Determining How Sec10 and the Exocyst Complex Regulate Ureter Development

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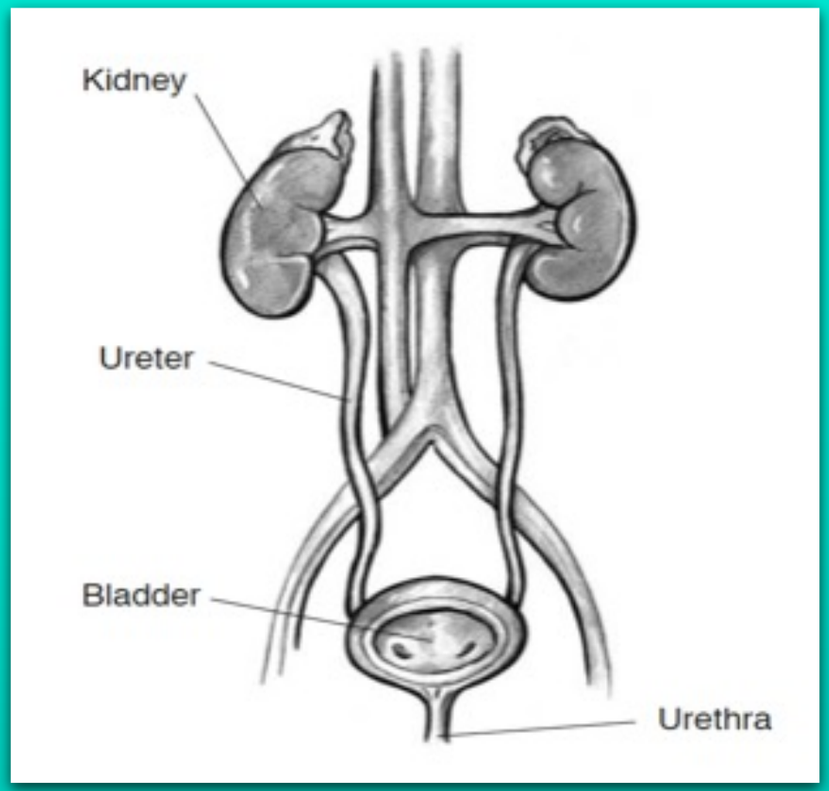
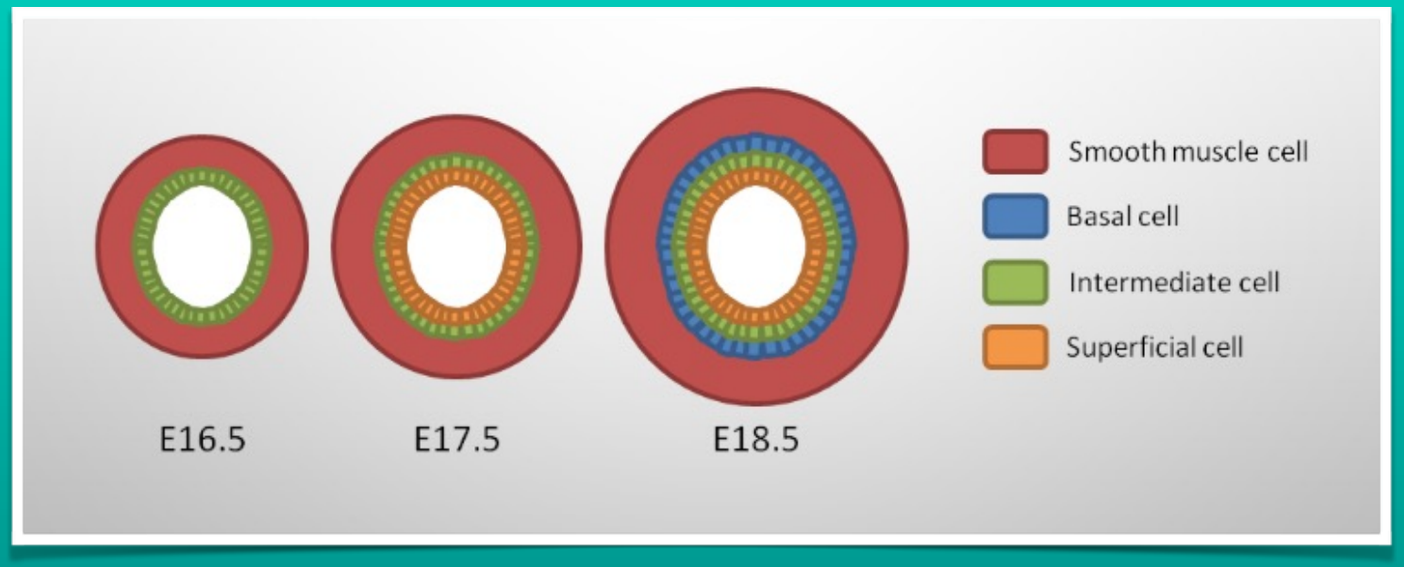
Background: Urinary System

- **Four main functions:**
  - Filtration
  - Reabsorption
  - Secretion
  - Excretion

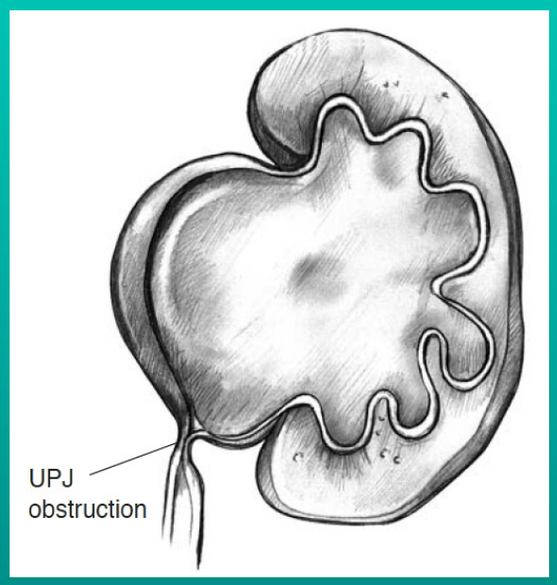
- **Kidney development**
  - Ureteric buds off from Wolffian duct
    - Ureteric bud branches proximally and distally
      - **Proximal** portion develops into kidney
      - **Distal** portion develops in the ureter





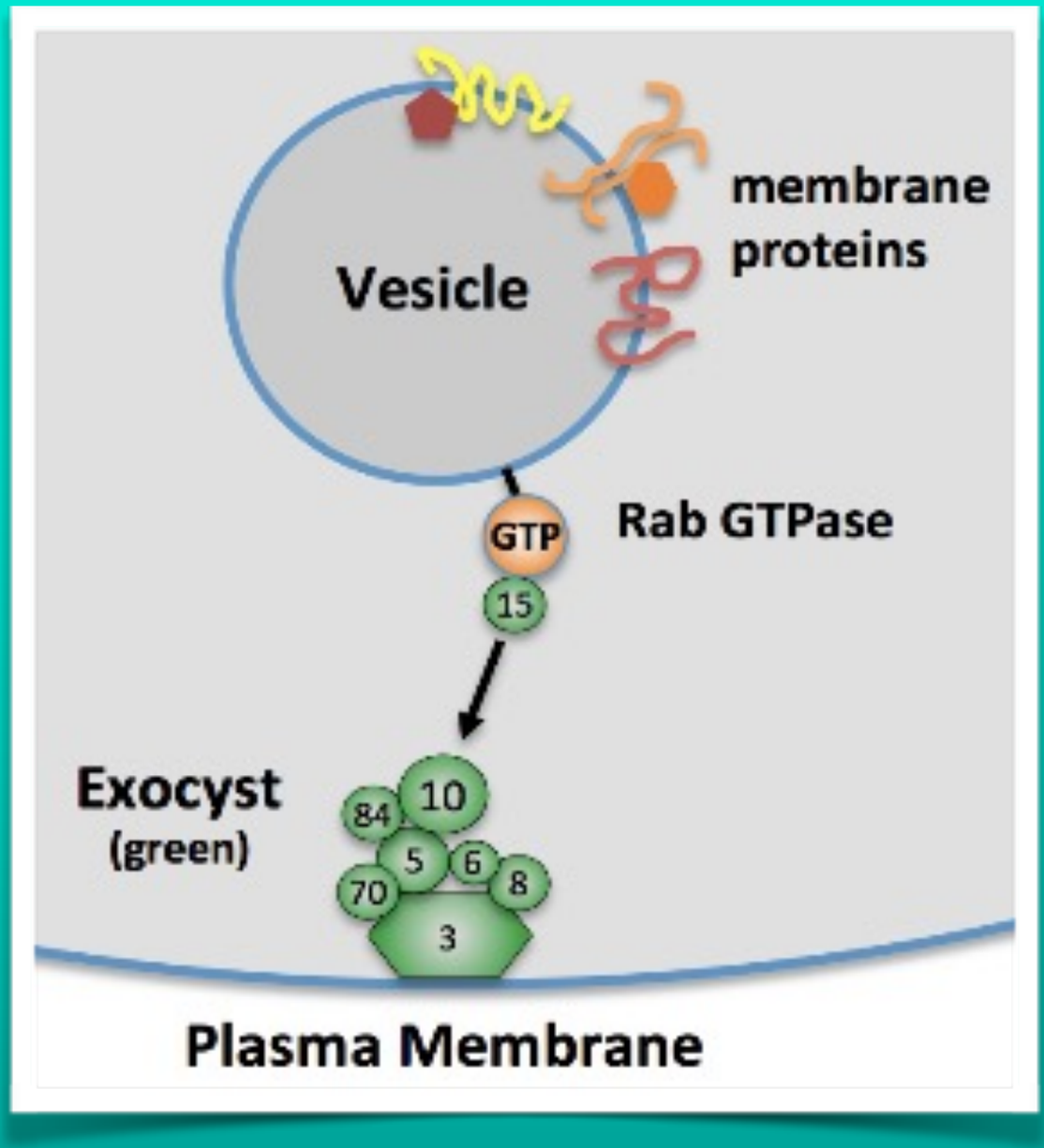
Background: Congenital Obstructive Nephropathy (CON)

- Most common cause of end stage renal disease (ESRD) and chronic kidney disease (CKD) in children
- Occurs in 1:500 births
- Leading cause of **CON** is ureteropelvic junction (UPJ) obstruction
  - Prevents urine outflow, causing **hydronephrosis**(swelling of the kidney due to urine accumulation in renal pelvis)
- Underlying cause is poorly understood



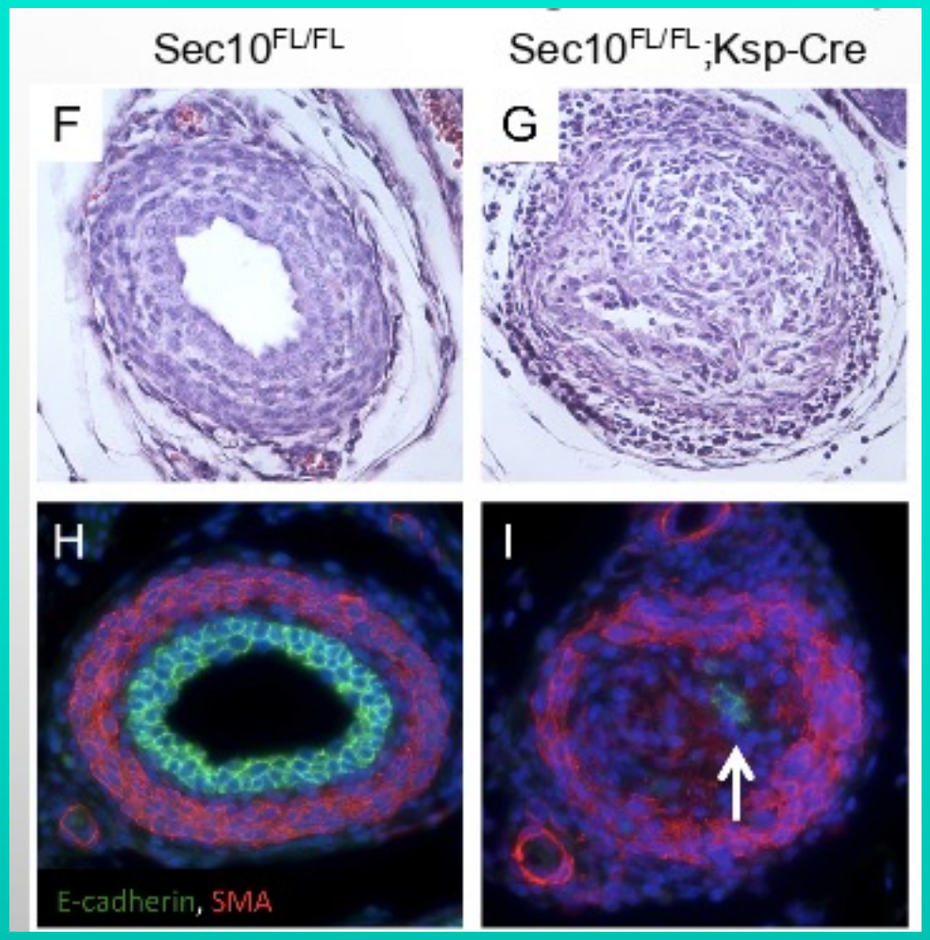
Background: Exocyst Complex

- Eight membered protein complex
  - Assists in epithelial cell growth, communication, and apical/basil polarity establishment
- *Sec10* central protein component
  - Attaches Sec15 to the exocyst complex on the plasma membrane
  - During stratification, urothelial cells die in absence of the exocyst complex



Lipschutz and Mostov, *Curr Biol*, 2002

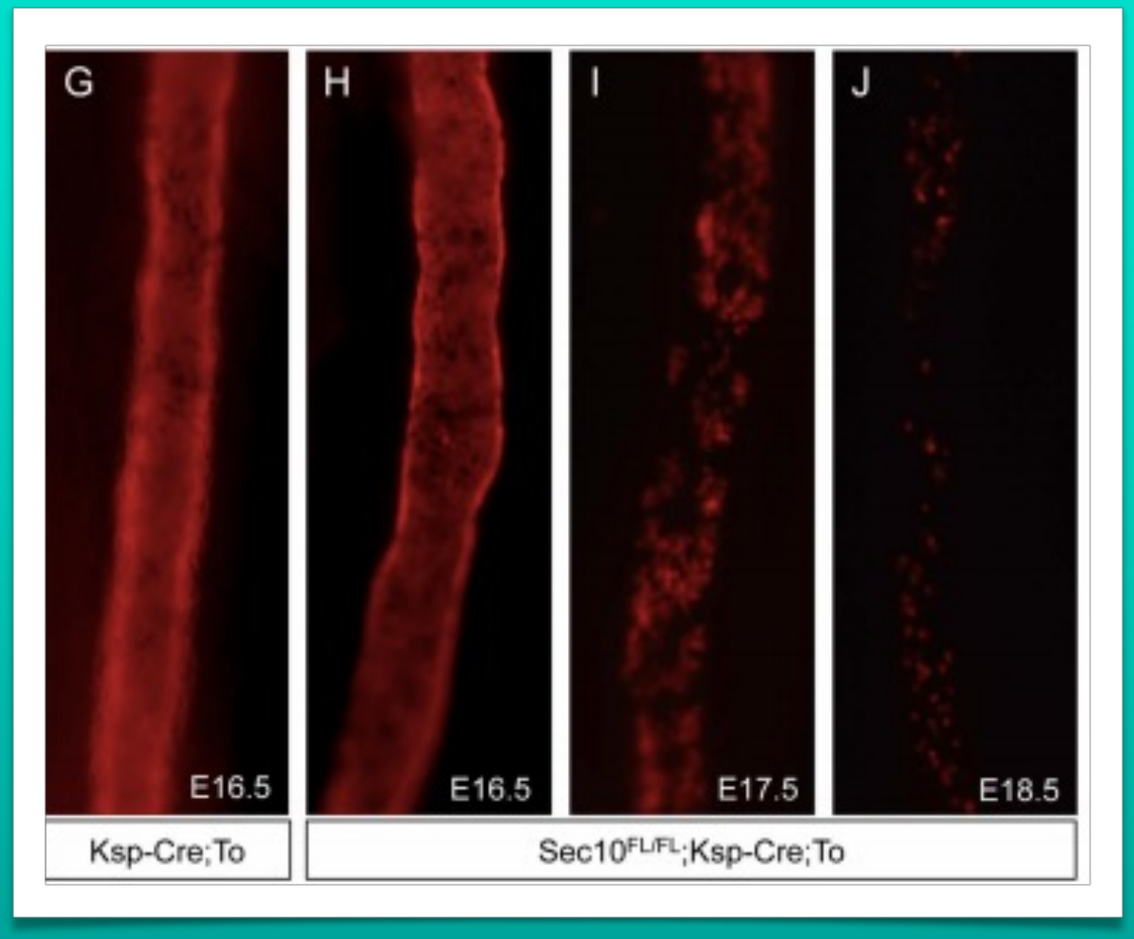
Background: CON in *Sec10* Urothelial Knockout



Fogelgren et al., *PLOS One,* 2015; Lee et al., Scientific Reports

- Progressive **loss of urothelial cells** after **E16.5** (embryonic day 16.5)

- Obstructed ureters shown in ***Sec10*** conditional knockout mice



Lee et al., *Scientific Reports,* 2016

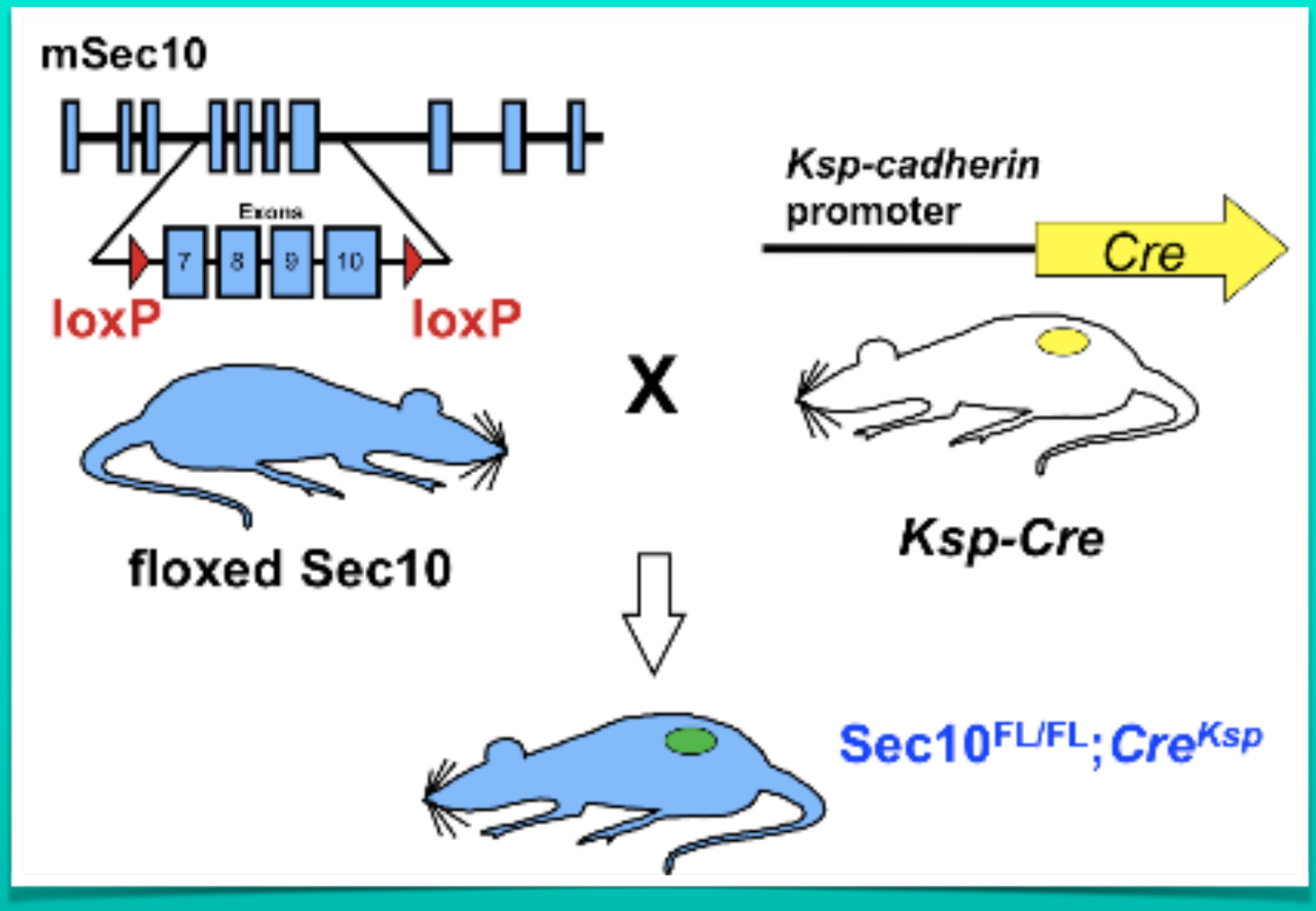
Hypothesis:

Abnormal cell signaling pathways may underlie cell death in affected epithelium

Research Question:

Which genes, of those associated with epithelial stress in mutant ureters, could be responsible for observed cell death?

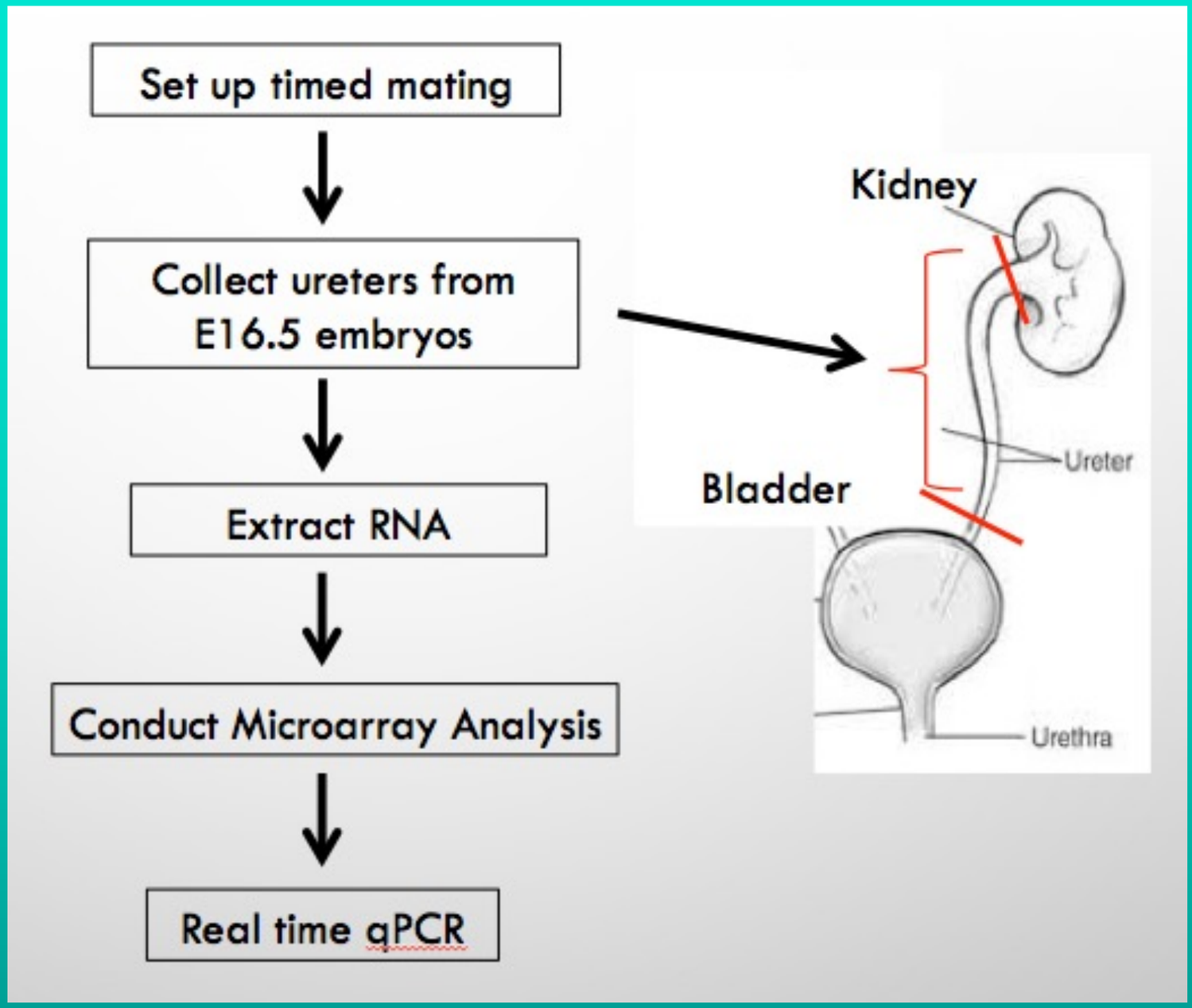
Methods:



Fogelgren et al., *PLOS One,* 2015; Lee et al., Scientific Reports

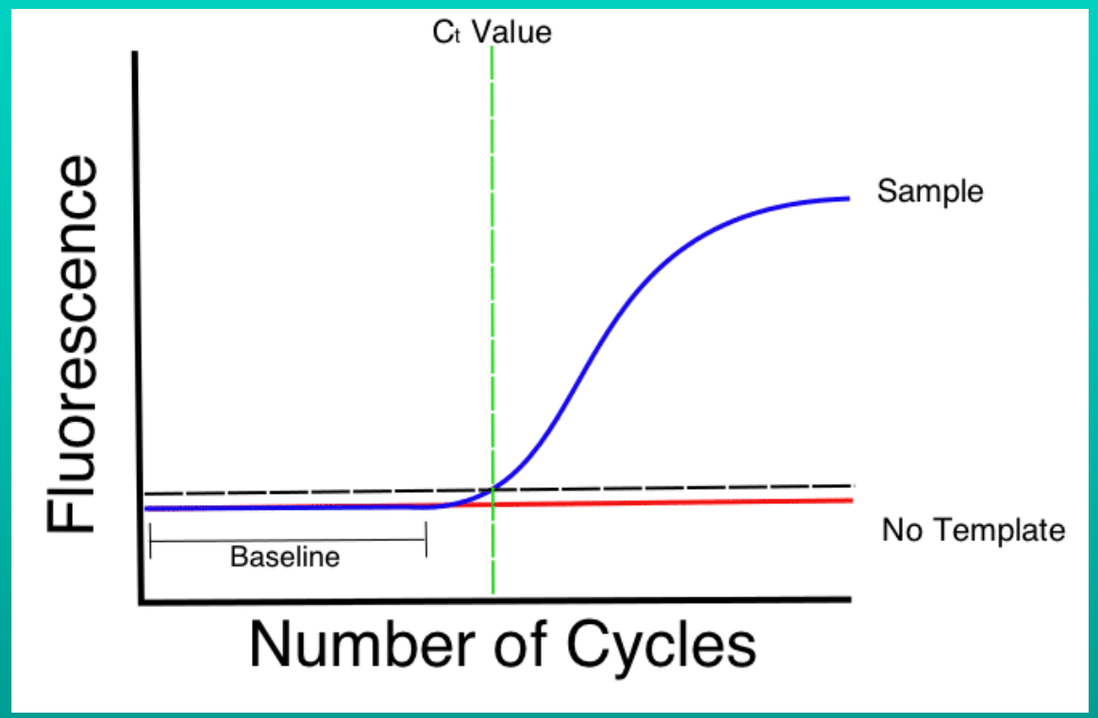
- *Sec10* conditional knockout mouse model created to model UPJO
  - Mouse husbandry and handling

Methods:


Set up timed mating
Collect ureters from E16.5 embryos
Extract RNA
Conduct Microarray Analysis
Real time qPCR
Kidney
Ureter
Bladder
Urethra

Methods:

- **Affymetrix microarray**
  - Transcriptome analysis software (TAC) to identify gene expression
    - Primers created from genes with the highest numbers of fold changes
- **Real time quantitative polymerase chain reaction (RT-qPCR)**
  - Using SYBR green probe
    - Binds to double stranded
  - Amplification and measurement



Affymetrix Microarray Analysis of E16.5 Ureters

- What molecular changes occur prior to cell death at E17.5?

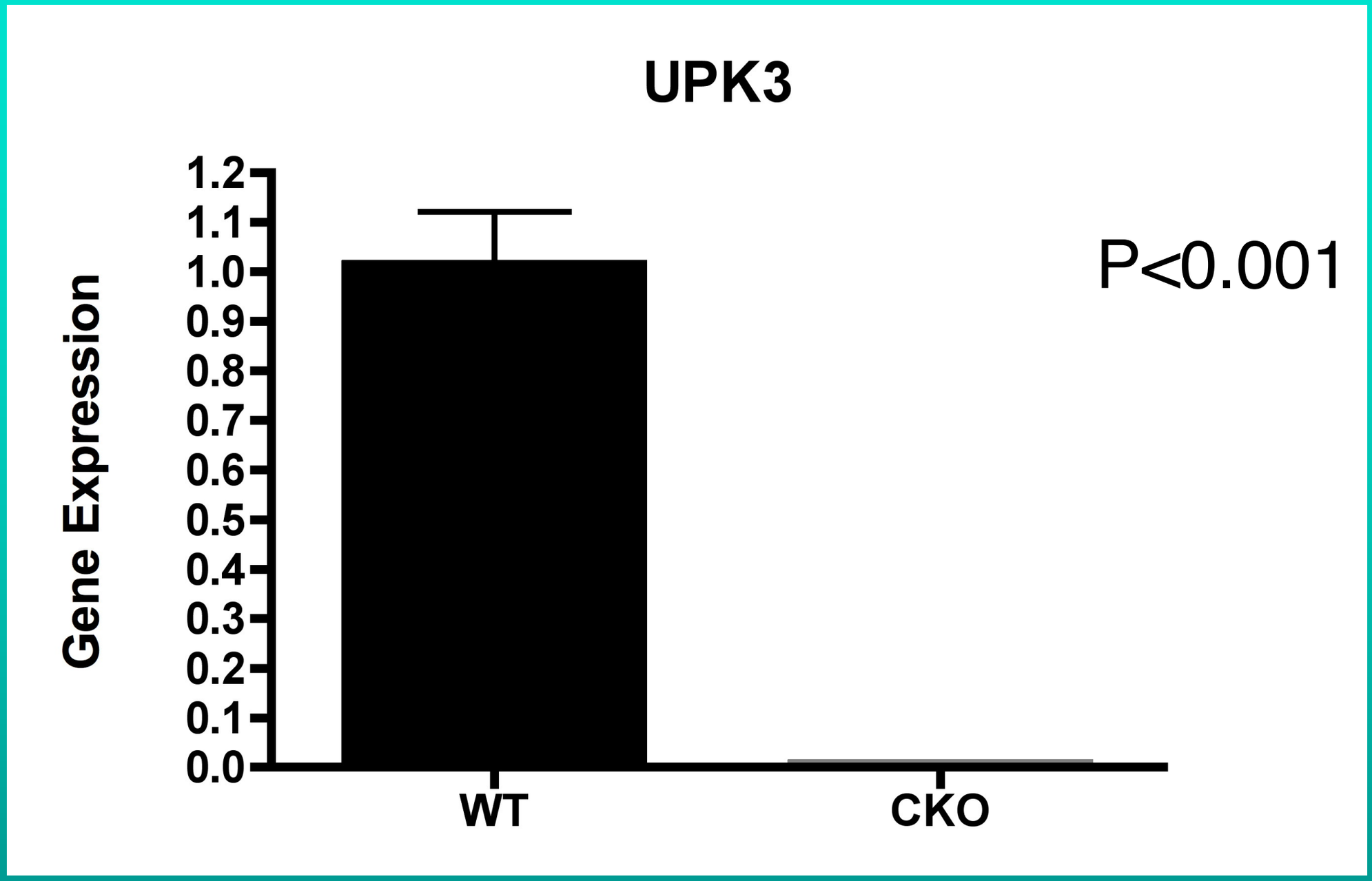
Table 1. Upregulated genes in E16.5 CKO vs WT ureters

Gene Name	Fold Change
Gprc5a	21.28
Tmem171	13.81
Efemp1	13.09
Krt14	9.62
Gadd45b	7.93
Epha2	7.55

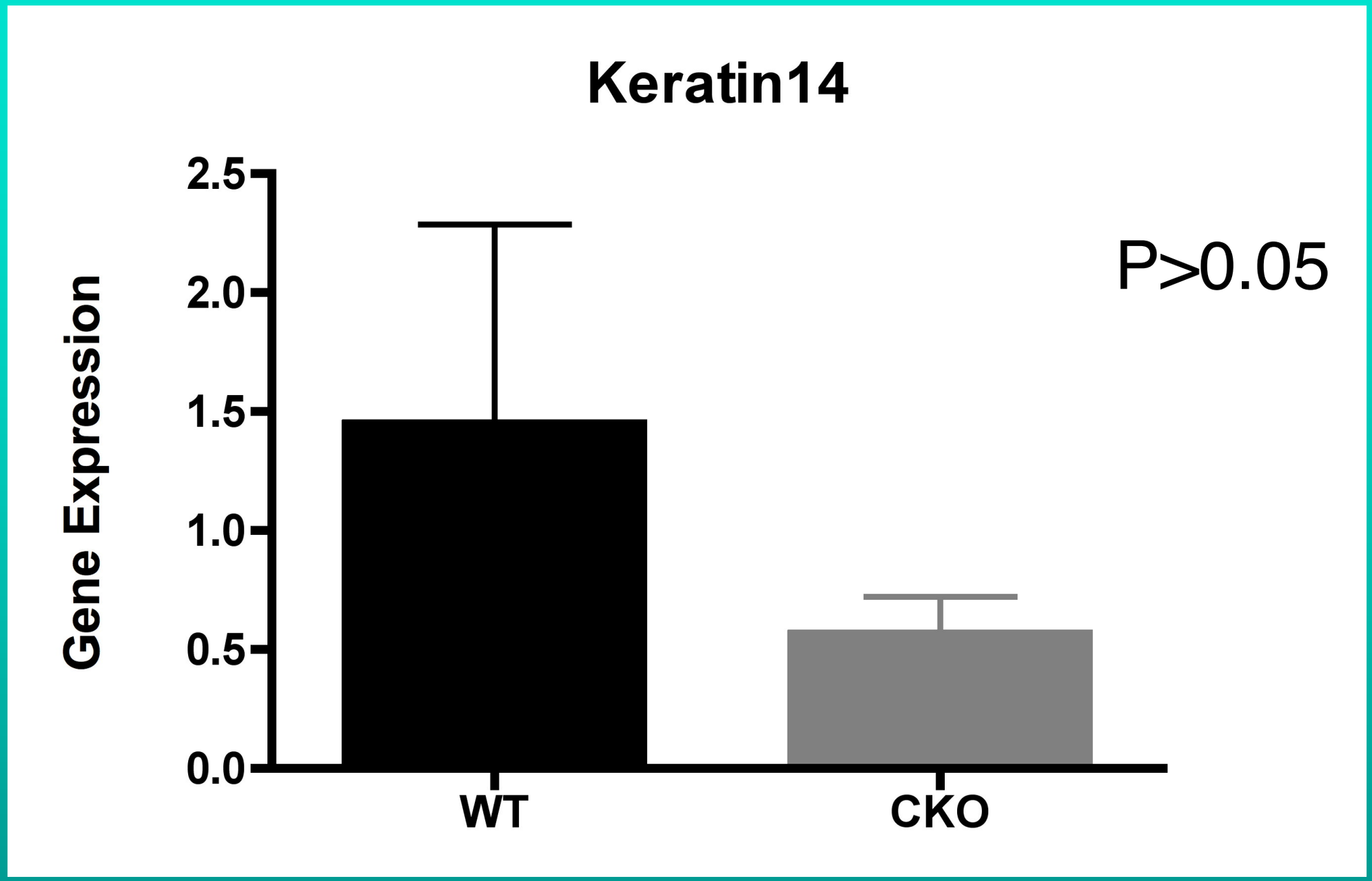
Table 2. Downregulated genes in E16.5 CKO vs WT ureters

Gene Name	Fold Change
Upk3a	-94.57
Sptssb	-26.5
Fxyd2	-24.97
Gsdmc2	-24.34
Tmem27	-19.9
Aqp3	-11.3

Results:


UPK3
Gene Expression
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
WT
CKO
P<0.001

Results:



Discussion:

- UPK3a significantly down-regulated in CKO ureters
    - Confirms integrity of the samples
- No significant difference in Keratin14 expression between WT and CKO ureters according to qPCR analysis
    - Does not confirm microarray data
- Other primers from Affymetrix Microarray were tested
    - Had difficulty optimizing primers

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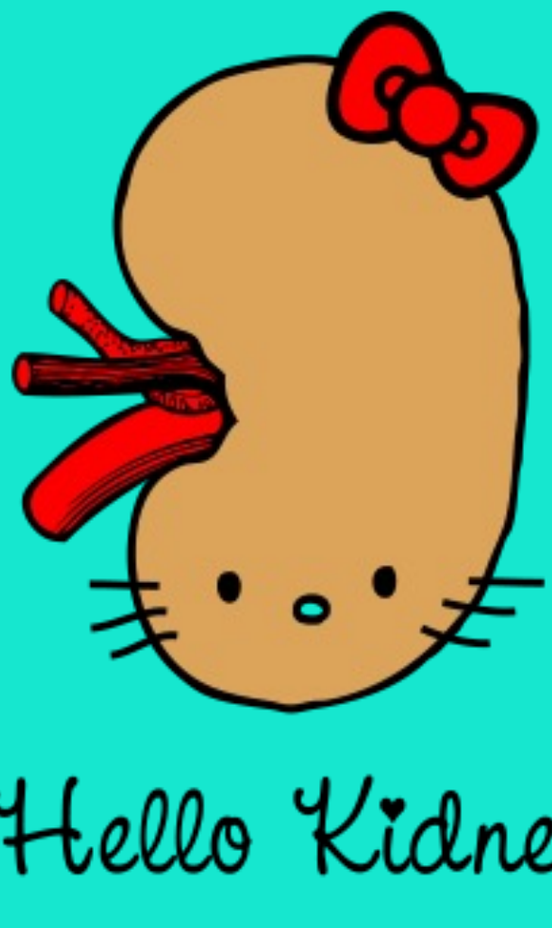

Questions?