Osr2-RFP Allele Characterization

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Findings:

RFP was readily detected in the kidney and gonads. We conclude this allele does exhibit the expected activity in the Osr2 expression domain.

Data:

Crosses

The Osr2-RFP strain is a RFP knock-in line. In order to characterize them, one questions was addressed:

1) Is the RFP expressed in the expected Osr2 domain?

We crossed B6 female mice with $Osr2^{RFP/+}$ males to obtain $Osr2^{RFP/+}$ embryos. The embryos were dissected on E15.5 to collect the urogenital system (UGS).

Three E15.5 litters of B6 were dissected on 5/5/2008,5/15/2008 and 5/23/2008 and litters of eight, nine and eleven embryos were isolated respectively. Whole embryos as well as dissected UGSs were viewed with a fluorescence microscope to detect RFP. Strong red fluorescence was visible in the head of the whole embryos, the testis, the genital ducts of both of male and female as well as the kidneys of the UGS (see Fig2)

Ten Osr2-RFP experimentals and ten wildtype controls from all of the E15.5 litter were embedded for frozen sectioning and 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: 347bp
DNA sequence (Osr2-F): 5' cgaggtcttggaggctaagg 3'
DNA sequence (Osr2-R) 5' ccctgaaggtggtccactgc 3'

Oligonucleotides: for targeted/transgenic allele Size: 260bp
DNA sequence (Osr2-F): 5' cgaggtcttggaggctaagg 3'
DNA sequence (RFP-R2) 5' accttgaagcgcatgaactc 3'
Amplifies from the RFP insert.

Rxn Buffer and Conditions: (25µl reaction)

10X GSB	2.5ul			
25mM dNTP	1ul	94ºC	3min	1 cycle
10uM primer F	1ul	94ºC	30sec	

10uM primer R1	1ul
10uM primer R2	1ul
DMSO	2.5ul
2-mercaptoethanol	0.125ul
Amplify Taq	0.3ul (5u/ul)
5x cresol red dye	2.5ul
Genomic DNA	1ul
Total volume	**25 ul**

60ºC	60sec	35cycles
72ºC	60sec	
72ºC	10min	1 cycle

10X Gitschier Buffer (GSB):
670 mM Tris, pH 8.8
166 mM Ammonium Sulfate
65 mM MgCl2
0.1% gelatin

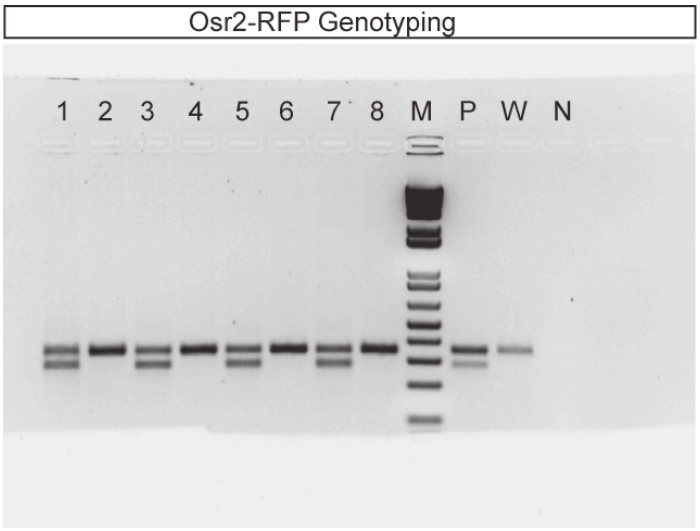
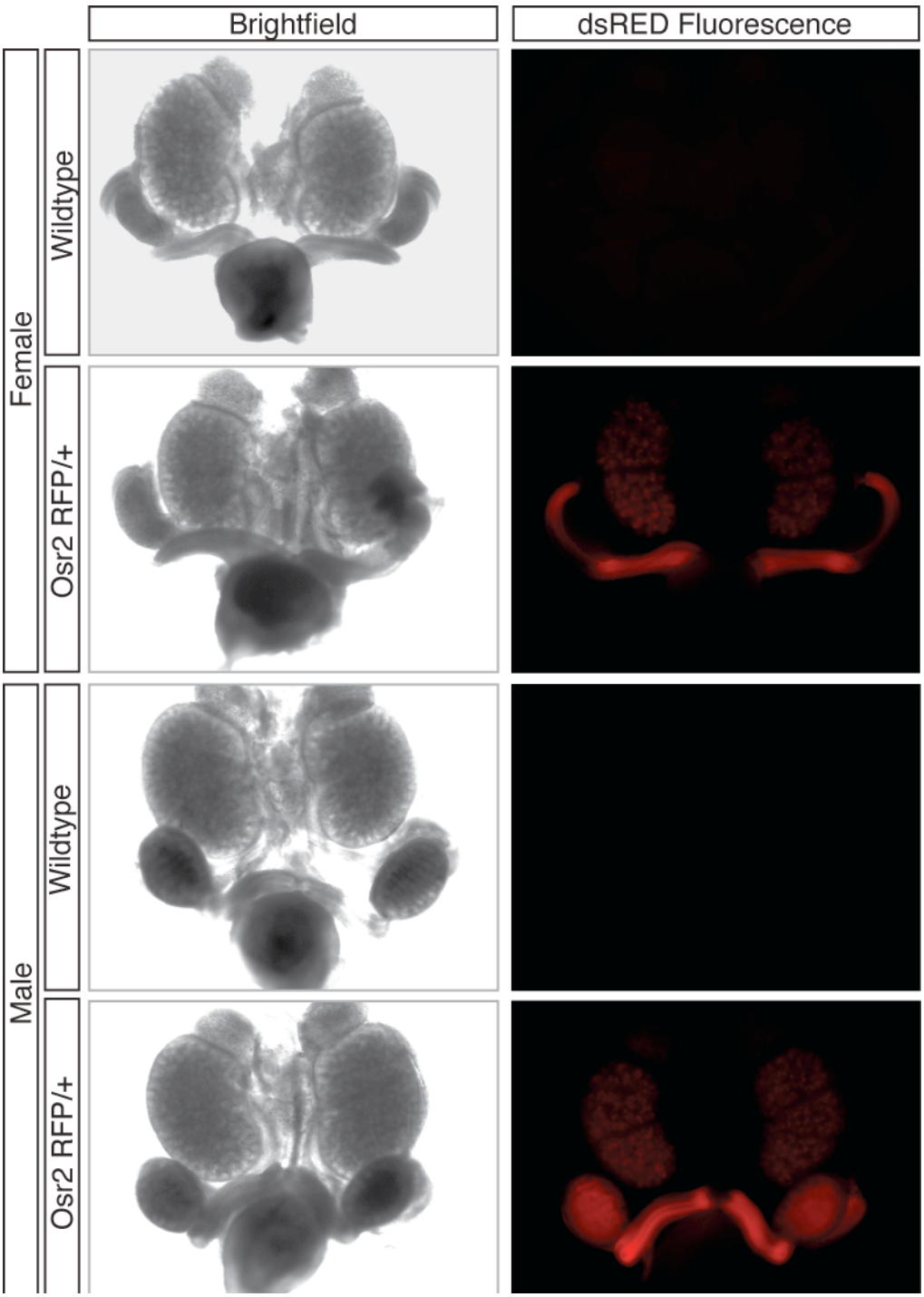


Figure 1. Osr2-RFP genotyping. No 1, 3, 5 and 7 are Osr2$^{RFP/+}$, No 2, 4, 6 and 8 are wildtype**.** M: Marker, P: positive control W: Wildtype control; N: Negative control

Native Fluorescence

Whole embryos as well as dissected UGSs were viewed with a fluorescence microscope to detect GFP. We observed the initial Osr2-RFP E15.5 litter above, and subsequent litters via direct fluorescence.

Figure 2 (below). Osr2-RFP direct fluorescence. Freshly dissected E15.5 UGSs where observed for RFP fluorescence. The genital ducts, the testis, and the kidneys showed RFP expression in the expected Osr2 domains.

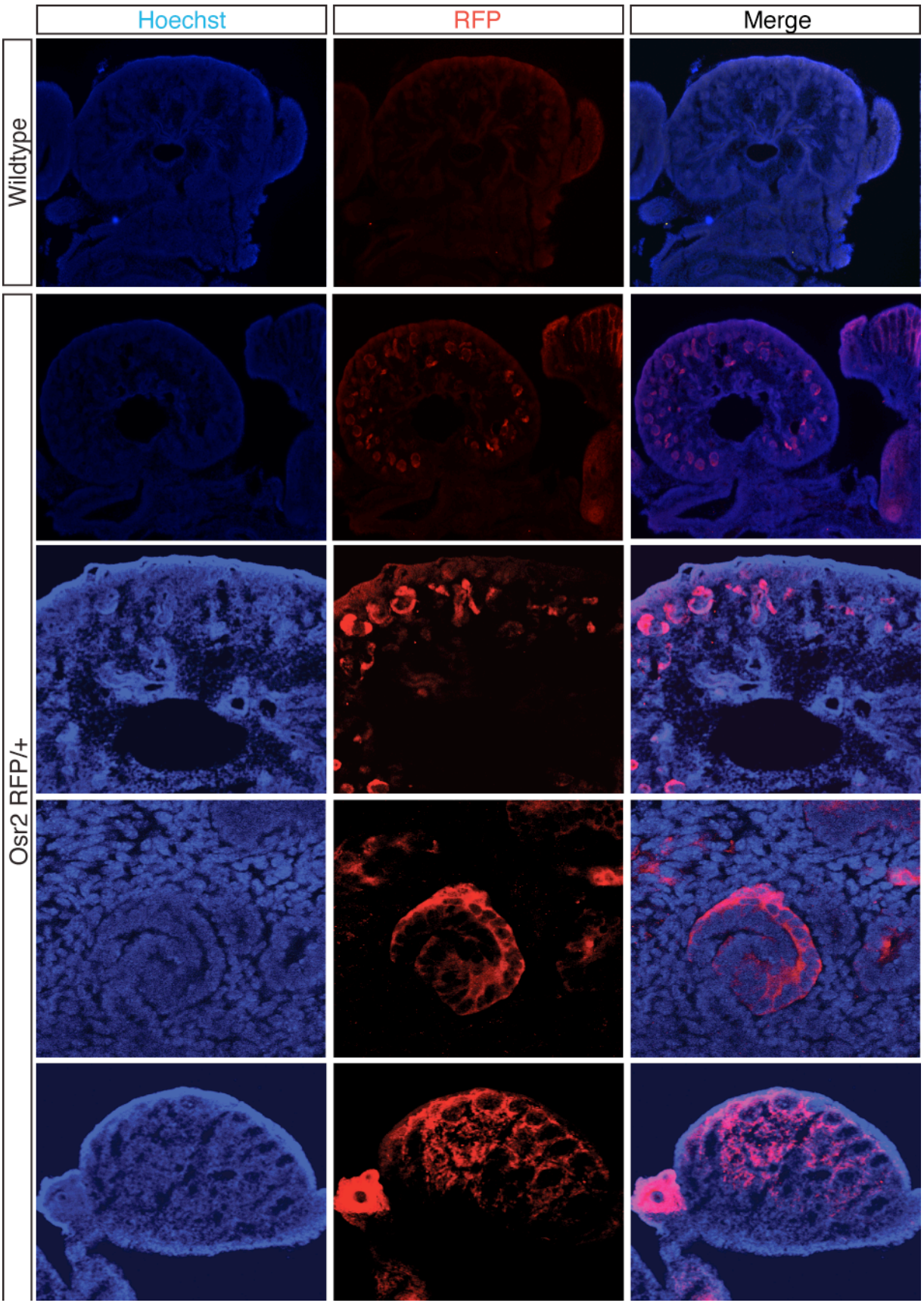

Brightfield
dsRED Fluorescence
Female
Wildtype
Osr2 RFP/+
Male
Wildtype
Osr2 RFP/+

Immunohistochemistry

Immunohistochemistry was performed to examine if the RFP allele was expressed in the expected Osr2 domain. Two each of $Osr2^{RFP/+}$ and wildtype UGSs (male and female) were assayed. RFP protein was assayed by staining with rabbit-anti-RFP. Anti-Cytokeratin staining was done to verify tissue quality for immunohistochemistry.

Whole UGSs were fixed in 4% paraformaldehyde at 4ºC for 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, and Rabbit-anti-RFP/mouse-anti-Cytokeratin, respectively. RFP (Rabbit, MBL, PM005 1:1000), Cytokeratin (Mouse IgG1, Sigma, C 2562, 1:500), for overnight at 4ºC. The secondary antibodies were Alexafluor 488 and 568 (Molecular probes).

Figure 3 (below). RFP protein is expressed in tubule structures and testis in $Osr2^{RFP/+}$ embryos. E15.5 $Osr2^{RFP/+}$ and wildtype kidneys are stained with anti-RFP (red). RFP is detected in the cortex of the developing kidney in forming nephron tubule structures (e.g. S-Shaped bodies, SB). Expression in SB is regionalized with higher protein expression levels in the more proximal regions of the SB (“lower limb”).


Hoechst
RFP
Merge
Wildtype
Osr2 RFP/+

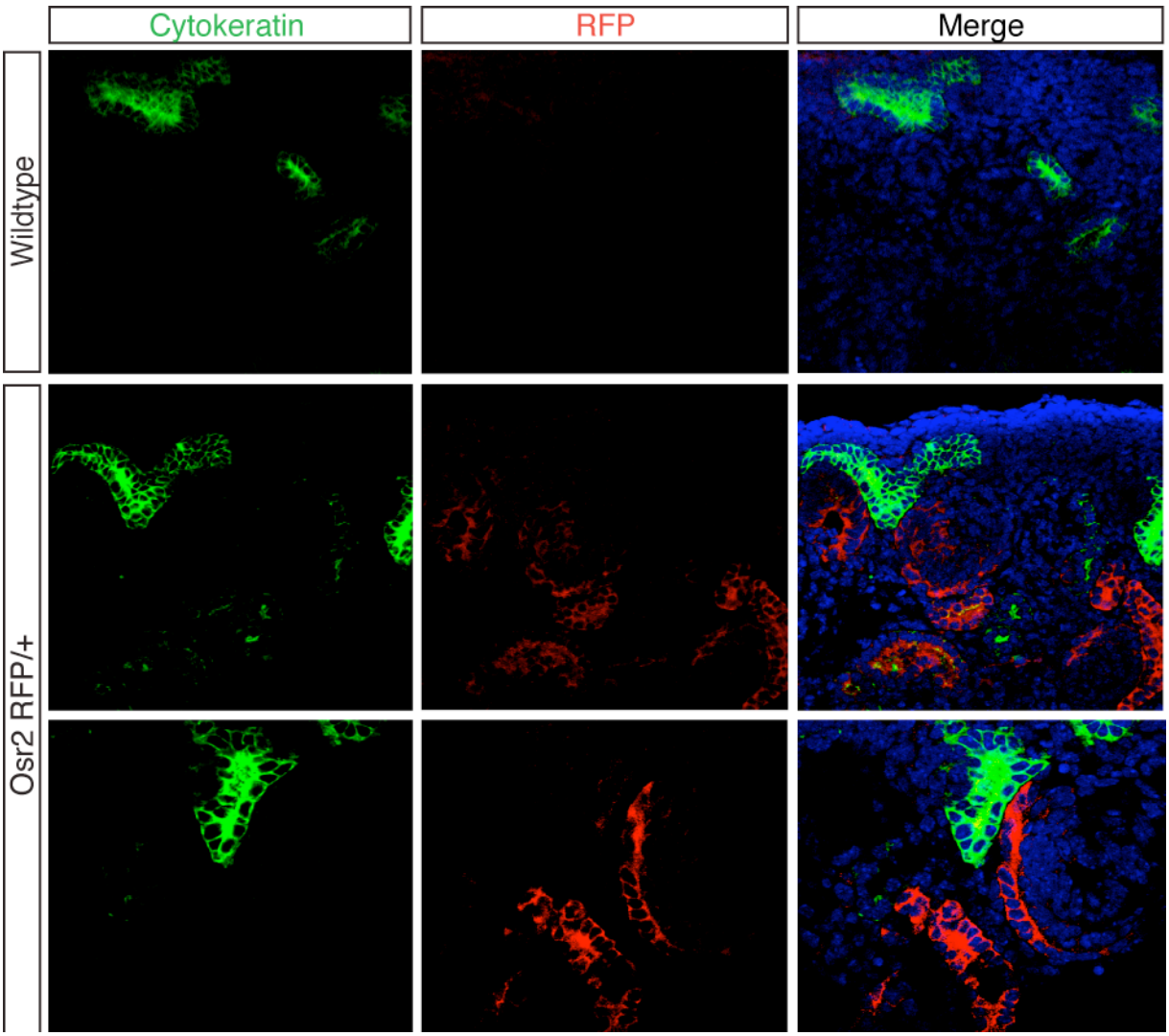


Figure 4. RFP protein was detected in Osr2$^{RFP/+}$ kidneys; no signal detected in wildtype control kidneys. Osr2$^{RFP/+}$ and wildtype kidneys are stained with anti-RFP and anti-Cytokeratin (Cytokeratin green, RFP red).