# Meox1-nuc-Tag-RFP-T-IRES-CE Allele Characterization

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## Findings: VALIDATED

Our analysis did not confirm activity of nuc-TagRFP-T under the regulation of Meox1. Native RFP-T expression was not detectable in wholemount and expression could not be visualized by immunohistochemistry. ß-galactosidase activity was detected upon tamoxifen induction: Cre dependant R26R LacZ expression was observed in Six2 positive cap mesenchyme progenitors and their nephron forming renal vesicle derivatives.

## Data:

### Crosses

The Meox1-nuc-TagRFP-T-IRES-CE strain is a BAC transgenic line with nuc-TagRFP-T expressed in the Meox1 domain: Mesenchyme homeobox 1.
Pronuclear injection of the BAC construct DNA into C57Bl6/DBA F1 embryos resulted in the birth of 39 pups of which 7 male and 6 females carried the transgene. The male founders were crossed to Rosa26R$^{lacZ/+}$ (R26R) mice and the urogenital system (UGS) was collected from 15.5 dpc embryos. Of the seven founders tested, six transmitted the transgene and six correctly expressed the nuc-TagRFP-T in the expected cell population: M12, M14, M17, M23, M30 and M32 (Table 1). Further analysis was carried out on the M30 line.

| Date of Birth | Pups Born | Founders | Founders Mated | Transmittal | Visible Reporter | Correct Reporter Activity | Antibody to Reporter |
|---|---|---|---|---|---|---|---|
| 8 Aug 2011 | 39 | 7M, 6F | M5 | No | na | na | na |
| | | | M12 | Yes | No | Yes | na |
| | | | M14 | Yes | No | Yes | na |
| | | | M17 | Yes | No | Yes | na |
| | | | M23 | Yes | No | Yes | na |
| | | | M30 | Yes | No | Yes | Yes (ß-gal) |
| | | | M32 | Yes | No | Yes | 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: 336 bp
DNA sequence (Forward): 5'-CCACGTGTTTGTGGAATTTG-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 |
| 10uM primer F | 1ul |
| 10uM primer R1 | 1ul |
| 5x cresol red dye | 5ul |
| Amplify Taq | 0.2ul (5u/ul) |
| Genomic DNA | 1ul |
| **Total volume** | **25 ul** |

| | | |
|---|---|---|
| 94ºC | 3min | 1 cycle |
| 94ºC | 30sec | 35cycles |
| 62ºC | 30sec | |
| 72ºC | 45sec | |
| 72ºC | 10min | 1 cycle |

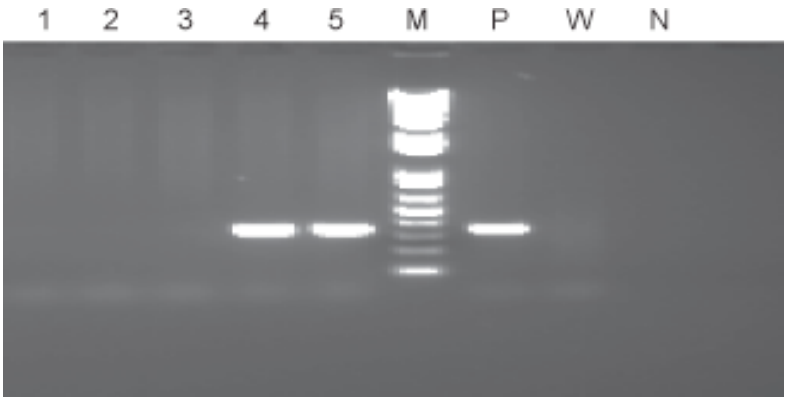


**Fig 1:** Number 4, and 5: Meox1$^{nuc\text{-}TagRFP\text{-}T/+}$,Rosa26R$^{lacZ/+}$, numbers 1,2, and 3: Rosa26R$^{lacZ/+}$, **P:** Meox1$^{nuc\text{-}TagRFP\text{-}T/+}$ Positive control, **W**: Wildtype, **N**: Negative control.

## 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

Meox1$^{nuc\text{-}TagRFP\text{-}T/+}$ male founders were mated to R26R$^{lacZ/+}$ females to generate Meox1$^{nuc\text{-}TagRFP\text{-}T/+}$ 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 cap mesenchyme and renal vesicle derivatives in Meox1$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ samples (Fig. 2 and 3).

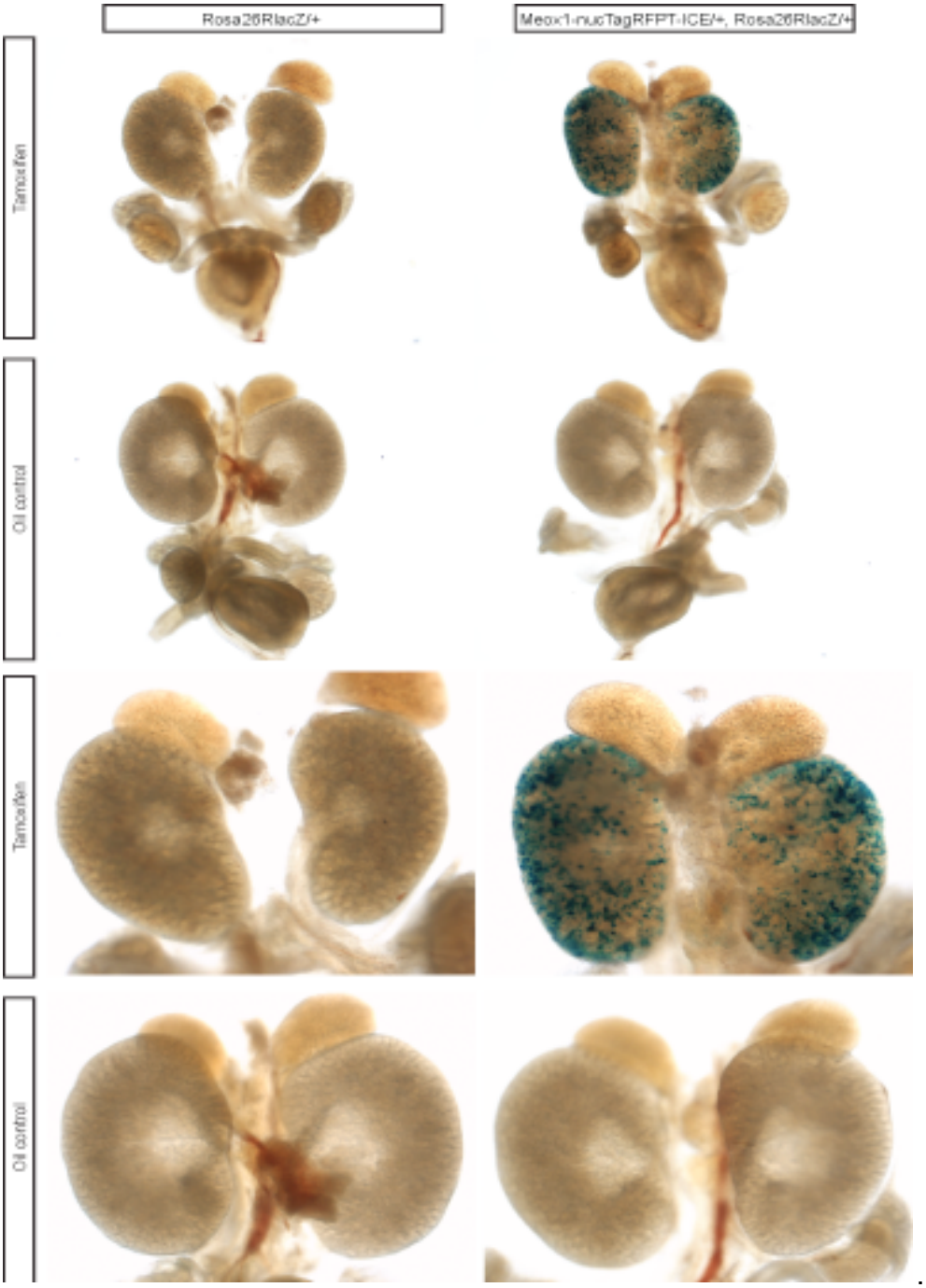


**Fig 2. Cre-dependent β-gal activity in Meox1$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ UGSs.** Tamoxifen injected at 11.5 and 13.5dpc resulted in ß-gal activity in the cap mesenchyme and renal vesicle derivatives of Meox1$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ but not R26R$^{lacZ/+}$ 15.5dpc UGS.

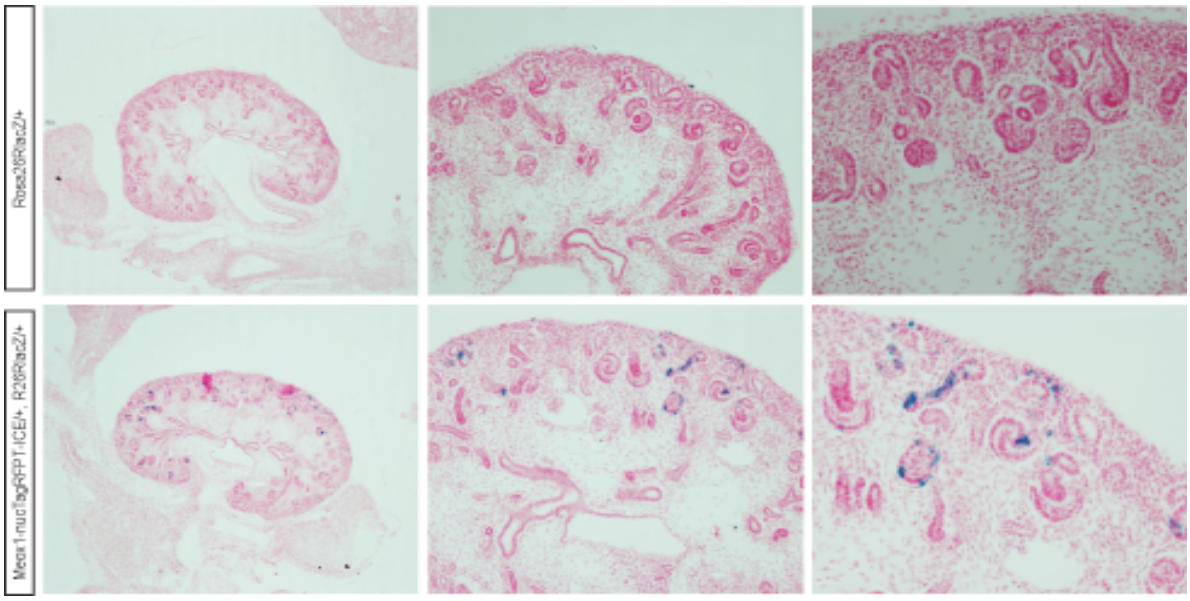


**Fig 3. Cre-dependent β-gal activity in Meox1$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ UGSs.** β-gal activity was detected within the cap mesenchyme nephron progenitors and early tubules in Meox1$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ 15.5 dpc kidneys upon induction with tamoxifen.

## 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): Rabbit-anti-β-gal/mouse-anti-Cytokeratin IgG1, mouse-anti-β-gal IgG2a/rabbit-anti-TagRFPT/mouse-anti-Cytokeratin IgG1, mouse-anti-β-gal IgG2a/rabbit-anti-Pax2 / mouse-anti-Cytokeratin IgG1and mouse-anti-β-gal IgG2a/rabbit-anti-Six2 / 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-488 | Invitrogen | 1/500 |
| Rabbit-anti-Pax2 | Covance | PRB-276P | 1/250 | Donkey-anti-rabbit-A555 | Invitrogen | 1/500 |
| Rabbit-anti-Six2 | Protein Tech Group, Inc | 11562-1-AP | 1/500 | Donkey-anti-rabbit-A555 | Invitrogen | 1/500 |
| mouse-anti-Cytokeratin IgG1 | Sigma | C2562 | 1/500 | Goat-anti-mouse IgG1-A647 | Invitrogen | 1/500 |
| Rabbit- anti β-gal | CAPPEL | 55976 | 1/20000 | Donkey-anti-rabbit-A488 | Invitrogen | 1/500 |

**Table 2. Summary of antibodies used to screen Meox1$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$ 15.5 dpc embryo sections.**

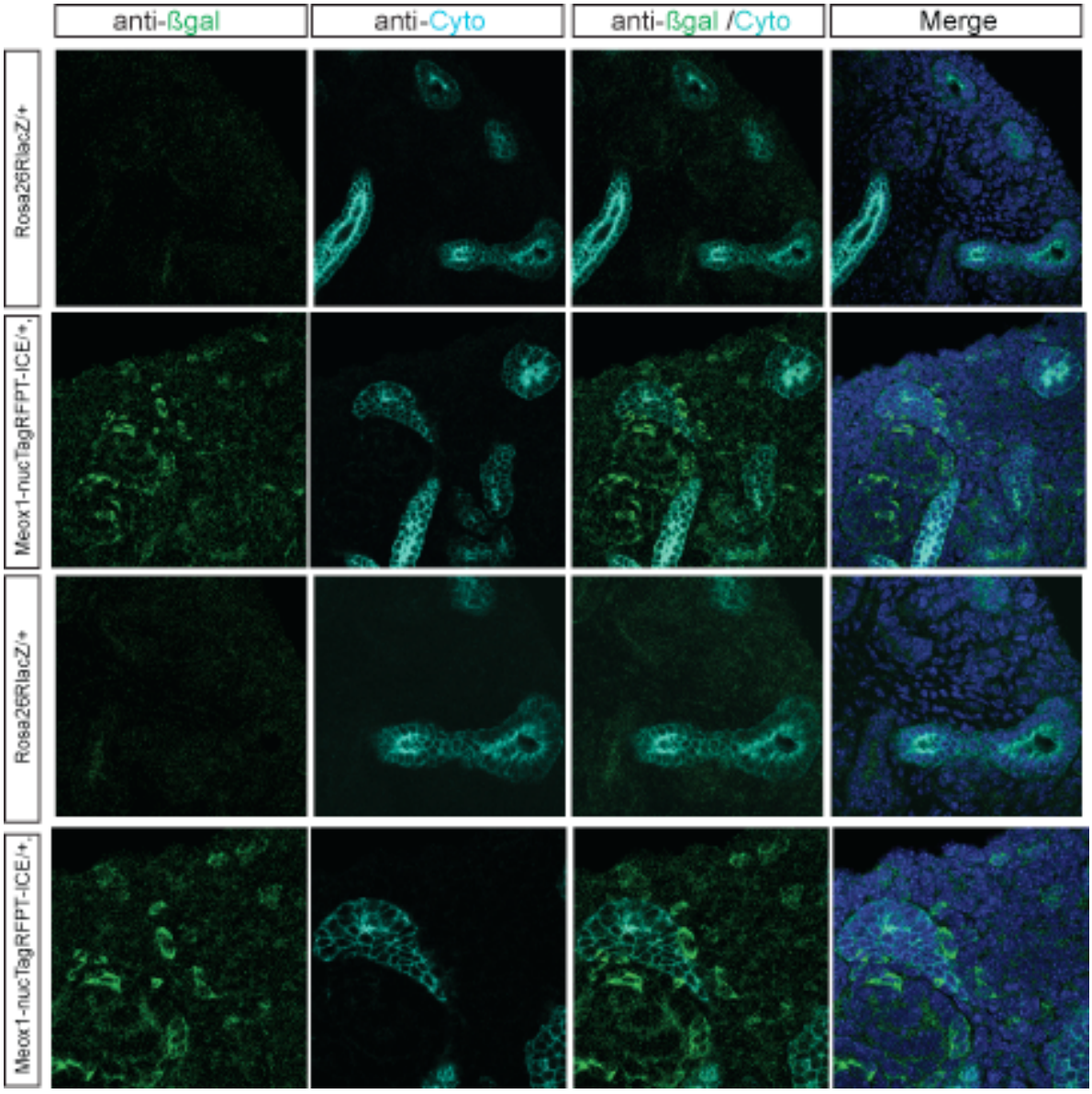


**Fig 4. ß-galactosidase detected in the renal vesicles in Meox1$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ tamoxifen injected kidneys.** Meox1$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$ kidneys were probed with anti-ß-gal, and anti-Cytokeratin IgG1 antibodies. A percentage of cap mesenchyme and renal vesicle derivatives are positive for ß-galactosidase activity upon induction by tamoxifen at 11.5 and 13.5 dpc.

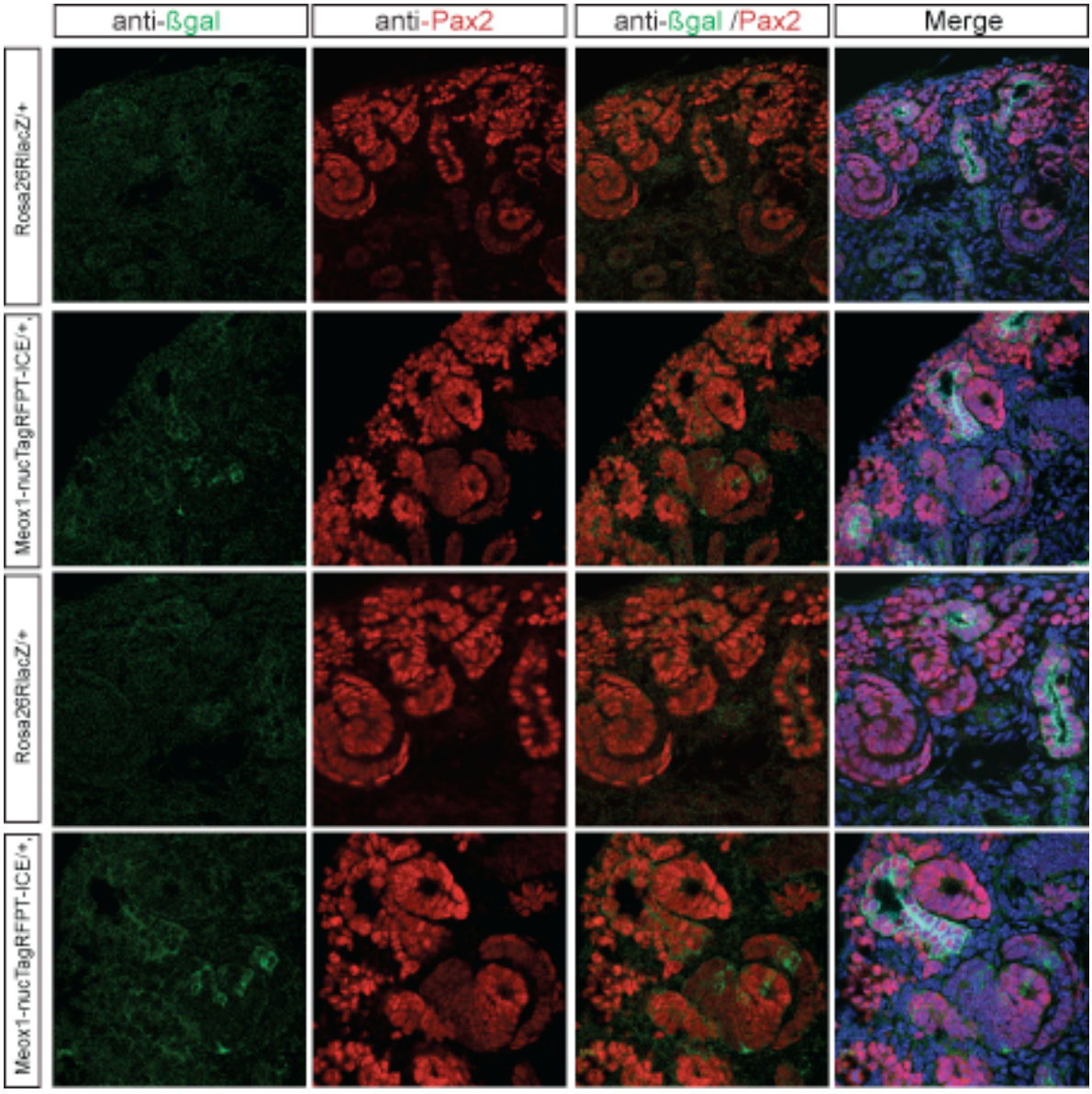


**Fig 5. ß-galactosidase and Pax2 positive cells in transitioning renal vesicles in Meox1$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ 15.5dpc kidneys.** Meox1$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$kidneys were probed with anti-ß-gal and anti-Pax2 antibodies. Pax2 is expressed in the pretubular aggregate, comma- and S-shaped bodies and in immature glomeruli.

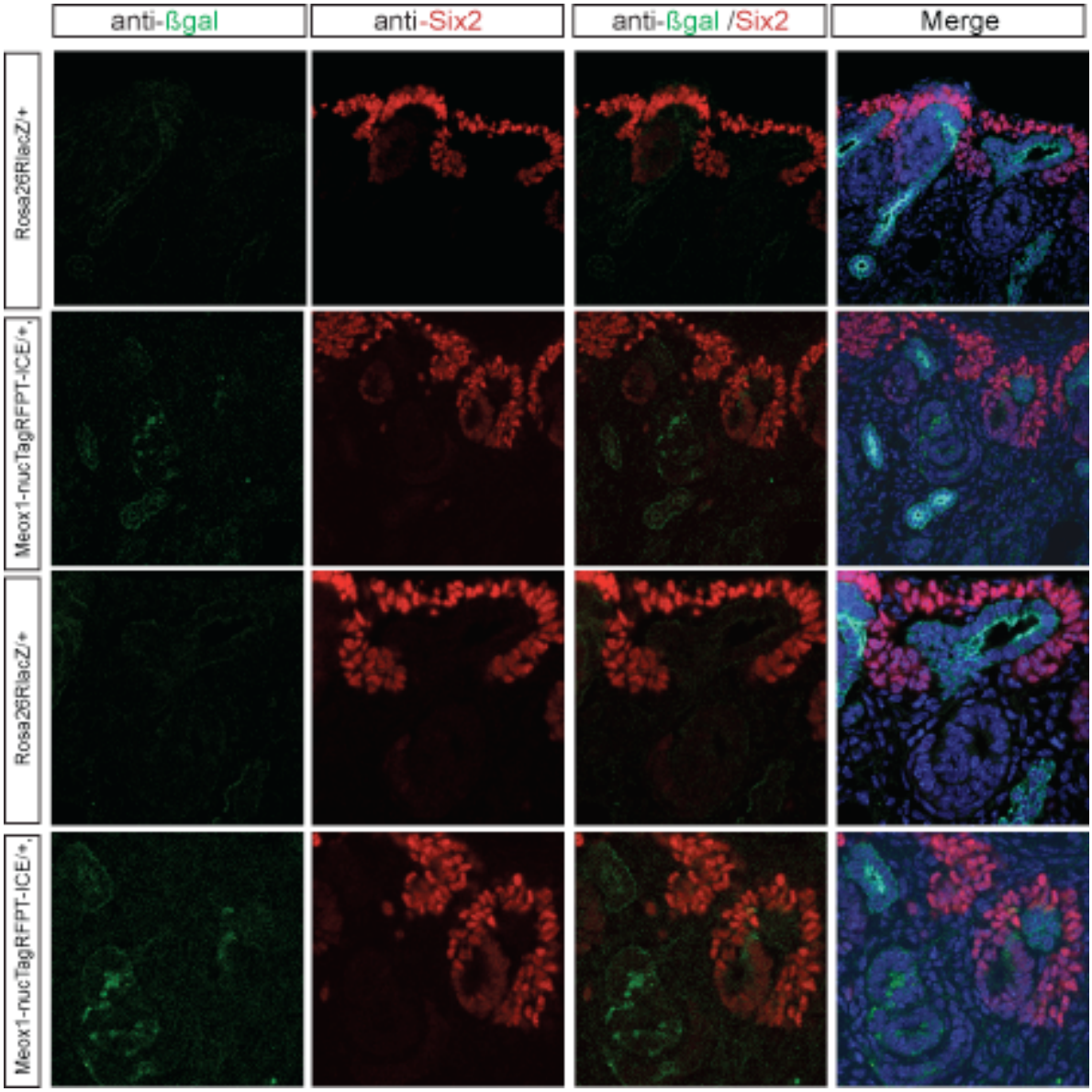


**Fig 6. ß-galactosidase and Six2 positive cells in transitioning renal vesicles in Meox1$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ 15.5dpc kidneys.** Meox1$^{nuc\text{-}TagRFP\text{-}T/+}$ R26R$^{lacZ/+}$ and R26R$^{lacZ/+}$kidneys were probed with anti-ß-gal and anti-Six2 antibodies. Six2 is expressed in the cap mesenchyme and transitioning renal vesicles.