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Supporting Information for

KIF2A deficiency causes early-onset neurodegeneration

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Supporting Materials and Methods, Figures S1 to S7 and their legends and Supporting references

Supporting Materials and Methods

Immunofluorescence

We used the following primary antibodies: Rabbit anti-Tbr1 (AbCam ab31940, 1:250); Chicken anti-Tbr1 (Millipore AB2261, 1:100); Rabbit anti-Cux1 (Proteintech 11733-1-AP, 1:250); Rat anti-Ctip2 (AbCam ab18465, 1:250); Rabit anti-GFAP (Millipore AB5804, 1:1000); Mouse anti-Satb2 (AbCam ab51502, 1:500); Guinea pig anti-Vglut2 (Millipore AB2251, 1:1000); Rabbit anti-Er81 (generous gift from, Silvia Arber, 1:1000); Rabbit anti-Tbr2 (AbCam ab23345, 1:250); Rabbit anti-Pax6 (Covance PRB-278P, 1:500); Rabbit anti-PHH3 (Cell Signaling 9701, 1:100); Rabbit anti-Ki67 (AbCam ab15580, 1:100); Rabbit anti-cleaved caspase 3 (Cell Signaling 9661, 1:200); Chicken anti-MAP2 (AbCam ab5392, 1:2000); Chicken anti-GFP (Aves-lab GFP-1020, 1:2000); Mouse anti-NeuN (Millipore MAB377, 1:1000); Rabbit anti-KIF2A (Proteintech 13105-1-AP, 1:500); Mouse anti-Rorβ (Perseus Proteomics Inc PP-N7927-00, 1:500); Rabbit anti-Calbindin D-28K (Swant CB38, 1:2000). Different AlexaFluor-conjugated secondary antibodies (Invitrogen, 1:800) were used. Nuclei were counterstained with DAPI (Sigma D9564) 100 µM for 5 min and the sections were mounted on slides with Mowiol.

In situ Hybridization (ISH), and Myelin and Nissl Staining

ISH was performed as described in (1) on vibratome sections (40 µm) mounted on adhesive slides (SuperFrost Ultra Plus™ ThermoScientific). Sections were treated twice for 10 min with RIPA buffer (NaCl 150mM, NP-40 1%, Na 0.5% deoxycholate, SDS 0.1%, EDTA 1mM pH 8.0, Tris 50mM pH 8.0 in distilled water) and postfixed 15 min with PFA 4% in PB 0.1M. They were then washed with PBS twice for 5 min each, treated with Triethanolamine buffer (Triethanolamine 100mM, Acetic acid pH 8.0 0.2%, Acetic anhydride 0.25% in distilled water) for 15 min and prehybridized for 1h with hybridization buffer (Formamide 50%, SSC, SDS 1%, heparin 50µg/ml, Yeast tRNA 50µg/ml in MiliQ water) at 65-70°C. Sections were incubated with the probes diluted in hybridization buffer (1µg/ml) ON at 68°C in a humidified chamber. They were subsequently washed twice with post-hybridization solution (50% formamide, SSC, tween-20 0.1% in distilled water) for one hour at 70°C, and twice with B1 buffer (Malic acid 100mM, NaCl 150mM, tween 0.1% in distilled water) for 5 min and 20 min. For immunological detection, slides were incubated during 1 hour at RT in B1 buffer containing 10% sheep serum, and ON at 4°C with Anti-Digoxigenin-AP antibody (Roche 11093274910, 1:2000) in B1 buffer and 10% sheep serum. The sections were washed three times in B1 buffer, 10 min each; 30 min in B2 buffer (Tris 100mM pH9.5, $MgCl_2$ 50mM, NaCl 100mM, tween-20 0.1% in distilled water), and incubated with an NBT-BCIP solution (Merck 11681451001). For *Kif2a* ISH, the riboprobe was generated using the following PCR primers: TTGAAAGGGGCATGGCTA (Forward), and AGAACTCCCCACACCCCACT (Reverse). For *Rorβ* (RZRβ) we used the probe described in (2). Luxol Fast Blue Staining for Myelin was performed on P21 mouse 40µm vibratome floating sections. The sections were incubated in with 1:1 alcohol/chloroform solution for 2 hours and stained ON at 56°C in Luxol fast blue solution (Luxol fast blue MBSN 0.1%, Glacial acetic acid 0.5% in 95% ethanol). The sections were rinsed in 95% ethanol and distilled water; and differentiated using a lithium carbonate solution (Lithium carbonate 0.05% in distilled water) for 30 seconds, and 70% ethanol for 30 seconds. Nissl staining was performed on vibratome sections (40 µm) previously mounted on adhesive slides (SuperFrost Ultra Plus™ ThermoFisher). Sections were dehydrated in ethanol solutions of increasing concentrations and incubated in absolute ethanol for 2 hours. The sections were stained with Cresyl violet (Merck 105235) solution (0.02% Cresyl violet, 0.2% sodium acetate, 0.3% acetic acid glacial in distilled water) for 2 h and differentiated in 95% ethanol. For ISH, Luxol blue and Nissl staining, sections were dehydrated in ethanol, incubated twice in toluene for 10 min, and mounted with Neo-Mount® medium (Merck 109016).

Western blotting (WB)

Mouse brain cortices were homogenized on ice in RIPA lysis buffer (ThermoFisher 89900), and protease inhibitors (Pierce™ ThermoFisher A32965); and centrifuged at 13,000 g for 15 min at 4°C. For Vglut1 and PSD95, cerebral cortices were homogenized in Synaptic Protein Extraction Reagent (Syn-PER™ ThermoFisher 87793) following the manufacturer's instructions. Protein quantification was performed with a BCA kit (Pierce™ ThermoFisher 23227). The supernatant was mixed with 4x

LDS Sample buffer (NuPAGE™, Invitrogen NP0007) and TCEP (ThermoFisher 77720) heated at 95 C for 5 min. An equal amount of proteins (10-20µg) was loaded on 8 or 4–12% Bis-Tris (Bolt™ ThermoFhiser NW00080 and NW04125), separated by MOPS running buffer (Bolt™ ThermoFisher B000102), and transferred to PVDF membranes (Millipore ISEQ00005). Membranes were blocked 1h at room temperature, with StartingBlock™ T20 (TBS) (ThermoFisher 37543) + 5% fat free milk (Sigma 70166), and incubated in the same blocking solution ON at 4°C with one of the following antibodies: Rabbit anti-acetylated tubulin (Sigma T6793; 1:2000); Mouse anti-alpha-tubulin (Sigma; T 6199, 1:5000); Rabbit anti-alpha-tubulin (AbCam ab18251, 1:5000); Chicken anti-GAPDH (Millipore AB2302, 1:5000); Mouse anti-βActin (AbCam ab6276, 1:5000); Rabbit anti-Polyglutamate chain (poly-E) (Adipogen AG-25B-0030-C050, 1:5000); Rabbit anti-CLASP1 (AbCam ab108620; 1:2000); Rat anti-EB3 (AbCam ab53360, 1:1000); Mouse anti-PSD95 (ThermoFisher MA1-045; 1:2000); Rabbit anti-Vglut1 (Synaptic Systems 135303, 1:2000); Mouse anti-Tau-1 (Millipore clone PC1C6, 1:5000); Rabbit anti-KIF2A (AbCam ab71160, 1:2000). Membranes were then incubated with HRP-coupled secondary antibodies (1:20000): Goat Anti-Mouse IgG (H+L) HRP (Dako P0447), Goat Anti-rabbit IgG (H+L) HRP (Cell Signaling #7074), Goat Anti-Rat IgG (H+L) HRP (Millipore AP136P0), Rabbit Anti-Chicken IgY (H+L) HRP (Sigma 12-341) and revealed using SuperSignal™ West Pico PLUS Chemiluminescent Substrate (ThermoFisher 34577). Membranes were imaged using Fusion Pulse platform (Vilber) and analyzed in Fiji (ImageJ). Values were normalized to GAPDH, Alpha-tubulin or β-Actin. Images of western blotting were cropped for presentation.

Electrophysiological recordings

Coronal slices were prepared from postnatal P21 control or *Emx1-cKO* mice. Briefly, the brain was quickly removed and transferred into ice-cold solution (in mM): 83 NaCl, 2.5 KCl, 1 NaH2PO4, 22 glucose, 26.2 NaHCO3, 0.5 CaCl2, 3.3 MgCSO4, 72 sucrose, pH 7.4 with 5% CO2/ 95% O2) and coronal slices (250 µm) were prepared using a vibratome (Leica VT1200). For recordings, brain slices were continuously perfused in a submerged chamber (Warner Instruments) at a rate of 3– 4 ml/min with (in mM): 127 NaCl, 2.5 KCl, 1.25 NaH2PO4, 25 NaHCO3, 1 MgCl2, 2 CaCl2, 25 glucose at pH 7.4 with 5% CO2/ 95% O2. Whole-cell patch-clamp recordings were done using borosilicate glass recording pipettes (resistance 3.5–5 MΩ, Sutter P-1000) using the following intracellular solution (in mM): 135 K-gluconate, 4 KCl, 10 HEPES, 4 Mg-ATP, 0.3 Na-GTP, 10 Na-phosphocreatine and 3 mg/ml biocytin (pH 7.3).
Whole cells recordings from visually identifiable pyramidal neurons in layer 2/3 of the primary somatosensory cortex were done using a double EPC-10 amplifier under control of Patchmaster v2 x 32 software (HEKA Elektronik, Lambrecht/Pfalz, Germany). Spontaneous EPSCs and IPSCs were recorded by whole-cell voltage-clamp recordings (Vm=−70 mV and Vm=0 mV, respectively). Currents were recorded at 20 Hz and low pass filtered at 3 kHz when stored. The series resistance was compensated to 75-85%. Spontaneous input was analyzed using Mini Analysis program (Synaptosoft). Cell intrinsic properties were measured from the same cells in whole-cell current clamp. Cells with series resistances above 20 MΩ were excluded for analysis. For all measurements, we performed four control and four *Emx1-cKO* littermate mice from four independent litters.

Immunocytochemistry and primary culture analysis

Hippocampal primary cultures were fixed in 4% PFA/ 0.1 M phosphate buffer (PB), pH 7.4, at room temperature (RT) for 10 min and washed 3 times for 10 min each in PBS. Coverslips were incubated 1h at RT in a blocking solution containing 4% bovine serum albumin (BSA, Amresco 9048-46-8), 10% goat serum (Sigma G9023), 0.2% Triton X-100 (Sigma T8787) in PBS, and ON at 4°C in the same blocking solution with the following antibodies: Chicken anti-MAP2 (AbCam ab5392, 1:2000); Mouse anti-Acetylated-tubulin (Sigma T6793, 1:2000), Rat anti-Tyrosinated-tubulin (AbCam ab6160, 1:2000), Mouse anti-PSD95 (ThermoFisher MA1-045; 1:2000); Rabbit anti-Vglut1 (Synaptic Systems 135303, 1:2000), Guinea pig anti-TRIM46 (Synaptic Systems 377005; 1:500), F-Actin (Alexa Fluor™ 647 Phalloidin, ThermoFisher). AlexaFluor-conjugated secondary antibodies (Invitrogen) were used at 1:800 dilution. Nuclei were counterstained with DAPI (Sigma D9564) and coverslips were mounted with Mowiol mounting medium for immunofluorescence. Sholl analysis and NeuronJ plugins were used to calculate the length and number of dendrites. Assessment of

the thickness of dendrites, number of axons per neurons, density of PSD95 and synaptic puncta was performed manually using the Fiji (ImageJ) software.

Supporting Figures

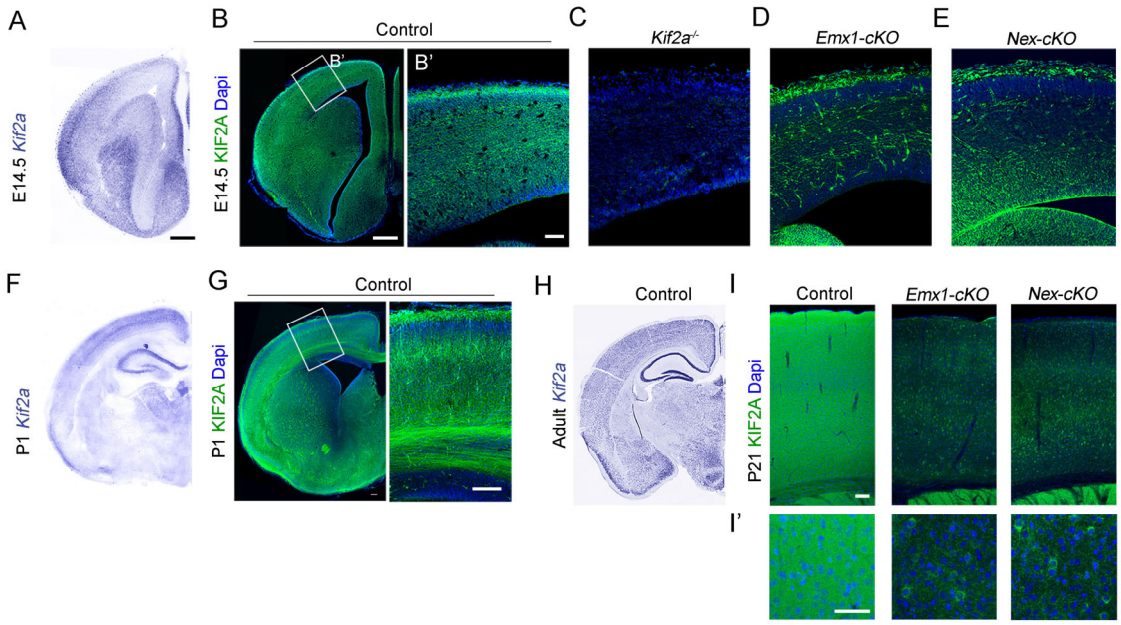


Figure S1. KIF2A expression in cerebral cortex. (A, B) E14.5 coronal sections hybridized with a *Kif2a* riboprobe (A) or stained with anti-KIF2A antibodies (B). (B') Magnification of the area boxed in B. (C-E) Coronal sections of E14.5 embryos from *Kif2a*$^{-/-}$ (C), *Emx1-cKO* (D), and *Nex-cKO* (E), immunolabelled with anti-KIF2A antibodies. (F, G) P1 coronal sections hybridized with a *Kif2a* riboprobe (F) or stained with anti-KIF2A antibodies (G). (H) *Kif2a in situ hybridization* showing the mRNA distribution in the adult. (I) KIF2A immunofluorescence at P21 in control, *Emx1-cKO*, and *Nex-cKO* mice. (I') Magnification of KIF2A staining in the cortex for each genotype. Scale bars: (A, B) 500µm, (B', G, I) 100µm, (I') 50µm.

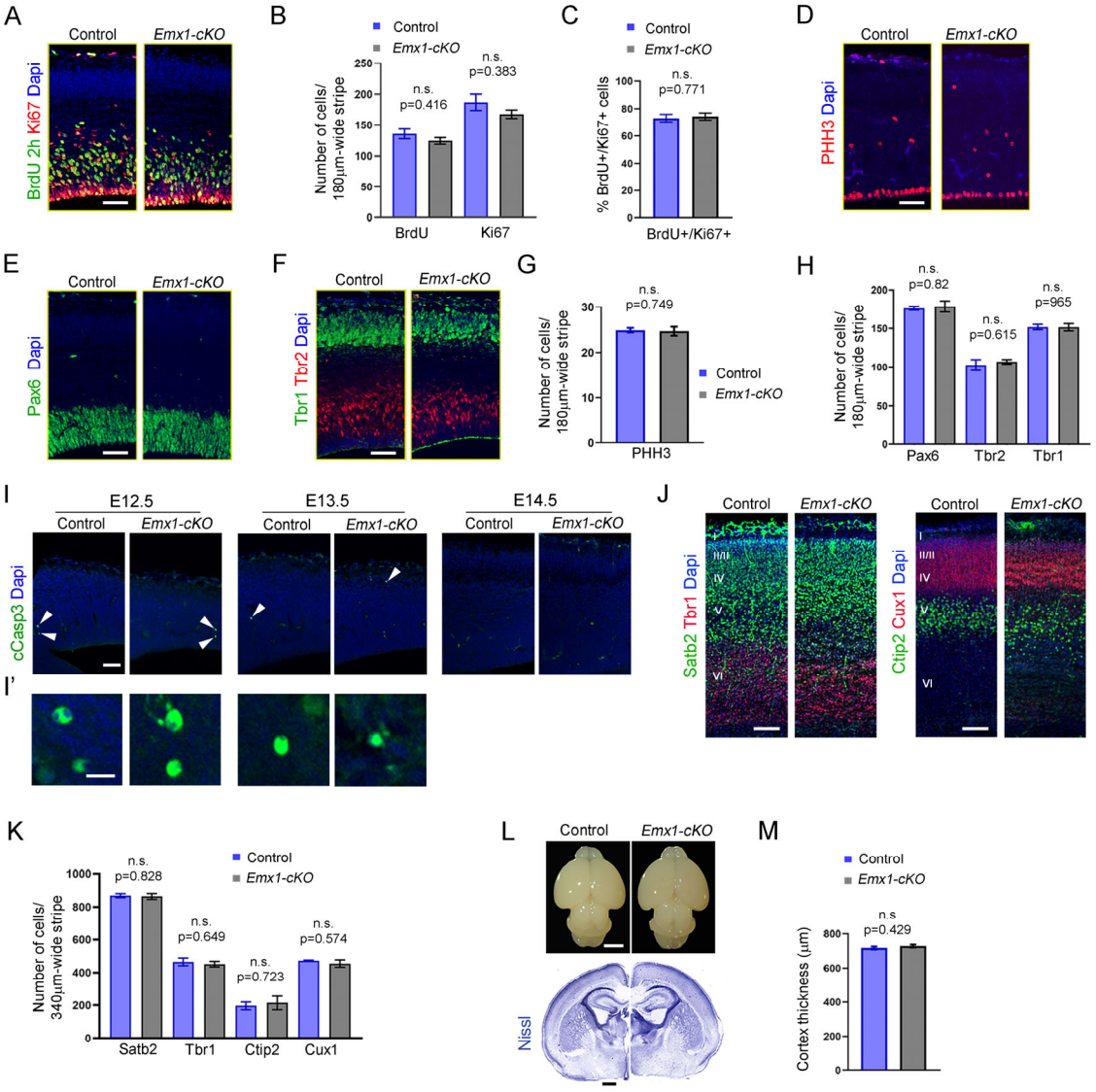


Figure S2. Conditional deletion of KIF2A does not alter proliferation of progenitors. (A) Coronal sections of E14.5 control (left) and *Emx1-cKO* (right) embryos immunolabelled for BrdU (injected 2h before sacrifice) and Ki67. (B) Average number of BrdU+ cells (control=135.9 ± 12.5; *Emx1-cKO*=124.4 ± 1.6; n=3 for each genotype) and Ki67+ cells (control=187.2 ± 17.7; *Emx1-cKO*=168 ± 8.4; n=3 for each genotype) per 180µm-wide stripe. (C) Ratio of BrdU+ cells in Ki67 population (control=73.1 ± 2.8; *Emx1-cKO*=74.3 ± 2.6; n=3 for each genotype). (D-F) Coronal sections of E14.5 control and *Emx1-cKO* embryos immuno-stained with PHH3 (cells in mitosis, D), Pax6 (apical radial glial, E), and Tbr2 and Tbr1 (intermediate progenitors and early postmitotic neurons respectively, F). (G, H) Number of cells per 180µm-wide stripe (PHH3+ cells: control=24.3 ± 0.3; *Emx1-cKO*=24.67 ± 0.6; Pax6+ cells: control=176.2 ± 2; *Emx1-cKO*=178.5 ± 7.1; Tbr2+ cells: control=102.7 ± 6.9; *Emx1-cKO*=178.5 ± 7.1; and Tbr1+ cells: control=151.8 ± 3; *Emx1-cKO*=151.5 ± 4.8; n=3 for each genotype). (I) cCasp3 immunostaining of brain coronal sections from control and *Emx1-cKO* at E12.5, E13.5, and E14.5. (I') Magnifications of cCasp3 positive cells indicated with arrowheads in I. (J) Coronal sections of control and *Emx1-cKO* neonates (P1) immunolabelled for Satb2, Tbr1, Ctip2 and Cux1. (K) Number of neurons per 340µm-wide stripe cortical area

(Satb2+ neurons: control=870.3 ± 12, *Emx1-cKO*=865.3 ± 17.9; Tbr1+ neurons: control=485.3 ± 23.4, *Emx1-cKO*=451.8 ± 17; Ctip2+ neurons: control=196.2±24, *Emx1-cKO*=214.6 ± 41.9; and Cux1+ neurons: control=474.2 ± 2.8; *Emx1-cKO*=455.9 ± 22.4; n=3 for each genotype). (L) P1 brains from control and *Emx1-cKO* pups (top) and coronal sections with Nissl staining (bottom). (M) Thickness of the somatosensory cortex in P1 animals (control=717.7 ± 8.2 μm; and *Emx1-cKO*=728.1 ± 8.6 μm; n=3 for each genotype). No gross anatomical abnormalities were detected in *Emx1-cKO* mice at birth. Scale bars: (A, D, E, F, I) 50 μm, (J) 100μm, (I') 10μm, (L) 2mm (top) and 500μm (bottom). Data are represented as mean ± SEM. Values were obtained by unpaired Student's t-test; n.s.: not significant, *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$.

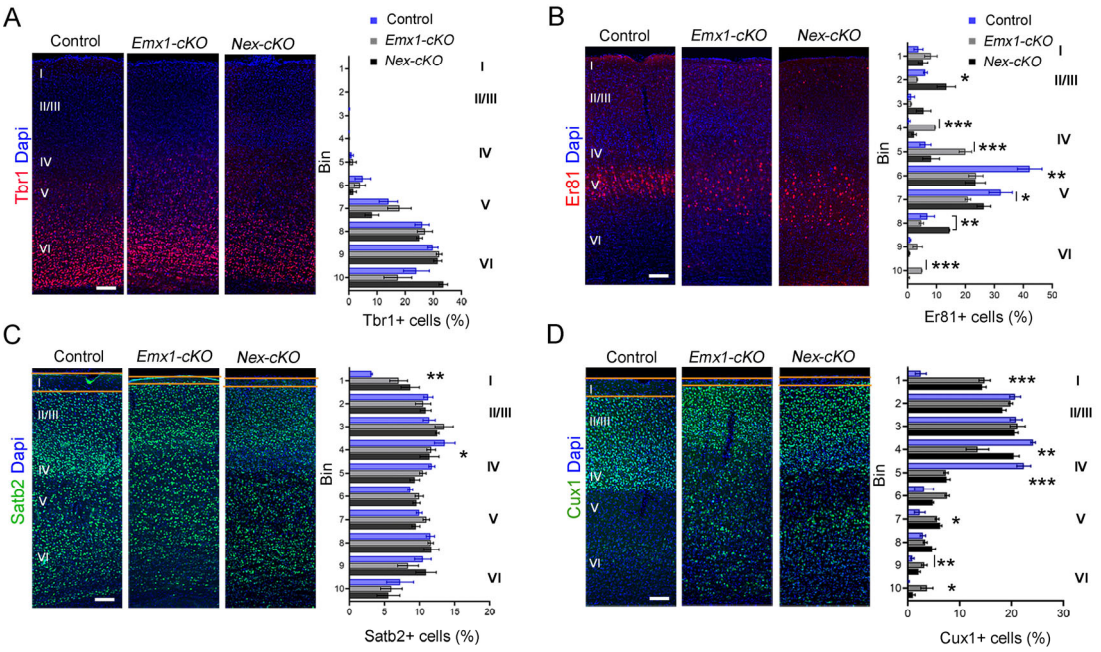
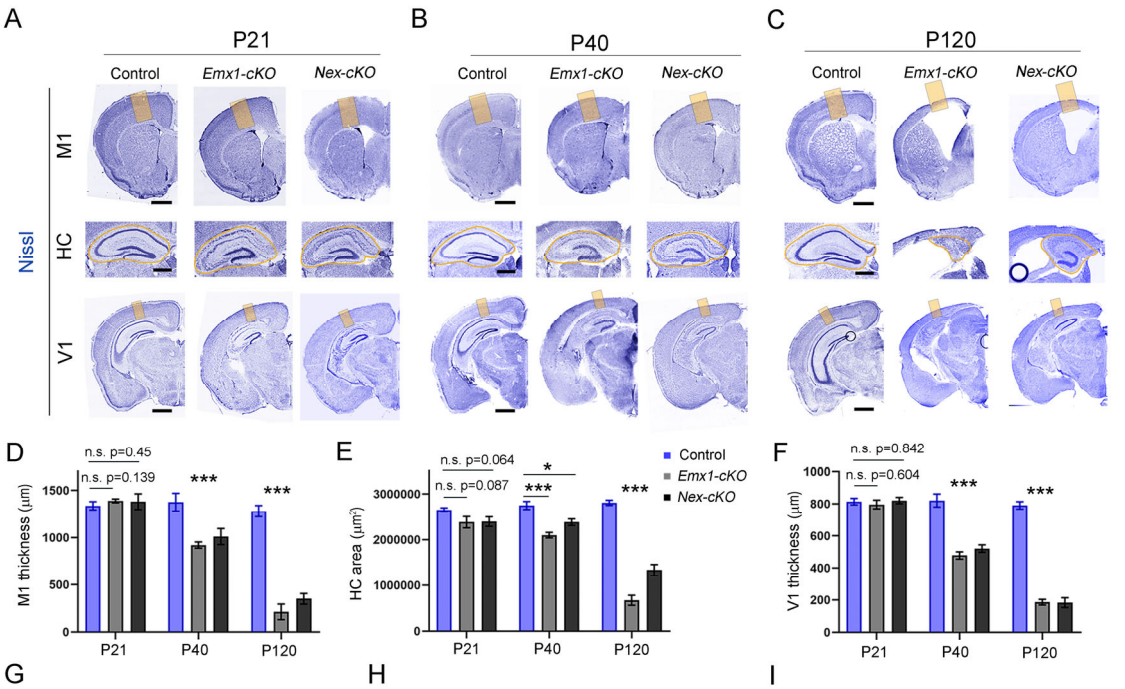


Figure S3. KIF2A is implicated in radial migration in the cortex in cell autonomous manner. (A) Immunofluorescence for Tbr1 (left) and distribution of Tbr1+ cells in cortical layers of the indicated genotypes at P21 (control n=4; *Emx1-cKO* n=3; *Nex-cKO* n=3). (B) Immunofluorescence for Er81 (left) and distribution of Er81+ cells in cortical layers at P21 (control n=3; *Emx1-cKO* n=3; *Nex-cKO* n=3). (C) Immunofluorescence for Satb2 (left) and distribution of Satb2+ cells in cortical layers at P21 (control n=3; *Emx1-cKO* n=3; *Nex-cKO* n=3). (D) Immunofluorescence for Cux1 (left) and distribution of Cux1+ cells in cortical layers at P21 (control n=5; *Emx1-cKO* n=4; *Nex-cKO* n=3). To calculate the distribution of each neuronal population, the cortex was subdivided into 10 equal bins and the percentage of positive neurons for each marker was calculated for each bin. Sale bars: (A-D) 100µm. Data are represented as mean ± SEM. Values were obtained by unpaired Student's t-test; *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$.



G

M1 (µm)	Control	*Emx1-cKO*	*Nex-cKO*
P21	1337 ± 25	1389 ± 11.4 (+3.84%)	1380 ± 37.1 (+3.17%)
P40	1375 ± 46	919.9 ± 18.8 (-33.06%)	1009 ± 42.1 (-26.55%)
P120	1280 ± 59	212.5 ± 82 (-83.43%)	349.9 ± 56 (-72.66%)

H

HC (µm²)	Control	*Emx1-cKO*	*Nex-cKO*
P21	(265 ± 4.6) E+04	(238 ± 12) E+04 (-10.20%)	(239 ± 9.3) E+04 (-9.73%)
P40	(275 ± 8.8) E+04	(210 ± 6) E+04 (-23.69%)	(238 ± 7.1) E+04 (-13.42%)
P120	(281 ± 9.9) E+04	(67.3 ± 18) E+04 (-76.06%)	(133 ± 21) E+04 (-52.53%)

I

V1 (µm)	Control	*Emx1-cKO*	*Nex-cKO*
P21	814.4 ± 20	796 ± 27 (-2.26%)	820.6 ± 20 (+0.75%)
P40	820.6 ± 40	477.9 ± 23 (-41.76%)	521.1 ± 23 (-36.5%)
P120	790.9 ± 39	186.6 ± 29 (-76.41%)	183.4 ± 52 (-76.82%)

Figure S4. Neuronal degeneration in KIF2A-deficient cerebral cortex. (A-C) Nissl staining of coronal sections at three rostral-caudal levels of the brain at P21 (A), P40 (B) and P120 (C). The yellow boxes designate the analyzed areas. (M1) primary motor cortex, (HC) hippocampus, and (V1) primary visual cortex. (D-I) Quantification of M1 thickness (D, G), HC area (E, H) and V1 thickness (F, I) at P21, P40 and P120. At least three animals per genotype and per stage were analyzed. Values are expressed as mean ± SEM. Scale bars: (A-C) 500µm for M1 and V1 and 250µm for HC. Data are represented as mean ± SEM. Values were obtained by unpaired Student's t-test; n.s.: not significant, *: P < 0.05, **: P < 0.01, and ***: P < 0.001.

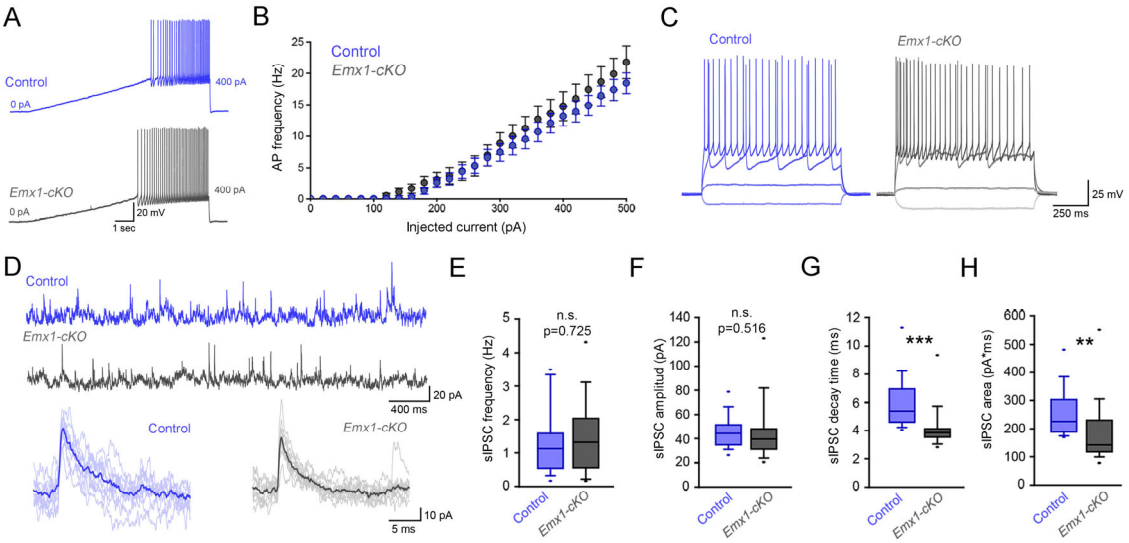


Figure S5. Rheobase and inhibitory synapses maintenance in KIF2A mutant pyramidal neurons at P21. (A) Example traces of ramp stimulation (from 0 to 400 pA over 10 sec) to determine rheobase values in control (blue) and *Emx1-cKO* (grey) neurons (control n=20 and *Emx1-cKO* n=27 neurons). (B) Firing rate (Hz) versus input current (pA) of control (blue) and cKO (grey) neurons (means ± SEMs for each genotype; control n=23 and *Emx1-cKO* n=26 neurons). (C) Representative membrane potential responses to consecutive hyperpolarizing (-80 pA) and depolarizing (40, 140 500 pA) current steps recorded in control (blue) and *Emx1-cKO* (grey) neurons. (D) Upper: example traces of sIPSCs in control (blue) and *Emx1-cKO* (grey) recordings. Lower: 10 superimposed individual (shaded) sIPSCs and the averaged (bold) sIPSC in control (blue) and *Emx1-cKO* (grey) neurons. (E) sIPSC frequency (control=1.34 ± 0.2 Hz; *Emx1-cKO*=1.48 ± 0.2 Hz). (F) sIPSC amplitude (control=44.79 ± 3.1 pA; *Emx1-cKO*=45.53 ± 5.1 pA). (G) sIPSC decay time (control=5.9 ± 0.4 ms; *Emx1-cKO*=4.2 ± 0.3 ms). (H) sIPSC area (control=254.13 ± 20.6 fC; *Emx1-cKO*=185.56 ± 24.2 fC). Sample size for E-H, control n=17 and *Emx1-cKO* n=21 neurons from 4 different animals. Data are represented as tukey plot (E-H). Values were obtained by unpaired Student's t-test; n.s.: not significant, *: P < 0.05, **: P < 0.01, and ***: P < 0.001.

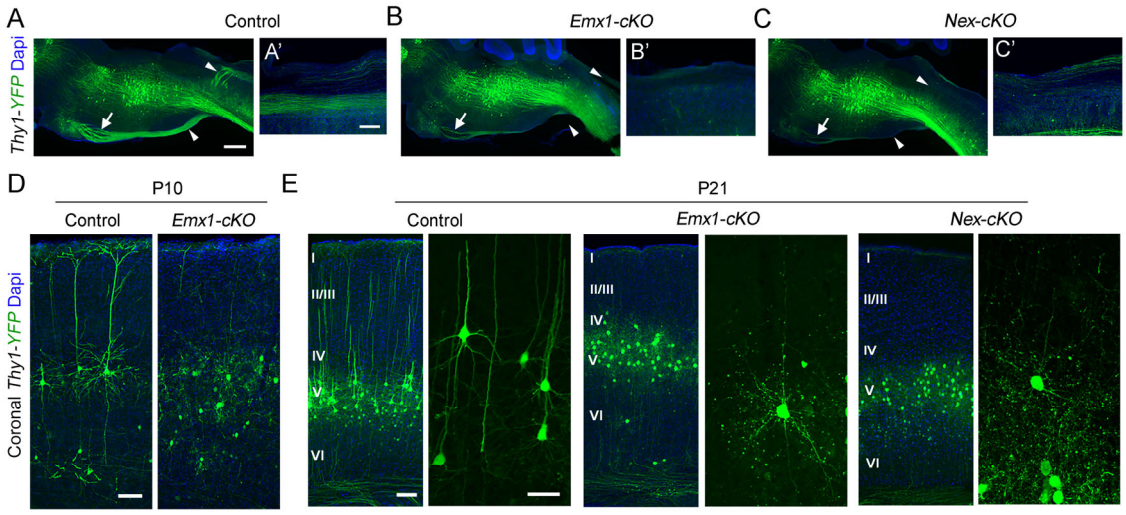


Figure S6. Wiring and morphology abnormalities in layer V neurons in *Kif2a cKO* mice.
(A-C) Hindbrain sagittal sections from control (A), *Emx1-cKO* (B), and *Nex-cKO* mice (C). Note the reduction of corticospinal tract (CST) (white arrow) and almost absence of the pyramidal decussation (white arrowheads) in *Emx1-cKO* and *Nex-cKO* mice. (A'-C') Spinal cord sagittal sections of the from control (A'), *Emx1-cKO* (B'), and *Nex-cKO* mice (C'). Axons of the CST were not detected in *Emx1-cKO* and *Nex-cKO* mice. (D, E) Coronal sections of P10 (D) and P21 (E) brains from control, *Emx1-cKO* and *Nex-cKO* mice at the level of the somatosensory cortex showing layer V pyramidal neurons labelled by *Thy1-YFP*. Scale bar: (A) 500μm, (A', D and E left panel) 100μm, (E right panel) 50μm.

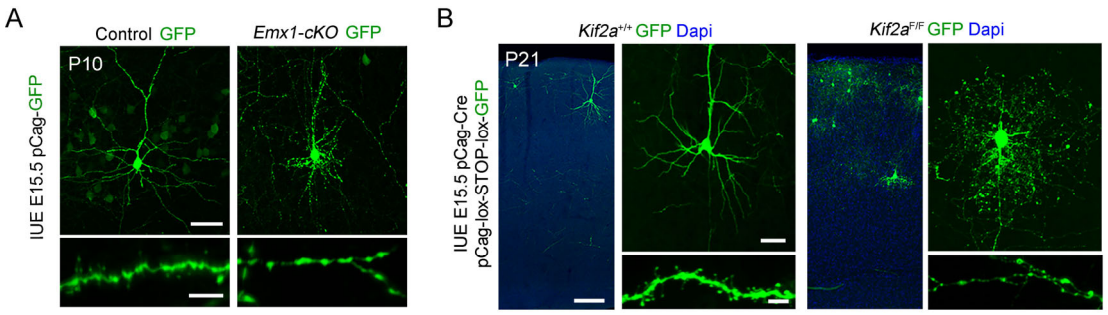


Figure S7. Morphology abnormalities in Layer II-III pyramidal neurons in KIF2A mutant mice. (A) Representative images of pyramidal neurons electroporated at E15.5 with GFP and imaged at P10. Lower panels are zooms on dendrites. Note the presence swellings and discontinuities in *Emx1-cKO* animals. (B) Coronal sections of P21 brains from *Kif2a*$^{+/+}$ and *Kif2a*$^{F/F}$ mice electroporated at E15.5 with pCag-Cre and pCag-lox-STOP-lox-GFP plasmids. Scale bars; (A) 50µm for the upper panel and 5µm for the lower panel, (B) 100µm for the left panel, 50µm for the right-top panel and 5µm for the right-bottom panel.

References

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