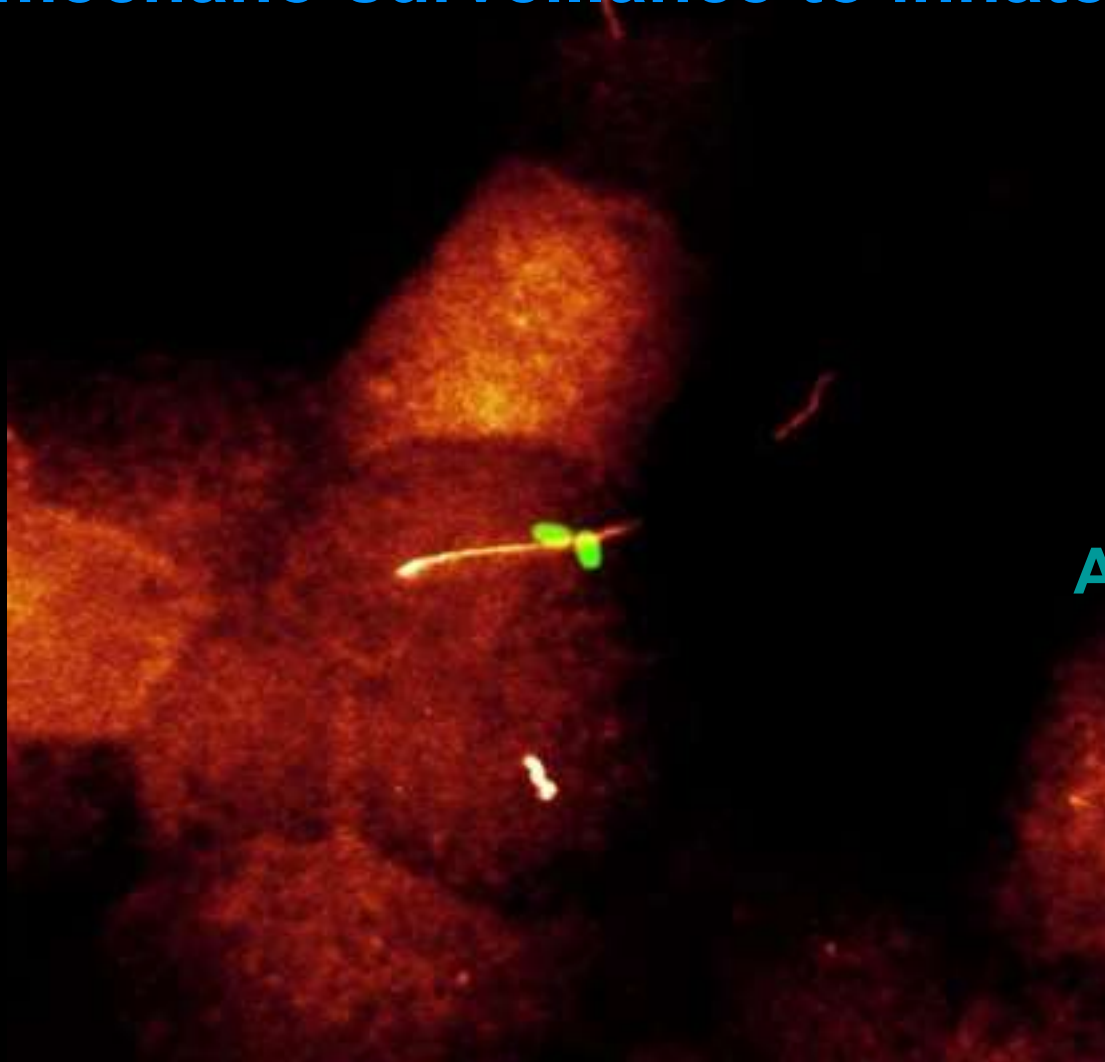


Novel functions of primary cilia in kidney homeostasis

From mechano-surveillance to innate immunity



Actualités Néphrologiques
21/05/24

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Explorations Fonctionnelles-Physiologie
INSERM U1151 / INEM - CKD team
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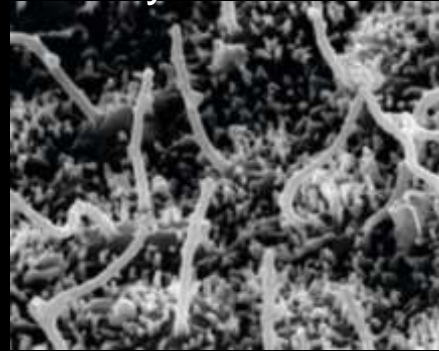
Primary cilia origin

Motile Cilia

Spermatozoids



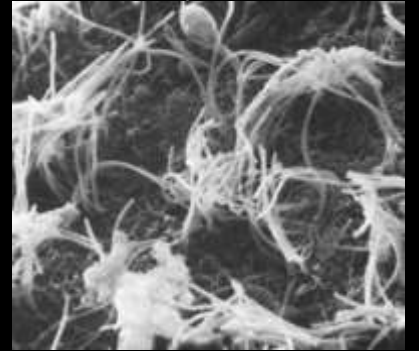
Embryonic node



Respiratory epith.



Ependymal cells

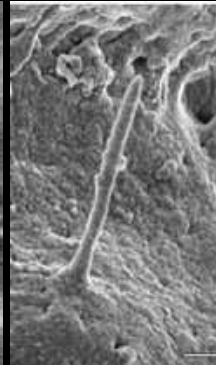


Primary cilia

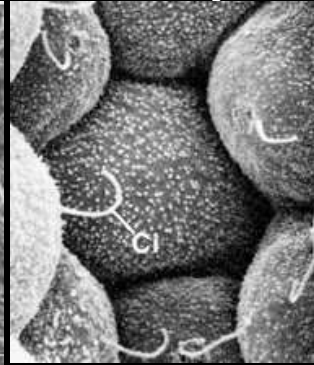
Neurons



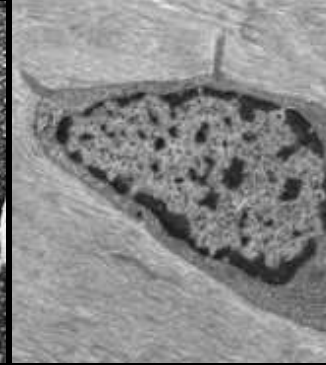
Heart



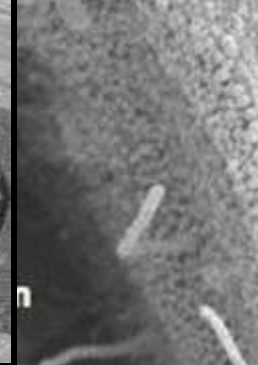
Kidney



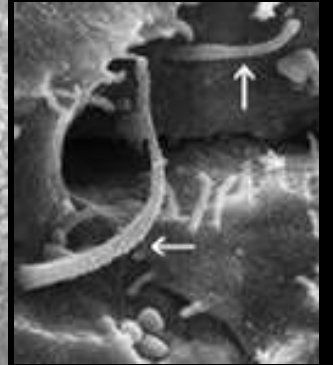
Bone



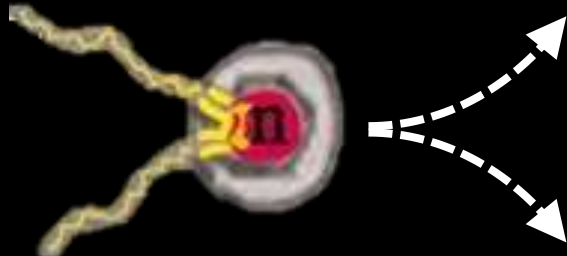
Liver



Pancreas



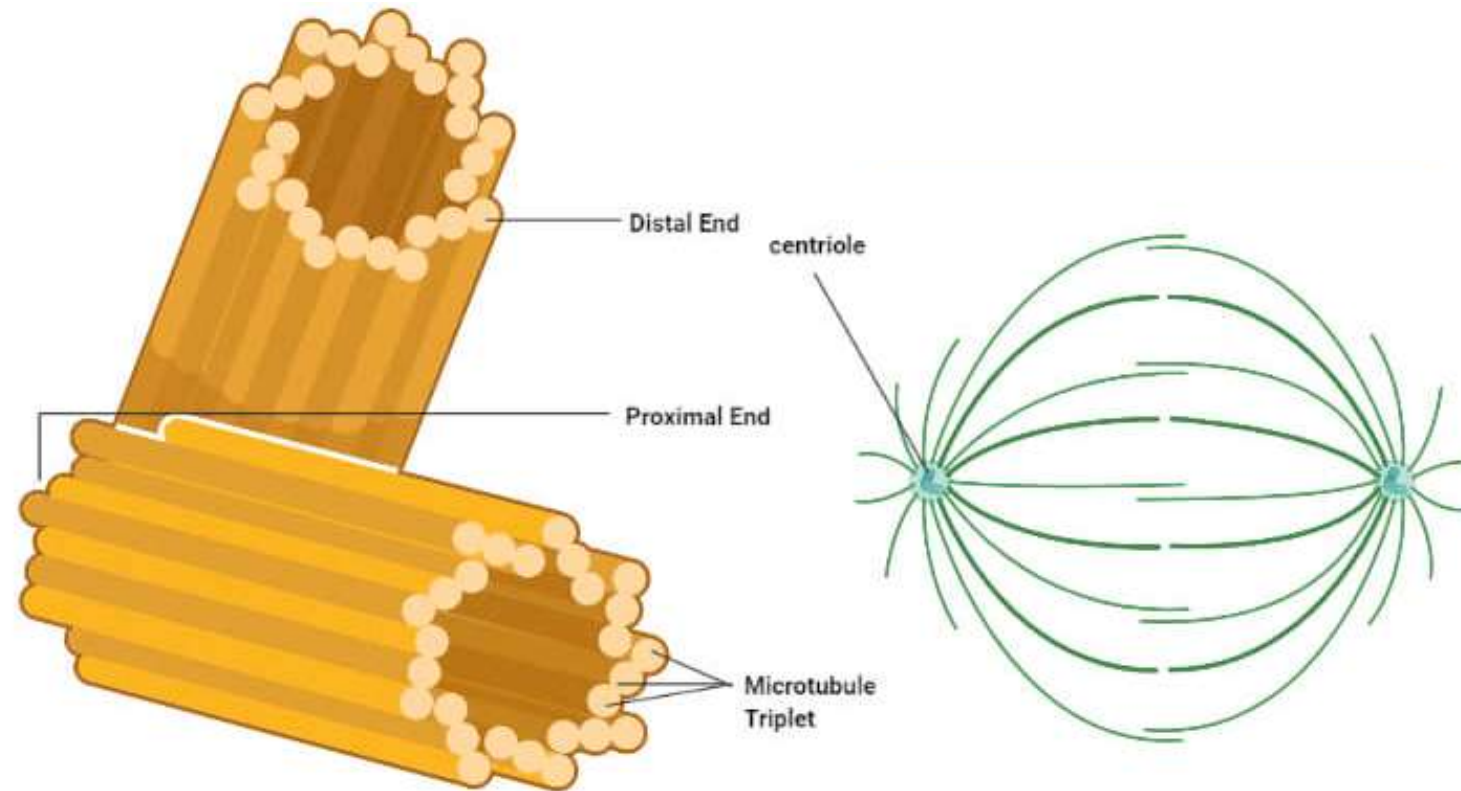
Last eucaryotes
common ancestry



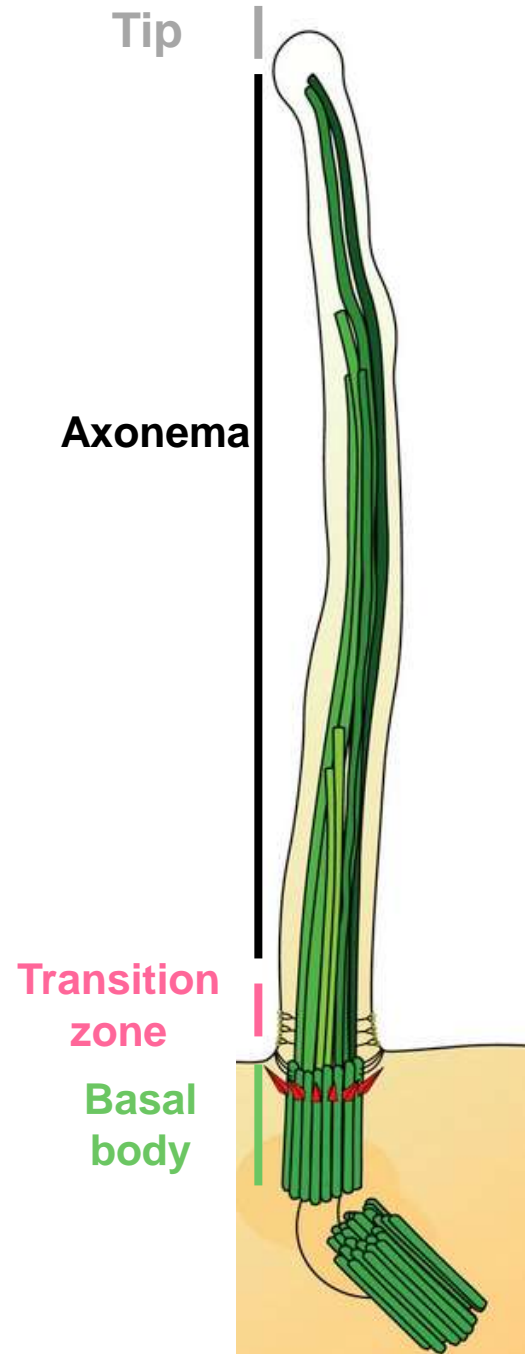
Margulis, 2006



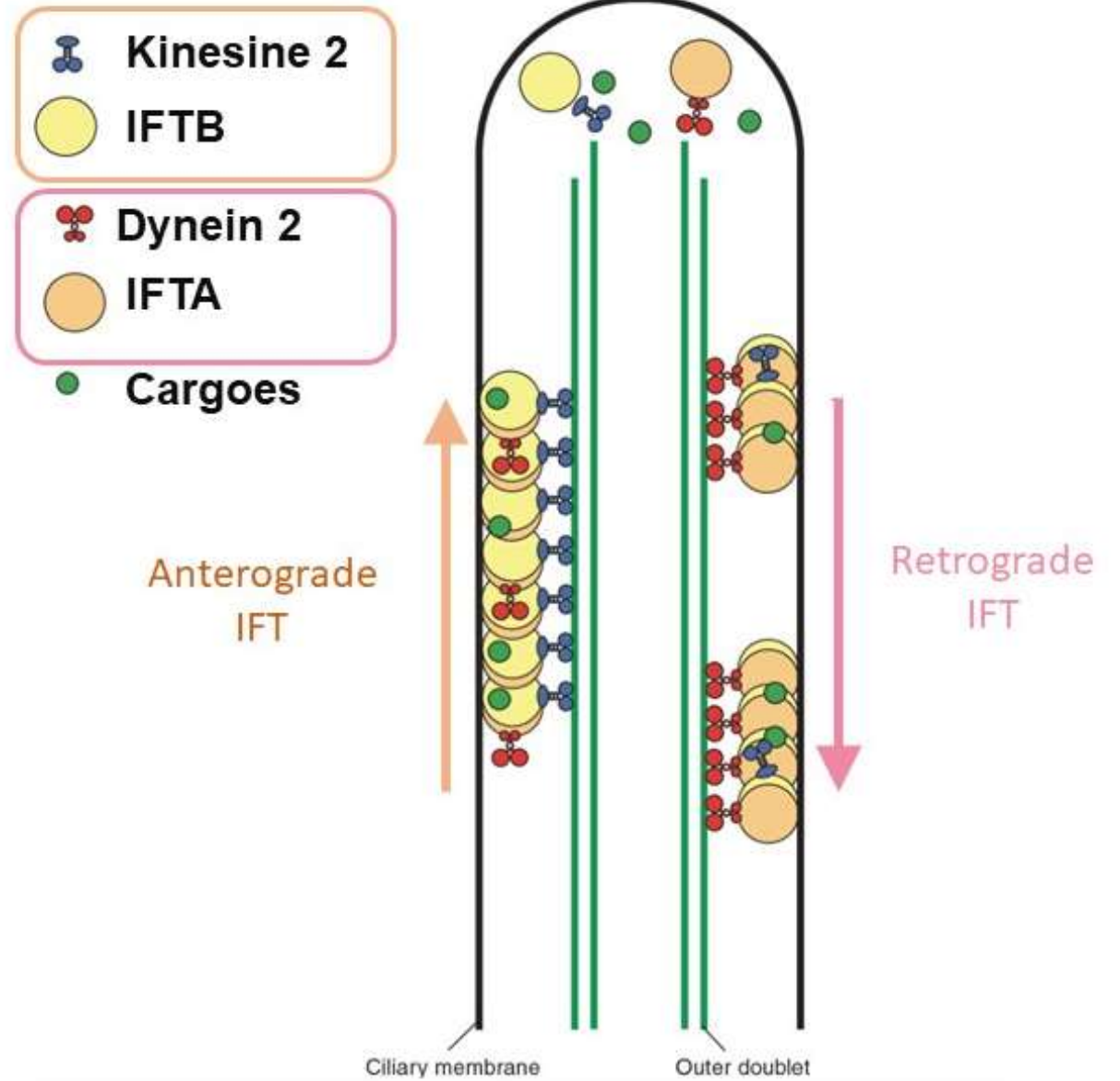
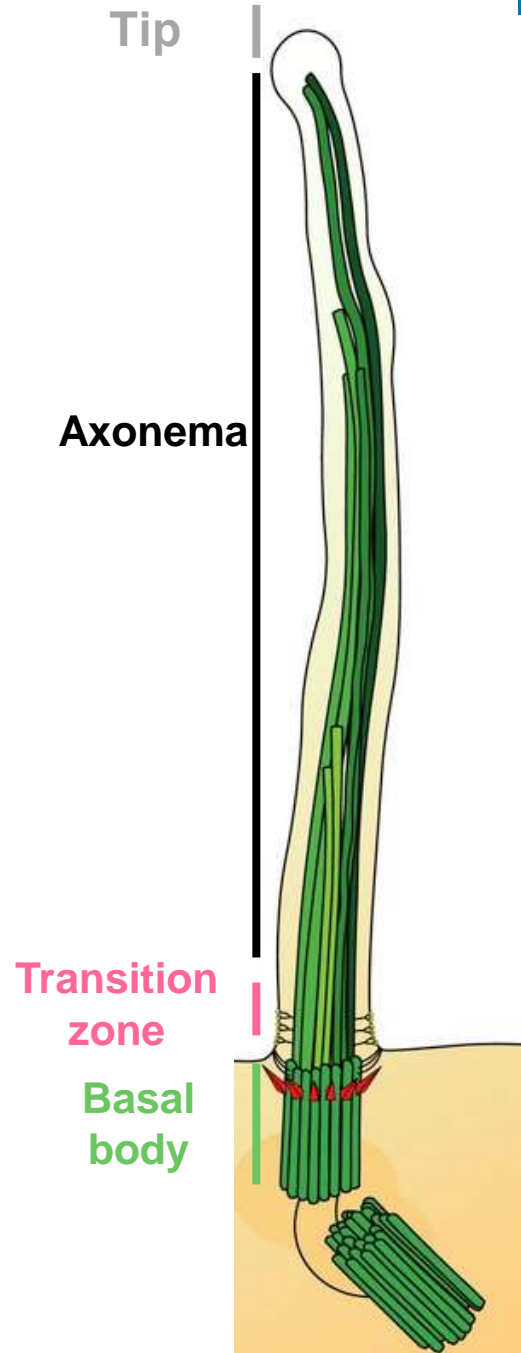
Cilia structure



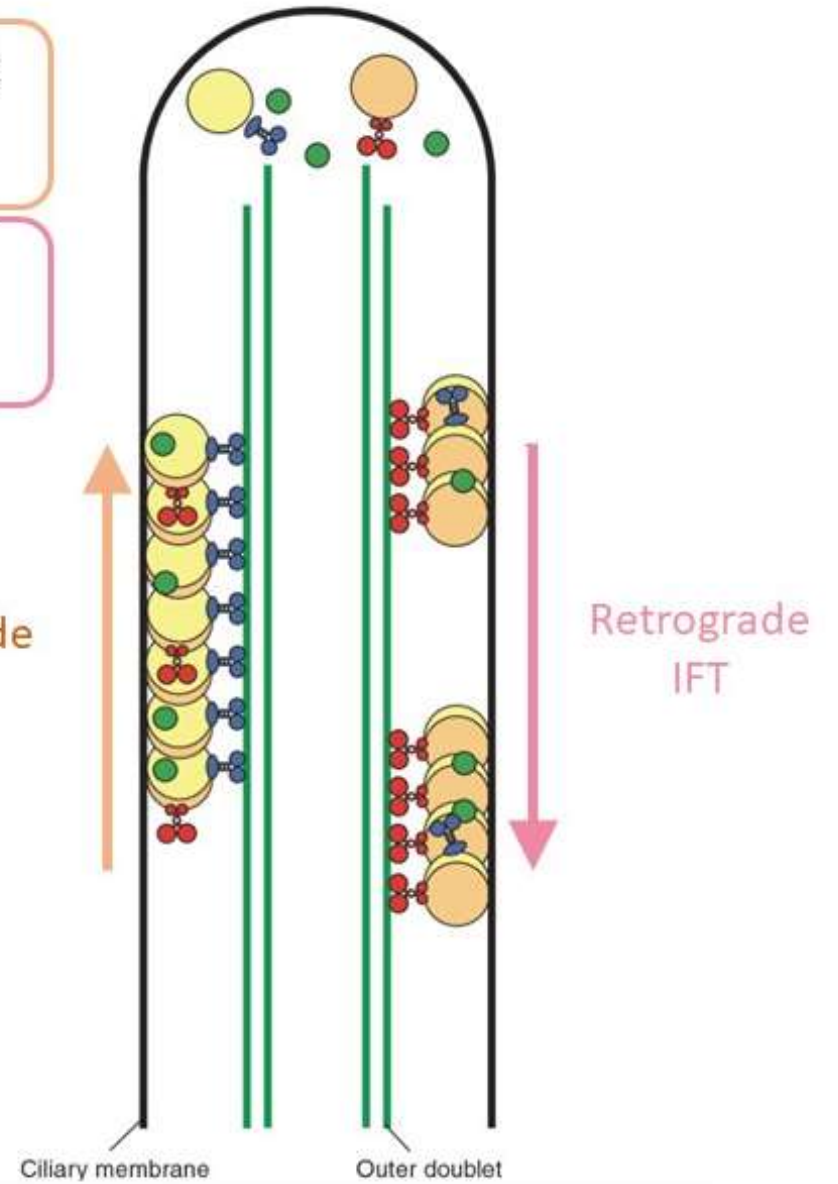
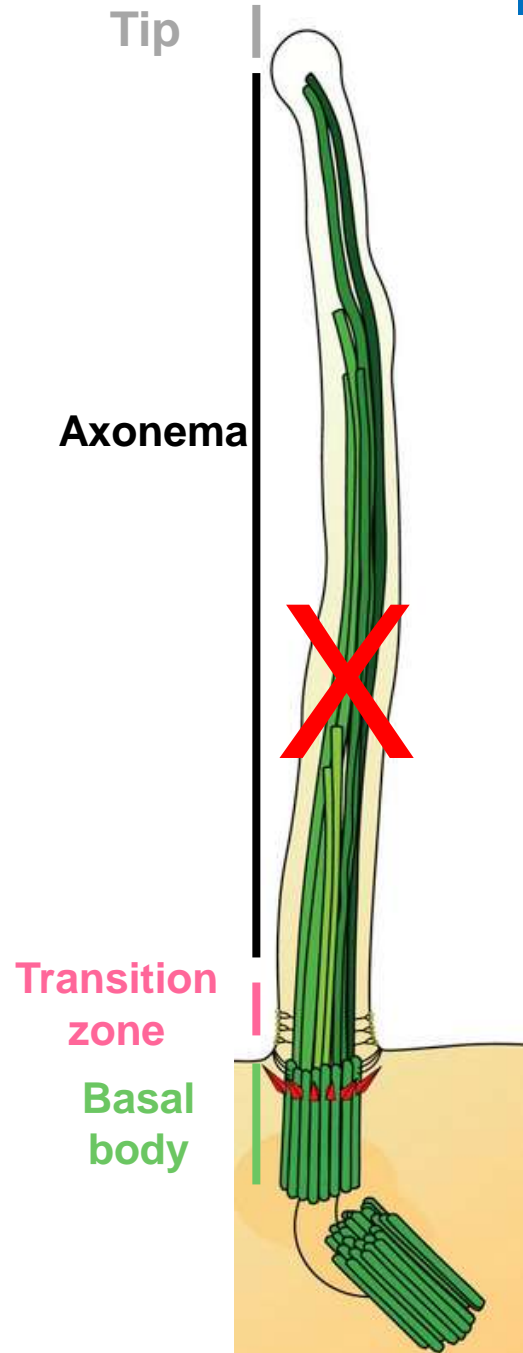
Cilia structure



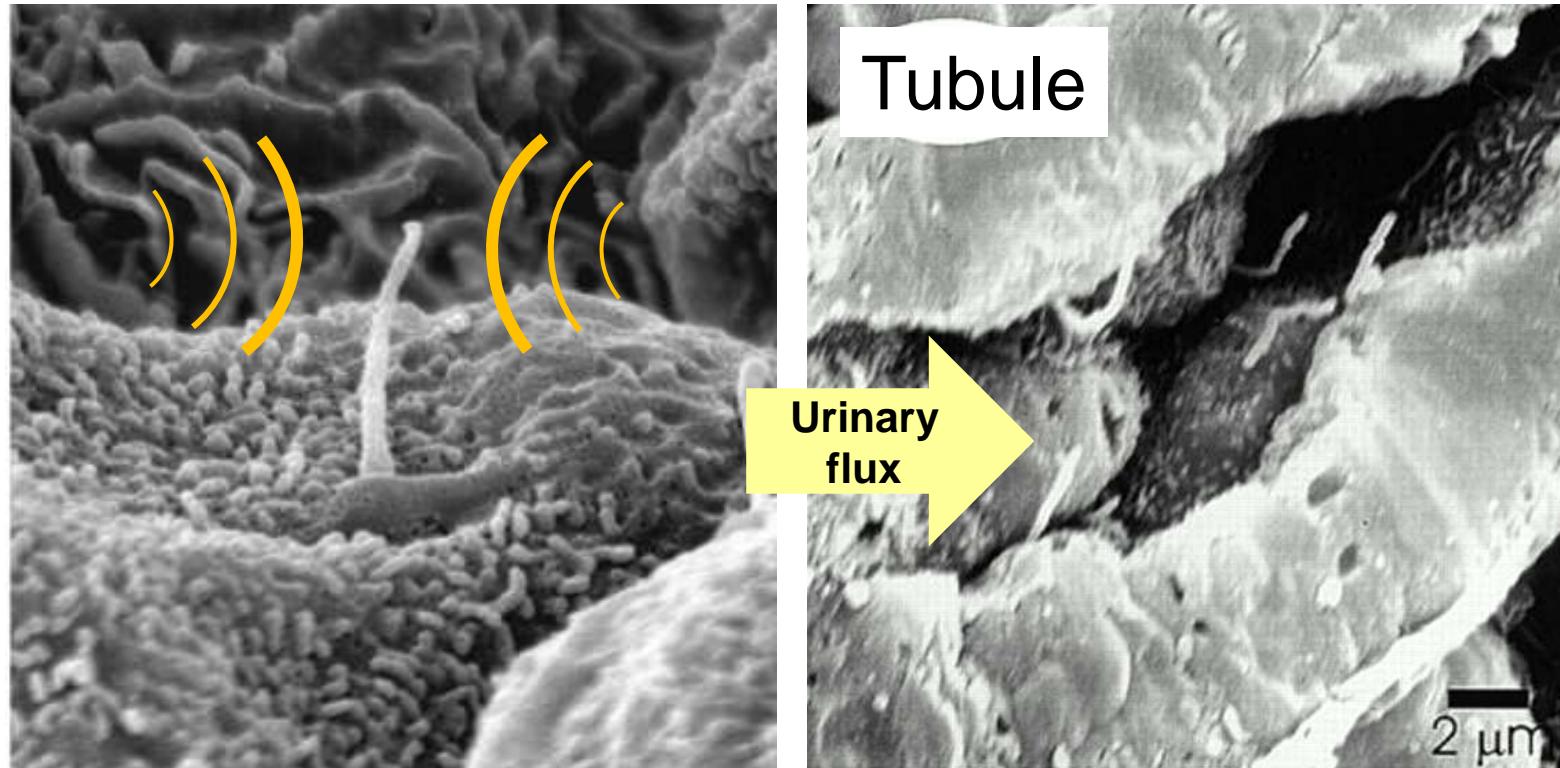
Intra-flagellar transport machinery



Intra-flagellar transport machinery



Primary cilium in the kidney

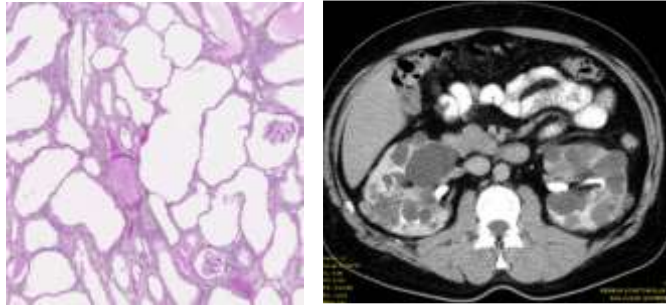


Signalling platform

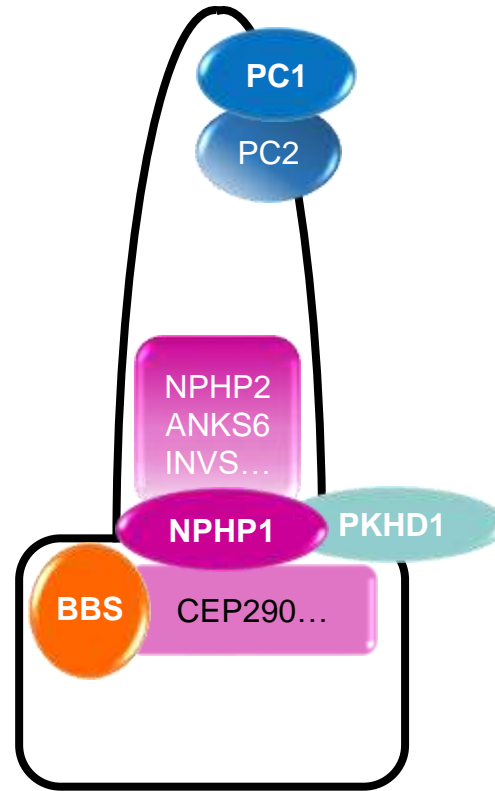
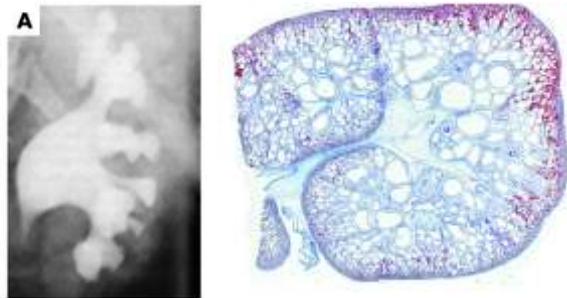
- Mechanosensor
- Chemosensor

Mutations in ciliary genes cause kidney diseases

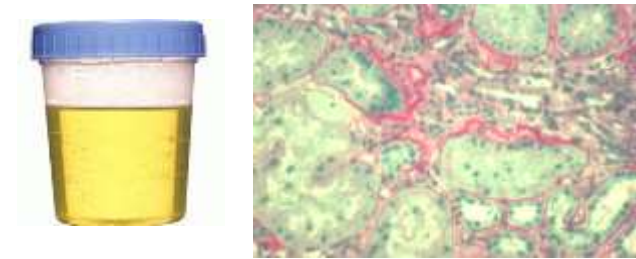
Autosomal Dominant Polycystic Kidney Disease



Bardet Biedl Syndrome



Nephronophthisis

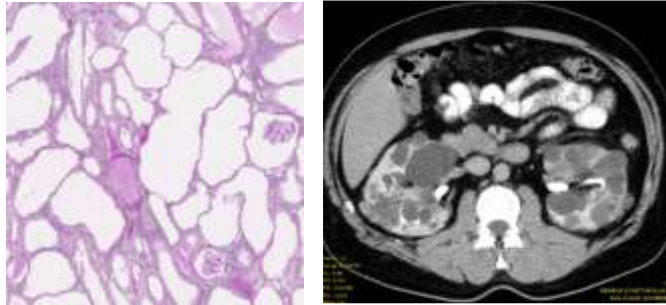


Autosomal Recessive Polycystic Kidney Disease

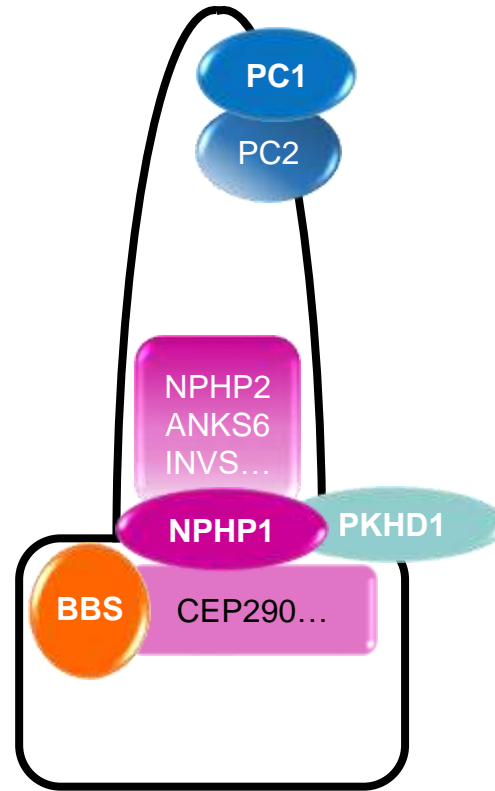
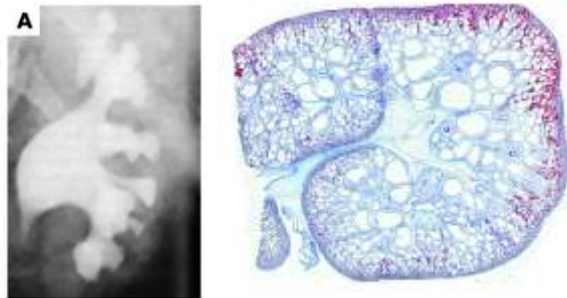


Mutations in ciliary genes cause kidney diseases

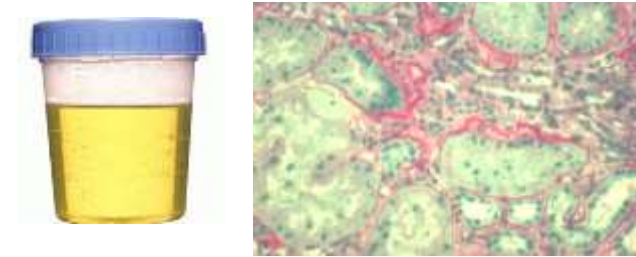
Autosomal Dominant Polycystic Kidney Disease



Bardet Biedl Syndrome



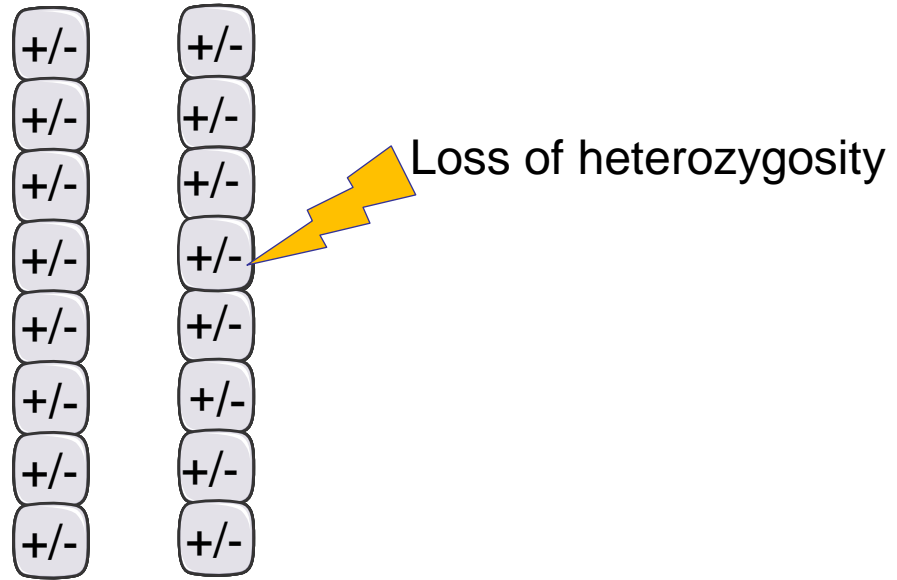
Nephronophthisis



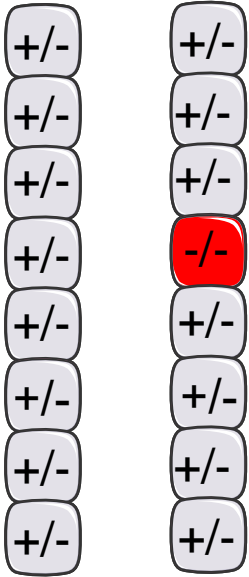
Autosomal Recessive Polycystic Kidney Disease



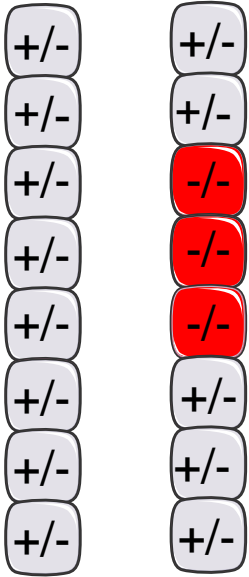
Model of cyst formation in ADPKD



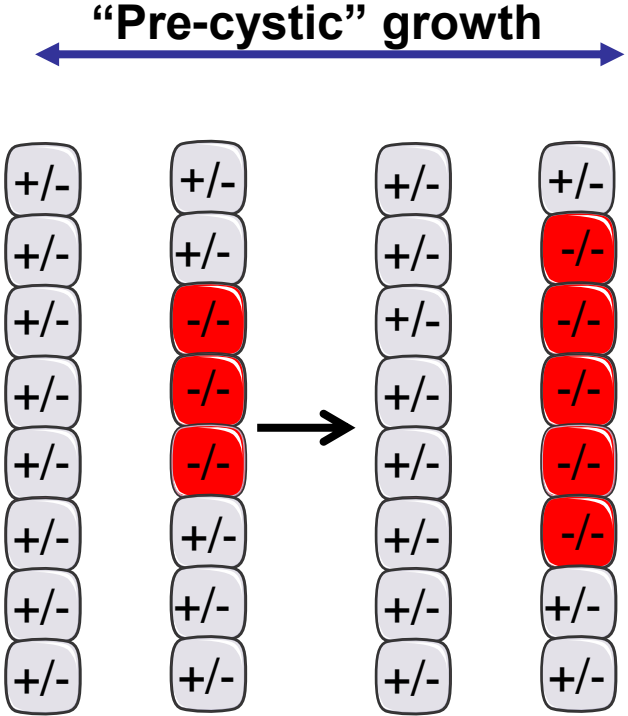
Model of cyst formation in ADPKD



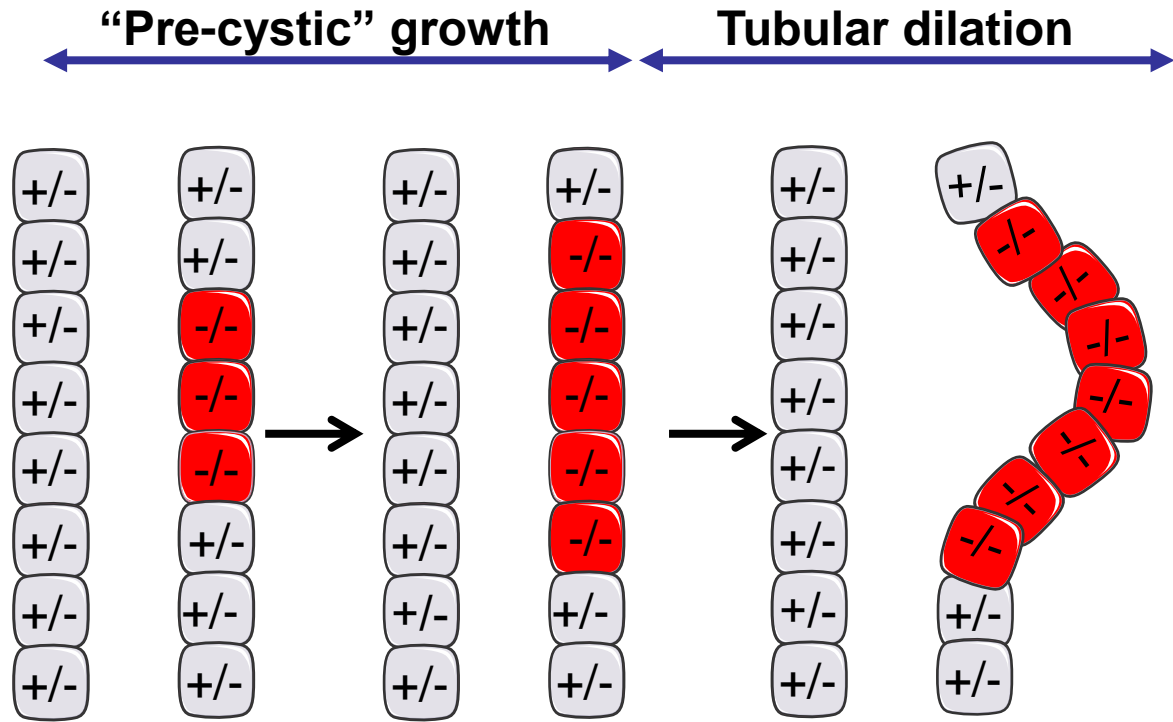
Model of cyst formation in ADPKD



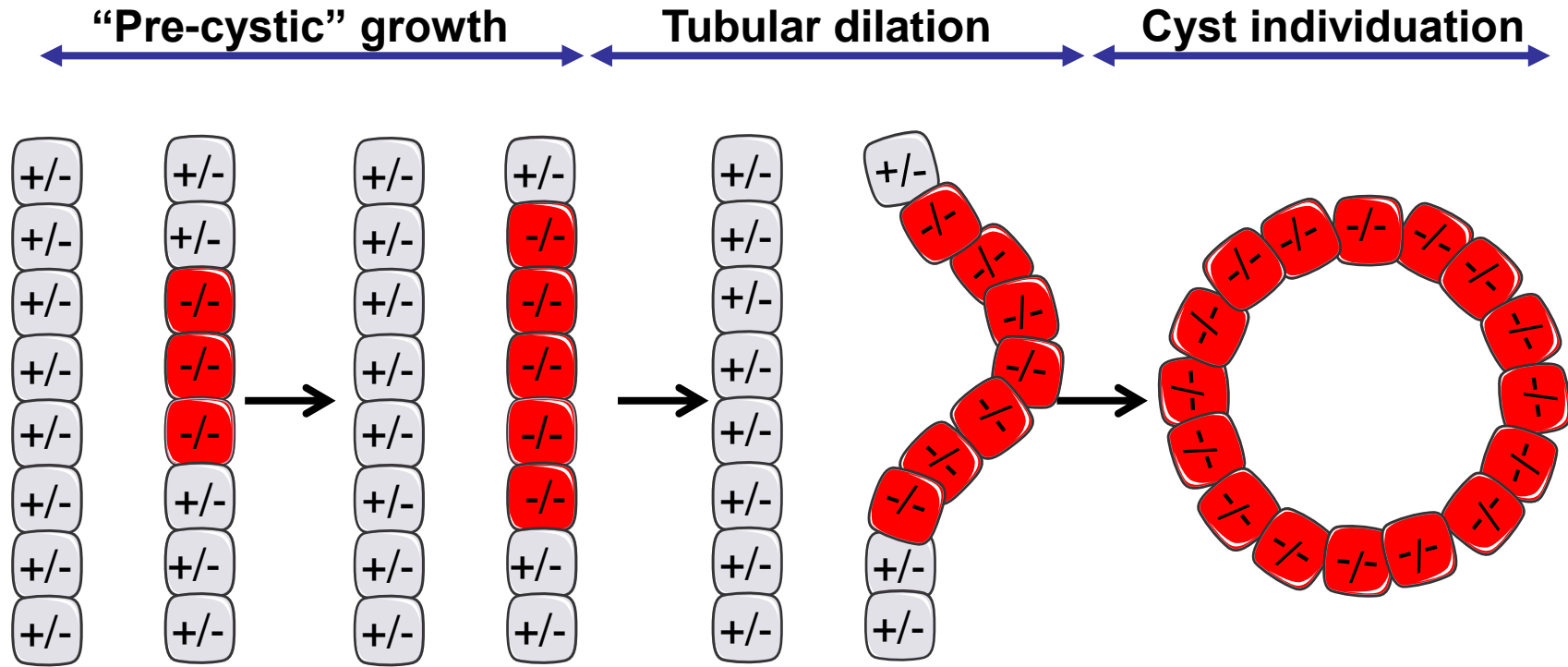
Model of cyst formation in ADPKD



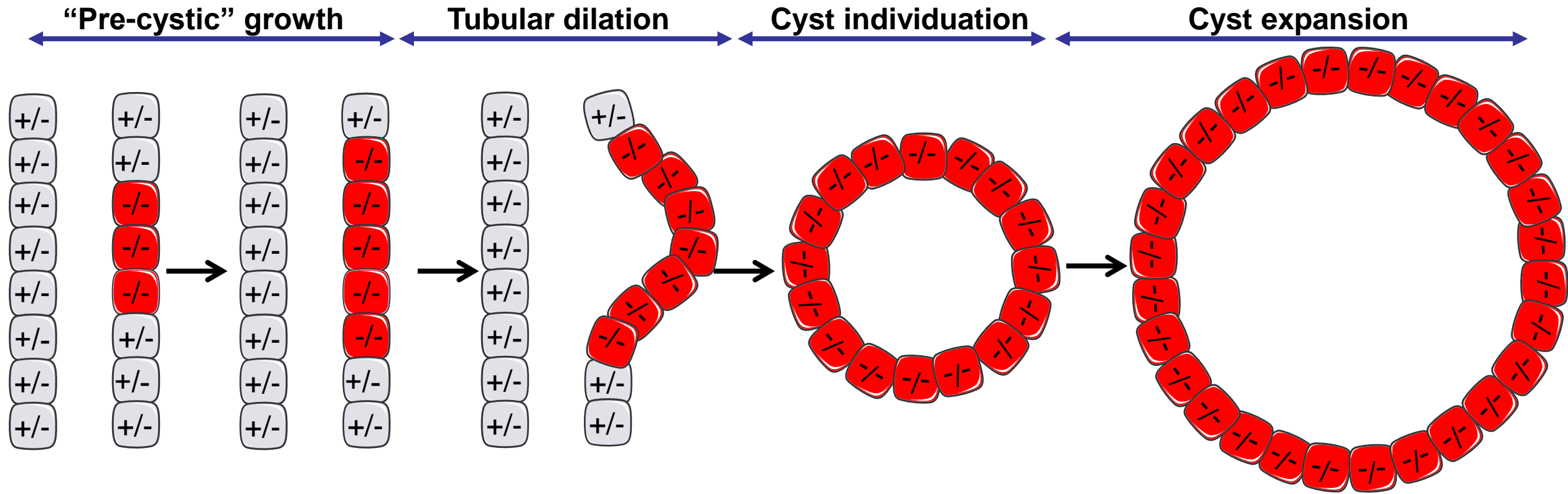
Model of cyst formation in ADPKD



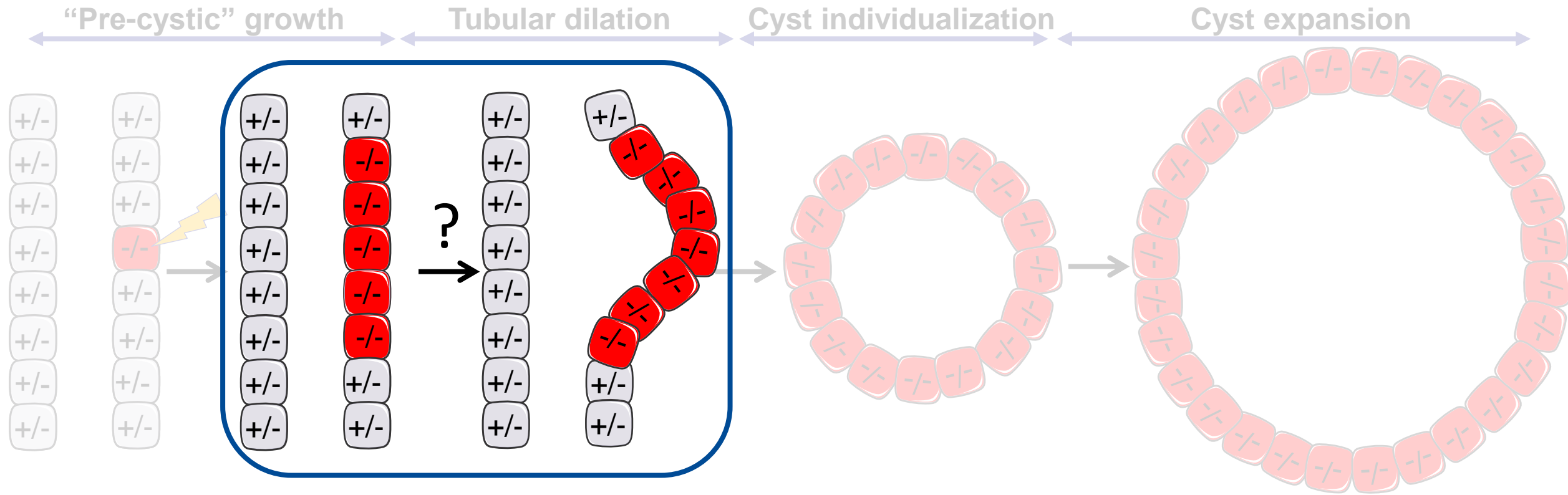
Model of cyst formation in ADPKD



Model of cyst formation in ADPKD



Question: how *Pkd1*^{-/-} tubules deform ?



- ✓ Primary cilia are needed
- ✓ Proliferation is required but is not sufficient
- ✓ Defects in planar cell polarity are not sufficient

Cilia to basement membrane signalling is a biomechanical driver of ADPKD

Manal Mazloun¹, **Brice Lapin**²⁻³, Amandine Viau³, Rushdi Alghamdi¹, Martine Burtin¹ **Pascal Houillier**^{4,5}, Amandine Aka¹, **E. Wolfgang Kühn**^{6,7}, Tilman Busch^{6,7}, Michael Köttgen^{6,7}, Camille Cohen¹, **Lydie Cheval**⁴, Gilles Crambert⁴, Marie-Christine Verpont⁸, Brigitte Lelongt⁸, **Stephanie Descroix**, Fabiola Terzi¹, **Sylvie Coscoy**² and **Frank Bienaimé**^{1,9}



Models: doxycycline-inducible and tubule-specific knock-out



Control

Pkd1^{Δtub}

Pkd1^{Δtub}; *Kif3a*^{Δtub}

D0

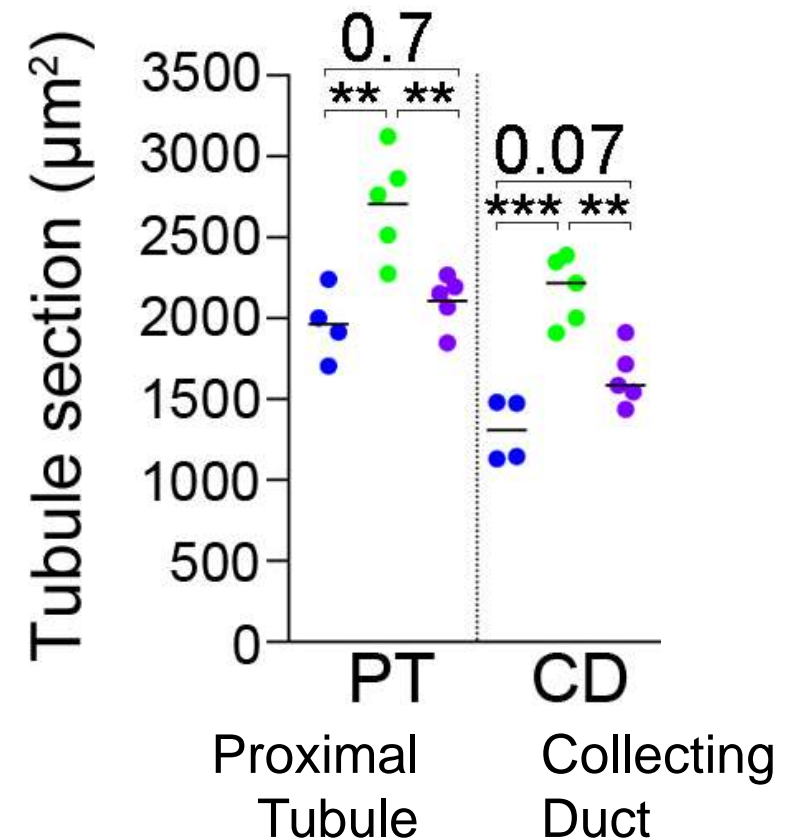
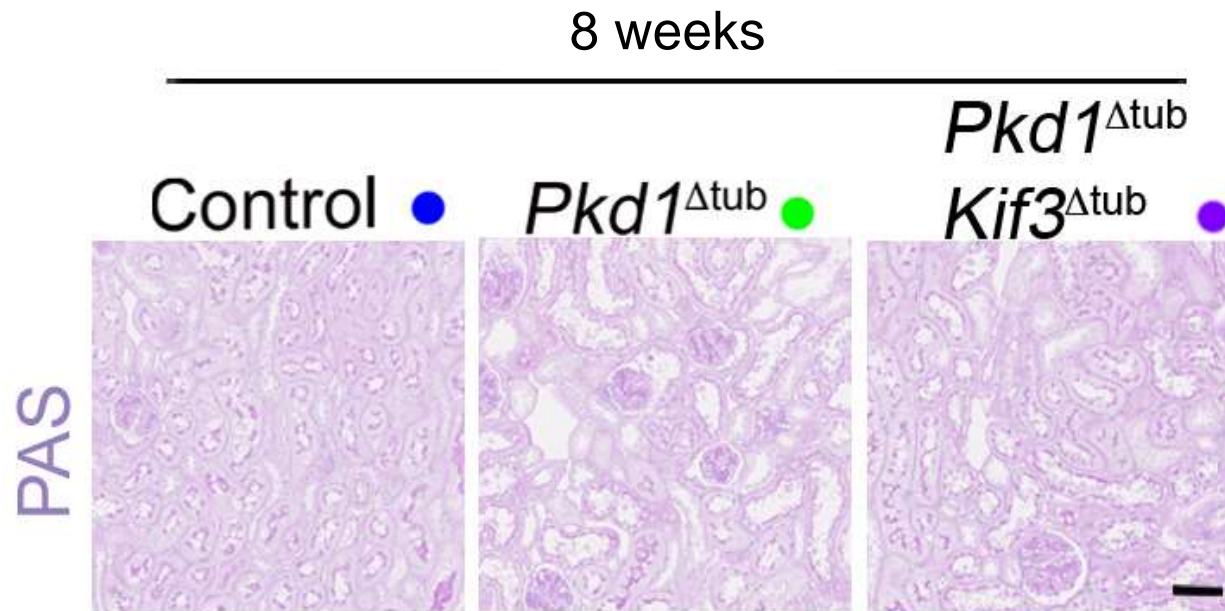
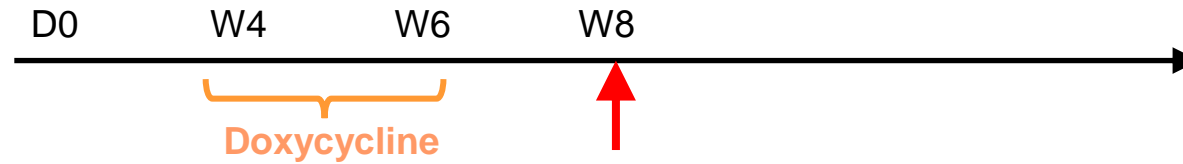
W4

W6

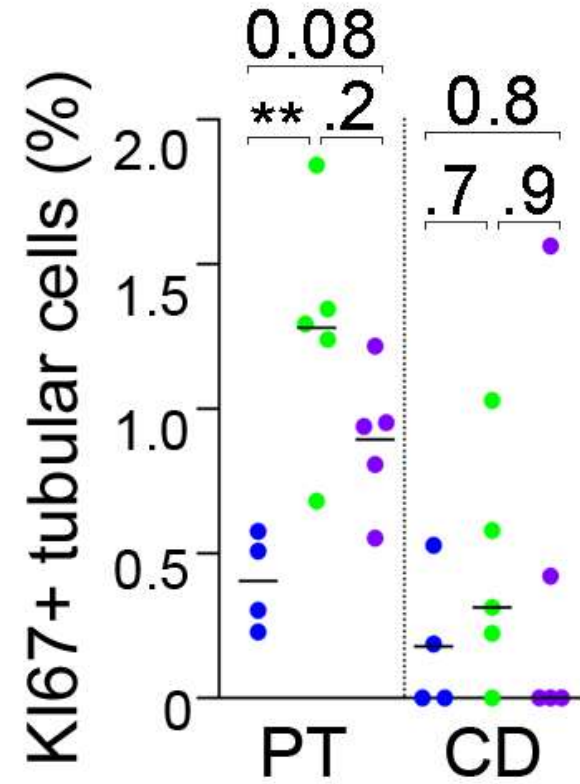
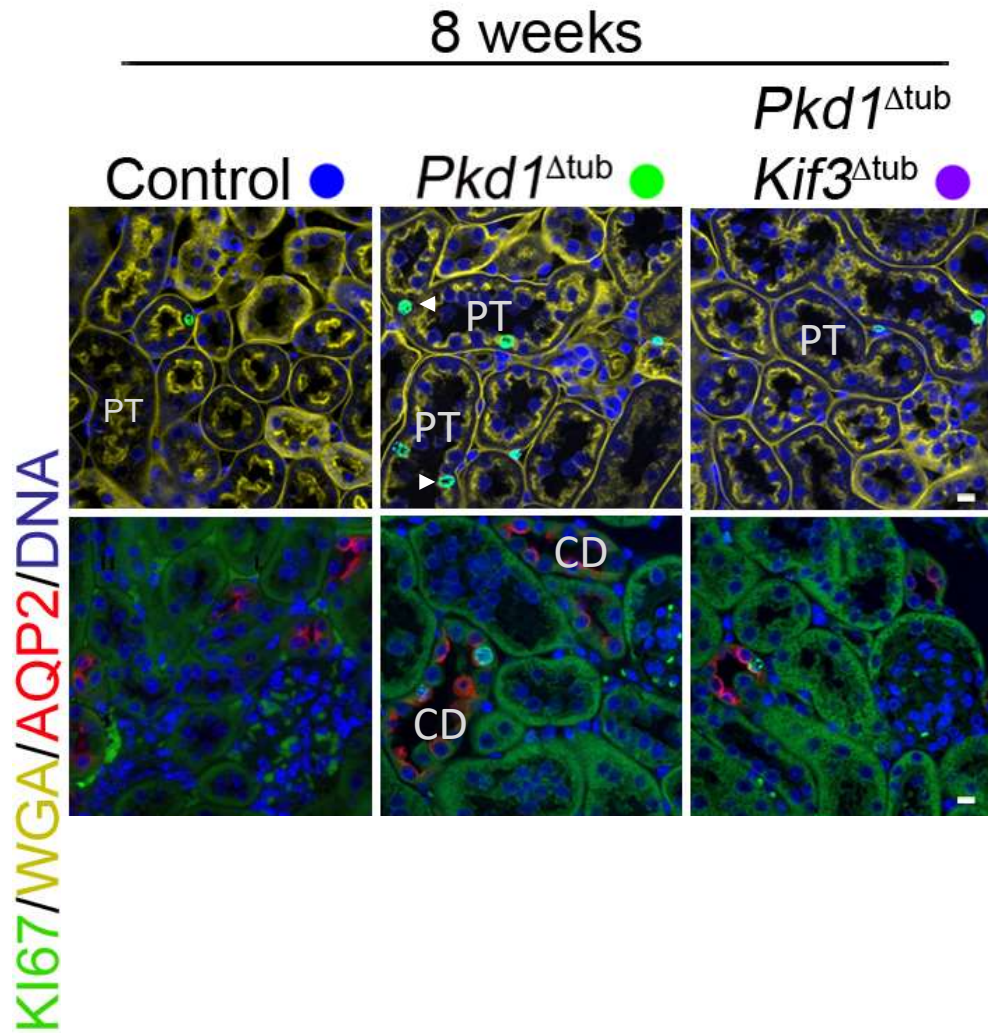


Doxycycline

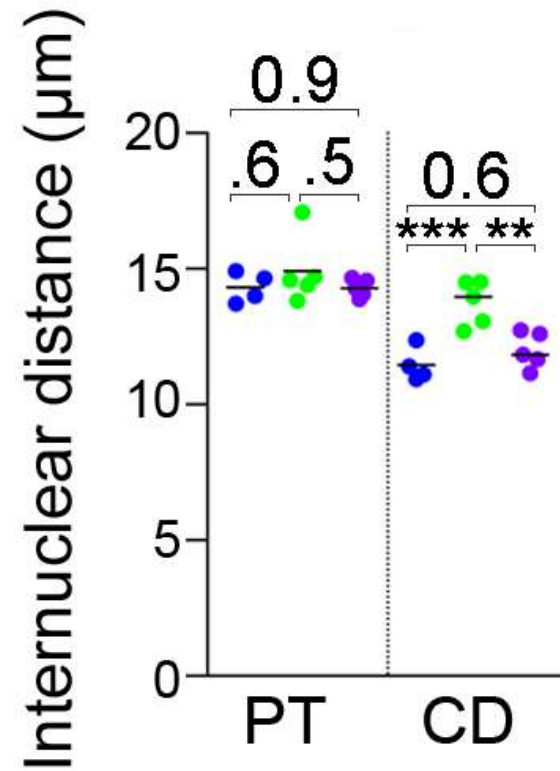
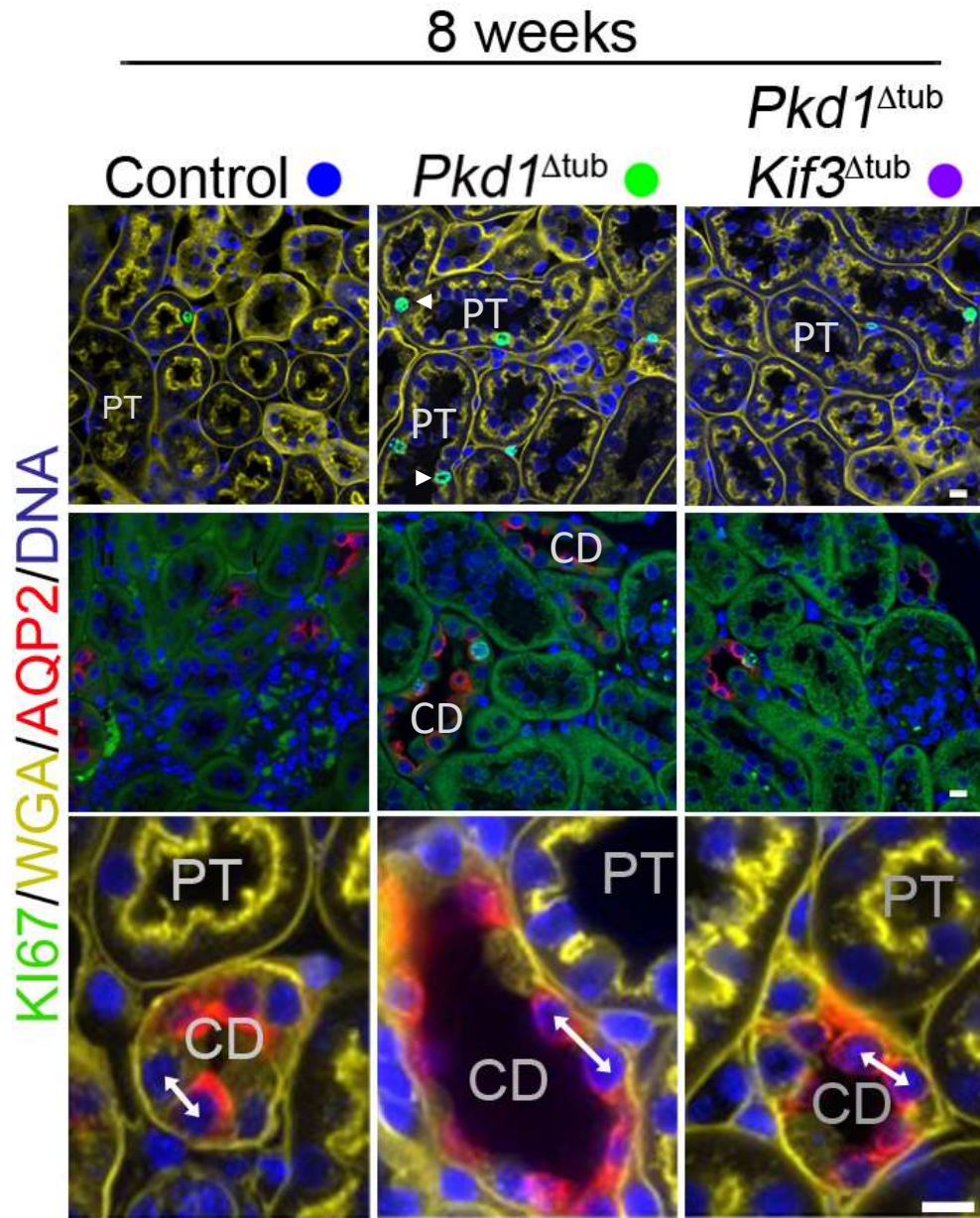
Pkd1 loss induces cilia-dependent tubule dilation in PT and CD...



... through different mechanisms



... through different mechanisms



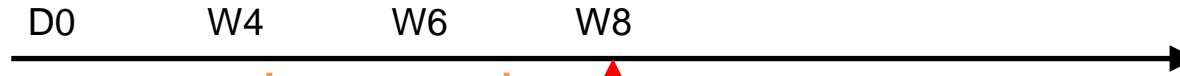
... through different mechanisms...



Control

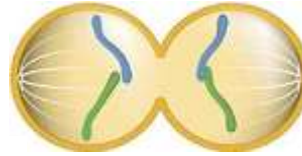
Pkd1^{Δtub}

Pkd1^{Δtub}; *Kif3a*^{Δtub}

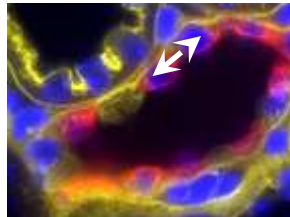


Doxycycline

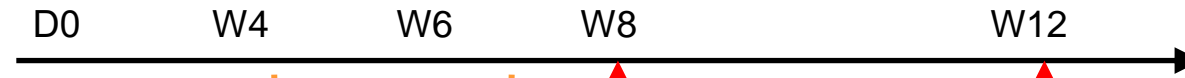
Proximal tubule



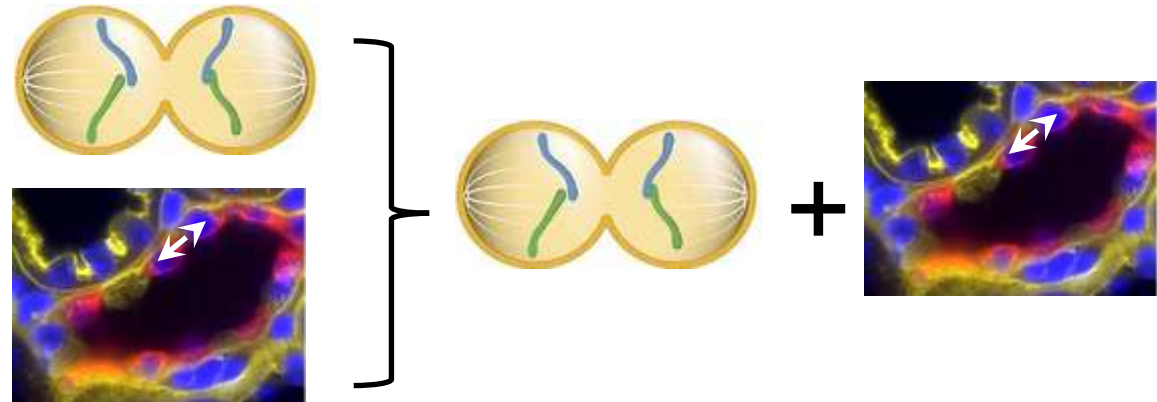
Collecting duct



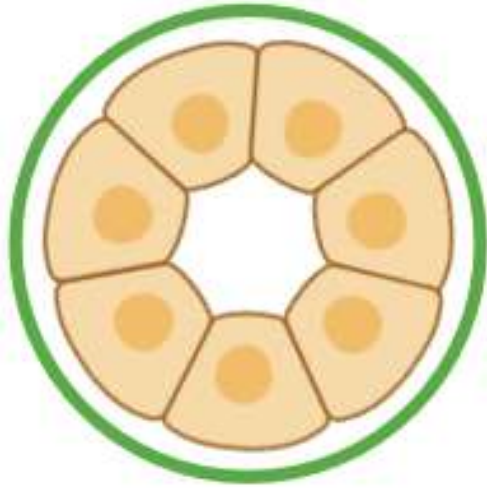
... which converges at later time points



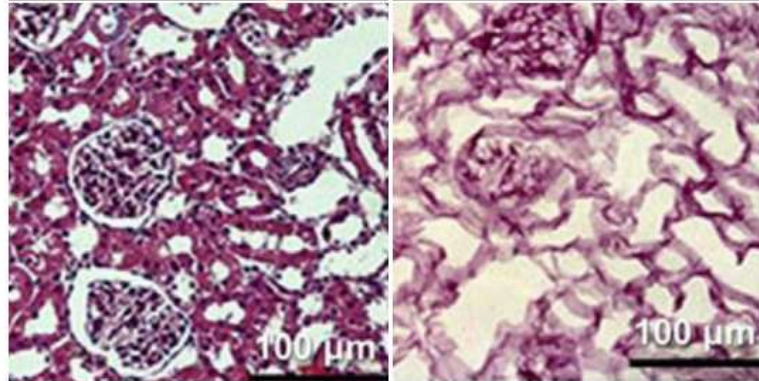
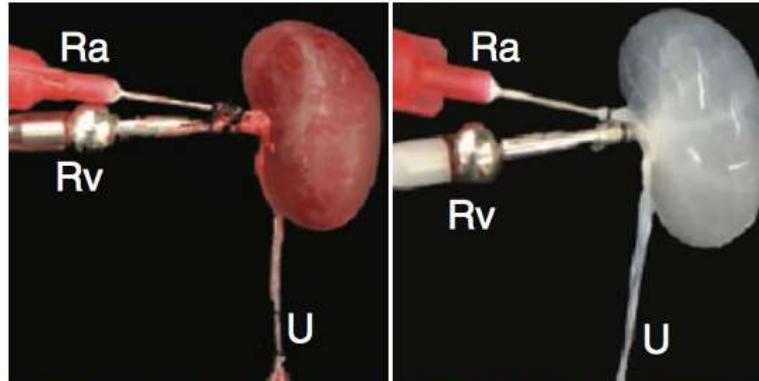
Proximal tubule
Collecting duct



Basement membrane dictates tubule mechanics

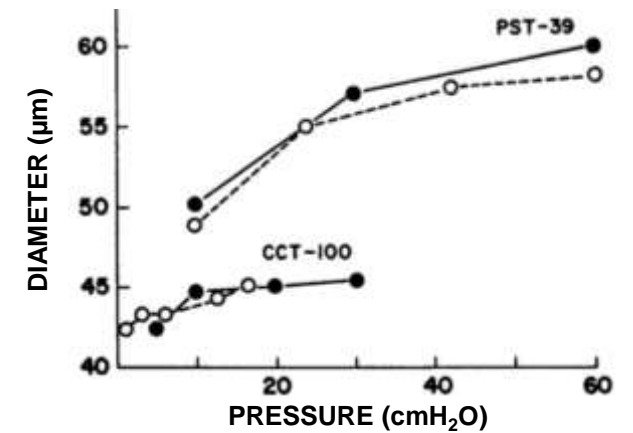
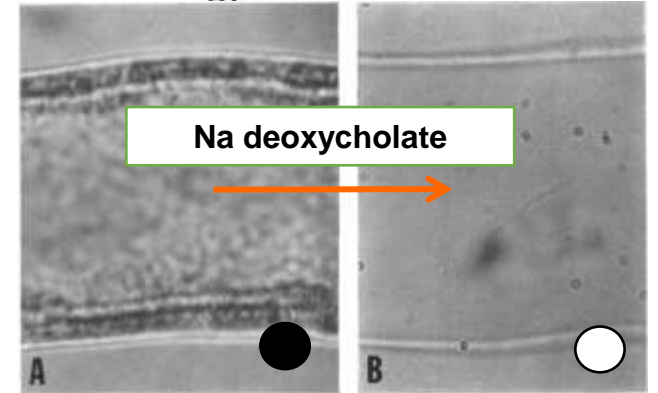
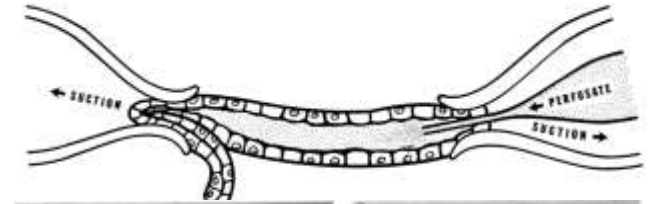


Kidney shape is maintained after decellularization



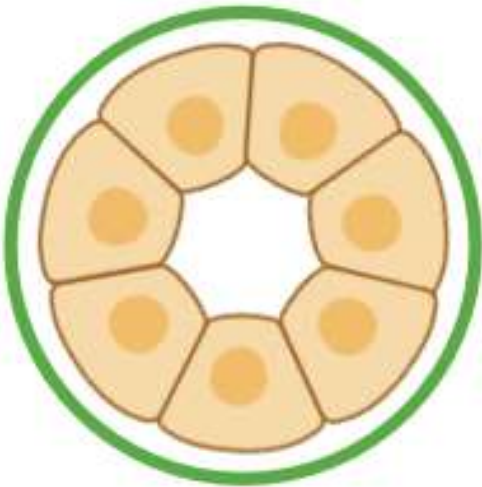
Song et al. Nat Med (2013)

Basement membrane dictates tubule mechanical properties



Grantham et al. JCI (1972)

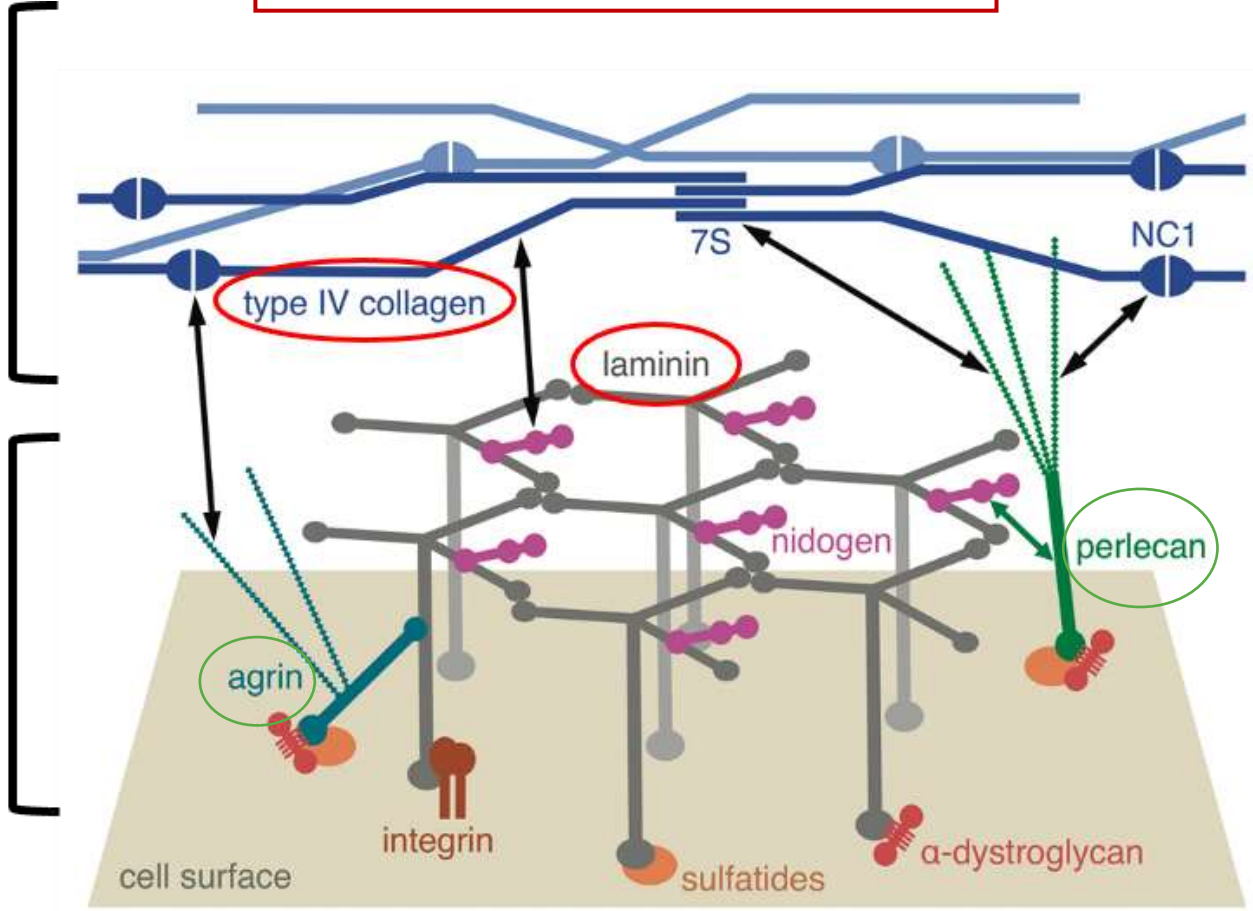
Basement membrane dictates tubule mechanics



Stabilizing structure

Cell-binding activity

RIGIDITY



SOFTNESS

Basement membrane dictates tubule mechanics

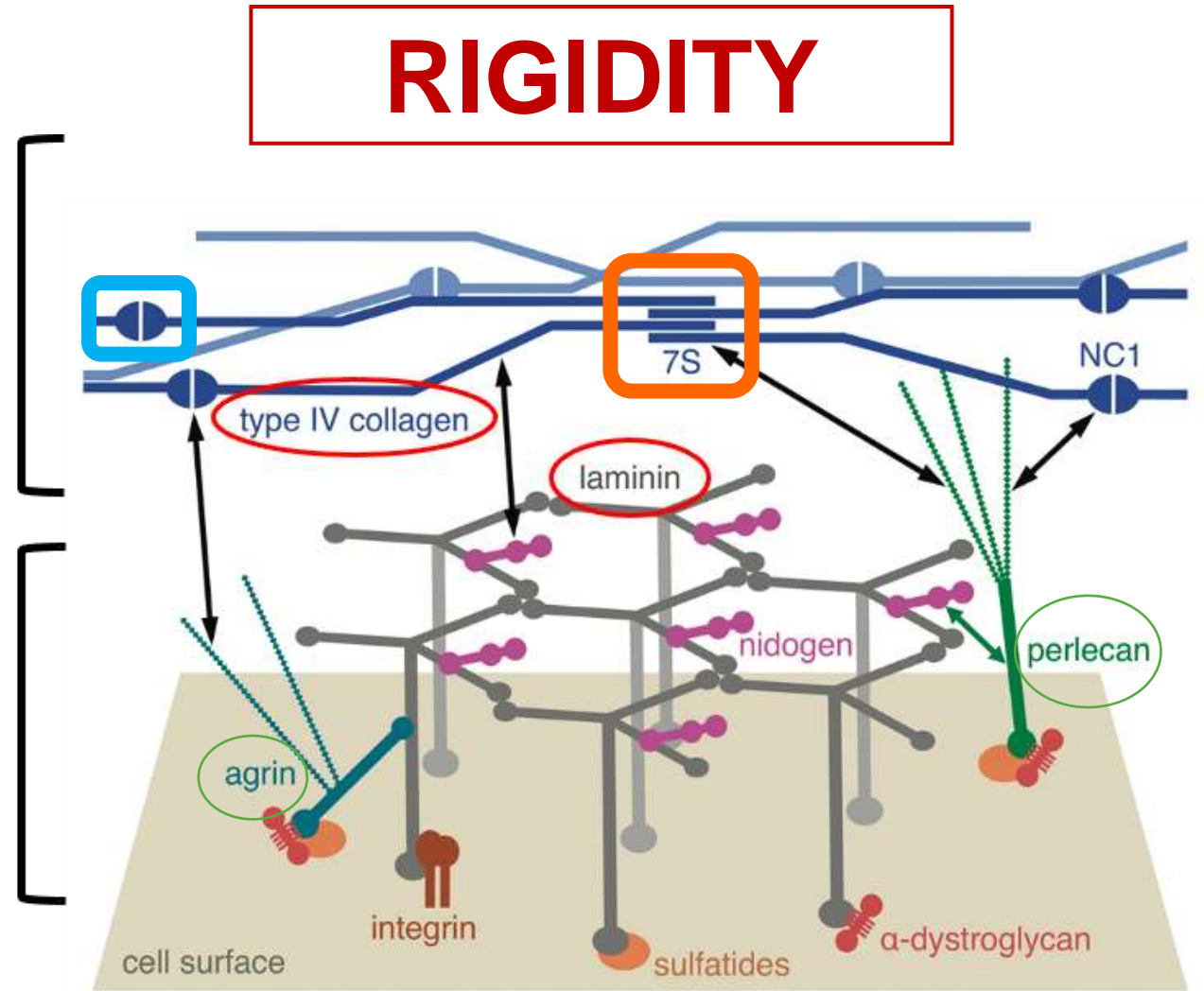
CROSS-LINKING (Collagène IV)

Peroxidasine :
Sulfilimine bounds

Lysyl oxidases :
lysine-hydroxylysine

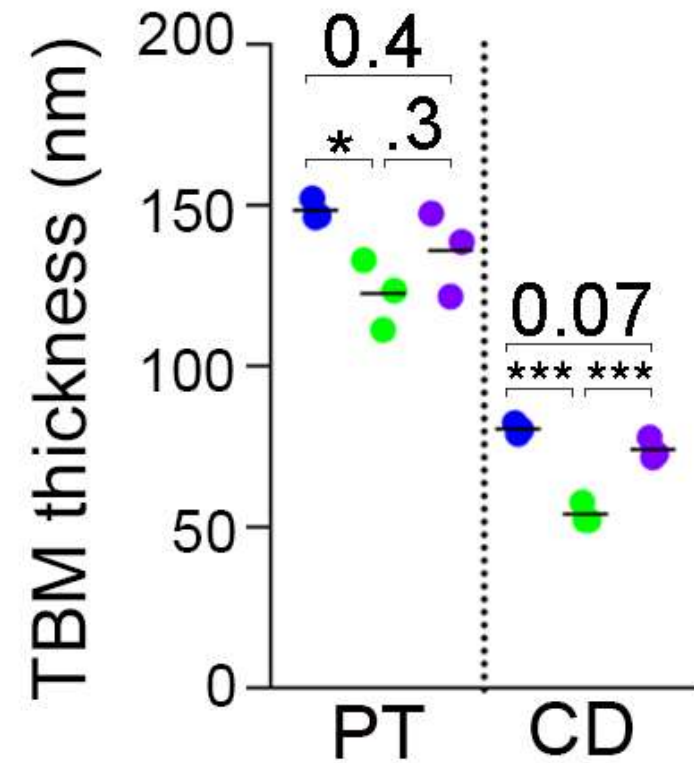
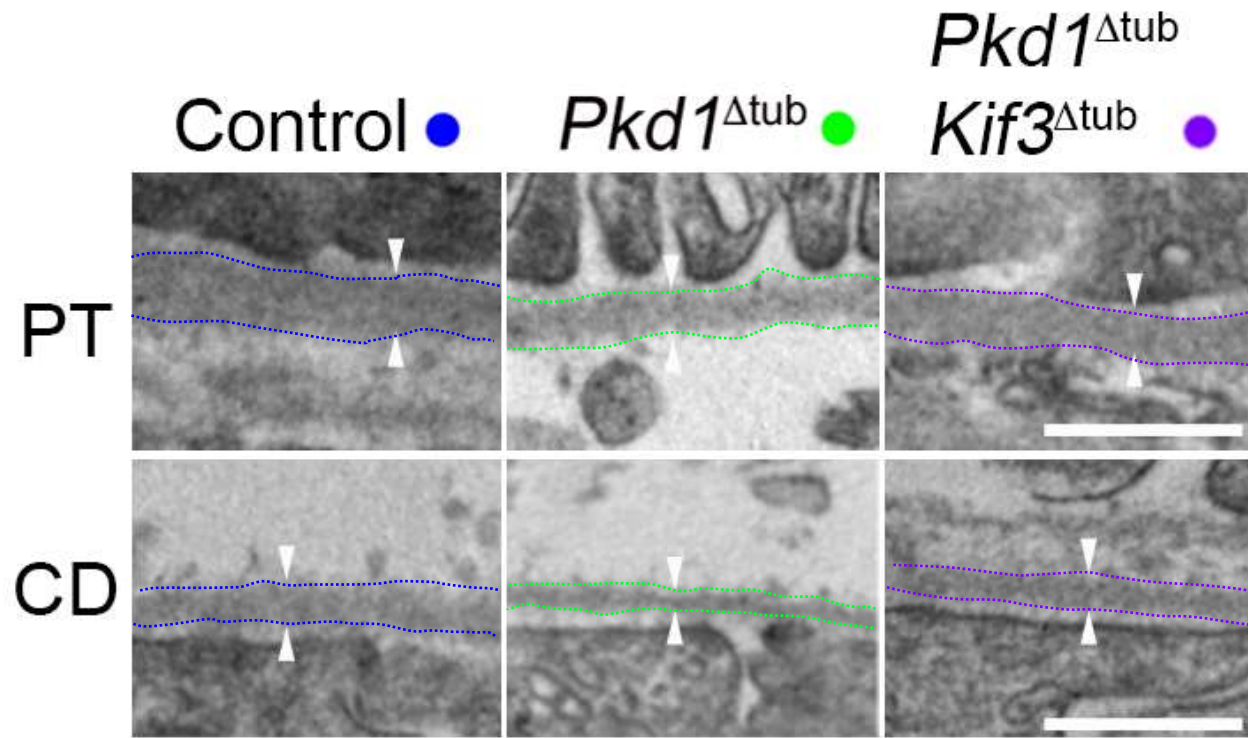
Stabilizing
structure

Cell-binding
activity



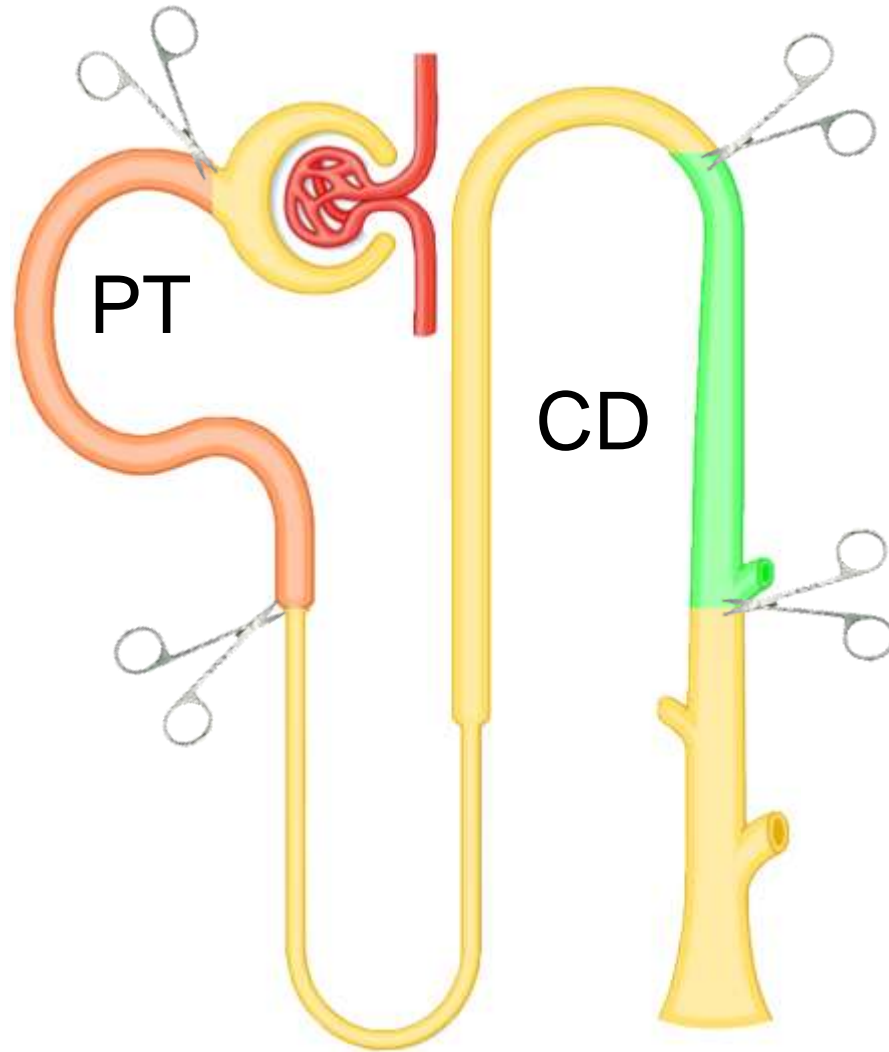
SOFTNESS

Pkd1 loss induces cilia dependent BM remodeling



Transcriptome profiling of isolated *Pkd1*^{-/-} tubules

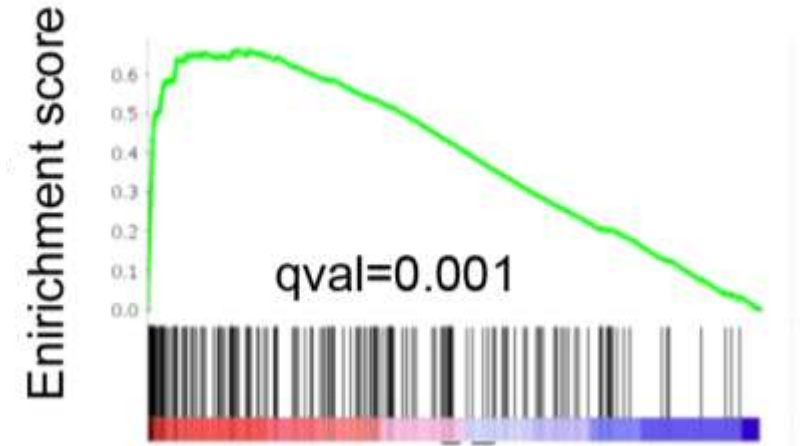
8 weeks
Control
Pkd1^{Δtub}



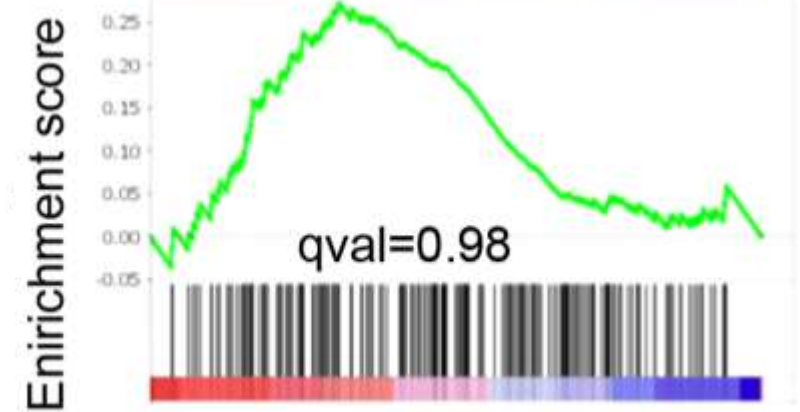
Segment specific
RNA-Seq

Proliferation Gene sets

PT

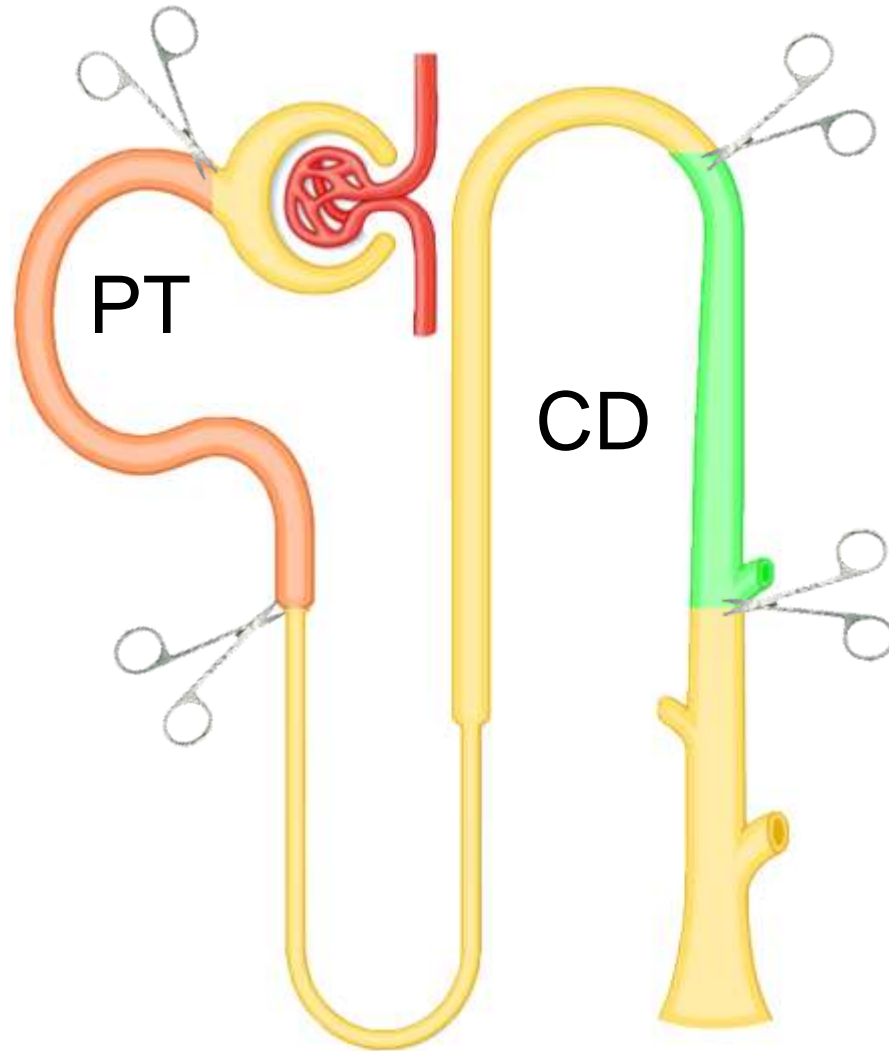


CD

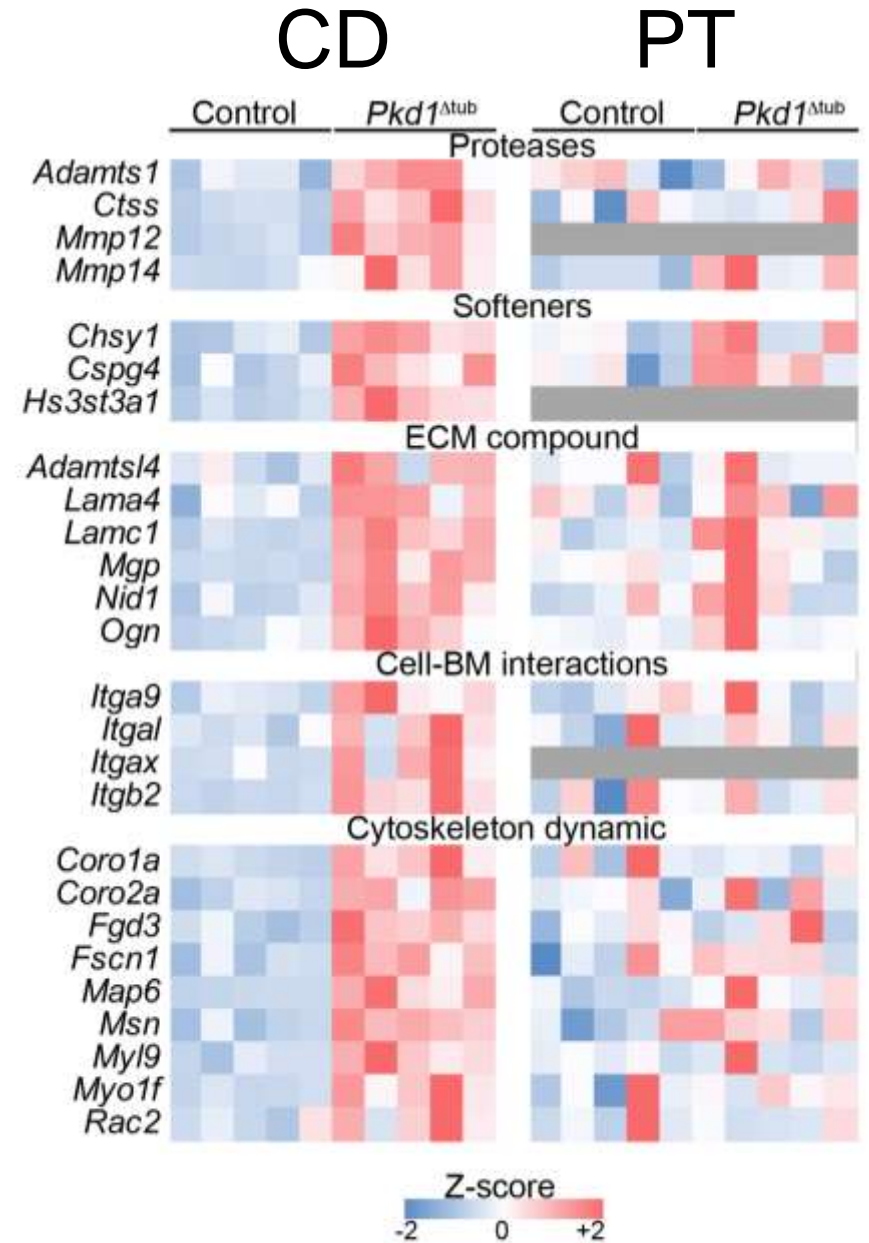


Transcriptome profiling of isolated *Pkd1*^{-/-} tubules

8 weeks
Control
Pkd1^{Δtub}



Segment specific
 RNA-Seq



Transcriptome profiling of isolated *Pkd1*^{-/-} tubules



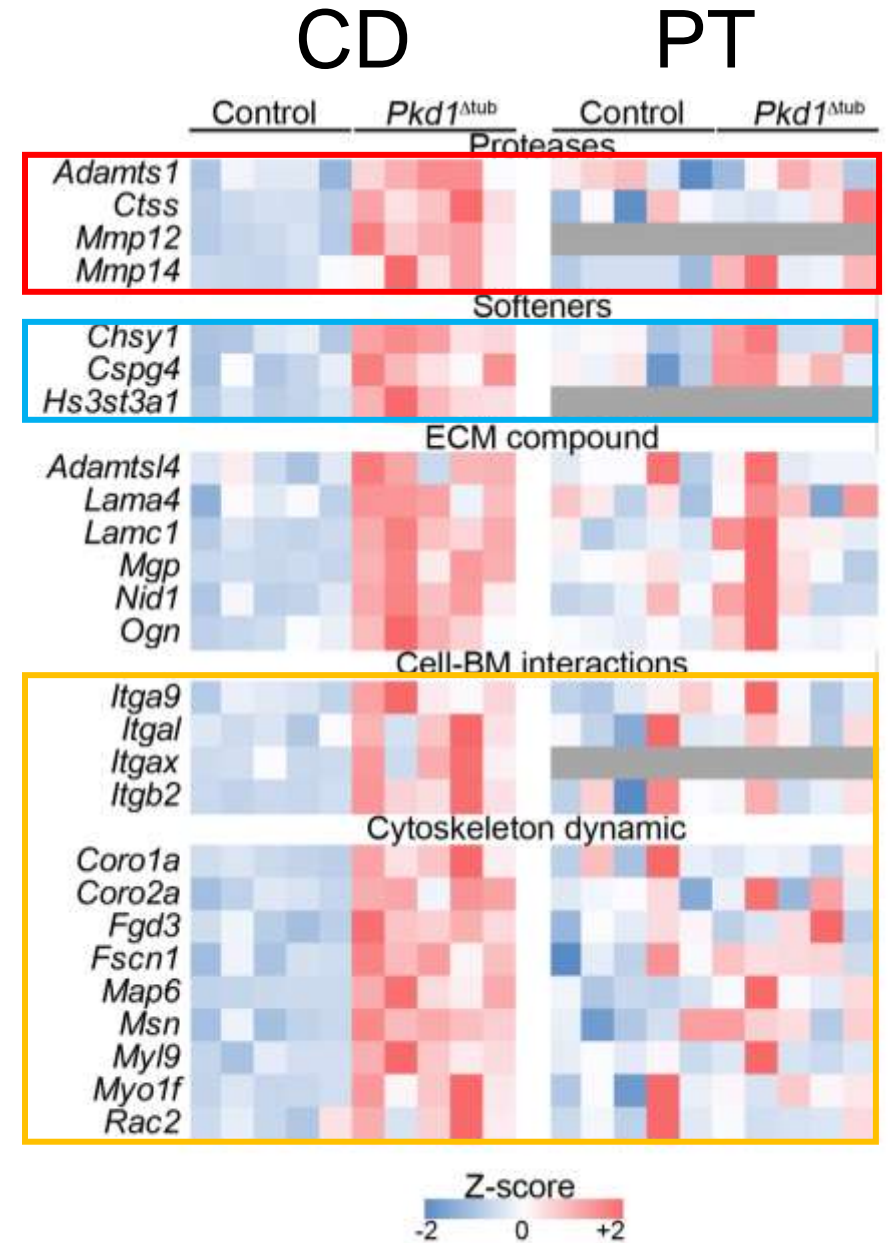
Proteases



Softeners



Traction forces

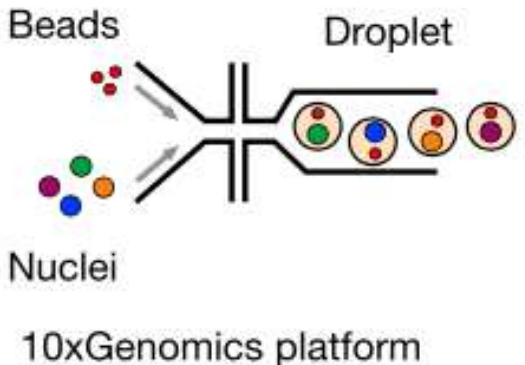


Conservation of BM remodelling signature in human ADPKD

Healthy kidney cortex

ADPKD cysts (cortical cups)

snRNA-seq or snATAC-seq

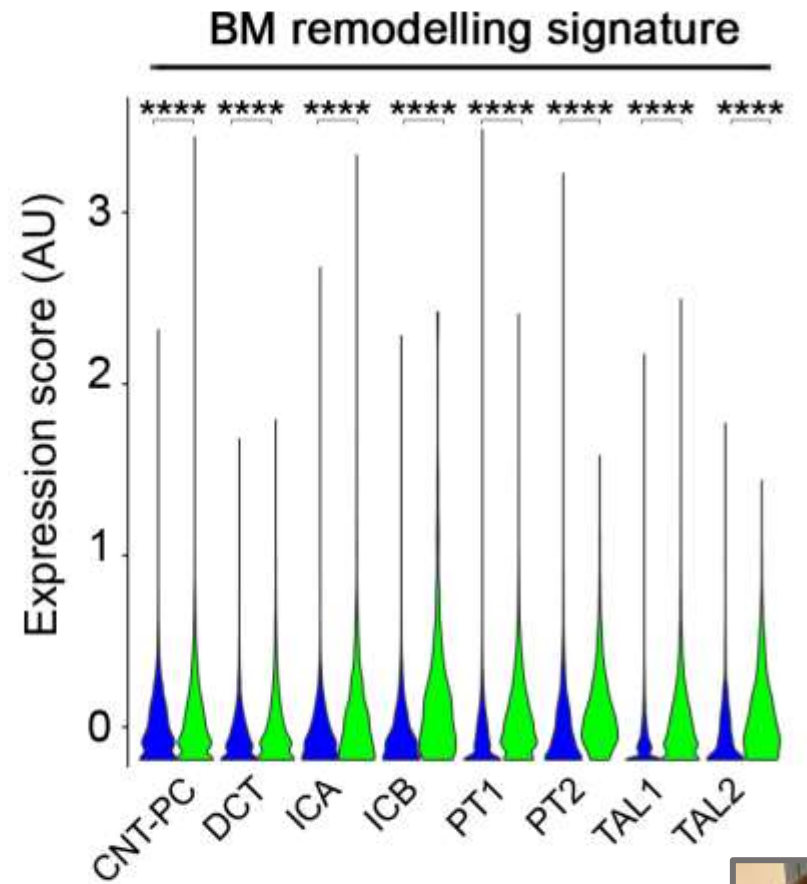
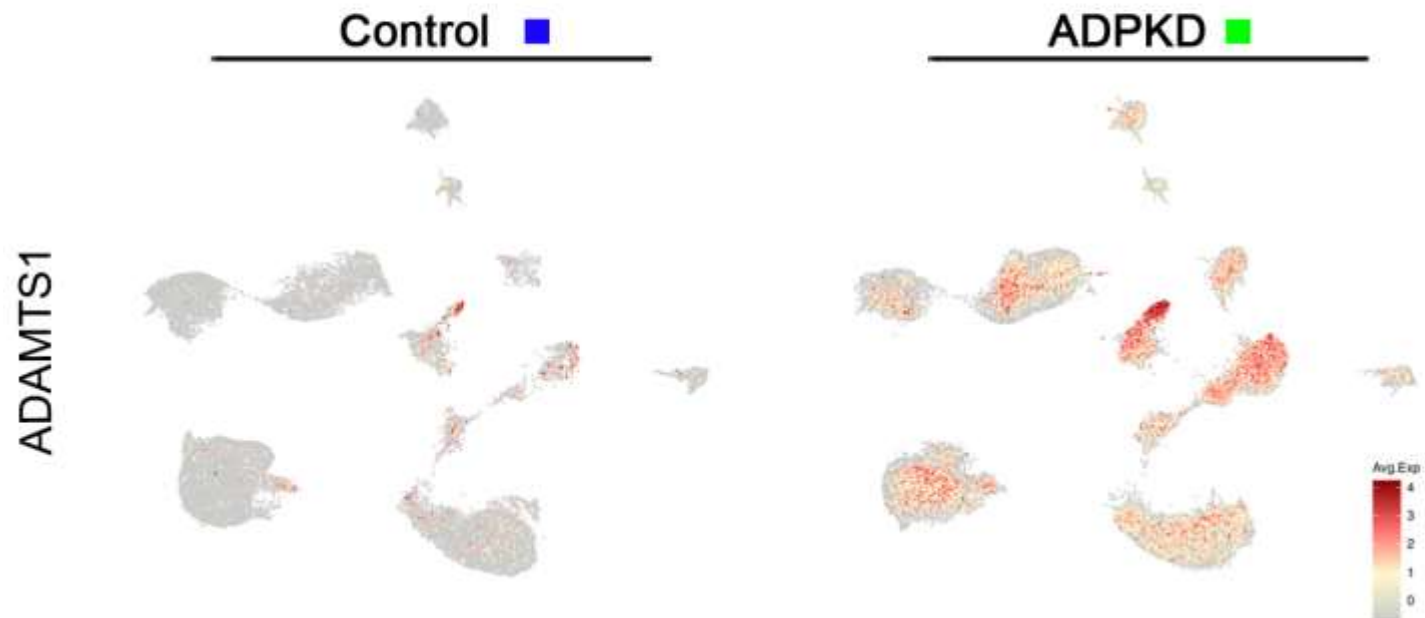
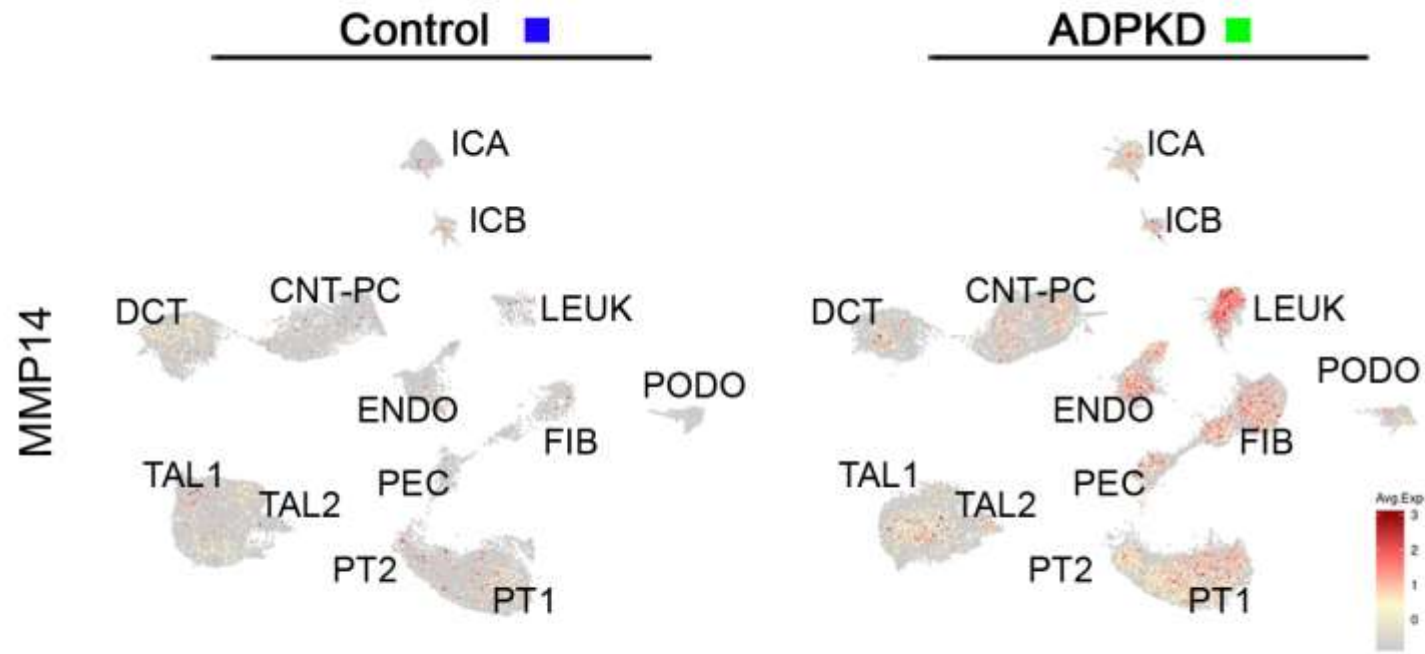


Data integration
(Harmony)

n=5

n=8

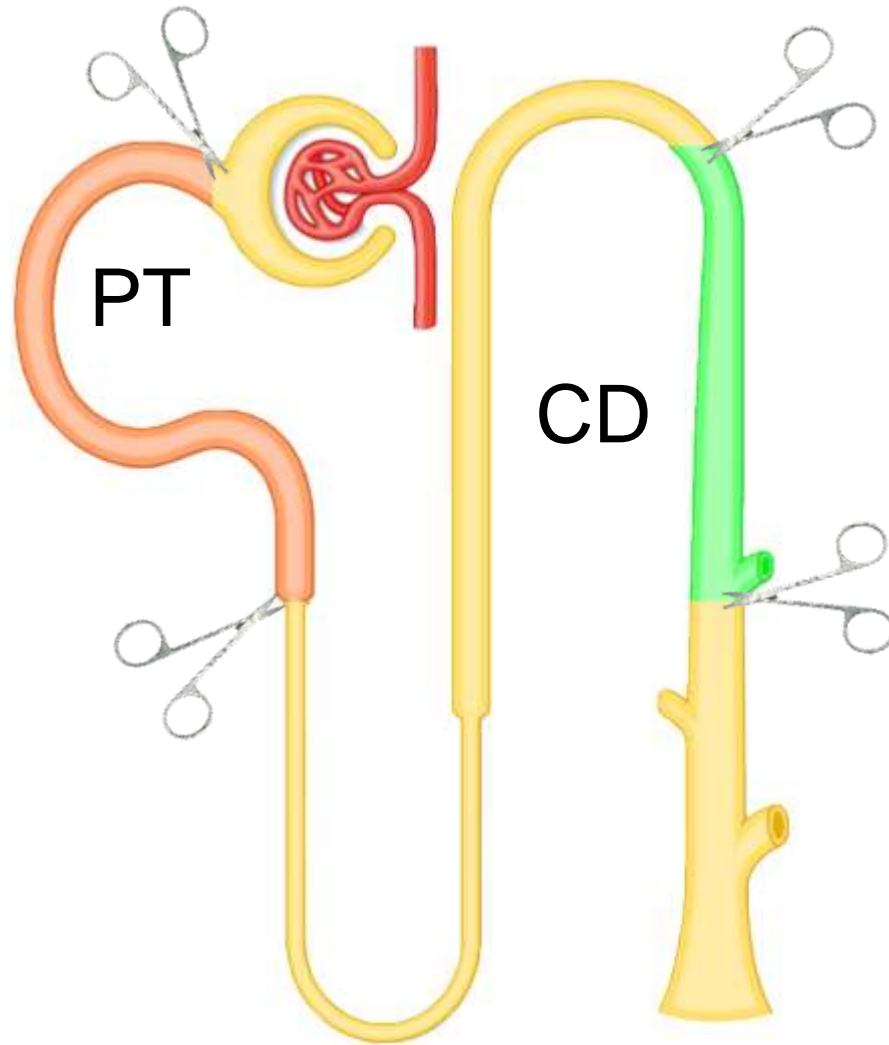
snRNAseq from human control and ADPKD kidneys (GSE185948)



C.Cohen

Does BM remodeling affects tubule mechanics ?

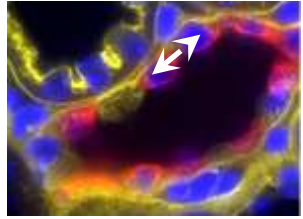
8 weeks
Control
Pkd1^{Δtub}



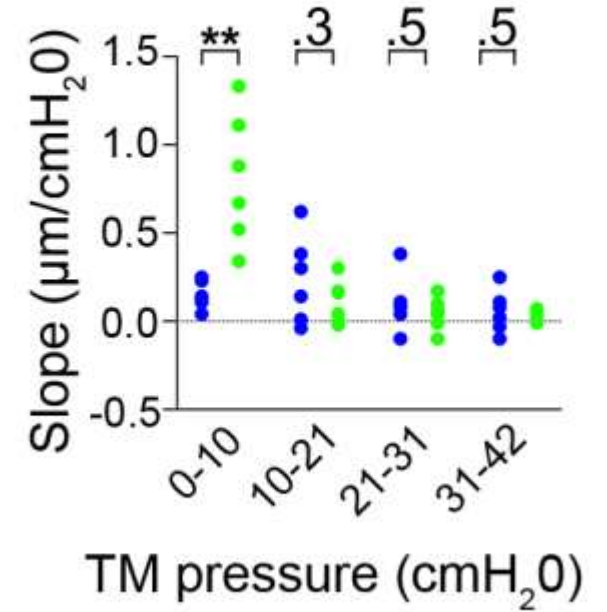
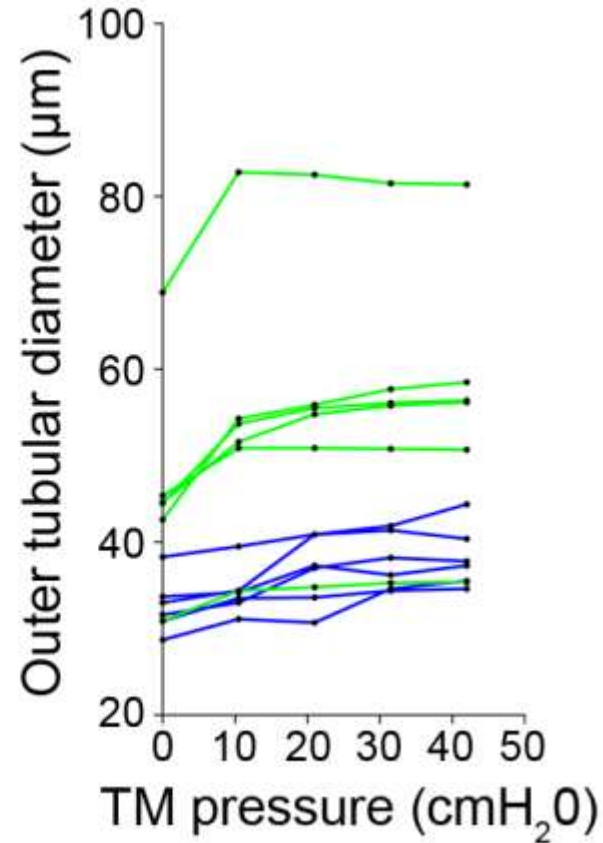
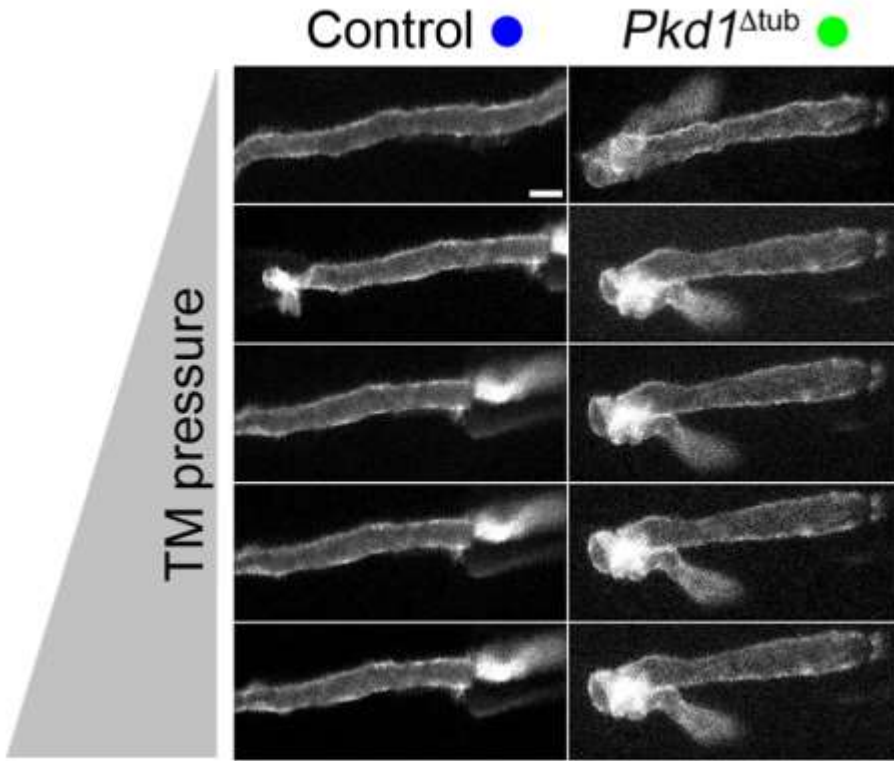
Pressure – diameter curves



Does BM remodeling affects tubule mechanics ?

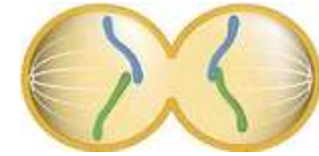


Collecting duct (8 weeks)

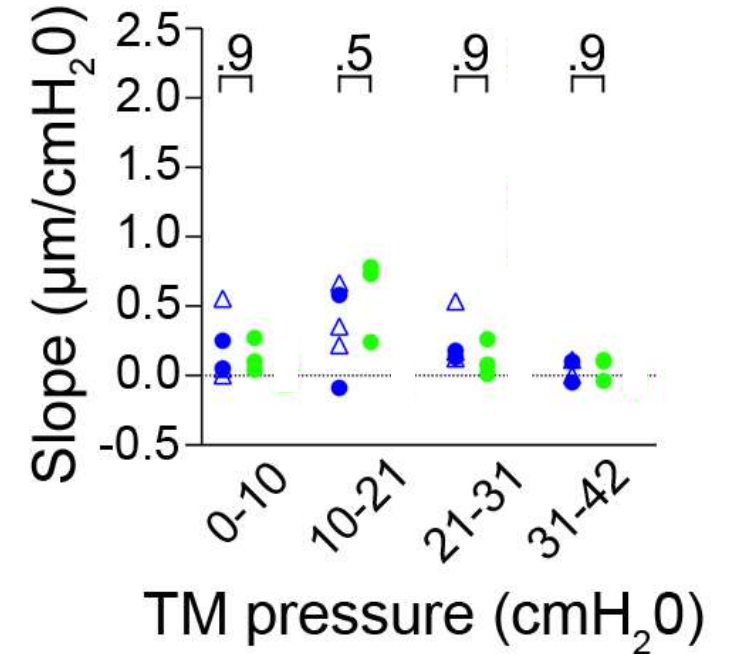
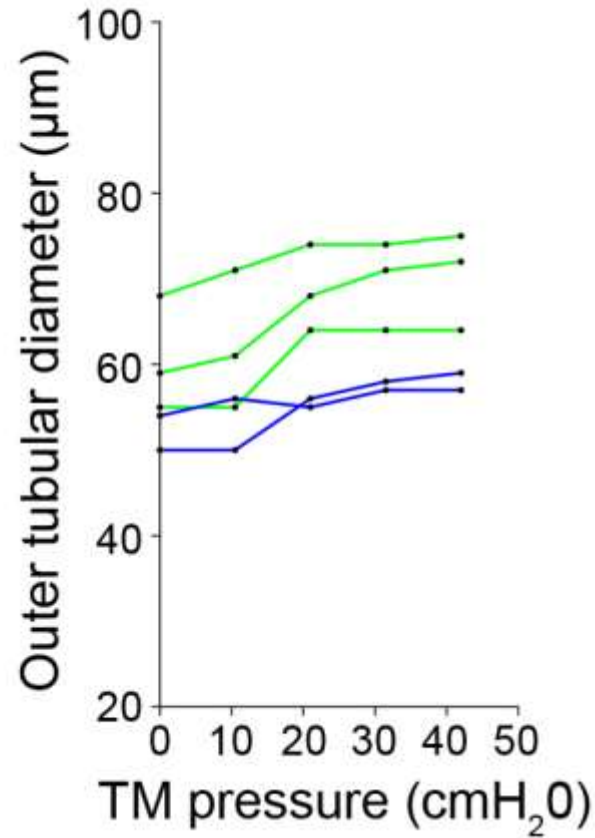
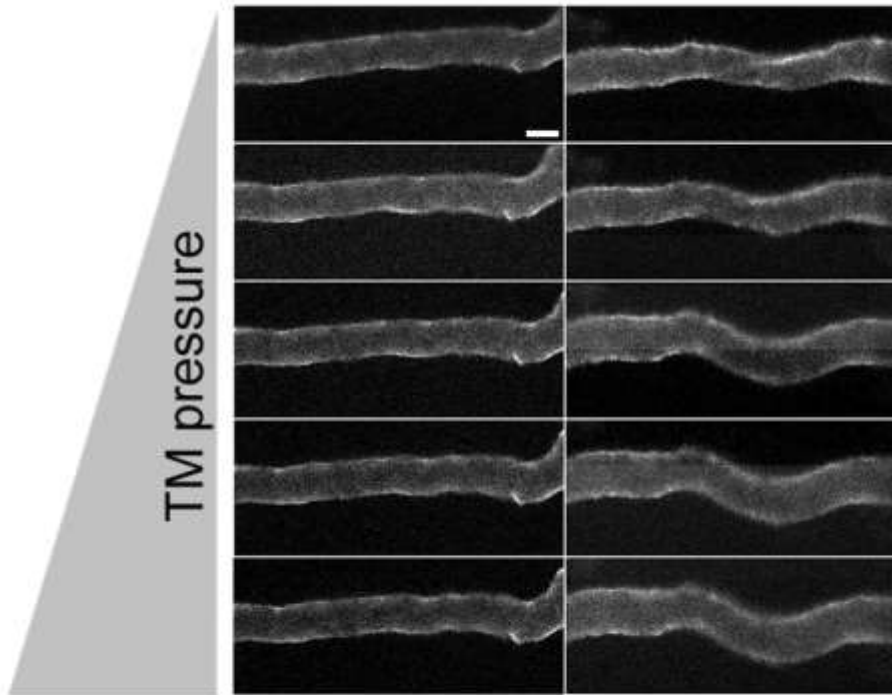


Does BM remodeling affects tubule mechanics ?

Proximal tubule (8 weeks)

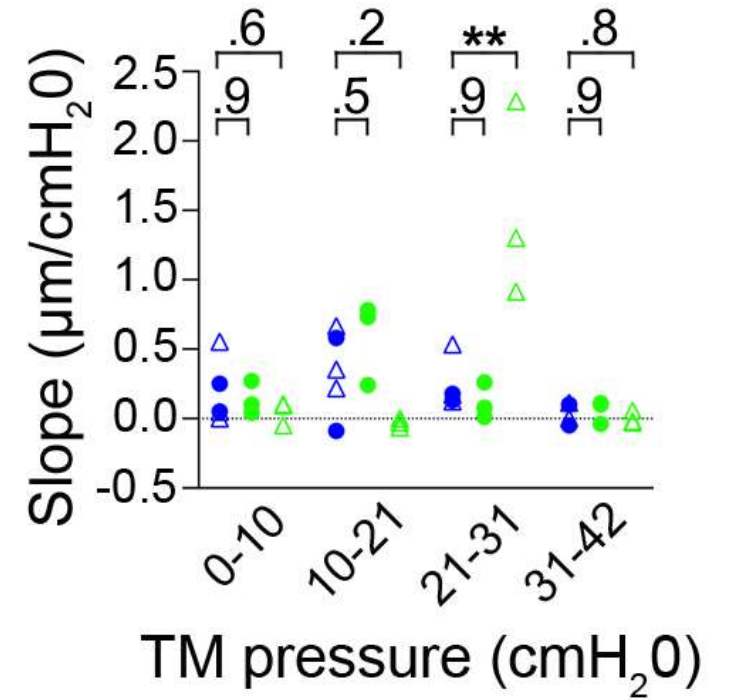
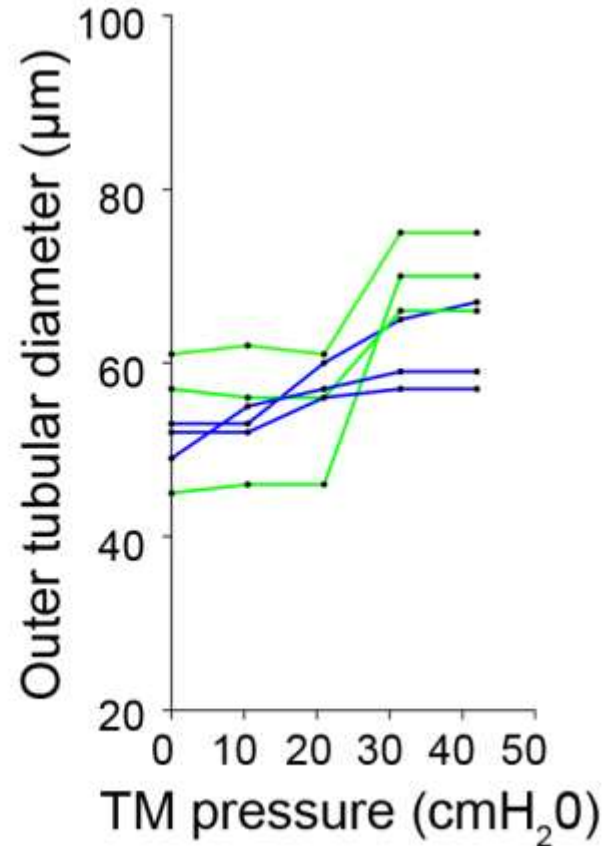
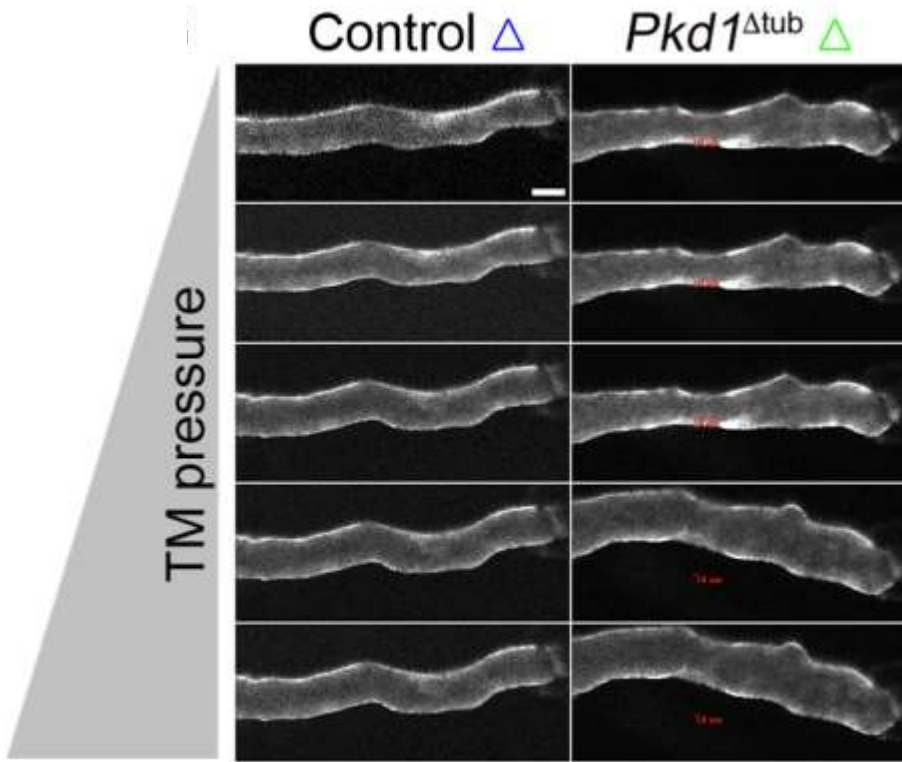
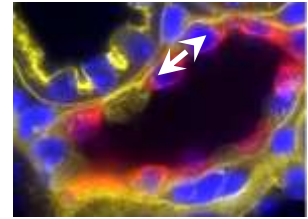
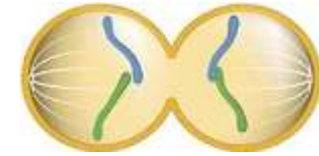


Control ● *Pkd1^{Δtub}* ●



Does BM remodeling affects tubule mechanics ?

Proximal tubule (12 weeks)

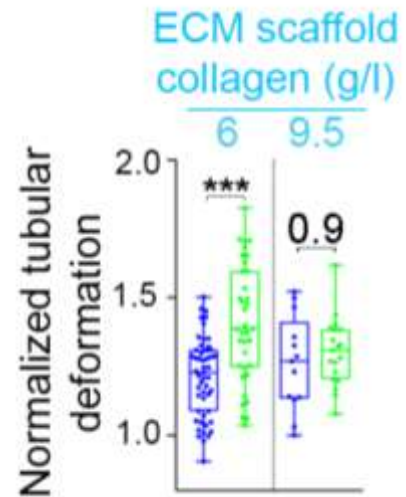
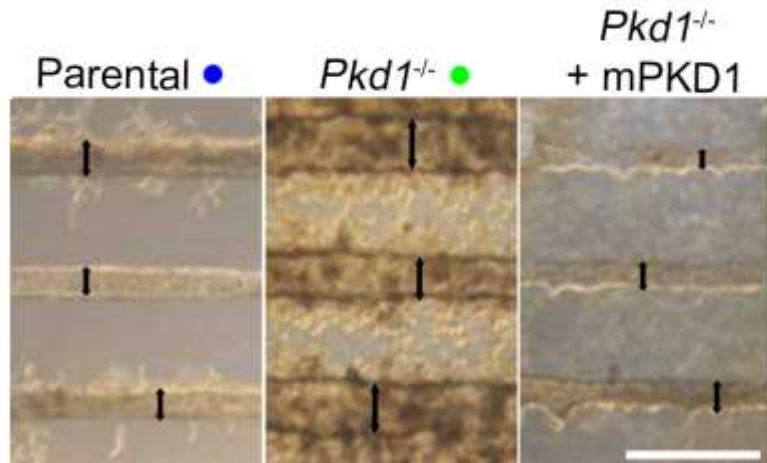


=> YES

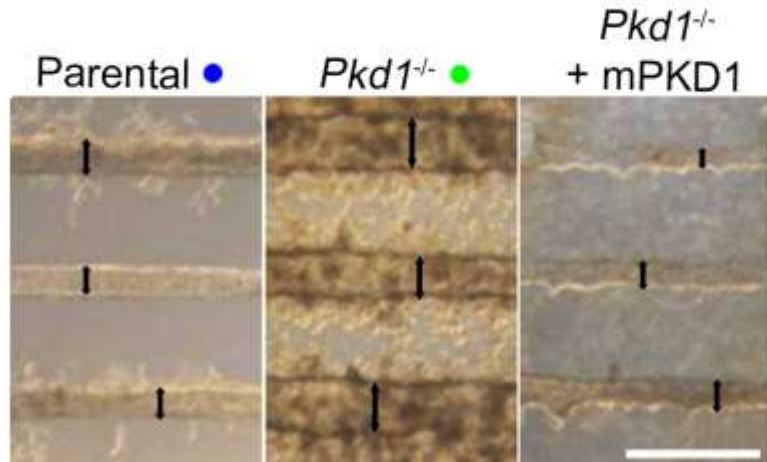


Does BM mechanics modulate cystogenesis ?

Does BM mechanics modulate cystogenesis ?



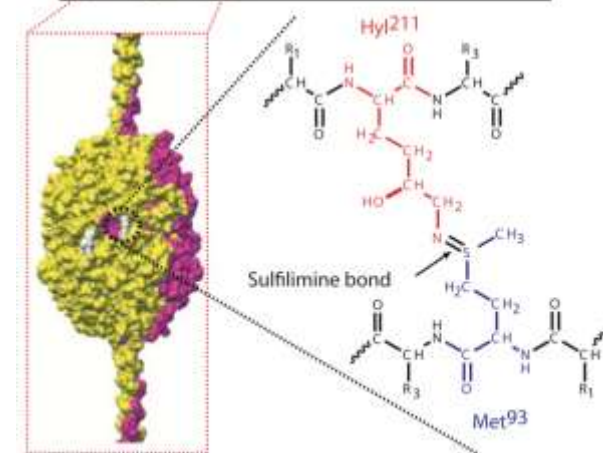
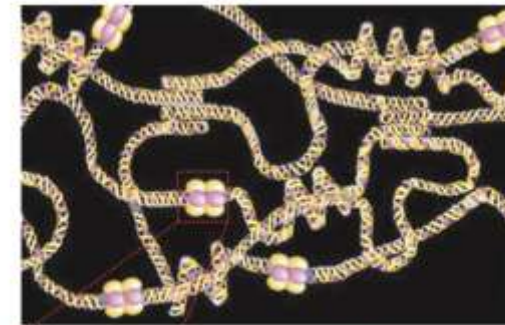
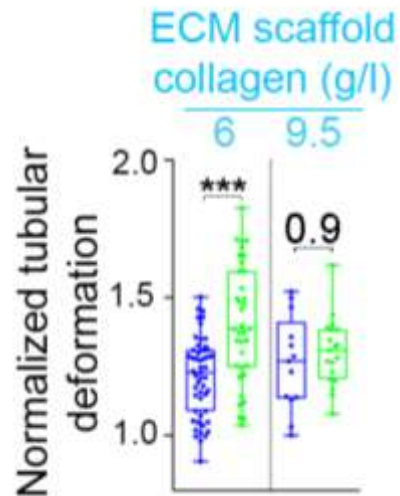
Does BM mechanics modulate cystogenesis ?



Peroxidasin KO mice (*Pxdn*^{-/-})



- ✓ Abolished sulfilimine cross-linking of collagen IV
- ✓ Increased TBM compliance



Miklos Geiszt

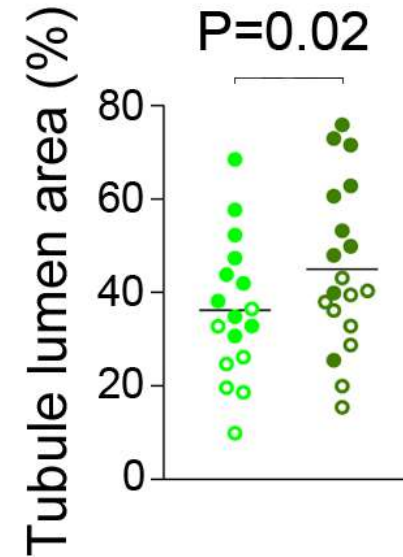
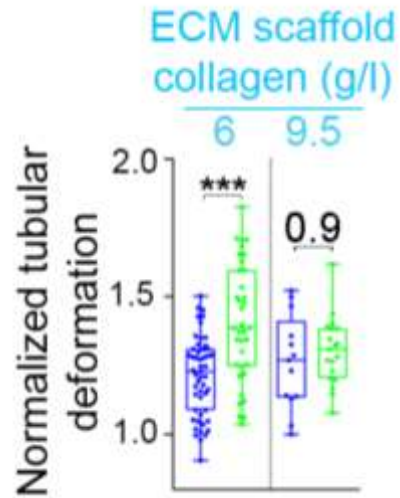
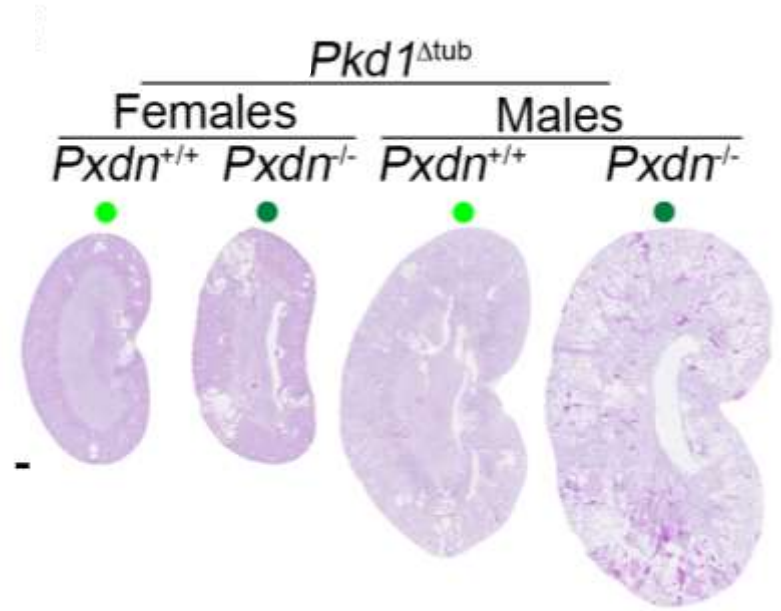
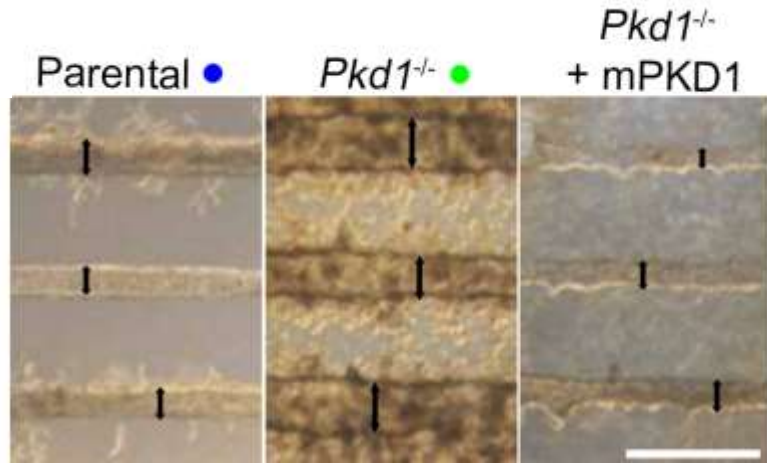


Bhave G *et al.* Am J Physiol Renal Physiol (2017)

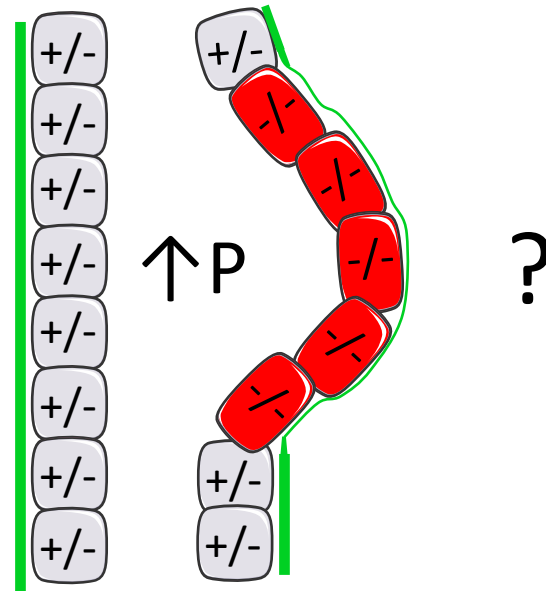
Vanacore R *et al.* Science (2009)

Bhave G *et al.* Nat Chem Biol (2012)

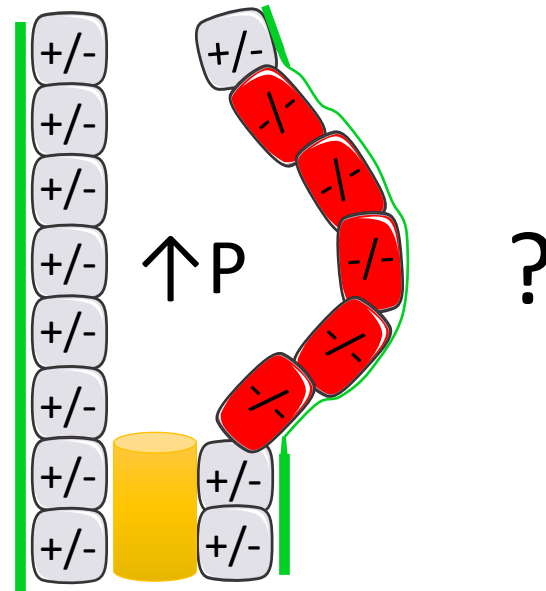
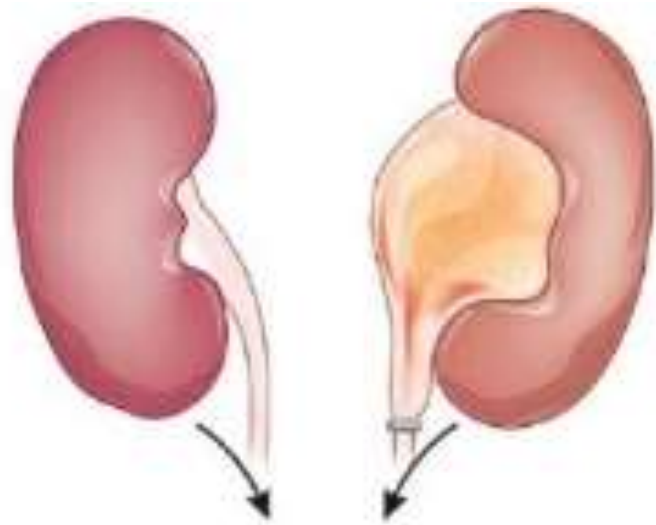
Does BM mechanics modulate cystogenesis ?



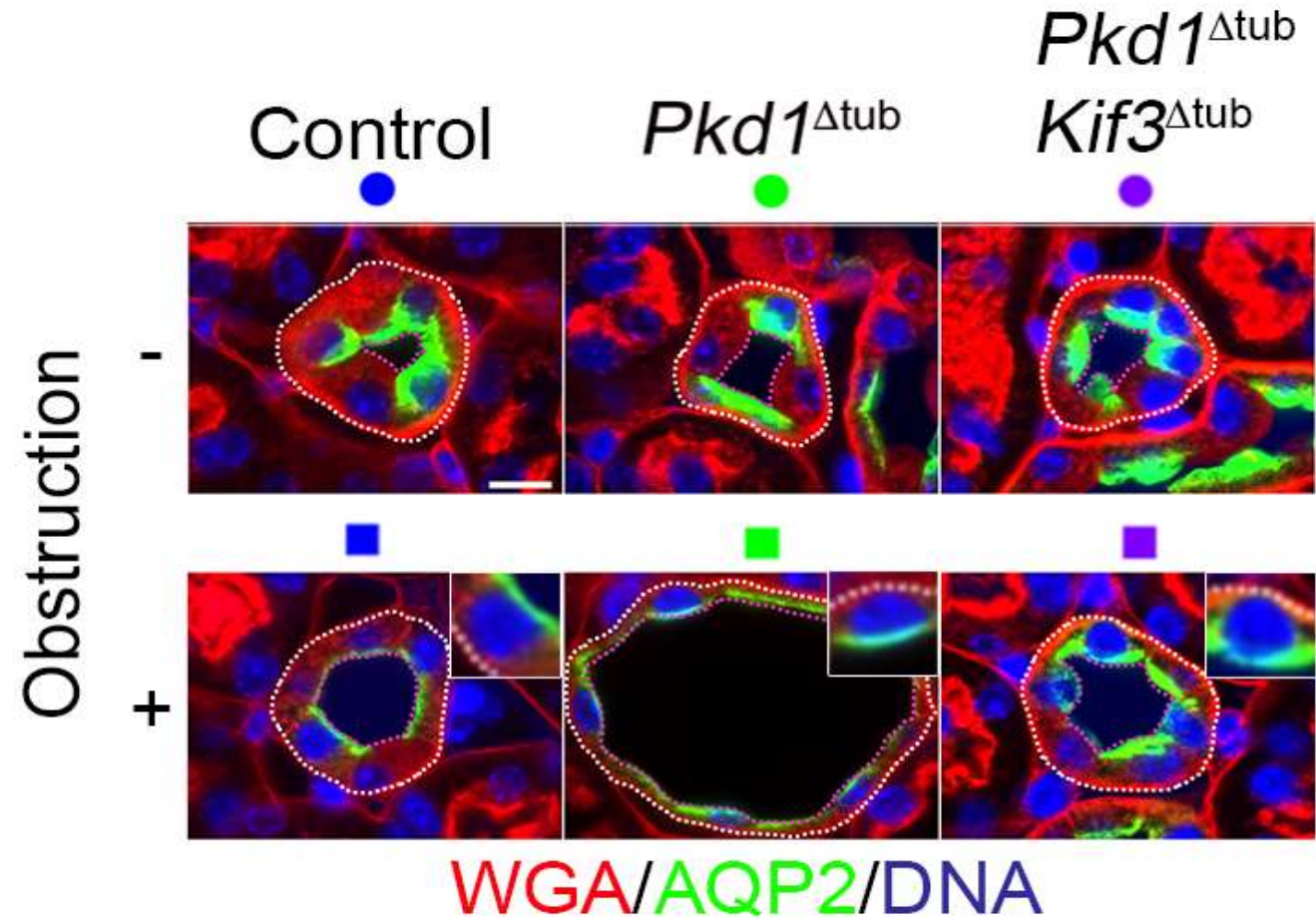
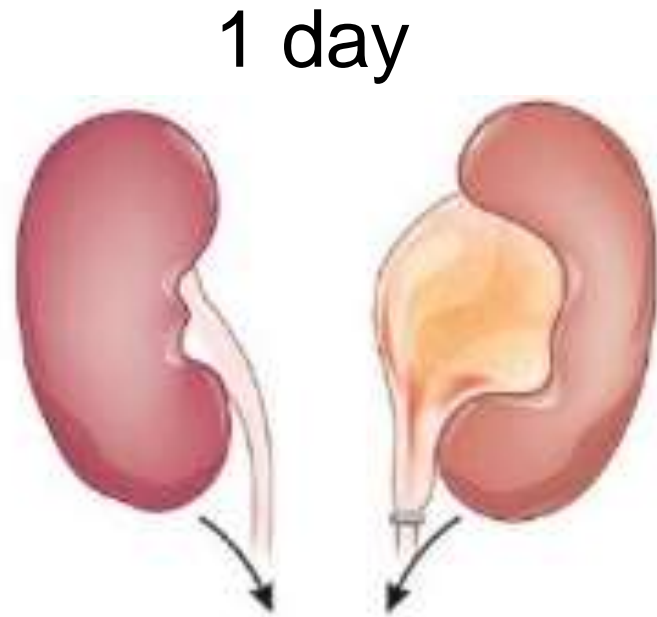
How intratubular pressure impacts tubule distension ?



How intratubular pressure impacts tubule distension ?

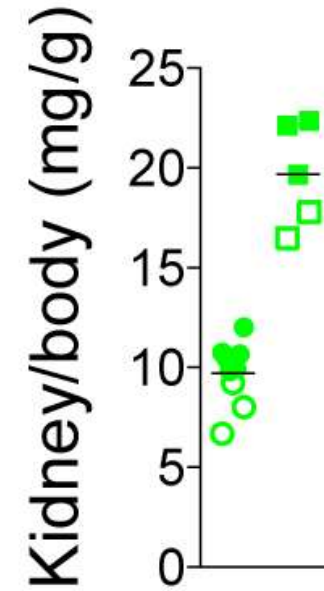
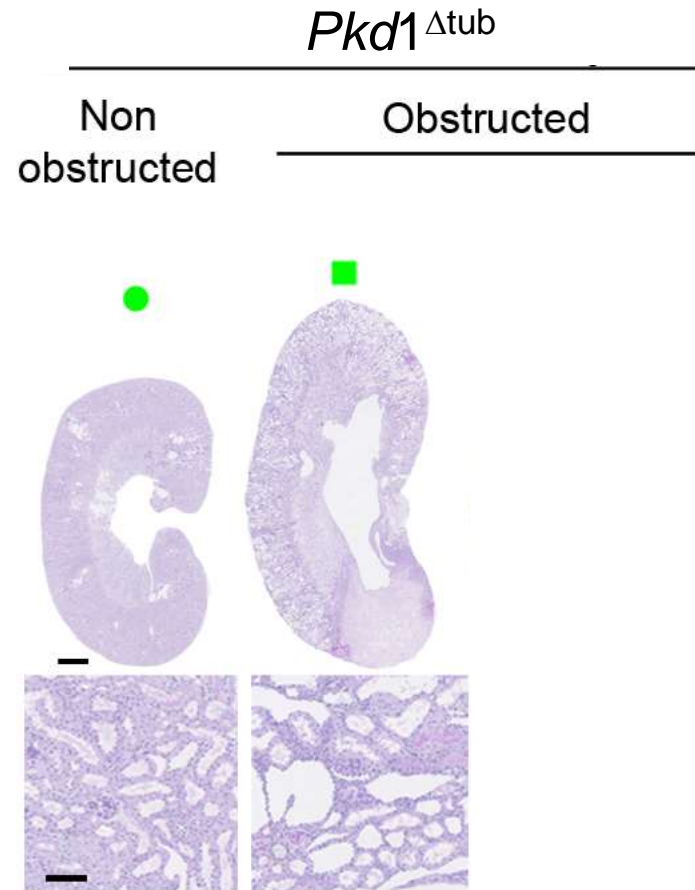
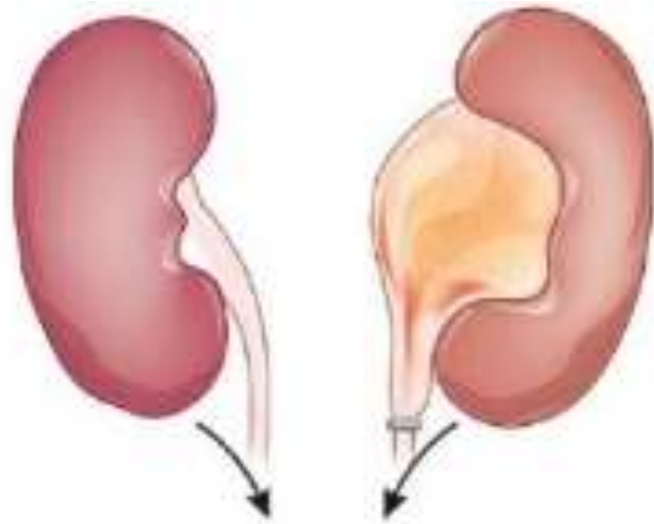


How intratubular pressure impacts tubule distension ?

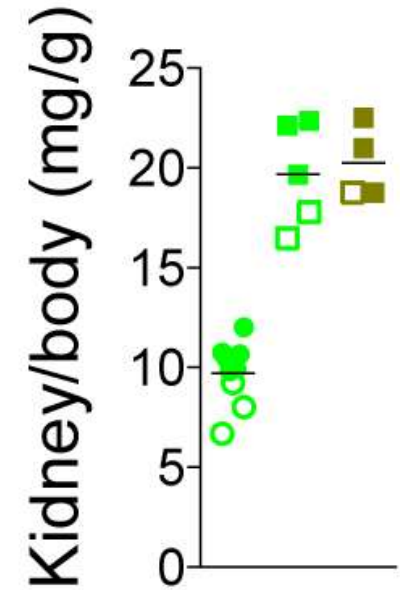
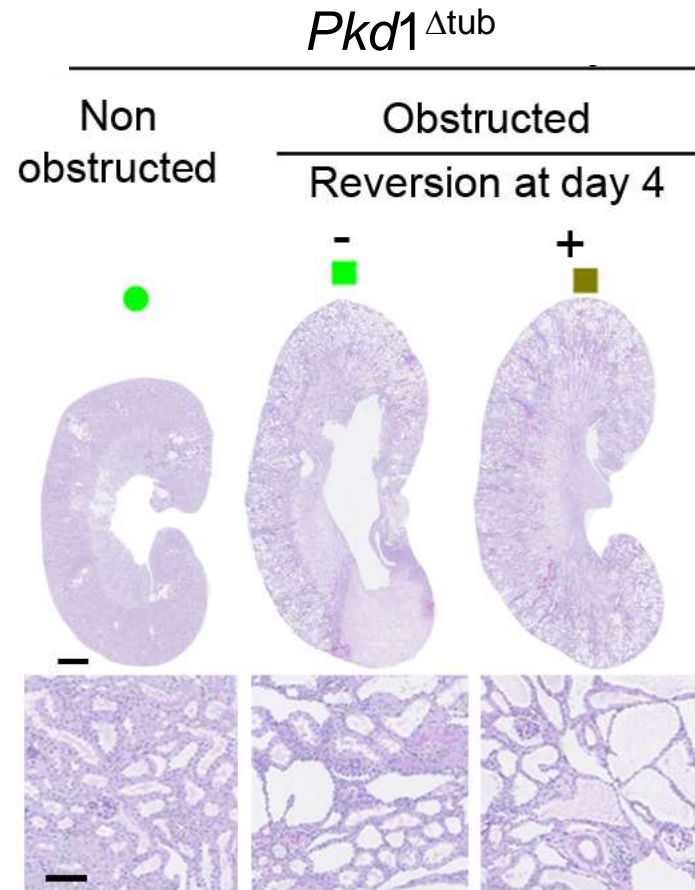
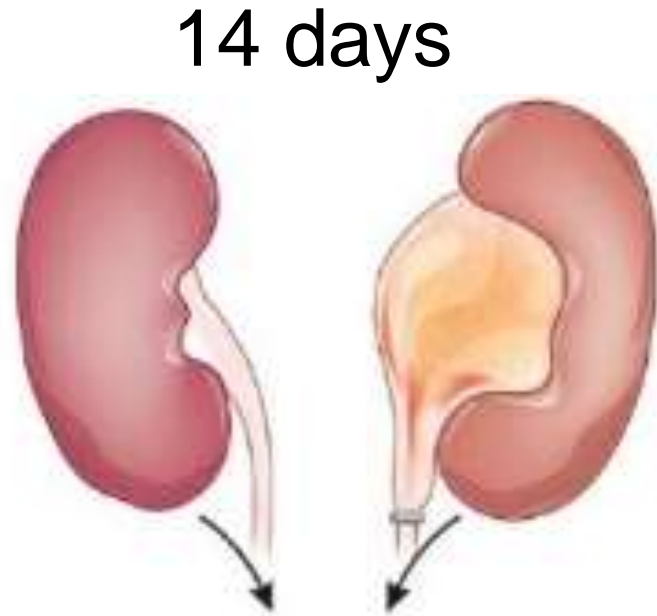


How intratubular pressure impacts tubule distension ?

14 days



How intratubular pressure impacts tubule distension ?

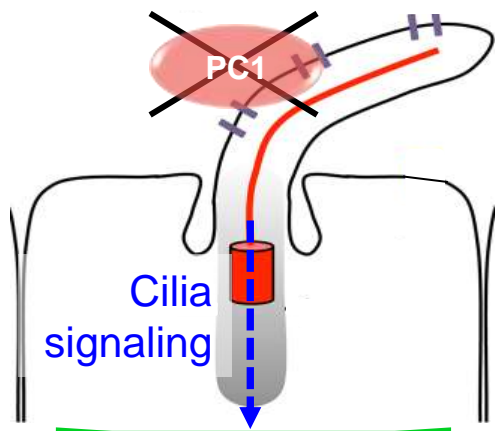


Obstruction precipitates irreversible cystogenesis



Tyre bulge model

Cilia dependent BM remodeling

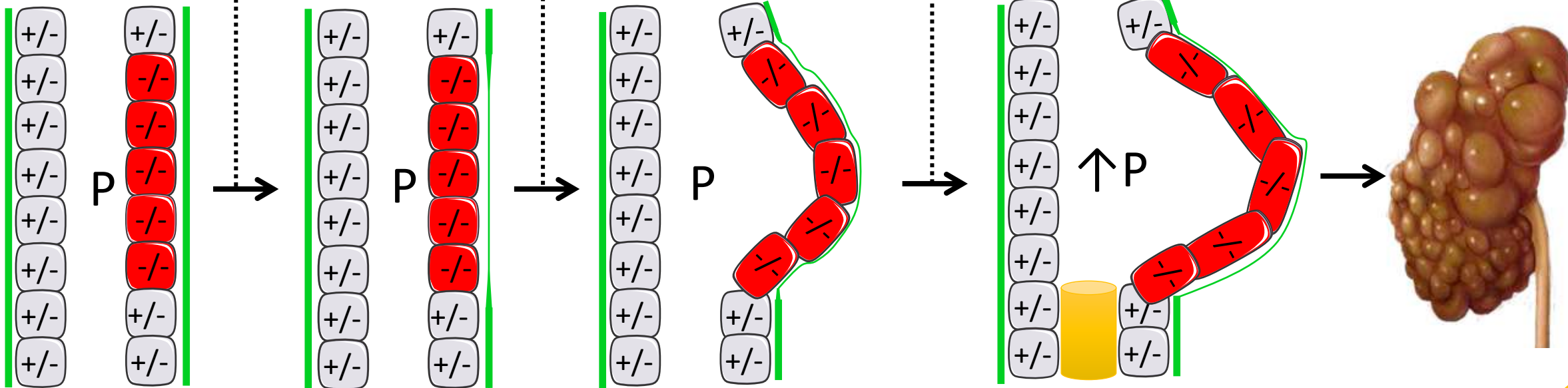
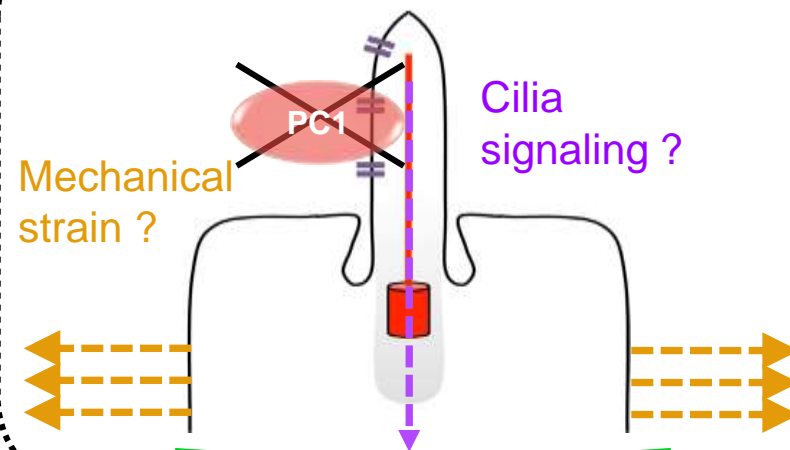


Tubule distension



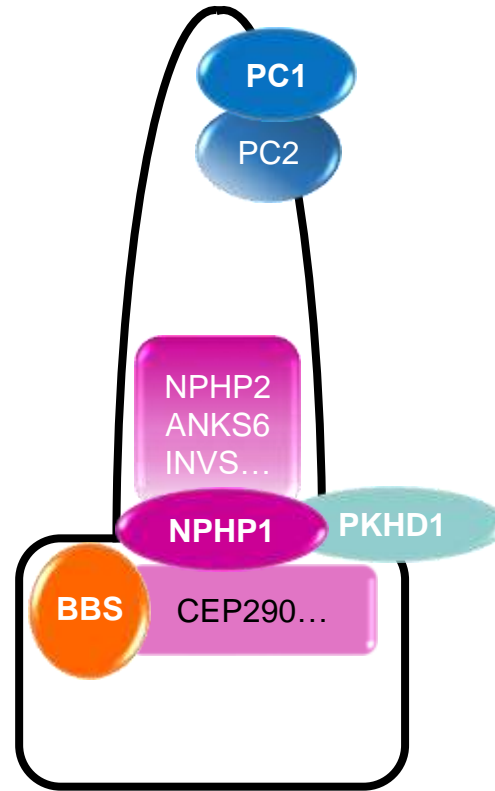
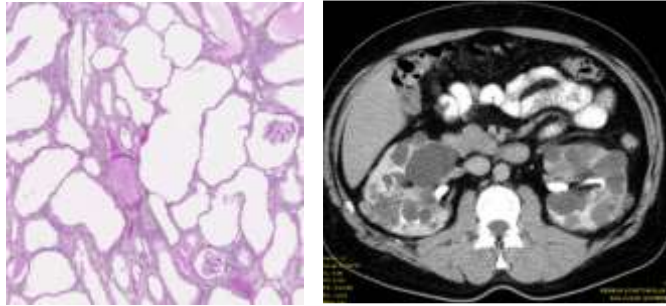
↑ cell stretch

Obstruction triggers cyst expansion

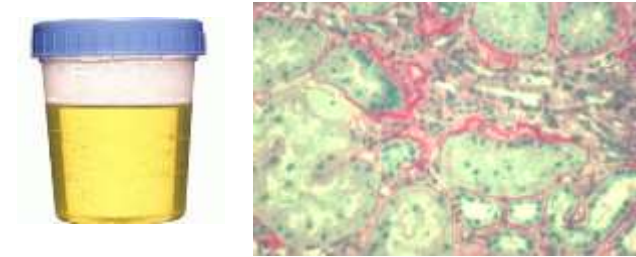


Mutations in ciliary genes cause kidney inflammation & fibrosis

Autosomal Dominant Polycystic Kidney Disease

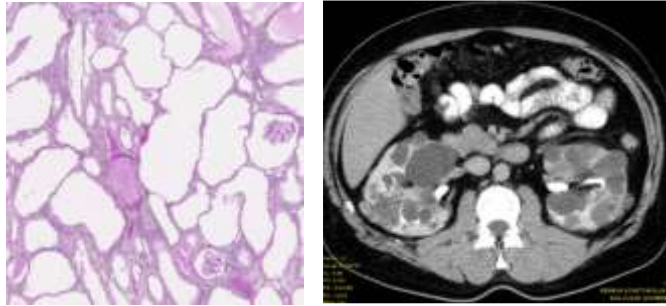


Nephronophthisis

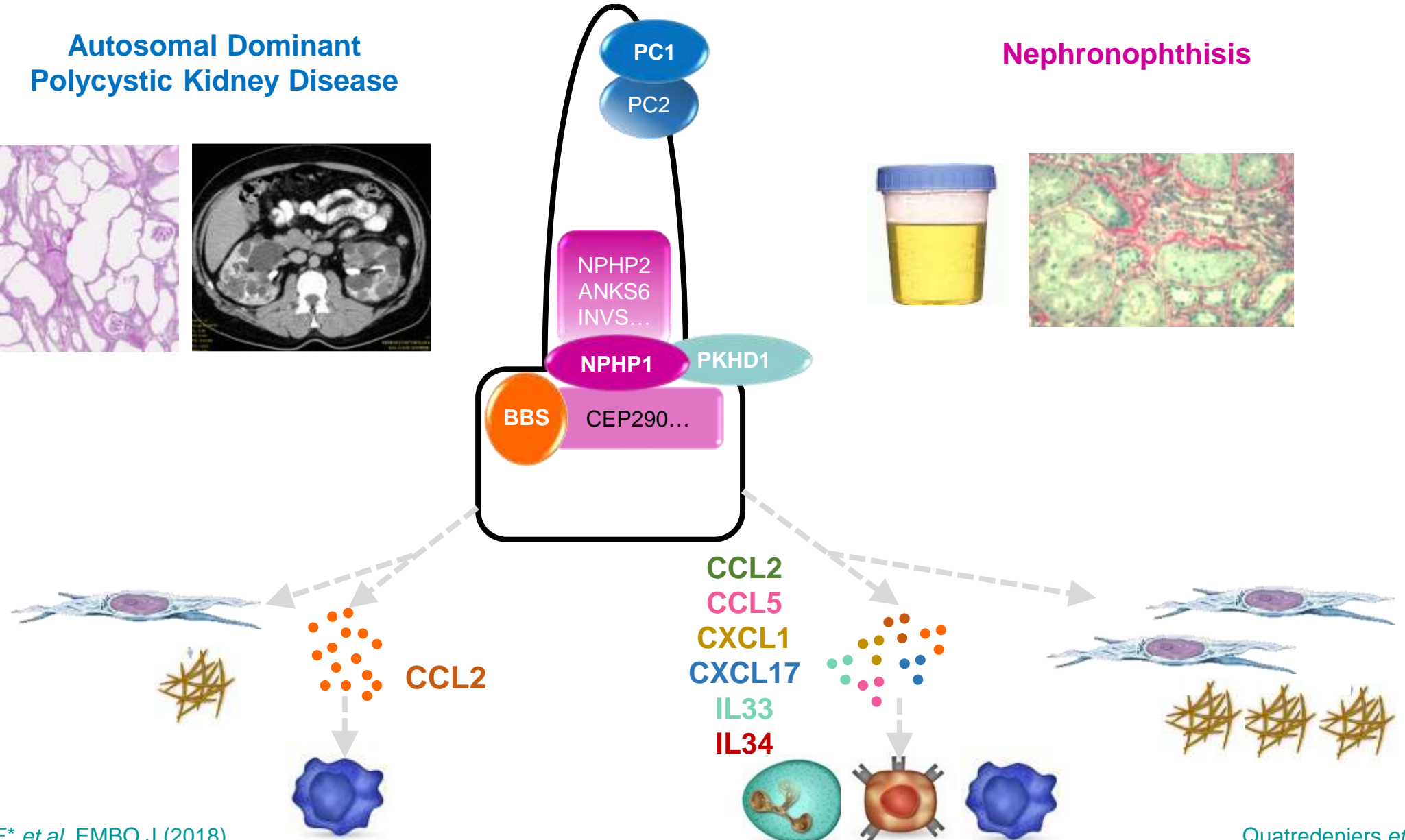
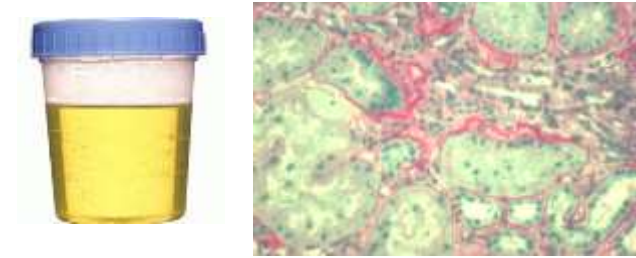


Mutations in ciliary genes cause kidney inflammation & fibrosis

Autosomal Dominant Polycystic Kidney Disease



Nephronophthisis

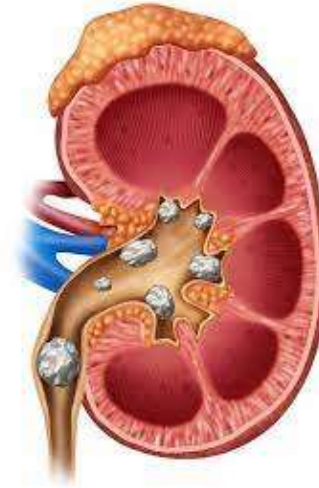
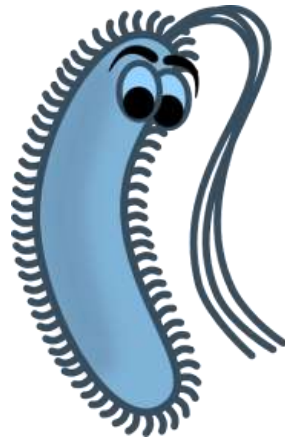


Question

Do primary cilia play physiological roles in the control of kidney inflammation and fibrosis ?

Question

Do primary cilia play physiological roles in the control of kidney inflammation and fibrosis ?



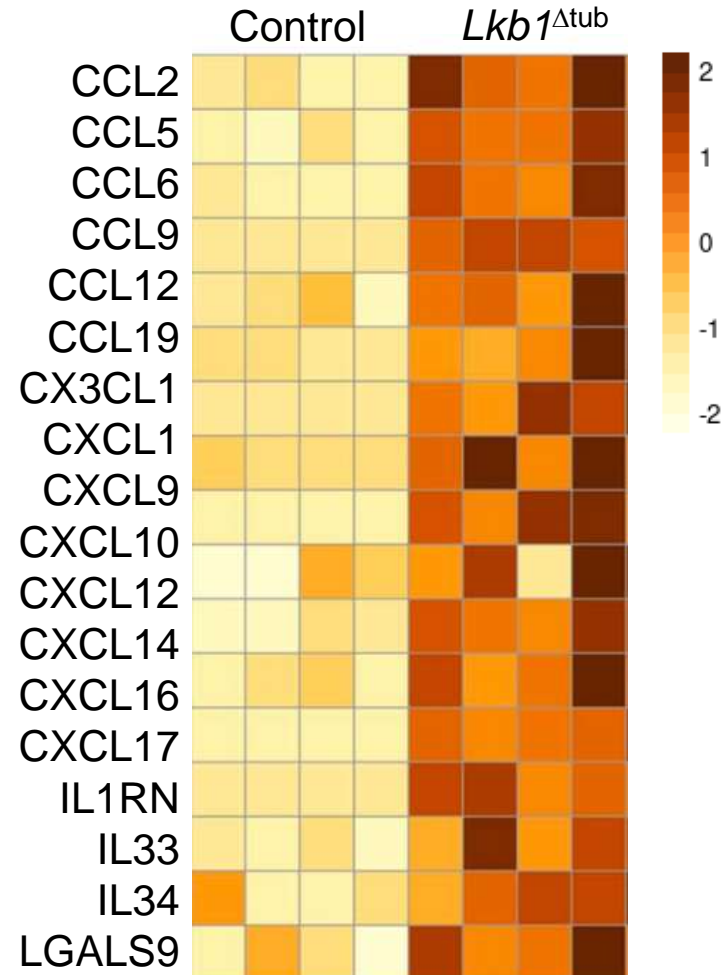
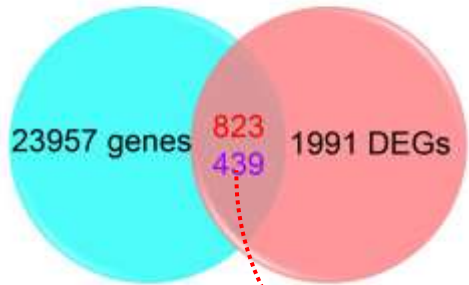
Tubular obstruction and infection evoke ciliopathy associated cytokine network



NPH models

Glis2
Dataset

Lkb1
Dataset

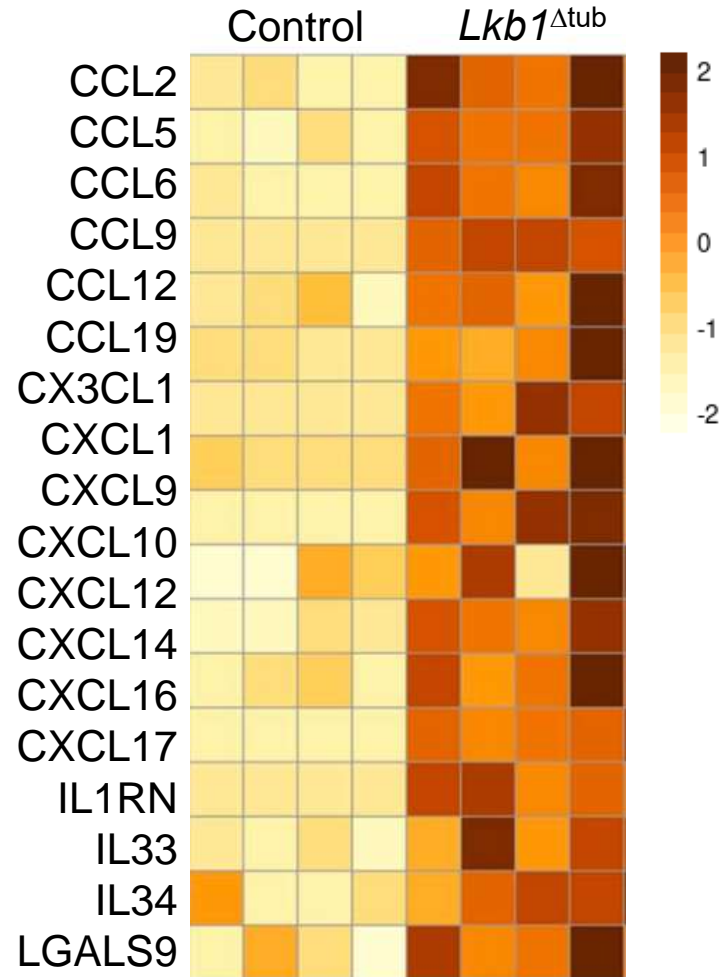
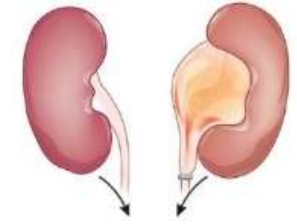
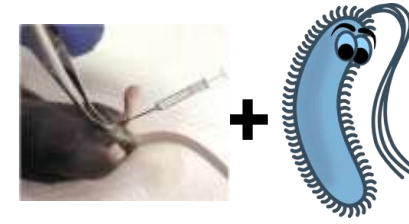
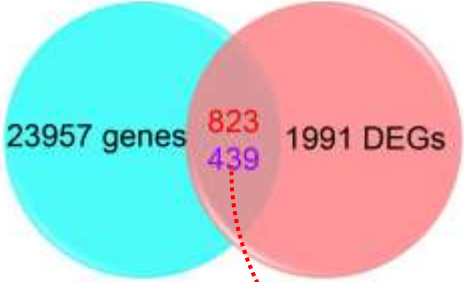


Tubular obstruction and infection evoke ciliopathy associated cytokine network

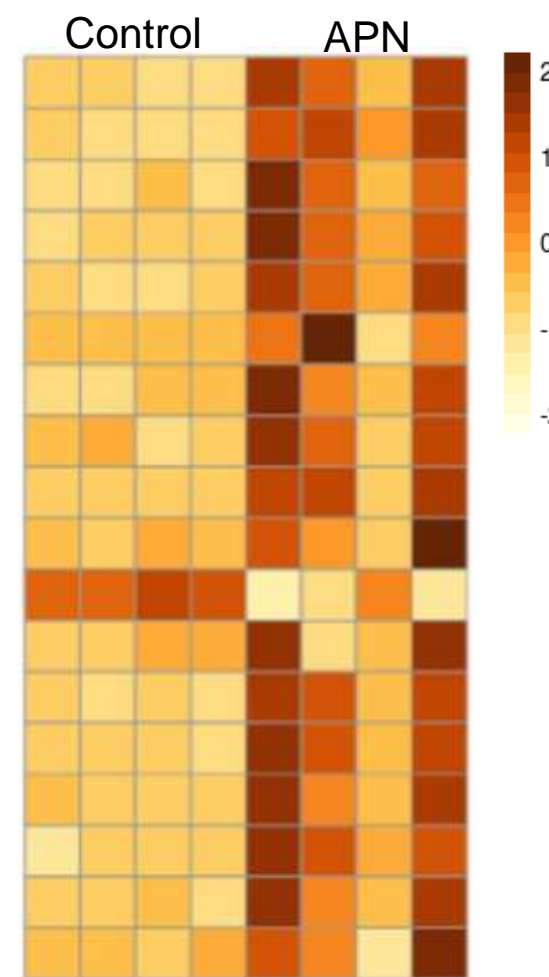


Glis2 Dataset

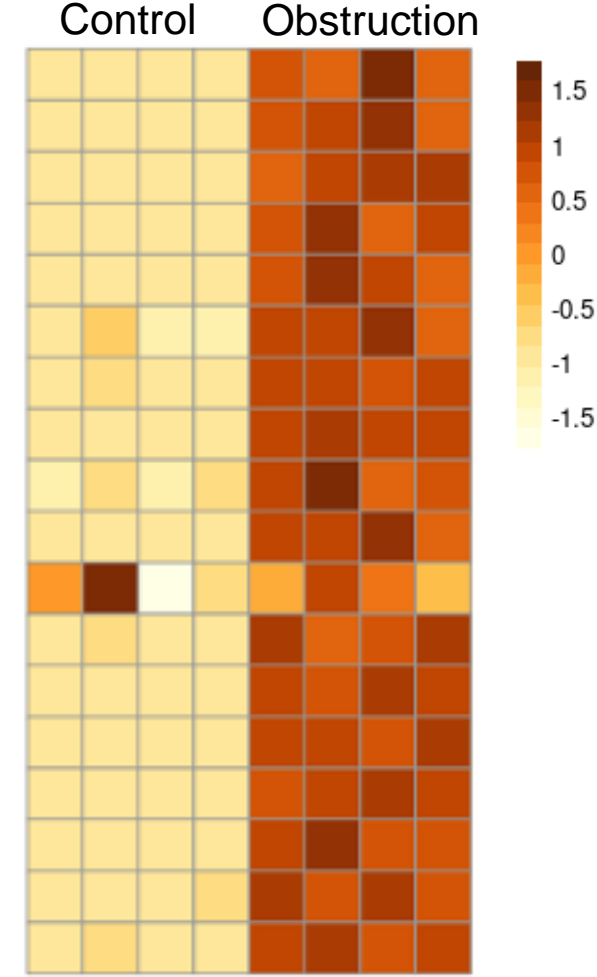
Lkb1 Dataset



Quatredenier. *Hum Mol Genet.* 2022

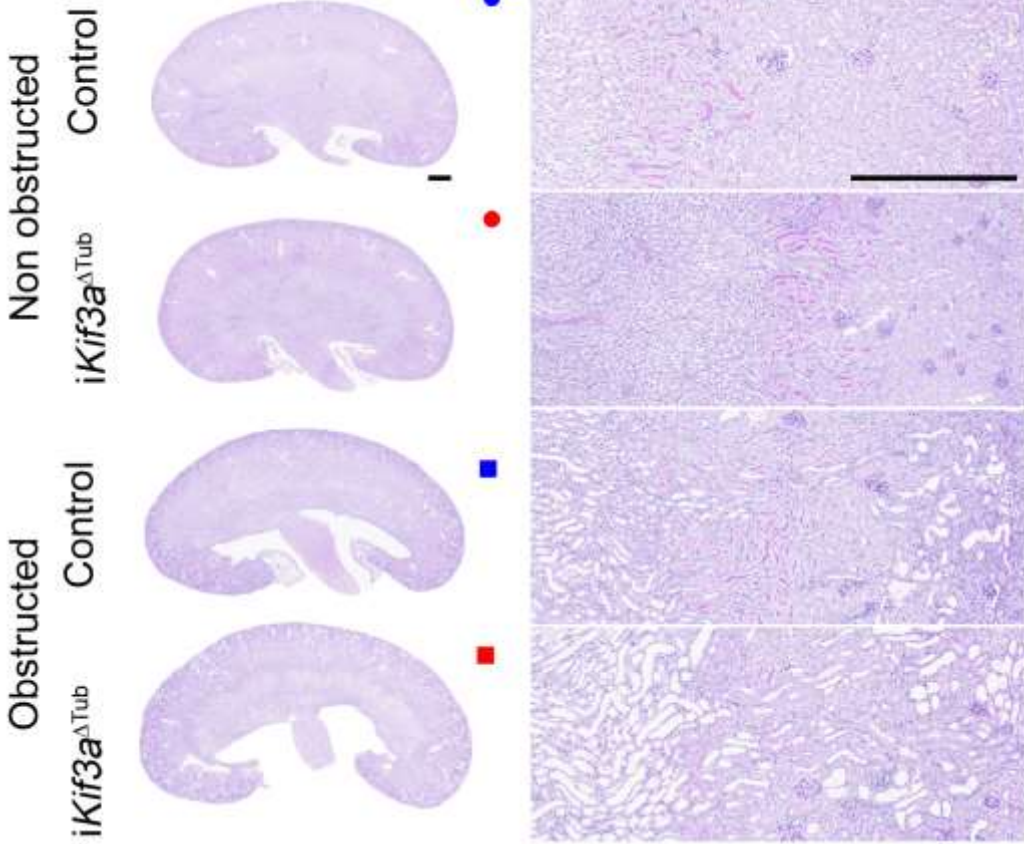


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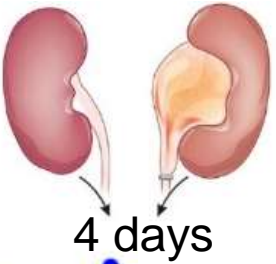


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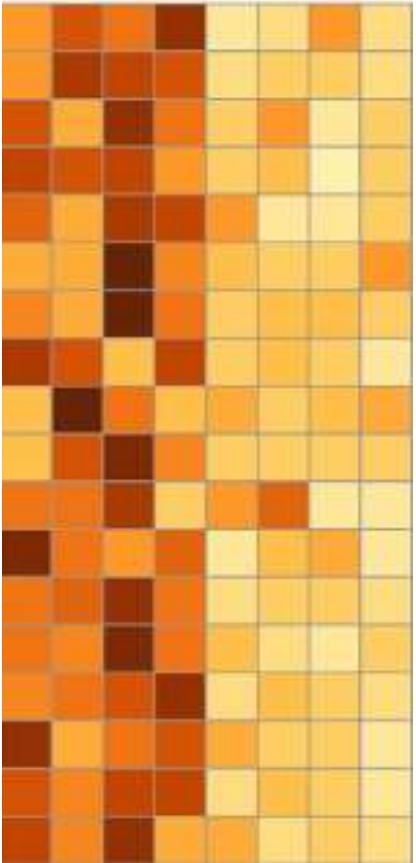
Loss of cilia reduce kidney fibro-inflammatory response to obstruction



Loss of cilia reduce kidney fibro-inflammatory response to obstruction

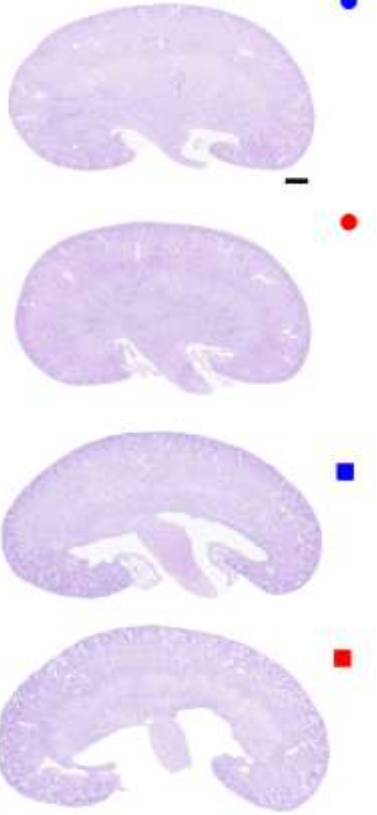


Obstruction
Cilia + Cilia -

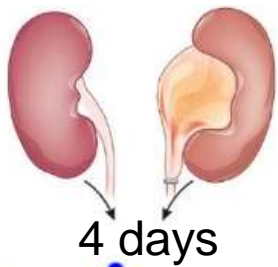


- CCL2**
- CCL5***
- CCL6
- CCL9
- CCL12
- CCL19
- CX3CL1**
- CXCL1***
- CXCL9
- CXCL10*
- CXCL12
- CXCL14**
- CXCL16**
- CXCL17****
- IL1RN****
- IL33**
- IL34***
- LGALS9

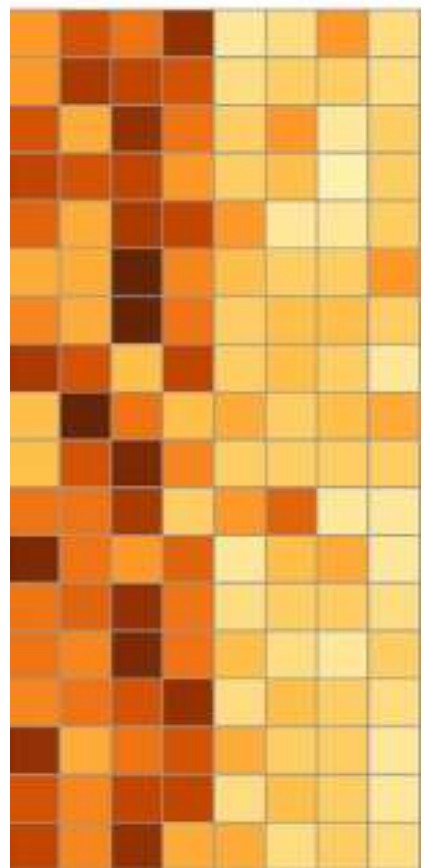
Non obstructed
Control
iKif3a^{ΔTub}
Obstructed
Control
iKif3a^{ΔTub}



Loss of cilia reduce kidney fibro-inflammatory response to obstruction

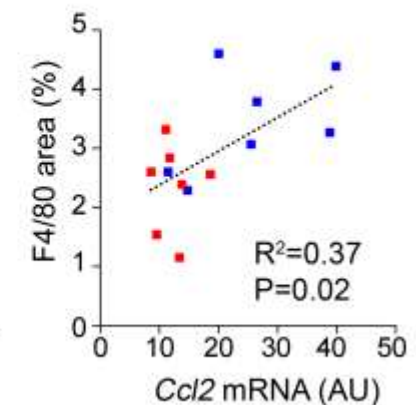
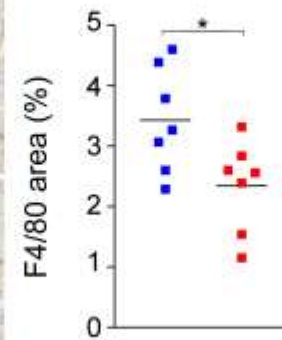
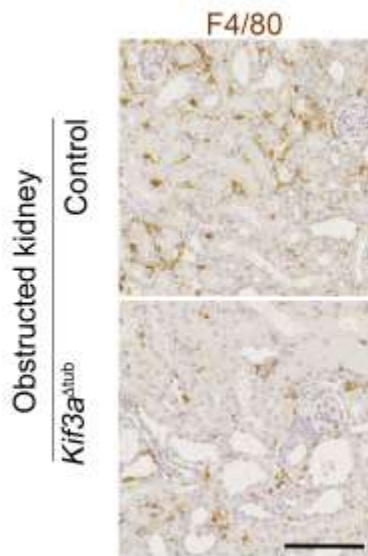
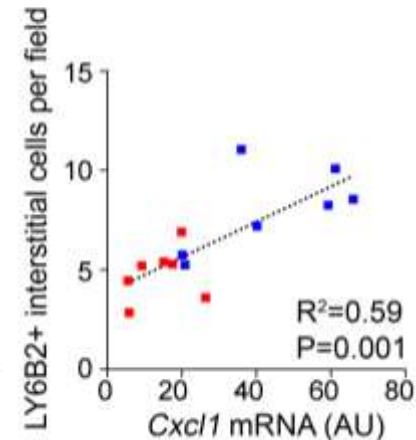
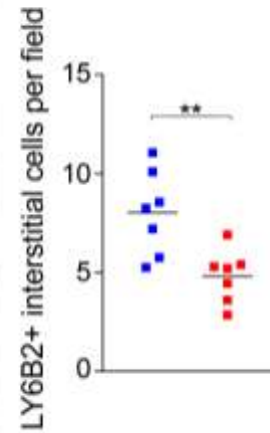
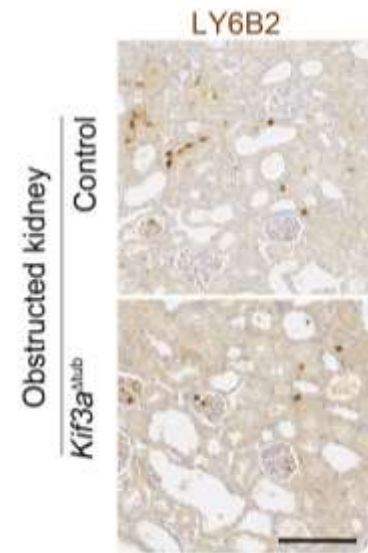
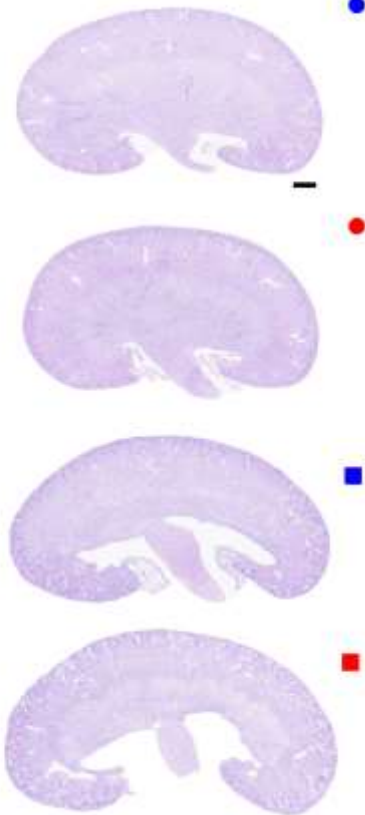


Obstruction
Cilia + Cilia -

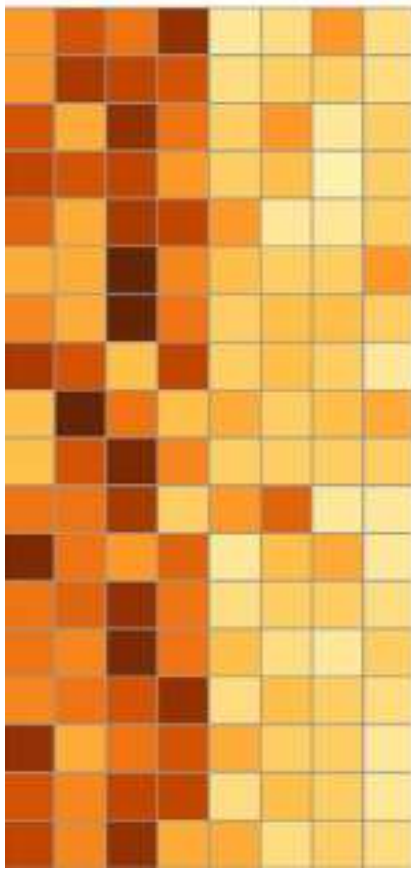
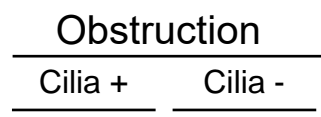
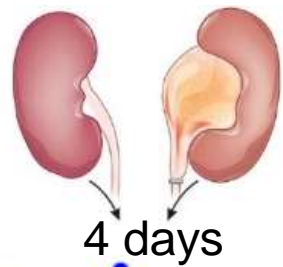


- CCL2**
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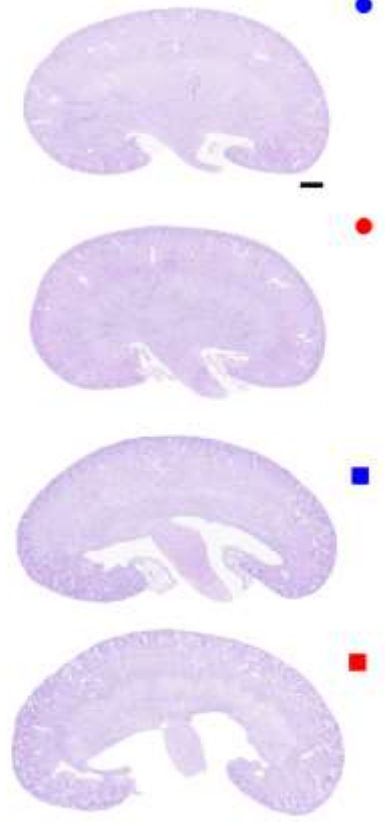
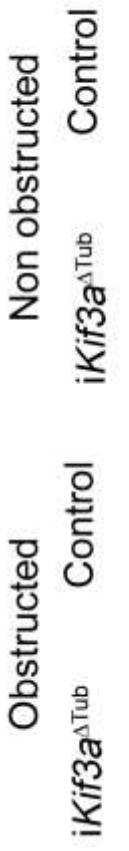
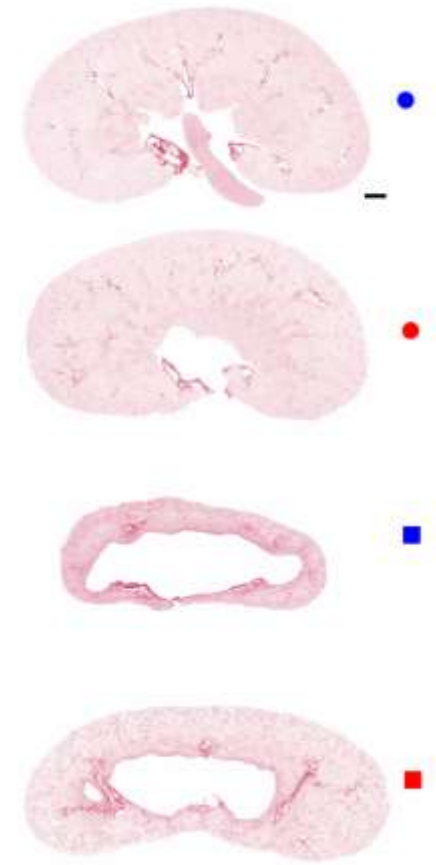
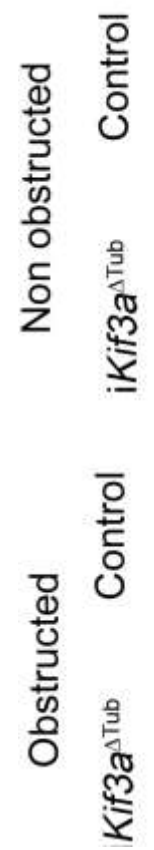
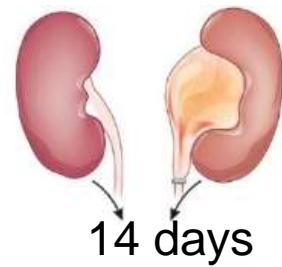
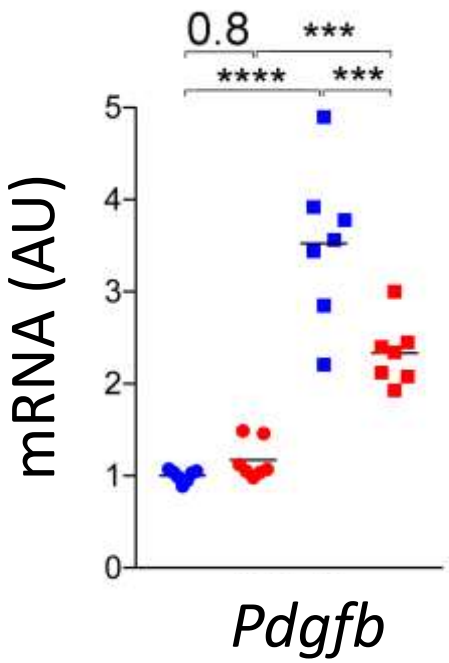
Non obstructed
Control
iKif3a^{ΔTub}
Obstructed
Control
iKif3a^{ΔTub}



Loss of cilia reduce kidney fibro-inflammatory response to obstruction

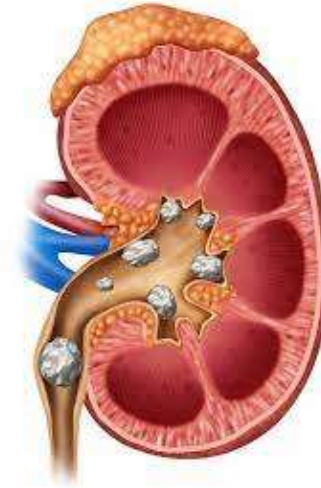
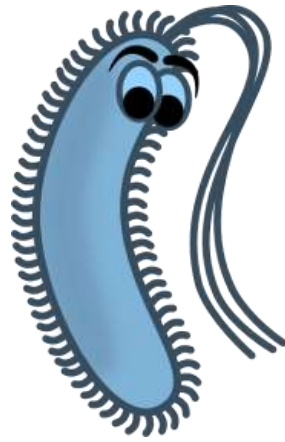


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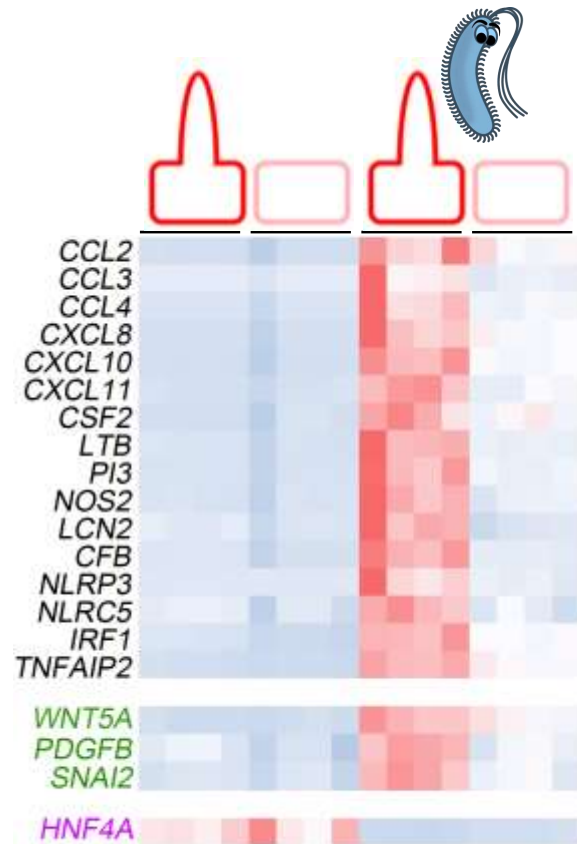
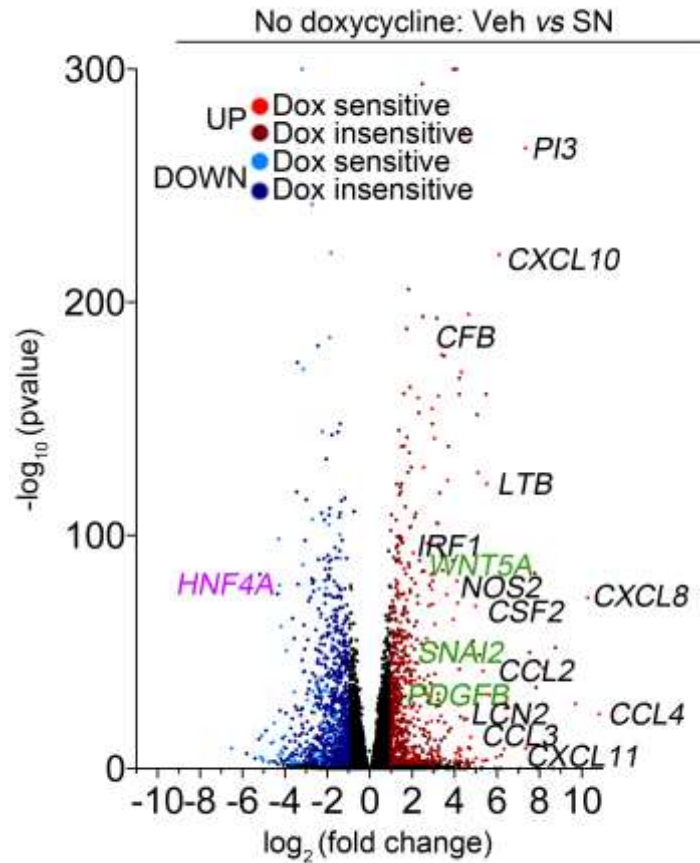
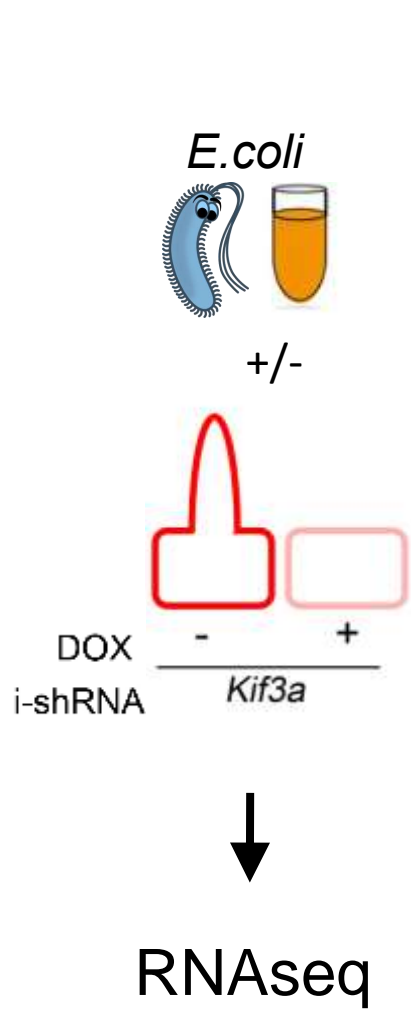


Question

Do primary cilia play physiological roles in the control of kidney inflammation and fibrosis ?



Primary cilia contribute to the fibro-inflammatory response to *E.coli* component

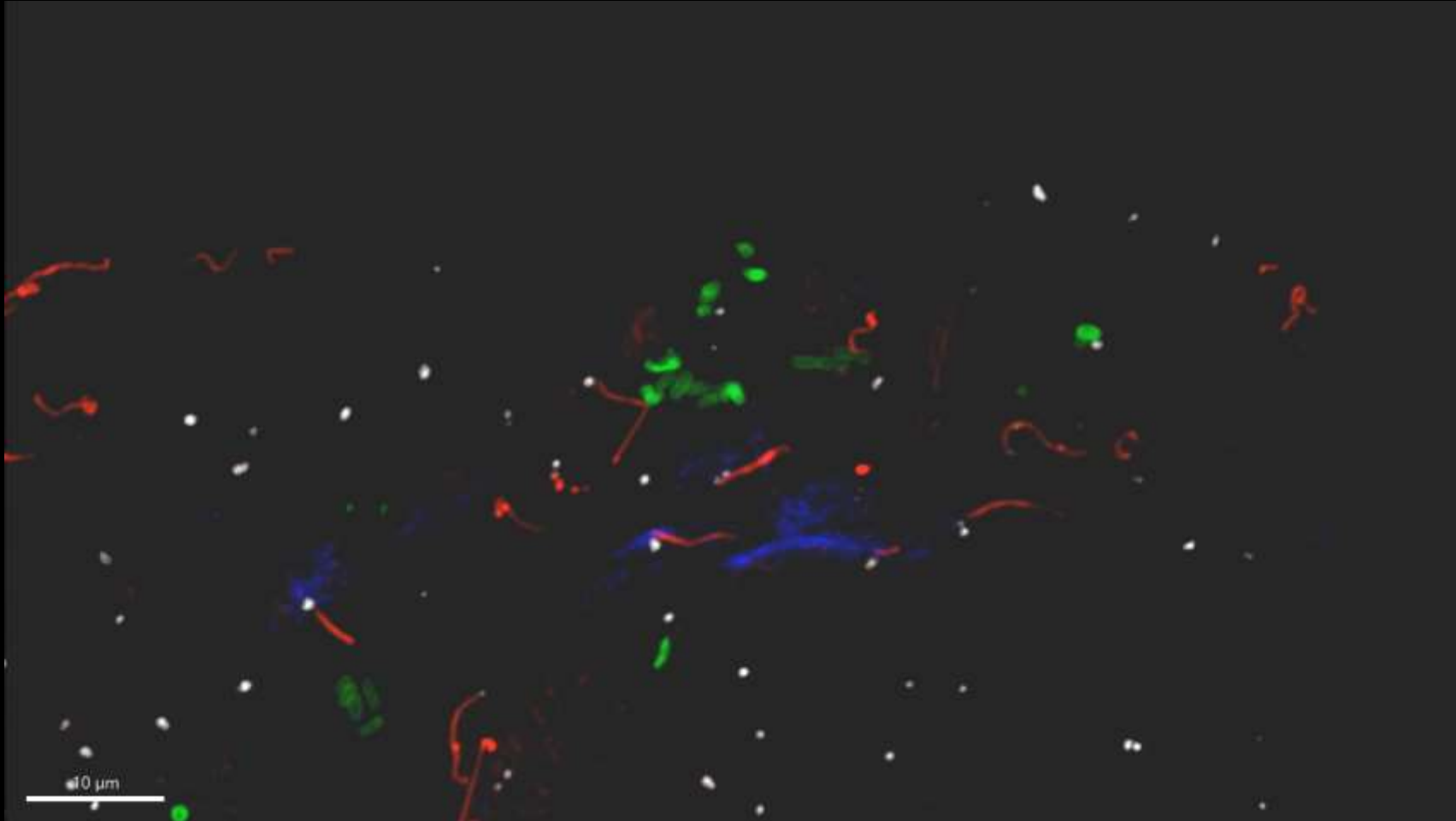


Enrichr

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RELB	30642670	7,9E-04	133
FOXM1	32153563	7,7E-03	75
SOX2	20726797	4,5E-06	73
ATF3	23680149	2,9E-05	62
TP63	17297297	1,4E-01	55
E2F7	22180533	1,4E-01	46
STAT5A	24692510	2,0E-04	46
PPARD	23176727	3,8E-02	42
OCT4	18692474	7,9E-04	39
FOXP1	22492998	3,2E-01	34
JUND	26020271	3,2E-03	32
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SMAD2	18955504	4,1E-03	30
SMAD3	18955504	4,1E-03	30

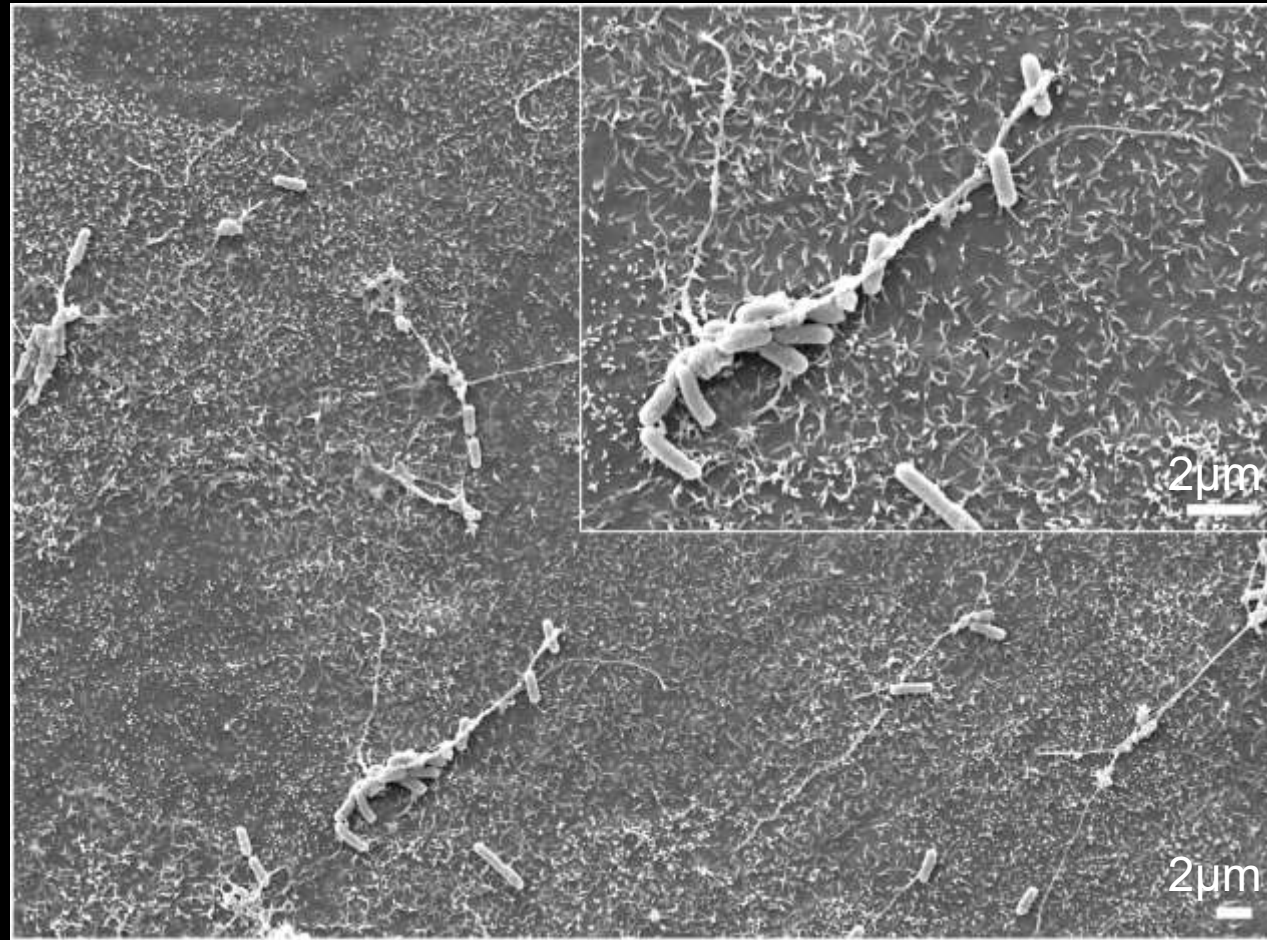
Cilia-bacteria interactions in acute pyelonephritis

AQP2/GFP/ γ TUB/AcTUB

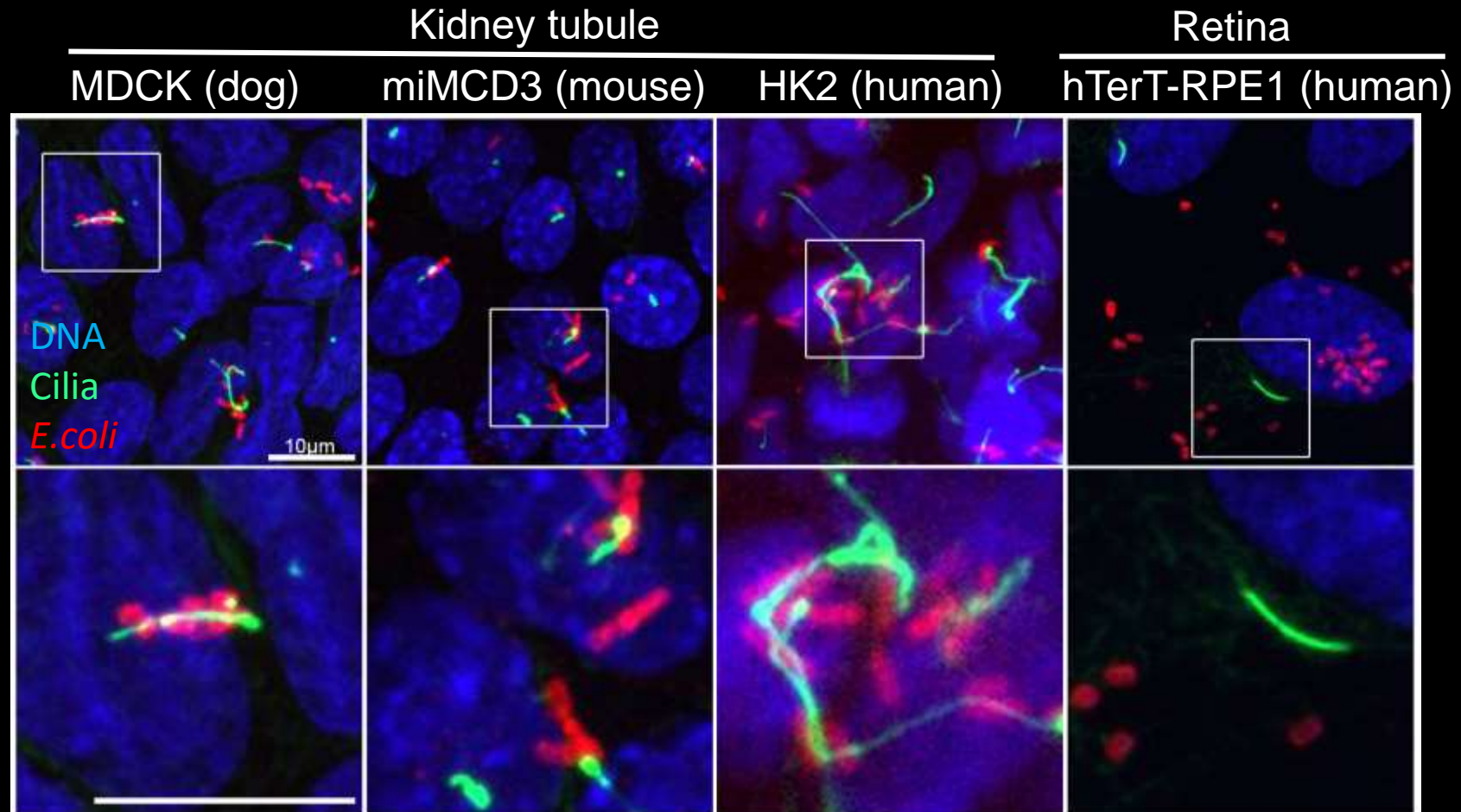


In vitro adhesion of uropathogenic *E.coli* to primary cilia

MDCK vs UTI89

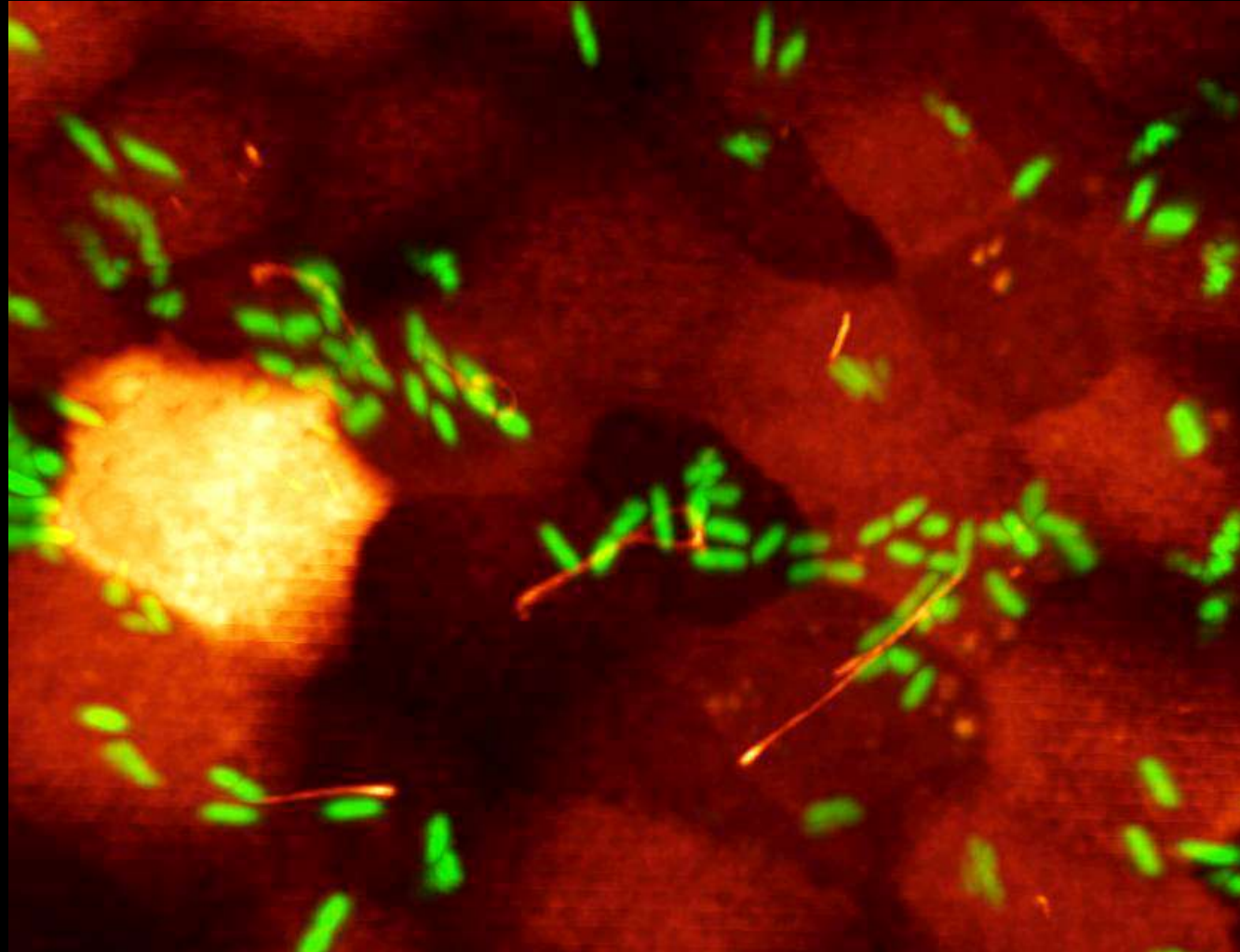


In vitro adhesion of uropathogenic *E.coli* to primary cilia



In vitro adhesion of uropathogenic *E.coli* to primary cilia

Infection with UTI89 MOI 1 for 3h
Then washed and imaged



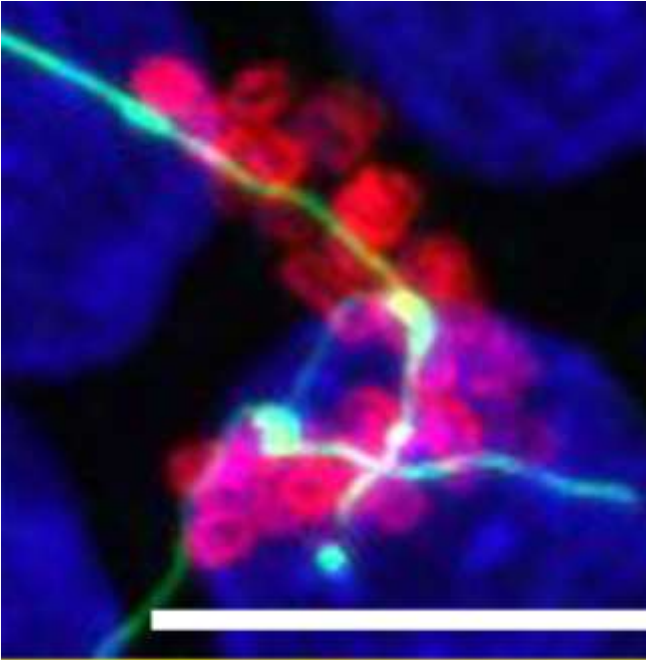
Cilia

E.coli

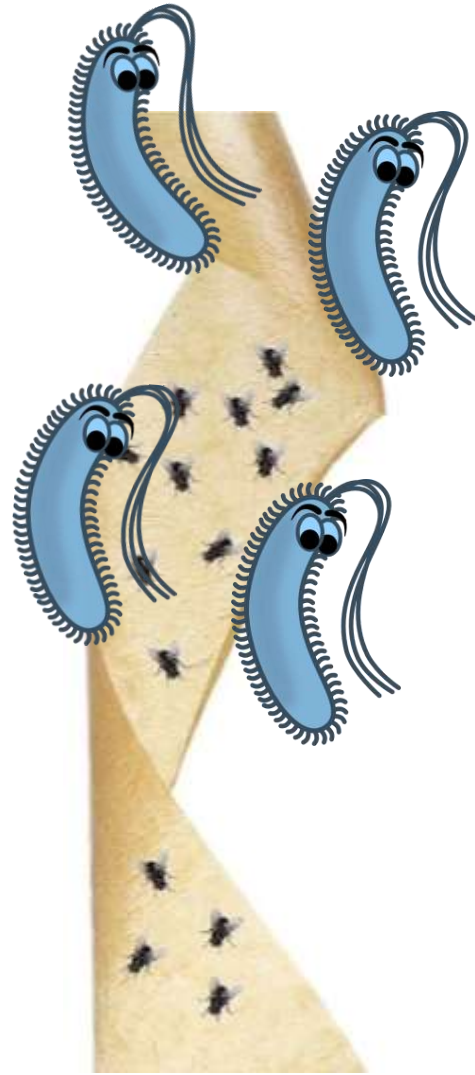
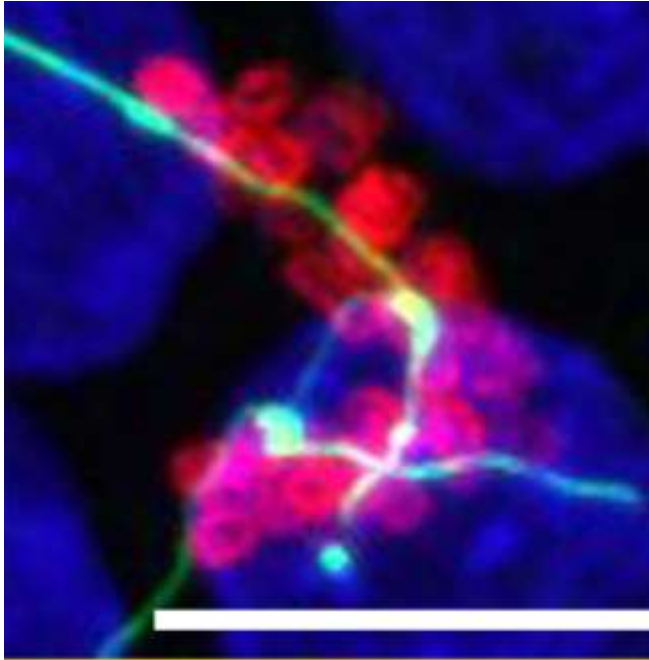
Frame interval: 3 minutes



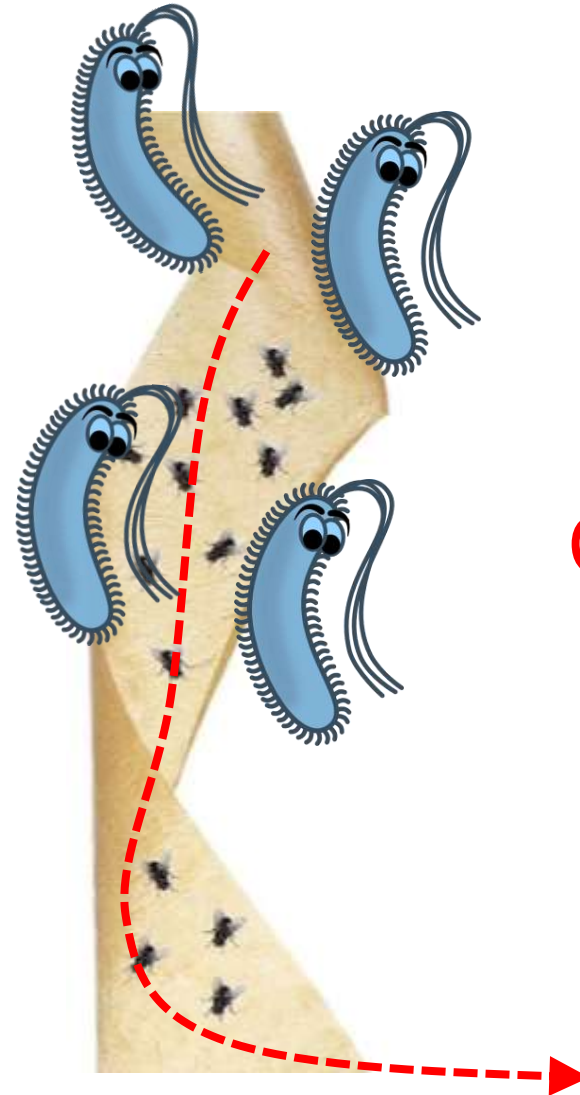
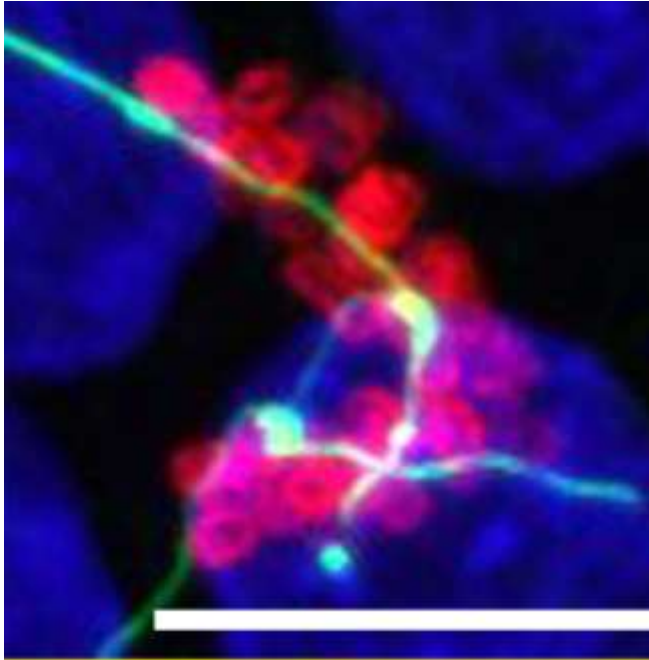
Proposed model



Proposed model



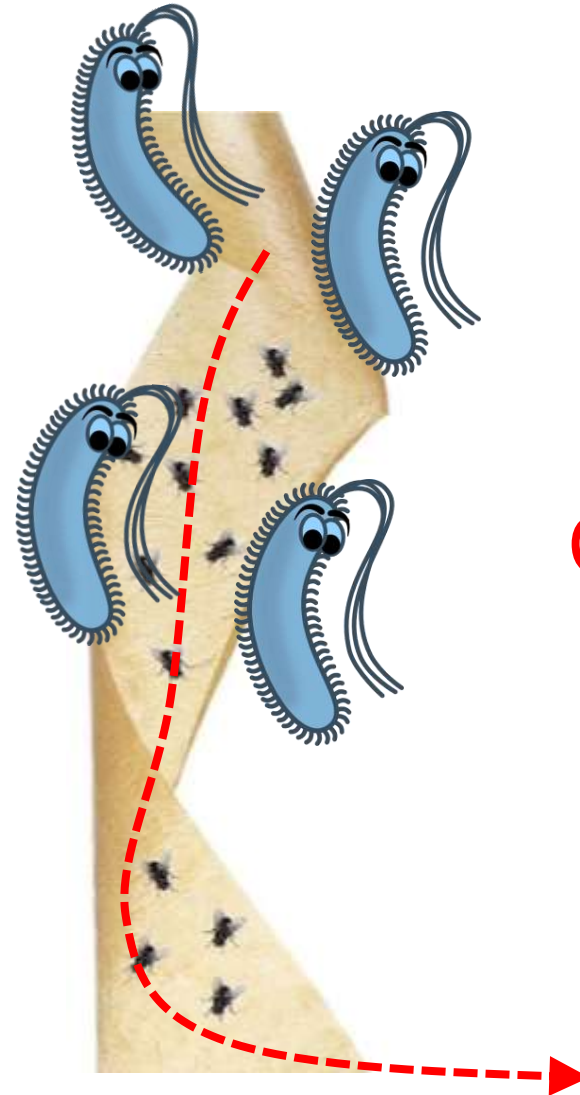
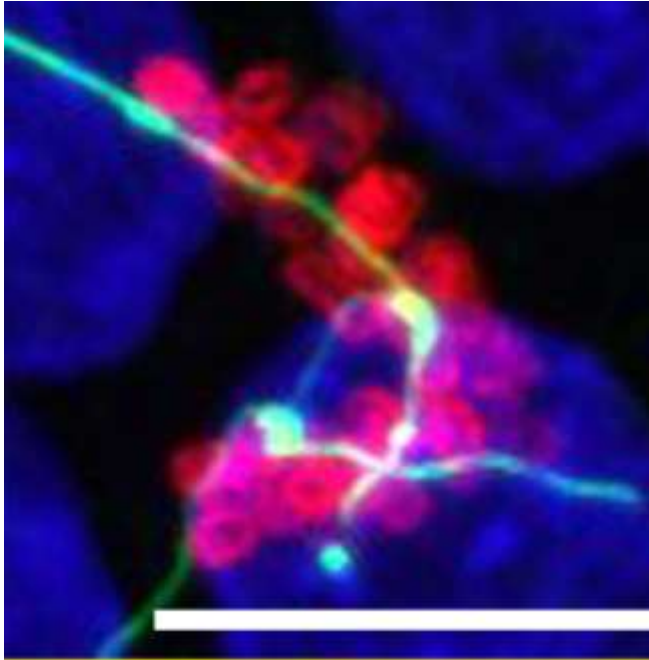
Proposed model



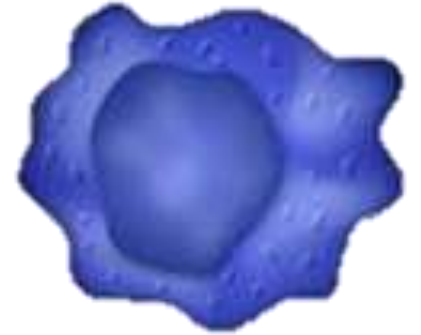
Chemokines



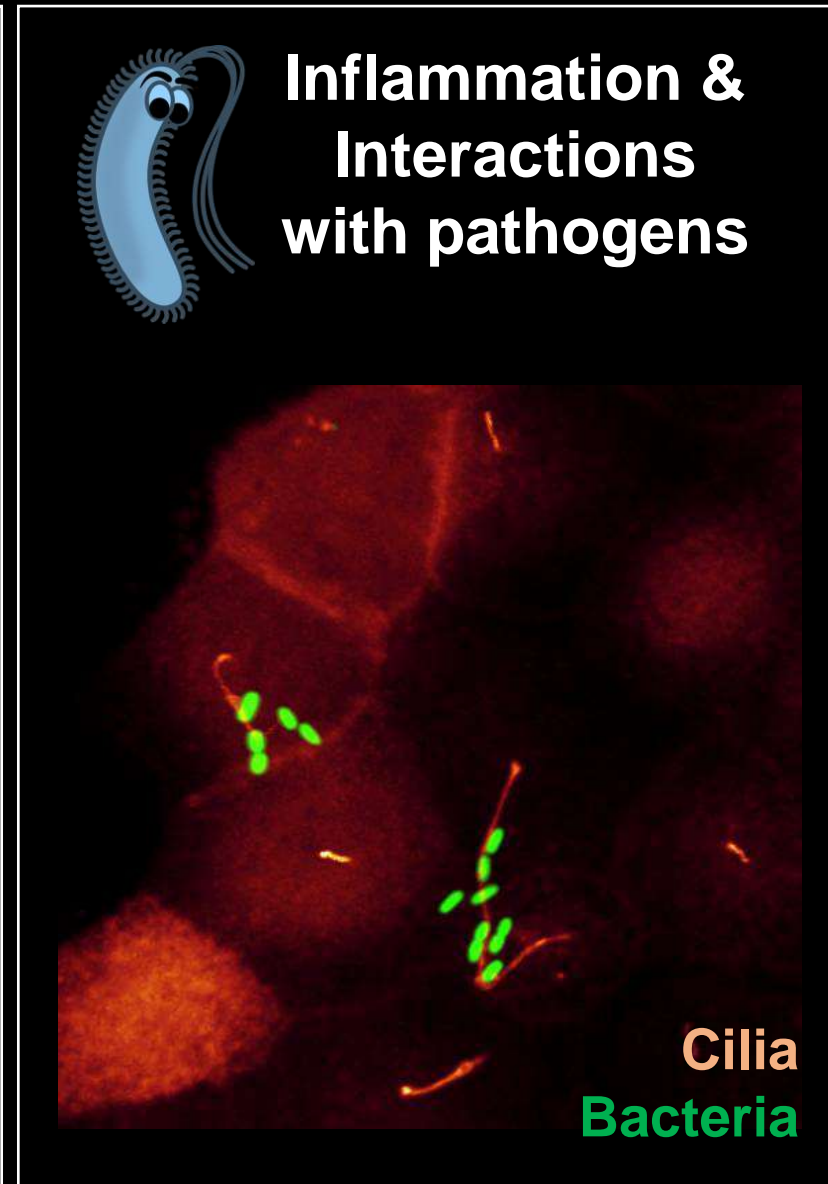
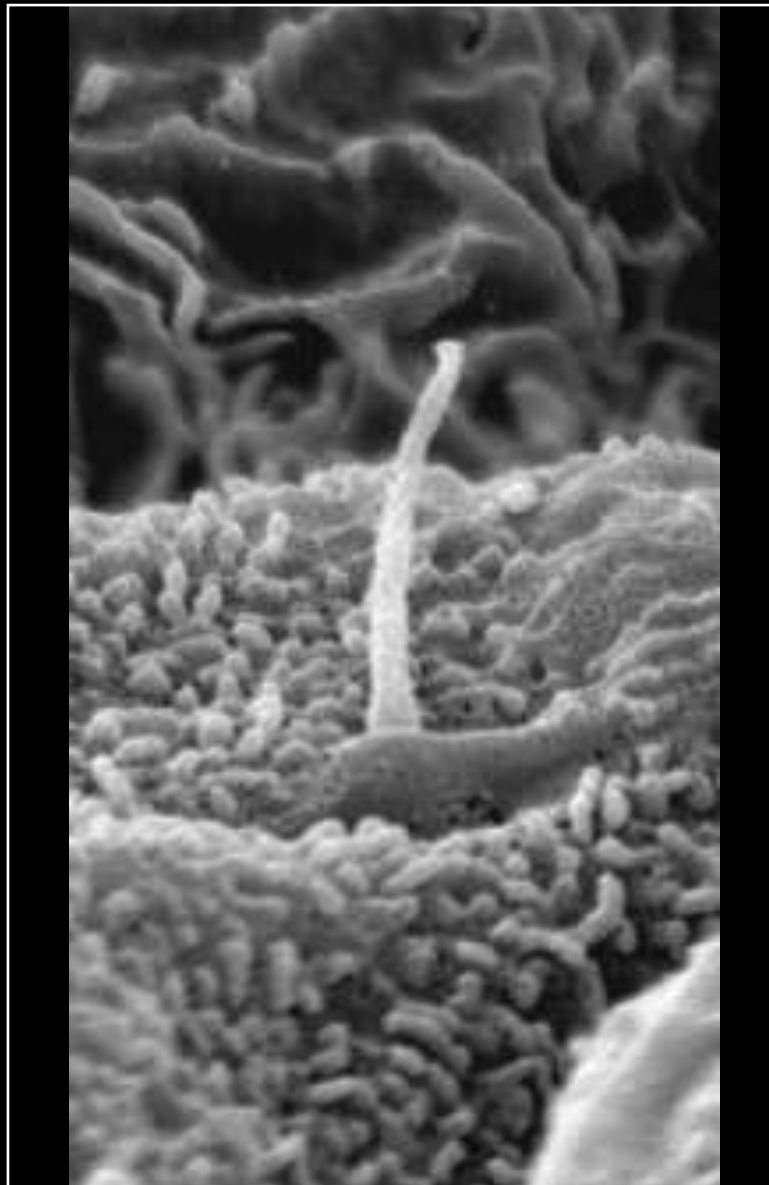
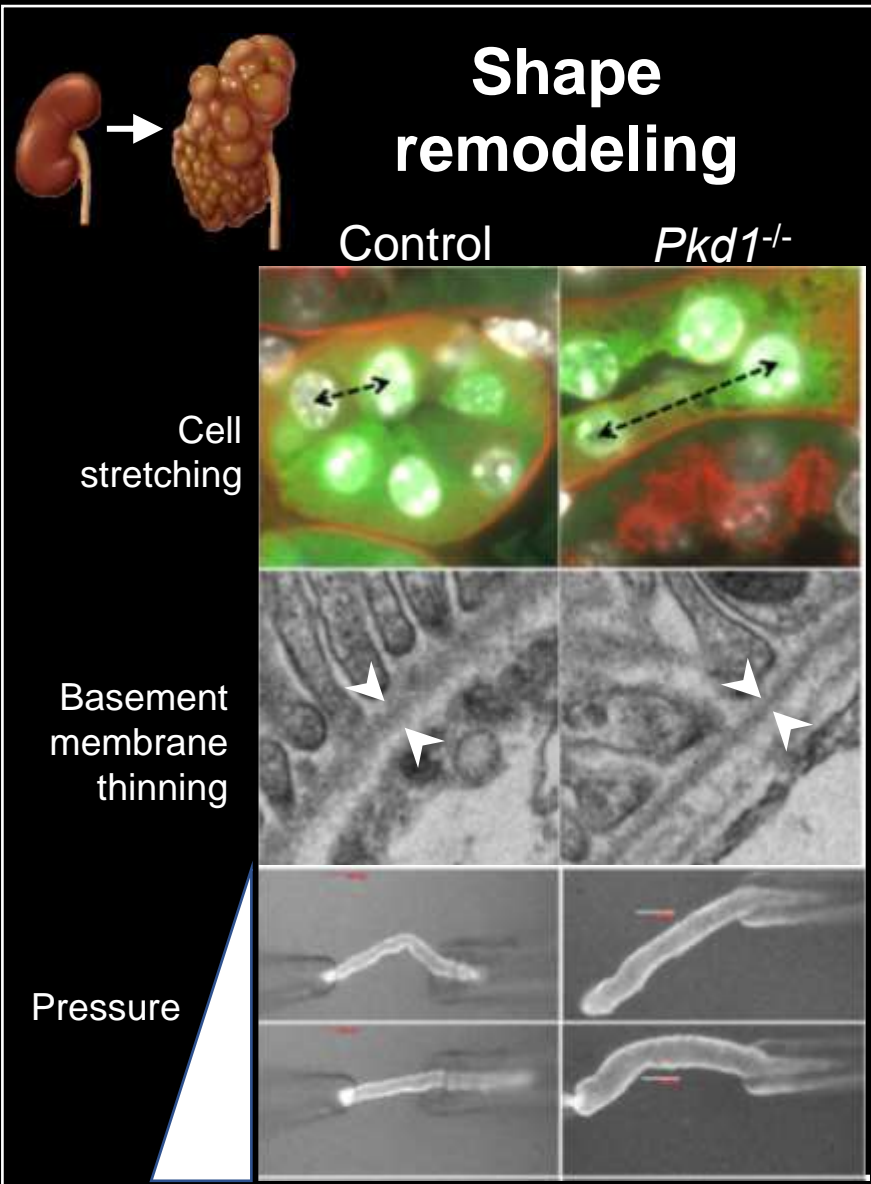
Proposed model



Chemokines



New functions of primary cilia



Acknowledgment

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