

Tshz3-GC Allele Characterization

Authors: Jinjin Guo, Jill A. McMahon, M. Todd Valerius, and Andrew P. McMahon

Created: 5 May 2011

Version: 3 – Final 09 Aug 2011

Findings: **VALIDATED**

Our analysis confirms the expression of eGFPCre at 15.5dpc under the regulation of Tshz3 in smooth muscle actin positive cells in the kidney. At 15.5dpc eGFPCre expression overlaps with Tshz3 in smooth muscle actin positive cell types and associated mesenchyme surrounding the ureteric epithelium. In addition, eGFPCre expression overlaps with Tshz3 positive cells in non-urogenital regions. However, we do not observe expression of the eGFPCre transgene in all Tshz3 positive cell types and some localized ectopic activity of the transgene is observed in urogenital structures.

Data:

Crosses

The Tshz3-GC strain is a BAC transgenic line with eGFPCre (GC) expressed in the Teashirt zinc finger homeobox 3 domain. Pronuclear injection of the BAC construct DNA into C57Bl6/DBA F1 embryos resulted in the birth of 60 pups of which 6 male and 9 females carried the transgene. Six male founders were crossed to Rosa26R^{lacZ/+} (R26R) females and the urogenital system (UGS) was collected from 15.5dpc embryos.

Construct	Date of Birth	Pups born	Founders	Founders mated	Transmittal	Visible Reporter	Antibody to reporter
Tshz3-GC	8/9/10	60	6M, 9F	M6	Yes	Yes	nd
				M7	Yes	Yes	nd
				M41	Yes	Yes	nd
				M43	Yes	Yes	Yes
				M44	Yes	Yes	nd
				M38	Yes	Yes	Yes

Table 1. Summary of founders crossed for transgenic characterization.

Genotyping

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

Oligonucleotides: for targeted/transgenic allele Size: 480 bp
 DNA sequence (forward): 5' - CCTCTGGAGCGATTGTCA -3'
 DNA sequence (reverse 2) 5' - GAACTTCAGGGTCAGCTTGC -3'
 Amplifies 5' arm into GFP sequence within GFP-Cre region.

Rxn Buffer and Conditions: (25 μ l reaction)

10X GSB	2.5ul			
25mM dNTP	1ul	94°C	3min	1 cycle
10uM primer F	1ul	94°C	30sec	
10uM primer R	1ul	56°C	30sec	35cycles
DMSO	2.5ul	72°C	45sec	
		72°C	10min	1 cycle
2-mercaptoethanol	0.125ul			
Amplify Taq	0.2ul (5u/ μ l)			
5x cresol red dye	5ul			
Genomic DNA	1ul			
Total volume	25 ul			

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

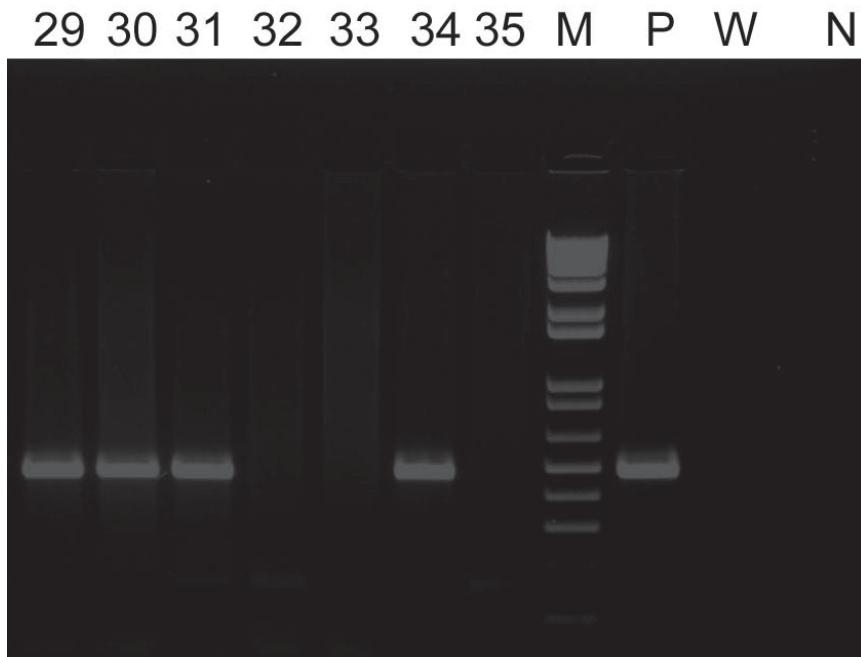


Fig1: Numbers 29, 30, 31 and 34 Tshz3^{GC/+} **P:** Tshz3^{GC/+} positive control,
W: Wildtype control, **N:** Negative control.

Native Fluorescence

15.5dpc UGSs were examined with a fluorescent microscope to view GFP expression. GFP can be seen in whole mount in the developing kidney and in the male and female reproductive track (Fig 2).

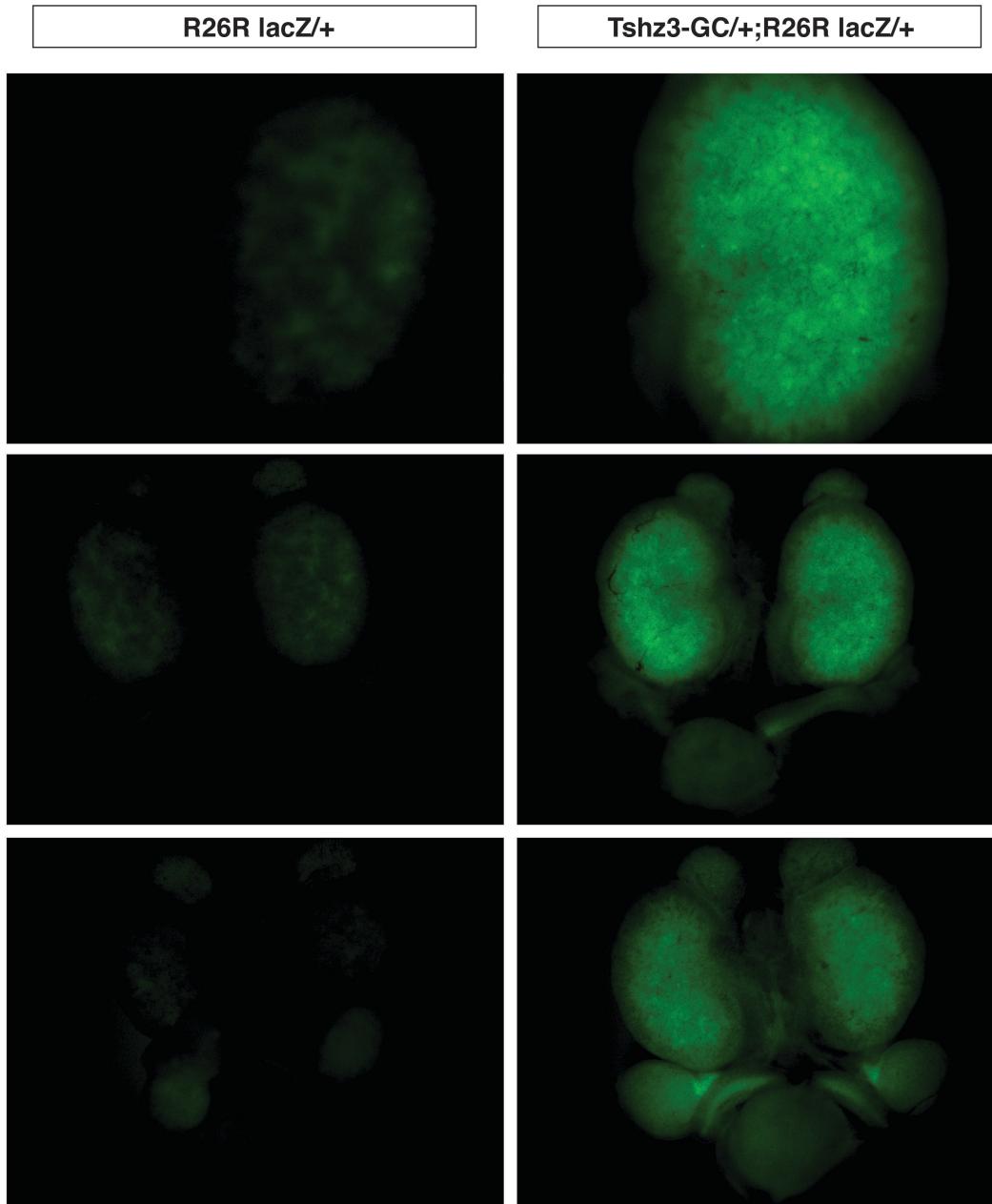


Fig 2. Wholemount GFP detection in 15.5dpc $Tshz3^{GC/+}$; $R26R^{lacZ/+}$ UGS.

GFP fluorescence was visible in developing 15.5dpc kidneys and in the male and female reproductive system.

Cre-recombinase Activity

We crossed $\text{Tshz3}^{\text{GC}/+}$ founder males with $\text{Rosa26R}^{\text{lacZ}/+}$ (R26R) female mice to obtain $\text{Tshz3}^{\text{GC}/+}; \text{R26R}^{\text{lacZ}/+}$ embryos. Dissected 15.5dpc UGS samples were stained with X-gal to assay for β -gal activity. Cre activity was detected in $\text{Tshz3}^{\text{GC}/+}; \text{R26R}^{\text{lacZ}/+}$ samples but not in R26R $^{\text{lacZ}/+}$ controls in the kidney and other structures within the UGS (Fig 3 & 4).

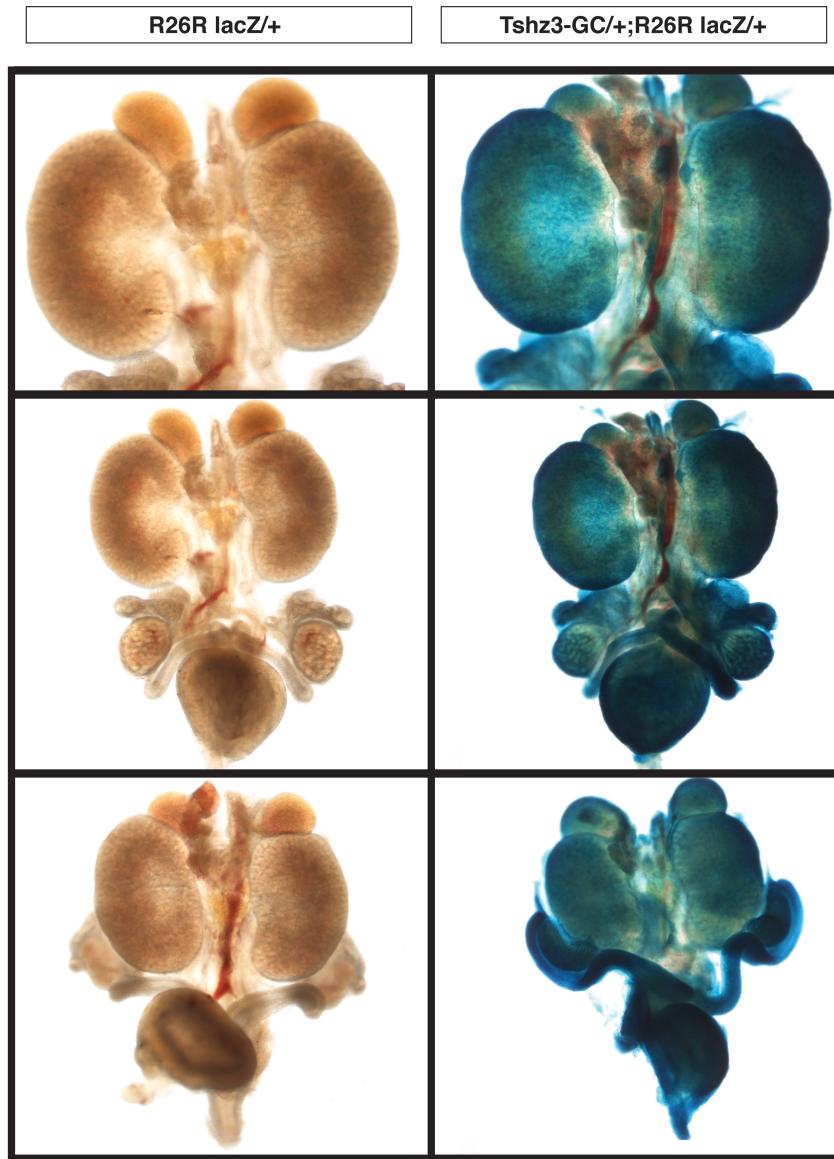


Fig 3. Cre-dependent β -gal activity in $\text{Tshz3}^{\text{GC}/+}; \text{R26R}^{\text{lacZ}/+}$ UGSs at 15.5 dpc. X-gal staining of $\text{Tshz3}^{\text{GC}/+}; \text{R26R}^{\text{lacZ}/+}$ embryos shows widespread β -gal activity in the UGS at 15.5 dpc

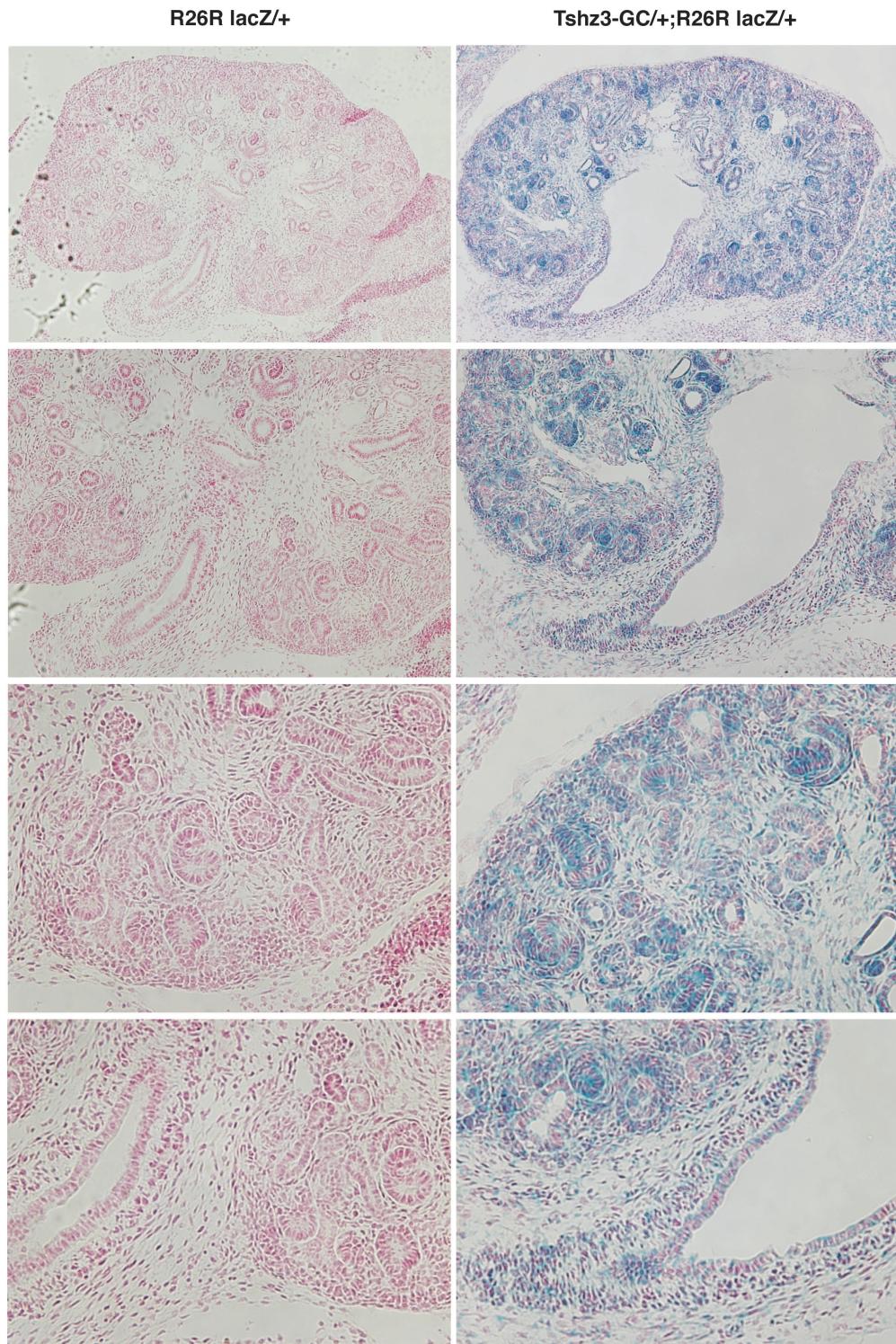


Fig 4. Cre-dependent β -gal activity in $Tshz3^{GC/+}$; $R26R^{lacZ/+}$ UGS at 15.5 dpc.

β -gal activity is apparent in $Tshz3^{GC/+}$; $R26R^{lacZ/+}$ kidney and ureteric epithelium as well as mesenchymal derivatives in other structures within the UGS.

Immunohistochemistry

Immunohistochemistry was performed to examine whether the eGFP^{Cre} allele was expressed in the expected Tshz3 domain. β-gal and GFP expression was examined in Tshz3^{GC/+}; R26R^{lacZ/+} and R26R^{lacZ/+} 15.5 dpc embryos. Whole UGSs were fixed in 4% paraformaldehyde at 4°C 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 16um and probed with:

Chicken anti-GFP /Rabbit anti bgal / mouse anti SMA IgG2a

Chicken anti-GFP /Rabbit anti Tshz3 / mouse anti SMA IgG2a

Chicken anti-GFP /Rabbit anti bgal / guinea pig anti Tshz3

Rabbit and guinea pig anti Tshz3 antibodies were generously provided by Dr Alistair N. Garratt, Max-Delbrück-Center for Molecular Medicine, Germany.

Primary Antibody	Company	Catalog #	Dilution	Secondary	Company	Dilution
Chicken-anti-GFP	Aves Labs, Inc	GFP-1020	1/500	Goat-anti-chicken-A488	Invitrogen	1/500
Rabbit-anti-b-gal	MP Biomedicals , LLC	55976	1/20,000	Donkey-anti-rabbit-A555	Invitrogen	1/500
Mouse anti-Actin, a-Smooth muuscle IgG2a	Sigma	A5228	1/500	Goat-anti-mouse IgG2a-A633	Invitrogen	1/250
Rabbit-anti-Tshz3	Dr Alistair N. Garratt	MDC-Germany	1/5000	Donkey-anti-rabbit-A555	Invitrogen	1/500
Guinea-anti-Tshz3	Dr Alistair N. Garratt	MDC-Germany	1/5000	Goat-anti-guinea-A488	Invitrogen	1/500

Table 2. Summary of antibodies used to screen Tshz3^{GC/+}; R26R^{lacZ/+} and R26R^{lacZ/+} 15.5 dpc embryo sections.

eGFP^{Cre} expression co-localizes with Tshz3 in smooth muscle actin positive cell types and associated mesenchyme surrounding the ureteric epithelium (Figs 5 & 6). However, we do not observe expression of the eGFP^{Cre} transgene in all Tshz3 positive cells. In addition, eGFP^{Cre} expression overlaps with Tshz3 positive cells in non-urogenital regions. β-gal expressing cells co-localize with a subset of GFP positive cells in the kidney and ureters in Tshz3^{GC/+}; R26R^{lacZ/+} 15.5dpc UGS, but not in R26R^{lacZ/+} (Fig. 7)

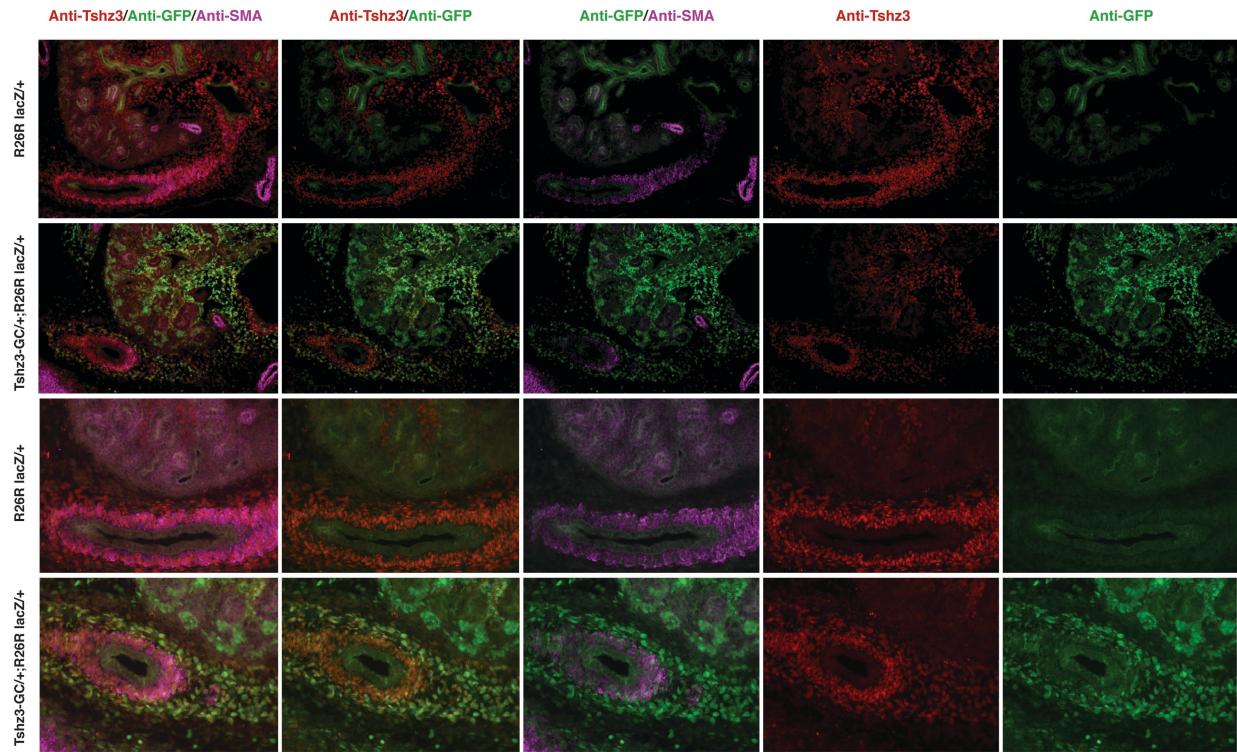


Fig 5. Tshz3 and GFP positive cells co-localize with Smooth muscle actin positive cells in ureteric epithelium in $Tshz3^{GC/+}$; $R26R^{lacZ/+}$ 15.5dpc UGS. $Tshz3^{GC/+}$; $R26R^{lacZ/+}$ and $R26R^{lacZ/+}$ kidneys probed with anti-Tshz3, anti-GFP and anti-Smooth muscle actin (SMA) antibodies confirm co-localization of GFP with Tshz3 positive cells, and cells expressing SMA. However, we do not observe expression of the eGFP Cre transgene in all Tshz3 positive cell types and some localized ectopic activity of the transgene is observed in urogenital structures.

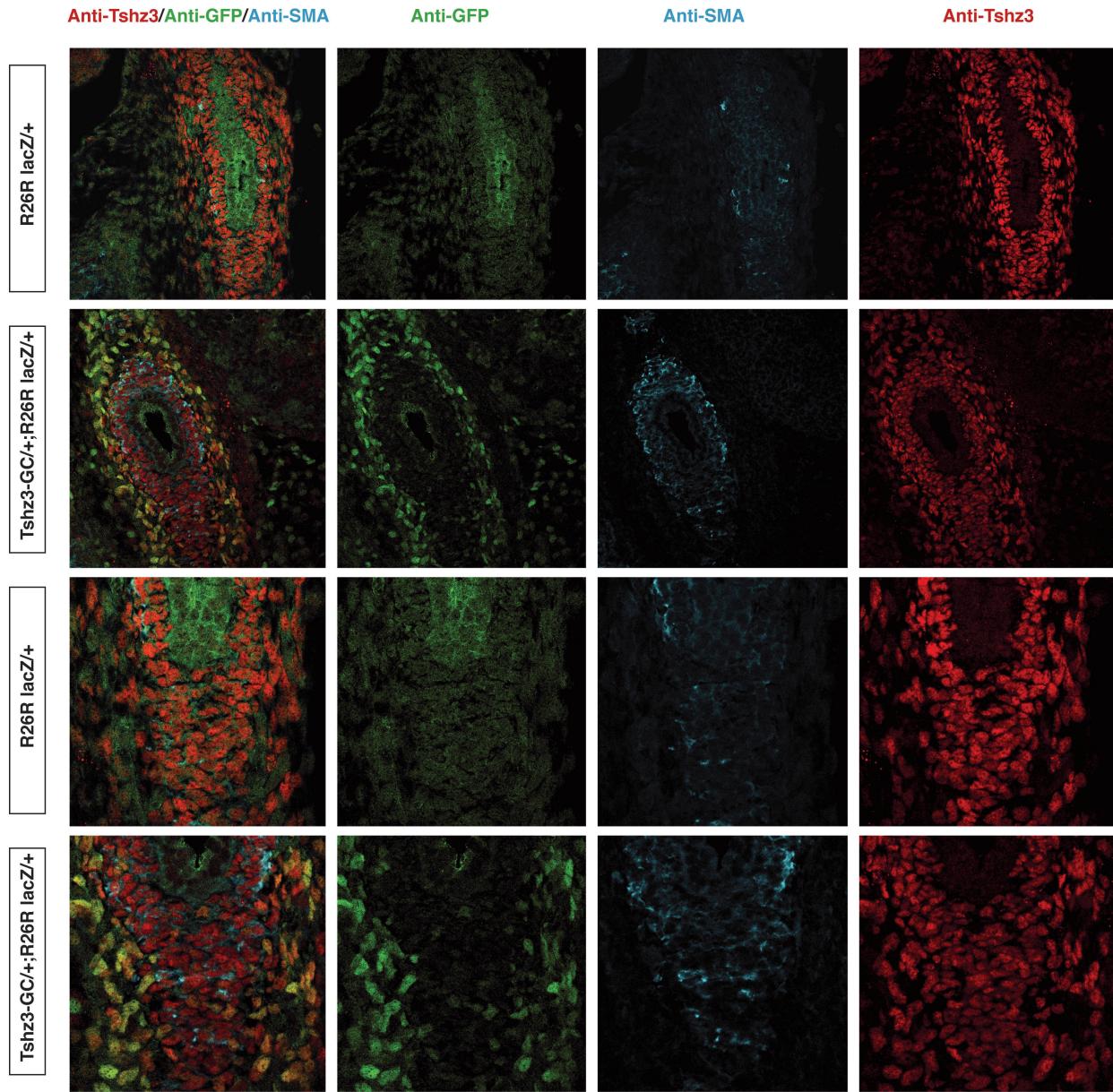


Fig 6. Tshz3 and GFP positive cells co-localize with Smooth muscle actin expressing cells in the ureter of Tshz3^{GC/+}; R26R^{lacZ/+} 15.5dpc UGS. Co-localization of GFP and a subset of Tshz3 positive cells were observed to overlap with SMA expressing cells in the ureters of Tshz3^{GC/+}; R26R^{lacZ/+} embryos. Additional non-overlapping GFP and Tshz3 positive cells were observed in adjacent mesenchymal derivatives of the ureteric epithelium.

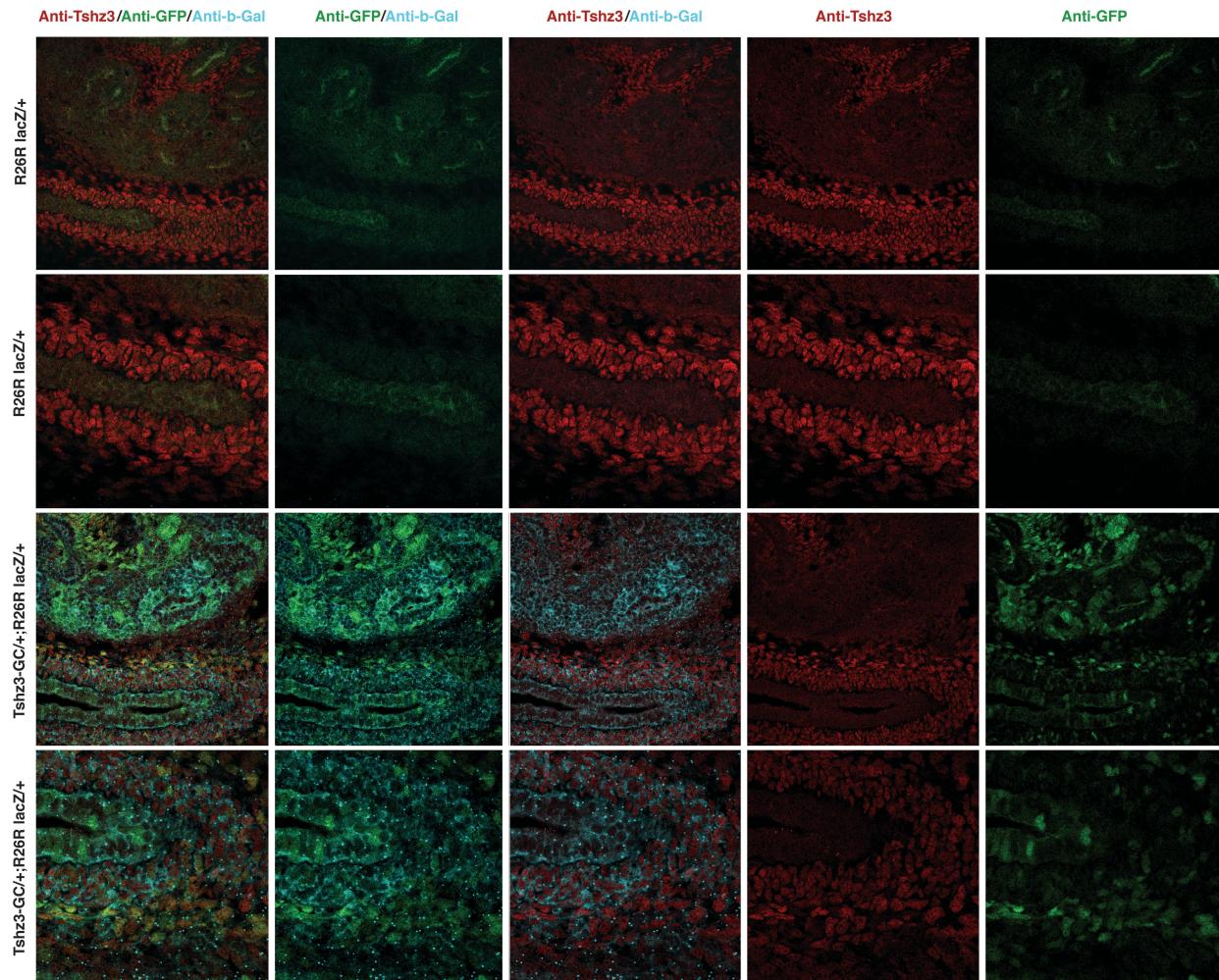


Fig 7. β -gal and GFP positive cells co-localize with Tshz3 cells in $Tshz3^{GC/+}$; $R26R^{lacZ/+}$ UGS at 15.5dpc. $Tshz3^{GC/+};R26R^{lacZ/+}$ and $R26R^{lacZ/+}$ UGS were probed with anti- β -gal, anti-GFP and anti-Tshz3 antibodies. Co-localization of GFP and β -gal positive cells was detected in the kidney and ureter, a percentage of the cells also expressed Tshz3.