nature portfolio

Corresponding author(s): Yu-Hui Wong

Last updated by author(s): Sep 7, 2024

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	Cutadapt (V1.9.1), Hisat2 (v2.0.1) and HT-seq (V 0.6.1) were used to process RNA-seq data.
Data analysis	EdgeR (V3.24.1) and Gene Set Enrichment Analysis (GSEA, v4.2.3) were used to analyze RNA-seq data.

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

RNA-sequencing data have been deposited at NCBI database with accession number GSE256393 and are publicly available as of the date of publication. Link: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?&acc=GSE256393

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	The study does not contain human participants or human data.
Population characteristics	The study does not contain human participants or human data.
Recruitment	The study does not contain human participants or human data.
Ethics oversight	The study does not contain human participants or human data.

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	We did not perform statistical methods to predetermine sample-size. For statistic calculation, our data are from at least 2 independent samples.
Data exclusions	No data exclusion in this study.
Replication	At least 2 replication was used for each assay.
Randomization	We included all the samples we collected for analysis.
Blinding	The study does not require blinding.

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

Methods

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

Antibodies

Antibodies used

Mouse monoclonal anti-Prtg [1D5], homemade, doi: 10.1523/JNEUROSCI.0473-10.2010
Mouse monoclonal anti-Prtg [2B3], GeneTex Cat# GTX83789; RRID: AB_10731789
Mouse monoclonal anti-Prtg [OTI2B3], OriGene Cat# TA501394; RRID: AB_11126435
Rabbit monoclonal anti-pSmad2 (Ser465/Ser467), Cell Signaling Technology Cat# 18338; RRID: AB_2798798
Rabbit polyclonal anti-pSmad2 (phospho Ser465/Ser467), GeneTex Cat# GTX133614; RRID: AB_2887051
Mouse monoclonal anti-Smad2/3, Santa Cruz Biotechnology Cat# sc-133098; RRID: AB_2193048
Rabbit monoclonal anti-Smad2, Cell Signaling Technology Cat# 5339; RRID: AB_10626777

Rabbit monoclonal anti-Smad4, Cell Signaling Technology Cat# 38454; RRID: AB_2728776
Rabbit polyclonal anti-HOXC10, GeneTex Cat# GTX118025; RRID: AB_11170594
Rabbit polyclonal anti-Oct4, GeneTex Cat# GTX101497; RRID: AB_10618784
Rabbit monoclonal anti-GDF11, Abcam Cat# ab124721; RRID: AB_10974143
Rabbit polyclonal anti-Flag tag, Sigma-Aldrich Cat# F7425; RRID: AB_439687
Rabbit polyclonal anti-HA tag, Proteintech Cat# 51064-2-AP; RRID: AB_11042321
Sheep polyclonal anti-Prtg, R&D Systems Cat# AF4919; RRID: AB_2172305
Goat polyclonal anti-TBX6, R&D Systems Cat# AF4744; RRID: AB_2200834
Mouse monoclonal anti-GAPDH, Thermo Fisher Scientific Cat# AM4300; RRID: AB_2536381
Rabbit monoclonal anti-pSmad2 (phospho S467), Abcam Cat# ab280888
Goat Anti-Mouse IgG Polyclonal antibody, Horseradish peroxidase Conjugated, Millipore Cat# AP124P; RRID: AB_90456
Goat anti-Rabbit IgG (H+L) Secondary Antibody, HRP, Thermo Fisher Scientific Cat# 31460; RRID: AB_228341
F(ab')2-Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 647, Invitrogen Cat# A48285; RRID: AB_2896349
Donkey anti-Sheep IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555, Invitrogen Cat# A21436; RRID: AB_2535857
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647, Invitrogen Cat# A31573; RRID: AB_2536183
Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 555, Invitrogen Cat# A21432; RRID: AB_2535853

Validation

All primary antibodies were validated by immunoblotting or immunofluorescence to ensure their specificity.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	The hiPSC line, NTUH-iPSC-02-02 (abbreviated to N2), was purchased from Bioresource Collection and Research Center of Food Industry Research and Development Institute, Taiwan. P19 embryonic carcinoma cells were purchased from Bioresource Collection and Research Center of Food Industry Research and Development Institute, Taiwan (BCRC Number: 60052).
Authentication	The N2 hiPSCs were stained with stem cell markers and alkaline phosphatase activity to ensure its pluripotency. P19 embryonic carcinoma cells were not authenticated.
Mycoplasma contamination	Mycoplasma contamination was not tested.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines found.

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	Transgene mouse C57BL/6
Wild animals	The study did not involve wild animals.
Reporting on sex	Sex was not considered in this study. The phenotype is not sex-based.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All procedures involving mice were conducted in strict accordance with the university guidelines. Ethical approval for animal experiments was obtained from the Institutional Animal Care and Use Committee of National Yang Ming Chiao Tung University.

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	Day 5 iPSC-PSM cells were rinsed with PBS and detached with TrypLE Express. The TrypLE enzyme was inactivated by diluted

Sample preparation	with PBS containing 3% BAS and removed by centrifugation. The concentration of cells was adjusted to 1e+6 cells/mL and 1 mL of cells was used for each assay. Cells were fixed with 4% PFA/PBS on ice for 15 minutes, followed by washing with TBST for two times and resuspended in 100 µL TBST. Permeabilization was performed by adding 900 µL prechilled 100% methanol to reach a concentration of 90% methanol and incubate at -20°C for 10 minutes. Cells were stored at -20°C before immunostaining. Immunostaining of cells was performed by pelleting and washed twice with TBST to remove methanol, followed by blocking in TBST containing 10% horse serum for 30 minutes at room temperature. Primary antibody was added directly to the solution after blocking and incubated for 45 minutes at room temperature. Cells were rinsed twice with TBST to remove unbound antibodies and then incubated with TBST containing 10% horse serum and secondary antibody for 45 minutes at room temperature. After immunostaining, cells were rinsed twice with TBST, resuspended in PBS and transferred into a test tube with cell strainer.
Instrument	Beckman CytoFLEX S (model no. B75442)
Software	CytExpert v 2.4
Cell population abundance	iPSC-PSM cell population was determined by TBX6 staining.
Gating strategy	Cell debris and clumps of multiple cells were removed by gating with FSC-A and SSC-A. iPSC-PSM cell population was determined by gating the fluorescence intensity of TBX6 staining.

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