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1 **Disease-specific oligodendrocyte lineage cells arise in multiple sclerosis**

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23

24 **One Sentence Summary:** Single-cell RNA-seq of a mouse model of multiple
25 sclerosis uncovers new oligodendrocyte populations, putative disease markers and
26 suggests new mechanisms underlying the pathogenesis of disease.

27

28

29

30 **Introductory paragraph (202 words)**

31 **Multiple Sclerosis (MS) is characterised by an immune system attack targeting**
32 **myelin, which is produced by oligodendrocytes (OLs). We performed single-cell**
33 **transcriptomic analysis of OL lineage cells from the spinal cord of mice induced**
34 **with experimental autoimmune encephalomyelitis (EAE), which mimics several**
35 **aspects of MS. We found unique OLs and OL precursor cells (OPCs) in EAE**
36 **and uncovered several genes specifically alternatively spliced in these cells.**
37 **Surprisingly, EAE-specific OL-lineage populations expressed genes involved in**
38 **antigen processing and presentation via major histocompatibility complex class I**
39 **and II (MHC-I and -II), and in immunoprotection, suggesting alternative**
40 **functions of these cells in a disease context. Importantly, we found that disease-**
41 **specific oligodendroglia are also present in human MS brains and that a**
42 **substantial number of genes known to be susceptibility genes for MS, so far**
43 **mainly associated with immune cells, are expressed in the OL lineage cells.**
44 **Finally, we demonstrate that OPCs can phagocytose and that MHC-II expressing**
45 **OPCs can activate memory and effector CD4+ T cells. Our results suggest that**
46 **OLs and OPCs are not passive targets but instead active immunomodulators in**
47 **MS. The disease-specific OL lineage cells, for which we identify several**
48 **biomarkers, may represent novel direct targets for immunomodulatory**
49 **therapeutic approaches in MS.**

50

51 **(main text: 3055 words)**

52 The adaptive immune system is currently thought to be the most likely aetiological
53 component for MS, although microglia have been suggested to also have a role^{1,2}. We
54 have shown that OLs, whose myelin is thought to be a passive target of the immune
55 system in MS, are heterogeneous in mouse^{3,4}. To investigate whether specific OL
56 populations are targeted in MS, we isolated single cells from the spinal cord of control
57 (treated with Complete Freund's Adjuvant, CFA) or EAE mice (Fig. 1a). Cells from
58 EAE mice were collected at the peak of the disease (score=3, indicating total
59 hindlimb paralysis, Fig. 1b) and we performed Smart-seq2⁵ single cell RNA-seq (Fig.
60 1a). OL-lineage cells were isolated by fluorescence activated cell sorting (FACS)
61 GFP+ cells from EAE-induced Pdgfra-H2B-GFP transgenic mice⁶ (enriching for
62 OPCs and “young” OLs, that had differentiated recently), and Pdgfra-Cre-LoxP-

63 GFP⁷, (containing mainly “old” OL-lineage cells labelled with GFP since early
64 development, but also “young” OLs and OPCs; Fig. 1a and Supplementary Fig. 1a).

65

66 EAE mice spinal cord cells segregated from CFA control mice, with a subset of
67 clusters uniquely found in EAE (Fig. 1c, Supplementary Fig. 1b,e). Clustering
68 analysis (GeneFocus pipeline, see Methods and Supplementary Fig. 2) revealed
69 thirteen OL lineage clusters, including four clusters of OPCs, one differentiation-
70 committed oligodendrocyte precursor (COP), one newly formed OL (NFOL) and
71 eight mature OL (MOLs; Fig. 1d and Supplementary Fig. 1b). We also identified
72 vascular and leptomeningeal cells (VLMCs)^{3,4}, consistent with the labelling of this
73 population with *Pdgfra*, and microglia-like cells (which, while they could also include
74 macrophages, we refer to as microglia; Fig. 1c and Supplementary Fig. 1b), which
75 could reflect transient *Pdgfra* expression in these cells, or phagocytosis of myelin-
76 associated GFP.

77

78 EAE-enriched OPC populations comprised OPCcyc (cycling), OPC2 and OPC3;
79 distinct from the OPC1 population found in healthy mice (Supplementary Fig. 1e) that
80 shares transcriptional profile similar to previously identified postnatal and adult
81 mouse OPCs^{3,4} (Fig. 1e and Supplementary Fig. 3d-f). Dimensionality reduction using
82 non-negative matrix factorization (NNMF) yielded a rank of 8 components (see
83 Methods). Removal of components 7 and 8 that correlated to S and G2 phase cell-
84 cycle genes revealed that OPCcyc can be further deconvoluted, suggesting that the
85 OPCcyc population describes a mixture of cell state transitions from OPC1, OPC2
86 and OPC3 (Supplementary Fig. 3). Comparison of OPC populations showed specific
87 expression of genes such as *Rph3a*, *Adora2b* in OPC1, *Phyhdl1*, *Sult1a1* in OPC2 and
88 *Slc14a1* in OPC3 (Fig. 1e and Supplementary Table 1). OPC2 and OPC3 clusters
89 displayed unique expression of *Fcgr2b*, *Mylk*, *Lgals1* (Fig. 1e, g, Supplementary Fig.
90 4a and Supplementary Table 1), when compared to OPC1, and increased levels of
91 *Myrf* (Fig. 1e), a transcription factor necessary for myelination⁸, and decreased levels
92 of *Hes1* and *Hes5* (Supplementary Fig. 4a), that keep OPCs in a progenitor state⁹. The
93 COPs and NFOLs expressing *Col20a1* (Fig. 1g) and *Syt4* (Supplementary Fig. 4a)
94 were mostly from EAE mice. Thus, OPCs undergo proliferation and differentiation in
95 EAE^{10,11}, but also transition to previously unidentified transcriptional states.

96

97 MOLs presented several markers identified in Marques et al.⁴, and could be
98 subcategorized accordingly (Fig. 1d, f and Supplementary Fig. 1c). EAE-associated
99 MOL lineage populations expressed unique genes, absent or very low expressed in
100 healthy controls. *Klk8*, *Itga8*, *Tlr3*, *Trim34a* were enriched in MOL1/2-EAE; *Plin4*,
101 *Sult1a1* and *Zfand4* in MOL5/6-EAE-a; and *S100a10*, *RNase4* and *Tnfrsf1a*, a MS
102 susceptibility gene,¹² in MOL5/6-EAE-b (Fig. 1f, g, Supplementary Fig. 4a and
103 Supplementary Table 1). We found disease-specific markers in OL lineage cells such
104 as *Igtp*, *Nlrc5*, and *Serpina3n* (Fig. 2b and Supplementary Fig. 4a). *Il12rb1* was
105 present in all MOL populations found in EAE mice (Fig. 1g). All OL lineage clusters
106 comprised cells from both transgenic mouse lines, with the exception of MOL5/6-
107 EAE-a, from *Pdgfra*-H2B-GFP mice, which might thus be composed of “young” OLs
108 (Supplementary Fig. 1e).

109

110 To identify gene-transcription trends, we decomposed the dataset into components
111 using NMF (Supplementary Fig. 4b) and found 19 robust gene modules, including
112 two that were uniquely associated with EAE: module 1, present in a subset of EAE
113 cells comprising genes related to interferon response pathways and MHC-I and -II
114 genes, and module 13 restricted to the MOL5/6-EAE-a population, which contained
115 genes such as *Plin4*, *Hif3a*, and *Fam107a* (Supplementary Fig. 4b). Similar results
116 were obtained when performing GO/Reactome analysis (Supplementary Fig. 4c and
117 5a, and Supplementary Table 3). We also uncovered 360 genes that were alternatively
118 spliced in EAE OL-lineage cells, with either exon exclusion or inclusion
119 (Supplementary Table 2). Gene Ontology (GO)/reactome analysis showed that genes
120 with alternatively spliced exons in EAE OPCs were related to regulation of
121 transcriptional elongation from RNA Pol II (exon exclusion), membrane trafficking
122 (exon inclusion), and RNA-splicing components (exon exclusion; Supplementary Fig.
123 5b and Supplementary Table 3). We also found evidence of alternative splicing in
124 EAE in several genes involved in myelination and MS, including the myelin genes
125 *Mbp* (as previously reported¹³), *Mobp*, *Pdgfa* and the *Ifih1* gene, in which MS-
126 associated polymorphisms have been found¹⁴ (Fig. 1.h, i Supplementary Fig. 5c and
127 Supplementary Table 2).

128

129 We performed RNAscope *in situ* hybridization (ISH) and immunohistochemistry
130 (IHC) in EAE spinal cords, against several markers of the identified clusters, such as

131 *Klk8*, which specifically marks MOL1/2-EAE (Fig. 1g) and *Hopx*, which marks
132 MOL2⁴. While control spinal cords exhibited sparse *Klk8* expression we observed a
133 strong induction of *Klk8* in a subset of *Hopx*⁺/*Sox10*⁺ MOL2 in EAE mice (Fig. 2a).
134 We also confirmed the presence of PLIN4 protein (enriched in MOL5/6 EAE-a) by
135 performing IHC on spinal cords and found elevated GFP/PLIN4 double positive cells
136 (from the *Pdgfra*-H2B-GFP mouse) and *Sox10*/PLIN4 (Supplementary Fig. 6a) in
137 EAE mice. Thus, *Klk8* and *Plin4* are new markers of subpopulations of EAE-
138 responsive MOLs.

139

140 All oligodendroglia in EAE displayed elevated *Serpina3n* expression (Fig. 2b),
141 encoding a serine protease inhibitor that has been shown to reduce EAE severity and
142 induce neuroprotection¹⁵. We confirmed the increase in *Serpina3n* in EAE by
143 RNAscope ISH, especially notable within *Sox10*⁺ cells (Fig. 2c). The expression of
144 this molecule reached such high levels in EAE, that we could no longer detect single
145 dots. Interestingly, we found expression of additional genes of the *Serpina3* family,
146 including *Serpina3h*, *Serpina3c*, *Serpina3i*, *Serpina3f* and *Serpina3g* specific to
147 MOL1/2-EAE (Fig. 2b), which could suggest that this population might be protected
148 against direct damage by T- or NK-cells. This population also expresses the gene
149 *Serping1* (Fig. 2b) encoding for an inhibitor of complement activation, another
150 possible mechanism of immunoprotection.

151

152 MHC-I genes such as *H2-K1*, *H2-D1*, *H2-T23*, *B2m*, and genes necessary for antigen
153 processing and binding to MHC-I molecules, such as *Psmb9*, *Tap1* and *Tap2* were
154 significantly increased upon EAE induction in OL lineage cells (Fig. 2d and
155 Supplementary Table 1). Thus, EAE OLs might be direct targets of cytotoxic T cells,
156 which is consistent with their expression of myelin epitopes. Surprisingly, however,
157 we found that OPCs also exhibited a robust increase in the expression of these
158 molecules (Fig. 2d), suggesting that OPCs might also be targeted during the disease,
159 despite not expressing myelin proteins. To validate the expression of MHC-I
160 molecules in oligodendroglia cells, we have performed RNAscope ISH and observed
161 an upregulation of *B2m* molecules in *Sox10*⁺ cells in EAE spinal cords as well as the
162 presence of *Psmb9* molecules in *Sox10*⁺ cells in EAE that were absent in *Sox10*⁺ cells
163 in control spinal cords (Fig. 2e).

164

165 MHC-II genes are thought to be restricted to microglia/macrophages in MS, and not
166 present in OLs in active lesions¹⁶. Strikingly, we found the expression of all key genes
167 required for a MHC-II mediated response, including *H2-aa*, *H2-ab1*, *H2-eb1*, *Cd74*
168 and *Ctss*, in specific subsets of OPCs and OLs (Fig. 3a). To confirm the presence of
169 these RNA transcripts in OL lineage cells, we performed RNAscope ISH in the EAE
170 and control spinal cords using probes targeting *Cd74* and *H2-Eb1* (for MHC-II), in
171 combination with *Aif1* (for microglia), *Sox10* (for OL lineage cells), *Klk6* (for MOL2)
172 and *Ptprz1* (for OPC) (Fig. 3b-d, Supplementary Fig. 6b, and Supplementary Videos
173 V1-V6). While we observed microglia processes enwrapping OLs (Supplementary
174 video V7), a small proportion of OLs/OPCs could be confidently assigned with
175 double positive *Cd74/Sox10* and few or no molecules for *Aif1* (Fig. 3d and
176 Supplementary Videos V1-V6). We also combined RNAscope ISH with
177 immunohistochemistry and found triple positive OPCs for GFP (from EAE Pdgfra-
178 H2B-GFP spinal cord), *Ptprz1* and *Cd74* (Fig. 3c and Supplementary video V2). We
179 also performed IHC for MHC-II and the OL markers OLIG2 and SOX10, and
180 identified double positive cells for OLIG2/MHC-II and SOX10/MHC-II
181 oligodendroglia in the spinal cord of EAE mice (Fig. 3e and Supplementary Fig. 6c).
182 We estimated that in the lesion areas of the white matter SOX10/MHC-II double
183 oligodendroglia constitute about 3,4% (+/-1,76 SD) of the SOX10⁺ cells. We
184 performed IBA-1 staining to distinguish between SOX10/MHC-II oligodendroglia
185 and MHC-II expressing macrophages/microglia and we could distinguish the two cell
186 types. Nevertheless, IBA-1 could still be observed in SOX10/MHC-II oligodendroglia
187 although with a lower expression, which could be due to induction of some *Aif1*
188 molecules (corresponds to IBA-1 protein) in oligodendroglia in EAE, by for instance
189 interferon-gamma (IFN γ)¹⁷. Control CFA-treated spinal cord OPCs/OLs did not
190 express MHC-II molecules neither at the RNA (RNAscope ISH) nor protein level
191 (IHC; Fig. 3b and data not shown).

192

193 We found several interferon responsive genes in MHC-II expressing OL/OPC
194 populations, such as *Ifih1*, *Iigp1*, *Trim34*, *Irf7*, *Irgm1*, *Irgm2*, *Igtp* and *Zbp1*
195 (Supplementary Fig. 7a). It has been shown that IFN γ can trigger endoplasmic
196 reticulum stress in OLs¹⁸. Indeed, we found many genes involved in protein
197 processing in endoplasmic reticulum differentially expressed in EAE (Supplementary
198 Fig. 7b). Transcription factors such as *Nlrc5*, a transactivator of the MHC-I¹⁹, was

199 expressed upon disease in all OL lineage cells, and *Ciita*, induced by the IFN γ
200 pathway and activator of MHC-II genes, was expressed in a subset of these cells
201 (Supplementary Fig. 4a). Thus, OL/OPC populations in the spinal cord appear to have
202 the capacity to activate MHC genes upon EAE induction.

203

204 To determine if these populations also occur in human MS, we performed IHC for
205 MHC-II and the OL markers OLIG2 and OLIG1 in postmortem brain tissue from two
206 MS patients. As in mouse model EAE, we observed cells in which immune cell-
207 derived MHC-II processes appear to touch/enwrap OLIG2⁺ nuclei (Supplementary
208 Fig. 6d arrowheads and Supplementary video V8) but also OLIG2 and OLIG1 cells
209 positive for MHC-II without any immune cell in their neighborhood (Fig. 3f and
210 Supplementary Fig. 6d arrows, and Supplementary video V9), indicating that indeed
211 human OLs can express adaptive immunity proteins in the context of MS.

212

213 Several MS susceptibility variants including MHC and autosomal non-MHC locus
214 and the associated putative susceptibility genes were recently described¹. Comparison
215 of these loci/genes with RNA-seq from bulk brain led to the inference that peripheral
216 immune system cells and brain resident microglia were the most likely cell types to
217 contribute to MS susceptibility¹. To examine if MS susceptibility genes were also
218 expressed in OL lineage cells in EAE, we analyzed the expression of the mouse
219 homologs for genes associated with the 200 non-MHC, 32 MHC loci, and 19 X-
220 chromosomal loci in our single-cell transcriptomics dataset (Fig. 3g and
221 Supplementary Fig. 6e and Supplementary Table S4). Interestingly, the MHC-II gene
222 *H2.Aa* (human HLA-DQA1) was enriched not only in microglia, but also in EAE-
223 derived OPCs and MOLs (Fig. 3g). Strikingly, several OL lineage cells expressed
224 non-MHC locus associated susceptibility genes (Fig. 3g and Supplementary Table
225 S4). This was particularly strong for OPCs (importantly both from control and EAE
226 mice) and MOL1/2-EAE populations (Fig. 3g). OPCs expressed 61% of these genes,
227 which was in the same range as microglia (67%). Moreover, while we found many
228 genes are equally expressed in all the populations, there were also genes enriched in
229 OL lineage cells (such as *Bcas1* and *Sox8*), MOLs (*Prr5l*), OPCs (such as *Kcnh8*,
230 *Pkia*, *Pitpnm2*) and microglia (such as *Plek*; Supplementary Table S4). Interestingly,
231 a subset of genes starts to be expressed and is upregulated in response to the disease
232 in MOL populations, in particular MOL1/2-EAE.

233

234 To investigate the mechanism triggering MHC-II expression in OL lineage cells and
235 the functional implications in the development of the disease, we performed co-
236 cultures of GFP⁺ OPCs (from Sox10Cre-GFP mice brains) and CD45⁺ immune cells
237 isolated from the spinal cord of EAE mice (Fig. 4a). After 72 hours of co-culture,
238 4.4% (+/-2.42 SD) of the total GFP⁺ OPCs in culture were positive for MHC-II (Fig.
239 4b). We also performed co-cultures of OPCs with CD45⁺ immune cells from CFA
240 mice (most likely comprising of microglia/macrophages and few invading leukocytes
241 from the periphery) and did not find any GFP/MHC-II double positive OPCs in these
242 conditions (Supplementary Fig. 8a). This indicates that factors secreted by EAE-
243 specific immune cells are inducing MHC-II expression in OPCs. Indeed, while MHC-
244 II expression has been reported to be excluded from OLs in MS¹⁶, expression of these
245 molecules in rat OPCs and OLs has been observed, upon dexamethasone and IFN γ
246 treatment²⁰. As CD4 lymphocytes such as Th1 and memory T cells are interferon-
247 producing cells, we hypothesized that the observed effect in the co-cultures was
248 mediated by lymphocyte derived IFN γ . As such, we treated OPCs with IFN γ (100
249 ng/ml), dexamethasone (1 μ M) and the combination of both for 3 days (Fig. 4c). We
250 observed the induction of expression of MHC-II molecules, in OPCs and OLs, only in
251 IFN γ treated cells, alone or in combination with dexamethasone, both at the RNA
252 level (RNAscope ISH) and protein level (shown by ICC; Fig. 4d and Supplementary
253 Fig. 8b-c). We did not find any positive cell for *Aif1* RNA molecules in our MACS
254 OPC primary cultures, nor IBA-1 positive cells, indicating that microglia were absent
255 and excluding the possibility that MHC-II RNA and protein were derived from
256 microglia processes. qPCR analysis further confirmed these results. While genes such
257 as *Plin4*, were not affected by IFN γ treatment but only to dexamethasone, we
258 observed a dramatic induction of interferon responsive genes (*Ifit2*, *Ifih1*) MHC-II
259 genes (*Cd74*) and MHC-II and I transactivators (*Ciita*, *Nlrc5*) (Fig. 4e). Thus, our
260 results indicate that MHC-II genes induction in EAE OL lineage cells is possibly
261 mediated by IFN γ .

262

263 To uncover the role of immunocompetent OPCs in the disease, we first investigated if
264 OPCs were capable of phagocytosis, by adding 1 μ m diameter fluorescent
265 microspheres to OPC cultures for 24h. OPCs could indeed uptake several

266 microspheres, with at least 48% of them exhibiting this capability (Fig. 4f). Moreover,
267 treatment with the phagocytosis inhibitor cytochalasin D at two different
268 concentrations for 24 hours, lead to a remarkable reduction in microsphere uptake
269 (Fig. 4f, g). IFN γ treatment did not alter OPC phagocytic capacity (Fig. 4g). To
270 further investigate whether OPCs can phagocytise myelin, we incubated OPCs for 6
271 hours with pHrodo-labeled myelin (a pH-sensitive fluorogenic dye that fluoresce red
272 in phagosomes²¹) and found that OPCs were able to uptake myelin as observed by the
273 red fluorescence staining of live OPCs (Fig. 4f). Altogether, these data demonstrate
274 that OPCs exhibit phagocytic activity and strongly suggests that OPCs are capable of
275 taking up myelin debris in a disease context.

276

277 Next, we addressed the impact of MHC-II expressing OPCs on the survival,
278 proliferation and cytokine production of CD4⁺ T cells derived from 2D2 mice, where
279 T cells express the T cell receptor for the MOG35-55 peptide²². OPCs (controls or
280 pre-treated with IFN γ and/or MOG35-55 peptide) were co-cultured for 72 hours with
281 either naïve, memory or effector T cells (naïve cells that had been pre-activated *in*
282 *vitro* in the presence of IL-12, acquiring a Th1 phenotype). T cells were subsequently
283 assessed by flow cytometry for survival (cell dead exclusion), proliferation (Ki67⁺),
284 as well as for expression of IFN γ and TNF (Fig. 4h). Co-culturing with non-
285 stimulated OPCs lead to an increase in numbers of surviving naïve, activated and
286 memory T cells. Survival was further enhanced in memory T cells when OPCs were
287 pre-treated in IFN γ and MOG peptide suggesting an MHC-II mediated effect (Fig. 4i,
288 Supplementary Figs. 8e and 9). Regarding proliferation, naïve CD4⁺ T cells seemed
289 unaffected by OPC co-culture (Fig. 4j and Supplementary Figs. 8e and 9), as
290 expected, since these cells have a high threshold of activation provided in secondary
291 lymphoid tissue. In contrast, we found a higher number of Ki67⁺ memory CD4⁺ T
292 cells when co-cultured with OPCs in the presence of MOG peptide. Similar effects
293 were observed for Th1 CD4⁺ T cells which proliferated more in the presence of OPCs
294 and MOG peptide. These results also suggest that the proliferation effect is via OPC-
295 MOG peptide presentation to memory and Th1 T cells. Of notice, the presence of
296 MOG peptide, not necessarily together with IFN γ , was enough to induce proliferation.
297 This indicates that production of IFN γ by memory and Th1 cells may provide a
298 positive regulatory feedback loop. We thus investigated if the increase in
299 survival/proliferation was accompanied by cytokine production. Indeed, memory

300 CD4⁺ T cells displayed more cells producing both TNF and IFN γ when co-cultured
301 with OPCs in the presence of MOG peptide (Supplementary Fig. 8d). In contrast, the
302 presence of OPCs per se triggered an increase in the number of Th1 CD4⁺ T cells
303 producing IFN γ and TNF (Supplementary Fig. 8d).

304

305 OLs are widely seen as a passive target of a dysregulated immune system, but there is
306 a growing body of evidence of immunomodulatory factors expressed in OLs such as
307 cytokines/chemokines and their receptors²³⁻²⁷. Our results indicate that both OPCs and
308 MOLs undergo a transcriptional overhaul during chronic inflammatory
309 demyelination. The selective expression of immunoprotective, innate and adaptive
310 immunity genes in OPCs and OLs in EAE suggests potential mechanisms of
311 protection and an immune function in the context of disease. Since several of these
312 genes are susceptibility genes for MS, the OL lineage might have a more central role
313 in the origin and progression of MS than previously thought.

314

315 OL cell death has been recently shown to lead to an adaptive auto-immune response²⁸,
316 which could imply an initial dysregulation in cells of the OL lineage as a triggering
317 event in MS and support the “inside-out” hypothesis for the aetiology of the disease²⁸.
318 Whether the new identified OL lineage cell states play a role on the aetiology of MS,
319 by acting as antigen presenting cells and triggering immunologic attack or reinforcing
320 the disease-initiating event from the periphery, or both, will require further
321 investigation. In either case, our results indicate that OL lineage cells may not simply
322 be passive targets of the immune system in MS, but rather central players which may
323 be targeted in therapeutic approaches for MS.

324 **Accession codes and data availability:** A web resource for browsing differential
325 gene expression data for the single cell data can be accessed at
326 <https://ki.se/en/mbb/oligointernode>. Raw data is deposited in GEO, accession number
327 GSE113973. Code used for single cell RNA-Seq analysis is available at
328 <https://github.com/Castelo-Branco-lab/GeneFocus>.

329

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357

358 **Author Contributions:** A.M.F., D.V.B and G.C.-B. conceived the project, designed
359 the study and interpreted results. A.M.F., S.M. and A.O.G-C. performed EAE model
360 and A.M.F and S.M. collected single cells to generate single-cell sequencing data.
361 D.V.B and E.A. performed computational analyses. A.M.F and M.M. designed,
362 performed and analyzed most *in vitro* OPC experiments and S. performed the
363 phagocytosis experiments together with A.M.F. A.O.G-C. and A.M.F. designed,
364 performed and analyzed all experiments involving co-cultures with immune cells.
365 S.J., A.W. and C.f.-C. provided the human postmortem MS brain tissue and
366 performed the human IHC analysis. D.P.V. provided support for computational

367 analysis. E.M.F. provided RNAscope ISH expertise and performed all videos. A.M.F,
368 D.V.B and G.C.-B. wrote the manuscript with feedback from all authors.

369

370 **Competing interests:**

371 The G.C-B research group has received funding from F. Hoffmann – La Roche, Ltd.
372 for another research project in the area.

373

374 **Additional information:** Reprints and permissions information is available at
375 www.nature.com/reprints. Readers are welcome to comment on the online version of
376 the paper. Correspondence and requests for materials should be addressed to G.C.-B.
377 (goncalo.castelo-branco@ki.se).

378

379

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381

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461

462 **Figure legends**

463 **Figure 1: Single cell RNA-sequencing of oligodendrocyte (OL) lineage cells in response**
464 **to experimental autoimmune encephalomyelitis (EAE) uncovers new disease-specific**

465 **populations and disease markers. a**, Schematic overview of the methodology used to
466 perform single-cell RNA-seq of the OL lineage cells. **b**, Clinical score of the diseased animals
467 used in the study (n=12 mice; data represented as mean \pm s.e.m.; only animals that reached
468 score 3 and one that reached score 2.5 were used in this study). **c**, t-SNE plots of all cells
469 sequenced showing the segregation of cells derived from Complete Freund's Adjuvant (CFA)
470 controls and EAE (n=4 biologically independent mouse spinal cord samples per condition;
471 total number of cells is 794 for controls and 971 for EAE). **d**, t-SNE plots of all cells
472 sequenced representing different cell populations within OL lineage cells. Mature
473 oligodendrocyte (MOL) identities were defined according to marker genes identified in ref. 4
474 (n=4 biologically independent mouse spinal cord samples per condition; total number of cells
475 is 745 for controls and 707 for EAE). **e-f**, Violin plots depicting the expression of specific
476 markers for OL precursor cells (OPC) (**e**) and for MOL clusters (**f**) (n=4 biologically
477 independent mouse spinal cord samples per condition; total number of cells is 116 for OPC
478 controls and 132 for OPC EAE and 626 for MOL controls and 575 for MOL EAE). Violin
479 plots are centered around the median with interquartile ranges and shape represents cell
480 distribution. **g**, t-SNE plots of disease-specific markers for OL lineage cells (n=4 biologically
481 independent mouse spinal cord samples per condition; total number of cells is 745 for
482 controls and 707 for EAE). **h**, Schematic representation of the exon 6 inclusion in the *Pdgfa*
483 gene in response to EAE. **i**, Violin plot representing the PSI (proportion of spliced isoform) in
484 controls (MOL2 Ct-a depicted in green) and EAE (MOL1/2 EAE depicted in purple) of the
485 alternative spliced exons in *Pdgfa* and *Mbp* genes. PSI=0 means totally excluded and PSI=1
486 totally included (n=4 biologically independent mouse spinal cord samples per condition; total
487 number of cells is 56 for MOL2 Ct-a and 49 for MOL1/2 EAE; *Pdgfa* ex6: p=0.0003176 and
488 *Mbp* ex2: p=5.323e-11 by two-sided Wilcoxon rank sum test with continuity correction;
489 Violin plots are centered around the median with interquartile ranges and shape represents
490 cell distribution. VLMCs - vascular and leptomeningeal cells; MiG1 - Microglia-like cells.

491

492 **Figure 2: Expression of immunoprotective and adaptive immunity genes in response to**
493 **EAE. a**, RNAscope ISH representing a spinal cord from CFA control and EAE mice marked
494 with probes for *Sox10*, *Klk8* and *Hopx*, markers of MOL1/2-EAE. Dashed boxes shown at
495 higher magnification highlight regions with higher densities of MOL1/2 cells. Arrows depict
496 triple positive cells in EAE and arrowheads depict *Hopx* positive OL lineage cells negative
497 for *Klk8*. Representative images, n=3 biologically independent mouse spinal cord samples.
498 Scale bars - 20 μ m **b**, Violin plots representing *Serpina3* family of genes in OL lineage cell
499 populations derived from CFA controls and EAE mice. (n=4 biologically independent mouse
500 spinal cord samples per condition; total number of cells is 745 for controls and 707 for EAE).

501 Violin plots are centered around the median with interquartile ranges and shape represents
502 cell distribution. **c**, RNAscope ISH representing a spinal cord from CFA control and EAE
503 mice marked with probes for *Sox10* and *Serpina3n*. Representative images, n=3 biologically
504 independent mouse spinal cord samples. Scale bars - 20µm. **d**, Violin plots representing MHC
505 class I related genes in OL lineage cell populations derived from CFA controls and EAE
506 mice. (n=4 biologically independent mouse spinal cord samples per condition; total number
507 of cells is 745 for controls and 707 for EAE). Violin plots are centered around the median
508 with interquartile ranges and shape represents cell distribution. **e**, RNAscope ISH
509 representing a spinal cord from CFA control and EAE mice marked with probes for *Sox10*,
510 and MHC-I related genes, *Psmb9* and *B2m*. Arrows depict triple positive cells, arrowheads
511 depict cells double positive for *Psmb9/B2m* and negative for *Sox10* that do not belong to the
512 OL lineage. Representative images, n=3 biologically independent mouse spinal cord samples,
513 scale bars - 20µm.

514

515 **Figure 3: Expression of MHC class II and MS susceptibility genes in the OL lineage cells**
516 **in response to EAE and in MS. a** Violin plots representing MHC class II related genes in
517 OL lineage cell populations derived from CFA controls and EAE mice. (n=4 biologically
518 independent mouse spinal cord samples per condition; total number of cells is 745 for
519 controls and 707 for EAE). Violin plots are centered around the median with interquartile
520 ranges and shape represents cell distribution. **b-d**, RNAscope ISH representing spinal cords
521 from CFA control and EAE mice marked with probes for MHC-II (*Cd74*), OL lineage cells
522 (*Sox10* and *Ptprz1/Pdgfra-H2B-GFP* specific for OPCs) and microglia (*Aif1*). Dashed boxes
523 shown at higher magnification represent both OL lineage cells expressing MHC-II
524 (arrowhead), and microglia cells expressing MHC-II (arrow). Representative images, n=3
525 biologically independent mouse spinal cord samples per condition, scale bars - 20µm. **e**,
526 Immunohistochemistry showing OL lineage cells (positive for *Sox10*) co-expressing MHC-II
527 protein, in a lesion in the spinal cord of EAE mice. Representative images, n=3 biologically
528 independent mouse spinal cord samples per condition, scale bars - 20µm. **f**,
529 Immunohistochemistry in human samples from two MS patients representing OLIG1 positive
530 OLs expressing MHC-II (arrows). Representative images, n=2 biologically independent
531 human brain samples. Scale bars - 25µm. **g**, Expression-based heat maps for the MS
532 susceptibility genes (from ref. 1) in microglia and OL lineage cells. MHC locus and non-
533 MHC locus related genes are represented in both microglia and all OL lineage cells
534 populations analyzed in this study.

535 **Figure 4: OPCs express MHC-II in response to interferon-γ, exhibit phagocytic capacity**
536 **and regulate T cell survival and proliferation. a**, Schematic representation of co-culture

537 between OPCs isolated from Sox10-Cre-GFP mice and CD45+ immune cells isolated from
538 EAE mice or CFA controls. **b**, Immunohistochemistry showing OL lineage cells (positive for
539 Sox10) co-expressing MHC-II protein, but not the microglia marker IBA1, upon co-culture
540 with CD45+ immune cells from EAE mice. Representative images, n=3 independent
541 experiments, scale bars - 20µm. **c**, Schematic representation of treatment of OPCs and OLs
542 with interferon-γ (100 ng/ml) and dexamethasone (1µM). **d**, OLs cultured with
543 dexamethasone and interferon-γ for 72h express MHC-II as represented by *Cd74/Sox10* and
544 MHC-II/CNP double staining in RNAscope ISH and immunocytochemistry, respectively.
545 Representative images, n=3 independent experiments. Scale bars - 20µm. **e**, qRT-PCR
546 analysis for MHC and interferon responsive genes on primary OPCs and OLs treated with
547 interferon-γ and dexamethasone, n=3 independent experiments per condition; data
548 represented as mean ± s.d. **f**, Uptake of pHrodo-labeled myelin and 1µm diameter fluorescent
549 microspheres by primary NG2+ OPCs after 6 and 24 hours, respectively, and upon treatment
550 with 50µM of cytochalasin D. Representative images, n=3 independent experiments. Scale
551 bars - 20µm. **g**, Quantification of the percentage of OPCs uptaking microspheres in the
552 presence or absence of IFN-γ (100 ng/ml) and upon treatment with 20 and 50µM of
553 cytochalasin D. n=3 independent experiments per condition; data represented as mean ± s.d.
554 **h**, Schematic representation of co-culture between primary OPCs, treated with interferon-γ
555 and/or MOG 35-55 peptide, and three different types of CD4 lymphocytes (naïve, memory
556 and activated Th1) from 2D2 transgenic mice, where a high proportion of CD4 T-
557 lymphocytes express a T-cell receptor specific for MOG35-55 peptide. **i-j**, Graph plots
558 obtained from FACS analysis of naïve, memory and activated Th1 2D2 CD4 T cells after 72
559 hours of co-culture with OPCs. General survival (dead cell exclusion marker) and
560 proliferation (CD4⁺ Vβ11⁺ Ki67⁺) were assessed. Numbers of cells in the live gate as well as
561 in the Ki67⁺ gate were estimated and reflect survival and proliferation since the same cell
562 numbers were seeded onto different wells. Plots represent the averages of the assessed values
563 for the different conditions divided by the values of the control (T cells only) for fold change
564 differences. n=7 independent experiments; data represented as mean ± s.e.m.

565 **ONLINE METHODS**

566 Information regarding methods used in this paper can be found below and also in the Life
567 Sciences Reporting Summary.

568

569 **Animals.** Mouse lines used in this study included C57BL/6NJ wild type (WT) mice, *Pdgfra*-
570 *Cre-LoxP-GFP*⁷, *Pdgfra-H2B-GFP* knock-in mice⁶, *Sox10Cre-LoxP-GFP*²⁹ and C57Bl/6 2D2
571 transgenic mice²². *Pdgfra-Cre-LoxP-GFP* mice are a strain of mice obtained originally by
572 crossing mice with Cre recombinase under the control of a *Pdgfra* genomic DNA fragment
573 (with a C57BL/6NJ genetic background; The Jackson Laboratories, CA, USA) with reporter
574 mice RCE:loxP-GFP (with CD1 background; Gord Fishell, NYU Neuroscience Institute)
575 to label the complete OL lineage. *Sox10-Cre-LoxP-GFP* mice are a strain of mice obtained
576 originally by crossing mice with Cre recombinase under the control of the *Sox10* promoter
577 (The Jackson Laboratories, CA, USA; with a C57BL/6 genetic background) with reporter
578 mice RCE:loxP-GFP (with CD1 background) to label the complete OL lineage. *Pdgfra*-
579 *H2B-GFP*, with a C57BL/6NJ background, presents an H2B-eGFP fusion gene expressed
580 under the promoter of the OPC marker, *Pdgfra*. Mice homozygous for the knock-in targeted
581 mutation have an embryonic lethal phenotype, with half of the embryos failing to survive past
582 embryonic day 12.5 and the remainder failing to survive beyond embryonic day 15.5
583 (<https://www.jax.org/strain/007669>). Only heterozygote mice were used, in which *Pdgfra* is
584 expressed mainly in OPCs but also in some extent in the early stages of OL differentiation,
585 due to GFP half-life. Animals were used in adult stage, between 10-12 weeks old and both
586 genders were included. The following light/dark cycle was used: dawn 6.00-7.00; daylight
587 07.00-18.00; dusk 18-00-19.00; night 19.00-06.00. A maximum of 5 adult mice per IVC-cage
588 of type II Allentown. Breedings were done with 1 male and up to 2 females. All experimental
589 procedures performed followed the guidelines and recommendations of local animal
590 protection legislation and were approved by the local committee for ethical experiments on
591 laboratory animals in Sweden (Stockholms Norra Djurförsöksetiska nämnd).

592

593 **Experimental Autoimmune Encephalomyelitis (EAE).** For the induction of chronic EAE,
594 animals were injected subcutaneously with an emulsion of MOG35-55 in complete Freud's
595 adjuvant (CFA; EK-2110 kit from Hooke Laboratories) followed by the administration of
596 pertussis toxin in PBS (0,2µg per animal), for two consecutive days (all according to
597 manufacturer's instructions). Spinal cords and cerebellum were collected at the peak/chronic

598 stage of the disease with clinical score=3 representing limp tail and complete paralysis of hind
599 legs. Animals that did not reach this clinical score were not analyzed in this study. Additional
600 animals were injected subcutaneously with a CFA emulsion (CK-2110 kit from Hooke
601 Laboratories) and analyzed as controls.

602

603 **Tissue dissociation for single-cell RNA sequencing, FACS analysis and sequencing data**
604 **processing.** Cells were isolated from the spinal cord of P90 *Pdgfra-H2B-GFP* and *Pdgfra-*
605 *Cre-LoxP-GFP* mice. Tissue was then dissociated into a single cell suspension, as previously
606 described in Marques et al 2016, with some modifications. Mice were perfused with
607 oxygenated cutting solution (87 mM, NaCl, 2.5 mM KCl, 1.25 mM NaH₂PO₄, 26 mM
608 NaHCO₃, 75 mM sucrose, 20 mM glucose, 1 mM CaCl₂, and 2 mM MgSO₄) and the brain
609 was quickly dissected and dissociated using the Adult brain dissociation kit from Miltenyi
610 (130-107-677) following the manufacturer's instructions (red blood cells removal step was
611 not included). After debris removal, cell suspension was filtered with 30µm filter (Partec) and
612 processed by FACS. Spinal cord single GFP⁺ cells were selected in a BD Influx sorter and
613 collected into a 384 plate for SmartSeq2, according to procedures described in⁵. SmartSeq2
614 raw data was processed according to procedures described in⁵. Smart-seq2 paired-end reads
615 were trimmed with cutadapt 1.8.0³⁰ and aligned with STAR 2.5.1b³¹ to mm10 genome
616 assembly, only uniquely mapped reads were used for downstream analyses.

617

618 **Primary OPC cell culture.** Mice brains from P7 pups were removed and dissociated in
619 single-cell suspensions using the Neural Tissue Dissociation Kit (P; Miltenyi Biotec, 130-
620 092-628) according to the manufacturer's protocol. OPCs were obtained with MACS with
621 CD140a microbeads following the manufacturer's protocol (CD140a Microbead kit, Miltenyi
622 Biotec 130-101-547). Alternatively to MACS, GFP⁺ OPCs derived from *Sox10-GFP* mice
623 were collected with FACS following brain dissociation. Cells were seeded in poly-L-lysine
624 (O/N; Sigma P4707) plus fibronectin (1h; Sigma F1141) coated dishes and grown on
625 proliferation media comprising DMEM/Gmax (ThermoFisher Scientific 10565018), N2
626 media (ThermoFisher Scientific 17502048), Pen/Strep (ThermoFisher Scientific 15140122),
627 NeuroBrew (Miltenyi 130-093-566), bFGF 20ng/ml (Peprotech 100-18B) and PDGF-AA
628 10ng/ml (Peprotech, 100-13A). For OPC differentiation, cells were left for 2 days in medium
629 without bFGF and PDGF-AA. Cells were treated either with Dexamethasone (1µM),
630 Interferon-gamma (100ng/mL; R&D, 485-MI-100) or the combination of the two for 72
631 hours. For the phagocytosis experiments, 1µL per ml of media of pHrodo-labeled myelin and

632 0.5µL per ml of media of Fluoresbrite® Polychromatic Red Microspheres 1µm (Polysciences,
633 Inc, 18660-5) were added to OPCs for 6h and 24h, respectively. Hoechst (ThermoFisher
634 Scientific, 62249) was added at a dilution 1:1000 to OPCs incubated with pHrodo-labeled
635 myelin for live-cell fluorescent staining of the DNA and nuclei. Phagocytosis inhibition
636 experiments were performed by adding two different concentrations of cytochalasin D
637 (Sigma, C8273) 20µM and 50µM in the presence or absence of Interferon-gamma
638 (100ng/mL) for 24 hours. Cells were thoroughly washed before fixation.

639 pHrodo-labeled myelin preparation: myelin sheaths were obtained by dissection and
640 mechanical homogenization of PBS perfused mouse brains. Pure myelin was then isolated by
641 several steps of density separation by ultra-centrifugation in sucrose solutions^{32,33}. After
642 several cleaning steps we incubated the purified myelin with pHrodo red (Thermo Fisher,
643 P36600) for 30 minutes following the manual instructions, followed by extensive washing in
644 PBS/Hepes.

645

646

647 **OPC/CD45+ EAE co-culture experiments.**

648 CFA and EAE mice were perfused in PBS and the spinal cords were collected. Immune cells
649 (lymphocytes, monocytes and macrophages) were then isolated using the adult Brain
650 Dissociation Kit (Miltenyi Biotec, 130-107-677) according to the manufacturer's protocol and
651 collected with MACS with CD45+ microbeads (CD45 Microbead kit, Miltenyi Biotec 130-
652 052-301) according to the manufacturer's protocol. CD45⁺ cells (150-200,000) were added on
653 top of cultured OPCs (300,000 cells) and incubated for 72hours.

654

655 **OPC/T cell co-culture experiments.**

656 Naïve and memory T cells were isolated from C57Bl/6 2D2 transgenic mice that express a
657 TCR specific for the MOG 35-55 peptide²². Splenocytes were isolated by tissue disruption
658 through a 100µm mesh and erythrocytes were lysed with ACK buffer (Invitrogen).
659 Splenocytes were depleted from all non-T cells using a naïve CD4⁺ T cell Isolation Kit (130-
660 104-453; Miltenyi), and the flow through was further sorted into CD44 low (naïve) and CD44
661 high (memory) cells using anti-CD44 beads from the same kit. Cells were frozen in FCS
662 containing 10% DMSO for subsequent experiments. Activated T cells were generated from
663 naïve cells stimulated in vitro with plate-bound anti-CD3 (BD; 555273; 2µg/ml) and soluble
664 anti-CD28 (BD; 553294; 1µg/ml) in the presence of 10ng/ml IL-12 (R&D Systems, 419-ML)
665 for 3 days, followed by 3 days expansion in 10ng/ml IL-2 (R&D Systems, 402-ML) and

666 cytokine starvation for 24h prior to co-culture (at the exception of one n which was not
667 deprived from cytokines).
668 OPCs (6 n's obtained with MACS and one n with FACS of GFP+ cells from Sox10-GFP
669 mice) were seeded at a density of 20,000 cells per well in a 96-well plate and pre-treated with
670 interferon-gamma (100ng/mL) and/or MOG 35-55 peptide (Anaspec, AS-60130-5) at
671 40µg/ml for 72hours. Cells were washed twice in PBS. Naïve, memory and activated T cells
672 were both seeded alone (with or without MOG 35-55 peptide at 40µg/ml) and on top of
673 control or stimulated OPCs at 2×10^5 cells/well in 96 well plates with MOG 35-55 peptide at
674 40µg/ml and incubated at 37°C. Media used for T cells as well as for T cell:OPC co-culture
675 was RPMI (Sigma, R8758), 10% FBS (v/v; Sigma F7524), Penicillin-Streptomycin (Sigma,
676 P4458, 100 U/ml). After 72h, cells were stimulated for 5h at 37°C in media containing
677 50ng/ml PMA (Sigma, P1585), 1µg/ml Ionomycin (Sigma, I0634) and 1µg/ml GolgiPlug
678 (BD, 555029), to boost cytokine secretion, followed by staining for CD4 PE-Dazzle
679 (BioLegend, 100566), Ki67 V450 (BD, 561281), anti-TCR Vbeta11 BV510 (BD, 743677),
680 TNF PE-Cy7 (eBioscience, 25-7423-82), IFNγ APC (BD, 554413) as well as dead cell
681 exclusion dye near-IR (Invitrogen, L10119). Surface staining at 4°C for 30 minutes was
682 followed by fixation and permeabilization using an intracellular staining kit (eBioscience, 88-
683 8824-00), followed by intracellular staining. For the relative assessment of cell survival and
684 proliferation, samples were resuspended in equal volume and acquired by fixed time with a
685 Gallios Flow Cytometer (Beckman Coulter) and analyzed using a Kaluza Flow Cytometry
686 Analysis Software (Beckman Coulter). Numbers of cells in the live gate as well as in the
687 Ki67-positive gate reflect survival and proliferation since the same cell numbers were seeded
688 onto different wells. All conditions were run in three biological replicates for each of the
689 seven experiments. Averages of the assessed values for the different conditions were divided
690 by the values of the control (T cells only) for fold differences.

691

692 **RNA extraction, cDNA synthesis and quantitative real-time PCR (qRT-PCR).** Spinal
693 cord and cerebellum were collected from WT control and EAE mice. OPCs and OLs from *in*
694 *vitro* cultures were collected in 700µl Qiazol. RNA was extracted with the miRNeasy
695 microkit (Qiagen, 217084) according to manufacturer's protocols. Contaminating DNA was
696 degraded by treatment of the samples with RNase-free DNase (Qiagen, 79254) in column.
697 0.35-1µg of RNA from each sample was reversed transcribed for 1h with the High-Capacity
698 cDNA Reverse Transcription Kit (Applied Biosystems, 4368813) including RNase inhibitor
699 (Applied Biosystems, N8080119). A reverse transcriptase negative (RT-) control was

700 included for each sample. Both the cDNA and the RT- were diluted 1:5 in RNase/DNase free
701 water for qRT-PCR.

702 qRT-PCR reactions were run on a StepOnePlus™ System (Applied Biosystems) in duplicate
703 and with RT- reactions to control for genomic DNA. Fast SYBR® Green Master Mix
704 (Applied Biosystems, 4385616) was used according to the manufacturer's instructions, each
705 PCR reaction had a final volume of 10µl and 1–2.5µl of diluted cDNA or RT-. The running
706 conditions were 20 seconds at 95°C, followed by 40 cycles of 3 seconds of 95°C and 30
707 seconds of 60°C, then 15 seconds at 95°C, 1 minute at 60°C and 15 seconds at 95°C. A
708 melting curve was obtained for each PCR product after each run, to control for primer dimers
709 and gene-specific peaks. *Tbp* and *Gapdh* were run as housekeeping genes. Relative standard
710 curves were generated for each gene to determine relative expression (CT values are
711 converted to arbitrary quantities of initial template per sample). Expression levels were then
712 obtained by dividing the quantity by the value of the geometric mean of the housekeeping
713 genes. PCR primers sequences (design according ³⁴) used are the following: *Pdgfa short* (F:
714 CGTCAAGTGCCAGCCTTC and R: GCACACTCCAGGTGTTCTC), *Pdgfa long* (F:
715 TGAAAGAGGTCCAGGTGAGG and R: CCTTTTCCTTTTCCGCTTTT), *Mbp exon2* (F:
716 GCTTCTTTAGCGGTGACAGG and R: CCTTGATACATGTGGCACAGC), *Mbp exon1* (F:
717 TGGCCACAGCAAGTACCAT and R: AGTCAAGGATGCCCGTGT), *Tbp* (F:
718 GGGGAGCTGTGATGTGAAGT and R: CCAGGAAATAATTCTGGCTCA), *Gapdh* (F:
719 GAGAAACCTGCCAAGTA and R: AGACAACCTGGTCCTCA), *Cd74* (F:
720 CTGGATGAAGCAGTGGCTCT and R: CCCAGGCCAGAAGATAGGTC), *Ciita* (F:
721 CTGGCACAGGTCTCTCCAGT and R: TACTGAGGCTGCTTGAAGGG), *Nlrc5* (F:
722 CCGTGGTACTCACATTTGCC and R: CCTTCGAGATCTCTGGGACA), *Ifit2* (F:
723 AAGGCAGAGGAAGAGGTTGC and R: GTCGCAGATTGCTCTCCAGT), *Ifih1* (F:
724 ATGTCTTGGACACTTGCTTCG and R: CTGACTCATTCCCGCTGTTT) and *Plin4* (F:
725 ACACAGTGGCCACAGGACTT and R: GGTCACCGTGTCTTAGTGC).

726

727 **Tissue processing for Immunofluorescence and RNAscope ISH.** For
728 immunohistochemistry and RNAscope ISH mice were perfused with PBS followed by 4%
729 PFA. Spinal cords from EAE and control mice were dissected and post-fixed with 4% PFA
730 for 1h, at 4°C. The tissues were embedded into OCT (Tissue-Tek), frozen in dry ice and
731 sectioned coronally (20 µm thickness). Sections were stored at –80°C.

732 For immunocytochemistry and RNAscope ISH in cultured cells, cells were fixed in 4%
733 formaldehyde for 10 minutes and washed in PBS.

734

735 **Immunohistochemistry and immunocytochemistry (mouse and human samples).** For
736 immunocytochemistry cells were incubated overnight at 4°C with the following primary
737 antibodies: CNP (Abcam ab6319, Mouse 1:200), MHC-II (anti-I-A/I-E; BD Bioscience rat
738 1:600), GFP (Abcam ab13970, Chicken 1:1000), NG2 (Millipore AB5320, Rabbit 1:200),
739 IBA-1 (Wako 019-19741, rabbit 1:400) in PBS/0.5%Triton/10% normal donkey serum
740 (Sigma, D9663). Cells were washed with PBS and then incubated for 2 hours with Alexa
741 Fluor-conjugated antibodies (Invitrogen).

742 Spinal cord sections were incubated overnight at 4°C in the following primary antibodies:
743 OLIG2 (R&D, Goat 1:200), SOX10 (R&D, Goat 1:100), PLIN4 (Sigma, Rabbit 1:200), IBA-
744 1 (Wako 019-19741, rabbit 1:400) and MHC-II (anti-I-A/I-E; BD Bioscience rat 1:600)
745 diluted in PBS/0.5% Triton/10% normal donkey serum. After washing the section with PBS,
746 secondary Alexa Fluor-conjugated antibodies (Invitrogen) diluted in PBS/0.5% Triton/10%
747 normal donkey serum were incubated for 2h at room temperature. Slides were mounted with
748 mounting medium containing DAPI (Vector, H-1200) and kept at 4°C until further
749 microscopic analysis. Images from sections were taken of the spinal cord using a Zeiss
750 LSM700 Confocal, and processed in ImageJ.

751 Human brain 4µm paraffin sections, from regions with high microglia activity (thus active
752 lesions) were dewaxed in a descending EtOH-row. After antigen retrieval and endogenous
753 peroxidase quenching, they were incubated over night at 4 degrees with the following primary
754 antibodies: OLIG1 (Abcam, 68105, 1:100), OLIG2 (Atlas, HPA003254, 1:200) and MHC-II
755 (Dako, M0775 1:100) diluted in TBS/0.3% Triton/20% normal horse serum. After washing
756 with TBS/0.001% Triton, they were incubated for 1 hour at room temperature with Goat F(ab)
757 Anti-Rabbit IgG H&L (HRP) and Goat F(ab) Anti-Mouse IgG H&L (HRP; ab7171 and
758 ab6823 abcam, 1:500). After washing in TBS/0.001% Triton, the fluorescence was visualized
759 with the tyramide kits from Perkin Elmer (NEL745B001KT and NEL744B001KT). The
760 human tissue used in this study comprised one female and one male, with ages of 38 and 50
761 years old both with secondary progressive multiple Sclerosis. No patient had specific disease
762 treatment. Post mortem MS tissue was provided via a UK prospective donor scheme with full
763 ethical approval from the UK Multiple Sclerosis Tissue Bank (MREC/02/02/39).

764

765 **RNAscope ISH.** RNAscope ISH was performed on cultured cells and spinal cord sections
766 from controls and EAE mice with probes for mouse *Cd74*, *H2-Eb1*, *Aif1*, *Sox10*, *Klk6*, *Hopx*,
767 *Ptpzr1*, *Serpina3n*, *B2m*, *Psmb9* and *Klk8* all purchased from ACD. RNAscope ISH protocol

768 for sections was performed following manufacturer's instructions with minor modifications
769 (ACD, RNAscope® Multiplex Fluorescent Detection Reagents v2 Cat. No.323110). Briefly,
770 sections were placed on a hot plate (100°C) with 1x target retrieval (Pretreatment Reagents
771 Cat. No. 322381 and 322000) for 5 min followed by 2 steps of washes of 2 min and 1 wash
772 with 100% ethanol for 2min. Protease IV was applied on top of the sections and incubated for
773 20min at RT followed by 2 washes of 2 min each. Probes were diluted 1:50 in the C1 probe,
774 hybridized for 2h at 40°C and washed twice in wash buffer (RNAscope® Wash Buffer
775 Reagents Cat. No. 310091). Amplification steps were performed by incubating with v2Amp1
776 (30min), v2Amp2 (30min) and v2Amp3 (15min) at 40°C with washes of 2x2min in between
777 steps (RNAscope® Multiplex Fluorescent Detection Reagents v2 Cat. No. 323110). Sections
778 were incubated v2HRP-C1 for 15min at 40°C and washed twice in wash buffer for 2min.
779 TSA conjugated fluorophores were diluted in 1:1500 in TSA buffer (RNAscope® Multiplex
780 TSA Buffer Cat. No. 322809) and incubated for 30min at 40°C followed by 2 washes of 2min
781 and HRP blocker incubation for 30min at 40°C. The last steps were performed subsequently
782 for v2HRP-C2 and v2-HRP-C3. To combine the RNAscope ISH with immunofluorescence
783 for GFP, sections were further blocked in block with 5% NDS in 0.3% PBS-Tx100, 1h, RT
784 and incubated with chicken anti-GFP (abcam, ab13970 1:200,) overnight at 4°C. Sections
785 were further incubated with goat anti-chicken (Alexa Fluor 488 1:500) for 2h at RT and with
786 DAPI (1:5000) for 2 min.

787

788 **Confocal Microscopy and cell counting.** Images from sections of both Immunofluorescence
789 and RNAscope ISH were acquired for the spinal cord using a Zeiss LSM700 Confocal, and
790 processed in Fiji/ImageJ. Images from the human tissue were acquired using a Leica SP8
791 Confocal, Images were processed in Fiji/ImageJ. Estimation of the percentage of the double
792 MHC-II/SOX10 positive cells in the spinal cord of EAE mice was performed by counting the
793 double positive cells present in the lesion areas of the white matter divided by the total
794 SOX10+ cells. The final percentage is represented as an average of 3 EAE mice. For the co-
795 cultures the number of GFP/MHC-II double positive cells were divided by the total number of
796 GFP+ cells. The final percentage is represented as an average of 3 independent experiments.
797 For estimation of the percentage of OPCs that phagocyte microspheres, only OPCs with more
798 than 10 microspheres surrounding the nucleus were considered as phagocytic OPCs. While
799 this might have led to an underestimation of phagocytic OPCs, this way we have avoided
800 counting OPCs with adherent and extracellular microspheres as phagocytic OPCs. Videos
801 were obtained from z-stacks after processing with Imaris image analysis software.

802

803 **Supplementary Video information**

804 **Movie 1: MOL2 cells express MHC-II genes.** RNAscope ISH representing a mouse spinal
805 cord from EAE mice marked with probes for *Cd74* (red dots) and *Klk6* (green dots). A double
806 positive cell is further highlighted in the video and represents an MOL2 cell expressing MHC-
807 II.

808 **Movie 2: OPCs express MHC-II genes.** RNAscope ISH representing a mouse spinal cord
809 from EAE mice marked with probes for *Cd74* (red dots), nuclear GFP (from *Pdgfra-H2B-*
810 *GFP* knock-in mice labeling OPCs) and *Ptprz1* (white dots). A triple positive cell is further
811 highlighted in the video and represents an OPC expressing MHC-II.

812 **Movie 3: OL lineage cells express MHC-II genes.** RNAscope ISH representing a mouse
813 spinal cord from EAE mice marked with probes for *Cd74* (red dots), *Sox10* (green dots) and
814 *Aif1* (white dots). A double positive cell is further highlighted in the video and represents an
815 OL lineage cell expressing MHC-II.

816 **Movie 4: OL lineage cells express MHC-II genes.** RNAscope ISH representing a mouse
817 spinal cord from EAE mice marked with probes for *Cd74* (red dots), *Sox10* (green dots) and
818 *Aif1* (white dots). A double positive cell is further highlighted in the video and represents an
819 OL lineage cell expressing MHC-II.

820 **Movie 5: OL lineage cells express MHC-II genes and few Aif1 molecules.** RNAscope ISH
821 representing a mouse spinal cord from EAE mice marked with probes for *Cd74* (red dots),
822 *Sox10* (green dots) and *Aif1* (white dots). At least 2 double positive cells are further
823 highlighted in the video and represent an OL lineage cells expressing MHC-II and few *Aif1*
824 molecules.

825 **Movie 6: OL lineage cells express MHC-II genes.** RNAscope ISH representing a mouse
826 spinal cord from EAE mice marked with probes for *H2.eb1* (red dots) and *Sox10* (green dots).
827 A double positive cell is further highlighted in the video and represents an OL lineage cell
828 expressing MHC-II.

829 **Movie 7: Microglia processes touch OL lineage cells.** RNAscope ISH representing a mouse
830 spinal cord from EAE mice marked with probes for *Cd74* (red dots), *Sox10* (green dots) and
831 *Aif1* (white dots). A microglia-derived process touching an OL lineage cell is further
832 highlighted in the video.

833 **Movie 8: MHC-II positive cells surround OL lineage cells in human MS patient samples.**
834 IHC performed in human brain tissue from one MS patient marked with antibodies for MHC-
835 II (white) and OLIG1 (green). A MHC-II positive cell that resides between two OLIG1

836 positive cells is further highlighted.

837 **Movie 9: OL lineage cells from human MS patient samples express MHC-II genes.** IHC
838 performed in human brain tissue from one MS patient marked with antibodies for MHC-II (white) and
839 OLIG1 (green). A double positive cell representing an OL lineage cell expressing MHC-II is further
840 highlighted in the video.

841

842

843 **Clustering analysis using GeneFocus pipeline.**

844 **Quality Control.** Cells were clustered using a custom-made approach, which we developed
845 and refer as GeneFocus. We generated an iterative Level 1 and Level 2 clustering pipeline,
846 taking advantage of diffusion mapping and spatial autocorrelation metrics to define relevant
847 genes and cell clusters. First cells were analysed and quality control (QC) filtering was
848 applied using the scater 1.6.0 in R. We applied cut-offs for the expression level 200000 total
849 FPKM and number of genes expressed 2500, resulting in 2304 and 1777 cells pre- and –post
850 QC respectively. Median FPKM values were 395721, and median gene counts were 3694
851 genes.

852

853 **Spatial Gene-filtering.** Cells were normalized (see supplemental code in Github) and feature
854 selection was performed using a support vector model from the e1071 R package
855 (<https://CRAN.R-project.org/package=e1071>). The resulting expression matrix was then inputted into
856 a custom pipeline performing iterative gene-filtering in the following manner: we converted
857 the expression matrix into a transition matrix using destiny 2.6.1 in R³⁵ with the input being
858 the first 30 principal components of the expression matrix. Subsequently, a distance matrix
859 was created from the transition matrix, after which the MoransI autocorrelation metric was
860 computed for each gene using the spdep 0.6-15 R package³⁶. Genes were filtered according to
861 the mean MoransI computed for all the genes in the expression matrix, and this mean MoransI
862 was set as a general threshold for subsequent gene filtering. After this initial filtering, a new
863 diffusion map was computed and we iteratively repeated this process until all genes within the
864 gene set remained above the determined threshold of spatial correlation set during the first
865 round of gene filtering. A similar approach was performed using the transition matrix as a
866 distance metric. A cut-off value was calculated using a support vector model that predicts a
867 mean distance as a function of the population size in which the gene is expressed and the
868 mean distance observed. We iterated the distance matrix until we obtained a gene set equal or
869 smaller than the covered gene set obtained from the MoransI metric. A joint diffusion map

870 was then created.

871

872 **Level 1 Clustering.** Level 1 clustering was established by estimating the ideal number of
873 clusters by silhouette width using the principal components of the transition matrix, from
874 which the number was estimated on the basis of an elbow plot. We estimated clusters using
875 the `factoextra` 1.0.5 package (<https://CRAN.R-project.org/package=factoextra>) and hierarchical
876 clustering using Wards metric. We determined 5 initial clusters representing OPCs and early
877 OLs, MOLs, VLMCs and pericytes, and microglial cells.

878

879 **Level 2 Clustering.** To determine more specific clustering and relevant genes we performed
880 the iterative gene filtering on each of the subclusters. The threshold for the MoransI metric
881 was established to be the mean MoransI of the gene set resulting from the second gene
882 filtering round. This resulted in a final gene set for each sub-cluster varying between 800-
883 1000 spatially correlated genes per Level 1 sub-cluster. Clusters were estimated by silhouette
884 width and the final clustering was evaluated using the resulting heatmap of the filtered genes.
885 For validation of the GeneFocus pipeline, we extracted the expression information from GEO
886 from³⁷ and were able to identify the disease-associated microglia cluster previously
887 described³⁷ (Supplementary Fig. 2). To achieve our final clustering result, we also removed
888 cells that were clustered in clusters with less than 3 cells, resulting in a total number of 1765
889 cells. These cells seemed to be doublets based on mixed expression profiles.

890

891 **Gene Modules.** To generate the gene-modules, we performed non-negative matrix
892 factorization, where ranks were established using a measure of mutual information with the
893 elbow method. We established that 30 ranks were the optimal rank to decompose the dataset
894 in. To only select robust components from the decomposed matrix, we performed PCA on the
895 components and established an optimal k number of clusters based on wards metric. All
896 component values falling within a cluster were summed after which the new concatenated
897 components were filtered based on a threshold of a Pearson correlation of 0.5 with any gene
898 in the dataset. This resulted in 15 components that revealed the major gene trends underlying
899 the data.

900

901 **Differential expression and pathway visualization**

902 Differential expression analysis was performed using the MAST R package v1.4.1³⁸. Pathway
903 visualization was performed using the clusterProfiler package v3.6.0³⁹.

904

905 **Comparison between OPCs in the current dataset and OPCs during development**⁴. The
906 R package MetaNeighbor was used, found at (<https://github.com/maggiemcrow/MetaNeighbor>).

907

908

909 **Gene ontology and pathway analysis.** Most significantly differentially expressed genes were
910 selected for each of the GO analyses. For each cell type, unique top differentially upregulated
911 genes in EAE and unique top differentially upregulated genes in controls were selected. For
912 comparisons between cell types, uniquely differentially expressed genes for each cell category
913 were selected. In order to make gene sets comparable, top 100 genes were selected. GO and
914 pathway analysis was performed with Cytoscape 3.5.1⁴⁰ plug-in ClueGO v2.3.3⁴¹ with
915 settings, GO Biological process (23.02.2017) and REACTOME pathways (01.03.2017),
916 showing only pathways with P-val ≤ 0.05 , minimum 5 genes per cluster, and default
917 settings.

918 In accordance with the NMF analysis, OPCs and OLs from EAE mice shared an enrichment
919 for genes involved in the positive regulation of adaptive immune response, positive regulation
920 of T cell mediated cytotoxicity, response to interferon and antigen processing and
921 presentation of endogenous peptides via MHC-I (Supplementary Fig. 4b). GO/Reactome
922 analysis for the enriched genes in individual clusters indicated that OPC2-EAE was enriched
923 in positive regulation of T cell mediated cytotoxicity, antigen presentation, among other
924 processes (Supplementary Fig. 5). MOL1/2-EAE exhibited enrichment in similar processes,
925 but also additional processes related with glycosylation (Supplementary Fig. 5), which has
926 been implicated in the activation of adaptive immune activation⁴². In contrast to these
927 populations, “young” (Pdgfra-H2B-GFP) MOL5/6-EAE-a exhibited less pronounced
928 upregulation in immune related GO-terms (Supplementary Fig. 5). Instead, this Plin4⁺ MOL
929 cluster is enriched in genes related to lipid modification, gliogenesis, and intrinsic apoptotic
930 signaling pathway (with anti-apoptotic genes *Bcl2l1*, *Cdkn1a* and pro-apoptotic genes *Bnip3*,
931 *Ppp1r13b*; Supplementary Table 1), among other categories (Supplementary Fig. 5).

932

933

934 **Alternative splicing analysis.** Alternatively spliced cassette exons were identified with BRIE
935 0.1.3⁴³ in the cell types clusters from Smart-seq2 data. Cassette exons annotations were
936 extracted from Gencode.vM12 from protein-coding genes according to the parameters in ⁴³.
937 For each of the cells classified as MOL1/2, MOL5/6 and OPC, the fraction of exon inclusion

938 was calculated (PSI), with default parameters. Then, for each cell type, cell to cell pair-wise
939 comparisons were performed, comparing EAE-assigned with control-assigned cells.
940 Significantly alternative spliced candidates were selected with the following parameters,
941 Bayes factor > 10, delta PSI between the two cells > 0.2 and a minimum number of
942 significant comparisons for each cell type (MOL12 >= 20, OPC >= 20 and MOL56 >= 100).
943 In order to get more specific alternatively spliced exons, the candidate events were selected
944 only when they were uniquely spliced in one direction for each cell type, for instance events
945 that are only skipped in EAE and events only included in EAE. Violin plots of selected events
946 were plotted using all the PSI values for that specific alternative exon in EAE and in control,
947 Wilcoxon rank sum test with continuity correction was used for significance test.
948 Visualization of the alternative spliced events junctions reads was done with the merged bam
949 files and the sashimi plot option in IGV⁴⁴.

950

951 **MS susceptibility genes.** Genes assigned to human MS susceptibility SNPs were transform to
952 *Mus musculus* annotation using BioMart⁴⁵. Human chrX associated SNPs from ref 2 were
953 assigned to hg19 GENCODEv19 gene annotations with Bedtools v2.25.0⁴⁶, with parameters
954 *windowbed -60000*. Recovered genes were transform to *Mus musculus* gene symbols using
955 BioMart.

956

957 **Ethics approval and consent to participate.** The collection of all animal samples was
958 performed according to the guidelines and recommendations of local animal protection
959 legislation and were approved by the local committee for ethical experiments on laboratory
960 animals in Sweden (Stockholms Norra Djurförsöksetiska nämnd). Human post mortem MS
961 tissue was provided via a UK prospective donor scheme with full ethical approval from the
962 UK Multiple Sclerosis Tissue Bank (MREC/02/02/39). All participants gave prospective pre-
963 mortem written consent for their brains to be banked and used for research.

964

965 **Code availability.** Code used for single cell RNA-Seq analysis is available at
966 <https://github.com/Castelo-Branco-lab/GeneFocus>.

967

968 **Data availability:** A web resource for browsing differential gene expression data for the
969 single cell data can be accessed at <https://ki.se/en/mbb/oligointernode>. Raw data is deposited
970 in GEO, accession number GSE113973. Code used for single cell RNA-Seq analysis is
971 available at <https://github.com/Castelo-Branco-lab/GeneFocus>.

972

973 **Supplementary Table information**

974 **Supplementary Table S1** – Differential gene expression and gene module associated with clusters
975 (level 1 and level 2 - oligodendrocytes) identified by single cell RNA-Seq in CFA and EAE mice. n=4
976 biologically independent mouse spinal cord samples per condition.

977 **Supplementary Table S2** - Splicing events and exon inclusion or exclusion events summarized by
978 cluster, in single cell RNA-Seq in CFA and EAE mice. n=4 biologically independent mouse spinal
979 cord samples per condition.

980

981 **Supplementary Table S3** - Differential genes expression between CTRL and EAE in MOLs and
982 OPCs and Gene Ontology terms for comparisons, enriched genes for each cluster, and alternatively
983 spliced genes between CTRL and EAE. n=4 biologically independent mouse spinal cord samples per
984 condition. All GO analysis were performed in cytoscape and $p < 0.05$; p values were calculated with
985 Bonferroni two-sided hypergeometric test.

986

987 **Supplementary Table S4** - Non-MHC MS susceptibility genes expressed in microglia and OPCs in
988 EAE and CTRL and significant differential expression across the major level 1 clusters.

989

990

991

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