



Around the Block: Neighborhoods in Kidney Health and Disease

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6-10-24

Disclosures

- NIH funding



KIDNEY PRECISION MEDICINE PROJECT



- Over 180 biopsies altruistically donated
- Large consortium with Recruitment sites, Tissue interrogation sites, Central Hub and Opportunity pool Awardees
- www.kpmp.org

EPIGENETICS

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

IMAGING

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

TRANSCRIPTOMICS

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

PROTEOMICS

- Regional Proteomics
- Spatial Proteomics



METABOLOMICS

- Spatial Metabolomics
- Spatial Lipidomics
- Spatial N-glycomics

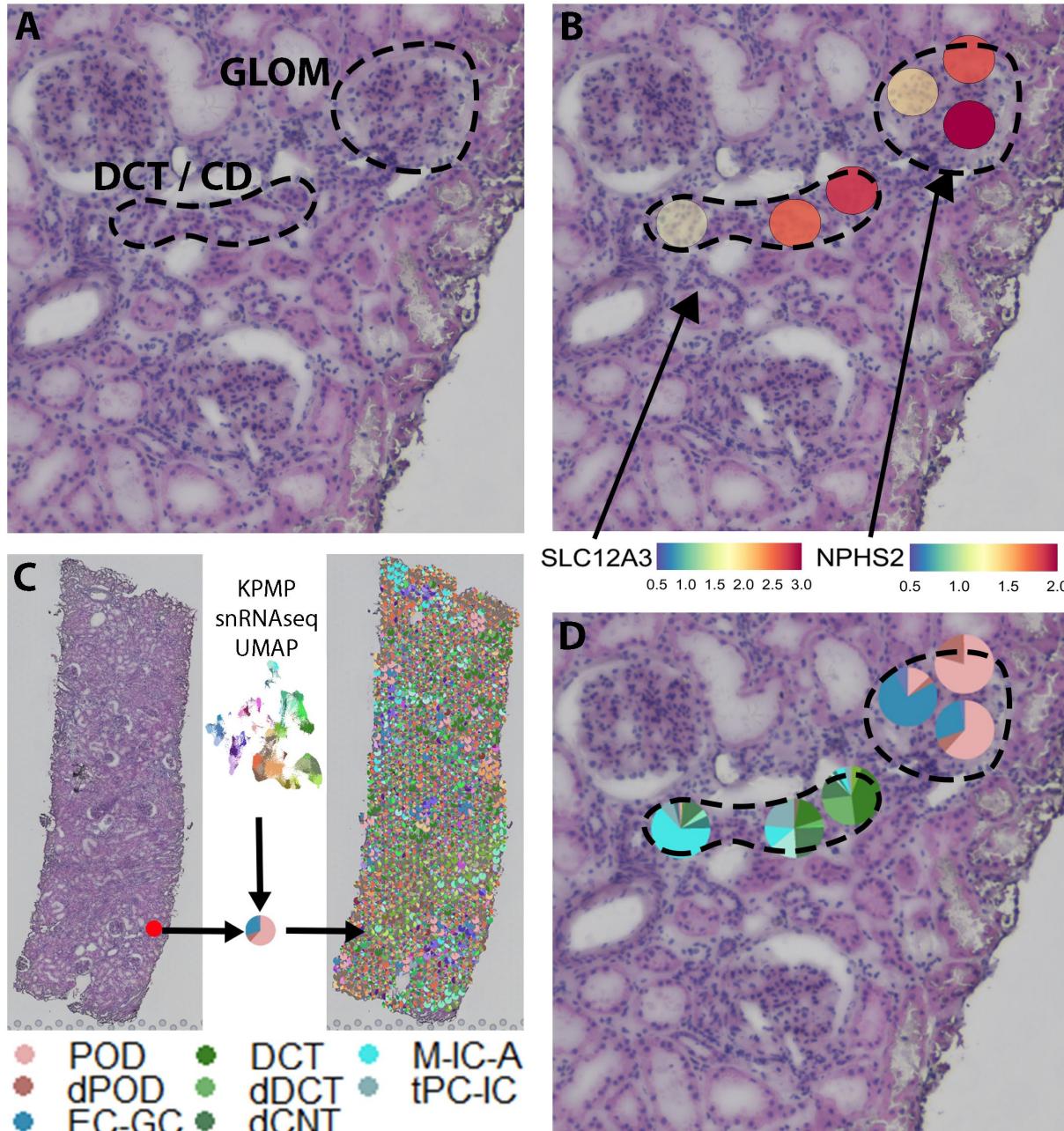


- Goal: develop an open and global platform to map healthy cells in the human body across multiple organs.
- Spatially anchor cell types within tissue samples and understand communication between cell types.
 - Direct co-registration
 - Alignment of neighborhoods in the analytic space

The image shows the HuBMAP website. At the top, there is a navigation bar with links for About, Data, News & Events, Member Services, and Data Portal. Below the navigation bar, the main content area features a section titled "What is HuBMAP?" with a brief description: "The Human BioMolecular Atlas Program is working to catalyze the development of a framework for mapping the human body at single cell resolution." A "Find Out More" button is located below this text. To the right, there is a video player window titled "HuBMAP Overview" showing a video thumbnail of a human figure with the text "HuBMAP" overlaid. The video player includes standard controls like play, pause, and volume, along with a "YouTube" link. At the bottom of the page, there is a summary of key statistics: 1039 Datasets, 1013 Samples, 86 Donors, and 21 Organs. There is also a "Explore our Data" button with a magnifying glass icon.

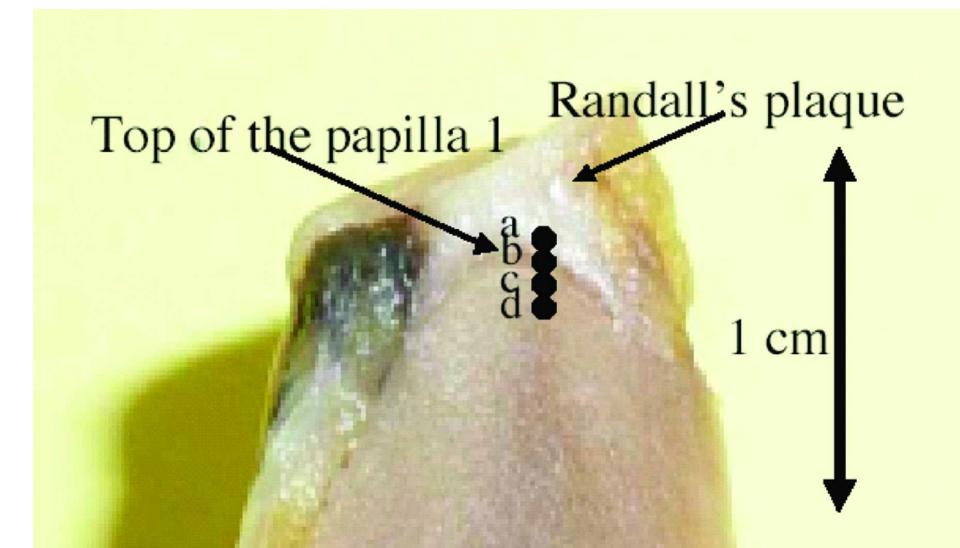
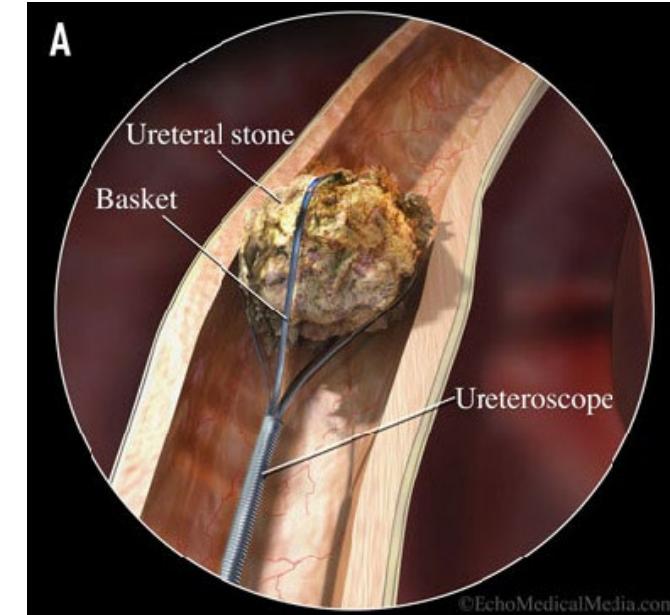
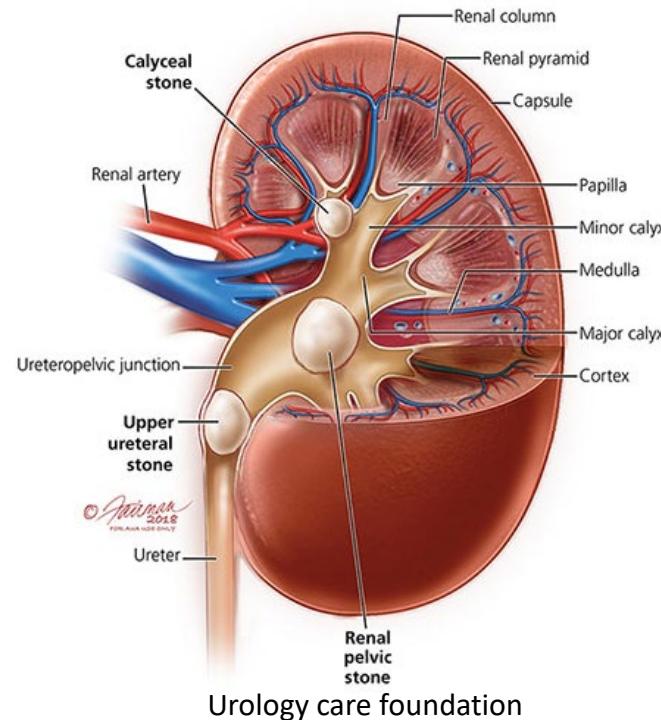
Why Spatial Transcriptomics?

- **The unique features of VSGE include:**
 - snRNAseq / scRNAseq provides cell type expression but needs spatial context
 - ST provides whole transcriptome mRNA expression with localization
 - Histological information from H+E staining on the same tissue section (not sequential sections)
 - Tissue economy: ~10 µm thickness of a core is used to study kidney biopsies
- **Applications**
 - Nephrolithiasis
 - Acute Kidney Injury and Chronic Kidney Disease

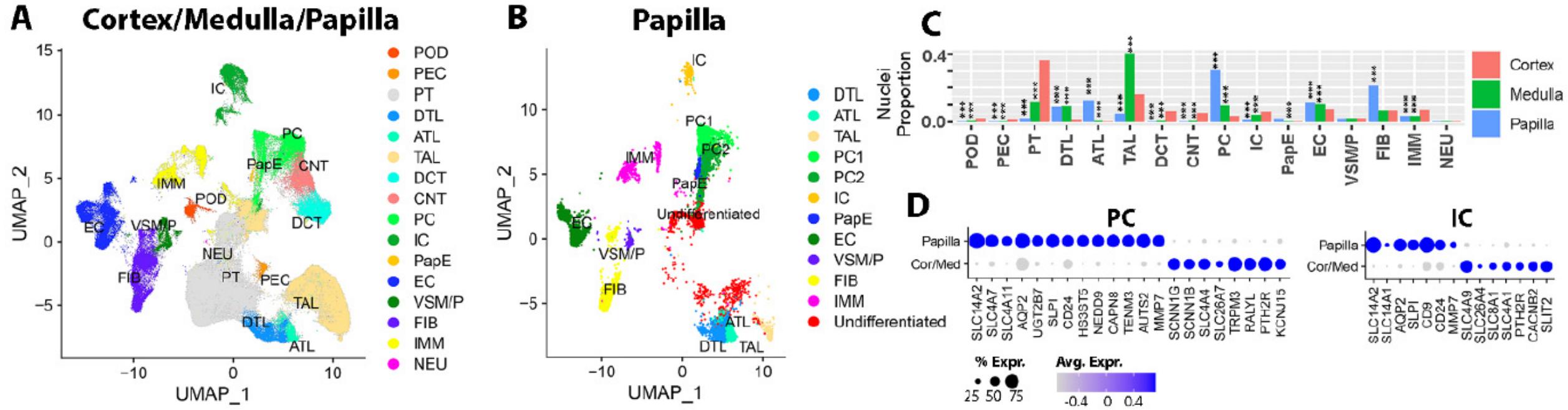


Nephrolithiasis

- Cost of \$5 billion annually
- 1 in 8 individuals over lifetime
- >70% Calcium Oxalate
- 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.

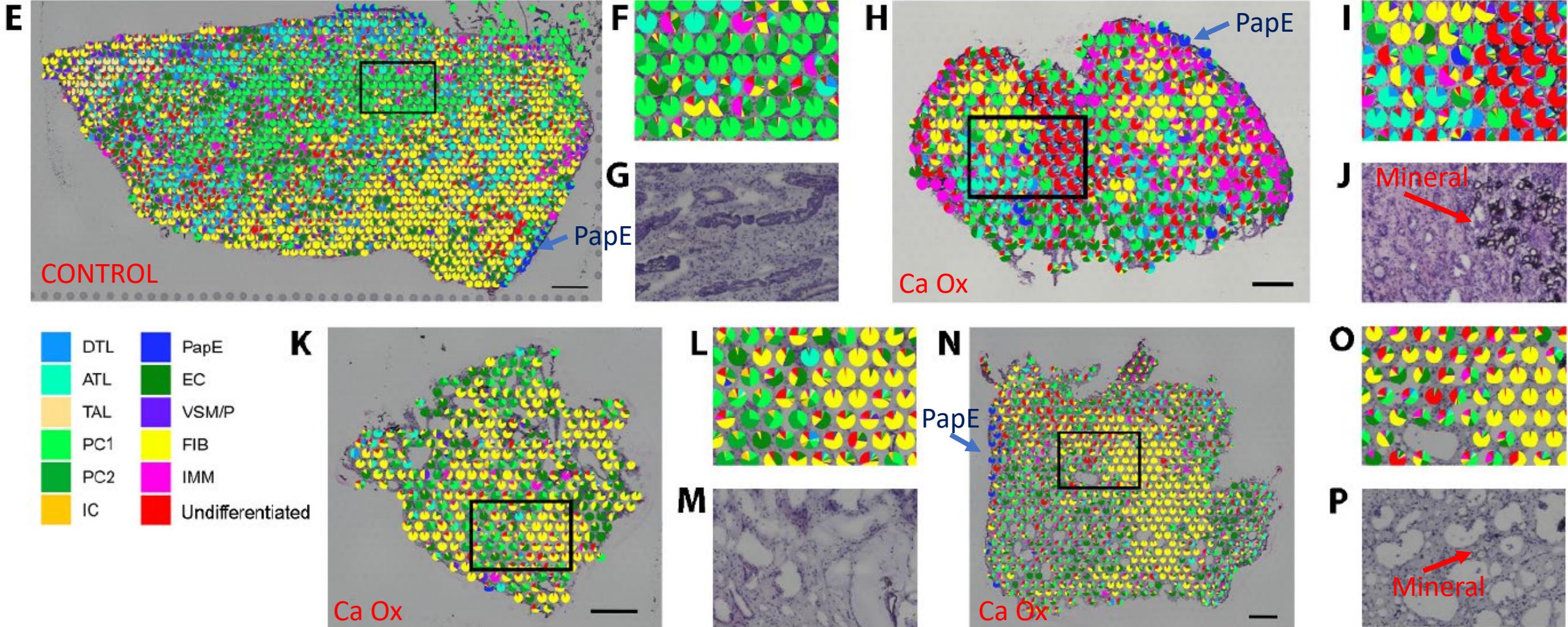


snRNAseq of Papilla

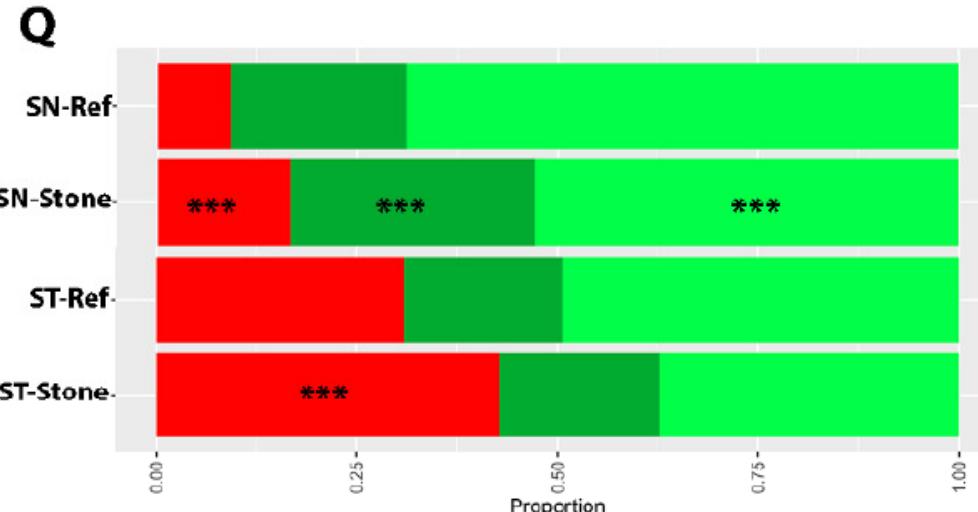


- 203,702 nuclei from kidney samples (includes cortex and medulla)
- 20,338 nuclei from papillary samples
 - Enriched for thin limb and principal cells (PCs)
 - Unique papillary PC cell expression pattern compared to medullary PCs.

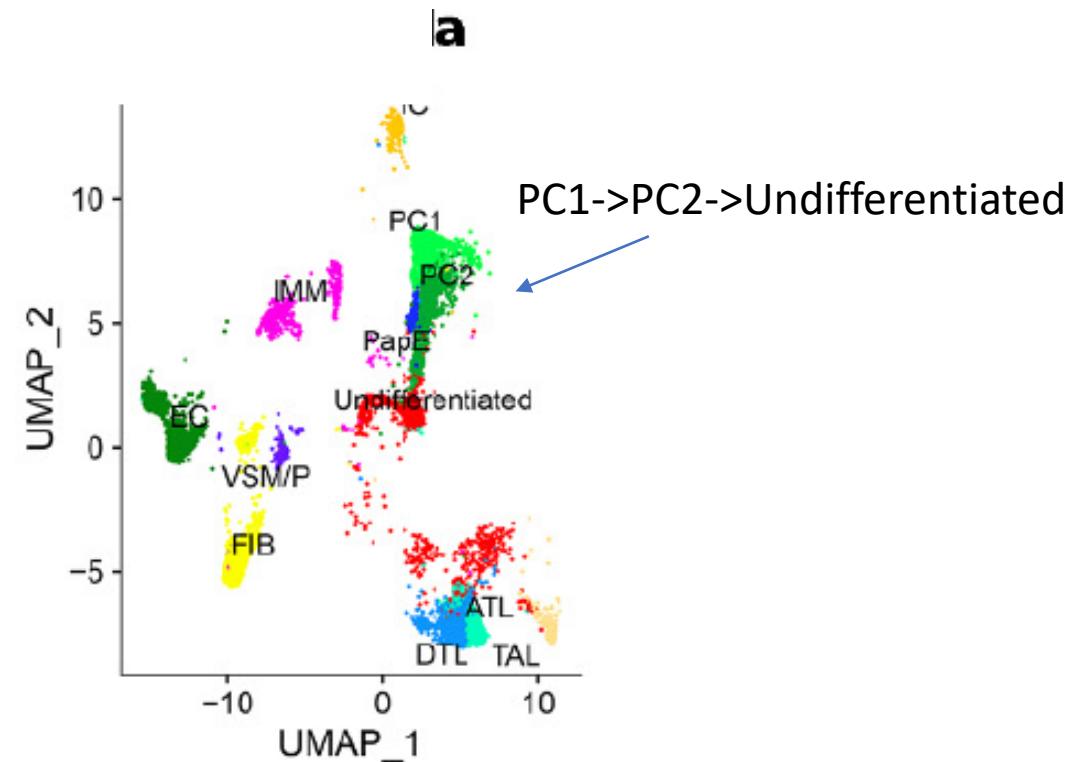
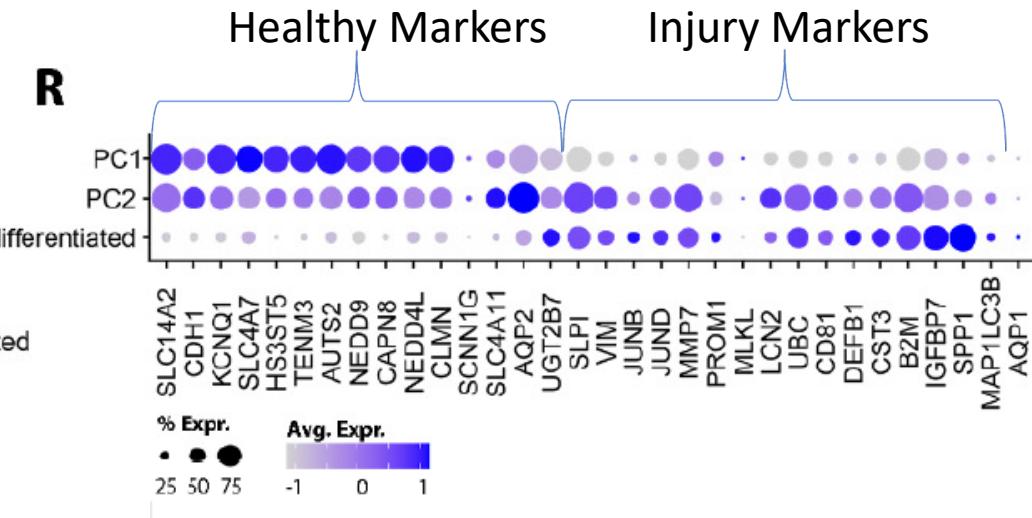
ST of the Papilla



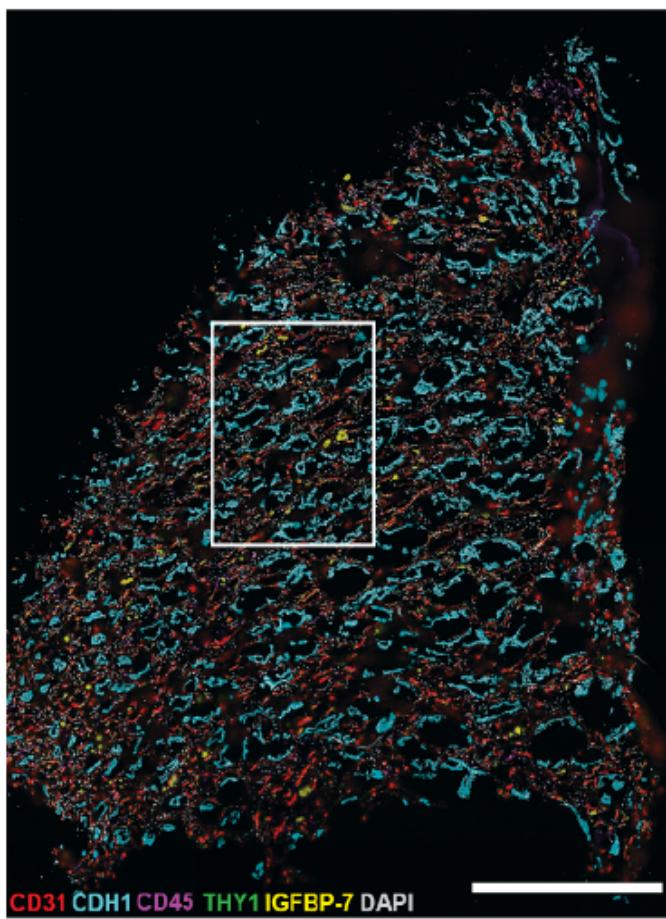
- An undifferentiated cell type was observed which overlies mineral deposition and colocalizes with immune and stromal cells.



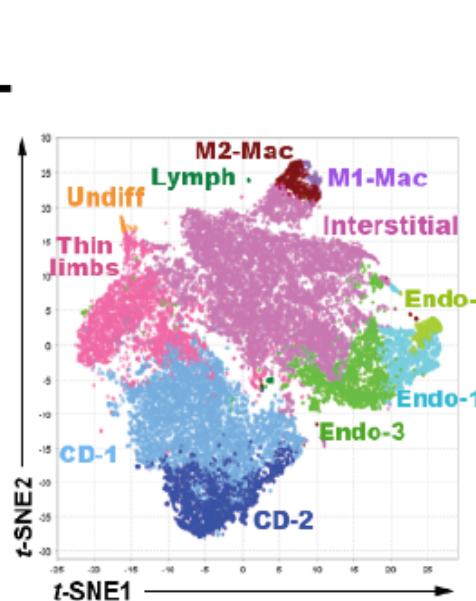
- Increase in proportion of injury (PC2) and undifferentiated clusters within stone forming papilla



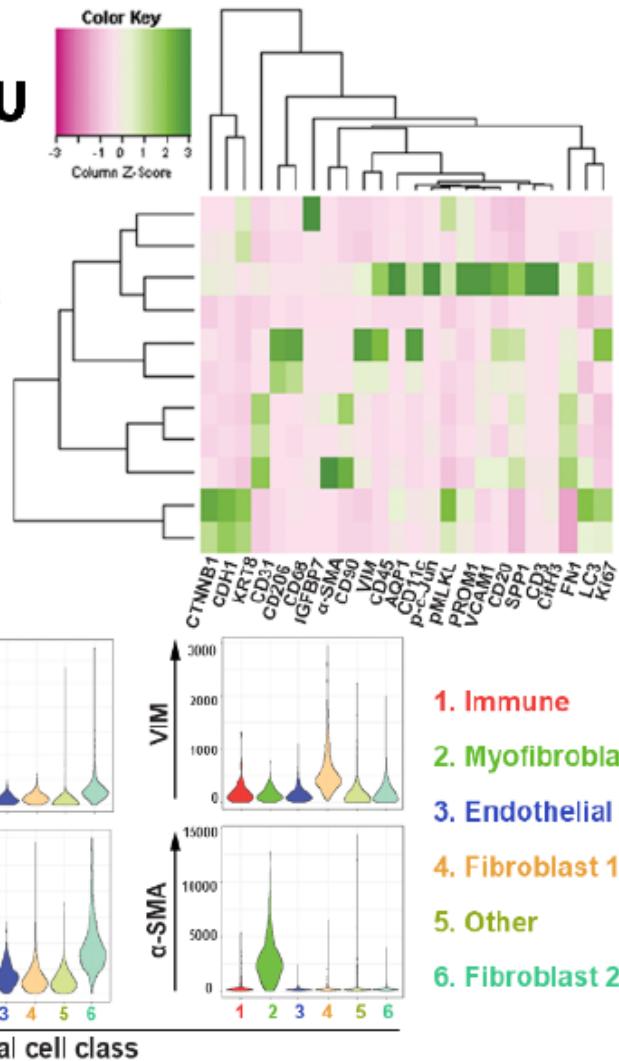
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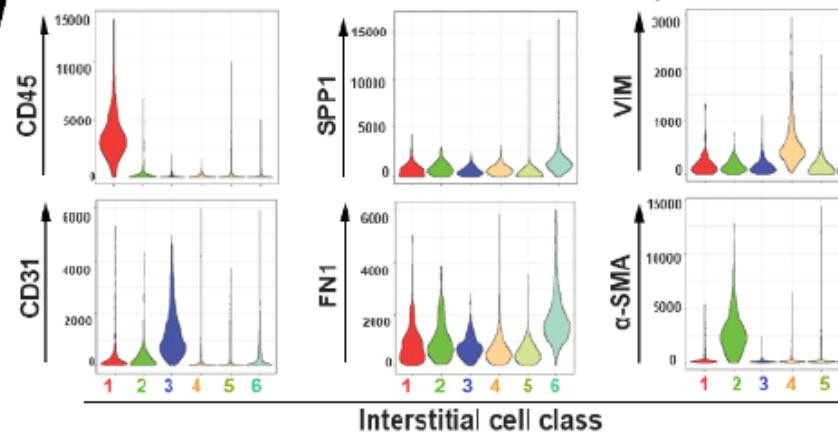
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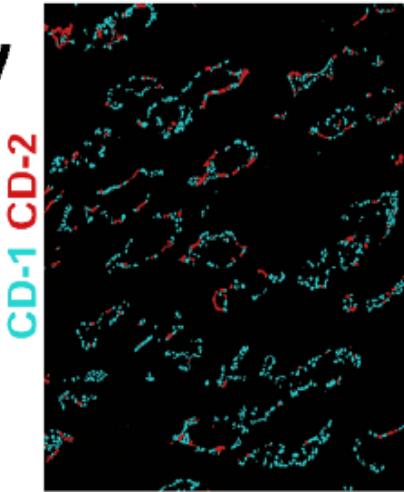
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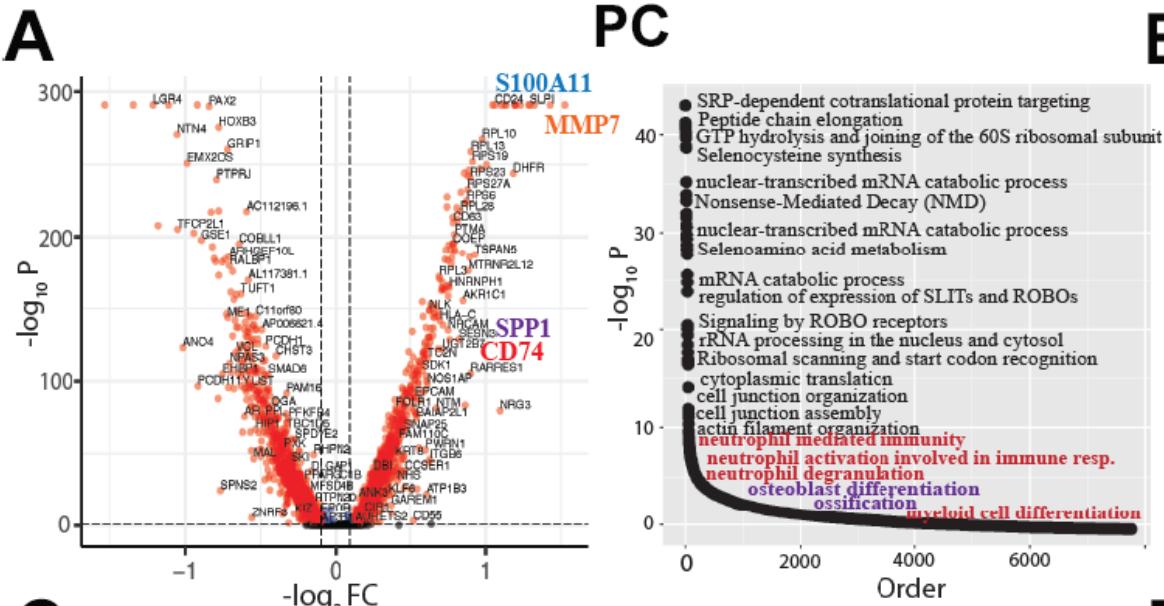
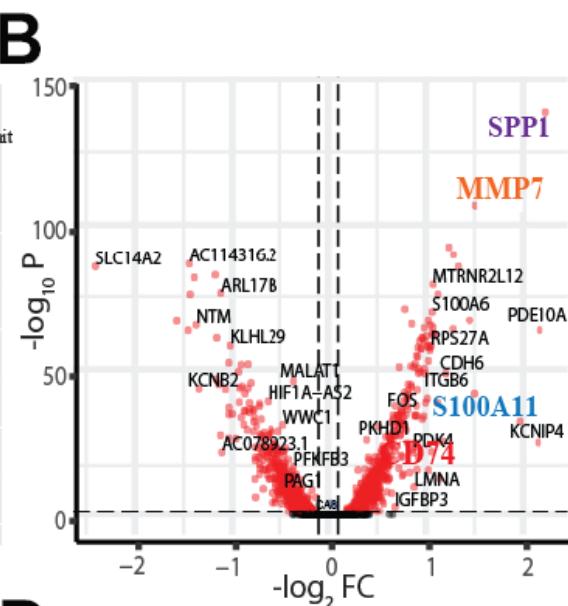
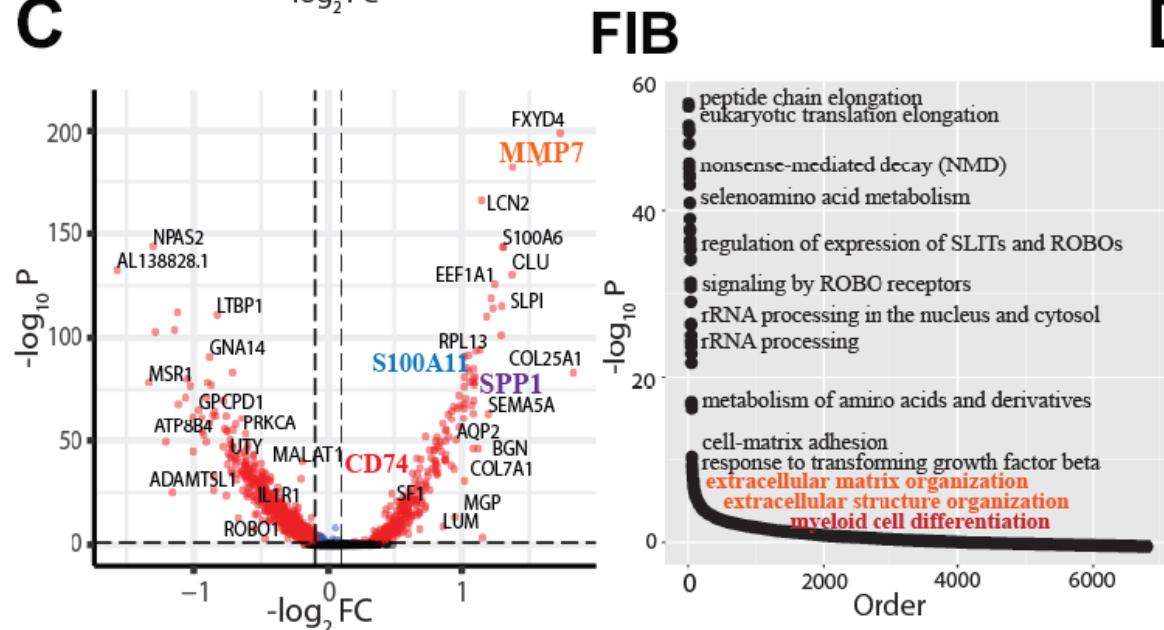
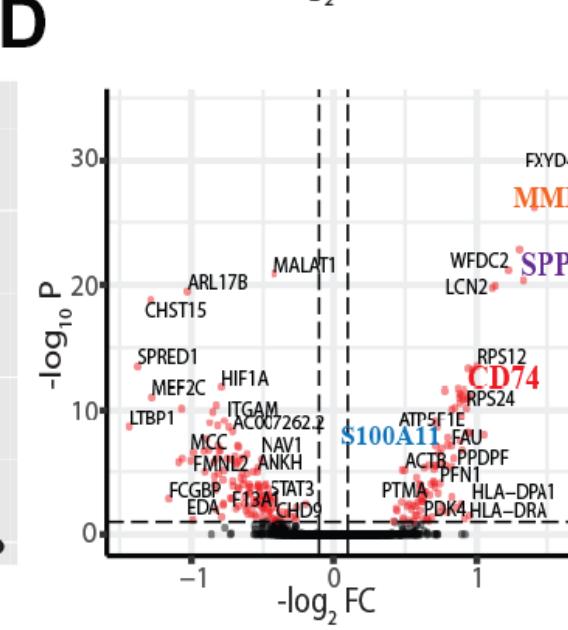
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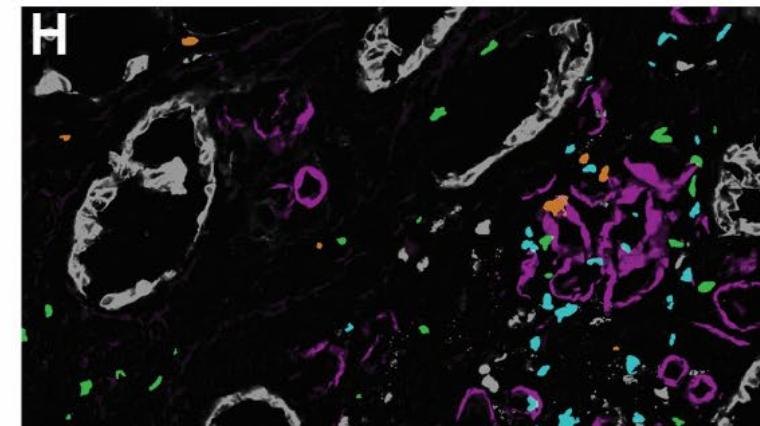
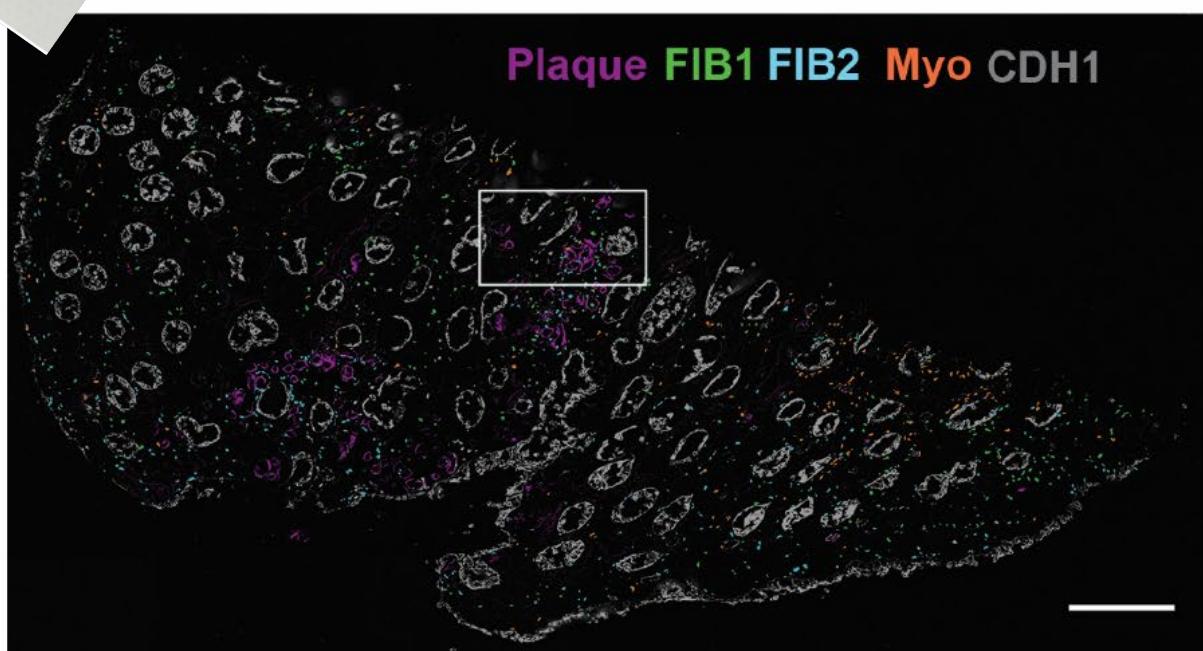
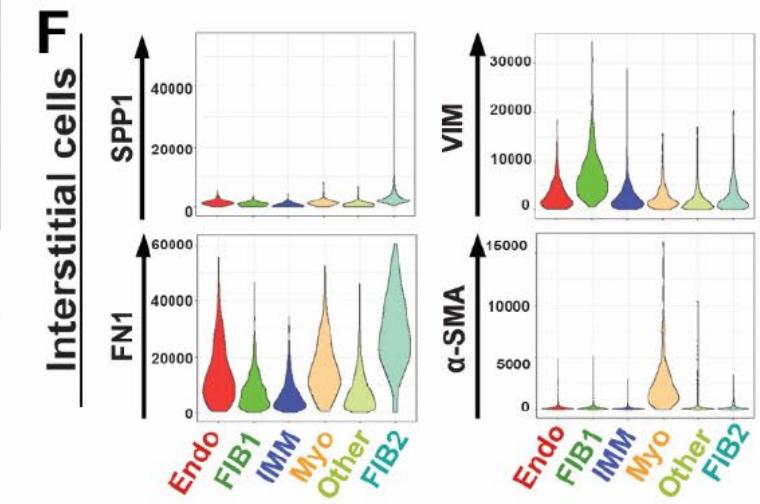
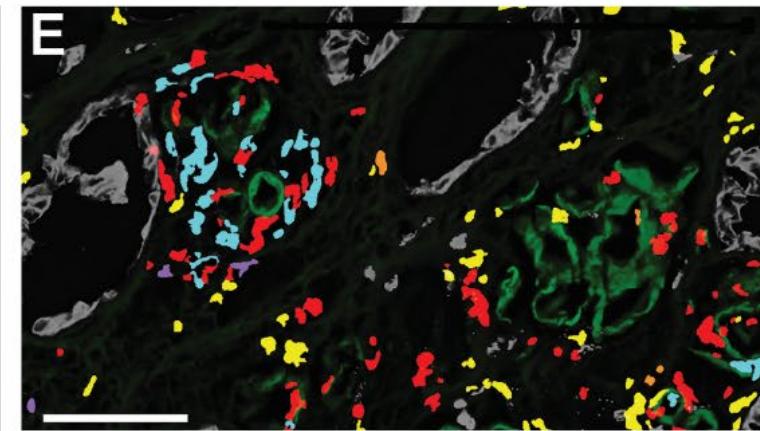
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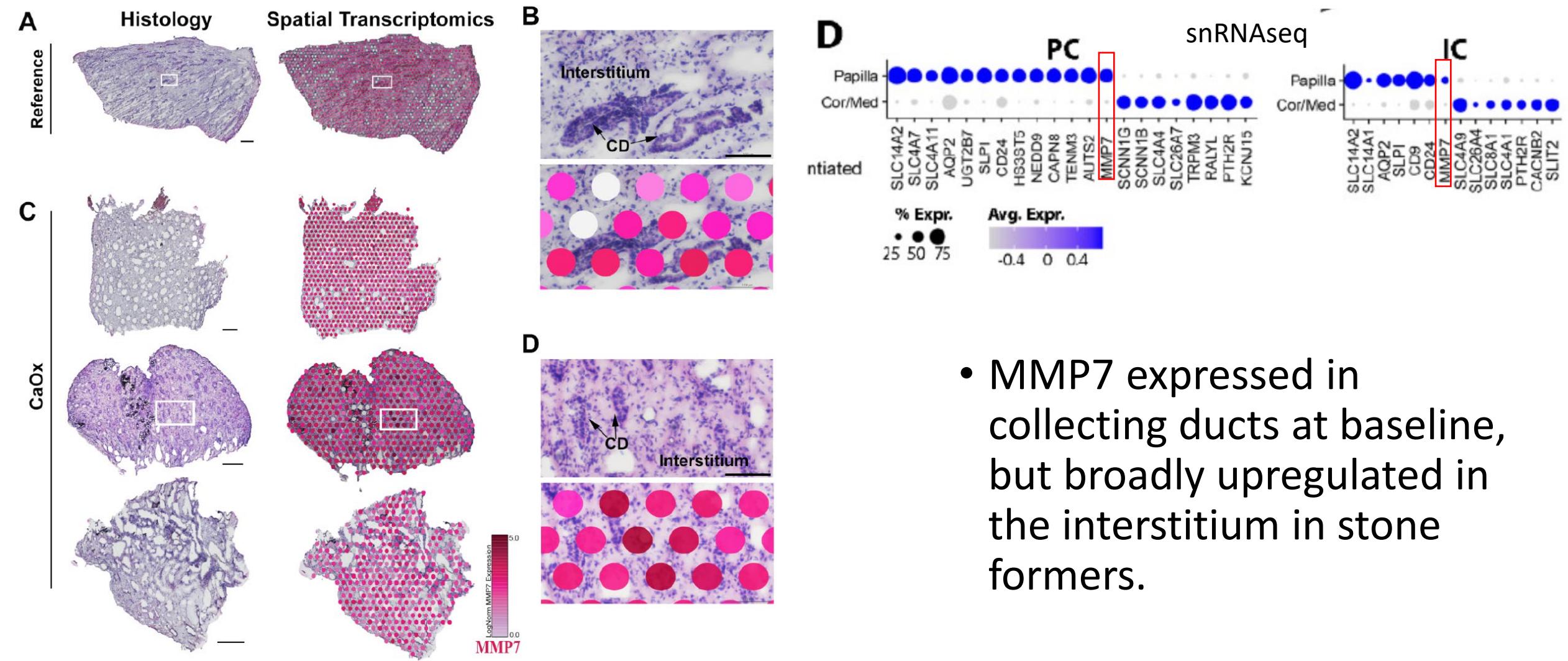
- Co-Detection by indexing (CODEX) imaging – 32 Abs
- Similar healthy and injured collecting duct (CD) populations were identified, aligning with principal cells (PC) of ST.

A**B****C****D**

- Immune cell pathways are upregulated in stone formers



- Immune and stromal colocalization with plaque
- Example of coregistration

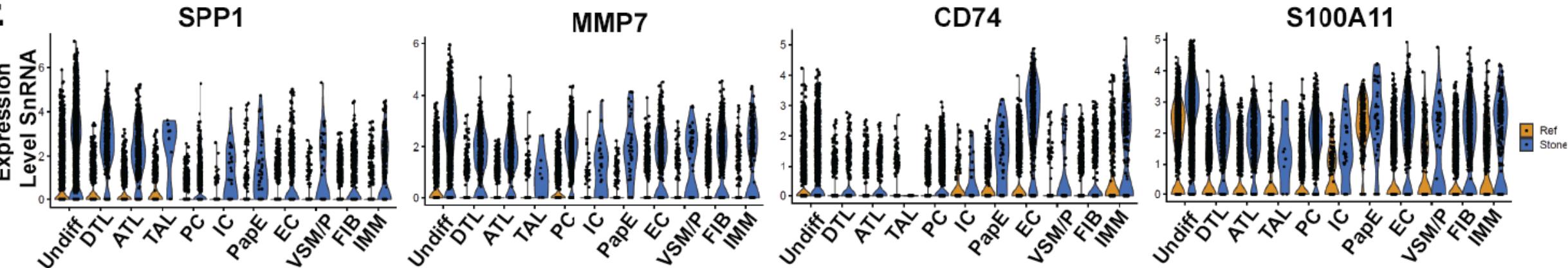


- MMP7 expressed in collecting ducts at baseline, but broadly upregulated in the interstitium in stone formers.

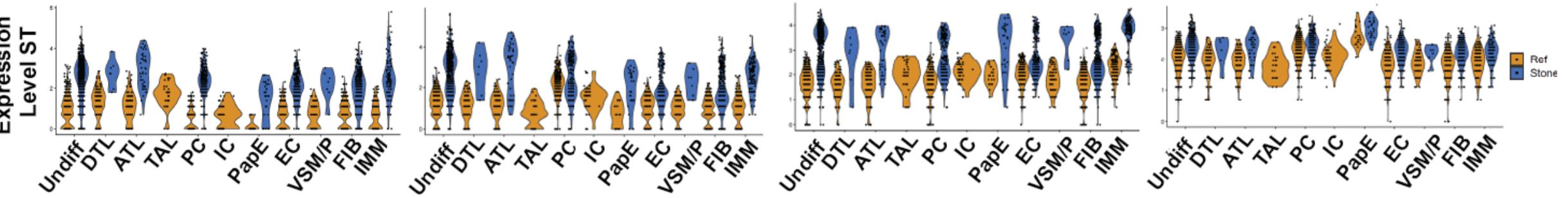
Figure 3. MMP7 expression in the papilla. Spatial transcriptomics analysis comparing control (Reference) and three different CaOx stone patient biopsies. In reference tissue, MMP7 expression is localized to collecting ducts. In stone disease, MMP7 expression is diffusely increased and encompasses various papillary cells and structures, which is consistent with the snRNAseq expression and the expression signature mapping on ST shown in Figure 2E and F. Scales bars in (A) and (C): 0.5 mm for reference and top stone sample, 0.25 mm for other 2 specimens; for (B) and (D): 0.1 mm.

snRNAseq and ST

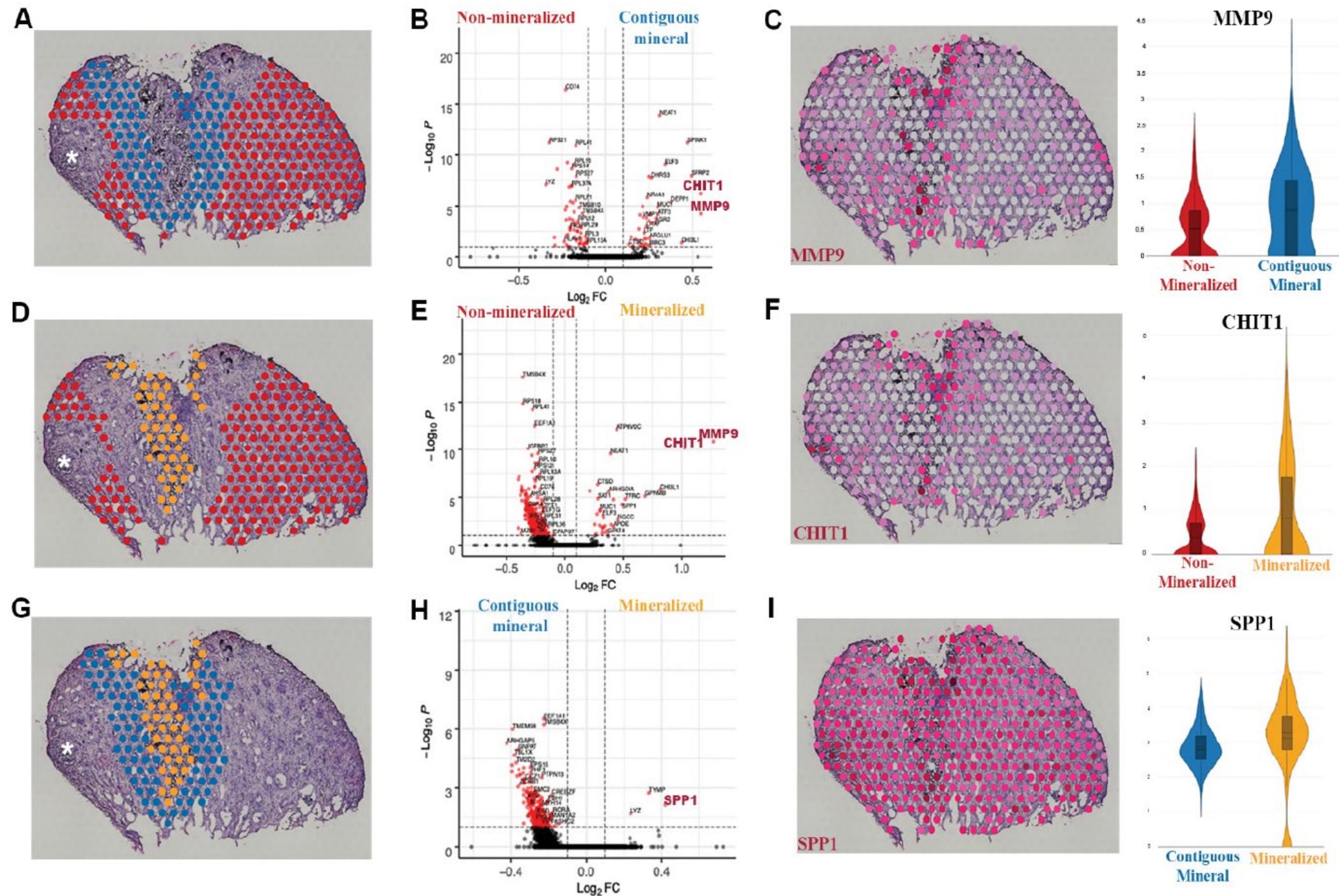
E



F



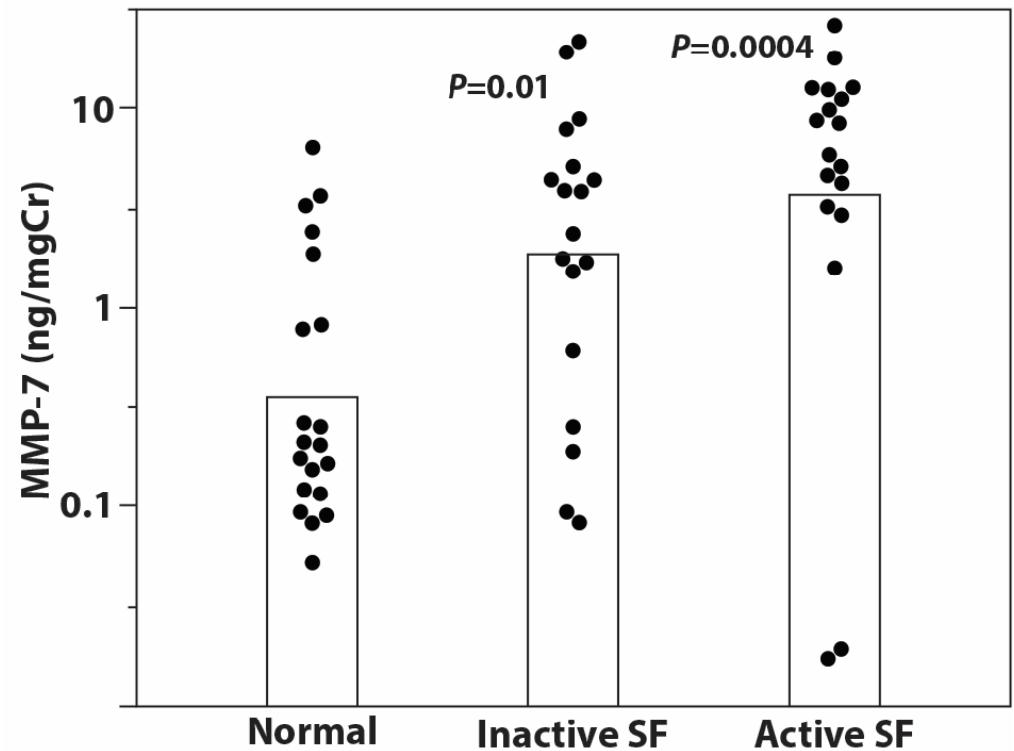
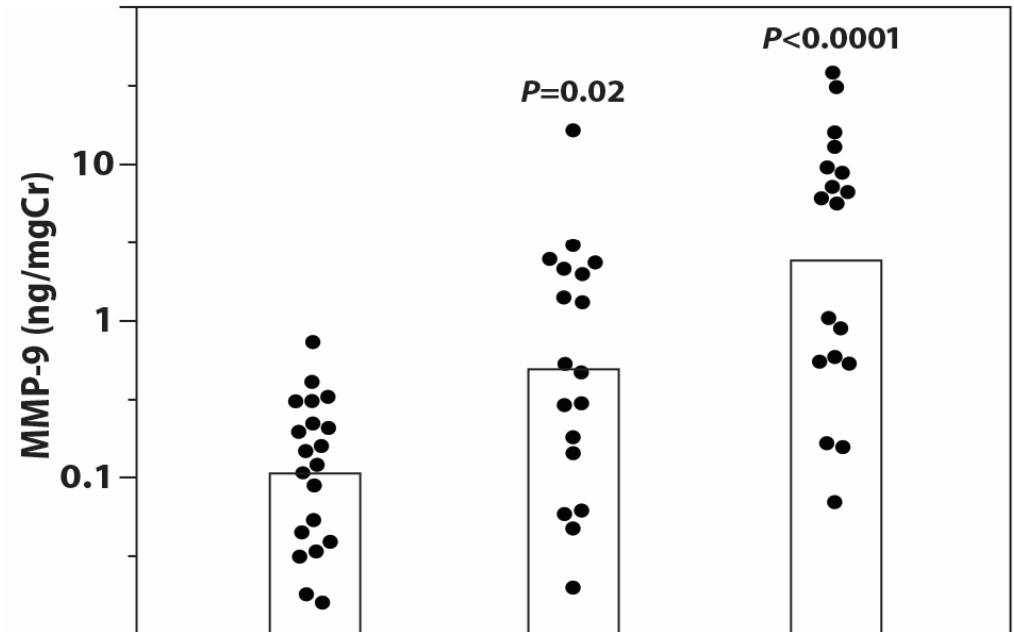
- snRNAseq and ST cell types have paralleled expression changes in stone formers compared to reference samples.
- Broad upregulation of MMP7 across all cell types



- Other markers of injury upregulated regionally in or near mineralization
 - MMP7 and MMP9 upregulated by ST.

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



Injury Signature Mapping in Chronic Kidney Disease (CKD) and Acute Kidney Injury (AKI)

CKD, AKI, and ESRD

- CKD -> AKI, AKI -> CKD
- CKD progression leads to End Stage Renal Disease (ESRD) where individuals need a transplant or dialysis.
- 698 million cases of CKD worldwide
- ~31 million in the US
- In the US, ~780,000 individuals have ESRD.
- Account for \$51 Billion annually or 9% of Medicare budget

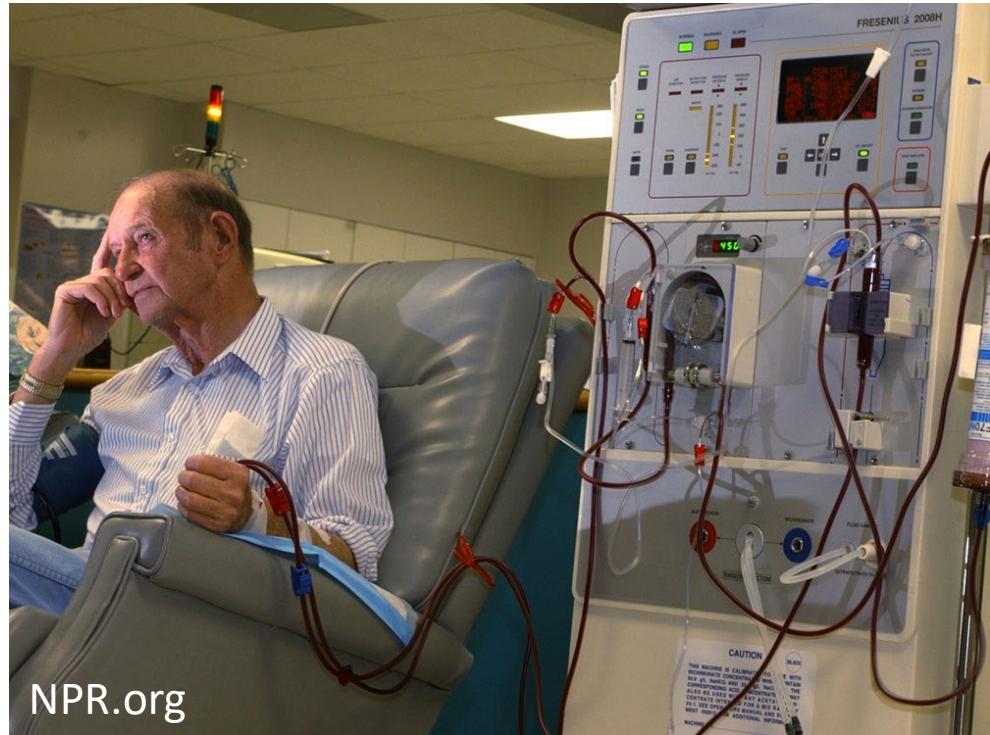
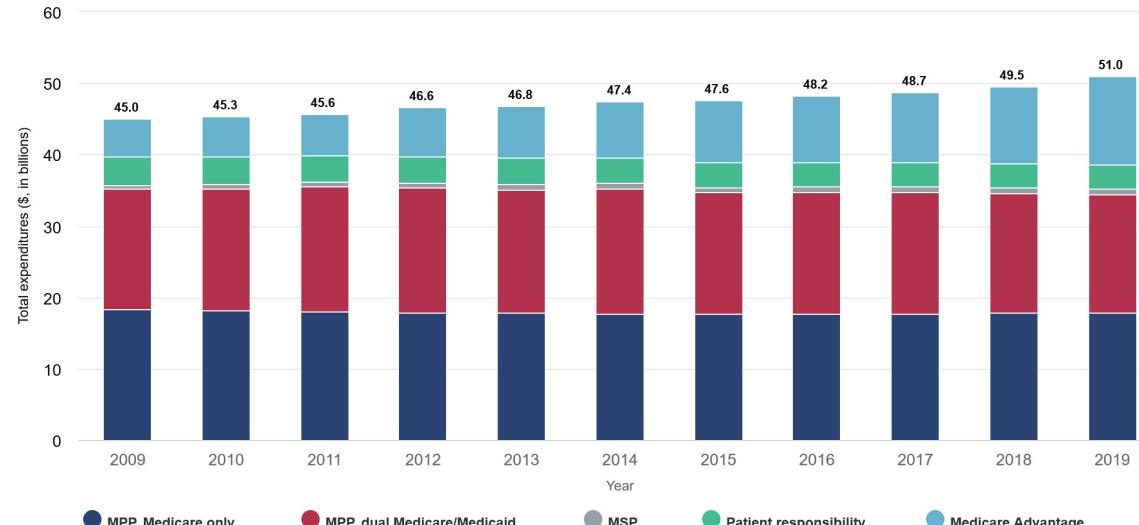
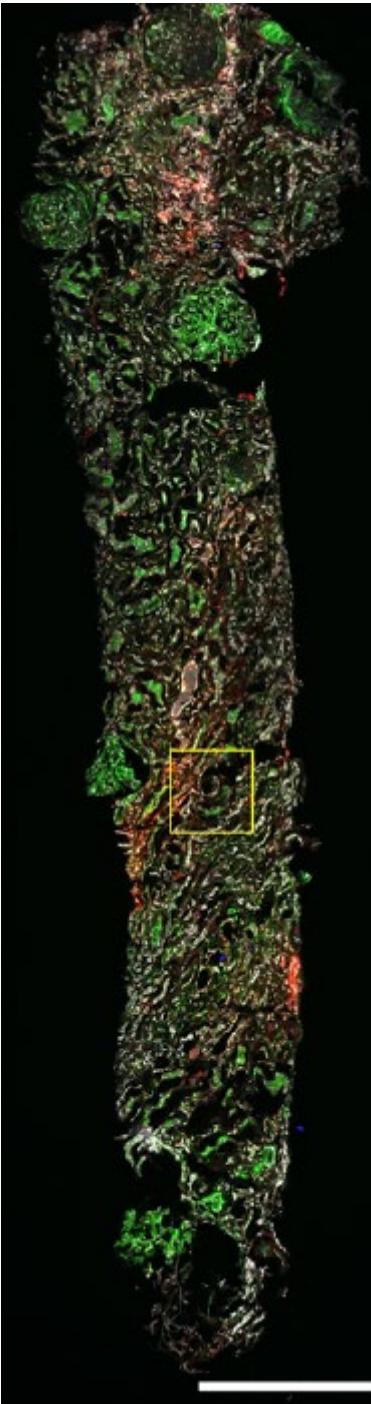
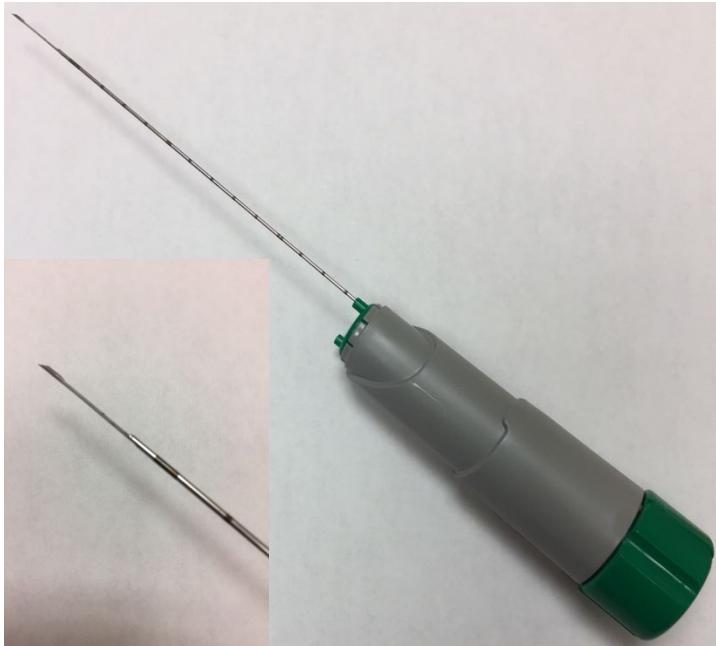


Figure 9.1 Inflation adjusted total spending for Medicare beneficiaries with ESRD, 2009-2019
Inflation adjusted



The Renal Biopsy

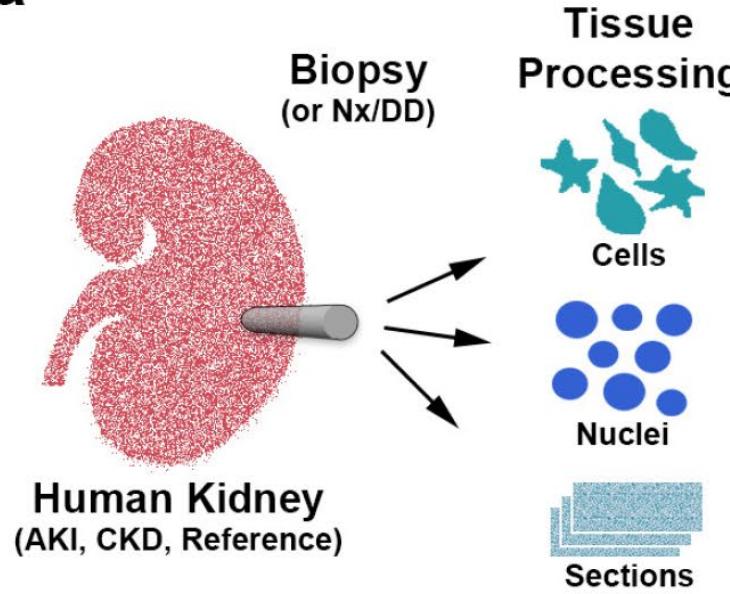
- Not typically performed in most CKD or AKI
- Performed when a clinical diagnosis is in question or to determine therapy
- KPMP – prospective cohort of altruistic kidney tissue donation in early CKD or AKI



Kidney Cell Atlas Overview: Technologies

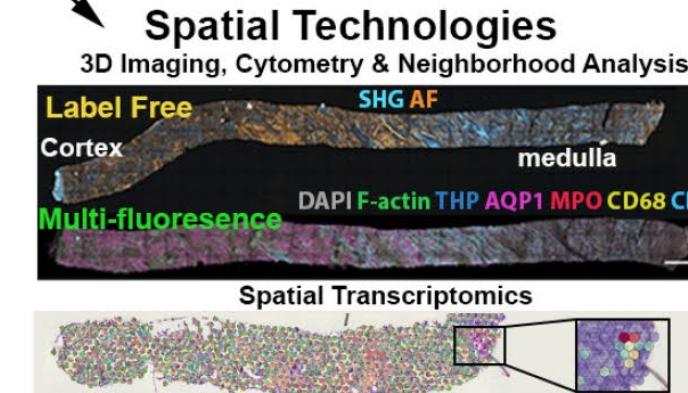
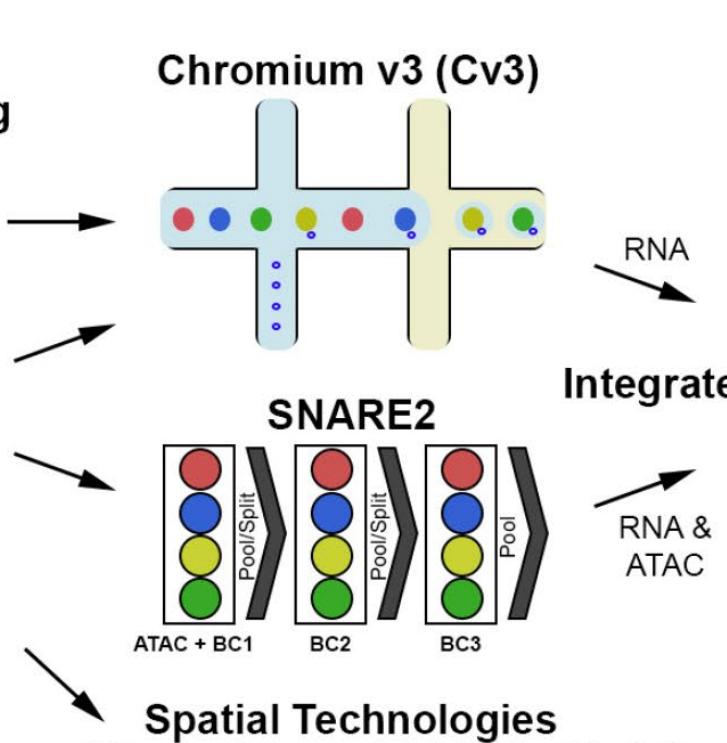
100 cell types defined, 6 cell states, 122 samples

a

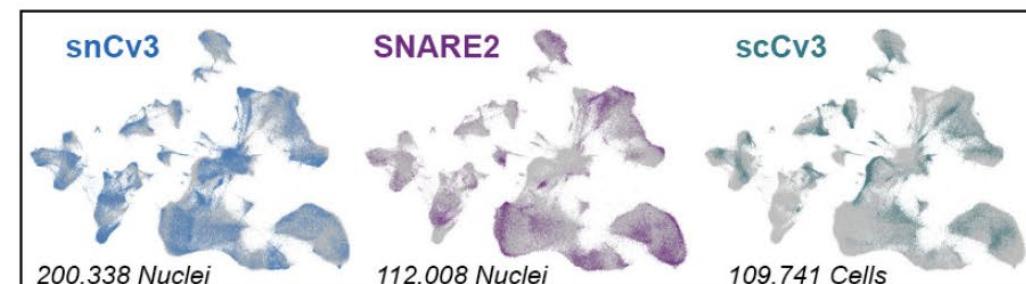
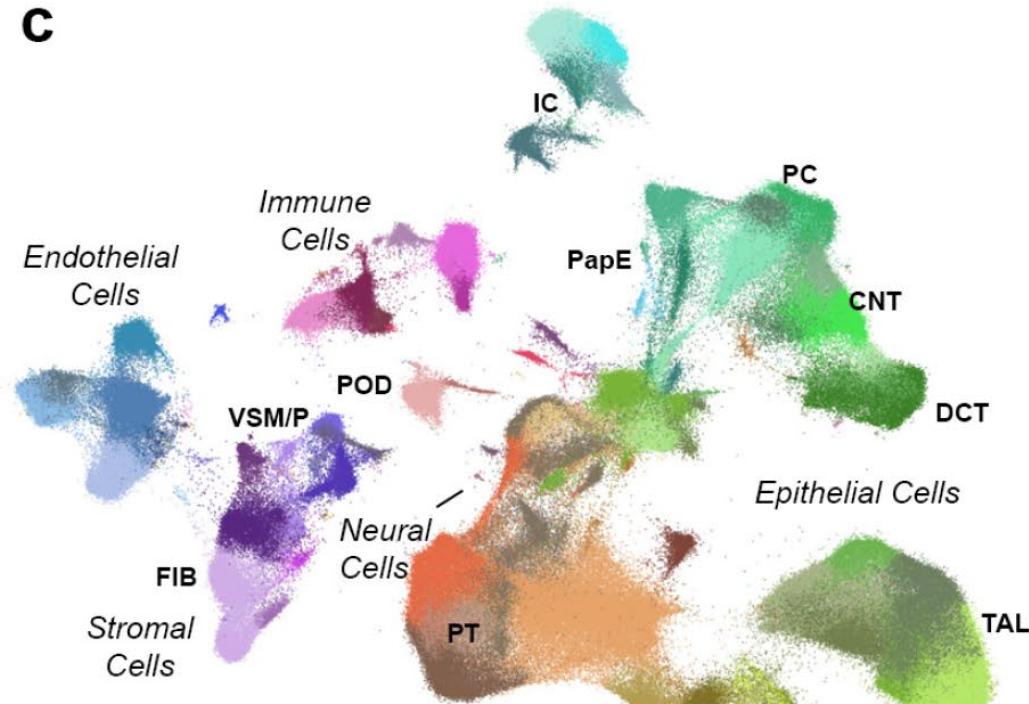


b

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



c





bioRxiv

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Kidney Cell Atlas Overview: Collaborators

An atlas of healthy and injured cell states and niches in the human kidney

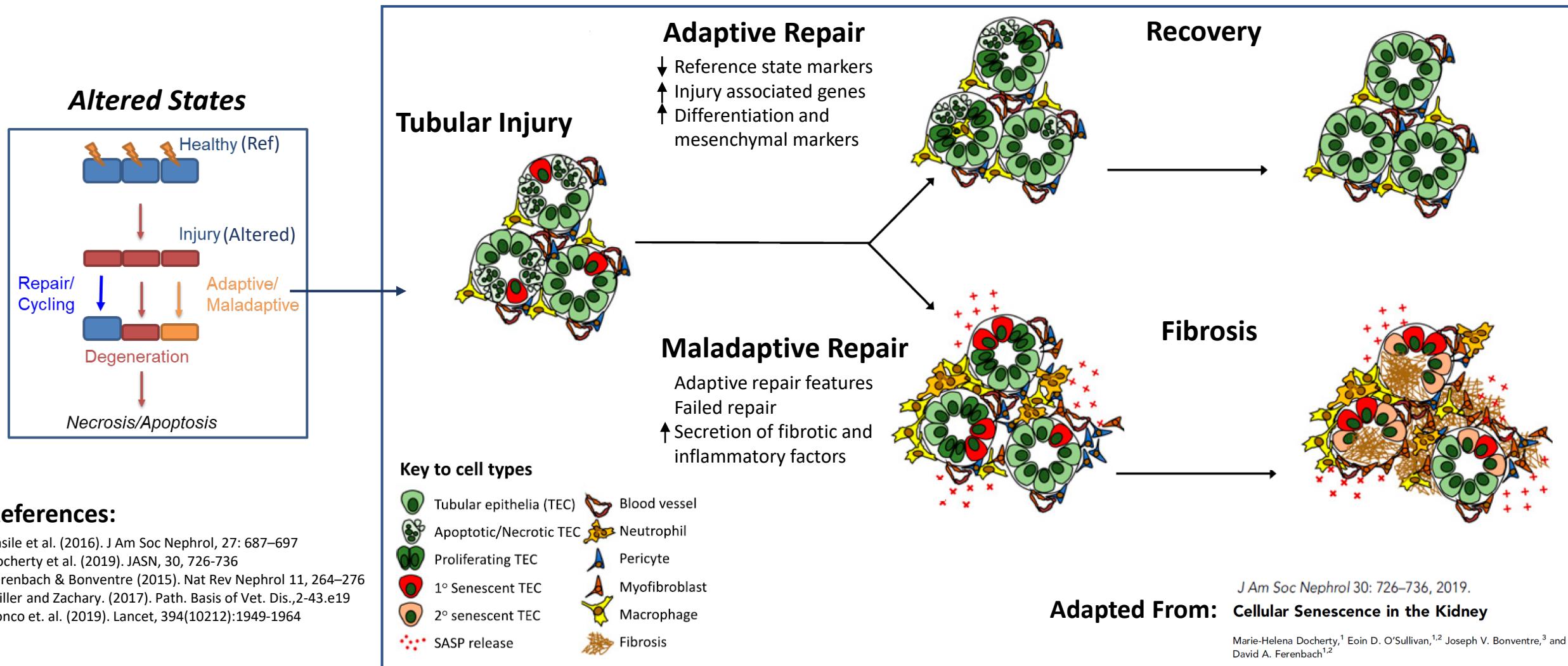
Blue B. Lake^{1*}, Rajasree Menon^{2*}, Seth Winfree^{3*}, Qiwen Hu^{4*}, Ricardo Melo Ferreira^{5*}, Kian Kalhor¹, Daria Barwinska⁵, Edgar A. Otto⁶, Michael Ferkowicz⁵, Dinh Diep¹, Nongluk Plongthongkum¹, Amanda Knoten⁷, Sarah Urata¹, Abhijit S. Naik⁶, Sean Eddy⁶, Bo Zhang⁷, Yan Wu¹, Diane Salomon⁷, James C. Williams⁵, Xin Wang⁴, Karol S. Balderrama⁸, Paul Hoover⁸, Evan Murray⁸, Anita Vijayan⁷, Fei Chen⁸, Sushrut S. Waikar⁹, Sylvia Rosas¹⁰, Francis P. Wilson¹¹, Paul M. Palevsky¹², Krzysztof Kiryluk¹³, John R. Sedor¹⁴, Robert D. Toto¹⁵, Chirag Parikh¹⁶, Eric H. Kim¹⁷, Evan Z. Macosko⁸, Peter V. Kharchenko⁴, Joseph P. Gaut¹⁸, Jeffrey B. Hodgin⁶, Michael T. Eadon⁵, Pierre C. Daghet^{5,‡}, Tarek M. El-Achkar^{5,‡}, Kun Zhang^{1,‡}, Matthias Kretzler^{6,‡}, Sanjay Jain^{7,18,‡}, for the KPMP consortium¹⁹.

DOI: 10.1101/2021.07.28.454201

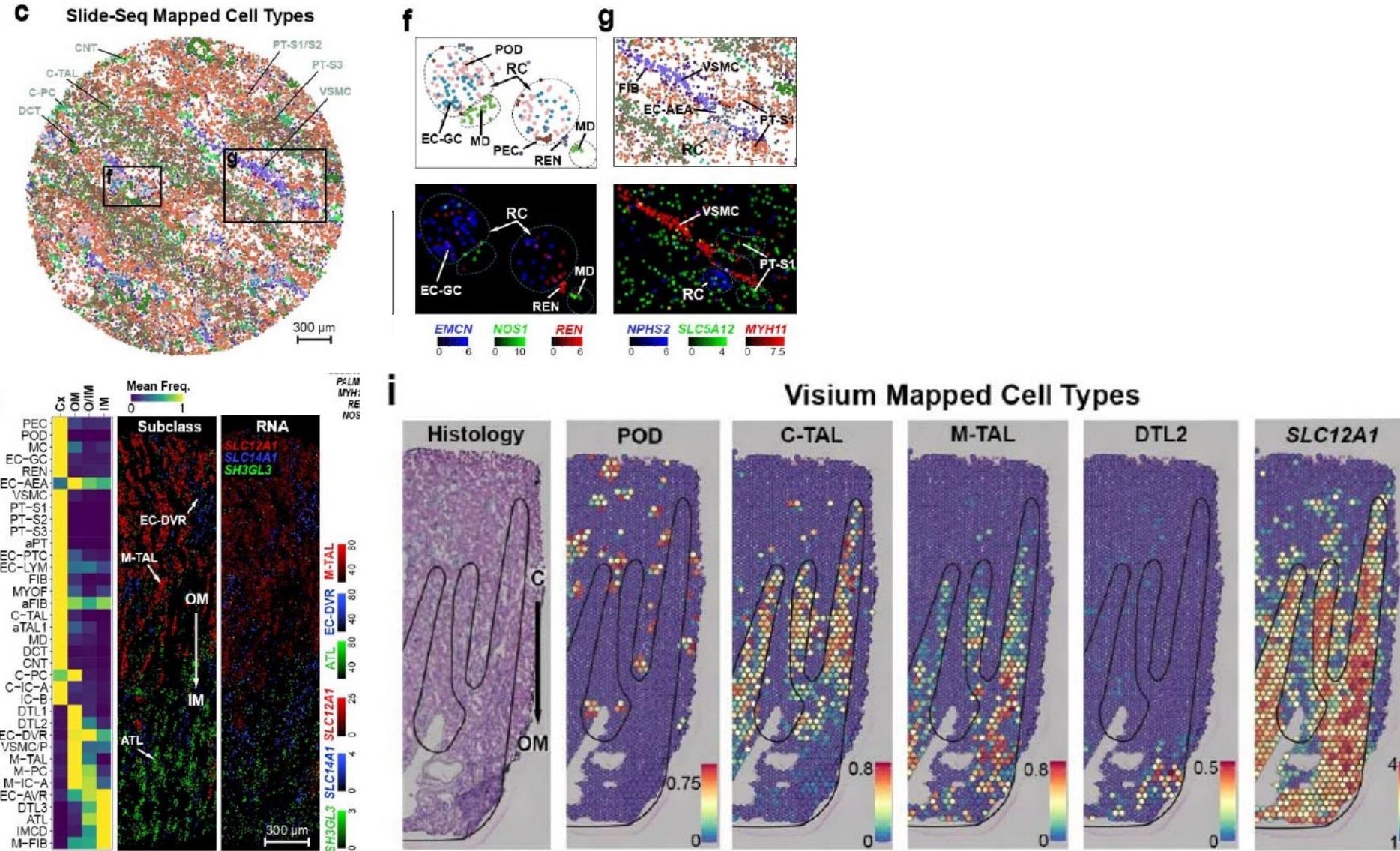
Assay	Sex		Condition		
	♂	♀	CKD	AKI	Ref
10X snRNA-Seq	19	17	13	10	13
10X scRNA-Seq	21	24	15	12	18
SNARE-Seq2	3	4	-	-	7
3D Imaging	6	9	9	6	-
Slide-seq2	5	1	-	-	6
Visium	12	10	10	6	6



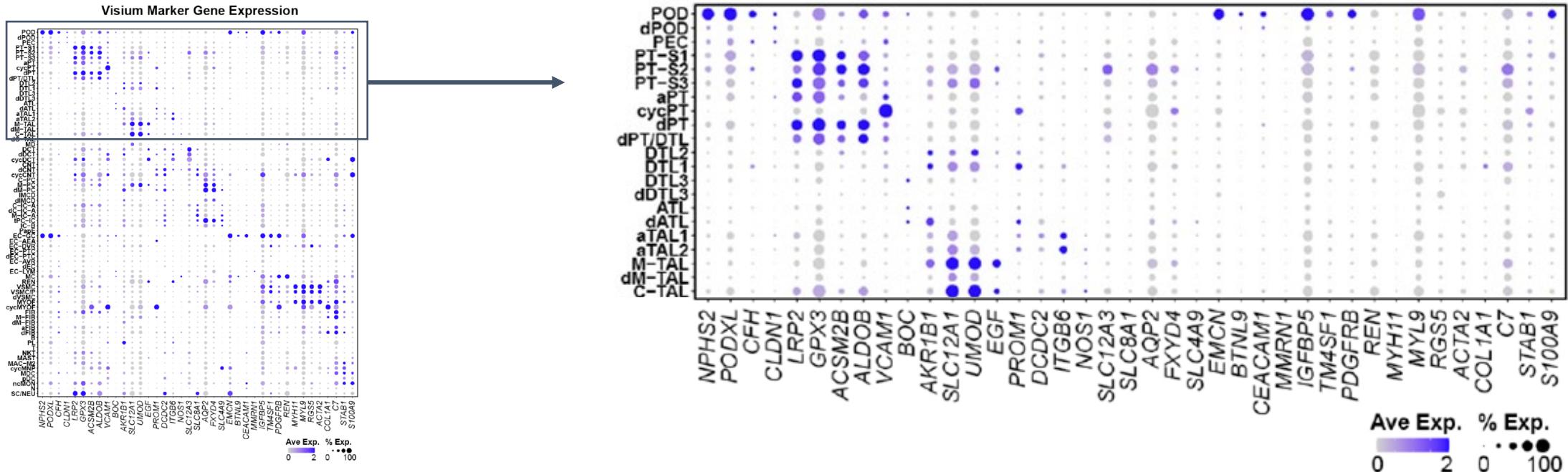
Defining Altered States in Injury



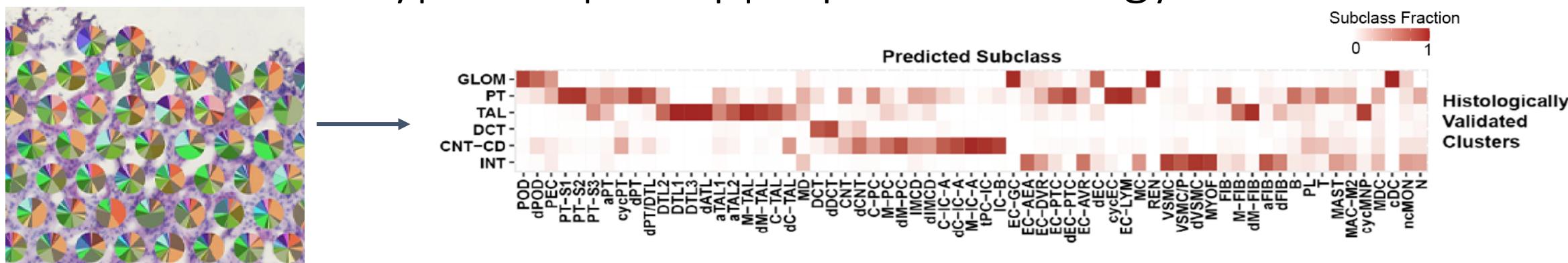
Spatial mapping of known cells

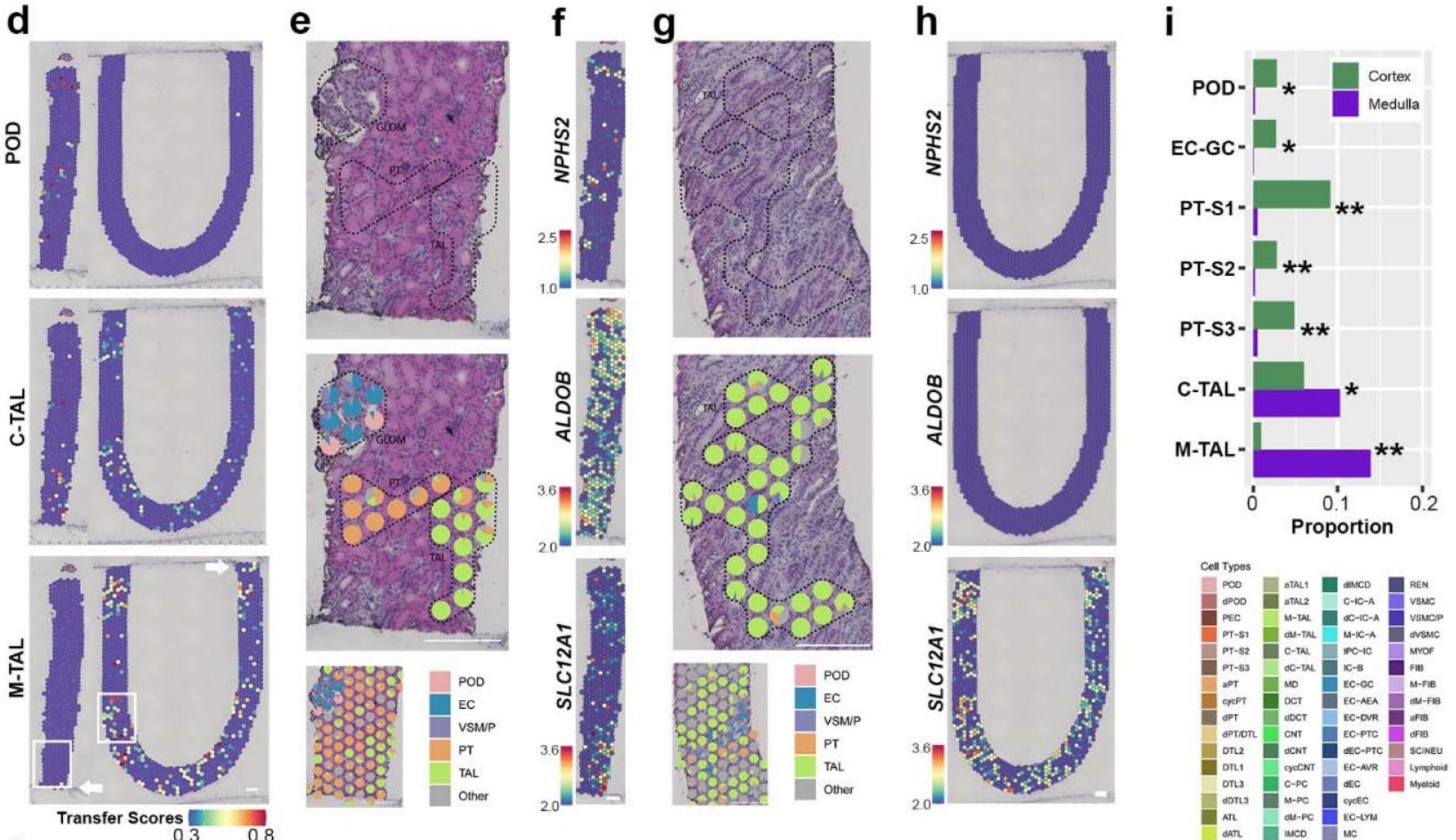


Cell types express expected markers

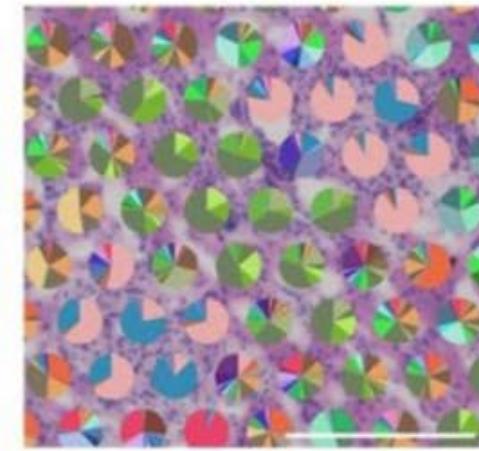
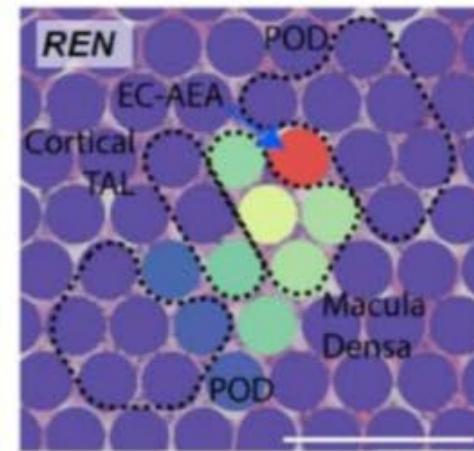
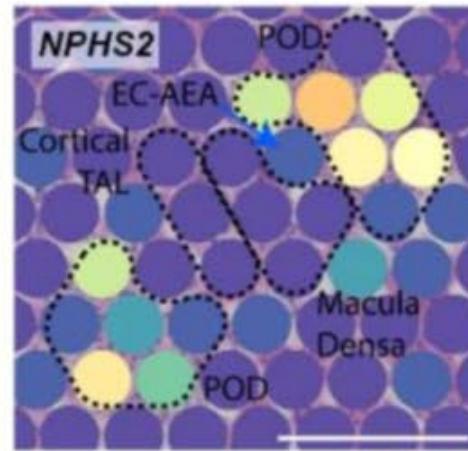
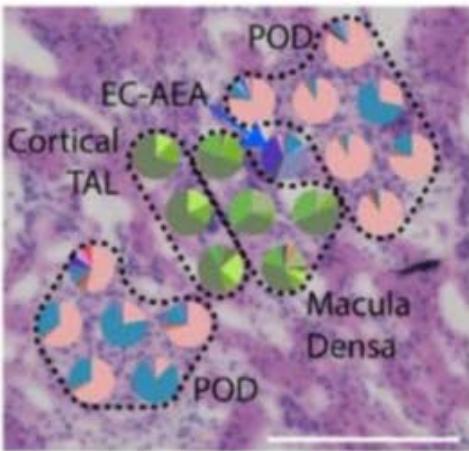
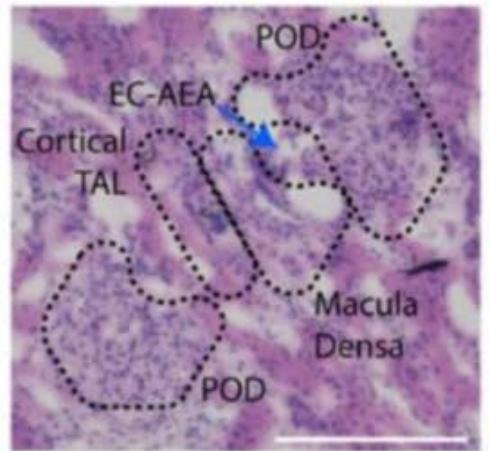


Cell types map to appropriate histology

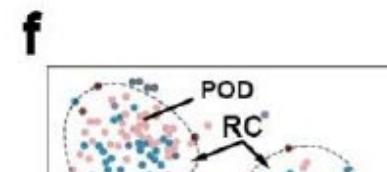
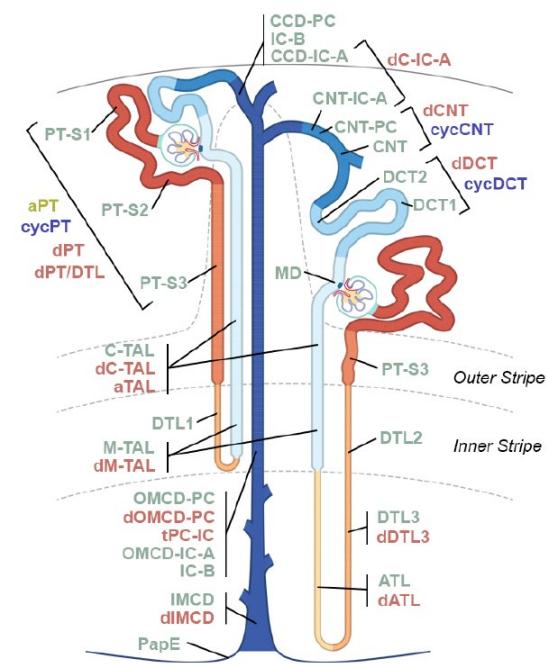




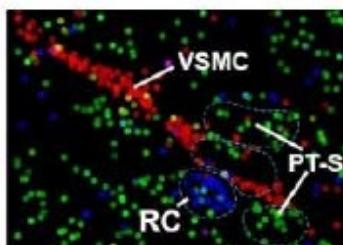
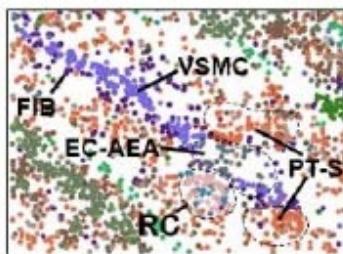
Renin producing cells



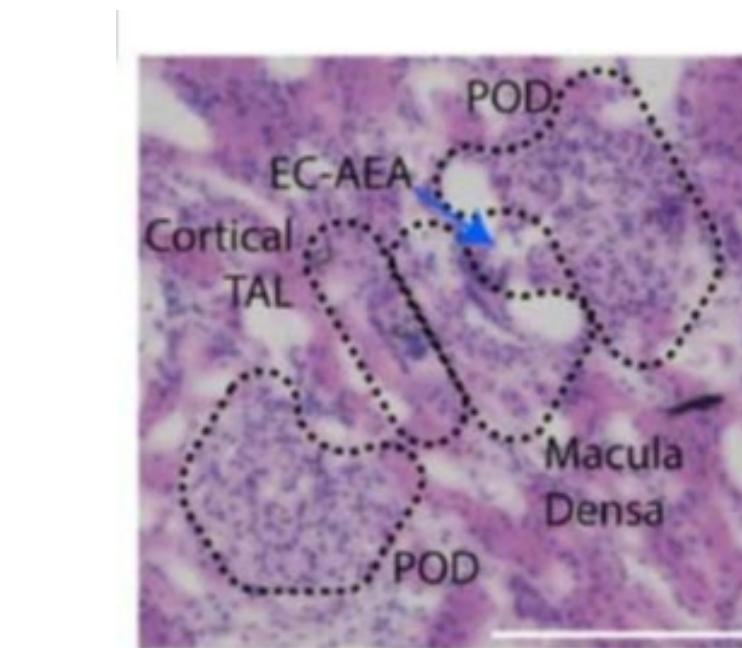
Nephron

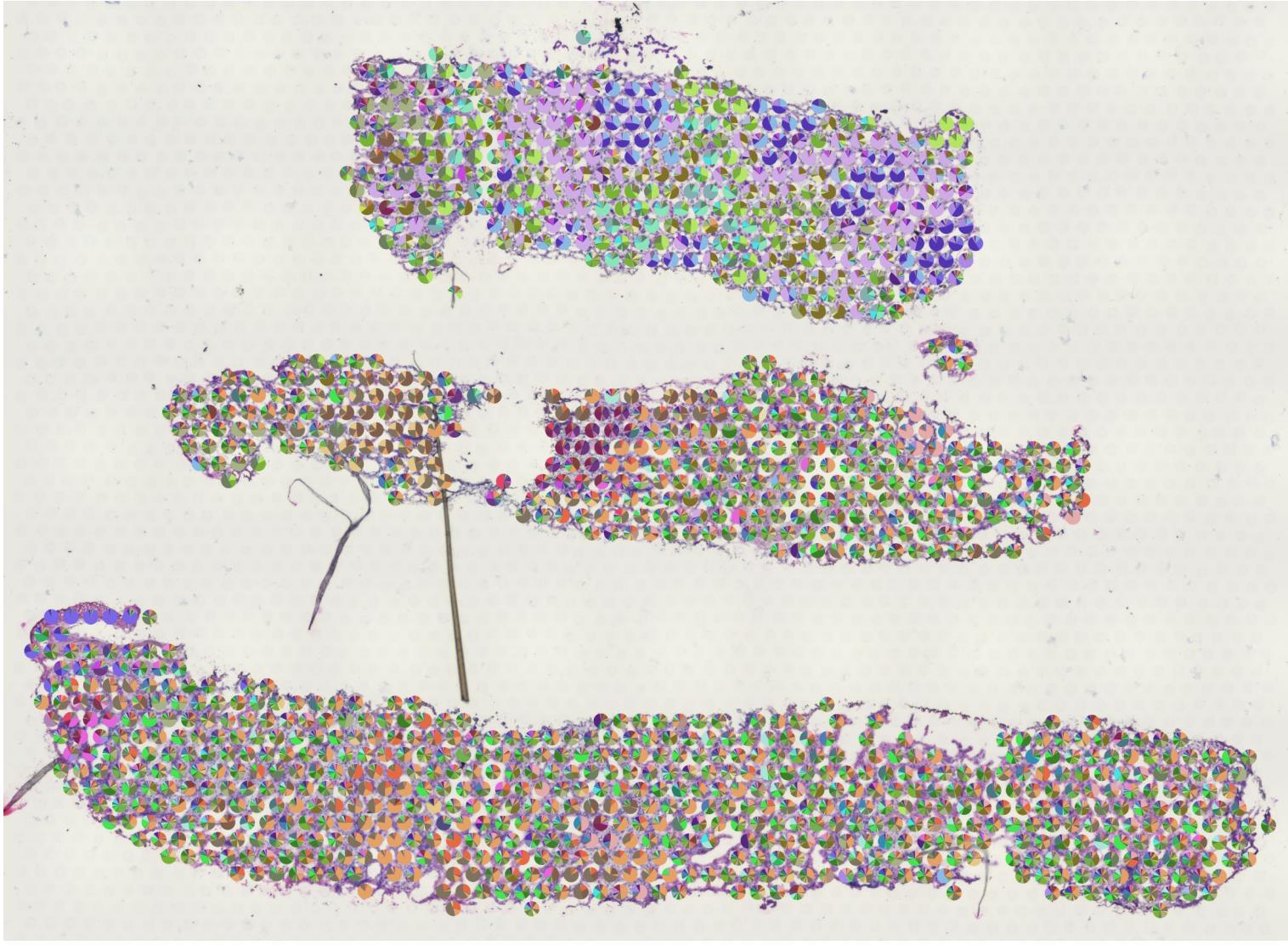


f



EMCN 0 6 *NOS1* 0 10 *REN* 0 6
NPHS2 0 6 *SLC5A12* 0 4 *MYH11* 0 7.5





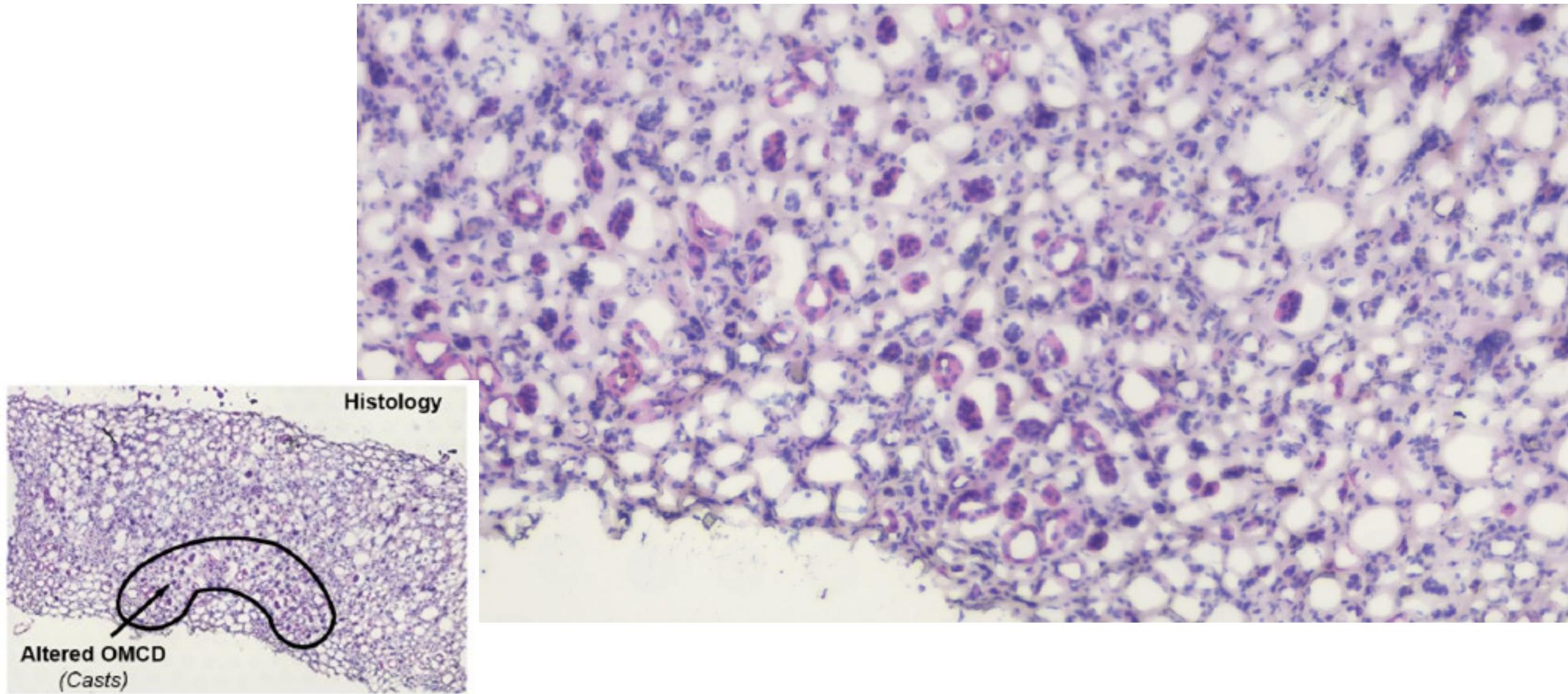
Subclass

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	

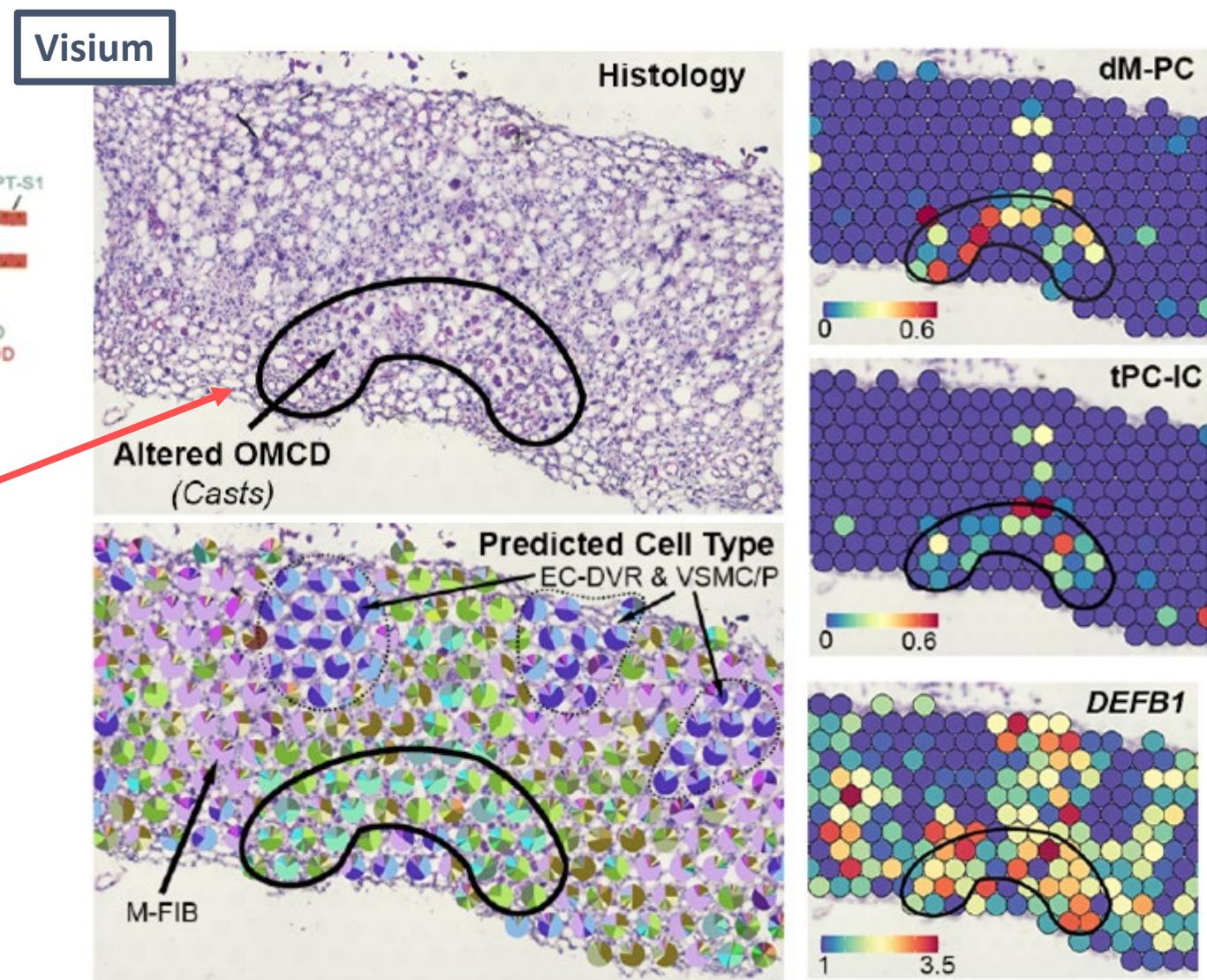
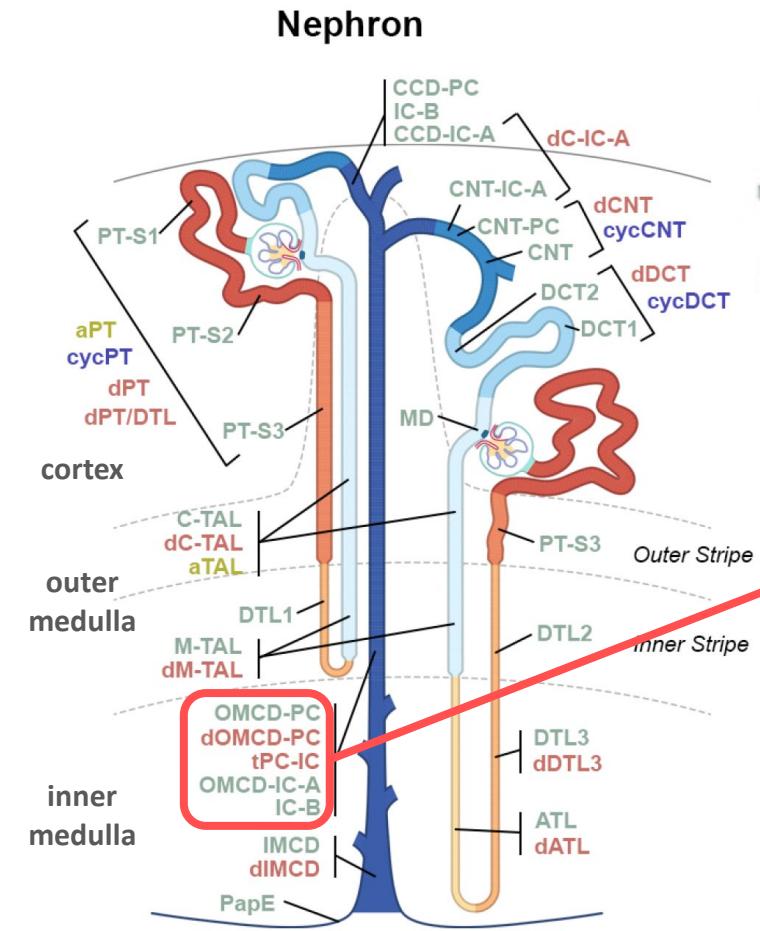


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Region of casts in the medulla of a DKD biopsy sample

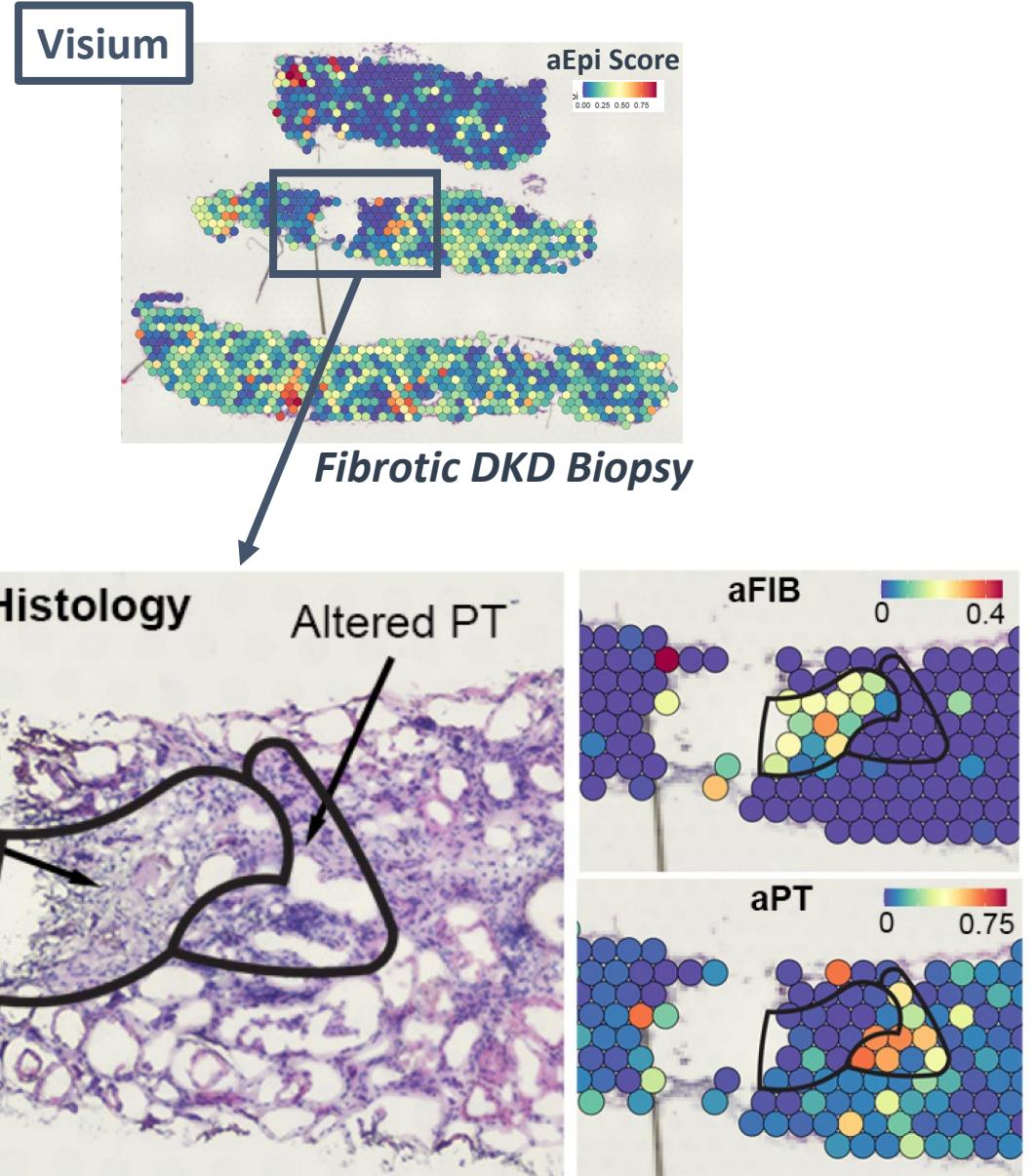
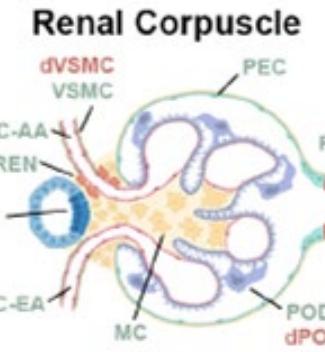
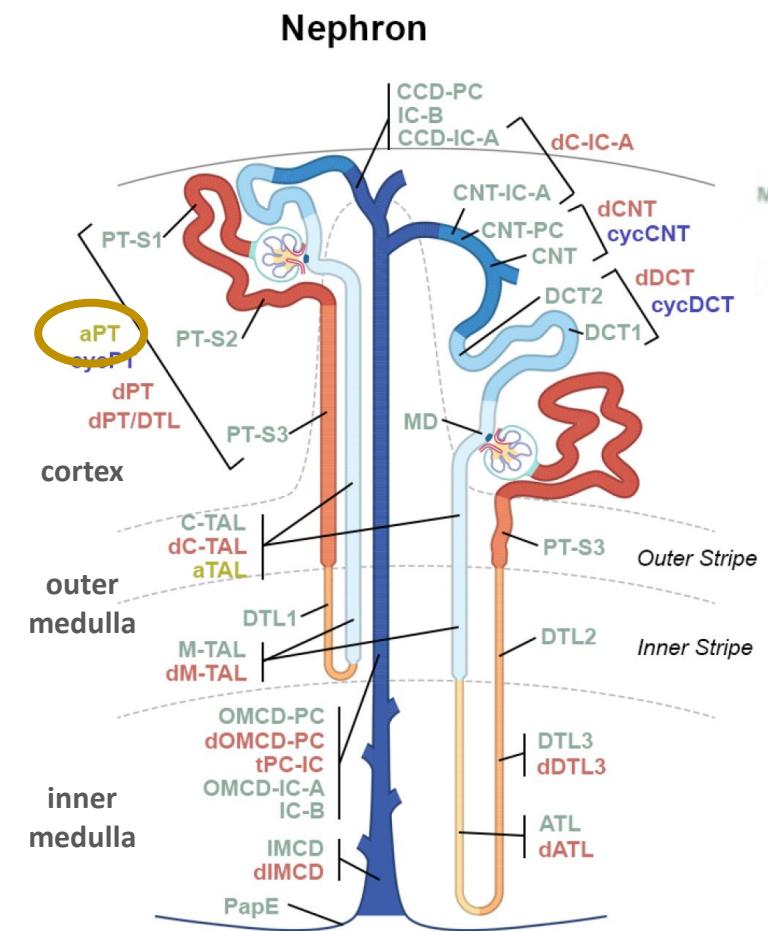


Degenerative States



Ricardo Melo Ferreira

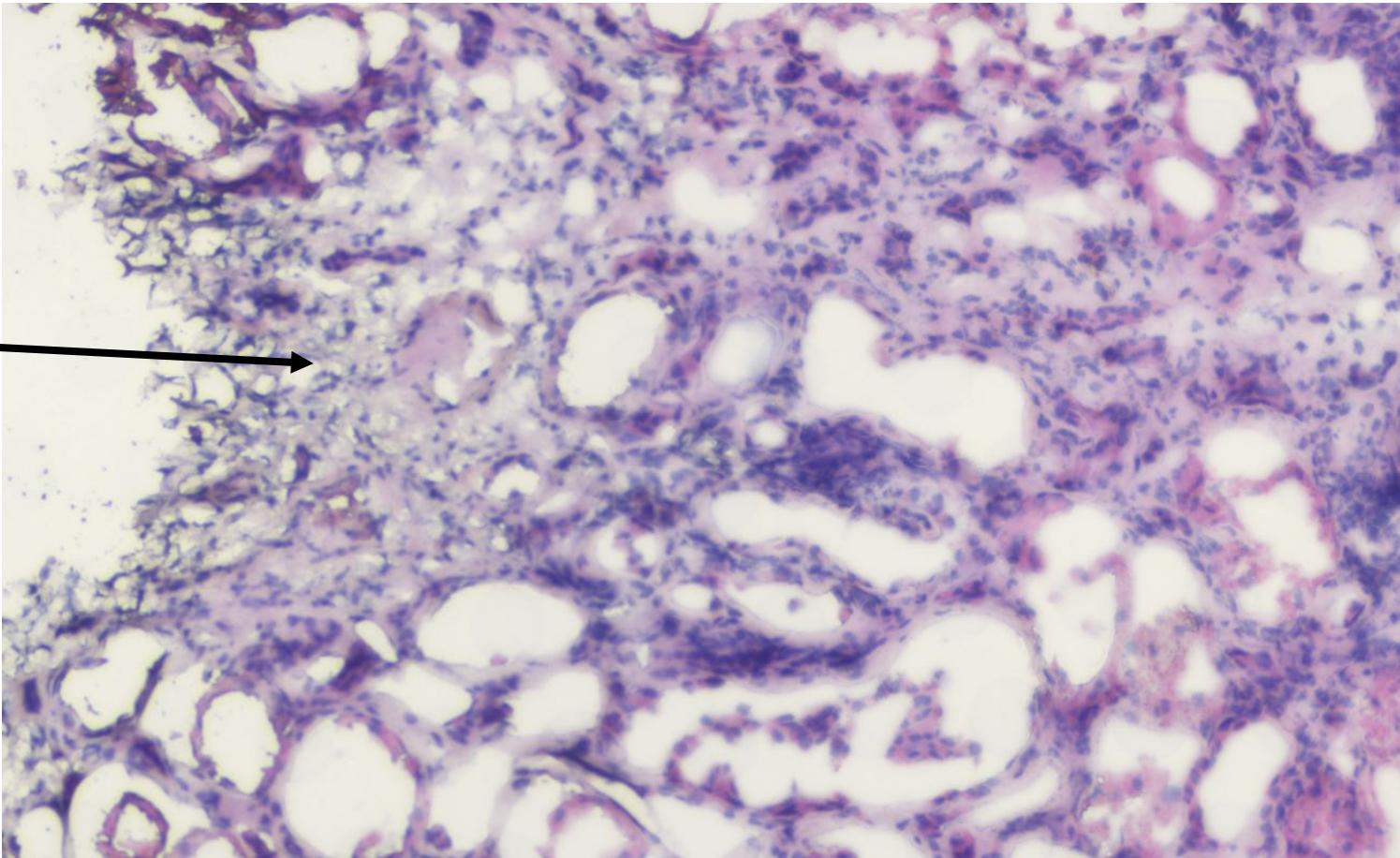
Adaptive Repair States



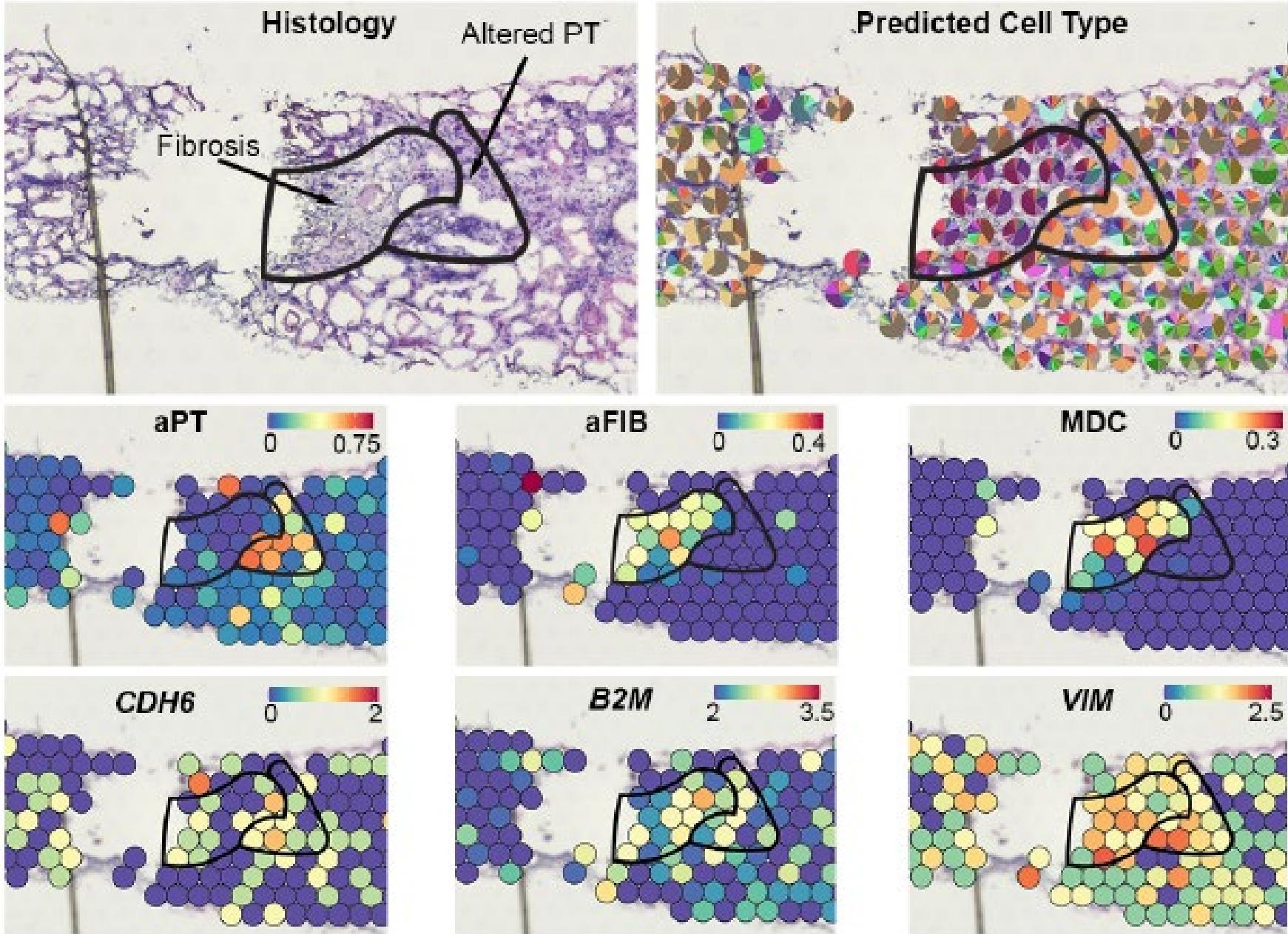
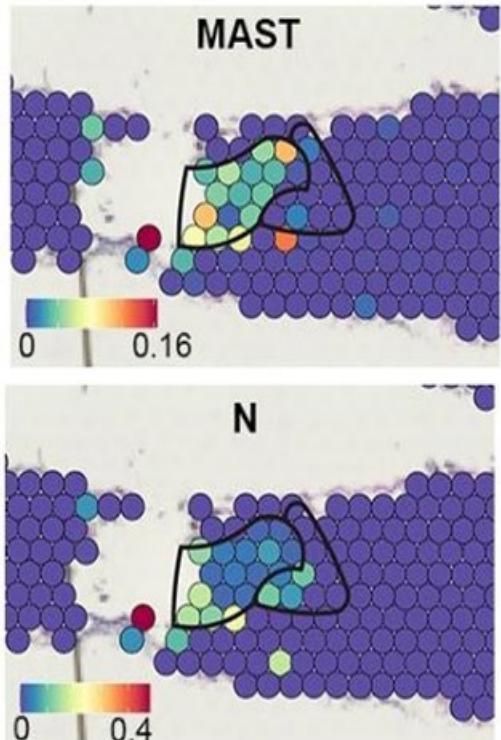
Ricardo Melo Ferreira

Fibrosis and injured tubules in the DKD sample

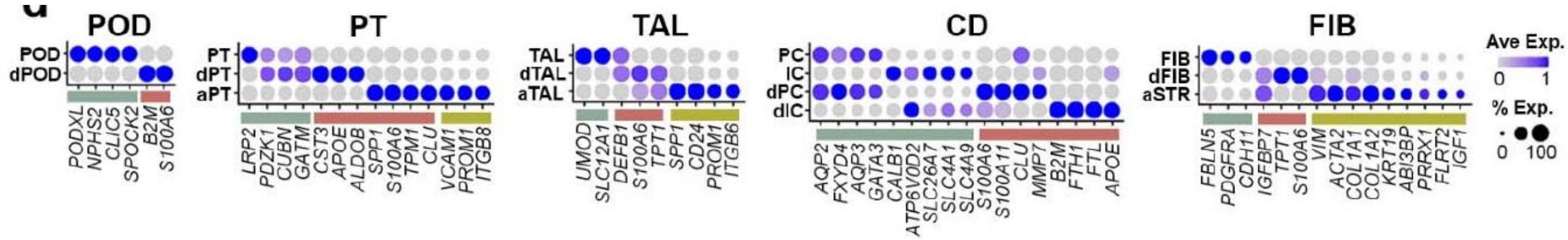
Fibrotic
Region



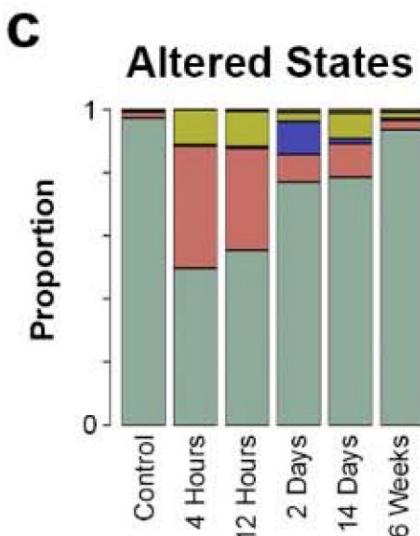
In this fibrotic region, we see adaptive fibroblasts (aFIB) and immune cells.



Altered cell states lose marker gene expression

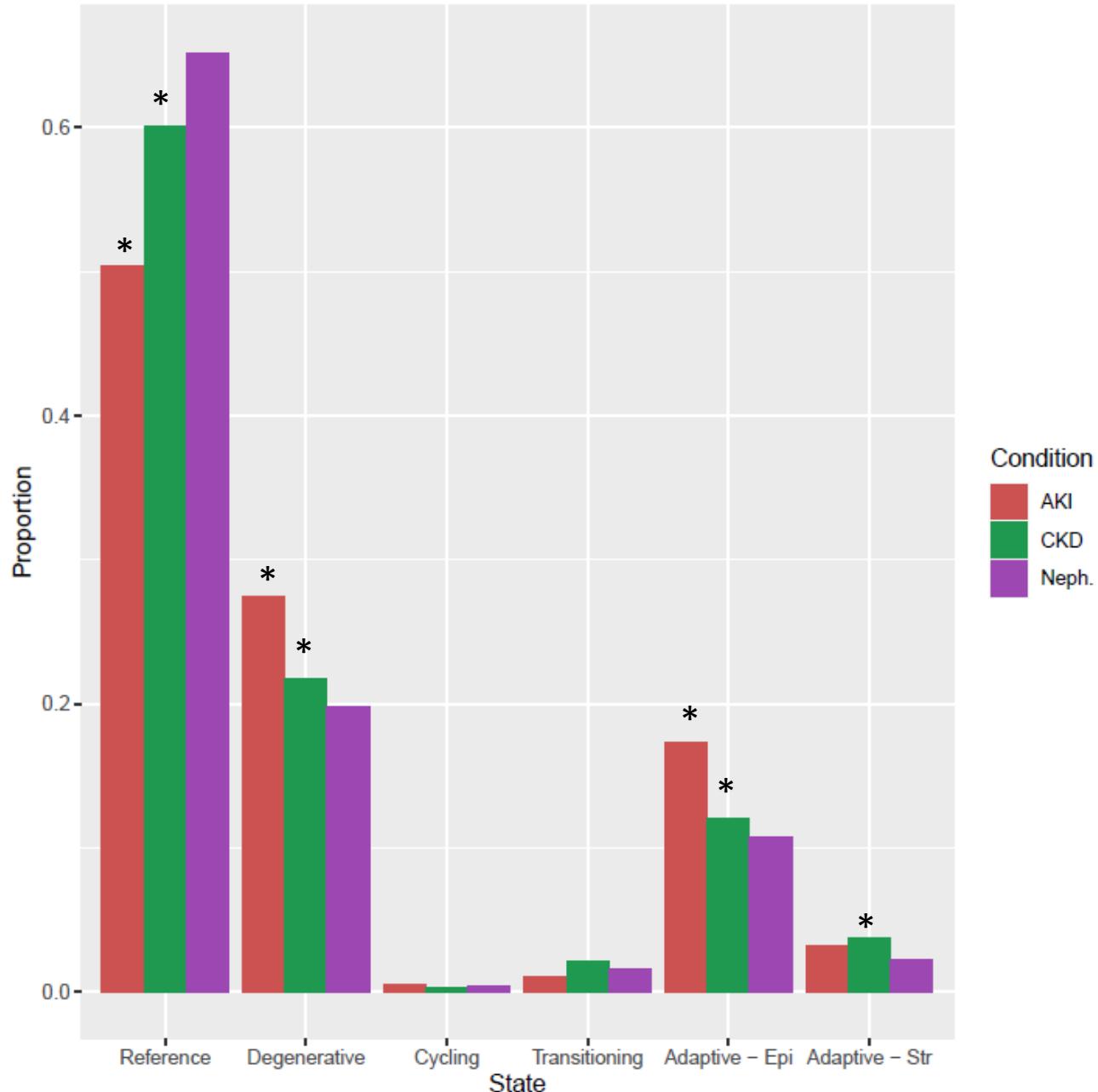


- Mouse model

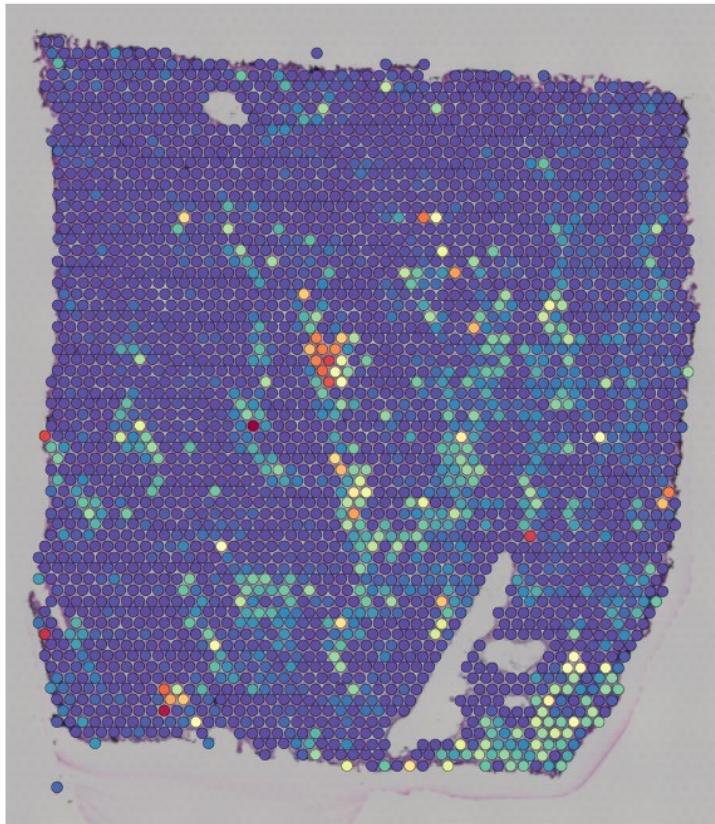
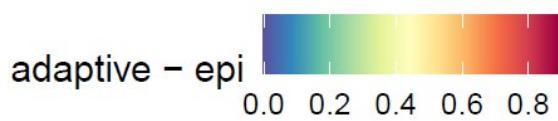


Cell State Mapping

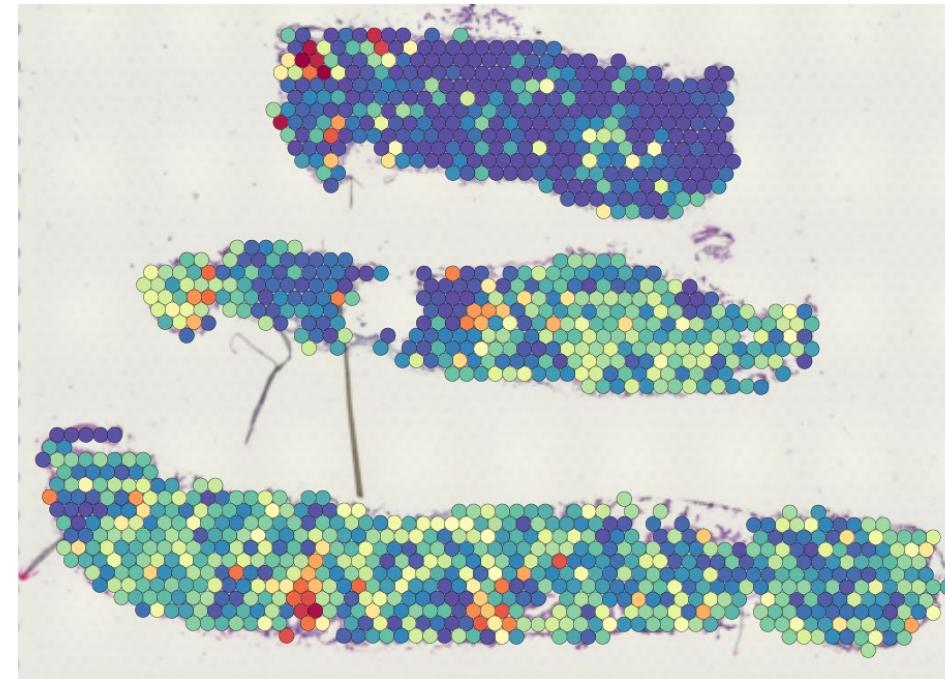
- 23 samples
- 6 nephrectomies
- 6 AKI (ATN) samples
- 11 CKD samples
- Adaptive and degenerative cell states are more common in AKI and CKD samples.



Cell Type + State proportions

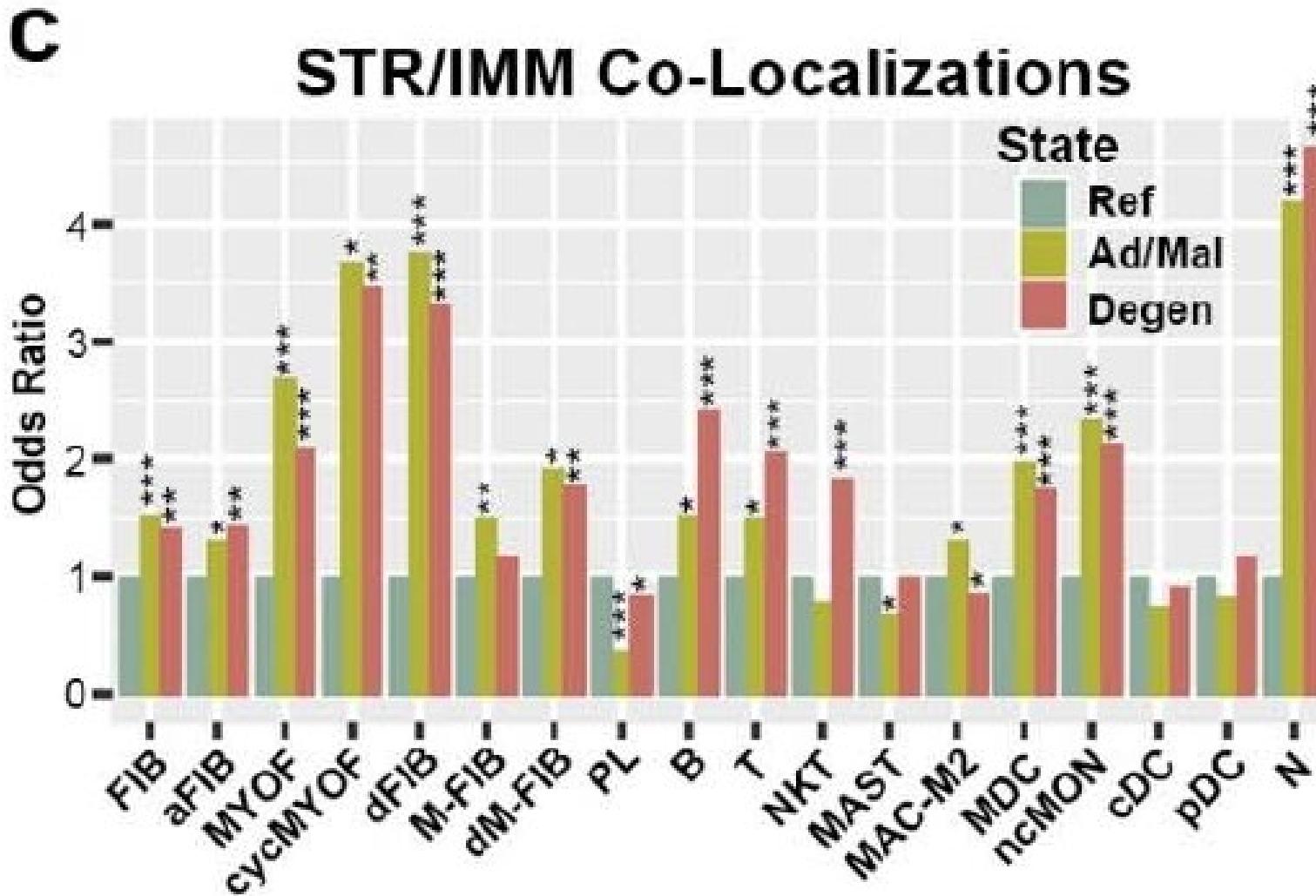


Reference Nephrectomy



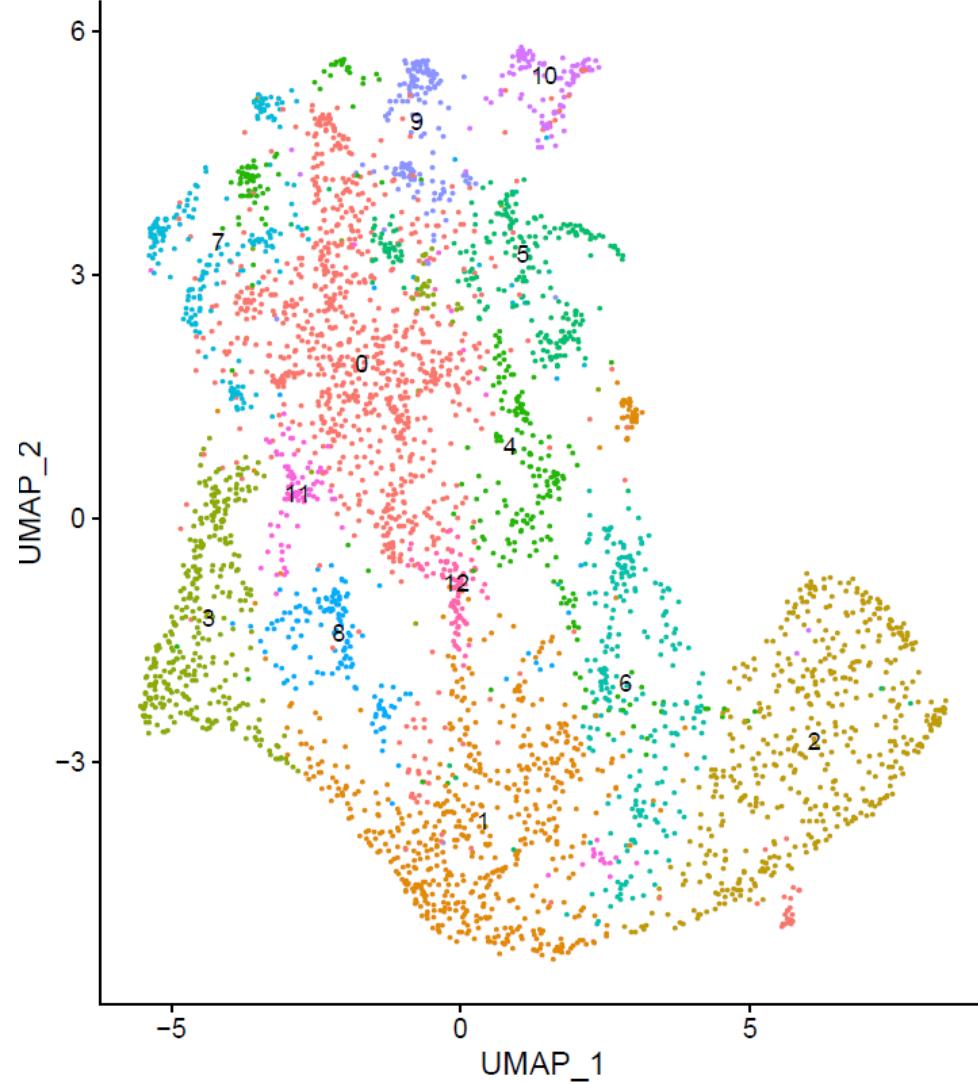
Fibrotic DKD biopsy sample

All epithelial cells

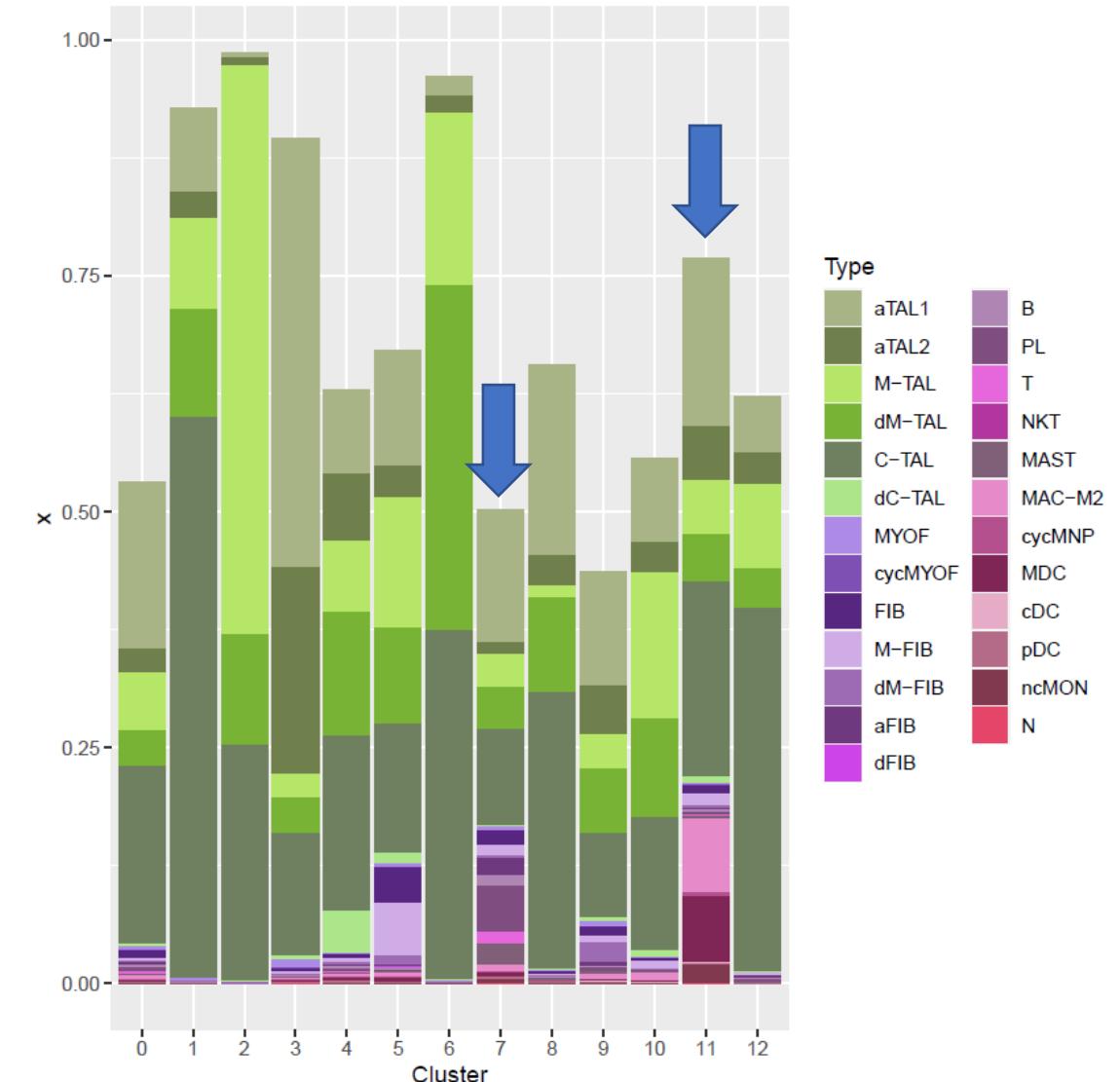


TAL Neighborhood clustering

(Clusters based on colocalized Seurat Transfer scores)



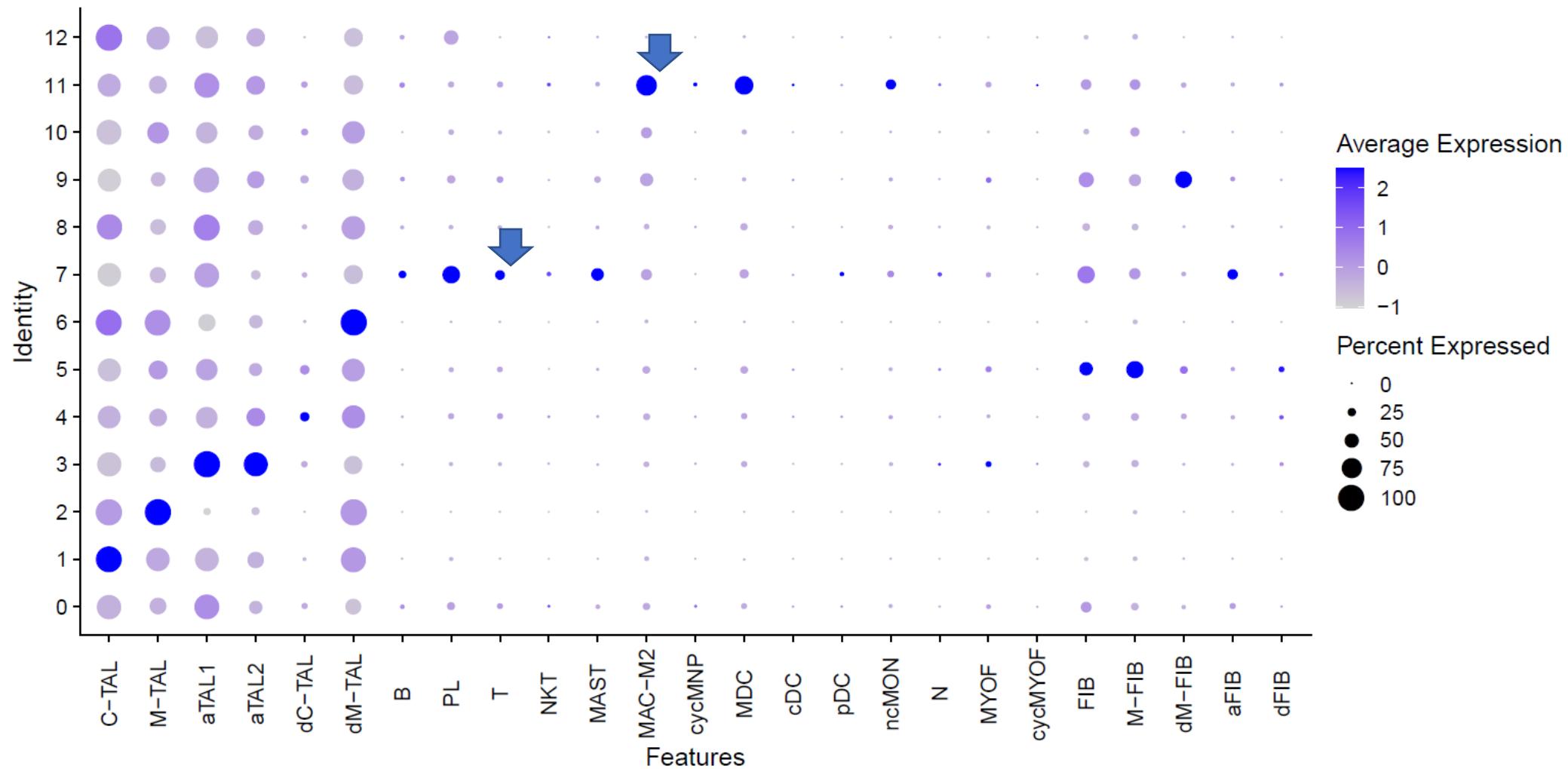
0
1
2
3
4
5
6
7
8
9
10
11
12



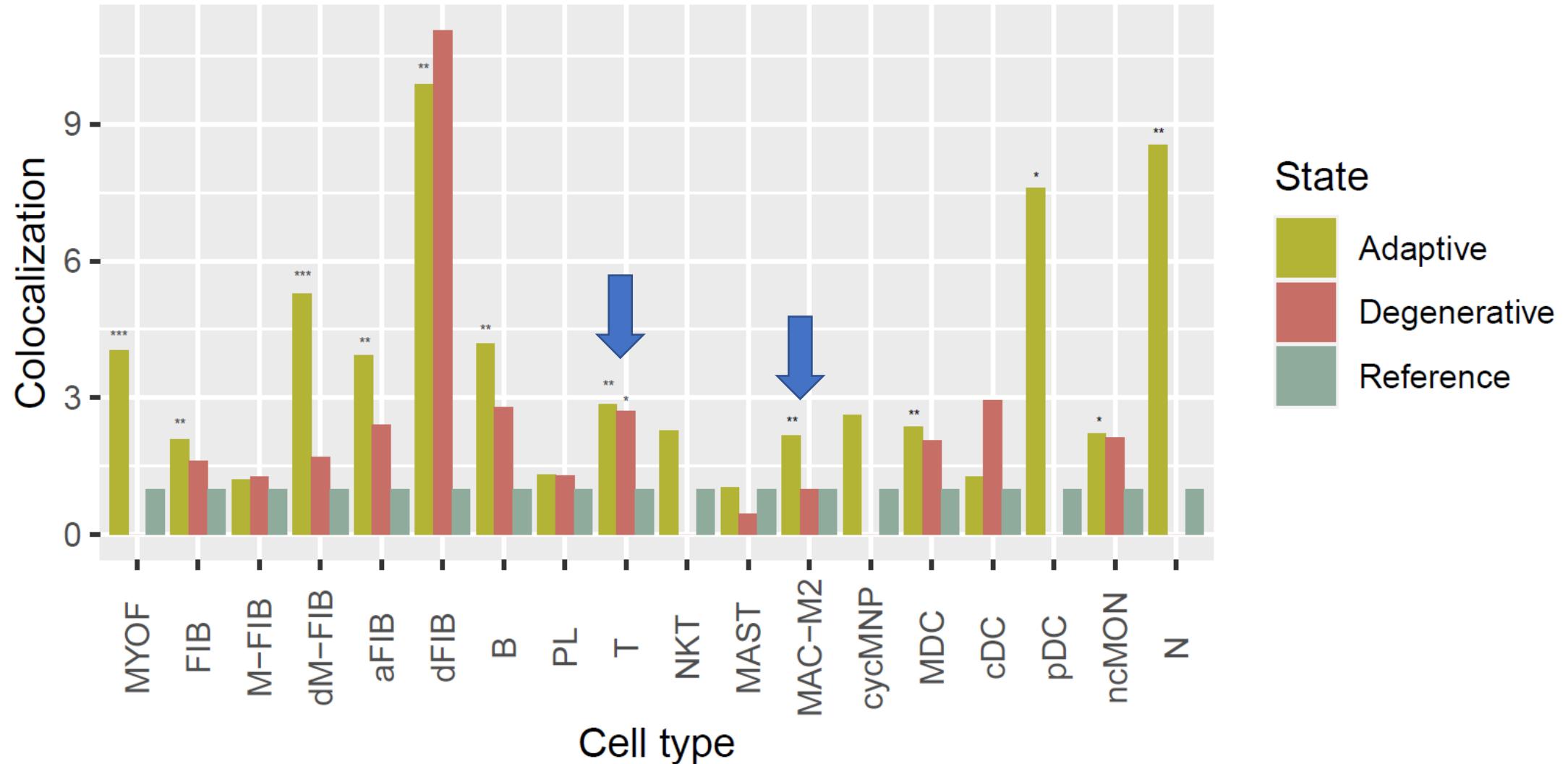
Type

aTAL1	B
aTAL2	PL
M-TAL	T
dM-TAL	NKT
C-TAL	MAST
dC-TAL	MAC-M2
MYOF	cycMNP
cycMYOF	MDC
FIB	cDC
M-FIB	pDC
dM-FIB	ncMON
aFIB	
dFIB	N

13 Neighborhoods



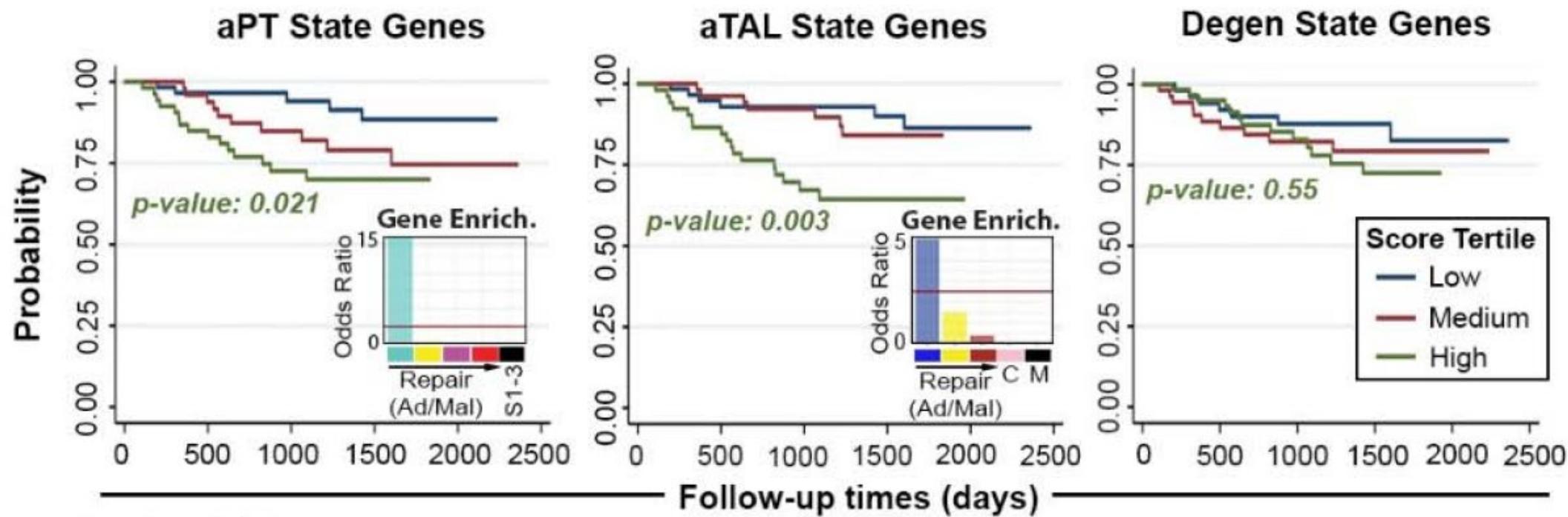
Immune cells colocalize with adaptive TAL cell state



e

NEPTUNE Cohort Survival Curves

(Composite ESRD or 40% Decline in eGFR)



Number at risk

65	53	37	27	1	0
65	43	32	20	1	0
63	44	30	17	0	0

65	48	34	29	2	0
66	49	39	17	0	0
62	43	26	18	0	0

64	45	33	20	1	0
64	44	32	21	1	0
65	51	34	23	0	0

Acknowledgments

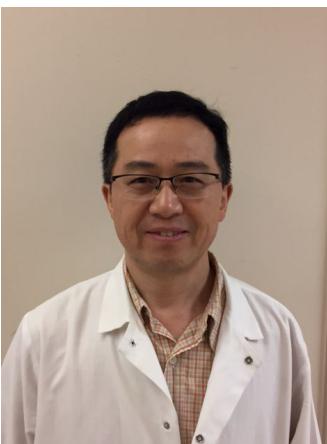


- IU

- Pierre Dagher
- Tarek Ashkar
- Ricardo Melo Ferreira
- Daria Barwinska
- Debora Gisch
- Mahla Asghari
- Yinghua Cheng
- Seth Winfree
- Ken Dunn
- Michael Ferkowicz
- Timothy Sutton
- Katherine Kelly



Ricardo Melo Ferreira



Yinghua Cheng



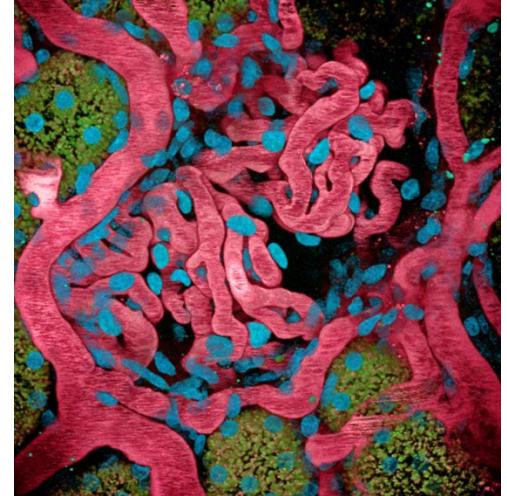
KIDNEY PRECISION
MEDICINE PROJECT



Debora Gisch



Mahla Asghari



**Indiana O'Brien Center for
Advanced Microscopic Analysis
3D Tissue Imaging Core**

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