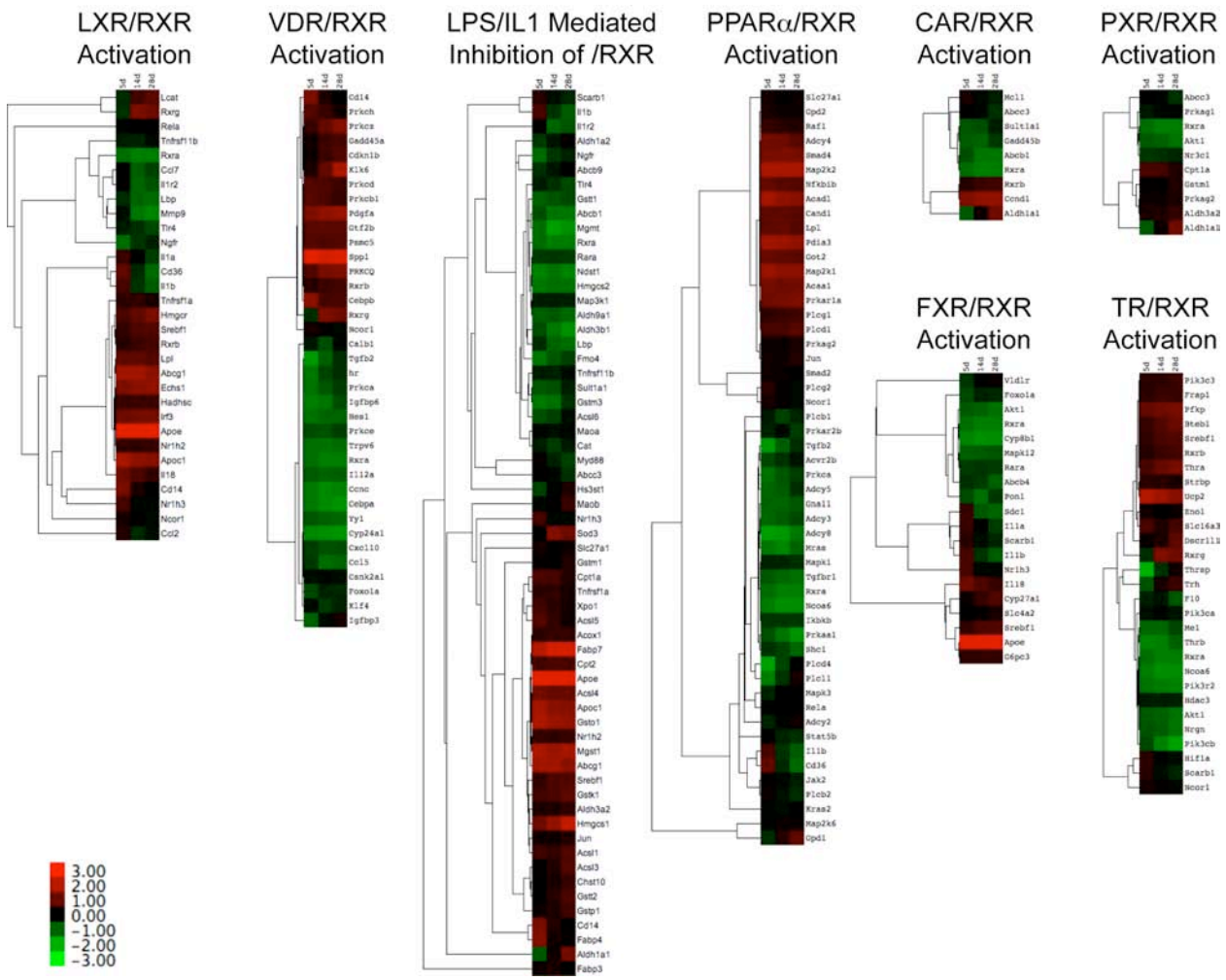
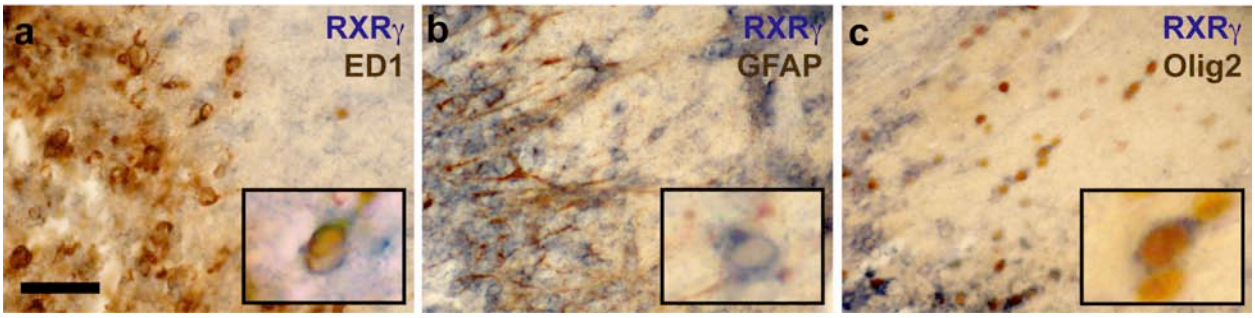
Retinoid X receptor gamma signaling accelerates CNS remyelination

Jeffrey K. Huang, Andrew A. Jarjour, Brahim Nait Oumesmar, Christophe Kerninon, Anna Williams, Wojciech Krezel, Hiroyuki Kagechika, Julien Bauer, Chao Zhao, Anne Baron-Van Evercooren, Pierre Chambon, Charles ffrench-Constant, Robin J. M. Franklin

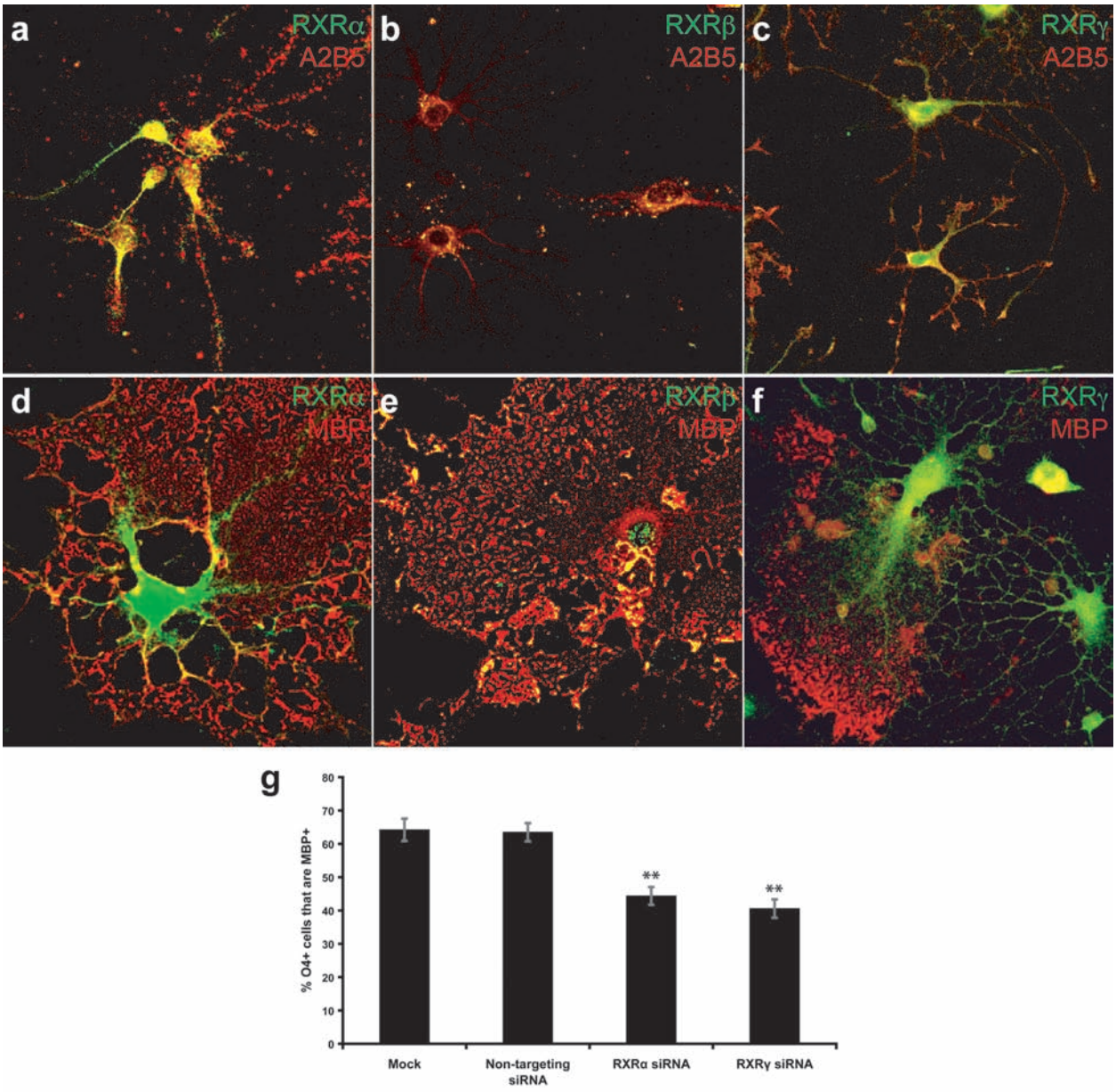
SUPPLEMENTARY MATERIAL



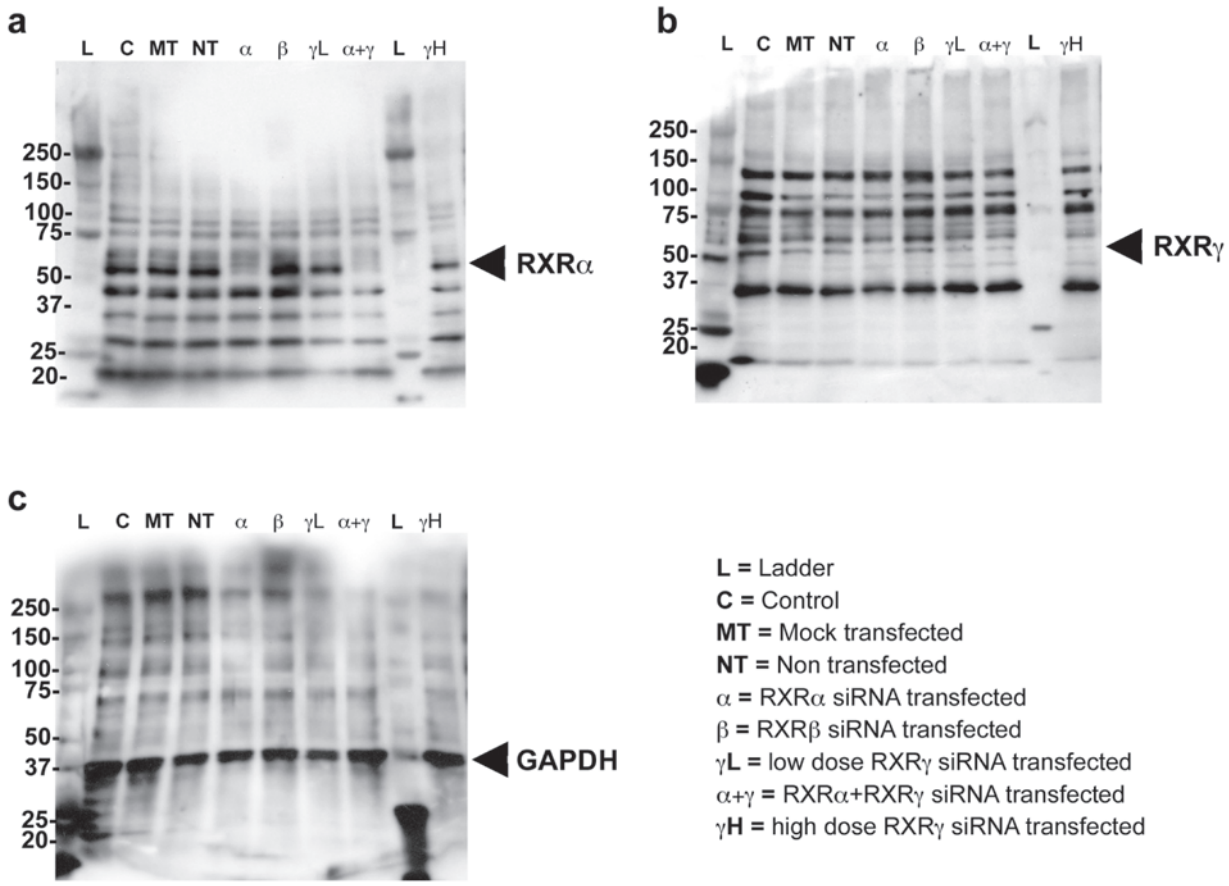
Supplementary Figure 1. Graphical analysis of IPA identified genes associated with RXR signaling. Total differentially expressed genes from 3 postlesion time points were analyzed and those associated with each RXR activation pathways were clustered by hierarchical clustering and visualized by Java TreeView.



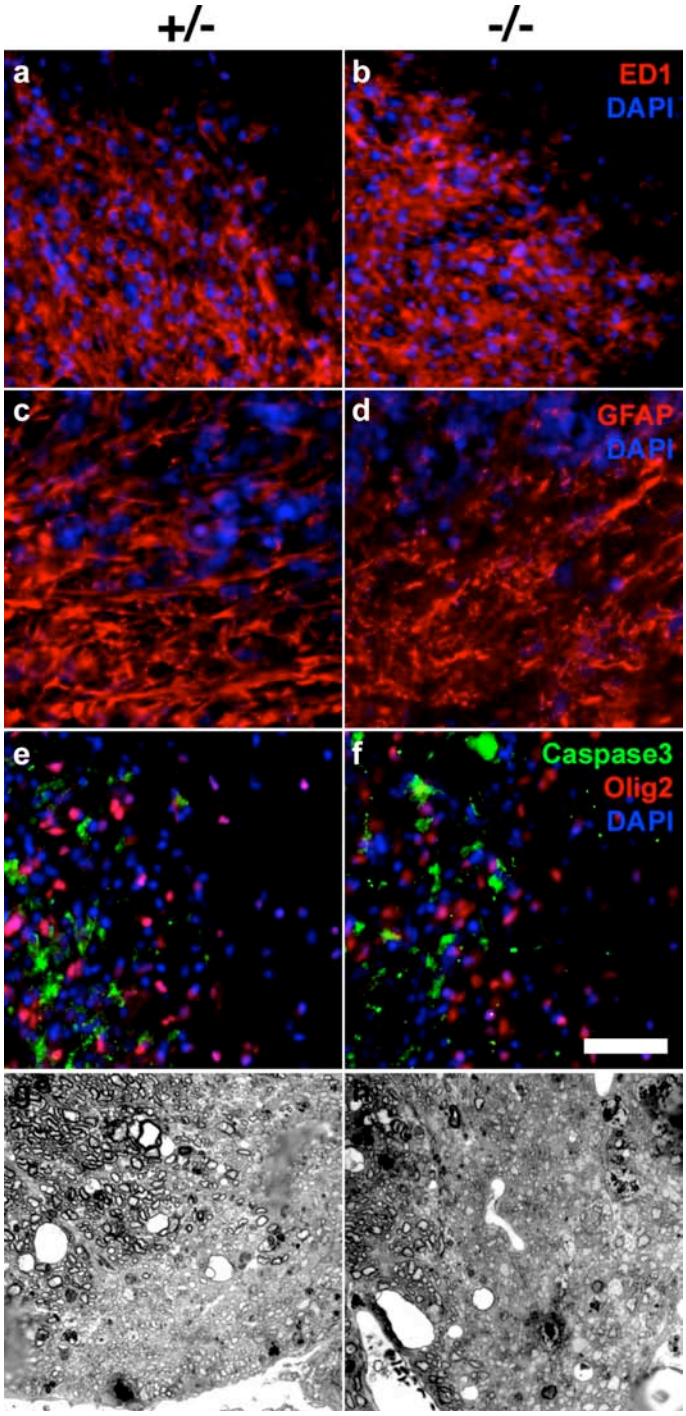
Supplementary Figure 2. *Rxrg* expression in remyelinating lesions. *In situ* hybridization against *Rxrg* followed by immunoperoxidase staining on 14 dpl CCPs with (**a**) ED1, (**b**) GFAP, and (**c**) OLIG2 was performed. *Rxrg* was detected in ED1$^+$ macrophage, GFAP$^+$ astrocytes, and Olig2$^+$ oligodendrocyte lineage cells. Insets are enlarged images of cells expressing *Rxrg*. Scale bar = 50 μm.



Supplementary Figure 3. Decreased oligodendrocyte differentiation after RXR-γ knockdown. Immunostaining of (**a, d**) RXR-α, (**b, e**) RXR-β, and (**c, f**) RXR-γ co-labeled with anti-O4 at 1 day and anti-MBP at 3 days *in vitro* show high RXR-α and RXR-γ expression, and relatively low RXR-β expression in oligodendrocyte lineage cells. (**g**) Percentage of $O4^+$ MBP^+ cells following transfection with siRNAs against RXR-α or RXR-γ. Mean values ± s.e.m. are displayed. **P < 0.005 vs. control, Student's t-test.



Supplementary Figure 4. Full length blot showing siRNA knockdown of RXR-α and RXR-γ. OPC lysates labeled with antibodies against (**a**) RXR-α, (**b**) RXR-γ, and (**c**) GAPDH.



Supplementary Figure 5. *Rxrg*$^{+/-}$ and $^{-/-}$ mouse analysis. Immunostaining for (**a, b**) ED1, (**c, d**) GFAP reveal no obvious difference in macrophage or astrocyte recruitment to lesions between +/- and -/- animals at 15 dpl. Immunostaining for (**e, f**) Caspase 3 and Olig2 reveals no obvious difference in oligodendrocyte lineage cells under going apoptosis in lesion at 15 dpl. Scale bar = 50 μm. (**g, h**) Semi-thin resin sections of mouse spinal cords at 30 dpl reveal no obvious difference in the extent of remyelination.

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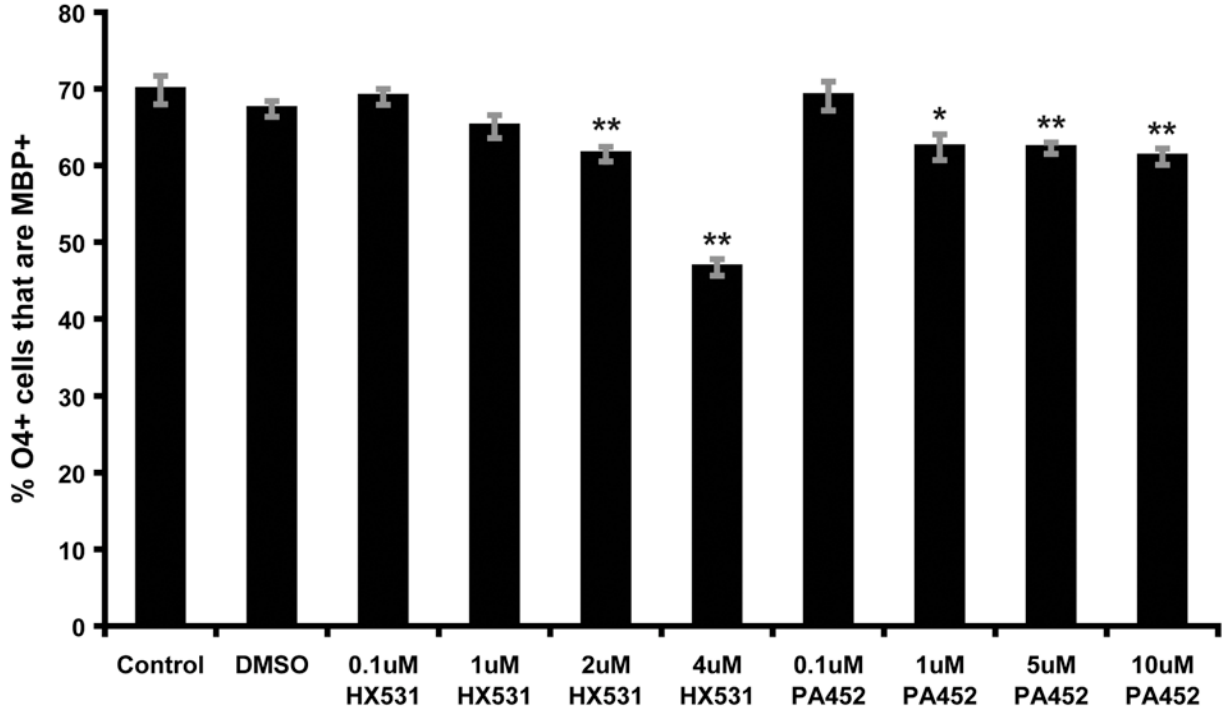
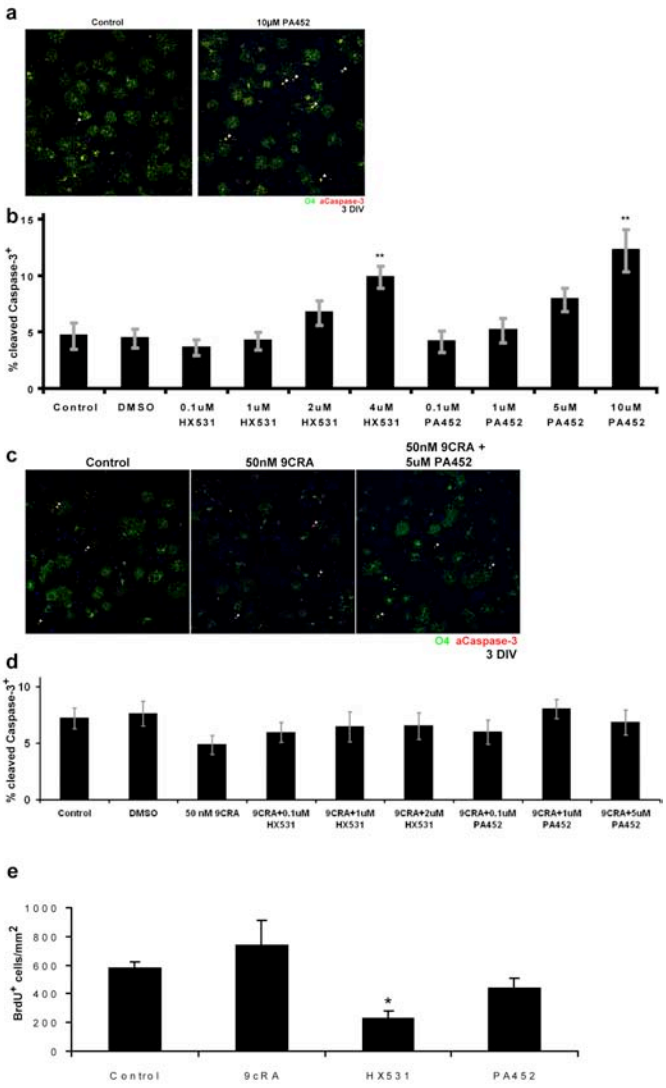


Supplementary Figure 6. Decreased oligodendrocyte differentiation after RXR antagonist treatment. Percentage of $O4^+$ cells that are also MBP^+ were analyzed following treatment with increasing concentrations of either HX531 or PA452. Mean values ± s.e.m. are displayed. *P < 0.05 vs. control, **P < 0.005 vs. control, Student's t-test.



Supplementary Figure 7. Apoptosis and proliferation count. Extent of cell death using anti-caspase 3 was determined in culture oligodendrocytes without treatment or with treatment with antagonists at 3 days *in vitro*. (**a**) At 10 μm PA452, there was a significant increase of oligodendrocytes under going apoptosis compared to control. (**b**) An analysis of the percentage of caspase3^{+} cells at different antagonist concentrations shows that neither HX531 nor PA452 influenced cell survival at the concentrations used for oligodendrocyte differentiation analysis. Cell death count only significantly increased at 4 μm HX531 and 10 μm PA452. (**c, d**) Analysis of caspase3 activity in 9cRA treated cultures revealed that 50 nM 9cRA or 50 nM 9cRA + up to 5 μm PA452 did not influence oligodendrocyte survival. (**e**) BrdU labeling for 16 hours at day 2 after demyelination and 9cRA or antagonist (2 μm HX531 or 5 μm PA452) treatment in *ex vivo* cerebellar slice cultures. There was a significant decrease in cell proliferation in HX531 treated cultures, but no significant difference between control and 9cRA or control and PA452 treated cultures. N = 2, 5 slices per factor. Mean values ± s.e.m. are displayed. *P < 0.05, **P < 0.01, Student's t-test.

SUPPLEMENTARY TABLE LEGENDS

Supplementary Table 1. Total genes differentially expressed between 5, 14 and 28 days post CCP demyelination.

Supplementary Table 2. Gene list used for IPA analysis.

Supplementary Table 3. Active signaling networks found between 5 and 14 dpl.

Supplementary Table 4. Total genes differentially expressed between 5 and 14 dpl ($P < 0.05$) used for volcano plot.

Supplementary Table 5. Assessment of known nuclear receptors in the CNS remyelination transcriptome.

Supplementary Table 6. IPA identified RXR associated pathways from the remyelination transcriptome.

Supplementary Table 7. Clinical data of the MS cases and classification of the lesions.

SYMBOL	Differentially expressed (P<0.05)	Nonpermissive heterodimer	Adj P-Val	SYNONYM
Cxr				
Nr1h2			0.438719873	LXRbeta
Nr1h3	✓		0.006468102	LXRalpha
Nr1h4				Fxr
Nr2f1	✓		0.003907145	Tfcoup1
Nr2f2			0.426103081	Tfcoup2
Nr2f6			0.08818271	Ear2
Nr4a2	✓		0.002880986	Nurr1
Ppara				
Ppard				
Pparg				
Pxr				
Rara		✓	0.859072094	
Rara				
Rarb		✓	0.61727044	
Rarb				
Rarg		✓		
Rxra	✓		0.039560609	
Rxrb	✓		0.006203785	
Rxrg	✓		0.002255554	
Thra	✓	✓	0.021245057	
Thrb	✓	✓	0.015554236	
Vdr		✓		

Supplementary Table 5. Assessment of known nuclear receptors in the CNS remyelination transcriptome.

Cases	Sex	Age (years)	PMD (h)	Course	Active	Chronic silent	Shadow plaque	PPWM	Topography
MS3132	F	65	20	SP		1	1	1	White matter, temporal lobe
MS3603	M	60	22	RR	1	1		1	Subcortical white matter, occipital lobe (internal)
MS7914	F	74	45	SP	2	3		3	Periventricular area, left frontoparietal region
Controls									
3861	F	74	49						
4984	M	70	30						
2468	F	66	43						

PMD, postmortem delay; PP, primary progressive; SP, secondary progressive; RP, relapsing progressive; ND, not determined.

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