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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	Confocal images were acquired on an Olympus FV3000 confocal microscope using Olympus Fluoview software. Flow cytometry data were acquired on a Beckman Coulter Cytoflex flow cytometer using BD Biosciences FACSDiva software. qPCR data were acquired on a Thermo Fisher QuantStudio 5 qPCR machine using QuantStudio Design and Analysis software. Deep sequencing was performed on a NovaSeq 6000 sequencer using NovaSeq Control Software. Electrophysiological signals were amplified and digitized using the Multiclamp 700B amplifier and DigiData 1440 digitizer (Molecular Devices) followed by data acquisition and recording with pCLAMP 10 software.
Data analysis	Confocal image analysis was performed using Imaris and ImageJ software. Flow cytometry data analysis was performed using FlowJo software. qPCR data analysis and statistical tests were performed on Microsoft Excel and GraphPad Prism software. Deep sequencing datasets were analyzed in Python, and further details regarding specific computational analyses of sequencing datasets are provided in the respective sections of the Methods statement. Electrophysiological data were analyzed using ClampFit software (Axon Instruments). Code availability: Computational scripts used in this study to analyze sequencing datasets are available on GitHub: https://github.com/lohlaboratory/ane-pne.

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

Bulk-population RNA-sequencing, single-cell RNA-sequencing, and OmniATAC-sequencing datasets generated as part of this study are available at the NCBI Gene Expression Omnibus (GEO): accession numbers GSE286146 (OmniATAC-sequencing), GSE286147 (bulk RNA-sequencing), and GSE286148 (single-cell RNA-sequencing). These datasets are grouped under SuperSet accession number GSE286214.

An interactive web browser to explore single-cell RNA-sequencing data of the hPSC-derived cell-types generated in this study is available: https://anglohlabs.shinyapps.io/dundes_jokhai_lohlab/.

Computational scripts used in this study to analyze sequencing datasets are available on GitHub: https://github.com/lohlaboratory/ane-pne.

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 sex and gender was not relevant to this study.
Reporting on race, ethnicity, or other socially relevant groupings	Reporting on race, ethnicity, or other socially relevant groupings was not relevant to this study.
Population characteristics	Population characteristics were not relevant to this study.
Recruitment	Recruitment was not relevant to this study.
Ethics oversight	hPSC experiments at Stanford University were performed under the regulatory oversight of the Stem Cell Research Oversight (SCRO) committee. Mouse experiments at Stanford University were performed under the regulatory oversight of the Administrative Panel on Laboratory Animal Care (APLAC) committee. Zebrafish experiments at University of California San Francisco were performed under the regulatory oversight of the Institutional Animal Care and Use Committee (IACUC). According to local institutional policies, ethical approval was not required for studies of early chicken and acorn worm embryos.

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	Sample sizes were based on those typically used in past studies from our laboratory.
Data exclusions	No samples or individual data points were excluded from the analyses, except for qPCR technical replicates that failed.
Replication	Experiments were repeated adequately to ensure reproducibility, and the number of experiments is generally described either in the figure legends or the Methods section.
Randomization	No randomization of samples to experimental groups was performed.
Blinding	Data collection and analysis were not performed blind to the conditions of the experiments.

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

Rabbit anti-TFAP2A (for immunostaining) Abcam ab108311, concentration 1:250
Mouse anti-GATA3 (for immunostaining) Thermo Fisher MA1-028, concentration 1:1000
Mouse anti-PAX3 (for immunostaining) R&D Systems mAB2457, concentration 1:50
Goat anti-SOX2 (for immunostaining) R&D Systems AF2018, concentration 1:100
Rabbit anti-RFP (for immunostaining) Rockland 600-401-379, concentration 1:500
Goat anti-5-HT (for immunostaining) Immunostar 20079, concentration 1:500
Goat anti-OTX2 (for immunostaining) R&D Systems AF1979, concentration 1:200
Mouse anti-LHX2 (for immunostaining) Invitrogen MA5-15834, concentration 1:2000
Mouse anti-EN1 (for immunostaining) DSHB 4611, concentration 1:35
Goat anti-HNF1B (for immunostaining) R&D Systems AF3330, concentration 1:250
Rabbit anti-MAFB (for immunostaining) Sigma HPA005653, concentration 1:800
Rabbit anti-HOXA3 (for immunostaining) Novus NBP1-83234, concentration 1:100
Rabbit anti-SIX3 (for immunostaining) Rockland 600-401-A26S, concentration 1:1000
Mouse anti-PAX6 (for immunostaining) Santa Cruz Biotechnology sc81649, concentration 1:50
Rabbit anti-NKX2.1 (for immunostaining) Abcam ab76013, concentration 1:250
Rabbit anti-OLIG3 (for immunostaining) Sigma HA018303, concentration 1:1000
Rabbit anti-OLIG2 (for immunostaining) Millipore AB9610, concentration 1:500
Mouse anti-TAU/MAPT (for immunostaining) Millipore MAB3420, concentration 1:1000
Rabbit anti-TBR1 (for immunostaining) Abcam ab31940, concentration 1:2000
Rabbit anti-DLX1 (for immunostaining) Thermo Fisher PA5-28899, concentration 1:1000
Mouse anti-VGLUT2 (for immunostaining) Thermo Fisher MA5-31378, concentration 1:200
Rabbit anti-VGAT (for immunostaining) Synaptic 131 003, concentration 1:1000
Rabbit anti-CHT/SLC5A7 (for immunostaining) Abcam ab56074, concentration 1:100
Mouse anti-ISL1/2 (for immunostaining) DSHB 39.4D5, concentration 1:500
Rabbit anti-TLX3 (for immunostaining) Thermo Fisher PA5-83146, concentration 1:500
Rabbit anti-NANOG (for immunostaining) Abcam AB21624, concentration 1:200
Rabbit anti-GBX2 (for immunostaining) Proteintech 21639-1-AP, concentration 1:1000
Mouse anti-Beta-catenin (for immunostaining) Abcam Ab305261, concentration 1:50
Human anti-NANOG-APC (for intracellular flow cytometry) Miltenyi 130-105-079, concentration 1:11
Mouse anti-SOX2-PE (for intracellular flow cytometry) BD Biosciences 245610, concentration 1:5
Rabbit anti-Gbx2 (for Western Blot) Thermo Fisher PA5-66953, concentration 1:1000
Rabbit anti-Vinculin (for Western Blot) Cell Signaling Technology E1E9V, concentration 1:1000
Donkey anti-goat Alexa 647 Thermo Fisher A21447, concentration 1:500
Donkey anti-guinea pig Alexa 488 Jackson ImmunoResearch 706-545-148, concentration 1:500
Donkey anti-mouse Alexa 555 Thermo Fisher A32773, concentration 1:500
Donkey anti-mouse Alexa 647 Thermo Fisher A31571, concentration 1:500
Donkey anti-rabbit Alexa 555 Thermo Fisher A-31572, concentration 1:500
Donkey anti-rabbit Alexa 647 Jackson ImmunoResearch 711-607-003, concentration 1:500
Donkey anti-goat Alexa 488 Thermo Fisher A32814, concentration 1:500

Validation

All antibodies are commercially available and have been validated by the companies from which they were acquired.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

H1 hESCs, from WiCell (WA01)

Cell line source(s)	H7 hESCs, from WiCell (WA07) H9 hESCs, from WiCell (WA09) SUN004.1 hiPSCs, from Hiromitsu Nakauchi's laboratory SUN004.2 hiPSCs, from Hiromitsu Nakauchi's laboratory HES3 MIXL1-GFP hPSCs, from Andrew Elefanty's, Edouard Stanley's, and Elizabeth Ng's laboratories WTC AAVS1-CAG-GCaMP6f hiPSCs, from Bruce Conklin's laboratory
Authentication	Cell lines used in this study were not genomically authenticated.
Mycoplasma contamination	Cell lines were generally tested on a regular basis for mycoplasma contamination and tested negative.
Commonly misidentified lines (See ICLAC register)	No lines from this register 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	Gbx2CreER/+ mice were originally developed by, and generously provided by, James Li's laboratory (JAX #022135) and maintained on a CD1 background, heterozygous for the targeted allele. Mice carrying the Gbx2CreER allele were identified by PCR as described by Jackson Laboratory. Sox2CreER/+ mice1were originally developed by Konrad Hochedlinger's laboratory, were obtained from Jackson Laboratory (JAX #017593), and maintained on a C57BL/6 background, heterozygous for the targeted allele. Mice carrying the Sox2CreER allele were identified by PCR as described by Jackson Laboratory. ROSA26-CAG-LoxP-STOP-LoxP-tdTomato ("Ai14") mice were originally developed by the Allen Brain Institute, were obtained from Jackson Laboratory (JAX #007914), and were maintained homozygous for the targeted allele. ROSA26-Confetti mice were originally developed by Hans Clevers's laboratory, were obtained from Jackson Laboratory (JAX #017492), and were respectively maintained as homozygous for the targeted allele. Tcf/Lef:H2B-GFP mice were originally developed by Anna-Katerina Hadjantonakis's laboratory, were obtained from the Jackson Laboratory (#013752), and maintained on a CD1 background, heterozygous for the targeted allele. These mice were shared by Teni Anbarchian and Roel Nusse.
Wild animals	Acorn worms (Saccoglossus kowalevskii) were caught from the wild from Waquoit Bay, MA.
Reporting on sex	The biological sex of mouse, chicken, zebrafish, and acorn worm embryos was not determined.
Field-collected samples	This study did not include field-collected samples.
Ethics oversight	Mouse experiments at Stanford University were performed under the regulatory oversight of the Administrative Panel on Laboratory Animal Care (APLAC) committee. Zebrafish experiments at University of California San Francisco were performed under the regulatory oversight of the Institutional Animal Care and Use Committee (IACUC). According to local institutional policies, ethical approval was not required for studies of early chicken and acorn worm embryos.

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

Plants

Seed stocks	No plants were used in this study.
Novel plant genotypes	No plants were used in this study.
Authentication	No plants were used 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	hPSCs and hPSC-derived ectoderm cells were dissociated by incubating them in TrypLE Express (Thermo Fisher) for 5 minutes at 37 ºC. The resulting cell suspension was diluted 1:10 in FACS Buffer, which comprised 0.5% BSA (Thermo Fisher) and 2 mM EDTA (Thermo Fisher) in PBS, and subsequently pelleted at 300 x g. Intracellular protein staining for flow cytometry was conducted using the Inside Stain Kit (Miltenyi Biotec) as per manufacturer's instructions. Alternatively, live cells were analyzed, in which case no fixation or permeabilization was used.
Instrument	Flow cytometry was performed on a Beckman Coulter CytoFlex analyzer (Stanford Stem Cell Institute FACS Core).
Software	Flow cytometry data were acquired on a Beckman Coulter Cytoflex flow cytometer using BD Biosciences FACSDiva software. For data analysis, cells were gated in FlowJo software based on forward and side scatter area parameters, followed by height and width parameters for doublet discrimination.
Cell population abundance	Cell population abundance varied for each cell type on which flow cytometry was performed. The exact cell population abundance for each cell type is reported in detail in this study.
Gating strategy	An example of the flow cytometry gating strategy used for live cells is shown in the Source Data.

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