Tracking Polycystin2 *In Vivo* Using a Novel Engineered *Pkd2-HALO* Allele

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Abstract

Background: ADPKD, one of the leading causes of end stage renal disease, is due to mutations in the genes *PKD1* or *PKD2*, which encode for polycystin1 or polycystin2, respectively. While the genes involved were identified several decades ago, research is ongoing to understand their functions and how disruption leads to renal cyst development. Studies focused on trafficking and localization of polycystin2 (PC2) are hindered by the difficulty in antibody generation, low-level expression of the endogenous protein, and inability to track the protein in live cells.

Methods: To expand the reagents available to the PKD research community and accelerate studies of PC2 function, the PKD RRC used CRIPSR/Cas9 to target HALO to the C-terminus of the endogenous mouse *Pkd2* gene.

Results: Mice homozygous for the engineered *Pkd2*-HALO allele are viable and have no overt renal cysts at three months of age indicating that the PC2-HALO protein is functional. Initial studies using western blot from tissues harvested from heterozygous mice showed expression of the PC2-HALO protein in brain and kidney lysates. Additionally, we have begun testing the functionality of the HALO ligand *in vivo* and in primary cells cultured from the mouse line. We confirmed, using a HALO-ligand in heterozygous primary cultured cells, the presence of fluorescent labeling of the fusion protein in the primary cilium of the renal epithelium. Additional characterization of this novel mouse resource is ongoing.

Conclusion: This mouse will provide an invaluable resource to analyze endogenous PC2 function, trafficking, and turnover in live cells and *in vivo*.

Pkd2-HALO Mice Do Not Develop Renal Cysts

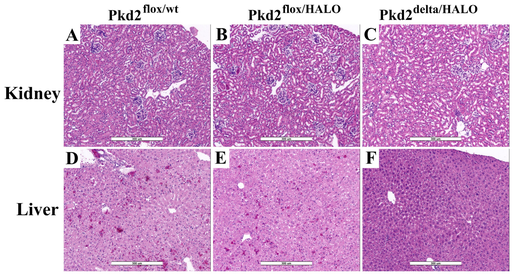

Figure 1: H&E-stained sections of kidney (A-C) and liver (D-F) from adult mice. The *Pkd2*$^{delta/HALO}$ sample also carried the ubiquitous CAGG-CreER transgene. *Pkd2* deletion was induced by IP injection of tamoxifen at postnatal day 5. No cyst development occurred in the *Pkd2*$^{delta/HALO}$ kidney indicating the PC2-HALO protein is functional. Additionally, mice homozygous for the *Pkd2*^{HALO} allele are viable and fertile with no overt phenotypes (not shown).

Live Labeling in Primary Kidney Cells

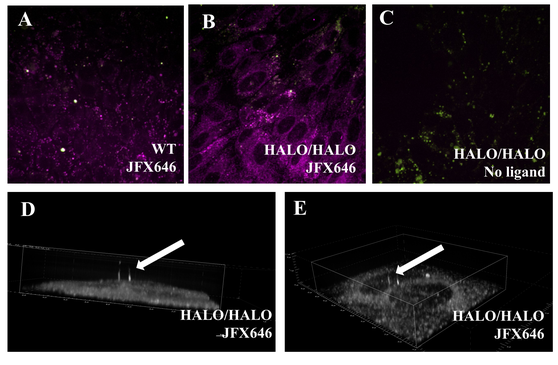

Figure 2: PC2-HALO localization in primary renal cell cultures. Renal epithelial cells were isolated from WT or *Pkd2*$^{HALO/HALO}$ mice and treated with JFX646 ligand (purple in A-C; white in D-E) to test live PC2-HALO labeling *in vitro*. PC2-HALO labeled cells showed an ER-like pattern (B, D and E). Labeling was still present after 24 hours (D and E). Labeled projections (arrows), likely primary cilia, were visible extending apically from cultured cells (D and E). No specific signal was seen in WT cells (A) or *Pkd2*$^{HALO/HALO}$ not incubated with ligand. Images were taken using the 488 laser to distinguish autofluorescence (green) from ligand.

PC2-HALO Protein Expression

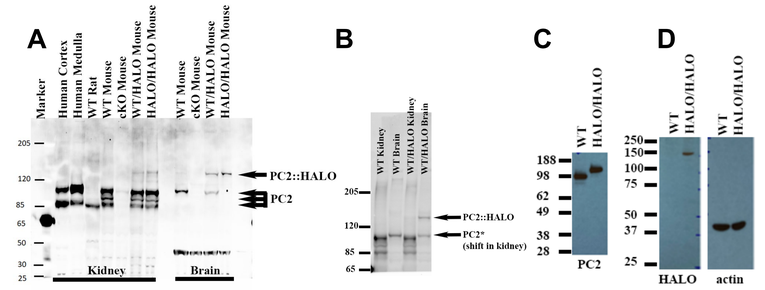

Figure 3: Western blot analysis of PC2-HALO protein expression. Total protein was isolated from kidney and brain (A and B) or primary renal epithelial cell cultures (C and D). In the kidney (A left side; B), PC2 antiserum showed expression of multiple bands that were absent in conditional knockout samples. A faint band corresponding to PC2-HALO was present only in heterozygous and homozygous Pkd2^{HALO} samples. In the brain, PC2 and PC2-HALO were seen at the expected size with none of the additional bands observed in the kidney. It is unclear whether the three bands seen in mouse kidney lysates are due to processing *in vivo* or during protein isolation, but a small decrease in PC2 size is visible when compared to brain lysate (B). In primary renal epithelial cell cultures, a single band is seen at the predicted size of PC2 or PC2-HALO. Presence of the PC2-HALO fusion protein was verified using anti-HALO antiserum (mAb; Promega). Actin was used to confirm similar loading of primary cell lysates.

In Vivo Labeling in the Choroid Plexus

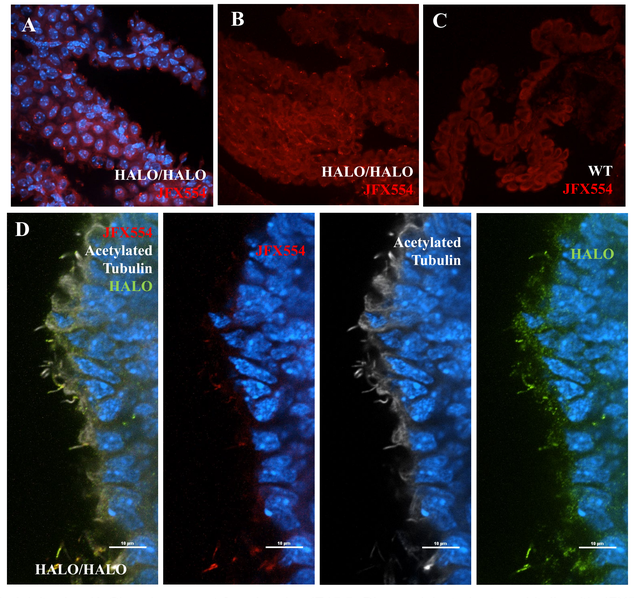

Figure 4: Adult mice (A-C) and pregnant female mice (E15.5; D) were injected retro-orbitally with JFX554 ligand prior to tissue isolation. Choroid plexus was isolated, fixed in 4% PFA and either directly visualized on a Nikon confocal spinning disk microscope, or whole-mount stained with antibodies (D) then visualized. In the *Pkd2*$^{HALO/HALO}$ adult (A and B), the ligand is readily detected in the cilia of the choroid plexus. No ciliary-localization is visible in WT mice injected with ligand (C). In the developing embryo, ligand-conjugated PC2-HALO-positive cilia (red, JFX554; white, acetylated tubulin) are visible on the cells of the choroid plexus. To confirm that the ligand seen is PC2-HALO, the choroid plexus was also stained with anti-HALO antiserum (green, pAb, Promega).

In Vivo Labeling in the Kidney

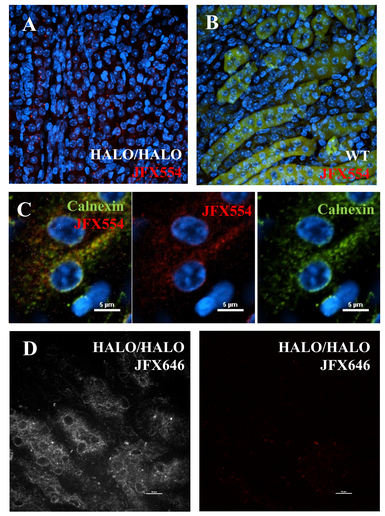

Figure 5: Adult mice were injected with JFX554 (A-C), JFX646 (D), or JFX552 (E) ligand prior to tissue isolation. (A and B) Ligand (red) was more prominently visible in the medulla only in *Pkd2*$^{HALO/HALO}$ mice. Green fluorescence represents kidney autofluorescence. (D) PC2-HALO-conjugated ligand in *Pkd2*$^{HALO/HALO}$ kidney cryosections partially colocalizes with the ER marker calnexin (green) in agreement with the known localization of PC2 within the ER. To confirm that the ER-like staining pattern is also seen using the JFX646 ligand and is not due to autofluorescence, cryosections from JFX646 injected *Pkd2*$^{HALO/HALO}$ kidneys were imaged for both far red (left) and red (right) fluorescence. PC2-HALO-ligand signal was not observed in the red channel. (E) To determine if the red foci colocalized with renal cilia, sections from JF552-injected *Pkd2*$^{HALO/HALO}$ kidneys were stained with Alexa646-conjugated anti-acetylated tubulin antibodies. While the ligand foci are near cilia, they do not overlap.

In Vivo Labeling in the Developing Kidney

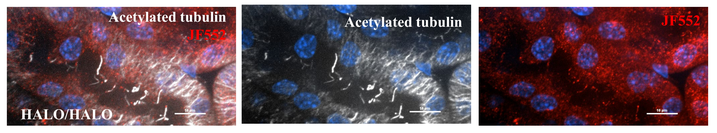

Figure 6: PC2-HALO localization in E15.5 kidney. A timed pregnant female was injected with JF552 ligand 3 hours prior to tissue isolation. Kidneys were isolated and fixed in 4% PFA, permeabilized, and incubated with Alexa646-conjugated acetylated tubulin antibodies. In contrast to the adult kidney (Fig 5), HALO ligand localized to cilia within the developing tubules in addition to punctate staining in an ER pattern.

Conclusions

- We have generated a mouse with insertion of a HALO tag at the C-terminus of Pkd2
- *Pkd2*$^{delta/HALO}$ and *Pkd2*$^{HALO/HALO}$ mice are viable and do not develop renal cysts
- HALO ligand can be added to renal primary cells isolated from the kidney of *Pkd2*$^{HALO/HALO}$ to visualize PC2 localization
- In primary kidney cells, PC2-HALO localizes to puncta within the cytoplasm and in extensions off the apical surface and co-localize with Arl13b (not shown)
- By western blot, PC2-HALO fusion protein is detected in the brain and primary cell culture. Preliminary data suggests C-terminal processing occurs in the kidney.
- *In vivo* labeling of *Pkd2*$^{HALO/HALO}$ demonstrates the utility of this novel mouse model to study Polycystin2 function and trafficking *in vivo*
- Visit the PKD-RRC website (PKD-RRC.org) for this and other PKD resources

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