Upk1b-RFP Allele Characterization

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Findings:

RFP is detected in the expected tissues. We conclude this allele does exhibit the expected activity.

Data:

Crosses

The Upk1b-RFP strain is a RFP knock-in line. In order to characterize them, we assayed for RFP expressed in the expected Upk1b domain.

We crossed B6 female mice with Upk1b-RFP males to obtain Upk1b-RFP/+ embryos. The embryos were dissected on E15.5 to collect the urogenital system (UGS). Two E15.5 litters were dissected and eighteen embryos were isolated. Whole embryos as well as dissected UGSs were viewed with a fluorescence microscope to detect RFP. A strong red fluorescence was visible in the embryos as well as the bladder and gonads of the UGSs (Fig2). Eight Upk1b-RFP/+ embryos, and eight wildtype control littermates were embedded for frozen sectioning and subsequent immunohistochemistry.

Genotyping

Tail samples of the embryos were collected and incubated in tail digestion buffer overnight at 55ºC. PCR was performed as per the protocol below and the PCR products were run on a 1.5% agarose gel (Fig1).

Oligonucleotides: for Wt allele — Size: 425 bp
DNA sequence (forward): 5' `agtcctgtgatggctgaccg` 3'
DNA sequence (reverse1) 5' `aggcactataaaggggaacg` 3'

Oligonucleotides: for targeted/transgenic allele — Size: ~275 bp
DNA sequence (forward): 5' `agtcctgtgatggctgaccg` 3'
DNA sequence (reverse 2) 5' `cttgatgacgtcctcggagg` 3'

Rxn Buffer and Conditions: (25μl reaction)

10X PCR	2.0ul
1.25mM dNTP	3.2ul
0.25ug/ul primer F	1ul
0.25ug/ul primer R1	1ul
0.25ug/ul primer R2	1ul
Amplify Taq	0.3ul (5u/ul)
5x cresol red dye	2.5ul
Genomic DNA	1ul
Total volume	**20 ul**

94ºC	3min	1 cycle
94ºC	30sec	35cycles
60ºC	60sec	
72ºC	60sec	
72ºC	10min	1 cycle

10X PCR Buffer
500 mM KCl
100 mM Tris-HCl, pH 8.4
15 mM MgCl2
200ug/ml gelatin (Fluka # 48322)

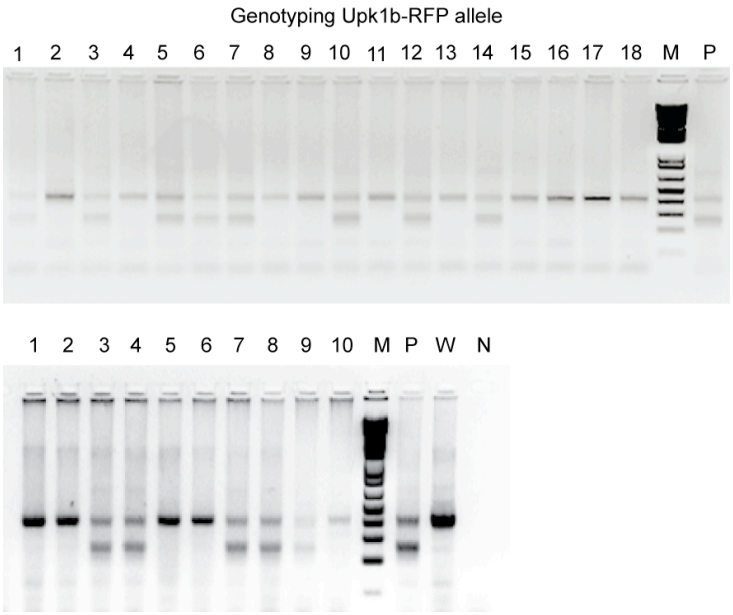

Figure 1: Upk1b-RFP genotyping Upper gel - No 1, 3, 5 6, 7, 10, 12 and 14 Upk1b$^{RFP/+}$, No 2, 4, 8,9,11,13,15,16,17 and 18 Wildtype. Lower gel – No 3, 4, 7, 8, and 9 are Upk1b$^{RFP/+}$. **M:** Marker, **P**: Positive control, **W**: Wildtype control, **N**: Negative control

Native Fluorescence

Whole embryos as well as dissected UGSs were viewed with a fluorescence microscope to detect RFP. At E15.5, RFP expression was restricted to the expected Upk1b expression domain.

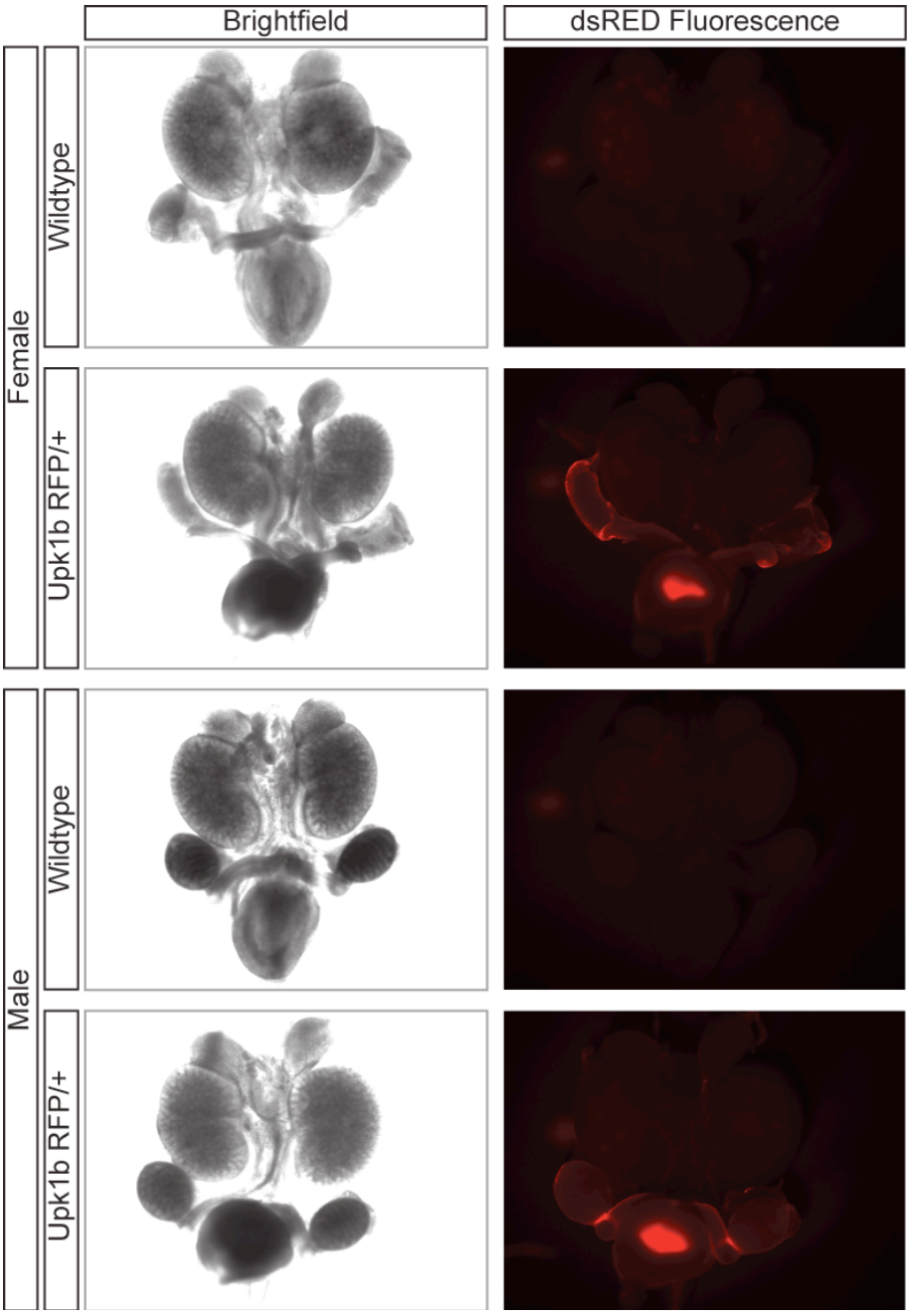

Figure 2. Wholemount RFP detection in E15.5 Upk1b$^{RFP/+}$ UGS samples. Strong RFP fluorescence was visible in dissected UGSs. The expression was limited to the Upk1b domain.

Immunohistochemistry

Immunohistochemistry was performed to examine if the RFP allele was expressed in the expected Upk1b domain. Two of each Upk1b-RFP/+; and wildtype UGSs (male and female) were assayed. RFP protein were assayed by staining with Rabbit-anti-RFP.

Whole UGSs were fixed in 4% paraformaldehyde at 4ºC 2 hours, washed 3 times in PBS, equilibrated in 30% sucrose overnight and then embedded in OCT and flash frozen on dry-ice. The UGSs were sectioned at 20um and stained with Rabbit-anti-RFP, (Rabbit, MBL, PM005 1:1000),for overnight at 4ºC. The secondary antibodies were Alexafluor 488 (Molecular probes).

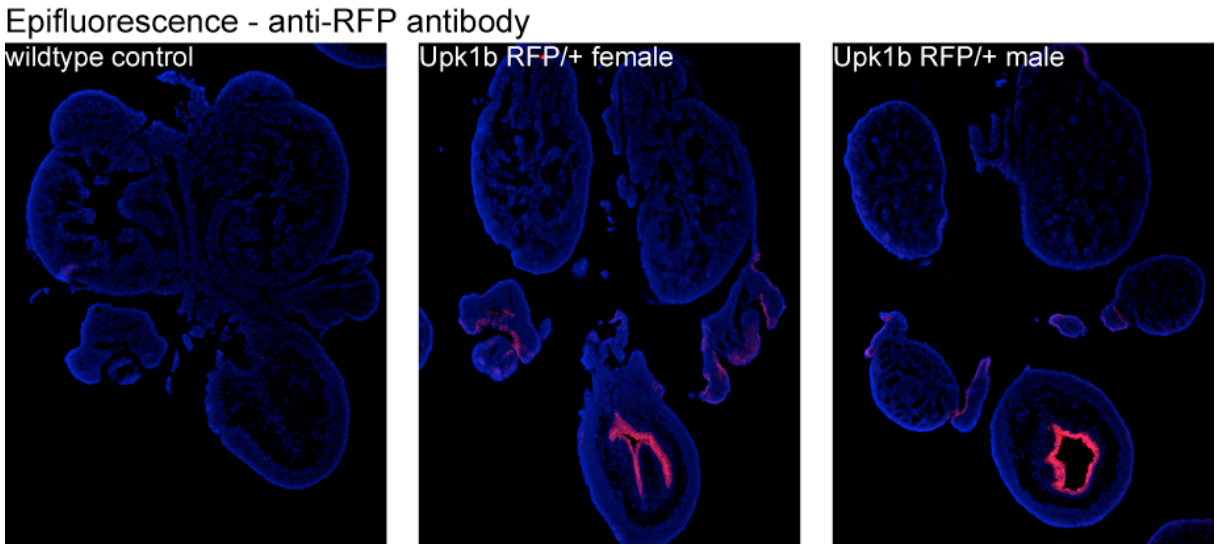

Figure 3. RFP signal is detected in the urothelium of the bladder, as well as the gonads of Upk1bRFP/+ samples.

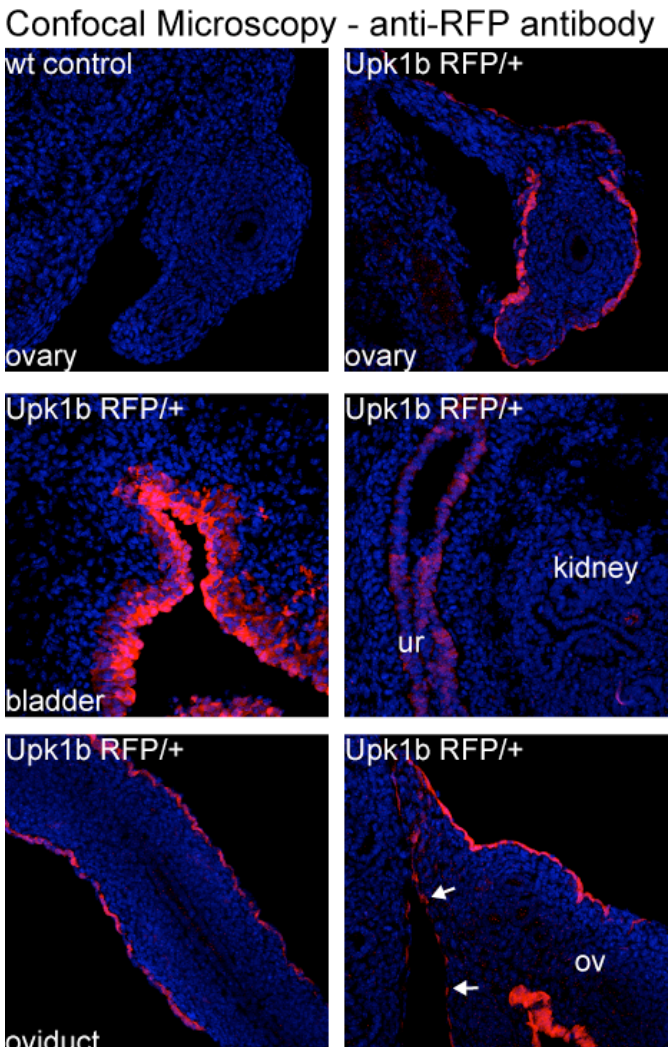

Figure 4. RFP expression in the bladder and gonads. RFP is detected by IHC in the surface cell layer of the oviduct and ovary. Note the non-contiguous expression in the thin cell layer of the ovary (arrows). RFP is also detected in the urothelium of the bladder and the ureter, though weaker in the ureter.

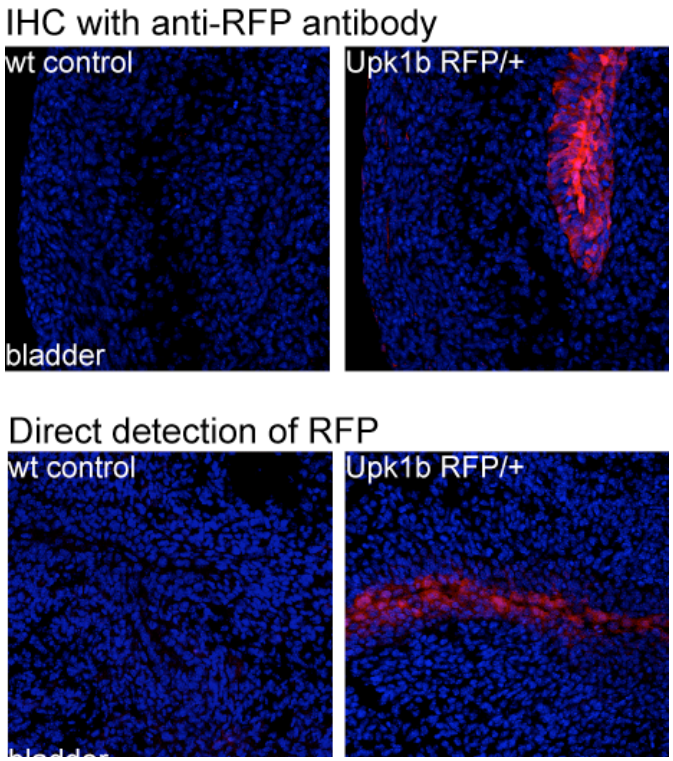

Figure 5. Comparison of direct RFP visualization to immunohistochemical detection using antiRFP antibody. As noted in Fig4, RFP expression in the ureter is weaker than in the bladder urothelium. Here tested the ability to detect native RFP with confocal microscopy. We show native RFP can be directly detected without the need for IHC, thought he signal is stronger using antibodies.