nature portfolio

Corresponding author(s): Tianlin Cheng, Zilong Qiu

Last updated by author(s): Oct 4, 2023

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	Quantitative real-time PCR data were collected by StepOnePlus RealTime PCR System (Applied Biosystems; No.4376600); Western blotting data were captured by Chemiluminescence Apparatus (AI600, GE); Double-positive mouse cortical neuronal cells were screened and collected through Fusion Flow Cytometer (FACSAria Fusion) and Moflo XDP Flow Cytometer (Beckman Coulter) for in vitro base editing efficiency identification; Laser-dissected cortical tissues were collected from mouse prefrontal cortex by utilizing CellCut Plus instrument (Molecular-Machines & Industries) for in vivo base editing efficiency identification; Confocal images were captured by Olympus VS120 high-throughput fluorescence microscope; Nikon NiE-A1 plus upright confocal fluorescence microscope; Nikon TiE-A1 plus inverted confocal fluorescence microscope; Da Hua high-definition video camera for video recording of behavioral performance. We quantified sanger sequencing data by calculating in EditR analysis (https://moriaritylab.shinyapps.io/editr_v10/; v.1.0.10).
Data analysis	All confocal images were analysed by Image J (v.1.51n); The behavioral performance (open field test, elevated plus maze, novel object recognition, three-chamber test, Barnes maze) were analyzed via Ethovision XT software (Noldus, v.11.5, Wageningen, Netherlands, RRID:SCR_000441) and the other behavioral data (self-grooming and scratching, social intruder test) were analyzed blinded to test mouse genotype by investigators based on the video recording. All statistical analysis were conducted by using GraphPad Prism (version: v.6.01) and OriginPro (version: v.2019b).

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

The datasets used and/or analyzed during the current study are available from the lead contact on reasonable request.

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	Sample sizes were not predetermined. Sample size is indicated in the figure legend for each experiment. Experiments were performed at least three times independently. Individual test animals were handled for several days in advance. The sample size was determined based on previous experience and reports for each experiment to yield high power to detect specific effects.
Data exclusions	We obeyed followed criterions for data exclusions.For samples that follow a normal distribution, if an individual data point does not fall within the 95% confidence interval of the sample, it will be excluded. Additionally, prior to conducting animal experiments, such as behavioral test, the health status of the experimental animals is evaluated. If a mouse exhibits health conditions such as extensive hair loss or severe wounds, it will be excluded from the experiment before it begins. To be mentioned, we performed outlier significance detection by using the Grubb's test and found a significant outlier value in the novel object recognition experiment of male Mef2c L35P heterozygous mice. Therefore, we made a reasonable decision to exclude that data point.
Replication	All experiments were reliably reproduced in independent biological samples (quantitative real-time PCR, western blotting), independent cells from independent mice (neuronal morphology, sparse labelling) or mice (behavior, immunofluorescence, immunohistochemistry, western blotting). All numbers of replicates (n) are indicated in the figure legends.
Randomization	Animals were chosen according to correct genotypes. Specifically, animal pairs from the same litters were compared to decrease variance in age and rearing conditions. Each experiment contained animals from at least two different litters to ensure that the differences between genotypes can be observed in mice from different litters.
Blinding	During the conduction and analyses of sparse labelling and neuronal morphological analysis, the investigators were blind to the genotypes of the individual animals. For in vivo immunohistochemistry and behavioral experiments, the investigator was blind to the experimental groups during the analysis. To be mentioned, the investigators were totally blind to the group allocation during data collection and analysis.

Behavioural & social sciences study design

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

Study description	*Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).*
Research sample	*State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.*
Sampling strategy	*Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*
Data collection	*Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*
Timing	*Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample*

Timing	*cohort.*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Non-participation	*State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*
Randomization	*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

Ecological, evolutionary & environmental sciences study design

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

Study description	*Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.*
Research sample	*Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.*
Sampling strategy	*Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Reproducibility	*Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.*
Randomization	*Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.*
Blinding	*Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Did the study involve field work? ☐ Yes ☒ No

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
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	The primary antibodies used in this study and dilutions were listed as followed : anti-MEF2A+MEF2C (1:1000; No.ab64644; host species: rabbit; Abcam, Cambridge, UK), and to be mentioned, ab64644 has been discontinued, ab197070 was suggested to be as a possible replacement (1:1000; No.197070; host species: rabbit; Abcam, Cambridge,

UK);
anti-FLAG (1:1000; No.M20008L; host species: mouse; Abmart, Shanghai, China);
anti-GAPDH (glyceraldehyde-3-phosphate dehydrogenase) (1:10000; No.ab8245; host species: mouse; Abcam, Cambridge, UK);
anti-GFP (green fluorescent protein) (1:1500; No.E022030; host species: rabbit; Earth Life Sciences Inc., California, USA);
anti-MAP2 (1:1000; MAB3418, host species: mouse; Millipore, Massachusetts, USA);
anti-PV (parvalbumin) (1:1000; No.MAB1572, host species: mouse; Millipore, Massachusetts, USA);
anti-SST (Somatostatin) (1:500; No.SC74556, host species: mouse; Santa Cruz Biotechnology, Santa Cruz de la Sierra, Spain);
anti-SpCas9 (1:500; NBP2-36440, host species: mouse; Novus Biologicals, Littleton, Colorado, USA);
anti-SpCas9 (1:500; No.ab204448; host species: rabbit; Abcam, Cambridge, UK);
anti-RFP (1:800; 600-401-379, host species: rabbit; Rockland Immunochemicals, Philadelphia, Pennsylvania, USA);
anti-NeuN (1:1500; ABN91, host species: chicken; Millipore, Massachusetts, USA)
anti-DAPI (4',6-diamidino-2-phenylindole) (1:1000; No. 28718-90-3, Sigma-Aldrich, Missouri, USA).
Donkey anti-rabbit IgG Alexa 488 (1:1000; A32790; Thermo Fisher Scientific, Massachusetts, USA);
Donkey anti-rabbit IgG Alexa 555 (1:1000; A32794; Thermo Fisher Scientific, Massachusetts, USA);
Donkey anti-mouse IgG Alexa 488 (1:1000; A32766; Thermo Fisher Scientific, Massachusetts, USA);
Donkey anti-mouse IgG Alexa 555 (1:1000; A32773; Thermo Fisher Scientific, Massachusetts, USA);
Donkey anti-mouse IgG Alexa 647 (1:1000; A32787; Thermo Fisher Scientific, Massachusetts, USA);
Donkey anti-chicken Alexa Fluor 488 (1:1000; No.20166; Biotium, California, USA);
Donkey anti-rabbit HRP-conjugated (1:2000; NA934; GE Healthcare, Shanghai, China);
Donkey anti-mouse HRP-conjugated (1:2000; NA931; GE Healthcare, Shanghai, China);

Validation

The primary antibodies used in this study and dilutions were listed as followed :
anti-MEF2A+MEF2C (1:1000; No.ab64644; host species: rabbit; Abcam, Cambridge, UK), and to be mentioned, ab64644 has been discontinued, ab197070 was suggested to as a possible replacement (1:1000; No.197070; host species: rabbit; Abcam, Cambridge, UK)—available for Flow Cyt (Intra), WB, IHC-P, ICC/IF; Representative reference: Harrington AJ et. al., MEF2C regulates cortical inhibitory and excitatory synapses and behaviors relevant to neurodevelopmental disorders. Elife (2016). PMID: 27779093;
anti-FLAG (1:1000; No.M20008L; host species: mouse; Abmart, Shanghai, China)—available for WB, IHC, IF, IP, ELISA; Representative reference: Cao Xu et. al., Degradation of MONOCULM 1 by APC/C(TAD1) regulates rice tillering. Nature Communication. 2012 Mar 20;3:750. PMID: 22434193;
anti-GAPDH (glyceraldehyde-3-phosphate dehydrogenase) (1:10000; No.ab8245; host species: mouse; Abcam, Cambridge, UK)—available for WB, ICC/IF; Representative reference: Franco A et. al., Correcting mitochondrial fusion by manipulating mitofusin conformations. Nature 540:74-79 (2016). PMID: 27775718;
anti-GFP (green fluorescent protein) (1:1500; No.E022030; host species: rabbit; Earth Life Sciences Inc., California, USA)—available for IHC/IF, WB,IP;
anti-MAP2 (1:1000; MAB3418, host species: mouse; Millipore, Massachusetts, USA)—available for IHC, WB; Representative reference: Bershteyn, M et. al., Cell-autonomous correction of ring chromosomes in human induced pluripotent stem cells. Nature 507: 99-103 (2014). PMID: 24413397;
anti-PV (parvalbumin) (1:1000; No.MAB1572, host species: mouse; Millipore, Massachusetts, USA)—available for ICC, IH(P), WB; Representative reference: Cooke, SF et. al., Visual recognition memory, manifested as long-term habituation, requires synaptic plasticity in V1. Nature neuroscience 18: 262-71 (2015). PMID: 25599221;
anti-SST (Somatostatin) (1:500; No.SC74556, host species: mouse; Santa Cruz Biotechnology, Santa Cruz de la Sierra, Spain)—available for WB,IP, IHC/IF, Elisa; Representative reference: Saxena, P., et. al., A programmable synthetic lineage-control network that differentiates human IPSCs into glucose-sensitive Insulin-secreting b-like cells. Nature communication 7: 11247 (2016). PMID: 27063289;
anti-SpCas9 (1:500; NBP2-36440, host species: mouse; Novus Biologicals, Littleton, Colorado, USA)—available for WB, IB, ICC/IF, IHC, IHC-Fr, IP, IHC-WhMt, KO; Representative reference: Aaron M Savage, et. al., tmem33 is essential for VEGF-mediated endothelial calcium oscillations and angiogenesis. Nature communication 10 (1):732 (2019). PMID: 30760708;
anti-SpCas9 (1:500; No.ab204448; host species: rabbit; Abcam, Cambridge, UK)—available for ICC/IF,IP,Flow Cyt,WB; Representative reference: Chaverra-Rodriguez D et al. Targeted delivery of CRISPR-Cas9 ribonucleoprotein into arthropod ovaries for heritable germline gene editing. Nat Commun 9:3008 (2018). PMID: 30068905;
anti-NeuN (1:1500; ABN91, host species: chicken; Millipore, Massachusetts, USA)—available for WB, IHC, IP, ICC;
anti-RFP (1:800; 600-401-379, host species: rabbit; Rockland Immunochemicals, Philadelphia, Pennsylvania, USA)—available for FC; IF; IHC; Representative reference: Maria Andres-Alonso, et. al., SIPA1L2 controls trafficking and local signaling of TrkB-containing amphisomes at presynaptic terminals. Nature communication 10 (1):5448 (2019). PMID: 31784514;
anti-DAPI (4',6-diamidino-2-phenylindole) (1:1000; No. 28718-90-3, Sigma-Aldrich, Missouri, USA)—available for IHC/IF; Representative reference: Bejerano-Sagie M. et. al., A checkpoint protein that scans the chromosome for damage at the start of sporulation in Bacillus subtilis. Cell 125: 679-690 (2006). PMID: 16713562.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293 cells (Cell Bank of the Chinese Academy of Sciences (Shanghai, China)).
Authentication	Cells were authenticated by using STR analysis.
Mycoplasma contamination	The cell line tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were applied in this study.

Palaeontology and Archaeology

Specimen provenance	*Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the*

Specimen provenance	*issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.*
Specimen deposition	*Indicate where the specimens have been deposited to permit free access by other researchers.*
Dating methods	*If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

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

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Experimental mice included both males and females, between the ages of 1-4 months.Mef2c L35P knock-in mouse was designed and constructed by CRISPR/Cas9 mediated homologous recombination strategy on the genetic background of C57BL/6 mouse by Biocytogen Co.,Ltd. All mice were bred onto a C57BL/6J background. All experimental mice were bred in a pathogen freepathogen-free (PF) unit under constant temperature (approximately 22 degree centigrade), humidity (approximately 55%RH), ventilation and automatic circadian rhythm on the condition of a 12h light/dark cycle (light from 7 a.m. to 7 p.m., dark from 7pm to 7 am) with food and water provided ad libitum.
Wild animals	No wild animals were used in this study.
Field-collected samples	We collected no field samples in this study.
Ethics oversight	All procedures were approved by the Animal Care and Use Committee of the Center for Excellence in Brain Science & Intelligence Technology, Chinese Academy of Sciences, Shanghai, China. The use and care of animals were in accordance with the guidelines of this committee.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	We collected and sequenced blood genomes acquired from one aged two years and half boy with ASD related phenotypes as well as his parents with Xinhua hospital affiliated to Shanghai Jiaotong University School of Medicine and the patient clinically diagnosed with Rett-like syndrome was based on the definition of Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition. Additionally, on the aspects of bioinformatics analysis of the MEF2C variants discovered and identified in this article, the longest of MEF2C transcript isoform was used as a reference sequence (NM_001193347.1, NP_001180276.1).
Recruitment	The patient mentioned in this study voluntarily sought medical treatment, and through ADOS behavioral analysis, it was determined that he exhibited behaviors characteristic of autism. This behavioral analysis adheres to the latest DSM-V clinical diagnostic manual, and does not involve the patient's subjective judgment, but is the result of objective analysis by professional medical institutions. It should be mentioned that we did not actively recruit volunteers during this process.
Ethics oversight	The genetic information collected from the ASD patient is approved by the Ethic Committee of Xinhua hospital, Shanghai Jiao Tong University School of Medicine (XHEC-C-2019-076).

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☒	☐	Public health
☒	☐	National security
☒	☐	Crops and/or livestock
☒	☐	Ecosystems
☒	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☒	☐	Demonstrate how to render a vaccine ineffective
☒	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐	Increase transmissibility of a pathogen
☒	☐	Alter the host range of a pathogen
☒	☐	Enable evasion of diagnostic/detection modalities
☒	☐	Enable the weaponization of a biological agent or toxin
☒	☐	Any other potentially harmful combination of experiments and agents

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*
Files in database submission	*Provide a list of all files available in the database submission.*
Genome browser session (e.g. UCSC)	*Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

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	Neuronal cells co-transfected with sgRNA-mCherry and AeCBE-EGFP plasmids were digested into a single-cell suspension and collected before acquisition on a flow cytometer at DIV4. Electroporation was conducted in primary cortical neurons in vitro and the neurons were electroporated with 3 μg of each plasmid mediated by specialized electroporation solution mixed of solution I, made of C10H14N5Na2O13P3 · H2O (A2383, sigma) and MgCl2 · 6H2O (M2393, sigma), and solution II, made of KH2PO4 (p5655, sigma), NaHCO3 (S5761, sigma) and glucose (G6152, sigma), at a proportion of 1:50.
Instrument	Fusion Flow Cytometer (FACSAria Fusion) and Moflo XDP Flow Cytometer (Beckman Coulter).
Software	Summit (version 5.2.0) (Dako Cytomation)
Cell population abundance	Positive cells abundance was dependent on FACS screening condition and EGFP/mCherry double positive cells were at the proportion of approximately 2-3%.
Gating strategy	Negative control (cells with neither EGFP or mCherry fluorescent proteins) were used to establish gates. Gates were applied to collect double positive cells expressing both EGFP and mCherry for subsequent genome DNA extraction, PCR amplification of target sequence and sanger sequencing analysis.

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

Magnetic resonance imaging

Experimental design

Design type	*Indicate task or resting state; event-related or block design.*
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)	*Specify: functional, structural, diffusion, perfusion.*
Field strength	*Specify in Tesla*
Sequence & imaging parameters	*Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*
Area of acquisition	*State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	*Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*
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	*Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*

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: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference
(See Eklund et al. 2016)

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

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
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.