

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

The sequencing reads were undertaken with MGISEQ T7 platform.

Data analysis

For spatial transcriptomics, we used SAW(v7.1) and BSTMatrix (v2.3) to generate spatial gene expression matrices. Data normalization, scaling, and bins clustering were processed using the R package Seurat(v4.0.6). We used CARD to transfer of cell-type annotations from scRNA-seq to spatial transcriptomics and infer correlations in cell-type proportions across spatial locations between pairs of cell types.

For the raw sequencing data processing, we used the DNBelab C Series scRNA analysis software(v2.0.5) ad CellRanger (v7.0.1) software to align scRNA-seq/snRNA-seq data to the chicken, turtle and duck reference genome. We used Seurat(v4.0.6) to create a Seurat object and the the R packages DoubletFinder (v2.0.3) to remove low-quality cells and doublets respectively.

For the cell clustering, we first used the R package Seurat(v4.0.6) to perform normalization and scaling. Harmony (v0.1.1) was used to correct four batch effects (sources, methods, platforms, and individuals).

For the pseudotime trajectory analysis, we used the R package Monocle2 (v2.26) and Slingshot (v2.12) to infer the state transition of the cell types/subtypes.

For the cellular communication analysis, we used the R package CellChat (v1.6.1) .

For the analysis of similarity of cell types across amniotes, we used MetaNeighbor(v1.24.0) to systematically assess the transcriptional similarity between cell types across species.

For the integration of transcriptomic data of immune cells from different species, we used “SelectIntegrationFeature” function of Seurat (v4.0.6). Integration anchors were identified by “FindIntegrationAnchors” function(reduction= “CCA” and normalization.method = “SCT”).

Weighted gene co-expression network analysis (WGCNA) was performed with functions in the WGCNA R package(v1.73)

For the differentially expressed genes analysis between species, MAST(v1.30.0) was used.

For the gene regulatory network analysis, GENIE3(v1.26.0) and SCENIC(v1.12) were applied to infer the gene regulatory network and calculate the TF activity scores.

For the function enrichment analysis, we used David (<https://david.ncicrf.gov/summary.jsp>) and Metascape (<https://metascape.org>).

All codes for processing of the single-cell RNA-sequencing data have been deposited to GitHub and available through this URL: <https://github.com/chicken123cc/singlecell.chicken> and at Zenodo (10.5281/zenodo.18795716).

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 scRNA-seq generated in this study have been deposited in the the Gene Expression Omnibus (GEO) repository under accession code PRJNA1125639 and PRJNA1128021(<https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1125639>; <https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1398958>). The spatial transcriptomic profiling of chicken embryo have been deposited in China National GeneBank DataBase under accession code CNS1441136 (https://db.cngb.org/data_resources/sample/CNS1441136). Processed datasets and spatial transcriptomic profiles of the chicken bursa of Fabricius and cecal tonsil have been deposited in the National Genomics Data Center (<https://ngdc.cncb.ac.cn/search/all?q=PRJCA059001>).

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

NA

Reporting on race, ethnicity, or other socially relevant groupings

NA

Population characteristics

NA

Recruitment

NA

Ethics oversight

NA

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](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

In total, we analyzed single-cell and single-nucleus RNA-seq data covering 35 tissues from 16 chickens, together with spatial transcriptomic data from a chicken embryo, chicken bursa of Fabricius, and cecal tonsil. We also profiled scRNA-seq data from 3 turtles (14 tissues) and 2 ducks (3 tissues).

Data exclusions

No tissue sample was excluded in our analysis process. For cells/nuclei in our analysis, cells were excluded for downstream analysis if the number of detected genes was below 500 or exceeded the 95th percentile threshold, or if the percentage of mitochondrial genes exceeded than 15%.

Replication	In this study, the samples were grouped by tissues. For the single-cell data, chicken tissues had at least three biological replicates, duck tissues two replicates, and turtle tissues at least two replicates. For the spatial transcriptomic data, one biological replicate was used per tissue.
Randomization	Randomization was not relevant in this study. The newly generated data in this study was used after data exclusions listed above. Samples were grouped by tissues.
Blinding	The blinding study design may be not applicable in this study. All the newly generated data in this study were from healthy individuals and were analyzed using a uniform pipeline.

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		Methods	
n/a	Involved in the study	n/a	Involved in the study
☒	☐ Antibodies	☒	☐ ChIP-seq
☒	☐ Eukaryotic cell lines	☒	☐ Flow cytometry
☒	☐ Palaeontology and archaeology	☒	☐ MRI-based neuroimaging
☐	☒ Animals and other organisms		
☒	☐ Clinical data		
☒	☐ Dual use research of concern		
☒	☐ Plants		

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	This study did not involve the laboratory animals.
Wild animals	This study did not involve the wild animals.
Reporting on sex	Three male and two female Hy-Line Brown embryos (approximately 13-21 days) and two male and six female adult Hy-Line Brown chickens (Gallus gallus, approximately 12-14 weeks old) were purchased from Yangling Julong Poultry Company Limited and used for sample collection. Two female Peking ducks (Anas platyrhynchos, approximately 24 weeks old) were purchased from Beijing Nankou Duck Breeding Technology Company Limited and used for sample collection. Three adult red eared slider turtles (Trachemys scripta elegans, approximately 5 years old) were acquired from Hunan Ruimeng Technology Company Limited.
Field-collected samples	In this study, tissue samples were collected, including Heart, Liver, Oviduct, Uterus, Cerebrum, Cerebellum, Thymus, Gizzard, Leg muscle, Pancreas, Cecal tonsil, Cecum, Lung, Testis, Kidney, Esophagus, Bursa of Fabricius, Proventriculus, Trachea, Bone, Bone marrow, Spleen, Skin, Retina, Whole blood, Duodenum, Jejunum, Ileum, Breast muscle, Blood vessel, Crop, Adrenal gland, Adipose.
Ethics oversight	All animal experiments were approved by the Institutional Animal Care and Use Committee of Hunan Agricultural University (approval number: 2024-159), and all procedures were carried out in accordance with the approved study protocol. The sampling process was rigorously monitored by members of the Institutional Animal Care and Use Committee of Hunan Agricultural University.

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

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA