New oligodendrocytes exhibit more abundant and accurate myelin regeneration than those that survive demyelination

Sarah A. Neely1,2, Jill M. Williamson1,2, Anna Klingseisen1,2, Lida Zoupi2,3, Jason J. Early1,2, Anna Williams2,3 and David A. Lyons1,2

Oligodendrocytes that survive demyelination can remyelinate, including in multiple sclerosis (MS), but how they do so is unclear. In this study, using zebrafish, we found that surviving oligodendrocytes make few new sheaths and frequently mistarget new myelin to neuronal cell bodies, a pathology we also found in MS. In contrast, oligodendrocytes generated after demyelination make abundant and correctly targeted sheaths, indicating that they likely also have a better regenerative potential in MS.

Loss of myelin (demyelination) is a feature of debilitating disorders of the central nervous system, including MS, but after demyelination, oligodendrocyte progenitor cells (OPCs) can produce new oligodendrocytes that remyelinate axons. Indeed, therapeutic strategies aimed at promoting otherwise limited, variable and age-depreciating remyelination in MS have focused on enhancing oligodendrogenesis1–3. However, recent studies have indicated that oligodendrocytes that survive demyelination can also contribute to remyelination2,4–6, potentially offering additional opportunities to promote remyelination. Nonetheless, it remains unclear to what extent surviving oligodendrocytes can contribute to remyelination in vivo.

Results

A novel zebrafish model to study remyelination. To compare remyelination by surviving and newly generated oligodendrocytes, we developed a zebrafish model of demyelination (Extended Data Fig. 1 and Methods). To induce myelin damage, we treated Tg(mbp:TRPV1-tagRFPt) animals with 10μM capsaicin (csn) for 2 h at 4 days post-fertilization (dpf). This led to extensive demyelination, as assessed by the transgenic reporter Tg(mbp:EGFP-CAAX) and transmission electron microscopy (TEM) (Extended Data Figs. 1f and 2). TEM analyses revealed a 95% reduction in myelinated axon number in the dorsal tract of the spinal cord just 1 day post-treatment (dpt) (Extended Data Fig. 2a–f). Assessment of Tg(mbp:EGFP-CAAX) and quantification of TEM images indicated rapid remyelination, with no significant difference in myelinated axon number between control and csn-treated zebrafish by 3 dpt (Extended Data Figs. 1f and 2g–j). The myelin present at 3 dpt surrounded similarly sized axons in the same regions in control and csn-treated animals, confirming remyelination (Extended Data Fig. 2g–j). Furthermore, axons at this time point showed both thick (>3 wraps) and thin (≤3 wraps) myelin, with those in the csn-treated animals having a higher proportion of thinly myelinated profiles (Extended Data Fig. 2k,l), recapitulating a common feature of remyelination: thin myelin sheaths. Despite the initial extensive (95%) loss of myelin, we saw a relatively modest reduction in total oligodendrocyte number, with only 33% fewer oligodendrocytes in csn-treated Tg(mbp:TRPV1-tagRFPt) animals at 3 hours post treatment (hpt) (Extended Data Figs. 2b and 3a–c), indicating that many oligodendrocytes survived demyelination. In line with ongoing remyelination, oligodendrocyte number increased over time (Extended Data Fig. 3b–f). Therefore, this model allows investigation of remyelination by both surviving and newly generated oligodendrocytes.

Surviving oligodendrocytes exhibit limited remyelination. To follow the fate of individual surviving oligodendrocytes, we mosaically labeled them with mbp:EGFP-CAAX (Methods) and imaged them before demyelination, 3 hpt, 1 dpt and after remyelination at 3 dpt (Fig. 1a). We imaged 37 oligodendrocytes with complete myelin sheath loss at 3 hpt, which led to production of myelin debris (Fig. 1a and Extended Data Fig. 4a) and an associated macrophage/microglial response (Extended Data Fig. 4b–d). Of these 37 oligodendrocytes (analyzed in 37 zebrafish), 14 cells underwent cell death, 12 of which died by 1 dpt. The remaining 23 oligodendrocytes were present at 3 dpt, confirming that oligodendrocytes can survive demyelination (Fig. 1a). Of these surviving cells, 22 made myelin (Fig. 1a,b), indicating that oligodendrocytes either rapidly die or make new myelin. Surviving oligodendrocytes made myelin sheaths of normal length but made very few, producing only two per cell on average, compared with 18 before demyelination (Fig. 1c,d). Furthermore, 13 of 22 surviving oligodendrocytes mistargeted newly made myelin, as evidenced by the appearance of myelin profiles surrounding cell bodies (Fig. 1a,h–i), as reported in other contexts29. By contrast, only one oligodendrocyte exhibited evidence of myelin mistargeting before demyelination (Fig. 1e), indicating that extensive myelin mistargeting is a pathological feature of remyelination by surviving oligodendrocytes. We also observed that new sheaths and mistargeted myelin profiles were localized closer to the oligodendrocyte cell body than before demyelination (Fig. 1f,g), suggesting that these cells have restricted process dynamics.

Surviving oligodendrocytes exhibit limited dynamic process activity. To study the dynamics of remyelination, we imaged an additional 23 surviving oligodendrocytes present at 1 dpt in 23 zebrafish and at 3 dpt, 5 dpt and 7 dpt. All 23 made myelin, with nine of 23 (39%) making sheaths only, 12 of 23 (52%) making...
sheaths and mistargeted myelin and two of 23 (9%) making mistargeted myelin only (Fig. 2). Most bona fide sheaths were present at 1 dpt, with sheath number per surviving cell remaining largely constant throughout the analyses (Fig. 2d,h–j), reflecting previously documented rapid sheath formation by single oligodendrocytes9. However, 16 new sheaths were made across seven cells after 1 dpt, 11 of which formed from pre-existing processes (Fig. 2a,b,i). Once formed, new sheaths extended along axons, completing their...
Myelin is mistargeted to neuronal cell bodies in MS. To assess whether myelin mistargeting is a feature of human disease, we analyzed postmortem motor cortex samples from five people with MS and five people without MS (Fig. 3 and Extended Data Fig. 6). We focused on the gray matter because its lower density of myelin allows discernment of individual oligodendrocytes and myelin sheaths. We co-stained MS tissue with either proteolipid protein (PLP) or CNPase to label oligodendrocytes and myelin sheaths and NeuN to label neurons and assessed subpial demyelinated lesions, perilesional areas and adjacent normal-appearing gray matter (Fig. 3 and Extended Data Fig. 6). This revealed many aberrant-appearing myelinating profiles enwrapping NeuN-labeled neuronal cell bodies (Fig. 3a–f and Extended Data Fig. 6b), with the number of PLP + myelinated neuronal cell bodies in MS perilesion sites, where remyelination is thought to take place1, almost 100-fold higher compared to control (non-MS) gray matter and normal-appearing gray matter in MS (Fig. 3h). Overall, these data indicate that myelin mistargeting is a previously unappreciated feature of MS gray matter pathology. Given the appearance of myelin mistargetting in areas of prospective remyelination, we asked whether this might be a general feature of remyelination or specific to oligodendrocytes that survive demyelination.

Fig. 2 | Myelin sheath dynamics of surviving oligodendrocytes after demyelination in the Tg(mbp:TRPV1-tagRFPt) zebrafish model. a–c, Confocal images of zoomed regions of oligodendrocytes that survive demyelination and form new sheaths from pre-existing processes (a and b) and de novo sheath formation (c) imaged over time at 1 dpf, 3 dpf, 5 dpf and 7 dpf. Arrows indicate locations of processes, and arrowheads highlight the locations of newly formed myelin sheaths. Scale bars, 10 µm. d, Quantification of the number of sheaths produced per oligodendrocyte by the same cells after demyelination over time at 1 dpf (median = 2.00, IQR = 1.00–2.00), 3 dpf (median = 1.00, IQR = 1.00–2.00), 5 dpf (median = 1.00, IQR = 1.00–2.00) and 7 dpf (median = 1.00, IQR = 1.00–2.00). e, Quantification of the number of mistargeted myelin profiles produced per oligodendrocyte after demyelination over time at 1 dpf (median = 0.00, IQR = 0.00–1.00), 3 dpf (median = 0.00, IQR = 0.00–1.00), 5 dpf (median = 0.00, IQR = 0.00–1.00) and 7 dpf (median = 0.00, IQR = 0.00–1.00). f, Quantification of the number of mistargeted myelin profiles produced per oligodendrocyte after demyelination over time at 1 dpf (median = 0.00, IQR = 0.00–1.00), 3 dpf (median = 0.00, IQR = 0.00–1.00), 5 dpf (median = 0.00, IQR = 0.00–1.00) and 7 dpf (median = 0.00, IQR = 0.00–1.00). g, Quantification of the number of mistargeted myelin profiles produced per oligodendrocyte after demyelination over time at 1 dpf (median = 0.00, IQR = 0.00–1.00), 3 dpf (median = 0.00, IQR = 0.00–1.00), 5 dpf (median = 0.00, IQR = 0.00–1.00) and 7 dpf (median = 0.00, IQR = 0.00–1.00). h, Quantification of the number of mistargeted myelin profiles produced per oligodendrocyte after demyelination over time at 1 dpf (median = 0.00, IQR = 0.00–1.00), 3 dpf (median = 0.00, IQR = 0.00–1.00), 5 dpf (median = 0.00, IQR = 0.00–1.00) and 7 dpf (median = 0.00, IQR = 0.00–1.00), 1 dpf versus 3 dpf, P > 0.9999; 1 dpf versus 5 dpf, P > 0.9999; 1 dpf versus 7 dpf, P > 0.9999; 3 dpf versus 5 dpf, P > 0.9999; 3 dpf versus 7 dpf, P > 0.9999; and 5 dpf versus 7 dpf, P > 0.9999. Friedman test with Dunn’s multiple comparisons test. Data are presented as median with IQR in n = 23 zebrafish from 23 zebrafish. i, Quantification of the average distance of myelin structures to the oligodendrocyte cell body for myelin sheaths (median = 11.97 µm, IQR = 7.36–16.24) and mistargeted myelin structures (median = 5.43 µm, IQR = 4.13–14.86) in surviving myelinating oligodendrocytes at 3 dpf (P = 0.3627). Two-tailed Mann–Whitney test. j, Quantification of the number of mistargeted myelin profiles of surviving oligodendrocytes pre-treatment (median = 0.00, IQR = 0.00–1.00) and 3 dpf (median = 0.00, IQR = 0.00–1.00). k, Quantification of the average distance of myelin (sheaths and mistargeted myelin) to the oligodendrocyte cell body for the same oligodendrocytes pre-treatment (median = 66.12 µm, IQR = 43.14–94.86) in surviving myelinating oligodendrocytes at 3 dpf (P = 0.3627). Two-tailed Mann–Whitney test.
Newly generated oligodendrocytes exhibit extensive remyelination. Given the limited contribution of surviving oligodendrocytes to the extensive remyelination observed by 3 dpt, we next assessed the contribution of individual newly generated oligodendrocytes to remyelination (Extended Data Fig. 7 and Methods). We found that they made an average of 25 sheaths per cell, thus over ten-fold more myelin than surviving cells (Extended Data Fig. 7b). Furthermore, almost all sheaths made were correctly targeted to axons (509 correctly targeted sheaths versus seven mistargeted myelin profiles across 20 newly generated cells), demonstrating that myelin mistargeting is a feature of remyelination by surviving oligodendrocytes.

Together, our data indicate that the potential and accuracy for remyelination by newly generated cells is far greater than that of oligodendrocytes that survive demyelination (Extended Data Fig. 8).

Discussion
Our observation of limited remyelination by surviving oligodendrocytes is in line with recent evidence from studies of remyelination in the rodent cortex, but it remains to be determined if surviving oligodendrocytes can be manipulated to exhibit more robust sheath formation—for example, to reinstate dynamic process extension. Nonetheless, surviving cells can support the elongation of sheaths...
along axons in line with the ability of mature oligodendrocytes to support sheath growth\textsuperscript{14–16}. Therefore, if demyelination results in the presence of lingering myelinating processes, substantial remyelination might ensue. In contrast to surviving cells, newly generated oligodendrocytes have an extensive capacity for remyelination— as in mammals, for example\textsuperscript{6,17}—begging the question of whether remyelination efficiency in MS might be related to oligodendrocyte diversity\textsuperscript{18}, including the relative number of surviving and newly generated oligodendrocytes.
made oligodendrocytes. The presence of surviving oligodendrocytes might even impair remyelination, given that cell death can stimulate the homeostatic generation of new oligodendrocytes from OPCs\(^{33,33}\). If oligodendrocyte death is indeed essential to triggering regeneration from OPCs, then it might be worth considering targeted destruction of surviving cells, to allow activation of OPCs with better remyelinating potential.

In summary, our analyses reveal limited and aberrant remyelination by oligodendrocytes that survive demyelination and suggest that therapeutic strategies to promote remyelination through the generation of new oligodendrocytes might be the most promising approach for demyelinating disorders, such as MS.

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/s41593-021-01009-x.

Received: 8 June 2020; Accepted: 22 December 2021; Published online: 14 February 2022

References
1. Thompson, A. J., Baranzini, S. E., Geurts, J., Hemmer, B. & Ciccarelli, O. Multiple sclerosis. *Lancet* 391, 1622–1636 (2018).
2. Franklin, R. J. M., Frisén, J. & Lyons, D. A. Revisiting remyelination: towards a consensus on the regeneration of CNS myelin. *Semin. Cell Dev. Biol.* 116, 3–9 (2020).
3. Cole, K. L. H., Early, J. J. & Lyons, D. A. Drug discovery for remyelination and treatment of MS. *Glia* 65, 1565–1589 (2017).
4. Yeung, M. S. Y. et al. Dynamics of oligodendrocyte generation in multiple sclerosis. *Nature* 666, 538–542 (2019).
5. Duncan, I. D. et al. The adult oligodendrocyte can participate in remyelination. *Proc. Natl Acad. Sci. USA* 115, E11807–E11816 (2018).
6. Baczek, C. M. et al. Motor learning promotes remyelination via new and surviving oligodendrocytes. *Nat. Neurosci.* 23, 819–831 (2020).
7. Klingseisen, A. et al. Oligodendrocyte neurofascin independently regulates both myelin targeting and sheath growth in the CNS Article oligodendrocyte neurofascin independently regulates both myelin targeting and sheath growth in the CNS. *Dev. Cell* 51, 730–744 (2019).
8. Almeida, R. G. et al. Myelination of neuronal cell bodies when myelin supply exceeds axonal demand. *Curr. Biol.* 28, 1296–1305 (2018).
9. Czopka, T., ffrench-Constat, C. & Lyons, D. A. Individual oligodendrocytes have only a few hours in which to generate new myelin sheaths invivo. *Dev. Cell* 25, 599–609 (2013).
10. Auer, E., Vagionitis, S. & Czopka, T. Evidence for myelin sheath remodeling in the CNS revealed by in vivo imaging. *Curr. Biol.* 28, 549–559 (2018).
11. Harboe, M., Torvund-Jensen, J., Kjaer-Sorensen, K. & Laursen, L. S. Ephrin-A1-EphA4 signaling negatively regulates myelination in the central nervous system. *Glia* 66, 934–950 (2018).
12. Wolf, R. M., Wilkes, J. J., Chao, M. V. & Resh, M. D. Tyrosine phosphorylation of p190 RhoGAP by Fyn regulates oligodendrocyte differentiation. *J. Neurobiol.* 49, 62–78 (2001).
13. Albert, M., Antel, J., Brück, W. & Stadelmann, C. Extensive cortical remyelination in patients with chronic multiple sclerosis. *Brain Pathol.* 17, 129–138 (2007).
14. Snidero, N. et al. Myelin membrane wrapping of CNS axons by P(3,4,5) P3-dependent polarized growth at the inner tongue. *Cell* 156, 277–290 (2014).
15. Hughes, E. G., Orthmann-Murphy, J. L. & Bergles, D. E. Myelin remodeling through experience-dependent oligodendrogenesis in the adult somatosensory cortex. *Nat. Neurosci.* 21, 696–708 (2018).
16. Hill, R. A., Patel, K. D., Goncalves, C. M., Grutzendler, J. & Nishiyama, A. Modulation of oligodendrocyte generation during a critical temporal window after NG2 cell division. *Nat. Neurosci.* 17, 1518–1529 (2014).
17. Orthmann-Murphy, J. et al. Remyelination alters the pattern of myelin in the cerebral cortex. *eLife* 9, e56621 (2020).
18. Jakel, S. et al. Altered human oligodendrocyte heterogeneity in multiple sclerosis. *Nature* 566, 543–547 (2019).
19. Kirby, B. B. et al. In vivo time-lapse imaging shows dynamic oligodendrocyte progenitor behavior during zebrafish development. *Nat Neurosci.* 9, 1506–1511 (2006).
20. Hughes, E. G., Kang, S. H., Fukaya, M. & Bergles, D. E. Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain. *Nat. Neurosci.* 16, 668–679 (2013).

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 America, Inc. 2022
NaturE NEurOScIENcE

Methods

Zebrafish lines and maintenance. All zebrafish were maintained under standard conditions in the Queen's Medical Research Institute BVS Aquatics facility at the University of Edinburgh. Studies were carried out with approval from the UK Home Office and in accordance with United Kingdom legislation, project licenses 70/8436 and PP5285280. Adult animals were kept on a 14-h light and 10-h dark cycle. Embryos were kept at 28.5 °C in 10 mM HEPES-buffered E3 embryo medium or conditioned aquatic water with methylene blue. Larval zebrafish were analyzed between 4 dpf and 9 dpf, before the onset of sexual differentiation in zebrafish.

The following transgenic zebrafish (Danio rerio) lines were used in this study: Tg(mbp:TRPV1-tagRFP) (generated for this study; see details below), Tg(mbp:EGFP), Tg(mbp:EGFP-CAXA)10, Tg(nbt:dsred) and Tg(mpeg1:EGFP)11. Throughout the text and figures, 'Tg' denotes stable, germline-inserted transgenic line.

Generation of the Tg(mbp:TRPV1-tagRFP) line. Given the animals’ advantages for longitudinal live imaging of myelination at high resolution in vivo12, we designed a zebrafish model to follow the fate of single oligodendrocytes after demyelination. We wanted to generate a model in which we could induce extensive demyelination but where a substantial number of affected oligodendrocytes might survive. We reasoned that we might be able to induce primary disruption to myelin sheaths by generating a transgenic system in which we drove cationic influx into myelin. This was based on our observation that high-amplitude, long-duration Ca2+ transients prefigure myelin sheath retraction during development13 and that excitotoxic influx of cations can mimic myelin pathology in mammals14–16. To do so, we generated the Tg(mbp:TRPV1-tagRFP) transgenic line, in which the cation-permeable TRPV1 channel of rats was expressed in myelinating oligodendrocytes downstream of the myelin basic protein (mbp) promoter of zebrafish (Extended Data Fig. 1). Importantly, unlike the rat ortholog of the protein, zebrafish TRPV1 channels are not cns sensitive17, and treatment of zebrafish with cns alone had no observable adverse effects on myelin or oligodendrocytes (Extended Data Fig. 1d,e). The principle of this system has previously been used to selectively ablate neurons in zebrafish18.

The mbp:TRPV1-tagRFP plasmid was generated by first making a pME-TRPV1-tagRFP entry vector plasmid. To do this, the TRPV1-tagRFP sequence was amplified by polymerase chain reaction (PCR) from the (uelt1GAL4VP16,4xUAS:TRPV1-RFPT) plasmid used by ref. 27, using the primers E3 embryo medium between imaging sessions. Zebrafish were screened at 4 dpf for isolated oligodendrocytes and, when identified, were imaged over time by identifying their position along the spinal cord, relative to the nearest somite as well as their position relative to other oligodendrocytes in the surrounding area labeled with the Tg(mbp:TRPV1-tagRFP) and Tg(mbp:EGFP-CAXA) transgenes. As not all oligodendrocytes die after initial demyelination, we were able to carefully assess relative positions using both tagRFP and EGFP-CAXA reporters (Extended Data Fig. 4a). Newly generated oligodendrocytes were carefully identified based on the appearance of oligodendrocytes labeled with the tagRFP and EGFP-CAXA reporters in areas previously devoid of fluorescence.

Image analysis. Image processing and analysis was performed in Fiji (ImageJ). Figure panels were produced using Fiji and Adobe Illustrator. For figures, maximum intensity projections of z-stacks were made, and a representative x–y area was cropped. All zebrafish images represent a lateral view of the spinal cord, anterior to the left and dorsal on top. For most of the spinal cord, the spinal cord was imaged with Xenius spinning disc confocal microscope (VAST-SDCM)19.

Single oligodendrocyte imaging. Zebrafish were screened at 4 dpf for isolated oligodendrocytes and, when identified, were imaged over time by identifying their position along the spinal cord, relative to the nearest somite as well as their position relative to other oligodendrocytes in the surrounding area labeled with the Tg(mbp:TRPV1-tagRFP) and Tg(mbp:EGFP-CAXA) transgenes. As not all oligodendrocytes die after initial demyelination, we were able to carefully assess relative positions using both tagRFP and EGFP-CAXA reporters (Extended Data Fig. 4a). Newly generated oligodendrocytes were carefully identified based on the appearance of oligodendrocytes labeled with the tagRFP and EGFP-CAXA reporters in areas previously devoid of fluorescence.

And incubated, with microwave stimulation, first in primary fixative (4% paraformaldehyde + 2% glutaraldehyde in 0.1 M sodium cacodylate buffer) and then in secondary fixative (2% osmium tetroxide in 0.1 M sodium cacodylate/1% glutaraldehyde). Samples were then stained en bloc with saturated uranyl acetate solution and dehydrated in an ethanol and acetone series, both with microwave stimulation. Samples were embedded in EMBed-812 resin (Electron Microscopy Sciences) and sectioned using a Reichert–Jung Ultracut Microtome. Sections were cut at similar somite levels by inspection of blocks under a dissection microscope and stained in uranyl acetate and Sato lead stain. TEM images were taken with a Philips CM120 BioTwin TEM. The Photometor tool in Adobe Photoshop was used to automate image registration and tiling. To assess axon diameter axonal areas were measured in ImageJ.
includes details on samples used. Tissue blocks of 2 cm × 2 cm × 1 cm were collected, fixed, dehydrated and embedded in paraffin blocks. Then, 4-μm sequential sections were cut and stored at room temperature. Gray matter MS lesions were identified using anti-PLP immunostaining.

**Human postmortem brain tissue immunohistochemistry.** Paraffin sections were rehydrated, washed in PBS and microewed for 15 min in Vector Unmasking Solution for antigen retrieval (H-330, Vector). For colorimetric immunohistochemistry, endogenous peroxidase and alkaline phosphatase activities were blocked for 10 min using Bioral solution (SP-6000, Vector). Sections were then blocked with 2.5% normal horse serum (S-5212, Vector) for 1 h at room temperature. Primary antibodies were incubated in antibody diluent solution (003118, Thermo Fisher Scientific) overnight at 4°C in a humidified chamber. Horse peroxidase or alkaline phosphatase (AP)-conjugated secondary antibodies (Vector) were applied for 1 h at room temperature. Staining development was performed using either DAB HRP substrate kit or Vector Blue substrate kit (both from Vector) according to the manufacturer’s instructions.

**Human postmortem brain tissue immunofluorescence.** For immunofluorescence, sections were incubated with AutoFluorescence Eliminator Reagent (2160, MERCK-Millipore) for 1 min and briefly washed in 70% ethanol after antigen retrieval. The sections were subsequently incubated with Image-IT FX Signal Enhancer (136933, Thermo Fisher Scientific) for 30 min at room temperature, washed and blocked for 1 h with 10% normal horse serum and 0.3% Triton-X 100 in PBS. Primary antibodies were diluted in antibody diluent solution (as above) and placed on sections overnight at 4°C in a humidified chamber. The next day, the sections were incubated with Alexa Fluor secondary antibodies (Thermo Fischer Scientific, 1:1000) for 1 h at room temperature and counterstained with Hoechst for the visualization of the nuclei. Primary antibodies used: rabbit polyclonal IgG antibody to NeuN (104225, Abcam, 1:1000), mouse monoclonal IgG2A antibody to myelin PLP (clone PLPC1, MAB388, Merck Millipore, 1:1000) and mouse monoclonal IgG2b antibody to CNPase (2',3'-cyclic nucleotide 3' phosphodiesterase, CL287, Amersham, 1:1000, Atlas Antibodies, 1:1000). All slides were mounted using Mowiol mounting medium (475904, Merck Millipore).

For fluorescent immunohistochemistry, secondary antibodies used: goat anti-mouse IgG2a cross-adsorbed secondary antibody, Alexa Fluor 568, A-21134, RRID: AB_2535773, Thermo Fisher Scientific; 1:1000 goat anti-mouse IgG2b cross-adsorbed secondary antibody, Alexa Fluor 568, A-21144, RRID: AB_2535780, Thermo Fisher Scientific 1:1000; and goat anti-rabbit IgG (H+L) secondary antibody, Alexa Fluor 488 conjugate, A-11008, RRID: AB_143165, Thermo Fisher Scientific 1:1000.

For chromogenic immunohistochemistry, secondary antibodies used: ImmPRESS HRP horse anti-mouse Polymer Detection Kit, peroxidase (MP-7402-15), Vector Laboratories; and ImmPRESS-AP anti-rabbit Ig (AP) Polymer Detection Kit (MP-5401), Vector Laboratories.

All listed secondary antibodies were ready-to-use antibody kits, and no specific dilution was used.

For chromogenic immunohistochemistry signal detection, the following were used:
- DAB Substrate Kit, peroxidase (HRP), with nickel (3,3′-diaminobenzidine), SK-4100, Vector Laboratories; and Vector Blue Substrate Kit (AP), SK-5300, Vector Laboratories.

Entire sections were imaged using the Zeiss Axiostar Slide scanner, and all quantifications were performed using Zeiss ZEN lite imaging software. For all cases, the whole gray matter area of the section was investigated focusing on perilesion areas in MS cases and in corresponding cortical layers in control tissue. Cell densities are presented as immune-positive cells per cm². z-stack images of the fluorescent-labeled samples were acquired with the LSM 880 confocal microscope equipped with Airyscan capture and a >20 objective (Zeiss Plan-Apochromat ×20 dry, NA=0.8). z-stacks were acquired with an optimal z-step according to the experiment.

**Reproducibility and sample size selection.** For zebrafish data, no statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications[1,2]. For human data, samples were randomly chosen for each group. We used donor tissue from both sexes randomly distributed in each group (Supplementary Table 2). The age of the donors, the postmortem interval and, in the case of MS samples, disease duration, were also randomly chosen. MS samples were selected based on the presence of at least one demyelinated lesion in the gray matter. Data collection and analysis could not be performed blinded to the conditions of the experiments, but, in the case of human immunohistochemistry analysis, the whole tissue section was analyzed so as not to introduce any regional bias. The results were validated by two independent researchers. No points were excluded.

**Statistics and reproducibility.** Graphs and statistical tests were carried out using GraphPad Prism. After screening to identify transgene-positive zebrafish, larvae were randomly assigned to treatment or control groups. Analysis of in vivo data was carried out blinded before treatment group was revealed. Data were averaged per biological replicate (n represents the number of zebrafish or humans) unless stated otherwise in figure legends. All single oligodendrocytes, which were imaged over a time course, were analyzed to make sure that the same cell was captured over time; where this was not the case, the oligodendrocyte was excluded for analysis. No data points were excluded from analysis due to variability.

For oligodendrocyte counts and electron microscopy analysis, findings are reported from single experimental run, from multiple zebrafish per condition. Data from Figs 2 and 3 characterizing single surviving oligodendrocytes are taken from at least three experimental runs. Data documenting oligodendrocytes that did not undergo demyelination, in Extended Data Fig. 5, is from two experimental runs.

Data were tested for normal distribution using the D’Agostino–Pearson test or the Shapiro–Wilk test. The variance of the data was assessed using the F test for variance. Indicated P values are from two-tailed unpaired t-tests, two-tailed unequal t-test with Welch’s correction, two-tailed paired t-tests, Wilcoxon signed-rank tests or Mann–Whitney tests. To compare more than two groups, a one-way ANOVA with Tukey’s multiple comparisons test, a Kruskal–Wallis test with Dunn’s multiple comparisons test, a mixed-effects analysis or a Friedman test was used. A difference was considered statistically significant when P <0.05. Throughout the figures, P values are indicated as follows: not significant (NS) P >0.05, *P<0.05, **P<0.01 and ***P<0.001. All data are shown as mean ± s.d. where data are normally distributed or median with interquartile range (IQR) (25th percentile and 75th percentile) where data are not normally distributed. Details of statistical test used, precise P values and n values for each comparison are detailed in the figure legends.

**Data analysis.** The following programs were used for data analysis: Fiji ImageJ versions 1.52p and 1.53c, Microsoft Excel 365, GraphPad Prism 9, Adobe Illustrator 2021 and Adobe Photoshop 2021.

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

**Data availability**
The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Code availability**
No code was used in this manuscript.

**References**
21. Almeida, R. G., Czopka, T., French-Constant, C. & Lyons, D. A. Individual axons regulate the myelinating potential of single oligodendrocytes in vivo. *Development* **138**, 4443–4451 (2011).
22. Ellett, F., Pase, L., Hayman, J. W., Andrianopoulos, A. & Lieschke, G. J. mpeg1 promoter transgenes direct macrophage-lineage expression in zebrafish. *Blood* **27**, e49–e56 (2011).
23. Bin, J. M. & Lyons, D. A. Imaging myelination in vivo using transparent animal models. *Brain Plast.* **3**, 29 (2016).
24. Baraban, M., Koudelka, S. & Lyons, D. A. Ca2+ activity signatures of myelin sheath formation and growth in vivo. *Nat. Neurosci.* **21**, 19–25 (2018).
25. Hamilton, N. B., Kolodziejczyk, K., Kougioumtzidou, E. & Attwell, D. Proton-gated Ca2+-permeable TRP channels damage myelin in conditions mimicking ischaemia. *Nature* **529**, 523–527 (2016).
26. Paz, P. M. & Lyons, D. A. Annual review of neuroscience calcium signaling in the oligodendrocyte lineage: regulators and consequences. *Annu. Rev. Neurosci.* **43**, 163–186 (2020).
27. Chen, S., Chiu, C. N., McArthur, K. L., Fetcho, J. R. & Prober, D. A. TRP channel mediated neuronal activation and ablation in freely behaving zebrafish. *Nat. Methods* **13**, 147–150 (2016).
28. Wuan, K. M. et al. The Tol2kit: a multisite gateway-based construction kit for Tol2 transposon transgenesis constructs. *Dev. Dyn.* **236**, 3088–3099 (2007).
29. Kartunen, M. J. et al. Regeneration of myelin sheaths of normal length and thickness in the zebrafish CNS correlates with growth of axons in caliber. *PLoS ONE* **12**, e0178058 (2017).
30. Early, J. & et al. An automated high-resolution in vivo screen in zebrafish to identify chemical regulators of myelination. *eLife* **7**, e35156 (2018).
31. Nicaise, A. M. et al. Cellular senescence in progenitor cells contributes to diminished remyelination potential in progressive multiple sclerosis. *Proc. Natl Acad. Sci. USA* **116**, 9030–9039 (2019).
32. Lloyd, A. F. et al. Central nervous system regeneration is driven by microglia necroptosis and repopulation. *Nat. Neurosci.* **22**, 1046–1052 (2019).

**Acknowledgements**

We thank C. ffrench-Constant, E. Hughes and the Lyons Laboratory for feedback, the Bioresearch & Veterinary Services aquatics facility for fish care, S. Mitchell for electron microscopy assistance and C. Melendez-Vasquez for suggesting the ROCK experiment. This work was supported by Wellcome Trust Senior Research Fellowships (102836/Z/13/Z and 214244/Z/18/Z), a Medical Research Council Project Grant (MR/P016272/1) and an MS Society Innovative Grant (95) to D.A.L. S.A.N. and J.M.W. were supported by the Wellcome Trust Four-Year Ph.D. Program in Tissue Repair (grant 108906/Z/15/Z) and J.M.W. by a University of Edinburgh Ph.D. Tissue Repair Studentship Award (MRC Doctoral Training Partnership MR/K501293/1). L.Z. and A.W. were supported by an MS Society UK Centre grant.

**Author contributions**

S.A.N., J.M.W. and D.A.L. conceived the project. S.A.N., J.M.W., A.K. and J.J.E. designed and performed the in vivo experiments. L.Z. and A.W. designed and performed the human tissue experiments. S.A.N. and D.A.L. co-wrote the manuscript, edited by all. D.A.L. managed the project.

**Competing interests**

The authors declare no competing interests.

**Additional information**

Extended data is available for this paper at [https://doi.org/10.1038/s41593-021-01009-x](https://doi.org/10.1038/s41593-021-01009-x).

Supplementary information The online version contains supplementary material available at [https://doi.org/10.1038/s41593-021-01009-x](https://doi.org/10.1038/s41593-021-01009-x).

Correspondence and requests for materials should be addressed to David A. Lyons.

Peer review information *Nature Neuroscience* thanks Bruce Appel, Jennifer Orthmann-Murphy, and the other, anonymous reviewer(s) for their contribution to the peer review of this work.

Reprints and permissions information is available at [www.nature.com/reprints](http://www.nature.com/reprints).
Extended Data Fig. 1 | Characterisation of the Tg(mbp:TRPV1-tagRFPt) zebrafish model. (a) Schematic illustrating the Tg(mbp:TRPV1-tagRFPt) demyelination model made using Biorender. The rat ortholog of the TRPV1 channel is expressed in myelinating oligodendrocytes and is activated by addition of csn which drives cation influx. Zebrafish TRPV1 channels are insensitive to csn, therefore csn treatment specifically results in damage to myelinating glia which express the rat ortholog of the TRPV1 channel. (b) Confocal images of myelinating oligodendrocytes in the Tg(mbp:EGFP; mbp:TRPV1-tagRFPt) zebrafish line at 4dpf showing oligodendrocytes co-expressing EGFP and tagRFPt in the merged image. Scale bar, 20 μm. (c) Brightfield images of a zebrafish containing the Tg(mbp:TRPV1-tagRFPt) transgene (TRPV1+ve), or wildtype siblings without the Tg(mbp:TRPV1-tagRFPt) transgene (TRPV1-ve) which show no developmental differences at 4dpf. Scale bars, 500 μm. (d and e) Confocal images of the (d) Tg(mbp:EGFP-CAAX) line and (e) the Tg(mbp:EGFP) line pre-treatment (indicated here as pre-t) at 4dpf, and 3hpt. Zebrafish not containing the Tg(mbp:TRPV1-tagRFPt) transgene show no disruption to myelin or oligodendrocytes following a 2 hour treatment of 10 μM csn. Scale bars, 20 μm. (f) Confocal images of myelin visualised in Tg(mbp:EGFP-CAAX; mbp:TRPV1-tagRFPt) zebrafish, with control (DMSO) and csn treated animals in top and bottom panels respectively pre-treatment (pre-t) at 4dpf, 3hpt, 1dpt and 3dpt. Scale bar, 20 μm.
Extended Data Fig. 2 | Csn treatment induces severe demyelination in the Tg(mbp:TRPV1-tagRFPt) zebrafish model. (a) Transmission electron microscopy images of DMSO and csn treated Tg(mbp:TRPV1-tagRFPt) zebrafish at 1dpt show numerous large calibre myelinated axons (blue) in DMSO treated animals and numerous large calibre unmyelinated axons (yellow) in csn treated animals. Scale bar, 1µm. (b) Quantification of the number of myelinated axons >0.4 µm diameter in the dorsal spinal cord at 1dpt in DMSO (median = 20.00, IQR = 16.50-22.00) versus csn (median = 0.00, IQR = 0.00-1.50) treated zebrafish, p<0.0001. Unpaired two-tailed t-test with Welch’s correction. N=5 zebrafish per condition. Data are presented as median with IQR. (c) Quantification of the number of unmyelinated axons >0.4 µm diameter in the dorsal spinal cord at 1dpt in DMSO (mean = 10.00 ± 5.24 SD) versus csn (mean = 27.40 ± 9.61 SD) treated zebrafish, p=0.0075. Unpaired two-tailed t-test. N=5 zebrafish per condition. Data are presented as mean ± SD. (d) Quantification of the number of axons >0.4 µm diameter in the dorsal spinal cord at 1dpt in DMSO (mean = 29.40 ± 8.26 SD) versus csn (mean = 28.00 ± 9.95 SD) treated zebrafish, p=0.8148. Unpaired two-tailed t-test. N=5 zebrafish per condition. Data are presented as mean ± SD. (e) Quantification of the number of axons with >3 myelin wraps in the dorsal spinal cord at 1dpt in DMSO (median = 20.00, IQR = 15.00-20.00) versus csn (median = 0.00, IQR = 0.00-1.50) treated zebrafish, p=0.0079. Two-tailed Mann-Whitney test. N=5 zebrafish per condition. Data are presented as mean ± SD. (f) Quantification of the number of axons with ≤3 myelin wraps in the dorsal spinal cord at 1dpt in DMSO (median = 1.00, IQR = 0.00-3.00) versus csn (median = 0.00, IQR = 0.00-0.00) treated zebrafish, p=0.1667. Two-tailed Kolmogorov-Smirnov test. N=5 zebrafish per condition. Data is presented as median with IQR. (g) Transmission electron microscopy images of DMSO and csn treated Tg(mbp:TRPV1-tagRFPt) zebrafish at 3dpt show numerous large calibre myelinated axons (>3 myelin wraps highlighted in blue, ≤3 myelin wraps highlighted in orange) and unmyelinated axons (highlighted in yellow) in DMSO and csn treated animals. Scale bar, 1µm. (h) Quantification of the number of myelinated axons >0.4 µm diameter in the dorsal spinal cord at 3dpt in DMSO (mean = 24.20 ± 9.83 SD) versus csn (mean = 20.83 ± 5.78 SD) treated zebrafish, p=0.4963. Unpaired two-tailed t-test. N=5 DMSO treated zebrafish, N=6 csn treated zebrafish. Data are presented as mean ± SD. (i) Quantification of the number of unmyelinated axons >0.4 µm diameter in the dorsal spinal cord at 3dpt in DMSO (mean = 24.20 ± 9.83 SD) versus csn (mean = 20.83 ± 5.78 SD) treated zebrafish, p=0.4963. Unpaired two-tailed t-test. N=5 DMSO treated zebrafish, N=6 csn treated zebrafish. Data are presented as mean ± SD. (j) Quantification of the number of axons >0.4 µm diameter in the dorsal spinal cord at 3dpt in DMSO (mean = 23.20 ± 6.76 SD) versus csn (mean = 30.17 ± 3.97 SD) treated zebrafish, p=0.0616. Unpaired two-tailed t-test. N=5 DMSO treated zebrafish, N=6 csn treated zebrafish. Data are presented as mean ± SD. (k) Quantification of the number of axons with >3 myelin wraps in the dorsal spinal cord at 3dpt in DMSO (median = 16.00, IQR = 15.50-27.00) versus csn (median = 8.50, IQR = 3.75-11.50) treated zebrafish, p=0.0152. Two-tailed Mann-Whitney test. N=5 DMSO treated zebrafish, N=6 csn treated zebrafish. (l) Quantification of the number of axons with ≤3 myelin wraps in the dorsal spinal cord at 3dpt in DMSO (median = 4.00 ± 3.24 SD) versus csn (median = 12.50 ± 5.01 SD) treated zebrafish, p=0.0099. Unpaired two-tailed t-test. N=5 DMSO treated zebrafish, N=6 csn treated zebrafish.
Extended Data Fig. 3 | Csn treatment induces minimal oligodendrocyte loss in the Tg(mbp:TRPV1-tagRFPt) model. (a) Confocal images of myelinating oligodendrocytes visualised in Tg(mbp:EGFP; mbp:TRPV1-tagRFpt) zebrafish, with control (DMSO) and csn treated animals in top and bottom panels respectively pre-treatment (pre-t) at 4dpf, 3hpt, 1dpt and 3dpt. Scale bar, 20 µm. (b–f) Quantification of myelinating oligodendrocyte number in DMSO and csn treated Tg(mbp:EGFP; mbp:TRPV1-tagRFpt) zebrafish over time. (b) Pre-treatment DMSO (mean = 58.60 ± 13.23 SD) versus csn (mean = 57.20 ± 7.64 SD), p = 0.7753. Data are presented as mean ± SD. (c) 3hpt DMSO (mean = 65.00 ± 14.93 SD) versus csn (mean = 44.11 ± 10.36 SD), p = 0.0041. Data are presented as mean ± SD. (d) 1dpt DMSO (mean = 76.90 ± 14.14 SD) versus csn (mean = 61.70 ± 7.39 SD), p = 0.0075. Data are presented as mean ± SD. (e) 3dpt DMSO (mean = 99.00 ± 15.93 SD) versus csn (mean = 85.70 ± 11.83 SD), p = 0.0444. Data are presented as mean ± SD. (f) 5dpt DMSO (mean = 104.5 ± 12.95 SD) versus csn (mean = 108.5 ± 15.10 SD), p = 0.5328. (b–f) Unpaired two-tailed t-tests. Pre-treatment N = 10 zebrafish per treatment group, 3hpt N = 8 (DMSO) and 9 (csn) treated zebrafish, 1dpt N = 10 zebrafish per treatment group, 3dpt N = 11 zebrafish (DMSO) and 10 zebrafish (csn), 5 dpt N = 10 zebrafish per treatment group. Data are presented as mean ± SD. Each data point represents total (dorsal + ventral) oligodendrocyte number analysed per imaged area per zebrafish.
Extended Data Fig. 4 | Characterisation of single oligodendrocyte loss and myelin debris phagocytosis following demyelination in the Tg(mbp:TRPV1-tagRFPt) zebrafish model. (a) Confocal images of a single oligodendrocyte labelled with mbp:EGFP-CAAX in the Tg(mbp:TRPV1-tagRFPt) line pre-treatment (pre-t) at 4dpf, and 3hpt. An example of oligodendrocyte cell death is demonstrated here by the disappearance of a tagRFP+ve cell body following csn treatment in the same zebrafish before and after demyelination, whilst 2 tagRFP+ve oligodendrocytes which survive demyelination are seen neighbouring it at 3hpt. Arrows indicate the location of the oligodendrocyte cell body which undergoes cell death, or where it was prior to demyelination. Scale bar, 20 µm. (b) Confocal images of microglia / macrophage engulfment of myelin debris following demyelination at 1dpt. Arrowheads highlight the location of myelin debris engulfment. Scale bar, 20 µm. (c) Quantification of the number of mpeg+ve cells (macrophages / microglia) in a 4-somite section of the spinal cord at pre-treatment (mean = 1.38 ± 0.74 SD), 1dpt (mean = 2.14 ± 1.07 SD), 2dpt (mean = 3.14 ± 0.38 SD), 3dpt (mean = 2.57 ± 1.90 SD) and 4dpt (mean = 3.00 ± 1.29 SD) (where treatment was a DMSO control). Pre-t vs 1dpt p = 0.7196, pre-t vs 2dpt p = 0.0505, pre-t vs 3dpt p = 0.3106, pre-t vs 4dpt p = 0.0844, 1dpt vs 2dpt p = 0.5190, 1dpt vs 3dpt p = 0.9597, 1dpt vs 4dpt p = 0.6588, 2dpt vs 3dpt p = 0.8930, 2dpt vs 4dpt p = 0.9994, 3dpt vs 4dpt p = 0.9597. Ordinary one-way ANOVA with Tukey's multiple comparison test. Pre-treatment N = 8 zebrafish, 1-4dpt N = 7 zebrafish. Data are presented as mean ± SD. (d) Quantification of the number of mpeg+ve cells (macrophages / microglia) in a 4-somite section of the spinal cord at pre-treatment (mean = 1.56 ± 1.015D), 1dpt (mean = 7.88 ± 2.75 SD), 2dpt (mean = 10.38 ± 3.42 SD), 3dpt (mean = 9.12 ± 2.48 SD) and 4dpt (mean = 6.63 ± 1.92 SD) (where treatment was a demyelinating csn treatment). Pre-t vs 1dpt p < 0.0001, pre-t vs 2dpt p < 0.0001, pre-t vs 3dpt p < 0.0001, pre-t vs 4dpt p = 0.0294, 1dpt vs 2dpt p = 0.2586, 1dpt vs 3dpt p = 0.8394, 1dpt vs 4dpt p = 0.8394, 2dpt vs 3dpt p = 0.8394, 2dpt vs 4dpt p = 0.0294, 3dpt vs 4dpt p = 0.2586. Ordinary one-way ANOVA with Tukey's multiple comparison test. Pre-treatment N = 9 zebrafish, 1-4dpt N = 8 zebrafish. Data are presented as mean ± SD.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | ROCK inhibitor treatment further increases myelin mistargeting by surviving oligodendrocytes in the Tg(mbp:TRPV1-tagRFPt) zebrafish model. (a) Confocal images of single oligodendrocytes which have not undergone demyelination treated with DMSO (control) or Y27632 ROCK inhibitor and imaged at 4dpf. Scale bar, 20 µm. (b) Confocal images of single surviving oligodendrocytes followed over time from prior to demyelination (csn treatment) at 4dpf through to 3dpt. Following demyelination oligodendrocytes were treated with either DMSO (control) or Y27632 ROCK inhibitor. Scale bars, 20 µm. (c) Quantification of the number of sheaths produced in oligodendrocytes which have not been demyelinated in control (mean = 16.08 ± 5.73 SD) and Y27632 ROCK inhibitor (mean = 19.35 ± 4.08 SD) treated zebrafish, p = 0.0386. Unpaired two-tailed t-test. N = 24 oligodendrocytes from 24 zebrafish (control), N = 20 oligodendrocytes from 20 zebrafish (Y27632). Data are presented as mean ± SD. (d) Quantification of the average sheath length (µm) produced in oligodendrocytes which have not been demyelinated in control (mean = 28.62 ± 7.18 SD) and Y27632 ROCK inhibitor (mean = 27.26 ± 6.91 SD) treated zebrafish, p = 0.5308. Unpaired two-tailed t-test. N = 24 oligodendrocytes from 24 zebrafish (control), N = 20 oligodendrocytes from 20 zebrafish (Y27632). Data are presented as mean ± SD. (e) Quantification of the number of mistargeted myelin profiles produced in oligodendrocytes which have not been demyelinated in control (median = 0.00, IQR = 0.00-0.00) and Y27632 ROCK inhibitor (median = 0.00, IQR = 0.00-0.00) treated zebrafish, p > 0.9999. Two-tailed Mann-Whitney test. N = 24 oligodendrocytes from 24 zebrafish (control), N = 20 oligodendrocytes from 20 zebrafish (Y27632). Data are presented as median with IQR. (f) Quantification of the number of sheaths produced per oligodendrocyte following demyelination in control (median = 3.00, IQR = 1.50-3.00) and Y27632 ROCK inhibitor (median = 4.00, IQR = 1.00-4.50) treated zebrafish, p = 0.5964. Two-tailed Mann-Whitney test. N = 5 oligodendrocytes from 5 zebrafish (control), N = 9 oligodendrocytes from 9 zebrafish (Y27632). Data are presented as median with IQR. (g) Quantification of the number of mistargeted myelin profiles produced per oligodendrocyte following demyelination in control (median = 1.00, IQR = 1.00-2.00) and Y27632 ROCK inhibitor (median = 3.00, IQR = 1.50-4.75) treated zebrafish, p = 0.0414. Unpaired two tailed t-test with Welch’s correction. N = 5 oligodendrocytes from 5 zebrafish (control), N = 8 oligodendrocytes from 8 zebrafish (Y27632). Data are presented as mean ± SD.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Mistargeted myelin profiles are present in remyelinating lesions in motor cortex tissue from people with MS. (a) Low magnification image of chromogenic immunohistochemistry for proteolipid protein (PLP - brown) and NeuN (blue) in human MS motor cortex. Outline of quantified areas shown with lesion area highlighted in red, perilesion area highlighted in green, normal appearing grey matter (NAGM) in purple and white matter indicated by ‘WM’ in white. Images 1 and 2 show examples of quantified areas in 2 different human MS motor cortex samples. Scale bars, 2000 µm. (B and C) High magnification images of chromogenic immunohistochemistry for proteolipid protein (PLP - brown) and NeuN (blue) in human MS motor cortex. (b) Images 1-9 show example images of PLP+ve wrapped NeuN+ve cells (myelinated neuronal cell bodies). Images 1 and 3-9 scale bars, 20 µm. Image 2 scale bar, 10 µm. (c) Images 1-5 show example images of PLP+ve wrapped NeuN-ve cells (oligodendrocytes). Scale bars, 20 µm. (d) Fluorescent immunohistochemistry for NeuN (white), PLP (red) and Hoechst (nuclei-blue) in human MS motor cortex. Arrows indicate the location of PLP+ve wrapped NeuN+ve Hoechst+ve cells (myelinated neuronal cell body). Scale bar, 20 µm. (e) Images 1-5 show example images of CNPase+ve wrapped NeuN+ve cells (myelinated neuronal cell bodies). Scale bars, 20 µm. (f) Fluorescent immunohistochemistry for NeuN (green), CNPase (magenta) and Hoechst (nuclei-blue) in human MS motor cortex. Arrows indicate the location of CNPase+ve wrapped NeuN+ve Hoechst+ve cells. Scale bar, 20 µm.
Extended Data Fig. 7 | Extensive remyelination by newly generated oligodendrocytes in the Tg(mbp:TRPV1-tagRFPt) zebrafish model. (a) Confocal images of csn treated zebrafish with oligodendrocytes newly generated after demyelination. Arrows show position of oligodendrocyte cell bodies. Scale bars, 20 µm. (b) Quantification of the total myelin produced per oligodendrocyte (calculated by multiplying number of sheaths per oligodendrocyte by the average sheath length per oligodendrocyte) (mean = 521.5 ± 138.30 SD), versus the same oligodendrocytes 3dpt (mean = 4718 ± 26.57 SD) and by newly differentiated oligodendrocytes at 3dpt (mean = 491.80 ± 199.10 SD). Pre-treatment versus surviving p < 0.0001, pre-treatment versus newly differentiated p = 0.8271, surviving versus newly differentiated p < 0.0001. Ordinary one-way ANOVA with Tukey’s multiple comparison test. N = 15 oligodendrocytes from 15 zebrafish (pre-treatment and surviving). N = 20 oligodendrocytes from 11 zebrafish (newly differentiated). Data are presented as mean ± SD.
Extended Data Fig. 8 | Summary Schematic. Summary schematic outlining the responses of oligodendrocytes which survive demyelination and those newly generated after demyelination made using Biorender.
Reporting Summary

Nature Portfolio 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 Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on 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 statistical parameters 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

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

| Data collection | Zen black 2.3 (Zeiss) |
|-----------------|-----------------------|
| Data analysis   | Fiji Image J version 1.52p and 1.53c, Microsoft Excel 365, Graph Pad Prism9, Adobe Illustrator 2021, Adobe Photoshop 2021. |

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The data that support the findings of this study are available from the corresponding author upon reasonable request.
### Field-specific reporting

Please select the one below that is 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/documents/nr-reporting-summary-flat.pdf](http://nature.com/documents/nr-reporting-summary-flat.pdf)

### Life sciences study design

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

| Sample size | For zebrafish data: this is the first study of documenting demyelination and remyelination in vivo using the Tg(mbp:TRPV1-tagRFPt) model. Therefore, it was not possible to do power calculations prior to the study, as the nature of oligodendrocyte survival and myelin sheath production were unknown in this model. No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications (Klingseisen et al., 2019). For our analyses of global effects of demyelination we imaged 8-11 zebrafish per condition. For our electron microscopy analysis we imaged 5-6 zebrafish per condition. For our analysis of single oligodendrocyte responses to demyelination we imaged a total of 60 zebrafish. For manipulations to surviving oligodendrocyte we imaged 5-9 zebrafish per condition, and for manipulations to control single oligodendrocytes we imaged 20-24 zebrafish. For newly made oligodendrocytes we imaged a total of 11 zebrafish per condition. Experimental differences were deemed statistically significant using standard significance analysis, as documented throughout.

| Data exclusions | No data were excluded due to variability. Single oligodendrocytes were only excluded when it was not possible to capture the same oligodendrocyte over time, or when myelin became too overlapping to quantify. For human data no data points were excluded.

| Replication | For zebrafish data: oligodendrocyte counts and electron microscopy analysis findings are reported from a single experimental run, from multiple zebrafish per condition. Data from figures 2 and 3 characterising single surviving oligodendrocytes are taken from at least 3 experimental runs, data documenting oligodendrocytes which did not undergo demyelination in Figure S5 is from 2 experimental runs. To increase the reproducibility between experiments all larval zebrafish were grown up in the same incubator to avoid any differences due to developmental stage between experiments. During live imaging analyses, experimental and control animals were imaged in an alternating pattern (per experiment) to ensure no confounding effects of developmental stage between groups.

| Randomization | For zebrafish data: following screening to identify transgene positive zebrafish, larvae were randomly assigned to treatment or control groups. For human data: samples were randomly chosen for each group. We used donor tissue from both sexes randomly distributed in each group (see Supplementary Table 2). The age of the donors, the post-mortem interval, and in the case of MS samples disease duration was also randomly chosen. The selected MS samples were selected based on the presence of at least one demyelinated lesion in the grey matter.

| Blinding | For zebrafish data: analysis of in vivo data was carried out blinded before treatment group was revealed. For human data: data collection and analysis could not be performed blind to the conditions of the experiments but in the case of human IHC analysis the whole tissue section was analysed so as not to introduce any regional bias. The results were validated by two independent researchers.

### Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

#### Materials & experimental systems

| Involved in the study |
|---|
| n/a |
| ☐ Antibodies |
| ☒ Eukaryotic cell lines |
| ☒ Palaeontology and archaeology |
| ☒ Animals and other organisms |
| ☒ Human research participants |
| ☒ Clinical data |
| ☐ Dual use research of concern |

#### Methods

| Involved in the study |
|---|
| n/a |
| ☒ ChiP-seq |
| ☒ Flow cytometry |
| ☒ MRI-based neuroimaging |

#### Antibodies

Primary antibodies used: rabbit polyclonal IgG antibody to NeuN (104225, Abcam, 1:100), mouse monoclonal IgG2A antibody to myelin Proteolipid Protein (clone PLPC1, MAB388, MERCK-Millipore, 1:100) and mouse monoclonal IgG2b antibody to CNPase (2',3'-Cyclic Adenosine Monophosphate Phosphodiesterase A) (1:500).
cyclic nucleotide 3’ phosphodiesterase, CL2887, AMAb91072, Atlas antibodies, 1:1000). Secondary antibodies used: Alexa Fluor secondary antibodies (Thermo Fischer Scientific, 1:1000).

Secondary antibodies used: Goat anti-Mouse IgG2a Cross-Adsorbed Secondary Antibody, Alexa Fluor 568, A-21134, RRID: AB_2535773 Thermo Fisher Scientific
Goat anti-Mouse IgG2b Cross-Adsorbed Secondary Antibody, Alexa Fluor 568, A-21144, RRID: AB_2535780 Thermo Fisher Scientific
Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor® 488 conjugate, A-11008, RRID: AB_143165 Thermo Fisher Scientific

Secondary antibodies used: Goat anti-Mouse IgG2a Cross-Adsorbed Secondary Antibody, Alexa Fluor 568, A-21134, RRID: AB_2535773 Thermo Fisher Scientific
Goat anti-Mouse IgG2b Cross-Adsorbed Secondary Antibody, Alexa Fluor 568, A-21144, RRID: AB_2535780 Thermo Fisher Scientific
ImmPRESS® HRP Horse Anti-Mouse IgG Polymer Detection Kit, Peroxidase (MP-7402-15) Vector Laboratories
ImmPRESS-AP Anti-Rabbit Ig (alkaline phosphatase) Polymer Detection Kit (MP-5401) Vector Laboratories

These are ready to use antibody kits. No specific dilution was used. Also for chromogenic IHC signal detection:

DAB Substrate Kit, Peroxidase (HRP), with Nickel, (3,3’-diaminobenzidine) SK-4100 Vector Laboratories
Vector® Blue Substrate Kit, Alkaline Phosphatase (AP) SK-5300 Vector Laboratories

Validation
Primary antibodies were validated by the manufacturer:
file:///C:/Users/medusauser/Desktop/datasheet_NeuN.pdf
https://www.atlasantibodies.com/api/print_datasheet/AMAb91072.pdf
https://www.merckmilitipore.com/GB/en/product/Anti-Myelin-Proteolipid-Protein-Antibody-CT-clone-PLPC1,MM_NF-MAB388-100UG#overview

Additionally myelin antibodies in the human tissue were validated by comparing the signal with other myelin antibodies such as MBP, and myelin histological stainings such as Luxol Fast Blue. NeuN was validated using antibodies against other neuronal markers and additionally compared with hematoxylin or cresyl violet histological stainings to verify the neuronal morphology of the positive cells.

The following antibodies have also already been used in the following published papers:
NeuN (Zoupi et al., 2021)
CNP (Jäkel et al., 2019)
PLP (Kokjohn et al., 2013 and Pedraza et al., 2014)

Animals and other organisms

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

Laboratory animals
Zebrafish (danio rerio) were used for experiments before the onset of sexual differentiation. A mix of stable transgenic lines and transient transgenic animals generated by injection of transgenes at the one cell stage were used in this study. The stable transgenic lines that were used include: Tg(mbp:TRPV1-tagRFP) (generated for this study), Tg(mbp:EGFP), Tg(mbp:EGFP-CAAX), Tg(nbt:dsred) and Tg(mpeg1:EGFP). Transient transgenic animals were injected with the following constructs: mbp:EGFP-CAAX. Adult zebrafish, up to two years of age, were used for breeding purposes. Larval zebrafish were analysed between 4 - 11 dpf, before the onset of sexual differentiation in zebrafish. Details of stages used for individual experiments is detailed in the main text and or figures and legends.

Wild animals
No wild animals were used in the study.

Field-collected samples
No field-collected samples were used in this study.

Ethics oversight
Studies were carried out with approval from the UK Home Office and according to its regulations, under project licenses 70/8436 and PPS258250.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics
For the analysis of Multiple Sclerosis (MS) tissue the study includes post-mortem brain tissue (motor cortices) from 5 people with MS and 5 people without MS (controls). We used donor tissue from both sexes randomly distributed in each group (see Supplementary Table 2). The age of the donors the post-mortem interval and in the case of MS samples disease duration was also randomly chosen. The selected MS samples were selected based on the presence of at least one demyelinated lesion in the grey matter.

Recruitment
The tissue was obtained from an accredited UK tissue bank. MS samples were selected on the basis of the presence of lesions. No participant compensation.

Ethics oversight
Post-mortem brain tissue (motor cortices) from MS patients and controls without neurological defects were provided by a UK prospective donor scheme with full ethical approval from the UK Multiple Sclerosis Society Tissue Bank (MREC/02/2/39).

Note that full information on the approval of the study protocol must also be provided in the manuscript.