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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	No software was used.
Data analysis	The clean reads of resequencing data were mapped onto the chicken reference genome (GRCg6a) with the Burrows-Wheeler Aligner (BWA v.0.7.17r1188). Mapping results were sorted and de-duplicated using SAMtools v.1.3.1, and were reordered and calibrated for the highest quality mapping reads using GATK v3.8. PLINK v1.90 package was implemented to control the quality of SNPs. UnifiedGenotyper of GATK v3.8 software was implemented to calling SNPs of the high quality mapping data from 90 different breeds of chickens. SNPs with genotype missing rate < 30% were imputed using the Beagle v5.0 procedure. VCFtools v0.1.16 was uesd for filtering SNPs. SNPs were annotated by SNPEff v 4.1 program. Association analyses of egg production traits were performed using the MLM in the GCTA v1.92.2. haplotype-based GWAS was performed based on MLM using R package lme4qtl v0.2.266. The CCA was calculated by cancor function in R package stats. Individual-based NJ tree was constructed by using PHYLIP v.3.69, and visualized using MEGA v10.0. The parameter R2 for LD was calculated for pairwise SNPs using PopLDdecay v3.41. FST and log2(π-ratio) approachs implemented in vcftools v.0.1.16. Clean reads of RNA-seq were aligned to the chicken galGal 6 reference genome using HISAT2 v2.2.1. A co-expression network analysis was performed using the WGCNA v1.72-1 package in R. DESeq2 v4.0.2 were used for differential expression analysis of transcriptome data. Cytoscape v3.7.1 was used for visualization of molecular network construction.

SPSS 20.0 was used to test the statistical significance of two or more groups.
"https:// github.com/marcus-seldin/QENIE" were an analytical method that has been reported for the identification of key endocrine factors regulating egg production via inter-tissue crosstalk.

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 resequenced raw data from 888 Gushi hens were deposited in the Genome Sequence Archive (GSA) with accession number PRJCA021392 (https://ngdc.cncb.ac.cn/gsa/search?searchTerm=PRJCA021392). The RNA-seq raw data of 5 tissue types were deposited in NCBI Sequence Read Archive with accession number PRJNA893445 (https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA893445) and PRJNA953784 (https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA953784). All other data supporting the findings of this study are available within the article and its Supplementary Information files. A reporting summary for this Article is available as a Supplementary Information file. Source data are provided with this paper.

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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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	900 non-duplicate individuals of Gushi hens with multiple egg-laying phenotypes were used to trace the genetic signals association with egg production traits. According to the phenotype statistics of 13 egg production traits, most of them followed normal distributions. According to the theory of statistics, sample size is depended on the population standard deviation (SD) of the trait, confidence coefficient and allowable error by simple random sampling. The maximum SD in egg production traits are 12.62. The sample size 260 could be acceptable under the conditions with the confidence level 99% and the allowable error 2. The sample size 900 is large enough to ensure the accuracy of data

analysis.
A total of 80 samples from five tissue types (hypothalamus, pituitary, ovary, liver and abdominal fat) of 43-week-old Gushi hens from high- (GS43wH, n=8) and low-yield group (GS43wL, n=8) were collected for RNA-Seq analysis. Previous studies have shown that when the number of biological replicates was ≥ 3, the detection rate of low-expressed differential genes could be improved (Genome Biol, doi: 10.1186/gb-2013-14-9-r95; Nat Biotechnol, doi: 10.1038/nbt.2450). The 16 samples from each tissue in this study ensured the relative accuracy of WGCNA analysis results.

Data exclusions

In 900 Gushi hens for genome-wide association study, 12 individuals were excluded according to the quality control condition: sample call rate >97%, SNP call rate < 95%, minor allele frequency (MAF)<0.01 and Hardy-Weinberg equilibrium P<1×10-6.
Two RNA samples of hypothalamus that failed quality inspection before building the mRNA libraries were removed.

Replication

We conducted three separate tests, and all tests at replication were successful.

Randomization

The sixteen 43-week-old hens used for transcriptome sequencing were grouped according to high or low egg production, while pullets at 12 weeks of age were randomized for liver tissue-specific overexpression tests.

Blinding

In this study, samples for multi-tissue transcriptome sequencing were divided into high-yield group and low-yield group according to egg number of 43-week-old Gushi hens to screen the hub genes that affect egg production. If blinding was applied to individuals with a limited sequencing sample size, genes that contributed more to the egg-laying phenotypes might not be obtained.

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

Antibody	supplier name	catalog number	as applicable
anti-TFPI2	Homemade antibody		Western Blot
anti-CYP11A1	Cusabio (Wuhan, China)	PA006389LA01HU	Western Blot
anti-CYP19A1	ABclonal (Wuhan, China)	A12684	Western Blot
anti-STAR	ABclonal (Wuhan, China)	A16432	Western Blot
anti-GAPDH	Proteintech (Wuhan, China)	60004-1-Ig	Western Blot
anti-GnRH1	Cloud-clone (Wuhan, China)	PAA843Ga01	Immunofluorescence
anti-FSHR	ABclonal (Wuhan, China)	A3172	Immunofluorescence

Validation

anti-CYP11A1, anti-CYP19A1 and anti-STAR (J Anim Sci Biotechnol, doi: 10.1186/s40104-022-00697-0)
anti-GAPDH (Nature, doi: 10.1038/s41586-022-05510-6)
anti-GnRH1 (ENVIRON RES, doi: 10.1016/j.envres.2014.07.006)
anti-FSHR (J INTEGR AGR, doi.org/10.1016/j.jia.2023.06.038)
anti-TFPI2 (J INTEGR AGR, doi.org/10.1016/j.jia.2023.06.038)

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Chicken primary hypothalamic neuron cells from 17-day-old chicken embryos that have not been sexed.
Chicken pituitary cells from 17-day-old chicken embryos that have not been sexed.
Chicken pituitary cell culture from the stratum granulosum of the 6-12 mm follicles of 30-week-old hens.

Authentication

The authenticity of primary cell culture of chicken gonad axis was confirmed by cellular morphology and immunofluorescence identification of marker genes.

Mycoplasma contamination

The cell lines were not tested for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

None

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	pcDNA3.1-EGFP expression vector and pcDNA3.1-3×flag expression vector, which inculde CMV enhancer promoter and SV40 for stable and transient expression at high levels in animal cell, were purchased from Invitrogen. Liver-specific overexpression recombinant AAV9 vector of chicken APOA4 (AAV9-TBG-APOA4-ZsGreen) and abdominal fat-specific overexpression recombinant AAV9 vector (AAV9-FABP4-ANGPTL2-ZsGreen) were constructed, packaged, and purified by Hanbio Biotechnology (Shanghai, China). The experimental animals used in this study were hens of Gushi chicken, a dual-purpose egg and meat type indigenous breed (NLPGRC 2011), among which the 43-week-old hens were used for transcriptome sequencing and the 12-week-old hens were used for liver tissue-specific overexpression test in vivo. NLPGRC (National Livestock and Poultry Genetic Resources Commission). 2011. Annals of Livestock and Poultry Genetic Resources of China: Annals of Poultry. China Agriculture Press, China. (in Chinese).
Wild animals	The study did not involve wild animals.
Reporting on sex	Only female was considered in this study design, because the egg production traits are sex-limited traits and can only be manifested in hens.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal experimental protocols were approved by the Institutional Animal Care and Use Committee of Henan Agricultural University (protocol number 11-0085). The methods were carried out in accordance with the approved guidelines.

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

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*