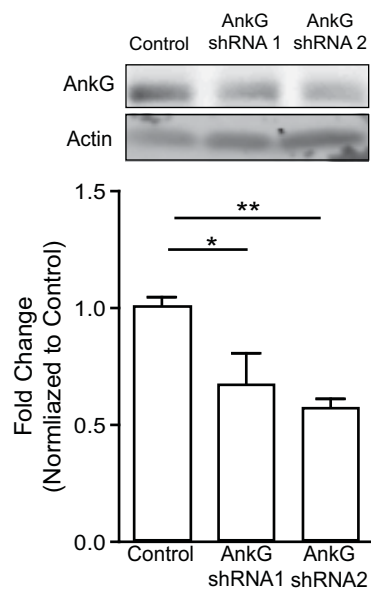
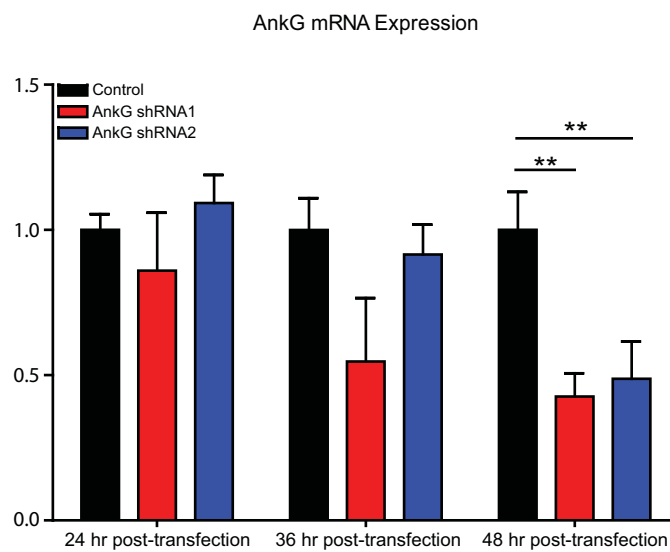
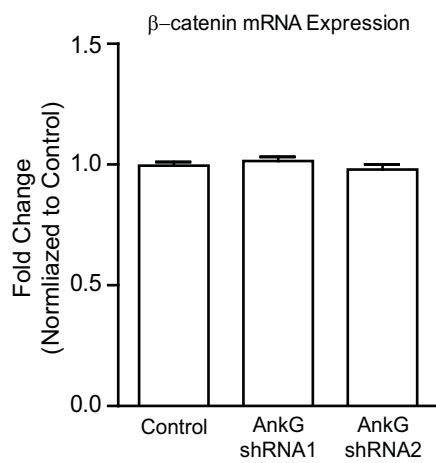
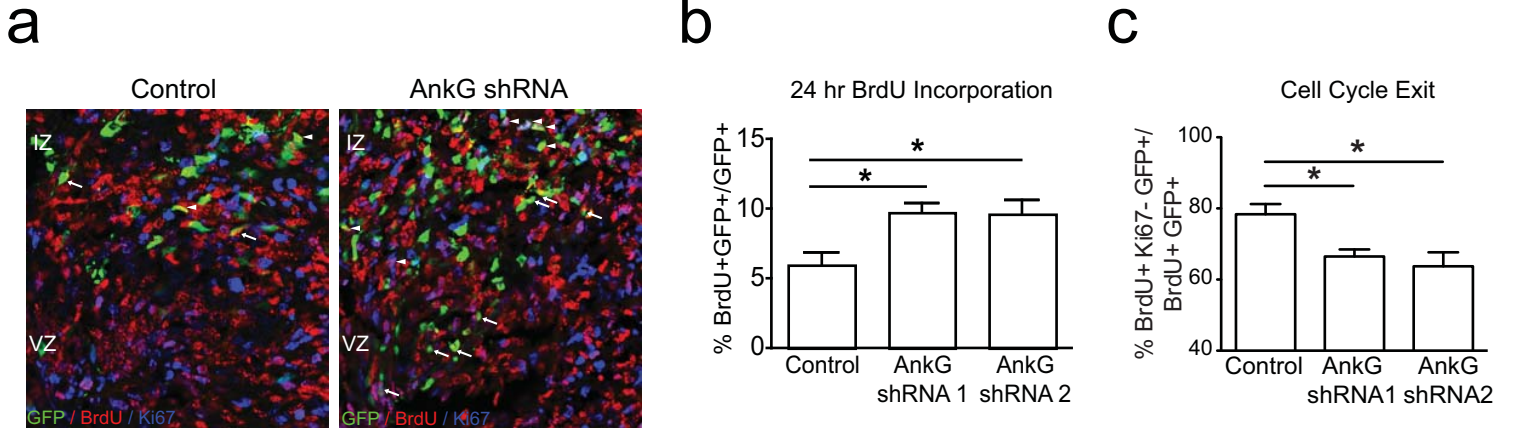


**a****b****c**

**Supplementary Figure 1: Ankyrin-G shRNAs specifically knocks-down ankyrin-G protein and mRNA levels, and does not change  $\beta$ -catenin mRNA levels. a)** Ankyrin-G shRNAs significantly lowered ankyrin-G protein expression compared to control shRNA in P19 cell line assessed by Western blotting (Control and shRNA2, n=4; shRNA1, n=3). **b)** Ankyrin-G shRNAs significantly lowered ankyrin-G mRNA levels compared to control shRNA 48 hours after transfection in P19 cell line assessed by qPCR analysis. (Control and shRNA1, n=10; shRNA2, n=9). **c)**  $\beta$ -catenin mRNA levels were not changed after ankyrin-G knockdown, consistent with  $\beta$ -catenin protein levels (Control and shRNA1, n=7; shRNA2, n=6). All analyses, one-way analysis of variance (one-way ANOVA) followed by Dunnett's Multiple Comparison Test, except panel (d) where Unpaired t-test is used; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001



**Supplementary Figure 2: Ankyrin-G knockdown reduces neural progenitor proliferation**

**in developing cortex a)** Images of E16 mouse cortices electroporated at E13 with non-targeting (left panel, Control) and ankyrin-G-directed small hairpin (right panel, AnkG shRNA) and GFP expression plasmid. Images were stained for GFP (green), BrdU (red) and Ki67 (blue). Arrows indicate BrdU, GFP double-positive cells, and arrowheads indicate Ki67, BrdU, GFP triple positive cells. **b)** Ankyrin-G knockdown resulted in increased BrdU incorporation (Control, n=4; shRNA1 and shRNA2, n=7). **c)** Ankyrin-G knockdown decreased cell cycle exit (Control, n=3; shRNA1, n=8 and shRNA2, n=4). All analyses, one-way analysis of variance (one-way ANOVA) followed by Dunnett's Multiple Comparison Test; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001

**Supplemental Table 1: DNA construct and primer sequences**

**Gene expression analysis.**

<b>Gene-specific primers</b>	<b>Forward sequence (5'-3')</b>	<b>Reverse sequence (5'-3')</b>
<i>ANK3</i>	AGTGAAGAGCCAAAGGAGAAG	TCAGAATCAAACCTCCCTCGTG
<i>Ctnnb1</i>	GCTATTCCACGACTAGTTCAGC	AGCTCCAGTACACCCTTCTAC
<i>Actb</i>	AGCCATGTACGTAGCCATCC	CTCTCAGCTGTGGTGGTGAA

**Knockdown assays.**

<b>Gene-specific shRNA</b>	
Ankyrin-G shRNA1	CCTGCTCATAGGAAGAGGAAA
Ankyrin-G shRNA2	CCGCCTGGTAAAGAGACATAA

*ANK3*, ankyrin-G (NM\_170730.1); *Ctnnb1*,  $\beta$ -catenin (NM\_007614); *Actb*,  $\beta$ -actin (NM\_007393).

Ankyrin-G shRNA1 (TRCN0000090056); ankyrin-G shRNA2 (TRCN0000090054).