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One Sentence Summary: Single-cell RNA-seq of a mouse model of multiple

sclerosis uncovers new oligodendrocyte populations, putative disease markers and

suggests new mechanisms underlying the pathogenesis of disease.

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-seq2[5] 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 mice[6] (enriching for

62   OPCs and "young" OLs, that had differentiated recently), and Pdgfra-Cre-LoxP-

63 GFP[7], (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, *Phyhd1*, *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 myelination[8], and decreased levels

92 of *Hes1* and *Hes5* (Supplementary Fig. 4a), that keep OPCs in a progenitor state[9]. 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 NNMF (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 reported[13]), *Mobp*, *Pdgfa* and the *Ifih1* gene, in which MS-
126   associated polymorphisms have been found[14] (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 MOL2[4]. 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 neuroprotection[15]. 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

MHC-II genes are thought to be restricted to microglia/macrophages in MS, and not present in OLs in active lesions[16]. Strikingly, we found the expression of all key genes required for a MHC-II mediated response, including *H2-aa*, *H2-ab1*, *H2-eb1*, *Cd74* and *Ctss*, in specific subsets of OPCs and OLs (Fig. 3a). To confirm the presence of these RNA transcripts in OL lineage cells, we performed RNAscope ISH in the EAE and control spinal cords using probes targeting *Cd74* and *H2-Eb1* (for MHC-II), in combination with *Aif1* (for microglia), *Sox10* (for OL lineage cells), *Klk6* (for MOL2) and *Ptprz1* (for OPC) (Fig. 3b-d, Supplementary Fig. 6b, and Supplementary Videos V1-V6). While we observed microglia processes enwrapping OLs (Supplementary video V7), a small proportion of OLs/OPCs could be confidently assigned with double positive *Cd74*/*Sox10* and few or no molecules for *Aif1* (Fig. 3d and Supplementary Videos V1-V6). We also combined RNAscope ISH with immunohistochemistry and found triple positive OPCs for GFP (from EAE Pdgfra-H2B-GFP spinal cord), *Ptprz1* and *Cd74* (Fig. 3c and Supplementary video V2). We also performed IHC for MHC-II and the OL markers OLIG2 and SOX10, and identified double positive cells for OLIG2/MHC-II and SOX10/MHC-II oligodendroglia in the spinal cord of EAE mice (Fig. 3e and Supplementary Fig. 6c). We estimated that in the lesion areas of the white matter SOX10/MHC-II double oligodendroglia constitute about 3,4% (+/-1,76 SD) of the SOX10$^+$ cells. We performed IBA-1 staining to distinguish between SOX10/MHC-II oligodendroglia and MHC-II expressing macrophages/microglia and we could distinguish the two cell types. Nevertheless, IBA-1 could still be observed in SOX10/MHC-II oligodendroglia although with a lower expression, which could be due to induction of some *Aif1* molecules (corresponds to IBA-1 protein) in oligodendroglia in EAE, by for instance interferon-gamma (IFNγ)[17]. Control CFA-treated spinal cord OPCs/OLs did not express MHC-II molecules neither at the RNA (RNAscope ISH) nor protein level (IHC; Fig. 3b and data not shown).

We found several interferon responsive genes in MHC-II expressing OL/OPC populations, such as *Ifih1*, *Iigp1*, *Trim34*, *Irf7*, *Irgm1*, *Irgm2*, *Igtp* and *Zbp1* (Supplementary Fig. 7a). It has been shown that IFNγ can trigger endoplasmic reticulum stress in OLs[18]. Indeed, we found many genes involved in protein processing in endoplasmic reticulum differentially expressed in EAE (Supplementary Fig. 7b). Transcription factors such as *Nlrc5*, a transactivator of the MHC-I[19], was

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199   expressed upon disease in all OL lineage cells, and *Ciita*, induced by the IFNγ

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 described[1]. 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 susceptibility[1]. 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 particular 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 MS[16], expression of these

245 molecules in rat OPCs and OLs has been observed, upon dexamethasone and IFNγ

246 treatment[20]. 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 IFNγ. As such, we treated OPCs with IFNγ (100

249 ng/ml), dexamethasone (1µM) 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 IFNγ 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 IFNγ 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 IFNγ.

262

263 To uncover the role of immunocompetent OPCs in the disease, we first investigated if

264 OPCs were capable of phagocytosis, by adding 1µm 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). IFNγ 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 phagosomes[21]) 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 peptide[22]. OPCs (controls or

280   pre-treated with IFNγ 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 IFNγ 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 IFNγ 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 IFNγ, was enough to induce proliferation.

297   This indicates that production of IFNγ 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

9

CD4^{+} T cells displayed more cells producing both TNF and IFNγ when co-cultured with OPCs in the presence of MOG peptide (Supplementary Fig. 8d). In contrast, the presence of OPCs per se triggered an increase in the number of Th1 CD4^{+} T cells producing IFNγ and TNF (Supplementary Fig. 8d).

OLs are widely seen as a passive target of a dysregulated immune system, but there is a growing body of evidence of immunomodulatory factors expressed in OLs such as cytokines/chemokines and their receptors[23-27]. Our results indicate that both OPCs and MOLs undergo a transcriptional overhaul during chronic inflammatory demyelination. The selective expression of immunoprotective, innate and adaptive immunity genes in OPCs and OLs in EAE suggests potential mechanisms of protection and an immune function in the context of disease. Since several of these genes are susceptibility genes for MS, the OL lineage might have a more central role in the origin and progression of MS than previously thought.

OL cell death has been recently shown to lead to an adaptive auto-immune response[28], which could imply an initial dysregulation in cells of the OL lineage as a triggering event in MS and support the "inside-out" hypothesis for the aetiology of the disease[28]. Whether the new identified OL lineage cell states play a role on the aetiology of MS, by acting as antigen presenting cells and triggering immunologic attack or reinforcing the disease-initiating event from the periphery, or both, will require further investigation. In either case, our results indicate that OL lineage cells may not simply be passive targets of the immune system in MS, but rather central players which may be targeted in therapeutic approaches for MS.

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

Acknowledgements

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.

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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:**

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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

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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**

13

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 ± 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; MiGl - 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 - 20μm **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 mice[6], Sox10Cre-LoxP-GFP[29] and C57Bl/6 2D2

571 transgenic mice[22]. 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 mMCaCl2, 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 in[5]. SmartSeq2

614   raw data was processed according to procedures described in[5]. Smart-seq2 paired-end reads

615   were trimmed with cutadapt 1.8.0[30] and aligned with STAR 2.5.1b[31] 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 peptide[22]. 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 naive 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 $2x10^5$ 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), IFNg 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: GCACACTCCAGGTGTTCCTC), *Pdgfa long* (*F:*

715 TGAAAGAGGTCCAGGTGAGG and R: CCTTTTCCTTTTCCGCTTTT), *Mbp exon2* (F:

716 GCTTCTTTAGCGGTGACAGG and R: CCTTGTACATGTGGCACAGC), *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   GTCGCAGATTGCTCTCCAGT),   *Ifih1*   (F:

724 ATGTCTTGGACACTTGCTTCG and R: CTGACTCATTCCCGCTGTTT) and *Plin4* (F:

725 ACACAGTGGCCACAGGACTT and R: GGTCACCGTGTCCTTAGTGC).

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 4ºC. 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.

21

734

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

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

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

RNAscope ISH. RNAscope ISH was performed on cultured cells and spinal cord sections from controls and EAE mice with probes for mouse *Cd74*, *H2-Eb1*, *Aif1*, *Sox10*, *Klk6, Hopx, 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 R[35] 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 package[36]. 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

25

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 from[37] and were able to identify the disease-associated microglia cluster previously
887 described[37] (Supplementary Fig. 2). To achieve out 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 v1.4.1[38]. Pathway
903 visualization was performed using the clusterProfiler package v3.6.0[39].

26

904

Comparison between OPCs in the current dataset and OPCs during development[4]**.** The

R package MetaNeighbor was used, found at (https://github.com/maggiecrow/MetaNeighbor).

907

908

Gene ontology and pathway analysis. Most significantly differentially expressed genes were

selected for each of the GO analyses. For each cell type, unique top differentially upregulated

genes in EAE and unique top differentially upregulated genes in controls were selected. For

comparisons between cell types, uniquely differentially expressed genes for each cell category

were selected. In order to make gene sets comparable, top 100 genes were selected. GO and

pathway analysis was performed with Cytoscape 3.5.1[40] plug-in ClueGO v2.3.3[41] with

settings, GO Biological process (23.02.2017) and REACTOME pathways (01.03.2017),

showing only pathways with P-val <= 0.05, minimum 5 genes per cluster, and default

settings.

In accordance with the NNMF analysis, OPCs and OLs from EAE mice shared an enrichment

for genes involved in the positive regulation of adaptive immune response, positive regulation

of T cell mediated cytotoxicity, response to interferon and antigen processing and

presentation of endogenous peptides via MHC-I (Supplementary Fig. 4b). GO/Reactome

analysis for the enriched genes in individual clusters indicated that OPC2-EAE was enriched

in positive regulation of T cell mediated cytotoxicity, antigen presentation, among other

processes (Supplementary Fig. 5). MOL1/2-EAE exhibited enrichment in similar processes,

but also additional processes related with glycosylation (Supplementary Fig. 5), which has

been implicated in the activation of adaptive immune activation[42]. In contrast to these

populations, "young" (Pdgfra-H2B-GFP) MOL5/6-EAE-a exhibited less pronounced

upregulation in immune related GO-terms (Supplementary Fig. 5). Instead, this Plin4^{+} MOL

cluster is enriched in genes related to lipid modification, gliogenesis, and intrinsic apoptotic

signaling pathway (with anti-apoptotic genes *Bcl2l1*, *Cdkn1a* and pro-apoptotic genes *Bnip3*,

Ppp1r13b; Supplementary Table 1), among other categories (Supplementary Fig. 5).

932

933

Alternative splicing analysis. Alternatively spliced cassette exons were identified with BRIE

0.1.3[43] in the cell types clusters from Smart-seq2 data. Cassette exons annotations were

extracted from Gencode.vM12 from protein-coding genes according to the parameters in [43].

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 IGV[44].

950

951 **MS susceptibility genes**. Genes assigned to human MS susceptibility SNPs were transform to

952 *Mus musculus* annotation using BioMart [45]. Human chrX associated SNPs from ref 2 were

953 assigned to hg19 GENCODEv19 gene annotations with Bedtools v2.25.0[46], 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

Supplementary Table information

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

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

980

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

986

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

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991

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1034
1035

a

Pdgfra-Cre-LoxP-GFP
Pdgfra-H2B-GFP

Dissociation to single cell suspension

FACS sorting

Data analysis

EAE

MOG
CFA

Control

PBS
CFA

spinal cord

Laser

single cell smartseq2

GFP⁻   GFP⁺

OPCs
OLs
VLMCs
Microglia

b

spinal cord collection

EAE score

Days post induction

c

Control
EAE

OPCs

VLMC

MiGl

tSNE

tSNE dim2

d

OPCs

MOL5/6 Ct-b   MOL2

MOL
Ct-a

MOL3
EAE

MOL5/6 EAE-c

OLs

MOL5/6 EAE-a

e

OPCs

Normalized Counts

Rph3a, Adora2b, Pbyhd1, Sult1a1, Slc14a1, Mylk, Myrf, Mki67, Bub1, Pdgfra

OPC1 OPC2 OPC3 OPC_Cycling

f

MOLs

Normalized Counts

Fbln, Slc9b2, Itga8, Tlr3, Sult1a1, Zfand4, S100a10, Rnase4

MOL2 Ct-a MOL5/6 Ct-a MOL5/6 Ct -b MOL1/2 EAE MOL5/6 EAE-a MOL5/6 EAE-b MOL5/6 EAE-c MOL3 EAE

Control
EAE enriched

g

Fcgr2b   OPCs EAE

Col20a1   OPCs EAE

COP/NFOL

Plin4

MOL5/6 EAE-a

Klk8   MOL1/2 EAE

Hif3a   OPC2

MOL5/6 EAE-a

Il12rb1

h

Pdgfa

Control

EAE

13894469   13898309   13899149   13899989

i

PSI

p= 0.0003176

Control
EAE

p= 5.323e- 11

Pdgfa
ex6

Mbp
ex2

a

CFA control

EAE

DAPI Sox10 Klk8 Hopx

b

Serpins

Control ┄┄┄
EAE enriched ───

Normalized Counts

Serpina3n
Serpina3h
Serpina3c
Serpina3i
Serpina3f
Serpina3g
Serping1

OPC1
MOL2 Ct-a
MOL5/6 Ct-a -b
MOL5/6 Ct -b
OPC2
OPC3
OPC Cyc
COPs
NFOLs
MOL1/2 EAE
MOL5/6 EAE-a
MOL5/6 EAE-b
MOL5/6 EAE-c
MOL3 EAE

c

CFA control

EAE

DAPI Sox10 Serpina3n

d

MHC-I

Control ┄┄┄
EAE enriched ───

Normalized Counts

Tap1
Psmb9
B2m
H2.K1
H2.D1
H2.T23

OPC1
MOL2 Ct-a
MOL5/6 Ct-a
MOL5/6 Ct-b
OPC2
OPC3
OPC Cyc
COPs
NFOLs
MOL1/2 EAE
MOL5/6 EAE-a
MOL5/6 EAE-b
MOL5/6 EAE-c
MOL3 EAE

e

CFA control

EAE

DAPI Sox10 Psmb9 B2m

a MHC-II

Control ······
EAE enriched ——

Cd74
Ctss
H2.Aa
H2.Ab1
H2.Eb1
H2.DMa

Normalized Counts

OPC1
MOL2 Ct-a
MOL5/6 Ct-a
MOL5/6 Ct-b
OPC2
OPC3
OPC Cyc.
COPs
NFOLs
MOL1/2 EAE
MOL5/6 EAE-a
MOL5/6 EAE-b
MOL5/6 EAE-c
MOL3 EAE

b CFA control

DAPI Cd74 Sox10 Aif1

c EAE

DAPI Cd74 GFP Ptprz1

d EAE

DAPI Cd74 Sox10 Aif1

Sox10   Aif1

Cd74   DAPI

e EAE

DAPI MHC-II SOX10 IBA1

DAPI   IBA1

MHC-II   SOX10

f Human MS

Brain pat. 1

Hoechst MHC-II | Hoechst OLIG1 | Hoechst OLIG1 MHC-II

Brain pat. 2

g MHC locus associated susceptibility genes

Rnf39
Trim31
H2.Eb2
Lta
Nfkbil1
Lst1
H2.Aa
Agpat1

OPC   MOL Ct   MOL EAE   MGli

Non-MHC locus associated susceptibility genes

OPC   MOL Ct   MOL EAE   MGli

Group
Ct
EAE

OPC1
OPC2
OPC3
OPC_Cycling
COP
NFOL
MOL2 Ct
MOL5/6 Ct-a
MOL5/6 Ct-b
MOL1/2 EAE
MOL5/6 EAE-a
MOL5/6 EAE-b
MOL5/6 EAE-c
MOL3 EAE

a

Sox10Cre::GFP
P7 brain
→ MACS CD140a+ → OPCs → Co-culture 72h → Immune cells ← MACS CD45+ ← WT MOG EAE or C / FA controls, adult EAE spinal cord

b

Sox10 Cre GFP
MHC-II
IBA1
DAPI Sox10Cre:GFP MHC-II IBA1

c

WT / Sox10Cre::GFP
P7 brain
→ MACS CD140a+ → OPCs / OLs, Dexamethasone Interferon-γ

d

Primary OLs

Control
ICC — DAPI MHC-II CNP
RNAscope — DAPI Cd74 Sox10

Dexamethasone + Interferon-γ
ICC — DAPI MHC-II CNP
RNAscope — DAPI Cd74 Sox10

e

Primary OPCs
- Control
- Dexamethasone
- Interferon-γ
- Dexamethasone + Interferon-γ

normalized mRNA
Cd74 Ciita Nlrc5 Ifit2 Ifih1 Plin4

Primary OLs
normalized mRNA
Cd74 Ciita Nlrc5 Ifit2 Ifih1 Plin4

f

Primary OPCs

Hoechst
pHrodo-labeled myelin

Control — DAPI Microspheres 1µm NG2 / DAPI Microspheres 1µm
Cytochalasin D 50µM — DAPI Microspheres 1µm NG2 / DAPI Microspheres 1µm

g

% phagocytic OPCs
- DMSO
- Cytochalasin D 20µM
- Cytochalasin D 50µM

Control Interferon-γ

h

CD4 T cells 2D2 Tg spleen
CD3/CD28+; IL12 treatment
Naive Memory Activated (Th1)

OPC Pre-treatment
Ct
IFN-γ
MOG
IFN-γ + MOG

Co-culture 72 hours +/- MOG → FACS analysis of T-cells

i

Survival (live cells) — fold change relative to T cells

Naive 2D2 CD4 T cells
Memory 2D2 CD4 T cells
Activated (Th1) 2D2 CD4 T cells

- - MOG OPCs OPCs IFN-γ OPCs MOG OPCs IFN-γ MOG

j

Proliferation (Ki67+) — fold change relative to T cells

- - MOG OPCs OPCs IFN-γ OPCs MOG OPCs IFN-γ MOG