

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	A total of 1139 avian genome assemblies were retrieved from the NCBI database, covering the period from January 2018 to May 2024. All high-throughput sequencing was performed on the Illumina platforms.
Data analysis	Software and web server including: MAFFT (v7.490), CRISPResso2 (v2.0.40), R2R (v1.0.7), BWA (v0.7.17), IQ-TREE 2 (v2.3.5), BLAST+ (v2.15.0), Trim Galore (v0.6.10), RepeatMasker (v4.2.1), CD-HIT (v4.8.1), ORFfinder (v0.4.3), InterProScan (v5.56-89.0), WebLogo (v3.6.0), BBMap (v39.08), TVBOT (https://www.chipshot.online/tvbot.html), RNAfold (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) and RNAalifold (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAalifold.cgi). Analytic code for the sequencing data is publicly available at Github: https://github.com/YanpingHu/avian_R2 .

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 sequencing data involved in this research have been submitted to GSA-Human (Genome Sequence Archive for Human) — a platform under the Beijing Institute of Genomics, Chinese Academy of Sciences (available at <https://ngdc.cncb.ac.cn/gsa-human/>), and the accession number is HRA013312.

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	-
Reporting on race, ethnicity, or other socially relevant groupings	-
Population characteristics	-
Recruitment	-
Ethics oversight	-

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	Sample sizes are indicated in the figure legends in accordance with previous literature and standard in the field of genome engineering.
Data exclusions	No data were excluded.
Replication	Replication times are indicated in the figure legends. Experiments in mammalian cells were performed with at least 3 biological replicates to ensure reproducibility.
Randomization	Mammalian cells were maintained under identical culture conditions, and no randomization was applied.
Blinding	Mammalian cells were maintained under identical culture conditions, and blinding was not applied.

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-FMC63 scFv Monoclonal Antibody, Alexa Fluor 647 (BioSwan Lab, Cat. No. 300402, M19H clone, Lot. No. 241111)
Validation	https://www.bioswan.com/index.php?m=search&c=index&a=init&siteid=1&q=300402

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	HEK293T (CL-0005, Pricella); primary human foreskin fibroblasts (HFF-23003, the Chinese National Stem Cell Resource Center); primary human skeletal muscle (HSKM) myoblasts (A12555, Gibco).
Authentication	HEK293T cell line was authenticated by the supplier using STR analysis. Primary human foreskin fibroblasts and primary human skeletal muscle (HSKM) myoblasts were authenticated by the supplier via morphological analysis.
Mycoplasma contamination	Cell lines were not further tested for mycoplasma contamination after purchase.
Commonly misidentified lines (See ICLAC register)	All cell lines used in this study are not in this list.

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	Female C57BL/6J mice (8–10 weeks old) were obtained from the breeding core of the Animal Center, Institute of Zoology, Chinese Academy of Sciences (Beijing, China). The animals were housed in groups in ventilated cages within a barrier facility maintained under specific pathogen free (SPF) conditions, subjected to a 12:12 h light/dark photoperiod at 20–26°C and 30%–60% relative humidity. Autoclaved standard rodent chow and water were provided ad libitum.
Wild animals	No wild animals were used in this study.
Reporting on sex	Female
Field-collected samples	-
Ethics oversight	All animal experiments were approved by the Institutional Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences (IOZ, CAS), and the reference number for approval is IOZ-YSB-2025-29.

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

Plants

Seed stocks	No plants were used in this study.
Novel plant genotypes	No plants were used in this study.
Authentication	No plants were used in this study.

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	Adherent cells (HEK293T, HFF, and HSKM myoblasts) were detached with 0.25% trypsin (Gibco) at 37 °C. The cells were then resuspended in PBS and transferred to tubes for flow cytometric analysis. Suspension cells (T and NK cells) were centrifuged at 300 g for 5 min and resuspended in PBS. The resuspended cells were transferred to flow tubes for flow cytometric analysis.
Instrument	BD FACSAria Fusion (Beckman Coulter) and BD LSRFortessa (Beckman Coulter).
Software	Data collection: BD FACSAria Fusion (Beckman Coulter) and BD LSRFortessa (Beckman Coulter) Flow Cytometer software. Data analysis: FlowJo(v10.8.1) and GraphPad Prism 8.0.1.
Cell population abundance	The percentage of positive cell populations was quantified by flow cytometry based on gating strategies and presented as the proportion of total or parent cells.
Gating strategy	For all cell types, live cells were gated by FSC-A and SSC-A and then single cells were gated by FSC-H and FSC-A. For efficiency analysis, GFP+ cells were gated by SSC-A and FITC-A; For dual-fluorescence reporter, GFP+/mCheery+ cells were gated by FITC-A and PE-Texas Red-A.

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