Experimental Procedure:

Cloning of constructs:

(1) pCAG-dnBMPR1b: chicken dnBMPR1b originally cloned into an avian retroviral backbone (RCAS-dnBMPR1b) (Kawakami et al., 1996) and obtained as a gift from Prof. Clifford J. Tabin, Harvard Medical School, Boston, USA, was sub-cloned at ClaI sites into a modified pCAG backbone (Gupta and Sen, 2015). (4) pCAG-BMPRIIΔLIMK: human BMPRIIΔLIMK from pCDNA-BMPRIIΔLIMK (gift from Prof. Samantha Butler, University of California Los Angeles, LA, USA) (Phan et al., 2010) was further sub-cloned into the modified pCAG backbone (Gupta and Sen, 2015) between ECoRI and NotI sites . (3) pCAG-Smurf1: mouse Smurf1 cDNA originally cloned into pCMV-SPORT6 (Gene bank accession no. BC029097, Mouse full length cDNA clone library) was further sub-cloned into modified pCAG backbone (Gupta and Sen, 2015) at ECoRV sites. (4). pCAG-mCherry: mCherry cDNA was subcloned in to modified pCAG backbone between ECoRI- NotI sites.

Immunohistochemistry:

Mouse forebrain sections at different developmental stages were post-fixed in 4% paraformaldehyde (PFA) in PBS, permeabilized with PBT (0.2% triton in 1xPBS) followed by blocking in 5% goat serum at room temperature for 1 hour. Sections were further incubated with the primary antibodies overnight at 4^0C followed by incubation with their respective secondary antibody at room temperature. Fluorescence signal was developed by using following secondary antibodies: anti-mouse (Cat.no. 111-585-003, Jackson ImmunoResearch Laboratories Inc.), anti-rabbit (Cat. No. 111-545-003, Jackson ImmunoResearch Laboratories Inc.), anti-goat (Cat.no. 705-165-003, Jackson ImmunoResearch Laboratories Inc.) and anti-chicken (Cat.no.103-165-155, Jackson ImmunoResearch Laboratories Inc.).

Quantification:

For quantification, each electroporated embryo analysed was considered as a biological replicate (n) and for each biological replicate, three to five consecutive brain sections were analysed which was considered as technical replicates (N). GFP positive cells from all of the technical replicate corresponding to each biological replicate (n) were counted and the mean was calculated. For cell polarity analysis, the GFP positive neuron with the Golgi localization through the apical dendrite was considered as normal cell while the cell with random Golgi localization within the cell body was considered as abnormal cell. For quantification of dendritic branches, the mean number of primary, secondary and tertiary branches were calculated for ~ 50 GFP positive neurons per animal (n = 3). The mean and standard deviation plotted on the graph were calculated from the mean of all the biological replicates. Quantification data is represented as % Mean ± Standard deviation.

Cell-based assay for testing efficacy of constructs used:

293T cells were used for a cell-based assay to test the efficacy of the constructs used. Cells plated in a 24-well plate were transfected with 1µg DNA corresponding to the following constructs: 1) pCAG-dnBMPR1b + pCAG-GFP, 2) pCAG-BMPRIIΔLIMK+ pCAG-GFP and 3) pCAG-Smurf1+ pCAG-GFP using TurboFect transfection reagent (Cat no. R0531, Thermo Fisher Scientific). The control set of cells were transfected with pCAG-GFP alone. After 24 hours of transfection, immunocytochemistry was performed for detection of pSmad1/5/8 and p-Cofilin in each set of cells as described previously. For each construct, the transfection was done in triplicates (n=3) and nearly 300 cells were counted from each replicate to quantify the percentage of GFP positive cells that were either pSmad1/5/8 positive and/or p-Cofilin positive. Quantification data is represented as % Mean ± Standard deviation.

Figure S1.

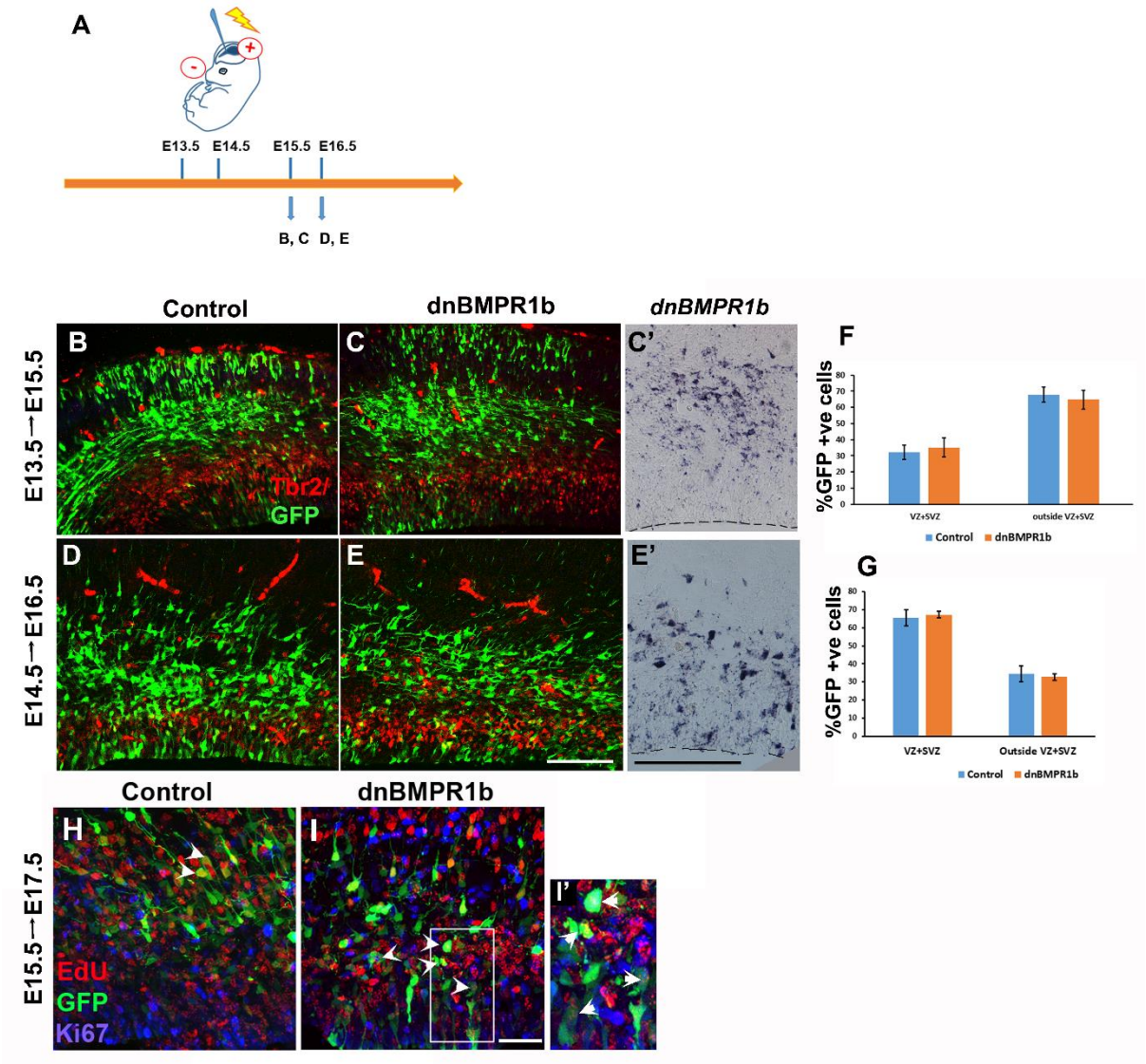


Figure S1: (A) Schematic for experimental design. (B-E) Immunohistochemistry for Tbr2 in the cortex electroporated with pCAG-GFP and pCAG-dnBMPR1b at E13.5 analysed at E15.5 (B and C) and electroporated at E14.5 and analysed at E16.5 (D and E). (C' and E') mRNA in situ hybridization for detecting the transcript of dnBMPR1b in the cortical sections 48 hours after electroporation performed at E13.5 (C') and E14.5 (E'). (F and G) Quantification of GFP positive cells in the VZ-SVZ and outside the VZ-SVZ demarcated by Tbr2 expression domain in the cortex electroporated at E13.5 (F) and E14.5 (G). (H, I and I') Images of the cortices

electroporated with pCAG-GFP (H) and pCAG-dnBMPR1b (I, I') showing Ki67, GFP and EDU triple positive cells (white arrowheads). Quantification data is represented as % Mean ± STD (n=3), *p<0.05 and **p<0.005. Scale bar: 100µm (B-E) and 50 µm (H and I).

Figure S2.

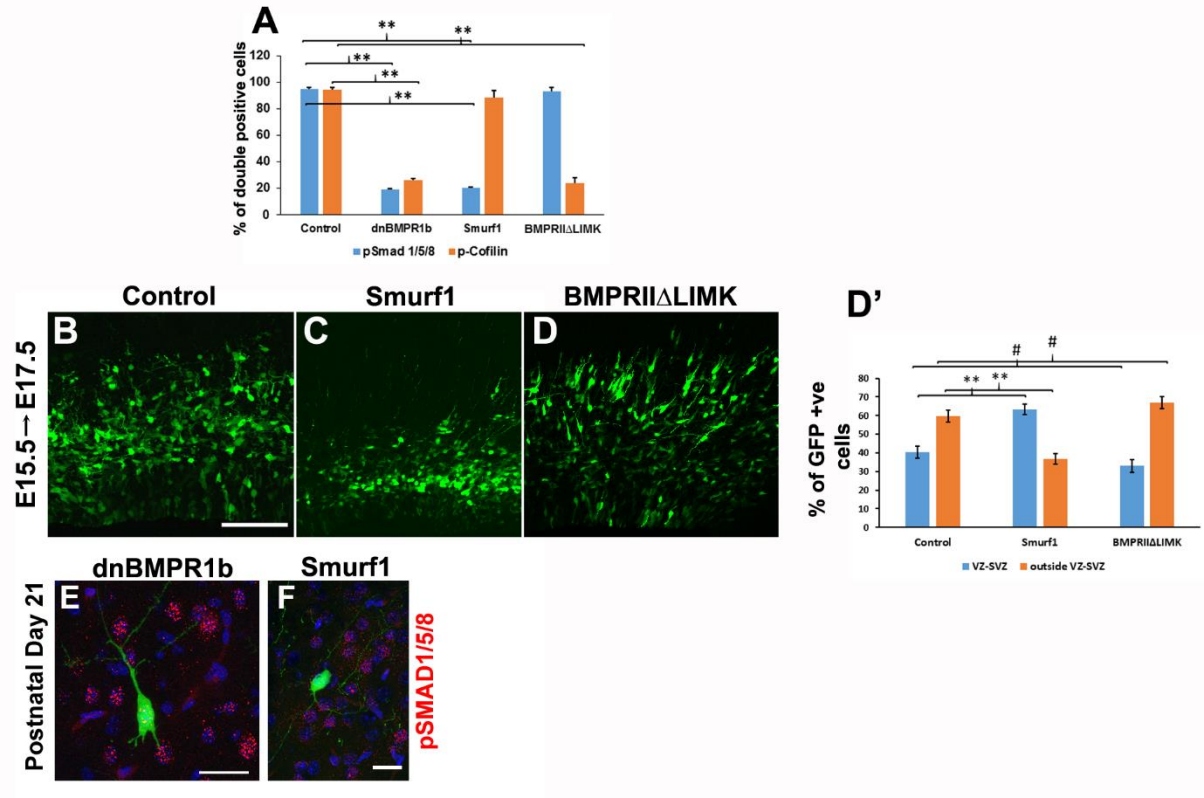
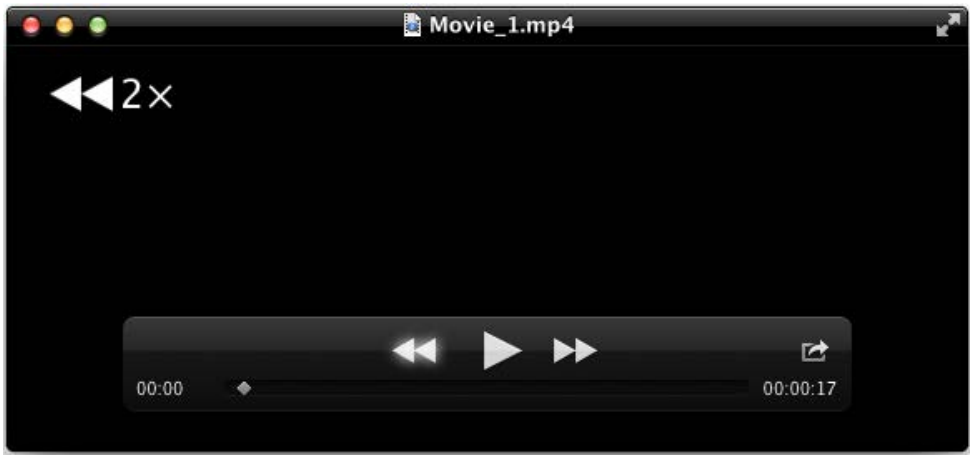


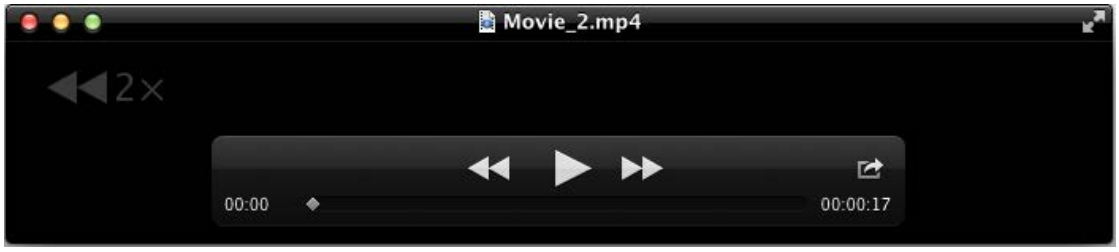
Figure S2: (A) Quantification showing GFP-pSMAD1/5/8 and GFP-p-Cofilin double positive cells transfected with pCAG-GFP (Control), pCAG-dnBMPR1b, pCAG-Smurf1 and pCAG-BMPRIIΔLIMK along with pCAG-GFP. Quantification data is represented as % mean ± STD. **p<0.005. (B-D) Image of the cortex overexpressing GFP (B), Smurf1 (C) and

BMPRIIΔLIMK (D) showing the distribution of GFP positive cells in the VZ-SVZ and the IZ in the cortex at E17.5. (D') Quantification of the distribution of GFP positive cells in the cortex expressing GFP, Smurf1 and BMPRIIΔLIMK at E17.5. (E and F) immunostaining showing pSMAD1/5/8 in the cortex overexpressing dnBMPR1b (E) and Smurf1 (F) at P21. Scale bar: 100 µm (B-D) and 20 µm (E-F).



Movie 1

A multi-angle rotational view of GFP positive cell from the control cortex at P6 showing the Golgi localization within the GFP positive domain.



Movie 2

A multi-angle rotational view of GFP positive cell from the dnBMPR1b electroporated cortex at P6 showing random Golgi localization in the cell body.

References:

GUPTA, S. & SEN, J. 2015. Retinoic acid signaling regulates development of the dorsal forebrain midline and the choroid plexus in the chick. *Development,* 142**,** 1293-8.

KAWAKAMI, Y., ISHIKAWA, T., SHIMABARA, M., TANDA, N., ENOMOTO-IWAMOTO, M., IWAMOTO, M., KUWANA, T., UEKI, A., NOJI, S. & NOHNO, T. 1996. BMP signaling during bone pattern determination in the developing limb. *Development,* 122**,** 3557-66.

PHAN, K. D., HAZEN, V. M., FRENDO, M., JIA, Z. & BUTLER, S. J. 2010. The bone morphogenetic protein roof plate chemorepellent regulates the rate of commissural axonal growth. *J Neurosci,* 30**,** 15430-40.