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 multiple 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 two genotype (++ 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

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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
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 streptomycin + 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 database1 [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 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 T2xRealStar 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

1 https://string-db.org/
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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, TGFβ2, 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−ΔΔCt 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
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

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            |
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 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; FGFR2 MGAT4A; ASPN; ENO2; FGF1; MASP1; ABHD14B; SMOC2; BMP2; PRKCB; PTPRR; TG; LOC105605699; NTN1; SH3GL2; MMP2; FGFR2 |
| Enzyme-linked receptor protein signaling pathway (GO:0007167) | biologica_process | 0.000188 | LOC101104157; TGFA; FGFI; ROR1; BMP2; PTPRR; FAM83G; FGFR2 |
| 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
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.

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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: CAAGTTCCACGGCAGTCATCA R: GTTCAGGGCCCATCACAAA                                         | 60                  | 248             |
| SMOC2         | F: AGGAGCGAGGCAAGGATAG R: CCCAGGTACTCTCTTCGTTG                                           | 60                  | 178             |
| RPL9          | F: TGATTCAAGGACCCACACCA R: AGCCTGCTGAACCTTGTCCTT                                       | 60                  | 92              |
| KIAA1217      | F: TCTGACTACCCGGCCTCCTC R: AATCGAGGTCTCTGGTGGT                                          | 60                  | 117             |
| GCSAML        | F: CCATGCCTATGCCCATCCTC R: AATCGAGGTCTCTGGTGGT                                          | 60                  | 90              |
| CCND2         | F: GAGTCCCAACTCCGAGACC R: AGGCTTGATGGAGAGGTCGG                                          | 60                  | 131             |
| BGI_novel_G000328 | F: ACCAGATCCAGGTGCAAGGA R: GTGCTACACCTCTGGTGAN                                              | 60                  | 112             |
| BGI_novel_G000272 | F: CACCCAGTCTCTCAAGCCCATC R: CTTGAGAGGCAACAGGTCAC                                           | 60                  | 111             |
| BGI_novel_G000077 | F: CTCTATGTTGAGGCAAGGA R: ACCTGCGATGAGTAGGAGAGA                                         | 60                  | 118             |
| BGI_novel_G000167 | F: TACAGCAGGCTTCAATTGGGAG R: GCAATGTCGCCGAAACAGTC                                         | 60                  | 100             |
| BGI_novel_G000094 | F: GTCCTTTCTCAAACCAACCCGCT R: GAAGGTGAGGAGTGCTCAAGAG                                    | 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     | ADAMTLS1 | –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     | ADGR1L4  | –1.20263   | 7.95E-23         | 2.87E-23        |
| 100169940     | ADRA1A   | 2.116488   | 2.15E-12         | 1.18E-12        |
| 101118077     | AMOT     | –1.35155   | 6.97E-49         | 1.38E-49        |
| 101103426     | ANKH     | –1.20703   | 9.59E-50         | 1.87E-50        |
| 101118240     | AK5      | 2.245889   | 1.64E-22         | 5.97E-23        |
| 101114182     | ALDH1L2  | –1.15317   | 2.14E-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   | 9.59E-50         | 1.87E-50        |
| 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).

| Gene ID | Gene Name | Log2 Fold Change | p-Value | FDR |
|---------|-----------|------------------|---------|-----|
| 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 |
| 100694900 | CNTFR | 1.9483 | 3.35E-10 | 2.11E-10 |
| 101121906 | COL16A1 | 2.003593 | 0 | 0 |
| 101107002 | COL1A1 | 2.486829 | 0 | 0 |
| 101120648 | COL1A2 | 2.61291 | 0 | 0 |
| 101121906 | COL1A2 | -2.03838 | 0 | 0 |
| 101114118 | CPED1 | -1.81146 | 5.33E-154 | 3.83E-155 |
| 101124277 | CPVL | 1.50768 | 1.54E-255 | 6.76E-257 |
| 101122057 | CRABP1 | 2.690728 | 5.27E-123 | 4.72E-125 |
| 101108913 | CRLF1 | -1.35131 | 2.65E-09 | 1.71E-09 |
| 101120245 | CRMP1 | 2.57079 | 5.24E-45 | 1.10E-45 |
| 101115999 | CSRP3 | 2.39143 | 1.94E-08 | 1.37E-08 |
| 101109111 | CTSZ | -1.84485 | 0 | 0 |
| 1010211145 | CXCL12 | 2.275621 | 1.71E-41 | 3.87E-42 |
| 101117308 | DES | 1.38575 | 0 | 0 |
| 101104867 | DILG2 | 1.134938 | 3.19E-11 | 1.89E-11 |
| 101105194 | DNA1H8 | -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 | DTXI | 2.415081 | 5.20E-33 | 1.43E-33 |
| 101118013 | DYNCL1H | -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 | ESRI | -2.32715 | 1.07E-163 | 7.21E-165 |
| 101100757 | 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 |
| Gene Symbol | Description | Fold Change | p-value (FDR) | p-value (Benjamini) |
|-------------|-------------|-------------|---------------|---------------------|
| FKBP1B      | -1.47371    | 2.71E-19    | 1.11E-19      |
| FUT4        | 1.210481    | 2.93E-26    | 9.56E-27      |
| GABRA2      | 1.693626    | 2.36E-25    | 7.93E-26      |
| GCSAML      | 2.823459    | 4.55E-214   | 2.41E-215     |
| GJB3        | -1.06392    | 1.37E-45    | 2.85E-46      |
| GLP1R       | -2.39932    | 0           | 0             |
| GLRX        | 2.529433    | 2.65E-42    | 5.90E-43      |
| GM2A        | -1.2398     | 0           | 0             |
| GPR150      | 4.82933     | 1.48E-214   | 3.91E-215     |
| HAND2       | 3.02998     | 1.73E-89    | 1.09E-90      |
| HIC1        | 1.651137    | 1.73E-89    | 2.09E-90      |
| HPDL        | 1.212106    | 1.85E-06    | 1.53E-06      |
| IGF2BP2     | 2.056694    | 5.31E-18    | 2.30E-18      |
| IL23A       | 1.99717     | 6.51E-60    | 1.09E-60      |
| ISLR        | 1.713184    | 3.54E-44    | 7.58E-45      |
| ITGB7       | -1.17549    | 9.70E-59    | 1.66E-59      |
| JAKMIP2     | 1.977513    | 1.41E-17    | 6.19E-18      |
| KCN4E       | -1.39623    | 1.70E-67    | 2.57E-68      |
| KCNH2       | 1.051297    | 9.47E-14    | 4.93E-14      |
| KCNMA1      | -1.20744    | 3.16E-70    | 4.59E-71      |
| KIAA1217    | -1.01894    | 3.00E-275   | 1.18E-276     |
| KIAA1644    | -1.7647     | 2.34E-209   | 1.27E-210     |
| KRT18       | 4.235439    | 0           | 0             |
| LAMB1       | 1.962775    | 0           | 0             |
| LDLRAD4     | 1.160491    | 9.36E-27    | 3.01E-27      |
| LGI3        | 2.762524    | 0.000126383 | 0.000127882   |
| LIN7A       | 1.791821    | 1.23E-18    | 5.15E-19      |
| LLGL2       | -1.38372    | 4.72E-38    | 1.14E-38      |
| LOC101102344| 1.723412    | 3.43E-05    | 3.26E-05      |
| LOC101103182| 1.938455    | 0           | 0             |
| LOC101104157| -1.99095    | 3.49E-14    | 1.78E-14      |
| LOC101105348| -1.2266     | 1.21E-146   | 9.21E-148     |
| LOC101107369| 1.133884    | 2.42E-12    | 1.34E-12      |
| LOC101108322| 9.728631    | 0           | 0             |
| LOC101109212| -1.414      | 2.65E-66    | 4.08E-67      |
| LOC101110116| 5.287297    | 6.96E-83    | 8.92E-84      |
| LOC101110649| 5.150004    | 1.15E-05    | 1.04E-05      |
| LOC101110973| 1.86542     | 7.64E-56    | 1.36E-56      |
| LOC101113911| 1.960252    | 1.04E-08    | 7.22E-09      |
| LOC101114033| 2.108251    | 4.68E-05    | 4.51E-05      |
| LOC101114167| 1.452539    | 1.99E-20    | 7.87E-21      |
Table S4. (Continued).

| Gene ID     | Gene Symbol | FDR1     | FDR2     | FDR3     |
|-------------|-------------|----------|----------|----------|
| 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| 1.29E-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   | OTOF        | 2.806467 | 8.87E-05 | 8.81E-05 |
| 101113827   | PARD3B      | -1.06528 | 9.33E-68 | 1.40E-68 |
| 101104239   | PAR1M       | -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   | PIKT        | -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).

| Genbank Accession | Description | Fold Change | p-value | Adjusted p-value |
|-------------------|-------------|-------------|---------|------------------|
| 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    | 4.31E-10| 4.12E-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).

| Gene symbol | Gene symbol | Log2FoldChange | p-value | q-value |
|-------------|-------------|----------------|---------|---------|
| 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  | 5.30E-130 |
| 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       | 4.1115194      | 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  |
| 10110712    | 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  | 5.84E-110 |
| 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   | SMO2C       | -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).

|     | Gene   | Log2FC | P-value 1 | P-value 2 |
|-----|--------|--------|-----------|-----------|
| 443445 | THRBP  | -2.16111 | 1.63E-16  | 7.47E-17  |
| 101102841 | TMEM119 | -1.16371 | 4.10E-177 | 2.56E-178 |
| 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  |
| 10113935 | 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 |   | 3.18297  | 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.
| Gene Symbol | EC Number | Ensembl ID | Cutoff | P-Value | Log2 Fold Change |
|-------------|-----------|------------|--------|---------|-----------------|
| BMPR1B      |           | 9940.ENSOARP000000019107 | 0.596   |         |                 |
| PCOLCE      |           | 9940.ENSOARP000000017807 | 0.595   |         |                 |
| TG          |           | 9940.ENSOARP000000060201 | 0.592   |         |                 |
| PDGFB       |           | 9940.ENSOARP000000017621 | 0.587   |         |                 |
| HAND2       |           | 9940.ENSOARP00000016468 | 0.584   |         |                 |
| AXIN2       |           | 9940.ENSOARP000000014760 | 0.581   |         |                 |
| TCF7        |           | 9940.ENSOARP000000012643 | 0.578   |         |                 |
| PLA2G2F     |           | 9940.ENSOARP000000005387 | 0.565   |         |                 |
| PDGFB       |           | 9940.ENSOARP000000005233 | 0.567   |         |                 |
| TG          |           | 9940.ENSOARP000000005602 | 0.566   |         |                 |
| BMP2        |           | 9940.ENSOARP000000012891 | 0.564   |         |                 |
| IL6         |           | 9940.ENSOARP000000012891 | 0.564   |         |                 |
| CXCL12      |           | 9940.ENSOARP000000031183 | 0.56   |         |                 |
| BMP2        |           | 9940.ENSOARP000000001450 | 0.56    |         |                 |
| IL6         |           | 9940.ENSOARP000000001450 | 0.555   |         |                 |
| LG13        |           | 9940.ENSOARP00000010757 | 0.546   |         |                 |
| LPCAT2      |           | 9940.ENSOARP000000001523 | 0.536   |         |                 |
| COL9A2      |           | 9940.ENSOARP0000004078  | 0.535   |         |                 |
| QRFPR       |           | 9940.ENSOARP00000010970 | 0.524   |         |                 |
| PTGER4      |           | 9940.ENSOARP00000008957 | 0.52    |         |                 |
| PTGER4      |           | 9940.ENSOARP0000000817   | 0.517   |         |                 |
| HIC1        |           | 9940.ENSOARP00000015911 | 0.515   |         |                 |
| F2R         |           | 9940.ENSOARP000000001289 | 0.515   |         |                 |
| SLC39A8     |           | 9940.ENSOARP00000008957 | 0.509   |         |                 |
| EFEMP1      |           | 9940.ENSOARP00000002158 | 0.505   |         |                 |
| KNCMA1      |           | 9940.ENSOARP00000001494 | 0.505   |         |                 |
| ADAMTS1L    |           | 9940.ENSOARP0000002849 | 0.503   |         |                 |
| PARM1       |           | 9940.ENSOARP00000010237 | 0.502   |         |                 |
| AEBP1       |           | 9940.ENSOARP00000002158 | 0.501   |         |                 |
| IL6         |           | 9940.ENSOARP0000000311  | 0.501   |         |                 |
| SLC27A2     |           | 9940.ENSOARP00000012643 | 0.495   |         |                 |
| SEMA3G      |           | 9940.ENSOARP00000002849 | 0.493   |         |                 |
| TBX3        |           | 9940.ENSOARP0000003666 | 0.491   |         |                 |
| MYB         |           | 9940.ENSOARP00000003565 | 0.491   |         |                 |
| MMP2        |           | 9940.ENSOARP00000012419 | 0.488   |         |                 |
| MMP2        |           | 9940.ENSOARP0000000311  | 0.485   |         |                 |
| OSMR        |           | 9940.ENSOARP00000009907 | 0.482   |         |                 |
| NFPM        |           | 9940.ENSOARP00000017666 | 0.479   |         |                 |
| BPI        |           | 9940.ENSOARP00000003565 | 0.478   |         |                 |
| BMP2        |           | 9940.ENSOARP0000008846  | 0.477   |         |                 |
| AXIN2       |           | 9940.ENSOARP00000011102 | 0.474   |         |                 |
| UNC5C       |           | 9940.ENSOARP00000012700 | 0.472   |         |                 |
| Gene | Accession | Expression | p-value | FDR | fold change |
|------|-----------|------------|---------|-----|-------------|
| IL6  | PTGER4    | 4890356    | 9940.ENSOARP00000012891 | 0.471 |
| RGS4 | CCND2     | 4889364    | 9940.ENSOARP00000011810 | 0.468 |
| GPR150 | QRFPR | 4895524    | 9940.ENSOARP00000018833 | 0.466 |
| AXIN2 | SFRP2    | 4893771    | 9940.ENSOARP00000016613 | 0.457 |
| NR2F1 | PITX1     | 4894976    | 9940.ENSOARP00000017946 | 0.457 |
| GABRA2 | MGMT    | 4893781    | 9940.ENSOARP00000016624 | 0.456 |
| TNFSP13B | CXCL12  | 4884047    | 9940.ENSOARP00000006026 | 0.456 |
| IL6  | ADCYAP1   | 4890356    | 9940.ENSOARP00000012891 | 0.452 |
| NR4A1 | TGFB2     | 4895468    | 9940.ENSOARP00000018468 | 0.449 |
| CELSR2 | SFRP2    | 4897372    | 9940.ENSOARP00000012014 | 0.447 |
| ASPN | EFEMP1     | 4886641    | 9940.ENSOARP00000008846 | 0.445 |
| ENO2 | NEFH       | 4883672    | 9940.ENSOARP00000005623 | 0.444 |
| BMP2 | AXIN2      | 4896979    | 9940.ENSOARP00000012014 | 0.442 |
| MATN2 | LOX      | 4882270    | 9940.ENSOARP00000004078 | 0.438 |
| BMPRI1B | TGFB2   | 4895420    | 9940.ENSOARP00000018416 | 0.436 |
| ADAMTS14 | LOX    | 4884578    | 9940.ENSOARP00000006598 | 0.435 |
| NERG1 | PTER       | 4890435    | 9940.ENSOARP00000012976 | 0.435 |
| IL6  | TGFB2     | 4890356    | 9940.ENSOARP00000012891 | 0.431 |
| TGFB2 | COL1A1    | 4889927    | 9940.ENSOARP00000012891 | 0.43 |
| TG   | ESRI       | 4884042    | 9940.ENSOARP00000006021 | 0.429 |
| QRFPR | PTGER4    | 4893369    | 9940.ENSOARP00000016182 | 0.429 |
| HIC1 | NTM        | 4893123    | 9940.ENSOARP00000015914 | 0.426 |
| IL6  | SERPINB1   | 4890356    | 9940.ENSOARP00000012891 | 0.424 |
| IGF2BP2 | PRKCB   | 4898771    | 9940.ENSOARP00000022089 | 0.422 |
| MAS1P1 | F2R      | 4898713    | 9940.ENSOARP00000022021 | 0.419 |
| KCNA1 | PLA2G4A   | 4887575    | 9940.ENSOARP00000009868 | 0.419 |
| ASPN | COL1A1    | 4886641    | 9940.ENSOARP00000008846 | 0.418 |
| GLP1IR | ADCYAP1  | 4894662    | 9940.ENSOARP00000017600 | 0.418 |
| FAP  | LOX       | 4884671    | 9940.ENSOARP00000006698 | 0.418 |
| LGI3 | SEMA3G     | 4888797    | 9940.ENSOARP00000011197 | 0.416 |
| MYB  | PITX2     | 4893191    | 9940.ENSOARP00000015988 | 0.416 |
| MYB  | PITX1     | 4893191    | 9940.ENSOARP00000015988 | 0.416 |
| PLXND1 | SIK1     | 4891945    | 9940.ENSOARP00000014629 | 0.416 |
| CHLI | EMO2      | 4887458    | 9940.ENSOARP00000009744 | 0.414 |
| BMP2 | HAND2     | 4896979    | 9940.ENSOARP00000020124 | 0.414 |
| IL6  | TNFRSF21   | 4890356    | 9940.ENSOARP00000012891 | 0.413 |
| FAP  | CXCL12     | 4884671    | 9940.ENSOARP00000006698 | 0.411 |
| CRLF1 | CNTFR     | 4889778    | 9940.ENSOARP00000012255 | 0.406 |
| MYB  | IL6       | 4893191    | 9940.ENSOARP00000015988 | 0.406 |
| PLA2G4A | LOX    | 4886338    | 9940.ENSOARP0000008524 | 0.403 |