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	Three-dimensional microCT images were created in Bone J (Version 1.2.1) using the Optimized Threshold Plugin for Image J.
Data analysis	Statistical analysis was performed using GraphPad PRISM (GraphPad Software, La Jolla,CA). scRNAseq analysis utilized Seurat/R, Harmony-based batch correction, MATLAB/scGEAToolbox

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

Data Availability: RNA-seq data generated in this study have been deposited in GEO under accession number GSE297901. Source data are provided with this paper. The datasets used and analyzed during the current study available from the corresponding author on reasonable request.

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	power test: A minimum sample size of 8 was determined via power analysis (1-β = 0.8; α = 0.05).
Data exclusions	No data was excluded
Replication	The regeneration studies were replicated independently 6 times each with greater than 64 digit samples (32 experimental and 32 control) over a period of 10 years.
Randomization	Random allocation based on growth factor treatment of entire litter. For genetic studies, mice were allocated into experimental groups based on genotype.
Blinding	Investigators were not blinded to group allocation during data collection and/or analysis as it was not relevant since the regenerative outcomes were anatomically based and compared to a highly reproducible failed regeneration response.

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	Primary antibodies: anti-CXCR4 (rat monoclonal, R&D MAB21651; 1:100), anti-RFP (Goat polyclonal, Origene AB1140-100; 1:1200), anti-ColX (rabbit polyclonal, Abcam 58632; 1:500), anti-ColII (rabbit polyclonal, Abcam ab34712; 1:200), anti-Acan (rabbit polyclonal,

EMD Millipore; AB1030; 1:300). Secondary antibodies: Alexa Fluor 568 goat anti-rabbit IgG (Invitrogen; A11011, 1:500), Alexa Fluor 568 goat anti-chicken IgG (Invitrogen, A11041, 1:500), Alexa Fluor goat anti-rabbit 488 IgG (Invitrogen, A11008, 1:500), Alexa Fluor 568 donkey anti-rabbit IgG (Invitrogen, A10042, 1:500), Alexa Fluor donkey anti-goat 488 IgG (Invitrogen, A11055, 1:500).

Validation

The induced digit regeneration response has been well documented, and immunohistochemical staining has been described in the neonate (Yu et al. 2012; Lee et al. 2013; Yu et al., 2019) and adult (Dawson et al. 2017) responses. Starting with the manufacturer's recommended protocol, positive and negative control sections (e.g. anti-collagen 2 on a cartilage sample verses a muscle sample) are used to determine specificity, optimization of antigen retrieval method (no retrieval, heat-induced epitope retrieval, or proteinase K retrieval), and antibody dilution. Primary antibody detection utilizes secondary antibodies (Alexa Fluor® 488 or 568). Using Slidebook software (Intelligent Imaging Innovation Inc., Denver, CO) background subtraction of the autofluorescent signal is performed to ensure the integrity of the Alexa Fluor® signal, or imaging using multiple fluorescent filters was performed to identify background signal from authentic signal.

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	Mus Musculus: Outbred CD1 (Crl:CD1(ICR), male and female mice were mated to obtain neonates for amputation and growth factor studies. Mice were obtained from Jackson Labs and bred in house at the Texas A&M Institute of Genomic Medicine. For lineage tracking: Prg4-GFP-CreERT2 mouse strain (Prg4tm1(GFP/cre/ERT2)Ab/J; JAX stock #022757) and the tomato reporter Ai9 line (Gt(ROSA)26Sortm9(CAG-tdTomato)Hze/J; JAX Stock #007909). These strains were obtained from Jackson Labs and bred in house at the Texas A&M Institute of Genomic Medicine.
Wild animals	N/A
Reporting on sex	Experiments were conducted on litters of neonatal mice blind to sex. There was a regenerative outcome in 100% of the mice compared to 0% of controls. Sex of the resulting mice was not recorded, however the data indicate that no sex based differences in results were observed.
Field-collected samples	N/A
Ethics oversight	Texas A&M University Institutional Animal Care and Use Committee. oversight Protocol number - AUP IACUC 2020-0267

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Plants

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