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# Introduction to 10X Visium and Xenium spatial transcriptomics technologies and applications to kidney disease

Ricardo Melo Ferreira

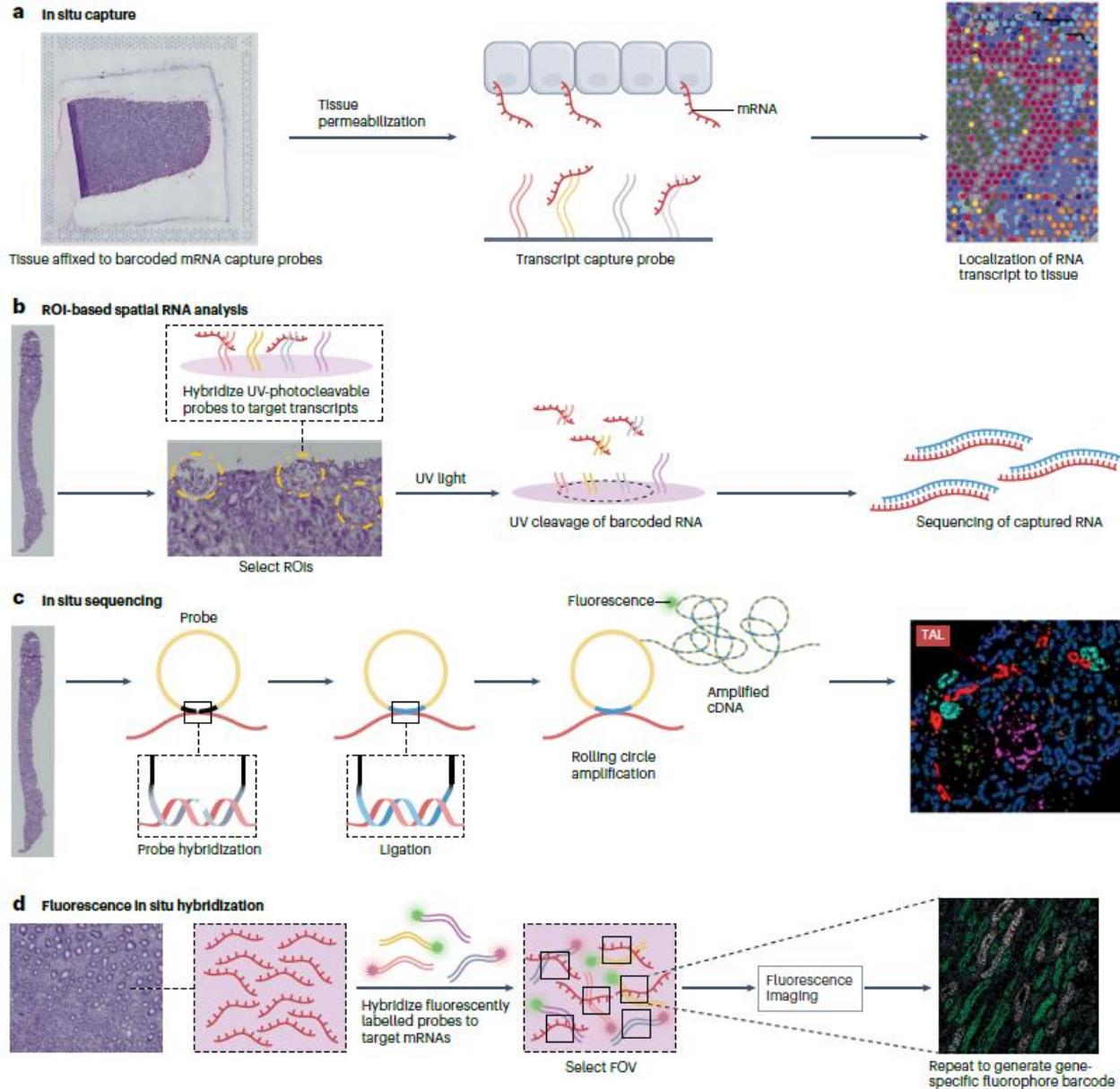
Indiana University School of Medicine

# Disclosures

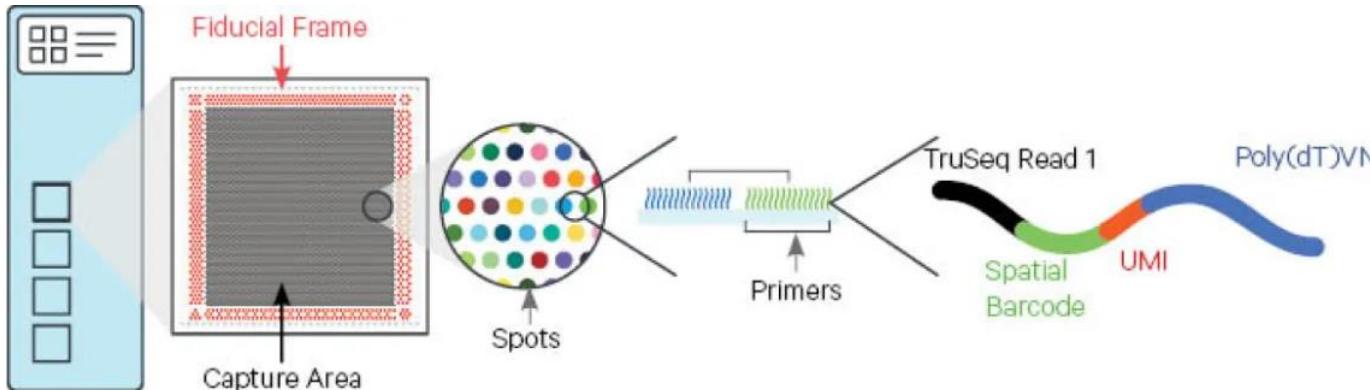
- NIH Funding
- DCI Funding

# Technologies overview

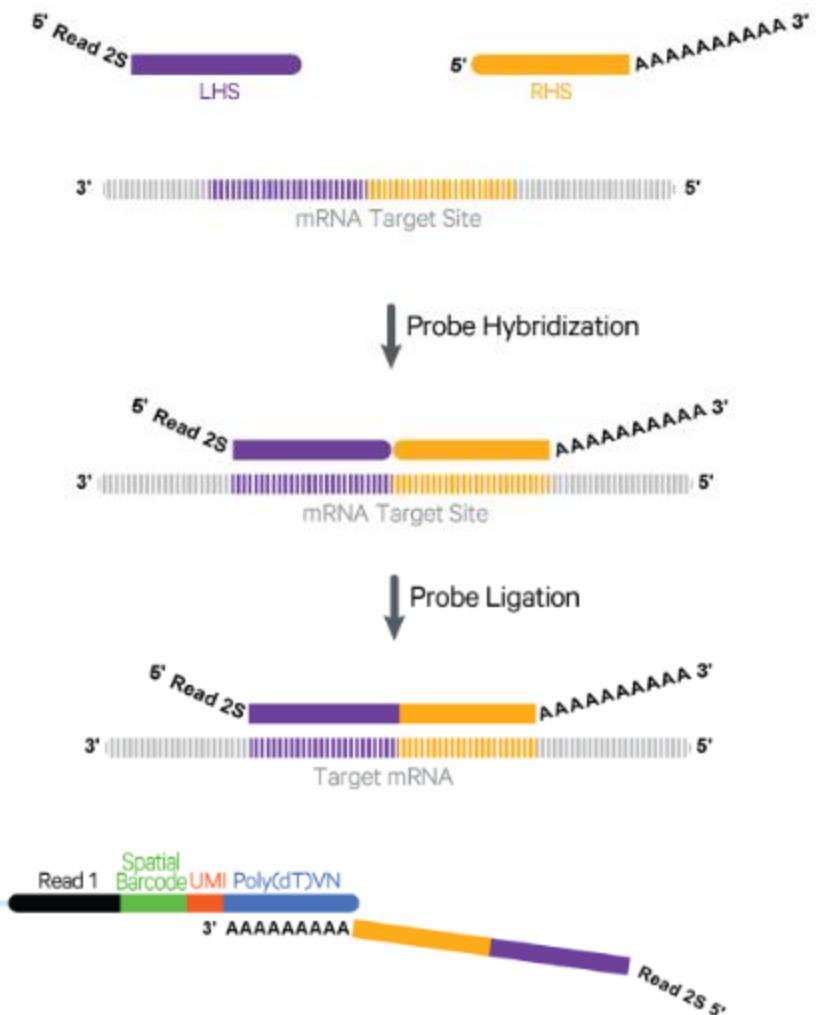
- Localization of RNA over tissue morphology.
- Two main groups are sequencing and imaging based.
- We will focus on 10X Visium and Xenium



# 10X Visium



## FFPE & CytAssyt



### Advantages:

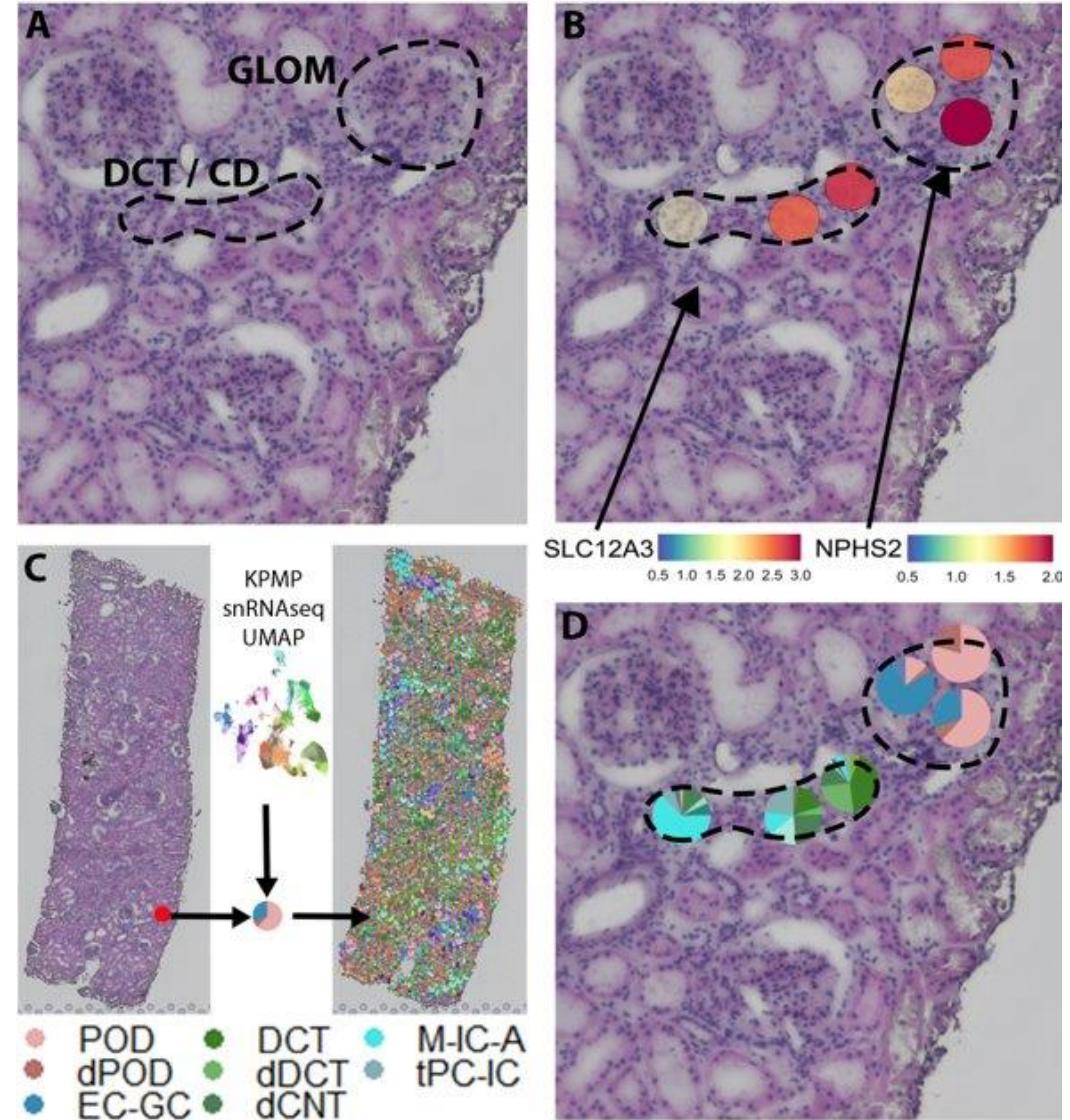
- Whole transcriptome
- FFPE: 18,536 genes targeted by 54,018 probes.
- Brightfield or IF image (histology preservation in FFPE)

### Disadvantages:

- Spots with 55 µm in diameter, 100 µm apart

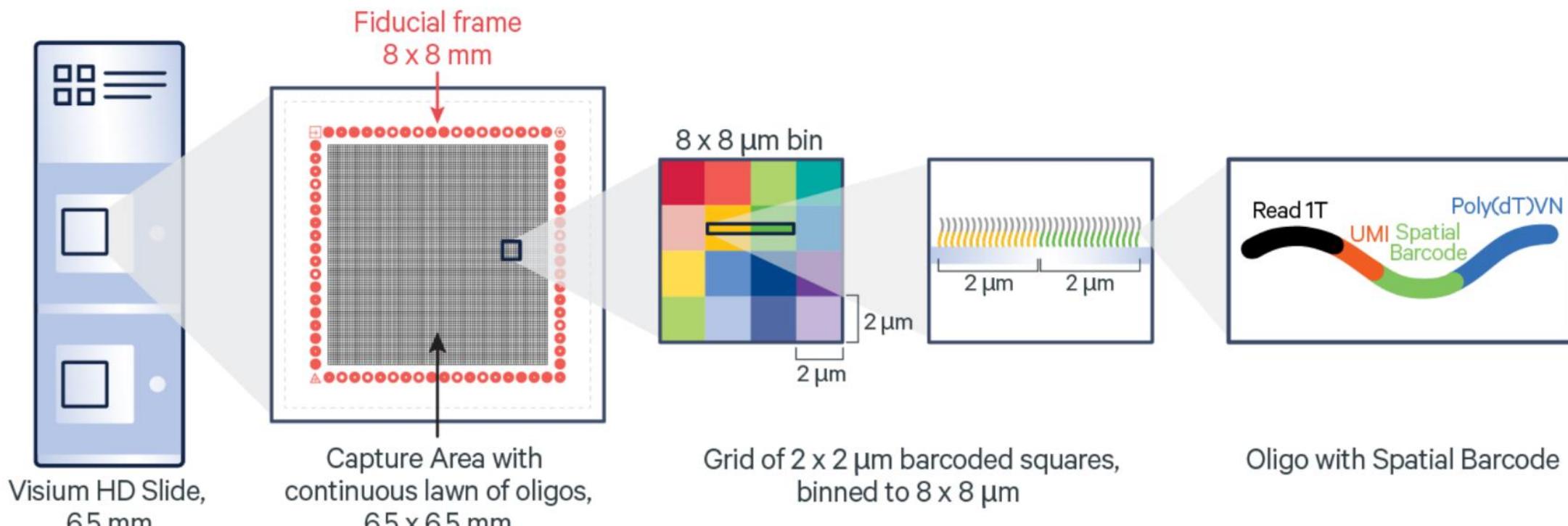
# Cell type deconvolution

- Each spot covers multiple cells
- Cell type proportion is derived from a reference dataset
- Multiple methods available: Seurat; RCTD; CellTrek; Cell2Location; CARD; Tangram; SPOTlight, etc...



Melo Ferreira R, Gisch D, Eadon MT  
*Curr Opinion Neph Hypertension* 2022

# 10X Visium HD



## Advantages:

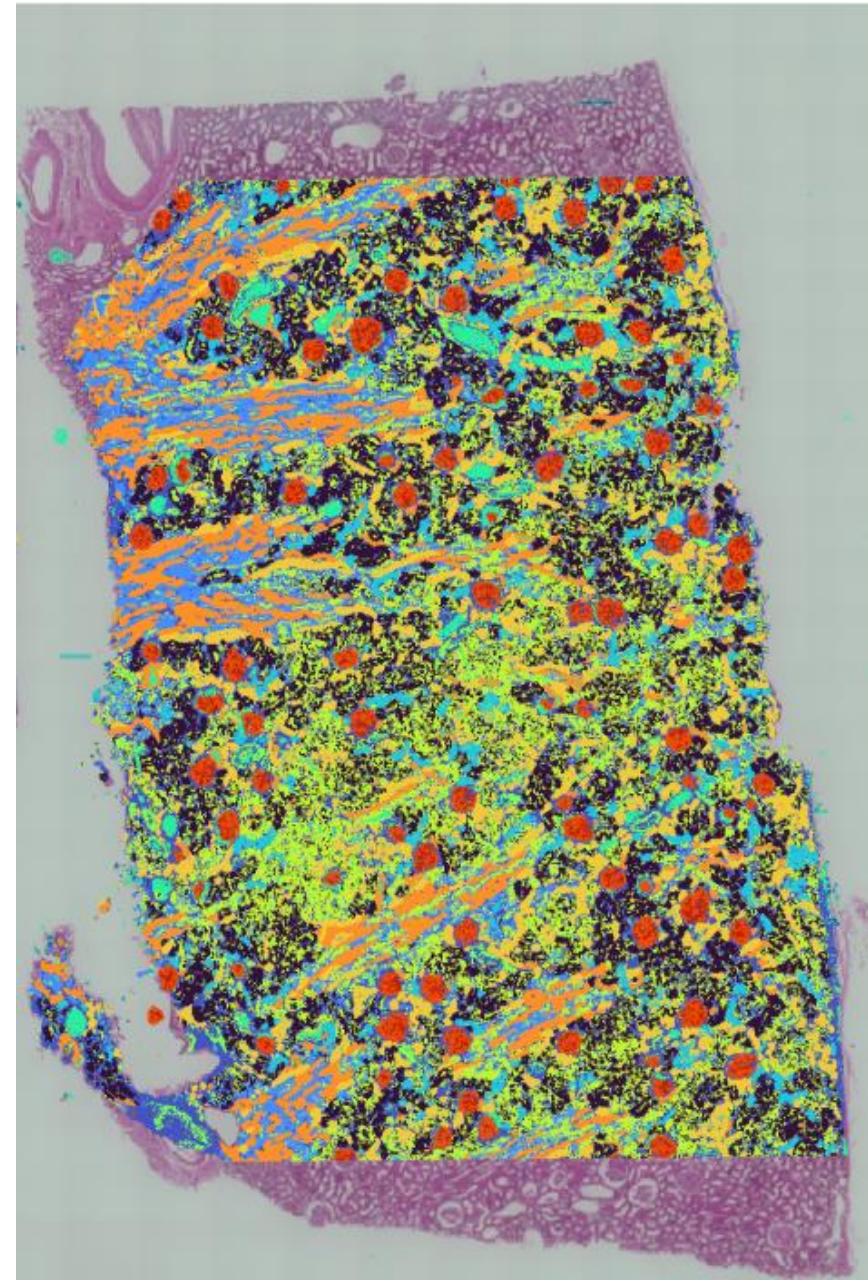
- Resolution of 2  $\mu\text{m}$

## Disadvantages:

- Only probes
- Cost
- Cell segmentation

# Unsupervised Clustering (8 μm)

	Markers	Cell type
Cluster 1	PRODH2, SLC22A8	PT-S1
Cluster 2	SERPINA1, APOE	adaptive / degenerative PT
Cluster 3	STAB1, CD14, CD163	Macrophages
Cluster 3	COL1A1, COL1A2	Fibroblast
Cluster 4	AQP2	Collecting Duct - Principal cell
Cluster 4	CLNQ	Collecting Duct - Intercalated cell
Cluster 5	SLC7A13, SLC22A7	PT-S3
Cluster 6	SLC12A1, UMOD	TAL
Cluster 7	SLC12A3, TRPM6	DCT
Cluster 8	NPHS1, NPHS2, PODXL	Podocytes
Cluster 9	No clear markers, low expression	Luminal space, to be excluded
Cluster 10	PLAT, EGCW2, SOST	Glomerular capillaries
Cluster 11	NTRK3, MY11	VSMC



- Cluster 1
- Cluster 2
- Cluster 3
- Cluster 4
- Cluster 5
- Cluster 6
- Cluster 7
- Cluster 8
- Cluster 9
- Cluster 10
- Cluster 11

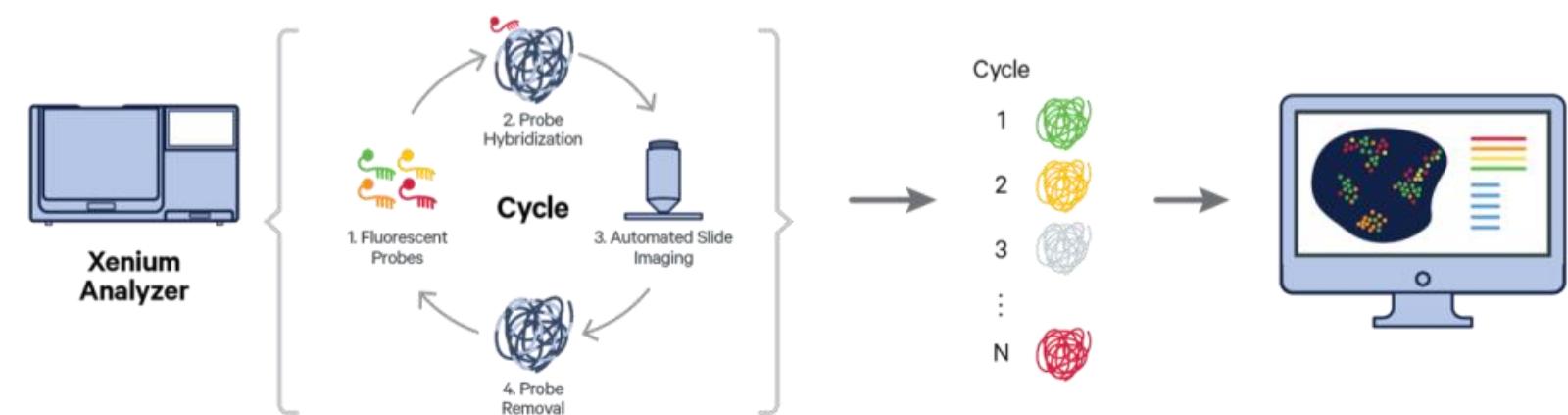
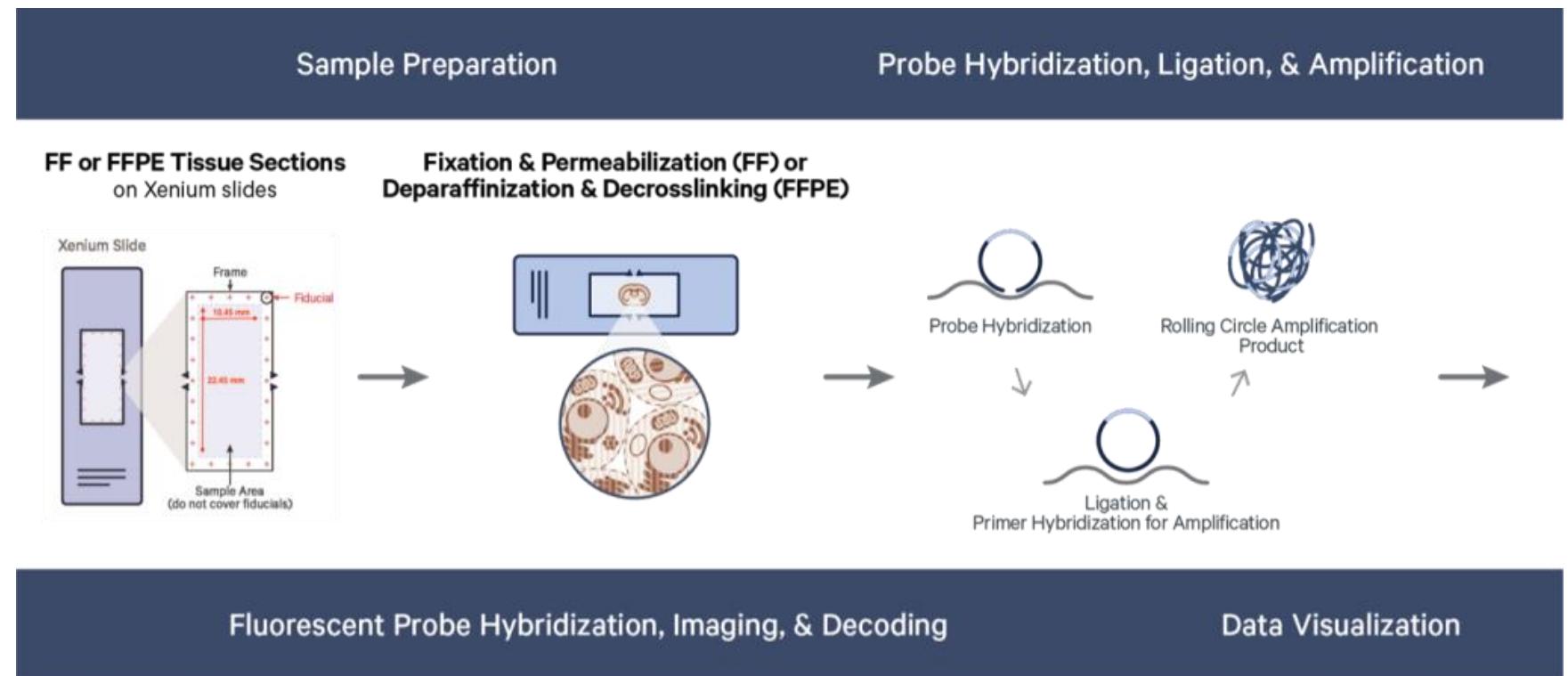
# Xenium

## Advantages:

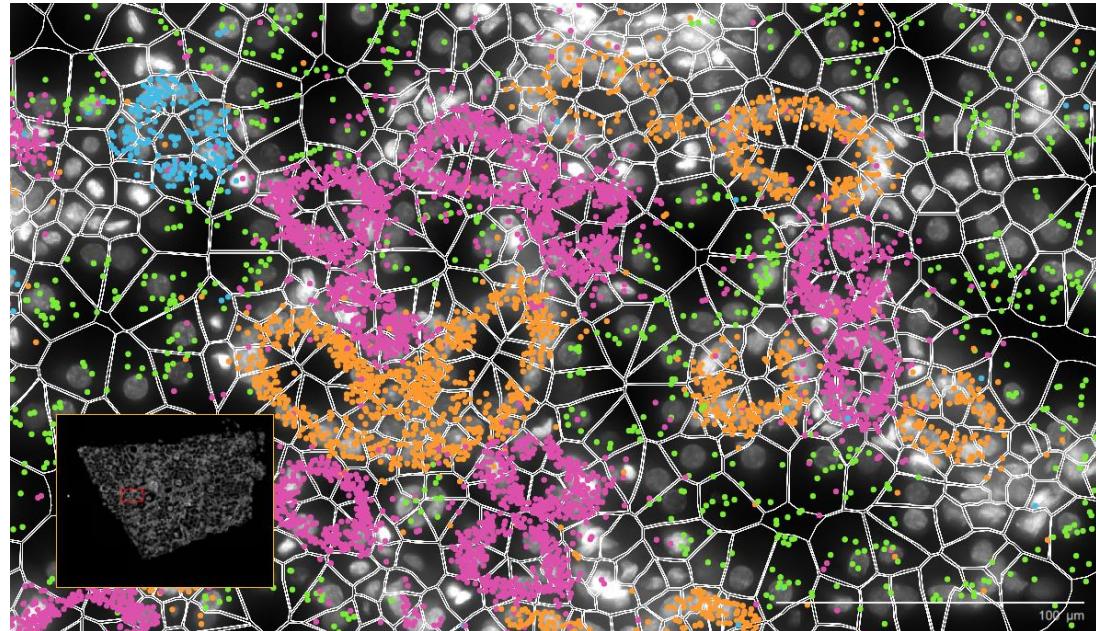
- Subcellular resolution
- Histology
- Optional membrane and cytoplasm staining

## Disadvantages:

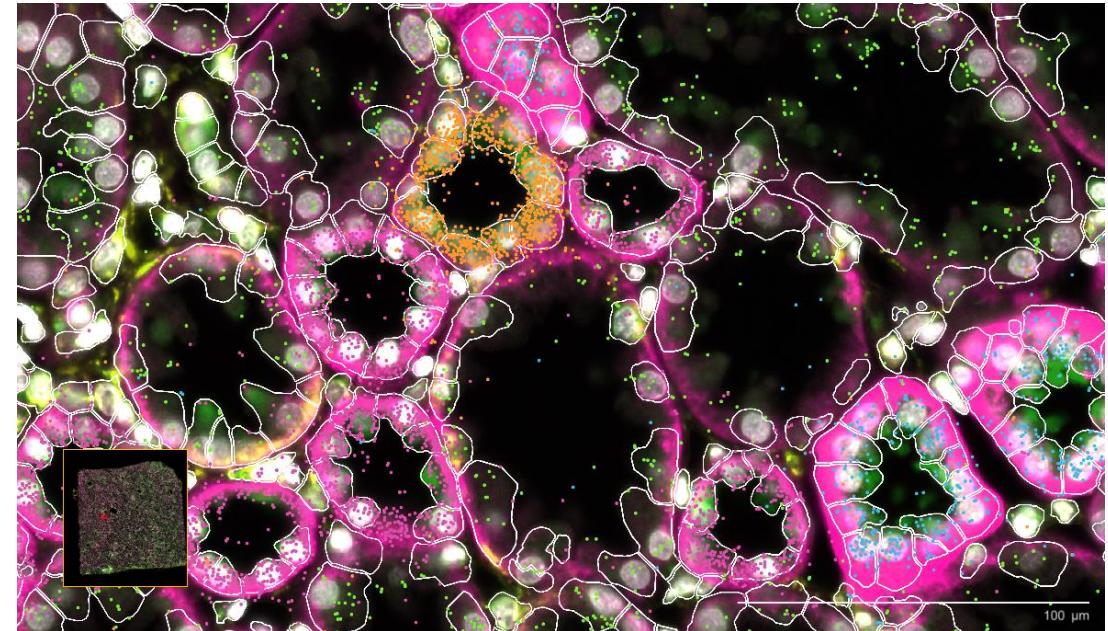
- Limited probes
- Segmentation might be challenging



# Cell segmentation



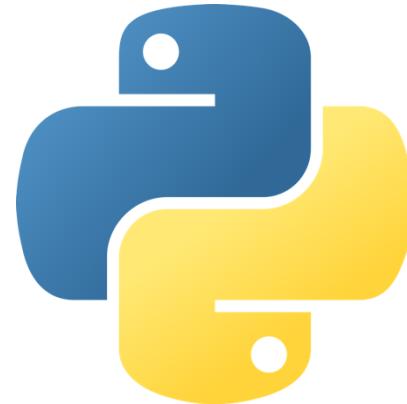
- AQP2
- LRP2
- SLC12A3
- UMOD



# Tools



- Seurat
- singleCellExperiment
- Giotto



- scVerse
- Scanpy
- Squidpy

# Seurat spatialobject

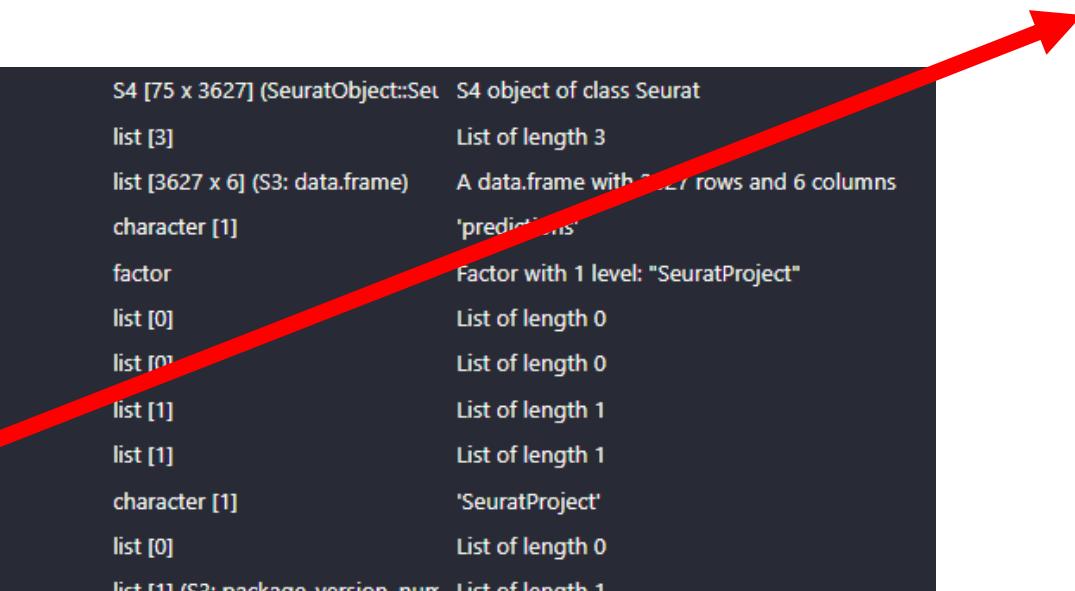
spatial.obj	S4 [75 x 3627] (SeuratObject)	S4 object of class Seurat
assays	list [3]	List of length 3
meta.data	list [3627 x 6] (S3: data.frame)	A data.frame with 3627 rows and 6 columns
active.assay	character [1]	'predictions'
active.ident	factor	Factor with 1 level: "SeuratProject"
graphs	list [0]	List of length 0
neighbors	list [0]	List of length 0
reductions	list [1]	List of length 1
images	list [1]	List of length 1
project.name	character [1]	'SeuratProject'
misc	list [0]	List of length 0
version	list [1] (S3: package_version, numr	List of length 1
commands	list [2]	List of length 2
tools	list [0]	List of length 0



assays	list [3]	List of length 3
Spatial	S4 [36601 x 3627] (SeuratObject)	S4 object of class Assay5
SCT	S4 [17433 x 3627] (Seurat::SCTAs	S4 object of class SCTAssay
predictions	S4 [75 x 3627] (SeuratObject::Ass	S4 object of class Assay

# Seurat spatial object

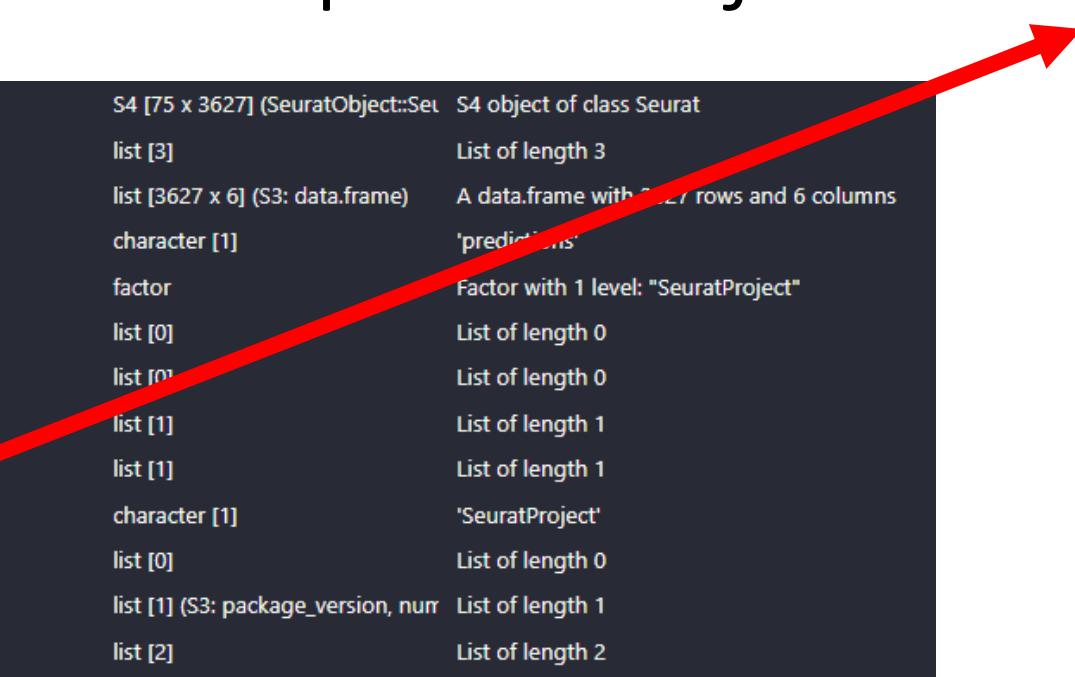
spatial.obj	S4 [75 x 3627] (SeuratObject::Seurat)	S4 object of class Seurat
assays	list [3]	List of length 3
meta.data	list [3627 x 6] (S3: data.frame)	A data.frame with 3627 rows and 6 columns
active.assay	character [1]	'predictions'
active.ident	factor	Factor with 1 level: "SeuratProject"
graphs	list [0]	List of length 0
neighbors	list [0]	List of length 0
reductions	list [1]	List of length 1
images	list [1]	List of length 1
project.name	character [1]	'SeuratProject'
misc	list [0]	List of length 0
version	list [1] (S3: package_version, numr	List of length 1
commands	list [2]	List of length 2
tools	list [0]	List of length 0



images	list [9]	List of length 9
slice1	S4 [557 x 600] (Seurat::VisiumV2)	S4 object of class VisiumV2
slice1.2	S4 [556 x 600] (Seurat::VisiumV2)	S4 object of class VisiumV2
slice1.3	S4 [556 x 600] (Seurat::VisiumV2)	S4 object of class VisiumV2
slice1.4	S4 [557 x 600] (Seurat::VisiumV2)	S4 object of class VisiumV2
slice1.5	S4 [556 x 600] (Seurat::VisiumV2)	S4 object of class VisiumV2
slice1.6	S4 [600 x 556] (Seurat::VisiumV2)	S4 object of class VisiumV2
slice1.7	S4 [600 x 557] (Seurat::VisiumV2)	S4 object of class VisiumV2
slice1.8	S4 [600 x 557] (Seurat::VisiumV2)	S4 object of class VisiumV2
slice1.9	S4 [600 x 557] (Seurat::VisiumV2)	S4 object of class VisiumV2

# Seurat spatial object

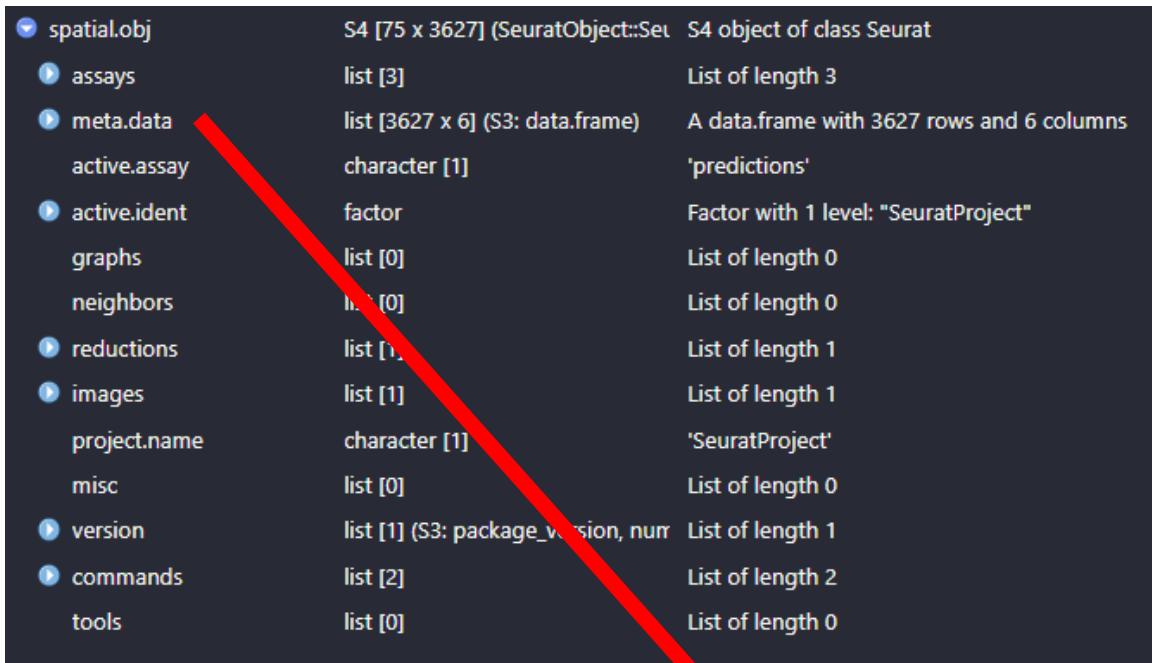
spatial.obj	S4 [75 x 3627] (SeuratObject::Seurat)	S4 object of class Seurat
assays	list [3]	List of length 3
meta.data	list [3627 x 6] (S3: data.frame)	A data.frame with 3627 rows and 6 columns
active.assay	character [1]	'predictions'
active.ident	factor	Factor with 1 level: "SeuratProject"
graphs	list [0]	List of length 0
neighbors	list [0]	List of length 0
reductions	list [1]	List of length 1
images	list [1]	List of length 1
project.name	character [1]	'SeuratProject'
misc	list [0]	List of length 0
version	list [1] (S3: package_version, nurn	List of length 1
commands	list [2]	List of length 2
tools	list [0]	List of length 0



images	list [1]	List of length 1
slice1	S4 [600 x 557] (Seurat::VisiumV2)	S4 object of class VisiumV2
image	double [600 x 557 x 3]	0.506 0.592 0.592 0.592 0
scale.factors	list [4] (S3: scalefactors)	List of length 4
molecules	list [0]	List of length 0
boundaries	list [1]	List of length 1
centroids	S4 (SeuratObject::Centroids)	S4 object of class Centroids
cells	character [3627]	'AAACAAGTATCTCCCA-1' 'AAC
nsides	integer [1]	0
radius	double [1]	85.838
theta	double [1]	0
coords	double [3627 x 2]	6953 7982 2816 6144 6603 821
bbox	double [2 x 2]	1549 1097 9596 8757
proj4string	S4 (sp::CRS)	S4 object of class CRS
assay	character [1]	'Spatial'
key	character [1]	'slice1_'

# Seurat spatial object

```
spatial.obj      S4 [75 x 3627] (SeuratObject::Seurat) S4 object of class Seurat
  assays          list [3]                         List of length 3
  meta.data       list [3627 x 6] (S3: data.frame) A data.frame with 3627 rows and 6 columns
  active.assay    character [1]                   'predictions'
  active.ident   factor                          Factor with 1 level: "SeuratProject"
  graphs          list [0]                         List of length 0
  neighbors       list [0]                         List of length 0
  reductions      list [1]                         List of length 1
  images          list [1]                         List of length 1
  project.name   character [1]                   'SeuratProject'
  misc            list [0]                         List of length 0
  version         list [1] (S3: package_version, numr List of length 1
  commands        list [2]                         List of length 2
  tools           list [0]                         List of length 0
```



The screenshot shows the RStudio interface with several tabs open in the session bar:

- prepare\_objects.R ×
- merged.obj ×
- merged.obj@meta.data** × (highlighted by a red arrow)
- spatial.obj ×
- spatial.obj@meta.data ×
- code\_workshop.Rmd ×
- list\_samples ×

The main area displays a data frame with the following columns and rows:

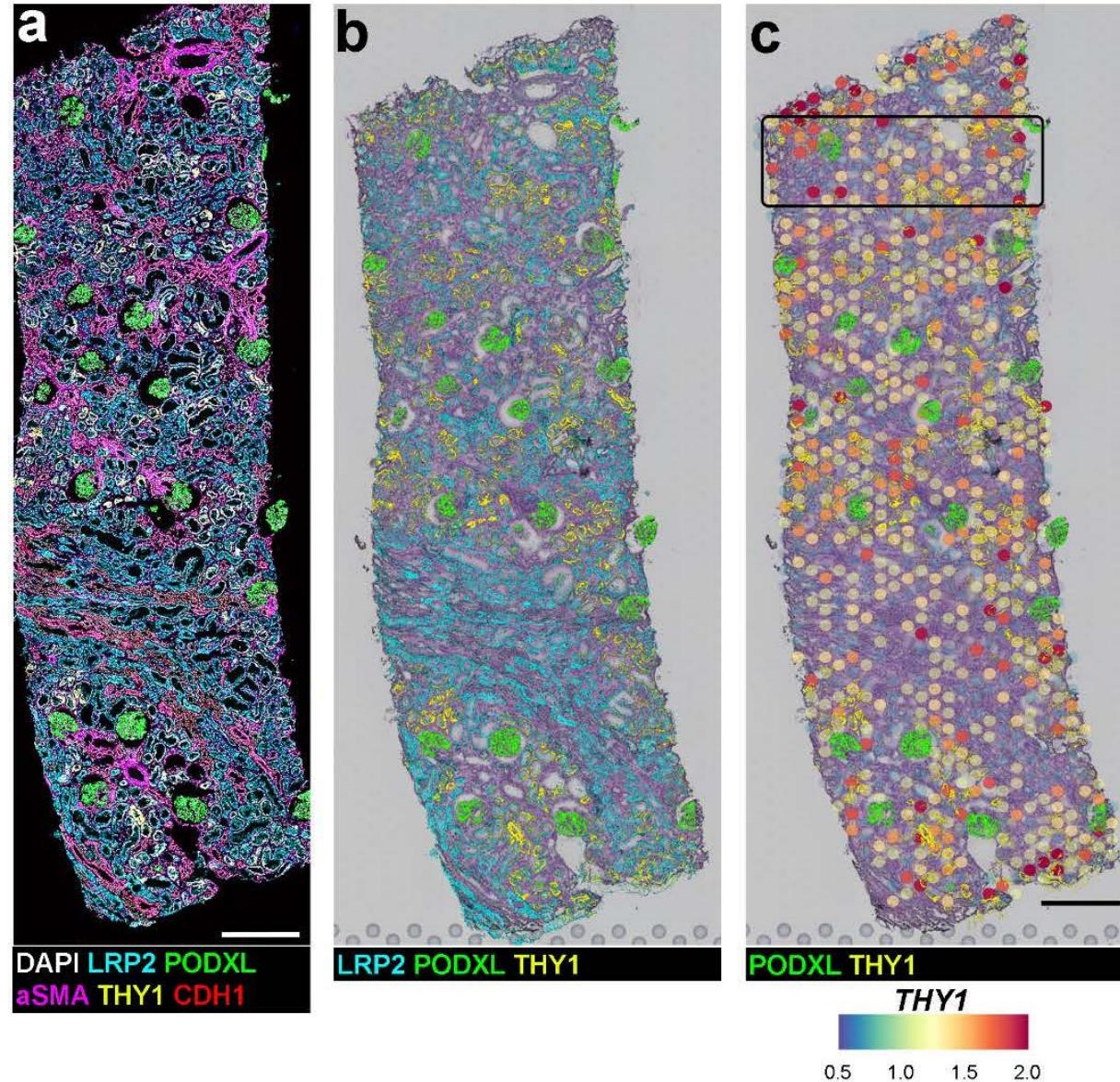
	orig.ident	nCount_Spatial	nFeature_Spatial	glom	nCount_SCT	nFeature_SCT	cca_clusters	seurat_clusters	glom_condition
AAACAAGTATCTCCA-1_1	V10S14-086_XY01_21	column 1: character	2410	3935	no_glom	7799	3665	4	4
AAACAGCTTCAGAAG-1_1	V10S14-086_XY01_21-0055		4930	2159	no_glom	6128	2147	3	3
AAACAGGGTCTATT-1_1	V10S14-086_XY01_21-0055		15798	4256	no_glom	7707	3267	10	10
AAACCCGAACGAAATC-1_1	V10S14-086_XY01_21-0055		8907	3042	no_glom	7557	3021	10	10

# Applications in kidney disease

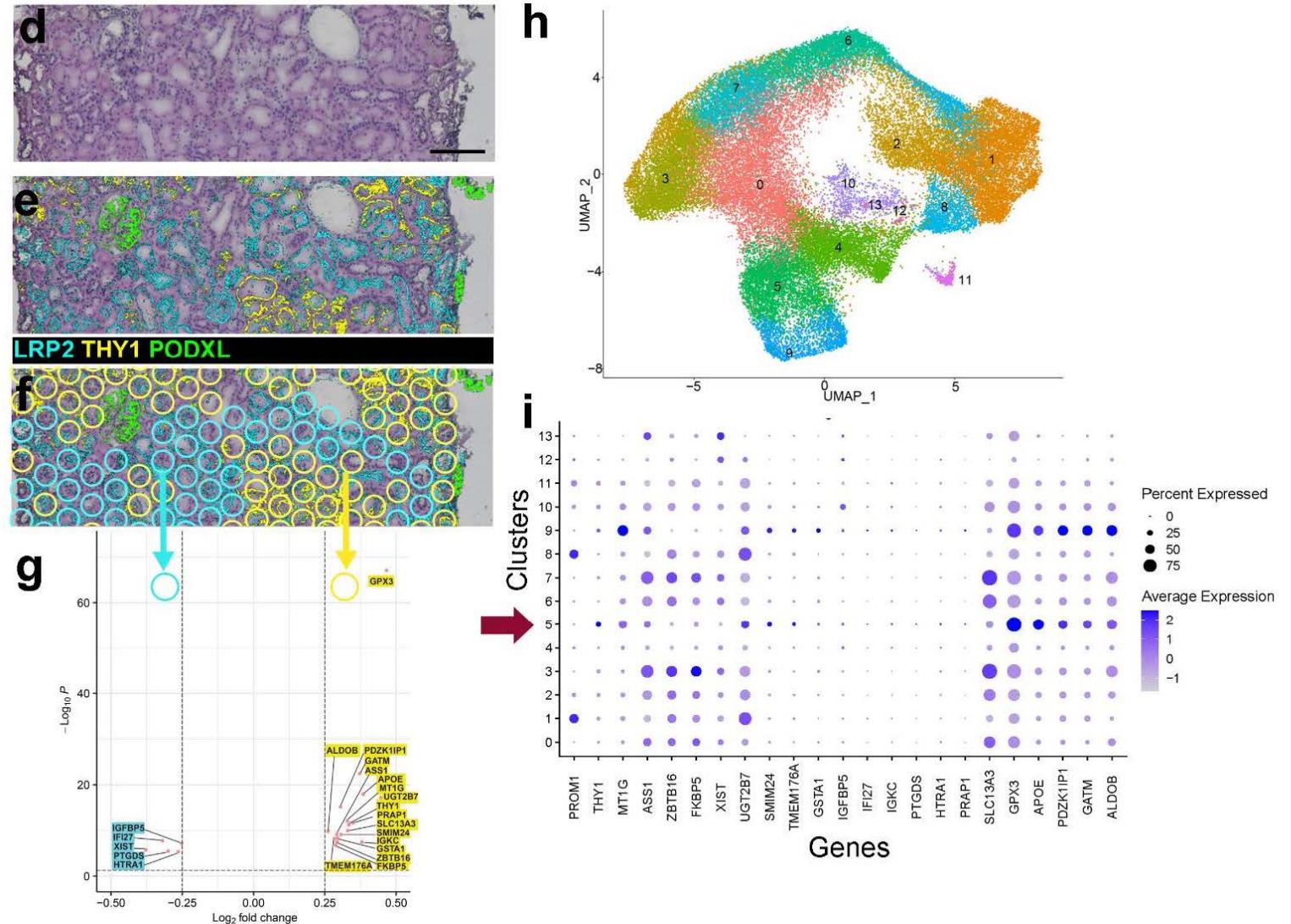
Integration of spatial multiplexed protein imaging and transcriptomics in the  
human kidney tracks the regenerative potential timeline of proximal tubules  
Asghari, Sabo, Barwinska *et al.* bioRxiv

# THY1: alignment with sequential sections

# Identification of Proximal Tubule subpopulation



# Identification of Proximal Tubule subpopulation



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

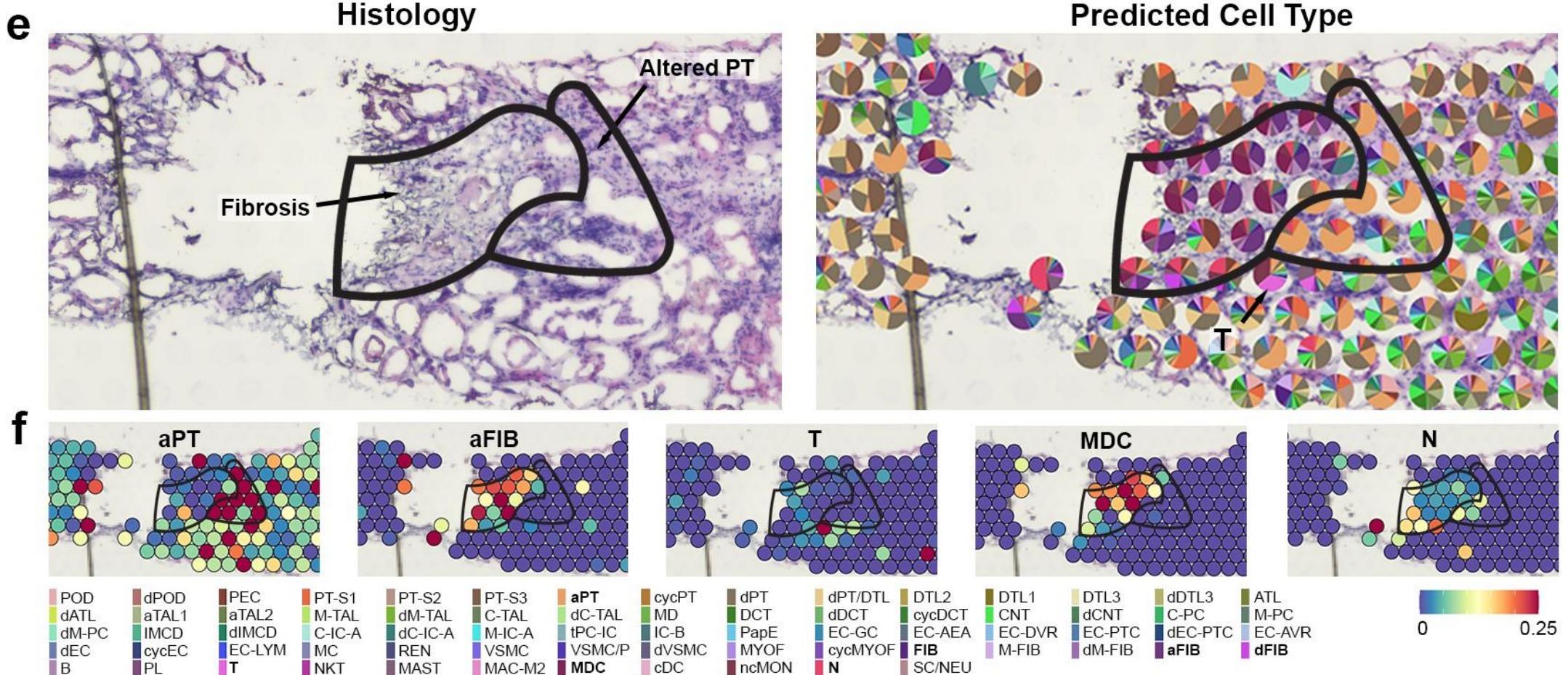
[Blue B. Lake](#), [Rajasree Menon](#), [Seth Winfree](#), [Qiwen Hu](#), [Ricardo Melo Ferreira](#), [Kian Kalhor](#), [Daria Barwinska](#), [Edgar A. Otto](#), [Michael Ferkowicz](#), [Dinh Diep](#), [Nongluk Plongthongkum](#), [Amanda Knoten](#), [Sarah Urata](#), [Laura H. Mariani](#), [Abhijit S. Naik](#), [Sean Eddy](#), [Bo Zhang](#), [Yan Wu](#), [Diane Salamon](#), [James C. Williams](#), [Xin Wang](#), [Karol S. Balderrama](#), [Paul J. Hoover](#), [Evan Murray](#), [Jamie L. Marshall](#), [Teia Noel](#), [Anitha Vijayan](#), [Austin Hartman](#), [Fei Chen](#), [Sushrut S. Waikar](#), [Sylvia E. Rosas](#), [Francis P. Wilson](#), [Paul M. Palevsky](#), [Krzysztof Kiryluk](#), [John R. Sedor](#), [Robert D. Toto](#), [Chirag R. Parikh](#), [Eric H. Kim](#), [Rahul Satija](#), [Anna Greka](#), [Evan Z. Macosko](#), [Peter V. Kharchenko](#), [Joseph P. Gaut](#), [Jeffrey B. Hodgin](#), [KPMP Consortium](#), [Michael T. Eadon](#)✉, [Pierre C. Dagher](#)✉, [Tarek M. El-Achkar](#)✉, [Kun Zhang](#)✉, [Matthias Kretzler](#)✉ & [Sanjay Jain](#)✉

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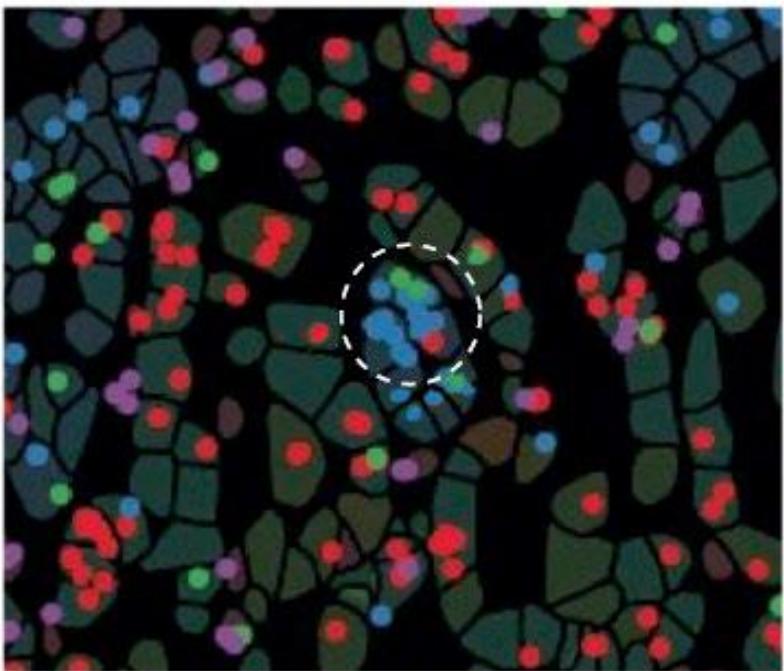
[Nature](#) **619**, 585–594 (2023) | [Cite this article](#)

# Kidney atlas: Cell Type colocalization

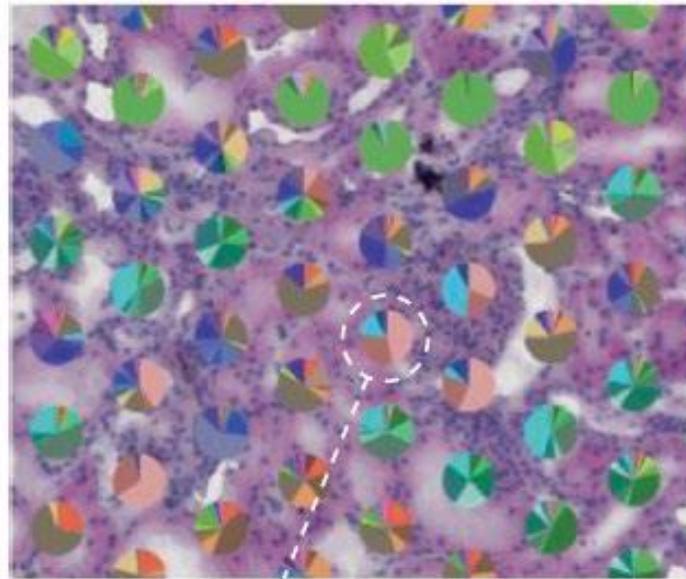
# Transcriptomic localization of cell states associated with injury



# Neighborhood Analysis

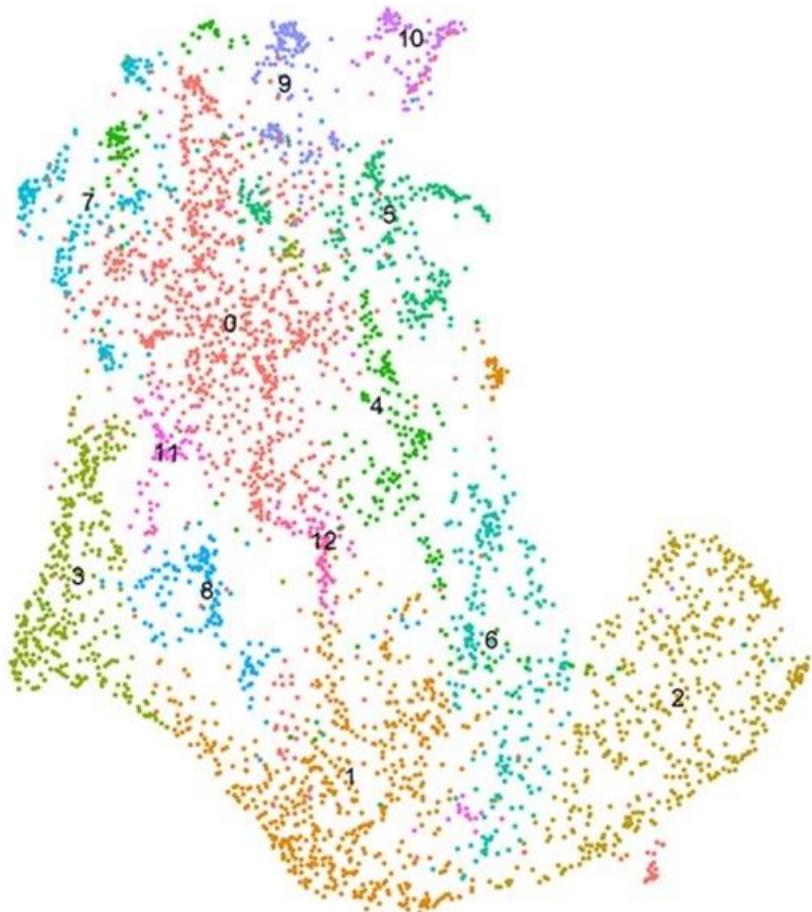


A neighbourhood defined  
by a radius around a centroid

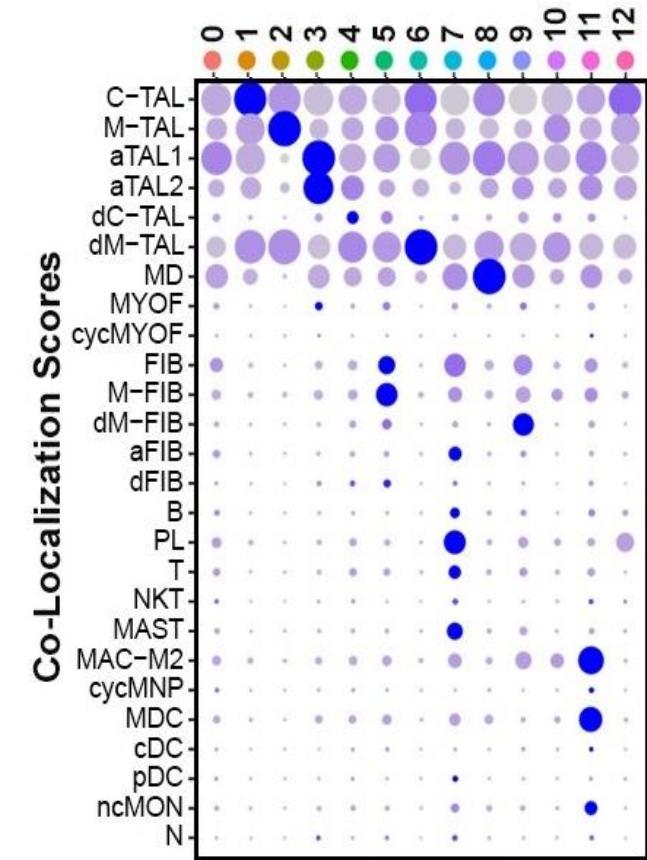
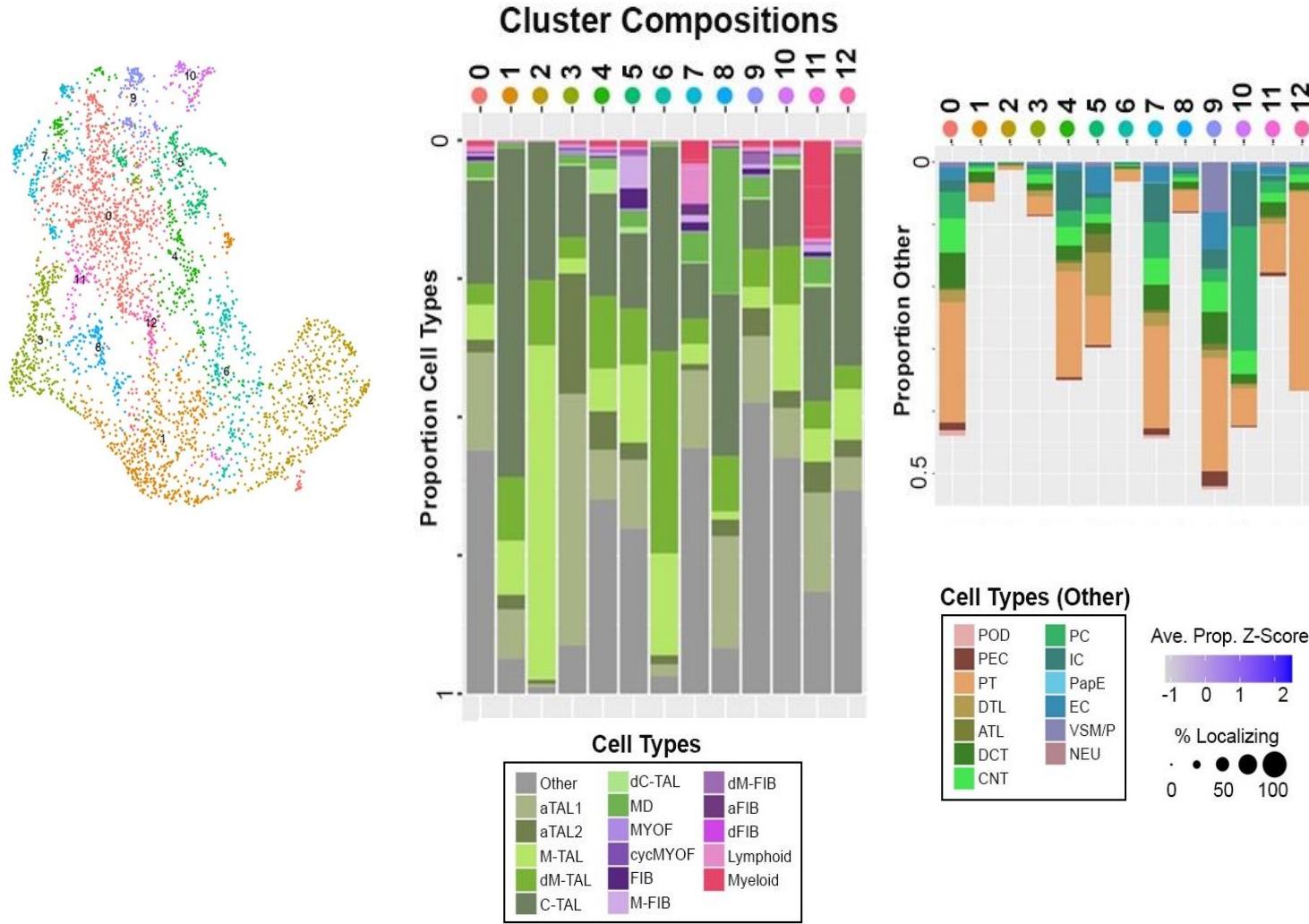


Components  
of the spot  
become the  
neighbourhood

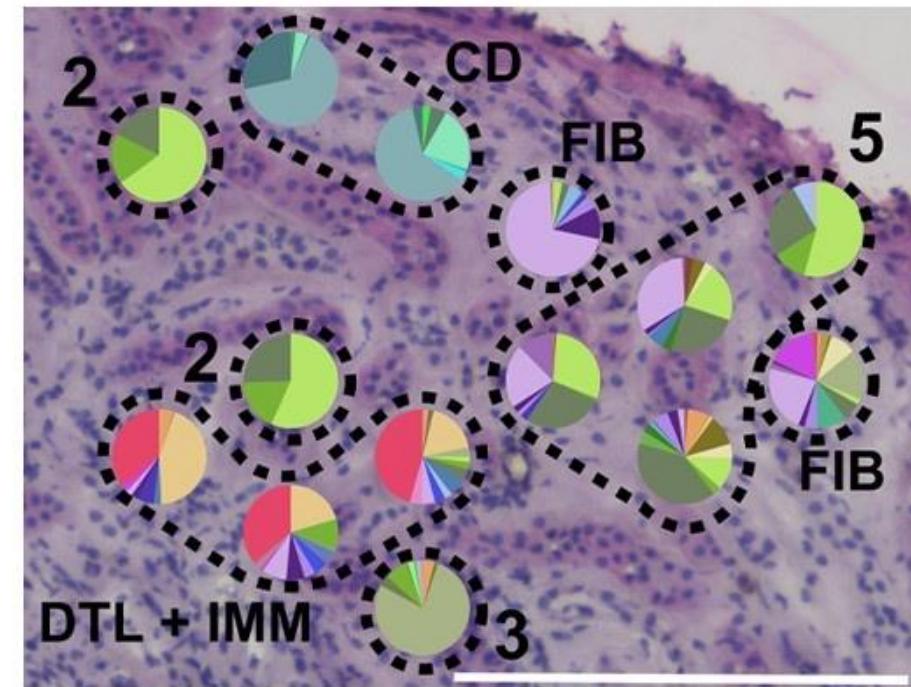
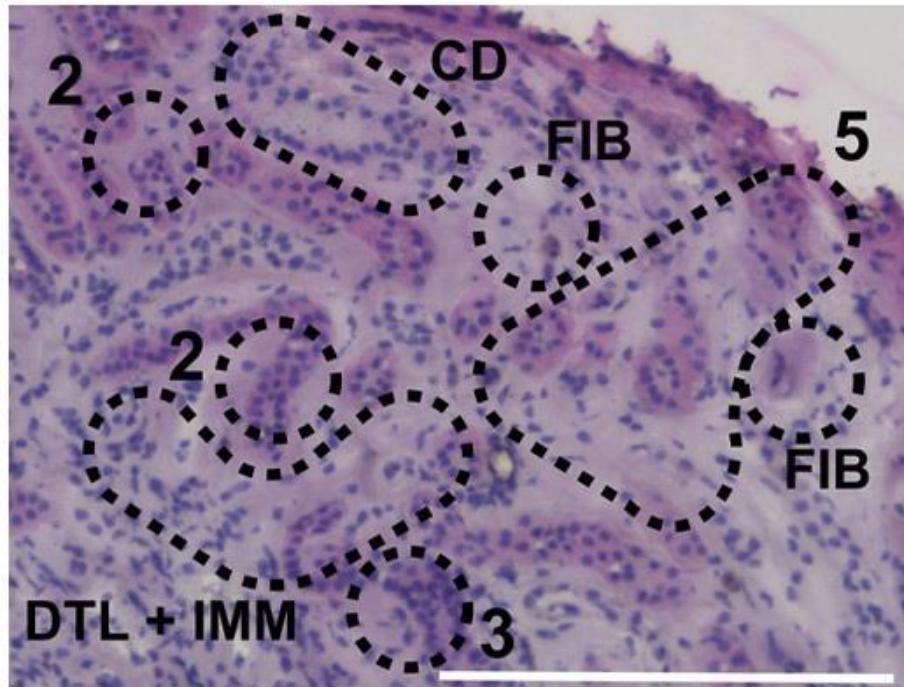
# Transcriptomic signature of TAL niches



# Transcriptomic signature of TAL niches



# Transcriptomic signature of TAL niches



Cell Type
aTAL1
aTAL2
M-TAL
dM-TAL
C-TAL
dC-TAL
MD
MYOF
cycMYOF
FIB
M-FIB
dM-FIB
aFIB
dFIB
Lymphoid
Myeloid
Other

## The chromatin landscape of healthy and injured cell types in the human kidney

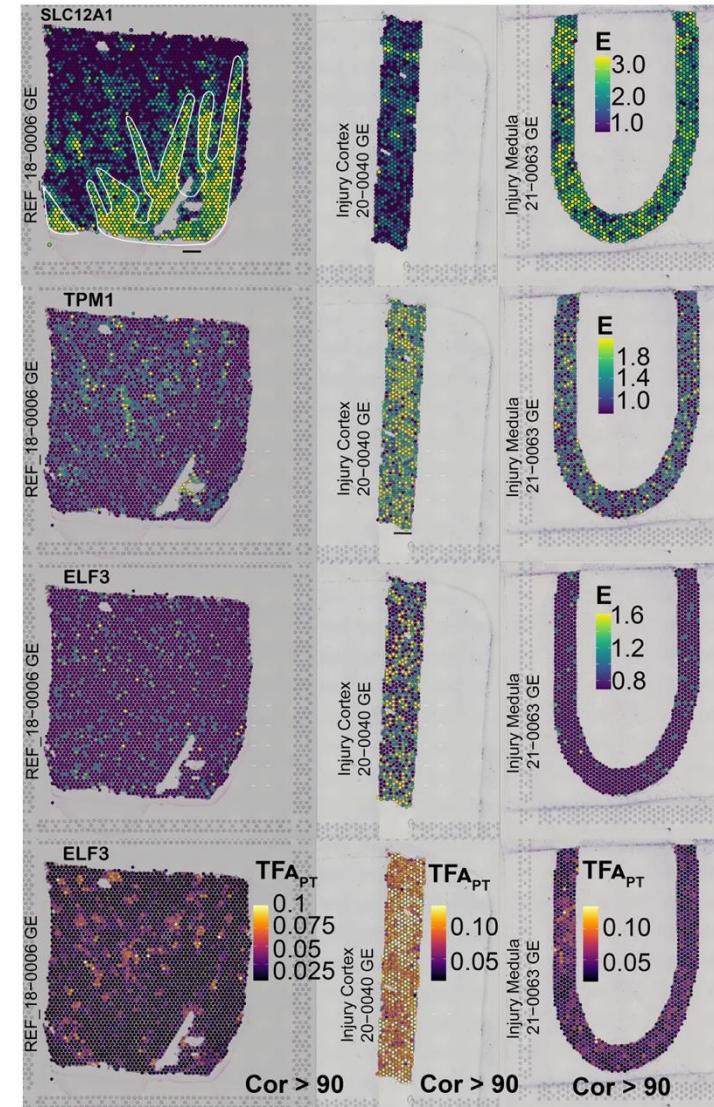
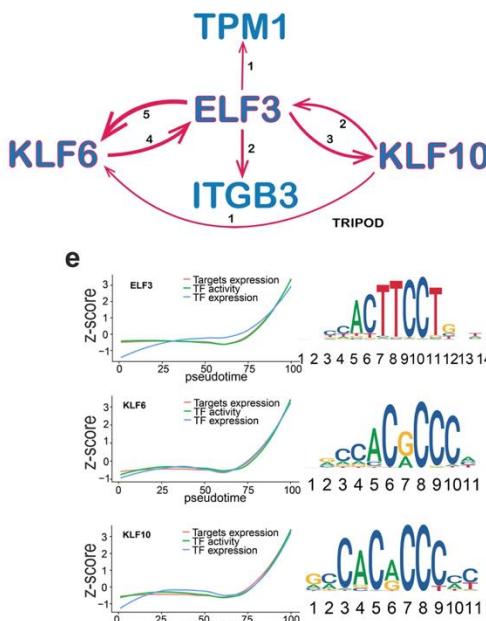
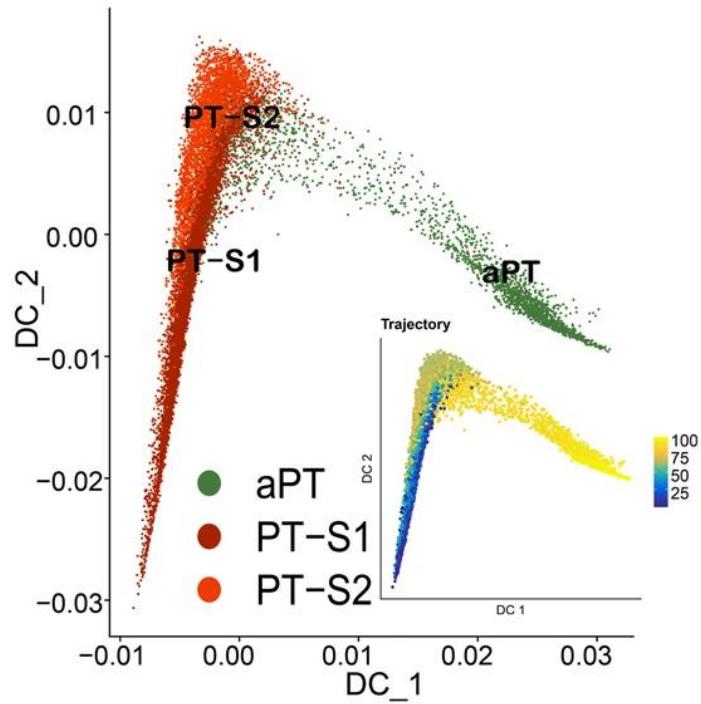
[Debora L. Gisch](#), [Michelle Brennan](#), [Blue B. Lake](#), [Jeannine Basta](#), [Mark S. Keller](#), [Ricardo Melo Ferreira](#),  
[Shreeram Akilesh](#), [Reetika Ghag](#), [Charles Lu](#), [Ying-Hua Cheng](#), [Kimberly S. Collins](#), [Samir V. Parikh](#), [Brad H. Rovin](#), [Lynn Robbins](#), [Lisa Stout](#), [Kimberly Y. Conklin](#), [Dinh Diep](#), [Bo Zhang](#), [Amanda Knoten](#), [Daria Barwinska](#), [Mahla Asghari](#), [Angela R. Sabo](#), [Michael J. Ferkowicz](#), [Timothy A. Sutton](#), [Katherine J. Kelly](#), [Jan H. De Boer](#), [Sylvia E. Rosas](#), [Krzysztof Kiryluk](#), [Jeffrey B. Hodgin](#), [Fadhl Alakwa](#), [Seth Winfree](#), [Nichole Jefferson](#), [Aydin Türkmen](#), [Joseph P. Gaut](#), [Nils Gehlenborg](#), [Carrie L. Phillips](#), [Tarek M. El-Achkar](#), [Pierre C. Dagher](#), [Takashi Hato](#), [Kun Zhang](#), [Jonathan Himmelfarb](#), [Matthias Kretzler](#), [Shamim Mollah](#), [the Kidney Precision Medicine Project \(KPMP\)](#), [Sanjay Jain](#)✉, [Michael Rauchman](#)✉ & [Michael T. Eadon](#)✉

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[Nature Communications](#) 15, Article number: 433 (2024) | [Cite this article](#)

# Epigenetic atlas: Validation of Transcription Factors

# Modelling Transcription factor network



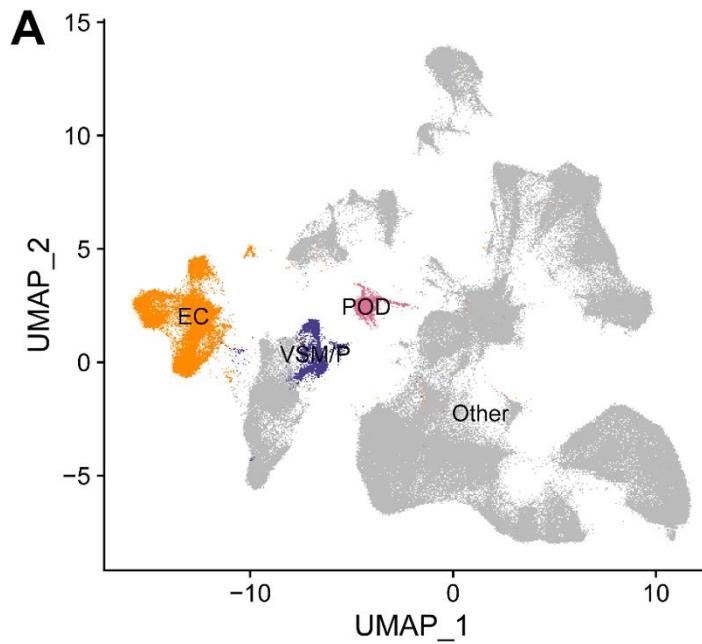
**A MEF2C transcription factor network regulates proliferation of glomerular endothelial cells in diabetic kidney disease**

✉ Ricardo Melo Ferreira, ✉ Debora L. Gisch, ✉ Carrie L. Phillips, ✉ Ying-Hua Cheng, ✉ Maansi Asthana, ✉ Blue B. Lake, ✉ William S. Bowen, Fang Fang, ✉ Mahla Ashgari, ✉ Angela Sabo, ✉ Daria Barwinska, ✉ Michael J. Ferkowicz, ✉ Robert D. Toto, ✉ John R. Sedor, ✉ Sylvia E. Rosas, Petter Bjornstad, Jeffrey B Hodgin, ✉ Charles E. Alpers, ✉ Pinaki Sarder, Jonathan Himmelfarb, ✉ Jennifer A. Schaub, ✉ Viji Nair, ✉ Seth Winfree, ✉ Timothy A. Sutton, Katherine J. Kelly, The Kidney Precision Medicine Project, ✉ Matthias Kretzler, ✉ Sanjay Jain, ✉ Tarek M. El-Achkar, ✉ Pierre C. Dagher, ✉ Michael T. Eadon

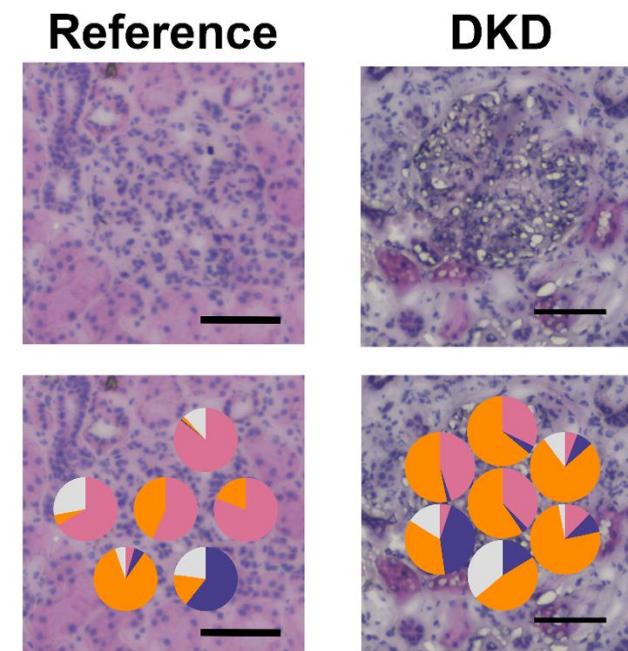
doi: <https://doi.org/10.1101/2024.09.27.615250>



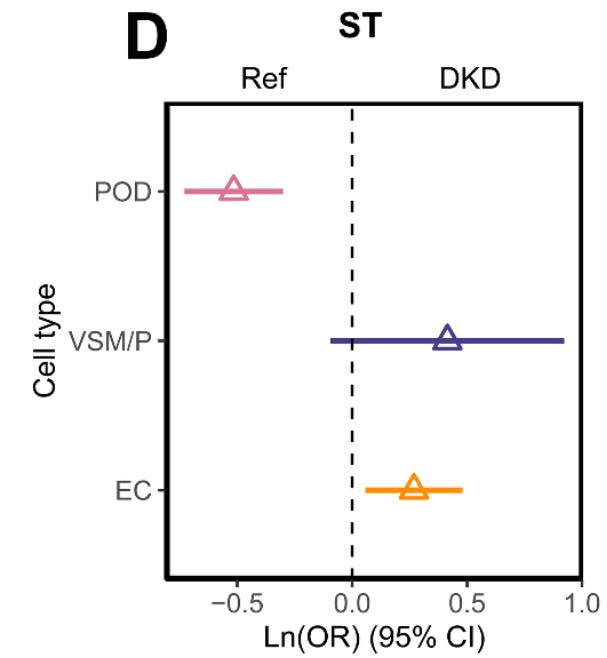
# Diabetic Kidney Disease: Using Visium to characterize single cell; validation with Xenium; correlation with histopathology



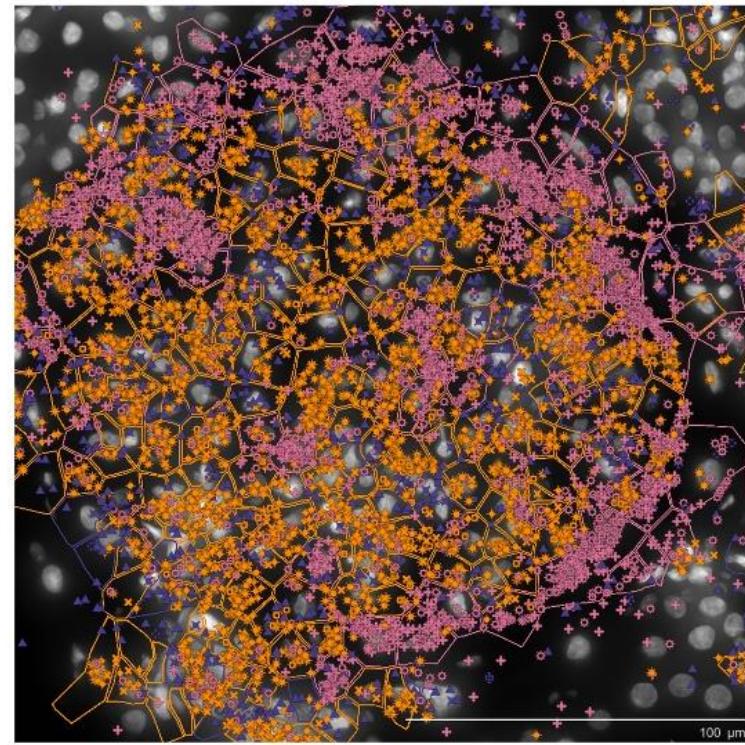
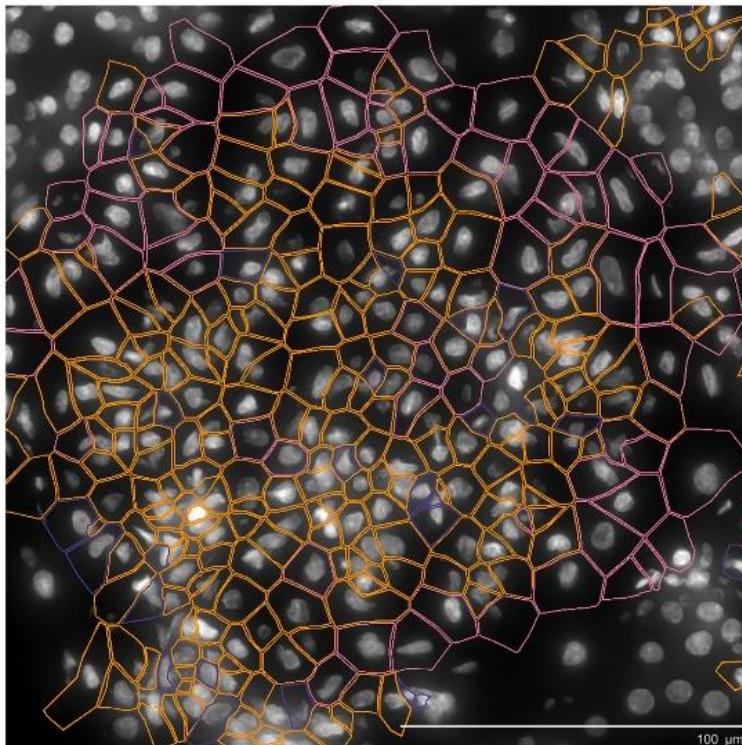
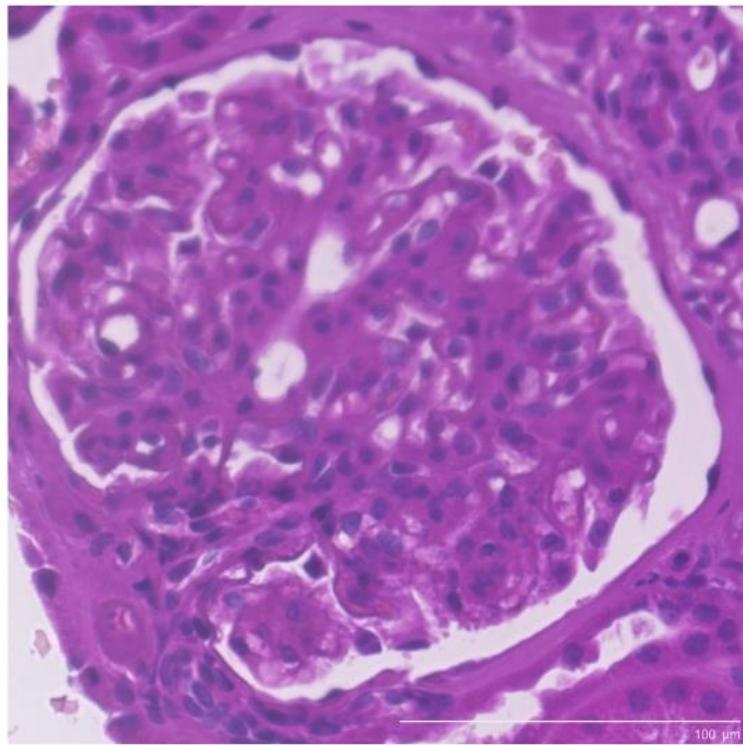
Subset of potential glomerular cells



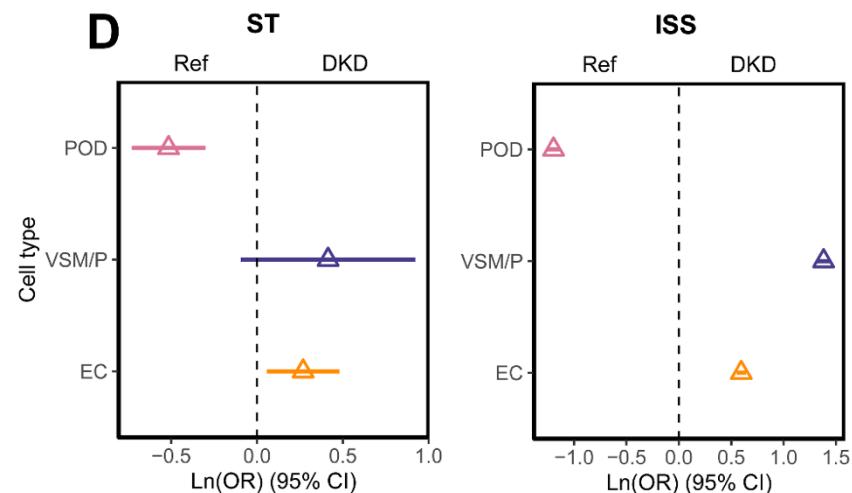
Label transfer suggests difference in glomerular cell type composition



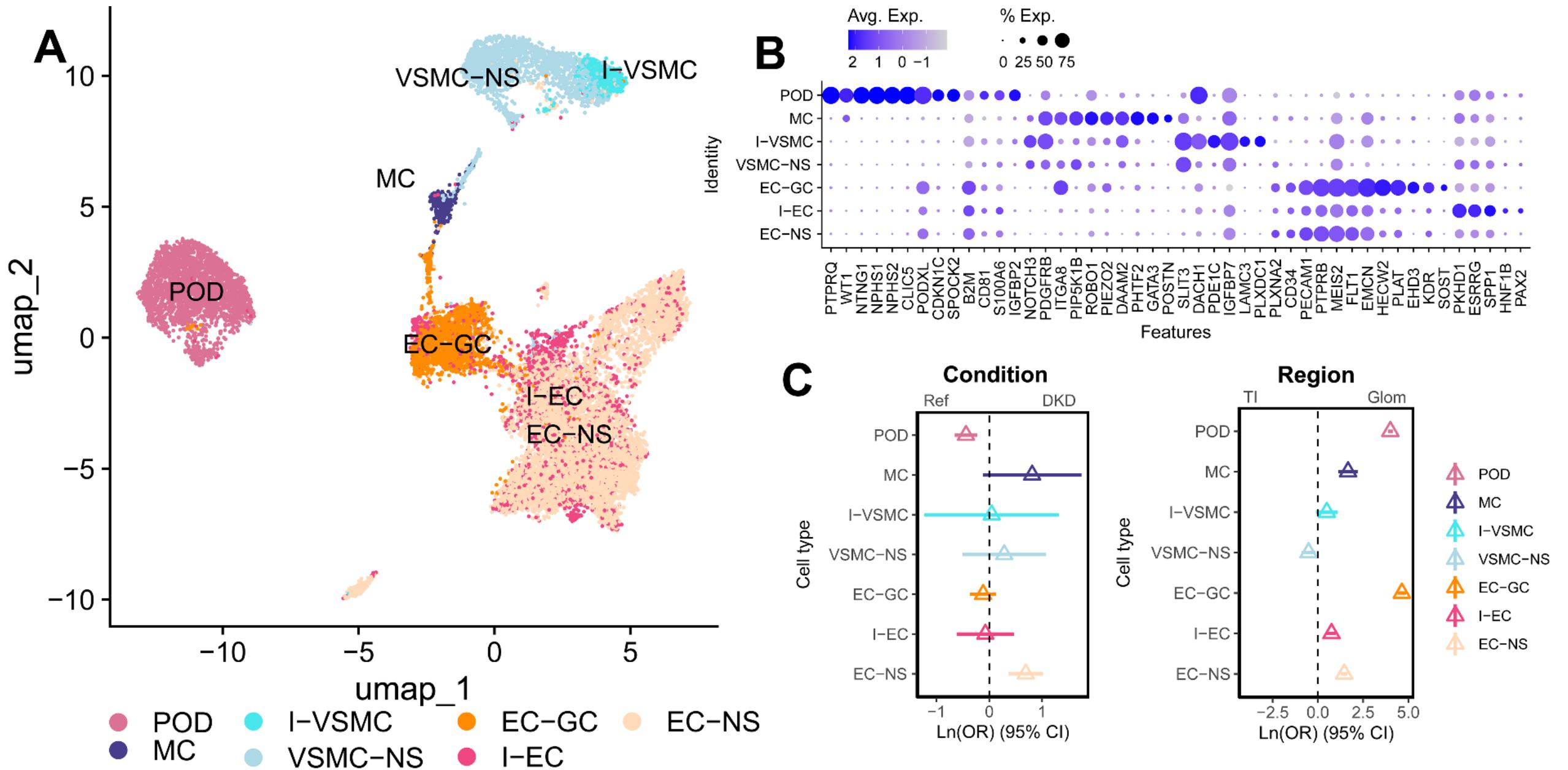
Reduction in podocyte signature. Increase in VSM/P + EC.



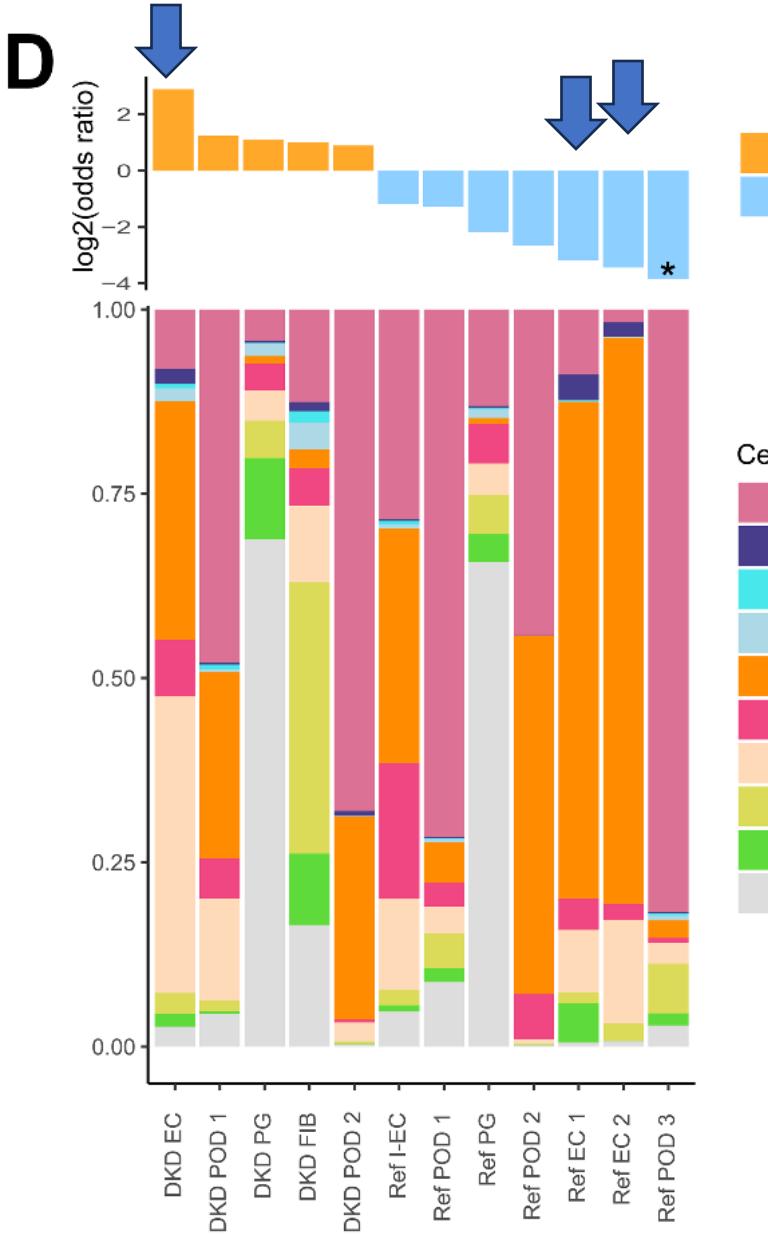
- ◆ NPHS2
- ◆ PODXL
- ◆ PIEZO2
- ◆ ROBO1
- ◆ TAGLN
- ◆ CD34
- ◆ FLT1
- ◆ HECW2
- ◆ PECAM1

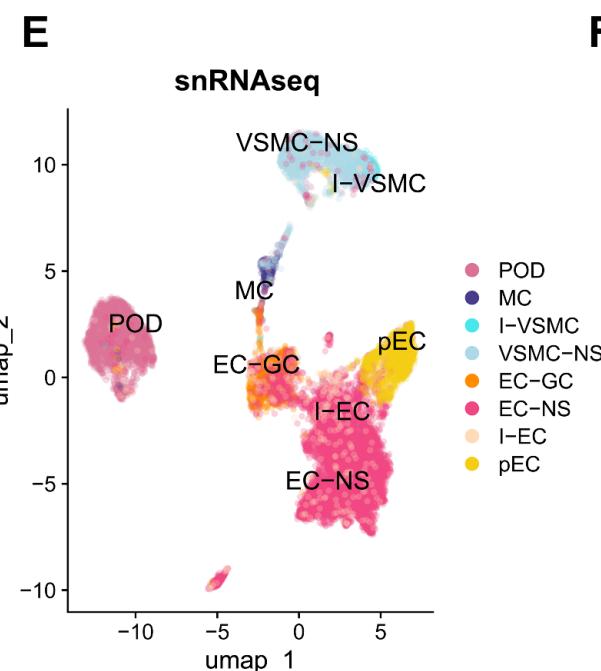
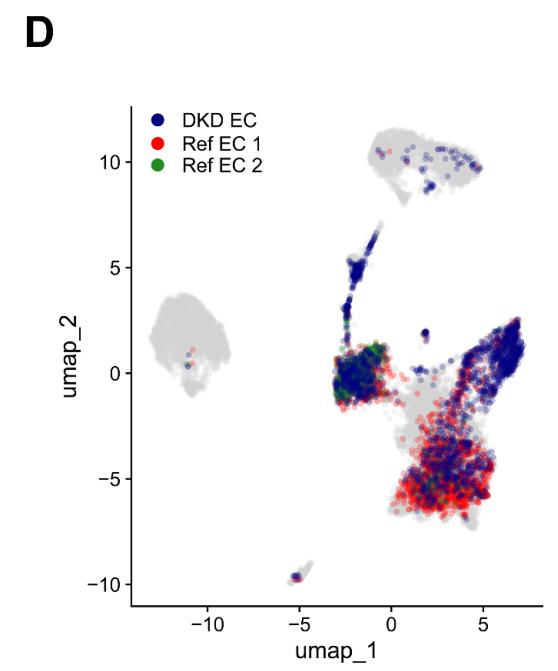
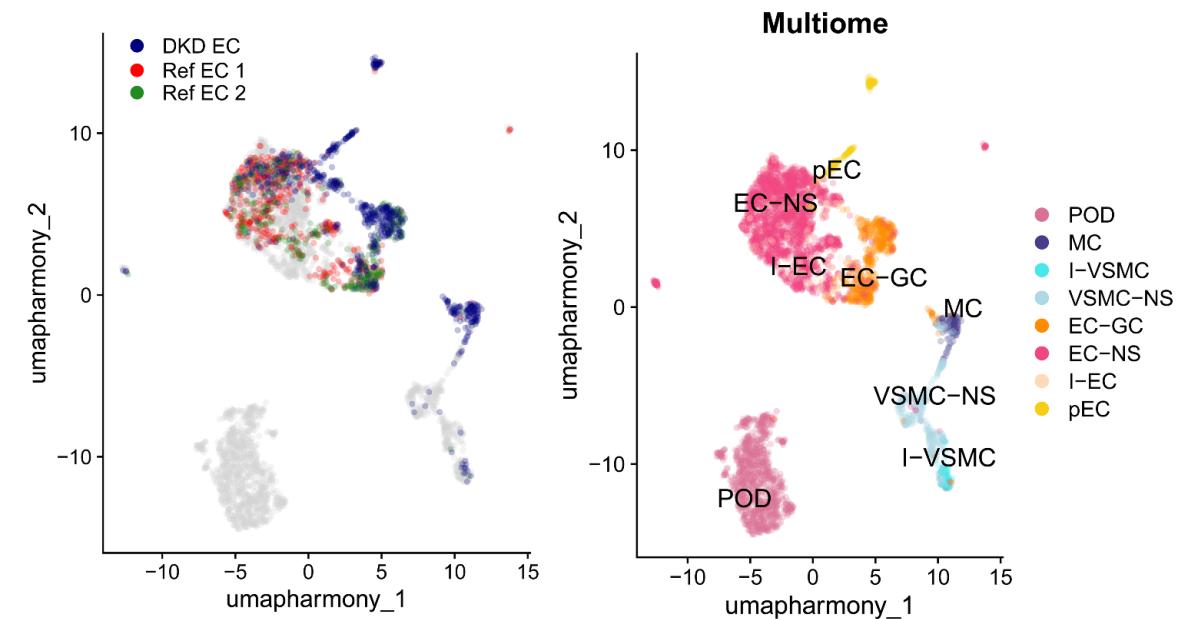


# Evaluated specific MC/VSM and EC subtypes



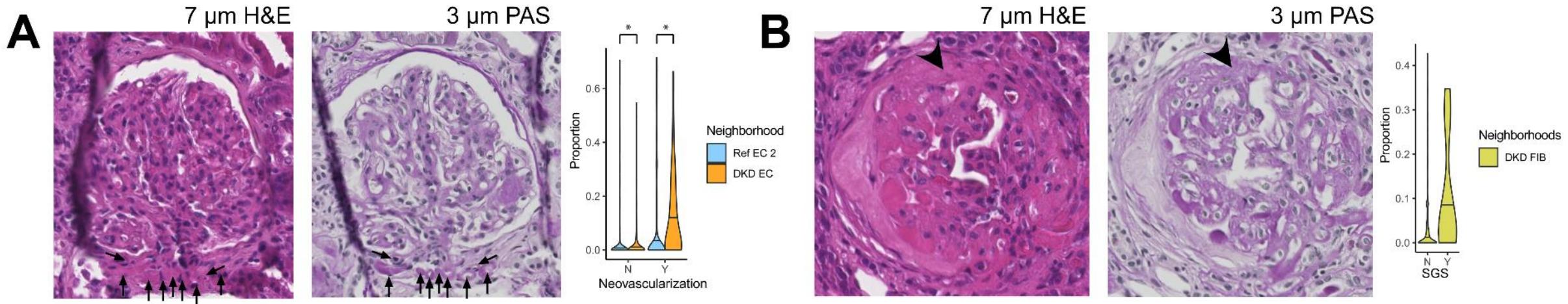
# Neighborhood analysis





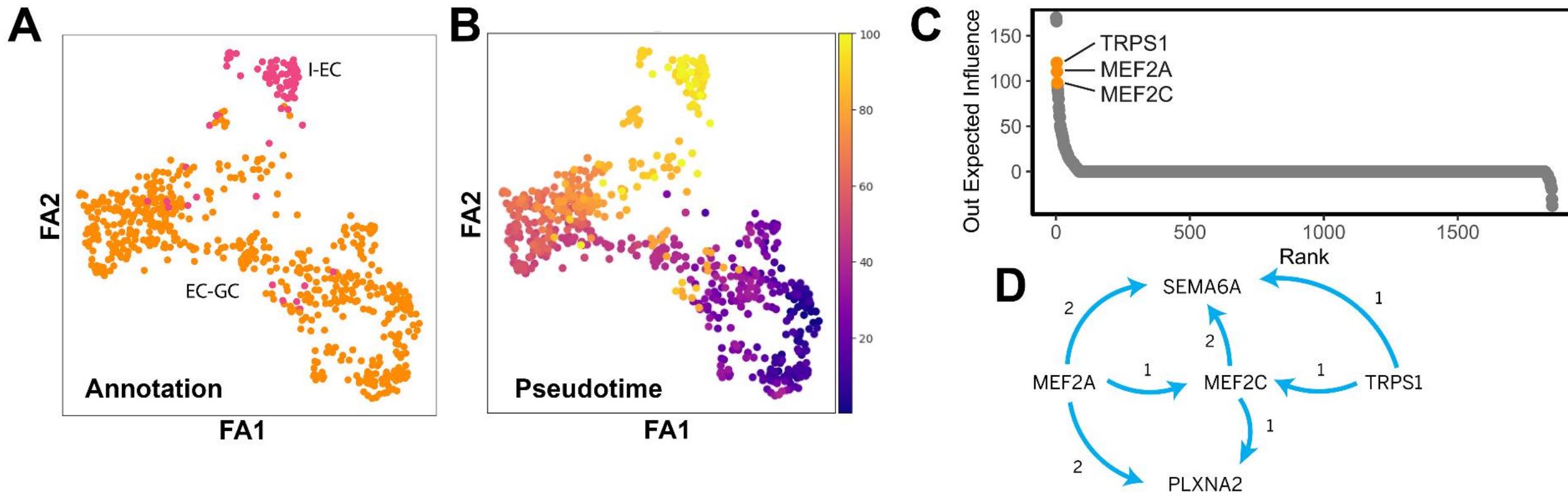
- Back-mapped ST neighborhood information (reverse label transfer)
- Determined which endothelial sub-cluster is in diabetic glomeruli.
- Called it pEC for proliferative endothelial cell.

# Histology



- Scored every glomerulus based on histology
- Molecularly defined neighborhoods align with expected histology

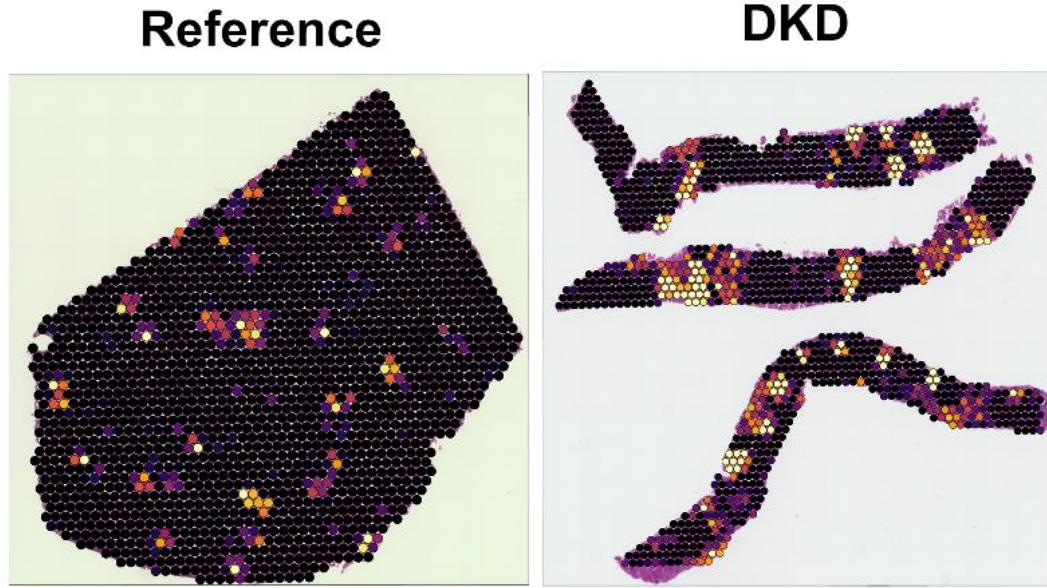
# Trajectory of glomerular capillary endothelial cell to degenerative endothelial cell



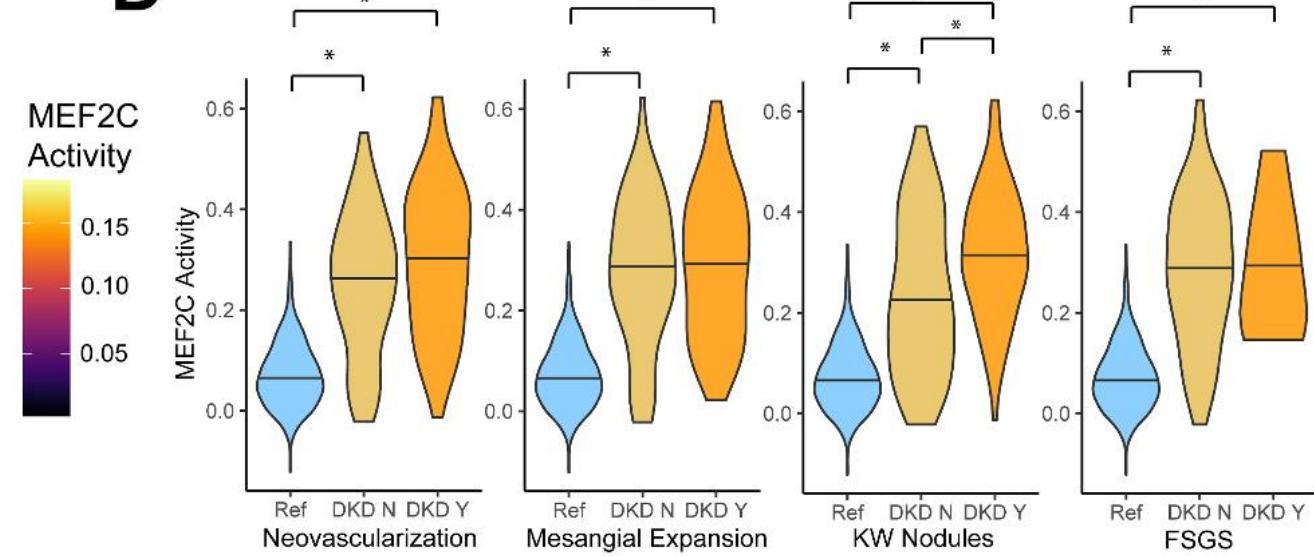
Transcription Factor Network with high centrality

# *MEF2C* activity mapping

C



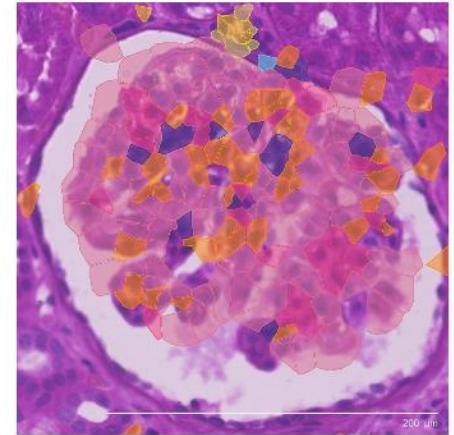
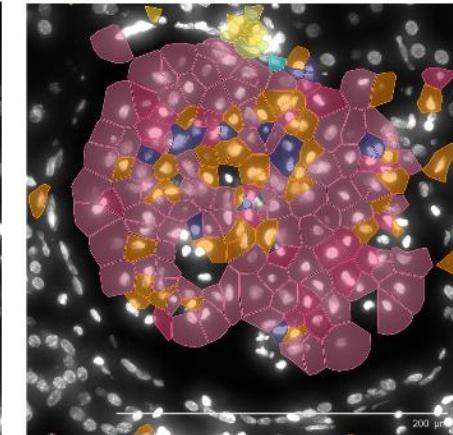
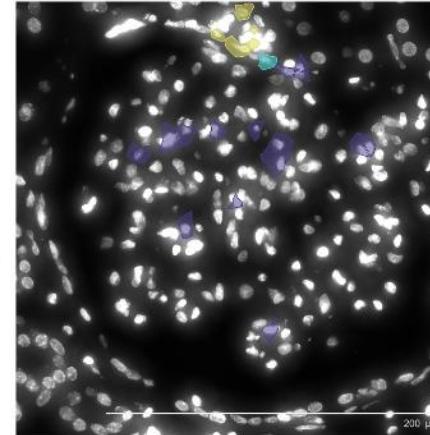
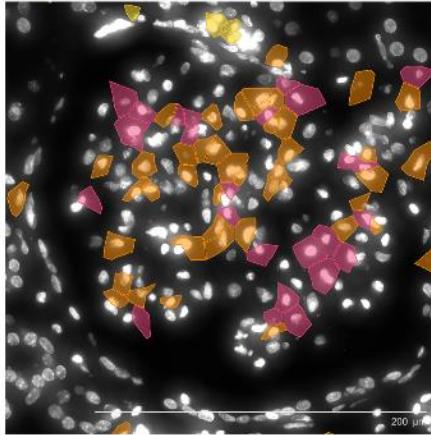
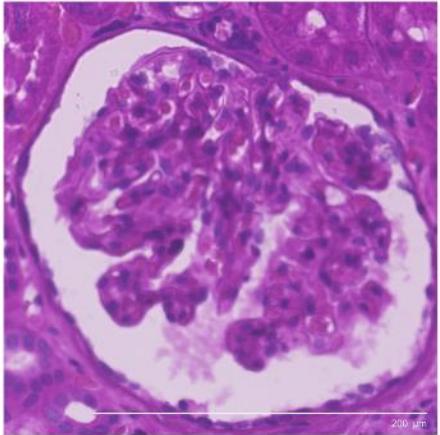
D



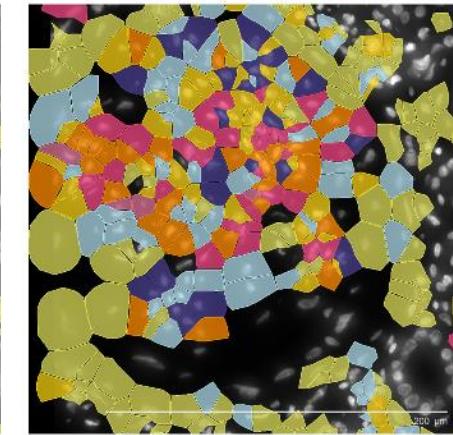
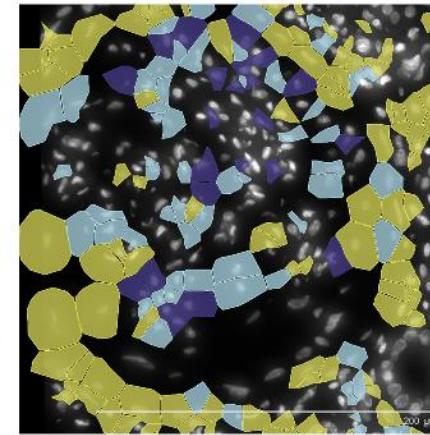
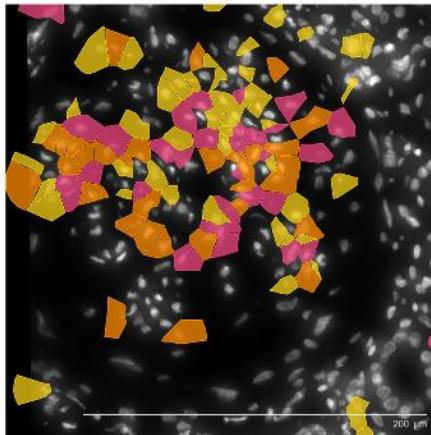
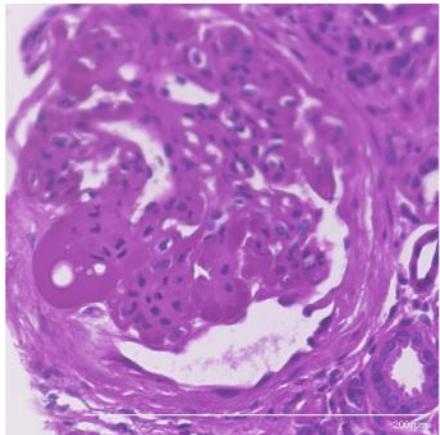
- *MEF2C* activity increased in DKD and upregulated further in nodular glomeruli.

# Spatial trajectories of diseased cells

Reference



DKD



EC-GC prEC  
dEC

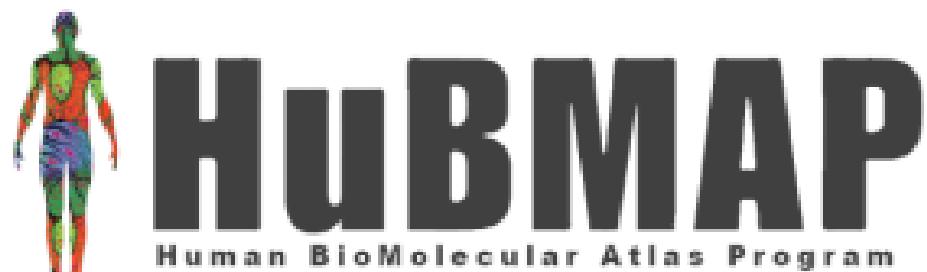
MC VSMC-NS  
dVSMC FIB

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  - NIDDK P50 DK133943 (PCEN)
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Questions?