Cited-1 nuc-TagRFP-T Allele Characterization

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Findings: VALIDATED

Our analysis confirms the expression of nuc-TagRFP-T under the regulation of Cited1 in cap mesenchyme cells apposing the ureteric buds in 15.5 dpc kidneys and in the male reproductive system. At 15.5dpc nuc-TagRFP-T co-localizes with Six2 expressing cells at the tips of the ureteric buds but not in the mesenchymal population of pre-tubular aggregate cells. Analysis of the expression in the male reproductive system was not included in this characterization.

Data:

Crosses

The Cited1 nuc-TagRFP-T strain is a BAC transgenic line with nuc-TagRFP-T expressed in the Cited1 domain: Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain 1. Pronuclear injection of the BAC construct DNA into C57Bl6/DBA F1 embryos resulted in the birth of 67 pups of which 9 male and 8 females carried the transgene. The male founders were crossed to C57Bl6 females and the urogenital system (UGS) was collected from 15.5 dpc embryos. Of the nine founder males tested, seven transmitted the transgene and three correctly expressed the nuc-TagRFP-T in the expected cell population: M26, M28 and M40 (Table 1). Further analysis was carried out on the M26 line.

Date of Birth	Pups born	Founders	Founders mated	Transmittal	Visible Reporter	Correct Reporter Activity	Antibody to Reporter
31-May-10	67	9M, 8F	M26	Yes	Yes	Yes	Yes
			M28	Yes	Yes	Yes	Yes
			M50	No	*nd*	*nd*	*nd*
			M53	Yes	No	*nd*	*nd*
			M66	Yes	No	*nd*	*nd*
			M40	Yes	Yes	Yes	*nd*
			M9	Yes	No	*nd*	*nd*
			M42	No	nd	*nd*	*nd*
			M6	Yes	Yes	No	*nd*

Table 1. Transmission analysis of male 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: ~458bp
DNA sequence (forward): 5'-ACCCCGACTTGGAATTTTCT-3'
DNA sequence (reverse 2) 5'-TTCTGCTGCCGTACATGAAG-3'
Amplifies 5' arm into TagRFP-T sequence within TagRFP-T

Rxn Buffer and Conditions: (25µl reaction)

10X GSB	2.5ul
25mM dNTP	1ul
10uM primer F	1ul
10uM primer R	1ul
DMSO	2.5ul
2-mercaptoethanol	0.125ul
Amplify Taq	0.3ul (5u/ul)
5x cresol red dye	2.5ul
Genomic DNA	1ul
Total volume	**25 ul**

94ºC	3min	1 cycle
94ºC	30sec	35cycles
56ºC	60sec	
72ºC	90sec	
72ºC	10min	1 cycle

10X Gitschier Buffer (GSB):
670 mM Tris, pH 8.8
166 mM Ammonium Sulfate
65 mM MgCl2
0.1% gelatin

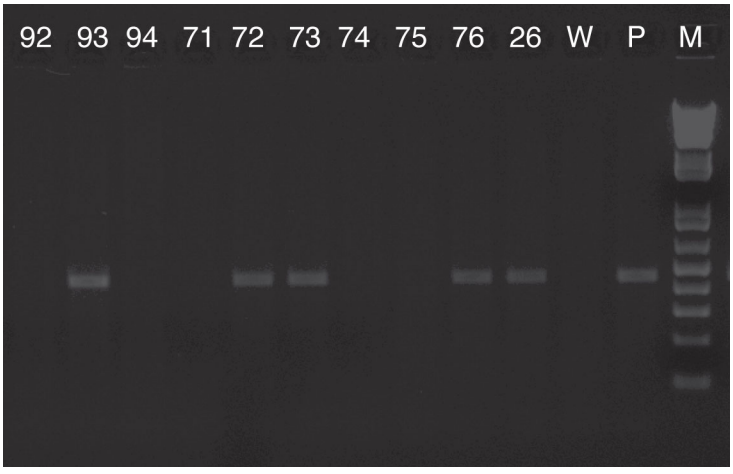

Fig 1. Numbers 93, 72, 73, 76 and 26 Cited $1^{nuc\text{-}TagRFP\text{-}T/+}$, numbers 92, 94, 71, 74 & 75 Wildtype, **P:** Cited $1^{nuc\text{-}TagRFP\text{-}T/+}$ positive control; **W**: Wildtype control; **N**: Negative control.

Native Fluorescence

15.5dpc UGSs were examined with a fluorescent microscope to view TagRFP-T expression. TagRFP-T can be seen in whole mount in the developing cap mesenchyme in the kidney and in the male reproductive track (Fig 2).

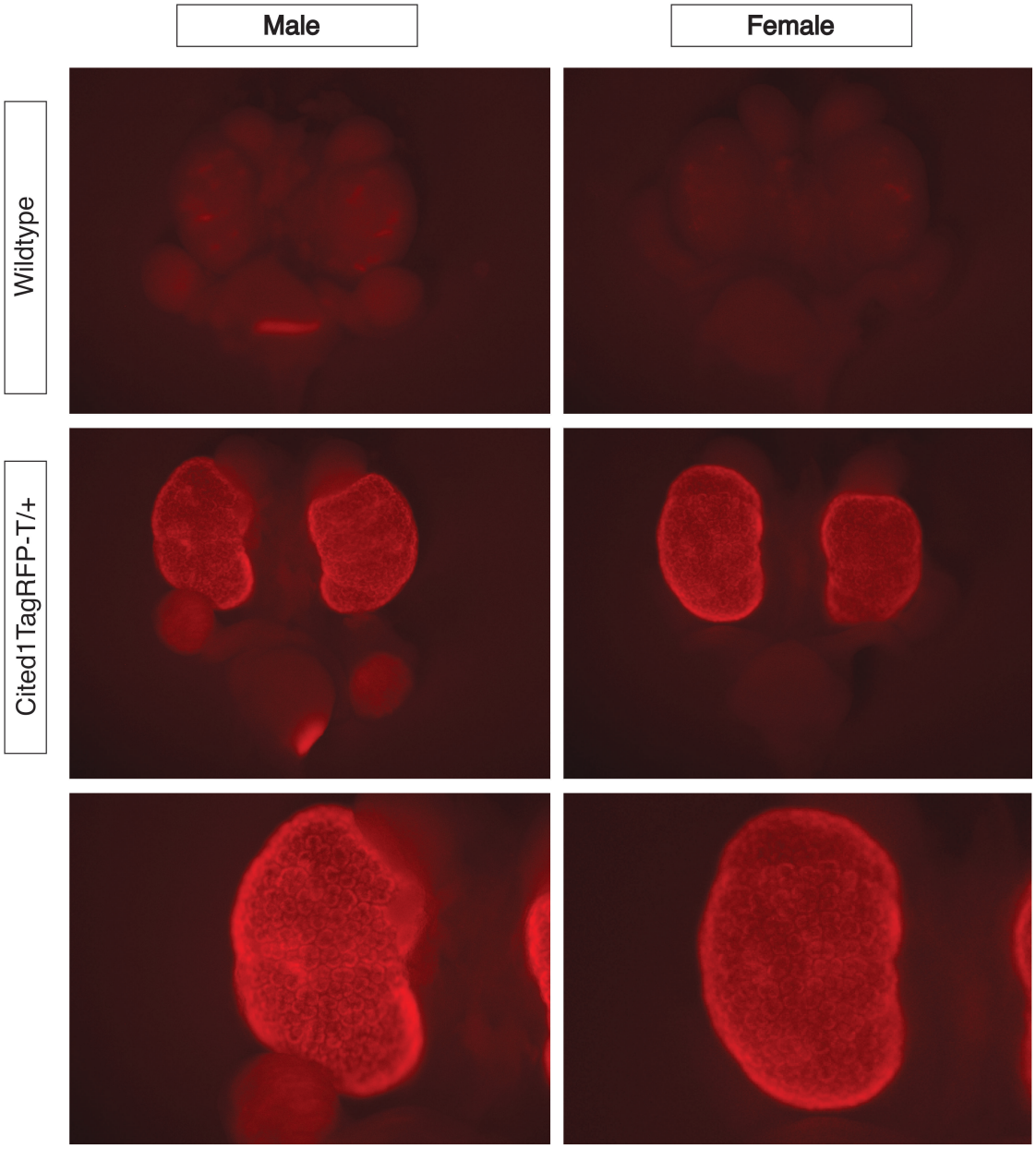

Fig 2. Wholemount nuc-TagRFP-T detection in 15.5dpc Cited1$^{nuc\text{-}TagRFP\text{-}T/+}$ UGS. Nuc-TagRFP-T fluorescence was visible in the cap mesenchyme cells of the nephrogenic zone in 15.5dpc kidneys and the male reproductive system. Nuc-TagRFP-T expression was limited to the Cited1 domain.

Immunohistochemistry

Immunohistochemistry was performed to examine whether TagRFP-T was expressed in the expected Cited1 domain. Nuc-TagRFP-T expression was examined by probing Cited1$^{nuc\text{-}TagRFP\text{-}T/+}$ 15.5dpc embryos with rabbit-anti-Cited1, rabbit-anti-TagRFP-T and rabbit-anti-Six2 antibodies and comparing these positive cell populations to native nuc-TagRFP-T expression. Co-localization of native nuc-TagRFP-T and cells positive for the Cited1 and TagRFP-T antibodies were observed in the cap mesenchyme cells surrounding the tips of the ureteric buds (Fig 3 and 4).

The population of nuc-TagRFP-T expressing cells observable by native fluorescence is apparently contained within the domain visualized with anti-Six2 antibodies (Fig 5). To clarify the extent of the overlapping domains Cited1$^{nuc\text{-}TagRFP\text{-}T/+}$ male mice were mated to Six2$^{TGC/+}$ females. The Six2-TGC mouse strain is a BAC transgenic line expressing GFP:Cre in the Six2 domain. Cited1$^{nuc\text{-}TagRFP\text{-}T/+;}$; Six2$^{TGC/+}$ 15.5dpc embryos were probed with rabbit-anti-TagRFP-T and chicken-anti-GFP antibodies. Anti-TagRFP-T and anti-GFP positive cells co-localize in cap mesenchyme cells surrounding the tips of the ureteric bud, but not with GFP positive cells delineating the Six2 population in pre-tubular aggregates undergoing mesenchyme to epithelial transition (Fig 5 and 6).

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 16μm and probed with: rabbit anti-TagRFP-T/mouse-anti-Cytokeratin, rabbit-anti-Cited1/mouse-anti-Cytokeratin, rabbit-anti-Six2/mouse-anti-Cytokeratin, rabbit-anti-Six2/mouse-anti-Cytokeratin and rabbit-anti-TagRFP-T/ Chicken-anti-GFP/mouse-anti-Cytokeratin respectively, incubated overnight at 4ºC and detected with secondary antibodies under the conditions listed in (Table 2).

Primary Antibody	Company	Catalog #	Dilution	Secondary	Company	Dilution
Rabbit-anti-TagRFP-T	Evrogen	AB234	1/500	Donkey-anti-rabbit-A555	Invitrogen	1/500
Rabbit-anti-Cited1	Lab Vision	RB-9219	1/500	Donkey-anti-rabbit-A488	Invitrogen	1/500
Rabbit-anti-Six2	Covance Custom polyclonal	HM6123	1/500	Donkey-anti-rabbit-A488	Invitrogen	1/500
Chicken-anti-GFP	Aves Labs, Inc	GFP-1020	1/500	Goat-anti-chicken-A488	Invitrogen	1/500
Mouse-anti-Cytokeratin IgG1	Sigma	C2562	1/500	Goat-anti-mouse IgG1-A647	Invitrogen	1/500

Table 2. List of primary and secondary antibodies used for analysis.

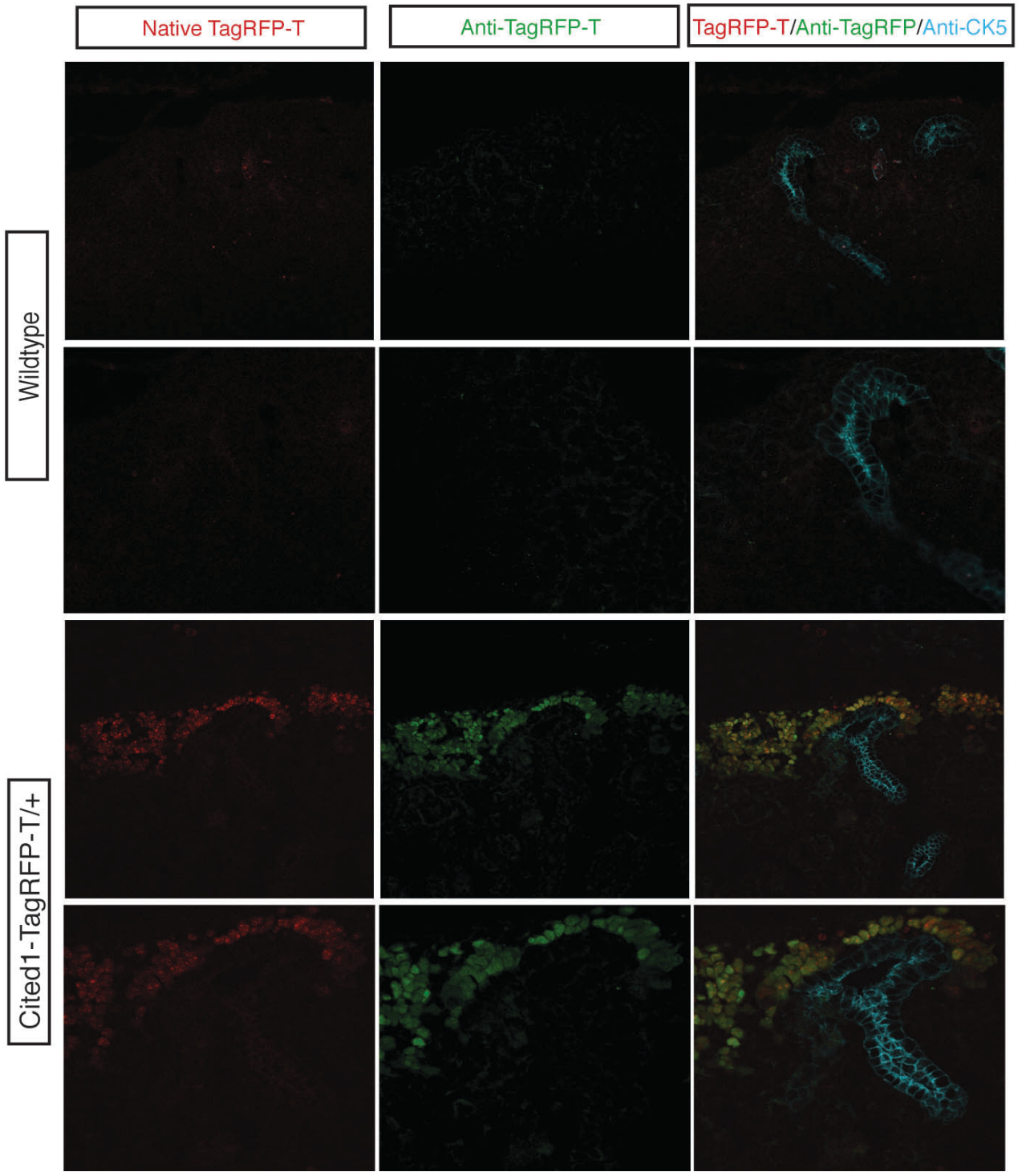

Fig 3. TagRFP-T fluorescent cells apposing the ureteric bud, co-localize with cells detectable with TagRFP-T antibody. Cited1$^{nuc\text{-}TagRFP\text{-}T}$ 15.5 dpc kidneys were probed with anti-TagRFP-T and anti-Cytokeratin 5 antibodies. Co-localization of native TagRFP-T fluorescent cells and cells positive for anti-TagRFP-T antibody are observable in the cap mesenchyme at the tips of the ureteric buds (Cytokeratin positive).

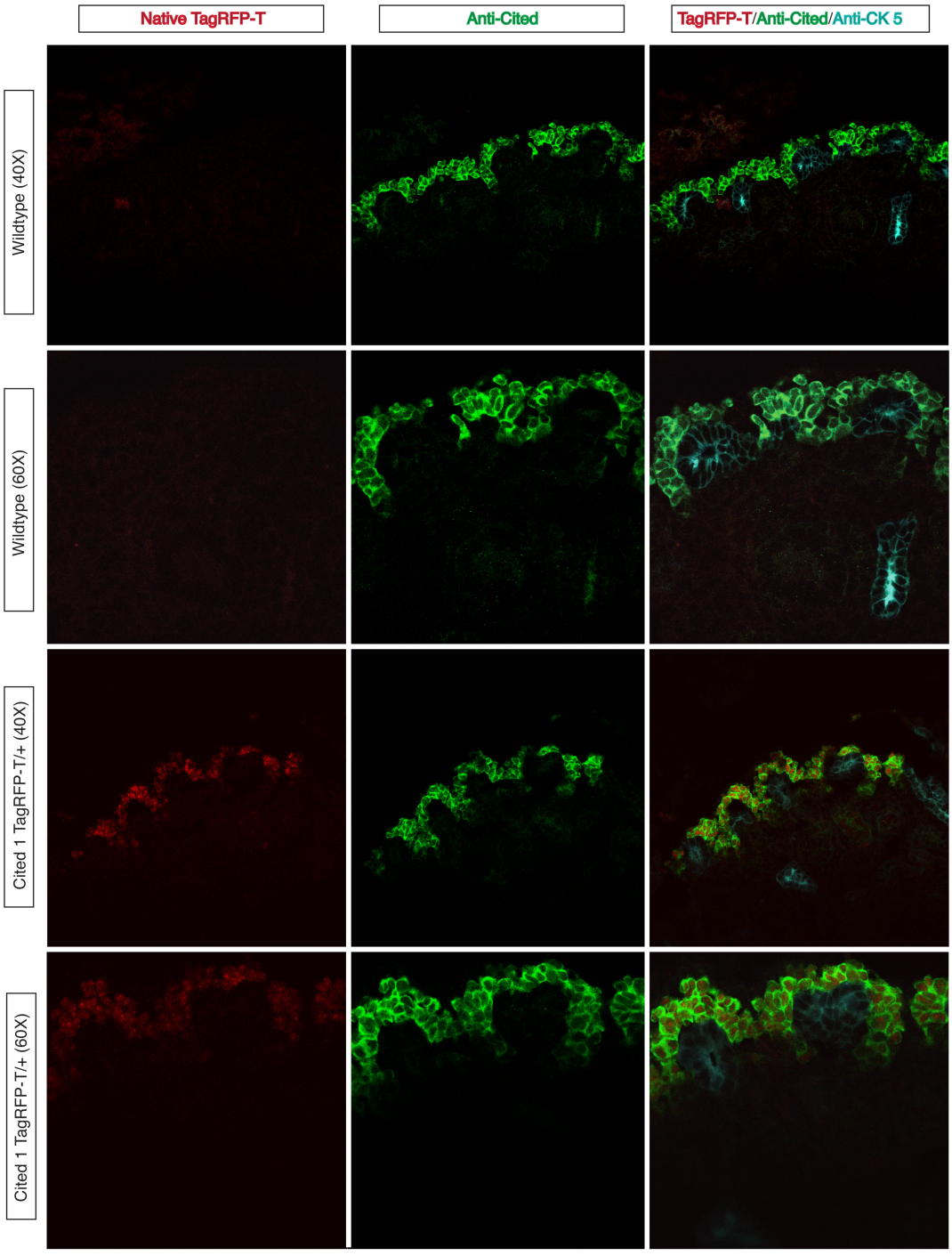

Fig 4. TagRFP-T fluorescent cells in the cap mesenchyme co-localize with cells detectable with anti-Cited 1 antibody. Cited1$^{nuc\text{-}TagRFP\text{-}T}$ 15.5 dpc kidneys were probed with anti-Cited1 and anti-Cytokeratin 5 antibodies. Co-localization of native TagRFP-T fluorescent cells and cells positive for anti-Cited1 antibody are observable in the cap mesenchyme at the tips of the ureteric buds (Cytokeratin positive).

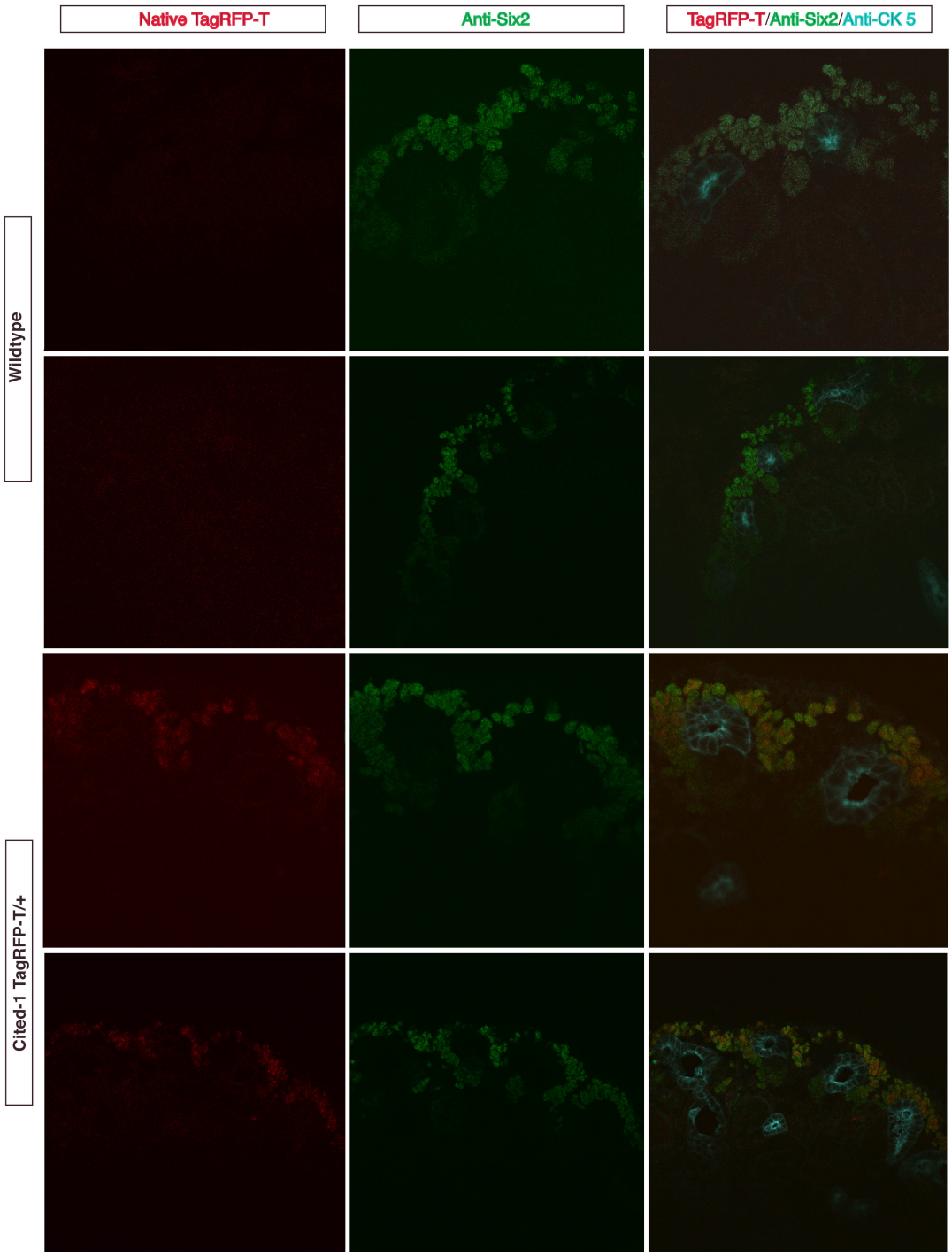

Fig 5. TagRFP-T fluorescent cells in the cap mesenchyme co-localize with cells detectable with anti-Six2 antibody. Cited1$^{nucTagRFP-T}$ 15.5 dpc kidneys were probed with anti-Six2 and anti-Cytokeratin 5 antibodies. Co-localization of native TagRFP-T fluorescent cells and cells positive for anti-Six2 antibody are observable in the cap mesenchyme at the tips of the ureteric buds (Cytokeratin positive) but not in the Six2 positive pre-tubular aggregates.

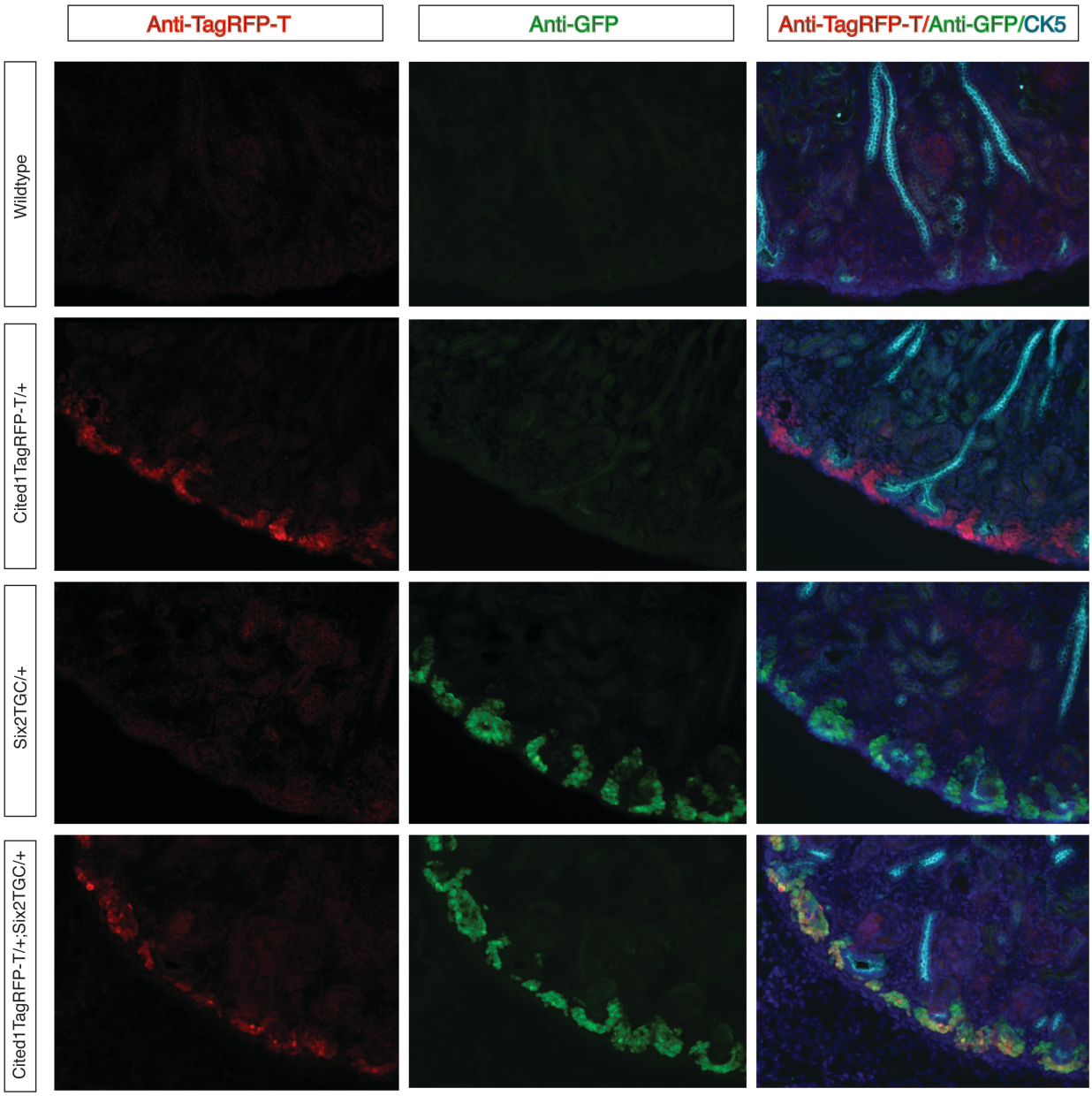

Fig 6. TagRFP-T expressing cells in the cap mesenchyme co-localize with a subpopulation of cells expressing GFP in Cited1$^{nuc\text{-}TagRFP\text{-}T}$; Six2$^{TGC/+}$ 15.5 dpc kidneys. UGSs were probed with anti-TagRFP-T, anti-GFP and anti-Cytokeratin 5 antibodies. Co-localization of TagRFP-T and GFP positive cells were observable in the cap mesenchyme at the tips of the ureteric buds (Cytokeratin positive) but not in the Six2 positive pre-tubular aggregates.

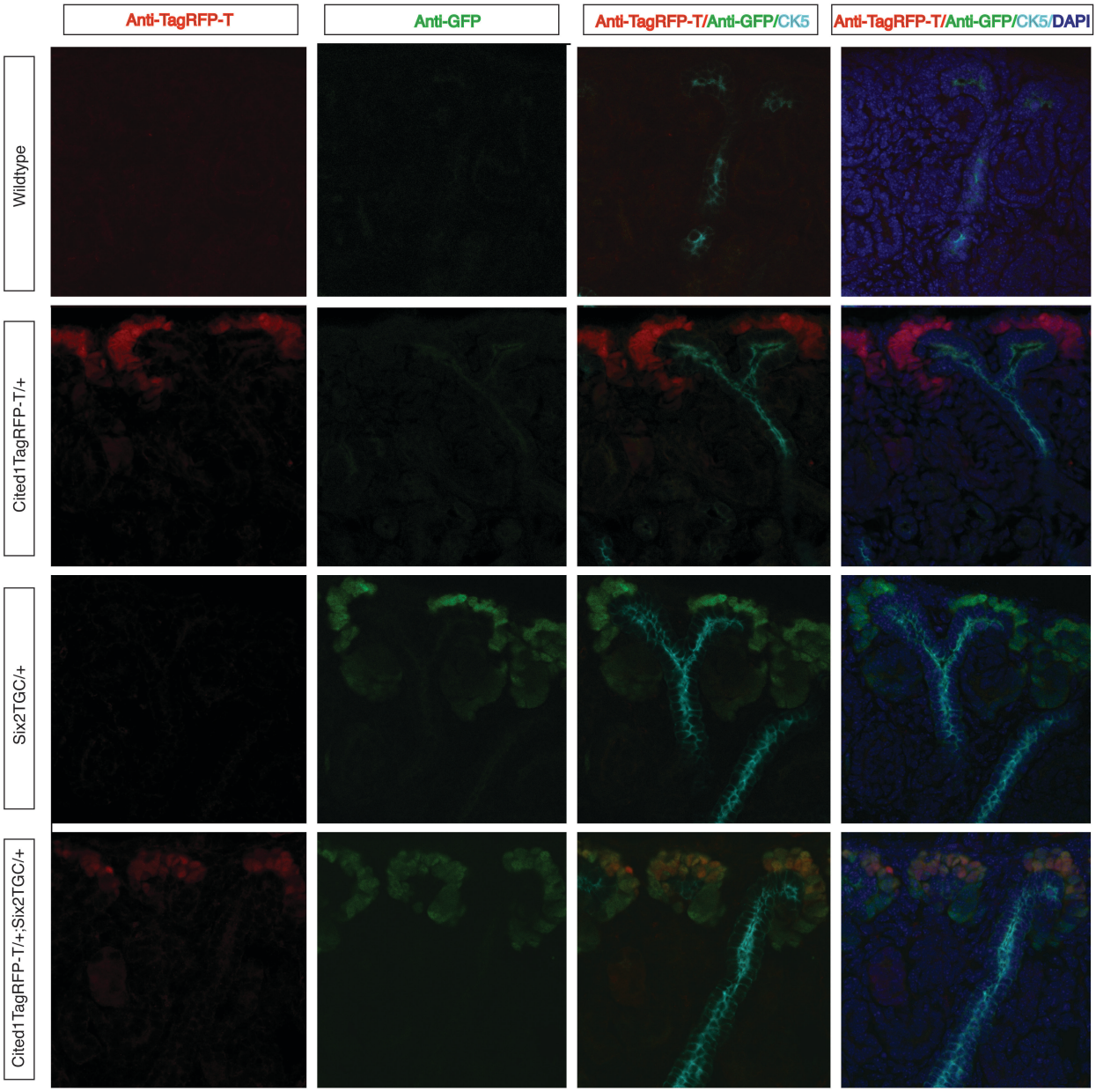

Fig 7. TagRFP-T expressing cells in the cap mesenchyme co-localize with a subpopulation of cells expressing GFP in Cited1$^{nucTagRFP-T}$; Six2$^{TGC/+}$ 15.5 dpc kidneys. UGSs were probed with anti-TagRFP-T, anti-GFP and anti-Cytokeratin 5 antibodies. Co-localization of TagRFP-T and GFP positive cells were observable in the cap mesenchyme at the tips of the ureteric buds (Cytokeratin positive) but not in the Six2 positive population of cells undergoing mesenchyme to epithelial transition.