

Ψ

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

Ricardo Melo Ferreira

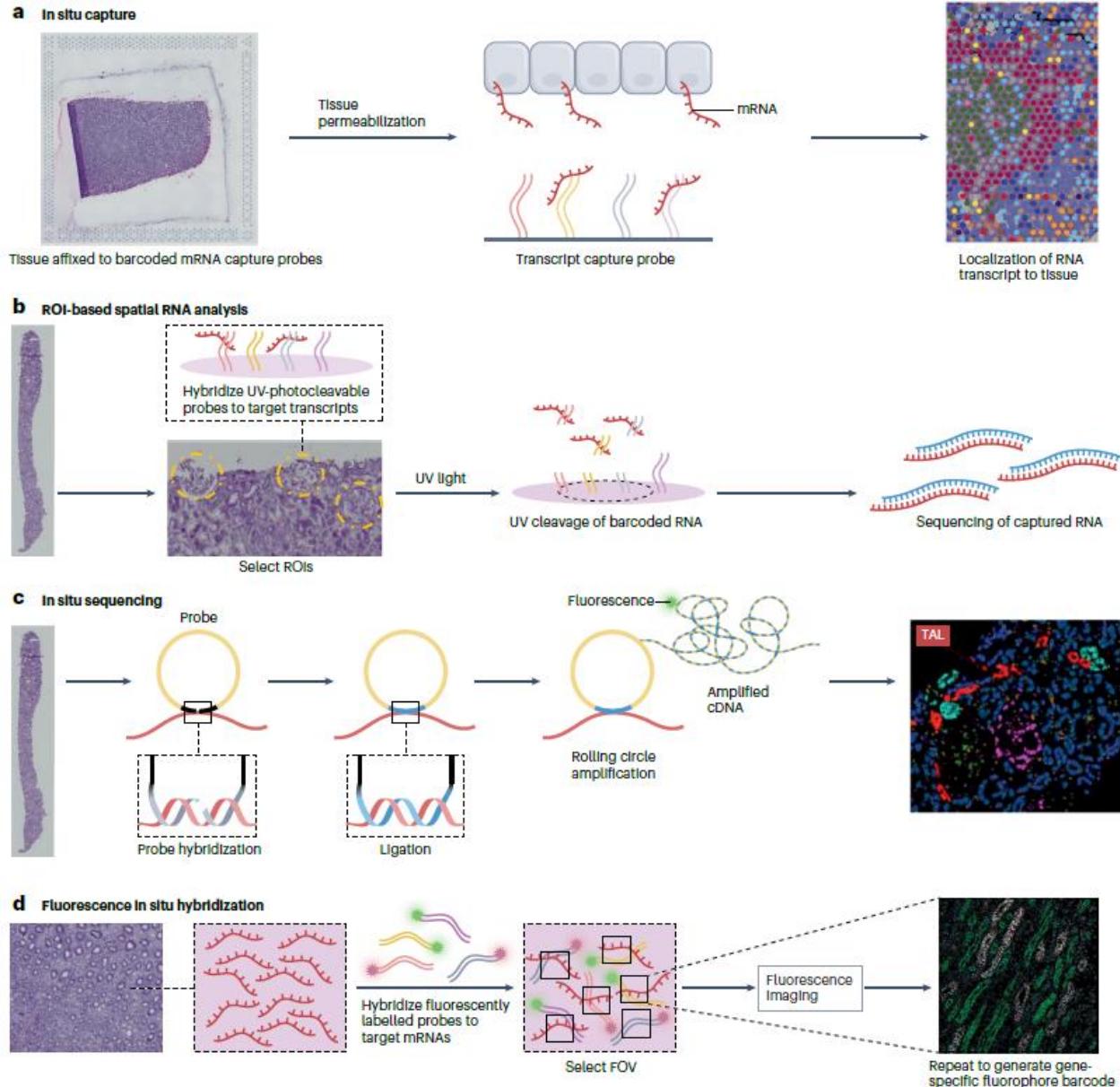
Indiana University School of Medicine

Disclosures

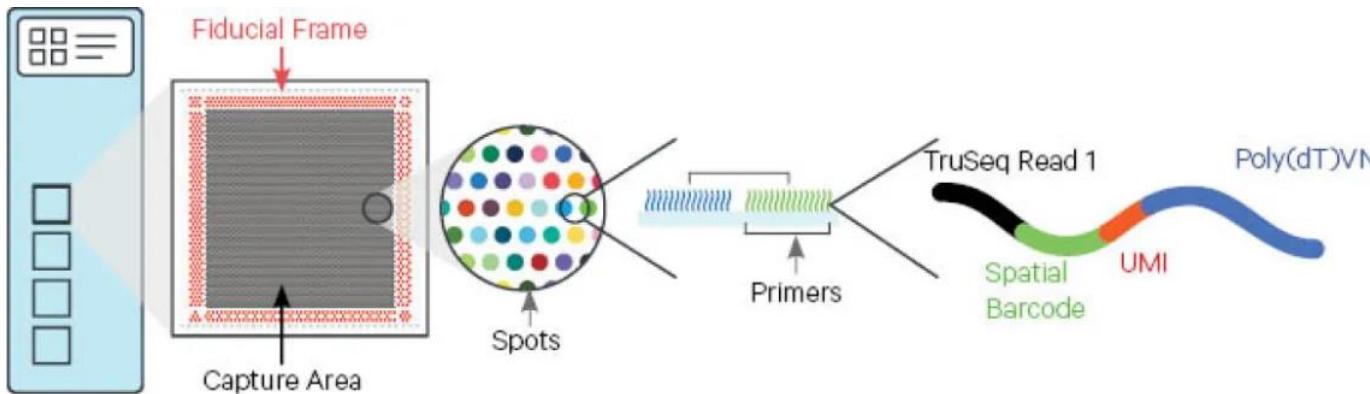
- NIH Funding
- DCI Funding

Technologies overview

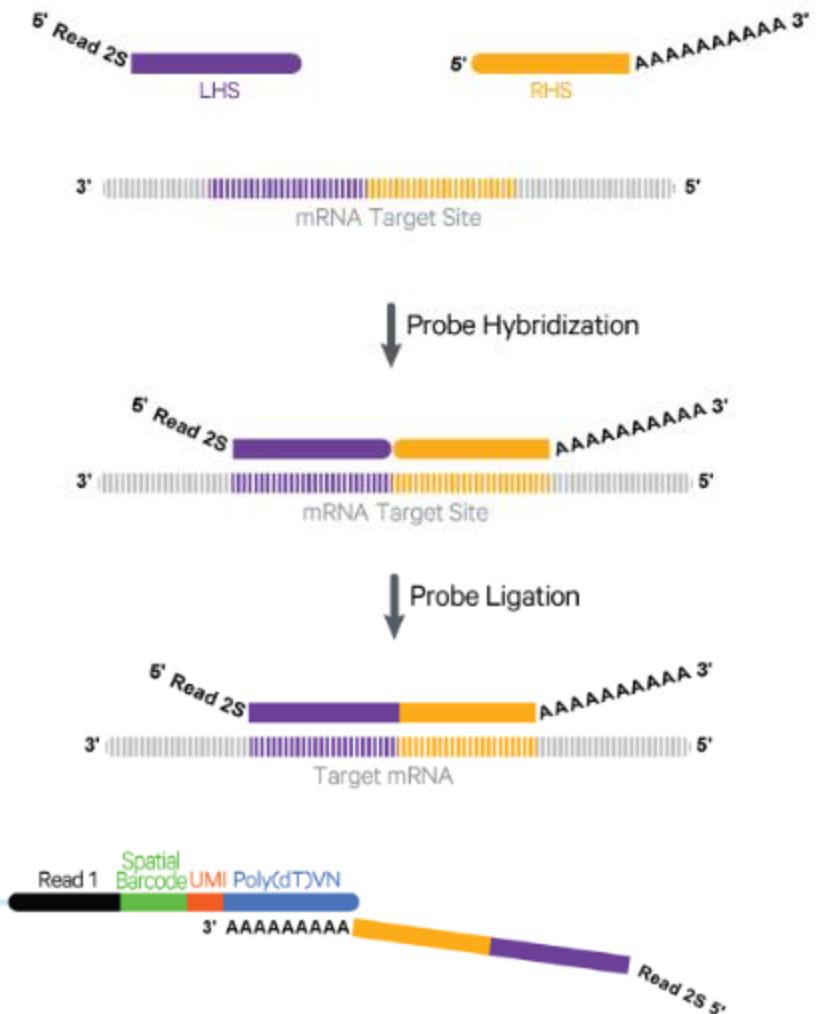
- Localization of RNA over tissue morphology.
- Two main groups are sequencing and imaging based.
- We will focus on 10X Visium and Xenium



10X Visium



FFPE & CytAssyt



Advantages:

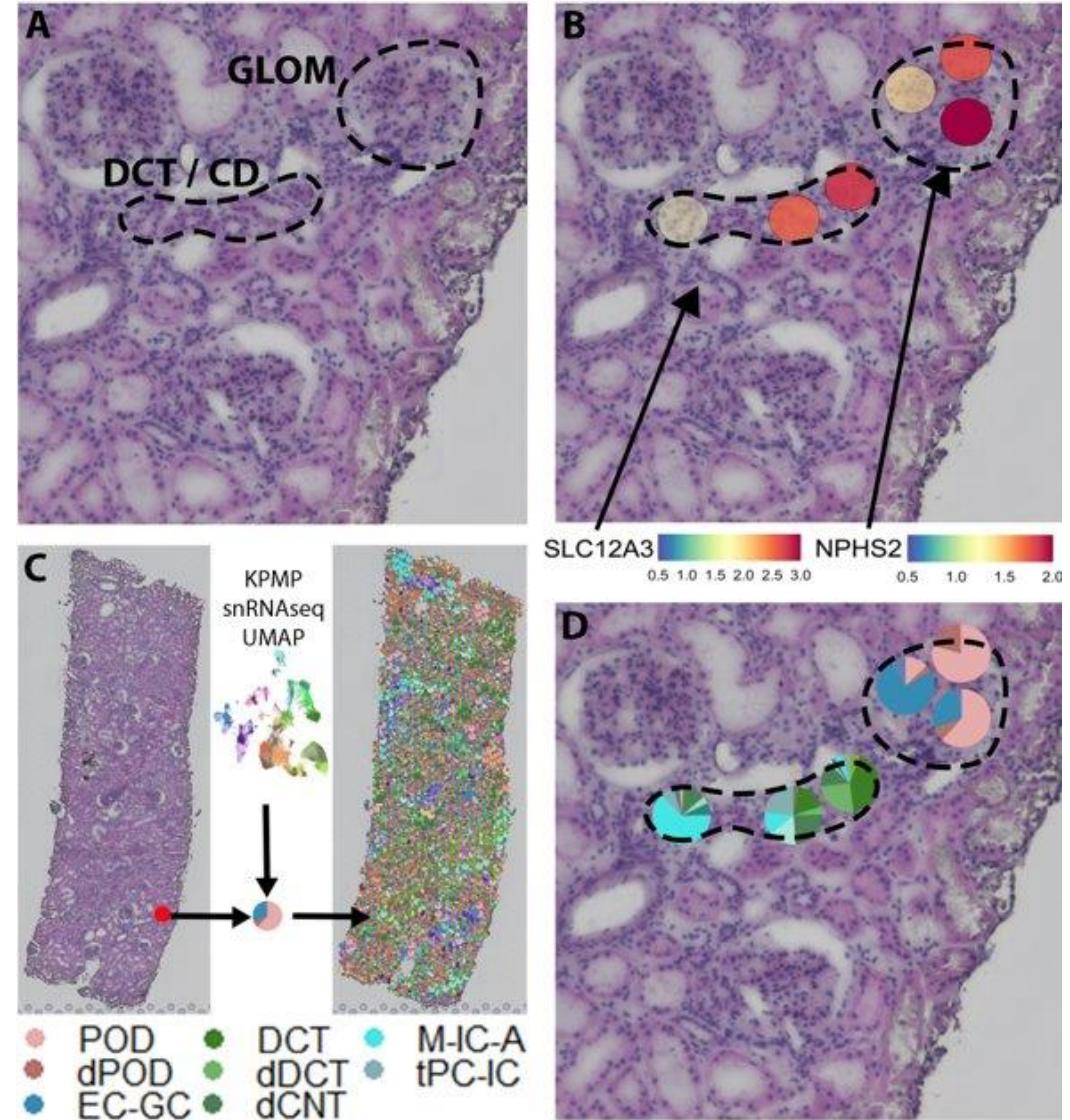
- Whole transcriptome
- FFPE: 18,536 genes targeted by 54,018 probes.
- Brightfield or IF image (histology preservation in FFPE)

Disadvantages:

- Spots with 55 µm in diameter, 100 µm apart

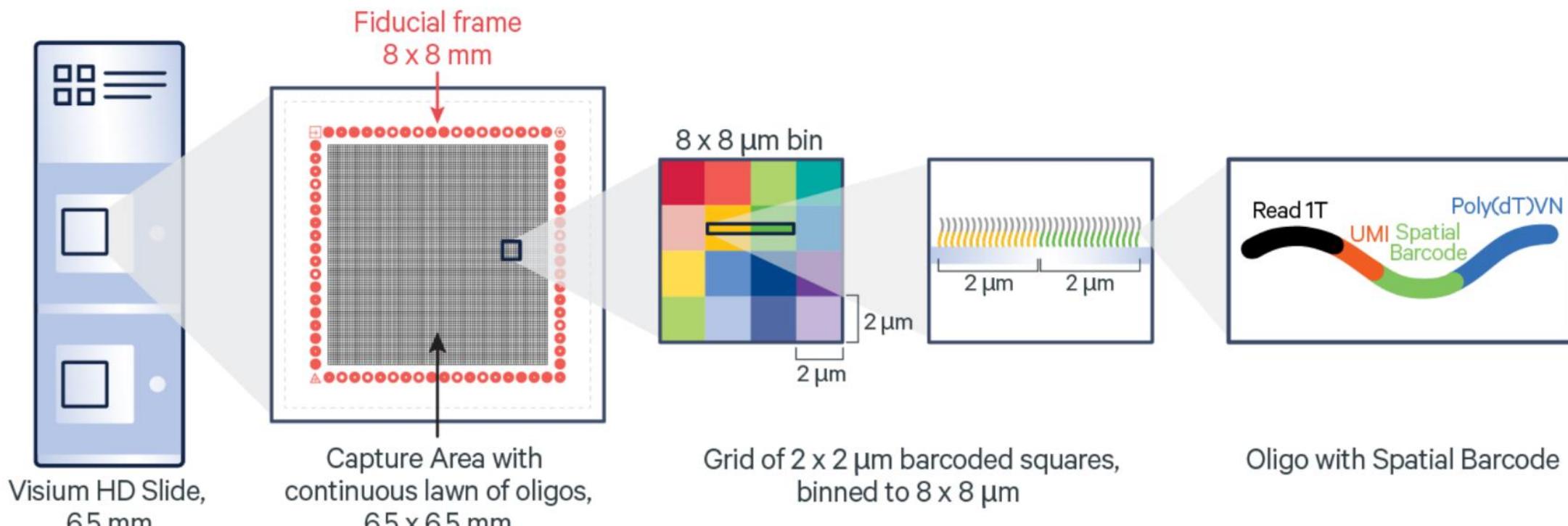
Cell type deconvolution

- 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:

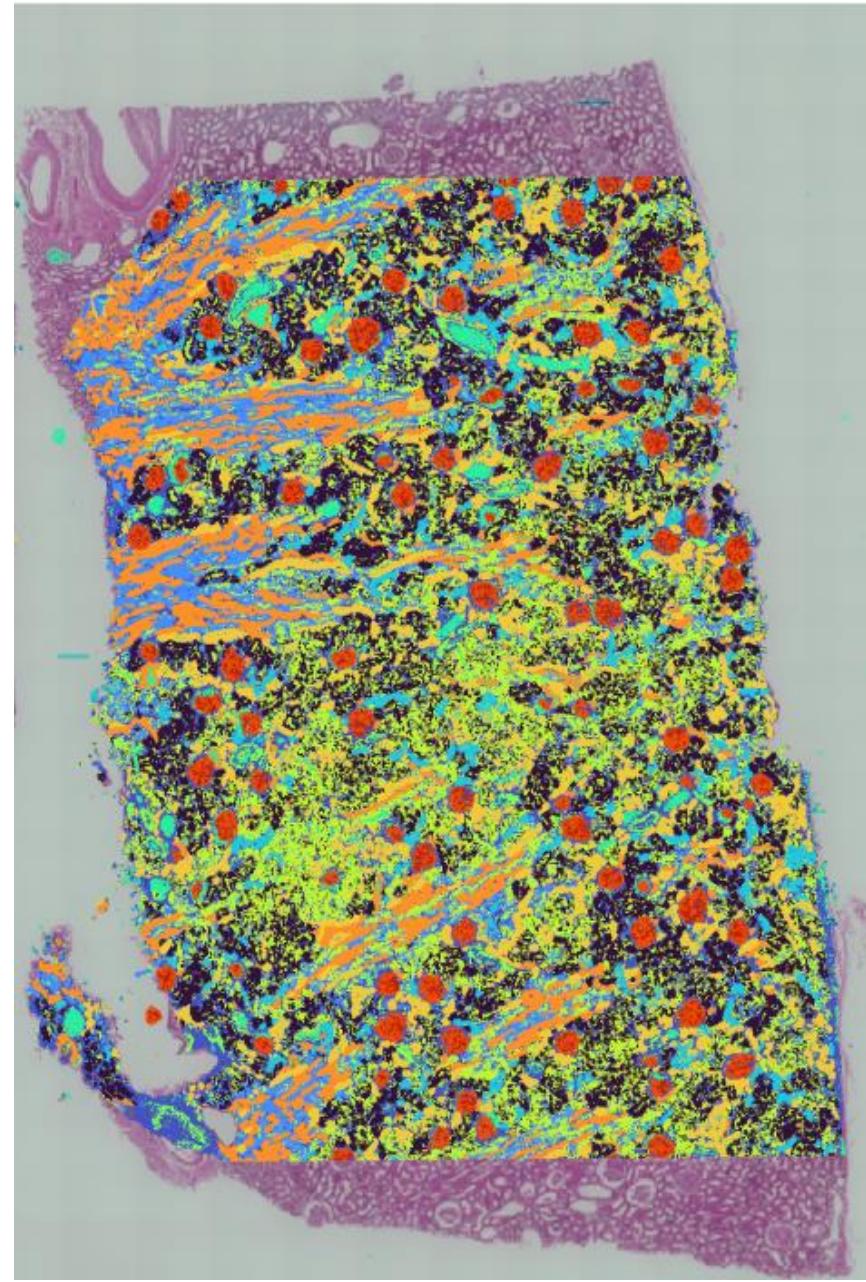
- Resolution of 2 μm

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

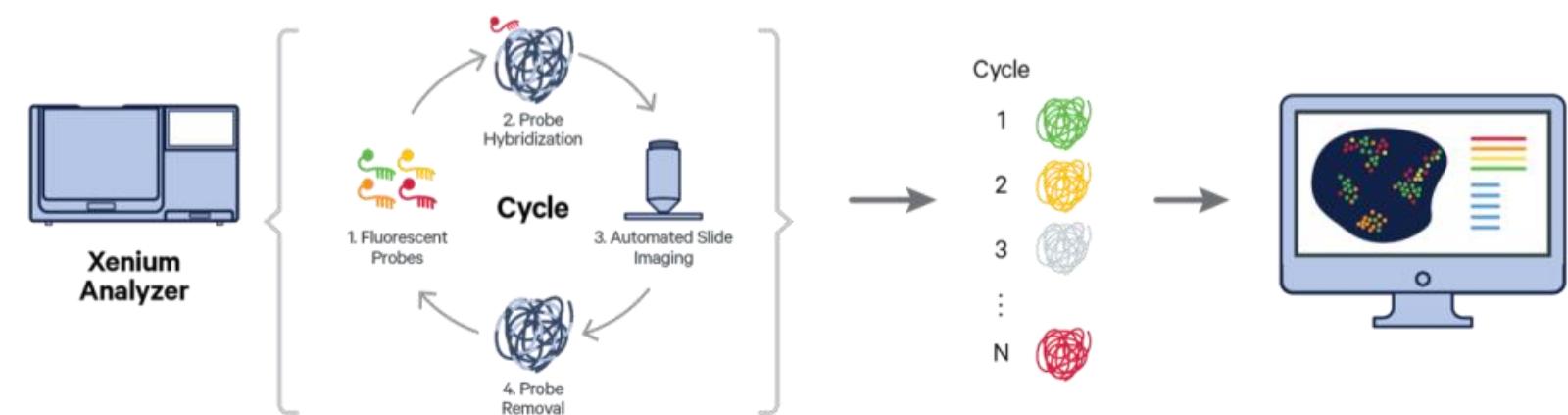
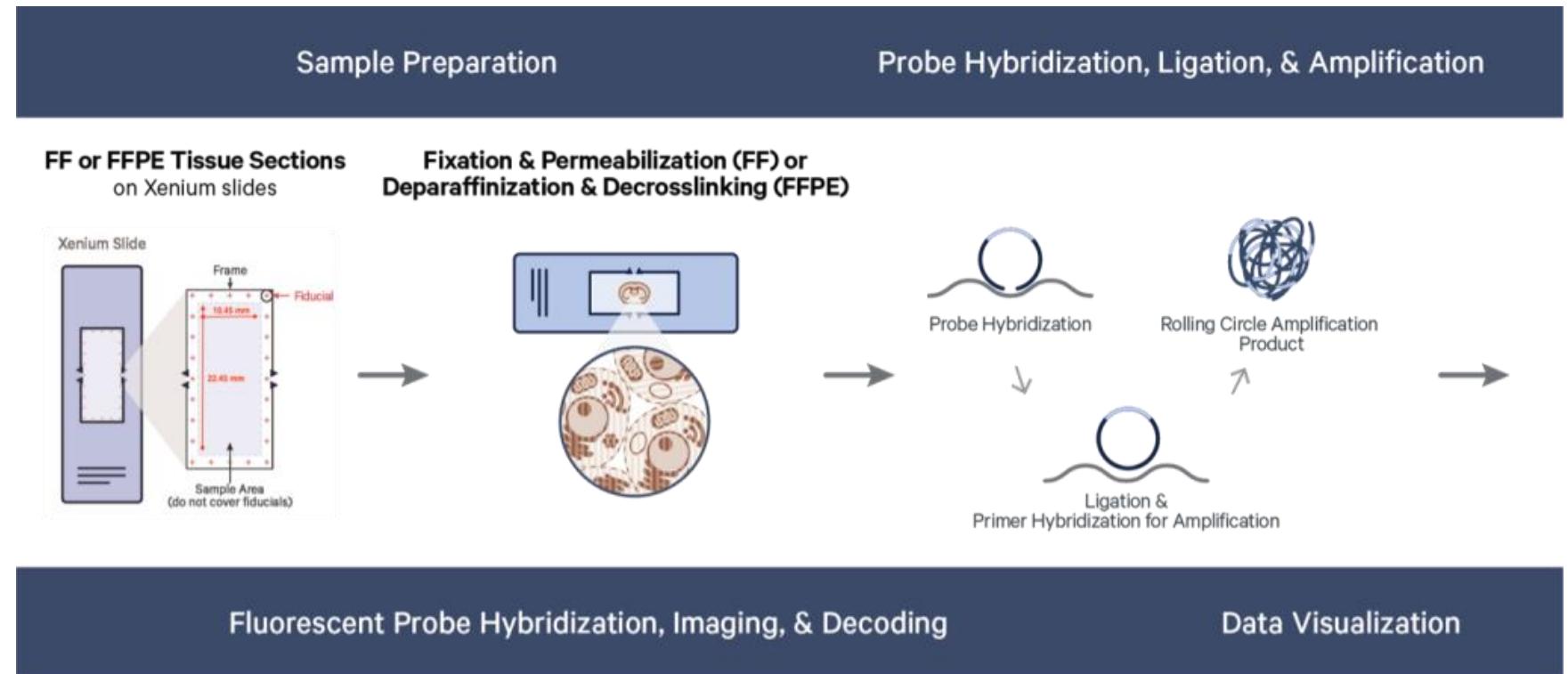
Xenium

Advantages:

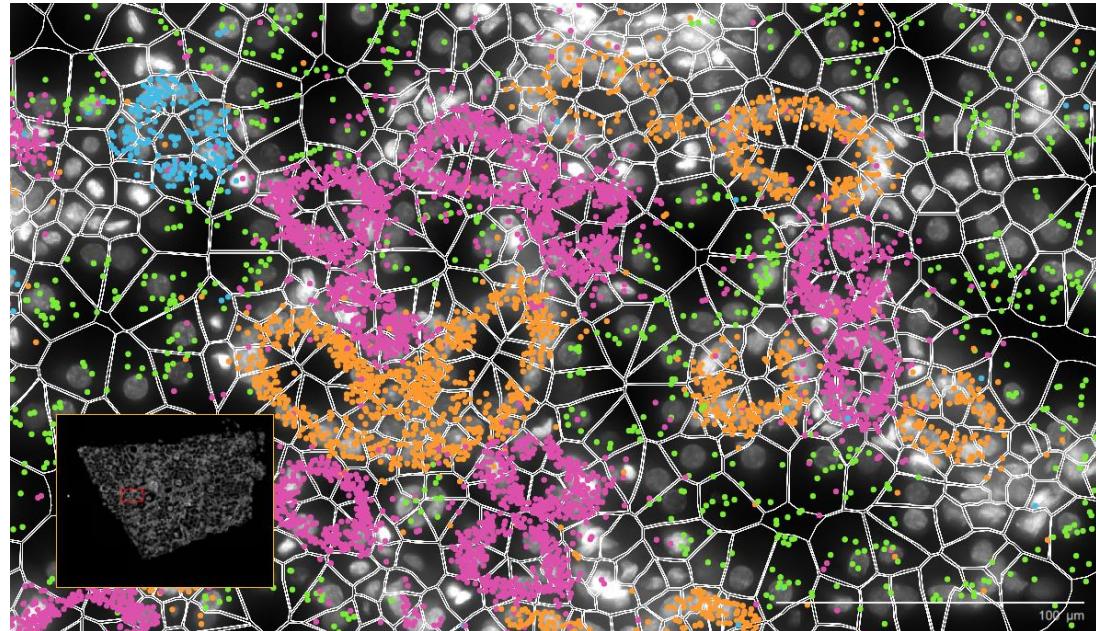
- Subcellular resolution
- Histology
- Optional membrane and cytoplasm staining

Disadvantages:

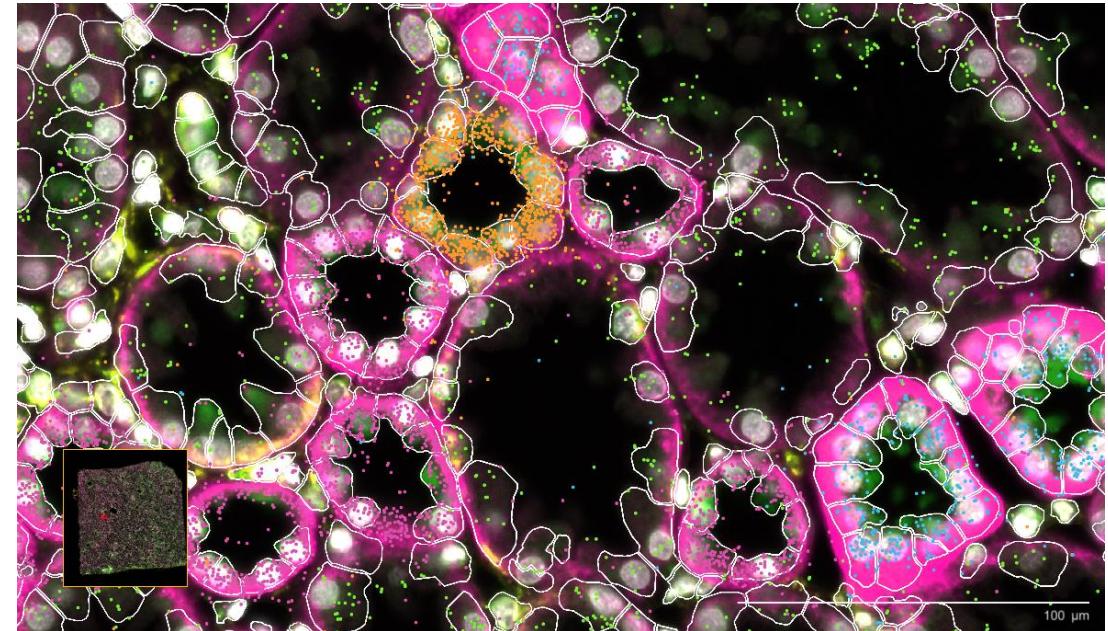
- Limited probes
- Segmentation might be challenging



Cell segmentation



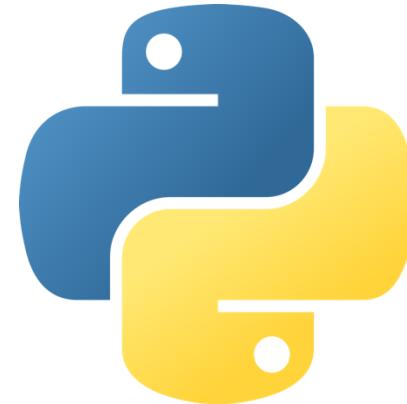
- AQP2
- LRP2
- SLC12A3
- UMOD



Tools



- Seurat
- singleCellExperiment
- Giotto



- scVerse
- Scanpy
- Squidpy

Seurat spatialobject

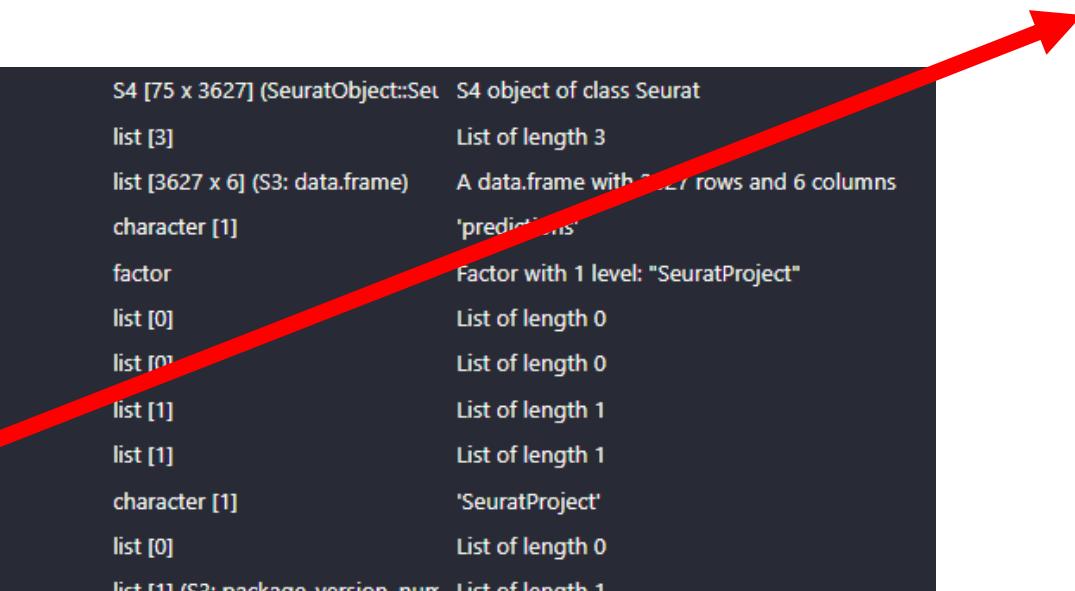
spatial.obj	S4 [75 x 3627] (SeuratObject)	S4 object of class Seurat
assays	list [3]	List of length 3
meta.data	list [3627 x 6] (S3: data.frame)	A data.frame with 3627 rows and 6 columns
active.assay	character [1]	'predictions'
active.ident	factor	Factor with 1 level: "SeuratProject"
graphs	list [0]	List 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

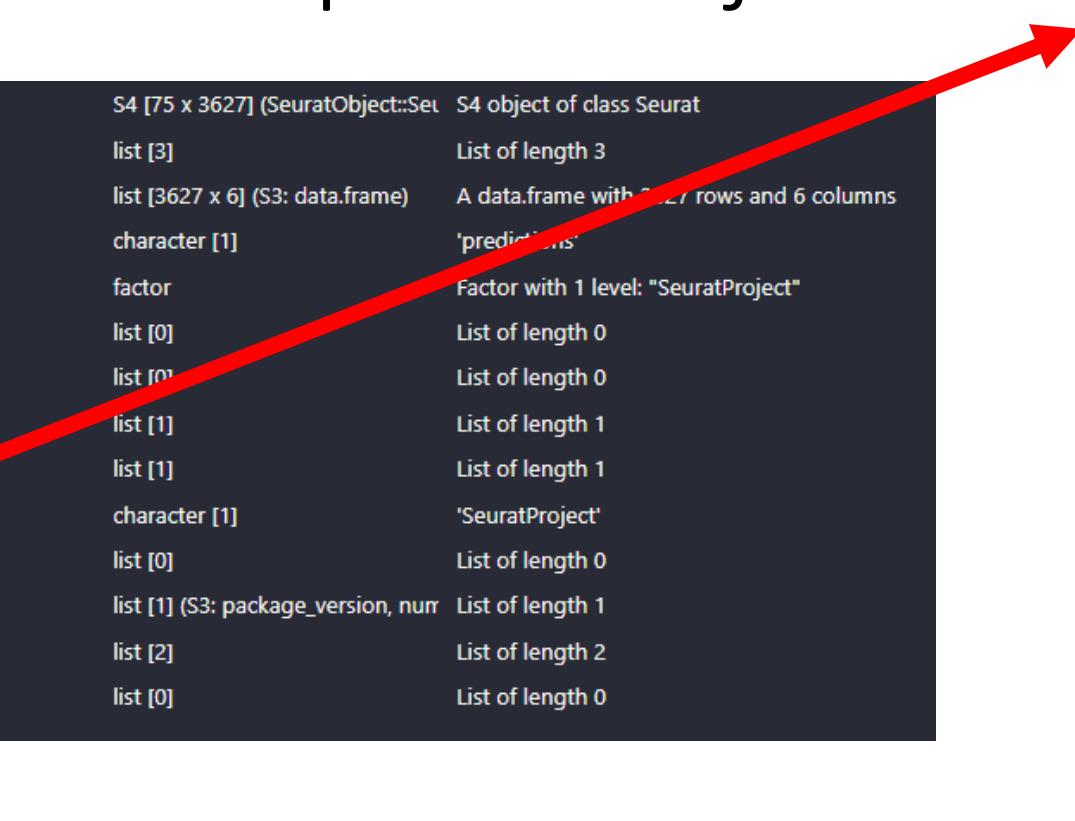
spatial.obj	S4 [75 x 3627] (SeuratObject::Seurat)	S4 object of class Seurat
assays	list [3]	List of length 3
meta.data	list [3627 x 6] (S3: data.frame)	A data.frame with 3627 rows and 6 columns
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active.ident	factor	Factor with 1 level: "SeuratProject"
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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

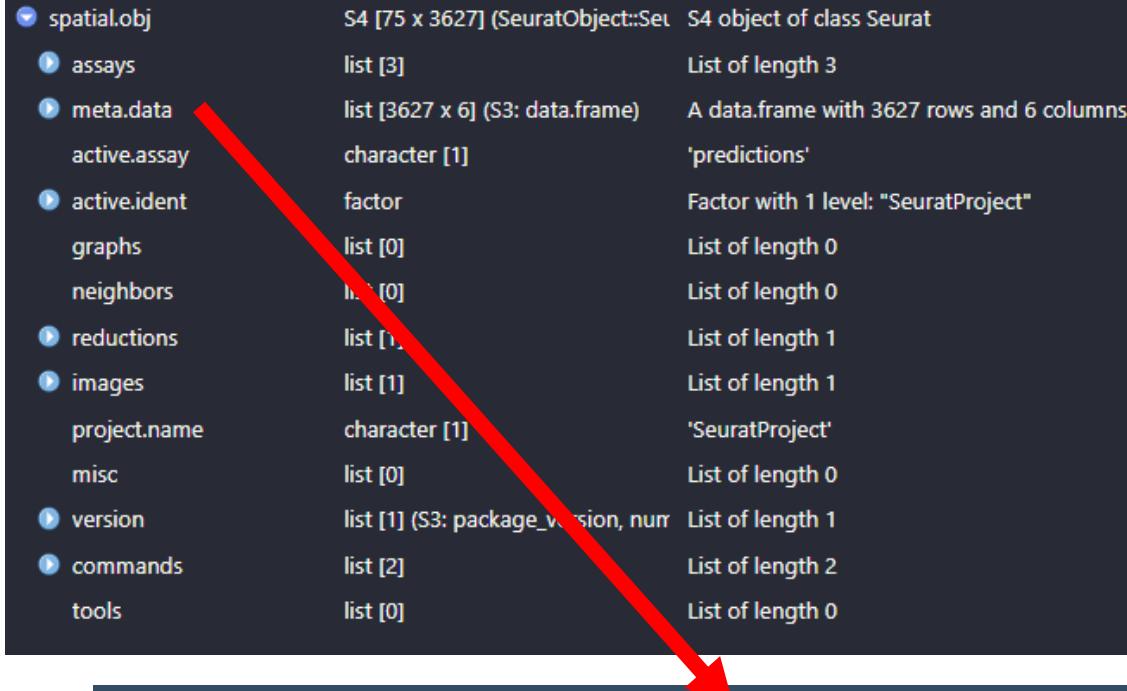
spatial.obj	S4 [75 x 3627] (SeuratObject::Seurat)	S4 object of class Seurat
assays	list [3]	List of length 3
meta.data	list [3627 x 6] (S3: data.frame)	A data.frame with 3627 rows and 6 columns
active.assay	character [1]	'predictions'
active.ident	factor	Factor with 1 level: "SeuratProject"
graphs	list [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:

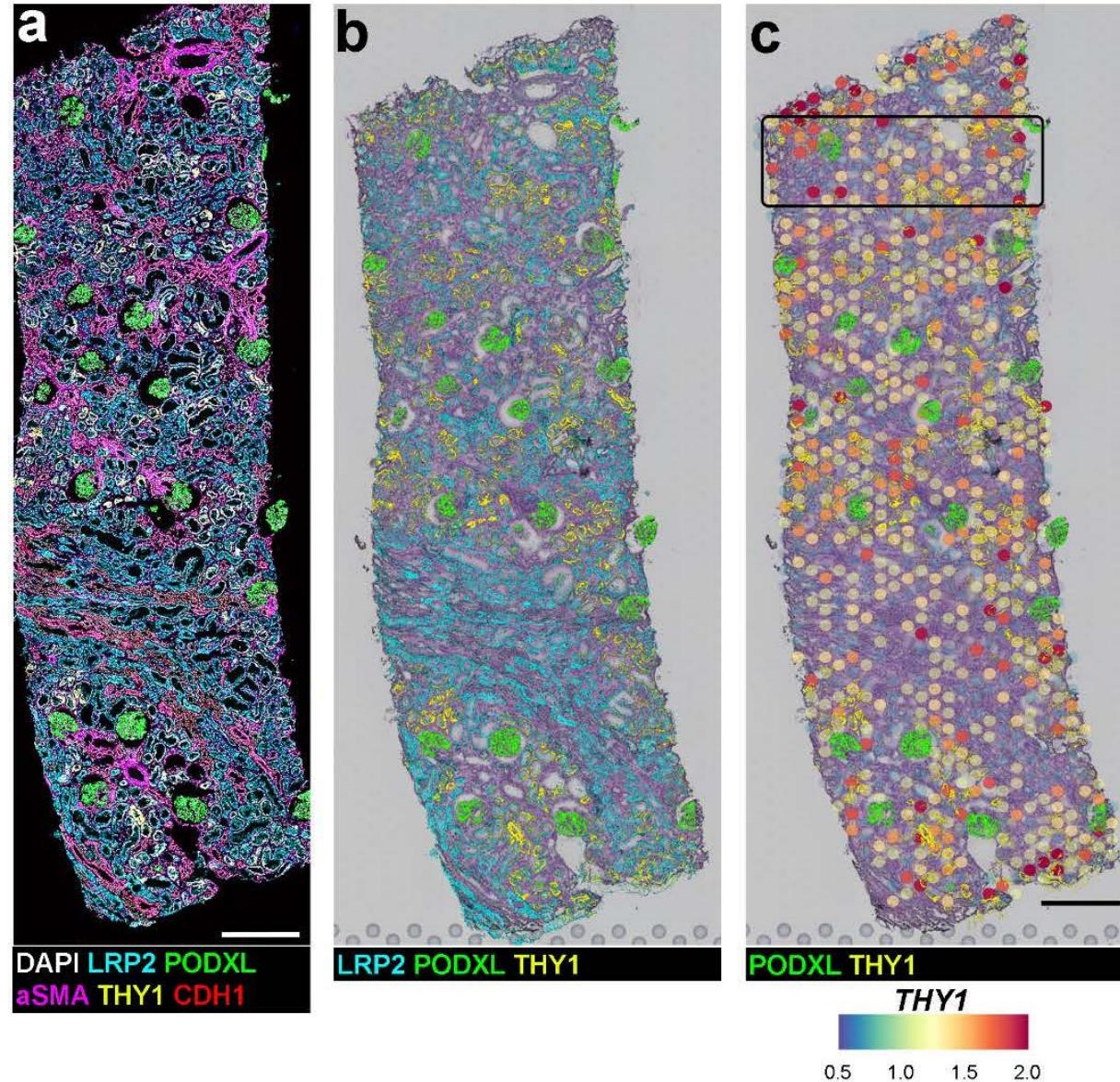
	orig.ident	nCount_Spatial	nFeature_Spatial	glom	nCount_SCT	nFeature_SCT	cca_clusters	seurat_clusters	glom_condition
AAACAAGTATCTCCA-1_1	V10S14-086_XY01_21	column 1: character	2410	3935	no_glom	7799	3665	4	4
AAACAGCTTCAGAAG-1_1	V10S14-086_XY01_21-0055		4930	2159	no_glom	6128	2147	3	3
AAACAGGGTCTATT-1_1	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

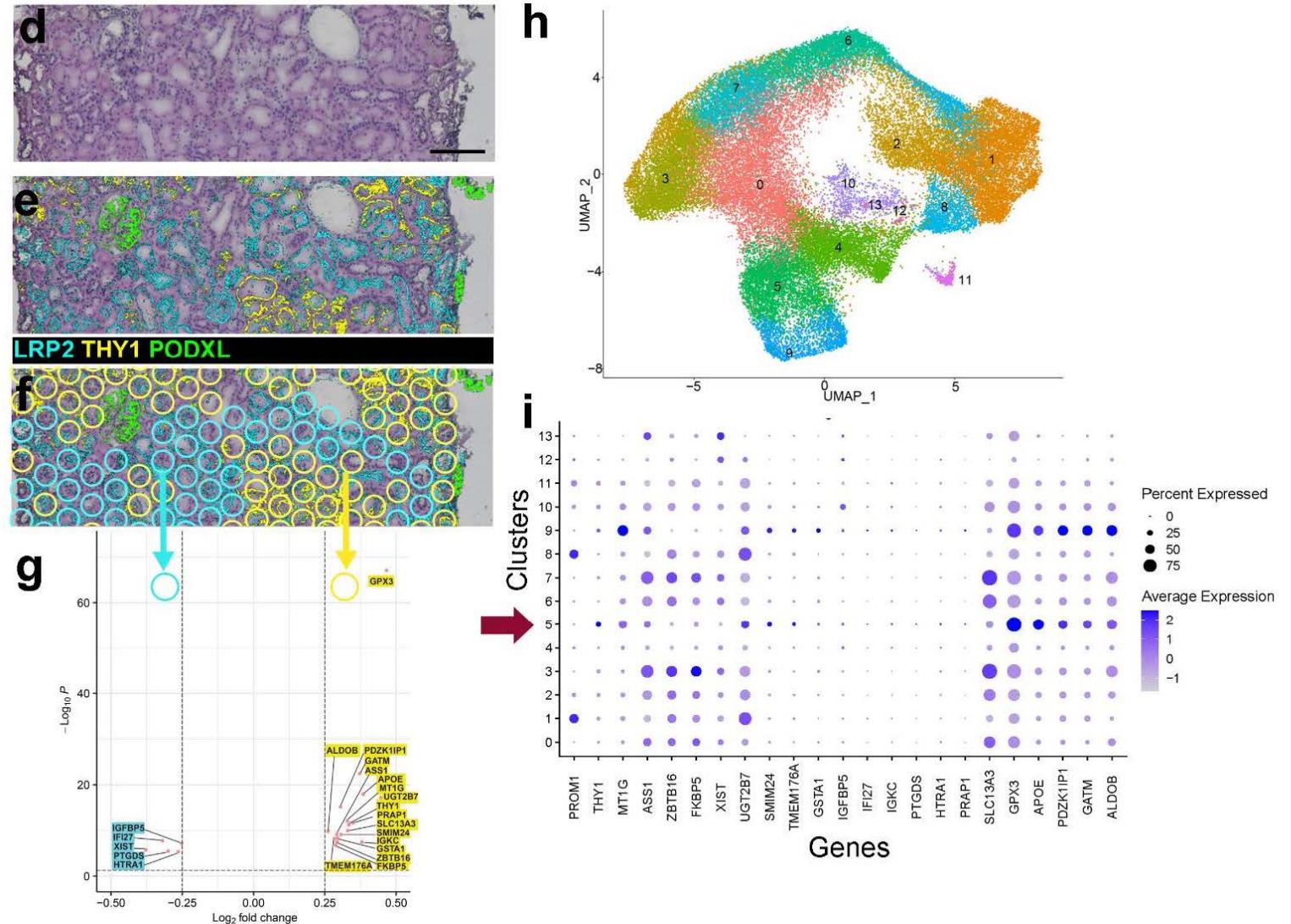
Integration of spatial multiplexed protein imaging and transcriptomics in the
human kidney tracks the regenerative potential timeline of proximal tubules
Asghari, Sabo, Barwinska *et al.* bioRxiv

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

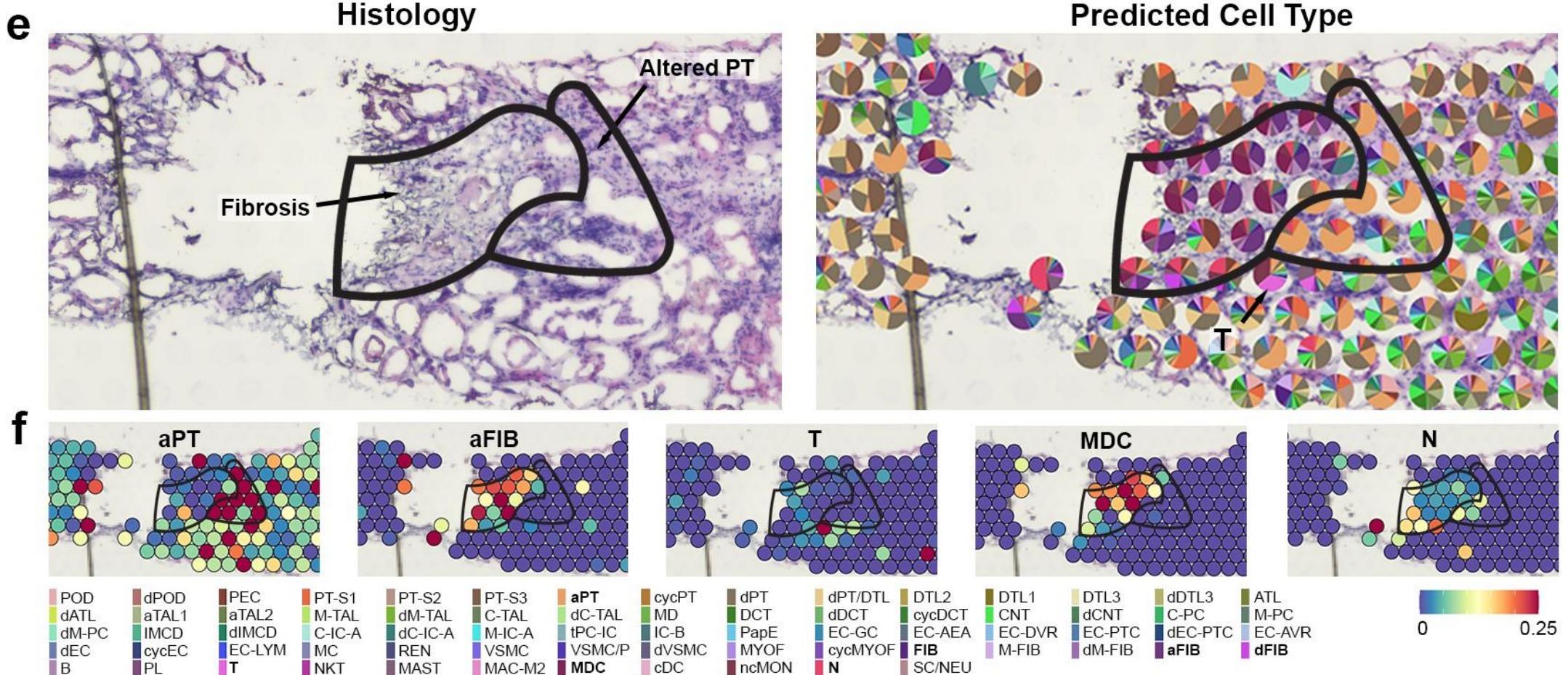
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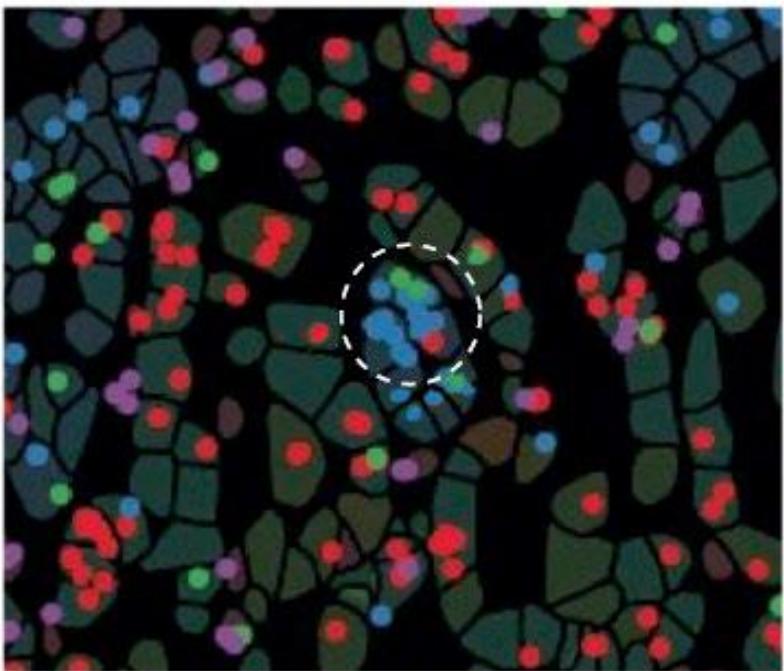
[Nature](#) **619**, 585–594 (2023) | [Cite this article](#)

Kidney atlas: Cell Type colocalization

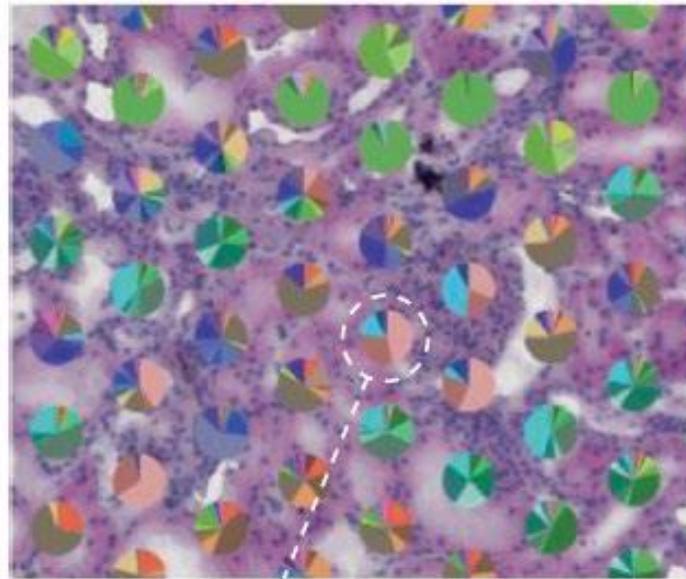
Transcriptomic localization of cell states associated with injury



Neighborhood Analysis

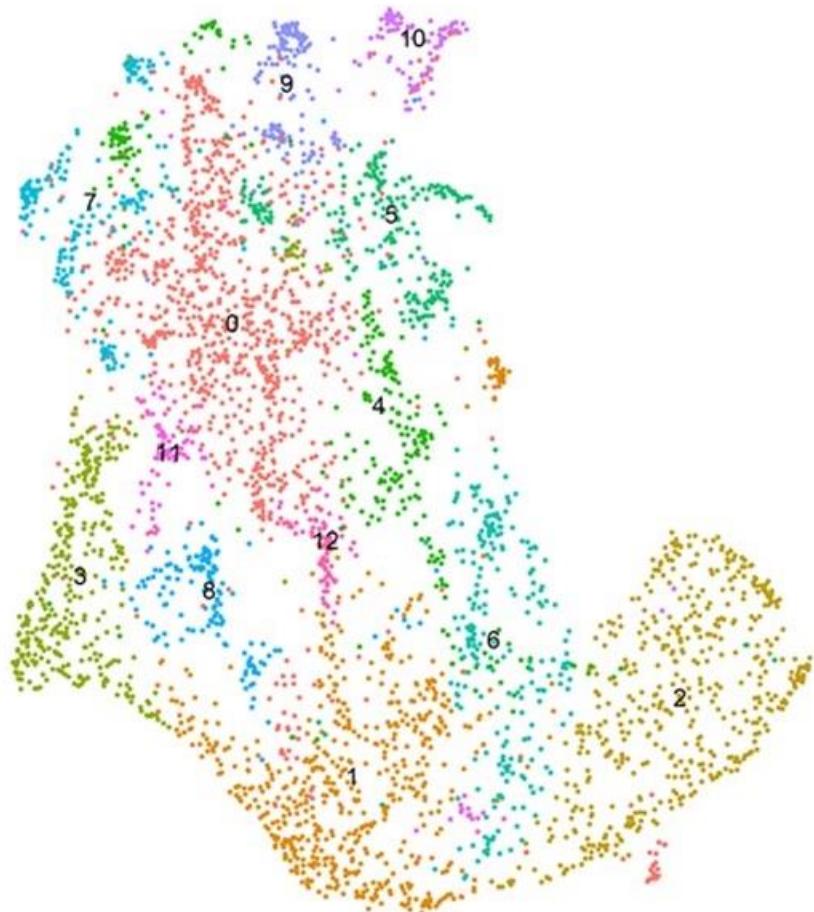


A neighbourhood defined
by a radius around a centroid

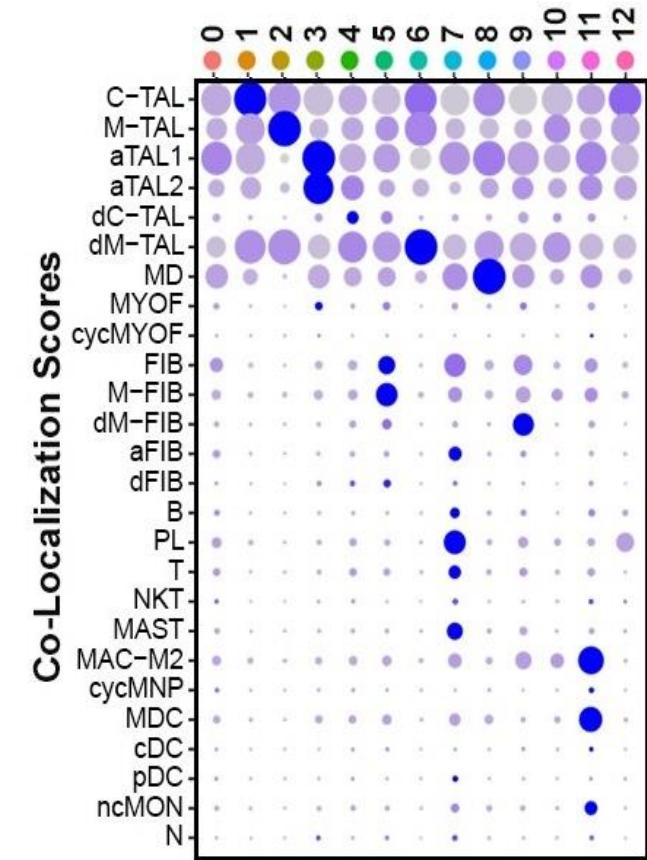
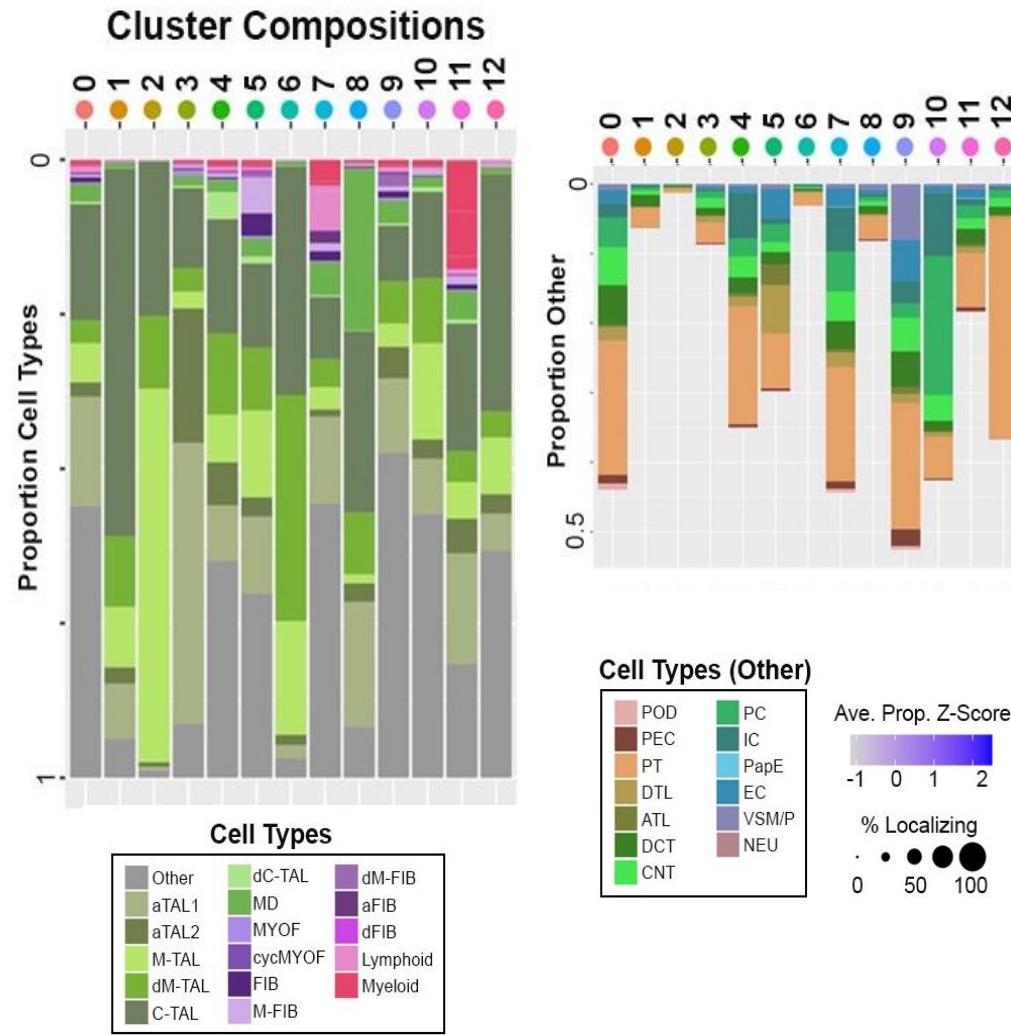
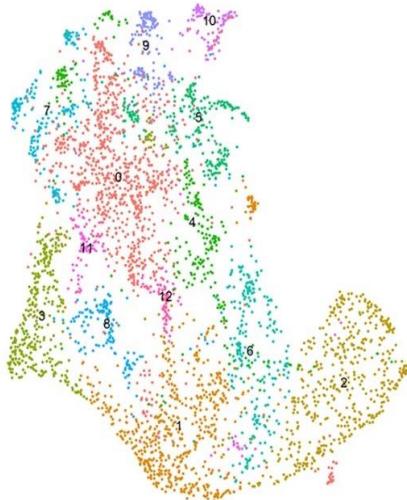


Components
of the spot
become the
neighbourhood

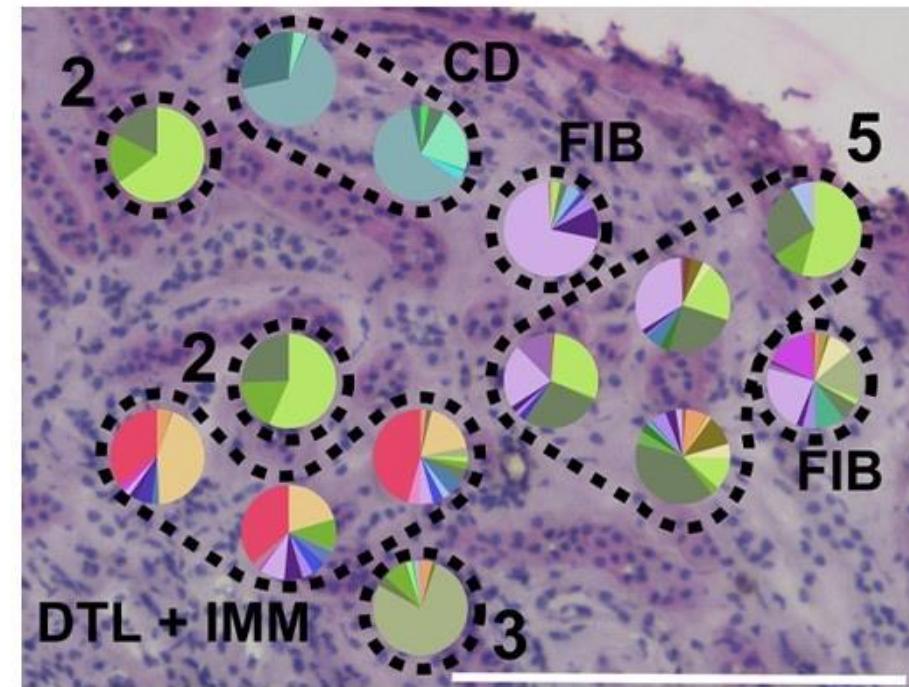
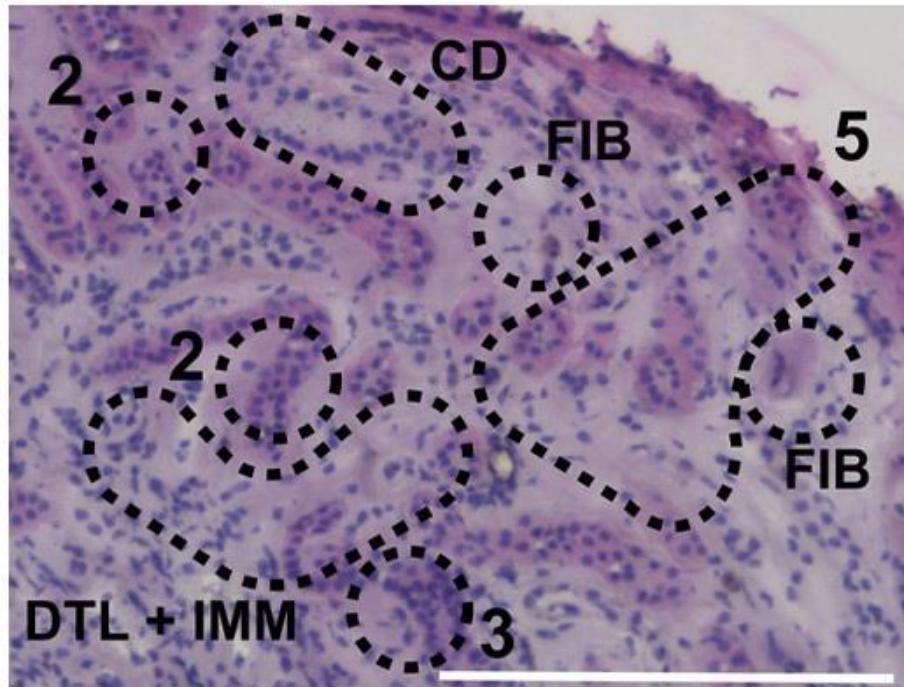
Transcriptomic signature of TAL niches



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

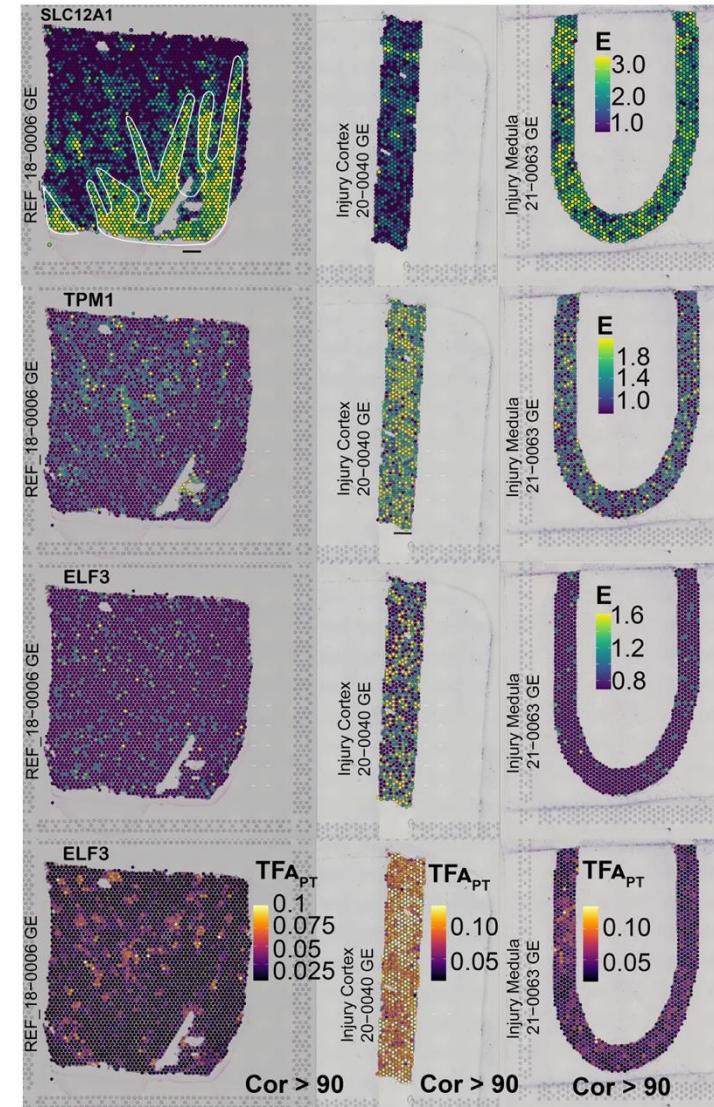
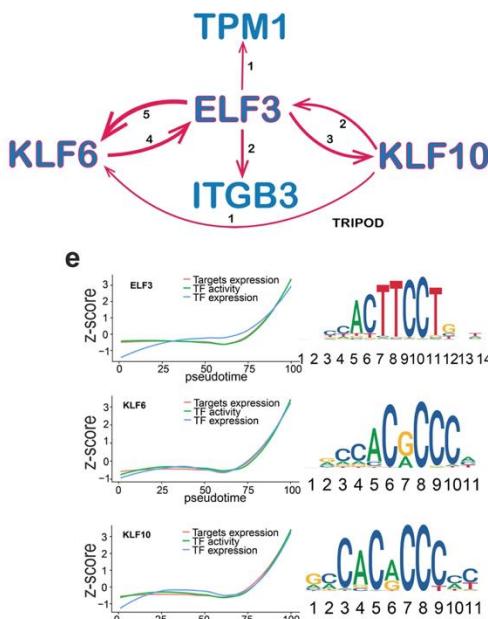
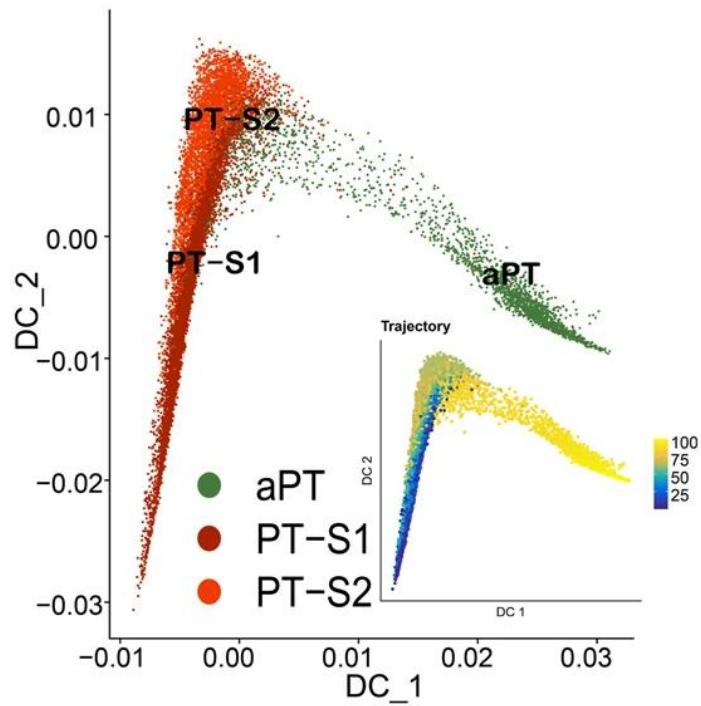
[Debora L. Gisch](#), [Michelle Brennan](#), [Blue B. Lake](#), [Jeannine Basta](#), [Mark S. Keller](#), [Ricardo Melo Ferreira](#),
[Shreeram Akilesh](#), [Reetika Ghag](#), [Charles Lu](#), [Ying-Hua Cheng](#), [Kimberly S. Collins](#), [Samir V. Parikh](#), [Brad H. Rovin](#), [Lynn Robbins](#), [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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Epigenetic atlas: Validation of Transcription Factors

Modelling Transcription factor network



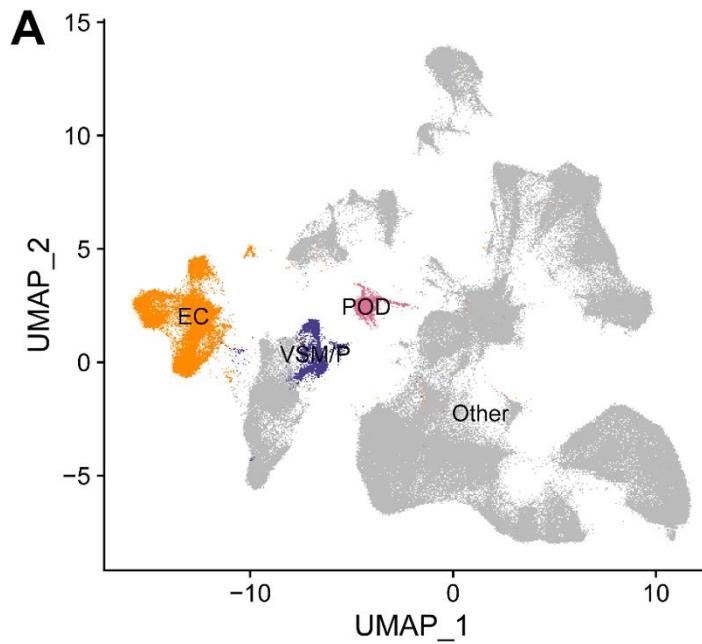
A MEF2C transcription factor network regulates proliferation of glomerular endothelial cells in diabetic kidney disease

✉ 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

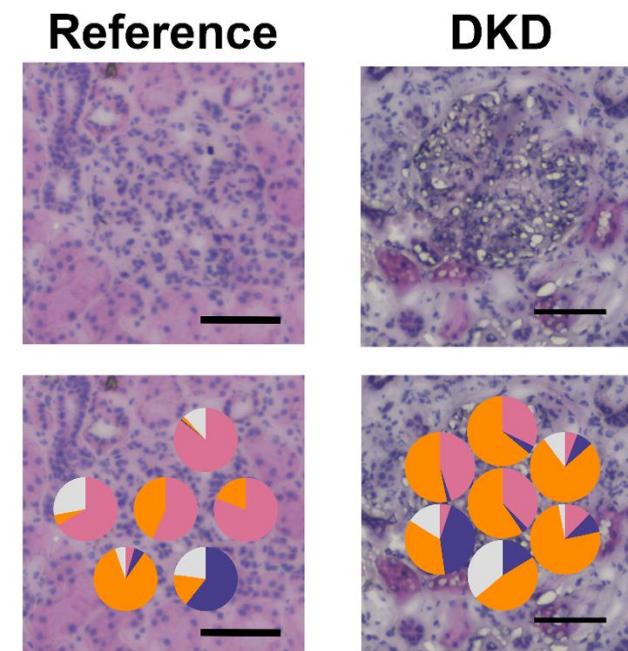
doi: <https://doi.org/10.1101/2024.09.27.615250>



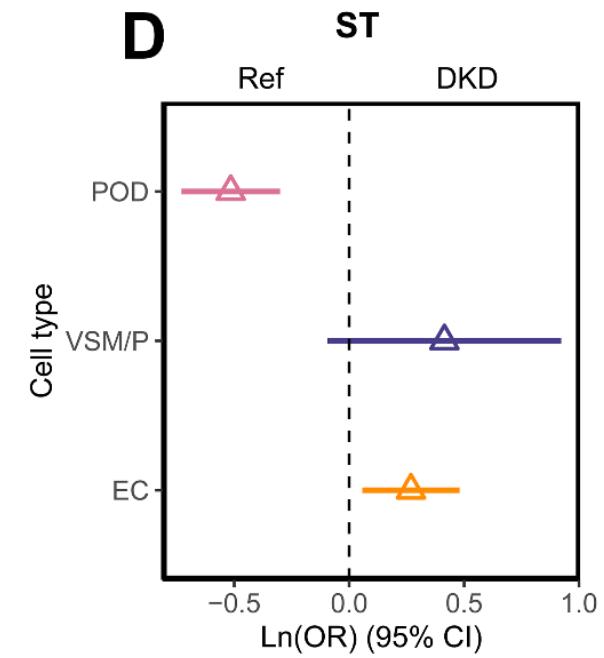
Diabetic Kidney Disease: Using Visium to characterize single cell; validation with Xenium; correlation with histopathology



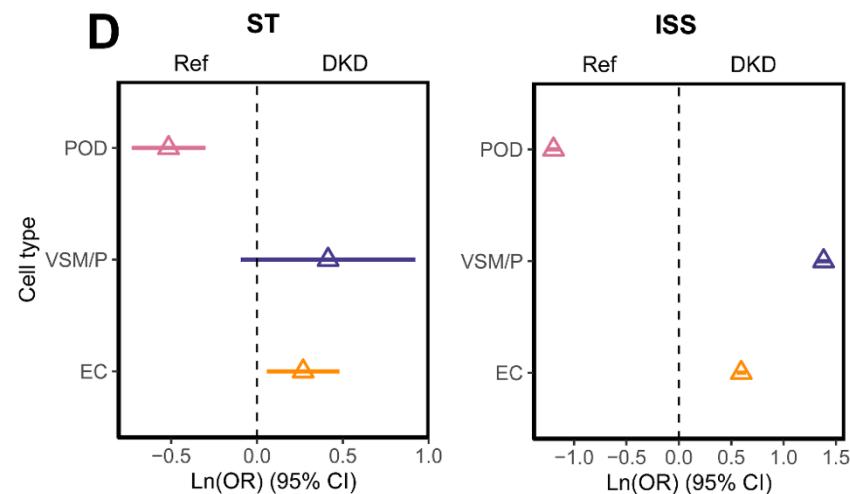
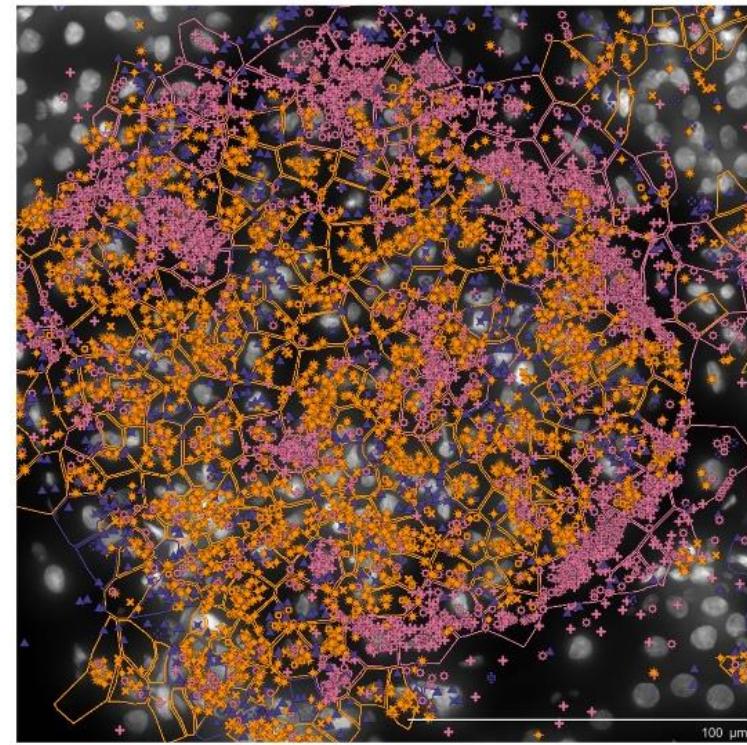
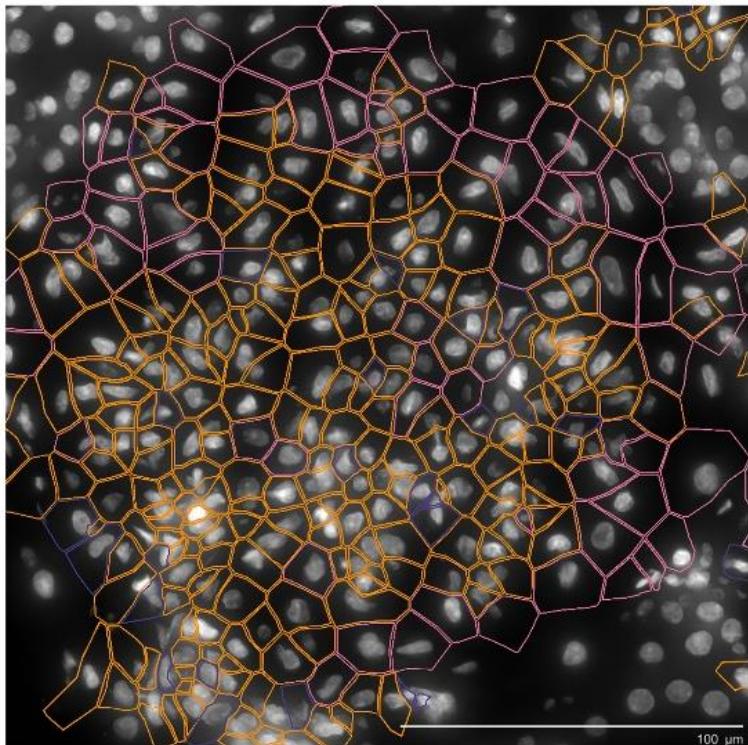
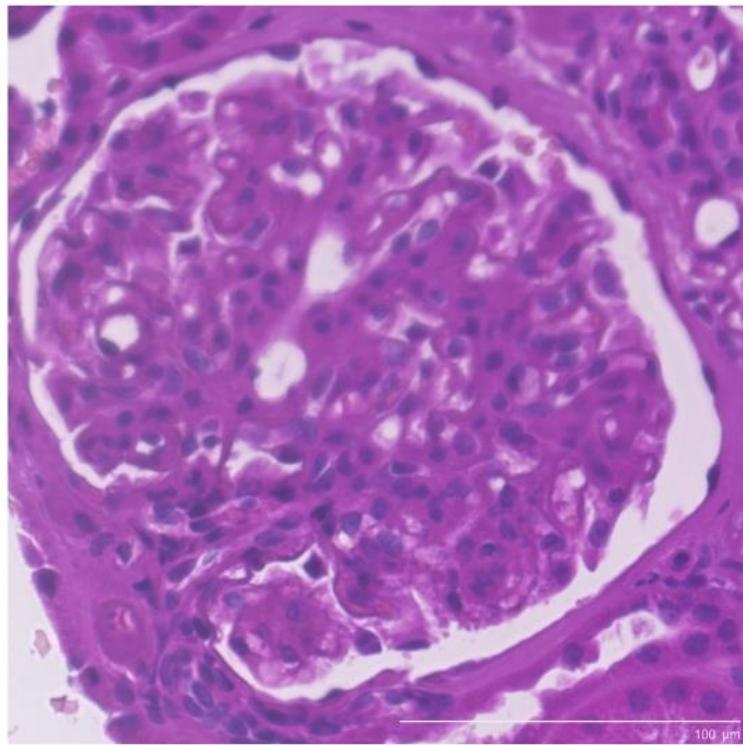
Subset of potential glomerular cells



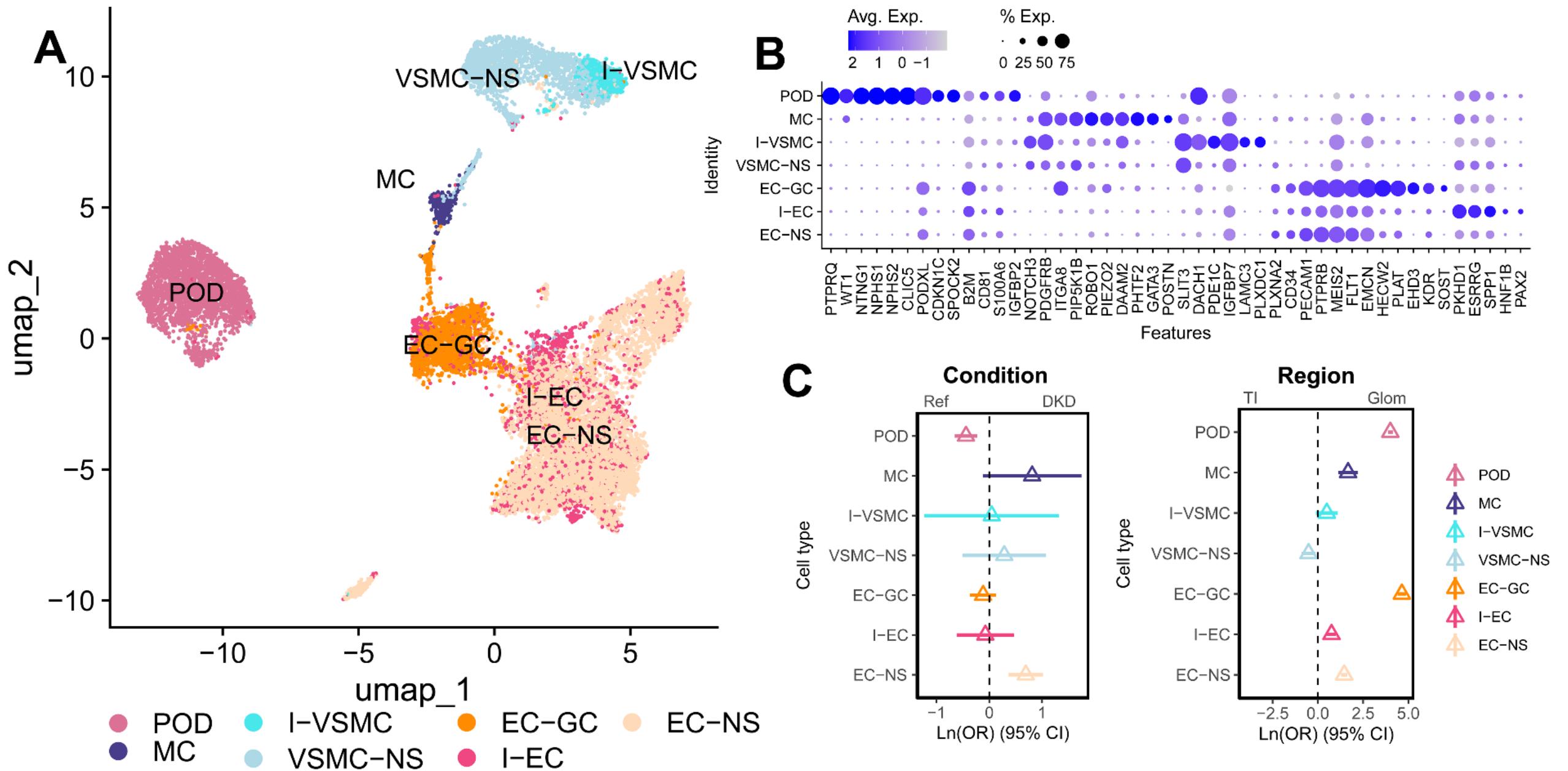
Label transfer suggests difference in glomerular cell type composition



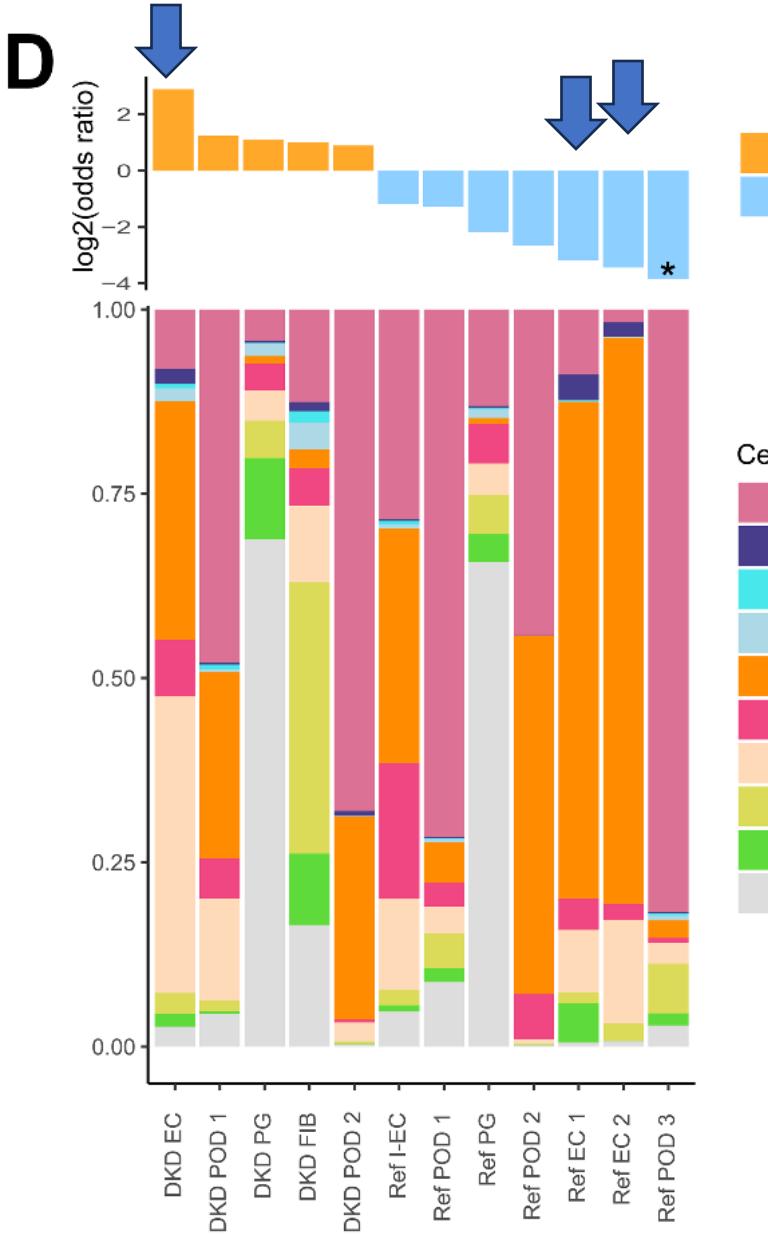
Reduction in podocyte signature. Increase in VSM/P + EC.

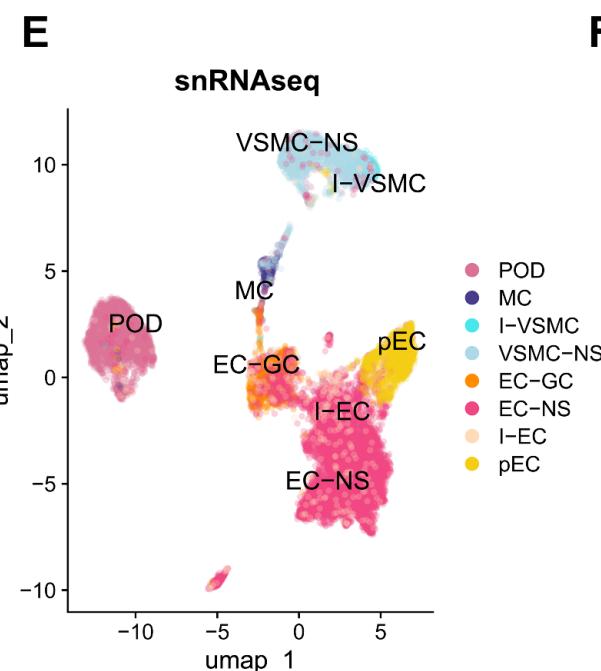
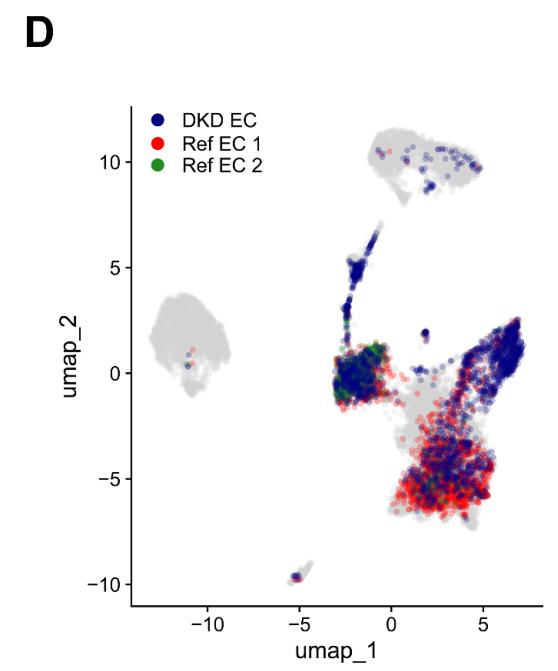
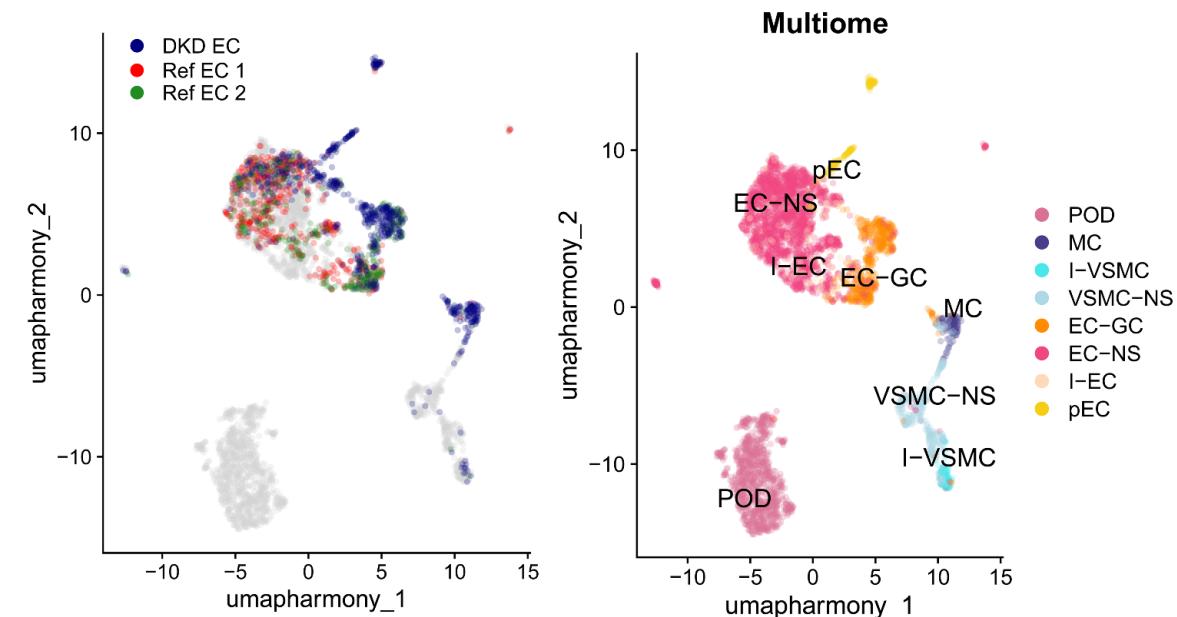


Evaluated specific MC/VSM and EC subtypes



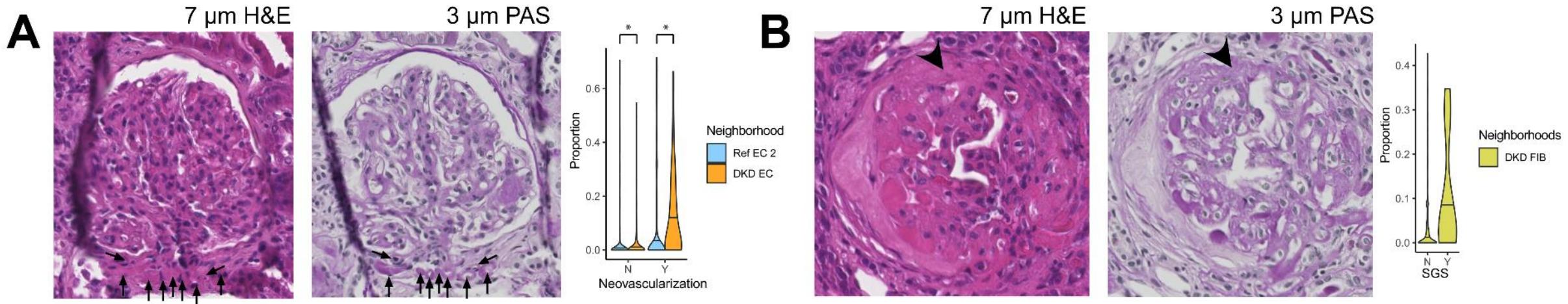
Neighborhood analysis





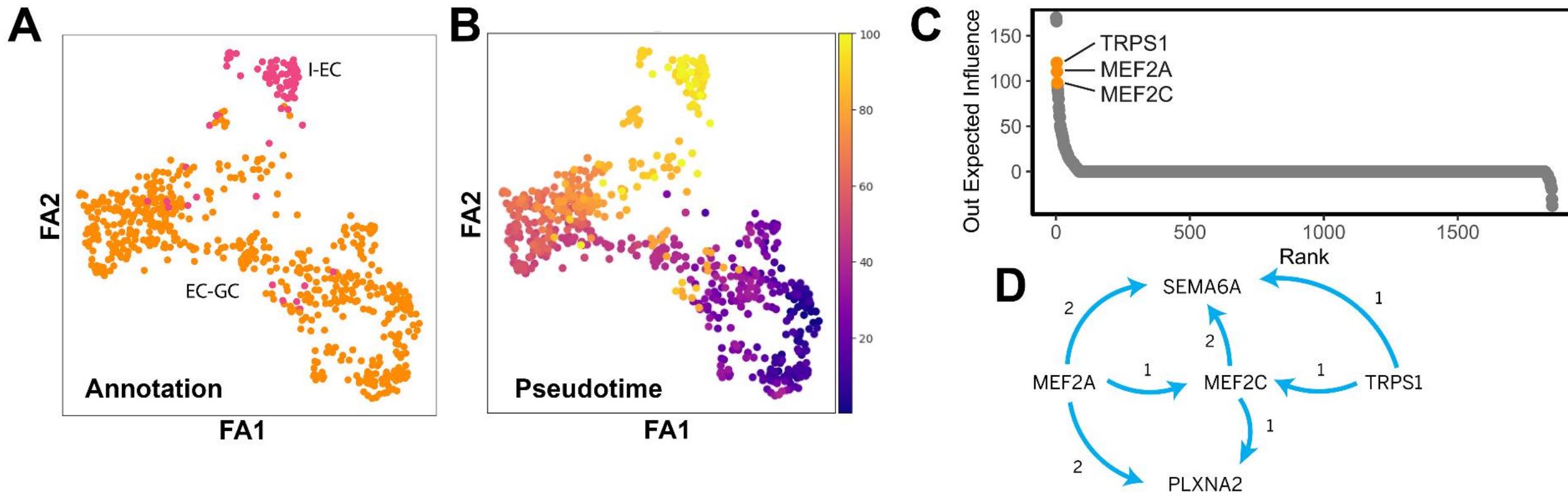
- Back-mapped ST neighborhood information (reverse label transfer)
- Determined which endothelial sub-cluster is in diabetic glomeruli.
- Called it pEC for proliferative endothelial cell.

Histology



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

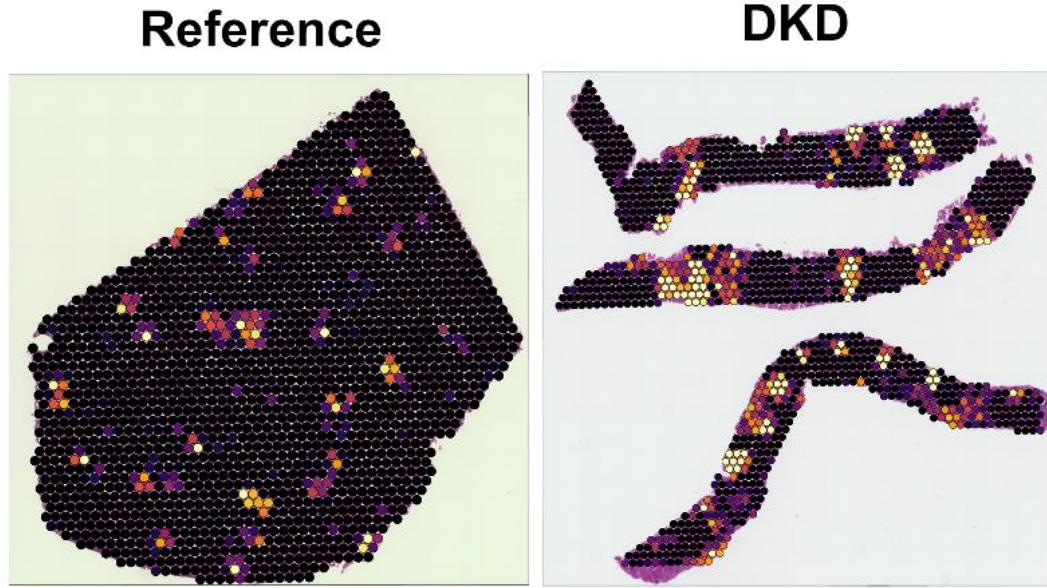
Trajectory of glomerular capillary endothelial cell to degenerative endothelial cell



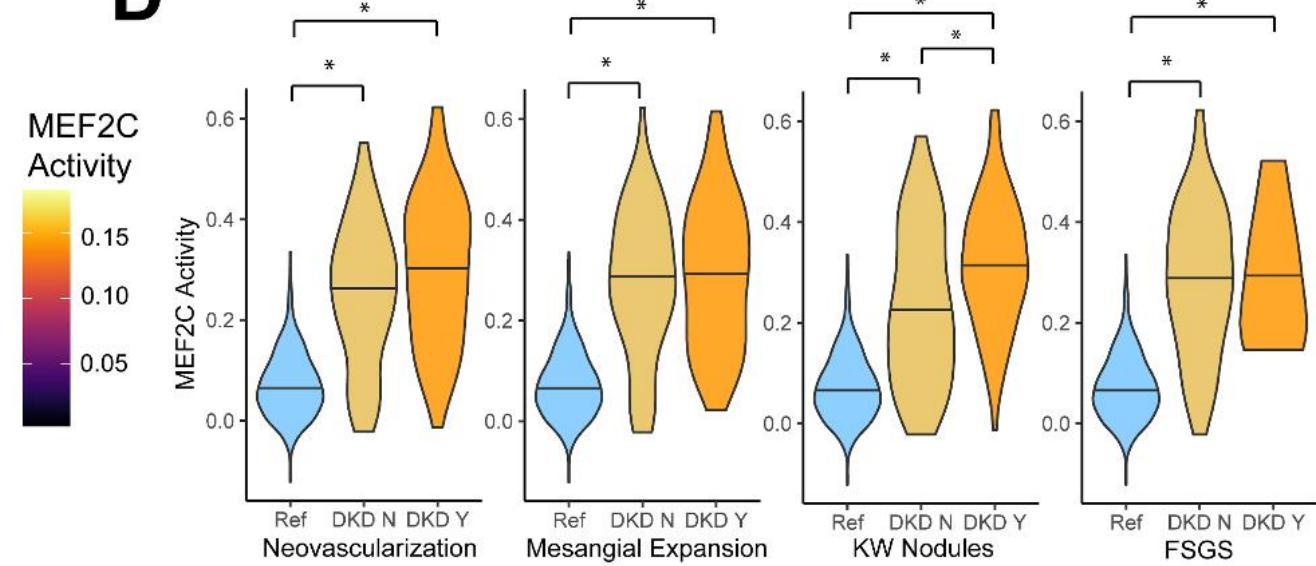
Transcription Factor Network with high centrality

MEF2C activity mapping

C



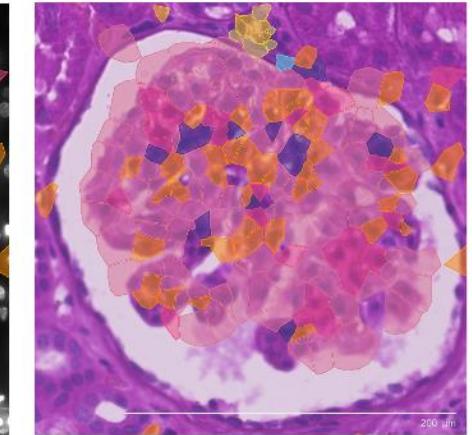
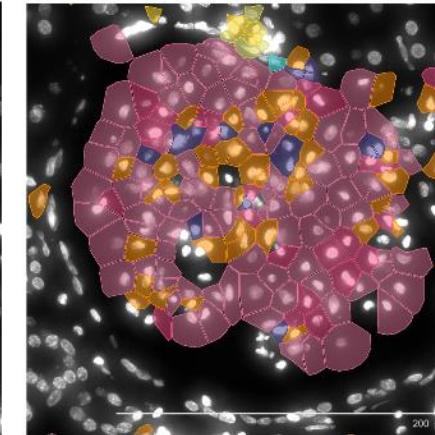
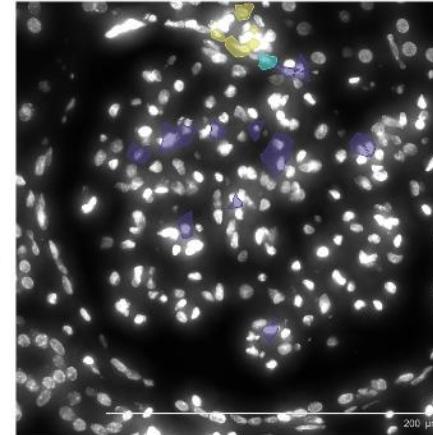
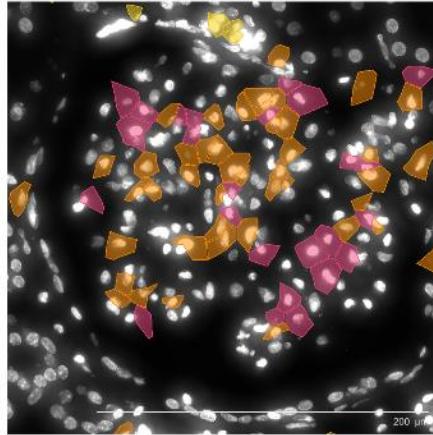
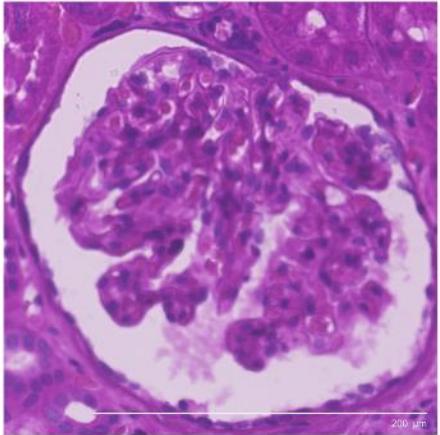
D



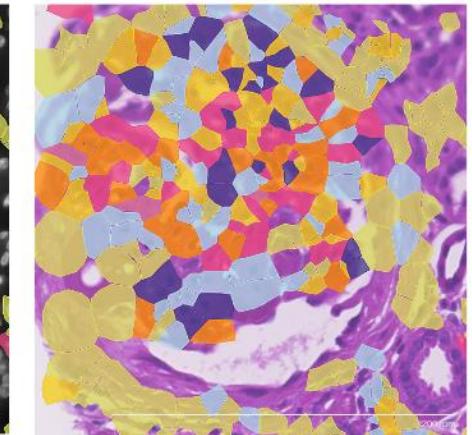
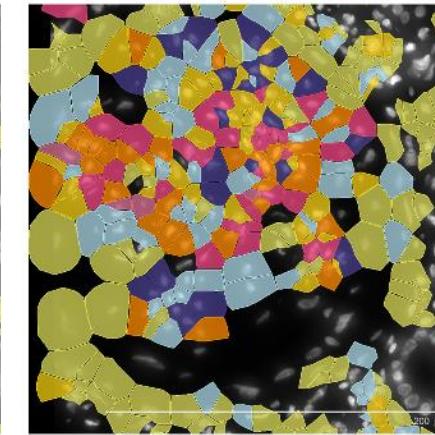
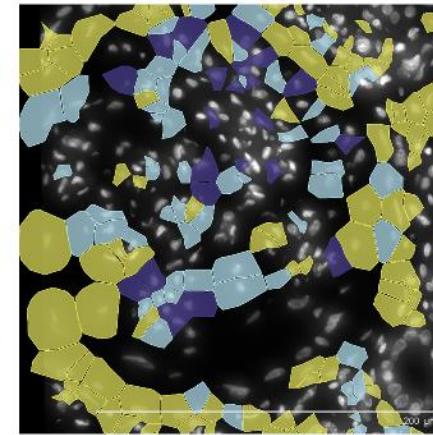
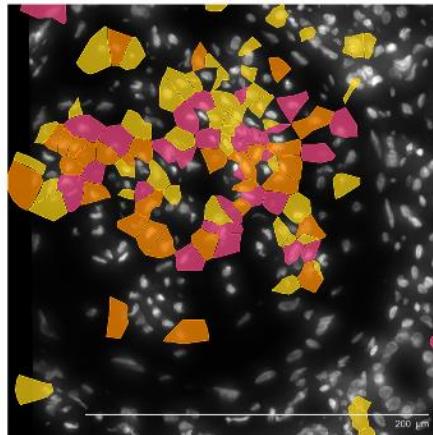
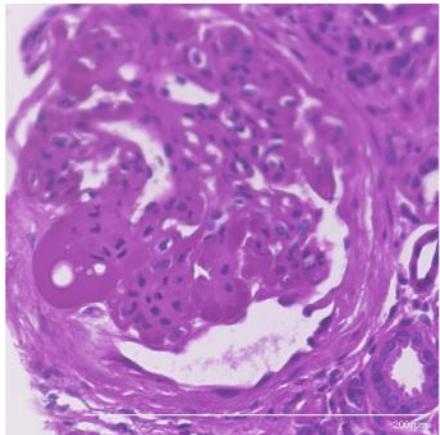
- *MEF2C* activity increased in DKD and upregulated further in nodular glomeruli.

Spatial trajectories of diseased cells

Reference



DKD



EC-GC prEC
dEC

MC VSMC-NS
dVSMC FIB

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Questions?