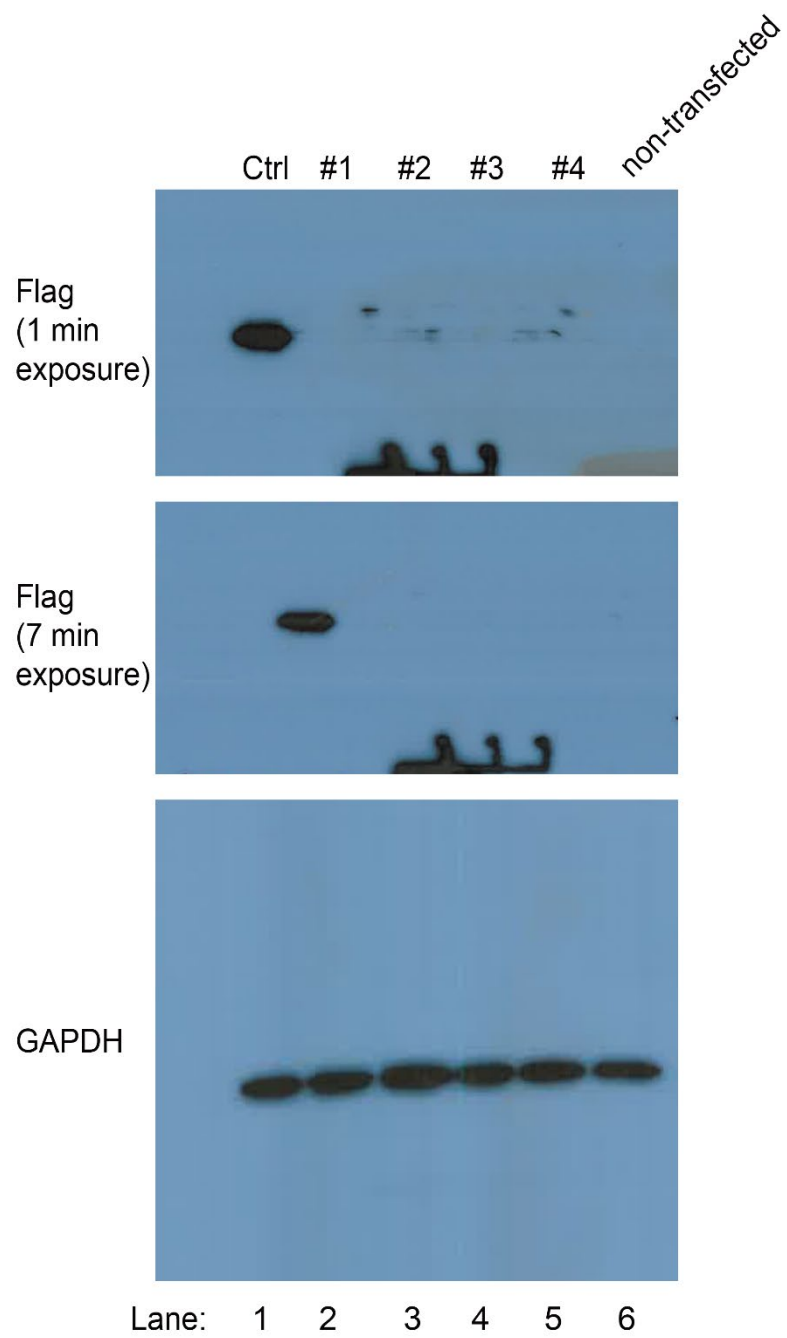




Unmodified full-length blots of Supplementary Figure 1A.

Supplementary materials for

***EXOSC10* haploinsufficiency causes primary microcephaly by
derepression of Sonic hedgehog signalling**

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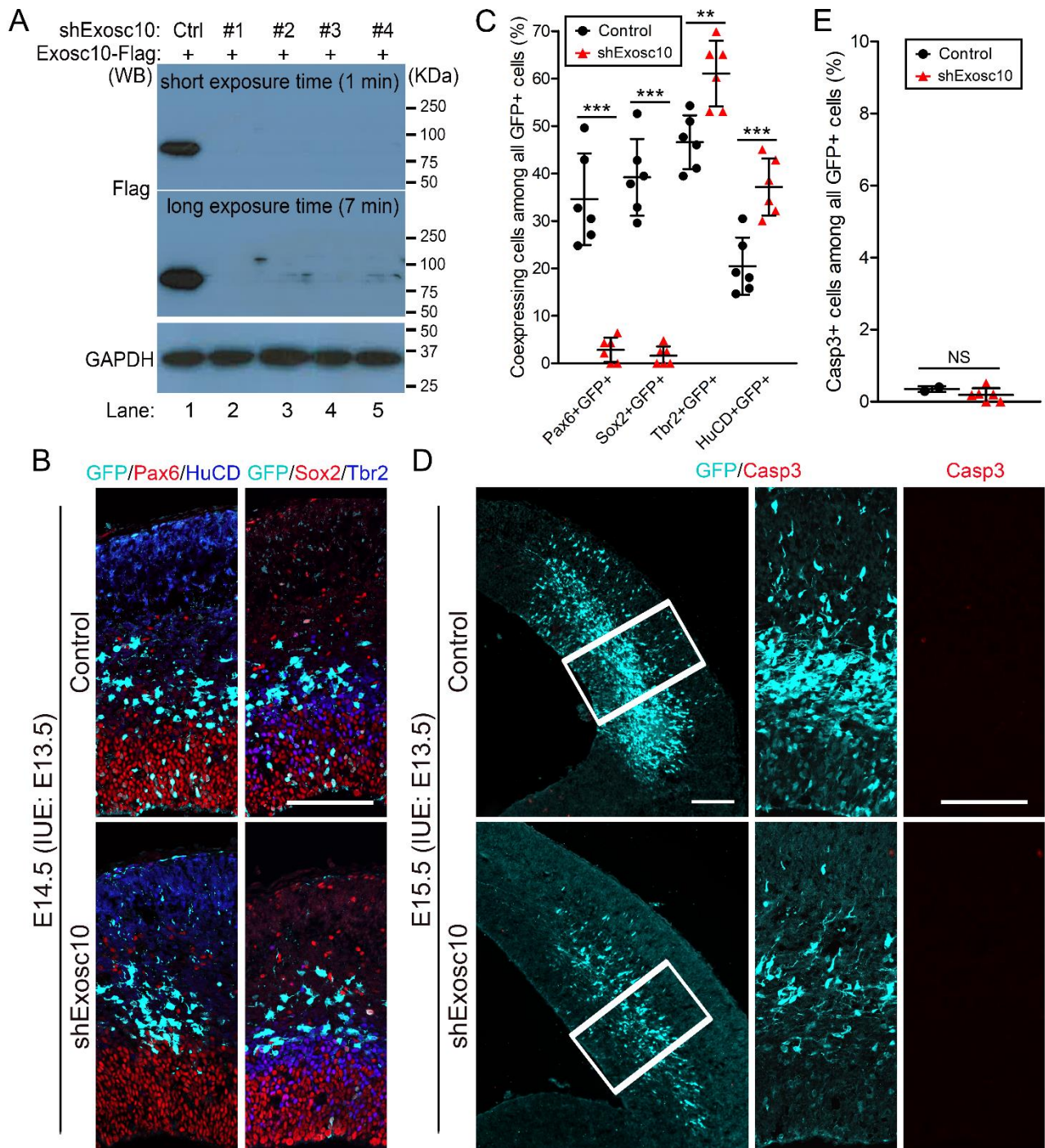
The PDF file includes:

Supplementary Figures 1-7 with legends

Legends for Supplementary Tables 1-7

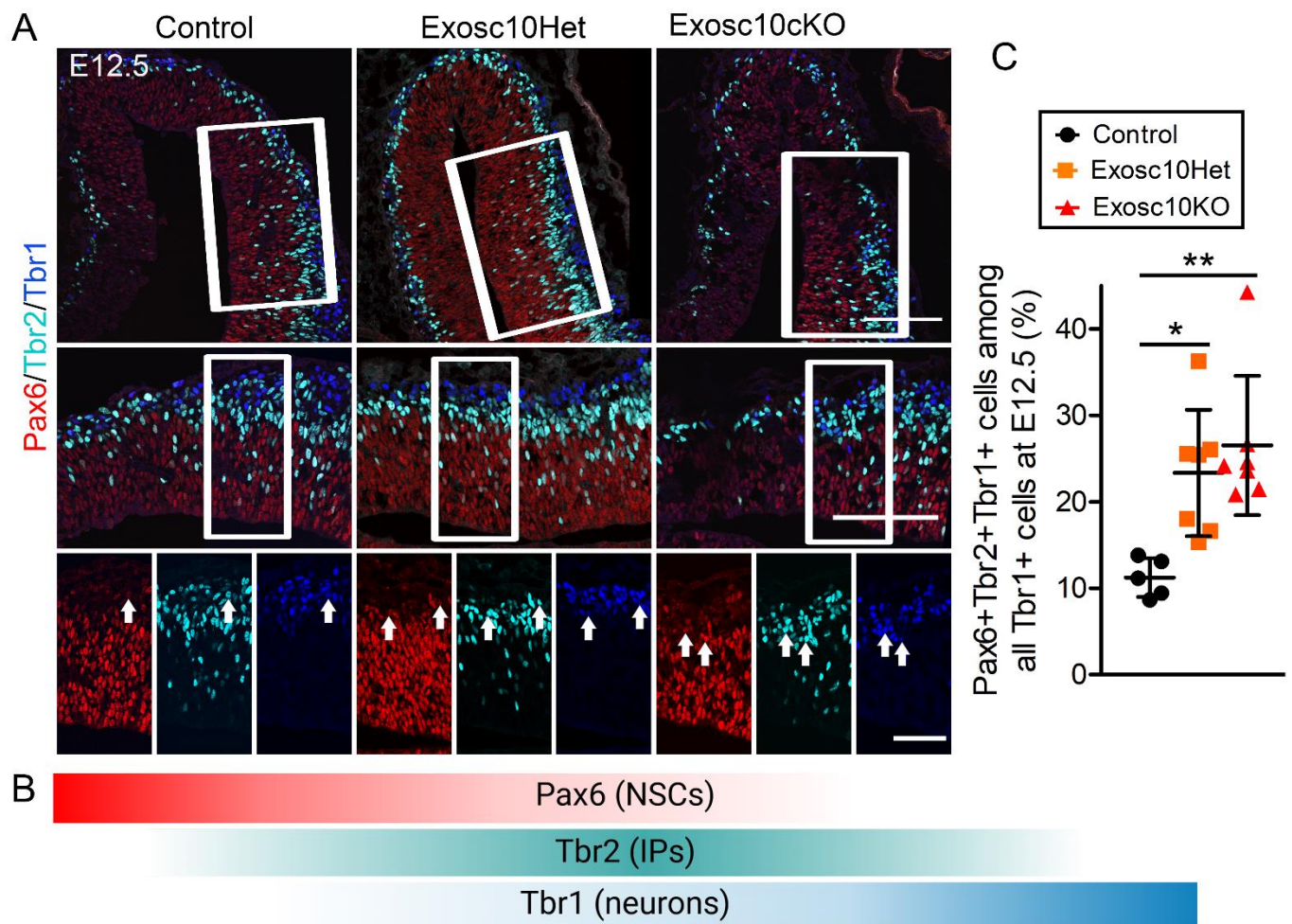
Other Supplementary material for this manuscript includes the following:

Supplementary Tables 1-7

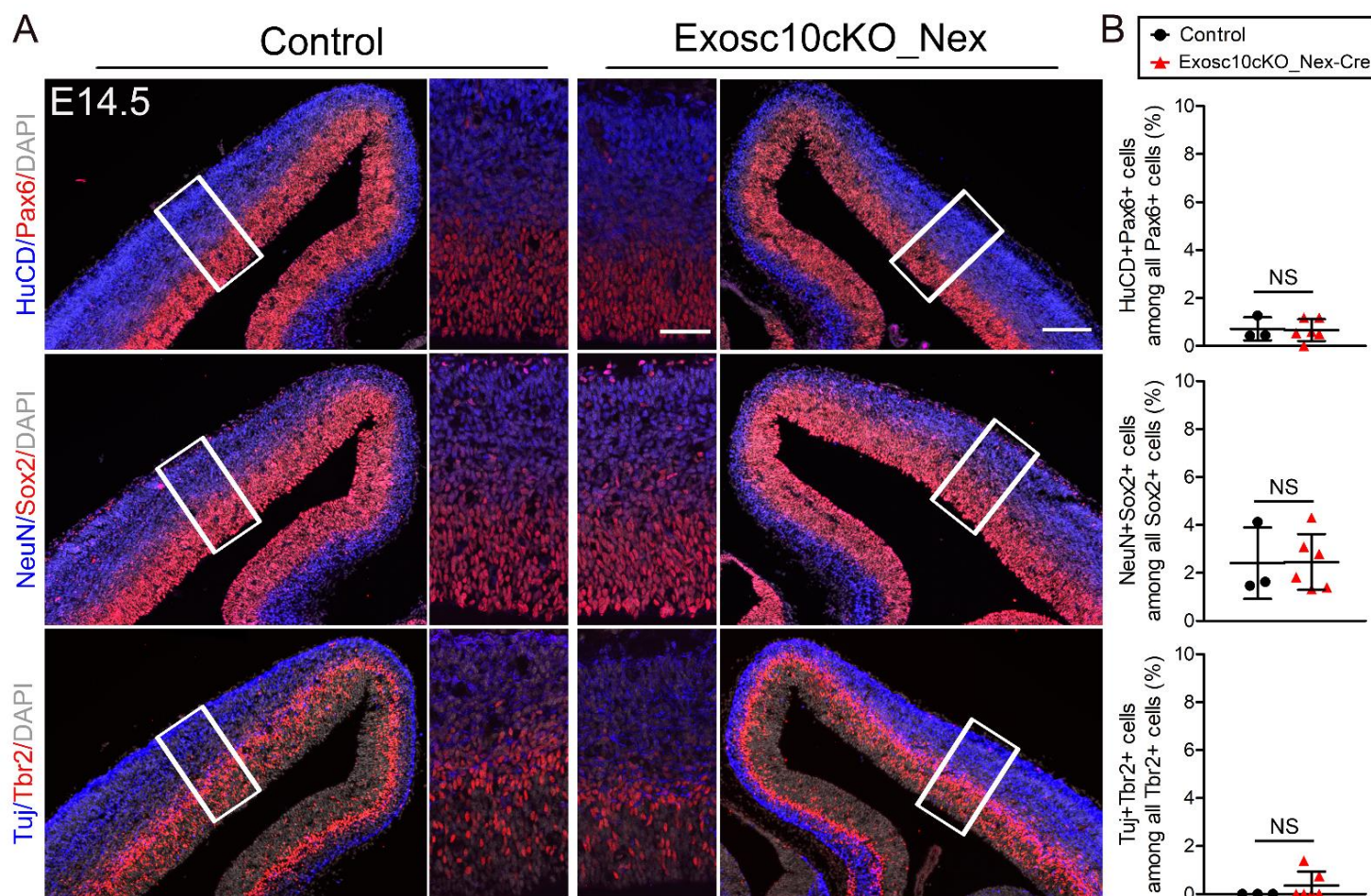


Supplementary Figure 1 Premature neurodifferentiation but no apoptosis in embryonic cortical cells upon focal deletion of Exosc10. (A) *In vitro* knockdown validation of Exosc10 expression with shExosc10. HEK293T cells were transfected with plasmids encoding Exosc10, together with shControl or shExosc10 plasmids. (B) Double immunohistochemical analyses with antibodies that specifically mark progenitors (Pax6, Sox2 and Tbr2) and neurons (HuCD) in the E14.5 cortex after *in utero* electroporation (IUE) at E13.5 using shExosc10 or a control eGFP-construct. Cells containing the electroporated construct are labelled with GFP. (C) Quantification of GFP-positive cells expressing progenitor genes and neuronal genes in the

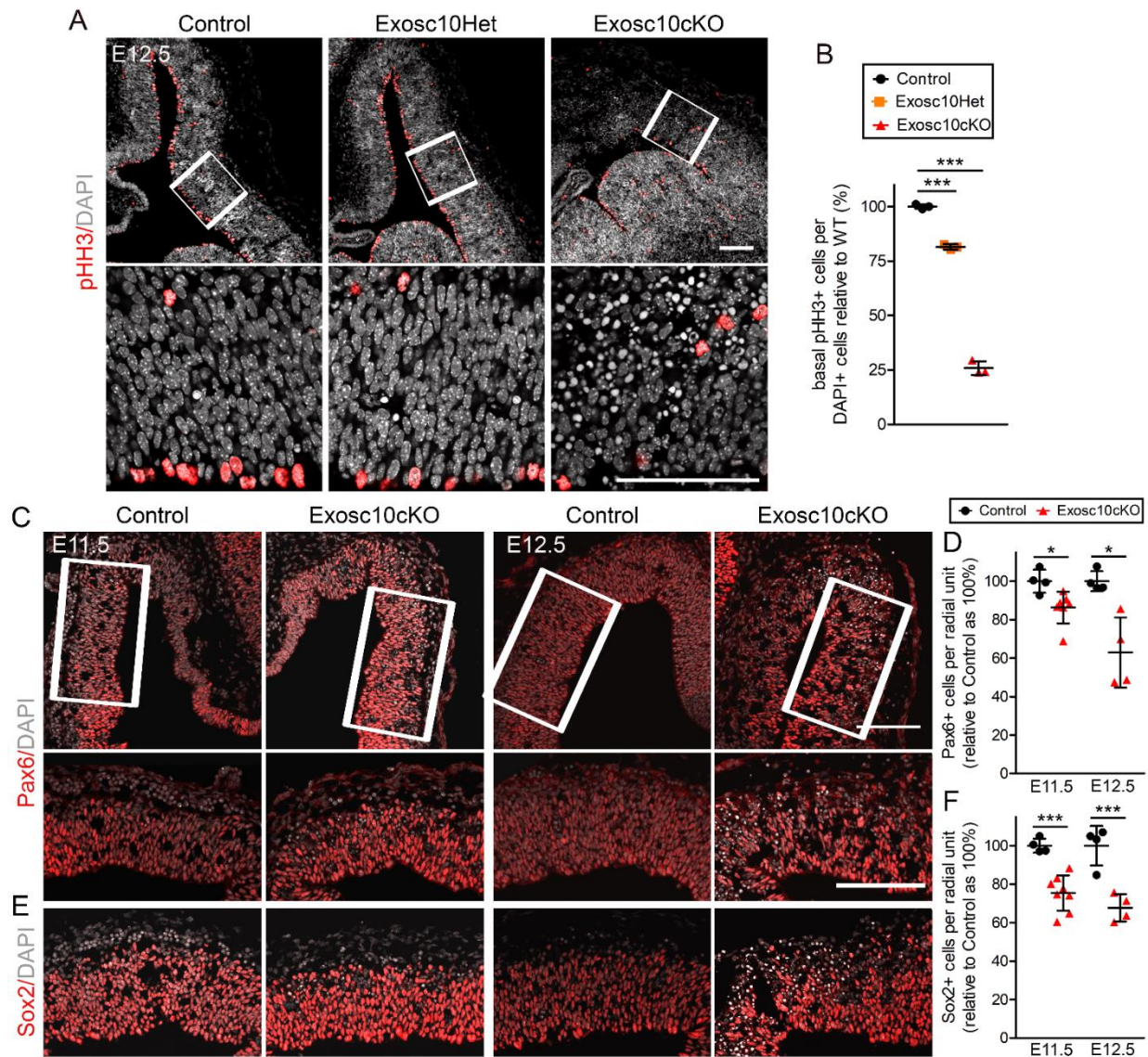
shExosc10-electroporated cortices relative to control. **(D)** Immunohistochemical analysis for the apoptosis marker caspase 3 (Casp3) in the E15.5 cortex after IUE at E13.5 using shExosc10 or a control eGFP-construct. Cells containing the electroporated construct are labelled with GFP. **(E)** Quantification of GFP-positive cells expressing Casp3 in the shExosc10-electroporated cortices relative to control. Student's t test (two-tailed) was performed. Values are reported as means $\pm$ STD, $**P < 0.01$, $***P < 0.001$; NS, not significant, $N = 6$ (C), $N \geq 3$ (E). Scale bars: 100 μm .



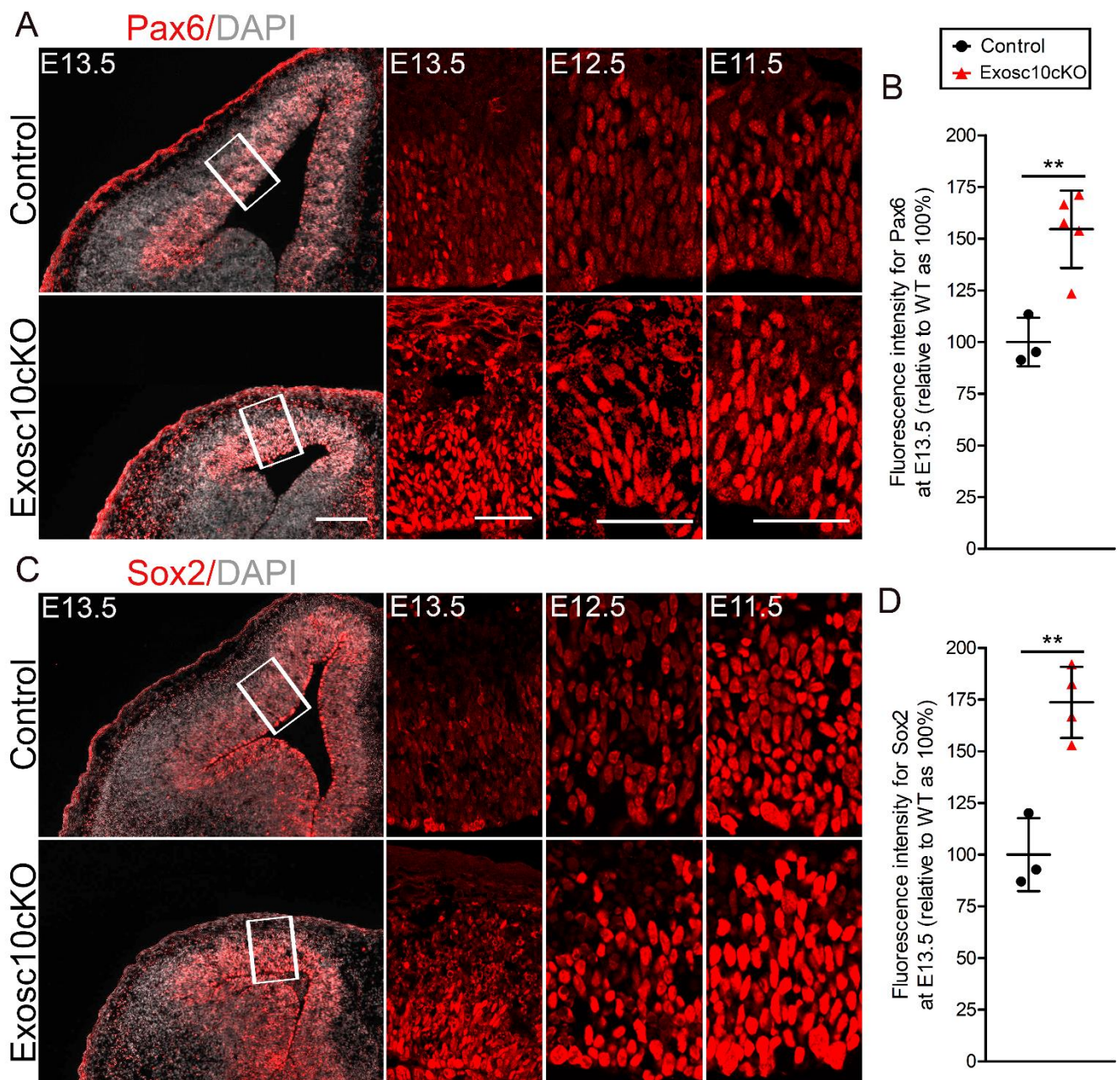
Supplementary Figure 2 Triple-expression of NSC-, IP-, and neuronal markers in embryonic Exosc10Het and Exosc10cKO cortices. (A) Immunohistochemistry showing cortical triple-expression of the NSC marker Pax6, the IP specific marker Tbr2 and the neuronal marker Tbr1 in coronal sections of Exosc10cKO, Exosc10Het and control at E12.5. Lower panel images are each higher magnifications of fields from upper images indicated by white frames. (B) Sketch showing the normal expression patterns of Pax6, Tbr2 and Tbr1 during the process of neurogenesis. (C) Quantification of Tbr1+ cells triple-expressing Pax6, Tbr2 and Tbr1 in the Het, cKO and control cortex at E12.5. Tukey's HSD (Honestly Significant Difference) was used to compare all three biological groups (Exosc10cKO, Exosc10Het and control). Values are reported as means $\pm$ STD, * $P < 0.05$, ** $P < 0.01$, $N \geq 5$. Scale bars: 200 μ m and 100 μ m (higher magnification).



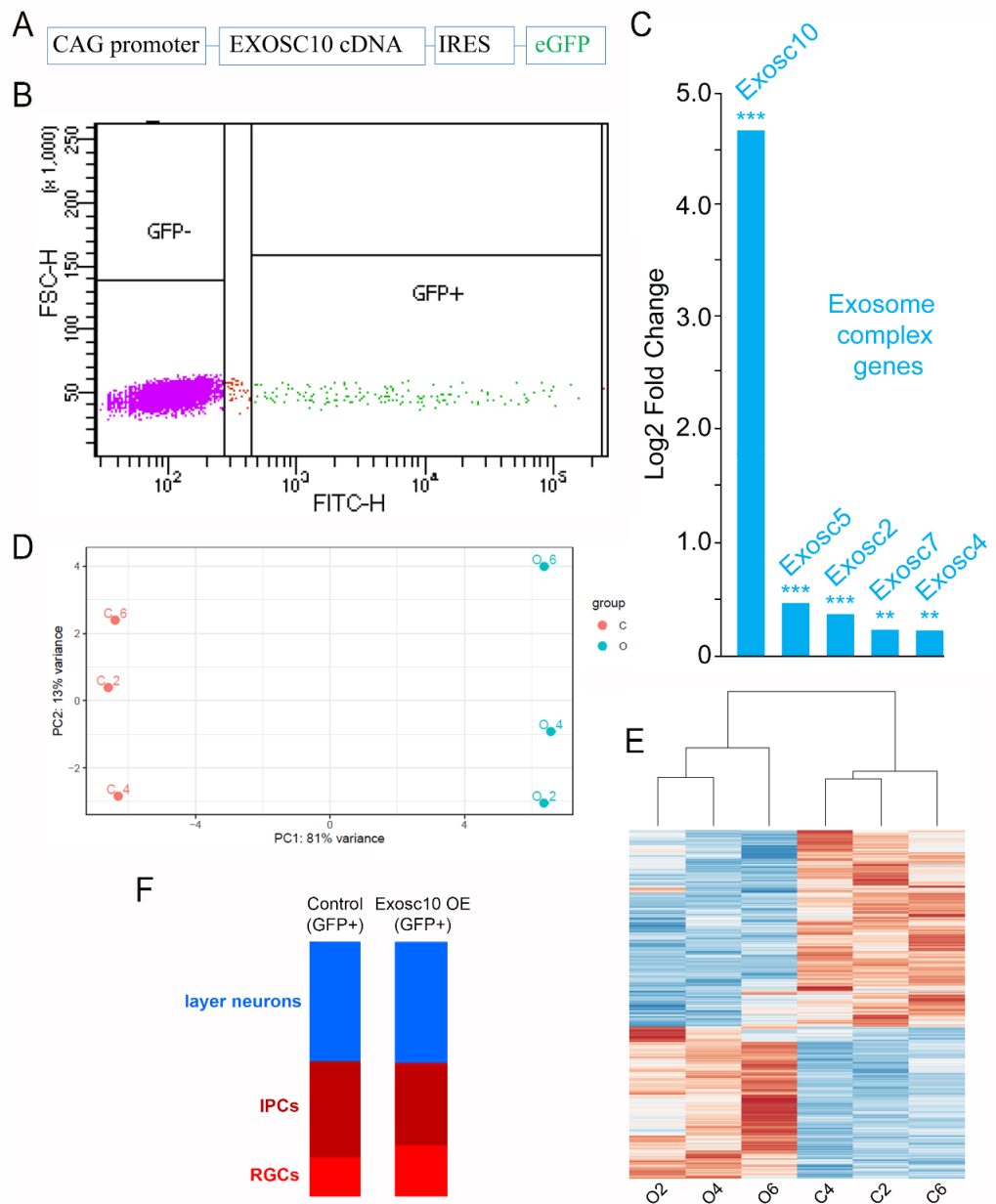
Supplementary Figure 3 No changes in expression of progenitor and neuronal markers upon neuron-specific loss of Exosc10. (A) Immunohistochemistry showing cortical expression of progenitor specific markers (Pax6, Sox2, Tbr2) and neuronal markers (HuCD, NeuN, Tuj) in coronal sections of Exosc10cKO_Nex-Cre mutants and control at E14.5. Middle Images are higher magnifications of fields indicated by the white frame. (B) Quantifications of progenitor cells co-expressing progenitor and neuronal markers in Exosc10cKO_Nex-Cre cortex at E14.5 relative to control. Student's t test was performed. Values are reported as means $\pm$ STD, NS, not significant, $N \geq 3$. Scale bars: 200 μ m and 50 μ m (higher magnification).



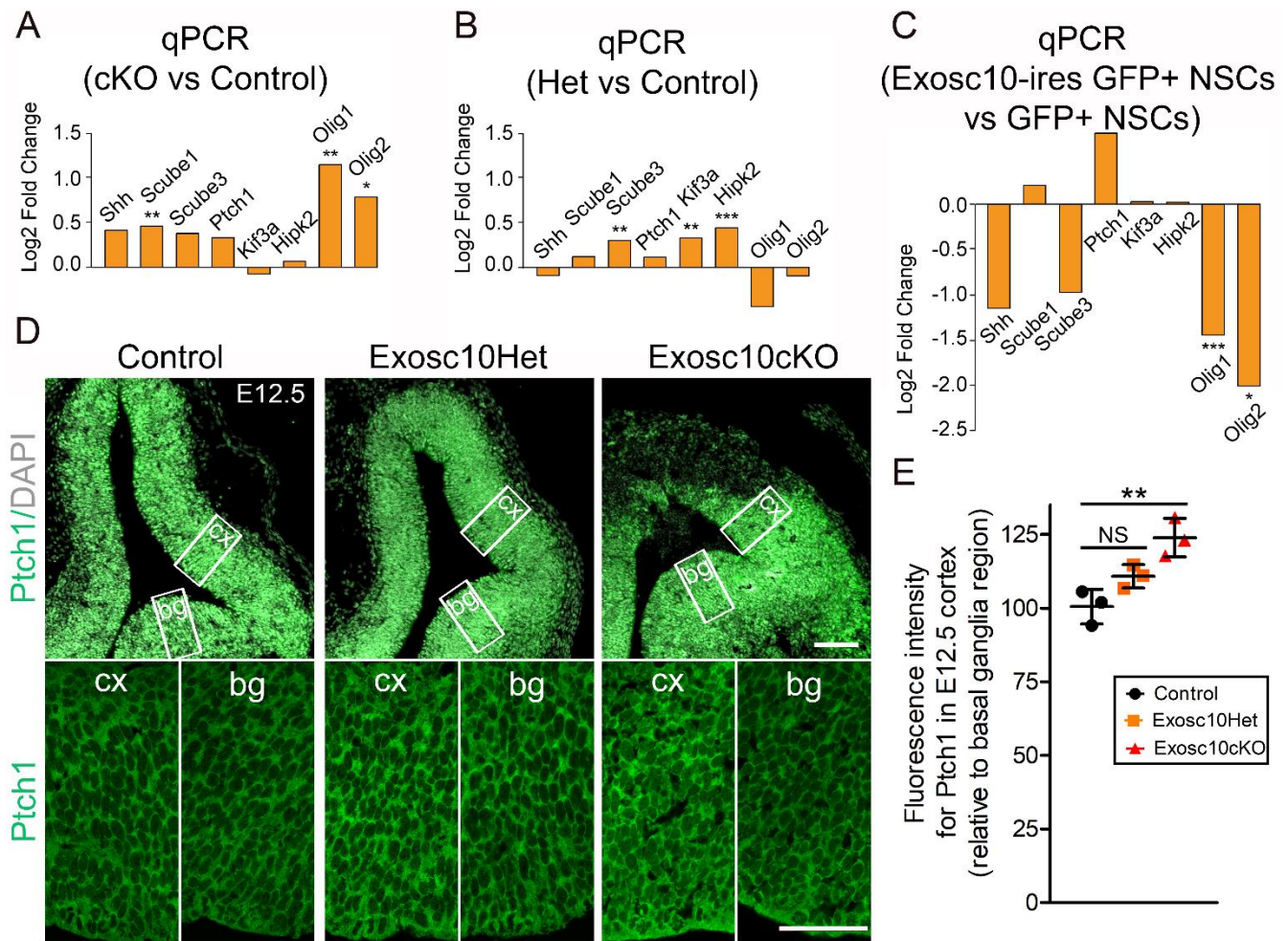
Supplementary Figure 4 Reduced number of mitotic cells and NSCs in embryonic Exosc10-deficient cortices. (A) Immunohistochemistry showing cortical expression of phospho-histone H3 (pHH3) in coronal sections of Exosc10Het, Exosc10cKO and control at E12.5. Lower images are higher magnifications of fields as indicated by the white frame. (B) Quantification of pHH3+ cells in the whole dorsal cortex of Exosc10Het and Exosc10cKO cortex at E12.5 relative to control. (C, E) Immunohistochemistry showing cortical expression of NSC specific markers Pax6 (C) and Sox2 (E) in coronal sections of Exosc10cKO and control at E11.5 and E12.5. Lower images are higher magnifications of fields as indicated by the white frame. (D, F) Quantification of NSC markers Pax6 (B) and Sox2 (D) in Exosc10cKO cortex at E11.5 and E12.5 relative to control. The cells were counted in a defined region (radial unit) in the dorsal cortical area. Tukey's HSD (B) and Student's t test (D, F) was performed. Values are reported as means $\pm$ STD, * P < 0.05, ** P < 0.01, *** P < 0.001, $N \geq 3$ (B), $N \geq 4$ (D, F). Scale bars: (A) 100 μ m, (C, E) 200 μ m.



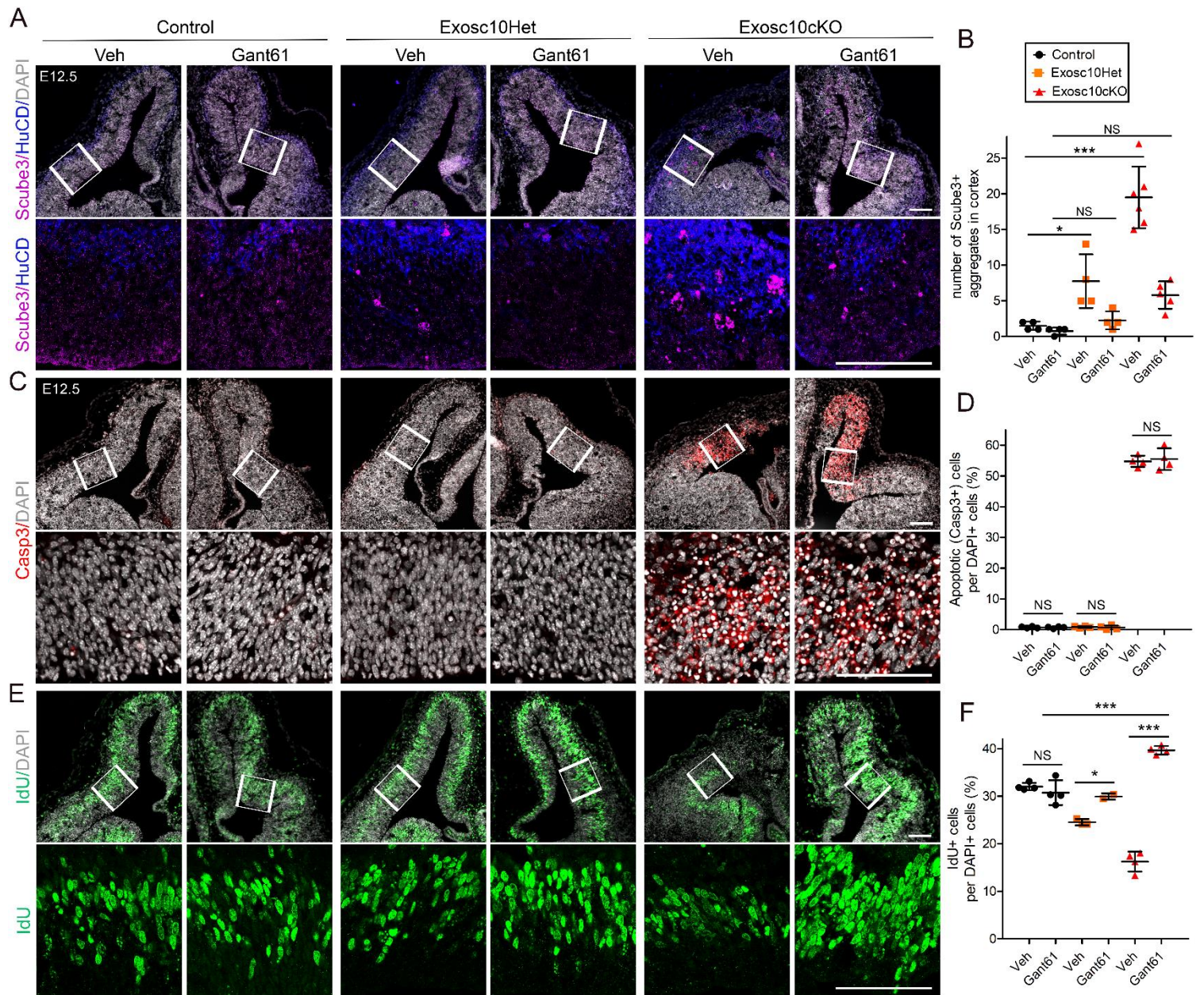
Supplementary Figure 5 Stronger expression of NSC markers in embryonic Exosc10cKO cortices. (A, C) Immunohistochemistry showing cortical expression of NSC specific markers Pax6 (A) and Sox2 (C) in coronal sections of Exosc10cKO and control at E11.5, E12.5 and E13.5. Right images are higher magnifications of fields as indicated by the white frame. (B, D) Quantification of fluorescence intensity of NSC markers Pax6 (B) and Sox2 (D) in Exosc10cKO cortex at E11.5 and E12.5 relative to control. Student's t test was performed. Values are reported as means $\pm$ STD, $**P < 0.01$, $N \geq 3$. Scale bars: 200 μ m and 50 μ m (higher magnifications).



Supplementary Figure 6 IUE-FACS-RNA-seq experiment to study the effects of Exosc10 overexpression on neuronal differentiation. (A) Sketch of the plasmid used to introduce Exosc10 overexpression via IUE. (B) Representative plot showing sorting gates for GFP+ and GFP- cells from E13.5 mouse cortex. (C) Log2 Fold Change of expression levels of exosome complex genes in Exosc10 overexpressing cells versus control (GFP-only expressing cells). Significance levels for each gene are shown in comparison to control. (D, E) PCA score plot (D) of RNA-seq of Exosc10 overexpressing cells versus control and corresponding heatmap (E) showing enrichment patterns of sorted cell populations. (F) Cell type fractions of RNA-seq samples from Exosc10 overexpressing and control cells estimated by deconvolution using publicly available E12.5 mouse dorsal forebrain single-cell RNA-seq data. O, Exosc10 overexpressing cells; C, control cells. Student's t test was performed, ** $P < 0.01$, *** $P < 0.001$, $N = 3$.



Supplementary Figure 7 Exosc10 suppresses Shh signaling. (A, B) Quantitative PCR (qPCR) to confirm upregulation of Shh pathway transcripts in E12.5 Exosc10cKO (A) and Exosc10Het cortex (B) vs control. Significance levels are shown for transcripts upregulated significantly ($P < 0.05$) in comparison to control. (C) qPCR to confirm downregulation of Shh pathway transcripts in E13.5 Exosc10 OE cells versus control. Significance levels are shown for transcripts downregulated significantly ($P < 0.05$) in comparison to control (GFP-only expressing cells). (D) Immunohistochemistry showing cortical expression of the Shh receptor Ptch1 in coronal sections of Exosc10cKO, Exosc10Het and control at E12.5. Lower images are higher magnifications of dorsal cortex (cx) and basal ganglia (bg) area as indicated by the white frames. (E) Quantification of fluorescence intensity in E12.5 cx relative to bg as internal control. Student's t test (A–C) and Tukey's HSD (E) were performed. Values are reported as means $\pm$ STD, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, $N = 4$ (A, B), $N = 3$ (C, E). Scale bars: 100 μ m and 50 μ m (higher magnifications).



Supplementary Figure 8 Differential effects of Shh signaling inhibition on Scube3 expression, apoptosis and proliferation in Exosc10Het and Exosc10cKO cortices. (A, C, E) Immunohistochemical analysis of coronal cortical sections from Exosc10cKO, Exosc10Het, and control embryos at E12.5 following daily administration of either an empty vehicle or GANT61 starting from E9.5. Staining includes Scube3 and the neuronal marker HuCD (A), the apoptosis marker caspase 3 (Casp3) (C), and IdU, which was injected 1 hour prior to analysis (E). Lower images are higher magnifications of dorsal cortex as indicated by the white frame. (B) Quantification of Scube3⁺ aggregates in E12.5 cortex after empty vehicle or Gant61 injection. (D) Quantification of apoptotic cells at E12.5 after empty vehicle or Gant61 injection. (F) Quantification of IdU⁺ cells at E12.5 after empty vehicle or Gant61 injection. Tukey's HSD

was performed. Values are reported as means $\pm$ STD, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, NS, not significant, $N \geq 4$ (B, D), $N \geq 3$ (F). Scale bars: 100 μm .

Legends for Supplementary Tables

Supplementary Table 1 Patient information

Supplementary Table 2 Genes differently expressed in E12.5 Exosc10cKO_*Emx1*-Cre cortex, including Gene Ontology categories (for Fig. 5A)

Supplementary Table 3 Genes differently expressed in E12.5 Exosc10Het_*Emx1*-Cre cortex, including Gene Ontology categories (for Fig. 5B)

Supplementary Table 4 Common Gene Ontology categories for genes differently expressed in Exosc10Het and Exosc10cKO cortex and genes involved in Shh signalling and patterning identified in both groups (for Fig. 5C, D and Fig. 7D)

Supplementary Table 5 Overlap between upregulated genes in RNA-Seq of E12.5 Exosc10Het cortex and transcripts bound by Exosc10 in RIP-Seq with E12.5 cortex, including Gene Ontology categories (for Fig. 5E, F)

Supplementary Table 6 Genes differently expressed in Exosc10 OE cells in E13.5 cortex, including Gene Ontology categories (for Fig. 6G, H, I)

Supplementary Table 7 Statistical analyses