Novel roles for PKD genes in the reproductive tract and in stromal cells of the kidney

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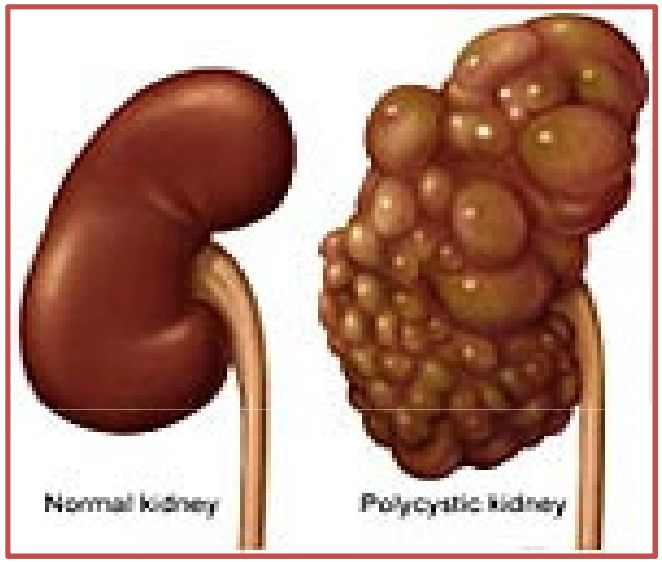
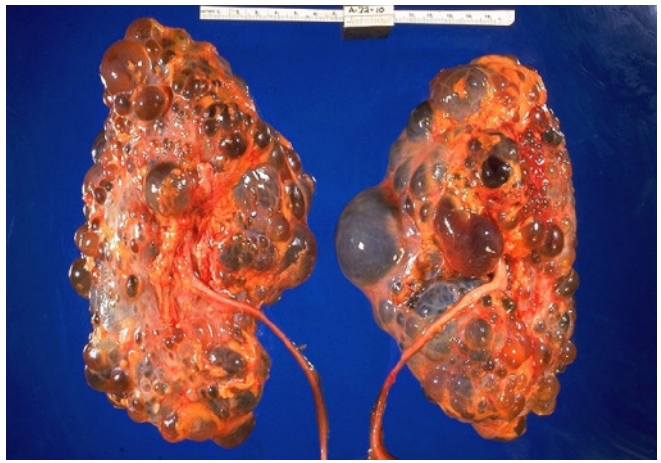
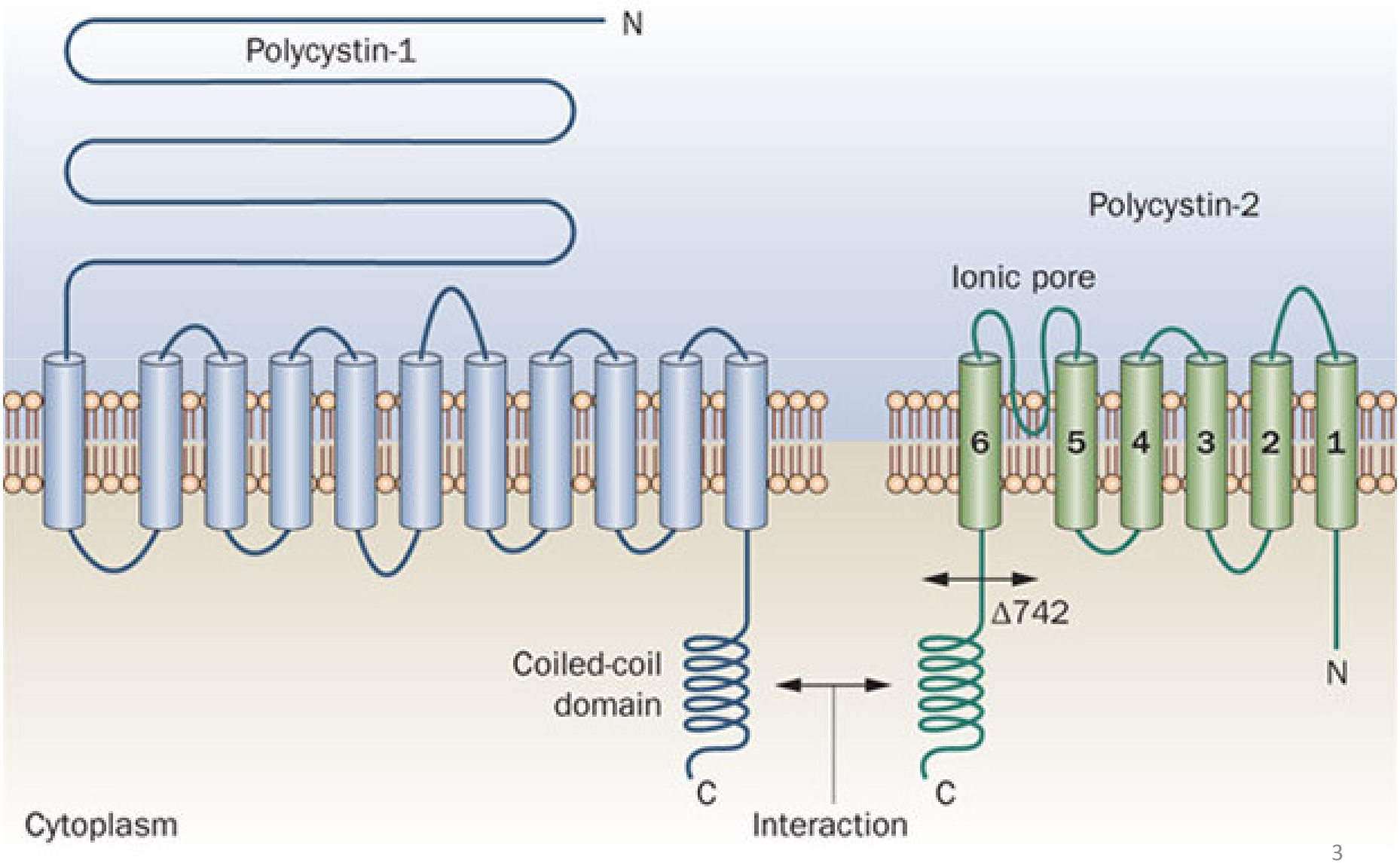
Some facts about Autosomal Dominant Polycystic Kidney Disease (ADPKD)

- Epidemiology: Incidence of 1:500 to 1:1000 live births, affecting all ethnicities
- Etiology*: Pkd1* mutations account for over 85% of ADPKD cases, *Pkd2* mutations account for approximately 15% of ADPKD.
- Clinical signs: Progressive development of cysts, enlargement of kidneys and progressive loss of renal function
- Over half of the cases proceed to end stage renal disease by fifth to sixth decades
- Treatment: dialysis and kidney transplantation
- Infertility can be a problem



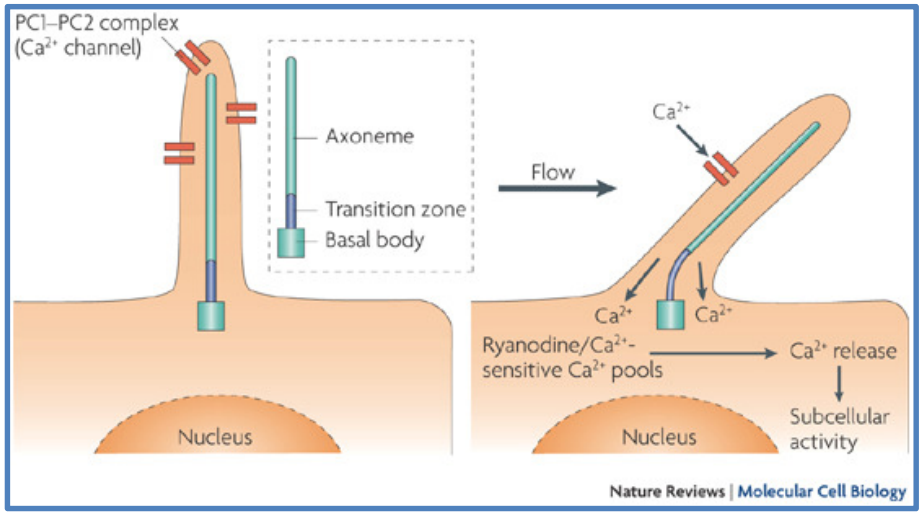


Structures of Polycystin1 and Polycystin2



Functions of PCs

- Polycystin-2 serves as a **calcium channel**
- Polycystin-1 and polycystin-2 act as **complex** (interact at the coiled-coil domains) to regulate calcium inflow in many cell types
- **Mechanosensing role** of polycystins in primary cilia of epithelial cells



PC regulated cellular events

- Cytoskeleton dynamics
- Cell adhesions
- Cellular signaling: Wnt signaling, mTOR, et al.
- Cell proliferation and apoptosis
- Metabolism changes

Genetic explanation of ADPKD

- **The "Second hit" theory**: a germline mutation in one allele plus a second hit mutation on the other allele causing loss of heterozygosity in a ADPKD gene initiates cyst formation from a single epithelial cell
- Accumulation of mutations throughout lifetime explains slow progression of the disease
- Modulators (a "third hit") might also play a role in variations of the disease

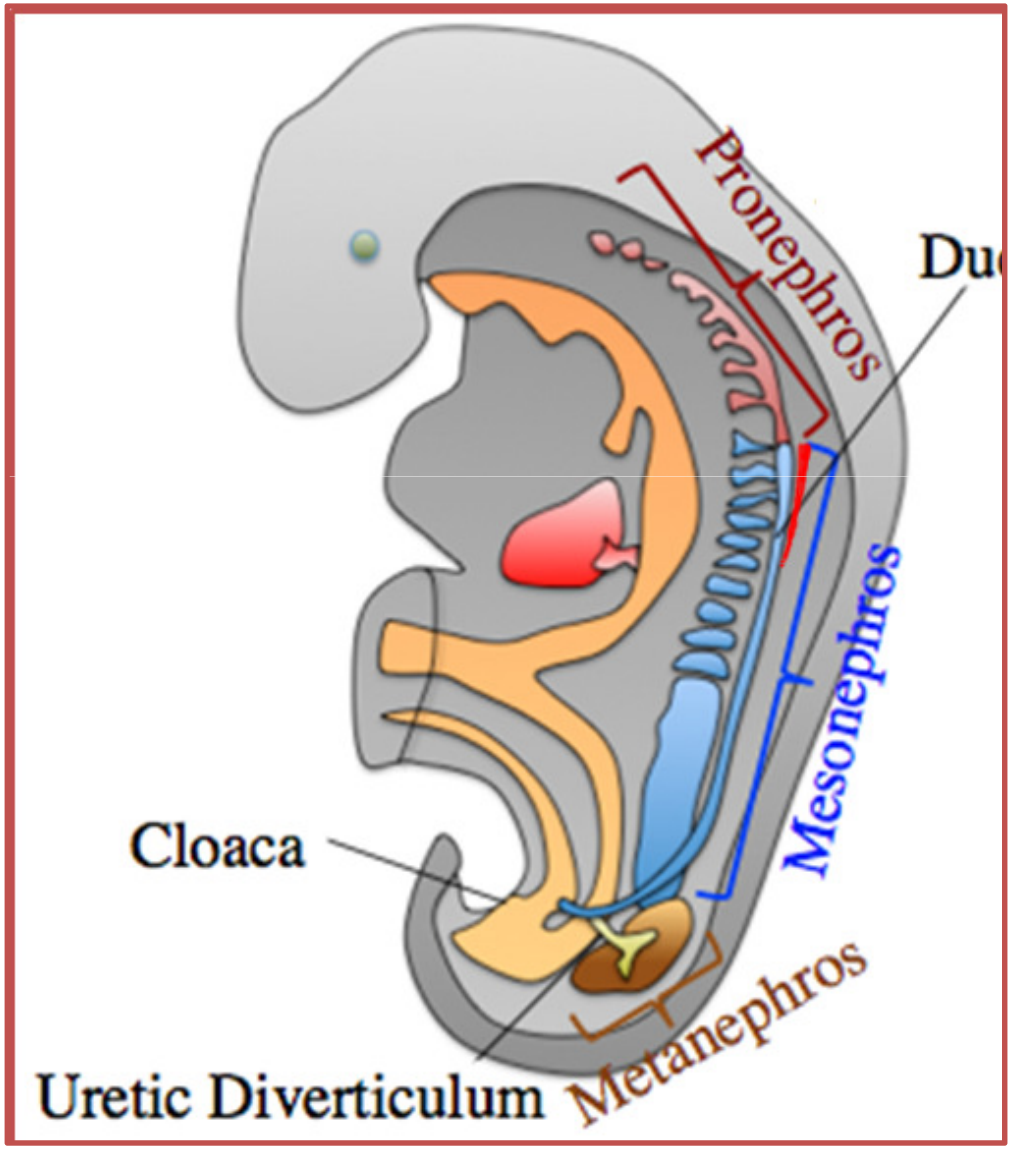
ADPKD Projects

1. **ADPKD genes (*Pkd1* and *Pkd2*) in male reproductive system development**
2. **Mechanisms of cyst development in ADPKD**: Although causative roles for ADPKD gene mutations are firmly established, the mechanisms for disease progression and variations remain to be determined.

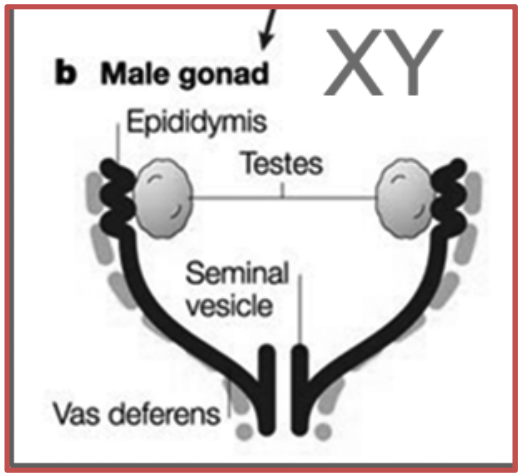
ADPKD genes in reproductive tract

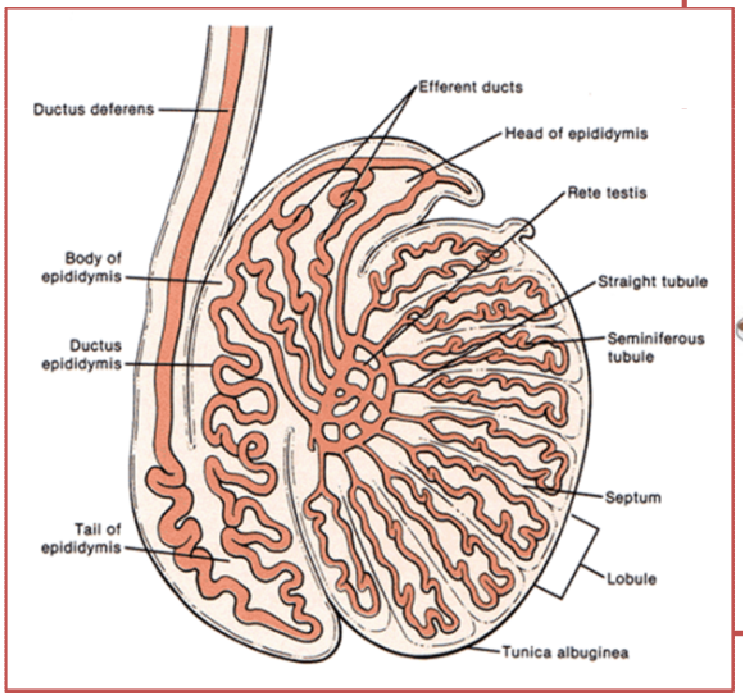
- ***ADPKD* genes (*Pkd1* and *Pkd2*) in male reproductive system development**
  - Required for mesonephric development, including epididymis, efferent ducts, testis
  - Disruption of Tgf-beta/Bmp and Wnt signaling

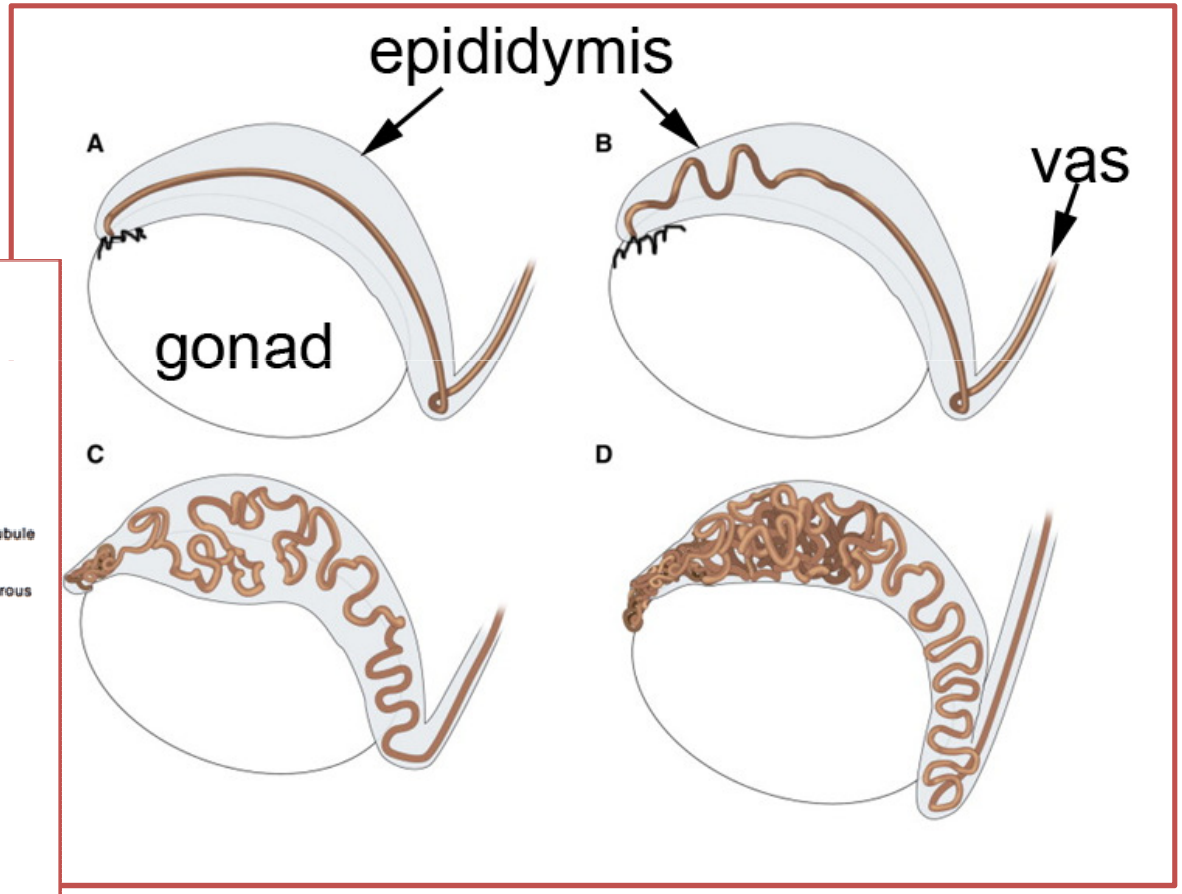
Development of Mesonephros and Gonad



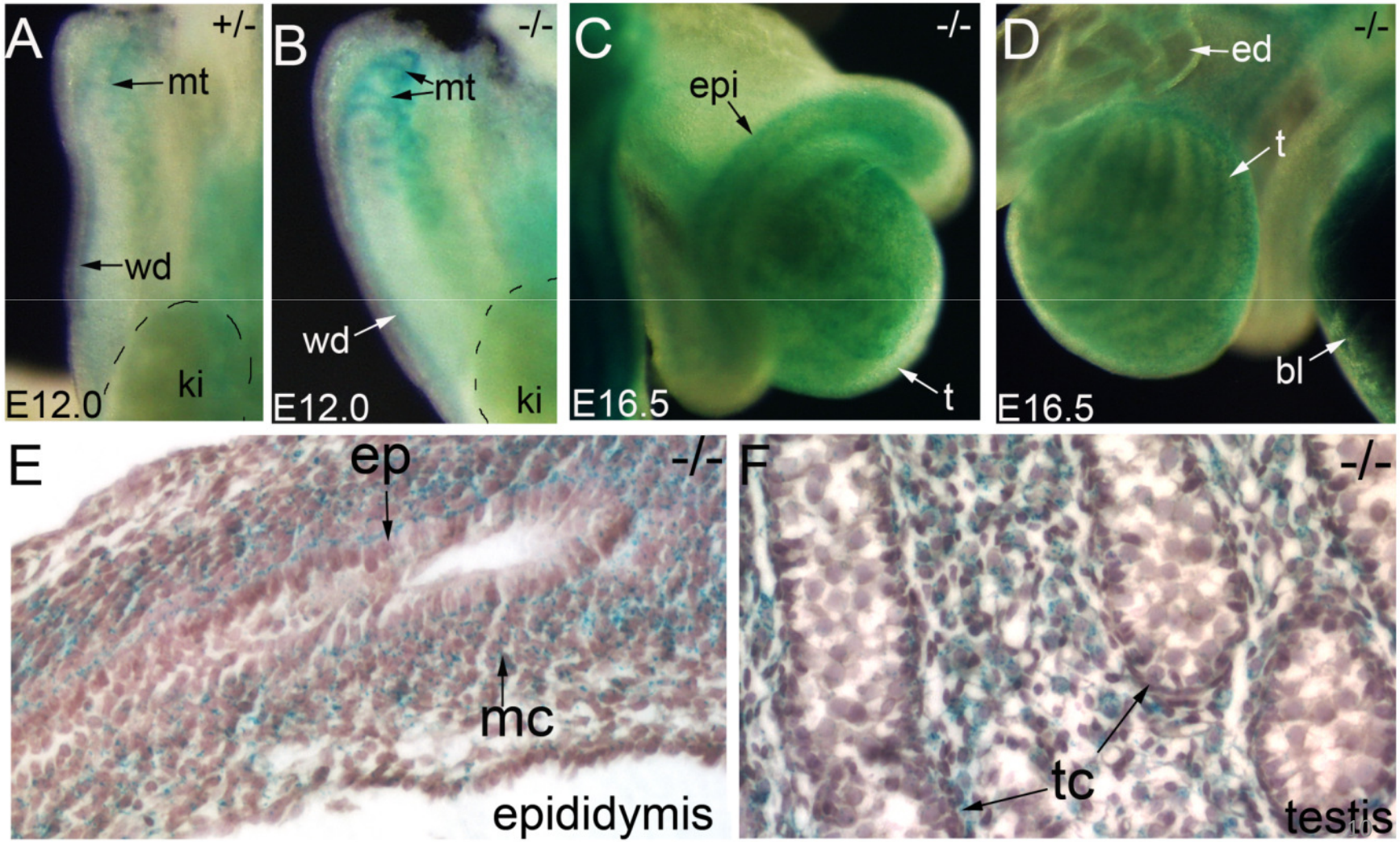
Development of the male reproductive system



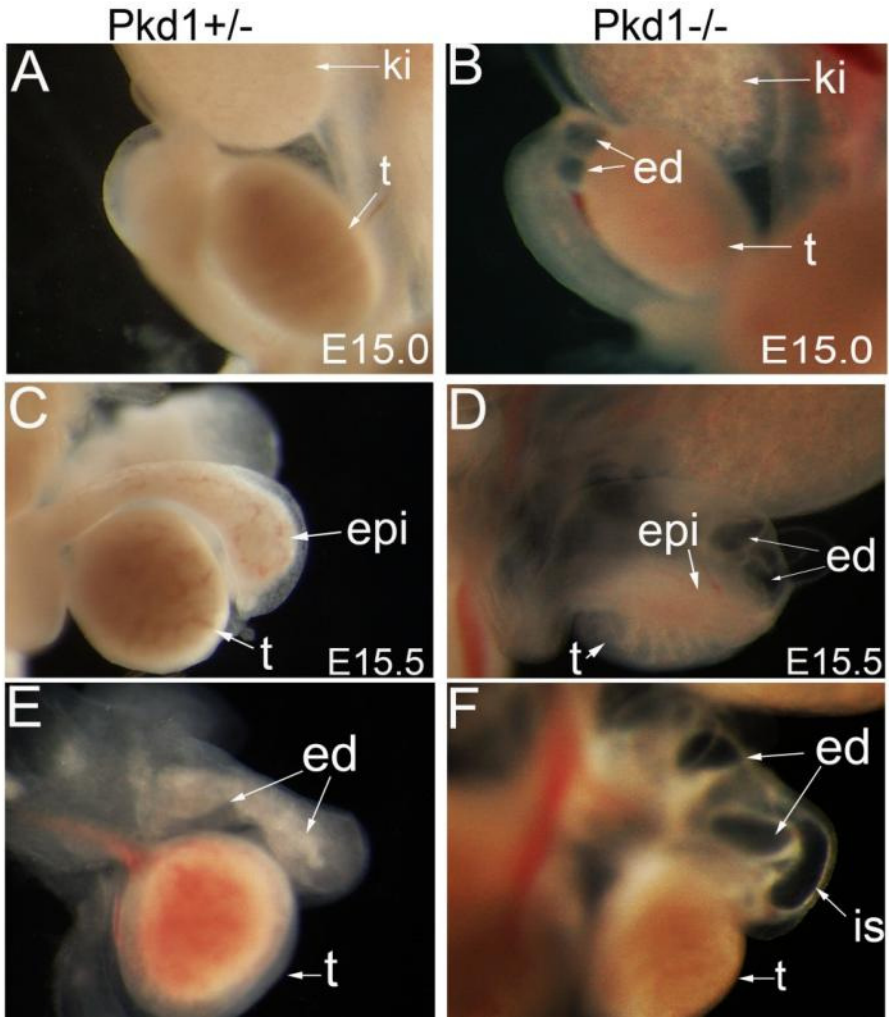


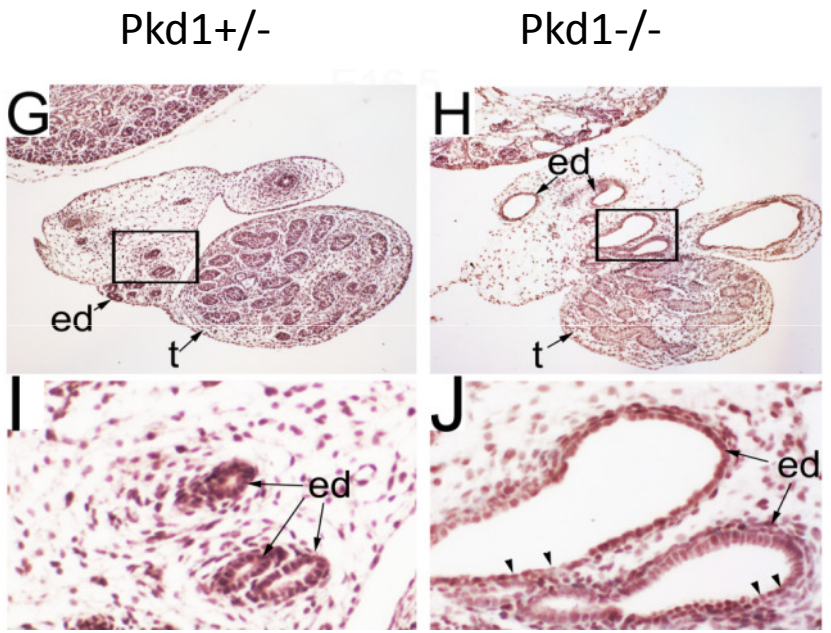


Pkd1 expression in developing male reproductive system



Efferent duct dilation and cystogenesis in *Pkd1*$^{LacZ/LacZ}$ (*Pkd1*$^{-/-}$)mice





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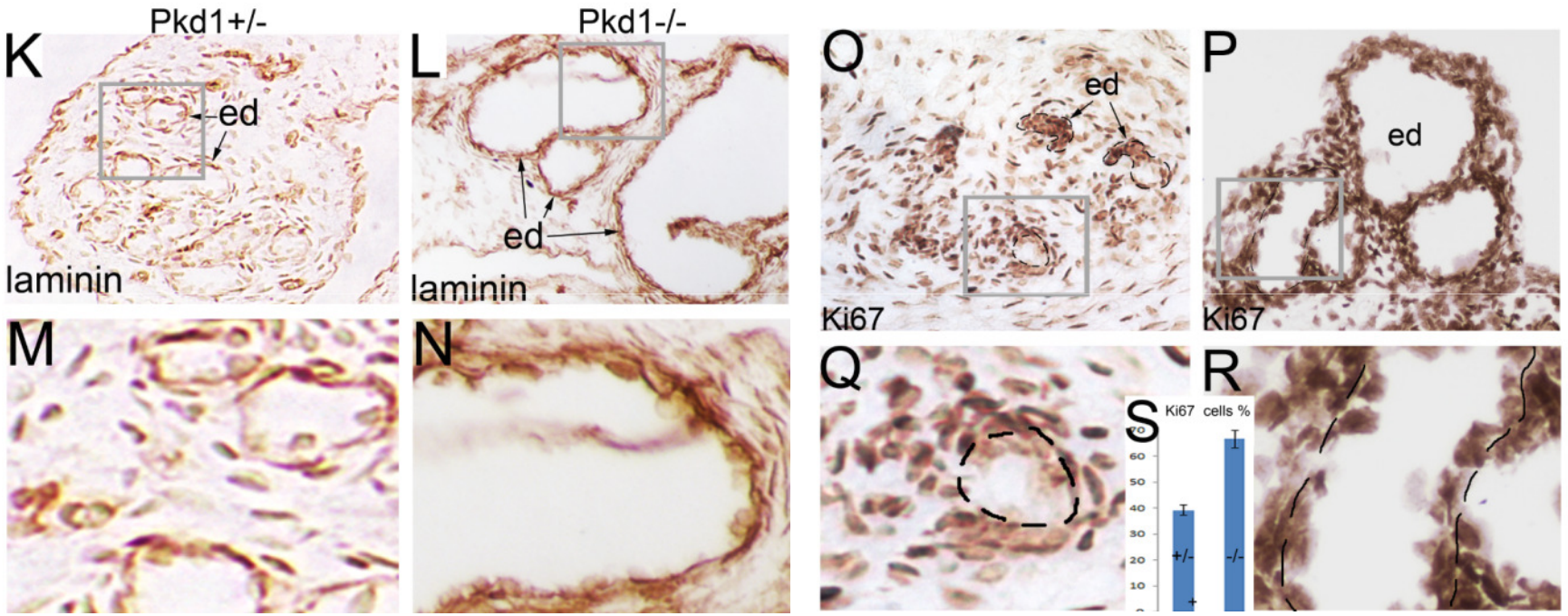
Changes in basement membrane and proliferation rate



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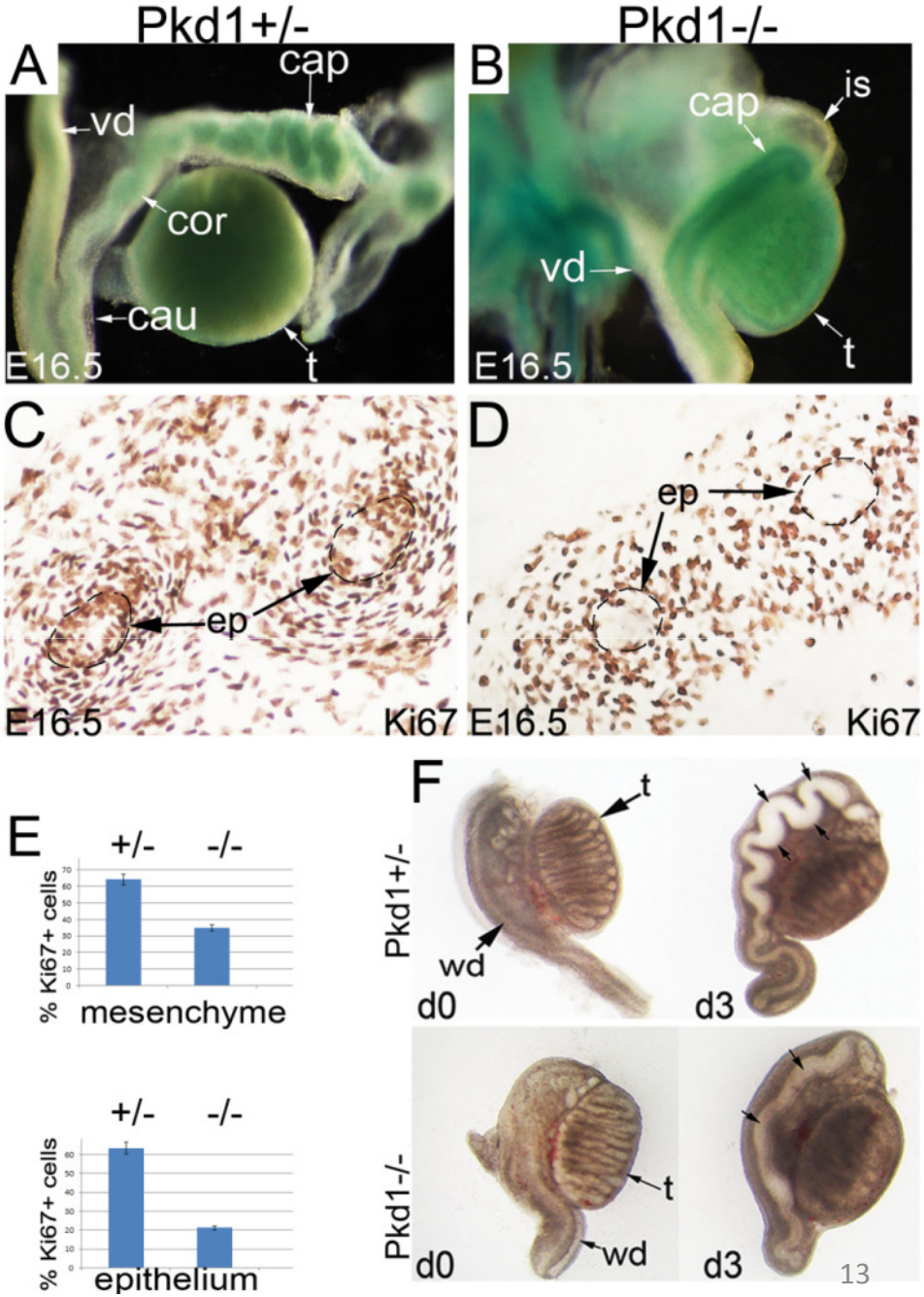
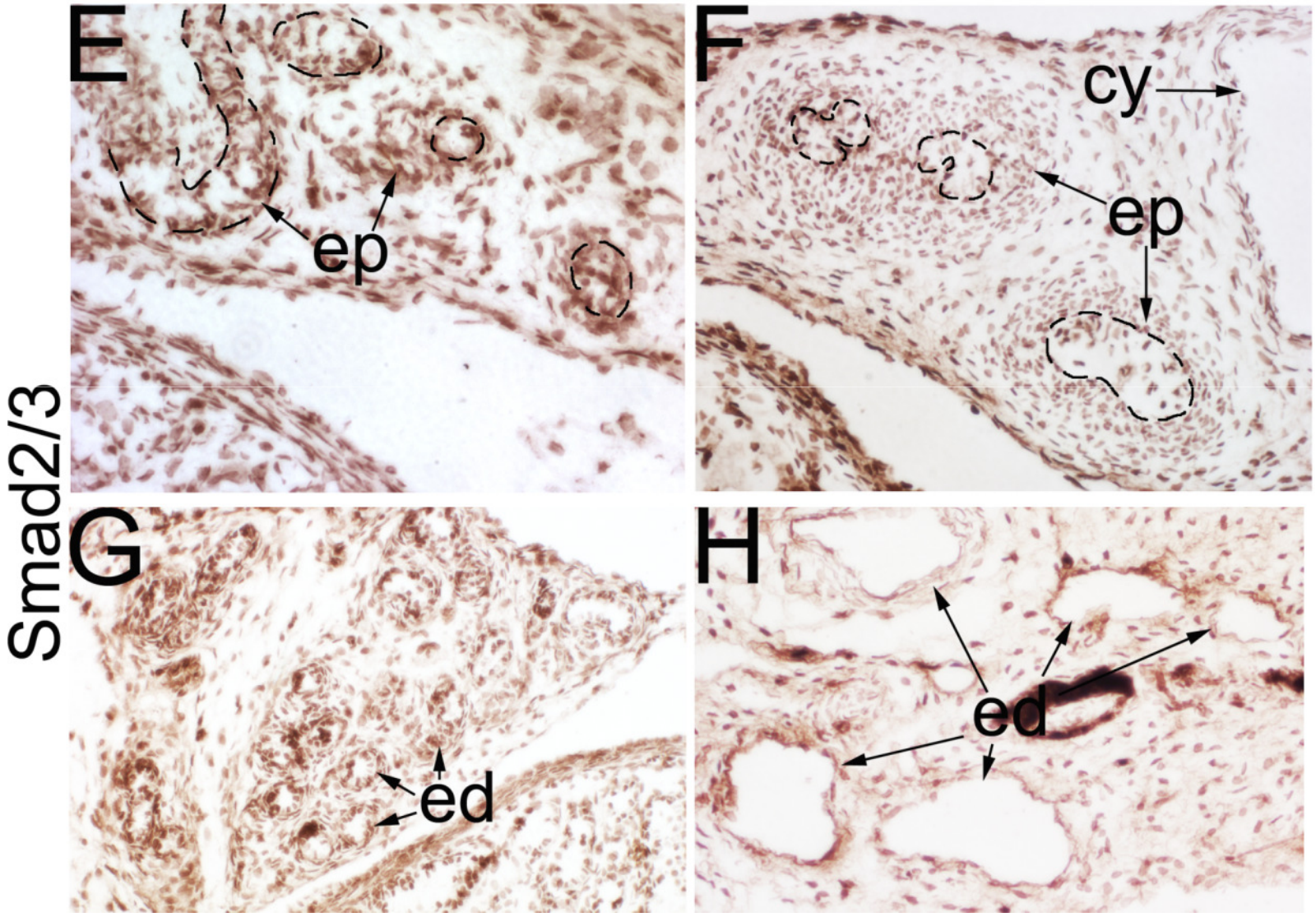
Epididymis defects in *Pkd1*$^{-/-}$ mice

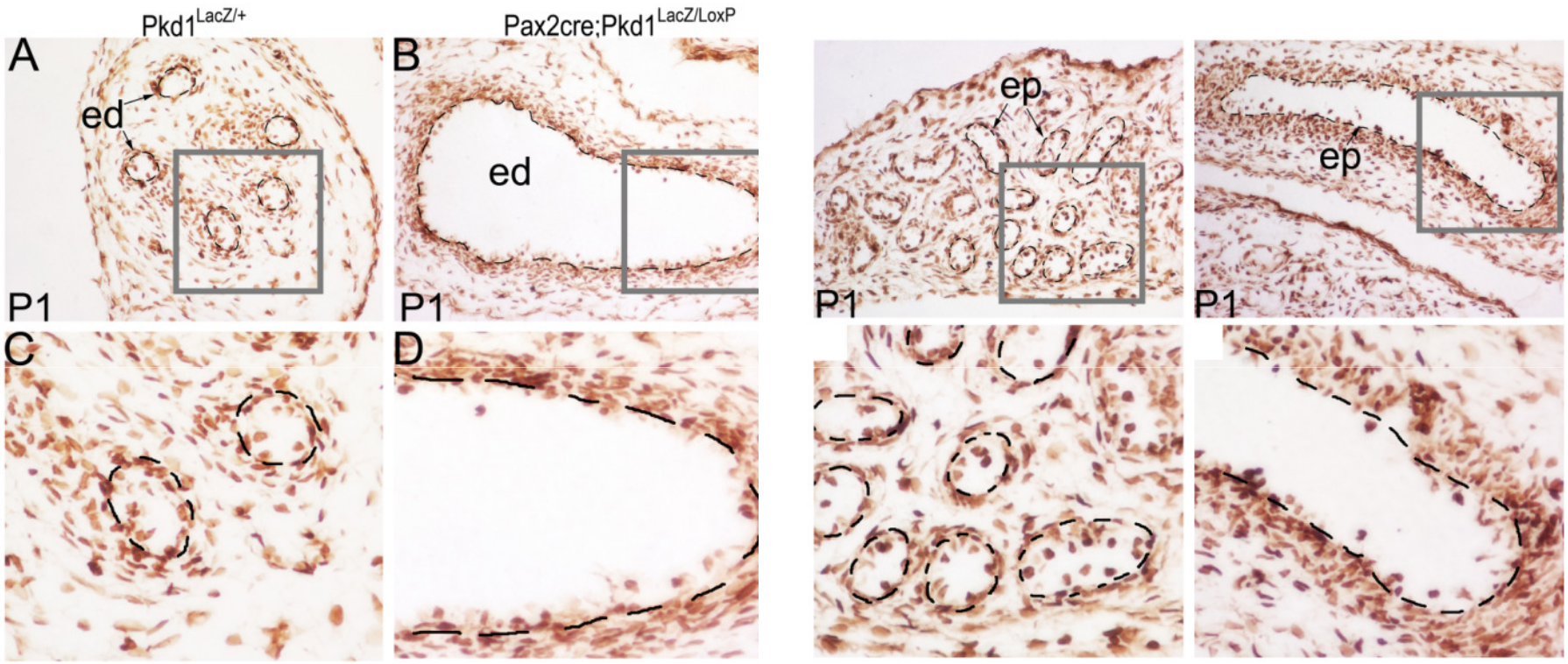
- Lack of coiling
- Reduced proliferation
- Lack of hormone responsiveness

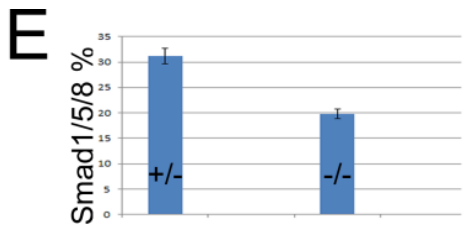


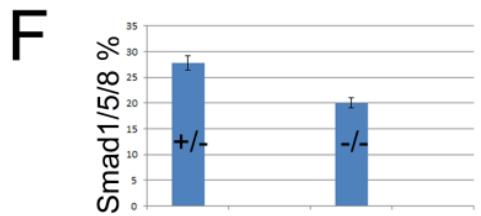
Decreased Tgf-β signaling



Decreased Bmp signaling







Summary 1

PC-1 deficient mice showed reproductive system defects in males, including:

- Efferent duct dilation
- Coiling defect and dilation of epididymis
- Phenotypic change of epithelium: columnar cells to highly proliferating flattened cells
- Abnormal Tgf-β/Bmp signaling
- Pkd2 knockout mice show similar features, but also show atypical testicular cords

New insights from genetic engineered mouse models

- Conditional inactivation of ADPKD genes in adulthood or late postnatal development only leads to focal cyst formation in mice, failing to model human ADPKD
- Cilium ablation or disrupting cilium-dependent calcium inflow, which is regulated by PC-1and PC-2, does not elicit cyst formation in both mice and zebrafish, challenging the “cilium polycystin theory”
- Other mechanisms likely contribute to cystogenesis

Stromal compartment in ADPKD

- Stromal compartment defects are common in ADPKD patients

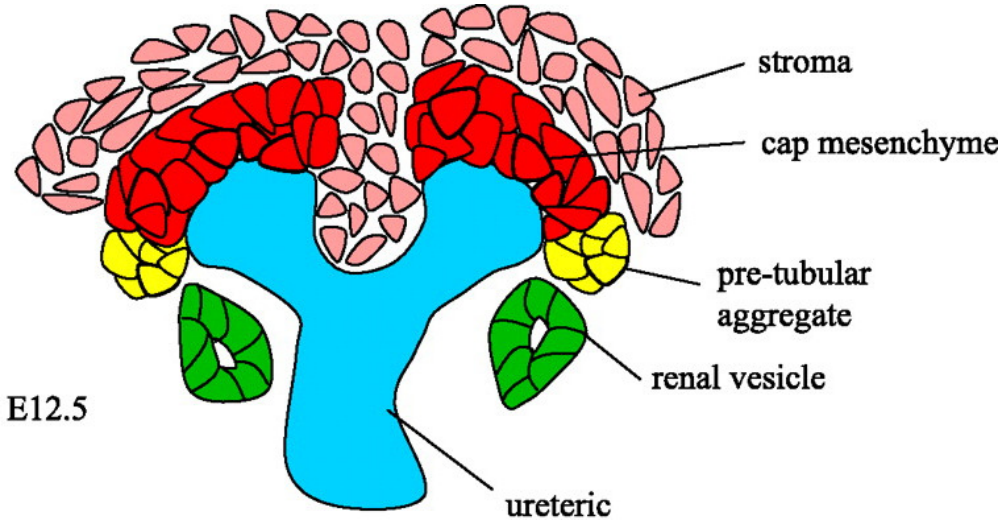
  -Apoptosis

  -ECM changes, fibrosis, basement membrane thickness

- *Pkd1* is expressed in undifferentiated mesenchyme during embryogenesis
- Little is known is about functional roles of ADPKD genes in renal stromal compartment

Does disruption of PKD genes in the stromal compartment initiate cystogenesis?

The renal stromal cells



- **Renal cell lineages during development:**
- **Cap mesenchyme-epithelial cells**: tubules and CDs
- **Endothelial progenitors(hemangioblasts)**: endothelia of renal vasculature
- **Stromal cells and Stromal cell derivatives**: mesangial cells, interstitial fibroblasts, smooth muscle cells, pericytes
- glomerular mesangial stalk, interstitium, vascular wall, smooth muscle

Stromal cell-specific deletion of *Pkd1*

- Using *Foxd1*$^{CreEgfp/+}$ mice to delete ADPKD genes from stromal cell derivatives

- *Foxd1*: a transcription factor regulating stromal cell development in the kidney (non-epithelial cells)

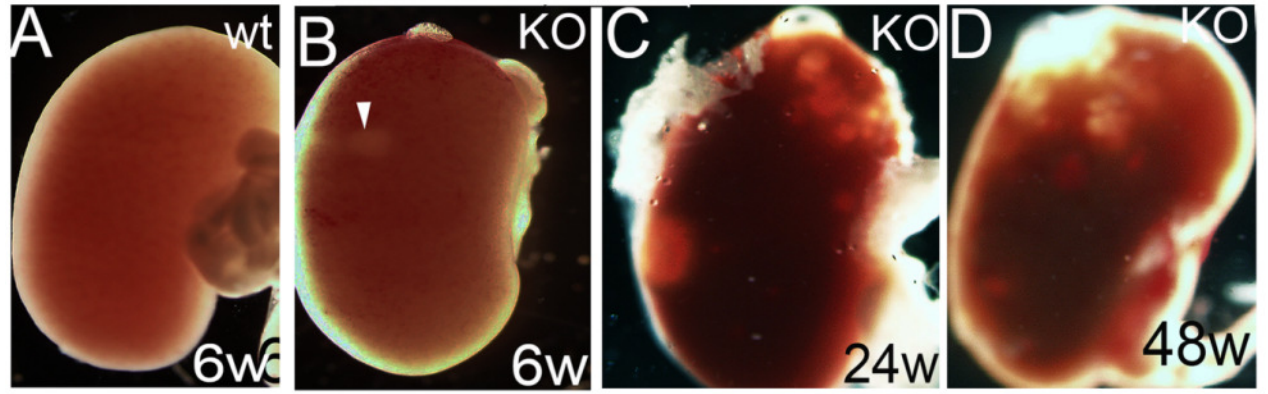
Experimental Approaches

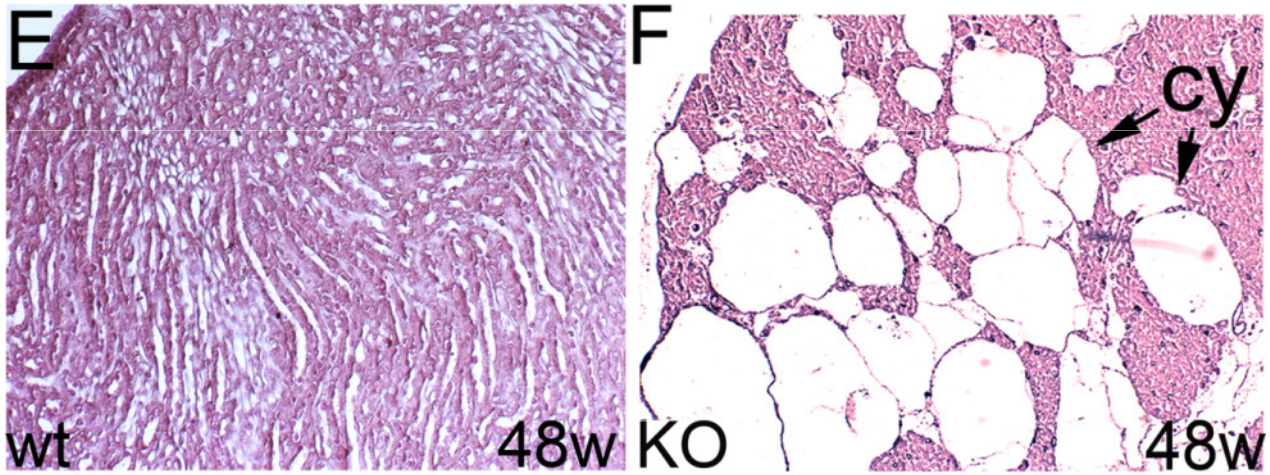
- $Foxd1^{CreEgfp/+}$ mice (Jackson Lab)
- $Pkd1^{LacZ/+}$ mice and $Pkd1^{loxp/loxp}$ mice (Hopkins PKD Core)
- $Foxd1^{CreEgfp/+};Pkd1^{LacZ/+}$ double heterozygous mice are fertile and healthy with normal life span. Minor defects exist in the kidneys, including rare cysts, varied tubule dimension
- $Foxd1^{CreEgfp/+};Pkd1^{LacZ/LoxP}$ mutant mice

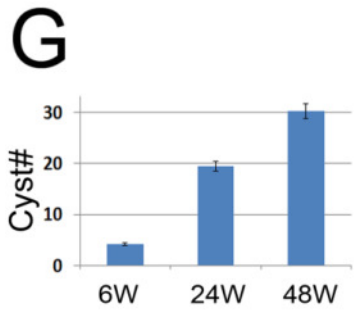
Mutant mice - characteristics

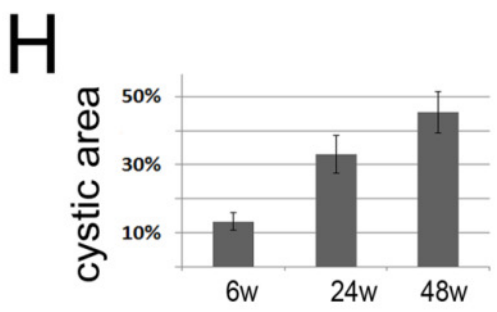
- *Foxd1*$^{CreEgfp/+;}$*Pkd1*$^{LacZ/LoxP}$ survived to adulthood
- A significant number of mutant mice survived beyond 1 year old now
- Altered craniofacial development: domed head, short snout, malocclusion
- Infertile

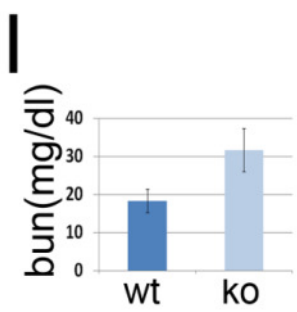
Progressive cyst formation in adulthood



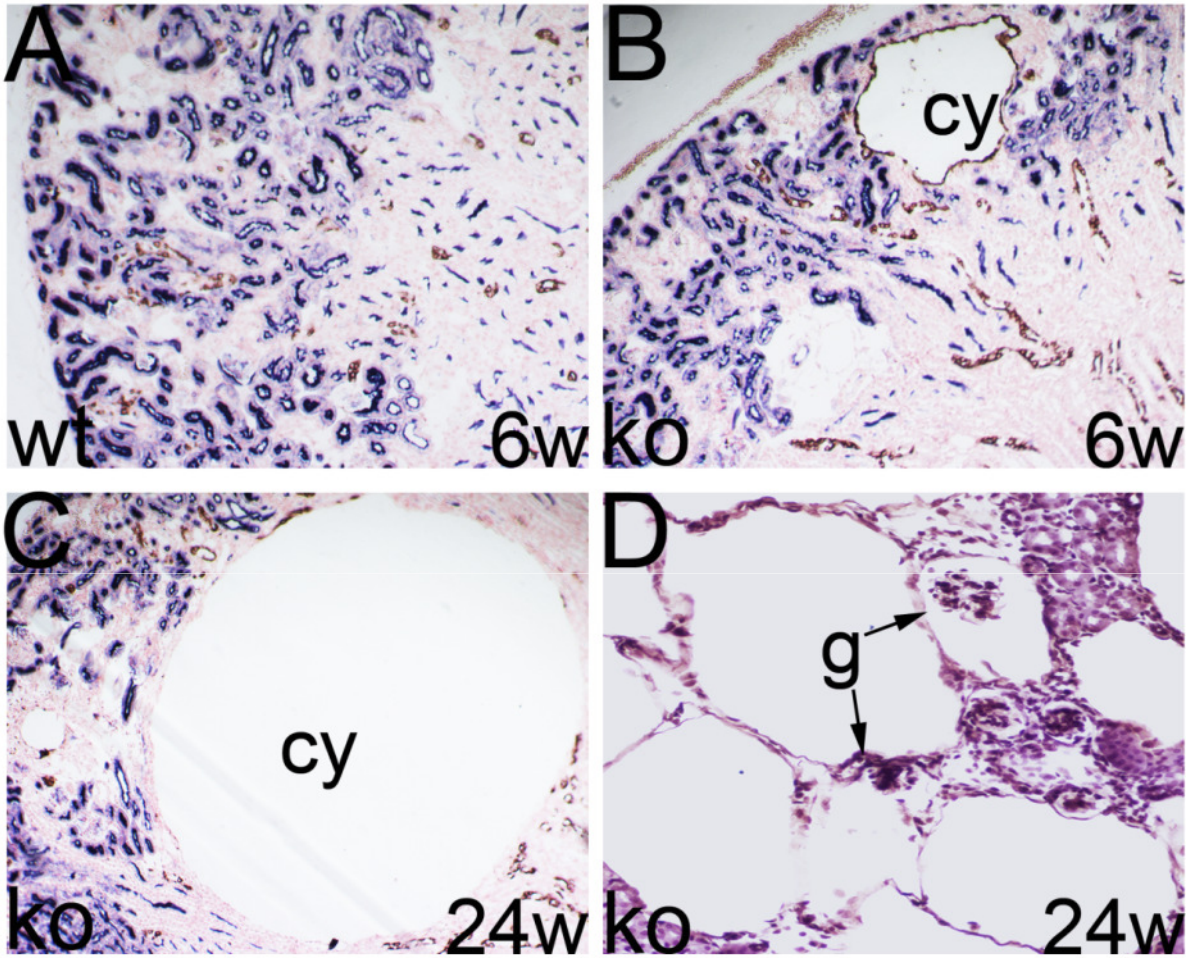




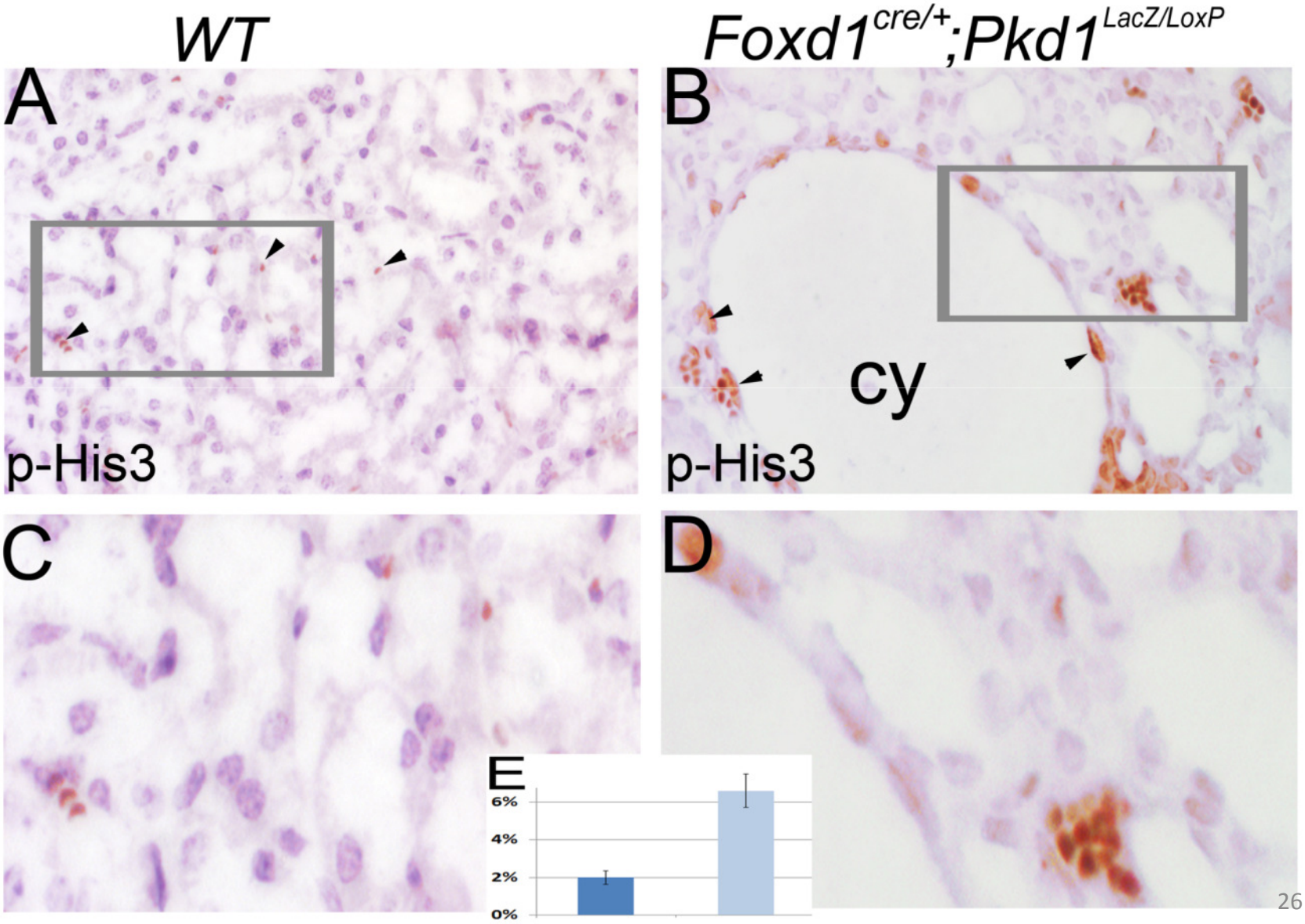




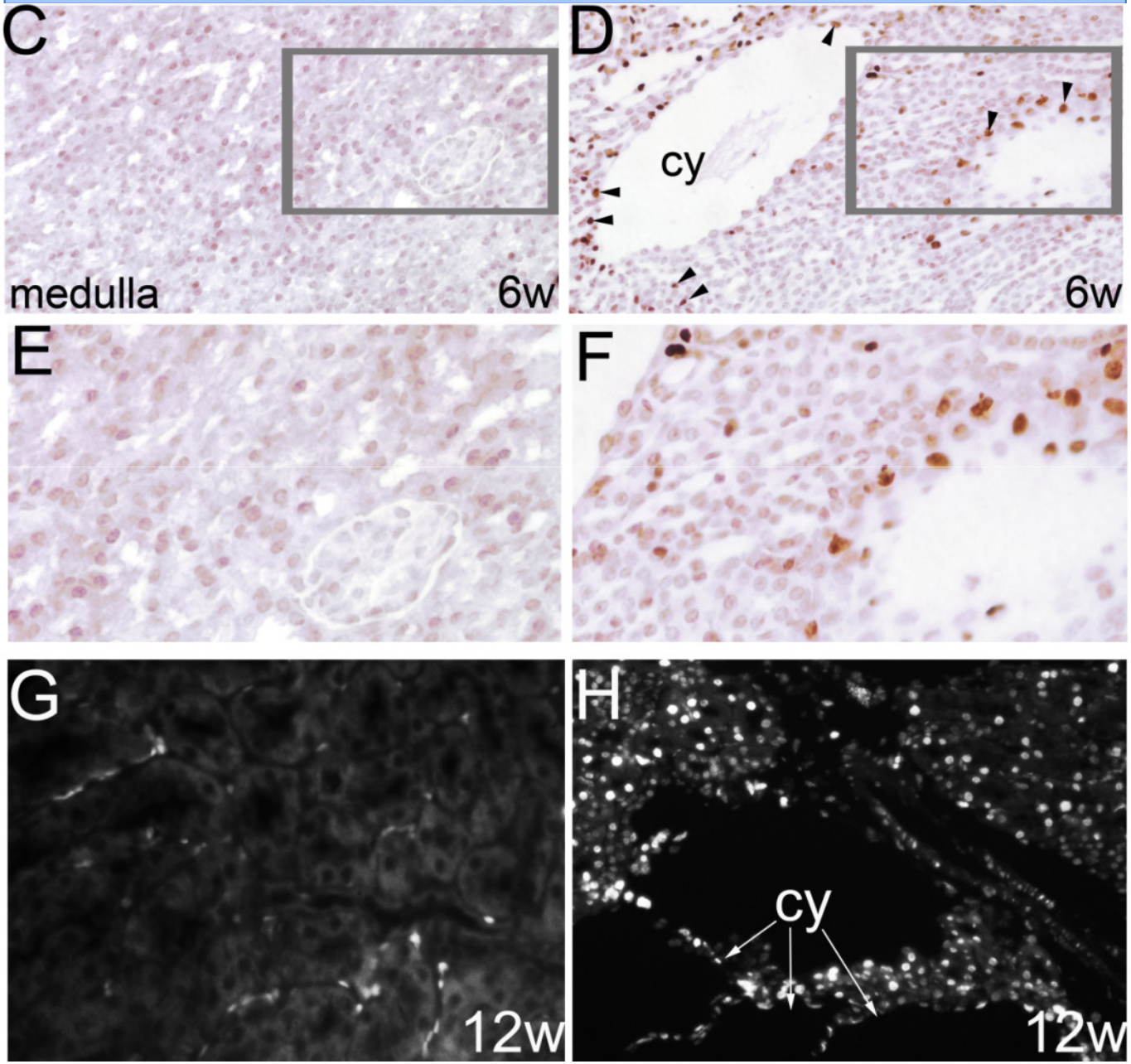
Cyst origins



Proliferation-phospho Histone H3

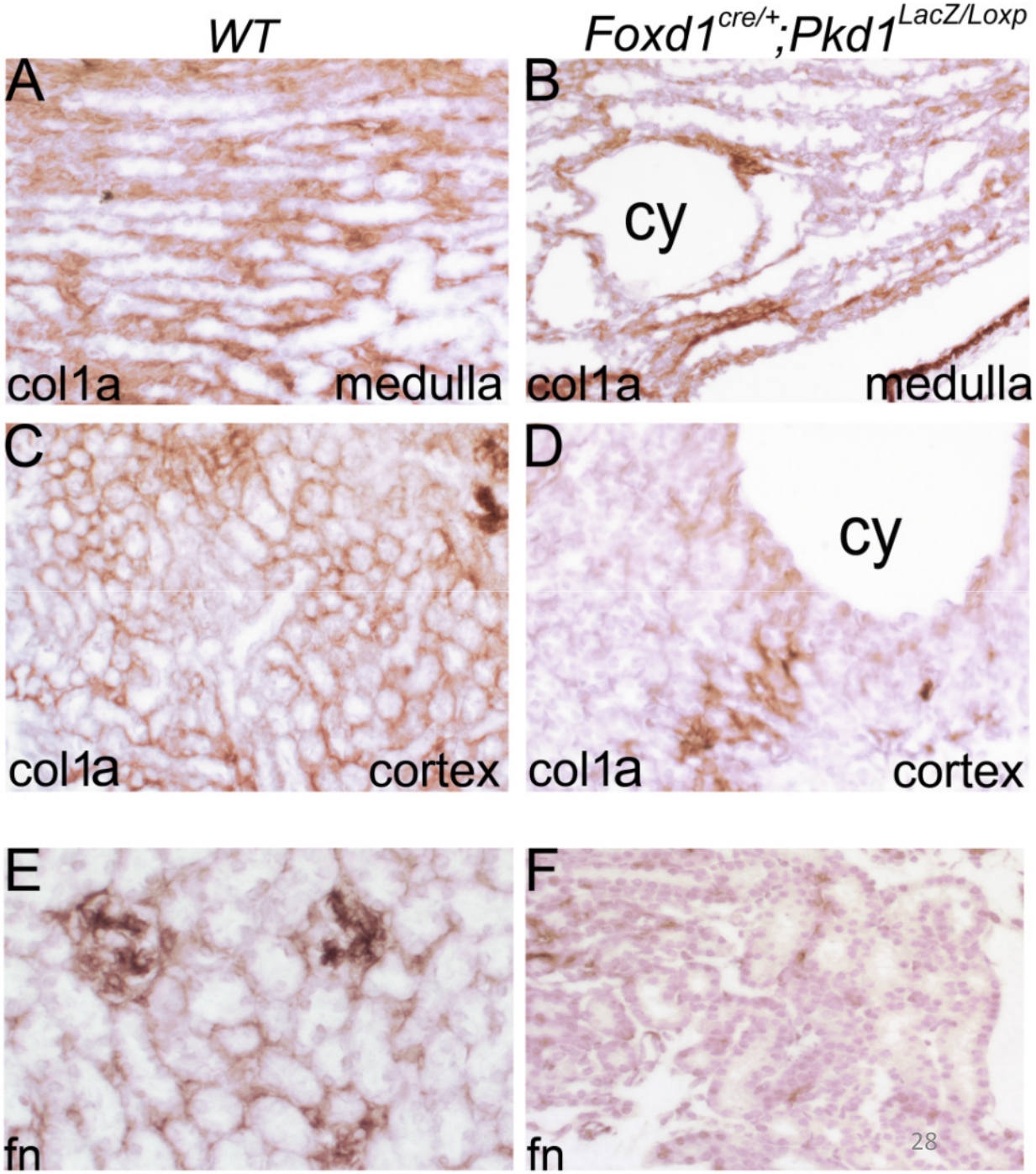


Apoptosis

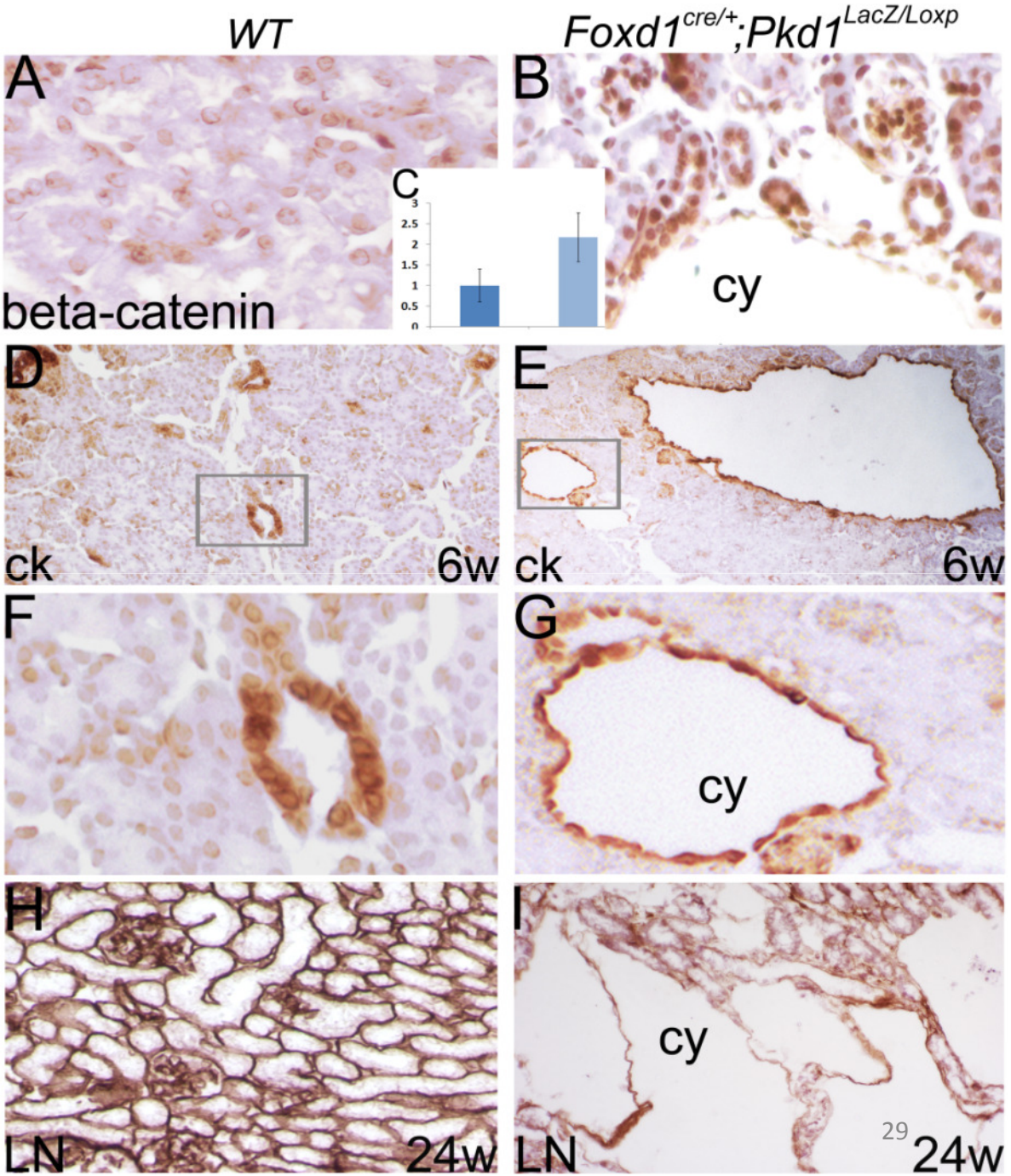


ECM

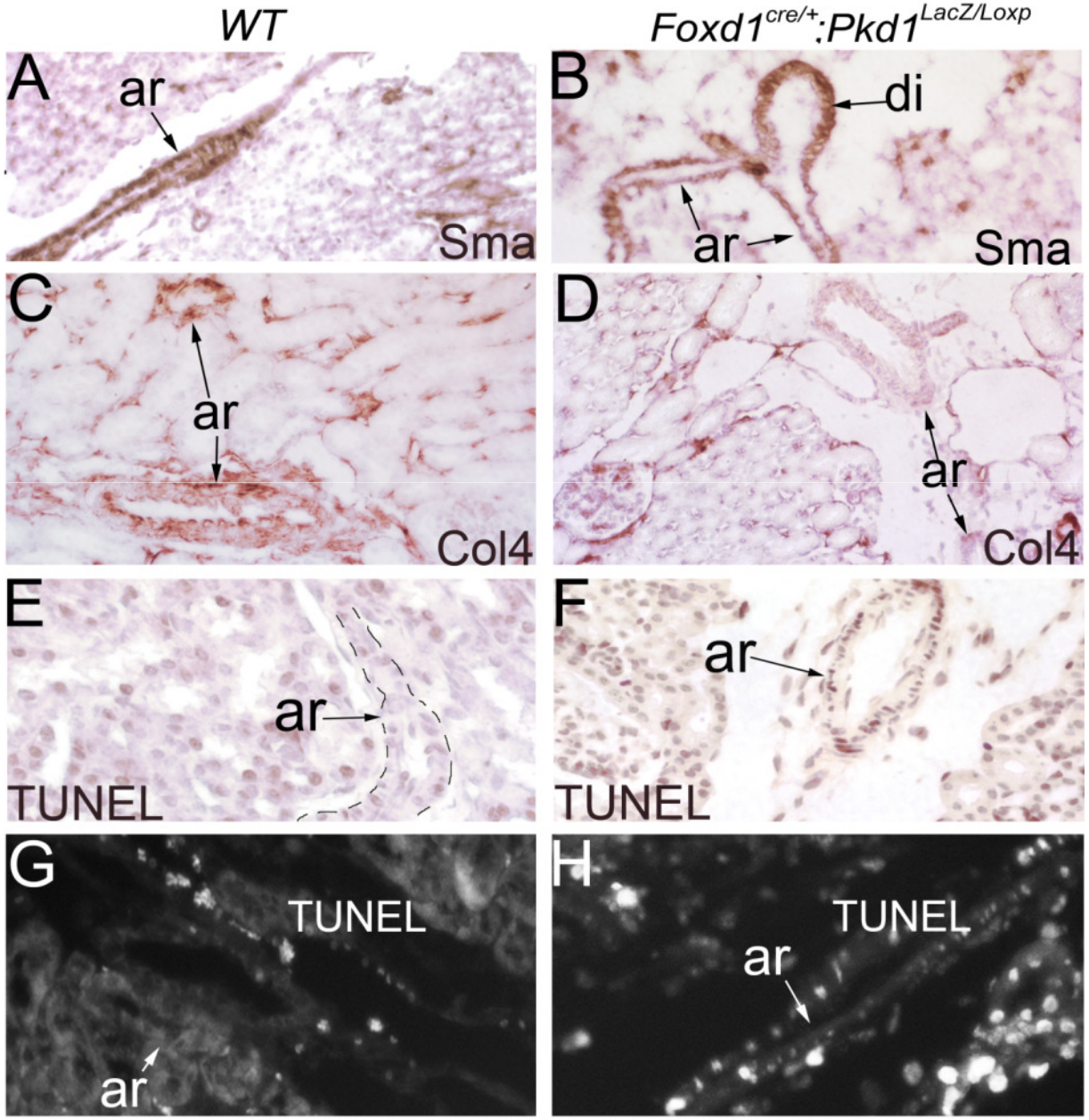
- Decreased ECM components in mutants
- Col1a: collagen type 1
- fn: fibronectin



- Epithelial changes
- -Increased Wnt
- - Flat epithelial cells
- -Disruption of BM at an advanced stage



Vascular changes



Summary 2

- Disruption of *Pkd1* in renal stromal cells leads to progressive cystogenesis in adulthood, modeling the clinical course of human ADPKD
- Disruption of *Pkd1* in renal stromal cells induces a spectrum of cellular and vascular changes seen in ADPKD.
- Polycystin deficiency in the stromal compartment might contribute significantly to renal changes of ADPKD.

Acknowledgments

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Thanks!