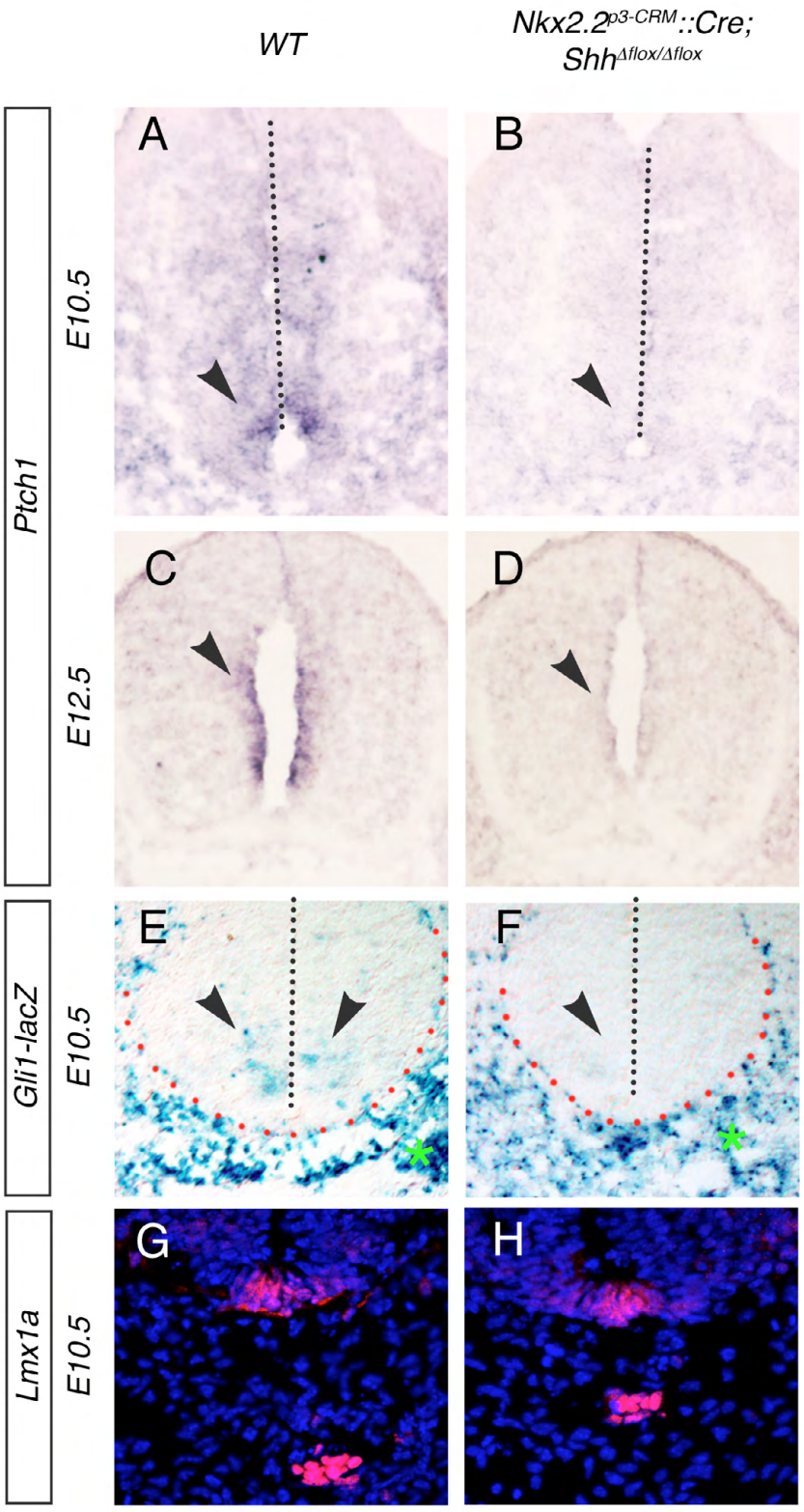

Fig. S1. Shh target gene expression changes in the neural tube of *Shh*$^{\Delta FP/\Delta FP}$ embryos. (**A-D**) *Ptch1* expression is reduced at both E10.5 and E12.5 (arrowheads). (**E**,**F**) β-gal expression in a targeted *Gli1*^{lacZ} reporter line shows weaker expression in the ventral spinal cord in *Shh*$^{\Delta FP/\Delta FP}$ embryos (arrowheads) at E10.5. (**G**,**H**) Lmx1a expression is not altered in the FP at E10.5. Section in H is at a more rostral level than that in G in the thoracic region.

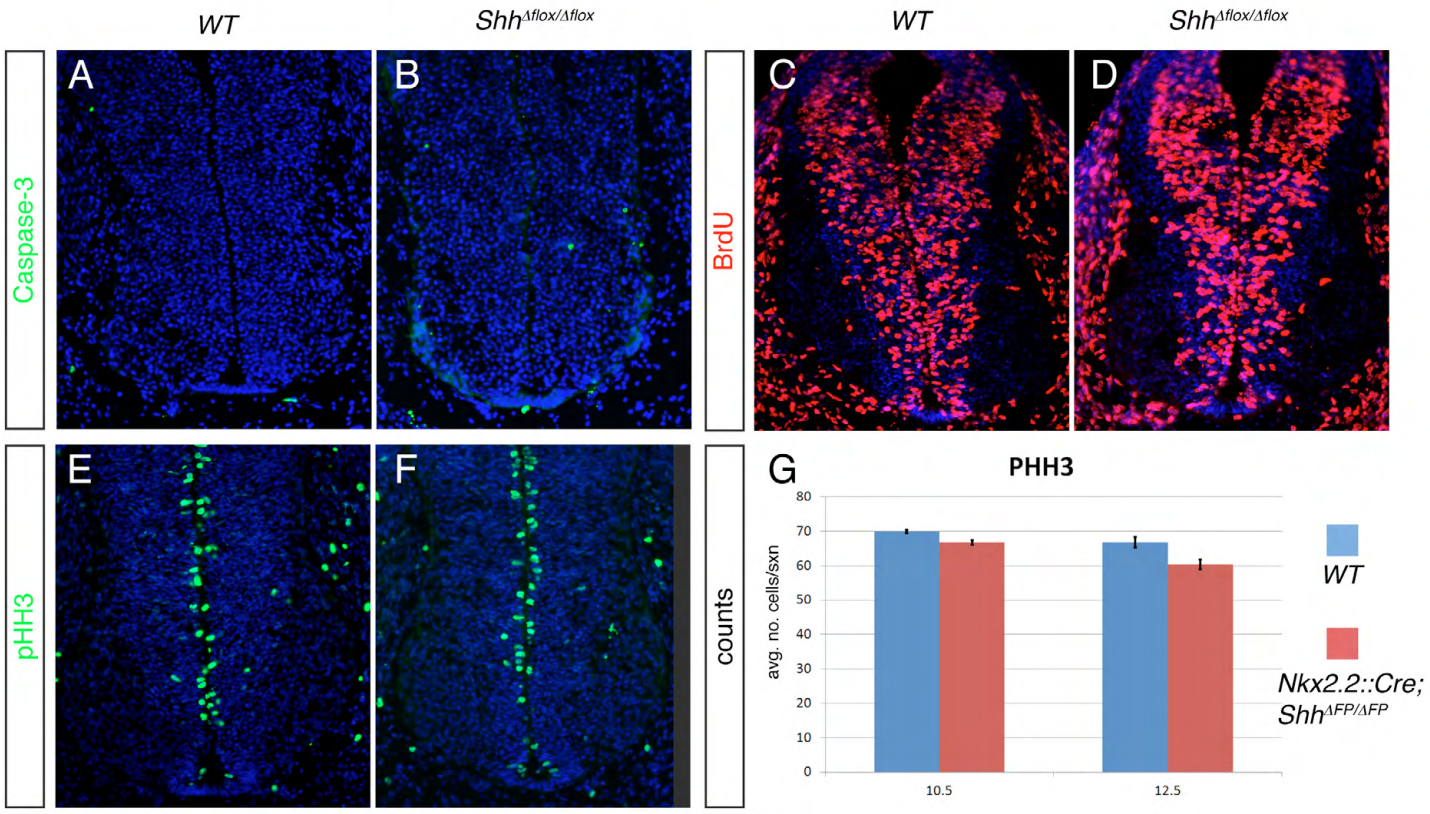

Fig. S2. Analysis of cell proliferation and cell death in *Shh*$^{\Delta FP/\Delta FP}$ embryos. (**A**,**B**) Activated caspase 3 expression is not elevated in conditional mutants compared with WT. (**C-G**) No significant differences were seen in BrdU incorporation rates or pHH3 expression in *Nkx2.2*$^{p3\text{-}CRM}$*::Cre;Shh*$^{\Delta FP/\Delta FP}$ conditional mutants at E10.5 (A-F) and E12.5 (G) compared with WT.

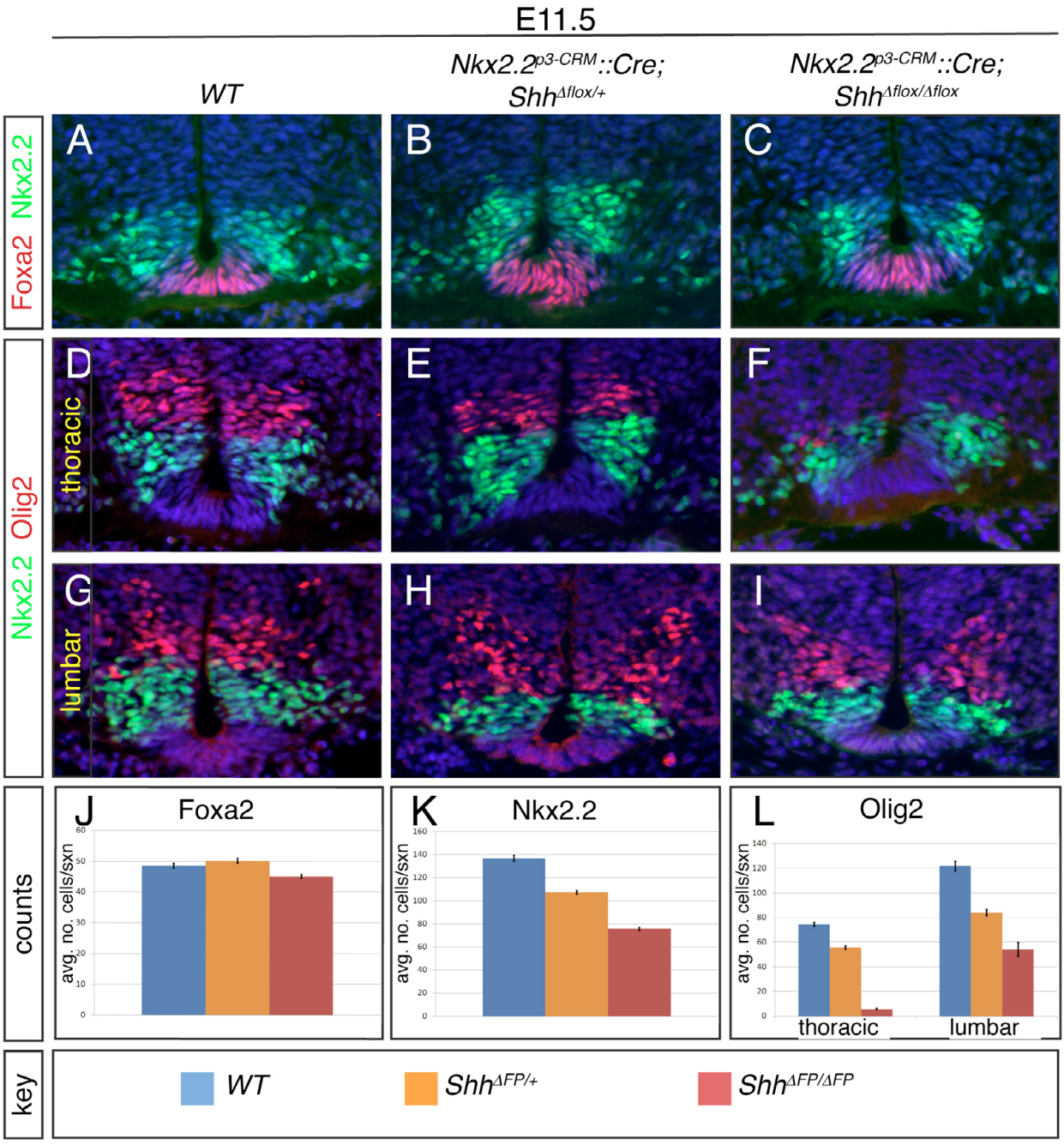

Fig. S3. Altered progenitor domain marker protein expression in *Shh*$^{\Delta FP/\Delta FP}$ embryos at E11.5. (**A-C**) Foxa2 and Nkx2.2 expression in WT (A), *Shh*$^{\Delta FP/+}$ heterozygotes (B) and *Shh*$^{\Delta FP/\Delta FP}$ homozygotes (C). (**D-I**) Nkx2.2 and Olig2 expression in thoracic (D-F) and lumbar (G-I) regions. (**J-L**) Quantification of marker expression.

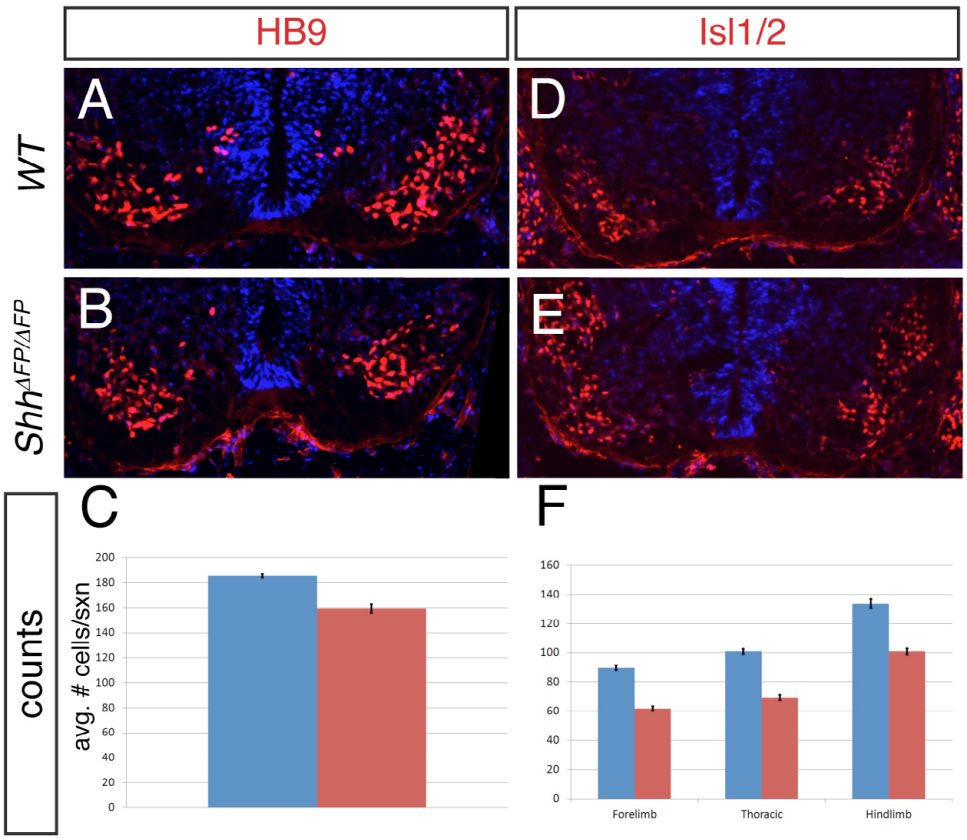

Fig. S4. Reduction in motoneuron formation in *Shh*$^{\Delta FP/\Delta FP}$ embryos. Staining for postmitotic motoneuron markers HB9 (**A-C**) and Isl1/2 (**D-F**) in E12.5 embryos shows that reduced numbers of cells are generated.

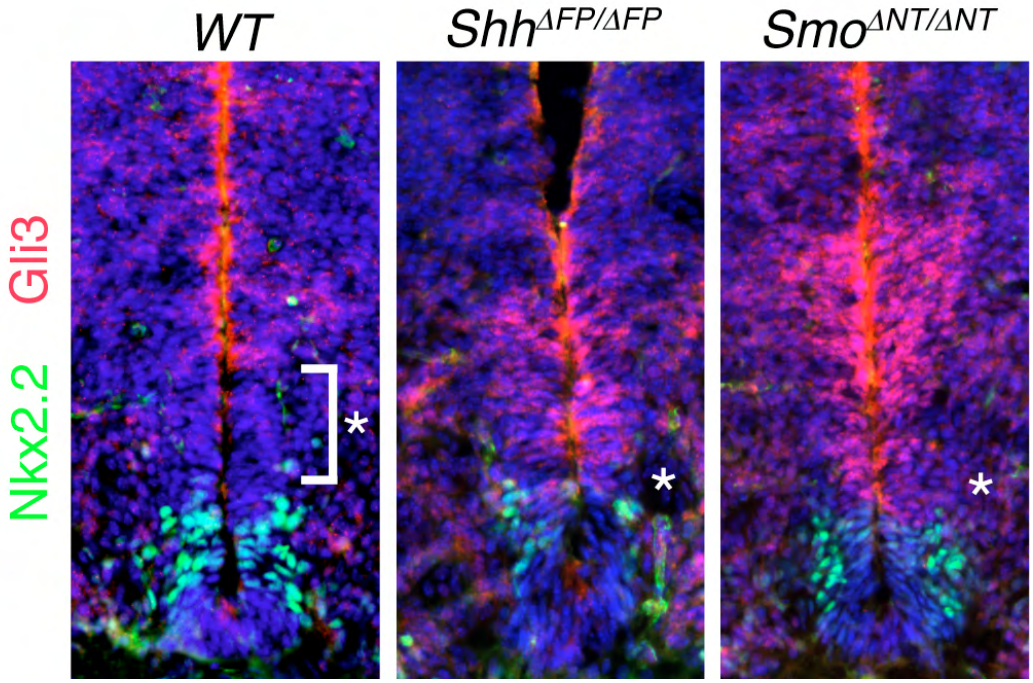

Fig. S5. Gli3 expression shifts ventrally in *Shh*$^{\Delta FP/\Delta FP}$ and *Smo*$^{\Delta NT/\Delta NT}$ embryos. E11.5 embryos stained for Gli3 and Nkx2.2 (marking the p3 domain) show a ventral shift in the expression boundary of Gli3 into the pMN domain (brackets, asterisks).

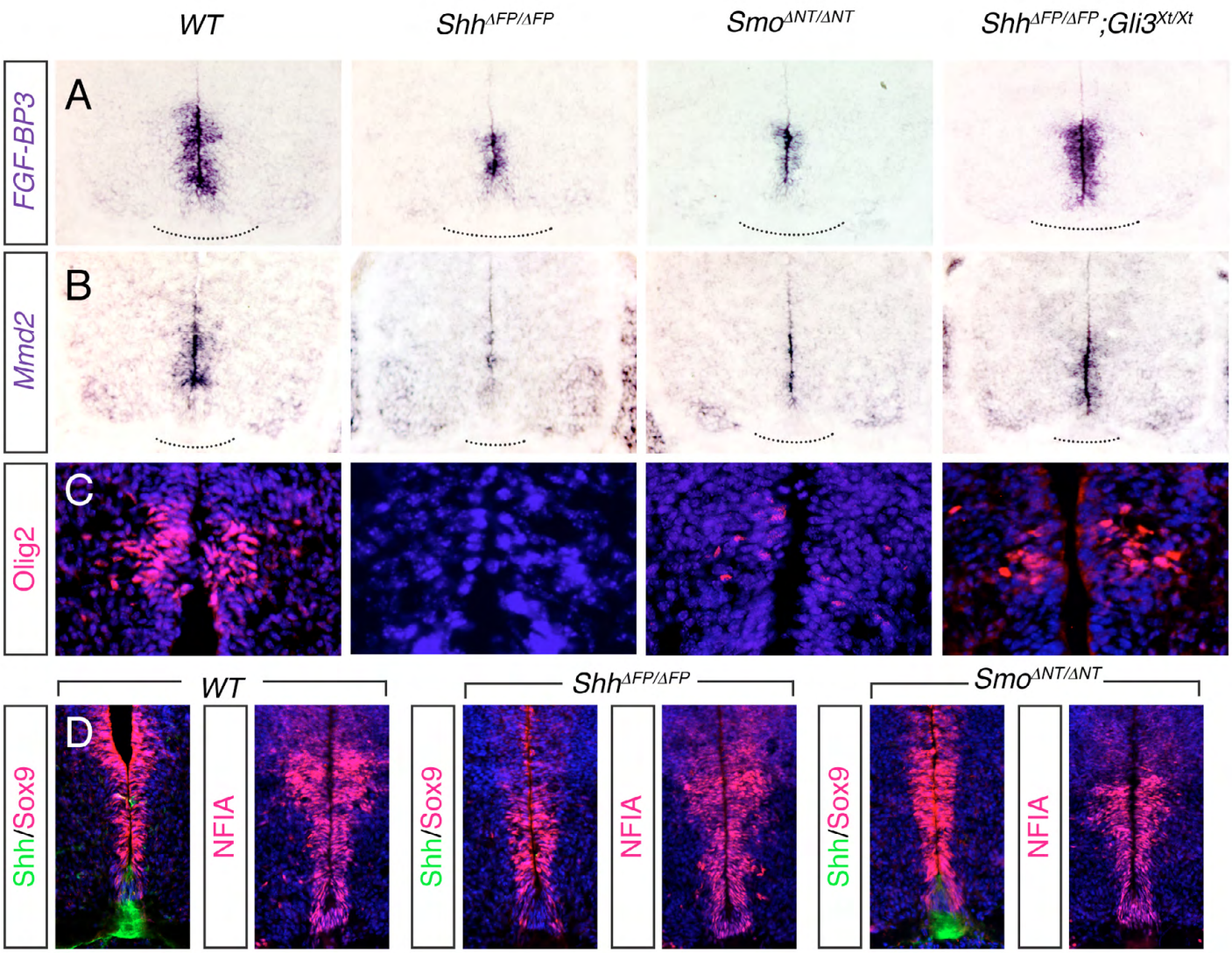

Fig. S6. Changes in the expression of specific glial progenitor genes in mutant embryos identifies novel Shh-Gli target genes. (**A**,**B**) Staining for Fgfbp3 and Mmd2 in WT, *Shh*$^{\Delta FP/\Delta FP}$, *Smo*$^{\Delta NT/\Delta N}$ and *Shh*$^{\Delta FP/\Delta FP}$*;Gli3*$^{xt/xt}$ mutant embryos at E12.5. Expression of both genes is reduced or absent in *Shh*$^{\Delta FP/\Delta FP}$ and *Smo*$^{\Delta NT/\Delta NT}$ embryos but is restored in *Shh*$^{\Delta FP/\Delta FP}$*;Gli3*$^{xt/xt}$ mutants. (**C**) Expression of Olig2, a gene that appears to be primarily regulated by Gli3 derepression, is shown for comparison. (**D**) Sox9 and Nfia expression in *Shh*$^{\Delta FP/\Delta FP}$ and *Smo*$^{\Delta NT/\Delta NT}$ embryos is not altered. Shh is shown in some panels for comparison.

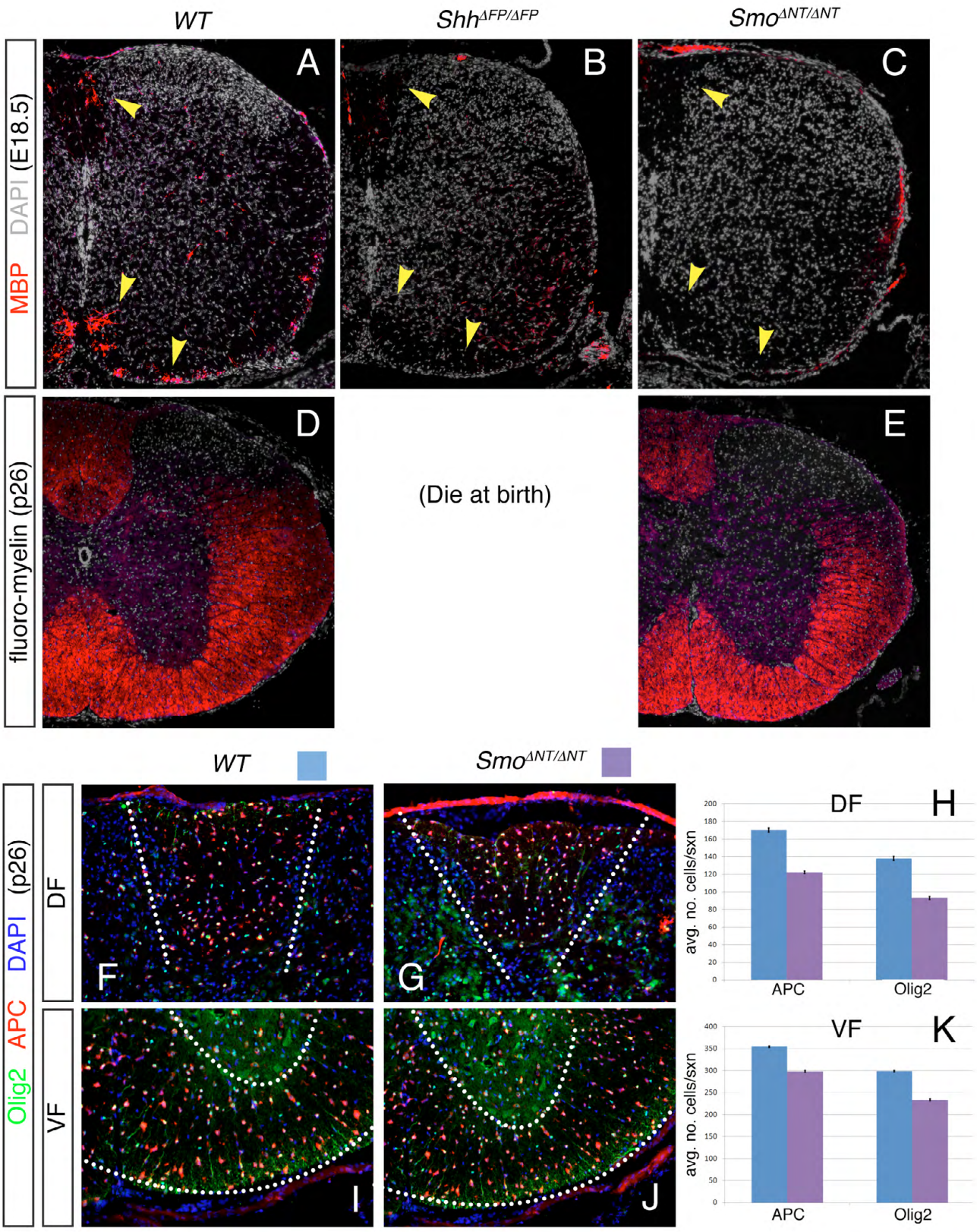

Fig. S7. Expression of differentiated OL marker protein expression in *Shh*$^{\Delta FP/\Delta FP}$ and *Smo*$^{\Delta NT/\Delta NT}$ embryos at E18.5 and P26. (**A-C**) Myelin basic protein (Mbp) expression in WT (A), *Shh*$^{\Delta FP/\Delta FP}$ (B) and *Smo*$^{\Delta NT/\Delta NT}$ (C) embryos shows reduced staining in both the VF and DF in mutant embryos (arrowheads). (**D**,**E**) Fluoromyelin expression is similar in WT (D) and *Smo*$^{\Delta NT/\Delta NT}$ (E) mice at P26. (**F-K**) Analysis of differentiated OL marker protein expression in P26 *Smo*$^{\Delta NT/\Delta NT}$ mice. Both Olig2 and Apc are reduced in both the DF and VF at this stage but to a lesser degree than at embryonic stages.

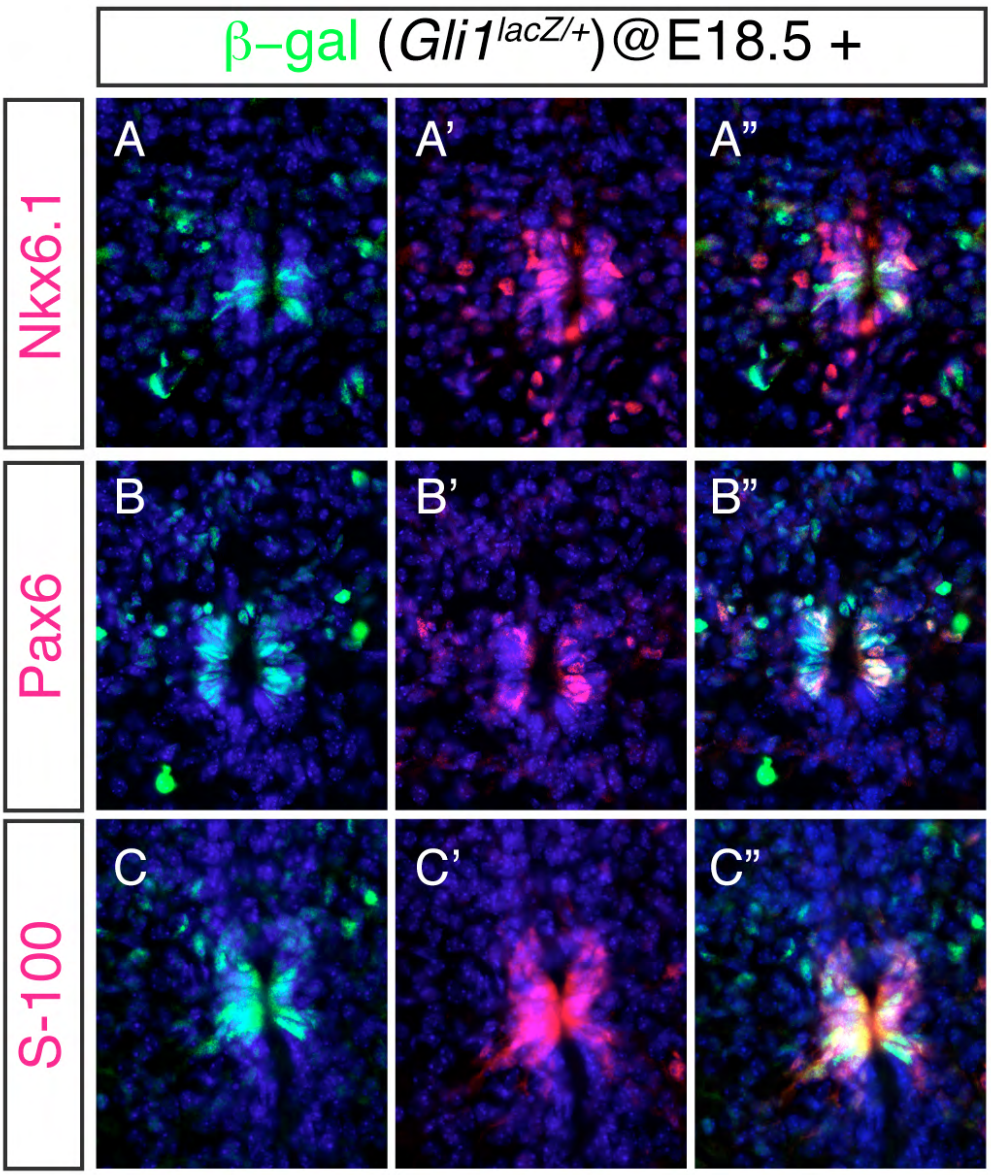

Fig. S8. Co-expression of Nkx6.1, Pax6 and S-100 in Gli1-lacZ^{+} VZ cells at E18.5. (**A-C″**) All three markers show strong overlap with β-gal (from a *Gli1^{lacZ}* knock-in line) in VZ cells (yellow). (A-C) β-gal; (A′-C′) marker; (A″-C″) merge; all with DAPI.