Akr1b7-TagRFP-T BAC Transgene Characterization

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Created: 10 May 2010
Updated: 24 May 2010
Submitted: 6 June 2010
Version: final
Tags: &kmap&mousestrains&gudmap&characterization & Akr1b7-TagRFP-T
New Fig 6 added March 2011

Findings: VALIDATED

Our analysis has confirmed expression of TagRFP-T cells in the adrenal glands and in association with the intrarenal arteries of the kidney. Potential expression in non-kidney components of the urogenital system were not characterized but have been reported (Taragnet C et al, 1988, J Reprod Fertil 83:835-842). Renal vasculature associated expression is observed in Smooth Muscle Actin positive cells that are closely associated with Flk 1 and PECAM positive intrarenal arteries. The expression of the Akr1b7-TagRFP-T is very similar to the expression of the Renin gene described in (Jones CA et als, 2000 Physiol Genomics 4:75-81) and may indicate a co-localization in the expression domains of the two genes. This possibility is being followed up.

Data:

Crosses

The Akr1b7-TagRFP-T strain is a BAC transgenic line with TagRFP-T expressed in the Akr1b7 domain. Pronuclear injection of the BAC construct DNA into C57Bl6/DBA F1 embryos resulted in the birth of 50 pups of which 5 male and 10 females carried the transgene. The male founders were crossed to C57Bl6 females and the urogenital system (UGS) was collected from 15.5 dpc embryos. Three founder males transmitted the transgene: M9, M10 and M46. Akr1b7$^{TagRFP\text{-}T/+}$ embryos from three litters collected for each founder exhibited similar expression patterns for the TagRFP-T, subsequent analysis was carried out on founder M9. The TagRFP-T reporter was expressed at high levels in the adrenal glands and in the medullary arterioles.

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: ~385bp
DNA sequence (forward): 5'- `ttctgcccagatagcctttg` -3'
DNA sequence (reverse 2) 5'- `cctcgaccaccttgattctc` -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	
58.5ºC	60sec	35cycles
72ºC	90sec	
72ºC	10min	1 cycle

10X PCR Buffer
500mM KCL
100mM Tris-HCl pH8.4
15mM MgCl2
200ug/ml gelatin, swine

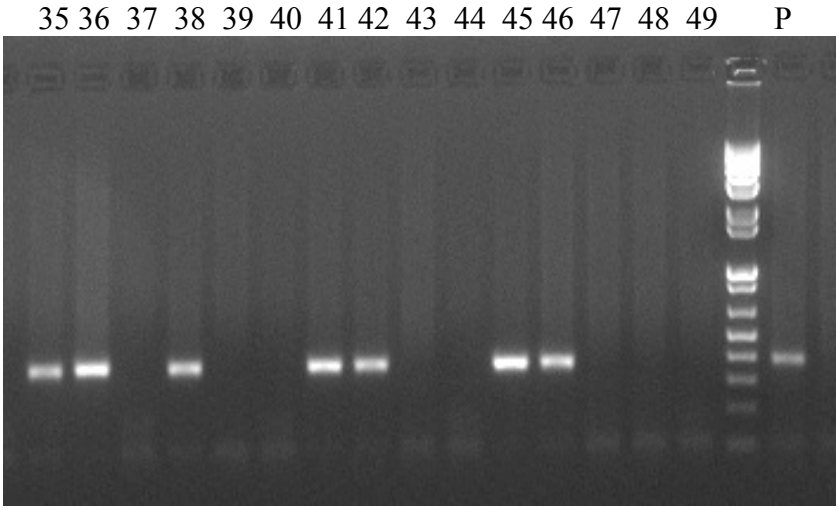

Fig1: Akr1b7-TagRFP-T genotyping. Founders 35,36,38,41,42,45 and 46 Akr1b7$^{TagRFP-T/+,}$ P**:** Akr1b7-TagRFP-T BAC DNA

Native Fluorescence

Whole embryos as well as dissected UGSs were examined with a fluorescent microscope to view TagRFP-T expression. In 10 Akr1b7$^{TagRFP-T/+}$ F1 embryos and 20 Akr1b7$^{TagRFP-T/+}$ F2 embryos TagRFP-T was expressed strongly in the adrenal glands and in the endothelial cells of the medullary vasculature.

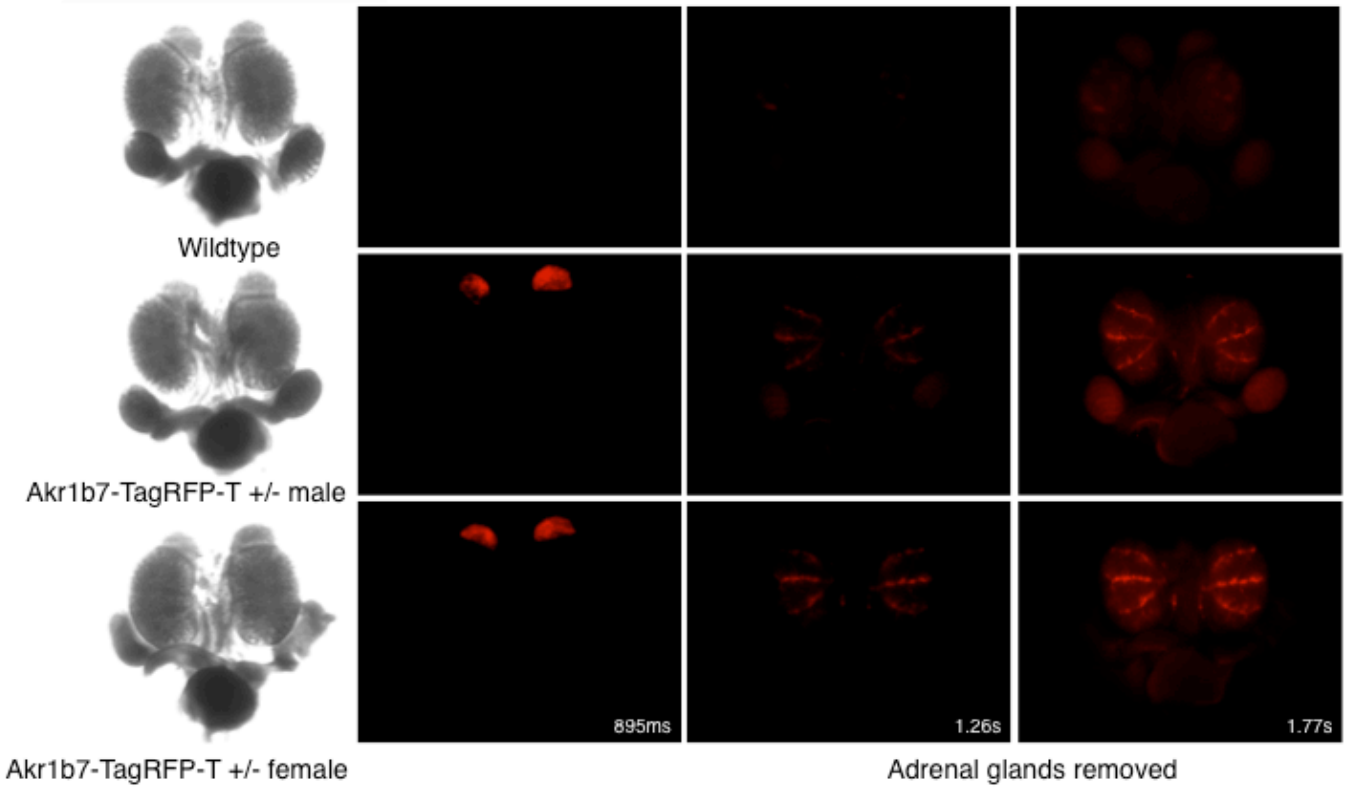

Fig 2. Wholemount TagRFP-T detection in 15.5dpc Akr1b7$^{TagRFP-T/+}$ UGS.
Strong TagRFP-T fluorescence was visible in the adrenal glands from dissected UGSs. Expression in the medullary vasculature was more apparent after removal of the adrenal glands. TagRFP-T expression was limited to the Akr1b7 domain.

Immunohistochemistry Immunohistochemistry was performed to examine whether the TagRFP-T allele was expressed in the expected Akr1b7 domain. UGS samples from 15.5 dpc Akr1b7 TagRFP-T +/- embryos and wildtype litter mates were examined.
Whole UGSs were fixed in 4% paraformaldehyde at 4ºC for 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 stained with either; rabbit-anti-tRFP/rat-anti-PECAM/mouse-anti-Cytokeratin, rabbit-anti-tRFP/rat-anti-Flk1/mouse-anti-Cytokeratin, or rabbit-anti-tRFP/mouse-anti-Actin, α-Smooth Muscle/ chicken-anti-Laminin. Anti-tRFP (Rabbit, Evrogen AB234,1:500); anti-Actin, α-Smooth Muscle

(Mouse IgG2a, Sigma, A5228, 1: 1000); anti-Flk1 (Rat, BD Pharmingen, 555307, 1:1000); anti-PECAM (Rat, BD Pharmingen, 553370, 1:1000);anti- Laminin (Chicken, Abcam, ab14055, 1:500); anti-Cytokeratin (Mouse IgG1, Sigma, C 2562, 1:500) were incubated overnight at 4ºC and detected with secondary antibodies Alexafluor 488, 568, 633, and 647 (Molecular probes) as indicated in the figure.

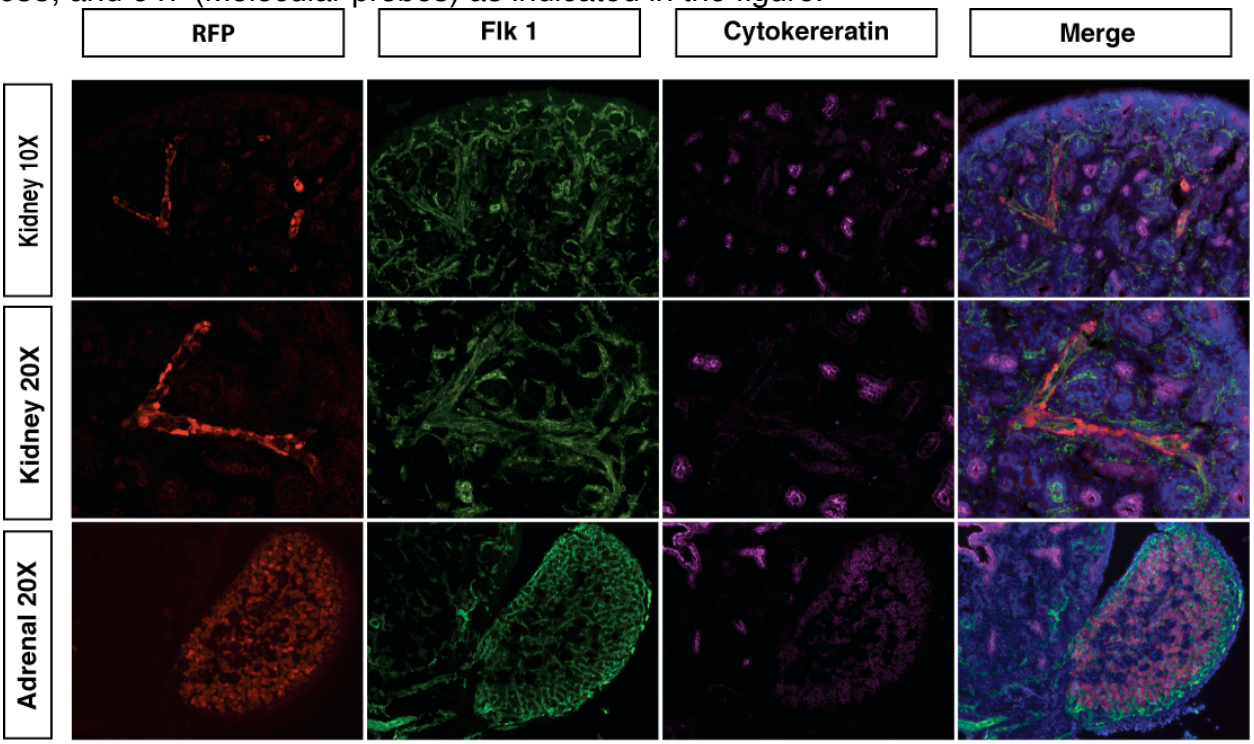

Fig 3. TagRFP-T signal is detected strongly in the adrenal glands and in the medullary vasculature of Akr1b7$^{TagRFP-T/+}$ embryos. 15.5 dpc UGS from Akr1b7$^{TagRFP-T/+}$ embryos probed with anti-TagRFP-T, anti-Flk 1(endothelial cell), cytokeratin antibodies, DAPI nuclear staining.

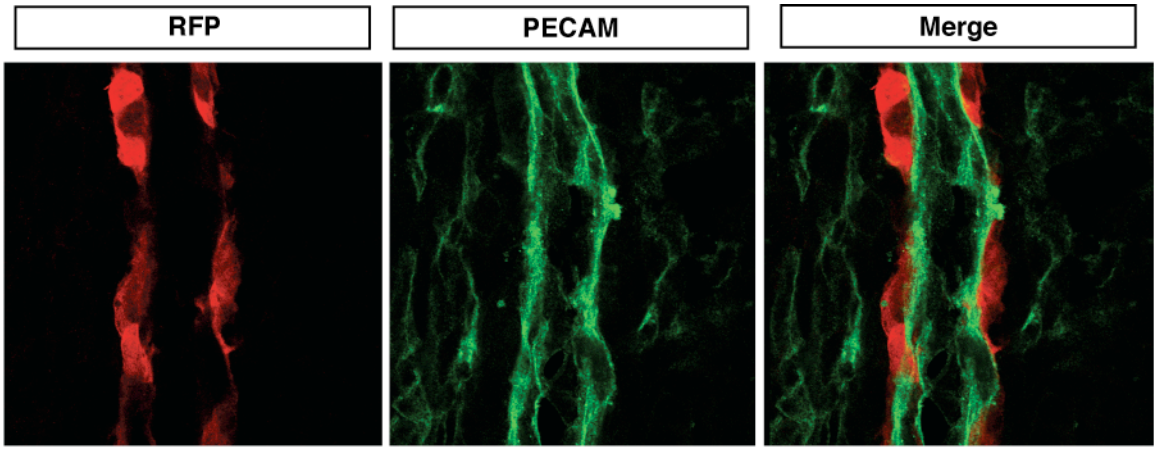

Fig 4. TagRFP-T positve cells are closely associated but distinct from PECAM positive medullary arterioles in the kidneys of 15.5 dpc Akr1b7$^{TagRFP-T/+}$ embryos.

Kidneys from Akr1b7$^{TagRFP-T/+}$ embryos probed with anti-TagRFP-T, and anti-PECAM (endothelial cell) antibodies show close apposition of the TagRFP-T positive cells with PECAM positive endothelial cells.

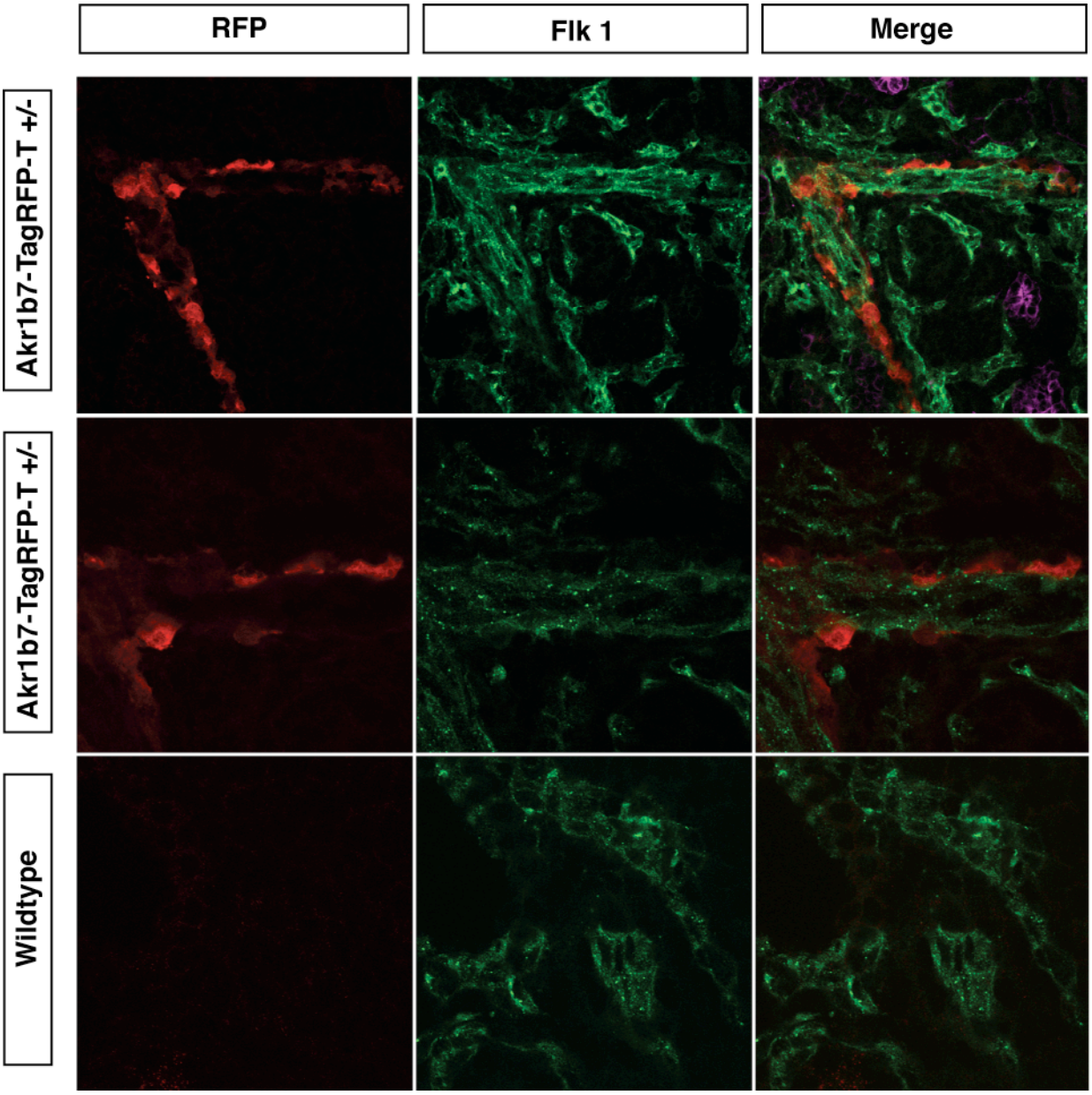

Fig 5. TagRFP-T signal is associated with the medullary vasculature in the kidneys of 15.5 dpc Akr1b7$^{TagRFP-T/+}$ embryos. Kidneys from Akr1b7$^{TagRFP-T/+}$ and wildtype embryos probed with anti-TagRFP-T, anti-Flk 1 (endothelial cells) and anti-Cytokeratin antibodies show a close association of the TagRFP-T expressing cells with Flk 1 positive endothelial cells of the medullary arterioles.

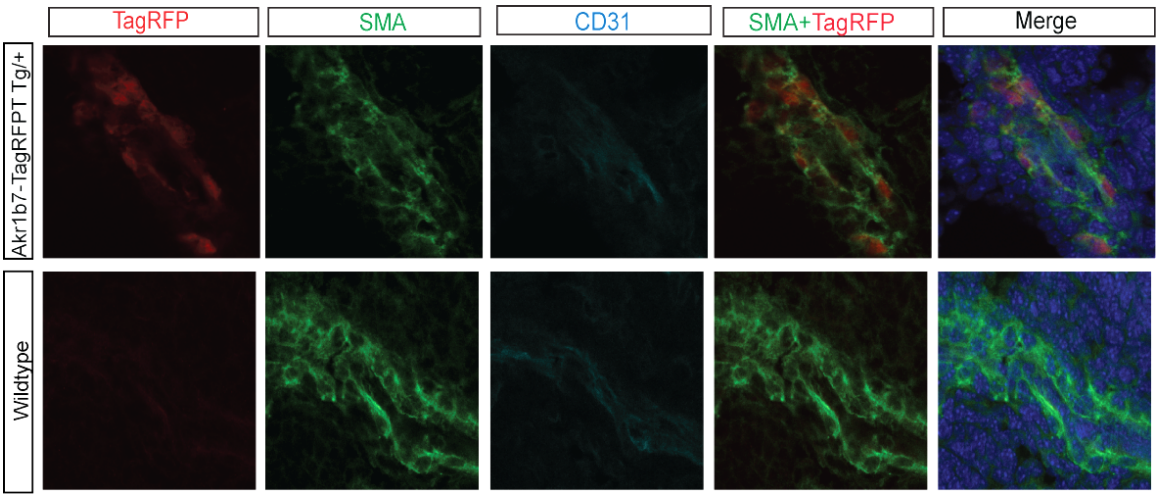

Fig 6. TagRFP-T signal is detected in close association with endothelial cells of the medullary vasculature in the kidney of 15.5 dpc Akr1b7$^{TagRFP-T/+}$ embryos. Kidneys from Akr1b7$^{TagRFP-T/+}$ and wildtype embryos probed with anti-TagRFP-T, anti-PECAM (endothelial cell) and anti-Smooth Muscle Actin antibodies show a coexpression of the TagRFP-T and Smooth Muscle Actin in non endothelial cells closely associated with the medullary arterioles.