nature portfolio

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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	All the data were collected using software provided by the equipment manufacturer. The developed platform utilized custom designed software, which can be accessed at https://github.com/MHKim-software/HITS-Bio.git
Data analysis	Data were analyzed using Prism v8.4.2. (GraphPad) and Image J v1.54 (NIH) for image analysis. MicroCT data were analyzed using Avizo 3D 2021.2 software (FEI Company).

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 results are available in the article and its Supplementary files or from the corresponding author upon reasonable request. Source data are

provided with this paper. The developed platform in this work utilized custom designed software, which can be accessed at GitHub (https://github.com/MHKim-software/HITS-Bio.git).

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

Life sciences study design

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

Sample size	Sample sizes were not predetermined based on statistical methods, but were chosen according to the exceeding standards of the field (at least three independent biological replicates for each condition), which generated significant statistical analysis.
Data exclusions	Data were not excluded from analysis.
Replication	Biological replications are as indicated in figure legends. All attempts at replications were successful.
Randomization	No randomization was performed in this study. The data were based on quantitative readouts that cannot be influenced by experimenter bias. In comparisons, all samples were prepared and treated identically. For imaging, random fields of view were selected.
Blinding	Bony bridging scoring based on microCT images were performed in a blinded manner to avoid observation bias. All other data sets are based on objectively measurable data. Blinding does not affect these data values.

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

Mouse anti-RUNX2 (runt-related transcription factor 2) antibody (1:20, ab76956; Abcam)
Rabbit anti-Sp7/Osterix (OSTERIX) antibody (1:100; ab209484, Abcam)
Rabbit anti-Von Willebrand Factor (VWF) (1:100, 27186-1-AP, Proteintech)
Sheep anti-Fibronectin (1:100, AF1918, R&D Systems)
Rabbit anti-bone sialoprotein (BSP) antibody (1:100, ab52128, Abcam)
Mouse anti-CD31 primary antibody (1:100, ab9498, Abcam)
Mouse anti-Aggrecan (BC-3) antibody (1:50, MA3-16888; Thermo Fisher Scientific)
Rabbit anti-Col-II antibody (1:100, Cat. No. ab34712, Abcam)
Mouse anti-procollagen 1 N-Peptide (P1NP) antibody (1:250, MA5-51183, Invitrogen)
Mouse anti- osteocalcin (OCN) antibody (1:200, 33-5400, Invitrogen)

Donkey anti-Sheep IgG (H+L) Antibody, Alexa Fluor 594, (1:200, A11016, Invitrogen)
Goat Anti Rabbit IgG H&L (Alexa Fluor® 488) (1:200, ab150077, abcam)
Goat Anti-Mouse IgG H&L (Alexa Fluor® 647) (1:200, ab150115, abcam)
Goat anti-rabbit IgG (H + L) Alexa Fluor 647 antibodies (1:200, A21245, Invitrogen)
Goat anti-mouse IgG (H + L) Alexa Fluor 488 (1:200, A11017 Invitrogen)
Alexa Fluor 568 Phalloidin (1:200, A12380 Fisher Scientific)
Phalloidin-iFluor™ 488 Conjugate (1:200, 20549, Cayman Chemical Company)
DAPI (4',6-diamidino-2-phenylindole) (D1306, Invitrogen)

Validation

Validation statements available from manufacturer.
RUNX2- https://www.abcam.com/en-us/products/primary-antibodies/runx2-antibody-2b9-ab76956#drawer=publications
OSTERIX - https://www.abcam.com/en-us/products/primary-antibodies/sp7-osterix-antibody-ab22552#drawer=publications
VWF- https://www.ptglab.com/Products/vwf-Antibody-27186-1-AP.htm#publications
Fibronectin - https://www.rndsystems.com/products/human-rat-fibronectin-antibody_af1918#product-citations
BSP - https://www.abcam.com/en-us/products/primary-antibodies/bone-sialoprotein-antibody-ab52128#drawer=publications
CD31- https://www.abcam.com/en-us/products/primary-antibodies/cd31-antibody-jc-70a-ab9498#drawer=publications
Aggrecan - https://www.thermofisher.com/antibody/product/Aggrecan-Antibody-clone-BC-3-Monoclonal/MA3-16888
Coll-II - https://www.abcam.com/en-us/products/primary-antibodies/collagen-ii-antibody-ab34712#drawer=publications
P1NP - https://www.thermofisher.com/antibody/product/Procollagen-I-N-Peptide-Antibody-clone-C8-Monoclonal/MA5-51183
OCN - https://www.thermofisher.com/antibody/product/Osteocalcin-Antibody-clone-OC4-30-Monoclonal/33-5400

Anti-sheep AF594 - https://www.thermofisher.com/antibody/product/Donkey-anti-Sheep-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11016
Anti-rabbit AF488 - https://www.abcam.com/en-us/products/secondary-antibodies/goat-rabbit-igg-h-l-alexa-fluor-488-ab150077#drawer=publications
Anti-mouse AF647- https://www.abcam.com/en-us/products/secondary-antibodies/goat-mouse-igg-h-l-alexa-fluor-647-ab150115#drawer=publications
Anti-rabbit AF647 - https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21245
Anti-mouse AF488 - https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Secondary-Antibody-Recombinant-Superclonal/A28175
Phalloidin 568 - https://www.thermofisher.com/order/catalog/product/A12380
Phalloidin 488 - https://www.caymanchem.com/product/20549/phalloidin-ifluor%E2%84%A2-488-conjugate
DAPI - https://www.thermofisher.com/order/catalog/product/D1306

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Induced pluripotent stem cells (iPSCs, IMR90C4, Wi Cell Research Institute), Human adipose-derived stem cells (hADSCs, PT-5006, Lonza), MDA-MB-231 (MDA; gift from Dr. Danny Welch, University of Kansas), human lung fibroblasts (HLFs; CC2512, Lonza), human umbilical vein endothelial cells (HUVECs; C2519A, Lonza), human dermal fibroblasts (HDFs; PCS-202-012, ATCC).

Authentication

None of the cell lines were authenticated.

Mycoplasma contamination

Cell lines were not tested for mycoplasma contamination but no indication of contamination was observed.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

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

Inbred immunodeficient RNU athymic rats (both male and female), acquired at 5 weeks of age from Charles River Laboratories International, Inc. and cared for and aged at our animal facility until the age of 12 weeks.

Wild animals	No wild animals were used in this study.
Reporting on sex	In accordance with NIH guidelines on the inclusion of sex as a biological variable (SABV), this study utilized a total of 14 rats, comprising an equal distribution of both sexes with 7 males and 7 females.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	Procedures were approved by the Institutional Animal Care and Use Committee (IACUC, protocol #46591) of Penn State University.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A