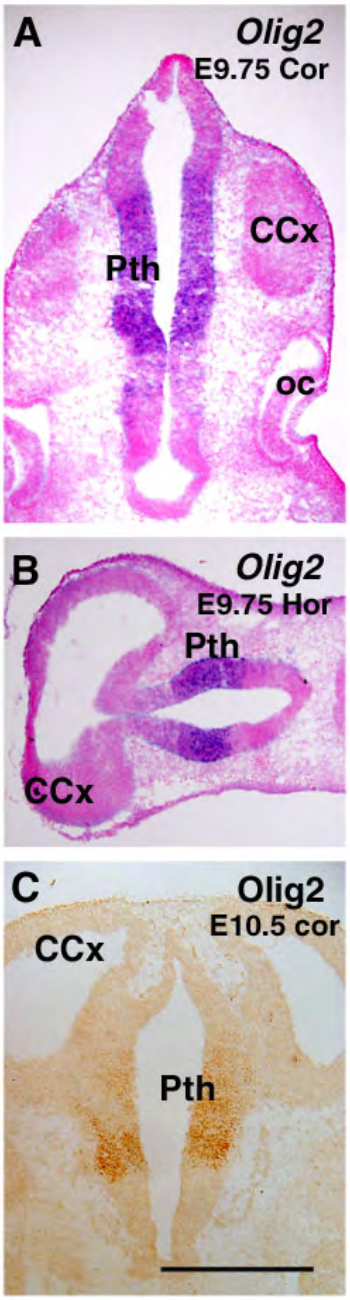
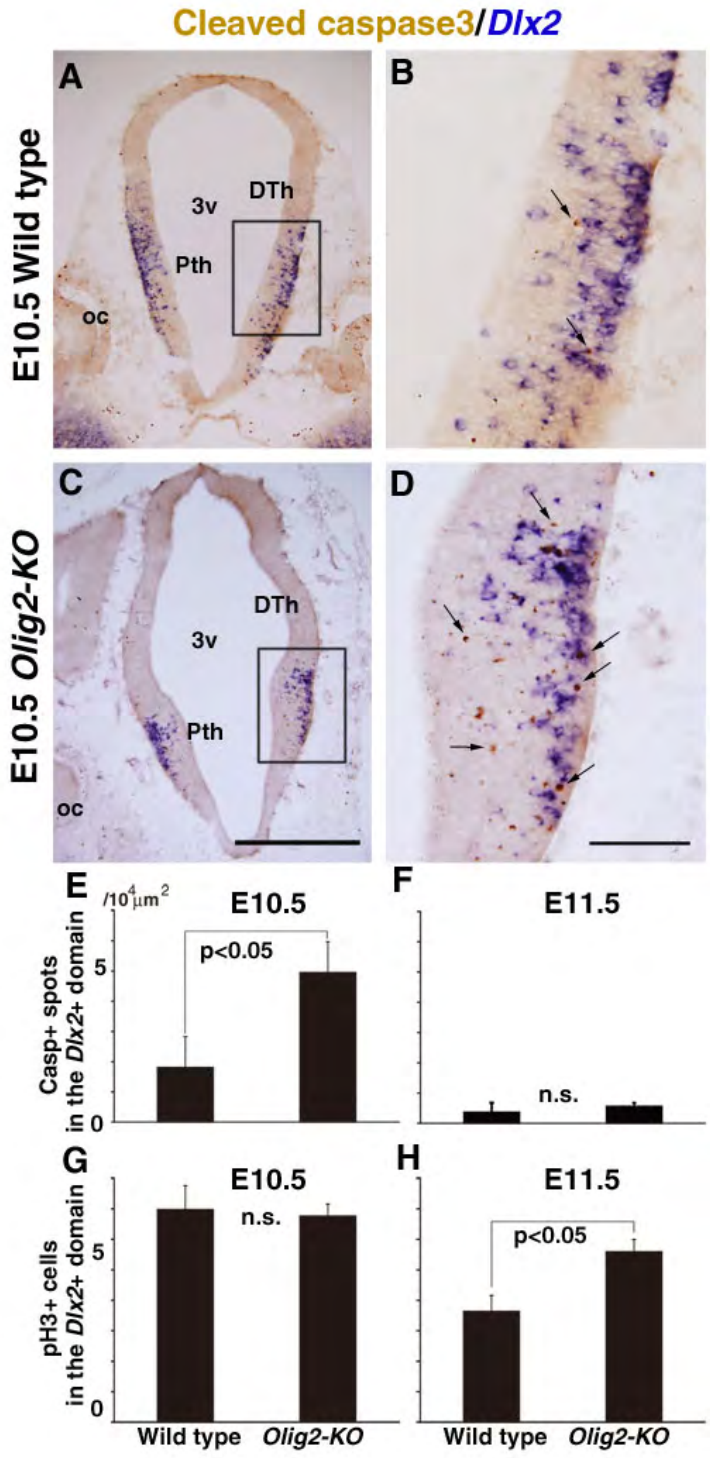
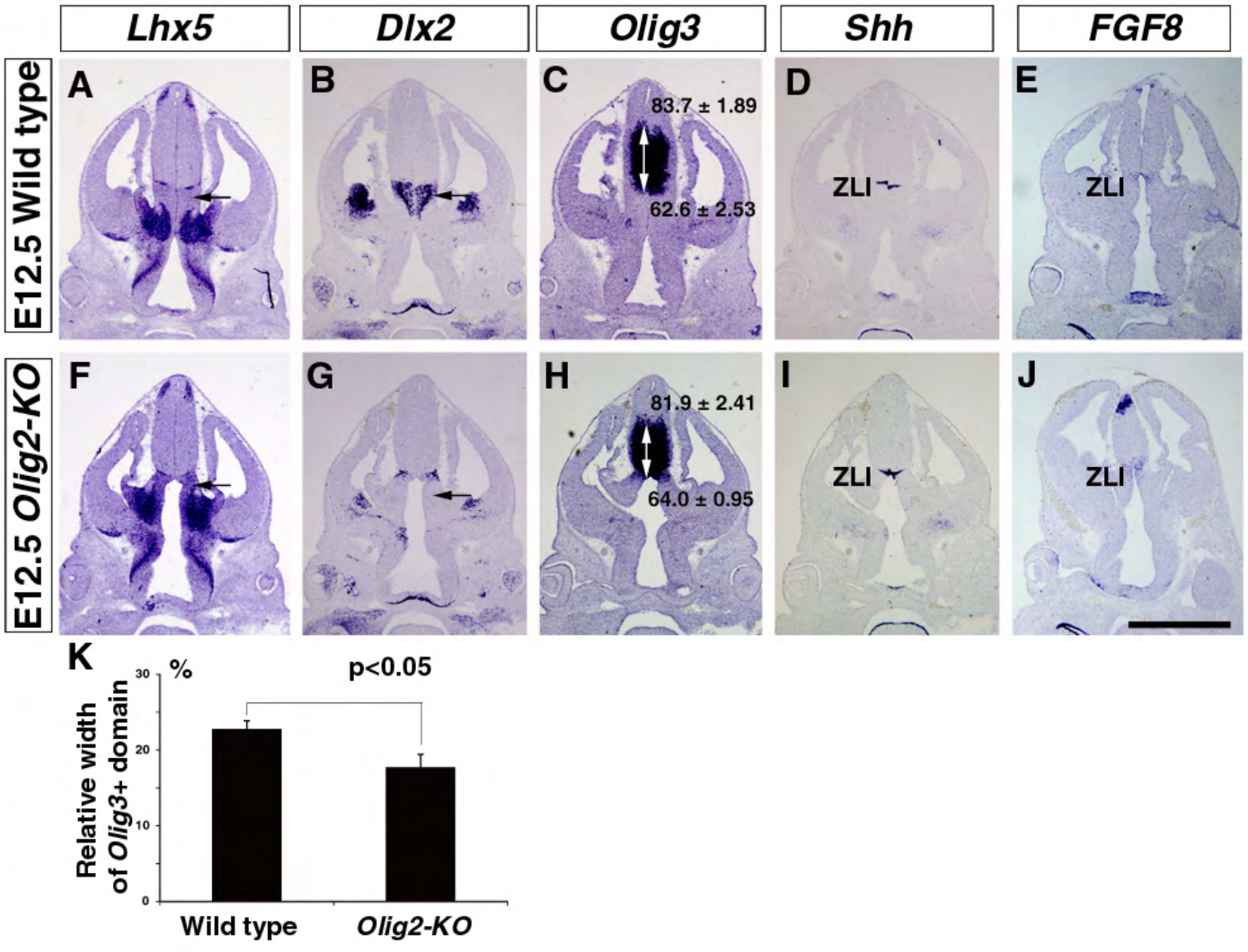


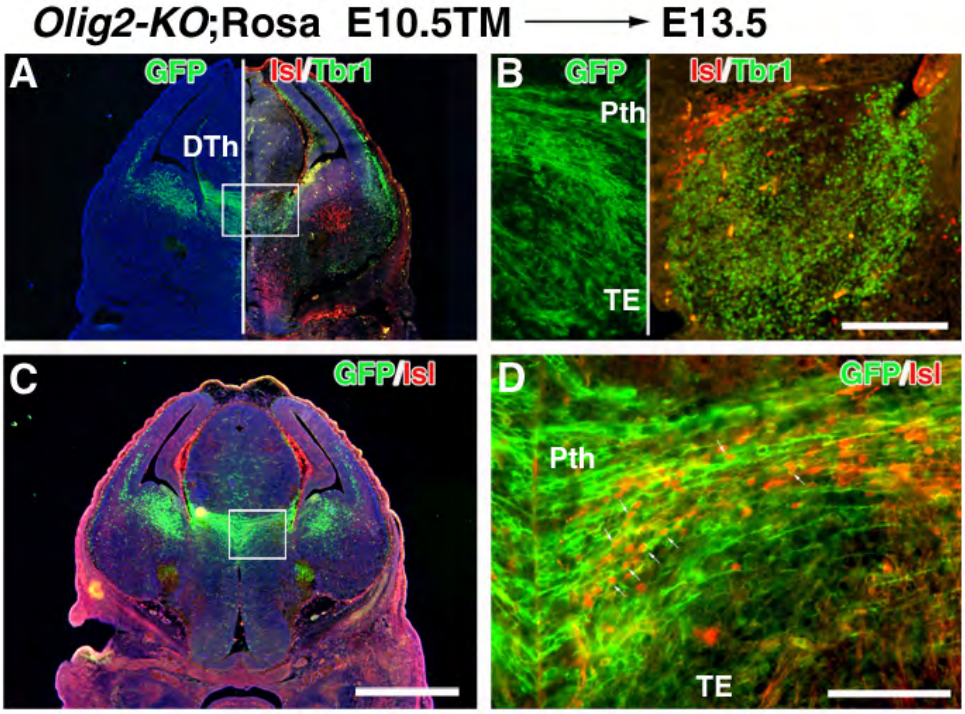
Supplementary Fig. 1 Expression of Olig2 in early diencephalon development
Expression of Olig2 was examined in the E9.75 (A,B) and E10.5 (C) diencephalon with *Olig2* ISH (dark blue) and Olig2 IHC (brown), respectively. A and C are coronal sections and B is a horizontal section. Note that Olig2 mRNA or protein is expressed in the diencephalon of these stages. Red in A and B is nuclear fast red for counter-staining. CCx, cerebral cortex. oc, optic cup. Pth, prethalamus. Bar in C = 500μm.



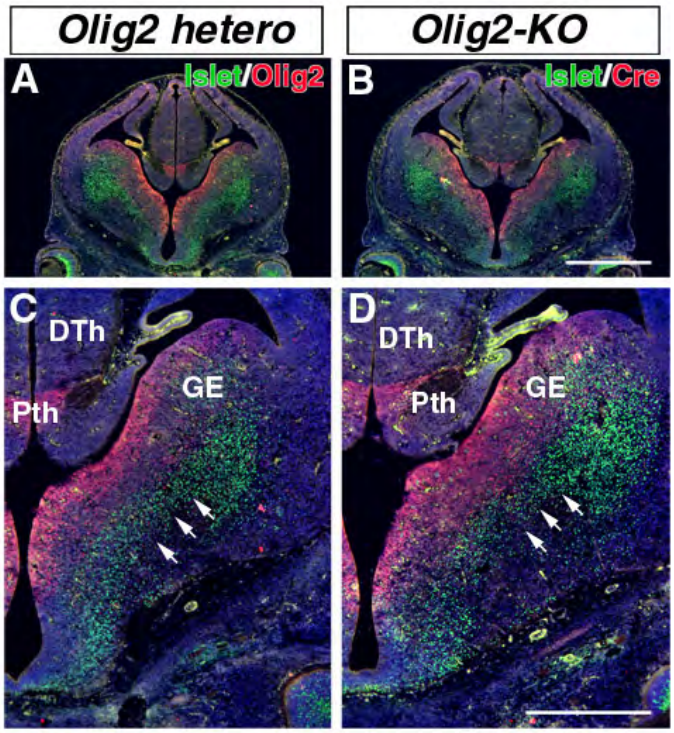
Supplementary Fig. 2 Apoptosis and proliferation in the prethalamus of *Olig2-KO* mice
A-D: E10.5 diencephalon of normal control (A,B) and *Olig2-KO* (C,D) was double-stained with *Dlx2* ISH and cleaved (activated) caspase-3 IHC. Caspase-3+ spots (arrows) were observed in the diencephalon, including the Dlx2+ prethalamus, and were more abundant in the *Olig2-KO* than in the control prethalamus. 3v, third ventricle. oc, optic cup. Bar in B = 200μm; in D = 100μm. E, F: Density of cleaved caspase-3+ spots in the E10.5 and E11.5 prethalamus. G,H: Density of pH3+ mitotic cells in the E10.5 and E11.5 prethalamus.



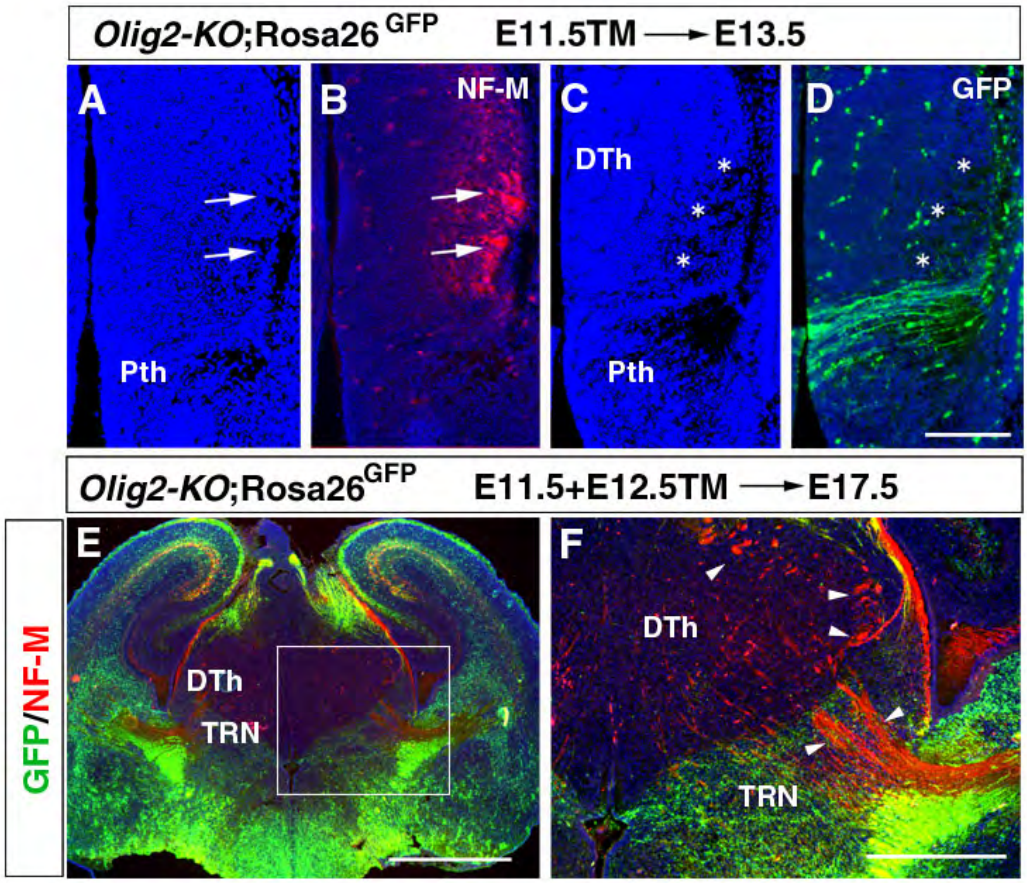
Supplementary Fig. 3 Expression of transcription factors and morphogen molecules in the E12.5 diencephalon
A-E: E12.5 wild-type. F-J: E12.5 *Olig2-KO*. Arrows in A, B, F, G indicate the prethalamus which shows hypoplasia. A, F: *Lhx5* is expressed in the dorsal border of the prethalamus and thalamic eminence while the main part of the prethalamus is devoid of *Lhx5* expression. In the *Olig2-KO* diencephalon, *Lhx5*-negative region is much smaller (F) than the control (A). B, G: *Dlx2* expression in the prethalamus. C, H: *Olig3* is mainly expressed in the dorsal thalamus. Double-direction arrows in images indicate an extend of Olig3+ domain, and numbers indicate the relative position of the dorsal and ventral borders, which are unchanged in *Olig2-KO* mouse (see text), while the relative width of the *Olig3*-expressing domain is slightly reduced in the *Olig2-KO* mouse (K). D, I: *Shh* expression is observed in the zona limitans intrathalamica (ZLI) in both wild-type and *Olig2-KO* mice. E, J: *FGF8* expression is mostly restricted to the rostralmost part of the diencephalon and ZLI shows very weak expression of *FGF8*, which is similar between wild-type and *Olig2-KO*. Bar = 1mm.



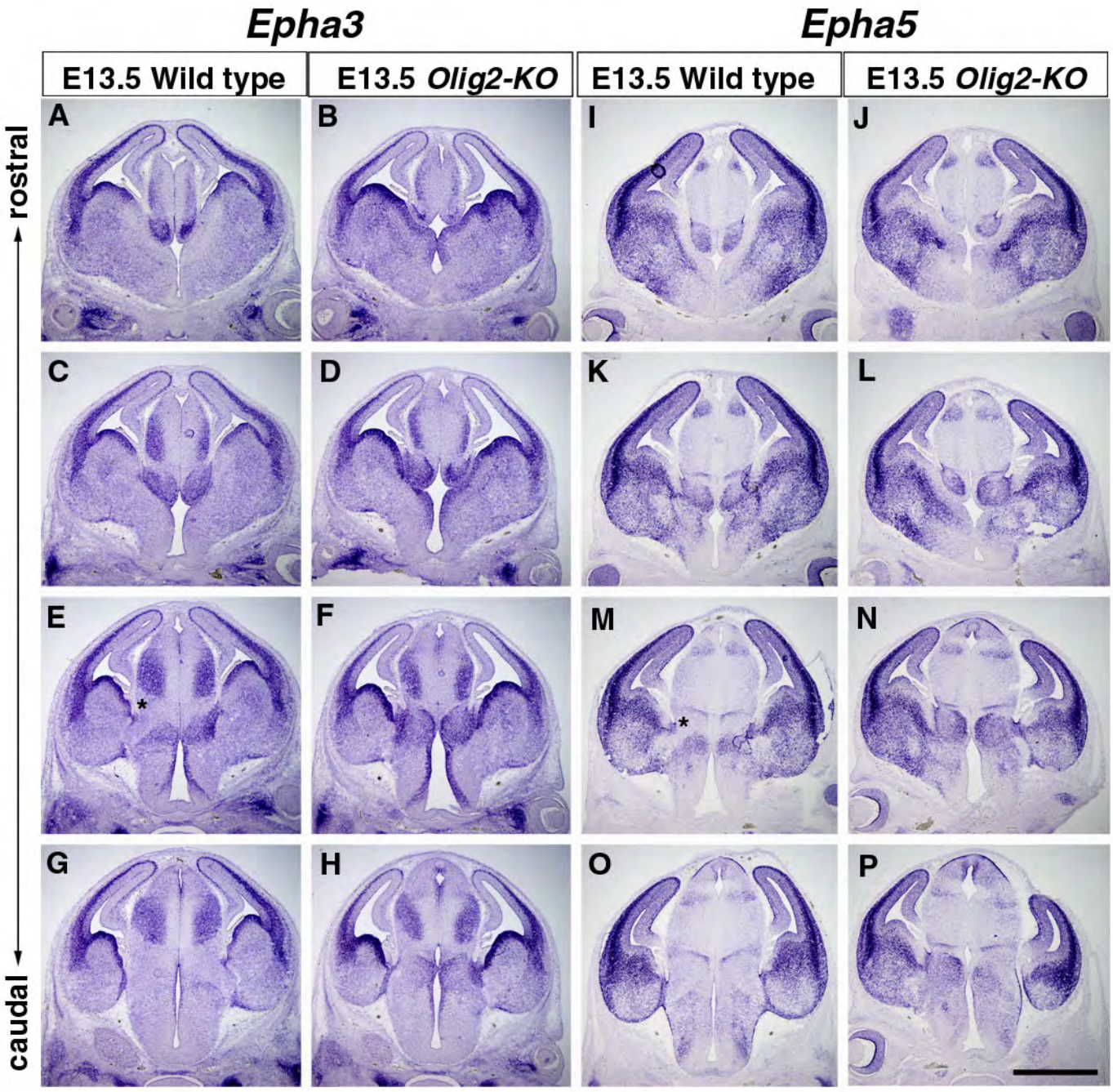
Supplementary Fig. 4 Dual phenotype of Olig2 lineage cells in the Olig2-KO diencephalon
E13.5 *Olig2-KO*;Rosa26^{GFP} mouse diencephalon with tamoxifen treatment at E10.5. Boxed areas in A and C are magnified in B and D, respectively. A, B: Composite pictures in which left and right halves are adjacent sections immunostained with GFP (green in left half), Islet1/2 (red in right half), and Tbr1 (green in right half) IHC. Note that GFP+ area shows Tbr1 and Islet1/2 expression. C, D: A section double-stained with anti-GFP and anti-Islet1/2 antibodies. Arrows in D indicate double-labeled cells. The results, together with those in Fig. 3, elucidate that Olig2 lineage cells in the *Olig2-KO* diencephalon are dual phenotypes, both prethalamus and thalamic eminence. Bars in C = 1mm; in D = 200μm.



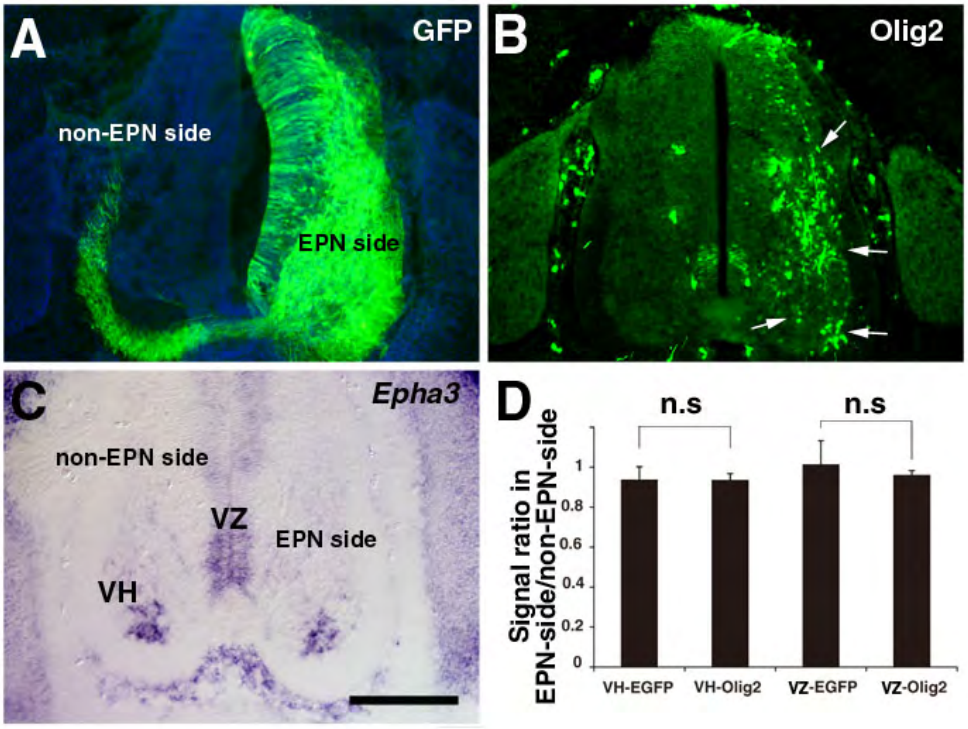
Supplementary Fig. 5 Normal arrangement of Islet1/2+ cells including corridor cells in the *Olig2-KO* ventral telencephalon
E13.5 forebrain is double-stained with Islet1/2 (green) and Olig2 (red in A and C) or CreER (red in B and D). Islet1/2+ cells are aligned dorsolaterally (arrows in C and D) in the ventral telencephalon, which contains corridor cells, in both Olig2 heterozygous and KO mice. Bars in B = 1 mm; in D =500μm.



Supplementary Fig. 6 Distribution of Olig2 lineage cells and axons in the prethalamus
A-D: E13.5 dorsal thalamus of the *Olig2-KO*;Rosa26^{GFP} mouse with E11.5 TM treatment is stained by NF-M (B) or GFP (D) immunohistochemistry. Nuclear staining with Hoechst reveals small cell-free zones (arrows in A) that are filled with NF-M+ axons (B). However, when the adjacent sections are stained with anti-GFP antibody, which labels Olig2 lineage cells and processes, GFP+ processes are observed in the prethalamus (Pth) and the marginal zone of the dorsal thalamus (DTh in C), but not in cell-free zones in the dorsal thalamus. E, F: E17.5 diencephalon of the *Olig2-KO*;Rosa26^{GFP} with tamoxifen treatment at E11.5 and E12.5. Most of the NF+ axons (red) in the dorsal thalamus are not labeled with GFP, so that disorganized axons are not extended from Olig2 lineage neurons. Bar in D = 200μm; in E = 1mm; in F = 500μm.



Supplementary Fig. 7 Details of *Epha3* and *Epha5* expression in the E13.5 diencephalon
Rostral to caudal arrangement of E13.5 forebrain stained with *Epha3* ISH (A-H) or with *Epha5* ISH (I-P) in the wild-type and *Olig2-KO* mice. Note that prethalamus in the wild-type is devoid of *Epha3* and *Epha5* expression that continues to the ventral telencephalon (asterisks in E and M). Such a *Epha*-negative region is missing in the *Olig2-KO* mouse. E, F, M and N are the same picture to Fig. 7A-D. Bar = 1mm.



Supplementary Fig. 8 No effect of ectopic Olig2 on *Epha3* expression
Olig2 expression vector was electroporated to the E3 chick neural tube together with EGFP expression vector, and the spinal cord was analyzed at E6 to identify whether *Epha3* expression was affected by ectopic overexpression of Olig2. A, B: Serial sections of the E6 spinal cord with co-electroporation of GFP and Olig2. Ectopic Olig2 was indicated by arrows, which includes ventral horn (VH). C: *EphA3* expression was observed in the ventral VZ and VH. D: Ratio of signal intensity between electroporated (EPN) side (Olig2 and GFP or GFP alone) and non-electroporated (non-EPN) side was nearly 1, which means that *Epha3* expression was unaffected by ectopic Olig2. Bar in C = 200μm.