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Reporting Summary

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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	No software was used.
Data analysis	CHOPCHOP v2 and Primer-BLAST were used for designing quide RNAs and primers. Analyses were performed using STAR aligner (v2.7.2b and v2.7.11a), DESeq2 (v1.24.0), R (v4.1 and v4.4.0), vsn (v3.52.0), Cutadapt (v4.4 and v4.6), TargetScan (v7.0 and v6.0), UMI-tools (v1.1.4), Bowtie2 (v2.5.1), minimap2 (v2.27-r1193), Sniffles2 (v2.2), samtools (v1.2), BLAST+ (v2.4), edgeR (v4.2.0), Clustal Omega, Primer-BLAST, IGV, and a modified Ribocutter pipeline (2021 preprint version). Gene annotations and ortholog mappings were retrieved using BioMart (Ensembl releases 106, 109, and 113). Custom codes developed in the study are available at https://github.com/amirshahr/MIR2954.

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

Raw sequencing data (mRNA-seq, small RNA-seq, DNA-seq, and Ribo-seq) have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1079296 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1079296) . Processed data for mRNA-seq and small RNA-seq are available in the Gene Expression Omnibus (GEO) repository under the same BioProject accession. Dosage sensitivity scores are available from Zenodo (zenodo.org/

records/6347673). The list of ohnologs is available from the OHNOLOGS database (ohnologs.curie.fr). Gene phylogenetic ages are available from GenTree (gentree.ioz.ac.cn). Genome assemblies, 3′ UTR sequences, and 1:1 orthologs were retrieved from Ensembl releases 109–113 (ensembl.org) and BioMart (ensembl.org/biomart/martview/64183b5d28d21d3d657ef39fbb9ad5b2). The following genome assemblies were used: chicken (Gallus gallus) bGalGal1.mat.broiler.GRCg7b, GRCg6a, and galGal4; zebra finch (Taeniopygia guttata) bTaeGut1_v1.p; crocodile (Crocodylus porosus) CroPor_comp1; ostrich (Struthio camelus) ASM69896v1; human (Homo sapiens) GRCh38.p14; and mouse (Mus musculus) GRCm39.

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 determine sample size. Sample size was based on the number of individuals available (see Supplementary Table 2).
Data exclusions	One batch of eggs was not used due to incubator malfunction.
Replication	Each tissue type from each embryo was represented by 3 replicates derived from 3 individuals, respectively. All attempts at replication were successful.
Randomization	Individuals collected from heterozygous parents were ensured a random distribution of the dissected embryos in terms of genotype and sex.
Blinding	The collection of samples and estimation of phenotypes were performed blindly, with the experimenter unaware of the individuals' genotype and sex.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	The primordial germ cells were derived from chicken embryo at embryonic day 2.5.
Authentication	The cell-lined that was used in this study was authenticated based on it appearance and its ability to propagate in the host's gonads.
Mycoplasma contamination	No test for Mycoplasma contamination was performed on the cell lines. The chicken lines used to derive PGCs had been routinely tested and were free of avian mycoplasma.
Commonly misidentified lines (See ICLAC register)	None

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Both male and female adults and embryos of domestic chickens (Gallus gallus domesticus, Hy-Line and Red Junglefowl breeds) were used in this study. Adult chickens, aged between 6 and 24 months, were used for breeding purposes and for generation of riboseq libraries. Chicken embryos were collected at embryonic days 3, 4, 5, 7, 12, 13, and 14. In addition, one adult female mouse brain (Mus musculus, strain: CD-1, RjOrl:SWISS; RRID:MGI:5603077) was used.
Wild animals	No wild animals were used in the study.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	All animal procedures were conducted in compliance with national and international ethical guidelines and regulations. Mouse experiments were approved by the local animal welfare authorities at Heidelberg University Interfaculty Biomedical Research Facility (T-64/17). Chicken experiments were conducted under UK Home Office license PP9565661 and approved by the Roslin Institute Animal Welfare and Ethical Review Board Committee, and Linköping Council for Ethical Licensing of Animal Experiments (288-2019). Mice (Mus musculus, strain: CD-1, RjOrl:SWISS, RRID:MGI:5603077) were purchased from Janvier Labs (France) and euthanized by cervical dislocation. All chicken (Lohmann white; Gallus gallus) management, maintenance, and embryo manipulation followed the relevant regulatory guidelines.

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