Expanded View Figures

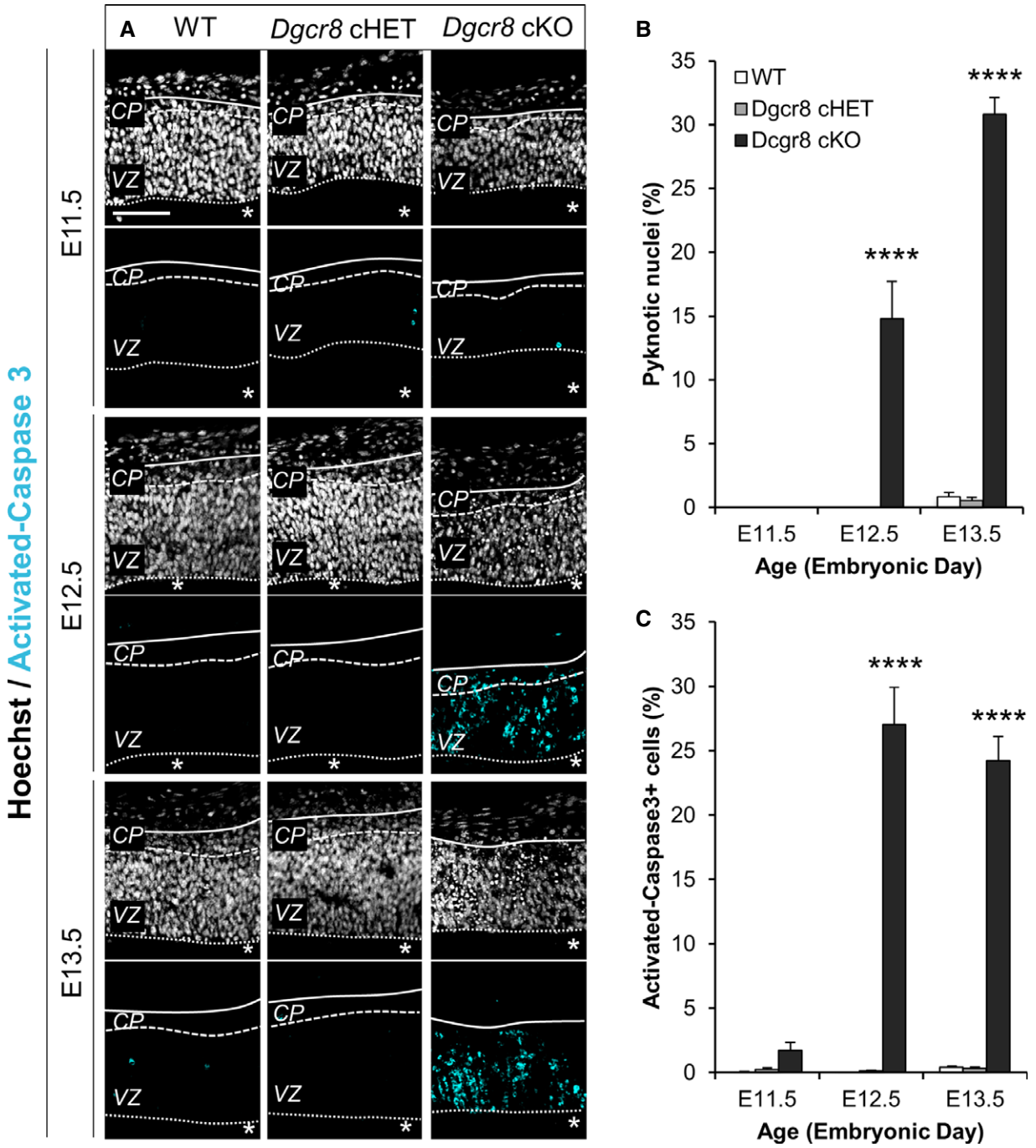


Figure EV1.

Figure EV1. Increased apoptosis in the dorsal telencephalon of *Dgcr8* cKO embryos from E12.5.

A Fluorescence microscopy images of coronal cryosections through the dorsal telencephalon of E11.5–E13.5 WT, *Dgcr8* cHET, and cKO mouse embryos stained for nuclear DNA with Hoechst (white) and anti-activated caspase-3 (cyan) antibody. Scale bar: 50 μm. Asterisks indicate the ventricular lumen. Solid and dashed lines indicate cortex boundaries. Cortical plate (CP); ventricular zone (VZ).

B Quantification of pyknotic nuclei in the cortical wall, expressed as a percentage of the total Hoechst-stained nuclei.

C Quantification of activated caspase-3$^+$ cells in the cortical wall, expressed as a percentage of the total Hoechst-stained nuclei.

Data information: Bars are mean ± SEM of three embryos per condition (18 counted fields per condition). Two-way ANOVA followed by Tukey's *post hoc* test, ****$P < 0.0001$.

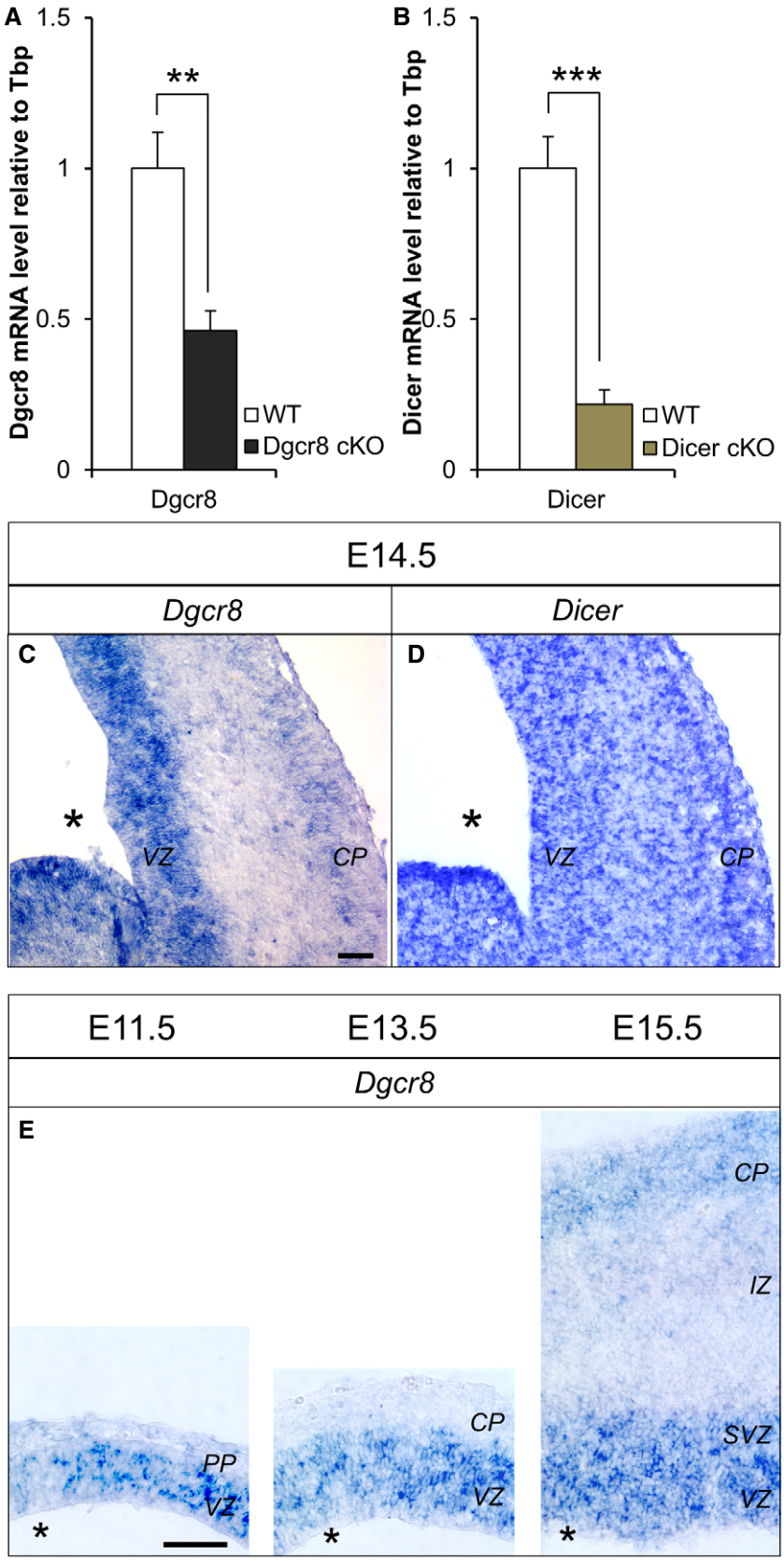


Figure EV2. Quantification of *Dicer* and *Dgcr8* transcript levels in WT and cKO cortices and their expression patterns in WT cortices.

A, B qRT–PCR for *Dgcr8* (A) or *Dicer* (B) transcripts in sorted (Tomato$^+$) cells from E13.5 WT, *Dgcr8*, or Dgcr8 cKO cortices. Bars are mean ± SEM of three embryos per condition (18 counted fields per condition). Unpaired Student's *t*-test, $^{**}P < 0.01$; $^{***}P < 0.001$.

C–E *In situ* hybridization of probes for *Dgcr8* (C, E) and *Dicer* (D) in coronal cryosections through the telencephalon of WT embryos at the indicated developmental times. Scale bars: 500 μm (C, D) and 200 μm (E). Asterisks indicate the ventricular lumen. Solid and dashed lines indicate cortex boundaries. Preplate (PP); cortical plate (CP); subventricular zone (SVZ); ventricular zone (VZ).

Figure EV3. Increased proportion of Tbr1$^+$ neurons and no change in total apical and basal progenitors proportions in E13.5 *Dgcr8* cKO cortices.

A–C Immunofluorescence microscopy of coronal cryosections through the dorsal telencephalon of E13.5 WT (A), *Dgcr8* cHET (B), and cKO (C) littermate mouse embryos, showing Pax6 (red) staining.

D Quantification of the proportion of Pax6$^+$ apical progenitors in the images shown in (A–C), expressed as a percentage of the total Hoechst-stained nuclei (not shown).

E–G Immunofluorescence microscopy of coronal cryosections through the dorsal telencephalon of E13.5 WT (E), *Dgcr8* cHET (F), and cKO (G) littermate mouse embryos, showing Tbr2 (magenta) staining.

H Quantification of the proportion of Tbr2$^+$ basal progenitors in the images shown in (E–G), expressed as a percentage of the total Hoechst-stained nuclei (not shown).

I–K Immunofluorescence microscopy of coronal cryosections through the dorsal telencephalon of E13.5 WT (I), *Dgcr8* cHET (J), and cKO (K) littermate mouse embryos, showing Tbr1 (green) staining.

L Quantification of the proportion of Tbr1$^+$ neurons in the images shown in (I–K), expressed as a percentage of the total Hoechst-stained nuclei (not shown).

M, N Immunostaining for Nestin$^+$ radial glia cells through the dorsal telencephalon of E13.5 WT (M) and *Dgcr8* cKO (N) littermate mouse embryos. White arrowheads indicate the disruption of the apical surface organization of the radial glia.

Data information: Scale bars: 50 μm. Asterisks indicate the ventricular lumen. Solid and dashed lines indicate cortex boundaries. Cortical plate (CP); subventricular zone (SVZ); ventricular zone (VZ). Apoptotic nuclei are excluded from all quantifications. Bars are mean ± SEM of three embryos per condition (18 counted fields per condition). One-way ANOVA, ****$P < 0.0001$; n.s., not significant.

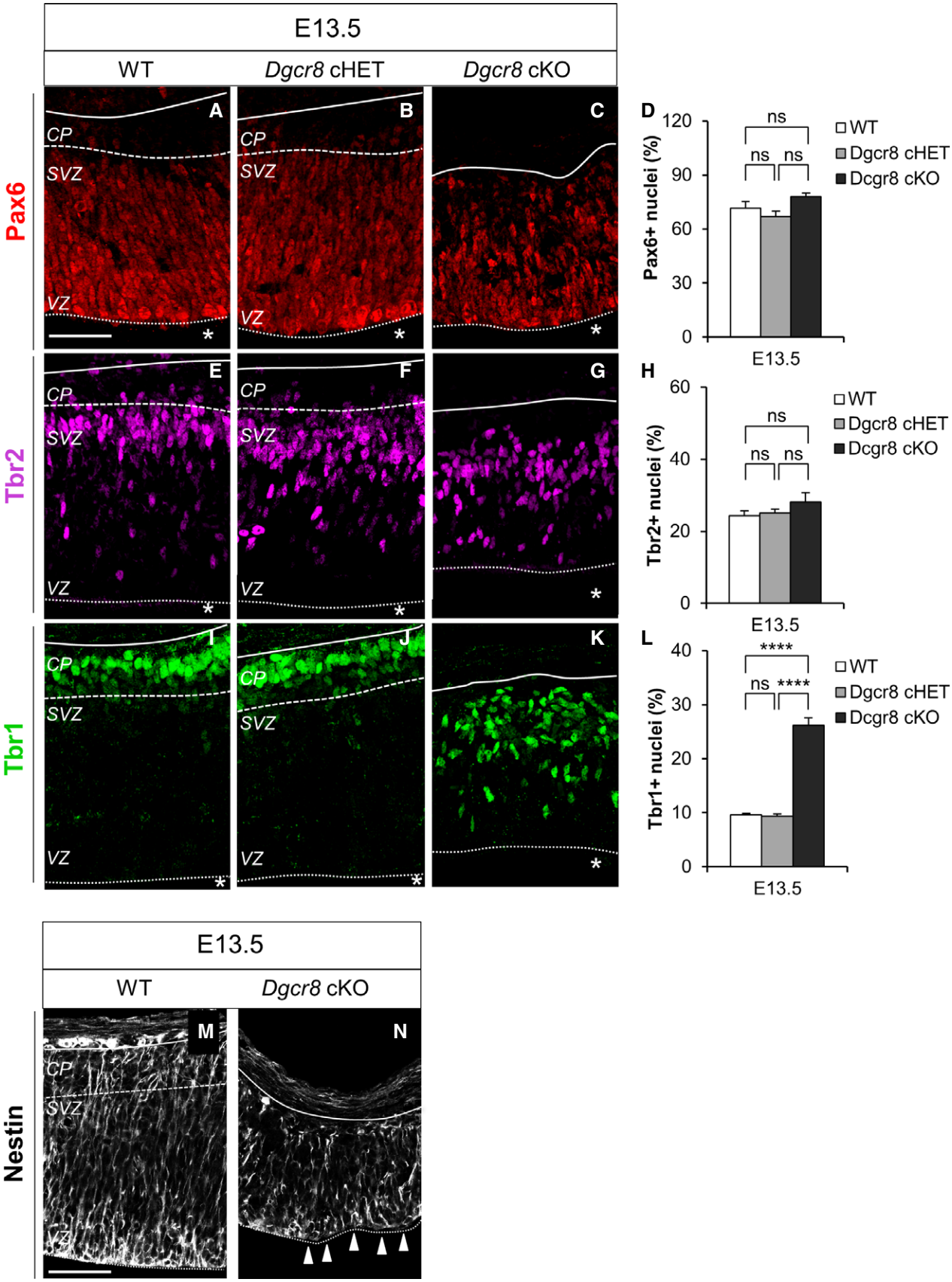
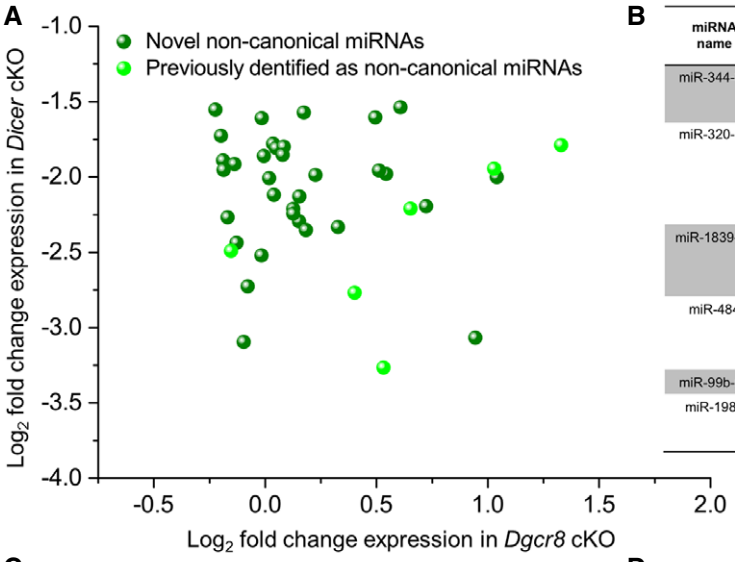

E13.5
WT
Dgcr8 cHET
Dgcr8 cKO
Pax6
Tbr2
Tbr1
Nestin
CP
SVZ
VZ
A
B
C
D
E
F
G
H
I
J
K
L
M
N
Pax6+ nuclei (%)
Tbr2+ nuclei (%)
Tbr1+ nuclei (%)
ns
****
Dcgr8 cKO

Figure EV3.

Figure EV4. Classification of DGCR8 and/or DICER-responsive miRNAs in deep-sequenced small RNA libraries from E13.5 WT, *Dgcr8*, and *Dicer* cKO cortices.

A, B Deep-sequencing $\log_2$ fold-change data of Dicer-responsive, DGCR8 non-responsive (i.e., non-canonical) miRNAs. Axes indicate read counts from cKO library over read counts from WT library. Reads are normalized to snoRNA reads in each library. Light green points (A) and table in (B) indicate previously identified non-canonical miRNAs.

C–H Representative read counts of the non-canonical miRNAs identified in this study. Data are the mean of 2 libraries per condition; error bars indicate the mean variation of the reads in libraries from the two cortices (SEM). One-way ANOVA, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; n.s., not significant.



B

miRNA name	Class	Log$_2$ fold change *Dicer* cKO	Log$_2$ fold change *Dgcr8* cKO	Sample type	PubMed evidences
miR-344-3p	shRNA	-1.79	1.33	ES	Babiarz et al., 2008; Castellano et al., 2013
miR-320-3p	shRNA	-1.94	1.03	ES, Brain	Wang et al., 2007; Babiarz et al., 2008; Babiarz et al., 2011; Moltzahn et al., 2011; Castellano et al., 2013
miR-1839-5p	snoRNA	-2.21	0.65	ES, Brain	Babiarz et al., 2008; Babiarz et al., 2011; Castellano et al., 2013
miR-484	shRNA	-2.77	0.40	ES	Babiarz et al., 2008; Moltzahn et al., 2011; Castellano et al., 2013
miR-99b-5p		-2.49	-0.15	ES	Wang et al., 2007
miR-1983	tRNA shRNA	-3.27	0.53	ES	Babiarz et al., 2008; Castellano et al., 2013

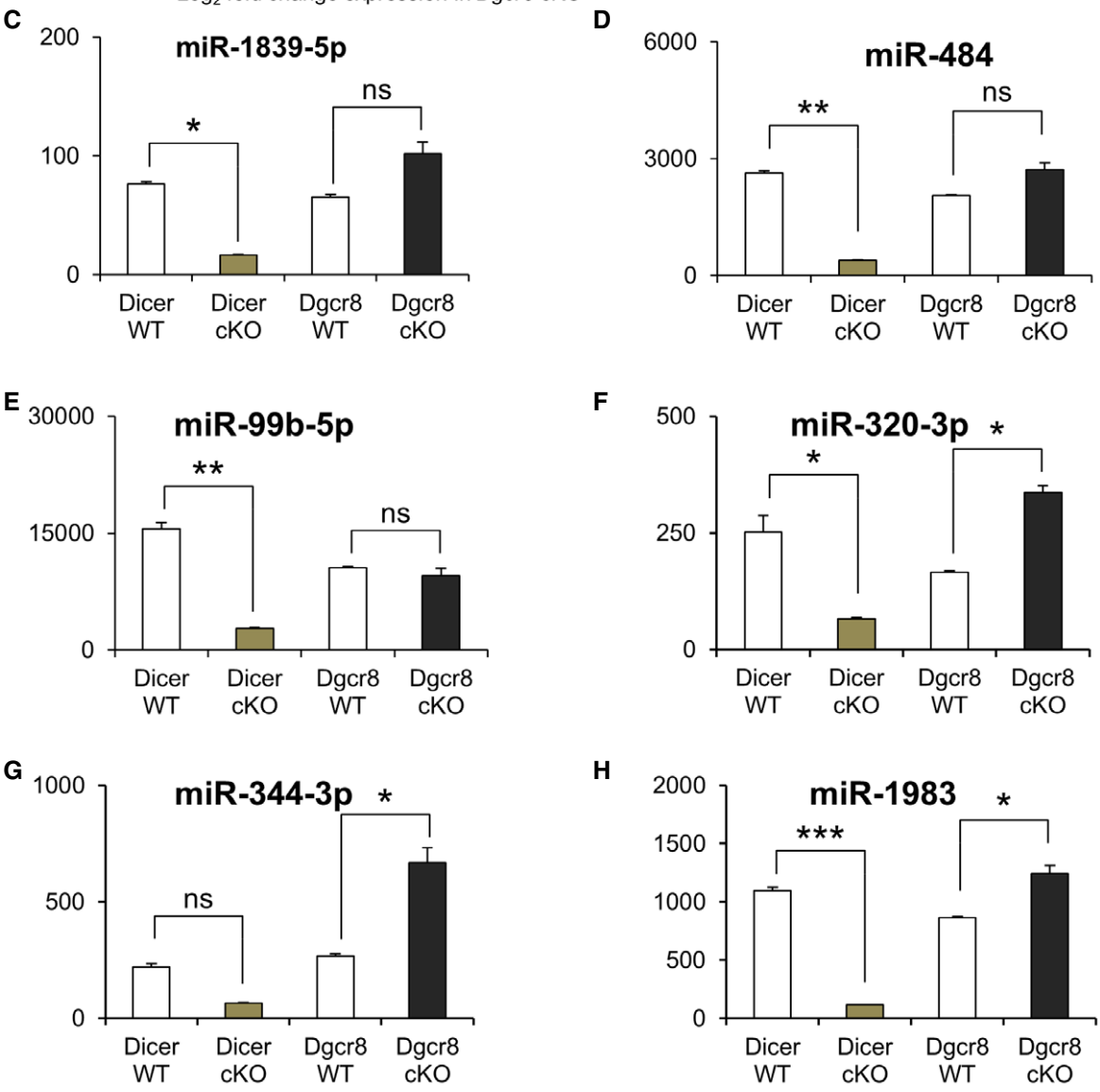


Figure EV4.

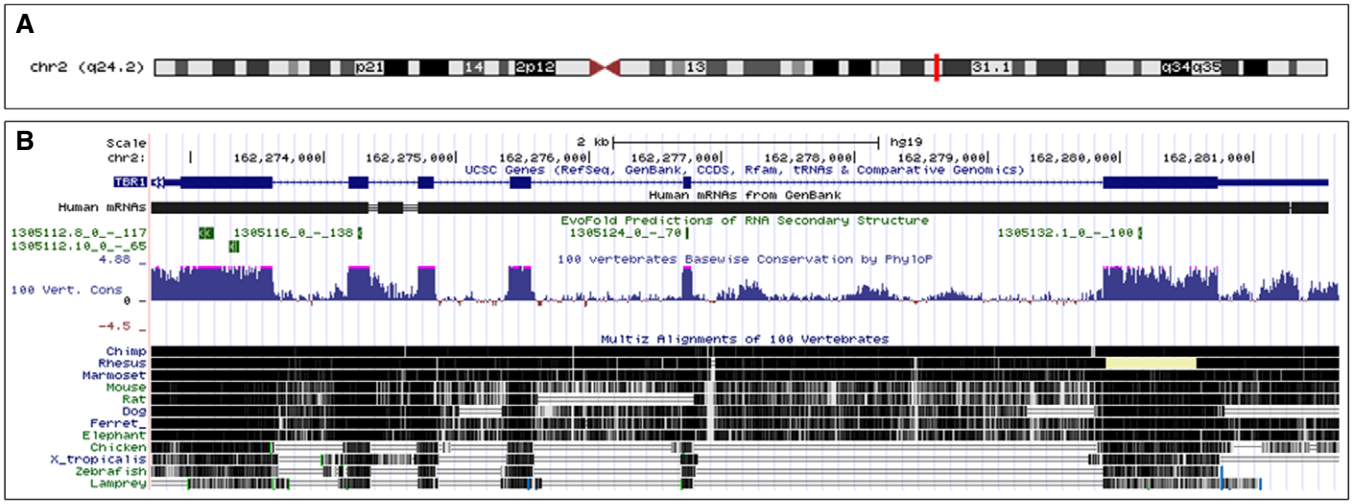
Figure EV5. Evolutionary conservation of the hairpins in the *Tbr1* transcript.

A, B Location of the *Tbr1* gene on human chromosome 2 (A, red line), and *Tbr1* mRNA (B, black solid line; GenBank), position of the 5 predicted hairpins (B, green boxes; EvoFold), and evolutionary conservation of the 5 hairpins in vertebrates (B, blue; PhyloP) and multiple alignment (black) of vertebrate *Tbr1* genes (B, black lines).

C Sequences of the 5 predicted hairpins (HPs) subject of this study (mouse).

D Length of the 5 HPs and their position in the *Tbr1* mRNA.

E Schematic representation of the psiCheck-2 luciferase plasmids empty or containing the different Tbr1 HPs.



C HP#	Sequence (5' – 3'), mouse
1	GGAGAGAAGTTCACCTTTGAAAAAAATTACCAGGGGGATGACGAATCAGTCAGATACAGACAATTTTCCTGACTCCAAGGACTCACCAGGGGACGTCCAGAGAAGTAAACTCTCTCC
2	GCCATGTTCCCGTACCCCAGCCAGCACGGACCGGCGCATCCCGCCTTCTCCATCGGCAGCCCCAGTCGCTACATGGC
3	GTGATTTTGGCGGATCCCAATCAC
4	AACCCCTTTGCAAAAGGATT
5	GGTTCGTCACGCCGGCCAACAACC

D HP#	Position (from ATG)	Exon	Length (#nt)
1	135	1	117
2	361	1	77
3	762	2	24
4	1150	5	20
5	1448	6	24

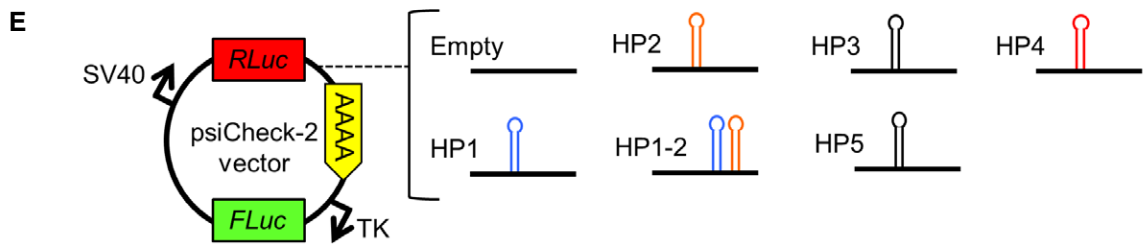


Figure EV5.