Corresponding author(s): Esteban O. Mazzoni
Shaun Mahony

Reporting Summary

Nature Research 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 Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☒	☐	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	RNA-seq, ChIP-seq, ATAC-seq, and sc-RNA-seq data was collected by Illumina NextSeq 500 sequencer. Confocal images of embryoid bodies and neurons were taken by Leica SP5 Confocal microscope. Live Acquisition software package (Till Photonics) was used to collect calcium imaging data.

Data analysis	SeqUnwinder Kakumanu et al., 2017 GREAT McLean et al., 2010 MultiGPS Mahony et al., 2014 DNAShapeR Chiu et al., 2016 Bowtie Langmead et al., 2009 DESeq2 Love et al., 2014 Tophat Kim et al., 2013 Rsubread Liao et al., 2013 Matlab- MATLAB and Statistics Toolbox Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States. Panther (version 13.1) - http://pantherdb.org Bedtools - Quinlan et al., 2016 EdgeR - Robinson et al. 2009 RefSeq - O'Leary et al. 2016

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

All data produced for this study are available under GSE114176.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications (Velasco et al., 2017; Mazzoni et al., 2013). Biologically independent cell differentiations were used as replicates. We used 3 independent biological replicates (n=3) for Ascl1 and Neurog2 ChIP-seq experiments. The rest of the ChIP-seq experiments, all ATAC-seq experiments, antibody stainings, and calcium imaging were performed in 2 independent biological replicates. Most of the RNA-seq experiments were performed in 2 biological replicates except iAscl1 48 (n=5) and EB t0 (n=5) because of the availability of additional replicates performed in the past for other studies.
Data exclusions	None
Replication	All attempts at replication were successful. For all experiments, replication was achieved through multiple independent cell differentiations.
Randomization	Experiments were not randomized and we did not perform any in vivo studies. We compared control and test samples through treatment and genetic information.
Blinding	The blinding of investigators were not performed but different people analyzed the same data.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used

Mouse monoclonal anti-V5_Thermo Fisher Scientific_Cat# R960-25_RRID:AB_2556564_1:5000 dilution
Rabbit polyclonal anti-β3-TUBULIN_Sigma_Cat# T2200_RRID:AB_262133_1:2000 dilution.
Sheep polyclonal anti-EBF2_R&D systems_Cat# AF7006_RRID:AB_10972102_5 ug for ChIP
Sheep polyclonal anti-ONECUT2_R&D systems_Cat# AF6294_RRID:AB_10640365_5 ug for ChIP
Goat polyclonal anti-NGN2_Santa Cruz_Cat# SC-19233_RRID:AB_2149513_5 ug for ChIP
Rabbit polyclonal anti-MASH1 (Ascl1)_abcam_Cat# ab74065_RRID:AB_1859937_5 ug for ChIP
Rabbit polyclonal anti-HA_abcam_Cat# ab9110_RRID:AB_307019_1:5000 dilution
Mouse monoclonal anti-FLAG_Sigma_Cat# F1804_RRID:AB_262044_1:500 dilution
Chicken polyclonal anti-MAP2_abcam_Cat# ab5392_RRID:AB_2138153_1:1000 dilution
Mouse monoclonal anti-NEUROFILAMENT_DSHB_Cat# 2H3_RRID:AB_531793_1:1000 dilution
Goat polyclonal anti-BRN2_Santa Cruz_Cat# SC-6029_RRID: AB_2167385_5 ug for ChIP
Rabbit polyclonal anti-H3K27ac_abcam_Cat# ab4729_RRID:AB_2118291_5 ug for ChIP
Goat anti-chicken 488 AlexaFluor_Invitrogen_Cat# A-11039_RRID:AB_142924_1:1000 dilution
Goat anti-mouse 647 Alexa Fluor_Invitrogen_Cat# A-21236_RRID:AB_141725_1:1000 dilution
Goat anti-rabbit 568 Alexa Fluor_Invitrogen_Cat# A-11036_RRID:AB_143011_1:1000 dilution

Validation

Antibodies are validated by using uninduced (mouse embryonic stem cells) cells as negative controls and comparison of the antibody staining/ChIP with the ones performed with tagged proteins. Additional validation and reports from the manufacturer and peer-reviewed articles can be found on manufacturer's websites.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Inducible cell lines were generated using the inducible cassette exchange (ICE) method that was previously described in Iacovino, M. et al. Inducible cassette exchange: a rapid and efficient system enabling conditional gene expression in embryonic stem and primary cells. Stem Cells 29, 1580–1588 (2011).

Authentication

We genotyped the cell lines.

Mycoplasma contamination

These cell lines used in the study were not tested for mycoplasma. However, the parental cell line that was used to generate these inducible lines are routinely checked in the lab and have not been found positive for mycoplasma.

Commonly misidentified lines
(See ICLAC register)

None of the lines used were misidentified.

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.

To review GEO accession GSE114176: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE114176

Files in database submission

HL7T3BGXY_n01_ba48.fastq.gz
HL7T3BGXY_n01_ba49.fastq.gz
HFW7KBGXY_n01_ba63.fastq.gz
HJY7YBGX2_n01_ba65.fastq.gz
C7KY3ACXX_l08n01_sv106.351000000040810.fastq.gz
C7KY3ACXX_l08n01_sv107.351000000040897.fastq.gz
HFW7KBGXY_n01_ba64.fastq.gz
C7KY3ACXX_l08n01_sv110.351000000409f3.fastq.gz
HJY7YBGX2_n01_ba77.fastq.gz
HJY7YBGX2_n01_ba78.fastq.gz

HJY7YBGX2_n01_ba76.fastq.gz
bgm3_ACAGTG_L002_R1_001.fastq.gz
C5WCJACXX_l06n01_bgm6.3410000000bfd5.fastq.gz
H7KNHBGX3_n01_ba83.fastq.gz
H7KNHBGX3_n01_ba84.fastq.gz
H3FWTBGX5_n01_ba126.fastq.gz
H3FWTBGX5_n01_ba127.fastq.gz
HL7T3BGXY_n01_ba50.fastq.gz
HFW7KBGXY_n01_ba59.fastq.gz
H3FWTBGX5_n01_ba128.fastq.gz
H3FWTBGX5_n01_ba129.fastq.gz
H7KNHBGX3_n01_ba67.fastq.gz
HL2NTBGX3_n01_ba108.fastq.gz
H7KNHBGX3_n01_ba85.fastq.gz
HL2NTBGX3_n01_ba107.fastq.gz
H3FWTBGX5_n01_ba130.fastq.gz
H3FWTBGX5_n01_ba131.fastq.gz
HL7T3BGXY_n01_ba51.fastq.gz
HFW7KBGXY_n01_ba61.fastq.gz
H3FWTBGX5_n01_ba133.fastq.gz
H3FWTBGX5_n01_ba134.fastq.gz

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

12h Ascl1 and Neurog2 ChIP-seq experiments were performed in 3 biological replicates. Rest of the ChIP-seq experiments are performed in 2 biological replicates.

Sequencing depth

File(s) Read length Paired or single-end seq Sequenced reads Uniquely mapped reads
HL7T3BGXY_n01_ba48.fastq.gz 51 single 41214096 30324495
HL7T3BGXY_n01_ba49.fastq.gz 51 single 37113165 27261442
HFW7KBGXY_n01_ba63.fastq.gz 76 single 34172197 26232594
HJY7YBGX2_n01_ba65.fastq.gz 50 single 47389148 26908039
C7KY3ACXX_l08n01_sv106.35100000040810.fastq.gz 51 single 42745488 32304763
C7KY3ACXX_l08n01_sv107.35100000040897.fastq.gz 51 single 60891637 34923424
HFW7KBGXY_n01_ba64.fastq.gz 76 single 32221513 25339660
C7KY3ACXX_l08n01_sv110.351000000409f3.fastq.gz 51 single 21141657 15391085
HJY7YBGX2_n01_ba77.fastq.gz 50 single 47382123 36758251
HJY7YBGX2_n01_ba78.fastq.gz 50 single 41435336 33111788
HJY7YBGX2_n01_ba76.fastq.gz 50 single 33191686 26401326
bgm3_ACAGTG_L002_R1_001.fastq.gz 51 single 29254740 22068673
C5WCJACXX_l06n01_bgm6.3410000000bfd5.fastq.gz 51 single 52494545 39539853
H7KNHBGX3_n01_ba83.fastq.gz 75 single 39374378 30086226
H7KNHBGX3_n01_ba84.fastq.gz 75 single 40159227 30518273
H3FWTBGX5_n01_ba126.fastq.gz 75 single 35918702 29038259
H3FWTBGX5_n01_ba127.fastq.gz 75 single 29446502 22765267
HL7T3BGXY_n01_ba50.fastq.gz 51 single 30042940 25695212
HFW7KBGXY_n01_ba59.fastq.gz 76 single 22333815 18954309
H3FWTBGX5_n01_ba128.fastq.gz 75 single 33047029 26099022
H3FWTBGX5_n01_ba129.fastq.gz 75 single 35249661 26716434
H7KNHBGX3_n01_ba67.fastq.gz 75 single 35428163 28605942
HL2NTBGX3_n01_ba108.fastq.gz 75 single 33487953 26778366
H7KNHBGX3_n01_ba85.fastq.gz 75 single 44875183 34995379
HL2NTBGX3_n01_ba107.fastq.gz 75 single 39157095 29516002
H3FWTBGX5_n01_ba130.fastq.gz 75 single 36018293 28555170
H3FWTBGX5_n01_ba131.fastq.gz 75 single 26957572 23011879
HL7T3BGXY_n01_ba51.fastq.gz 51 single 34287101 29026034
HFW7KBGXY_n01_ba61.fastq.gz 76 single 37968301 32408097
H3FWTBGX5_n01_ba133.fastq.gz 75 single 33712513 25428696
H3FWTBGX5_n01_ba134.fastq.gz 75 single 37727645 30308287

Antibodies

anti-Ascl1(abcam, ab74065), anti-Neurog2 (Santa Cruz, SC-19233), anti-HA (abcam, ab9110) anti-Brn2 (Santa Cruz, SC-6029), anti-Ebf2 (R&D, AF7006), anti-Onecut2 (R&D, AF6294), anti-H3K27ac (abcam, ab4729)

Peak calling parameters

All ChIP-seq fastq files were aligned to the mouse genome (version mm10) using Bowtie (1.0.1) with options "-q --best --strata -m 1 --chunkmbs 1024". Only uniquely mapped reads were considered for further analysis. MultiGPS was used to define transcription factor DNA binding events. A q-value cutoff of 0.01 (assessed using binomial tests and Benjamini-Hochberg multiple hypothesis test correction), was used to call statistically significant binding events. Differential binding analysis between time points (12hr vs 48hr) or factor inductions (iAscl1 vs iNeurog2) was also performed using MultiGPS, which calls EdgeR internally. Differentially bound sites are defined as those that display significantly greater read enrichment levels (minimum 1.5-fold, q-value < 0.05) as determined by EdgeR's negative binomial generalized linear models applied to MultiGPS' per-replicate count data (TMM normalized). Shared binding events are defined as those that are called in both conditions, and not displaying significant differences in read enrichment level. To account for some differences in the

	numbers of peaks called for Neurog2 and Ascl1, some analyses of differential and shared binding restrict analysis to the top 10,000 most ChIP-enriched binding events for each of those transcription factors.
Data quality	We assessed the quality of the sequencing reads using FastQC and assessed library complexity using custom scripts
Software	SeqUnwinder Kakumanu et al., 2017 ChIPEnrich Welch et al., 2014 MultiGPS Mahony et al., 2014