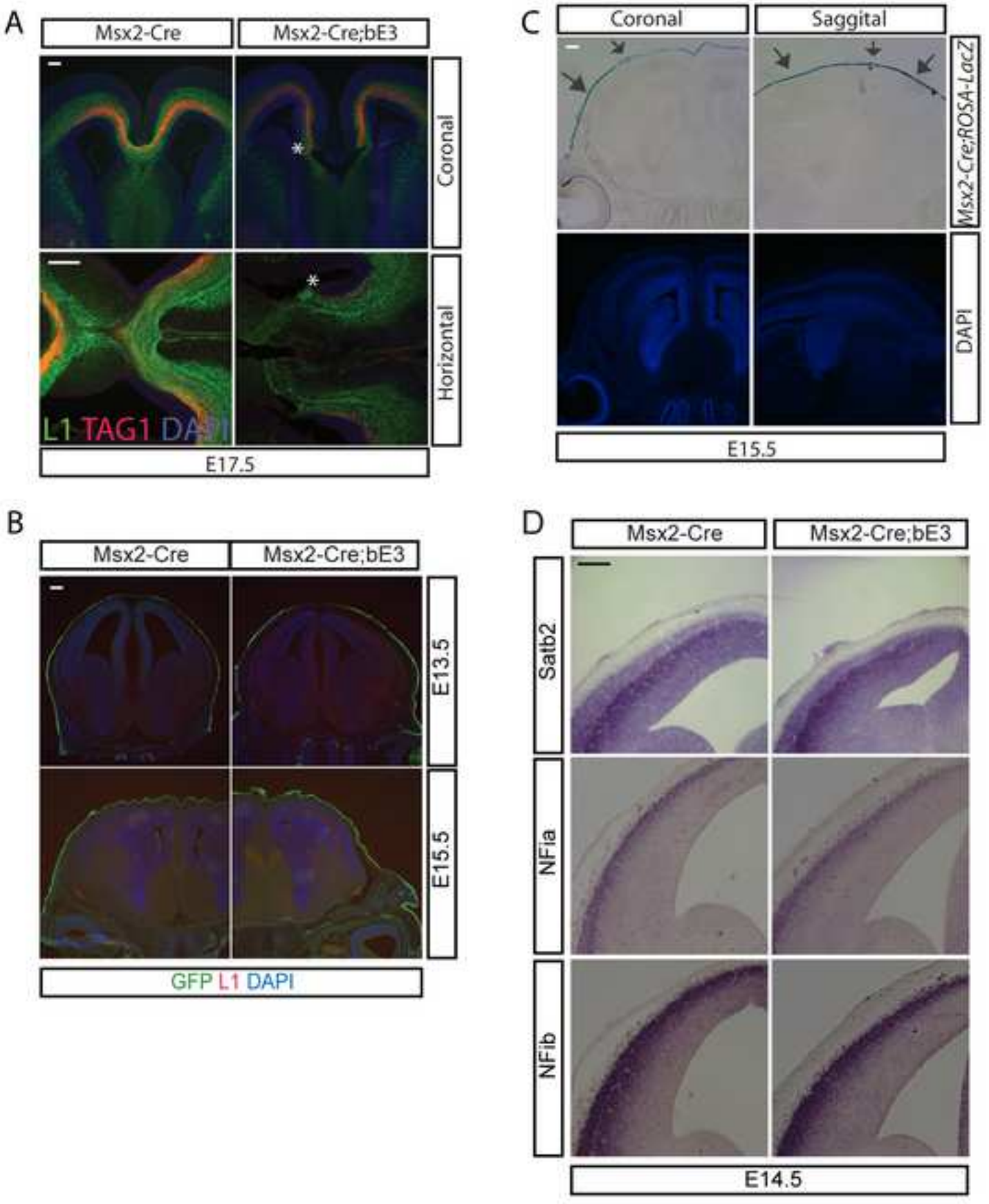
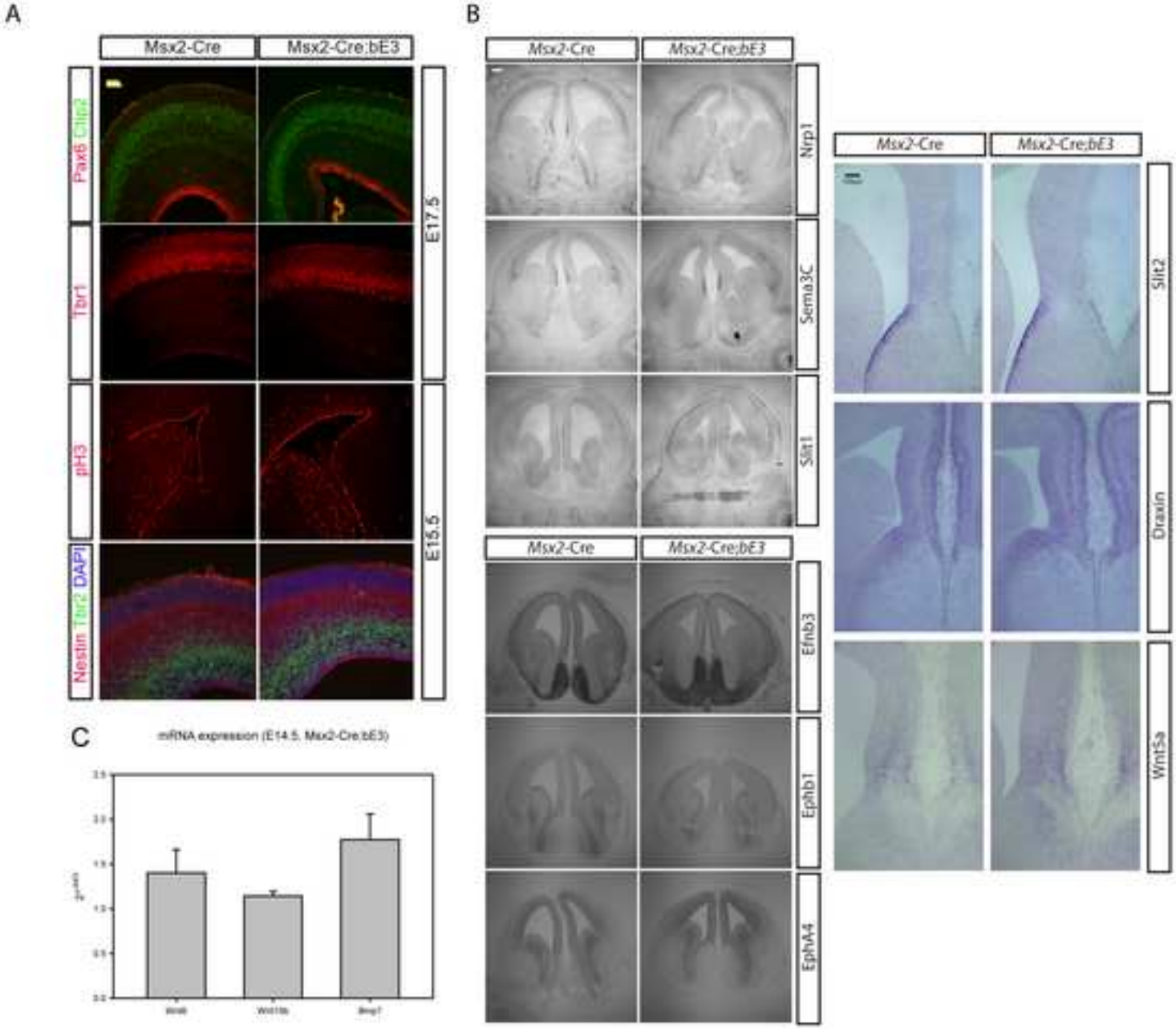
Supplemental Figure 1



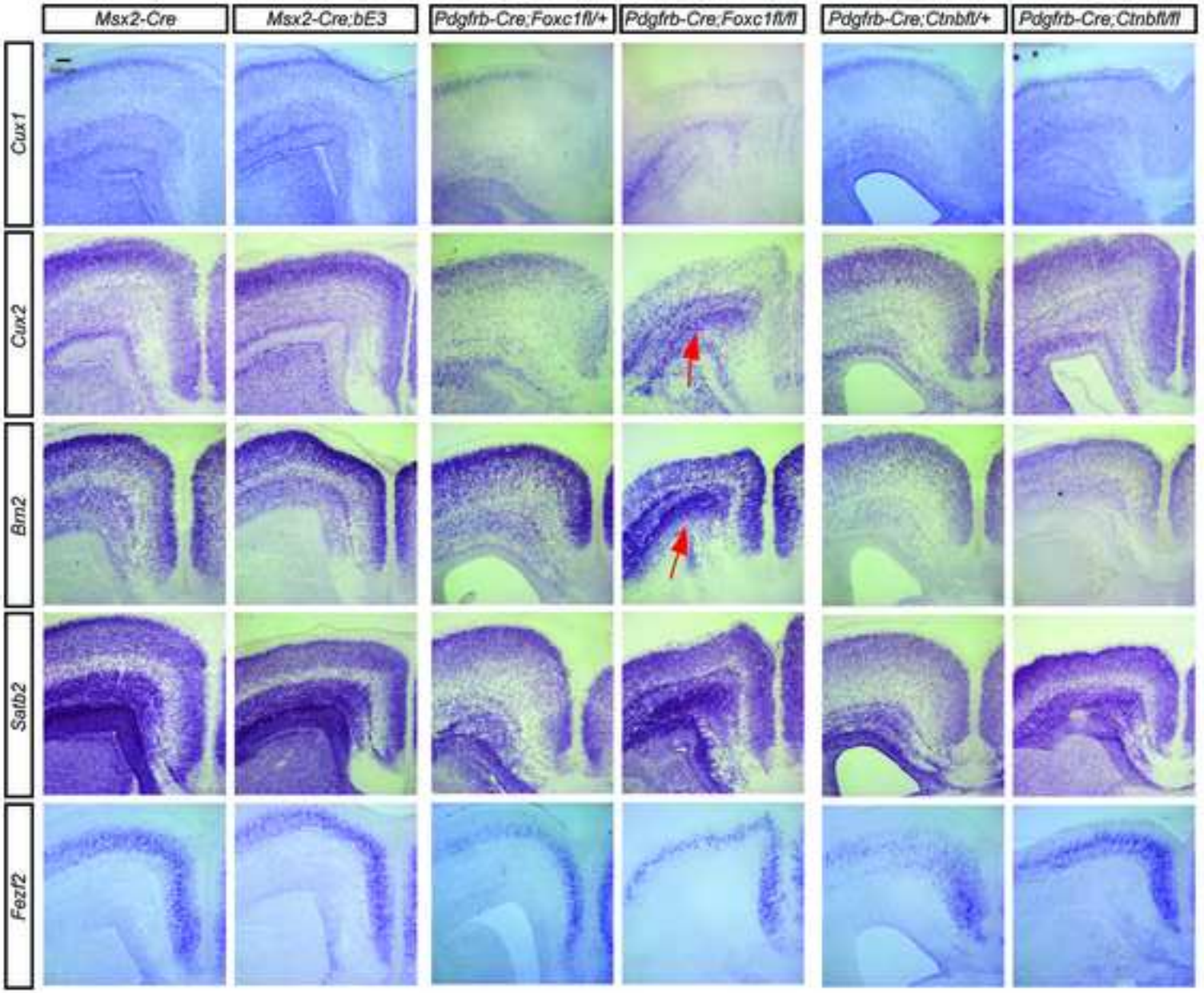
Supplemental Figure 1

Supplemental Figure 2

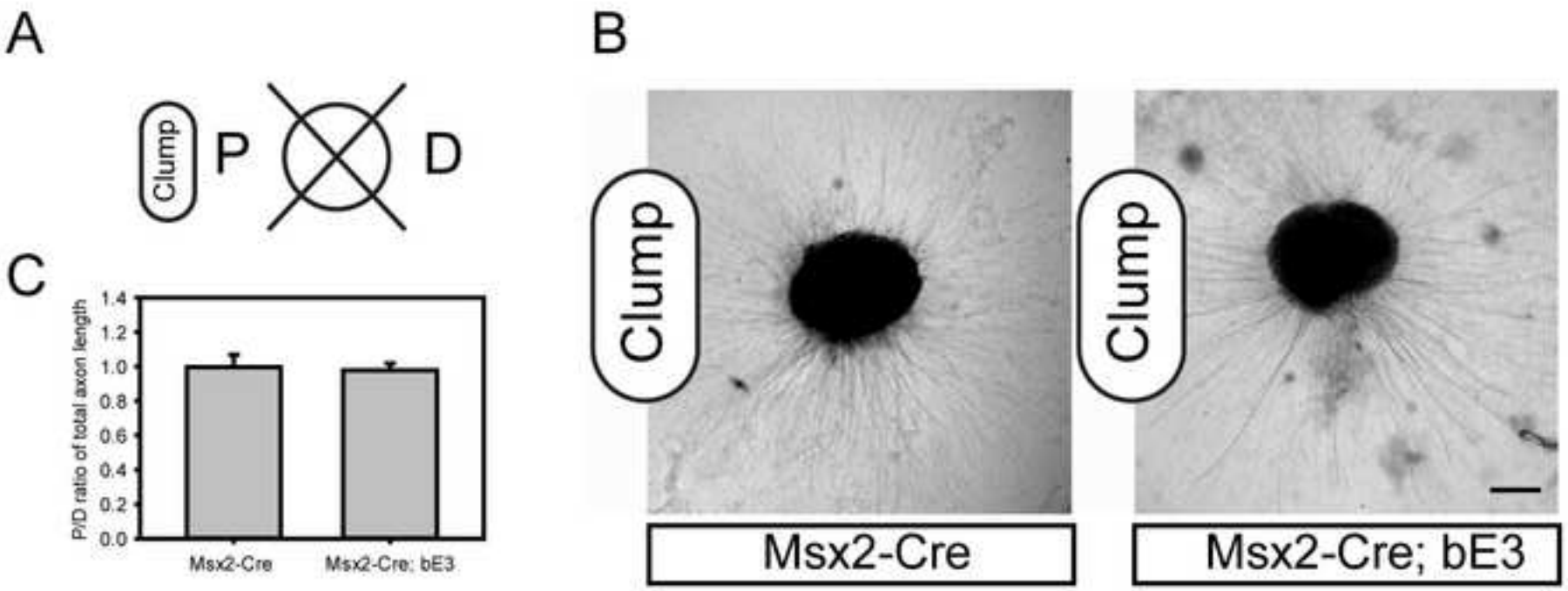


Supplemental Figure 2

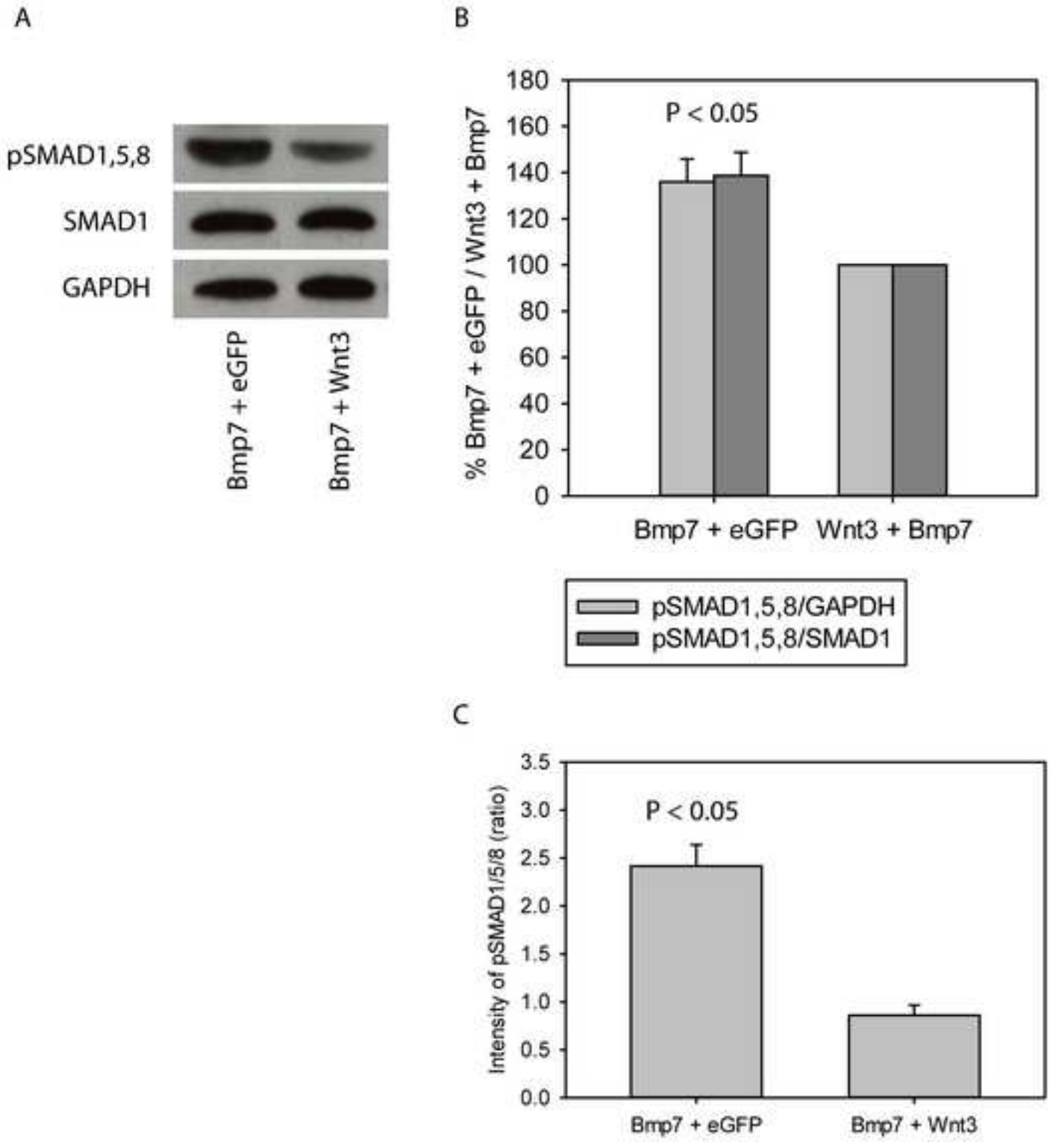
Supplemental Figure 3



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5

Supplemental Detailed Methods Section

Animals

Mice carrying the floxed exon 3 allele of *β-Catenin* (*Ctnnb1*$^{lox(ex3)}$) (Harada et al., 1999) were kept on a CD1 genetic background and mice carrying the loss of function allele of *β-Catenin* (*Ctnnb1*$^{lox(lof)}$) (Brault et al., 2001), floxed *Foxc1* (*Foxc1*^{lox}) (Hayashi and Kume, 2008) and transgenic *Pdgfrβ-Cre* (Foo et al., 2006) mouse lines were maintained in a mixed background. *Msx2*-Cre transgenic line was kindly provided by Dr. Gail Martin (UCSF) and maintained in the CD1 background (after back crossing for >10 generations). CRE recombinase reporter lines (ROSA-YFP, ROSA-LacZ) were obtained from the Jackson Laboratory (Bar Harbor, Maine). We generated experimental mice by crossing male mouse carrying both *Cre* transgene and one copy of the floxed allele to the female mouse carrying homozygous floxed alleles, except in the case of *Msx2-Cre*;*Ctnnb1*$^{lox(ex3)}$ which we obtained experimental mice by crossing an *Msx2-Cre* male mouse to a female mouse carrying a single copy of *Ctnnb1*$^{lox(ex3)}$. The day of vaginal plug was considered to be embryonic day 0.5 (E0.5). Fourteen mutant and littermate control embryos were analyzed to study the corpus callosum defect of Msx2-Cre crossed embryos. Mouse colonies were housed at the University of California, San Francisco, in accordance with UCSF IACUC guidelines. Timed pregnant CD1 mice were used for *in utero* electroporation at E13.5 and embryos were collected at E14.5, E16.5 and E17.5 for further analysis. Timed pregnant mice were subcutaneously injected with BrdU (Roche) dissolved in saline (10 mg/ml) at the dose of 50 mg/kg animal for birth-dating analysis.

In utero electroporation

Timed pregnant CD1 mice were purchased from Charles River for surgery done according to IACUC approved protocols at UCSF. Briefly, the CD1 pregnant females were anaesthetized with Nembutal. The uterine horns were exposed and embryos were injected with 1mg/ml DNA in TE into the lateral ventricle unless otherwise stated. Electroporation was conducted at 32V, 50 ms, 950 ms with five pulses. All DNA constructs were cloned into the pCIG2-IRES-EGFP vector from Dr. Franck Polleux (Hand et al., 2005) by basic DNA cloning techniques. For *Bmpr1a* variants, K261R mutation for DN-*Bmpr1a*, Q233D for CA-*Bmpr1a* were used. All cDNA constructs contain full-length cDNA from Open Biosystems. For all electroporation experiments multiple litters (at least three) were obtained for each construct and high-expressing, properly targeted electroporations were collected to get a minimum of N=6 embryos were examined and the results were uniform.

Immunostaining, *in situ* hybridization and quantitative RT-PCR

Embryos were collected at noon of embryonic days. Collected brains were fixed in 4% paraformaldehyde (PFA)/PBS overnight and cryo-protected in 20-30% sucrose/PBS for additional day. OCT-embedded tissues were processed in a cryostat at 12µm sections for immunostaining and 20µm sections for *in situ* hybridization. Primary antibodies used for the immunostaining are guinea pig anti-K5 (American Research Products, 1:100), mouse anti-NF-M (Bagri et al., 2002),

chicken anti-GFP (Aves Labs, 1:1000), rat anti-L1 (Millipore, 1:2000), mouse anti-TAG1 (Developmental Studies Hybridoma Bank at the University of Iowa, 1:500), rabbit anti-Calretinin (Chemicon, 1:2000), rabbit anti-pSMAD1/5/8 (Cell Signaling Technology, 1:100), rabbit anti-BLBP (Chemicon, 1:500), rabbit anti-GFAP (Dako, 1:500), mouse anti-NeuN (Chemicon, 1:200), rabbit anti-pH3 (Santa Cruz Biotechnology, 1:200), rabbit anti-Tbr1 (Abcam, 1:1000), rabbit anti-Tbr2 (Abcam, 1:200), mouse anti-Nestin (Abcam, 1:200), rabbit anti-Pax6 (Covance, 1:200), rat anti-Ctip2 (Abcam, 1:1000), rabbit anti-Zic (gift from J. Aruga, RIKEN Institute, 1:1500), rabbit anti-Ki67 (LabVision, 1:200), goat anti-FOXC2 (Santa Cruz, 1:100), mouse anti-Reelin (Chemicon, 1;1000), rabbit anti-LEF1 (Cell Signaling Technology, 1:100). Templates for RNA probes used *in situ* hybridization were mostly purchased from Open Biosystems: *Wnt6, Wnt10b, BMP7, Wnt3, Dan, Gdf5, Lef1, Dll1, Axin2, Satb2, NFia, NFib* except *Cxcr4, Sdf1, Raldh2* which were stated in our recent publications (Siegenthaler et al., 2009). All experiments were done by comparing control and mutant sections stained on the same slides to minimize variation. To measure the size of the corpus callosum, L1-stained sections were used. Using the Ruler Tool of the Adobe Photoshop, we measured the thickness of L1 staining in the midline and used littermate control embryos to make a ratio of the size. Seven embryos from each group were used for the experiment. For in situ hybridization, slides were warmed to room temperature and treated with proteinase K (50 ug/ml) for 2 min, and fixed with 4% PFA for 10 min. Acetylation was performed using 0.25% acetic anhydride in 0.1 M triethanolamine, pH 8.0, for 10 min, followed by three PBS washes. Slides were incubated with hybridization buffer [50% formamide, 5x SSC, 0.3 mg/ml yeast tRNA, 100 mg/ml heparin, 1x Denhardt's, 0.1% Tween 20, 0.1% CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate), 5 mM EDTA] for 10 min at 65°C, followed by overnight incubation with a digoxigenin-labeled probe (500 ng/ml). Five high-stringency washes were performed with 0.2x SSC at 65°C. Slides were then incubated with horseradish alkaline phosphatase (AP)-conjugated anti-digoxigenin and NBT (nitroblue tetrazolium)/BCIP (5-bromo-4-chloro-indolyl phosphate) (Roche) for the detection of signals. To double-stain Calretinin proteins on the sections which were first detected with RNA probes by *in situ* hybridization, signals for the antigen were amplified using TSA kits (Invitrogen). We performed X-gal staining of a ROSA-LacZ reporter line as stated in the previous publication (Siegenthaler et al., 2009). Images were acquired at the Nikon Imaging Center at UCSF using an upright Nikon C1 spectral confocal microscope equipped with 405, 488 and 561 nm lasers with 10x, 20x and 63x objective.

Quantitative RT-PCR (qPCR) was conducted using total RNA purified from two E14.5 embryonic brain tissues using 7500 Fast Real-Time PCR System (Applied Biosystems). Primer pairs were Wnt6_F (ATGTGGACTTCGGGGATGAGA), Wnt6_R (GCCTCGTTGTTGTGCAGTTG), Wnt10b_F (GAAGGGTAGTGGTGAGCAAGA), Wnt10b_R (GGTTACAGCCACCCCATTCC), Bmp7_F (GACCTTCCAGATCACAGTCT), Bmp7_R (tcctcagaagcccagatggt), and GAPDH_F (GGCTGGCATTGCTCTCAATG), GAPDH_R (GTGGGTGGTCCAGGGTTTCT). Three independent qPCR was done using a SYBR Green PCR master mix (Applied Biosystems) and the ddCt value was used to present fold differences.

Co-culture of COS7 cells and cortical neurons

We transfected COS7 cells with *Wnt3* and *BMP7* expressing pCIG2-IRES-EGFP vectors with Lipofectamine 2000 (Invitrogen) 2-day before collecting medial cortical tissues from E14.5 CD1 embryos. Cortical neurons were co-plated with trypsinized COS7 cells on poly-*L*-lysine coated cover slides. Cells were fixed in 4% PFA/PBS solution and double-stained with anti-Calretinin and anti-GFP antibodies to label Calretinin+ cingulate neurons and transfected COS7 cells respectively. Numbers of Calretinin+ neurons containing neurites on COS7 cells and neurons with neurites passing COS7 cells were counted from three independent experiments.

Statistics

For the pair-wise analysis of cell counting from co-culture of cingulate neurons and COS7 cells, Student's *t*-test was used to evaluate the significance. Error bars depict +/- SEM.

Explant culture

Explants (~500 μm width) were embedded in a rat-tail collagen gel matrix containing a resuspension of meningeal tissues that were obtained from E15.5 control and mutant embryos. Explants were maintained at 37 °C with 5% CO2 for 16 h in B-27 supplemented neurobasal medium (Invitrogen) before fixation. For quantification, the total length of axons was measured in the proximal and distal quadrants.

Western blot

CD1 mice at E13.5 were electroporated with Bmp7 + eGFP or Bmp7 + Wnt3 (1:2) and the electroporated areas of the E17.5 medial cortex were collected and analyzed by Western blot using protein extracts pooled from five embryos. Forty μg of protein extracts were separated and immunoblotted with a NUPAGE SDS-PAGE system (Invitrogen). Signals determined by anti-pSMAD1/5/8, anti-SMAD1, and anti-GAPDH (all 1:000, Cell Signaling Technology) were analyzed with the NIH ImageJ software and plotted using data from three independent experiments. N = 3.

Supplemental Figure Legends

Supplemental Figure 1. Skin-restricted expression of *Msx2*-Cre. **A**) Coronal and horizontal sections of control and mutant embryos show L1 and TAG-stained callosal axons at E17.5. * = failure of the corpus callosum. **B**) Double-immunostaining with GFP (green) and L1 (red) antibodies in E13.5 and E15.5 embryos from *Msx2*-Cre mice crossed with ROSA-YFP reporter mice shows skin-specific recombination of *Msx2*-Cre. **C**) Coronal and sagittal sections of ROSA-LacZ CRE reporter lines show *Msx2-Cre* expression in the skin and the dermal condensate at E15.5. Arrows indicate the X-gal signal in the skin where Msx2-Cre drives recombination. **D**) *In situ* hybridization of cortical projection neuron specific markers, *Satb*2, *NFia* and *NFib* in the WT and *Msx2*-Cre; *Ctnnb1*$^{lox(ex3)}$ cortex at E14.5. All *in situ* hybridizations were performed three

times on different samples and the immochemistry performed 6 times on different samples. Scale bars = 200 μm.

Supplemental Figure 2. Normal growth of cortical projection neurons born at the time of *Msx2*-Cre expression. **A**) Proliferation and differentiation of neurons born at the time of *Msx2*-Cre expression are presented with staining markers such as Pax6 (E17.5), Nestin (E15.5) (ventral neuronal progenitor), pH3 (E15.5) (M-phase cell; red) and Tbr2 (E15.5) (basal neuronal progenitor) and projection neurons with Tbr1, Ctip2 (E17.5) (green). **B**) *In situ* hybridization of axon guidance molecules at E14.5 from control and *Msx2-Cre*; *Ctnnb1*$^{lox(gof)}$embryos. **C**) Graph depicts the quantitation of three independent qRT-PCR results for Wnt6, Wnt10b, and Bmp7 expression at E14.5. All *in situ* hybridizations were performed three times on different samples and the immochemistry performed 6 times on different samples. Scale bars = 200 μm.

Supplemental Figure 3. Cortical layer markers in mice with excess meninges and mid-corticogenesis loss of meninges. *In situ* hybridization at E17.5 for layer specific markers (Cux1, Cux2, Brn2, Satb2 and Fezf2) showing that the expanded population of upper layer neurons in *Pdgfrβ*-Cre*; Foxc1*$^{lox/lox}$ mutants (red arrows). Representative images are presented from three independent experiments. All *in situ* hybridizations were performed three times on different samples. Scale bar = 100 μm.

Supplemental figure 4: Meningeal effects on cingulate explant axon outgrowth. **A**) A diagram of the collagen explant assay. **B**) Explants of E15.5 CD1 cingulate cortex were cocultured for 16 hours next to a meningeal aggregate obtained from *Msx2*-Cre or *Msx2*-Cre; *Ctnnb1*$^{lox(gof)}$embryos. Proximal/Distal (P/D) ratio was derived from measuring the total length of axons in the proximal and distal quadrants of the explants to determine a soluble effect of meninges on cingulate axon guidance. **C**) Quantification of the total axon length P/D ratio. Error bars represent standard error of the mean. Scale bar = 200 μm. N = 3.

Supplemental Figure 5: Immunoblot of pSMAD1/5/8 using electroporated medial cortical tissues. **A**) A representative image of Western blot analysis shows pSMAD1/5/8, SMAD1, and GAPDH expression from E17.5 cortical tissues electroporated with BMP7 + eGFP or Bmp7 + Wnt3 for four days. **B**) A plot shows the relative increase of pSMAD1/5/8 expression normalized to the level of SMAD1 or GAPDH in the BMP7 + eGFP electroporated tissues (experiment was performed 3 times using samples pooled from five embryos). These results were statistically significant $P<0.05$ using Student's T-test. **C**) A plot shows the increase of pSMAD1/5/8 staining signal intensities of the electroporated cortex normalized to the non-electroporated contralateral cortex from immunostaining experiments (N = 5).