# $Srd5a2^{F2aeGFPT2aCE}$ Allele Characterization

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## Findings: VALIDATED

Our analysis confirms activity of CreErt2 under the regulation of Srd5a2. Robust expression of Cre dependent tdTomato expression was observed in the prostate, seminal vesicles and other components of the male reproductive tract and the ovary in 8-12 week old mice following tamoxifen injection at P5 and in the adult. CreErt2 activity in the prostate and seminal vesicles was confirmed by immunohistochemistry. Tamoxifen induced tdTomato cells co-localize with Acta2+ (SMA) cell types and were closely apposed to Krt5+ but not Krt8+ cells in the prostate and seminal vesicles. eGFP expression was not detectable in either embryos or adults by direct fluorescence or immunohistochemical procedures.

## Data:

### Crosses

The $Srd5a2^{F2aeGFPT2aCE}$ (hereafter designated as $Srd5a2^{G2aCE}$) strain is a JM8A3.N1.C2 ES cell derived knock-in of eGFP and CreErt2 into the Srd5a2 (steroid 5-alpha reductase 2). Three targeted, non-conditional ES cell clones were obtained from the Knockout Mouse Project (EUCOMM) consortium. (http://www.mousephenotype.org/data/alleles/MGI:2150380/tm2e(EUCOMM)Hmgu/)
The clones were screened for chromosome number and clone HEPD0799_3_G07, which displayed an acceptable modal 40 chromosome karyotype (17 of 20 cells scored), was modified by dual-recombinase mediated cassette exchange (dRMCE) to generate the targeted allele (Figure 1).
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 Srd5a2 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).

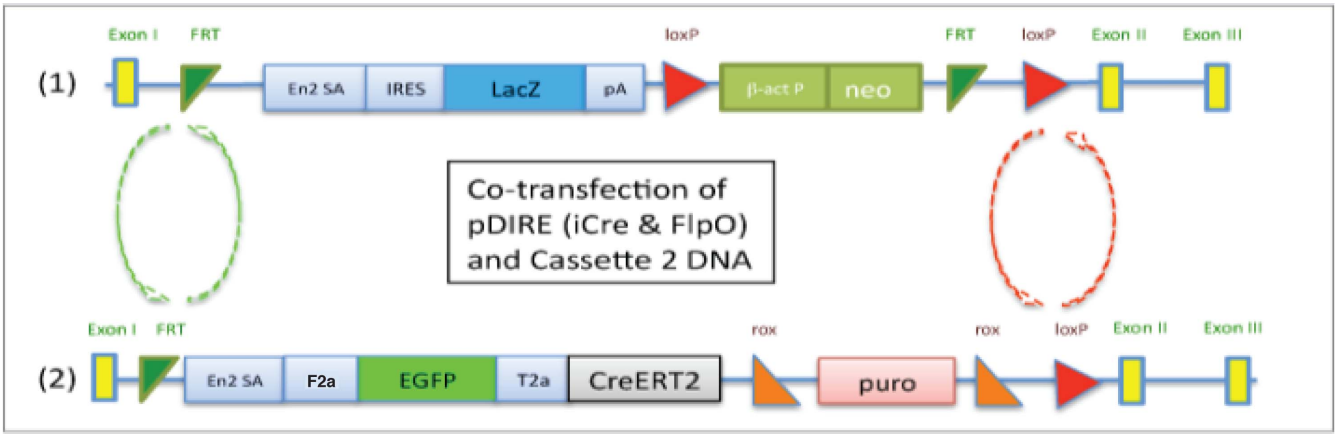



**Figure 1.** RCME strategy. Modified from: Osterwalder, M., et al. Dual RMCE for efficient re-engineering of mouse mutant alleles. Nat Methods. 2010 Nov;7(11):893-5.

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 8-12 wk old mice post Tamoxifen induction. Of the ten chimeras tested, 4 (M2, M3, M6A, M10A) 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 |
|---|---|---|---|---|---|---|---|
| Srd5a2-G2aCE M1 | 32 | 80 | 37 | 0 | No | - | |
| Srd5a2-G2aCE M2 | 32 | 80 | 56 | 5 | Yes | Undetectable | Yes |
| Srd5a2-G2aCE M3 | 32 | 65 | 63 | 2 | Yes | Undetectable | Yes |
| Srd5a2-G2aCE M4 | 32 | 65 | 27 | 0 | No | - | - |
| Srd5a2-G2aCE M5A | 20 | 80 | 0 | - | - | - | - |
| Srd5a2-G2aCE M6A | 20 | 70 | 63 | 31 | Yes | Undetectable | Yes |
| Srd5a2-G2aCE M7A | 20 | 70 | 38 | 0 | No | - | - |
| Srd5a2-G2aCE M8 | 32 | 50 | 27 | 0 | No | - | - |
| Srd5a2-G2aCE M9A | 20 | 70 | 0 | - | - | - | - |
| Srd5a2-G2aCE M10A | 20 | 30 | 58 | 1 | Yes | Undetectable | 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 (Figure 2).

Oligonucleotides: for targeted/transgenic allele (3' arm) Size: 483 bp

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

DNA sequence (reverse) 5-CTGGAGGGTCTGATTGGTTG-3'

Rxn Buffer and Conditions: (25$\mu$l reaction)

| | |
|---|---|
| 10X PCR Buffer | 2.5ul |
| 1.25mM dNTP | 4ul |
| 10uM primer F | 1ul |
| 10uM primer R | 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 |
| 60ºC | 30sec | |
| 72ºC | 45sec | |
| 72ºC | 10min | 1 cycle |

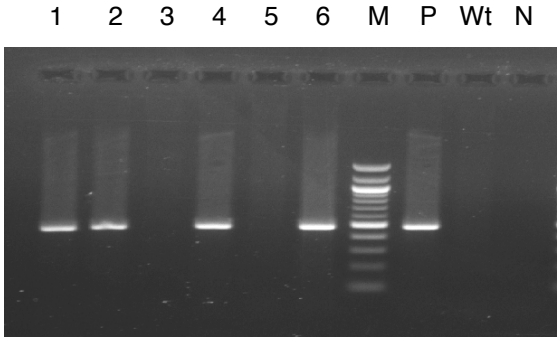



**Figure 2:** Number 1, 2, 4 & 6: Srd5a2$^{G2aCE/+}$, 3 & 5: Wildtype

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

## Endogenous Fluorescence

Dissected UGSs were examined with a fluorescent microscope to view eGFP expression. No eGFP activity was detected in Srd5a2 $^{G2aCE\ /+}$;R26R$^{tdTomato/+}$ embryos or adults.

## Cre-recombinase Activity

Srd5a2 $^{G2aCE\ /+}$ male chimeras were mated to R26R$^{lacZ/lacZ}$ and R26R $^{tdTomato/tdTomato}$ females to generate Srd5a2 $^{G2aCE\ /+}$ R26R$^{lacZ/+}$ or R26R$^{tdTomato/+}$ pups and adults. In order to activate ß-galactosidase (β-gal) or tdTomato reporter expression, P5 pups were injected intraperitoneally with tamoxifen in corn oil (1X 2mg to 40g body weight) and the tissues were assayed at 8 wks. In addition, 8 wk old adult mice were injected with tamoxifen in corn oil (4X 4mg per injection) and the tissues were assayed at day 9-10; 1-2 days after the final injection. Tamoxifen dependent Cre activity was detected in prostate and seminal vesicles and in the ovary in Srd5a2 G2aCE /+; R26R tdTomato/+ samples (Figure 3-7).

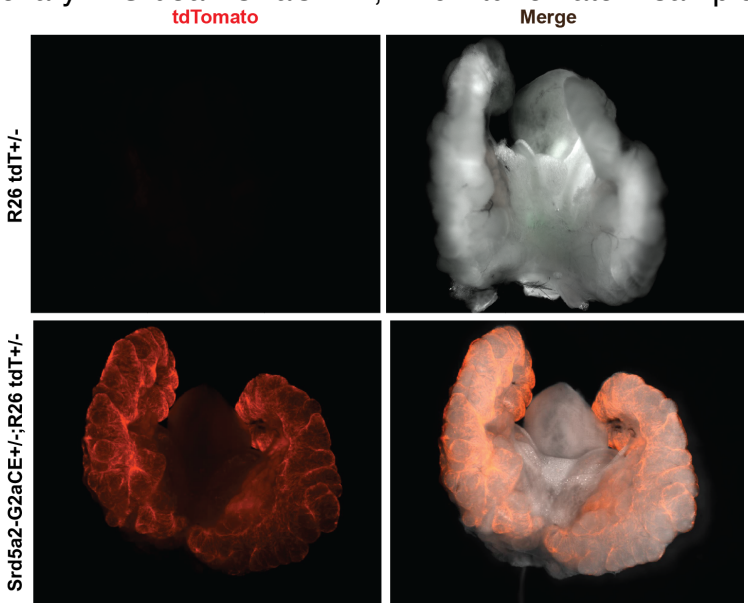



**Figure 3.** Tamoxifen dependant tdTomato positive cells observed in the seminal vesicles of Srd5a2-G2aCE+/-;R26tdT+/- 8 week old mice after a single injection (2mg/40g body weight) at P5. Prostate tissue was not examined in this experiment.

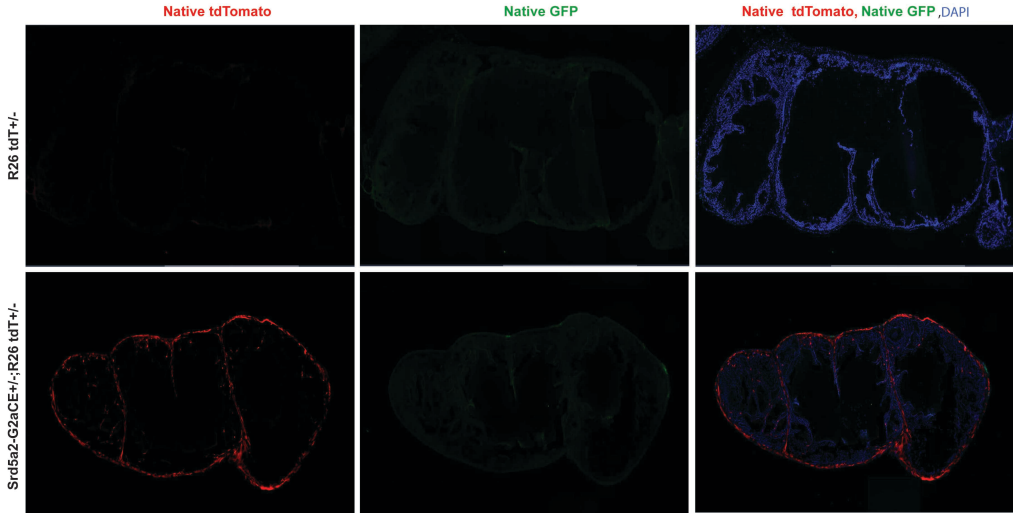



**Figure 4.** Sections of tdTomato positive Srd5a2-G2aCE+/-;R26tdT+/-seminal vesicles in 8 week mice after a single injection of tamoxifen at post natal day 5 (2mg/40g body weight), reveals the tdTomato positive cells reside in a thin layer of cells surrounding the lumen.

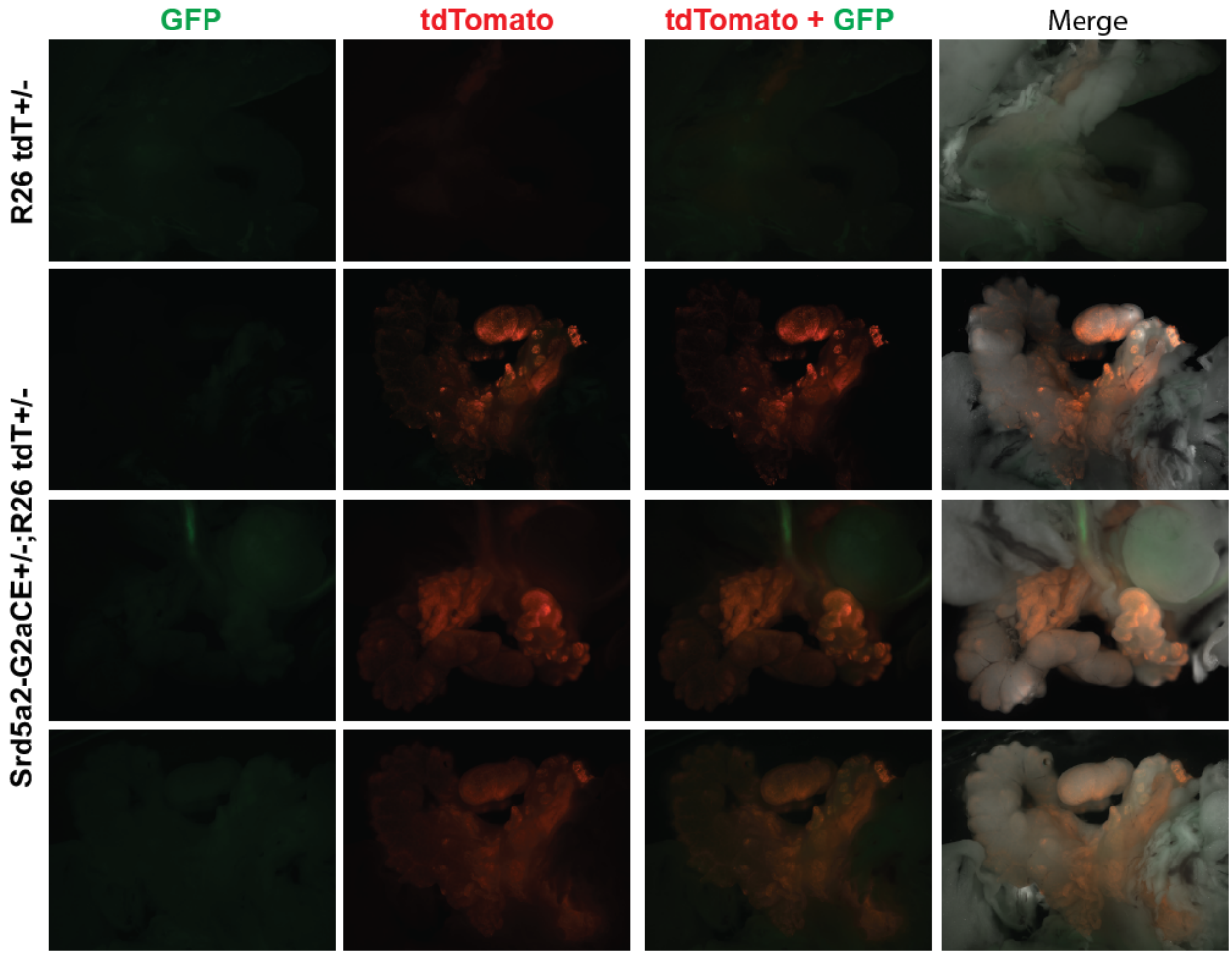



**Figure 5.** Tamoxifen dependant tdTomato positive cells present in the prostate and seminal vesicles of Srd5a2-G2aCE+/-;R26tdT+/- 8 week old mice after 4 injections of tamoxifen (4mg/injection) in 8 wk old adult mice. Injection schedule: post natal day 1, 3, 5 & 7, collection day 9.

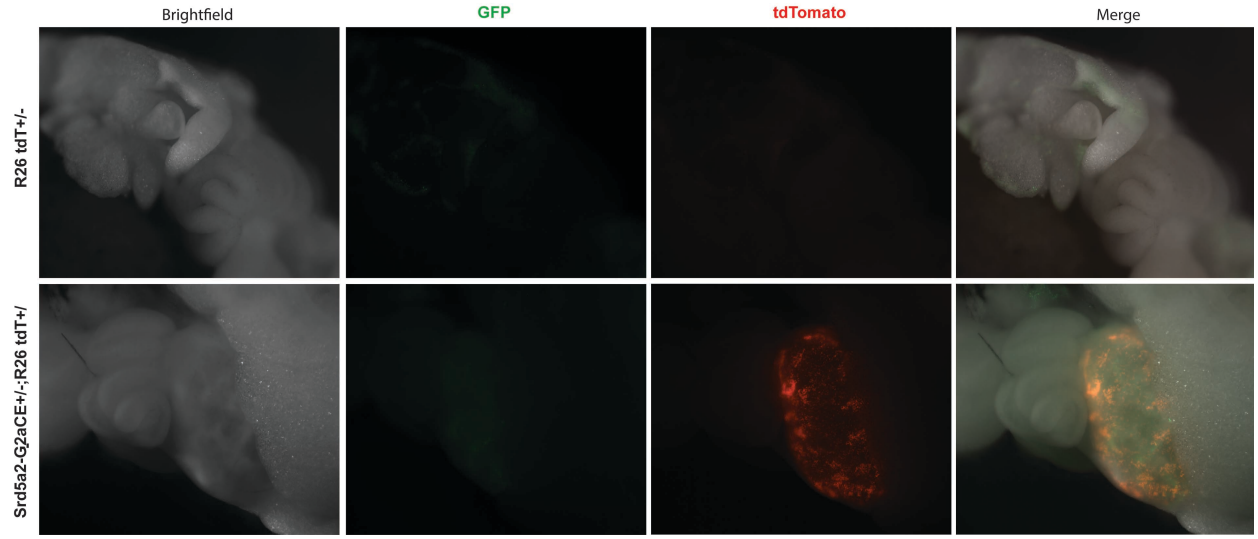



**Figure 6**. Tamoxifen dependant tdTomato positive cells can be seen in the ovary of Srd5a2-G2aCE+/-;R26tdT+/- 8 week old mice after 4 injections of tamoxifen (4mg/injection) in the adult. Injection schedule: day 1, 3, 5, 7, collection day 10.

## Immunohistochemistry

Whole UGSs were fixed in 4% paraformaldehyde at 4ºC for 45 minutes, 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).

| Primary Antibody | Company | Catalog # | Dilution | Secondary | Company | Dilution |
|---|---|---|---|---|---|---|
| Chicken IgY anti GFP | Aves Lab | GFP-1020 | 1/500 | Goat anti-chicken IgGA633 | Invitrogen | 1/500 |
| Rabbit anti Keratin 5 | Covance | PRB-160p | 1:1000 | Goat anti-rabbit IgG A488 | Invitrogen | 1/500 |
| Mouse IgG2a anti-Actin (α-Smooth Muscle) | Sigma | A5228 | 1/2000 | Goat anti-mouse IgG2a A488 | Invitrogen | 1/500 |
| Mouse-anti-Cytokeratin IgG1 | Sigma | C2562 | 1/500 | Goat anti-mouse IgG1-A647 | Invitrogen | 1/500 |
| Rat-anti-E-Cadherin | Sigma | U3254 | 1/1000 | Goat anti-rat IgG-A488 | Invitrogen | 1/500 |

**Table 2.** Summary of antibodies used to screen Srd5a2$^{G2aCE/+}$; R26R$^{tdTomato/+}$ P5 and adult UGS sections.

200-400 reporter cells for each target tissue (prostate and seminal vesicles) were counted in high-resolution confocal images of sections probed with anti-smooth muscle actin, anti-Krt5 or anti-Krt8 antibodies. A percentage of the tamoxifen dependent tdTomato+ cells colocalized with smooth muscle actin and were closely apposed but appear to be distinct from Keratin 5 expressing cells in the Srd5a2 $^{G2aCE/+}$;R26RtdTomato/+ prostate and seminal vesicles. Keratin 8 expressing cells were identified in a separate cell population from the tdTomato positive cells.

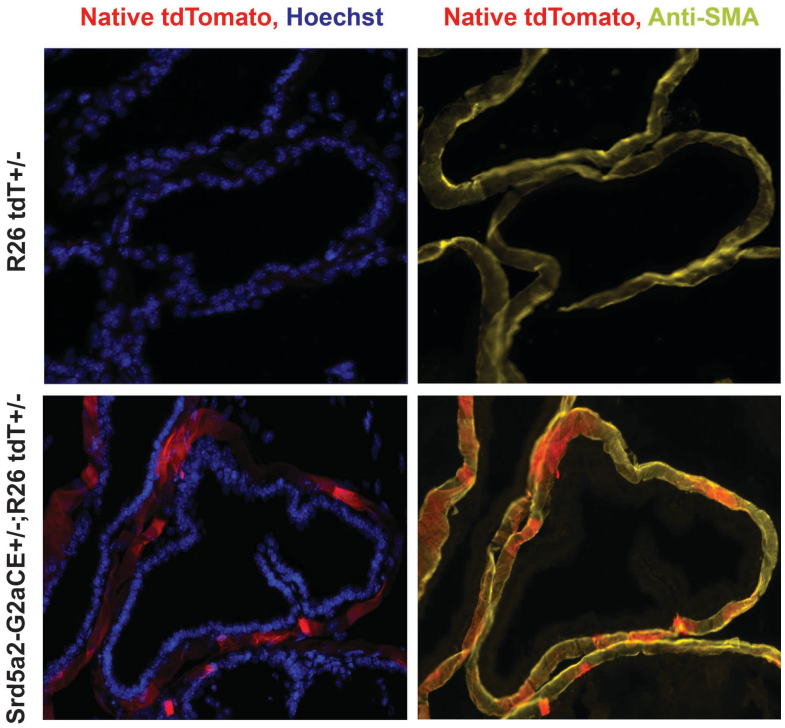
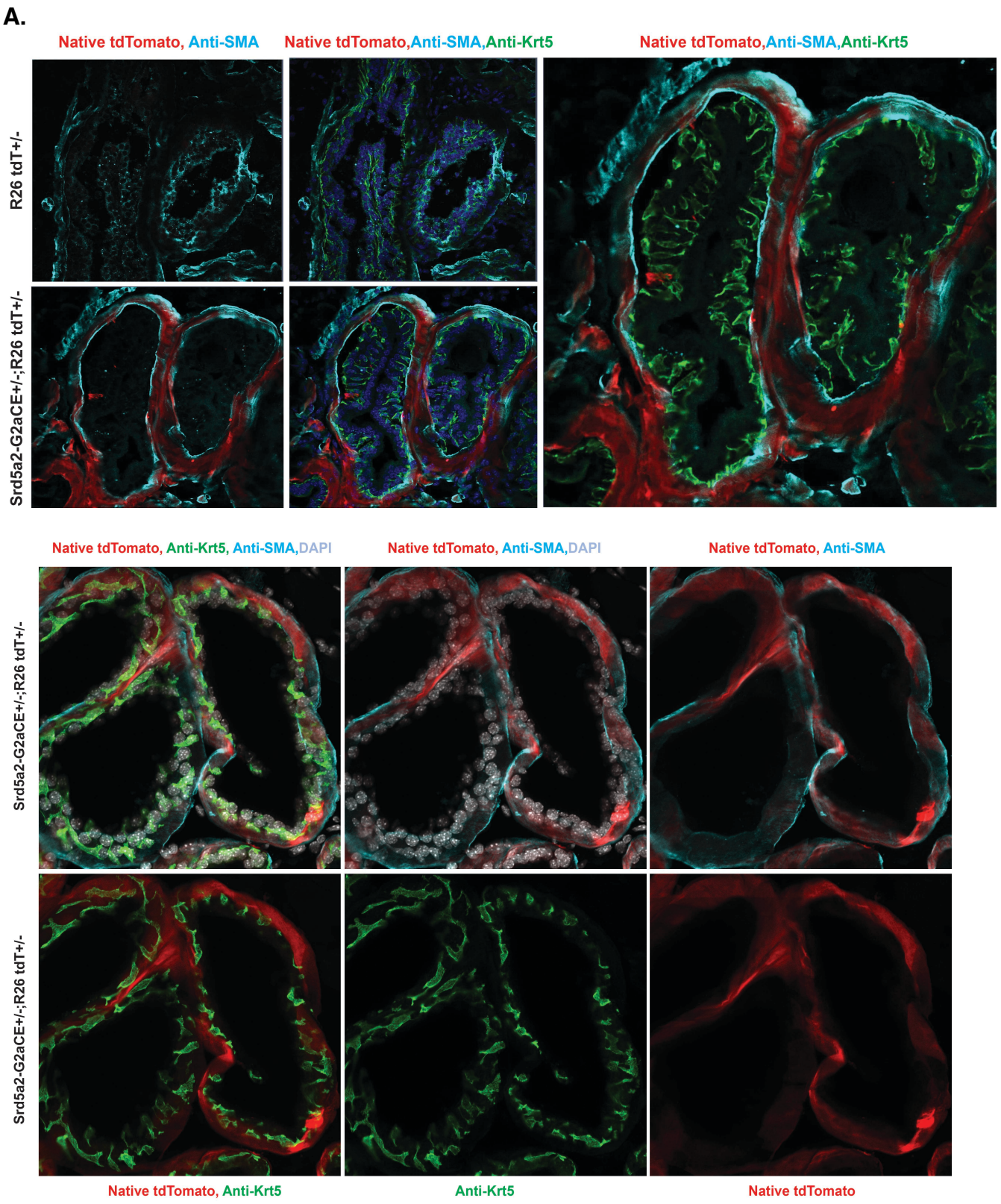



**Figure 7**. A percentage of tamoxifen dependent tdTomato positive cells in Srd5a2 $^{G2aCE/+}$;R26RtdTomato/+ prostate co-localize with Acta2 (SMA) expressing cells in 8 week old mice. Injection schedule: day 1, 3, 5 & 7, collection day 9.


A.
Native tdTomato, Anti-SMA
Native tdTomato,Anti-SMA,Anti-Krt5
Native tdTomato,Anti-SMA,Anti-Krt5
R26 tdT+/-
Srd5a2-G2aCE+/-;R26 tdT+/-
Native tdTomato, Anti-Krt5, Anti-SMA,DAPI
Native tdTomato, Anti-SMA,DAPI
Native tdTomato, Anti-SMA
Srd5a2-G2aCE+/-;R26 tdT+/-
Srd5a2-G2aCE+/-;R26 tdT+/-
Native tdTomato, Anti-Krt5
Anti-Krt5
Native tdTomato

**B.**

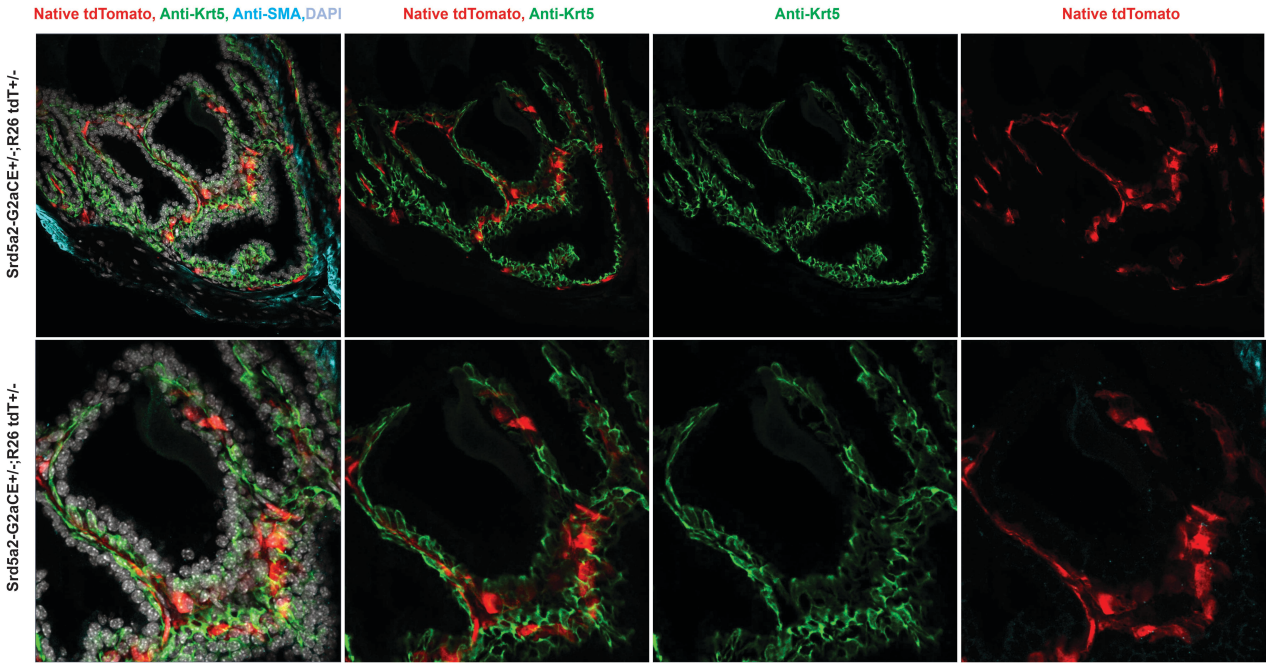



**Figure 8**. A percentage of tamoxifen dependent tdTomato positive cells in Srd5a2 $^{G2aCE\ /+}$;R26RtdTomato/+ prostate (A) and seminal vesicle (B) co-localize with Acta2 (SMA) , though many cells do not show high levels of Acta2 (SMA). tdTomato positive cells closely appose Krt5+ epithelial cells in 8 week old mice. Injection schedule: day 1, 3, 5 & 7, collection day 9.

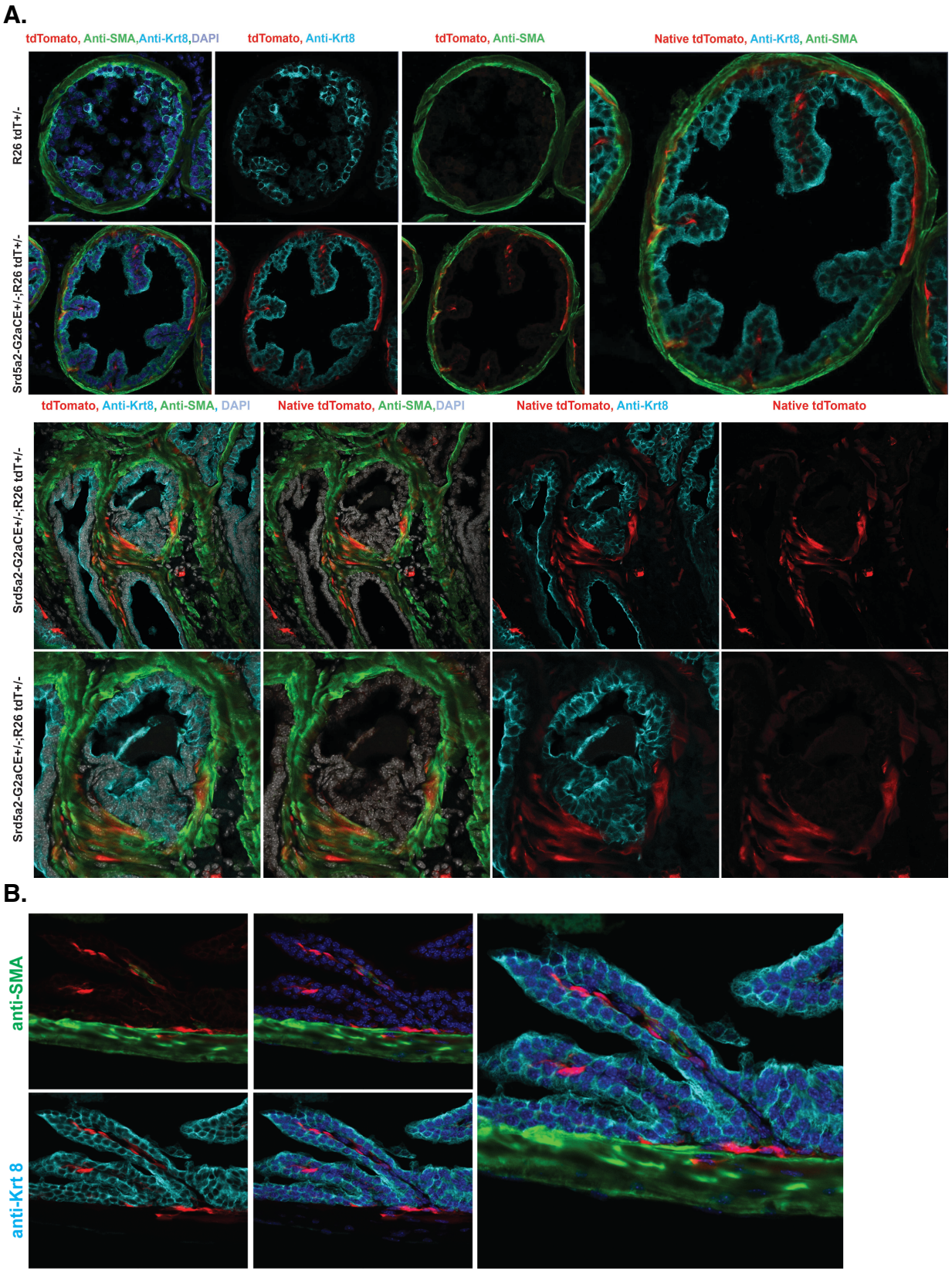



**Figure 9.** TdTomato fluorescence was detected in the prostate gland (A) and seminal vesicle (B), a percentage of the tdTomato positive cells co-localize with Acta2 (SMA) positive cells though some tdT+ cells do not show high levels of Acta2 (SMA). tdTomato expressing cells do not co-localize with Krt8+ epithelial cells in 8 week old mice. Injection schedule: day 1, 3, 5 & 7, collection day 9.