

Supplementary Information

Loss of symmetric cell division of apical neural progenitors drives *DENND5A*-related developmental and epileptic encephalopathy

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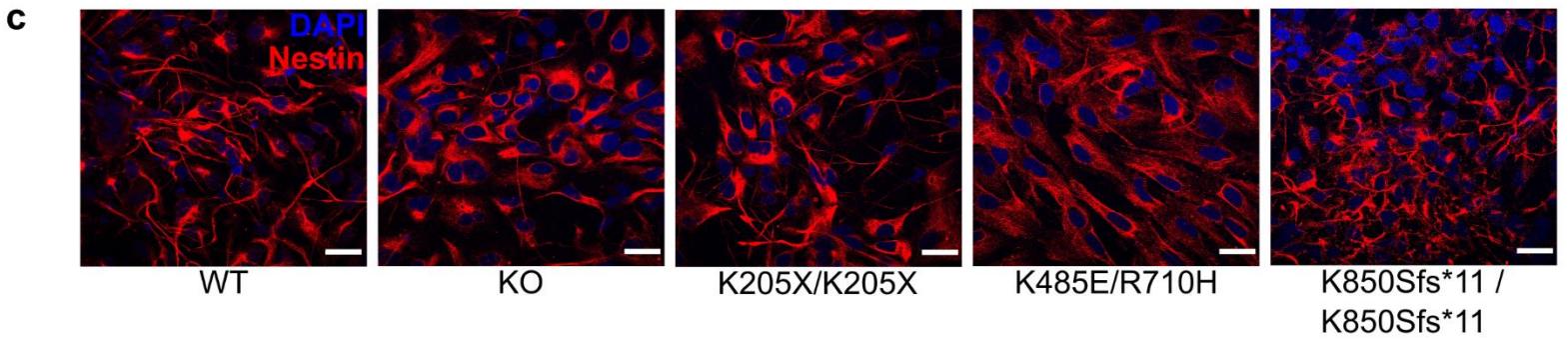
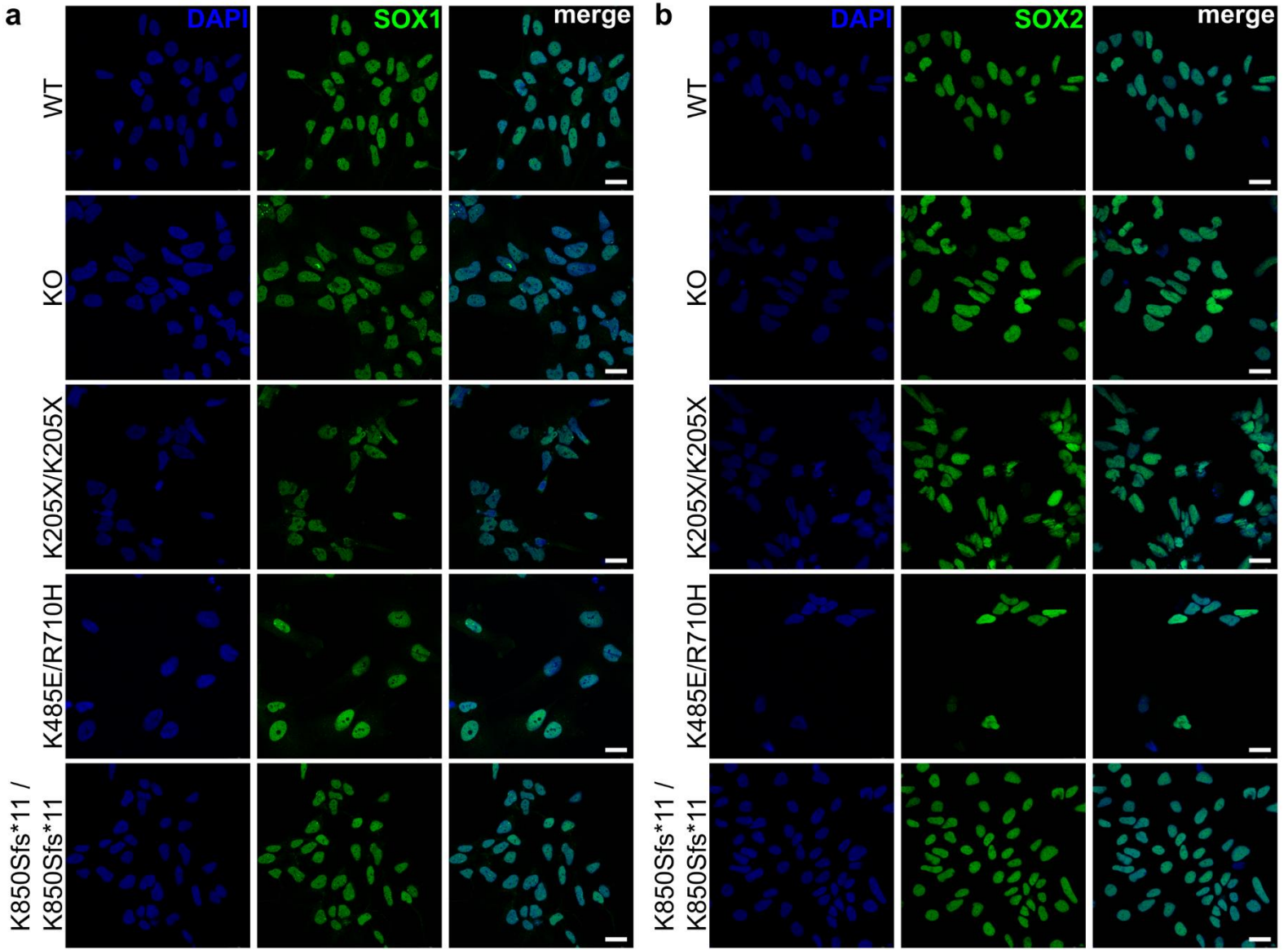
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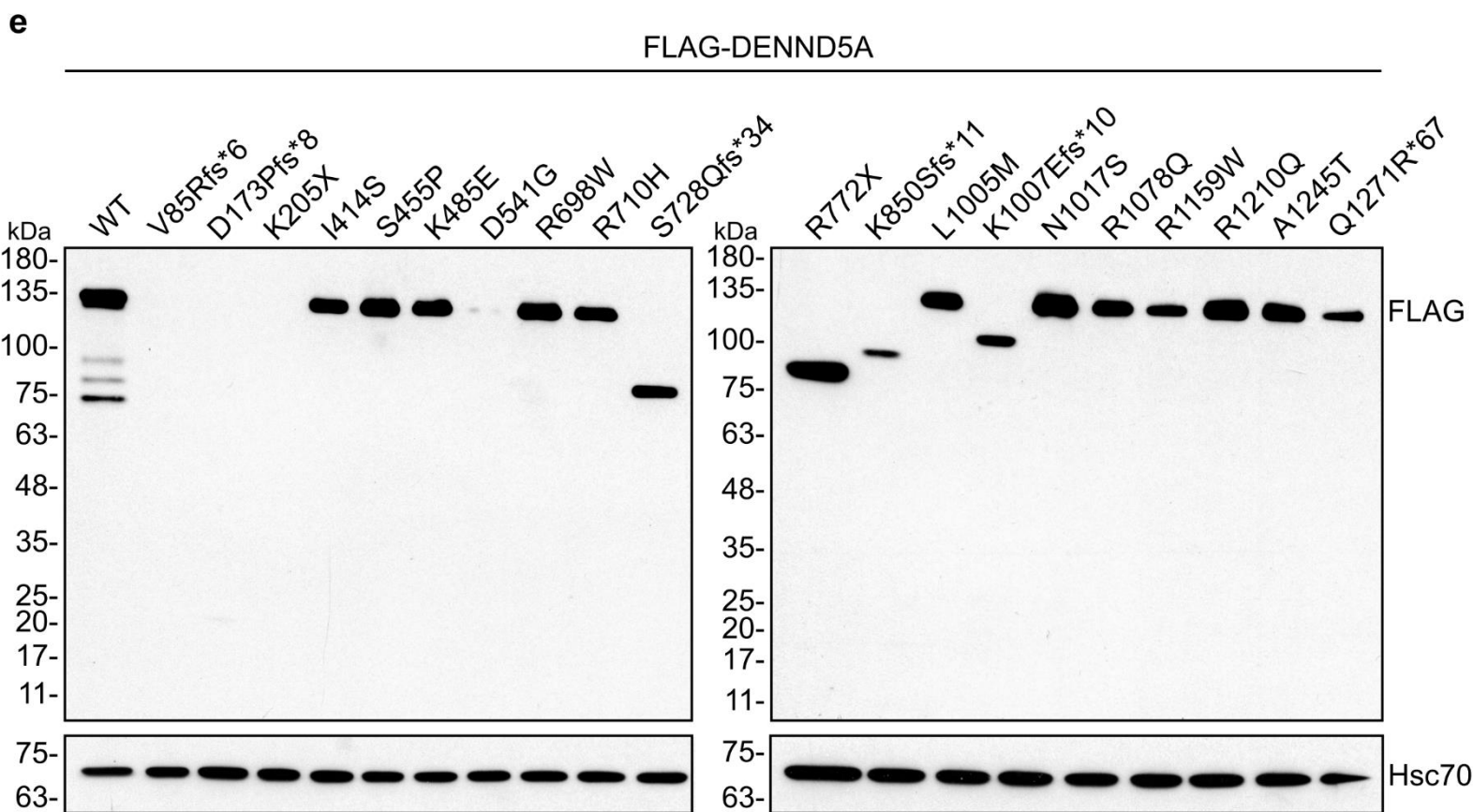
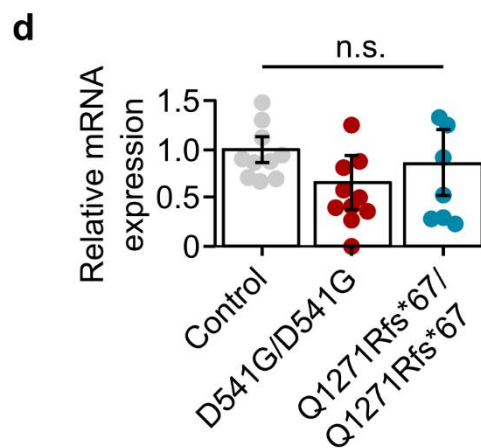
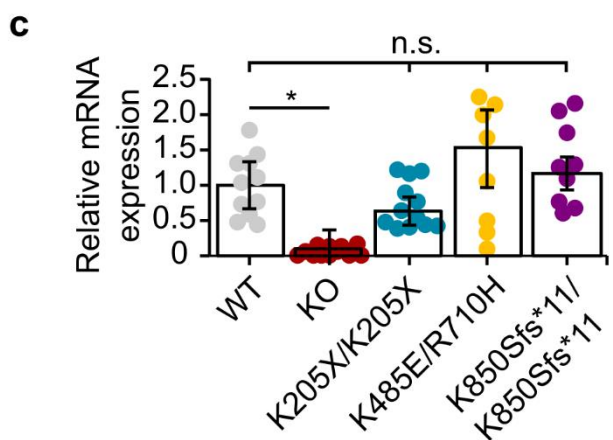
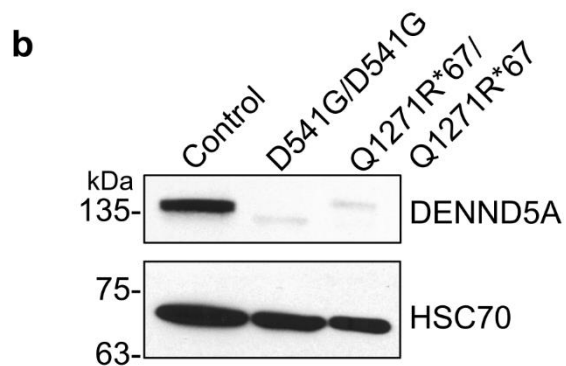
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1. Supplementary Figures

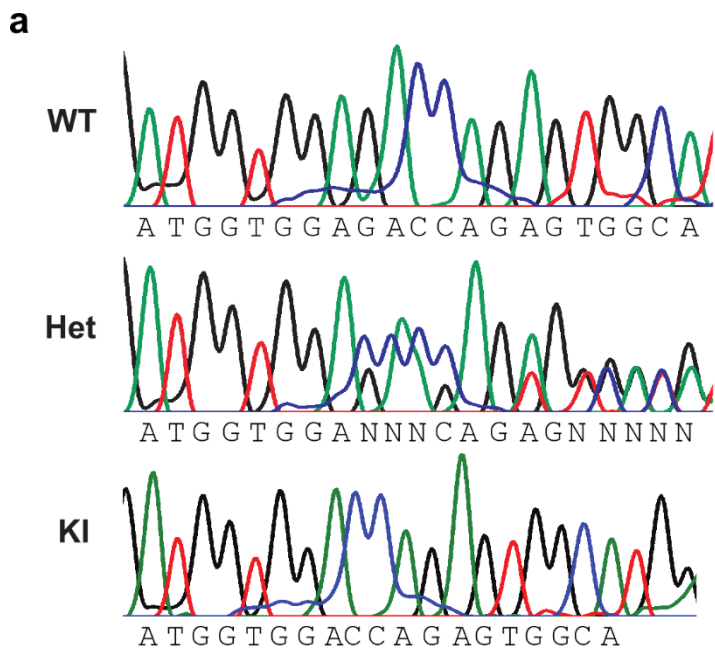


Supplementary Figure 1: All established NPC lines express neural progenitor-specific markers. hiPSCs differentiated into NPCs express **a**, SOX1 (green); **b**, SOX2 (green), and **c**, Nestin (red). Blue = DAPI. Scale bars = 20 μm . Results were reproduced each time NPCs were generated, for a total of 8 independent experiments.



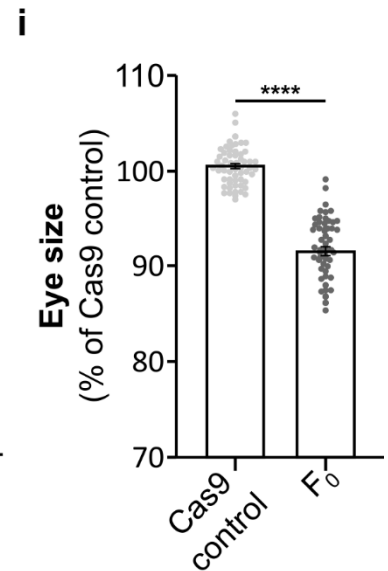
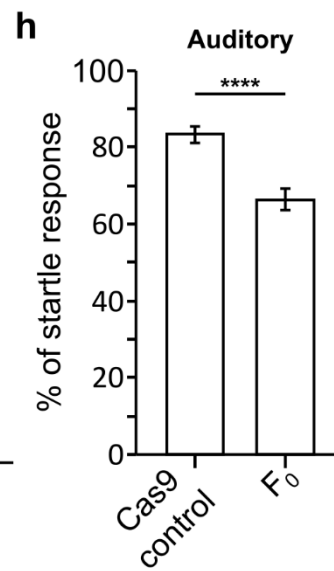
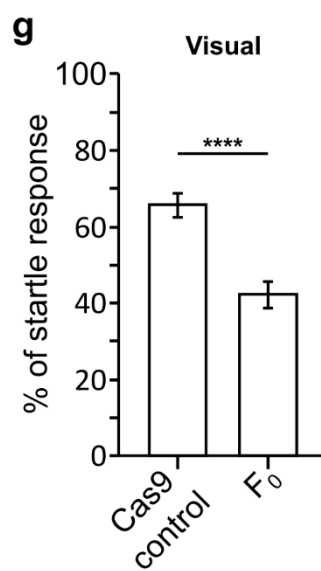
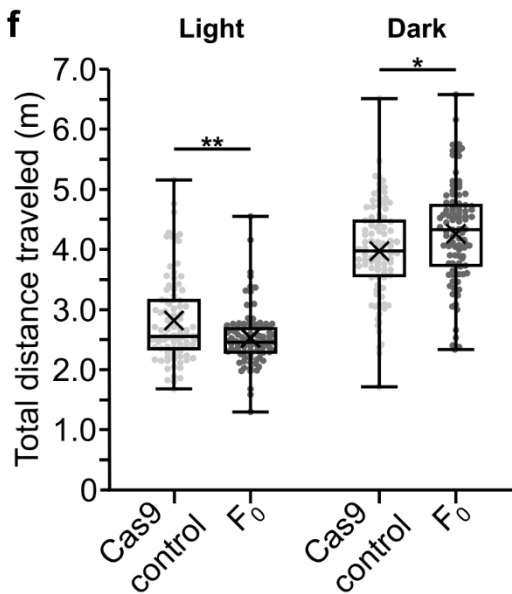
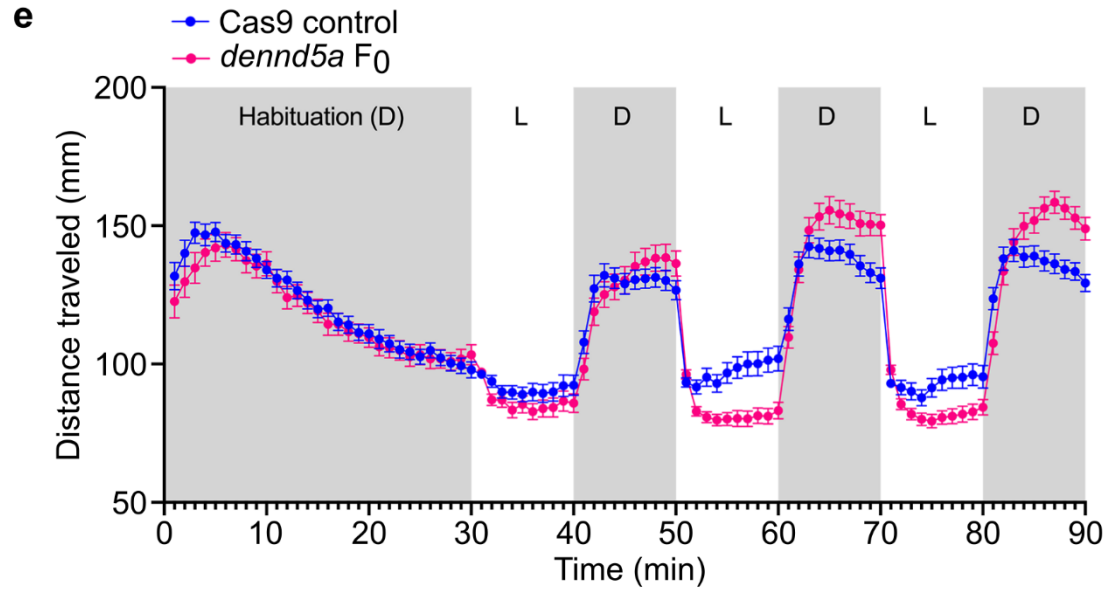
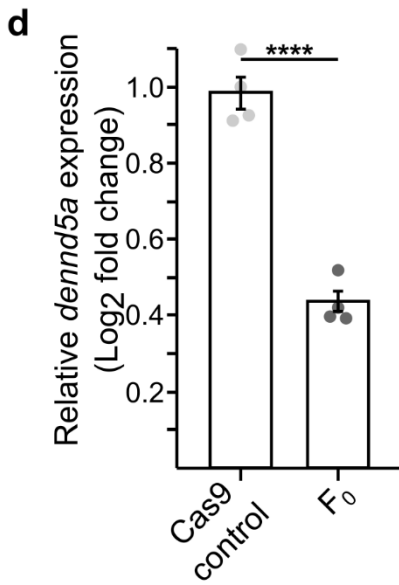
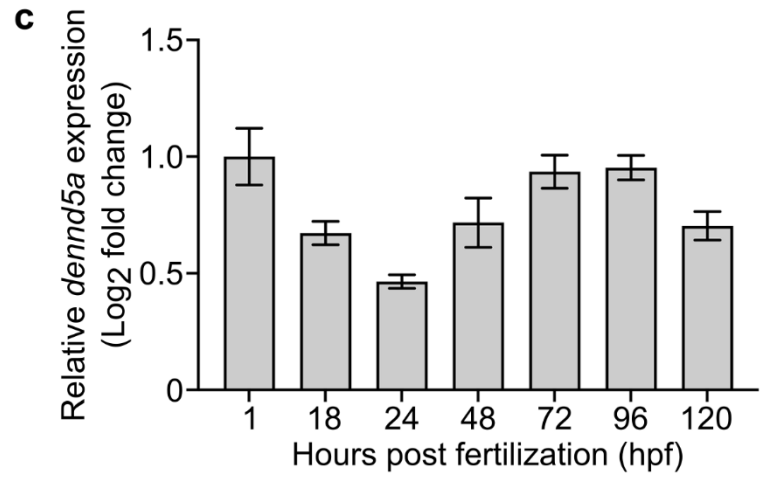
Supplementary Figure 2: DENND5A expression varies depending on the variant.

DENND5A protein expression in **a**, NPCs and **b**, lymphoblasts. Results were reproduced in 3 independent experiments. **c**, Relative *DENND5A* mRNA expression measured by RT-qPCR in NPCs. Measurements were made with 4 technical replicates on $n = 3$ independent samples. Data are mean \pm SEM analyzed via Kruskal-Wallis tests with Bonferroni-corrected pairwise comparisons ($H(4) = 21.75, p = .0002$). **d**, Relative *DENND5A* mRNA expression measured by RT-qPCR in lymphoblasts. Measurements were made with 4 technical replicates on $n = 3$ independent samples. Data are mean \pm SEM analyzed via Kruskal-Wallis tests with Bonferroni-corrected pairwise comparisons ($H(2) = 2.451, p = .294$). **e**, Overexpression of FLAG-DENND5A mutagenized to contain several variants influences protein stability and expression levels in HEK293T. Results were reproduced in 3 independent experiments. Source data for each panel are provided as a Source Data file.

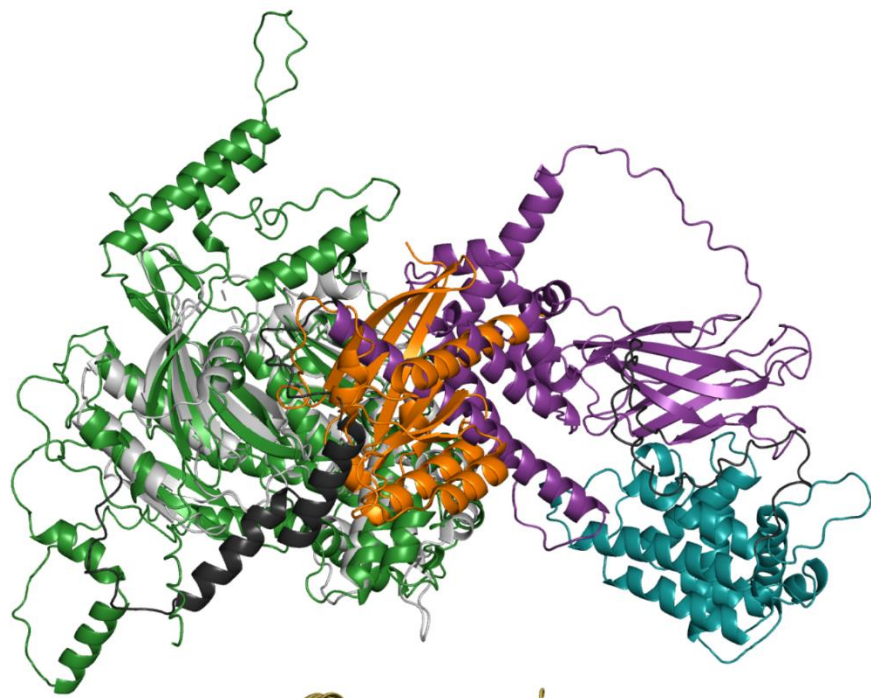


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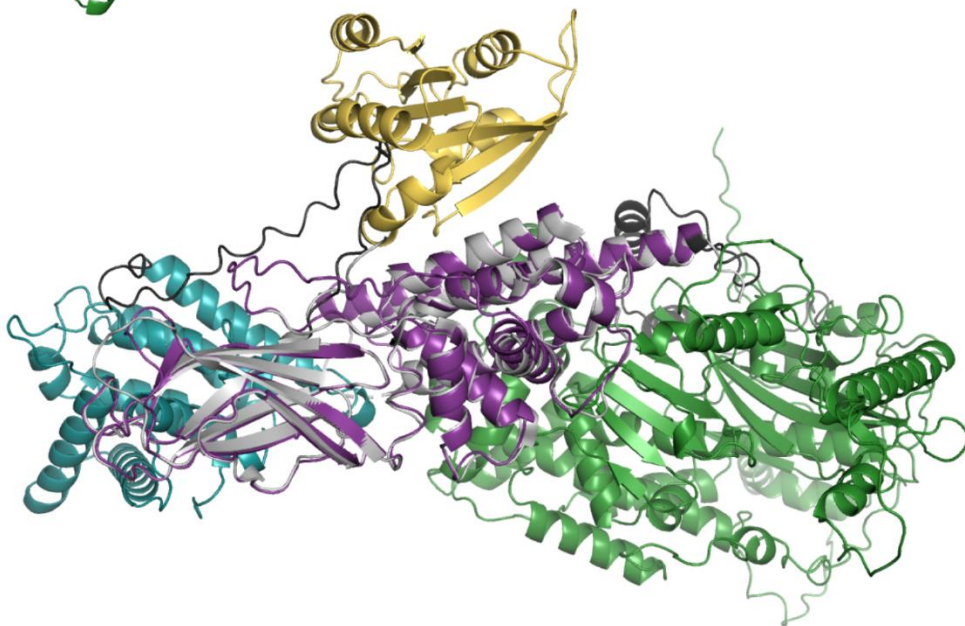
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(NM_021494)	[508	GATGGTGGAGACCAGAGTGGC						
<i>Homo sapiens</i>	[508	GATGACAGAGACCAGAGCAGC						
(NM_015213)	[170	D	D	R	D	Q	S	S



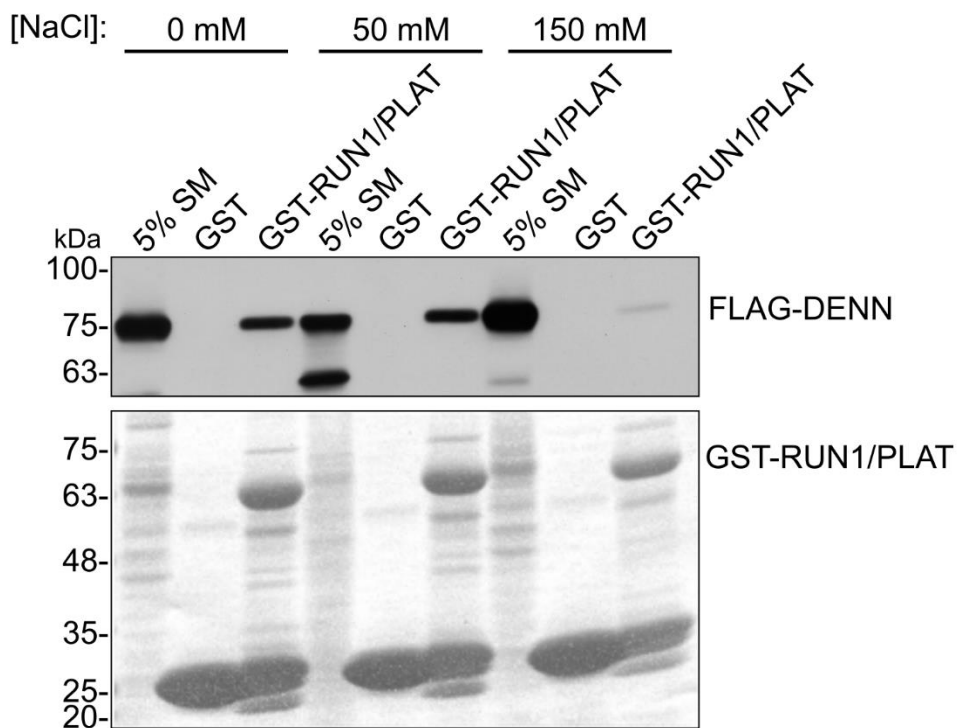
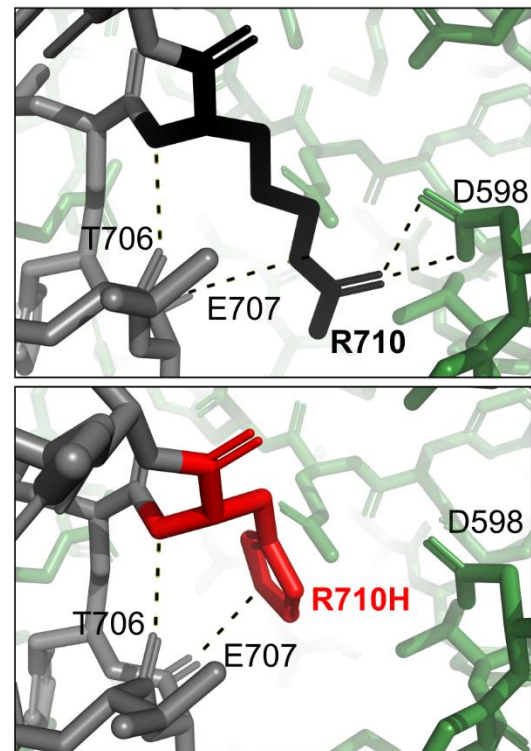
Supplementary Figure 3: Features of *DENND5A* transgenic animals make them valid models to study *DENND5A*-related DEE. **a**, Sample chromatograms demonstrating *DENND5A* DNA sequences in WT, heterozygous (Het), and knock-in (KI) mice. **b**, DNA and amino acid sequence alignment between human and mouse *DENND5A* sequences. Highlighted base pairs indicate bases deleted using CRISPR/Cas9. **c**, The temporal expression of zebrafish *dennd5a* mRNA by RT-qPCR at different developmental stages from 3 experiments performed with technical triplicates. Expression levels were normalized to the *18S* housekeeping gene and compared to 1 hpf embryos. Data are mean \pm SD. **d**, Expression of *dennd5a* mRNA in Cas9 controls and *dennd5a* F₀ knockouts detected by RT-qPCR at 5 dpf. 4 experiments were performed with technical triplicates. Data are mean \pm SEM analyzed via two-tailed student's *t*-test ($t(6) = 10.706, p = .000039$). **e**, Locomotor activities of zebrafish larvae at 5 dpf with $n = 96$ larvae for each group. Data are mean \pm SEM. D = Dark period, L = light period. **f**, Quantification of distance traveled by each larva during the cycles of light or dark periods, analyzed via two-tailed Mann-Whitney U test (light; $Z = -2.81, p = .005$) and two-tailed student's *t*-test (dark; $t(190) = -2.438, p = .016$) with $n = 96$ larvae for each group. Each dot represents one larva. **g**, Visual startle response in $n = 143$ larvae at 6 dpf. Data are mean \pm SEM analyzed via two-tailed Mann-Whitney U test ($Z = -4.957, p = 7.15 \times 10^{-7}$). **h**, Acoustic evoked behavioral response in $n = 134$ larvae at 6 dpf. Data are mean \pm SEM analyzed via two-tailed Mann-Whitney U test ($Z = -4.947, p = 7.53 \times 10^{-7}$). **i**, Quantification of eye size in $n = 60$ larvae. Each dot represents one larva. Data are mean \pm SEM analyzed via two-tailed Welch's *t*-test ($t(96.016) = 17.831, p = 3.16 \times 10^{-32}$). Source data for (**d-i**) are provided as a Source Data file.

a

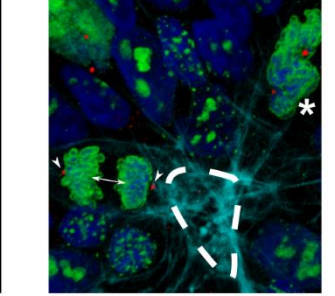
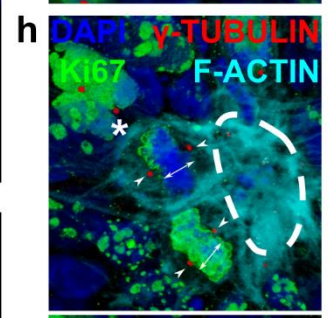
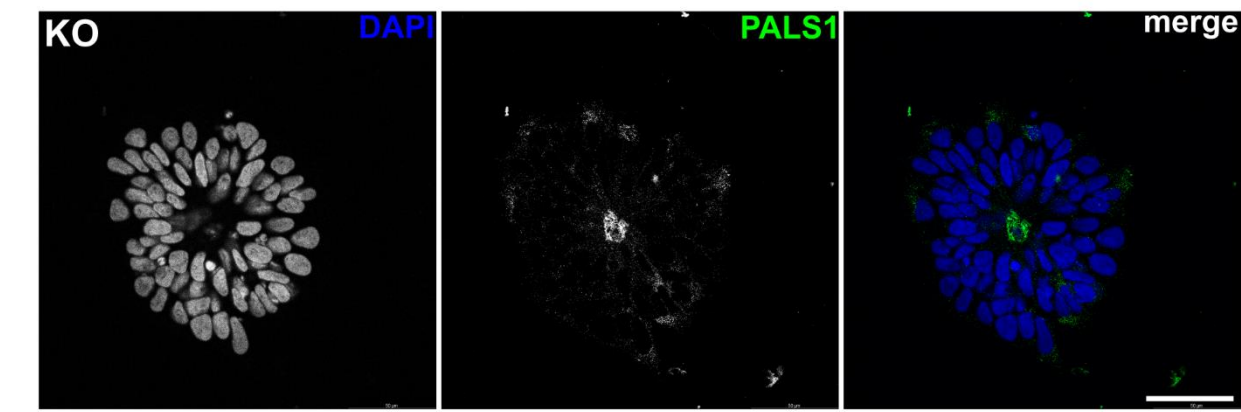
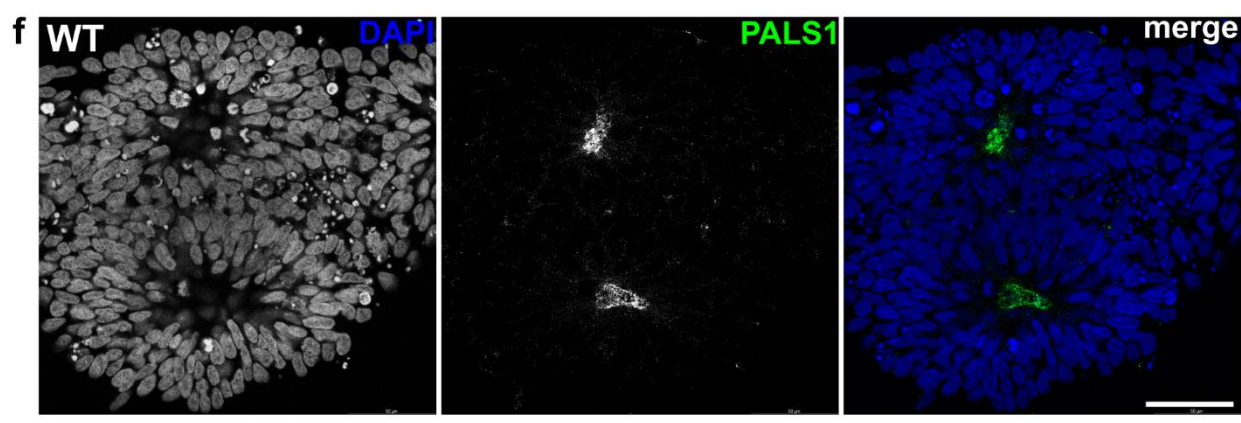
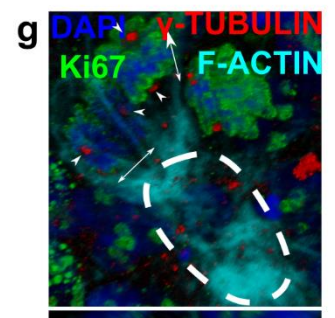
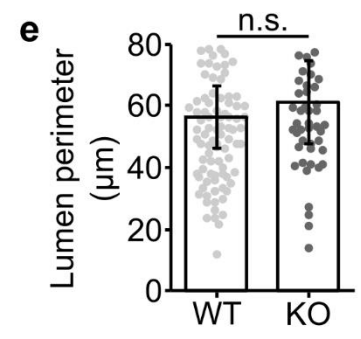
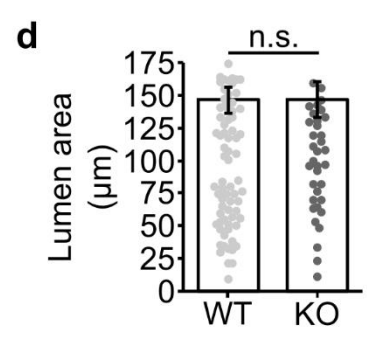
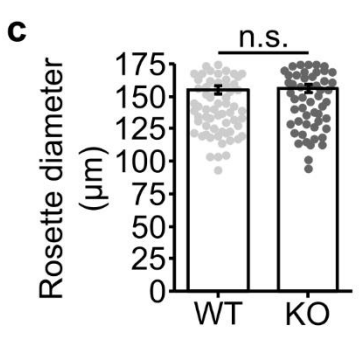
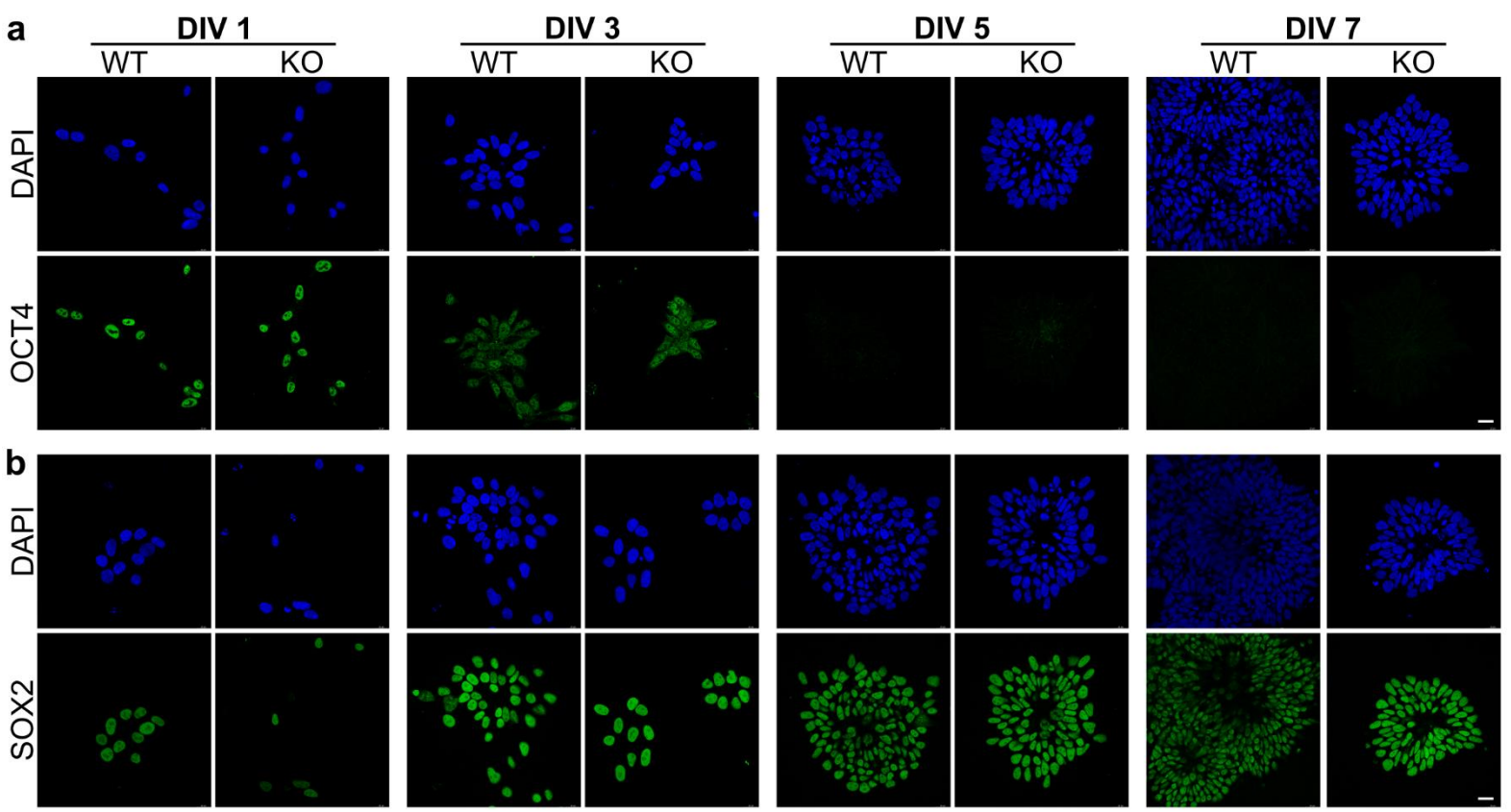
DENN (DENND5A)
 RUN1/PLAT
 Linker regions
 RUN2
 DENN (DENND1B)
 Rab35

b

DENN (DENND5A)
 RUN1/PLAT
 Linker regions
 RUN2
 RUN1/PLAT
 Rab6A

c**d**

Supplementary Figure 4: Analysis of the predicted DENND5A structure indicates intramolecular interactions may regulate other protein-protein interactions. **a**, Structural alignment between the predicted DENND5A structure and PDB:3TW8 (gray, yellow) **b**, Structural alignment between the predicted DENND5A structure and PDB:3CWZ (gray, yellow) **c**, Pulldown experiment showing binding capacity between GST-RUN1/PLAT and FLAG-DENN domains of DENND5A under varying NaCl concentrations. Results were reproduced in 2 independent experiments. Uncropped blots are provided as a Source Data file. **d**, The R710H variant found in the cohort and within the region that interacts with PALS1/MUPP1 results in the removal of two hydrogen bonds with D598 of the DENN domain. Dotted lines indicate hydrogen bonds.



Supplementary Figure 5: WT and *DENND5A* KO neural rosettes differ in density and cell division properties, but not in marker expression or size. Expression of **a**, OCT4 and **b**, SOX2 during neural rosette development. Blue = DAPI, green = OCT4/SOX2. Scale bars = 20 μ m. Results were reproduced in 2 independent experiments **c**, Average diameter of individual rosettes. $n = 159$ rosettes were analyzed from 2 independent experiments. Data are mean \pm SEM and analyzed via two-tailed student's *t*-test ($t(157) = -0.182, p = .856$). **d**, Average lumen area of rosettes. $n = 294$ rosettes were analyzed from 2 independent experiments. Data are mean \pm SEM and analyzed via two-tailed Mann-Whitney U test ($Z = -0.091, p = .928$). **e**, Average lumen perimeter of rosettes. $n = 294$ rosettes were analyzed from 2 independent experiments. Data are mean \pm SEM and analyzed via two-tailed Mann-Whitney U test ($Z = -0.917, p = .359$). **f**, PALS1 staining (green) shows an apical localization in both WT and KO neural rosettes. Scale bars = 50 μ m. Results were reproduced in 2 independent experiments. **g**, 3D-rendered images of apical progenitors of WT neural rosettes. Blue = DAPI, green = Ki67, red = α -tubulin, cyan = F-actin. Arrowheads indicate centrosomes, arrows indicate orientation of cell divisions, dotted lines indicate the lumen. Results were reproduced in 2 independent experiments. **h**, 3D-rendered images of apical progenitors of KO neural rosettes. Blue = DAPI, green = Ki67, red = α -tubulin, cyan = F-actin. Arrowheads indicate centrosomes, arrows indicate orientation of cell divisions, asterisks indicate abnormally condensed chromatin, dotted lines indicate the lumen. Results were reproduced in 2 independent experiments. Source data for (c-e) are provided as a Source Data file.

2. Supplementary Methods

Motor skills scoring system

Item	Scoring
Able to reach/grasp objects	+1 if positive
Able to roll over	+1 if positive
Able to sit with support	+1 if positive OR is able to sit without support
Able to sit without support	+1 if positive
Able to stand with support	+1 if positive OR is able to stand without support
Able to stand without support	+1 if positive
Able to walk with support	+1 if positive OR is able to walk without support
Able to walk without support	+1 if positive
Muscle tone or spasm problems	+1 if negative for all (hyperreflexia, spastic tetraplegia, clonus, and current hyper/hypotonia)
Motor regression after seizure	+1 if negative AND could perform one of the above behaviors in past
TOTAL	10

Scoring system used for quantifying motor abilities. A low score reflects minimal motor abilities, a high score indicates a high degree of motor capabilities. If a child's ability to do a skill is unknown, it is counted as positive.

Neurological phenotype scoring system

Item	Scoring
Seizures	+1 if positive
Reduced volume (cerebral or supratentorial parenchymal volume loss)	+1 if positive
Cerebellum abnormalities (hypoplastic vermis, reduced volume)	+1 if positive
Thalamus abnormalities (thalami fusion or reduced volume, massa intermedia prominence)	+1 if positive
Basal ganglia abnormalities (dysplasia or reduced volume)	+1 if positive
Calcifications	+1 if positive
Ventricle or CSF abnormalities	+1 if positive
White matter abnormalities (reduced corpus callosum or other white matter tract volume, delayed myelination or hyperintensity)	+1 if positive
Hemorrhage or ischemic event	+1 if positive
Cortical visual impairment	+1 if positive
TOTAL	10

Scoring system used for quantifying neurological phenotypes. A low score corresponds to few neurological abnormalities, a high score indicates many neurological abnormalities.

Communication skills scoring system

Item	Scoring
Smiles	+1 if positive
Eye contact	+1 if positive
Points at objects/people	+1 if positive
Babbles	+1 if positive OR if speaks in at least single words
Uses PECS board	+1 if positive OR if speaks in at least single words
Speaks in single words	+1 if positive OR if speaks in at least short phrases
Speaks in short phrases	+1 if positive OR if speaks in sentences
Speaks in sentences	+1 if positive
Language regression after seizure	+1 if negative AND if had language skills in past
Receptive language delay	+1 if negative AND at least babbles
TOTAL	10

Scoring system used for quantifying communication abilities. A low score reflects minimal communication ability, a high score reflects more advanced language and communication abilities.

Comorbidities scoring system

Item	Scoring
Chronic constipation	+1 if positive
Autism spectrum disorder (formally diagnosed or clinically suspected)	+1 if positive
Psychiatric disorders (ADHD, anxiety)	+1 if positive
Behavioral disorders or abnormalities (self-injury, poor sleep, hyperphagia)	+1 if positive
Lung or breathing abnormalities (restrictive lung disease, asthma)	+1 if positive
Cardiac abnormalities (ventricular/atrial septal defects, arrhythmia)	+1 if positive
Blindness	+1 if positive
Obesity	+1 if positive
Bone abnormalities (low density or osteoporosis, scoliosis, vertebral fusion, posterior fossa abnormality)	+1 if positive
GERD	+1 if positive
TOTAL	10

Scoring system used for quantifying neurological phenotypes. A low score corresponds to few comorbidities, a high score indicates many comorbidities.

3. Supplementary Note

Members of the Undiagnosed Diseases Network include:

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