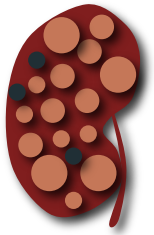


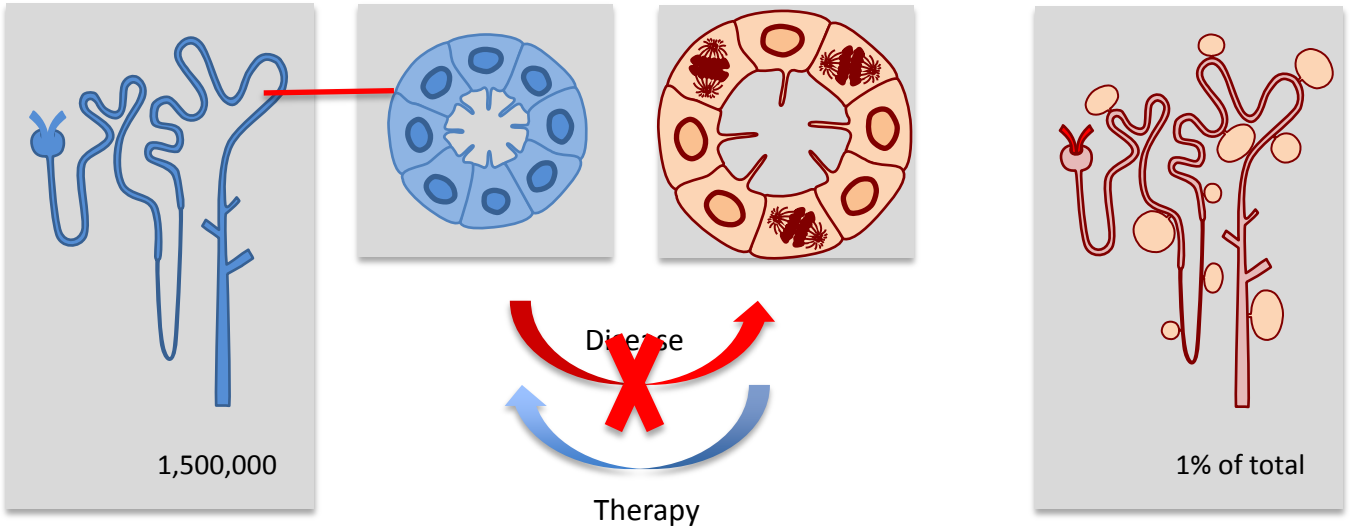
Deducing the decision-making process of a kidney cell

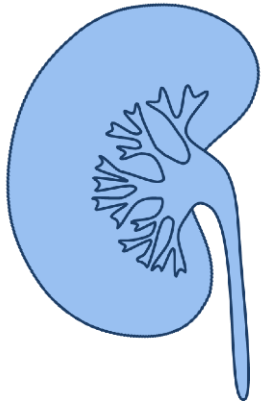
December 13, 2015
Chan Woon Chueng Memorial Fund Award Lecture
1st International Congress of Chinese Nenphrologists, Hong Kong

Jing Zhou (周晶), M.D., Ph.D.

Harvard Center for Polycystic Kidney Disease Research
Harvard Medical School
Brigham and Women's Hospital

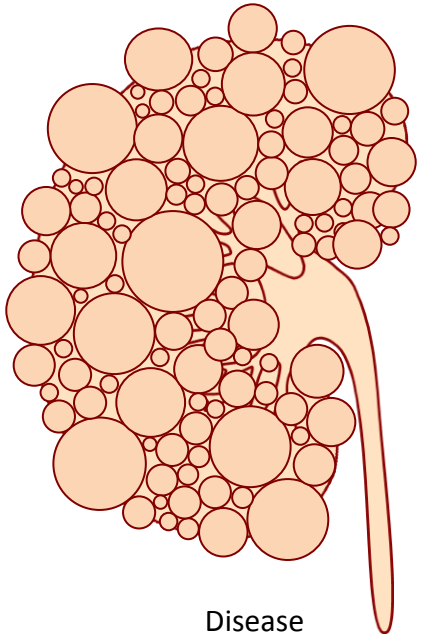
jzhou@bwh.harvard.edu





Normal Human Kidney

150 g
180 L blood



Disease

7,500 g

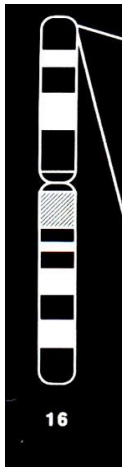
Morphologic transformation

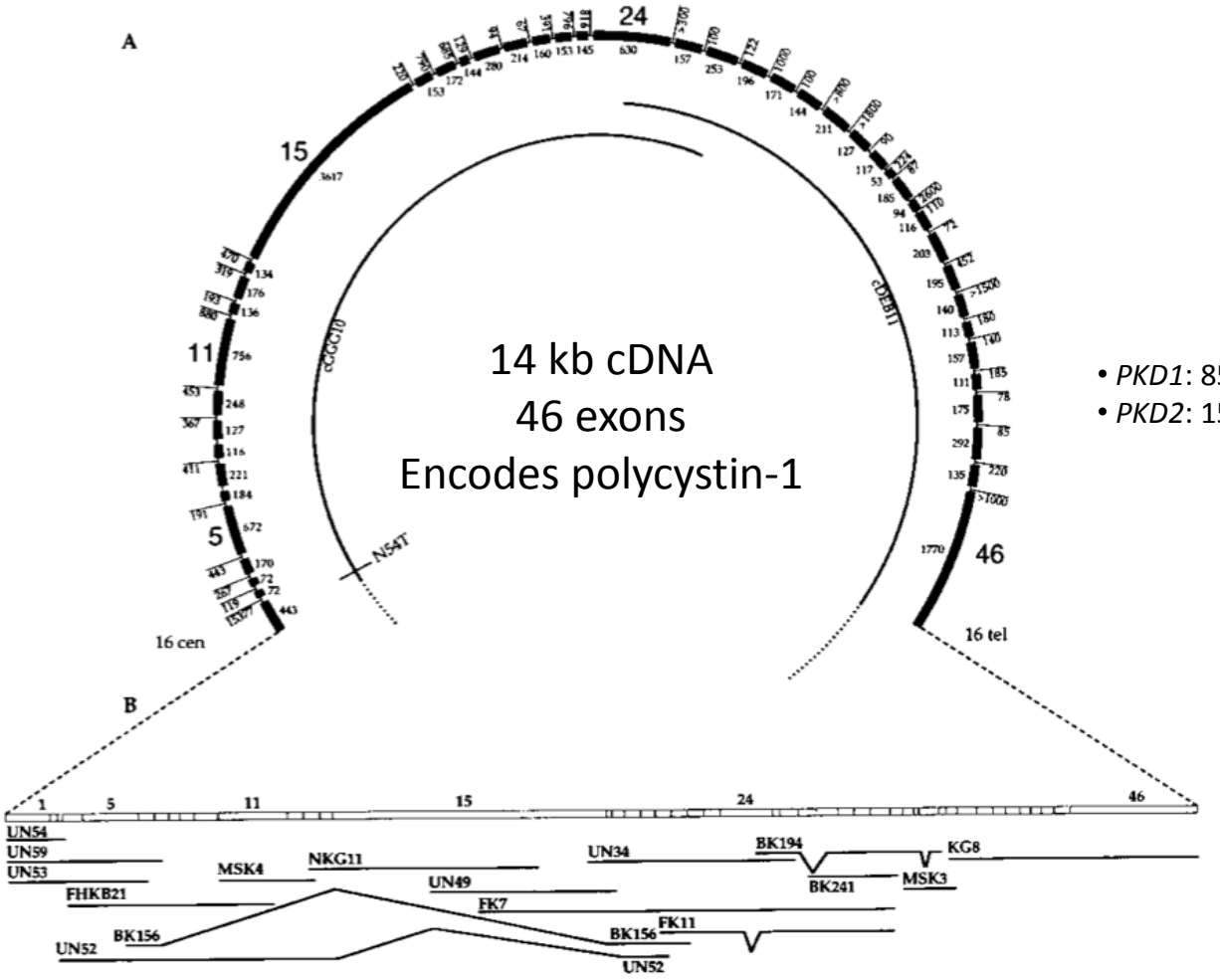
ADPKD Kidney
显性多囊肾

Autosomal Dominant Polycystic Kidney Disease **(ADPKD)**

- Caused by mutations in either *PKD1* or *PKD2* genes, respectively encoding polycystin-1 (PC1) and -2 (PC2)
- Affects 1 in 500-1,000 live births, the most common life-threatening monogenic genetic disease, the leading genetic cause of kidney failure
- Adult-onset
- Progressive development and enlargement of epithelial lined, fluid filled cysts in the kidney, also in the liver
- There is no effective treatment for PKD (2 trials)

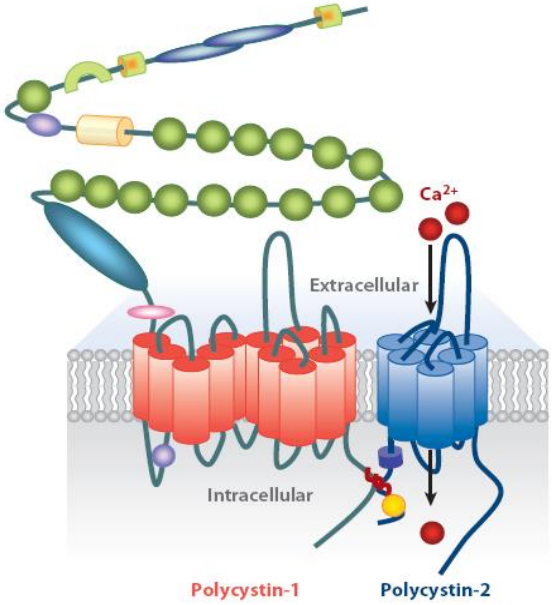
Mutation in the *PKD1* Gene is Most Common



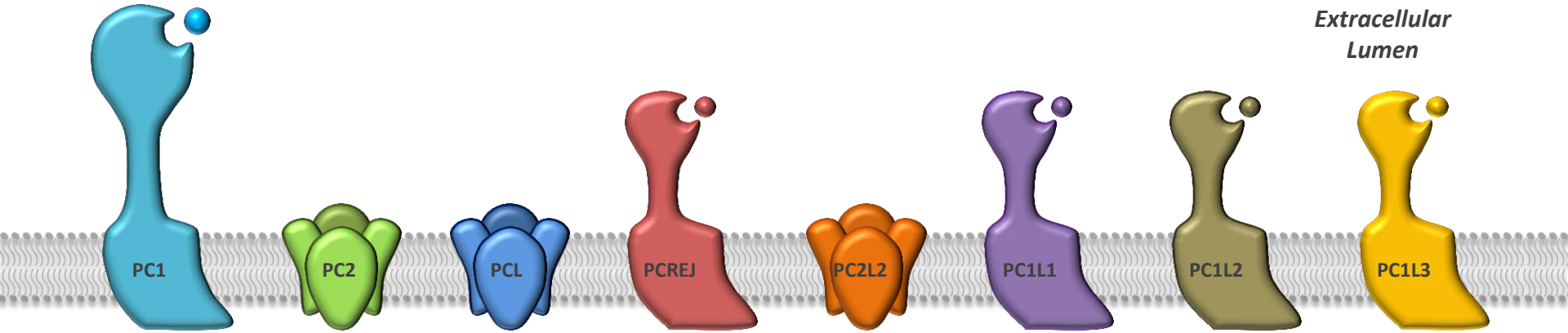


- *PKD1*: 85%, severe, earlier ESRD
- *PKD2*: 15%, mild, later ESRD

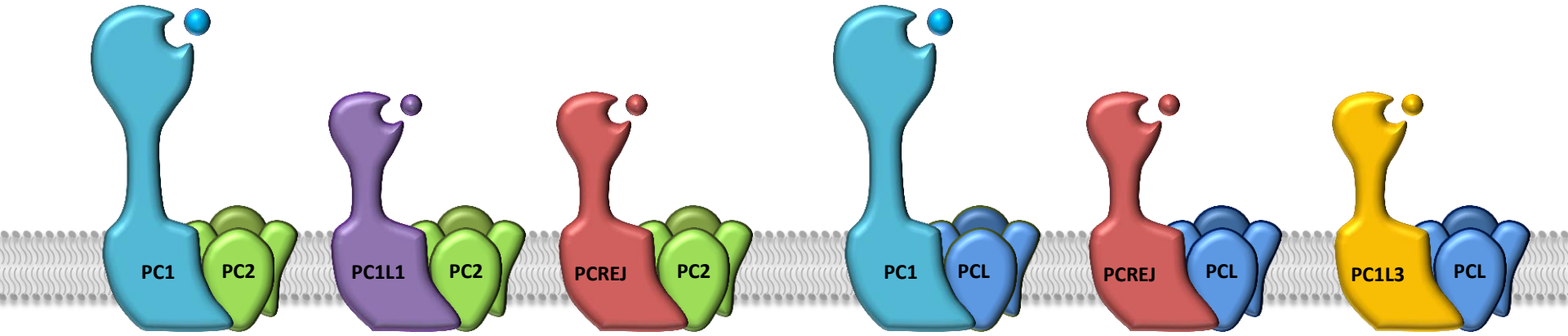
International Consortium, Cell, 1995



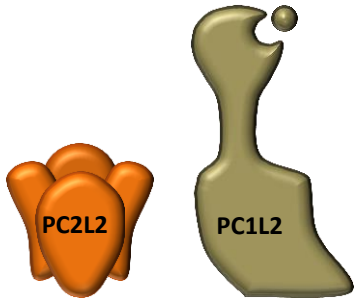
Polycystin Protein Family



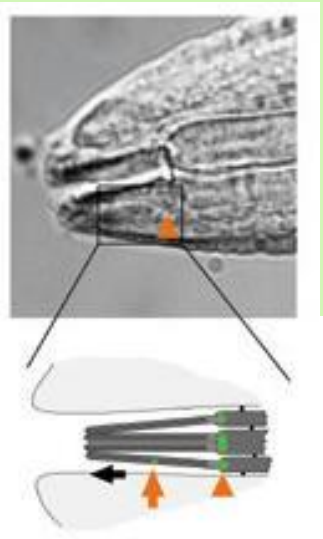
Polycystin Protein Family

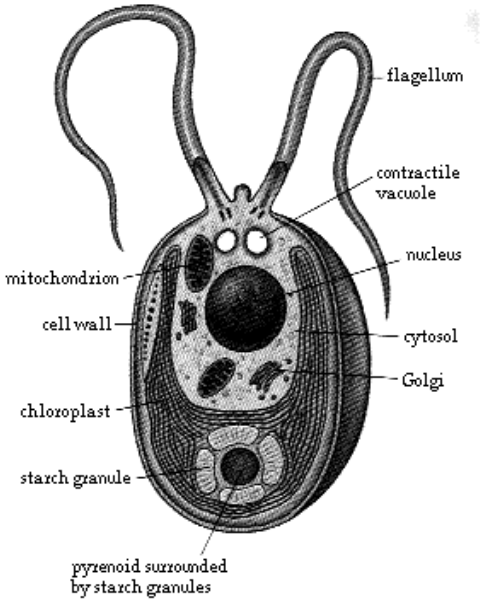


Tubule morphogenesis **L-R asymmetry** **Fertilization** **Fertilization** **Sour sensation?**

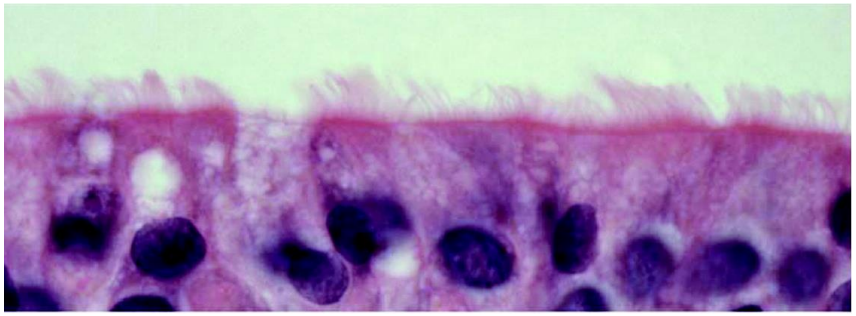


Cilia

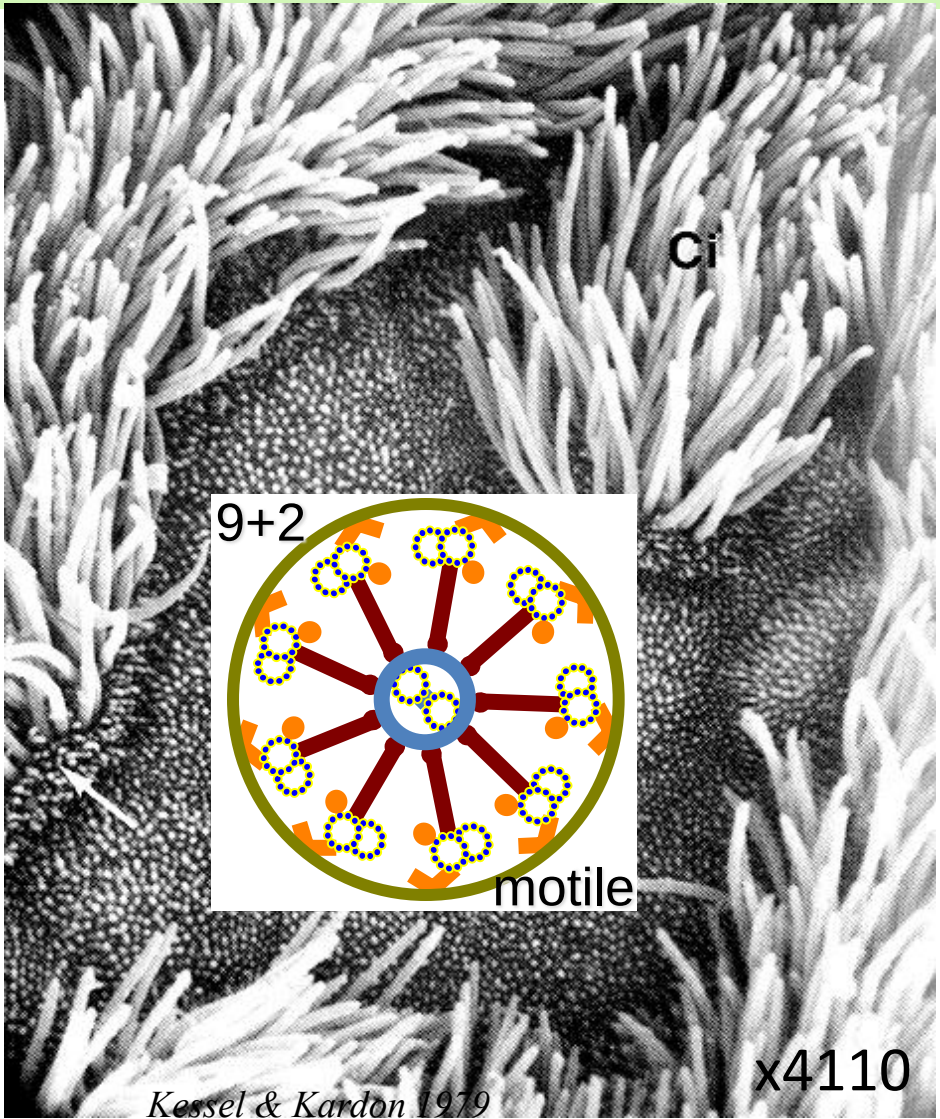



flagellum
contractile vacuole
nucleus
mitochondrion
cell wall
cytosol
Golgi
chloroplast
starch granule
pyrenoid surrounded by starch granules

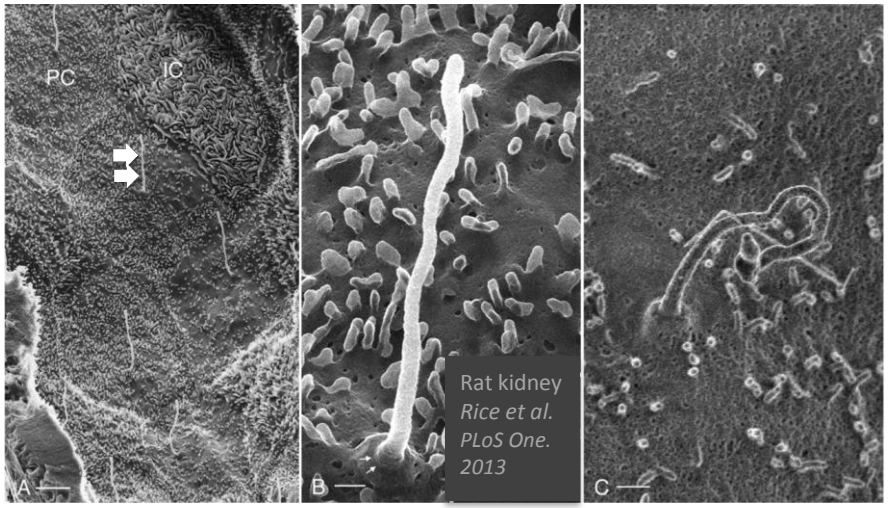
Cilia in the Respiratory System



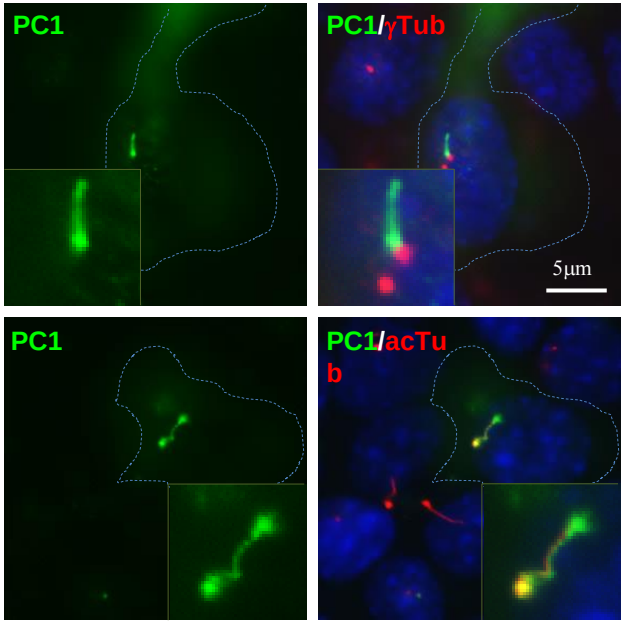
Lung cilia

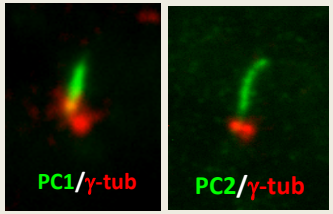


PC1 Goes to the Primary Cilia in Kidney Epithelial Cells

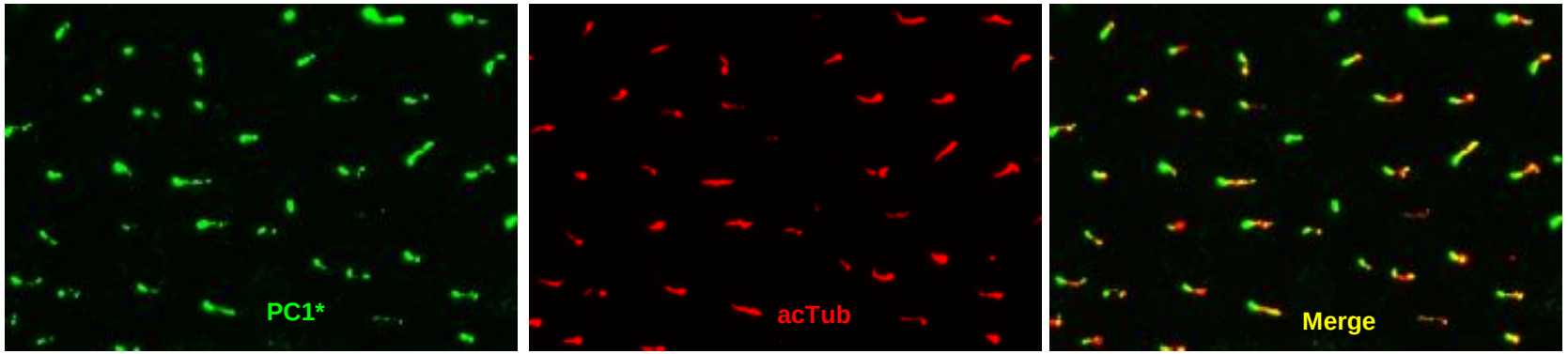


Rat kidney
Rice et al. PLoS One. 2013





Nauli et al. Nature Genetics 2003



IMCD3/YFP-PC1-AviTag (*surface staining)

Su et al. Hum Mol Genet., 2014

~25% human mutations are missense mutations

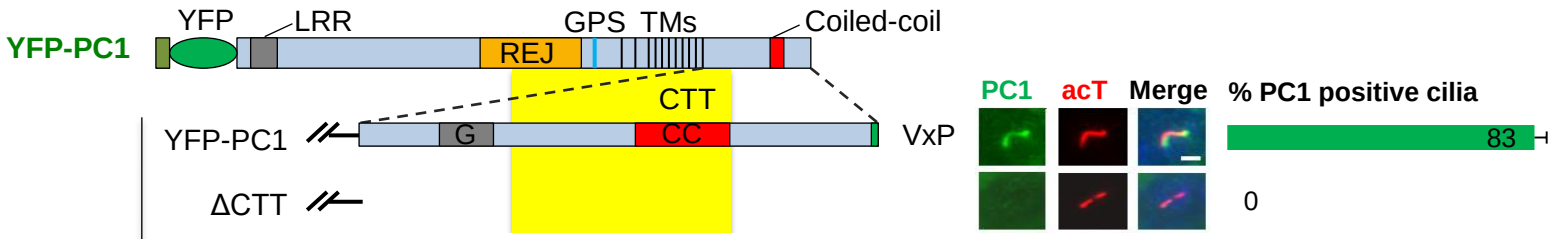
Hot questions:

How does PC1 get to cilia?

Does mutant PC1 traffic to cilia?

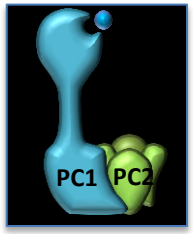
These patients are candidates for personalized therapy
e.g. chaperone therapy

Systemic analysis revealed that multiple sequences in PC1 C-terminal tail (CTT) contribute to ciliary targeting of full-length PC1

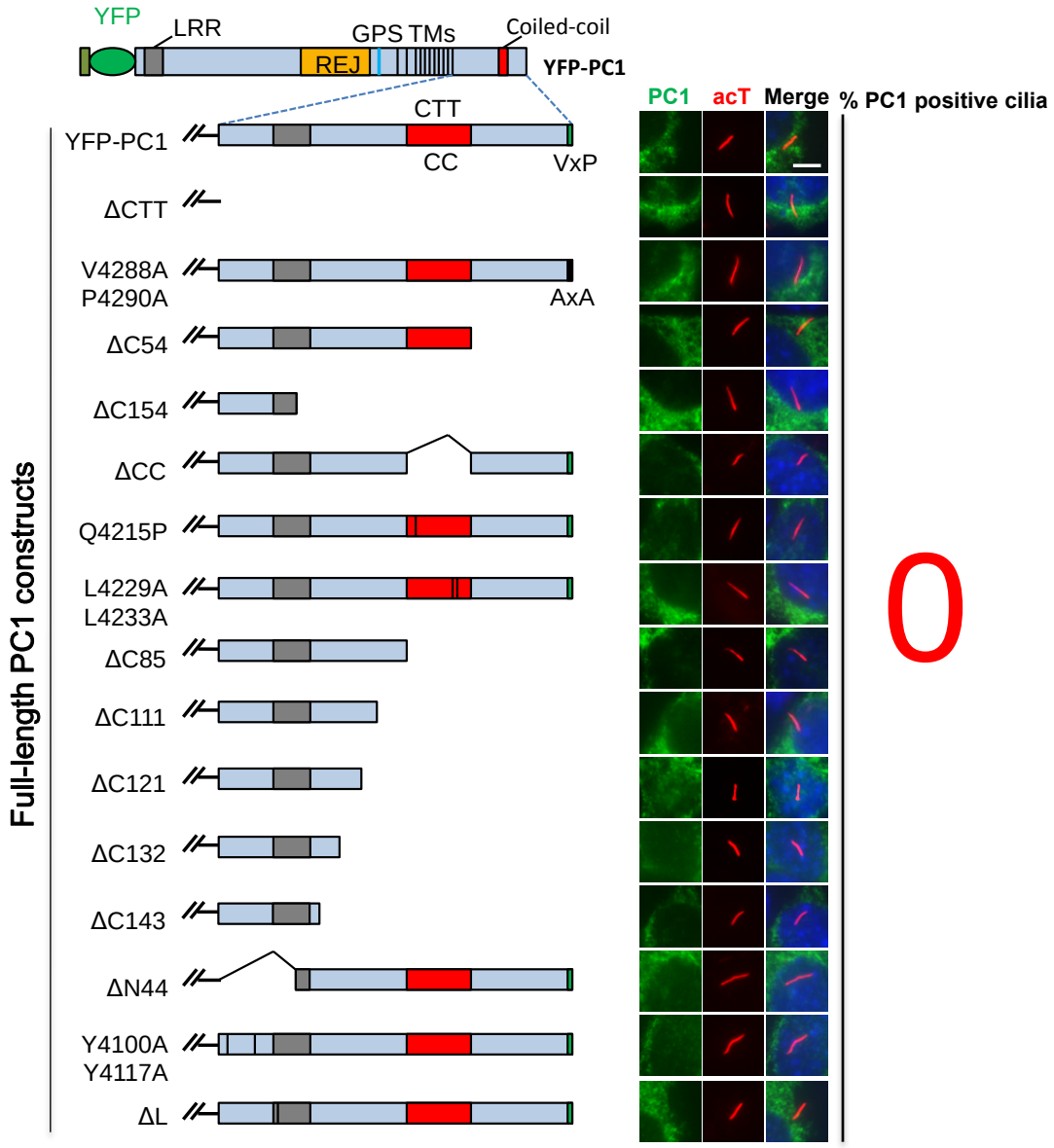


Full-length PC1 (~14 kb cDNA) constructs

Su et al. J Cell Sci., 2015



PC1 needs PC2 to get to cilia



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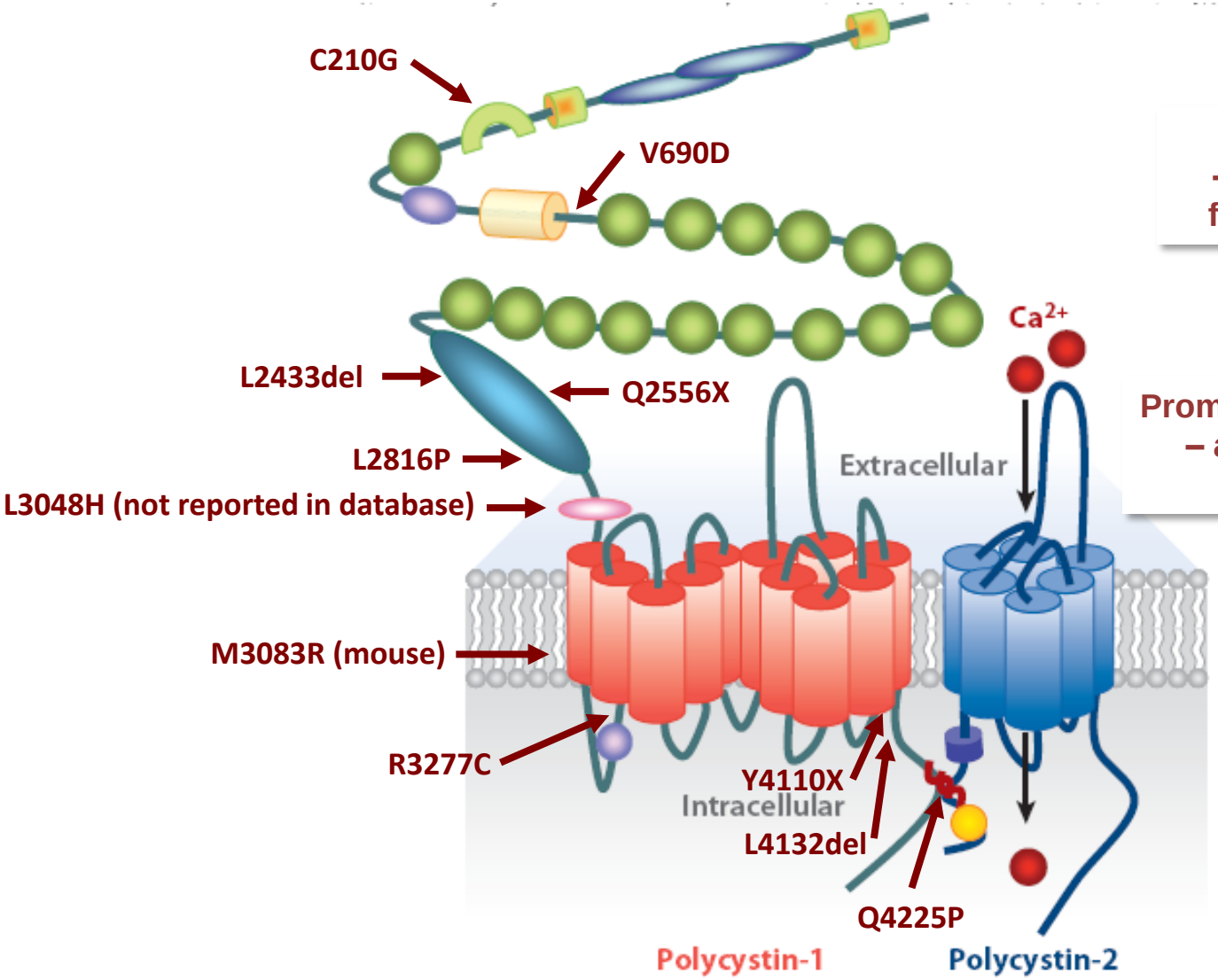
Su et al. J Cell Sci., 2015

Pathogenic mutations affecting PC1 trafficking to cilia



Ciliary trafficking – a potential new assay for PKD potein function

Promoting ciliary trafficking – a potential new therapy for PKD

Su et al. Hum Mol Genet., 2014
Cai et al. JCI, 2014
Su et al. J Cell Sci. 2015

Cystic Kidney Disease and Ciliopathy

Clinical phenotypes associated with ciliopathies

	AD	MKS	BBS	JBTS	JATD	OFD1	MKKS	SLS	NPH	LCA
Retinopathy	-	+	+	+	–	–	–	+	+	+
Polydactyly	-	+	+	+	–	+	+	–	–	–
Kidney disease	+	+	+	+	+	+	–	+	+	–
Situs inversus	?	+	+	+	–	–	–	+	–	–
Mental retardation/developmental delay	-	+	+	+	–	+	–	–	+	+
Hypoplasia of cerebellum	-	+	+	+	–	+	–	–	+	–
Hydrometrocolpos	-	–	+	–	–	–	+	–	–	–
Obesity	-	–	+	+	–	–	–	–	–	–
Hepatic dysfunction	+	+	+	+	–	–	–	+	+	–

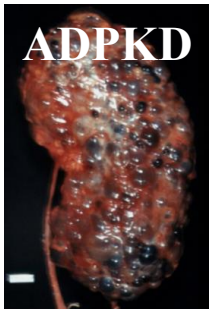
JATD, Jeune syndrome; OFD1, orofaciodigital syndrome 1; MKKS, McKusick-Kaufman syndrome; SLS, Senior-Loken syndrome.

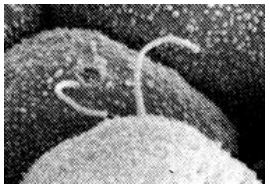
MKS, Meckel–Gruber syndrome; LCA, Leber congenital amaurosis; MKKS, McKusick-Kaufman syndrome

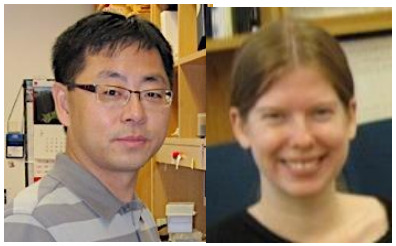
Zaghloul and Katsanis. J Clin Invest (2009) vol. 119 (3) pp. 428-37

All these genes are localized to cilia, basal bodies or centrosomes
Mutations in the same gene may cause different diseases
The same disease may be caused by mutations in different genes

ADPKD Proteins interact with other Ciliopathy Proteins

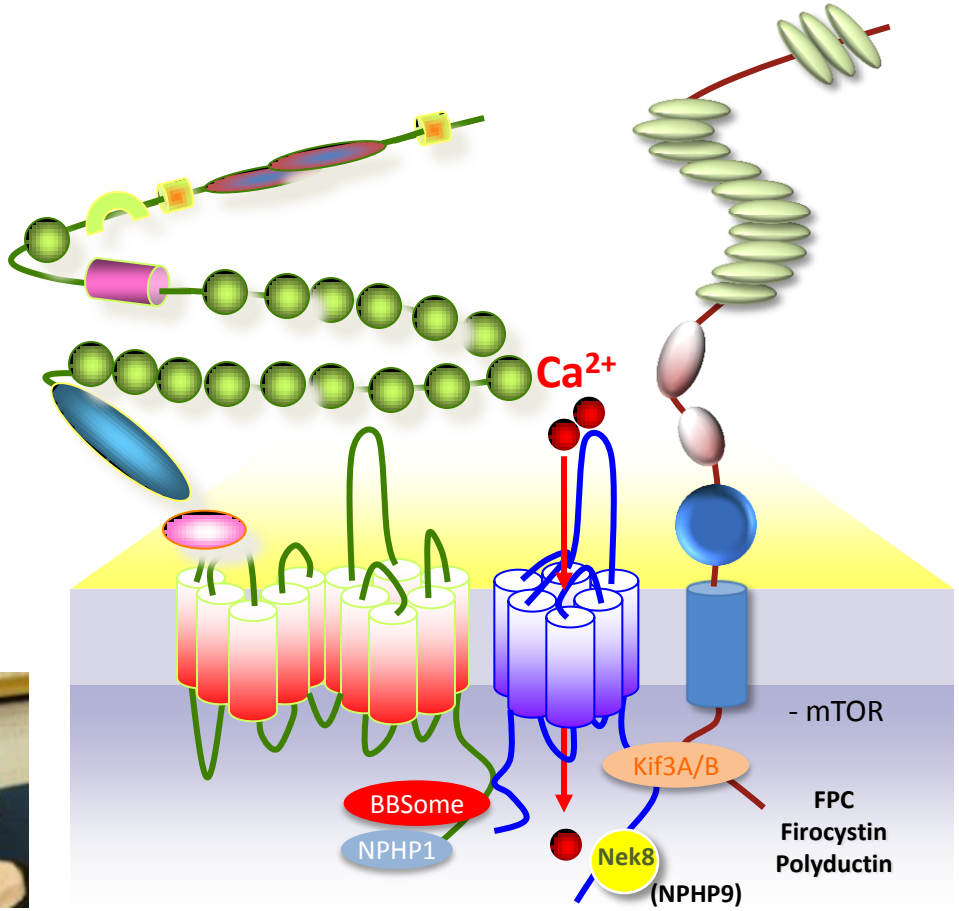






Xuefeng Su Kaitlin Driscoll

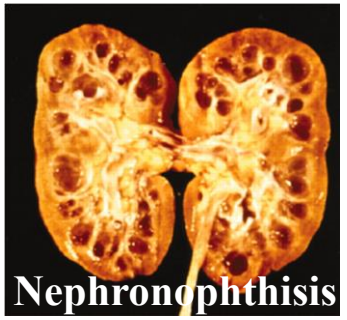
Su et al., HMG, 2014

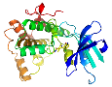


Wang et al. JASN 2004

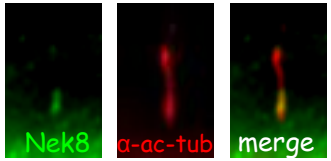
Wang et al. Mol Cell Biol 2007

Wang et al. PLoS One 2014





NPHP9



Sohara et al. JASN 2008

The Bardet Biedl Syndrome

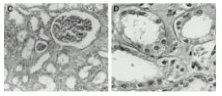
Nineteen genes identified:

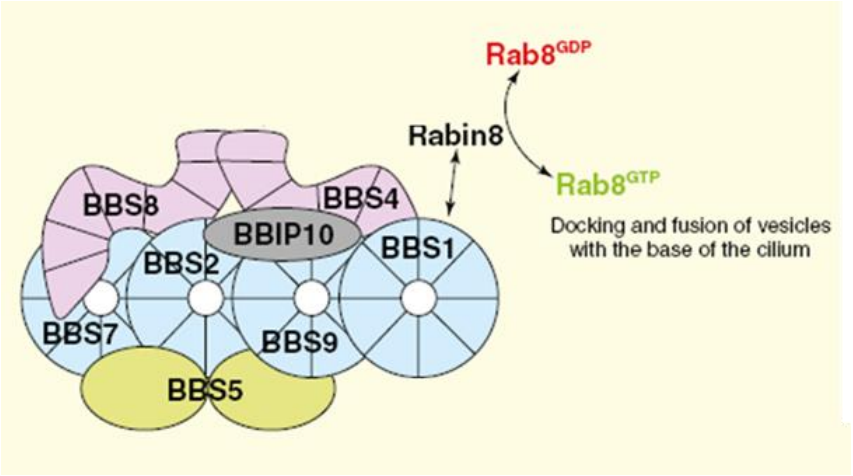
- *BBS1*
- *BBS2*
- *ARL6/BBS3*
- *BBS4*
- *BBS5*
- *MKKS/BBS6*
- *BBS7*
- *TTC8/BBS8*
- *B1/BBS9*
- *BBS10*
- *TRIM32/BBS11*
- *BBS12*
- *MKS1/BBS13*
- *CEP290/BBS14*
- *WDPCP/BBS15*
- *SDCCAG8/NPHP10/BBS16*
- *LZTFL1/BBS17*
- *BBSIP1/BBSIP10/BBS18*
- *IFT27/BBS19*

Six Primary Features:

- Postaxial polydactyly
- Rod-cone dystrophy
- Truncal obesity
- Learning disabilities
- Hypogonadism/genital abnormalities
- Renal anomalies

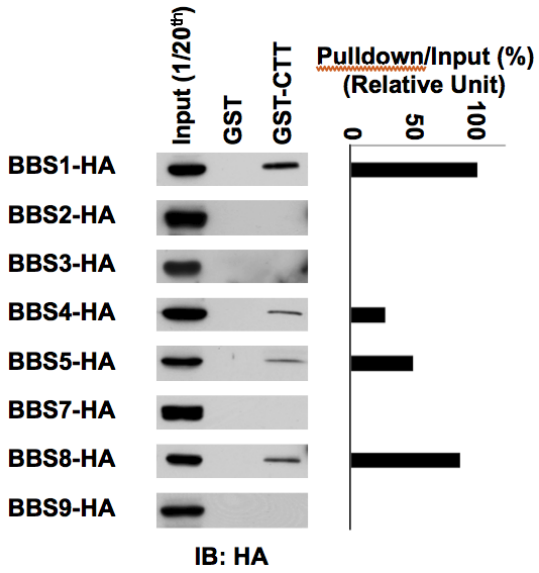
Fig. 1 : Loss of secondary sexual characters

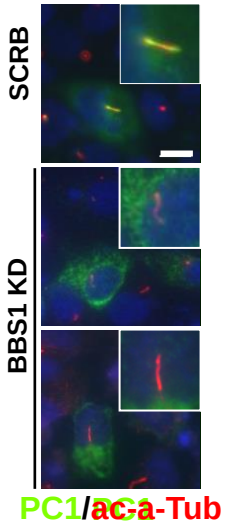


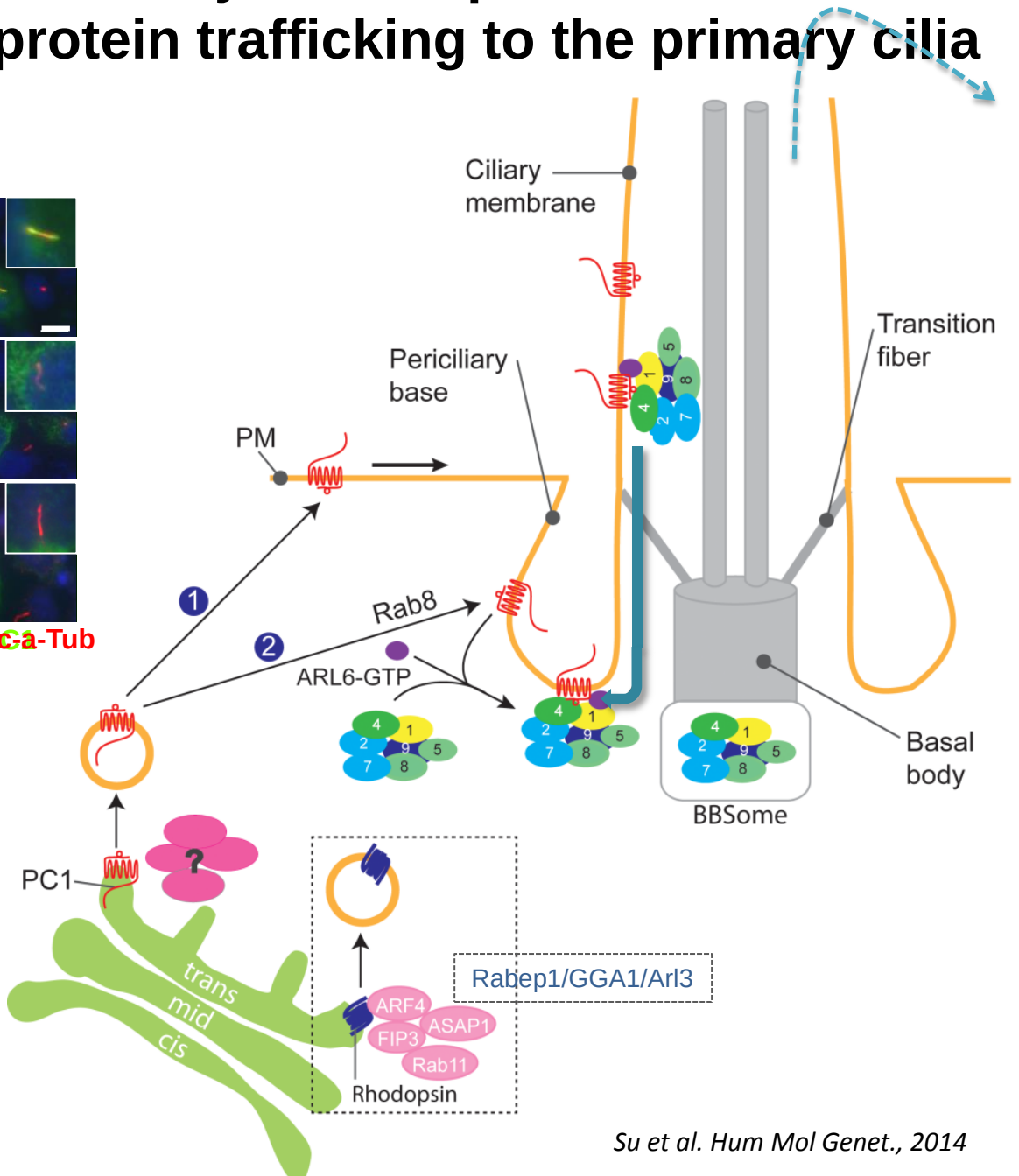


Nachury, Current Biology19, 2009, R472-R473

Bardet Biedl syndrome proteins modulate ADPKD protein trafficking to the primary cilia

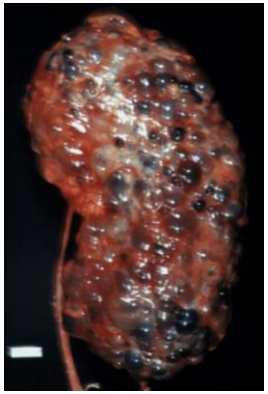




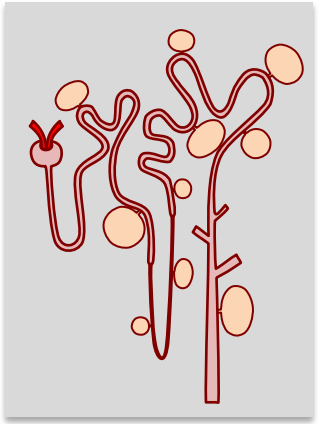


Su et al. Hum Mol Genet., 2014

Cystogenesis Mechanisms in ADPKD



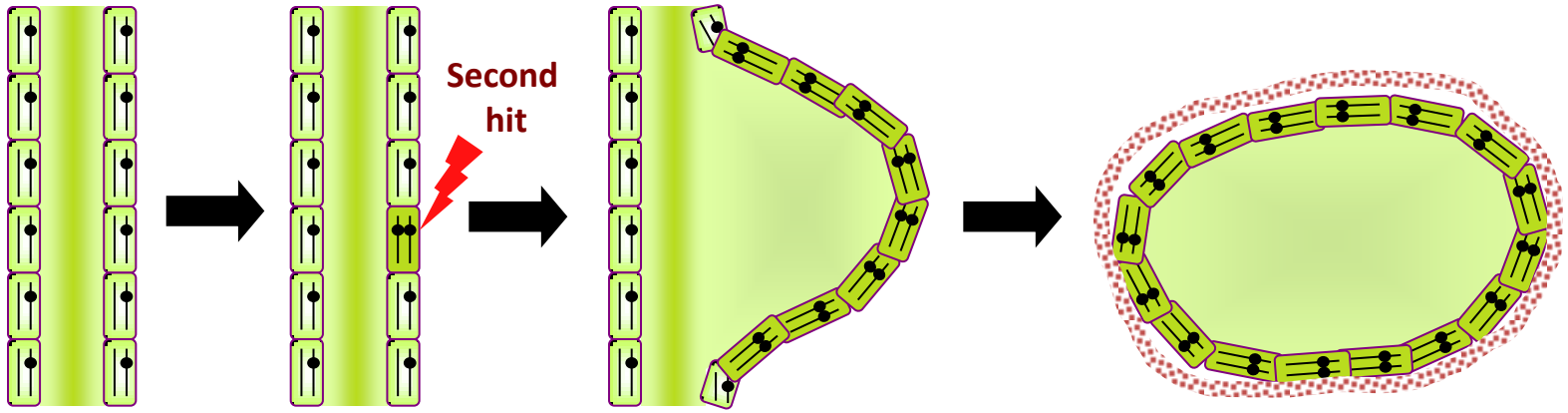
ADPKD
- **< 5% of nephrons**



ADPKD
- **Focal cysts in a nephron**

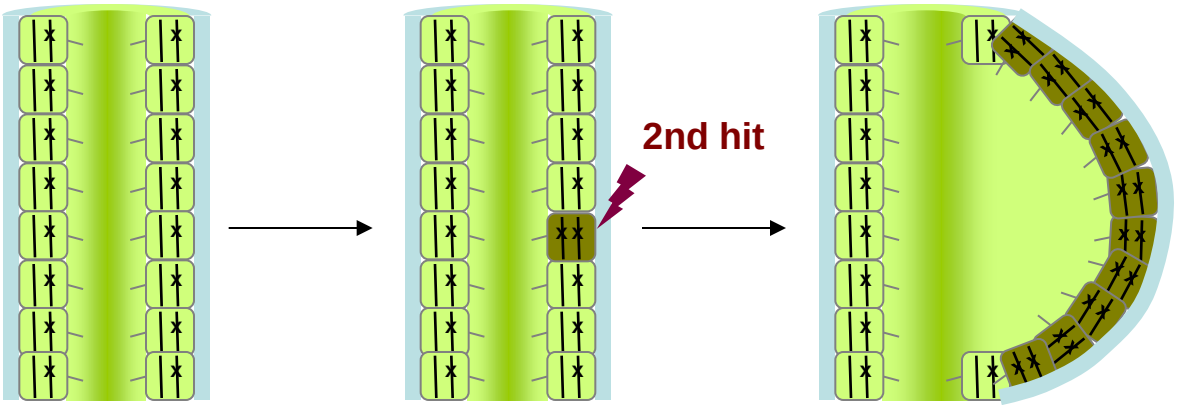
"Two-Hit" Hypothesis

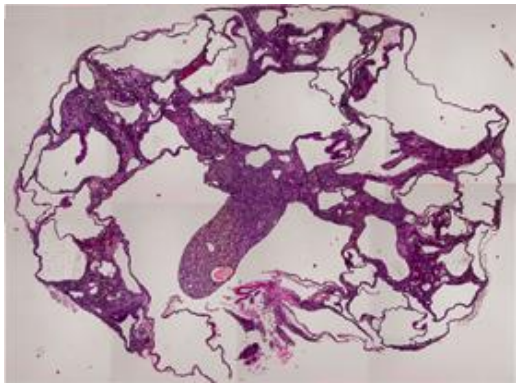
Reeders, Nature Genetics, 1992



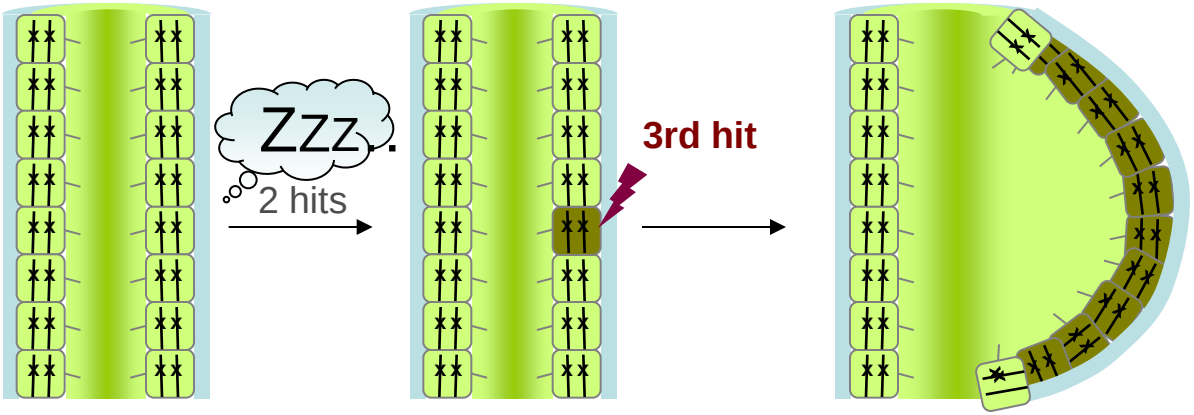
“Two-Hit” v.s. “Three-Hit” Model of Cytogenesis

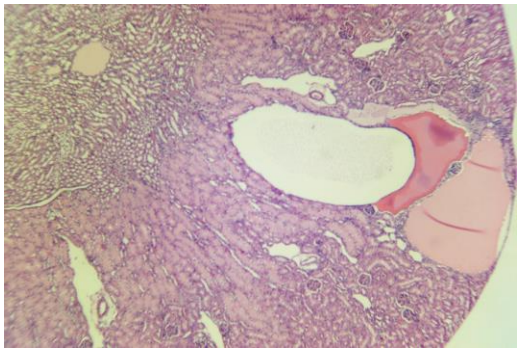
“two hits” **In developing kidney**

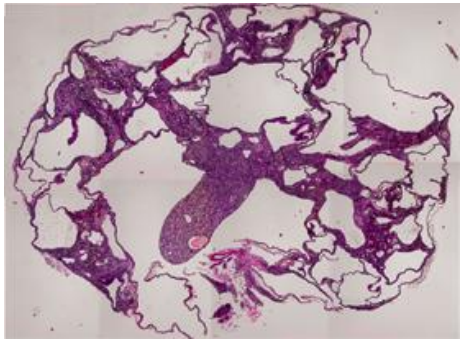




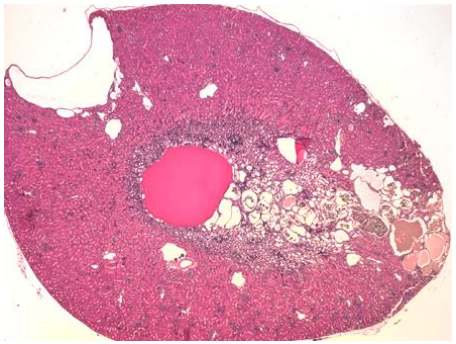
“three hits” **In mature kidney**







***Pkd1* neonatal CT inactivation**



***Pkd1* adult CT inactivation**

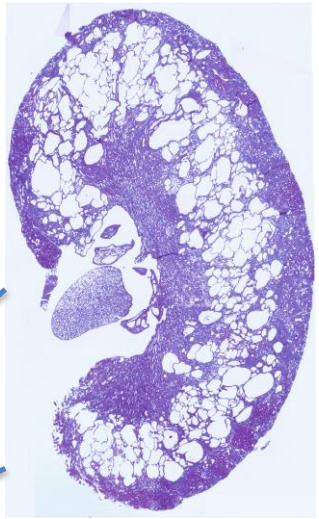
Takakura et al. JASN 2008

Takakura et al. Hum Mol Genetics 2009

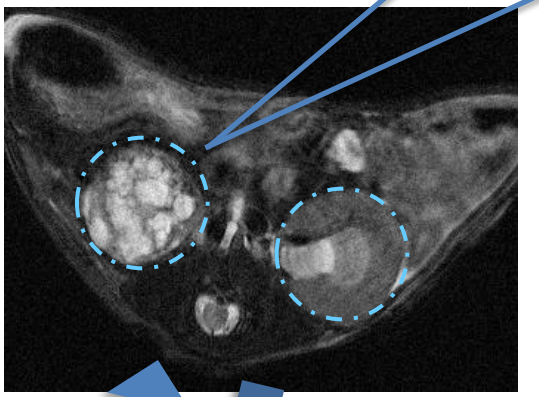
Ayumi Takakura

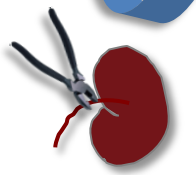
"A genetic or non-genetic event that reactivates renal developmental programs or triggers cellular repair/cell proliferation (activation of an oncogene, inactivation of a tumor suppressor gene, exposure to cellular stress, toxin, or injury) is required for rapid cyst formation."

"Third Hit" Hypothesis

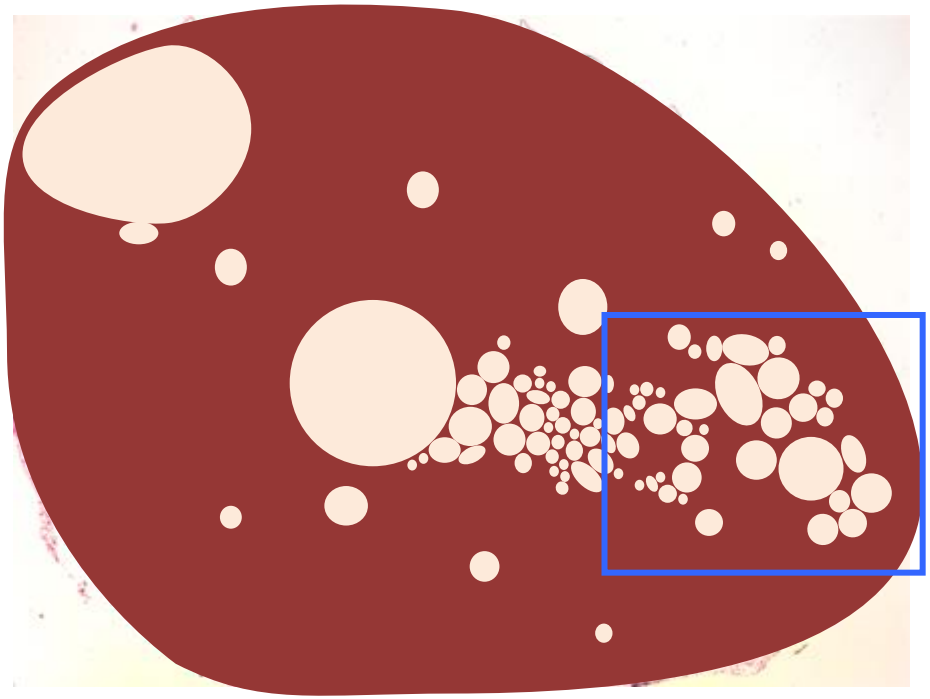


Renal injury is a 3rd hit

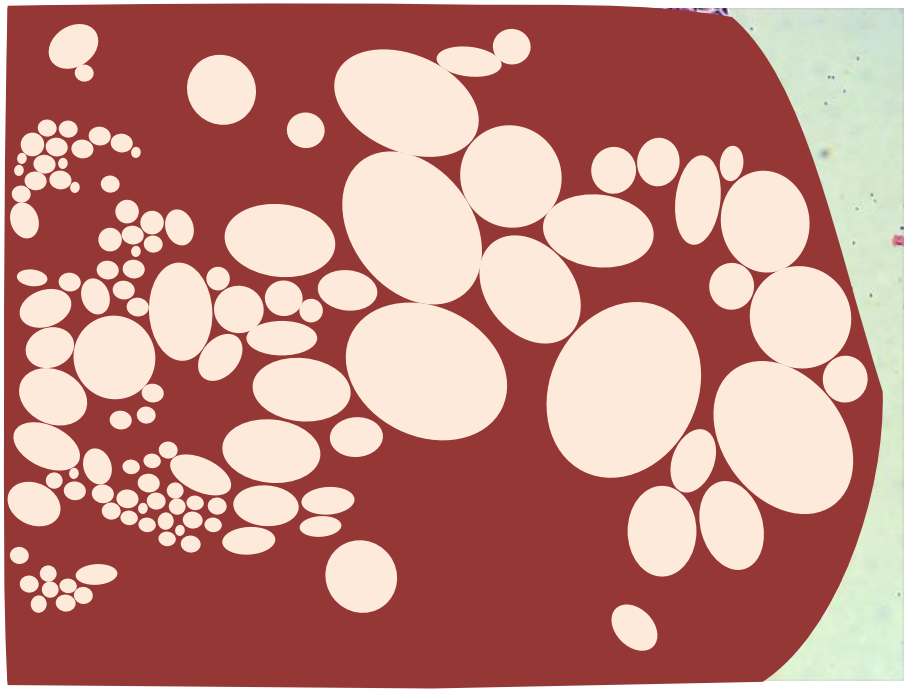


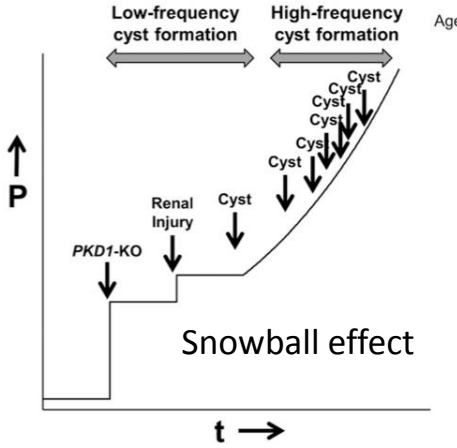


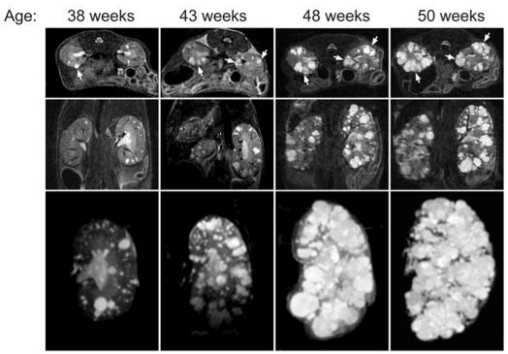
Pkd1 Knockout in Mature Kidney Causes Focal Cyst Formation



Takakura et al. JASN 2008

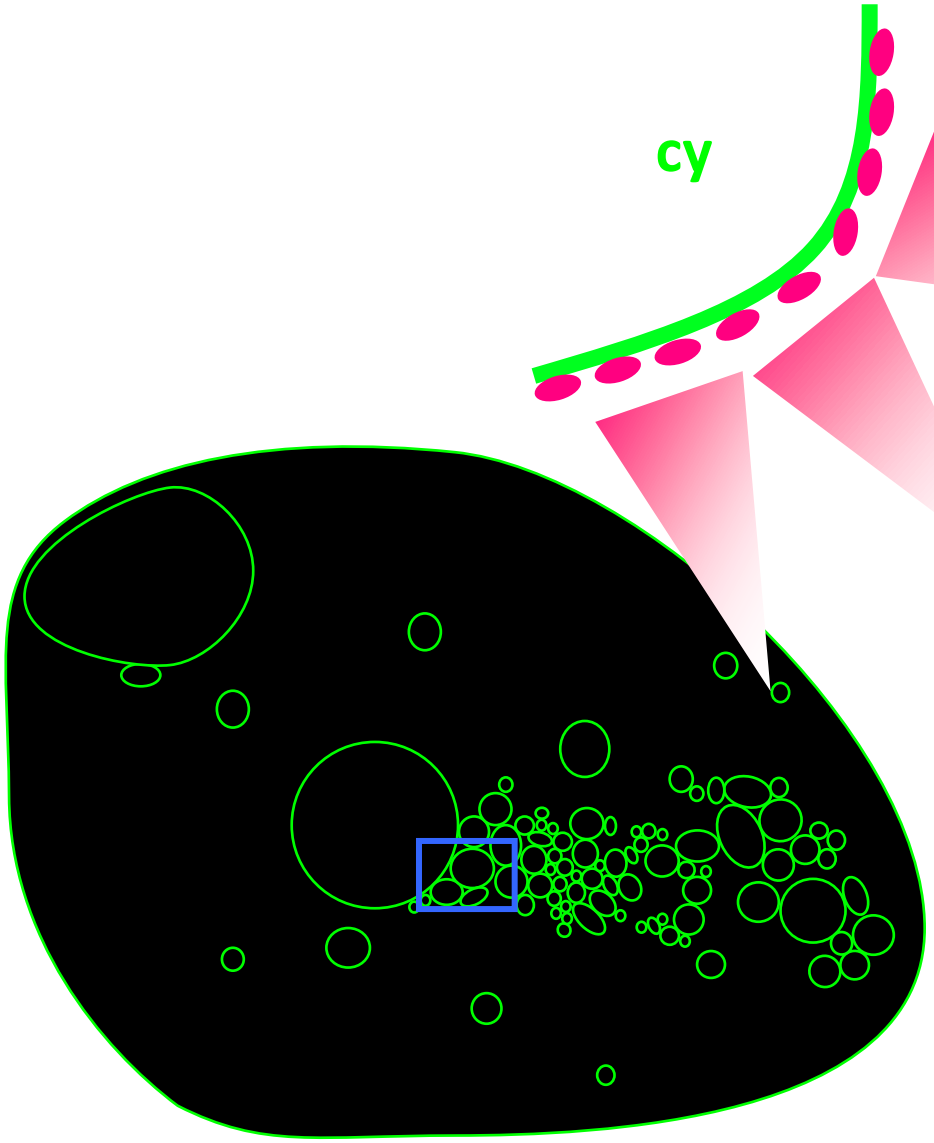


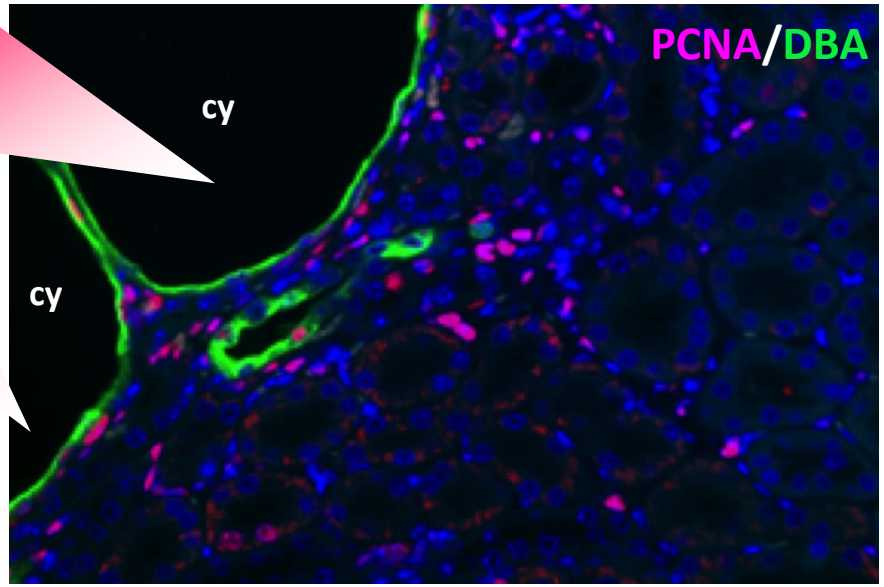




Leonhard et al. JASN 2015

Increased cell proliferation next to cysts



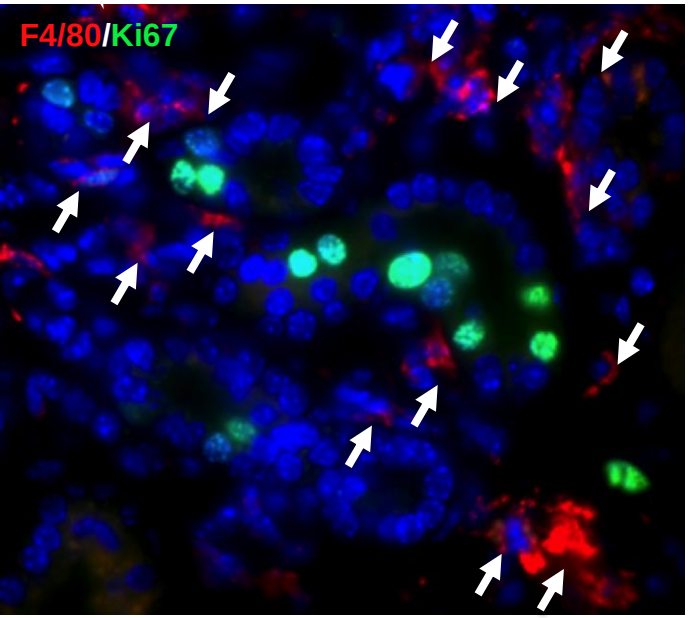


PCNA - cell proliferation marker
DBA – collecting tubule marker

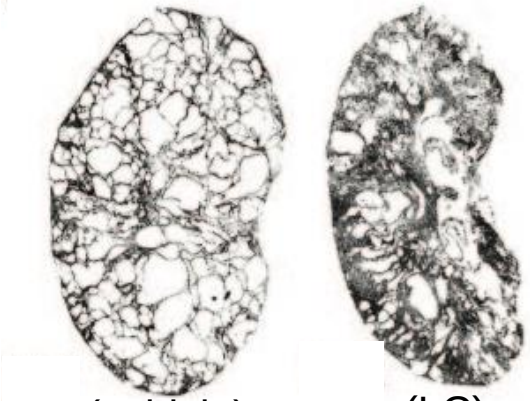
- chemokine gradient, paracrine cell-cell signaling?
- Mechanical stress/pressure-dependent proliferation?

Takakura et al. JASN, 2008

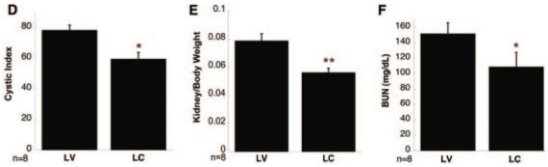
Cell Proliferation, Macrophages, and PKD



Takakura et al. JASN, 2008

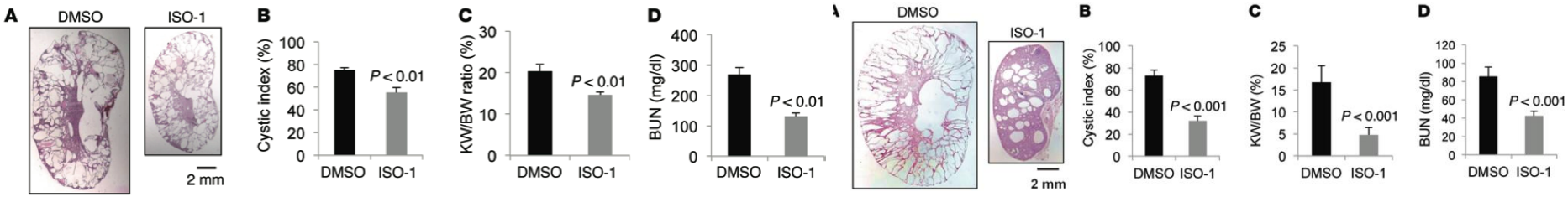


(vehicle) (LC)



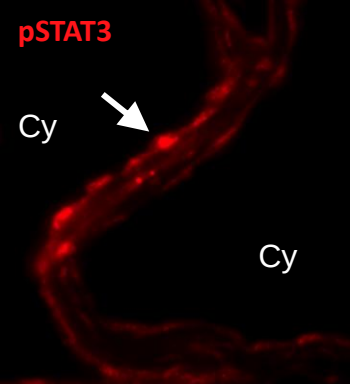
Depletion of macrophages by liposomal clodronate (LC) inhibits cyst formation in *Pkd1*- and *Pkd2*-disease

Karihaloo et al. JASN 2011



Isoxazoline (ISO-1) inhibits microphage migration inhibitory factor (MIF) and reduces cyst formation in *Pkd1*-disease

Chen et al. JCI 2015

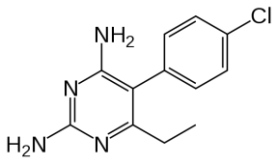


Discovery of Anti-Parasitic Compound Pyrimethamine Inhibits STAT3 and Slows PKD Progression

Chemical Screen

↓

Pyrimethamine
(乙胺嘧啶)

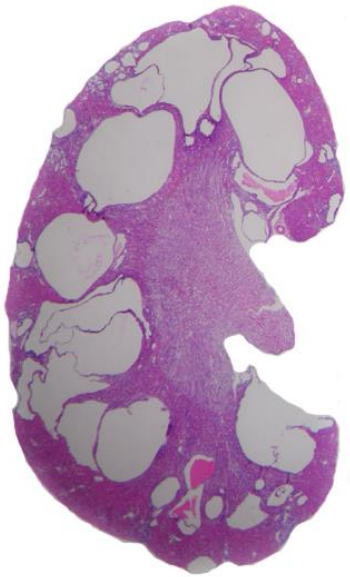


Used for
Malaria and HIV

⊥

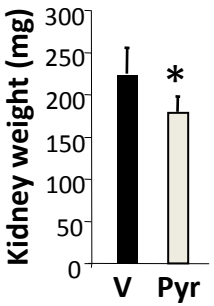
STAT3

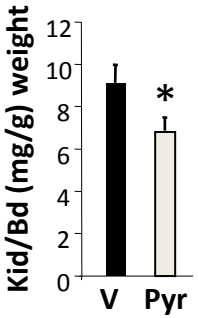
Takakura et al, HMG 2011

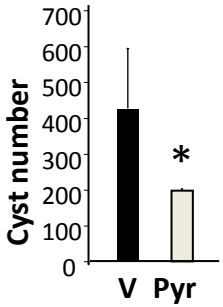


Vehicle

Pyrimethamine

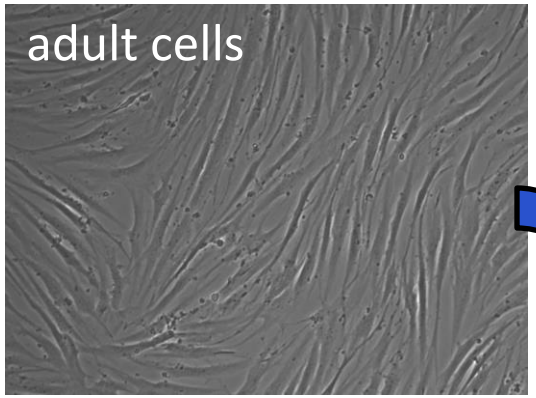






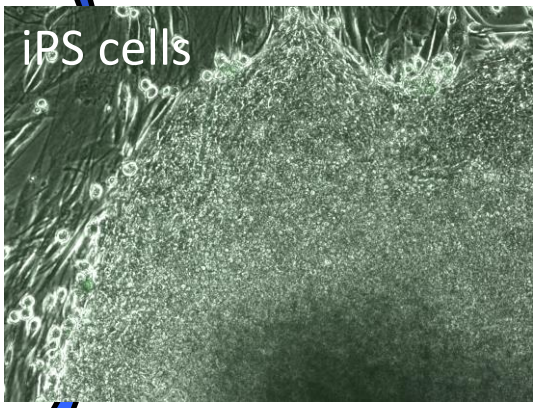
V: Vehicle-treated IKO kidney, **Pyr**: Pyrimethamine-treated IKO kidney

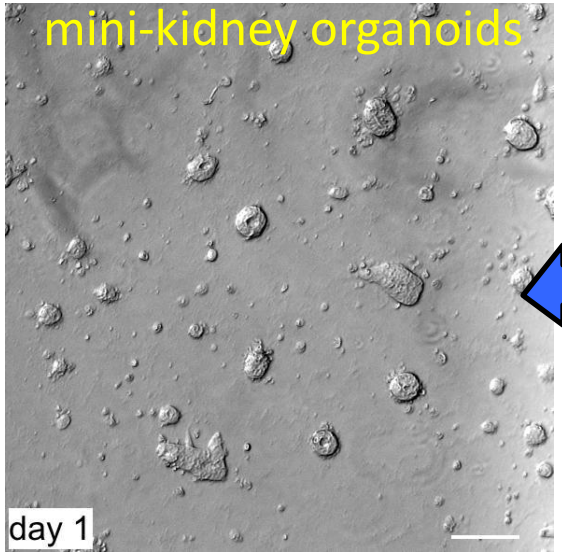
New kidney tissue from patient stem cells



Stem cell genes and growth signals

(e.g. urine sample)





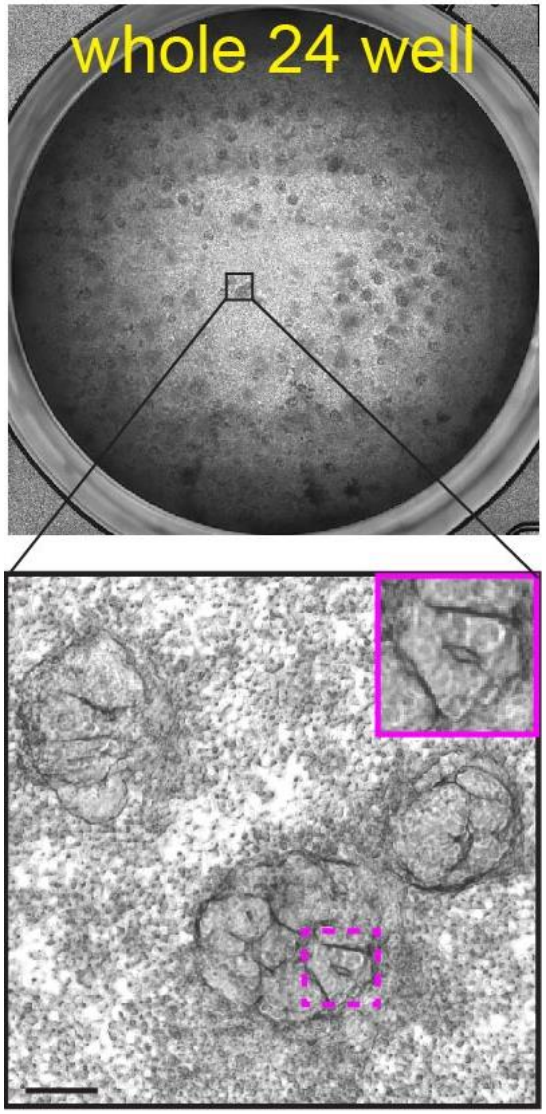
Time frame ~ 1 year

gene-corrected transplant (immunocompatible)

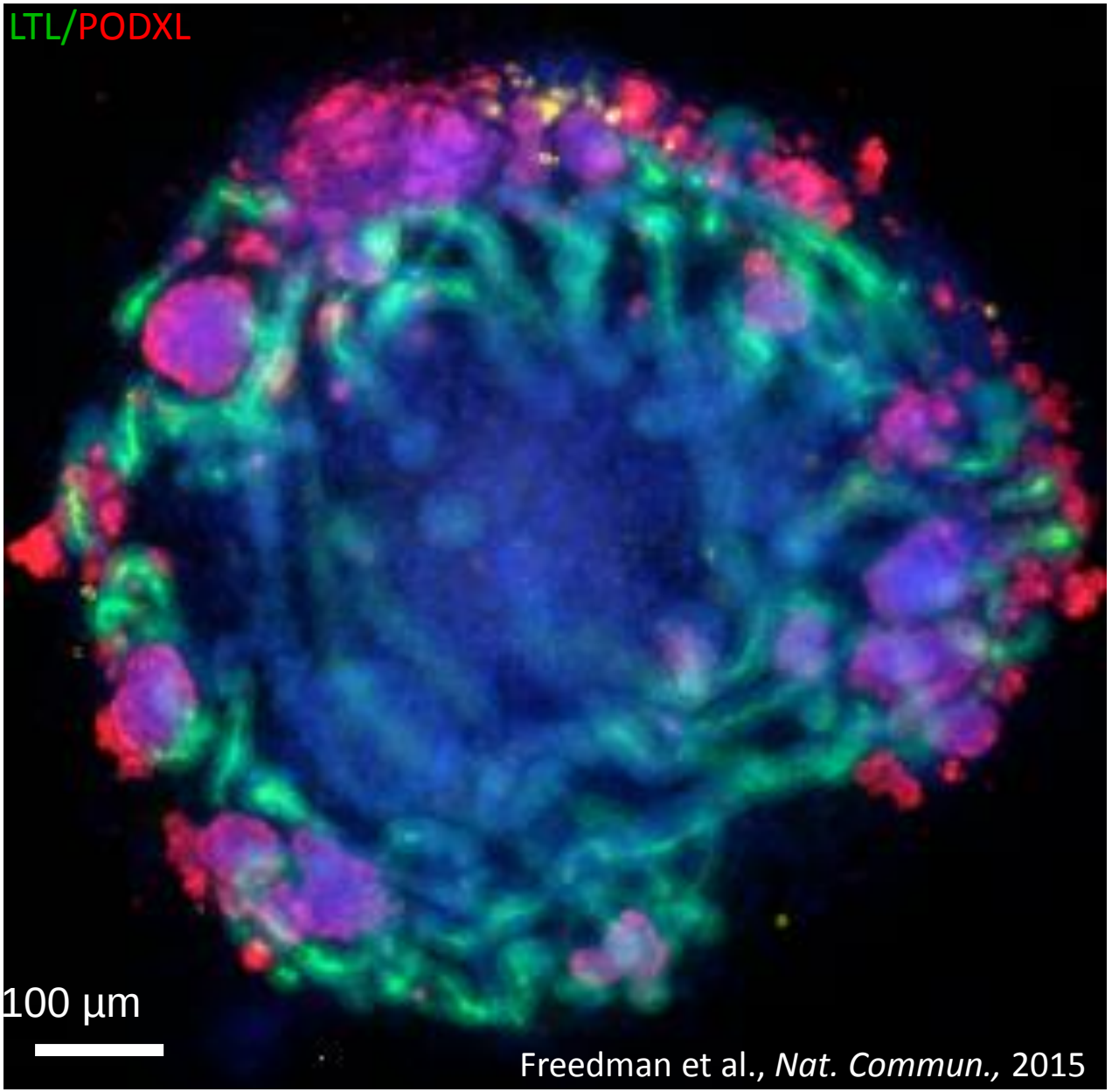
disease modeling
drug discovery

Beno Freedman

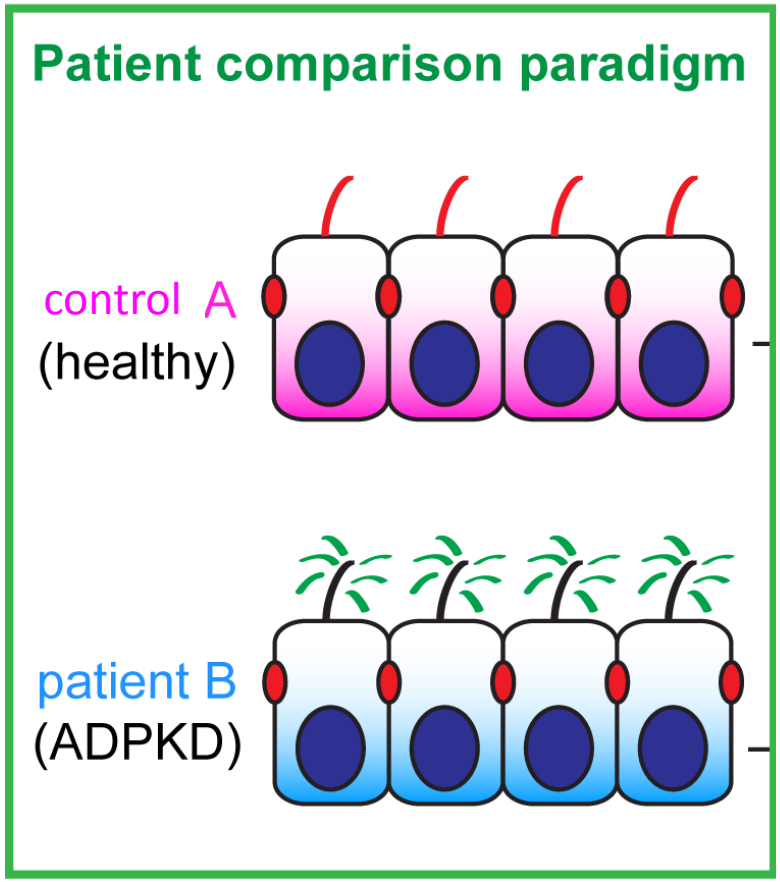
Each well contains numerous kidney organoids



~90 organoids/well



CRISPR mutants for PKD disease genes



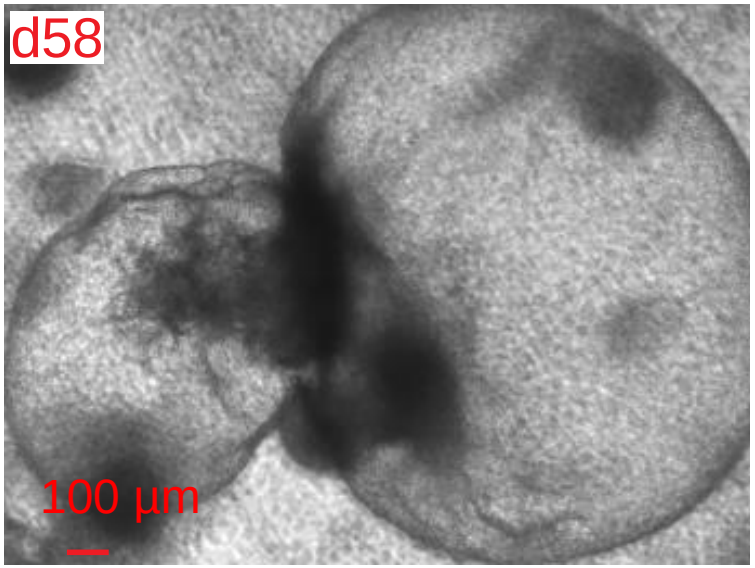
cilium with PC2

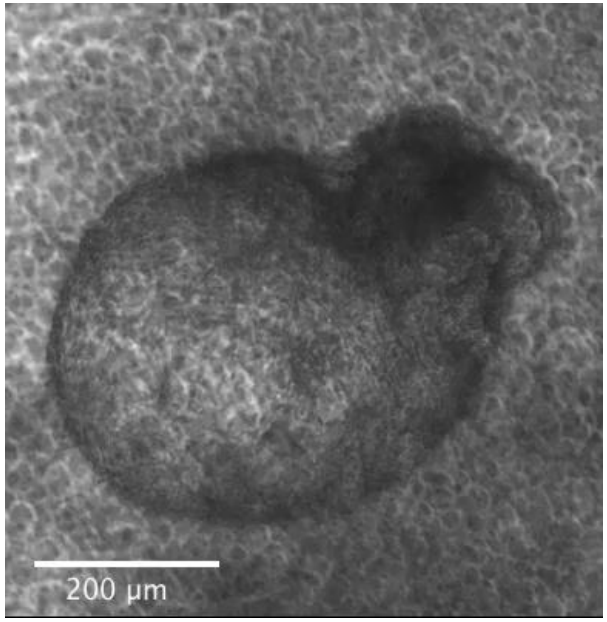
epithelial cell-cell junction

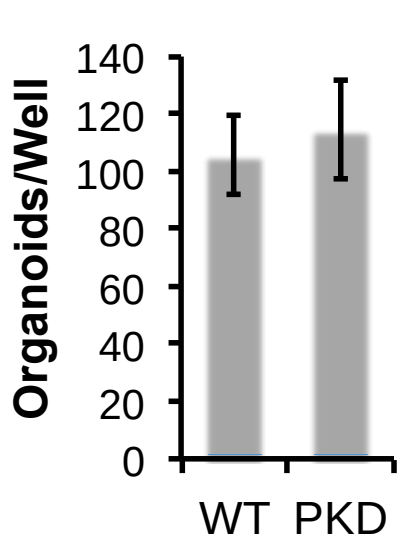
cilium without PC2 (aberrant signaling)

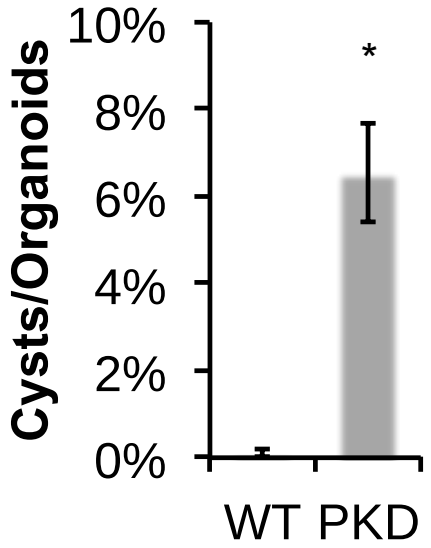
Freedman, *Biomark. Insights,* in press

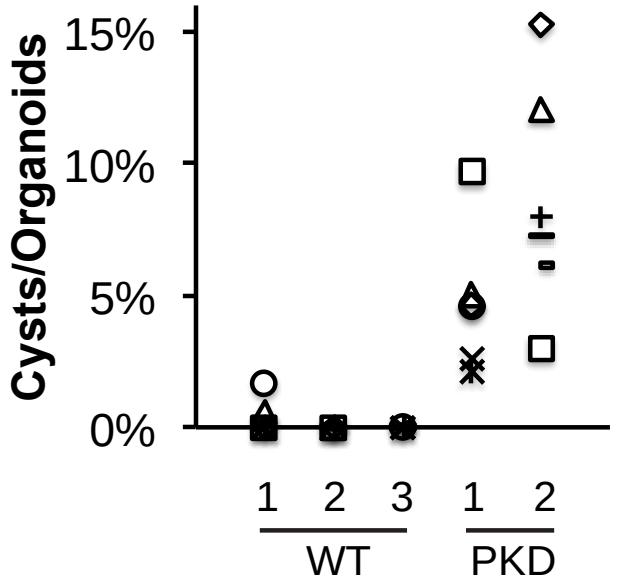
PKD$^{-/-}$ organoids form cysts from tubular cells



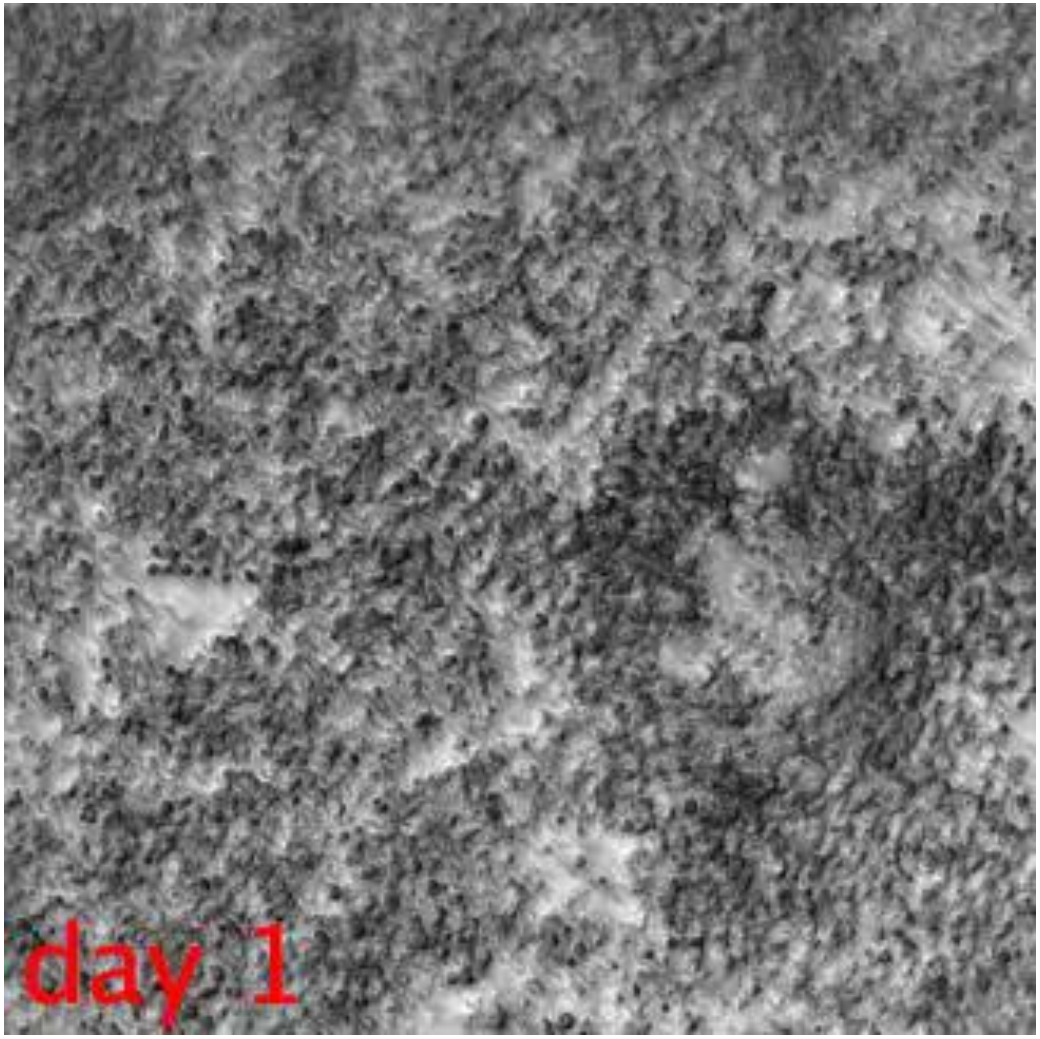






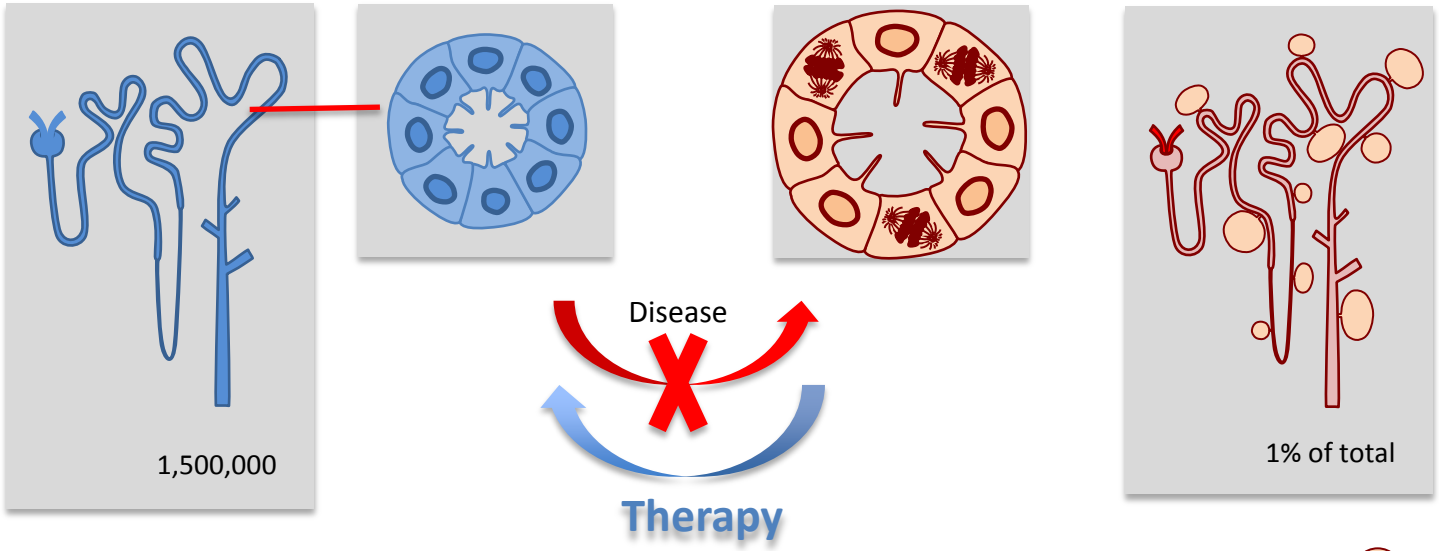


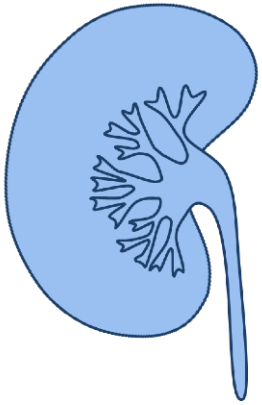
Live imaging of PKD cyst formation from tubules



Freedman et al., *Nat. Commun.,* 2015

Summary





Normal Human Kidney

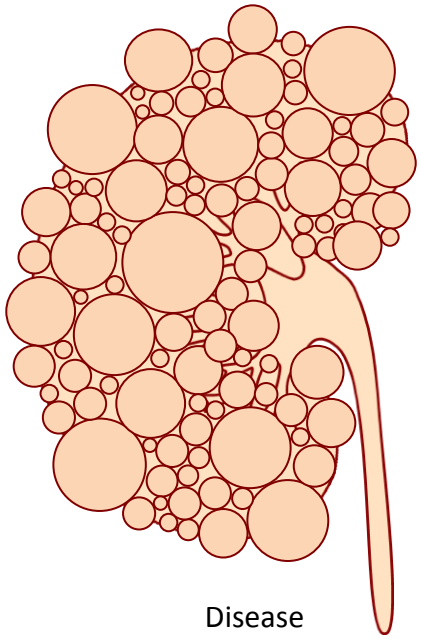
150 g
180 L blood
1.5-2 L urine

Modulating the 3rd hit
e.g. anti-inflammation therapy

Modulating protein trafficking
Chaperone therapy

Gene editing
Stem cell therapy

…



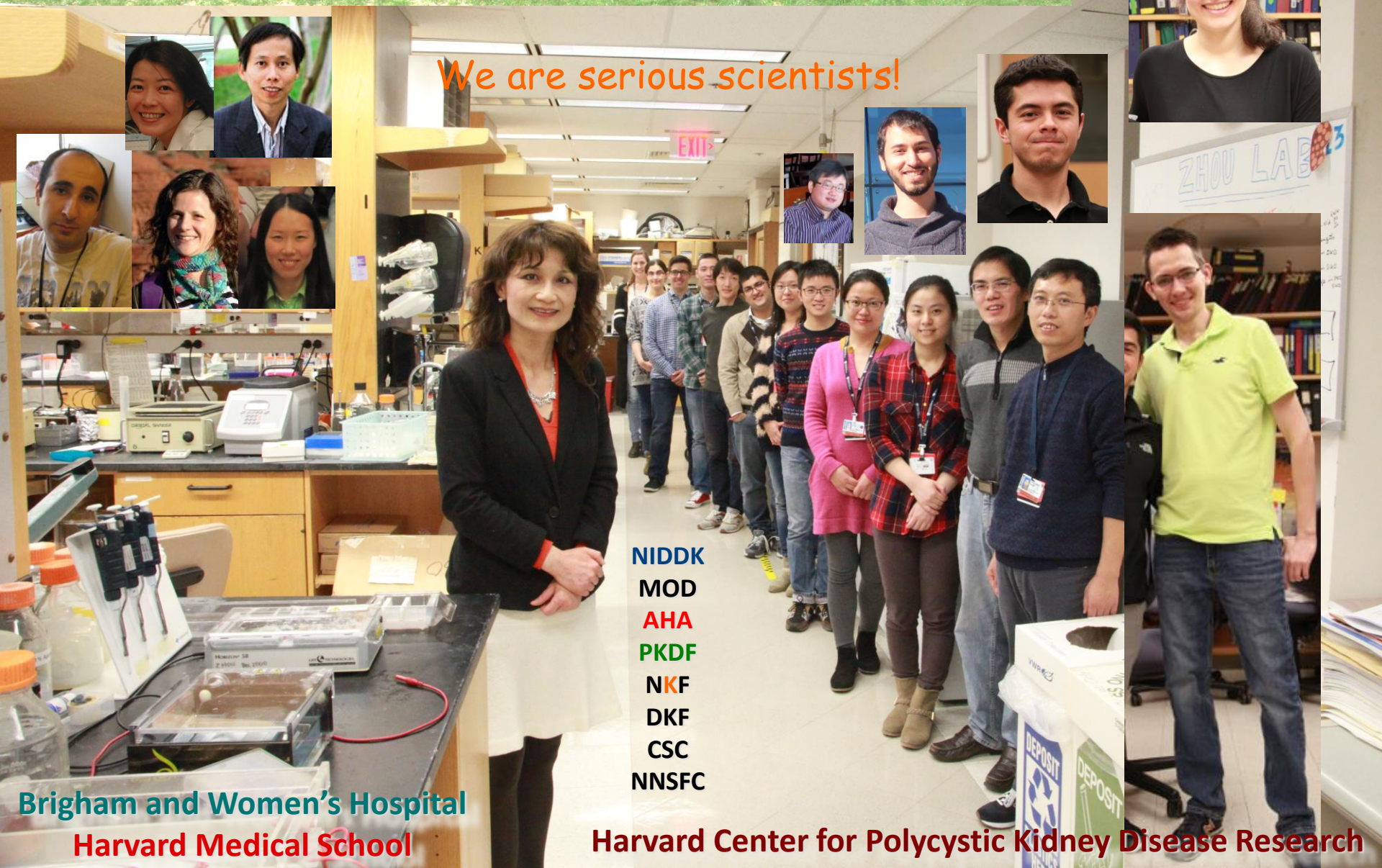
Disease
7,500 g

Harvard Center for Polycystic Kidney Disease Research

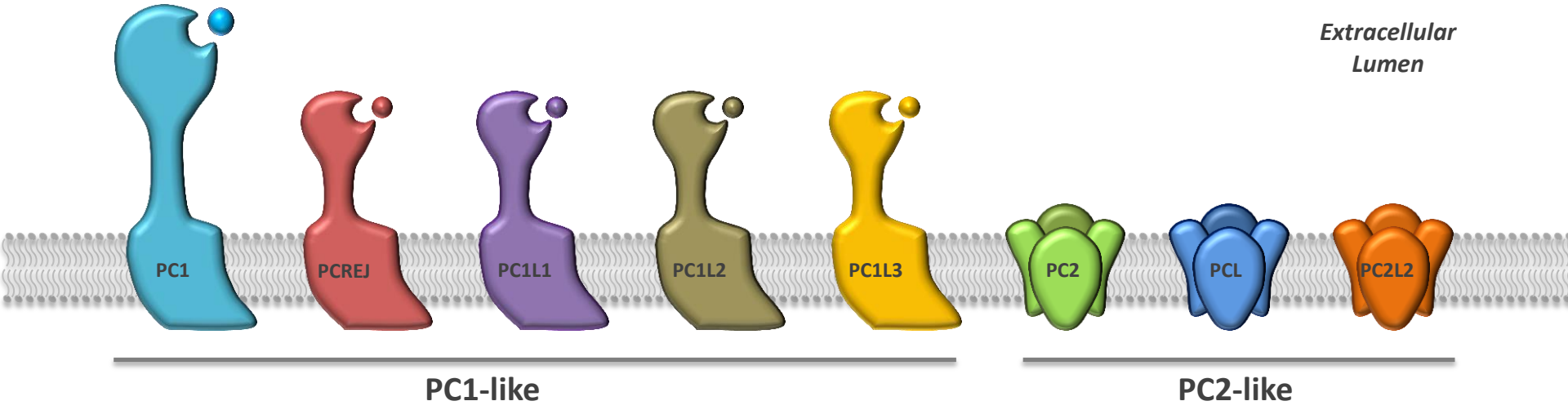
National Institutes of Health (NIDDK)

Active!


Lab


We are serious scientists!
EXIT
NIDDK
MOD
AHA
PKDF
NKF
DKF
CSC
NNSFC
Brigham and Women's Hospital
Harvard Medical School
Harvard Center for Polycystic Kidney Disease Research

Polycystin Protein Family

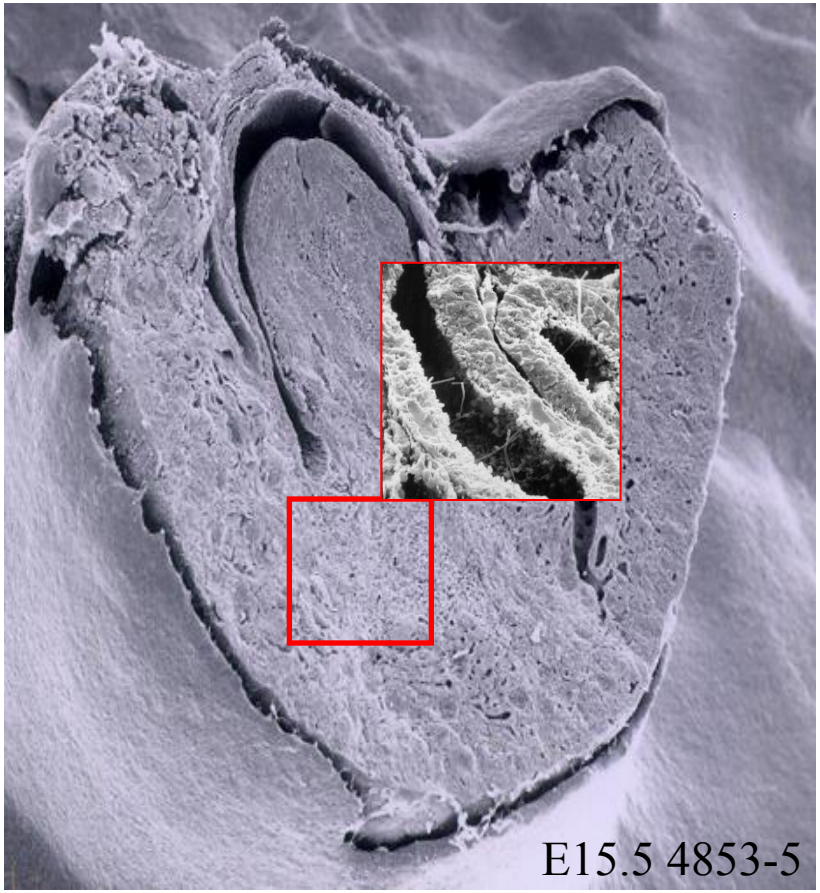


Structural Integrity of Primary Cilia

in mouse ortholog of human disease

Surya Nauli

normal



Pkd1 Knockout