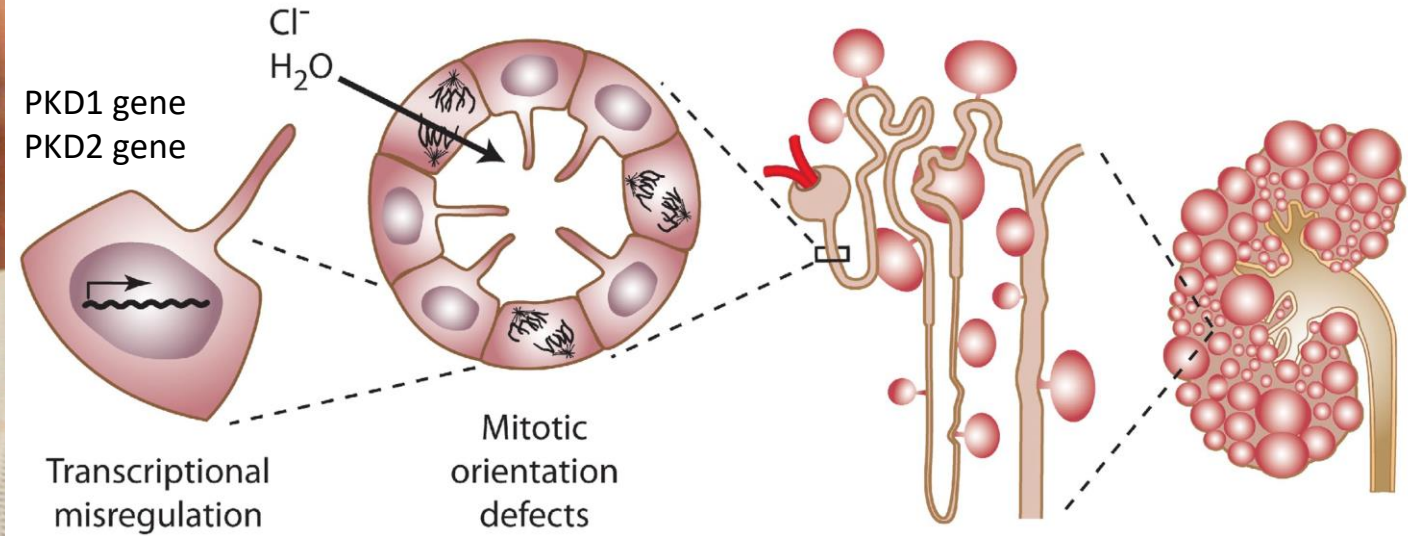


# Single Cell Transcriptomic Analysis to Define Cellular Heterogeneity in Human ADPKD

Yoshiharu Muto<sup>1</sup>, Eryn E. Dixon<sup>3</sup>, Chidambaram Ramachandran<sup>2</sup>, Andrew J. King<sup>2</sup>, Stephen L. Seliger<sup>3</sup>, Owen M. Woodward<sup>3</sup>, Paul A. Welling<sup>4</sup>, Terry J. Watnick<sup>3</sup>, Benjamin D. Humphreys<sup>1</sup>

1. Washington University School of Medicine, St. Louis, MO, United States.
2. Chinook Therapeutics, Inc., Vancouver, BC, Canada.
3. University of Maryland School of Medicine, Baltimore, MD, United States.
4. Johns Hopkins School of Medicine, Baltimore, MD, United States.

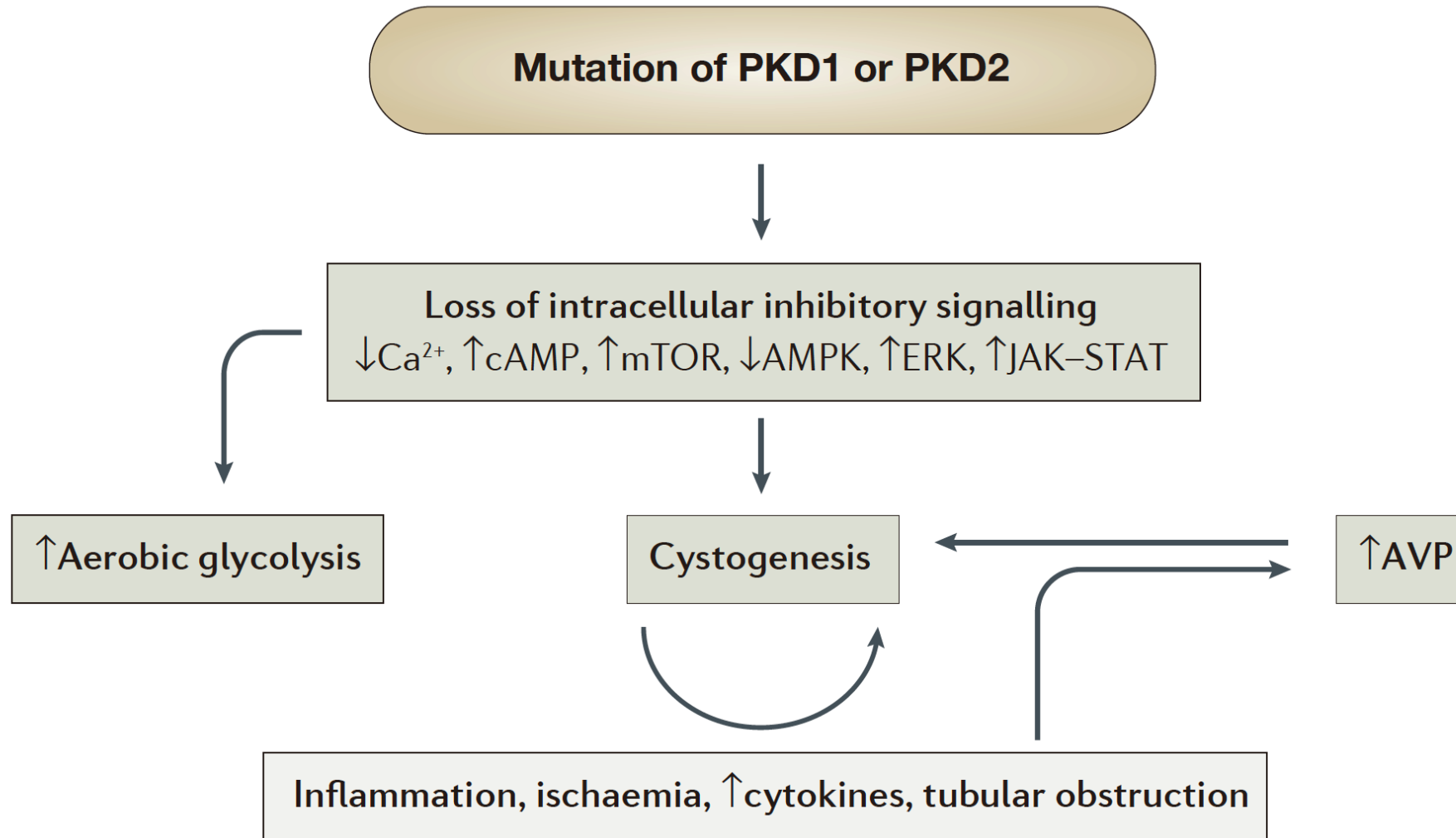
# Polycystic kidney disease : an inherited disorder with multiple cysts in the kidney



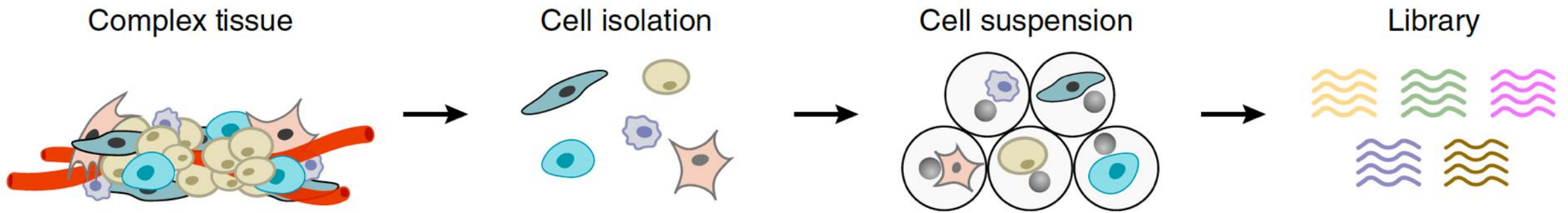
J. Cell Biol., 2010, Nov 15;191(4):701-10

<https://www.nih.gov/news-events/news-releases/two-drugs-are-no-more-effective-one-treat-common-kidney-disease>

Many cell-type-specific signaling pathways were altered in PKD



Single-cell transcriptomic analysis is a suitable method to understand the heterogeneity in the complex tissues



Cell, 2015, May 21;161(5):1202-1214.

Sample ID	Date	L/R	Kidney size	Sex	Age	Location of cyst
PKD1	10.11.17	right	1079 grams	female	NA	cortical cup
PKD2	11.9.16	left	803 grams	female	53	cortical cup
PKD3	7.29.19	left	1207 grams	female	58	cortical cup
PKD4	4.6.17	left	2061 grams	male	54	cortical cup
PKD5	7.6.18	right	1094 grams	female	61	cortical cup
PKD6	8.30.18	left	3170 grams	male	35	cortical cup
PKD7	4.9.18	right	2100 grams	male	42	cortical cup
PKD8	7.13.17	right	1531 grams	male	47	cortical cup

PKD1



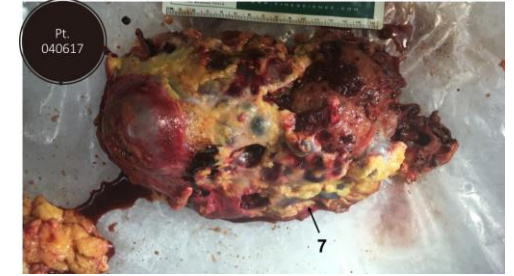
PKD2



PKD3



PKD4



PKD5



PKD6



PKD7

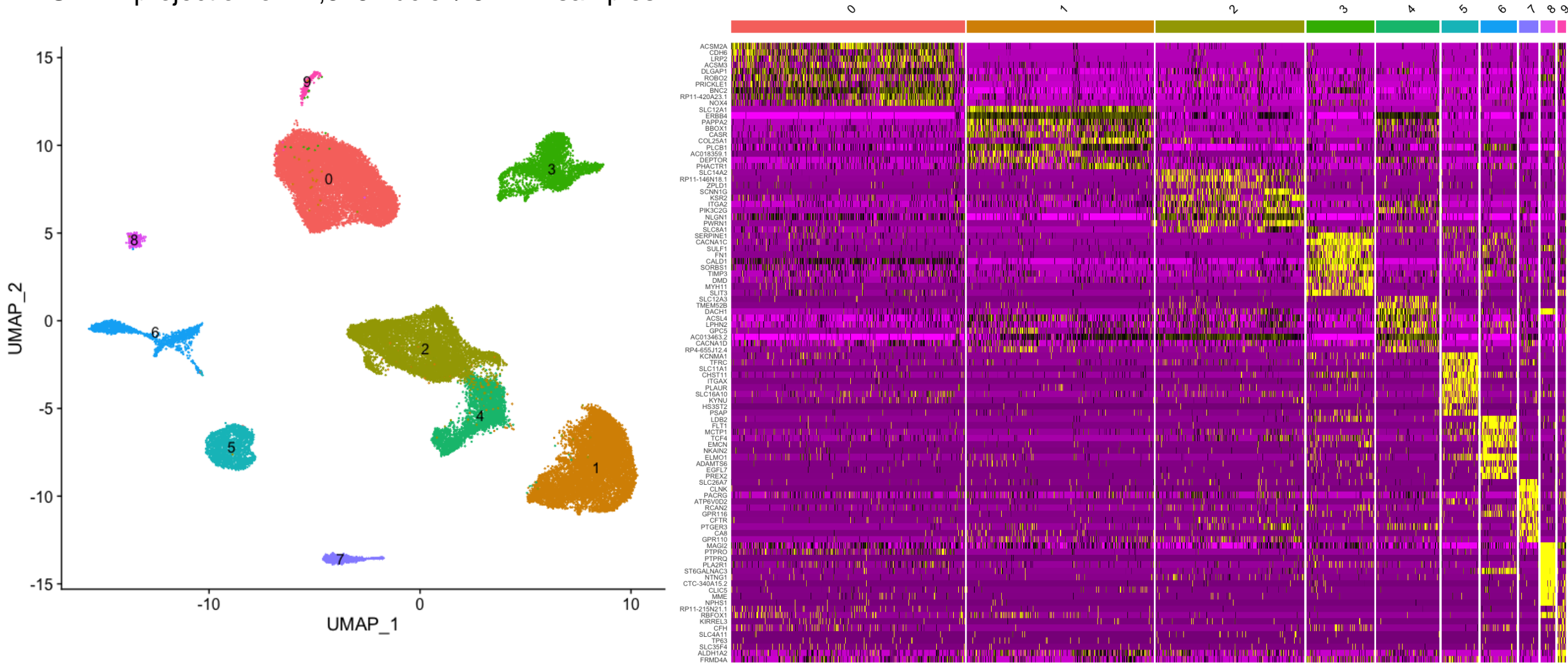


PKD8



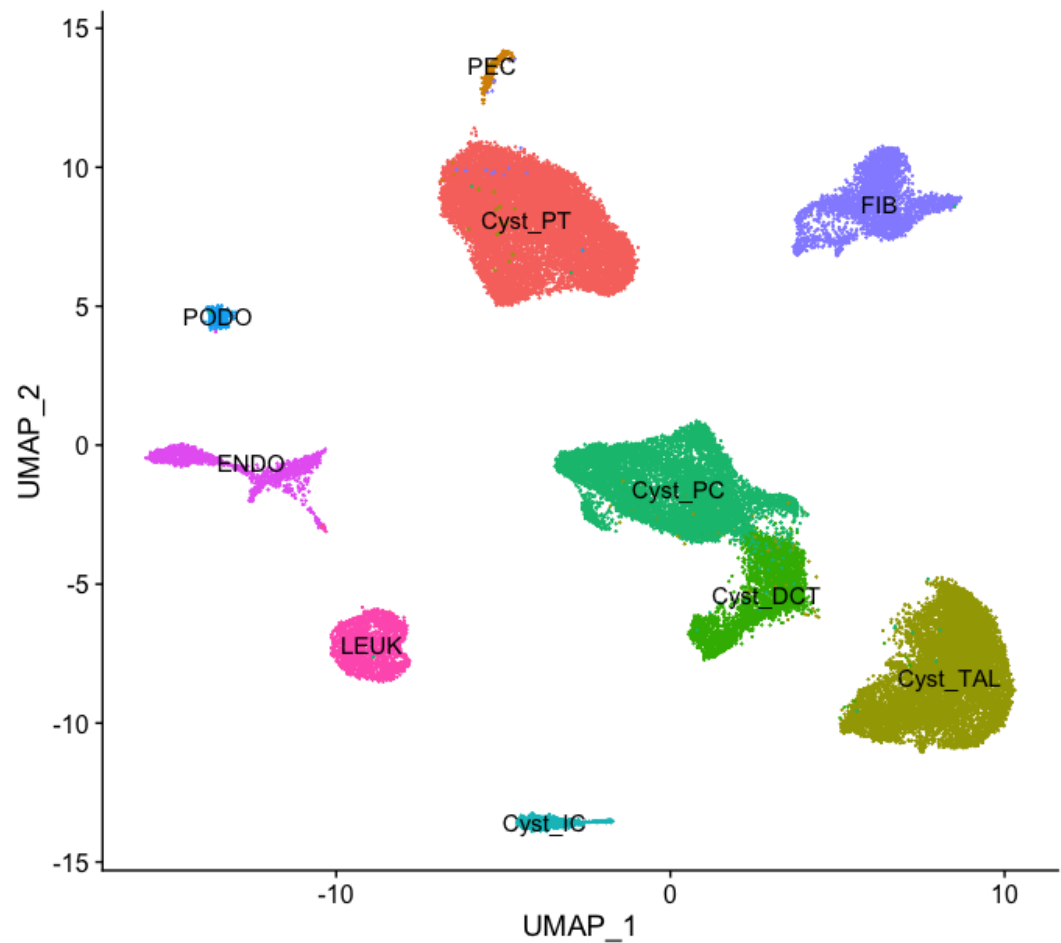
# snRNA-seq on 8 PKD samples (4 female / 4 male) revealed cellular heterogeneity in the PKD kidneys

## UMAP projection of 42,545 nuclei / 8 PKD samples

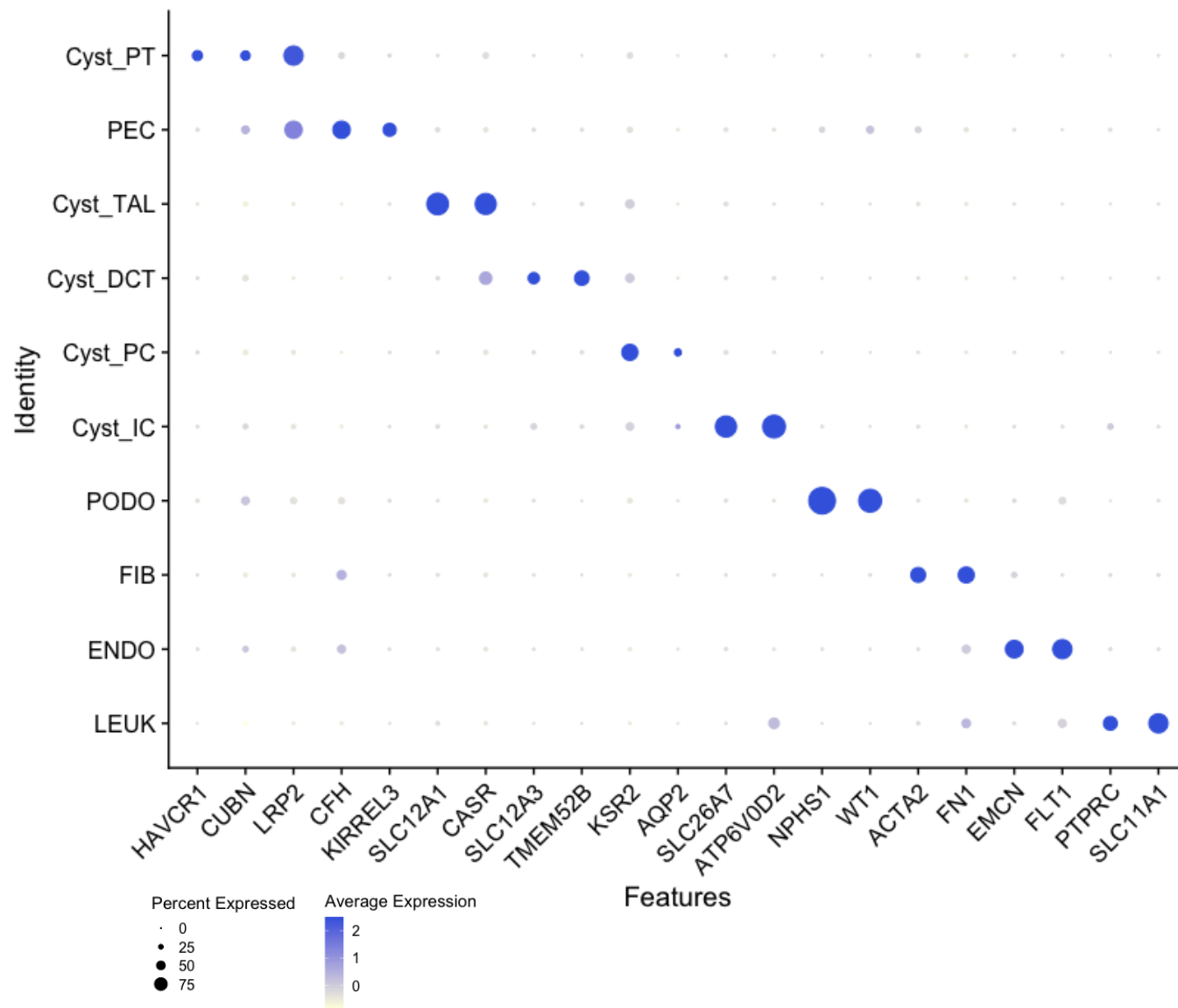


# PKD kidney cells maintain expression of lineage markers for healthy kidney cell types

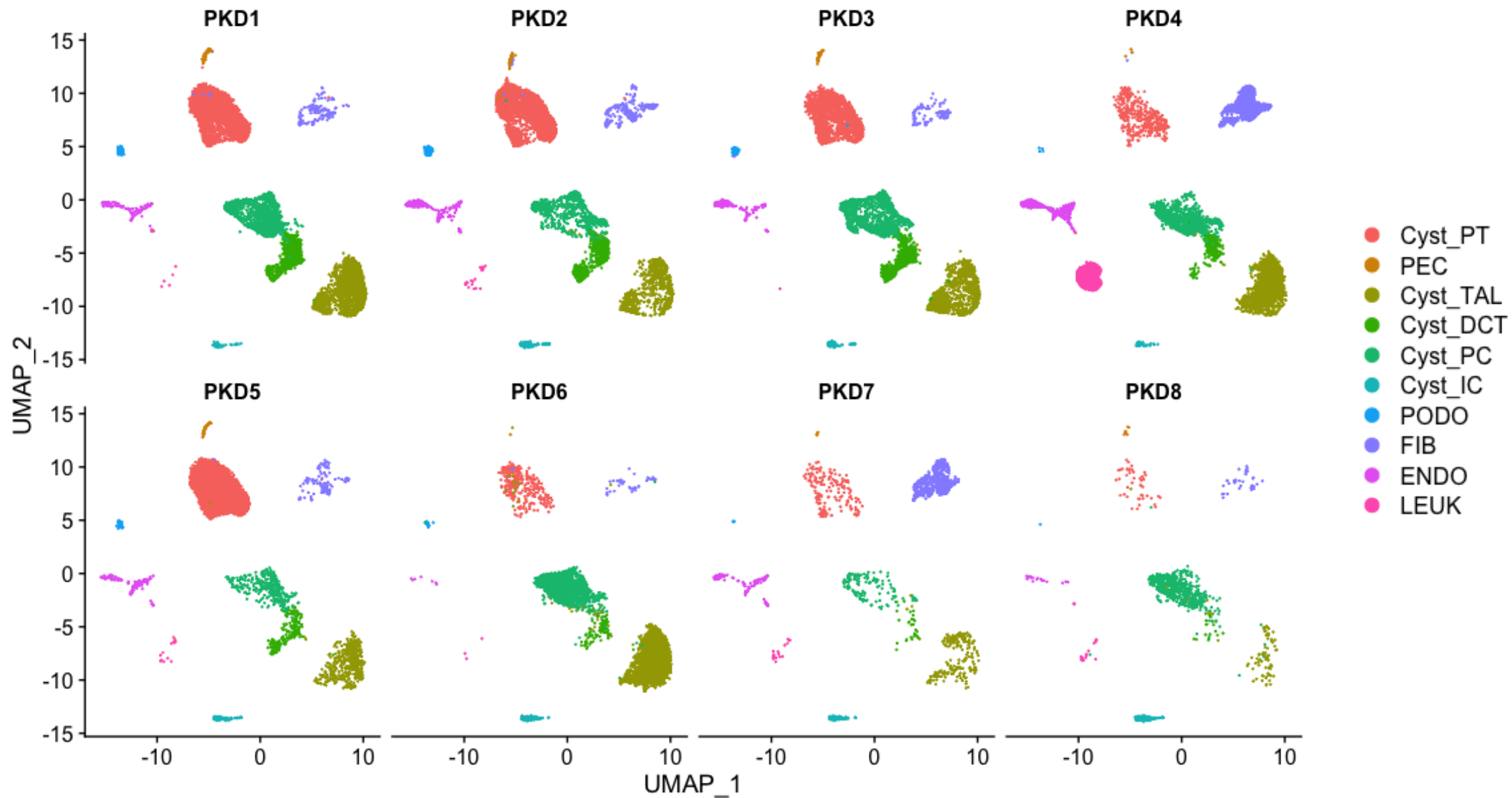
UMAP projection of 42,545 nuclei / 8 PKD samples



Gene expression in each cell type



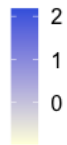
Major cell types are detected in each of the PKD sample



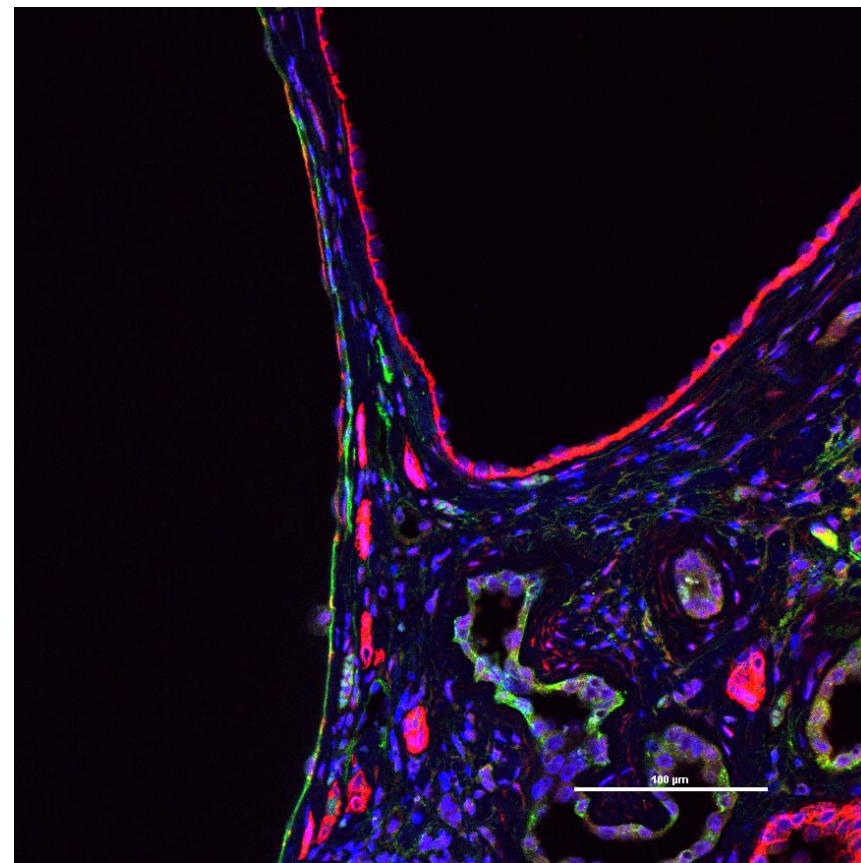
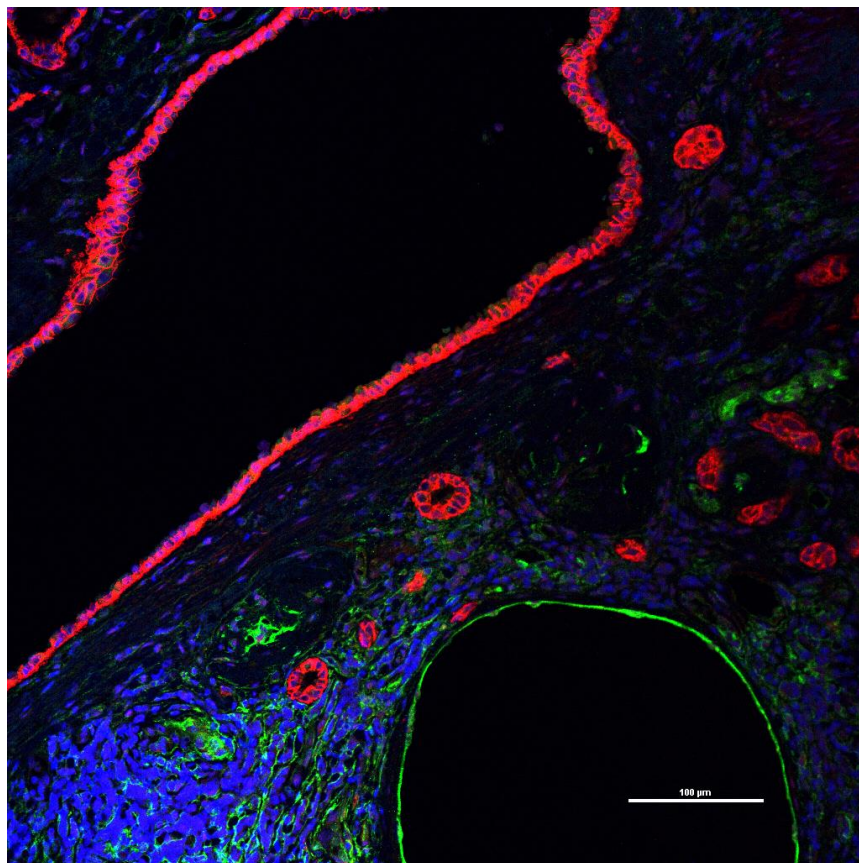
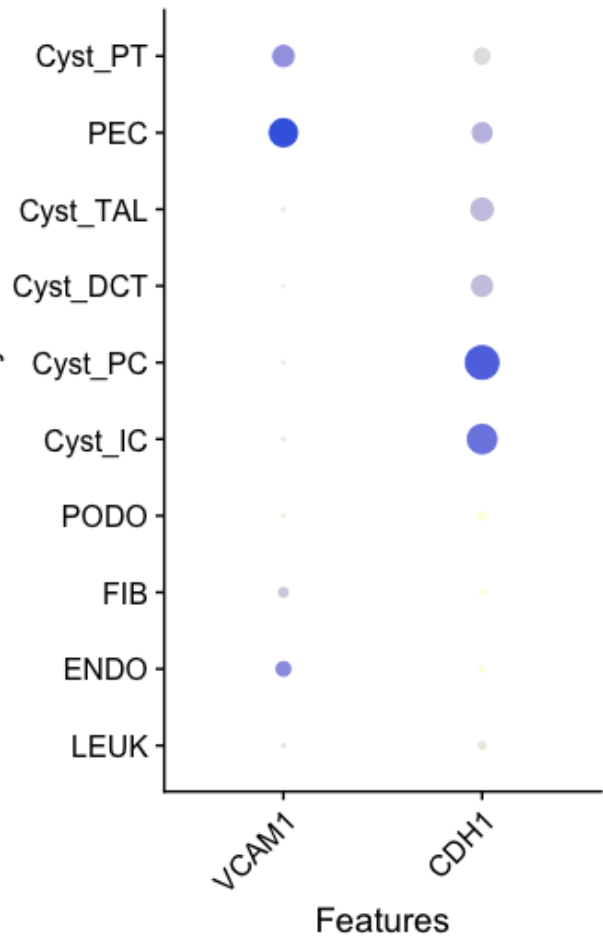


# Validation of cellular heterogeneity in cyst wall with immuno-staining approach

Average Expression

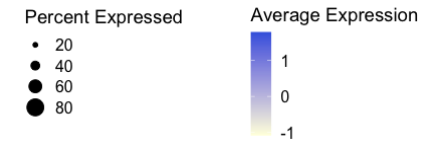
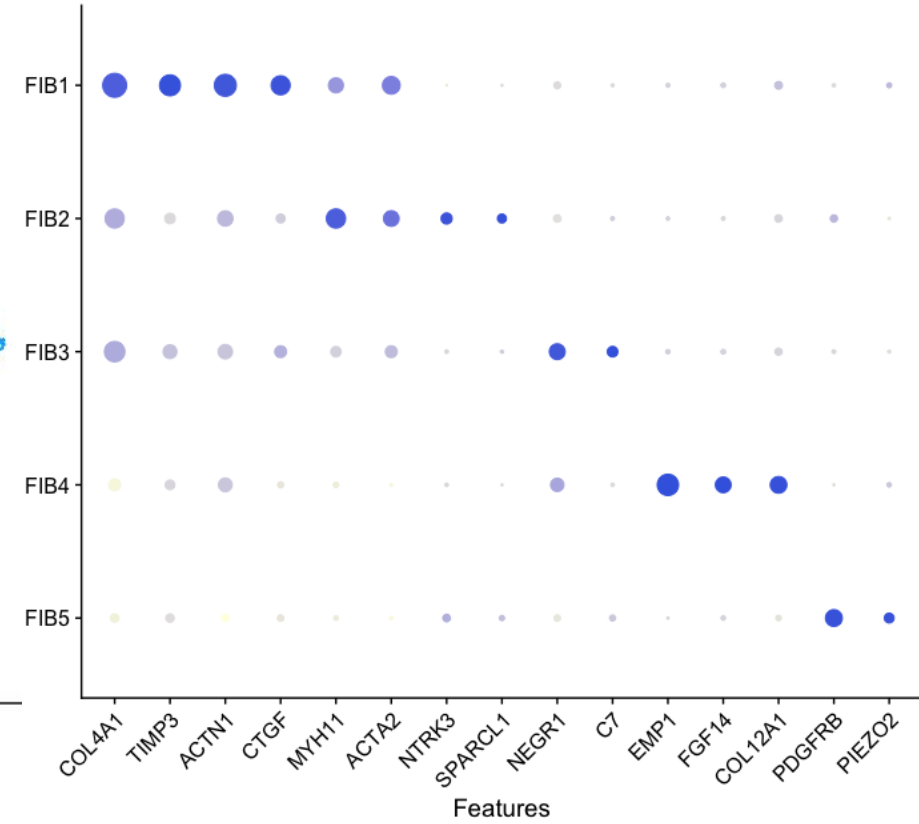
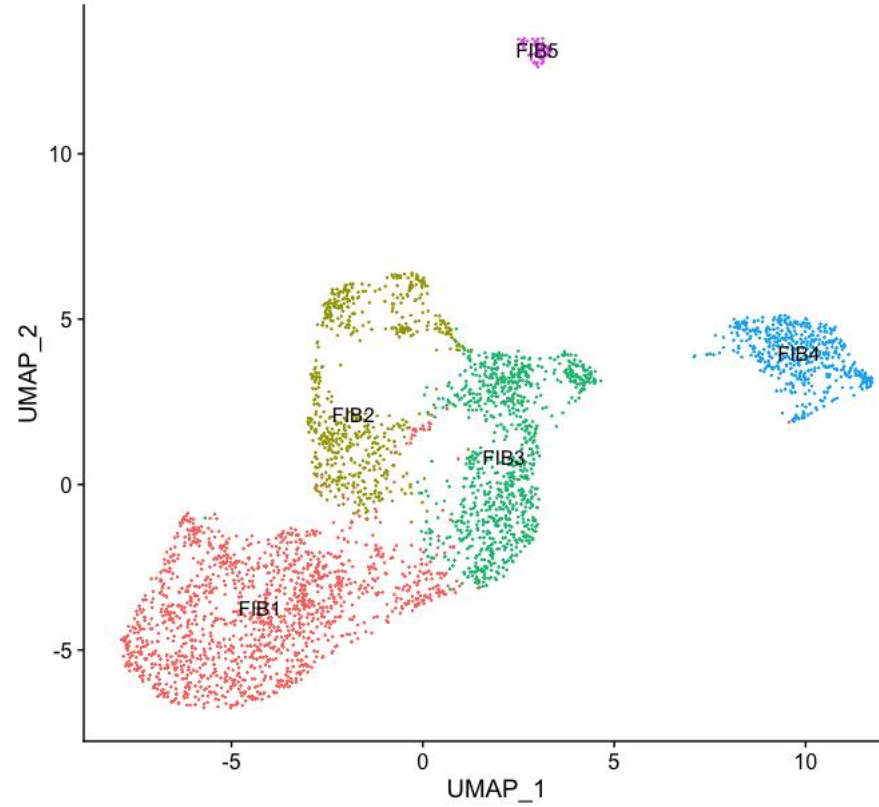
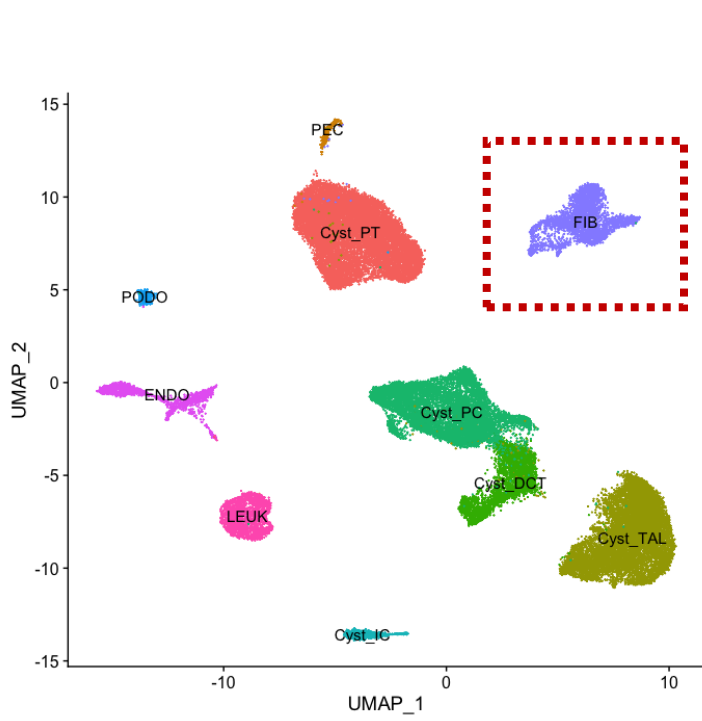


VCAM1/CDH1

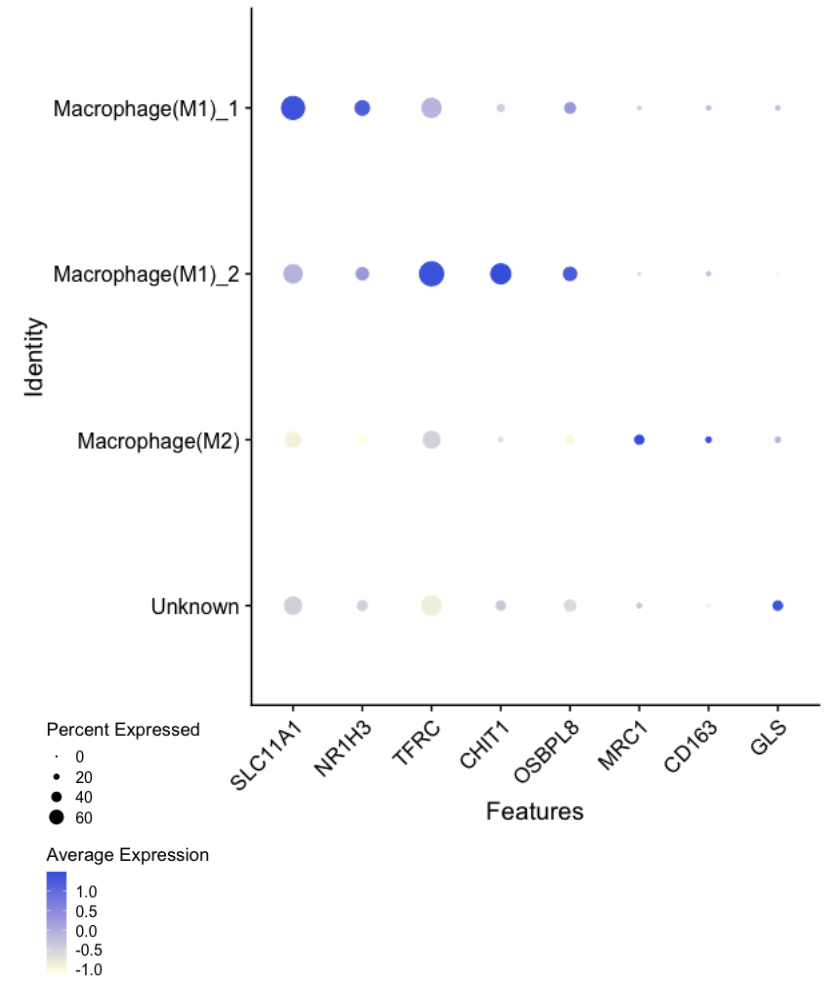
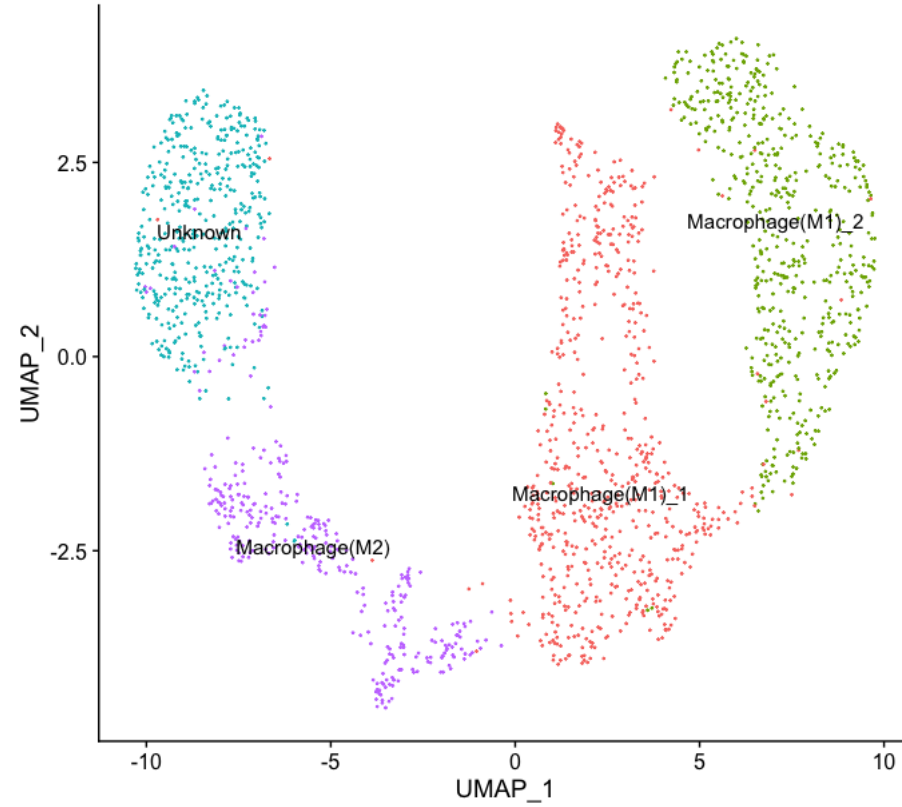
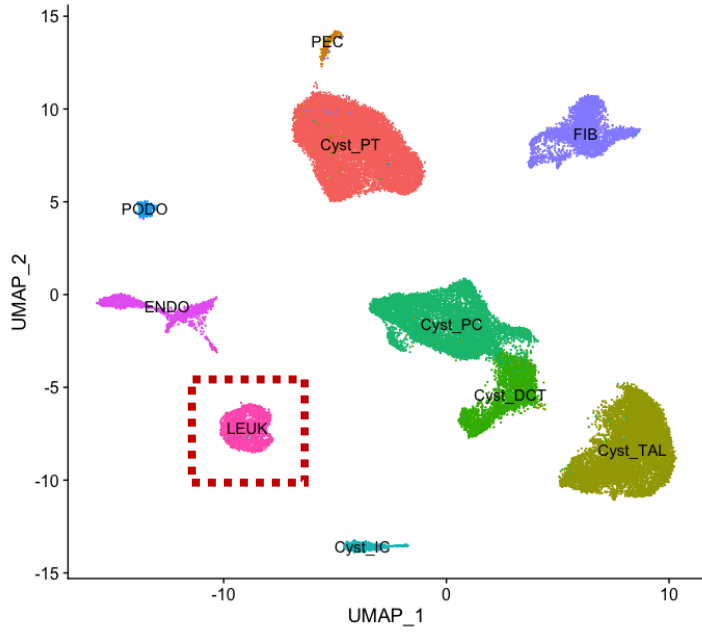




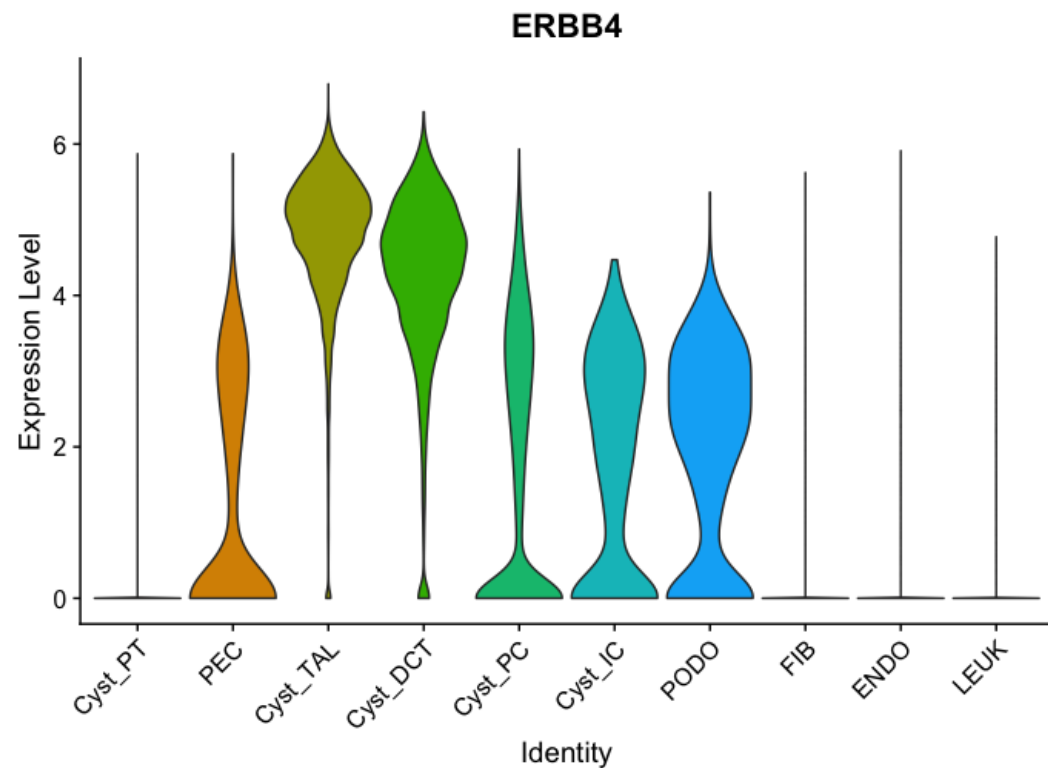
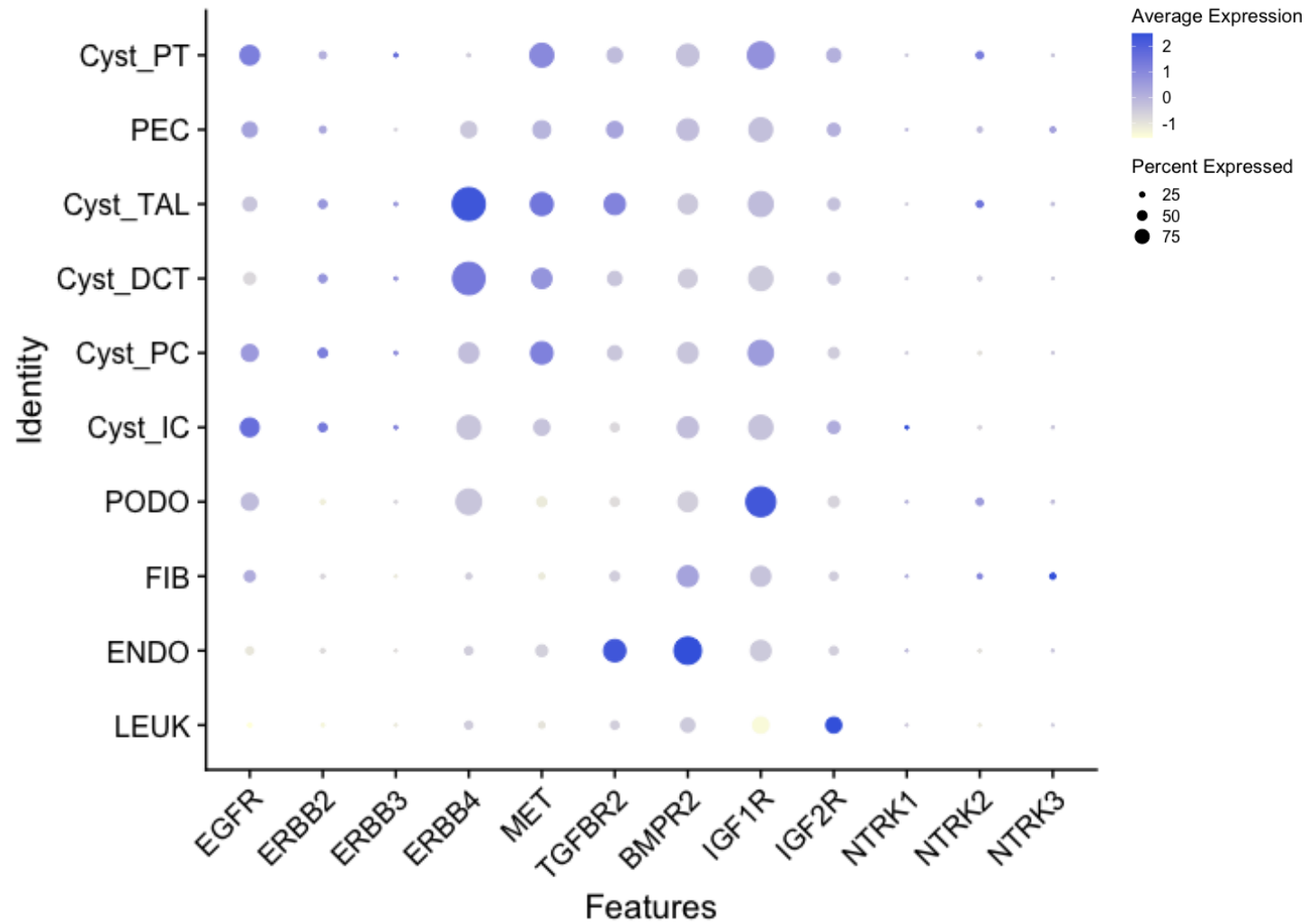
# Heterogeneity of fibroblasts in PKD dataset



# Heterogeneity of macrophages in PKD dataset

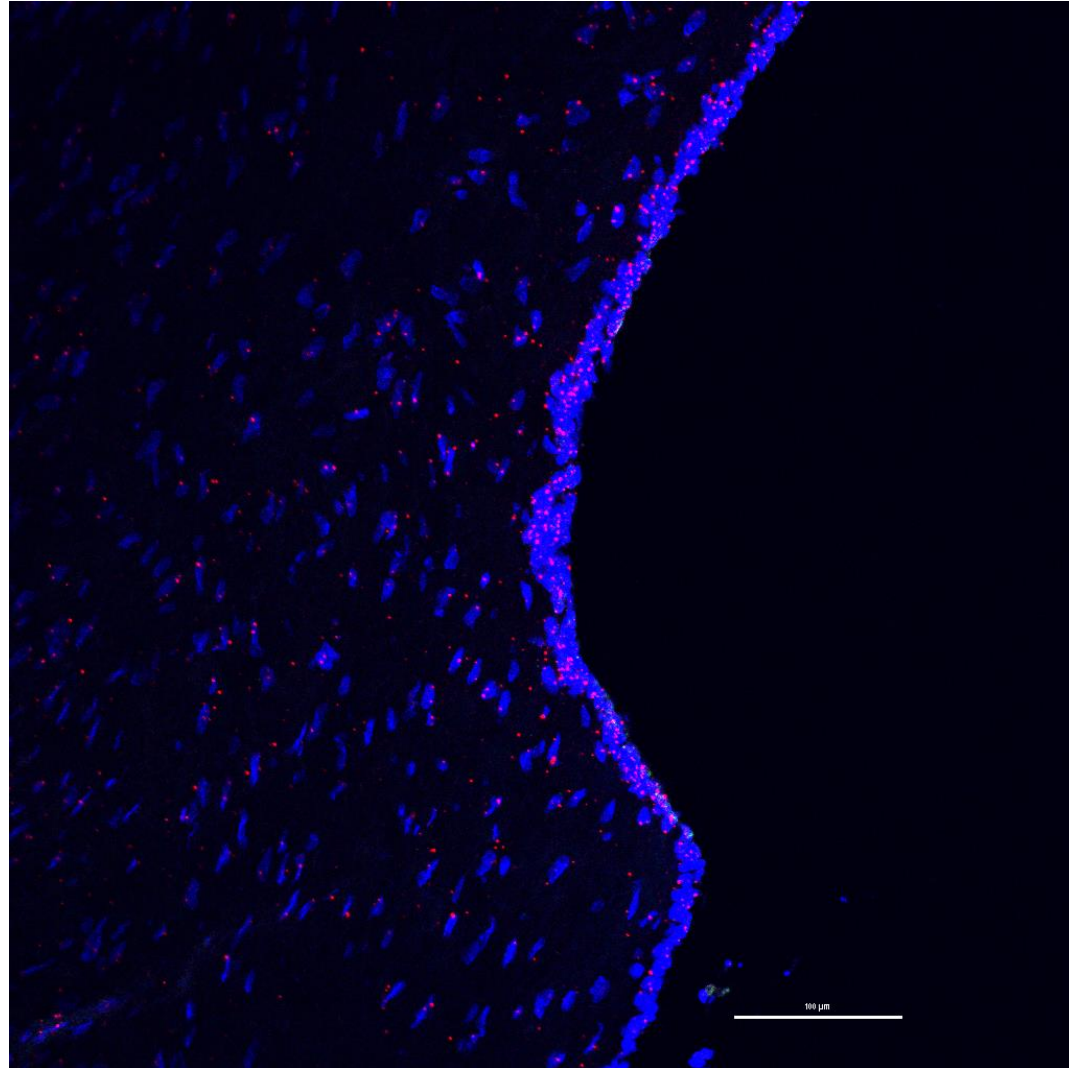


# ERBB4 expression is dominant in distal nephron

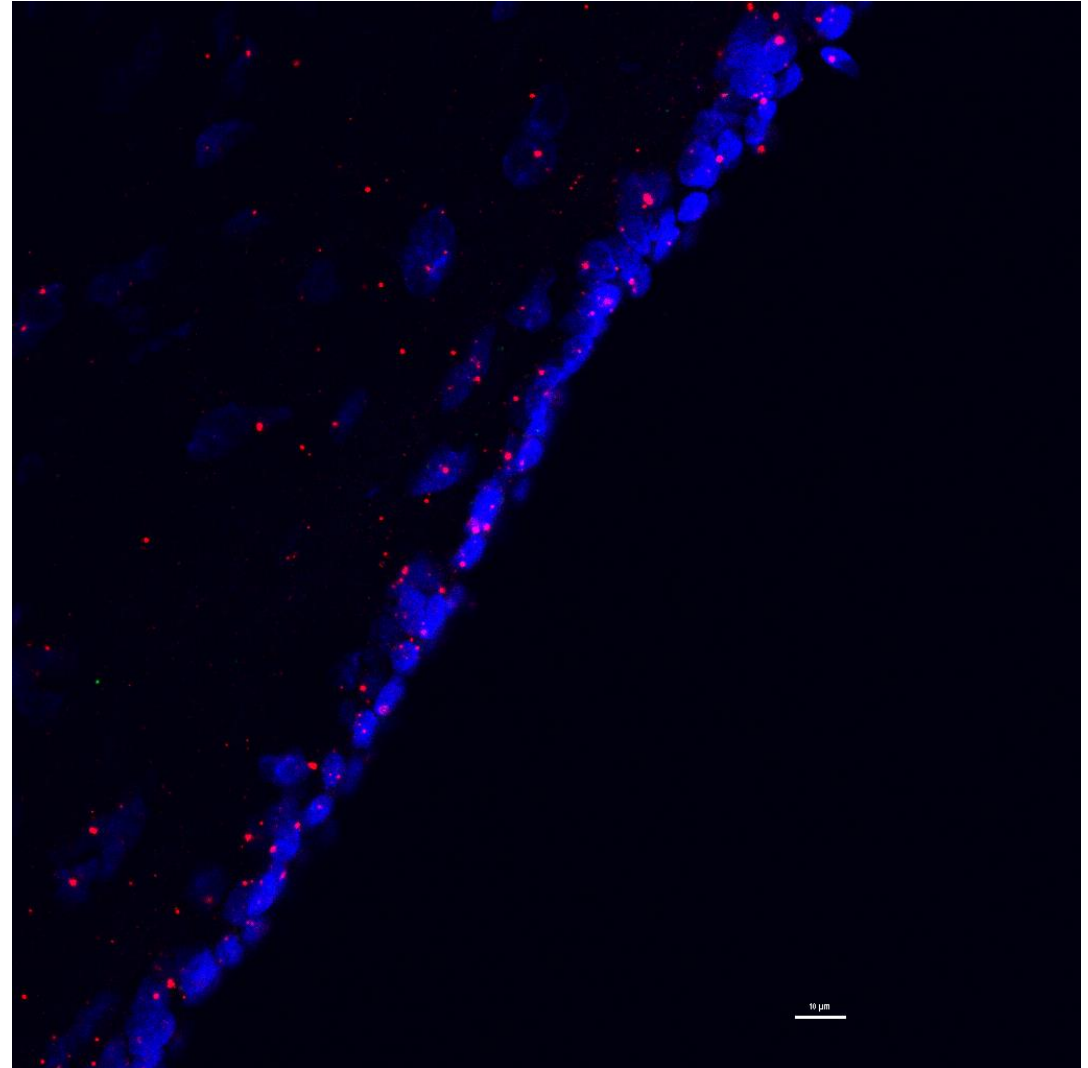


# ERBB4 is dominantly expressed in the cyst lining cells

ERBB4 (x20)



ERBB4 (x60)



Cell-to-cell communication prediction suggested that ERBB4 interacts with various ligands in PKD

FIB → TAL

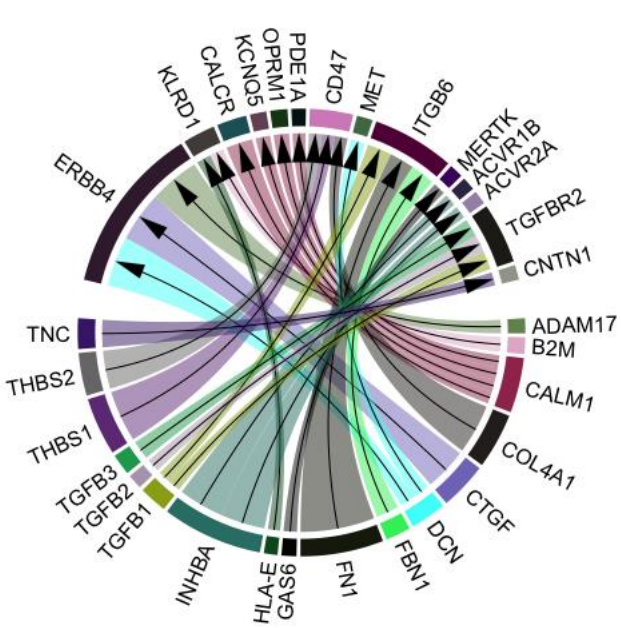
LEUK → TAL

Cyst\_PT → TAL

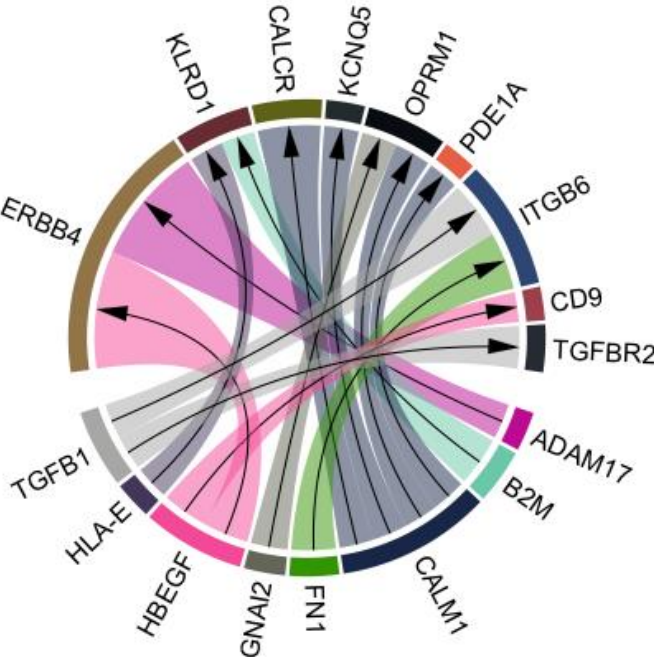
Thick ascending limb

Thick ascending limb

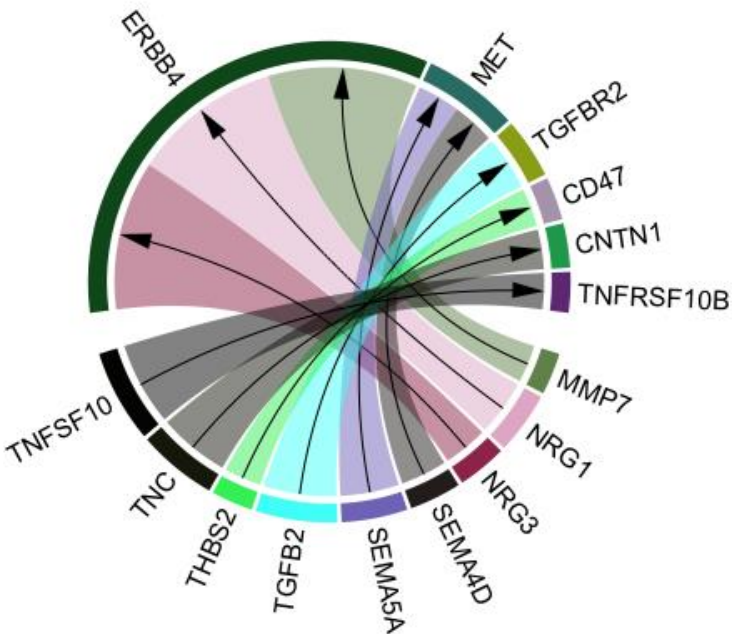
Thick ascending limb



Fibroblast



Leukocyte



Proximal tubules

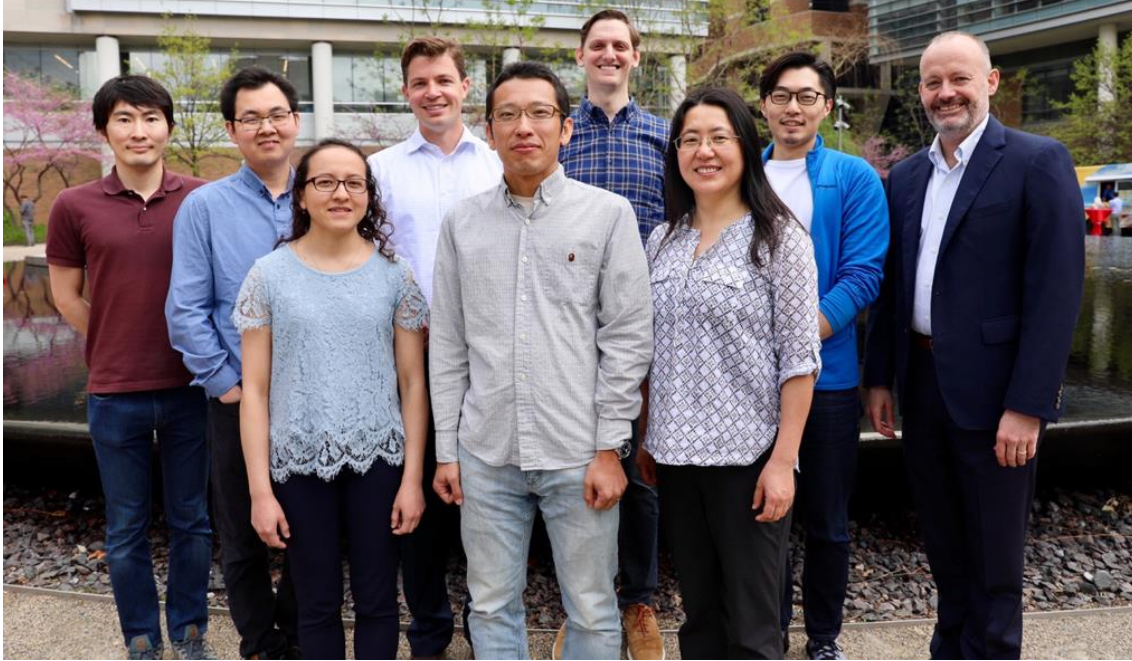
## Conclusion

snRNA-seq was successfully performed on the frozen, surgically-removed PKD kidney samples.

snRNA-seq revealed the previously unrecognized cellular heterogeneity and complexity of cyst. Various cellular compositions are observed in cyst samples.

Single cell analysis revealed cell-type-specific activations of signaling pathways including ERBB4, and diversity of cell-to-cell communications in PKD cysts.





Funding support



**CHINOOK**  
THERAPEUTICS

**Chinook Therapeutics**

Chidambaram Ramachandran

Andrew J. King

**Humphreys lab**

Benjamin D. Humphreys

Andrew F Malone

Haikuo Li

Jeffrey Koenitzer

Monica C. Panesso

Shayna Bradford

Yoshiharu Muto

Eryn E. Dixon

Haojia Wu

Lucy Fan

Nicolas Ledru

Yasuhiro Yoshimura

**University of Maryland School of Medicine**

Stephen L. Seliger

Owen M. Woodward

Paul A. Welling

Terry J. Watnick

**Johns Hopkins School of Medicine**

Paul A. Welling