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Myelin Regulatory Factor Drives Remyelination in Multiple Sclerosis

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Corresponding Author:	Wolfram Tetzlaff University of British Columbia Vancouver, British Columbia CANADA	
Corresponding Author Secondary Information:		
Corresponding Author's Institution:	University of British Columbia	
Corresponding Author's Secondary Institution:		
First Author:	Greg J. Duncan	
First Author Secondary Information:		
Order of Authors:	Greg J. Duncan	
	Jason R. Plemel	
	Peggy Assinck	
	Sohrab B. Manesh	
	Fraser G.W. Muir	
	Ryan Hirata	
	Matan Berson	
	Jie Liu	
	Michael Wegner	
	Ben Emery	
	G.R. Wayne Moore	
Order of Authors Secondary Information:		
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	Multiple Sclerosis Society of Canada (EGID 2810)	Dr. Wolfram Tetzlaff
Abstract:	Remyelination is limited in the majority of multiple sclerosis (MS) lesions despite the presence of oligodendrocyte precursor cells (OPCs) in most lesions. This observation has led to the view that a failure of OPCs to fully differentiate underlies remyelination failure. OPC differentiation requires intricate transcriptional regulation, which may be disrupted in chronic MS lesions. The expression of few transcription factors have been	

differentially compared between remyelinating lesions and lesions refractory to remyelination. In particular, the oligodendrocyte transcription factor myelin regulatory factor (MYRF) is essential for myelination during development, but its role during remyelination and expression in MS lesions is unknown. To understand the role of MYRF during remyelination, we genetically fate mapped OPCs following lysolecithin-induced demyelination of the corpus callosum in mice and determined that MYRF is expressed in new oligodendrocytes. OPC-specific *Myrf* deletion did not alter recruitment or proliferation of these cells after demyelination, but decreased the density of new glutathione S-transferase π positive oligodendrocytes. Subsequent remyelination, in both the spinal cord and corpus callosum is highly impaired following *Myrf* deletion from OPCs. Individual OPC-derived oligodendrocytes, produced in response to demyelination, showed little capacity to express myelin proteins following *Myrf* deletion. Collectively, these data demonstrate a crucial role of MYRF in the transition of oligodendrocytes from a premyelinating to a myelinating phenotype during remyelination. In the human brain, we find that MYRF is expressed in NogoA and CNP-positive oligodendrocytes. In MS, there was both a lower density and proportion of oligodendrocyte lineage cells and NogoA+ oligodendrocytes expressing MYRF in chronically demyelinated lesions compared to remyelinated shadow plaques. The relative scarcity of oligodendrocyte lineage cells expressing MYRF in demyelinated MS lesions demonstrates, for the first time, that chronic lesions lack oligodendrocytes that express this necessary transcription factor for remyelination and supports the notion that a failure to fully differentiate underlies remyelination failure.

[Click here to view linked References](#)

1 **Title: Myelin Regulatory Factor Drives Remyelination in Multiple Sclerosis**

2 **Authors:** Greg J. Duncan^{1,2}, Jason R. Plemel⁶, Peggy Assinck^{1,3}, Sohrab B. Manesh^{1,3}, Fraser G.W. Muir^{1,4}, Ryan
3 Hirata¹, Matan Berson¹, Jie Liu¹, Michael Wegner⁸, Ben Emery^{9,10}, G.R. Wayne Moore^{1,4,7} and Wolfram Tetzlaff^{1,2,5}.

4 **Affiliations:** ¹ International Collaboration on Repair Discoveries (ICORD), University of British Columbia (UBC),
5 Vancouver, BC, Canada

6 ² Department of Zoology

7 ³ Graduate Program in Neuroscience

8 ⁴ Pathology and Laboratory Medicine

9 ⁵ Department of Surgery

10 ⁶ Department of Clinical Neurosciences, Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta Canada

11 ⁷ Vancouver Hospital and Health Sciences Centre

12 ⁸ Institut für Biochemie, Emil-Fischer-Zentrum, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen,
13 Germany.

14 ⁹ School of Medicine, Jungers Center for Neurosciences Research, Oregon Health and Science University, U.S.A.

15 ¹⁰ Department of Anatomy and Neuroscience, University of Melbourne, Australia.

16 ***Corresponding Author:**

17 Wolfram Tetzlaff, MD, PhD.

18 John and Penny Ryan British Columbia Leadership Chair in Spinal Cord Research

19 Professor, Departments of Zoology and Surgery

20 Director ICORD (International Collaboration on Repair Discoveries)

21 University of British Columbia

22 Blusson Spinal Cord Centre

23 818 West 10th Avenue

24 Vancouver, BC, V5Z 1M9

25 *Phone:* 604 675 8848

26 *Fax:* 604 675 8820

27 *Email:* tetzlaff@icord.org

28

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39 **Abstract**

40 Remyelination is limited in the majority of multiple sclerosis (MS) lesions despite the presence of oligodendrocyte
41 precursor cells (OPCs) in most lesions. This observation has led to the view that a failure of OPCs to fully
42 differentiate underlies remyelination failure. OPC differentiation requires intricate transcriptional regulation, which
43 may be disrupted in chronic MS lesions. The expression of few transcription factors have been differentially
44 compared between remyelinating lesions and lesions refractory to remyelination. In particular, the oligodendrocyte
45 transcription factor myelin regulatory factor (MYRF) is essential for myelination during development, but its role
46 during remyelination and expression in MS lesions is unknown. To understand the role of MYRF during
47 remyelination, we genetically fate mapped OPCs following lysolecithin-induced demyelination of the corpus
48 callosum in mice and determined that MYRF is expressed in new oligodendrocytes. OPC-specific *Myrf* deletion did
49 not alter recruitment or proliferation of these cells after demyelination, but decreased the density of new glutathione
50 *S*-transferase π positive oligodendrocytes. Subsequent remyelination, in both the spinal cord and corpus callosum is
51 highly impaired following *Myrf* deletion from OPCs. Individual OPC-derived oligodendrocytes, produced in
52 response to demyelination, showed little capacity to express myelin proteins following *Myrf* deletion. Collectively,
53 these data demonstrate a crucial role of MYRF in the transition of oligodendrocytes from a premyelinating to a
54 myelinating phenotype during remyelination. In the human brain, we find that MYRF is expressed in NogoA and
55 CNP-positive oligodendrocytes. In MS, there was both a lower density and proportion of oligodendrocyte lineage
56 cells and NogoA+ oligodendrocytes expressing MYRF in chronically demyelinated lesions compared to
57 remyelinated shadow plaques. The relative scarcity of oligodendrocyte lineage cells expressing MYRF in
58 demyelinated MS lesions demonstrates, for the first time, that chronic lesions lack oligodendrocytes that express this
59 necessary transcription factor for remyelination and supports the notion that a failure to fully differentiate underlies
60 remyelination failure.

61

62 **Keywords:** remyelination, multiple sclerosis, MYRF, oligodendrocyte, Cre-loxP

63

64

65 **Introduction**

66 Multiple sclerosis (MS) is characterized by inflammatory CNS demyelination and is one of the most
67 common causes of chronic motor disability in young adults [60]. Remyelination occurs in MS [49, 51], which is
68 sufficient to restore conductance in experimental models [55] and potentially protect axons from degeneration [16,
69 26, 32, 44]. However, remyelination is often incomplete [46] with a lower percentage of remyelinating shadow
70 plaques relative to demyelinated plaques at all ages [20] despite fewer acute inflammatory lesions with disease
71 chronicity [19, 36]. While oligodendrocyte precursor cells (OPCs) and some premyelinating oligodendrocytes are
72 found within chronically demyelinated MS lesions [7, 34, 61, 62], these lesions contain few oligodendrocytes
73 capable of remyelination. This has led to the hypothesis that a failure of OPCs to fully differentiate causes
74 remyelination failure in MS [14, 15, 34]. Oligodendrocyte differentiation requires intricate transcriptional regulation
75 during development, but the role of many key transcription factors remain untested during remyelination [12]. We
76 hypothesized that remyelination failure could result if the chronic lesion environment inhibits the expression of
77 essential transcription factor(s) needed for oligodendrocyte differentiation or myelin gene expression.

78 Myelin regulatory factor (MYRF) is a transcription factor expressed in oligodendrocytes [5, 6, 11] and is
79 essential for developmental myelination [11]. MYRF can directly bind putative enhancer sequences of myelin genes,
80 such as *Mbp* and *Plp*, to induce their expression [5] and together with the transcription factor Sox10, synergistically
81 promote myelin gene expression [11, 25]. Genetic deletion of *Myrf* during development results in a near complete
82 failure to differentiate into late-stage myelinating oligodendrocytes yet does not overtly affect the specification of
83 OPCs [11]. Therefore, *Myrf* deletion during development mirrors what is observed in chronically demyelinated MS
84 lesions; OPCs are maintained within the CNS but cannot fully differentiate. At this time, no study has investigated
85 the role of MYRF during remyelination nor its expression in MS lesions.

86 Here, we examined the function of MYRF during remyelination. By genetically fate mapping OPCs
87 following lyssolecithin (LPC) demyelination in the corpus callosum of mice, we found that MYRF was expressed in
88 new oligodendrocytes. When *Myrf* was deleted in OPCs, their recruitment and proliferation within the lesion were
89 not affected, but their capacity to differentiate into new oligodendrocytes was greatly diminished. OPC
90 differentiation stalled at the premyelinating stage with recombined cells unable to express myelin proteins,
91 ultimately inhibiting remyelination in both the corpus callosum and in the spinal cord. In human white matter,
92 MYRF was expressed in Sox10+ oligodendrocyte lineage cells, which often co-labelled with the mature

93 oligodendrocyte marker NogoA. In the centres of chronic active MS lesions, there was a reduction in the density of
94 MYRF+Sox10+ cells relative to both shadow plaques and normal-appearing white matter (NAWM). Additionally,
95 fewer Sox10+ oligodendrocyte lineage cells, and Sox10+NogoA+ oligodendrocytes expressed MYRF in the lesion
96 centres relative to shadow plaques, indicating these cells lacked expression of a necessary transcription factor for
97 differentiation and myelination. Collectively, these data demonstrate that *Myrf* is essential for remyelination in the
98 rodent CNS and its failure to be expressed in oligodendrocytes in chronic MS lesions is associated with
99 remyelination failure.

100

101 **Materials and Methods**

102 *Transgenic Mice and Experimental Design*

103 *Myrf*^{fl/fl} mice [11], which have LoxP inserted around exon 8 of *Myrf*, were crossed with PDGFR α -CreERT2
104 mice [52]. The Cre-mediated recombination of *Myrf* in the *Myrf*^{fl/fl} mice was predicted to result in the production of
105 a truncated protein that lacks the DNA-binding domain found in exon 8 and the C-terminus of the protein due to a
106 frame shift, ultimately making the protein non-functional [11, 40]. *Myrf*^{fl/fl} PDGFR α -CreERT2 (P-*Myrf*^{fl/fl}) mice
107 were used to induce selective recombination and *Myrf* deletion in a portion of platelet-derived growth factor receptor
108 α (PDGFR α)⁺ cells and *Myrf*^{fl/wt} PDGFR α -CreERT2 (P-*Myrf*^{fl/wt}) littermates were used as controls. *Myrf*^{fl/fl} mice
109 demonstrate no phenotype following recombination compared to mice wildtype for *Myrf* [11, 40]. For genetic fate
110 mapping experiments, *Myrf*^{fl/fl} PDGFR α -CreERT2 lines were subsequently crossed with Rosa26R-eYFP (YFP) Cre-
111 inducible reporter mice [56] or ROSA26-mGFP (mT/mG) [42] to induce, via Cre-mediated recombination,
112 cytoplasmic or membrane-tethered fluorescence, respectively. Both Cre and inducible reporter genes were
113 heterozygous in all experiments. All animals were genotyped prior to experiments via standard protocols. *Myrf*^{fl/fl}
114 mice were also crossed with PLP-CreERT2 and Rosa26-YFP mice to produce *Myrf*^{fl/wt} and *Myrf*^{fl/fl} PLP CreERT2
115 Rosa26-YFP mice to induce recombination in oligodendrocytes following tamoxifen injection.

116 *Tamoxifen administration*

117 Tamoxifen (T5648, Sigma) was dissolved in corn oil (C8267, Sigma) at 20mg/ml. P-*Myrf*^{fl} mice received
118 intraperitoneal injections (100mg/kg) once daily for five days starting two days prior to lyssolecithin demyelination.
119 For *Myrf*^{fl/wt} PLP CreERT Rosa26 YFP and *Myrf*^{fl/fl} PLP CreERT Rosa26 YFP mice, 4-hydroxytamoxifen (H7904,
120 Sigma) was dissolved in corn oil at 10mg/mL and 1mg was administered by intraperitoneal injection once per day

121 for five consecutive days. Tamoxifen improves the speed of remyelination at relatively low doses (0.5-2.0
122 mg/kg)[22] and results in cellular stress at high doses (75mg/kg) indicated by the upregulation of the Atf3
123 transcription factor [9]. To control for tamoxifen-mediated effects, all mice were treated with the same tamoxifen
124 regiment except for a group of mice for qPCR and another for lesion size analysis at 3 days post lesion (DPL) which
125 were injected with corn oil alone. We found that tamoxifen had no effect on lesion size at 3 DPL (tamoxifen treated
126 $0.473 \pm 0.099 \text{ mm}^2$ versus non-tamoxifen treated $0.376 \pm .027 \text{ mm}^2$, $P=0.355$ Student's T-Test) suggesting that
127 tamoxifen does not alter the susceptibility to lysolecithin-mediated demyelination.

128 *Real-time quantitative PCR*

129 2mm^3 blocks of the anterior corpus callosum and cortex were collected from P-Myrf^{fl/fl} and P-Myrf^{fl/wt} mice
130 12 days after the last tamoxifen or oil injection and flash frozen. RNA was extracted and reversed transcribed as
131 previously described [48]. Primers for recombinant *Myrf* [31] were normalized relative to β -actin and CT values were
132 determined by automatic baseline and auto-threshold. $\Delta\Delta\text{CT}$ method was used to compare relative gene expression
133 between groups [38].

134 *Lysolecithin demyelination*

135 1% lysophosphatidylcholine (lysolecithin L1381, Sigma) was dissolved in sterile phosphate-buffered saline
136 (PBS) by sonication. Mice were deeply anaesthetized using a 3% isoflurane-oxygen mixture (Baxter) and a small
137 hole was drilled with a dentist drill to allow for the insertion of a glass capillary attached to a 5 μL Hamilton syringe
138 into the brain tissue. A total of 2 μL was injected at a rate of 50nL/min by a pump (Precision Scientific Instruments)
139 into the corpus callosum at the coordinates 1.4mm anterior to bregma, 1mm lateral of bregma and 2.1mm deep from
140 the cortical surface. The glass capillary was retained in place for at least five minutes post injection to reduce reflux
141 along the needle track. Mice received buprenorphine twice daily to alleviate pain (0.03mg/kg) for the first two days
142 post-surgery and 1mL Ringer's solution. The overlying skin was sutured. For lysolecithin demyelination of the
143 cervical spinal cord, surgeries were conducted as above with the following differences. A laminectomy of the C4
144 vertebrae was performed with fine surgical rongeurs. Injections were placed at a 30 degree angle relative to vertical
145 and just lateral to the midline in the dorsal column to a depth of 0.5mm ventral to the dorsal surface. A total of
146 0.5 μL of lysolecithin was injected at a rate of 50nL/min. The overlying musculature and skin were sutured.

147 *Tissue processing*

148 For immunohistochemical analysis of tissue, mice were deeply anaesthetized and transcardially perfused
149 with PBS followed by 4% paraformaldehyde (04042, Fisher Scientific). Spinal cords or brains were dissected and
150 postfixed in paraformaldehyde for either eight hours or overnight for brains. Tissue was then cryoprotected in
151 ascending sucrose solutions before being embedded in OCT, frozen, and stored at -80° Celsius. Tissue was sectioned
152 coronally at 20µm thickness using a cryostat (HM-525, Thermo Scientific) and sections were thaw-mounted onto
153 slides (12-550-15, Fisher Scientific).

154 To prepare tissue for electron microscopy, mice were deeply anaesthetized then perfused with 0.01M PBS
155 before receiving 1% glutaraldehyde (16221, Electron Microscopy Sciences) with 4% paraformaldehyde at 4° C. The
156 area of demyelination was immediately dissected into 1mm³ cubes and postfixed in 2% glutaraldehyde. Tissue was
157 washed three times in 0.1M cacodylate buffer with 5.3mM CaCl₂ before being placed in 1% osmium tetroxide
158 (19190, Electron Microscopy Sciences) with 1.5% potassium ferrocyanide (BDH) for 1.5 hours. Tissue was then
159 dehydrated through ascending alcohol washes before embedding in Spurr resin.

160 *Immunohistochemistry*

161 Slides were thawed prior to staining and rehydrated with PBS. Sections were blocked using 10% donkey
162 serum dissolved in PBS with 0.1% Triton. Primary antibodies (Supplementary Table 1) were applied overnight at
163 room temperature in a humid chamber, washed, then appropriate donkey Dylight or Alexa Fluor secondary
164 antibodies (Jackson ImmunoResearch Laboratories, Inc) were applied for two hours. Slides were subsequently
165 washed and coverslipped using Fluoromount-G (0100-01, Southern Biotech). Prior to myelin stains, delipidation
166 was performed using ascending and descending ethanol washes followed by PBS washes before the blocking step.

167 *Cell counts and quantifications on mouse tissue*

168 Imaging on mice was performed on a Zeiss Axio-Observer M1 inverted spinning disc confocal microscope
169 using Zen 2011 or Zen 2 software. Tiled confocal merged images of the entire demyelinated zone in the corpus
170 callosum were captured with a 40x oil immersion objective (numerical aperture 1.3) using a distance of 1µm
171 between the individual optical sections. For cell counts, the lesion was defined by either the absence or obvious
172 damage of myelin based on myelin basic protein (MBP) or 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNP)
173 stains. The lesion area was defined by the presence of the astrocyte scar (increased GFAP+ density) for the analysis
174 of the contribution of recombined cells to remyelination and analyses of the number of nodes of Ranvier. Five to
175 seven sections were analyzed per animal beginning with the lesion epicenter. For analyses of the oligodendrocyte

176 density in the uninjured CNS, the corpus callosum contralateral to the lysolecithin injection were examined for
177 PDGFR α + and glutathione s-transferases pi isoform (GST π)⁺ cells in the area dorsal of the ventricle.

178 To quantify node of Ranvier density systematic random sampling was conducted in the lesion and nodes of
179 Ranvier were counted in 50 μ m x 50 μ m areas. Mature nodes of Ranvier were defined as punctate clusters of
180 Ankyrin-G (AnkG) flanked by two punctate Caspr-positive paranodes [41]. Typically, over 1000 nodes were
181 counted over 5-6 sections per animal. The density of SMI312⁺ axons were thresholded on ImageJ (NIH) and
182 quantified as a percentage of lesion area. The volume of demyelinated tissue in P-Myrf^{fl/fl} YFP and P-Myrf^{fl/wt} YFP
183 was examined by manually outlining the area of the intact corpus callosum that was MBP-negative.

184 To determine the area of demyelination that had been remyelinated by recombined cells in Myrf^{fl/fl}
185 PDGFR α CreERT2 mT/mG and Myrf^{fl/wt} PDGFR α CreERT2 mT/mG mice, the lesion was imaged for GFP, MBP
186 and GFAP. The ImageJ plugin ‘RG2B Colocalization’ was used to determine the area of GFP and MBP
187 colocalization within the manually defined GFAP⁺ lesion.

188 *Electron microscopy*

189 Resin blocks with lysolecithin lesions were sampled every 250 μ m to determine the lesion epicenter. At the
190 epicenter, 1 μ m semithin sections were cut on an ultramicrotome (Ultracut E, Reichert-Jung) and stained briefly in a
191 1% toluidine blue and 2% borax solution then coverslipped with Permount (SP15, Fisher Scientific). Rank analysis
192 was performed [30] by two blinded raters on images of semithin sections of the whole dorsal column taken with a
193 63x oil immersion objective (numerical aperture 1.3) on a Zeiss, Axio Imager.M2 microscope. Each section was
194 scored independently for the presence of thinly myelinated axons. When discrepancies in the ranking occurred, the
195 average score was taken. For electron microscopy, ultrathin (90nm) sections of the lesion epicenter were collected
196 and stained with Reynold’s lead citrate and uranyl acetate to enhance contrast then imaged at 10000-12500x primary
197 magnification on a Zeiss EM910.

198 *Human tissue analysis*

199 Human brain tissue was used with the approval of the UBC Clinical Research Ethics Board of the
200 University of British Columbia (H01-70430). Patient information as well as the number of lesions analyzed for non-
201 MS controls and MS patients is displayed in Supplementary Table 2. Sections (5 μ m) from formalin-fixed paraffin-
202 embedded tissue were obtained on microtome (1512, Leitz) and all lesions were stained on adjacent slides with luxol
203 fast blue (LFB), and immunohistochemically for class II human leukocyte antigen (HLA II), MBP and CNP for

204 lesion classification according to histological criteria [4, 59]. Only lesions within the subcortical or periventricular
205 white matter were examined. NAWM was examined at least 1.0 cm distant from a lesion in an area lacking
206 inflammation. For immunohistochemical staining, slides were first deparaffinized then antigen retrieval was
207 performed by heating slides in pH 6.0 10mM sodium citrate buffer for ten minutes. Blocking was performed in 10%
208 normal donkey serum in PBS Tween. Primary antibodies were applied overnight in a humid chamber before
209 washing and applying appropriate Alexa Fluor conjugated secondary antibodies (Jackson ImmunoResearch
210 Laboratories) at 1:200 for two hours. Slides were washed with PBS then stained with 0.30% (0.15% in slides with
211 NogoA) Sudan black in 70% ethanol (4197-25-5, Sigma) for three minutes before being washed again in PBS and
212 coverslipped with Fluoromount-G. An adjacent control slide for each lesion was stained simultaneously with
213 secondary antibodies, Hoechst (1:10000) and Sudan black but lacked primary antibodies for comparison of
214 background fluorescence. Cells were considered positive only if their fluorescence was substantially higher than
215 background fluorescence as assessed on control slides imaged with the same exposure settings. Systematic random
216 sampling was used within the lesion area manually defined on the Zen 2 software by Sudan black staining and cross-
217 referenced with adjacent slides with HLA II and LFB staining. At least twenty 100 μ m x 100 μ m areas were
218 examined in all lesions. Chronic lesions had 60 100 μ m x 100 μ m areas counted to increase the likely of detecting
219 oligodendrocyte lineage cells.

220 *Statistical Analyses*

221 Statistical analyses were conducted on Statistical Package for Social Sciences software (IBM) and
222 Graphpad (Prism) version 6.0. Parametric statistics were used assuming data met requirements for normality, tested
223 by the Shapiro-Wilk test. For comparisons between two normally distributed groups, Student's t-test was used with
224 or without the Welch correction depending on whether assumptions of the homogeneity of variance were met,
225 analyzed by with Levene's test. The Mann-Whitney U-test was used to compare P-Myrf^{fl/fl} to controls when the data
226 was not normally distributed or was ordinal. A one-way ANOVA followed by Tukey's or Tamhane's *post hoc* test
227 depending on whether there was equal homogeneity of variance between groups was used to compare three or more
228 groups at one time point. For comparisons of cell counts between groups at different time points post-lysolecithin
229 injection, a two-way ANOVA was run followed by Tukey's *post hoc* test. A unit was considered an individual
230 animal or MS lesion. Individuals performing surgeries, cell counts and imaging were blinded to mouse genotype by
231 a third party. For all tests, statistical significance was obtained if P<0.05 and all statistical tests were two-tailed. Data

232 are presented as the mean \pm standard error of the mean. On graphs ns = not statistically significant, * = $P \leq 0.05$, ** =
233 $P \leq 0.01$, *** $P \leq 0.001$ unless otherwise specified.

234

235 **Results**

236 *MYRF is expressed in new oligodendrocytes during remyelination*

237 The role of many oligodendrocyte transcription factors remains unstudied during remyelination, including
238 MYRF. To investigate if MYRF is expressed in new oligodendrocytes during remyelination, we fate mapped OPCs
239 using a tamoxifen-inducible Cre reporter line (Rosa26-eYFP) [56] in conjunction with an OPC inducible Cre-
240 recombinase (PDGFR α -CreERT2) [52]. Mice were heterozygous for the *Myrf* floxed allele (*Myrf*^{fl/wt}). *Myrf* is
241 haplosufficient and its function in oligodendrocytes is unchanged versus mice with two copies of the gene [11]. To
242 induce reproducible demyelination, mice received an injection of lysolecithin into the genu of the corpus callosum
243 (Fig. 1a). Remyelination following lysolecithin demyelination involves a stereotypic evolution [13]. OPCs
244 proliferate and are recruited in the first 5 DPL, which is followed by prominent oligodendrocyte differentiation
245 between 5-10 DPL and remyelination by ~14 DPL (Fig. 1a). Lysolecithin was very toxic to oligodendrocyte lineage
246 cells and the majority of MYRF+ cells were lost at 3 DPL (Fig. 1b). However, by 7 and 14 DPL, MYRF+ cells
247 increased in density relative to 3 DPL (Fig. 1c). Recombined yellow fluorescent protein positive (YFP+) cells rarely
248 expressed MYRF at 3 DPL ($10.28 \pm 4.16\%$) but by 7 DPL $37.5\% \pm 4.00\%$ of recombined cells expressed MYRF.
249 To identify the cells expressing MYRF during remyelination, we assessed co-immunoreactivity of YFP with CC1
250 for oligodendrocytes (Fig. 1e) and PDGFR α for OPCs (Fig. 1f). While MYRF was not found expressed in OPCs
251 (Fig. 1f), new oligodendrocytes (YFP+CC1+) were frequently observed to express nuclear MYRF staining at 7 DPL
252 in P-*Myrf*^{fl/wt} YFP mice (Fig. 1e). Collectively, MYRF+ cells were lost after lysolecithin-induced demyelination,
253 and OPCs differentiate into new oligodendrocytes that express MYRF during remyelination.

254

255 *Inducible deletion of Myrf from OPCs is sufficient to reduce MYRF expression within new oligodendrocytes*

256 To uncover MYRF's function during remyelination, *Myrf*^{fl/fl} PDGFR α CreERT2 Rosa26-eYFP mice (P-
257 *Myrf*^{fl/fl} YFP) were produced to delete *Myrf* and visualize OPCs and their progeny when tamoxifen had been
258 administered. New oligodendrocytes (YFP+CC1+) in P-*Myrf*^{fl/fl} YFP mice were less likely to express MYRF (Fig.
259 1e, g). Total MYRF density in P-*Myrf*^{fl/fl} YFP mice declined by 32% at 7 DPL and 45% at 14 DPL relative to

260 controls (Supplementary Fig. 1S e). The MYRF antibody used here, raised against the N-terminus, should be
261 capable of detecting the predicted truncated, non-functional protein product of the recombined allele. Nevertheless,
262 the protein produced appears to be unstable and is only weakly detected in most recombined oligodendrocytes ([31]
263 and Supplementary Fig. S1, 2, Fig. 1e, g). To explicitly determine if the inducible knockout effectively recombined
264 *Myrf*, we examined the relative expression using a PCR primer sequence specific to recombined *myrf* mRNA
265 lacking exon 8 [31]. P-Myrf^{fl/fl} mice had increased levels of recombined *myrf* in the brain compared to P-Myrf^{fl/fl}
266 mice without tamoxifen (Fig. 1h). Taken together, conditional deletion of *Myrf* in OPCs was effective at reducing
267 MYRF expression within new oligodendrocytes during remyelination.

268
269 *MYRF is not required for recruitment or proliferation of OPCs but is crucial for their complete maturation into new*
270 *oligodendrocytes following demyelination.*

271 OPC recruitment is crucial for timely remyelination [47], and OPC proliferation is required for sustained
272 oligodendrogenesis [53]. To determine directly if OPC recruitment or proliferation was altered by *Myrf* deletion,
273 sections from P-Myrf^{fl/fl} YFP and P-Myrf^{fl/wt} YFP mice were examined for PDGFR α and the cell proliferation
274 marker Ki67 to label cells in active stages of the cell cycle [54]. Both the non-recombined PDGFR α + OPCs and
275 recombined subpopulation expressed Ki67 after lyssolecithin demyelination (Fig. 2a, d, e). *Myrf* deletion from OPCs
276 did not alter the density of OPCs (Fig. 2b), the density of proliferating OPCs (Fig. 2c), the overall percentage of
277 proliferating OPCs (Fig. 2f) or proliferation within recombined OPCs (Fig. 2g). Therefore, MYRF is not required
278 for OPC proliferation or recruitment to demyelinated lesions.

279 To determine how *Myrf* deletion from OPCs affects their subsequent differentiation and maturation during
280 remyelination, we first examined whether recombined cells continued to express the OPC marker PDGFR α or have
281 differentiated and express CC1 (Fig. 3a). At 3, 7 and 14 DPL the percentage of recombined cells that expressed CC1
282 or PDGFR α did not differ between P-Myrf^{fl/fl} YFP and P-Myrf^{fl/wt} mice (Fig. 3b, c). Given that CC1 was expressed
283 early after lyssolecithin-induced demyelination before the onset of significant remyelination (Supplementary Fig.
284 S3), we reasoned that MYRF might be dispensable for early differentiation of oligodendrocytes into a
285 premyelinating phenotype, but crucial for the expression of later markers of oligodendrocyte differentiation. At 7
286 DPL, a time point in which CC1+ cells were abundant within the lesion, staining with the oligodendrocyte marker
287 GST π , indicates that GST π + cells are largely absent from the lesion (Supplementary Fig. S3). However, by 14 DPL,

288 GST π ⁺ cells were found throughout the lesion (Fig. 3d), suggesting GST π labels a later stage of oligodendrocyte
289 development relative to CC1. Recombined cells from P-Myrf^{fl/fl} YFP and P-Myrf^{fl/wt} YFP mice were examined for
290 GST π and CNP expression at 7 and 14 DPL (Fig. 3d, e, f). In P-Myrf^{fl/fl} YFP mice, a lower percentage of
291 recombined cells expressed CNP (Fig 3g) and GST π (Fig. 3h) relative to controls at 14 DPL. There was also an
292 overall decrease in the total density of GST π ⁺ cells in P-Myrf^{fl/fl} YFP mice, despite recombination in only a portion
293 of OPCs (Fig. 3i). Thus during remyelination, markers expressed later in the differentiation of OPCs into
294 oligodendrocytes, like GST π , were increasingly diminished relative to early differentiation markers like CC1
295 following *Myrf* deletion.

296 Decreased expression of GST π could not be accounted for by increased differentiation of OPCs into other
297 cell types including astrocytes, as *Myrf* deletion did not increase the proportion of cells expressing GFAP during
298 remyelination and over 95% of recombined cells have nuclear Olig2 suggesting they remain within the
299 oligodendrocyte lineage (Supplementary Fig. S4). In sections from a complimentary cohort of P-Myrf^{fl/fl} and P-
300 Myrf^{fl/wt} mice lacking the Rosa26-eYFP inducible reporter, sections were co-stained with Olig2 in combination with
301 stage-specific markers of oligodendrocyte maturation to ensure that changes in OPC and oligodendrocyte densities
302 could not be attributable to labelling of CC1 or PDGFR α in other cell types (Supplementary Fig. S5). Like in
303 inducible reporter-positive mice, we found no change in OPC recruitment (Supplementary Fig. S5 b, e), or the onset
304 of differentiation (Olig2+CC1+ cells) at 5 DPL but a decrease in Olig2+ density and Olig2+CC1+ oligodendrocytes
305 at 10 DPL (Supplementary Fig. S5 d, f). Collectively, these data indicate MYRF is not required for OPC
306 proliferation, recruitment or initial differentiation but is required for the expression of late-stage oligodendrocyte
307 markers during remyelination.

308

309 *Myrf* deletion from OPCs leaves new oligodendrocytes prone to apoptosis

310 Increased apoptosis occurs in the optic nerve of Myrf^{fl/fl} Olig2 Cre mice during development [11] and in the
311 adult spinal cord following inducible *Myrf* deletion from mature oligodendrocytes [31]. To determine whether the
312 absence of *Myrf* increases apoptosis of oligodendrocytes following demyelination, we examined cleaved caspase-3
313 (CCasp3) expression in P-Myrf^{fl/fl} and P-Myrf^{fl/wt} mice (Fig. 4a, b). We found no overall difference in CCasp3+ cell
314 density between P-Myrf^{fl/fl} and P-Myrf^{fl/wt} mice at either 5 or 10 DPL (Fig. 4c), likely because the majority of the
315 apoptotic cells co-labelled with the pan-leukocyte marker CD45 (Fig. 4b) or the microglial/macrophage marker Iba1

316 (Fig. 4d, e). However, occasional CC1+CCasp3+ cells were observed (Fig. 4f). The density of CC1+
317 oligodendrocytes undergoing apoptosis was increased by approximately 4-fold at 10 DPL in P-Myrf^{fl/fl} mice (Fig.
318 4g). Similarly, the percentage of oligodendrocytes that were CCasp3+ was higher at 10 DPL (Fig. 4h). However,
319 oligodendrocytes comprised the minority of apoptotic cells, even in P-Myrf^{fl/fl} mice (Fig. 4i). The truncated protein
320 predicted to be produced in P-Myrf^{fl/fl} mice is unlikely to be directly apoptotic to oligodendrocytes as recombined
321 oligodendrocytes persist in Myrf^{fl/fl} PLP-CreERT2 mice for weeks following *Myrf* deletion (Supplementary Fig. 2S
322 and [31]). During remyelination, oligodendrocytes are overproduced [21], similar to developmental myelination, and
323 compete for axonal-derived cues necessary for survival [2, 3]. New oligodendrocytes lacking *Myrf* undergo
324 apoptosis, presumably due to impaired stabilization, ensheathment or access to axonal-derived cues. Collectively,
325 these data suggest impairing the later stages of oligodendrocyte differentiation by deleting *Myrf* leaves
326 oligodendrocytes vulnerable to apoptosis during remyelination.

327

328 *Myrf* deletion from OPCs does not induce overt demyelination, astrogliosis, or inflammation in the first two weeks
329 following recombination

330 There is a continual production of new oligodendrocytes in adulthood [29, 52]. *Myrf* deletion from OPCs
331 inhibits the formation of new oligodendrocytes during motor learning [40, 63] and could plausibly induce
332 demyelination by impairing the differentiation of new oligodendrocytes in the healthy CNS. To determine if *Myrf*
333 deletion resulted in demyelination in the healthy CNS during the first two weeks, we assessed myelin status by
334 staining the uninjured side of the corpus callosum contralateral to lysolecithin injection with the myelin protein
335 MBP (Fig. 5a, a', e, e'). We observed no overt signs of demyelination of the uninjured corpus callosum 14 DPL in
336 P-Myrf^{fl/fl} YFP mice and GST π + oligodendrocytes were readily observed (Fig. 5b, b', f, f'). Electron microscopy
337 revealed compact myelination in both control and P-Myrf^{fl/fl} YFP mice at 14 DPL in the uninjured corpus callosum
338 and spinal cord (Fig. 6c). Astrogliosis (Fig. 5c, c', g, g') was not observed nor clustering of microglia or obvious
339 changes in their morphology (Fig 5d, d', h, h'), in either P-Myrf^{fl/fl} YFP or P-Myrf^{fl/wt} YFP mice. The total density of
340 GST π + oligodendrocytes (Fig. 5k) and PDGFR α + OPCs (Fig. 5l) did not differ at 14 DPL in the contralateral non-
341 injected corpus callosum. However, when the recombined subpopulations were examined for GST π (Fig. 5i) or
342 PDGFR α (Fig. 5j), a higher percentage were found to express PDGFR α in P-Myrf^{fl/fl} YFP mice compared to P-
343 Myrf^{fl/wt} YFP mice (Fig. 5m). Recombined cells in P-Myrf^{fl/fl} YFP mice rarely expressed GST π (2.41% \pm 0.83%),

344 whereas in P-Myrf^{fl/wt} YFP mice, 15.10% ± 3.83% expressed GSTπ. Thus, *Myrf* deletion from OPCs prevents the
345 formation of new late-stage oligodendrocytes in the healthy brain as well as the demyelinated brain, but does not
346 result in overt demyelination, astrogliosis or inflammation during the first two weeks after deletion.

347

348 *MYRF is essential for remyelination*

349 *Myrf* deletion lowered the density of new GSTπ+ oligodendrocytes in response to demyelination, and as a
350 consequence should reduce the efficiency of remyelination. We examined remyelination in semithin sections of the
351 dorsal column 14 DPL in P-Myrf^{fl/fl} and P-Myrf^{fl/wt} mice (Fig. 6a). The spinal cord has larger calibre axons relative
352 to the corpus callosum allowing remyelinated axons to be easily distinguished based on their thinner myelin. This is
353 in contrast to the corpus callosum, which has many smaller axons (< 1μm) that do not always demonstrate thinner
354 myelin during remyelination [1]. In the spinal cords of control mice, numerous thinly myelinated axons, suggestive
355 of remyelination, were found (Fig. 6b, c). In contrast, there was a scarcity of thinly myelinated axons in P-Myrf^{fl/fl}
356 mice (Fig. 6b, c) that was confirmed by blinded rank analysis (Fig. 6d). We also examined the presence of MBP
357 staining in the corpus callosum to determine the extent of demyelination following lysolecithin injection in P-
358 Myrf^{fl/fl} YFP and P-Myrf^{fl/wt} YFP mice. (Fig. 6e, f). The volume of demyelination (area lacking MBP staining) did
359 not differ at 3 or 7 DPL, indicating *Myrf* deletion from OPCs did not leave the callosum more susceptible to myelin
360 loss. However, by 14 DPL there was a larger demyelinated area in P-Myrf^{fl/fl} YFP mice compared to controls (Fig.
361 6e) suggestive of impaired remyelination. Thus, in two cohorts of animals, in two different regions of the CNS,
362 MYRF was crucial for effective remyelination.

363 Remyelination can restore conductance [55], likely in part through clustering of sodium channels [18].
364 Clustering of sodium channels at nodes of Ranvier does not typically occur in the absence of oligodendrocytes and
365 myelination in the CNS [39]. To assess whether *Myrf* deletion from OPCs impaired the restoration of nodes of
366 Ranvier in lysolecithin-demyelinated lesions in the corpus callosum, we stained tissue with AnkG—to identify the
367 sodium channel scaffolding protein at the nodes—and Caspr to label paranodes. We counted nodes of Ranvier as
368 those with punctate Caspr surrounding AnkG staining (Fig. 6i) [41]. *Myrf* deletion reduced the density of nodes of
369 Ranvier within the lesion (Fig. 6g). Less axons within the lesion could also diminish node of Ranvier density, but no
370 differences in the density of SMI312+ axons were observed (Fig 6h). Thus, these data suggest a reduction of node of
371 Ranvier density is due to reduced oligodendrogenesis and not due to axon loss.

372 To examine axon-oligodendrocyte contact and remyelination directly in recombined cells, we crossed P-
373 *Myrf*^{fl/fl} and P-*Myrf*^{fl/wt} mice with a reporter line (mT/mG) that expressed Cre-inducible membrane-anchored GFP
374 (Fig. 7a) [42]. The membrane-anchored inducible GFP allows for the visualization of ensheathment and myelination
375 of axons by new oligodendrocytes [29]. At 28 DPL in P-*Myrf*^{fl/wt} mT/mG mice, OPCs were recruited to the lesion,
376 differentiated, and extended processes that co-label with MBP (Fig. 7b, d, e). In contrast, recombined cells in P-
377 *Myrf*^{fl/fl} mT/mG mice increased in density near the lesion but rarely co-label with MBP (Fig. 7b, d, e). There was a
378 large reduction in the capacity of recombined cells to produce myelin at 28 DPL (Fig 7c). While non-recombined
379 cells produced myelin normally in P-*Myrf*^{fl/fl} mT/mG mice, this was not sufficient to compensate for the recombined
380 cells and resulted in incomplete remyelination even at 28 DPL (Fig. 7c). Taking advantage of the larger axon calibre
381 in the spinal cord relative to the corpus callosum, we examined ensheathment and myelination of individual axons
382 by new oligodendrocytes in the dorsal column. While new oligodendrocytes (CC1+GFP+) were found to wrap
383 axons and produced MBP in control P-*Myrf*^{fl/wt} mT/mG mice following demyelination (Fig. 7f, g) new
384 oligodendrocytes in P-*Myrf*^{fl/fl} mT/mG mice occasionally ensheathed axons but failed to express MBP (Fig. 7f, h).
385 These data demonstrate that MYRF is required for the expression of myelin proteins in new oligodendrocytes.
386 Notably, *Myrf* deletion from OPCs does not prevent the formation of myelinating Schwann cells (P0+) from
387 recombined cells following demyelination in the spinal cord (Supplementary Fig. S6).

388

389 *MYRF expression within oligodendrocytes is correlated with successful remyelination in MS*

390 Remyelination often fails in MS [20, 46]. The expression of few transcription factors has been differentially
391 compared in human tissue between remyelinated ‘shadow plaques’ and chronically demyelinated lesions. Given the
392 crucial role of MYRF in rodent remyelination, we examined MYRF and the oligodendrocyte-lineage marker Sox10
393 [58, 64] in both the healthy white matter and in periventricular and subcortical white matter lesions (Fig. 8a, b).
394 MYRF was expressed in Sox10+ cells (Fig. 8c). Faint MYRF immunoreactivity was observed along blood vessels
395 or in myelin sheaths/debris. However, MYRF was not typically detected in Iba1+ microglia or GFAP+ astrocytes in
396 NAWM (Supplementary Fig. S7). In NAWM, MYRF was expressed in cells with strong NogoA expression
397 (Supplementary Fig. S8), CNP+ cells in remyelinated shadow plaques (Fig 8d), and in NogoA+ cells within the
398 active rims and centres of chronic lesions (Fig. 8e). CNP and NogoA are established markers of differentiated

399 oligodendrocytes in human tissue [34, 35, 50], and our data demonstrate that MYRF is expressed in
400 oligodendrocytes in the human CNS.

401 To assess whether MYRF expression is associated with remyelination in MS, we quantified the density of
402 Sox10+MYRF+ cells within lesions and NAWM. The density of both Sox10+ oligodendrocyte lineage cells and
403 Sox10+MYRF+ oligodendrocytes were reduced in the centre of chronic lesions relative to shadow plaques, chronic
404 active lesion rims and NAWM, indicating a depletion of both oligodendrocyte lineage cells and MYRF-expressing
405 oligodendrocytes within chronic lesions (Fig. 8f, g Supplementary Fig. S9). The percentage of Sox10+ cells
406 expressing MYRF was also reduced in chronic lesion centres relative to shadow plaques and NAWM (Fig. 8h). We
407 next examined NogoA, Sox10 and MYRF staining in chronic active lesions, NAWM and shadow plaques. Fewer
408 Sox10+ cells expressed strong NogoA in chronic active lesion centres relative to shadow plaques (Supplementary
409 Fig. S8 b), suggesting an accumulation of OPCs relative to oligodendrocytes. However, there was also a decreased
410 percentage of Sox10+ strongly NogoA+ oligodendrocytes expressing MYRF within chronic active lesion centres
411 relative to both chronic lesion rims, shadow plaques, and NAWM (Fig. 8i), indicating there was also a population of
412 differentiated oligodendrocytes unable to express detectable MYRF specifically in lesion centres. Collectively, the
413 increased density and capacity of oligodendrocytes to express MYRF in areas of remyelination demonstrates that
414 MYRF is associated with successful remyelination in the MS lesions examined.

415

416 **Discussion**

417 Many transcription factors crucial for developmental myelination remain poorly characterized during
418 remyelination, including MYRF. Using an inducible deletion of *Myrf* from OPCs concurrent with a focal
419 demyelinating lesion, we demonstrated that MYRF is not expressed in OPCs in the healthy or demyelinated CNS,
420 and their proliferation and recruitment to demyelinated lesions is not altered by *Myrf* deletion. However, genetic fate
421 mapping revealed that in the absence of *Myrf*, OPCs initially differentiate but are unable to robustly express late-
422 stage oligodendrocyte markers or myelin proteins. Thus, *Myrf* deletion from OPCs stalls their differentiation at the
423 premyelinating stage during remyelination (Supplementary Fig. S10). In human white matter, Sox10+NogoA+
424 oligodendrocytes were found to be positive for MYRF protein expression. We encountered fewer Sox10+MYRF+
425 cells in chronically demyelinated lesions. Additionally, a lower proportion of oligodendrocyte lineage cells
426 expressed MYRF in chronic lesion centres compared to shadow plaques or NAWM indicating a strong association

427 of MYRF expression with remyelination in MS. Collectively, our findings implicate MYRF in orchestrating myelin
428 regeneration in both the rodent and human CNS.

429
430 *Oligodendrocyte lineage cells in chronic MS lesions lack expression of the transcription network required for*
431 *myelination*

432 In chronic MS lesions, we find a deficiency of MYRF-expressing oligodendrocytes relative to shadow
433 plaques and NAWM. MYRF together with Sox10 constitute an essential regulatory network that drives myelin gene
434 expression [25]. The lack of MYRF expression in Sox10+ cells from chronic lesions could be a result of an
435 accumulation of OPCs relative to oligodendrocytes, supporting the notion that remyelination failure results from
436 impaired OPC differentiation [34, 61, 62]. Accordingly, we find a lower percentage of Sox10+ cells express strong
437 NogoA within chronic lesions centres relative to NAWM, suggestive of a failure to initially differentiate. However,
438 we also detect a population of Sox10+NogoA+ oligodendrocytes unable to express MYRF within chronic lesion
439 centres. NogoA is not typically expressed in OPCs [35], suggesting that these cells have differentiated but fail to
440 express MYRF and would, therefore, be unable to remyelinate. These data raise the possibility that even if OPCs are
441 able to initially differentiate, the inhibitory environment of the chronic MS lesions examined may prevent MYRF
442 expression, the later stages of differentiation, and subsequent remyelination. In mice, we demonstrated that MYRF is
443 crucial for the survival of newly generated oligodendrocytes, so an inability to express MYRF in oligodendrocytes
444 may leave them vulnerable to apoptosis. Over time, this could contribute to the severe depletion of oligodendrocytes
445 observed in most chronic MS lesions.

446 Nonetheless, in all chronic active lesions examined occasional Sox10+MYRF+ cells are found, suggesting
447 there is a population of oligodendrocytes expressing the necessary transcription factors for remyelination, yet these
448 cells are apparently unable to successfully remyelinate these lesions. This finding is in accordance with previous
449 research which indicates a population of myelin proteolipid protein expressing (PLP+) oligodendrocytes fail to
450 radially wrap axons and successfully remyelinate in the majority of chronically demyelinated lesions [7].
451 Collectively, these data imply remyelination failure in chronic lesions may be *multifactorial*. OPC differentiation
452 failure, which we define as the inability transition from a proliferative OPC to post-mitotic oligodendrocyte, along
453 with myelination failure, or the inability to wrap axons and deposit myelin, may both contribute to remyelination
454 failure. Conduction deficits in axons of chronically demyelinated lesions may inhibit effective differentiation [21]

455 and/or stabilization of oligodendrocyte wraps and subsequent myelination [24]. Additionally, inhibitory substrates
456 on axons such as polysialylated-neural cell adhesion molecule[8], myelin debris [33, 48], extracellular molecules
457 like fibronectin [57] and chondroitin sulfate proteoglycans (CSPGs) [30] may inhibit remyelination. Together these
458 and other factors could leave oligodendrocyte lineage cells in chronic MS lesions unable to express MYRF and
459 subsequently remyelinate axons.

460 Eliciting MYRF expression within oligodendrocyte lineage cells of chronic lesions may be a crucial step to
461 promote remyelination in MS. A greater understanding of the signaling pathways that induce MYRF expression
462 within premyelinating oligodendrocytes and the inhibitory influence of chronic MS lesions on these pathways will
463 be of critical importance for designing new therapeutics to overcome remyelination failure. Deletion of extracellular
464 signal-regulated kinases 1/2 (ERK1/2) in oligodendrocytes reduced the expression of *myrf* during development and
465 in the healthy CNS [27]. Conversely, sustained activation of the ERK1/2 increases *myrf* expression and reinitiates
466 myelination in quiescent oligodendrocytes in the uninjured and demyelinated CNS [28]. The antifungal agent
467 miconazole, which results in the sustained phosphorylation of ERK1/2 in OPCs, has been shown to accelerate
468 remyelination [43]. Targeting the phosphorylation of ERK1/2 with miconazole or other compounds may stimulate
469 MYRF expression and possibly overcome the inhibitory milieu of the chronic lesion and enhance remyelination.

470

471 *The Role of MYRF in Remyelination Broadly Recapitulates Developmental Myelination*

472 During both developmental myelination and remyelination, OPCs proceed through the same stages of
473 maturation from OPC to myelinating oligodendrocyte [12, 17]. This process requires the differential expression of
474 transcription factors at distinct stages of maturation [10]. We find that during remyelination, MYRF was expressed
475 with the onset of differentiation at the premyelinating stage after the downregulation of the OPC mitogen receptor
476 PDGFR α , and nearly concurrent with the expression of CC1. Given that MYRF is not expressed in OPCs during
477 remyelination, it is not surprising its deletion does not alter OPC recruitment or proliferation. Recombined cells
478 lacking MYRF can initially differentiate but have reduced expression of the more mature markers GST π and CNP
479 and ultimately fail to express the myelin protein MBP. Thus, during remyelination, MYRF has a unique combination
480 of characteristics amongst oligodendrocyte transcription factors; it is only expressed after initial OPC differentiation,
481 regulates the transition from premyelinating to myelinating oligodendrocyte, and is crucial for myelin gene
482 expression.

483 *Myrf* deletion from OPCs will be a useful tool to examine the extent and mechanisms by which remyelination
484 protects axons

485 Oligodendrocytes have been theorized to support axonal survival [44]. While many studies [23, 37, 45]
486 provide strong support for an essential role of oligodendrocytes in the health of axons, little *causative* evidence
487 exists that oligodendrocyte remyelination is sufficient to preserve axons during inflammatory demyelination. This is,
488 in large part, due to the difficulty of decoupling oligodendrogenesis and remyelination from inflammation and other
489 degenerative processes during demyelination. Several therapies targeting remyelination in MS are entering clinical
490 trials, and it will be crucial to determine the relative effectiveness and timeframe by which remyelination may
491 prevent axon loss following inflammatory demyelination.

492 P-Myrf^{fl/fl} mice may be an excellent model to assess the sufficiency and mechanisms by which remyelination
493 protects axons following demyelination. Lesions in P-Myrf^{fl/fl} mice resemble those of chronic MS lesions, in that
494 these lesions contain OPCs and premyelinating oligodendrocytes, but few oligodendrocytes capable of
495 remyelinating axons. Inducible *Myrf* deletion from OPCs does not result in overt signs of demyelination, reactive
496 astrogliosis, or inflammatory lesions during the timeframe of our study, which could confound an interpretation of
497 the role of remyelination on axonal health. MYRF is also not expressed in OPCs nor alters their proliferation or
498 recruitment to areas of demyelination. Given that non-recombined cells can remyelinate normally in P-Myrf^{fl/fl} mice,
499 a higher recombination efficiency of *Myrf* from OPCs would have been ideal. A second PDGFR α CreERT2 line
500 developed independently to the one used in this study has a higher recombination efficiency throughout the CNS
501 [29]. This line combined with autoimmune or cuprizone demyelination should result in long-term demyelination and
502 thus be a suitable tool to assess the efficacy, rate, and mechanisms by which new oligodendrocytes and
503 remyelination may protect axons from degeneration and enhance recovery following remyelination failure.

504 Collectively, our work demonstrates that MYRF is essential for successful remyelination by acting as a
505 master regulator crucial for the transition of oligodendrocytes from a premyelinating to myelinating phenotype. We
506 establish, for the first time, that chronic MS lesions lack oligodendrocytes that express this necessary transcription
507 factor for remyelination. Eliciting MYRF expression in oligodendrocyte lineage cells may be essential for
508 overcoming remyelination failure in MS.

509

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519

520 **Compliance with ethical standards**

521 All animal experiments were approved by the University of British Columbia Animal Care Committee, in
522 accordance with the guidelines of the Canadian Council on Animal Care.

523

524 **Conflict of interest**

525 The authors declare that they have no competing interests.

526

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538

539 **Figure Legends**

540 **Fig. 1** MYRF is expressed in new oligodendrocytes following focal demyelination. **a** Schematic of the transgenic
541 mice used, lysolecithin injection location and experimental timeline. **b** Micrographs of MYRF and YFP expression
542 in demyelinating lesions at 3, 7 and 14 DPL in P-Myrf^{fl/wt} YFP mice. Dashed line demarcates the approximate lesion
543 boundary. **c** Quantification of MYRF+ cell density demonstrates increased density in P-Myrf^{fl/wt} YFP mice between
544 3 and 7 DPL ($P=0.013$) and 3 and 14 DPL ($P<0.001$). **d** Quantification indicating an increase in the percentage of
545 recombined cells (YFP+) which are also MYRF+ by 7 DPL ($P=0.001$) and 14 DPL ($P<0.001$) relative to 3 DPL. **e**
546 Micrographs demonstrating that some recombined cells have differentiated and express the mature oligodendrocyte
547 marker CC1, which often co-label with MYRF (arrowheads) in P-Myrf^{fl/wt} YFP mice 7 DPL. In P-Myrf^{fl/fl} YFP
548 mice, many recombined cells have either faint or undetectable expression of MYRF in newly differentiated
549 oligodendrocytes (arrows). **f** Micrograph indicating MYRF is not expressed in PDGFR α + cells in lesions or adjacent
550 to lesion boundaries (dashed line). **g** Quantification demonstrating the percentage of recombined oligodendrocytes
551 (CC1+YFP+) that express MYRF is reduced at 7 DPL ($P<0.001$), and 14 DPL ($P=0.016$) in P-Myrf^{fl/fl} YFP relative
552 to P-Myrf^{fl/wt} YFP mice. **h** Quantification using primers specific for recombined *myrf* (lacking exon 8) shows there
553 is an increase in expression within the brain of P-Myrf^{fl/fl} mice treated with tamoxifen ($P=0.036$) compared to P-
554 Myrf^{fl/fl} mice that did not receive tamoxifen. Two-way ANOVA followed by Tukey's *post hoc* test in **c**, **g** and
555 Tamhane's *post hoc* test in **d**. $n=4-6$ mice per group per timepoint in **c**, **d** and **g**. Kruskal-Wallis test followed by
556 Dunn's test, $n=3-4$ per group in **h**. Scale bars are 50 μm in **b** and 20 μm in **e** and **f**

557 **Fig. 2** MYRF is not required for OPC recruitment or proliferation in demyelinated lesions. **a** Representative
558 photomicrographs of lesion epicentre stained for CNP and Ki67 or YFP and PDGFR α in P-Myrf^{fl/wt} YFP and P-
559 Myrf^{fl/fl} YFP mice 3 DPL. Dashed line demarcates approximate lesion boundary. **b** Quantification of PDGFR α + cell
560 density indicates there is no difference between P-Myrf^{fl/fl} YFP and P-Myrf^{fl/wt} YFP mice at any time point. **c**
561 Quantification demonstrating Ki67+PDGFR α + cell density does not differ between P-Myrf^{fl/wt} YFP and P-Myrf^{fl/fl}
562 YFP mice at 3, 7 or 14 DPL, but declines between 3 DPL and 7 DPL in both groups ($P\text{-Myrf}^{\text{fl/wt}} \text{ YFP } P<0.001$, $P\text{-Myrf}^{\text{fl/fl}} \text{ YFP } P=0.002$). **d** Single optical confocal section demonstrating co-labelling between YFP, PDGFR α , and
564 Ki67 (arrowheads) in P-Myrf^{fl/wt} YFP and **e** P-Myrf^{fl/fl} YFP mice. Arrows indicate YFP+PDGFR α +Ki67- cells. **f**
565 Quantification indicating the percentage of PDGFR α + cells which express Ki67 does not differ between P-Myrf^{fl/fl}

566 and P-Myrf^{fl/wt} mice at any time point, but declines between 3 and 7 DPL in both P-Myrf^{fl/wt} YFP ($P<0.001$) and P-
567 Myrf^{fl/fl} YFP ($P<0.001$) mice. **g** Quantification demonstrating the percentage of recombined OPCs
568 (YFP+PDGFR α +) that are Ki67+ does not differ between P-Myrf^{fl/wt} YFP and P-Myrf^{fl/fl} YFP mice at any time point
569 examined but declines between 3 and 7 DPL in both P-Myrf^{fl/wt} YFP ($P<0.001$) and P-Myrf^{fl/fl} YFP ($P<0.001$) mice.
570 Scale bars are 50 μ m in **a** and 10 μ m in **d**, and **e**. Two-way ANOVA followed by Tukey's *post hoc* test for **b**, **c**, **f**, **g**.
571 $n=4-6$ mice per group per timepoint.

572 **Fig. 3** *Myrf* deletion does not alter the transition from an OPC to premyelinating oligodendrocyte, but inhibits the
573 later stages of oligodendrocyte differentiation during remyelination. **a** Photomicrographs of a single optical confocal
574 section of the lesion at 7 DPL. Recombined cells within lesions express either CC1 (arrowheads) or PDGFR α
575 (arrows), in both P-Myrf^{fl/wt} YFP and P-Myrf^{fl/fl} YFP mice. **b** Quantification demonstrating the percentage of
576 recombined cells that are CC1+ does not differ at any time point examined between P-Myrf^{fl/wt} YFP and P-Myrf^{fl/fl}
577 YFP mice but increases in both groups between 3 and 14 DPL (*P-Myrf^{fl/wt} YFP* $P<0.001$, *P-Myrf^{fl/fl} YFP* $P<0.001$). **c**
578 Quantification of demonstrating the percentage of recombined cells that are PDGFR α + does not differ between P-
579 Myrf^{fl/wt} YFP and P-Myrf^{fl/fl} YFP mice at any time point examined; but the percentage of cells declines in both
580 groups between 3 and 14 DPL (*P-Myrf^{fl/wt} YFP* $P=0.003$, *P-Myrf^{fl/fl} YFP* $P=0.002$). **d** Representative
581 photomicrograph of lesion epicentre 14 DPL in both P-Myrf^{fl/wt} YFP and P-Myrf^{fl/fl} YFP mice stained for YFP,
582 GST π and CNP. Dashed lines demarcate lesion boundaries. **e** Single optical section demonstrating considerable co-
583 labelling between YFP, GST π (arrowheads) and CNP in P-Myrf^{fl/wt} YFP mice 14 DPL. **f** Recombined cells in P-
584 Myrf^{fl/fl} YFP mice rarely express GST π . **g** Quantification demonstrating that the percentage of recombined cells that
585 are CNP+ ($P=0.021$) and **h** GST π + ($P<0.001$) declines in P-Myrf^{fl/fl} YFP compared to P-Myrf^{fl/wt} YFP mice at 14
586 DPL **i** Quantification indicating the total GST π + cell density is reduced within the lesion at 14 DPL in P-Myrf^{fl/fl}
587 YFP compared to P-Myrf^{fl/wt} YFP mice ($P=0.043$). All statistical comparisons in **b**, **c**, **g**, **h**, **i** used a two-way
588 ANOVA followed by Tukey's *post hoc* test, $n=4-6$ mice per group per timepoint. Scale bars are 20 μ m in **a**, **e** and **f**
589 and 50 μ m in **d**

590 **Fig. 4** Oligodendrocytes are more prone to apoptosis following *Myrf* deletion from OPCs during remyelination. **a**
591 Representative photomicrographs of demyelinated lesion stained for MBP and CCasp3 at 5 and 10 DPL in both P-
592 Myrf^{fl/wt} and P-Myrf^{fl/fl} mice. Dashed line demarcates approximate lesion boundary. **b** Micrograph of the lesion

593 epicentre stained with CD45 and CCasp3 in P-Myrf^{fl/wt} and P-Myrf^{fl/fl} mice at 10 DPL. The majority of CCasp3+
594 cells associate with CD45. **c** Quantification of total CCasp3+Hoechst+ (apoptotic) cell density. **d** Single optical
595 confocal section demonstrating that CCasp3+ is expressed in CD45+ cells (arrowhead), and many cells also express
596 the microglia/macrophage marker Iba1 in both P-Myrf^{fl/wt} and **e** P-Myrf^{fl/fl} mice. **f** Single optical confocal section at
597 10 DPL in P-Myrf^{fl/fl} mice, with single channels of Hoechst, CC1 and CCasp3. Arrowhead indicates
598 CC1+Hoechst+CCasp3+ cell. **g** Quantification indicating CC1+CCasp3+ density is higher in P-Myrf^{fl/fl} compared to
599 P-Myrf^{fl/wt} mice at 10 DPL ($P=0.039$). **h** Quantification demonstrating increased percentage of CC1+ cells are
600 CCasp3+ in P-Myrf^{fl/fl} compared to P-Myrf^{fl/wt} mice at 10 DPL ($P=0.028$). **i** Quantification revealing an increase at
601 10 DPL in the percentage of apoptotic cells that express CC1 in P-Myrf^{fl/fl} relative to P-Myrf^{fl/wt} mice ($P=0.028$).
602 Two-way ANOVA followed by Tukey's *post hoc* test to determine individual group differences for **c**, **g**, **h** and **i**.
603 $n=4-5$ mice per group per timepoint. Scale bars are 50 μ m in **a**, 20 μ m in **b** and 5 μ m in **d**, **e**, and **f**

604 **Fig. 5** *Myrf* deletion from OPCs does not induce overt demyelination, astrogliosis or inflammation but reduces the
605 number of new oligodendrocytes in the healthy brain two weeks following tamoxifen injection. P-Myrf^{fl/wt} YFP and
606 P-Myrf^{fl/fl} YFP mice were examined on the contralateral side of the corpus callosum (CC) to the lysolecithin lesion
607 14 DPL. Representative photomicrographs of staining with **a**, **a'** MBP, **b**, **b'** GST π , **c**, **c'** GFAP and **d**, **d'** Iba1.
608 Enlarged images of **e**, **e'** MBP, **f**, **f'** GST π , **g**, **g'** GFAP and **h**, **h'** Iba1. **i** Single merged confocal optical section in
609 the corpus callosum contralateral to lysolecithin lesion in P-Myrf^{fl/wt} mice 14 DPL demonstrating occasional
610 YFP+GST π + oligodendrocytes (arrowhead). Single channel micrograph showing **i'** YFP and **i''** GST π . **j** Single
611 merged optical section in the corpus callosum contralateral to the lysolecithin lesion of P-Myrf^{fl/wt} mice 14 DPL
612 demonstrating co-labelling between YFP and PDGFR α (arrowheads). Arrows indicate PDGFR α + cells which did
613 not recombine (YFP-). Single channel image showing **j'** YFP and **j''** PDGFR α . Quantification showing no
614 difference in cell density of **k** (GST π +) and **l** (PDGFR α +) cells in the contralateral side of the corpus callosum
615 between P-Myrf^{fl/wt} and P-Myrf^{fl/fl} mice. **m** Quantification demonstrating recombined cells are unable to differentiate
616 into CNP+ ($P=0.027$) or GST π + ($P=0.032$) oligodendrocytes and a greater percentage remain PDGFR α + ($P=0.016$)
617 in P-Myrf^{fl/fl} YFP relative to P-Myrf^{fl/wt} YFP mice. Student's T-Test in **k** and **l** and Mann Whitney U Test in **m**, $n=4-$
618 5 mice per group. Scale bars are 100 μ m in (**a-d**) and (**a'-d'**) and 20 μ m in (**e-h**), (**e'-h'**), **i**, **i'**, **i''** and **j**, **j'**, **j''**

619 **Fig. 6** *Myrf* deletion from OPCs inhibits remyelination. **a** Schematic demonstrating the location of lysolecithin
620 injections into the dorsal column of the C4 spinal cord and location of semi-thin and electron micrographs. **b**
621 Semithin sections at the C4 spinal cord level stained with toluidine blue from P-Myrf^{fl/wt} and P-Myrf^{fl/fl} mice. There
622 are few myelinated axons in P-Myrf^{fl/fl} mice. **c** Electron micrographs of the uninjured and 14 DPL C4 spinal cord in
623 P-Myrf^{fl/wt} and P-Myrf^{fl/fl} mice. **d** Ranking analysis in P-Myrf^{fl/fl} and P-Myrf^{fl/wt} mice, demonstrates there is less
624 remyelination in P-Myrf^{fl/fl} mice ($P=0.030$). **e** Quantification of the volume of spared tissue that is MBP-negative
625 within the corpus callosum. At 14 DPL, there is a reduction in the MBP-negative volume in P-Myrf^{fl/wt} YFP relative
626 to P-Myrf^{fl/fl} YFP mice ($P=0.028$). **f** Overview of lesion epicentre in the corpus callosum stained with MBP at 3, 7,
627 and 14 DPL in P-Myrf^{fl/wt} YFP and P-Myrf^{fl/fl} YFP mice. MBP is expressed throughout the corpus callosum at 14
628 DPL in P-Myrf^{fl/wt} YFP in contrast to P-Myrf^{fl/fl} YFP mice. **g** Quantifications indicating node of Ranvier density in
629 the corpus callosum is reduced ($P=0.047$) but **h** SMI312+ axon staining does not differ between P-Myrf^{fl/wt} YFP and
630 P-Myrf^{fl/fl} YFP mice at 14 DPL ($P=0.754$) in the corpus callosum. **i** Example micrograph of a subset of lesion
631 demonstrating notably fewer punctate Caspr flanking AnkG, in P-Myrf^{fl/fl} YFP relative to P-Myrf^{fl/wt} YFP mice at 14
632 DPL. Mann Whitney U Test used in **d**, **e** and **g**, Student's T-test in **h**. $n=4-6$ mice per group per time point. Scale
633 bars are 50 μ m in **b** and **f**, 20 μ m **i** and 2 μ m in **c**

634 **Fig. 7** New oligodendrocytes are unable to effectively remyelinate in P-Myrf^{fl/fl} mT/mG mice. **a** Schematic of the
635 transgenic lines used and experimental timeline. All mice were perfused 28 DPL. **b** Overview of lysolecithin lesion
636 in the corpus callosum stained for GFP and MBP in P-Myrf^{fl/wt} mT/mG and P-Myrf^{fl/fl} mT/mG mice. **c**
637 Quantification demonstrating reductions in the amount of myelin generated by recombined cells within the lesion
638 (GFP+MBP+) in P-Myrf^{fl/fl} mT/mG relative to P-Myrf^{fl/wt} mice (green bars, Mann Whitney U test, $P=0.006$) but
639 myelin generated by non-recombined cells (GFP-MBP+) does not change (clear portion of bars, T-Test, $P=0.236$).
640 There is less overall MBP in P-Myrf^{fl/fl} mT/mG mice in the lesion (total of bars, T-Test, $P=0.020$). $n=6$ mice per
641 group. **d** Single optical section of the corpus callosum with axons in cross section reveals co-labelling of GFP with
642 MBP in P-Myrf^{fl/wt} mT/mG mice but almost no GFP+MBP+ sheaths in P-Myrf^{fl/fl} mT/mG mice. **e** Processes from
643 CC1+GFP+ oligodendrocytes (arrowheads) are seen to align along myelinated fibers and co-label with MBP
644 (yellow) in P-Myrf^{fl/wt} mT/mG mice adjacent to lesion epicentre but CC1+GFP+ cells rarely have processes which
645 co-label with MBP in P-Myrf^{fl/fl} mT/mG mice. **f** Single optical confocal coronal section taken at the C4 level of the
646 spinal cord of P-Myrf^{fl/wt} mT/mG and P-Myrf^{fl/fl} mT/mG mice stained with SMI312, MBP, GFP and CC1. Arrows

647 indicate GFP+CC1+ cell bodies. **g** Enlarged image of individual oligodendrocyte processes from P-Myrf^{fl/wt} mT/mG
648 mice. Within the lesion, numerous GFP+ process surround axons and co-label with MBP indicative of new
649 myelination. **h** Occasional GFP+CC1+ oligodendrocytes wrap axons in P-Myrf^{fl/fl} mT/mG mice, but rarely express
650 MBP. In **g** and **h** arrowheads demarcate GFP+MBP+ myelin, and arrows indicate GFP+ processes wrapping axons
651 that are MBP-negative. Scale bars are 50µm in **b**, 10 µm in **d**, **e** and **f** and 5µm in **g** and **h**

652 **Fig 8** Successful remyelination in MS lesions is associated with MYRF expression in oligodendrocytes. **a**
653 Micrographs of human MS lesions and NAWM stained with luxol fast blue (LFB). Demyelination is observed in
654 active and chronic active plaques, whereas faint LFB staining is present in shadow plaques. **b** Class II HLA
655 reactivity is perilesional in chronic active plaques, and found throughout the lesion in active plaques. **c** Co-labelling
656 between MYRF and the oligodendrocyte lineage marker Sox10 is seen in NAWM. MYRF is primarily nuclear, as
657 indicated by co-labelling with Hoechst. **d** Single optical confocal section demonstrating MYRF is expressed in
658 CNP+ cells within shadow plaques (arrowheads). Arrows indicate CNP+MYRF-negative cells. **e** Example
659 micrographs of single optical confocal sections in chronic active lesion centres and rims demonstrating MYRF is
660 typically expressed in Sox10+NogoA+ oligodendrocytes (arrowhead). Arrows denote Sox10+NogoA+ cells which
661 lack MYRF. **f** Less Hoechst+Sox10+ cells are observed in the centre of chronic active lesions relative to active
662 lesions ($P=0.042$), chronic active rim ($P<0.001$), shadow plaques ($P=0.026$), NAWM ($P<0.001$), and non-MS
663 ($P<0.001$). **g** Less Hoechst+Sox10+MYRF+ cells in chronic lesion centres compared to all other groups (*active*
664 *lesions* $P=0.016$, *chronic active rim* $P=0.002$, *shadow plaques* $P=0.012$, *NAWM* $P<0.001$, and *non-MS* $P<0.001$). **h**
665 The percentage of Hoechst+Sox10+ cells that express MYRF is reduced in chronic active lesions relative to shadow
666 plaques ($P=0.041$), NAWM ($P<0.001$) and non-MS white matter ($P<0.001$). **i** The percentage of Sox10+NogoA+
667 oligodendrocytes which express MYRF is reduced in chronic active lesion centres relative to NAWM ($P<0.001$),
668 shadow plaques ($P<0.001$), and chronic active rims ($P<0.001$). Horizontal lines with vertical dashes above
669 quantifications in **f-i** indicate all statistically significant *post hoc* tests relative to the group with the larger vertical
670 line. One-way ANOVA followed by Tukey's *post hoc* for **f**, **g**, **h**, **i**. Scale bars are 500 µm in **a** and **b**, 50µm in **c**,
671 20µm in **d** and 10µm in **e**. * = statistical significance

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673 **References**

674 1 Bai CB, Sun S, Roholt A, Benson E, Edberg D, Medicetty S, et al. (2016) A mouse model for testing
675 remyelinating therapies. *Exp Neurol* 283: 330-340 Doi 10.1016/j.expneurol.2016.06.033

676 2 Barres BA, Raff MC (1999) Axonal control of oligodendrocyte development. *The Journal of cell*
677 *biology* 147: 1123-1128

678 3 Barres BA, Raff MC (1994) Control of Oligodendrocyte Number in the Developing Rat Optic-
679 Nerve. *Neuron* 12: 935-942 Doi 10.1016/0896-6273(94)90305-0

680 4 Bo L, Mork S, Kong PA, Nyland H, Pardo CA, Trapp BD (1994) Detection of MHC class II-antigens
681 on macrophages and microglia, but not on astrocytes and endothelia in active multiple sclerosis
682 lesions. *Journal of neuroimmunology* 51: 135-146

683 5 Bujalka H, Koenning M, Jackson S, Perreau VM, Pope B, Hay CM, et al. (2013) MYRF Is a
684 Membrane-Associated Transcription Factor That Autoproteolytically Cleaves to Directly Activate
685 Myelin Genes. *PLoS biology* 11: Doi 10.1371/journal.pbio.1001625

686 6 Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, Christopherson KS, et al. (2008) A
687 transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for
688 understanding brain development and function. *J Neurosci* 28: 264-278 Doi
689 10.1523/JNEUROSCI.4178-07.2008

690 7 Chang A, Tourtellotte WW, Rudick R, Trapp BD (2002) Premyelinating oligodendrocytes in
691 chronic lesions of multiple sclerosis. *The New England journal of medicine* 346: 165-173 Doi
692 10.1056/NEJMoa010994

693 8 Charles P, Hernandez MP, Stankoff B, Aigrot MS, Colin C, Rougon G, et al. (2000) Negative
694 regulation of central nervous system myelination by polysialylated-neural cell adhesion
695 molecule. *Proc Natl Acad Sci U S A* 97: 7585-7590 Doi 10.1073/pnas.100076197

696 9 Denk F, Ramer LM, Erskine EL, Nassar MA, Bogdanov Y, Signore M, et al. (2015) Tamoxifen
697 induces cellular stress in the nervous system by inhibiting cholesterol synthesis. *Acta*
698 *neuropathologica communications* 3: 74 Doi 10.1186/s40478-015-0255-6

699 10 Emery B (2010) Transcriptional and post-transcriptional control of CNS myelination. *Current*
700 *opinion in neurobiology* 20: 601-607 Doi 10.1016/j.conb.2010.05.005

701 11 Emery B, Agalliu D, Cahoy JD, Watkins TA, Dugas JC, Mulinyawe SB, et al. (2009) Myelin gene
702 regulatory factor is a critical transcriptional regulator required for CNS myelination. *Cell* 138:
703 172-185 Doi 10.1016/j.cell.2009.04.031

704 12 Fancy SP, Chan JR, Baranzini SE, Franklin RJ, Rowitch DH (2011) Myelin regeneration: a
705 recapitulation of development? *Annu Rev Neurosci* 34: 21-43 Doi 10.1146/annurev-neuro-
706 061010-113629

707 13 Fancy SP, Harrington EP, Yuen TJ, Silbereis JC, Zhao C, Baranzini SE, et al. (2011) Axin2 as
708 regulatory and therapeutic target in newborn brain injury and remyelination. *Nat Neurosci* 14:
709 1009-1016 Doi 10.1038/nn.2855

710 14 Fancy SP, Kotter MR, Harrington EP, Huang JK, Zhao C, Rowitch DH, et al. (2010) Overcoming
711 remyelination failure in multiple sclerosis and other myelin disorders. *Exp Neurol* 225: 18-23 Doi
712 10.1016/j.expneurol.2009.12.020

713 15 Franklin RJ, Ffrench-Constant C (2008) Remyelination in the CNS: from biology to therapy. *Nat*
714 *Rev Neurosci* 9: 839-855 Doi 10.1038/nrn2480

715 16 Franklin RJ, Ffrench-Constant C, Edgar JM, Smith KJ (2012) Neuroprotection and repair in
716 multiple sclerosis. *Nature reviews Neurology* 8: 624-634 Doi 10.1038/nrneuro.2012.200

717 17 Franklin RJ, Hinks GL (1999) Understanding CNS remyelination: clues from developmental and
718 regeneration biology. *Journal of neuroscience research* 58: 207-213

719 18 Freeman SA, Desmazieres A, Simonnet J, Gatta M, Pfeiffer F, Aigrot MS, et al. (2015)
720 Acceleration of conduction velocity linked to clustering of nodal components precedes
721 myelination. *Proc Natl Acad Sci U S A* 112: E321-328 Doi 10.1073/pnas.1419099112

722 19 Frischer JM, Bramow S, Dal-Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M, et al. (2009)
723 The relation between inflammation and neurodegeneration in multiple sclerosis brains. *Brain*
724 132: 1175-1189 Doi 10.1093/brain/awp070

725 20 Frischer JM, Weigand SD, Guo Y, Kale N, Parisi JE, Pirko I, et al. (2015) Clinical and pathological
726 insights into the dynamic nature of the white matter multiple sclerosis plaque. *Annals of*
727 *neurology* 78: 710-721 Doi 10.1002/ana.24497

728 21 Gautier HO, Evans KA, Volbracht K, James R, Sitnikov S, Lundgaard I, et al. (2015) Neuronal
729 activity regulates remyelination via glutamate signalling to oligodendrocyte progenitors. *Nat*
730 *Commun* 6: 8518 Doi 10.1038/ncomms9518

731 22 Gonzalez GA, Hofer MP, Syed YA, Amaral AI, Rundle J, Rahman S, et al. (2016) Tamoxifen
732 accelerates the repair of demyelinated lesions in the central nervous system. *Sci Rep* 6: 31599
733 Doi 10.1038/srep31599

734 23 Griffiths I, Klugmann M, Anderson T, Yool D, Thomson C, Schwab MH, et al. (1998) Axonal
735 swellings and degeneration in mice lacking the major proteolipid of myelin. *Science* 280: 1610-
736 1613

737 24 Hines JH, Ravanelli AM, Schwindt R, Scott EK, Appel B (2015) Neuronal activity biases axon
738 selection for myelination in vivo. *Nat Neurosci* 18: 683-689 Doi 10.1038/nn.3992

739 25 Hornig J, Frob F, Vogl MR, Hermans-Borgmeyer I, Tamm ER, Wegner M (2013) The transcription
740 factors Sox10 and Myrf define an essential regulatory network module in differentiating
741 oligodendrocytes. *PLoS genetics* 9: e1003907 Doi 10.1371/journal.pgen.1003907

742 26 Irvine KA, Blakemore WF (2008) Remyelination protects axons from demyelination-associated
743 axon degeneration. *Brain* 131: 1464-1477 Doi 10.1093/brain/awn080

744 27 Ishii A, Furusho M, Dupree JL, Bansal R (2014) Role of ERK1/2 MAPK signaling in the
745 maintenance of myelin and axonal integrity in the adult CNS. *J Neurosci* 34: 16031-16045 Doi
746 10.1523/JNEUROSCI.3360-14.2014

747 28 Jeffries MA, Urbanek K, Torres L, Wendell SG, Rubio ME, Fyffe-Maricich SL (2016) ERK1/2
748 Activation in Preexisting Oligodendrocytes of Adult Mice Drives New Myelin Synthesis and
749 Enhanced CNS Function. *J Neurosci* 36: 9186-9200 Doi 10.1523/JNEUROSCI.1444-16.2016

750 29 Kang SH, Fukaya M, Yang JK, Rothstein JD, Bergles DE (2010) NG2+ CNS glial progenitors remain
751 committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration.
752 *Neuron* 68: 668-681 Doi 10.1016/j.neuron.2010.09.009

753 30 Keough MB, Rogers JA, Zhang P, Jensen SK, Stephenson EL, Chen T, et al. (2016) An inhibitor of
754 chondroitin sulfate proteoglycan synthesis promotes central nervous system remyelination. *Nat*
755 *Commun* 7: 11312 Doi 10.1038/ncomms11312

756 31 Koenning M, Jackson S, Hay CM, Faux C, Kilpatrick TJ, Willingham M, et al. (2012) Myelin gene
757 regulatory factor is required for maintenance of myelin and mature oligodendrocyte identity in
758 the adult CNS. *J Neurosci* 32: 12528-12542 Doi 10.1523/JNEUROSCI.1069-12.2012

759 32 Kornek B, Storch MK, Weissert R, Wallstroem E, Stefferl A, Olsson T, et al. (2000) Multiple
760 sclerosis and chronic autoimmune encephalomyelitis: a comparative quantitative study of
761 axonal injury in active, inactive, and remyelinated lesions. *Am J Pathol* 157: 267-276 Doi
762 10.1016/S0002-9440(10)64537-3

763 33 Kotter MR, Li WW, Zhao C, Franklin RJ (2006) Myelin impairs CNS remyelination by inhibiting
764 oligodendrocyte precursor cell differentiation. *J Neurosci* 26: 328-332 Doi
765 10.1523/JNEUROSCI.2615-05.2006

766 34 Kuhlmann T, Miron V, Cuo Q, Wegner C, Antel J, Bruck W (2008) Differentiation block of
767 oligodendroglial progenitor cells as a cause for remyelination failure in chronic multiple
768 sclerosis. *Brain* 131: 1749-1758 Doi 10.1093/brain/awn096

769 35 Kuhlmann T, Remington L, Maruschak B, Owens T, Bruck W (2007) Nogo-A is a reliable
770 oligodendroglial marker in adult human and mouse CNS and in demyelinated lesions. *J*
771 *Neuropath Exp Neur* 66: 238-246

772 36 Kutzelnigg A, Lucchinetti CF, Stadelmann C, Bruck W, Rauschka H, Bergmann M, et al. (2005)
773 Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain* 128: 2705-
774 2712 Doi 10.1093/brain/awh641

775 37 Lappe-Siefke C, Goebbels S, Gravel M, Nicksch E, Lee J, Braun PE, et al. (2003) Disruption of *Cnp1*
776 uncouples oligodendroglial functions in axonal support and myelination. *Nature genetics* 33:
777 366-374 Doi 10.1038/ng1095

778 38 Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time
779 quantitative PCR and the 2(T)(-Delta Delta C) method. *Methods* 25: 402-408 Doi
780 10.1006/meth.2001.1262

781 39 Mathis C, Denisenko-Nehrbass N, Girault JA, Borrelli E (2001) Essential role of oligodendrocytes
782 in the formation and maintenance of central nervous system nodal regions. *Development* 128:
783 4881-4890

784 40 McKenzie IA, Ohayon D, Li H, de Faria JP, Emery B, Tohyama K, et al. (2014) Motor skill learning
785 requires active central myelination. *Science* 346: 318-322 Doi 10.1126/science.1254960

786 41 Miron VE, Boyd A, Zhao JW, Yuen TJ, Ruckh JM, Shadrach JL, et al. (2013) M2 microglia and
787 macrophages drive oligodendrocyte differentiation during CNS remyelination. *Nat Neurosci* 16:
788 1211-1218 Doi 10.1038/nn.3469

789 42 Muzumdar MD, Tasic B, Miyamichi K, Li L, Luo L (2007) A global double-fluorescent Cre reporter
790 mouse. *Genesis* 45: 593-605 Doi 10.1002/dvg.20335

791 43 Najm FJ, Madhavan M, Zaremba A, Shick E, Karl RT, Factor DC, et al. (2015) Drug-based
792 modulation of endogenous stem cells promotes functional remyelination in vivo. *Nature* 522:
793 216-220 Doi 10.1038/nature14335

794 44 Nave KA (2010) Myelination and support of axonal integrity by glia. *Nature* 468: 244-252 Doi
795 10.1038/nature09614

796 45 Nguyen T, Mehta NR, Conant K, Kim KJ, Jones M, Calabresi PA, et al. (2009) Axonal protective
797 effects of the myelin-associated glycoprotein. *J Neurosci* 29: 630-637 Doi
798 10.1523/JNEUROSCI.5204-08.2009

799 46 Patrikios P, Stadelmann C, Kutzelnigg A, Rauschka H, Schmidbauer M, Laursen H, et al. (2006)
800 Remyelination is extensive in a subset of multiple sclerosis patients. *Brain* 129: 3165-3172 Doi
801 10.1093/Brain/Awl217

802 47 Piaton G, Aigrot MS, Williams A, Moyon S, Tepavcevic V, Moutkine I, et al. (2011) Class 3
803 semaphorins influence oligodendrocyte precursor recruitment and remyelination in adult
804 central nervous system. *Brain* 134: 1156-1167 Doi 10.1093/brain/awr022

805 48 Plemel JR, Manesh SB, Sparling JS, Tetzlaff W (2013) Myelin inhibits oligodendroglial maturation
806 and regulates oligodendrocytic transcription factor expression. *Glia* 61: 1471-1487 Doi
807 10.1002/Glia.22535

808 49 Prineas JW, Barnard RO, Kwon EE, Sharer LR, Cho ES (1993) Multiple sclerosis: remyelination of
809 nascent lesions. *Annals of neurology* 33: 137-151 Doi 10.1002/ana.410330203

810 50 Prineas JW, Kwon EE, Goldenberg PZ, Ilyas AA, Quarles RH, Benjamins JA, et al. (1989) Multiple
811 sclerosis. Oligodendrocyte proliferation and differentiation in fresh lesions. *Laboratory*
812 *investigation; a journal of technical methods and pathology* 61: 489-503

813 51 Raine CS, Wu E (1993) Multiple sclerosis: remyelination in acute lesions. *J Neuropathol Exp*
814 *Neurol* 52: 199-204

815 52 Rivers LE, Young KM, Rizzi M, Jamen F, Psachoulia K, Wade A, et al. (2008) PDGFRA/NG2 glia
816 generate myelinating oligodendrocytes and piriform projection neurons in adult mice. *Nat*
817 *Neurosci* 11: 1392-1401 Doi 10.1038/nn.2220

818 53 Schneider S, Gruart A, Grade S, Zhang Y, Kroger S, Kirchhoff F, et al. (2016) Decrease in newly
819 generated oligodendrocytes leads to motor dysfunctions and changed myelin structures that
820 can be rescued by transplanted cells. *Glia* 64: 2201-2218 Doi 10.1002/glia.23055

821 54 Scholzen T, Gerdes J (2000) The Ki-67 protein: from the known and the unknown. *Journal of*
822 *cellular physiology* 182: 311-322 Doi 10.1002/(SICI)1097-4652(200003)182:3<311::AID-
823 JCP1>3.0.CO;2-9

824 55 Smith KJ, Blakemore WF, McDonald WI (1979) Central Remyelination Restores Secure
825 Conduction. *Nature* 280: 395-396 Doi 10.1038/280395a0

826 56 Srinivas S, Watanabe T, Lin CS, Williams CM, Tanabe Y, Jessell TM, et al. (2001) Cre reporter
827 strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC*
828 *developmental biology* 1: 4 Doi 10.1186/1471-213X-1-4

829 57 Stoffels JM, de Jonge JC, Stancic M, Nomden A, van Strien ME, Ma D, et al. (2013) Fibronectin
830 aggregation in multiple sclerosis lesions impairs remyelination. *Brain* 136: 116-131 Doi
831 10.1093/brain/aws313

832 58 Stolt CC, Rehberg S, Ader M, Lommes P, Riethmacher D, Schachner M, et al. (2002) Terminal
833 differentiation of myelin-forming oligodendrocytes depends on the transcription factor Sox10.
834 *Genes & development* 16: 165-170 Doi 10.1101/gad.215802

835 59 van der Valk P, De Groot CJ (2000) Staging of multiple sclerosis (MS) lesions: pathology of the
836 time frame of MS. *Neuropathology and applied neurobiology* 26: 2-10

837 60 Wingerchuk DM, Weinshenker BG (2000) Multiple sclerosis: epidemiology, genetics,
838 classification, natural history, and clinical outcome measures. *Neuroimaging Clin N Am* 10: 611-
839 624 ,vii

840 61 Wolswijk G (2002) Oligodendrocyte precursor cells in the demyelinated multiple sclerosis spinal
841 cord. *Brain* 125: 338-349

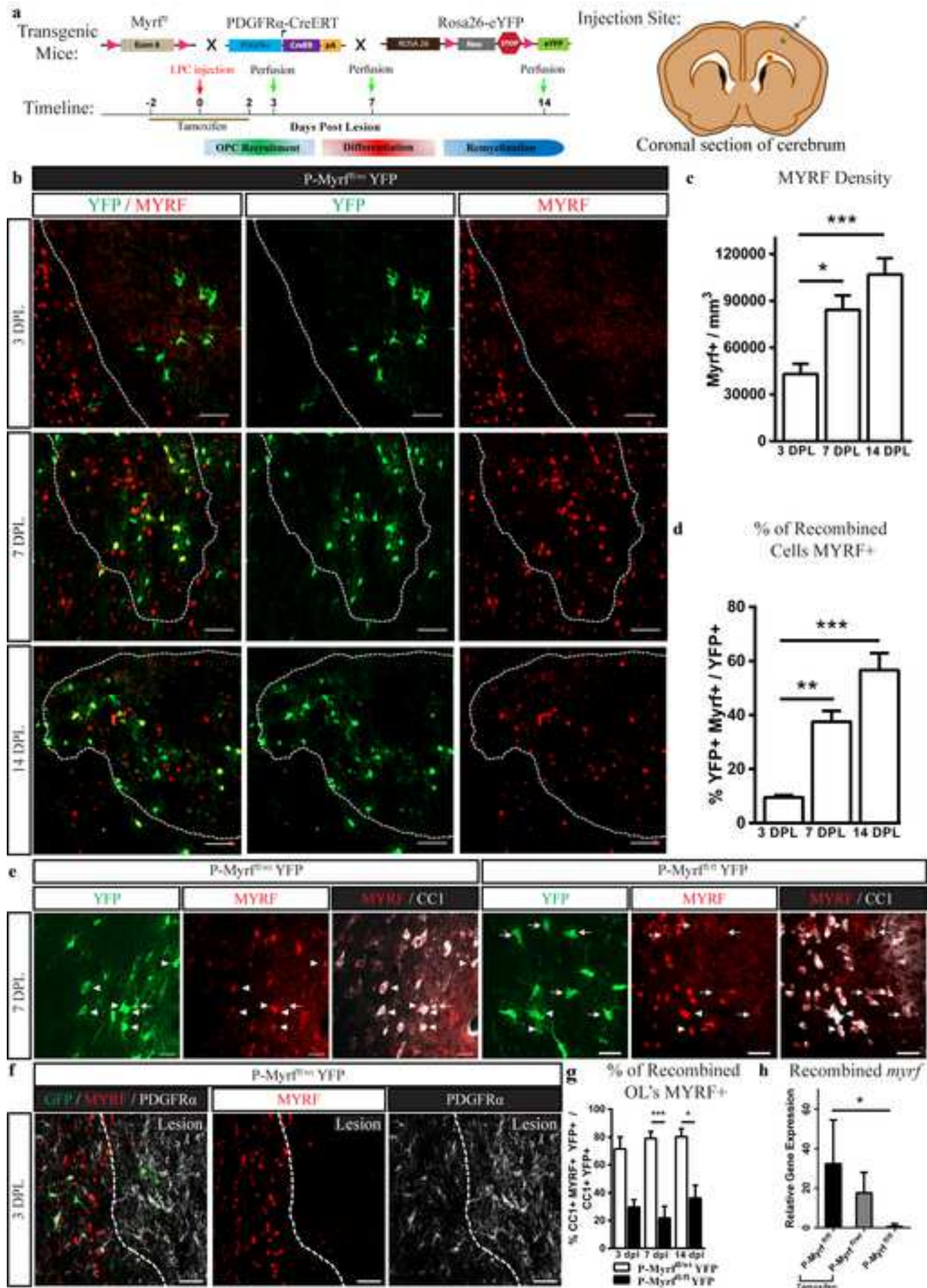
842 62 Wolswijk G (2000) Oligodendrocyte survival, loss and birth in lesions of chronic-stage multiple
843 sclerosis. *Brain* 123 (Pt 1): 105-115

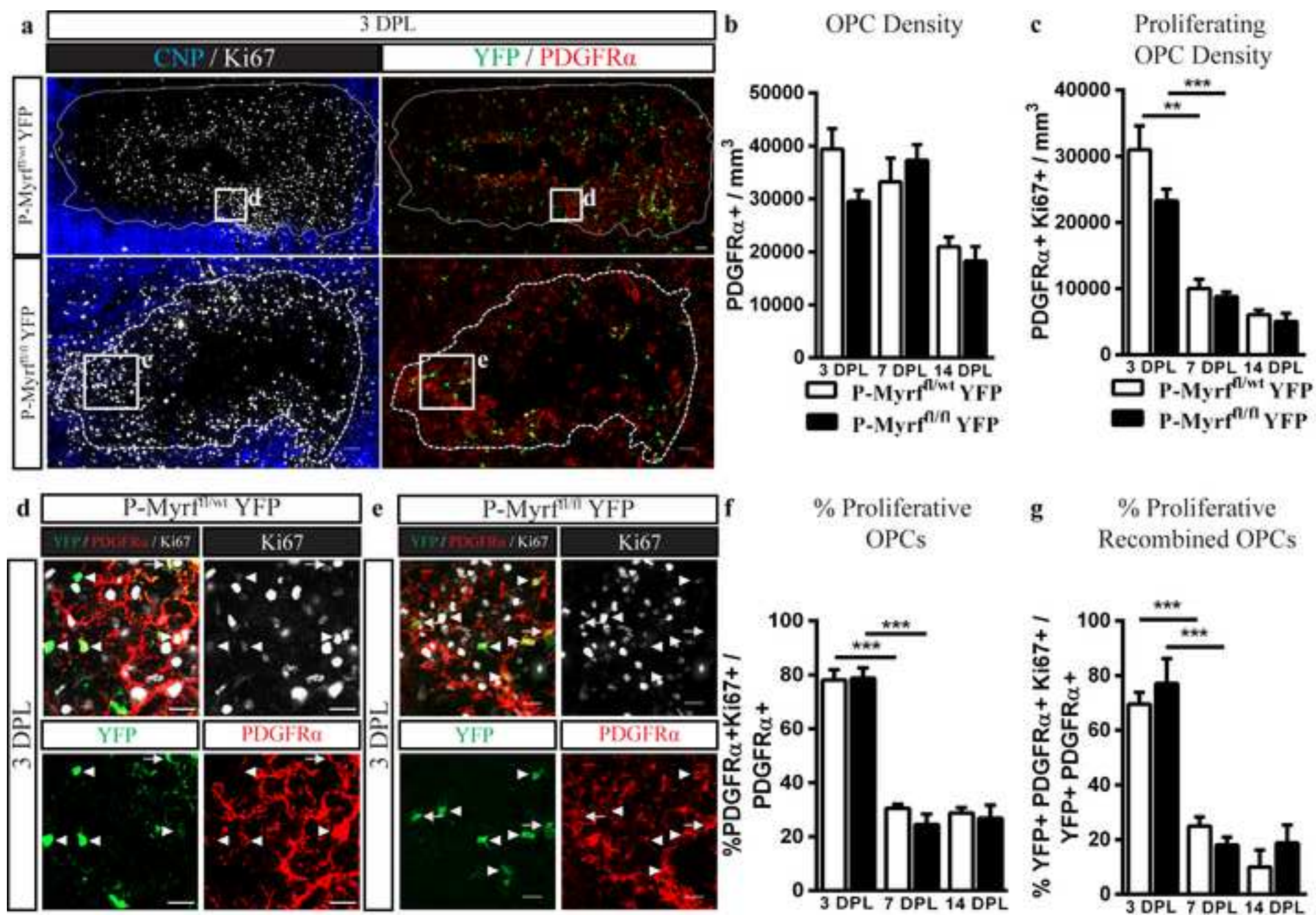
844 63 Xiao L, Ohayon D, McKenzie IA, Sinclair-Wilson A, Wright JL, Fudge AD, et al. (2016) Rapid
845 production of new oligodendrocytes is required in the earliest stages of motor-skill learning. *Nat*
846 *Neurosci* 19: 1210-1217 Doi 10.1038/nn.4351

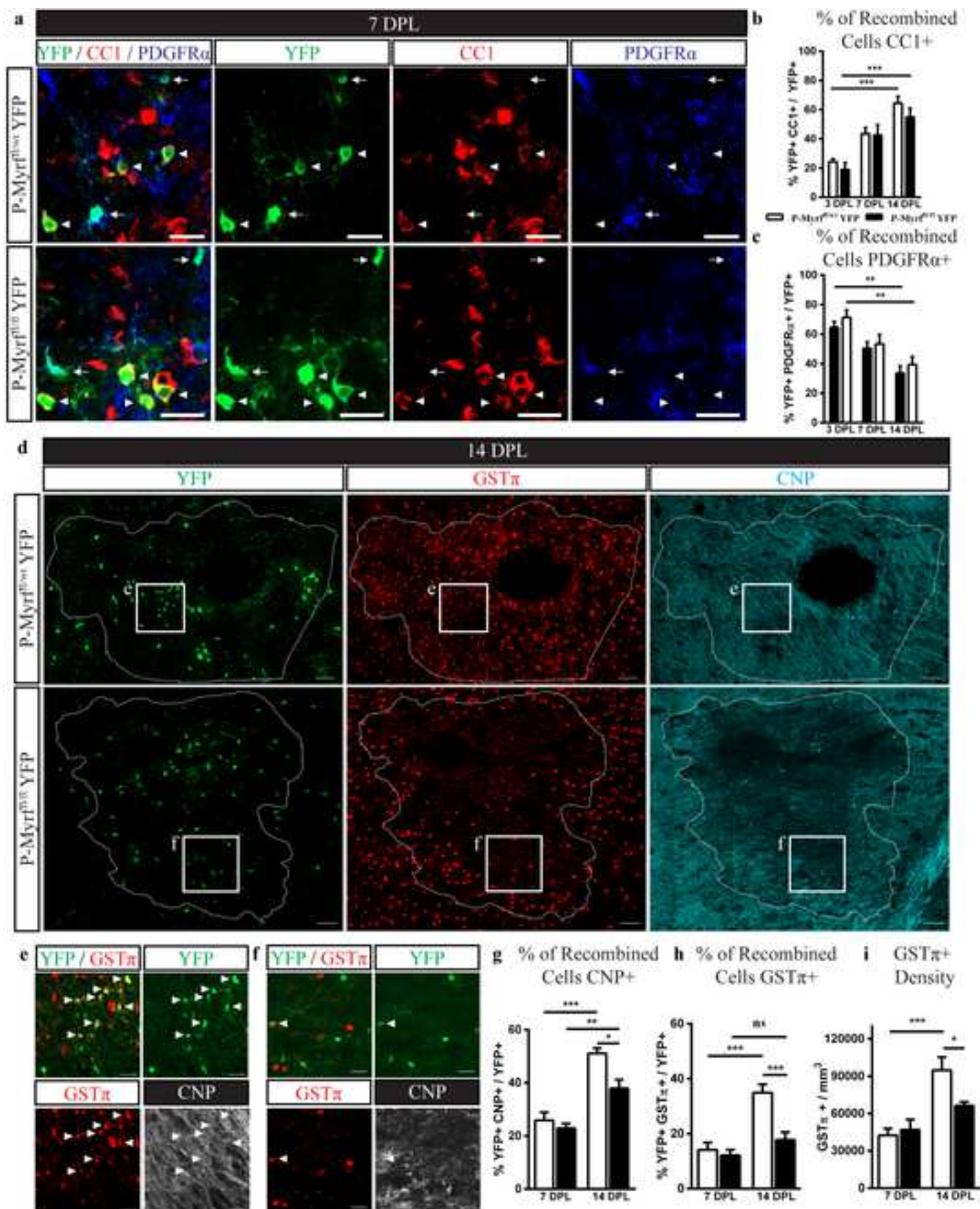
847 64 Yeung MSY, Zdunek S, Bergmann O, Bernard S, Salehpour M, Alkass K, et al. (2014) Dynamics of
848 Oligodendrocyte Generation and Myelination in the Human Brain. *Cell* 159: 766-774 Doi
849 10.1016/j.cell.2014.10.011

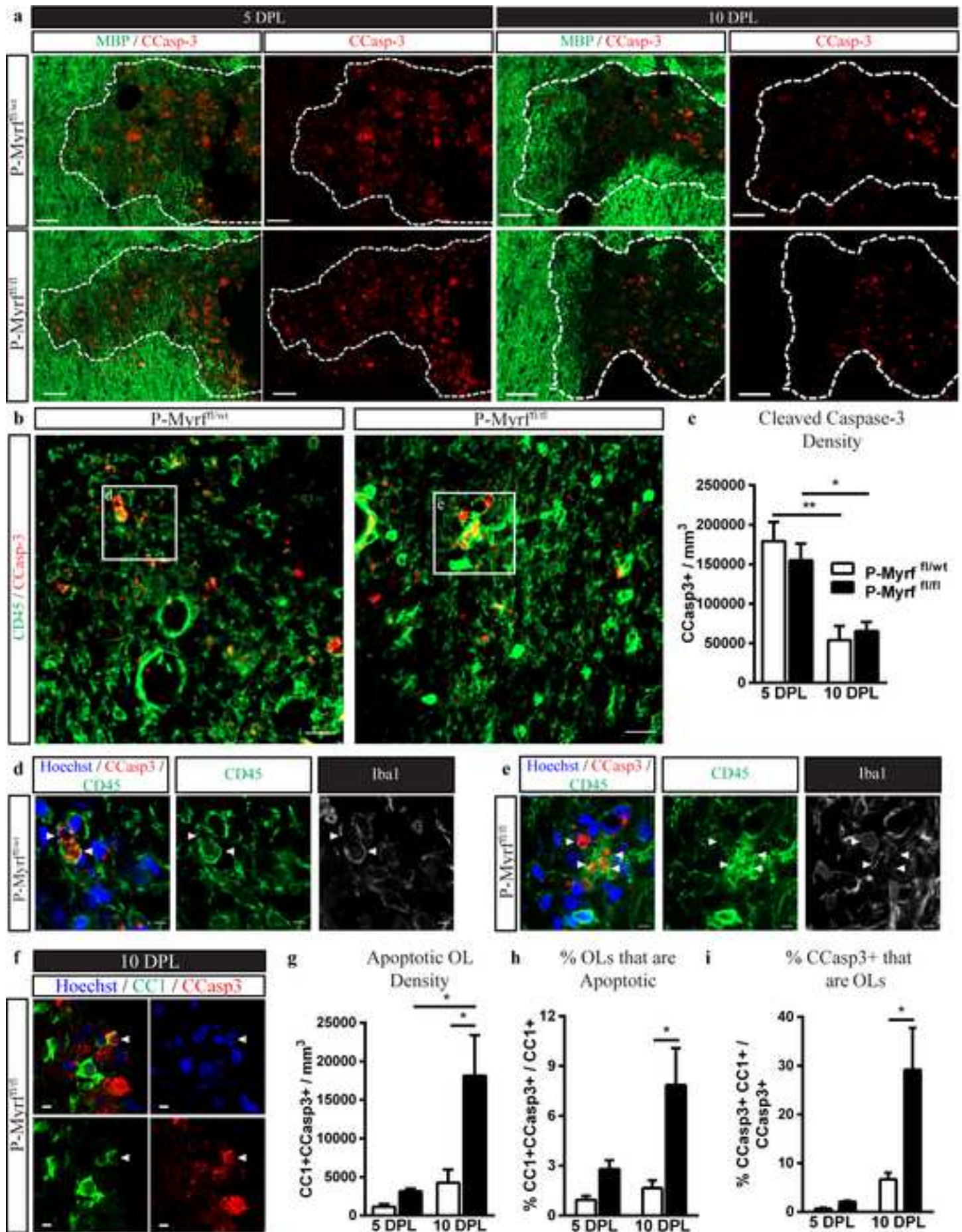
850

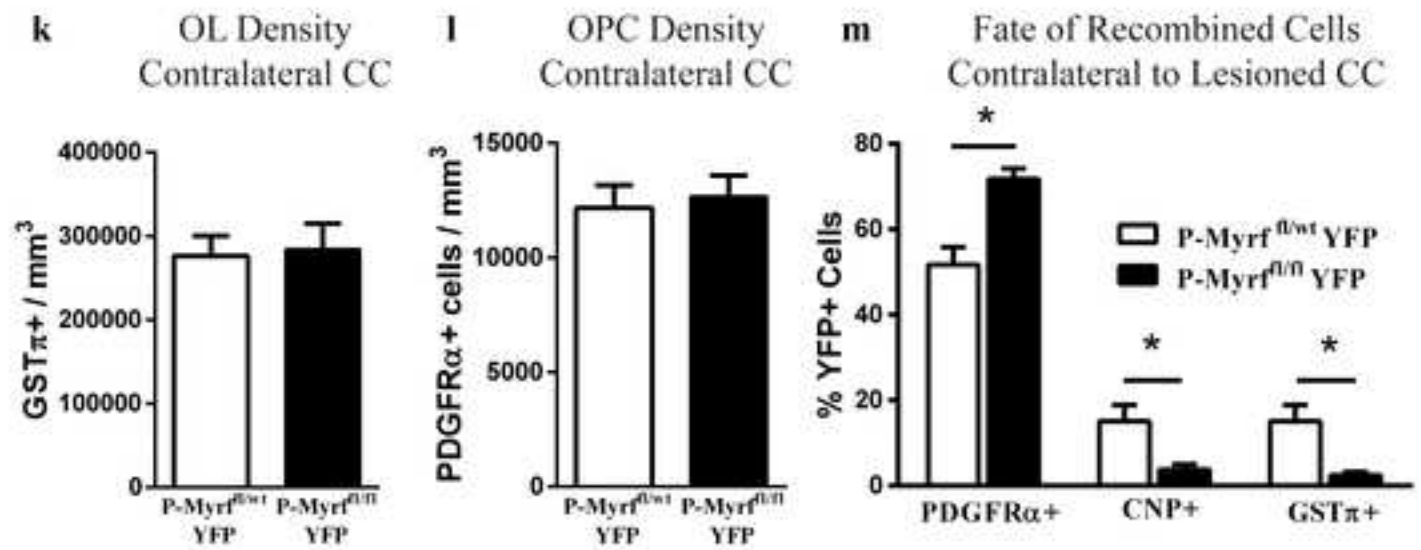
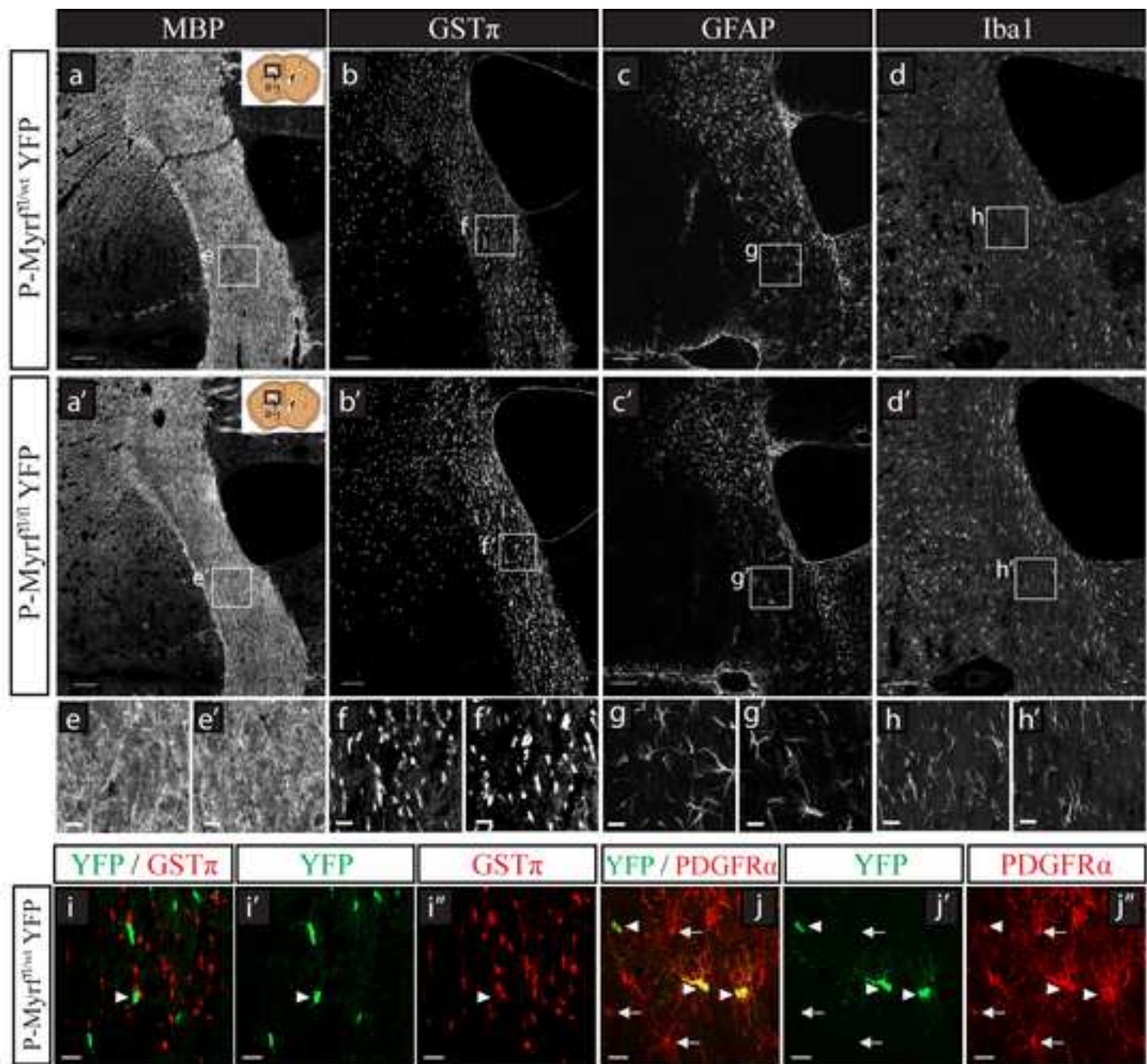
851

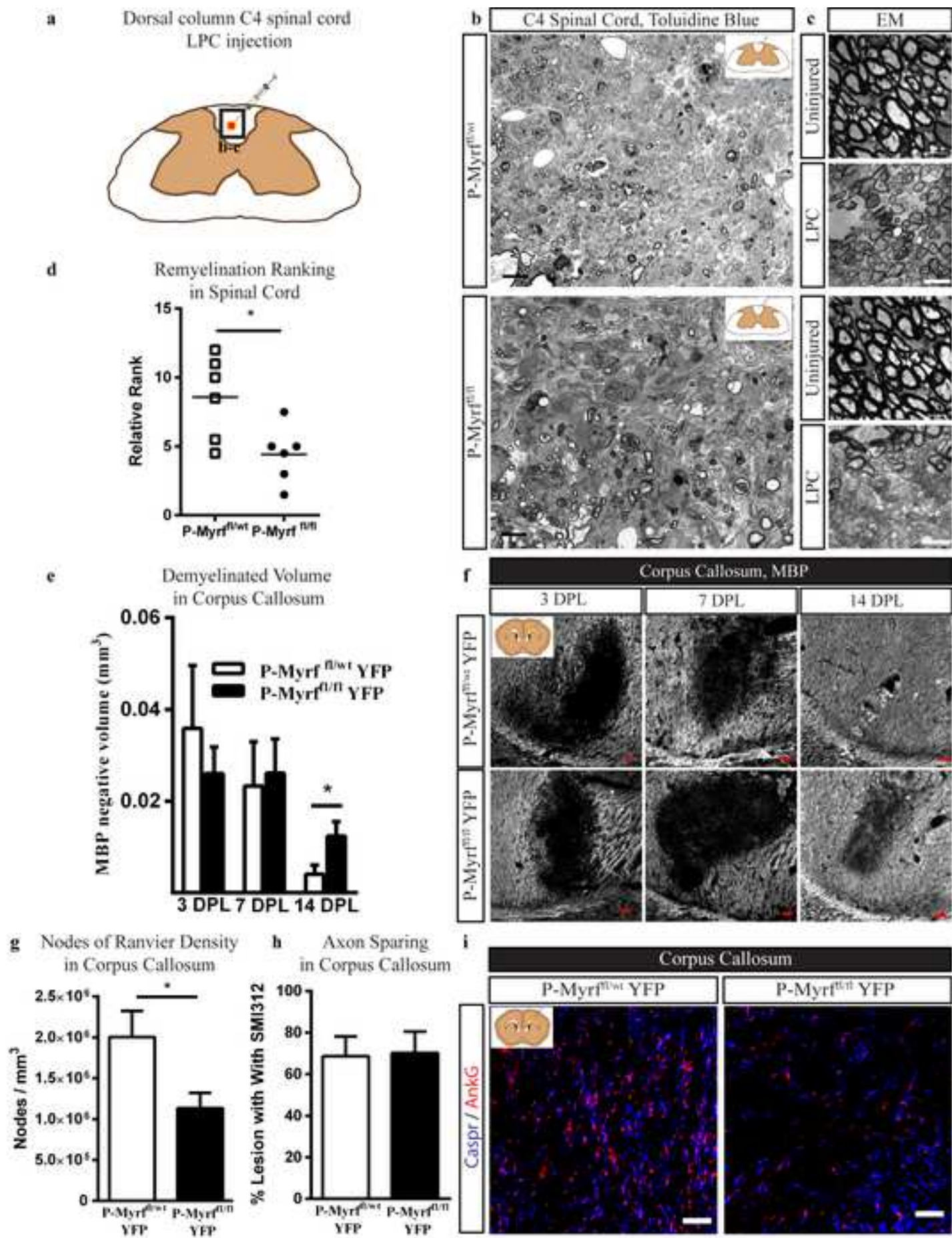


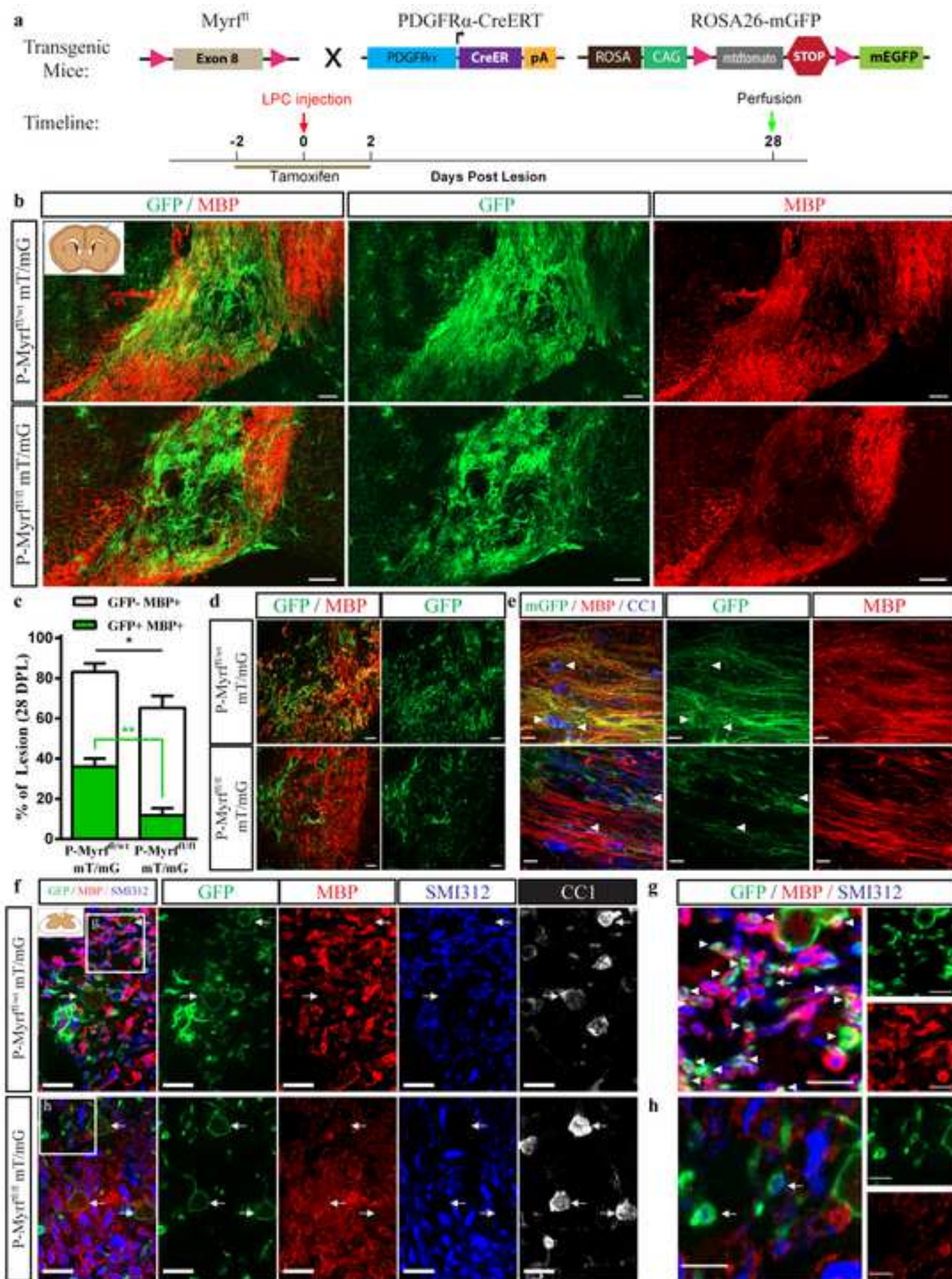


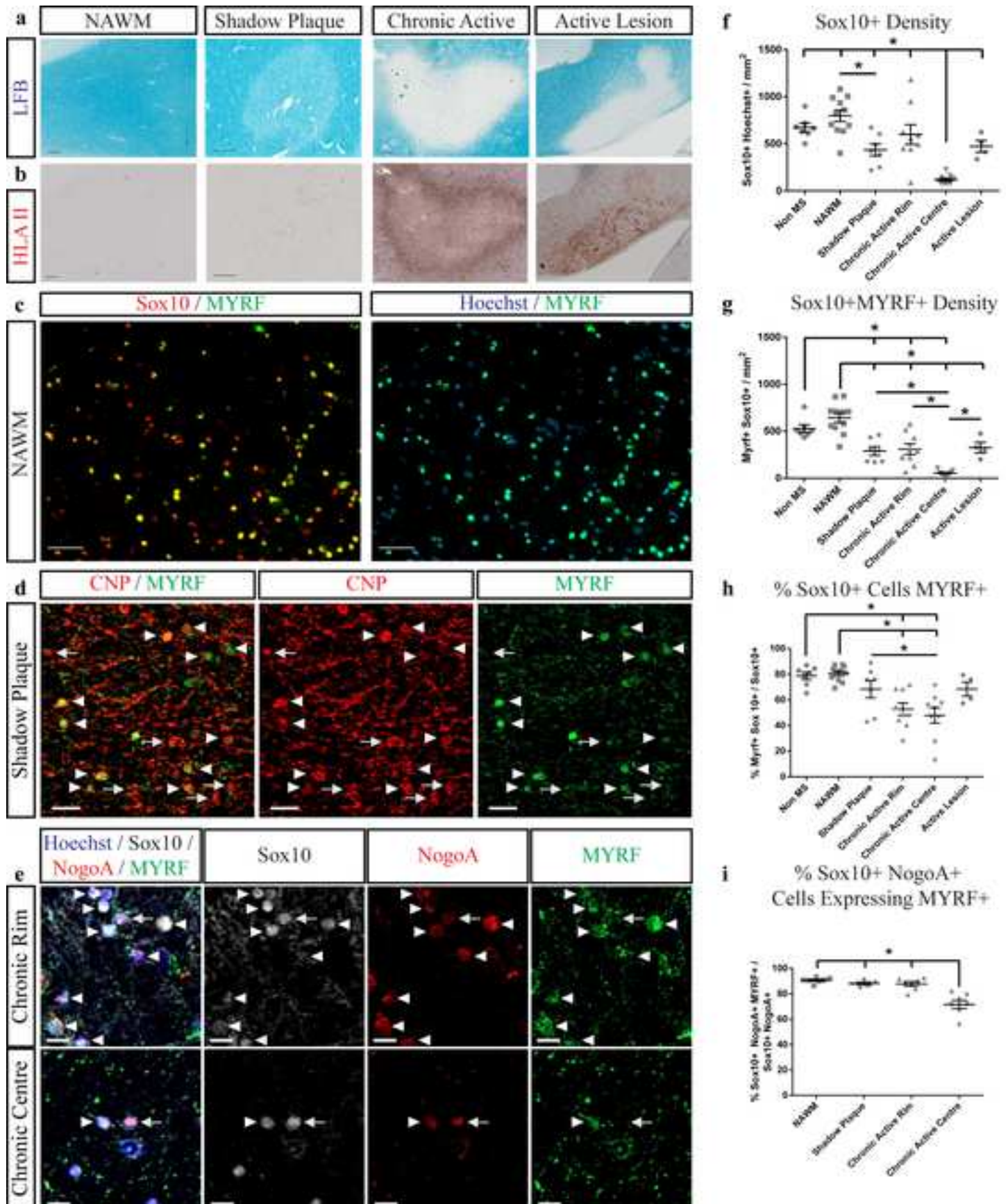














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