nature portfolio

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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	WGS and MPAS/snMPAS were performed on Illumina instruments using their propriertary platform and DRAGEN platform (WGS only).
Data analysis	Mutect2 (v4.0.4.0 from GATK), MosaicHunter (single-mode, v 1.0), GATK (v 4.0.4), DeepMosaic (v 1.0.1), MosaicForecast (v 8-13-2019), Strelka2 (v 2.9.2), gnomAD (v 2.1.1), FlowJo (Ashland, Oregon), Bismark (v 0.23.1), Snakemake (v 6.12.3), BEDTools (v 2.30.0), Python (v 3.10), R (4.1.3), MEGA (v11.0.13), MUSCLE (v1), Cell Ranger (v6.0.2), Seurat (v4.0.5), fastqc (v0.11.8), cutadapt (v1.16), GRCh38 human genome and gencode (v27) gtf anntotation using STAR (v2.6.0c), SAMtools (v1.7), Picard (v2.20.7), qualimap (v2.2.2-dev), featureCounts (v2.0.0), rsem (v1.3.1), r-pvclust (v2.2.0), ComplexHeatmap (v2.16.0), r-ggplot2 (v3.4.3), maftools (v2.16.0), r-umap (v0.2.10.0), SAMBAMBA (v0.7.0). Scripts for variant filtering are provided on GitHub (https://github.com/shishenyxx/Human_Inhibitory_Neurons).

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

Raw whole genome sequencing and massive parallel amplicon sequencing data (MPAS) and single nucleus MPAS (snMPAS) are available through SRA (accession number: PRJNA799597) and NDA (accession number: study 919) for ID01 and ID05. The 300× WGS panel of normal is available on SRA (accession number: PRJNA660493).
human_g1k_v37 reference: http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/reference/
gnomAD: https://gnomad.broadinstitute.org/
Human Multiple Cortical Areas SMART-seq dataset: https://portal.brain-map.org/atlases-and-data/rnaseq/human-multiple-cortical-areas-smart-seq
Details and codes for the data processing and annotation are provided on GitHub (https://github.com/shishenyxx/Human_Inhibitory_Neurons).

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	We use already expired postmortem organs from donated cadavers, which are not considered as human subjects.
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	ID01 were donated from a 70-year-old female, cause of death was 'global geriatric decline' with a contributing cause of 'post-surgical malabsorption'. ID05 were donated from a 73-year-old female, medical history indicated 'knee replacement, fractured pelvis, hernia, fractured fibula, hypothyroidism, empyema, pulmonary arterial hypertension, and scleroderma'. Both donors were documented to be of European ancestry. Organs were collected within a 26-hour postmortem interval for both donors (ID01: 24 hrs, ID05: 26 hrs). Prior medical history showed no signs of neurological, psychiatric or cancer diseases for either, and tested negative for infection with HIV, Hepatitis B, or COVID-19.
Recruitment	no participants were recruited.
Ethics oversight	According to 45 CFR 46.102(e)(1), The use of human anatomical cadaver specimens of ID01 and ID05 are exempt from oversight of the University of California, San Diego Human Research Protections Program (IRB) but are subject to oversight by the University of California, San Diego Anatomical Materials Review Committee (AMRC). This study was overseen and approved by the AMRC. The approval number is 106135. Donors met AMRC qualifications: (i) Obtain information or biospecimens through intervention or interaction with the individual, and uses, studies, or analyzes the information or biospecimens; or (ii) Obtains, uses, studies, analyzes, or generates identifiable private information or identifiable biospecimens.

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

Life sciences study design

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

Sample size	This is a pioneer descriptive study with limited expectations. The number of individuals included was limited by the availability of biological sample (due to postmortem interval, history of disease, etc). Samples from both hemispheres and all neocortical lobes as well as non-brain organs were included for ID01 and ID05.
Data exclusions	We did not exclude any generated sequencing data, but filtered detected variants as described in the methods.

Replication	This study consists two cadavers ID01 and ID05.
Randomization	This is a descriptive study, and no ramdomization was performed. Cadavers with short PMI are very rare, and it is very difficult to collect enough number of cadavers for randomization. We collected any of cadavers meeting our criteria.
Blinding	No groups were allocated by the scientists.

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	NEUN Alexa Fluor 488 (Clone A60, 1:2,500; Millipore Sigma, MAB377, RRID:AB_2298772), TBR1 (1:1,000; abcam, ab31940, RRID:AB_2200219), DLX1 (1:200; Atlas Antibodies, HPA045884, RRID:AB_10960361), COUPTFII (1:400; Novus Biologicals, PP-H7147-00, RRID:AB_1964214), DARPP32 (1:400; abcam, ab40801, RRID:AB_731843), goat anti-rabbit Alexa 647 (1:4,000; ThermoFisher Scientific, A21244, RRID:AB_2535812) and donkey anti-mouse Alexa 647 (1:4000; ThermoFisher Scientific, A32787, RRID:AB_2762830).
Validation	The validation results of all antibody specificity to formaldehyde-fixed nuclei was published in PMID:35444276 or 24561062. We further validated the performance of DLX1, COUPTFII and TBR1 antibodies under the MFNS condition through single-cell RNAseq or bisulfite sequencing in this study.

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	<Formaldehyde-fixed nuclear preparation for sorting> For DARPP32/NEUN dual staining with BG nuclei, frozen Cau and Put of ID01 were homogenized in 1% formaldehyde in Dulbecco's phosphate-buffered saline (DPBS, Corning) using a motorized homogenizer (Fisherbrand PowerGen 125), then incubated on a rocker at room temperature for 10 min, quenched with 0.125 M glycine at room temperature on a rocker for 5 min, then centrifuged at 1,100×g in a swinging bucket centrifuge. The following steps were all performed on ice except where indicated. Homogenates were washed twice with NF1 buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 5mM MgCl2, 0.1M sucrose, 0.5% Triton X-100 in UltraPure water) and centrifuged at 1,100×g for 5 min at 4°C in a swinging bucket centrifuge. Next, pellets were resuspended in 5 ml NF1 buffer and Dounce homogenized 5x in a 7 ml Wheaton Dounce Tissue Grinder (DWK Life Sciences) using a 'loose' pestle. After 30 minutes of incubation on ice, homogenates were Dounce homogenized 20x with a 'tight' pestle and filtered through a 70 µm strainer. To remove myelin debris, homogenates were overlaid on a sucrose cushion (1.2M sucrose, 1 M Tris-HCl pH 8.0, 1 mM MgCl2, 0.1 M DTT) and centrifuged at 3,200×g for 30 min with acceleration and brakes on 'low'. Pellets of nuclei were washed with NF1 buffer and centrifuged at 1,600×g for 5 min and stored at -80°C, same as documented. <Nuclear preparation for MFNS or unfixed nuclei sorting> For the MFNS protocol, approximately 200 mg of freshly frozen tissue stored at -80°C was prepared, and subsequent

procedures were conducted using solutions maintained at 4°C. The prepared tissue was homogenized in 300 µl of lysis buffer (composed of 10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.1% NP-40, and 1 mM Dithiothreitol in nuclease-free water) while kept on ice. Next, additional 9.7 ml of lysis buffer was added to the homogenate, and the mixture was incubated on ice for 5 minutes. The homogenate was then passed through a 70 µm cell strainer (FALCON, 352350) and centrifuged at 1100g for 5 minutes at 4°C. The supernatant was discarded, and the remaining pellet was gently resuspended and washed with 10 ml of sorting buffer (containing 1% bovine serum albumin, 1 mM EDTA, and 10 mM HEPES in 1x HBSS solution). For the density gradient centrifugation step, the resuspended pellet in 25% Iodixanol solution (OptiPrep™, Millipore D1556) was layered onto a 29% Iodixanol cushion. The centrifugation was carried out at 10,000g with a swinging bucket rotor, employing low acceleration and braking, at 4°C for 40 min, the pellet resuspended in 80% methanol prechilled and stored at -20°C for at least 30 minutes before further use. For samples to perform single-nucleus transcriptome and PTA with ResolveOME, brain tissue from participant ID05 were homogenized in 1 ml of ice-cold NIB (composed of 0.25 M sucrose, 25 mM KCl, 5 mM MgCl2, 10 mM Tris pH 7.5, 100 mM DTT, and 0.1% Triton X-100) and subjected to homogenization, incubated on a rocker for 5 min at 4 °C and then centrifuged at 1,000g employing low acceleration and braking in a swinging-bucket centrifuge, and the pellet was reconstituted in 0.5 ml of sorting buffer and filtered through a 70-um strainer. Nuclei in the flow-through were immediately subjected to staining.

For pellets of defrosted and homogenized brain nuclei were washed twice in sorting buffer and then re-suspended in 0.2 ml sorting buffer and incubated overnight at 4°C. The following antibodies were used: NEUN Alexa Fluor 488 (1:2,500; Millipore Sigma, MAB377), TBR1 unconjugated (1:1,000; Abcam, ab31940), DLX1 (1:200; Atlas Antibodies, HPA045884), COUPTFII (1:400; Novus Biologicals, PP-H7147-00), and DARPP32 (1:400; Abcam, ab40801). The following day, nuclei were washed with staining buffer and in case an unconjugated antibody was used, nuclei were stained subsequently for 30 minutes with goat anti-rabbit Alexa 647 (1:4,000; ThermoFisher Scientific, A21244) for TBR1, DLX1 and DARPP32, or donkey anti-mouse Alexa 647 (1:4000; ThermoFisher Scientific, A32787) for COUPTFII. Stained nuclei were washed one more time with staining buffer and passed through a 70 µm strainer. Immediately before the sort, nuclei were stained with 0.5 µg/ml DAPI. For single-nucleus transcriptome and whole-genome amplification with ResolveOME, nuclei were stained with Propidium Iodide (1:20; Invitrogen, BMS500PI). Nuclei for the cell type of origin were sorted either on a MoFlo Astrio EQ sorter (Beckman Coulter), BD FACSAria II (Becton-Dickinson), or BD InFlux Cytometer (Becton-Dickinson) similar to previous documentation17. At least 1000 methanol-fixed or >50,000 formaldehyde-fixed sorted nuclei were pooled in each tube. Sorted nuclei were pelleted in staining buffer at 1,600×g for 10 minutes. Nuclei for DNA extraction and bisulfite sequencing were stored at -80℃. FANS data was visualized using FlowJo v10 software (Ashland, Oregon). Following MPAS (see below) sorted populations were deemed to be of sufficient overall quality if at least 95% variants were sequenced above >1,000×.

Instrument

Nuclei for the cell type of origin were sorted either on a MoFlo Astrio EQ sorter (Beckman Coulter), BD FACSAria II (Becton-Dickinson), or BD InFlux Cytometer (Becton-Dickinson) similar to previous documentation (PMID 35444276).

Software

FANS data was visualized using FlowJo v10 software (Ashland, Oregon).

Cell population abundance

Single-cell RNAseq or bisulfite sequencing was performed to validate cell type identity.

Gating strategy

For the population level sorting, we selected of DAPI positive nuclei, gating on singlets. Excitatory and inhibitory neurons were sorted by using TBR1 and DLX1 or COUPTFII antibodies. For single nuclei sorting, doublets were excluded and nuclei positive for PI were further gated into NeuN+ populations.

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