

S100b-GCE Allele Characterization

Authors: Jinjin Guo, Jill McMahon, M. Todd Valerius, and Andrew P. McMahon

Created: 5 October 2010
Updated: 12 October 2010

Version: 3 - Final
Submitted: 22 October 2010

Findings: **VALIDATED**

Our analysis confirmed the expression of eGFP^{CreER}T² cells at 15.5dpc under the regulation of S100b in chondrocytes in developing bone and in neural cells in the dorsal root ganglia (DRG). Cre dependent β-galactosidase (β-Gal) activity is observed in a subset of the GFP positive cells in bone and to a lesser extent in the DRG upon Tamoxifen treatment. GFP positive cells co-localize with S100b expressing cells in bone and DRG.

Data:

Crosses

The S100b-GCE strain is a BAC transgenic line with eGFP^{CreER}T² (GCE) expressed in the S100b domain. Pronuclear injection of the BAC construct DNA into C57Bl6/DBA F1 mouse embryos resulted in the birth of 68 pups of which 6 male and 6 females carried the transgene. Four male founders were crossed to Rosa26R^{lacZ/+} (R26R^{lacZ/+}) females and 15.5dpc embryos were collected, one male proved to be sterile. Although, three founder males transmitted the transgene: M22, M56 and M40, no native GFP fluorescence was detectable in whole mount in S100b^{GCE/+} embryos. Subsequent analysis was carried out on M22 and M56.

We crossed M22 and M56 S100b^{GCE/+}; males with R26R^{lacZ/+} female mice to obtain S100b^{GCE/+}; R26R^{lacZ/+} embryos. In order to activate β-galactosidase (β-gal) reporter expression from the R26R^{lacZ/+} allele, an intraperitoneal injection of Tamoxifen in corn oil (1mg to 40g body weight) was injected into pregnant 13.5dpc mice. A control group was injected with the same volume of corn oil. Embryos from Tamoxifen induced females were dissected at 15.5-16.5dpc and Tamoxifen dependant β-gal activity was observed in the chondrocytes but not in neurons in the peripheral nervous system. By doubling the volume of Tamoxifen injected and injecting the pregnant females at 11.5 and 13.5dpc, a small number of β-gal positive cells were observed in neurons in DRG and an increased number of β-gal positive cells in embryonic bone from the M22 line at 15.5dpc.

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 (Fig1).

Oligonucleotides: for targeted/transgenic allele Size: 338bp

DNA sequence (forward): 5'- TGGGGACATAGAAGGGACAG -3'
 DNA sequence (reverse) 5'- GAACTTCAGGGTCAGCTTGC -3'
 Amplifies 5' arm into GFP sequence within the GFP-Cre region.

Rxn Buffer and Conditions: (25 μ l reaction)

10X GSB	2.5ul					
25mM dNTP	1ul	94°C	3min	1 cycle		
10uM primer F	1ul	94°C	30sec			
10uM primer R	1ul	56°C	30sec	35cycles		
DMSO	2.5ul	72°C	45sec			
		72°C	10min	1 cycle		
2-mercaptoethanol	0.125ul					
	0.2ul					
Amplify Taq	(5u/ μ l)					
5x cresol red dye	5ul					
Genomic DNA	1ul					
Total volume	25 ul					

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

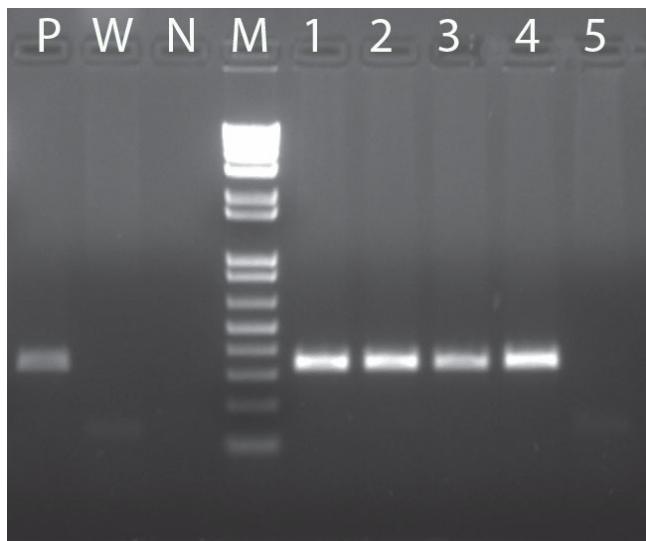


Fig1: Numbers 1-4: S100b^{GCE/+}; Number 5: Wild type **P**: S100b^{GCE/+} positive control; **W**: wild type control; **N**: negative control.

Native Fluorescence

Whole embryos as well as isolated bones and sagittal slices from 15.5dpc neural tubes were examined with a fluorescent microscope to view GFP expression. However, GFP was not detectable under these conditions.

Cre-recombinase Activity

Dissected 15.5dpc embryo samples were stained with X-gal to assay for β -gal activity. Tamoxifen dependent Cre activity was detected in S100b^{GCE/+}; R26R^{lacZ/+} samples (Fig. 2 and 3).

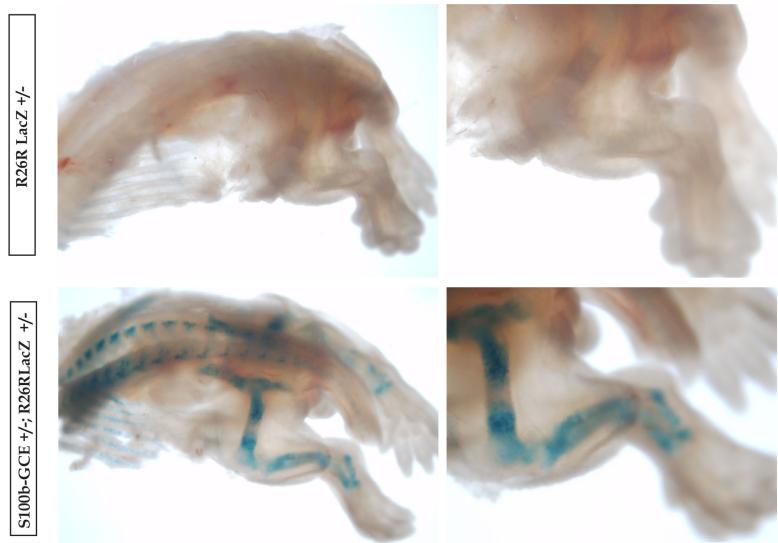


Fig 2. Cre-dependent β -gal activity in S100b^{GCE/+}; R26R^{lacZ/+} embryos. An injection of 2mg/40g Tamoxifen induces β -gal activity in chondrocytes in S100b^{GCE/+}; R26R^{lacZ/+} 15.5dpc embryos.

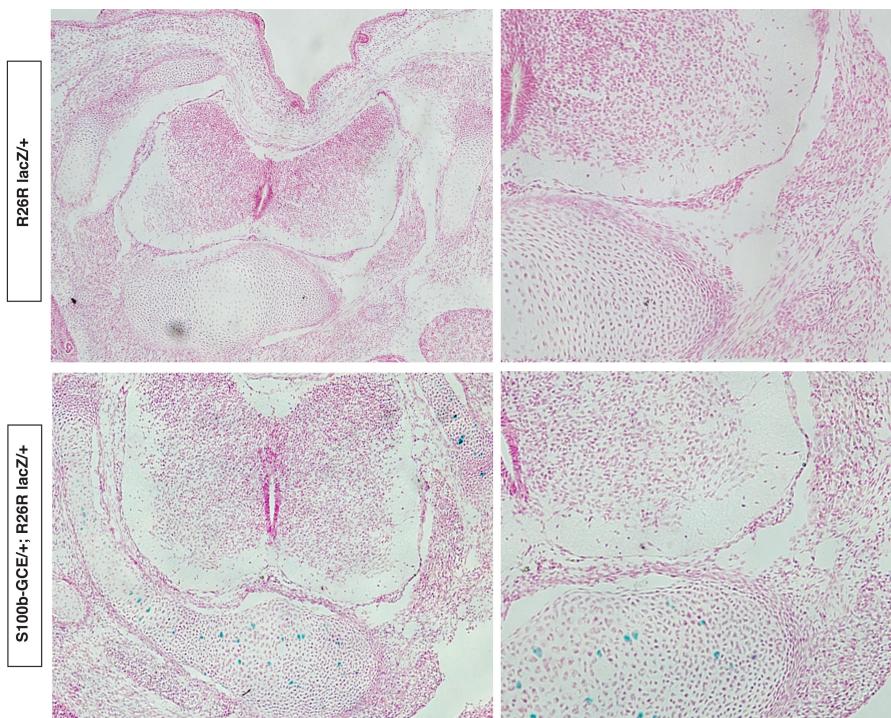


Fig 3. Cre-dependent β -gal activity in S100b^{GCE/+}; R26R^{lacZ/+} embryos. An injection of 2mg/40g Tamoxifen induces β -gal activity in chondrocytes in S100b^{GCE/+}; R26R^{lacZ/+} but not DRG in 15.5dpc embryos.

Immunohistochemistry

Immunohistochemistry was performed to examine if the eGFP^{CreER}^{T2} allele was expressed in the expected S100b domain. To test for Cre function, 15.5-16.5dpc S100b^{GCE/+}; R26R^{lacZ/+} and R26R^{lacZ/+} embryos from Tamoxifen injected mice and S100b^{GCE/+}; R26R^{lacZ/+} corn oil control embryos were assayed. Co-localization of GFP and S100b expression was examined by probing with rabbit anti-S100b, chicken-anti-GFP and rat anti-neurofilament antibodies (Figs. 4, 5, and 6). GFP and β-gal were examined by probing with rabbit anti-β-gal, chicken-anti-GFP and rat anti-neurofilament antibodies (Figs 4, 7 and 8).

Embryos 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 embryos were sectioned at 16um and probed with either: rabbit-anti-S100b/chicken-anti-GFP/Rat-anti-Neurofilament or rabbit-anti-β-gal/Chicken-anti-GFP. GFP (Chicken, Aves Labs, Inc, GFP-1020, 1:500); β-gal (Rabbit, MP Biomedicals, LLC, 55976, 1: 10000), S100b (Rabbit, Dako tytommation, Z0311, 1:500), Neurofilament (Rat, DSHB, 2H3, 1:20) were incubated overnight at 4°C and detected with secondary antibodies Alexafluor 488, 555, 633, and 647 (Molecular probes) as indicated in the figure.

GFP was detected in both S100b^{GCE/+}; R26R^{lacZ/+} Tamoxifen injected and S100b^{GCE/+}; R26R^{lacZ/+} corn oil control embryos in the chondrocytes and DRG. β-gal positive cells colocalized with a subset of GFP positive cells in S100b^{GCE/+}; R26R^{lacZ/+} Tamoxifen injected embryos (Figs 4-8).

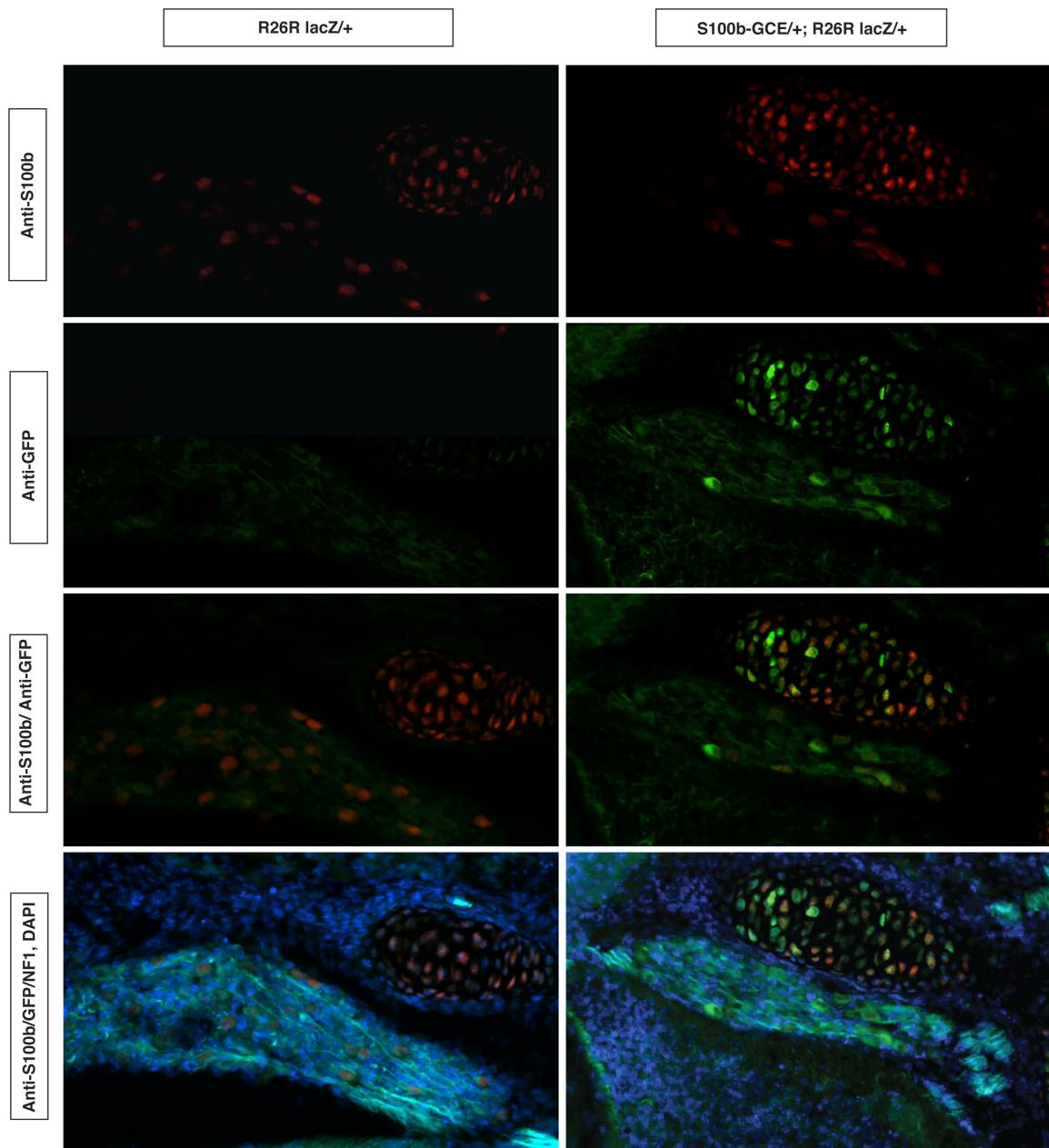


Fig 4. GFP is detected in a subset of S100b positive chondrocytes and DRG in S100b^{GCE/+}; R26R^{lacZ/+} embryos at 15.5dpc. S100b^{GCE/+}; R26R^{lacZ/+} and R26R^{lacZ/+} embryos were probed with anti-GFP, anti-S100b and anti-Neurofilament antibodies.

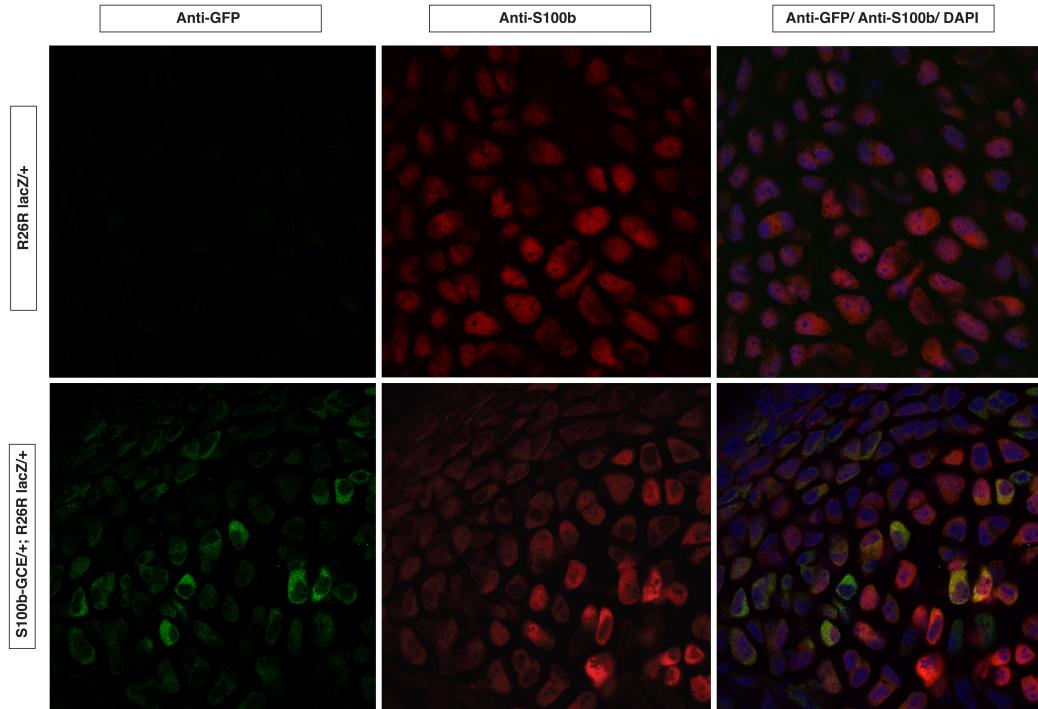


Fig 5. GFP detected in a subset of S100b positive chondrocytes in S100b^{GCE/+}; R26R *lacZ*^{+/} embryos at 15.5dpc. S100b^{GCE/+}; R26R *lacZ*^{+/} and R26R *lacZ*^{+/} embryos were probed with anti-GFP, anti-S100b and anti-Neurofilament antibodies.

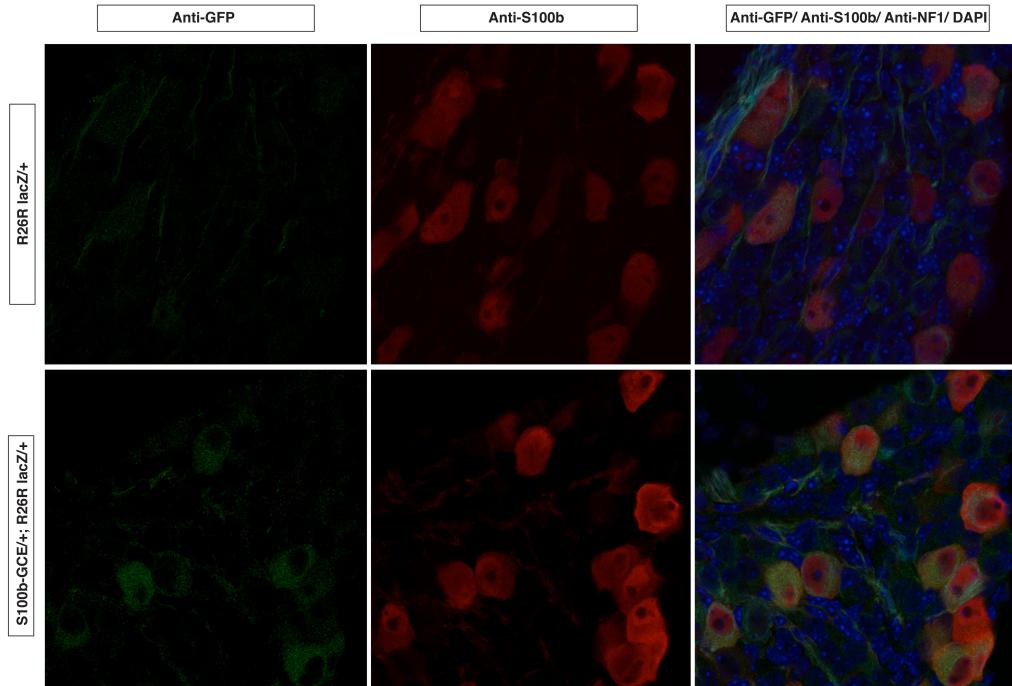


Fig 6. GFP detected in a subset of S100b positive neurons in DRG of S100b^{GCE/+}; R26R *lacZ*^{+/} embryos at 15.5dpc. S100b^{GCE/+}; R26R *lacZ*^{+/} and R26R *lacZ*^{+/} embryos were probed with anti-GFP, anti-S100b and anti-Neurofilament antibodies.

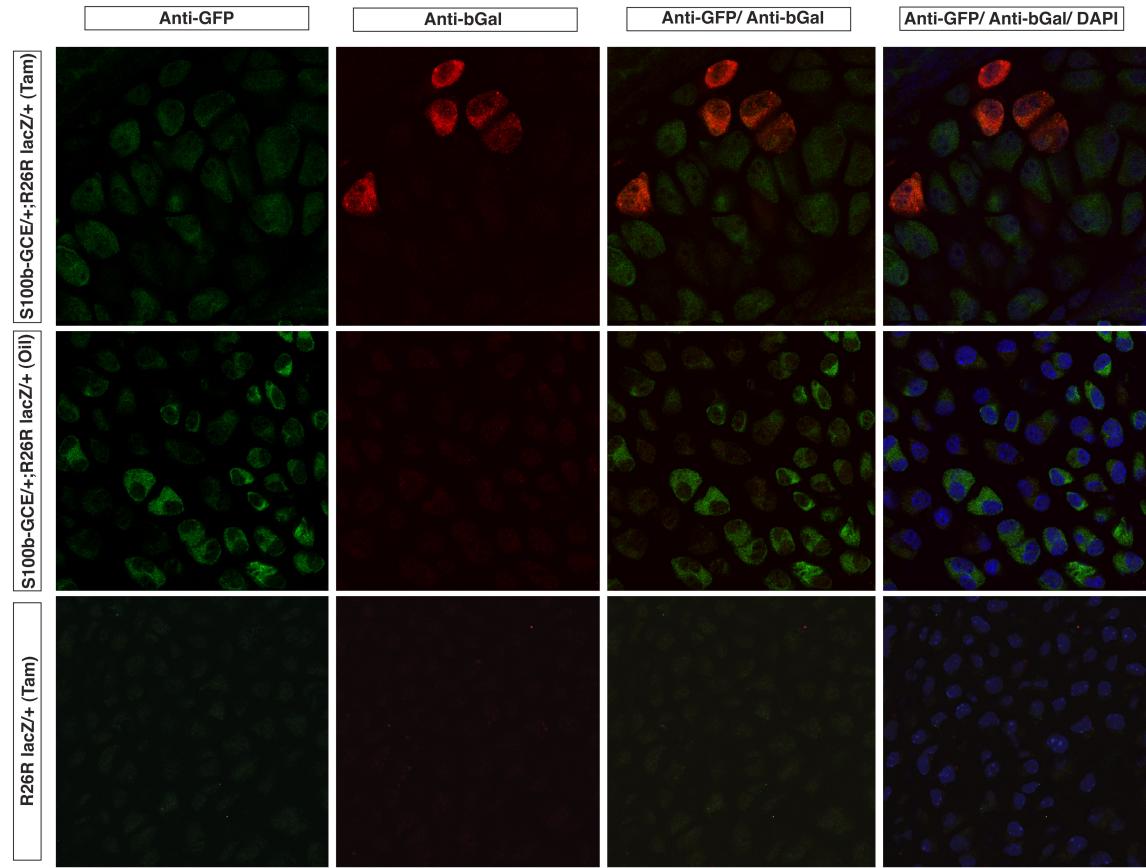


Fig 7. β -gal detected in a subset of GFP positive chondrocytes in Tamoxifen injected S100b^{GCE^{+/}}; R26R^{lacZ^{+/}} embryos at 15.5dpc. S100b^{GCE^{+/}}, R26R^{lacZ^{+/}}and R26R^{lacZ^{+/}}embryos were probed with anti-GFP, anti-S100b and anti-Neurofilament antibodies.

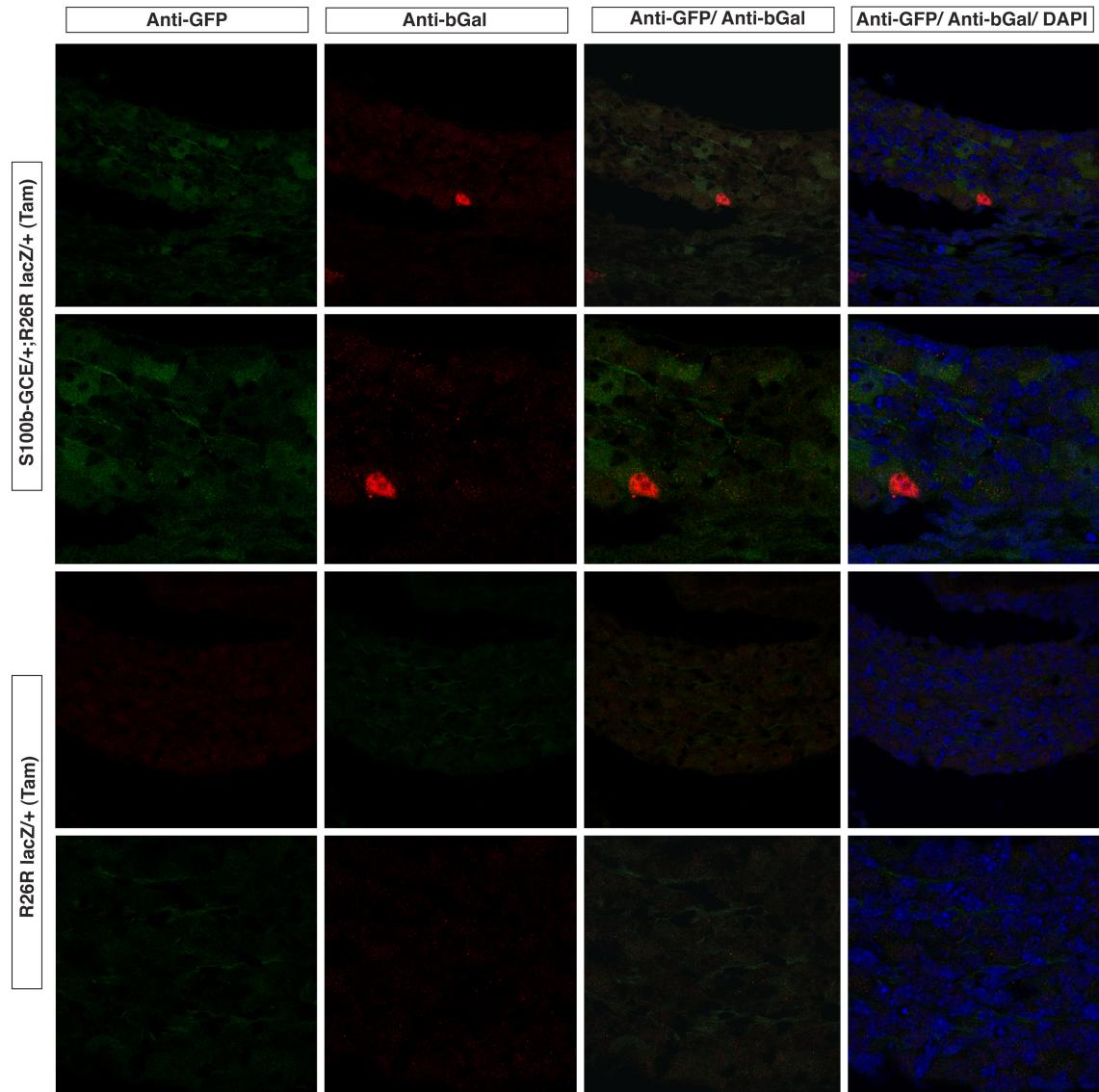


Fig 8. β -gal is detected in a subset of GFP positive neurons in DRG of Tamoxifen injected S100b^{GCE^{+/+}}; R26R^{lacZ/+} embryos at 15.5dpc. S100b^{GCE^{+/+}}; R26R^{lacZ/+} and R26R^{lacZ/+} embryos were probed with anti-GFP, anti-S100b and anti-Neurofilament antibodies.