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

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Polycystin-2 (PC2) is the protein encoded by the *Pkd2* gene, which when mutated results in the development of renal cysts. Previous work has shown that PC2 protein localizes to the primary cilium of renal epithelial cells but visualization and trafficking in live samples is not feasible with current tools. The objective for our research was to incorporate a Halo protein tag at the C-terminus of the *Pkd2* coding region to generate a PC2:Halo fusion protein expressed from the endogenous locus. This will enhance the ability to study PC2 localization and expression both *in vivo* and *in vitro*. We hypothesize that 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.

To assess the novel Pkd2Halo, we isolated kidney and liver from homozygous $Pkd2^{Halo/Halo}$ and wild type mice. Hematoxylin and eosin staining was done on the paraffin processed sections of $Pkd2^{fl/Halo}$, $Pkd2^{delta/Halo}$, and wildtype kidneys.

As expected, the homozygous kidneys, along with the $Pkd2^{fl/Halo}$, $Pkd2^{delta/Halo}$, had no observable cysts. The development of cysts would indicate that addition of the Halo tag altered PC2 normal protein function and would not be useful for *in vivo* studies. We will confirm a lack of cyst development over a longer time course (e.g., 2-6 months of age) to ensure a more-slowly progressing cystic phenotype is not present. Additionally, mRNA will be isolated and sequenced to verify that expression levels are unchanged and no novel alternative splicing occurs in the $Pkd2^{Halo}$ allele.

Overall, the $Pkd2^{Halo}$ allele appears functional, and based on ongoing experiments related to the addition of a fluorescent ligand to the halo tag, is indicative of being a valuable reagent in allowing researchers to follow PC2 *in vivo*.

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