Tackling Renal Cyst Formation Using a *Drosophila* Model of Polycystic Kidney Disease

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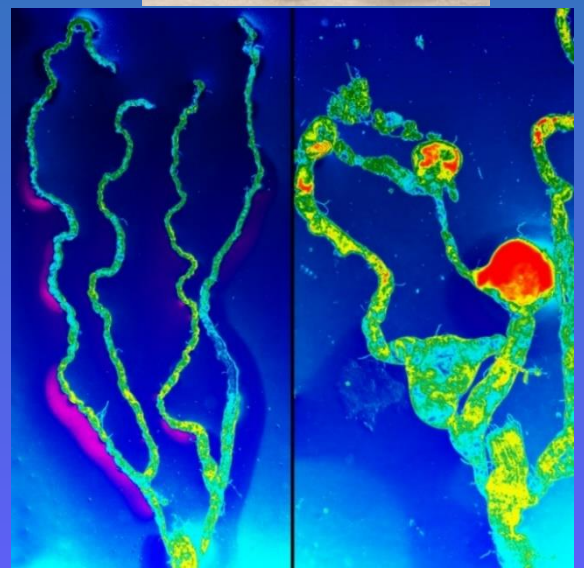
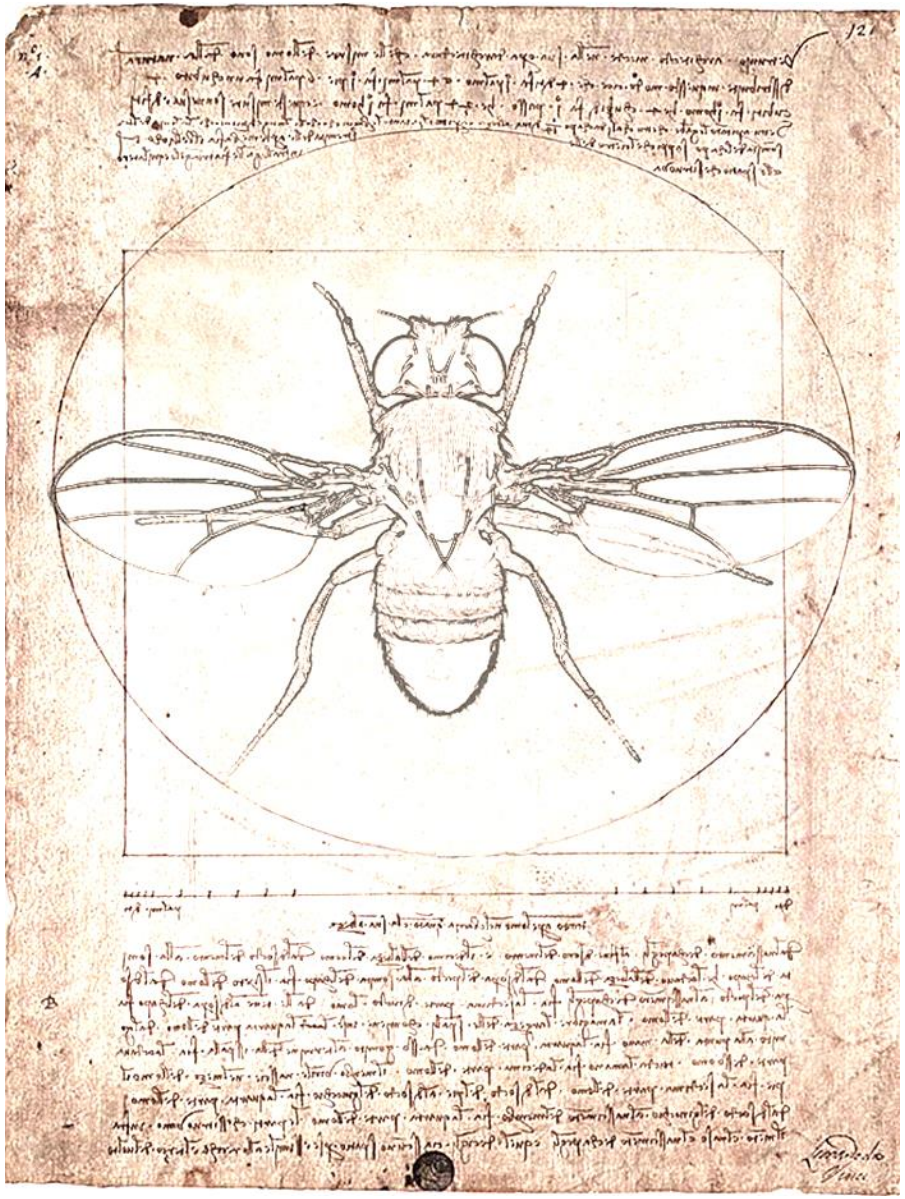
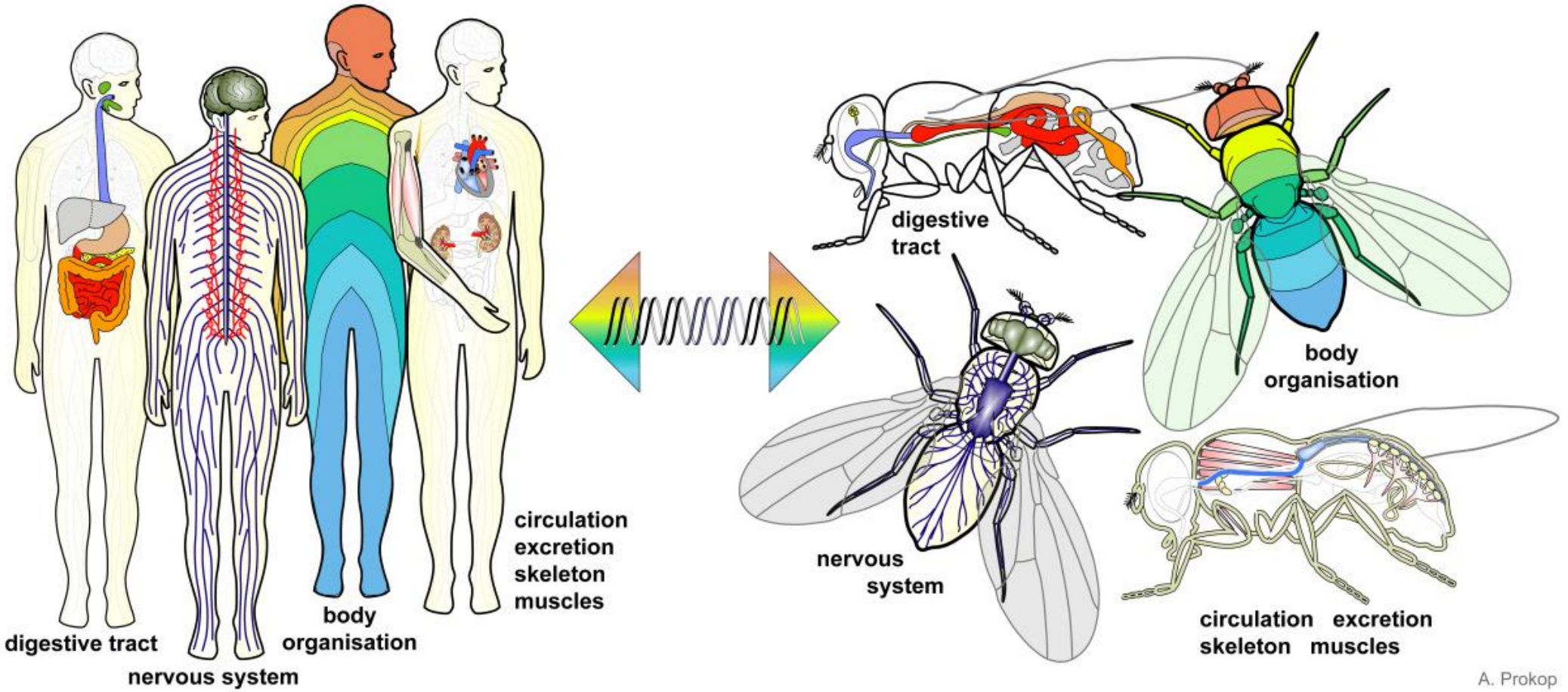


Today:

- *Drosophila* to model complex human renal physiology.
- Decipher conserved mechanisms of renal cyst formation and polycystic kidney disease.
- Drug discovery.



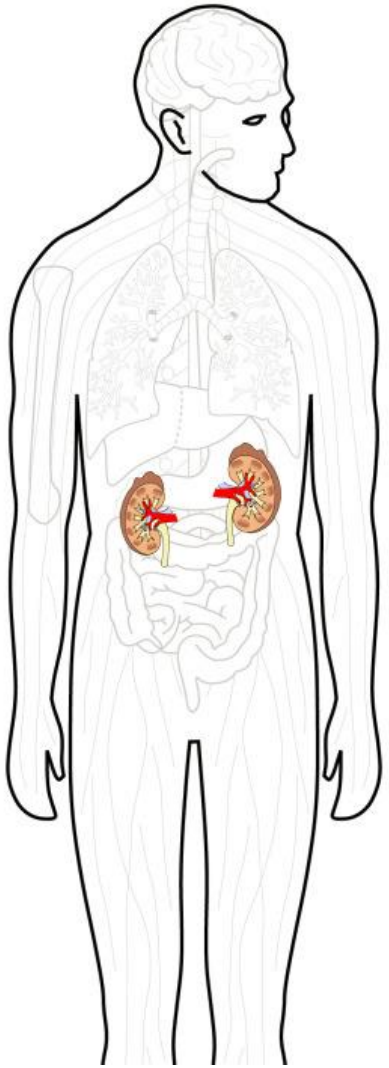
Fruit flies display streamlined versions of human organs and conserved gene regulation

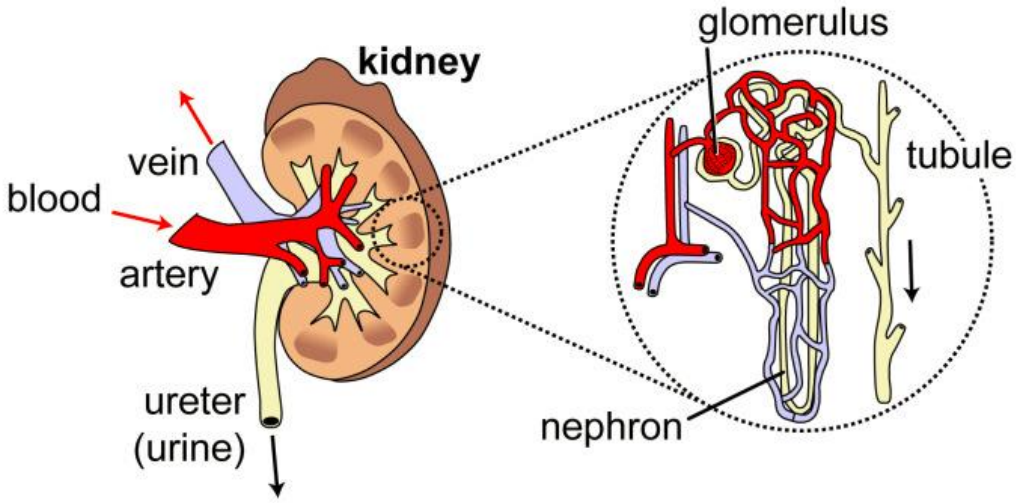


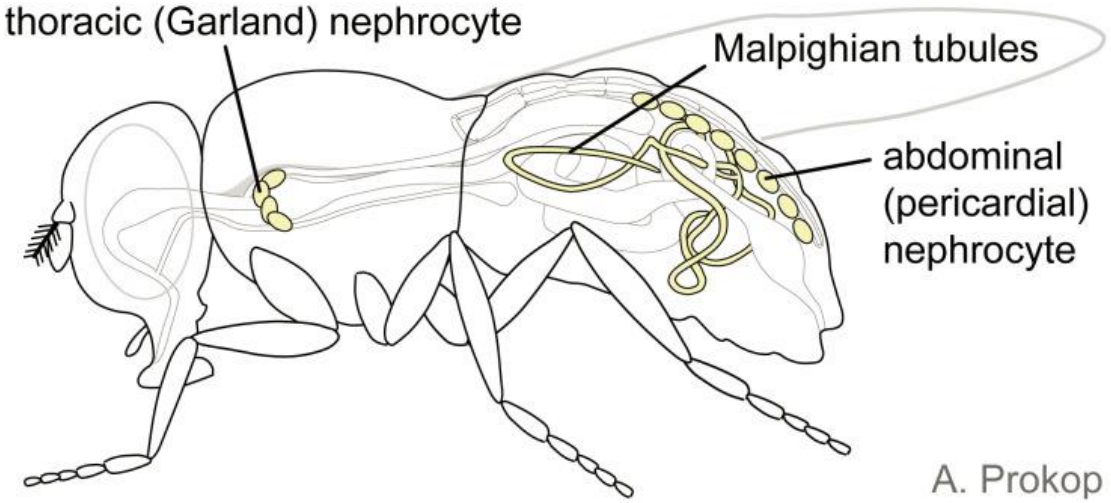
- **75% conservation of genes and pathways.**

Modeling renal function in the fly

The fly's simple renal anatomy enables cellular and biochemical studies precluded in vertebrate models.

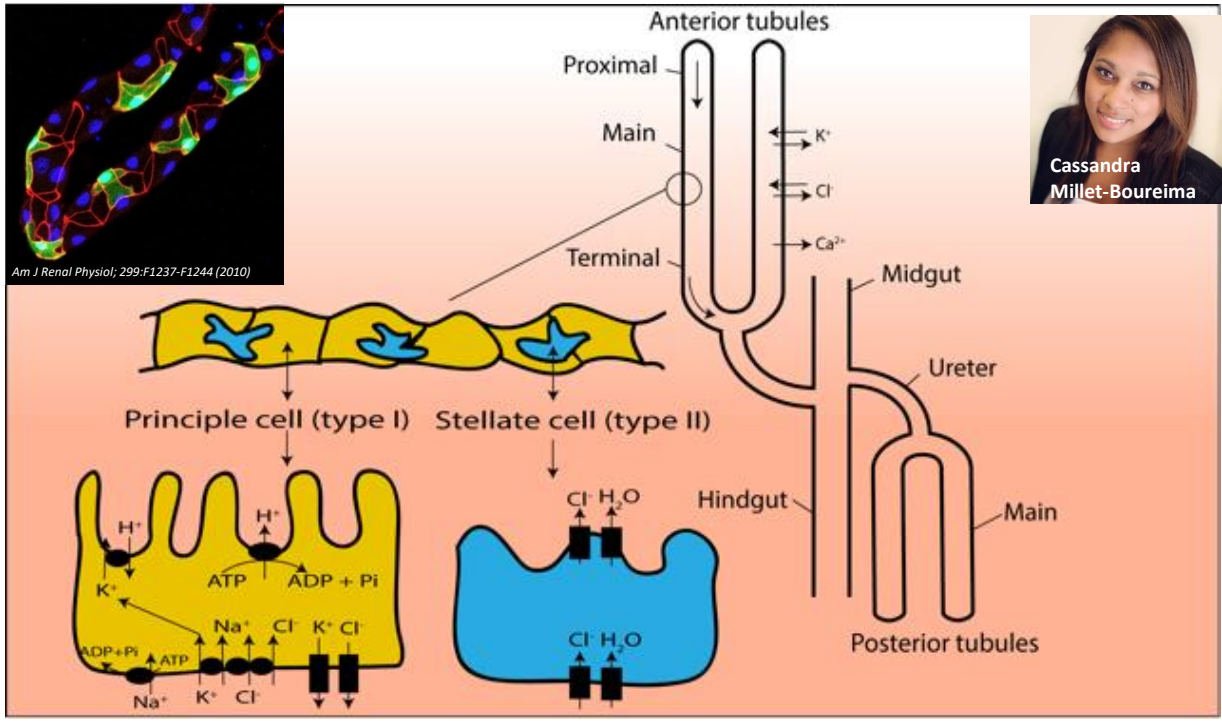


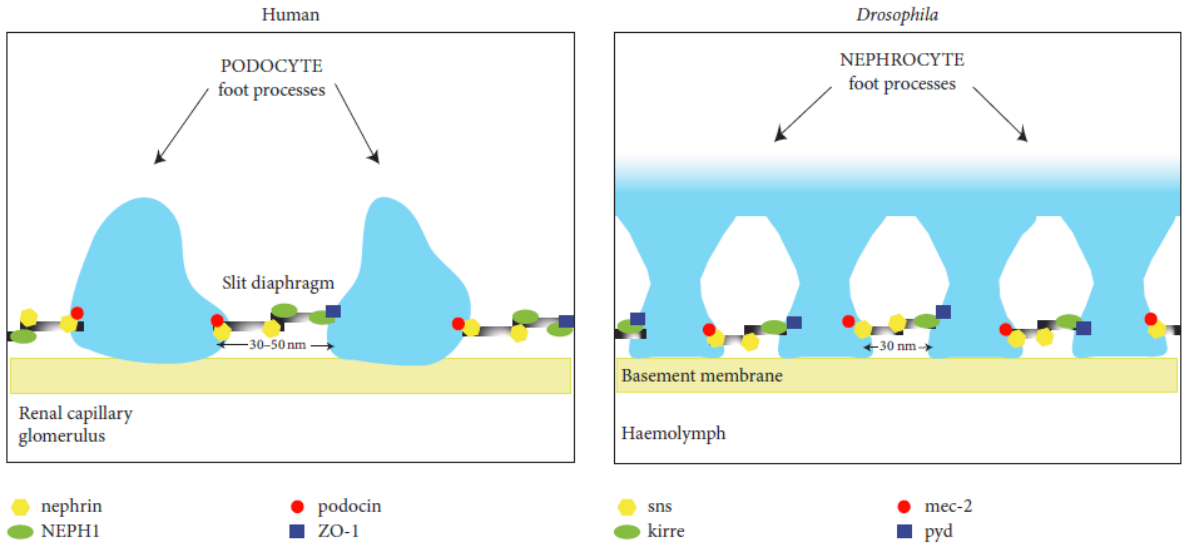




Fly models of renal disease and development

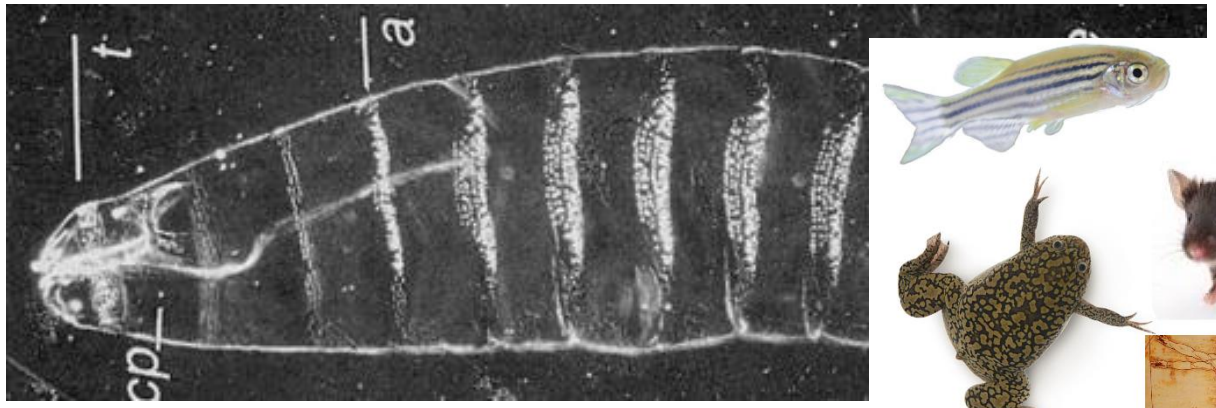
- Renal tubule development (reviewed in Millet-Boureima *et al*., 2018)
- Slit diaphragm function (reviewed in Millet-Boureima *et al*., 2018)
- Cytochrome P450, glutathione S transferase (Yang et al., 2007)
- V-ATPase/ATP6B1 (Allan *et al*., 2005)
- Nephrolithiasis (Ca oxalate, uric acid, Chi *et al*., 2015, Smith *et al*., 2000)
- PKD (Gamberi *et al*., 2017)

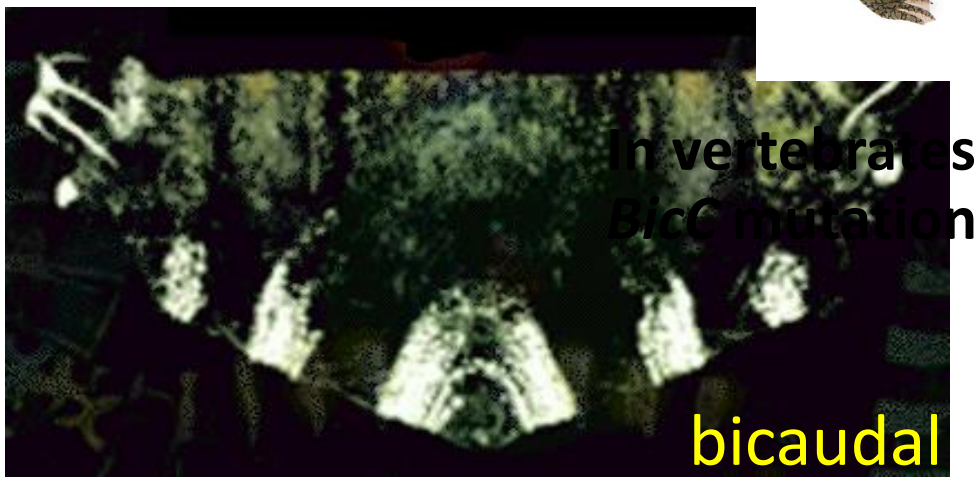




C. Millet-Boureima, J. Porras-Marroquin and C. Gamberi, *BioMed Res Intern* 2018, Article ID 5697436

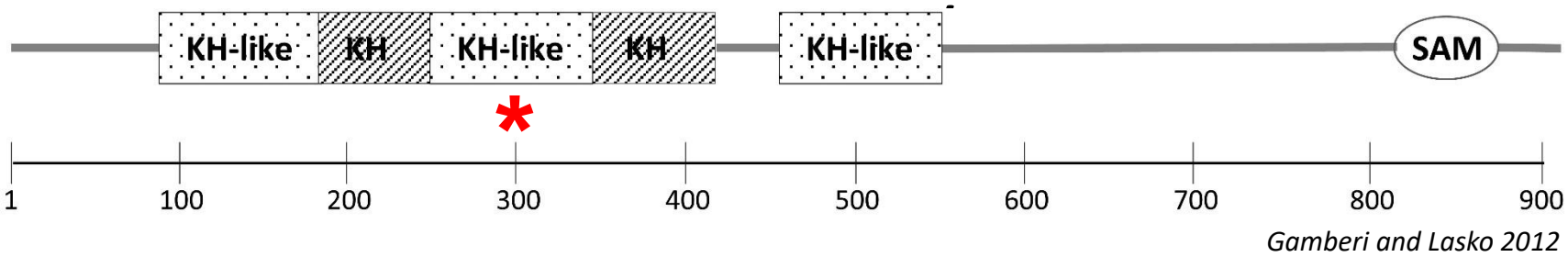
The *Bicaudal C* (*BicC*) gene is involved in embryonic patterning and renal function



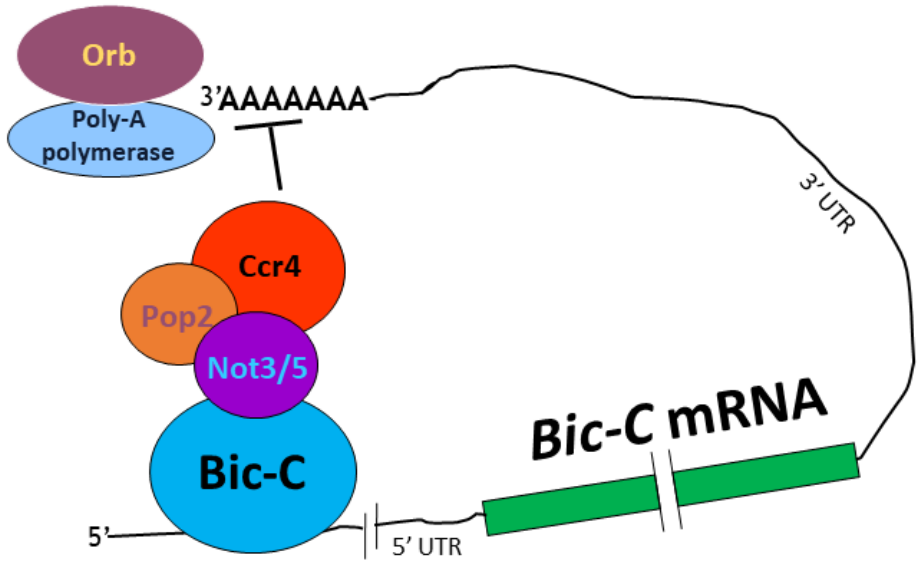


In vertebrates ,
***BicC* mutation induces renal cysts**

BicC is a conserved RNA binding protein with germline functions

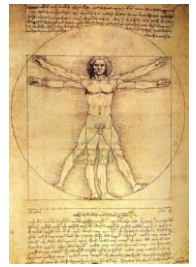


Gamberi and Lasko 2012

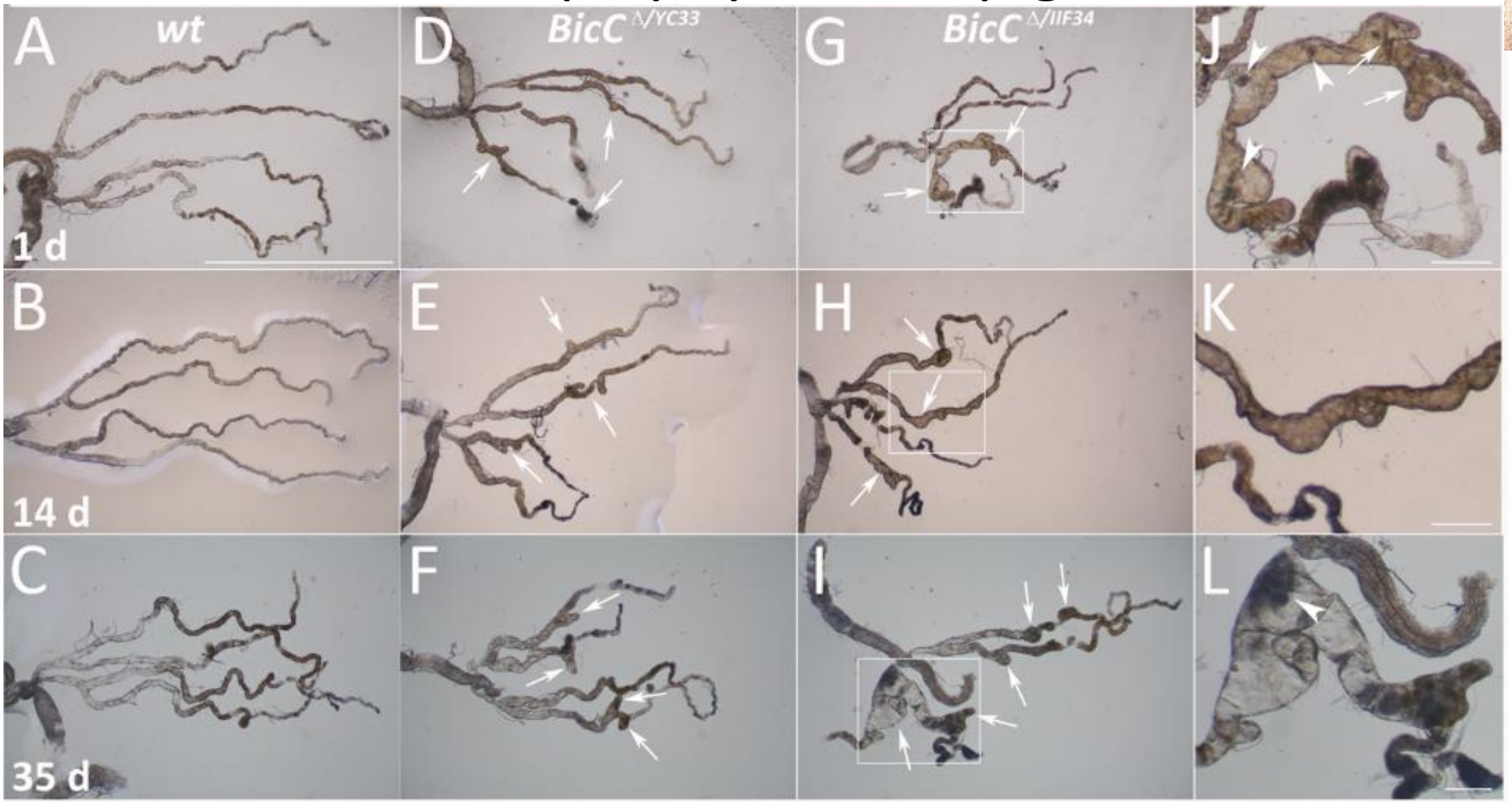


Mahone *et al. EMBO J.* 14: 2043 (1995); Saffman *et al. MCB 18*: 4855 (1998), Chicoine, … Gamberi *et al. Dev. Cell* 13:691-704 (2007); Gamberi and Lasko *Comp Funct Genomics* 2012:141386. doi: 10.1155/2012/141386 (2012).

BicC mutant flies develop PKD-like renal cysts

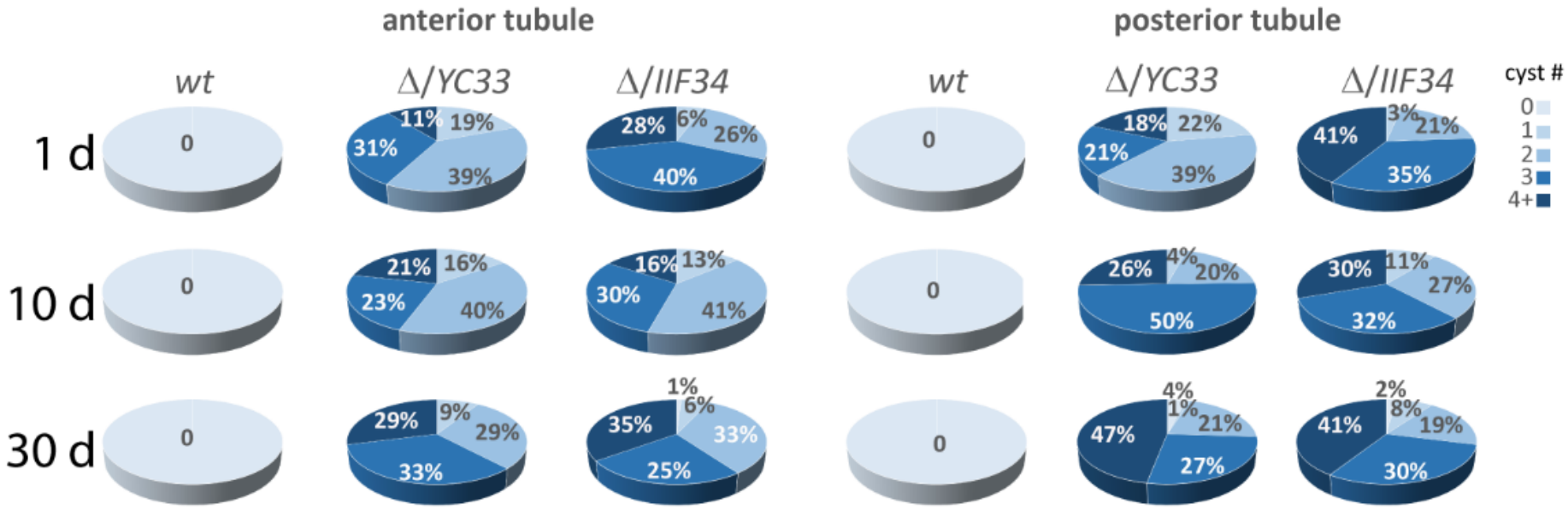


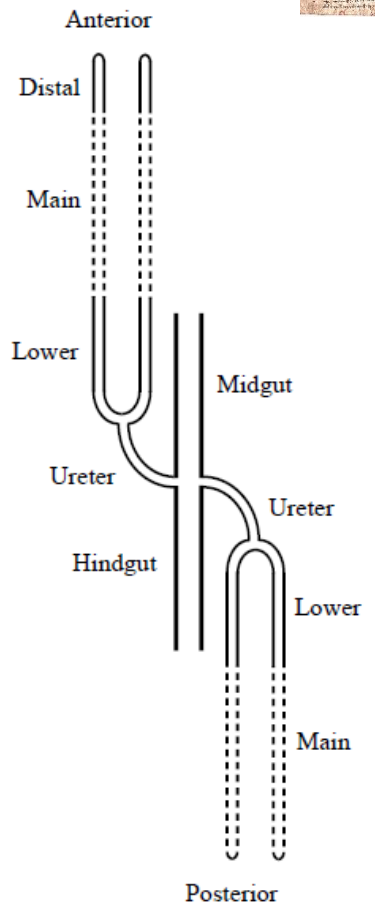
BicC mutant flies display cystic Malpighian tubules



Gamberi C., Hipfner D. R., Trudel M., Lubell W. D. 2017. *PLOS Genetics*, 2017 13(4):e1006694.

Cyst number increases over time





O'Donnell and Maddrell J Exp Biol 198: 1647-53 (1995)

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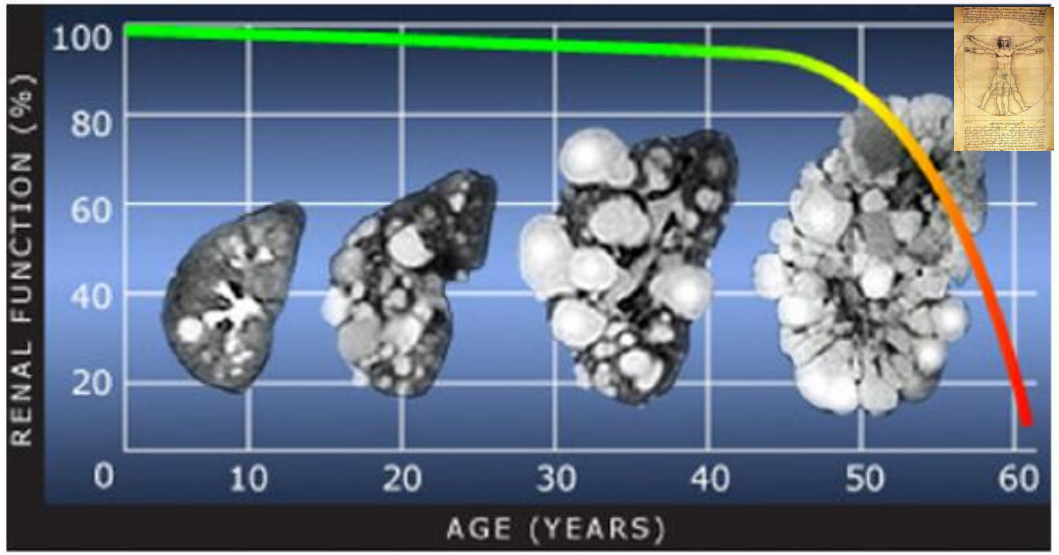
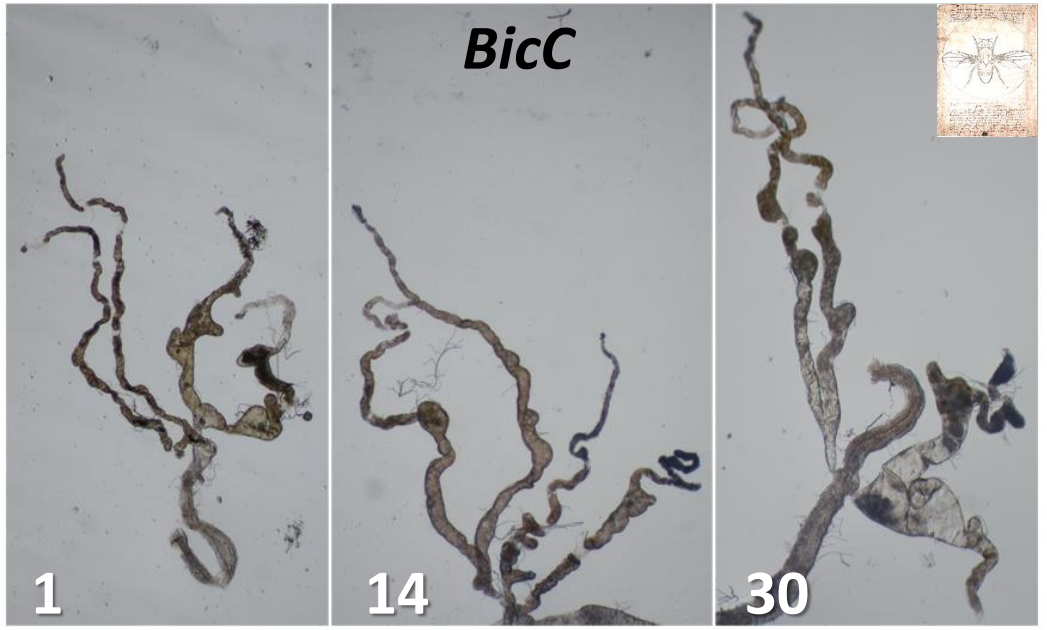
Polycystic kidney disease

- **ADPKD (autosomal dominant PKD):** 12.5 million people affected, *PKD1*, *PKD2*, possible high frequency of spontaneous mutations.
  - *PKD1* > polycystin (PC) 1, GPCR.
  - *PKD2* > polycystin 2, non-selective Ca^{++} channel, TRPP2 family.
  - *BicC*?

- **ARPKD (autosomal recessive PKD)**: *PKHD1*.
  - *PKHD1* > fibrocystin, transmembrane receptor.
  - *BicC*?

What is a cyst?
What causes cysts?



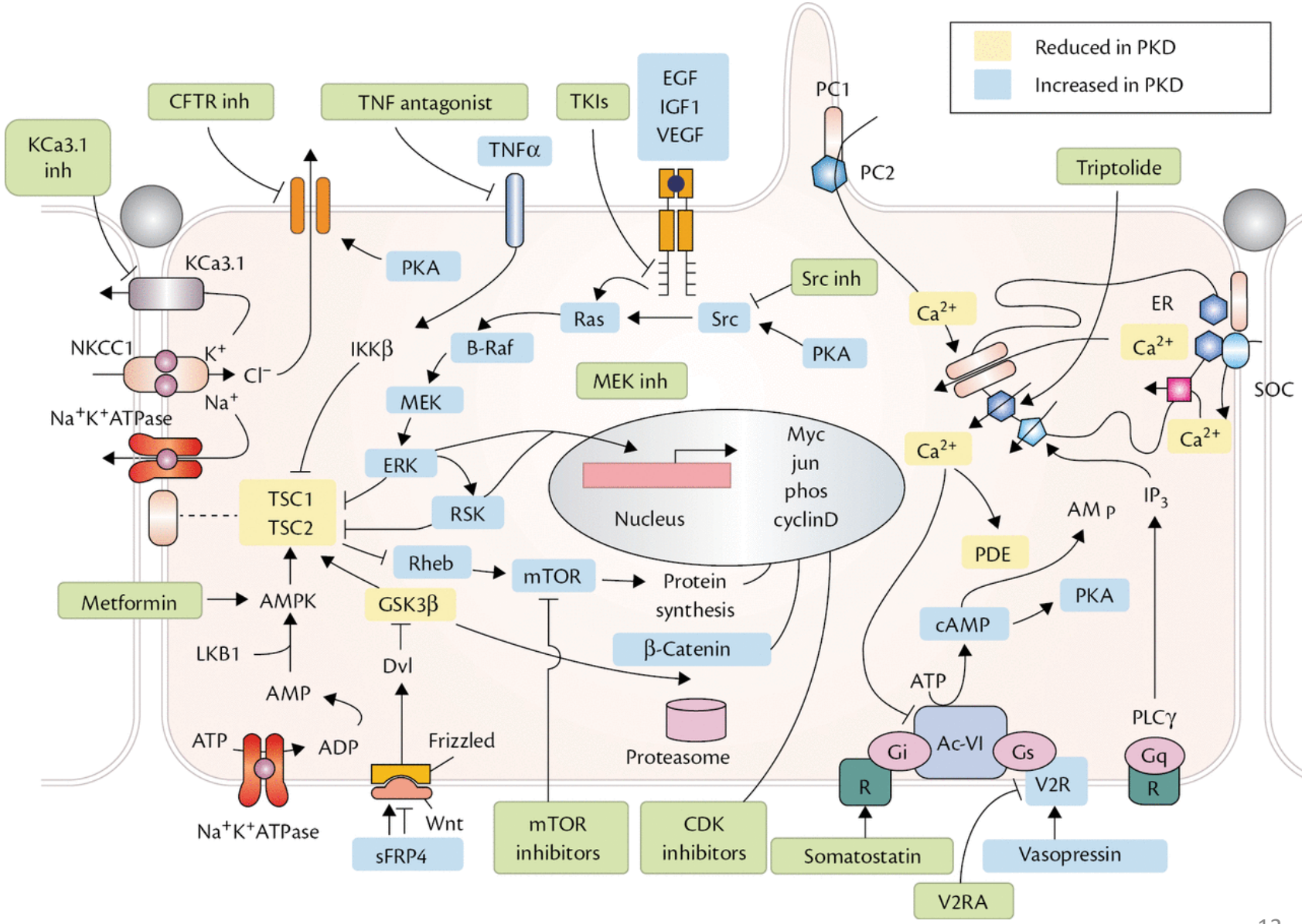


AGE (days)

Multiple signaling pathways are altered in PKD, unknown mechanisms

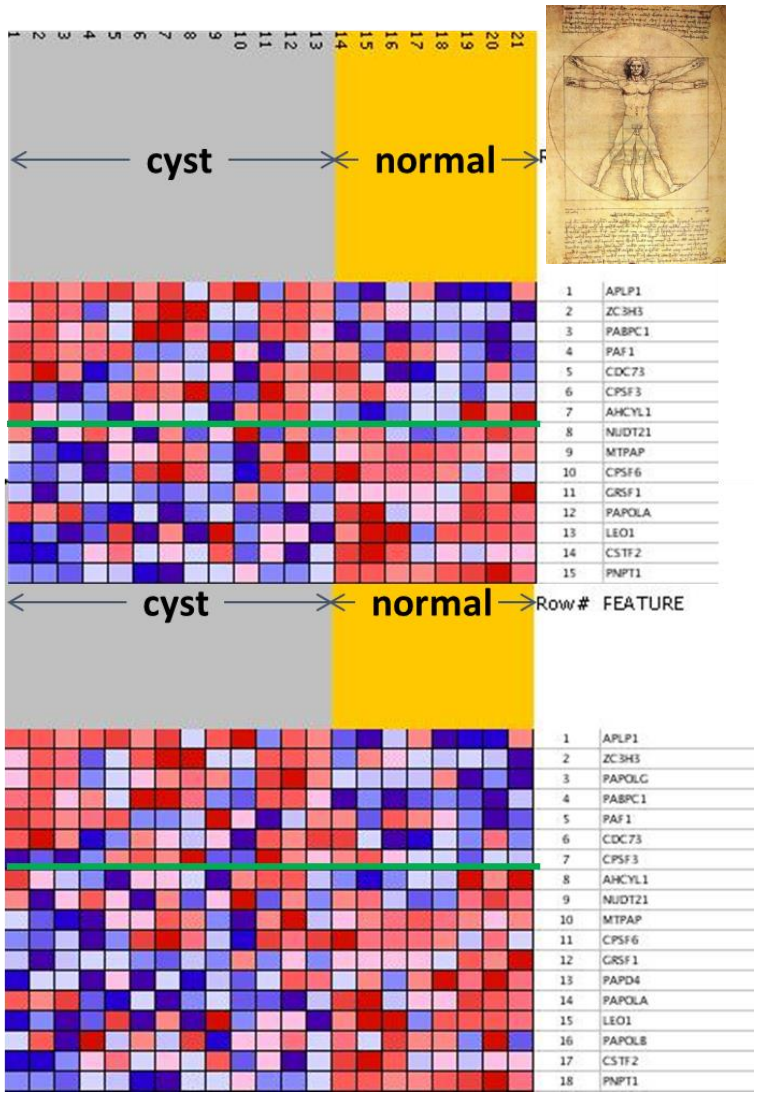
1- cell growth
2- cell polarity
3- fluid transport
4- secretion
5- Ca^{++} signaling, cAMP
6- …

Primary vs. secondary (compensatory) changes

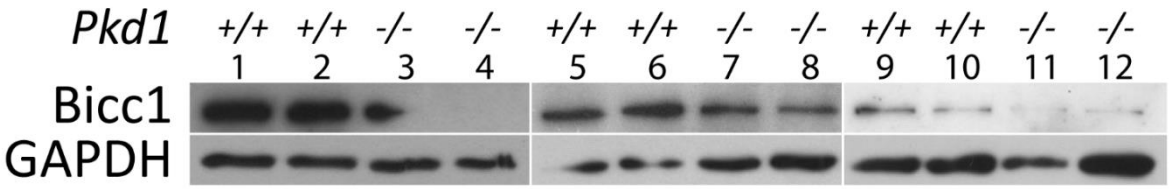


Wolfgang Kühn and Gerd Walz "The molecular basis of ciliopathies and cyst formation " Oxford Textbook of Clinical Nephrology (4 ed.)

PKD tissue displays reduced *BicC* function



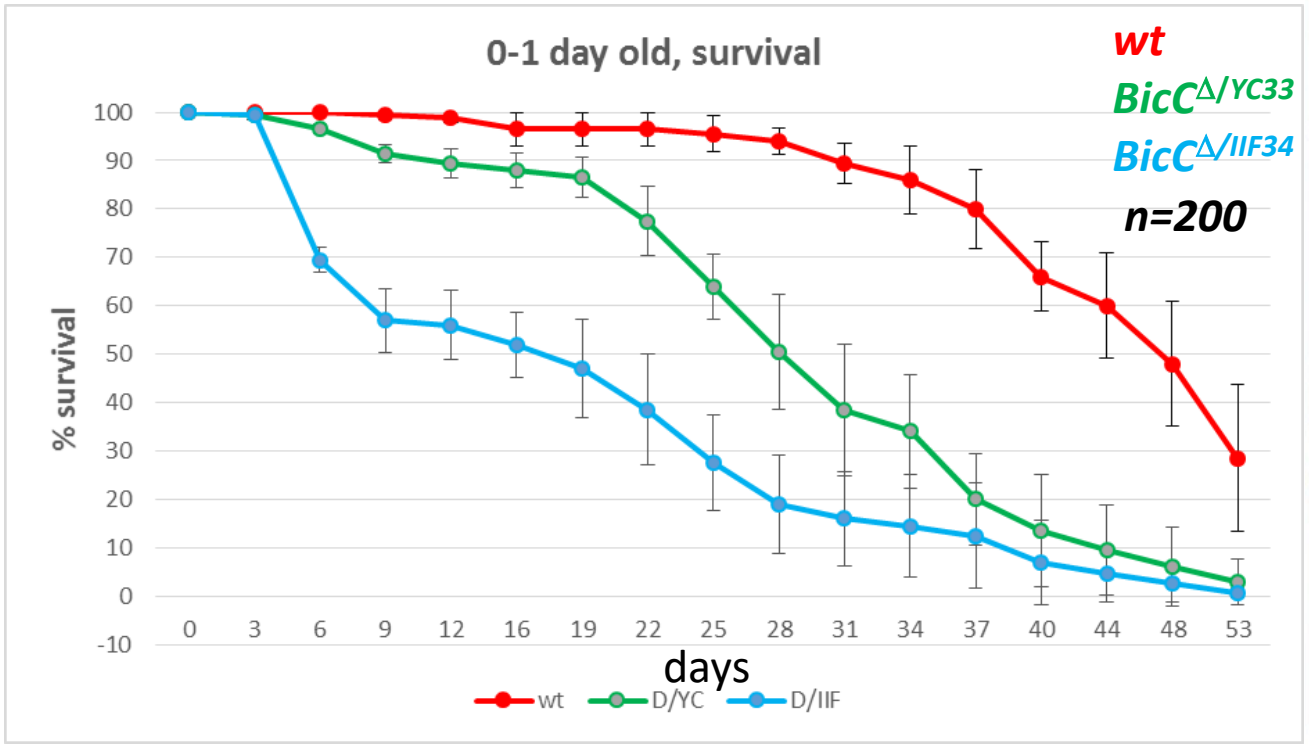
Decreased *BICC1* mRNA in cystic tissue of PKD patients (*p*=0.0196).

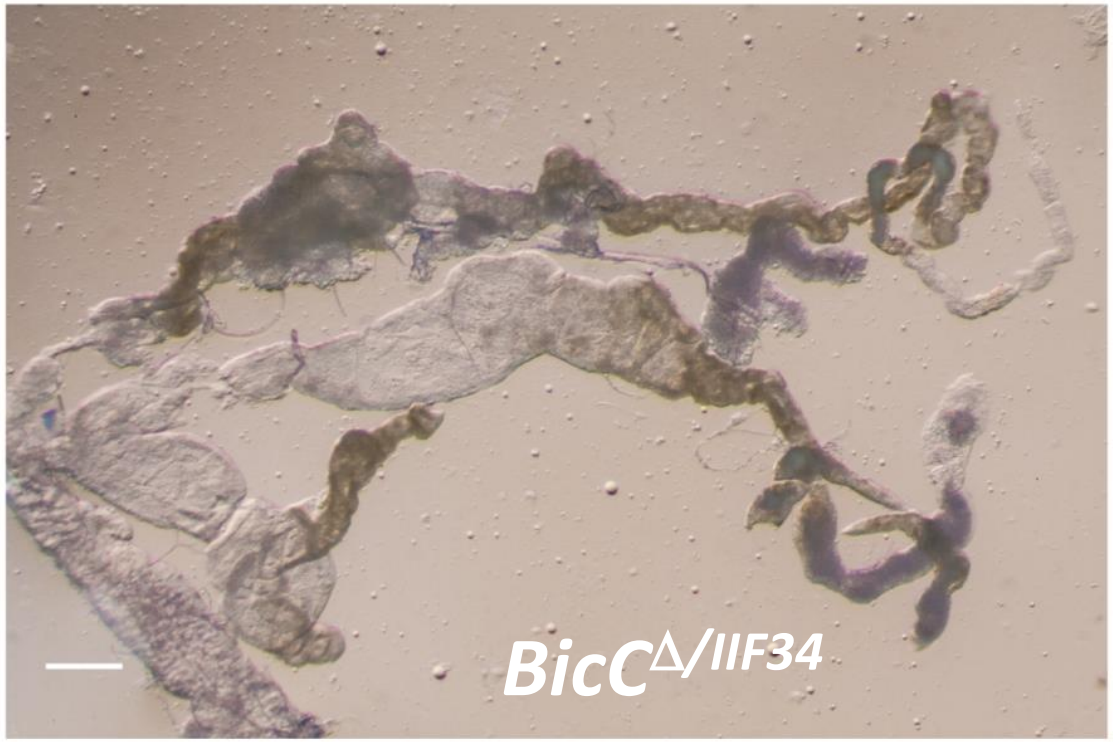


***BICC1* is genetically downstream of *PKD1*.**

Gamberi C., Hipfner D. R., Trudel M., Lubell W. D. 2017. *PLOS Genetics*, 2017 13(4):e1006694.

Shorter life span of *BicC* mutant flies





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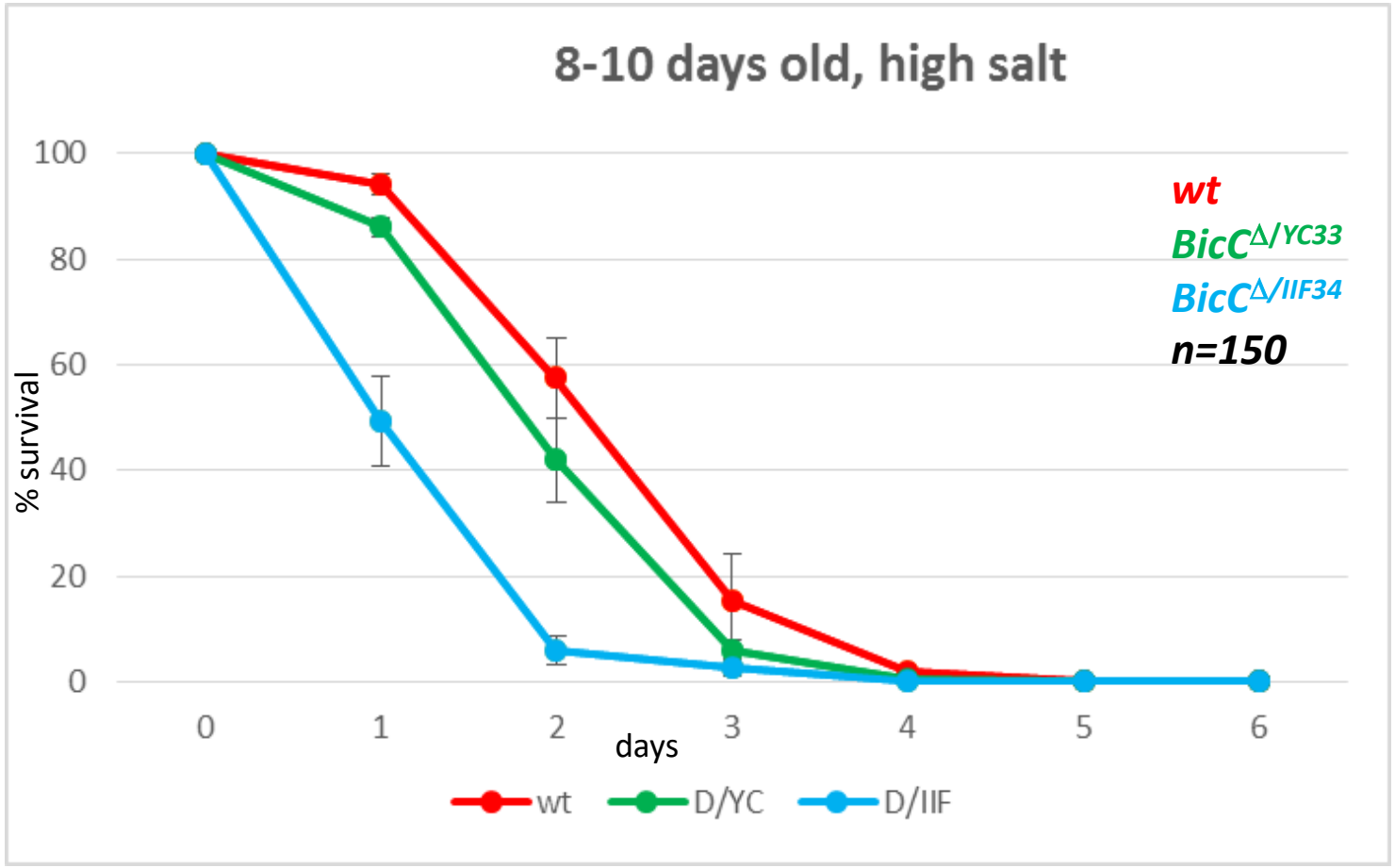
BicC mutant flies display impaired renal function



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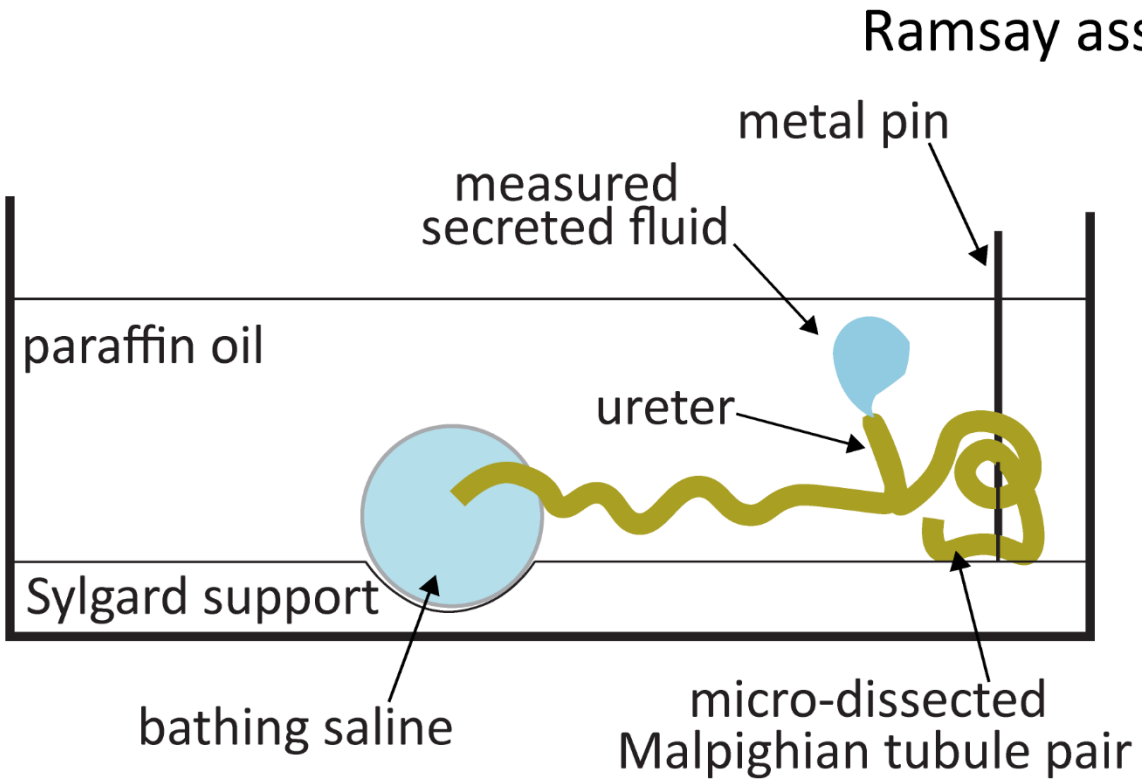
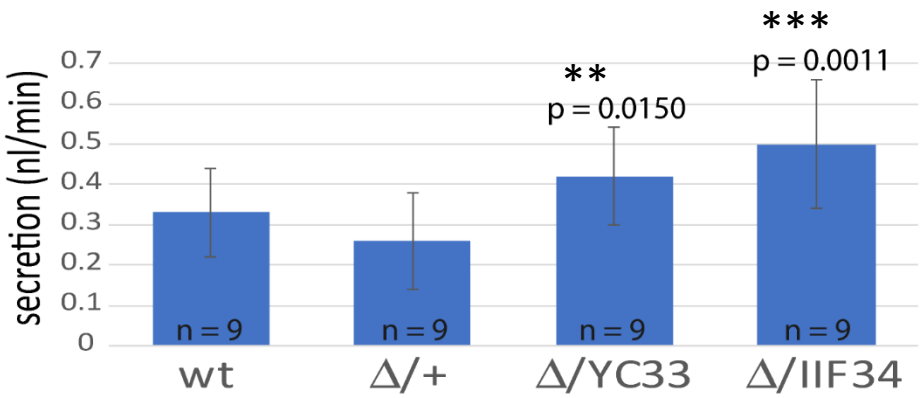
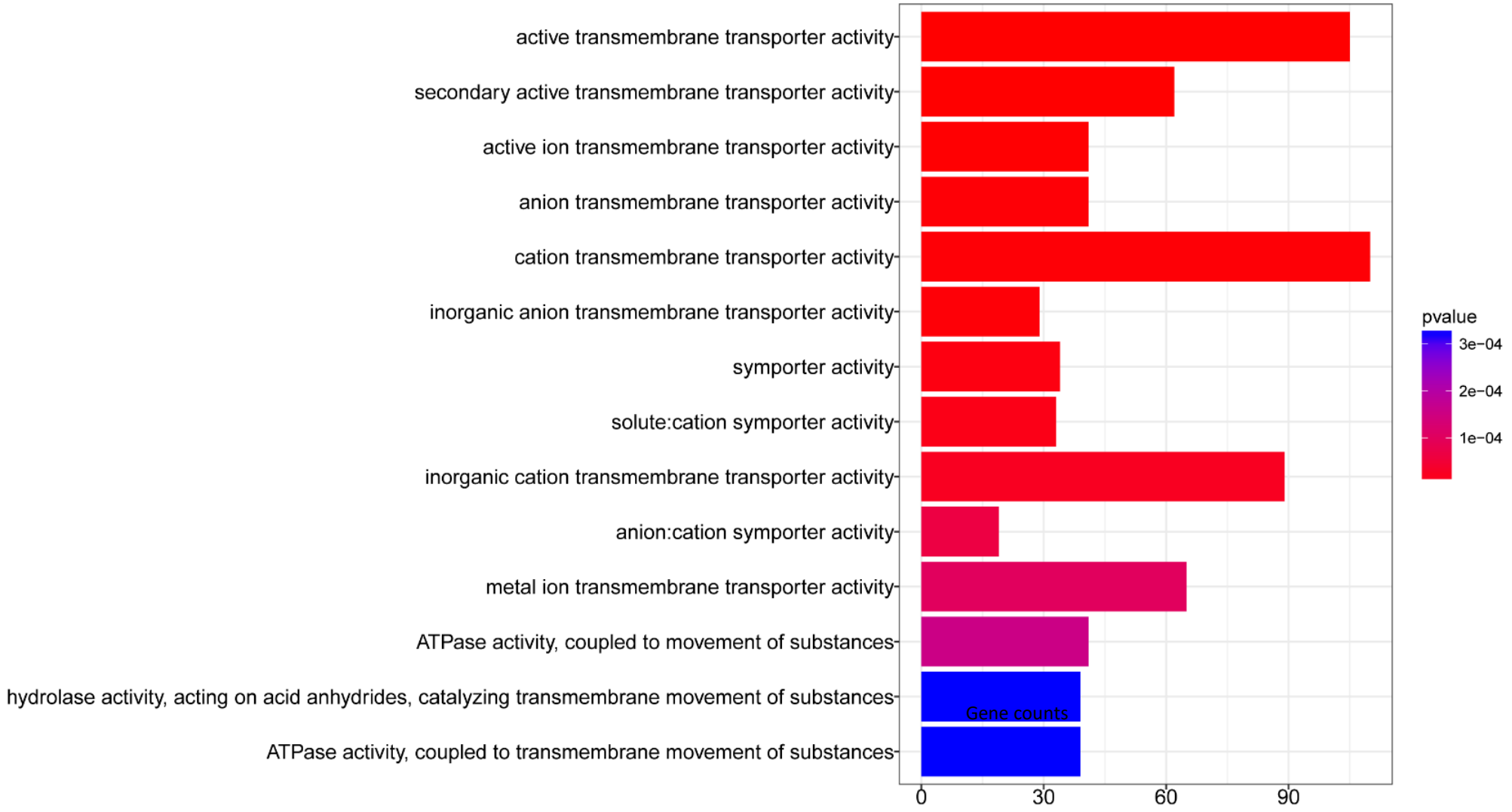




Comparative transcriptomics highlights key physiological changes in the *BicC* Malpighian tubules



Fulton D., Geoffrion N. and Gamberi C.

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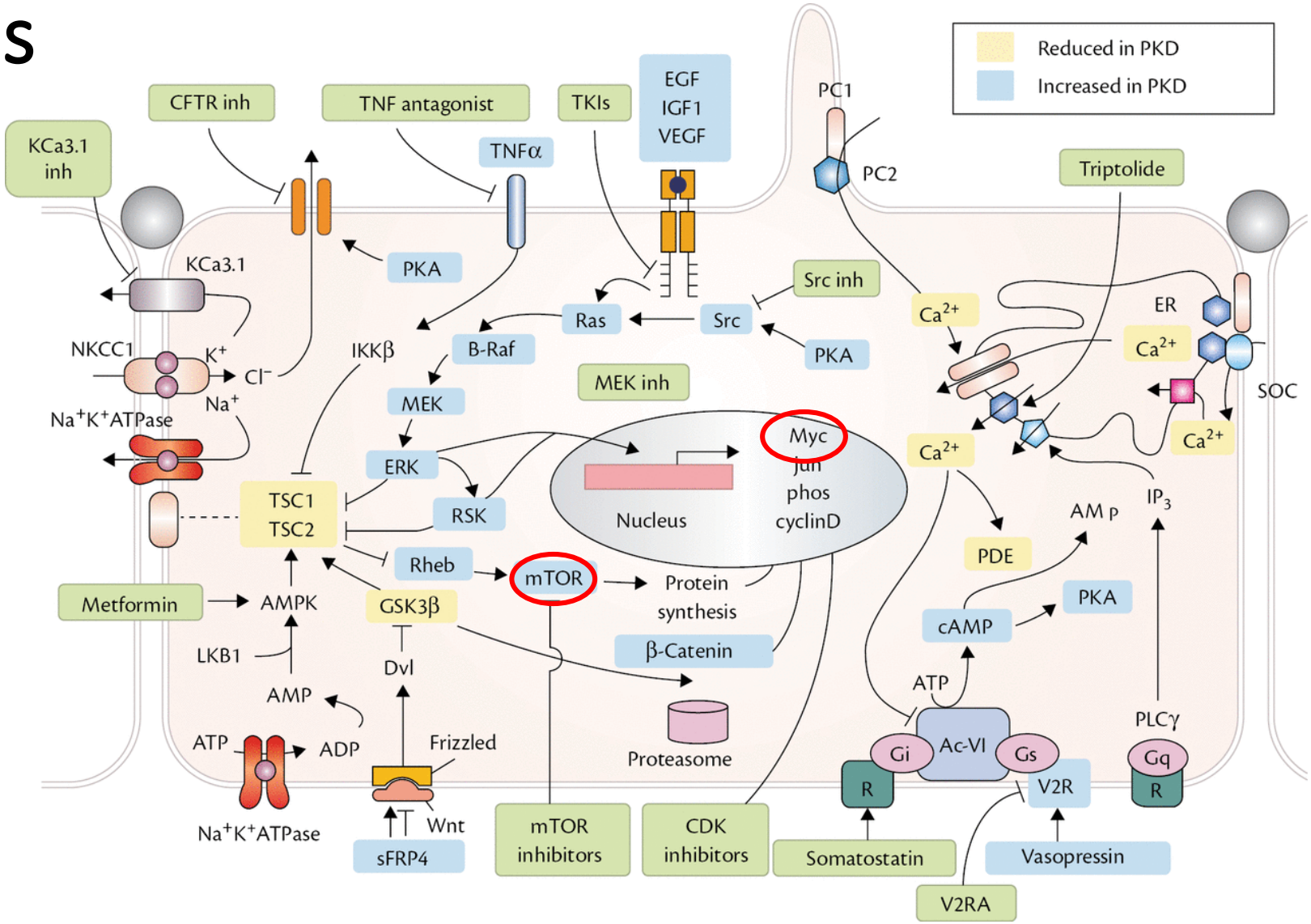
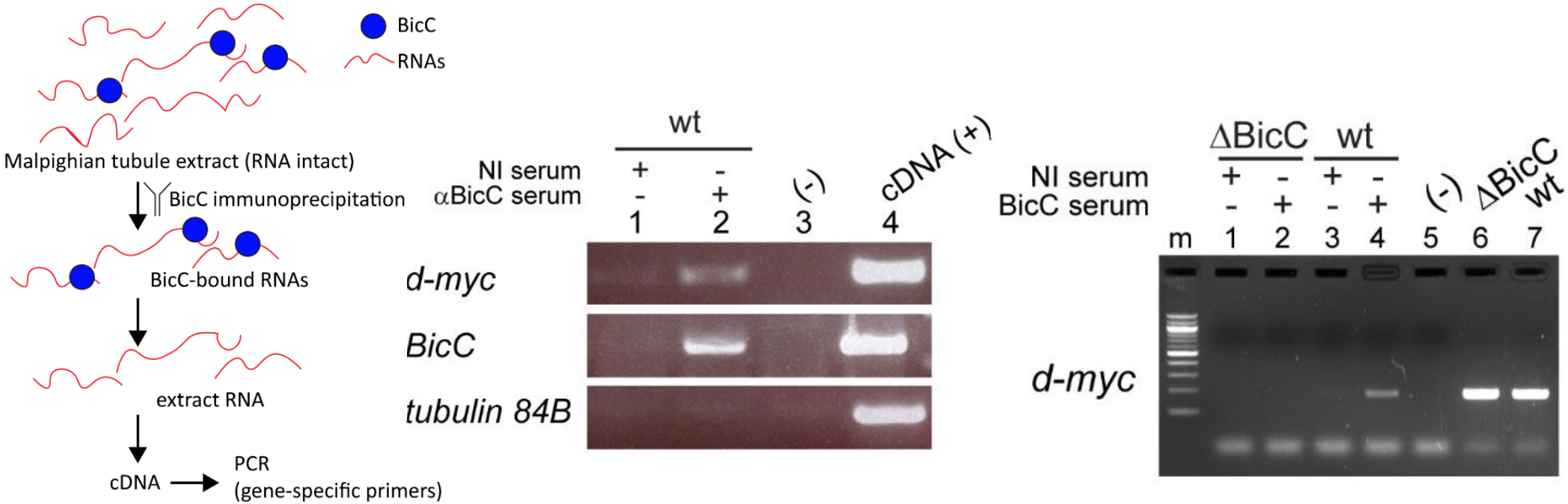
Primary vs. secondary (compensatory) changes



Wolfgang Kühn and Gerd Walz "The molecular basis of ciliopathies and cyst formation " Oxford Textbook of Clinical Nephrology (4 ed.)

BicC associates with *myc* mRNA in the renal tubules



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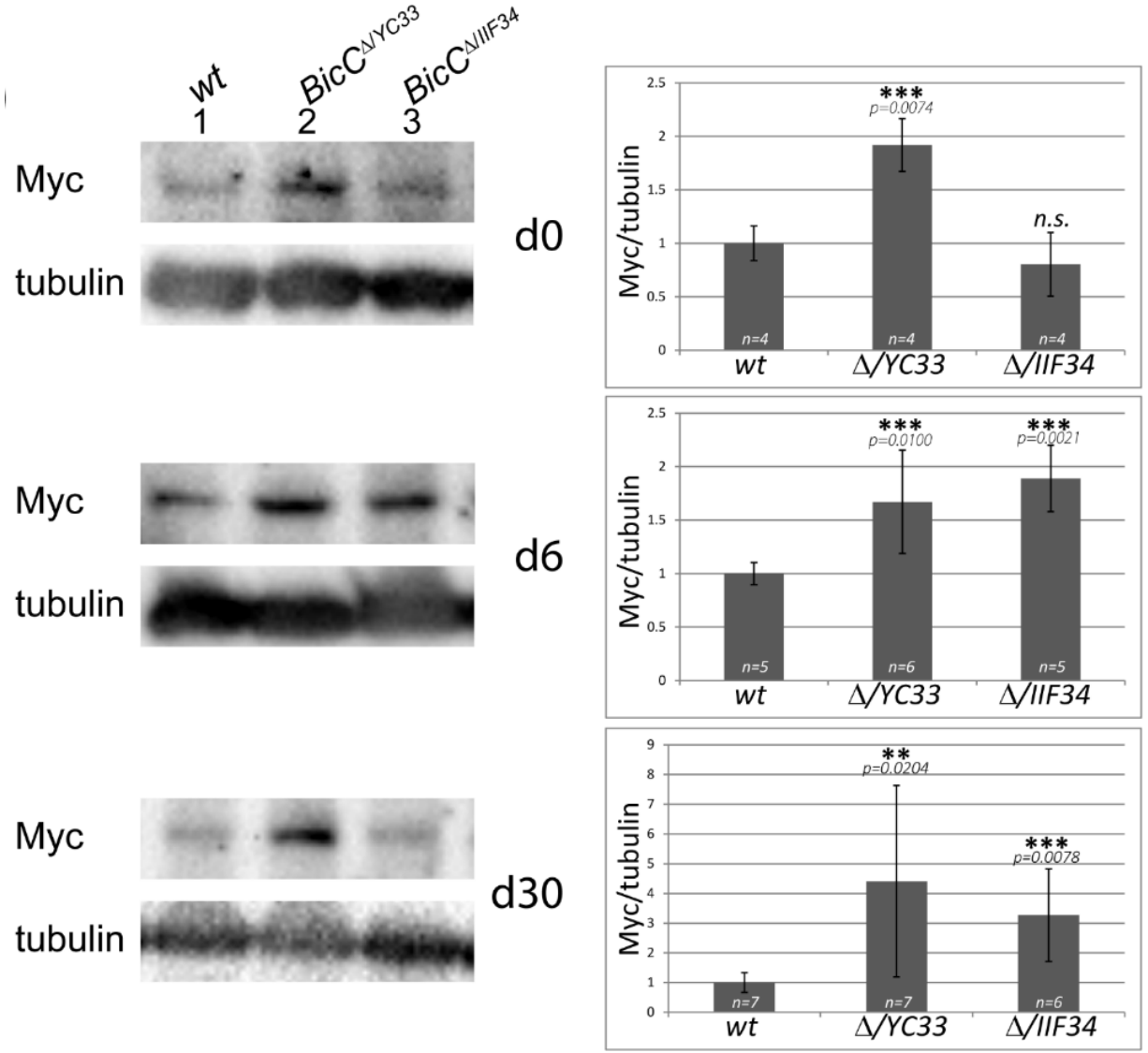
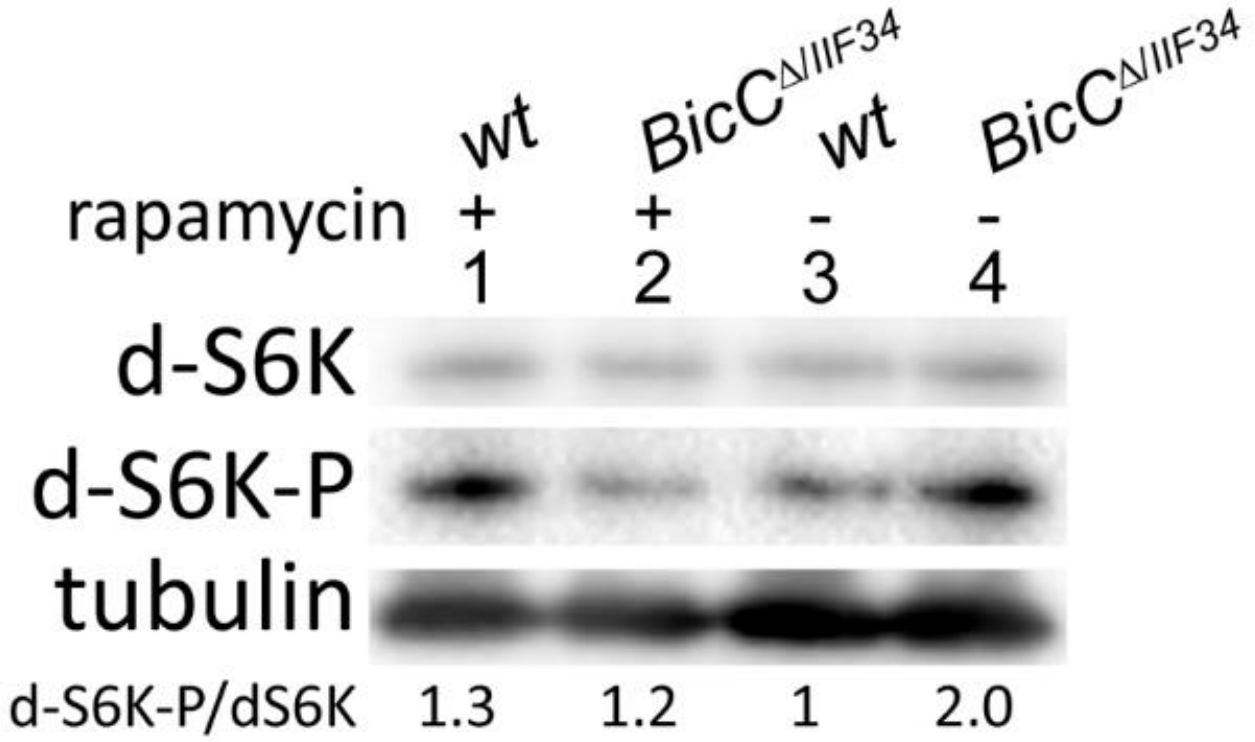
Excess Myc protein in *BicC* mutants



- ***BicC*** **renal tubules progressively accumulate Myc protein, similar to human PKD kidneys and proliferative diseases (*e.g.*, cancer).**

Gamberi C., Hipfner D. R., Trudel M., Lubell W. D. 2017. *PLOS Genetics*, 2017 13(4):e1006694.

TOR pathway activation in *BicC* mutants



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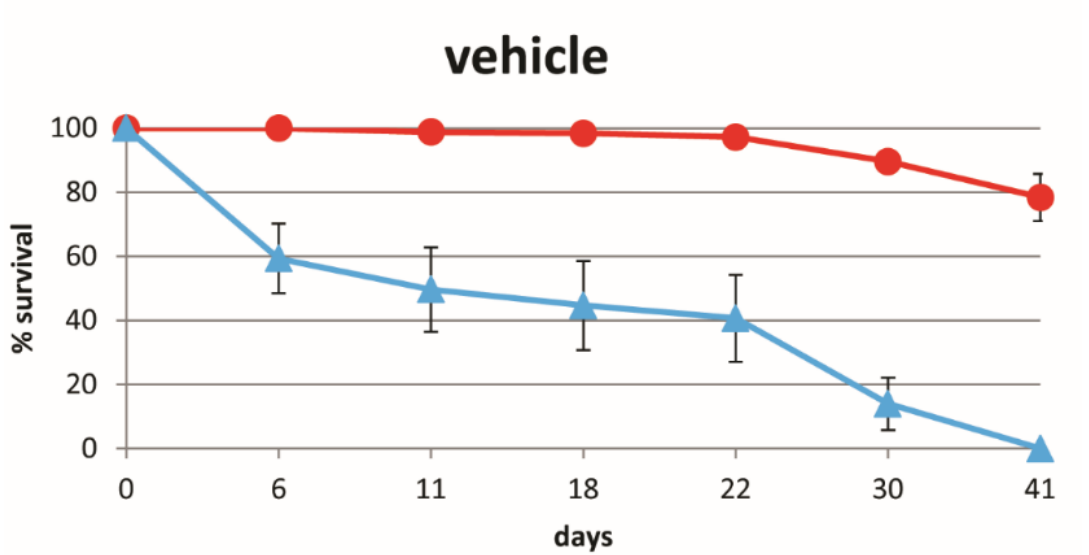
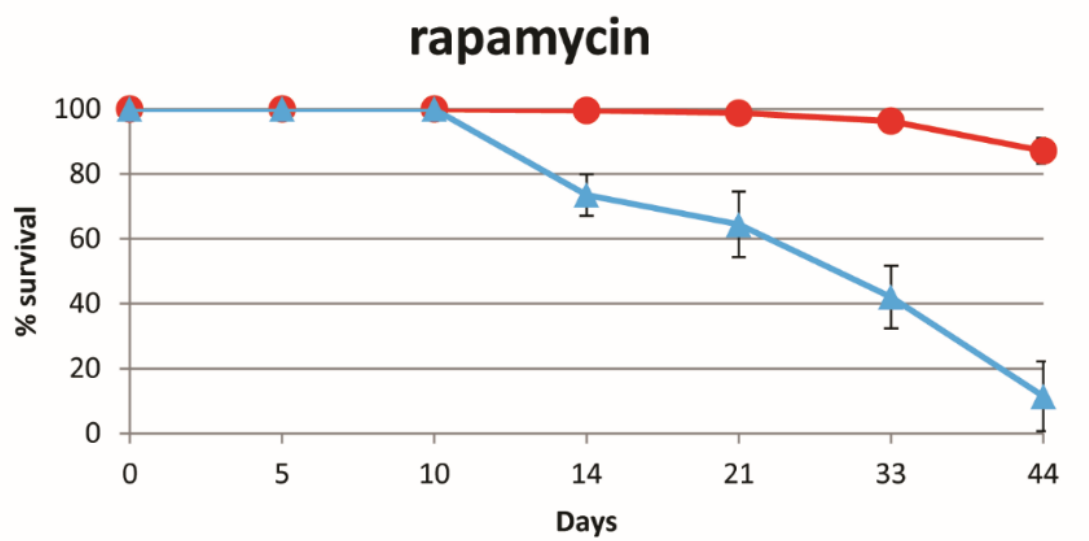
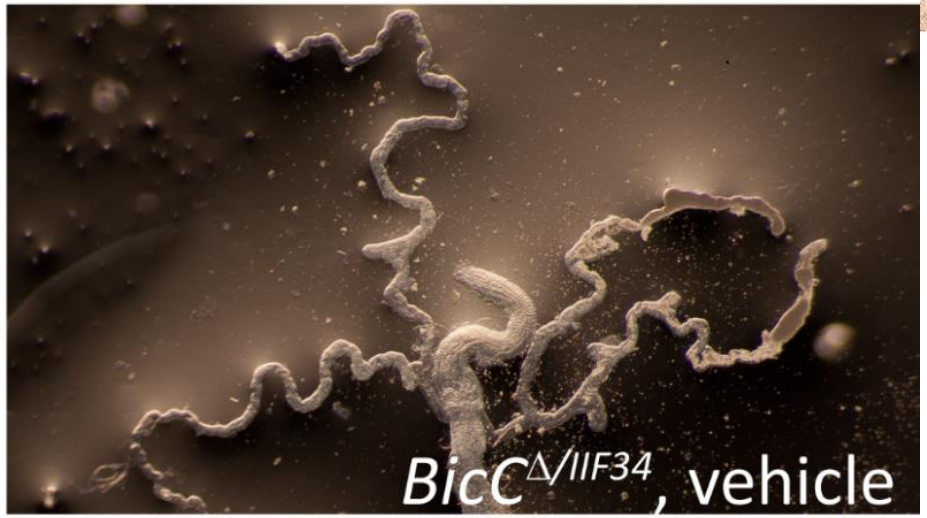
Temporary rescue by rapamycin

wt
BicC$^{\Delta/IIF34}$
n=200







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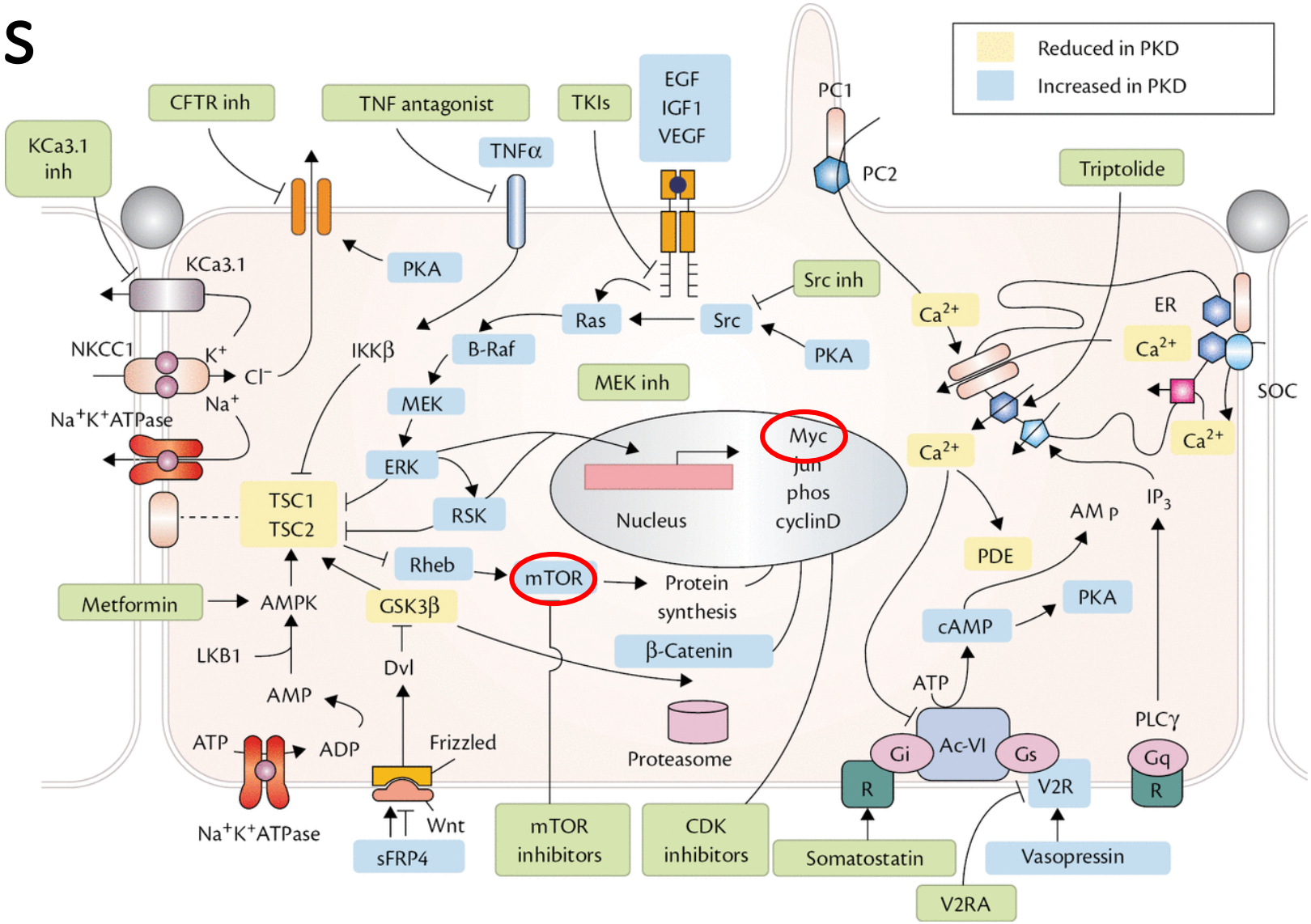
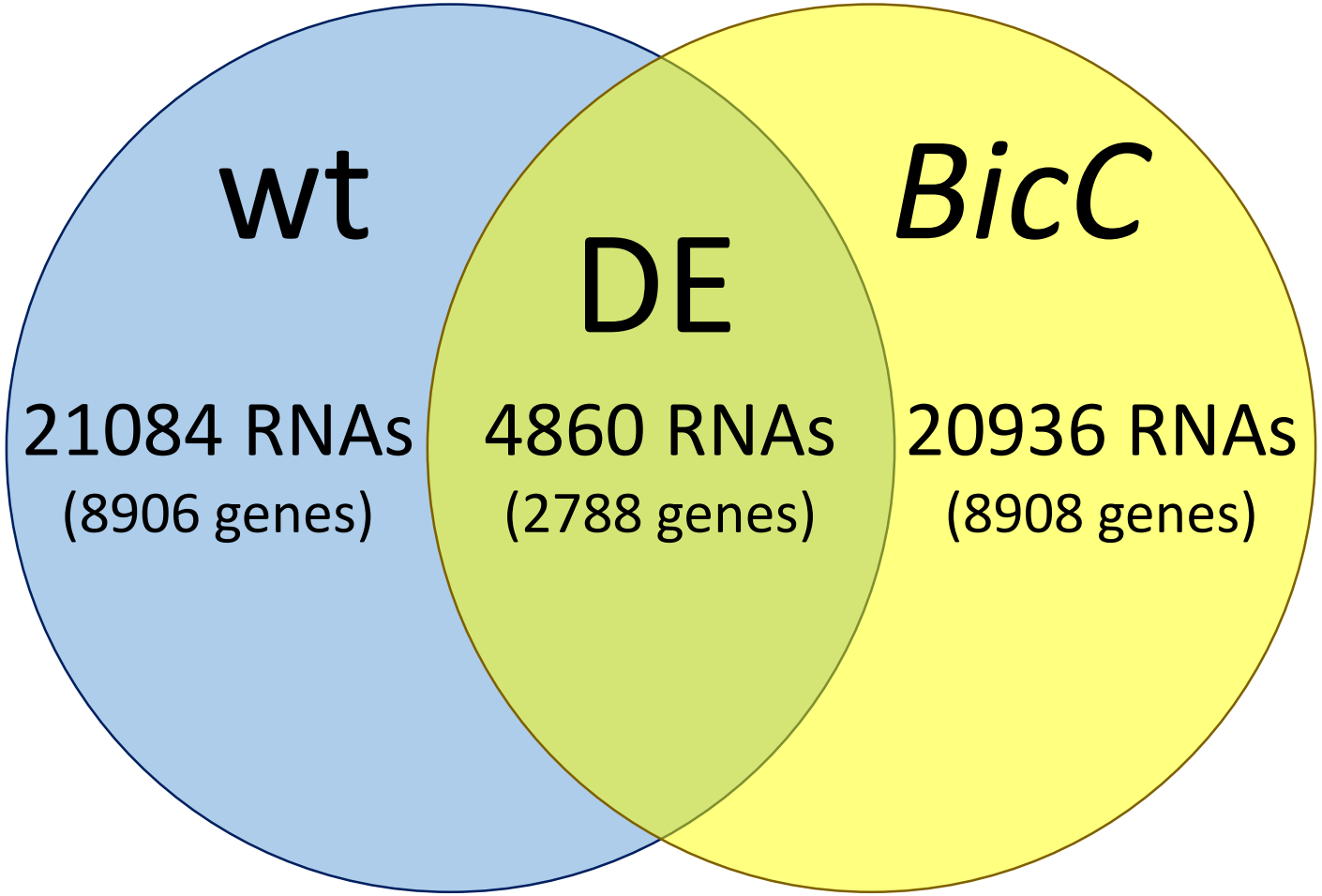


Wolfgang Kühn and Gerd Walz "The molecular basis of ciliopathies and cyst formation " Oxford Textbook of Clinical Nephrology (4 ed.)

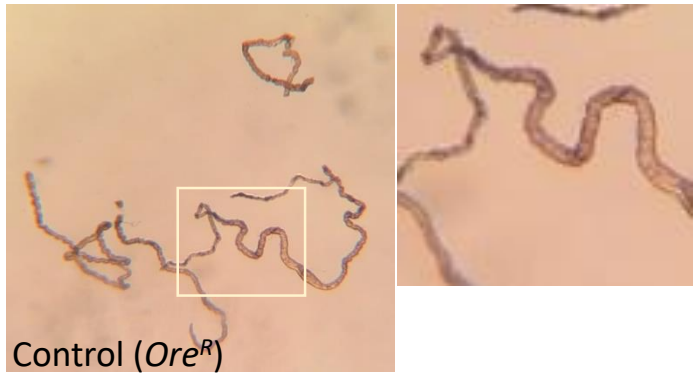
Transcriptomics of the *BicC* fly renal tubule highlights genome-wide changes of gene expression

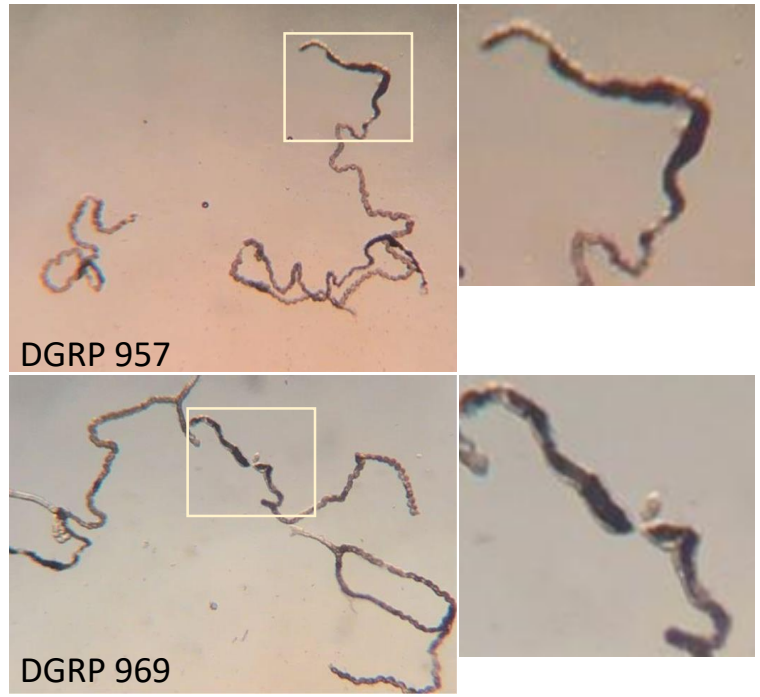


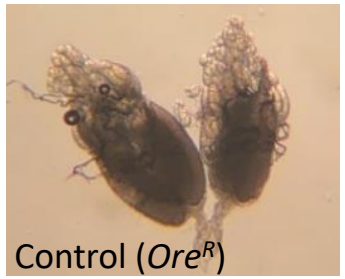
Fulton D., Geoffrion N., and Gamberi C.

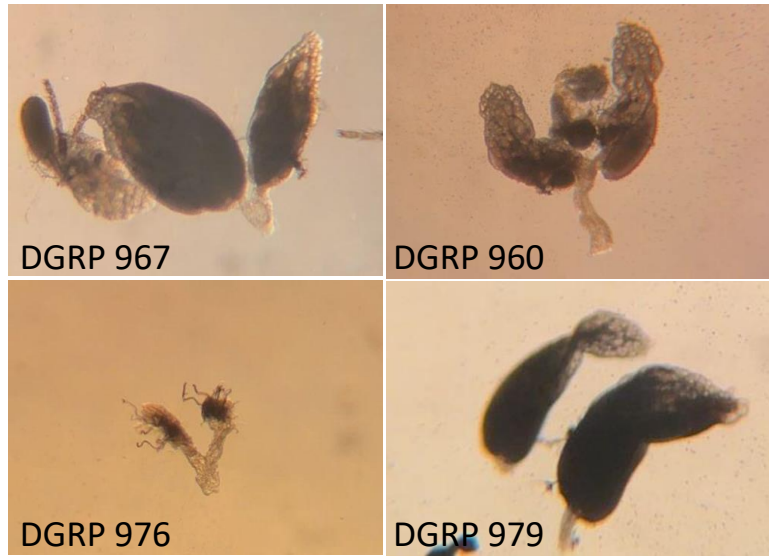
Ongoing genetic modifier screen to decipher the BicC network

- *Drosophila* Genetic Reference Panel (DGRP1, Mackay group, Clemson U.)
  - Over 2.5 million natural variants in 205 lines.
- Cystic phenotypes.
- Found ovarian phenotypes as well.
- Partially overlapping candidate lists.

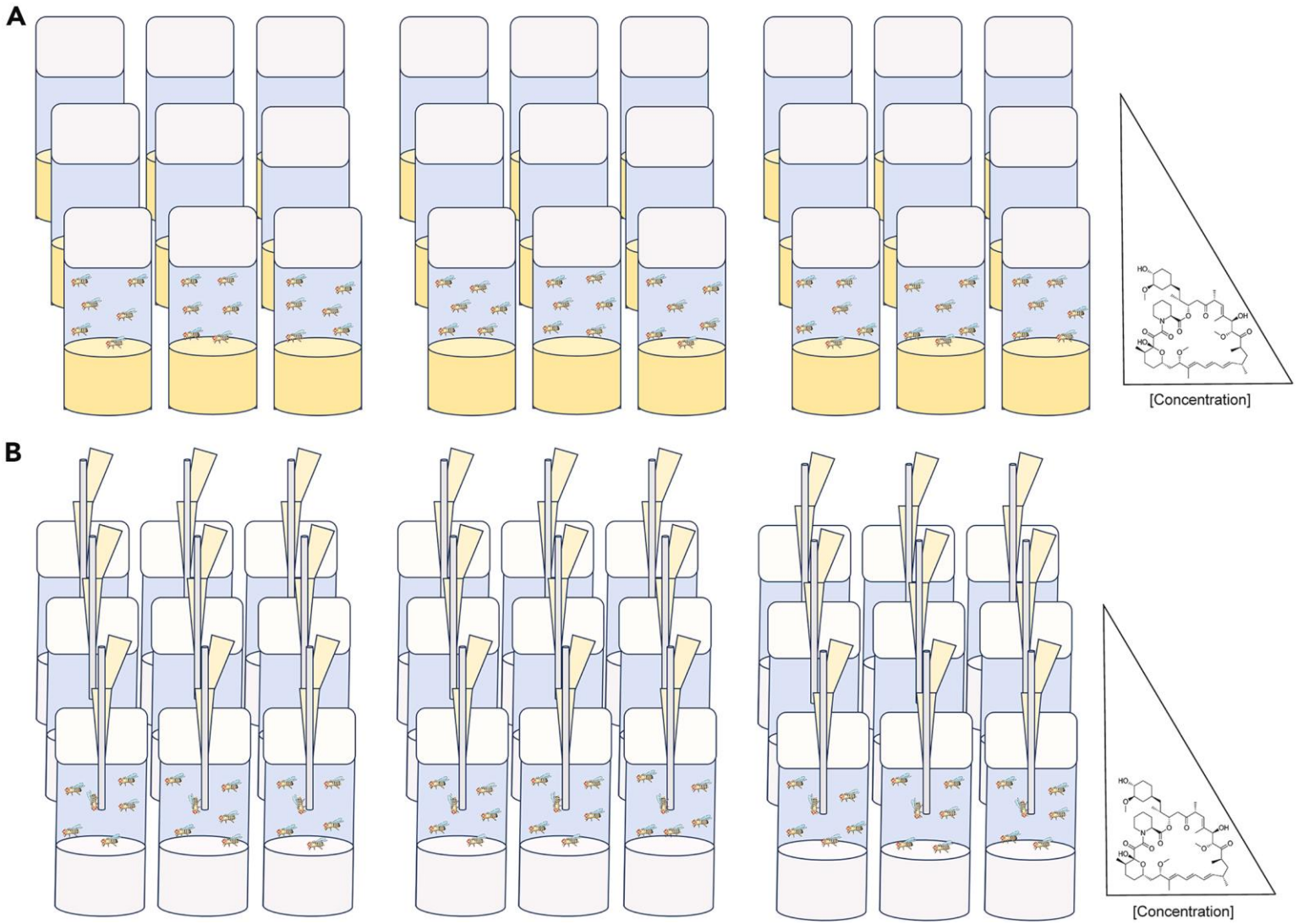


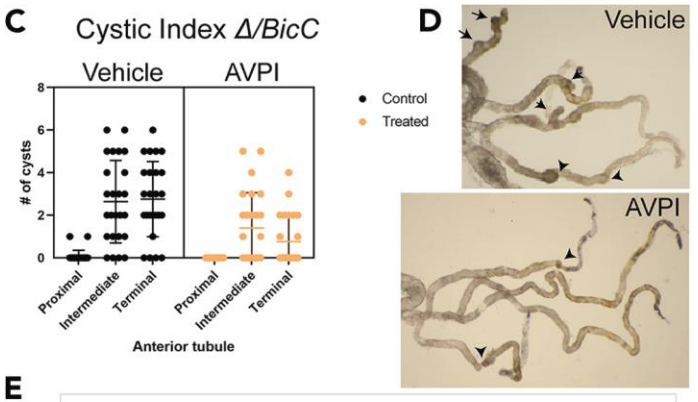


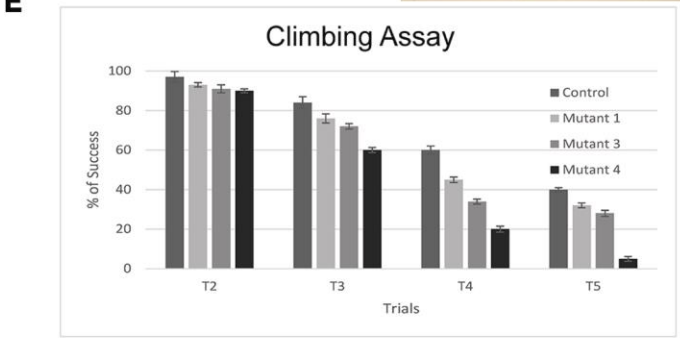


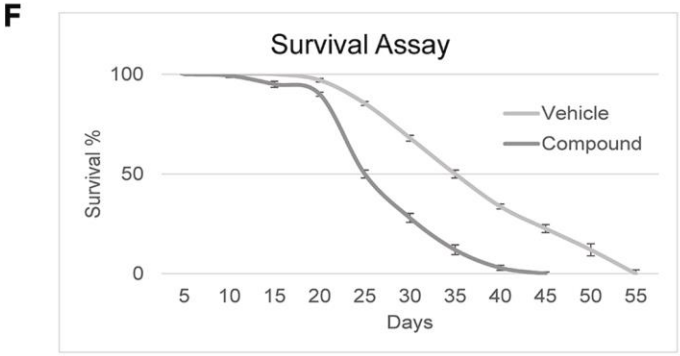


Fly pharmacology



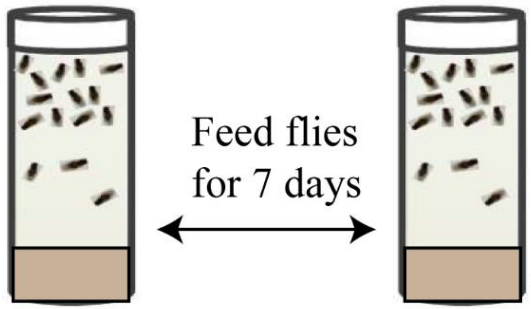


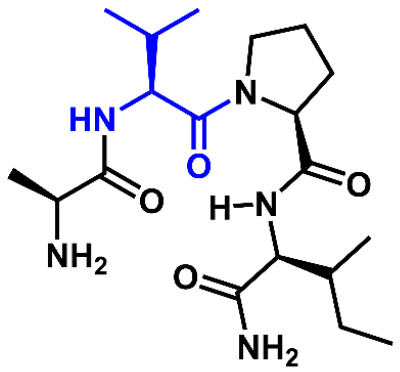




DeLoriea J, Millet-Boureima C, and Gamberi C. *STAR Protocols* 4(4):102747. 2023.

Cyst reduction in a PKD *Drosophila* model using Smac mimics





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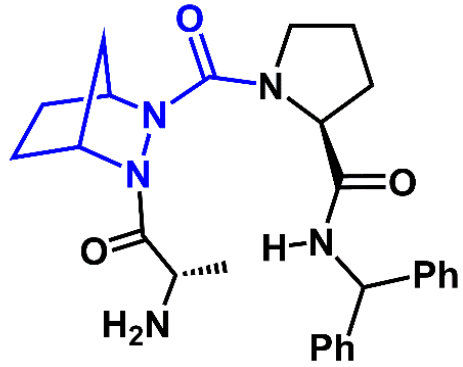

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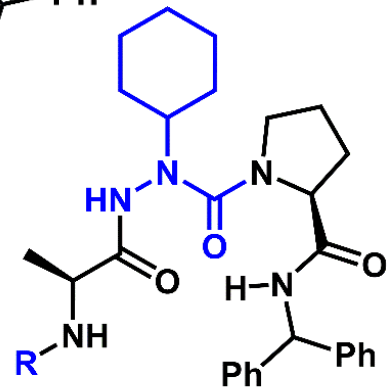


3 : R = H
4 : R = CH_3

Feed flies for 7 days

Smac-mimics or vehicle added to food

0-2

7-9

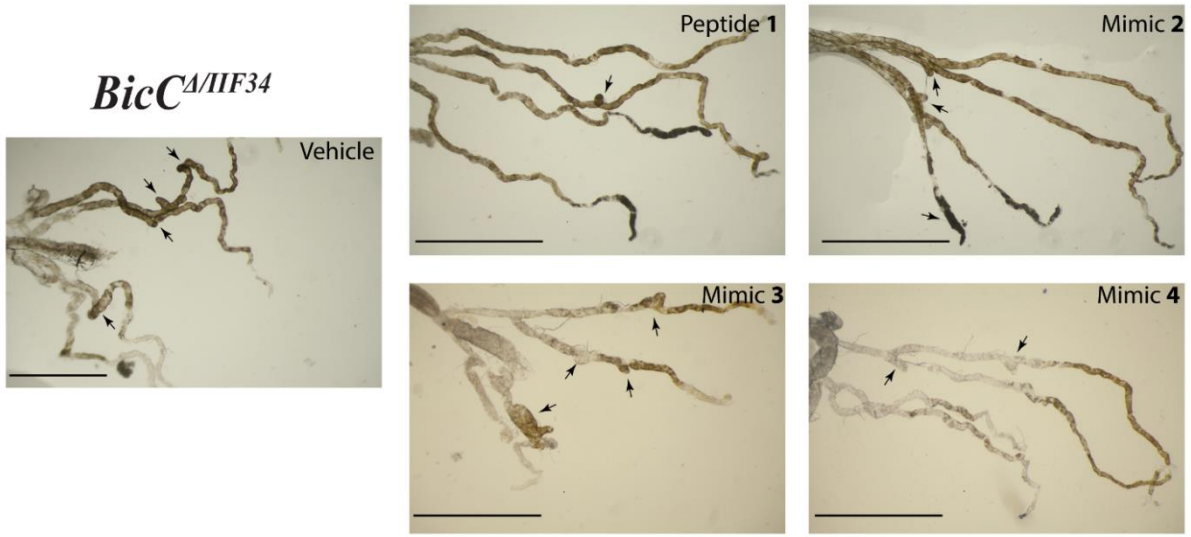
27-29 days

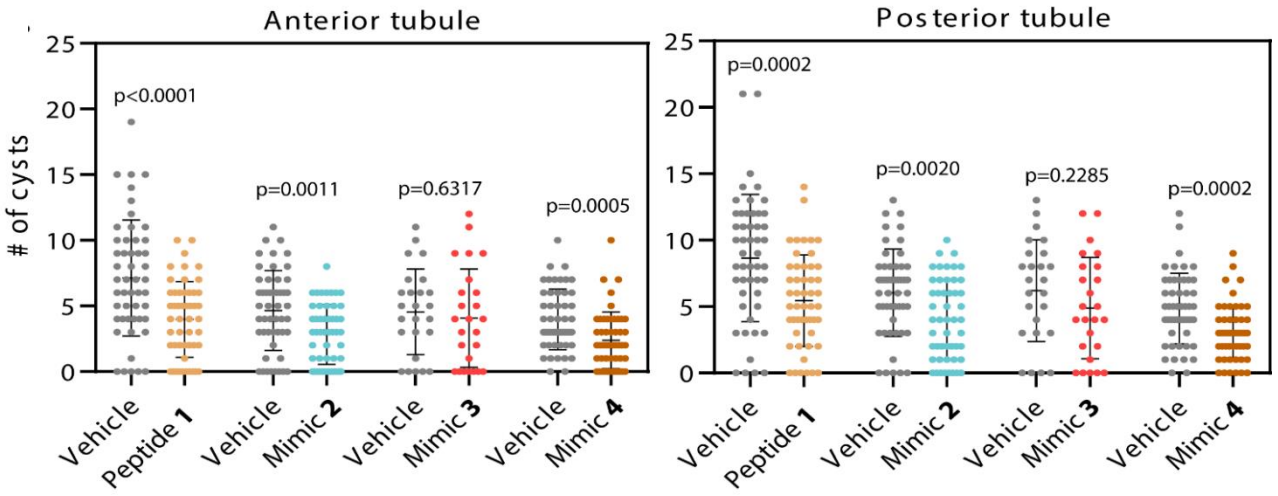
Flies added to vials with compound

Dissect tubules + score # of cysts

Millet-Boureima C., Chingle R., Lubell W.D., and Gamberi C. 2019. *Biomedicines*. 7(4), 82; doi:10.3390/biomedicines7040082.

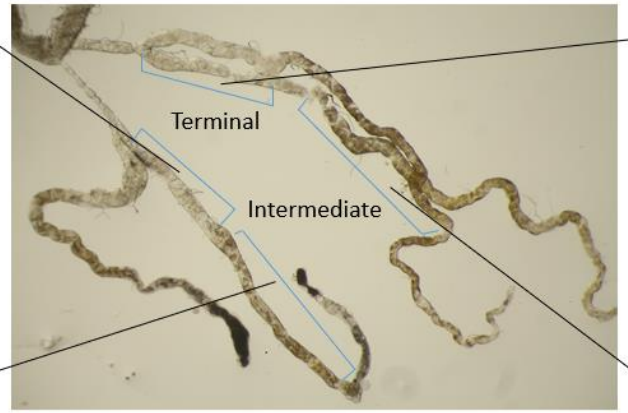
Cyst reduction and regional specificity of Smac mimics





Post. Term.
1 – 32%
2 – 32%
3 – *21%*
4 – 51%

Post. Interm.
1 – 41%
2 – *27%*
3 – *10%*
4 – *22%*

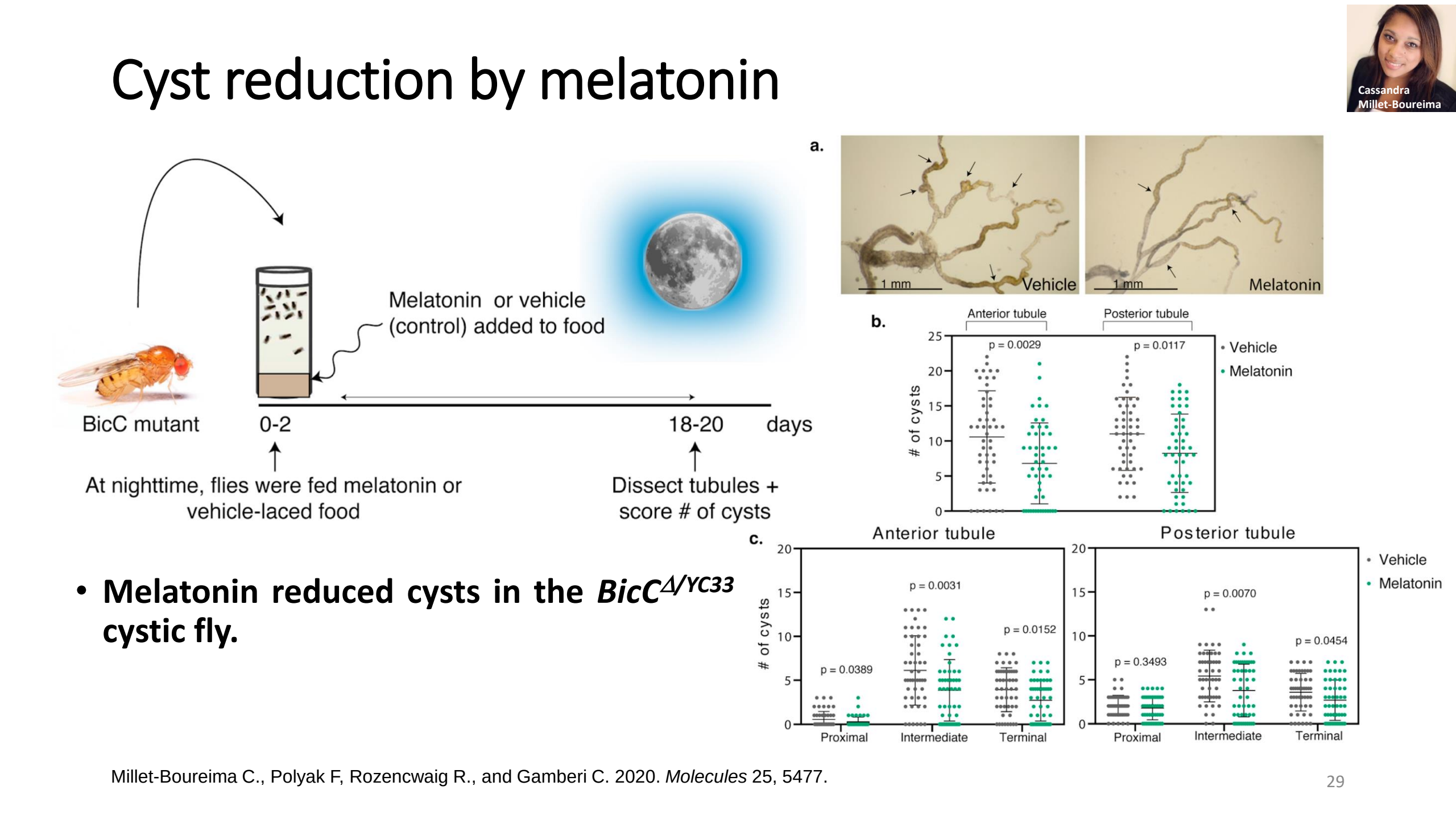


Ant. Term.
1 – 40%
2 – 37%
3 – *11%*
4 – 38%

Ant. Interm.
1 – 48%
2 – 41%
3 – *9%*
4 – 42%

Millet-Boureima C., Chingle R., Lubell W.D., and Gamberi C. 2019. *Biomedicines*. 7(4), 82; doi:10.3390/biomedicines7040082.

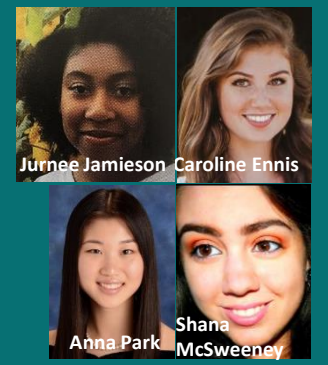
Cyst reduction by melatonin



- **Melatonin reduced cysts in the *BicC$^{\Delta/YC33}$* cystic fly.**

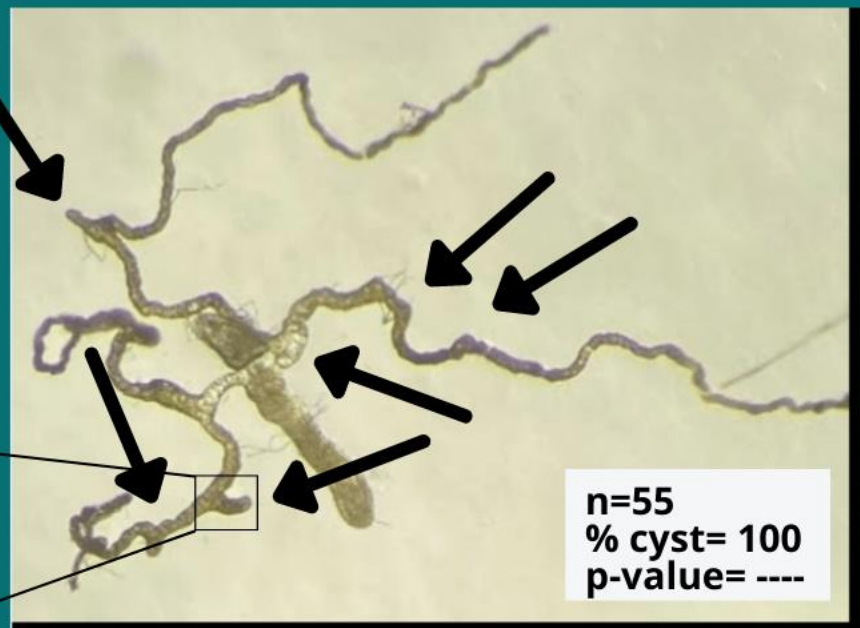
Millet-Boureima C., Polyak F, Rozencwaig R., and Gamberi C. 2020. *Molecules* 25, 5477.

Three Novel Cyst Reducing Compounds

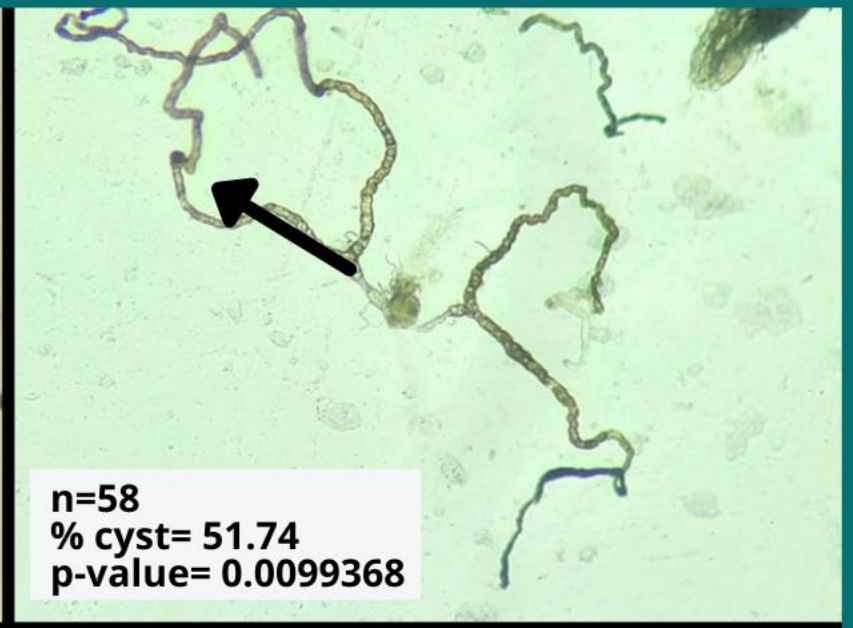


Δ/BicC^{IIF34}

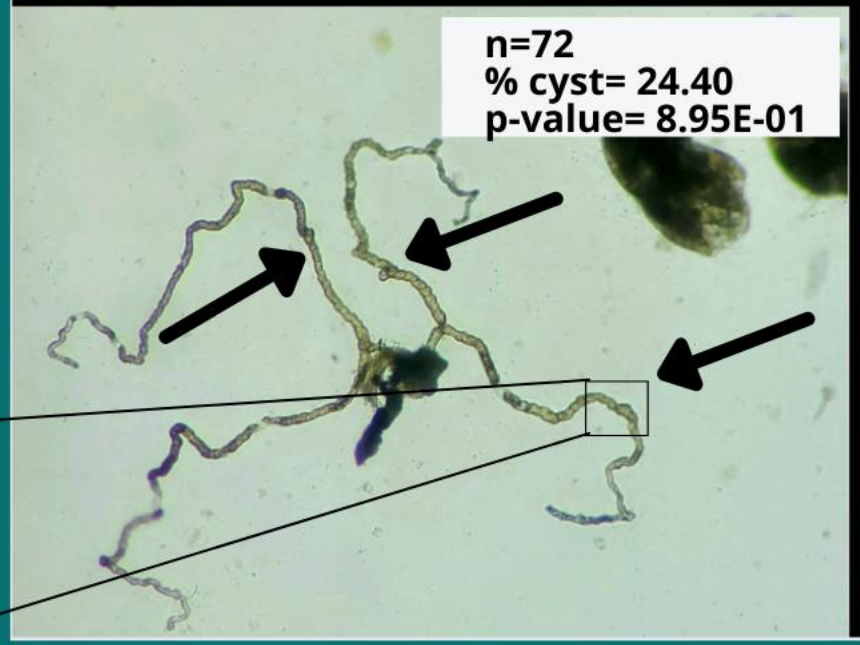
Vehicle



MT-0



MT-76



MT-145

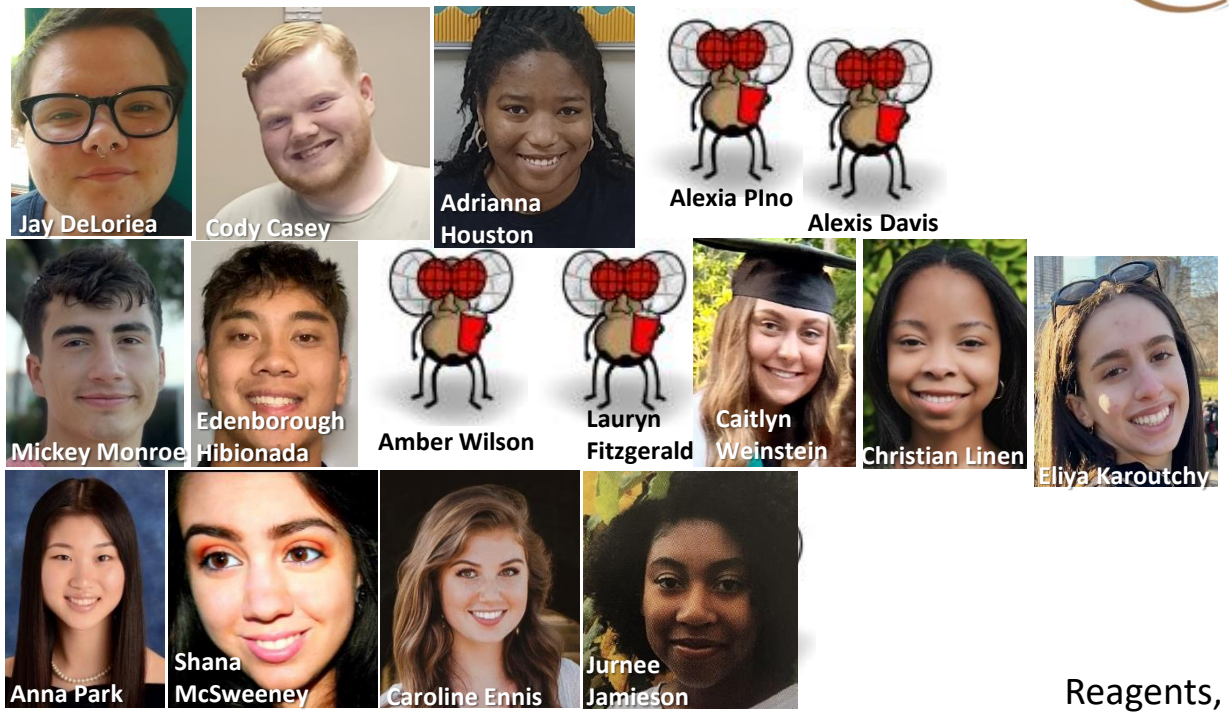
Conclusions

- ***BicC* mutant flies display PKD-like features.**
  - ***myc* RNA and TOR pathway upregulation.**
  - **Several pathways contribute to cyst formation in the *BicC* mutant.**
  - **Pharmacological response to rapamycin and Smac mimicry.**

- **The *BicC* model can give insight:**
  - **Core conserved mechanisms of renal cyst formation**
    - **(Molecular) genetics.**
  - **Chemical probing and drug discovery (fly pharmacology)**
    - **Rapamycin, Smac mimics (PoC), melatonin, MT compounds.**
  - **Genome-wide changes linked to renal cyst formation.**
  - **Genetic modifiers of the cystic phenotype.**
  - **Environmental modifiers of the cystic phenotype (e.g., microbiome, pollutants).**
  - **BicC tissue-specific function.**
  - **(Evolutionary conservation of) translational regulation.**

Acknowledgments

Gamberi laboratory



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Developmental Studies Hybridoma Bank
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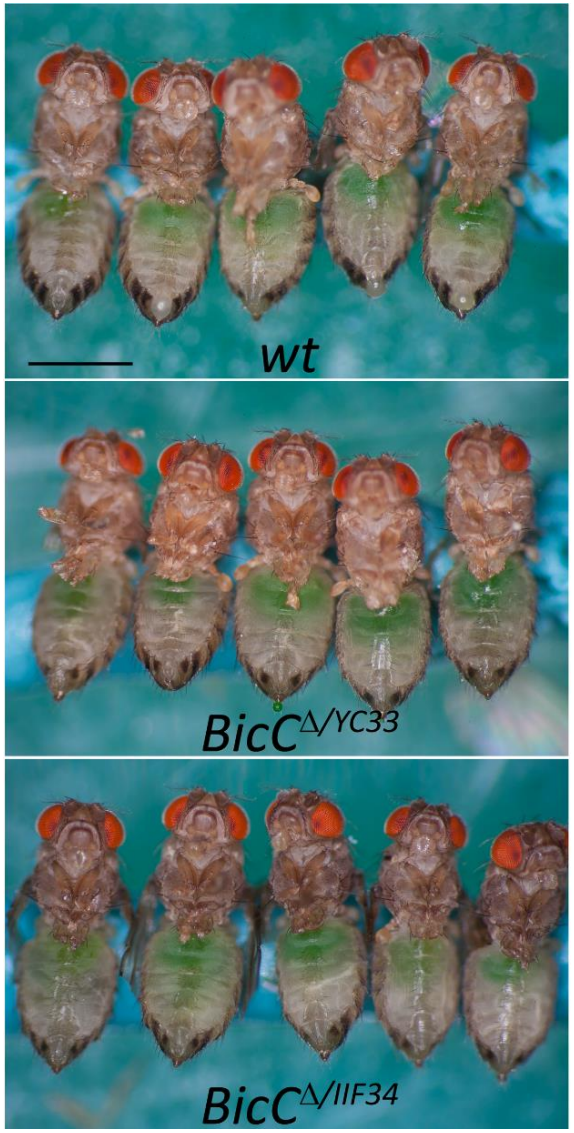
Bruce Edgar (DKFZ, Heidelberg)
Robert Eisenman (Fred Hutchinson Cancer Center)
M. Therrien (IRIC, Université de Montréal)

Drug administration and “patient” compliance

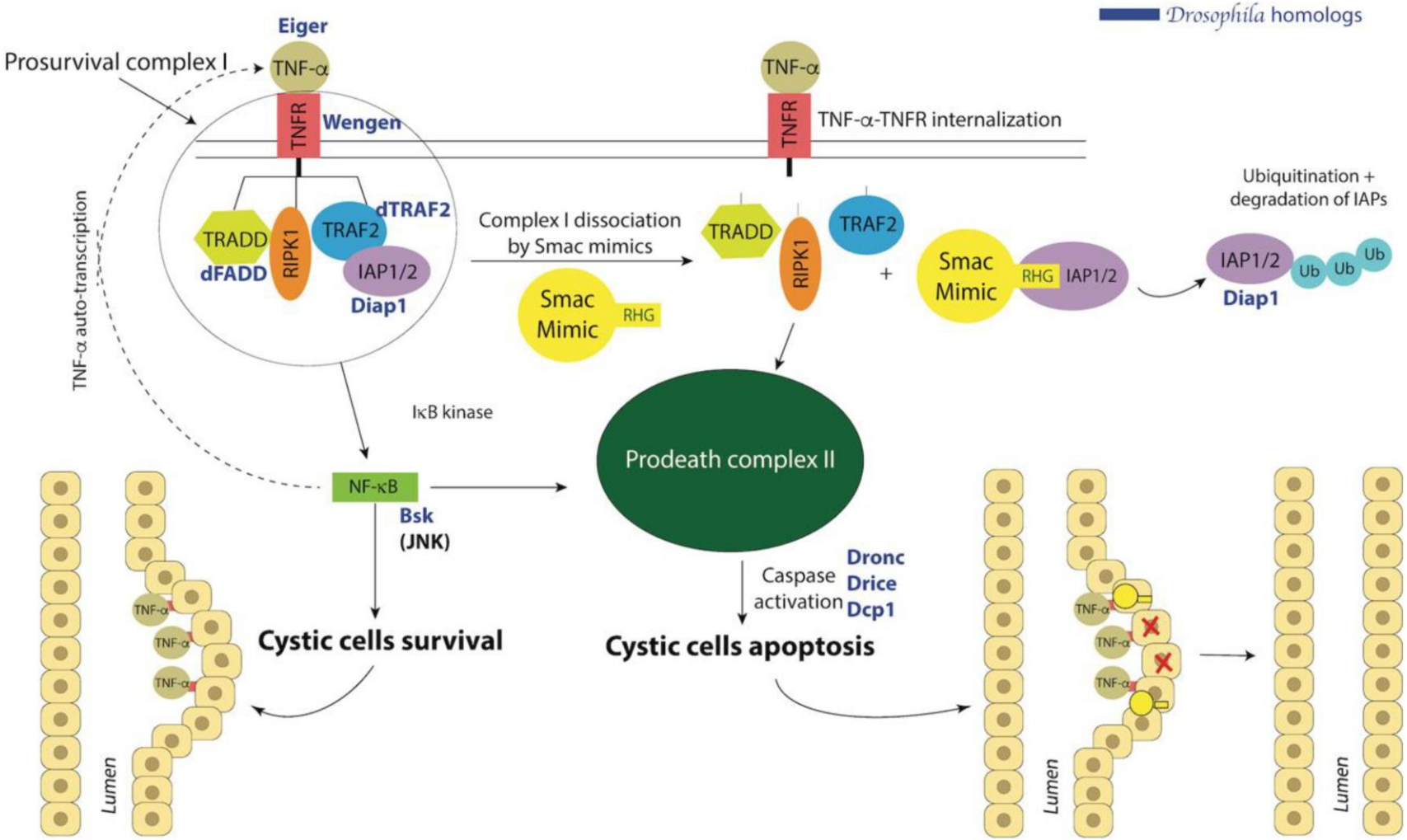
- **When drug is administered orally, it is mixed with an attractant (food, sugar) and a dye.**



Gamberi C., Hipfner D. R., Trudel M., Lubell W. D. 2017. *PLOS Genetics*, 2017 13(4):e1006694.

Cyst reduction using Smac mimics

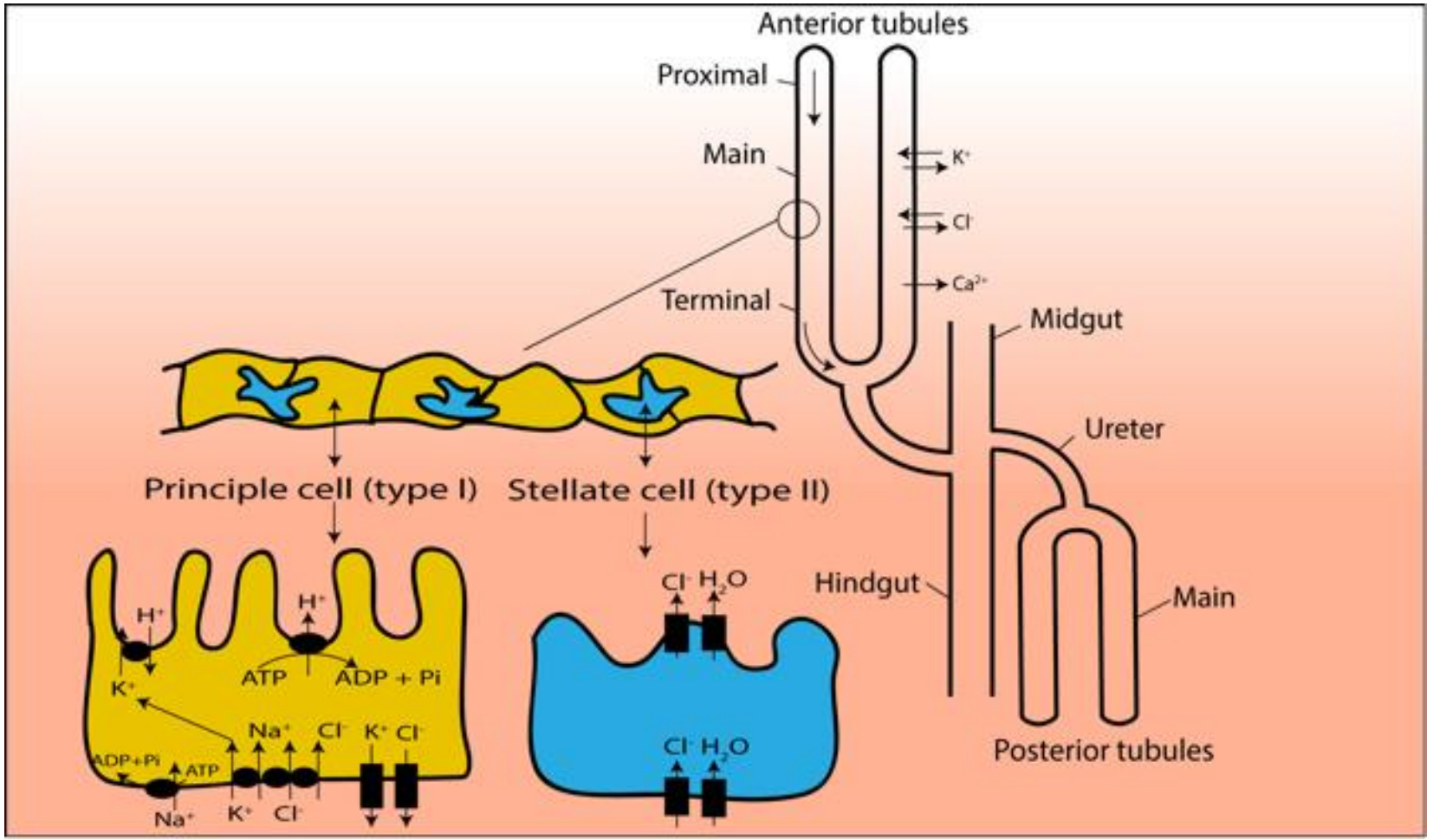
- **Birinapant analog GT13072 (mimic of the second mitochondrial-derived activator of caspases, Smac) reduced cysts in a murine PKD model.**



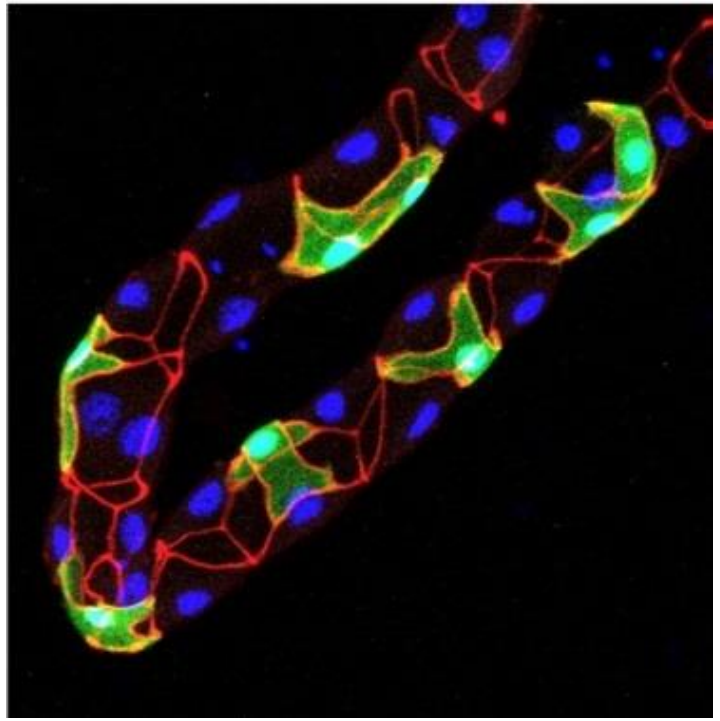
C. Millet-Boureima

Fan, L.X.et al. 2013. *JASN* 24, 2010-2022.
Millet-Boureima C., He S., Le T.B.U. and Gamberi C. 2021. *Int J Mol Sci* 22(8), 3918.

Simple cellular composition of the fly renal (Malpighian) tubule



C. Millet-Boureima, J. Porras-Marroquin and C. Gamberi, BioMed Res Intern Volume 2018, Article ID 5697436



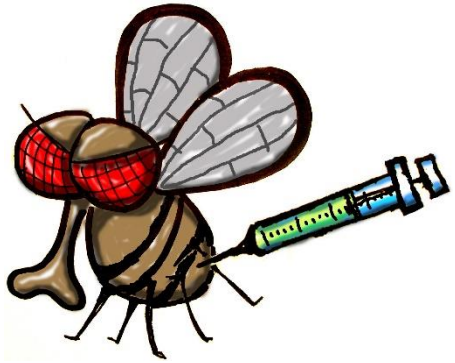
Dow J A T , Romero M F Am J Renal Physiol;299:F1237-F1244 (2010)

Studying complex Biology in flies

SPACE SCIENCE

STEM CELLS

AGING DEMENTIA

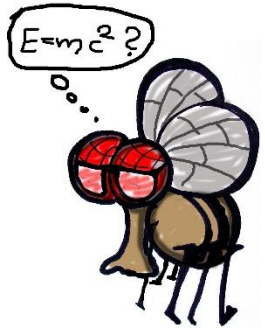


DRUG SCREENS

BEHAVIOUR

SLEEP

JET LAG



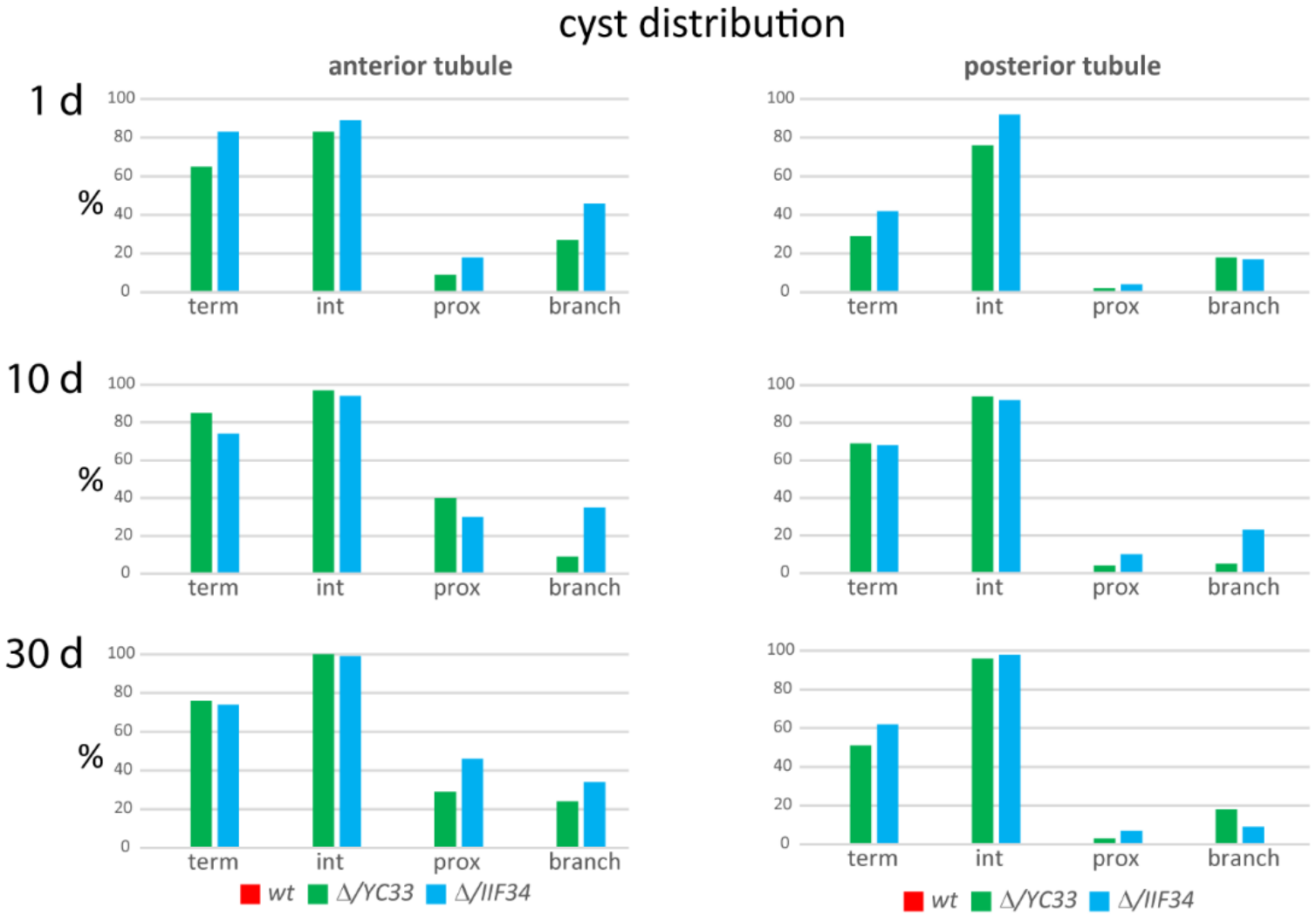
LEARNING AND MEMORY

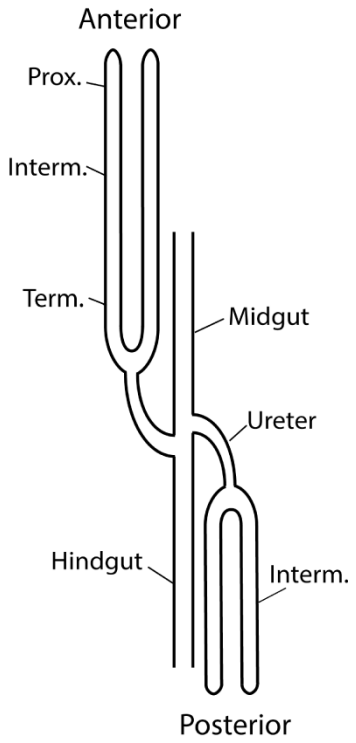
ALCOHOL

https://droso4schools.wordpress.com/why-fly/

Cyst distribution hints at physiological consequences

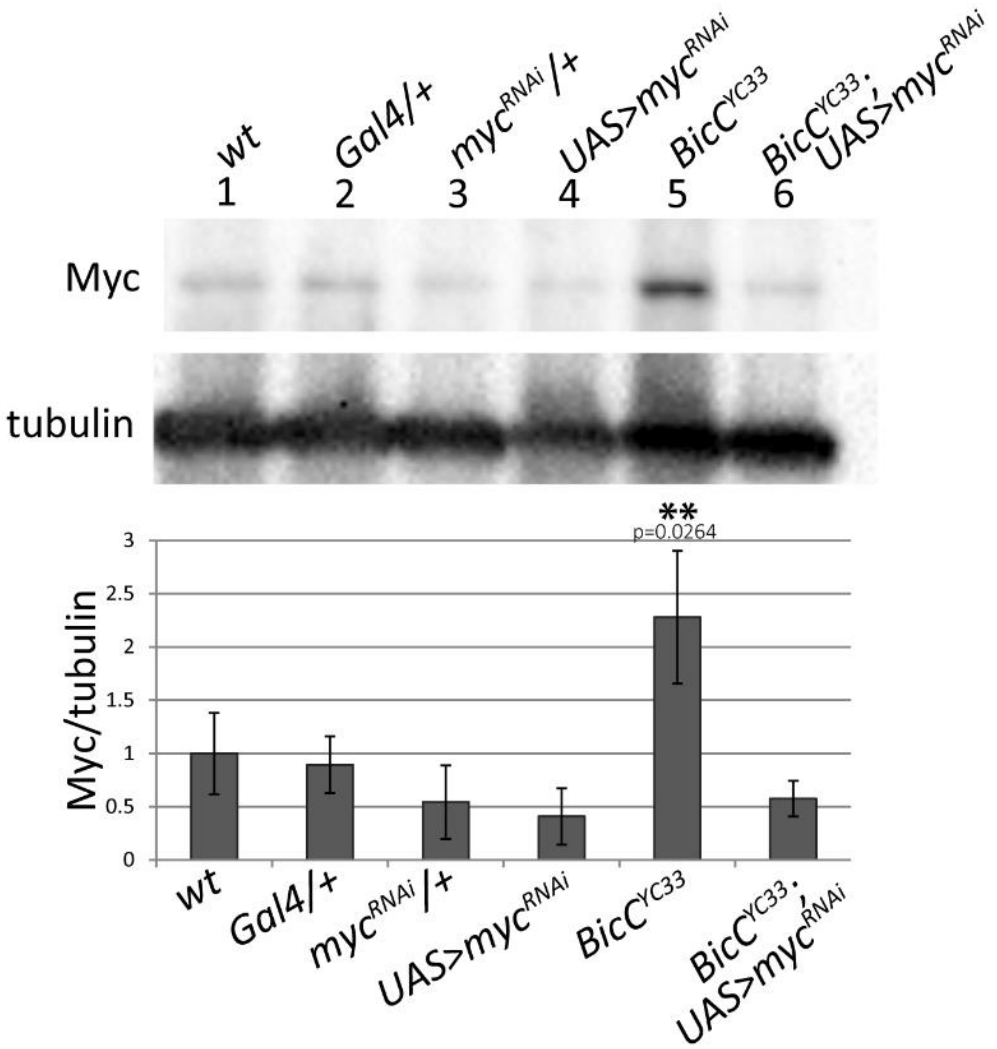
- **Alike PKD, cysts form in all regions of the *BicC* renal tubules and most frequently in the intermediate and terminal regions.**





Gamberi C., Hipfner D. R., Trudel M., Lubell W. D. 2017. *PLOS Genetics*, 2017 13(4):e1006694.

myc^{RNAi} rescue in *BicC* Malpighian tubules



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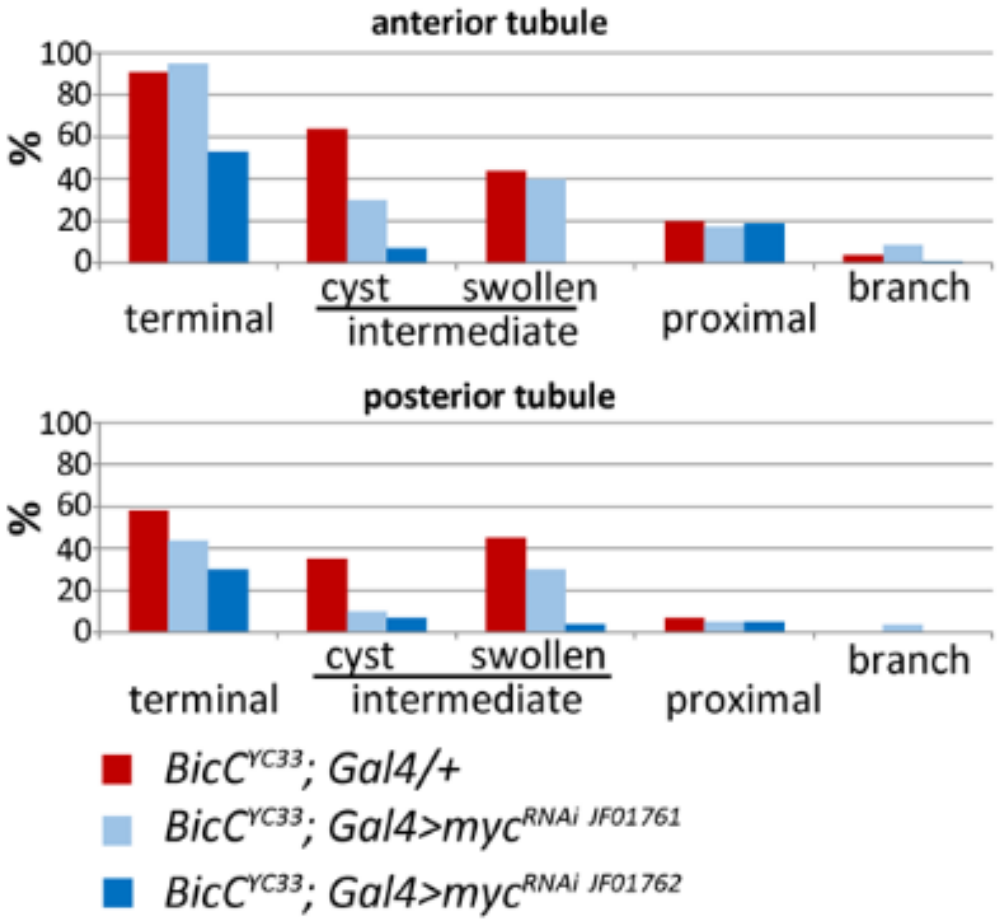

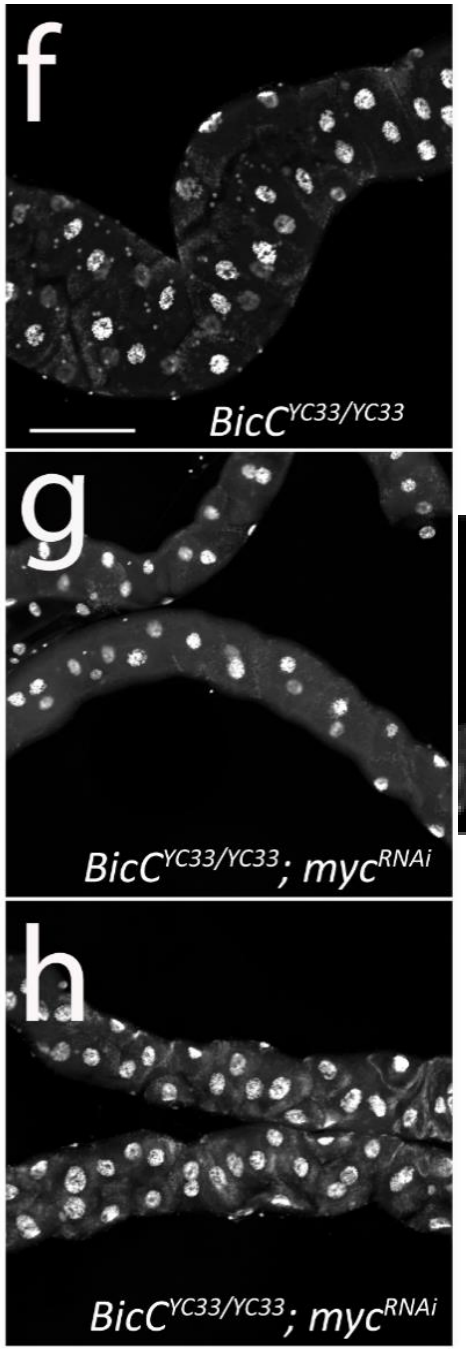

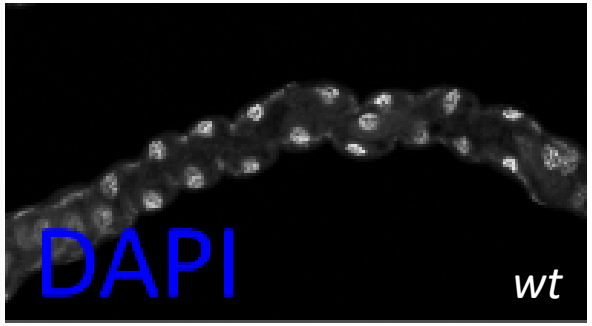

Gamberi C., Hipfner D. R., Trudel M., Lubell W. D. 2017. *PLOS Genetics*, 2017 13(4):e1006694.