FoxO6 affects Plxna4-mediated neuronal migration during mouse cortical development

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Supporting Information

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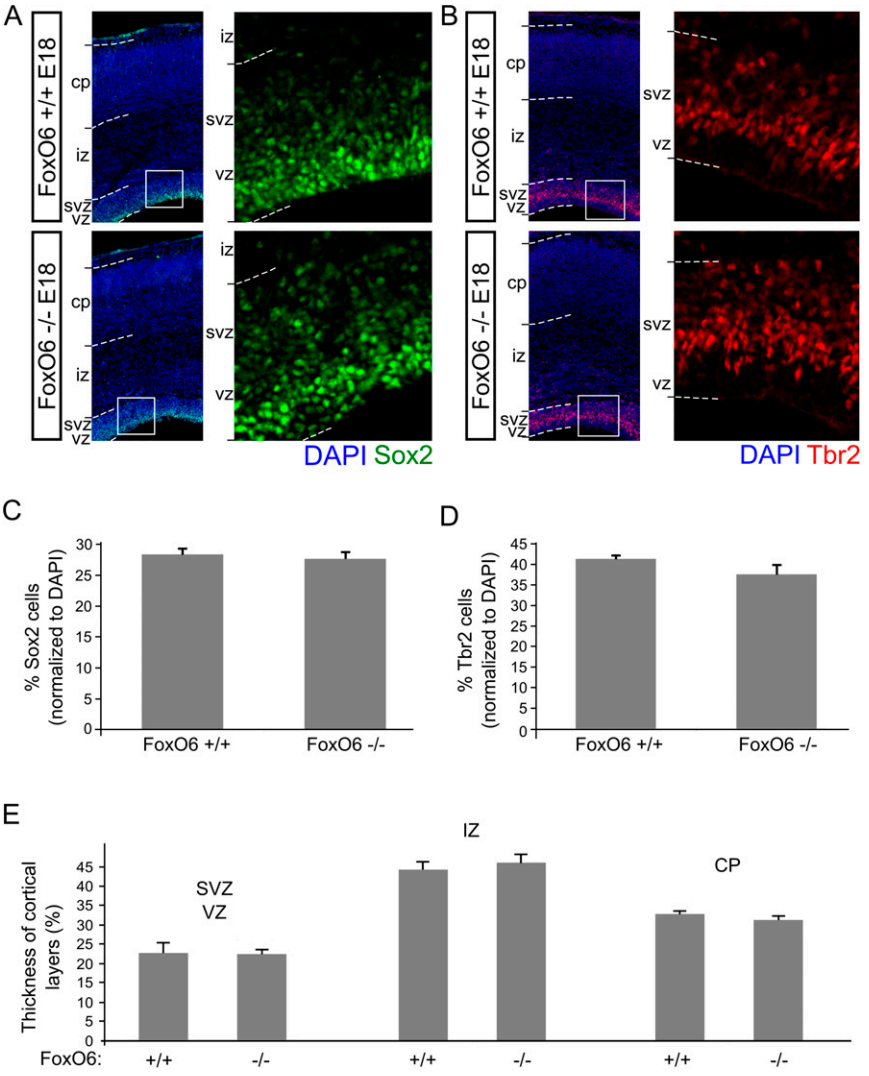

Fig. S1. Numbers of apical and basal progenitor cells are unchanged in *FoxO6*$^{-/-}$ cortices. (*A*) Immunostaining for Sox2 reveals no clear difference in the amount of multipotent apical progenitor cells in *FoxO6*$^{-/-}$ cortices. Cortices were counterstained with DAPI. (*B*) Immunostaining for Tbr2 reveals that the pool of basal progenitors is not clearly affected in *FoxO6*$^{-/-}$ 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*$^{+/+}$ and *FoxO6*$^{-/-}$ 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*$^{+/+}$ and *FoxO6*$^{-/-}$ 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*$^{+/+}$ and *FoxO6*$^{-/-}$ brains. The error bars show the SD. Two-tailed Student's *t* test; no significant differences were observed.

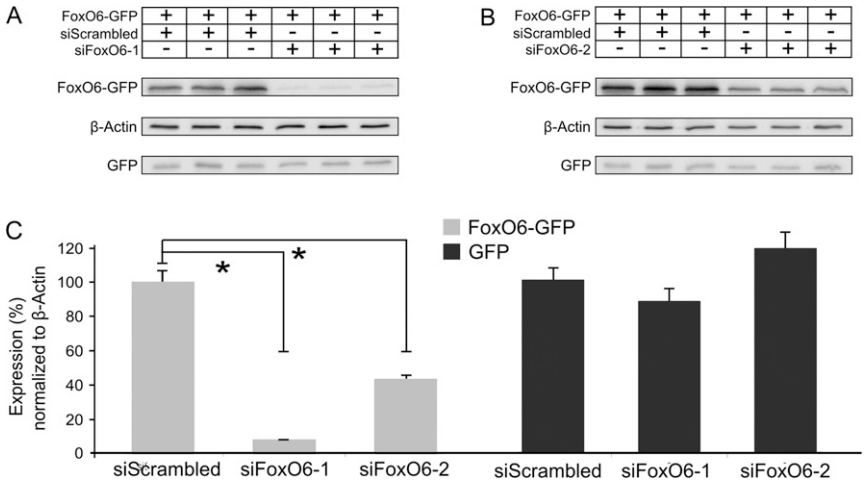

Fig. S2. siFoxO6-1 and siFoxO6-2 down-regulate FoxO6-GFP protein expression. (*A* and *B*) HEK293 cells were transfected with FoxO6-GFP and either siScrambled, siFoxO6-1, or siFoxO6-2. Western blot revealing down-regulation of *FoxO6-GFP* upon treatment with siFoxO6-1 and siFoxO6-2. β-Actin was used 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; $^*P < 0.05$.

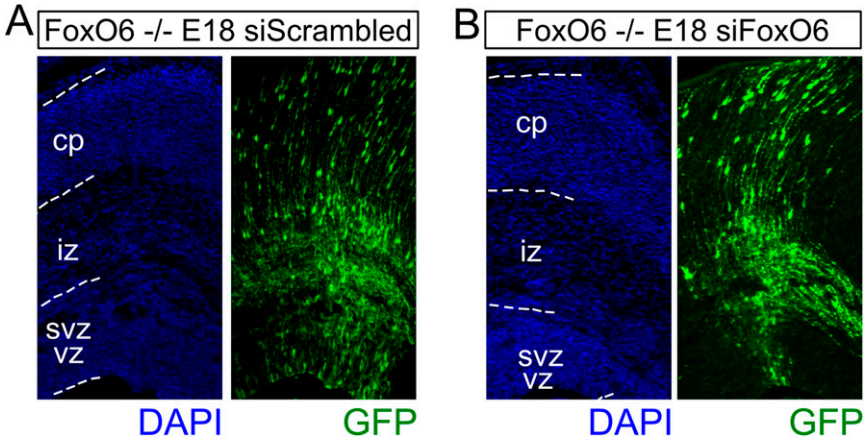

Fig. S3. siRNA-mediated down-regulation of *FoxO6* does not induce additional migration defects in *FoxO6*$^{-/-}$ cortices. (*A*) E18 *FoxO6*$^{-/-}$ cortices in utero electroporated with siScrambled at E14.5 and cotransfected with a *GFP* expression vector display altered neuronal distribution at E18. (*B*) E18 cortices in utero electroporated with siFoxO6 at E14.5 and cotransfected with a *GFP* expression vector did not display additional migration deficits.

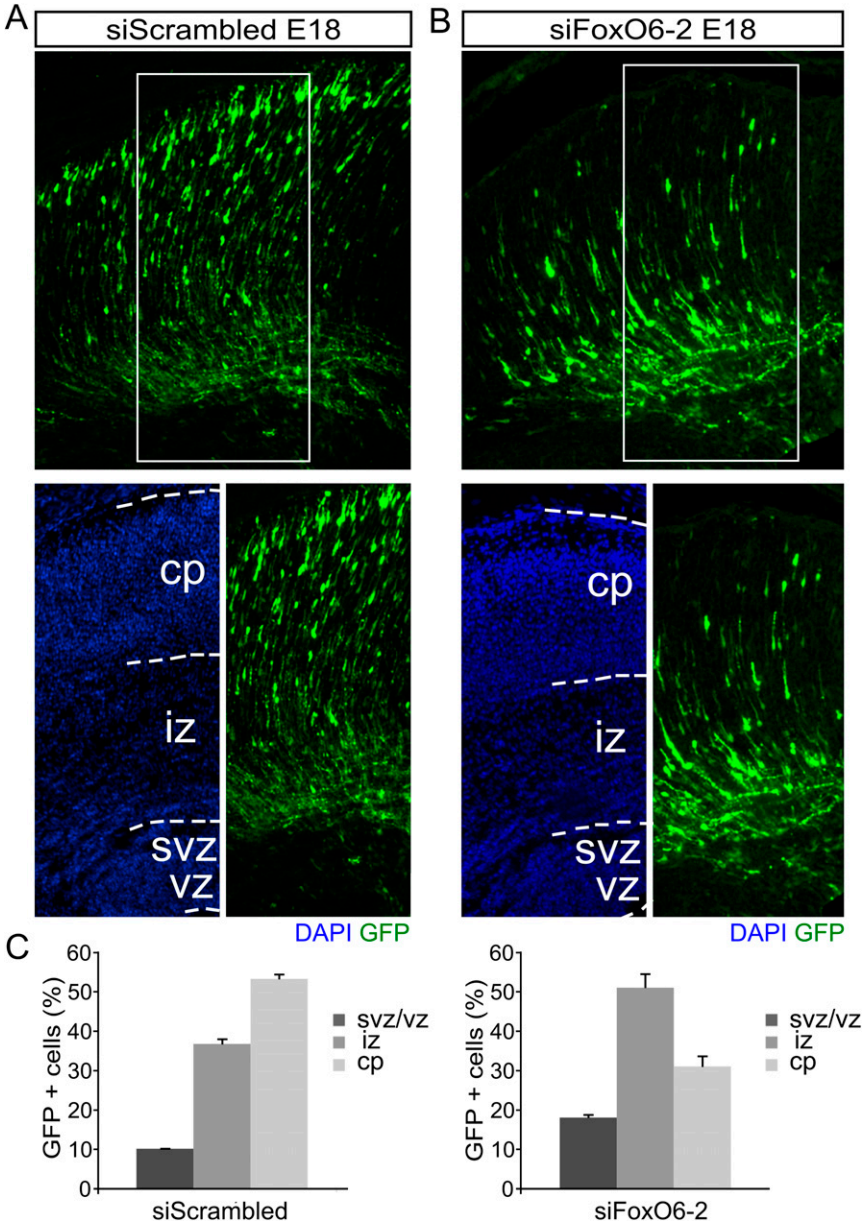

Fig. S4. Knockdown of *FoxO6* via siFoxO6-2 results in defective radial migration, confirming data of siFoxO6-1. (*A* and *B*) GFP protein expression at E18 in cortices in utero electroporated with siScrambled or siFoxO6-2, cotransfected with a *GFP* expression vector. siFoxO6-2 displays an altered distribution 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.

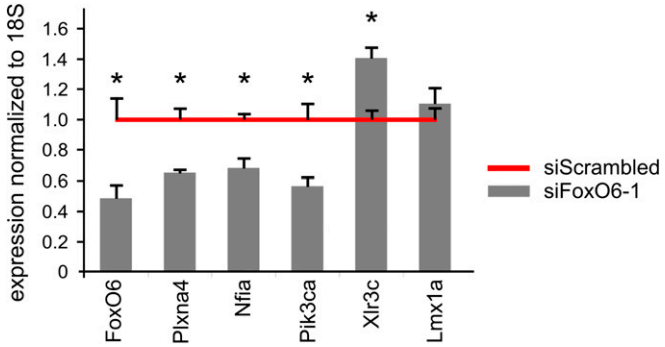

Fig. S5. Validation of a selection of genes as identified in the *FoxO6* knockdown genome-wide transcriptome analysis. In an independent set of experiments, cortices were in utero electroporated with siFoxO6-1 and cotransfected with a *GFP* expression vector, followed by FACsorting and RNA isolation. 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 *Xlr3c* was also confirmed. *Lmx1a* was used as an unchanged control. The error bars show the SD. Two-tailed Student's *t* test; $*P < 0.05$.

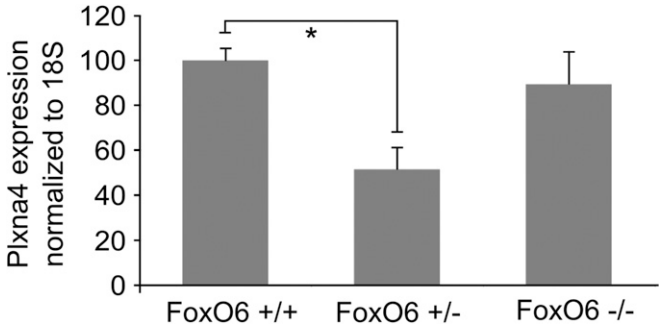

Fig. S6. *Plxna4* expression is significantly reduced specifically in $FoxO6^{+/-}$ FACsorted cortical cells. $FoxO6^{+/+}$, $FoxO6^{+/-}$, and $FoxO6^{-/-}$ cortices were in utero electroporated with *GFP* at E14.5, and labeled cells were FACsorted at E16.5 and followed by RNA isolation. *Plxna4* expression 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; $*P < 0.05$.

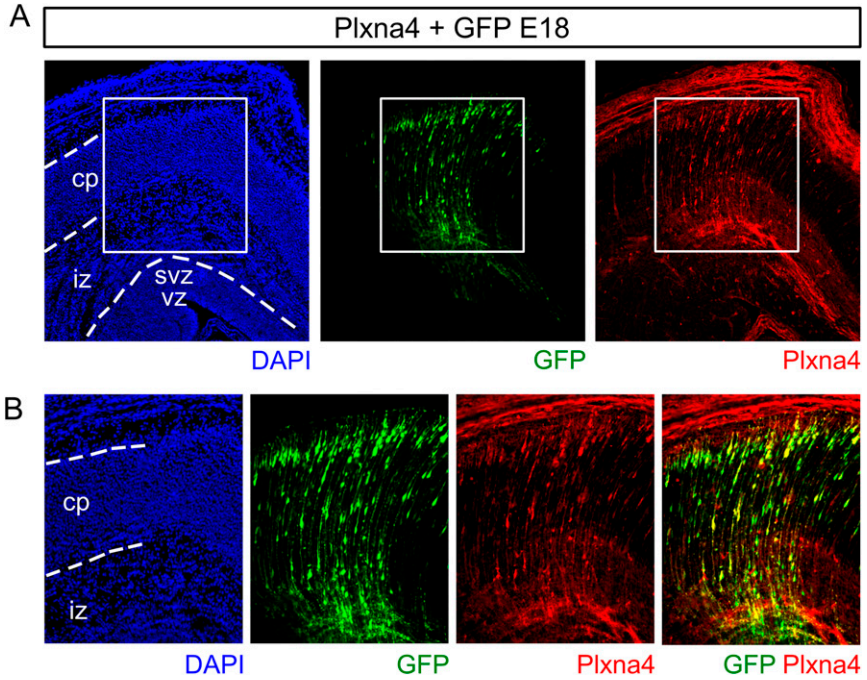

Fig. S7. Validation of Plxna4 protein overexpression after IUE with a *Plxna4* expression plasmid. (*A*) Cortices were electroporated with both *GFP* and *Plxna4* expression plasmids. Immunostaining for GFP and Plxna4 reveals that both genes are expressed in transduced cortical cells. (*B*) Magnifications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

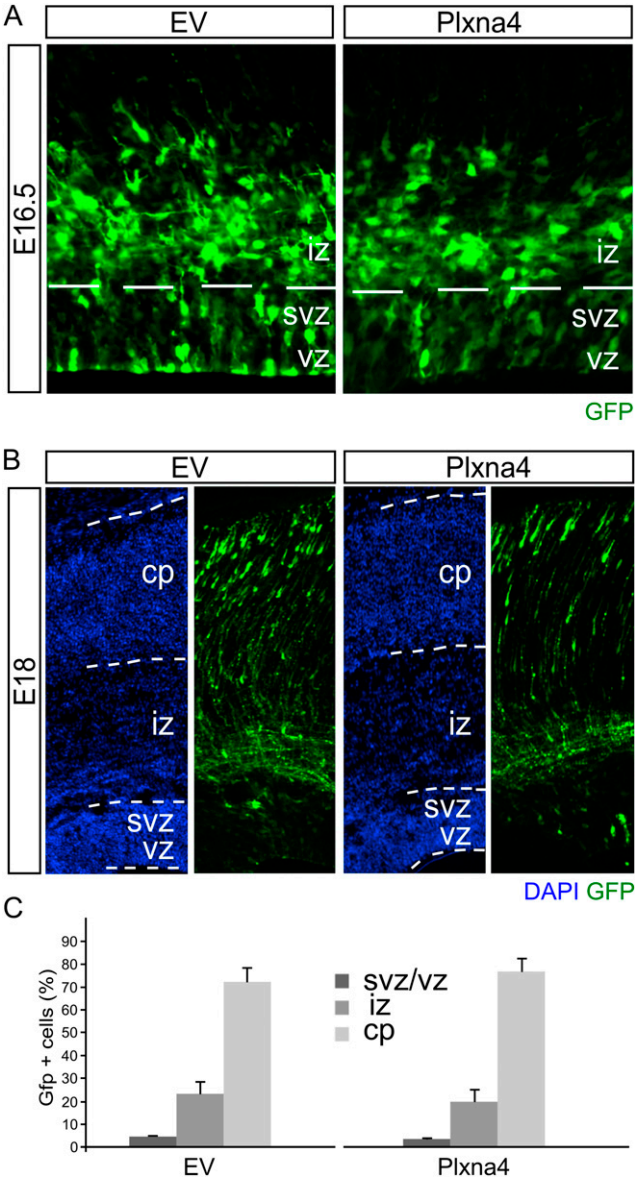

Fig. S8. Wild-type cortices overexpressing *Plxna4* are unaffected in cortical migration. (*A*) Cortical migration is unaffected at E16.5 in embryos in utero electroporated at E14.5 with a vector expressing *Plxna4* compared with empty vector control. (*B*) Cortical migration is unaffected at E18 in embryos in utero electroporated at E14.5 with a vector expressing *Plxna4* compared with empty vector control. (*C*) Quantification of duplicate experiments as shown in *B*. Ratios of GFP^+ cells between the (sub)ventricular zone, intermediate zone, and cortical plate are shown. The error bars show the SD.

Other Supporting Information Files

Table S1 (DOC)