Supplementary Materials and Methods

Generation of conditional knockout mice. Paxillin floxed (*Pxn*$^{F/F}$) and *Hic-5*$^{-/-}$ mice were generated by the Gene Targeting and Transgenic Facility of the University of Connecticut (Farmington, CT, USA). To generate *Pxn*$^{F/F}$ mice, a targeting vector was generated with exon assignment based on the Ensembl paxillin transcript (ENSMUST00000067268). The vector was constructed such that exons 2-5 were flanked with loxP sites. An Frt-PGK1-Neo-Frt positive selection cassette (Neomycin phosphotransferase under the phosphoglycerate kinase 1 promoter) was inserted 3' to exon 5. The targeting vector was introduced into 129/SvEv embryonic stem cells and homologous recombination was confirmed by G418 positive selection and diphtheria toxin A (DTA) negative selection. Aggregation was performed with CD1 morula and the resulting chimeric mice were screened for germline transmission of the targeting vector. To avoid the possibility of cryptic splice sites produced by Neo-cassette, ROSA26-Flp (flippase recombinase) mice were then bred with the germline chimeric mice to remove the PGK1-Neo cassette. *Hic-5*$^{F/F}$ mice were generated with a similar protocol and bred with *HPRT-Cre* mice to generate a germline deletion of exons 2-7 (Goreczny et al., 2016). Both the *Pxn*$^{F/+}$ and *Hic-5*$^{+/-}$ mice were maintained by backcross with C57BL/6J and intercrossed to generate homozygous mutants. Generation of *Nestin-Cre:Pxn*$^{F/F}$ mice. *Pxn*$^{F/+}$ mice were bred with *Nestin-Cre* transgenic mice (Tronche et al., 1999) which drives Cre-mediated recombination in the central nervous system by E10.5 (Graus-Porta et al., 2001). *Nes-Cre:Pxn*$^{F/+}$ offspring were then bred with *Pxn*$^{F/F}$ to generate homozygous conditional mutants (*Nes-Cre:Pxn*$^{F/F}$). The *Nes-Cre:Pxn*$^{F/F}$ are thus on a mixed C57BL/6J-129/Sv background. Generation of paxillin/Hic-5 double knockout mice. *Nes-Cre:Pxn*$^{F/F}$ mice were crossed with *Hic-5*$^{-/-}$ mice for two generations to achieve *Nes-Cre:Pxn*$^{F/F}$*Hic-5*$^{-/-}$ offspring. Genetic targeting of neurons. *NEX-Cre* mice (Eppig and Schroeder, 1989, Belvindrah et al., 2007) were crossed into *Pxn*$^{F/F}$ to delete paxillin in neurons, at the onset of cortical migration.

Mouse genotyping by PCR

In most cases, genomic DNA obtained from tail digest was used for genotyping. For example, the *Nes-Cre* transgene is expressed in the hair follicle thus allowing for genotyping of *Nes-Cre* conditionals using tail DNA (Li et al., 2003). However, confirmation of *NEX-Cre* mediated recombination required genomic DNA isolated from brain. Primers used for genotyping: Detection of paxillin (unrecombined allele): The forward primer 5'-gtttggggctggactctacc, and reverse primer 5'-tacagctaaggcatgtagag produce a band at 347 bp for the native paxillin

wildtype allele and a band at 443 bp for paxillin flox allele. Detection of the paxillin KO allele (recombined allele): The forward primer 5'-gtttggggctggactctacc and reverse primer 5'-tacagcgctgcacatagacg produce no product for wildtype allele and a 604 bp band for the paxillin KO allele. *Cre* transgenes (both *Nestin* and *NEX*) were detected with forward primer 5'-gcggtctggcagtaaaaactatc and reverse primer 5'-gtgaaacagcattgctgtcactt which produces ~100bp band. Detection of the *Hic-5* KO allele: The forward primer 5'-tcactcaatcctgcttgtgc and reverse primer 5'-catttatctcccacagtgtcc produce no product for the *Hic-5* wildtype allele and a 567 bp band for the *Hic-5* KO allele.

Expression analysis by microarray and *in situ* analysis

Data from a prior Affymetrix (Gene 1.0ST) microarray study of FAC sorted GFP-expressing neurons from E14.5 Tg(Eomes::eGFP)Gsat embryos (Cameron et al., 2012) were queried for the RMA expression levels of the focal adhesion adaptor proteins and controls. *In situ* hybridization: four probes were used (*Pxn, Hic-5, and Lpxn* anti-sense) as well as a control *Hic-5* sense probe. The probes were generated by PCR amplification of cDNA derived from whole embryos using the following primers: *Pxn* forward 5'-gacagcatgttggggagtct, *Pxn* rev 5'-ggtgttgagggctgaaatgt, *Hic-5* forward 5'-gtaaccaacccatccgacac, *Hic-5* reverse 5'-gctgagcatggaaatggttt, *Lpxn* forward 5'-aagaagccatactgccgaaa, *Lpxn* reverse 5'-ggctggggacagaatctatg. The PCR products were then cloned into pcDNA3.1 (Invitrogen) for subsequent linearization and digoxigenin-labeled probe synthesis. *In situs* were performed by UB-In Situ (Natick, MA) using cryosection from E15.5 mouse brains that were snap-frozen in isopentane.

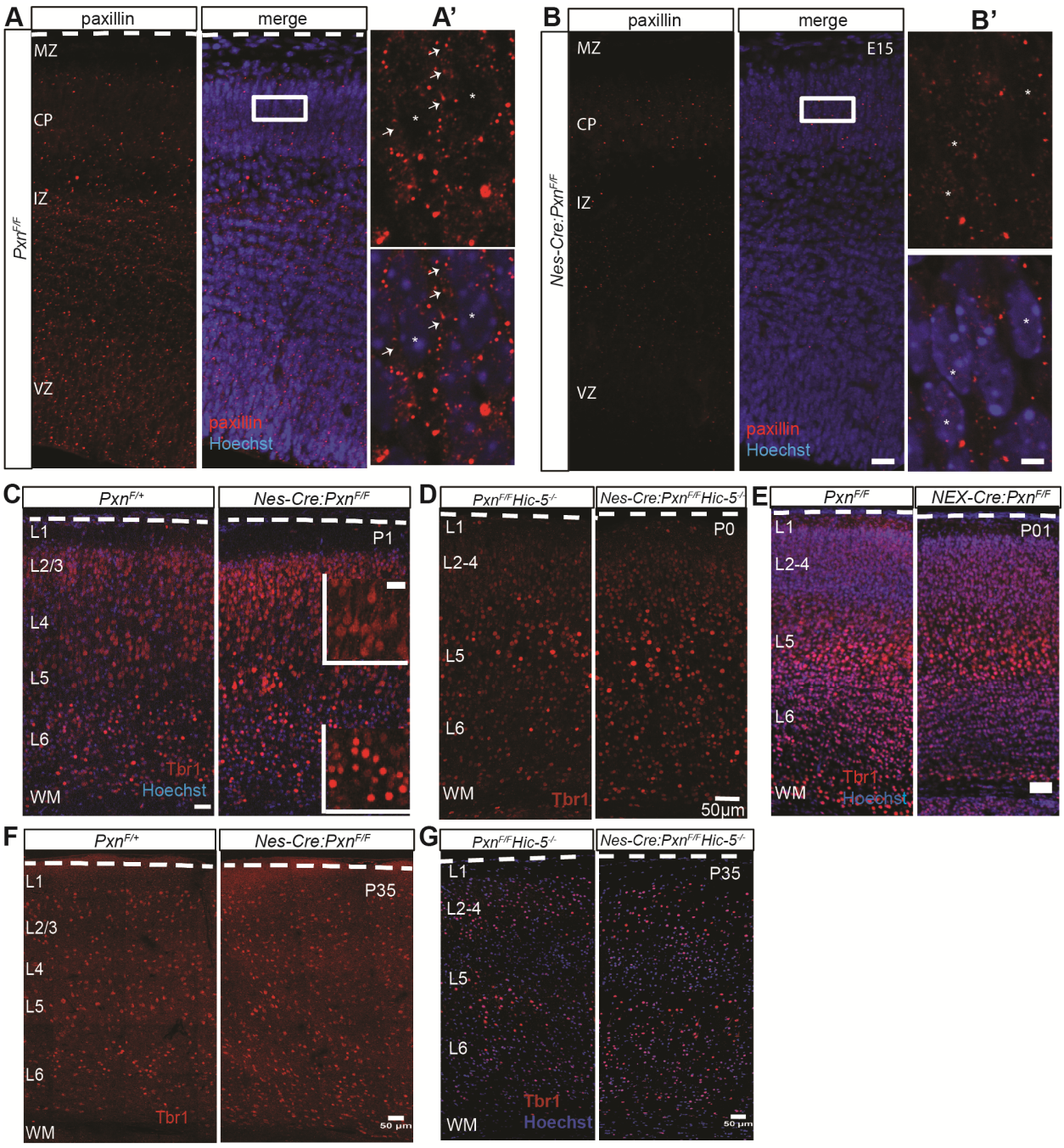

Fig. S1. Expression of paxillin and Tbr1 in the cortical sections. Representative images of paxillin (red) immunosignal on (A) wild type cortex and (B) mutant cortex at E15. Higher magnification image in the CP (box) showed that paxillin mostly found in the perinuclear and leading process area (arrows in A') and devoid of nucleus (asterisk in A'). Paxillin mutant has very faint signal that diffused non-specifically to the nucleus (asterisk in B'). (C-G) Representative images of Tbr1 staining for *Nes-Cre:Pxn$^{F/F}$*, *Nes-Cre:Pxn$^{F/F}$Hic-5$^{-/-}$, and NEX-*

Cre:Pxn$^{F/F}$ along with their littermate controls at different ages. Note: Only strong nuclear Tbr1+ neurons (usually in lower layer, inset C) were counted for quantification. Diffusely immunolabeled Tbr1 (usually in upper layer, inset) were excluded for quantification. Scale bars: 20 µm in B; 5 µm in B'; 50 µm in C-G; 20 µm in inset of C.

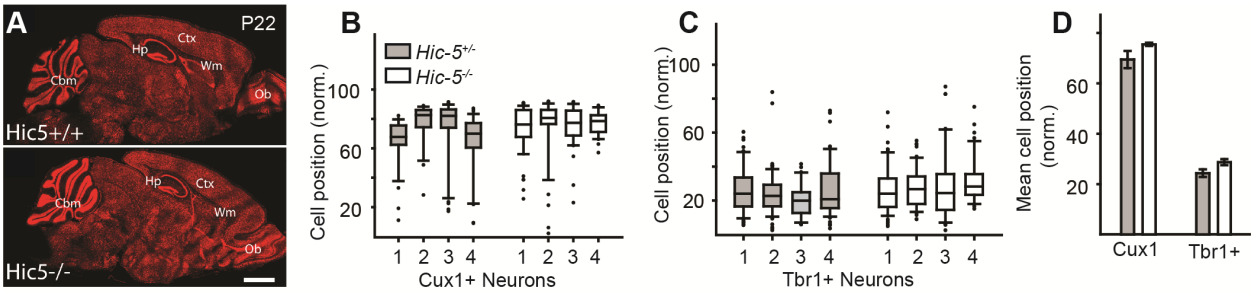

Fig. S2. Analyses of P22 *Hic-5*$^{-/-}$ mouse brain. (A) Sagittal section of Hic-5$^{+/+}$ and Hic-5$^{-/-}$ mouse brain stained with nuclear dye propidium iodide. (B) Distribution of Cux1+ neurons, and (C) Tbr1+ neurons in the cortex. (D) No differences in distribution were detected between genotypes for either marker. Box-and-whisker data: horizontal black line, median; horizontal red line, mean; box, 25th to 75th percentile; whisker, 10th to 90th percentile; circles, outliers. Error bars represent s.e.m. Data were analyzed using unpaired Student's *t*-test. Scale bar: 1300 µm.

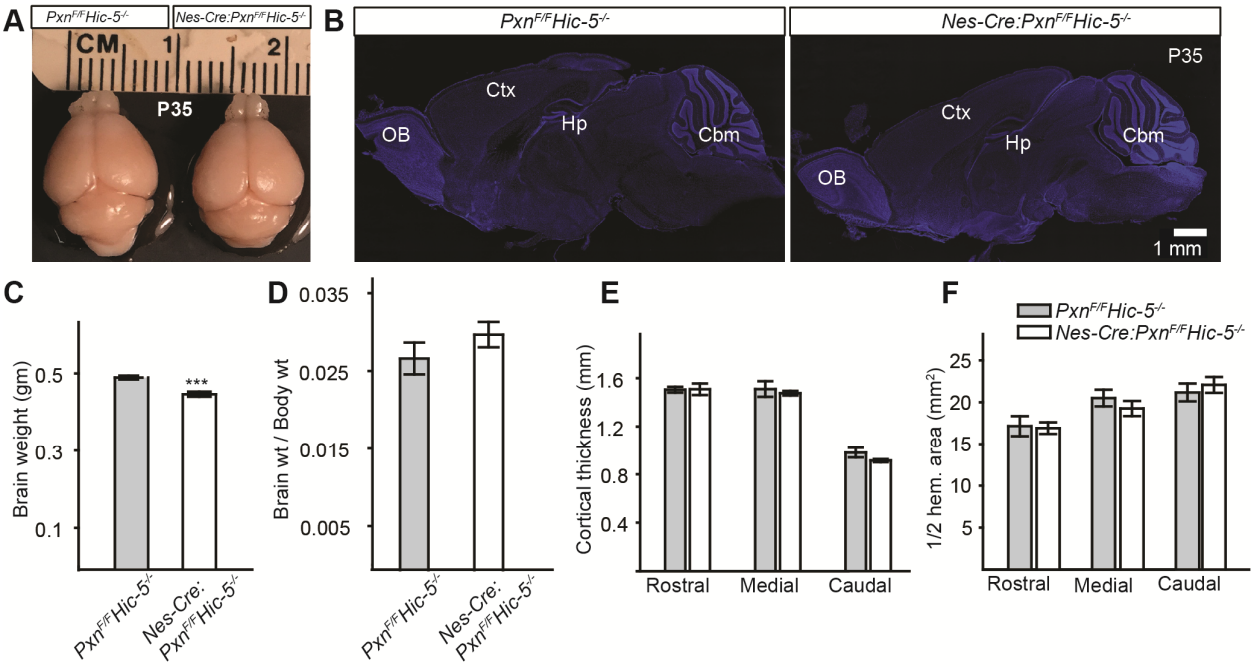

Fig. S3. Characterization of paxillin/Hic-5 dKO mouse brain. (A) Representative images of control (*Pxn$^{F/F}$Hic-5$^{-/-}$*) and littermate dKO (*Nes-Cre:Pxn$^{F/F}$Hic-5$^{-/-}$*) mouse brain at P35. (B) Sagittal sections stained with Hoechst 33342. (C) Post-fixation brain weights of mutants were significantly lower than littermate controls (n=6 for control, n=9 for dKO; p<0.001). (D) However, the brain weight to body weight ratio was not different between the genotypes (n=3 for control, n=6 for dKO). (E) The average cortical thickness was not different between genotypes (n=6 per group). (F) There was no difference between genotypes in the area of each half hemisphere from matched coronal sections (n=6 per group). Abbreviations: Cre-, *Pxn$^{F/F}$Hic-5$^{-/-}$*; Cre+, *Nes-Cre:Pxn$^{F/F}$Hic-5$^{-/-}$*. OB, olfactory bulb; Ctx, cortex; Hp, hippocampus; Cbm, cerebellum. Error bars represent s.e.m. Data were analyzed using unpaired Student's *t*-tests. ***p<0.001. Scale bar: 1 mm.

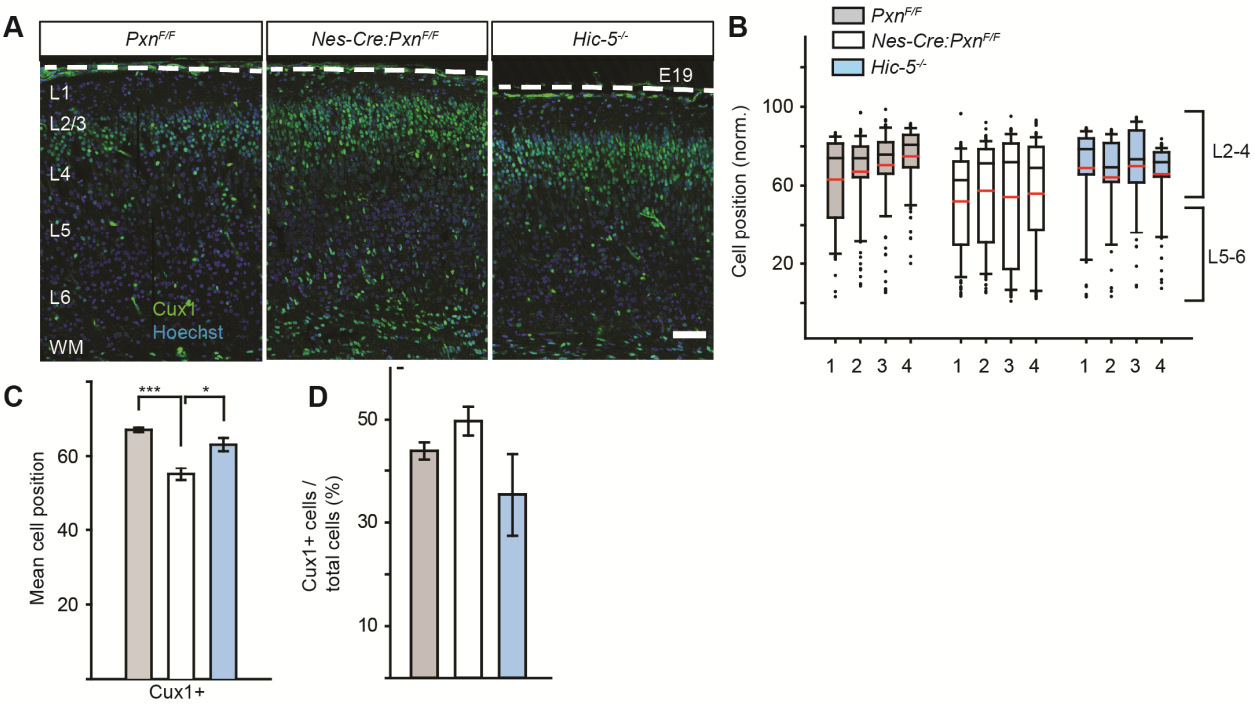

Fig. S4. Malpositioning of upper layer neurons is apparent prenatally in paxillin-deficient cortex. (A) Littermate control and *Nes-Cre:Pxn*$^{F/F}$ paxillin-deficient mutant immunostained for Cux1 (green) at E19. (B) Box-and-whisker plot distribution of Cux1+ neurons. Numbers on X-axis represent individual animal. (C) The mean position of Cux1+ neurons was 12% deeper in the paxillin mutant. There is no difference upper layer neuron position between *Pxn*$^{F/F}$ and *Hic-5*$^{-/-}$. (D) The total percentages of Cux1+ cells within the counting box were indistinguishable among the groups. Dashed white lines outline the pial surface. Box-and-whisker data: horizontal black line, median; horizontal red line, mean; box, 25th to 75th percentile; whisker, 10th to 90th percentile; circles, outliers. Error bars represent s.e.m. Data were analyzed using unpaired Student's *t*-test. *p<0.05. Abbreviations: MZ, marginal zone; WM, white matter. Scale bar: 50 µm.

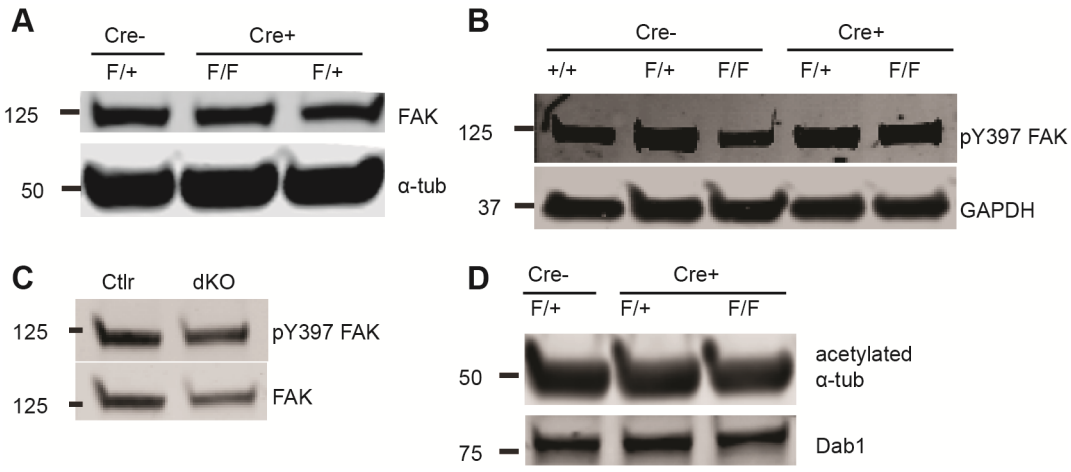

Fig. S5. Acetylated α–tubulin and FAK expression levels are unchanged in the paxillin deficient cortex. Western blot analyses of embryonic cortical lysate probed for (A) FAK (on E15), and (B) pY397 FAK (on E14). Note: the same GAPDH blot was used for Fig. 2C. (C) E14 cortical lysate analyzed for pY397 FAK and total FAK. (D) Acetylated α-tubulin. Ctlr = *Pxn*$^{F/F}$*Hic-5*$^{-/-}$, dKO = *Nes-Cre:Pxn*$^{F/F}$*Hic-5*$^{-/-}$. GAPDH, α-tubulin, and Dab1 are used as loading controls.

Table S1. Distribution of genotypes from the breeding *Pxn*$^{F/+}$ X *Nes-Cre:Pxn*$^{F/F}$

	Pxn$^{F/+}$	*Pxn*$^{F/F}$	*Nes-Cre:Pxn*$^{F/+}$	*Nes-Cre:Pxn*$^{F/F}$
Expected (%)	25	25	25	25
Observed (%)	24.7 (21/85)	25.8 (22/85)	25.8 (22/85)	23.5 (20/85)

Table S2. Distribution of genotypes from the breeding *Pxn*$^{F/F}$*Hic-5*$^{-/-}$ X *Nes-Cre:Pxn*$^{F/F}$*Hic-5*$^{-/-}$

	Nes-Cre:Pxn$^{F/F}$*Hic-5*$^{-/-}$	*Pxn*$^{F/F}$*Hic-5*$^{-/-}$
Expected (%)	50	50
Observed (%)	46% (46/100)	54% (56/100)

Table S3. Mean position of different classes of neurons across genotypes

Age/ Genotypes	P1			P35		
	Cux1	Tbr1	Tle4	Cux1	Tbr1	Tle4
Pxn$^{F/+}$	77.0% ± 1.5% (n=4) $^{\alpha, ***}$	25.6% ± 0.5% (n=4) $^{\alpha, n.s.}$	n.d.	61.9% ± 0.7% (n=4) $^{\alpha, n.s.}$	39.5% ± 1.8% (n=3) $^{\alpha, n.s.}$	21.9% ± 0.5% (n=5) $^{\alpha, n.s.}$
Pxn$^{F/F}$	n.d.	n.d.	30.3% ± 2.0% (n=3) $^{\delta, n.s.}$	n.d.	n.d.	n.d.
Nes-Cre:Pxn$^{F/+}$	72.6% ± 2.0% (n=3) $^{\gamma, n.s}$	26.4% ± 2.4% (n=3) $^{\gamma, n.s.}$	n.d.	57.9% ± 2.0% (n=3) $^{\gamma, n.s}$	32.8% ± 1.9% (n=3) $^{\gamma, n.s}$	20.8% ± 1.4% (n=4) $^{\alpha, n.s.}$
Nes-Cre:Pxn$^{F/F}$	62.9% ± 1.4% (n=5) $^{\beta, *}$	25.6% ± 2.1% (n=5) $^{\beta, n.s.}$	29.8% ± 2.2% (n=4)	58.0% ± 1.7% (n=4) $^{\beta, n.s.}$	40.8% ± 3.3% (n=4) $^{\beta, n.s.}$	21.0% ± 0.7% (n=5) $^{\alpha, n.s.}$

α, compared between *Pxn$^{F/+}$ vs Nes-Cre:Pxn$^{F/F}$*.

β, compared between *Nes-Cre:Pxn$^{F/+}$ vs Nes-Cre:Pxn$^{F/F}$*.

γ, compared between *Pxn$^{F/+}$ vs Nes-Cre:Pxn$^{F/+}$*.

δ, compared between *Pxn$^{F/F}$ vs Nes-Cre:Pxn$^{F/F}$*.

***p<0.001, *p<0.05. Data were analyzed using unpaired Student's *t*-test. n.s., not significant. n.d., not determined.

Table S4. Mean position of BrdU+ neurons

Age/Genotypes	E14.5	P1
Pxn$^{F/+}$	n.d.	n.d.
Pxn$^{F/F}$	53.1%±1.8% (n=4) $^{\delta,*}$	71.6%±3.5% (n=3) $^{\delta,*}$
Nes-Cre:Pxn$^{F/+}$	n.d	n.d
Nes-Cre:Pxn$^{F/F}$	48.4%±0.9% (n=5)	55.3±1.5% (n=3)

δ, compared between *Pxn$^{F/F}$ vs Nes-Cre:Pxn$^{F/F}$*.

***p<0.001, *p<0.05. Data were analyzed using unpaired Student's *t*-test. n.d., not determined.