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Supplemental Information

Cortical Neurogenesis Requires Bcl6-Mediated Transcriptional Repression of Multiple Self-Renewal-Promoting Extrinsic Pathways

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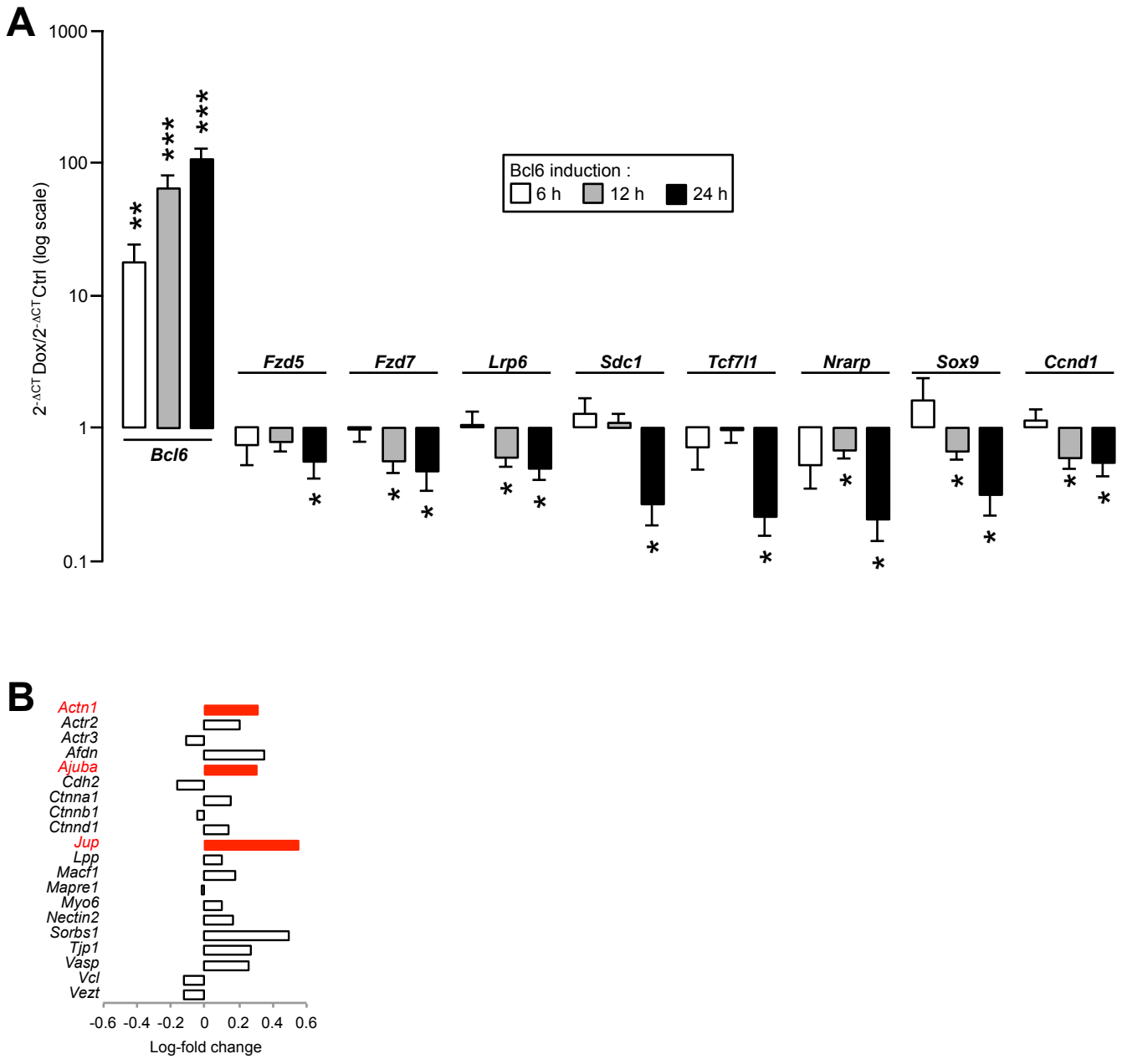
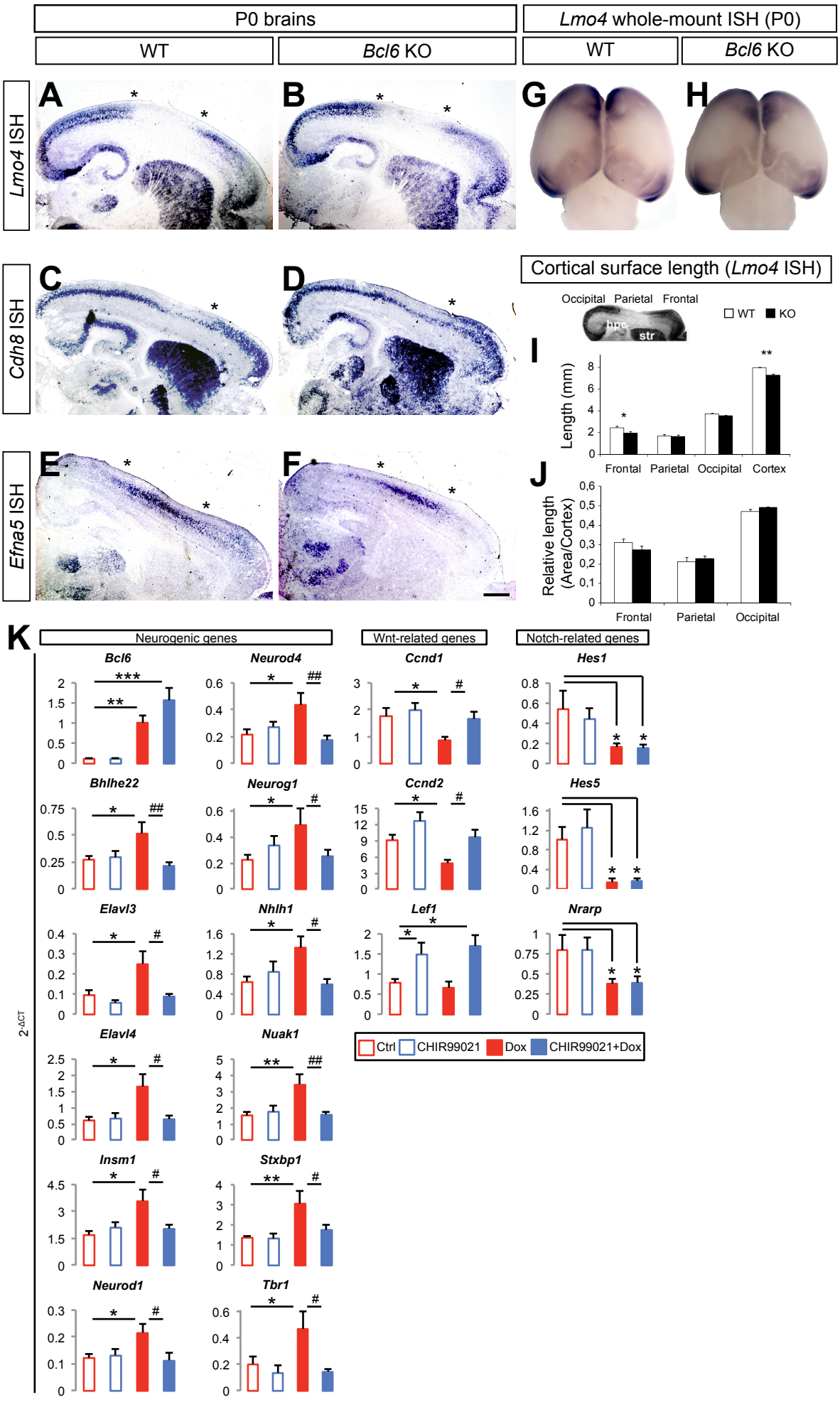
Supplementary Figure S1



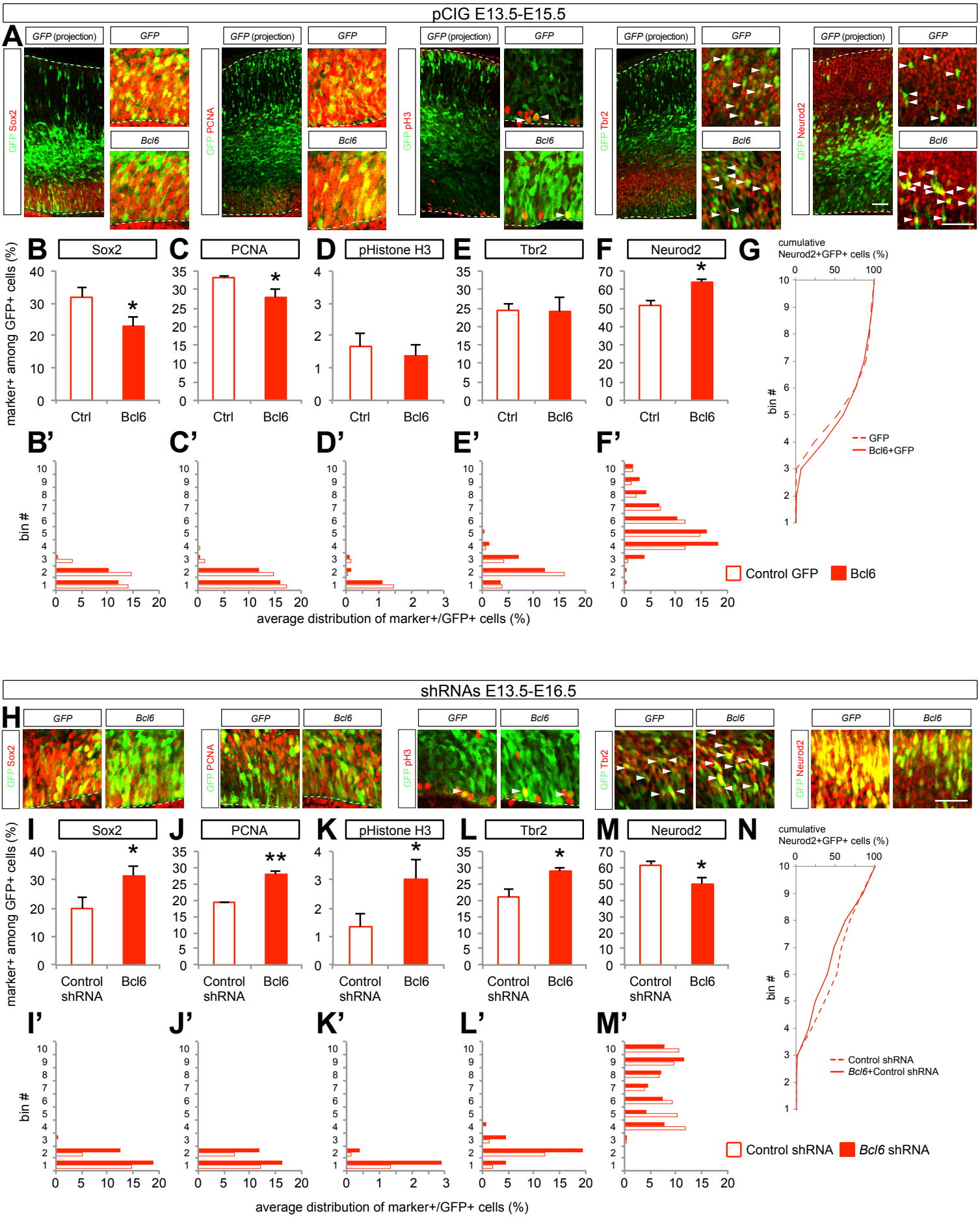
Supplementary Figure S1. Related to Figure 1. (A) Bcl6-mediated down-regulation of Wnt-related genes occurs before changes in cell identity markers. RT-qPCR analysis of *Bcl6* and additional Wnt-related genes in day 12 *in vitro* cortical progenitor cells treated with DMSO (Control) or doxycycline for 6, 12 or 24h. Data are presented as mean + s.e.m. of Dox/Ctrl absolute levels (*n* = 7-9 (6h), 27-30 (12h), and 9 (24h) from at least 3 independent differentiations for each group). * *P*<0.05, ** *P*<0.01, *** *P*<0.001. **(B) Bcl6 impact on adherens junction-related genes.** Histograms representing the log-fold change of the main genes involved in the formation/function of catenin-based adherens junctions following induction of Bcl6 expression in *in vitro* ES cell-derived cortical progenitors. Significantly overexpressed genes are indicated in red.

Supplementary Figure 2



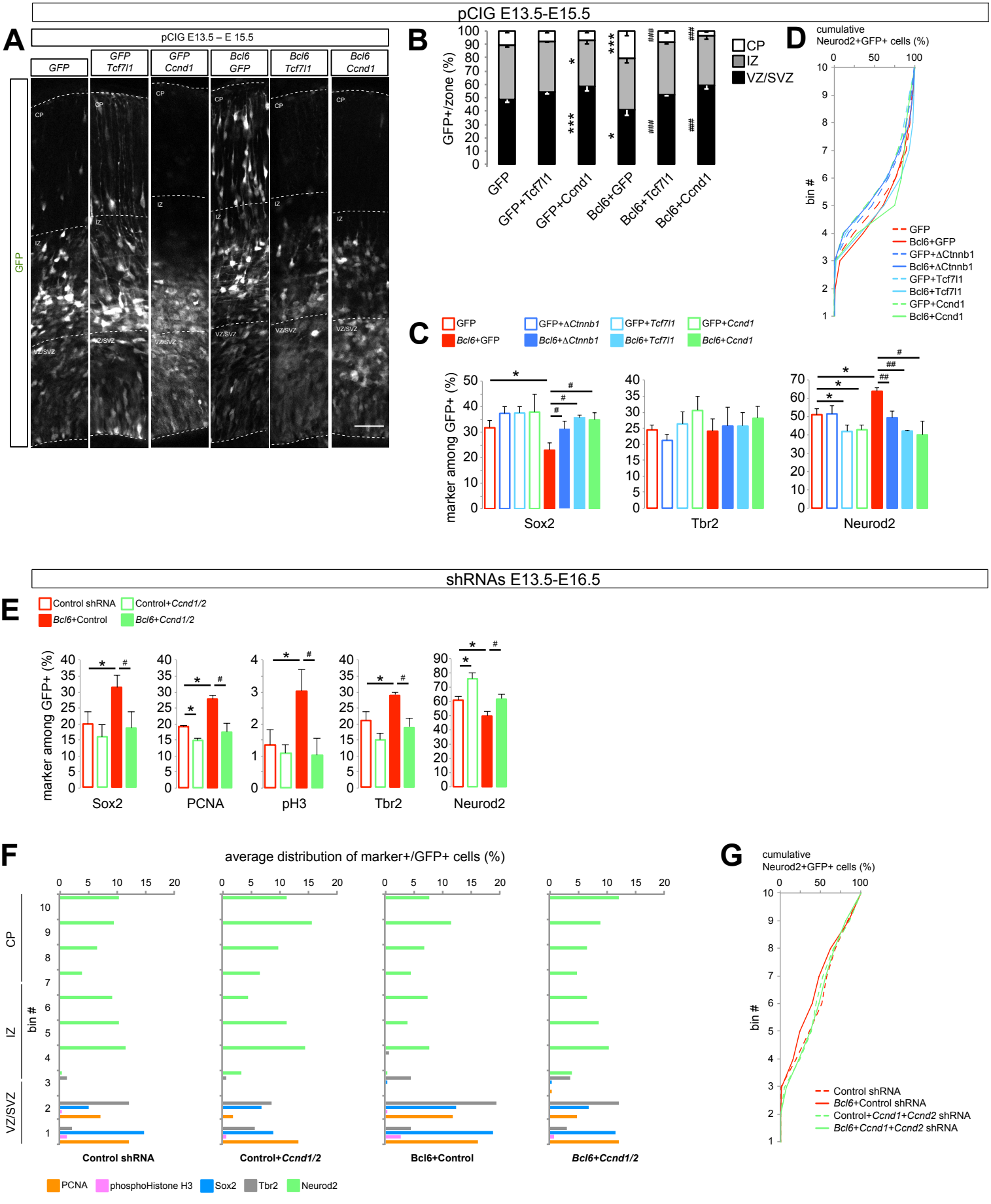
Supplementary Figure S2. Related to Figure 2. (A-J) Bcl6 is not required for proper establishment of primary cortical areas. (A-F) *In situ* hybridization on sagittal sections of wild-type and *Bcl6* KO brains at P0 with antisense probes for *Lmo4* (A-B), *Cdh8* (C-D) and *Efna5* (E-F). Scale bar, 200 μm. (G-H) Whole-mount *in situ* hybridization wild-type and *Bcl6* KO brains at P0 with an antisense probe for *Lmo4*. (I-J) Quantification of the absolute (I) or relative (J) lengths of the cortical surface of the frontal, parietal and occipital domains of wild-type and *Bcl6* KO cortices at P0 as delineated by borders of *Lmo4 in situ* hybridization on comparable sagittal sections (depicted in the scheme; hpc, hippocampus; str, striatum). Data are presented as mean + s.e.m. (n=5 (WT) and 6 (*Bcl6* KO) sections from 3 brains each). * $P<0.05$, ** $P<0.01$. **(K) Bcl6-mediated up-regulation of neurogenic genes is prevented by β-catenin overactivation using the GSK3 inhibitor, CHIR99021.** RT-qPCR analysis of neurogenic markers, Wnt targets and Notch targets in cortical progenitors derived from *Bcl6* A2 lox.Cre mouse ES cells (differentiation day 12) treated with DMSO (Control), CHIR99021, doxycycline and CHIR99021+doxycycline. Data are presented as mean + s.e.m. of absolute levels (n = 9 differentiations). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ Bcl6 overexpression (doxycycline) vs. control and $^{\#}$ $P<0.05$, $^{\#\#}$ $P<0.01$ CHIR+Dox vs. Bcl6 overexpression (doxycycline).

Supplementary Figure S3



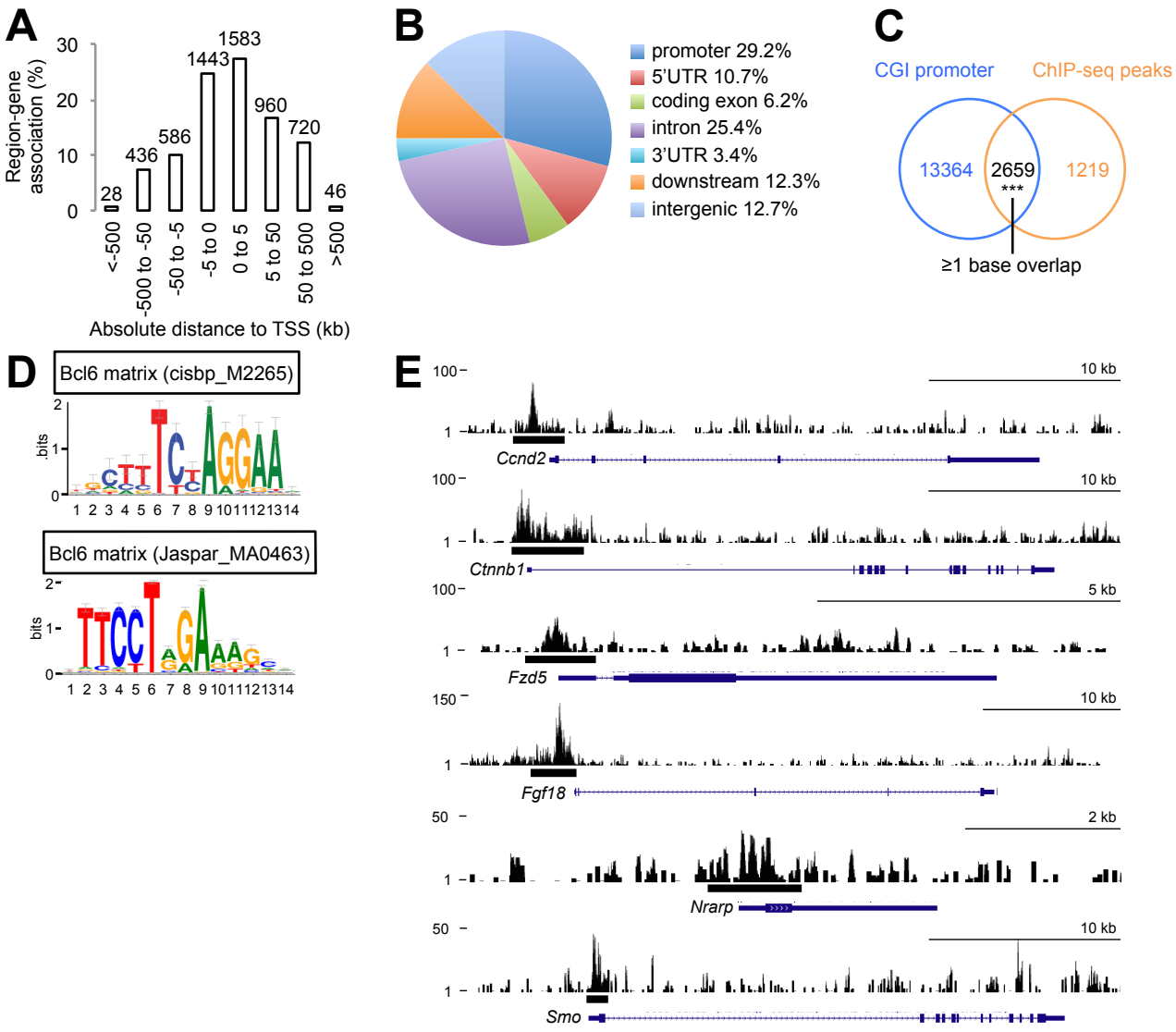
Supplementary Figure S3. Related to Figures 2 and 3. Characterization of Bcl6 neurogenic activity following its overexpression or knockdown using *in utero* electroporation. (A) Confocal images of immunofluorescence on coronal sections of E15.5 brains for cell fate and proliferation markers following *in utero* electroporation of control pCIG or pCIG-*Bcl6* at E13.5 using maximal intensity projection of Control z-stacks (whole cortical thickness) or a single z plan. Dashed lines represent the apical margin of the ventricular zone. Arrowheads point at double positive cells. Scale bars represent 50 μm. (B-F) Histograms show the percentage of Sox2+, PCNA+, pH3+, Tbr2+ and Neurod2+ cells among the GFP+ cells. Data are presented as mean + s.e.m. * $P<0.05$. (B'-F') Bin analysis of the average distribution of the markers+GFP+ cell populations quantified in B-F. (G) Assessment of neuronal migration behavior using bin analysis of cumulative Neurod2+GFP+ cells. (H) Single confocal z plan of immunofluorescence on coronal sections of E16.5 brains for cell fate and proliferation markers following *in utero* electroporation of scramble (control) or scramble+*Bcl6* shRNAs at E13.5. Dashed lines represent the apical margin of the ventricular zone. Arrowheads point at double positive cells. Scale bar, 50 μm. (I-M) Histograms show the percentage of Sox2+, PCNA+, pH3+, Tbr2+ and Neurod2+ cells among the GFP+ cells. Data are presented as mean + s.e.m. * $P<0.05$, ** $P<0.01$. (I'-M') Bin analysis of the average distribution of the markers+GFP+ cell populations quantified in I-M. (N) Assessment of neuronal migration behavior using bin analysis of cumulative Neurod2+GFP+ cells.

Supplementary Figure S4



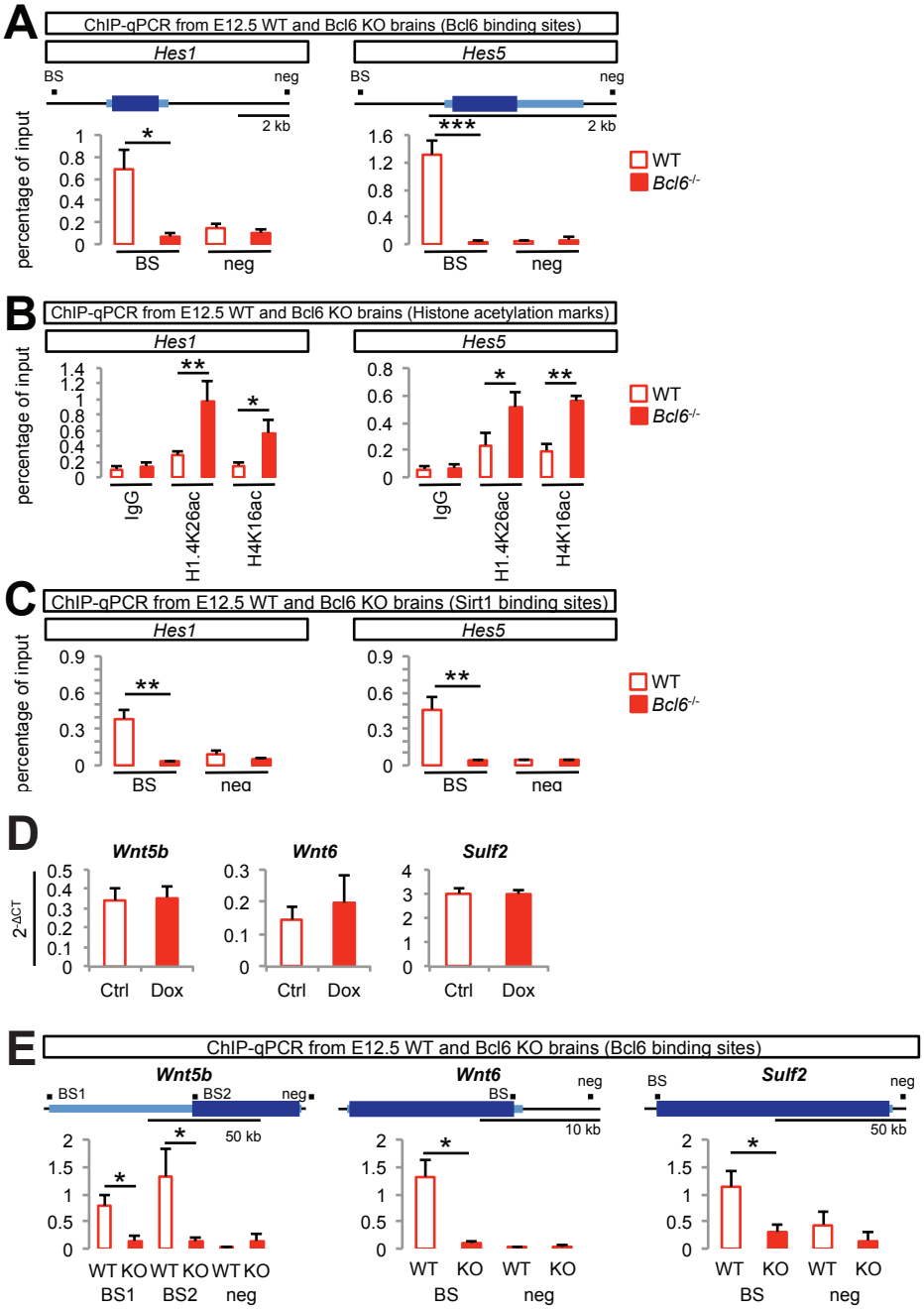
Supplementary Figure S4. Related to Figures 2 and 3. (A-D) Bcl6-elicited neurogenesis is blocked *in vivo* by β-Catenin, Tcf7l1 and Ccnd1 overexpression. . *In utero* electroporation of pCIG, pCIG+pCAG-*Δ(1-90)Ctnnb1*, pCIG+pEF1α-*Tcf7l1*, pCIG+pCAG-*Ccnd1*, pCIG-*Bcl6*+pCIG, pCIG-*Bcl6*+pCAG-Δ(1-90)*Ctnnb1*, pCIG-*Bcl6*+ pEF1α-*Tcf7l1*, or pCIG-*Bcl6*+pCAG-*Ccnd1* at E13.5. immunofluorescence was performed on coronal sections of E15.5 brains. (A) Representative images of Hoechst and GFP immunofluorescence performed on coronal sections of E15.5 brains. Dashed lines mark the basal and apical margins of the ventricular + subventricular zone (VZ+SVZ), intermediate zone (IZ) and cortical plate (CP). Scale bar, 50 μm. (B) Histograms show the percentage of GFP+ cells in VZ+SVZ, IZ and CP. Data are presented as mean + s.e.m. * P<0.05, ** P<0.01, *** P<0.001 vs. pCIG and ### P<0.001 vs. pCIG-*Bcl6*+pCIG. (C) Histograms show the percentage of Sox2+, Tbr2+ and Neurod2+ cells among the GFP+ cells. Data are presented as mean + s.e.m. * P<0.05 vs. pCIG+pCIG and # P<0.05, ## P<0.01 vs. pCIG-*Bcl6*+pCIG. (D) Assessment of neuronal migration behavior using bin analysis of cumulative Neurod2+GFP+ cells. Note that the quantifications in (C,D) for the GFP and Bcl6 groups are similar to those presented on Supplementary Figure S3B,E,F,G as all the conditions were performed simultaneously to compare phenotypes in littermate embryos. **(E-G) *Bcl6* knockdown is rescued *in vivo* by *Ccnd1* and *Ccnd2* shRNAs.** *In utero* electroporation of scramble (control), scramble+*Ccnd1*+*Ccnd2*, *Bcl6*+scramble and *Bcl6*+*Ccnd1*+*Ccnd2* shRNAs at E13.5. (E) Histograms show the percentage of Sox2+, PCNA+, phosphoHistone H3+, Tbr2+ and Neurod2+ cells among the GFP+ cells. * P<0.05 vs. Control and # P<0.05 vs. Bcl6+scramble shRNAs. Data are presented as mean + s.e.m. (F) Bin analysis of the average distribution of the markers+GFP+ cell populations quantified in A. (G) Assessment of neuronal migration behavior using bin analysis of cumulative Neurod2+GFP+ cells. Note that the quantifications for the control and Bcl6+scramble groups are similar to those presented on Supplementary Figure S3I-N as all the conditions were performed simultaneously to compare phenotypes in littermate embryos.

Supplementary Figure S5



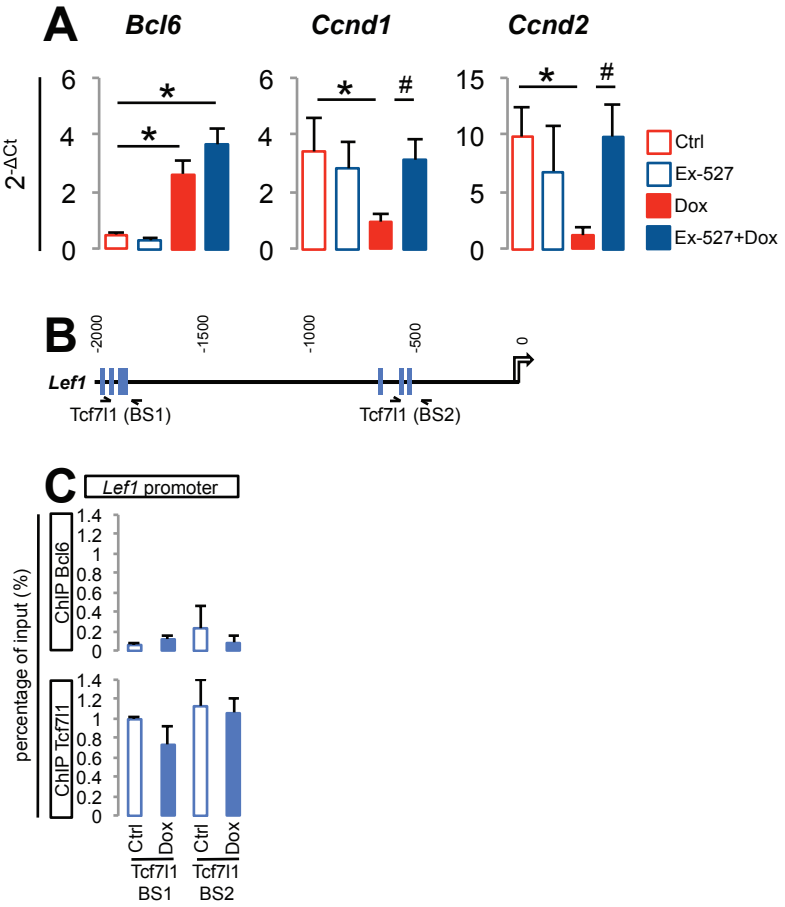
Supplementary Figure S5. Related to Figure 4. ChIP-seq analysis following Bcl6 induction in cortical progenitors derived from *Bcl6* A2 lox.Cre mouse ES cells (differentiation day 12, 24h Dox treatment). (A) Absolute distance of significant Bcl6-bound sequences to gene transcription starting sites. Numbers of region-gene associations are indicated on the histograms. (B) Pie chart localization of the significant Bcl6-bound sequences in the mouse genome. (C) Venn diagram of the overlap between the total number of CpG island promoters and the MACS-called peaks. *** *P*=9.999e-05 using a hypothesis testing for overlap analysis. (D) Significantly enriched Bcl6 matrices from the MACS-called peaks using i-cisTarget on a promoter-only database. Normalized Enrichment Score (NES) = 3.64 for the Bcl6 matrix Cisbp_M2265 and NES = 3.55 for the Bcl6 matrix Jaspar_MA0463. (E) Examples of profiles for Bcl6-predicted target genes related to Wnt, Notch, SHH, and FGF signaling. Black bars indicate MACS-called peaks and RefSeq genes from mm10 mouse genome assembly (https://genome.ucsc.edu) are represented in blue using 5' to 3' orientation.

Supplementary Figure S6



Supplementary Figure S6. Related to Figures 4 and 5. (A-C) Bcl6 binds to Notch targets, *Hes1* and *Hes5*, leading to chromatin remodeling. (A) ChIP-qPCR of Bcl6-binding sites on regulatory regions of Notch target genes, *Hes1* and *Hes5*, and negative control sites in E12.5 wild-type and *Bcl6*-/- telencephalon. Data are presented as mean + s.e.m. of input enrichment (n = 4). * $P<0.05$ and *** $P<0.001$. (B) ChIP-qPCR of histone marks H1.4K26ac and H4K16ac on Bcl6 binding sites on regulatory regions of *Hes1* and *Hes5* genes in E12.5 wild-type and *Bcl6*-/- telencephalon. Data are presented as mean + s.e.m. of input enrichment (n = 4). * $P<0.05$ and ** $P<0.01$. (C) ChIP-qPCR of Sirt1 on Bcl6 binding sites on regulatory regions of *Hes1* and *Hes5* genes in E12.5 wild-type and *Bcl6*-/- telencephalon. Data are presented as mean + s.e.m. of input enrichment (n = 4). ** $P<0.01$. **(D-E) Bcl6 also binds to some Wnt-related genes without inducing detectable transcriptional changes.** (A) RT-qPCR analysis of Wnt-related genes from DMSO- and doxycycline-treated Bcl6 A2 lox.Cre cells at day 12 of differentiation. Data are presented as mean + s.e.m. of absolute levels (n = 21 from 8 differentiations). (B) ChIP-qPCR validation of screened Bcl6-binding sites on regulatory regions and negative control sites of non-transcriptionally altered Wnt-related genes in E12.5 wild-type and *Bcl6*-/- telencephalon using a Bcl6 antibody. Data are presented as mean + s.e.m. of input enrichment (n = 4). * $P<0.05$.

Supplementary Figure S7



Supplementary Figure S7. Related to Figure 6. (A) Bcl6-elicited *Ccnd1/2* down-regulation is blocked by the Sirt1 inhibitor Ex-527. RT-qPCR analysis of the Bcl6 targets *Ctnnb1* and *Ccnd2* in day 12 *in vitro* ES cell-derived cortical progenitor cells (differentiation day 12, treated with DMSO (Control) or doxycycline ± Ex-527 for 24h). Data are presented as mean + s.e.m. of absolute levels (*n* = 12-18 from at least 3 independent differentiations). * $P<0.05$ vs. Control and # $P<0.05$ vs. Dox. **(B-C) Control ChIP-qPCRs of *in vitro* Tcf7l1 binding to the 5' regulatory region of the Wnt target gene *Lef1* upon Bcl6 induction.** (B) Schematic representation of the genomic region 2 kb upstream from *Lef1* transcription starting site showing putative Tcf7l1 binding sites as predicted by the Jaspar software (http://jaspar.genereg.net). The arrows represent the amplified regions by qPCR used to measure the enrichment following ChIP. (C) ChIP-qPCR analysis of the Tcf7l1 binding sites on the *Lef1* regulatory region in cortical progenitors derived from *Bcl6* A2 lox.Cre mouse ES cells (differentiation day 12, 24h DMSO (Ctrl) or Dox treatment) using Bcl6 and Tcf7l1 antibodies. Data are presented as mean + s.e.m. of input enrichment (n = 3 differentiations).