Failed regeneration of CNS myelin contributes to clinical decline in neuroinflammatory and neurodegenerative diseases, for which there is an unmet therapeutic need. Here we reveal that efficient remyelination requires death of pro-inflammatory microglia followed by repopulation to a pro-regenerative state. We propose that impaired microglia death and/or repopulation may underpin dysregulated microglia activation in neurological diseases, and we reveal therapeutic targets to promote white matter regeneration.

CNS remyelination reinstates axon health/function, yet fails in prevalent neurodegenerative disorders contributing to axon dysfunction/loss for which there is an unmet therapeutic need. These disorders (for example, multiple sclerosis, amyotrophic lateral sclerosis (ALS), spinal cord injury) are associated with chronic activation of resident immune cells, microglia. Resolution of proinflammatory microglia activation (iNOS+TNF+; CCL2+) via a transition to a pro-regenerative microglia state (Arg-1+ CD206+ IGF-1+) initiates remyelination. Remyelination is impaired when this transition is prevented (by depletion of pro-regenerative microglia), or when it fails, identified by prolonged proinflammatory microglia presence in aged mice and chronic human brain lesions. However, the mechanisms underpinning this transition in microglia activation remain unknown.

To reveal these mechanisms, we performed RNA sequencing of microglia isolated from focal demyelinated lesions of young adult mouse corpus callosum induced with the myelin toxin lysophosphatidyl choline (LPC), where regeneration occurs without concomitant damage and timing of microglia activation is defined (Fig. 1a). At peak proinflammatory (3 d post-LPC (dpl)) and pro-regenerative microglia activation (10 dpl), microglia were isolated by fluorescence-activated cell sorting (FACS) based on expression of CD11b, lack of expression of neutrophil/T-lymphocyte markers (Ly6G, CD3) and low expression of CD45 (gating strategy in Supplementary Fig. 1a). Cells did not express markers for border-associated macrophages or monocye-derived cells (Supplementary Fig. 1b). Of the 5,000 most highly expressed genes (including non-differentially expressed genes), 57.2% were shared and 21.4% were specific to each time point (Fig. 1b). Microglia expressed genes associated with microglia during developmental myelination or neurodegeneration (Supplementary Fig. 1c–i), some of which were significantly enriched at 10 dpl. A total of 1,020 genes were significantly differentially expressed between 3 and 10 dpl (P < 0.05) (Fig. 1c; log fold change in Supplementary Table 1), including genes involved in survival/proliferation (Birc5, Smad2, Ccnb1), anti-oxidant responses (Keap1), inflammation (Tnfsf11b, Jak2, Nfkbid, Cryba1, Gpmmb, Socs1, Cd40) and remyelination (Axl, Osm, Adam8), and associated with microglia in neurodegeneration (Msr1, Hidac5, Spy) (Supplementary Fig. 1j). Pathway analysis identified enrichment of chronic inflammation at 3 dpl, and anti-inflammatory responses/transcriptional regulators at 10 dpl (Supplementary Figs. 1k and 2 and Supplementary Table 2). Significantly upregulated genes at 10 dpl included those encoding proteins associated with remyelination/regeneration (Matn2, Osm, Fgf1, Cds300tf), myelination (Bmp1, Cd69, Fhbp5) and oligodendrocyte lineage responses (iron export (Cp), Wnt pathway inhibition (Nrt1)) (Fig. 1d and Supplementary Fig. 1i). Microglia at both time points showed engagement of phagocytic pathways, by Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis ('fat digestion' at 3 dpl; 'endocytosis' at 10 dpl; Fig. 1e). Gene Ontology enrichment (for example, 'degradation of lipoproteins' at 3 dpl; 'structural constituent of myelin sheath' at 10 dpl; Supplementary Fig. 2) and receptor expression (Fig. 1f), suggesting engulfment/breakdown of myelin debris throughout remyelination.

Cell death-associated pathways were enriched in microglia at 3 dpl, with (1) engagement of KEGG pathways ('axon guidance', 'colorectal cancer') associated with genes regulating cell death (Birc5, Smad2, Epdrh1b2) (Fig. 1e), (2) 'cell death & survival' identified as a major molecular and cellular function (Fig. 1g) due to regulation of genes associated with death (for example, TNF receptor signaling, TRAIL, p53; Supplementary Table 3), and (3) most transcriptional regulators upregulated at 3 dpl known to control cell death (Supplementary Table 2 and Supplementary Fig. 1m). To determine whether microglia undergo death after demyelination, we analyzed IBA-1+ cells in lesions, which were homogenous at 3 dpl, sparse at 7 dpl and clustered at 10 dpl (Fig. 1i), and decreased in density at

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Central nervous system regeneration is driven by microglia necroptosis and repopulation

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**Brief Communication**

**Genes associated with phagocytosis**

**Fig. 1 | Microglia death occurs during transition in activation following in vivo demyelination.** **a.** Microglia were isolated from focal LPC-demyelinated lesions of mouse corpus callosum at 3 and 10 dpl, representative of key time points of microglial activation during remyelination, for subsequent RNA sequencing. **b.** Overlap of top 5,000 genes expressed by microglia (including non-significantly differentially expressed) at 3 and 10 dpl, ranked by average fragments per kilobase of transcript per million (FPKM). **c.** Heat map of gene expression level per sample relative to average expression across all samples. Red represents higher expression and blue represents lower expression. **d.** Genes with a significant log2 fold change (P < 0.05) in 10-dpl versus 3-dpl microglia with known roles in regulating remyelination, myelination and the oligodendrocyte lineage, represented as mean FPKM (± s.e.m.). **e.** Ingenuity pathway analysis of significantly engaged molecular and cellular functions in microglia (P < 0.05, right-tailed Fisher’s exact test performed for upregulated and downregulated genes separately). **f.** Expression of genes associated with phagocytosis of myelin debris in microglia at 3 and 10 dpl, indicated as mean FPKM (± s.e.m.). No significance between time points, two-tailed paired Student’s t test (P = 0.0690, t = 1.371, d.f. = 2). **g.** IPA of significantly engaged KEGG pathways in microglia at 3 versus 10 dpl. KEGG pathways are represented on the y axis, and enrichment score on the x axis is represented as −log10(P) enrichment (P < 0.05, right-tailed Fisher’s exact test performed for upregulated and downregulated genes separately). **h.** Analysis of apoptosis markers Annexin-V-FITC and 7-AAD at 3, 7 and 10 dpl. The experiment was performed with three mice per time point. **i.** Flow cytometry plots of lesion-isolated microglia (CD11b-PE-Cy7+CD45-BV605+) positive for cell death markers Annexin-V-FITC and 7-AAD at 3, 7 and 10 dpl. The experiment was performed with three mice per time point. **j.** Mean proportion of all microglia that are Annexin-V+7-AAD+ at 3, 7 and 10 dpl ± s.e.m. **k.** Mean proportion of all microglia that are Annexin-V+7-AAD+ at 3, 7 and 10 dpl ± s.e.m. 

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**Note:** The images and tables accompanying the text provide visual representations of the data and analysis, including heat maps, fold change plots, and flow cytometry data. The figures and tables are crucial for understanding the detailed results and conclusions drawn from the study. The text provides context and interpretation of these visual data, emphasizing the role of microglia in the transition of activation following in vivo demyelination. The study highlights the importance of gene expression and cellular function in the remyelination process, offering insights into the mechanisms of microglial activation and phagocytic activity.
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7 dpl (Fig. 1h,i). Flow cytometric analysis of microglia (CD11b+ CD45+) revealed an increase in death (Annexin-V−7-AAD+) at 7 dpl (Fig. 1j,k; gating strategy, Supplementary Fig. 3a,c). Microglia isolated from sham-demyelinated mice at 7 d post-surgery were negative for Annexin-V and 7-AAD (Supplementary Fig. 3b), excluding that death occurred from injection/cell isolation. Thus, after demyelination and before onset of remyelination, microglia die during the transition from proinflammatory to pro-regenerative activation.

To investigate mechanisms regulating microglia death after demyelination, we first used ex vivo mouse organotypic cerebellar explants which mimic in vivo tissue microenvironments and remyelination (Supplementary Fig. 4a). Microglia (CD68+, PU.1+, IBA-1+) in LPC-demyelinated explants were decreased by 1 day post-injection, intermediate to peaks of proinflammatory microglia (iNOS+ CD68+; 0.5 dpl) and pro-regenerative microglia (Arg-1+ CD68+; 7 dpl) (Supplementary Fig. 4). Vehicle (PBS)-treated explants showed no demyelination or iNOS+ CD68+ cells, with myelin basic protein (MBP) immunoreactivity comparable to fully myelinated untreated slices (Supplementary Fig. 4g-i). Live incorporation of a marker of compromised membrane integrity (propidium iodide) confirmed microglia death before cell loss (18–24 h post-LPC [hpl]) and propidium iodide was present in most PU.1+ microglia nuclei by 24 hpl (Supplementary Fig. 4j–m). LPC did not have toxic effects on microglia as no cell loss occurred when primary microglia were treated overnight (Supplementary Fig. 5a). Live imaging of explants from microglia reporter mice (G3pr-eGFP) showed microglia rounding up and rupturing post-LPC, which was not observed in control (Supplementary Videos 1 and 2). Microglia were negative for apoptotic markers (cleaved caspase-3, TdT-mediated dUTP nick end labeling), and constitutively positive for pyroptosis marker cleaved caspase-1 even without demyelination (Supplementary Fig. 5b,c). We then investigated necroptosis, a programmed necrosis whereby a necroptosome complex composed of RipK1, RIPK3 and MLKL compromises membrane integrity. Necroptosis markers were expressed in IBA-1+ or CD68+ cells after demyelination before death: at 3 day in vivo (Fig. 2a–c and Supplementary Fig. 5d; IBA-1 and MLKL co-localization: 34.2% at 3 day versus 7.0% at 7 day) and 12 hpl in explants (Fig. 2d,e). CD68+ cells in sham-injected mice were RIPK3-negative (Fig. 2a). Several genes regulating necroptosis were enriched in the ‘cell death & survival’ Ingenuity Pathway Analysis (IPA) pathways (for example, Tnfsf10, Bcl3, Tnfrsf11b) (Supplementary Table 3). Lineage tracing of infiltrating monocytes via Ccr2-driven RFP expression confirmed that the majority of RIPK3+ cells in lesions in vivo were RFP- rather than monocyte-derived (RFP+), and CD68 strongly co-localized with microglial marker Tmem119 (Supplementary Fig. 6). We confirmed that microglia necroptosis is a common feature of remyelination by analyzing two additional in vivo demyelination models. In the cuprizone toxin-diet model, RIPK3+ and MLKL+ microglia were significantly increased at remyelination onset and decreased when complete (Supplementary Fig. 7a–d). Mining of published microglia transcriptomes from a model of chronic myelin injury to the spinal cord (MOG-induced autoimmune encephalomyelitis) indicated expression of Ripk3 and Mlkl at late stages of disease when remyelination occurs (Supplementary Fig. 7e). Therefore, microglia necroptosis is associated with remyelination, regardless of CNS region or mode of injury.

To determine the role of microglia necroptosis in remyelination, we used necrostatin-1, a small molecule which prevents necroptosome activity. At 1 day, necrostatin-1 treatment of demyelinated explants prevented loss of CD68+ microglia (Fig. 2f) and maintained iNOS+ CD68+ microglia (Fig. 2g–h), even at 7 day when the transition to the pro-regenerative phenotype would normally have taken place (Fig. 2g,h). We observed a decrease in iNOS+ CD68+ cell numbers by 14 dpl (Fig. 2g), indicating either delayed change in activation or apoptosis due to prolonged necroptosis inhibition. Necrostatin-1 significantly hindered remyelination at 7 and 14 dpl compared with vehicle control (Fig. 2h,l). This did not result from directly
inhibiting oligodendrocyte or neuronal necroptosis\textsuperscript{11,12}, as only \(\sim3\%\) of oligodendrocyte lineage cells (Olig2\textsuperscript{+}) or neurons (NeuN\textsuperscript{+}) were RIPK3\textsuperscript{+} post-LPC in explants (Supplementary Fig. 8a–d). Necrostatin-1 treatment of undemyelinated explants had no consequence on MBP immunoreactivity or iNOS expression in CD68\textsuperscript{+} microglia (Supplementary Fig. 8e,f). To determine whether microglia necroptosis is required for remyelination in vivo, we aimed to target necrostatin to macrophages by encapsulation in lipidd nanocapsules (LNCs), predicted to be preferentially taken up by phagocytes as shown with other lipid-rich nanoparticle formulations (Fig. 2k). Uptake specificity in lesions was verified by injection of 1,1\textsuperscript{′}-dioctadecyl-3,3,3\textsuperscript{′},3\textsuperscript{′}-tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt (DiD)-labeled LNCs, with \(>90\%\) of DiD clusters co-localized with IBA-1\textsuperscript{+} cells (Fig. 2l,n), significantly...
more than those co-localized with other phagocytic cells (3.9% in astrocytes (GFAP+; Fig. 2l,n) and 1.3% in oligodendrocyte lineage cells (CC1+, Olig1+; Fig. 2n and Supplementary Fig. 8g,h)). Three-dimensional (3D) reconstruction confirmed internalization of DiD LNCs within IBA-1+ cells (Fig. 2m and Supplementary Fig. 8h). At 3 dpl, necrotostatin-loaded LNCs inhibited microglia necroptosis as indicated by decreased MLKL in MLK1-1+ cells compared with vehicle (dimethylsulfoxide)-LNCs (Fig. 2o), reducing co-localization of MLK1 and IBA-1 from 45.11% to 20.93%. Necrotostatin-LNCs did not affect percentage of RIP3+ CD68+ cells (Supplementary Fig. 8i), as expected given that necrotostatin does not prevent RIPK3 expression but acts downstream to inhibit MLKL recruitment/activation. Necrotostatin-LNCs caused a relative increase in Tmem119+ cells compared with vehicle-LNCs, associated with increased percentage of CD68+ cells expressing iNOS and decreased percentage of those expressing Arg-1 (Fig. 2p). Increased microglia numbers were not due to proliferation, as Ki67+ PU.1+ cell number was significantly downregulated at 3 dpl in Necrotostatin-LNC-treated lesions relative to vehicle-LNC control, then negligible by 10 dpl (Supplementary Fig. 8j). Remyelination was impaired at 10 dpl following necrotostatin-LNC treatment, as indicated by reduced expression of early remyelination marker myelin-associated glycoprotein (MAG) (Fig. 2q,r). This was not due to accumulation of myelin debris (identified using MBP which is not yet expressed at this early stage of remyelination) which was equally cleared in both conditions (Supplementary Fig. 8k), consistent with RNA sequencing data suggesting phagocytic capacity of proinflammatory microglia (Fig. 1e and Supplementary Fig. 2). Altogether, these data demonstrate the requirement for microglia necroptosis for remyelination to occur, although we acknowledge the possibility that inhibiting necroptosis of a small percentage of other cell types may have also affected remyelination.

We next determined how microglia repopulate to the pro-regenerative phenotype following demyelination. We assessed Nestin expression, which identifies repopulating microglia following experimental depletion11-13. In vivo lesions showed increased co-localization of IBA-1 with Nestin from 3 dpl to 7 dpl, which was reduced by 10 dpl when the transition in microglia activation has taken place (Fig. 3a-c). Little to no Nestin was co-localized with IBA-1 in sham control, indicating expression by microglia largely during remyelination (Fig. 3a). Microglia repopulation following experimental depletion has been proposed to occur via: (1) de novo differentiation of CNS-resident Nestin+ cells14,15, or (2) proliferation of residual microglia which did not die11,16. However, these studies examined microglia repopulation in healthy gray matter, where the microenvironment may differ from injured white matter. To investigate microglia repopulation following demyelination, we induced focal lesions in mice in which Nestin promoter-driven tdTomato (tdT) expression is inducible (Nes-CreERT2:RCL-tdT), allowing label-ling of Nestin+ cells before demyelination. Of all tdT+ cells, the proportion that were CD11b+ CD45+ (gating, Supplementary Fig. 9a) increased from 3 dpl to 7 and 10 dpl (Fig. 3d,e and Supplementary Fig. 9b). Although the proportion of all CD11b+ CD45+ cells that were tdT+ increased at 7 dpl versus 3 dpl and sham control, these only represented <5% of total microglia (Fig. 3f,g), suggesting that repopulation in vivo is mediated primarily by residual microglia.

Microglia repopulation also occurred in the explant model (Supplementary Fig. 4c). Recombination in Nestin+ cells before LPC led to detection of some tdT+ IBA-1+ cells only during early repopulation (1-2 dpl), which were undetectable by 7 dpl (Supplementary

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**Fig. 3 | Microglia repopulation is associated with remyelination in mouse and human white matter and depends on type-1 IFN signaling.** a, In vivo lesions of the corpus callosum (dotted line) at 3, 7 and 10 dpl, along with sham PBS control (7 d post-surgery), stained for IBA-1 (green) and Nestin (red). Scale bar, 50 μm. Inset at 7 dpl demonstrates zoom-in co-localization of stains. The experiment was performed with three mice per time point or condition. b, Z-plane orthogonal view of 7 dpl in vivo lesioned corpus callosum stained for Nestin (red) and IBA-1 (green) demonstrates co-expression. The experiment was performed with three mice at 7 dpl. c, Mean percentage co-localization between IBA-1 and Nestin in vivo staining normalized to total IBA-1 signal ± s.e.m. *=P=0.0219 7 dpl versus 10 dpl (Kruskal–Wallis test, Dunn’s Multiple Comparison Post-test). N=3 mice per time point. d, Example plot of flow cytometric analysis of proportion of tdT+ cells within in vivo lesions that are CD11b+ CD45+. At 3, 7 and 10 dpl the experiment was performed with three mice per time point or condition. e, Average percentage of tdT+ cells ± s.e.m. that are CD11b+ CD45+ at 3, 7 and 10 dpl in vivo. *=P=0.0496 (1-way ANOVA with Tukey’s post-hoc test, t=5.376, d.f.=6). N=3 mice per time point. f, Average percentage of CD11b+ CD45+ cells ± s.e.m. that are tdT+ at 3, 7 and 10 dpl in vivo. N=3 mice per time point. g, Example plot of flow cytometric analysis of proportion of CD11b+ CD45+ cells that are tdT+ within in vivo lesions at 3, 7 and 10 dpl compared with sham PBS control. The experiment was performed with three mice per time point or condition. h, Mean density of MLKL+ microglia (CD68+) per mm2 in control, active, chronic inactive and remyelinated multiple sclerosis (MS) lesions. Individual data points represent separate lesions (see Supplementary Table 3). *=P=0.0226 active lesions versus control (two-tailed Mann–Whitney test). N=3 control tissues, 5 active lesions, 6 chronic inactive lesions and 8 remyelinated lesions. i, Mean density of RIP3+ microglia (CD68+) per mm2 in control, active, chronic inactive and remyelinated MS lesions. Individual data points represent separate lesions (see Supplementary Table 1). *=P=0.0357 active lesions versus control (two-tailed Mann–Whitney test). N=3 control tissues, 5 active lesions, 6 chronic inactive lesions and 5 remyelinated lesions. j, Mean density of Nestin+ PU.1+ cells per mm2 in control, active, chronic inactive and remyelinated lesions. Individual data points represent separate lesions (see Supplementary Table 3). *=P=0.0286 active lesions versus control (two-tailed Mann–Whitney test). N=3 control tissues, 4 active lesions, 4 chronic inactive lesions and 8 remyelinated lesions. k, Control brain tissue and active MS lesion immunostained for CD68 (blue) and RIP3 (red), counterstained with Hoechst (turquoise). Arrows indicate CD68+ RIP3+ cells. Scale bar, 20 μm. The experiment was performed on five active lesions and three control tissues. l, Control brain tissue and active MS lesion immunostained for PU.1 (blue) and Nestin (red), counterstained with Hoechst (turquoise). Arrows indicate PU.1+ Nestin+ cells. Scale bar, 20 μm. The experiment was performed on four active lesions and three control tissues. m, In vivo remyelinating lesions at 3, 7 and 10 dpl immunostained for CD68 (red) and IFNAR2 (green), counterstained with Hoechst (blue). Inset: rabbit primary isotype control. Scale bar, 10 μm. The experiment was performed on three mice per time point. n, In vivo remyelinating lesions at 3, 7 and 10 dpl immunostained for PU.1 (red) and P-Y701 (green), counterstained with Hoechst (blue). Scale bar, 25 μm. The experiment was performed on three mice per time point. o, Representative image of focal in vivo lesion at 7 dpl immunostained for PU.1 (red) and P-Y701 (green), counterstained with Hoechst (blue). White square corresponds to panel n. Scale bar, 25 μm. The experiment was performed on three mice per time point. p, Mean number of microglia (PU.1+) per field ± s.e.m. in explants at 1 and 7 dpl, treated with anti-IFNAR2 neutralizing antibody or IgG control. *=P=0.0098 Goat IgG 1 dpl versus 7 dpl (t=4.346, d.f.=8); *=P=0.0103 Goat IgG 7 dpl versus anti-IFNAR2 IgG 7 dpl (t=4.309, d.f.=8). NS, not significant. One-way ANOVA and Sidak’s multiple comparison test, N=3 mice per time point. q, Explants at 7 dpl treated with anti-IFNAR2 IgG or control IgG, immunostained for PU.1 (red) and counterstained with Hoechst (blue). Scale bar, 10 μm. The experiment was performed with three mice per condition. r, Mean remyelination index of explants at 7 dpl ± s.e.m. treated with anti-IFNAR2 IgG or control goat IgG. *=P=0.0252, two-tailed Student’s t test (t=3.485, d.f.=4). N=3 mice per condition. s, Explants at 7 dpl treated with anti-IFNAR2 IgG or control goat IgG, immunostained for MBP (red) and neurofilament-H (NF; green), showing healthy early remyelination in control and debris in anti-IFNAR2 IgG treatment. Scale bar, 10 μm. The experiment was performed with three mice per condition.
Fig. 9c–f); tdT⁺ cells expressed neural stem cell markers Musashi-1 and Sox-2, but not GFAP (Supplementary Fig. 9g). We assessed the contribution of residual microglia to repopulation in explants derived from Cx3cr1-CreER;RCL-tdT mice (Supplementary Fig. 9h), in which tdT labeled IBA-1⁺ microglia (Supplementary Fig. 9i) but not the oligodendrocyte lineage, previously suggested to express CX3CR1 (Supplementary Fig. 9j). The majority of repopulated IBA-1⁺ cells were tdT⁺ and therefore derived from residual microglia (Supplementary Fig. 9k–m), with 30% being Nestin⁺ at 2dpl (Supplementary Fig. 9n,o). Therefore, lineage tracing supports that microglia repopulation during remyelination occurs primarily from residual microglia.
To investigate necroptosis and remyelination in human white matter disease, we examined multiple sclerosis lesion subtypes: (1) active lesions, which have high densities of macrophages, positively correlated with remyelination and oligodendrocyte precursor abundance; (2) chronic inactive lesions, which have low potential for remyelination; and (3) fully remyelinated lesions (Supplementary Table 4). Although densities of CD68+ cells undergoing necroptosis (RIPK3+ and MLKL+; Fig. 3hi,k) or PU.1+ cells undergoing repopulation (Nestin+; Fig. 3j,l) were present in all multiple sclerosis lesion types, these were only significantly increased in active lesions compared with control. This may suggest abundance of cues for pro-remyelination microglial responses in an inflammatory environment.

We next investigated molecular pathways controlling microglia during remyelination. IPA analysis indicated regulation of type-1 interferon (IFN) signaling, with ‘Interferon signaling’ and ‘Role of JAK1, JAK2 and TYK2 signaling in Interferon signaling’ identified as top canonical pathways, and top predicted upstream regulators included IFNα/β, IFNAR, STAT1, IRF7 and IRF3 (P = 0.00013, 0.000089, 0.000045, 0.0071 and 0.000086, respectively). Microglia expressed genes encoding the IFNα/β receptor (Ifnar1, Ifnar2) and IFN-association genes linked with microglia during remyelination (Supplementary Fig. 10a,b), and CD68+ cells expressed IFNα/β receptor subunit 2 (IFNAR2) protein in vivo (Fig. 3m). IFN signaling, assessed by nuclear phospho-STAT1 (P-Y701), was only active at 7 dpl in vivo and was selective to 58 ± 7% of PU.1+ nuclei (Fig. 3mo), consistent with the largest microglia subpopulation in this model having an IFN signature. We assessed the role of type-1 IFN signaling using explants where microglia death and repopulation are temporally separated and can be investigated in isolation. Using a neutralizing antibody against IFNAR2 did not significantly affect PU.1+ microglia numbers at 1 dpl compared with IgG isotype control (Fig. 3p), therefore did not prevent microglia death. A significant increase in PU.1+ cells was observed from 1 dpl to 7 dpl in controls but not following IFNAR2 blockade (Fig. 3p), suggesting impaired microglia repopulation. PU.1+ cells were significantly reduced in anti-IFNAR2 IgG conditions relative to control at 7 dpl (Fig. 3pq), associated with decreased P-Y701 PU.1+ microglia (Supplementary Fig. 10c). Consequently, blocking IFNAR2 impaired early remyelination at 7 dpl relative to control (Fig. 3rs). Altogether, these data support a regenerative role for type-1 IFN signaling in regulating the repopulation of white matter microglia during efficient remyelination.

In summary, our data reveal that remyelination is driven by proinflammatory microglia necroptosis and repopulation to a regenerative state. Whereas necroptosis of other cell types is associated with demyelination and neurodegeneration, we show a regenerative role for necroptosis in shutting down proinflammatory microglial activation to support remyelination. Although previous studies identified the capacity of microglia to repopulate following experimental depletion in healthy aged, irradiated or neurodegenerating brain, we show that this feature can also serve to reconstitute microglia after naturally occurring death following white matter injury, while regulating microglia activation. We reveal that microglia repopulation during white matter remyelination is positively regulated by type-1 IFN signaling. This contrasts with its deleterious role in repopulated microglia selectively in gray matter following experimental depletion, highlighting CNS region-specific consequences of IFN signaling in microglia. We propose that targeting proinflammatory microglia death may represent a strategy to dampen chronic CNS white matter inflammation, and support a regenerative response to reinstate myelin integrity.

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References
1. Franklin, R. J. M. & French-Constant, C. Nat. Rev. Neurosci. 18, 753–769 (2017).
2. Frakes, A. et al. Nature 81, 1009–1023 (2014).
3. Miron, V. et al. Nat. Neurosci. 16, 1211–1218 (2013).
4. Kigerl, K. et al. J. Neurosci. 29, 13435–13444 (2009).
5. Wlodarczyk, A. et al. EMBO J. 36, 3292–3308 (2017).
6. Hagemeyer, N. et al. Acta Neuropathol. 134, 441–458 (2017).
7. Keren-Shaul, H. et al. Cell 169, 1276–1290 (2017).
8. Krasemann, S. et al. Immunity 47, 566–581 (2017).
9. Lewis, N., Hill, J., Juchem, K., Stefanopoulos, D. & Modis, L. J. Neuroinflamm. 277, 26–38 (2014).
10. Mei, F. et al. elife. 5, e18246 (2016).
11. Olengeim, D. et al. Cell Rep. 10, 1836–1849 (2015).
12. Caccamo, A. et al. Nat. Neurosci. 20, 1236–1246 (2017).
13. Bruttiger, J. et al. Immunity 43, 92–106 (2015).
14. Elmore, M. et al. Nature 82, 380–397 (2014).
15. Huang, Y. et al. Nat. Neurosci. 11, 530–540 (2018).
16. Hammond, T. R. et al. Immunity 55, 253–271 (2018).
17. Elmore, M. R. P. et al. Aging Cell 17, e12832 (2018).
18. Krukowski, K. et al. Sci. Rep. 8, 7857 (2018).
19. Rice, R. A. et al. Glia 65, 931–944 (2017).
20. Rubino, S. J. et al. Nat. Commun. 9, 4578 (2018).

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Author contributions
A.F.L. co-designed the study, carried out the experiments, analyzed and interpreted the data, and wrote the manuscript. C.L.D. carried out lesioning experiments, optimized lesion isolation protocols, assisted with flow cytometry, and performed experiments and analysis for RNA sequencing. R.K.H. assisted in lesioning experiments and optimized human tissue sampling and analysis protocols. Y.L., D.C. and A.D.R. developed and tested LNCs for microglia targeting. G.I. assisted with genotyping. A.D. and D.S. developed remyelination index quantification protocols. E.B. and A.W. provided cortical corpus callosum lesion tissue. J.C.R. provided guidance for experimental design. A.W. and J.W.P. co-supervised the project and assisted with experimental design and interpretation and manuscript editing. T.K. provided cuprizone tissue and edited the manuscript. A.W. provided human tissue neuropathological mapping. J.P. assisted in experimental design, data interpretation and manuscript editing. V.E.M. co-designed the study, supervised the project and guided experimental design, data interpretation and manuscript preparation.

Competing interests
A.F.L.’s salary and experiments for this study were co-funded by GlaxoSmithKline. J.C.R. was a full-time employee at GlaxoSmithKline at the time of the study.

Additional information
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Correspondence and requests for materials should be addressed to V.E.M.

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Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41593-019-0418-z.
Focal demyelinating lesion induction. Ten-week-old male C57BL/6 mice were anaesthetized with isoflurane before being stereotactically injected with 2 µl 1% lysophosphatidyl choline (LPC; v/v) into the corpus callosum. Control mice underwent the same procedure with a sham injection. Mice were allowed to recover before killing by perfusion-fixation at 3.7 and 10 dpl with 4% PFA for immunofluorescence, or 3.8% sodium citrate in PBS for flow cytometric analysis. The former were post-fixed overnight with 4% PFA, cryoprotected in sucrose and cryosectioned at 12-µm thickness. A minimum of three animals were analyzed per time point.

LNC formulation. Kolliphor HS15 (0.169 g), Lipoid (0.15 g; Lipoid GmbH), NaCl (0.0178 g), Labrafil (0.2056 g; Gattefosse SA) and water (0.592 g) were mixed under gentle magnetic stirring at 50 °C for 5 min. The solution was progressively heated (90 °C) and cooled (60 °C) three times. During the last cooling, cold water (20 °C) was added at 74 °C under high-speed stirring. Necrostatin-1-loaded LNCs were prepared by adding 25 µl necrostatin-1 stock solution (30 mg/ml in dimethylsulfoxide) during the last cooling of LNC preparation. Control LNCs were prepared following the same protocol using dimethylsulfoxide. Fluorescent LNCs (DiD; Thermofisher) were prepared with the same protocol using 220 µl DiD solution (1 mg/ml in absolute ethanol). The nanoparticles were filtered on a sterile 0.2-µm filter and stored at 4 °C until further use. The concentration of necrostatin-loaded LNC solution was 1 µmol necrostatin-1 and 126 mg/ml nanoparticles. Size, zeta (ζ)-potential and PDI of nanoparticles were measured using a Malvern Zetasizer Nano ZS (Malvern Instruments) (N = 3, n = 3). For the measurement of size and PDI, samples were diluted 1/100 (v/v) in water. For the measurement of ζ-potential, samples of 0.1% sodium chloride (NaCl) 10 mM were used. The encapsulation efficiency of necrostatin-1 was calculated using the following formula:

\[
\text{Encapsulation Efficiency} = \frac{\text{Necrostatin-1 total (g) - non-encapsulated Necrostatin-1 (g)}}{\text{Necrostatin-1 introduced in the formulation (g)}} \times 100
\]

Necrostatin-1 was extracted from necrostatin-1 LNC by dissolving in methanol at a ratio of 1:20 (v/v) (‘Necrostatin-1 total’). Necrostatin-1 was quantified by reversed-phase HPLC (Waters) with a Macherey-Nagel 125/4 Nucleosil 100-5 C18 column. A gradient mobile phase was composed of 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B). The gradient was as follows: for the first 2 min, the gradient was set at 80% A and 20% B. Then, A decreased to 60% for the following 3 min, and for the last 2 min A decreased to 20%. The flow was set for both solutions at 1 ml min⁻¹ with a volume injection of 20 µl and the detection wavelength set at 269 nm. To determine the percentage of non-encapsulated necrostatin-1, necrostatin-1 LNCs were centrifuged in a VIVAPSIN 500 with a membrane that possessed a 30-KD MWCO at 13,000 g, and 10 min, and the filtered solution was collected and quantified by reversed-phase HPLC. The properties of the LNCs were as follows: (1) necrostatin-1 LNCs: 54.2±0.8/0.5 PDI/–2.0 µv ζ-potential/263.8 µg/ml/100% encapsulation efficiency, (2) dimethylsulfoxide LNCs: 56.4±0.0/0.6 PDI/–1.7 µv ζ-potential/80% encapsulation efficiency, and (3) DiD LNCs: 64.1±0.0/0.0 PDI/–1.8 µv ζ-potential/14.7% (v/v) concentration. LNCs were stereotactically injected into the corpus callosum of adult mice at the time of lesioning, and mice were killed at 3 or 10 dpl. The 3D surface view in Slidebook 6 was used for 3D rendering to visualize DiD-LNC localization.

Microglia isolation and RNA extraction/sequencing. Lesioned corpus callosum of 8-12-week-old male C57Bl6 mice was homogenized using a 2-ml Dounce and filtrated (250-µm filter; Pierce). Following a spin at 600g for 5 min (with brake), cells were resuspended in 100% fetal bovine serum (FBS) and 33% Percoll (1:1), overlaid with 1 ml 10% FBS and spun for 15 min at 800 g at 4 °C without brake. The cell pellet was washed in FACS buffer and spun for 1 min at 600g at 4 °C, and incubated in anti-mouse CD16/32 Fc-block (Clone 93, BioLegend, 1:200) on ice for 10 min. Fluorescently conjugated antibodies CD11b-PerC (Clone M1/70, Invivogen, 1:100), CD45 BV605 (Clone 3D11, BioLegend, 1:200), Ly6G-PerCP Cy5.5 (Clone 1A8, BioLegend, 1:200) and CD3-APC (Clone 17A2, BioLegend, 1:200) were applied on ice for 30 min. Following centrifugation and filtration (30 µm), cells were sorted by flow cytometry into FBS-coated Eppendorf tubes on ice (BD FACSAria Fusion, 100-µm nozzle). Use of differential CD45 expression (on the logarithmic scale) to distinguish between microglia (lo) and infiltrating monocyte-derived macrophages (hi) in white matter injury has been previously validated using image analysis software and bone marrow chimeras. Cells were spun at 800g for 5 min, resuspended in RLT Plus buffer with β-mercaptoethanol and centrifuged at 10,000 r.p.m. for 2 min in QIAGenschafts tubes (Qiagen). RNA was extracted using the AllPrep DNA/RNA/miRNA kit (Qiagen) as per the manufacturer’s instructions. Complementary DNA production/library preparation was performed using the NuGEN Ovation RNAseq System v2 kit (NuGEN) by BG1 (Hong Kong). End Repair Mix was added to the amplified cDNA and incubated at 20°C for 30 min. AxyPrep Mag PCR Clean-up
Kit (Oxygen) was used to purify the end-repaired DNA, which was then combined with A-tailing Mix (Enzymatic) and incubated at 37°C for 30 min. Adapters (Invitrogen) were ligated to the adenylated 3’ ends DNA, and incubated at 16°C for 16 h. Insert size was used to select the adapter-ligated DNA fragments. Several rounds of PCR amplification with PCR Primer Cocktail (Invitrogen) and PCR Master Mix (New England Biolabs) were performed to enrich the adapter-ligated DNA fragments to produce the final library, purified using AsyPrep Mag PCR Cleanup kit. The final library average molecule length was determined using the Bioanalyzer 2100 using the DNA 1000 kit (Agilent) and was quantified by real-time quantitative PCR (qPCR) (TaqMan probe). cDNA was used to amplify the libraries to generate the cluster on the Flow Cell (HiSeq 4000, Illumina). An average of 40 million clean paired-end reads (read lengths approximately 100 base pairs) were achieved per sample, with >90% uniquely mapped reads. Data were processed to remove adapters and low-quality reads from raw reads.

Bioinformatics. Raw data analysis was carried out by Fios Genomics (Edinburgh, United Kingdom). RNAseq data were preprocessed and aligned to the mouse genome (GRCh38) using STAR aligner, and the numbers of mapped read-pairs per gene were quantified based on the GENCODE v.12 annotation. A total of six samples (three per time point) were quality control analyzed using the 'array design' feature in R Bioconductor. Data were normalized using trimmed mean from 30 million reads and transformed using Voom to log_{2} counts, per million with associated precision weights. Comparisons were undertaken using linear modeling using Limma package in Bioconductor to determine differentially expressed genes (P < 0.05); empirical Bayesian analysis for log2 fold change was applied. Differentially expressed genes were used for functional enrichment analysis by investigation of KEGG pathways (P < 0.05), Gene Ontology terms (P < 0.05), Venny 2.1 and IPA (P ≤ 0.05).

Real-time qPCR. Real-time qPCR was run using BioRad Custom PrimerPCR plates, per the manufacturer's instructions. Briefly, cDNA was synthesized using 5x iScript Advanced Reaction Mix and iScript Advanced Reverse Transcriptase plates, as per the manufacturer's instructions. Briefly, cDNA was synthesized using 5x iScript Advanced Reaction Mix and iScript Advanced Reverse Transcriptase (BioRad) at 46°C for 20 min then 95°C for 1 min. cDNA samples were mixed with 2x Taq Universal SYBR-green Supermix and iScript Reverse Transcriptase. The real-time qPCR was performed at 90°C for 10 min, 95°C for 1 min, 95°C for 15 sec (40 cycles) and 60°C for 60 sec (40 cycles). Data were analyzed using CFX manager and presented as 2^−ΔΔCt.

Immunofluorescent staining in vivo focal lesions. Sections of frozen tissue were air dried for 15 min before being permeabilized and blocked for 1 h (5% horse serum and 0.5% Triton X-100 in PBS), then incubated with primary antibodies overnight at 4°C in a humid chamber. Primary antibodies used include rabbit anti-TaB1 (Abcam, ab20064, 1:100), rabbit anti-CD68 (Abcam, ab53444, 1:100), rabbit anti-β-I-1 (Wako Chemicals, 019-7971-1, 1:500), rabbit anti-RP3K (Novus Biologicals, NB1P-77299, 1:100), rabbit anti-MLKL (Merck-Millipore, MABC604, 1:100), mouse anti-iNOS (BD Biosciences, 610329, 1:1000), goat anti-Arginase-1 (Santa Cruz Biotechnology, sc18355, 1:50), mouse anti-Nestin (Abcam, ab6142, 1:100), mouse anti-S100B (Abcam, ab1942, 1:100), rabbit anti-NF200 (BD Biosciences, MABS12434, 1:100), rabbit anti-CD68 (Abcam, ab53444, 1:100), rabbit anti-iNOS (BD Biosciences, MABC4095, 1:250), rabbit anti-iNOS (EMD Millipore, AB4892, 1:1000), mouse anti-OLIG1 (EMD Millipore, MAB5540, 1:100), mouse anti-PI-3 (Abcam, ab29045, 1:100) and rabbit anti-IFNAR2 (Abcam, ab56670, 1:100). Subsequent to washes in PBS, fluoroscently conjugated secondary antibodies were applied for 2h at 20–25°C in a humid chamber (rabbit anti-IgG IgG (Abcam, ab11034, 1:21206, 1:10042, 1:11131), goat anti-Cy+ (Abcam, ab11006, 1:1000, all at a concentration of 1:1000 (all from Invitrogen)). Slides were counterstained with Hoechst and coverslipped with Fluoromount-G (Southern Biotech). Images were acquired with an Olympus spinning disk confocal microscope with x20 or x60 objectives with a x60 objective with Slidebook software. Image intensity within lesions was quantified using Adobe Photoshop CS4 with respective background intensity outside the lesion subtracted.

Flow cytometry. Focal demyelinated lesions of the corpus callosum of 8–12-week-old C57Bliev were dissected out and homogenized with a 2 ml Dounce. A Percoll (Sigma-Aldrich) gradient was used to isolate cells from myelin debris. Sections of frozen tissue were air dried for 15 min before being permeabilized and blocked for 1 h (5% horse serum and 0.3% Triton X-100 in PBS), then incubated with primary antibodies overnight at 4°C in a humid chamber. Microglia/macrophages were detected with rat anti-CD68 (Abcam, ab53444, 1:100) and cell death was assessed using rabbit anti-MP3K (Novus Biologicals, NB1P-77299, 1:100) and rat anti-MLKL (Merck-Millipore, MABC604, 1:100). Following washes in PBS, fluoroscently conjugated secondary antibodies were applied (Invitrogen, 1:1000). Slides were counterstained with Hoechst and coverslipped with Fluoromount-G (Southern Biotech). Images were acquired with an Olympus spinning disk confocal microscope using a x60 objective with Slidebook software. Three animals were analyzed at each time point.

Human tissue. Post-mortem tissue from patients with multiple sclerosis and controls (cause of death was unrelated to multiple sclerosis) were obtained from the Institute of Neurology, Queen Square, London. The tissue was fixed in 10% formalin for 2 weeks, processed into paraffin wax and sliced into 6 μm thick sections, stained with H&E, and formalin-fixed paraffin-embedded brain sections were used for all experiments. Partial brain sections were deparaffinized using Histoclear (Fisher) and rehydrated using a gradient of ethanol concentrations, each for 5 min in the following order: 2×100%, 1×95%, 1×70% and 1×50%, before being washed in Tri-buffered saline (TBS) for 3×5 min washes. Slides were then placed in Vector Unmasking Solution under high heat and pressure for 20 min before a final TBS wash. Slides were permeabilized and blocked for 1 h (5% horse serum and 0.3% Triton X-100 in PBS) before incubation with primary antibodies overnight at 4°C in a humid chamber. Microglia/macrophages were detected with rat anti-CD68 (Abcam, ab53444, 1:100) and cell death was assessed using rabbit anti-MLKL (Novus Biologicals, NB1P-77299, 1:100) and rat anti-MLKL (Merck-Millipore, MABC604, 1:100). Following washes in PBS, fluoroscently conjugated secondary antibodies were applied (Invitrogen, 1:1000). Slides were counterstained with Hoechst and coverslipped with Fluoromount-G (Southern Biotech). Images were acquired with an Olympus spinning disk confocal microscope using a x60 objective with Slidebook software. Three animals were analyzed at each time point.

Statistics. Details on experimental design and reagents can be found in the Life Sciences Reporting Summary. For animal experiments, power was calculated by two-sided 95% confidence interval using the normal approximation method using OpenEpi software, and gave 88–100% power for all experiments. Animals were randomly selected for experimental groups, and littersmates were compared for all treatment groups. All manual cell counts for explant cultures, in vivo lesioned tissue and multiple sclerosis tissue were performed in a blinded manner. Data are represented as mean ± s.e.m. from a minimum of three mice (in vivo experiments) or replicates. Data distribution was assessed by one-way analysis of variance (ANOVA) test or t-test (for select comparisons between groups) for comparison post-test or 1-way analysis of variance (ANOVA) with Sidak’s post-test or Mann–Whitney test for ≤2 comparisons, one-sample t-test for analysis of normalized data or comparison to sham, and either Kruskal–Wallis test with Dunn’s Multiple Comparison post-test or 1-way analysis of variance (ANOVA) with Sidak’s post-test (for select comparisons between groups) for ≥3 comparisons. P values of ≤0.05 were considered statistically significant at a confidence interval of 95%. Data handling and statistical processing were performed using Microsoft Excel and GraphPad Prism 7 and 8 Software. Diagrams were created with BioRender software.

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 on request. The RNA sequencing data discussed in this
publication have been deposited in NCBI's Gene Expression Omnibus and are accessible through the series GSE118456; accession numbers are GSM3330371 (3-dpl microglia sample 1), GSM3330372 (3-dpl microglia sample 2), GSM3330373 (3-dpl microglia sample 3), GSM3330374 (10-dpl microglia sample 4), GSM3330375 (10-dpl microglia sample 5) and GSM3330376 (10-dpl microglia sample 6). Raw data (FPKM) are shown in Fig. 1d,f and Supplementary Figs. 1b,d,e,g,h,k,m and 10a,b. Processed data are shown in Fig. 1, Supplementary Figs. 1 and 2 and Supplementary Tables 1–3.

References
21. Oliveros, J. C. Venny. An interactive tool for comparing lists with Venn’s diagrams. http://bioinfogp.cnb.csic.es/tools/venny/index.html (2015).
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.

Statistics

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

| n/a | Confirmed |
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| ✓   | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
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| ✓   | 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. |
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| ✓   | 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

Slidebook 6 software was used for 3D rendering, confocal imaging and live-imaging. FACS and flow cytometry data was acquired using FlowJo version 9 and 10 software. Human tissue was acquired using the Zeiss Zen2 software.

Data analysis

Remyelination index was quantified using Volocity 6.3 software. Bioinformatics on RNA sequencing data was carried out using STAR aligner, Bioconductor (limma package), QIAGEN Ingenuity Pathway Analysis. Real time PCR data was analysed using CFX manager. MAG intensity was quantified using Adobe Photoshop CS4. Data handling was performed using Microsoft Excel and statistical analysis was performed using GraphPad prism 7 and 8. Power calculations were performed using OpenEpi website (www.openepi.com).

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. 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

RNA sequencing data is available on GEO as the series GSE118450, accession numbers are GSM3330371, GSM3330372, GSM3330373, GSM3330374, GSM3330375, GSM3330376. All Log2 fold changes are available as Supplementary Table 1. Raw data (FPKM) is shown in figures 1D,F; supplementary figures 1B, 1D, 1E, 1G, 1H, 1K, 1M, 10A, 10B.
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.

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Life sciences study design

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

| Sample size | For ex vivo studies, each repeat (N number) is represented as a mean of 3 brain slices from the same mouse, and 3-5 mice were used per time point throughout the manuscript. For in vivo studies, 3 mice were used per time point. A minimum of three biological repeats were used initially, then power calculations were performed to subsequently determine appropriate n numbers for significance. The range of power is (88-100%) is listed in the Methods under statistics. |
| Data exclusions | For human tissue analysis of Nestin+ PU.1+ cells, staining with blood vessel morphology was excluded from counts to avoid quantifying endothelial cells. This was a pre-established criteria prior to counting so no data was excluded from the manuscript. |
| Replication | All attempts at replication were successful. Data represents 3-5 biological repeats. |
| Randomization | Ex vivo slices and mice for in vivo studies were randomly assigned to experimental time points. |
| Blinding | All manual counts were performed in a blinded manner. Non-objective computational measurements such as co-localization measurements and flow cytometrical analysis did not require blinding. |

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 | Antibodies | Eukaryotic cell lines | Palaeontology | Animals and other organisms | Human research participants | Clinical data |
|---------------------------------|------------|----------------------|---------------|-----------------------------|---------------------------|-------------|
| n/a                             | ☑          | ☑                    |               |                             |                           |             |

| Methods | n/a | Involved in the study |
|---------|-----|-----------------------|
| ☑       | ChIP-seq | |
| ☑       | Flow cytometry | |
| ☑       | MRI-based neuroimaging | |

Antibodies

For human tissue staining, primary antibodies used include goat anti-CD68 (Santa Cruz Biotechnology, sc7082, 1:100), mouse anti-Nestin (Santa Cruz Biotechnology, sc23927, 1:100), goat anti-PU.1 (Santa Cruz Biotechnology, sc949, 1:100), rat anti-MLKL (Merck-Millipore, MABC604.1, 1:100) and rat anti-RIP3 (Novus Biologicals, NB1-79299, 1:100). For explants, primary antibodies used include rat anti-CD68 (Abcam, ab53444, 1:100), rabbit anti-IBA-1 (Wako Chemicals, 019-19741, 1:50), goat anti-PU.1 (Santa Cruz Biotechnology, sc949, 1:100), mouse anti-iNOS (BD Biosciences, 610329, 1:100), goat anti-Arginase-1 (Santa Cruz Biotechnology, sc18355, 1:50), rabbit anti-RIP3 (Novus Biologicals, NB1-79299, 1:100), rat anti-MLKL (Merck-Millipore, MABC604, 1:100), rabbit anti-cleaved Caspase-3 (BD Pharmingen, 559565, 1:100), mouse anti-cleaved Caspase-1 (Santa Cruz Biotechnology, sc22165, 1:100), rat anti-myelin basic protein (MBP; AbD Serotec, MCA409S, 1:250), chicken anti-neurofilament heavy chain (NF-H; EnC BioLink, CP-CF-NF-H, 1:10,000), mouse anti-Nestin (Abcam, ab6142, 1:100), rabbit anti-Olig2 (Merck, AB9610, 1:100), rabbit anti-GFAP (DAKO, 20334, 1:500), mouse anti-NeuN (Merck Millipore, MAB377, 1:100), rabbit anti-Musashi-1 (Abcam, ab52865, 1:100), mouse anti-Sox2 (Abcam, ab17380, 1:100), mouse anti-phospho-STAT1 (P-Y701) (Abcam, ab20045, 1:100), and secondary secondary antibodies anti-goat IgG (A21432, A11055), anti-rabbit IgG (A11034, A12106, A10042, A11011), anti-rat IgG (A21434, A11006, A21247), anti-mouse IgG (A31570, A21235, A31571, A21042), and anti-chicken IgG (A10399, 1:1000, Invitrogen). For FACS isolation, fluorescently-conjugated antibodies include CD11b-PeCy7 (Clone M1/70, Invitrogen, 1:1000), CD45-BV605 (Clone 30D11, Biolegend, 1:2000), Ly6G-PerCP Cy5 (Clone 1A8, BioLegend, 1:200), and CD3-APC (Clone 17A2, BioLegend, 1:200). For in vivo sections, primary antibodies used include rabbit anti-Tmem119 (Abcam, ab209064, 1:100), rat anti-CD68 (Abcam, As53444, 1:100), rabbit anti-IBA-1 (Wako Chemicals, 019-19741, 1:50), rabbit anti-RIP3 (Novus Biologicals, NB1-77299, 1:100), rat anti-MLKL (Merck-Millipore, MABC604, 1:100), mouse anti-iNOS (BD Bioscience, 610329, 1:100), goat anti-Arginase-1 (Santa Cruz Biotechnology, sc18355, 1:50), mouse anti-Nestin (Abcam, ab6142, 1:100), mouse anti-MAG (EMD Millipore, MAB1567, 1:100), rat anti-MBP (AbD Serotec, MCA409S, 1:250), rabbit anti-Ki67 (EMD Millipore, AB9260, 1:100), mouse anti-Olig1 (EMD Millipore, MAB5540, 1:100), mouse anti-phospho-STAT1 (P-Y701; Abcam,
ab29045, 1:100), rabbit anti-IFNAR2 (Abcam, ab56070, 1:100), and secondary antibodies anti-rabbit IgG (A11034, A21206, A10042, A11011) and anti-rat IgG (A21434, A11006, A21247) all at a concentration of 1:1000 (all from Invitrogen). For flow cytometry, fluorochrome-conjugated antibodies used include CD11b-PeCy7 (eBioscience, 25-0112-82, 1:100) and CD45-BV605 (Biolegend, 103139, 1:100). For primary microglia cultures, antibodies included mouse anti-iNOS (BD Biosciences, 610329, 1:100), goat anti-Arginase-1 (Santa Cruz Biotechnology, sc18355, 1:50), and rat anti-CD68 (Abcam, ab53444, 1:100) and fluorescently-conjugated secondary antibodies (Invitrogen, A21432, A21202, A21247, 1:1000).

Validation

All antibodies have been tested for reactivity against the appropriate species against which it was used in this study based on the specification sheets on the providers' websites. Antibodies against CD68, iNOS, Arginase-1, IBA-1, MBP, MAG, neurofilament, GFAP, Olig2, Ki67 were previously validated in Miron et al., 2013, Nature Neuroscience. PU.1 was validated in Askew et al., 2017, Cell Reports. Tmem119 was validated in Bennett et al., 2016, PNAS. FACS and flow antibodies were validated in Davies et al., 2019, Methods in Molecular Biology. All secondary antibodies used were tested in Miron et al., 2013 Nature Neuroscience and Dillenburg et al., 2018, Acta Neuropathologica.

Animals and other organisms

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

Laboratory animals

Species, strain, sex, and age are indicated in the methods section. All in vivo lesioning experiments were carried out on male C57Bl6J mice of 8-12 weeks old. All explants were derived from male and female P0-P3 CD1 mouse pups. Primary microglia were derived from P0-P3 male and female sprague-dawley rat pups. ARRIVE guidelines have been followed for reporting and included in the manuscript, such as ethical permissions, animal strains used, surgical protocols, methods of termination, all commercial providers for reagents, sex of animals, exact n numbers used, statistical information and p values, and relevance to human disease.

Wild animals

This study did not involve the use of any wild animals.

Field-collected samples

This study did not involve field-collected samples.

Ethics oversight

Experiments were performed under a UK Home Office project licence issued under the Animals (Scientific Procedures) Act.

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

This study used post-mortem tissue of human brain from non-neurological controls and people with MS. This included 4 non-neurological controls who died of carcinoma of the tongue, myelodysplastic syndrome/Rheumatoid arthritis, cardiac failure and ovarian cancer, with 3 males and 1 female ranging from 35 to 82 years old. The multiple sclerosis cases consisted of 7 cases (1 primary progressive and 6 secondary progressive), consisting of 3 males and 4 females ranging in age from 37 to 64 and with a disease duration ranging from 9 years to 27 years. All information pertaining to these tissues is listed in supplementary table 4.

Recruitment

The tissue was obtained via a UK prospective donor scheme with full ethical approval from the UK Multiple Sclerosis Tissue bank (MREC/02/2/39) and their use was in accord with the terms of the informed consents.

Ethics oversight

Post-mortem tissue from multiple sclerosis (MS) patients and controls that died of non-neurological causes were obtained via a UK prospective donor scheme with full ethical approval from the UK Multiple Sclerosis Tissue Bank (MREC/02/2/39) and their use was in accord with the terms of the informed consents.

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

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

Focal demyelinated lesions of the corpus callosum were dissected out and homogenised with a 2 ml dounce. A Percoll (Sigma-Aldrich) gradient was used to isolate cells from myelin debris. For cell isolation, cells were incubated in anti-mouse CD16/32 Fc-block (Clone 93, BioLegend, 1:200) on ice for 10 min. Fluorescently-conjugated antibodies CD11b-PeCy7 (Clone M1/70, Invitrogen, 1:100), CD45-BV605 (Clone 3D11, Biolegend, 1:200), Ly6G-PerCP Cy5 (Clone 1A8, BioLegend, 1:200), and CD3-APC (Clone 17A2, BioLegend, 1:200) were applied on ice for 30 min. Following centrifugation and filtration (30 μm), cells were sorted by flow cytometry into FBS-coated Eppendorf tubes on ice.
For staining, samples were blocked with Fc-block (LEAF-purified anti-mouse CD16/32 (Biolegend, 101321)), then incubated with fluorochrome-conjugated antibodies CD11b-PeCy7 (eBioscience, 25-0112-82, 1:100) and CD45-BV605 (Biolegend, 103139, 1:100) for 30 minutes on ice, followed by incubation with ‘FITC Annexin-V apoptosis detection kit with 7-AAD’ for 15 minutes at room temperature (Biolegend, 640922, 1:20). Following washes in buffer, samples were run (See methods; Flow Cytometry).

| Instrument          | LSR Fortessa 6 lasers (flow cytometry), BD FACS Aria Fusion (100 um nozzle; FACS) |
|---------------------|---------------------------------------------------------------------------------|
| Software            | FlowJo version 9 and 10 LLC.                                                   |
| Cell population abundance | Abundance of cell populations shown in Figure 1-J-K, 3D-G. Additional purity shown by RNA sequencing of FACS-isolated populations in Supplementary Figure 1. |
| Gating strategy     | Shown in Supplementary Figure 1A (for FACS), Supplementary Figure 3A (for Flow cytometry), Supplementary Figure 9A (for Flow cytometry), all show where boundaries were defined. |

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