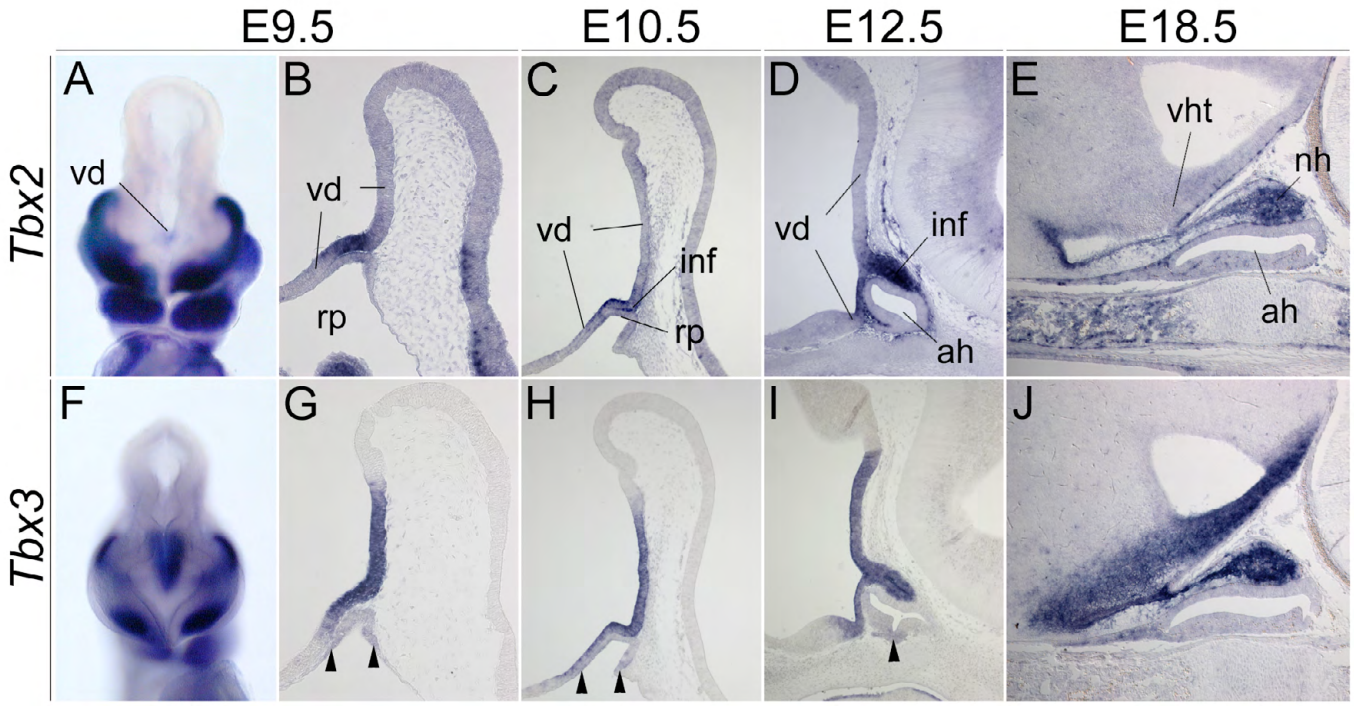


Fig. S1. Expression of *Tbx2* and *Tbx3* in the developing hypothalamus-pituitary axis. (**A-J**) Analysis by *in situ* hybridization of expression of *Tbx2* (A-E) and *Tbx3* (F-J) in whole heads (A,F) and on midsagital sections through the head (B-E,G-J) of wild-type embryos. Cranial is to the left. Expression of *Tbx2* is confined to a small sub-region in the midline of the ventral diencephalon, the infundibulum and the neurohypophysis. *Tbx3* expression overlaps with *Tbx2* but expands more posteriorly in the ventral diencephalon throughout all developmental stages analyzed. Black arrowheads in G-I indicate weak additional *Tbx3* expression in the ventral aspect of the developing adenohypophysis. ah, adenohypophysis; inf, infundibulum; nh, neurohypophysis; rp, Rathke's pouch; vd, ventral diencephalon; vht, ventral hypothalamus.

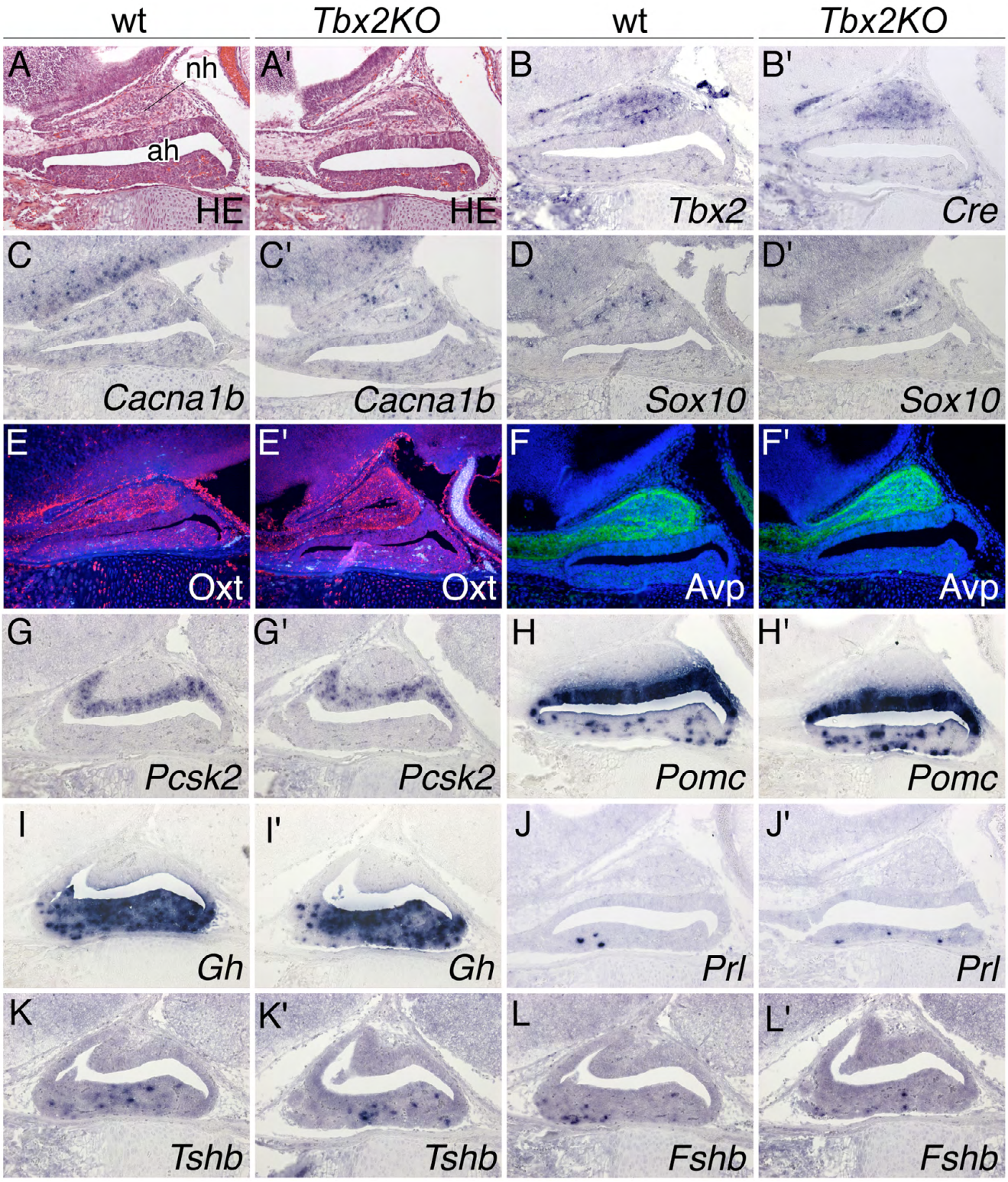


Fig. S2. Histological and molecular analysis of the pituitary gland in *Tbx2KO* embryos at E18.5. (**A-L'**) Midsagittal sections of E18.5 control (wt) and *Tbx2KO* embryos were analyzed by Hematoxylin and Eosin staining (HE), immunofluorescence of Oxt and Avp, and *in situ* hybridization of specific markers for the neurohypophysis (*Tbx2*, *Cacna1b*, *Sox10*) and for the endocrine lineages of the adenohypophysis (*Pcsk2* for melanotropes, *Pomc* for melanotropes and corticotropes, *Gh* for somatotropes, *Prl* for lactotropes, *Tshb* for thyrotropes, and *Fshb* for gonadotropes). All markers were unaltered in *Tbx2KO* embryos. ah, adenohypophysis; nh, neurohypophysis.

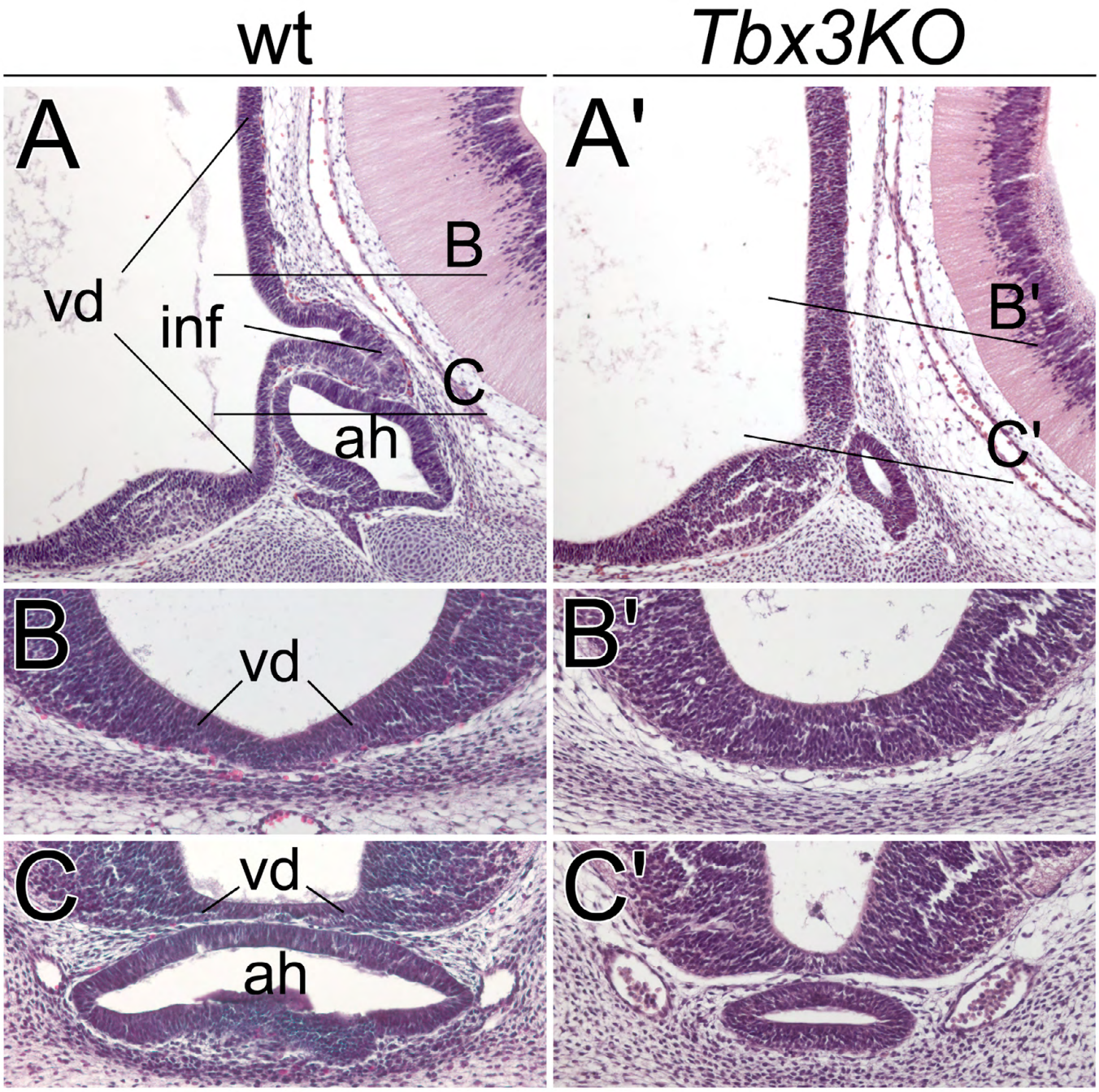


Fig. S3. Loss of *Tbx3* affects morphogenesis of the ventral diencephalon. (**A-C'**) Hematoxylin and Eosin staining of transverse sections of control (wt) and *Tbx3KO* embryos at E12.5. Section levels of B and C are indicated in A and of B′ asnd C′ in A′. ah, adenohypophysis; inf, infundibulum; vd, ventral diencephalon.

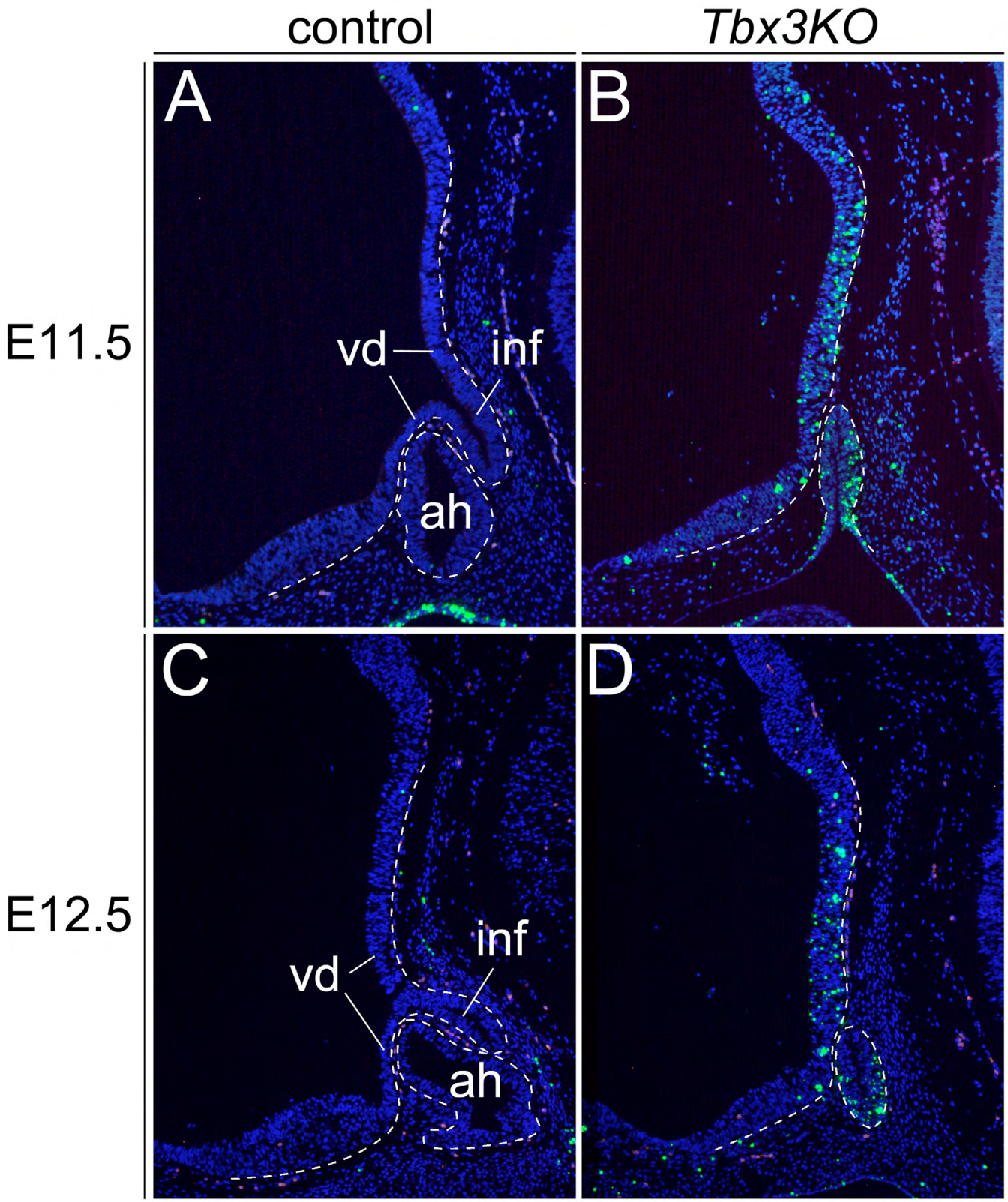


Fig. S4. Loss of *Tbx3* causes apoptosis in the ventral diencephalon and the developing adenohypophysis. (**A-D**) Analysis of cell death by the TUNEL assay on midsagittal sections of wild-type (control) and *Tbx3KO* embryos. Cranial is to the left. Stages and genotypes are indicated in the figure. Note the strong increase of apoptosis in the ventral diencephalon and Rathke's pouch in the mutant at both stages. ah, adenohypophysis; inf, infundibulum; vd, ventral diencephalon.

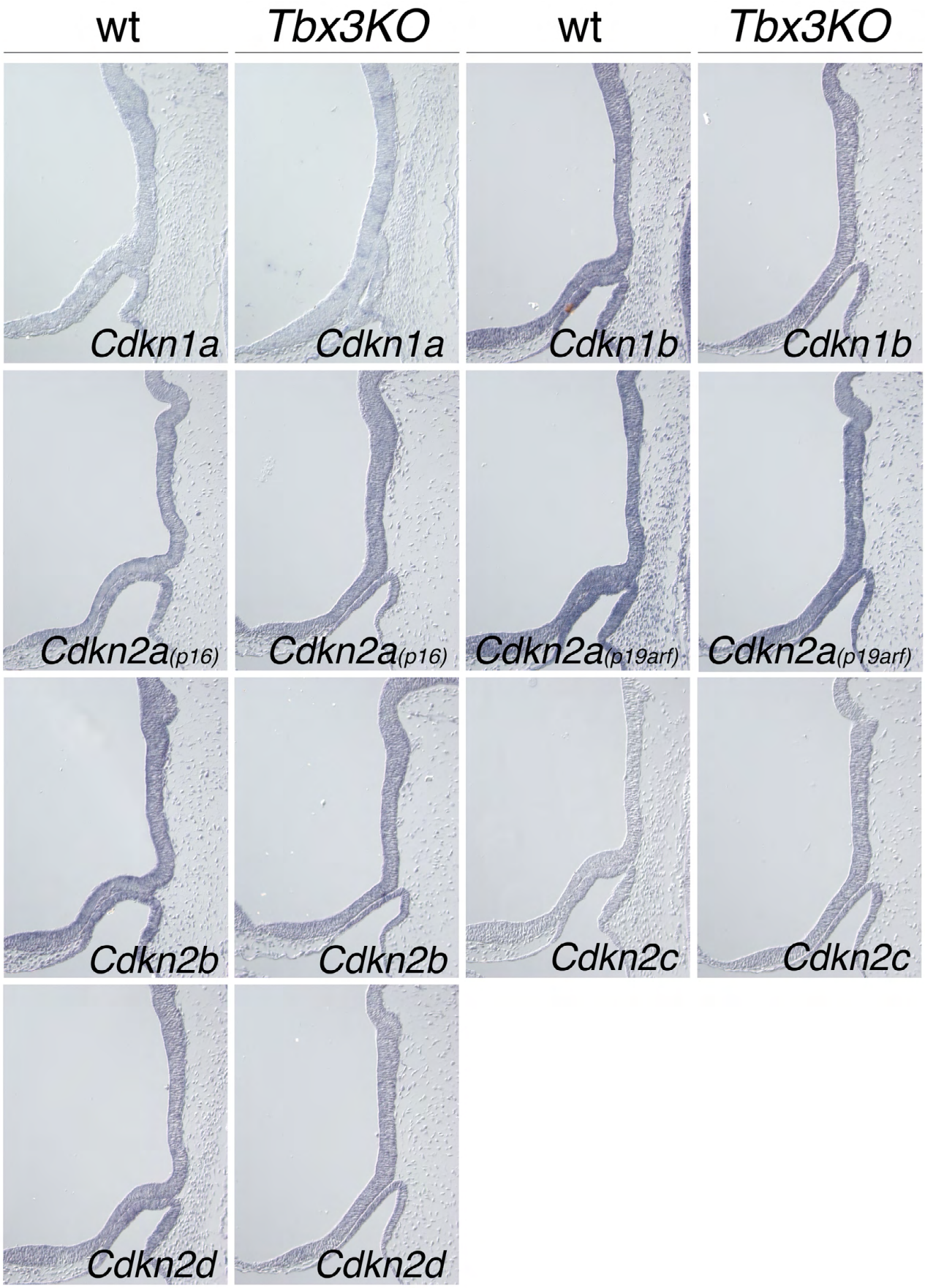


Fig. S5. Expression of cell cycle regulators in the developing ventral diencephalon in *Tbx3KO* embryos. *In situ* hybridization analysis on midsagittal sections of E10.5 control (wt) and *Tbx3KO* embryos. Cranial is to the left. All molecular markers are as indicated in the figure.

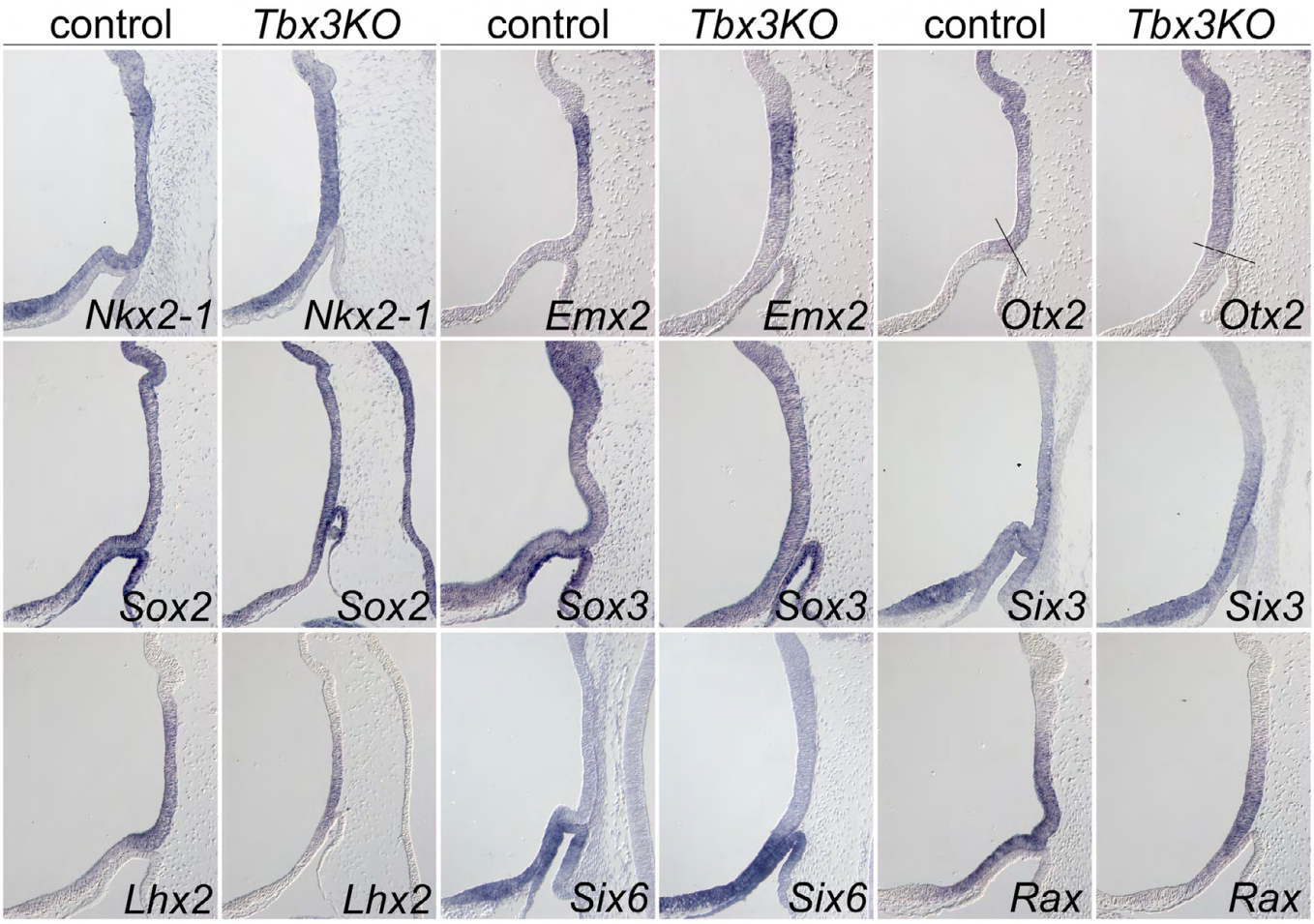
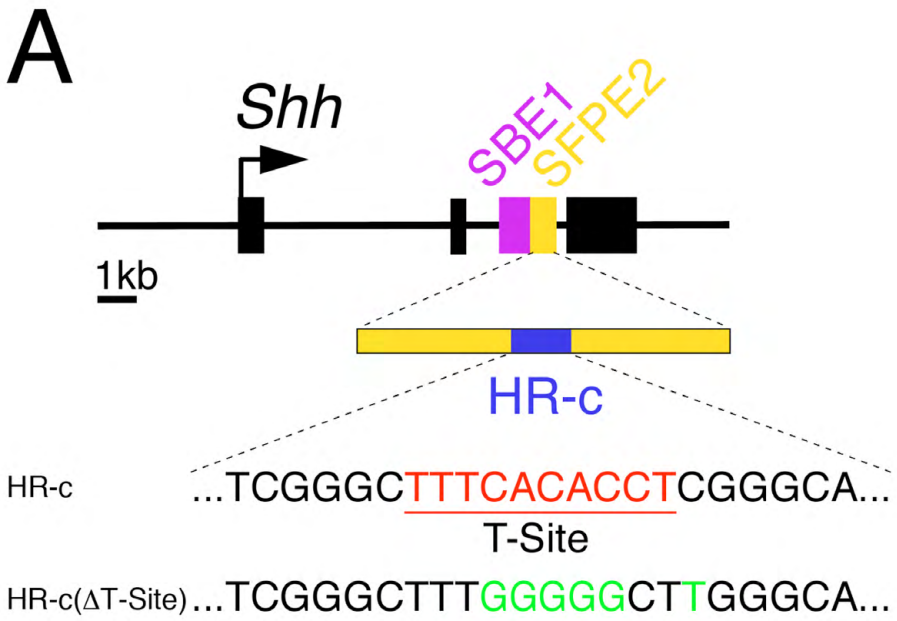
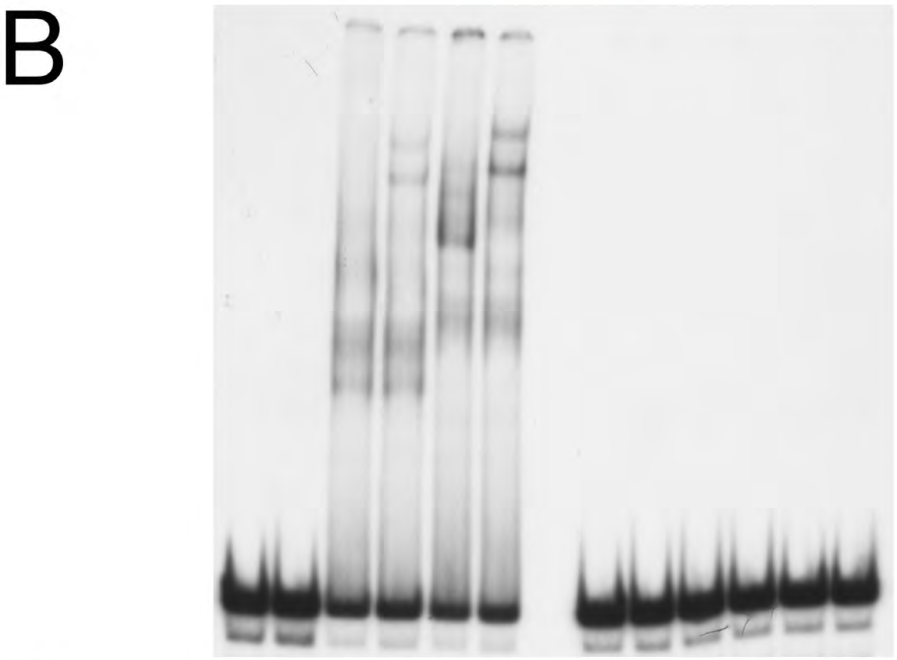


Fig. S6. Molecular analysis of the developing ventral diencephalon in *Tbx3KO* embryos. *In situ* hybridization analysis on midsagittal sections of E10.5 control and *Tbx3KO* embryos. Cranial is to the left. All molecular markers are as indicated in the figure.





HR-c	+	+	+	+	+	+							
HR-c(ΔT-Site)								+	+	+	+	+	+
unpr. Lysate		+							+				
Tbx2.Myc			+	+						+	+		
Tbx3.Myc					+	+						+	+
anti-Myc				+		+					+		+

Fig. S7. Tbx2 and Tbx3 bind to the regulatory element of *Shh* expression, *SFPE2*. (**A**) Schematic of the *Shh* locus including the conserved T-Site within the homology region c (*HR-c*) of the *SFPE2* element. Exons are depicted in black (adapted from Jeong and Epstein, 2003). The T-site in HR-c is marked in red. Point mutations in the T-site of a mutated *HR-c* fragment without T-Site, also termed *HR-c(ΔT-Site)* (Jeong and Epstein, 2003), are marked in green. (**B**) Electrophoretic mobility shift assay demonstrates binding of TBX2 and TBX3 to the wild-type *HR-c* DNA fragment of *SFPE2 in vitro* but not to one with a mutated T-Site [*HR-c(ΔT-Site)*]. *HR-c*, homology region c of *SFPE2*; *SBE*1, sonic hedgehog brain enhancer 1; *SFEP2*, sonic hedgehog floor plate enhancer 2.