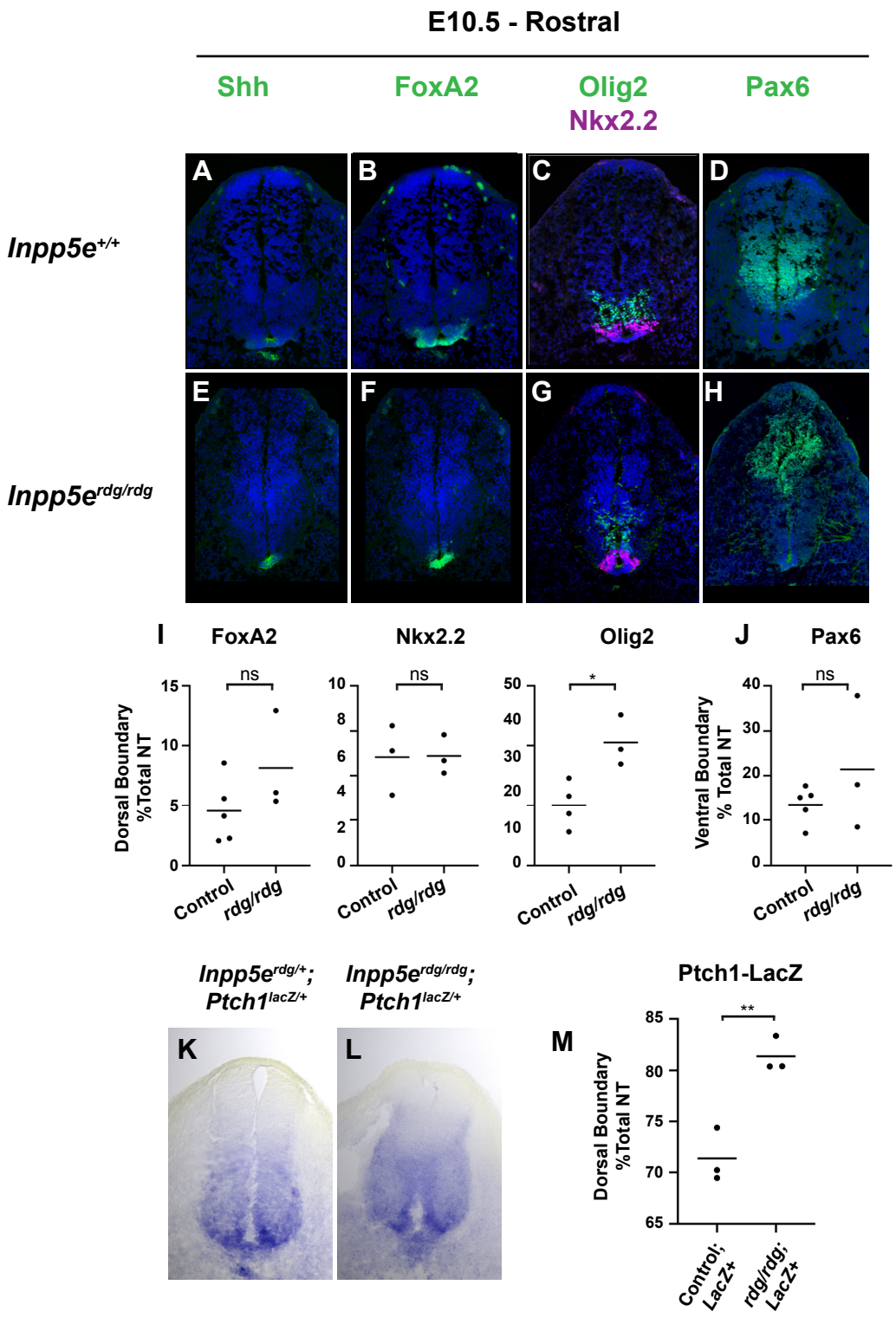

Figure S1. Response to Shh is varied along the rostral-caudal axis. Rostral (forelimb) sections of E10.5 neural tube. (A-C, E-G) Shh, FoxA2 and Nkx2.2 domains are comparable to control embryos (control n=5, *Inpp5e*$^{rdg/rdg}$ n=3). (C, G) Olig2 domain shows an expanded dorsal boundary. (I-J) Quantification of dorsal and ventral boundary as a percentage of total lumen distance. (K, L) Rostral (forelimb) neural tube sections of *Inpp5e*$^{rdg/+}$;*Ptch1*$^{LacZ/+}$ (n=3) and *Inpp5e*$^{rdg/rdg}$;*Ptch1*$^{LacZ/+}$ embryos (n=3) stained for β-galactosidase activity. (K-L) *Inpp5e*$^{rdg/rdg}$;*Ptch1*$^{LacZ/+}$ mutants display a dorsal expansion of β-galactosidase activity in the neural tube. (M) Quantification of K and L. Bar indicates mean of biological replicates. Analyzed by two tailed unpaired t-test with Welch's correction. *p<0.05, **p<0.01. ns, not significant.

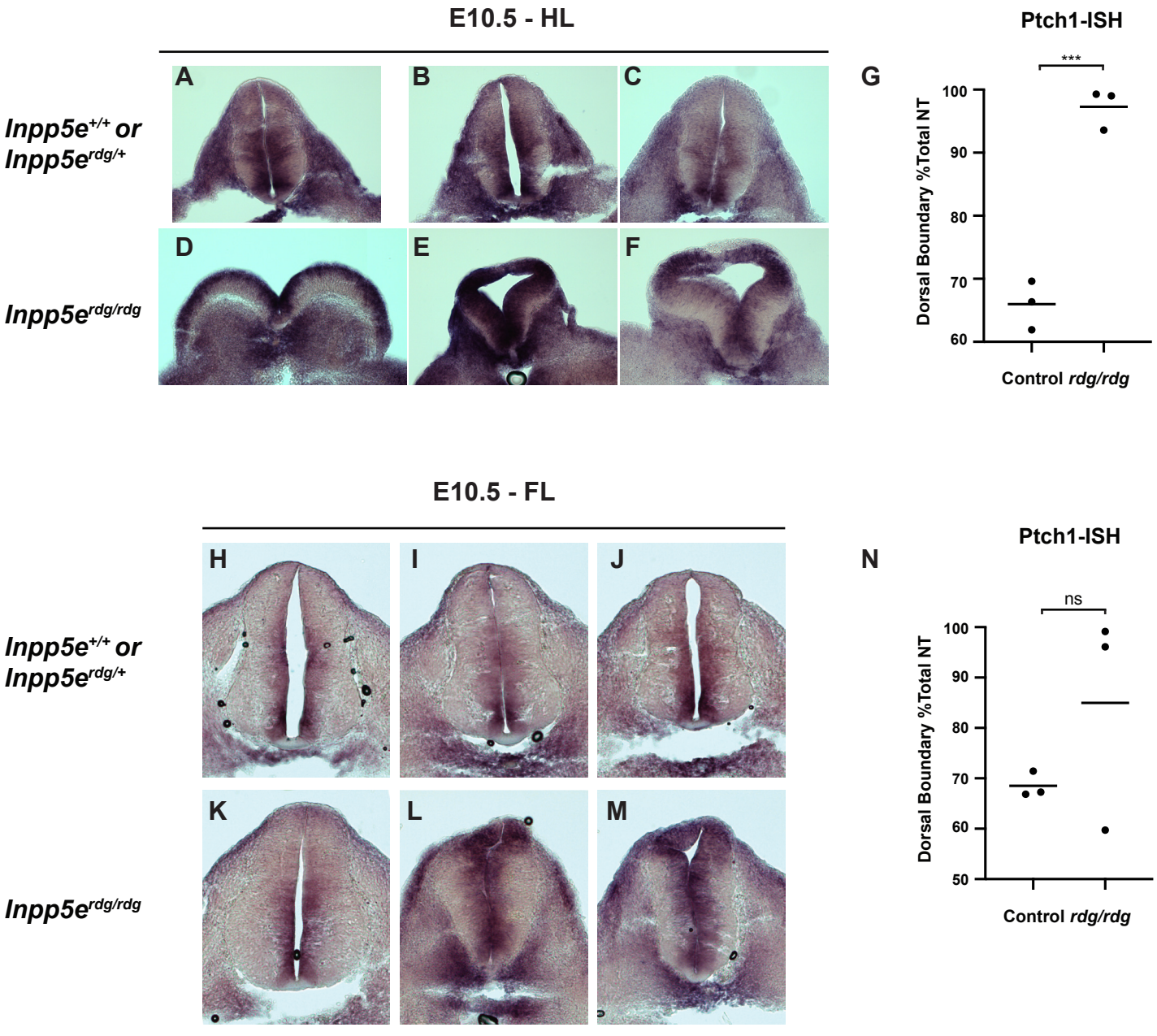

Figure S2. *Patched1* is expressed more dorsally in *Inpp5e*$^{rdg/rdg}$. *Patched1 in situ* hybridization of caudal (hindlimb) and rostral (forelimb) sections of E10.5 neural tube (control n=3, *Inpp5e*$^{rdg/rdg}$ n=3). (A-F) *Patched1* expression extends more dorsally in caudal sections of *Inpp5e*$^{rdg/rdg}$ embryos. (G) Quantification of dorsal boundary as a percentage of total lumen distance. (H-M) *Patched1* expression of rostral (forelimb) sections shows more variability in dorsal boundary. (M) Quantification of H-M. Bar indicates mean of biological replicates. Analyzed by two tailed unpaired t-test with Welch's correction. ***p<0.001. ns, not significant.

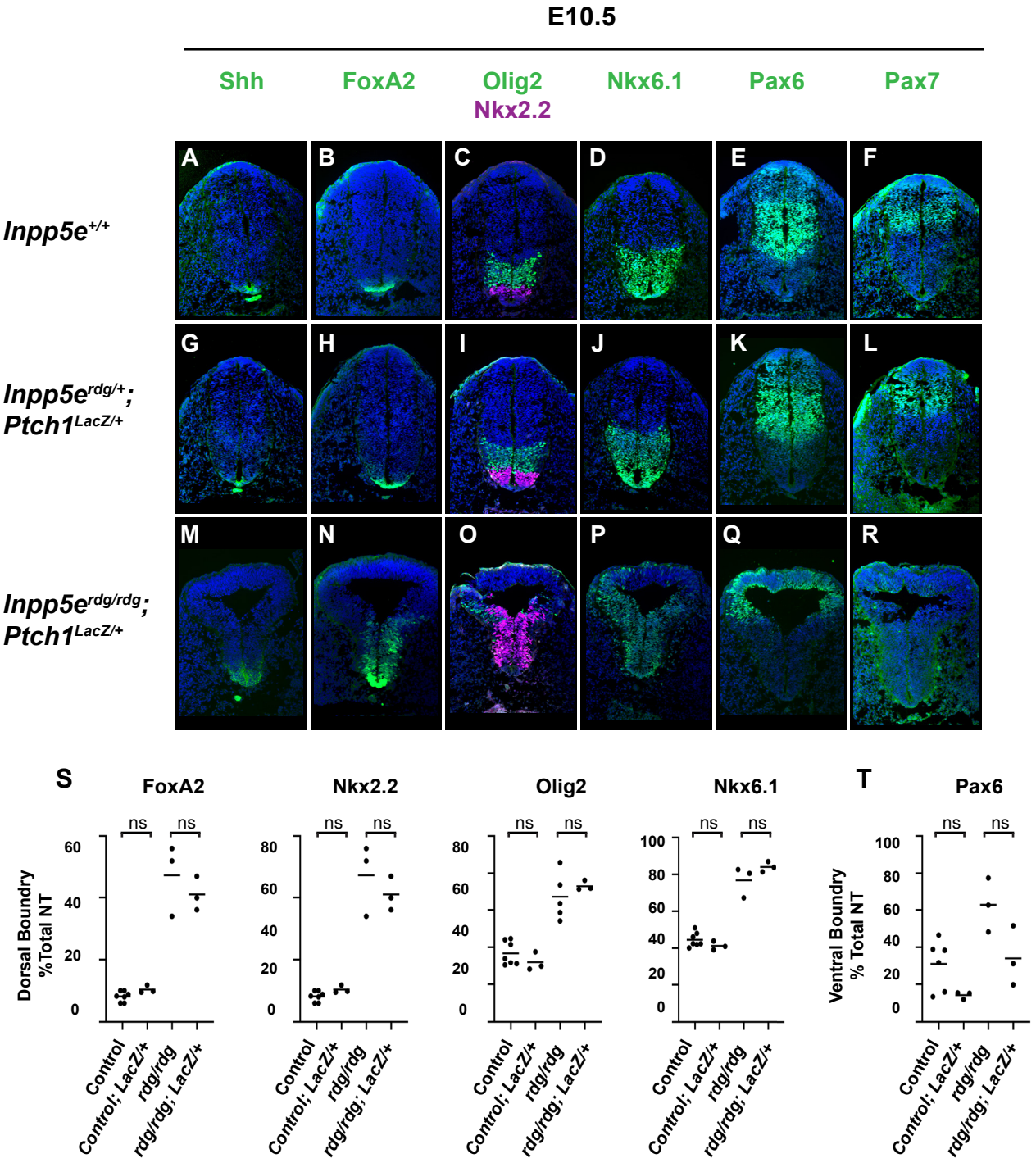

Figure S3. Ventral neural progenitor marker expression of *Inpp5e*$^{rdg/rdg}$; *Ptch1*$^{LacZ/+}$ mutant embryos is indistinguishable from *Inpp5e*$^{rdg/rdg}$ animals. Caudal (hindlimb) sections of E10.5 neural tube. (A-L) *Inpp5e*$^{rdg/+}$; *Ptch1*$^{LacZ/+}$ mutant embryos show similar expression of the cell markers indicated when compared to wild type (wild type n=2, *Inpp5e*$^{rdg/+}$; *Ptch1*$^{LacZ/+}$ n=3). (M-P) *Inpp5e*$^{rdg/rdg}$; *Ptch1*$^{LacZ/+}$ mutant embryos (n=3) show expanded expression of ventral cell fates compared to both wild type and *Inpp5e*$^{rdg/+}$; *Ptch1*$^{LacZ/+}$ mutant embryos. (Q) Ventral boundary of Pax 6 staining is dorsally shifted in *Inpp5e*$^{rdg/rdg}$; *Ptch1*$^{LacZ/+}$. (R) Pax7 staining is absent in *Inpp5e*$^{rdg/rdg}$; *Ptch1*$^{LacZ/+}$ embryos. (S-T) Quantitation of dorsal and ventral boundary as a percentage of total lumen distance. Bar indicates mean of biological replicates. Analyzed by ANOVA using Tukey correction for multiple comparisons. ns, not significant.

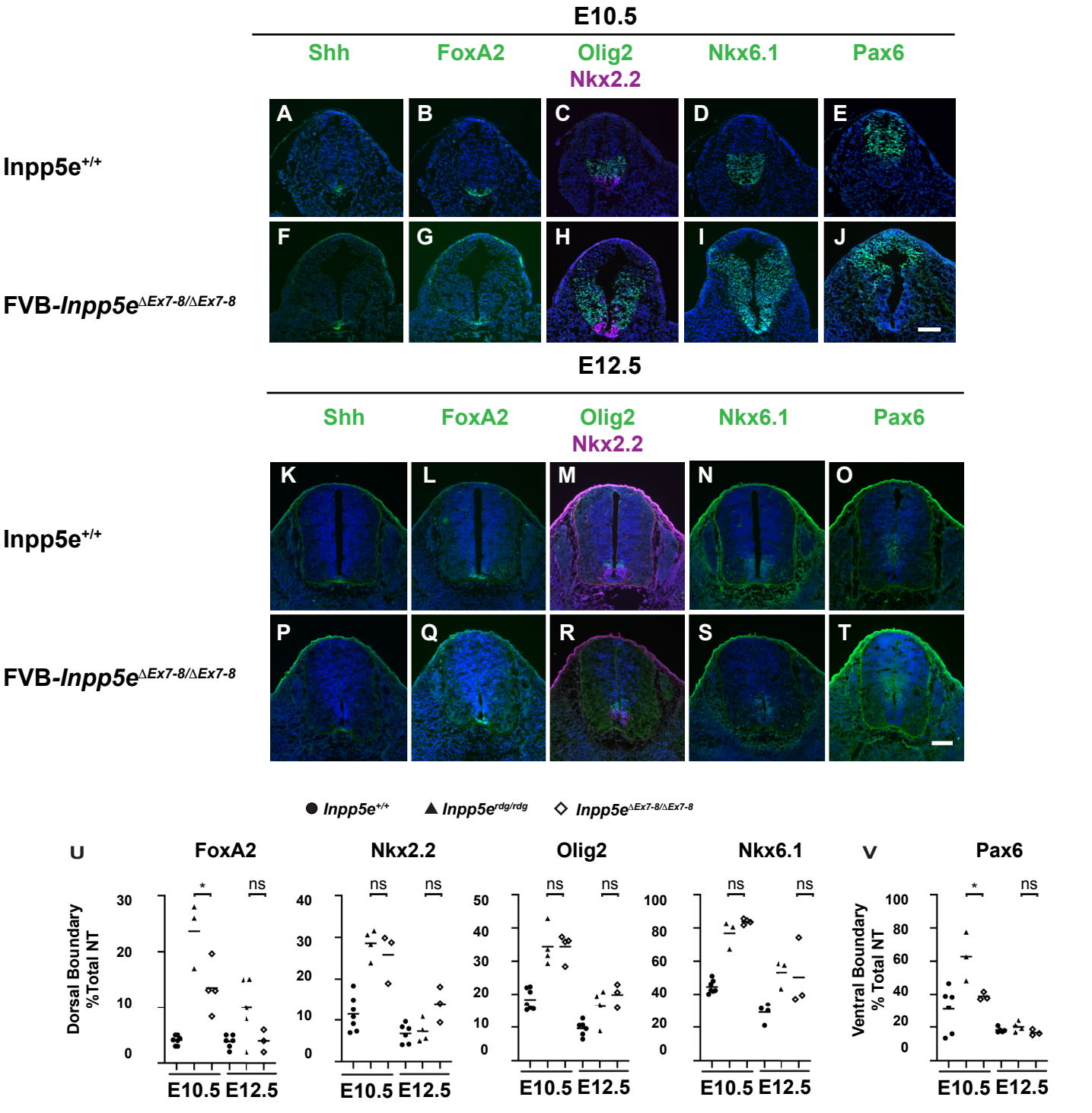

Figure S4. FVB-*Inpp5e*$^{\Delta Ex7\text{-}8/\Delta Ex7\text{-}8}$ embryos show expanded ventral cell fates at E10.5 and recovery at E12.5. Caudal (hindlimb) E10.5 and E12.5 neural tube sections stained with antibodies against indicated cell fates. (A-J) E10.5 FVB-*Inpp5e*$^{\Delta Ex7\text{-}8/\Delta Ex7\text{-}8}$ mutants show expanded ventral cell fates and Shh activity similar to *Inpp5e*$^{rdg/rdg}$ mutants (Fig. 1F-J) (control n=6, FVB-*Inpp5e*$^{\Delta Ex7\text{-}8/\Delta Ex7\text{-}8}$ n=4). (K-T) At E12.5, these expanded cell fates have returned to normal, with few cells scattered dorsally (control n=6, FVB-*Inpp5e*$^{\Delta Ex7\text{-}8/\Delta Ex7\text{-}8}$ n=3). Scale bars: 100μm. (U-V) Quantitation of dorsal and ventral boundary as a percentage of total lumen distance. Bar indicates mean of biological replicates analyzed by ANOVA using Tukey correction for multiple comparisons. Circles represent control, triangles indicate *Inpp5e*$^{rdg/rdg}$ and diamonds represent FVB-*Inpp5e*$^{\Delta Ex7\text{-}8/\Delta Ex7\text{-}8}$. *p<0.05. ns, not significant.

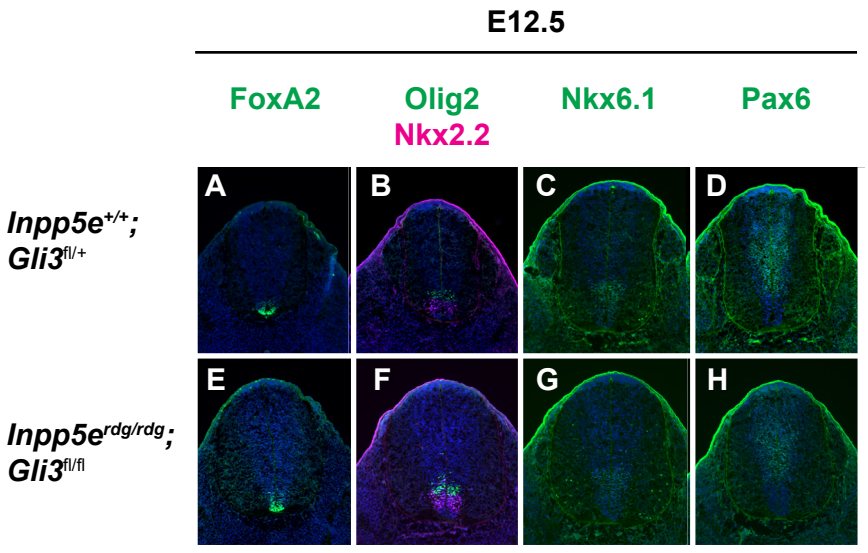

Figure S5. Gli3 background has no effect on *Inpp5e*$^{+/+}$ and *Inpp5e*$^{rdg/rdg}$ phenotype. Caudal (hindlimb) sections of E12.5 *Inpp5e*$^{+/+}$;*Gli3*$^{fl/+}$ (control) (A-D, n=2) and *Inpp5e*$^{rdg/rdg}$;*Gli3*$^{fl/fl}$ (E-H, n=2). Expression of markers in littermate control and *Inpp5e*$^{rdg/rdg}$;*Gli3*$^{fl/fl}$ sections is comparable to corresponding genotype in Fig 2.