

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                                                                                                                                                                                                                                                                                      |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A description of all covariates tested                                                                                                                                                                                                                     |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                        |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted<br><i>Give P values as exact values whenever suitable.</i>                     |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                                |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), 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 | BZ-X analyzer (Keyence) and Las-X 3.5.7 (Leica) software were used for acquiring immunohistochemistry images. PyDAQmx v1.4.5 and pypylon 1.7.2 were used for OEG acquisition. The open ephys GUI (v0.4.4.1) and Motion Sequencing acquisition software (kinect2-nidaq v0.2.3) were used to collect electrophysiological and behavioral data, respectively. All MRI was performed in vivo on a 7 T Bruker scanner (Stanford University) running ParaVision 360.3.5.                                                                                                                                                                                                                                                                                                                          |
| Data analysis   | The following custom- and publicly-available software packages were used for data analysis; implementation details are provided in the Methods section: ImageJ 1.53q, QuPath v0.5.1, CellPose 3.0.8.dev19+g0ce3653, Python 3.7 and 3.10, Motion Sequencing (MoSeq2-app 1.3.1, moseq2-extract 1.2.0, moseq2-model 1.2.0, moseq2-pca 1.2.0, moseq2-stat 0.6.0, moseq2-viz 1.3.0), NumPy 1.20 / 1.26.4, SciPy 1.7.0 / 1.15.1, scikit-learn 1.0.2 / 1.6.1, StatsModels 0.13.5, Pandas 1.3.5 / 2.2.3, Seaborn 0.12 / 0.13.2, Matplotlib 3.1.2 / 3.5.3 / 3.10.0, scikit-image 0.16.2, H5py 2.10 / 3.12.1, Dask 2.30, Bokeh 2.4.2 / 3.6.3, Plotly 4.14.3, Neo 0.14.0, and Numba 0.61.2. OEG analysis also used: Tiffiffle 2024.9.20, CalmAn v1.12.1, CMasher 1.9.2., MRtrix3 V.3.0.3, ANTs v.2.3.5 |

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](#)

Single-nucleus gene expression data are available under the Gene Expression Omnibus accession number GSE301194. Spatial transcriptomics data have been deposited at Zenodo (<https://doi.org/10.5281/zenodo.21138437>) and are available as of the date of the publication. The following public datasets were used for XCX graft snRNA-seq analysis: human genome sequence information from Ensembl ([http://ftp.ensembl.org/pub/release-98/fasta/homo\\_sapiens/dna/Homo\\_sapiens.GRCh38.dna.primary\\_assembly.fa.gz](http://ftp.ensembl.org/pub/release-98/fasta/homo_sapiens/dna/Homo_sapiens.GRCh38.dna.primary_assembly.fa.gz)) and human gene annotation from GENCODE ([http://ftp.ebi.ac.uk/pub/databases/genocode/Gencode\\_human/release\\_32/genocode.v32.primary\\_assembly.annotation.gtf.gz](http://ftp.ebi.ac.uk/pub/databases/genocode/Gencode_human/release_32/genocode.v32.primary_assembly.annotation.gtf.gz)); information on the mouse genome sequence is from Ensembl ([http://ftp.ensembl.org/pub/release-98/fasta/mus\\_musculus/dna/Mus\\_musculus.GRCh38.dna.primary\\_assembly.fa.gz](http://ftp.ensembl.org/pub/release-98/fasta/mus_musculus/dna/Mus_musculus.GRCh38.dna.primary_assembly.fa.gz)); the Allen Brain Institute generated human adult snRNA-seq data from the primary motor cortex (<https://portal.brain-map.org/atlas-and-data/rnaseq>; accessed May 2022); the Human Neural Organoid Cell Atlas (<https://datasets.cellxgene.cziscience.com/83b34db4-0319-44ee-b81d-815e3005be30.h5ad>; accessed March 2026); Wang et al. transplanted organoid snRNA (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE185472>; accessed February 2026). The following public datasets were used for mouse whole-brain snRNA-seq analysis: mouse genome sequence information from Ensembl ([https://ftp.ensembl.org/pub/release-110/fasta/mus\\_musculus/dna/Mus\\_musculus.GRCh39.dna.primary\\_assembly.fa.gz](https://ftp.ensembl.org/pub/release-110/fasta/mus_musculus/dna/Mus_musculus.GRCh39.dna.primary_assembly.fa.gz)); mouse brain scRNA-seq atlas from Zeisel et al. 2018 ([https://storage.googleapis.com/linnarsson-lab-loom/I5\\_all.loom](https://storage.googleapis.com/linnarsson-lab-loom/I5_all.loom); accessed March 2022). DWI data are available <https://osf.io/5sdx6/overview>, and related code [https://github.com/garikioitz/apallial\\_dmri](https://github.com/garikioitz/apallial_dmri). All other data are available as source datasheets or on zenodo.

## 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

*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*

### Reporting on race, ethnicity, or other socially relevant groupings

*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.*

### Population characteristics

*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*

### Recruitment

*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*

### Ethics oversight

*Identify the organization(s) that approved the study protocol.*

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 sizes were estimated empirically, based on previous studies (Birey et al., Nature 2017; Marton et al., Nature Neuroscience 2019; Pasca et al., Nature Medicine 2019, Khan et al., Nature Medicine 2020, Myura et al., Nature Biotechnology 2020, Revah et al., Nature 2022, Gschwind et al., Neuron 2023).

|                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|-----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | Mice were excluded if they presented morbidity and did not complete the core battery of behavioral tasks.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Replication     | Micrographs depict representative results and were repeated with the following n: Fig. 2n, o (3 mice); Fig. 2p (3 mice); Fig. 3b (3 mice); Fig. 5b (3 mice); Extended Data Fig. 1e (1 mouse/condition); Extended Data Fig. 3b (3 mice); Extended Data Fig. 3c (1 mouse); Extended Data Fig. 3f (3 mice); Extended Data Fig. 7d (3 sagittal sections/1 mouse, presence of subcortical fiber tracts replicated in coronal sections from 3 mice); Extended Data Fig. 7e,f (3 mice); Extended Data Fig. 7j (5 mice); Extended Data Fig. 7m,n (3 mice); Extended Data Fig. 7o (1 mouse); Extended Data Fig. 7p,q (3 mice); Extended Data Fig. 10a (4 mice); Extended Data Fig. 10b (2 mice); Extended Data Fig. 10c (4 mice); Extended Data Fig. 10d (2 mice). |
| Randomization   | Randomization was used in the stereological analysis of soma size. Images were acquired from an equally distributed grid throughout the tissue for downstream analysis. Randomization of subjects was performed to ensure both cell-lines were represented. Most analyses used the entire population, so randomization was not required.                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Blinding        | Data were collected and analyzed by investigators blinded to the group identity or automatically analyzed using Motion Sequencing.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |

## 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 type="checkbox"/>            | <input checked="" type="checkbox"/> MRI-based neuroimaging |

## Antibodies

### Antibodies used

Primary antibodies:

1. ChAT (AB144P, Millipore)
2. CTIP2 (ab123449, abcam)
3. GAD65/67 (AB1511, Chemicon)
4. GFAP (Z0334, DAKO)
5. GFP (A21311, Invitrogen)
6. HIF-1 $\alpha$  (NB100-479SS, Novus Biologicals)
7. HNA (ab191181, abcam)
8. HNA (235-1R, NeoBiotechnologies)
9. IBA1 (ab5076, Abcam)
10. NeuN (ab104225, Abcam)
11. NeuN (mab377, Millipore Sigma)
12. Netrin-G1 (sc-271774, Santa Cruz)
13. SATB2 (ab207040, abcam)
14. STEM121 (Y40410, Takara)
15. vGlut1 (guinea pig; from Jessel lab CU1328)

#### Secondary antibodies:

1. Donkey anti-mouse 405 (715-475-150; Jackson ImmunoResearch)
2. Donkey anti-rabbit 488 (A-21206; Invitrogen)
3. Donkey anti-rabbit 568 (A10042; Invitrogen)
4. Donkey anti-mouse 647 (A32787; Invitrogen)
5. Donkey anti-goat Plus 647 (A32849; Invitrogen)
6. Donkey anti-rabbit Plus 647 (A32795; Invitrogen)
7. Donkey anti-goat Cy3 (705-165-003 or 705-165-147; Jackson ImmunoResearch)
8. Donkey anti-guinea pig 405 (706-475-148; Jackson ImmunoResearch)
9. Donkey anti-rabbit 405 (711-475-152; Jackson ImmunoResearch)
10. Donkey anti-chicken 488 (703-545-155; Jackson ImmunoResearch)

### Validation

All antibodies have been validated and previously used for immunofluorescence in mouse or human. The strategies for validation and the number of studies using this antibody are from the company's website. Specific citations refer to relevant papers using the antibody.

#### Primary Antibodies

1. ChAT (AB144P; Millipore); <https://www.sigmaaldrich.com/US/en/product/mm/ab144p>

2. CTIP2 (ab123449; Abcam); <https://www.abcam.com/en-us/products/primary-antibodies/ctip2-antibody-25b6-ab123449>
3. GAD65/67 (AB1511; Chemicon); <https://www.sigmaaldrich.com/US/en/product/mm/ab1511>
4. GFAP (Z0334; DAKO); [https://www.agilent.com/store/en\\_US/Prod-Z033429-2/Z0334](https://www.agilent.com/store/en_US/Prod-Z033429-2/Z0334)
5. GFP (A21311; Invitrogen); <https://www.thermofisher.com/antibody/product/GFP-Antibody-Polyclonal/A-21311>
6. HIF-1 $\alpha$  (NB100-479SS; Novus Biologicals); [https://www.novusbio.com/products/hif-1-alpha-antibody\\_nb100-479ss](https://www.novusbio.com/products/hif-1-alpha-antibody_nb100-479ss)
7. HNA (ab191181; Abcam); <https://www.abcam.com/en-us/products/primary-antibodies/human-nuclear-antigen-antibody-235-1-ab191181>
8. HNA (235-1R; NeoBiotechnologies); <https://neobiotechnologies.com/product/human-nuclei-235-1r/>
9. IBA1 (ab5076; Abcam); <https://www.abcam.com/en-us/products/primary-antibodies/iba1-antibody-ab5076>
10. NeuN (ab104225; Abcam); <https://www.abcam.com/en-us/products/primary-antibodies/neun-antibody-1b7-ab104225>
11. NeuN (mab377; Millipore Sigma); <https://www.sigmaaldrich.com/US/en/product/mm/mab377>
12. Netrin-G1 (sc-271774; Santa Cruz); <https://www.scbt.com/p/netrin-g1-antibody-a-8>
13. SATB2 (ab207040; Abcam); <https://www.abcam.com/en-us/products/primary-antibodies/satb2-antibody-epr20038-ab207040>
14. STEM121 (Y40410; Takara); [https://www.takarabio.com/products/antibodies-and-elisa/primary-antibodies-and-elisas-by-research-area/stem-cell-research-antibodies/stem-antibodies?](https://www.takarabio.com/products/antibodies-and-elisa/primary-antibodies-and-elisas-by-research-area/stem-cell-research-antibodies/stem-antibodies?srsltid=AfmBOopqOE5ZBSLBKPh2kt_voLpWiWK4qvx09PHCywhmVRImu9RMBbN)
15. vGluT1 (guinea pig; from Jessel lab CU1328)

#### Secondary Antibodies

1. Donkey anti-mouse 405 (715-475-150; Jackson ImmunoResearch); <https://www.jacksonimmuno.com/catalog/products/715-475-150>
2. Donkey anti-rabbit 488 (A-21206; Invitrogen); <https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21206>
3. Donkey anti-rabbit 568 (A10042; Invitrogen); <https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-10042>
4. Donkey anti-mouse 647 (A32787; Invitrogen); <https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32787>
5. Donkey anti-goat Plus 647 (A32849; Invitrogen); <https://www.thermofisher.com/antibody/product/Donkey-anti-Goat-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32849>
6. Donkey anti-rabbit Plus 647 (A32795; Invitrogen); <https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32795>
7. Donkey anti-goat Cy3 (705-165-003 or 705-165-147; Jackson ImmunoResearch); <https://www.jacksonimmuno.com/catalog/products/705-165-003>
8. Donkey anti-guinea pig 405 (706-475-148; Jackson ImmunoResearch); <https://www.jacksonimmuno.com/catalog/products/706-475-148>
9. Donkey anti-rabbit 405 (711-475-152; Jackson ImmunoResearch); <https://www.jacksonimmuno.com/catalog/products/711-475-152>
10. Donkey anti-chicken 488 (703-545-155; Jackson ImmunoResearch); <https://www.jacksonimmuno.com/catalog/products/703-545-155>

## Eukaryotic cell lines

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

|                                                                      |                                                                                                                                                           |
|----------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cell line source(s)                                                  | 1208-2 (UCLA IRB#13-000296); 8119-1 (UCLA IRB#13-000296); 0524-1 (Stanford IRB-27795).                                                                    |
| Authentication                                                       | Genomic integrity was assessed by SNP microarray "GSAMD-24v2--0" and pluripotency was assessed by immunocytochemistry (Khan et al., Nature Medicine 2020) |
| Mycoplasma contamination                                             | All cell lines tested negative for Mycoplasma contamination.                                                                                              |
| Commonly misidentified lines<br>(See <a href="#">ICLAC</a> register) | No commonly misidentified cell lines were used in this study.                                                                                             |

## 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 | This study utilized both male and female Esco2fl/fl (JAX, B6N.129S-Esco2tm1.1Ge/J), Emx1-cre (JAX, B6.129S2-Emx1tm1(cre)Krl/J), C57BL/6J (JAX, B6.Cg-Prkdcscid/SzJ), and Swiss Webster (Charles River, Crl:CFW(SW), active dams custom ordered) mice. Mice were group housed with littermates and kept on a 12:12 light:dark cycle and at a temperature of 20-23C and 40-60% humidity.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Wild animals       | This study did not involve wild animals.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Reporting on sex   | Data were collected from both sexes. For unsupervised segmentation of spontaneous behavior with Motion Sequencing, males (n=43) and females (n=44) were modeled separately. Group x sex interactions were tested for all the directed behavioral tasks in Fig. 4 and Extended Data Fig. 9. We did not find any significant interactions, so data from male and female mice were pooled. These studies were not powered to detect modest effects of sex, but 3 out of 48 directed behavioral results (Catwalk Swing Duration, Y-maze distance, AC vertical time) revealed a main effect of sex and details are presented in Table S5. Sex x genotype effects were not estimable in the hypoxic injury experiments because the apallial group contained only female mice. Sex was therefore assessed in the two groups where it could be: a genotype x sex ANOVA restricted to control and XCX mice revealed no main effect of sex and no |

genotype  $\times$  sex interaction for any of the CatWalk measures in Fig. 5k–n and Extended Data Fig. 10f–i. Sexes were pooled for the analyses shown.

Field-collected samples This study did not involve samples collected from the field.

Ethics oversight For datasets generated by the authors: Prior to starting experiments, we sought approval from the Stanford Administrative Panel on Laboratory Animal Care (APLAC). Beyond this conventional review procedure, we requested higher-level consultation with bioethicists at Stanford who provided an extensive overview of implications. Soon after, we consulted with the Stanford Neuroscience Institute's Executive Committee that included leading neuroscientists. As we initiated these experiments and established the apallial model in the laboratory, we also confidentially sought feedback from several neuroscientists around the world. In parallel, Stanford University School of Medicine convened an external committee composed of neuroscientists, legal scholars, ethicists, and patient advocates to develop a framework for oversight of these studies. We presented this work to the committee on multiple occasions, and their feedback substantially informed our approach.

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

## Plants

Seed stocks 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.

Novel plant genotypes 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.

Authentication 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.

## Magnetic resonance imaging

### Experimental design

Design type Diffusion-weighted imaging (DWI), and susceptibility weighted imaging (SWI).

Design specifications Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

### Acquisition

Imaging type(s) Structural, 2D SWI

Field strength 7T

Sequence & imaging parameters 2D T2w Turbo RARE sequence was used to obtain transverse structural MRI for volume measurements (TR/TE = 4500/41 ms, FOV = 18 x 18 mm<sup>2</sup>, matrix = 258 x 258 yielding 70 x 70  $\mu$ m<sup>2</sup> in-plane resolution, slice thickness = 0.5 mm, RARE Factor = 8, NEX = 2, scan time = 4 min 3 sec). For some animals, 3D RAREvfl sequence was used (TR/TE = 1800/90.26 ms, FOV = 15 x 15 x 8 mm<sup>3</sup>, matrix = 150 x 150 x 80 yielding 0.100 mm isotropic resolution, RARE Factor = 43, NEX = 1.6, scan time = 10 min 33 sec with Compressed Sensing) and data are reconstructed with Smart Noise Reduction option. For high resolution anatomical T2W images for DWI co-registration, two RARE (Rapid Acquisition with Relaxation Enhancement) sequences were acquired per animal: (i) a low-resolution scan (200 x 200 x 500  $\mu$ m<sup>3</sup>) for co-registration reference, and (ii) a high-resolution scan (100 x 100 x 300  $\mu$ m<sup>3</sup>; RARE factor = 4, TR/TE = 4000/30 ms, 2 averages, flip angle = 180°) used as the primary anatomical reference for visualization and registration. 2D SWI data were acquired (TR/TE = 858.8/18 ms, FOV = 15 x 15 mm<sup>2</sup>, matrix = 280 x 280 yielding 54 x 54  $\mu$ m<sup>2</sup> in-plane resolution, slice thickness = 0.5 mm, NEX = 6, scan time 21 min 54 sec).

Area of acquisition Whole brain

Diffusion MRI ☒ Used ☐ Not used

Parameters Diffusion-weighted imaging (DWI) data were acquired with a 4-segment diffusion-weighted EPI sequence (Bruker: DtiEpi) using the following parameters: b = 1000 s/mm<sup>2</sup>, 40 non-collinear diffusion-encoding directions plus 5 b = 0 images (45 volumes total), diffusion timing  $\delta/\Delta$  = 4.5/10.3 ms, TR/TE = 3500/21 ms, in-plane resolution 150 x 150  $\mu$ m<sup>2</sup>, slice thickness 500  $\mu$ m, FOV = 16 x 15 mm<sup>2</sup>, matrix = 107 x 100, bandwidth = 250 kHz, second-order B<sub>0</sub> map shimming, three FOV saturation bands. The B<sub>0</sub> map was obtained prior to the sequence and first- and second-order shims were applied using mapshim. NiFTi formatted data were converted

from Bruker ParaVision format using dcm2niix.

## Preprocessing

|                            |                                                                                                                                                                                                                                         |
|----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Preprocessing software     | MRTrix3                                                                                                                                                                                                                                 |
| Normalization              | If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization. |
| Normalization template     | Allen Atlas for the control group                                                                                                                                                                                                       |
| Noise and artifact removal | Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).                                                                 |
| Volume censoring           | Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.                                                                                                                           |

## Statistical modeling & inference

|                                           |                                                                                                                                                                                                                  |
|-------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Model type and settings                   | Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation). |
| Effect(s) tested                          | Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.                                                   |
| Specify type of analysis:                 | <input type="checkbox"/> Whole brain <input type="checkbox"/> ROI-based <input checked="" type="checkbox"/> Both                                                                                                 |
| Anatomical location(s)                    | Describe how anatomical locations were determined (e.g. specify whether automated labeling algorithms or probabilistic atlases were used).                                                                       |
| Statistic type for inference              | Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.                                                                                                                  |
| (See <a href="#">Eklund et al. 2016</a> ) |                                                                                                                                                                                                                  |
| Correction                                | Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).                                                                                     |

## Models & analysis

|                                     |                                                                       |
|-------------------------------------|-----------------------------------------------------------------------|
| n/a                                 | Involved in the study                                                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Functional and/or effective connectivity     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Graph analysis                               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Multivariate modeling or predictive analysis |