

SUPPLEMENTARY

Supplementary Table S1. Grouping and numbering information

Month	Pituitary	Hypothalamus
6	T01 T03 T05 (Group1)	T02 T04 T06 (Group2)
18	T07 T09 T11 (Group3)	T08 T10 T12 (Group4)
30	T13 T15 T17 (Group5)	T14 T16 T18 (Group6)

Supplementary Table S2. Sequencing data and comparison results for each sample.

Samp le-ID	Total Reads	Mapped Reads	Uniq Mapped Reads	Multiple Map Reads	Reads Map to '+'	Reads Map to '-'	GC Content (%)	%≥Q3 0
T1	57,118 ,252	54,816,325 (95.97%)	54,042,135 (94.61%)	774,190 (1.36%)	27,353,429 (47.89%)	27,385,142 (47.94%)	48.21	94.70
T2	50,699 ,016	48,523,637 (95.71%)	47,830,518 (94.34%)	693,119 (1.37%)	24,215,110 (47.76%)	24,227,642 (47.79%)	48.48	94.39
T3	44,959 ,694	43,008,790 (95.66%)	42,405,126 (94.32%)	603,664 (1.34%)	21,455,108 (47.72%)	21,489,408 (47.80%)	48.02	94.27
T4	46,484 ,518	44,533,701 (95.80%)	43,884,485 (94.41%)	649,216 (1.40%)	22,209,686 (47.78%)	22,249,549 (47.86%)	48.19	94.25
T5	47,866 ,648	45,900,604 (95.89%)	45,231,684 (94.50%)	668,920 (1.40%)	22,896,323 (47.83%)	22,925,322 (47.89%)	48.10	94.19
T6	42,429 ,662	40,730,396 (96.00%)	40,122,977 (94.56%)	607,419 (1.43%)	20,321,568 (47.89%)	20,344,945 (47.95%)	48.57	94.79
T7	42,093 ,036	40,343,568 (95.84%)	39,783,217 (94.51%)	560,351 (1.33%)	20,117,091 (47.79%)	20,152,776 (47.88%)	48.31	94.55
T8	53,370 ,874	51,268,347 (96.06%)	50,525,761 (94.67%)	742,586 (1.39%)	25,568,874 (47.91%)	25,615,228 (47.99%)	48.07	94.64
T9	45,486 ,310	43,599,130 (95.85%)	42,928,790 (94.38%)	670,340 (1.47%)	21,756,027 (47.83%)	21,769,779 (47.86%)	48.37	94.61
T10	48,425 ,134	46,417,437 (95.85%)	45,678,600 (94.33%)	738,837 (1.53%)	23,138,023 (47.78%)	23,167,391 (47.84%)	48.70	94.64
T11	45,893 ,680	43,625,973 (95.06%)	42,757,833 (93.17%)	868,140 (1.89%)	21,709,308 (47.30%)	21,752,593 (47.40%)	49.17	94.26
T12	45,320 ,976	43,574,262 (96.15%)	42,972,962 (94.82%)	601,300 (1.33%)	21,740,072 (47.97%)	21,769,970 (48.04%)	48.41	94.77
T13	58,017 ,574	55,713,072 (96.03%)	54,950,762 (94.71%)	762,310 (1.31%)	27,807,615 (47.93%)	27,826,194 (47.96%)	48.20	94.48
T14	48,262 ,590	46,502,866 (96.35%)	45,896,580 (95.10%)	606,286 (1.26%)	23,220,420 (48.11%)	23,220,923 (48.11%)	48.00	94.48
T15	46,909 ,660	45,042,180 (96.02%)	44,412,368 (94.68%)	629,812 (1.34%)	22,471,758 (47.90%)	22,503,901 (47.97%)	48.15	94.31
T16	49,442 ,698	47,442,833 (95.96%)	46,694,886 (94.44%)	747,947 (1.51%)	23,662,959 (47.86%)	23,681,663 (47.90%)	48.34	94.68

T17	46,414 ,634	44,486,879 (95.85%)	43,857,110 (94.49%)	629,769 (1.36%)	22,190,044 (47.81%)	22,220,732 (47.87%)	47.61	94.56
T18	44,376 ,678	42,383,143 (95.51%)	41,791,234 (94.17%)	591,909 (1.33%)	21,136,472 (47.63%)	21,174,333 (47.72%)	47.89	94.78

Total reads: number of clean reads, calculated as single-ended.

Mapped reads: number of reads compared with the reference genome and their percentage in the clean reads.

Unique mapped reads: number of reads compared with the unique position of the reference genome and percentage in the clean reads.

Multiple map reads: Comparison of the number of reads at multiple locations in the reference genome and their percentage in clean reads.

Reads map to "+": number of reads compared with the positive strand of the reference genome and their percentage in clean reads. Reads map to "-": number of reads compared with the negative strand of the reference genome and their percentage in clean reads.

GC content: content of the clean data; GC is the percentage of G and C bases in the clean data to the total bases.

≥Q30%: percentage of bases with a clean data quality value ≥30.

Supplementary Table S3. Statistics of the variable shear event structure.

Event ID	Event Type	Gene ID	Chrom	Event Start	Event End	Event Pattern	Strand
1000001	TSS	gene100	AC_000158.1	9607340	9607574	9607574	+
1000002	TTS	gene100	AC_000158.1	9920035	9921259	9920035	+
1000003	SKIP_ON	gene100	AC_000158.1	9798277	9798333	9795702,9798277- 9798333,9811726	+
1000004	SKIP_OFF	gene100	AC_000158.1	9798277	9798333	97,957,029,811,726 9777740,9795535-	+
1000005	MSKIP_ON	gene100	AC_000158.1	9795535	9798333	9795702,9798277- 9798333,9811726	+
1000006	MSKIP_OFF	gene100	AC_000158.1	9795535	9798333	97,777,409,811,726 9777740,9795535-	+
1000007	SKIP_ON	gene100	AC_000158.1	9795535	9795702	9795702,9811726	+
1000008	SKIP_OFF	gene100	AC_000158.1	9795535	9795702	97,777,409,811,726	+
1000009	TSS	gene1006	AC_000158.1	133395622	133395742	133395622	-

Event ID: AS event number.

Event Type: AS event type.

Gene ID: gene number.

Chrom: chromosome number.

Event Star: AS event start position.

Event End: AS event end position.

Event pattern: AS event characteristics.

Strand: gene positive and negative chain information.

Supplementary Table S4. Primers for real-time quantitative PCR.

Target Name		Primer
PPP1R11	F	CAGAAAAGACAGAAGGGTGCC
	R	CGTTCCGAAGTTTGATGGTTA
TBX21	F	GCCTACCAGAATGCCGAGA
	R	TCAACGGAAGGATACATGGACT
SHANK2	F	ACATCTGGACCCTCTACAACC
	R	CTTCAGTTCCTTATCTCCACCT
PPP1R14A	F	ACATGCCCCGATGAGGTCAAC
	R	AGTCACTGGATTTTACGGCTT
HAPLN2	F	CCACCTACGCCCAGCAGTACC
	R	AGCCCCGCTTACACCAGTCCA
TEX15	F	CTCTTCAAGTATACGCGT
	R	TGTCTTTACTTTCTTCGGTT
CAMK4	F	TCTCTGGTCGCTTCTATCGCT
	R	CCTCCTCGTCACTCCT
ANGPTL7	F	ACGGAATAGCTATCGCCT
	R	GCAGCAGTTGTACCAGTAT
ORM1	F	GTCGGCTTTCTTTTACCTT
	R	CAAAGTGTCTCTGTCC
HSP90AB1	F	CACTCCAACCGCATCTACC
	R	CTCCTCTACCGTCACCTC
RORB	F	CCTGACATCCGTACCCAA
	R	ATTTCCGGTCATTGTTATCCCT
TBR1	F	GCCCTCCTCTATCAAGTCCAT
	R	TTTGGCCTGCTCGTAAAT
HSPB7	F	CTTCATGCGTCCCCACTCGG
	R	CCACAGCAAACCTCATAGGCGTCTC
NOG	F	ACCTCATCGAACACACGGACC
	R	AGCAGCGTCTCGTTCAGATCC
ORM1	F	GTCGGCTTTCTTTTACCTT
	R	CAAAGTGTCTCTGTCC
PTK2B	F	CCTGAAAGAAGACAAGACCAC
	R	CCATCCCCCTCGCTGTCC
AGO3	F	GGCATCTCAAGAACACCT
	R	TATCTCCGACACGATTGACC
RLF	F	GAGGCATTACAAACGCAC
	R	GCTCCTCCTTAATTTTGGT
CENPA	F	GCCCCCTTGCCGACTG
	R	AAATGCTTCTGGCGCCTC
LOC506989	F	TCAACTGCTGGCCCCTCG
	R	TGGTGTCTCTTCGTGCTA
ZNF282	F	ACATCCCAACGTACCCC
	R	CTGGTCCTCCTGCTTTATC

Supplementary Table S5. SNP site statistics.

Sample-ID	SNP Number	Genic SNP	Intergenic SNP	Transition	Transversion	Heterozygosity
T1	247,191	203,899	43,292	72.33%	27.67%	40.68%
T2	203,152	169,538	33,614	71.97%	28.03%	40.50%
T3	228,495	189,837	38,658	71.96%	28.04%	45.37%
T4	239,243	198,611	40,632	72.03%	27.97%	45.40%
T5	198,388	163,998	34,390	72.10%	27.90%	38.80%
T6	140,088	113,535	26,553	72.12%	27.88%	37.68%
T7	186,525	154,055	32,470	72.28%	27.72%	42.71%
T8	212,891	174,209	38,682	72.22%	27.78%	43.93%
T9	173,482	144,102	29,380	72.03%	27.97%	38.03%
T10	130,330	105,090	25,240	72.08%	27.92%	39.99%
T11	75,571	57,997	17,574	72.10%	27.90%	40.49%
T12	196,402	161,703	34,699	72.14%	27.86%	41.42%
T13	223,090	185,434	37,656	72.05%	27.95%	41.72%
T14	199,483	165,311	34,172	72.19%	27.81%	40.20%
T15	194,608	162,245	32,363	72.07%	27.93%	39.63%
T16	166,754	136,553	30,201	72.08%	27.92%	39.16%
T17	196,132	161,964	34,168	71.92%	28.08%	40.92%
T18	201,488	165,848	35,640	72.03%	27.97%	41.01%

SNP Number: total number of SNP sites.

Genic SNP: total number of SNP loci in the gene region.

Intergenic SNP: total number of SNP loci in the intergenic region.

Transition: percentage of conversion-type SNP sites in the total number of SNP sites.

Transversion: percentage of the number of transversion-type SNP sites in the total number of SNP sites.

Heterozygosity: percentage of the number of heterozygous SNP sites in the total number of SNP sites.

Supplementary Table S6. Numbers of differential genes.

Parameters	Group	DEGs total	DEGs up	DEGs down
DESeq EBSeq FDR<0.05 FC \geq 2	G3vsG1	18	4	14
	G5vsG1	18	11	7
	G5vsG3	3	3	0
	G4vsG2	36	24	12
	G6vsG2	12	7	5
	G6vsG4	3	0	3
	G1vsG2	402	258	144
	G3vsG4	6	0	6
	G5vsG6	5	2	3

Parameters: testing software used DESeq and EBSeq; the evaluation standards were FDR<0.05 and FC \geq 2.

Supplementary Table S7. New gene function annotation results

Annotated databases	New Gene Number
COG	179
GO	2,933
KEGG	2,012
KOG	896
Pfam	863
Swiss-Prot	1,032
eggNOG	2,585
Nr	4,053
All	4,072

Supplementary Table S8. Reproductive-related GO classification table involving new transcripts between groups

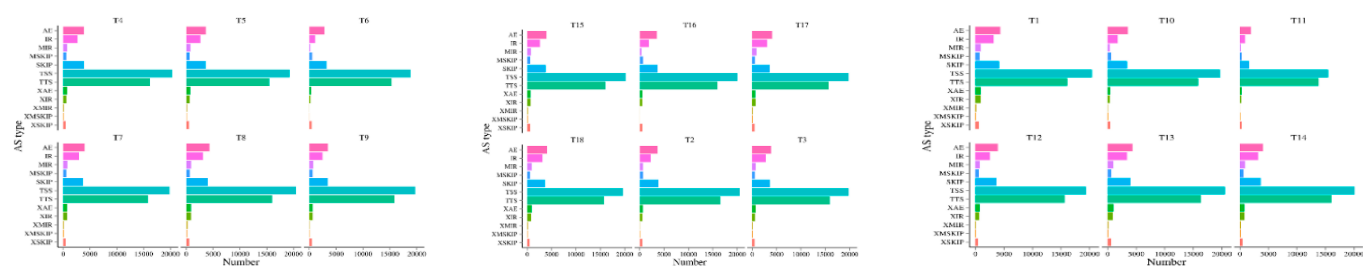
Group	GO annotation (Biological process)	GO Classification Number	New transcription	Differential Transcriptions
G3vsG1	reproduction	GO:0000003	56	7
	reproductive process	GO:0022414	56	7
G5vsG1	reproduction	GO:0000003	56	9
	reproductive process	GO:0022414	56	9
G5vsG3	reproduction	GO:0000003	56	8
	reproductive process	GO:0022414	56	8
G4vsG2	reproduction	GO:0000003	56	6
	reproductive process	GO:0022414	56	6
G6vsG2	reproduction	GO:0000003	56	6
	reproductive process	GO:0022414	56	6
G6vsG4	reproduction	GO:0000003	56	6
	reproductive process	GO:0022414	56	6

Supplementary Table S9. Comparison of pituitary hormone genes and related receptor expression levels

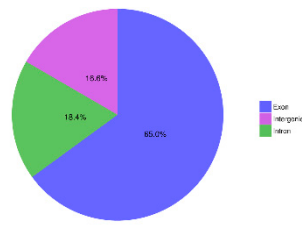
Gene	Description	FPKM in Groups						Ratio of groups in FPKM			
		G1	G2	G3	G4	G5	G6	G3/G1	G5/G1	G4/G2	G6/G2
Pomc	Proopiomelanocortin	3.635	4.983	18.480	6.265	4.875	4.385	5.084	1.341	1.257	0.880
PRL	Prolactin	0.007	0.035	0.018	0.087	0.076	0.068	2.405	10.255	2.485	1.957
LHb	Luteinizing hormone beta polypeptide	1.344	1.570	2.035	0.932	0.950	1.616	1.514	0.707	0.593	1.029
TSHb	Thyroid-stimulating hormone, β -subunit	0.029	0.056	0.066	0.026	0.033	0.123	2.261	1.155	0.459	2.180
Ghdc	GH3 domain containing	6.836	6.703	7.838	5.417	6.117	12.531	1.147	0.895	0.808	1.870
GHR	Growth hormone receptor	1.034	1.956	1.905	1.606	2.306	1.633	1.843	2.230	0.821	0.835
GHSR	Growth hormone secretagogue receptor	4.396	3.147	3.064	3.336	2.771	1.904	0.697	0.630	1.060	0.605
PRLR	Prolactin receptor	0.897	0.853	0.759	0.943	0.742	0.983	0.846	0.827	1.105	1.153

Supplementary Table S10. Specific functional transcription factors in the pituitary gland and hypothalamus

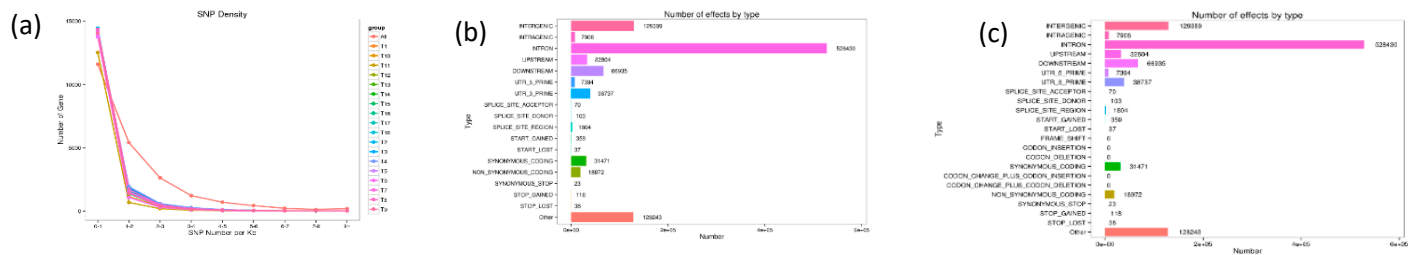
Gene	Description	FPKM in Groups						Ratio of groups in FPKM			
		G1	G2	G3	G4	G5	G6	G3/G1	G5/G1	G4/G2	G6/G2
LHX2	LIM homeobox 2	9.803	15.276	13.683	12.961	15.320	14.882	1.396	1.563	0.848	0.974
LHX6	LIM homeobox 6	0.155	4.952	0.195	1.106	0.151	1.579	1.258	0.972	0.223	0.319
DLX2	distal less homeobox 2	19.235	5.404	11.846	16.663	20.889	14.105	0.616	1.086	3.083	2.610
TBX2	T-box transcription factors	0.851	1.217	1.390	1.452	1.861	1.282	1.634	2.187	1.193	1.053
SOX9	SRY-box 9	3.426	5.672	3.967	2.663	4.789	3.787	1.158	1.398	0.470	0.668
SOX8	SRY-box 8	13.662	26.216	31.092	15.829	16.550	19.277	2.276	1.211	0.604	0.735
SOX2	SRY-box 2	38.963	41.830	37.558	37.433	46.780	42.536	0.964	1.201	0.895	1.017
GATA2	GATA binding protein 2	0.836	1.555	1.178	1.060	1.453	1.233	1.408	1.738	0.681	0.793



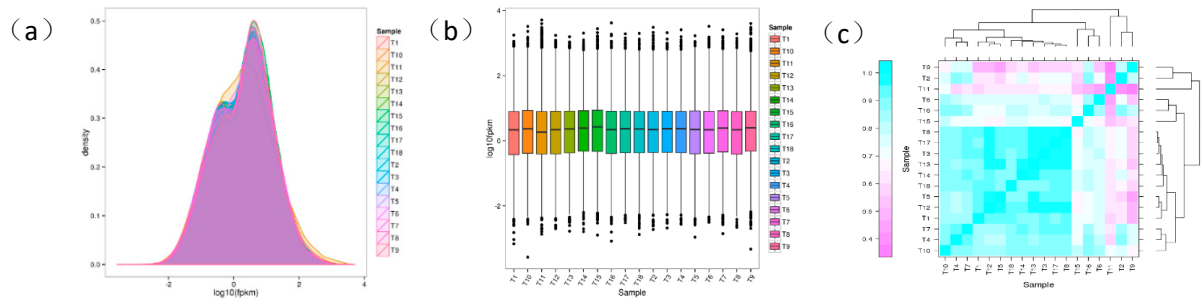
Supplementary Figure S1. Statistics for the variable splicing events.



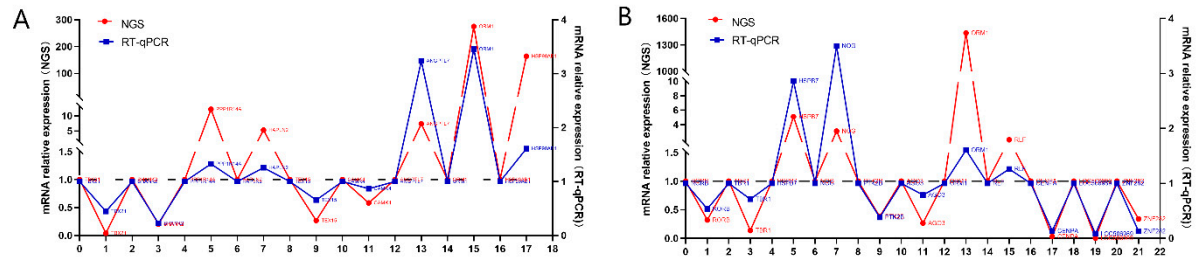
Supplementary Figure S2. Distribution of reads in different regions of the genome. Exons are blue; introns are green; intergenic regions are purple.



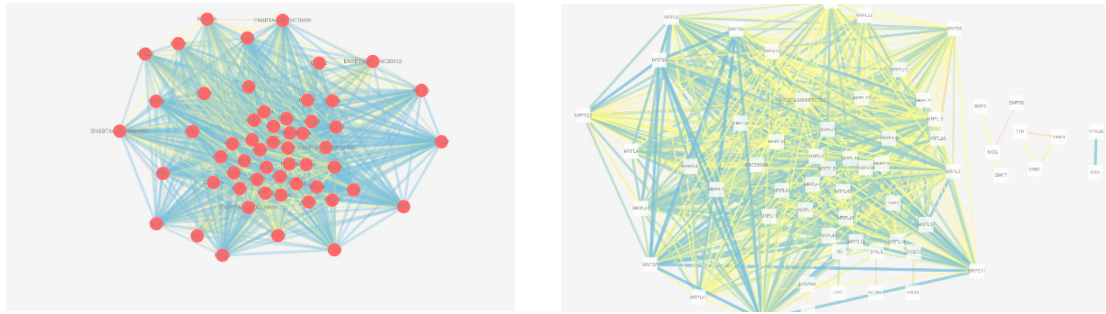
Supplementary Figure S3. SNP density map and annotation classification diagram. (a) SNP density map; annotation classification diagram. (b) SNP annotation classification diagram. (c) Indel annotation classification diagram. The vertical axis is the area or type where the SNP/InDel is located, and the horizontal axis is the number of categories.



Supplementary Figure S4. Sample gene expression and overall distribution. (a) Comparison chart of FPKM density distribution in each sample. (b) FPKM box line diagram of each sample. The graph measures the expression level of each sample from the overall discrete perspective of the expression level. (c) Heatmaps with clustering of analyzed samples.



Supplementary Figure S5. The results of RT-qPCR were consistent with the NGS. A was the pituitary gland, B was the hypothalamus, red was the results of NGS, blue was the results of RT-QPCR.



Supplementary Figure S6. Pituitary (left) and hypothalamus (right) DEGs network.