Supplementary Information

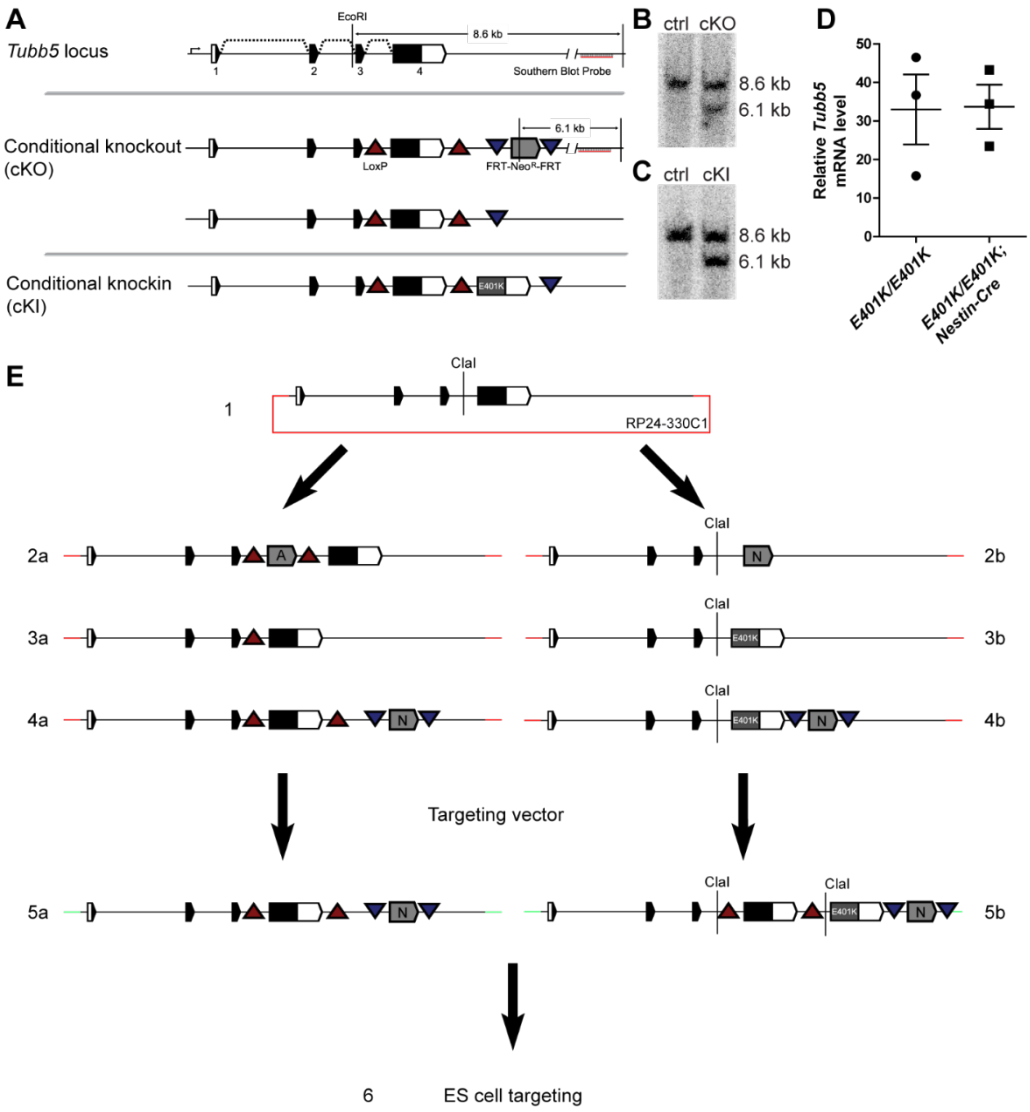

Fig. S1. (A) Schematic representation of the *Tubb5* locus, the conditional knockout allele before and after Flp- and Cre-recombination, and the conditional knockin allele. (B) Southern blot analysis of an ESC clone harboring the conditional knockout allele (ctrl=control, cKO=conditional knockout). (C) Southern blot analysis of an ESC clone harboring the conditional knockin allele (cKI=conditional knockin). (D) Quantification of the relative mRNA level of Tubb5 transcript, assessed in the embryonic cortex of *Tubb5(E401K/E401K)* and *Tubb5(E401K/E401K); Nestin-Cre* at E14.5. Homozygous knockin of the E401K mutation does not decrease transcript abundance. For the statistical analysis we employed a two-tailed Student's t-test. (E) Schematic representation of the generation of the two targeting constructs. Recombineering employed a BAC clone containing the *Tubb5* murine locus (RP24_330C1, 1). For the knockout, a LoxP-AmpR-LoxP (GeneBridges) construct was added upstream of Exon 4 (2a). Following Cre-recombinase mediated removal of the AmpR cassette (3a), a LoxP-FRT-NeoR-FRT cassette was added downstream of Exon4 (4a). For the knockin, Exon 4 was replaced by a NeoR cassette (2b) which was replaced by an E401K containing Exon 4 using negative selection (3b) followed by the addition of a NeoR cassette (4b). Both constructs were then transferred into the targeting vector. Utilizing an endogenous ClaI site a floxed version of Exon 4 (wild type) was added upstream of the mutated Exon 4 (5b). Targeting constructs (5a and 5b) were then used to target the endogenous *Tubb5* locus of ES cells (6).

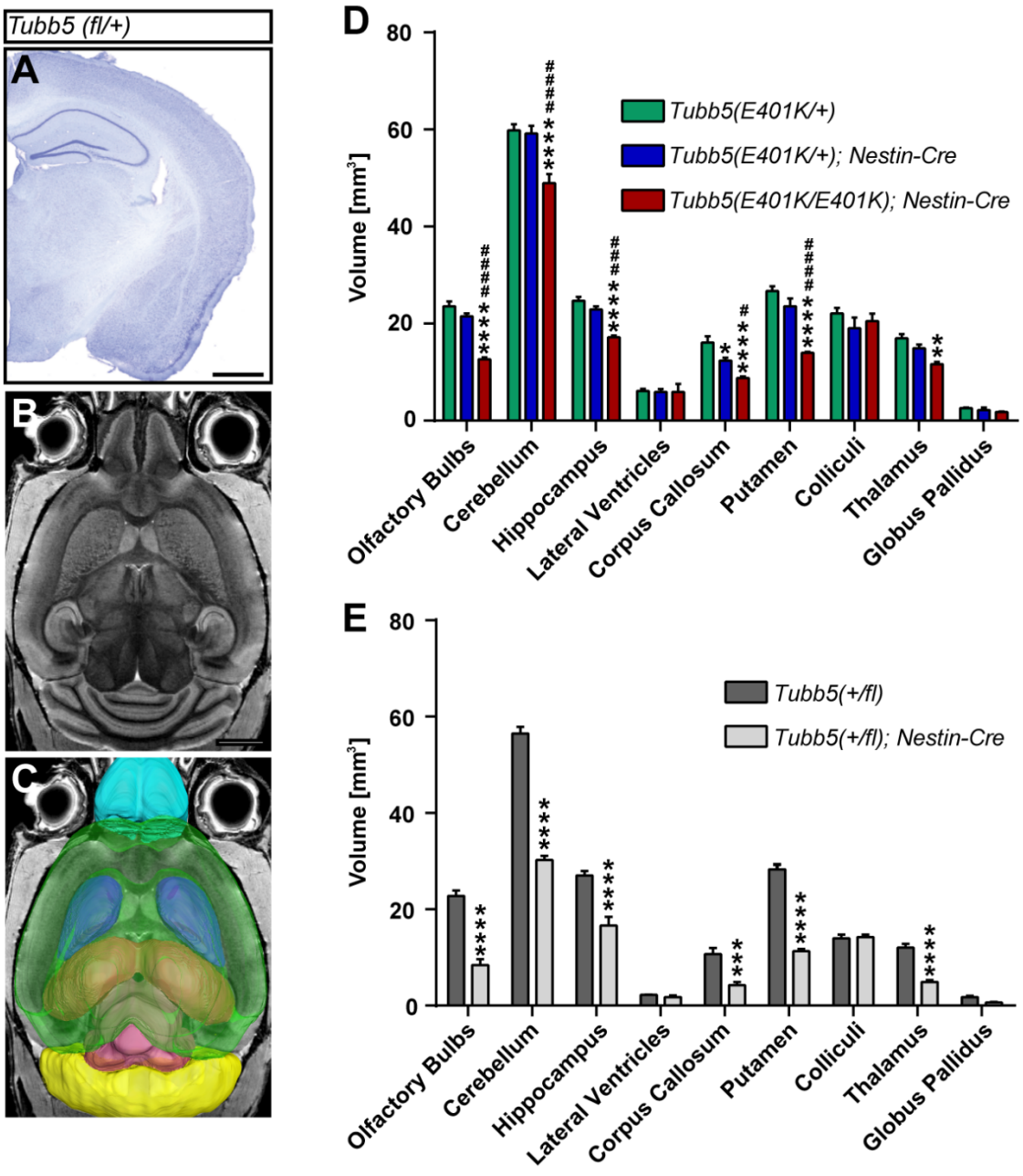

Fig. S2. Related to Fig. 1. (A) Nissl-stained, coronal section of the adult brain for *Tubb5(fl/+)*. (B) Axial magnetic resonance image (MRI) of a *Tubb5(fl/+)* mouse. (C) Same image as in B with 3D reconstructions of sampled brain regions following segmentation. Shown are olfactory bulbs (light blue), putamen (dark blue), hippocampus (red), cerebellum (yellow), the colliculi (light red) and the cortex (translucent green). (D-E) Quantifications of brain regions for the knockin (D) and knockout (E) mouse lines. Error bars show ± SEM. $*^{/\#}$P<0.05, $**^{/\#\#}$P<0.01, $***^{/\#\#\#}$P<0.001, $****^{/\#\#\#\#}$P<0.0001 (* and # in E indicate significance relative to *Tubb5(E401K/+)* and *Tubb5(E401K/+); Nestin-Cre*, respectively); for the statistical analysis shown in D-E we employed a two-way ANOVA with a Bonferroni post-test for multiple comparisons. Scale bars in A and B show 1000 µm and 2000 µm, respectively. n=3 for all conditions.

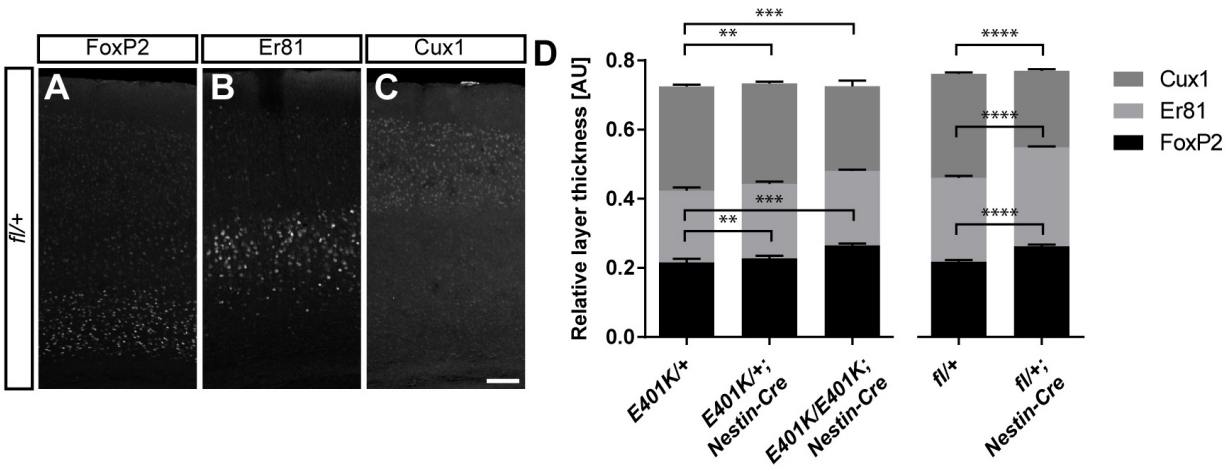

Fig. S3. Related to Fig. 2. (A-C) Control immunostaining of the *Tubb5(fl/+)* line for FoxP2 (A), Er81 (B) and Cux1 (C). These are littermate controls of the *Tubb5(fl/+); Nestin-Cre* animals show in Figure 2 (D, H, and L). (D) Relative cortical layer thickness for the knockin and knockout lines for FoxP2, Er81 and Cux1 positive layers. Graphs show mean ± SEM. **P<0.01, ***P<0.001, ****P<0.0001; for the statistical analysis shown in D we employed a two-way ANOVA with a Bonferroni post-test for multiple comparisons. Scale bar in C shows 100 µm. n=3 for all conditions.

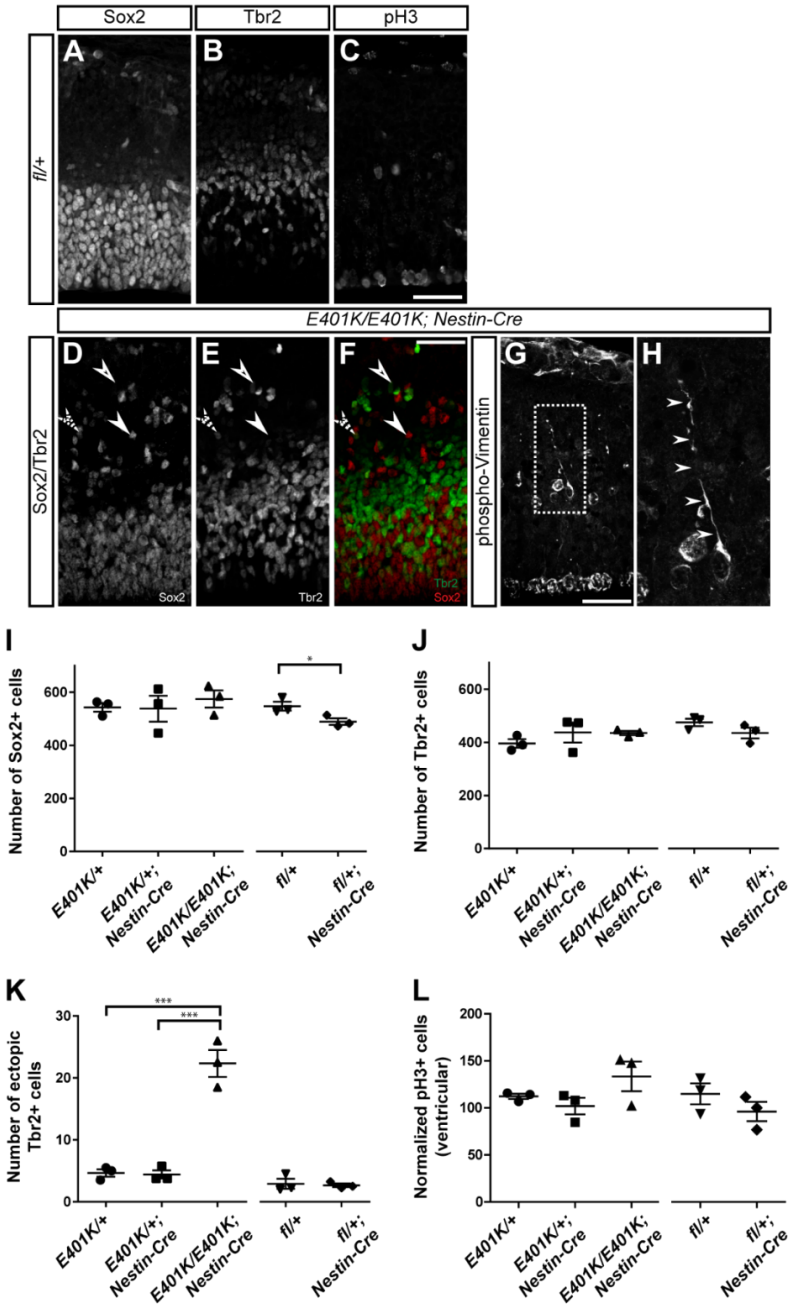

Fig. S4. Related to Fig. 3. (A-C) Immunostainings for Sox2 positive radial glial cells (A), Tbr2 positive intermediate progenitors (B) and pH3 positive mitotic cells (C) for the *Tubb5(fl/+)* embryonic cortex at E14.5. These are littermate controls of the *Tubb5(fl/+); Nestin-Cre* animals show in Figure 3 (D, H, and l). (D-H) Immunostainings for the *Tubb5(E401K/E401K); Nestin-Cre* embryonic cortex (E14.5) for Sox2 and Tbr2 (D-F) and phospho-Vimentin (G-H). D and E show grayscale images of F for Sox2 and Tbr2, respectively. Arrowheads in D-F indicate Tbr2 (open), Sox2 (closed) and double positive cells (open, dashed). G shows a phospho-Vimentin positive cell with a glial process and morphology consistent with an ectopic radial glial cell. H shows a magnification of the boxed region in G. Arrowheads indicate a putative basal process. (I-L) Quantification of the number of Sox2 positive (I), Tbr2 positive (J), Tbr2 positive ectopic (K), and the relative number of ventricular pH3 positive (L) cells for all genotypes. Error bars in I-L show the mean ± SEM. *P<0.05, ***P<0.001; for the statistical analysis shown in I-L we employed a one-way ANOVA with a Bonferroni post test for multiple comparisons for the knockin and a two-tailed Student's t-test for the knockout. Scale bars in C, F and G show 50 μm.

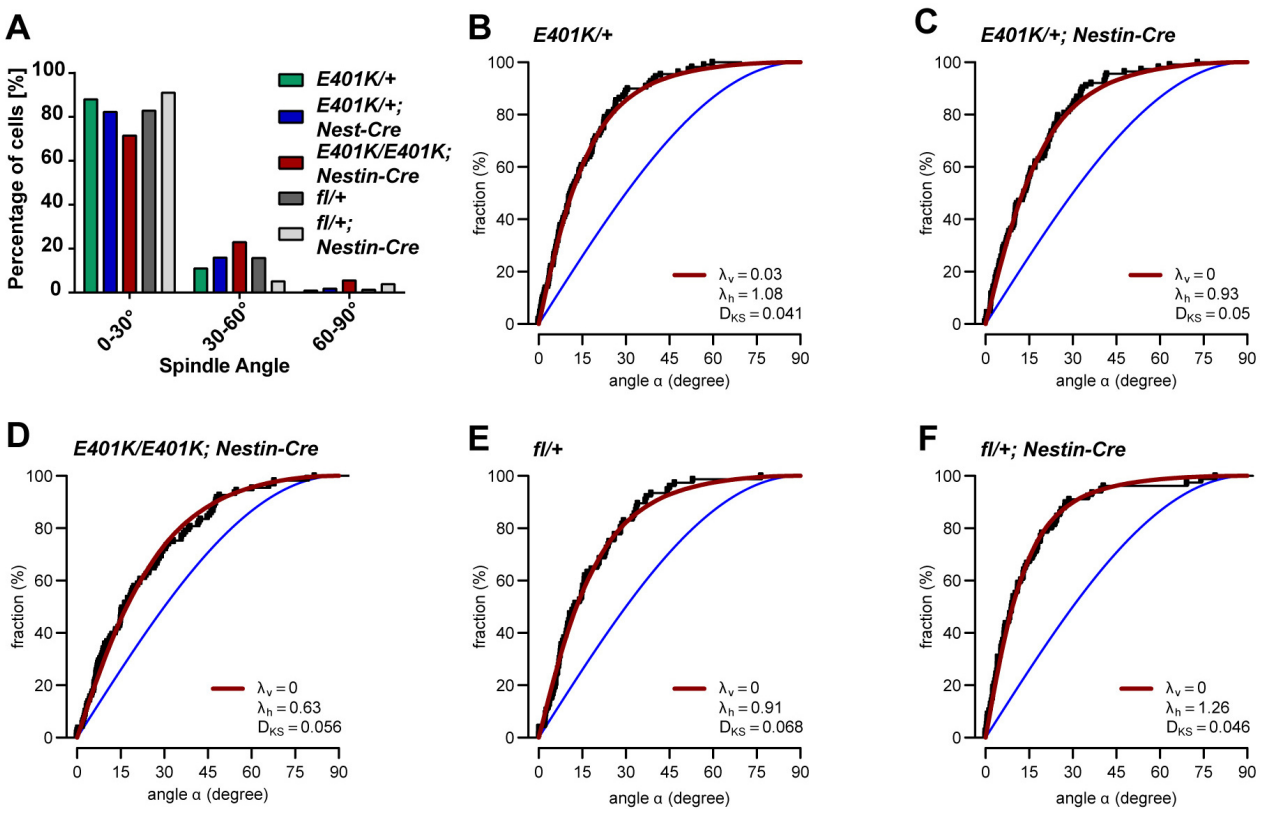

Fig. S5. Related to Fig. 3. (A) Quantification of the relative abundance of horizontal (0-30º), oblique (30-60º) and vertical (60-90º) spindle orientations for knockin and the knockout lines. Note the increase in oblique and vertical spindle orientations in the homozygous knockin (n=4 animals, n≥76 mitotic cells per condition). (B-F) Cumulative spindle angle plot for the data shown in A (1). λ_v and λ_h show enrichment for vertical and horizontal spindle orientations, respectively.

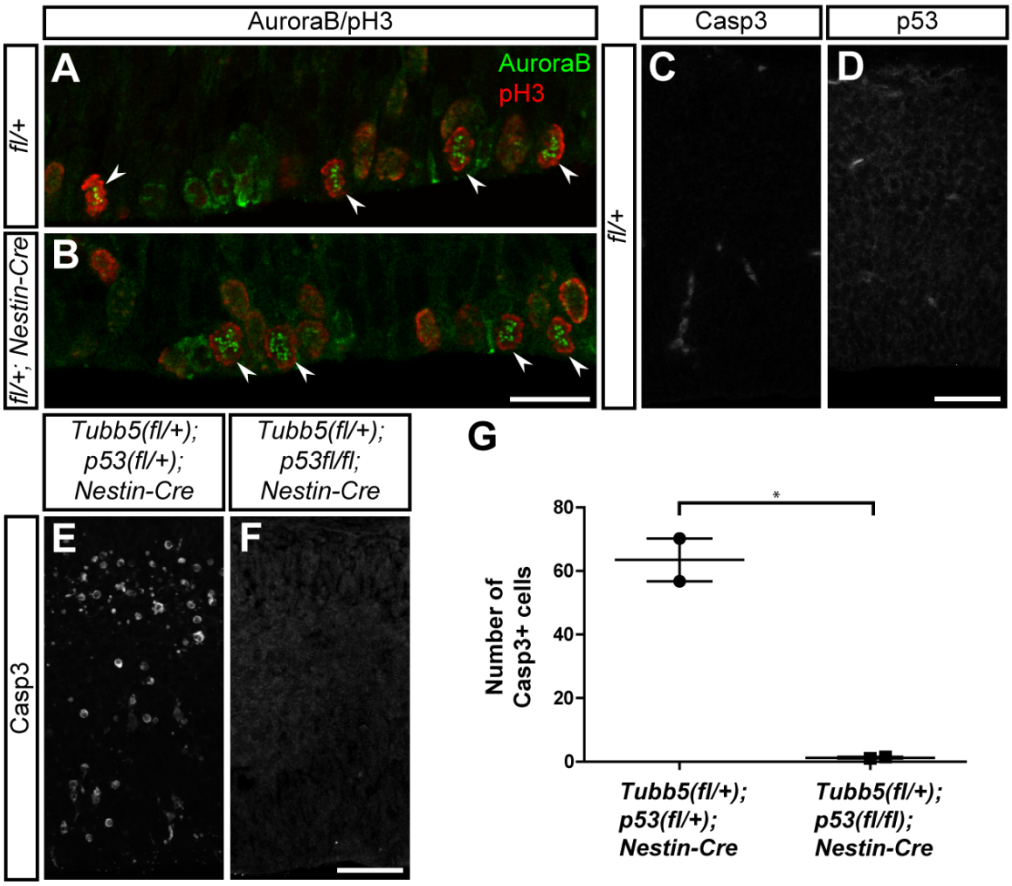

Fig. S6. Related to Fig. 4. (A-B) Immunstainings for AuroraB and pH3 for the heterozygous knockout line. Arrowheads indicate cells that were scored to be in metaphase. (C-D) Immunostainings for cleaved Caspase3 (C) and p53 (D) for the *Tubb5(fl/+)* embryonic cortex at E14.5. These are littermate controls for the data shown in Figure 4T and X. (E-F) Immunostainings for cleaved Caspase 3 for a *Tubb5(fl/+); p53(fl/+); Nestin-Cre* embryonic cortex at E14.5 and a *Tubb5(fl/+); p53(fl/+); Nestin-Cre* rescue. Note the absence of cleaved Caspase3 staining upon homozygous knockout of p53. (G) Quantification of the number of cleaved Caspase 3 positive cells for the genotypes shown in E and F. Error bars in G show the mean ± SEM. *P<0.05; for the statistical analysis shown in G we employed a two-tailed Student's t-test. Scale bars show 50 µm in D and F and 20 µm in B. n=3 for A-D; n=2 for E-G.

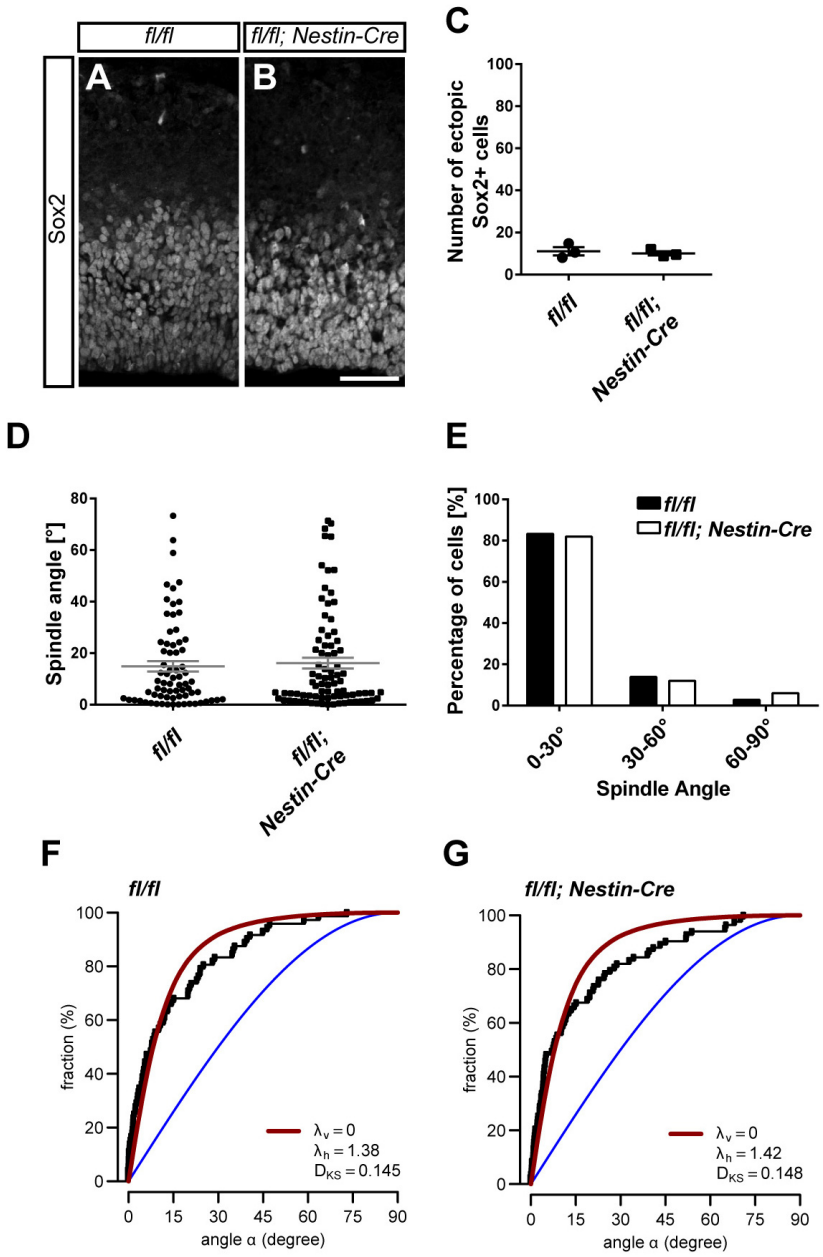

Fig. S7. Related to Fig. 5. (A-B) Immunostainings for the radial glia marker Sox2 for the homozygous knockout (*Tubb5(fl/fl); Nestin-Cre*) and a littermate control (*Tubb5(fl/fl)*). (C) Quantification of the number of ectopic Sox2 positive cells found per cortical image. (D-E) Quantification of spindle angle measurements for the homozygous knockout and littermate controls. There is no significant difference in average spindle orientation. Graphs show the summary of the total population of cells analyzed (n=3 animals, n≥72 mitotic cells per condition). (F-G) Cumulative spindle angle plot according to Jüschke and colleagues for the data shown in A and B (1). Error bars in C and D show ± SEM. For the statistical analysis shown in C-D we employed a two-tailed Student's t-test. Scale bar shows 50 µm in *B*.

Table S1. Primers

Tubb5_LoxP_F	TCTAGAACCTGGGACTATGG
Tubb5_LoxP_R	CACCACATCCAAGACAGAG
Cre_F	TCGCGATTATCTTCTATATCTTCAG
Cre_R	GCTCGACCAGTTTAGTTACCC
Tubb5_SB_F	CGGTGGCTTACTCTTAAGACTG
Tubb5_SB_R	CTTGAGTCATGGCTGGGGAG
Southern Blot Probe	CGGTGGCTTACTCTTAAGACTGgcagcaaagacaaatggagacatgtgaacaggactgcggag gtagattatgaccacaaagggctcagtgccctcgggaccctcatactgtagcgacttacccagggagggcaagtacc agcctcatccattctgacgctaagtccttagcctagctgctctaagaagtcacaaggcggttcccaggccctggcttgat tctgtgcctgaaatggatcccactaccgagtaggctgagctgaccagaagaatgcttcctgggtctcttccacgttgtgt atcccactggtggcgctggcttttctctctgactcaggccacccgCTCCCCAGCCATGACTCAAG

Table S2. Antibodies

Antibody	Supplier	Dilution	Blocking solution
FoxP2	Abcam (ab16046)	1:500	0.1% TX-100/PBS; 2% ds
Er81	Kind gift of the Jessel laboratory	1:3000	0.3% TX-100/PBS; 2% ds
Cux1	Santa Cruz (sc-6327)	1:100	0.1% TX-100/PBS; 2% ds
Sox2	Santa Cruz (sc-17320)	1:200	0.3% TX-100/PBS; 2% ds
Tbr2	Abcam (ab23345)	1:200	0.3% TX-100/PBS; 2% ds
Dcx	Santa Cruz (sc-8066)	1:100	0.3% TX-100/PBS; 2% ds
pH3	Millipore (06-570)	1:500	0.3% TX-100/PBS; 2% ds
N-Cadherin	Invitrogen (3B9)	1:500	0.3% TX-100/PBS; 2% ds
γ-Tubulin	Sigma-Aldrich (T6557-.2ML)	1:100	0.3% TX-100/PBS; 2% ds
p-Vimentin	MBL (D-076-3)	1:1000	0.3% TX-100/PBS; 5% BSA
Cleaved Casp3	Cell Signaling (9661S)	1:400	0.3% TX-100/PBS; 2% ds
p53	Leica Microsystems (P53-CM5P)	1:1000	0.3% TX-100/PBS; 2% ds
AuroraB	BD Biosciences (611083)	1:250	0.3% TX-100/PBS; 2% ds