Assessment of the Novel Pkd2Halo Allele for *In Vivo* Labelling and Study of the Polycystin-2 Protein

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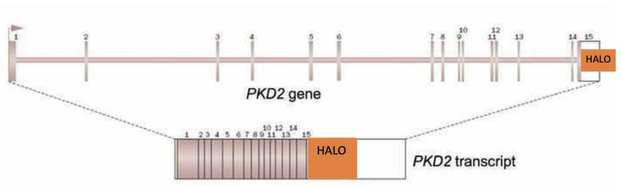
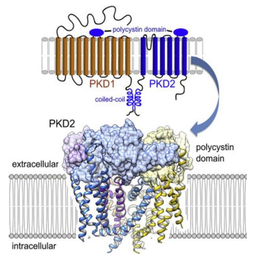
Introduction:

- Polycystin-2 (PC2) is a protein encoded for by the *Pkd2* gene and has been implicated in the development of renal cysts in human patients with ADPKD. Previous work suggests the localization of the protein was on the primary cilium and endoplasmic reticulum of renal epithelial cells. The objective was to use the Halo protein tag in the C-terminus of the coding region of *Pkd2* to make a PC2:Halo fusion protein, and then to study the localization of PC2:Halo fusion protein both *in-vivo* and *in-vitro*.



Adapted from: Balcells and Criach, 2010



Adapted from: Shen et al., 2016

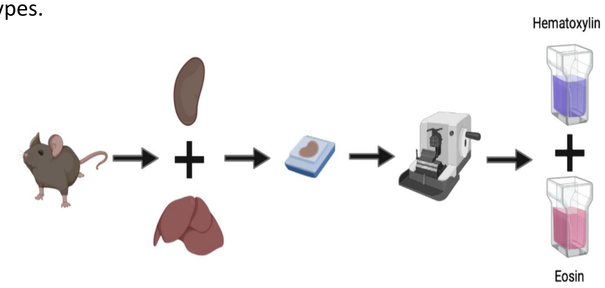
Hypothesis:

The insertion of the Halo tag will not interfere with PC2 function in mouse models nor result in a cystic phenotype; thus, the mice with the PC2:Halo fusion will resemble wildtype mice.

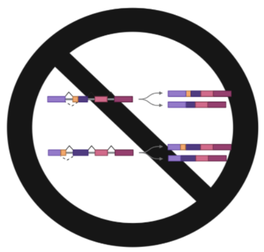
Methods:

- Kidney and liver tissues were isolated, embedded in paraffin, and sectioned into 8μm sections. Hematoxylin and Eosin staining was completed, and the tissues were imaged to assess histological phenotypes.

Genotypes
Pkd2 $^{Halo/Halo}$
Pkd2 $^{fl/Halo}$
Pkd2 $^{\Delta/Halo}$
Pkd2^{WT}



- mRNA was isolated and reverse transcriptase was used to generate cDNA to then assess for expression of wildtype and Halo alleles.



Assess for the absense of alternative splicing

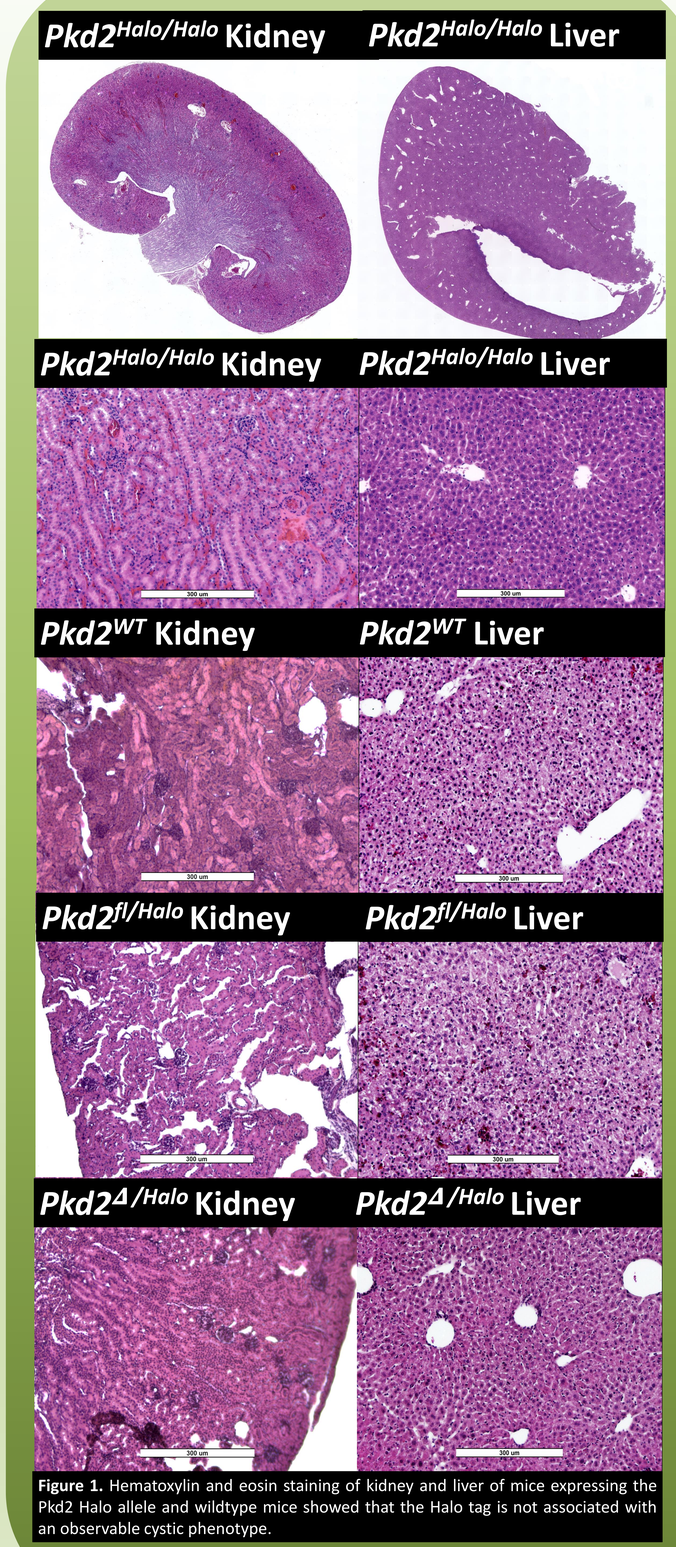


Figure 1. Hematoxylin and eosin staining of kidney and liver of mice expressing the Pkd2 Halo allele and wildtype mice showed that the Halo tag is not associated with an observable cystic phenotype.

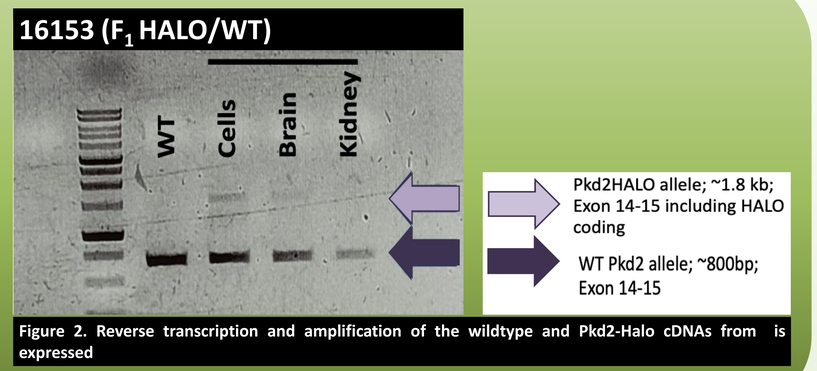


Figure 2. Reverse transcription and amplification of the wildtype and Pkd2-Halo cDNAs from is expressed

Results:

- There are no abnormal phenotypes observed in the *Pkd2*$^{Halo/Halo}$ kidney and liver tissues.
- Normal histology is also observed in the kidney and liver of *Pkd2*$^{\Delta/Halo}$ mice, which only express the PC2 Halo allele.
- There were no observable cysts in *Pkd2*$^{Halo/Halo}$, *Pkd2*$^{fl/Halo}$, *Pkd2*$^{\Delta/Halo}$, which indicates functionality of the PC2 Halo.
- The cDNA reverse transcribed from the mRNA shows that splicing occurs normally, and the gene is expressed.
- This was confirmed by sequencing the cDNA.

Conclusion:

- Based on current findings from the histology of kidney and liver tissues from mice with the PC2:Halo fusion protein, there is no cystic phenotype observable.
- The mRNA shows that no novel alternative splicing occurs in the *Pkd2*^{Halo} allele.
- Because the PC2:Halo fusion protein can be conjugated to a fluorescent ligand, it can allow for the following of the PC2 protein in live cells.
- Additionally, the PC2:Halo fusion protein could also be used with the fluorescent ligand to show trafficking.

Future Directions:

- Kidney and liver histology will be assessed in older mice to verify no cyst development from a slow-progressing phenotype.

Acknowledgements: NIH/NIDDK 1U54DK126087 (BKY)