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Supplementary Materials for

An atlas of regulatory elements in chicken: A resource for chicken genetics and genomics

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Figs. S1 to S16
Legends for tables S1 to S12
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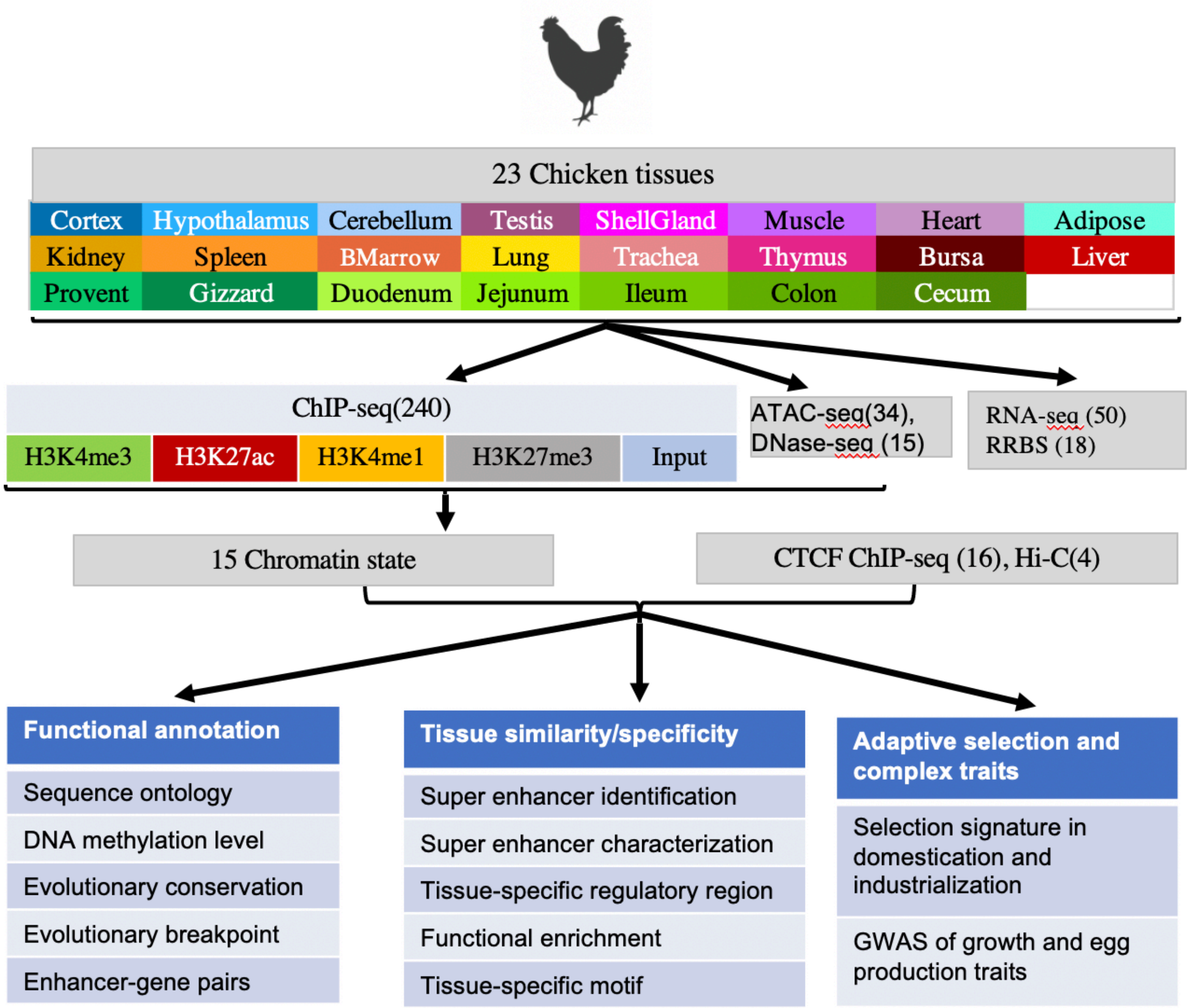
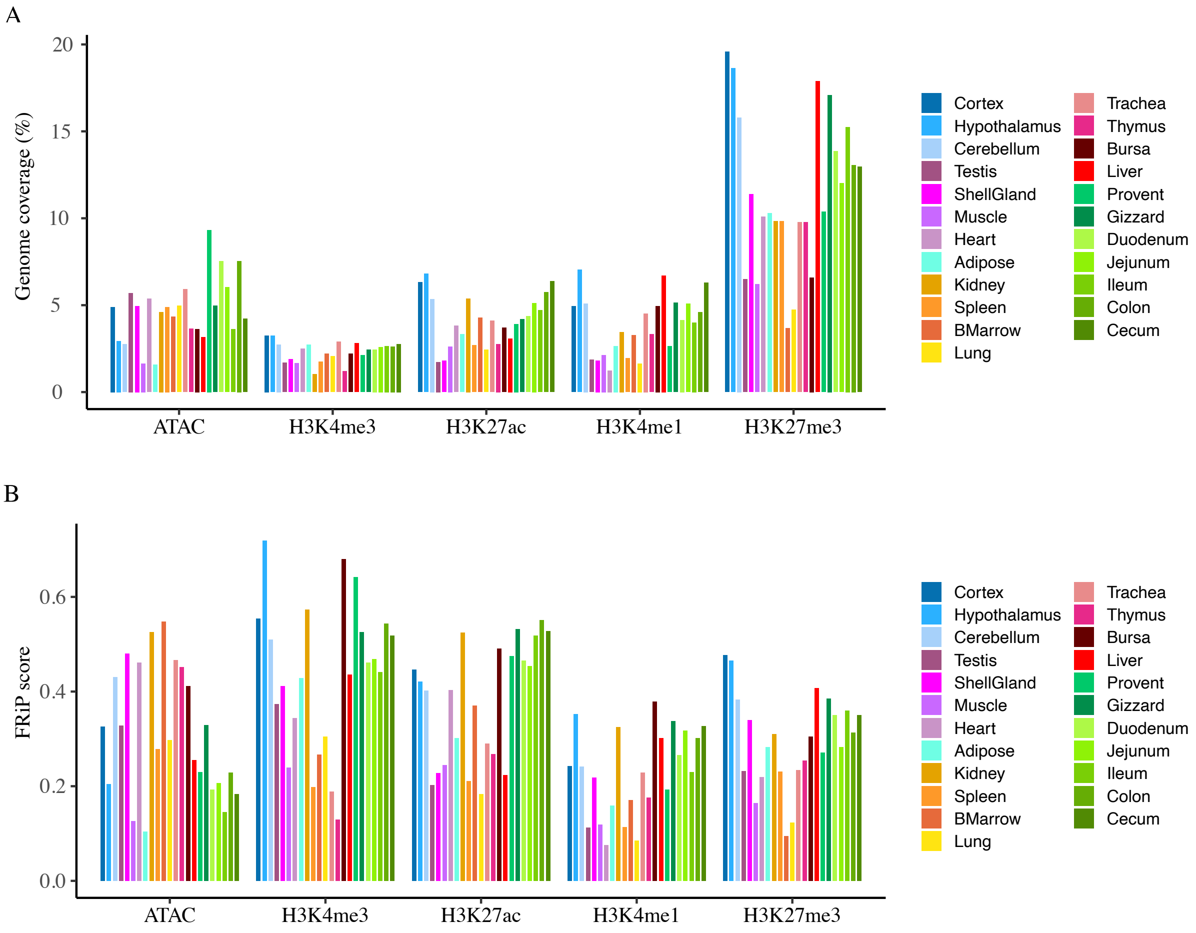
Other Supplementary Material for this manuscript includes the following:

Tables S1 to S12

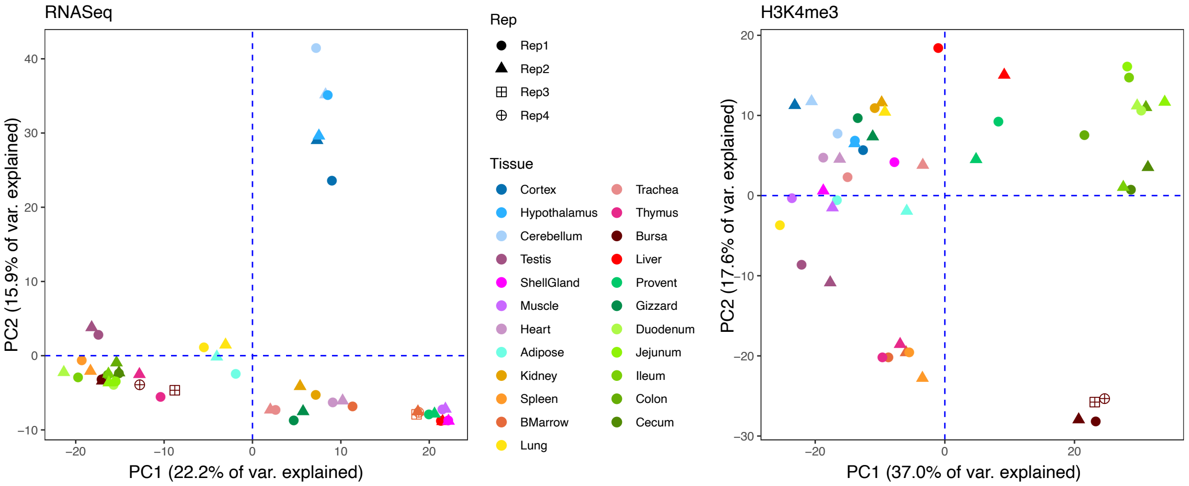
Supplementary Figures

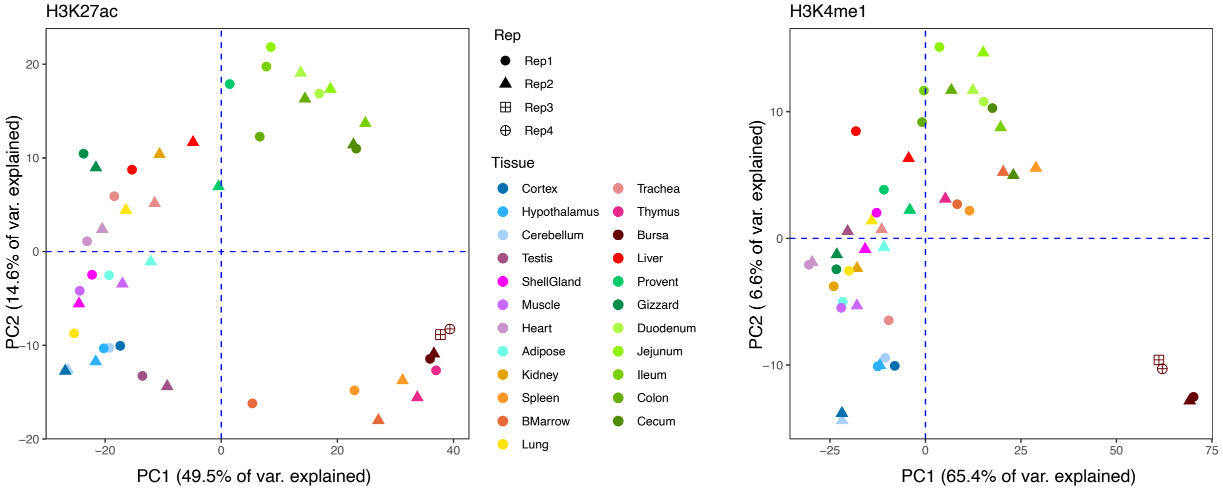


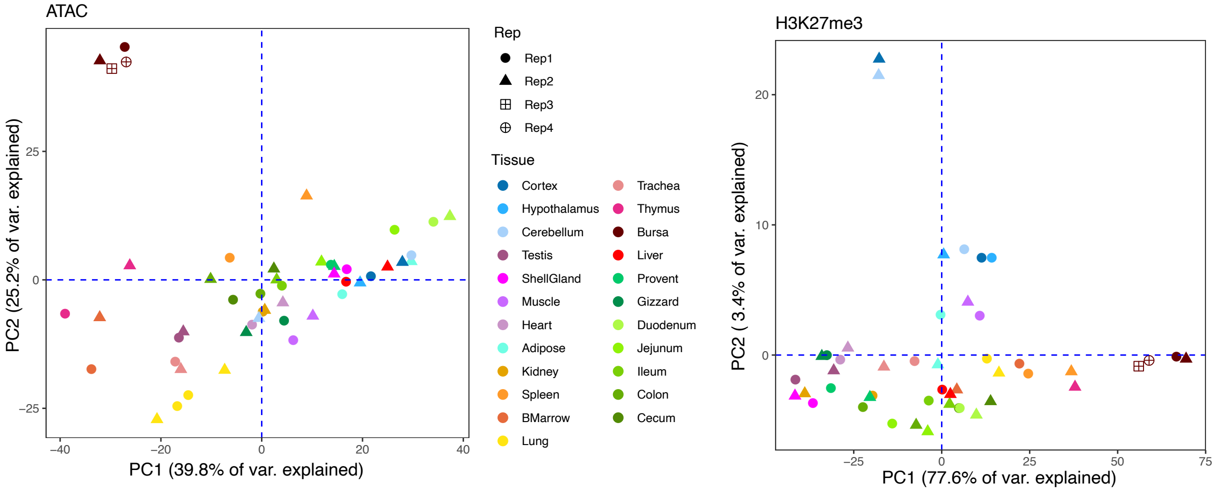
Supplementary Fig. 1. **The overview of the current study design.** This study collected and systematically integrated multi-omics data from 23 tissues in chickens, including ChIP-seq of histone modifications, ATAC-seq, DNase-seq, RNA-seq, RRBS, CTCF ChIP-seq, and Hi-C datasets. The numbers in the brackets are the number of samples for the corresponding assays.



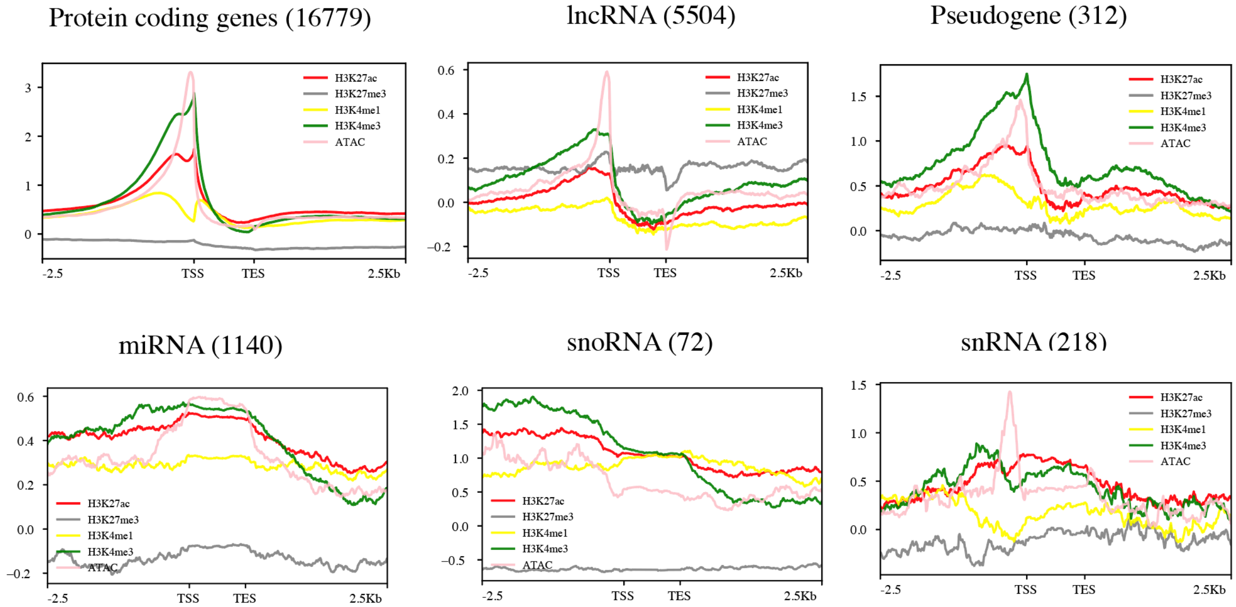
Supplementary Fig. 2. (**A**) The average genome coverage of peaks for each epigenetic marks in each tissue. (**B**) The average Fraction of Reads in Peaks (FRiP) for epigenetic marks across 23 tissues.


RNASeq
PC1 (22.2% of var. explained)
PC2 (15.9% of var. explained)
Rep
Rep1
Rep2
Rep3
Rep4
Tissue
Cortex
Hypothalamus
Cerebellum
Testis
ShellGland
Muscle
Heart
Adipose
Kidney
Spleen
BMarrow
Lung
Trachea
Thymus
Bursa
Liver
Provent
Gizzard
Duodenum
Jejunum
Ileum
Colon
Cecum
H3K4me3
PC1 (37.0% of var. explained)
PC2 (17.6% of var. explained)

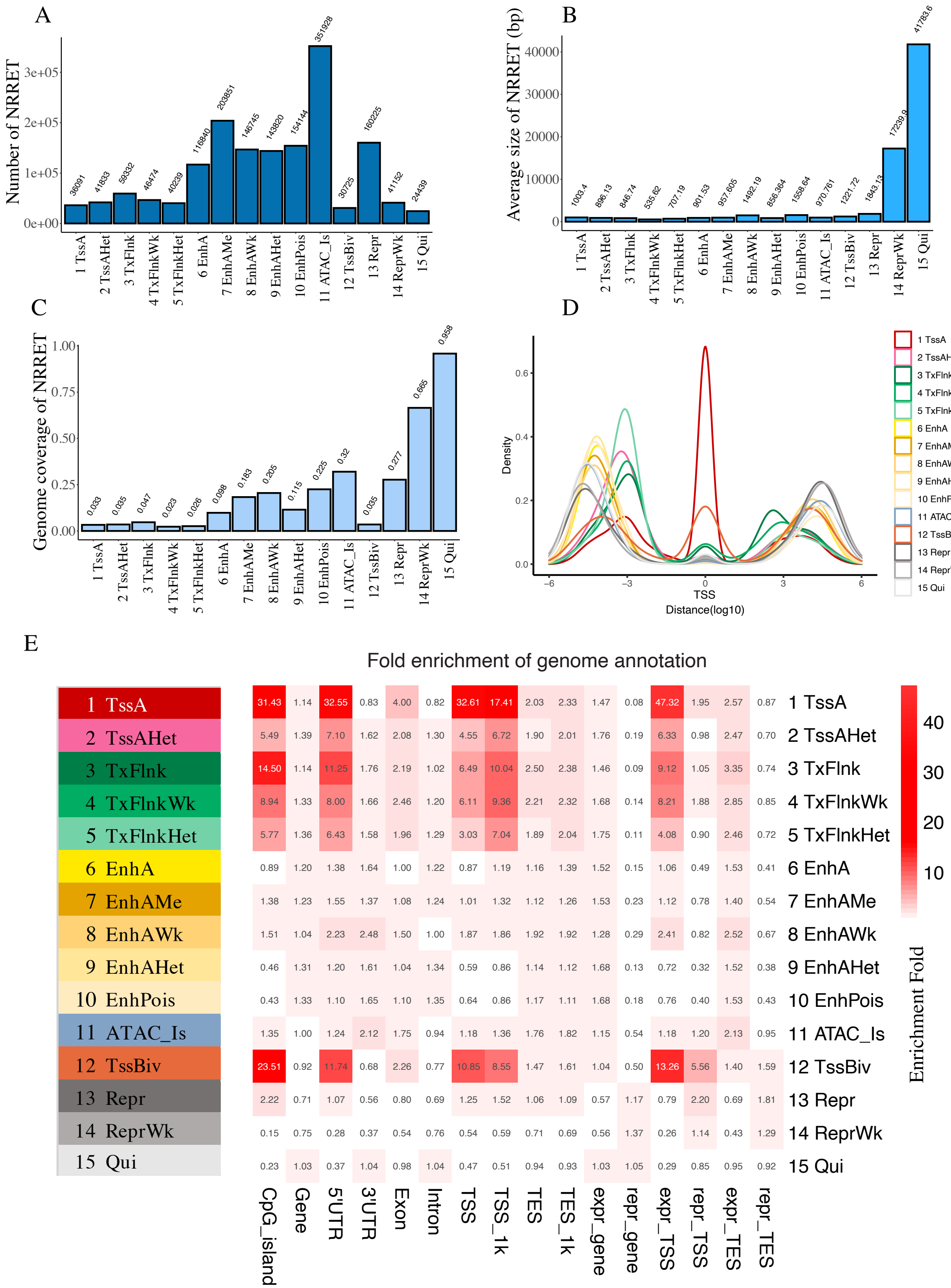

H3K27ac
PC1 (49.5% of var. explained)
PC2 (14.6% of var. explained)
Rep
Rep1
Rep2
Rep3
Rep4
Tissue
Cortex
Hypothalamus
Cerebellum
Testis
ShellGland
Muscle
Heart
Adipose
Kidney
Spleen
BMarrow
Lung
Trachea
Thymus
Bursa
Liver
Provent
Gizzard
Duodenum
Jejunum
Ileum
Colon
Cecum
H3K4me1
PC1 (65.4% of var. explained)
PC2 (6.6% of var. explained)


ATAC
PC1 (39.8% of var. explained)
PC2 (25.2% of var. explained)
Rep
Rep1
Rep2
Rep3
Rep4
Tissue
Cortex
Hypothalamus
Cerebellum
Testis
ShellGland
Muscle
Heart
Adipose
Kidney
Spleen
BMarrow
Lung
Trachea
Thymus
Bursa
Liver
Provent
Gizzard
Duodenum
Jejunum
Ileum
Colon
Cecum
H3K27me3
PC1 (77.6% of var. explained)
PC2 (3.4% of var. explained)

Supplementary Fig. 3. The principal component analysis (PCA) of samples based on five epigenetic marks and RNA-seq data in 23 chicken tissues. The legends for color and shape are the same across all six plots.



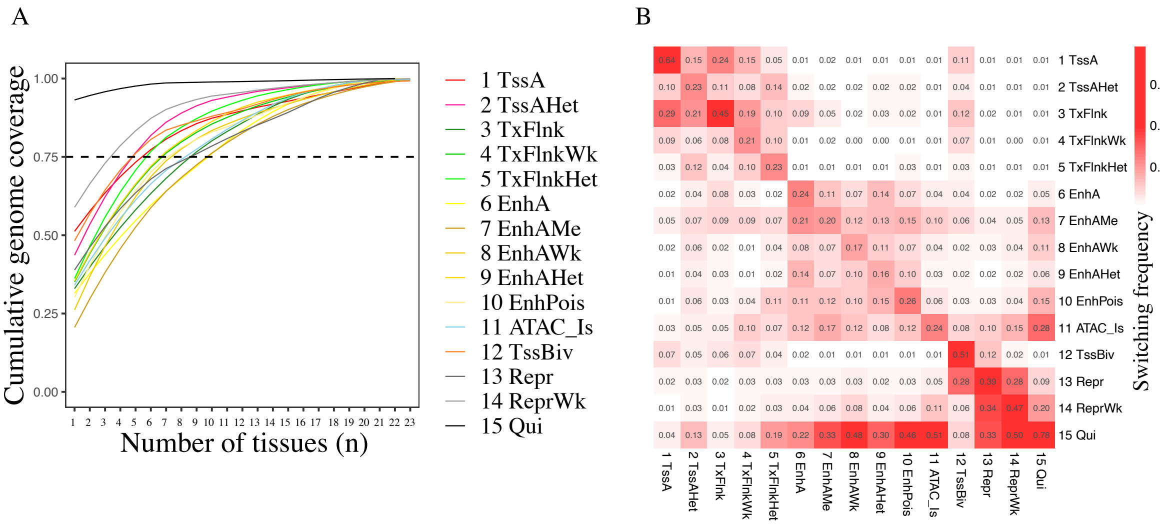
Supplementary Fig. 4. The distribution of normalized signals (y-axis) of epigenetic marks around ± 2.5Kb of six different gene types. These are including protein-coding genes, lncRNA, Pseudogene, miRNA, snoRNA and snRNA. TSS: transcription start site, TES: transcription end site. The numbers in the brackets are the number of corresponding genes.



	CpG_island	Gene	5'UTR	3'UTR	Exon	Intron	TSS	TSS_1k	TES	TES_1k	expr_gene	repr_gene	expr_TSS	repr_TSS	expr_TES	repr_TES	
1 TssA	31.43	1.14	32.55	0.83	4.00	0.82	32.61	17.41	2.03	2.33	1.47	0.08	47.32	1.95	2.57	0.87	1 TssA
2 TssAHet	5.49	1.39	7.10	1.62	2.08	1.30	4.55	6.72	1.90	2.01	1.76	0.19	6.33	0.98	2.47	0.70	2 TssAHet
3 TxFlnk	14.50	1.14	11.25	1.76	2.19	1.02	6.49	10.04	2.50	2.38	1.46	0.09	9.12	1.05	3.35	0.74	3 TxFlnk
4 TxFlnkWk	8.94	1.33	8.00	1.66	2.46	1.20	6.11	9.36	2.21	2.32	1.68	0.14	8.21	1.88	2.85	0.85	4 TxFlnkWk
5 TxFlnkHet	5.77	1.36	6.43	1.58	1.96	1.29	3.03	7.04	1.89	2.04	1.75	0.11	4.08	0.90	2.46	0.72	5 TxFlnkHet
6 EnhA	0.89	1.20	1.38	1.64	1.00	1.22	0.87	1.19	1.16	1.39	1.52	0.15	1.06	0.49	1.53	0.41	6 EnhA
7 EnhAMe	1.38	1.23	1.55	1.37	1.08	1.24	1.01	1.32	1.12	1.26	1.53	0.23	1.12	0.78	1.40	0.54	7 EnhAMe
8 EnhAWk	1.51	1.04	2.23	2.48	1.50	1.00	1.87	1.86	1.92	1.92	1.28	0.29	2.41	0.82	2.52	0.67	8 EnhAWk
9 EnhAHet	0.46	1.31	1.20	1.61	1.04	1.34	0.59	0.86	1.14	1.12	1.68	0.13	0.72	0.32	1.52	0.38	9 EnhAHet
10 EnhPois	0.43	1.33	1.10	1.65	1.10	1.35	0.64	0.86	1.17	1.11	1.68	0.18	0.76	0.40	1.53	0.43	10 EnhPois
11 ATAC_Is	1.35	1.00	1.24	2.12	1.75	0.94	1.18	1.36	1.76	1.82	1.15	0.54	1.18	1.20	2.13	0.95	11 ATAC_Is
12 TssBiv	23.51	0.92	11.74	0.68	2.26	0.77	10.85	8.55	1.47	1.61	1.04	0.50	13.26	5.56	1.40	1.59	12 TssBiv
13 Repr	2.22	0.71	1.07	0.56	0.80	0.69	1.25	1.52	1.06	1.09	0.57	1.17	0.79	2.20	0.69	1.81	13 Repr
14 ReprWk	0.15	0.75	0.28	0.37	0.54	0.76	0.54	0.59	0.71	0.69	0.56	1.37	0.26	1.14	0.43	1.29	14 ReprWk
15 Qui	0.23	1.03	0.37	1.04	0.98	1.04	0.47	0.51	0.94	0.93	1.03	1.05	0.29	0.85	0.95	0.92	15 Qui

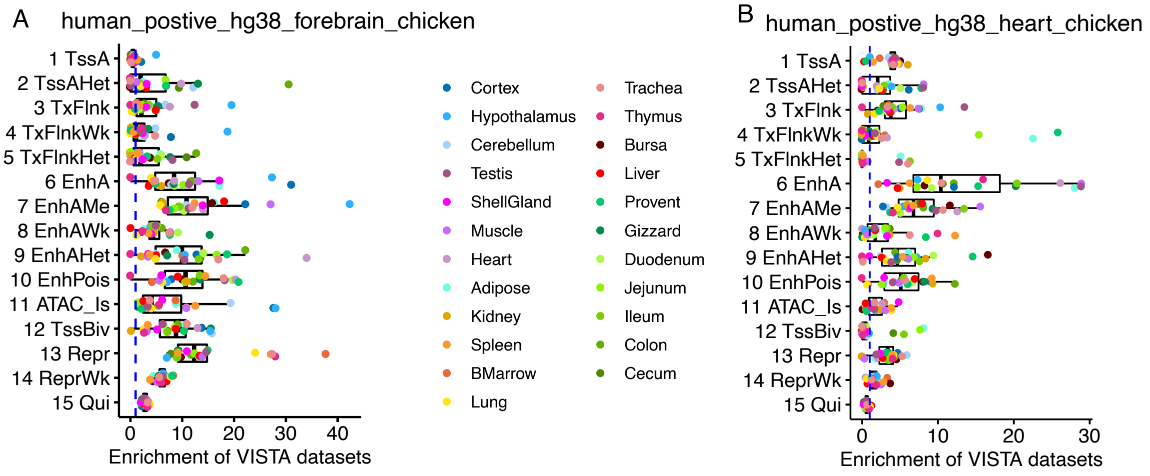
Supplementary Fig. 5. General summary and characterization of chromatin states across 23 chicken tissues. (A) The total number of non-redundant regulatory elements across all 23 tissues

(NRRET) for each chromatin state. (B) Average size of NRRET for each chromatin state. (C) Genome coverage (proportion, not percent) of NRRET in each chromatin state. **(D)** The distance of chromatin state to the TSS of the closest gene. (E). The average enrichment of chromatin states for genomic annotations across tissues, including CpG islands, genes, TSS/TES_1K (±1 kb around TSS and TES), expressed genes (TPM ≥ 0.1), and repressed genes (TPM < 0.1). TSS: transcription start site, TES: transcription end site.



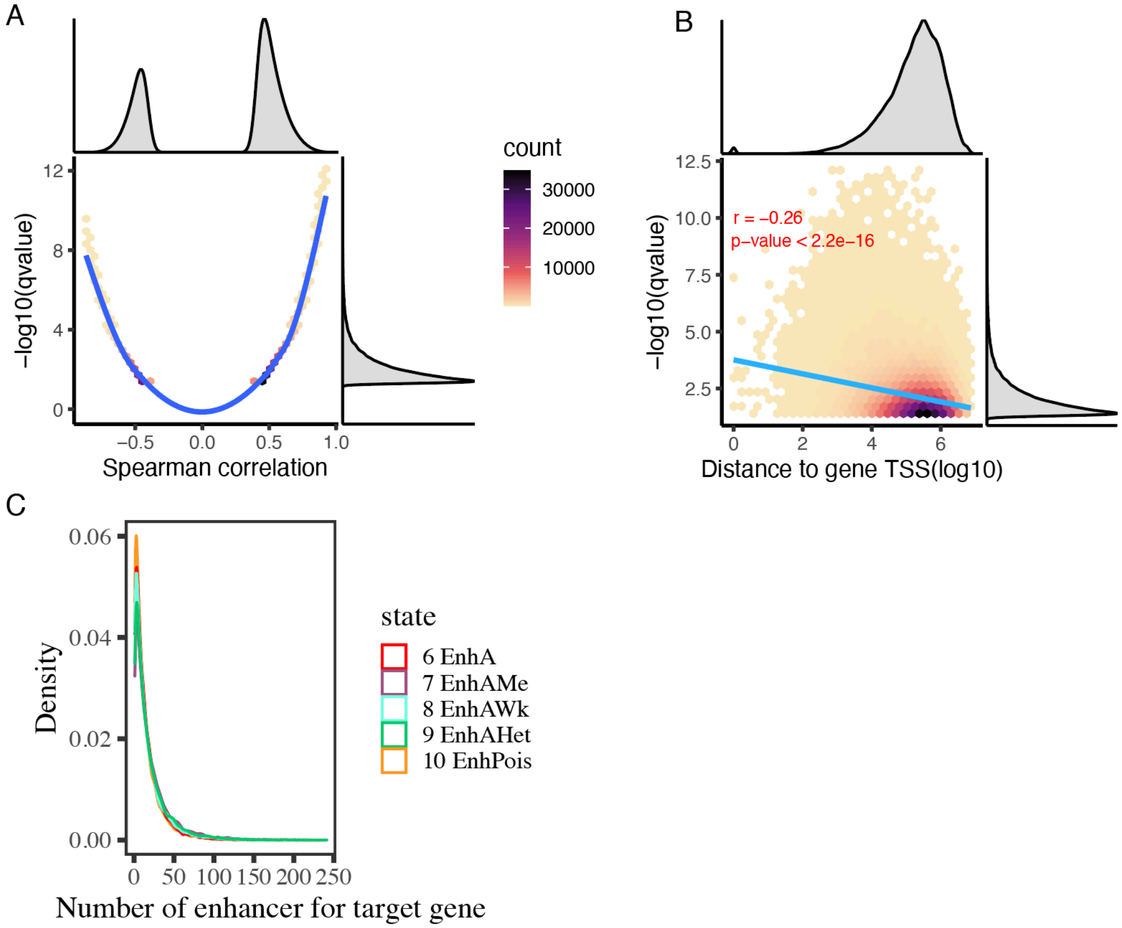
	1 TssA	2 TssAHet	3 TxFlnk	4 TxFlnkWk	5 TxFlnkHet	6 EnhA	7 EnhAMe	8 EnhAWk	9 EnhAHet	10 EnhPois	11 ATAC_Is	12 TssBiv	13 Repr	14 ReprWk	15 Qui
1 TssA	0.64	0.15	0.24	0.15	0.05	0.01	0.02	0.01	0.01	0.01	0.01	0.11	0.01	0.01	0.01
2 TssAHet	0.10	0.23	0.11	0.08	0.14	0.02	0.02	0.02	0.02	0.02	0.01	0.04	0.01	0.01	0.01
3 TxFlnk	0.29	0.21	0.45	0.19	0.10	0.09	0.05	0.02	0.03	0.02	0.01	0.12	0.02	0.01	0.01
4 TxFlnkWk	0.09	0.06	0.08	0.21	0.10	0.01	0.02	0.00	0.00	0.01	0.01	0.07	0.01	0.00	0.01
5 TxFlnkHet	0.03	0.12	0.04	0.10	0.23	0.01	0.01	0.01	0.01	0.03	0.01	0.03	0.01	0.01	0.01
6 EnhA	0.02	0.04	0.08	0.03	0.02	0.24	0.11	0.07	0.14	0.07	0.04	0.04	0.02	0.02	0.05
7 EnhAMe	0.05	0.07	0.09	0.09	0.07	0.21	0.20	0.12	0.13	0.15	0.10	0.06	0.04	0.05	0.13
8 EnhAWk	0.02	0.06	0.02	0.01	0.04	0.08	0.07	0.17	0.11	0.07	0.04	0.02	0.03	0.04	0.11
9 EnhAHet	0.01	0.04	0.03	0.01	0.02	0.14	0.07	0.10	0.16	0.10	0.03	0.02	0.02	0.02	0.06
10 EnhPois	0.01	0.06	0.03	0.04	0.11	0.11	0.12	0.10	0.15	0.26	0.06	0.03	0.03	0.04	0.15
11 ATAC_Is	0.03	0.05	0.05	0.10	0.07	0.12	0.17	0.12	0.08	0.12	0.24	0.08	0.10	0.15	0.28
12 TssBiv	0.07	0.05	0.06	0.07	0.04	0.02	0.01	0.01	0.01	0.01	0.01	0.51	0.12	0.02	0.01
13 Repr	0.02	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.05	0.28	0.39	0.28	0.09
14 ReprWk	0.01	0.03	0.01	0.02	0.03	0.04	0.06	0.08	0.04	0.06	0.11	0.06	0.34	0.47	0.20
15 Qui	0.04	0.13	0.05	0.08	0.19	0.22	0.33	0.48	0.30	0.46	0.51	0.08	0.33	0.50	0.78

Supplementary Fig. 6. Tissue-sharing patterns of chromatin states. (**A**) The variability of chromatin states across 23 chicken tissues based on the cumulative fraction of genome coverage (proportion, not percent). Dashed line = 0.75. (**B**) The average switching patterns of chromatin states between tissues.

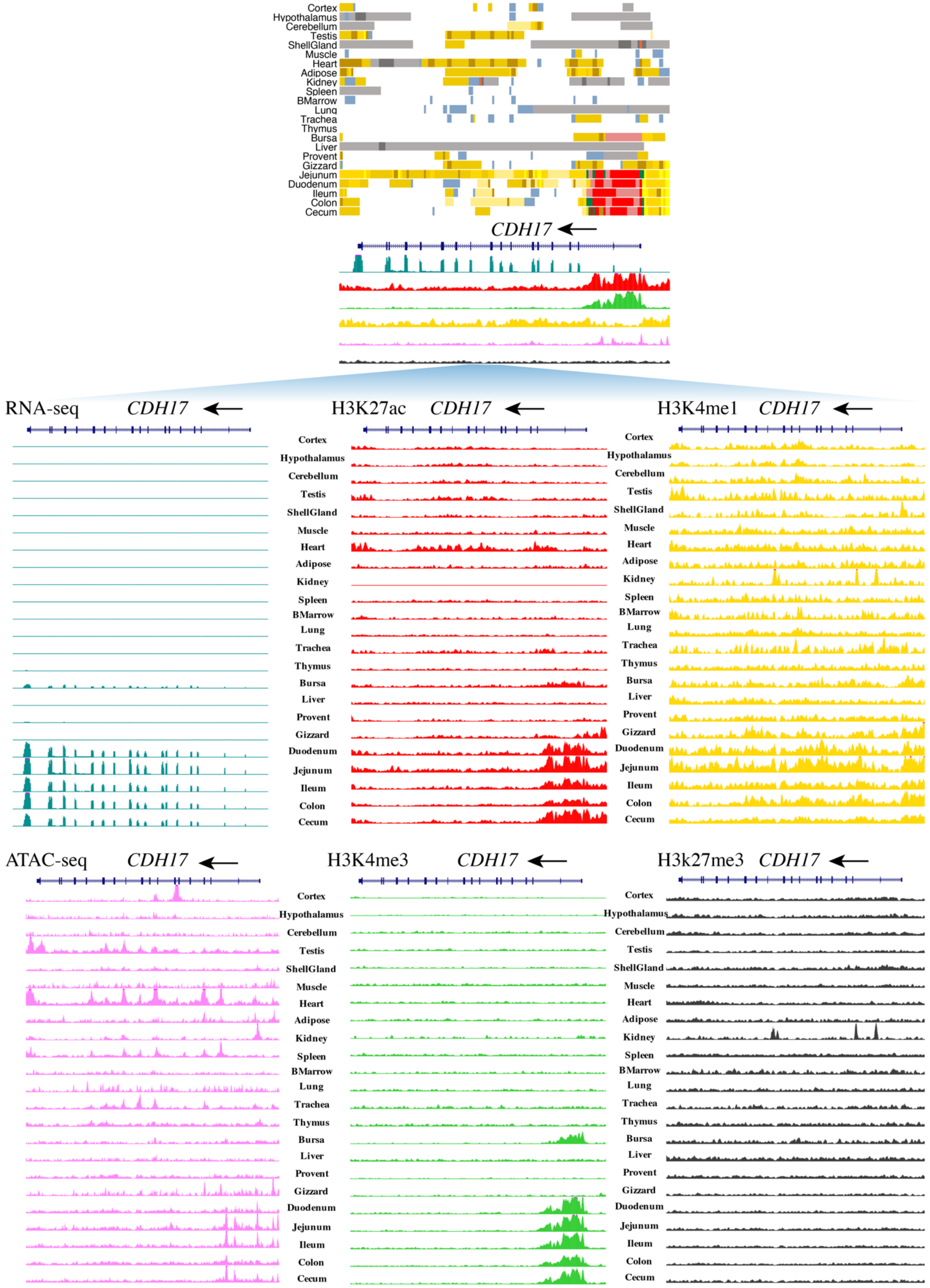


Supplementary Fig. 7. The enrichment of VISTA enhancers in chicken chromatin states. (A) The enrichment of human forebrain enhancers in chromatin states of 23 chicken tissues. The

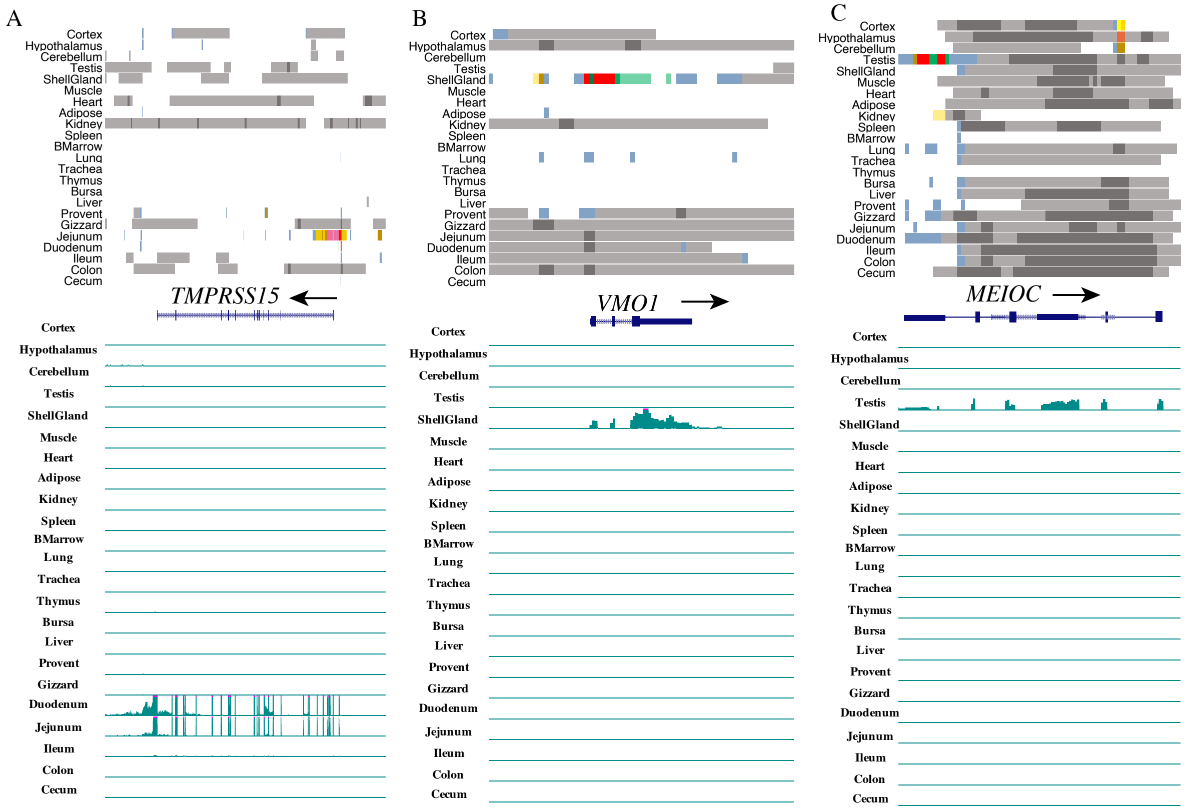
VISTA forebrain specific enhancer had the highest enrichment at the enhancer (EnhA, EnhAMe) of brain tissues (Cortex, Hypothalamus, Cerebellum). (B) The enrichment of human heart enhancers in chromatin states of 23 chicken tissues. VISTA heart specific enhancers had the highest enrichment at the enhancer (EnhA, EnhAMe) of heart.



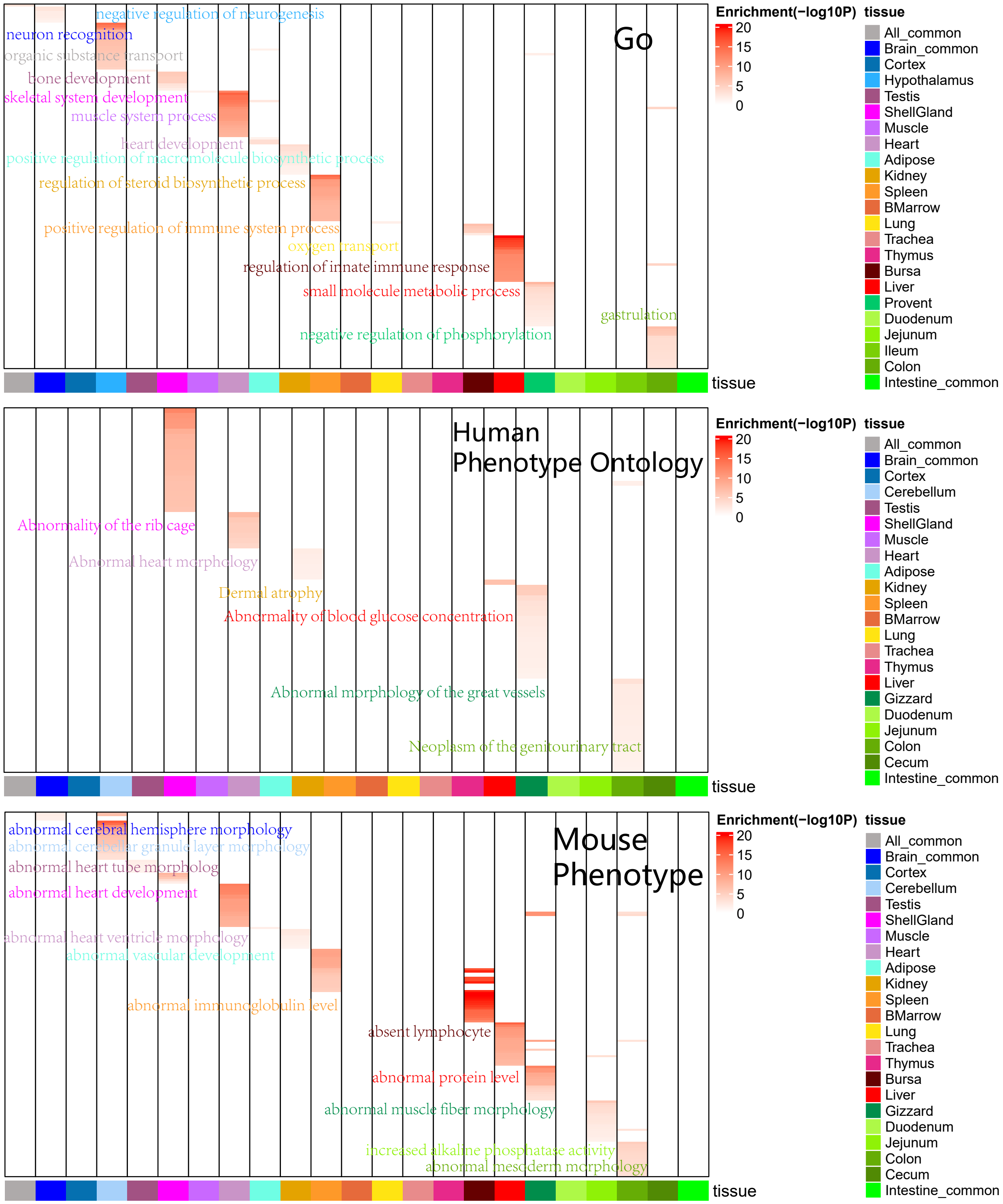
Supplementary Fig. 8. General summary of enhancer-gene pairs. (**A**) The relationship between Spearman correlations of H3K27ac signal and gene expression and the corresponding *p* values of enhancer-gene pairs. (**B**) The Spearman correlation between the distance of enhancers to their target genes and the confident *p* values of the enhancer-gene pairs. (C) The distribution of number of enhancers for target gene.


Cortex
Hypothalamus
Cerebellum
Testis
ShellGland
Muscle
Heart
Adipose
Kidney
Spleen
BMarrow
Lung
Trachea
Thymus
Bursa
Liver
Provent
Gizzard
Jejunum
Duodenum
Ileum
Colon
Cecum
CDH17
RNA-seq CDH17
H3K27ac CDH17
H3K4me1 CDH17
Cortex
Hypothalamus
Cerebellum
Testis
ShellGland
Muscle
Heart
Adipose
Kidney
Spleen
BMarrow
Lung
Trachea
Thymus
Bursa
Liver
Provent
Gizzard
Duodenum
Jejunum
Ileum
Colon
Cecum
ATAC-seq CDH17
H3K4me3 CDH17
H3k27me3 CDH17
Cortex
Hypothalamus
Cerebellum
Testis
ShellGland
Muscle
Heart
Adipose
Kidney
Spleen
BMarrow
Lung
Trachea
Thymus
Bursa
Liver
Provent
Gizzard
Duodenum
Jejunum
Ileum
Colon
Cecum

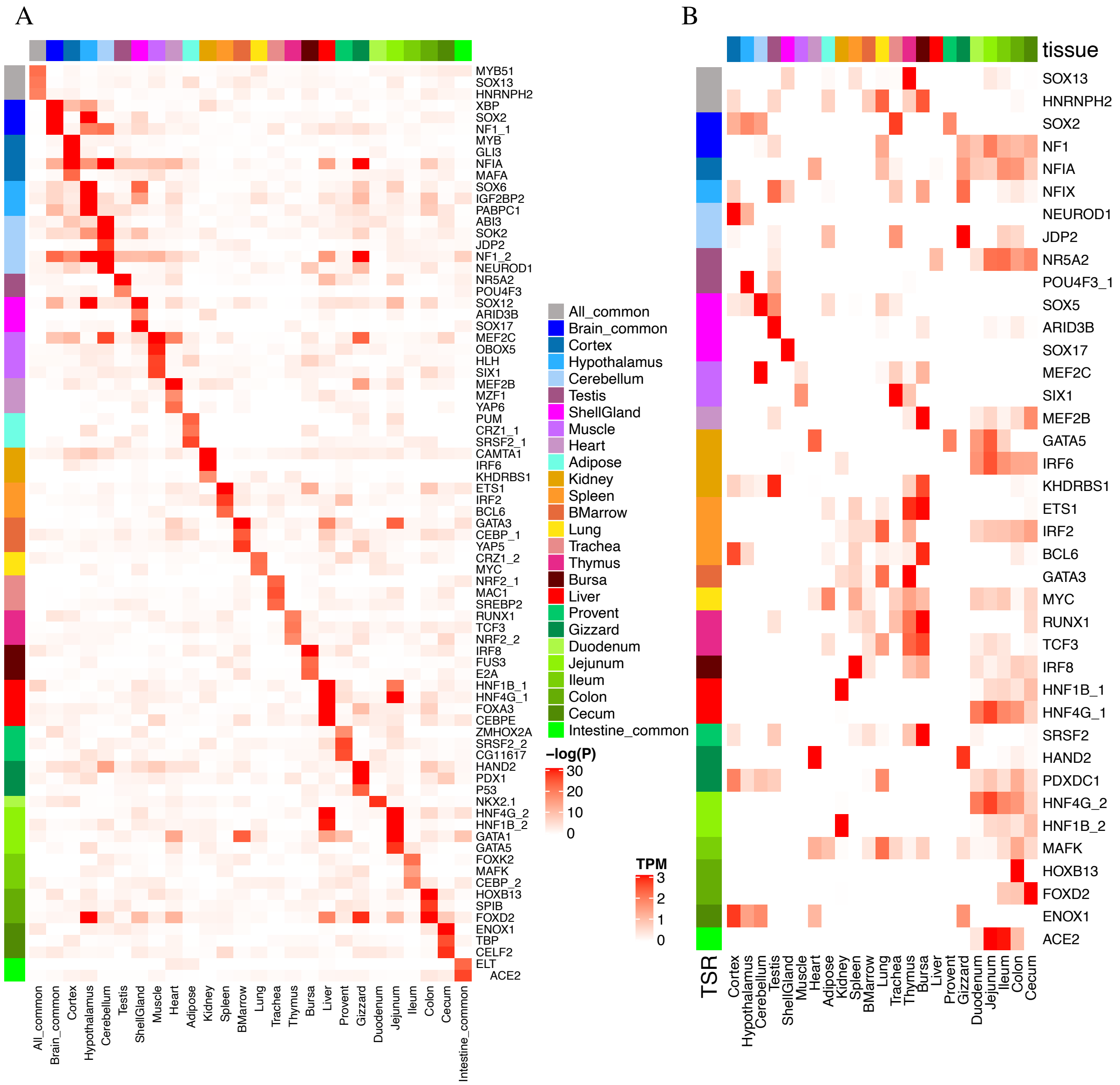
Supplementary Fig. 9. Chromatin states around *CDH17* gene (chr2:125,878,923-125,908,898, galGal6) in 23 chicken tissues. Vertical scale of UCSC tracks shows the normalized signal from 0 to 500 for RNA-seq, 0 to 150 for H3K27ac and H3K4me3, and 0 to 100 for other marks and ATAC-seq.



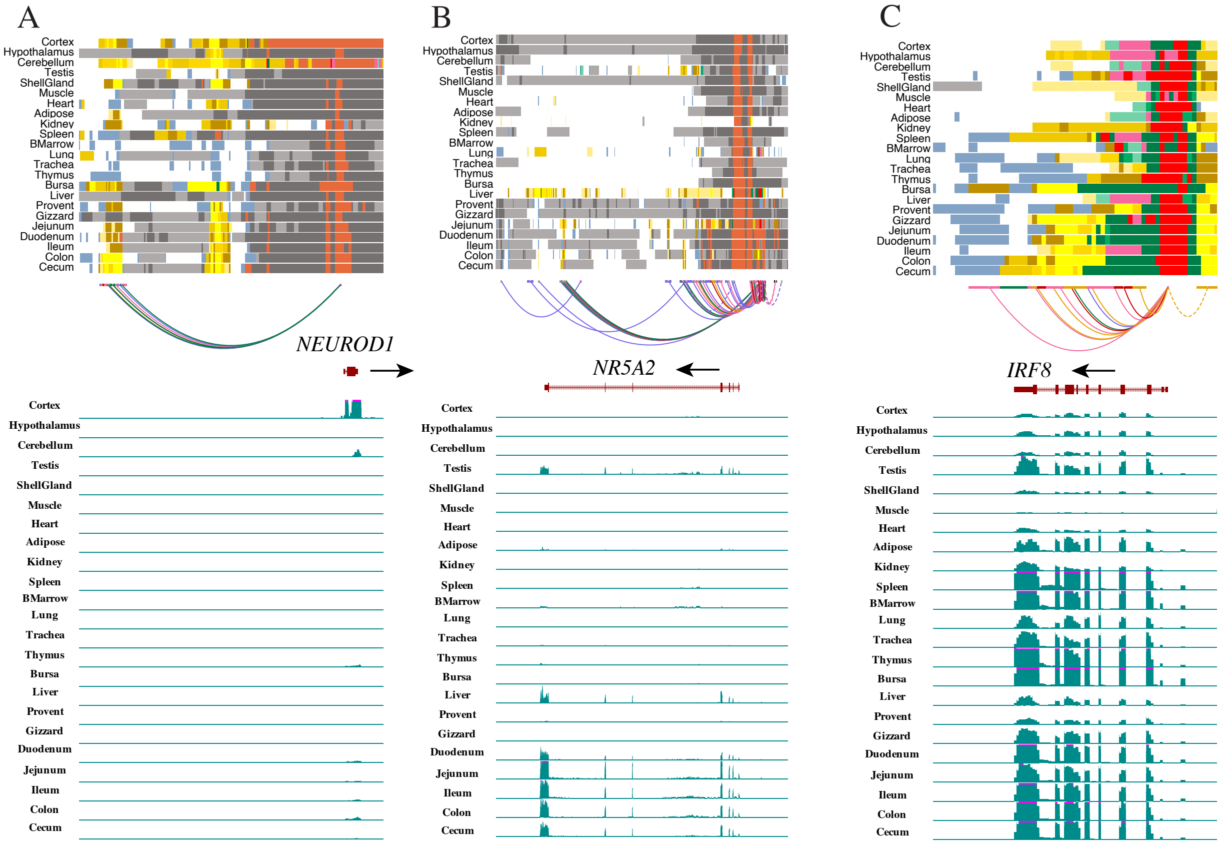
Supplementary Fig. 10. Examples of genes regulated by chromatin states. (**A**) Chromatin states and gene expression (normalized read counts) around Transmembrane Serine Protease 15 (*TMPRSS15)* (chr1:100,929,822-101,014,556). (B) Chromatin states and gene expression around Vitelline Membrane Outer Layer 1 Homolog (*VM01*) (chr1:182,057,442-182,069,414). (C) Chromatin state and gene expression around Meiosis Specific with Coiled-Coil Domain (MEIOC) (chr27:3,604,663-3,618,779), which is a germ-cell specific factor conserved in most metazoans. Vertical scale of UCSC tracks (bottom) shows the normalized read counts from 0 to 100 for RNA-seq.



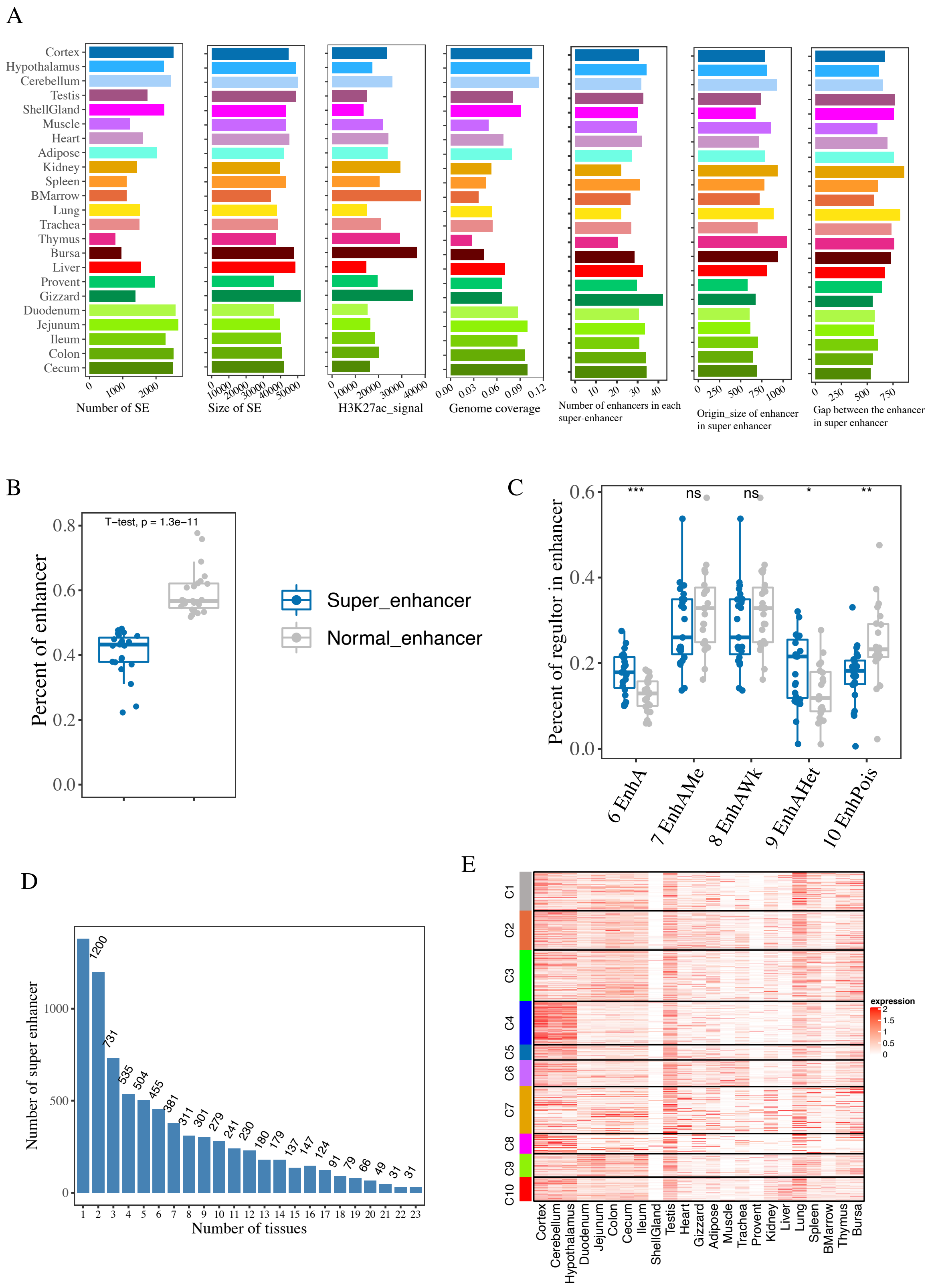
Supplementary Fig. 11. GO function, human phenotype, and mouse phenotype in conserved (human and chicken) tissue-specific enhancer. The columns represent 26 modules of EnhAs. The rows represent GO term, human phenotypes and mouse phenotype. Notes within the heatmap summarized nearby GO term, enriched phenotypes and mouse phenotype, with the color of the text indicating the corresponding tissue.



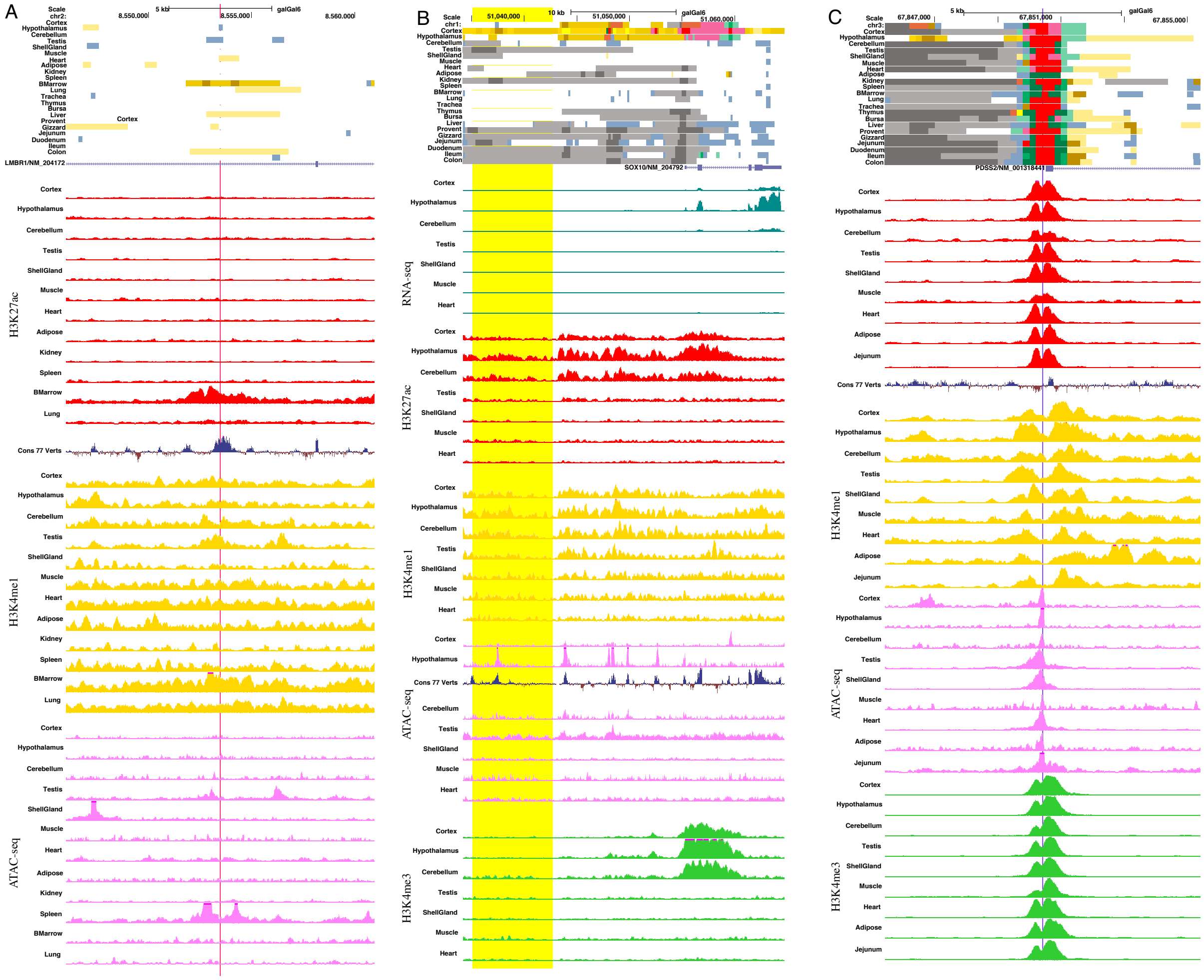
Supplementary Fig. 12. Motif enrichment of tissue-specific strong enhancers (EnhAs). (A), The enrichment of major motif across 23 tissues. The *P* value was generated by HOMMER. (B) The expression level (TPM) of corresponding transcriptional factors in 23 tissues.



Supplementary Fig. 13. Examples of transcriptional factors (TF) binding to tissue-specific strong enhancers (EnhAs). (A) NEUROD1 (chr7:14,223,290-14,263,375). (B) NR5A2 (chr8:1,968,961-2,079,138), (C) IRF8 (chr11:17,586,856-17,599,302). Vertical scale of UCSC tracks (bottom) shows the normalized read counts from 0 to 100 for RNA-seq.

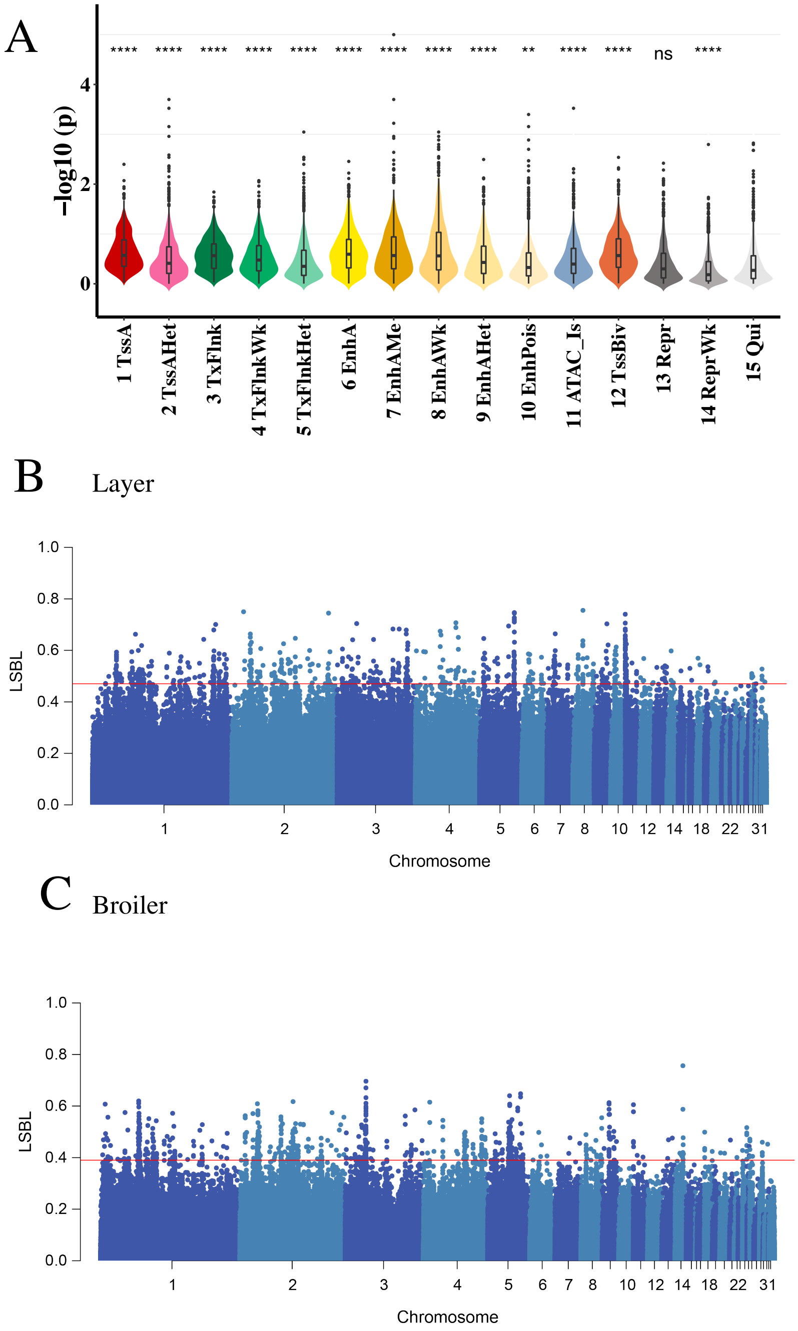


Supplementary Fig. 14. Summary of super-enhancers in chicken. (**A**) The distribution of number, length, H3K27ac signal intensity, and genome coverage of super-enhancers, as well as number of normal enhancers, length of normal enhancers and gap between normal enhancers per super-enhancer across 23 tissues. (**B**) The proportion of enhancers in super-enhancers and normal enhancers. (**C**) The percent of each of five enhancer types in super-enhancers and normal enhancers. (**D**) The number of super-enhancers shared across tissues. (**E**) The expression (normalized TPM) of super-enhancer target genes in each cluster.



Supplementary Fig. 15. Causal variants within regulator elements. (A) The chromatin state and epi-peak around a potential causal SNP (SNP (chr2:8553470G>T, rs80659072) of Polydactyly. The red line means where the SNP located. (B) The chromatin state and epi-peak around a deletion (g.51035106_51042744delins) occurred the upstream of SOX10 which influences Dark brown/yellow plumage in chicken (chr1:51,034,163-51,064,718). The highlight region means the deletion region. (C) The chromatin state and epi-peak around a single base change (chr3:67850419C>G, rs316090093) that results in the Silky/Silkie (chr3:67,845,419-67,855,418). The blue line means where the SNP located. For (A) and (B), Vertical scale of UCSC tracks shows the normalized read counts from 0 to 100 for RNA-seq, 0 to 150 for H3K27ac, and 0 to 50 for

H3K4me1, 0 to 150 for H3K4me3, 0 to 100 for H3K27me3; for (C) Vertical scale of UCSC set auto-scale.



Supplementary Fig. 16. GWAS signal and selection signature. **(A)** GWAS signal enrichment of 15 chromatin states across 23 tissues for 44 complex traits in chicken. The statistical significance of comparisons between chromatin states were calculated by two-sided *t*-test using “15 Qui” as a reference. ***$P < 0.001$. Whiskers show 1.5× interquartile range. Black points were outliers. The Manhattan plot of selection signatures (LSBL) in Layer (**B**) and in Broiler (**C**).

Supplementary Table:

Supplementary Table 1. The 377 genome-wide sequencing datasets using in this study
Supplementary Table 2. The summary of data quality of all the ChIP-seq of epi-marks, control, ATAC-seq, RRBS and RNA-seq data sets of chicken
Supplementary Table 3. the number, genome coverage and size of chromatin state in 23 tissues.
Supplementary Table 4. The genes in chicken specific evolution breakpoint regions
Supplementary Table 5. The gene ontology (GO) enrichment of three kinds of genes with different number of enhancers
Supplementary Table 6. The number and size of tissue specific regulator elements in 23 tissues
Supplementary Table 7. The top 10 GO functional enrichment of genes within super-enhancer of each cluster
Supplementary Table 8. The chicken likely (known) causal variants and their regulators
Supplementary Table 9. The data summary of chicken GWAS used in this study
Supplementary Table 10. The selective sweeps (LSBL) in chicken domestication by using GGJ (G. g. jabouillei) and GGM (G. g. murghi) as outgroups
Supplementary Table 11. The selective sweeps (LSBL) in Broiler and Layer breeding
Supplementary Table 12. The enrichment of tissue specific enhancer in selection signature

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