

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

Image Studio Lite LI-COR BioSciences https://www.licor.com/bio/products/software/image_studio_lite/; RRID: SCR_01421
LAS AF Leica Microsystems <http://www.leica-microsystems.com/>; RRID: SCR_013673

Data analysis

Image analysis:
MATLAB MathWorks <https://uk.mathworks.com/products/matlab.html>; RRID:SCR_001622
Imaris 7.5.2 Bitplane <http://www.bitplane.com/>; RRID: SCR_007370
Fiji ImageJ 1.53t <https://fiji.sc/>
Huygens Essential version 23.10 (Scientific Volume Imaging, The Netherlands, <http://svi.nl>)

Transcriptomics pipeline:

R/Bioconductor package DESeq2 R DESeq2 RRID:SCR_015687
R/Bioconductor package ClusterProfiler R ClusterProfiler RRID:SCR_016884
org.Mm.eg.db org.Mm.eg.db RRID:SCR_023488
NextFlow RNASeq pipeline SeqeraLabs <https://www.nextflow.io/example4.html>

Electrophysiology: MiniAnalysis SynaptoSoft, Decatur, GA, USA Mini Analysis Program RRID:SCR_002184

Statistical analysis: GraphPad 9.3.1 GraphPad Software <https://www.graphpad.com/> RRID:SCR_000306

All quantification methods using custom scripts are described in Method Details.

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](#)

All transcriptomic data used will be made publicly available via the Gene Expression Omnibus upon acceptance.

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

N/A

Reporting on race, ethnicity, or other socially relevant groupings

N/A

Population characteristics

N/A

Recruitment

N/A

Ethics oversight

N/A

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

No statistical method was used to predetermine sample size. Sample sizes were determined empirically according to our previous experiences and the review of similar experiments in literature (Favuzzi et al., 2019). At least n= 3 mice was used for all samples.

Data exclusions

Outlier cells for perisomatic inputs and in situ hybridization quantifications were identified using the ROUT Method with Q = 1% (GraphPad 9.3.1), and were excluded from the original dataset.

Replication

3 biological replicates per condition were used for RNASeq.
Each experiment involved multiple independent litters.
In order to ensure reproducibility, we provided detailed methods and reported the number of mice and neurons included in each dataset as well as error bars and p-values for all experiments.

Randomization

Both male and female animals were randomly allocated to experimental groups. Different genetic manipulations (control versus Cdh12 or Cdh13 KD) were randomly determined.

Blinding

Data collection and analysis were not performed blind to the conditions of the experiments. Blinding was made difficult due to the nature of the experiment (specific injections and dissection). We used the same protocols and custom-written codes for collecting and analyzing data.

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
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Primary antibodies

Mouse anti-NeuN - Sigma-Aldrich MAB377
 Rabbit anti-NeuN - Millipore ABN78
 Goat anti-CB1R - Frontier Institute CB1-Go-Af450-1
 Mouse anti-CB1R - SySy 258011
 Goat anti-mCherry - Antibodies-Online ABIN1440057
 Rabbit anti-DsRed - Clontech 632496
 Mouse anti-Syt2 - ZFIN ZDB-ATB-081002-25
 Guinea pig anti-RFP - SySy 390004
 Rabbit anti-tagRFP - EvrogenAB233
 Chicken anti-PV - SySy 195 006
 Chicken anti-GFP - Aves Lab GFP-1020
 Rabbit anti-GFP - Molecular Probes A11122

Secondary antibodies

Donkey anti-rabbit 405 - Abcam Ab175652
 Goat anti-rabbit 405 - Abcam Ab175651
 Goat anti-mouse IgG1 488 - Molecular Probes A-21121
 Goat anti-mouse IgG2a 488 - Molecular Probes A-21131
 Goat anti-mouse IgG2b 488 - Molecular Probes A-21141
 Goat anti-chicken 488 - Molecular Probes A-11039
 Donkey anti-goat 488 - Molecular Probes A-11055
 Donkey anti-rabbit 555 - Molecular Probes A-31572
 Donkey anti-goat 555 - Molecular Probes A-21432
 Goat anti-mouse IgG2b 555 - Molecular Probes A-21147
 Goat anti-mouse IgG2a 647 - Molecular Probes A-21241
 Goat anti-mouse IgG2b 647 - Molecular Probes A-21242
 Donkey anti-mouse 647 - Molecular Probes A-31571
 Goat anti-rat Cy5 - Molecular Probes A-10525
 Goat anti-chicken 568 - ThermoFisher A-11041
 Goat anti-chicken biotin - Vector BA-9010
 Streptavidin 555 - Molecular Probes S32355
 Chicken anti-HA HRP - Abcam Ab1190
 Mouse anti-actin HRP - Sigma A3854
 Further information can be found in the Methods section.

Validation

All antibodies were validated by the companies we obtained them from prior to purchase. Additionally, the antibodies have been validated in previous published work (del Pino et al., 2017; Favuzzi et al., 2019; Exposito-Alonso et al., 2020; Bernard et al., 2022).

Eukaryotic cell lines

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

Cell line source(s)

HEK293FT - ThermoScientific Cat# R70007
 B7GG - Dr. T. Karayannis lab (Brain Research Institute, University of Zurich)
 BHK-EnvA - The Salk Institute of Biological Sciences
 BHK-EnvB - The Salk Institute of Biological Sciences
 HEK293-TVA - Dr. T. Karayannis lab (Brain Research Institute, University of Zurich)
 HEK293-TVB - The Salk Institute of Biological Sciences

Authentication

None of the cell lines used were authenticated

Mycoplasma contamination

The cell lines were not tested for mycoplasma contamination

Commonly misidentified lines
(See [ICLAC](#) register)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

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	Tlx3-Cre (C57BL/6J background), Rico lab (Gerfen, Paletzki, & Heintz, 2013) Nex-Cre (C57BL/6J background), Rico lab (Goebbels, Bormuth, Bode, Hermanson, & Schwab, 2006) C57BL/6J, Charles River 000664; RRID: IMSR_JAX:000664 CD1, Charles River Tlx3-Cre and Nex-Cre were maintained in a C57BL/6 background (Charles River Laboratories). Animals were housed in groups of up to five littermates and maintained under standard, temperature controlled, laboratory conditions. Mice were kept on a 12:12 light/dark cycle and received water and food ad libitum.
Wild animals	The study did not involve wild animals.
Reporting on sex	Both male and female animals were used, results are not displayed according to sex.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal procedures were approved by the ethical committee (King's College London) and conducted in accordance with European regulations, and Home Office personal and project licenses under the UK Animals (Scientific Procedures) 1986 Act.

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>

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☐ All plots are contour plots with outliers or pseudocolor plots.
- ☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Non-fixed, samples prepared on the day of the experiment from mouse somatosensory cortex
Instrument	FACS Aria II and III, Becton Dickinson - 85 nozzle
Software	FACSDiva software for acquisition
Cell population abundance	Abundance of the relevant population: 3.4% IT, 1.2% ET. The purity of the samples were determined by sorting again the fluorescence negative neuronal population. The percentage of purity was 99.5%.

Gating strategy

We designed our gating strategy according to the following:

- FCS and SSC parameters were adjusted in order to sort L5 pyramidal neurons with a cell body > 20um

- Doublets were excluded, only singlets were kept

- The live fraction of L5 IT and L5 ET neurons retrogradely labeled with red fluorescent retrobeads (RB555) was finally gated based on DAPI and PE-A intensity such that DAPI negative and RB555 positive cells were sorted.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.