Title

Sox2 in the adult rat sensory nervous system

Authors

Taro Koike, Taketoshi Wakabayashi, Tetsuji Mori, Yasuharu Takamori, Yukie Hirahara and Hisao Yamada

Department of Anatomy and Cell Science, Kansai Medical University, 2-5-1 Shinmachi, Hirakata-City, Osaka, 573-1010, Japan

Corresponding author

Hisao Yamada

Email: yamada@hirakata.kmu.ac.jp

Tel: +81-72-804-2300

Fax: +81-72-804-2309

Abstract

SRY (sex-determining region Y)-box 2 (Sox2) is a member of the Sox family transcription factors. In the central nervous system, Sox2 is expressed in neural stem cells from neurogenic regions, and regulates stem-cell proliferation and differentiation. In the peripheral nervous system, Sox2 is found only in immature and dedifferentiated Schwann cells, and is involved in myelination inhibition or N-cadherin redistribution. In the present immunohistochemical study, we found that Sox2 is also expressed in other cells of the adult rat peripheral nervous system. Nuclear Sox2 was observed in all satellite glial cells, non-myelinating Schwann cells and the majority of terminal Schwann cells that form lamellar corpuscles and longitudinal lanceolate endings. Sox2 was not found in myelinating Schwann cells and terminal Schwann cells of subepidermal free nerve endings. Satellite glial cells exhibit strong Sox2 immunoreactivity, whereas non-myelinating Schwann cells show weak immunoreactivity. RT-PCR confirmed the presence of *Sox2* mRNA, indicating the cells are likely Sox2 expressors. Our findings suggest that the role of Sox2 in the peripheral nervous system may be cell-type-dependent.

Keywords

Sox2; Peripheral nervous system; Satellite glial cell; Schwann cell; Immunohistochemistry; Rat

Introduction

The SRY (sex determining region Y-box) (Sox) gene family encodes transcription factors characterized by HMG-type (high mobility group) DNA binding domains (Gubbay et al. 1990). Sox2 is a member of the SoxB1 family and regulates cell differentiation in various tissues (Sarkar and Hochedlinger. 2013). In early development, Sox2 deletion causes lethality due to failure of the inner cell mass to form epiblasts and differentiate into trophectoderm (Avilion et al. 2003). In cancerous tissues, Sox2 is involved in tumor growth, promoting cancer cell proliferation and invasion in lung squamous cell carcinoma, esophageal squamous cell carcinoma, glioma, melanoma and Merkel cell carcinoma (Bass et al. 2009; Ikushima et al. 2009; Laga et al. 2010).

In addition to a role in early development, Sox2 plays an important role in the central nervous system (CNS). Sox2 is a known neural stem cell marker and is found in the neurogenic region, specifically, embryonic neuroepithelial cells, the periventricular germinal (or subventricular) zone, and the adult hippocampal subgranular zone. Within the neurogenic region, Sox2 maintains the neural stem cell state by controlling proliferation and differentiation (Ferri et al. 2004; Bani-Yaghoub et al. 2006; Favaro et al. 2009). In the peripheral nervous system (PNS), Sox2 is observed in neural crest stem cells, regulating their migration, proliferation and differentiation (Wakamatsu et al. 2004; Cimadamore et al. 2011). However, Sox2 protein and its mRNA have not been observed in the dorsal root ganglion (DRG) (Aquino et al. 2006; Li et al. 2007). In adult rat, dedifferentiated Schwann cells (SCs) are reported to re-express Sox2, thereby inhibiting myelination and regulating N-cadherin localization (Sarkar and Hochedlinger. 2013).

Aside from these studies, localization of Sox2 protein and its mRNA in the adult PNS has not been examined in other cell types, for example non-myelinating and terminal SCs that

contribute to sensory receptor organ formation. Therefore in the present study, we identified Sox2 immunopositive cells in the adult rat PNS, and used RT-PCR to confirm *Sox2* mRNA expression.

Materials and Methods

Animals

Eight week old male Wistar rats (250g body weight) obtained from SHIMIZU laboratory supplies (Kyoto, Japan) were used. The Animal Ethics Committee of Kansai Medical University approved all experimental protocols, and all studies were performed in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985).

Tissue preparation

Rats were deeply anesthetized with sodium pentobarbital (50 mg/kg i.p.). Animals were perfused transcardially with 0.1M phosphate buffer (PB, pH7.4), followed by 4% formaldehyde (FA) in PB. Lumber DRG, sciatic nerves (harvested from the middle thigh), the hind paw pad and ear were removed and immersed in fixative for 12 hours at 4°C. After cryoprotection with 20% sucrose in PB for 12 hours at 4°C, tissues were embedded in OCT compaund and frozen with CO_2 gas. Cryostat sections of appropriate thickness (5, 20 or 35 μM) were prepared. Sections were stored at 4°C in 0.1M phosphate buffered saline (PBS) with 0.02% sodium azide until use.

Immunohistochemistry

Sections were rinsed in PBS containing 0.3% Triton-X-100 (PBST), and incubated in primary antibodies diluted with PBST for 12 hours at 4°C. The primary antibodies used were: goat anti-Sox2 (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit anti-Sox2 (1:400; Chemicon, Temecula, CA, USA), rabbit anti-glutamine synthetase (GS) (1:10000; Sigma, St. Louis, MO, USA), mouse anti-S100 beta subunit protein (1:1000; Sigma), rabbit anti-S100 (1:10000; DakoCytomation, Glostrup, Denmark), mouse anti-nestin (1:400; Chemicon), mouse anti-myelin basic protein (MBP) (1:1000; UltraClone Ltd., Cambridge, UK), mouse anti-p75 (1:2000; Abcam, Cambridge, UK), goat-anti CGRP (1:200; Abcam) and rabbit anti-neurofilament 200 (NF200) (1:400; Sigma). We predominantly used the Santa Cruz antibody for Sox2 staining. After washing three times in 0.3% PBST for 10 minutes at 20°C, sections were incubated in Cy2, Cy3 and/or Cy5 labeled secondary antibodies specific to appropriate animals (1:200; Jackson ImmunoResearch, West Grove, PA, USA). After washing three times in PBST, sections were mounted in medium containing 100 mM DTT, 5 μg/ml Hoechst dye 33258 (Nacalai Tesque Inc., Kyoto, Japan), 50% glycerol and PBS. Fluorescent images were captured using a confocal microscope (LSM 510-META, Carl Zeiss, Oberkochen, Germany) with objective lens (Plan-APOCHROMATO 63× oil).

For Sox2 signal intensity measuring, DRG, sciatic nerve, pad skin and ear skin were harvested from 3 animals, individually. Fluorescence images of Sox2 were captured under same exposure condition using a fluorescence microscope (E600, Nikon, Tokyo, Jpan) with objective lens (Plan-Fluor 40×). Total Sox2 signal intensity and total pixel number were measured at nucleic area of appropriate glial cells (see results) using the image analysis software, MetaMorph (Molecular Devices, Sunnyvale, CA, USA). The signal intensity/pixel was calculated in each glial cell type.

Semi-thin sections

Sciatic nerve cross sections (50 μm) were incubated in rabbit anti-p75 antibody (1:2000), diluted in PBST, for 48 hours at 4°C. After washing three times in PBST, sections were incubated in biotinylated anti-rabbit IgG antibody (1:200; Vector Lab, Burlingame, CA, USA) for 4 hours at 20°C. Next, samples were washed thoroughly in PBST and incubated with avidin-biotin complex (ABC) (Vector Lab) for 3 hours at 20°C. ABC-peroxidase was visualized by incubation in 0.05% diaminobenzidine in 0.05M Tris-HCl buffer (pH 7.6) and 0.06% H_2O_2, for 10 minutes. Sections were PBS washed, reacted with 1% OsO_4 for 1 hour and then washed with distilled water, before dehydrating in ascending acetone and embedding in Epon (Lubeak 812). Semi-thin sections (1 μm) were counter stained with toluidine blue and observed using a light microscope (E1000M, Nikon) with objective lens (Plan-Apo 100× oil).

Control immunohistochemistry

Primary goat anti-Sox2 IgG antibody (1:1000; Santa Cruz) was reacted with five-fold (by weight) blocking peptide (Santa Cruz) for 12 hours at 4°C before use. Sections were then reacted with primary antibody containing blocking peptide for 12 hours at 4°C. After washing with PBST three times for 10 minutes at 20°C, sections were incubated in biotinylated rabbit anti-goat IgG antibody (1:200; Vector Lab) diluted in PBST, for 2 hours at room temperature, washed three times in PBST for 10 minutes, and incubated in ABC solution (Vector Lab). Peroxidase was visualized by incubating in 0.05% diaminobenzidine in 0.05M Tris-HCl buffer (pH 7.2) and 0.06% H_2O_2, for 7 minutes. Sections were then mounted, counterstained in hematoxylin, dehydrated with ascending ethanol and coverslipped. Sections were observed using a light microscope (E1000M, Nikon).

PCR

Total RNA was extracted from DRG, the sciatic nerve, foot pad and ear using Sepasol-RNA I Super G (Nacalai Tesque Inc.), according to the manufacturer's instructions. RNA was reverse-transcribed into cDNA using the QuantiTect reverse transcription kit (Qiagen K.K., Tokyo, Japan). Reverse transcriptase (RT) negative samples were used as controls. PCRs were performed in a final volume of 50 µl with 0.25 M forward (5′-ACCGGCGGCAACCAGAAGAACAG-3′) and reverse (5′-GCGCCGCGGCCGGTATTTAT-3′) primers (amplifying a 263 bp product), or forward (5′-AGAACCCCAAGATGCACAAC-3′) and reverse (5′-ATGTAGGTCTGCGAGCTGGT-3′) primers (amplifying a 466 bp product), cDNA template and GoTaq master mix (Promega Corporation, Madison, WI, USA). The PCR reaction conditions used were: an initial denaturation for 4 minutes at 95°C, followed by 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. PCR products were electrophoresed in 2% agarose gels and visualized with GelRed (Biotium, Inc., Hayward, CA, USA). PCR product identity was confirmed by sequencing.

Results

Sox2-immunopositive cell distribution

We found two types of glial cells with the exception of SCs. They exhibited nuclear Sox2-immunoreactitvity (Fig.1). One glial cell type is well known, namely satellite glial cells (SGCs), flattened cells that cover the somata of both small and large DRG neurons (Fig. 1a-c). All of them were Sox2 immunopositive. The other glial cell type (*see discussion*) covered large caliber axons not insulated by myelin sheath. In general, large caliber axons are covered with myelinating SCs, and immunohistochemically identifiable with anti-MBP antibody,

however MBP immunoreactivity was absent in these cells (Fig. 1d-f). To identify the initial location of these glial cells, large neurons and their large caliber axons were immunohistochemically stained with anti-NF200, and myelin sheaths with anti-MBP. Tracing NF-200-positive axons from their somata to the periphery, identified non-myelinated axons proximal to neuronal somata (Fig. 1g-i). These non-myelinated axons were covered by glial cells (Fig. 1j-l). All of these non-myelinating glial cells in DRG were Sox2 immunopositive.

Previous work has shown p75 is expressed in cells of the non-myelinating SC lineage (Zorick et al. 1996). In transverse sciatic nerve sections, all p75 positive cell nuclei showed Sox2 immunoreactivity (Fig. 2a-c). In addition, pre-embedded p75 semi-thin sections, identified small pored structures in p75-positive cytoplasm (Fig. 2d), thereby identifying p75 positive cells as non-myelinating SCs possessing fine axons. Within DRG, myelinating SCs did not show Sox2 immunoreactivity (Fig. 1d-f).

In the present study, we used anti-nestin and -S100 antibodies to detect terminal SCs. As shown by Su *et al*. (2013), glial cells in the adult PNS are nestin positive. Moreover sensory receptor organs are typically composed of an axon terminal covered by terminal SCs. We examined terminal SCs of lamellar corpuscles, longitudinal lanceolate endings, circumferential nerve endings and free nerve endings. Lamellar corpuscles were observed in dermal papillae of the foot pad skin, and composed of an axon terminal with more than one terminal SC. Sox2 immunoreactivity was observed in the majority of nuclei within terminal SCs (Fig. 3a-c). Longitudinal lanceolate endings were situated around hair follicles, and circumferential nerve endings rarely observed at collar regions of longitudinal lanceolate endings in ear skin. We found the nuclei of terminal SCs sheathing longitudinal lanceolate endings expressed Sox2, but not those surrounding circumferential nerve endings (Fig. 3d-f). Free nerve endings located at the sub-epidermis, were observed in the foot pad and auricle

skin. Sox2 was not found in terminal SCs of free nerve endings (Fig. 3g-h).

Sox2-immunoreactivity intensity was compared between SGCs, non-myelinating SCs and terminal SCs (Fig. 4). Sections harvested from each tissue were simultaneously stained and captured under the same exposure conditions. SGC nuclei around neurons and non-myelinating glial cells (described above) were strongly stained. Non-myelinating SC nuclei showed very weak Sox2-immunoreactivity. Sox2 immunoreactivity in lamellar corpuscles and longitudinal lanceolate endings was stronger than non-myelinating SCs.

To confirm whether the tissues examined were intact or damaged, we identified inflammatory and apoptotic cells, because it is known that dedifferentiated SCs re-express Sox2 in injury sites (Le et al. 2005). A small number of CD45 positive cells, representing leukocytes, were scattered in DRG and sciatic nerve. In the skin, many epidermal dendritic cells were CD45 positive. However, no abnormal leukocyte accumulation was observed in any tissue examined (Online Resource 1, 2). Using TUNEL staining to detect apoptopic cells, TUNEL positive cells were detected in the epidermal granular layer and hair follicles, regions with apoptopic cells under normal conditions. TUNEL positive cells were not found in either DRG or sciatic nerve (Online Resource 1, 2).

RT-PCR results

RT-PCR was performed on DRG, the sciatic nerve, foot pad and ear to determine the presence of *Sox2* mRNA. Only a single 263 bp PCR product was detected in each tissue sample. We also observed single PCR products using another primer pair (Fig. 5a). No PCR product was observed in RT negative samples. (Online Resource 1, 3). Sequencing of PCR products confirmed identical nucleotide sequences to the designed sequence.

Antibody specificity

Antibody immunoreactivity was absent when goat anti-Sox2 antibody was preabsorbed with blocking peptide (Fig. 5b, c), demonstrating reliability of our immunohistochemical results. Our immunohistochemical findings using goat (Santa Cruz) and rabbit (Chemicon) anti-Sox2 antibodies were identical (Fig. 5d, e).

Discussion

We have identified, for the first time, Sox2 immunopositive cells in the adult rat sensory nervous system. Sox2 was observed in SGCs, and non-myelinating and terminal SCs of longitudinal lanceolate endings and lamellar corpuscles. Sox2 immunoreactivity intensity was strongeat in SGCs and weak in non-myelinating SCs. No immunoreactivity was found in DRG neurons and terminal SCs of free or circumferential nerve endings. Simultaneous Sox2 protein and mRNA localization corroborates the likelihood these cells are Sox2-expressors.

In the adult CNS, Sox2 is expressed in neural stem/progenitor cells, ependymal cells and neurons located in the septum, thalamus and striatum (Ferri et al. 2004). We found that in the adult PNS, Sox2 is not expressed in DRG neurons but is found in specific glial cell populations, suggesting that in the sensory nervous system, Sox2 has a different physiological role than it does in the brain.

Sox2 immunopositive cell identification

Our immunohistochemical findings are reliable because: (1) two different Sox2-antibodies, obtained from different manufacturer's, show the same results; (2) antibody specificity was confirmed with peptide neutralization; (3) the same results were obtained with both fluorescent- and enzyme-labeled immunohistochemistry; (4) *Sox2* mRNA was confirmed

by RT-PCR; and (5) accumulation of leucocytes or apoptotic cells was not found. Thus, Sox2 is expressed in the normal adult rat PNS.

Sox2-cell distribution

Our findings in DRG are in disagreement with previous studies, which show no Sox2 mRNA in rat (Li et al. 2007), or protein in mouse (Aquino et al. 2006). This discrepancy may be due to differing RT-PCR and immunohistochemistry conditions used in the studies.

Currently, S100 protein, glial fibrillary acidic protein and GS are known SGC markers; however they are not SGC specific markers because SCs are also stained (Woodham et al. 1989; Miller et al. 2002; Lazzarini. 2004; Hanani. 2005). Therefore, we identified SGCs morphologically, and found 2 glial cell types in DRG. One was a general SGC covering the neuronal soma. The other cell type wrapped around NF200 positive axons, proximal to neuronal somata. All recent studies on SGCs have examined the first type of SGC. However, Pannese (1960) has shown two SGC types in the mammalian DRG (including adult rat), specifically perisomatic SGCs covering the neuronal cell body, and a periaxonal SGCs, wrapping the initial axonal projection of DRG neurons without myelin sheaths. This suggests that the second group of glial cells we have described are SGCs.

Sox2 expression has been observed in developing PNS ganglia using $Sox2^{\beta\text{-}geo}$ mice (Zappone et al. 2000). Prospective SGCs express Sox2 during DRG development (Wakamatsu et al. 2004; Aquino et al. 2006). Our results indicate Sox2 is expressed throughout the lifetime of both SGC types as in the enteric nervous system (Heanue and Pachnis 2011).

Sox2 expression is found in immature SCs from mouse sciatic nerve. Sox2 expression gradually decreases with development, although it is still present in adult sciatic

nerve (Le et al. 2005). To date, the cells expressing Sox2 in adult sciatic nerve were not known. We immunohistochemically identified Sox2 positive cells in adult rat sciatic nerve as non-myelinating SCs, although expression levels are low (Fig. 4c, d). Myelinating SCs do not show Sox2-immunoreactivity. Both myelinating and non-myelinating SCs are known to arise from immature SCs (Jessen and Mirsky. 2005), and express Sox2 (Wakamatsu et al. 2000). Our results indicate that Sox2 expression is maintained in the non-myelinating, but not myelinating, SC lineage.

In skin, Sox2 is expressed in skin derived stem cells at dermal papillae and Merkel cells (Biernaskie et al. 2009, Driskell et al. 2009). Our histological observation discriminated terminal SCs from these cells, as the glial cells exhibited appropriate morphologies (Fig. 3).

Terminal SCs cover the axon terminal and are elements of sensory receptor organs. Lamellar corpuscles are well distributed in dermal papillae in glabrous skin, and longitudinal lanceolate endings located at hair follicles (Jirmanova et al. 1997; Cauna. 1969; Munger and Halata. 1983). We found that Sox2 is expressed in almost all terminal SCs of these two receptor organs. In contrast, Sox2 was not observed in terminal SCs of sub-epidermal and circumferential nerve endings. Sub-epidermal nerve endings are known to be free nerve endings (Cauna. 1973), and circumferential nerve endings are also considered free nerve endings (Kruger et al. 1981). Thus, our results indicate that terminal SCs of free nerve endings are Sox2 negative, and that Sox2 is present in SCs found between restricted types of sensory receptor organs.

Sox2 significance

Neurogenesis in the DRG is controversial. An increase in neuronal number during postnatal maturation has been reported (Popken and Farel. 1997; Farel. 2002; Farel. 2003),

although studies also report no change (La Forte et al. 1991; Pover et al. 1994). It has been suggested that the increased neuronal number is caused by maturation of post-mitotic immature cells, as BrdU positive neurons have not been observed in DRG (Ciaroni et al. 2000; Farel. 2003). However, recent *in vitro* experiments show BrdU positive neurons and neurospheres in postnatal mouse and adult rat DRG (Namaka et al. 2001; Li et al. 2007). In neurogenic regions of developing or adult animals, Sox2 is expressed in neural stem and progenitor cells, and thereby maintains the stem cell state (Graham et al. 2003; Ferri et al. 2004; Bani-Yaghoub et al. 2006, Shu et al. 2007). Neural progenitor cells require Sox2 at an early stage of differentiation, promoting DRG expression of proneural bHLH genes, including *NGN1* and *Mash1* (Cavallaro et al. 2008; Cimadamore et al. 2011). We found Sox2 expression in SGCs of adult rat DRG, corroborating the previous finding that SGCs have the potential to generate neurospheres (Li et al. 2007), although it is still unclear whether neurogenesis occurs in adult DRG.

Recently, roles for Sox2 in the adult PNS were reported. Sox2 is a known negative regulator of myelin-related genes, via Krox20 (Le et al. 2005; Parkinson et al. 2008). EphB signaling regulates N-cadherin distribution though Sox2, arranging dedifferentiated myelinating SCs for axonal regeneration (Parrinello et al. 2010). Sox2 regulates $p27^{Kip1}$, inhibiting inner pillar cell proliferation in neonatal and adult mouse cochlea (Liu et al. 2012). Thus within the PNS, Sox2 plays a different role in each cell type. Moreover, we found Sox2 expression patterns and level differed among glial cell types in the sensory nervous system. These differing expression patterns suggest that Sox2 plays a cell specific role.

In conclusion, we have identified Sox2 in PNS glial cells, Sox2 was observed in SGCs, and non-myelinating and specific terminal SCs. Although, Sox2 was expressed in

non-myelinating glial cells, it preferentially localized to less differentiated cells in DRG and sciatic nerve, and intricately-shaped, highly differentiated terminal SCs in sensory receptor organs. These results indicate that the role of Sox2 is different in distinct glial cell populations.

Acknowledgments

This study was supported by a Grant D2 from Kansai Medical University (T.K.), and a Grant-in-Aid for Scientific Research C (No. 24500876) from the Japan Society for Promotion of Science (H.Y.)

Reference

Aquino JB, Hjerling-Leffler J, Koltzenburg M, Edlund T, Villar MJ, Ernfors P (2006) In vitro and in vivo differentiation of boundary cap neural crest stem cells into mature Schwann cells. Exp Neurol 198:438-449

Avilion AA, Nicolis SK, Pevny LH, Perez L, Vivian N, Lovell-Badge R (2003) Multipotent cell lineages in early mouse development depend on SOX2 function. Genes Dev 17:126-140

Bani-Yaghoub M, Tremblay RG, Lei JX, Zhang D, Zurakowski B, Sandhu JK, Smith B, Ribecco-Lutkiewicz M, Kennedy J, Walker PR, Sikorska M (2006) Role of Sox2 in the development of the mouse neocortex Dev Biol 295; 52-66

Bass AJ, Watanabe H, Mermel CH, Yu S, Perner S, Verhaak RG, Kim SY, Wardwell L, Tamayo P, Gat-Viks I, Ramos AH, Woo MS, Weir BA, Getz G, Beroukhim R, O'Kelly M, Dutt A, Rozenblatt-Rosen O, Dziunycz P, Komisarof J, Chirieac LR, Lafargue CJ, Scheble V, Wilbertz T, Ma C, Rao S, Nakagawa H, Stairs DB, Lin L, Giordano TJ, Wagner P, Minna JD, Gazdar AF, Zhu CQ, Brose MS, Cecconello I, Jr UR, Marie SK, Dahl O, Shivdasani RA, Tsao MS, Rubin MA, Wong KK, Regev A, Hahn WC, Beer DG, Rustgi AK, Meyerson M (2009) SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. Nat Genet 41:1238-1242

Biernaskie J, Paris M, Morozova O, Fagan BM, Marra M, Pevny L, Miller FD (2009) SKPs derive from hair follicle precursors and exhibit properties of adult dermal stem cells. Cell Stem Cell 5:610-623

Cauna N (1969) The fine morphology of the sensory receptor organs in the auricle of the rat. J Comp Neurol 136:81-98

Cauna N (1973) The free penicillate nerve endings of the human hairy skin. J Anat 115:277-288

Cavallaro M, Mariani J, Lancini C, Latorre E, Caccia R, Gullo F, Valotta M, DeBiasi S, Spinardi L, Ronchi A, Wanke E, Brunelli S, Favaro R, Ottolenghi S, Nicolis SK (2008) Impaired generation of mature neurons by neural stem cells from hypomorphic Sox2 mutants Development 135(3); 541-557

Ciaroni S, Cecchini T, Cuppini R, Ferri P, Ambrogini P, Bruno C, Del Grande P (2000) Are there proliferating neuronal precursors in adult rat dorsal root ganglia? Neurosci Lett 281:69-71

Cimadamore F, Fishwick K, Giusto E, Gnedeva K, Cattarossi G, Miller A, Pluchino S, Brill LM, Bronner-Fraser M, Terskikh AV (2011) Human ESC-Derived Neural Crest Model Reveals a Key Role for SOX2 in Sensory Neurogenesis. Cell Stem Cell 8:538-551

Driskell RR, Giangreco A, Jensen KB, Mulder KW, Watt FM (2009) Sox2-positive dermal papilla cells specify hair follicle type in mammalian epidermis. Development 136:2815-2823

Farel PB (2002) Sensory neuron addition in juvenile rat: time course and specificity. J Comp Neurol 449:158-165

Farel PB (2003) Late differentiation contributes to the apparent increase in sensory neuron number in juvenile rat. Brain Res Dev Brain Res 144:91-98

Favaro R, Valotta M, Ferri AL, Latorre E, Mariani J, Giachino C, Lancini C, Tosetti V, Ottolenghi S, Taylor V, Nicolis SK (2009) Hippocampal development and neural stem cell maintenance require Sox2-dependent regulation of Shh. Nat Neurosci 12:1248-1256

Ferri AL, Cavallaro M, Braida D, Di Cristofano A, Canta A, Vezzani A, Ottolenghi S, Pandolfi PP, Sala M, DeBiasi S, Nicolis SK (2004) Sox2 deficiency causes neurodegeneration and impaired neurogenesis in the adult mouse brain. Development 131:3805-3819

Graham V, Khudyakov J, Ellis P, Pevny L (2003) SOX2 functions to maintain neural progenitor identity. Neuron 39:749-765

Gubbay J, Collignon J, Koopman P, Capel B, Economou A, Munsterberg A, Vivian N, Goodfellow P, Lovell-Badge R (1990) A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. Nature 346:245-250

Hanani M (2005) Satellite glial cells in sensory ganglia: from form to function. Brain Res Brain Res Rev 48:457-476

Heanue TA, Pachnis V (2011) Prospective identification and isolation of enteric nervous system progenitors using Sox2. Stem Cells 29:128-140

Ikushima H, Todo T, Ino Y, Takahashi M, Miyazawa K, Miyazono K (2009) Autocrine TGF-beta signaling maintains tumorigenicity of glioma-initiating cells through Sry-related HMG-box factors. Cell Stem Cell 5:504-514

Jessen KR, Mirsky R (2005) The origin and development of glial cells in peripheral nerves. Nat Rev Neurosci 6:671-682

Jirmanova I, Dubovy P, Zelena J (1997) Regeneration of tactile lamellar corpuscles of the rat after postnatal freeze injury. Anat Embryol (Berl) 195:363-374

Kruger L, Perl ER, Sedivec MJ (1981) Fine structure of myelinated mechanical nociceptor endings in cat hairy skin. J Comp Neurol 198:137-154

La Forte RA, Melville S, Chung K, Coggeshall RE (1991) Absence of neurogenesis of adult rat dorsal root ganglion cells. Somatosens Mot Res 8:3-7

Laga AC, Lai CY, Zhan Q, Huang SJ, Velazquez EF, Yang Q, Hsu MY, Murphy GF (2010) Expression of the embryonic stem cell transcription factor SOX2 in human skin: relevance to melanocyte and merkel cell biology. Am J Pathol 176:903-913

Miller R (2004) Schwann Cell Development. In: Lazzarini RA (ed) Myelin Biology and Disorders 1, 2nd edn. Elsevier, USA, pp329-370

Le N, Nagarajan R, Wang JY, Araki T, Schmidt RE, Milbrandt J (2005) Analysis of congenital hypomyelinating Egr2Lo/Lo nerves identifies Sox2 as an inhibitor of Schwann cell differentiation and myelination. Proc Natl Acad Sci USA 102:2596-2601

Li HY, Say EH, Zhou XF (2007) Isolation and characterization of neural crest progenitors from adult dorsal root ganglia. Stem Cells 25:2053-2065

Liu Z, Walters BJ, Owen T, Brimble MA, Steigelman KA, Zhang L, Mellado Lagarde MM, Valentine MB, Yu Y, Cox BC, Zuo J (2012) Regulation of p27Kip1 by Sox2 maintains quiescence of inner pillar cells in the murine auditory sensory epithelium. J Neurosci 32:10530-10540

Miller KE, Richards BA, Kriebel RM (2002) Glutamine-, glutamine synthetase-, glutamate dehydrogenase- and pyruvate carboxylase-immunoreactivities in the rat dorsal root ganglion and peripheral nerve. Brain Res 945:202-211

Munger BL, Halata Z (1983) The sensory innervation of primate facial skin. I. Hairy skin. Brain Res 286:45-80

Namaka MP, Sawchuk M, MacDonald SC, Jordan LM, Hochman S (2001) Neurogenesis in postnatal mouse dorsal root ganglia. Exp Neurol 172:60-69

Pannese E (1960) Observations on the morphology, submicroscopic structure and biological properties of satellite cells (s.c.) in sensory ganglia of mammals. Z Zellforsch Mikrosk Anat 52:567-597

Parkinson DB, Bhaskaran A, Arthur-Farraj P, Noon LA, Woodhoo A, Lloyd AC, Feltri ML, Wrabetz L, Behrens A, Mirsky R, Jessen KR (2008) c-Jun is a negative regulator of myelination. J Cell Biol 181(4):625-634

Parrinello S, Napoli I, Ribeiro S, Digby PW, Fedorova M, Parkinson DB, Doddrell RD, Nakayama M, Adams RH, Lloyd AC (2010) EphB signaling directs peripheral nerve regeneration through Sox2-dependent Schwann cell sorting. Cell 143:145-155

Popken GJ, Farel PB (1997) Sensory neuron number in neonatal and adult rats estimated by means of stereologic and profile-based methods. J Comp Neurol 386:8-15

Pover CM, Barnes MC, Coggeshall RE (1994) Do primary afferent cell numbers change in relation to increasing weight and surface area in adult rats? somatosense & mot res 11:163-167

Sarkar A, Hochedlinger K (2013) The sox family of transcription factors: versatile regulators of stem and progenitor cell fate. Cell Stem Cell 12:15-30

Suh H, Consiglio A, Ray J, Sawai T, D'Amour KA, Gage FH (2007) In vivo fate analysis reveals the multipotent and self-renewal capacities of Sox2+ neural stem cells in the adult hippocampus. Cell Stem Cell 1:515-528

Su PH, Chen CC, Chang YF, Wong ZR, Chang KW, Huang BM, Yang HY (2013) Identification and cytoprotective function of a novel nestin isoform, Nes-S, in dorsal root ganglia neurons. J Biol Chem 288:8391-8404

Wakamatsu Y, Endo Y, Osumi N, Weston JA (2004) Multiple roles of Sox2, an HMG-box transcription factor in avian neural crest development. Dev Dyn 229:74-86

Wakamatsu Y, Maynard TM, Weston JA (2000) Fate determination of neural crest cells by

NOTCH-mediated lateral inhibition and asymmetrical cell division during gangliogenesis. Development 127:2811-2821

Widera D, Heimann P, Zander C, Imielski Y, Heidbreder M, Heilemann M, Kaltschmidt C, Kaltschmidt B (2011) Schwann cells can be reprogrammed to multipotency by culture. Stem Cells Dev 20:2053-2064

Woodham P, Anderson PN, Nadim W, Turmaine M (1989) Satellite cells surrounding axotomised rat dorsal root ganglion cells increase expression of a GFAP-like protein. Neurosci Lett 98:8-12

Zappone MV, Galli R, Catena R, Meani N, De Biasi S, Mattei E, Tiveron C, Vescovi AL, Lovell-Badge R, Ottolenghi S, Nicolis SK (2000) Sox2 regulatory sequences direct expression of a (beta)-geo transgene to telencephalic neural stem cells and precursors of the mouse embryo, revealing regionalization of gene expression in CNS stem cells. Development 127:2367-2382

Zorick TS, Syroid DE, Arroyo E, Scherer SS, Lemke G (1996) The transcription factors SCIP and Krox-20 mark distinct stages and cell fates in Schwann cell differentiation. Mol Cell Neurosci 8:129-145

Figure Legends

Fig. 1 Confocal microscopy images of rat DRG. Sections were immunohistochemically stained. **a-c** Stacked images of a section stained with anti-GS (green) and goat anti-Sox2 (red) antibodies. Flattened SGCs (green) cover ganglion neurons (N) with thin cytoplasm (arrowheads). Sox2 is observed in the nuclei of all SGCs. **d-f** Stacked images of a section stained with anti-S100 (green), goat anti-Sox2 (yellow) and -MBP (red) antibodies. A non-myelinating glial cell (arrow) and myelinating SC (arrowhead) sheaths a large caliber axon adjacent to a neuron. Sox2 is found in the nuclei of non-myelinating glial cells, but not myelinating SCs. **g-i** Section stained with anti-NF200 (green) and -MBP (red) antibodies. Each image shows a different plane. An axon is traced according to the numbered arrowheads. The axon projects from a neuronal soma (N) (arrowhead 1) and is not covered with myelin sheath until arrow 4. **j-l** Section stained with anti-NF200 (red) and -S100 beta (green) antibodies. An axon is traced from the neuronal soma (N) according to the numbered arrowheads. The NF200 positive axon is covered by S100 positive glial cells. DNA, cyan. Scale bar 20 μm

Fig. 2 Stacked confocal images of rat sciatic nerve. **a-c** Sections stained with anti-p75 (green) and goat anti-Sox2 (red) antibodies. Nuclei of p75-positive cells show Sox2 immunoreactivity (arrows). **c** Merged image. DNA, cyan. Scale bar, 20 μm. **d** Pre-embedded semi-thin sections stained with anti-p75 (brown) antibody and toluidine blue. The arrow shows a non-myelinating SC. Small pores (arrowheads) are seen in the cytoplasm of the non-myelinating SC. Scale bar, 5 μm

Fig. 3 Stacked confocal images of rat sensory receptor organs. **a-f** Sections stained with

anti-nestin (green), -NF200 (red) and goat anti-Sox2 (magenta) antibodies. **g-i** Section stained with anti-S100 (green), -CGRP (red), and goat anti-Sox2 (red) antibodies. Terminal SCs are nestin or S100 positive, thick nerve fibers are NF200 positive, and free nerve endings are CGRP positive. **a-c** A lamellar corpuscle located apical of a dermal papilla. Nuclei of the corpuscle are Sox2 positive (arrowheads). **d-f** Lanceolate endings surrounding a hair follicle. Sox2 is detected within the nucleus of a terminal SC (arrowhead). **g-i** Section of free nerve endings. Terminal SCs do not express Sox2 (arrows). EP, epidermis. DP, dermal papilla. HF, hair follicle. DNA, cyan. Scale bar, 20 μm

Fig. 4 Comparison of Sox2 signal intensity between glial cell types. Signal intensity were strongest in SGCs, and weakest in non-myelinating SCs. Error bars, SD

Fig. 5 *Sox2* RT-PCR and antibody specificity controls. **a** Electrophoresed PCR products. A single band of the expected size (upper 466 bp, lower 263 bp) was observed. **b**, **c** Antibody absorption test in DRG. **b** Section stained with goat anti-Sox2 antibody. Many nuclei are stained (brown). **c** Section stained with goat anti-Sox2 antibody pre-incubated with an antigenic peptide. No reaction product is seen. Faint blue, hematoxylin. **d, e** Double immunohistochemistry using goat (d) and rabbit (e) anti-Sox2 antibodies. Positive reactions co-localize. Scale bar, 20 μm

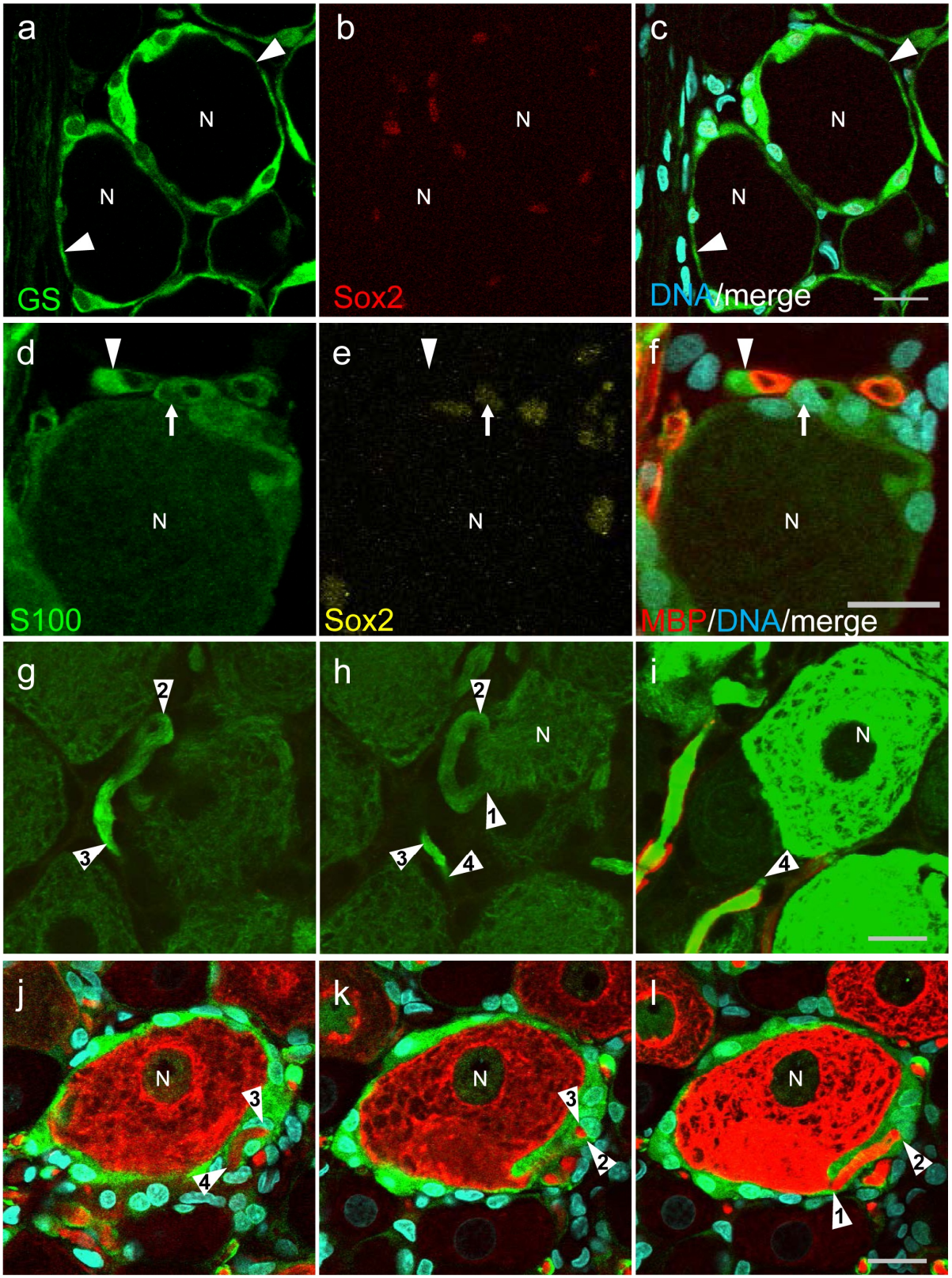


Fig. 1 Confocal microscopy images of rat DRG. Sections were immunohistochemically stained. **a-c** Stacked images of a section stained with anti-GS (green) and goat anti-Sox2 (red)

antibodies. Flattened SGCs (green) cover ganglion neurons (N) with thin cytoplasm (arrowheads). Sox2 is observed in the nuclei of all SGCs. **d**-**f** Stacked images of a section stained with anti-S100 (green), goat anti-Sox2 (yellow) and -MBP (red) antibodies. A non-myelinating glial cell (arrow) and myelinating SC (arrowhead) sheaths a large caliber axon adjacent to a neuron. Sox2 is found in the nuclei of non-myelinating glial cells, but not myelinating SCs. **g-i** Section stained with anti-NF200 (green) and -MBP (red) antibodies. Each image shows a different plane. An axon is traced according to the numbered arrowheads. The axon projects from a neuronal soma (N) (arrowhead 1) and is not covered with myelin sheath until arrow 4. **j-l** Section stained with anti-NF200 (red) and -S100 beta (green) antibodies. An axon is traced from the neuronal soma (N) according to the numbered arrowheads. The NF200 positive axon is covered by S100 positive glial cells. DNA, cyan. Scale bar 20 μm

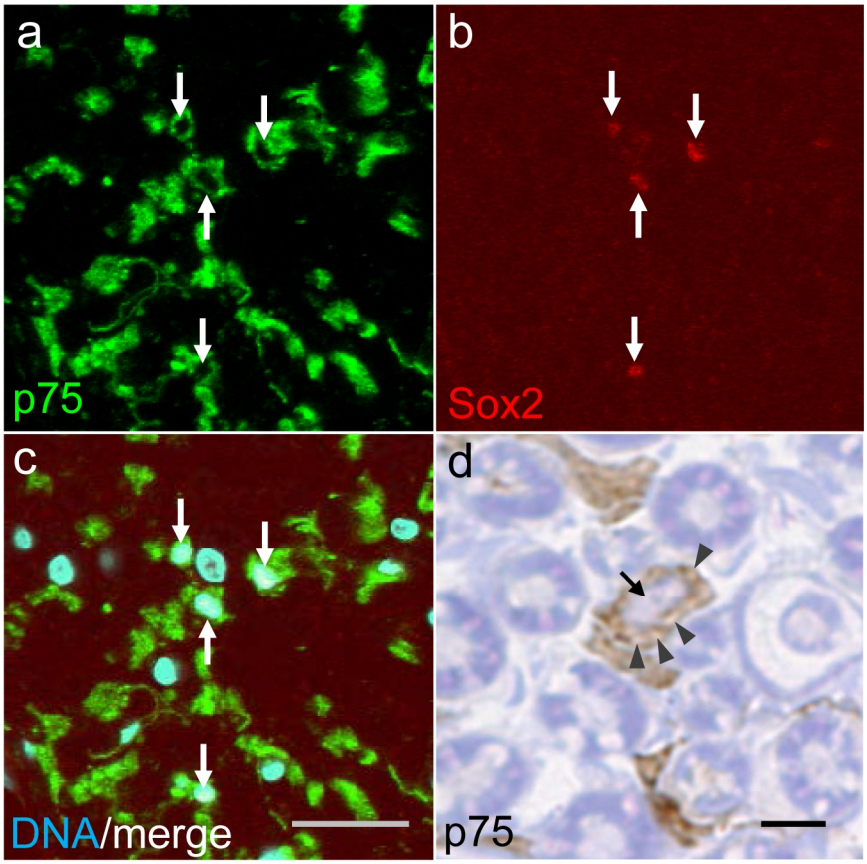


Fig. 2 Stacked confocal images of rat sciatic nerve. **a-c** Sections stained with anti-p75 (green) and goat anti-Sox2 (red) antibodies. Nuclei of p75-positive cells show Sox2 immunoreactivity (arrows). **c** Merged image. DNA, cyan. Scale bar, 20 μm. **d** Pre-embedded semi-thin sections stained with anti-p75 (brown) antibody and toluidine blue. The arrow shows a non-myelinating SC. Small pores (arrowheads) are seen in the cytoplasm of the non-myelinating SC. Scale bar, 5 μm

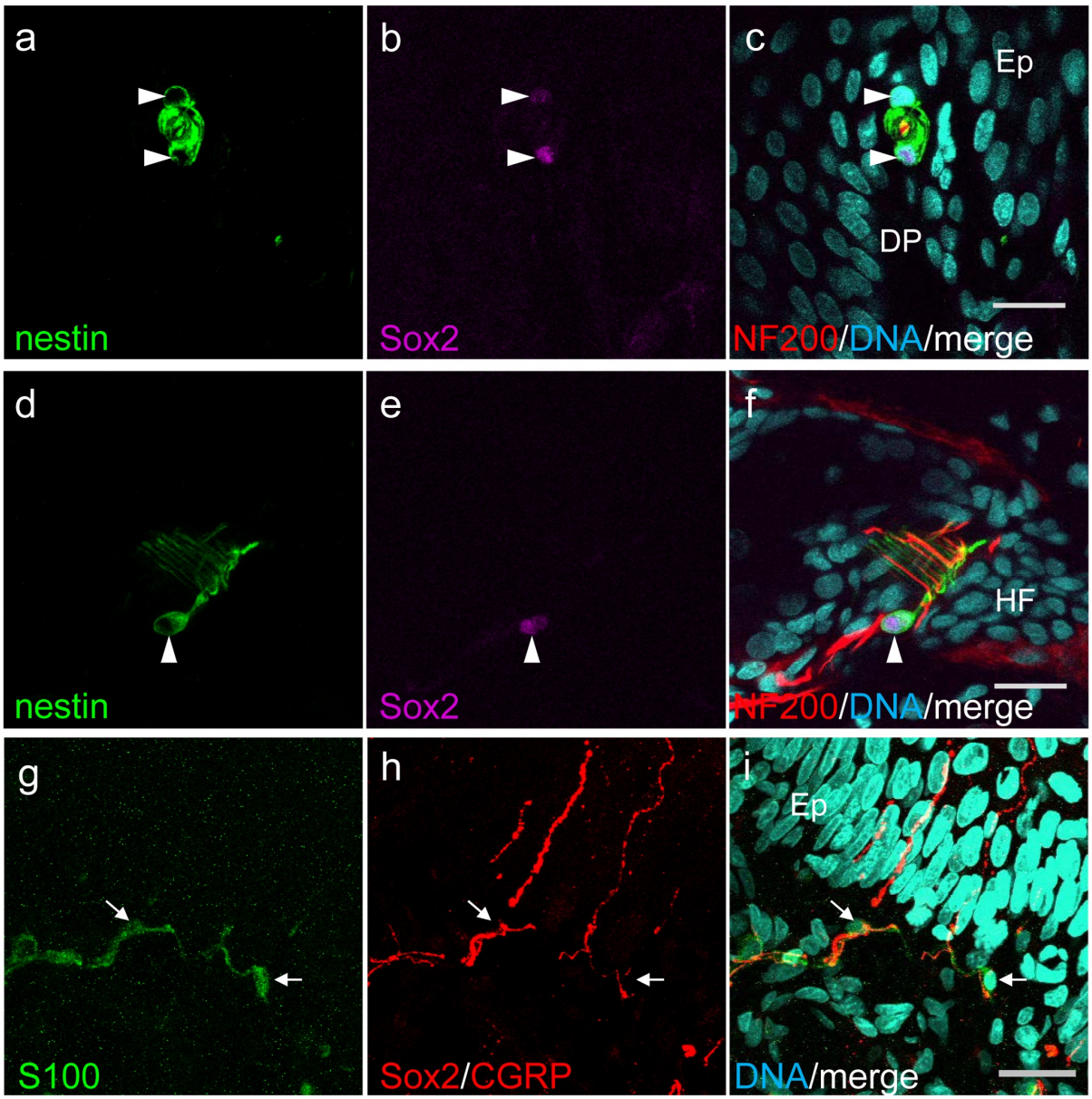


Fig. 3 Stacked confocal images of rat sensory receptor organs. **a-f** Sections stained with anti-nestin (green), -NF200 (red) and goat anti-Sox2 (magenta) antibodies. **g-i** Section stained with anti-S100 (green), -CGRP (red), and goat anti-Sox2 (red) antibodies. Terminal SCs are nestin or S100 positive, thick nerve fibers are NF200 positive, and free nerve endings are CGRP positive. **a-c** A lamellar corpuscle located apical of a dermal papilla. Nuclei of the corpuscle are Sox2 positive (arrowheads). **d-f** Lanceolate endings surrounding a hair follicle.

Sox2 is detected within the nucleus of a terminal SC (arrowhead). **g-i** Section of free nerve endings. Terminal SCs do not express Sox2 (arrows). EP, epidermis. DP, dermal papilla. HF, hair follicle. DNA, cyan. Scale bar, 20 μm

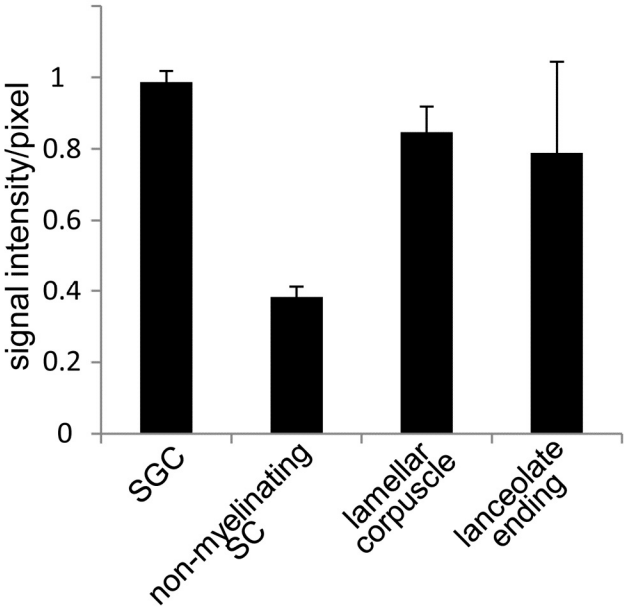


Fig. 4 Comparison of Sox2 signal intensity between glial cell types. Signal intensity were strongest in SGCs, and weakest in non-myelinating SCs. Error bars, SD

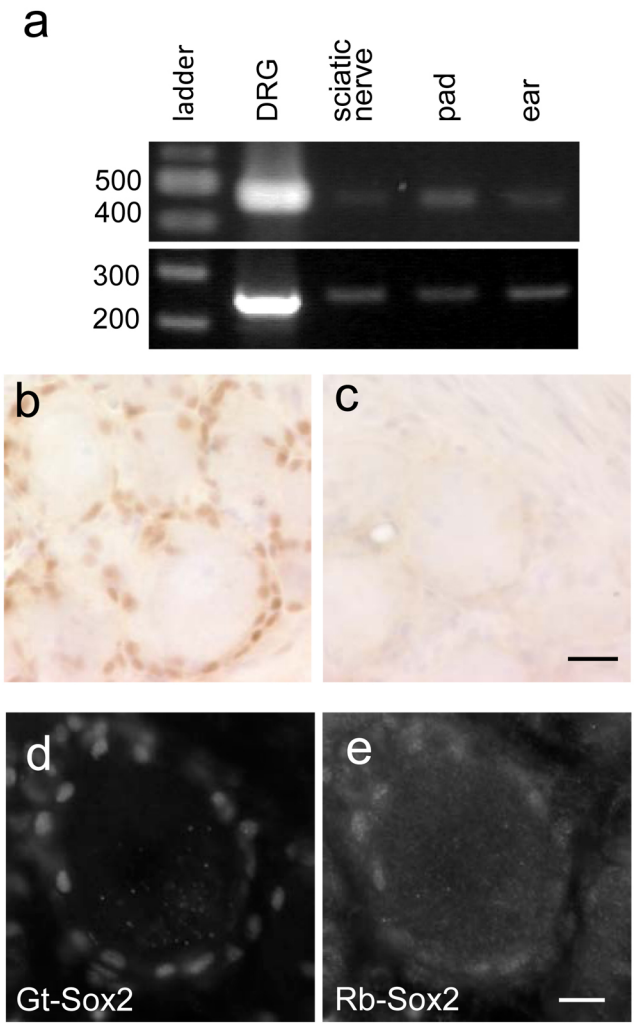


Fig. 5 *Sox2* RT-PCR and antibody specificity controls. **a** Electrophoresed PCR products. A single band of the expected size (upper 466 bp, lower 263 bp) was observed. **b**, **c** Antibody absorption test in DRG. **b** Section stained with goat anti-Sox2 antibody. Many nuclei are stained (brown). **c** Section stained with goat anti-Sox2 antibody pre-incubated with an antigenic peptide. No reaction product is seen. Faint blue, hematoxylin. **d, e** Double immunohistochemistry using goat (d) and rabbit (e) anti-Sox2 antibodies. Positive reactions co-localize. Scale bar, 20 μm

Sox2 in the adult rat sensory nervous system

Histochemistry and Cell Biology

Taro Kioke, Taketoshi Wakabayashi, Tetsuji Mori, Yasuharu Takamori, Yukie Hirahara and Hisao Yamada

Department of Anatomy and Cell Science, Kansai Medical University, 2-5-1 Shinmachi, Hirakatashi, Osaka 573-1010, Japan

E-mail: yamada@hirakata.kmu.ac.jp

Online Resource 1.

Antibody
Anti-CD45 antibody (1:3000; Biolegend, San Diego, CA)

TUNEL
In Situ Cell Death Detection Kit, TMR red (Roche Diagnostics, Tokyo, Japan)

Culture
NIH3T3 cells were seeded in culture dishes and cultured in DMEM (GIBCO, Tokyo, Japan) containing 10% FCS and penicillin/streptomycin (P/S) (GIBCO) at 37°C, 5% CO_2 for 2 days.
Brains from E14.5 mice were harvested in sphere forming medium (DMEM/F12 (1:1) (GIBCO) containing 1% P/S, B27 (20μl/ml, GIBCO), EGF (20ng/ml, Wako Pure Chemical Industries, Ltd., Tokyo, Japan), b-FGF (20ng/ml, Wako) and 0.135% glucose (Wako), and dissociated with a fire polished Pasteur pipette. Next, cells were passed through a 45-μm grid cell strainer, seeded in sphere forming medium and cultured in an incubator at 37°C, 5% CO_2 for 7 days.

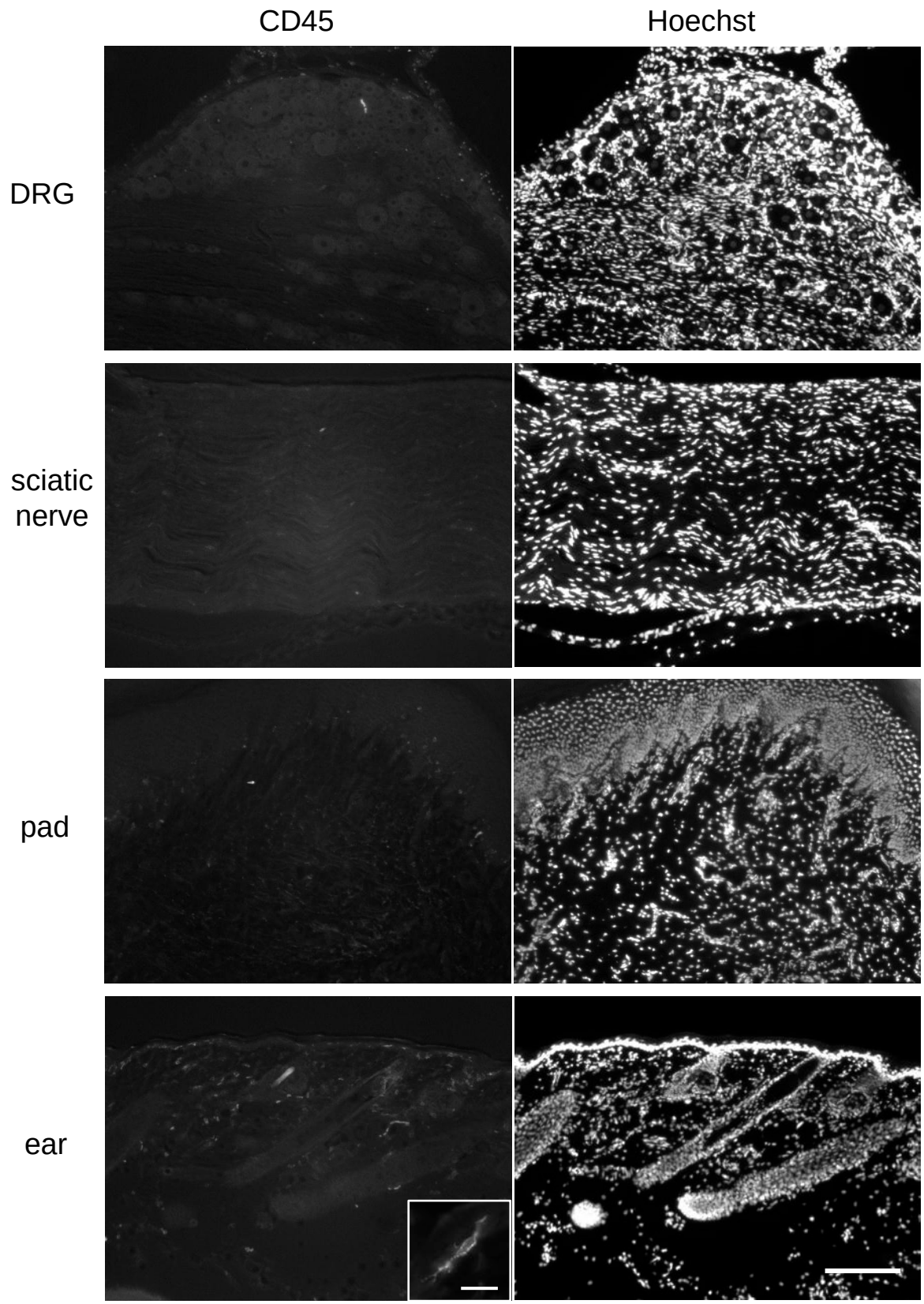
Sox2 in the adult rat sensory nervous system

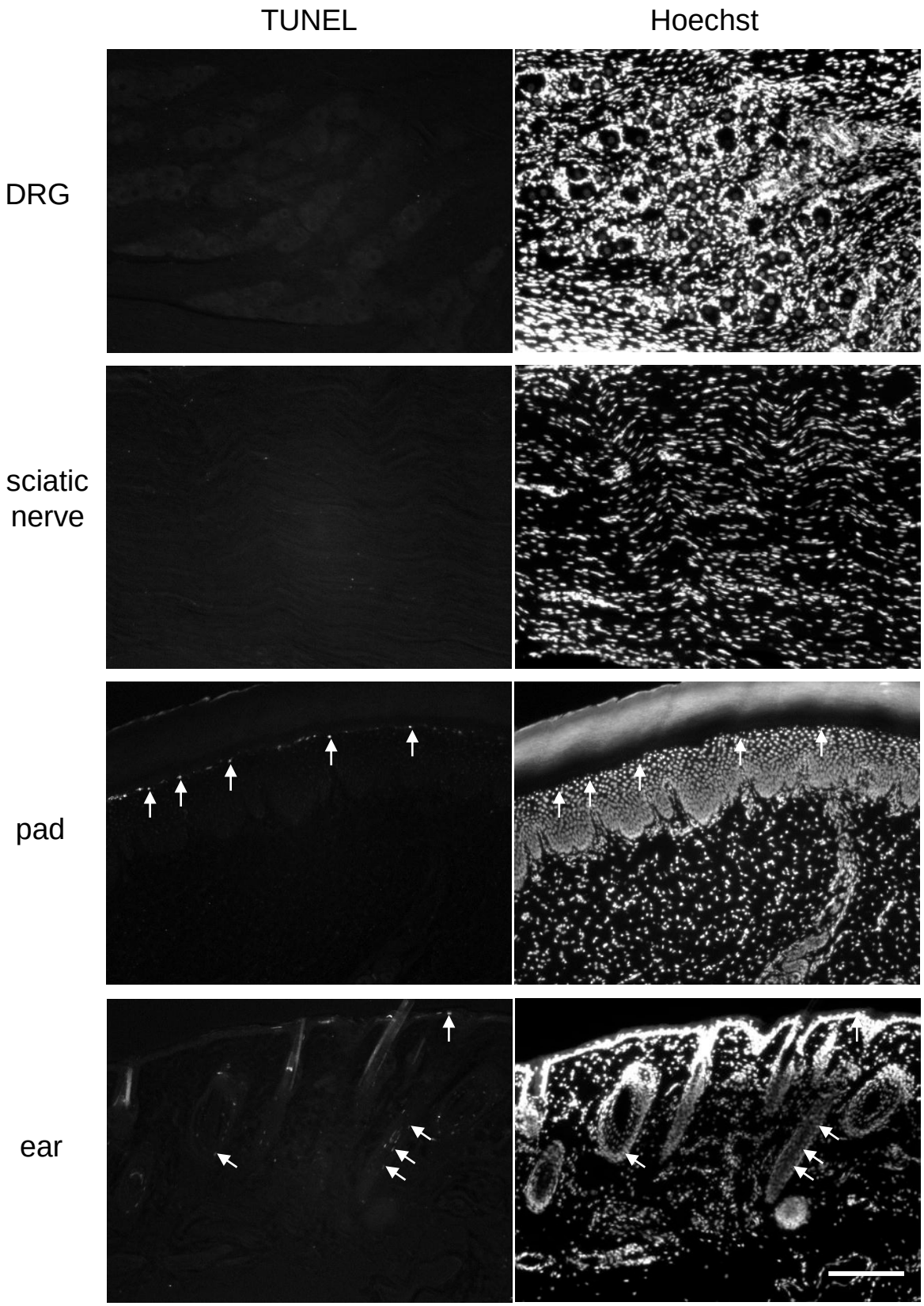
Histochemistry and Cell Biology

Taro Kioke, Taketoshi Wakabayashi, Tetsuji Mori, Yasuharu Takamori, Yukie Hirahara and Hisao Yamada

Department of Anatomy and Cell Science, Kansai Medical University, 2-5-1 Shinmachi, Hirakatashi, Osaka 573-1010, Japan

E-mail: yamada@hirakata.kmu.ac.jp





Online Resource2. Fluorescent images from each tissue. **a** Sections stained with anti-CD45 antibody. Few CD45 positive cells were dispersed in DRG or sciatic nerve. Epidermal dendritic cells in the pad and ear were CD45 positive (boxed area; Scale bar, 20 mm). **b** Sections stained using the TUNEL method. TUNEL positive cells are observed in the epidermal granular layer and hair follicles (arrows), but not in DRG or sciatic nerve. Scale bar, 100 mm

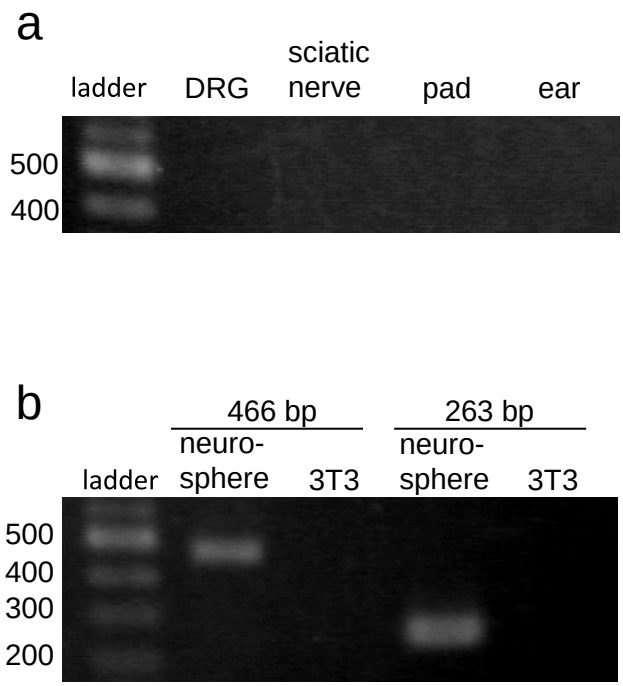
Sox2 in the adult rat sensory nervous system

Histochemistry and Cell Biology

Taro Kioke, Taketoshi Wakabayashi, Tetsuji Mori, Yasuharu Takamori, Yukie Hirahara and Hisao Yamada

Department of Anatomy and Cell Science, Kansai Medical University, 2-5-1 Shinmachi, Hirakatashi, Osaka 573-1010, Japan

E-mail: yamada@hirakata.kmu.ac.jp



Online Resource 3. RT-PCR of *Sox2* mRNA. **a** Electrophoresed PCR products. PCR products were not observed in reverse transcriptase negative samples. **b** Electrophoresed PCR products. Two set of primers were used. A single band is detected in the positive control (neurospheres), but no band is detected in the negative control (NIH3T3 cells).