Niche stiffness underlies the ageing of central nervous system progenitor cells

Michael Segel1,2, Björn Neumann1,2, Myfanwy F. E. Hill1,2, Isabell P. Weber3, Carlo Viscomi4, Chao Zhao1,2, Adam Young1,2, Chibeza C. Agley1, Amelia J. Thompson3, Ginez A. Gonzalez3,2, Amar Sharma1,2, Staffan Holmqvist1,5, David H. Rowitch1,5, Kristian Franze3, Robin J. M. Franklin1,2* & Kevin J. Chalut1,6*

Ageing causes a decline in tissue regeneration owing to a loss of function of adult stem cell and progenitor cell populations1. One example is the deterioration of the regenerative capacity of the widespread and abundant population of central nervous system (CNS) multipotent stem cells known as oligodendrocyte progenitor cells (OPCs)2. A relatively overlooked potential source of this loss of function is the stem cell ‘niche’—a set of cell-extrinsic cues that include chemical and mechanical signals3,4. Here we show that the OPC microenvironment stiffens with age, and that this mechanical change is sufficient to cause age-related loss of function of OPCs. Using biological and synthetic scaffolds to mimic the stiffness of young brains, we find that isolated aged OPCs cultured on these scaffolds are molecularly and functionally rejuvenated. When we disrupt mechanical signalling, the proliferation and differentiation rates of OPCs are increased. We identify the mechanoresponsive ion channel PIEZO1 as a key mediator of OPC mechanical signalling. Inhibiting PIEZO1 overrides mechanical signals in vivo and allows OPCs to maintain activity in the ageing CNS. We also show that PIEZO1 is important in regulating cell number during CNS development. Thus we show that tissue stiffness is a crucial regulator of ageing in OPCs, and provide insights into how the function of adult stem and progenitor cells changes with age. Our findings could be important not only for the development of regenerative therapies, but also for understanding the ageing process itself.

Fig. 1 | The CNS niche stiffens with ageing and the neonate niche restores the function of aOPCs. a, b, Representative images and quantifications of the proliferation and differentiation rates of transplanted nOPCs and aOPCs in nCNS (N = 4) at 14 days after transplantation. Throughout all figures, ratios on y-axes of graphs represent the ratio of the number of cells that express the relevant marker or markers in the numerator, over the number of cells expressing the relevant marker in the denominator. Blue arrows highlight example cells in which EdU (5-ethynyl-2’-deoxyuridine, a marker of cell proliferation) was detectable. OLIG2 is a marker for the oligodendrocyte lineage; CC1 is a marker for differentiated cells of the oligodendrocyte lineage. Here, and throughout the text, each individual point on the bar chart represents data from a single biological replicate, or the mean across biological replicates if noted as such. c, d, Representative images and quantifications of the proliferation and differentiation rates of aOPCs seeded onto decellularized nECM and aECM. e, f, Representative images and quantifications of proliferating and differentiating cells per square millimetre of caudal cerebellar peduncle (CCP) lesion cores 14 days after lesion and 7 days after direct injection of penicillinase or chABC into N = 4 aged females. g, h, Stiffness of brains at different ages determined by AFM indentation measurements. Each point represents the means of n = 3 sections from N = 3 animals. Regional mean stiffness values are calculated by mapping AFM measurements to brain slices. For all figures, error bars represent standard deviation over biological replicates; and P values are calculated by one-way analysis of variance (ANOVA) and indicated in the figures. Scale bars, 50 µM.
It is widely thought that a loss of exposure to growth factors underlies the quiescence of progenitor cells during ageing. To test this, we purified OPCs from neonatal and aged rats (nOPCs and aOPCs; Extended Data Fig. 1a) and cultured them in conditions known to enable the self-renewal of nOPCs. After long-term culture, while nOPCs showed high levels of proliferation, aOPCs showed very low levels (Extended Data Fig. 1c). We then hypothesized that ECM stiffness might play a role in OPC loss of function. First, we confirmed with atomic force microscopy (AFM) that the prefrontal cortex progressively stiffens with age (Fig. 1g, h). We then developed polycrylamide hydrogels to mimic the stiffness of neonatal and aged CNS (nCNS and aCNS), and found that aOPCs gained the capacity to both proliferate and differentiate at rates comparable to those of transplanted neonatal controls (Fig. 1a, b). By comparison, there were few proliferating progenitors in the CNS of aged littermates (aCNS; Extended Data Fig. 1d). Thus, aOPCs can become activated in the neonatal niche, but not in their native niche.

The niche is a factor in OPC ageing, therefore, we next addressed whether changes in the tissue microenvironment underlie the observed differences in the age state of OPCs. We seeded aOPCs on neonatal and aged decellularized brain extracellular matrix (nECM and aECM; Extended Data Fig. 1e–h). We found that aOPCs seeded on nECM showed a tenfold increase in proliferation rate and ability to differentiate compared with aOPCs seeded on aECM (Fig. 1c, d). Conversely, nOPCs lost their proliferative capacity when seeded on aECM (Extended Data Fig. 1i, j). These results indicate that the ageing ECM is important in impairing the function of OPCs. Thus, we hypothesized that digesting the ECM of aCNS using chondroitinase ABC (chABC) would activate aOPCs. To test the effect on differentiation in aged rats, we used a well-established model of focal areas of demyelination (see Supplementary Information). Following injection of chABC, there was a roughly threefold increase in both OPC proliferation and differentiation (Fig. 1e, f), further underlining the importance of the niche in OPC ageing.

We then hypothesized that ECM stiffness might play a role in OPC loss of function. First, we confirmed with atomic force microscopy (AFM) that the prefrontal cortex progressively stiffens with age (Fig. 1g, h). We then developed polycrylamide hydrogels to mimic the stiffening of the ECM with age. We invented these new hydrogels to decouple ECM composition and stiffness, enabling us to investigate cellular changes that result from mechanical signals alone. As expected, aOPCs proliferated and differentiated poorly on stiff hydrogels. However, the proliferation and differentiation of aOPCs plated on soft hydrogels increased more than tenfold (Fig. 2a, b and Extended Data Fig. 2). Meanwhile, nOPCs lost their capacity to proliferate and differentiate on stiff substrates (Extended Data Fig. 2b–e). Taken together, these results suggest that the ‘cellular activity’ of OPCs is predominantly regulated not by the age of the cell or by ECM chemistry, but by ECM stiffness.

To further investigate whether a soft environment pushes the identity of aOPCs closer to that of nOPCs, we performed RNA sequencing of aOPCs and nOPCs that were either acutely isolated or seeded on hydrogels. We found that, in terms of transcriptomics, aOPCs and nOPCs cultured on soft hydrogels resembled freshly isolated nOPCs more closely than did aOPCs and nOPCs cultured on stiff hydrogels (Fig. 2c). We also found that the cellular signalling pathways enriched in the transcriptomes of acutely isolated aOPCs overlap with pathways affected in ageing processes, such as metabolism, cell cycle, inflammation and DNA stability (Extended Data Fig. 3a–c). Furthermore, the
To further solidify the role of mechanics in OPC ageing, we next investigated the mechanotransduction of aOPCs by using inhibitors of actomyosin contractility (Extended Data Fig. 4a). We found that these inhibitors increased proliferation and differentiation rates five-fold when applied to aOPCs on stiff substrates (Fig. 2e, f and Extended Data Fig. 4b–e). We also investigated the effect of blocking actomyosin contractility in vivo (Extended Data Fig. 4f–k), as well as other potential mechanotransduction factors—such as the ‘mechanostat’ protein Lamin A/C—on the activity state of OPCs. These factors had varying levels of positive effects on the activity state of aOPCs (Extended Data Fig. 5). However, these are all relatively downstream factors; there -

To examine the role of PIEZO1 in OPC activity, we transfected Piezo1 short interfering RNA (siRNA) into aged rat OPCs, and found that they proliferated and differentiated four- to five-fold more than control cells on stiff hydrogels (Fig. 3d, e and Extended Data Fig. 6f, g). As PIEZO1 gives rise to calcium transients, we next used imaging to investigate whether substrate mechanics affects intracellular calcium dynamics. OPCs on stiff hydrogels regularly demonstrated calcium transients, whereas OPCs on soft hydrogels had virtually no calcium fluxes. Moreover, transfection with Piezo1 siRNA abolished calcium transients on stiff hydrogels (Extended Data Fig. 6h–j). We also found that treating aOPCs with a calcium-chelating agent caused an increase in the proportion of aOPCs entering the cell cycle (Extended Data Fig. 6k, l).

We next asked whether PIEZO1 could be silenced to eliminate the age-related loss of function of OPCs in vivo. To do this, we generated a CRISPR system based on the nucleas Cas9 that leads to an efficient knock-in of a DNA cassette producing overexpression of Piezo1 short hairpin RNA (shRNA) and green fluorescent protein RNA (GFP) (Extended Data Fig. 7a–g). We transfected Piezo1-targeting and non-targeting (control) constructs into nOPCs, and transplanted the cells into the aged prefrontal cortex. As predicted, the control nOPCs lost their capacity to proliferate in the aged cortex (Fig. 3f, g). On the other hand, OPCs expressing the Piezo1 knockdown continued to proliferate, indicating a high level of OPC activity despite the aged microenvironment.

We then investigated the role of PIEZO1 in regeneration. We did this by combining several in vivo genome-engineering strategies, first knocking GFP and a ribozyme-flanked Piezo1 guide RNA (gRNA) into the 3’-untranslated repeat (UTR) of the platelet-derived growth factor-α gene (Pdgfra) using non-homologous end-joining recombination (NHEJ), and then packaging this into a recombinant PHP-EB
Given that PIEZO1 reduces OPC function with age, we wondered what its purpose in OPCs might be. PIEZO1 provides negative feedback for proliferation, so it could potentially prevent an excess of OPCs during development. To test this, we synthesized a vector that expresses GFP and a ribozyme-flanked Piezo1 gRNA from the Ng2 promoter\(^8\) (Fig. 4d and Extended Data Fig. 9e). We injected this vector and a CMV–Cas9 vector into neonate mouse pups, finding high specificity and efficiency for OPCs (Extended Data Fig. 10a–g), thereby achieving cell-specific knockdown without using transgensics. We observed a fivefold increase in proliferation and twofold increase in the total density of OPCs, without a corresponding change in differentiation (Fig. 4e, f and Extended Data Fig. 10h–j), indicating that PIEZO1 plays a part in regulating OPC numbers. This suggests that PIEZO1-mediated mechanical signalling is essential for OPCs to interact appropriately with their niche during growth and development, but then becomes refractory to regeneration with ageing.

We have shown that adult CNS progenitor cells acquire properties that reflect the features of their mechanical environment. This age-related loss of function can be reversed either by softening the ECM or by manipulating the cells, through inhibition of PIEZO1, into functioning as if they are in a softer environment. We have also found that young progenitors transplanted into aged environments lose optimal regenerative properties, indicating that tissue mechanics are likely to be an important determinant of the success of cell-based therapies that aim to treat age-related diseases. Furthermore, we have identified a means by which to mitigate the negative response to the aged environment by decreasing the mechanical responsiveness of the CNS progenitor.
cells—namely, by using a new progenitor-cell-specific in vivo CRISPR strategy. Other ageing pathways should now be considered in the context of the mechanical microenvironment. Our findings raise the possibility that niche mechanics—not cell-intrinsic factors—may be a general factor driving ageing in other adult stem cell systems.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-019-1484-9.

Received: 7 April 2017; Accepted: 15 July 2019;
Published online 15 August 2019.

1. Goodell, M. A. & Rando, T. A. Stem cells and healthy aging. Science 350, 1199–1204 (2015).
2. Sim, F. J., Zhao, C., Penderis, J. & Franklin, R. J. M. The age-related decrease in CNS remyelination efficiency is attributable to an impairment of both oligodendrocyte progenitor recruitment and differentiation. J. Neurosci. 22, 2451–2459 (2002).
3. Gopinath, S. D. & Rando, T. A. Stem cell review series: aging of the skeletal muscle stem cell niche. Aging Cell 7, 590–598 (2008).
4. Swift, J. et al. Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. Science 341, 1240104 (2013).
5. Hinks, G. L. & Franklin, R. J. Delayed changes in growth factor gene expression during slow remyelination in the CNS of aged rats. Mol. Cell. Neurosci. 16, 542–556 (2000).
6. Tang, D. G., Tokumoto, Y. M., Apperly, J. A., Lloyd, A. C. & Raff, M. C. Lack of replicative senescence in cultured rat oligodendrocyte precursor cells. Science 291, 868–871 (2001).
7. Keough, M. B. et al. An inhibitor of chondroitin sulfate proteoglycan synthesis promotes central nervous system remyelination. Nat. Commun. 7, 11312 (2016).
8. He, L., Li, G., Huang, J., Samuel, A. D. T. & Perrimon, N. Mechanical regulation of stem-cell differentiation by the stretch-activated Piezo channel. Nature 555, 103–106 (2018).
9. Eisenhoffer, G. T. et al. Crowding induces live cell extrusion to maintain homeostatic cell numbers in epithelia. Nature 484, 546–549 (2012).
10. Li, J. et al. Piezo1 integration of vascular architecture with physiological force. Nature 515, 279–282 (2014).
11. McHugh, B. J. et al. Integrin activation by Fam38A uses a novel mechanism of R-Ras targeting to the endoplasmic reticulum. J. Cell Sci. 123, 51–61 (2010).
12. McHugh, B. J., Murdoch, A., Haslett, C. & Sethi, T. Loss of the integrin-activating transmembrane protein Fam38A (Piezo1) promotes a switch to a reduced integrin-dependent mode of cell migration. PLoS One 7, e40346 (2012).
13. Jäkel, S. et al. Altered human oligodendrocyte heterogeneity in multiple sclerosis. Nature 566, 543–547 (2019).
14. Suzuki, K. et al. In vivo genome editing via CRISPR/Cas9 mediated homology-independent targeted integration. Nature 540, 144–149 (2016).
15. Nissim, L., Perli, S. D., Frickin, A., Perez-Pinera, P. & Lu, T. K. Multiplexed and programmable regulation of gene networks with an integrated RNA and CRISPR/Cas toolkit in human cells. Mol. Cell 54, 698–710 (2014).
16. Chan, K. Y. et al. Engineered AAVs for efficient noninvasive gene delivery to the central and peripheral nervous systems. Nat. Neurosci. 20, 1172–1179 (2017).
17. Duncan, I. D., Brower, A., Kondo, Y., Curlee, J. F. Jr & Schultz, R. D. Extensive remyelination of the CNS leads to functional recovery. Proc. Natl Acad. Sci. USA 106, 6832–6836 (2009); correction 106, 12208 (2009).
18. Seilers, D. L., Marié, D. O. & Horner, P. J. Postinjury niches induce temporal shifts in progenitor fates to direct lesion repair after spinal cord injury. J. Neurosci. 29, 6722–6733 (2009).

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2019
Media used for OPC isolation and maintenance are as follows.

OPC medium (100 ml): This contains 100 ml of Dulbecco’s modified Eagle’s medium (DMEM)/F12 (Thermo Fisher; 11039-021), 2 ml sodium pyruvate (Sigma; P2256), and 13,465 mg 3-(N-morpholino)propanesulfonic acid (MOPS; Sigma, M1254). Medium was pH adjusted to 7.30 and filtered sterilized (Millipore; SCVPU02RE).

MWB washing buffer. This buffer contains 10× PBS (Thermo Fisher; 70011-044), 2 mM sodium pyruvate, 2 mM EDTA (Thermo Fisher; 15575-020) and 10 µg ml⁻¹ insulin.

Generating polycrystalline gels of varying stiffness. Glass coverslips were washed sequentially in distilled water, 70% ethanol (Sigma; 458836) and 0.2 M sodium hydroxide. Bottom coverslips were air-dried and pre-coated in 1.2% bind silane (Sigma; GE17-1330-01) in 95% ethanol and 5% glacial acetic acid (Scientific Fisher; 64-19-7), then air dried and polished with lint-free cloths. Top coverslips were submerged in 15% Sigma-cote (Sigma; SL2-25ML) diluted in chloroform (Sigma; 288306) and incubated for 1 hour. Top coverslips were removed from the Sigma-cote solution and polished with a lint-free cloth. To make a soft hydrogel, we combined 7% acrylamide (Sigma; A4058), 6% bis-acrylamide (Sigma; A14672), and 48 mM 6-acrylamidohexanoic acid in distilled water. To make stiff hydrogels, we combined 14% acrylamide and 12% bis-acrylamide with 48 mM 6-acrylamidohexanoic acid. (The incorporation of 6-acrylamidohexanoic acid is a novel step that allows for the independent control of ECM tethering and hydrogel stiffness in our so-called StemBond hydrogels.) Finally, 0.004 g ml⁻¹ tetramethylrhodamine (TEMED; Sigma; T9281) and 0.001 g ml⁻¹ ammonium persulfate (Sigma; A3678) were added to the acrylamide solution. Acrylamide solution was rapidly pipetted onto the bottom glass coverslips and the hydrophobic top coverslip was placed on top. After 5 min, the top coverslip was removed with a scalpel and the bottom coverslip with the now polymerized hydrogel was washed twice in methanol. The hydrogels were 90% hydrated and stored in PBS.

To activate the hydrogels, we incubated them in 10 mM 4-morpholineethanesulfonic acid (MES) hydrate (Sigma; M5287) with 500 mM NaCl (Sigma; S9888) in distilled water (pH 6.1) for 10 min. To activate the functional group in the hydrogels, we incubated the gels for 30 min in 480 mM N-hydroxysuccinimide (Sigma; 130672) combined with 200 mM N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (Sigma; E7750) added to the MES hydrate solution. The gels were washed once in 60% methanol in PBS and then covered overnight in 50 µg ml⁻¹ laminin (Sigma, L2200) in pH 8.2 HEPES (Sigma; H3375).

Atomic force microscopy. Rats of different ages—neonate (around 7 days post-natum), young adult (about 120 days), and aged adult (around 455 days)—were anesthetized with 5% isoflurane and killed by intraperitoneal injection of a lethal dose of pentobarbital sodium (Euthatal). The brain was dissected out and placed into cold slicing artificial cerebrospinal fluid (a-CSF). One half of the brain was then glued onto a vibratome platform (VT1000 S; Leica Microsystems) using superglue. Coronal brain sections, 500-µm thick, were cut in cold a-CSF bubbled with 95% O₂ and 5% CO₂ using one half of a Gillette 7 O’Clock double-edged razor blade. The frequency was set to 12 Hz and the forward speed to about 50 µm s⁻¹. Brain sections were transferred to a Cell-Tak–coated (Corning; 354240) 35-mm glass-bottom Petri dish and covered with cold measuring artificial cerebrospinal fluid (m-aCSF). The samples were mounted on an inverted microscope (Zeiss; Axio Observer.A1) and constantly perfused with fresh m-aCSF bubbled with 95% O₂ and 5% CO₂.

For blebbistatin treatment, fresh aged CNS was vibrated as above and transferred into a 24-well plate containing m-aCSF and either dimethylsulfoxide (DMSO) or 5 µM blebbistatin. The slices were then transferred into an incubator for 30 min and measured.

AFM indentation measurements were performed as described previously. In brief, force–distance curves were recorded using a JPK Nanowizard Cellhesion 200 (JPK Instruments AG) in a raster scan (step size = 100–200 µm) using tipless silicon cantilevers (rat brains: Arrow-TTL, spring constant = 0.01–0.05 N m⁻¹; hydrogels: Sicon-TL, spring constant = 0.2–0.3 N m⁻¹; both from NanoWorld) with polystyrene beads (diameter = 37 µm; microParticles GmbH) glued to them. The maximum force was set to 7 nN for brain measurements and 0.15 nN for hydrogels, and the approach speed to 10 µm s⁻¹. Images were taken using a SCMOS camera (Zyla 4.2. Andor) mounted on a Zeiss Axio Zoom.V16 on top of the AFM setup. Using a custom algorithm 20, we analysed data for maximum indentation by fitting the force–distance curves to the Hertz model 21–23.

\[
F = \frac{3}{2} E \left(1 - \nu^2\right) \frac{h^2}{3} = \frac{1}{3} K_1 (h/k)^2
\]

where \(F\) is the applied force, \(E\) is Young’s modulus, \(\nu\) is Poisson’s ratio, \(r\) is the radius of the probe, \(\delta\) is the indentation depth, and \(K_1 (1 - \nu^2)\) is the apparent elastic modulus. Intact brain and decellularized brain curvatures were analysed for the full indentation depth at \(F = 7\) nN, and curves from hydrogel measurements for an indentation depth of 0.5 µm. The shear modulus \(G\) of the polycrystalline gels was calculated using 24:

\[
G \approx \frac{1}{2} E \left(1 - \nu^2\right) \frac{3h}{2k^2}
\]
for the Quantitect reverse transcription kit and primer extension analysis, 75 µL of 0.01% ethidium bromide into the caudal cerebral peduncles of aged female rats (15 months old) as described27. Seven days post-injection, 4 µL of 50 µM 1′-CHBr2C (Sigma, C3667), 50 µM 1′-chloropropylcytosine (Sigma, C3667) and 50 µM 1′-aminopropylcytosine (Sigma, C3667) were added to the injection well. Immediately after OPC isolation, RNA was isolated from purified OPCs using the Directzol RNA MicroPrep kit (Zymo Research; R2061). RNA was stored at −80 °C. Complementary DNA was generated from the RNA according to the QuantiTect reverse transcription kit’s instructions (Qiagen; 205310). For reverse transcription (RT)–quantitative polymerase chain reaction (qPCR), pre-designed primers (the table of primers in Supplementary Information) were used at a concentration of 400 nM and an efficiency of greater than roughly 98%, determined for each primer pair using serial dilutions of OPC cDNA. cDNA, primers, and SYBR Green PCR master mix (Qiagen; 204141) were combined according to the kit’s instructions, and RT–qPCR and melting-curve analysis were performed on Life Technologies’ QuantiStudio 6 Flex Real-Time PCR System.

Immediately after OPC isolation, protein was isolated from purified OPCs using the CellLytic M (Sigma; C2978) protein-extraction solution and a protease inhibitor (Sigma; P8340). Integrated whole-protein content was measured using a BSA gradient kit (Bio-Rad; 500-0206), and gradient intensity was quantified using a Tecan Infinite 200 Pro Microplate Reader. Ten micrometers of isolated protein was combined with 1/5 × 106 LDS sample buffer (Thermo Fisher; B007) and brought to 95 °C for 10 min, then with the SeeBlue protein ladder (Thermo Fisher; LC5925) run on Bolt 4–12% Bis-Tris Plus gels (Thermo Fisher; NW04120BOX) in MES buffer (Thermo Fisher; B0002) for 35 min at 165 V. Protein was transferred for 90 min at 100 V to a polyvinylidene difluoride (PVDF) membrane (Millipore; IPVSN07852) in transfer buffer (Bio-Rad; 161-0732) with 20% methanol (Sigma; 322415). PVDF membranes were blocked for 30 min with 50% Odyssey blocking buffer (Lico; 927–40100) in Tris-buffered saline (TBS; Thermo Fisher; BP24711) and 0.1% Tween-20 (Sigma; P2287). Primary antibodies were added at the proper dilution (see the table of antibodies in Supplementary Information) to the blocking buffer, and membranes were left overnight in primary antibodies and blocking buffer at 4 °C. Membranes were washed twice in TBS with Tween-20 for 10 min each wash, and near-infrared species-appropriate secondary antibodies were added (see the table of antibodies in Supplementary Information). Membranes were stained for 2 hours, washed twice in TBS with 0.1% Tween-20, and imaged on the Licor Odyssey Fc.

Transplantation of labelled OPCs into neonates. Neonatal OPCs (at P1–P3) and aOPCs (at 14–18 months of age) were isolated in parallel using the MACS isolation protocol described above. AOPCs and OPCs were labeled with Bovine 2.0 CMV–GFP (Thermo Fisher; B10838) as per the manufacturer’s guidelines, and 300,000 cells were transplanted into the prefrontal cortex of P1–P3 neonate rat pups using published coordinates26. For proliferation analysis, nine days following the original transplantation, 75 µg g−1 EdU (Abcam; ab146186) was injected intraperitoneally 14–16 hours before perfusion fixation.

In vivo demyelinating focal lesions and small-molecule delivery. Bilateral focal demyelinating white matter lesions were created by injecting 4 µL of 0.01% ethidium bromide into the caudal cerebral peduncles of aged female rats (15 months old) as described27. Seven days post-injection, 4 µL of 50 µM 1′-CHBr2C (Sigma, C3667), 50 µM 1′-chloropropylcytosine (Sigma, P3089) or 5 µM mblleblatin was injected into the lesion site using animal-specific injection-site coordinates. For proliferation analysis, 75 µg g−1 EdU was injected intraperitoneally 14–16 hours before perfusion fixation.

Mouse spinal-cord lesions were created by injecting 1% lysolceithin in PBS into the ventral white-matter tract of the spinal cord, as described28. For proliferation analysis, 75 µg g−1 EdU was injected intraperitoneally 14–16 hours before perfusion fixation.

For histological cryosections, animals were perfusion-fixed with 4% paraformaldehyde at 21 days post-injection.

sRNAs, modified mRNA synthesis and transfection. From cDNA, primers and the target sequences were generated using the T7 promoter on the 5′-end of the Lmnb1 and Lmna transcripts and the Kozak sequence, and a standard 35-cycle PCR was performed according to the Phusion polymerase kit (Thermo Fisher; F5305). From the PCR product, we synthesized the RNA using the synthesized DNA and HiScribe T7 ARCA mRNA kit with tailing (NEB E2060S), with the addition of 5-methylcytine (Trilink; N-1014) and pseudouridine (NEB; 1019). To determine the fragment size, the synthesis of the OLIG2–activated transcription factor SOX10 was used to identify oligodendrocyte lineage cells. For siRNA transfection of one well of a 96-well plate, each well being plated with 10,000 cells, we combined 0.5 pmol of siRNA (GE; D-001960-01-05) with...
1.5 μl of Opti-MEM media (Thermo Fisher; 31985062). In a separate tube, we combined 1.5 μl of Opti-MEM media with 0.15 μl of lipofectamine RNAiMAX reagent (Thermo Fisher; 13778030). The diluted RNAiMAX was then combined with the diluted RNA and incubated at room temperature for 20 min. Following the incubation, 3 μl of the transfection combination was added to each well.

For modified mRNA transfection of one well of a 96-well plate, each plated with 10,000 cells per well, 25 ng per well of modified RNA was combined with 1.25 μl of Opti-MEM (Thermo Fisher; 31985062). In a separate tube, 1.25 μl of Opti-MEM media was combined with 0.0375 μl of lipofectamine Messenger Max reagent (Thermo Fisher; 13778030). The diluted lipofectamine was then combined with the diluted RNA and incubated at room temperature for 5 min. Following the incubation, 2.5 μl of the transfection combination was added to each well.

Generation of decellularized CNS scaffold. CNS tissue from neonatal rats (P7) and aged rats (between 14 and 18 months of age) was dissected and immediately vibrated in 500-μM coronal sections in HALF medium on ice. Brains were decellularized using an protocol adapted from previous reports22. Sections were immediately flash-frozen at −80°C in distilled water. Sections were rapidly thawed at 37°C. Individual sections were transferred to a 24-well plate with 4% sodium deoxycholate (Sigma; D7650) for 2 hours and placed on an orbital shaker at 105 r.p.m. Sections were washed in 1× PBS with 1% penstrep (Sigma; P4333) for 15 min on the orbital shaker. PBS was removed and 3% Triton X-100 in PBS was added onto the sections, which were again shaken for 1 hour on the orbital shaker at 105 r.p.m. Triton X-100 was then removed and replaced with PBS. These steps (sodium deoxycholate to PBS to Triton X to PBS) were repeated three times. Finally, the sections were transferred into an 8-μm-pore cell-culture insert (Corning; 353097) coated with poly-γ-lysine (Sigma; P6407), and incubated overnight in PBS with 1% penstrep with DNase I (Sigma; 11284932001). The following day, the sections were washed three times more with PBS with 1% penstrep, and finally the sections were incubated in OCP media with growth factors. Freshly isolated OPGs were pipetted into the insert.

Calcium imaging. Cells were loaded with 1 μM Rhod-2-am (ab142780) as per the manufacturer’s guidelines for 30 min. Cells were washed twice in PBS, supplied with fresh media with growth factors, and equilibrated in the incubator for a further 20 min. Images were acquired every 20 seconds for 10 min to examine spontaneous calcium flux.

Generation, transfection and transplantation of Cas9 vectors. Competent meningioma cells were generated from ZYC10135ST2 meningioma cell line (System Biosciences; MN900A-1). Using Phusion polymerase (Thermo Fisher; F5305), PCR fragments with 20-base-pair overlaps using the primers listed below and the pSPCas9(BB)-2A-GFP and pAI14-GFP-NL-MC plasmids were assembled into NEBBuilder HiFi DNA assembly (NEB, E2621S) and gel extracted (Qiagen; 28704). pSPCas9(BB)-2A-GFP (PX458) was a gift from F. Zhang (Addgene plasmid 48138); pAI14-GFP-NL-MC was a gift from J. Belmonte (Addgene plasmid 87114). Plasmids were generated as described23 and plasmids were isolated with a Midi kit (Machery-Nagel; 740410.10). Plasmid sequences were confirmed using Sangencer sequencing.

Capped Cas9 mRNA with modified base pairs (TriLink; L-7206). Tub3b targeting CRISPR RNA (crRNA):trans-activating crRNA (tracrRNA; Dharmacon), and minicircle constructs containing reverse-strand Tub3b target sequence were transfected into MACS-seorted NPCs 24 hours after isolation using lipofectamine LTX with plus reagent (Thermo Fisher; 15338100). To test whether vector was successfully knocked-in, we mixed a U6 forward primer along with a 5′-loop of gRNA with modified base pairs (TriLink; L-7206), Tubb3 mRNA with modified base pairs (TriLink; L-7206), and the gRNA qPCR primer was designed using the SMARTer Stranded Total RNA-Seq kit/Pico Input Mammalian kit with multiplexed barcodes (Takara; 635005). We performed 150-base-pair paired-end directional sequencing on an Illumina HiSeq 4000.

Multiplexed samples were filtered, aligned to the rat University of Santa Cruz (UCSC) rn6 assembly, normalized and quantified using Trimmomatic, Hisat2, Stringtie and Ballgown as described24. Dendrogram clustering, r-distribut- isticated stochastic neighbour embedding, gene-set-enrichment analysis and heatmaps were generated in ipython notebook using the libraries pandas, matplotlib, numpy, seaborn, gseapy and scikit-learn.

We carried out analysis of pre-existing single-cell sequencing data using the open source single-cell-sequencing database https://castelobranco.shinyapps.io/ MScInt槌CA_1.8 using the table of primers in Supplementary Information.

Transfection of mouse embryonic fibroblasts and western blot. We seeded 5 × 105 mouse embryonic fibroblasts (MEFs) in a six-well plate and cultured the cells in 10% fetal bovine serum (FBS) in DMEM/F12. On day 3, cells were dissociated with TrypLE Express and nucleofected in the 4-d nucleofector system (Lonza) using the p4 nucleofector kit with 3 μg of each DNA plasmid construct (Lonza; V4XP-4012). The medium was changed at 48-hour intervals and protein was extracted 5 days after electroporation. Western blots were performed as above.

In vivo animal work. C57BL/6 mice and Sprague–Dawley rats were obtained from C. River (Margate, UK). The animals were maintained as breeding or age- ing colonies in a standard facility for rodents at the University of Cambridge (individually vented cages, controlled temperature and humidity, and a 12-hour light/dark cycle) until the age required, at least 10 days before experiments. The animals were randomly divided into control and treatment groups. Both sexes were used. The experiments and analyses were conducted blindly. No statisti- cal methods were used to predetermine sample size. This research has been regulated under the Animals (Scientific Procedures) Act 1986, Amendment Regulations 2012, following ethical review by the University of Cambridge Animal Welfare and Ethical Review Body (AWERB). All procedures were conducted strictly following the relevant protocols defined in Home Office Project Licence PC00C0F291.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Raw and processed sequencing data have been deposited at the National Center for Biotechnology Information (NCBI) with the expression omnibus (GEO) with accession number GSE133886 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE133886). Source data include final quantifications from in vivo ani- mal work.
19. Koser, D. E., Moeendarbary, E., Hanne, J., Kuerten, S. & Franze, K. CNS cell distribution and axon orientation determine local spinal cord mechanical properties. *Biophys. J.* **108**, 2137–2147 (2015).

20. Christ, A. F. et al. Mechanical difference between white and gray matter in the rat cerebellum measured by scanning force microscopy. *J. Biomech.* **43**, 2986–2992 (2010).

21. Franze, K. et al. Spatial mapping of the mechanical properties of the living retina using scanning force microscopy. *Soft Matter* **7**, 3147–3154 (2011).

22. Hertz, H. Über die Berührung fester elastischer Körper. *J. Reine Angew. Math.* **92**, 156–171 (1881).

23. Koser, D. E. et al. Mechanosensing is critical for axon growth in the developing brain. *Nat. Neurosci.* **19**, 1592–1598 (2016).

24. Moshayedi, P. et al. Mechanosensitivity of astrocytes on optimized polyacrylamide gels analyzed by quantitative morphometry. *J. Phys. Condens. Matter* **22**, 194114 (2010).

25. Boudou, T. et al. An extended modeling of the micropipette aspiration experiment for the characterization of the Young’s modulus and Poisson’s ratio of adherent thin biological samples: numerical and experimental studies. *J. Biomech.* **39**, 1677–1685 (2006).

26. Khazipov, R. et al. Atlas of the postnatal rat brain in stereotaxic coordinates. *Front. Neuroanat.* **9**, 161 (2015).

27. Woodruff, R. H. & Franklin, R. J. M. Demyelination and remyelination of the caudal cerebellar peduncle of adult rats following stereotaxic injections of lysolipid, ethidium bromide, and complement/anti-galactocerebroside: a comparative study. *Glia* **25**, 216–228 (1999).

28. Jeffery, N. D. & Blakemore, W. F. Remyelination of mouse spinal cord axons demyelinated by local injection of lysolipid. *J. Neurocytol.* **24**, 775–781 (1995).

29. De Waele, J. et al. 3D culture of murine neural stem cells on decellularized mouse brain sections. *Biomaterials* **41**, 122–131 (2015).

30. Kay, M. A., He, C.-Y. & Chen, Z.-Y. A robust system for production of minicircle DNA vectors. *Nat. Biotechnol.* **28**, 1287–1289 (2010).

31. Challis, R. C. et al. Widespread and targeted gene expression by systemic AAV vectors: production, purification, and administration. Preprint at https://doi.org/10.1101/246405 (2018).

32. Pertea, M., Kim, D., Pertea, G. M., Lee, J. T. & Salzberg, S. L. Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nature Protocols* **11**, 1650–1667 (2016).

Acknowledgements We thank D. Morrison for technical assistance and E. Paluch for helpful discussions and help with the manuscript. The work was supported by European Research Council (ERC) grant 772798 (to K.J.C.) and 772426 (to K.F.); the UK Multiple Sclerosis Society (to R.J.M.F.); Biotechnology and Biological Sciences Research Council (BBSRC) grant BB/M008827/1 (to K.J.C and R.J.M.F.) and BB/N006402/1 (to K.F.); the Adelson Medical Research Foundation (R.J.M.F. and D.H.R.); an EMBO Long-Term Fellowship ALTF 1263-2015 and European Commission FP7 actions LTFCOFUND2013, GA-2013-609409 (to I.P.W.); a Royal Society University Research Fellowship (to K.J.C.); and a core support grant from the Wellcome Trust and Medical Research Council (MRC) to the Wellcome Trust–MRC Cambridge Stem Cell Institute.

Author contributions M.S., R.J.M.F. and K.J.C. designed the study and wrote the manuscript. R.J.M.F and K.J.C. supervised the study. M.S., B.N., C.V., C.Z., M.F.E.H. and G.A.G. carried out animal experiments and quantifications, including transplantations and in vivo CRISPR experiments. M.S., I.P.W., K.F. and A.J.T. designed, performed and analysed the AFM experiments. A.Y. carried out the molecular biology associated with experiments. B.N., A.S. and M.S. carried out the in vitro OPC culturing experiments. B.N. developed and optimized the protocol for isolating neonatal and aged in vitro OPCs. M.S., S.H. and D.H.R performed the RNAscope imaging and analysis. C.C.A and K.J.C. invented the hydrogels.

Competing interests The authors declare no competing interests.

Additional information Supplementary information is available for this paper at https://doi.org/10.1038/s41586-019-1484-9. Correspondence and requests for materials should be addressed to R.J.M.F. or K.J.C.

Peer review information Nature thanks D. Discher, M. Lutolf and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Reprints and permissions information is available at http://www.nature.com/reprints.
Extended Data Fig. 1 | Dynamics of OPC activation, in vitro and in vivo.

a, Representative flow-cytometry analysis of MACS-purified OPCs confirms that we are able to isolate a pure population of OLIG2\(^+\) NG2\(^+\) OPCs (indicated by percentages) from both neonatal and aged brains. NG2 is a marker specific for OPCs in the neonatal and adult CNS.

b, EdU labelling of OPCs after 7 and 14 days in vitro. Scale bar, 25 \(\mu\)M. ‘GF’ indicates the growth factors FGF and PDGF.

c, Quantifications of \(N = 3\) replicates of neonatal and aOPCs in proliferation conditions on PDL-coated tissue-culture plastic after five days, showing that aOPCs proliferate poorly even in the presence of growth factors.

d, Representative image of white matter labelled with OLIG2, EdU and CC1, from a 16-month-old female, showing that aOPCs also proliferate poorly in vivo. Scale bar, 50 \(\mu\)M. DAPI is used to stain nuclear DNA.

e, Overview of the decellularization protocol.

f, DAPI staining following the decellularization protocol shows no remaining nuclear DNA, indicating complete cell removal. Scale bar, 200 \(\mu\)M. g, Rat brains of different ages (neonatal or aged) were decellularized, fixed, and stained for chondroitin sulphate proteoglycans (CSPGs), showing that the ECM remains intact following decellularization. Scale bar, 20 \(\mu\)M. h, The recellularization protocol. OPCs (grey cells with tendrils) are MACS-purified using the OPC surface marker A2B5, then cultured for five days in proliferation conditions. A subset of the brain ECM cultures are fixed with paraformaldehyde, and the remainder are placed into differentiation conditions for a further five days. The coloured, connected circles represent the molecule paraformaldehyde.

i, Representative images and a quantification of nOPCs seeded on neonatal and aged matrix, showing that nOPCs on aECM proliferate poorly. Panels a, d, f, g portray representative quantifications or images from \(N = 3\) or more biological replicates.
Extended Data Fig. 2 | OPCs grown in progressively stiff environments lose their proliferation and differentiation capacity. a, Mean shear moduli determined by AFM of our fabricated 'soft' and 'stiff' hydrogels. b, c, MACS-purified nOPCs (stained for EdU and Sox10) cultured on stiff hydrogels lose their ability to proliferate following five days in proliferation conditions. nOPCs cultured on soft hydrogels, however, continue to proliferate. Scale bar, 40 µm. d, e, Similarly, nOPCs cultured on stiff hydrogels differentiate into oligodendrocytes (staining for OLIG2 and MBP) very inefficiently following five days in differentiation conditions. Conversely, nOPCs differentiated on soft hydrogels differentiate efficiently into oligodendrocytes. Scale bar, 100 µm. f, g, Representative images and quantifications from \( N = 3 \) replicates of EdU-labelled OPCs, seeded at \( 0.5 \times, 1 \times \) and \( 2 \times \) cell-seeding densities on increasingly stiff hydrogels, after 120 hours in culture show cell-density-independent, stiffness-dependent OPC activation. Scale bar, 100 µM. h, i, Labelling and quantifications from \( N = 3 \) viability assays of neonatal and aged OPCs on soft and stiff hydrogels after 48 hours in culture with propidium iodide (PI, a stain for DNA), showing that the stiffness effect does not affect cell death. The y-axis shows ratios of PI-stained cells to total cells. In all Extended Data Figures, averages represent means of biological replicates; error bars represent standard deviations; and \( P \) values are calculated by one-way ANOVA.
Extended Data Fig. 3 | Gene-expression profiling show stiffness-driven changes in OPCs. a–c, Gene-set enrichment analysis (GSEA) reveals a number of ageing-related pathways that are differentially regulated between cultured aOPCs (a) and nOPCs (c) grown on soft hydrogels versus those grown on stiff hydrogels, and between aged and neonatal freshly harvested OPCs (b). HIF-1, hypoxia-inducible factor-1; mTOR, mammalian target of rapamycin; NOD, nucleotide-binding oligomerization domain; STAT, signal transducer and activator of transcription; TGF-β, transforming growth factor-β; TNF, tumour necrosis factor. d, Volcano plot of genes that are differentially expressed between aOPCs cultured on soft versus stiff hydrogels. Red dots show the 1,300 significantly upregulated expressed genes as determined from N = 3 biological replicates per condition (P < 0.05). e, Heatmaps showing the log2 FPKM expression of the 25 genes with the highest fold increase in expression between N = 3 biological replicates of aOPCs cultured on soft versus stiff hydrogels (left), and of the 25 genes with the highest fold increase in expression between aOPCs cultured on soft versus stiff hydrogels (right). ECM-related genes such as Dab1, Acan and Plxnd1 were upregulated in aOPCs cultured on stiff hydrogels, while cell-cycle and DNA-repair genes such as Cdk1, Cdk1 and Sirt7, OPC-activation genes such as Etv1, and Hippo-pathway genes such as Rassf2 were amongst the most upregulated genes in aOPCs cultured on soft hydrogels. All genes shown are significantly differentially expressed with a P value of 0.05 or less. f, Venn diagram showing that similar gene sets to those enriched in neonates are also enriched in nOPCs grown on soft hydrogels. g, Specific genes involved in genomic and epigenomic stability and in the activation of OPCs are upregulated both in nOPCs and in aOPCs grown on soft hydrogels.
Extended Data Fig. 4 | Small molecules modulating the cell cytoskeleton promote the proliferation of aOPCs. a, Using the 96-well-plate format, the GE Incell 2000 and a cell profiler for quantification, we optimized the dosing and timing of the small molecules Y27632 (‘Y27’) and blebbistatin (‘Bleb’) in order to identify the small-molecule conditions that maximize aOPC proliferation. Averages represent mean proportion of EdU+OLIG2+ cells for N = 3 biological replicates. b, Representative images and quantifications from N = 3 biological replicates of the rates of proliferation of nOPCs cultured on soft and stiff hydrogels in the presence of 5 µM blebbistatin, showing that treatment with blebbistatin promotes the proliferation of OPCs similarly to soft hydrogels. Scale bar, 50 µm. c, Representative images and quantifications of N = 3 adult OPCs on soft hydrogels treated with blebbistatin or DMSO (as a control) show no change in the rates of proliferation, indicating that there is no stiffness-independent effect of blebbistatin. Scale bar, 50 µM. 

Extended Data Fig. 4 | Small molecules modulating the cell cytoskeleton promote the proliferation of aOPCs. a, Using the 96-well-plate format, the GE Incell 2000 and a cell profiler for quantification, we optimized the dosing and timing of the small molecules Y27632 (‘Y27’) and blebbistatin (‘Bleb’) in order to identify the small-molecule conditions that maximize aOPC proliferation. Averages represent mean proportion of EdU+OLIG2+ cells for N = 3 biological replicates. b, Representative images and quantifications from N = 3 biological replicates of the rates of proliferation of nOPCs cultured on soft and stiff hydrogels in the presence of 5 µM blebbistatin, showing that treatment with blebbistatin promotes the proliferation of OPCs similarly to soft hydrogels. Scale bar, 50 µm. c, Representative images and quantifications of N = 3 adult OPCs on soft hydrogels treated with blebbistatin or DMSO (as a control) show no change in the rates of proliferation, indicating that there is no stiffness-independent effect of blebbistatin. Scale bar, 50 µM. d, Representative images and quantifications of N = 3 adult OPCs on soft hydrogels treated with blebbistatin or DMSO (as a control) show no change in the rates of proliferation, indicating that there is no stiffness-independent effect of blebbistatin. Scale bar, 50 µM. 

Extended Data Fig. 4 | Small molecules modulating the cell cytoskeleton promote the proliferation of aOPCs. a, Using the 96-well-plate format, the GE Incell 2000 and a cell profiler for quantification, we optimized the dosing and timing of the small molecules Y27632 (‘Y27’) and blebbistatin (‘Bleb’) in order to identify the small-molecule conditions that maximize aOPC proliferation. Averages represent mean proportion of EdU+OLIG2+ cells for N = 3 biological replicates. b, Representative images and quantifications from N = 3 biological replicates of the rates of proliferation of nOPCs cultured on soft and stiff hydrogels in the presence of 5 µM blebbistatin, showing that treatment with blebbistatin promotes the proliferation of OPCs similarly to soft hydrogels. Scale bar, 50 µm. c, Representative images and quantifications of N = 3 adult OPCs on soft hydrogels treated with blebbistatin or DMSO (as a control) show no change in the rates of proliferation, indicating that there is no stiffness-independent effect of blebbistatin. Scale bar, 50 µM. d, Representative images and quantifications of N = 3 adult OPCs on soft hydrogels treated with blebbistatin or DMSO (as a control) show no change in the rates of proliferation, indicating that there is no stiffness-independent effect of blebbistatin. Scale bar, 50 µM. 

Extended Data Fig. 4 | Small molecules modulating the cell cytoskeleton promote the proliferation of aOPCs. a, Using the 96-well-plate format, the GE Incell 2000 and a cell profiler for quantification, we optimized the dosing and timing of the small molecules Y27632 (‘Y27’) and blebbistatin (‘Bleb’) in order to identify the small-molecule conditions that maximize aOPC proliferation. Averages represent mean proportion of EdU+OLIG2+ cells for N = 3 biological replicates. b, Representative images and quantifications from N = 3 biological replicates of the rates of proliferation of nOPCs cultured on soft and stiff hydrogels in the presence of 5 µM blebbistatin, showing that treatment with blebbistatin promotes the proliferation of OPCs similarly to soft hydrogels. Scale bar, 50 µm. c, Representative images and quantifications of N = 3 adult OPCs on soft hydrogels treated with blebbistatin or DMSO (as a control) show no change in the rates of proliferation, indicating that there is no stiffness-independent effect of blebbistatin. Scale bar, 50 µM. 

Extended Data Fig. 4 | Small molecules modulating the cell cytoskeleton promote the proliferation of aOPCs. a, Using the 96-well-plate format, the GE Incell 2000 and a cell profiler for quantification, we optimized the dosing and timing of the small molecules Y27632 (‘Y27’) and blebbistatin (‘Bleb’) in order to identify the small-molecule conditions that maximize aOPC proliferation. Averages represent mean proportion of EdU+OLIG2+ cells for N = 3 biological replicates. b, Representative images and quantifications from N = 3 biological replicates of the rates of proliferation of nOPCs cultured on soft and stiff hydrogels in the presence of 5 µM blebbistatin, showing that treatment with blebbistatin promotes the proliferation of OPCs similarly to soft hydrogels. Scale bar, 50 µm. c, Representative images and quantifications of N = 3 adult OPCs on soft hydrogels treated with blebbistatin or DMSO (as a control) show no change in the rates of proliferation, indicating that there is no stiffness-independent effect of blebbistatin. Scale bar, 50 µM. 

Extended Data Fig. 4 | Small molecules modulating the cell cytoskeleton promote the proliferation of aOPCs. a, Using the 96-well-plate format, the GE Incell 2000 and a cell profiler for quantification, we optimized the dosing and timing of the small molecules Y27632 (‘Y27’) and blebbistatin (‘Bleb’) in order to identify the small-molecule conditions that maximize aOPC proliferation. Averages represent mean proportion of EdU+OLIG2+ cells for N = 3 biological replicates. b, Representative images and quantifications from N = 3 biological replicates of the rates of proliferation of nOPCs cultured on soft and stiff hydrogels in the presence of 5 µM blebbistatin, showing that treatment with blebbistatin promotes the proliferation of OPCs similarly to soft hydrogels. Scale bar, 50 µm. c, Representative images and quantifications of N = 3 adult OPCs on soft hydrogels treated with blebbistatin or DMSO (as a control) show no change in the rates of proliferation, indicating that there is no stiffness-independent effect of blebbistatin. Scale bar, 50 µM.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | The composition of the nuclear lamina of OPCs changes both with ageing and in response to niche stiffness.

a, qPCR of OPCs reveals a loss of Lmnb1 expression and gain of Lmna expression with ageing. Values represent averages of OPCs from $N = 3$ animals for each time point and are the log$_2$ change in cycle threshold ($\Delta$CT) values normalized to the expression of Tbp, which is expressed in cells throughout the body. b, Representative images of in vivo cerebellar grey-matter cryosections from $N = 3$ biological replicates confirm changes in nuclear lamina that occur with ageing. Scale bar, 50 $\mu$m. White arrows highlight representative Ng2-expressing OPCs at each age. c, Representative western blot of Lamin B1 (LMNB1) and Lamin C (LMNC) proteins from freshly isolated OPCs of different ages confirms the qPCR data. ACTB is actin-$\beta$, a reference protein; p4 to p810 are protein fragments. Similar results were obtained for $N = 3$ biological replicates for each age group. d, RNA-sequencing data for nuclear Lmnb1 and Lmnb2 in neonatal and aged OPCs show low levels of expression of Lmnb2 in both age groups. e, f, Western blot quantifications of laminins from aged OPCs grown on soft and stiff hydrogels. g, Representative images from $N = 3$ biological replicates of changes in nuclear lamina in OPCs on hydrogels of different stiffnesses. Scale bar, 50 $\mu$m. h, Representative image from $N = 3$ biological replicates of red fluorescent protein (RFP)-conjugated non-targeting siRNA shows a high efficiency of siRNA transfection in vitro. Scale bar, 100 $\mu$m. i, qPCR on adult OPCs 48 hours after transfection with siRNAs, showing efficient knockdown of Lmna and Fak1 (also known as Ptk2) expression. Values represent averages of OPCs from $N = 3$ animals and are the log$_2$ $\Delta$ACT values normalized to Tbp. j, k, Representative images and quantifications of the proliferation of $N = 3$ aOPCs in growth factors on stiff hydrogels following transfection with siRNAs for Lmna and Fak1. Scale bars, 50 $\mu$m. l, A representative image from $N = 3$ biological replicates of GFP-encoding mRNA in neonatal OPCs shows high-efficiency transfection. Scale bar, 100 $\mu$m. m, Representative image from $N = 3$ biological replicates, showing efficient transfection, high translation and proper protein localization of Lamin C in aOPCs. Scale bar, 25 $\mu$m. n, qPCR data five days post-transfection from RNA isolated from transfected OPCs. Means represent log$_2$ $\Delta$ACT means across $N = 2$ biological replicates. o, p, Representative images and quantifications of $N = 3$ replicates in nOPCs on soft hydrogels show loss of proliferative capacity 120 hours after Lmnc mRNA overexpression but not after GFP overexpression. Scale bar, 100 $\mu$m.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | PIEZO1, which mediates calcium flux, is highly expressed in OPCs but not other cells of oligodendrocyte lineage. 

**a**, Representative images of aOPCs on soft and stiff hydrogels, showing that PIEZO1 is expressed in rat OPCs in vitro. Scale bar, 100 µm. 

**b**, Western blot for PIEZO1 in acutely isolated OPCs shows a modest increase in protein expression from neonates to adults. 

**c, d**, Representative images and quantifications of in situ hybridizations for Piezo1 and Pdgfra in aged mouse cortex using RNAscope, showing expression of Piezo1 in aged mouse OPCs. A negative control is included. Scale bar, 10 µm. 

**e**, t-distributed stochastic neighbour embedding (t-SNE) plots from a single-cell-sequencing study of human cells\(^\text{13}\) show that Pdgfra/Olig2 co-expressing OPCs from the adult CNS also highly express Piezo1 in adult white matter. 

**f, g**, Representative images and quantifications of aOPCs transfected with a control siRNA ('Scramble') or with a Piezo1 siRNA and placed in proliferation conditions for five days, indicating no stiffness-independent effect of PIEZO1. Scale bar, 100 µm. 

**h**, Representative Rhod-2-AM-stained live-cell images from \(N = 3\) biological replicates of aOPCs on soft and stiff hydrogels transfected with siScramble or siPiezo1. 

**i**, Representative traces from \(N = 3\) biological replicates of individual cells fluxing with calcium (\(\Delta F_{\text{intensity}}\)) over 270 seconds. Fluorescence was normalized to the maximum fluorescence intensity per cell over the acquisition time. 

**j**, Quantifications of the proportion of cells that fluxed calcium once or more throughout the 540-second image-acquisition period, showing that either seeding OPCs on soft hydrogels or overexpressing PIEZO1 inhibits the calcium flux. 

**k, l**, Representative images and proliferation quantifications from \(N = 3\) biological replicates of aOPCs cultured in proliferation conditions for five days on a stiff hydrogel in the presence of 5 µM BAPTA (which chelates intracellular calcium), showing a boost in proliferation with calcium chelation. Scale bar, 100 µm.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | In vivo Piezo1-knockdown strategies. **a**, Diagram showing CAS9-mediated genomic manipulation using in vitro transcribed (IVT) gRNA, Cas9 mRNA and in-house-made minicircle vectors overexpressing Piezo1-targeting shRNAs. This CAS9-mediated knock-in of shRNA–GFP has the benefit of producing a characterizable monotonic knockdown across the pool of cells expressing the GFP. **b**, qPCR data from RNA isolated from transfected OPCs, 48 hours after transfection with either the Piezo1 siRNA or the Piezo1 shRNA construct, both show an approximately 80% knockdown of Piezo1 mRNA. Means represent log2 ΔΔCT means from $N = 3$ biological replicates. **c**, Representative images from $N = 3$ biological replicates show high rates of co-transfection of the minicircle with Cas9 mRNA and IVT gRNA. Scale bar, 25 µM.

**d, e**, PCR design and appropriate fragment length of the correctly knocked-in minicircle fragment construct, representative of results from $N = 3$ replicates. **f, g**, Representative images and quantifications show that CAS9-mediated knock-in of shPiezo1 fragments in aOPCs on stiff hydrogels phenocopies the effect of the Piezo1 siRNA in aOPCs. Scale bars, 100 µM. **h**, In order to knock down Piezo1 in endogenous OPCs in the aged mouse, we developed the strategy outlined here. A NHEJ-mediated knock-in inserts a construct into a gene specific to a given cell type. This construct contains a ribozyme-flanked second gRNA, targeting CAS9-mediated gene knockdown to a second locus. KO, knockout; PA, polyadenylation sequence. The rectangle and triangle represent the gRNA target sequence.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | A nested CRISPR system efficiently labels OPCs with GFP and subsequently mutates the Piezo1 locus.

a, b, Representative images and quantifications of cells co-expressing GFP and OLIG2 across multiple regions of the CNS. Fewer than 5% of GFP-expressing cells express a marker other than OLIG2. Data taken from N = 3 control knockdown animals. Scale bars, 50 µm. c, RT–PCR shows Cas9 and GFP mRNA expression from whole-brain homogenate in N = 3 animals. d, A schematic and a DNA gel of N = 2 biological replicates of the Pdgfra locus following CRISPR/CAS9-mediated knock-in of a GFP transgene confirms construct knock-in in the correct position. KD, knockdown; NT, non-targeting gRNA (control construct); P1, forward primer for PCR; P2 reverse primer; ø symbol, uninjected control animal PCR. e, Outline of our experimental strategy to confirm the efficacy of our in vivo nested CRISPR approach. In brief, 5 × 10¹¹ of each viral vector was tail-vein-injected into 18-month-old animals. At 35 days, brains were dissociated, and cells were FACS-sorted on the basis of their expression of Pdgfra and Olig2. Confirmatory PCR was carried out, as well as a surveyor assay to detect off-target small insertions and deletions (indels).

f, A representative FACS plot from N = 3 biological replicates shows gating of sorted PDGFRA⁺ OLIG2⁺ OPCs from whole mouse brain. g, h, A representative agarose gel from a surveyor assay using T7 endonuclease I (g), and quantifications from N = 4 18-month-old animals (h), showing an indel rate of roughly 35% specific to FACS-sorted oligodendrocyte-lineage cells. i, Quantification of the off-target indels from N = 3 FACS-sorted OPCs from brains three weeks after AAV infection. N.D., not detected. j, k, qPCR results showing ∆∆CT from N = 3 biological replicates of PDGFRA/OLIG2-sorted cells from the in vivo infected aged CNS show a 75% reduction of Piezo1 indel-spanning mRNA and high expression of Piezo1 gRNA. l, Representative image from N = 3 biological replicates of GFP-plasmid electroporation in MEFs, showing a high efficiency of transfection. Scale bar, 50 µm. m–o, Schematic, western blot and quantifications of PIEZO1 protein levels in MEFs five days after electroporation of the Pdgfra-knock-in and Piezo1 gRNA construct. Quantifications represent averages from N = 3 replicate transfections for the PIEZO1 targeting and the non-targeting in vivo CRISPR constructs.
Extended Data Fig. 9 | In vivo knockdown of PIEZO1 in an aged lesion enhances OPC regeneration. a, b. Representative images and box-and-whisker-plot quantification of white matter lesion (below the white line) stained only for OLIG2 show increased OPC infiltration into the lesion site following Piezo1 knockdown. c, d. Fluoromyelin staining and quantifications of the ratio of lesion area with positive fluoromyelin staining from \( N = 3 \) biological replicates show increased myelin deposition in Piezo1-knockdown animals. e. In order to show the role of PIEZO1 in development, we generated an additional in vivo CRISPR system. The diagram depicts GFP and a ribozyme-flanked Piezo1 gRNA under the control of the OPC-specific Cspg4 promoter. The square next to the triangle on the PIEZO1 genomic locus represents the gRNA target sequence. Scale bars, 100 µm.
Extended Data Fig. 10 | A cell-type-specific CRISPR-mediated knockout of Piezo1 in OPCs during development increases both OPC proliferation and total cell number. a, Outline of our experimental strategy to confirm the efficacy of our in vivo CRISPR approach. In brief, $5 \times 10^{10}$ of each viral vector were tail-vein-injected into P1 pups. At 35 days, brains were dissociated, and cells were sorted by FACS on the basis of their expression of Pdgfra and Olig2. b, Representative FACS plot shows gating of a sorted PDGFRA$^+$ OLIG2$^+$ OPC population from whole mouse brain. c, An agarose gel from a surveyor assay using T7 endonuclease I (c), and quantifications from $N = 3$ P35 neonatal pups (d), showing a roughly 30% indel rate specific to oligodendrocyte-lineage cells. e, Representative images from $N = 3$ biological replicates, showing transgene specificity for OLIG2-expressing OPCs. f, qPCR results showing $\Delta \Delta CT$ from $N = 3$ biological replicates of PDGFRA$^+$ OLIG2$^+$ sorted cells from in vivo infected neonatal CNS, showing a roughly 55% reduction of Piezo1 indel-spanning mRNA relative to PDGFRA$^+$ OLIG2$^+$ sorted cells. Representative lower-power images showing EdU labelling of PDGFRA/OLIG2$^+$ expressing cells in corpus callosum. Scale bar, 100 $\mu$m. g, Quantifications of PIEZO1 protein levels in MEFs five days after electroporation of the Piezo1 gRNA construct under the control of the Cspg4 promoter. Quantifications are from $N = 3$ biological replicates. h–j, Representative images and quantifications from $N = 3$ animals showing the total density of oligodendrocyte-lineage cells, as labelled by OLIG2/PDGFRA or OLIG2/CC1 co-expression, in P35 mouse corpus callosum following Piezo1 knockdown. Scale bars, 100 $\mu$m.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

| n/a | Confirmed |
|-----|-----------|
| ☐   | ☒        |

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated
- Clearly defined error bars
- State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

**Data collection**

No software was used.

**Data analysis**

- Trimomatic, Hisat2, Stringtie, and Ballgown using a previously published protocol (Pertea et al., Nature Protocols, 2016). Data was processed and visualised with ipython notebook using the libraries pandas, matplotlib, numpy, seaborn, gseapy, and scikit-learn.
- AFM data processing was done using algorithms previously described (Christ et al., Journal of Biomechanics, 2010).
- Most image processing and analysis was done using FIJI.
- Image analysis was also performed using CellProfiler using standard co-localization parameters.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

If the paper is accepted, sequencing data will be uploaded to NCBI.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No sample-size calculations were done Sample size was deemed sufficient based on the consistency of differences between assayed conditions. |
| Data exclusions | No data was excluded. |
| Replication | All attempts at replication for were successful. |
| Randomization | The experiments were not randomized. When possible, each experimental setup used sex-matched littermates. |
| Blinding | All processing was performed blindly. This is not noted in current manuscript but will be in any potential revision. |

Reporting for specific materials, systems and methods

Materials & experimental systems

- n/a Involved in the study
- ☒ ☒ Unique biological materials
- ☒ Antibodies
- ☒ Eukaryotic cell lines
- ☐ Palaeontology
- ☒ Animals and other organisms
- ☒ Human research participants

Methods

- n/a Involved in the study
- ☒ ChIP-seq
- ☐ Flow cytometry
- ☒ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

All plasmids will be made available upon acceptance of work.

Antibodies

Antibodies used

- Goat α Olig2 R&D AF2418
- Rabbit α Pdgfra Cell Signaling 3174
- Rabbit α HA-Tag Cell Signaling C29F4
Rabbit α Olig2  ab9610
Rabbit α Ng2  MAB5384
Rat α MBP  MCA409S
Rabbit α Lmnb1  ab16048
Goat α Lmna/c  sc-20681
Mouse α Actin  556321
Mouse α CC1  MABC200
Goat α Sox10  sc-17342
Rabbit α Piezo1  15939-1-AP
Mouse α Anti-Chondroitin Sulfate  CB035
Rabbit α GFP ab290
Chicken α GFP Iab13970
Mouse α A2B5 MAB312
Alexa Fluor 594 Goat α Mouse IgM Thermo Fisher A-21044
Alexa Fluor 488 Donkey α Rabbit Millipore A21206
Alexa Fluor 594 Donkey α Rabbit Molecular Probes A21207
Alexa Fluor 594 Donkey α Goat Thermo Fisher A-11058
Alexa Fluor 647 Donkey α Mouse Thermo Fisher A-31571
IRDye800CW D anti-Goat Li-Cor 926_32214
IRDye600CW D anti-Rabbit Li-Cor 926_38073

Validation
Rabbit Olig2 is validated on Millipore’s website for being suitable for use in rat tissue and is widely used (Huang et al., Nature Neuroscience, 2011)
Rabbit Ng2 is validated on Millipore’s website for being suitable for use in rat tissue and is widely used (Miron et al., Nature Neuroscience, 2013).
Rat MBP is validated on Serotec’s website for being suitable for use in rat tissue and is widely used (Miron et al., Nature Neuroscience, 2013).
Rabbit Lmnb1 is validated on Abcam’s website for being suitable for use in rat tissue and is widely used (Dou et al., Nature, 2015).
Goat Lmna is validated on Santa Cruz website for being suitable for use in rat tissue and is widely used (Denais et al., Science, 2016).
Mouse HRP-actin is validated on Sigma’s website for being suitable for use in rat and is very widely used.
Mouse CC1 is widely used for rat tissue (Huang et al., Nature Neuroscience, 2011).
Goat Sox10 is validated for use in rat on Santa Cruz’s website and is widely used (Huang et al., Nature Neuroscience, 2011).
Rabbit Piezo1 from Proteintech is widely used for Piezo1 in zebrafish and mouse (Eisenhoffer et al., Nature, 2011), though not validated for rat. In our hands, however, it showed one distinct, correctly sized, band on our western blot (see Fig S11c).
Mouse Chondroitin Sulfate is verified for use in rat on Sigma’s website and is widely used (Moon et al., Nature Neuroscience, 2001).
Chicken GFP from Abcam is a very widely used antibody.
Mouse A2BS is widely used for rats, is validated as such on Millipore’s website, and is a standard antibody for the OPC field (Huang et al., Nature Neuroscience, 2011).
Rabbit Pdgfra Monoclonal from Cell Signaling (FACS: Bamidele et al., Cellular and Molecular Gastroenterology and Hepatology, 2018; IHC: Montagne et al., Nature Medicine, 2018).
Goat Olig2 from R&D regularly used and validated (IHC: Osipovitch et al., Cell Stem Cell, 2018).
Rabbit HA antibody from Cell Signalling (IHC validated from Zetsche et al., Nature Biotechnology, 2017)
Rabbit GFP is very well cited antibody (IHC in Dou et al., Nature, 2015).

Eukaryotic cell lines
Policy information about cell lines
Cell line source(s) HEK293T and Mouse Embryonic Fibroblast cells derived from Cambridge University’s Stem Cell Institute Tissue Culture Core Facility
Authentication The Stem Cell Institute Tissue Core Facility at Cambridge University validated the authenticity of these cell lines.
Mycoplasma contamination Cells prior to receipt were tested for Mycoplasma.
Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | Animals were Sprague Dawley rats. The neonates reported in this paper are all under the age of P15 and are of mixed sex. The aged rats reported in this study are all over the age of 14 months and are of different sexes depending on the study (the exact ages and sex are reported for each study).

For AAV work and in situ, animals were C57/Bl6 mice bred at our ‘in-house’ breeding facility. Aged mice were 18 months of age while neonates were p0/p1. Aged mice were all females while neonates were of mixed-sex. |
| Wild animals | This study does not involve wild animals |
| Field-collected samples | This study does not involve field collected samples. |

Flow Cytometry

Plots

Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ’group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Flow cytometry analysis of primary isolated OPCs or whole brain was performed by fixing OPCs or cells in suspension for 20 minutes in 4% PFA. Following fixation, cell suspension was spun at 800g for 5 minutes, and the PFA was aspirated. The cell pellet was re-suspended in 0.1% Triton X-100 and 5% donkey serum in PBS and placed for 30 minutes on ice. The suspension was spun again and incubated in primary antibody diluted 1:300 overnight at 4°C. The cell suspension was washed twice by spinning the cell suspension, incubating it in 0.1% Triton X-100 in PBS for 10 minutes, and spinning again. After the second wash-spin, the cell pellet was re-suspended in secondary antibodies diluted 1:500 in 0.1% Triton X-100 and 5% donkey serum diluted in PBS and incubated on ice for 2 hours. The cell suspension was wash twice again for 10 minutes each and re-suspended in PBS.

Instrument

Attune NxT Flow Cytometer (Thermo Fisher) for flow cytometry. BD FACs ARIA II for FACs sorting.

Software

Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.

Cell population abundance

~5% of cells from whole brain were Pdgfra+/Olig2+ double-positive OPCs.

Gating strategy

Gating was done after doing flow on negatively stained controls and after single color stained laser compensation.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.