Supplementary Figures

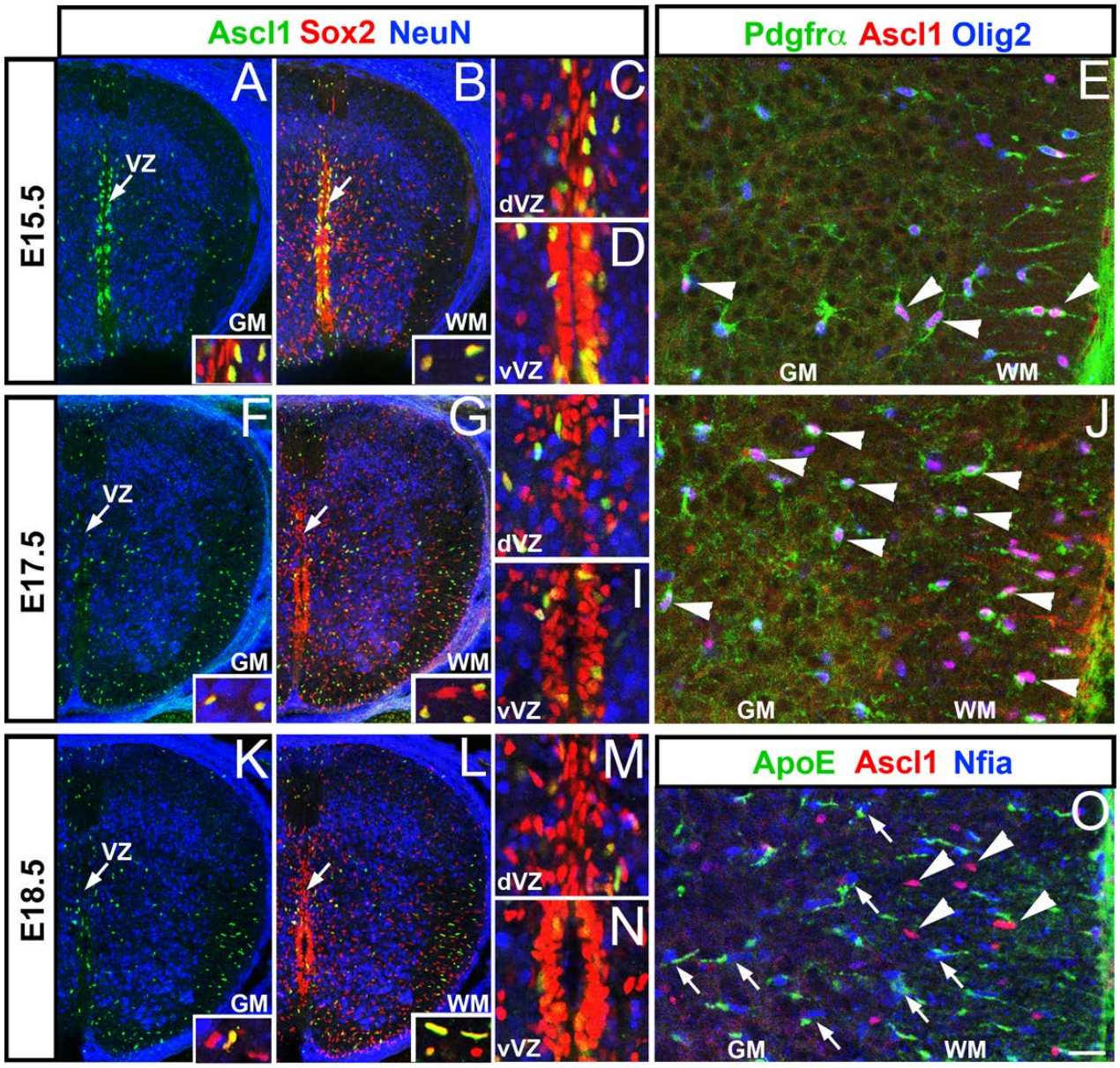


Figure S1. Temporal expression of Ascl1 during gliogenesis in the spinal cord
Immunofluorescence for Ascl1 and glial cell markers on E15.5, E17.5, and E18.5 thoracic spinal cord sections. **(A-E)** At E15.5, Ascl1 is expressed in a single cell layer lining the lateral edge of the VZ (A-D), as well as in Sox2+ (insets, A,B) and Olig2+;Pdgfrα+ OPCs (arrowheads, E) in the GM and WM. **(F-O)** By E17.5 and E18.5, Ascl1 is expressed in very few cells in the VZ (H,I,M,N) and in Sox2+ (insets, F,G,K,L) and Olig2+;Pdgfrα+ OPCs (arrowheads, J) in the GM and WM, but it is not express in Nfia+;ApoE+ IAPs (arrows, O). Note that Ascl1+;Nf1a+ is ApoE- (arrowheads, O). Scale bar is 100μm for A,B,F,G,K,L, and 25μm for all others.

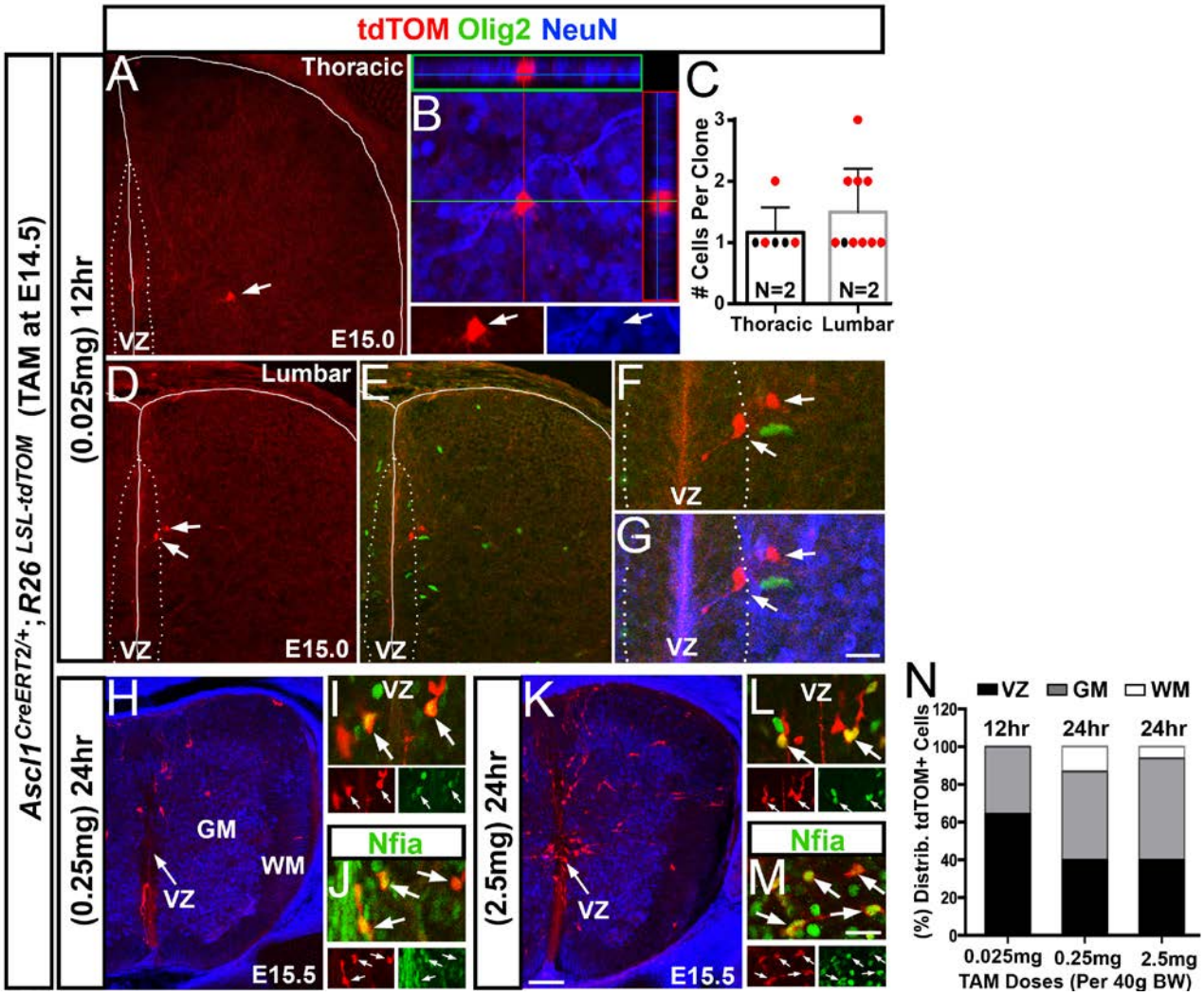


Figure S2. Low dose tamoxifen administration at E14.5 sparsely labels Ascl1-lineage glial cells in the spinal cord

Immunofluorescence on spinal cord sections of *Ascl1*$^{CreERT2/+}$;*R26*$^{LSL\text{-}tdTOM}$ mice treated with varying doses (0.025-2.5mg/40g body weight) of tamoxifen at E14.5. tdTOM expression is detected using anti-dsRed.**(A,B,D-G)** 12 hours post low dose tamoxifen administration shows that one or two cells were sparsely labeled in or near the VZ (arrows, D-G), or in the GM (arrows, A,B), and they are NeuN-;Olig2-. **(C)** Quantification of the number (mean±SD) of cells per clone found in the thoracic and lumbar region of the spinal cord. Red dots represent clones that were found in or near the VZ (as in D-F) and black dots are clones found in GM (A,B). **(H-M)** 24 hours post intermediate and high dose tamoxifen shows that all tdTOM+ cells are Nfia+ (arrows, J,M), but some in the VZ and GM are also Olig2+ (arrows, I,K). Scale bar is 50µm for A,D,E,I,K; 17µm for B,F,G; 100µm for H,K; and 25µm for insets in I,J,L and M. **(N)** Quantification of the percentage distribution of tdTOM+ cells in or adjacent to the VZ, in the GM, and WM from low to high tamoxifen doses.

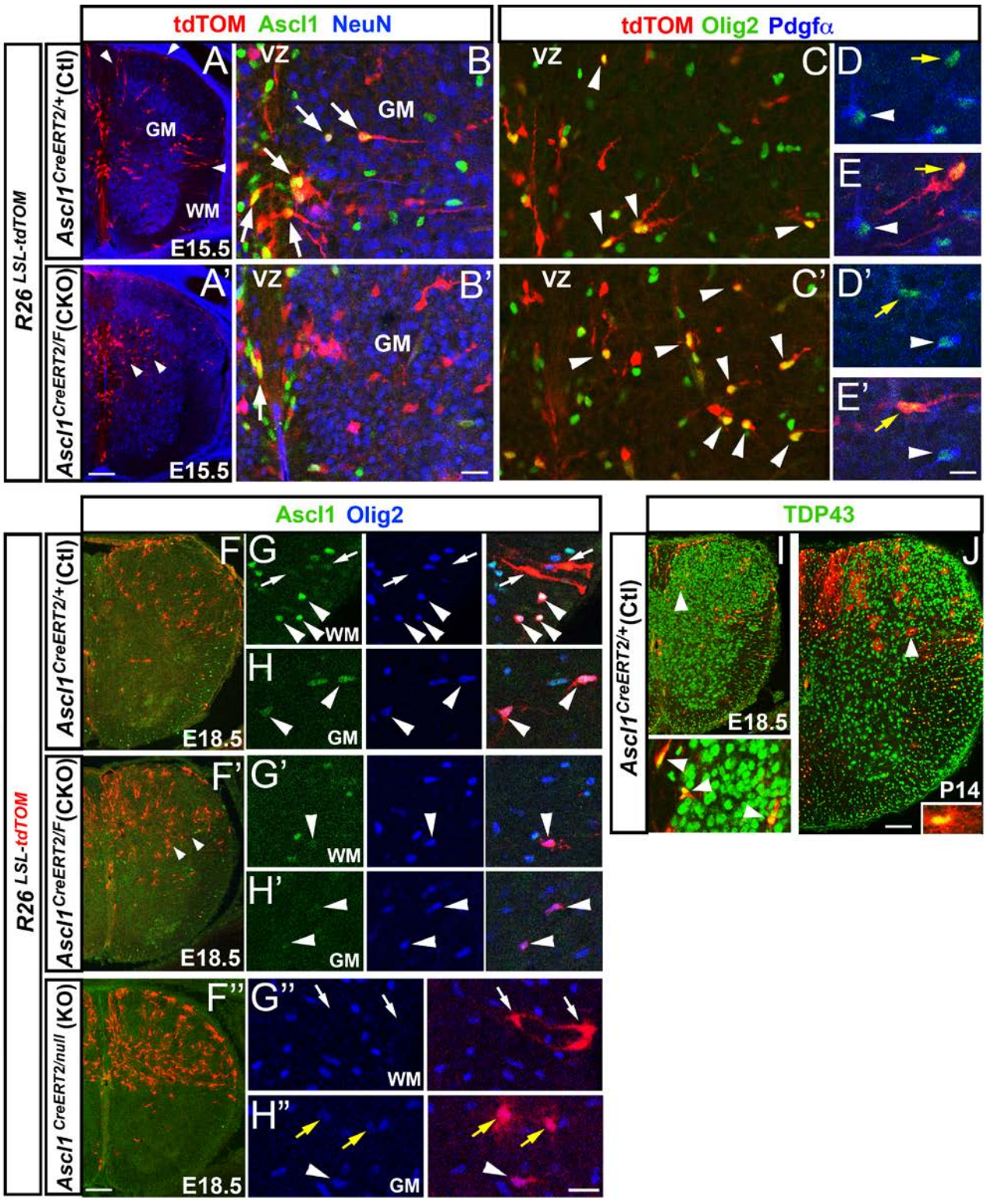
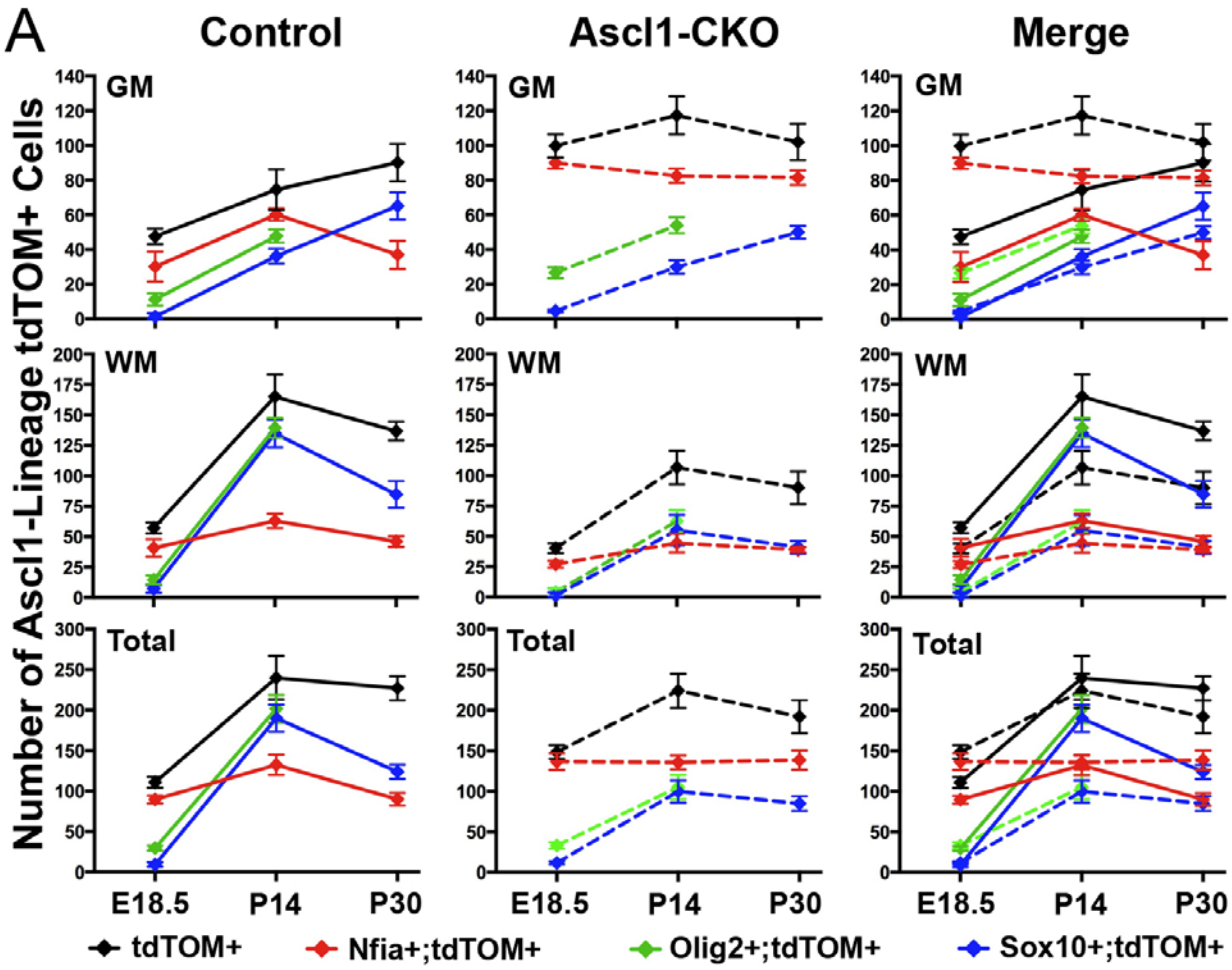


Figure S3. *Ascl1*^{CreERT2} efficiently deletes the *Ascl1*^{FL} allele during gliogenesis in the spinal cord.

Immunofluorescence on E15.5 (A-E,A'-E'), E18.5 (F-I, F'-H', F"-H") and P14 (J) thoracic spinal cord sections of *Ascl1*$^{CreERT2/+}$;*R26*$^{LSL\text{-}tdTOM}$ control, *Ascl1*$^{CreERT2/FL}$;*R26*$^{LSL\text{-}tdTOM}$ CKO, or *Ascl1*$^{CreERT2/null}$;*R26*$^{LSL\text{-}tdTOM}$ KO mice treated with tamoxifen at E14.5. **(A-E)** E15.5 control spinal

cord showing tdTOM+ (via anti-dsRed) cells in the VZ, GM, and WM (arrowheads, A). Some tdTOM+ cells in the VZ and GM continue to express Ascl1 (arrows, B) and Olig2 but are negative for Pdgfrα (yellow arrow, D,E), whereas surrounding Olig2+ (tdTOM-) cells are Pdgfrα+ (arrowheads, D,E). **(A'-E')** E15.5 *Ascl1* CKO spinal cord showing tdTOM+ cells in the VZ and medial GM (arrowheads, A'), the majority of which no longer express Ascl1, indicating a successful deletion of the *Ascl1*^{Fl} allele. The number of tdTOM+ cells that are Olig2+; Pdgfrα- are also increased in the GM (arrowheads C and yellow arrows D,E).**(F-H)** E18.5 control spinal cords showing Ascl1 continues to be expressed in Olig2+;tdTOM+ cells in the GM and WM (arrowheads, F,H) **(F'-H')** Ascl1 expression is no longer detected in Olig2+;tdTOM+ cells in the GM and WM of *Ascl1* CKO (arrowheads, F',G'). **(F"-H")** E18.5 *Ascl1* KO spinal cord showing the presence of Olig2+;tdTOM+ cells with astrocyte morphology in the GM (yellow arrows, H") but no Olig2+;tdTOM+ cells in the WM (F",G"). **(I-J)** Co-localization of TDP43 (a pan nuclear marker) and tdTOM was used for quantifying the total number of tdTOM+ labeled cells (insets). Scale bar is 100µm for A,A',F'-F"',I,J; 25µm for B,B',C,C',G-G",H-H", and 16.6µm for D,D',E,E', and insets in I,J.



B

(%) Distrib. Ascl1-Lineage tdTOM+ Cells

		% tdTOM+		% Sox10+;tdTOM+		% Nfia+;tdTOM+	
		Ctl	CKO	Ctl	CKO	Ctl	CKO
P14	GM	33 ± 5	**54 ± 6** *	14 ± 4	13 ± 5	22 ± 4	**35 ± 5** *
	WM	67 ± 5	**46 ± 6** *	52 ± 11	**24 ± 9** *	25 ± 6	19 ± 6
P30	GM	40 ± 6	52 ± 6	20 ± 7	18 ± 6	23 ± 4	**46 ± 11** *
	WM	60 ± 6	48 ± 6	39 ± 7	**20 ± 9** *	20 ± 5	19 ± 5

$$\text{(\%) average} \pm \text{SD} = \left(\frac{\text{\# marker+;tdTOM+ Cells}}{\text{Total tdTOM+ Cells}} \right) \quad *p<0.05$$

Figure S4. Number and ratio of Ascl1-lineage glial cells in the GM and WM of control and *Ascl1*-CKO spinal cords

Number **(A)** and distribution **(B)** of total tdTOM+ cells for astrocyte (Nfia+;tdTOM+) and oligodendrocyte (Olig2+;tdTOM+ and Sox10+;tdTOM+) lineage cells in the GM and WM of thoracic spinal cord of control and *Ascl1* CKO at E18.5, P14, and P30. Statistical analysis was performed using Student's *t*-test. Error bars are mean±SD.

Table S1. Source and dilution of antibodies

Primary Antibodies	Source & Catalogue Number	Dilution
Chicken Anti-GFP	Chemicon, AB16901	1:500
Goat Anti-Adolase C	Santa Cruz Biotechnology, (D-14):SC-12066	1:100
Goat Anti-Apolipoprotein E	Chemicon, AB947	1:2,000
Goat Anti-Sox10	R&D Systems, AF2864	1:20
Guinea Pig Anti-Ascl1	Kim et al., 2008, TX518	1:10,000
Guinea Pig Anti-Olig2	Wichterle et al., 2002	1:5,000
Mouse Anti-CC1 (or APC)	Calbiochem, OP80	1:100
Mouse Anti-GFAP	Sigma, G3893	1:500
Mouse Anti-NeuN	Chemicon, MAB377	1:1,000
Rabbit Anti-dsRed	Clontech, 632496	1:500
Rabbit Anti-NFIA	Dr. B. Deneen, Baylor College of Medicine	1:5,000
Rabbit Anti-Olig2	Millipore, AB9610	1:1,000
Rabbit Anti-RFP Booster	Chromotek, Atto594ts	1:500
Rabbit Anti-Sox2	Millipore, AB5603	1:1,000
Rabbit Anti-TDP43	Dr. G. Yu, UTSW Medical Center	1:5,000
Rat Anti-PDGFRa (APA5)	BD Pharmigen, 558774	1:100