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 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
- 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 were 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 possible.
- 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 | Illumina HiSeqTM 2500, Illumina HiSeqX Tenplatform, CFX-Connect Real-time PCR detection system, HD FACS LS RFortessa, Fluorescence inverted microscope system(Olympus, BX51), Confocal microscope system(Olympus, FV1200), Immersion microscope system(Olympus, BX41) |
|-----------------|------------------------------------------------------------------------------------------------------------------|
| Dataanalysis    | GraphPad Prism 7.0, FlowJo, eXpress, DESeq, Bowtie2, HISAT2, HTSeq-count, R packages (ggplot2, Pca3d and Pheatmap), Burrows-Wheeler Aligner, ‘astp’, ‘R’ |

For manuscripts utilizing custom algorithms or software that are 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 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

The datasets generated and analyzed during the current study are available from the corresponding author 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 based on previously published work and preliminary studies. For in vitro experiments, no sample size calculation was performed and we generally included three samples per condition. For in ovo experiments, sample size calculation was based on the multiple sample tests(n≥3). Microsatellite detection, transcriptome and genome sequencing experiments were generally performed in biological duplicates. The consistently very high correlation between these replicates, suggests that this is sufficient.

Data exclusions
No samples were excluded from the analysis except for two samples in the transcriptome sequencing that failed to build the library.

Replication
All replication attempts were successful. For each experiment, duplicates are noted in the legend. Briefly: for in vivo studies, at least three biologically independent replicates of separate experimental combinations are statistically processed (unless otherwise specified); for in vitro studies, biologically independent samples are plated in triplicate (as technical replicates). Sequencing experiments are also highly repeatable.

Randomization
For animal experiments, the fertilized eggs were randomly assigned into different groups before treatment.

Blinding
In all of experiments, the measurement of the sample was performed blindly by other colleagues. The data collection were blinded to groups assignments. Data analysis were done by a person who was blinded to genotypes and treatments of samples. Analyses of RNA-Seq and Whole-genome resequencing were performed by the biotech company who were blinded to the genotypes.

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 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.

Field work, collection and transport

Field conditions
Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).

Location
State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).

Access & import/export
Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).

Disturbance
Describe any disturbance caused by the study and how it was minimized.

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

| Involved in the study | n/a |
|-----------------------|-----|
| Antibodies            | ☑   |
| Eukaryotic cell lines | ☑   |
| Palaeontology and archaeology | ☑   |
| Animals and other organisms | ☑   |
| Human research participants | ☑   |
| Clinical data         | ☑   |
| Dual use research of concern | ☑   |

Methods

| Involved in the study | n/a |
|-----------------------|-----|
| ChIP-seq              | ☑   |
| Flow cytometry        | ☑   |
| MRI-based neuroimaging | ☑   |
Antibodies

In this study, the following antibodies were used for flow cytometry analysis, Western blotting and immunofluorescence staining. The details of the antibodies are as follows.

- SSEA1 (R&D, Minneapolis, USA, IC2155T),
- MVH (Abcam, Cambridge, UK, ab13840),
- CKIT (Thermo Fisher Scientific, Shanghai, China, 14-1172-81),
- GFP (CWBio, Beijing, China, CW0087M),
- β-actin (CWBio, Beijing, China, CW0096M),
- Goat anti-Rabbit IgG FITC Conjugated (CWBio, Shanghai, China, CW0114S), Goat anti-Mouse IgG H & L (Abcam, Cambridge, UK, ab6768),
- Goat anti-rabbit antibody (CWBio, Beijing, China, CW0107S),
- Goat anti-mouse antibody (CWBio, Beijing, China, CW0102S),
- Goat anti-Rat IgG (Proteintech, Chicago, USA, SA00003-11),
- Goat anti-mouse IgG (Proteintech, Chicago, USA, SA00003-12)

Validation

In this study, we verified the availability of these antibodies in chickens through the following methods:

1. Preliminary experiments, and then used these antibodies in formal experiments.
2. The results from previous publications from our lab. (Zhang Z, Elsayed A K, Shi Q, et al. Crucial Genes and Pathways in Chicken Germ Stem Cell Differentiation[J]. Journal of Biological Chemistry, 2015, 290(21):13605.)
3. (1) Manufacture provided validation on the same species, relevant information on the antibodies are available on the manufacturers’ websites.
   - The species reactivity of SSEA1 (R & D, Minneapolis, USA, ic2155t) are human and mouse. (https://www.novusbio.com/products/ssea-1-antibody-mc-480_ic2155t#datasheet)
   - The species reactivity of MVH (Abcam, Cambridge, UK, ab13840) are mouse, rat, cow, human, pig, platypus, which can be used in WB, IHC-P and IHC-Fr. (https://www.abcam.com/ddx4--mvh-antibody-ab13840.html)
   - CKIT (Thermo Fisher Scientific, Shanghai, China, 14-1172-81) has been reported that it can be applied to fish, guinea pig, human, mouse, rat and can be used in flow cytometric analysis, immunoprecipitation, immunoblotting (WB), and immunohistochemical staining of frozen tissue sections. (https://www.thermofisher.com/cn/zh/antibody/product/CD117-c-Kit-Antibody-clone-ACK2-Monoclonal/14-1172-82)
   - The species reactivity of Goat anti mouse IgG H & L (Abcam, Cambridge, UK, ab6768) is mouse, which can be used in Immunomicroscopy, Flow Cyt, IHC-P, IHC-Fr, ICC/IF and ELISA. (https://www.abcam.com/goat-mouse-igg-hl-trtct-ab6768.html)
   - GFP (cwbio, Beijing, China, cw0087m) specifically recognizes some mutants of GFP and EGFP. It is an affinity purified rabbit polyclonal antibody IgG, which can be used in Western blot and ELISA. (https://www.cwbiotech.com/goods/index/id=10107)
   - β-actin (cwbio, Beijing, China, cw0096m) is a mouse monoclonal antibody IgG2b, which can be used in Western blot test by using species of human, rat, mouse, rabbit, pig, chicken and sheep. (https://www.cwbiotech.com/goods/index/id/10113)
   - Goat anti rabbit IgG FITC Conjugated (cwbio, Shanghai, China, cw0114s) is rabbit IgG, which can be used in Immunofluorescence. (Item removed by company)
   - Goat anti rabbit antibody (cwbio, Beijing, China, cw0107s) is rabbit IgG, which can be used in Western blot, flow cytometry and ELISA. (https://www.cwbiotech.com/goods/index/id/10121)
   - Goat anti mouse antibody (cwbio, Beijing, China, cw0102s) is a mouse IgG, which can be used in Western blot, IHC and ELISA. (https://www.cwbiotech.com/goods/index/id/10118)
   - Goat anti rat IgG (protein, Chicago, USA, sa00003-11) can be used in IF, flow and Cyt. (https://www.ptglab.com/Products/Goat-anti-rat-IgG-(H-L)-FITC-conjugate-secondary-antibody.htm)
   - Goat anti mouse IgG (protein, Chicago, USA, sa00003-12) can be used in IF, flow and Cyt. (https://www.ptglab.com/products/fluorescein-FITC-conjugated-AffiniPure-Goat-Anti-Human-IgG-H-L-secondary-antibody.htm)

Palaeontology and Archaeology

Specimen provenance

Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).

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

We specify the sex of laboratory animals used in the reporting summary:

In this study, we used the fertilized eggs of black feather Langshan Chicken (both male and female) to isolate chicken embryo fibroblasts at 9-11 days after hatching. We also used the fertilized eggs of white plymouth rock chickens, both male and female were used as recipients for transplantation of iPSC at 2.5 days after hatching. Besides, we used the fertilized eggs of Langshan Chicken (both male and female) to isolate chicken embryonic stem cells on day 0 and primordial germ cells on day 4.5 after hatching. For the chickens (both male and female) produced in this study, we observed their feather color at birth and recorded their weight changes. When they were 1 month old, we collected their pterygoid vein blood for microsatellite testing and genome sequencing.

### Wild animals

This study did not involve wild animals.

### Field-collected samples

In this study, the samples collected in the field mainly included observing the feather color of the chickens when they were born in our lab incubator (humidity 60%, temperature 38.5°C), and weighing them at different growth stages in the experimental chicken house (lighting condition: from 7 AM to 12 PM; Humidity: 55%-60%; Temperature: week1, 32-36°C, Reduce by 3-4°C every week and stop at 21°C at week 4; Adequate Ventilation). And when chickens were one month old, the pterygoid vein blood was collected and used for microsatellite testing and genome sequencing. All these experiments were carried out in the experimental chicken house.

### Ethics oversight

The procedures involving animals and their care conformed to the U.S. National Institute of Health guidelines (NIH Pub. No. 85–23, revised 1996). The experiments were conducted under the approval of the Ethics Committee of Yangzhou University for Laboratory and Experimental Animals (SYXK(Su)2016-0020). The chickens used in this study were obtained from the Institute of Poultry Science, Chinese Academy of Agriculture Sciences. All animals were housed in the Animal Facility of Yangzhou University. The use and care of animals complied with the guidelines of the Animal Advisory Committee at Yangzhou University. The ethics application was approved by the Ethics Committee of Yangzhou University.

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

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write “See above.”

### Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

### Ethics oversight

Identify the organization(s) that approved the study protocol.

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 non-pathogen 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

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

In this study, the cells used for flow cytometry analysis mainly included iPS cells and iPGC induced by different systems. The source of cells has been described in detail in the method section. The detailed steps for sample preparation are as follows. Cells harvested from different induction days were blocked with blocking buffer (PBS containing 10% fetal bovine serum) (Gibco, New York, USA, 10270-106) for 2 h at 37°C. Samples were incubated with antibodies against cell surface epitopes (SSEA1, R&D, Minneapolis, USA, IC215ST, 1:100-1000; CVH, abcam, Cambridge, UK, ab13840,1: 100; CKIT, Thermo Fisher Scientific, Shanghai, China, 14-1172-81,1: 100,) at 4°C overnight, and washed with PBS containing 0.1% Tween-20 (Solarbio, Beijing, China, TB220) for three times, followed by fluorescence coupled secondary antibody (Goat Anti-Rabbit IgG FITC Conjugated, CWBIO, Shanghai, China, CW01145, 1: 100; Goat Anti-Mouse IgG H & L (TRICT), abcam, Cambridge, UK, ab6786, 1: 100) incubation at 37°C for 2 h. Then the cells were washed with PBS containing 0.1% Tween-20 for three times. The staining signal was analyzed by FACS LSRFortessa (BD Biosciences, USA) with a minimum of 10^4 events in each experiment.

Instrument

FACS LSRFortessa (BD Biosciences, USA)

Software

Flowjo Version10

Cell population abundance

In this study, we did not sort the cells for flow cytometry, but only detected the proportion of positive cells. Moreover, the cells we detected were iPS cells induced by CEF reprogramming and iPGC cells induced by different systems. These cells are heterogeneous and the cell composition is complex, so we did not detect the abundance of these cells.

Gating strategy

For all flow cytometry experiments, forward scattering and side scattering measurements were used to pre-gate the measured cell population, and then the side scatter height and width measurements were used to gate individual cells. The boundary between “positive” and “negative” stained cell populations is distinguished by the unstained sample.

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

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, MNIST, 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

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.