

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 [Authors & Referees](#) and the [Editorial Policy Checklist](#).

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
- An indication of 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 statistics 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
- Clearly defined error bars
State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on [statistics for biologists](#) may be useful.

Software and code

Policy information about [availability of computer code](#)

Data collection

Data analysis

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/reviewers upon request. 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

All raw sequencing data for all RNAseq libraries (Figure 3) and merged reference transcriptomes are available online (NCBI BioProject# PRJNA497902). Sequences of in situ probe templates for Figures 1B, 1C, 2A, and 2C are available through GenBank accession codes found in the methods.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf](https://www.nature.com/authors/policies/ReportingSummary-flat.pdf)

Life sciences study design

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

Sample size	For chicken RNAseq replicates, a pre-determined number of a cells was collected per kit instructions for the SMART-seq v4 ultra low input RNA kit (Cranial Rep 1= 1534 cells, Cranial Rep 2= 1530 cells, Cranial Rep 3= 1527 cells; Trunk Rep 1= 1500, Trunk Rep 2= 721, Trunk Rep 3= 958). For lamprey dissections, approximately 100 embryos were dissected for each replicate of each axial level for RNAseq libraries. No statistical tests were used to determine sample size. The sample size provided enough RNA for subsequent library preparation.
Data exclusions	No datasets were excluded from this analysis.
Replication	Chicken RNAseq libraries were collected in triplicate for biological replicates. Lamprey RNAseq libraries were collected with replicates, as well, for biological replicates. All attempts at replication were successful. For in situ hybridization, embryos were pooled from different breeding pairs (fish), brooding stocks (skates), or embryo batches (lamprey) to ensure replication of results in multiple fixed collections. All in situ expression patterns were replicated 100% over multiple rounds of in situs.
Randomization	Chicken eggs obtained from a local chicken farm were incubated in multiple incubators over different days to account for inter-batch variability. Lamprey tissues were collected from different breeding events to account for inter-batch variability.
Blinding	Different batches of chicken embryos were separately incubated in different incubators and electroporated with fresh DNA reporter construct solution. Different breeding pairs for lamprey were used for tissue collections to blind for batch variability. For all trunk libraries, dissections were made at the same somitic levels and not based on reporter expression to ensure blinding in sample collection.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Unique biological materials
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants

Methods

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used

Validation

Animals and other organisms

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

Laboratory animals

Wild animals

Wild animals

Commission, in cooperation with its partners at the USGS Hammond Bay Biological Station, USFWS Marquette Biological Station, and the Department of Fisheries and Oceans, Canada. They were sent overnight in chilled, oxygenated water to the lamprey facility at the California Institute of Technology, where they were maintained under the parameters set in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, with protocols approved by the Institutional Animal Care and Use Committees of the California Institute of Technology (lamprey, Protocol #1436-17). After spawning, the captive adult lamprey died of natural causes.

Field-collected samples

Lamprey embryos were produced by in vitro fertilization at the California Institute of Technology lamprey facility, using captive gravid lamprey (*Petromyzon marinus*) provided by the Great Lakes Fishery Commission, in cooperation with its partners at the USGS Hammond Bay Biological Station, USFWS Marquette Biological Station, and the Department of Fisheries and Oceans, Canada. Lamprey were maintained with a water temperature of 10-18 degrees C on a 15:9h light:dark cycle.

All skate embryos were collected from wild-caught brood stock housed at the Marine Resources Centre of the Marine Biological Laboratory (MBL) in Woods Hole. Eggs were maintained in a flow-through seawater system with a water temp of 15 degrees C, on a 12h:12h light:dark cycle. Prior to fixation, all embryos were euthanized with an overdose of buffered MS-222 (1g/L in seawater). All embryos collection was performed in accordance with protocols approved by the MBL Institutional Animal Care and Use committee.

Fertilized chicken eggs were obtained from a local farm in Sylmar, CA. Developing chicken embryos were maintained at a temperature of 37 degrees C.

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

Chicken embryos were dissected in Ringers and washed thrice in chilled 1x PBS. The tissues were dissociated in Accumax (Innovative Cell Technologies, Inc.) for 15 minutes at 37°C.

Instrument

Sony SY3200 Cell Sorter

Software

WinList from Verity Software House

Cell population abundance

Collected cells were obtained and analyzed on a hemocytometer for fluorescence and viability to ensure 100% purity.

Gating strategy

Gating was assigned according to standard protocols. Clear differentials between GFP+ and GFP- populations were observed.

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