| Identifier | MAF | PED | PEDOT | TRANSCRIPT | PEDOB | RECESSIVE |
|------------|-----|------|--------|------------|--------|-----------|
| c.3440dupC | c.3440dupC | p.Ala1148Cysfs*20 | p.Ala1148Cysfs*20 | COL4A1 and COL4A2 long isoforms & COL4A3, COL4A4, and COL4A5 short isoforms | COL4A1 and COL4A2 long isoforms & COL4A3, COL4A4, and COL4A5 short isoforms |

**Birth History**

- **Mode of delivery:**
  - Trio WES
  - Trio WES
  - Trio WES
  - Trio WES
  - Duo ES

- **Pregnancy history:**
  - n.d.
  - Significant PV bleeding
  - Borderline gestational diabetes mellitus
  - n.d.
  - 22q FISH - normal

- **Birth gestational age:**
  - n.d.
  - 21 weeks
  - 41 weeks
  - 40 weeks
  - 38 weeks

- **Birth Weight:**
  - n.d.
  - 440g
  - 3.20 kg (-0.58 SD)
  - 3.69 kg (.26 SD)
  - 2.73 kg (0.1 SD)

- **Birth length:**
  - n.d.
  - 26cm
  - n.d.
  - 50cm (-0.06SD)
  - 45 cm (-0.06SD)

- **Birth head circumference:**
  - n.d.
  - 20.8cm
  - n.d.
  - n.d.
  - n.d.

- **Biological Sex:**
  - Male
  - Male
  - Male
  - Male
  - Male

- **Age at last evaluation:**
  - 3 months
  - 21 gestational weeks
  - 17 years
  - 30 years
  - 3 years 6 months

- **Head Circumference:**
  - 36.2 cm (-3.4SD)
  - n/a
  - n.d.
  - 55.5cm (0.27SD)
  - n.d.

- **Neuromuscular:**
  - n.d.
  - Arthrogryposis
  - Not present
  - Learning disability
  - n.d.

- **Epilepsy/Seizures:**
  - n.d.
  - Not present
  - Not present
  - Epilepsy
  - Not present

- **Ventricular abnormality:**
  - Ventriculomegaly
  - n.d.
  - Colpocephaly
  - Hydrocephalus
  - Mild ventriculomegaly

- **White matter abnormality:**
  - Decreased volume
  - Poor grey-white differentiation
  - Not present
  - n.d.
  - Not present

- **Corpus Callosum abnormality:**
  - Thin
  - Agenesis
  - n.d.
  - Thin
  - n.d.

- **Cortical abnormality:**
  - Cerebral atrophy
  - Bilateral schizencephaly
  - Polymicrogyria
  - Polymicrogyria
  - n.d.

- **Ophthalmologic:**
  - Strabismus
  - n/a
  - Not present
  - Divergent left strabismus, bilateral nystagmus
  - Not present

- **Dysmorphic features:**
  - Narrow palpebral fissure, retrognathia/micrognathia,
    short neck, short philtrum, bitemporal narrowing,
    high narrow palate, hypotelorism
  - Slightly low set left ear, long flat philtrum
  - Not present
  - Synophrys, brachydactyly, growth retardation

- **Congenital Heart Defects:**
  - n.d.
  - Not present
  - Not present
  - n.d.
  - Not present

- **GI/GU abnormalities:**
  - Ileus/megacolon
  - Absent stomach bubble
  - Not present
  - n.d.
  - Normal abdominal ultrasound and blood chemistry

**Systemic Abnormalities**

- **Genetic:**
  - n.d.
  - Not present
  - Not present
  - n.d.
  - Not present

- **General:**
  - n.d.
  - Not present
  - Not present
  - n.d.
  - Not present

- **Neurologic:**
  - n.d.
  - Not present
  - Not present
  - n.d.
  - Not present

- **Ophthalmologic:**
  - Strabismus
  - n/a
  - Not present
  - Divergent left strabismus, bilateral nystagmus
  - Not present

- **Dysmorphic features:**
  - Narrow palpebral fissure, retrognathia/micrognathia,
    short neck, short philtrum, bitemporal narrowing,
    high narrow palate, hypotelorism
  - Slightly low set left ear, long flat philtrum
  - Not present
  - Synophrys, brachydactyly, growth retardation

- **Congenital Heart Defects:**
  - n.d.
  - Not present
  - Not present
  - n.d.
  - Not present

- **GI/GU abnormalities:**
  - Ileus/megacolon
  - Absent stomach bubble
  - Not present
  - n.d.
  - Normal abdominal ultrasound and blood chemistry
Figure S1 (Qian et al.)
**Figure S2** (Qian et al.)

### Table C

| MUpro Prediction results using SVM and sequence information | R721C | P949S | A1559V | R1624C | R1602W |
|------------------------------------------------------------|-------|-------|--------|--------|--------|
| Decrease stability                                        | Decrease stability | Decrease stability | Decrease stability | Decrease stability | Decrease stability |
| Delta Delta G                                              | -0.65820316 | -0.95141304 | -0.12075156 | -0.76832272 | 0.43446018 |
| MUpro prediction result using neural network               | Decrease stability | Decrease stability | Decrease stability | Decrease stability | Decrease stability |
| Confidence Score                                           | -0.743334995 | -0.85870411 | -0.705808984 | -0.905943529 | -0.99632671 |
Figure S3 (Qian et al.)

(A) Mouse E16.5

B

Validation of shRNA KD efficiency in N2A cells

(C) Mouse E13.5-17.5 IUE Scrambled shRNA

D

Mouse E13.5-17.5 IUE Control

E

Laminar distribution of electroporated neurons

Figure S3 (Qian et al.)
Figure S4 (Qian et al.)
## Figure S5 (Qian et al.)

### Table A

| KO line name | Parent line | Guide Target              | Indel % |
|--------------|-------------|---------------------------|---------|
| D17          | PGP1        | CGGCCCTGATGGCTTGTCGA      | 99      |
| N19          | PGP1        | CGGCCCTGATGGCTTGTCGA      | 99      |
| D2           | 280         | CCAGCACCACGACCCAGCTG      | 99      |
| G4           | 280         | CCAGCACCACGACCCAGCTG      | 99      |

### Table B

- **PQP1 (Parent line)**
- **D17 (KO clone1)**
- **N19 (KO clone2)**
- **D2 (KO clone1)**
- **G4 (KO clone2)**

### Table C

- **D17**
- **N19**
- **D2**
- **G4**
Figure S6 (Qian et al.)

A

| Day 0 | Day 1 | Day 7 | Day 14 | Day 42 | Day 60 |
|-------|-------|-------|--------|--------|--------|
| mTeSR+ | AWEB+ Dorso+ A83 | N2+ CHIR+ S8 | N2 B27 | B27+ cAMP |
| Shaking culture | Shaking culture | Shaking culture |
| Matrigel (embed) | Matrigel (dissolved) |

B

Day 0
10k cell EB
Day 1
EB Day 6
Organoid Day 14
Organoid Day 35
Organoid Day 85

C

Day 70 KI67 CTIP2 TBR2

D

Day 45 KIF26A CTIP2 PAX6

E

Day 60
RNAscope
DAPI KIF26A RORB NEUN

F

WT Day 60 KIF26A SATB2 CTIP2 DAPI

KO Day 60 KIF26A SATB2 CTIP2 DAPI

G

WT Day 60 SOX2 PH3 TBR2 DAPI
KO Day 60 SOX2 PH3 TBR2 DAPI

H

%PHT+ TBR2 in VZ

I

division axis
cleavage
angle
apical(ventricular) surface

Figure S6 (Qian et al.)
Figure S7 (Qian et al.)
Figure S8 (Qian et al.)
SUPPLEMENTARY TABLE

Table S1. Clinical Information of Presented Subjects. Related to Figure 1 and S1.
SUPPLEMENTARY FIGURES

Figure S1. Mapping and Sequencing of KIF26A Pathogenic Variants; Related to Figure 1.

(A-E) Confirmation and segregation analysis of KIF26A variants in unaffected and affected individuals of enrolled families using Sanger sequencing.

(F) Sequence alignment of the amino acids surrounding the mutations from human KIF26A and orthologs across species, showing high degree of conservation.

(G) Predicted helices and strands of KIF26A protein architecture. Unannotated regions display conserved and transiently ordered islands, See STAR Methods. The locations of compound heterozygous mutations in the human KIF26A sequence are noted by asterisks above the domain graphic, and with the exception of R721C that is located in the motor domain, all fall in the unstructured chain between D2 and D3.

(H) Predicted structures of globular domains in KIF26A protein. Domain D1 is a compact 3-helix bundle with a cluster of 4 Cys residues at one end that likely coordinate Zn2+, that structurally resembles the rare IMA1 module that has been seen in nuclear envelope membrane proteins (like yeast IMA1 or human TMEM210). Domain D2 is the centrally-located and microtubule-binding motor domain that closely matches other kinesin structures (here, for comparison is PDB 4BN2, for KIF15, with bound Mg2+ and ADP). The C-terminal domain D3 has a helix-loop-helix fold that maps to the region of KIF26A that interacts with FAK.
**Figure S2. Functional Characterization of KIF26A Patient Variants; Related to Figure 1.**

(A) HEK293T cells transfected with WT and variant KIF26A with a mCherry fluorescent tag, co-immunostained for KIF26A and microtubule marker β3 Tubulin. KIF26A expression is only detectable in transfected mCherry+ cells, consistent with the low baseline endogenous expression of KIF26A in HEK293T cells. Scale bar = 10 µm.

(B) Microtubule depolymerization assay on SHSY5Y cells transfected with WT and variant KIF26A. Transfected SHSY5Y cells were treated with microtubule depolymerizer Nocodazole (10µM for 15 min) before fixation. Diffused staining patterns of Acetylated Tubulin and β3 Tubulin indicated microtubule depolymerization. Scale bar = 10 µm.

(C) Machine-learning based prediction of the impact of patient missense variants to protein stability using MUpro Predictor (Cheng et al., 2006). Prediction of the sign of energy change using Support Vector Machines (SVM) and neural networks: method used, effects of mutation on protein stability, and a confidence score between -1 and 1 to measure the confidence of the prediction. A score less than 0 means the mutation decreases the protein stability. The smaller the score, the more confident the prediction is. Conversely, a score more than 0 means the mutation increases the protein stability. The bigger the score, the more confident the prediction is.

(D) Representative images of acetylated tubulin (AceTub) and detyrosinated tubulin (DtTub) immunostaining for SHSY5Y transfected with scrambled shRNA and KIF26A shRNA1 and shRNA2. GFP-labeled KIF26A shRNA1 and 2 transfected cells have reduced fluorescent intensity than neighboring untransfected cells. Only transfected cells are analyzed in (E). Scale bar = 10 µm.

(E) Quantification for the fluorescent intensity of acetylated tubulin and detyrosinated tubulin in transfected cells normalized to the area of the cell body. Values represent Mean ± S.D. (n = 34 cells for scrambled, 25 cells for KIF26A shRNA1 and 2 from 10 areas of views. Student’s t-test, ***, p < 0.0005).
Figure S3. Additional Characterization of Kif26a in Embryonic Mouse Brain. Related to Figure 3.

(A) Kif26a is expressed in the CP and IZ of E16.5 mouse cortex, overlapping with neuronal markers CTIP2 and SATB2. Insets show magnified view. Scale bar = 200µm, inset = 100µm.

(B) Validation of Kif26a knockdown with qPCR in transfected N2A cells. Values represent Mean ± S.D. (student’s t-test: ***, p < 0.0005).

(C) Representative images of In utero electroporation (IUE) of control scrambled shRNA (left) and Kif26a shRNA (right) in mouse cortex at E13.5 and analyzed at E17.5 (E13.5-17.5). Insets of magnified view show electroporated cells are SATB2⁺ neurons. Scale bar = 100µm (top), = 50µm (bottom).

(D) IUE of control plasmid (scrambled shRNA) and CMV-human KIF26A overexpression (OE) plasmid into mouse cortex at E13.5 and analyzed at E17.5. Insets of magnified view show electroporated cells are SATB2⁺ neurons. Scale bars = 100µm.

(A) Quantification of the laminar distributions of electroporated cells in the cortex, similar to Figure 3C. The cortex was evenly divided into 10 bins from basal (bin 1) to apical (bin 10) surfaces and the cell distribution was normalized by the total number of electroporated cells in the analyzed area. Values represent Mean ± S.D. (n = 7 brains for control, n = 6 for hKIF26A OE).
Figure S4. *Kif26a* KD Does Not Affect Cell Death. Related to Figure 3.

(A) Representative images showing cell death in the cortex after IUE labeled by TUNEL staining at E13.5-17.5. Scale bar = 100µm.

(B) Quantification of the dead cell density labeled by TUNEL in electroporated mouse brains at E13.5-17.5. Values represent Mean ± S.D. (n = 5 brains; Student's t-test, N.S., no significant difference).

(C) Representative images showing cell death in the cortex after IUE labeled by TUNEL staining at E13.5-15.5. Bottom show magnified. Scale bar = 100µm, top; = 50 µm bottom.

(D) Quantification of the dead cell density labeled by TUNEL in mouse brains electroporated with scrambled shRNA or *Kif26a* shRNA at E13.5-15.5, and E15.5 mouse brains without injection and electroporation. Values represent Mean ± S.D. (n = 7 brains for scrambled, 5 brains for *Kif26a* KD, 4 brains for no injection. Student's t-test, N.S., no significant difference).
Figure S5. *KIF26A* KO iPSC Line Characterization. Related to Figure 4.

(A, B) Summary table (A) and target sequence validation (B) of successful introduction of frame-shift mutation in *KIF26A* KO iPSC lines. Sanger sequence view showing edited and wild-type (control) sequences in the region around the guide sequence. The horizontal black underlined region represents the guide sequence. The horizontal red underline is the PAM site. The vertical black dotted line represents the actual cut site.

(C) KO lines have normal karyotype after edit examined by KaryoStat.
Figure S6. Characterization of KIF26A KO Forebrain Organoids. Related to Figures 5.

(A) Schematic summary of the protocol to generate forebrain organoids. AWEB, AggreWell™ Embryonic Body (EB) Formation Media; CHIR, CHIR99021; SB, SB431542. See STAR Methods for details.

(B) Representative phase contrast images for different stages of forebrain organoid differentiation. Scale bar length is indicated on the images.

(C) Representative tiled confocal images of forebrain organoid at Day 70. Dashed lines delineates the boundaries separating CP, SVZ and VZ. Scale bar = 100µm.

(D) Representative image showing KIF26A expression in neuron but not progenitors in forebrain organoid. Scale bar = 100µm.

(E) In situ hybridization for KIF26A on Day 60 WT organoid. Scale bar = 100 µm.

(F) KIF26A KO organoids have normal neurogenesis. Scale bars = 100µm.

(G) Representative images of PH3 immunostaining in WT and KO organoids. Scale bars = 100µm.

(H) Quantitative analysis of density of dividing SOX2+ TBR2- vRG (top) and TBR2+ IPC (bottom) visualized by PH3 immunostaining in the of WT and KO organoids. Values represent Mean ± S.D. (n = 10 organoids. Student’s t-test, N.S., no significant difference).

(I) Quantitative analysis of vRG cleavage angle in the VZ of WT and KO organoids. Cleavage angle is measured as the angle of dividing cell division axis against the apical surface of the VZ. Values represent Mean ± S.D. (Same samples as H).
Figure S7. Additional Characterization of Radial Migration and FAK Inhibition in Organoids. Related to Figures 5 and 6.

(A, B) Representative images showing EdU-labeled neuron neurons express KIF26A in WT organoid (A) but not in KO organoid (B). Inset show magnified view of the CP area. Inset width = 50µm. Scale bars = 100µm.

(C) Representative images showing the laminar distribution of EdU labeled cells in Day 70+8 WT and KO forebrain organoids. Scale bars = 50µm.

(D) Phosphorylated FAK (pFAK Tyr576/577) is higher in the CP of KO organoids than WT organoids. Scale bar = 20µm.

(E) Representative images showing apoptosis in Day 60 WT (left) and KO (right) organoids treated with DMSO or 0.5 µM GSK2256098 (GSK) for 8 days. Dashed lines delineate the borders between the CP, SVZ and VZ. Scale bar = 100µm.

(F) Quantification of the density of apoptotic cells in the VZ, SVZ and CP layers of WT and KO organoids with GSK or DMSO treatment. Values represent Mean ± S.D. (n = 8 organoids from two pairs of isogenic lines. Student’s t-test, *** p < 0.0005; N.S., no significant difference).
Figure S8. Additional Bioinformatic Analyses of Brain Organoid ScRNAseq. Related to Figure 7.

(A) Graph-based clustering of single cells from WT and KO brain organoids (left) with cell type identity transferred (right) from human fetal cortex atlas (Fan et al., 2018; Nowakowski et al., 2016; Polioudakis et al., 2019; Zhong et al., 2018) based on transcriptome similarities.

(B) Correlation matrix comparing clusters annotated in brain organoids and cell types in human fetal cortex atlas.

(C) Single cells from different libraries integrate well between ages (Day 60 and Day 90) and cell lines (WT and KO).

(D) The cell-type compositions across libraries (one organoid per library) are similar to each other, demonstrating consistency between individual organoids. KIF26A KO did not significantly alter the cell-type composition.

(E) Volcano plot showing differentially expressed genes (DEGs) between WT and KO cells in maturing excitatory neurons. Significant DEGs with adjusted p-value < 0.05, and Log_2 fold change > 0.25 or < -0.25, are shown in blue. Selected DEGs involved in neuronal survival and apoptosis are highlighted in red.

(F) Venn diagrams showing the overlap of significant (adjusted p-value < 0.05) DEGs in migrating and maturing excitatory neurons. Selected DEG with known involvement in neuronal survival and apoptosis are highlighted.

(G) Network plots for GSEA across migrating and maturing excitatory neurons between KO and WT. G2M_CHECKPOINT, E2F_TARGETS and MYC_TARGETS_V1 are the only three significantly changed (adjusted p-value < 0.05) pathways among the 50 Hallmark pathways. For each pathway, and the number of genes in each pathway is represented by node size. Nodes are connected by lines that represent individual genes in the data set which are common to multiple nodes.