

Fig. S1. Mcl1 is expressed in embryonic NPCs during cortical neurogenesis. (A) Mcl1 protein expression in NPCs 24 hours following *in vitro* transfection with Ctl, Mcl1 and mt Mcl1 constructs. Non-transfected cell lysates (no vector) were also loaded as controls. (B) Endogenous Mcl1 expression in an E14 mouse brain. (C) Representative high-magnification photomicrographs of transfected cells in the dorsomedial cortex in Ctl-, Mcl1- and mt Mcl1-electroporated brains. (D) TUNEL staining of an E14 non-transfected brain. Inset shows low magnification of the cortical hemisphere. (E,F) TUNEL staining of (E) Ctl- and (F) mt Mcl1-transfected brains at 24 h.p.e. Green, GFP⁺ cells; red, TUNEL⁺ cells.

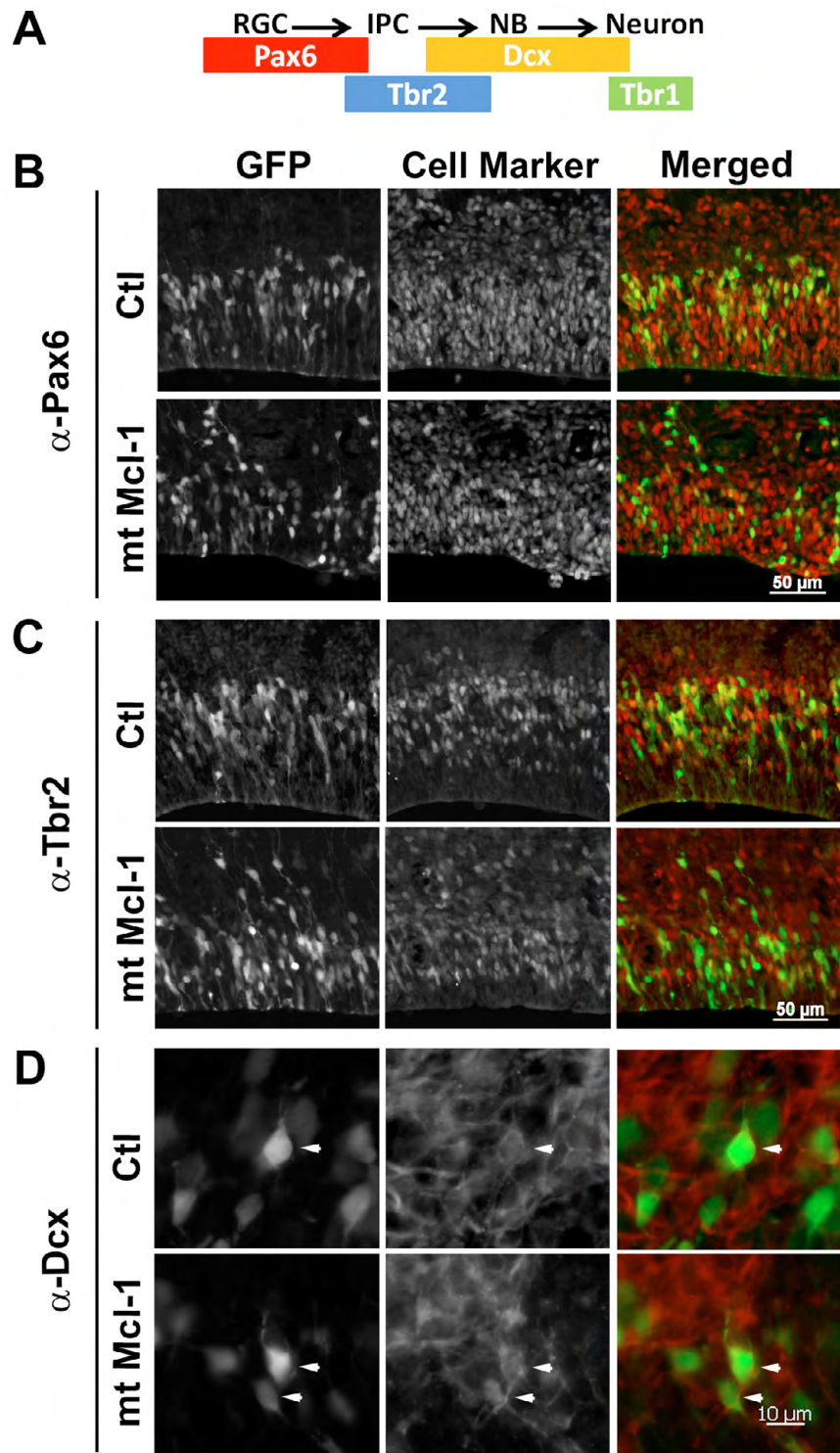


Fig. S2. Mcl1 promotes premature NPC differentiation in the embryonic brain. (A) Schematic showing the overlap in transcription factor expression during the transition from radial glial cell (RGC) to intermediate progenitor cell (IPC) to neuroblast (NB) to neuron (N). (B-D) E13 mouse embryos were *in utero* electroporated and collected at 20 h.p.e. and brain sections immunostained with antibodies to (B) Pax6, (C) Tbr2 and (D) Dcx. Note that higher magnification was used to observe GFP⁺ Dcx⁺ double-labeled cells. Green, GFP⁺ cells; red, Pax6⁺, Tbr2⁺ or Dcx⁺ cells.

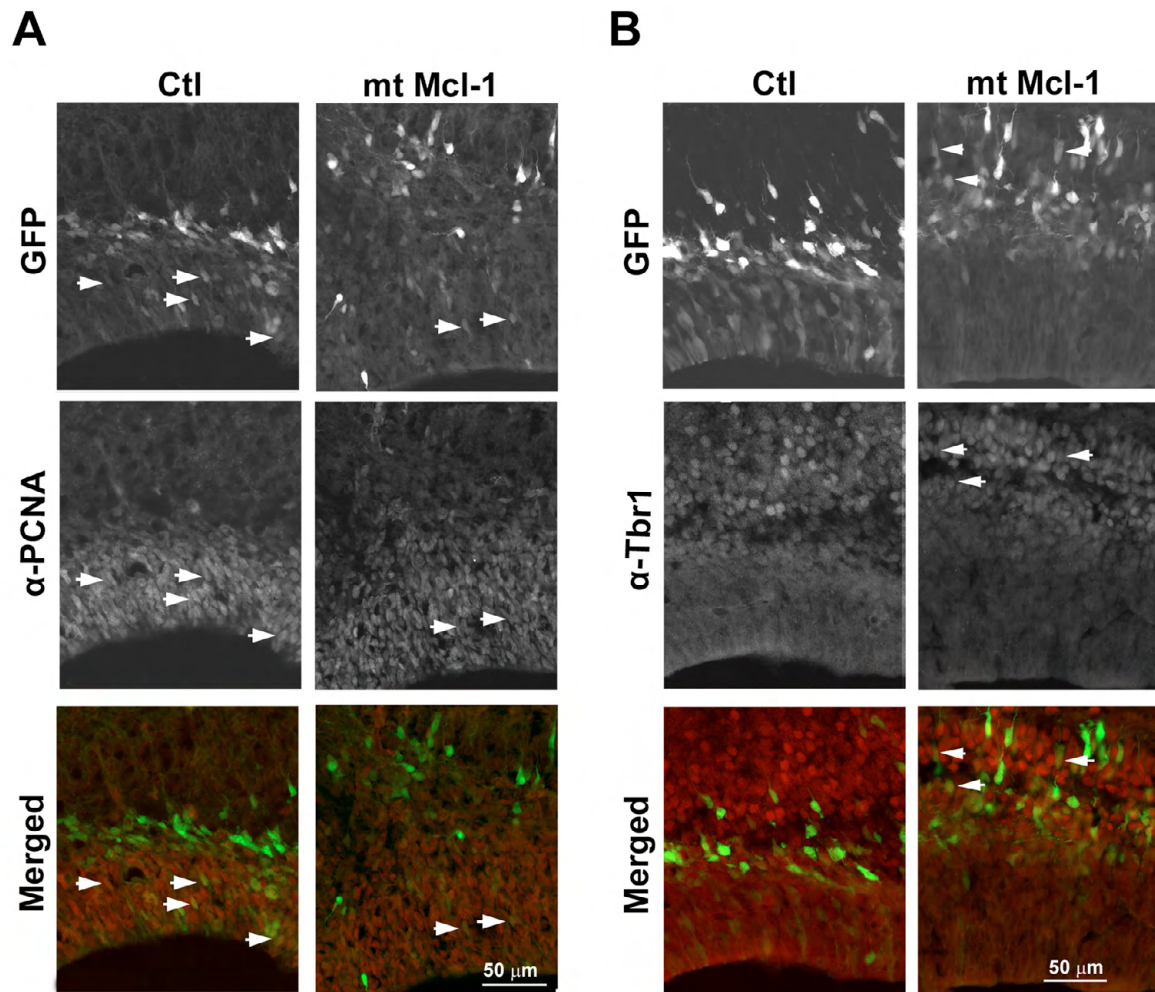


Fig. S3. Mcl1 promotes premature NPC terminal mitosis in the embryonic brain. E13 mouse embryos were *in utero* electroporated and collected at 48 h.p.e. and immunostained with antibodies to (A) PcnA and (B) Tbr1. Green, GFP⁺ cells; red, PCNA⁺ or Tbr1⁺ cells.

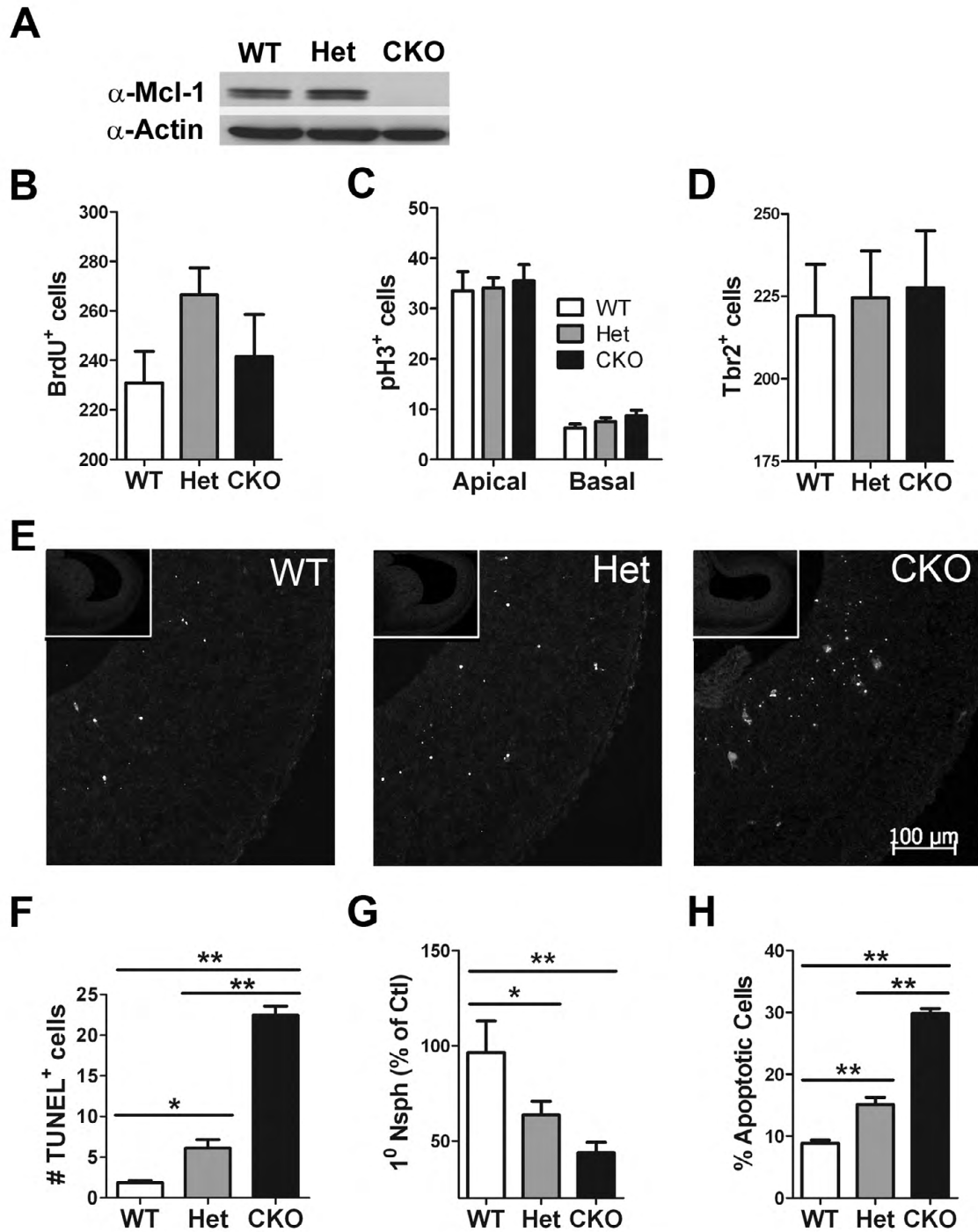


Fig. S4. Mcl1 loss-of-function *in vivo* results in apoptosis. (A) Mcl1 protein expression in NPCs from Mcl1 wild-type (WT), heterozygous (Het) and conditional knockout (CKO) embryos. Actin was used as a loading control. (B) Quantification of BrdU⁺ cells following a 4-hour BrdU pulse in Mcl1 WT, Het and CKO embryos at E14 ($n=4$ embryos/genotype). (C,D) Quantification of (C) PH3⁺ cells, a marker of cells in M phase, and of (D) Tbr2⁺ cells in the cortices of E14 Mcl1 WT, Het and CKO littermates. ($n=5$ embryos/genotype). (E) TUNEL staining of E14 Mcl1 WT, Het and CKO embryos. Cortical hemispheres are shown at low magnification in insets. (F) Quantification of TUNEL-positive cells revealed significantly higher levels of apoptosis in Mcl1 CKO and Het embryos. (G) The neurosphere assay was performed with NPCs cultured from E12 Mcl1 WT, Het and CKO NPCs and after 7 days *in vitro* the number of primary neurospheres was quantified. $n=13$ WT, $n=23$ Het, $n=24$ CKO. (H) Quantification of apoptotic cells in E12 Mcl1 WT, Het and CKO NPC cultures after 3 days *in vitro*. $n=5$ embryos/genotype. Means were analysed by one-way ANOVA and Tukey's post hoc test (B,D,F-H) or two-way ANOVA (C). * $P<0.05$, ** $P<0.01$. Error bars indicate s.e.m.

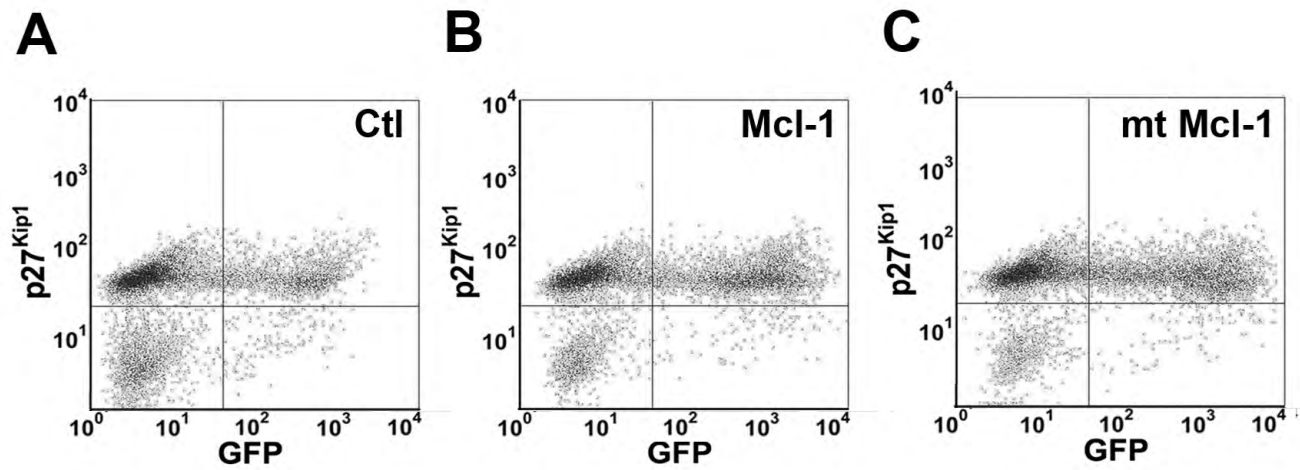


Fig. S5. Mcl1 upregulates $p27^{Kip1}$ in NPCs. FACS analysis was performed 24 h.p.t. on NPCs transfected with (A) Ctl, (B) Mcl1 and (C) mt Mcl1 plasmids. Analysis was performed on 20,000 gated GFP⁺ cells. $n=3$ /treatment.

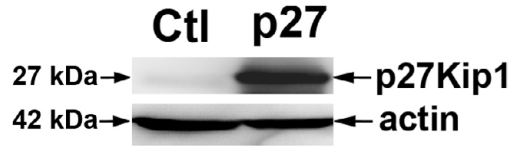
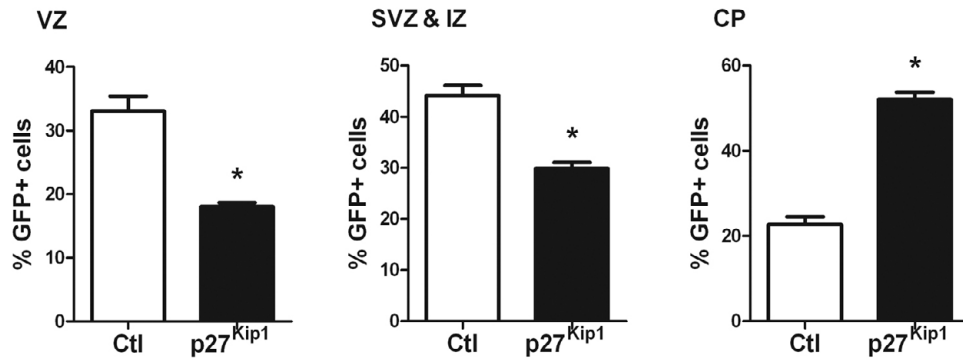
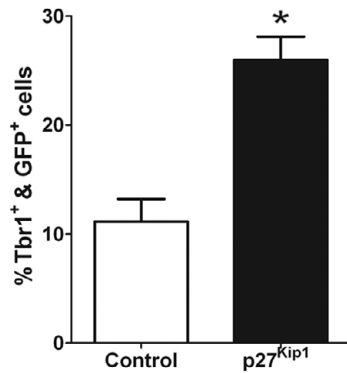
A**B****C**

Fig. S6. p27^{Kip1} promotes NPC terminal mitosis similar to Mcl1. (A) Western blot analysis of p27^{Kip1} expression in HEK 293A cells at 48 h.p.t. with pCIG2 empty vector (control, Ctl) and pCIG2-p27^{Kip1} constructs. (B) Quantification of the percentage of GFP⁺ cells located in the VZ, SVZ and CP at 48 h.p.e. in Ctl- and p27^{Kip1}-electroporated brains. *n*=3 embryos/treatment. (C) Quantification of the percentage of Tbr1⁺ GFP⁺ double-labeled neurons at 48 h.p.e. in Ctl- and p27^{Kip1}-electroporated brains. *n*=3 embryos/treatment. Means were analysed by *t*-test. **P*<0.05. Error bars indicate s.e.m.