CNS fibroblasts form a fibrotic scar in response to immune cell infiltration

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Fibrosis is a common pathological response to inflammation in many peripheral tissues and can prevent tissue regeneration and repair. Here, we identified persistent fibrotic scarring in the CNS following immune cell infiltration in the experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis. Using lineage tracing and single-cell sequencing in EAE, we determined that the majority of the fibrotic scar is derived from proliferative CNS fibroblasts, not pericytes or infiltrating bone marrow-derived cells. Ablating proliferating fibrotic cells using cell-specific expression of herpes thymidine kinase led to an increase in oligodendrocyte lineage cells within the inflammatory lesions and a reduction in motor disability. We further identified that interferon-gamma pathway genes are enriched in CNS fibrotic cells, and the fibrotic cell-specific deletion of Ifngr1 resulted in reduced fibrotic scarring in EAE. These data delineate a framework for understanding the CNS fibrotic response.
Here, we show that extensive fibrotic scar formation occurs following immune cell infiltration, which contributes to disease severity. This fibrotic scar is found in every neuroinflammatory lesion and lasts for months following lesion formation. Using lineage tracing and single-cell sequencing, we demonstrate that the scar forms largely from the proliferation and migration of CNS fibroblasts, and that inhibiting CNS fibroblast proliferation leads to decreased fibrotic scarring and reduced disability in the chronic stages of disease. We further show that interferon (IFN)-γ signaling regulates the amplitude of fibrotic scar formation, identifying a potential therapeutic target to modulate levels of scar formation.

**Results**

**Immune cell infiltration drives CNS fibrosis.** To determine if a fibrotic scar forms following neuroinflammation, we induced EAE in Col1a1-GFP mice and examined both Col1 protein expression and the number and localization of Col1a1-GFP+ cells in the spinal cord. In healthy samples, Col1 protein and Col1a1-GFP+ cells were found in the meninges and associated with large parenchymal vessels (Fig. 1a and Extended Data Fig. 1a). In EAE, a robust Col1+ fibrotic scar was found in every lesion examined starting at 5 days after symptom onset (5 d PSO) and remained for the duration of the experiment (60 d PSO; Fig. 1a and Extended Data Fig. 1b–d). Col1 deposition coincided with a massive increase in the number of Col1a1-GFP+ cells throughout the parenchymal lesions that peaked at 10 d PSO and maintained their numbers during the experiment (Fig. 1a,b and Extended Data Fig. 1e,f). Fibrotic scar formation and expansion of Col1a1-GFP+ cells were observed secondary to the initial influx of immune cells and the onset of motor symptoms (Fig. 1b–d and Extended Data Fig. 1b,d), suggesting that fibrosis is likely a response to, rather than a cause of, immune cell infiltration and symptom initiation.

We further immunostained fibrotic spinal cords with a series of cellular markers to determine which markers colocalize with the Col1a1-GFP+ fibrotic cells. Col1a1-GFP+ cells were immuno-reactive for both platelet-derived growth factor receptors α and β (αPDGFRα and βPDGFRβ), which often mark fibroblasts in peripheral organs. The Col1a1-GFP+ cells did not stain positive for markers of astrocytes (SOX9 and GFAP), microglia/macrophages (IBA1 and CD11b) or mural cells (NG2 and desmin; Fig. 1e). Additionally, while Col1a1-GFP+ cells increased substantially during disease, there was not a comparable increase in desmin+ cells in the lesions over time (Fig. 1c,d). Staining for OLG2, a marker for oligodendrocyte lineage cells, demonstrated that these cells were outside the Col1 stained region, suggesting that this scar may block oligodendrocyte lineage cells from entering the lesion site and repairing demyelinated axons (Fig. 1e). In contrast to the clear lamination of the fibrotic and glial scars that occurs following SCI, we observed intermixing of GFAP reactive astrocyte processes and Col1a1-GFP+ fibrotic cells (Extended Data Fig. 1c). These results demonstrate that a robust Col1+ fibrotic scar forms in response to immune cell infiltration, and the Col1-secreting cells increase in the lesion over time and are immunoreactive for both PDGFRα and PDGFRβ.

To determine the relative contributions of inflammation and demyelination to fibrotic scarring, we inhibited inflammation in EAE using fingolimod (FTY720) which prevents immune cell exit from lymph nodes and is used as a treatment for MS in human patients. Mice administered saline following EAE had robust demyelination, motor symptoms, fibrotic scarring and Col1a1-GFP+ cell expansion, whereas mice administered FTY720 did not experience motor symptoms or demyelination and had no discernable fibrotic scar or increase in scar-forming cells (Fig. 1f–h). Thus, inhibition of immune cell infiltration in the EAE model reduced both demyelination and fibrotic scarring. To further delineate the roles of inflammation and demyelination, we looked for fibrotic scarring in mice with the cuprizone and lyssolecithin (LPC) models of demyelination. Through immunostaining for Col1 and infiltrating immune cells, we found that mice administered cuprizone, which does not lead to widespread CNS immune cell infiltration, did not have fibrotic scarring in the areas of demyelination, while mice administered LPC, which does lead to immune cell entry into demyelinated areas, did have a fibrotic scar (Extended Data Fig. 2). Together, these experiments suggest that fibrotic scarring is associated with demyelination driven by immune cell infiltration.

**CNS fibroblasts overwhelmingly form the fibrotic scar in neuroinflammation.** Conflicting studies have reported that CNS fibrotic scar formation is derived from either the expansion of Col1-expressing CNS fibroblasts or pericytes turning on the expression of collagen. Single-cell RNA sequencing (RNA-seq) of vascular cells in the CNS has demonstrated that there are indeed distinct populations of pericytes (along capillaries and post-capillary venules) and fibroblasts (associated primarily with large vessels); however, it is likely that these cells have been confused with each other in the literature as both cell types express PDGFRβ.

To determine the identity of the scar-forming cells, we performed lineage tracing experiments with the Rosa-Isl1-tdTomato reporter mouse line mated to tamoxifen-inducible Cre transgenic lines: NG2CreER<sup>TM</sup> to label mural cells (pericytes and vascular smooth muscle cells (vSMCs)) and oligodendrocyte precursor cells (OPCs), aSMACreER<sup>T2</sup> to label vSMCs and Col1a2CreERT to label cells that express Col1 at rest. We injected mice from each strain with tamoxifen at 6 weeks of age to induce expression of tdTomato within the specific cell populations and induced EAE at 12 weeks of age. We collected spinal cords at 10 d PSO to assess the presence of any cells that were downstream of the genetically labeled NG2, aSM or Col1a2 cells within the Col1+ fibrotic scar. We observed a 70-fold increase in the number of Col1a2CreERT-labeled cells in the injury site that colocalized with the Col1+ fibrotic scar, without a similar increase in the NG2CreER<sup>TM</sup> or aSMACreER<sup>T2</sup> traced cells (Fig. 2a–d). We then bred the Col1a2CreER<sup>T2</sup> reporter mouse with the Col1a1-GFP mice and found that over 90% of GFP+ cells were tdTomato+ in healthy mice and over 80% in EAE mice (Extended Data Fig. 3c,d). Additionally, less than 5% of Col1a2CreERT-labeled cells were NG2+ (Extended Data Fig. 3e,f). This demonstrates that the vast majority of fibrotic scarring cells arose from the...
expansion of cells that expressed Col1 at rest, but not the proliferation of mural cells (vSMCs/pericytes) or other cell populations turning on the expression of Col1.

To determine whether the CNS fibrotic cells are derived from the bone marrow, we transplanted wild-type mice with bone marrow from either Col1a1-GFP or UBC-GFP (pan-cellular ubiquitous

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**Figure a**

No EAE  
EAE D30 PSO  
EAE D5 PSO  
EAE D10 PSO  
EAE D60 PSO

**Figure b**

No. of GFP+ cells in lesion

**Figure c**

Col1-GFP Desmin

**Figure d**

Col1-GFP PDGFRα  
Col1-GFP PDGFRβ  
Col1-GFP SOX9  
Col1-GFP IBA1  
Col1-GFP OLIG2  
Col1-GFP NG2

**Figure e**

Saline  
FTY720

**Figure g**

Col1a1-GFP cells per area (µm²)

**Figure h**

Col1a1-GFP area (µm²)
expression of GFP) mice. Recipients of UBC-GFP bone marrow displayed massive infiltration of GFP+ cells into EAE lesions, confirming that donor bone marrow derived immune cells can infiltrate the CNS in this transplantation model. Col1a1-GFP bone marrow recipients displayed a robust Col1+ fibrotic scar but had no Col1a1-GFP+ cells in lesions. These results indicate that Col1-expressing fibrotic cells were derived from the host and not bone marrow-derived fibrocytes or immune cells that migrated into EAE lesions (Fig. 2e).

To further evaluate the cellular identities of Col1-secreting cells in healthy and EAE mice, we performed single-cell RNA-seq of GFP+ cells from Col1a1-GFP mice without EAE or with EAE at 5–7 d PSO. The cells clustered into eight clusters (0–7), which could be subdivided into three major classes that were clearly distinct on the uniform manifold approximation and projection (UMAP) plot: class 1 (clusters 0, 2, 3, 4 and 6), class 2 (clusters 1 and 5) and class 3 (cluster 7; Fig. 3b). Cells from both healthy and EAE mice were found in each class of cells and each cluster within the class, suggesting that no unique cell population turns on the expression of Col1a1 in EAE. This is consistent with lineage tracing results demonstrating that fibroblastic cells result from cells that express Col1 in health. Class 1 and class 2, representing 98% of cells, were characterized as fibroblasts, and class 3 as stromal cells using SingleR, a computational method for unbiased cell-type recognition of single-cell datasets using thousands of bulk RNA-seq reference datasets from all organs (Fig. 3b,d)34. Indeed, the expression of pericyte- and vSMC-specific genes was low in clusters 0–6 (classes 1 and 2), whereas canonical fibroblast-specific genes were highly expressed in these clusters that make up 98% of the Col1a1-GFP+ cells. Cluster 7 expressed a combination of pericyte- and vSMC-specific genes (Fig. 3e and Extended Data Fig. 4d)30. Although Col1a1-GFP+ mural cells were identified in cluster 7, these represented a minority (<2%) of the Col1-producing cells, and this cluster displayed the lowest Col1 expression of any of the clusters. These data, combined with NG2CreERT2 and aSMACreER2 lineage tracing studies, demonstrate that mural cells are not major contributors to fibrotic scar formation.

The proportions of each cell population were similar in healthy mice compared to EAE mice, with small increases in class 1 clusters (0, 2, 3 and 4) and decreases in class 2 clusters (1 and 5) in EAE (Fig. 3a–c). However, within clusters, genes indicative of activated fibroblasts were expressed more highly in EAE cells, indicating that there was a shift towards a more activated fibroblast in EAE (Fig. 3h). Proliferation analysis of the cells revealed that each cluster had some...
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Fig. 3 | Scar-forming cells have the transcriptional profile of fibroblasts at the single-cell level. a–d, Col1α1-GFP+ cells from spinal cords of healthy mice (n = 3 samples with two to three spinal cords each) and mice with EAE 5–7 d PSO (n = 2 samples with two spinal cords each) were transcriptionally profiled at the single-cell level and clustered using Seurat v3. a, UMAP plot with the sample identity (healthy versus EAE) labeled for each cell. b, UMAP plot of the clustering analysis revealed eight clusters that could be subdivided into three classes: class 1 (clusters 0, 2, 3, 4 and 6), class 2 (clusters 1 and 5) and class 3 (cluster 7). c, Pie charts showing the percentage of each cluster relative to the total number of cells in healthy mice or mice with EAE. d, UMAP plot showing each cell labeled with its cellular identity determined using SingleR and the Immgen reference dataset. e, Violin plots of the expression levels of pericyte-, vSMC- and fibroblast-specific genes per cluster. f, UMAP plot showing the transcriptional cell cycle identity per cell. g, Bar graphs showing the percentage of transcriptional signatures of the different steps of the cell cycle in each cluster. h, Dot plot of selected genes that are expressed at greater levels in mice with EAE than in healthy mice, in at least one cluster.

proportion of cells with a transcriptional profile indicative of the S and G2/M phases of the cell cycle, with cells in cluster 2 having the largest proportion of cells in the G2/M stage (Fig. 3f,g). Cluster 0 had the most enriched expression of ECM proteins such as collagens, and cluster 3 was enriched for the transcription factors Fos, Fosb and Junb, which are expressed in activated fibroblasts and required for stretch-induced ECM production (Extended Data Fig. 4c,5,36). These transcription factors were most highly expressed in cells in this cluster from EAE (Fig. 3h). Therefore, these data indicate that two classes of Col1+ fibroblasts produce the fibrotic scar, which can be divided into clusters that define specific states of these cells, such as dividing and actively producing high levels of ECM.

Taken together, these lineage tracing and single-cell RNA-seq studies indicate that fibrotic cells arise overwhelmingly from the
For these experiments, we examined primary OPCs seeded in cell culture wells treated with poly-L-lysine (PLL) alone, PLL + Col1, PLL + fibronectin and PLL + laminin to compare the effects of Col1 to those of ECM proteins of the basement membrane. We found that Col1 did not have an effect on cell proliferation or differentiation, but significantly decreased OPC migration across a transwell insert (Extended Data Fig. 6). In contrast, the basement membrane matrix proteins fibronectin and laminin both significantly increased migration, which corresponds to the migration of OPCs along CNS vessels during development19.

These results suggest that fibrosis impairs the ability of reparative myelin-forming oligodendrocyte lineage cells to migrate into the lesions. However, ablation of proliferative fibrotic cells alone does not promote axonal remyelination or full symptomatic recovery in EAE mice. We found a profound loss of axons positive for neurofilaments within lesions in both control and IHTK mice, suggesting that although more oligodendrocyte lineage cells were able to enter the lesion, there was a lack of healthy axons for myelination (Extended Data Fig. 5e). Further, electron microscopy showed that both control and IHTK mice had significantly less myelinated axons than healthy, wild-type mice (Fig. 4j,k). Therefore, combining anti-fibrotic approaches with therapeutics to maintain axon integrity might prove synergistic in the treatment of diseases such as MS.

Interferon-gamma signaling regulates the amplitude of fibrotic scarring. To gain deeper insight into the molecular mechanisms of neuroinflammatory fibrosis, we performed bulk RNA-seq on Col1a1-GFP+ cells sorted from spinal cords of healthy mice, mice with EAE at 5 and 10 d PSO, and whole spinal cord homogenates from healthy mice. Genes including Col1a1, Col1a2 and Col3a1 were highly enriched in both the healthy and EAE GFP+ cells compared to the whole spinal cord, and their expression continued to increase during EAE (Extended Data Fig. 7). When comparing Col1a1-GFP+ cells in healthy and EAE mice, there were more significantly differentially expressed genes at 5 d PSO (2,516 upregulated and 2,278 downregulated) than at 10 d PSO (1,414 upregulated and 1,122 downregulated). Many of the genes highly upregulated at 5 d PSO are involved in inflammatory signaling, and their expression largely peaked at 5 d PSO and moderately decreased at 10 d PSO (Fig. 5a and Extended Data Fig. 7). A smaller subset of genes was increased at 5 d PSO and continued to increase at 10 d PSO, and included many genes encoding collagen subunits (Extended Data Fig. 7). These data suggest that Col1+ cells turn on inflammatory pathways early in disease progression when the most cell migration occurs and continue depositing ECM once their expansion is complete.

Pathway analysis identified IFN-γ signaling is enriched in EAE fibrotic cells compared to the whole spinal cord (Extended Data Fig. 7d). We found that fibrotic cells expressed IFN-γ receptors (Ifngr1 and Ifngr2) and downstream signaling molecules (Jak1, Jak2, Stat1, Stat5a and Stat5b) in both healthy and EAE mice, and upregulated IFN-γ target genes CXCL9 and CXCL10 following EAE induction. These studies further identify potential fibroblast cell states specifically associated with neuroinflammatory fibrotic scar formation.

### Fig. 4 | Reducing fibrotic scar formation reduces disease severity in EAE.

**a**, Spinal cord sections from fHTK mice and littermate controls 30 d after EAE induction were stained for Olig2 (green), DAPI (blue) and CD11b (top; red) to label immune cells or CD31 (bottom; red) to label endothelial cells. **b**, Quantification of the percentage of the area of immune infiltration, denoted by CD11b, that was Col1+ (± s.e.m.; **P < 0.0001 by Student’s two-tailed t-test; n = 21 control and 19 fHTK). **c**, Quantification of the total lesion size, denoted by CD11b staining (± s.e.m.; n = 21 control and 19 fHTK; P = 0.33 by Student’s two-tailed t-test). **d**, EAE score for the fHTK mice and controls up to 30 d after EAE induction (± s.e.m.; **P < 0.05 and P < 0.0012 using a paired t-test). **e**, Pie charts depicting the percentages of control and fHTK mice that were paralyzed at day 24 and day 30 after EAE induction. **f**, Spinal cords from control and fHTK mice were stained for Olig2 (green), CD11b (red) and DAPI (blue). The white dashed line marks the area of the CD11b+ lesion. **g**, The number of Olig2+ cells per CD11b+ lesion was quantified comparing the fHTK and control mice (± s.e.m.; n = 21 control and 19 fHTK; *P = 0.038 by Student’s one-tailed t-test). **h**, Spinal cord sections from fHTK mice and controls at 30 d after EAE induction were stained for FluoroMyelin (red) and DAPI (blue). The white dashed line marks the area of the CD11b+ lesion. **i**, Quantification of the percentage of the total white matter area that was positive for FluoroMyelin (± s.e.m.; n = 21 control and 19 fHTK; P = 0.96 by Student’s two-tailed t-test). **j**, Electron microscopy images of spinal cord sections from healthy wild-type mice and from fHTK mice and controls 30 d after EAE induction. **k**, Quantification of the number of myelinated axons in the electron microscopy images from the three groups shown in **j**, per 3,000× picture frame (n = 4 per group; **P = 0.0002 and ***P = 0.0012 using a one-way ANOVA with multiple comparisons). Scale bars: 100 μm (immunofluorescence images) and 2 μm (electron microscopy images).
(Fig. 5b,c)40–42. In the single-cell sequencing dataset, IFN-γ pathway genes were expressed throughout each of the different clusters of both class 1 and class 2 fibroblasts (Fig. 5d). IFN-γ target genes Cxcl9 and Cxcl10 were expressed mainly in class 1 cluster 2, which is the cluster with the highest proportion of actively dividing cells, suggesting that this IFN-γ signaling may regulate fibroblast...
proliferation. Taken together, these data reveal that CNS fibroblasts express the machinery to respond to IFN-γ signaling, but this signaling cascade is only induced following EAE. To determine which cells in the EAE lesion are secreting IFN-γ, we performed single-cell sequencing on whole spinal cords from EAE mice. IFN-γ was highly expressed in a cluster of cells that were identified as T cells (Fig. 5e). These data suggest that during EAE, T cells secrete IFN-γ signals to CNS fibroblasts.

The role of IFN-γ signaling in fibrosis in peripheral tissues has been debated. To determine whether IFN-γ signaling in fibroblasts is necessary for CNS fibrotic scar formation, we selectively deleted Ifngr1 from fibroblasts before EAE induction. Mice with fibrotic cell-specific Ifngr1 deletion (Ifngr1<sup>fl/fl</sup>; Col1a2CreER<sup>T</sup> (Ifnγ-)) and littermate controls (Ifngr1<sup>fl/fl</sup>) were injected with tamoxifen at 6 weeks of age, induced with EAE at 12 weeks of age, collected 30 d after EAE induction and analyzed for the extent of fibrotic scar formation. IfNγ-mice had a significant reduction in fibrotic scar formation (Fig. 5a,b), although not to the extent of the reduction in the cell ablation paradigm. There were no differences in motor disability, lesion size or myelination between groups (Fig. 6c–f). To determine whether IFN-γ is sufficient for scar formation, we analyzed tissue from mice administered cuprizone for 5 weeks with astrocyte-specific overexpression of IFN-γ<sup>γ</sup>. We found no fibrotic scar in the areas of demyelination, suggesting that an induction of IFN-γ is not sufficient for scar formation (Extended Data Fig. 2b,c). Together, these experiments confirm that IFN-γ signaling in CNS fibroblasts regulates the amplitude of fibrotic scarring, but on its own is not sufficient to initiate fibrotic scarring.

Discussion

In this study, we demonstrate that a dense, Col1<sup>+</sup> fibrotic scar forms in the spinal cord following immune infiltration in mouse models of demyelination and plays a role in regulating disease severity. Combined with previous characterizations of fibrotic scarring following SCI, our data suggest that there is a robust fibrotic response to both injury and inflammation in the CNS, and that activation and proliferation of CNS fibroblasts may be a common response across many different neurological disorders. This has wide implications for our understanding of the pathophysiology and repair of a wide array of neurological diseases, and should stimulate future research into the fibrotic response in various human neurological and neurodegenerative diseases.

Using lineage tracing and single-cell sequencing, we illustrate that this fibrotic scar is derived overwhelmingly from the proliferation and migration of CNS fibroblasts found in the meninges and surrounding large blood vessels in healthy mice. In contrast, previous studies reported that a subtype of pericytes forms the fibrotic scar following SCI. These studies used a GlastCreER reporter to label scar-forming cells and denoted these cells as type A pericytes due to their expression of PDGFRβ and perivascular localization. As these qualities are also true of fibroblasts, it is possible that some type A pericytes are fibroblasts. Indeed, Glast (<SIC1a3>) is strongly expressed in fibroblasts in addition to astrocytes and pericytes. Our lineage tracing experiments suggest that the scar originates from cells that express Col1 in health, but not NG2<sup>+</sup> or aSMA<sup>+</sup> cells, and our single-cell sequencing data classify the vast majority of these cells as fibroblasts. We did find that a small proportion (2%) of collagen-expressing cells in healthy and EAE mice expressed typical markers of mural cells (vSMCs/pericytes). While these cells had lower collagen transcript expression than fibroblasts, we cannot formally rule out their contribution to fibrosis. The proportion of this scar that comes from CNS fibroblasts in the meninges compared to those surrounding large blood vessels remains unknown, but we hypothesize that fibroblasts from both regions may play a role in scar formation. Techniques such as in vivo, two-photon imaging could be used to further delineate the roles of CNS fibroblasts from different regions.

Interestingly, when we ablated proliferating fibroblastic cells, although the fibrotic scar was greatly diminished, there was still Col1 deposition largely restricted to the area around blood vessels. This remaining Col1 deposition may derive from the incomplete recombination of the Col1a2CreER<sup>T</sup> used in the ablation paradigm, as scar tissue deposition by CNS fibroblasts that aren’t actively proliferating and/or scar deposition by vascular cells such as endothelial cells, pericytes or vSMCs. A recent study found that in EAE, endothelial cells take up myelin debris, which leads to an endothelial→mesenchymal transition and an upregulation in the expression of ECM proteins<sup>4</sup>. While we did not find robust Col1a1-GFP reporter expression in CNS endothelial cells (Extended Data Fig. 4a), it is possible that CNS endothelial cells express Col1 protein without turning on the Col1a1-GFP promoter, thus leading to perivascular Col1 accumulation. Interestingly, we found a small proportion of mural cells secrete Col1, suggesting that these cells may regulate the perivascular Col1 deposition.

Fibrotic scarring could potentially influence the course of neuroinflammatory disease by restricting immune cell trafficking into inflammatory lesions or by preventing tissue repair and regeneration. We found that reducing fibrotic scar formation by preventing fibroblast cell proliferation decreases motor disability and increases the number of oligodendrocyte lineage cells in the lesion in the chronic stages of disease without affecting immune cell entry or myelination. Additionally, collagen proteins inhibit OPC migration in vitro. This suggests that fibrotic scarring limits the ability of cells with repair potential (oligodendrocyte lineage cells) from entering the demyelinating lesion; however, these cells are still not capable of remyelination. This lack of remyelination may stem from either the presence of OPC differentiation inhibition cues or axon degeneration that occurs in the EAE model. Indeed, we found robust axon degeneration in this EAE model (Fig. 4j,k and Extended Data Fig. 5e). Therefore, combining therapeutics that inhibit scar formation with those that preserve axon integrity may synergize to enhance repair following neuroinflammation. As there are no changes in myelination after reducing fibrotic scarring, it is not completely clear how reducing the fibrotic scar decreases the EAE score in the chronic stages of disease.

This study also implicates IFN-γ signaling in CNS fibrotic scar formation, unveiling a new molecular mechanism that could be of interest for CNS disorders with scar tissue deposition. As Ifngr1 deletion from fibroblastic cells did not completely ablate the fibrotic...

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**Fig. 5 | Fibrotic cells upregulate interferon-gamma pathway genes in EAE.** 
- **a.** Heat map of the expression levels of the top 100 differentially expressed genes by false discovery rate (FDR) in Col1a1-GFP<sup>+</sup> cells from EAE mice at 5 and 10 d PSO (CNS fibro EAE D5 and D10) compared to Col1a1-GFP<sup>+</sup> cells from healthy mice (CNS fibro healthy). 
- **b.** mRNA levels in counts per million (CPM) from different gene expression of whole spinal cord tissue (n = 2) and CNS fibro healthy, CNS fibro EAE D5 and CNS fibro EAE D10 (n = 3 each; ±s.e.m., *FDR < 0.05 and **FDR < 0.01 for white spinal cord; *FDR < 0.05 and ***FDR < 0.01 for CNS fibro healthy). 
- **c.** Spinal cord sections from Col1a1-GFP mice without (left) or with EAE (right) cells were stained with CXCL10 (red). 
- **d.** Violin plots from the single-cell sequencing dataset of Col1a1-GFP<sup>+</sup> cells described in Fig. 3, depicting the total RNA counts for genes in the IFN-γ pathway. 
- **e.** UMAP plot of individual cells sequenced from the whole spinal cord of a wild-type mouse with EAE 4 days PSO with their assigned cell-type identity using SingleR and the Immgene reference dataset (left). IFN-γ expression is indicated in blue (right) and is found predominantly in T cells in EAE. NK, natural killer. Scale bars: 100 μm.
scar in neuroinflammatory lesions, other pathways are also likely involved in fibrotic scar formation. Additionally, IFN-γ is mainly secreted by adaptive immune cells, and thus this signal may be utilized to amplify fibrotic scar formation when adaptive immunity is involved in neuroinflammatory lesion formation. Pathways involved in fibrosis in peripheral tissues such as the transforming growth factor-β pathway and Wnt pathway could also play a role in CNS fibrosis following a variety of triggers. Our RNA-seq dataset of Col1+ cells in healthy and EAE samples will be widely useful to understand other drivers of CNS scar formation, and could influence treatment options for SCI, stroke and other neurological injuries and inflammatory diseases with fibrotic scarring.
**Fig. 6** | Interferon-gamma signaling regulates scar formation following neuroinflammation. 

**a**, Spinal cord sections from fibrotic cell-specific IFN-γ knockout mice (fIFN-γ, IFN-γ^fl/fl; Col1a2CreER^) and littermate controls (IFN-γ^fl/fl) stained for Coll (green), CD11b (red) or CD31 (red), and DAPI (blue).

**b**, Quantification of the amount of fibrotic scar covering the lesion (±s.e.m., **P** = 0.0039 by Student’s two-tailed t-test; n = 14 control and 15 fIFN-γ).

**c**, EAE score for the fIFN-γ mice and controls up to 30 d after EAE induction (±s.e.m., n = 14 control and 15 fIFN-γ mice).

**d**, Quantification of the CD11b^+ immune cell area in the control and fIFN-γ groups 30 d after EAE immunization (±s.e.m., **P** = 0.61 by Student’s two-tailed t-test; n = 14 control and 15 fIFN-γ).

**e**, Quantification of the percentage of the total white matter area that was FluoroMyelin-positive for the control and fIFN-γ groups (±s.e.m., **P** = 0.44 by Student’s two-tailed t-test; n = 14 control and 15 fIFN-γ mice).

**f**, Quantification of OLIG2^+ cells per CD11b^+ lesion area between the control and fIFN-γ groups (±s.e.m., **P** = 0.76 by Student’s two-tailed t-test; n = 14 control and 15 fIFN-γ mice).

**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/s41593-020-00770-9](https://doi.org/10.1038/s41593-020-00770-9).

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Methods

Experimental model and subject details. Col1a1-GFP mice (C57BL/6 background) were used to label Col1-producing cells for imaging and FACS analysis. Rosa-lsl-tdTomato mice (Charles River Laboratories, 007909; C57BL/6 background) were crossed with Col1a2CreERT + mice (Jackson Labs, 025967; C57BL/6 background), NG2CreERT + mice (Jackson Labs, 008538; C57BL/6 background) or SMACcreERT + mice (previously described) for lineage tracing analysis. B6.129S7-Hprt1tm10Ivl/J (Har+Mmcu) (MMRRC 010860; UC; C57BL/6 Try c-Brd mixed background) were crossed to Col1a2CreERT mice for the IFN-γ mechanistic studies. UCP-GFP (004353) reporter mice and CD45.1 (002014) mice used in bone marrow transplant experiments were purchased from Jackson Labs. GFAP/TTA mice on the C57BL/6 background were mated with TRE/IFN-γ on the C57BL/6 background to produce GFAP/TTA;TRE/IFN-γ double transgenic mice and were maintained in the Popko laboratory for cryopreservation studies. Animal protocols were approved by the Institutional Animal Care and Use Committee at the University of California, San Diego (UCSD), University of California, San Francisco (UCSF) and Northwestern University, and we have followed all ethical regulations in the use of mice for this study. Male mice were used for IHT experiments as the gene is X-linked, and for the Col1a1-GFP regional quantifications in healthy samples. Female mice were used for the bone marrow transplant studies, and the Col1a1-GFP cell number in EAE quantifications. Male and female mice were used for all other experiments. Mice were used between 2 and 6 months of age unless otherwise noted and were housed in a temperature-controlled environment (68 to 72 °F for UCSD and 40–70% humidity) with a 12-h light/dark cycle.

Experimental autoimmune encephalomyelitis. EAE was induced through two subcutaneous injections of myelin oligodendrocyte glycoprotein in Freud's adjuvant and an intraperitoneal (i.p.) injection of pertussis toxin (PTX; Hoeke Laboratories, ER-2110). At 24 h after the initial injection, a second i.p. injection of PTX was administered. We used 120–160 mg PTX for each dose based on the manufacturer's instructions. Mice were induced at 10–12 weeks of age unless notified otherwise. The following EAE score system was used to assess motor outcomes:

0.5 – tip of tail limp
1 – whole tail limp
2 – mouse does not instantly turn over when flipped
2.5 – weakly ambulate
3 – mouse dragging backside
3.5 – paralysis of one hind limb
4 – paralysis of both hind limbs
5 – moribund

Mice were scored every day following the induction of EAE and until tissue collection was performed.

FTY720 injections. Col1a1-GFP mice were injected with either saline or 2 mg kg−1 FTY720 ip. starting on the day of EAE induction and continuing daily until tissue was collected on days 8–10 PSo for the saline mice.

Cuprizone model of demyelination. Cuprizone model of demyelination. Male and female mice were used for all other experiments. Mice were used as bone marrow donors. UBC-GFP mice express enhanced GFP transgene with an aSmaCreERT2 (2C Zeiss) and digital camera (Axiolab Hr2, Carl Zeiss) using the AxioVision software (Axiovisio 4.8 v.2.0) and contrasted using Adobe Photoshop. For all fluorescence experiments, tissue from multiple mice was stained and representative images were chosen for publication. For light-sheet microscopy images, mice were perfused with 100 μl of a tomato lectin (Vector Laboratories, DL-1177) in DPBS before PFA, and spinal cord tissue was embedded and incubated overnight with 4% PFA followed by 24 h fixation. Myelin was then cleared in a solution of 8% SDS, 10% N-butyldiethanolamine, 3% 1-thioglycolic in PBS for 72 h and then imaged on a Zeiss 71 light-sheet microscope at the UCSD School of Medicine Microscopy Core. For confocal images, Col1a1-GFP mouse spinal cords stained with CD31 were imaged on a Leica SP8 confocal microscope at the UCSD School of Medicine Microscopy Core.

Lineage tracing. All mice received i.p. injections of 2 mg tamoxifen in sterile corn oil for three consecutive days at 6 weeks of age to induce tdTomato expression. EAE was induced at 12 weeks of age as described above. Mice were scored based on their EAE score and tissue harvest done above and tissue was collected 18 PSo and stained for collagen I. The total collagen area for each section was traced using ImageJ 1.52a, and the number of tdTomato+ reporter cells within this area was quantified and compared between groups. This number was normalized to the average of the number of reporter cells per white matter area for each of the reporters in age-matched healthy mice (Col1a2CreERT analysis: n = 7 healthy and n = 3 EAE; NG2CreERT analysis: n = 10 healthy and n = 3 EAE; SmacCreERT analysis: n = 4 healthy and n = 7 EAE). Male and females were used in all groups.

Bone marrow transplantation. Female 12-week-old Col1a1-GFP or UBC-GFP mice were used as bone marrow donors. UBC-GFP mice express enhanced GFP under the direction of the human ubiquitin C promoter. GFP is expressed in all tissues examined, is uniform within cell lineages and remains constant throughout development and in injury paradigms (JAX: 004353). Female CD45.1 mice were used as recipients for cell transplantation. This strain carries the CD45.1 pan-leukocyte marker to distinguish donor-derived cells from recipient CD45.2 cells. Recipient mice were irradiated with 900 rad, with a split dose, 3 h apart using a cesium source. Purified donor cells (4 × 10⁶) from bone marrow were injected intravenously with 200,000 spleen helper cells, and hematopoietic reconstitution was monitored in the peripheral blood based on either GFP or CD45.1 expression. Recipients with ≥99% donor chimera were considered reconstituted. Note no GFP+ cells were observed in the blood from Col1a1-GFP recipient mice. Transplanted mice were kept on antibiotic-containing food for 2 weeks. All mice were maintained at UCSF in accordance with protocols approved by the Institutional Animal Care and Use Committee.

Tissue dissociation and FACS. To obtain single-cell suspensions of CNS fibroblasts, spinal cords from Col1a1-GFP mice were dissected, chopped with a no. 10 blade and enzymatically dissociated with papain (Worthington Biochemical, LK003176; one vial per sample) containing DNase (109878) and DNase (125 U ml−1) and a second enzymatic digestion with 1.0 mg ml−1 collagenase type II (Worthington Biochemical, LS001476) and 0.4 mg ml−1 neuraminidase protease (Worthington Biochemical, LS02104) at 35 °C for 30 min. Next, myelin-specific removal was performed using myelin removal beads (MACS Miltenyi Biotec, 130-096-433) and LS columns (MACS Miltenyi Biotec, 130-042-401) on a MidiMACS separator (MACS Miltenyi Biotec, 130-042-300). Samples were then blocked with rat IgG (Sigma-Aldrich, IB015; 1:100) for 25 min on ice. The cell pellets were resuspended in buffer containing
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DAPC1 at a 1.5:1 dilution and rat anti-CD31 Alexa Fluor 647 (Molecular Probes, A14716) at a 1:100 dilution and incubated in the fridge for 30 min. Suspensions were washed twice with buffer and live, CNS fibroblasts were FACs sorted into Triple (Invitrogen, 15596026) based on GFP fluorescence using an Aria II sorter at the Flow Cytometry Core at the VA Hospital in California. Forward scatter and side scatter analysis were also used as gates to limit the sorting to single, live cells using FACSDiva v8 software.

Single-cell sequencing. Tissue was dissociated and FACs sorted as described above into PBS + 0.05% BSA. Three samples were collected from healthy, Col1a1-GFP mice, each containing the Col1a1-GFP cells from two to three spinal cords. Two EA samples were collected, each containing the Col1a1-GFP cells from a spinal cord from a Col1a1-GFP mouse at 3 d PSO and a spinal cord from a Col1a1-GFP mouse at 7 d PSO. For the whole spinal cord in EA group, 1 spinal cord from a wild-type mouse with EAE at 4 d PSO was sorted to generate gene count data and then analyzed using Seurat v3. We used the transwell method for differential expression analysis, with a min.pct of 0.25 and logfc threshold of 0.25. SingleR v1.4 was used to annotate cells using the Immgen reference dataset.

CNS fibrotic scar prevention and interferon-gamma receptor deletion. All mice received i.p. injections of 2 mg tamoxifen in sterile corn oil for three consecutive days at 6 weeks of age to induce Cre expression. EAE was induced at 12 weeks of age in males and females. Mice were collected for the HTK experiment, males were used as the HTK gene is X-linked. For the HTK experiment, males were used as the HTK gene is X-linked. One of the control mice died during EAE, so this data was not included in the histogram quantifications. For the IFN-γ deletion experiments, tamoxifen and EA were administered in males and females. Mice were collected 30 d after EA induction (n = 14 control and 15 IFN-γ). Electron microscopy. Control and HTK mice at day 30 after EA induction and healthy C57BL/6 mice were anesthetized through an i.p. injection of a ketamine/xylazine cocktail and then perfused transcardially with 2% PFA and 0.5% BSA. Three samples were collected from healthy, Col1a1-GFP female at 3 months of age. RNA was purified from the FACs-sorted fibroblasts using the Qiagen RNA Isolation Microkit. The RNA was then analyzed for purity and concentration at the UCSF IGM Core using a Agilent Bioanalyzer. Stranded, cDNA libraries were made using the SMARTer Stranded Total RNA-Seq Kit (Pico Input Mammalian; Clontech) and then the samples were sequenced on an Illumina HiSeq 4000 for 100 cycles to produce paired-end reads. Sequence reads were aligned to Ensemble mm9 v67 mouse genome using TopHat v2.0.11 and Bowtie 2 v2.2.1 using the parameters: -m 2 -a 5. The resulting files were then sorted using SAMTools v0.19 and count tables generated using HTSeq 0.6.1. Differential expression analysis and statistical analysis including P-values and FDR were performed using DESeq2. Heat maps were generated using heatmapper software (https://software.broadinstitute.org/morpheus/) and clustered using hierarchical clustering with a metric of one minus the Pearson correlation. Quantification and statistical analysis. EAE time-course quantifications. To calculate the number of Col1a1-GFP cells in each lesion over time, tissue sections from Col1a1-GFP mice with EA were stained for CD45. CD45 areas were traced using ImageJ, and the number of Col1a1-GFP cells within this area were counted at different time points throughout EAE. To calculate the number of pericites and T cells in EA lesion over time, tissue sections from Col1a1-GFP or C57BL/6 mice induced with EA and collected at different time points PSO were stained with antibodies against CD4 and CD8 followed by Cy3 and Cy5, respectively, to determine the lesion location owing to increased cell density. The number of desmin, CD4 or CD8 cells within the lesion was counted for each mouse using ImageJ. Between three and six mice were quantified per group, with at least four spinal cord sections (two thoracic and two lumbar) analyzed from each mouse. Lysolcithin and cuprizone scar tissue quantifications. To quantify the area of demyelination that was Col1+ in the LPC mouse model, spinal cord cross sections from four mice at 7 d after LPC administration were stained for Col1 and FluoroMyelin. The total Col1+ area and FluoroMyelin+ area were quantified using ImageJ. Between three and six mice were quantified per group, with at least four spinal cord sections (two thoracic and two lumbar) analyzed from each mouse.
Lesion areas were traced using DAPI in ImageJ, and the number of CD4+ cells per lesion, tissue sections were stained for OLIG2 and CD11b. CD11b areas were traced using ImageJ, and the number of OLIG2+ cells within this area was counted and compared between groups. To calculate the percentage of OLIG2+ cells that were CC1⁺, sections were stained with OLIG2 and CC1 using the Vector Labs Mouse on Mouse Basic Kit (BMK-2202). DAPI was then administered to all slides and used to determine the lesion location due to increased cell density. The percentage of OLIG2+ cells also positive for CC1 within the lesion was counted for each mouse using ImageJ. To calculate the number of CD4+ and CD8+ cells per lesion, tissue sections were stained for CD4 or CD8. Lesion areas were traced using DAPI in ImageJ, and the number of CD4+ or CD8+ cells within this area was counted and compared between groups. To calculate the number of axons in the lesions of control and HfTK mice, tissue sections were stained for CD11b and neurofilament heavy polypeptide. Quantifications were performed using Cell Profiler. For all quantifications, at least six spinal cord sections (three thoracic and three lumbar) were analyzed from each mouse.

Col1a1-GFP regional analysis. Brains and spinal cords of four male adult Col1a1-GFP mice were collected, sectioned and stained for CD31 in red, and smooth muscle actin in far red. The length of the total vasculature and smooth muscle actin-positive vasculature and the number of GFP+ cells associated with the vasculature for the different brain regions were quantified using ImageJ.

Col1a2CreER analysis. All mice were injected with tamoxifen, induced with EAE, and tissue was collected and sectioned in the same way as the lineage training mice. For the Col1a1-GFP:Col1a2CreER overlap analysis, six spinal cord cross sections per mouse were imaged, and the percentage of Col1a1-GFP+ cells that were also positive for the tdTomato reporter was calculated using ImageJ (n=5 healthy and 4 EAE). For the Col1a2CreER;NG2 overlap analysis, spinal cord sections from Col1a2CreER mice were stained for NG2. At least six spinal cord sections were imaged for each mouse, and the number of Col1a2CreER-label labeled cells positive for NG2 and the number of Col1a2CreER-labeled cells negative for NG2 were counted using ImageJ (n=4 healthy).

Statistics. The statistics for each experiment are described in the figure legends. All error bars represent the s.e.m., and n values refer to the number of animals used in the experiment. Animals were assigned to groups based on genotype when appropriate or at random when mice of the same genotype were split into groups. Investigators were blinded to the data collection and experimental analysis. Sample sizes were selected based on variability of the measurement and values of difference between conditions. Data distribution was assumed to be normal. Statistics were calculated using Prism 7 and Microsoft Excel 2013.

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

Data availability
All RNA-seq and single-cell sequencing raw and processed data files have been uploaded to the Gene Expression Omnibus and can be accessed using the following accession codes: GSE135186 for single-cell transcriptional profile of Col1a1-GFP+ cells in healthy and EAE samples; GSE135044 for transcriptional profile of Col1a1-GFP+ CNS cells in healthy and EAE samples; and GSE135185 for single-cell transcriptional profile of a mouse spinal cord in EAE.

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Author contributions
C.E.D., T.A. and R.D. designed experiments and wrote the manuscript. C.E.D. performed and analyzed lineage tracing, single-cell and bulk RNA-seq, fibrosis reduction and IFN-γ experiments, and performed immunostaining and microscopy on EAE spinal cords. D.A. analyzed single-cell sequencing data. E.A.H. quantified the location of Col1a1-GFP+ cells in healthy and disease samples. R.N.S. analyzed fibrosis reduction experiments. K.K.H. performed and analyzed OPC in vitro experiments. L.F. quantified the number of pericytes and immune cells in lesions over time. Y.C. and B.P. performed cuprizone experiments. T.A., C.O.L. and K.M.C. performed and analyzed bone marrow transplant and lineage tracing experiments. G.A.W. analyzed RNA-seq experiments, and S.P.I.F performed LPC surgeries.

Competing interests
The authors declare no competing interests.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Col1a1-GFP cell localization in health and following EAE. a, Analysis of the number of Col1a1-GFP+ cells per total vascular length and smooth muscle actin (SMA) + vascular length in different CNS regions in healthy adult mice. CP = choroid plexus, SC = spinal cord white or grey matter, ± s.e.m., n = 4, Col1a1-GFP mice. b-d, Spinal cord sections from wild type mice in health or with EAE at 2, 5 or 10 days PSO were stained with Col1 (red), DAPI (blue) and CD11b (b, green), GFAP (c, green), or CD4 (d, green) Scale bars = 100 µm. e, Light sheet microscopy image of a Col1a1-GFP mouse in health (left) and one with EAE (right) 10 days PSO perfused with tomato lectin and optically cleared, scale bars = 200 µm. f, Confocal microscopy images of Col1a1-GFP spinal cords from health (left) and EAE (right) stained for CD31 in red, scale bars = 10 µm.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Fibrotic scarring is present in the LPC, but not cuprizone, model of demyelination. a, Spinal cord sections from mice either 5, 7, or 14 days post LPC injection into the spinal cord were stained for either Col1 (green) and FluoroMyelin (red) or myelin basic protein (MBP) (green) and Cd11b (red). b, Brain sections in the area of the corpus callosum from GFAP/tTA;TRE/IFN-γ mice following cuprizone administration. GFAP/tTA;TRE/IFN-γ mice received doxycycline starting from birth, and half of the mice were taken off of doxycycline at 6 weeks of age (-dox) to induce the expression of IFN-γ in the CNS. Both the +dox and -dox groups were divided into two groups. The first group (3 wk) was given cuprizone for 3 weeks and then analyzed at the end of the 3 week period. The second group (8 wk) was given cuprizone for 5 weeks and then analyzed 3 weeks after completion of the cuprizone administration. All brains were stained for either Col1 (green) and FluoroMyelin (red) or myelin basic protein (MBP) (green) and Cd11b (red). Scale bar = 100 µM. c, Quantification of the proportion of the demyelinated area that is Col1+ from mice 7 days post LPC injection (n = 4) or mice 3 weeks post cuprizone administration with and without doxycycline (n = 3 each), ± s.e.m.
Extended Data Fig. 3 | Col1a2CreER<sup>T</sup> and NG2CreER<sup>Tm</sup> reporter expression. Spinal cords of Col1a2CreER<sup>T</sup>;Rosa-tdTomato (a) or NG2CreER<sup>Tm</sup>;Rosa-tdTomato (b) mice in health or EAE 10 d PSO were stained with CD31 in green and DAPI in blue. Scale bars = 100 µm. c, Spinal cords of Col1a2CreER<sup>T</sup>;Rosa-tdTomato;Col1a1-GFP mice in health or 10 days EAE PSO were imaged for both reporters. The percentage of GFP<sup>+</sup> cells that were also positive for the tomato reporter in health and EAE is quantified in (d), ± s.e.m., n = 5 health, 4 EAE. e, Col1a2CreER<sup>T</sup>;Rosa-tdTomato mice were stained with NG2 in red, and the proportion of Col1a2CreER<sup>T</sup><sup>+</sup> cells that were NG2<sup>+</sup> and NG2<sup>−</sup> were quantified in (f), ± s.e.m., n = 4. Scale bars = 100 µm.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Single-cell sequencing: cell purification and cluster expression. a, Sample FACS plots of the purification of Col1a1-GFP+ cells used for the single-cell sequencing analysis of Col1a1-GFP+ cells in health and EAE. b, UMAP plot of the single-cell RNA-seq dataset of Col1a1GFP+ cells in health and EAE with the individual sample identity labeled for each cell. c, Heat map depicting the expression of the 10 most differentially expressed genes in each cluster based on the logFC of the dataset. d, Heat map depicting the expression levels of genes specific to the labeled cell types, Olig = oligodendrocyte.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Effects of reducing fibrotic scar formation on immune cell infiltration, oligodendrocyte lineage cell populations and axon numbers. 

**a.** Spinal cord sections from fHTK mice and controls were stained for DAPI (blue) and periostin (green), ER-TR7 (green), Col3 (green) and CD11b (red), or GFAP (green) and Cd11b (red).

**b.** Spinal cord sections from fHTK mice and controls were stained for CD4 (red, left) and DAPI (blue) and the number of CD4+ cells per lesion area was compared between groups (right), \( p = 0.38 \) by Student's two-tailed t-test, ± s.e.m., \( n = 21 \) control and 19 fHTK.

**c.** Spinal cord sections from fHTK mice and controls were stained for CD8 (red, left) and DAPI (blue) and the number of CD8+ cells per lesion area was compared between groups (right), ± s.e.m., \( p = 0.22 \) by Student's two-tailed t-test, \( n = 21 \) control and 19 fHTK.

**d.** Spinal cord sections from fHTK mice and controls were stained for OLIG2 in green and CC1 in red, and the percent of OLIG2+ cells that were also CC1+ was quantified, ± s.e.m., \( p = 0.26 \) by Student's two-tailed t-test.

**e.** Spinal cord sections from fHTK mice and controls were stained for neurofilament heavy polypeptide (NF) in green and CD11b in red, and the number of healthy, blebed and total axons was quantified, ± s.e.m.. Scale bars = 100 µm.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Coll1 reduces OPC migration, but not proliferation or differentiation, in vitro. 
a. Representative images of OLIG2 (green) and PDGFRα (red) staining with EdU labeling (cyan) in rat OPC cultures on PLL, laminin, fibronectin, or collagen I after a 2 hour incubation in 10 uM EdU. 
b. Quantification of the percentage of EdU+ OPCs (OLIG2+PDGFRα+) for cultures represented in a. ± s.e.m., n = 9 replicates. 
c. Representative images of MBP (red) staining in rat OPC cultures on PLL, laminin, fibronectin, or collagen I three days after removal of PDGF-AA. Cell nuclei detected with DAPI (blue). 
d. Quantification of MBP+ cells over total cells (DAPI+) for cultures represented in c. ± s.e.m., n = 9 replicates. 
e. Representative images of PDGFRα (green) staining with DAPI in rat OPC cultures that had migrated through transwells coated with PLL, laminin, fibronectin, or collagen I following a 24 hour incubation. 
f. Quantification of PDGFRα+ cells on the underside of each transwell for the cultures represented in e. ± s.e.m., n = 9 replicates. Data displayed represent 3 replicates of 3 samples, each containing cells from three pooled postnatal day-7 rats. Comparisons were performed using one-way ANOVA with Sidak’s post hoc tests, ****p < 0.0001, ***p < 0.001, *p < 0.05. Scale bars = 100 µm.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | RNA sequencing analysis of Col1a1-GFP⁺ cells from the spinal cord in health and EAE. a-c, MA plots comparing the transcriptome of CNS fibroblasts in health with whole spinal cord tissue (a), CNS fibroblasts in health with CNS fibroblasts EAE D5 (b) or D10 (c) PSO with red dots signifying genes with FDR < 0.1. d, Pathway analysis using DAVID Bioinformatics Resources 6.8, NIAID/NIH, GOTERM_BP_DIRECT for genes with a log2 fold change greater than 2 for CNS fibroblasts EAE D5 PSO compared to CNS fibroblasts in health. e, CPM of collagen genes from the bulk sequencing of whole spinal cord tissue (Whole SC, n = 2), CNS fibroblasts from health (CNS Fibro Health, n = 3) CNS fibroblasts 5 days PSO (CNS Fibro EAE D5, n = 3) and 10 days PSO (CNS Fibro EAE D10, n = 3), ± s.e.m., *FDR < 0.05, **FDR < 0.01 to Whole SC, #FDR < 0.05, ##FDR < 0.01 to CNS Fibro Health. f, Heat map of the expression levels of the top genes differentially expressed in CNS Fibro Health (each expressed at least 1 CPM in each control sample) compared to the whole spinal cord by FDR.
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Software and code

Policy information about availability of computer code

Data collection  no software was used

Data analysis  Single Cell: CellRanger 2.1.1, Seurat v3, SingleR 1.4
  RNAseq: Tophat v 2.0.11, Bowtie 2 v 2.2.1, SAMtools v.0.1.19, HTSeq-0.6.1, DESeq2
  Heat maps: Morpheus (https://software.broadinstitute.org/morpheus/)
  Pathway analysis: DAVID BIOINFORMATICS (https://david.ncifcrf.gov/)
  Statistics: Prism 7, Microsoft Excel 2013
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All RNA sequencing and single-cell sequencing raw and processed data files have been uploaded to GEO and can be accessed using the following accession codes: GSE135186 for Single Cell Transcriptional Profile of Col1a1GFP+ cells in health and EAE, GSE135044 for Transcriptional Profile of Col1a1GFP+ CNS cells in health and EAE, and GSE135185 for single cell transcriptional profile of mouse spinal cord in EAE.
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**Life sciences study design**

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| Sample size | Sample sizes were selected based on variability of the measurement and values of difference between conditions. |
|-------------|----------------------------------------------------------------------------------------------------------|
| Data exclusions | No data were excluded from the analysis. |
| Replication | To verify the reproducibility of the findings RNA sequencing results from each biological sample of each condition were compared and determined to have similar expression patterns. All experiments requiring the quantification of cellular properties in tissue sections contained many tissue sections for at least 3 mice. All attempts at replication were successful. |
| Randomization | Animals where assigned to various experimental groups at random. |
| Blinding | Investigators were blinded to groups during data collection and experimental analysis. |

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**Antibodies**

Primary antibodies used were: Col1 abcam ab21286 1:750, CD11b Bio-Rad MCA711 1:1000, CD45 Bio-Rad MCA1031 1:1000, GFAP abcam ab7260 1:500, CD31 BD Biosciences 553370 1:1000, PDGFRb ebioscience 14-1402-82 clone APBS 1:500, PDGFRa BD Biosciences 558774 clone APAS 1:500, Sox9 abcam ab185966 1:500, Iba1 Wako 019-19741 1:500, Olig2 EMD Millipore AB8610 1:500, NG2 EMD Millipore MAB5384 1:500, CD4 ebioscience 16-004108 1:1000, CD31 abcam ab7778 1:500, Periostin R&D Systems MAB3548 clone 345613, ER-TR7 Novus NB100-64932 1:1000, CD8 clone 53-6.7 eBioscience 14-0081-82 1:1000, Actin, α-smooth muscle Sigma-Aldrich A2547 1:500, Neorufilament heavy polypeptide abcam ab8135 1:1000, Desmin abcam ab8135 1:1000, GFAP abcam ab7260 1:1000, CC1 Calbiochem OP80 1:500, CXCL10 abcam ab9938 1:1000, MBP Bio-Rad/Serotec MCA409S, CD31 AF647 Molecular Probes A14716 1:100. Slides were then washed with D-PBS and incubated at room temperature for 1.5 hours with the following secondary antibodies, all 1:1000 in D-PBS: Goat-anti-Rabbit-Alexa 488 (ThermoFisher A11034), Goat-anti-Rat-Alexa 488 (ThermoFisher A11006), Goat-anti-Rabbit-Alexa 594 (ThermoFisher A11006), Goat-anti-Rabbit-Alexa 594 (ThermoFisher A11006), Goat-anti-Rabbit-Alexa 594 (ThermoFisher A11006), Goat-anti-Rabbit-Alexa 594 (ThermoFisher A11006).

**Validation**

Antibody were validated by manufacturers or in published studies:

- CD31 (clone MEC 13.3, BD Pharmingen 553370), Baldwin, H. S. et al. Platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31): alternatively spliced, functionally distinct isoforms expressed during mammalian cardiovascular development. Development 120, 2539-2553 (1994).
- PDGFRb (clone APBS, ebioscience 14-1402-82), He, L. et al. Analysis of the brain mural cell transcriptome. Sci Rep 6, 35108, doi:10.1038/srep35108 (2016).
- Collagen I (Abcam ab21286), Abcam site WB for purified mouse tendon collagen. https://www.abcam.com/collagen-i-antibody-ab21286.html#description_images_3.
- Collagen III (Abcam ab7778), Briss, B. K. et al. Type III Collagen Directs Stromal Organization and Limits Metastasis in a Murine Model of Breast Cancer. Am J Pathol 185, 1471-1486, doi:10.1016/j.ajpath.2015.01.029 (2015), Abcam https://
CD11b (Bio-Rad MCA711), Rosen H, Gordon S. Monoclonal antibody to the murine type 3 complement receptor inhibits adhesion of myelomonocytic cells in vitro and inflammatory cell recruitment in vivo. J Exp Med. 1987 Dec 1;166(6):1685-701. doi: 10.1084/jem.166.6.1685, https://www.bio-rad-antibodies.com/monoclonal/mouse-cd11b-antibody-sc6-mca711.html?f=purified#references.

CD45 (Bio-Rad MCA1031), Lee DC, Rizer J, Selenica ML, Reid P, Kraft C, Johnson A, Blair L, Gordon MN, Dickey CA, Morgan D. LPS-induced inflammation exacerbates phospho-tau pathology in Tg4510 mice, https://www.bio-rad-antibodies.com/monoclonal/mouse-cd45-antibody-yw62-3-mca1031.html?f=purified#references.

GFAP (abcam ab7260), https://www.abcam.com/gfap-antibody-ab7260.html, cited in 424 references.

PDGFRα (BD Biosciences 558774 clone APA5), Takakura N, Yoshida H, Kunisada T, Nishikawa S, Nishikawa SI. Involvement of platelet-derived growth factor receptor-alpha in hair canal formation. J Invest Dermatol. 1996; 107(5):770-777.

Sox9 (abcam ab185966), https://www.abcam.com/sox9-antibody-epr14335-78-ab185966-references.html#active-tab, cited in 29 references and tested by manufacturer in Western blot, ICH and IF.

Iba1 (Wako 019-19741), reference list from manufacturer: https://labchem-wako.fujifilm.com/us/category/docs/01213_doc02.pdf.

Olig2 (EMD Millipore AB9610), Hambardzumyan, D; Becher, OJ; Rosenblum, MK; Pandolfi, PP; Manova-Todorova, K; Holland, EC, Genes & development 22 436-48 2008, https://www.emdmillipore.com/US/en/product/Anti-Olig-2-Antibody,MM_NF-AB9610#anchor_REF.

NG2 (EMD Millipore MAB5384), http://www.emdmillipore.com/US/en/product/Anti-NG2-Chondroitin-Sulfate-Proteoglycan-Antibody-clone-132.38,MM_NF-MAB5384-I-100UG#documentation, tested by manufacturer in Western blot and immunofluorescence.

CD4 (eBioscience 16-0041081), Tang Y, Xu X, Guo S, Zhang C, Tang Y, Tian Y, Ni B, Lu B, Wang H. An increased abundance of tumor-infiltrating regulatory T cells is correlated with the progression and prognosis of pancreatic ductal adenocarcinoma. PLoS One. 2014 Mar 17;9(3):e91551.

Periostin (R&D Systems MAB3548), Nakama, T., Yoshida, S., Ishikawa, K., Kubo, Y., Kobayashi, Y., Zhou, Y., ..., Ishibashi, T. (2017). Therapeutic Effect of Novel Single-Stranded RNAi Agent Targeting Periostin in Eyes with Retinal Neovascularization. Molecular therapy. Nucleic acids, 6, 279–289.

ER-TR7 (Novus NB100-64932), Mello Coelho, V. d., Bunbury, A., Rangel, L. B., Giri, B., Weeraratna, A., Morin, P. J., ... Taub, D. D. (2009). Fat-storing multilocular cells expressing CCR5 increase in the thymus with advancing age: potential role for CCR5 ligands on the differentiation and migration of preadipocytes. International journal of medical sciences.

CD8 (clone 53-6.7 eBioscience 14-0081-82), https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&products_subtype=antibody_primary&productid=14-0081-82&version=86 cited in 352 references and tested by manufacturer using flow cytometry.

Actin, α-smooth muscle (Sigma-Aldrich A2547), Zhou J1, Lhoták S, Hilditch BA, Austin RC. Activation of the unfolded protein response occurs at all stages of atherosclerotic lesion development in apolipoprotein E-deficient mice. Circulation. 2005 Apr 12;111(14):1814-21.

Neurofilament heavy polypeptide (abcam ab8135) https://www.abcam.com/neurofilament-heavy-polypeptide-antibody-ab8135.html cited in 47 references and tested by manufacturer in Western blot and ICH.

Desmin abcam ab8592 1:1000 specificity tested using immunoblots by abcam on samples from mouse skeletal muscle and heart tissue, cited in 76 publicaions.

CC1 Calbiochem OP80 1:500 tested by manufacturer using cerebellum tissue as a positive control and MOPC 21 cells as a negative control, cited in many publications including McTigue, D.M., et al. 2001. J. Neurosci. 21, 3392, which is used as an application reference for immunofluorescence.

CXCL10 abcam ab9938 1:1000 specificity tested by manufacturer using a Western blot probing for recombinant mouse CXCL10 protein at a concentration gradient as a positive control, cited in 5 publications.

MBP Bio-Rad/Serotec MCA4095 https://images.bio-rad-antibodies.com/datasheets/datasheet-MCA4095.pdf?_ga=2.52809320.1934310161.1597964419-826248813.1597964419 cited in 34 publications.

CD31 AF647 Molecular Probes A14716 1:100 tested by manufacturer in two separate flow cytometry experiments on BALB/c splenocytes.
Animals and other organisms

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

Laboratory animals
- Col1a1GFP mice from David Brenner (C57BL/6 background) were used to label Col1 producing cells for imaging and FACS sorting analysis. Rosa-Isl-tomato mice, Charles River Laboratories 007909 (C57BL/6 background), were crossed to Col1a2CreERT mice, Jackson Labs 029567 (C57BL/6 background), Ngn2CreERT mice, Jackson Labs 008538 (C57BL/6 background), or aSMACreERT2 mice for lineage tracing analysis. B6;129S7-Hprt1tm2(Pgk1-Pac/Tk)Brd/Mmucd, MMRRRC 010860-UCD (C57BL/6Try c-Brd mixed background), (lox-stop-lox-HTK) mice were crossed to Col1a2CreERT for the fibroblasts ablation experiments. Ifngr1fl/fl mice, Jackson Labs 025394 (C57BL/6 background), were crossed to Col1a2CreERT mice for the IFN mechanistic studies. UBC-GFP (004353) reporter mice and CD45.1 (002014) mice used in bone marrow transplant (BMT) experiments were purchased from Jackson Labs. GFAP/tTA mice on the C57BL/6 background were mated with TRE/IFN- on the C57BL/6 background to produce GFAP/tTA;TRE/IFN- double transgenic mice and were maintained in the Popko lab for cuprizone studies. Male mice were used for fHTK experiments as the gene is X linked and for the Col1a1GFP regional quantifications in health. Female mice were used for the bone marrow transplant studies and and the Col1a1GFP cell number in EAE quantifications. Male and female mice were used for all other experiments. Mice were used between 2 and 6 months of age unless otherwise noted, and were housed in a temperature controlled environment (68 to 72 degrees Fahrenheit for UCSD and 40-70% humidity) with a 12 hour light/dark cycle.

Wild animals
- The study did not involve wild animals.

Field-collected samples
- The study did not involve samples collected in the field.

Ethics oversight
- All experiments were performed under IACUC approval at UCSF, UCSD and Northwestern.

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: Homogenates of spinal cord were enzymatically and mechanically dissociated for FACS. Sorting was based on positive selection for GFP fluorescent protein and negative selection with anti-CD31-A647 and dead cell markers.
- Instrument: BD biosciences FACS Aria II
- Software: FACSDiva v8
- Cell population abundance: Purity of collagen-expressing cells was determined post-RNA sequencing by confirming expression levels of collagen genes.
- Gating strategy: From the starting tissue homogenate, FSC/SSC was adjusted to isolate singlet cells then followed by live cell-DAPI/negative selection-647 dump. Gating for collagen-expressing cells (GFP) was set conservatively.

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