Transcriptome sequencing analysis of ovarian granulosa cells in FecB hybrid ewe lamb

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Abstract: The reproductive trait of livestock is part of the most important economic traits in animal husbandry. FecB is a major gene for multiplet traits in sheep. However, studies on FecB in lamb are not clear and comprehensive. The 1-month ewe lamb of F1 generation cross between Booroola Merino sheep and Xinji fine wool sheep was superovulated. Transcriptome sequencing was performed on ovarian granulosa cells of the FecB gene wild homozygous type (++ genotype) and FecB gene mutant heterozygous type (B+ genotype). We found 306 DEGs in the ovarian granulosa cells of the two genotype (170 genes more highly in B+ genotype and 136 genes more highly in ++ genotype). These genes are mainly involved in the biological processes of the extracellular matrix, extracellular region, negative regulation of multicellular organismal process, negative regulation of secretion by cell, receptor binding, and also significantly enriched in the Kyoto Encyclopedia of Genes and Genomes pathway of MAPK signaling pathway, PI3K-Akt signaling pathway, and Wnt signaling pathway. BMP2 will be presumed to be a key gene associated with the regulation of the reproductive performance of the FecB gene in lambs. This experiment provided a reference for the discussion of the regulatory mechanism of the FecB gene to ovarian granulosa cells in ewe lambs.

Key words: FecB, ewe lamb, ovarian granulosa cells, transcriptome sequencing

1. Introduction

A746G mutation of BMPR1B gene was originally found on chromosome 6 of Booroola Merino sheep. This mutation caused an amino acid substitution of glutamine into arginine, and the number of ovulation in sheep increased significantly. Thus, A746G mutation of BMPR1B gene was named FecB by the Committee on Genetic Nomenclature of Sheep and Goats [1–4]. At present, FecB gene has been studied more in adult ewes. Many researchers have a tendency to suggest that increased ovulation rate in sheep carrying the FecB mutation is related to a reduced rate of atresia [5–7]. However, the mechanism for FecB gene regulating the reproductive performance still remains unknown in ewe lamb.

The Xinji fine wool sheep is a kind of fine wool breed cultivated in China. It is an important genetic resource in the national fine wool gene bank. However, the reproduction rate of Xinji fine wool sheep is only 110–125%, which is a typical single breed [8]. At present, the FecB gene was not found in the Xinji fine wool sheep [9]. Therefore, to breed Xinji fine wool sheep prolificacy strain, we used Booroola merino sheep with FecB gene as the

male parent to cross-breed with Xinji fine wool sheep in combination with the Juvenile in vitro embryo technology (JIVET) in our previous study [10,11]. In this study, transcriptome sequencing was used to explore two kinds of FecB genotype ovarian granulosa cells in 1-month-old lambs, providing an experimental basis for the study of sheep reproduction mechanism.

2. Materials and methods

2.1. Animal care

This study was carried out in strict accordance with relevant guidelines and regulations by the Ministry of Agriculture of the People's Republic of China. The Animal Ethics Committee approved the protocol of this study of Jilin Academy of Agricultural Sciences (AWEC2017A01, 9 March 2017).

2.2. Measurement of animals

FecB gene detection in 1-month ewe lambs, which were hybrid lambs between Booroola Merino Sheep and Xinji fine wool sheep by PCR-RFLP. Three lambs without the FecB gene and three lambs with the FecB gene hybrid were

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selected and superovulated with muscle injection: daily injection of 100 IU FSH at 7:30 and 19:30, three consecutive days, the first injection of FSH and one-time injection of PMSG 100 IU (Ningbo Sansheng Pharmaceutical co., LTD).

2.3. Obtained and cultured cells

At 12 h after the last FSH injection, the abdomen of the lamb was cut about 5 cm along the midline, the ovaries were exposed to the abdomen, and the follicles were punctured and absorbed with a 10 mL syringe containing 1 mL follicle buffer (H199 + 2% ESS + 50 mg/mL Sodium Heparin + 100 μg/mL sterptomycin + 100 IU/mL penicillin, Sigma). Oocytes were picked up under stereoscopic microscope. The remaining follicular fluid was centrifuged and the supernatant was discarded. Ovarian granulosa cells were washed three times with PBS containing 1% cyclamate and streptomycin. Ovarian granulosa cells were cultured in 35 mm medium containing 10% fetal bovine serum Gemini Bio-Products, Mexico

2.4. Total RNA isolation and sequencing

After the cells were grown, the culture medium was discarded, and cells were washed three times with PBS. All ovarian granulosa cells were collected by adding Trizol in Petri dishes, and total RNA was extracted with Trizol reagent (Invitrogen, USA) according to manufacturer protocols. The total RNA quality and concentration were detected using nucleic acid protein detector (Quawell, USA). The total RNA was used to construct a single stranded circular DNA library (Figure S1). Finally, the transcriptome sequencing of ovarian granulosa cells was performed on the BGISEQ-500 platform.(Shenzhen Huada gene technology co., ltd)

2.5. Data analyses

We removed reads containing adapter, reads containing ploy-N, and low quality reads from raw data with trimmomatic, and clean reads were obtained. Bowtie2 was used to compare clean reads to the reference gene sequence (Oar_v4.0), and then RSEM was used to calculate the expression levels of genes and transcripts. DEGseq method was used to calculate the differentially expressed genes (DEGs) among different samples. The "phyper" function in R software was used to analyze the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of differential genes. Finally, the protein interaction network was mapped for BMPR1B gene and 306 DEGs by Cytoscape 3.7.1 on STRING database[1] [12].

2.6. Quantitative real-time PCR

To verify RNA-seq results, we randomly selected ten genes from the DEGs and detected the expression level of each

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[1] https://string-db.org/

gene using quantitative real-time PCR (qPCR). The first-strand cDNA was synthesized using Transcriptor cDNA Synthesis Kit 2 (Roche, Germany), and the RT-qPCR was performed using T2×RealStar Green Fast Mixture (Genstar, China). The amplification conditions were 95 °C for 3 min, followed by 40 PCR cycles: 95 °C for 10 s, 60 °C for 15 s, 72 °C for 30 s and it was performed on Light Cycler® 480 System (Roche, USA). The primer sequences were shown in Table S1.

3. Results

3.1. PCR-RFLP

FecB gene detection in 1-month ewe lamb which was hybrid lambs between Booroola Merino Sheep and Xinji fine wool sheep. Six healthy lambs were selected, including 3 ++ genotype (W1, W2, W3) and 3 B+ genotype (H1, H2, H3)(Figure 1).

3.2. Quality control of transcriptome sequencing

Six samples were measured on the BGISEQ-500 platform, and each sample produced 6.30 GB of data on average. To ensure the reliability of the results, the reads containing the connectors was removed from the original sequencing data. Reads with unknown bases (N) were more than 5%. Low-quality reads were removed (reads with a mass value less than ten accounting for more than 20% of the total base number were defined as low-quality reads (Table S2). Subsequently, we classified and mapped the clean reads to the sheep reference genome assembly (Oar_v4.0). The average mapping ratio of samples to genomes was 88.98% (Table S3). We identified 13,537 transcripts, 17,142 known genes, 610 novel genes, and 647 transcripts of novel coding protein.

3.3. Analysis of gene expression differences

Genes with a different multiple of more than twice and a Q value ≤0.001 were significantly differentially expressed. After further deletion of discredited data, we obtained 306 DEGs between ovarian granulosa cells of ++ genotype and B+ genotype (Table S4). The expression level of 170 genes was significantly upregulated, and the expression level of 136 genes was significantly downregulated (Figure 2).

3.4. GO annotation, KEGG pathway, and PPI

GO functional classification and enrichment analysis were conducted according to the results of DEGs (Figures S2 and S3). The DEGs were significantly enriched to 19 molecular function items, 8 cellular component items, and 201 biological process items. The DEGs are mainly involved in such functions as protein binding, response to stimulus, and multicellular organic process. GO analysis on the sets of genes found to be more highly expressed in B+ genotype ovarian granulosa cells (170 genes) versus
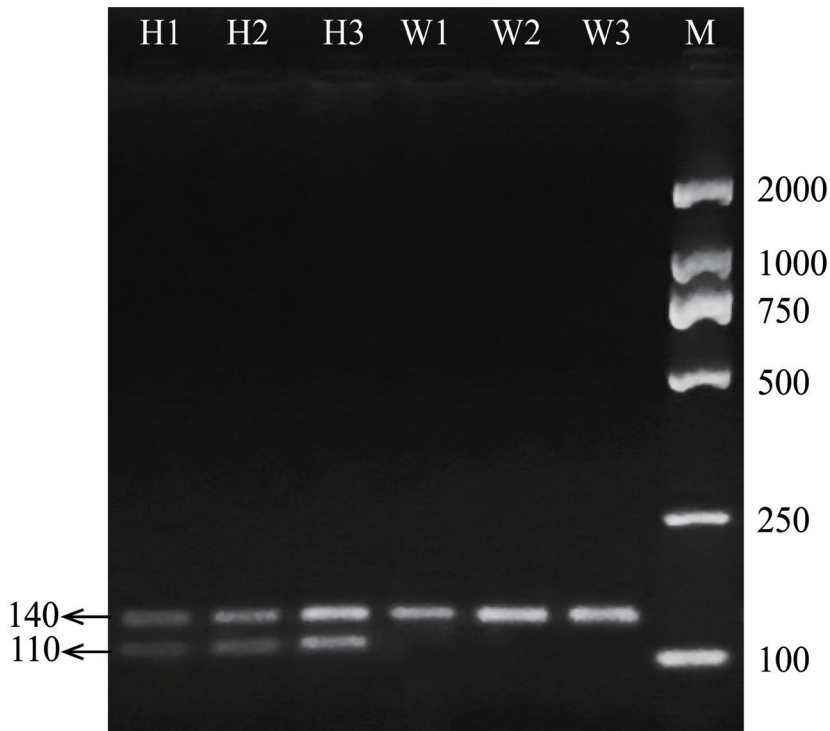
Figure 1. FecB genotyping of six hybrid sheep with PCR-RFLP. H represents FecB gene mutant heterozygous type (B+ genotype). W represents FecB gene wild homozygous type (++ genotype). M is 2000 bp marker.

those in ++ genotype (136 genes) are shown in Tables 1 and 2, respectively. Moreover, upregulated genes TCF7, TCF21, PITX2 and downregulated genes BMP2, FGF1, and PTPRR were enriched to multiple entries. It is speculated that these six genes are closely related to FecB gene.

The KEGG pathway annotation classification and enrichment analysis of DEGs were conducted (Figure S4). The bubble graph shows the top 20 GO terms with the smallest Q value. The enrichment results of KEGG pathway of their DEGs were shown in Figure 3. The top 10 significantly enriched pathways are shown in Table 3. The DEGs mainly involve ovarian diseases, follicular development, and embryogenesis signaling pathways: MAPK signaling pathway, PI3K-Akt signaling pathway, Wnt signaling pathway, axon guidance, and ribosome.

Protein interaction analysis was performed on BMPR1B gene and 306 DEGs. Finally, a major protein interaction network was obtained (Figure 4, Table S5). The protein interaction network is associated with 95 DEGs. Among them, five genes, IL6, COL1A1, LOX, BMP2, and ESR1, have strong interaction with other genes, and the node degree of interaction is not less than 6. Besides, BMP2, TGFB2, and FKBP1B genes directly interact with BMPR1B genes. They may be key genes for FecB to regulate reproductive traits in lambs

3.5. qPCR verification

To validate RNA-seq results, we detect gene expression levels using the qPCR analysis by $2-\Delta\Delta Ct$ method. The result of comparison between qPCR and RNA-sequencing are presented in Figure 5. Correlation analysis shows strong consistency with an R2 of 0.906, and all selected DEGs showed similar expression patterns.

4. Discussion

In this study, among the 306 DEGs, only 35 DEGs had a difference multiple of more than two times. The difference between the two types of ovarian granulosa cells is small. This may be explained by a similar genetic relationship in experimental animals. Additionally, since the experimental animals are from the hybrid generation, and there are no FecB gene homozygous (BB genotype) individuals, the experimental results lack a strong control group.

Through GO functional annotation analysis, we found that the protein binding of molecular function has the largest number of DEGs, which is 60. This result is in agreement with the early trend of the ovulation mechanism affecting the FecB gene. The previous study showed that FecB mutations enhance the inhibitory effect of FKBP-12 on BMPR1B activity, and the sensitivity of cells to BMPR1B-specific ligands may decrease, which
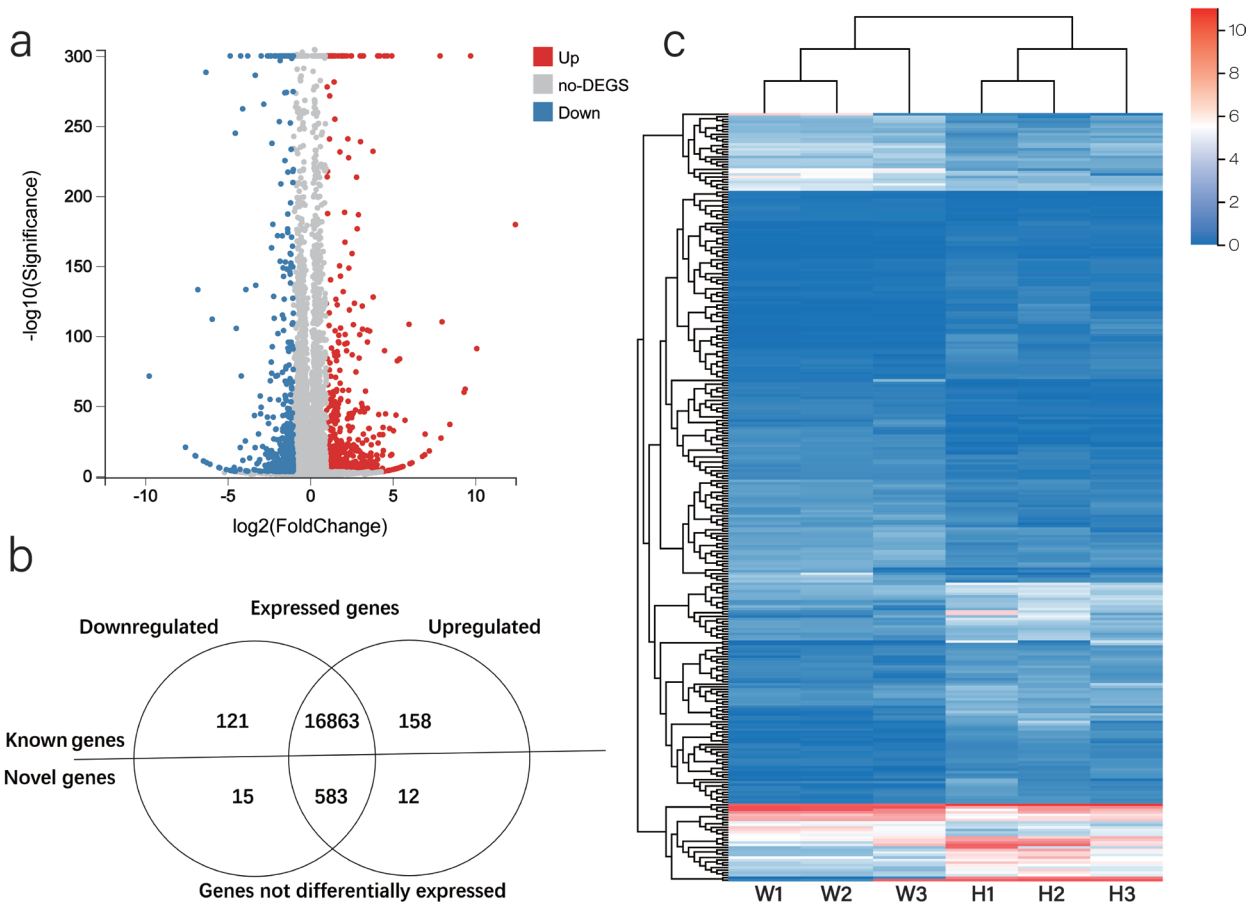
Figure 2. The mRNA expression profile changes in ovarian granulosa cells of B+ genotype and ++ genotype. (a) Volcano plot indicating up- and downregulated mRNAs further deletion of discredited data. (b) Venn diagram summarizing sequencing analysis results in ovarian granulosa cells of B+ genotype and ++ genotype. (c) Heat map showing hierarchical clustering of altered mRNAs. Up- and downregulated genes are in red and blue, respectively.

Table 1. Gene ontology analysis of genes more highly expressed in B+ type ovarian granulosa cells.

Gene ontology term	Gene ontology level	P-value	Genes
Digestive tract Morphogenesis (GO:0048546)	biological_process	2.77E-05	*SFRP2; TCF7; TCF21; PITX2*
Embryonic digestive tract development (GO:0048566)	biological_process	2.17E-05	*TCF7; TCF21; PITX2; TGFB2*
Regulation of collagen metabolic process (GO:0010712)	biological_process	8.25E-05	*F2R; FAP; RGCC*
Glutathione-homocystine Transhydrogenase activity (GO:0047139)	molecular_function	8.00E-05	*GLRX; LOC105601854*
Embryonic digestive tract morphogenesis (GO:0048557)	biological_process	5.54E-05	*TCF7; TCF21; PITX2*

may eventually lead to a series of changes in cells, such as differences in signal transduction intensity and transcript expression [13–15].

It was reported that FecB mutations in adult ewe are associated with BMP4 and GDF5 which are two natural ligands of BMPR1B [6]. Ovarian granulosa cells from

Table 2. Gene ontology analysis of genes more highly expressed in ++ type ovarian granulosa cells.

Gene ontology term	Gene ontology level	P-value	Genes
Extracellular region (GO: 0005576)	cellular_component	8.27E-05	*NPVF; KCNMA1; CTSZ; LOC101104157; LOC101105348; GM2A; MGAT4A; ASPN; ENO2; FGF1; MASP1; ABHD14B; SMOC2; BMP2; PRKCB; PTPRR; TG; LOC105605699; NTN1; SH3GL2; MMP2; FGF2; MLN*
Enzyme-linked receptor protein signaling pathway (GO:0007167)	biologica_process	0.000188	*LOC101104157; TGFA; FGF1; ROR1; BMP2; PTPRR; FAM83G; FGF2*
Spectrin binding (GO:0030507)	molecula_function	0.000303	*ADD2; DYNC1I1*
Negative regulation of heart rate (GO: 0010459)	biologica_process	0.000645	*ADRA1A; FKBP1B*
Extracellular region part (GO:0044421)	cellular_component	0.000758	*KCNMA1; CTSZ; LOC101104157; LOC101105348; GM2A; MGAT4A; ASPN; ENO2; FGF1; MASP1; ABHD14B; SMOC2; BMP2; PRKCB; PTPRR; LOC105605699; NTN1; SH3GL2; MMP2; SLPI*
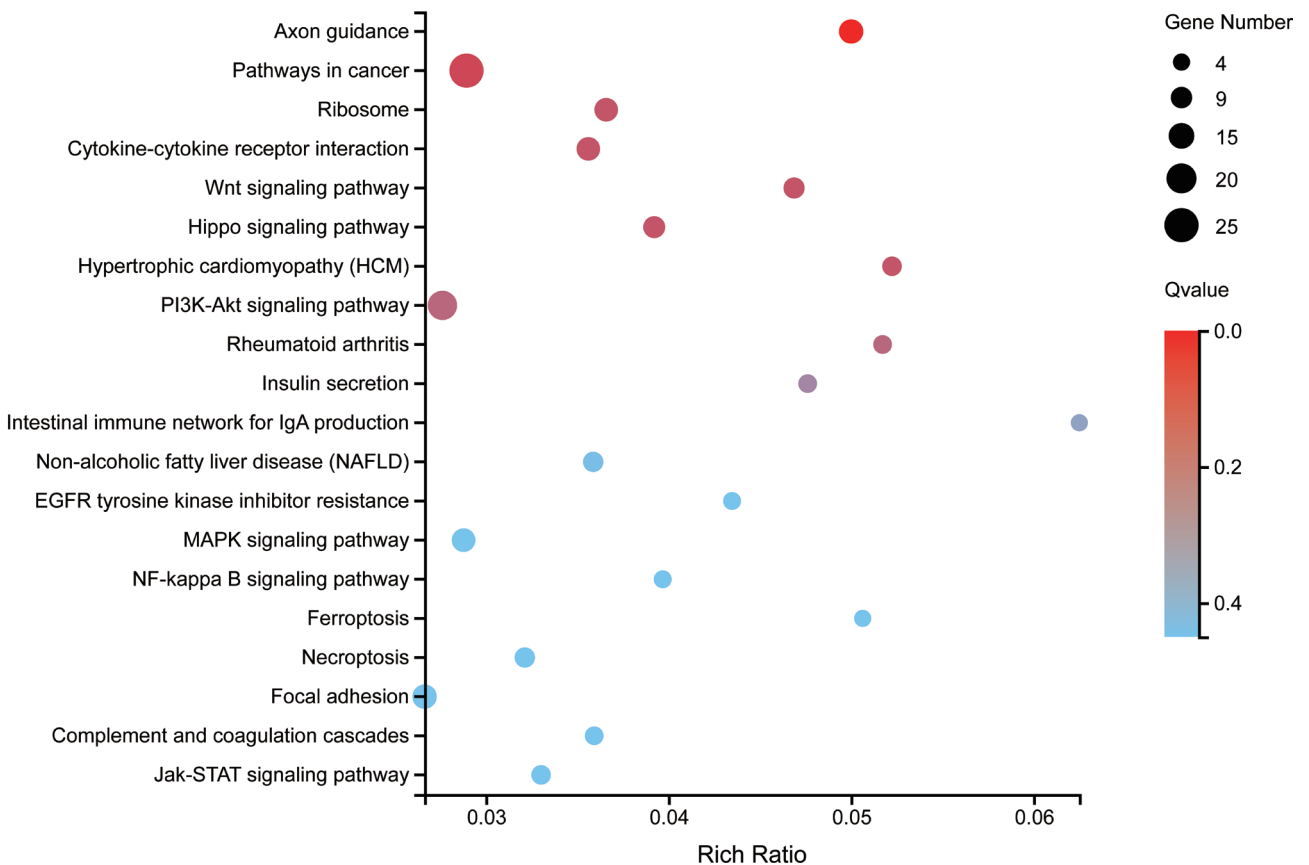
Figure 3. KEGG analysis bubble diagram of gene expression differences. The X-axis represents the enrichment ratio (Rich ratio = Term candidate gene num/Term gene num). The Y-axis represents KEGG pathway. The size of the bubble represents the number of genes annotated on a KEGG pathway, the color represents the enrichment Q value, and the darker the color, the smaller the Q value. Bubble graph shows the top 20 GO terms with the smallest Q value.

BB genotype ewes were less responsive than granulosa cells from B+ genotype ewes to the inhibitory effect on steroidogenesis of GDF5 and BMP4 [16,17]. Additionally, BMP2 was also suggested to be another potential ligand of BMPR1B. The BMP system mainly regulates the growth and development of follicles by promoting the production

Table 3. Genes of top 10 significantly enriched pathways.

| Pathway name	P-value	Gene
Axon guidance (ID: ko04360)	0.000159	SHF; NFATC4; ISLR; SLIT2; NTN1; PARD3B; UNC5C; CRMP1; NTN4; SEMA3G; CXCL12; SLITRK2
Pathways in cancer (ID: ko05200)	0.001255	LOC105606059; CCBE1; TCF7; ESR1; TRAF5; LAMB1; PTGER4; TGFB2; F2R; CCND2; LOC105608895; IL6; C1QTNF6; FGF1; PDGFB; FGF2; IL23A; MMP2; PRKCB; AXIN2; LOC105605056; BMP2; TGFA; CRLF1; CXCL12
Ribosome (ko03010)	0.004146	RPL6; RPS3A; LOC101107369; LOC101108322; LOC101109212; LOC101110649; LOC101114033; LOC101120489; RPL30
Cytokine-cytokine receptor interaction (ko04060)	0.005133	IL23A; CNTFR; OSMR; CRLF1; TNFRSF21; BMP2; LOC101120093; CCL17; CXCL12; IL6; TGFB2; TNFSF13B
Wnt signaling pathway (ko04310)	0.002548	NFATC4; TCF7; CCND2; SFRP2; SERPINF1; PRKCB; AXIN2; NKD1; WISP1
Hippo signaling pathway (ko04390)	0.005372	CCND2; TCF7; DLG2; AMOT; FGF1; AXIN2; PARD3B; BMP2; LLGL2; TGFB2
Hypertrophic cardiomyopathy (HCM) (ko05410)	0.004198	PRKAG3; ITGB7; CACNA1C; DES; FAM13C; IL6; TGFB2;
PI3K-Akt signaling pathway (ko04151)	0.007962	CCND2; C1QTNF6; LAMB1; F2R; OSMR; TGFA; FGF1; MYB; ITGB7; NR4A1; CCBE1; TNC; COL9A2; FGF2; IL6; COL1A1; COL1A2; PDGFB
Rheumatoid arthritis (ko05323)	0.008251	IL23A; LOC101120093; CXCL12; IL6; TGFB2; TNFSF13B
Insulin secretion (ko04911)	0.012115	KCNMA1; ADAMTS14; CACNA1C; PRKCB; ADCYAP1; GLP1R

of estradiol, inhibiting the synthesis of progesterone, and controlling the differentiation and maturation of ovarian granulosa cells, thereby affecting the reproductive performance of multiple embryos of female animals [18]. Our results suggest a stronger regulatory relationship between FecB mutation and BMP2 gene in lambs.

At present, studies have shown that the FecB gene had no effect on the live weight and average daily weight gain of the lambs. The B+ ewe lamb tended to achieve puberty more rapidly than the ++ ewe lamb until 10 months of age [19]. In studies of adult ewes, the FecB gene was identified to increase ovulation and decrease follicle diameter [20]. Single copy FecB gene has no significant effect on multiple ovulation [21]. Interestingly, in our late JIVET study. The oocyte of the lamb with ++ genotype had better in vitro development ability than the lamb with B+ genotype. The average number of the oocyte, maturation rate, culture rate, cleavage rate, and blastula rate of the ++ lamb were all higher than those of the B+ lamb [22]. This suggests that the FecB gene has an effect on exogenous hormone response in ewe lamb. We will take a closer look at this aspect in future studies.

The number of antral follicles in the ovine fetal ovaries began to appear at 135 days of gestation, and the number of antral follicles in 4–8 days after birth was the largest in a lifetime. A large number of antral follicles are latched during development. A stable number of antral follicles are formed on the ovary until sexual maturity [23,24]. In addition, as the primary steroid secreting cells in the follicle, ovarian granulosa cells, which synthesize estrogen, progesterone and androgens, have vital roles in follicular growth and atresia [25,26]. Studies have shown that the decreased quality or increased apoptosis of granulosa cells can affect the secretion of hormones and cytokines, and even affect the development of oocytes and subsequent embryo quality [11,27–29]. In addition, the FecB alters follicular development from the onset of follicular formation [30]. However, in our study, there was no difference in mRNA expression levels of the apoptotic genes in the two genotypes of granulosa cells, such as P53, Fas, Bcl-2, and Myc.

In summary, this is a novel study comparing DEGs of ovarian granulosa cells in ewe with different FecB genotypes. The effect of FecB on the reproductive performance of lambs is unclear. We speculate that FecB gene may have no significant effect on ovarian granulosa cell apoptosis in lambs and more likely to think that the FecB gene affects the accumulation of primal follicles on
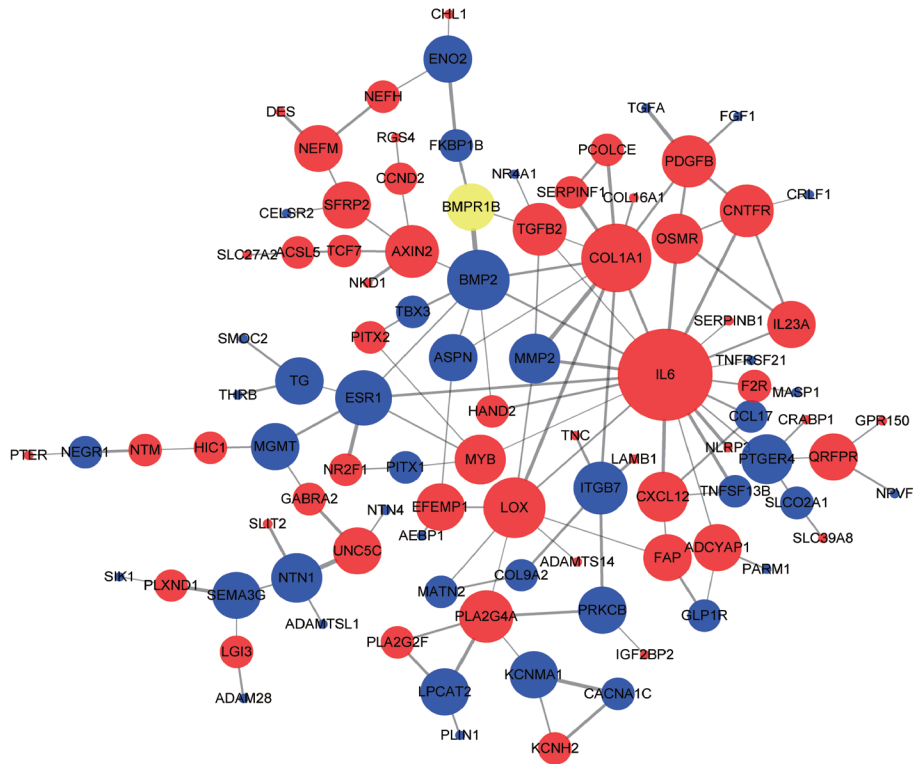
Figure 4. Protein interaction networks of the DEGs in ovarian granulosa cells of B+ genotype and ++ genotype with Cytoscape. The protein interaction network is associated with 95 DEGs. Node size represents the degree. Red nodes indicate upregulated genes, blue nodes indicate downregulated genes, and yellow nodes indicate BMPR1B which is not differentially expressed between B+ genotype and ++ genotype. Edge size represents the combine-score.
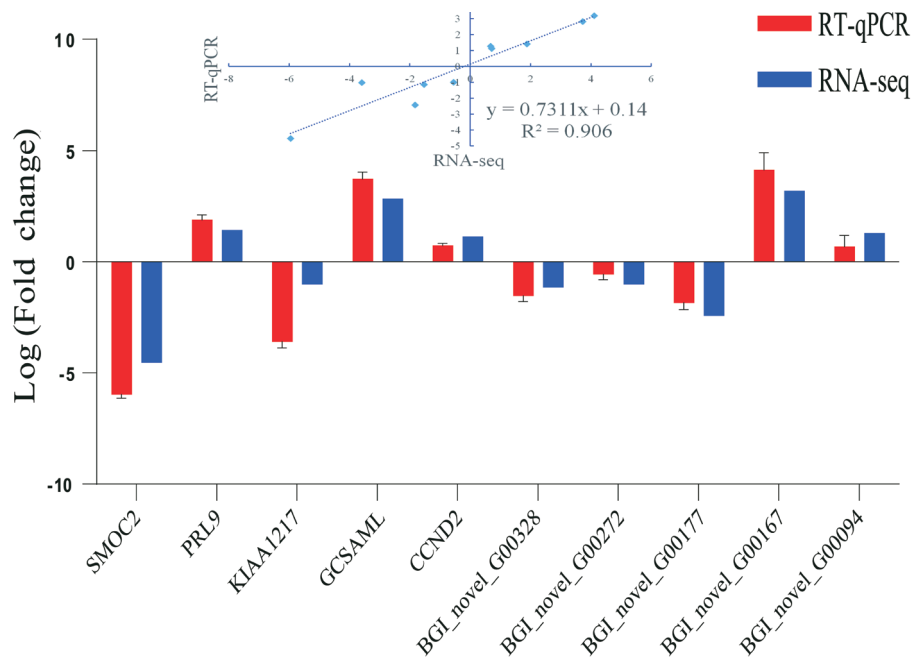
Figure 5. The comparison of transcript expression in terms of fold change as measured by RNA-sequencing and RT-qPCR. Verification of 10 DEGs by RT-qPCR. Red bars indicate RT-qPCR results, blue bars indicate RNA-seq results. The expression levels of 5 upregulated and 5 downregulated mRNAs were consistent with the sequencing data. Correlation analysis revealed strong concordance with an R2 of 0.906.

the ovaries of ewe lambs. Our study generated sequencing information of considerable value for further research reproductive performance in ewe lamb.

Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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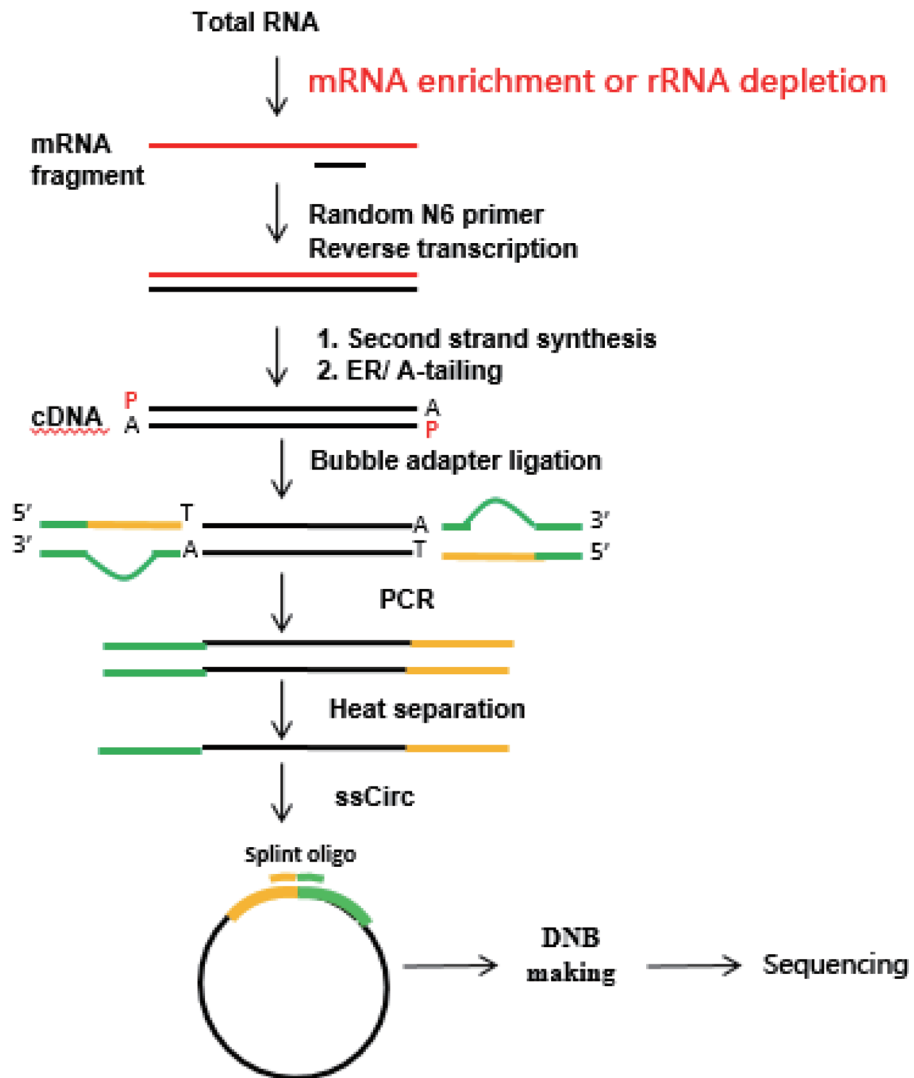
Figure S1. Flow chart of single stranded circular DNA library construction.
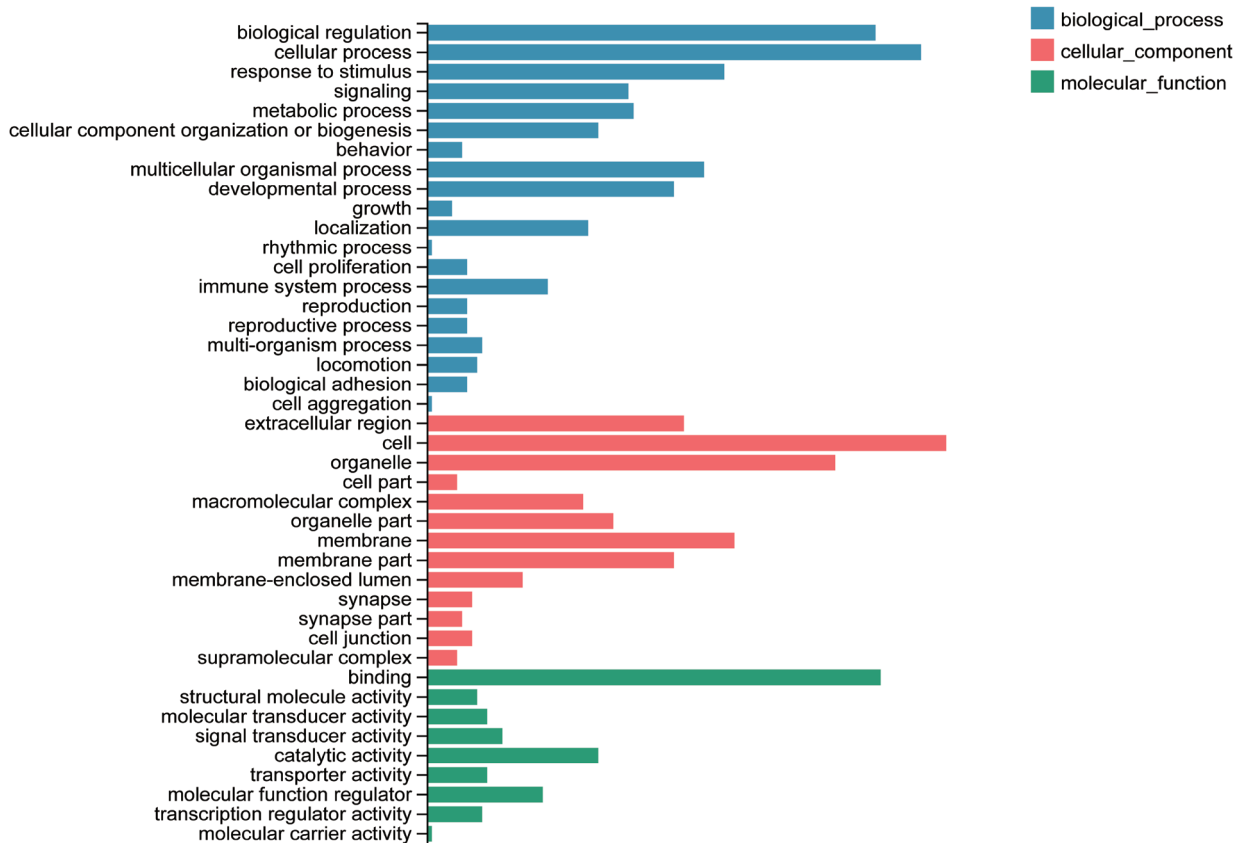
Figure S2. GO classification of the DEGs in granulosa cells of ++ genotype and B+ genotype. The X-axis represents the number of genes annotated to GO entries, and the Y-axis represents the GO functional classification. The blue bar represents molecular function, the red bar represents cellular component, and the green bar represents biological process.
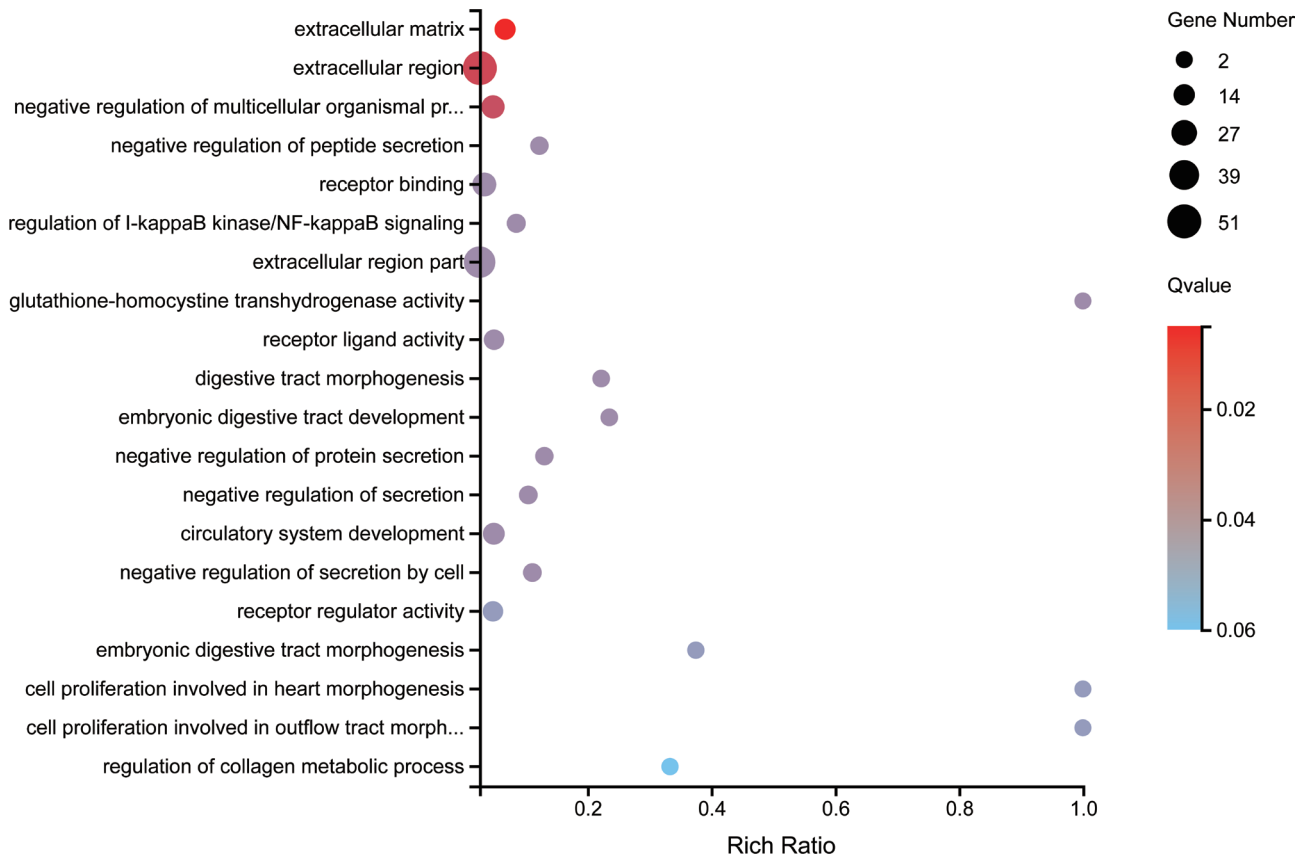
Figure S3. CO analysis bubble diagram of the DEGs in granulosa cells of ++ genotype and B+ genotype. The X-axis represents the enrichment ratio (Rich Ratio = Term candidate gene num/Term gene num). The Y-axis represents GO term. The size of the bubble represents the number of genes annotated on a GO term, the color represents the enrichment Q value; and the darker the color, the smaller the Q value. Bubble graph shows the top 20 GO terms with the smallest Q value.
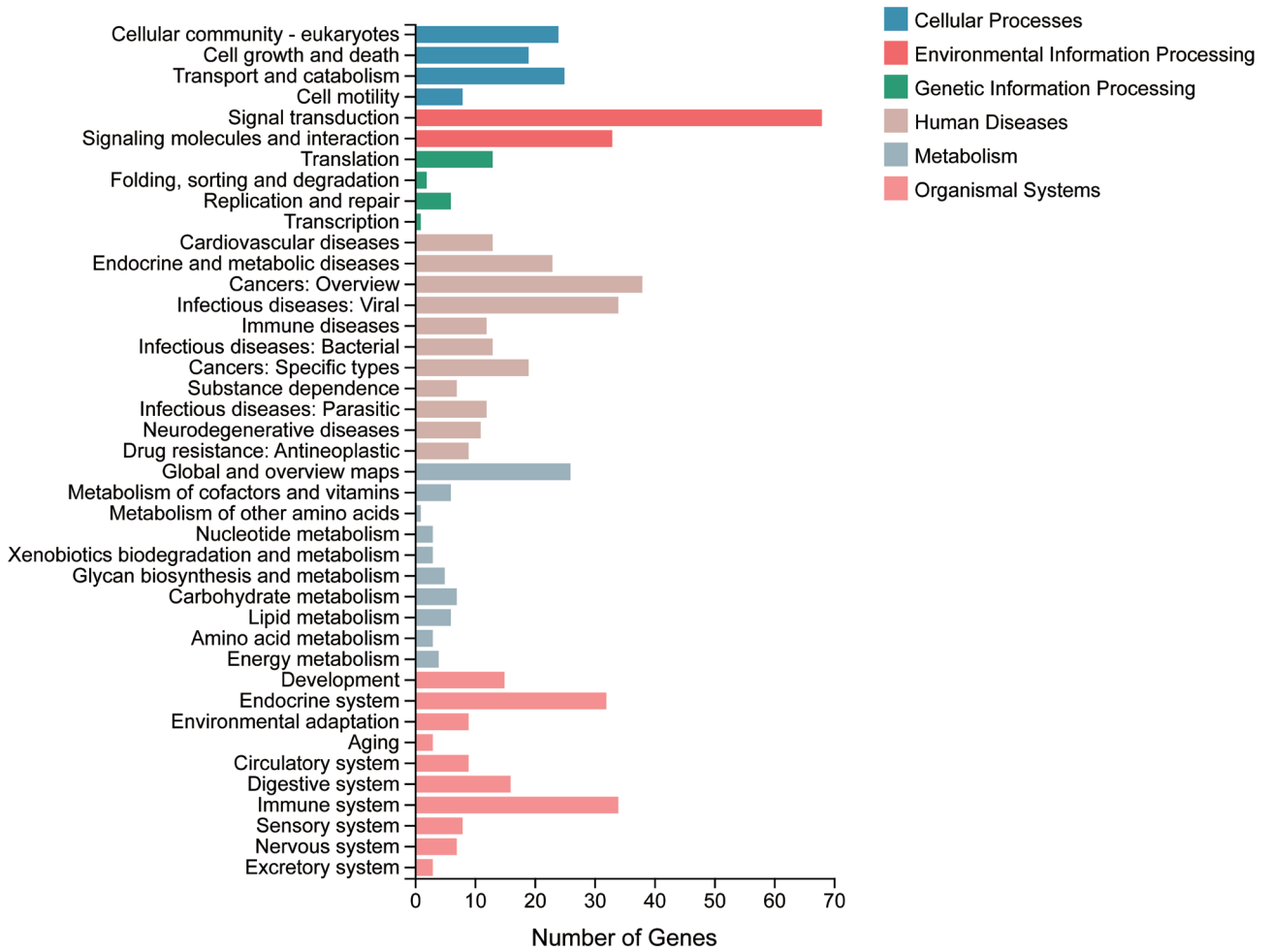
Figure S4. KEGG classification of the DEGs in granulosa cells of ++ genotype and B+ genotype. The X-axis represents the number of genes annotated to GO entries, and the Y-axis represents the KEGG pathway classification.

Table S1. Primer sequence information table.

Gene	Primer sequence (5'-3')	Annealing temp (°C)	Product size/pb
GAPDH	F:CAAGTTCCACGGCACAGTCA R:GGTTCACGCCCATCACAAA	60	248
SMOC2	F: AGGAGCCGAGGCAAGGATAG R: CCCCAGGTACTCCTTCGTGT	60	178
RPL9	F: TGATTCAGCAAGCCACCACA R: AGCCTGCTGAACTGTTCCTT	60	92
KIAA1217	F: TCTGACTACCGCATCCTCCT R: AAATCGAGGCTCTGTTCGGT	60	117
GCSAML	F: CCATGCCTATGCCCATCCTC R: GTCACCACAAAATGGGGCAG	60	90
CCND2	F: GAGTCCCAACTCCGAAGACC R: AGGCTTGATGGAGTTGTCGG	60	131
BGI_novel_G000328	F: ACCAGATCCACGTGCAGAAG R: GTGCTACCACCTTCGCTGAT	60	112
BGI_novel_G000272	F: CACCCAGTCCTCAAGCCATC R: CCTTGAGAGGCACAGGTCAC	60	111
BGI_novel_G000077	F: CTCTATGGTTGAGGCCGGAG R: ACCTGCCAGATGTAGGGAGAA	60	118
BGI_novel_G000167	F: TACAGCAGGGTTCATTGGGC R: GCATGATGCCCGAAACAGTC	60	100
BGI_novel_G000094	F: GTCCCTTTCCTAAACCACGCT R: GAAGGGTGAGGAGTCCAAGAG	60	136

Table S2. Summary of the transcriptome sequencing data.

Sample	Total raw reads(M)	Total clean reads(M)	Total clean bases(Gb)	Clean reads Q20(%)	Clean reads Q30(%)	Clean reads ratio(%)
W1	67.68	64.75	6.48	97.05	89.49	95.67
W2	70.19	65.63	6.56	96.88	88.4	93.5
W3	57.72	55.55	5.55	97.44	90.38	96.23
H1	66.2	62.98	6.3	96.68	88.72	95.13
H2	67.68	64.4	6.44	97.27	89.38	95.15
H3	67.68	65	6.5	97	89.47	96.03

Table S3. Results of RNA-Seq read mapping.

Sample	Total clean reads (M)	Total mapping (%)	Uniquely mapping (%)
W1	62.98	88.48	55.8
W2	64.4	89.34	62.61
W3	65	89.34	58.29
H1	64.75	88.62	58.25
H2	65.63	88.29	60.54
H3	55.55	89.82	60.68

Table S4. The DEGs list between B+ and ++ sheep.

Gene ID	Symbol	log2 (H/W)	Q value (W-vs-H)	P-value (W-vs-H)
101114818	ABHD14B	−1.05674	9.10E-165	6.06E-166
101118240	ACOT11	−1.06452	2.94E-05	2.77E-05
101121475	ACSL5	3.288142	1.97E-46	4.06E-47
101120058	ADAM28	−1.146	8.07E-07	6.49E-07
101106305	ADAMTS14	1.692262	0	0
101122835	ADAMTSL1	−1.96222	2.74E-172	1.76E-173
443337	ADCYAP1	3.040523	1.24E-46	2.55E-47
101106757	ADD2	−2.0535	3.52E-34	9.33E-35
101109070	ADGRG2	1.665059	6.51E-21	2.52E-21
101103604	ADGRL4	−1.20263	7.95E-23	2.87E-23
100169940	ADRA1A	−1.26629	6.21E-80	8.17E-81
101119132	AEBP1	−1.26206	0	0
101122182	AIF1L	−1.11781	5.18E-64	8.15E-65
101121984	AK5	2.245889	1.64E-22	5.97E-23
101114182	ALDH1L2	−1.15317	1.24E-53	2.26E-54
101107577	ALS2CR12	2.116488	2.15E-12	1.18E-12
101108810	AMOT	−1.35155	6.97E-49	1.38E-49
101103426	ANKH	−1.20703	0	0
101118077	AP3M2	−2.95753	9.59E-50	1.87E-50
101112046	ARNTL2	1.682595	7.47E-87	9.23E-88
101110236	ARSI	3.82051	1.42E-232	6.86E-234
101108992	ARX	1.178684	2.19E-76	2.99E-77
101108885	ASPN	−1.38562	1.41E-22	5.12E-23
101117857	ATP11A	−2.51083	0	0
101109815	AXIN2	2.646841	6.31E-23	2.27E-23
101119388	B4GALNT1	1.159804	1.59E-05	1.45E-05
101119849	BASP1	1.985053	0	0
101114560	BCL2A1	3.372571	4.15E-15	2.02E-15
101119363	BICC1	1.447965	1.29E-39	3.02E-40
101117688	BMP2	−2.30128	2.64E-238	1.24E-239
101105767	C12H1orf115	−1.06972	0	0
101115904	C15H11orf96	3.584075	1.31E-34	3.43E-35
101105949	C16H5orf49	1.090696	1.97E-05	1.82E-05
101102801	C1QTNF6	1.491321	2.68E-50	5.18E-51
101116016	C26H8orf4	2.216694	4.58E-90	5.47E-91
101111429	C2H2orf88	1.473522	6.45E-47	1.32E-47
101122031	C7H14orf37	−1.36205	0	0
101114711	C9H8orf34	2.426763	1.85E-17	8.17E-18
101116562	CACNA1C	−2.17081	2.74E-113	2.68E-114
101107141	CADPS	1.384088	6.48E-34	1.73E-34
101117553	CCBE1	−1.5597	5.95E-127	5.23E-128
101108991	CCDC160	1.754	6.85E-10	4.41E-10

Table S4. (Continued).

101120186	CCL17	−6.30402	5.20E-289	1.96E-290
100147799	CCND2	1.121823	0.000129986	0.000131795
101105681	CD79A	1.451372	0.000316474	0.00033654
101103712	CD8B	1.653534	6.30E-123	5.71E-124
101107570	CDH23	−1.06441	3.45E-18	1.48E-18
101103606	CELSR2	−1.33359	1.12E-21	4.20E-22
106990096	CFH	5.759733	1.87E-40	4.32E-41
101113017	CHL1	3.060532	1.67E-239	7.81E-241
101123239	CHRNA3	3.130778	2.00E-52	3.73E-53
101115407	CHRNA5	1.360842	1.26E-07	9.49E-08
101113104	CHRNA7	4.520493	4.05E-90	4.83E-91
100885760	CNTFR	1.9483	3.35E-10	2.11E-10
101107002	COL16A1	2.003593	0	0
443483	COL1A1	2.486829	0	0
443512	COL1A2	2.51291	0	0
101121906	COL9A2	−2.03838	0	0
101111418	CPPED1	−1.81146	5.33E-154	3.83E-155
101112477	CPVL	1.50768	1.54E-255	6.76E-257
101122057	CRABP1	2.690728	5.27E-124	4.72E-125
101108913	CRLF1	−1.35131	2.56E-09	1.71E-09
101102645	CRMP1	2.37079	5.24E-45	1.10E-45
101115999	CSRP3	2.39143	1.94E-08	1.37E-08
101101911	CTSZ	−1.84485	0	0
101121145	CXCL12	2.275621	1.71E-41	3.87E-42
101117308	DES	1.38575	0	0
101104867	DLG2	1.134938	3.19E-11	1.89E-11
101105194	DNAH8	−1.64239	1.29E-34	3.39E-35
101116636	DOCK8	−1.15992	1.85E-67	2.79E-68
101102070	DPYS	3.170785	4.79E-122	4.38E-123
101109123	DTX1	2.415081	5.20E-33	1.43E-33
101118013	DYNC1I1	−1.1308	3.89E-24	1.35E-24
101120648	EFEMP1	1.698123	0	0
101109164	ENO2	−1.34172	9.80E-188	5.80E-189
101120026	ENPP5	−1.43749	0	0
101106481	EPB41L3	−2.57896	9.71E-43	2.14E-43
443228	ESR1	−2.32715	1.07E-163	7.21E-165
101105757	F2R	2.757969	8.45E-48	1.71E-48
101122713	FAM129A	−1.07423	0	0
101119609	FAM13C	1.343467	3.51E-40	8.16E-41
106991420	FAM83G	−1.45124	2.67E-44	5.71E-45
101106492	FAP	2.863846	3.21E-177	2.00E-178
101109353	FGF1	−1.10487	6.17E-63	9.87E-64
443306	FGF2	−1.52773	8.97E-08	6.67E-08
101115568	FIBIN	1.471571	0	0

Table S4. (Continued).

101108378	FKBP1B	−1.47371	2.71E-19	1.11E-19
101104089	FUT4	1.210481	2.93E-26	9.56E-27
101121265	GABRA2	1.693626	2.36E-25	7.93E-26
105613513	GCSAML	2.823459	4.55E-214	2.41E-215
101107396	GJB3	−1.06392	1.37E-45	2.85E-46
541609	GLP1R	−2.39932	0	0
100147797	GLRX	2.529433	2.65E-42	5.90E-43
101105496	GM2A	−1.2398	0	0
105607633	GPR150	4.829239	1.48E-34	3.91E-35
101120384	HAND2	3.029984	4.87E-14	2.49E-14
105616456	HIC1	1.839645	1.67E-17	7.37E-18
101104698	HOOK1	1.651137	1.73E-89	2.09E-90
101119084	HPDL	1.212106	1.85E-06	1.53E-06
101115925	IGF2BP2	2.056694	5.31E-18	2.30E-18
100462692	IL23A	3.804066	5.38E-05	5.22E-05
443406	IL6	3.686886	4.77E-17	2.14E-17
101110908	INPP1	1.199717	6.51E-60	1.09E-60
101110878	ISLR	1.713184	3.54E-44	7.58E-45
101109956	ITGB7	−1.17549	9.70E-59	1.66E-59
101115029	JAKMIP2	1.977513	1.41E-17	6.19E-18
101120989	KCNE4	−1.39623	1.70E-67	2.57E-68
105615177	KCNH2	1.051297	9.47E-14	4.93E-14
100270685	KCNMA1	−1.20744	3.16E-70	4.59E-71
101105016	KIAA1217	−1.01894	3.00E-275	1.18E-276
101107952	KIAA1644	−1.7647	2.34E-209	1.27E-210
101108117	KRT18	4.235439	0	0
101105743	LAMB1	1.962775	0	0
101107751	LDLRAD4	1.160491	9.36E-27	3.01E-27
101122581	LGI3	2.762524	0.000126383	0.000127882
101104472	LIN7A	1.791821	1.23E-18	5.15E-19
101120426	LLGL2	−1.38372	4.72E-38	1.14E-38
101102344	LOC101102344	1.723412	3.43E-05	3.26E-05
101103182	LOC101103182	1.938455	0	0
101104157	LOC101104157	−1.99095	3.49E-14	1.78E-14
101105348	LOC101105348	−1.2266	1.21E-146	9.21E-148
101107369	LOC101107369	1.133884	2.42E-12	1.34E-12
101108322	LOC101108322	9.728631	0	0
101109212	LOC101109212	−1.414	2.65E-66	4.08E-67
101110116	LOC101110116	5.287297	6.96E-83	8.92E-84
101110649	LOC101110649	5.150004	1.15E-05	1.04E-05
101110973	LOC101110973	1.86542	7.64E-56	1.36E-56
101113911	LOC101113911	1.960252	1.04E-08	7.22E-09
101114033	LOC101114033	2.108251	4.68E-05	4.51E-05
101114167	LOC101114167	1.452539	1.99E-20	7.87E-21

Table S4. (Continued).

101108820	MGAT4A	−1.30203	7.55E-35	1.97E-35
101106897	MGMT	−1.05406	3.75E-14	1.92E-14
443473	MLN	−2.68271	2.76E-16	1.28E-16
443115	MMP2	−2.15411	0	0
101117552	MRO	3.15621	0	0
101117691	MT1C	2.009614	2.49E-132	2.12E-133
101109886	MYB	1.414258	4.32E-41	9.88E-42
101107472	NEFH	2.340083	5.38E-10	3.44E-10
101112452	NEFM	3.832163	1.82E-128	1.59E-129
101119516	NEGR1	−3.88221	7.18E-134	6.03E-135
101118617	NFATC4	3.361506	2.92E-61	4.83E-62
101115536	NFE2L3	−1.4493	1.04E-78	1.39E-79
101116855	NKD1	4.128396	3.18E-24	1.10E-24
101107699	NLRP3	1.000525	5.26E-124	4.71E-125
100127217	NPVF	−1.49338	1.72E-153	1.25E-154
101121774	NR2F1	1.586863	2.20E-12	1.21E-12
101113222	NR4A1	−1.15962	4.76E-159	3.29E-160
101118143	NTM	2.354767	3.52E-149	2.63E-150
105607800	NTN1	−1.03174	3.00E-218	1.56E-219
780511	NTN4	−1.22362	0	0
101118362	NYNRIN	1.611798	5.65E-83	7.23E-84
101112387	OLFML2A	1.59431	7.12E-27	2.28E-27
101111399	OSBPL10	−1.02013	1.83E-91	2.14E-92
101105948	OSMR	1.66294	2.51E-20	9.96E-21
101112665	OTOR	2.806467	8.87E-05	8.81E-05
101113827	PARD3B	−1.06528	9.33E-68	1.40E-68
101104239	PARM1	−1.79364	0	0
101118052	PCDH18	1.647492	1.80E-46	3.71E-47
101104033	PCOLCE	1.746105	3.94E-101	4.27E-102
101118773	PDE1B	1.813178	3.69E-232	1.79E-233
443545	PDGFB	1.182186	8.50E-28	2.66E-28
101109187	PI15	4.506234	0	0
101105203	PIEZO2	2.611909	1.87E-45	3.90E-46
101103908	PIRT	−1.46146	2.80E-77	3.80E-78
101119560	PITX1	−2.57325	0	0
101116988	PITX2	1.809226	1.89E-32	5.26E-33
101106664	PLA2G2F	9.403674	9.90E-63	1.59E-63
101111472	PLA2G4A	3.0113	1.31E-101	1.42E-102
100135431	PLIN1	−1.6198	9.59E-12	5.50E-12
101110619	PLK2	1.317969	0	0
101106801	PLXND1	1.427877	6.12E-61	1.02E-61
101118098	POLM	−1.42724	1.45E-54	2.62E-55
100142674	PRKAG3	−1.2262	1.00E-36	2.50E-37
101118410	PRKCB	−2.28118	5.35E-93	6.17E-94

Table S4. (Continued).

101115187	LOC101115187	−2.42716	1.05E-05	9.43E-06
101115773	LOC101115773	2.492493	8.19E-06	7.26E-06
101116336	LOC101116336	1.181607	6.49E-11	3.91E-11
101116391	LOC101116391	−1.49368	3.73E-26	1.22E-26
101116756	LOC101116756	1.036408	8.41E-23	3.04E-23
101116975	LOC101116975	−1.25554	3.77E-47	7.68E-48
101117112	LOC101117112	2.936931	3.05E-187	1.81E-188
101117184	LOC101117184	3.24122	0	0
101117232	LOC101117232	2.434256	1.35E-06	1.11E-06
101117946	LOC101117946	2.977086	6.42E-85	7.99E-86
101118175	LOC101118175	−1.69736	6.71E-10	4.31E-10
101119456	LOC101119456	−2.24572	2.09E-180	1.28E-181
101120093	LOC101120093	3.483794	5.27E-49	1.04E-49
101120489	LOC101120489	1.40421	4.65E-58	8.05E-59
101120702	LOC101120702	3.05831	1.19E-08	8.28E-09
101121518	LOC101121518	5.435824	2.66E-84	3.34E-85
101121593	LOC101121593	2.784641	6.15E-75	8.50E-76
101122717	LOC101122717	−4.19746	0	0
105601854	LOC105601854	2.615814	2.20E-21	8.38E-22
105604795	LOC105604795	−1.0891	5.79E-08	4.23E-08
105604847	LOC105604847	−1.03853	1.80E-12	9.92E-13
105604929	LOC105604929	12.44483	3.13E-180	1.92E-181
105605056	LOC105605056	−1.09931	7.54E-22	2.81E-22
105605699	LOC105605699	−1.67887	3.37E-172	2.17E-173
105605750	LOC105605750	−2.51095	1.07E-13	5.55E-14
105606059	LOC105606059	−1.13787	5.89E-07	4.68E-07
105606075	LOC105606075	−1.01702	1.70E-10	1.05E-10
105608895	LOC105608895	−1.0921	1.29E-24	4.42E-25
105609492	LOC105609492	1.151917	1.55E-17	6.83E-18
105615158	LOC105615158	−2.2239	1.07E-07	8.02E-08
105616100	LOC105616100	−1.47829	1.66E-08	1.17E-08
106991049	LOC106991049	−3.65296	1.74E-13	9.17E-14
101112220	LOX	1.408839	0	0
101115816	LPCAT2	−2.8021	3.11E-266	1.27E-267
101103927	LRFN5	1.384746	2.19E-40	5.07E-41
101119304	LTC4S	−6.92598	6.70E-15	3.31E-15
105603011	MAGEL2	1.134388	2.59E-18	1.10E-18
101117094	MAP3K8	1.109344	2.91E-24	9.99E-25
101111065	MASP1	−1.22233	4.77E-05	4.60E-05
101107106	MATN2	−1.08686	8.97E-05	8.91E-05
780509	MB	3.101923	5.63E-15	2.77E-15
101111297	MEDAG	1.828494	6.87E-67	1.05E-67
101118811	MEIS3	2.326858	2.56E-36	6.45E-37
101118099	MEST	1.090902	3.31E-35	8.59E-36

Table S4. (Continued).

101102782	PSD3	−1.40524	1.60E-148	1.20E-149
101105738	PSD4	−3.01242	9.14E-58	1.59E-58
101109988	PTER	1.250993	7.98E-141	6.38E-142
101104343	PTGER4	−1.18202	1.04E-17	4.53E-18
101121162	PTPRR	−1.26423	1.84E-20	7.30E-21
100145863	QRFPR	4.166054	1.61E-44	3.44E-45
101106414	RARG	−1.00732	7.23E-117	6.91E-118
101107654	RARRES1	1.195699	3.54E-272	1.41E-273
101120281	RCAN2	3.480995	2.26E-96	2.53E-97
101108061	RGCC	1.944598	5.70E-24	1.98E-24
101103743	RGS16	1.50541	1.73E-86	2.15E-87
100381246	RGS4	1.557746	1.10E-42	2.44E-43
101122698	RGS6	−1.59718	1.58E-104	1.65E-105
101109659	ROR1	−2.19899	6.14E-129	5.30E-130
106990098	RPL30	4.115194	0	0
100135440	RPL6	1.127658	0	0
101106110	RPS3A	1.909948	0	0
101120982	SBK1	−1.23793	3.70E-08	2.67E-08
101106194	SDCBP2	−2.99177	4.82E-45	1.01E-45
101111838	SEMA3G	−2.22313	1.12E-42	2.49E-43
101110712	SERPINB1	1.039251	1.39E-278	5.39E-280
100192425	SERPINF1	2.573544	1.81E-44	3.86E-45
101122973	SETD6	2.324052	3.07E-23	1.10E-23
100302355	SFRP2	6.00116	5.76E-109	5.84E-110
106990245	SH3GL2	−3.068	0.000326256	0.000347616
101111716	SHF	−1.14016	4.90E-234	2.34E-235
101122497	SIK1	−1.18723	1.46E-36	3.67E-37
101105818	SLC25A41	1.211113	8.92E-22	3.33E-22
101108576	SLC27A2	2.961746	2.74E-09	1.83E-09
101108488	SLC39A8	2.075526	0	0
101105033	SLC6A6	−1.32535	0	0
654334	SLCO2A1	−1.85967	7.69E-254	3.38E-255
100125611	SLIT2	2.62959	1.06E-108	1.08E-109
101118672	SLITRK2	1.462101	4.24E-282	1.64E-283
641306	SLPI	−1.15022	1.11E-17	4.86E-18
101116580	SMOC2	−4.52073	1.57E-245	7.20E-247
100302330	SPRN	1.17647	5.80E-60	9.74E-61
101109366	SYNJ2	−1.59156	6.73E-23	2.43E-23
101110875	TBX3	−1.62509	2.51E-149	1.87E-150
101106947	TCF21	3.019461	4.74E-18	2.04E-18
101101980	TCF7	1.79157	9.52E-151	7.03E-152
101122868	TG	−1.66664	7.51E-28	2.35E-28
101106508	TGFA	−2.35866	0	0
554322	TGFB2	3.478893	8.23E-105	8.57E-106

Table S4. (Continued).

443445	THRB	−2.16111	1.63E-16	7.47E-17
101102841	TMEM119	−1.16371	5.68E-30	1.67E-30
101107614	TMEM92	1.43623	2.55E-10	1.60E-10
101105606	TMPPE	−1.15877	2.59E-09	1.73E-09
101118337	TNC	2.480419	0	0
101107794	TNFAIP8L3	1.660836	1.07E-24	3.64E-25
101117284	TNFRSF21	−1.35234	4.10E-177	2.56E-178
780517	TNFSF13B	−1.08468	3.37E-05	3.21E-05
101119408	TRAF5	1.041971	7.61E-10	4.91E-10
101113935	UNC5C	1.461466	1.82E-11	1.06E-11
101104704	UPK1B	1.066661	4.28E-188	2.53E-189
101120271	USH1C	−1.01142	2.97E-06	2.51E-06
101111451	WIPF3	−1.20163	7.22E-145	5.57E-146
101120418	WISP1	−1.53782	0	0
101107710	ZDHHC14	−1.22655	1.34E-24	4.58E-25
101121179	ZFPM2	2.806467	2.01E-21	7.62E-22
105611419		4.040523	1.43E-15	6.86E-16
BGI_novel_G000005		−1.16754	7.26E-35	1.90E-35
BGI_novel_G000032		1.402206	0	0
BGI_novel_G000056		2.12456	2.97E-12	1.65E-12
BGI_novel_G000077		−4.8303	0	0
BGI_novel_G000094		1.266137	1.03E-20	4.05E-21
BGI_novel_G000099		4.954366	5.11E-16	2.39E-16
BGI_novel_G000167		3.182297	1.67E-105	1.73E-106
BGI_novel_G000177		−2.42029	2.16E-55	3.86E-56
BGI_novel_G000193		−1.41075	1.17E-15	5.55E-16
BGI_novel_G000219		−1.0539	0.000545168	0.000599006
BGI_novel_G000232		1.27394	3.00E-06	2.54E-06
BGI_novel_G000252		−1.6453	1.69E-10	1.05E-10
BGI_novel_G000254		1.283702	1.00E-05	8.98E-06
BGI_novel_G000256		−1.23512	1.55E-26	5.02E-27
BGI_novel_G000272		−1.00088	1.09E-300	3.98E-302
BGI_novel_G000325		−4.46344	4.26E-106	4.41E-107
BGI_novel_G000328		−1.14782	4.25E-18	1.83E-18
BGI_novel_G000334		1.384875	0.000135928	0.00013826
BGI_novel_G000346		1.328241	1.40E-33	3.77E-34
BGI_novel_G000414		1.214777	1.15E-16	5.24E-17
BGI_novel_G000430		−2.31909	4.29E-74	5.96E-75
BGI_novel_G000435		−1.33719	2.34E-45	4.90E-46
BGI_novel_G000529		−1.34808	7.49E-09	5.16E-09
BGI_novel_G000574		1.130778	6.85E-06	6.02E-06
BGI_novel_G000580		−1.37829	1.51E-08	1.06E-08
BGI_novel_G000584		5.515758	6.12E-07	4.87E-07
BGI_novel_G000626		−1.06403	0.000743133	0.000830093

Table S5. Protein interaction networks of the DEGs in granulosa cells of ++ genotype and B+ genotype.

Node1	Node2	Node1 string internal id	Node2 string internal id	Node1 external id	Node2_external id	Combined score
UNC5C	NTN1	4895174	4881149	9940.ENSOARP00000018162	9940.ENSOARP00000002849	0.984
BMP2	BMPR1B	4896979	4895420	9940.ENSOARP00000020124	9940.ENSOARP00000018416	0.982
MMP2	COL1A1	4896287	4883317	9940.ENSOARP00000019362	9940.ENSOARP00000005233	0.9
NR2F1	ESR1	4894976	4881800	9940.ENSOARP00000017946	9940.ENSOARP00000003565	0.83
CACNA1C	KCNMA1	4891414	4887575	9940.ENSOARP00000014034	9940.ENSOARP00000009868	0.825
PLXND1	SEMA3G	4891945	4882861	9940.ENSOARP00000014629	9940.ENSOARP00000004730	0.809
PDGFB	TGFA	4894680	4889454	9940.ENSOARP00000017621	9940.ENSOARP00000011905	0.803
IL6	OSMR	4890356	4888216	9940.ENSOARP00000012891	9940.ENSOARP00000010565	0.749
IL6	CXCL12	4890356	4881453	9940.ENSOARP00000012891	9940.ENSOARP00000003183	0.745
IL6	NLRP3	4890356	4885990	9940.ENSOARP00000012891	9940.ENSOARP00000008143	0.74
FKBP1B	ENO2	4896058	4883672	9940.ENSOARP00000019107	9940.ENSOARP00000005623	0.739
LPCAT2	PLA2G4A	4896341	4886338	9940.ENSOARP00000019423	9940.ENSOARP00000008524	0.738
COL1A1	LOX	4883317	4878813	9940.ENSOARP00000005233	9940.ENSOARP00000000311	0.737
IL6	TNFSF13B	4890356	4884047	9940.ENSOARP00000012891	9940.ENSOARP00000006026	0.733
SERPINF1	COL1A1	4892530	4883317	9940.ENSOARP00000015280	9940.ENSOARP00000005233	0.732
MMP2	IL6	4896287	4890356	9940.ENSOARP00000019362	9940.ENSOARP00000012891	0.724
PCOLCE	COL1A1	4894846	4883317	9940.ENSOARP00000017807	9940.ENSOARP00000005233	0.723
CACNA1C	KCNH2	4891414	4879913	9940.ENSOARP00000014034	9940.ENSOARP00000001494	0.698
SLIT2	NTN1	4883231	4881149	9940.ENSOARP00000005140	9940.ENSOARP00000002849	0.696
IL6	CNTFR	4890356	4887609	9940.ENSOARP00000012891	9940.ENSOARP00000009907	0.693
NKD1	AXIN2	4895590	4893771	9940.ENSOARP00000018607	9940.ENSOARP00000016613	0.687
PRKCB	ITGB7	4895884	4894913	9940.ENSOARP00000018920	9940.ENSOARP00000017878	0.685
DES	NEFM	4898403	4888321	9940.ENSOARP00000021677	9940.ENSOARP00000010680	0.682
UNC5C	GABRA2	4895174	4893781	9940.ENSOARP00000018162	9940.ENSOARP00000016624	0.68
PRKCB	PLA2G4A	4895884	4886338	9940.ENSOARP00000018920	9940.ENSOARP00000008524	0.673
NTM	NEGR1	4892409	4890435	9940.ENSOARP00000015144	9940.ENSOARP00000012976	0.662
ITGB7	LAMB1	4894913	4885636	9940.ENSOARP00000017878	9940.ENSOARP00000007749	0.658
LPCAT2	PLA2G2F	4896341	4887768	9940.ENSOARP00000019423	9940.ENSOARP00000010080	0.65
IL6	ESR1	4890356	4881800	9940.ENSOARP00000012891	9940.ENSOARP00000003565	0.647
ITGB7	COL1A1	4894913	4883317	9940.ENSOARP00000017878	9940.ENSOARP00000005233	0.643
NEFM	NEFH	4888321	4882722	9940.ENSOARP00000010680	9940.ENSOARP00000004583	0.631
MGMT	ESR1	4893120	4881800	9940.ENSOARP00000015911	9940.ENSOARP00000003565	0.622
PDGFB	OSMR	4894680	4888216	9940.ENSOARP00000017621	9940.ENSOARP00000010565	0.618
ITGB7	TNC	4894913	4884368	9940.ENSOARP00000017878	9940.ENSOARP00000006368	0.618
BMP2	COL1A1	4896979	4883317	9940.ENSOARP00000020124	9940.ENSOARP00000005233	0.611
COL9A2	ITGB7	4898440	4894913	9940.ENSOARP00000021716	9940.ENSOARP00000017878	0.61
IL6	COL1A1	4890356	4883317	9940.ENSOARP00000012891	9940.ENSOARP00000005233	0.61
GLP1R	FAP	4894662	4884671	9940.ENSOARP00000017600	9940.ENSOARP00000006698	0.602
PDGFB	CNTFR	4894680	4887609	9940.ENSOARP00000017621	9940.ENSOARP00000009907	0.6
OSMR	IL23A	4888216	4887625	9940.ENSOARP00000010565	9940.ENSOARP00000009923	0.6
IL23A	CNTFR	4887625	4887609	9940.ENSOARP00000009923	9940.ENSOARP00000009907	0.6

Table S5. (Continued).

FKBP1B	BMPR1B	4896058	4895420	9940.ENSOARP00000019107	9940.ENSOARP00000018416	0.596
PCOLCE	SERPINF1	4894846	4892530	9940.ENSOARP00000017807	9940.ENSOARP00000015280	0.595
TG	THRB	4884042	4882885	9940.ENSOARP00000006021	9940.ENSOARP00000004757	0.592
PDGFB	FGF1	4894680	4880325	9940.ENSOARP00000017621	9940.ENSOARP00000001941	0.587
HAND2	IL6	4893636	4890356	9940.ENSOARP00000016468	9940.ENSOARP00000012891	0.584
AXIN2	TCF7	4893771	4892067	9940.ENSOARP00000016613	9940.ENSOARP00000014760	0.581
TCF7	ACSL5	4892067	4890131	9940.ENSOARP00000014760	9940.ENSOARP00000012643	0.578
PLA2G2F	PLA2G4A	4887768	4886338	9940.ENSOARP00000010080	9940.ENSOARP00000008524	0.575
PDGFB	COL1A1	4894680	4883317	9940.ENSOARP00000017621	9940.ENSOARP00000005233	0.567
TG	SMOC2	4884042	4883460	9940.ENSOARP00000006021	9940.ENSOARP00000005387	0.565
BMP2	IL6	4896979	4890356	9940.ENSOARP00000020124	9940.ENSOARP00000012891	0.564
IL6	IL23A	4890356	4887625	9940.ENSOARP00000012891	9940.ENSOARP00000009923	0.564
CXCL12	CCL17	4881453	4878662	9940.ENSOARP00000003183	9940.ENSOARP00000000145	0.56
BMP2	TBX3	4896979	4884666	9940.ENSOARP00000020124	9940.ENSOARP00000006691	0.555
IL6	CCL17	4890356	4878662	9940.ENSOARP00000012891	9940.ENSOARP00000000145	0.546
LGI3	ADAM28	4888797	4888394	9940.ENSOARP00000011197	9940.ENSOARP00000010757	0.545
LPCAT2	PLIN1	4896341	4890047	9940.ENSOARP00000019423	9940.ENSOARP00000012552	0.54
COL1A1	COL16A1	4883317	4879615	9940.ENSOARP00000005233	9940.ENSOARP00000001172	0.536
COL9A2	MATN2	4898440	4882270	9940.ENSOARP00000021716	9940.ENSOARP00000004078	0.535
QRFPR	NPVF	4893369	4888592	9940.ENSOARP00000016182	9940.ENSOARP00000010970	0.524
PTGER4	SLCO2A1	4887776	4886742	9940.ENSOARP00000010089	9940.ENSOARP00000008957	0.52
PTGER4	CRABP1	4887776	4879281	9940.ENSOARP00000010089	9940.ENSOARP00000000817	0.517
HIC1	MGMT	4893123	4893120	9940.ENSOARP00000015914	9940.ENSOARP00000015911	0.515
F2R	IL6	4895199	4890356	9940.ENSOARP00000018191	9940.ENSOARP00000012891	0.515
SLC39A8	SLCO2A1	4891550	4886742	9940.ENSOARP00000014183	9940.ENSOARP00000008957	0.509
EFEMP1	LOX	4880522	4878813	9940.ENSOARP00000002158	9940.ENSOARP00000000311	0.505
KCNMA1	KCNH2	4887575	4879913	9940.ENSOARP00000009868	9940.ENSOARP00000001494	0.505
ADAMTSL1	NTN1	4892445	4881149	9940.ENSOARP00000015184	9940.ENSOARP00000002849	0.503
PARM1	ADCYAP1	4893518	4887910	9940.ENSOARP00000016341	9940.ENSOARP00000010237	0.502
AEBP1	EFEMP1	4894477	4880522	9940.ENSOARP00000017400	9940.ENSOARP00000002158	0.501
IL6	LOX	4890356	4878813	9940.ENSOARP00000012891	9940.ENSOARP00000000311	0.501
SLC27A2	ACSL5	4899203	4890131	9940.ENSOARP00000022561	9940.ENSOARP00000012643	0.495
SEMA3G	NTN1	4882861	4881149	9940.ENSOARP00000004730	9940.ENSOARP00000002849	0.493
TBX3	PITX2	4884666	4881888	9940.ENSOARP00000006691	9940.ENSOARP00000003666	0.491
MYB	ESR1	4893191	4881800	9940.ENSOARP00000015988	9940.ENSOARP00000003565	0.491
MMP2	TGFB2	4896287	4889927	9940.ENSOARP00000019362	9940.ENSOARP00000012419	0.488
MMP2	LOX	4896287	4878813	9940.ENSOARP00000019362	9940.ENSOARP00000000311	0.485
OSMR	CNTFR	4888216	4887609	9940.ENSOARP00000010565	9940.ENSOARP00000009907	0.482
NEFM	SFRP2	4888321	4880158	9940.ENSOARP00000010680	9940.ENSOARP00000001766	0.479
BMP2	ESR1	4896979	4881800	9940.ENSOARP00000020124	9940.ENSOARP00000003565	0.478
BMP2	ASPN	4896979	4886641	9940.ENSOARP00000020124	9940.ENSOARP00000008846	0.477
AXIN2	CCND2	4893771	4888713	9940.ENSOARP00000016613	9940.ENSOARP00000011102	0.474
UNC5C	NTN4	4895174	4890180	9940.ENSOARP00000018162	9940.ENSOARP00000012700	0.472

Table S5. (Continued).

IL6	PTGER4	4890356	4887776	9940.ENSOARP00000012891	9940.ENSOARP00000010089	0.471
RGS4	CCND2	4889364	4888713	9940.ENSOARP00000011810	9940.ENSOARP00000011102	0.468
GPR150	QRFPR	4895524	4893369	9940.ENSOARP00000018533	9940.ENSOARP00000016182	0.468
AXIN2	SFRP2	4893771	4880158	9940.ENSOARP00000016613	9940.ENSOARP00000001766	0.466
NR2F1	PITX1	4894976	4892954	9940.ENSOARP00000017946	9940.ENSOARP00000015732	0.465
GABRA2	MGMT	4893781	4893120	9940.ENSOARP00000016624	9940.ENSOARP00000015911	0.457
TNFSF13B	CXCL12	4884047	4881453	9940.ENSOARP00000006026	9940.ENSOARP00000003183	0.456
IL6	ADCYAP1	4890356	4887910	9940.ENSOARP00000012891	9940.ENSOARP00000010237	0.452
NR4A1	TGFB2	4895468	4889927	9940.ENSOARP00000018468	9940.ENSOARP00000012419	0.449
CELSR2	SFRP2	4897372	4880158	9940.ENSOARP00000020556	9940.ENSOARP00000001766	0.447
ASPN	EFEMP1	4886641	4880522	9940.ENSOARP00000008846	9940.ENSOARP00000002158	0.445
ENO2	NEFH	4883672	4882722	9940.ENSOARP00000005623	9940.ENSOARP00000004583	0.444
BMP2	AXIN2	4896979	4893771	9940.ENSOARP00000020124	9940.ENSOARP00000016613	0.442
MATN2	LOX	4882270	4878813	9940.ENSOARP00000004078	9940.ENSOARP00000000311	0.438
BMPR1B	TGFB2	4895420	4889927	9940.ENSOARP00000018416	9940.ENSOARP00000012419	0.436
ADAMTS14	LOX	4884578	4878813	9940.ENSOARP00000006598	9940.ENSOARP00000000311	0.435
NEGR1	PTER	4890435	4887343	9940.ENSOARP00000012976	9940.ENSOARP00000009623	0.435
IL6	TGFB2	4890356	4889927	9940.ENSOARP00000012891	9940.ENSOARP00000012419	0.431
TGFB2	COL1A1	4889927	4883317	9940.ENSOARP00000012419	9940.ENSOARP00000005233	0.43
TG	ESR1	4884042	4881800	9940.ENSOARP00000006021	9940.ENSOARP00000003565	0.429
QRFPR	PTGER4	4893369	4887776	9940.ENSOARP00000016182	9940.ENSOARP00000010089	0.429
HIC1	NTM	4893123	4892409	9940.ENSOARP00000015914	9940.ENSOARP00000015144	0.426
IL6	SERPINB1	4890356	4880779	9940.ENSOARP00000012891	9940.ENSOARP00000002447	0.424
IGF2BP2	PRKCB	4898771	4895884	9940.ENSOARP00000022089	9940.ENSOARP00000018920	0.422
MASP1	F2R	4898713	4895199	9940.ENSOARP00000022021	9940.ENSOARP00000018191	0.419
KCNMA1	PLA2G4A	4887575	4886338	9940.ENSOARP00000009868	9940.ENSOARP00000008524	0.419
ASPN	COL1A1	4886641	4883317	9940.ENSOARP00000008846	9940.ENSOARP00000005233	0.418
GLP1R	ADCYAP1	4894662	4887910	9940.ENSOARP00000017600	9940.ENSOARP00000010237	0.418
FAP	LOX	4884671	4878813	9940.ENSOARP00000006698	9940.ENSOARP00000000311	0.418
LGI3	SEMA3G	4888797	4882861	9940.ENSOARP00000011197	9940.ENSOARP00000004730	0.416
MYB	PITX2	4893191	4881888	9940.ENSOARP00000015988	9940.ENSOARP00000003666	0.416
MYB	PITX1	4893191	4892954	9940.ENSOARP00000015988	9940.ENSOARP00000015732	0.416
PLXND1	SIK1	4891945	4889590	9940.ENSOARP00000014629	9940.ENSOARP00000012049	0.416
CHL1	ENO2	4887458	4883672	9940.ENSOARP00000009744	9940.ENSOARP00000005623	0.414
BMP2	HAND2	4896979	4893636	9940.ENSOARP00000020124	9940.ENSOARP00000016468	0.414
IL6	TNFRSF21	4890356	4889976	9940.ENSOARP00000012891	9940.ENSOARP00000012473	0.413
FAP	CXCL12	4884671	4881453	9940.ENSOARP00000006698	9940.ENSOARP00000003183	0.411
CRLF1	CNTFR	4889778	4887609	9940.ENSOARP00000012255	9940.ENSOARP00000009907	0.406
MYB	IL6	4893191	4890356	9940.ENSOARP00000015988	9940.ENSOARP00000012891	0.406
PLA2G4A	LOX	4886338	4878813	9940.ENSOARP00000008524	9940.ENSOARP00000000311	0.403