

Wnt10b^{F2aeGFPT2aCE} Allele Characterization

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Findings: **VALIDATED**

Our analysis confirms activity of CreERT2 under the regulation of Wnt10b in the prostate, epididymis, bladder and skin at postnatal day 5 (P5). Very low endogenous eGFP expression was detectable in the skin from P5. CreERT2 activity in the prostate and bladder was confirmed by immunohistochemistry. Tamoxifen induced tdTomato cells colocalized with Keratin 5 positive cells in the prostate but not in the bladder at P5. Upon induction with tamoxifen at P3, robust Cre dependent tdTomato expression was observed in 12-week adult prostate and epididymis.

Data:

Crosses

The Wnt10b^{F2aeGFPT2aCE} (hereafter designated as Wnt 10b^{G2aCE}) strain is a JM8A1.N3 ES cell derived knock-in of eGFP and CreERT2 into the Wnt10b (wingless-type MMTV integration site family, member 10b). Two Targeted Non-Conditional (Promotor Driven Cassette) ES cell clones were obtained from the Knockout Mouse Project (KOMP) consortium.

(http://www.mousephenotype.org/martsearch_ikmc_project/martsearch/ikmc_project/46459). The clones were screened for chromosome number and clone EPD0314_2_A09, which displayed an acceptable modal 40 chromosome karyotype (19 of 20 cells scored), was modified by dual-recombinase mediated cassette exchange (dRMCE) to generate the targeted allele (see Figure 1 below).

A two-vector system was optimized in collaboration with EUCOMMTOOLS scientists at the Sanger (www.knockoutmouse.org/about/eucommtools) to give rise to a gene-targeting event in which a transcript encoding eGFP and CreERT2 is produced from the Wnt10b locus. The resulting transcript is predicted to lead to the production of individual polypeptides for each of these protein products due to the failure of amino acid incorporation where the translating ribosome encounters viral target sequences upstream of eGFP (F2a) and CreERT2 (T2a).

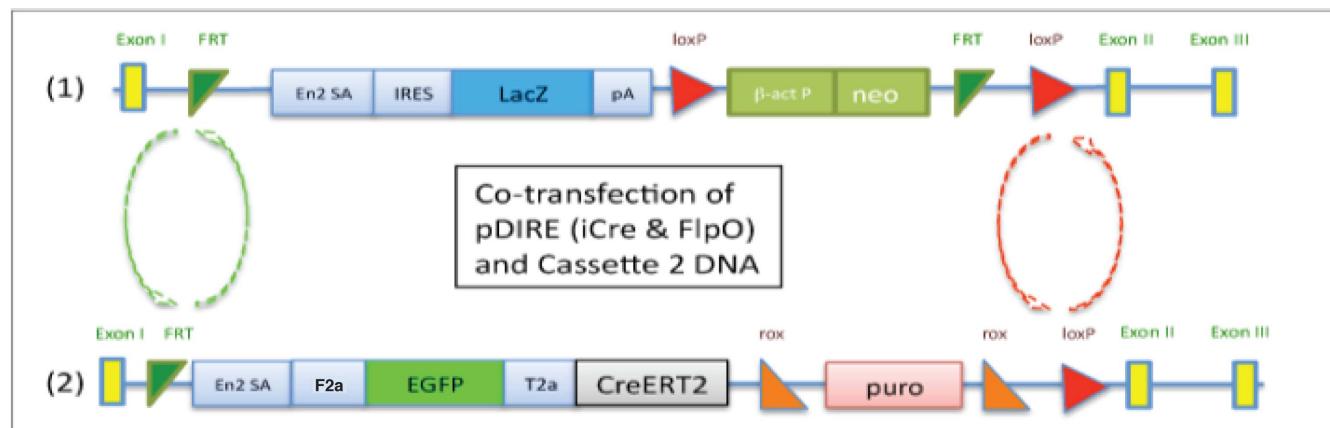


Figure 1. RCME strategy. Modified from: Osterwalder, M., et al. Dual RMCE for efficient re-engineering of mouse mutant alleles. Nat Methods. 2010 Nov;7(11):893-5.

Three correctly targeted clones were screened again by chromosome counting to increase the likelihood of germ line transmission and two clones with > 80% of cells displaying 40 chromosomes were injected into albino B6(Cg)-Tyr<c-2J>/J donor blastocysts. Male chimeras were mated to R26R^{lacZ/lacZ} and R26R^{tdTomato/tdTomato} female mice and the urogenital system (UGS) was collected from 11.5dpc embryos, P5 pups and 12wk adults. Of the eight chimeras tested, one (M7) transmitted the transgene. Although the mice demonstrated activity of CreERT2 in the expected cell population no endogenous eGFP was detected (Table 1).

| Line | Clone | % Chim | Embryos | # Tg | GLT | Visible GFP | Cre Activity |
|------------------|-------|--------|---------|------|-----|--------------|--------------|
| Wnt10b-G2ACE M1 | 28 | 90 | 61 | 0 | No | - | - |
| Wnt10b-G2ACE M2 | 28 | 85 | 65 | 0 | No | - | - |
| Wnt10b-G2ACE M3 | 28 | 80 | 0 | - | - | - | - |
| Wnt10b-G2ACE M7 | 28 | 80 | 7 | 3 | Yes | Undetectable | Yes |
| Wnt10b-G2ACE M8 | 28 | 80 | 35 | 0 | No | - | - |
| Wnt10b-G2ACE M4A | 15 | 80 | 0 | - | - | - | - |
| Wnt10b-G2ACE M5A | 15 | 70 | 41 | 0 | No | - | - |
| Wnt10b-G2ACE M6A | 15 | 60 | 32 | 0 | No | - | - |

Table 1. Transmission analysis of founders

Genotyping

Tail samples of the 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 (Figure 2).

Oligonucleotides: for targeted/transgenic allele (3' arm) Size: 525 bp

DNA sequence (forward): 5'-GGCATTATTAAAGTTAGGCGCG-3'

DNA sequence (reverse) 5'-CCAGCATGGAGAAGGGAGAAA-3'

Rxn Buffer and Conditions: (25µl reaction)

| | | | | | |
|---------------------|---------------|------|-------|----------|--|
| 10X PCR Buffer | 2.5ul | | | | |
| 1.25mM dNTP | 4ul | 94°C | 3min | 1 cycle | |
| 10uM primer F | 1ul | 94°C | 30sec | | |
| 10uM primer R | 1ul | 60°C | 30sec | 35cycles | |
| 5x cresol red dye | 5ul | 72°C | 45sec | | |
| Amplify Taq | 0.2ul (5u/ul) | 72°C | 10min | 1 cycle | |
| Genomic DNA | 1ul | | | | |
| Total volume | 25 ul | | | | |

1 2 3 4 5 6 7 8 9 10 M P Wt N

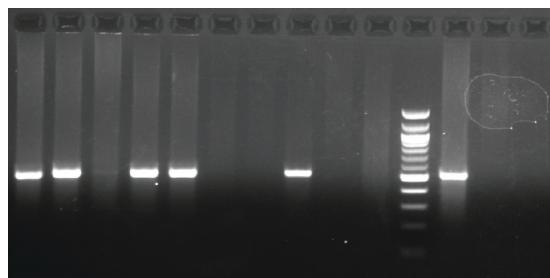


Figure 2: Number 1, 2, 4, 5 and 8: Wnt10b^{G2aCE/+}, numbers 3,6,7,9 and 10: Wildtype

M: DNA Marker, **P:** Positive control, **Wt:** Wildtype, **N:** Negative control.

Endogenous Fluorescence

Whole embryos as well as dissected UGSs were examined with a fluorescent microscope to view eGFP expression. Very low levels of endogenous eGFP were detected in the skin of $\text{Wnt10b}^{\text{G2aCE}+/+};\text{R26R}^{\text{tdTomato}+/+}$ P5 pups (Figure 4).

Cre-recombinase Activity

$\text{Wnt10b}^{\text{G2aCE}+/+}$ male chimeras were mated to $\text{R26R}^{\text{lacZ/lacZ}}$ and $\text{R26R}^{\text{tdTomato/tdTomato}}$ females to generate $\text{Wnt10b}^{\text{G2aCE}+/+};\text{R26R}^{\text{lacZ}+/+}$ or $\text{R26R}^{\text{tdTomato}+/+}$ embryos/pups/adults. In order to activate β -galactosidase (β -gal) or tdTomato reporter expression, pregnant females/pups and adults were injected intraperitoneally with tamoxifen in corn oil (1X 2mg to 40g body weight) and the tissues were assayed at 11.5,P5 and 12 wks. A control group was injected with the same volume of corn oil. Tamoxifen dependent Cre activity was detected in prostate, cells in the epididymis, skin and bladder in $\text{Wnt10b}^{\text{G2aCE}+/+};\text{R26R}^{\text{tdTomato}+/+}$ samples (Figure 3-7).

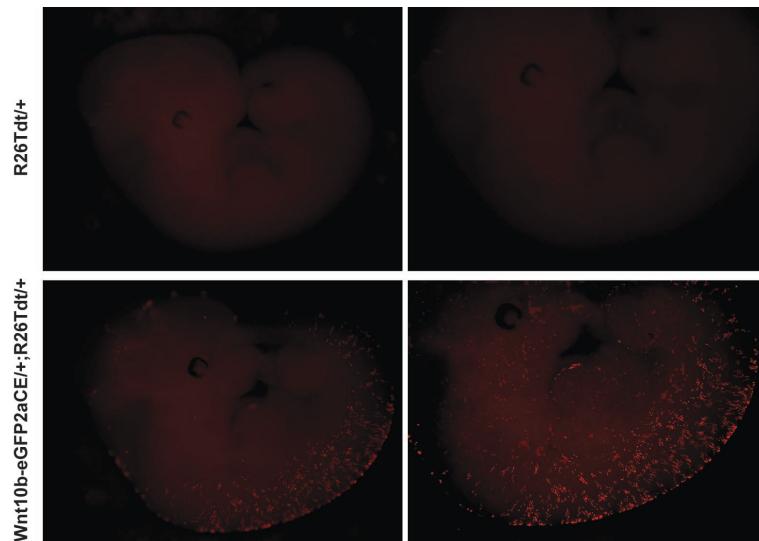


Figure 3. Cre-dependent tdTomato activity in $\text{Wnt10b}^{\text{G2aCE}+/+};\text{R26R}^{\text{tdTomato}+/+}$ 11.5dpc embryo. Cre activity was detected in the skin of 11.5dpc embryos following tamoxifen injection at 9.5dpc.

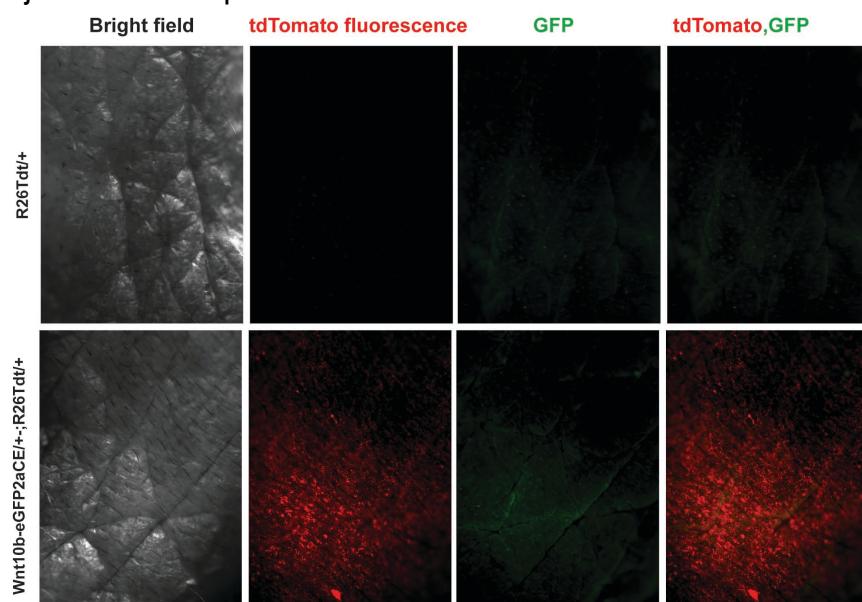


Figure 4. eGFP detection and Cre activity in P3 $\text{Wnt10b}^{\text{G2aCE}+/+};\text{R26R}^{\text{tdTomato}+/+}$ skin. Low levels of endogenous eGFP activity were observed in skin of $\text{Wnt10b}^{\text{G2aCE}+/+};\text{R26R}^{\text{tdTomato}+/+}$

mice. Robust tamoxifen dependent tdTomato fluorescence was observed in P5 skin cells following tamoxifen injection at P3.

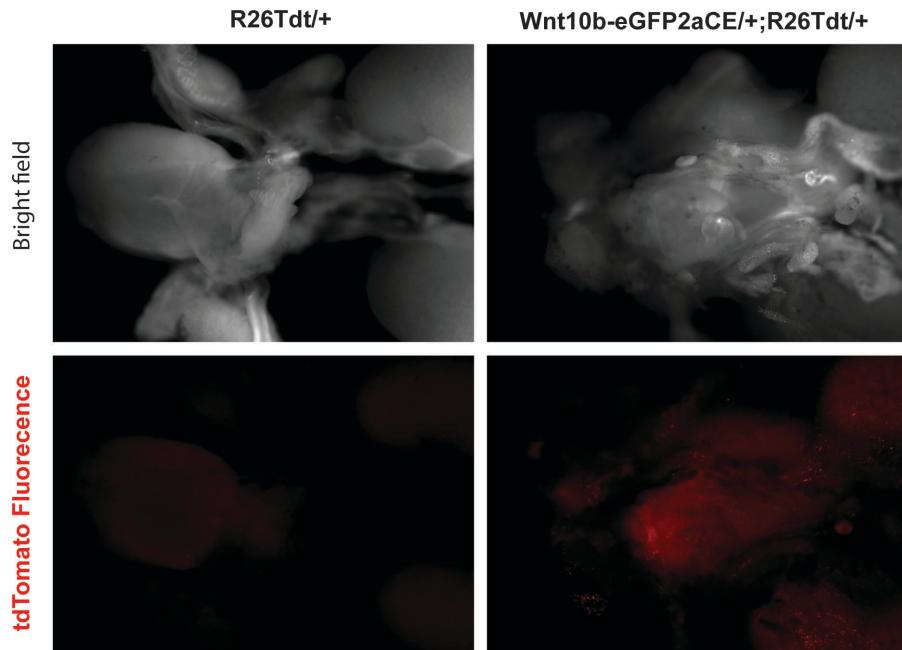


Figure 5. Cre activity visualized at P5 in $\text{Wnt10b}^{\text{G2aCE}+/+}; \text{R26R}^{\text{tdTomato}+/+}$ prostate.

Tamoxifen dependent tdTomato fluorescence was observed in prostate cells upon injection with tamoxifen at P3 collection at P5.

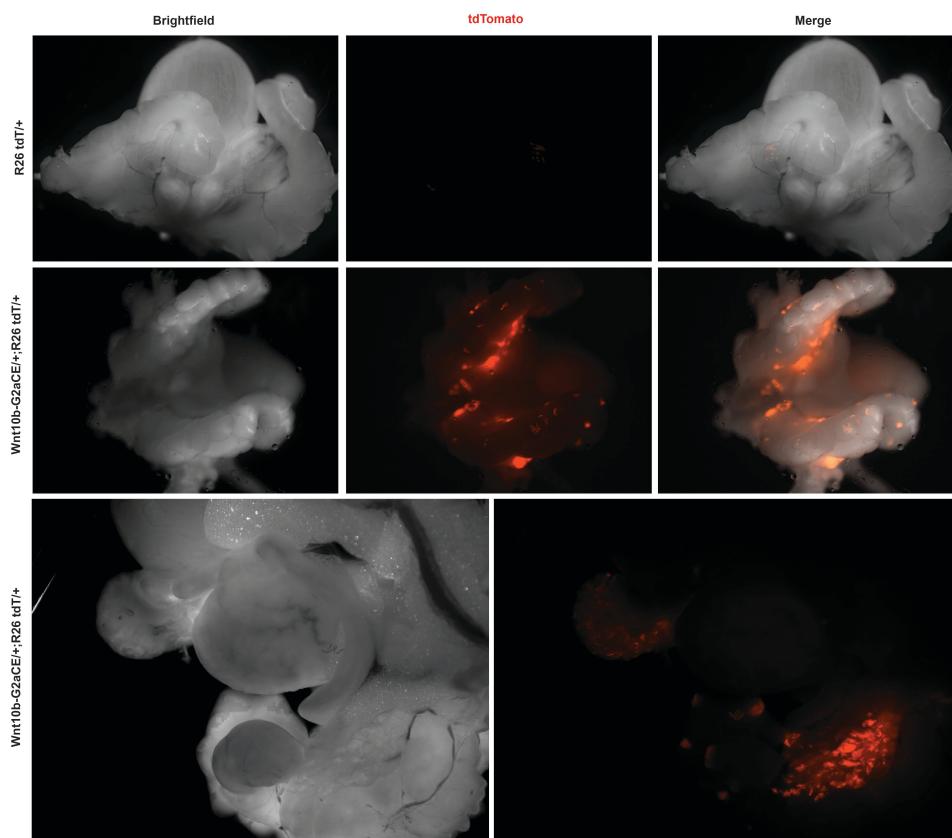


Figure 6. $\text{Wnt10b}^{\text{G2aCE}+/+}; \text{R26R}^{\text{tdTomato}+/+}$ labeling of prostate cells at P3 shows retention of labeled cells within the adult prostate at 12 weeks of age.

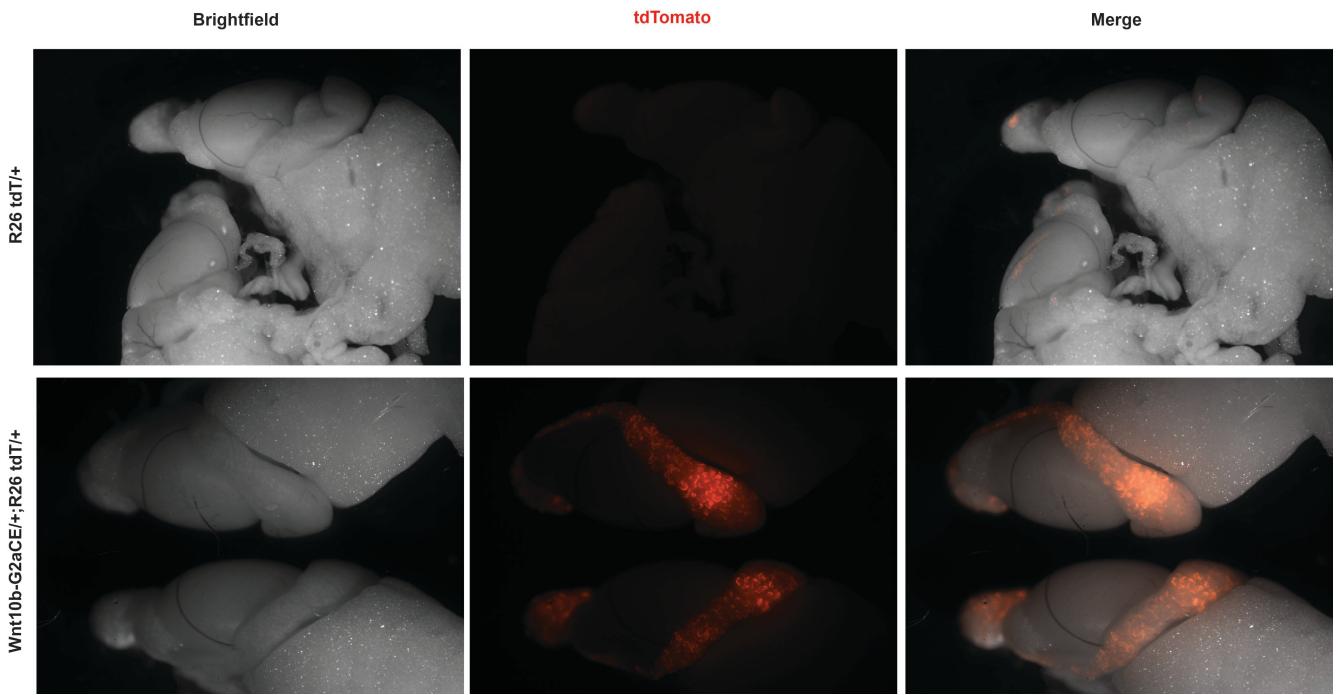


Figure 7. *Wnt10b*^{G2aCE/+}; *R26R*^{tdTomato/+} labeling at P3 shows retention of labeled cells within the adult epididymis at 12 weeks of age. TdTomato fluorescence was observed in the adult epididymis following tamoxifen injection at P3.

Immunohistochemistry

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 12um and probed with the antibodies listed in (Table 2).

Tamoxifen dependent tdTomato+ cells in *Wnt10b*^{G2aCE/+}; *R26R*^{tdTomato/+} colocalize with Keratin 5 expressing cells in the prostate but not in the bladder.

| Primary Antibody | Company | Catalog # | Dilution | Secondary | Company | Dilution |
|--|----------|-----------|----------|-----------------------------|------------|----------|
| Chicken IgY anti GFP | Aves Lab | GFP-1020 | 1/500 | Goat anti-chicken IgG-A488 | Invitrogen | 1/500 |
| Rabbit anti Keratin 5 | Covance | PRB-160p | 1:1000 | Donkey anti-rabbit IgG A488 | Invitrogen | 1/500 |
| Mouse IgG2a anti-Actin (α-Smooth Muscle) | Sigma | A5228 | 1/2000 | Goat anti-mouse IgG2a A633 | Invitrogen | 1/500 |

Table 2. Summary of antibodies used to screen *Wnt10b*^{G2aCE/+}; *R26R*^{tdTomato/+} P5 UGS sections.

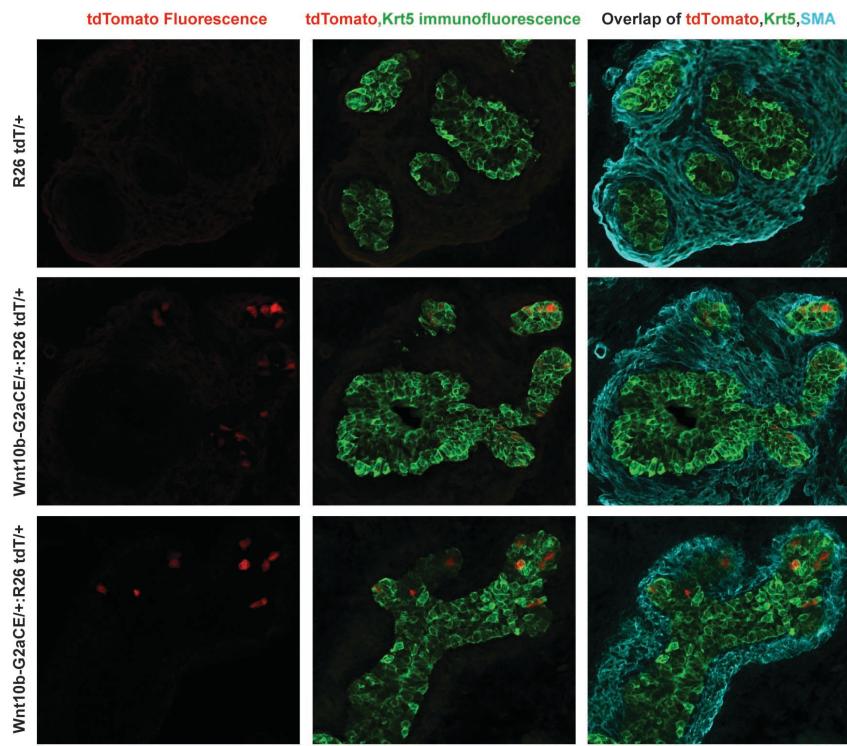


Figure 8. Tamoxifen dependent Cre activity in $\text{Wnt10b}^{\text{G2aCE}/+}$; $\text{R26R}^{\text{tdTomato}/+}$ P5 prostate colocalized with Keratin 5 expressing cells. TdTomato fluorescence was observed in a percentage of Krt⁺ cells within the P5 prostate following tamoxifen injection at P3.

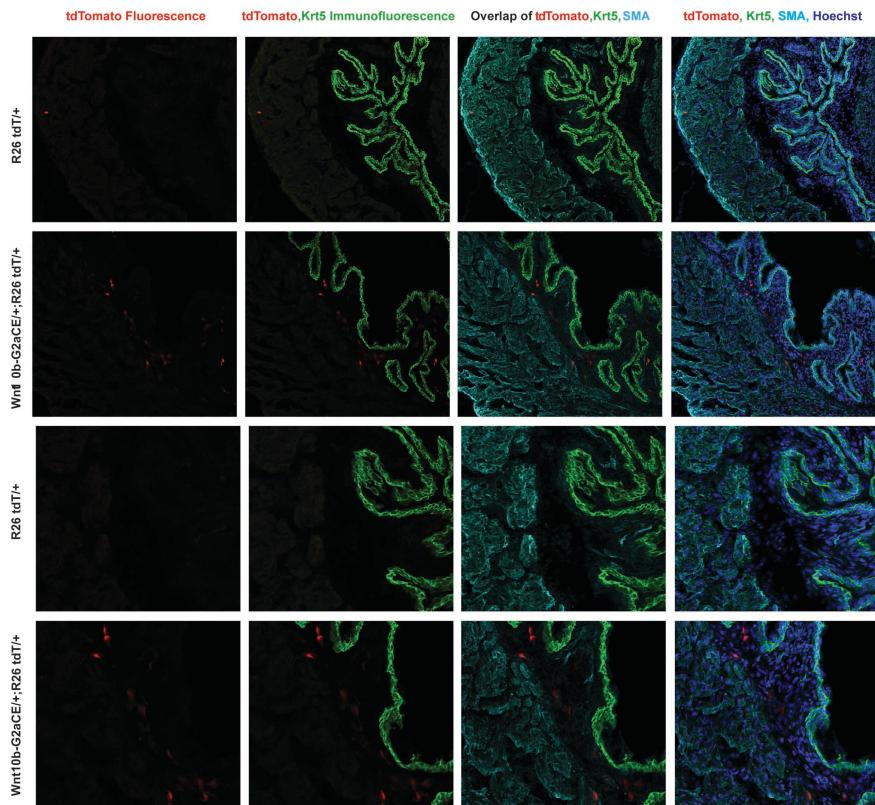


Figure 9. Tamoxifen dependent Cre activity in $\text{Wnt10b}^{\text{G2aCE}/+}$; $\text{R26R}^{\text{tdTomato}/+}$ P5 bladder epithelium adjacent to Keratin 5 expressing cells. TdTomato fluorescence was observed in a population of cells underlying the bladder epithelium, distinct from Smooth muscle actin and Krt 5 positive cells, within the P5 bladder following tamoxifen injection at P3