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	Capture and maximum intensity projection of confocal images: Zen Black 2.3 SP1 (Carl Zeiss), Zen Blue 3.7 (Carl Zeiss) Capture of Leica steremicroscopy images: LAS V4.8 (Leica) Computed Tomography (CT) imaging: Micro-CT system Latheta LCT-200 (Hitachi Aloka Medical Ltd.) Downloading sequence data: wget (version 1.14, GNU), fastq-dump (version 2.9.0, NCBI) Barcoded single-cell cDNA library synthesis and sequencing: Chromium Single Cell 3' Reagent Kits v3.1 Dual Index (10X Genomics) and Hiseq platform (Illumina)
Data analysis	Measurement of limb skeletal elements: fiji software (ImageJ) Measurement of joint interspace distance: cellSens software (Olympus) 3D rendering of Computed Tomography slices: VGStudio MAX2.0 software (Volume Graphics GmbH) Analysis of joint fusion in Computed Tomography images: DICOM viewer (RadiAnt) Processing and de-multiplexing raw single cell sequencing data: Cell Ranger version 7.1.0 (10× Genomics) Analyses of single cell sequencing data: Seurat R package v4.3.0

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

The authors declare that all data supporting the findings of this study are available within the article, Supplementary Data Information Files and Zenodo (doi: 10.5281/zenodo.11127881) or from the corresponding author upon reasonable request.
Chicken forelimb single cell RNA sequence data of stage 24 (SRA accession numbers: SRR14570167 to SRR14570174) and stage 27 (SRA accession numbers: SRR14570175 to SRR14570178) were downloaded from the Sequence Read Archive (SRA).
Single cell RNA sequence data that support the findings of this study have been deposited in the DNA Data Bank of Japan (DDBJ) under accession codes DRA014432 and DRA017391.

Human research participants

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

Reporting on sex and gender	The study did not involve human research participants.
Population characteristics	See above.
Recruitment	See above.
Ethics oversight	See above.

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	No statistical methods were used to predetermine sample size. The sample size was chosen empirically to provide sufficient data for comparison.
Data exclusions	For in vivo experiments: no data was excluded from the analysis. An exception was made for chicken electroporation, in which any embryos presenting malformations were not used. In addition, chicken embryos that were not immobilised following DMB treatment were discarded.
Replication	All attempts of replication were successful.
Randomization	Chicken embryos were staged and then randomly allocated into control or experimental groups.
Blinding	Investigators were not blinded during data collection and/or analysis.

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

Primary: anti-MYH1E Antibody (MF-20 clone; hybridoma cells have been provided by the Developmental Studies Hybridoma Bank), anti-cleaved caspase-8 antibody (9664, Cell signaling technology), anti-8-oxoguanine antibody (ab64548, Abcam)
Secondary: goat anti-mouse IgG, HRP-conjugated antibodies (HAF007, Funakoshi), goat anti-rabbit IgG Alexa Fluor-488 conjugated antibody (ab150077Abcam) ,goat anti-mouse IgG Alexa Fluor-488 conjugated antibody (Invitrogen), sheep anti-fluorescein-AP antibody (Roche)

Validation

The MF-20 antibody is widely used as a muscle marker in avians and have been extensively validated (https://dshb.biology.uiowa.edu/MF-20). We have validated our MF-20 supernatant by ensuring that the staining was specific to muscle tissue in all samples. The anti-cleaved caspase-8 antibody (more than 5,000 citations were introduced in https://www.cellsignal.jp/products/primary-antibodies/cleaved-caspase-3-asp175-5a1e-rabbit-mab/9664) and anti-8-oxoguanine antibody are widely used as a active caspase-8 marker and DNA damage marker (more than 20 citations were introduced in https://www.citeab.com/antibodies/771610-ab64548-anti-oxoguanine-8-antibody-2q2311), respectively.

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

Commercially available, White Leghorn chicken (Gallus gallus) eggs were incubated at 38 °C and staged as described before. Fertilised emu (Dromaius novaehollandiae) eggs were purchased from Kakegawa Kachoen and Okhotsk Emu Pasture, incubated at 36.5 °C and staged as described. Wing samples of adult emu were provided from Tokyo Nodai Bioindustry Corporation.

Wild animals

The study did not involve wild animals.

Reporting on sex

The sex of the animals was unknown.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

All animal work was performed in accordance with guidelines for animal experiments of the Tokyo Institute of Technology, Kumamoto University, and The Jikei University School of Medicine, and the experimental protocols were approved by the committees of Tokyo Institute of Technology and Kumamoto University.

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