Aberrant oligodendroglial-vascular interactions disrupt the blood-brain barrier, triggering CNS inflammation

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Disruption of the blood–brain barrier (BBB) is critical to initiation and perpetuation of disease in multiple sclerosis (MS). We report an interaction between oligodendroglia and vasculature in MS that distinguishes human white matter injury from normal rodent demyelinating injury. We find perivascular clustering of oligodendrocyte precursor cells (OPCs) in certain active MS lesions, representing an inability to properly detach from vessels following perivascular migration. Perivascular OPCs can themselves disrupt the BBB, interfering with astrocyte endfoot and endothelial tight junction integrity, resulting in altered vascular permeability and an associated CNS inflammation. Aberrant Wnt tone in OPCs mediates their dysfunctional vascular detachment and also leads to OPC secretion of Wif1, which interferes with Wnt ligand function on endothelial tight junction integrity. Evidence for this defective oligodendroglial-vascular interaction in MS suggests that aberrant OPC perivascular migration not only impairs their lesion recruitment but can also act as a disease perpetuator via disruption of the BBB.

MS is an autoimmune disease of the CNS associated with demyelination and axonal loss that eventually leads to neurodegeneration, and exhibits many of the features of an inflammatory autoimmune disorder including breakdown of the BBB. BBB disruption is one of the earliest cerebrovascular abnormalities in MS, allowing unrestricted access of immune cells and blood-borne components into the CNS which play a central role in demyelination and axonal damage1–7. Regeneration of damaged myelin can occur in MS, involving a recruitment of migrating OPCs into areas of demyelination from surrounding normal-appearing white matter (NAWM) followed by their differentiation into mature oligodendrocytes8–10, but remyelination is not durable and can fail at either of these stages11–13. Recurrence of BBB breakdown may occur at the same or different locations within intervals of weeks or months, with lesions developing irregularly with additional phases of BBB leakage, immunologically mediated demyelination, and axonal transection. Contributors to BBB dysregulation during the disease are not fully understood. We report here a pathological finding in MS, suggesting a dysfunctional interaction between OPCs and the vasculature, and question its cause and its implication.

Results
Aberrant perivascular clustering of OPCs in MS. MS is characterized by immune infiltration of cells across the BBB into brain parenchyma, and perivascular accumulation of inflammatory cells. Analysis in certain active MS (Fig. 1a and Supplementary Table 1) lesions, however, identifies perivascular clusters of cells around CD31+ vasculature (Fig. 1b–j), which separate from markers for inflammatory cells (Fig. 1d). These perivascular clusters express the oligodendroglial marker OLG2 (Fig. 1e,f,i) and the marker NKX2.2 (Fig. 1l). They also express the Wnt pathway marker RNF43 (Fig. 1g and Supplementary Fig. 1a–c), previously shown to be a target of Wnt in OPCs that is activated under high Wnt tone14,15, and have the typical bipolar morphology of OPCs. Perivascular OPC clusters were not seen in NAWM (Fig. 1n) or normal-appearing gray matter (NAGM) (Fig. 1m) from the MS patients analyzed, and while isolated OPCs are often seen in close association with blood vessels in the core of chronic MS lesions (Fig. 1j,k), the finding of perivascular clustering is significantly more frequent in active inflammatory areas (Fig. 1o). Cluster frequency, appearance, and size were variable, presumably reflecting significant differences in MS lesion age and history (Fig. 1o and Supplementary Table 1).

OPC perivascular clustering represents a defective single cell migration. The underlying cause of this pathological finding in MS is unclear. OPCs are known to associate intimately with vasculature during a developmental phase, where they use blood vessels as a critical physical scaffold for single cell migration16. They maintain the ability in adulthood to be activated and migrate significant distances in response to injuries, and we find that they retain this capacity for single cell perivascular migration in remyelination (Fig. 2a–d and Supplementary Figs. 2–4). There is a markedly increased association of individual PDGFRα+ (Fig. 2c; 1 d versus 0 d P = 9.66 × 10−7) and Olig2+ OPCs with blood vessels at lesion borders at early times during murine remyelination of spinal cord and corpus callosum (Fig. 2a–c and Supplementary Fig. 3a,b). Live imaging of these OPCs in lesioned adult NG2creERT/Tomato mice, following intracardiac infusion of fluorescein-lectin for vessel labeling, identifies migration at lesion edges limited to an early lesion time window (Fig. 2d and Supplementary Figs. 3d–h and 4). Motile OPCs responding to...
demyelination at 1.5-days post lesioning (dpl) lesion edges demonstrate single cell perivascular migration, with directed crawling motility along (Fig. 2d, Supplementary Fig. 3h, and Supplementary Video 1) and jumping toward/between (Supplementary Fig. 3d,h and Supplementary Video 2) vasculature, while tending to move in the direction of the injury (Supplementary Fig. 3g).

OPCs use single cell perivascular migration for their recruitment into areas of demyelination, and their perivascular clustering is therefore not a feature that is seen in wild-type (WT) murine white matter injury. OPC association with blood vessels during murine developmental migration is mediated by a Wnt-driven upregulation of Cxcr4 in OPCs 13, and the finding of individual perivascular OLIG2

\[\text{OLIG2}^{+}\text{cells}\] expressing \[\text{CXCR4}\] in human white matter injury (Supplementary Fig. 3i) suggests a conserved mechanism of attraction to the endothelium. To address whether the perivascular clustering of OPCs in MS represents a dysfunctional migration, and an involvement for overactive Wnt signaling in this aberrant vascular interaction, we performed focal demyelination in adult inducible

Fig. 1 | Perivascular clustering of OPCs in MS. a, Schematic of human MS showing lesions throughout the brain. MS cases assessed are outlined in Supplementary Table 1. b, Low magnification of MS Case 1 active lesion, with Luxol fast blue (LFB) to assess demyelination and LN3 immunohistochemistry to assess inflammatory cell activity. c, Boxed area in b shows area of magnified view in c, and from where images c-g were taken. d, Magnified region from Case 1 showing a cluster of cells (unfilled arrow) which do not colocalize with inflammatory marker LN3 (filled arrows). e-g, Clustered perivascular OLIG2

\[\text{OLIG2}^{+}\text{cells}\] (e and f, arrows) and clustered RNF43

\[\text{RNF43}^{+}\text{cells}\] (g, arrow) in Case 1 active lesion. h,i, Clustered OLIG2

\[\text{OLIG2}^{+}\text{cells}\] (arrows) on blood vessels in active lesions of MS Cases 2 (h) and 3 (i). j, MS Case 4 chronic active lesion, with boxed areas showing regions where images from k and l were taken. k, Chronic core of lesion showing hypocellularity and limited LN3

\[\text{LN3}^{+}\text{activity}\]. Example of isolated OLIG2

\[\text{OLIG2}^{+}\text{cell}\] (arrow) in close association with blood vessel. l, Active edge of chronic active lesion with significant LN3

\[\text{LN3}^{+}\text{inflammatory activity}\] and perivascular clustering of NKX2.2

\[\text{NKX2.2}^{+}\text{cells}\] (arrows). m,n, Clustering of OLIG2

\[\text{OLIG2}^{+}\text{cells}\] is not seen in NAGM or NAWM from these patients. o, Frequency of OLIG2

\[\text{OLIG2}^{+}\text{clusters}\] seen in NAGM, NAWM, and areas of chronic inactive and active lesions from the patients described in Supplementary Table 1. Data were analyzed by one-way ANOVA. The measure of center represents the mean. Active versus Chronic, \(P = 0.04\); Active versus NAWM, \(P = 0.003\); Active versus NAGM, \(P = 0.003\). Scale bars, 2.2 mm (b), 90 \(\mu\)m (c, k left panel, l left panel), 20 \(\mu\)m (d-i, m, n, k right panel, l right panel), and 750 \(\mu\)m (j). *\(P < 0.05\), **\(P < 0.01\).
PDGFRα-creERT2:APCloxP/loxP and Olig2-cre:APCloxP/loxP mice, which have excessive Wnt activation in OPCs due to the conditional loss of the obligate pathway repressor adenomatous polyposis coli (APC). We find that OPCs accumulate as perivascular clusters at 10-dpl lesion edges during remyelination in both PDGFRα-creERT2:APCloxP/loxP (Fig. 2f) and Olig2-cre:APCloxP/loxP (Fig. 2g) mice. These clusters are not seen in control littermate remyelination (Fig. 2a,b,c), and have excessive Wnt tone evidenced by significantly increased Axin2 messenger RNA expression (Supplementary Fig. 5a–d) and GFP expression in PDGFRα-creERT2:APCloxP/loxP:Rosa-GFP (Fig. 2h and Supplementary Fig. 5e,f). We find reduced OPC numbers at early 3-dpl time points in lesion centers in both PDGFRα-creERT2:APCloxP/loxP (Supplementary Fig. 6a,c) (P = 0.0003) and Olig2-cre:APCloxP/loxP mice (Supplementary Fig. 6a,b) (P = 0.0013), without effects on OPC proliferation (Supplementary Fig. 6e–h), suggesting that an increased OPC association with vasculature (Supplementary Fig. 6d), in the form of perivascular clusters (Fig. 2e), leads to a reduced ability for proper recruitment to the lesion core. These results indicate that OPC perivascular clustering following demyelination represents an aberrant OPC migration on vessels and is mediated by defective Wnt signaling.

**OPC perivascular clusters disrupt the BBB.** We questioned how this dysfunctional OPC perivascular migration and vascular...
detachment failure affects the integrity of the underlying vessel. Injury models themselves cause significant vascular damage. We therefore required a non-injury model to identify the specific effects of an OPC perivascular cluster in the absence of any injury-induced vascular disruption. We used Olig2-cre:APC\textsuperscript{Stop/Stop} mice, which have defective OPC vascular detachment postnatally, with perivascular accumulations appearing in a non-injury setting that closely resemble the clusters seen in both human white matter injury (Supplementary Fig. 7) and following demyelination in PDGFRa\textsuperscript{creERT2}:APC\textsuperscript{Stop/Stop} and Olig2-cre:APC\textsuperscript{Stop/Stop}. In this non-injury setting, we find that aberrant oligodendroglial–vascular interactions interfere with several aspects of BBB integrity. OPC perivascular clusters interfere with astrocyte–vascular interactions by altering the positioning of astrocyte endfeet on vasculature. Use of Aldh11i1-GFP mice (which label astrocytes and their endfeet) (Fig. 3a), shows that, when crossed into Olig2-cre:APC\textsuperscript{Stop/Stop} mice, there are significant astrocyte endfoot placement deficits (P = 1.56 × 10\textsuperscript{-2}) in areas where OPCs are clustered around blood vessels (Fig. 3b,c,g). Three-dimensional (3D) reconstruction of astrocytes and OPCs on vessels in Olig2-cre:APC\textsuperscript{Stop/Stop};TiTomato:Aldh11i1-GFP mice suggests a competition for space on the vessel surface and a physical displacement of astrocyte processes from vasculature at sites of OPC clustering (Fig. 3d–f). This is also seen as significant gaps in staining for Aquaporin-4 (Aqp4) (Fig. 3h–i and Supplementary Fig. 8) (P = 1.47 × 10\textsuperscript{-2}), a water channel highly enriched in astrocyte endfeet at the BBB. 3D reconstruction of perivascular clusters from 10-dpl lesion edges in PDGFRa\textsuperscript{creERT2}:APC\textsuperscript{Stop/Stop} mice following focal demyelination (Supplementary Fig. 9) suggests a similar mechanism of endfoot displacement, whereby the processes of perivascular clustered OPCs can actually exist between endothelial cells (ECs) and overlying astrocyte endfeet.

In addition to astrocyte disruption, OPC perivascular clusters also cause EC dysfunction. Staining for plasmalemma vesicle-associated protein (PLVAP), a structural component of endothelial fenestrae, but gaps in staining for claudin5 (Cldn5) (Fig. 4a–c), a structural component of EC tight junctions, identifies areas of non-intact BBB around OPC clusters (Fig. 4a–c). In contrast to astrocyte coverage, pericyte coverage of vessels seems unaffected by OPC clustering (Supplementary Fig. 10), but does not rule out functional deficits in these cells. The effects on astrocyte endfoot placement and EC tight junction integrity suggested possible effects on the barrier function of the BBB. We assessed leakage of the soluble 340-kDa serum glycoprotein fibrinogen from vessels, as well as leakage of a 10-kDa dextran-tetramethylrhodamine tracer 6 hours post tail vein injection. Both of these are detectable only within the lumen of vessels in control mice (Fig. 4d,g), but there are significant increases in the amount seen in CNS parenchyma around OPC clusters (arrows in Fig. 4e,h) in Olig2-cre:APC\textsuperscript{Stop/Stop} mice (Fig. 4f, P = 0.0026; Fig. 4i, P = 6.46 × 10\textsuperscript{-4}), suggesting extravasation of blood components and further supporting the notion that BBB integrity is affected at these areas.

OPC perivascular clusters trigger a CNS inflammation. OPC effects on the barrier function of the BBB trigger a CNS inflammatory reaction. We found increased numbers of cells expressing Iba1 (which stains microglia and macrophages) which have a less processed and more globose morphology (Supplementary Fig. 11a, b). Pronounced CD11c and F4/80 staining in cells around perivascular OPCs (Fig. 5a,b,d and Supplementary Fig. 11b), which is not present in control littermate mouse brains (Fig. 5a,b,d and Supplementary Fig. 11a), also identifies activated microglia/macrophages. These cells expressed high levels of iNOS rather than Arg-1 (Supplementary Fig. 11b), suggesting an M1-polarized population. Use of Cx3cr1-GFP:CCR2-RFP mice crossed into the Olig2-cre:APC\textsuperscript{Stop/Stop} suggests that this represents predominantly a microglial activation (Fig. 5c and Supplementary Fig. 11c,d) rather than recruitment of activated macrophages from the circulation. However, small numbers of RFP\textsuperscript{+} macrophages (in the CCR2-RFP mice, Supplementary Fig. 11d, e) and CD3-expressing T cells, including CD4 and CD8 subsets, were observed within the perivascular clusters (Fig. 5e–g), suggesting extravasation of inflammatory cells into surrounding brain parenchyma in addition to protein leakage. Effects on the barrier function of the BBB lead to injury to both surrounding axons and perivascular OPCs. SM132\textsuperscript{+} axonal spheroids, indicating swellings of damaged axons, are significantly increased (P = 0.0286) and identify axonal degeneration adjacent to perivascular clusters (Fig. 5h–j). OPCs themselves also undergo significant cell death within clusters (P = 0.0009) (Fig. 5l–n), leading to a resolution over time of the aggregations (Fig. 5k and Supplementary Fig. 12a–c), with reinvestment of astrocyte endfeet (Fig. 3c and Supplementary Fig. 12b) on blood vessels and reduction in microglial activation (Supplementary Fig. 12d).

To further assess the contribution of OPC perivascular clusters as a cause of BBB disruption, we performed an in vivo pharmacological blockade of the chemokine mediating their vascular association. Postnatal treatment of Olig2-cre:APC\textsuperscript{Stop/Stop} intravenously with the Cxcr4-Sdf1 inhibitor AMD3100 leads to significant reversal of OPC cluster size on vasculature (P = 1.04 × 10\textsuperscript{-2}) (Supplementary Fig. 13a–c). This led to concomitant reductions in the number of vessels lacking Aqp4 (P = 0.0001) (Supplementary Fig. 13g–i) and endfoot coverage (P = 0.0008) (Supplementary Fig. 13d–f), and significant normalization of fibrinogen extravasation (P = 0.0023) (Supplementary Fig. 13j–l) and CD11c\textsuperscript{+} cells (P = 0.0044) (Supplementary Fig. 13m–o). These results identify a requirement for the physical presence of OPC clusters on blood vessels for effects on vascular integrity, and show that failure of OPC detachment from vasculature can itself disrupt the BBB and trigger CNS inflammation, in an otherwise non-inflammatory setting.

Wnt inhibitory factor 1 (Wif1) production by Wnt-activated perivascular OPCs disrupts EC tight junctions. OPC perivascular clusters cause disruption of cells at the vascular surface, and their physical presence on vessels is required for BBB effects. We questioned how these perivascular OPCs might further contribute to effects on the barrier function of the BBB via local effects resulting from physical proximity to the endothelium. Excessive Wnt signaling in OPCs mediates their perivascular accumulation. To uncover factors upregulated in clustered perivascular OPCs that might have effects on neighboring endothelium, we performed RNA sequencing (RNAseq) profiling of spinal cord white matter from postnatal day 4 (P4) and P9 Olig2-cre:APC\textsuperscript{Stop/Stop} mice (Supplementary Fig. 14a). Differential expression analysis compared with APC\textsuperscript{Stop/Stop} controls identified 270 genes significantly altered (false discovery rate (FDR) < 0.05) at P4 (43 upregulated and 227 downregulated genes in Olig2-cre:APC\textsuperscript{Stop/Stop}) (Supplementary Fig. 14b), and 4,505 genes at P9 (2,092 up and 2,413 down in Olig2-cre:APC\textsuperscript{Stop/Stop}). We identified Wif1 as the most upregulated secreted factor in this tissue at both time points (FDR < 2.13 × 10\textsuperscript{-4} at P4, FDR < 3.79 × 10\textsuperscript{-8} at P9) (Supplementary Fig. 14c). We found Wif1 mRNA expression markedly increased in Wnt-activated perivascular OPCs in spinal cord (Fig. 6a and Supplementary Fig. 15g; P = 2.67 × 10\textsuperscript{-4} and corpus callosum (Fig. 6b,d) of Olig2-cre:APC\textsuperscript{Stop/Stop} mice, in cultured OPCs from these same mice (Fig. 6c; P = 5.43 × 10\textsuperscript{-4}), and in WT OPCs treated with Wnt3a (Fig. 6c; P = 0.0093). Wif1 protein was also significantly increased on western blot in OPCs isolated from Olig2-cre:APC\textsuperscript{Stop/Stop} compared with controls (Supplementary Fig. 15a,b) (P = 0.0286). WIF1 is also expressed by OPCs in MS lesions. We find WIF1 mRNA expression in OLIG2\textsuperscript{+} clusters in MS (Fig. 6e,f), and evidence of WIF1 protein expressed by cells around the vasculature in active MS lesions (Fig. 6g and Supplementary Fig. 1d). Wif1 is directly upregulated by Wnt signaling in a number of cell types, acting as a negative feedback control mechanism of
It is a secreted protein that functions as a paracrine inhibitor of the pathway by binding, and inhibiting the activity of, extracellular Wnt ligands. As Wnt ligands are important for the induction and maintenance of barrier properties in ECs, establishing a paracrine loop that maintains the BBB, we reasoned that high local production of Wif1 by Wnt-activated perivascular OPCs might affect EC tight junction integrity. We found that treatment of primary CNS ECs with Wif1 significantly reduced their expression of Cldn5 tight junctions (Fig. 6h-i and Supplementary Fig. 15c,d), and counteracted the effects of Wnt
ligands ($P = 3.03 \times 10^{-6}$) in inducing barrier markers in these cells (Fig. 6h,i,m and Supplementary Fig. 15c,d). This was accompanied by a reduction in Wnt tone in ECs (evidenced by significantly reduced $Axin2$ expression, $P = 0.0338$), and increased expression of the endothelial fenestrae marker PLVAP ($P = 1.46 \times 10^{-11}$) (Fig. 6m). Similar effects on tight junction formation, barrier marker expression, and Wnt tone in ECs were observed when treated with conditioned medium from Wnt hyperactive (Olig2-cre:APC$^{loxP/loxP}$) OPCs (Fig. 6j,k,n and Supplementary Fig. 15e,f). Selective depletion of secreted Wif1 protein from this conditioned medium, using anti-Wif1 antibody-mediated pull down (Fig. 6l), prevented these effects on endothelium (Fig. 6j,k,n and Supplementary Fig. 15e,f),
Fig. 5 | OPC perivascular clusters trigger a CNS inflammation. a,b. Marked upregulation of CD11c expression in P9 Olig2-cre:APC\textsuperscript{loxP/loxP} brain compared with controls around OPC perivascular clusters (stained with DAPI), quantified in b (n=4 animals; P=0.009). Data were analyzed by unpaired two-sided Student’s t-test. c. As CD11c can detect some other immune cells in addition to activated microglia and macrophages, we made use of CX3CR1-GFP:CCR2-RFP mice (which label microglia green and macrophages red) crossed into Olig2-cre:APCloxP/loxP mice, identifying these cells as predominantly activated microglia around OPC clusters. d. Frequency of F4/80-expressing cells at different postnatal times in CC in Olig2-cre:APCloxP/loxP mice versus controls (n=6 animals at each time; all statistical analyses compare Olig2-cre:APCloxP/loxP with controls at each time; P9, P=2.28 × 10\textsuperscript{-6}; P12, P=1.94 × 10\textsuperscript{-8}; P16, P=1.39 × 10\textsuperscript{-8}; P30, P=0.5490). Data were analyzed by unpaired two-sided Student’s t-test. e,f. Extravasation of small numbers of CD2\textsuperscript{+}, CD4\textsuperscript{+}, and CD8\textsuperscript{+} T cells within OPC perivascular clusters in P9 Olig2-cre:APCloxP/loxP mouse brain (f), which are not seen in APC\textsuperscript{loxP/loxP} controls (e). g. Quantification of CD3\textsuperscript{+} cell numbers per perivascular cluster in Olig2-cre:APCloxP/loxP mice versus controls (n=6 animals; P=0.0006). Data were analyzed by unpaired two-sided Student’s t-test. h,j. SMI32\textsuperscript{+} (non-phosphorylated neurofilament) axonal spheroids colocalize with the neurofilament marker NF200 (NF), indicating swellings of damaged axons adjacent to an OPC perivascular cluster (arrows in l, stained with DAPI) in Olig2-cre:APCloxP/loxP P9 brain, which are not seen in controls (h). i. Quantification of SMI32\textsuperscript{+}/NF200\textsuperscript{+} axonal dots within 100 μm distance of clusters in Olig2-cre:APCloxP/loxP P9 corpus callosum (CC) versus similar areas in APC\textsuperscript{loxP/loxP} mice (n=4 animals; P=0.0286). Data were analyzed by unpaired two-sided Student’s t-test. k. Quantification of OPC perivascular cluster size in Olig2-cre:APCloxP/loxP:TdTomato CC at times between P9 and P30 (n=6 animals; all statistical analyses compared with P9; P12 versus P9, P=0.3846; P16 versus P9, P=0.0007; P20 versus P9, P=3.37 × 10\textsuperscript{-4}; P30 versus P9, P=2.55 × 10\textsuperscript{-4}). Data were analyzed by unpaired two-sided Student’s t-test. l. TdT-mediated dUTP nick end labeling (TUNEL) staining in P9 mouse brain colocalizes with PDGF-Rx (in Olig2-cre:APCloxP/loxP mice) (l) and with TdTomato (in Olig2-cre:APCloxP/loxP:TdTomato mice, arrows in m), indicating OPC death within perivascular clusters. m. Percentage of TdTTomato\textsuperscript{+} cells that are TUNEL\textsuperscript{+} in perivascular clusters versus non-clusters in Olig2-cre:APCloxP/loxP:TdTomato mice (n=6 animals; P=0.0009). Data were analyzed by unpaired two-sided Student’s t-test. Scale bars, 10 μm (l), 20 μm (all other panels). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. Values are mean ± s.d.
identifying it as the mediator of these effects on tight junction marker expression and Wnt pathway tone in ECs. Wif1 also functionally regulates the barrier properties of endothelium (Fig. 6a), as Wif1 treatment of ECs in an in vitro BBB permeability assay significantly increases the permeability to a 10-kDa dextran-tetramethylrhodamine dye and significantly counteracts the action of Wnt ligands to reduce barrier permeability. Evidence of WIF1 expression in Olig2+ clusters in human white matter injury (Fig. 6e-f) suggests that high local production of this Wnt inhibitor by perivascular OPCs may also contribute to dysfunction of EC barrier properties in MS.

**Discussion**

Recruitment of OPCs into areas of demyelination is a critical initial step for successful myelin regeneration, and dysfunction of this process contributes to remyelination failure in MS12. We show here that OPCs utilize single cell perivascular migration on microvessels for recruitment into murine demyelinated injury. This perivascular migration following injury appears to be a recapitulation of their developmental migration, where OPCs require a vascular scaffold for their dispersal through the CNS13. They show a significantly increased association with vasculature at lesion edges at early time points during remyelination, along with morphological changes more akin to their developmental counterparts. Live imaging studies in murine remyelination suggest a limited time window of OPC migration into lesions around 1.5 dpl, with the majority of OPC numbers in lesions made up from proliferation once they have migrated into the intrasellar environment.

Completion of OPC perivascular migration requires their detachment from vessels to allow for oligodendrocyte differentiation and interaction with axonal targets. We find in several MS cases, in lesion areas with active inflammation, that OPCs can be found clustered on vasculature, representing a defect in single cell perivascular migration and inability to detach from blood vessels. We do not see this OPC perivascular clustering in WT murine remyelination, and it therefore seems to represent a feature that distinguishes human from murine white matter injury. This OPC perivascular clustering is mediated by aberrant Wnt tone in remyelination, as evidenced in PDGFRα-creERT2:APC<sup>cre-mediated</sup> and Olig2-cre:APC<sup>cre-mediated</sup>. Importantly, it both affects proper OPC dispersal into remyelinating lesions and exposes them to further damage from blood-borne inflammatory insults.

Failure of OPC detachment from vasculature following migration represents a pathological oligodendrogial–vascular interaction and detrimentally affects the underlying vessel. We find in both development and remyelination that OPC perivascular clusters disrupt other cells at the microvessel surface, causing physical displacement of astrocyte endfeet from vessels, with the processes of clustered OPCs seeming to exist between endothelium and overlying astrocyte endfeet. While pericyte coverage seems unaltered, it will be important to assess how these cells might be functionally affected, considering critical roles in formation of the BBB<sup>7</sup> and emerging roles in CNS remyelination<sup>9,10</sup>. To determine the specific effects of these OPC clusters on the integrity of underlying vasculature, we had to make use of a non-injury model, as injury models by their very nature induce significant vascular disruption. We find, in an otherwise non-inflammatory setting, that OPC perivascular clusters themselves cause endothelial disruption, and BBB integrity defects that trigger a subsequent CNS inflammation. Vascular integrity defects lead to damage to surrounding axons and perivascular OPCs, identifying OPC clusters themselves as contributors to pathology.

Pharmacological reversal of OPC perivascular clustering with AMD3100 (which inhibits the OPC–vascular Cxcr4/Sdf1 interaction without altering OPC Wnt tone) in vivo in Olig2<sup>cre-mediated</sup> shows that these clusters are required for detrimental BBB effects, and moreover that their physical presence on vessels is necessary for these effects, presumably through disruption of cells at the vascular surface. In addition, we identify Wif1, previously reported to disrupt the BBB in Wnt-driven medulloblastoma<sup>8</sup>, as a factor secreted by OPCs in Wnt-driven perivascular clusters that may contribute to endothelial dysfunction in MS. Wif1 secreted by Wnt-activated OPCs acts to counteract the effects of Wnt ligands in promoting endothelial tight junction markers, acts to increase permeability of endothelium, and also acts to reduce Wnt tone in ECs. Considering that Wnt tone in CNS vessels has been implicated in the restoration of...
of BBB integrity in MS, and also in limiting immune cell infiltration into the CNS\textsuperscript{22}, the local production of Wif1 by dysregulated perivascular OPCs could further contribute to pathology in MS. There may be a variety of contributors to BBB dysregulation in MS that are not fully understood. It will be important to establish the contribution of this aberrant oligodendroglial–vascular interaction to the failed OPC recruitment seen in many MS lesions, and to perpetuation of disease through ongoing disruption of the BBB.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and
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Author contributions

J.N. and S.P.J.F conceived the study. J.N. and S.P.J.F designed the experiments and analyzed the data. J.N. performed most of the experiments. S.P.J.F performed experiments in human MS tissue. H.H.T. assisted with live imaging experiments. K.K.H. assisted with tissue processing and staining. N.H. and G.Y. assisted with 3D reconstruction of confocal images. S.E.B. and K.K. performed analysis of the mRNAseq data. L.X. contributed to discussion. J.R.C. helped design some of the experiments and contributed to discussion. J.N. and S.P.J.F wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Methods

Mice. Animal husbandry and procedures were performed according to University of California at San Francisco guidelines under Institutional Animal Care and Use Program (IACUC)-approved protocols. Mouse identifiers are in the main text and figure legends. Both mouse sexes were used in this study. Mice were randomly assigned to the different experimental groups.

NG2-CreERT;tdTomato. This mouse has been described previously10. We used 8-week-old mice to label OPCs in slice cultures. Before slice culture, the mice were given tamoxifen (7.5-648, Sigma) gavage with 1 mg of tamoxifen twice a day for 5 consecutive days (10 mg in total).

Olig2-cre. A multi-functional mouse line was constructed previously11 by inserting fva, an avian-specific retroviral receptor, and an IRES-cre recombinase cassette into the endogenous Olig2 locus by homologous recombination. This Olig2-cre allows for cre-mediated activity in oligodendrocyte lineage cells.

PDGFRα-creERT2. These mice have been described previously12. A tamoxifen inducible cre recombinase gene was inserted into exon 2. These mice were used to cross with APCloxPloxP mice. To knock out APC, the mouse was given tamoxifen gavage as described above.

APCloxPloxP. These mice have been described previously13. A conditional (loxP- flanked) allele of APC was provided by Dr. R. Fodde (Leiden University). Through intercrosses with Olig2-cre or PDGFRα-creERT2 recombinase we can achieve conditional knockdown of APC in OPCs. We used a breeding strategy of crossing either Olig2-creAPCloxPloxP or PDGFRα-creERT2APCloxPloxP mice with APCloxPloxP mice, thus generating Olig2-creAPCloxPloxP or PDGFRα-creERT2APCloxPloxP offspring and APCloxPloxP control littermates. Control littermates are written as APCloxPloxP in figures.

Aldh1l1-GFP. These mice have been described previously14. The diffuse expression pattern of the Aldh1l1-GFP throughout the CNS is consistent with the endfeet in our study.

Cx3cr1-GFP:CCR2-RFP. These mice have been described previously28. The expression pattern of the Aldh1L1-GFP throughout the CNS is consistent with the endfoot in our study.

Live imaging of remyelination in slice culture. Demyelination was produced in mouse spinal cords in vivo, as described above. Live imaging was performed on slices taken from spinal cord lesions at various times after lyssolecithin-induced demyelination in vivo. Images shown in Fig. 2 and Supplementary Figs. 2 and 3 were from slices of cord taken at 1.5 dpi. To label the shape of the vasculature, we injected Fluorescent-lectin (100 µl, Vector Labs DL-1174) via tail vein injection. After 10 min, allowing dye circulation, spinal cords were immediately transferred to ice-cold artificial cerebrospinal fluid. Spinal cords were then embedded in 5% low-melting-point agarose in artificial cerebrospinal fluid, and vibratome-sliced at 300 µm. Slices were transferred onto Millicell-CM slice culture inserts (Millipore) over culture medium (FluoroBrite DMEM with 25% horse serum, 1% N2 supplement, 1% penicillin and streptomycin) in glass-bottom 6-well plates and incubated at 37°C, 5% CO₂, for at least 1 h. The plates were then transferred to an inverted Leica TCS SP5X confocal with an on-stage incubator (while streaming 5% CO₂, 5% O₂), and imaging with a x10 immersion objective (numerical aperture = 0.3) at specified interval and total imaging time in texts with intermittent repositioning of the focal planes. Lesion boundaries were assessed using DAPI staining following the completion of live imaging. The time-lapse images were processed in Imaris (Bitplane).

Human MS. Human MS postmortem tissue blocks were provided by the UK Multiple Sclerosis Tissue Bank at Imperial College London. All tissues were collected following fully informed consent by the donors via a prospective donor scheme following ethical approval by the London Multicentre Research Ethics committee (MREC 02/2/39). MS lesions were characterized as described previously15, using Luxol fast blue to assess demyelination, SMI-31 immunohistochemistry to assess preservation of axons, and I3 immunohistochemistry to assess loss of inflammatory cell-associated neurofilaments. SMI31 to assess florid parenchymal and perivascular inflammatory cell infiltration, myelin fragmentation, and demyelination with indistinct margins were classified as active plaques. Chronic active plaques were classified as those with extensive demyelination, well-demarcated borders, and abundant inflammatory cells at the lesion edge. Chronic inactive plaques were classified as those with extensive demyelination and well-demarcated borders, but few or no inflammatory cells in any part of the demyelinated area. Chronic lesions quantified in Fig. 1o and Supplementary Fig. 1a were chronic inactive areas. Areas of NAWM and NAGM from each patient were also assessed. Areas of NAGM and NAWM did not demonstrate increased inflammatory activity, but it is not possible to completely rule out other subtle pathology in these areas. Cases assessed are described in Supplementary Table 1.

Dextran-tetramethylrhodamine tracer to assess in vivo barrier function of BBB. Dextran-tetramethylrhodamine tracer (Dextran-Tetramethylrhodamine, 10 kDa, D8187, Thermofisher) was injected into mice through tail vein injection, and allowed to circulate for 6 h. Brain and spinal cord were then dissected immediately and fixed by immersion fixation overnight in 4% paraformaldehyde (PFA) to immobilize the tracers at the end of the experiment. The dextran-tetramethylrhodamine tracer leakage was assessed within frozen sections.

In vitro BBB permeability assay. Primary cultured ECs were cultured on the apical surface of 3-µm pore size, collagen type I and fibronectin precoated Transwell filter inserts (CLS3472, Corning). After treatments, 100 mg ml⁻¹ l-κda dextran-tetramethylrhodamine was added to the upper chamber. The EC in vitro barrier was then incubated at 37°C for 1 h. The relative fluorescence intensity of Rhodamine that had passed across the barrier into the lower chamber was measured with a FilterMax F5 microplate reader. Results shown are the mean fluorescence intensity of three independent experiments. The permeability of a blank insert was used as the positive control.

OPC culture and collection of conditioned medium. Mouse OPCs were isolated by immunopanning from PrAPCloxPloxP mouse and Olig2-creAPCloxPloxP mouse cortices as previously described10. Briefly, mouse brain cerebral hemispheres were minced and dissociated with papain at 37°C for 60 min. After trituration, cells were incubated at room temperature sequentially on three immunopanning dishes: Ran-2, GalC, and O4. The purified mouse OPCs were plated onto 10-cm dishes and cultured for another 4 d. The cultured medium was filtered with a 0.22-µm filter (Millipore), then collected as conditioned medium for downstream experiments or stored at −80°C. The conditioned medium from APCloxPloxP control OPC cultures was labeled as WT-CM; the conditioned medium from Olig2-creAPCloxPloxP cultures was labeled as APC-CM.

Wif1 depletion from conditioned medium and Wif1 enzyme-linked immunosorbent assay (ELISA). To deplete Wif1 from OPC conditioned medium, PrAPCloxPloxP control or Olig2-creAPCloxPloxP OPC conditioned medium was incubated with Wif1 antibody (1:100, rabbit, ab155101, Abcam) at 4°C overnight followed by pull down with agarose beads (sc-2003, Santa Cruz). BSA was used as the non-antibody control. The purified Wif1-depleted conditioned media were then used for EC treatment. To clarify the concentrations of Wif1 protein in the conditioned media before and after Wif1 depletion, four groups of conditioned media (WT-CM + BSA, Olig2-creAPCloxPloxP CM + BSA, WT-CM + Ab, and Olig2-creAPCloxPloxP CM + Ab) were examined using the Wif1-specific ELISA kit (mouse WIF1 PicoKine ELISA Kit, EK1523, Boster) according to the manufacturer’s instructions.
The optical density values were determined by measuring the absorbance at 450 nm using the microplate reader (Bio-RAD, Model 680). Independent experiments were performed in triplicate.

**Western blot.** Western blot was also used to quantify Wif1 and Claudin-5 expression. Protein samples were separated on 10% SDS–PAGE gels, transferred to nitrocellulose membranes, and probed with antibody against Wif1 (1:1,000, rabbit, ab155101, Abcam) or Claudin-5 (1:500, mouse, 352588, ThermoFisher). Mouse heart tissue lysate was used as the positive control. Quantification of band intensity was analyzed using the Image-Pro Plus software.

**EC culture and treatment.** For in vitro EC treatment, primary cultured rat ECs were isolated from P14 Sprague Dawley rat cortices as previously described, and cultured on collagen type I and fibronectin precoated coverslips for immunostaining and for quantitative PCR (qPCR). Cultured ECs were treated with recombinant Wif1 (10 ng/ml; ab208465, Abcam), recombinant Wnt3a (100 ng/ml−1, 315-20, Propeptide), or Wif1 with Wnt3a for 2 d. PBS was used as a control. To test the effects of conditioned media on ECs, cultured ECs were treated with conditioned media with or without Wnt3a depletion (25% fresh medium +75% conditioned medium) for 2 d.

**qPCR.** Total RNA was isolated using RNeasy Plus Mini Kit (74134, Qiagen). qPCR was performed with the C1000 Touch Real-time PCR Detection System (Bio-Rad) and GoTaq qPCR Master Mix (Promega). The oligonucleotide primers, amplification procedure, and melt curve analysis were performed. For each sample, independent repeats were performed in triplicate.

**mRNAseq of Olig2-cre:APCloxP/loxP mice.** Whole spinal cord was dissected from three Olig2-cre:APCloxP/loxP mice and three APC−/− littermate control mice at both P4 and P9 time points. An RNAseq library was prepared using QuantSeq kit (Lexogen) according to manufacturer’s instructions and sequenced on an Illumina HiSeq 4000. Sequence reads were mapped to the mouse genome reference (GRCm38) with genome annotation (M14) and we counted number of reads using STAR aligner v2.5.a. A differential expression analysis was performed on read counts using DESeq2. Based on PCA (principle component analysis) plot (Supplementary Fig. 14a) and a sample-to-sample distance heatmap, we removed outlier samples that were clustered with different conditions. Pairwise comparisons were performed between Olig2-cre:APCloxP/loxP and APC−/− littermate controls, and between P4 and P9. We obtained FDR-adjusted P value and log2 fold change. Genes were selected by significance threshold of FDR <0.05, log2 (fold change) >1, or log2 (fold change) <−1.

**Quantifications.** **OPC quantifications.** For the OPC–vessel association analysis, PDGFRA/CD31 double staining was used to detect the association of OPCs with blood vessels. The percentage of PDGFRA+ cells with their cell bodies directly in contact with blood vessels was quantified.

To quantify OPC process and blood vessel correlation, we used the Imager-Pro Plus 5 software to measure the value of the angle between OPC leading process and blood vessel. This measurement was only done for OPCs in which the cell body was on a blood vessel and the leading process was clearly seen. Oriana software was used to draw the vector map for summarizing the correlation of OPC and blood vessel. For the OPC leading process direction analysis, we used the Imager-Pro Plus 5 software to draw a vector between the OPC cell body and the end of the leading process in the direction of the OPC’s leading process. This leading process direction analysis was only done for OPCs in which the cell body was on a blood vessel and the leading process was clearly seen. Oriana software was used to draw the vector map for summarizing the directionality of OPC migration.

For the qualification of OPC migration in slice cultures, the directionality of OPC migration was measured by Imager-Pro Plus 5 and Oriana as described previously. Speed of migration was measured by dividing the total length of OPC movement by the total time. Vector maps show the directions of OPC movement.

For OPC cluster quantification, a ‘cluster’ constituted 4+ cells in cell body contact.

**Quantifications of astrocytic endfeet coverage.** To quantify Aldh11L1-GFP astrocytic endfeet coverage, images of areas with blood vessels covered by OPC clusters in Olig2-cre:APCloxP/loxP;Aldh11L1-GFP mice and blood vessels with no clusters in similar positions in APC−/−;Aldh11L1-GFP control mice were captured for analysis. When the GFP+ process was wrapped on/along CD31+ vessels, it was considered an astrocytic endfoot. Quantifications of length of blood vessels and GFP+ endfeet were done using Image-Pro Plus 5 software. AQP4+ endfeet coverage quantification was performed in a similar way.

**Quantifications of fibrinogen and dye leakage.** To identify vascular leakage of fibrinogen or dye, we performed CD31 staining of blood vessels to mark out the edges of vasculature. To quantify the extent of leakage in Olig2-cre:APCloxP/loxP, we measured the fibrinogen or dye immunoactive areas outside of these blood vessels. Similar areas in non-cpe APC−/− mice were used as control. The fluorescent area quantifications were measured as described below.

**Quantification of Cldn5/PLVAP distribution.** To quantify blood vessel distribution levels of Cldn5 versus PLVAP, double staining of Cldn5/PLVAP was performed in non-cpe APC−/− control mice and Olig2-cre:APCloxP/loxP mice. Regions with OPC perivascular clusters were imaged in Olig2-cre:APCloxP/loxP alongside comparable areas in control APC−/− mice. The lengths of Cldn5 and PLVAP positive endothelium in these imaged areas were measured using Image-Pro Plus 5 software.

**Quantification of pericyte coverage.** To quantify the pericyte coverage, double staining of CD31/PDGFRα was performed in non-cpe APC−/− control mice and Olig2-cre:APCloxP/loxP mice. The length of blood vessels covered with pericytes was measured using Image-Pro Plus 5 software.

**Fluorescent intensity and area quantifications.** To quantify fluorescent-positive area, intensity, and western blot–positive band, the positive areas were automatically selected in Image-Pro Plus 5 software. The areas of interest were separated by setting a threshold at least two times the background. Cell counting and fluorescence intensity analyses were conducted on six randomly chosen fields for each sample using an Image-Pro Plus image analysis system.

**Statistical analysis.** Statistical significance between groups was determined using GraphPad Prism software. The unpaired t-test was used to determine the significance between two experimental groups. One-way analysis of variance (ANOVA) was used to determine the significances among three or four groups. Data distribution was assumed to be normal, but this was not formally tested. A probability of P < 0.05 was considered statistically significant. All significant statistical results are indicated within the figures with the following conventions: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Error bars represent standard deviation. No statistical methods were used to predetermine sample sizes. The sample size per group was determined from previous publications with similar methodology: Control mice and their littermate mutant mice were collected from different litters, and randomly selected for each experiment. For lesioning experiments using adult control mice, those mice were randomly assigned into different time points as indicated. Investigators were blinded to group allocation during data analysis. All experiments were performed at least three times, and the findings were replicated in individual mice and cell cultures in each experiment.

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

**Data availability**

The data that support the findings of this study are available from the corresponding author upon request. R code used for the mRNAseq analysis can be found on the following github page: https://github.com/baranzini-lab/RNAseq_QuantSeq_Fancy. Raw sequence data (fastq) for the mRNAseq data are available on DASH data share (https://doi.org/10.7272/Q63N2IKB).

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

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

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

| R code used for the mRNAseq analysis can be found on the following github page. | https://github.com/baranzini-lab/RNAseq_QuantSeq_Fancy |

Data analysis

| Microscopy images were prepared using ImageJ/Fiji and Image-Pro plus 6. Live imagings were assembled using Imaris software. Statistical analyses were performed using Prism software. Software used for the mRNAseq data analysis was as follows: Trim Galore v0.4.4 with CutAdapt v1.14, FastQC v0.11.6, STAR aligner v2.5.0a, R version 3.5.1, R packages: Bioconductor version 3.7, DESeq2_1.20.0, ggplot2_3.1.0, RColorBrewer_1.1-2, pheatmap_1.0.10, readr_1.2.1. |

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- A description of any restrictions on data availability

The data that support the findings of this study are available from the corresponding author upon request. Raw sequence data (fastq) for the mRNAseq data are available on DASH data share (https://doi.org/10.7272/Q63N21KB).

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Life sciences study design
All studies must disclose on these points even when the disclosure is negative.

| Sample size | No statistical method was used in deciding sample sizes. The sample size per group was determined from previous publications with similar methodologies. |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | In the mRNAseq experiment, we removed one outlier sample from APC^fl/fl at P4 and one from Olig2cre:APC^fl/fl at P9 since those samples did not cluster with the same experimental group on PCA analysis. No data were excluded from any other analyses. |
| Replication | For each animal experiment, at least three sections were analyzed per animal and at least 4 animals were used per condition. For in vitro endothelial cell treatments, all conditions were performed in triplicate. All attempts at replication were successful. |
| Randomization | Control mice and their littermate mutant mice were collected from different litters, and randomly selected for each experiment. For lesioning experiments using adult control mice, those mice were randomly assigned into different time points as indicated. |
| Blinding | Investigators were blinded to group allocation during data analysis. |

Reporting for specific materials, systems and methods

Materials & experimental systems

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

Methods

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

Antibodies

Antibodies used

- PDGFRA (1:200, rat, 558774, BD Biosciences) (http://www.bdbiosciences.com/us/applications/research/stem-cell-research/ectoderm-markers/mouse/purified-rat-anti-mouse-cd140a-apa5/p/558774);
- PDGFRA (1:8000, rabbit, gift from W. Stallcup, Sanford Burnham Prebys) (this antibody is used in our previous study http://science.sciencemag.org/content/351/6271/379.long);
- Olig2 (1:2000, rabbit, gift from C.D. Stiles, Harvard) (this antibody is used in our previous study http://science.sciencemag.org/content/351/6271/379.long);
- CD31 (1:200, rat, 553370, BD Biosciences) (http://www.bdbiosciences.com/us/applications/research/stem-cell-research/cancer-research/mouse/purified-rat-anti-mouse-cd31-mec-133/p/553370);
- CD31 (1:200, rabbit, ab28364, Abcam) (https://www.abcam.com/CD31-antibody-ab28364.html);
Animals and other organisms

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

Laboratory animals

Mice
Animal husbandry and procedures were performed according to UCSF guidelines under IACUC approved protocols. The following mouse strains were used:

1) NG2-CreERT:TdTomato
This mouse has been described previously (X. Zhu et al., Age-dependent fate and lineage restriction of single NG2 cells. Development 138, 745-753 (2011)). We used 8-week old mice to label OPCs in slice cultures. Before slice culture, the mice were given tamoxifen (T-5648, Sigma) gavage with 1mg of tamoxifen twice a day for 5 consecutive days (10mg in total). We used 8-week old male and female mice.

2) Olig2-cre
A multi-functional mouse line was constructed previously (U. Schuller et al., Acquisition of granule neuron precursor identity is a critical determinant of progenitor cell competence to form Shh-induced medulloblastoma. Cancer Cell 14, 123-134 (2008).) by inserting tva, an avianspecific retroviral receptor, and an IRES-cre recombinase cassette into the endogenous Olig2 locus by homologous recombination. This Olig2-cre allows for cre mediated activity in oligodendrocyte lineage cells. We used P10 and adult male and female mice.

3) PDGFRalpha-CreERT2
These mice have been described previously (L. E. Rivers et al., PDGFRA/NG2 glia generate myelinating oligodendrocytes and p sinform projection neurons in adult mice. Nat Neurosci 11, 1392-1401 (2008)). A tamoxifen inducible cre recombinase gene was inserted into exon 2. These mice were used to cross with APC floxed mice. In order to knock out APC, the mouse was given tamoxifen gavage as described above. We used adult male and female mice.

4) APC floxed
These mice have been described previously (E. C. Robanus-Maandag et al., A new conditional Apc-mutant mouse model for colorectal cancer. Carcinogenesis 31, 946-952 (2010)). A conditional (floxed) allele of APC (adenomatous polyposis coli) provided by Dr. R. Fodde (Leiden University). Through intercrosses with Olig2-cre or PDGFRalpha-CreERT2 recombinase we can achieve conditional knockout of APC in OPCs. We used P10 and adult male and female mice. We used a breeding strategy of crossing either Olig2cre:APCfl/fl or PDGFRα-CreERT2:APCfl/fl mice with APCfl/fl mice, thus generating Olig2cre:APCfl/fl or PDGFRα-CreERT2:APCfl/fl offspring and APCfl/fl control littermates. Control littermates are written as ‘APCfl/fl’ in figures.

5) Aldh1f1-GFP
These mice have been described previously (J. D. Cahoy et al., A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. J Neurosci 28, 264-278 (2008)). The...
diffuse expression pattern of the Aldh1L1-GFP throughout the CNS is consistent with pan-astrocyte expression, which allows us to visualize the astrocyte processes in our study. We used P10 male and female mice.

6) Cx3cr1-GFP:CCR2-RFP
These mice have been described previously (M. Mizutani et al., The fractalkine receptor but not CCR2 is present on microglia from embryonic development throughout adulthood. J Immunol 188, 29-36 (2012)). The Cx3cr1-GFP specifically labels microglia in the CNS, and CCR2-RFP labels macrophages. By using this reporter mouse, we are able to distinguish infiltrating macrophages from resident microglia within tissue sections. We used P10 male and female mice.

Wild animals
We did not use any wild animals

Field-collected samples
We did not collect any field samples