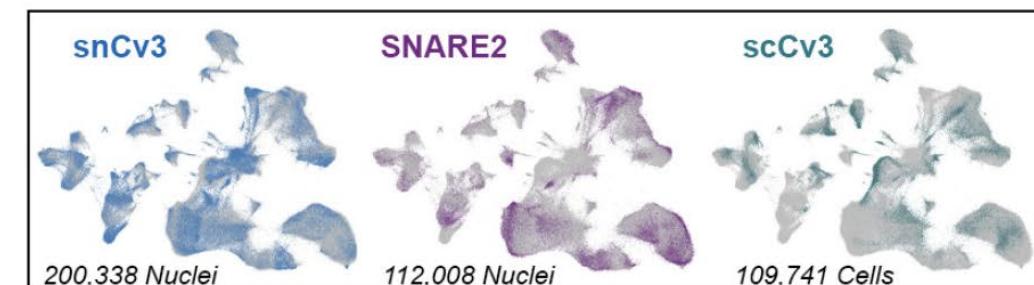
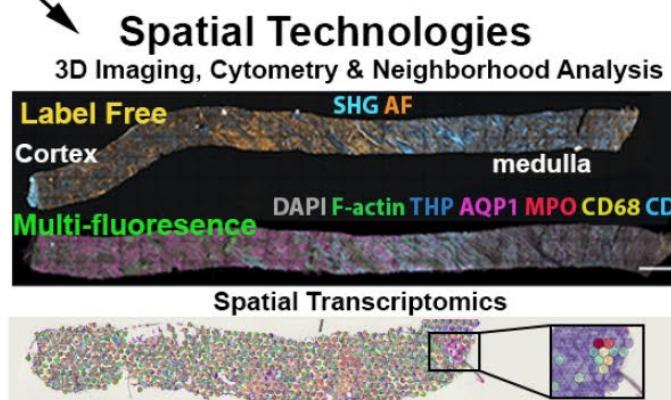


c

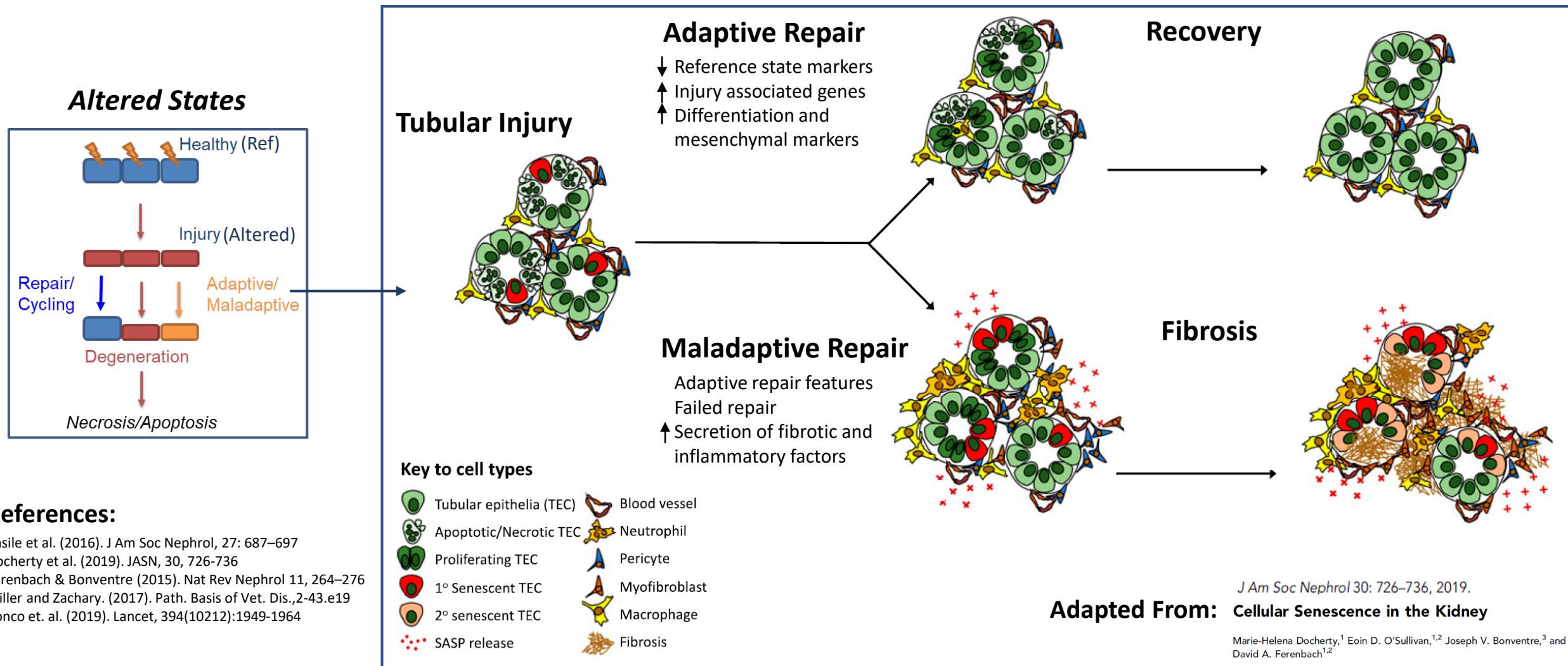


b

Assay	Sex		Condition		
	♂	♀	CKD	AKI	Ref
10X snRNA-Seq	19	17	13	10	13
10X scRNA-Seq	21	24	15	12	18
SNARE-Seq2	3	4	-	-	7
3D Imaging	6	9	9	6	-
Slide-seq2	5	1	-	-	6
Visium	12	10	10	6	6

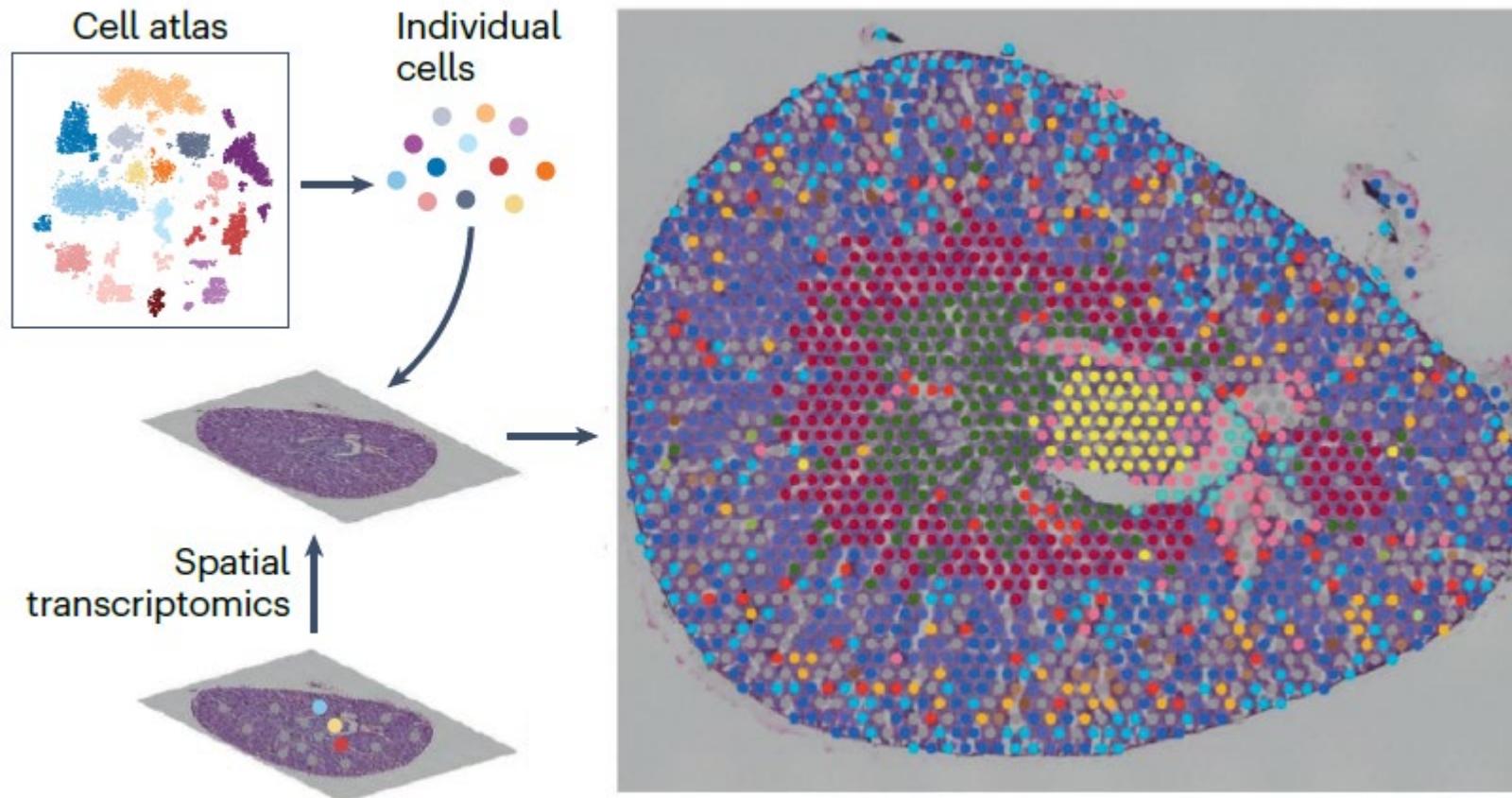


Defining Altered States in Injury



Label Transfer

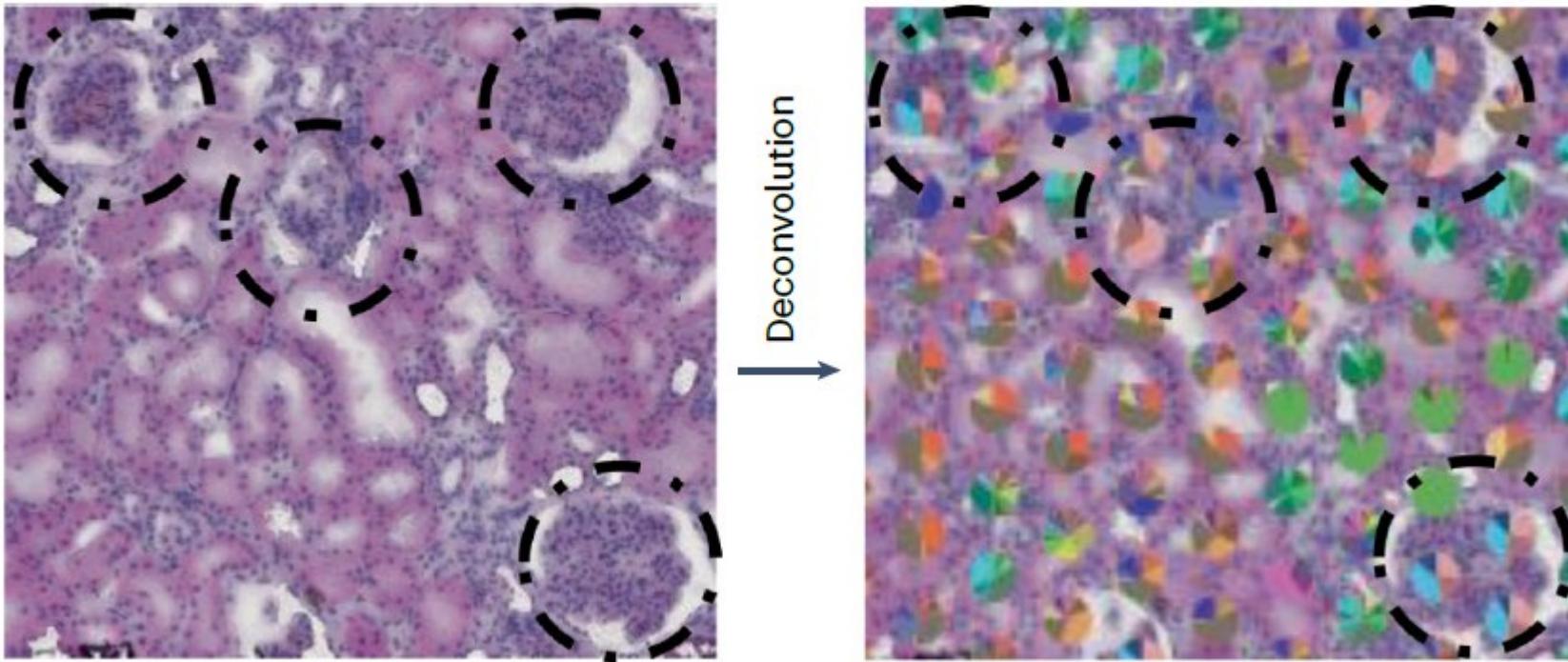
a Transfer of scRNA-seq cluster labels to ST cells or spots



Deconvolution

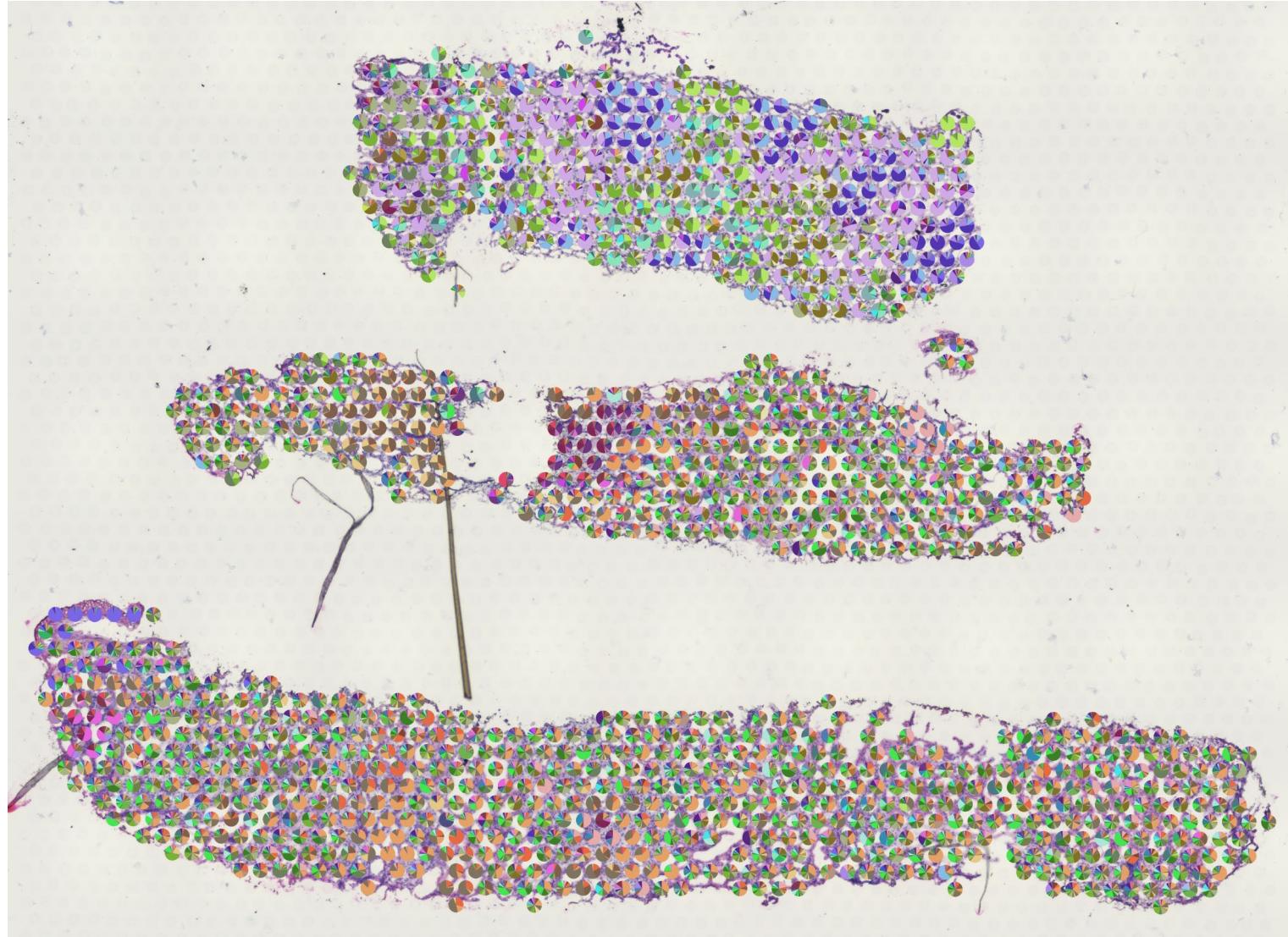
Useful when probes overly 2 or more cells

C Cell type deconvolution when indicated



Tips:

- There are an abundance of deconvolution methodologies
- Performance varies across tissue types
- Align your deconvolution with orthogonal validation (histology or protein)



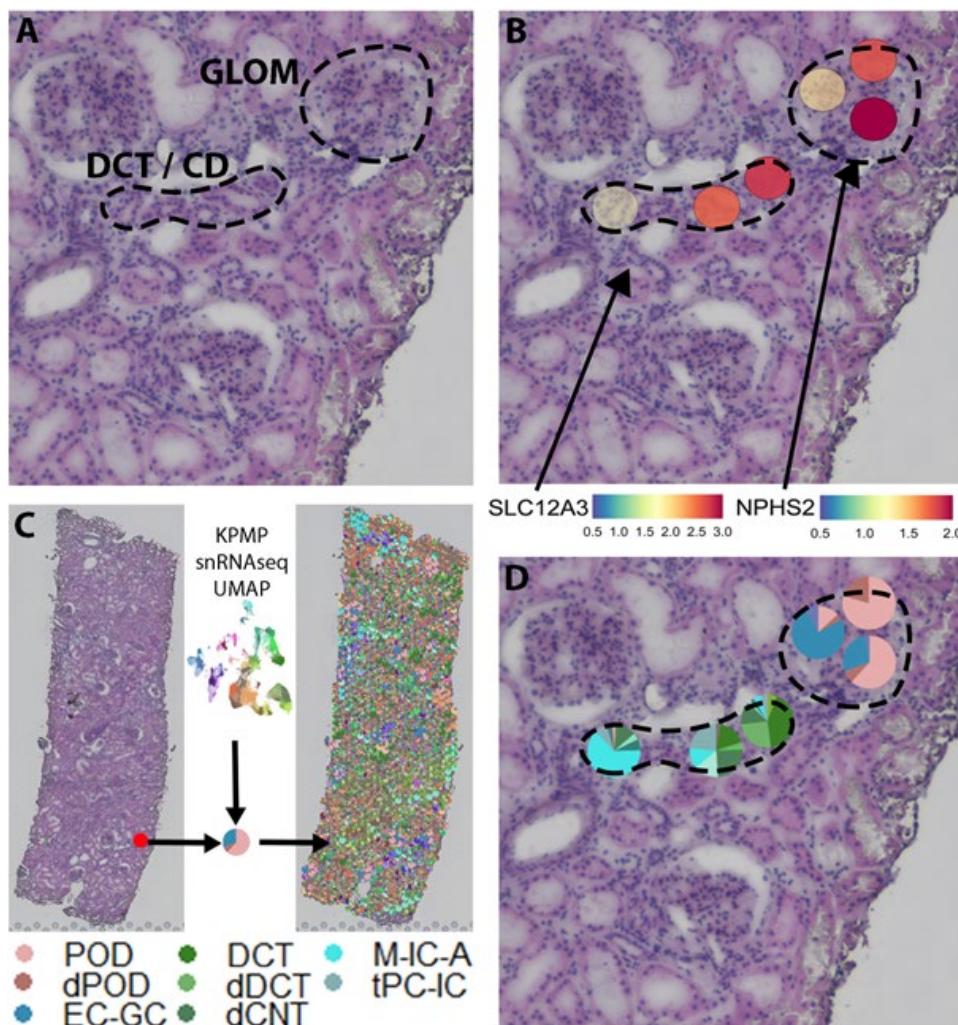
Subclass

POD	M-TAL	tPC-IC	cycMYOF
dPOD	dM-TAL	IC-B	FIB
PEC	C-TAL	PapE	M-FIB
PT-S1	dC-TAL	EC-GC	dM-FIB
PT-S2	MD	EC-AEA	aFIB
PT-S3	DCT	EC-DVR	dFIB
aPT	dDCT	EC-PTC	B
cycPT	cycDCT	dEC-PTC	PL
dPT	CNT	EC-AVR	T
dPT/DTL	dCNT	dEC	NKT
DTL2	C-PC	cycEC	MAST
DTL1	M-PC	EC-LYM	MAC-M2
DTL3	dM-PC	MC	MDC
dDTL3	IMCD	REN	cDC
ATL	dIMCD	VSMC	ncMON
dATL	C-IC-A	VSMC/P	N
aTAL1	dC-IC-A	dVSMC	SC/NEU
aTAL2	M-IC-A	MYOF	

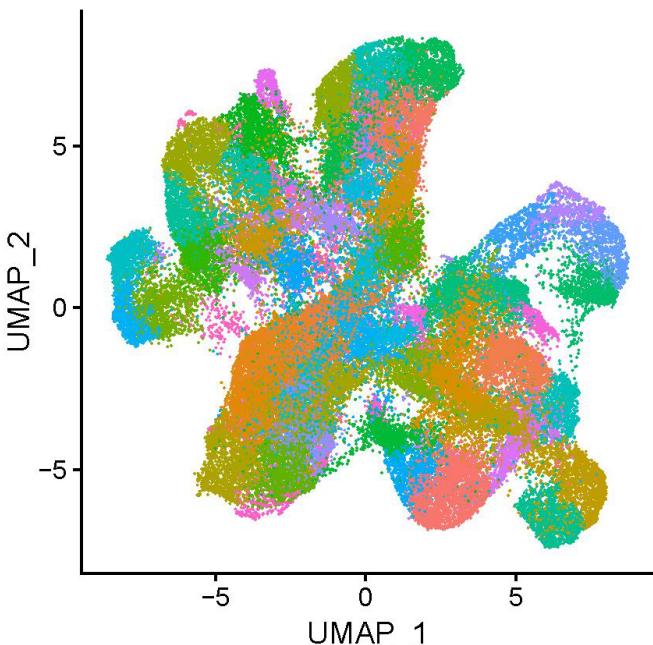
71 cell types mapped within tissue



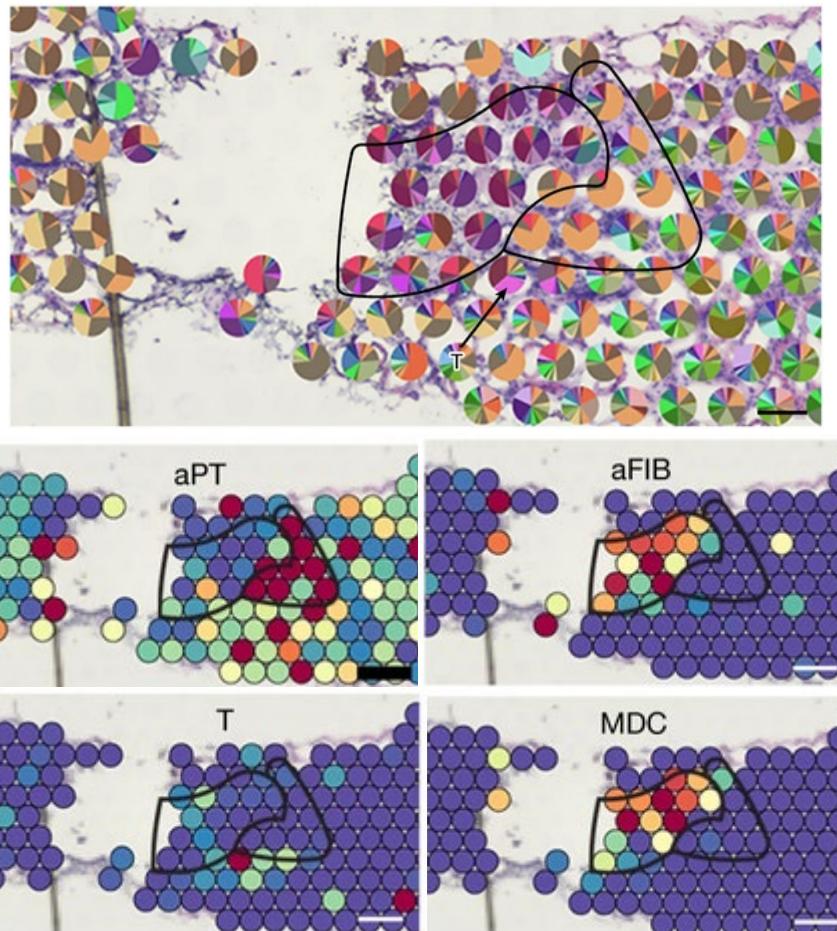
KPMP sc/snRNA-seq mapping



Map / deconvolute cell types from sn/scRNA-seq



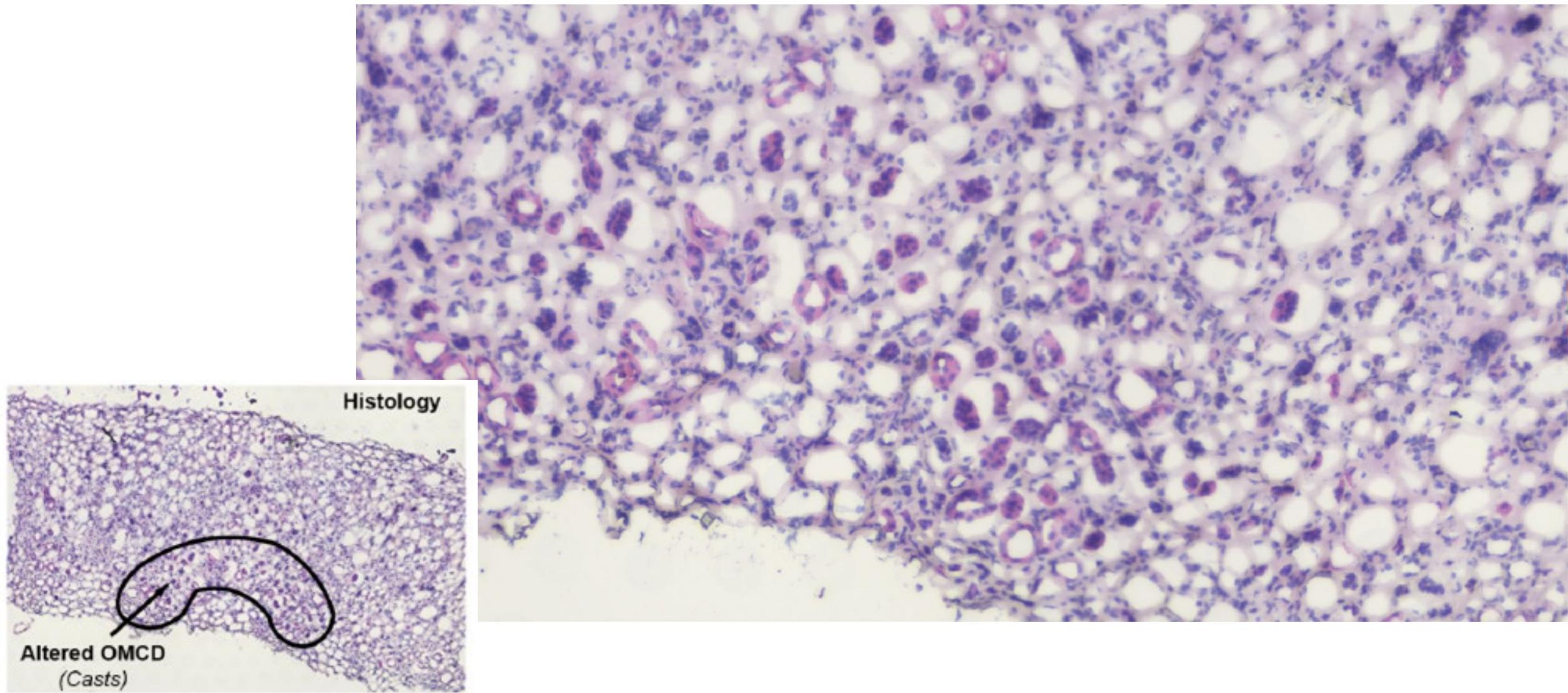
71 clusters from >70K spots in all samples based on cell type proportion



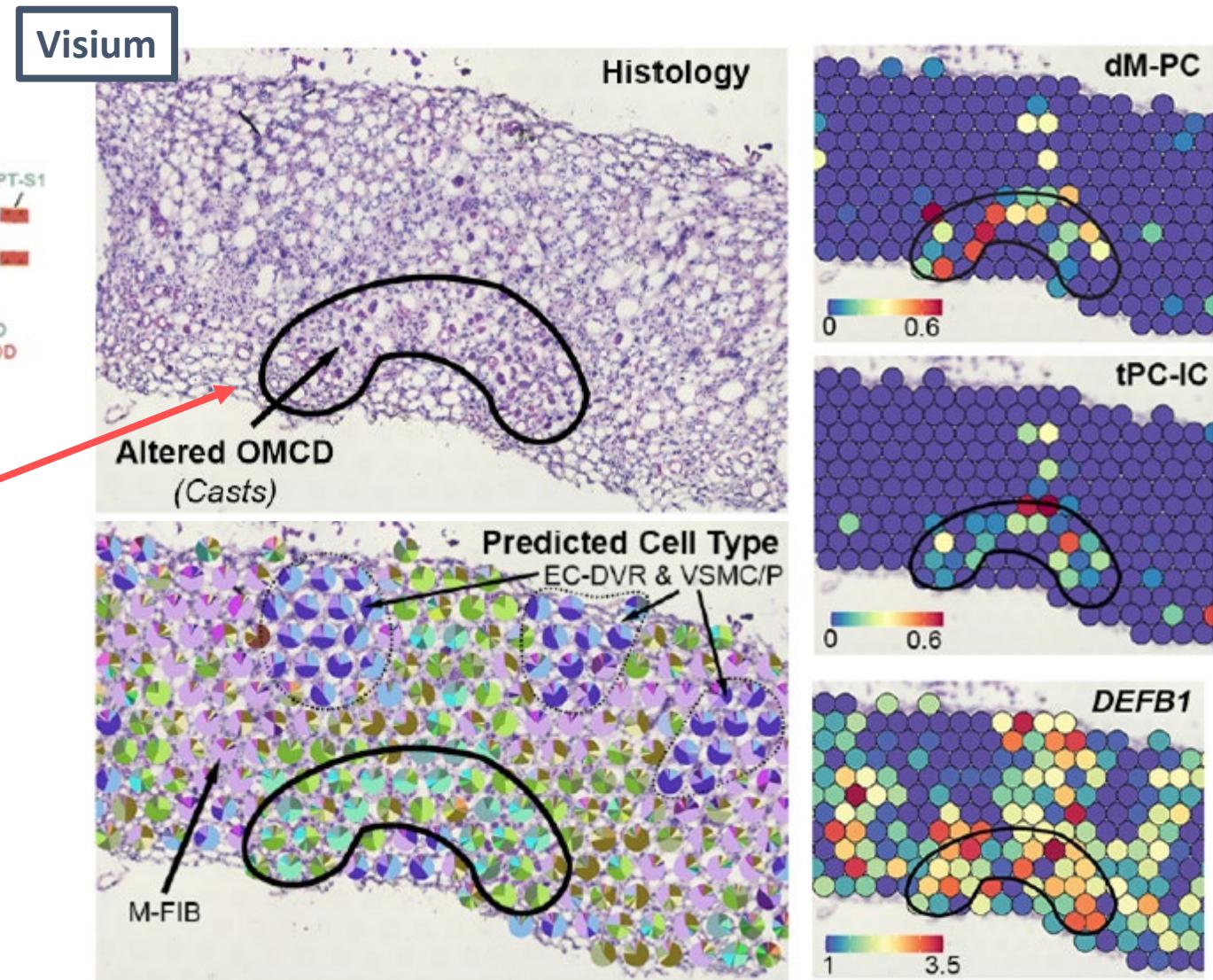
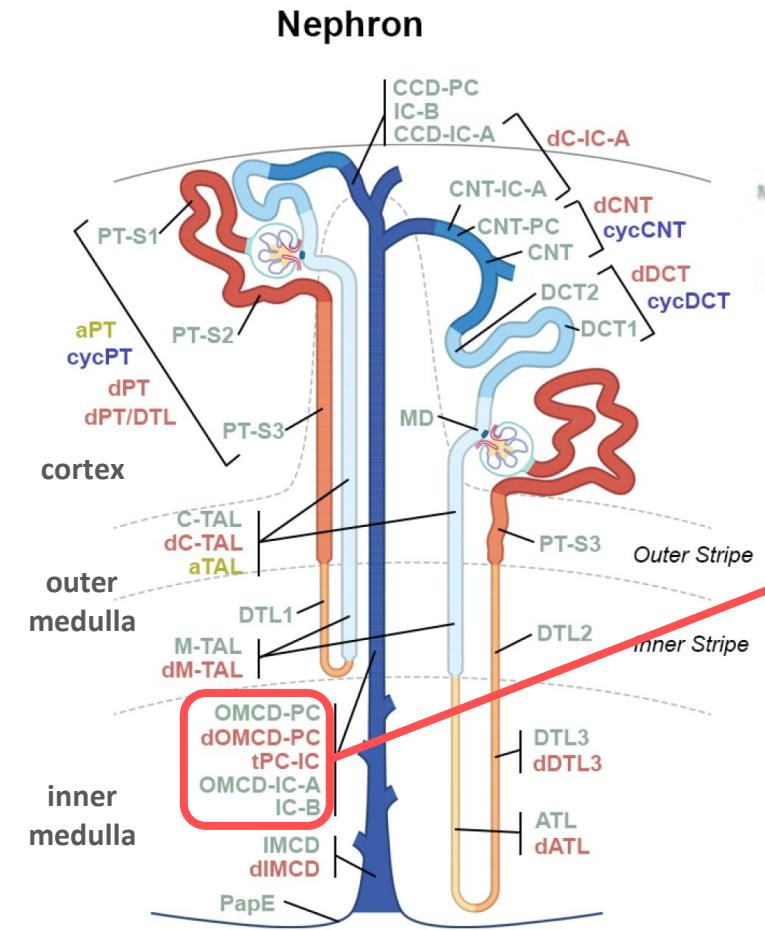
Neighborhoods of colocalizing injured epithelial, stromal and immune cells defined by Visium ST

- *Neighborhoods allow quantification / comparison of cell patterns across all biopsies*
- *Several genes in these neighborhoods are associated with decline in kidney function*

Region of casts in the medulla of a disease biopsy sample

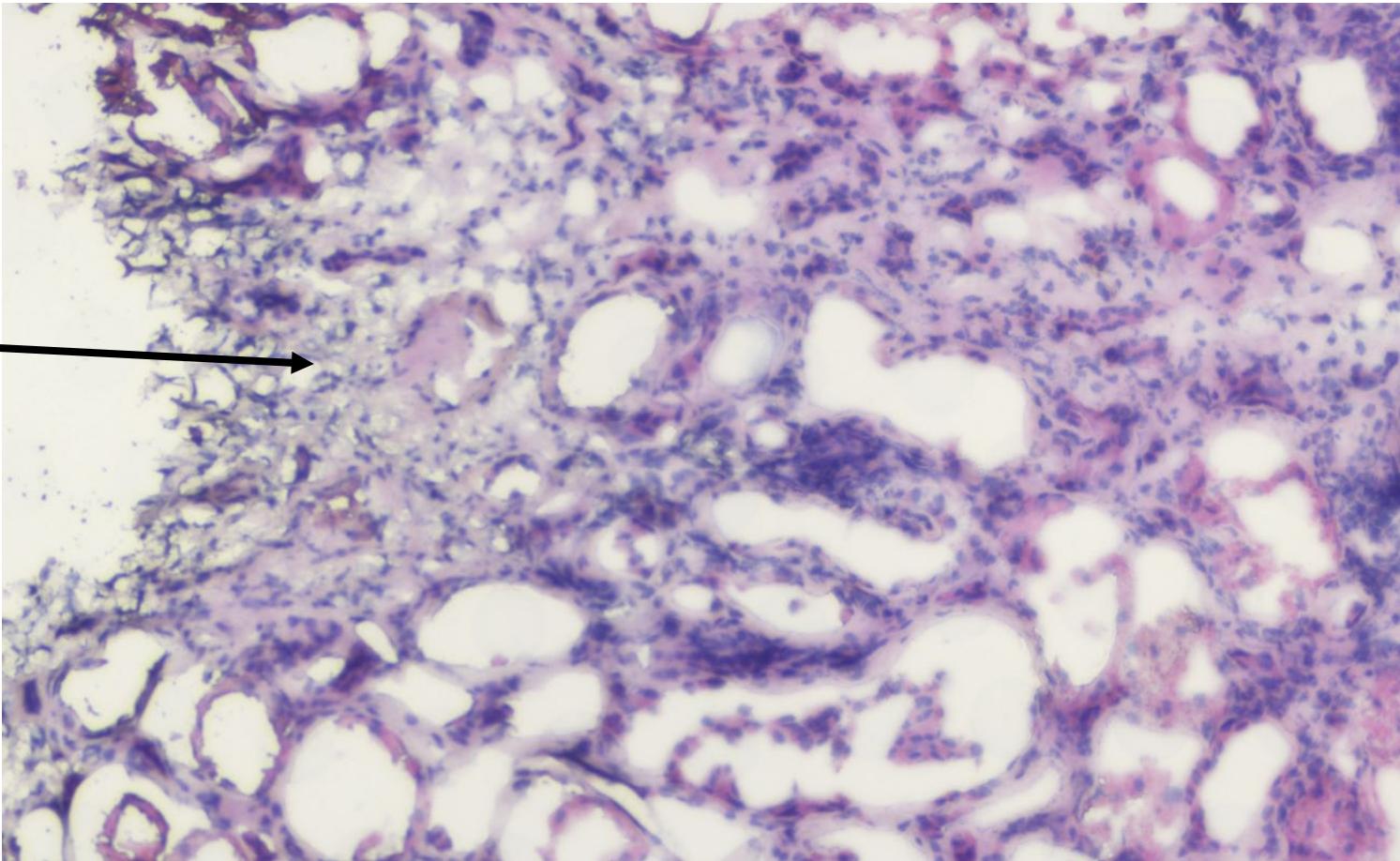


Degenerative State

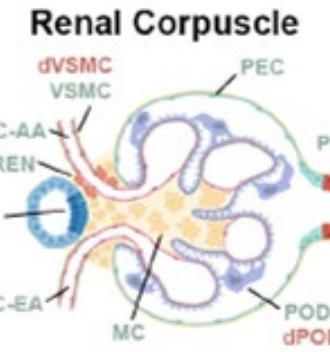
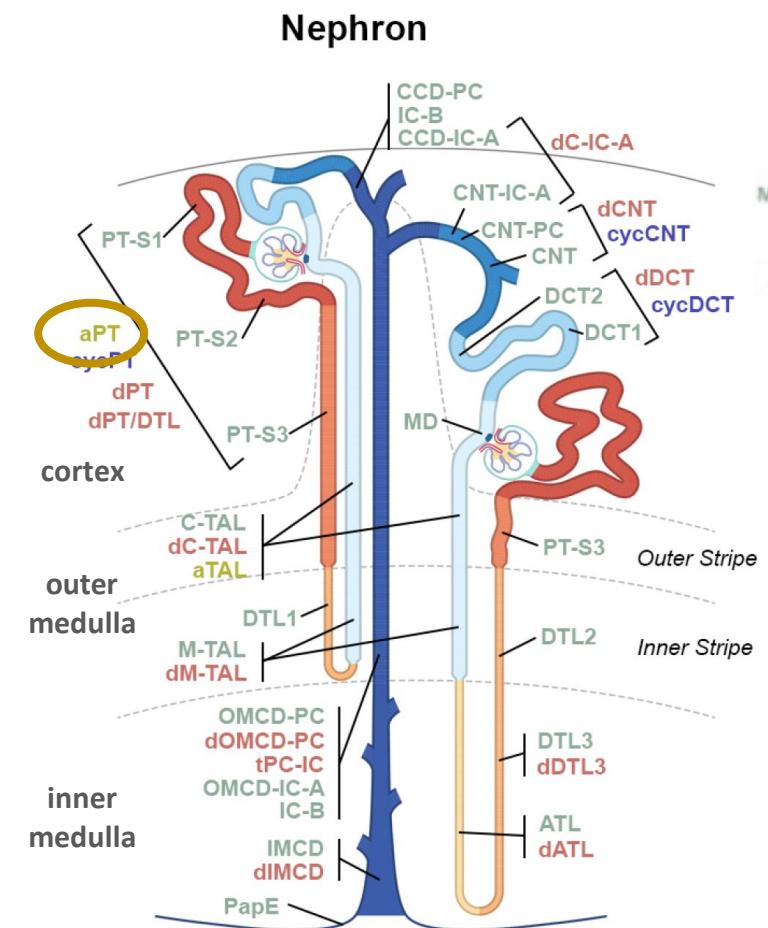


Fibrosis and injured tubules in the DKD sample

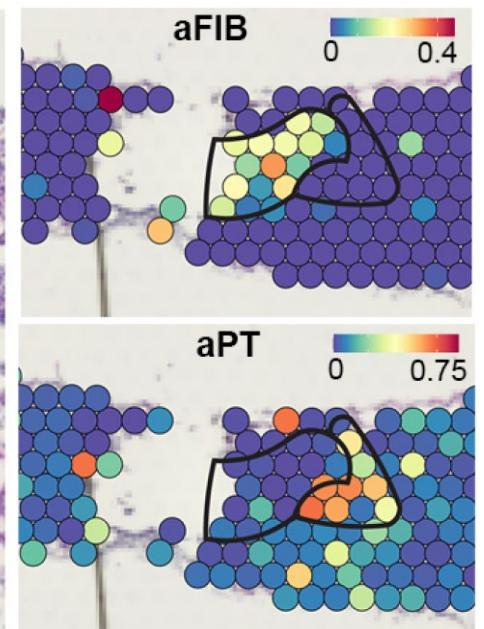
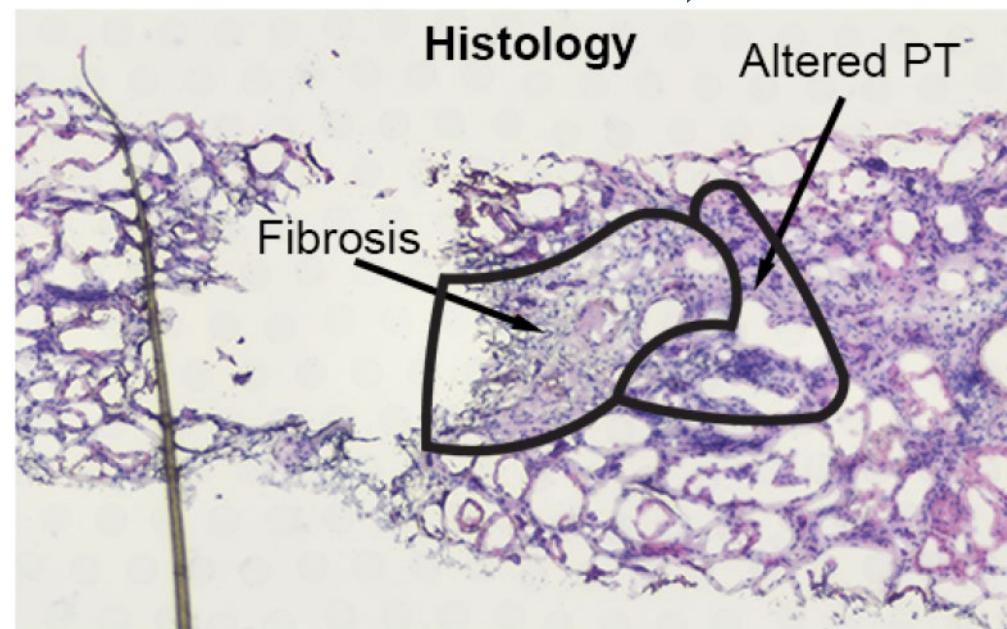
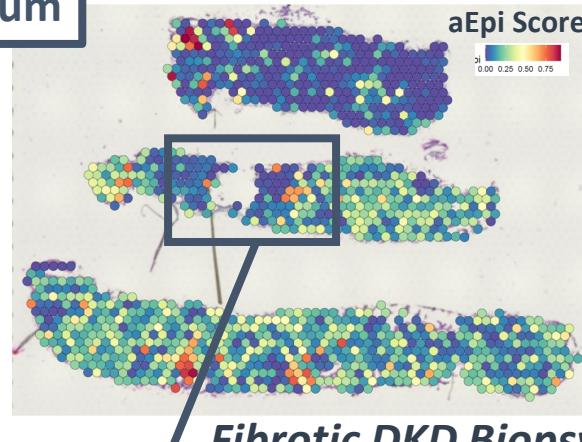
Fibrotic
Region



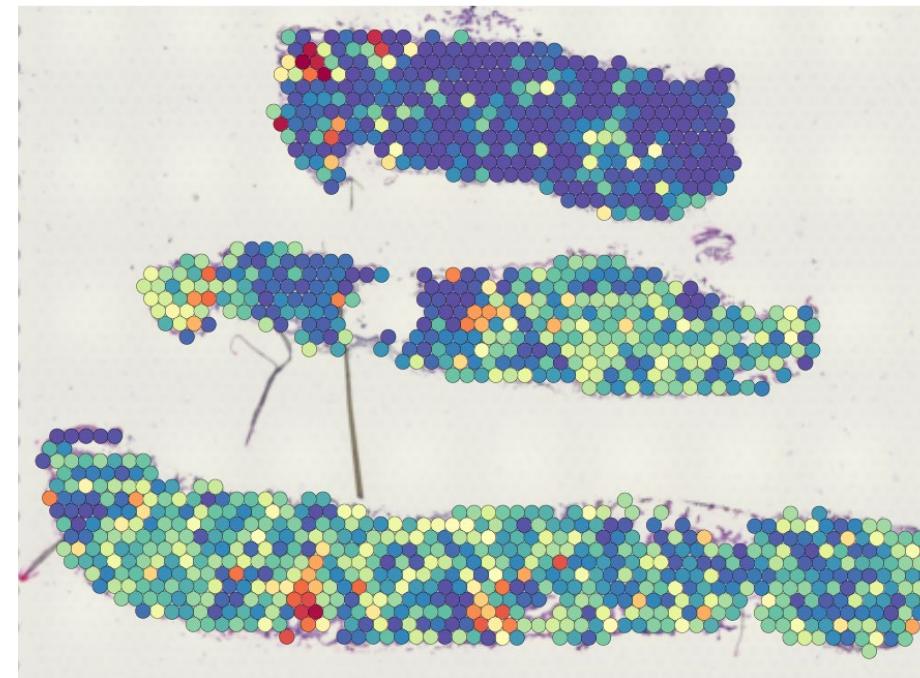
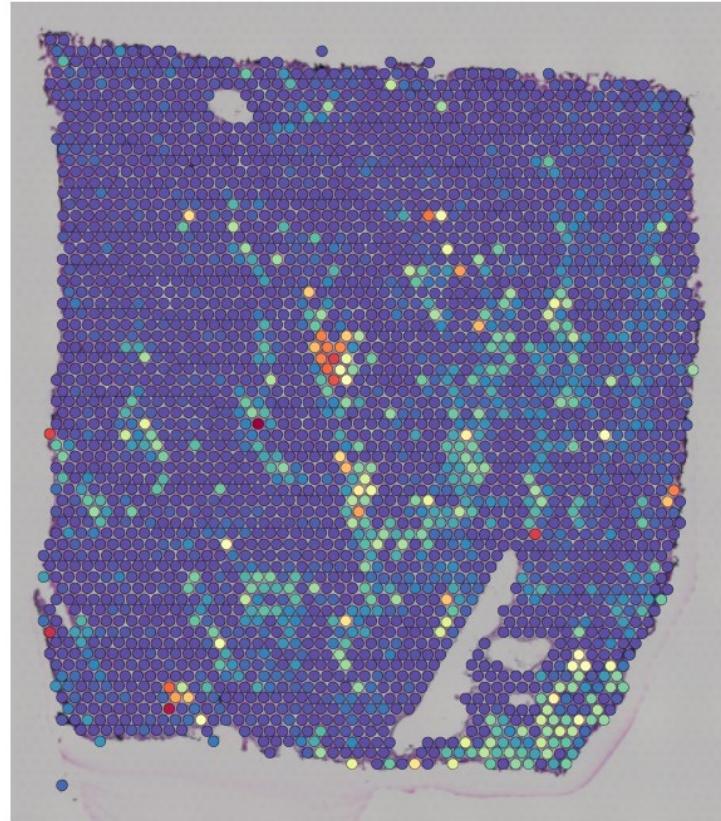
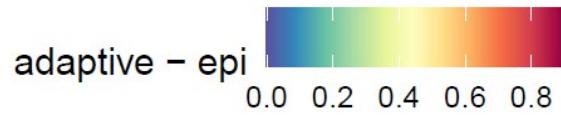
Adaptive Repair State



Visium

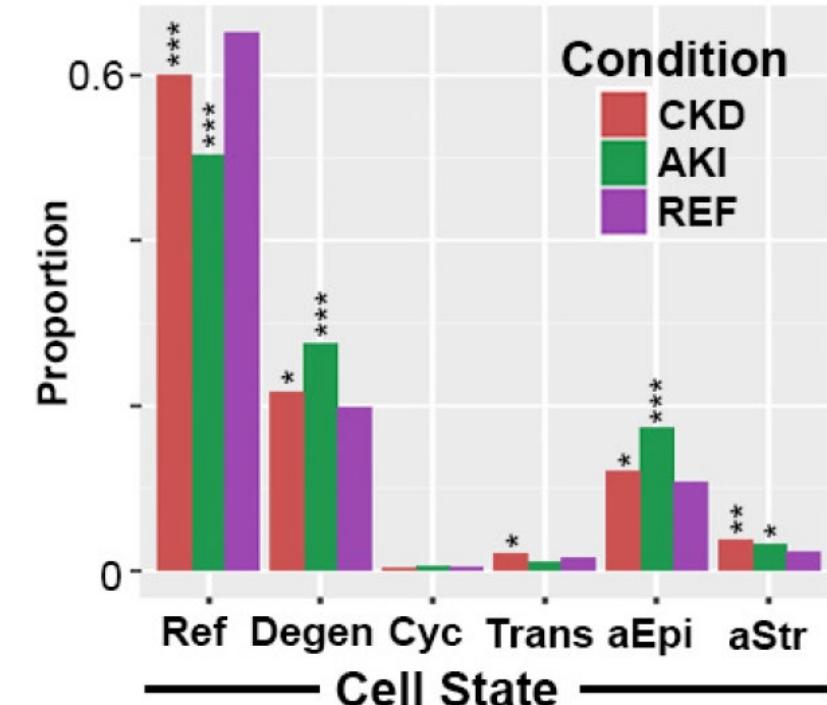


Cell State Mapping



Adaptive and degenerative cell states are more common in kidney disease samples (N = 23).

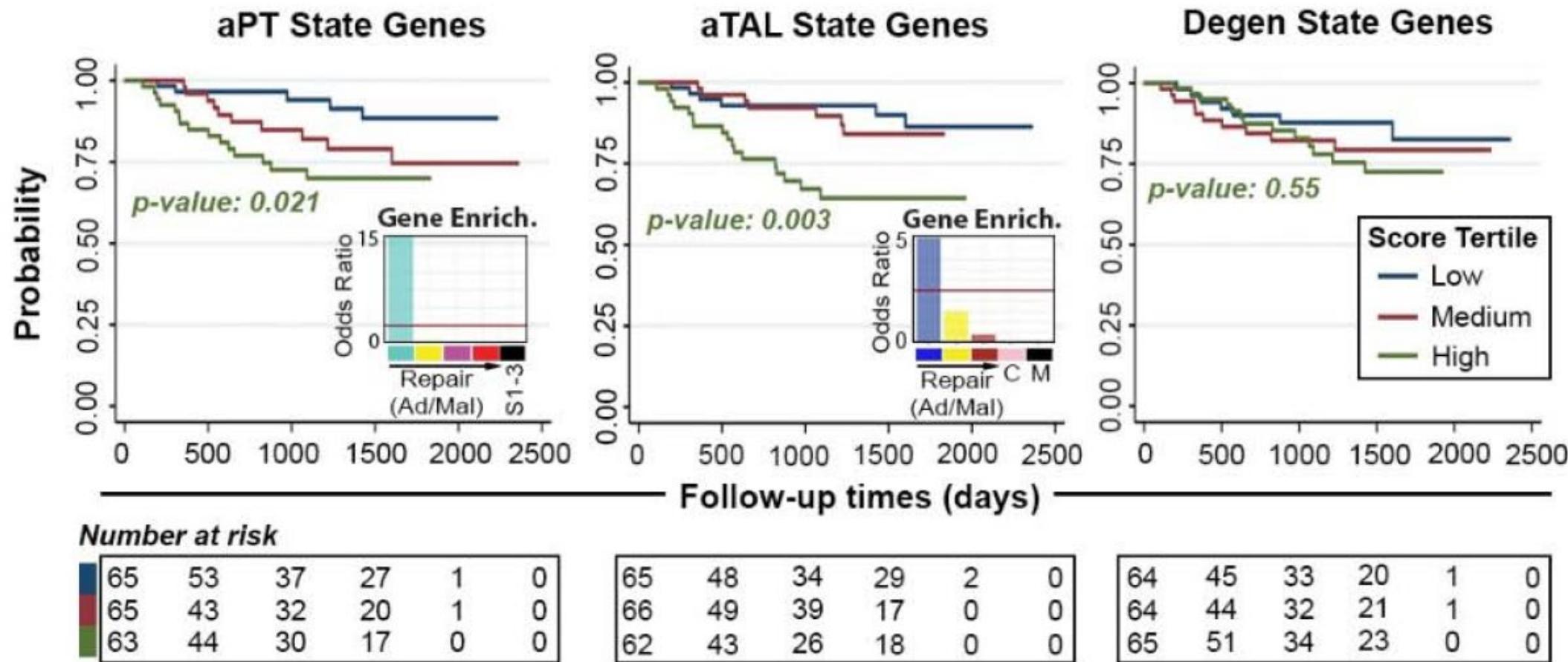
Visium Altered States



Outcomes

NEPTUNE Cohort Survival Curves

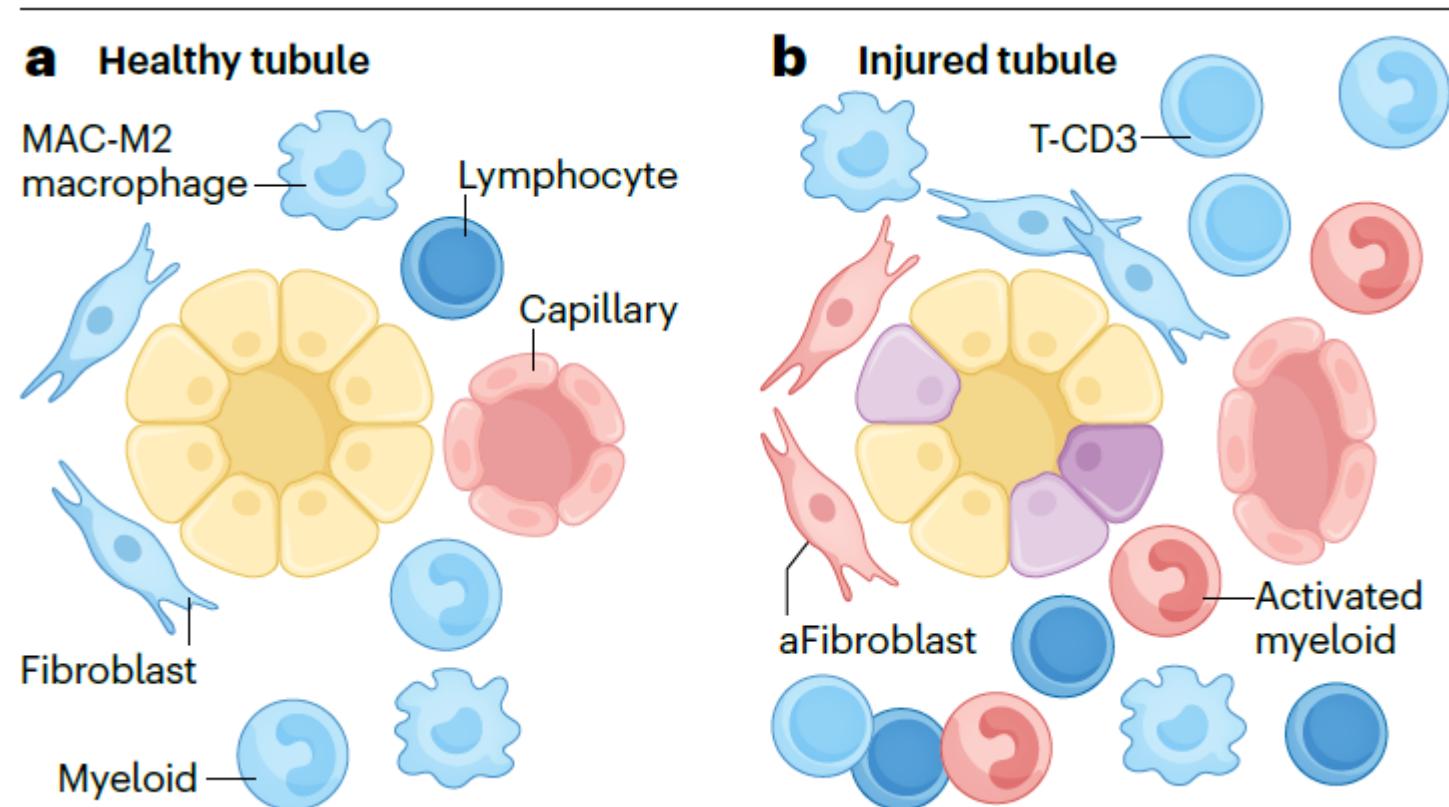
(Composite ESRD or 40% Decline in eGFR)



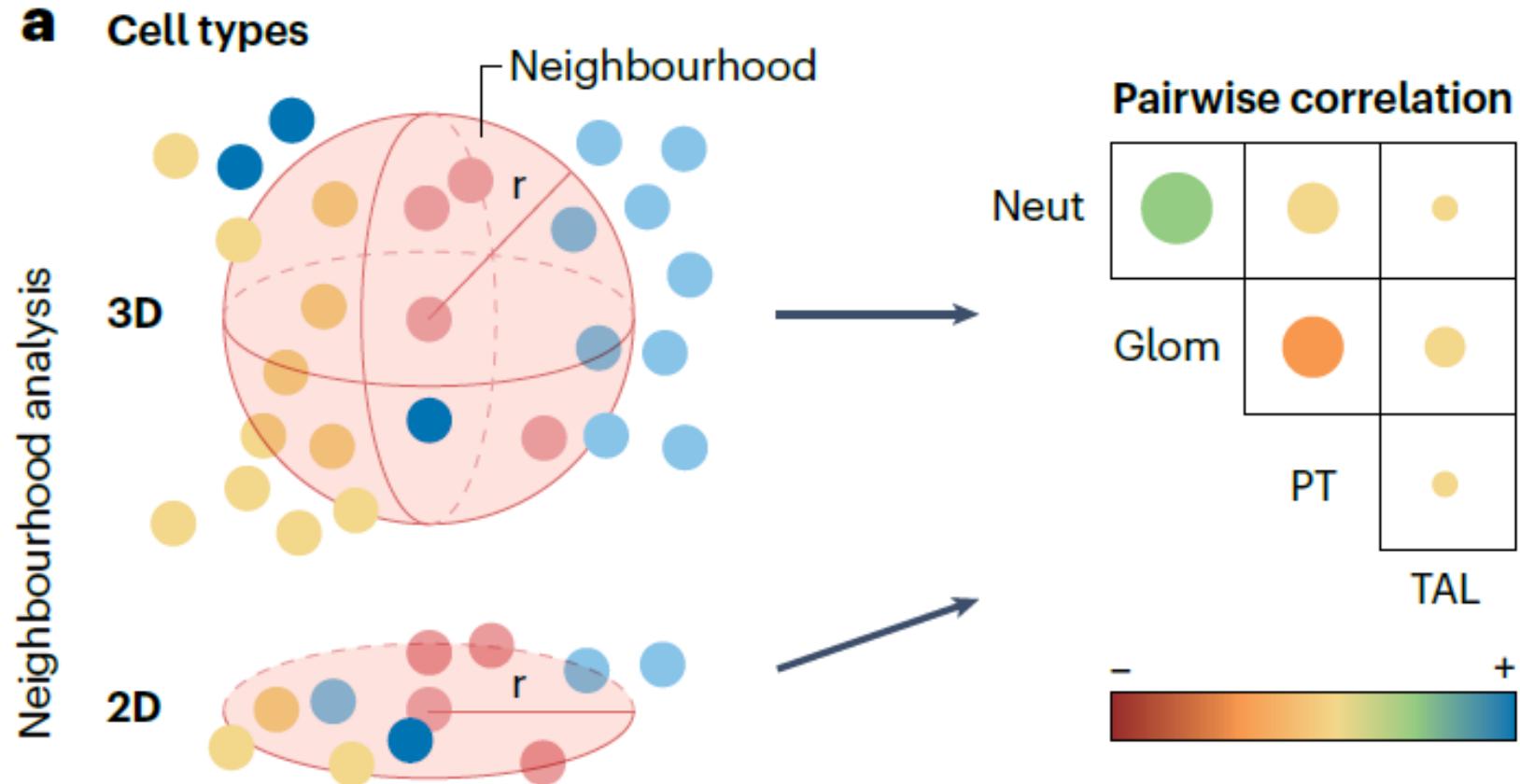
- In bulk kidney mRNASeq data of the NEPTUNE cohort, adaptive injury states associate with kidney disease progression.

Neighborhoods

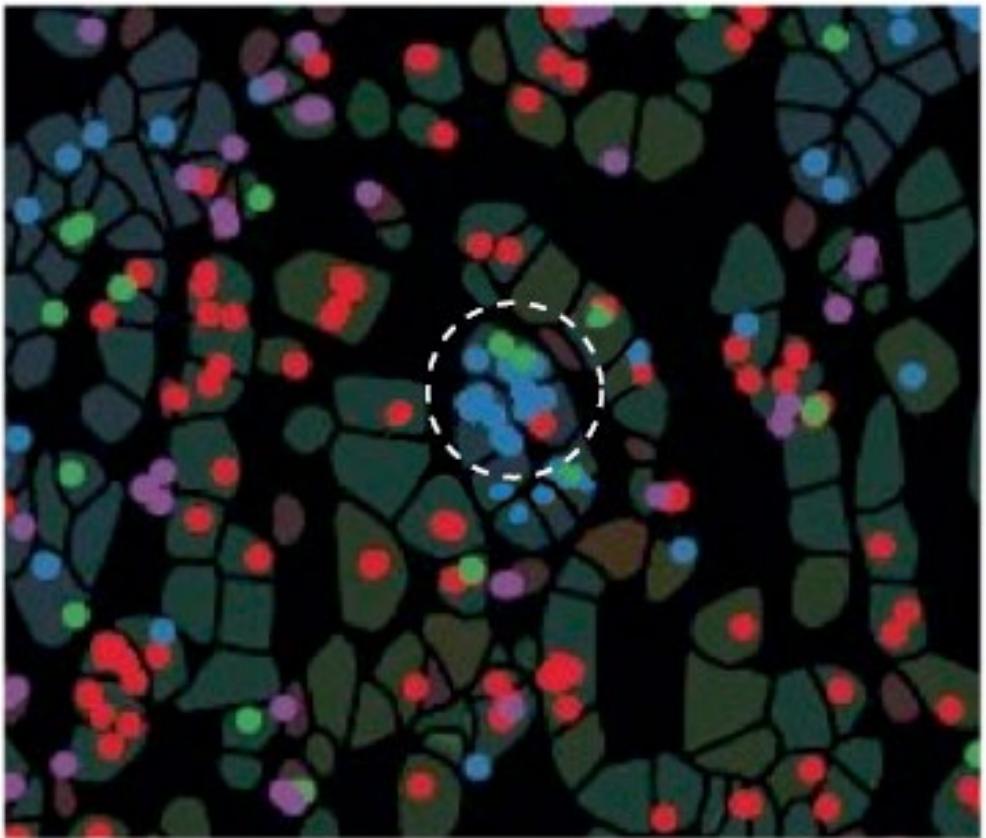
Neighborhood example – disease state



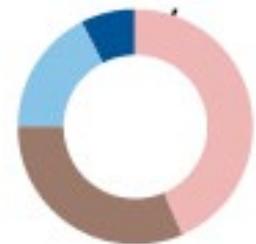
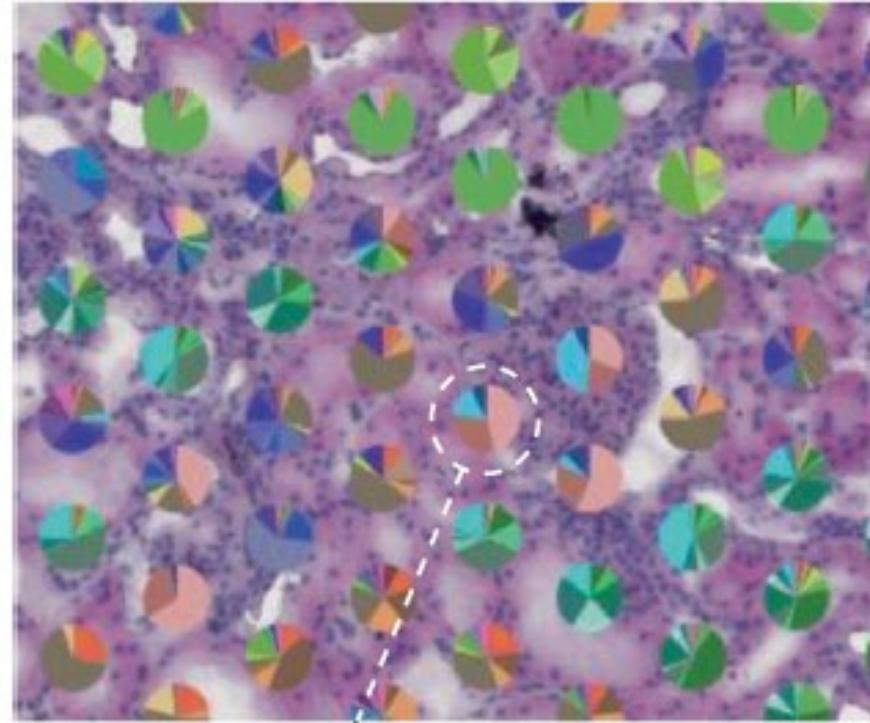
Neighborhood Analysis



Neighborhood Analysis



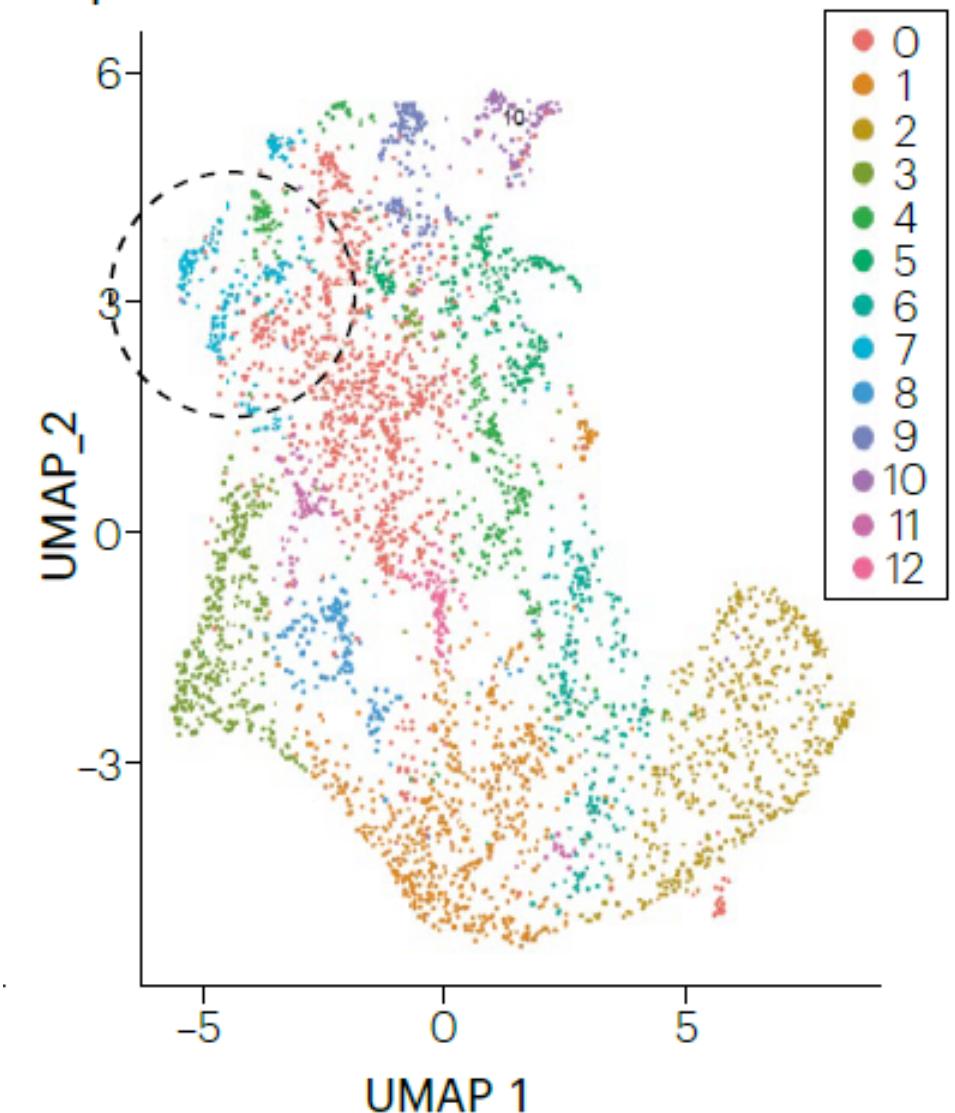
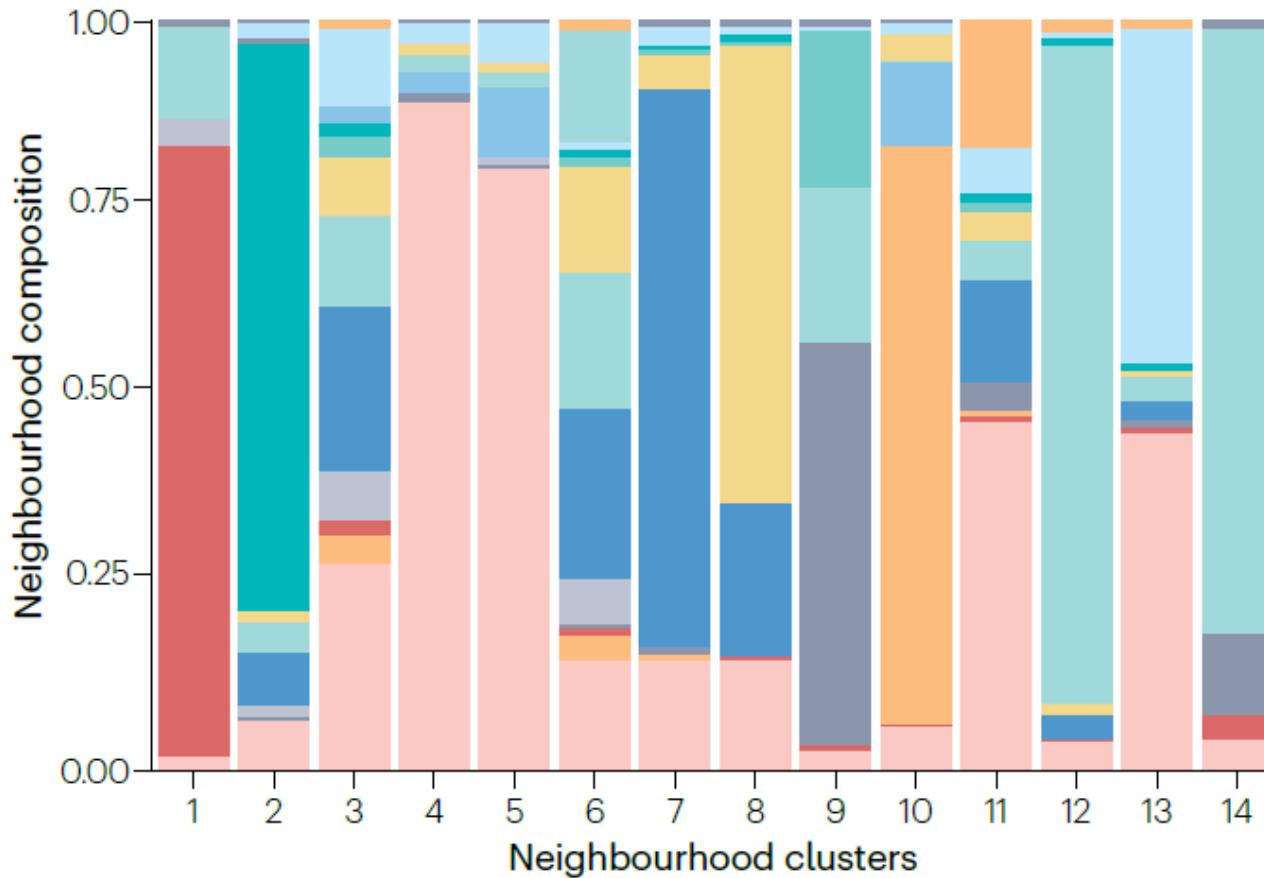
A neighbourhood defined
by a radius around a centroid



Components
of the spot
become the
neighbourhood

Neighborhood Components + Clustering

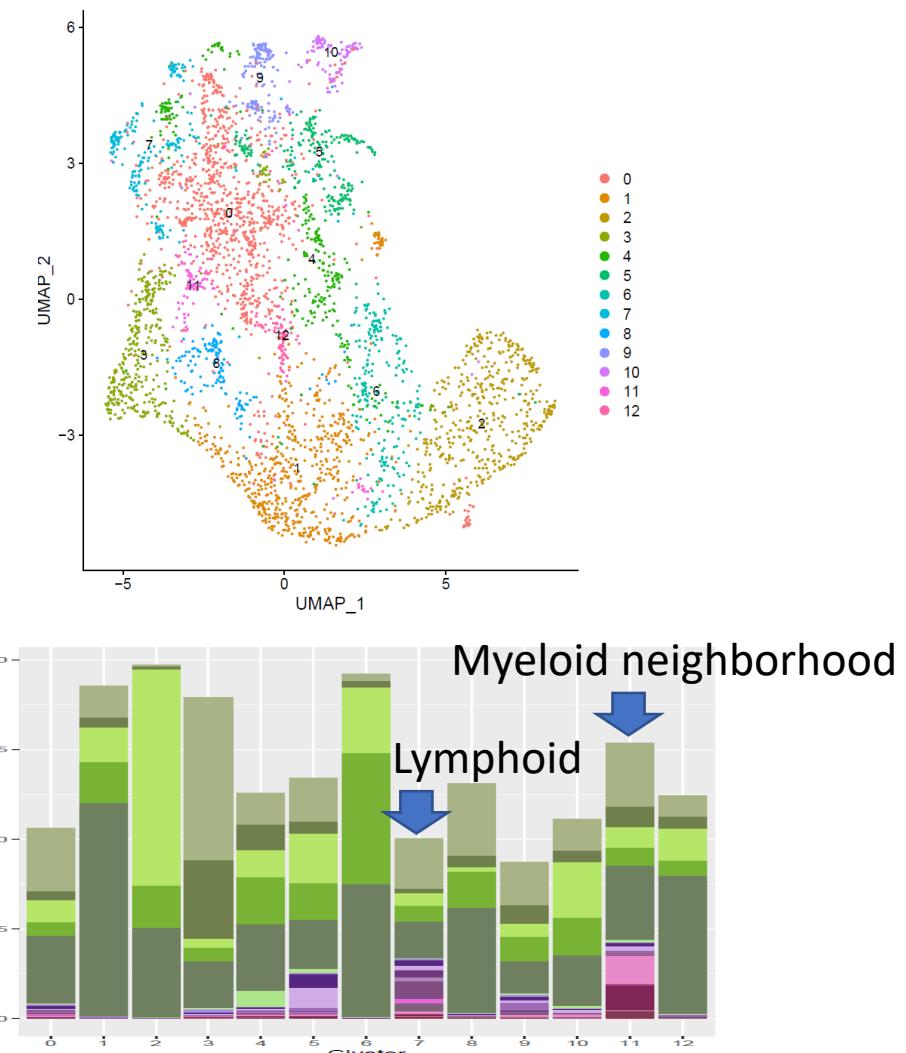
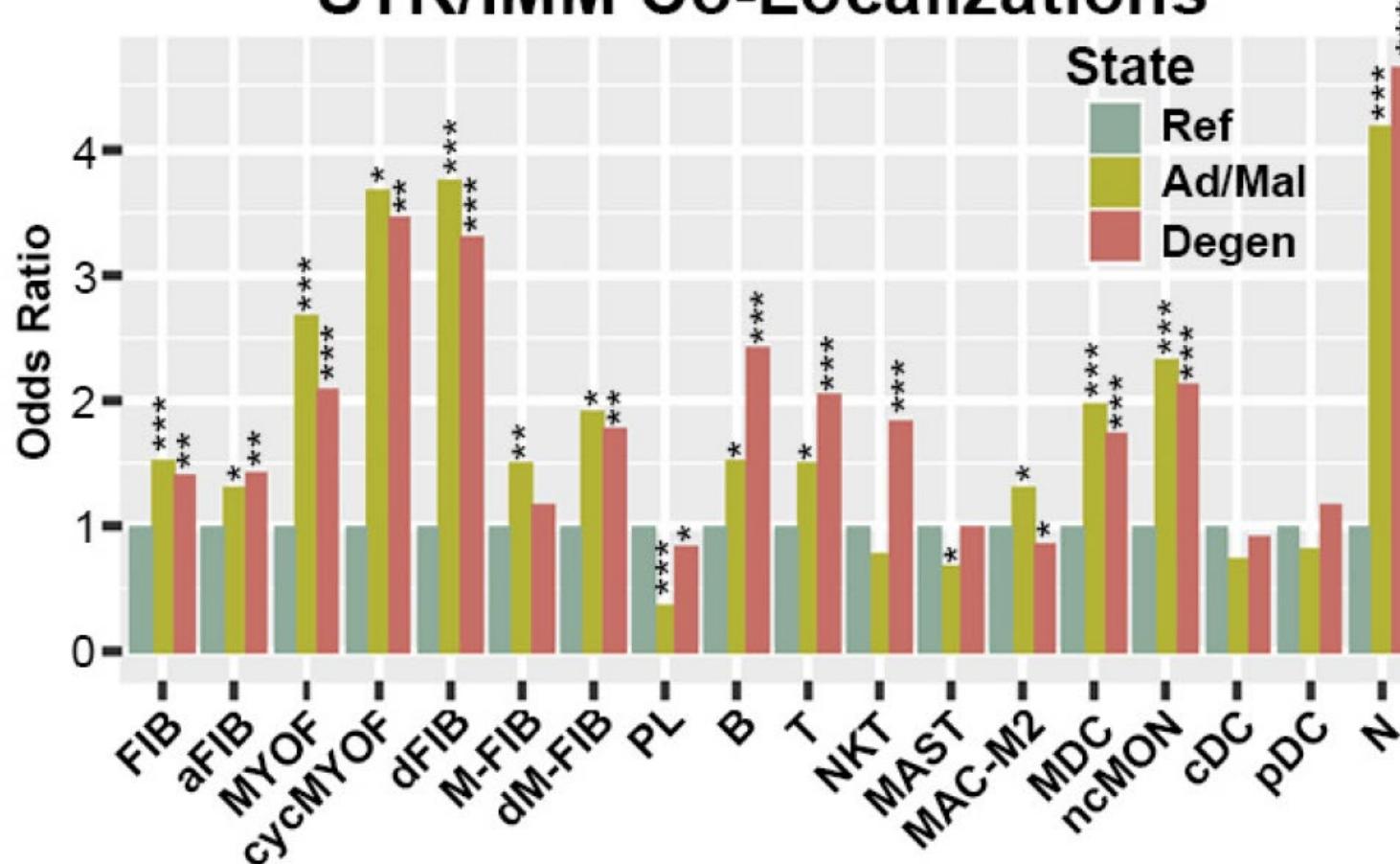
e Neighbourhood cluster composition



Epithelial cell co-localization with stromal and immune cells

C

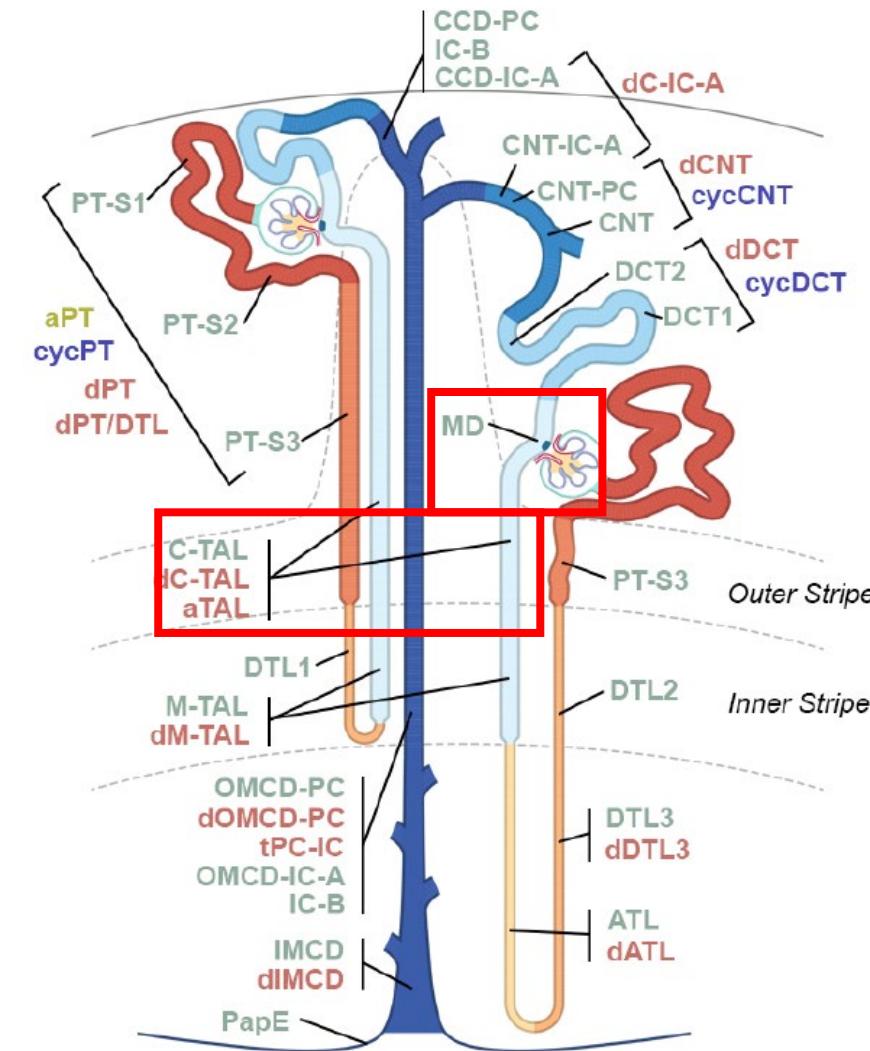
STR/IMM Co-Localizations



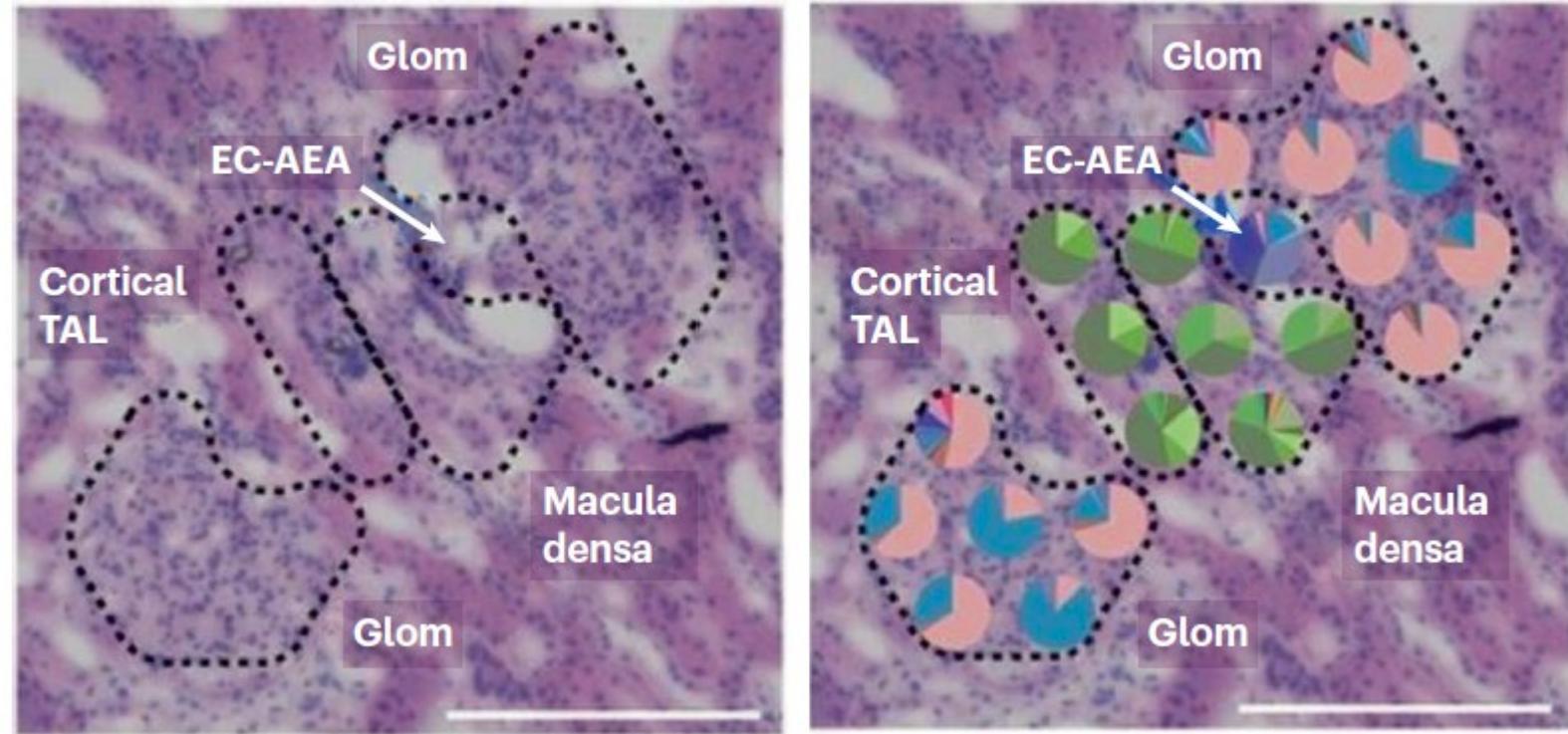
- ST reveals altered cell state signature colocalizes with immune cell infiltration. Each spot = a neighborhood.

Neighborhood example 2: Anatomic

Nephron



C



Spatial Variable Gene Analysis

Spatial Variable Gene Analysis

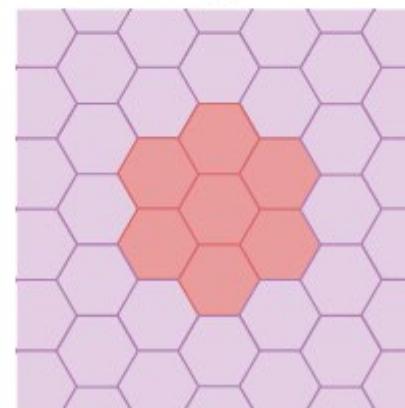
BSP: Useful for cross-cutting injury features like hypoxia

b Determination of spatially variable genes

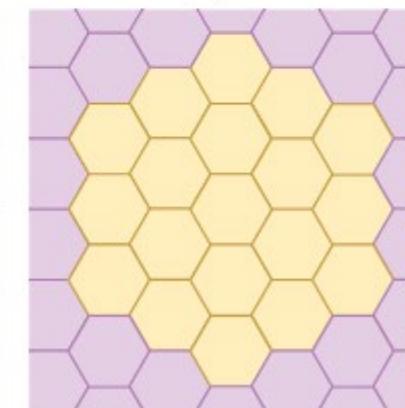


Focal region of injury with spatially variable expression determined by two sets of radii

Small patch



Big patch

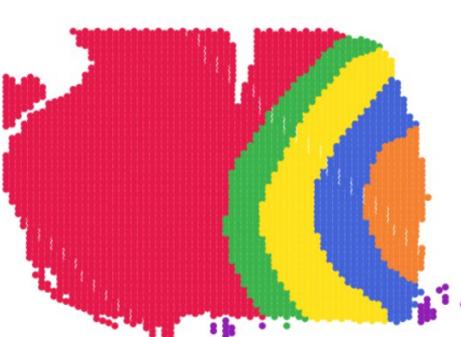
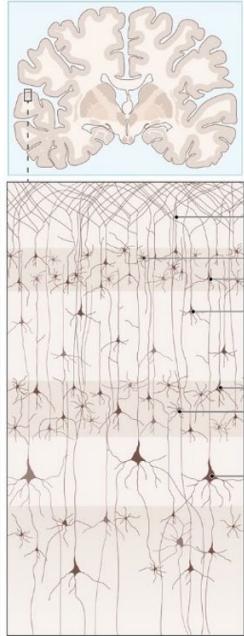


Juexin Wang

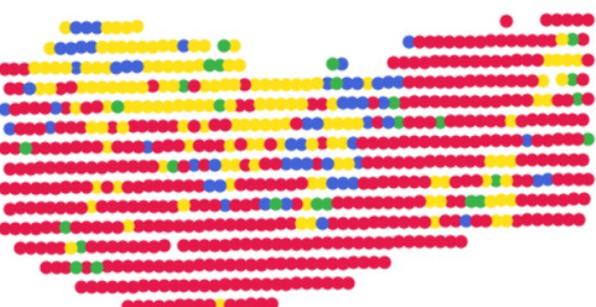
Relation Equivariant Graph Neural Networks

Brain

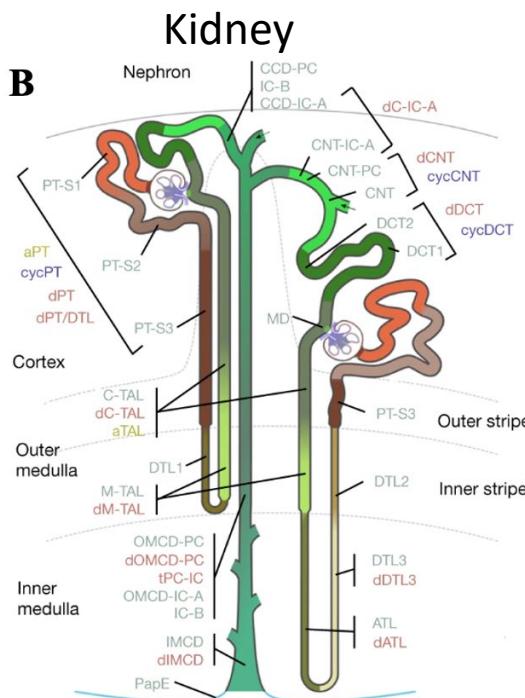
A



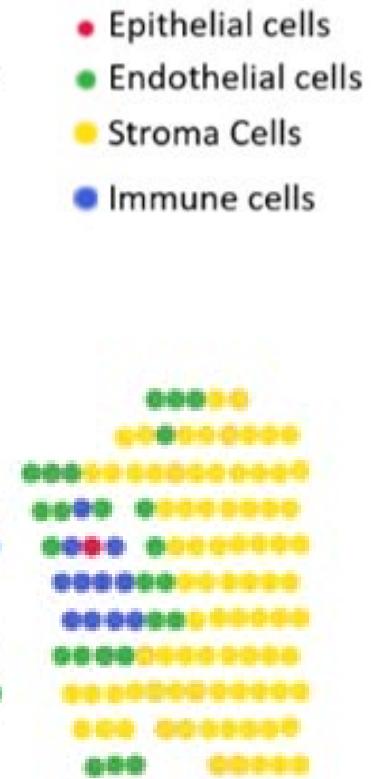
Large homogenous structures



Mosaic, stochastic structures

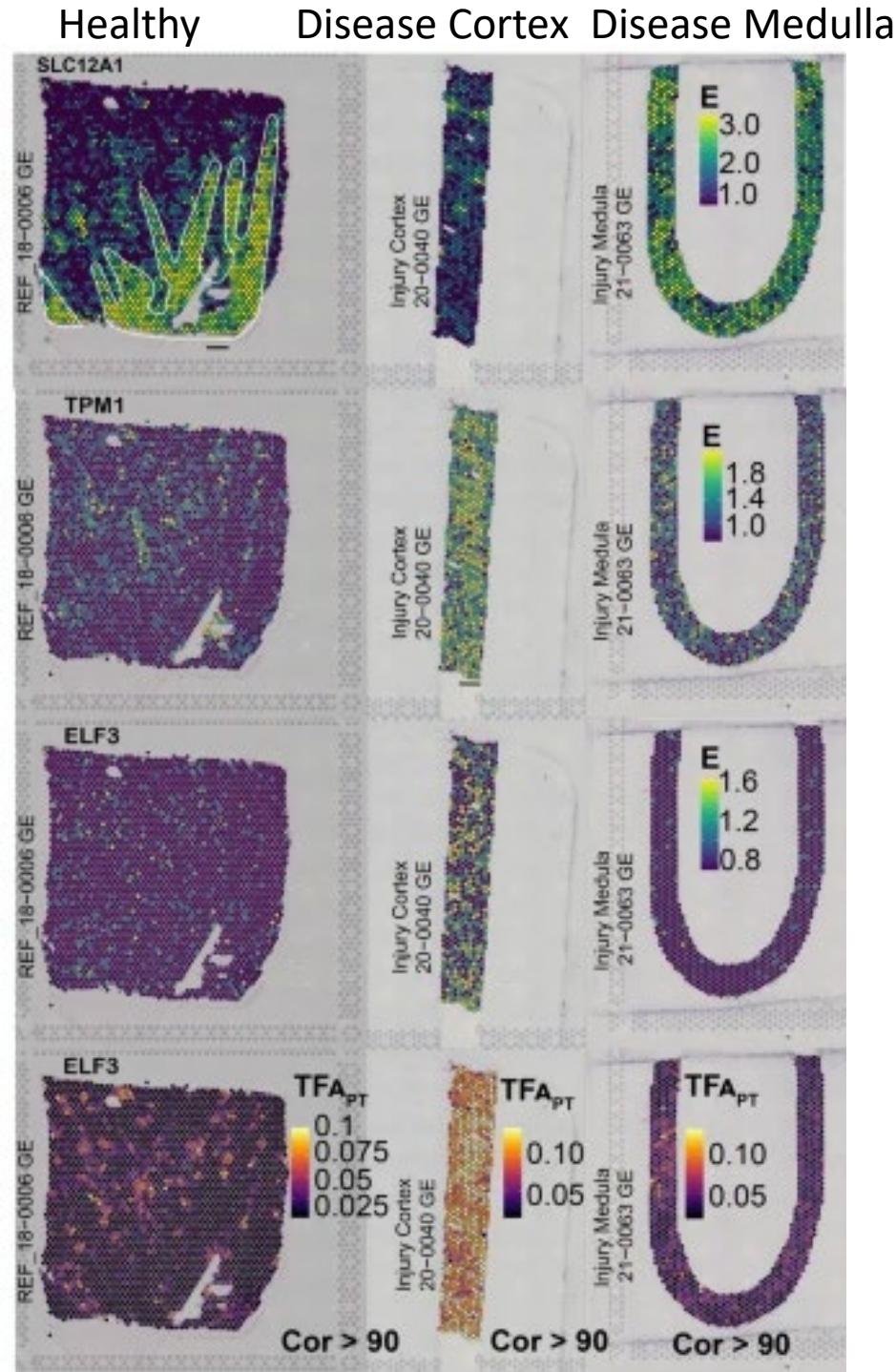
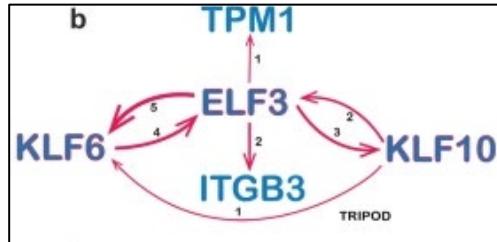
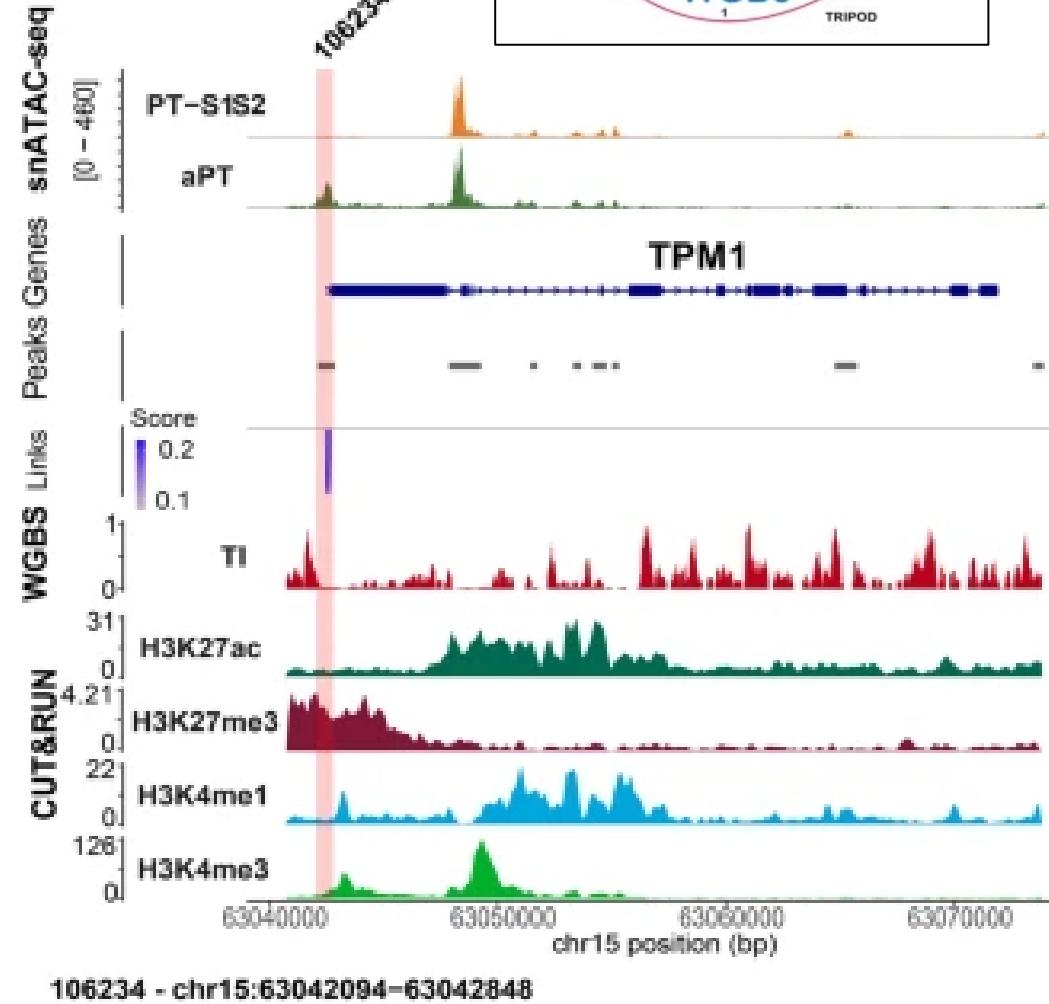


REGNN
ARI = 0.58



TF Network Mapping

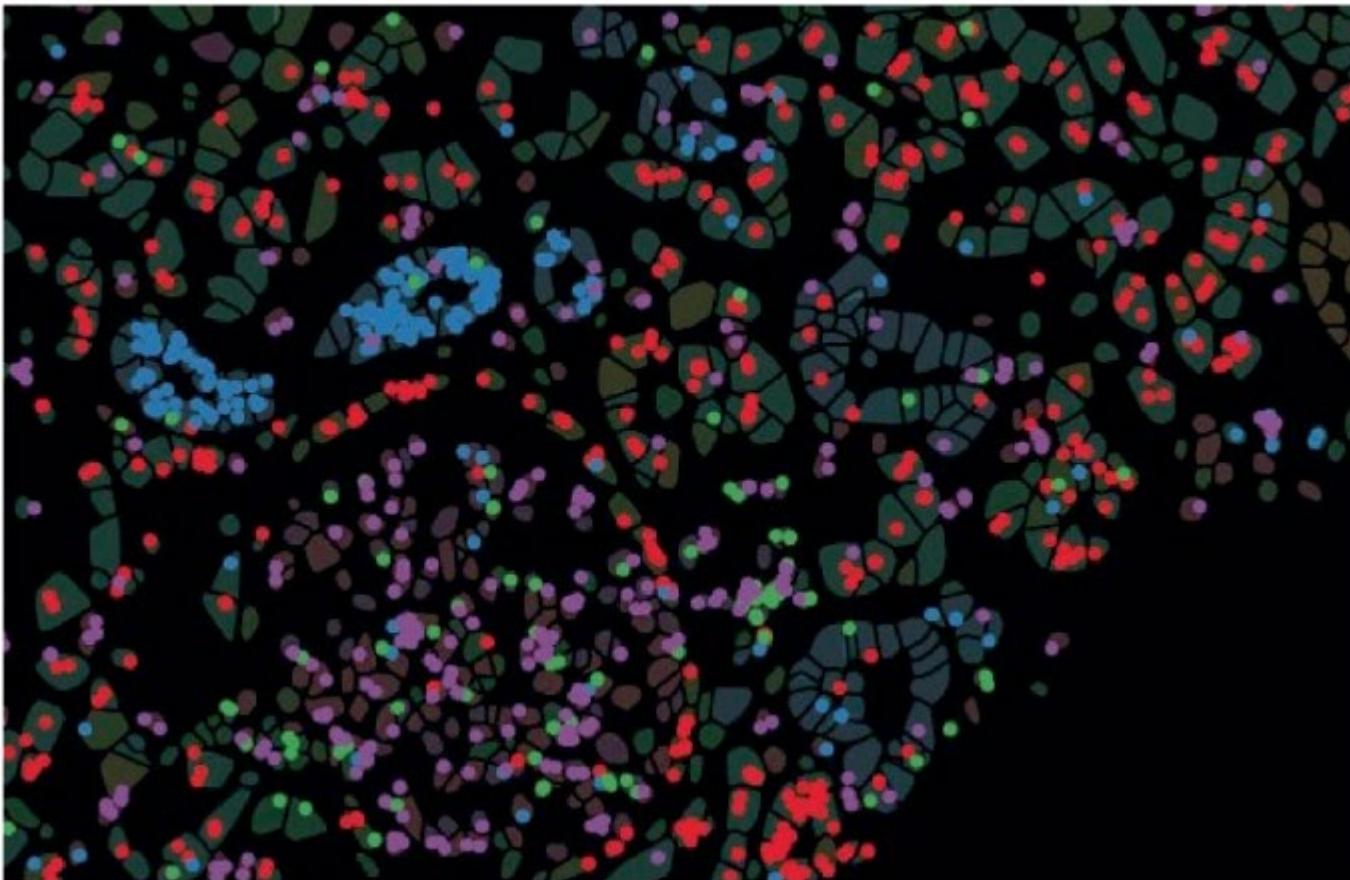
Gene: TPM1 TF: ELF3



Debora Gisch

Segmentation

d Segmentation of single cells



Tips:

- Cellpose is a leading approach
- Proprietary segmentation available in 10x platforms
- Use of cell membrane and cytoplasm markers improve accuracy.

Histologic Annotation with AI

Histologic Annotation using AI

- <https://github.com/spborder/FUSION/blob/main/README.md>
- <http://fusion.hubmapconsortium.org/>

The screenshot shows the FUSION web application. At the top, there is a dark header bar with the FUSION logo, the text "FUNCTIONAL UNIT STATE IDENTIFICATION AND NAVIGATION WITH WSI", and four buttons: "User Survey", "Cell Cards", "Lab Website", and "User Feedback". Below the header, there is a navigation bar with three buttons: "MENU", "REGISTERED USER LOGIN", and "SIGN UP FOR USABILITY STUDY". A welcome message "Welcome: fusionguest" is displayed. The main content area features a large "Welcome to FUSION" heading, a small illustration of a microscope, and a call to action "Or, Select a topic below to view more information". On the left, there is a "Getting Started" section with a link to "FUSION INTRODUCTION" and a list of topics: "FUSION Introduction" and "Preprocessing Steps". On the right, there is a sidebar with a portrait of a man, Samuel Border, and text identifying him as a "PH.D. STUDENT, BIOMEDICAL ENGINEERING" and his research as "Computational Pathology".

FUSION
FUNCTIONAL UNIT STATE IDENTIFICATION AND NAVIGATION WITH WSI

User Survey Cell Cards Lab Website User Feedback

MENU REGISTERED USER LOGIN SIGN UP FOR USABILITY STUDY

Welcome: fusionguest

Welcome to FUSION

Or, Select a topic below to view more information

Click the Menu button above to see other pages

Getting Started: Select a category below to view tutorial slides

FUSION INTRODUCTION

- FUSION Introduction
- Preprocessing Steps

UF College of Medicine UNIVERSITY OF FLORIDA

UF Intelligent Critical Care Center UNIVERSITY OF FLORIDA

FUSION

PH.D. STUDENT, BIOMEDICAL ENGINEERING

Samuel Border

Research: Computational Pathology

**FUSION**

FUNCTIONAL UNIT STATE IDENTIFICATION AND NAVIGATION WITH WSI

[User Survey](#)[Cell Cards](#)[Lab Website](#)[User Feedback](#)

A

Manual Annotation Control

Heatmap Overlays Colorbar

Whole Slide Image Viewer

Tools

Overlays Cell Compositions Cell Graphics Morphological Clustering

download data

use this tab for controlling properties of FTU & spot overlays

SELECT CELL FOR OVERLaid HEATMAP VIEWING

Main_CellTypes: >>> Prostinal Tissue

Select A Cell Type Value

EXPERIMENT SUBTYPE

Select... ADD SUB TYPE

Adjust Transparency of Heatmap

FTU Filter by Overlay Value

FTU Boundary Color Picker

Cortical Intraductal Medullary Intraductal Glomeruli
Sclerotic Glandular Tubules Arteries and Arterioles Spots
Squamous Histology Activate Windows

B

Inputs:
- Whole Slide Image (WSI)
- Omics data

WSI → FTU Segmentation → Pathomic Feature Extraction → DSA → Spatial Aggregation → User Interaction → ...

Omics → Spatial Data Extraction → Pathomic Feature Extraction → DSA → Spatial Aggregation → User Interaction → ...

Key

- Plugin
- Process
- Server
- Frontend

User Annotations

Detailed description: The top section, labeled 'A', shows a screenshot of a digital pathology software interface. It displays a whole slide image of tissue with various cell types highlighted in different colors (red, blue, green, yellow). A yellow heatmap overlay is applied to a specific region, and a color bar at the bottom indicates values from 0.00 to 1.00. To the right of the image are several toolbars and dropdown menus for 'Tools' like 'Overlays', 'Cell Compositions', and 'Morphological Clustering'. Below these are sections for 'download data', 'SELECT CELL FOR OVERLaid HEATMAP VIEWING', 'EXPERIMENT SUBTYPE', 'Adjust Transparency of Heatmap', 'FTU Filter by Overlay Value', 'FTU Boundary Color Picker', and a color palette. The bottom section, labeled 'B', is a flowchart illustrating the Fusion pipeline. It starts with 'Inputs: - Whole Slide Image (WSI) - Omics data'. These feed into two parallel processes: 'FTU Segmentation' and 'Spatial Data Extraction'. Their outputs both feed into 'Pathomic Feature Extraction'. This leads to a 'DSA' (Data Storage and Analysis) step, which then feeds into 'Spatial Aggregation'. Finally, 'Spatial Aggregation' leads to 'User Interaction'. A 'Key' on the right defines four components: 'Plugin' (square), 'Process' (rectangle), 'Server' (cylinder), and 'Frontend' (oval). Arrows indicate the flow of data between these components.

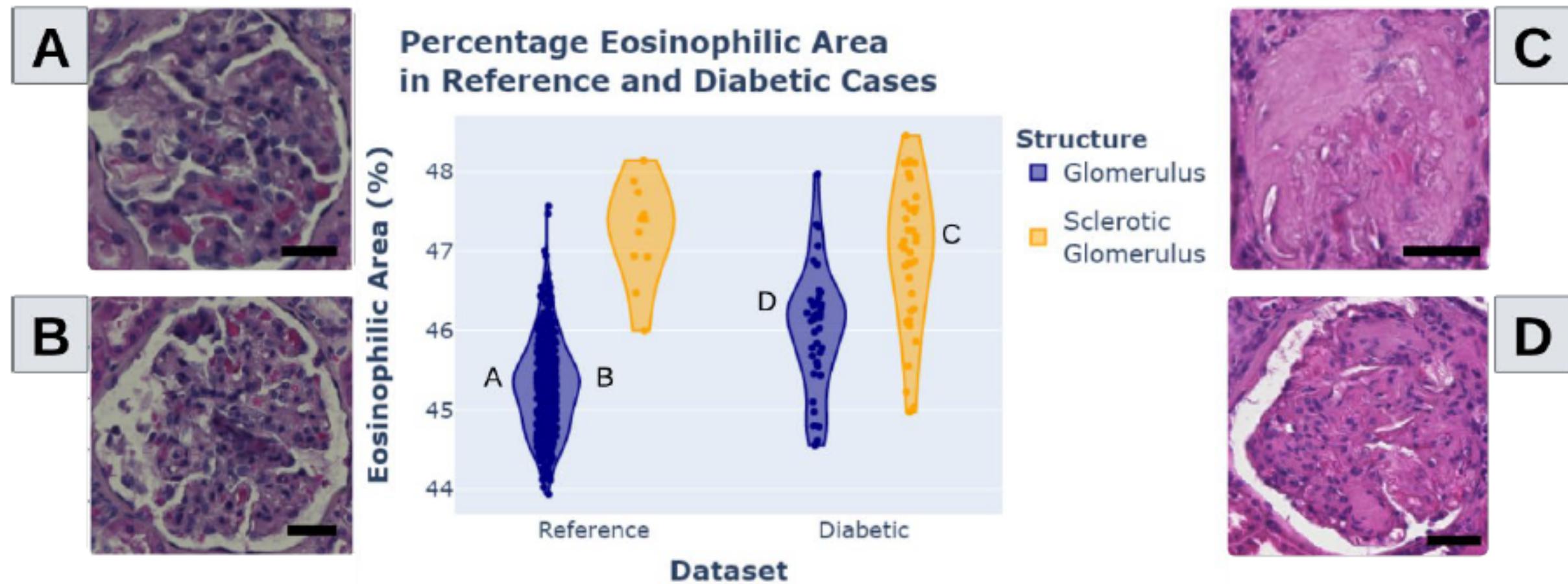
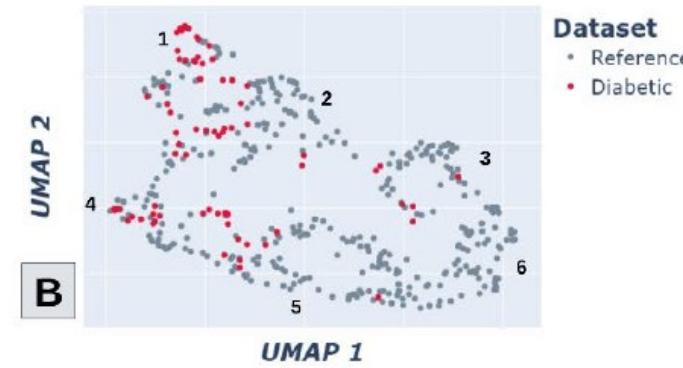


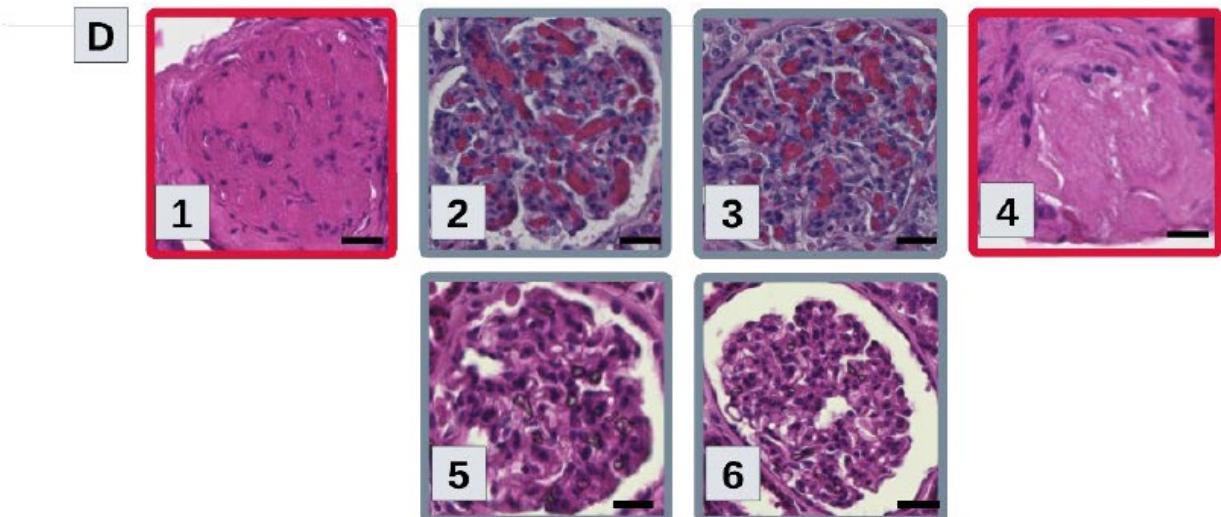
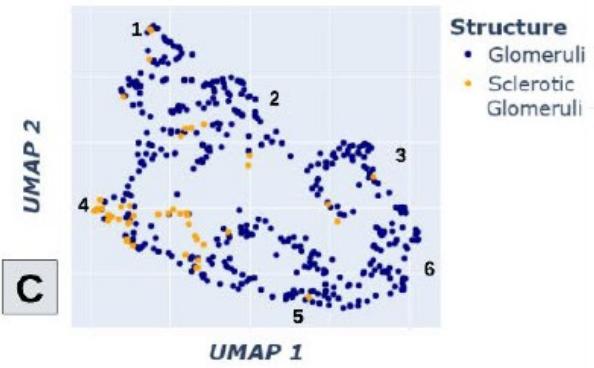
Fig. 3. Local comparison of digital histology images using a single feature. As an example, glomerular hypertrophy is compared in diabetes and reference. Percentage eosinophilic area between normal and sclerotic glomeruli from diabetic and reference kidney tissues. (A-D) Example glomeruli selected from the feature distribution where indicated. Dots represent the individual glomeruli. Sclerosis increases eosinophilic area by reducing open capillary lumens and Bowman's space. Diabetic kidney disease, in the absence of sclerosis, increases the eosinophilic area through hypertrophy and the expansion of the mesangium. Scalebar indicates 25 μm .



**UMAP of Glomeruli
Reference and Diabetic**



**UMAP of Glomeruli
Reference and Diabetic**



- Clustering based on multiple pixel level features

Orthogonal Protein Data

Nephrolithiasis

- 1 in 8 individuals over lifetime
- Diagnosis:
 - CT scan - radiation
 - Hematuria and pain – non-specific
- Form by attachment to a nidus called Randall's plaque
- Papilla samples acquired surgically from stone formers and matched with nephrectomy controls.



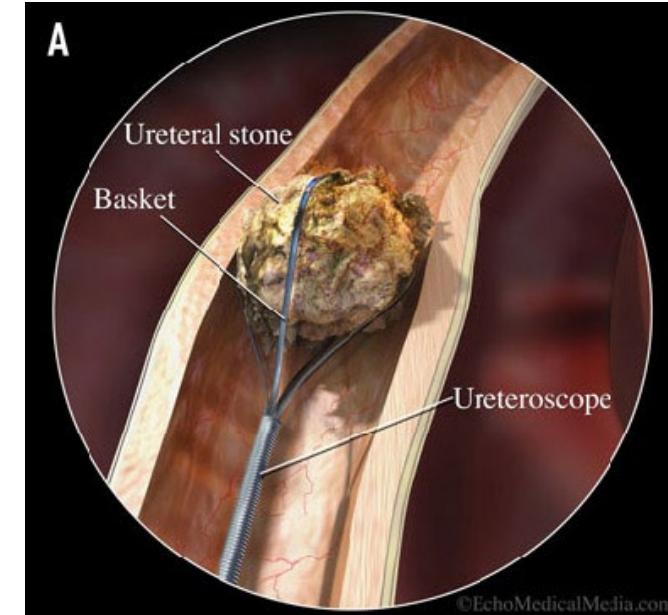
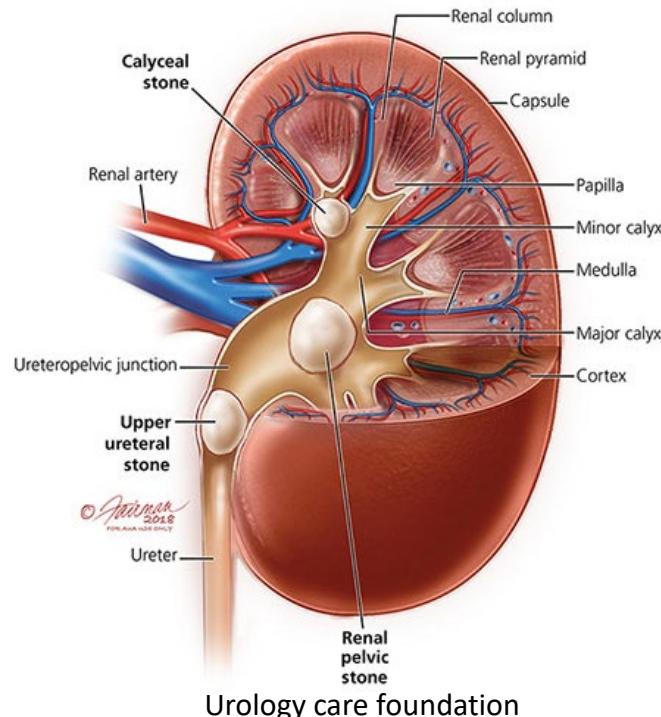
Tarek El-Achkar



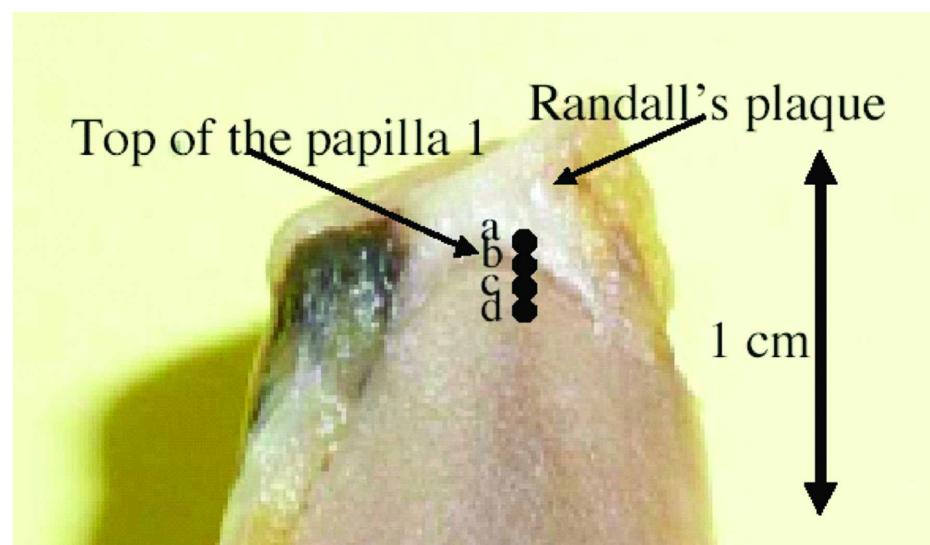
James Williams



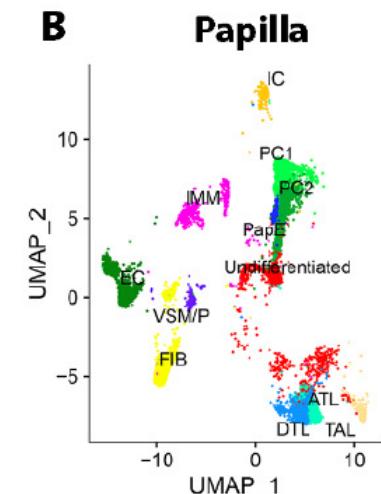
Sanjay Jain



Advances.massgeneral.org

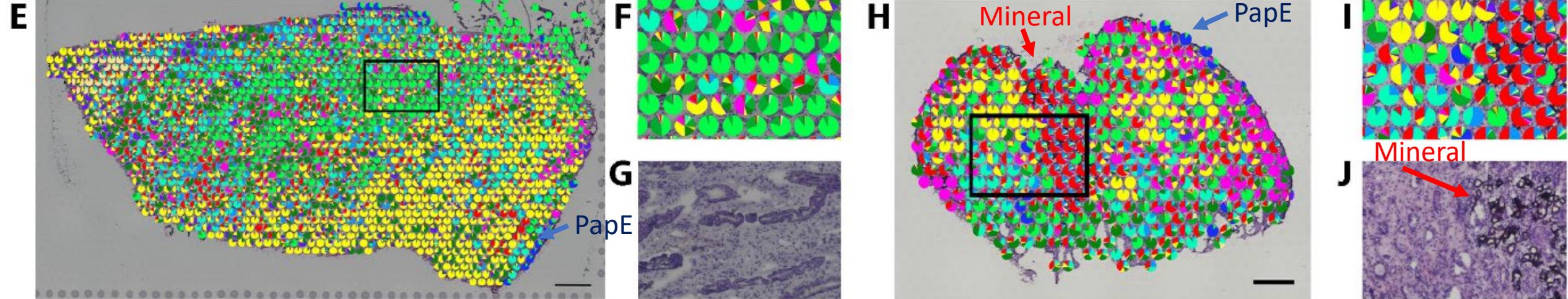


Carpentier X, et. al Journal of Synchrotron Radiation Volume 17 | Part 3 | March 2010 | Pages 374-379

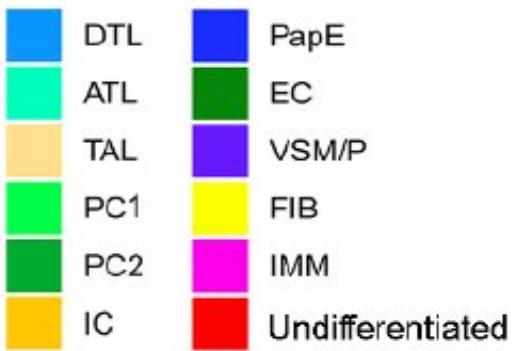


20,338 nuclei from papillary samples

10x ST of the Renal Papilla in Health and Disease

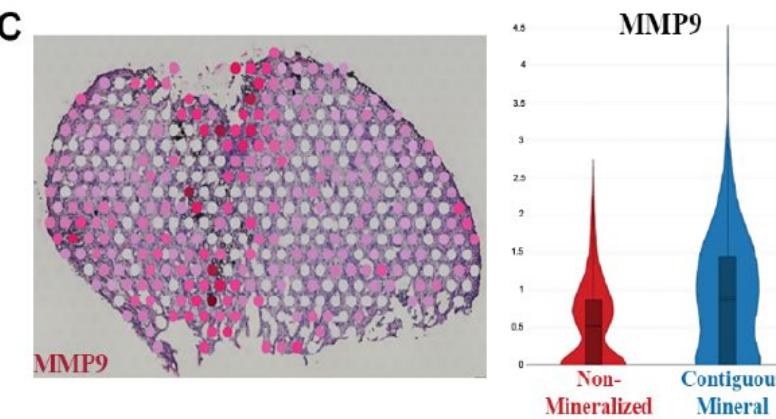
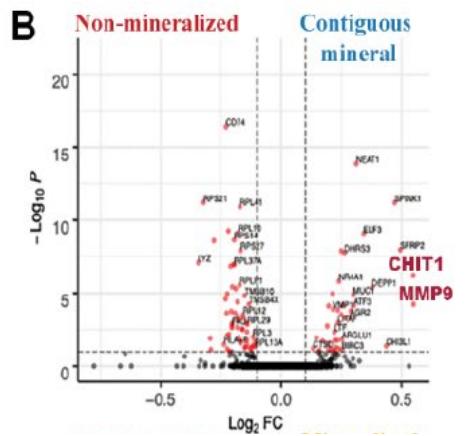
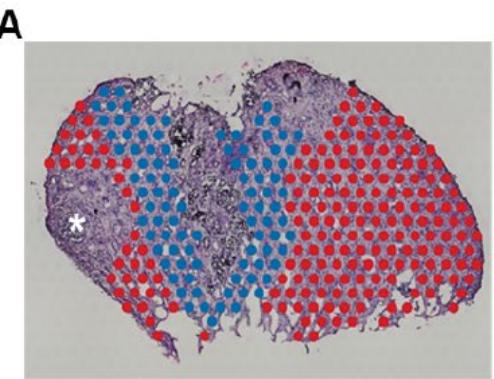
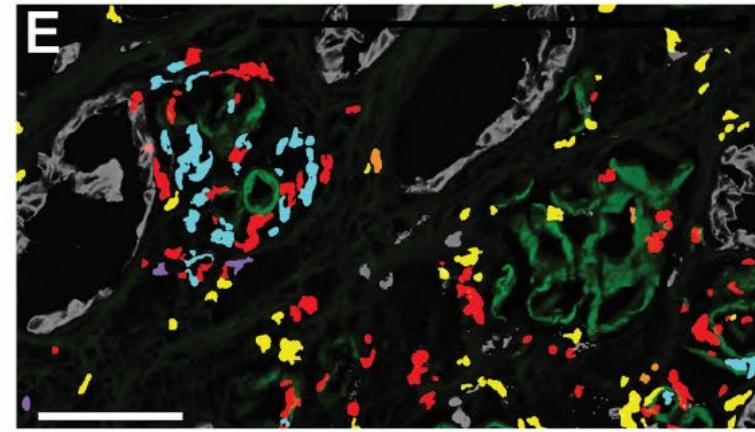
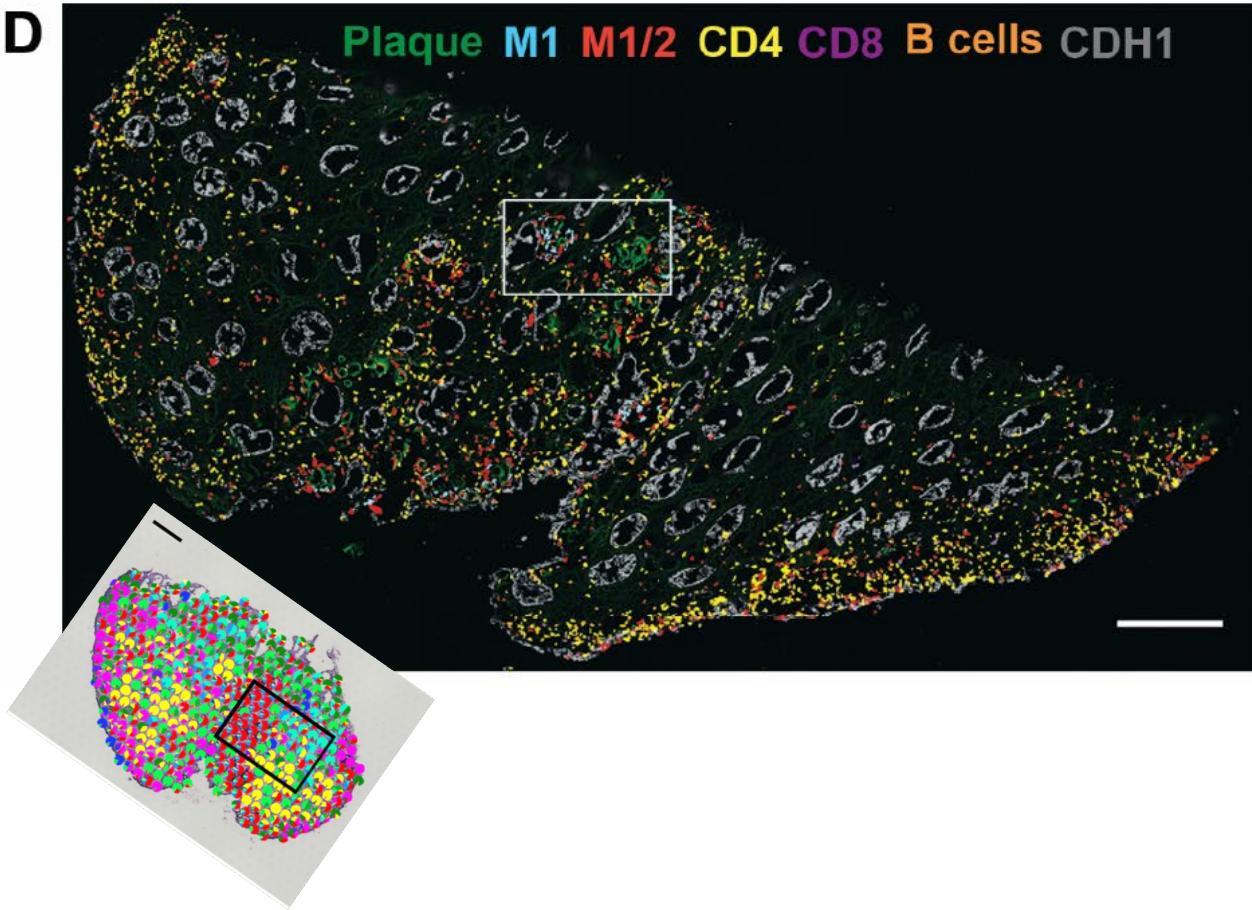


Reference



An undifferentiated cell type was observed which overlies mineral deposition and colocalizes with immune and stromal cells.

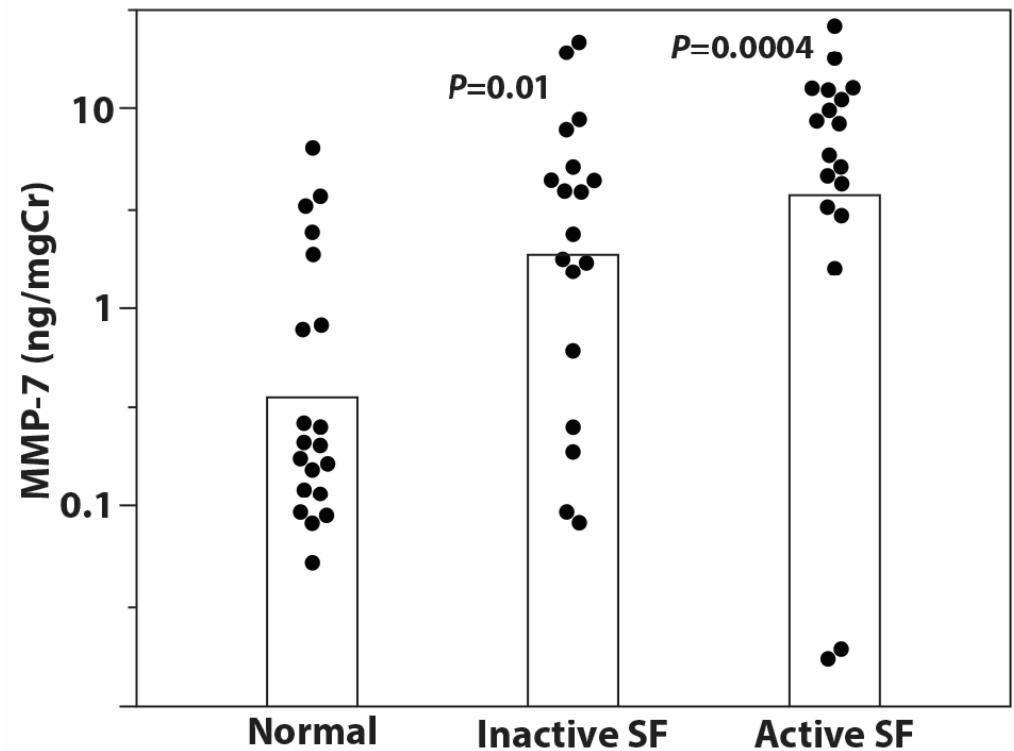
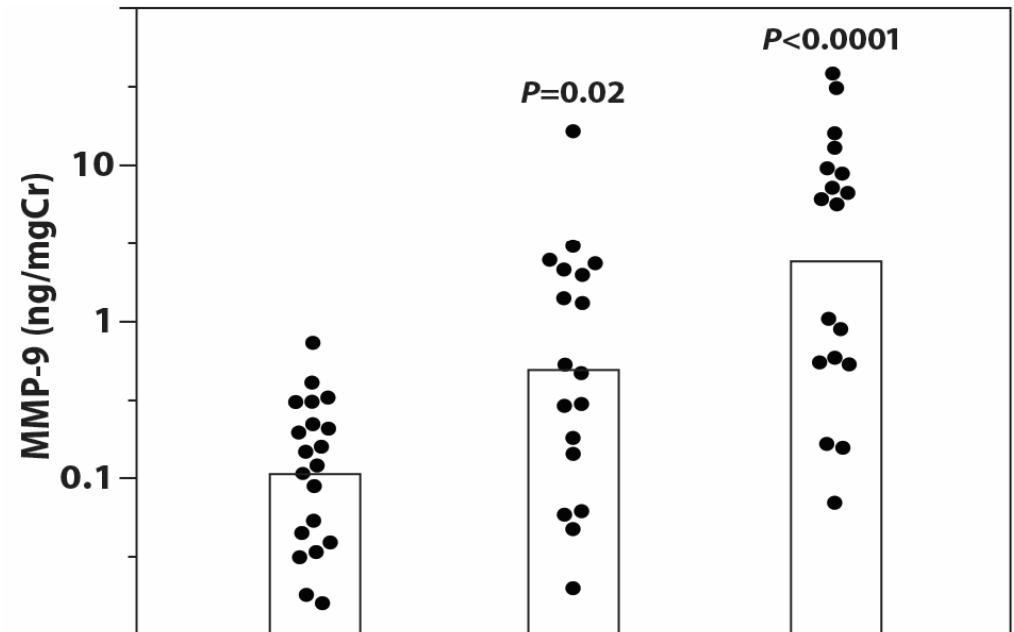
- Immune and stromal colocalization with plaque
 - Co-Detection by indexing (CODEX) imaging – 32 Abs



MMP9 upregulated
regionally in or near
mineralization

Urinary biomarker of active stone disease

- In controls (N=20), inactive stone formers (N=18), and active stone formers (N=18)...
- MMP7 and MMP9 increase in the urine in active stone formers.
- Non-invasive screen without CT scan or ED visit



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3D Tissue Imaging Core**

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