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Supplementary Materials for

Adult rat myelin enhances axonal outgrowth from neural stem cells

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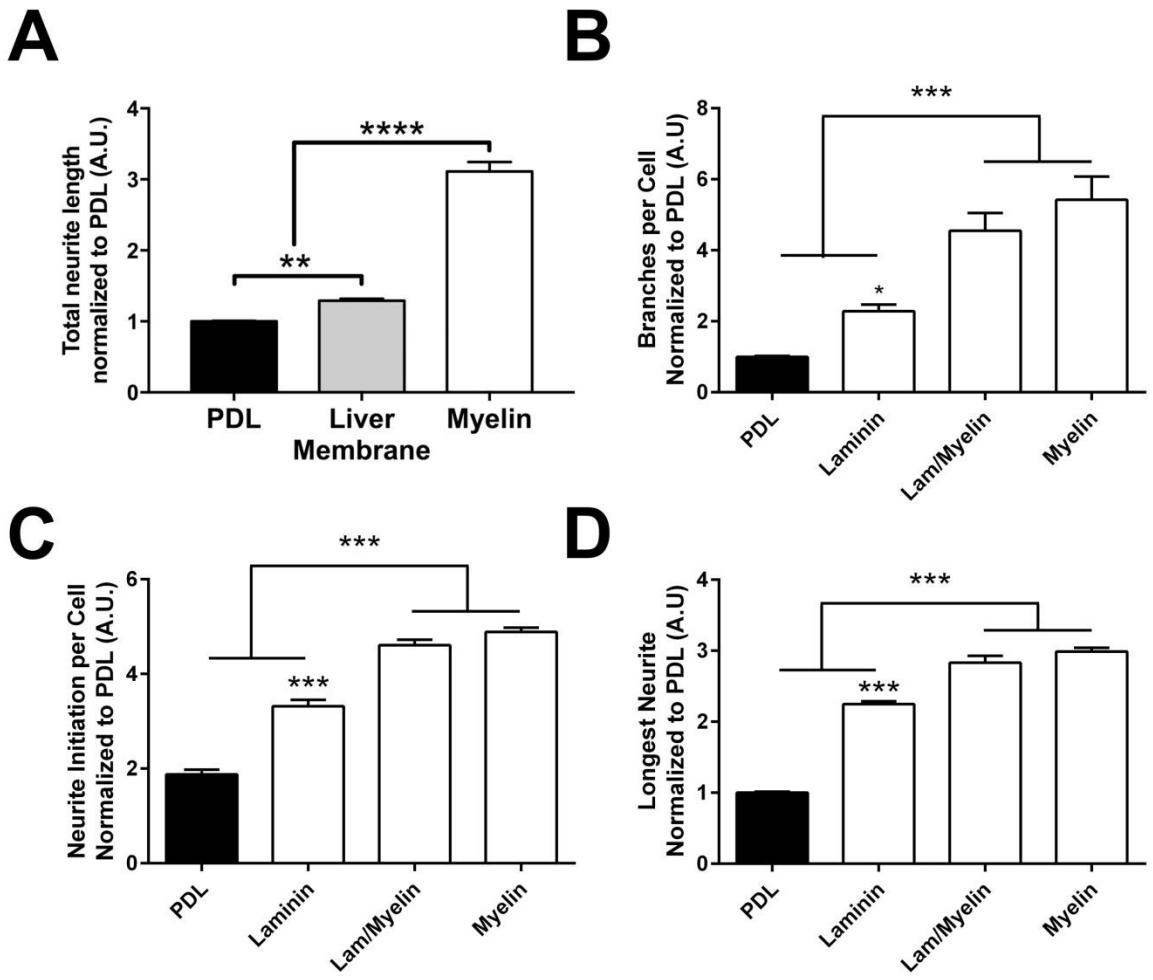


Figure S1: NPC-derived axon growth is stimulated by a myelin substrate in vitro.

Enhanced neurite outgrowth (βIII-tubulin label) from rat E14 spinal cord-derived NPCs on myelin substrates after 48 hours in vitro. (**A**) Neurite outgrowth stimulation comparing non-specific membranes from liver tissue to myelin membranes, (**B**) Neurite branching, (**C**) neurite initiation per cell, (**D**) longest neurite. All values are normalized to the PDL condition for each individual experiment. Show are Mean ± SEM; ***p < 0.001 One-way ANOVA, with *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, post-hoc Tukey's; n = 3 embryos, n = 4 wells per embryo.

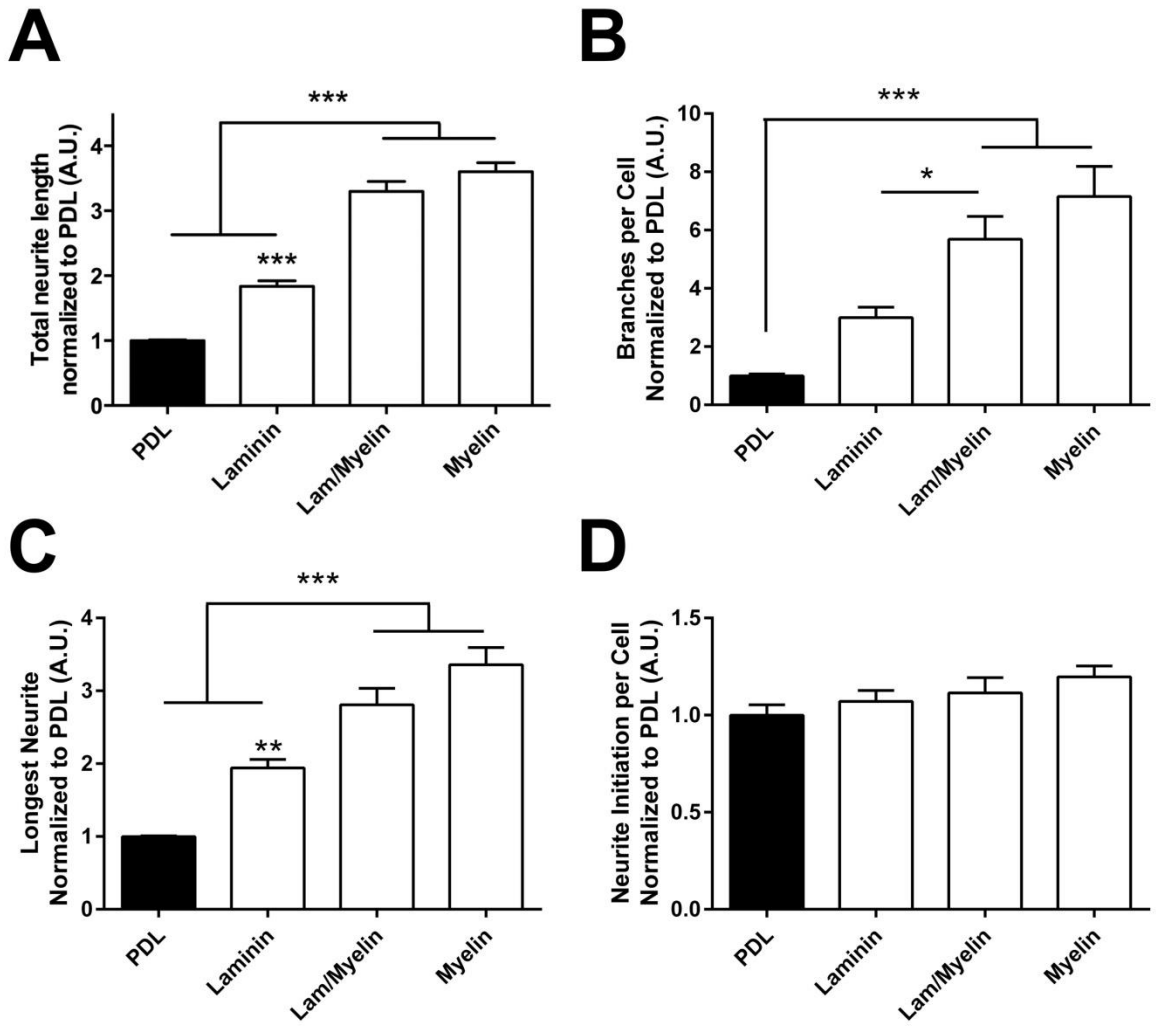


Figure S2: Mouse E12 spinal cord–derived NPC neurite growth is stimulated by a myelin substrate in vitro.

Enhanced neurite outgrowth (βIII-tubulin label) from mouse E12 spinal cord-derived NPCs on myelin substrates after 48 hours in vitro. (**A**) Neurite length per cell, (**B**) Neurite branching per cell, (**C**) longest neurite and (**D**) neurite initiation. All values are normalized to the PDL condition for each individual experiment. Show are Mean ± SEM; ***p < 0.001 One-way ANOVA, with *p < 0.05, **p < 0.01, ***p < 0.001, post-hoc Tukey's; n = 3 embryos, n = 4 wells per embryo

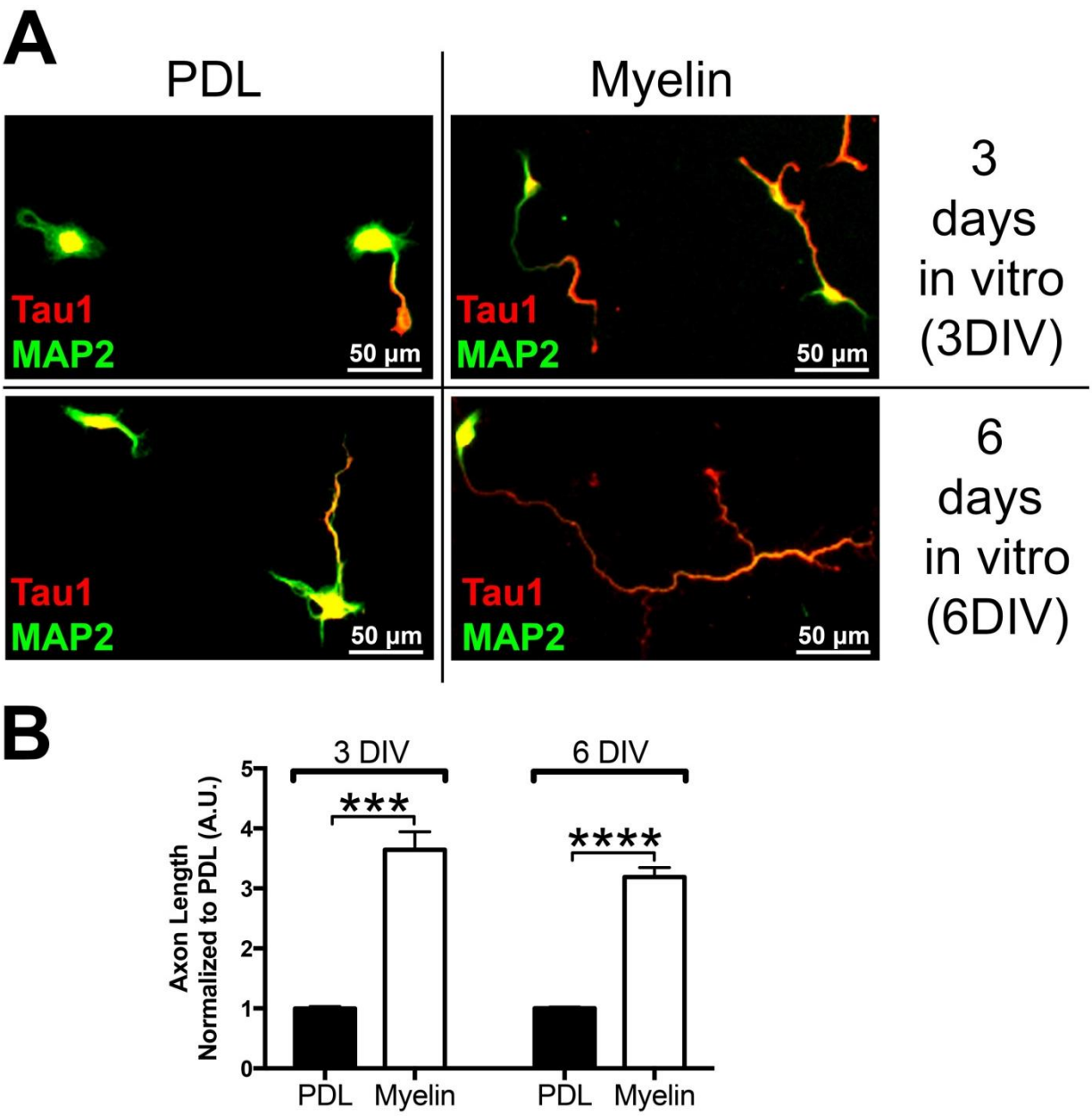


Figure S3: Axon (Tau1) growth is stimulated by a myelin substrate in vitro.

(A-D) E14 spinal cord-derived NPCs exhibit stimulation of *axon* growth on myelin substrates identified by the axonal marker Tau1 and the dendritic marker MAP2 after 3 and 6 days in vitro (DIV). (**B)** Quantification of axonal growth stimulation via Tau1 labeling. Values are normalized to the PDL condition for each individual experiment. Show are Mean ± SEM; ***p < 0.001, ****p < 0.0001, two-tailed t-test; n = 3 embryos, n = 4 wells per embryo. Scale bar: A-D, 100 µm.

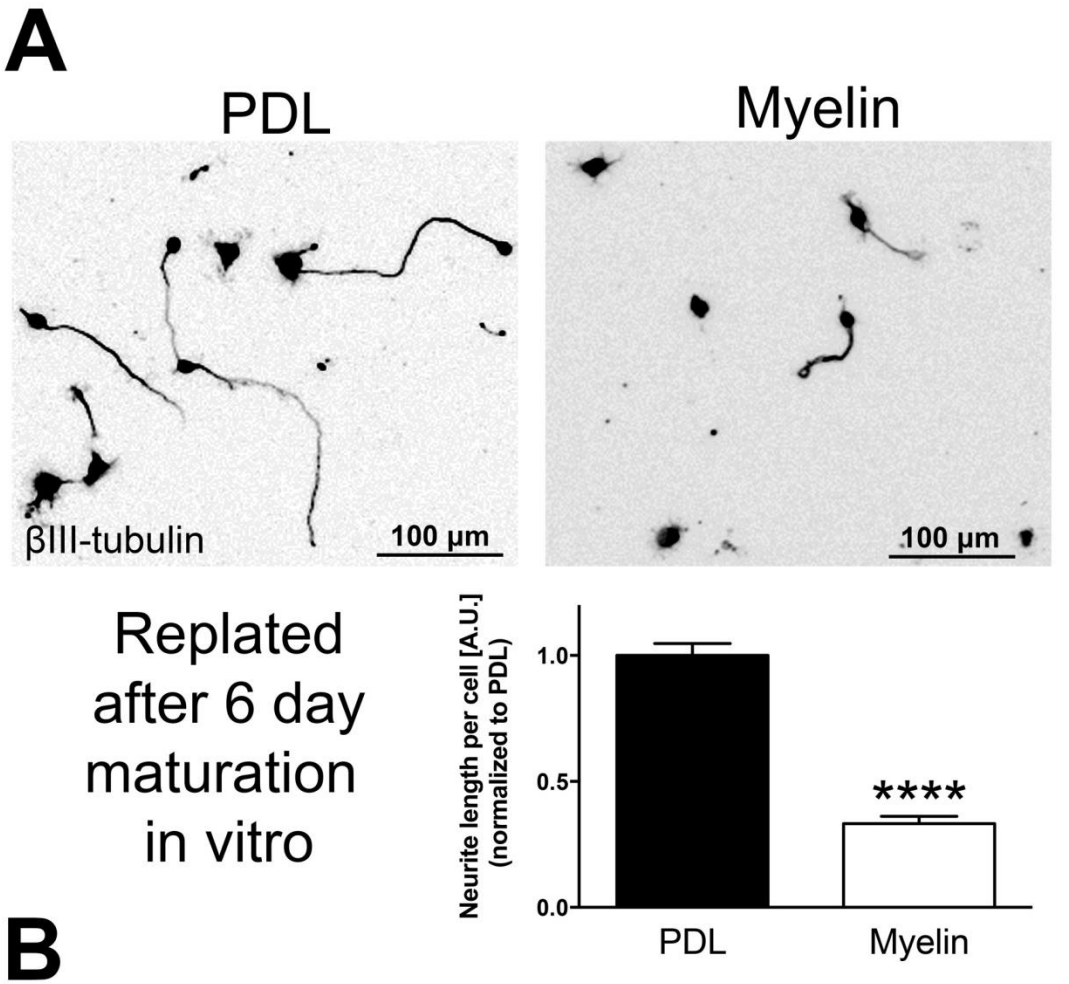

A
PDL
Myelin
βIII-tubulin
100 µm
100 µm
Replated after 6 day maturation in vitro
Neurite length per cell [A.U.] (normalized to PDL)
1.0
0.5
0.0
****
PDL
Myelin
B
DIV 1
BrdU/DAPI/βIII-tubulin
50 µm
DIV 3
BrdU/DAPI/βIII-tubulin
50 µm
DIV 5
BrdU/DAPI/βIII-tubulin
50 µm
BrdU/βIII-tubulin double positive cells [%]
5
4
3
2
1
0
DIV 1
DIV 3
DIV 5

Figure S4: E14 rat spinal cord–derived NPCs lose their ability to be stimulated by myelin upon in vitro maturation.

(A) E14 spinal cord-derived NPCs were cultured on PDL and matured for 6 days in vitro (6DIV), paralleling the developmental timeframe from E14 to E20. After trypsinization and replating, in vitro "matured" NPCs show significant neurite outgrowth inhibition on myelin compared to PDL substrates. Values are normalized to the PDL condition for each individual experiment (****$p < 0.0001$, two-tailed t-test; n = 3 individual experiments). (**B)** E14 spinal cord-derived NPCs were cultured on PDL and fixed after 1, 3 and 5 days in vitro. **BrdU** was added to the culture medium at time of plating to label nuclei that underwent mitosis during the culture period. The nuclear label **DAPI** was used to label all cells in culture and the neuronal marker, and **βIII-tubulin** was used to identify the neuronal population. Non-neuronal cells that underwent mitosis co-label for **DAPI** and **BrdU** (white arrowheads) and neuronal cells that underwent mitosis co-label for **DAPI** and **BrdU** and **βIII-tubulin** (black arrowheads). Quantification shows that fewer than 3% of cells labeled for BrdU were neuronal (n = 3 embryos, n = 3 wells per embryo). Mean ± SEM. Scale bar: A, 100 μm; B, 50 μm.

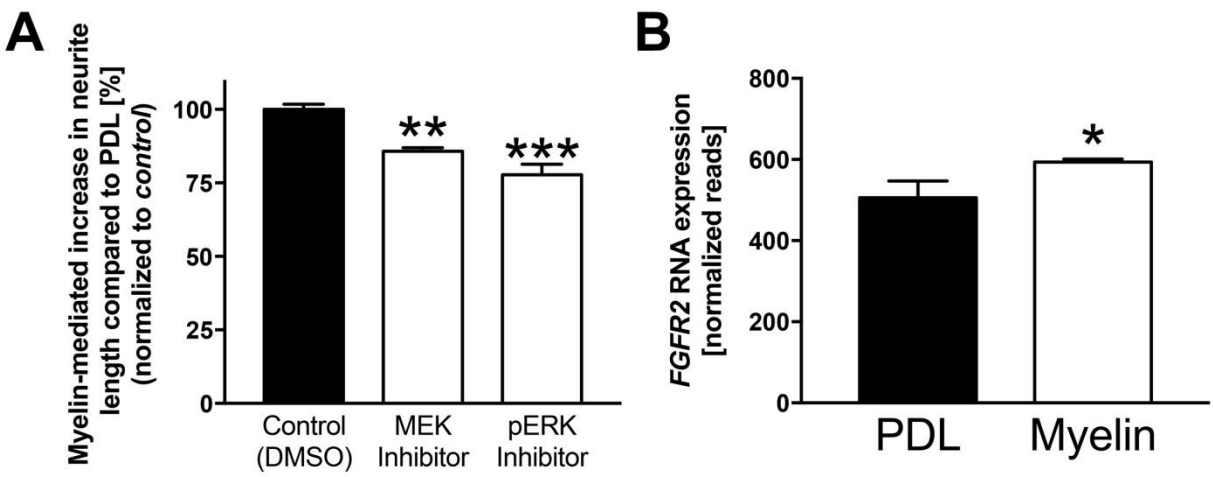
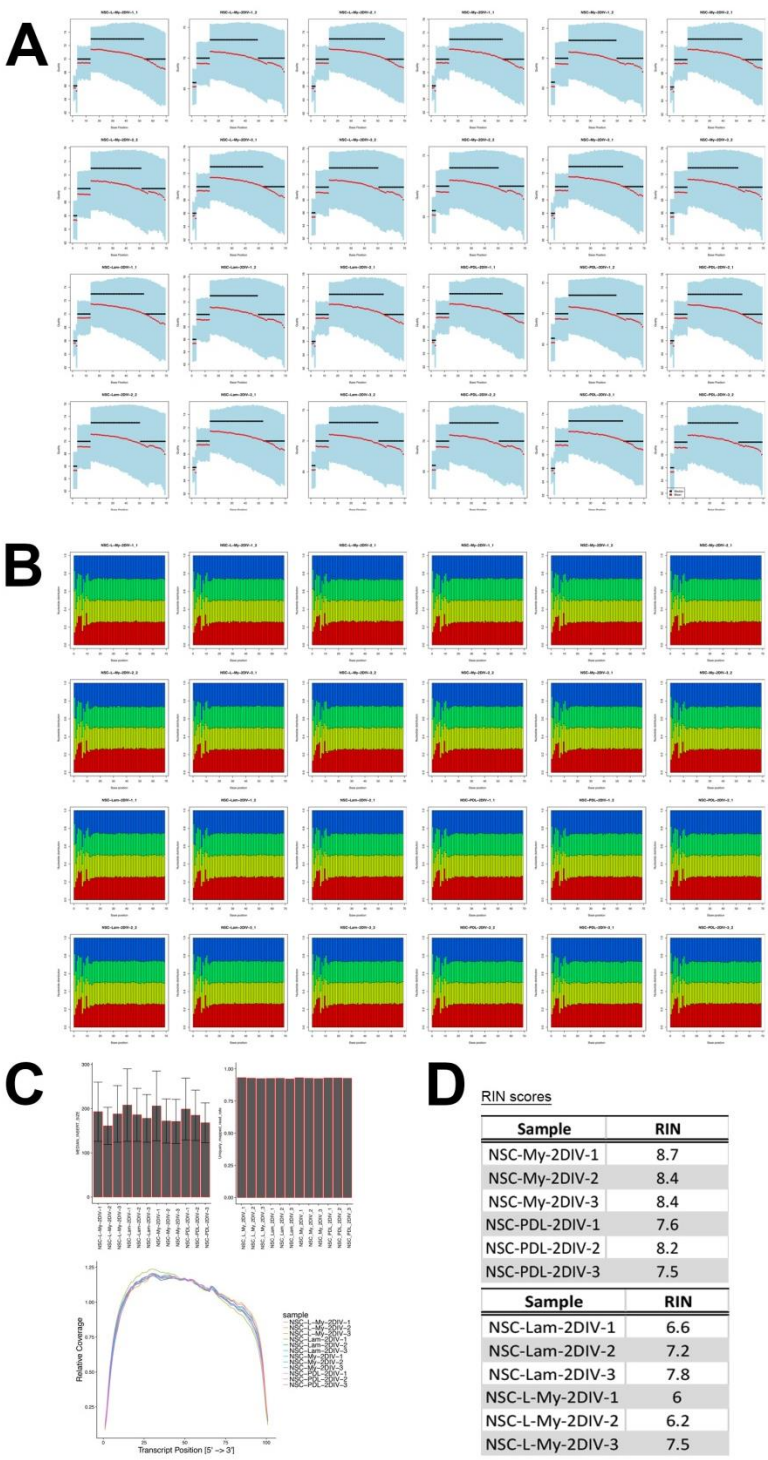


Figure S5: Growth-dependent mechanisms involving *pERK*.

(A) Rat E14 spinal cord-derived NPCs were plated on PDL and myelin substrates and cultured in the presence of DMSO (control), ERK-kinase-(MEK)-inhibitor (10 μg/ml; PD98059) or ERK-inhibitor (12.5 μM; CAS1049738) for 24 hours. Both inhibitors showed partial blockade (14% and 22%, respectively) of myelin-mediated stimulation compared to DMSO, indicating that p*Erk* is an essential, but partial contributor to the stimulatory effects of myelin. Values are normalized to the PDL condition for each individual experiment (***$p < 0.001$, one-way ANOVA, with **$p < 0.01$, ***$p < 0.001$ post-hoc Tukey's; n = 4 individual experiments). Mean ± SEM. **(B)** *FGFR2,* which has been reported to potentially mediate Negr1-dependent activation of p*ERK (29),* is significantly increased upon myelin stimulation as shown by RNA-seq. ($p = 0.05$, one-tailed t-test; n = 3 individual experiments). Mean ± SEM.



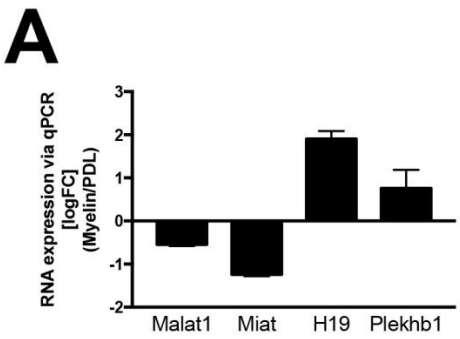
RIN scores

Sample	RIN
NSC-My-2DIV-1	8.7
NSC-My-2DIV-2	8.4
NSC-My-2DIV-3	8.4
NSC-PDL-2DIV-1	7.6
NSC-PDL-2DIV-2	8.2
NSC-PDL-2DIV-3	7.5

Sample	RIN
NSC-Lam-2DIV-1	6.6
NSC-Lam-2DIV-2	7.2
NSC-Lam-2DIV-3	7.8
NSC-L-My-2DIV-1	6
NSC-L-My-2DIV-2	6.2
NSC-L-My-2DIV-3	7.5

Figure S6: Quality measures of RNA-seq.

Several measurements showed consistent quality across samples were conducted for consistency of RNA-sequencing data. Consistency of RNA-sequencing quality: **(A)** base quality distribution, **(B)** nucleotide composition, and **(C)** insert sizes, percentage of uniquely aligned reads (>90%) and transcript coverage. Plots in (A) and (B) represent paired-end reads for each sample separately. Error bars in (C) represent ± SD**. (D)** RNA integrity number (RIN) for the RNA samples.



RNAseq Data

Gene name	refSeq	Rank	logFC_Mye_vs_PDL	FDR_Mye_vs_PDL
Malat1	NR_002847	22	-0.903049544	0.00024071
Miat	NR_033657	153	-1.34474975	1.03E-09
H19	NR_001592	2702	0.983220962	0.096013131
Plekhb1	NM_013746	6941	5.603426205	3.45E-33

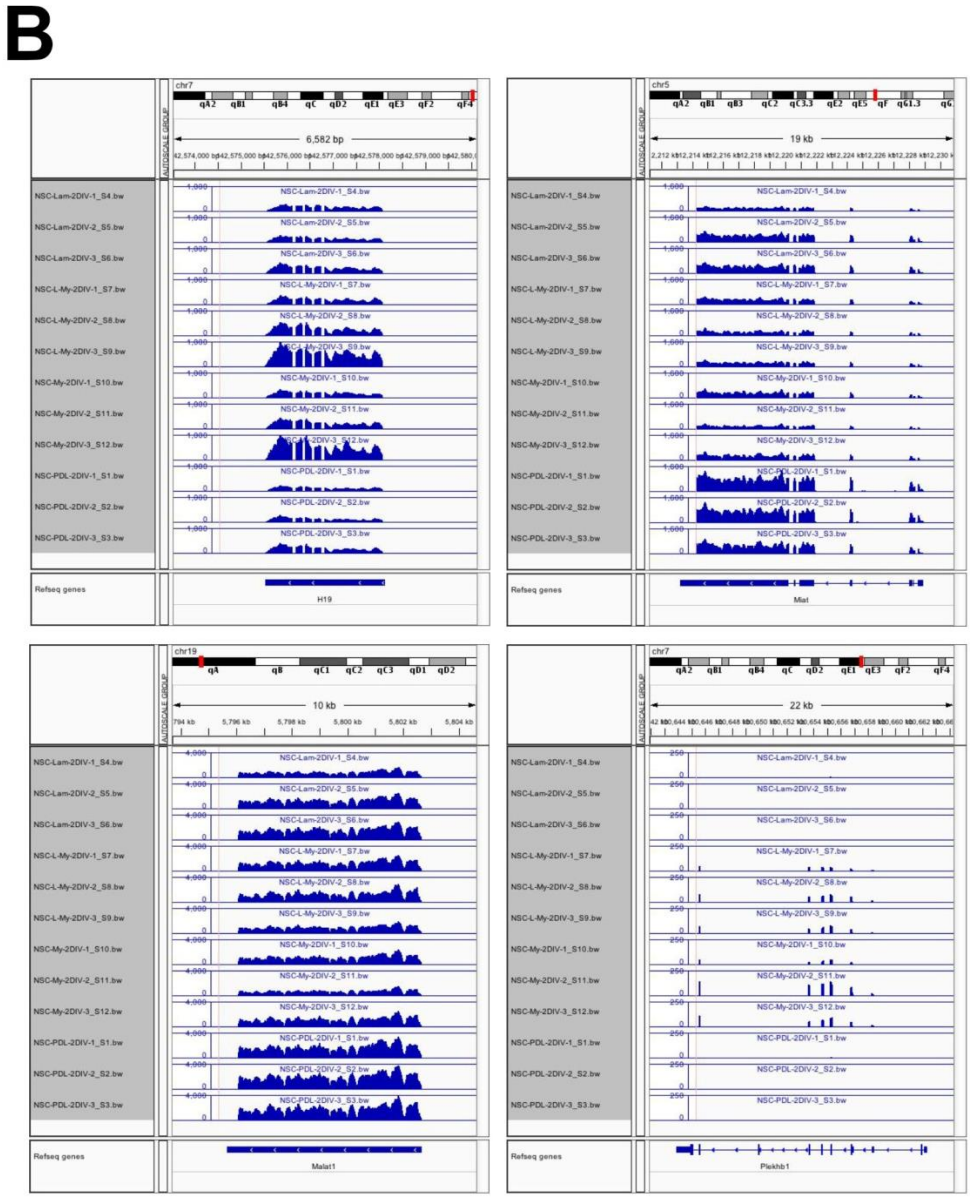


Figure S7: qPCR verification of RNA-seq.

(A) qPCR validation of selected differentially expressed genes from RNAseq analysis. Mean ± SEM log-2 fold changes, n = 3 individual experiments. **(B)** Integrative Genomics Viewer (IGV) RNA-seq screenshots for the selected genes.

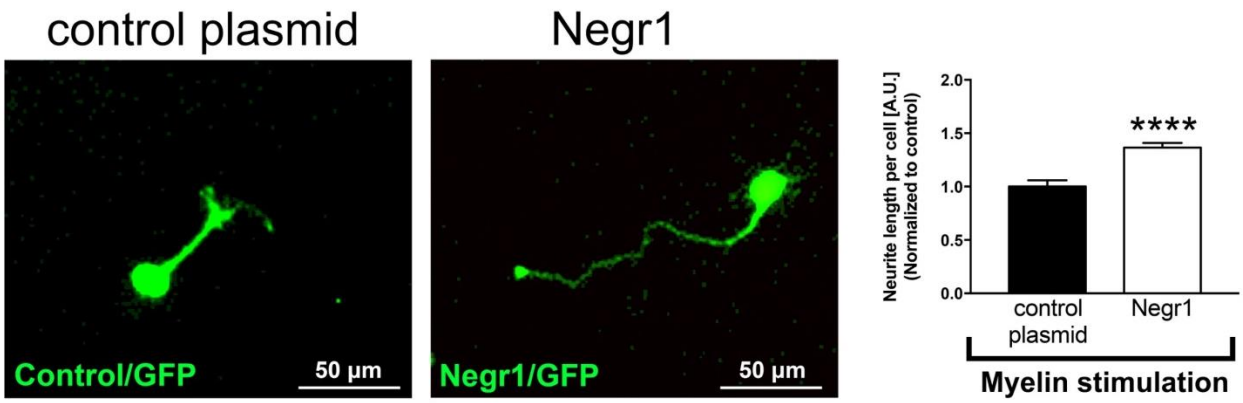


Figure S8: Overexpression of Negr1 in mature spinal cord–derived NPCs increases neurite growth on a myelin substrate in vitro.

Rat E14 spinal cord-derived NPCs were co-electroporated with either Negr1-pcDNA3 + Pmax-GFP (Lonza) or with empty pcDNA3 backbone (*30*) + Pmax-GFP and plated on PDL. After maturation for 6 days in culture, cells were trypsinized, re-plated on myelin substrates, and neurite extension of GFP+ neurons in both conditions was quantified 24 hours later. Values are normalized to the control condition for each individual experiment (****$p < 0.0001$, two-tailed t-test; n = 3 individual experiments). Mean ± SEM. Scale bar: 100 µm.

Table S1: Individual level data for experiments with *n* < 20.

Figure 1 D

GM	WM		
701	1622		
161	1099		
383	2123		
48	521		

Figure 3J

PDL	Laminin	Laminin Myelin	Myelin
0.9376485	1.968279	3.364525	3.513651
1.003122	1.875667	3.21026	3.690671
1.05923	2.165765	2.783476	3.65955
0.9974955	1.857898	2.94977	2.768584
1.020199	1.898934	2.957377	3.053421
0.982305	1.809032	2.931887	3.033966
0.9638977	1.714552	2.549689	2.770257
0.9776784	1.825356	2.59493	2.614839
1.058424	1.953561	2.446282	2.885765

Fig.4B

PDL	10ug/ml	20ug/ml	30ug/ml
0.9799908	0.222083	0.163167	0.149773
1.020009		0.178827	0.134477
1.018551	0.371738	0.262109	0.184733
0.981449	0.467662	0.252328	0.159128
1.035904	0.341421	0.267268	0.148537
0.9640962	0.375682	0.258209	0.162933

Fig5C

	Control			+ Myelin		
hours	Mean	SEM	N	Mean	SEM	N
0.5	0.5166	0.06072	2	0.55	0.136	3
3	0.4759	0.0715	2	0.9546	0.1906	3
6	0.4409	0.01013	2	0.9515	0.1573	3
24	0.2695	0.01696	4	0.2926	0.05762	4
48	0.2462	0.06515	4	0.2829	0.02972	4

Figure 5B

	PDL			Laminin			Myelin		
hours	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
0.5	1	0	2	0.6848	0.1338	2	1.492	0.38	2
3	1	0	2	1.055	0.04201	3	1.796	0.129	3
6	1	0	2	1.093	0.128	3	1.466	0.3682	3

Figure 5D

PDL	Naive	Boiled	Proteinase K
1.004397	3.055143	1.922036	0.911368
1.083399	3.29691	1.980274	0.904209
0.912204	3.678771	2.06404	0.865971
			0.850135
0.983615	2.66178	1.839425	1.214888
1.044202	2.61882	1.764552	1.089457
0.998229	2.756785	1.761174	1.070655
0.973954	2.760554	1.709298	1.033241
0.974684	3.636772	2.383122	1.340591
1.018555	3.604411	2.531465	1.330772
0.916681	3.383844	1.954139	1.206385
1.09008	3.666773	2.048139	1.313252

Figure 5F raw data

	Control			+ Myelin		
hours	Mean	SEM	N	Mean	SEM	N
0.5	10.55797	0.3816391	3	5.990297	1.210558	3
3	11.73929	0.6161627	3	5.328002	0.5687032	3
6	10.15037	1.470427	3	3.621894	0.5267618	3
24	6.931765	0.9638297	3	9.044109	2.059074	3
48	7.991506	0.7468404	3	9.640215	1.484095	3

Figure 5G

DMSO				Forskolin			
PDL	Laminin	Myelin	Lam/Myl	PDL	Laminin	Myelin	Lam/Myl
0.914039081	2.798402529	4.022326345	3.427652209	1.193460724	3.161371588	3.158684659	3.950069609
0.993232388	2.918873556	3.992127317	3.48571051	1.259842826	3.168001592	2.674425027	3.929865034
1.038261184	3.134685348	3.741525362	3.248612638	1.151562927	3.39316188	3.318049887	3.889184456
1.054467347	2.95328393	3.897618722	3.680405602	1.070979234	3.458980084	2.685526234	3.934167909

Figure 7B

E12 PDL	E12 Myelin	E15 PDL	E15 Myelin	E19 PDL	E19 Myelin
0.962608019	1.076999532			1.707628473	0.720516638
1.122590164	1.332655324			0.67064382	0.806379589
0.914801818	1.812845204			0.621727707	0.936394995
1.055780045	1.240629436	1.251251107	1.217191748		
0.86875541	1.56164858	0.97225715	0.968916196		
1.075464544	1.032746195	0.776491743	1.606931022		

Figure 7E

Negr1-neg PDL	Negr1-pos PDL
86.35244	91.42979
91.93753	99.05585
90.13136	83.73885
95.50204	86.39191
90.37185	67.12975
96.93829	109.8909
99.24283	128.5095
104.7811	122.8953
112.7978	113.1423
104.644	92.29341
106.2745	99.73518
143.0425	115.8456
93.08077	86.19056
109.6377	100.361
118.3879	89.92833
122.3344	113.4618

Figure 7F

ko myl	wt myl
86.47428	68.27435
92.11978	46.43345
66.78403	73.0576
63.29745	82.45737
65.84101	222.3244
74.50852	95.94683
62.02673	97.14731
52.77027	93.04443
79.56451	94.60319
77.5761	93.89897
80.1557	88.08913
54.23563	106.5503
75.5246	77.37109
71.31109	69.37274
78.4692	67.93879
78.9018	70.37634
	139.3821
	141.4391
	154.8743
	117.4182

Figure 3I

PDL	Laminin	Laminin Myelin	Myelin
0.969891	3.25272	1.756619	1.1233
1.030109	3.114737	1.812364	1.0613
1.037479	3.228576	1.76192	1.0069
0.9625212	3.188884	1.685545	0.9903
0.9344046	3.371454	1.566497	0.928
1.065595	3.799289	1.397923	0.9751
0.9757862	3.428959		0.7519
1.024215	2.92167	1.108344	0.7751
1.023651	3.90078	1.342802	0.8749
0.9763499	3.94464	1.466743	0.8718
1.043299	3.883072	1.335682	0.8248
0.9567009	4.032176	1.258662	0.8744

Fig.4D

E14	E15	E16	E17	E18	E19
3.513651	3.179068	2.724788	2.1553	1.654383	1.146782
3.690671	3.060253	2.815954	2.0525	1.879611	1.364649
3.65955	3.40315	2.698492	2.1567	1.798982	1.283699
			2.0698		1.215537
2.768584					
3.053421					
3.033966					
2.770257					
2.614839					
2.885765					

Figure 5E

PDL	WT	MAG KO	Nogo KO	OMgp KO	AG/Nogo K	Triple KO
1.039438	5.111193	5.731461	5.2818	5.301224	3.345964	5.212978
1.048731	4.820433	4.018408	4.4814	4.864947	3.982004	4.480123
0.9118301	2.501642	2.528305	3.7035		4.003647	3.224748
0.9161536	3.516953	3.769743	3.1537	3.266012	2.976243	2.807553
1.030493	3.703214	3.21242	3.0963	3.053938	3.542002	3.712954
1.053354	3.960066	3.381776	3.0685	3.256917	3.561254	3.662236
1.030644	3.225423	2.950324	2.4975	3.608754	3.641268	
0.9397038	3.213254	3.342328	2.5208	2.998343	2.703547	2.848555
1.029652	3.732924	3.493861	3.1531	3.682022	3.54506	3.237879
1.015983	2.73848	2.542627	2.4571		1.620304	
0.9858687	2.852688	2.667689	1.5962		2.402569	
0.9981484	2.983647	2.502067	2.3343		2.627571	

Figure 7A

PDL	Laminin	Lam/My	Myelin
5421.1	5991.544	6830.179	6586.994
4847.9	5746.436	7044.501	6529.317
5100.9	5596.07	5945.586	6041.53

Figure 7D

PDL	Laminin	Lam/My	Myelin
1	1.8338		1.854418
1	1.1169		2.65145
1	1.0553	1.623358	2.054507
1	2.3946	5.224469	3.978405
1	1.2769	2.048755	1.240277

Figure S1B

PDL	Laminin	Lam/Myelin	Myelin
1.036805	2.974649	6.844672	7.446768
0.9327578	2.248227	6.511783	8.099982
1.030437	3.142875	4.990945	7.958589
1.013797	2.531725	4.694995	4.687332
1.014571	2.548718	4.732308	5.17731
0.9716306	2.103575	4.601326	5.145464
0.901384	1.584791	2.914891	3.495752
0.965476	1.665377	2.92851	3.216261
1.133139	1.769764	2.734036	3.596031

Figure S1C

PDL	Laminin	Lam/Myelin	Myelin
1.503185	2.72379	4.464111	4.667819
1.62685	2.733883	4.403545	4.786535
1.447552	2.981279	3.996645	4.880961
1.877129	3.706865	5.070045	5.035917
1.914371	3.578411	4.980132	5.24639
2.172535	3.292302	5.00317	5.336112
2.125754	3.531801	4.490929	4.593424
2.163162	3.650732	4.522177	4.631714
2.089062	3.659744	4.541465	4.828947

Figure S1D

PDL	Laminin	Lam/Myelin	Myelin
0.968717	2.226842	2.703598	2.784066
0.997264	2.072961	2.567502	2.917486
1.03402	2.289064	2.376214	2.813211
0.980925	2.274325	3.13499	2.899399
1.022243	2.391152	3.19475	3.198653
0.996832	2.352504	3.174733	3.15986
0.943506	2.076361	2.819913	3.10993
0.970897	2.211519	2.836121	2.852723
1.085597	2.345921	2.692174	3.148058

Figure S2A

PDL	Laminin	Lam/Myelin	Myelin
1.022281	2.171786	3.252658	3.837268
0.9419227	2.6056	3.207879	4.430448
1.035796	2.235878	1.618051	2.247527
1.027782	2.053363	3.725694	3.578325
1.009762	1.834936	3.454604	4.288897
0.9624555	1.919756	3.416583	3.878588
0.9403325	1.613241	1.911724	2.339585
1.011017	1.646502	2.649237	2.999243
1.04865	1.701562	2.632786	3.580889

Figure S2B

PDL	Laminin	Lam/Myelin	Myelin
1.291745	3.407408	7.61128	9.22094
0.785278	5.131862	8.130276	11.88669
0.922965	3.693468	2.706113	4.847388
1.046447	3.329358	8.183002	7.693183
1.028441	2.736833	7.334257	10.18953
0.925108	2.966447	6.603623	8.675414
0.766294	1.905429	2.349286	2.974168
1.33081	2.065459	4.360406	4.388721
0.902889	1.736307	3.931937	4.545713

1.039438	2.811903	3.705822	5.111195
1.048731	2.072876	4.914915	4.820434
0.9118304	2.10251	3.898753	2.501643
0.9161535	1.475095	3.146789	3.516952
1.030493	1.636621	3.271878	3.703213
1.053353	1.628759	3.174637	3.960066
1.030644			3.225423
0.9397036			3.213254
1.029652			3.732923
1.02762	1.594245	3.466608	3.505965
0.9723803	1.468289	3.735374	3.974663

Figure S2C

PDL	Laminin	Lam/Myelin	Myelin
1.030357	2.127674	3.020882	3.643303
0.962145	2.648937	3.089812	4.161821
1.007497	2.162693	1.753581	2.314597
1.028513	2.041152	3.659352	3.673543
1.005079	1.810385	3.4715	4.189525
0.966407	1.901218	3.377709	3.743196
0.981753	1.597459	1.987859	2.409643
1.013891	1.588638	2.469688	2.751496
1.004356	1.596018	2.452187	3.33983

1.007616	1.494366	3.301108	3.439279
0.9923841	1.474494	3.74481	3.547771
1.00045	1.377546	3.63923	3.503495
0.9995495	1.625126	3.411883	3.535678

Figure S2D

PDL	Laminin	Lam/Myelin	Myelin
0.839159	0.8885576	0.935184	1.00452
0.792792	0.9529179	0.88395	1.10002
1.368048	1.47329	1.654501	1.50415
1.014303	1.092331	1.226685	1.115212
0.992839	1.081372	1.118964	1.287987
0.992858	1.095168	1.158197	1.272972
0.966551	1.00353	0.868511	0.997745
0.998618	0.9832631	1.095006	1.153555
1.034831	1.06801	1.087719	1.336714

Figure S3

3div map2 growth (PDL)	3div MAP2 growth (myelin)	3div tau1 growth (PDL)	3div tau1 growth (myelin)	6div map2 growth (PDL)	6div MAP2 growth (myelin)	6div tau1 growth (PDL)	6div tau1 growth (myelin)
0.989969737	1.644371379	0.941470072	4.220923003	0.978846935	1.733180137	0.992810379	3.217336881
1.004221172	1.834989318	1.034257547	3.218849347	1.033590464	1.819612707	0.982711321	3.331300809
1.005809091	1.804107287	1.02427238	3.497064824	0.987562601	1.847605139	1.0244783	3.020434773

Figure S4B

% neuronal BrdU of DAPI 1DIV	% neuronal BrdU of DAPI 3DIV	% neuronal BrdU of DAPI 5DIV
1.972872513	1.323043005	1.388888333
1.223582197	3.953147854	4.702413369
1.130653272	3.448275862	2.496329515

Figure S5A

increase in neurite length [%] CAS1049738	increase in neurite length [%] DMSO	increase in neurite length [%] PD98059
73.82872844	103.6300807	87.28094053
73.72911881	98.66832767	88.02618711
75.06386814	97.13605546	84.67144505
88.39839405	100.5655362	83.25872637