

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](#).

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

☐	☒	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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒	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 <i>Give P values as exact values whenever suitable.</i>
☒	☐	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.

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

Zeiss LSM 710
ZEN 2010
BD LSR II flow cytometer and FACSDiva
API 4000 tandem mass spectrometer
7900HT Real Time PCR System
Axion BioSystems Maestro Pro
Illumina NextSeq 550
NovaSeq 6000

FLOWJO software
ImageJ
Analyst software (v1.6.2)
Sequence Detection System software (v2.4)
Matlab R2012a
Axion BioSystems Neural Metrics and Plotting Tool software
GraphPad Prism 9
10x Genomics Cell Ranger
Seurat (version 4.0.2) toolkit in R (version 4.0.1)
DoubletFinder (version 2.0.3)

STAR aligner
FeatureCounts (Subread v1.6.3)
DESeq2 in R (v4.3.2)

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

Data

Policy information about [availability of data](#)

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

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

Single nuclei RNA sequencing data in this study have been deposited in Gene Expression Omnibus under accession code GSE313106.

Bulk RNA sequencing data in this study have been deposited in EBI biostudies <https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16232>.

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

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

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

Data exclusions

Replication

Randomization

Blinding

Behavioural & social sciences study design

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

Study description	Not applicable
Research sample	Not applicable
Sampling strategy	Not applicable
Data collection	Not applicable
Timing	Not applicable
Data exclusions	Not applicable
Non-participation	Not applicable
Randomization	Not applicable

Ecological, evolutionary & environmental sciences study design

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

Study description	Not applicable
Research sample	Not applicable
Sampling strategy	Not applicable
Data collection	Not applicable
Timing and spatial scale	Not applicable
Data exclusions	Not applicable
Reproducibility	Not applicable
Randomization	Not applicable
Blinding	Not applicable

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
☒	☐ 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	anti-ACTN2, Sigma, A7811 anti-BRN3A, Merck, MAB1585
-----------------	--

anti-CTNT, Abcam, ab45392
 anti-CD45, Abcam, Ab8216
 anti-CD68, Synaptic Systems, HS-460-008
 anti-DBH, Sigma, HPA070789
 anti-HCN4, Abcam, ab69054
 anti-Neurexin 1, Sigma, HPA071400
 anti-NF (H), Synaptic Systems, 171 106
 anti-NF (H), Biolegend, 822601
 anti-PAX6, Biolegend, 901301
 anti-PDGFRb, Cell Signalling, 28E1
 anti-PECAM1, DAKO, M0823
 anti-Peripherin, NOVUS, NBP1-05423
 anti-Phalloidin, Invitrogen, A12379
 anti-PHOX2B, Santa Cruz, sc-376997
 anti-Synapsin 1, Synaptic Systems, 106 011
 anti-TH, Synaptic Systems, 213 004
 anti-VACHT, Synaptic Systems, 139 103
 anti-VGLUT1, NanoTag, N1602-Abb635P
 anti-chicken Alexa Fluor 488, Invitrogen, A10039
 anti-rabbit Alexa Fluor 546, Invitrogen, A11035
 anti-mouse Alexa Fluor 546, Invitrogen, A11074
 anti-guinea pig Alexa Fluor 633, Invitrogen, A21052

Validation

All antibodies used were validated by the respective manufacturer. Antibody specificity were assessed with a positive and negative control.

Eukaryotic cell lines

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

Cell line source(s)

Human induced pluripotent stem cell lines:
 RUCDRi002-A: Male, obtained from Institute of Pharmacology and Toxicology, University Medical Center Goettingen.
 RUCDRi002-A-71: Male, obtained from Institute of Pharmacology and Toxicology, University Medical Center Goettingen.
 UMGi093-A: Male, obtained from Lukas Cyganek (Stem Cell Unit, University Medical Center Goettingen).
 BIHi001-A-2: Male, obtained from Sebastian Diecke (Max Delbrück Center, Berlin).
 MDCi052_A26: Male, generated by Sebastian Diecke (Max Delbrück Center, Berlin).
 MDCi052_A27: Male, generated by Sebastian Diecke (Max Delbrück Center, Berlin).
 MDCi052_A30: Male, generated by Sebastian Diecke (Max Delbrück Center, Berlin).
 MDCi052_A31: Male, generated by Sebastian Diecke (Max Delbrück Center, Berlin).

Authentication

Cell lines that are well-established:
 RUCDRi002-A: <https://hpscreg.eu/cell-line/RUCDRi002-A>
 RUCDRi002-A-71: <https://www.sciencedirect.com/science/article/pii/S1873506124000151?via%3Dihub>
 UMGi093-A: <https://sfb1002.med.uni-goettingen.de/production/cellmodel/cell-line/view?tab=internal&line=125>
 BIHi001-A-2: https://www.cellosaurus.org/CVCL_CONJ

Cell lines established in the paper:
 MDCi052_A26: Sub-clone of RUCDRi002-A, mutation was validated by PCR and Sanger Sequencing. Pluripotency analysis and 3 germ layer differentiation were performed.
 MDCi052_A27: Sub-clone of RUCDRi002-A, mutation was validated by PCR and Sanger Sequencing. Pluripotency analysis and 3 germ layer differentiation were performed.
 MDCi052_A30: Sub-clone of RUCDRi002-A, mutation was validated by PCR and Sanger Sequencing. Pluripotency analysis and 3 germ layer differentiation were performed.
 MDCi052_A31: Sub-clone of RUCDRi002-A, mutation was validated by PCR and Sanger Sequencing. Pluripotency analysis and 3 germ layer differentiation were performed.

Mycoplasma contamination

All cell lines were routinely checked for mycoplasma contaminations and were tested negative.

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

No misidentified lines have been used in this study.

Plants

Seed stocks	Not applicable
Novel plant genotypes	Not applicable
Authentication	Not applicable

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	SNOs were dissociated into single cells using Collagenase Type I and Accutase dissociation reagent. Digested cells were fixed in 4% formaldehyde at room temperature for 15 min and stained for FACS. iEHMs were lysed with EZ prep nuclei lysis buffer and the pellet was resuspended in Nuclei Suspension buffer. Lysate was strained through 40 uM Flowmi Cell Strainers into FACS tube and stained. Cardiomyocytes were dissociated into single cells using Accutase dissociation reagent. Digested cells were fixed in cold 70% ethanol. Cells were subsequently stained for FACS.
Instrument	Cells were measured with BD LSR II Flow cytometer or BD FACSaria III.
Software	Data were analyzed with FACSDiva and FLOWJO.
Cell population abundance	Abundance of cell population is determined as a % of the parent population gating (gated after FSC/SSC plot and Hoechst-A/SSC-A). Isotype control were used to distinguish between the negative and positive signals.
Gating strategy	Cells were gated by the FSC/SSC plot and further gated by Hoechst-A/SSC-A or 7-AAD/SSC-A signal. Isotype control were used to distinguish between negative and positive signals.
☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.	