Supplementary Materials and Methods

Immunostaining

Mouse brains were dissected in PBS and fixed overnight in 4% paraformaldahyde in PBS at 4°C. Brains were then cryoprotected using 30% sucrose in PBS overnight at 4°C and embedded in OCT for cryosectioning. Frozen sections were washed with 0.2% Triton X-100 in TBS (TBST) and incubated in the blocking solution (3% normal donkey serum in TBST) for 1 hour at room temperature. Sections were incubated with primary antibodies (Table S1) diluted in the blocking solution overnight at 4°C, washed with TBST, and incubated with DyLight- or Alexa Fluor-conjugated secondary antibodies (Jackson ImmunoResearch) diluted at 1:1000 in the blocking solution for 2 hours at room temperature. Sections were counterstained with DAPI, washed in TBST, and mounted in ProLong Gold antifade reagent (Invitrogen). For BrdU detection, sections were first treated with 1N HCl at 45°C for 30 minutes, washed with TBST, and processed as above using an anti-BrdU antibody. For costaining using an anti-BrdU antibody and other primary antibodies, sections were first stained with the other primary antibodies, cross-linked in 4% paraformaldahyde in PBS for 20 minutes at room temperature, then stained with the anti-BrdU antibody.

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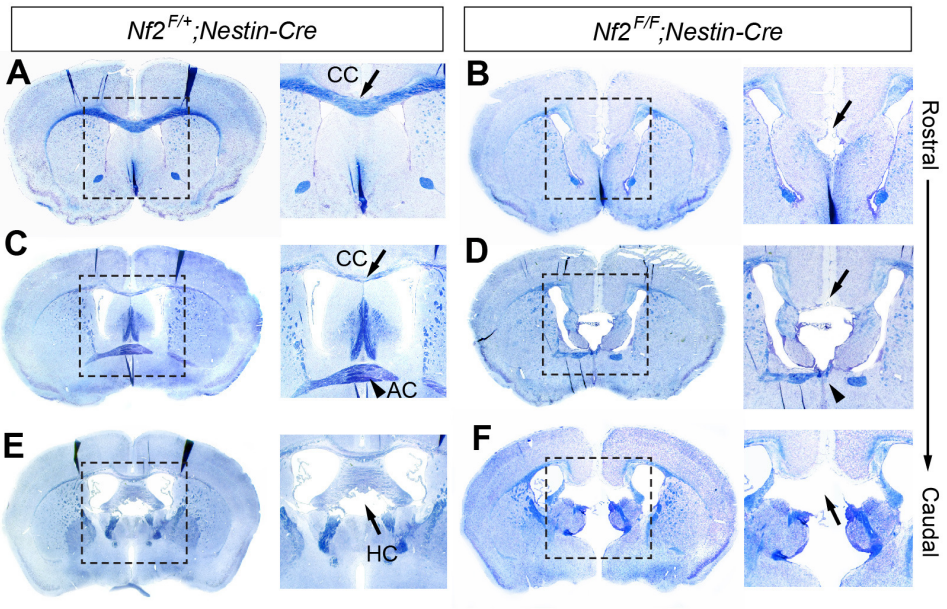

Fig. S1. Deleting *Nf2* with *Nestin-Cre* leads to agenesis of forebrain commissures. Luxol blue staining of myelinated axons (blue) and cresyl violet staining of neuronal cell bodies (purple) showing agenesis of the corpus callosum (CC) (A–D, arrow), anterior commissure (AC) (C,D, arrowhead), and hippocampal commissure (HC) (E,F, arrow) in 2-month–old *Nf2$^{F/F}$;Nestin-Cre* mice. *n*=3. A magnified view of the boxed region is shown in the image to the right.

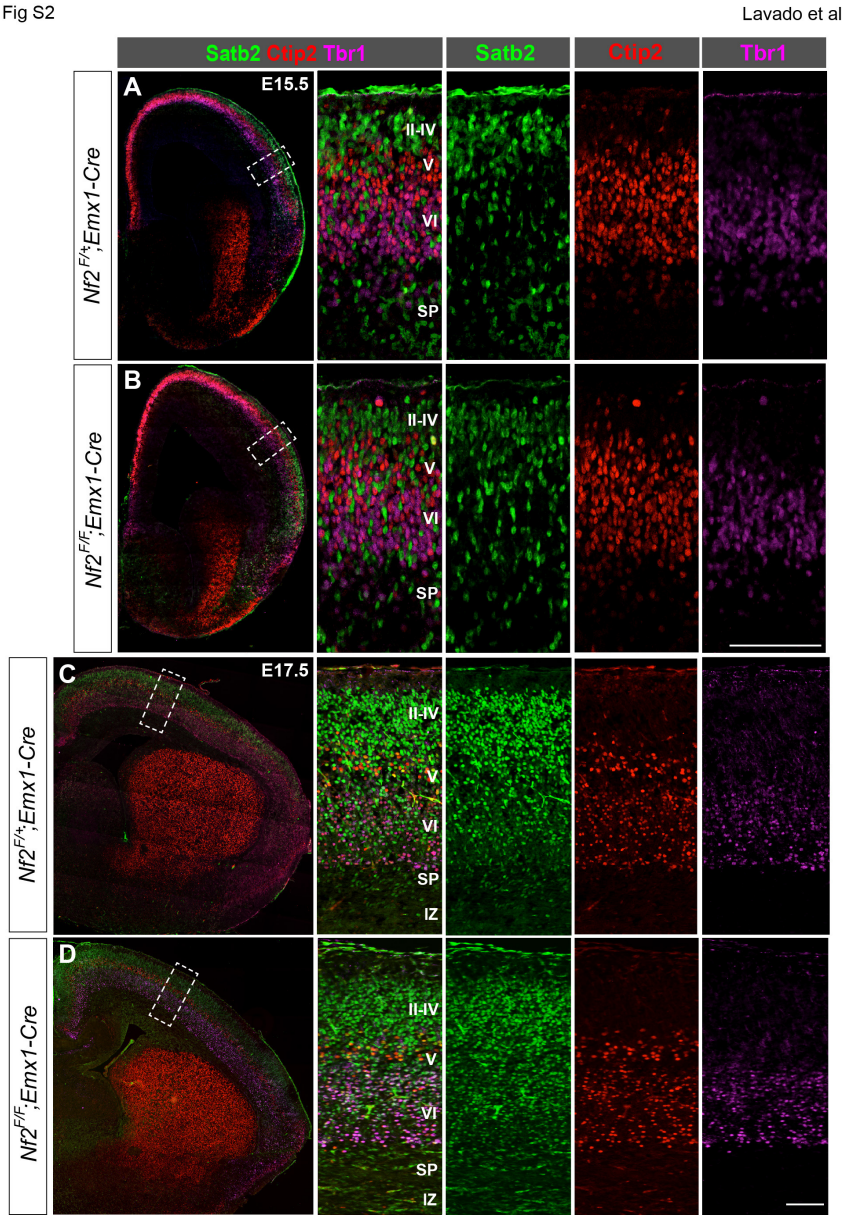

Fig. S2. Specification and production of callosal neurons are unaffected in *Nf2* mutants. Co-immunostaining showing proper expression of the callosal neuron–specific marker Satb2 in *Nf2*$^{F/F}$*;Emx1-Cre* neocortex and its proper laminar organization at E15.5 and E17.5. Images in the left column show low-magnified views. Regions in dashed boxes are enlarged in images to the right. SP: subplate; IZ: intermediate zone. Scale bars: 50 μm.

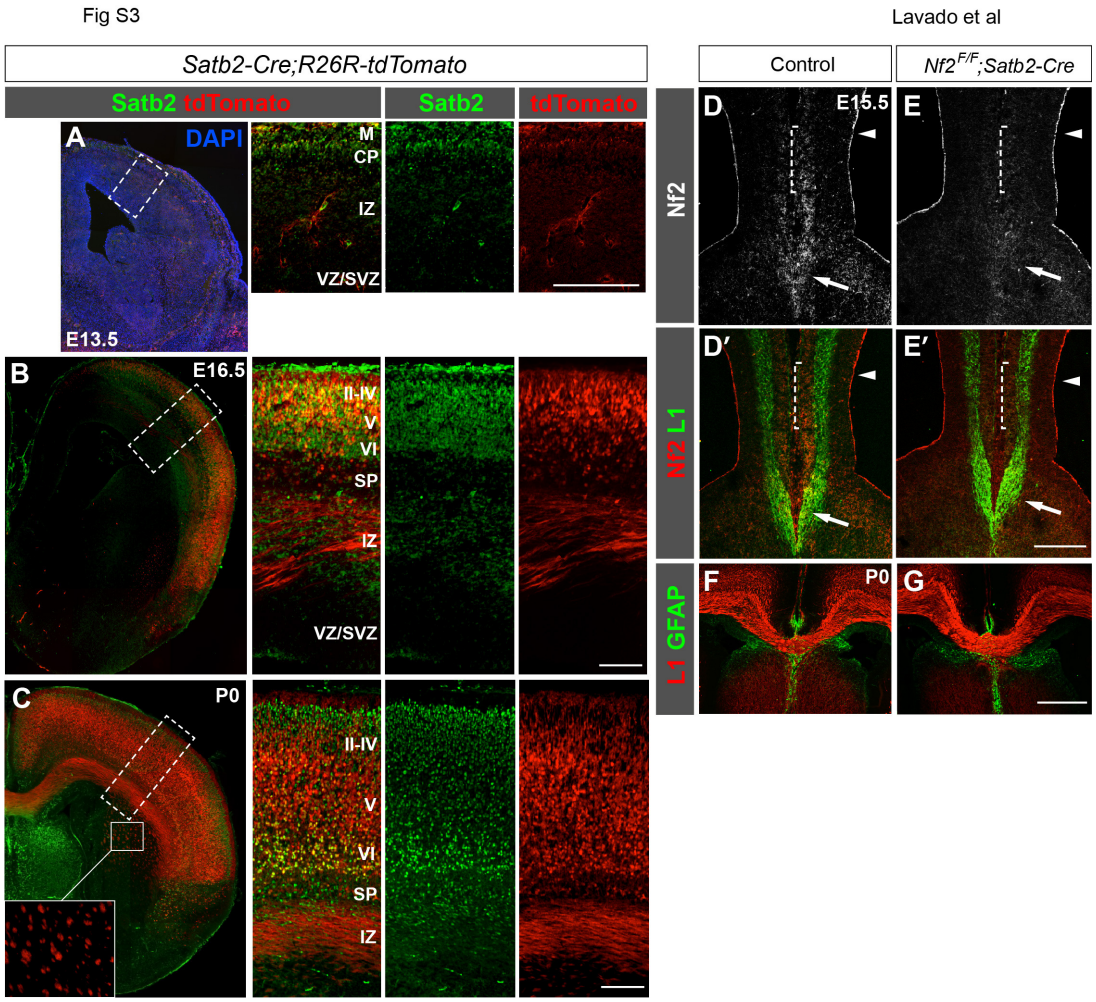

Fig. S3. Deleting *Nf2* in callosal neurons by using *Satb2-Cre* does not affect corpus callosum formation. (**A–C**) Co-immunostaining of Satb2 and tdTomato shows tdTomato expression in Satb2^{+} callosal neurons but not in ventricular zone and subventricular zone (VZ/SVZ) progenitor cells. TdTomato expression depends on Cre-mediated excision of the *LSL* (*loxP-stop-loxP*)-cassette in the *R26R-tdTomato* allele. As a consequence, tdTomato expression lags slightly behind Satb2 expression, which is likely why most Satb2^{+} cells at E13.5 are tdTomato-negative (A), as are the uppermost layer of Satb2^{+} cells at P0 (C). Inset in C shows a magnified view of the internal capsule, which also contains tdTomato-labeled axons. CP: cortical plate; IZ: intermediate zone; M: meninges; SP: subplate. (**D–E′**) In E15.5 *Nf2$^{F/F}$;Satb2-Cre* brains, Nf2 immunoreactivity in the cortical plate (dashed bracket) and callosal axons (arrow) is eliminated but that in the ventricular surface (arrowhead) is unperturbed. (**F**,**G**) The corpus callosum forms normally in P0 *Nf2$^{F/F}$;Satb2-Cre* brains. Scale bars: 50 μm in A,B,C; 200 μm in E′; 500 μm in G.

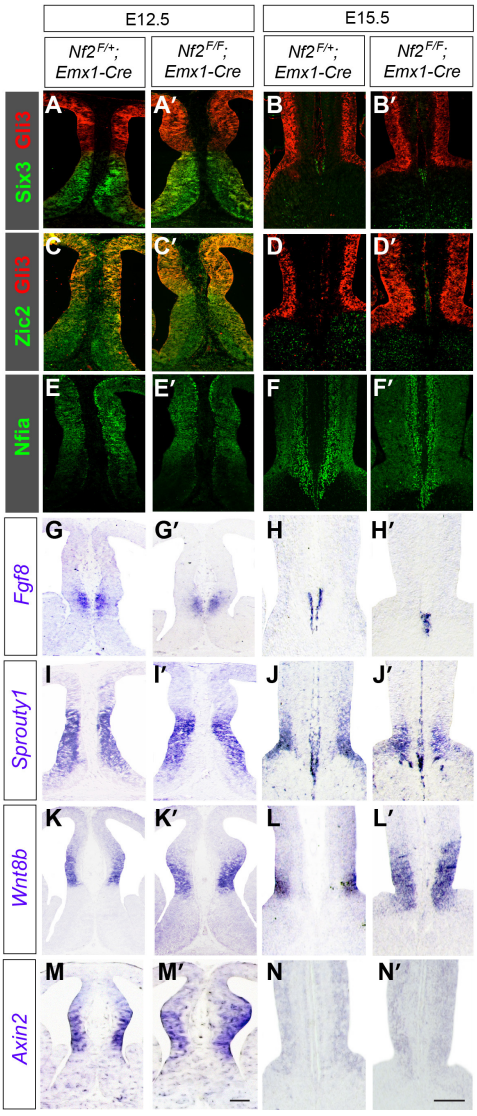

Fig. S4. Patterning of the corticoseptal boundary is grossly normal in *Nf2* mutants. Immunostaining and *in situ* hybridization show proper expression of Six3, Zic2, Nfia (transcription factors that delineate subdomains of the commissural plate), Gli3, *Fgf8* (molecules required for corticoseptal boundary patterning), *Sprouty1*, *Wnt8b*, and *Axin2* in *Nf2*$^{F/F}$*;Emx1-Cre* midline at E12.5 and E15.5. The *Wnt8b*$^{+}$ region is elongated dorsoventrally in E15.5 *Nf2*$^{F/F}$*;Emx1-Cre* midline (L′). Scale bars: 200 μm.

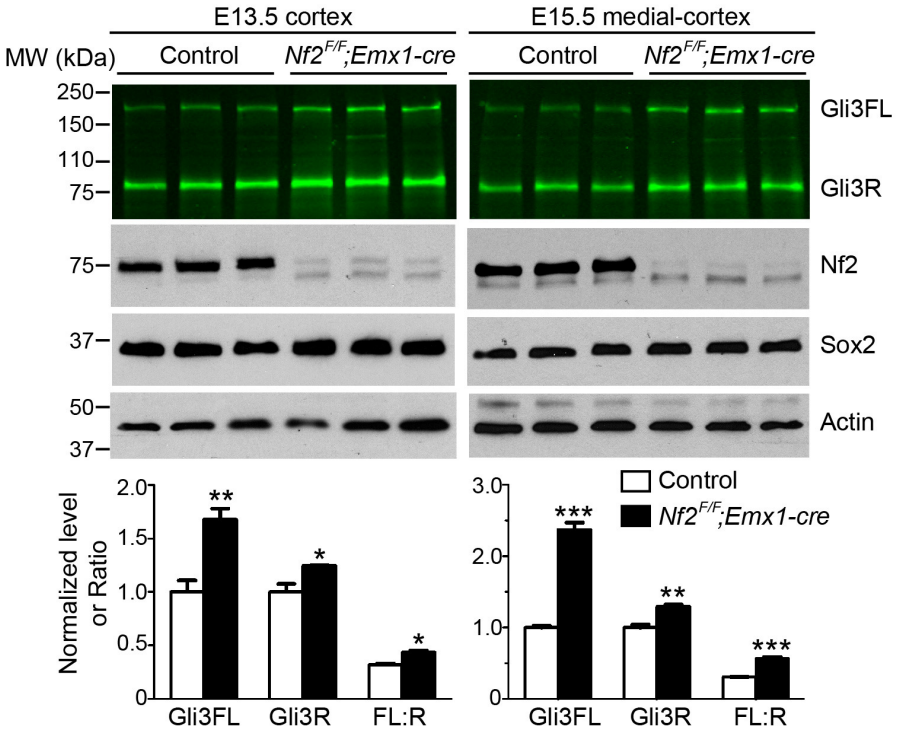

Fig. S5. Gli3 protein levels are altered in *Nf2* mutants. Quantitative western blot analyses using an antibody against the N-terminus of Gli3 detect both the full-length activator form (Gli3FL) and cleaved repressor form (Gli3R). Graphs show normalized levels of Gli3FL and Gli3R, with the levels of each form in controls set as 1, and the ratio of Gli3FL to Gli3R (FL:R). n=3 embryos per genotype, *P<0.05, **P<0.01, ***P<0.001. Because Gli3 expression is mostly restricted to neural progenitor cells, the neural progenitor marker Sox2 was used as a loading-control in addition to Actin.

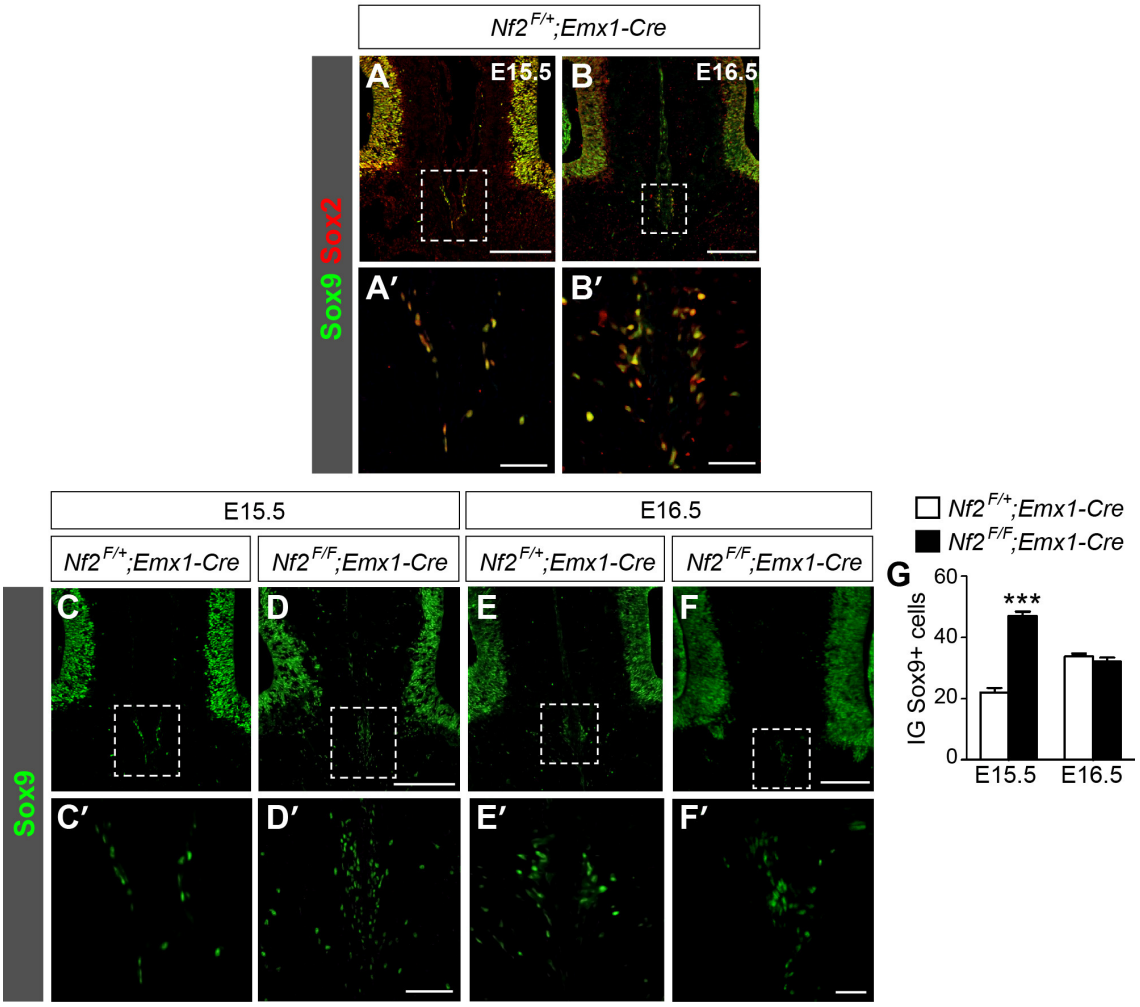

Fig. S6. The number of indusium griseum glia is transiently increased in *Nf2* mutants. (**A–C**) Co-immunostaining shows the Sox2 antibody and Sox9 antibody label the same population of cells at the indusium griseum. (**C–G**) The number of indusium griseum (IG) Sox9^{+} cells is increased in *Nf2$^{F/F}$;Emx1-Cre* embryos at E15.5 (*n*=4) but is similar to that in controls at E16.5 (*n*=3). ***P<0.001. Scale bars: 200 μm in A,B,D,F; 50 μm in A′,B′,D′; 20 μm in F′.

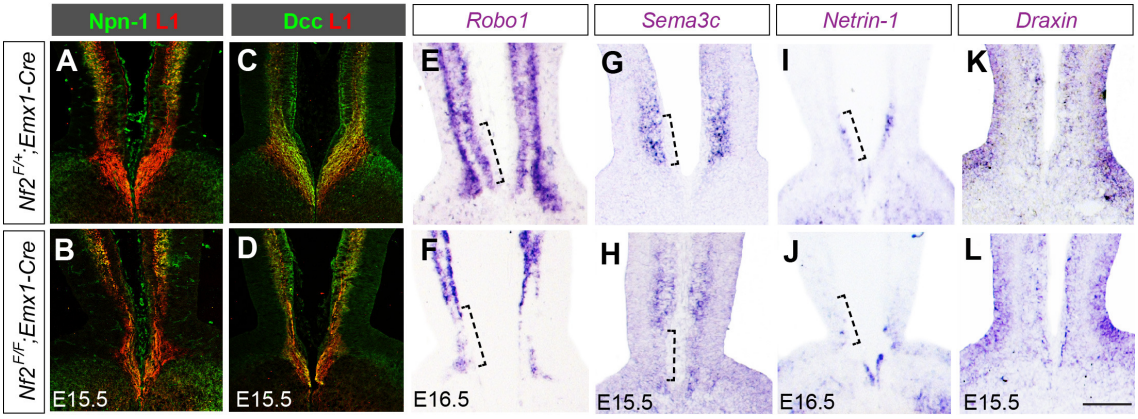

Fig. S7. Proper expression of axon guidance molecules in *Nf2* mutants. Expression patterns of guidance receptors Neuropilin-1(Npn-1), Dcc, and Robo1 and guidance cues Sema3c, Netrin-1, and Draxin are normal in *Nf2*$^{F/F}$*;Emx1-Cre* midline region. The apparent reduction of *Robo1*, *Sema3c*, and *Netrin-1 in situ* signals (dashed brackets) in *Nf2*$^{F/F}$*;Emx1-Cre* midline is likely due to reduction of the Calretinin^{+} guidepost neurons that express these genes. Scale bar: 200 μm.

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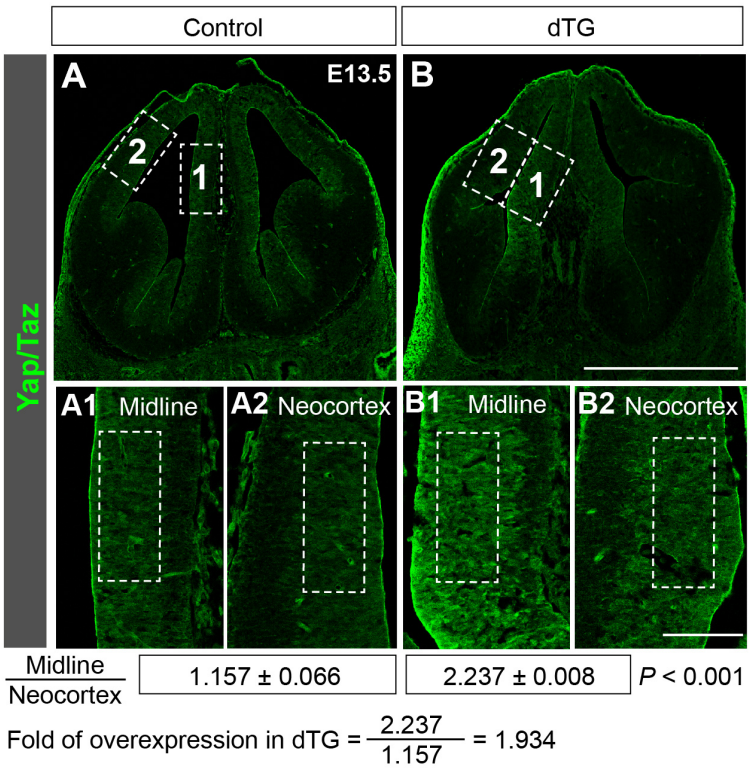

Fig. S8. Overexpressing YAP using *TetO-YAP1* and *Axin2-rtTA* double transgenic system. (**A**,**B**) Quantification of the level of YAP overexpression by measuring the fluorescence intensity of Yap/Taz immunostaining signals. To control for staining variations between sections, the intensity at the midline region (dashed box 1) was normalized to that at the neocortex region (dashed box 2) of the same section. The ratio of fluorescence intensity at the midline region and the neocortex region is shown below the confocal images. *n*=3 embryos per genotype, 6 sections per embryo. Scale bars: 1mm in B; 200 μm in B2. (**C**) Quantitative western blot analysis of E16.5 medial-cortex tissues.

Table S1. Primary antibodies for immunostaining and western blotting.

Antibody	Host species	Vendor	Catalog #	Dilution in immunostaining	Dilution in western blot
Merlin/Nf2	rabbit	Santa Cruz	sc-332	1:200 and amplification with Invitrogen TSA kit	
Nf2	rabbit	Sigma	HPA003097	1:500	1:1000
L1	rat	Millipore	MAB5272	1:500	
GFAP	rabbit	Dako	Z0334	1:1000	
Satb2	mouse	Abcam	ab51502	1:100	
Ctip2	rat	Abcam	ab18465	1:250	
Tbr1	rabbit	Abcam	ab31490	1:200	
Tbr1	rabbit	Millipore	AB10554	1:500	
Sox2	goat	Santa Cruz	sc-17320	1:100	
Sox2	rabbit	Cell Signaling	3728		1:500
Sox9	rabbit	Millipore	ab5535	1:500	
BrdU	rat	Accurate	OBT00306	1:1000	
Calretinin	rabbit	Thermo	RB-9002-P0	1:500	
Calretinin	rabbit	Millipore	AB149	1:5000	
Npn-1	goat	R&D	AF566	1:500	
Dcc	mouse	BD Pharmigen	554223	1:100	
Gli3	goat	R&D	AF3690	1:100	1:500 (ECL); 1:25 (LI-COR)
Zic2	rabbit	Millipore	AB15392	1:1000	
Tbr2	rat	eBiosciences	14-4875-82	1:500	
Tbr2 (Alexa-647-conjugated)	mouse	eBiosciences	51-4875-80	1:100	
Nf1-A	rabbit	Active motif	39398	1:1000	
Yap/Taz	rabbit	Cell Signaling	8418	1:500	
Yap	mouse	Sigma	WH0010413 M1		1:1000 (ECL); 1:100 (LI-COR)
Six3	rabbit	from G. Oliver		1:500	
GFP	chick	Aves	GFP-1020	1:1000	
Actin	mouse	Ambion	AM4302		1:40000 (ECL); 1:2000 (LI-COR)

Table S2. In situ probes

Probe	Sequence	Starts	Size (bp)
Slit2	Starts in position 4340 of rat Slit2 (NM_022632.2)	5’-TTACGTAGGAGGTATGCCTG	1600
Robo-1	Starts in position 250 of rat Robo-1 (NM_022188.1)	5’-CCCGCCACCCTCAACTGTAA	1000
Netrin-1	Starts in position 4264 of mouse Netrin-1 (NM_008744.2)	5’-TGTAGCAAATAACATCCAGC	760
Sema3C	Starts in position 1671 of mouse Sema3C (NM-013657.5)	5’-AGCAACAGTTGTACGTGAGC	700
Draxin	Full cDNA, mouse (IMAGE clone 6853328)	5’-GAGCAGCCTCCTGCCACCCG	5161
Fgf8	Full cDNA of transcript variant 4, with partials 5’-UTR and 3’-UTR, mouse (NM_001166363.1)	5’-CCCGCTCCGCGCTGAGCTGC	800
Sprouty1	Full cDNA, mouse (Addgene 22091)	5’-CCGCAGCCAGAGCTCTGCGG	1500
Wnt8b	Full cDNA, mouse (IMAGE clone 615408 moved into pCMV-SPORT2)	5’-TTCATTTCACCACCCCTTAA	478
Axin2	Starts in position 350 of mouse Axin2 (NM_015732.4)	5’-ATGAGTAGCGCCGTGTTAGT	2322

Table S3. Quantitative RT-PCR primers

Primer	Sequence	Note
mouse Axin2 forward	AAGTGTCTCTACCTCATTTTCCG	
mouse Axin2 reverse	TCCAGTTTCAGTTTCTCCAGC	
mouse Slit2 forward	GATCTCTTTAACCCCTGCCAG	
mouse Slit2 reverse	TCCCTTATCCGTTCCCCTC	
mouse Gusb forward	CACCCCTACCACTTACATCG	normalizer
mouse Gusb reverse	ACTTTGCCACCCTCATCC	normalizer
human SLIT2 forward	TCTGTTTAACCCATGCCAGG	
human SLIT2 reverse	TCTCTTATCCTTTCCCCTCGAC	
human WNT5A forward	TCGCCCAGGTTGTAATTGAAG	
human WNT5A reverse	TGAGAAAGTCCTGCCAGTTG	
human CYR61 forward	CAAGGAGCTGGGATTCGATG	
human CYR61 reverse	AAAGGGTTGTATAGGATGCGAG	
human GUSB forward	AGGTGATGGAAGAAGTGGTG	normalizer
human GUSB reverse	AGGATTTGGTGTGAGCGATC	normalizer