

**Supplementary Fig. 1:** The cortical hem is normal in Nfib-deficient mice.

The cortical hem was investigated in wildtype (A) and Nfib-deficient (B) E12 hemisected brains by *in situ* hybridization analysis of *Wnt2b* expression. Sagittal views of these brains demonstrated that the cortical hem (arrows in A and B) appeared morphologically normal in Nfib mutants when assayed for expression of this marker. Section *in situ* hybridization of E12 wildtype (C,E) and knockout (D,F) brains demonstrates that the expression of *Wnt3a* (C,D) and *Lhx2* (E,F) is also grossly normal in mice lacking Nfib. In C-F cortical hem is labeled with a closed arrowhead, while the medial wall of the cortex is labeled with an open arrowhead. Scale bar: A,B 500 µm, C-F 700 µm.

**Supplementary Fig. 2:** Cellular proliferation is unchanged in the hippocampus of Nfib-deficient mice between E13 and E15.

Phosphohistone H3 immunohistochemistry on wildtype (A,C,E) and Nfib-deficient mice (B,D,F) at E13 (A,B), E14 (C,D) and E15 (E,F) in the mouse hippocampus showed no statistically significant differences in the number of cells in prophase in the ventricular zone of mutants compared to wildtype littermates (G). Error bars indicate standard error of the mean. Scale bar: A,B 225 µm; C,D 200 µm; E,F 180 µm.

**Supplementary Fig. 3:** Nfib-deficient mice do not exhibit increased apoptosis during hippocampal development.

Cellular apoptosis was investigated using anti-cleaved caspase 3 immunohistochemistry at E14, E15 and E18 in both wildtype (A,C,E) and Nfib-deficient mice (B,D,F). The arrows in all panels show apoptotic cells in the hippocampus. Apoptotic cells were uncommon in all sections examined. Scale bar: A,B 200 µm; C,D 180 µm; E,F 80 µm.
**Supplementary Fig. 4:** Expression of β-galactosidase in *Nfib* heterozygote and knockout mice.

Coronal paraffin sections of *Nfib* heterozygote (*A,C*) or knockout (*B,D*) hippocampi at E17 demonstrating expression of the β-galactosidase reporter gene. The boxed regions in *A* and *B* are shown at a higher magnification in *C* and *D*, respectively. In the wildtype, β-galactosidase expression is present evenly throughout the developing hippocampus (*C*). Expression of β-galactosidase in the mutant is also widespread but, crucially, β-galactosidase is highly expressed by cells in ventricular zone of the hippocampus (arrowhead in *D*). Scale bar: *A,B* 200 μm; *C,D* 100 μm.