

# Krt20<sup>T2A-CRE-ERT2</sup> Allele Characterization

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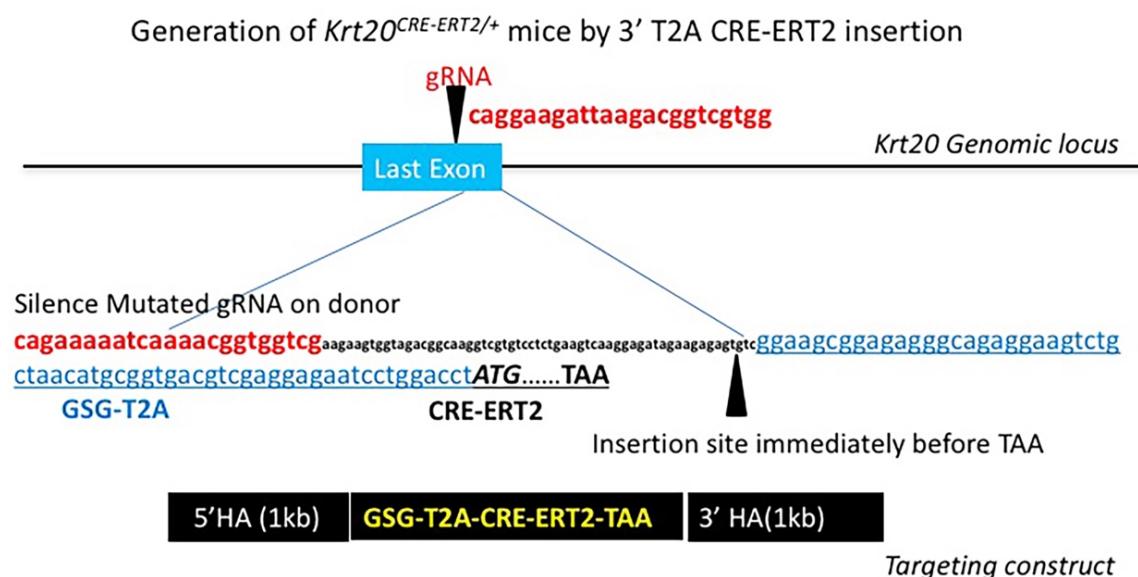
## Findings: VALIDATED

Our analysis confirms activity of CRE::ERT2 under the regulation of Krt20. Expression of Cre dependent tdTomato expression was observed upon induction in the bladder and intestine in postnatal day 23 (P23) pups following tamoxifen injection at P21. Cre inducible expression in the epithelium of the bladder and intestines was confirmed by immunohistochemistry, tdTomato+ cells co-localize with Krt20+ and E-Cadherin+ epithelial cells lining the lumen of the bladder and intestine. In the gut, these comprises most but not all differentiated cell types within intestinal villi. In the bladder, tdTomato+/KRT20+ cells are the most superficial Uroplakin III+ component of the bladder epithelial umbrella cell population.

## Data:

### Crosses

The Krt20<sup>T2A-CRE-ERT2</sup> strain is a CRISPR/ Cas9 mediated knock-in of T2A-CRE-ERT2 immediately before the stop codon of the *Krt20* (Keratin 20) gene in JM8.N4 ES cells. The targeted *Krt20* gene encodes a type I, acidic, intermediate filament in the gastric and intestinal mucosa. gRNAs were designed with the program: <http://crispr.mit.edu>. Annealed oligos containing the gRNA sites were cloned into BbsI sites of plasmid pSpCas9(BB)-2A-puro (Ran FA et al. Nature Protocol, 2013). The donor targeting construct was generated using GIBSON assembly with four PCR fragments: 5' 1-kb homologous arms (HA), T2A-CRE-ERT2-bGHpA DNA and 3' 1-kb homologous arms (HA), and linear pBluescript vector. A silent mutation was introduced into the 5' PCR fragment at the gRNA recognition site so that the final donor construct will not be cut by the gRNA. 5ug gRNA-Cas9 construct and 25ug donor targeting construct were transfected into C57BL/6 JM8.N4 ES cells (KOMP) with FugeneHD (Promega). The cells were kept in 2i media on gelatin coated plates during transfection for 48h followed by 48h 1.75ug/ml puromycin selection on MEF plates.



**Figure 1.** Diagram of the strategy adopted to generate CRISPR/Cas9 mediated knock-in of T2A-CRE-ERT2-bGHpA into the Krt20 locus of JM8.N4 ES cells.

Three correctly targeted clones were screened by chromosome counting to increase the likelihood of germ line transmission and two clones with > 80% of cells displaying a modal number of chromosomes were injected at Jackson Laboratory into albino B6(Cg)-Tyr<c-2J>/J donor blastocysts. Male chimeras were mated to albino B6(Cg)-Tyr<c-2J>/J female mice to determine coat color transmission and heterozygous progeny were confirmed by PCR. F1 males were sent to the McMahon Lab for characterization. Krt20<sup>T2A-CRE-ERT2</sup> males were mated to R26R <sup>tdTomato/tdTomato</sup> female mice and the urogenital system (UGS) and intestines were collected from P23 pups post tamoxifen induction. Three F1 males were tested, (M1, M2, M3) and transmitted the transgene (Table 1).

Line	Clone	GLT	Cre activity
Krt20 <sup>T2A-CRE-ERT2</sup> M1	19	Yes	Yes
Krt20 <sup>T2A-CRE-ERT2</sup> M2	19	Yes	Yes
Krt20 <sup>T2A-CRE-ERT2</sup> M3	19	Yes	Yes

**Table 1.** Transmission analysis of founders

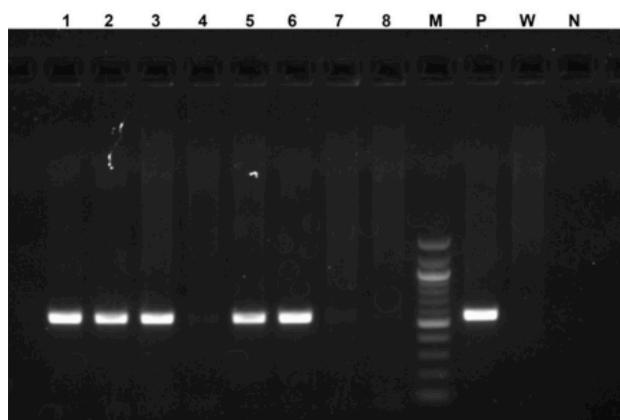
### Genotyping

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 Size: 548bp  
DNA sequence (forward): 5'- GATTTGGCACACCCCTATG-3'  
DNA sequence (reverse ) 5'- TCCCTGAACATGTCCATCAG-3'  
Amplifies 5' arm into Cre sequence.

#### Rxn Buffer and Conditions: (25μl reaction)

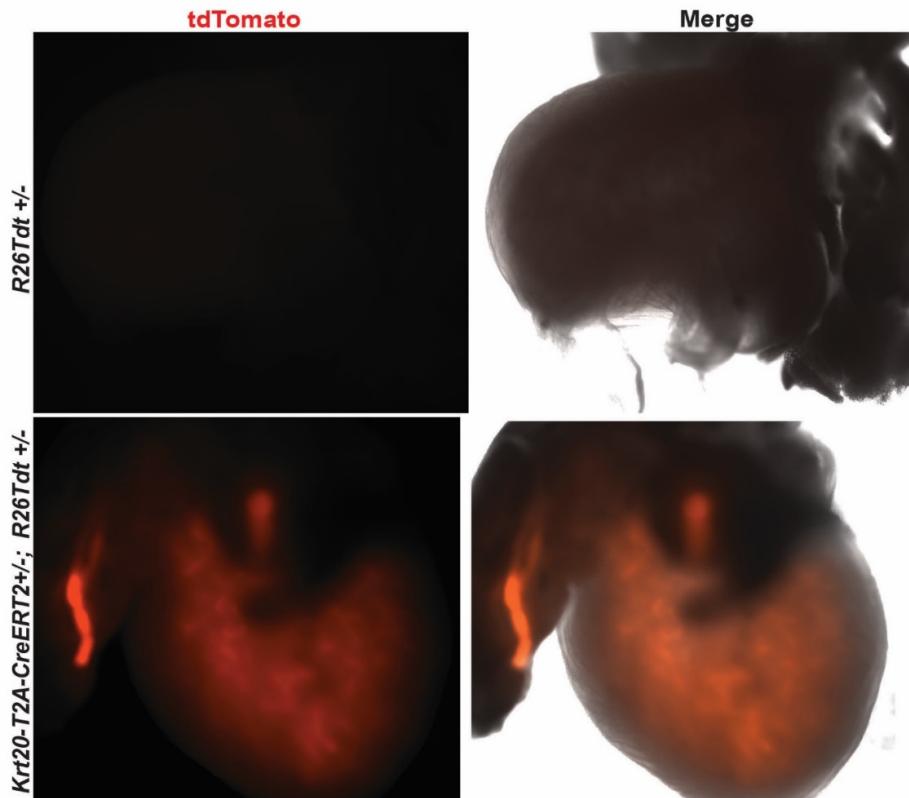
10X PCR Buffer	2.5ul			
1.25mM dNTP	4ul	94°C	3min	1 cycle
10uM primer F	1ul	94°C	20sec	
10uM primer R	1ul	60°C	20sec	35cycles
5x cresol red dye	5ul	72°C	45sec	
Taq polymerase	0.2ul (5u/uL)	72°C	10min	1 cycle
Genomic DNA	1ul			
<b>Total volume</b>	<b>25 ul</b>			



**Figure 2:** Lanes 1, 2, 3, 5 & 6 show pups that display the expected diagnostic PCR product of 548 bp for the targeted allele. **M:** DNA Marker, **P:** Positive control, **Wt:** Wildtype.

## Cre-recombinase Activity

Krt20<sup>T2A-CRE-ERT2</sup> male chimeras were mated to R26R<sup>tdTomato/tdTomato</sup> females to generate Krt20<sup>T2A-CRE-ERT2/+</sup>; R26R<sup>tdTomato/+</sup> pups. In order to activate tdTomato reporter expression, P21 pups were injected intraperitoneally with tamoxifen in corn oil (1X 2mg to 40g body weight) and the tissues were assayed at P23. Tamoxifen dependent Cre activity was detected in cells in the bladder and intestine in Krt20<sup>T2A-CRE-ERT2/+</sup>; R26R<sup>tdTomato/+</sup> samples (Figure 3-7).



**Figure 3.** Tamoxifen dependant tdTomato positive cells are observed in the bladder of Krt20<sup>T2A-CRE-ERT2/+</sup>; R26R<sup>tdTomato/+</sup> P23 day pups after a single injection at P21(2mg/40g body weight).

## Immunohistochemistry

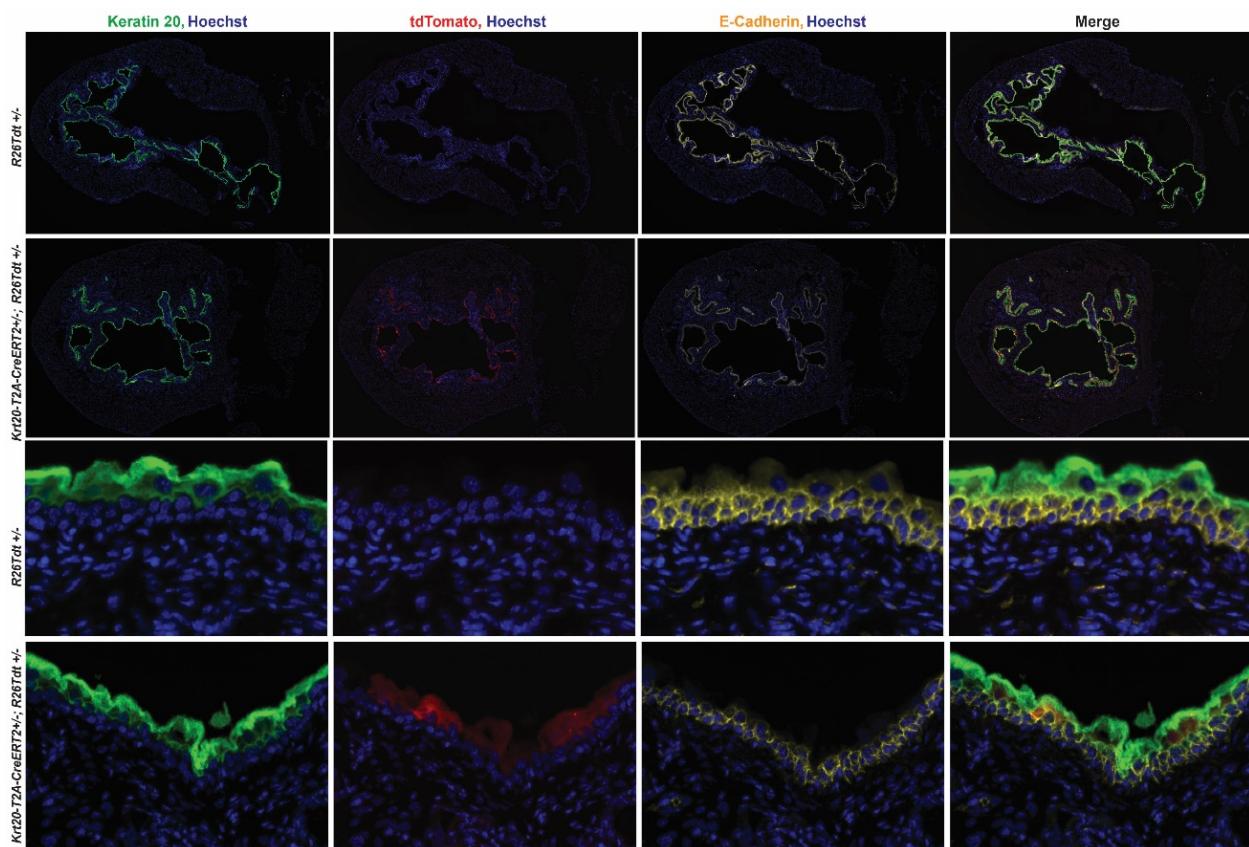
Bladder and intestines were fixed in 4% paraformaldehyde at 4°C for 1 hour, washed 3 times in PBS, equilibrated in 30% sucrose overnight and then embedded in OCT and flash frozen on dry-ice. The tissues were sectioned at 12um and probed with the antibodies listed in (Table 2).

Primary Antibody	Company	Catalog #	Dilution	Secondary	Company	Dilution
Mouse-anti-E-Cadherin	BD Biosciences	610182	1/250	Goat anti-mouse IgG2a-A647	Invitrogen	1/500
Rabbit anti keratin 20	Abcam	Ab118574	1:300	Donkey anti-rabbit Ig G A488	Invitrogen	1/500
Mouse-anti- keratin 20 IgG2a	Dako	M7019	1/200	Goat anti-mouse IgG2a-A488	Invitrogen	1/500
Rabbit anti- RFP	Rockland	600-401-379	1:2000	Donkey anti-rabbit Ig G A647	Invitrogen	1/500
Rabbit anti keratin 5	Covance	PRB-160p	1:1000	Donkey anti-rabbit Ig G A647	Invitrogen	1/500

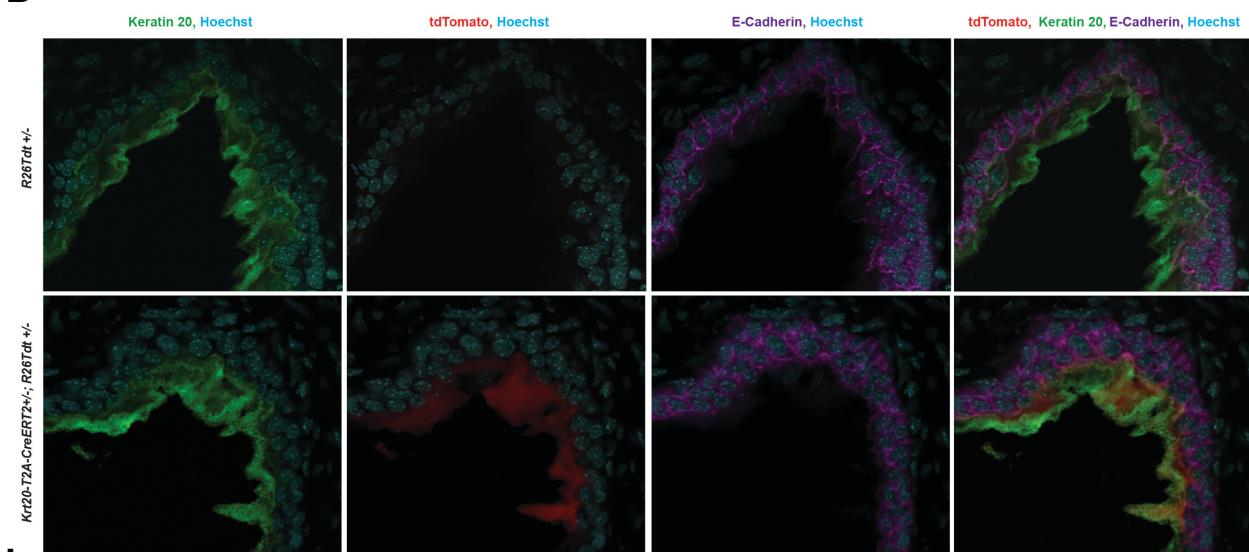
Mouse-anti- Uroplakin III IgG1	Fitzgerald	10R-U103a	1/500	Goat anti-mouse IgG1-A488	Invitrogen	1/500
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**Table 2.** Summary of antibodies used to screen Krt20<sup>T2A-CRE-ERT2/+</sup>; R26R<sup>tdTomato/+</sup> P23 bladder and intestine sections.

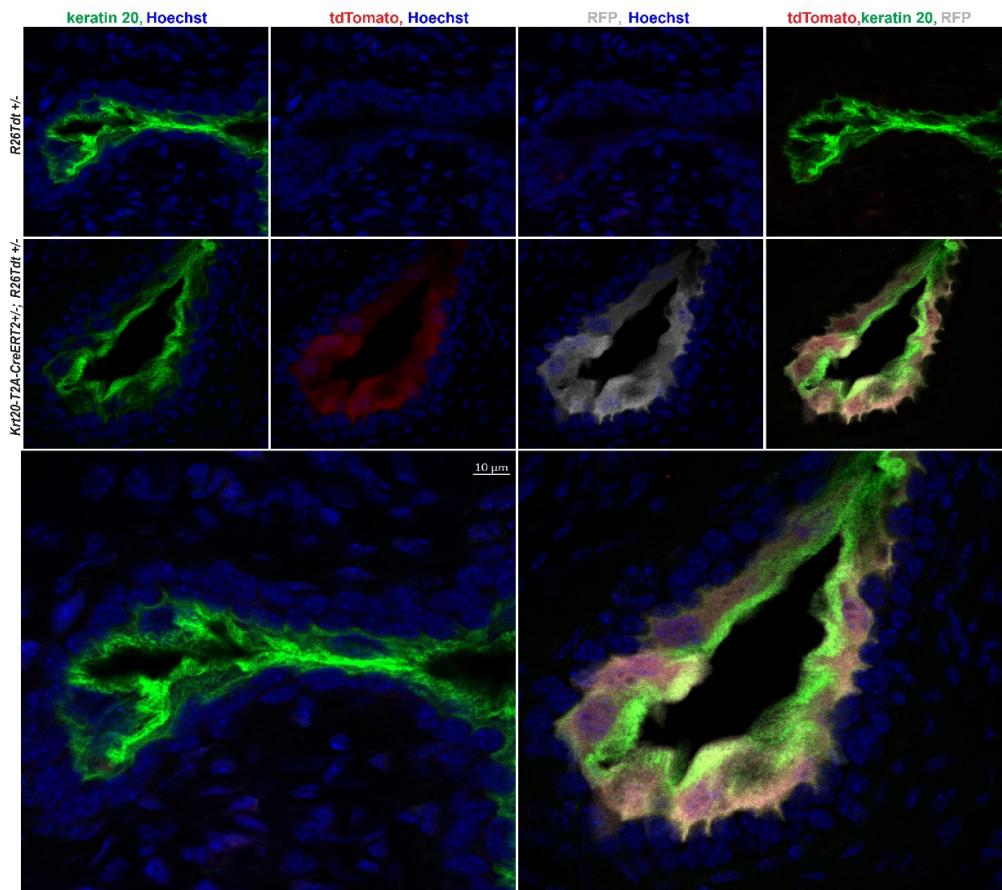
A.



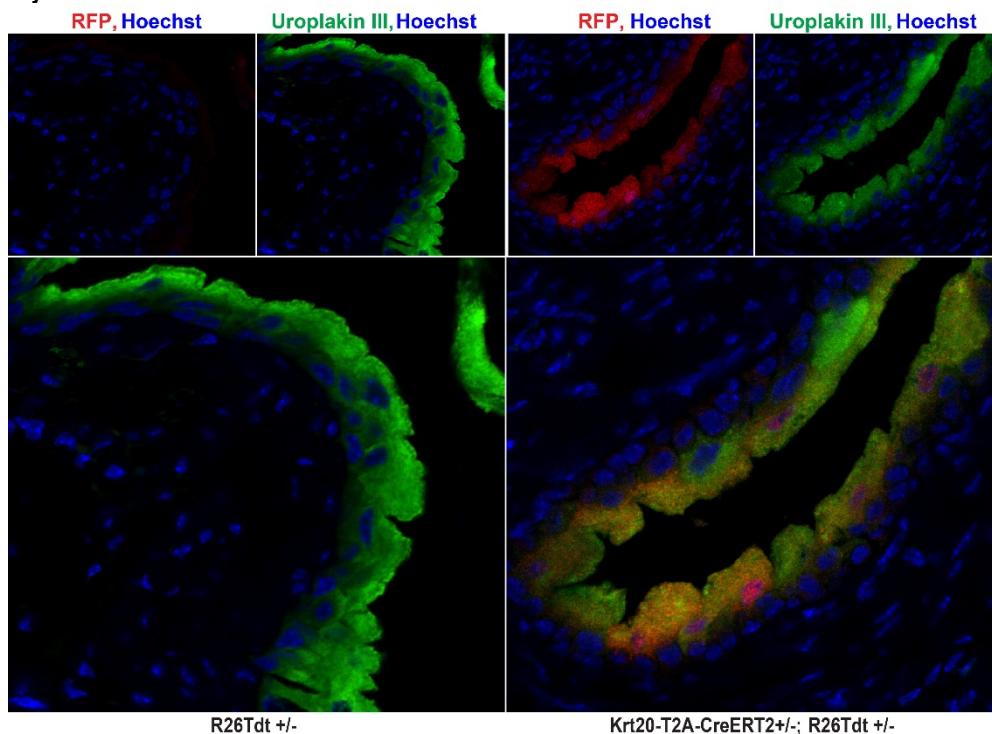
B



**Figure 4.** Tamoxifen dependent tdTomato positive cells in Krt20<sup>T2A-CRE-ERT2/+</sup>; R26R<sup>tdTomato/+</sup> bladder co-localize with Krt20+ and E-Cadherin+ epithelial cells lining the lumen of the bladder, but not with deeper E-Cadherin positive bladder epithelial cells, in P23 days pups: following tamoxifen injection at P21(A&B).

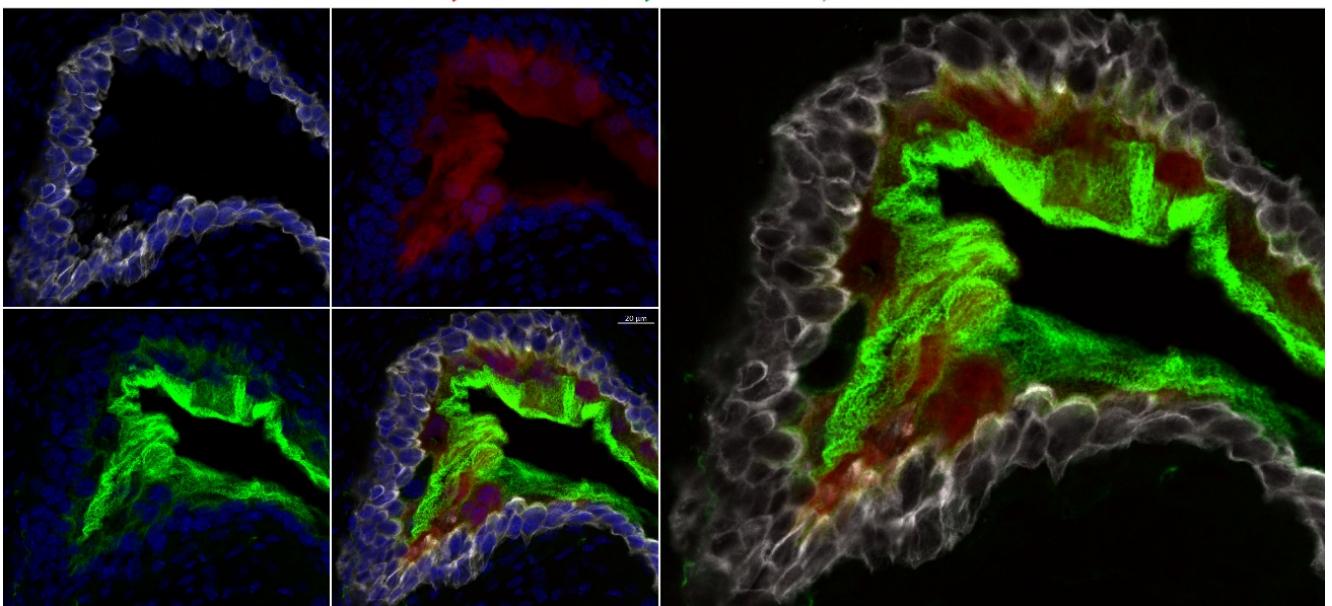


**Figure 5.** Tamoxifen dependent tdTomato positive cells in  $\text{Krt20}^{\text{T2A-CRE-ERT2/+}}$ ;  $\text{R26R}^{\text{tdTomato/+}}$  bladder co-localize with Krt20+ and RFP+ epithelial cells in P23 days pups: tamoxifen injection at P21.



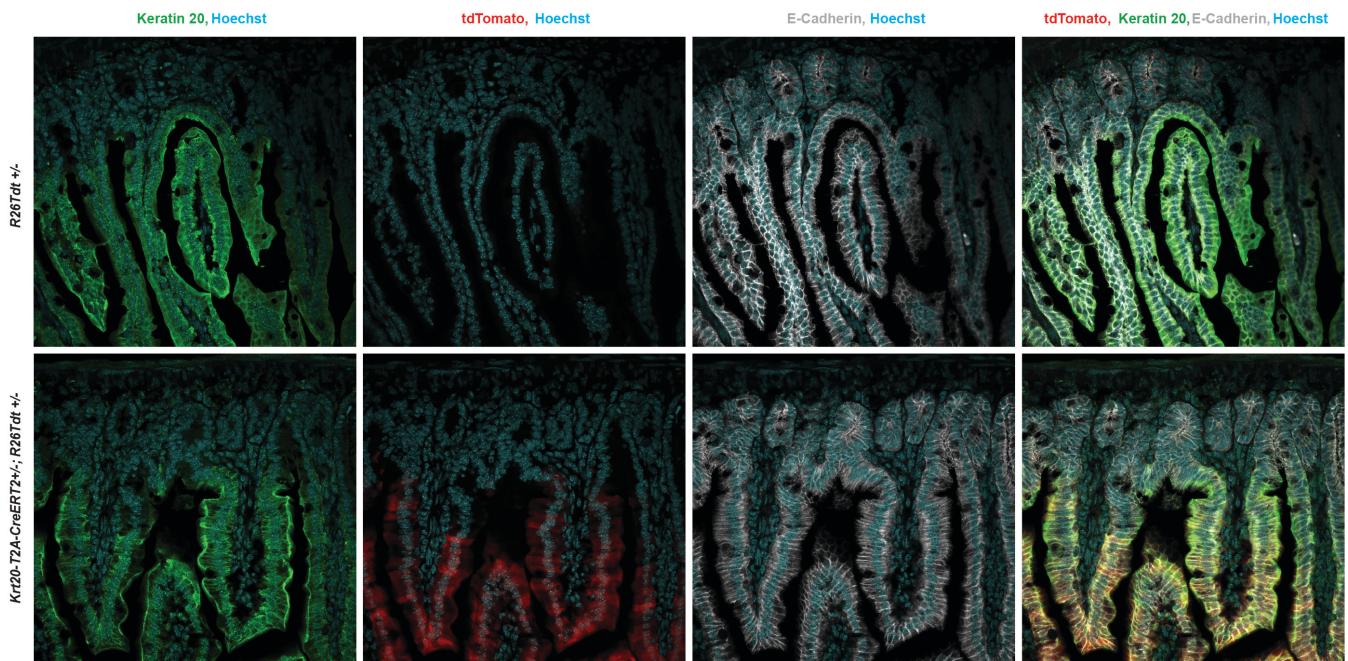
**Figure 6.** A percentage of RFP positive cells in  $\text{Krt20}^{\text{T2A-CRE-ERT2/+}}$ ;  $\text{R26R}^{\text{tdTomato/+}}$  bladder co-localize with Uroplakin III+ epithelial cells in P23 days pups: tamoxifen injection at P21.

**tdTomato, keratin 20, Keratin 5, Hoechst**



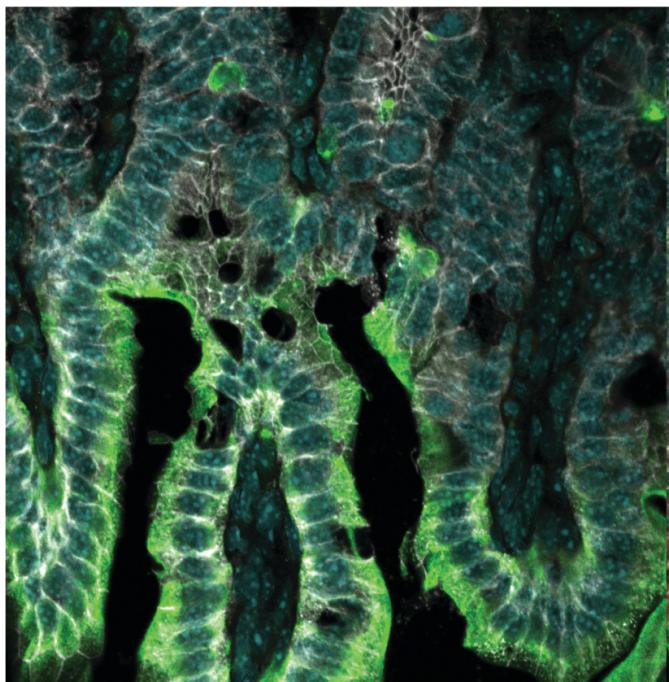
**Figure 7.** A percentage of tamoxifen dependent tdTomato positive cells in  $Krt20^{T2A-CRE-ERT2/+}$ ;  $R26R^{tdTomato/+}$  bladder co-localize with Krt20+ epithelial cells, but do not co-localize with Krt5+ epithelial cells in P23 days pups: tamoxifen injection at P21.

**A.**



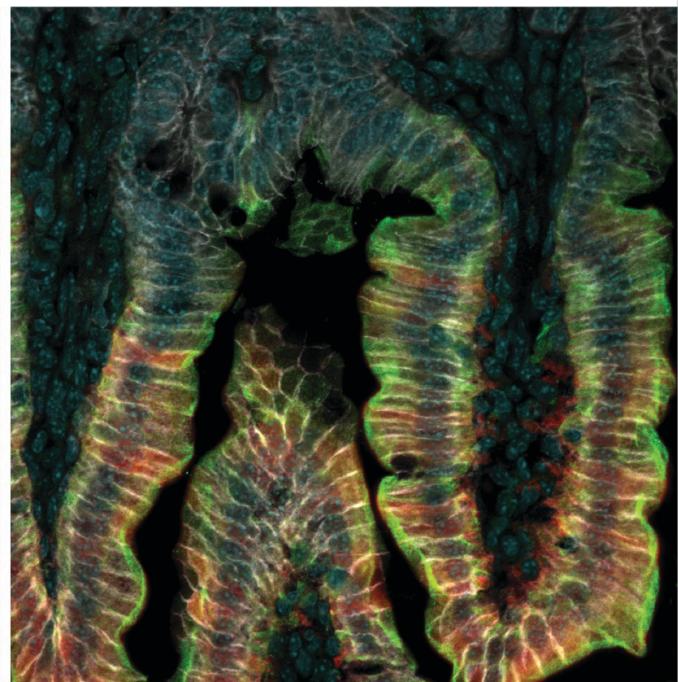
**B.**

Keratin 20, E-Cadherin, **tdTomato**, Hoechst



R26Tdt +/-

Keratin 20, E-Cadherin, **tdTomato**, Hoechst



Krt20-T2A-CreERT2 +/-; R26Tdt +/-

**Figure 8.** Tamoxifen dependent tdTomato positive cells in  $\text{Krt20}^{\text{T2A-CRE-ERT2}+/+}$ ;  $\text{R26R}^{\text{tdTomato}+/+}$  intestines, co-localize with Krt20+ and E-Cadherin+ epithelial cells of the villus facing the lumen, but not E-Cadherin+ cells without contact to the lumen in 23 days pups: tamoxifen injection at P21 (A&B).