Cyp11a1-GC Allele Characterization

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Created: 18 November 2008
Updated: 16 January 2009
Submitted: January 2009
Version: 2
Tags: &kmap &mousestrains &gudmap &characterization &Cyp11a1-gc

Findings: VALIDATED

This allele is expressing GFP-Cre in the expected cell populations and Cre-dependent R26R-LacZ expression is observed in those tissues. We conclude this allele does exhibit the expected activity.

Data:

Crosses

The Cyp11a1-GC strain is a GFP knock-in line. In order to characterize them, two questions were addressed:

1. Is the GFP-Cre cassette (GC) expressed in the expected Cyp11a1 domain?
2. Does Cre function as expected?

We crossed Rosa26R$^{lacZ/+}$ (R26R) female mice with Cyp11a1$^{GC/+}$ males to obtain Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$ embryos. The embryos were dissected on E15.5 to collect the urogenital system (UGS). To address the first question assay by GFP and answer the second question assay with β-gal reported.

Six E15.5 litters of the R26R$^{lacZ/lacz}$ female mice crossed with Cyp11a1$^{GC/+}$ males were dissected on 9/18/2008, 9/24/2008, 10/9/2008, 10/15/2008, 10/23/2008 and 10/24/2008 and fifty embryos were isolated. Whole embryos as well as dissected UGSs were viewed with a fluorescence microscope to detect GFP. However, no green fluorescence was visible.

Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$ UGSs as well as R26R$^{lacZ/+}$ littermates were for X-gal staining and immunohistochemistry as needed.

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: 376 bp
DNA sequence (forward): 5' `gagctgcctgccagtgtttg` 3'
DNA sequence (reverse1) 5' `ggacctaggactgctagtag` 3'

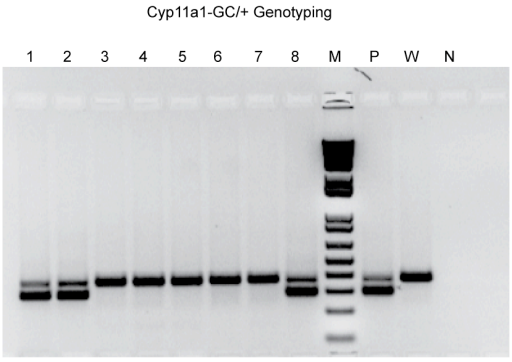
Oligonucleotides: for targeted/transgenic allele — Size: 296 bp
DNA sequence (forward): 5' `gagctgcctgccagtgtttg` 3'
DNA sequence (reverse 2) 5' `gtccagctcgaccaggatgg` 3'

Rxn Buffer and Conditions: (25μl reaction)

10X GSB	2.5ul
25mM dNTP	1ul
10uM primer F	1ul
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**

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



No 1, 2 and 8 Cyp11a1-GC/+; No 3, 4, 5, 6 and 7 Wildtype; M: Marker; P: Cyp11a1-GC/+ positive control; W: Wildtype control

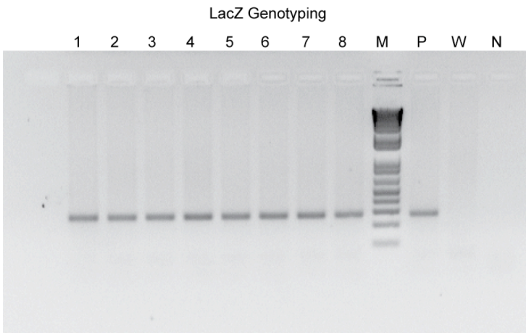


Figure 1. Cyp11a1-GC genotyping No 1, 2 and 8 are Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$; No 3, 4, 5, 6 and 7 are R26R$^{lacZ/+}$; M: Marker; P: Cyp11a1$^{GC/+}$ positive control; W: R26R$^{lacZ/+}$ control N: negative control.

Native Fluorescence

Whole embryos as well as dissected UGSs were viewed with a fluorescence microscope to detect GFP. However, GFP was not detectable under these conditions.

Cre-recombinase Activity

Three male and four female Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$ UGSs as well as R26R$^{lacZ/+}$ littermates were stained with X-gal to assay for X-gal activity. X-gal activity was detected in all of male and female adrenal gland, all male testes, one in three male kidneys and

one in four female kidneys (four different experiments). No Bgal activity was detected in R26R$^{lacZ/+}$ controls. These results are shown in Figure 2.

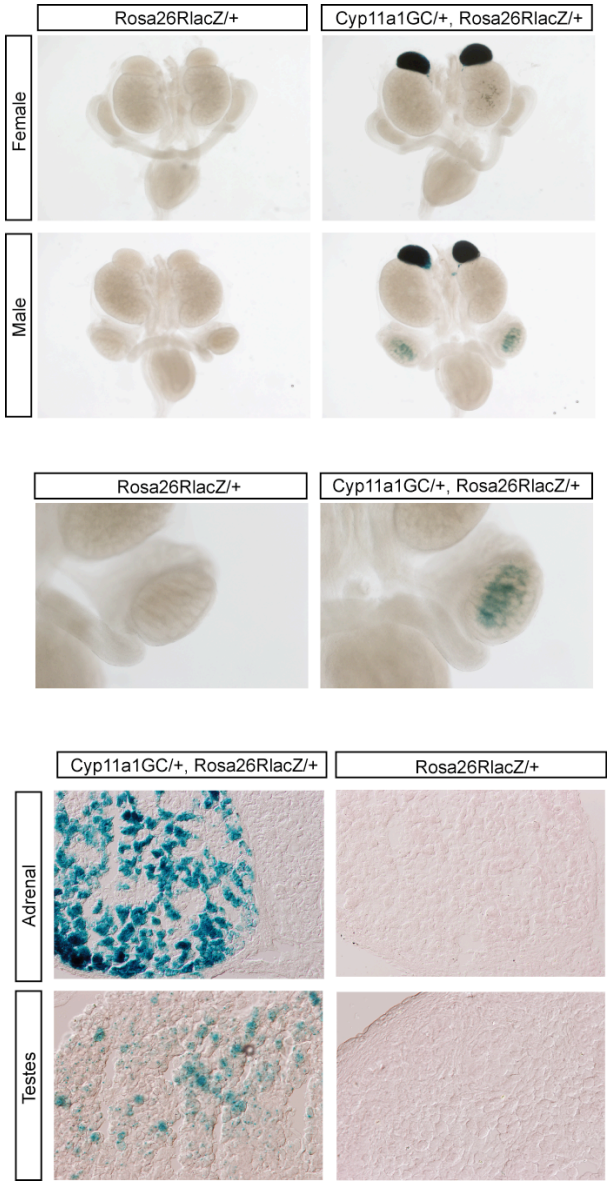


Fig2. Cre- β-gal activity in Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$ UGSs. β-gal activity was detected in all of the Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$ UGSs. Not in R26R$^{lacZ/+}$ littermates UGGs.

Upper two rows: Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$ littermates UGSs were stained with X-gal, (X-gal Blue), at low magnification.

Middle row: Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$ littermates UGSs were stained with X-gal, (X-gal Blue), at high magnification.

Lower two rows: Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$ littermates UGS of the sections were stained with X-gal, (X-gal Blue).

Immunohistochemistry

Immunohistochemistry was performed to examine if the GC allele was expressed in the expected Cyp11a1 domain. Two of each male and female of Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$ experimentals and R26R$^{lacZ/+}$ littermate controls from two litters were assayed, respectively. GFP protein was assayed by staining with chicken-anti-GFP. To test for Cre function, β-gal and GFP expression were examined by double staining with rabbit-anti-b-gal and chicken-anti-GFP. To test for the GFP-Cre cassette expressed in the expected Cyp11a1 domain, GFP and Cyp11a1 (i.e. P450) protein were examined by double staining with rabbit-anti- rat cytochrome P450 side chain cleavage antibody and chicken-anti-GFP.

Whole UGSs were fixed in 4% paraformaldehyde at 4ºC 2 hours, washed 3 times in PBS, equilibrated in 30% sucrose overnight, embedded in OCT and flash frozen on dry ice. The UGSs were sectioned at 20um and stained with rabbit-anti-b-gal/chicken-anti-GFP/mouse-anti-Cytokeratin, rabbit-anti-Laminin/chicken-anti-GFP, and rabbit-anti-cytochrome p450/chicken-anti-GFP, respectively. GFP (Chicken, Aves Labs, Inc, GFP-1020, 1:500); beta-gal (Rabbit, MP Biomedicals, LLC, 55976, 1: 20000); cytochrome P450 side chain cleavage antibody (Chemicon International, AB 1294, 1:200); Laminin (Rabbit, Sigma, L9393, 1:300) Cytokeratin (Mouse IgG1, Sigma, C 2562, 1:500) were incubated overnight at 4ºC and detected with secondary antibodies Alexafluor 488, 568, 647 (Molecular probes) as indicated in the figures 3, 4, and 5.

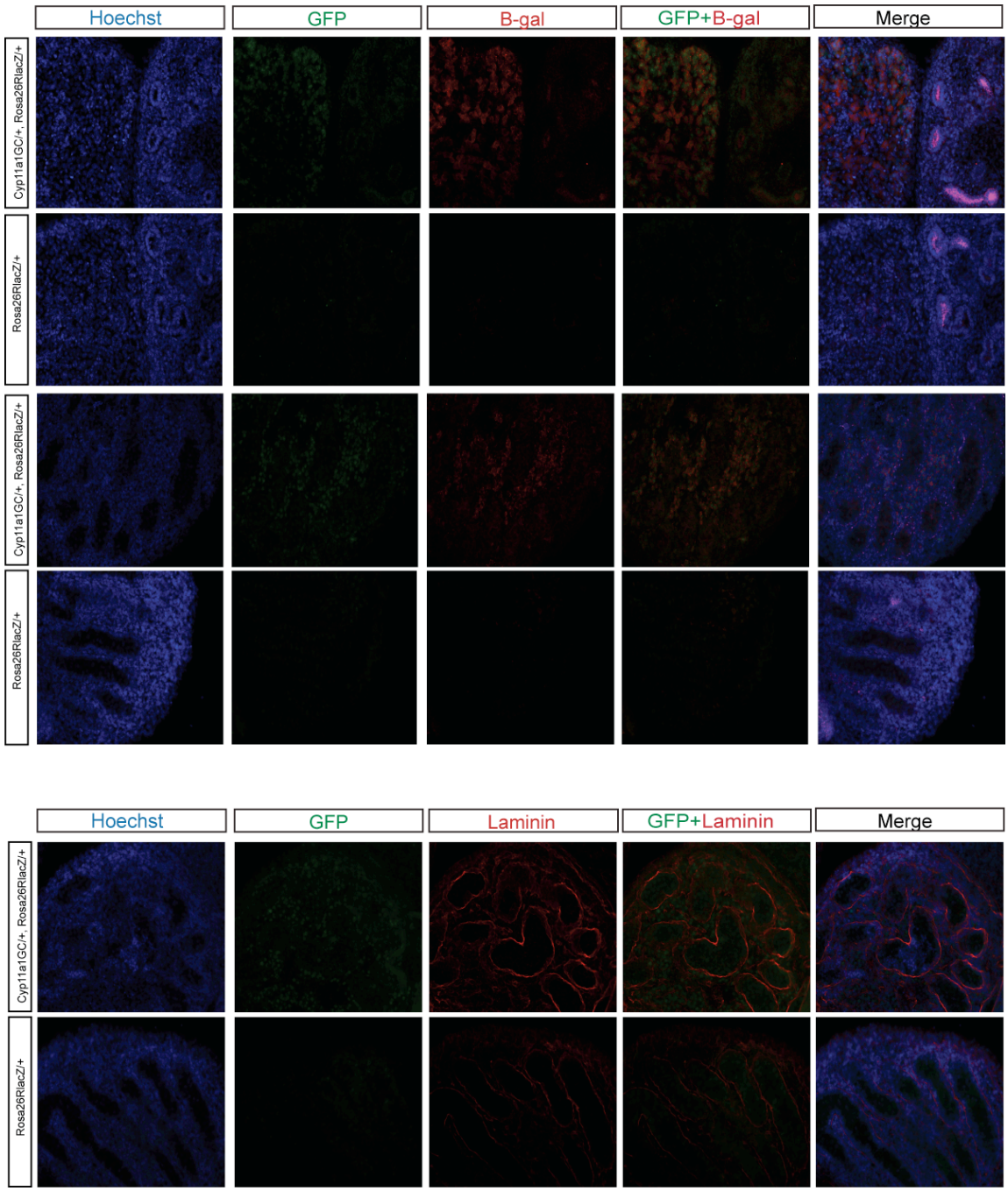


Figure 3. GFP protein detected by antibody staining . GFP and Bgal protein was detected in Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$ kidneys, no GFP and Bgal was detected in R26R$^{lacZ/+}$ littermates control kidneys.

Upper four rows: Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$ kidneys and R26R$^{lacZ/+}$ littermates control kidneys are stained with anti- b-gal and anti-GFP, (b-gal red, GFP Green).

Lower two rows: Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$ kidneys and R26R$^{lacZ/+}$ littermates control kidneys are stained with anti- Laminin and anti-GFP, (Laminin red, GFP Green).

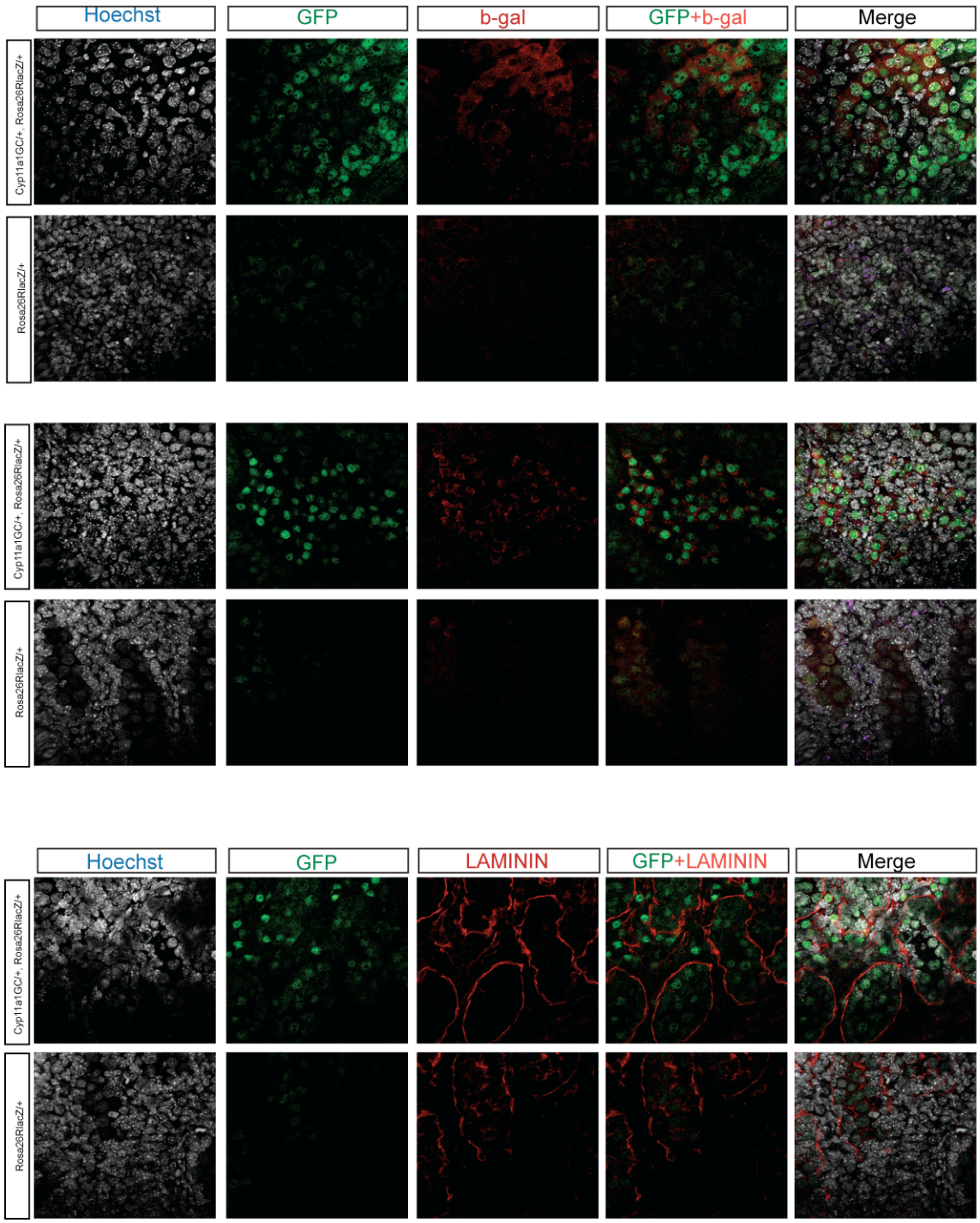


Figure 4. GFP protein detected by antibody staining (Confocal). GFP and Bgal protein was detected in Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$ kidneys, no GFP and Bgal was detected in R26R$^{lacZ/+}$ littermates control kidneys.

Upper four rows: Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$ kidneys and R26R$^{lacZ/+}$ littermates control kidneys are stained with anti- b-gal and anti-GFP, (b-gal red, GFP Green).

Lower two rows: Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$ kidneys and R26R$^{lacZ/+}$ littermates control kidneys are stained with anti- Laminin and anti-GFP, (Laminin red, GFP Green).

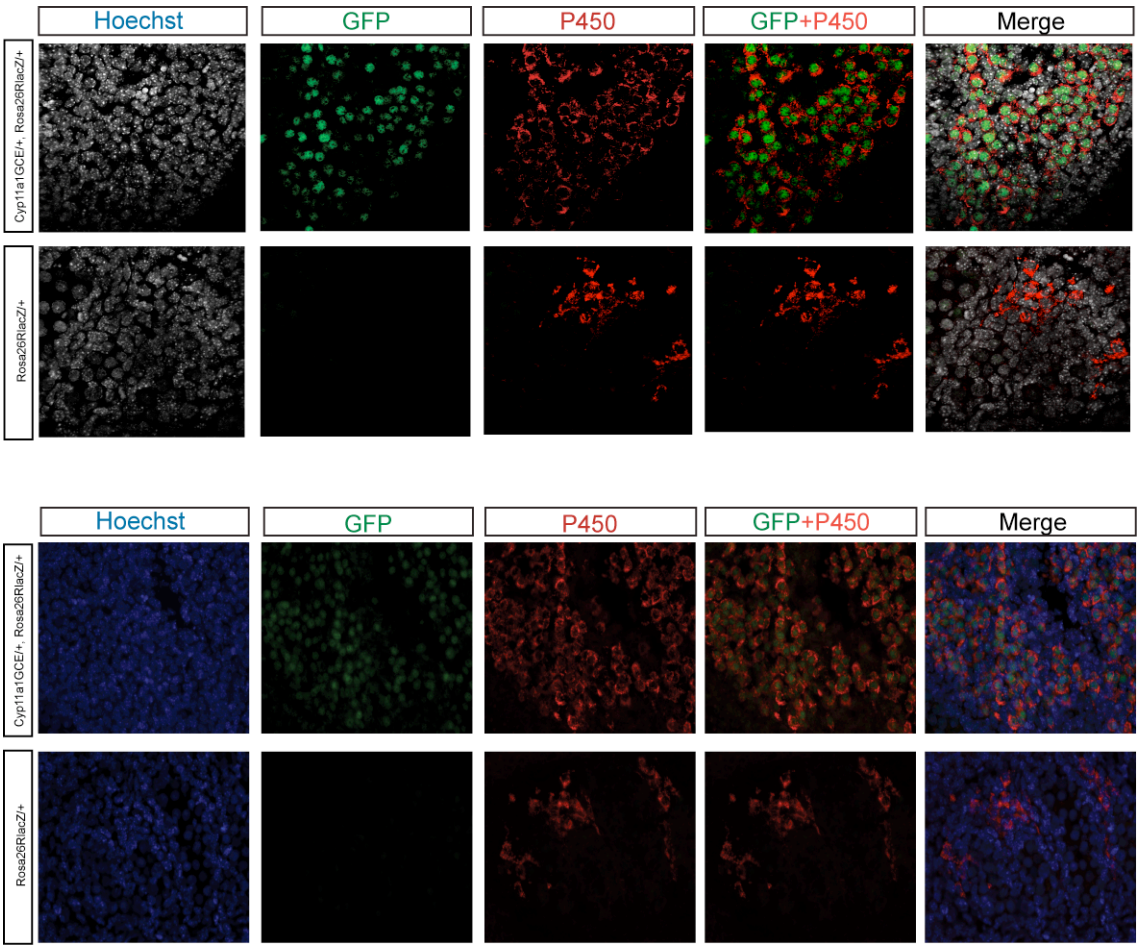


Figure 5. GFP protein detected by antibody staining. GFP was detected in the Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$ testis; cytochrome P450 was detected in both of the Cyp11a1$^{GC/+}$; R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$ littermate control testis. GFP and cytochrome P450 were colocalized in the Leydig cells of the Cyp11a1$^{GC/+}$;R26R$^{lacZ/+}$ testis. The upper two rows were collected by confocal microscopy. The lower to rows were collected by epifluorescence.