Supplementary Information

Canine dorsal root ganglia satellite glial cells represent an exceptional cell population with astrocytic and oligodendrocytic properties

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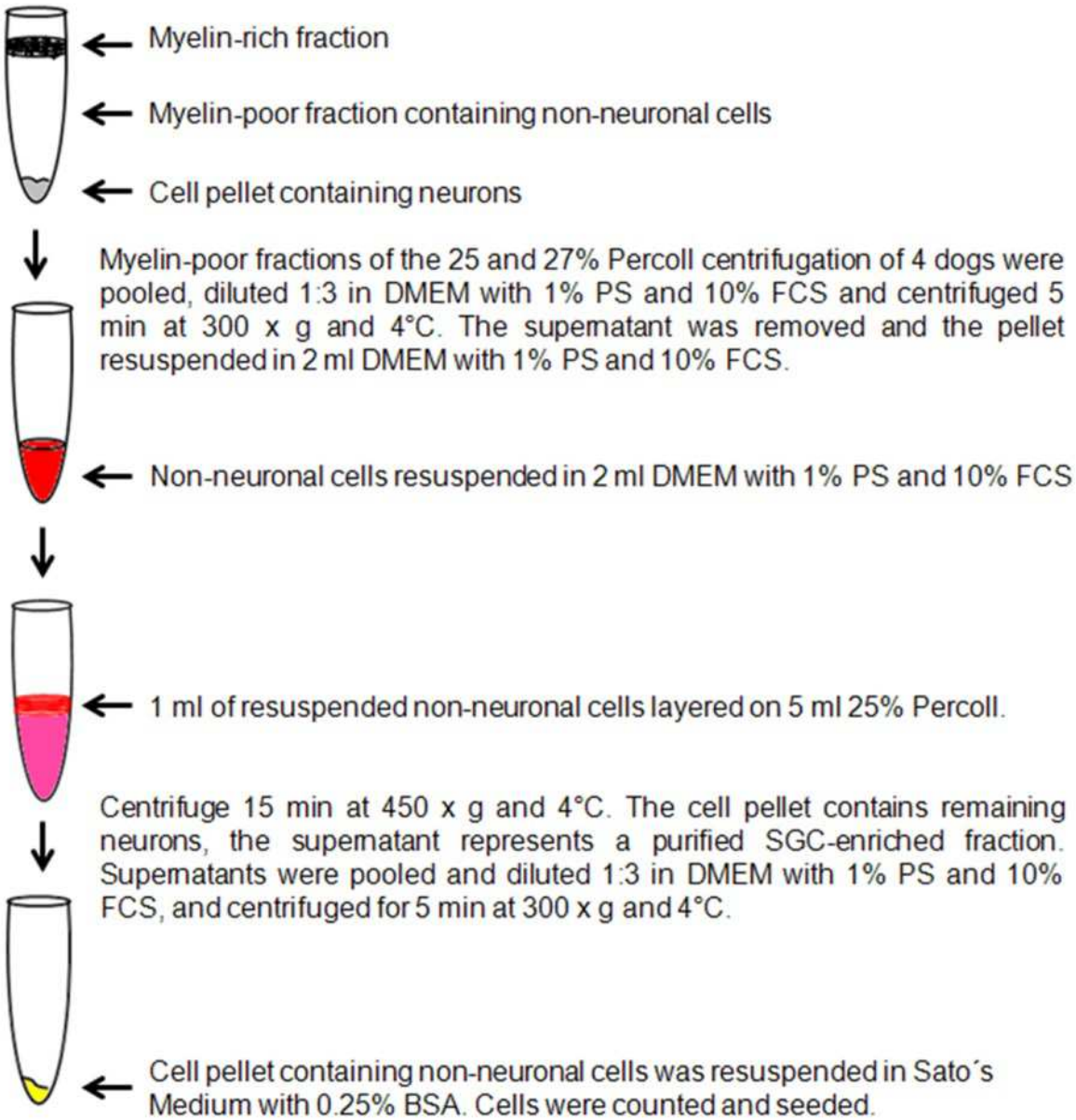
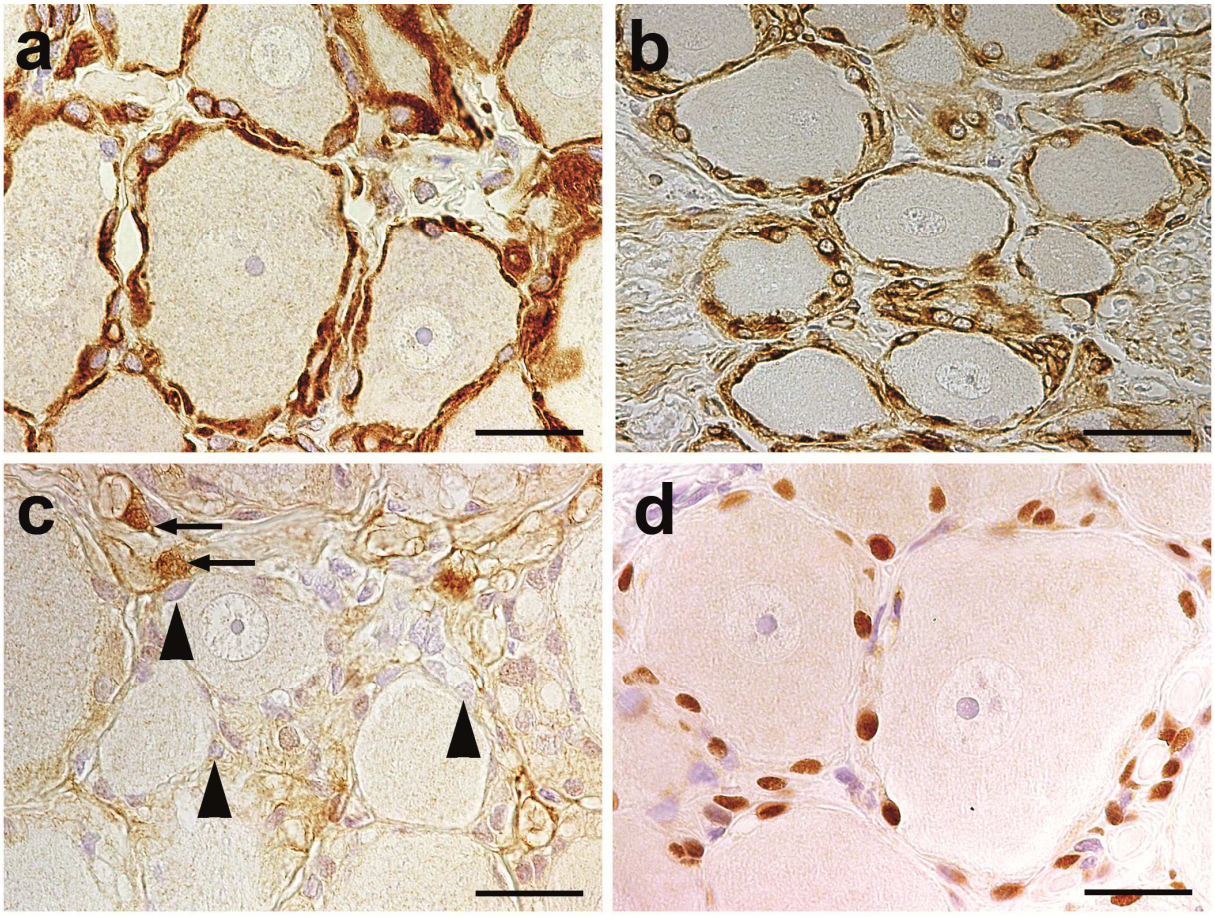
Supplementary Fig. S1: Preparation of a pooled satellite glial cell (SGC)-enriched cell fraction of 4 dogs. Isolated dorsal root ganglia from one dog were splitted on 2 tubes for digestion and subsequent 25% and 27% Percoll centrifugation. About 4 ml of the myelin-poor fraction were collected per tube (in total approximately 16 ml for each dog). For 1:3 dilution, the 16 ml were pipetted in a 50 ml Falcon tube and filled with Dulbecco's modified eagle medium (DMEM) with 1% Penicillin/Streptomycin (PS) and 10% fetal calf serum (FCS) to a volume of 48 ml (4 tubes for 4 dogs). The following centrifugation (5 min at 300 x g and 4°C) leads to a cell pellet containing SGCs, other non-neuronal cells, and small to moderate numbers of neurons. The cell pellet of one dog was resuspended in 2 ml DMEM with 1% PS and 10% FCS. This suspension was used to resuspend consecutively cell pellets of the other dogs and finally contains the non-neuronal cells of four dogs in 2 ml DMEM with 1% PS and 10% FCS. Two tubes containing 5 ml 25% Percoll were prepared, layered with 1 ml of the SGC-enriched resuspension, and centrifuged (15 min at 450 x g and 4°C). The resulting cell pellet contains the remaining neurons. About 4.5 ml of the supernatant were collected from the two tubes, pooled in a 50 ml Falcon tube, and diluted 1:3 by adding 18 ml DMEM with 1% PS and 10% FCS. The following centrifugation (5 min at 300 x g and 4°C) results in a cell pellet containing SGC and other non-neuronal cells, which was resuspended in Sato´s Medium with 0.25% bovine serum albumin (BSA). The cell content was evaluated using a Neubauer chamber.


Myelin-rich fraction
Myelin-poor fraction containing non-neuronal cells
Cell pellet containing neurons
Myelin-poor fractions of the 25 and 27% Percoll centrifugation of 4 dogs were pooled, diluted 1:3 in DMEM with 1% PS and 10% FCS and centrifuged 5 min at 300 x g and 4°C. The supernatant was removed and the pellet resuspended in 2 ml DMEM with 1% PS and 10% FCS.
Non-neuronal cells resuspended in 2 ml DMEM with 1% PS and 10% FCS
1 ml of resuspended non-neuronal cells layered on 5 ml 25% Percoll.
Centrifuge 15 min at 450 x g and 4°C. The cell pellet contains remaining neurons, the supernatant represents a purified SGC-enriched fraction. Supernatants were pooled and diluted 1:3 in DMEM with 1% PS and 10% FCS, and centrifuged for 5 min at 300 x g and 4°C.
Cell pellet containing non-neuronal cells was resuspended in Sato´s Medium with 0.25% BSA. Cells were counted and seeded.

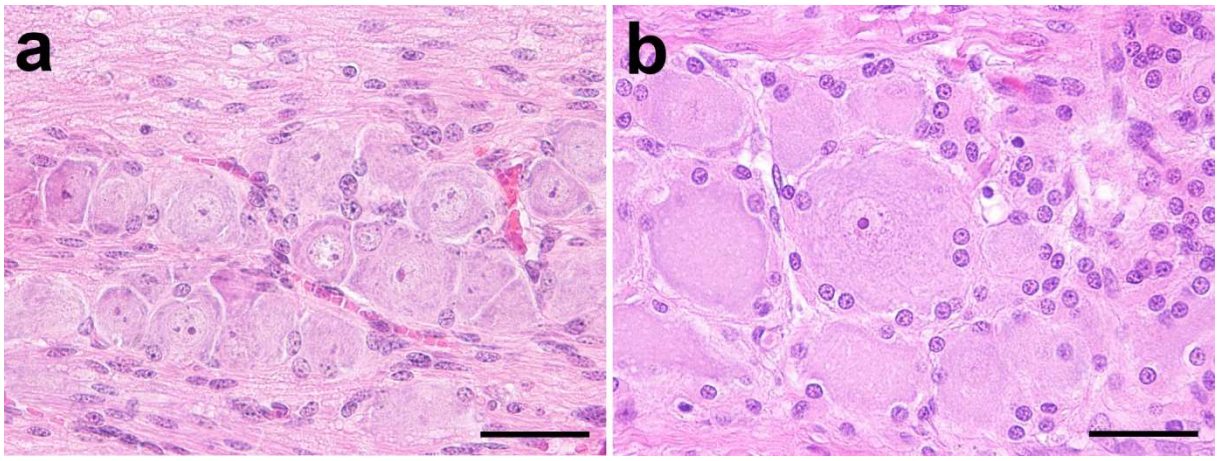
Supplementary Fig. S2:

Dorsal root ganglion of a 1-year-old beagle dog **(a, c, d)** and a 4-year-old northern plains gray langur (*Semnopithecus entellus*) **(b)**. The majority of canine **(a)** and simian **(b)** satellite glial cells (SGCs) expressed vimentin. Few canine SGCs also expressed S-100 **(c)**. Most of canine SGCs showed a strong intranuclear expression of Sox2 **(d)**. Bars, 40 µm.



Supplementary Fig. S3:

Dorsal root ganglion of a 4-month-old mouse **(a)** and a 4-year-old northern plains gray langur (*Semnopithecus entellus*) **(b)**. Multiple neurons surrounded by a satellite glial cell sheath. Note few fibroblasts and capillaries in the interstitial stroma. Hematoxylin and eosin staining. Bars, 40 µm.



Supplementary Fig. S4:

Dorsal root ganglion of a female 22-month-old beagle dog. Immune transmission electron microscopy for the intermediate filament glial fibrillary acidic protein (GFAP). **(a)** Morphology of $GFAP^+$ satellite glial cells *in vitro*. The marked area is shown in a higher magnification in "**b**". Bar, 1 µm. **(b)** Higher magnification of the marked area in "**a**" showing the cytoplasmic GFAP filaments labeled with gold particles (arrows). Bar, 200 nm.