

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 (<i>n</i>) 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> 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 <i>d</i> , Pearson's <i>r</i>), 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	WGBS sequencing was performed on an Illumina NextSeq 500 instrument.
Data analysis	Bismark v0.23.1 (https://doi.org/10.1093/bioinformatics/btr167) was used to align DNA reads. For analysis of genomic features (e.g. promoters, gene bodies, 5Kbp tiles), CpG methylation rates were computed assuming a binomial model as previously (https://doi.org/10.1038/s41586-019-1825-8). To generate the Circos plot, the pycircos library was used.

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](#)

All data supporting the findings of this study are available within the paper and its Supplementary Information.

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 N/A

Reporting on race, ethnicity, or other socially relevant groupings N/A

Population characteristics N/A

Recruitment N/A

Ethics oversight N/A

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 For the analysis of Mendelian segregation of alleles, samples size was determined by power analysis using the following site <http://biomath.info/power/chsq1gp.htm>. Sufficient animals were used in order to detect a 50% reduction in expected frequency, using power of 0.8 and alpha 0.05. Others samples sizes were not predetermined as the magnitude of effect was unknown prior to the experiment.

Data exclusions No data were excluded from analyses.

Replication All experiments were repeated as described in the figure legends or materials and methods and for each experiment all attempts of replication were successful. Different biological replicates were performed on separated days, with samples collected from separated cell culture batches or independent biological samples collected from different animals on different days and processed on different days. Samples were allocated to groups based upon subsequent genotyping. Experiments were reproducible across all repeats. All replication data are included and confirm the original findings.

Randomization No randomization was involved as groups are based upon the genotype of the animal/emrbyo.

Blinding The investigators were blind to the genotypes of mice and relied solely on identification numbers throughout the study.

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-OCT4 (1:200, catalog no. ab181557; Abcam); anti-TFAP2C (1:100, catalog no. sc-12762; Santa Cruz); anti-SOX17 (1:200, catalog no. AF1924; R&D); Donkey anti-rabbit IgG (Alexa Fluor 594, catalog no. A21207; Invitrogen); Donkey Anti-Goat IgG H&L (Alexa Fluor 488, catalog no. ab150129; Abcam); Donkey anti-Mouse IgG (H&L) (Alexa fluor 594, catalog no. R37115; Life Technologies); anti-PLZF (1:200, sc-28319) and anti-Ki67 (1:200, ab16667); swine anti-rabbit (1:200, catalog no. P0339, Dako) or anti-mouse horseradish peroxidase (HRP)-conjugated immunoglobulins (1:200, catalog no. P0339, Dako); anti-GFP (1:500, catalog no. GF090RI; Nacalai); anti-GFP (1:2,000, catalog no. ab13970); anti-phospho-Histone H2A.X (Ser139) (1:1,000, catalog no. 05-636; Millipore); anti-cleaved caspase 3 (1:400, catalog no. 9661; Cell Signaling Technology); anti-MVH (ab27591); anti-RPA (1:100, catalog no. 2208; Cell Signalling Technology); anti-cyclin B1 (1:100, catalog no. 4138; Cell Signalling Technology); anti-phospho-histone 3 (1:200, catalog no. 9701; Cell Signalling Technology); goat anti-rat Alexa Fluor 488 (1:1000, catalog no. A11006, Thermo Fisher Scientific), goat anti-chicken Alexa Fluor 488 (1:1000, catalog no. A11039, Thermo Fisher Scientific), goat anti-mouse Alexa Fluor 594 (1:1000, catalog no. A11032, Thermo Fisher Scientific), goat anti-rabbit Alexa Fluor 594 (1:1000, catalog no. A21429; Thermo Fisher Scientific), goat anti-rat Alexa Fluor 594 (1:1000, catalog no. A21209; Thermo Fisher Scientific); anti-SSEA1 conjugated to Alexa Fluor 647 (1:100, catalog no. MC-480; Biolegend); anti-CD4 (clone H129.19, BD Pharmingen), CD3e (clone 145-2C11, eBioscience), Ly-6G/Gr-1 (clone RB6-8C5, eBioscience), CD11b/Mac-1 (clone M1/70, BD Pharmingen), CD45R/B220 (clone RA3-6B2, BD Pharmingen), Fcε R1α (clone MAR-1, eBioscience), CD8a (clone 53-6.7, BD Pharmingen), CD11c (clone N418, eBioscience); TER-119 (clone Ter119, BD Pharmingen), anti-c-Kit (PerCP-Cy5.5, clone 2B8, eBioscience), anti-Sca-1 (PE-Cy7, clone D7, eBioscience); anti-CD48 (FITC, clone HM48-1, BioLegend), anti-CD41 (FITC, clone MWReg30, BD Pharmingen), anti-CD150 (APC, clone TC15-12F12.2, BioLegend); anti-CD45R/B220 (PE, clone RA3-6B2, BD Pharmingen); anti-IgM (APC, clone II/41, BD Pharmingen); anti-TER-119 (APC, clone Ter-119, BD Pharmingen); anti-CD71 (PE, clone C2, BD Pharmingen); anti-CD11b/Mac-1 (APC, clone M1/70, BD Pharmingen); anti-Ly-6G/Gr-1 (PE, clone RB6-8C5, eBioscience); CD4 (FITC, clone H129.19, BD Pharmingen); CD8a (PE, clone 53-6.7, BD Pharmingen).
Validation	All antibodies used were published previously

Eukaryotic cell lines

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

Cell line source(s)	The hiPSC cell line (BTAG 585b1-868) was a kind gift from Prof. Mitunori Saitou (Institute for the Advanced Study of Human Biology, Kyoto, Japan). All other lines were generated in this study
Authentication	<i>Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.</i>
Mycoplasma contamination	All lines were mycoplasma free
Commonly misidentified lines (See ICLAC register)	<i>Name any commonly misidentified cell lines used in the study and provide a rationale for their use.</i>

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	All animal experiments performed in this study were approved by the Medical Research Council's Laboratory of Molecular Biology animal welfare and ethical review body and conform to the UK Home Office Animal (Scientific Procedures) Act 1986 (License no. PP6752216). All mice were maintained under specific pathogen-free conditions in independently ventilated cages (GM500; Techniplast) on Lignocel FS-14 spruce bedding (IPS) and provided with environmental enrichment (fun tunnel, chew stick and Enviro-Dri nesting material (LBS)) at 19-23°C. Mice fed Dietex CRM pellets (SpecialDietService) ad libitum with light from 7:00 a.m. to 7:00 p.m. No mice used in this study were wild and no field-collected samples were used. All mice were maintained on an isogenic C57BL/6j background. Embryos were examined at various developmental stages from E8.5 to E13.5 as indicated in the text. Female mice used in timed-mating experiments were aged between 6-25 weeks. The investigators were blinded to the genotypes of all mice throughout the study and data were acquired by relying entirely on identification numbers. The Stella-GFP (Tg(Dppa3/EGFP)6-25Masu) allele (MGI ID: 5519126) was a kind gift from Azim Surani. B6;129P2-Polktm1.1Rsky/J allele (MGI ID: 2445458) and the B6.Cg-Polqt1Jcs/J allele (MGI ID: 2155399) have been described previously. GOF18-GFP(Tg(Pou5f1-EGFP)2Mnn) (MGI ID: 3057158) JAX (stock ID: 004654) mice were purchased from The Jackson Laboratory.
Wild animals	No wild animals were used
Reporting on sex	Reproductive tissues were examined therefore males and females were treated separately as detailed in the text
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	All animal experiments undertaken in this study were approved by the Medical Research Council's Laboratory of Molecular Biology animal welfare and ethical review body and the UK Home Office under the Animal (Scientific Procedures) Act 1986 (license no. PP6752216).

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

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

Human PGCLC: Day 4 aggregates were collected and dissociated with 0.25% trypsin-EDTA (Life Technologies, 25200056) for 10 min at 37°C under agitation. Cells were washed with PBS containing fetal bovine serum (FBS) and 0.1% bovine serum albumin (BSA) before being subjected to centrifugation. Dissociated cells were then resuspended in FACS buffer (PBS, 0.1% BSA) and filtered by a cell strainer (BD Biosciences).

Mouse PGC: the entire embryo (E8.5-10.5) or the developing urogenital ridges (E11.5-13.5) were isolated from embryos and placed into 500 µL or 150 µL pre-warmed trypsin solution (2.5 µg.mL⁻¹ trypsin (Gibco), 25 mM Tris, 120 mM NaCl, 25 mM KCl, 25 mM KH₂PO₄, 25 mM glucose, 25 mM EDTA, pH 7.6) respectively, and incubated at 37°C for 10 min. Subsequently, 1 µL of Benzonase endonuclease (Millipore) was added and the sample gently disaggregated by pipetting and incubated for 5 min at 37°C. The trypsin was inactivated by adding 1 mL of PBS/5% v/v fetal bovine serum and centrifuged at 3,300 rpm for 10 min. The sample was resuspended in 100 µL of anti-SSEA1 conjugated to Alexa Fluor 647 (catalog no. MC-480; Biolegend) diluted 1:100 in PBS/2.5% v/v fetal bovine serum and incubated for 10 min at room temperature. Samples were diluted by adding 300 µL of PBS/2.5% v/v fetal bovine serum and passed through a 70 µm filter.

Instrument

hPGCLC quantification: Aria III, BD Biosciences
mPGC quantification: ECLIPSE analyzer (Sony Biotechnology)

Software

Data was processed using FlowJo version 10

Cell population abundance

N/A

Gating strategy

For hPGCLC quantification, cells were gated using FSC/SSC and hPGCLCs defined using eGFP and tdTomato reporters. For mPGC quantification, cells were gated using FSC/SSC and PGCs defined as (APC-SSEA+GFP+).

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