FoxO6 affects Plxna4-mediated neuronal migration during mouse cortical development


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Fig. S1. Numbers of apical and basal progenitor cells are unchanged in FoxO6<sup>−/−</sup> cortices. (A) Immunostaining for Sox2 reveals no clear difference in the amount of multipotent apical progenitor cells in FoxO6<sup>−/−</sup> cortices. Cortices were counterstained with DAPI. (B) Immunostaining for Tbr2 reveals that the pool of basal progenitors is not clearly affected in FoxO6<sup>−/−</sup> cortices. Cortices were counterstained with DAPI. (C) Quantification of the number of Sox2-positive cells for a fixed region covering the VZ and SVZ in FoxO6<sup>+/+</sup> and FoxO6<sup>−/−</sup> cortices. Data were normalized against the number of DAPI cells in that region. (D) Quantification of the number of Tbr2-positive cells for a fixed region covering the VZ and SVZ in FoxO6<sup>+/+</sup> and FoxO6<sup>−/−</sup> cortices. Data were normalized against the number of DAPI cells in that region. (E) Quantification of the average width of the subventricular zone/ventricular zone, intermediate zone, and cortical plate. Previously performed DAPI counterstaining was used to identify and measure the width of the indicated layers in E18 FoxO6<sup>+/+</sup> and FoxO6<sup>−/−</sup> brains. The error bars show the SD. Two-tailed Student’s t test; no significant differences were observed.
Fig. S2. siFoxO6-1 and siFoxO6-2 down-regulate FoxO6-GFP protein expression. (A and B) HEK293 cells were transfected with FoxO6-GFP and either siScrambled, siFoxO6-1, or siFoxO6-2. Western blot revealing down-regulation of FoxO6-GFP upon treatment with siFoxO6-1 and siFoxO6-2. β-Actin was used as a loading control. GFP was used to show that the siRNAs target FoxO6 and not GFP. (C) Densitometric analysis of the Western blot analysis; n = 3 vs. n = 3. The error bars show the SD. Two-tailed Student’s t test; *P < 0.05.

Fig. S3. siRNA-mediated down-regulation of FoxO6 does not induce additional migration defects in FoxO6−/− cortices. (A) E18 FoxO6−/− cortices in utero electroporated with siScrambled at E14.5 and cotransfected with a GFP expression vector display altered neuronal distribution at E18. (B) E18 cortices in utero electroporated with siFoxO6 at E14.5 and cotransfected with a GFP expression vector did not display additional migration deficits.
Fig. S4. Knockdown of FoxO6 via siFoxO6-2 results in defective radial migration, confirming data of siFoxO6-1. (A and B) GFP protein expression at E18 in cortices in utero electroporated with siScrambled or siFoxO6-2, cotransfected with a GFP expression vector. siFoxO6-2 displays an altered distribution of GFP-positive cells at E18 compared with siScrambled (control) electroporated cortices. (C) Quantification of duplicate experiments as shown in A and B. Ratios of GFP+ cells between the ventricular zone, intermediate zone, and cortical plate are shown. Note the reduction in cortical plate-positioned neurons upon treatment with siFoxO6-2, resembling the phenotype observed with siFoxO6-1. The error bars show the SD.
Validation of a selection of genes as identified in the FoxO6 knockdown genome-wide transcriptome analysis. In an independent set of experiments, cortices were in utero electroporated with siFoxO6-1 and cotransfected with a GFP expression vector, followed by FACsorting and RNA isolation. Genes significantly regulated according to the transcriptome data were investigated by qPCR. Significant down-regulation of FoxO6, Plxna4, Nfia, and Pik3ca was confirmed. Significant up-regulation of Xlrc3 was also confirmed. Lmx1a was used as an unchanged control. The error bars show the SD. Two-tailed Student’s t test; *P < 0.05.

**Fig. S6.** Plxna4 expression is significantly reduced specifically in FoxO6+/− FACsorted cortical cells. FoxO6+/−, FoxO6+/−, and FoxO6−/− cortices were in utero electroporated with GFP at E14.5, and labeled cells were FACsorted at E16.5 and followed by RNA isolation. Plxna4 expression was measured by qPCR. Only in FoxO6+/− animals was a significant down-regulation of Plxna4 observed. The error bars show the SD. Two-tailed Student’s t test; *P < 0.05.

**Fig. S7.** Validation of Plxna4 protein overexpression after IUE with a Plxna4 expression plasmid. (A) Cortices were electroporated with both GFP and Plxna4 expression plasmids. Immunostaining for GFP and Plxna4 reveals that both genes are expressed in transduced cortical cells. (B) Magnifications showing GFP and Plxna4 colocalization.
**Other Supporting Information Files**

Table S1 (DOC)