Lfng-nuc-Tag-RFP-T-IRES-CE Allele Characterization

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Findings: VALIDATED

Our analysis confirms the expression of nuc-TagRFP-T under the regulation of Lfng in renal vesicles and early tubules at 15.5dpc. Native RFP-T expression was not detectable in wholemount but could be visualized by immunohistochemistry. Upon induction with tamoxifen Cre dependant R26R LacZ expression was observed in a subset of Lhx-1 and Jag 1 positive cells in developing renal vesicles transitioning to comma- and S-shaped bodies.

Data:

Crosses

The Lfng-nuc-TagRFP-T-IRES-CE strain is a BAC transgenic line with nuc-TagRFP-T expressed in the LFNG domain: Lfng O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase. Lfng is a member of the fringe gene family, which also includes manic and radical fringe. Members of the fringe family are thought to act in the Notch signaling pathway to define boundaries during embryonic development. Pronuclear injection of the BAC construct DNA into C57Bl6/DBA F1 embryos resulted in the birth of 29 pups of which 3 male and 2 females carried the transgene. The male and female founders were crossed to Rosa26R$^{lacZ/+}$ (R26R) mice and the urogenital system (UGS) was collected from 15.5 dpc embryos. Of the five founders tested, three transmitted the transgene and three correctly expressed the nuc-TagRFP-T in the expected cell population: M8, M25 and F3 (Table 1). Further analysis was carried out on the M8 line.

Date of Birth	Pups Born	Founders	Founders Mated	Transmittal	Visible Reporter	Correct Reporter Activity	Antibody to Reporter
16 Mar 2011	29	3M, 2F	M8	Yes	No	Yes	Yes
			M25	Yes	No	Yes	Yes
			F3	Yes	No	Yes	Yes
			F29	No	na	na	na
			M16	Infertile	na	na	na

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: 311 bp
DNA sequence (Forward): 5'-CCAAGGCTAGCAGCCAATTA-3'
DNA sequence (Reverse) 5'-GTGCCCTCCATGTACAGCTT-3'
Amplifies 5' arm into RFP sequence.

Rxn Buffer and Conditions: (25μl reaction)

10X PCR Buffer	2.5ul			
1.25mM dNTP	4ul	94ºC	3min	1 cycle
10uM primer F	1ul	94ºC	30sec	
10uM primer R	1ul	62ºC	30sec	35cycles
5x cresol red dye	5ul	72ºC	45sec	
Amplify Taq	0.2ul (5u/ul)	72ºC	10min	1 cycle
Genomic DNA	1ul			
Total volume	25 ul			

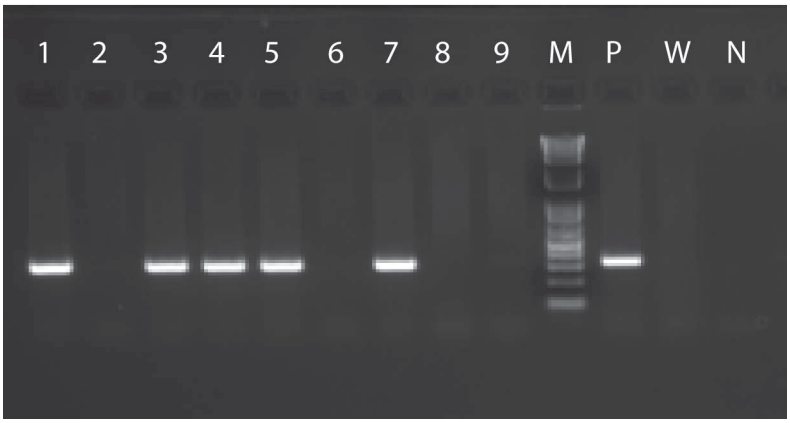

Fig 1: Number 1, 3-5, and 7: Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$,Rosa26R$^{lacZ/+}$, numbers 2, 6, 8 and 9: Rosa26R$^{lacZ/+}$, **P:** Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$ Positive control, **W**: Wildtype control, **N**: Negative control.

Native Fluorescence

Whole embryos as well as dissected UGSs were examined with a fluorescent microscope to view RFP-T expression. However, RFP-T was not detectable under these conditions.

Cre-recombinase Activity

Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$ male founders were mated to R26R$^{lacZ/+}$ females to generate Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ embryos. In order to activate ß-galactosidase (β-gal) reporter expression from the R26R$^{lacZ/+}$allele, an intraperitoneal injection of tamoxifen in corn oil (2X 2mg to 40g body weight) was injected into pregnant mice at 11.5 and 13.5dpc. 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 was detected in S-shaped bodies and renal vesicles derivatives in Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ samples (Fig. 2 and 3).

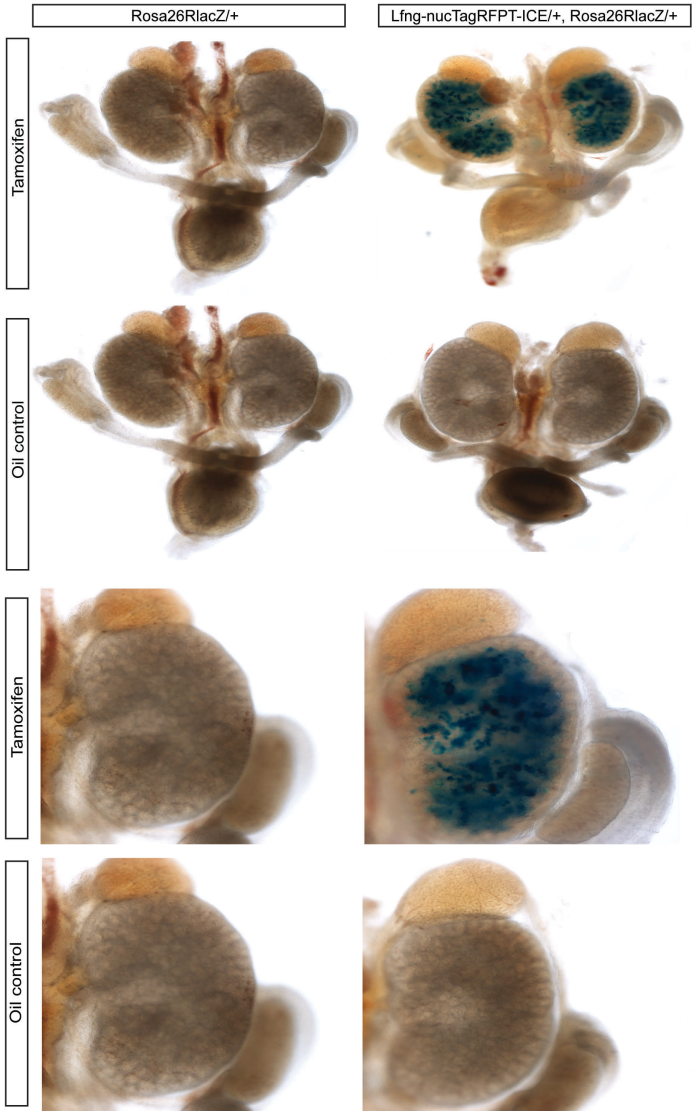

Fig 2. Cre-dependent β-gal activity in Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ UGSs. Tamoxifen injected at 11.5 and 13.5dpc resulted in ß-gal activity in the nephrogenic zone of Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ but not in R26R$^{lacZ/+}$ 15.5dpc UGS.

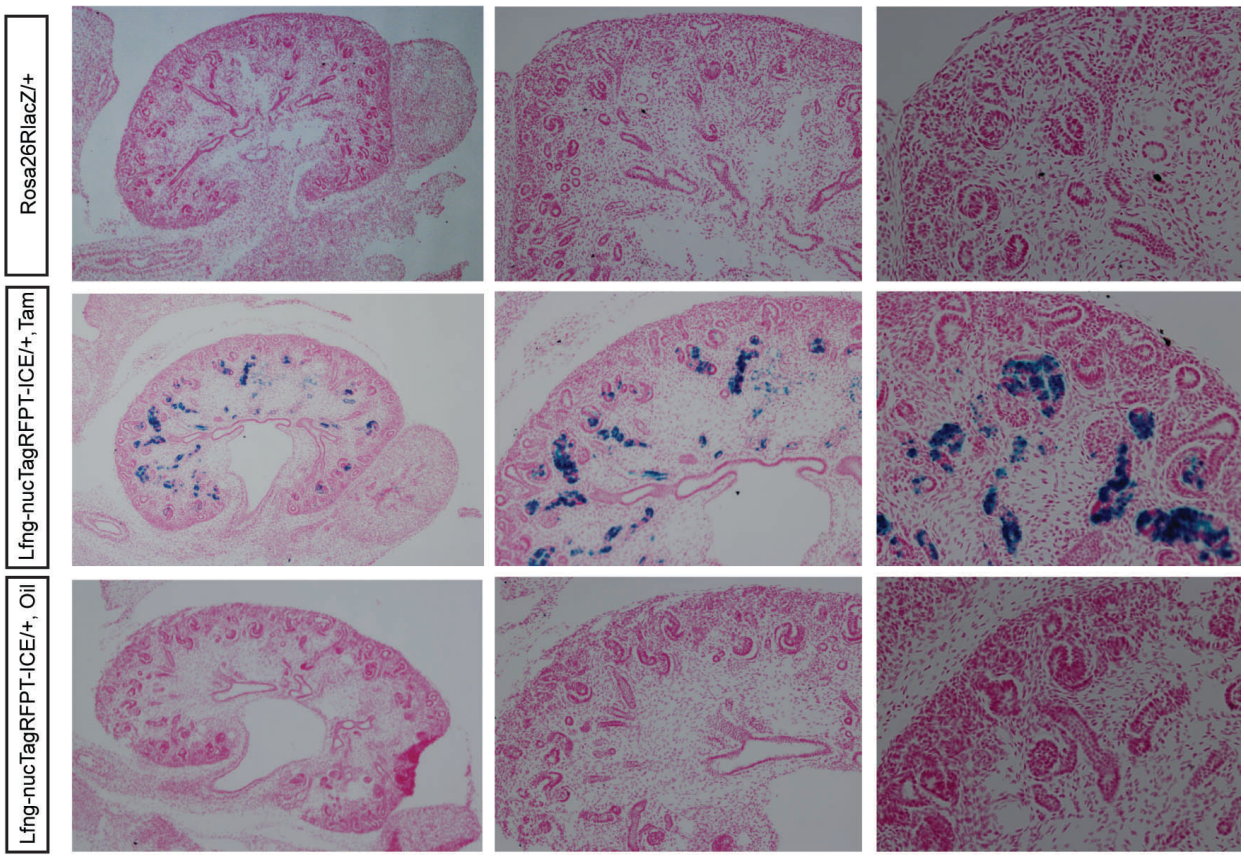

Fig 3. Cre-dependent β-gal activity in Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ UGSs. β-gal activity was detected in maturing renal vesicles-S shaped bodies and in early tubules in Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ 15.5 dpc kidneys upon induction with tamoxifen.

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): goat-anti-β-gal/rabbit-anti-TagRFPT/rat-anti-E-Cadherin, goat-anti-β-gal/rabbit-anti-TagRFPT /mouse-Lhx 1 IgG1 and goat-anti-β-gal/rabbit-anti-TagRFPT /rat-anti-Jagged1 IgG2a.

Primary Antibody	Company	Catalog #	Dilution	Secondary	Company	Dilution
Rabbit-anti-TagRFPT	Evrogen	AB234	1/500	Donkey-anti-rabbit-A555	Invitrogen	1/500
Goat anti β-gal	Biogenesis	4600-1409	1/500	Donkey-anti-goat-A488	Invitrogen	1/500
Rat anti Jagged1 IgG2a	DSHB	TS1.15H	1/20,000	Donkey-anti-rat-A488	Invitrogen	1/500
Mouse anti Lhx1 IgG1	DSHB	4F2	1/50	Goat-anti-mouse IgG1-A488	Invitrogen	1/500
Rat anti E-cadherin	Sigma	U3254	1/1000	Chicken-anti-rat-A647	Invitrogen	1/500

Table 2. Summary of antibodies used to screen Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$ 15.5 dpc embryo sections.

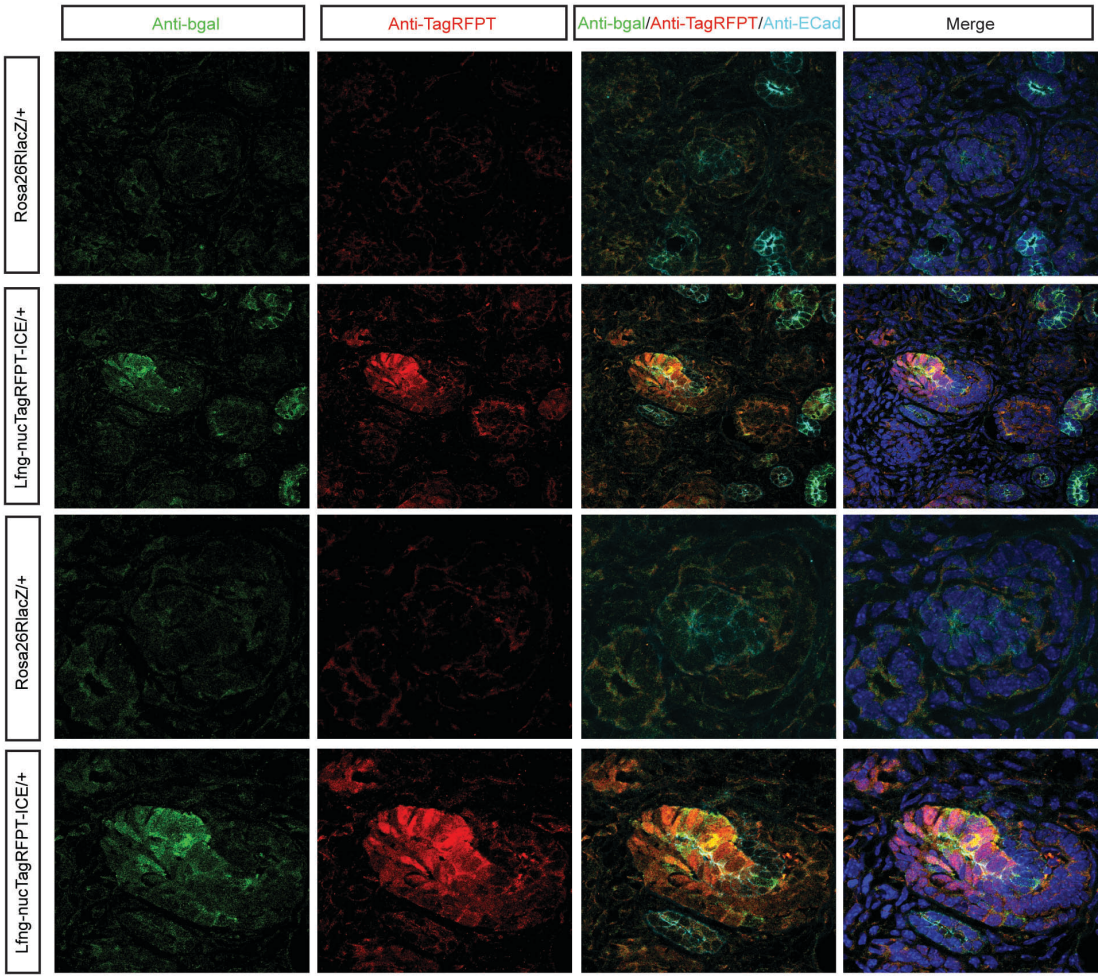

Fig 4. ß-galactosidase detected in the renal vesicles in Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ tamoxifen injected kidneys. Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$ kidneys were probed with anti-ß-gal, anti-TagRFPT and anti-E Cadherin antibodies. Co-localization of TagRFPT and tamoxifen-dependent ß-gal expression is detected in renal vesicles during transition to the S-shaped body.

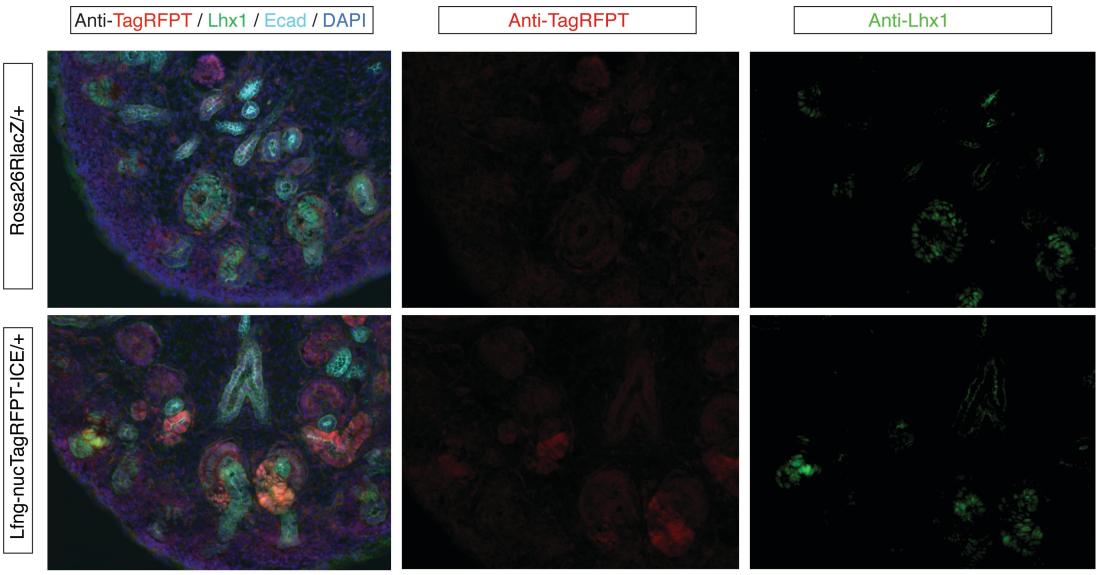

Fig 5. Lhx1 and TagRFPT positive cells in transitioning renal vesicles in Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ 15.5dpc kidneys. Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$kidneys were probed with anti-Homeobox protein Lim1 (Lhx1) and anti-TagRFPT antibodies. Lim1 is expressed in the pretubular aggregate, comma- and S-shaped bodies and in immature glomeruli.

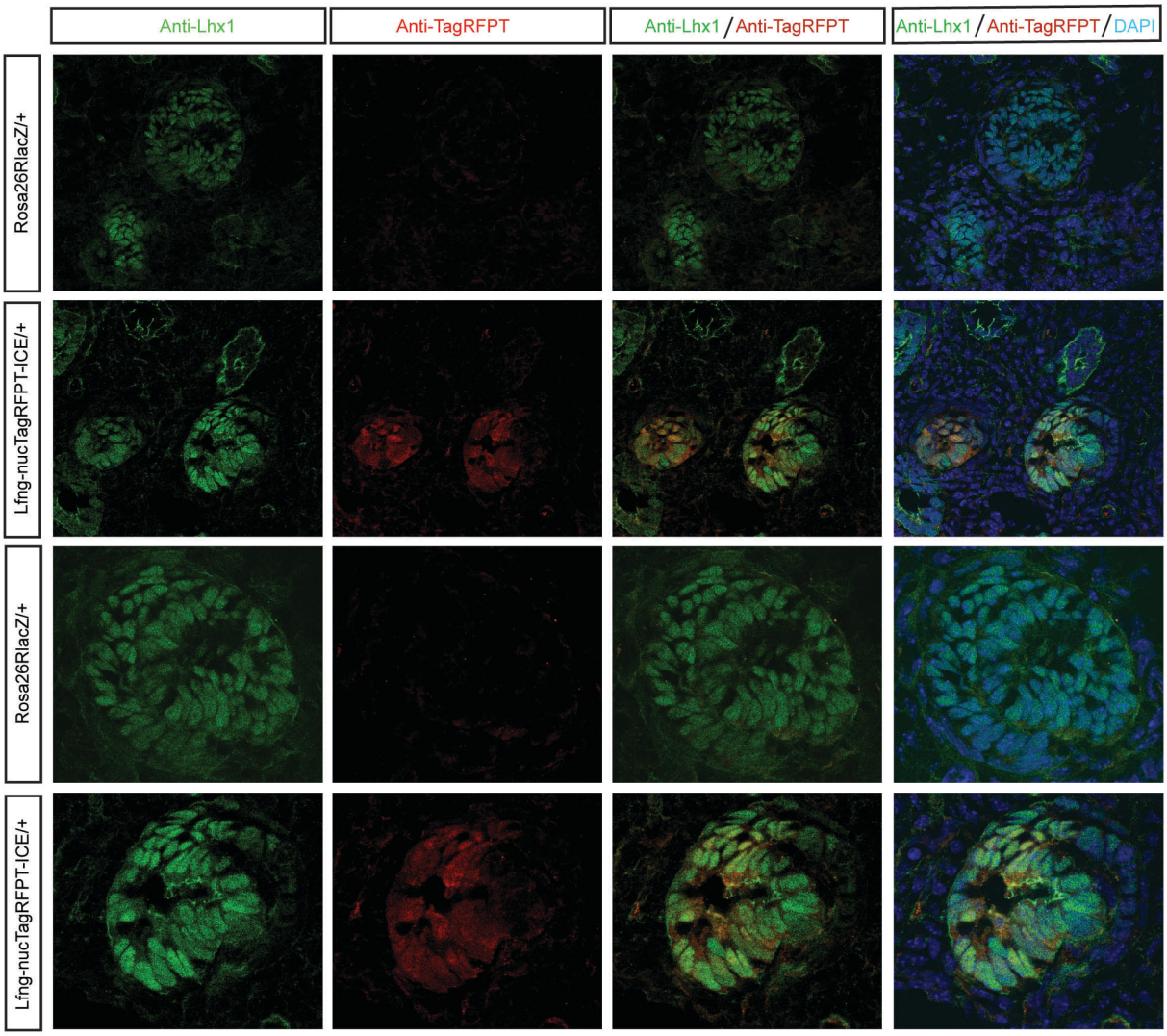

Fig 6. Lhx1 and TagRFPT positive cells in transitioning renal vesicles in Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ 15.5dpc kidneys. Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$kidneys were probed with anti-Homeobox protein Lim1 (Lhx1) and anti-TagRFPT antibodies. TagRFPT cells co-localize with a percentage of Lhx1 positive cells in transitioning renal vesicles.

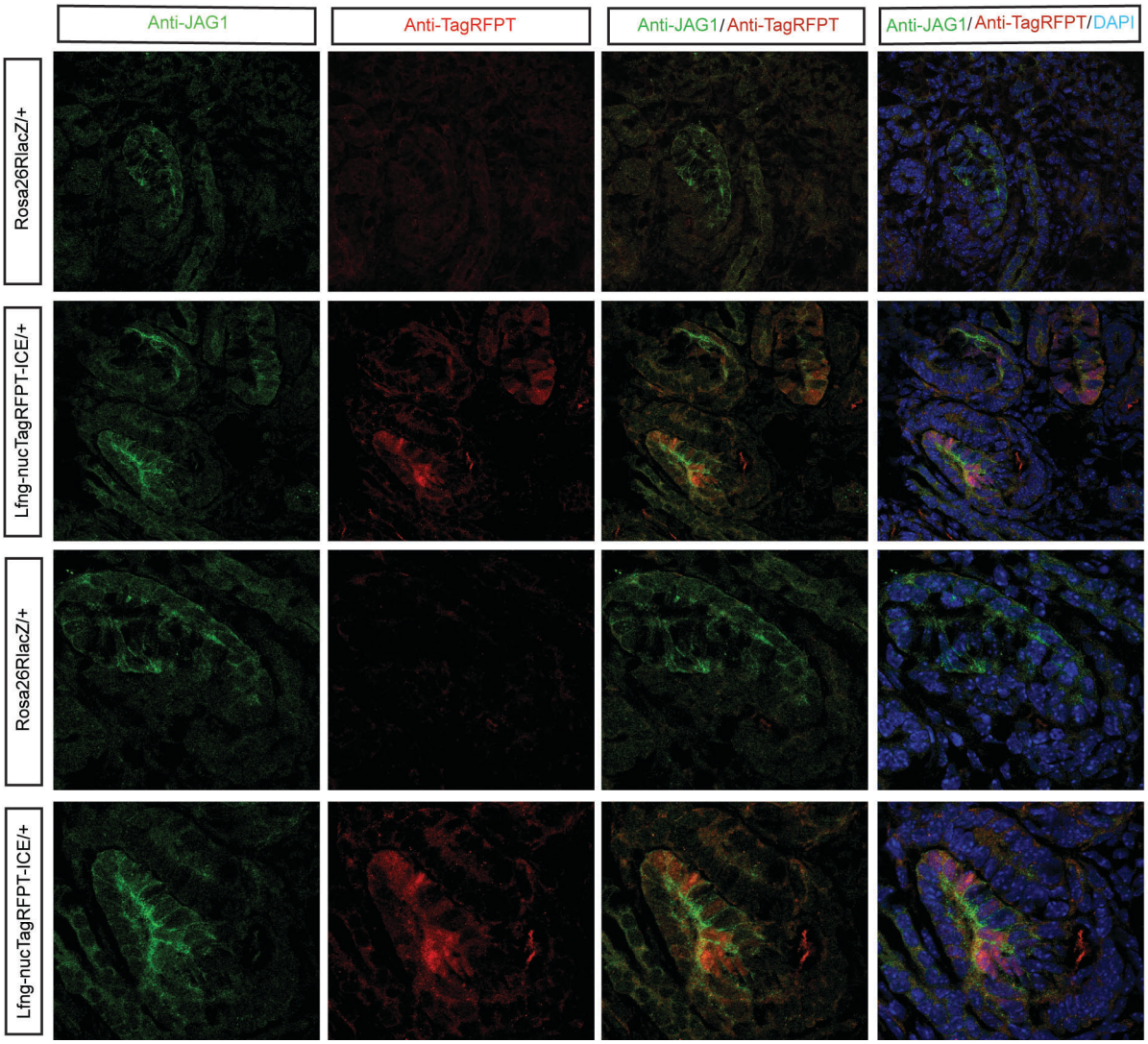

Fig 6. Jag1 and TagRFPT positive cells in transitioning renal vesicles in Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ 15.5dpc kidneys. Lfng$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$kidneys were probed with anti-Jag1 (Notch ligand) and anti-TagRFPT antibodies. Jag 1 is expressed in renal vesicles and derivatives including the middle segment of the S-shaped bodies a position thought to contain proximal tubule precursor cells. TagRFPT cells co-localize with a percentage of Jag1 positive cells in transitioning renal vesicles.