



Contribution of transcriptomics in nephropathology

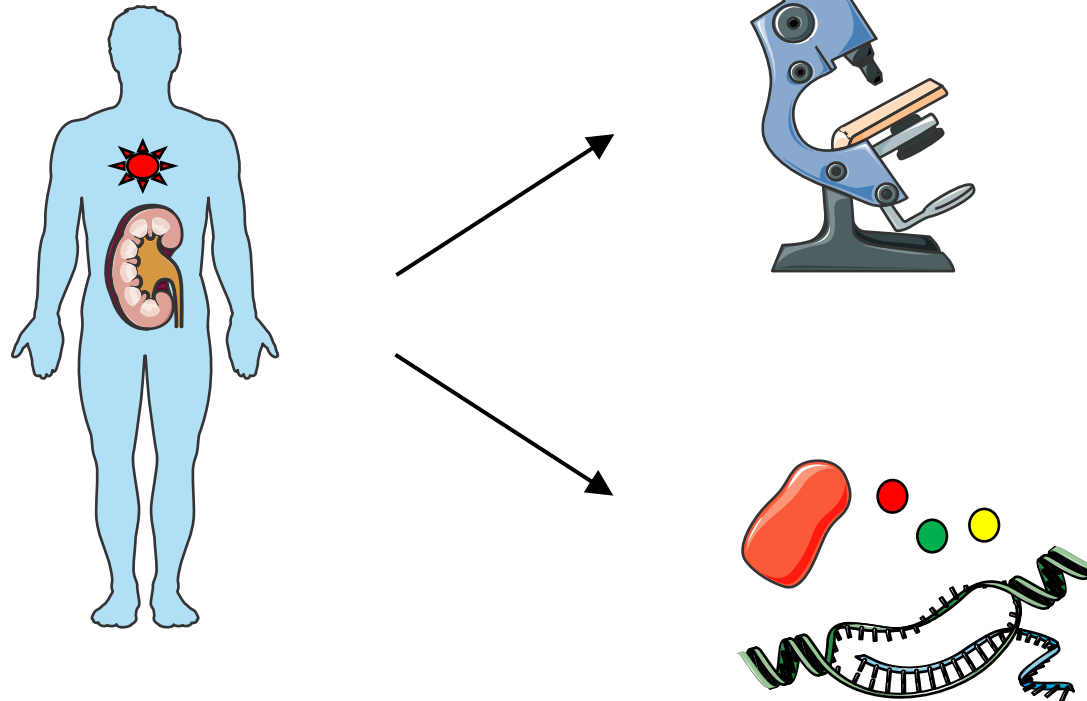
P. ISNARD

Hôpital Necker – Enfants Malades et Hôpital Robert Debré
Université de Paris

Introduction (1)

The traditional tasks of the pathologist :

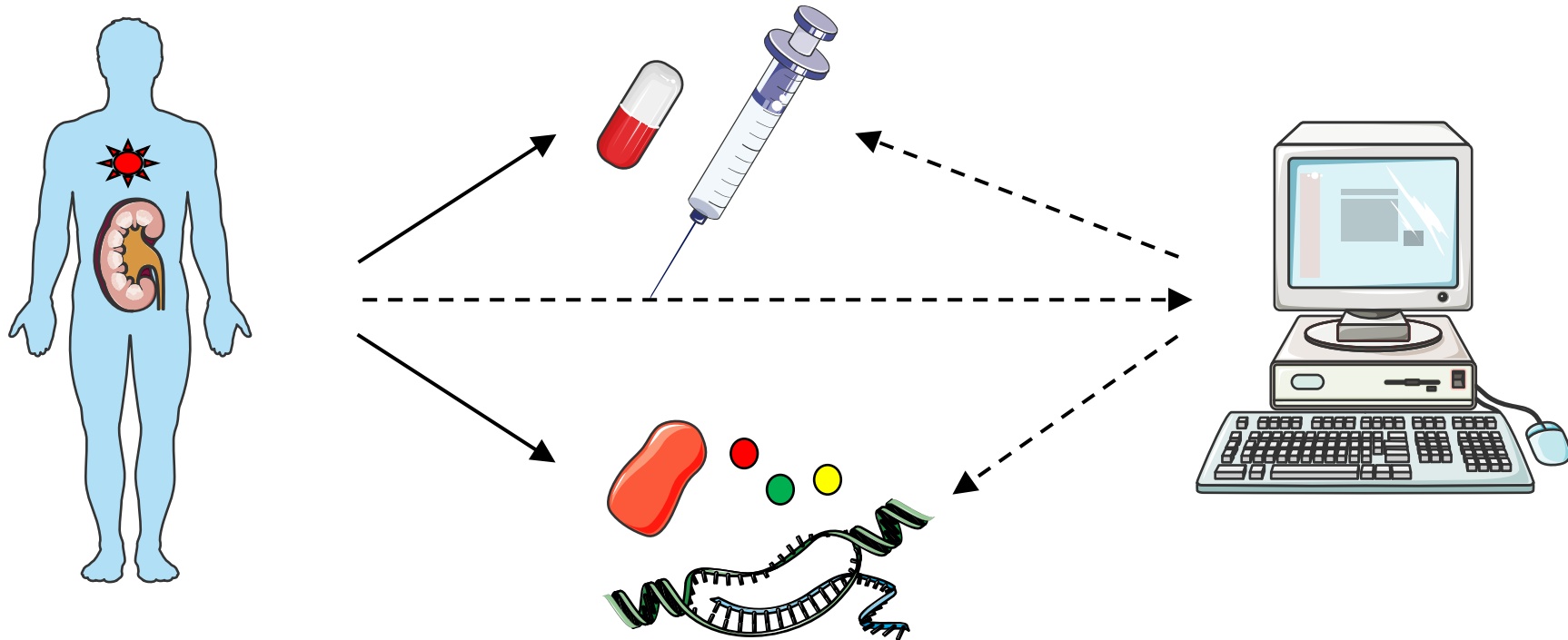
- Correct diagnosis of diseases
- Understand pathophysiology



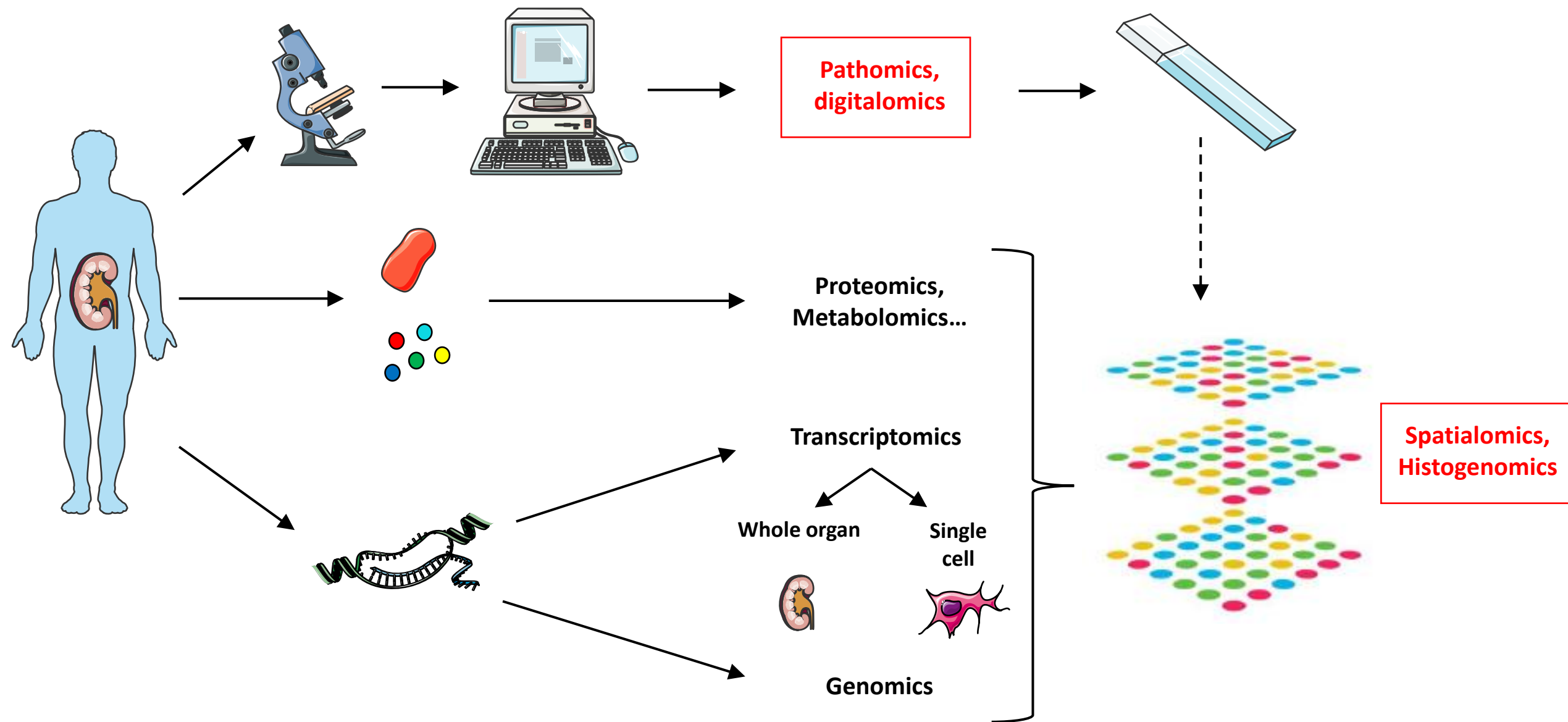
Introduction (2)

Personalized medicine

Collecting and analyzing the tremendous amounts of medical data



The concept of Pathomics



Whole Kidney Transcriptomics

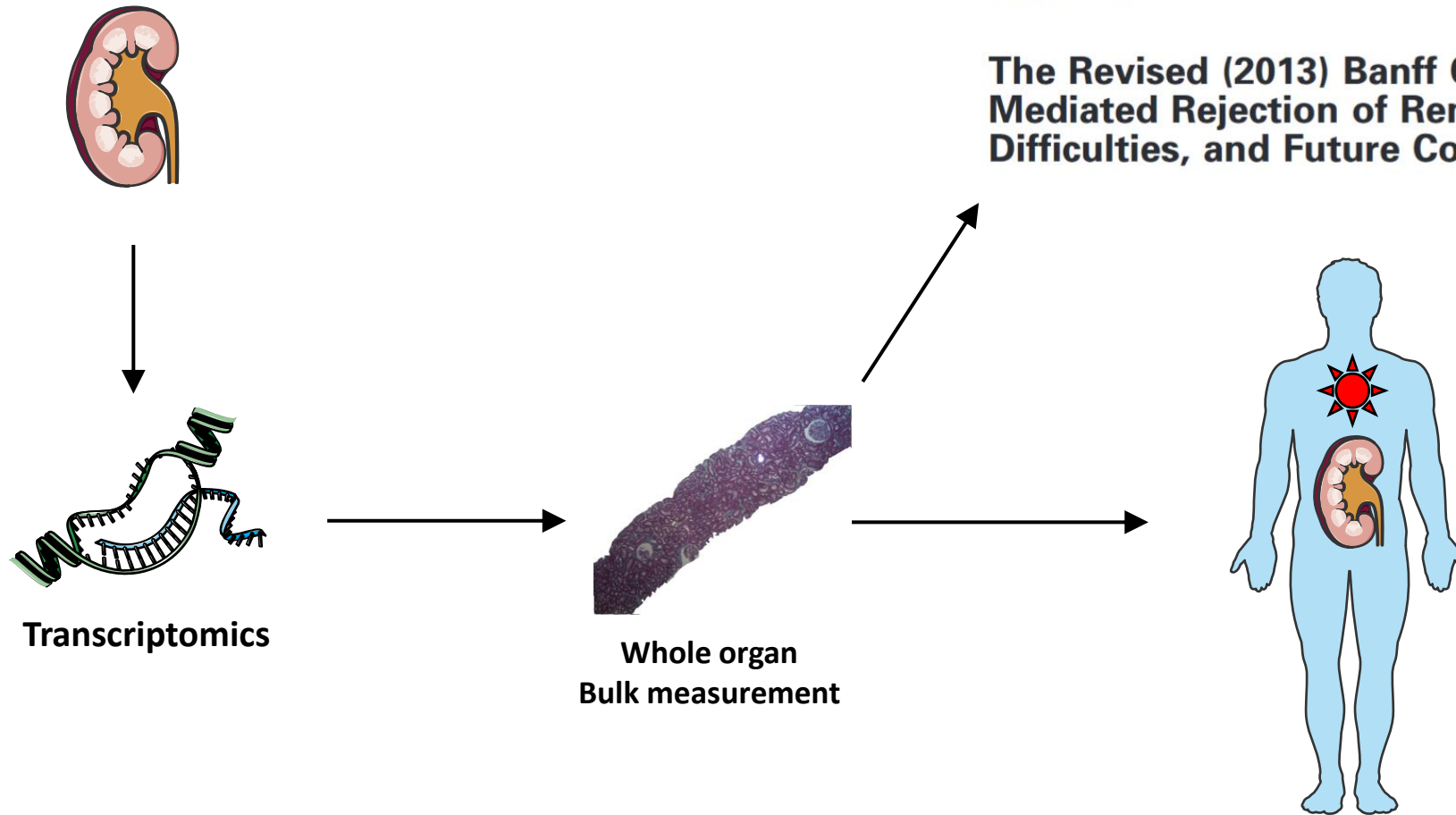
American Journal of Transplantation 2016; 16: 1352–1357
Wiley Periodicals Inc.

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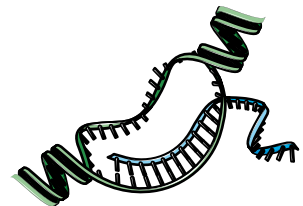
Minireview

doi: 10.1111/ajt.13661

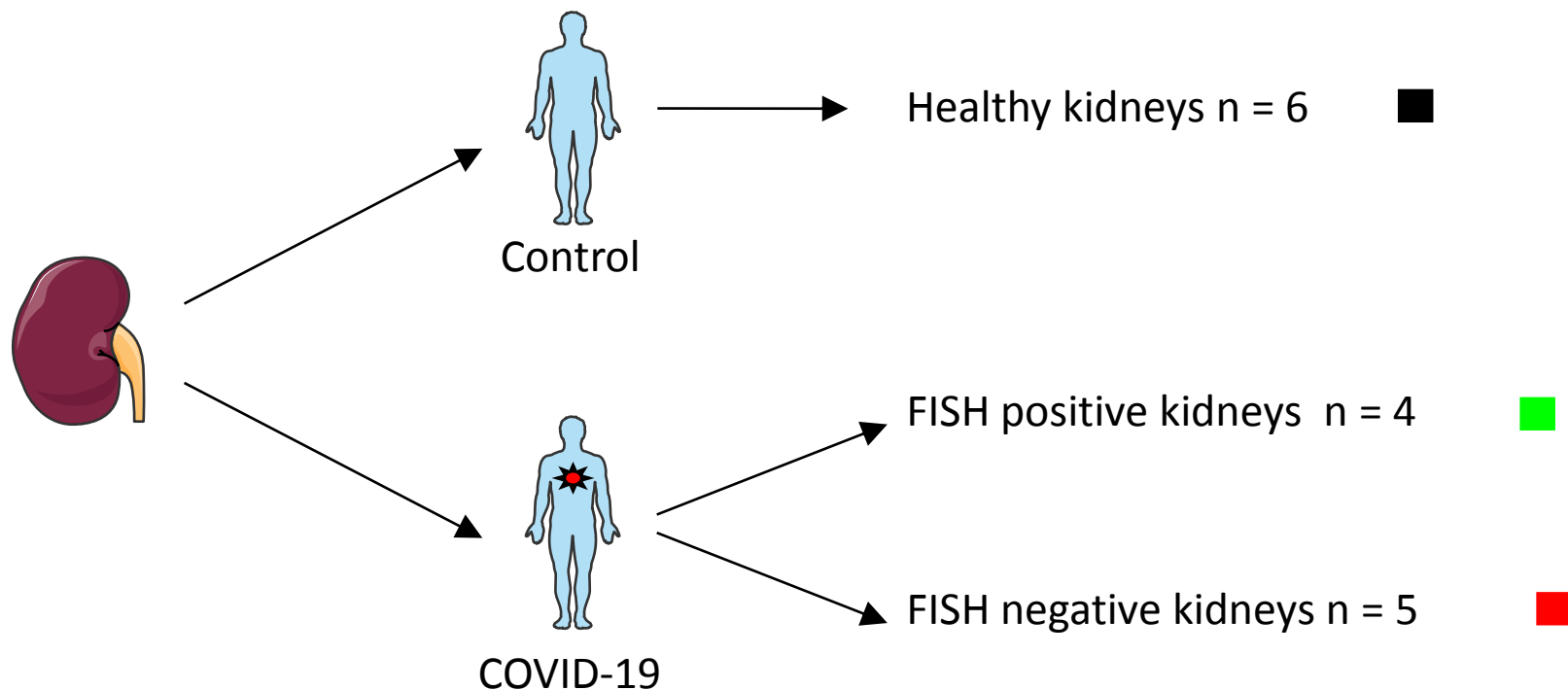
The Revised (2013) Banff Classification for Antibody-Mediated Rejection of Renal Allografts: Update, Difficulties, and Future Considerations



Understanding the renal pathology of SARS-CoV-2

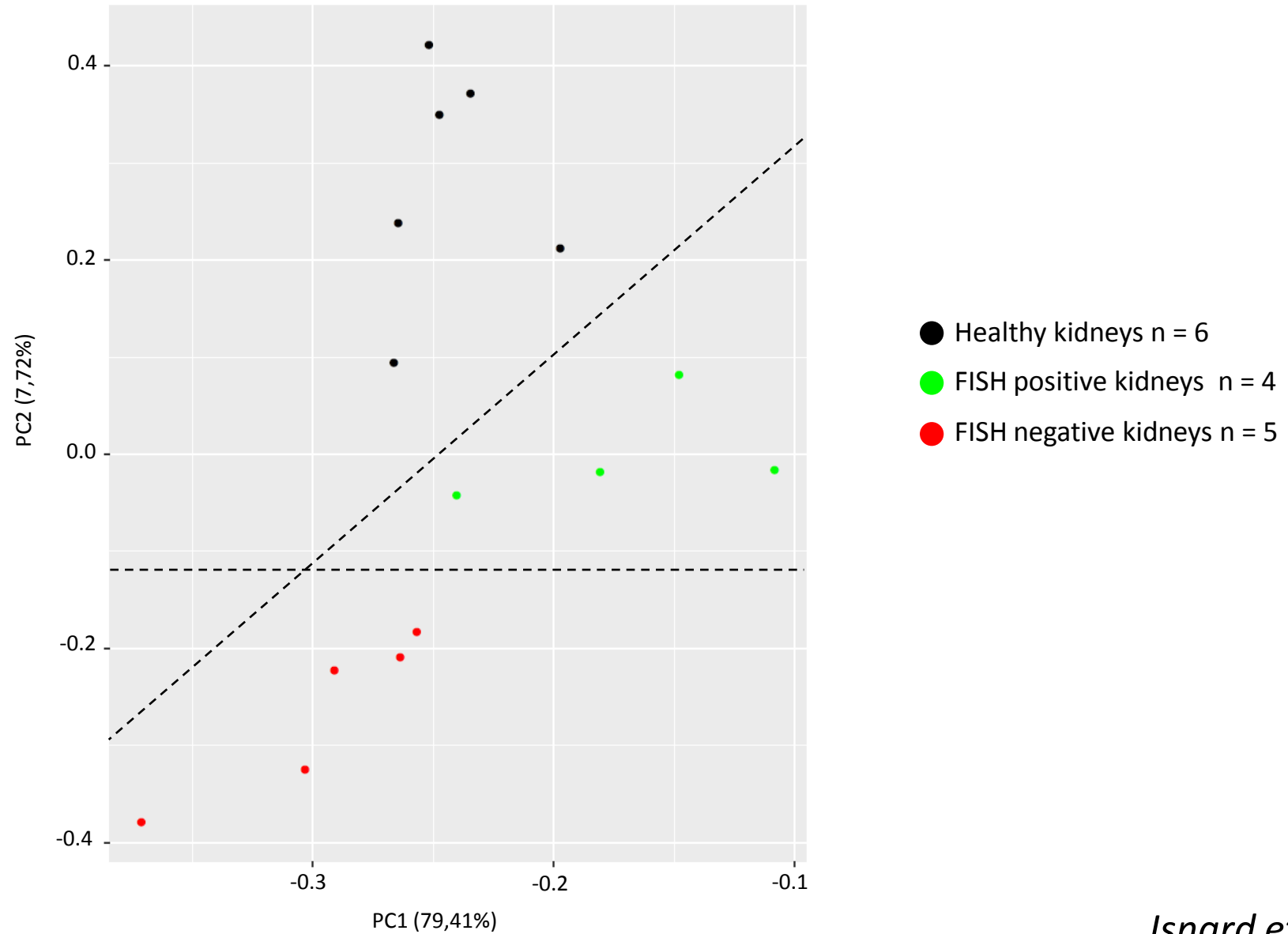


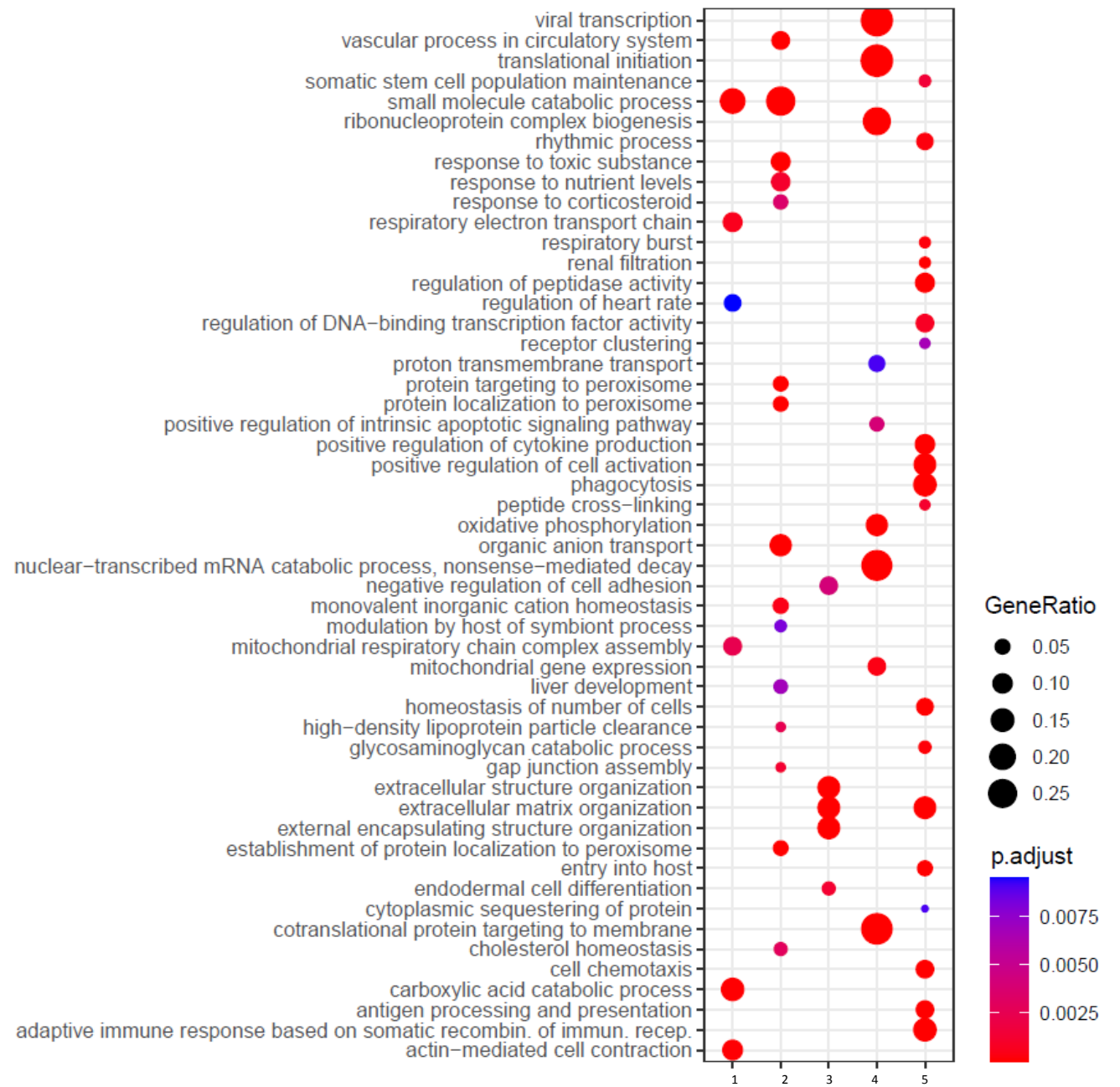
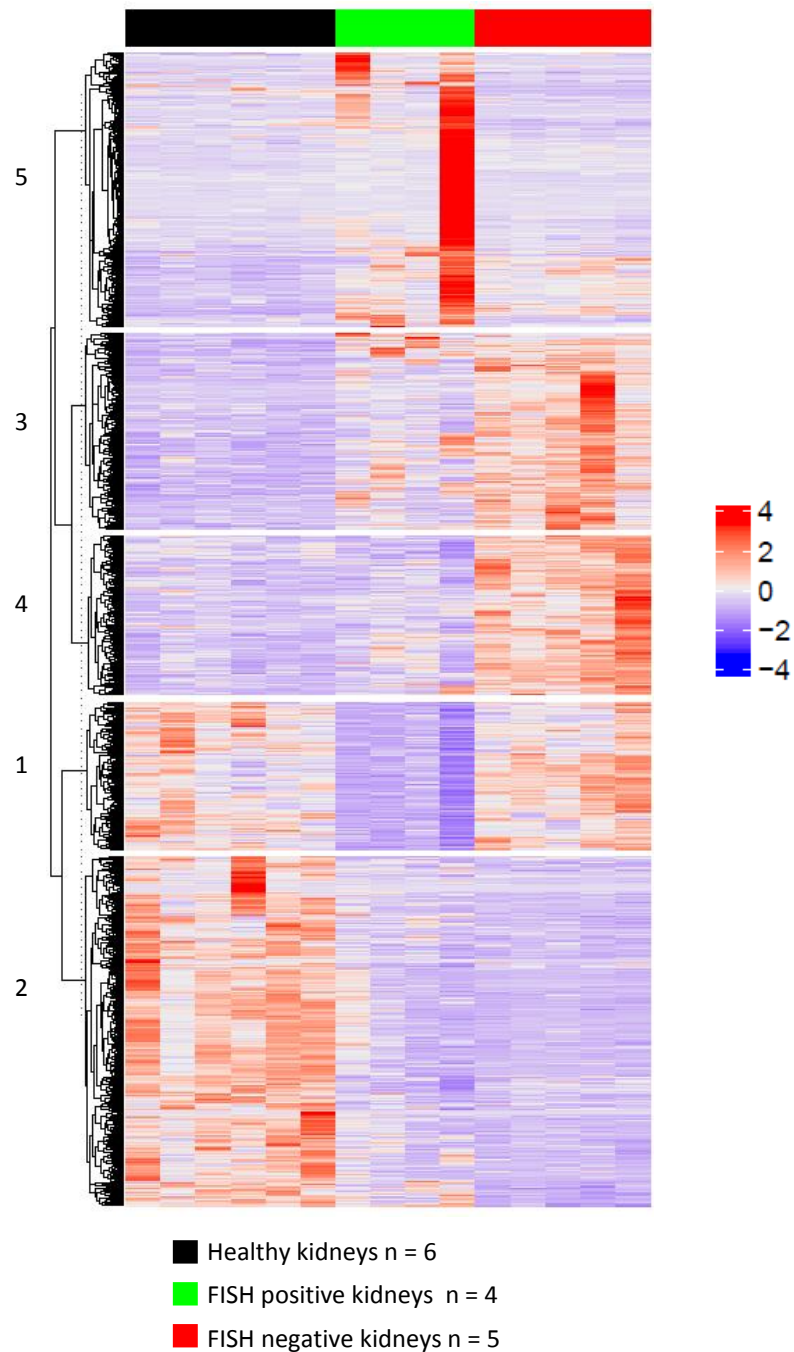
Understanding the renal pathology of SARS-CoV-2



RNAseq analysis of COVID-19 Kidneys

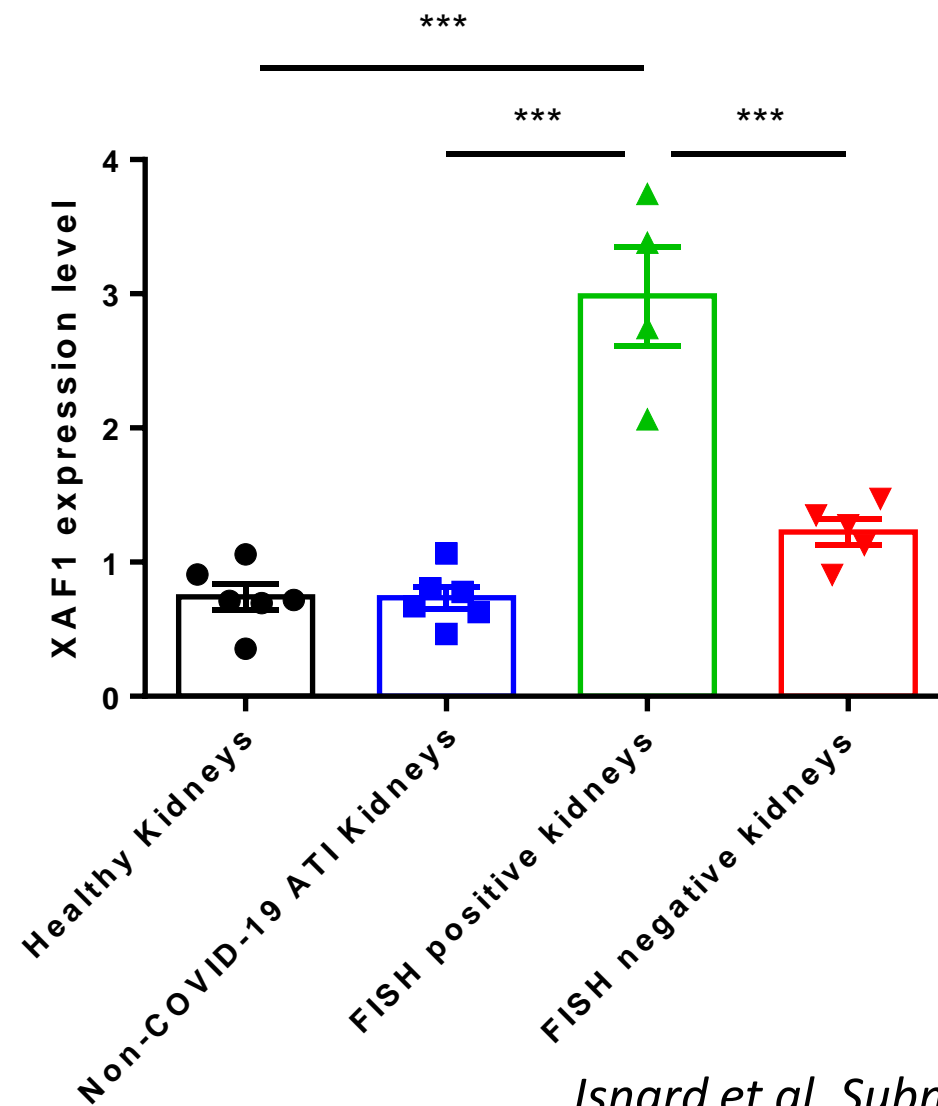
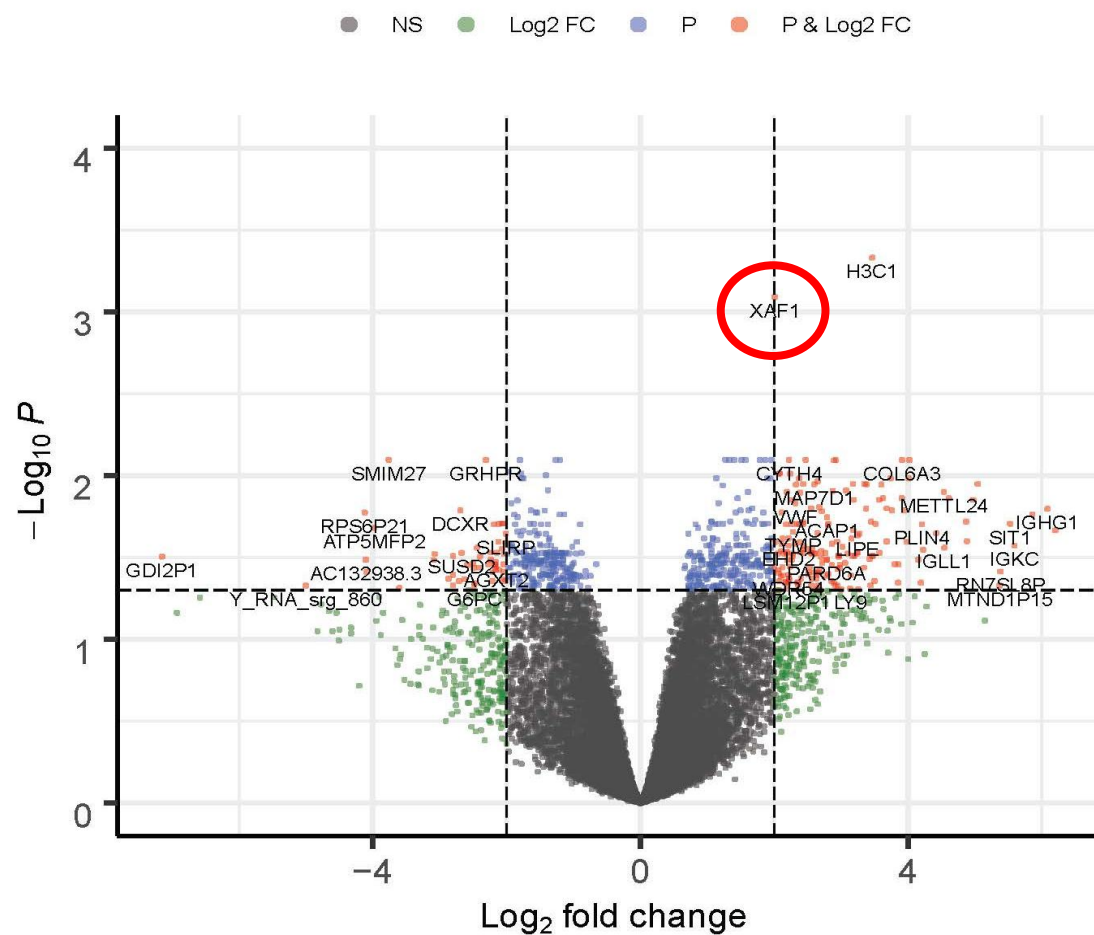
SARS-CoV-2 renal infection elicits a specific molecular signature



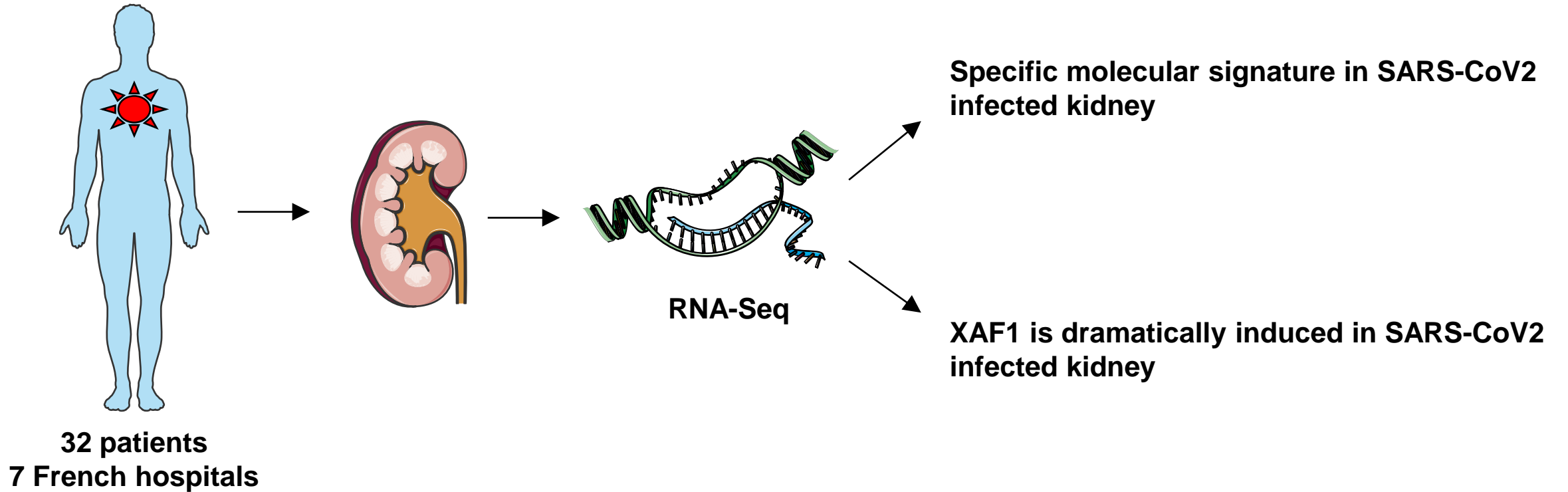


XAF1 is dramatically induced in SARS-CoV2 infected kidneys

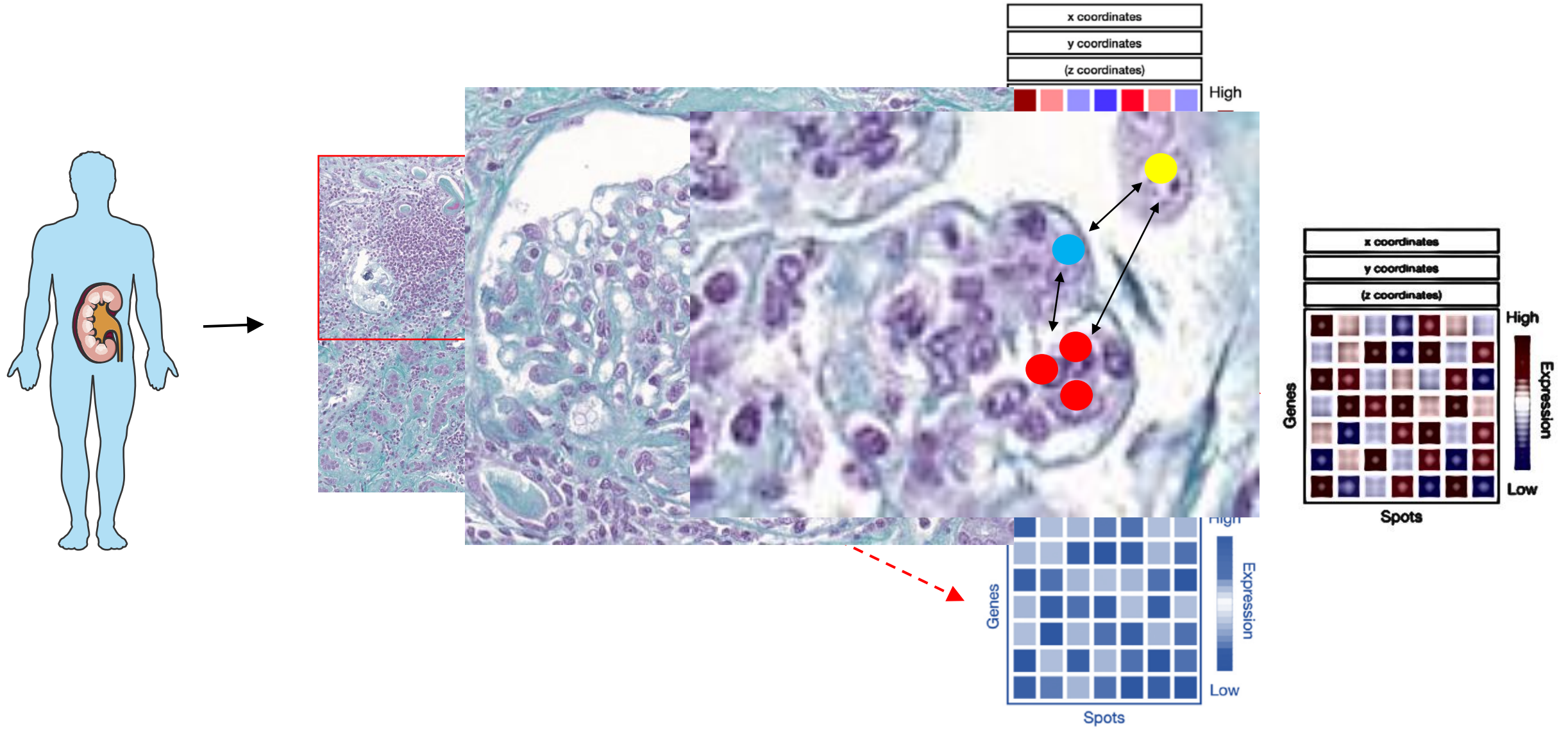
FISH positive ATI kidneys vs healthy kidneys

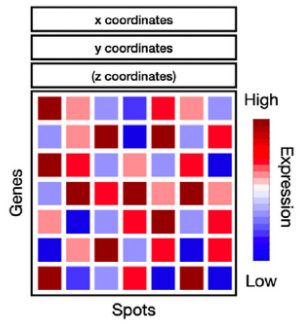


Conclusion

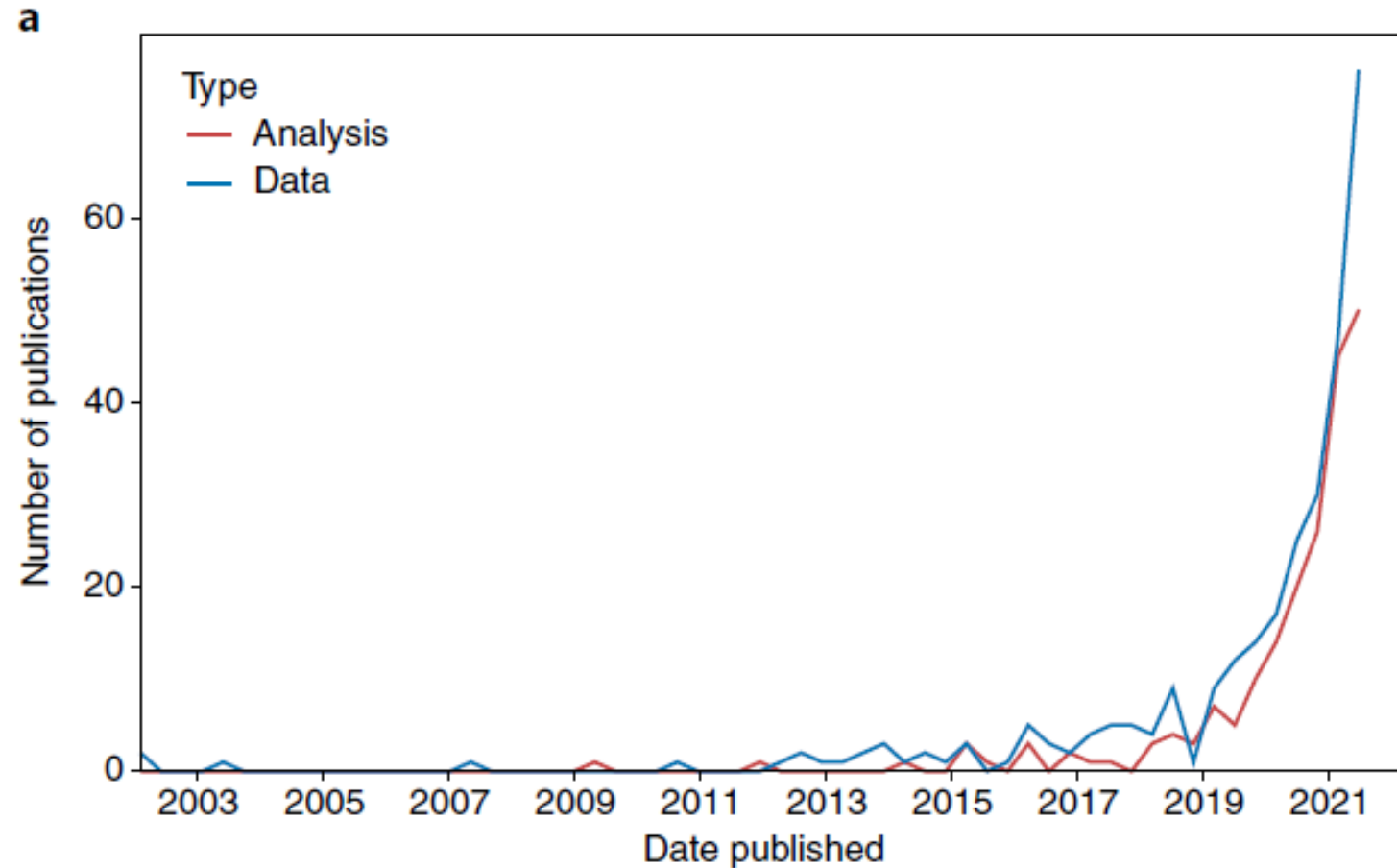


Exploring kidney pathology using spatial transcriptomics (1)



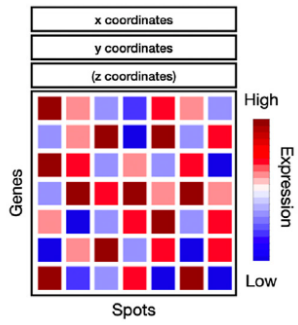


Exploring kidney pathology using spatial transcriptomics (2)

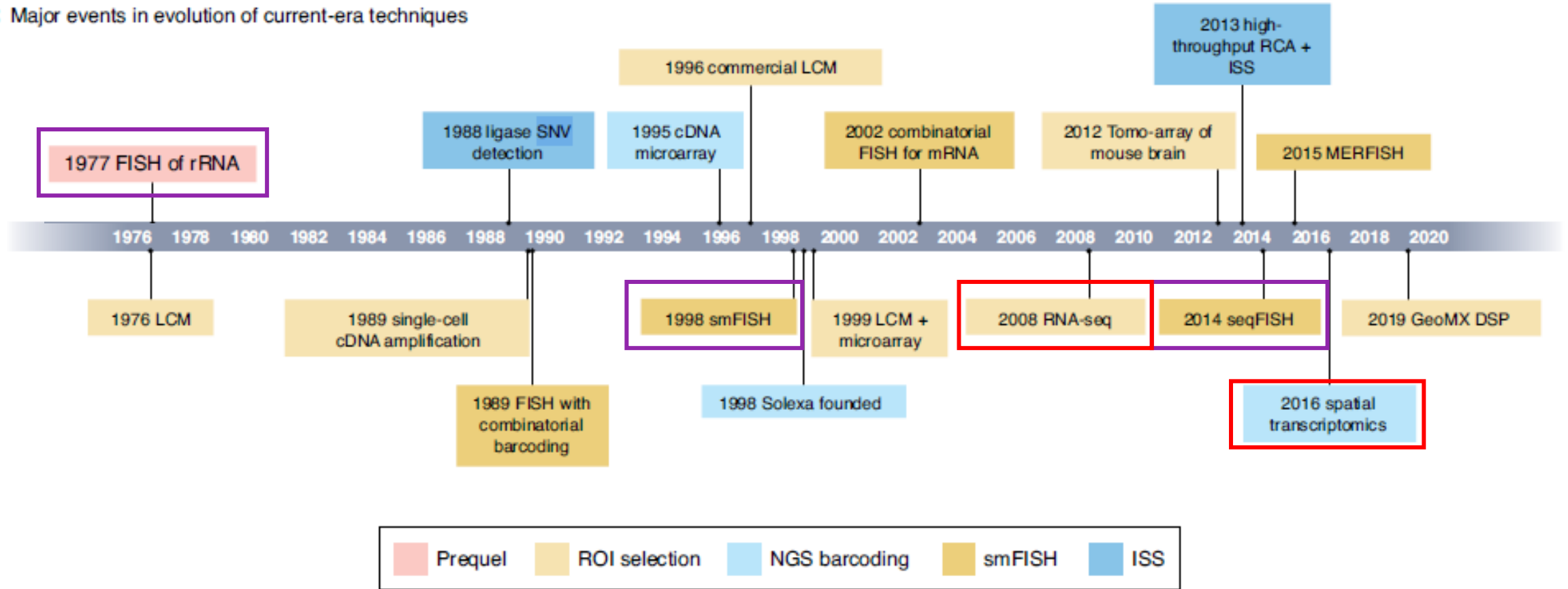


Moses et al. Nature Methods, March 2022

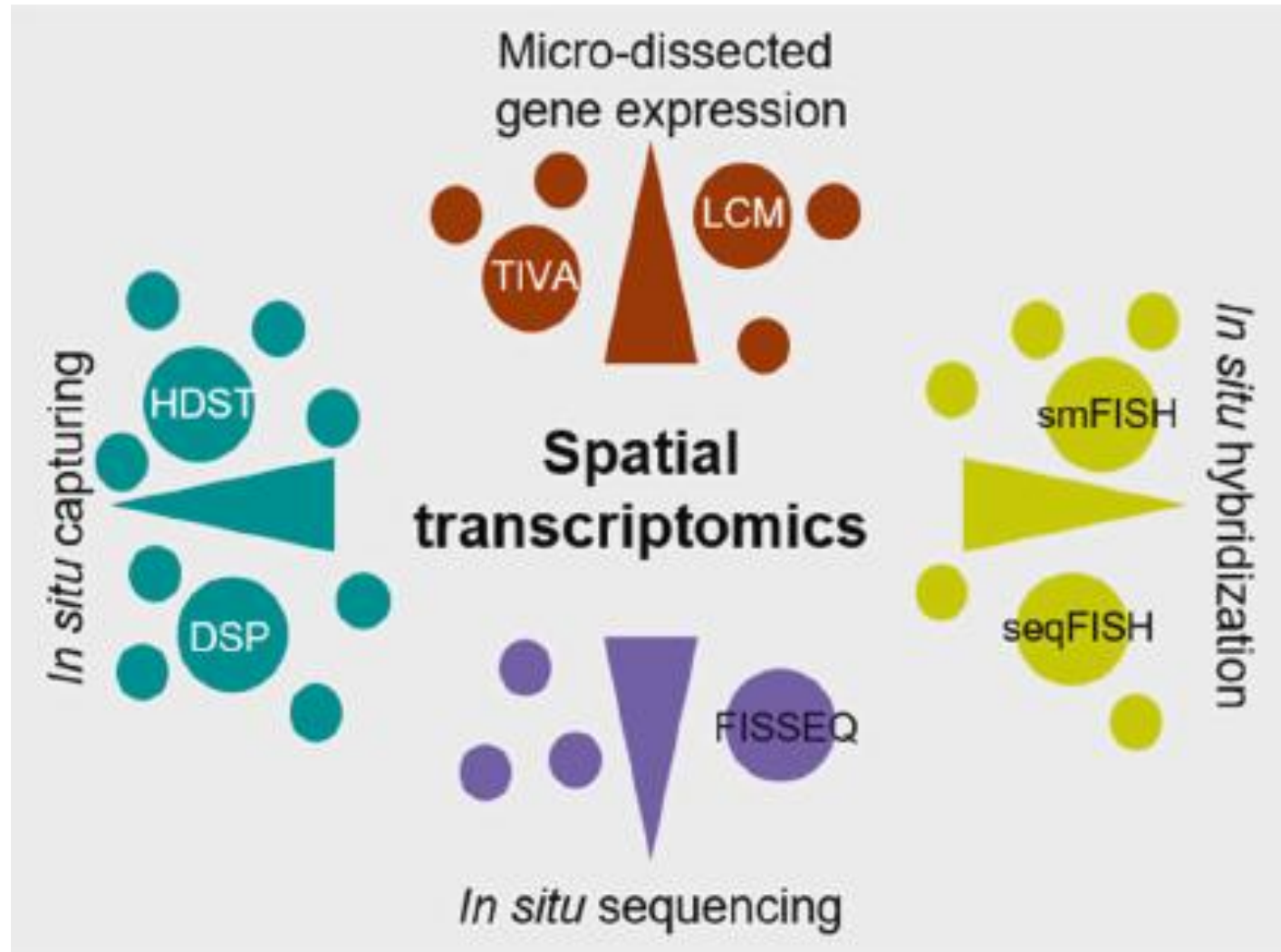
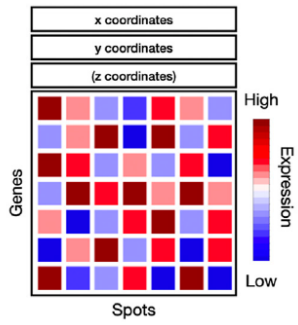
Exploring kidney pathology using spatial transcriptomics (4)

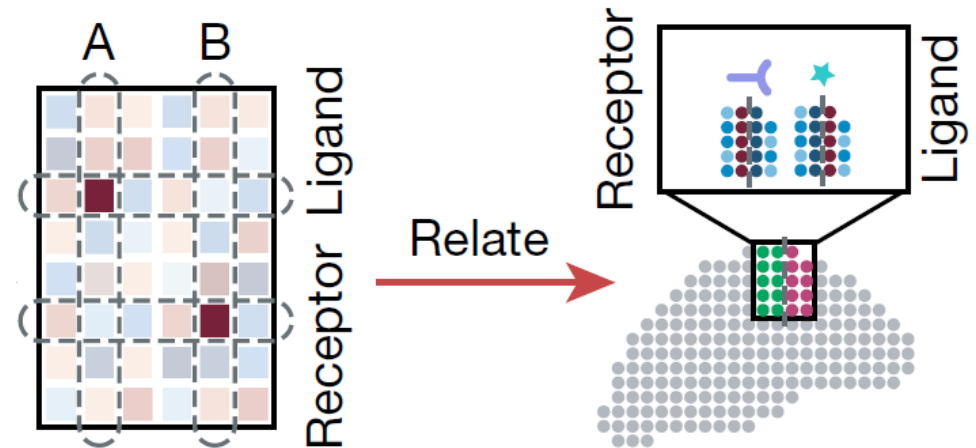
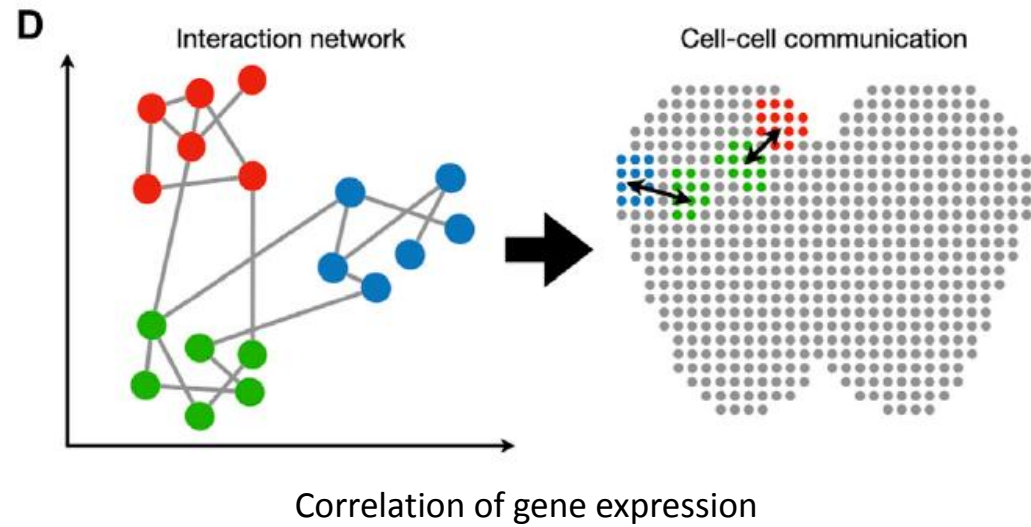
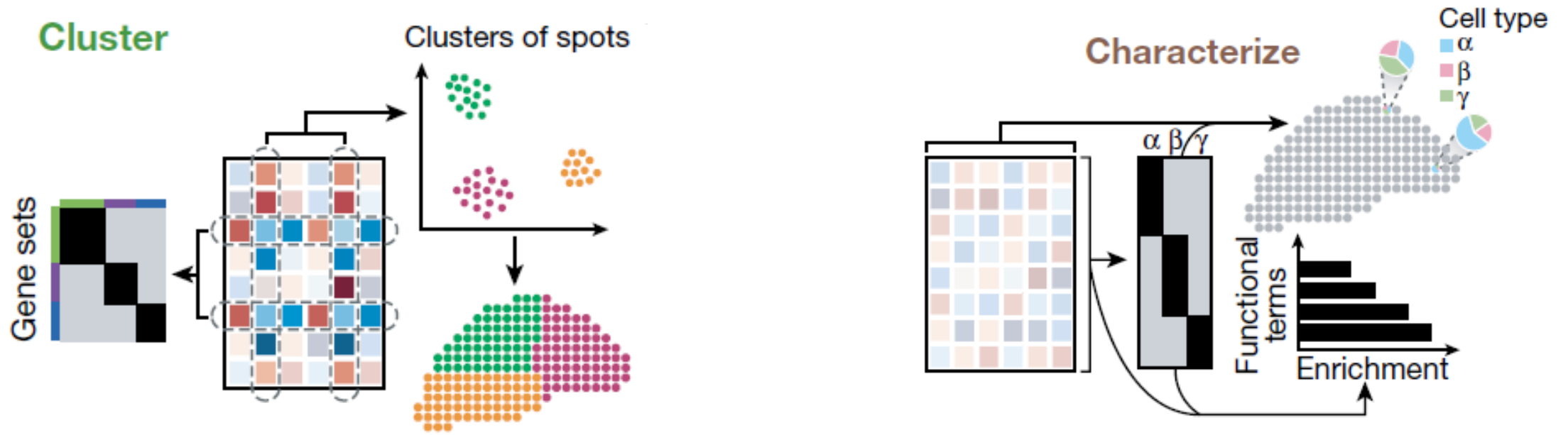


c Major events in evolution of current-era techniques



Exploring kidney pathology using spatial transcriptomics (5)

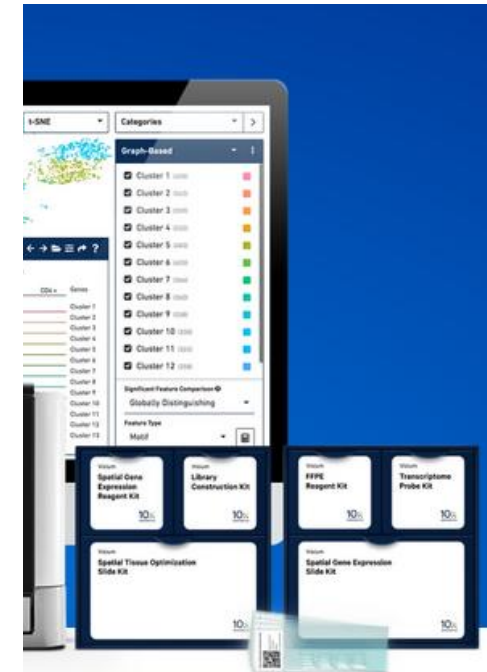
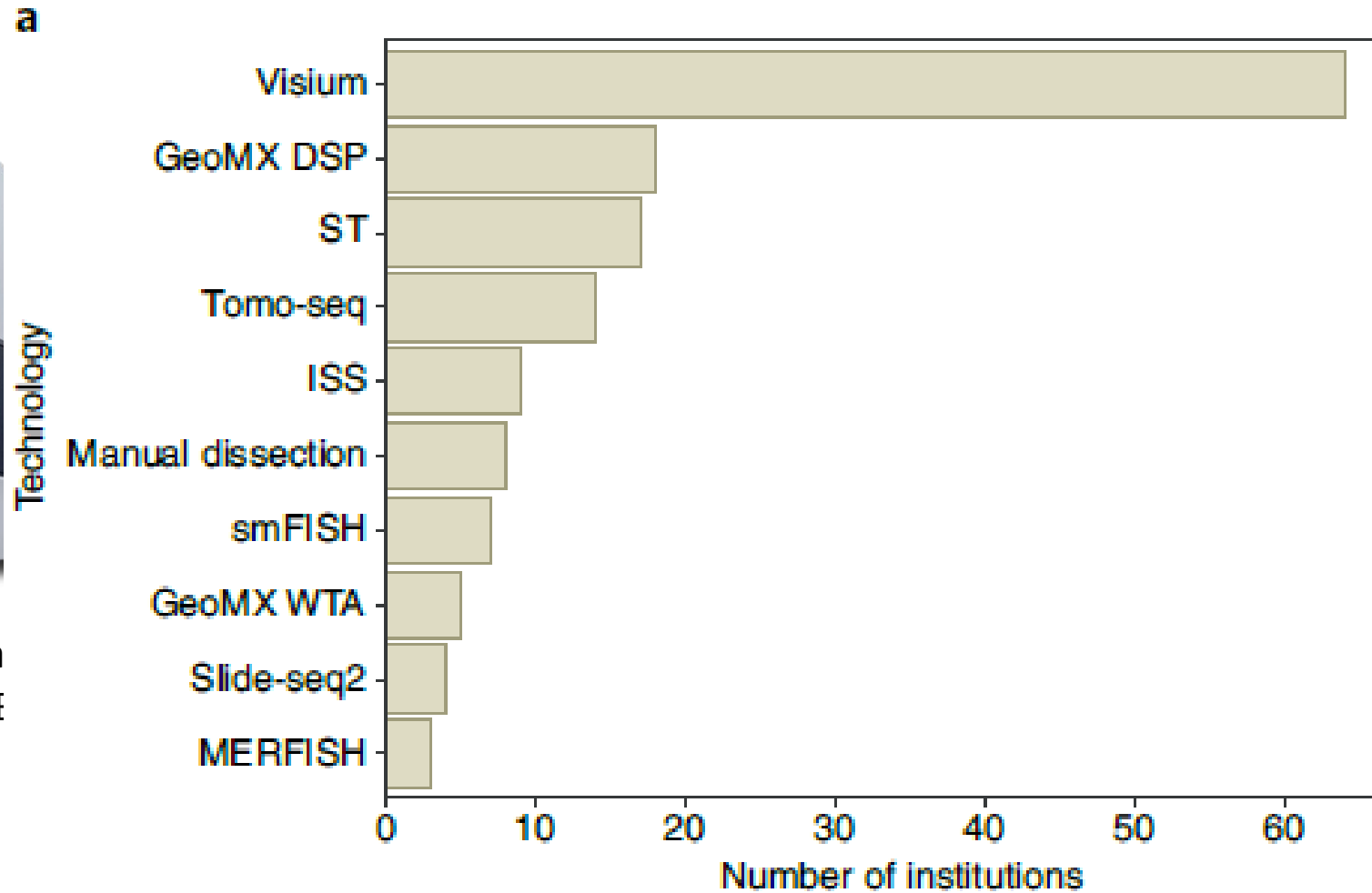




Exploring kidney pathology using spatial transcriptomics (7)

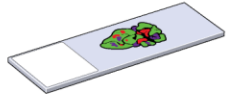


GeoMx® Digital Spa
Spatially Resolved Transcriptomics



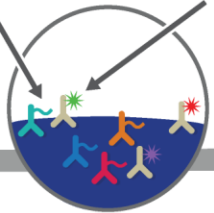
GeoMx DSP Workflow

① Stain



UV-photocleavable
oligos

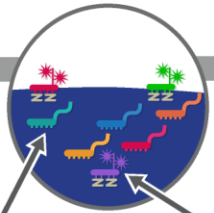
Fluorescent
antibodies



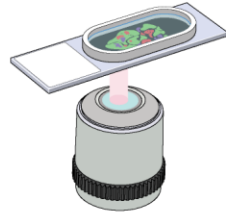
RNA

UV-photocleavable
oligos

RNAscope
probes



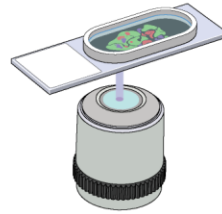
② Select ROI



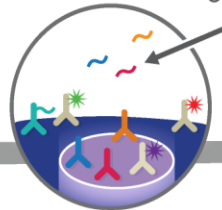
Customize your regions
of interest.



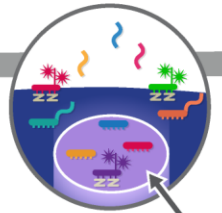
③ UV-Cleave



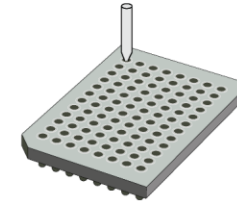
photocleaved
oligos ready for
collection



UV light projected
onto ROI from below

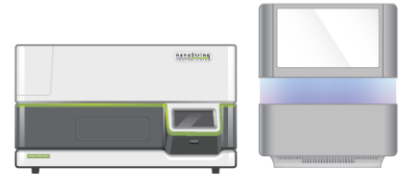


④ Collect & Dispense

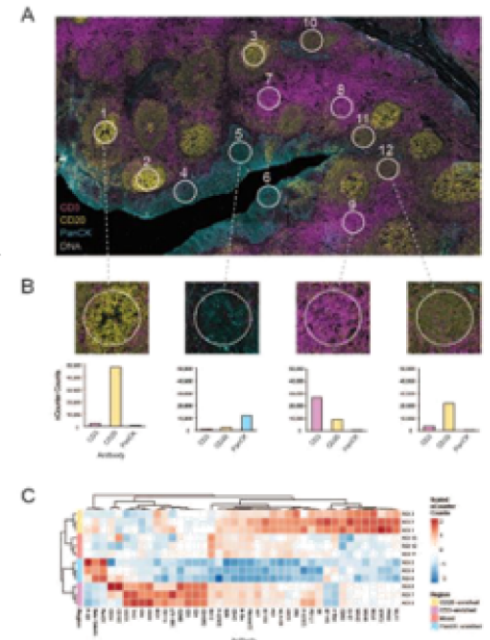


⑤ Repeat for each ROI

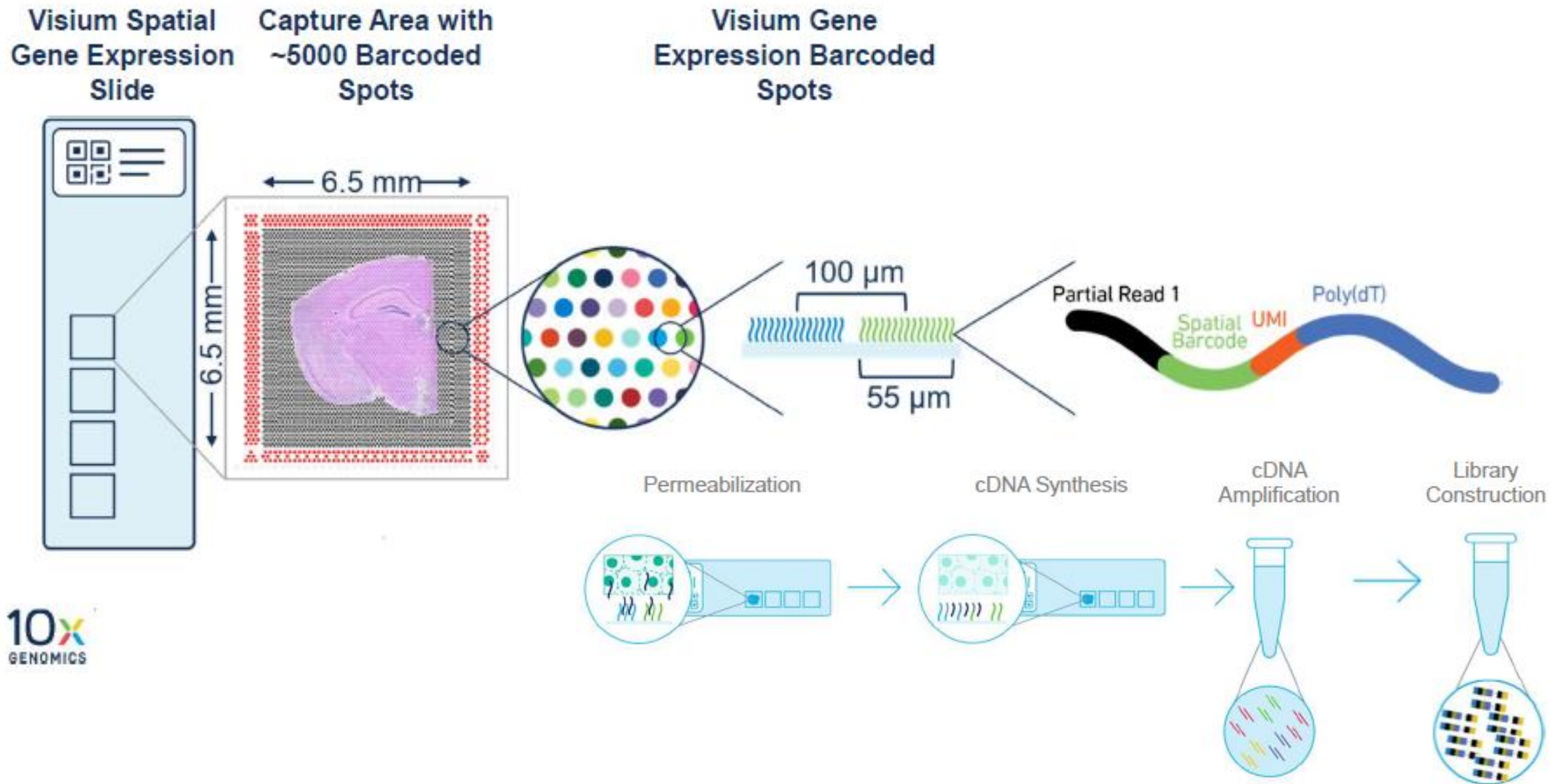
⑥ Count



Rich data sets of biology,
region by region.



10X Genomics Visium ST Workflow



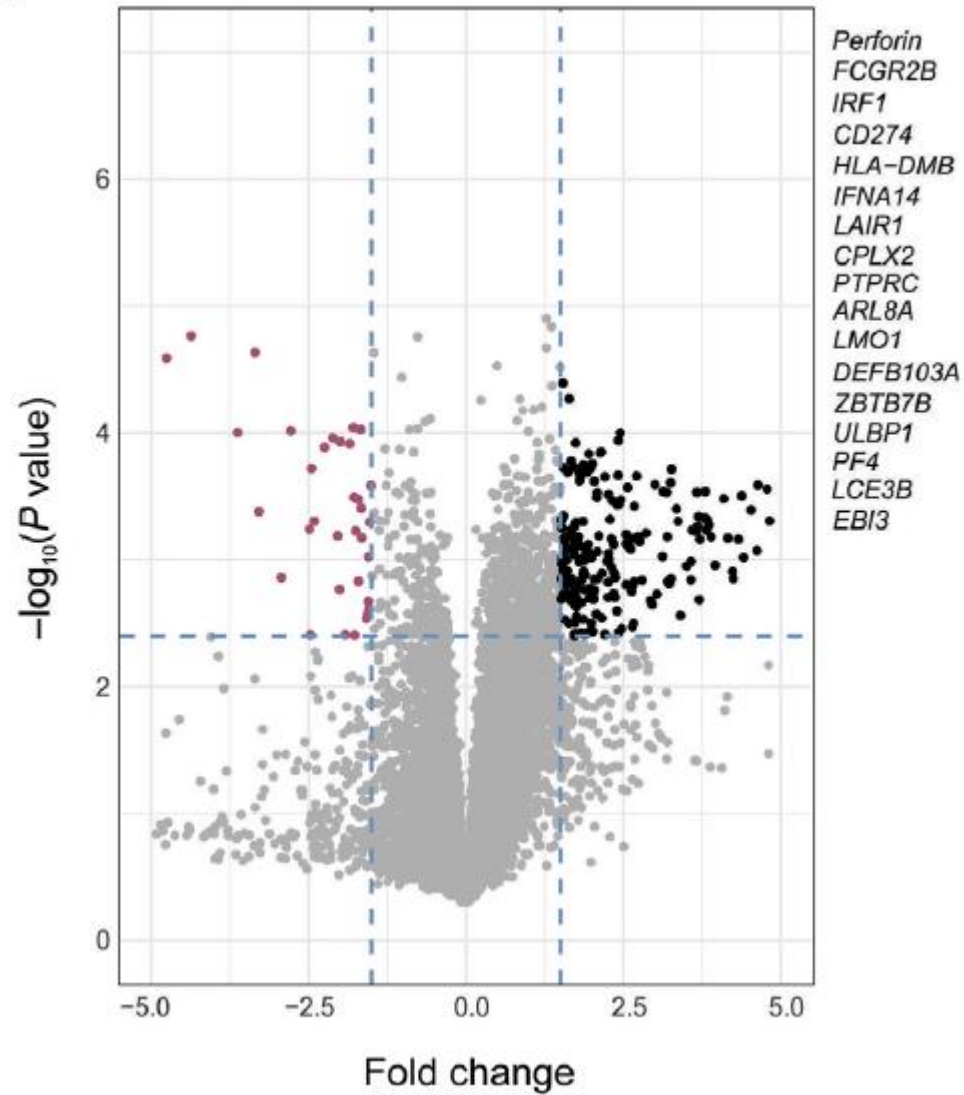
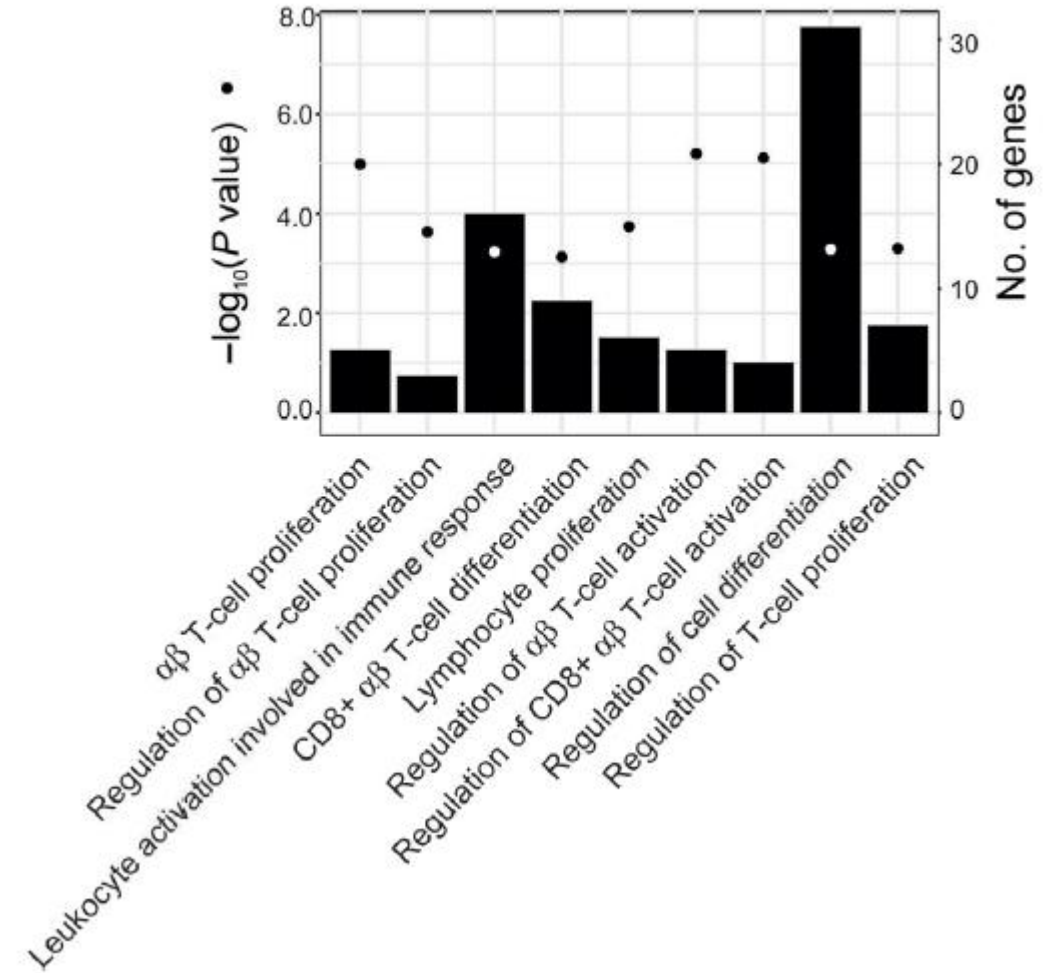
The spatially resolved transcriptional profile of acute T cell-mediated rejection in a kidney allograft



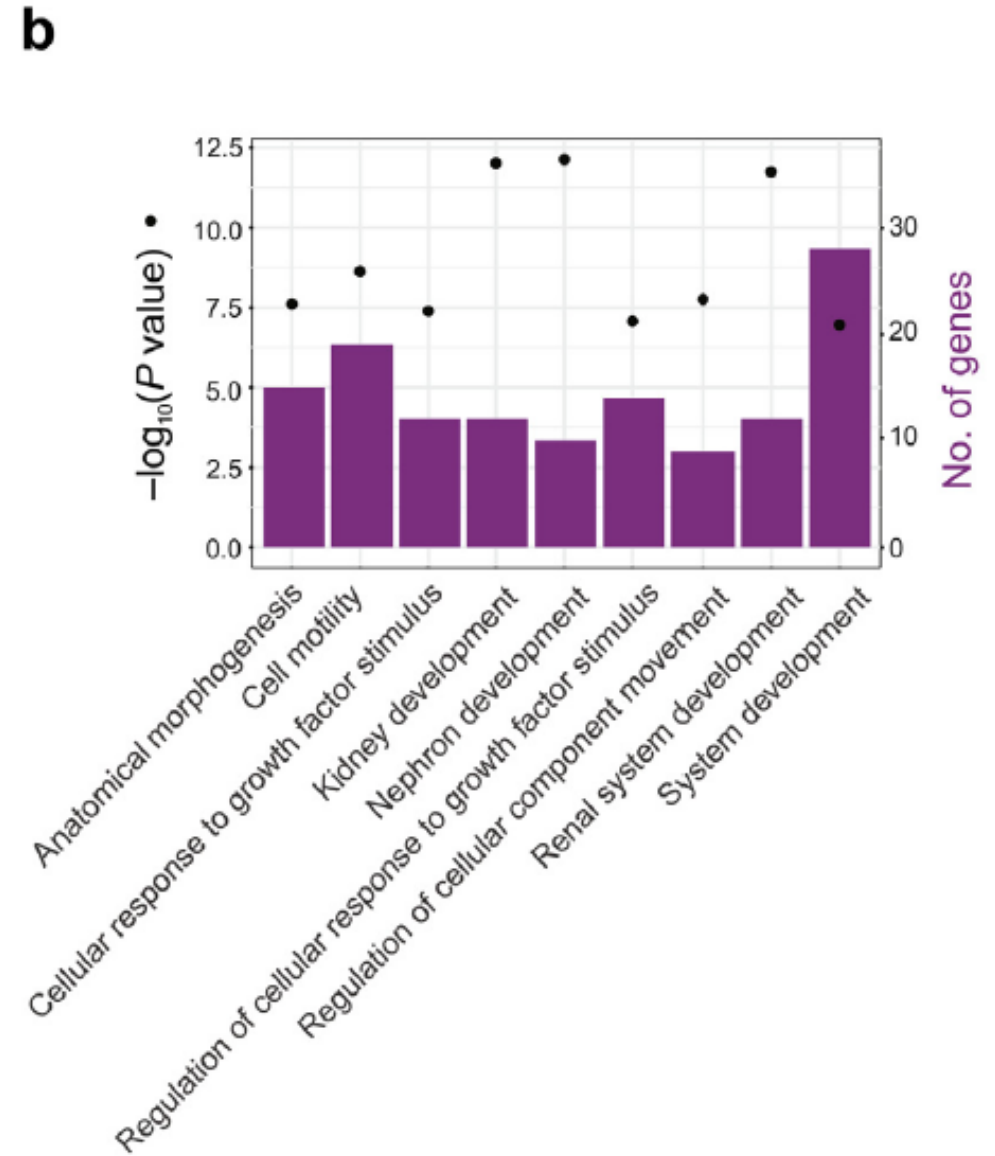
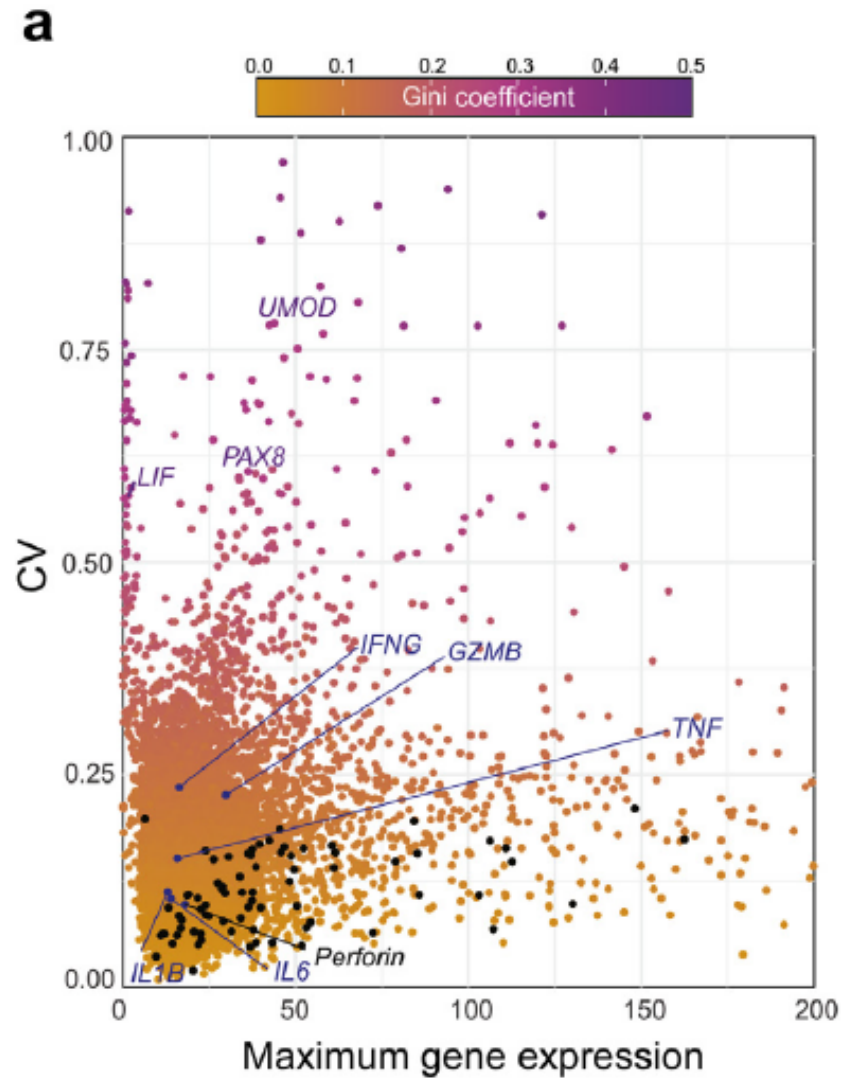
Fadi Salem^{1,8}, Laura Perin^{2,3,8}, Sargis Sedrakyan^{2,3}, Andrea Angeletti⁴, Gian Marco Ghiggeri⁴, Maria Cristina Coccia⁵, Marty Ross⁶, Miguel Fribourg^{7,9} and Paolo Cravedi^{7,9}

¹Department of Pathology and Molecular and Cell Based Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA;

²GOFARR Laboratory for Organ Regenerative Research and Cell Therapeutics in Urology, Saban Research Institute, Division of Urology, Children's Hospital Los Angeles, Los Angeles, California, USA; ³Department of Urology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA; ⁴Nephrology, Dialysis and Transplantation Unit, Giannina Gaslini Scientific Institute for Research, Hospitalization and Healthcare, Genoa, Italy; ⁵Pathological Anatomy Unit, Giannina Gaslini Scientific Institute for Research, Hospitalization and Healthcare, Genoa, Italy; ⁶NanoString Technologies Inc., Seattle, Washington, USA; and ⁷Department of Medicine, Translational Transplant Research Center, Icahn School of Medicine at Mount Sinai, New York, New York, USA

a**b**

Higher expression of inflammatory genes in tubular regions of interest (ROIs) in the acute cellular rejection (ACR) biopsy.



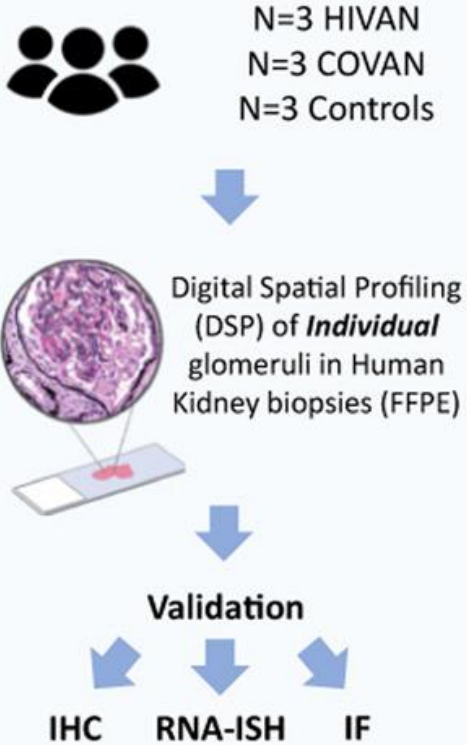
High variability in the kidney development transcriptional program across interstitial areas in a cellular rejection

Digital spatial profiling of collapsing glomerulopathy

Kelly D. Smith^{1,2,4}, David K. Prince², Kammi J. Henriksen³, Roberto F. Nicosia¹, Charles E. Alpers^{1,2} and Shreeram Akilesh^{1,2,4}

¹Department of Laboratory Medicine and Pathology, University of Washington, Seattle, Washington, USA; ²Kidney Research Institute, Seattle, Washington, USA; and ³Department of Pathology, University of Chicago, Chicago, Illinois, USA

Methods and cohort

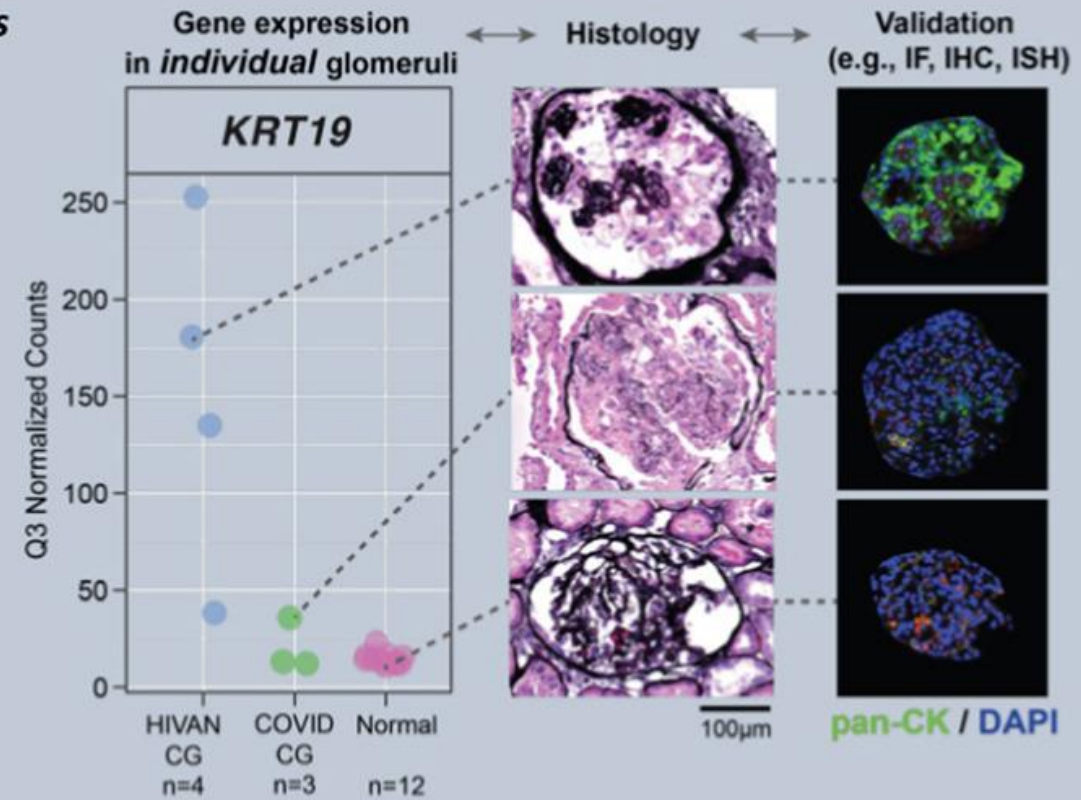


Analysis questions

- Is there a universal mechanism of **collapsing glomerulopathy**?
- Is collapsing glomerulopathy in **HIVAN and COVAN** driven by the same mechanism?
- Are there differences in signatures of normal and collapsing glomeruli within the same biopsy?
- What are the protein-protein interaction networks?

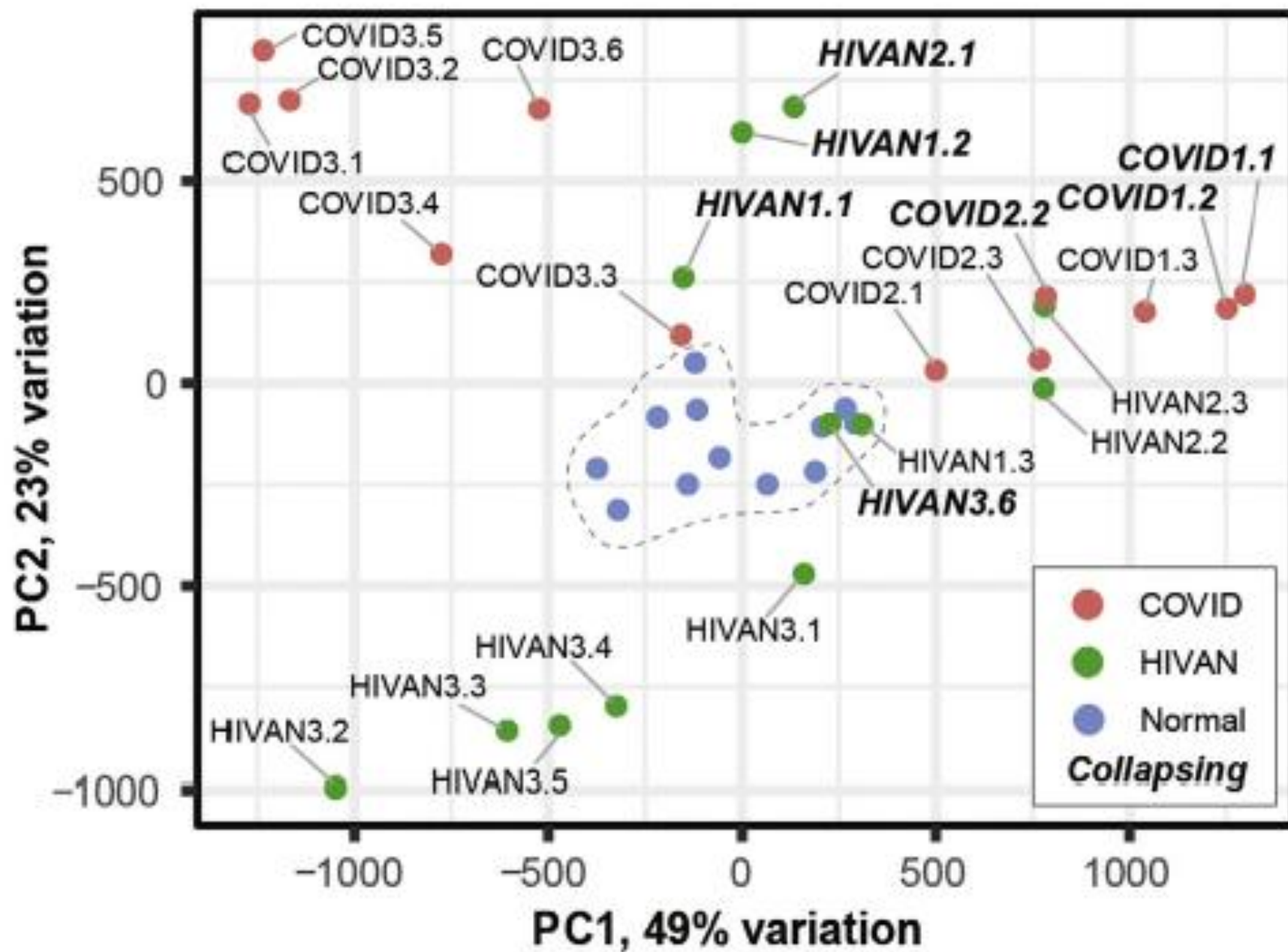


Findings

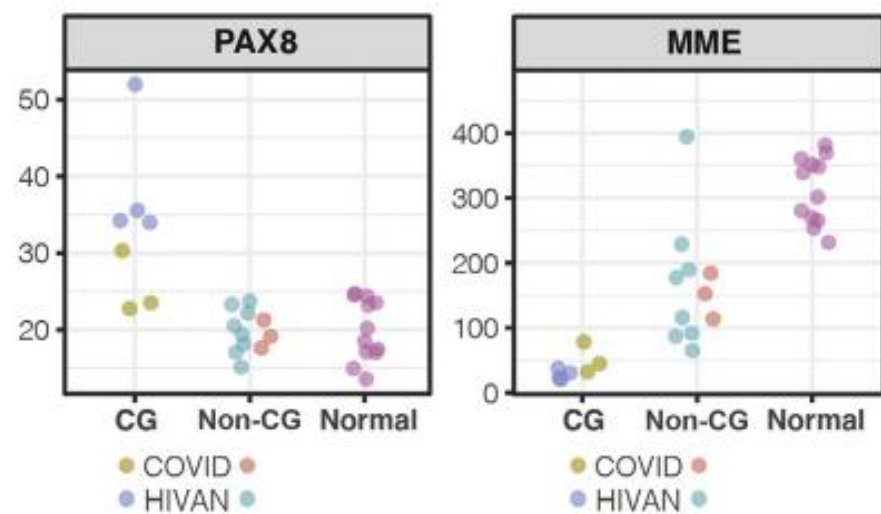


DSP enables **DIRECT** correlation of histopathology to validated gene expression signatures

CONCLUSION: Digital spatial profiling can generate individual glomerular transcriptomes from single FFPE tissue sections of clinically procured human kidney biopsies without prior optimization. This enables direct correlation of histopathology with molecular signatures of disease.

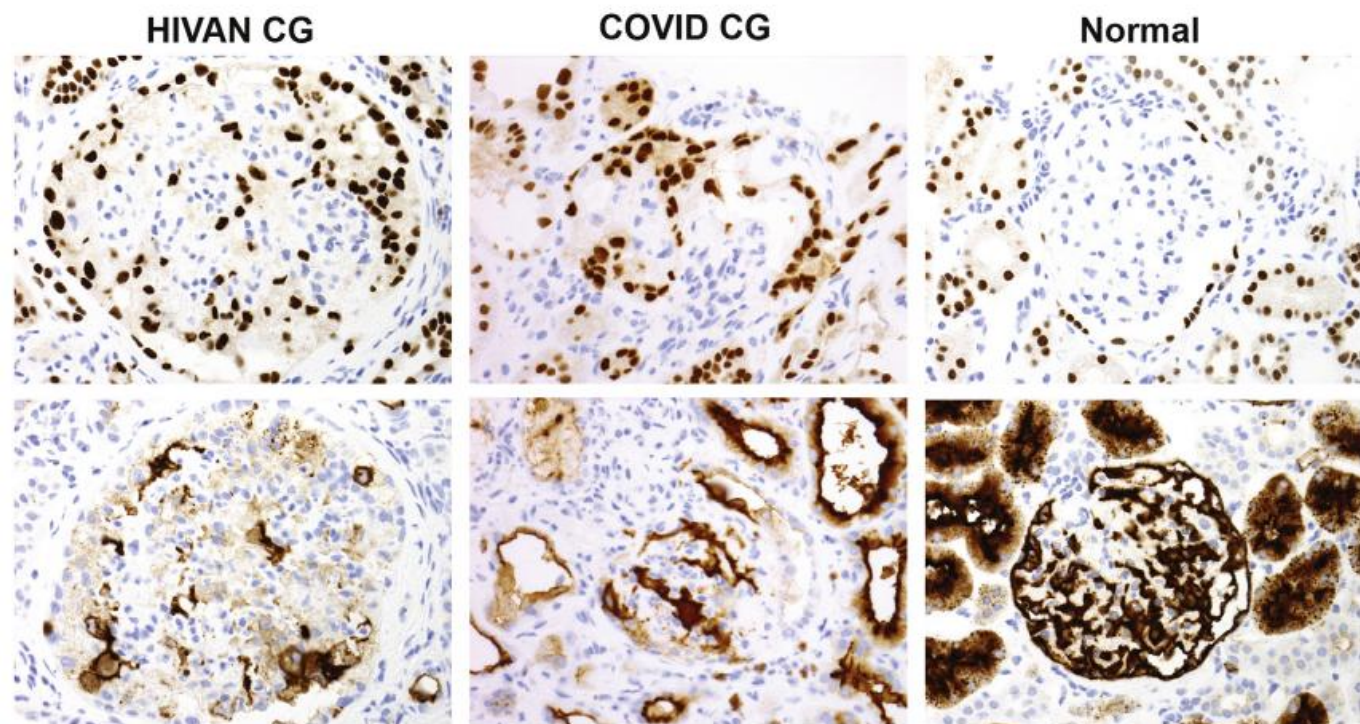


Q3 normalized counts

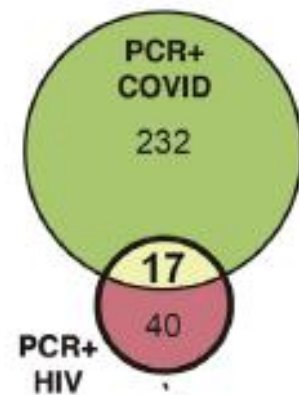
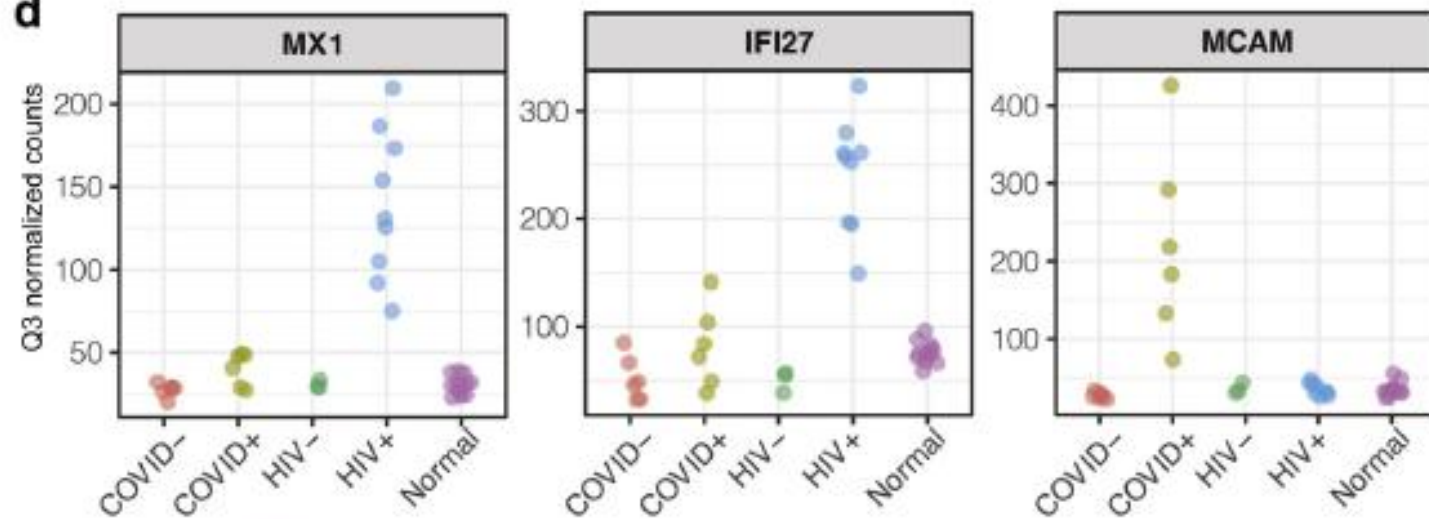
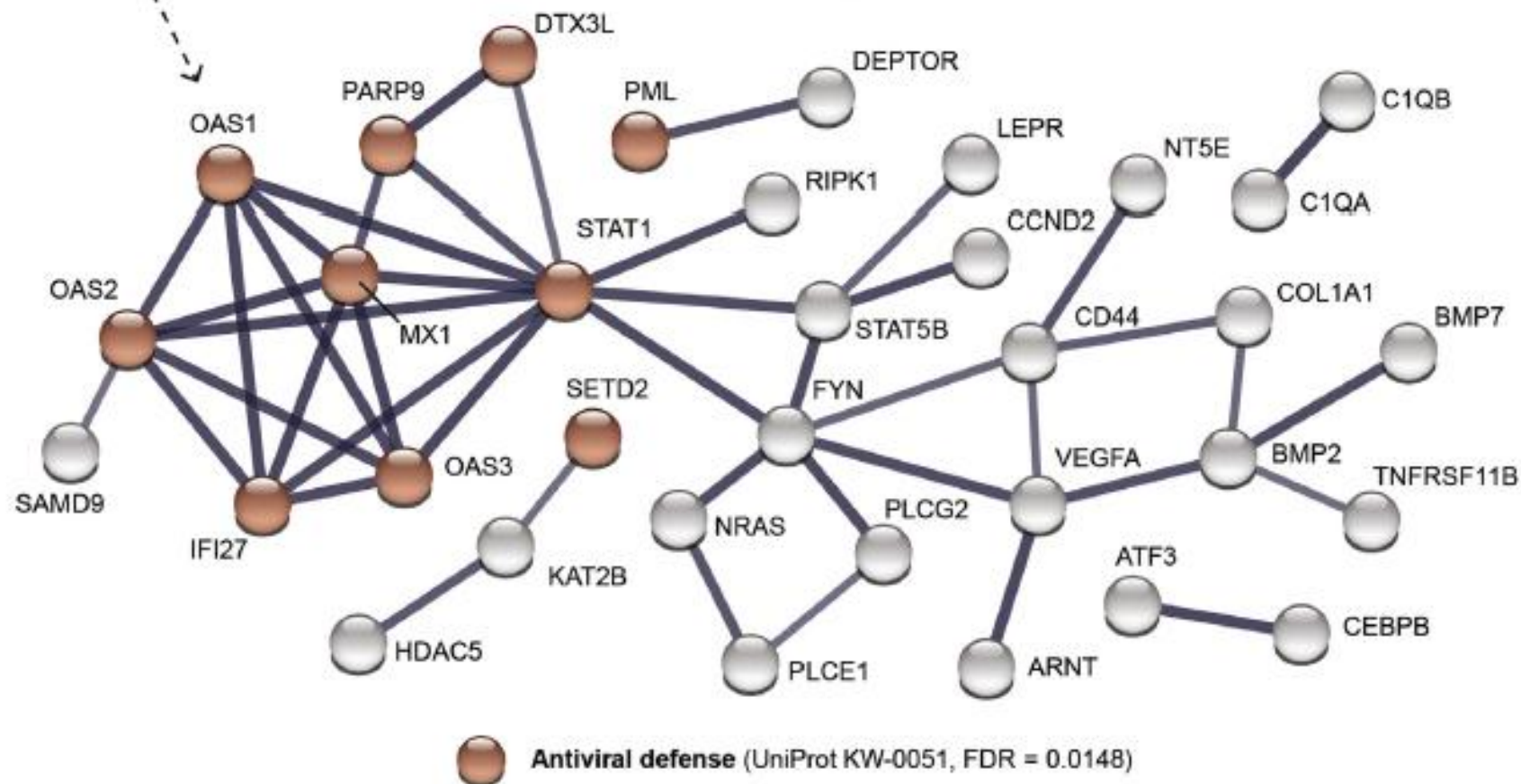


PAX8

MME



50µm

c**d****e**

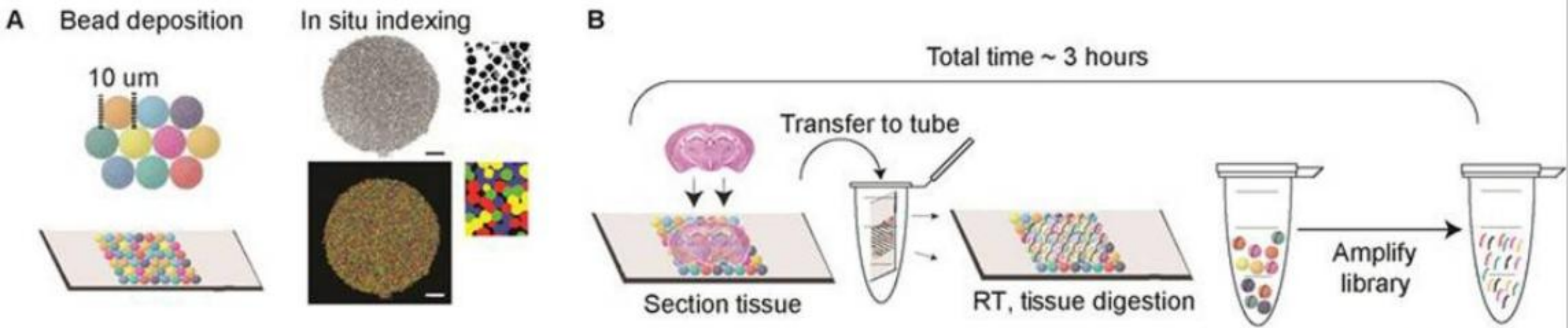


Article

High-resolution Slide-seqV2 spatial transcriptomics enables discovery of disease-specific cell neighborhoods and pathways

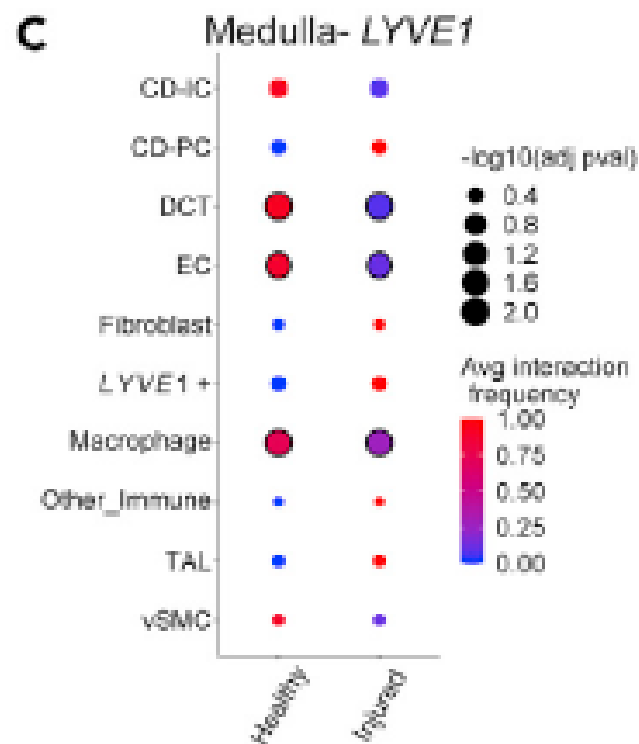
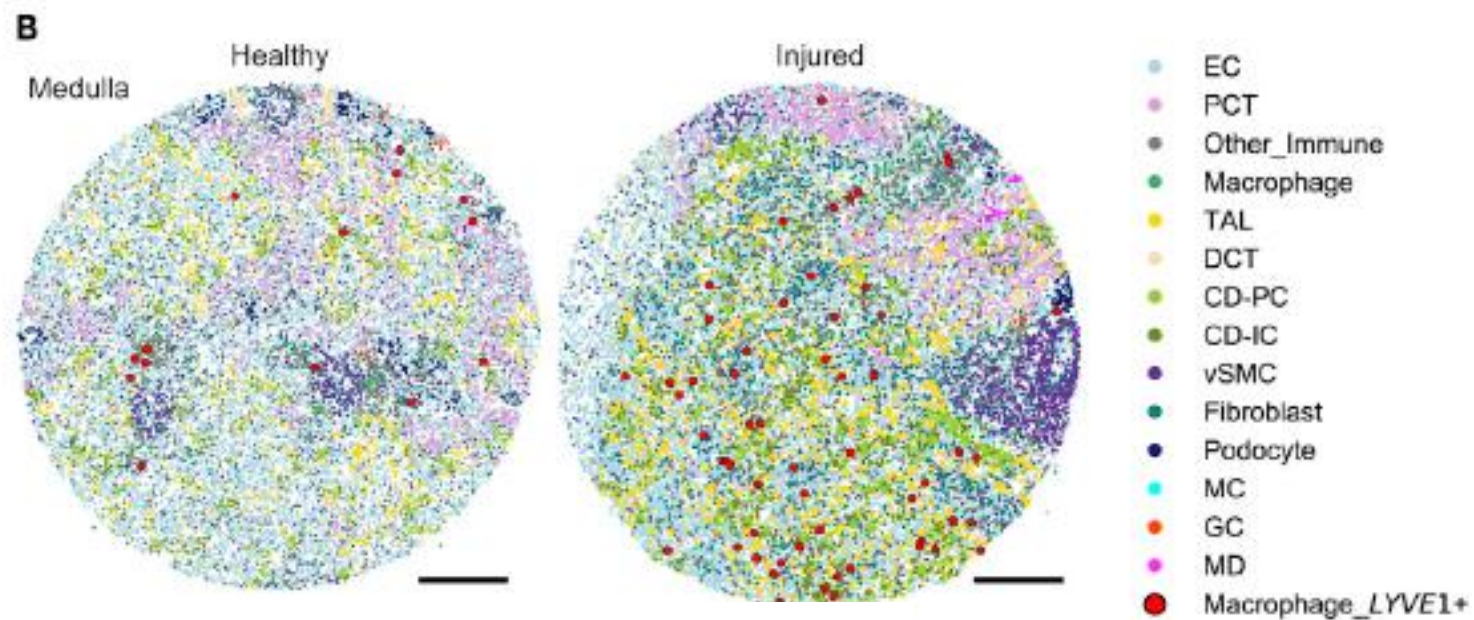
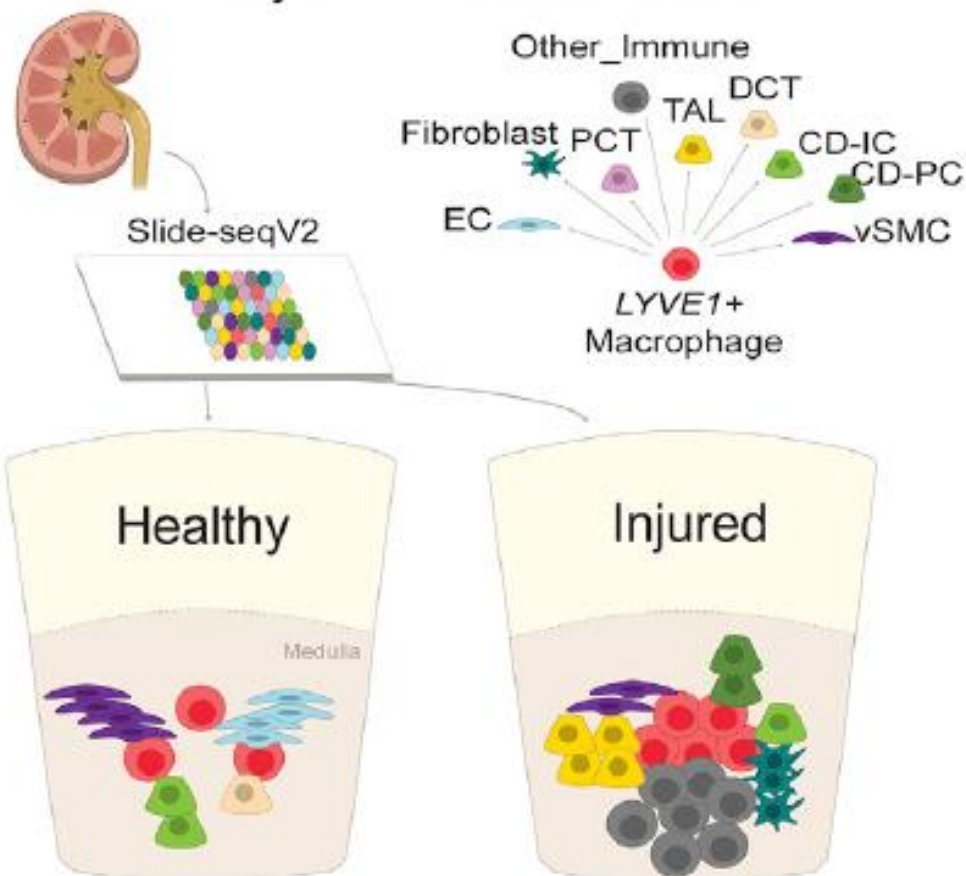
Jamie L. Marshall,
Teia Noel, Qingbo
S. Wang, ..., Evan
Z. Macosko, Fei
Chen, Anna Greka

Slide-SeqV2 workflow

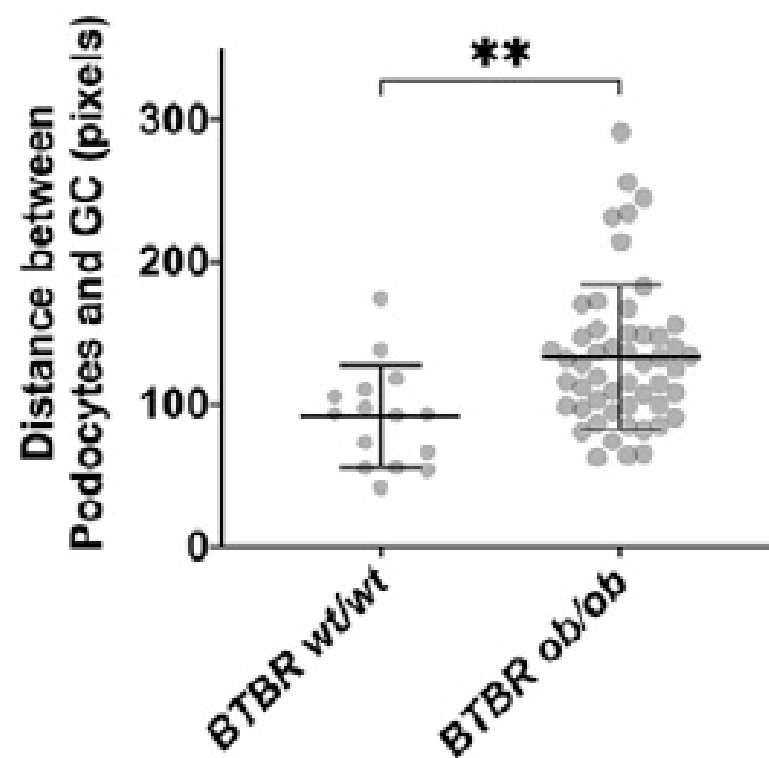
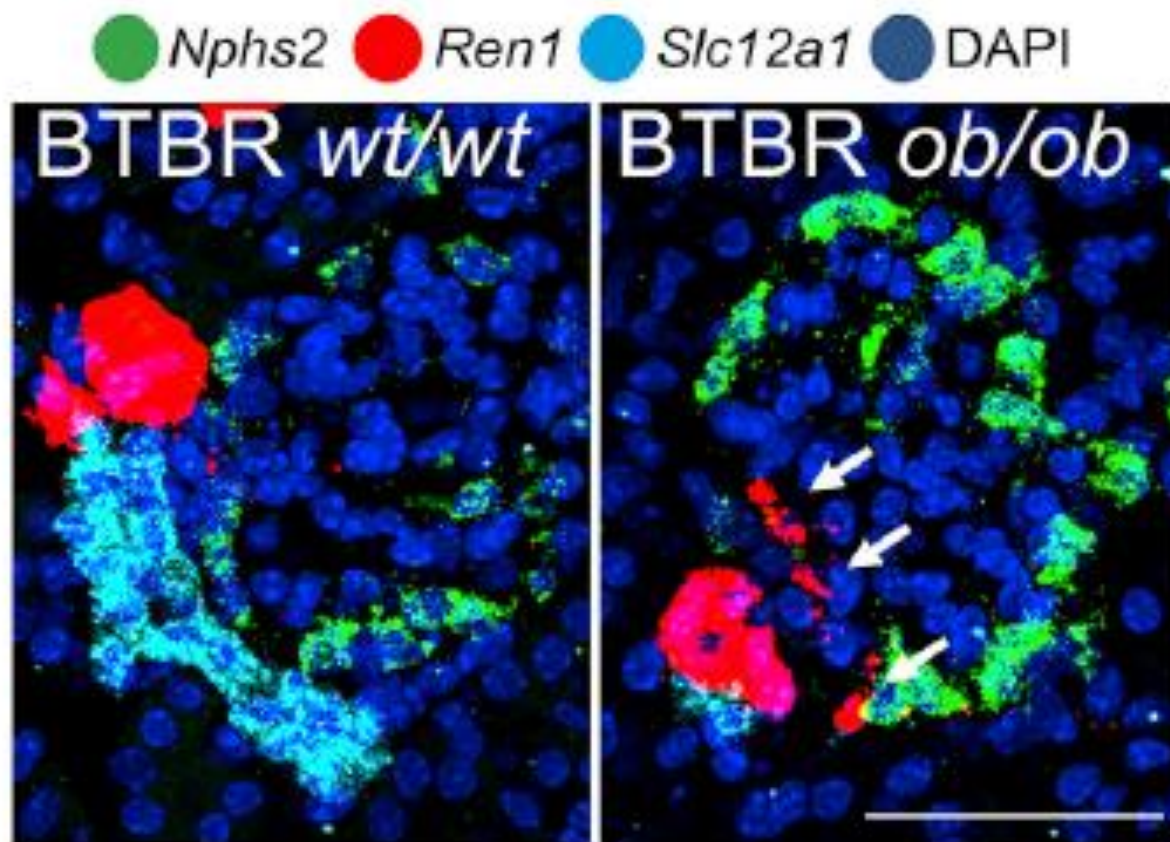


Rodriques et al. Science March 2020.

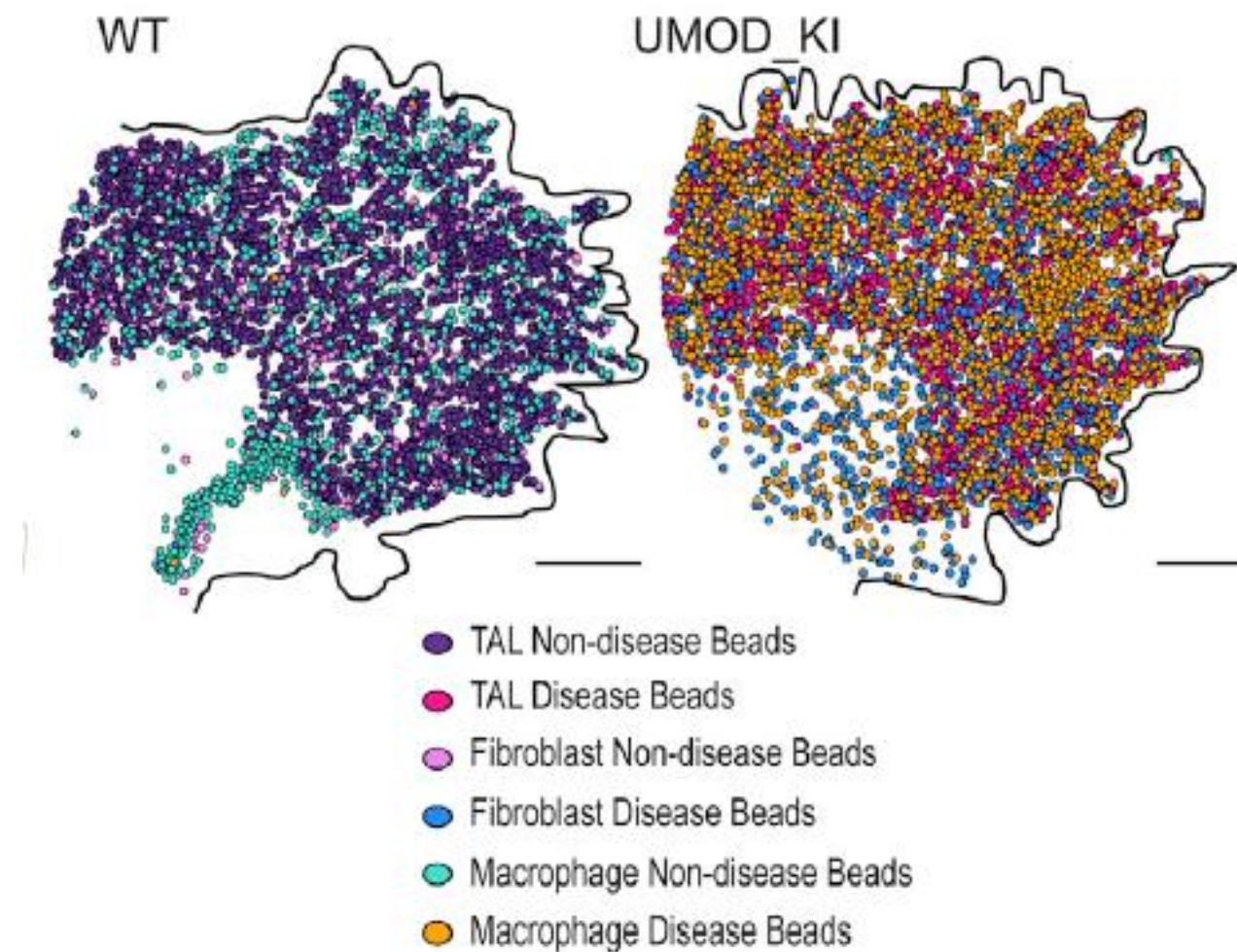
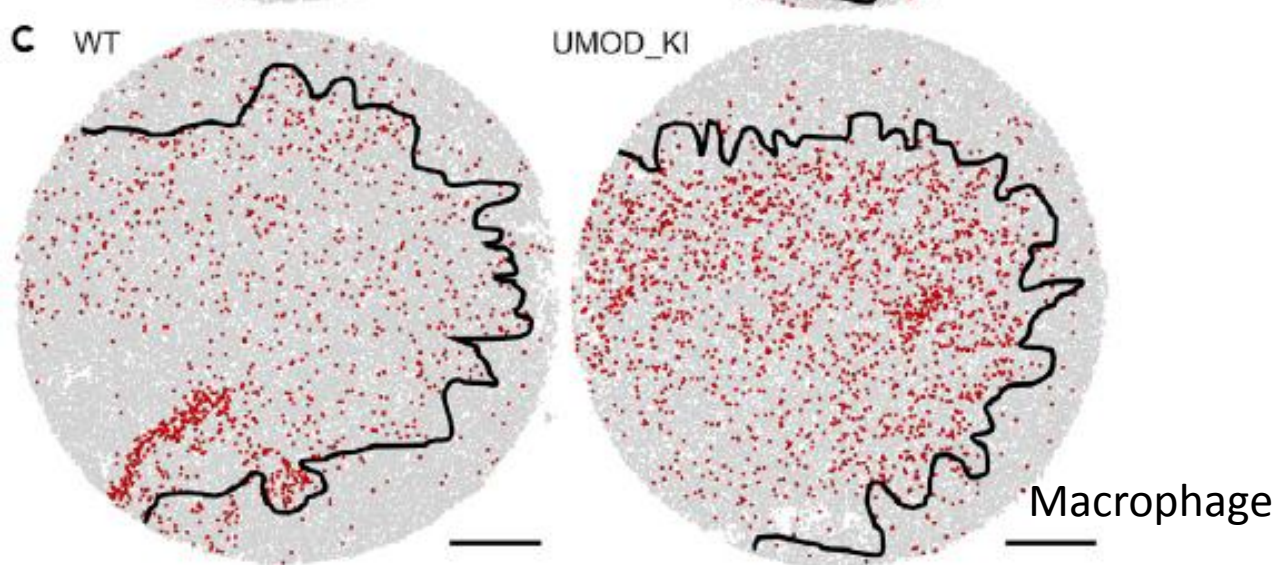
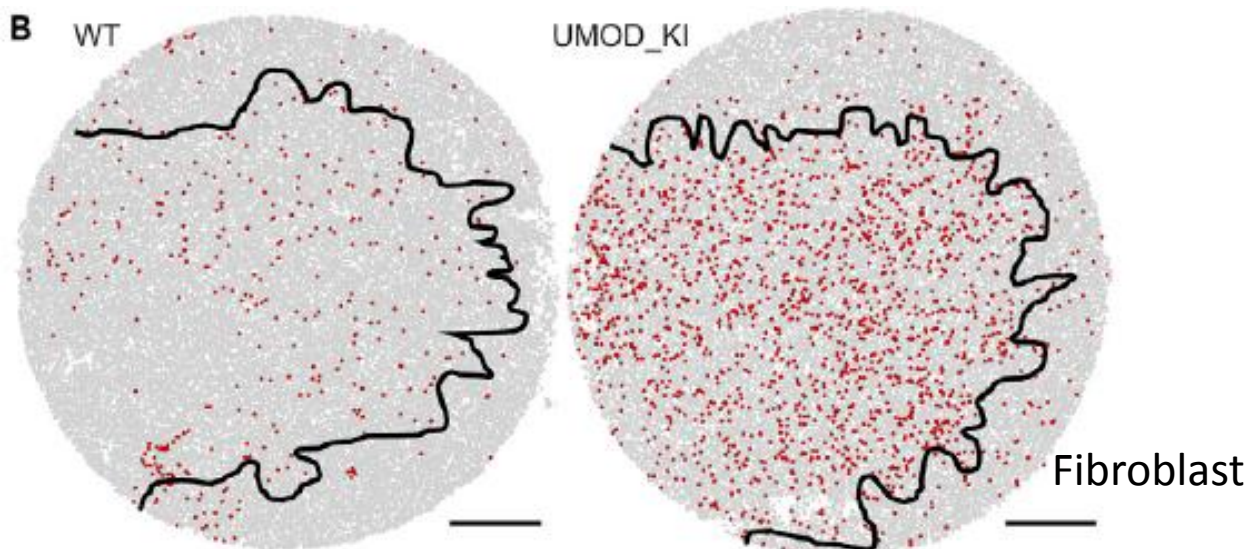
LYVE1+ Macrophage Neighborhoods in Injured Human Medulla

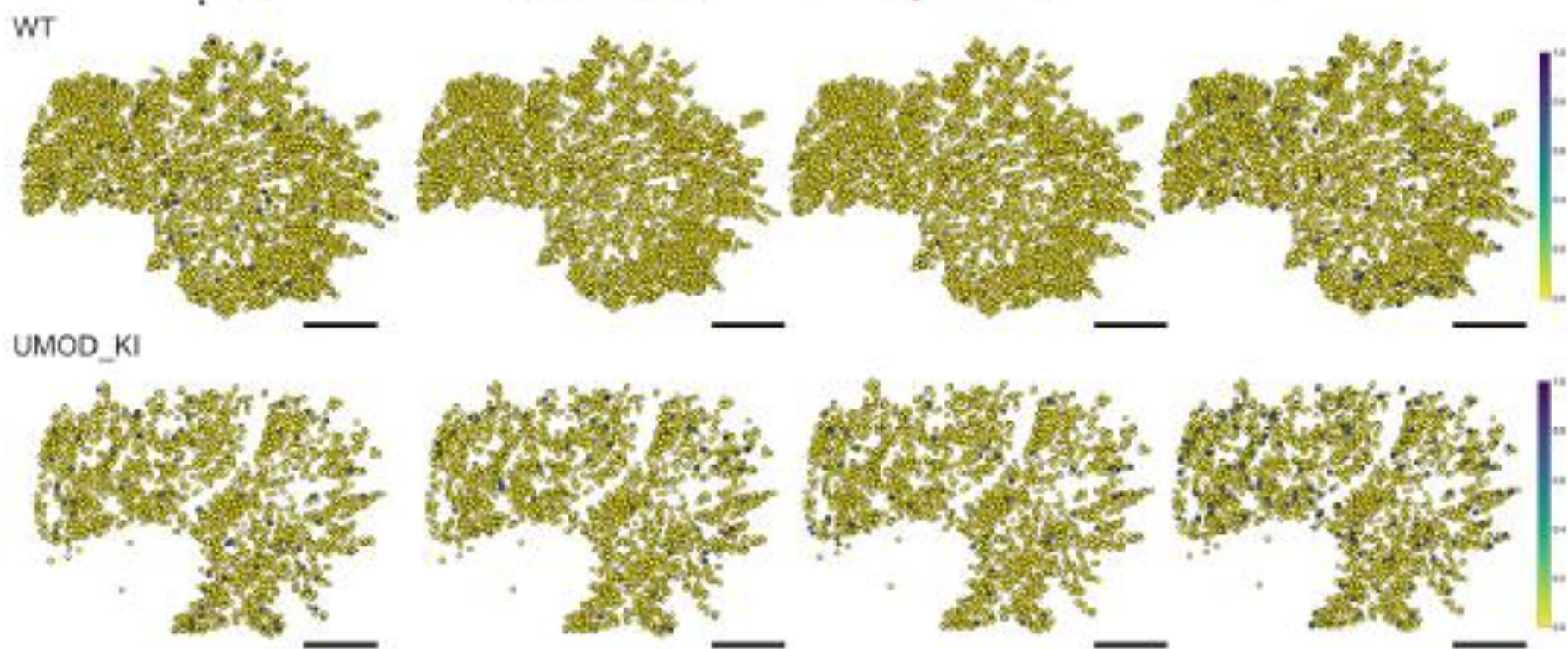
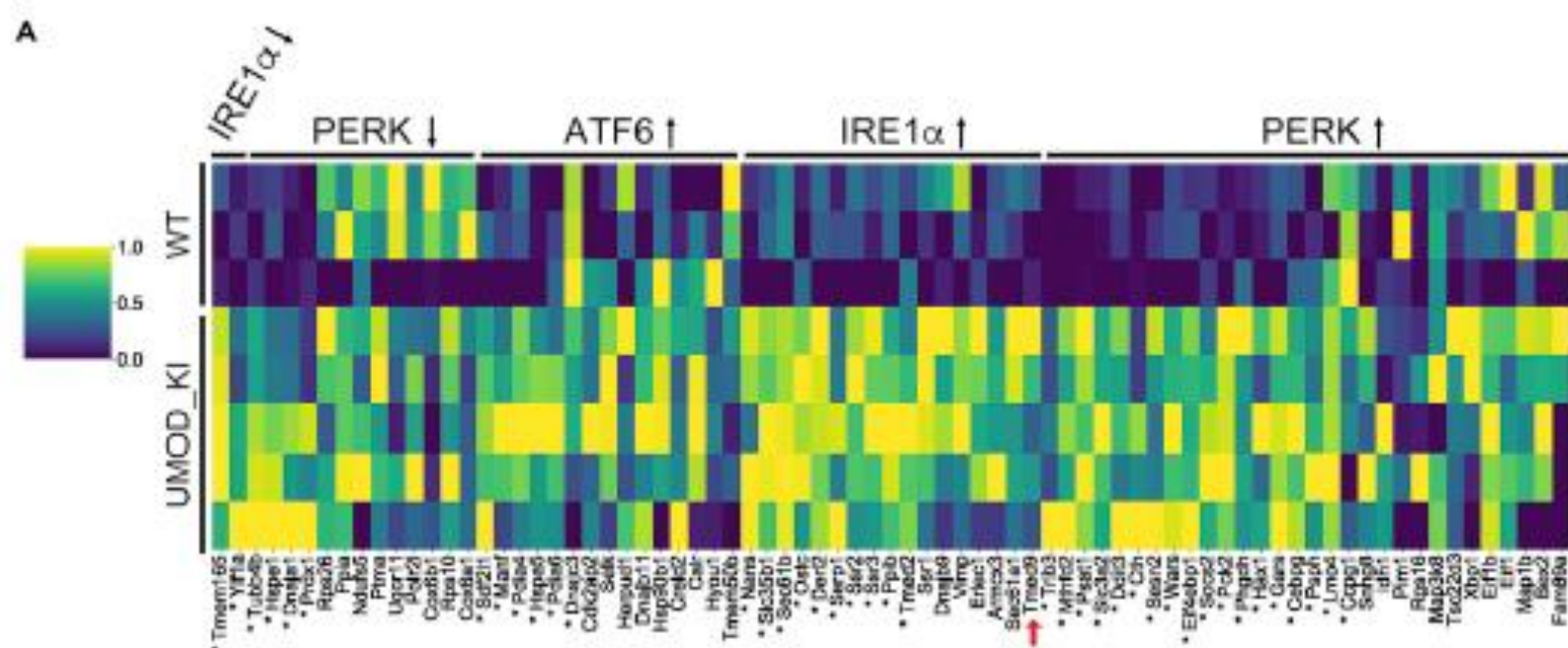


Re-organized Blood Flow Apparatus in Mouse Diabetic Kidney Disease

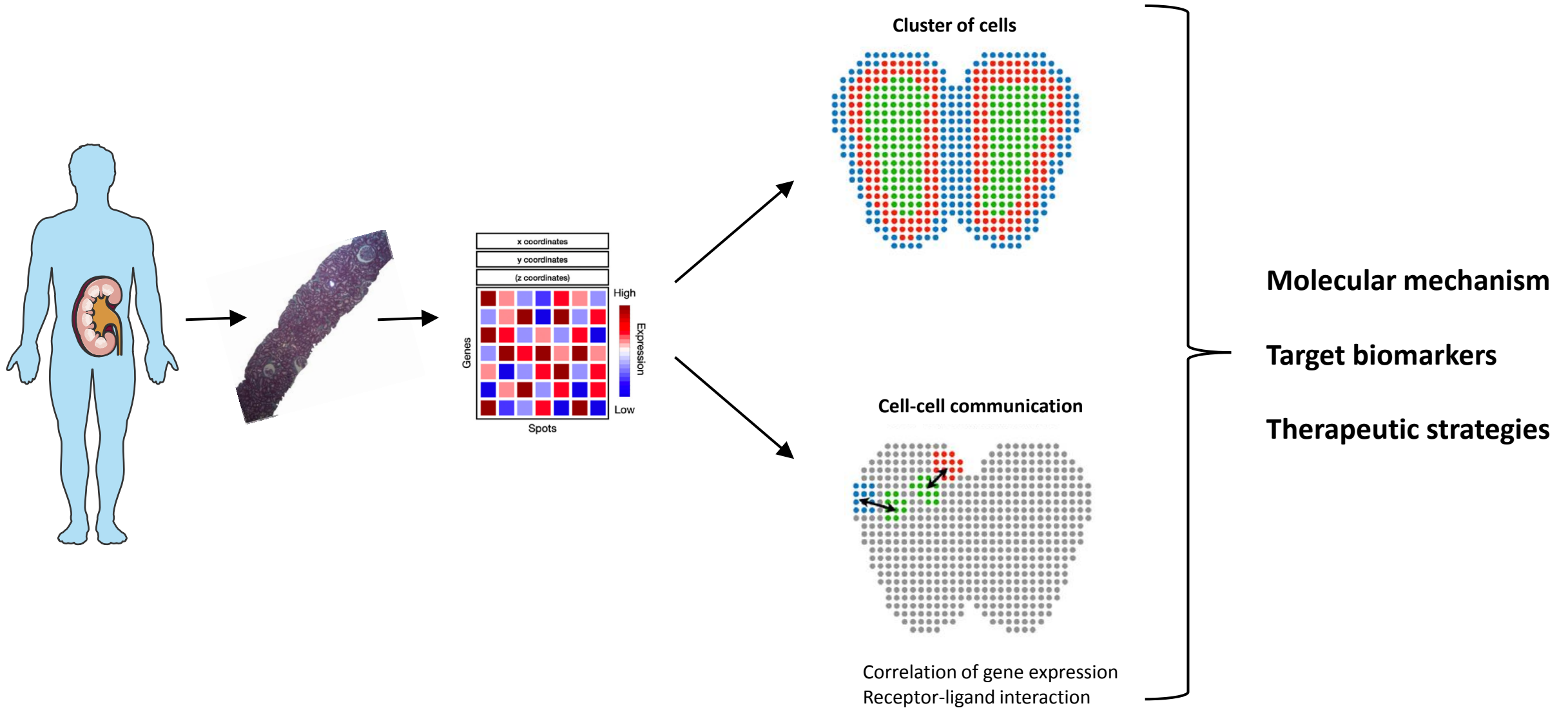


Trem2⁺ Cell Neighborhoods and Transcriptional Signatures of Disease in Mouse Medullary Toxic Proteinopathy

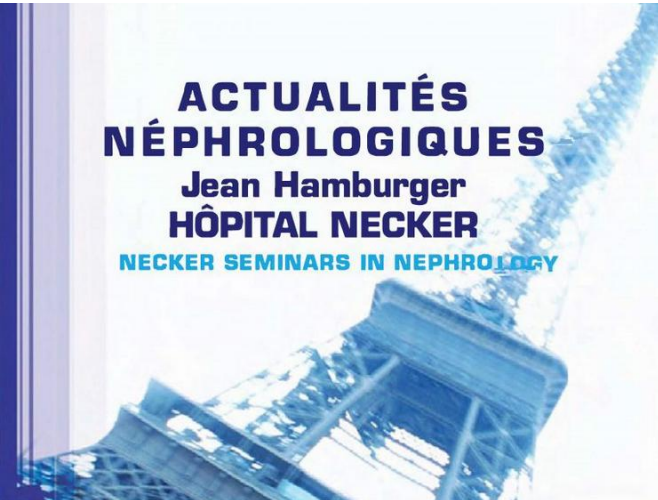




Conclusion



Remerciements



Laboratoire Anatomopathologie Necker

Equipe Fabiola Terzi

Service de Néphrologie et Transplantation rénale Necker