

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- n/a Confirmed
- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
 - ☒ ☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
 - ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
 - ☒ ☐ A description of all covariates tested
 - ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
 - ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
 - ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
 - ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
 - ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
 - ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection pCLAMP 10 software (Molecular Devices)

Data analysis Electrophysiological recordings were analyzed using IgorPro (v6) and AxoGraph (v X) and organized using Graph Pad Prism 9.0.0. Imaging data was analyzed by Imaris (9.10.0), and organized with the KNIME Analytics Platform 4.7.1. Statistical comparisons were performed by Graph Pad Prism 9.0.0 or R 4.4.0. For illustration, representative images were processed with the FIJI software package version 2.14.0/1.54f. MD simulations: the following software packages were used: VMD 1.93, Gromacs 2021.2, AlphaFold2 (01 JUL 21), Matplotlib 3.1.3, MDAnalysis 2.4.2.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Data availability statement: All data supporting the findings of this study are available within the paper and in Source Data Tables 1-4. MD simulation data are available in <https://zenodo.org/uploads/14554776>. All other primary data will be made available on request from the corresponding authors. Publicly available databases used in this study include GeneMatcher (<https://genematcher.org>), MetaDome web server (<https://stuart.radboundumc.nl/metadome/>), ClinVAR (<https://www.ncbi.nlm.nih.gov/clinvar>), Genome Aggregation Database (gnomAD; <http://gnomad.broadinstitute.org>), OMIM (<http://www.omim.org>), UCSC Genome Browser (<https://genome.ucsc.edu>), Ensembl (<https://www.ensembl.org>), and UniProt (<https://www.uniprot.org>).

Code availability statement: Code has not been developed in this manuscript.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	The sex of identified patients is indicated in supplemental table 1, no further sex or gender-related analysis was made.
Reporting on race, ethnicity, or other socially relevant groupings	We do not report on the race, ethnic affiliation, or other socially relevant groupings in our patient cohort.
Population characteristics	Our study population includes patients with neurodevelopmental features, ranging in age from 11 months to 32 years, who underwent exome or genome sequencing on a clinical or research basis at academic institutes or diagnostic labs worldwide. Informed consent was obtained from parents or guardians of the affected individuals, with approval from local institutional review boards. De-identified genetic variants, clinical data, and facial photographs (shown in Figures 3-5 and Supplementary Figures 1 and 2) were obtained from collaborating institutions based on these informed consents.
Recruitment	Individuals included in this study underwent exome or genome sequencing on diverse sequencing platforms on a clinical or research basis in academic institutes or diagnostic labs worldwide. Using GeneMatcher1 and personal communication with colleagues enabled us to assemble clinical and genetic details on a total of 48 individuals with de novo or inherited heterozygous or biallelic variants in UNC13A (NM_001080421.3), including two previously published cases harboring c.154G>A, p.(E52K) [case 74 of Lionel et al., 20182], and c.4379C>T, p.(A1460V) [case UPN-0740 of 3] variants with further follow up information.
Ethics oversight	This study was performed as part of a research study approved by the ethics commission of the Canton of Zurich (ID PB_2016-02520 [SIV 11/09]). In addition, for each patient, ethical approvals and informed consent forms from parents or guardians were obtained by the respective research teams and institutions for data use and publication, including photographs or videos where applicable. Participants did not receive compensation.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size calculations for each of the recorded parameters were not performed prior to these experiments. In general, for the electrophysiological experiments presented, we were interested in detecting large and therefore likely biologically significant effects, i.e. differences in sample means of 0.8 to 1 times SD, corresponding to a 'Large effect' according to Cohen. Sample sizes (n) to resolve such effects are $\geq 17-26$ for each group for a statistical power of 0.8 and a significance level of 0.05. For all experiments, n was ≥ 19 .
Data exclusions	In electrophysiological experiments, all data which fulfilled the quality criteria (e.g. leak current etc) was included in the analysis. No 'outliers detection' or related procedures were applied.
Replication	Data was collected from two or more cultures. In the vast majority of experiments, data was independently obtained by two experimenters

Replication	(Figure 3 - in different laboratories, in Figures 4-7 by different experimenters), to ensure reproducibility of the observations. Approximately equal numbers of WT and Munc13-1-variant recordings were obtained during each measurement day.
Randomization	Randomization was not used in this study, as it is not relevant in the experiments described here.
Blinding	Blinding was not used in this study. Instead, for each of the variants, recordings were performed by two or three independent experimenters and across two or three different labs, and all phenotypes were confirmed.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	<p>Antibody; Source; Dilution used; RRID; Identifier; clone ID (when relevant)</p> <p>Ab 1. Mouse monoclonal GFP; Merck Millipore; 1:250; AB_94936; MAB3580, Clone ID: N/A</p> <p>Ab 2. Rabbit polyclonal VGLUT1; Synaptic Systems; 1:1000; AB_887877; 135 302</p> <p>Ab 3. Guinea pig polyclonal Shank 2; Synaptic Systems; 1:250; AB_2619861; 162 204</p> <p>Ab 4. Chicken polyclonal MAP2; Novus Biologicals; 1:1000; AB_2138178; NB300-213</p> <p>Secondary Abs for immunostaining</p> <p>Ab 5. Goat anti-Mouse Alexa 488; Thermo Fisher; 1:2000; AB_2534088; A11029</p> <p>Ab 6. Goat anti-Rabbit Alexa 633; Thermo Fisher; 1:2000; AB_141419; A21071</p> <p>Ab 7. Goat anti-Guinea Pig Alexa 568; Abeam; 1:2000; AB_2864763; Ab1 75714</p> <p>Ab 8. Goat anti-Chicken Alexa 405; Abeam; 1:1000; AB_2890171; Ab175674</p> <p>Primary antibodies for Western Blot analysis</p> <p>Ab 9. Polyclonal rabbit anti-FLAG; Sigma-Aldrich; 1:2000; AB_439687; F7425</p> <p>Ab 10. Monoclonal mouse anti- Green Fluorescent Protein (1E4); Enzo Life Sciences; 1:1000; ADI-SAB-500-E; Clone ID: 1E4</p> <p>Secondary antibodies for Western Blot analysis</p> <p>Ab 12. Peroxidase AffiniPure Goat Anti-Mouse IgG (H+L), Jackson immunoResearch; 1:5000; AB_2307392; 115-035-146</p> <p>Ab 13. Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L), Jackson immunoResearch; 1:30000 AB_2307391; 111-035-144</p>
Validation	<p>Ab 1. Mouse monoclonal GFP; Merck Millipore; 1:250; AB_94936; MAB3580</p> <p>Recognizes a protein tag</p> <p>Website information: Antibody validated for use in ELISA, IC, IH & WB.</p> <p>In this study: immunostaining signal was absent in a non-transfected control (Figure 2c, 2e)</p> <p>Ab 2. Rabbit polyclonal VGLUT1; Synaptic Systems; 1:1000; AB_887877; 135 302</p> <p>Website information: Antibody was validated using KO samples, citations can be found in https://sysy.com/product/135302#list</p> <p>Ab 3. Guinea pig polyclonal Shank 2; Synaptic Systems; 1:250; AB_2619861; 162 204</p> <p>Website information: Antibody was validated using KO samples (PMID: 2997098)</p> <p>Ab 4. Chicken polyclonal MAP2; Novus Biologicals; 1:1000; AB_2138178; NB300-213</p> <p>Website information: Knockdown Validated (PMID: 32294442). More publications in https://www.novusbio.com/products/map2-antibody_nb300-213?srsltid=AfmBOorEtC2sqRWpEaMtjX36c2y8L3tkHsKuvStTIAstIKx6DkunwKBB#reviews-publications</p> <p>Ab 9. Polyclonal rabbit anti-FLAG; Sigma-Aldrich; 1:2000; AB_439687; F7425</p> <p>Recognizes a protein tag</p> <p>In this study: validated in this study by targeting a non-transfected control using western blot (Supplementary figure 4b)</p> <p>Ab 10. Monoclonal mouse anti- Green Fluorescent Protein (1E4); Enzo Life Sciences; 1:1000; ADI-SAB-500-E</p> <p>Recognizes a protein tag</p> <p>In this study: validated in this study by targeting a non-transfected control using western blot (Supplementary figure 4b)</p>

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	HEK293T (ATCC-CRL-3216), human embryonic kidney, commercially available
Authentication	The cell line was not authenticated.
Mycoplasma contamination	Mycoplasma contamination was not detected.
Commonly misidentified lines (See ICLAC register)	Not relevant

Animals and other research organisms

Policy information about [studies involving animals; ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	<p>Mice: We used mice at embryonic day 18 (E18) to prepare primary hippocampal neuronal cultures</p> <p>Strain: Unc13a/b double knock-out mice (MGI Unc13atm1Bros and Unc13btm2Bros) - made in the lab of co-authors of this study (Dr. Nils Brose). Adult mice were kept under IVC/SPF conditions, at 12h/12h light/dark cycle, at room temperature of 22 +/-2°C, and humidity levels of 55 +/-10%.</p> <p>C. elegans: Control strains used: N2, unc-13(nu641). unc-13(nu641) harbors a C-terminal mScarlet in the unc-13 locus. CRISPRmodified strains were outcrossed at least four times and the relevant genomic region was sequenced to confirm the target mutation.</p>
Wild animals	No wild animals were used
Reporting on sex	<p>Mice: Sex was not considered and both female and male embryonic mice were used to make cultures</p> <p>C. elegans: hermaphrodite worms were used</p>
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	The use of the Unc13a/b knockout mice were approved by the responsible local government organizations in Germany (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit; 33.19-42502-04-15/1817 and 33.19-42502-04-20/3589, and Landesamt für Gesundheit und Soziales; G106/20)

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	<i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>
Novel plant genotypes	<i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i>
Authentication	<i>Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.</i>