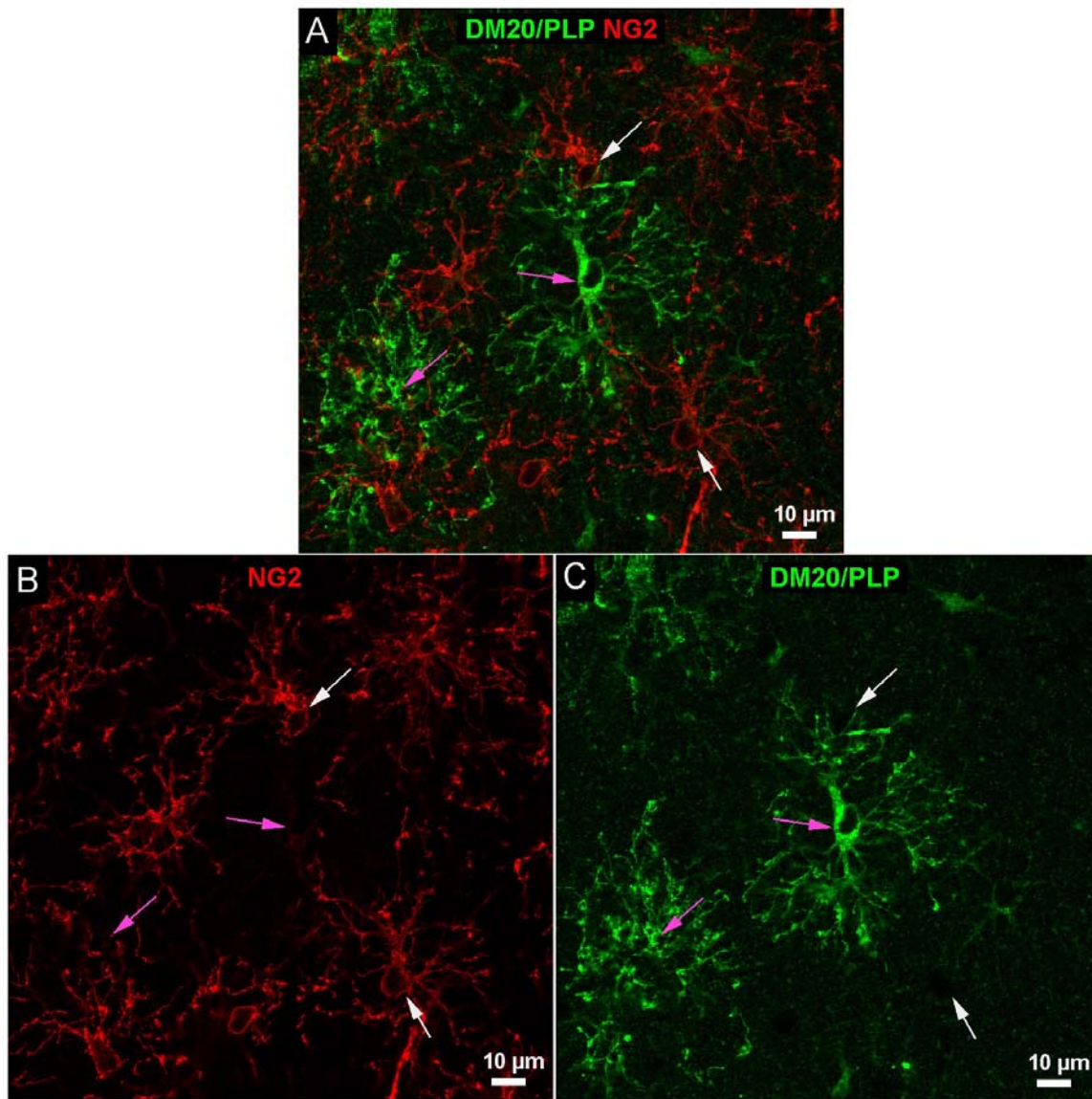
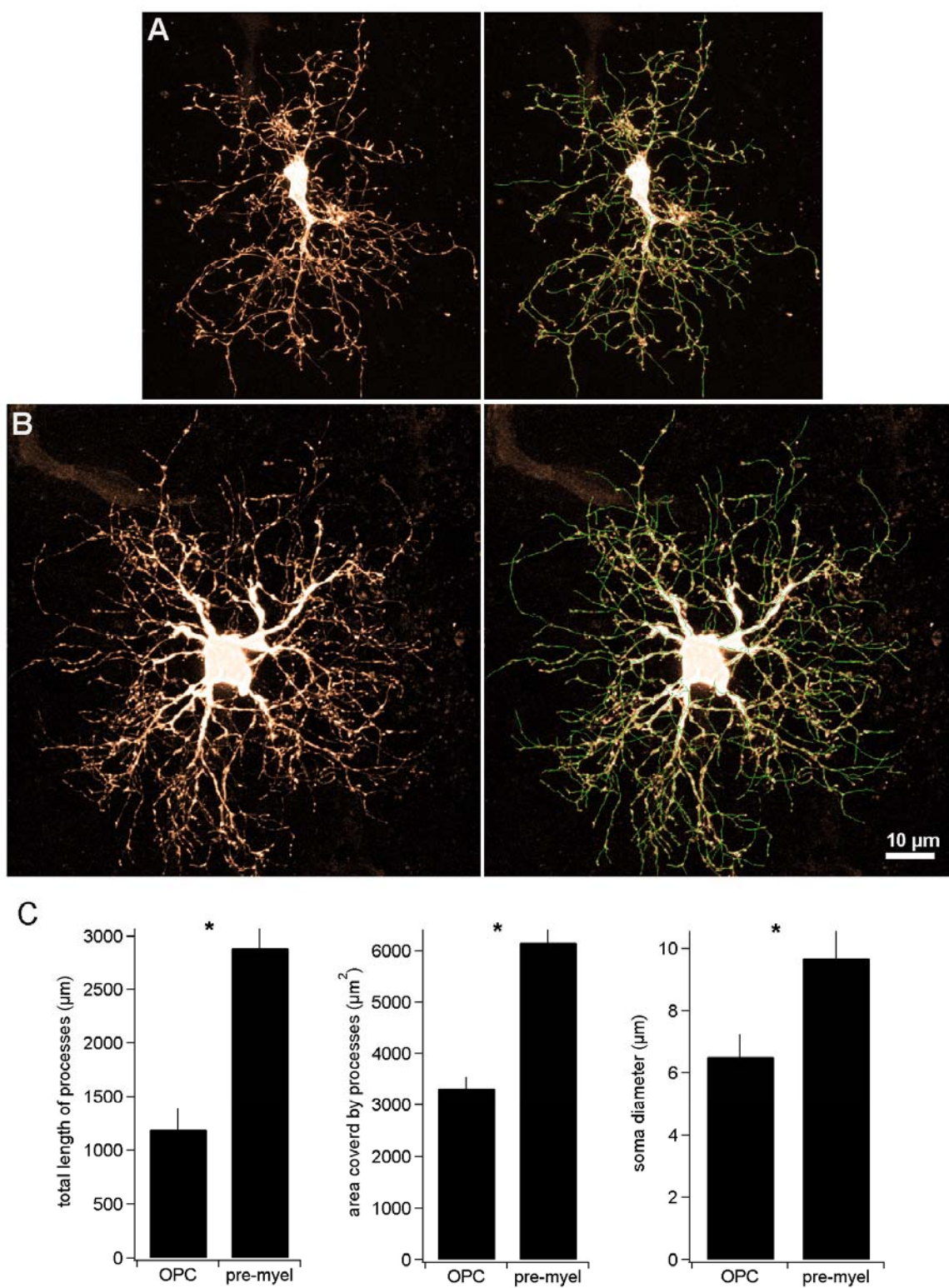




Suppl. Figure 1

Suppl. Figure 1. Pre-myelinating oligodendrocytes do not express NG2.

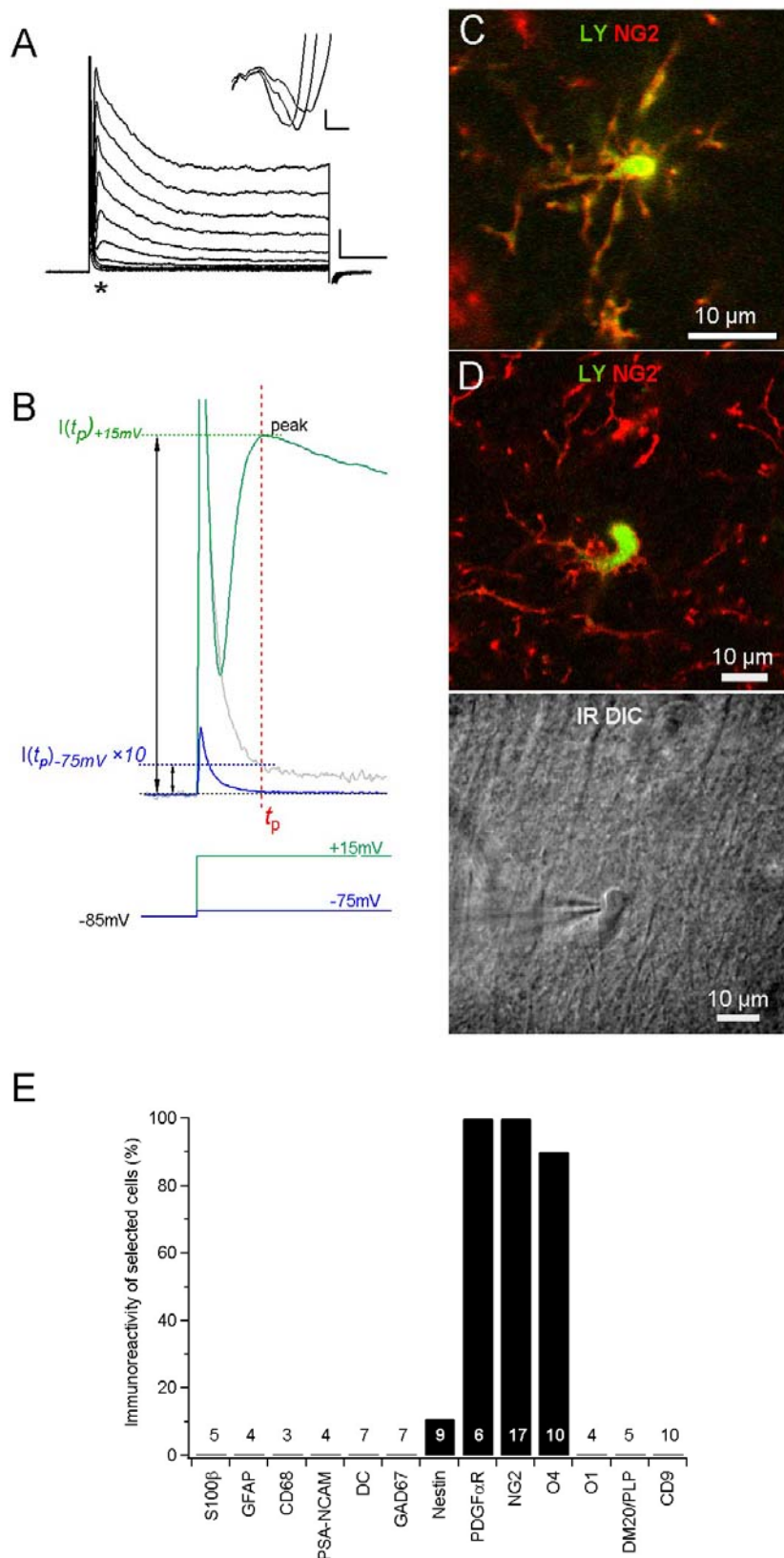
- A) Double staining against DM20/PLP (Alexa555, green) and NG2 (Alexa488, red) in CA1 stratum radiatum at P14. Pre-myelinating oligodendrocytes (purple arrows) and OPCs (white arrows) are largely distinct populations of cells.
- B) NG2 channel of the compound image shown in A. Note that the labelled pre-myelinating oligodendrocytes (purple arrows) are not NG2 positive.
- C) DM20/PLP channel of the compound image shown in A. Vice versa, NG2 cells are not labelled by DM20/PLP antibodies.



Suppl. Figure 2

Supplementary figure 2. Morphometry of developing oligodendroglial cells.

- A) Left panel: maximum projection of a deconvolved confocal image stack of a Lucifer Yellow filled OPC. Note the typical polygonal shape of the soma. Right panel: as in A plus superimposed green lines which indicate how processes were traced (using the NeuronJ plugin of ImageJ). This scale bar in panel B also applies to this image.
- B) Left panel: maximum projection of a deconvolved confocal image stack of a pre-myelinating oligodendrocytes filled with Lucifer Yellow. Noteworthy, pre-myelinating oligodendrocytes showed significantly more processes, a larger soma and their tree of processes spans over a wider area. This cell stained positively for O1 (n=4). Right panel: green lines are superimposed to show how the tree of processes was traced for analysis.
- C) Summary bar graphs comparing the total process length, the area covered by all processes and the soma in diameter between OPCs (n = 4) and pre-myelinating oligodendrocytes (pre-myel, n = 4). All measurements have been obtained from maximum projections as shown in A and B. The soma diameter was calculated for each cell by measuring its minimum and maximum extent in two orthogonal directions and averaging the two values.



Suppl. Figure 3. Selection of cells for patch clamp analysis and verification of them as OPCs.

The aim of supplementary figure 3-8 is to illustrate how we selected oligodendrocyte precursor cells (OPCs) for patch clamp recordings based on simple criteria and how we verified the cells' identity by immunohistochemistry after dye-filling during recording ($n = 91$). Furthermore, we illustrate how astrocytes and microglial cells can be reliably identified based on their whole-cell current pattern.

During the initial search for a suitable cell in the live DIC video image we selected cells in stratum radiatum of the CA1 region with a small cell body showing no processes (cf supplementary figure 3D, lower panel). On the video screen the cell bodies typically were smaller than $10 \mu\text{m}$. In particular, we avoided large cell bodies and cells with one or more visible processes which may represent interneurons (as recorded for

supplementary figure 5) and other types of cells.

With this preselection we obtained two types of current patterns. The most frequently encountered current pattern was dominated by prominent voltage activated outward currents as shown in supplementary figure 3A (and in figure 6A). Based on this initial observation and based on previous work by the group of Bergles (Bergles et al., 2000) we speculated that the presence of large transient outward currents may reliably identify OPCs. We used a simple procedure to measure the amplitude of the outward current (supplementary figure 3B) and *hypothesised that all of those preselected cells in which the outward current exceeds the leak current at least 3-fold can safely be classified as OPCs*. We then subjected 91 cells, which fulfilled this criterion (ratio-criterion), to post-recording immunohistochemical staining protocols with various antibodies against cell-type specific markers. As can be seen in supplementary figure 3E, this population of cells is clearly identified as OPCs because it expresses NG2, O4 and PDGFalphaR and no markers of more mature oligodendroglial stages or of other glial lineages. Supplementary figures 3 to 6 illustrate examples of these stainings. We further confirmed that these cells do not express markers of immature or mature neurons (nestin, doublecortin, PSA-NCAM, GAD67, Fig. 3E, scans not shown).

The second type of current pattern, which we observed only occasionally (due to our preselection), did not show detectable amounts of voltage gated currents but only passive time-independent currents. Passive currents were either very pronounced or very tiny yielding either input resistance values of around 20 MOhm or 4 GOhm, respectively. The morphology of the cells revealed by dye filling suggested that the former group represent astrocytes and the latter group represent microglial cells. This classification was confirmed by astrocyte- and microglia-specific antibodies (supplementary figure 4, 5).

Supplementary figure 7 shows the distribution of our criterion value across the entire population of cells as well as the electrical passive properties of OPCs as opposed to astrocytes and microglial cells.

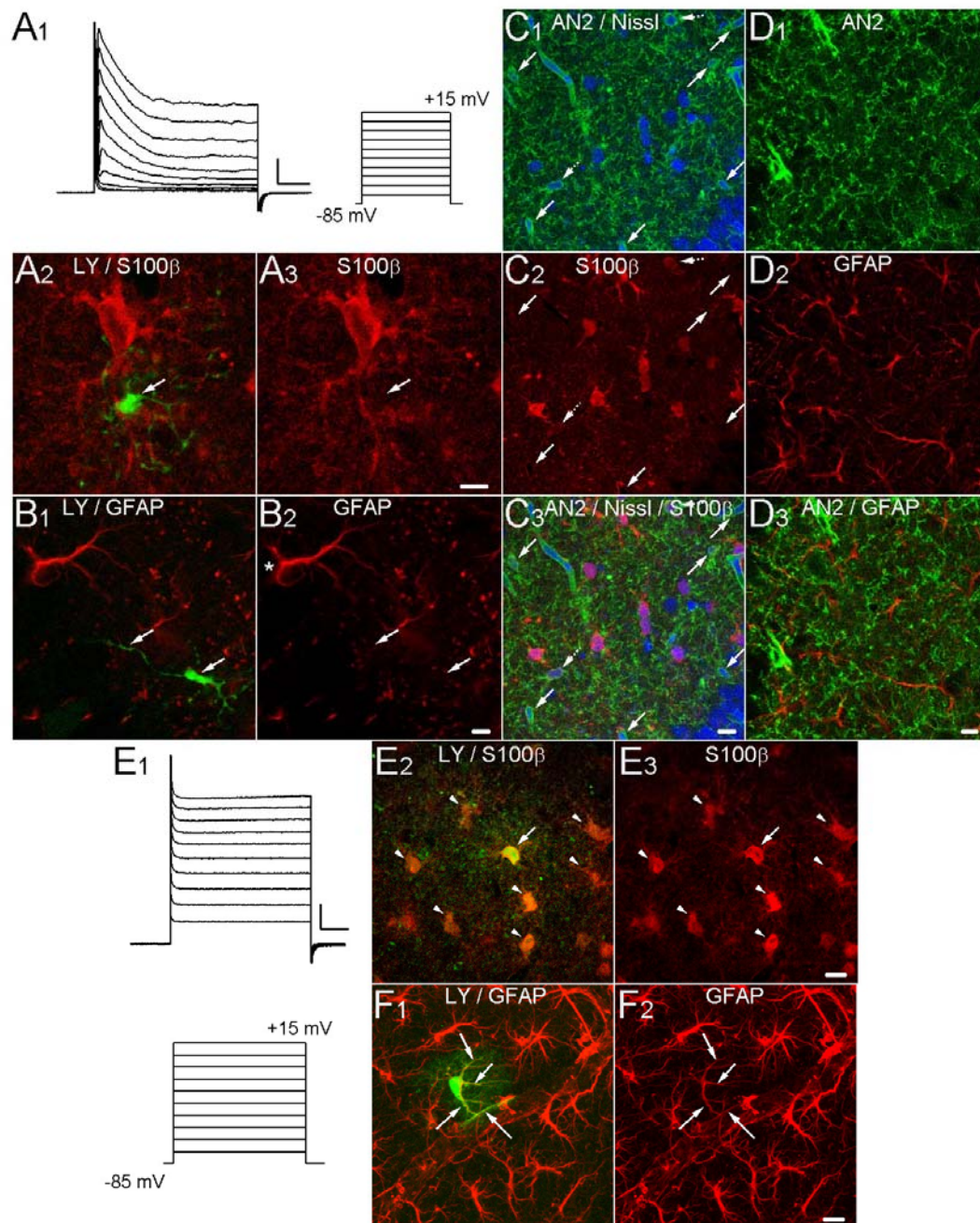
In the results section of the main part of this manuscript we describe the current pattern of pre-myelinating oligodendrocytes in which the outward currents also exceed the leak currents 3-fold. This seemingly contradicts our claim made in this supplementary material that the ratio-criterion identifies OPCs and that

out of 19 selected cells not one was stained with either O1, DM20/PLP or CD9 antibodies (supplementary figure 4E). However, it is important to note firstly, that our ratio-criterion includes the preselection of small cells without any processes and thereby avoids the large sized pre-myelinating oligodendrocytes and secondly, that pre-myelinating oligodendrocytes are much rarer than OPCs (at least 20-fold) and would therefore, even without preselection, only rarely be encountered. Similarly, most neurons fulfil the requirement of outward currents 3-fold larger than leak currents but are excluded by the preselection. Therefore, our ratio-criterion (which includes our preselection according to size and number of processes in the DIC picture) works very well to identify OPCs for recording, but it does not imply that there are no other types of cell in which the outward current exceeds the leak current at least 3-fold.

- A) Typical whole cell current pattern fulfilling the ratio-criterion and presumed to define an OPC in stratum radiatum of the CA1 region in the second postnatal week (capacitive currents are truncated for clarity, see supplementary figure 3A1 for the applied voltage step protocol). Note the prominent transient and sustained outward currents activated starting at around -40 mV. Outward currents are preceded by small inward currents resembling fast Na^+ currents (labeled with an asterisk, scale bar: 200 pA, 20 ms). Inset: A magnified view of the initial inward currents (leak subtracted) at the potentials -5, +5 and +15 mV (scale bar: 50 pA, 0.5 ms).
- B) Calculation of the criterion value for the electrophysiological identification of OPCs. *Upper panel*, the cell is held at -85 mV. Blue trace, current response to a 10 mV voltage step (from -85 to -75 mV). Green trace, current response to 100 mV voltage step (from -85 to +15 mV). Grey trace, current response to 10 mV voltage step (from -85 to -75 mV), multiplied by 10. t_p , the time point at which the voltage-gated outward currents peak. At this time point t_p , the ratio of the currents $I(t_p)_{+15\text{mV}} / [I(t_p)_{-75\text{mV}} \times 10]$ was calculated from a 100 mV and a 10 mV voltage steps, where $I(t_p)_{+15\text{mV}}$ is the peak amplitude of voltage-gated potassium current at +15 mV (100 mV step) and $[I(t_p)_{-75\text{mV}} \times 10]$ is the amplitude of a current at -75 mV (10 mV step), multiplied by a factor of 10. The value of this ratio roughly expresses the amplitude of the outward current with respect to the amount of leak current (at t_p capacitive currents have largely subsided) and thereby corrects for

differences in cell size (if assuming that larger cells display more leak current). If this ratio was larger than 3, cells were included into the presumed OPC group. In some cells we started the voltage steps at a holding potential of -80 mV instead of -85 mV. *Lower panel*, corresponding command voltages (colour-coded). Blue trace represents the 10 mV rectangular voltage step, green trace shows the 100 mV rectangular voltage step.

- C) During whole cell recordings cells were routinely filled with the tracer Lucifer Yellow (LY) allowing post-recording immunohistochemical staining (shown in the following supplementary figures 4 to 8). All cells selected with the ratio criterion shown in B) were labeled by anti-NG2 antibodies (17/17 cells) as illustrated by the dual channel confocal laser scan (LY, green; NG2, red, Cy3). The same cell as in A.
- D) *Upper panel*, another dual channel confocal laser scan (LY, green; NG2, red, Cy3) showing that selected cells express NG2. Same cell as shown in B). *Lower panel*, IR DIC image of the cell shown in the upper panel while being patch-clamped in the live hippocampal slice. The patch pipette approaches the cell from the left.
- E) Summary of postrecording immunostaining of cells selected by the ratio criterion as described above. The marker profile suggests that these cells are OPCs in the stage of pro-oligodendrocytes as defined by their reactivity to antibodies against NG2, O4 and PDGFalphaR. The small numbers denote the total numbers of cells investigated for each marker.



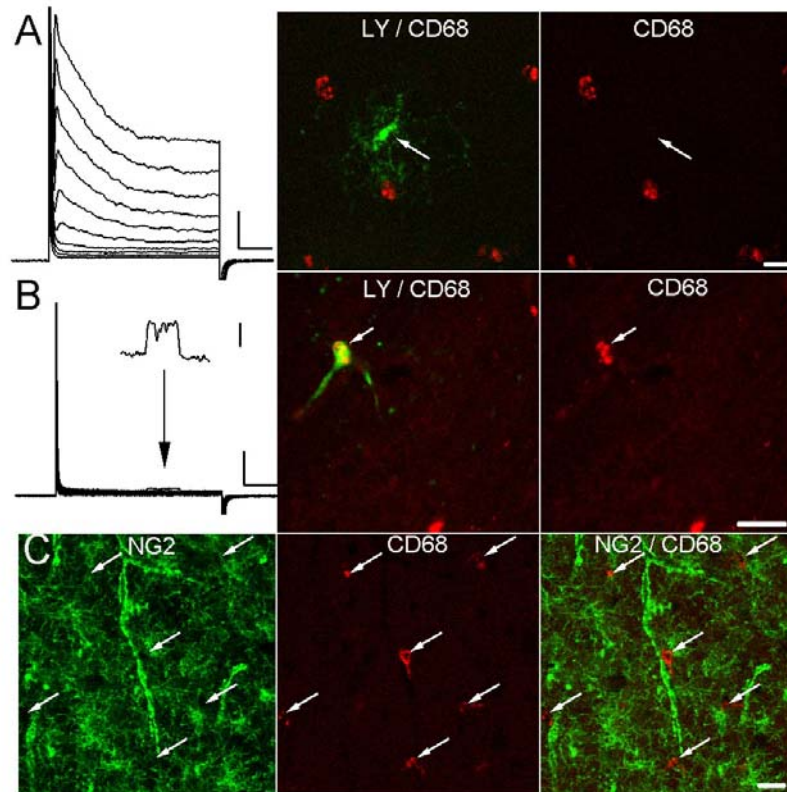
Suppl. Figure 4. Presumed OPCs are not labelled by astrocyte markers.

- A) A1: Whole cell current pattern of another cell fulfilling the presumed OPC criterion and showing prominent transient and sustained outward and small Na^+ currents. The standard voltage command waveform is shown on the right. This cell was recorded for 60 s only to avoid dilution of cellular S100 β content. Scale bars, 20 ms, 0.2 nA. Ratio cells are not labeled with astrocyte-identifying anti-S100 β antibodies. A2: dual channel laser scan of the Lucifer yellow (LY, green) filled cell shown in A (white arrow) together with a neighbouring S100 β + astrocyte (S100 β detected with

- Cy5, red). Note the larger size of the astrocyte and the close proximity of an astrocytic process with the recorded cell. A3 illustrates the S100 β related fluorescence only to show that the ratio cell (white arrow at the same position) does not co-localize with this marker. Scale bar, 5 μ m.
- B) Another presumed OPC filled with Lucifer yellow (green) and subjected to a staining for the astrocytic filament protein GFAP (Cy5, red). The soma and a process of the ratio cell are marked with white arrows and completely lack GFAP expression (cf B2). A GFAP positive astrocyte is indicated with an asterisk placed on the position of the nucleus. GFAP is typically found in the processes of an astrocyte and is only weakly expressed in the soma. Note again the larger size of the astrocytic soma and processes when compared to the ratio cell. Scale bar, 5 μ m.
- C) Double immunolabeling for AN2 (murine NG2 analogue, green, Alexa555) and S100 β (red, Cy5). Nuclear/cytoplasmic counterstain is shown in blue ("Nissl green") and reveals the pyramidal cell layer in the right bottom corner of the image. In P10 CA1 stratum radiatum S100 β and AN2 mark two largely distinct cell populations. AN2⁺ cells were identified as nuclei, which are surrounded by a rim of AN2 staining. Full arrows denote AN2⁺ positive cells, which completely lack S100 β . Dashed arrows indicate a few AN2⁺ cells expressing a low amount of S100 β . In general, AN2⁺ S100 β ⁺ cells appeared larger than AN2⁺ S100 β ⁻ cells. In 5 slices we counted 41 AN2⁺ and 57 S100 β ⁺ cells in CA1 stratum radiatum. In agreement with a previous report (Deloulme et al., 2004), only 22 % of AN2⁺ cells expressed low levels of S100 β at this age. Conversely, only 16 % of S100 β ⁺ cells were marked by AN2 antibodies. This overlap of AN2 and S100 β is expected since recent work shows that also certain developmental stages of oligodendroglial cells transiently express S100 β (Deloulme et al., 2004; Hachem et al., 2005; Vives et al., 2003). Note that AN2 (as well as NG2) antibodies strongly react with vascular cells outlining the course of capillaries. Scale bar, 10 μ m.
- D) Double immunolabeling for AN2 (green, Alexa555) and GFAP (red, FITC). Both markers label the processes of glial cells. However, apart from accidental crossings of processes, we could not detect any co-localisation which may suggest a cell expressing both GFAP and AN2. Scale bar, 10

μm.

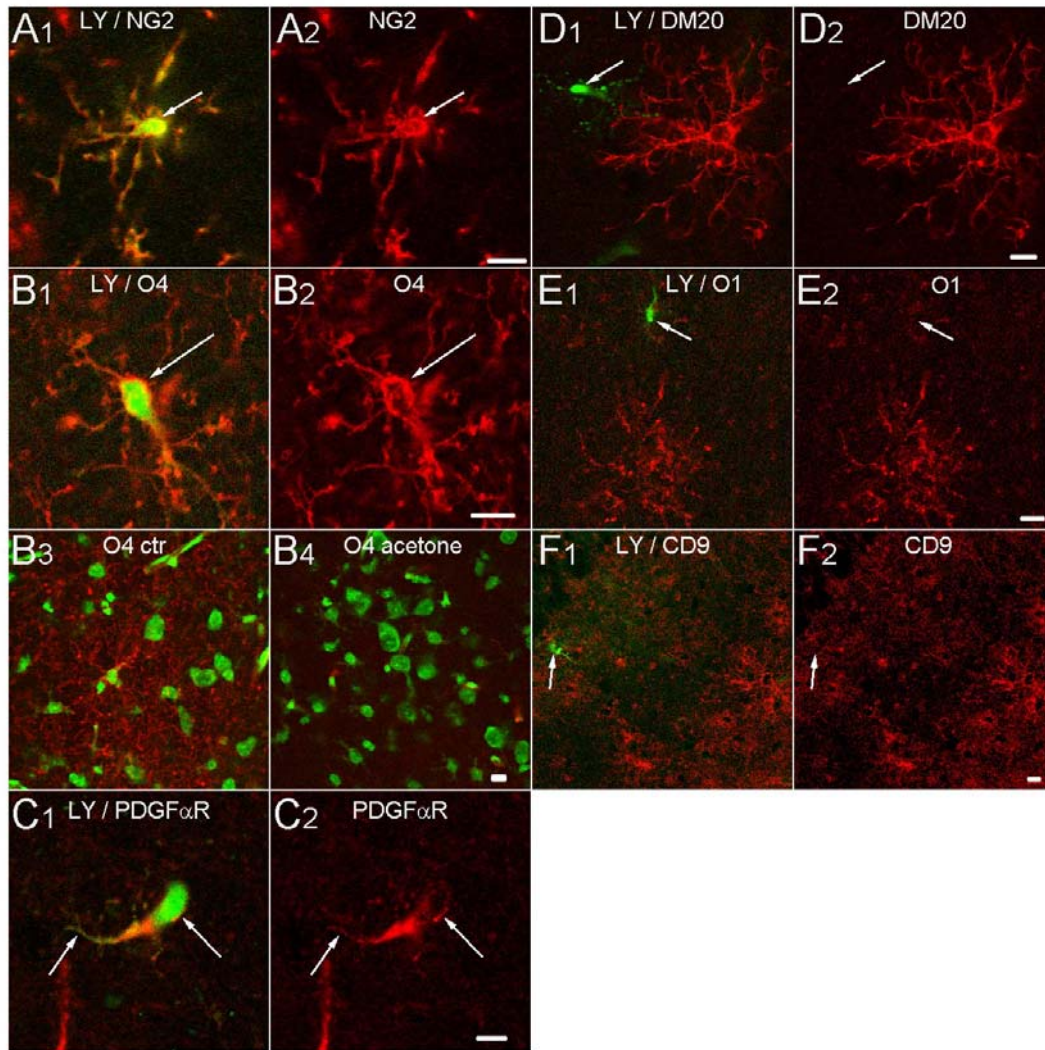
- E) *E1*: Astrocytes are electrophysiologically identified by an ohmic current pattern and large ungated leak currents. The whole cell current increases slightly sublinearly with increasing depolarization because of a slight rise of series resistance during the recording (as judged by the capacitive current response, here truncated for clarity). Scale bar, 200 pA, 20 ms. The lower panel shows the applied command voltage waveform. *E2+E3*: The cell shown in *E1* is marked by an arrow and was clearly stained by S100β antibodies (red, Cy5, 3 out of 4 cells with this current pattern were positive for S100β). During whole cell recording LY was transferred to numerous neighboring S100β+ astrocytes (arrowheads). Scale bar, 10 μm.
- F) *F1*: dual channel confocal scan illustrating a Lucifer yellow filled cell which showed a passive current pattern, as depicted in *E1*, and which strongly stained for GFAP. 6 out of 7 cells with this current pattern were clearly positive for GFAP. *F2*: GFAP channel only. Arrows indicate the position of the dye-filled cell and demonstrate the co-localisation with the filamentous protein. Scale bar, 10 μm. Note that the astrocytes shown in the panels E and F have been subjected to the same staining procedure as the presumed OPCs shown in *A* and in *B* and therefore provide a positive control for the post-recording immunostaining protocol.



Suppl. Figure 5. Presumed OPCs do not express microglial markers.

- A) Whole cell current pattern of a ratio-criterion cell (left panel) filled with LY and immunostained for the microglia marker CD68 following recording (middle and right panel, respectively). Note that recorded cell (arrow) lies in between positive microglial cells but is not labeled by CD68 antibodies (red, Alexa555). Note that in resting microglial cells, the CD68 antigen is primarily found on extra-nuclear, somatic organelles. Scale bar, 20 ms, 0.2 nA and 10 μ m.
- B) A non-ratio-criterion cell displaying a passive current pattern but with very few leak channels (left panel). Such cells are stained with antibodies against the CD68 microglial antigen. Microglial cells lack voltage activated channels, show a high input resistance (>1 GOhm, left panel) and a depolarized membrane potential (around -20 mV). Occasionally, single channel events can be observed (arrow). Inset shows an opening of a single putative BK channel at +20 mV. Middle and right panels show that this LY filled cell is labeled with anti-CD68 antibodies (red, Alexa555). 6 out of 6 cells with this ohmic current pattern and high input resistance stained positively for CD68. Scale bar, 20 ms, 0.2 nA (5 pA in inset) and 10 μ m.

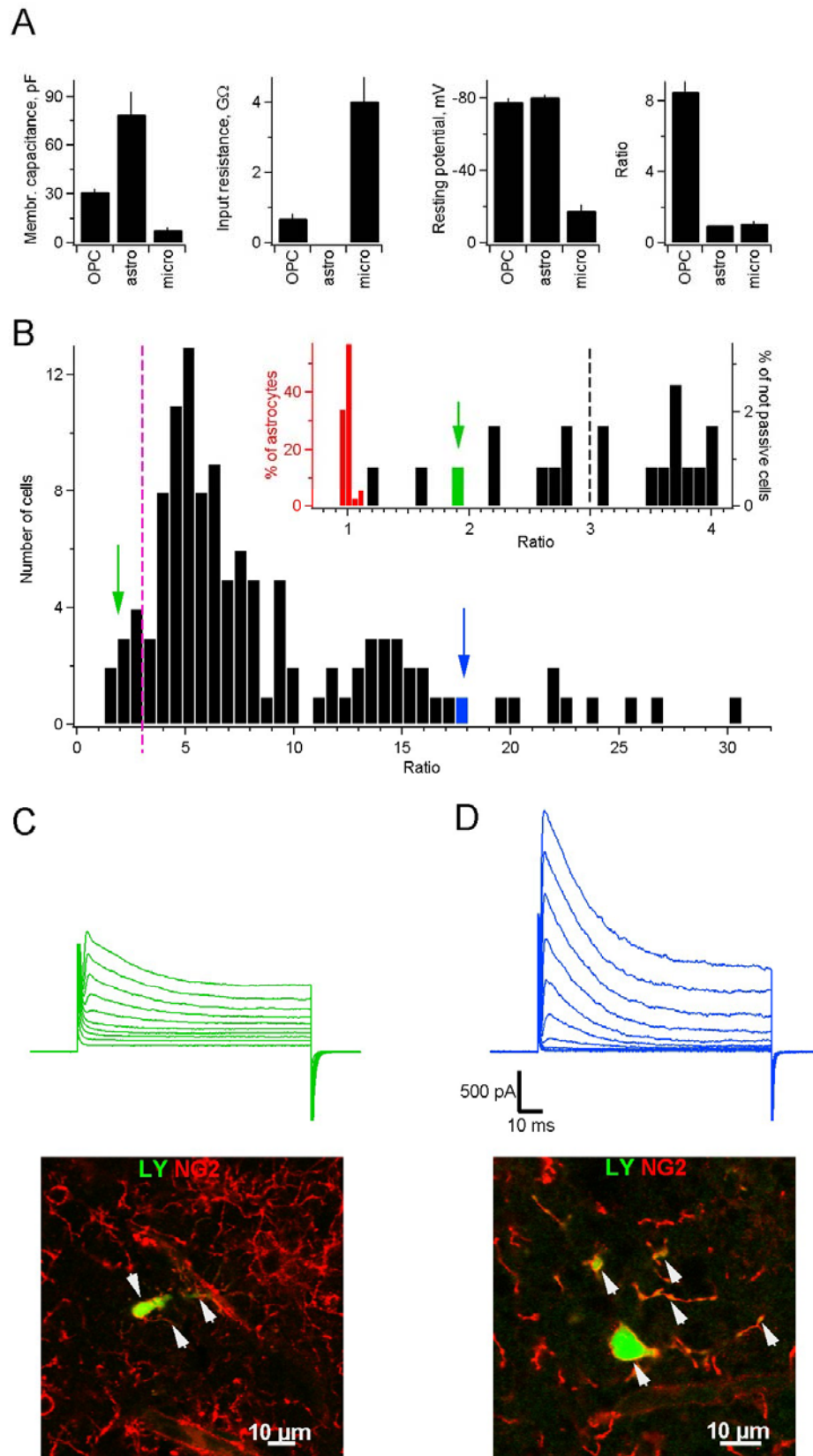
- C) Double labeling of NG2 (green, Cy2) and CD68 (red, Alexa555) in CA1 stratum radiatum of P10 mouse. Two distinct populations of cells are marked with no apparent overlap. Somata of microglial cells are indicated with arrows. Scale bar, 20 μm .



Suppl. Figure 6. Presumed OPCs are labelled by NG2, O4, PDGFαR but not by more mature markers of the oligodendroglial lineage.

- A) A1: Ratio-criterion cells express NG2 (LY, green, NG2 red). A2: as in A1 but only the NG2 channel is shown. Scale bar, 5 μ m.
- B) B1: Ratio-criterion cells (LY, green, arrow) co-express O4 (red, Cy3), a marker that characterizes more mature oligodendrocyte precursors (pre-oligodendrocytes). B2: O4 channel in isolation. Scale bar, 10 μ m. B3: O4 staining (red, Cy3) in CA1 stratum radiatum shown at a lower magnification (green, Nissl stain). B4: controlling the specificity of the O4 staining. The sulfatides labelled by O4 antibodies are known to be dissolved by acetone. We verified this property by briefly incubating hippocampal slices in acetone. While the nuclear stain (green) is preserved, the O4 staining is nearly entirely removed by this procedure. Scale bar, 10 μ m.

- C) *C1*: Ratio-criterion cells (LY, green, arrows) are strongly labeled by PDGF α R antibodies (red, Cy3). *C2*: red channel of the image shown in C1. Scale bar, 10 μ m.
- D) *D1*: Selected cells (LY, green, arrow) do not express DM20/PLP (red, Alexa555), which characterizes pre-myelinating and myelinating oligodendrocytes. Note the typical morphology of the DM20/PLP positive pre-myelinating oligodendrocyte next to the dye-filled OPC. The soma is larger and round and the processes show a higher degree of branching. *D2*: red channel of the image shown in D1. Scale bar, 10 μ m.
- E) *E1*: O1 antibodies (red, Cy3) do not mark cells selected according the ratio-criterion (LY, green). Note that O1 positive pre-myelinating oligodendrocytes already start to appear in hippocampus at P8 to P12 (lower half of the image, note that the soma of the O1 cell is out of plane). *E2*: red channel of the image shown in E1. Scale bar, 10 μ m.
- F) *F1*: Ratio-criterion cells (LY, green, arrow) do not express the tetraspanin CD9 (red, Alexa555). Cells stained by CD9 antibodies resemble premyelinating oligodendrocytes and are found in the right half of this image. Compare the example at the right part of the figure to the DM20+ cell in D. *F2*: red channel of the image shown in F1. Scale bar, 10 μ m.

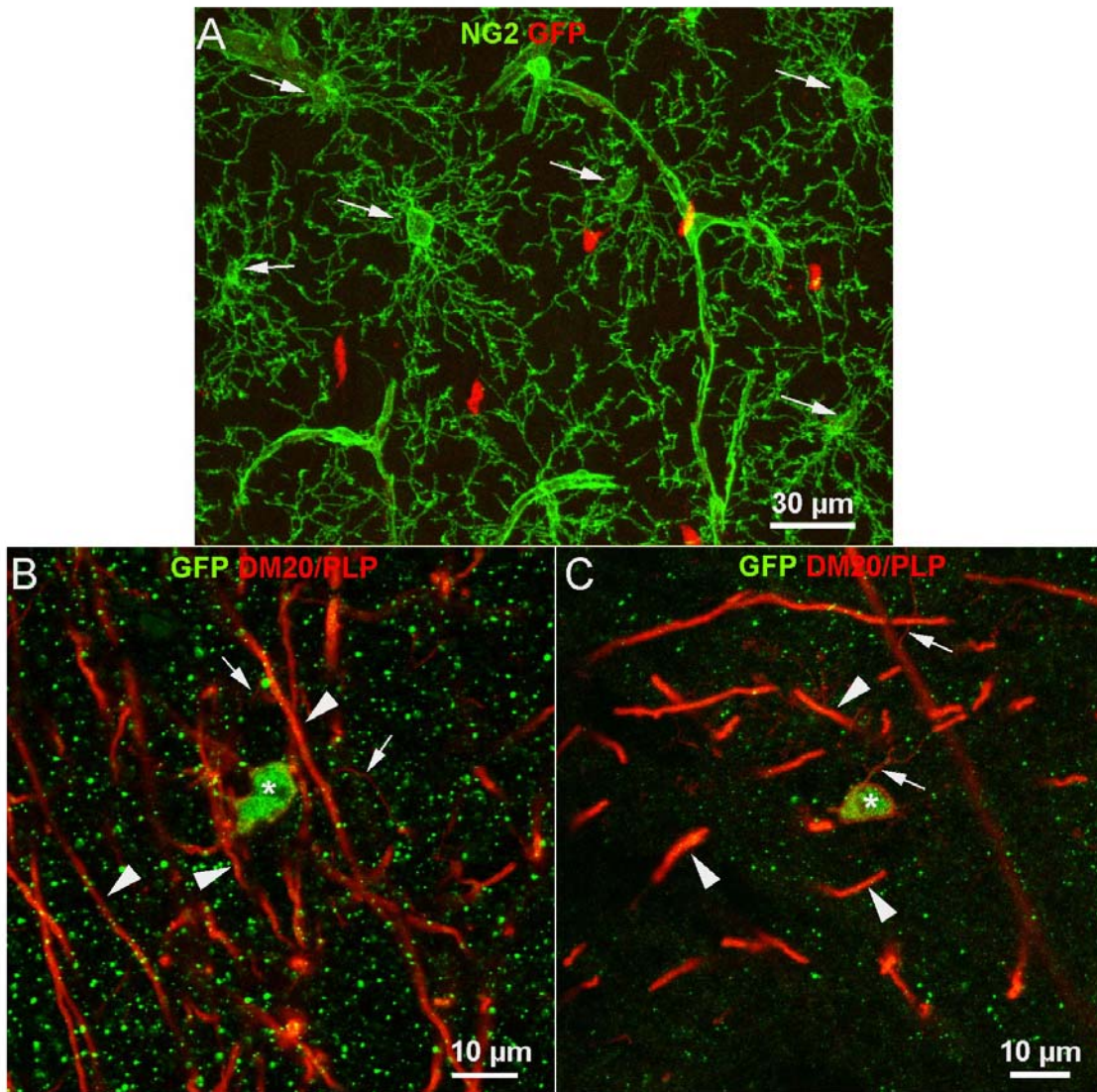


Suppl. Figure 7. Selection criterion well represents entire population of OPCs.

A) Passive properties and mean ratio value of OPCs (selected by ratio criterion, $n = 30$, random

selection out of the population shown in B), astrocytes (astro, n = 10) and microglial cells (micro, n = 8) recorded in hippocampal CA1 stratum radiatum of P8-P12 mice. Note that the surface area, as indicated by the membrane capacitance, is largest for astrocytes and smallest for microglial cells and the reverse is true for input resistance. Also note that in microglial cells a depolarised resting membrane potential is measured. The right-most panel clearly shows that the cells with a passive current pattern, astrocytes and microglial cells, are characterised by a ratio of unity.

- B) Histogram (bin width = 0.6) of the ratio values of all hippocampal glial cells recorded in this study (n = 116) which do not show a passive, ohmic current pattern, i.e. which possess voltage-gated K^+ currents. The dashed magenta line indicates ratio criterion value of 3 and shows that our selection criterion rejects only about 8% of all non-passive cells while the vast majority of cells of this population entered the analysis. The green and the blue arrows and bars denote two cells from both ends of the histogram which are NG2 positive and which are shown in C and D, respectively. *Inset*: ratio value histogram of astrocytes (red bars, bin width = 0.05) and non-passive glial cells (black bars, bin width = 0.1) shown at an x-axis scaling matched to the transition region of the two groups of cells. Note that the ratio values of the population of astrocytes are centred very tightly around a value of 1 and that there are only very few transitional/intermediate cells with a ratio value between 1 and 2, out of which one (green bar) was confirmed to be an NG2 positive OPC.
- C) *Top panel*: current pattern of an intermediate cell represented by the green bar in B. Voltage gated outward currents are clearly present but there also is a substantial passive current component. Leak subtraction reveals a fast sodium current of approximately 120 pA in this case (not shown). *Bottom panel*: the same cell filled with Lucifer Yellow (green) positively stained for NG2 (red, white arrows).
- D) *Top panel*: current pattern of a cell with a high ratio value and whose current pattern is dominated by voltage gated currents and nearly shows no leak current. *Bottom panel*: NG2 staining (red) of the same cell (green).



Suppl. Figure 8. In PLP-GFP transgenic mice, the expression of GFP is restricted to NG2 negative cells.

- A) Confocal laser scan of an anti-NG2 (Cy3, green) and anti-GFP (Cy2, red) double labelling in CA1 stratum radiatum of a 3-month-old PLP-GFP transgenic mouse. GFP positive cells (red cell bodies) do not express NG2. White arrows indicate the cell bodies of NG2 positive OPCs.
- B) and C) DM20/PLP and GFP double labelling in their CA1 area of a 12-month-old PLP-GFP mouse identifies GFP expressing cells as oligodendrocytes. The cell bodies of GFP expressing cells (asterisk) are surrounded by a rim of red immunostaining. Thin processes (white arrows) connect the cell bodies to myelin sheaths (white arrow heads).

Supplementary Material and Methods

Immunohistochemistry. The following primary antibodies were used throughout the study:

antigen	host	dilution	source	protocol	comment
AN2	rat	1:5	J.Trotter	1	
CD68	rat	1:100	Serotec	1	FA-11
CD9	rat	1:25	BD	4	KMC8
doublecortin	goat	1:50	Santa Cruz	1	C-18
GAD67	rabbit	1:250	Chemicon	2	
GAD67	mouse	1:100	Chemicon	1	
GFAP	rabbit	1:100	DAKO	1	
nestin	rabbit	1:250	R.McKay	1	#130
NG2	rabbit	1:500	B.Stallcup/Chem.	1	
O1	mouse	1:50	Chemicon	2	
O4	mouse	1:50	Chemicon	2	
PDGFR	rat	1:100	BD	1	APA5
PLP/DM20	rat	1:10	B.Zalc	3	AA3
PSA-NCAM	mouse	1:250	Chemicon	1	2-2B
S100b	rabbit	1:500	Swant	1	
GFP	rabbit	1:500	Invitrogen	1	
GFP	goat	1:200	ABCam	1	FITC labelled

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