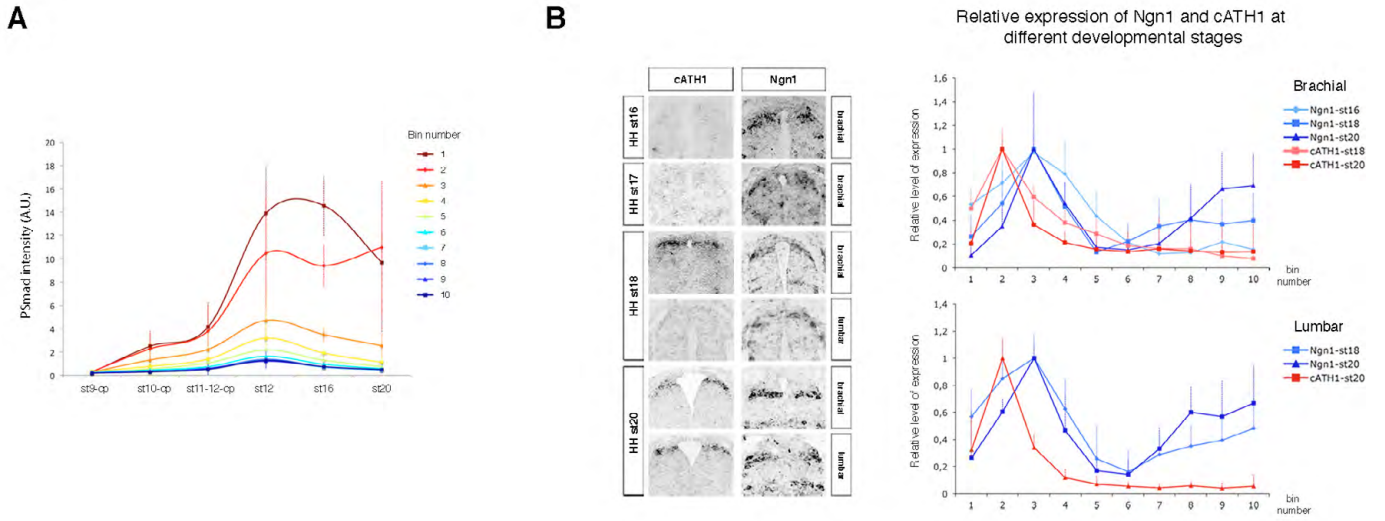


Fig. S1. Effect of concentration and duration of BMP on dorsal identities and BMP signalling. (A) Percentage of Olig3 (dP1-3) and Hnk1 (expressed in migratory neural crest cells) in explants exposed for 24 hours to the indicated concentrations of Bmp4. (B) Expression of chick Ath1 at the indicated time points in explants exposed to 8 ng/ml Bmp4 from the start of the experiment (solid line) or after the explants had been incubated in control media for 24 hours (dotted line). (C) Expression of Ngn1 and chick Ath1 at 24 hours in explants exposed to either 8, 32 or 128 ng/ml Bmp4 for 1 hour, or constantly to 8 ng/ml Bmp4. (D) Intracellular BMP signaling activity measured with BRE-luciferase in explants in the indicated conditions. Explants were exposed to control media (i), 8 ng/ml Bmp4 for 1 hour (iii) or 24 hours (iv). In (ii), explants in condition (iii) were washed three times, fresh medium was added and left for 1 hour on these explants before it was transferred to naive explants. BRE-luciferase activity measured in (iii) was significantly different from the activity measured in (i) and (ii) (asterisks), whereas conditions (i) and (ii) were not significantly different (Student's *t*-test).



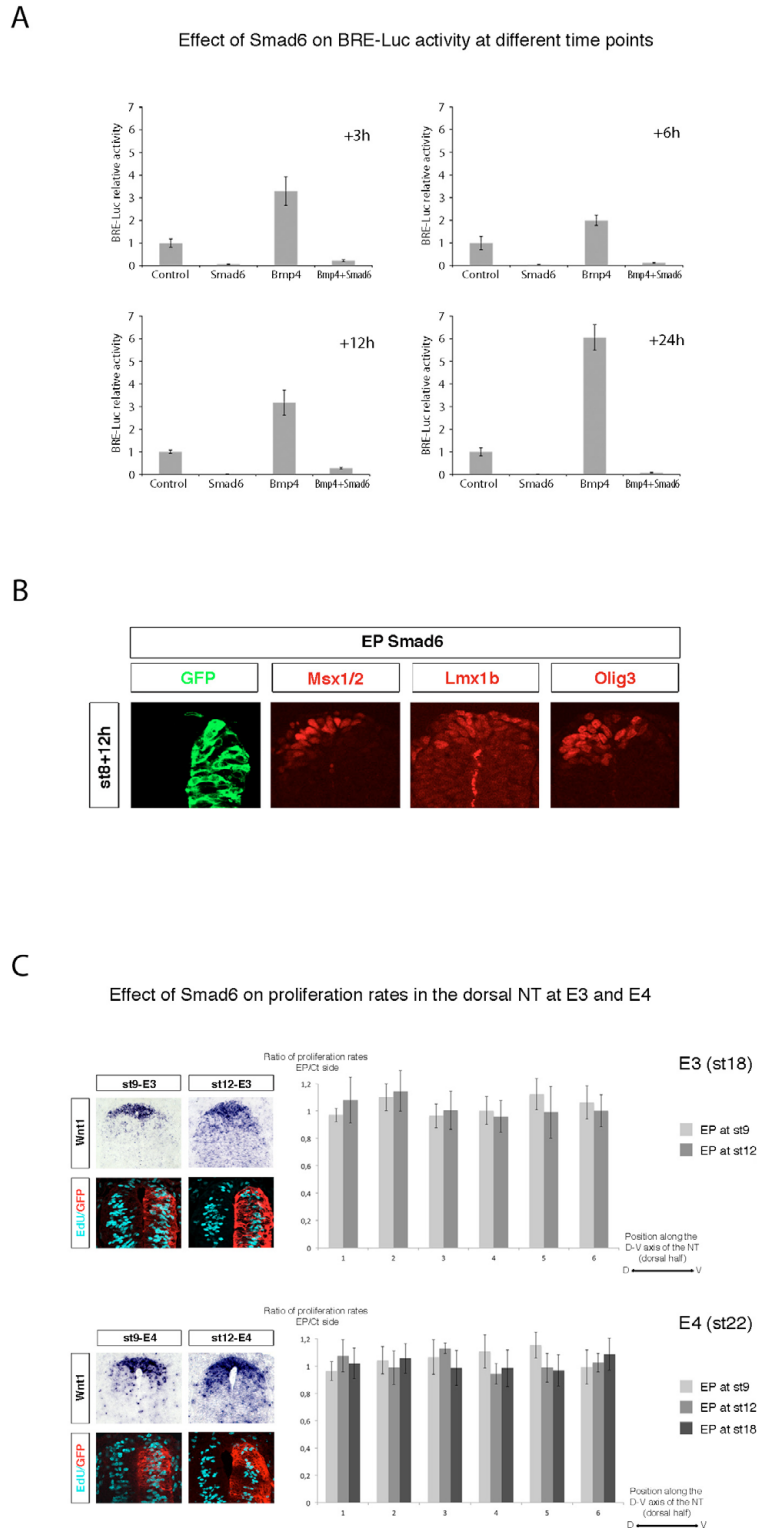


Fig. S3. Smad6 inhibits BMP signaling without affecting the proliferation rate of dorsal progenitors. (A) Smad6 inhibits endogenous and ectopic BMP activity. Relative BRE-Luc activity in embryos electroporated at stage 12 with an empty vector, Smad6, Bmp4 or Smad6+Bmp4. Embryos were incubated for 3, 6, 12 or 24 hours before harvesting and processing for luciferase assays. Bmp4 induces an increase in BRE-Luc activity within 3 hours of transfection. Smad6 inhibits both endogenous and Bmp4-induced BRE-Luc activity by over 90% within 3 hours of transfection. The Smad6-mediated blockade of BMP signaling was maintained at approximately the same level for at least 24 hours. (B) Expression of GFP (marking Smad6 transfected cells), Msx1/2, Lmx1b and Olig3 at brachial level of the NT 12 hours after EP of Smad6 at HH stage 8. (C) Expression of Wnt1 and proliferation rates at E3 and E4 in NT electroporated with Smad6 at HH stage 9, stage 12 and stage 18 (E3). The expression of Wnt1 appeared unaffected by transfection of Smad6. Embryos were incubated with EdU (a fluorescent thymidine analogue) for 1 hour before harvesting. The dorsal half of the neural tube was divided into six bins from dorsal (1) to ventral (6) and the proliferation rate was measured in each of them by normalising the number of EdU⁺ cells to the total number of progenitors. No significant difference was detected between the transfected and the control side.

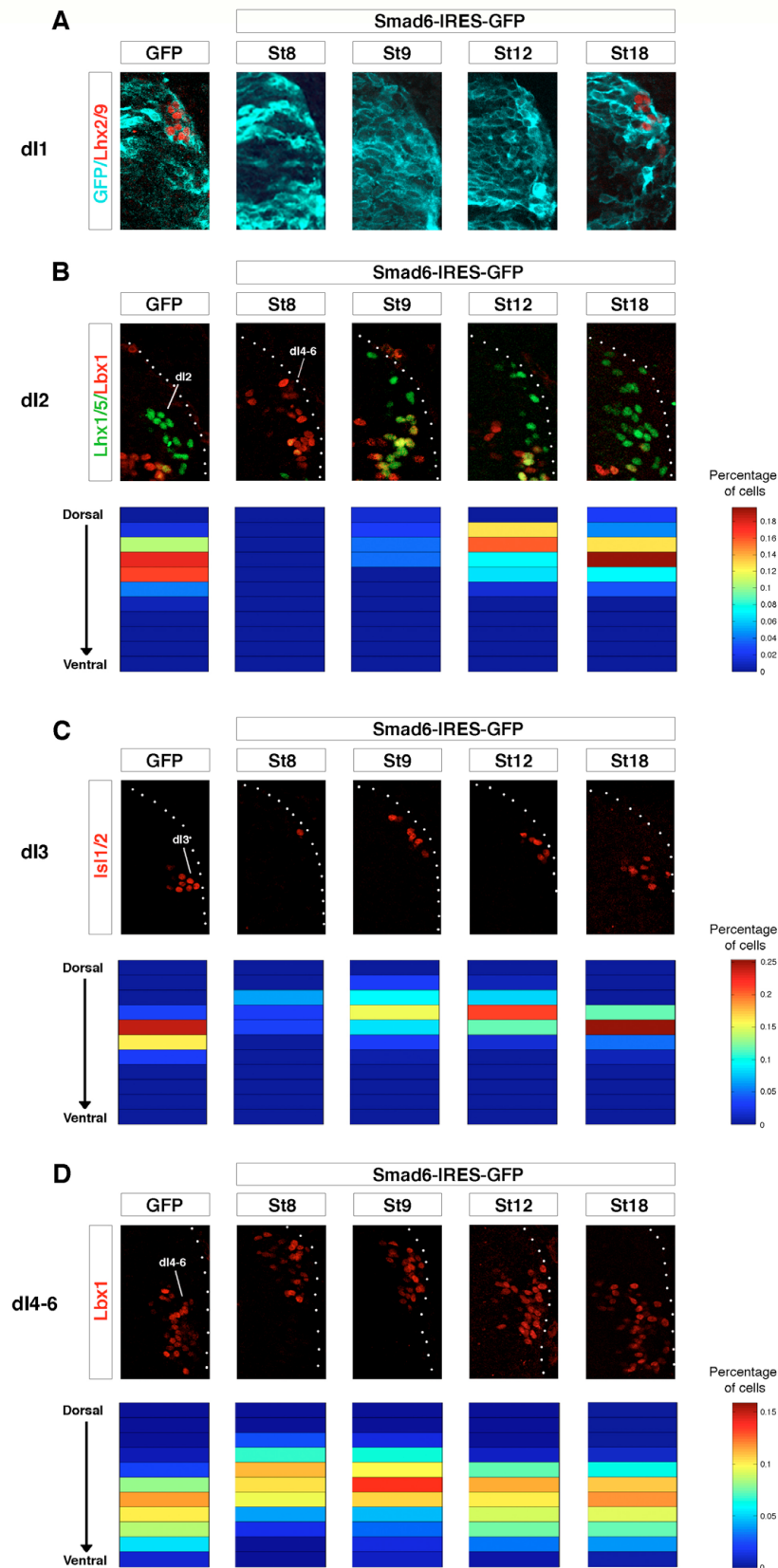


Fig. S4. Dorsal interneurons are dorsally shifted after blockade of BMP signaling. (A-D) Top panels: dl1 (A), dl2 (B), dl3 (C) and dl4-6 (D) were identified by immunostaining with specific markers after EP of either Smad6-IRES-GFP or an empty vector, expressing GFP only. Embryos were transfected at the indicated stages and analysed at E4. Images for each interneuron subtype were centred on the region of interest. dl2 and dl3 neurons are rarely found after Smad6 EP at HH stage 9 and stage 8, respectively. Thus, the corresponding pictures show specific examples in which these neurons could be detected but were dorsally shifted. Bottom panels: the distribution of dorsal interneurons is represented by the heat maps that indicate the proportion of each neuronal subtype in each bin of the dorsal NT (each bin represented 5% of the DV length of the NT). Transfection of GFP resulted in similar interneuron distributions at all stages.