Wnt11-myrTagRFP-IRES-CE Allele Characterization

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Findings: VALIDATED

Our analysis confirms the expression of myrTagRFP-IRES-CE under the regulation of Wnt11 in the ureteric tips and collecting duct system at 15.5dpc. Native TagRFP expression is detected in wholemount in the UGS and lung. Upon induction with tamoxifen Cre dependant R26R LacZ expression is observed in the ureteric buds and tree, the developing collecting ducts, male and female reproductive system and the lung. Expression of the transgene in tissue other then the kidney is not covered in this report.

Crosses

The Wnt11-myrTagRFP-IRES-CE strain is a BAC transgenic line with myrTagRFP-IRES-CE expressed in the Wnt11 domain: wingless-type MMTV integration site family, member 11. The Wnt family of genes, encode secreted signaling proteins involved in the regulation of cell fate and patterning during embryogenesis.
Pronuclear injection of the BAC construct DNA into C57Bl6/DBA F1 embryos resulted in the birth of 45 pups of which 10 males and 7 females carried the transgene. The male founders were crossed to Rosa26R$^{lacZ/+}$ (R26R) mice and the urogenital system (UGS) and lungs were collected from 15.5 dpc embryos. Of the six founders tested, six transmitted the transgene and all correctly express the myr-TagRFP in the expected cell population (Table 1). Further analysis was carried out on the M28 line.

Date of Birth	Pups born	Founders	Founders mated	Transmittal	Visible Reporter	Correct Reporter Activity	Antibody to Reporter
10-July-11	45	10M, 7F	M1	Yes	Yes	Yes	nd
			M2	Yes	Yes	Yes	Yes
			M5	Yes	Yes	Yes	nd
			M12	Yes	Yes	Yes	nd
			M16	Yes	Yes	Yes	Yes
			M28	Yes	Yes	Yes	Yes

Table 1. Transmission analysis of founders

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 targeted/transgenic allele Size: 375bp
DNA sequence (forward): 5'-CGACCCCTCCTTTGTAATTTG-3'
DNA sequence (reverse 2) 5'-CCTCGACCACCTTGATTCTC-3'
Amplifies 5' arm into myrTagRFP sequence within RFP-Cre region.

Rxn Buffer and Conditions: (25μl reaction)

10X GSB	**2.5ul**
25mM dNTP	**1ul**
10uM primer F	**1ul**
10uM primer R1	**1ul**
DMSO	**2.5ul**
2-mercaptoethanol	**0.125ul**
Amplify Taq	**0.2ul (5u/ul)**
5x cresol red dye	**5ul**
Genomic DNA	**1ul**
Total volume	**25 ul**

94ºC	3min	
94ºC	30sec	
54ºC	30sec	35cycles
72ºC	45sec	
72ºC	10min	

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

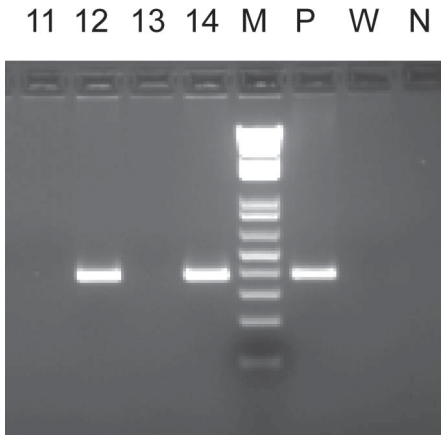

Fig 1: Number 12 & 14: Wnt11$^{myrTagRFP/+}$,Rosa26R$^{lacZ/+}$, numbers 11 & 13: Rosa26R$^{lacZ/+}$, **P:** Wnt11$^{myrTagRFP/+}$ Positive control, **W**: Wildtype control, **N**: Negative control.

Native Fluorescence

Whole embryos as well as dissected UGSs were examined with a fluorescent microscope to view myrTagRFP expression. TagRFP is seen in wholemount in the ureteric bud with stronger expression in the ureteric tips. The fluorescent reporter is also expressed in the developing mesonephros of male and female reproductive systems (Fig 2).

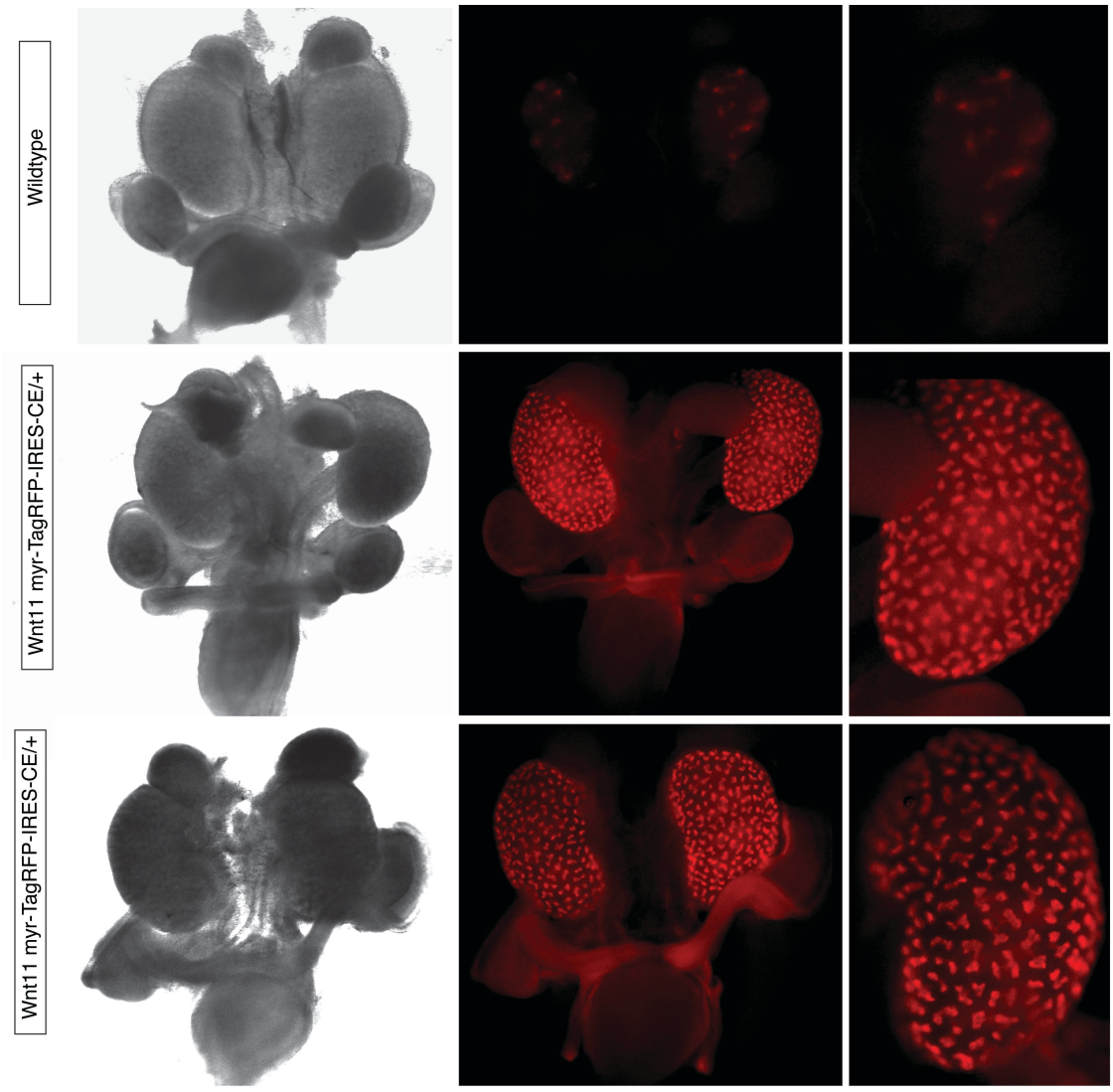

Fig 2: Wholemount myr-TagRFP detection in 15.5 dpc Wnt11$^{myr\text{-}TagRFP/+}$ UGS. TagRFP fluorescence is visible in the ureteric tips and tree in the nephrogenic zone in 15.5 dpc kidneys and in the male and female reproductive systems. TagRFP expression is limited to the Wnt 11 domain.

Cre-recombinase Activity

Wnt11$^{myr\text{-}TagRFP/+}$ male founders were mated to R26R$^{lacZ/+}$ females to generate Wnt11$^{myrTagRFP/+}$;R26R$^{lacZ/+}$ embryos. In order to activate ß-galactosidase (β-gal) reporter expression from the R26R$^{lacZ/+}$allele, two intraperitoneal injections of tamoxifen in corn oil (2mg to 40g body weight) were injected into pregnant 11.5 and 13.5dpc mice. A control group was injected with the same volume of corn oil. UGS samples were dissected at 15.5dpc and stained with X-gal to assay for β-gal activity. Tamoxifen dependent Cre activity is detected in the ureteric tree and tips and in the developing collecting duct as well as the male and female reproductive systems in Wnt11$^{myrTagRFP/+}$;R26R$^{lacZ/+}$ samples (Fig 3 and 4).

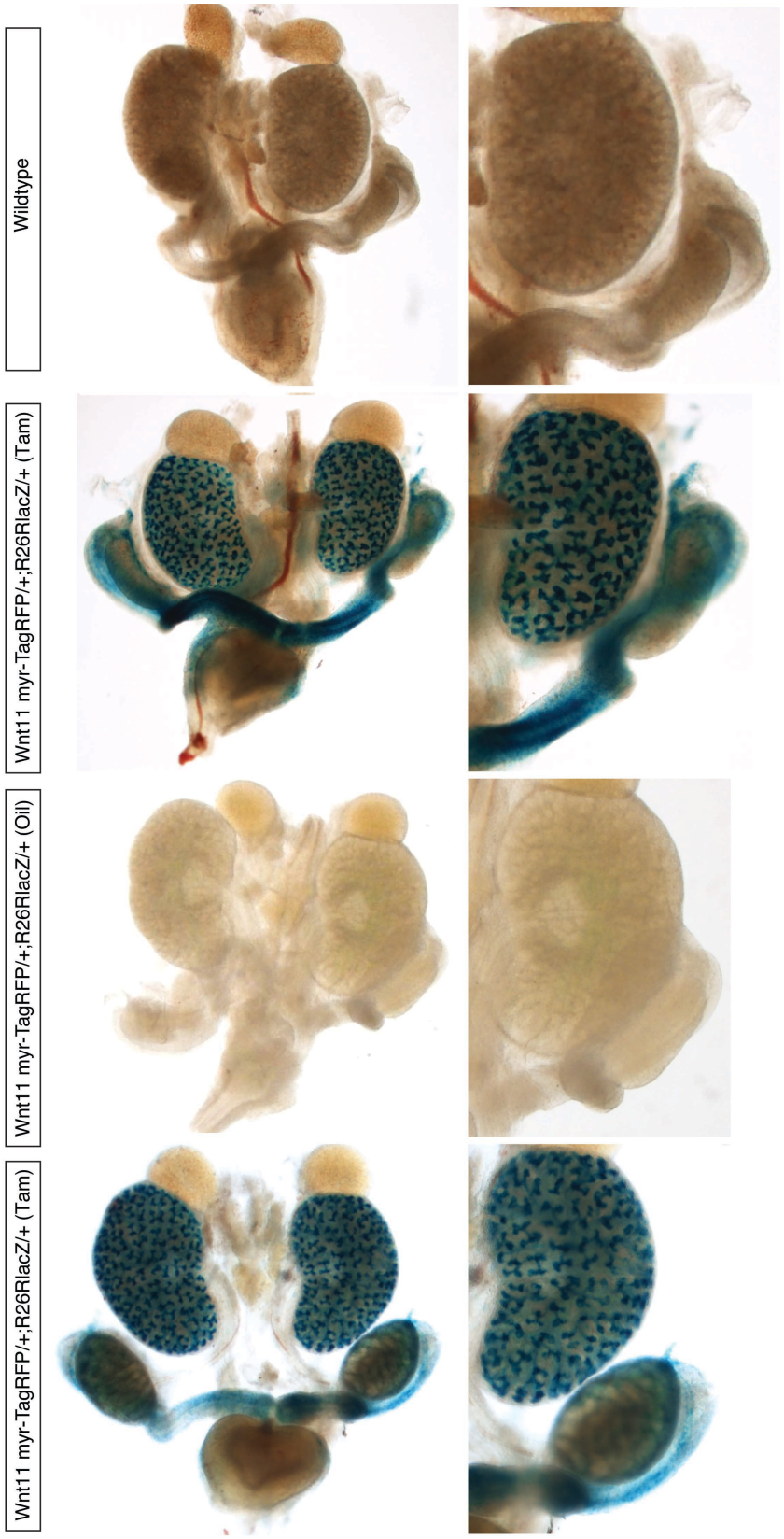

Fig 3. Cre-dependent β-gal activity in Wnt11$^{myrTagRFP/+}$;R26R$^{lacZ/+}$ UGSs. Tamoxifen injected at 11.5 and 13.5 dpc resulted in β-gal activity in the ureteric tips and tree of the kidney and in the male and female mesonephros of Wnt11$^{myrTagRFP/+}$;R26R$^{lacZ/+}$ embryos.

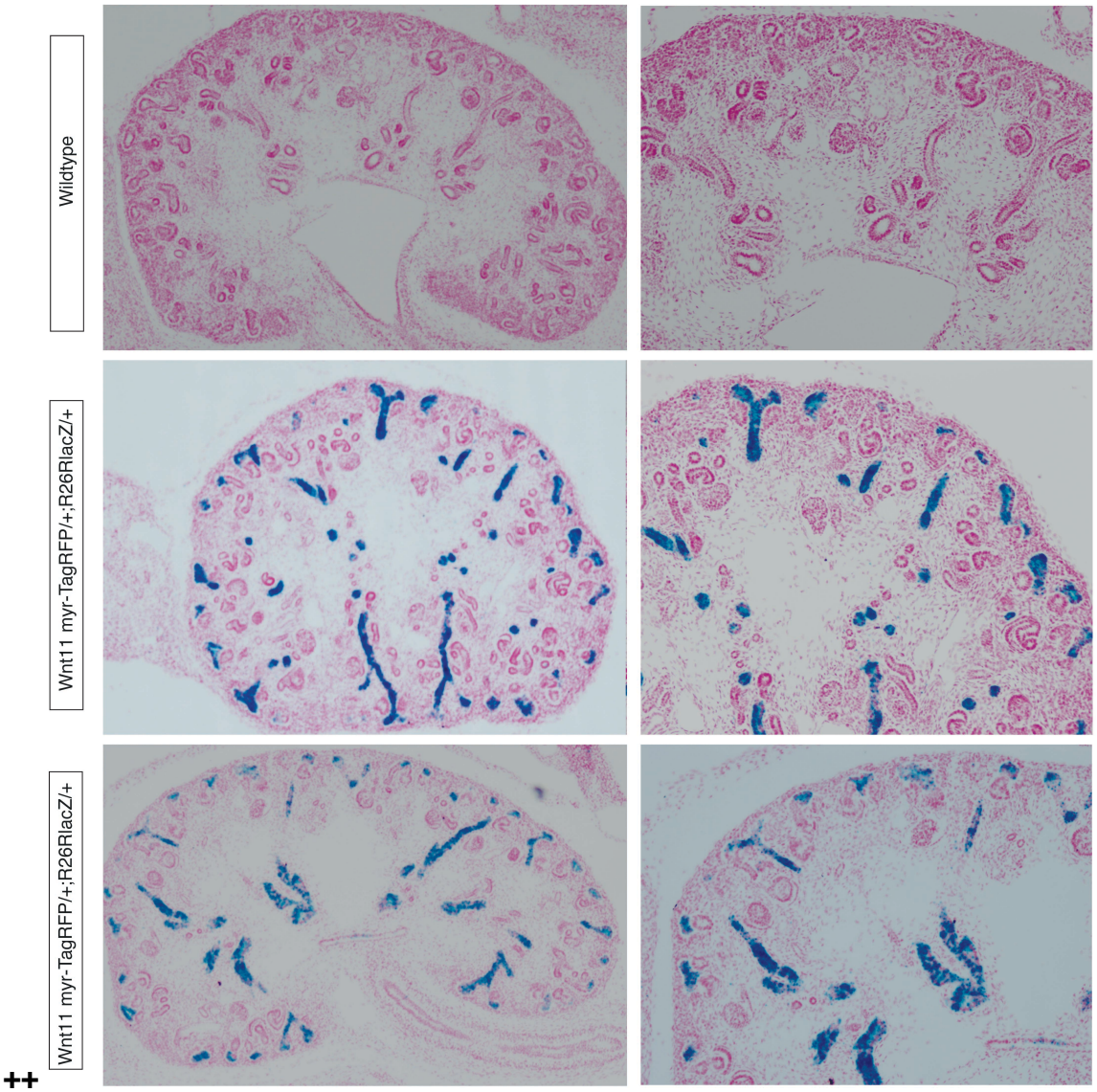

Fig 4. Cre-dependent β-gal activity in Wnt11$^{myrTagRFP/+}$;R26R$^{lacZ/+}$ UGSs.

β-gal activity is detected in ureteric buds and tips and in the developing collecting ducts in Wnt11$^{myrTagRFP/+}$;R26R$^{lacZ/+}$ 15.5 dpc kidneys upon induction with tamoxifen at 11.5 and 13.5 dpc.

Immunohistochemistry

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 16um and probed with the antibodies listed in (Table 2): Rabbit anti-TagRFPT (Goat anti rabbit A405 or Donkey anti-rabbit A555) /Mouse anti-β-gal IgG2a / Mouse anti-Cytokeratin IgG1, Rabbit anti-TagRFPT/ Mouse-anti-Cytokeratin IgG1, Rabbit anti-β-gal/ Mouse-anti-Cytokeratin IgG1.

Primary Antibody	Company	Catalog #	Dilution	Secondary	Company	Dilution
Rabbit-anti-TagRFPT	Evrogen	AB234	1/500	Donkey-anti-rabbit-A555	Invitrogen	1/500
Mouse anti-β-gal IgG2a	Promega	Z3781	1/1000	Goat-anti-mouse IgG2a-A488	Invitrogen	1/500
Rabbit-anti-β-gal	MP Biomedicals, LLC	55976	1/20000	Donkey-anti-rabbit-A488	Invitrogen	1/500
Mouse-anti-Cytokeratin IgG1	Sigma	C2562	1/500	Goat-anti-mouse IgG1-A633	Invitrogen	1/500
Rabbit-anti-TagRFPT	Evrogen	AB234	1/500	Goat-anti-rabbit-A405	Invitrogen	1/500

Table 2. Summary of antibodies used to screen Wnt11$^{myrTagRFP/+}$;R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$ 15.5 dpc embryo sections.

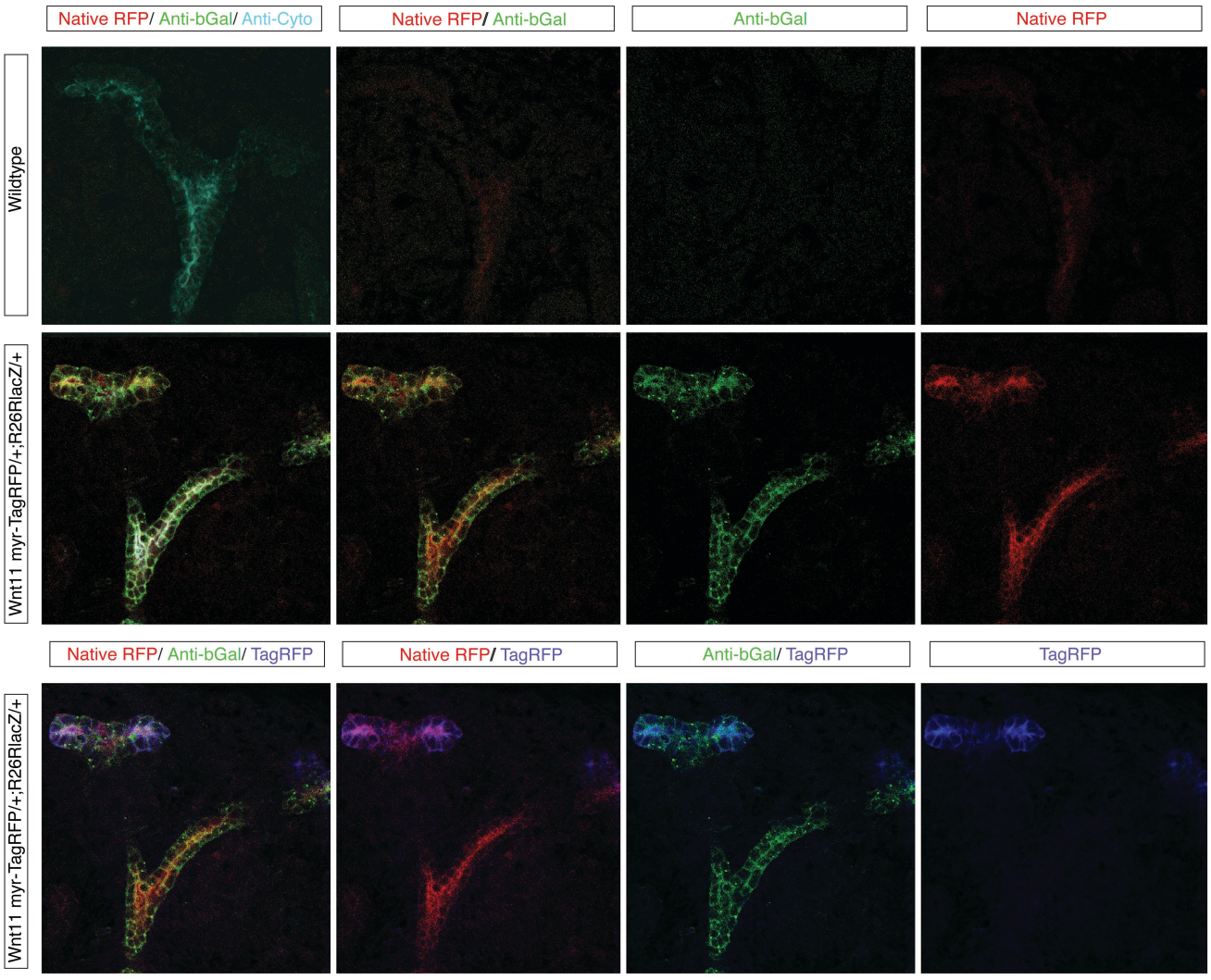

Fig 4. Comparison of native myrTagRFP with cells positive for anti TagRFP and ß-gal in Wnt11$^{myrTagRFP-T/+}$;R26R$^{lacZ/+}$ tamoxifen injected kidneys.
Wnt11$^{myrTagRFP/+}$;R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$ kidneys were probed with anti-ß-gal, anti-TagRFP and anti-Cytokeratin antibodies. Co-localization of ß-gal and native myrTagRFP positive cells are found in the ureteric tips and tree and in the developing collecting ducts. Cells positive for the TagRFP antibodies co-localize with the ß-gal positive cells in the ureteric tips.

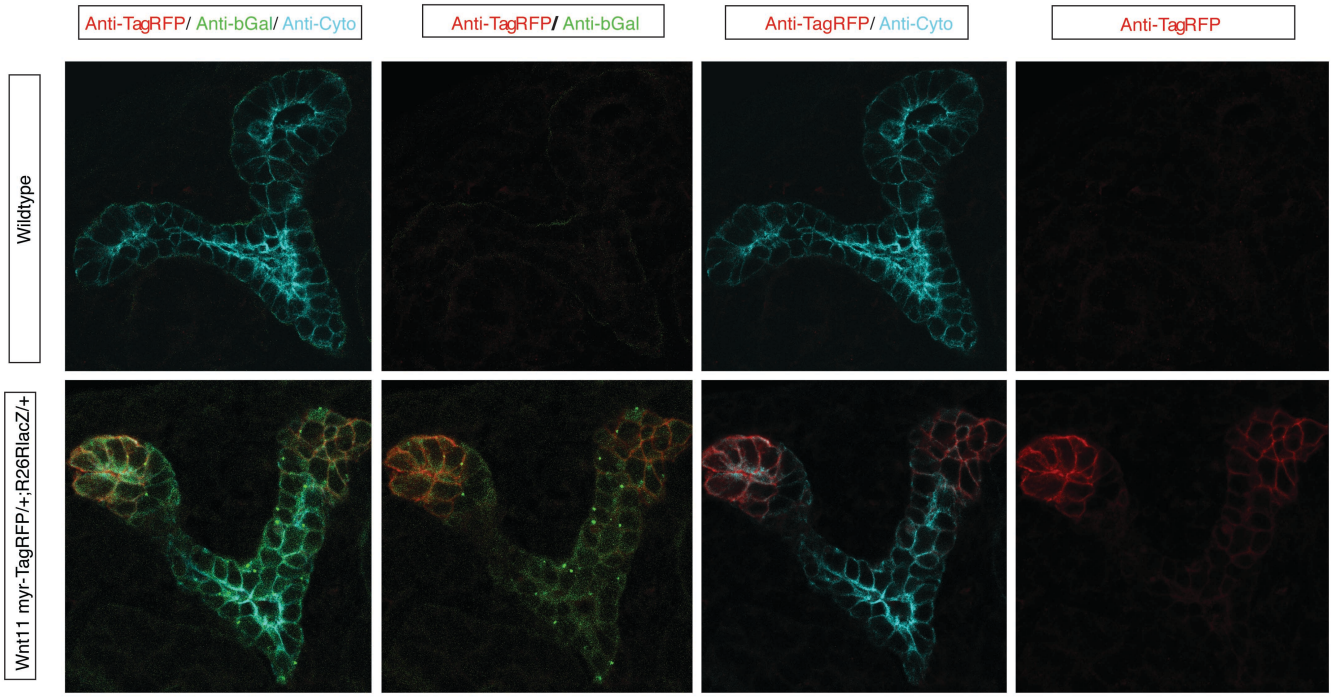

Fig 4. ß-galactosidase positive cells detected in Wnt11$^{myrTagRFP-T/+}$;R26R$^{lacZ/+}$ tamoxifen injected kidneys. Wnt11$^{myrTagRFP-T/+}$;R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$ kidneys were probed with anti-ß-gal, anti-TagRFP and anti-Cytokeratin antibodies. Co-localization of TagRFP and tamoxifen-dependent ß-gal expression is observed in the ureteric tips of the branching ureteric bud. ß-gal positive cells derived from the ureteric tips are detected in the ureteric tree and developing collecting ducts but are not positive for TagRFP.