

Figure S1. Expression patterns for Tgfb β and Activin ligands and Smad3 in embryonic mouse spinal cord. Spinal cord sections from C57BL/6 embryos, immunolabeled for Smad3 and Tgfb β and Activin ligands, and/or neuronal or oligodendrocyte lineage markers, at E12.5 (A-D) and E14.5 (E,F). (A) At E12.5, Smad3 protein was detected throughout the periventricular neuroepithelium, and included robust expression by Olig2⁺ OLP in the pMN domain (white arrow, shown inset at higher magnification), the floor plate (arrowhead), and neurons dorsolaterally (magenta arrow). The latter population corresponded to D11/D12 neurons which are Lim-1⁺, and which are similarly indicated by a magenta arrow in an adjacent section shown in panel (B). ‘V’ labels the ventricle. See also Figs.1A-C. (C,D) Distribution of mRNA for the ActB subunit *Inhbb* in E12.5 spinal cord. *Inhbb* localized ventrolaterally within the cord, to two distinct zones which displayed differential signal intensity (1,2, arrowed) (C). Both zones lay within the Mnr⁺ motoneuron domain, labeled in an adjacent section and similarly arrowed in (D). See also Fig.1H. (E,F) Expression patterns of Tgfb β 2 and 3 in E14.5 spinal cord. (E) By E14.5, Tgfb β 2 expression expanded beyond the periventricular neuroepithelium and dorsolateral neurons, to include more central areas of the spinal cord parenchyma. A representative area of central parenchyma is outlined, and shown below at higher magnification. In contrast, Tgfb β 3 remained largely confined to the ventral periventricular zone, and the floorplate (arrowhead), with additional weak signal localizing to the ventrolateral motor neuron domain (arrowed). See also Figs.1F,G. Data shown are representative of samples from four individuals in each case. Scalebars, (A-D) 50 μ m, (E,F) 100 μ m.

Figure S2. Distribution of ligand-binding Tgfb β and Activin receptors in postnatal mouse spinal cord. Distributions of Tgfb β 2 (A-C) and ActrIIB (D-F) in P5 C57BL/6 spinal cord. Data complement findings in Figure 5. (A,D) illustrate whole spinal cord sections co-stained with Olig2, with representative areas of immunoreactivity in white matter shown inset. (B,E) show co-staining with Pdgfra (OLP) or CC-1 (mature OL) at higher magnification. (C,F) show co-staining with NeuN. Indicating that oligodendrocyte lineage cells remained sensitive to both Tgfb β and Activin ligands in the postnatal period, at P5 both Tgfb β 2 and ActrIIB were widely distributed throughout the spinal cord, localizing to populations including Pdgfra⁺ OLP and mature CC-1⁺ OL, as well as to other lineages including NeuN⁺ neurons. Data are representative of samples from four individuals. Scalebars, (A,D) 100 μ m, (B,C,E,F) 10 μ m

Figure S3. Tgfb β 1, ActB and co-treatment of Oli-Neu cells elicit distinct patterns of canonical Smad-dependent and non-canonical MAP kinase signaling. (A) Primary rat OLP cultures grown in medium favoring proliferation and non-permissive for differentiation were fixed, immunolabeled for Tgfb β superfamily ligand-binding receptors and Olig2, and imaged by confocal microscopy. Cells were ubiquitously positive for Tgfb β 2, ActrIIB and Bmpr2. (B-J) Immunoblotting (B) and densitometric data (C-J) from Oli-Neu cells plated into serum-free media and exposed to 50ng/ml Tgfb β 1 and/or ActB for 15, 30 or 60min. Findings complement data from primary OLP in Figs.3A and 3B. (B,C) Tgfb β 1 or ActB alone each induced Smad3 phosphorylation at Ser423/425, although the effect of Tgfb β 1 was lost by 60min. However, the effect of ActB was stronger than the equivalent Tgfb β 1 concentration at all three timepoints, and this difference became more pronounced over time. In contrast, Tgfb β 1 activation of p42/44 MAP kinase (P-Thr202/Tyr204) was seen at 15min, whereas ActB treatment persistently reduced p42/44 phosphorylation (B,D). Notably, co-treatment with Tgfb β 1 plus ActB together produced a third distinct pattern, which combined increased levels of P-Smad3 (Ser423/425) similar to or beyond those induced by ActB alone, with increased phosphorylation of p42/44 MAP kinase beyond that induced by Tgfb β 1 alone at later timepoints (B-D). No significant changes were seen in levels of total Smad3 or p42/44 proteins (B,E,F). Neither ligand alone or in combination impacted activity of the Akt-Gsk3 signaling pathway, as measured by changes in levels of phosphorylated Akt (P-Thr308) or Gsk3 α/β (P-Ser21/9) (B,G-I). Smad3 phosphorylation within its linker region at Ser208 resulting from non-canonical pathway activation has been shown to alter its transcriptional activity, but no changes in Smad3 (P-Ser208) were detected in Tgfb β 1- or ActB-treated cultures (B,J). (K,L) Oli-Neu cultures plated into serum-free media were exposed to 50ng/ml Tgfb β 1, ActB or vehicle for 30min, then were subjected to co-immunoprecipitation using anti-Smad3 antibody or IgG control. HEK cells were used for comparison of cell type specificity. Immunoprecipitates and lysates were then subjected to immunoblotting and densitometric analysis. Blots of immunoprecipitates were probed for potential Smad3-interacting coactivators, and lysate blots were probed for actin (loading control). In Oli-Neu cells (but not HEK cells), Smad3 was found to associate with FoxH1/FAST (K), but not with other potential Smad-interacting factors, including ETF, Sp-1, Gli, TCF, or FoxO1. No differences in Smad3 binding to FoxH1/FAST were observed following ActB or Tgfb β 1 treatment (K,L). Data are representative of findings from 3 independent experiments in separate cultures. Scalebars, (A), 10 μ m.

Figure S4. Tgfb β 1, ActB and co-treatment of primary OLP elicit differential functional outcomes. (A-D) Immunoblotting (A,C) and densitometric data (B,D) from primary OLP plated into serum-free media and exposed to 0-50ng/ml ActB and/or Bmp4 for 30min. Findings complement Tgfb β 1, ActB and Bmp4 signaling data from primary OLP in Figs.3A and 3B, and from Oli-Neu cells in Figure S3. In OLP cultures, Bmp4 dose-dependently induced phosphorylation of Smads1/5 (P-Ser463/465) (A,B), whereas ActB induced Smad3 (P-Ser423/425) phosphorylation (C,D). Notably, while ActB or Tgfb β 1 did not alter Bmp4 Smad1/5 activation (A,B), Bmp4 dose-dependently abrogated ActB-induced Smad3 phosphorylation (C,D). (E-G) Morphometric analysis of primary OLP cultures grown in serum-free media in the presence of 50ng/ml Tgfb β 1, ActB, both or vehicle control for up to 5d. Cultures were harvested at 12h intervals and stained for Olig2 (lineage marker), Mbp (maturation), cleaved caspase-3 (apoptosis) and BrdU labeling following a 12h pulse (proliferation). Data shown complement findings in Figs.3E-J, in which results are expressed as raw cell counts. Here, the same data are expressed relative to total Olig2⁺ cell number. In controls, numbers of postmitotic mature Mbp⁺ cells progressively increased, proliferating progenitors decreased, and apoptosis gradually increased (E-G). Tgfb β 1 or ActB both significantly reduced the fraction (and total number) of apoptotic cells (E). Tgfb β 1 also increased the fraction and number of proliferating BrdU⁺Olig2⁺ cells (F), whereas ActB increased the fraction and number of mature Mbp⁺ oligodendrocytes (G). Co-treatment reduced apoptotic cell number more strongly than either ligand alone (E), and increased the fraction of BrdU⁺Olig2⁺ cells similar to Tgfb β 1 in isolation (F). Importantly, in cultures exposed to Tgfb β 1+ActB together, the fraction of Mbp⁺ cells was similar to that in ActB-treated cultures (G), but the overall Olig2⁺ population was much larger (see Fig.3H), thus the total number of Mbp⁺ cells was doubled in co-treated versus ActB-treated cultures (see Fig.3J). Data are representative of findings from at least 3 independent experiments in separate cultures. Statistics, (E-G) two-way ANOVA plus Bonferroni post test, *P<0.05, **P<0.01, ***P<0.001. Significant values shown are for co-treated versus control cultures.

Figure S5. Bmp4 treatment of primary OLP cultures restricts maturation. Morphometric analysis of primary OLP cultures grown in serum-free media in the presence of 50ng/ml Bmp4 or vehicle control for up to 5d. Cultures were harvested at 12h intervals and stained for Olig2, Mbp, cleaved caspase-3, and/or BrdU following a 12h pulse. Data complement results in Figs.3C and 3D. In vehicle controls, numbers of mature Mbp⁺ cells progressively increased, proliferating progenitors decreased, and apoptosis gradually increased. Compatible with previous reports (Miller et al., 2004; See et al., 2004), Bmp profoundly reduced the accumulation of Mbp⁺ mature oligodendrocytes in treated cultures (A,B), but had minimal effects on apoptosis (C) or proliferation (D). Data are representative of findings from 3 independent experiments in separate cultures. Statistics, two-way ANOVA plus Bonferroni post-test, *P<0.05, **P<0.01, ***P<0.001.

Figure S6. Tgfb1 and ActB promote neural progenitor viability *in vitro*. Adherent cultures of neural progenitors were grown in media favoring either proliferation (Egf) or specification and differentiation (growth factor-free), in the presence of 50ng/ml Tgfb1, ActB or vehicle. Cells positive for Nestin, Olig2, cleaved caspase-3, and BrdU labeling were quantified at 12h and 60h in proliferation medium (A,C,E), and at 96h in differentiation medium (B,D,F). ActB and Tgfb1 both reduced the number and fraction of apoptotic cleaved caspase-3⁺ cells under both culture conditions, although findings in proliferation media only reached significance at 60h (A,B). Neither ligand impacted the BrdU⁺ fraction in Egf-containing media (C), and neither was able to rescue proliferation in Egf-free media (D). Neither ligand had detectable effects on oligodendrocyte lineage cell number. No Olig2⁺ cells were observed in proliferation medium under any condition, with the population in these cultures remaining entirely Nestin positive and Olig2 negative (E). Under conditions favoring lineage specification and differentiation, at 96h approximately 30% of cells were Olig2⁺, and this was unchanged by ActB or Tgfb1 treatment (F). Data are representative of findings from 3 independent experiments in separate cultures. Statistics, (A,C,E) two-way ANOVA plus Bonferroni post-test, (B,D,F) Student's t test, *P<0.05, **P<0.01.

Figure S1

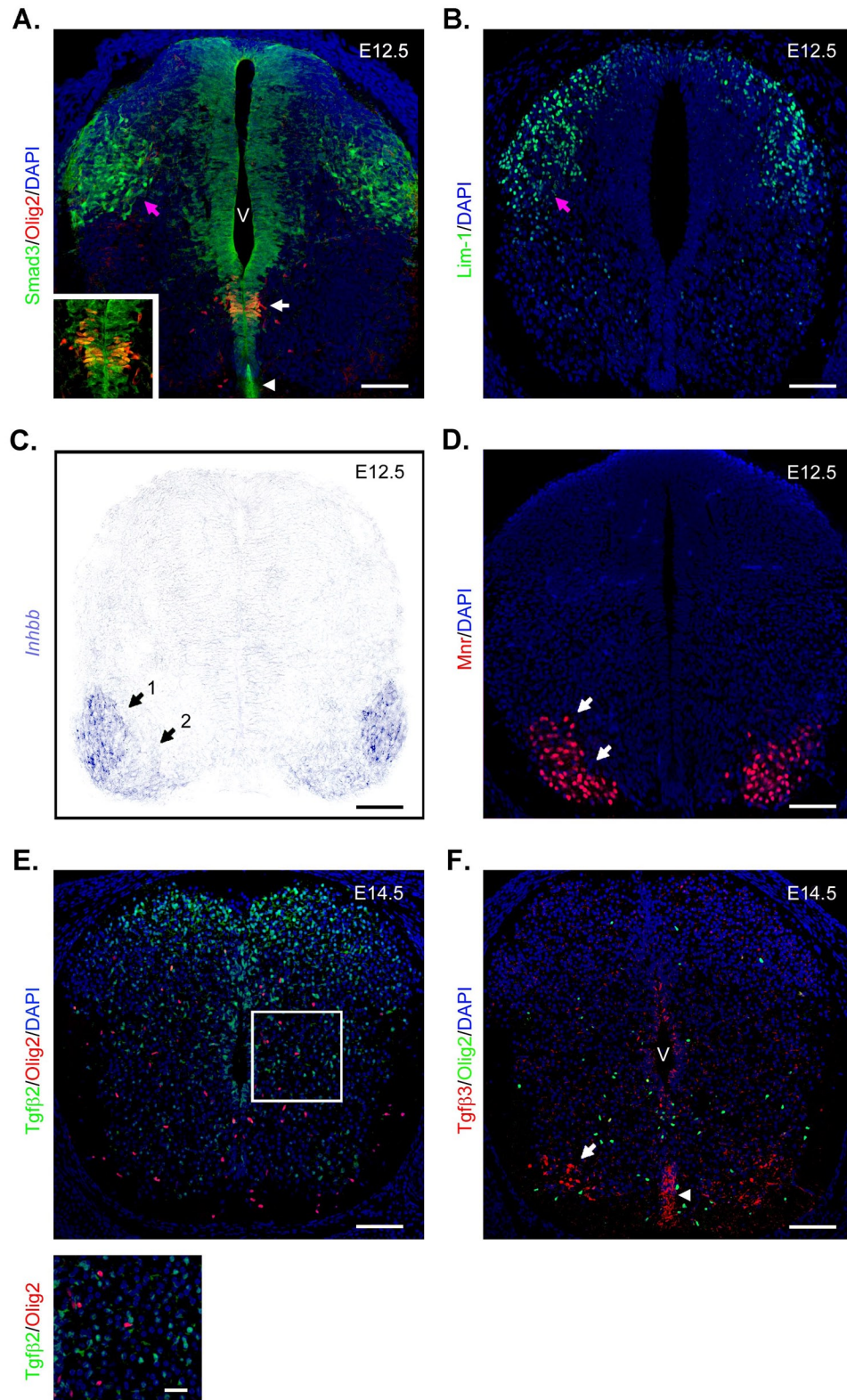


Figure S2

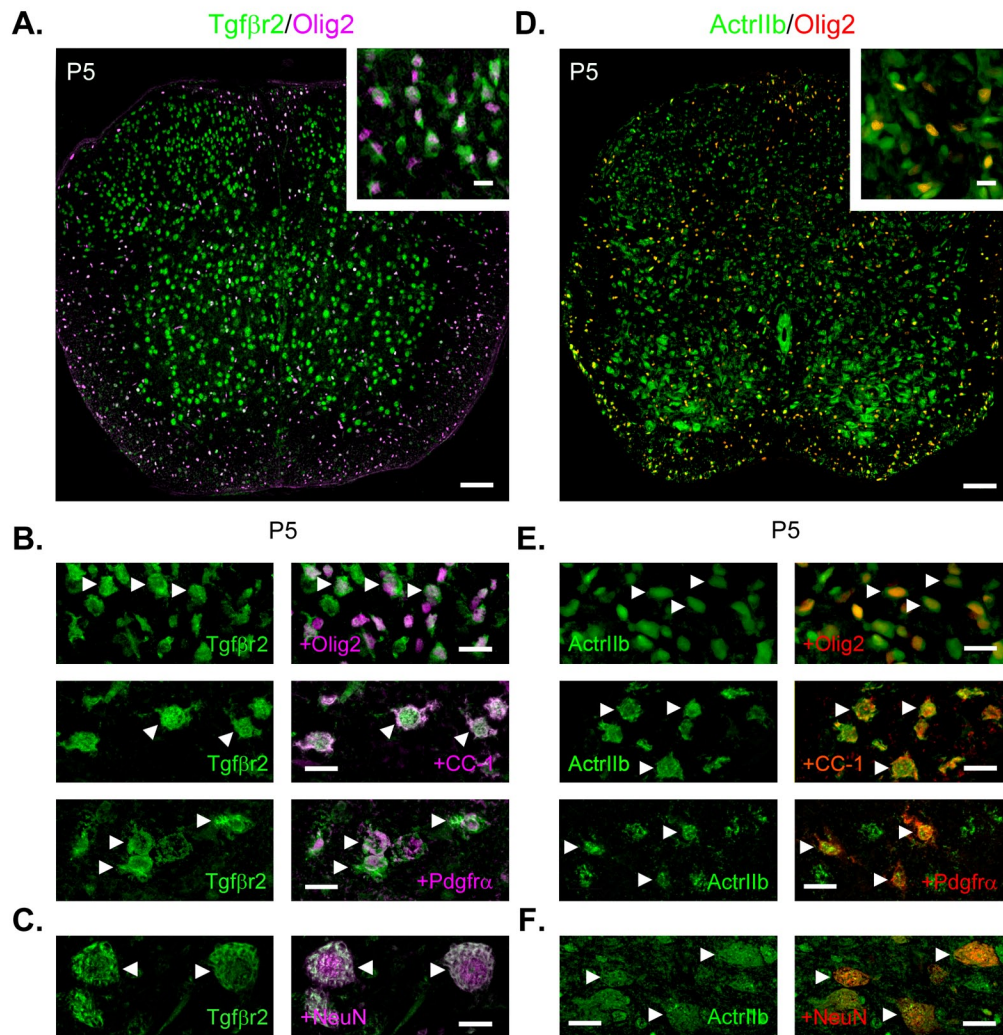


Figure S3

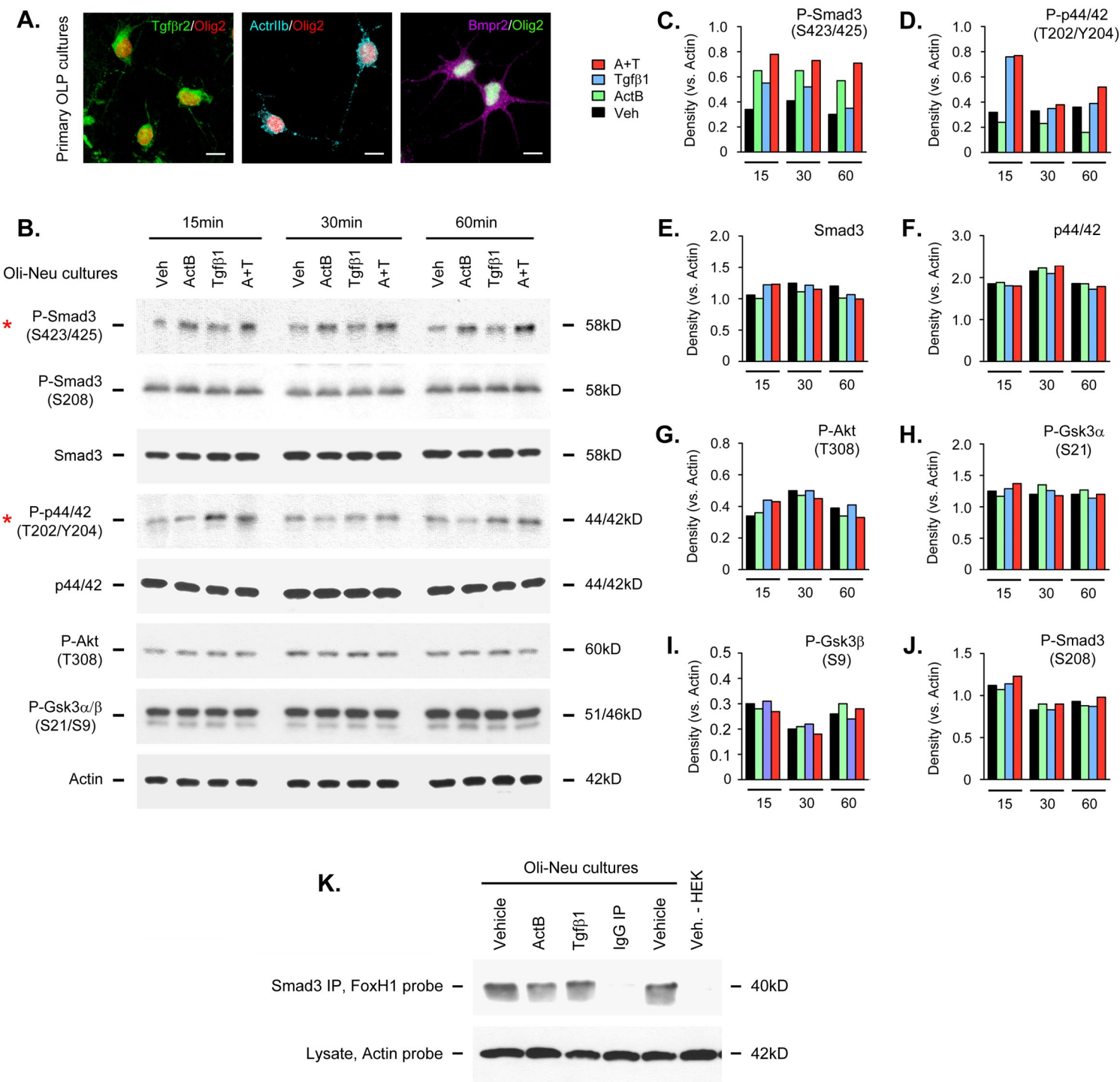


Figure S4

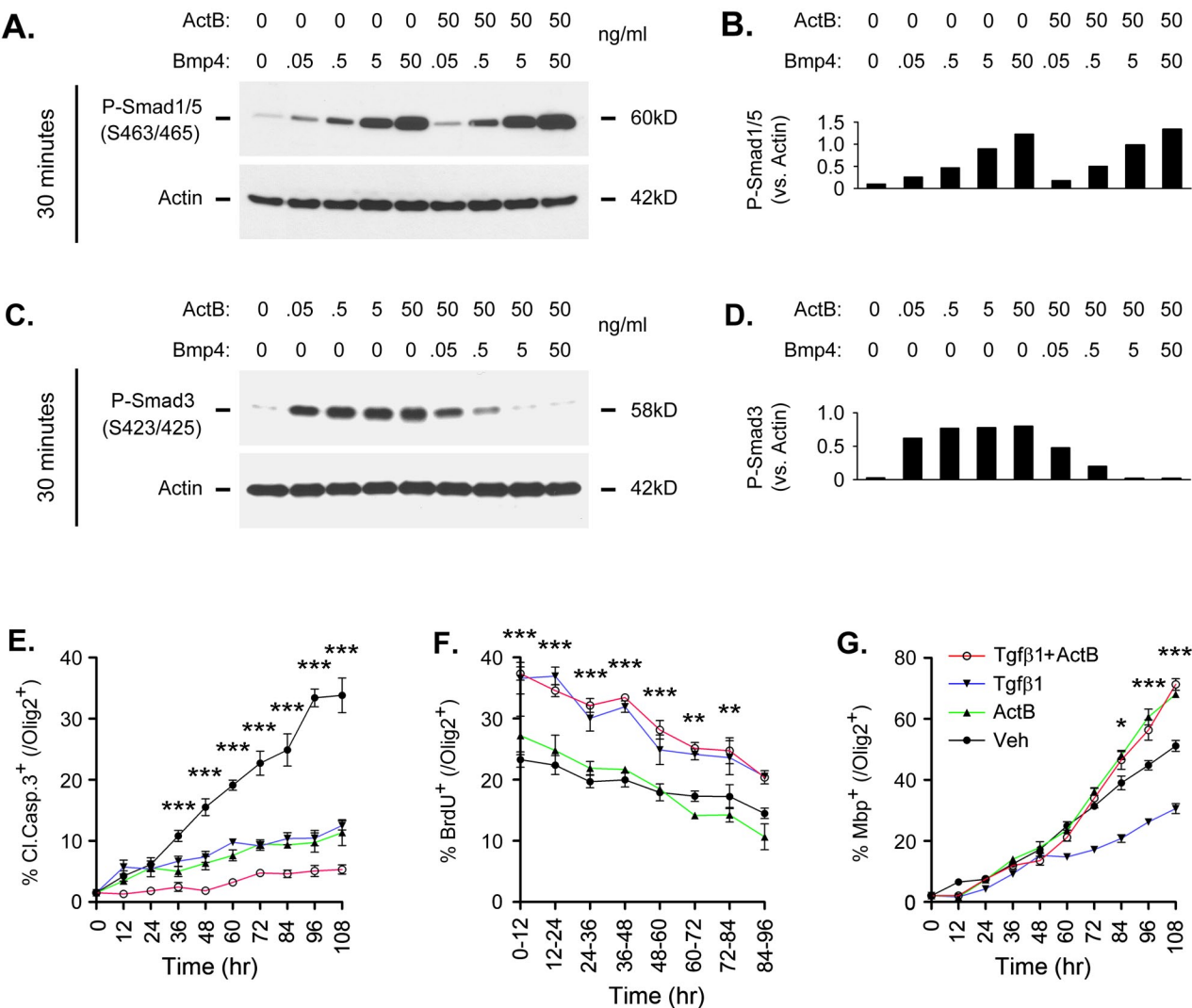


Figure S5

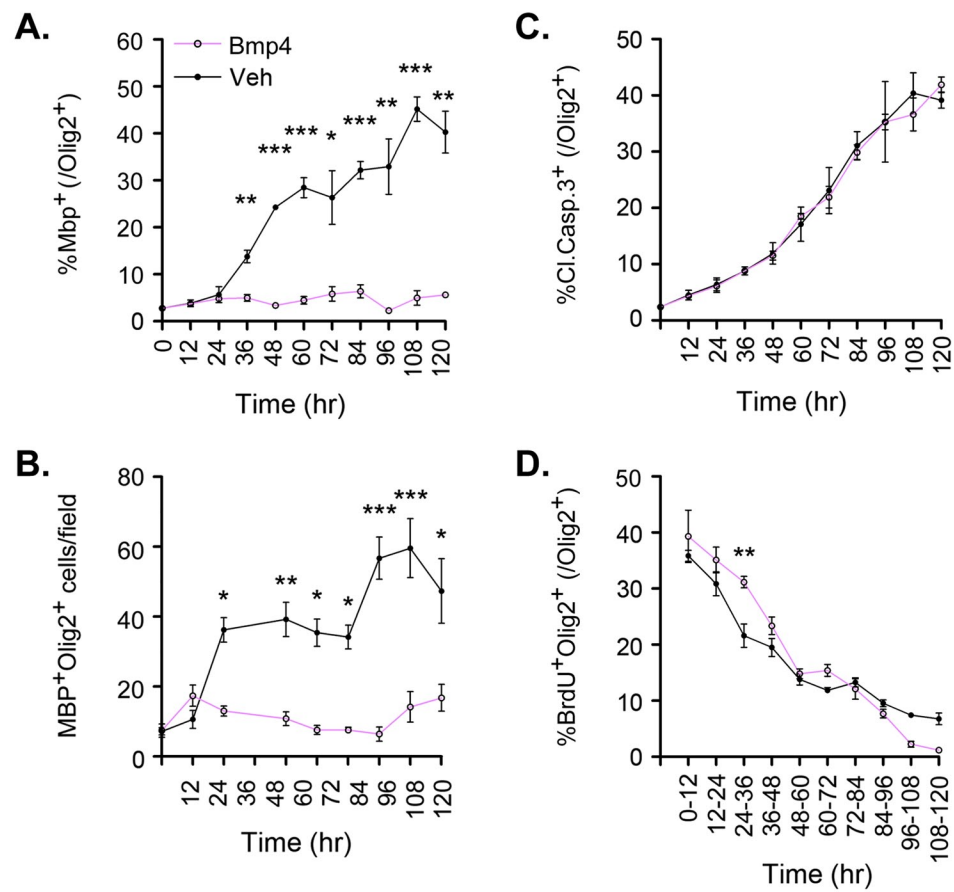


Figure S6

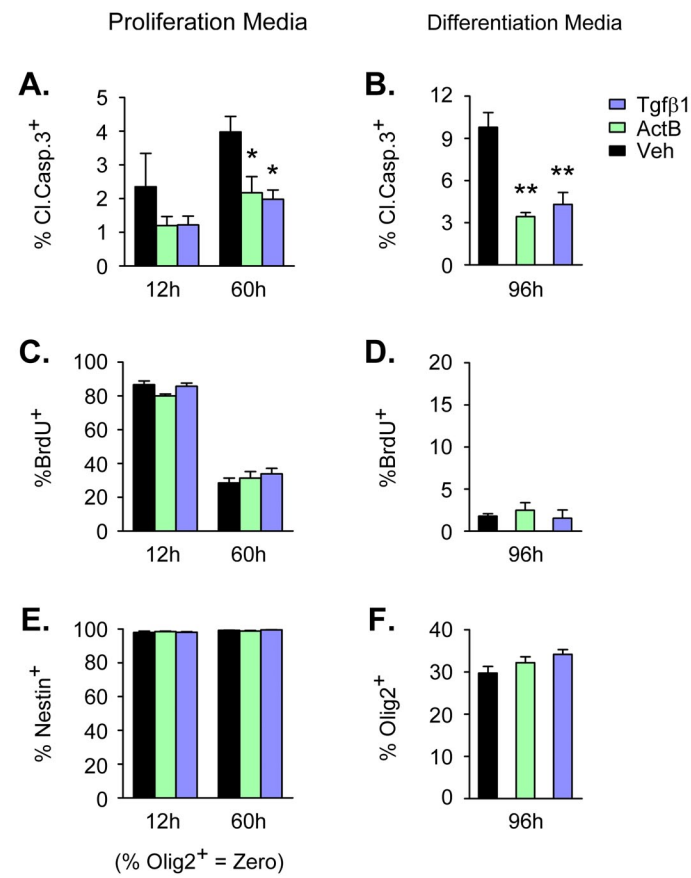


Table S1. Antibodies used at 1:100 dilution.

Antibody	Species	Company	Catalog #
ActrIIa (Acvr2a)	Rabbit	Ab Biotec	251303
ActrIIb (Acvr2b)	Rabbit	Ab Biotec	251300
CC-1 (Apc)	Rabbit	Abcam	Ab15270
Gfap	Rabbit	Abcam	Ab7260
Mbp	Mouse IgG1	Abcam	Ab62631
Cleaved Caspase-3	Rabbit	Cell Signaling	9661
p38 (Mapk14)	Rabbit	Cell Signaling	9212
Phospho-Gsk3 α/β (Ser21/9)	Rabbit	Cell Signaling	9331
Phospho-p38 (Thr180/Tyr182)	Rabbit	Cell Signaling	4511
Phospho-p44/42 (Mapk3/1; Thr202/Tyr204)	Rabbit	Cell Signaling	4370
Phospho-Smad1/5 (Ser463/465)	Rabbit	Cell Signaling	9516
Phospho-Smad2 (Ser465/467)	Rabbit	Cell Signaling	3108
Phospho-Smad3 (Ser423/425)	Rabbit	Cell Signaling	9520
Smad2	Rabbit	Cell Signaling	3122
Smad3	Rabbit	Cell Signaling	9523
Mbp	Rabbit	Dako	A0623
Isl2	Mouse IgG1	DSHB	51.4H9
Mnr (Mnx1)	Mouse IgG1	DSHB	81.5C10
BrdU	Chicken	Immunology Consultants	CBDU-65A
Cnp	Mouse IgG1	Millipore	MAB326
Ki67	Mouse IgG1	Millipore	MAB4190
Lim1 (Lhx1)	Rabbit	Millipore	AB3200
Nestin	Mouse IgG1	Millipore	MAB5326
NeuN (Rbfox3)	Mouse IgG1	Millipore	MAB377
NeuN	Rabbit	Millipore	ABN78
Olig2	Mouse IgG1	Millipore	MABN50
Olig2	Rabbit	Millipore	AB9610
Sox2	Mouse IgG1	Millipore	MAB4423
Sox9	Rabbit	Millipore	AB5535
Inhba	Rabbit	Proteintech	10651-1-AP
Inhbb	Rabbit	Proteintech	17577-1-AP
Mag	Rabbit	Salzer laboratory, NYU	N/A
Beta actin	Mouse IgG1	Santa Cruz Biotechnology	sc-47778
Bmpr2	Goat	Santa Cruz Biotechnology	sc-5683
Foxh1 (FAST)	Rabbit	Santa Cruz Biotechnology	sc-14031
Mouse IgG	Mouse IgG	Santa Cruz Biotechnology	sc-2025
Olig2	Goat	Santa Cruz Biotechnology	sc-19969
Pdgfra	Rabbit	Santa Cruz Biotechnology	sc-338
Rabbit IgG	Rabbit	Santa Cruz Biotechnology	sc-2027
Tgf β 1	Mouse IgG1	Santa Cruz Biotechnology	sc-52893
Tgf β 2	Rabbit	Santa Cruz Biotechnology	sc-90
Tgf β 3	Rabbit	Santa Cruz Biotechnology	sc-82
Tgf β 2	Rabbit	Santa Cruz Biotechnology	sc-400