

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 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

The BZ-X710 fluorescence microscope (Keyence) was used to take phase-contrast or immunostaining images. The AriaMX Real-Time PCR System (Agilent) was used to perform qRT-PCR analysis. The CytoFLEX S flow cytometer (Beckman Coulter) was used for flow-cytometry analysis. The Multiskan Sky Microplate Spectrophotometer (Thermo Fisher) was used to measure absorbance. The Amersham Imager 600 (Amersham) was used to detect the signals of WB, and the HITACHI H-7100S electron microscope was used for transmission electron microscopy analysis.

Data analysis

Microsoft Excel was used for analysis of qRT-PCR or ExLBM-based chemical screening data. AriaMX software (Agilent) was used for the analysis of qRT-PCR data. Trimmomatic (version 0.36), HISAT2 (v2.1.0), featureCounts software (Subread package), RUVSeq (Release 3.10), R prcomp function and hclust function (R ver 3.6.1) were used for the analysis of RNA-seq data. Adobe photoshop 2021 was used for the analysis of WB or immunostaining. BZ-X analyzer software (KEYENCE) was used for the analysis of ExLBM-based chemical screening. FlowJo software (FlowJo LLC) was used for the analysis of flowcytometry. GraphPad Prism 9 (GraphPad Software) was used for other data calculations and statistical analyses.

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 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 main data supporting the results in this study are available within the paper and its Supplementary Information. All data generated in this study, including source data and the data used to make the figures, are available from figshare with the identifier <https://doi.org/10.6084/m9.figshare.14888937>.

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 Although no statistical methods were used to predetermine sample size for the in vitro and in vivo analyses, we conducted preliminary experiments to estimate variances for each assay and to determine adequate sample sizes.

Data exclusions No data were excluded.

Replication We successfully reproduced all the data reported in the paper.

Randomization All animal experiments were performed using mice or rats with the same age and similar weights. We did not perform randomization.

Blinding To perform qRT-PCR or RNA-seq analyses, the data analyzer was blinded to group allocation.

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

☒ ☐ Human research participants

☒ ☐ Clinical data

☒ ☐ Dual use research of concern

Methods

n/a Involved in the study

☒ ☐ ChIP-seq

☐ ☒ Flow cytometry

☒ ☐ MRI-based neuroimaging

Antibodies

Antibodies used

PRRX1 (Novusbio, NBP2-13816, 1:200 for IHC, 1:2,000 for WB), COL1 (southern biotech, 1441-01, 1:200 for IHC), COL2 (ThermoFisher, MA1-37493, 1:200 for IHC), COLX (ThermoFisher, 14-9771-80, 1:200 for IHC), VIMENTIN (Abcam, ab16700, 1:200 for IHC), OCT4 (Cell Signaling Technology, 75462, 1:200 for IHC), NANOG (Cell Signaling Technology, 4903, 1:200 for IHC), SOX2 (Cell Signaling Technology, 23064, 1:200 for IHC), TRA-1-60 (ThermoFisher, 41-1000, 1:200 for IHC), SOX9 (MERK, AB5535, 1:200 for IHC), anti-human Nucleoli (abcam, ab190710, 1:200 for IHC), anti-rabbit Alexa Fluor 647 Fab2 (Cell Signaling Technology, 4414, 1:400 for IHC), anti-Goat IgG HRP (ThermoFisher, A15999, 1:400 for IHC), anti-Mouse IgG HRP (Cell Signaling Technology, 7076, 1:400 for IHC, 1:5,000 for WB), anti-Rabbit IgG HRP (Cell Signaling Technology, 7074, 1:400 for IHC, 1:5,000 for WB), ACTB (SANTA CRUZ BIOTECHNOLOGY, sc-47778, 1:2,000 for WB), CD90 (BD Pharmingen, 560747, 1:20 for flow cytometry), CD140B (BD Pharmingen, 560747, 1:20 for flow cytometry), SSEA4 (BD Pharmingen, 560747, 1:20 for flow cytometry), PE-Cy7-CD82 (BioLegend, 342109, 1:200 for flow cytometry), APC-CD82 (BioLegend, 342113, 1:200 for FACS), FITC-CD90 (BioLegend, 328107, 1:200 for FACS), BB700-CD140B (BD Pharmingen, 745822, 1:200 for FACS), anti-Mouse Alexa Fluor 488 (ThermoFisher, A28175, 1:1,000 for flow cytometry).

Validation

PRRX1 (Nouvusbio, NBP2-13816), COL1 (southern biotech, 1441-01), COL2 (ThermoFisher, MA1-37493), COLX (ThermoFisher, 14-9771-80), VIMENTIN (Abcam, ab16700), OCT4 (Cell Signaling Technology, 75462), NANOG (Cell Signaling Technology, 4903), SOX2 (Cell Signaling Technology, 23064), TRA-1-60 (ThermoFisher, 41-1000), SOX9 (MERK, AB5535), anti-human Nucleoli (abcam, ab190710), anti-rabbit Alexa Fluor 647 Fab2 (Cell Signaling Technology, 4414), anti-Goat IgG HRP (ThermoFisher, A15999), anti-Mouse IgG HRP (Cell Signaling Technology, 7076, 1:400 for IHC), anti-Rabbit IgG HRP (Cell Signaling Technology, 7074), ACTB (SANTA CRUZ BIOTECHNOLOGY, sc-47778), CD90 (BD Pharmingen, 560747), CD140B (BD Pharmingen, 560747), SSEA4 (BD Pharmingen, 560747), PE-Cy7-CD82 (BioLegend, 342109), APC-CD82 (BioLegend, 342113), FITC-CD90 (BioLegend, 328107), BB700-CD140B (BD Pharmingen, 745822), anti-Mouse Alexa Fluor 488 (ThermoFisher, A28175).

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)

414C2 hiPSCs, from the Center for iPS Cell Research and Application, Kyoto University.
1383D2 hiPSCs and the hESCs SEES4, SEES5, SEES6 and SEES7, from RIKEN BRC (Japan).
The COL2pathy-derived hiPSCs, including ACGII-1 hiPSCs (heterozygous 1-bp substitution [T>C] located at the acceptor site of exon 41 in COL2A1) and HCG-1 hiPSCs (heterozygous 1348G>C [Gly450Arg] mutation in exon 21 in COL2A1), from Dr. Noriyuki Tsumaki (Center for iPS Cell Research and Application, Kyoto University).
BM-hMSCs, from LONZA (Lot# 0000494678).

Authentication

All cell lines used in this study were authenticated by the distributors.

Mycoplasma contamination

We confirmed that all cell lines were negative for mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

For the subcutaneous transplantation of cartilaginous particles, NOD-SCID mice (CLEA, female, 5 weeks-old) were used. For the transplantation of cartilaginous particles into damaged articular cartilage, we used SCID rats (Tokyo University, female, 10 weeks-old).

Wild animals

The study did not use wild animals.

Field-collected samples

The study did not use samples collected from the field.

Ethics oversight

All animal experiments were approved by the Committee on Animal Experimentation of Okayama University, and performed in compliance with the University's Guidelines for the Care and Use of Laboratory Animals.

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

After dissociating with Accutase (Thermo Fisher), 1×10^5 cells were suspended with 100 μ l of 2% FBS/PBS and incubated with primary antibody (200 $\times$) for 1 h on ice. Then cells were washed and suspended in 100 μ l of 2% FBS/PBS containing secondary antibody (1,000 $\times$) for 30 min on ice. SOX9 was stained using Transcription Factor Buffer Set (BD) following manufacturer's instructions.

Instrument

Flowcytometry; CytoFLEX S flow cytometer (Beckman Coulter, Serial number: BB32105)
FACS; FACSAria III Cell Sorter (BD, Serial number: R648282170002)

Software

CytExpert (Beckman Coulter) was used for data collection and FlowJo software (FlowJo LLC) was used to analyse the obtained data. BD FACSDiva (BD) was used to identify and isolate the desired cell populations.

Cell population abundance

In the case of the PRRX1-tdTomato reporter line, the parental cell line or 414C2 was used as a negative control to define PRRX1-tdTomato-positive cells. In the case of immunostaining of surface antigens, cells stained with IgG control (for PE-Cy7-

CD82, APC-CD82, FITC-CD90, BB700-CD140B) or secondary antibody (for CD90 and CD140B) were used as a negative control to define cells positive for each surface antigen.

Gating strategy

To exclude doublet cells, the SSC-A/FSC-A fraction was sub-gated to FSC-A/FSC-H and then to the SSC-A/SSC-H fraction. Cells that were not stained with DAPI were used for further analysis.

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