Identification of key pathways and genes in polycystic ovary syndrome via integrated bioinformatics analysis and prediction of small therapeutic molecules

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Abstract

To enhance understanding of polycystic ovary syndrome (PCOS) at the molecular level, this investigation intends to examine the genes and pathways associated with PCOS by using an integrated bioinformatics analysis. Based on the expression profiling by high throughput sequencing data GSE84958 derived from the Gene Expression Omnibus (GEO) database, the differentially expressed genes (DEGs) between PCOS samples and normal controls were identified. We performed a functional enrichment analysis. A protein-protein interaction (PPI) network, miRNA-target genes and TF-target gene networks, were constructed and visualized, with which the hub gene nodes were identified. Validation of hub genes was performed by using receiver operating characteristic (ROC) and RT-PCR. Small drug molecules were predicted by using molecular docking. A total of 739 DEGs were identified, of which 360 genes were up regulated and 379 genes were down regulated. GO enrichment analysis revealed that up regulated genes were mainly involved in peptide metabolic process, organelle envelope and RNA binding and the down regulated genes were significantly enriched in plasma membrane bounded cell projection organization, neuron projection and DNA-binding transcription factor activity, RNA polymerase II-specific. REACTOME pathway enrichment analysis revealed that the up regulated genes were mainly enriched in translation and respiratory electron transport and the down regulated genes were mainly enriched in generic transcription pathway and transmembrane transport of small molecules. The top 10 hub genes (SAA1, ADCY6, POLR2K, RPS15, RPS15A, CTNN D1, ESR1, NEDD4L, KNTC1 and NGFR) were identified from PPI network, miRNA-target gene network and TF-target gene network. The modules analysis showed that genes in modules were mainly associated with the transport of respiratory electrons and signaling NGF, respectively. We find a series of crucial genes along with the pathways that were most closely related with PCOS initiation and advancement. Our investigations provide a more detailed molecular mechanism for the progression of PCOS, detail information on the potential biomarkers and therapeutic targets.

Keywords: polycystic ovary syndrome, expression profiling by high throughput sequencing, biomarkers, pathway enrichment analysis, differentially expressed gene
Introduction
Polycystic ovary syndrome (PCOS) is one of the most prevalent endocrine disorder around the world, with an estimated about one in 15 women worldwide [1]. PCOS exposes patients to a major psychosocial burden and is characterized by hyperandrogenism and chronic anovulation [2]. Diabetes, heart disease, obesity, non-alcoholic fatty liver disease and hypertension are the risk factors associated with PCOS [3–7]. Therefore, it is of prime importance to identify the etiological factors, molecular mechanisms, and pathways to discover novel diagnostic markers, prognostic markers and therapeutic targets for PCOS.

Numerous research strategies have recently investigated the molecular mechanisms of PCOS. High-throughput RNA sequencing technology has received extensive attention among these research strategies and has generated significant advances in the field of endocrine disorder with marked clinical applications ranging from molecular diagnosis to molecular classification, patient stratification to prognosis prediction, and discovery of new drug targets to response prediction [8]. In addition, gene expression profiling investigation on PCOS have been performed using high-throughput RNA sequencing, and several key genes and diagnostic biomarkers have been diagnosed for this syndrome, including the profiling of many of differentially expressed genes (DEGs) associated in different pathways, biological processes, or molecular functions [9]. Integrated bioinformatics analyses of expression profiling by high throughput sequencing data derived from different investigation of PCOS could help identify the novel diagnostic markers, prognostic markers and further demonstrate their related functions and potential therapeutic targets in PCOS.

Therefore, in the current investigation, the dataset (GSE84958) was then retrieved from the publicly available Gene Expression Omnibus database (GEO, http://www.ncbi.nlm.nih.gov/geo/) [10] to identify DEGs and the associated biological processes PCOS using comprehensive bioinformatics analyses. The DEGs were subjected to functional enrichment and pathway analyses; moreover, a protein-protein interaction (PPI) network, miRNAs - target gene regulatory network and TFs - target gene regulatory network were constructed to screen for key genes, miRNA and TFs. The aim of this investigation was to identify key genes and pathways in PCOS using bioinformatics analysis, and then to explore the molecular mechanisms of PCOS and categorize new potential diagnostic therapeutic biomarkers of PCOS. We anticipated that these investigations will provide further understanding of PCOS pathogenesis and advancement at the molecular level.

Materials and Methods
RNA sequencing data
Expression profiling by high throughput sequencing dataset GSE84958 was downloaded from NCBI-GEO, a public database of next-generation sequencing, to filter the DEGs between PCOS and normal control. The expression profiling by high throughput sequencing GSE84958 was based on GPL16791 platforms (Illumina HiSeq 2500 (Homo sapiens)) and consisted of 30 PCOS samples and 23 normal control.

Identification of DEGs
The limma [11] in R bioconductor package was used to analyze the DEGs between PCOS samples and normal control samples in the expression profiling by high throughput sequencing data of GSE84958. The adjusted P-value and \([\text{logFC}]\) were calculated. The Benjamini & Hochberg false discovery rate method was used as a correction factor for the adjusted P-value in limma [12]. The statistically significant DEGs were identified according to \(P<0.05\), and \([\text{logFC}] > 2.5\) for up regulated genes and \([\text{logFC}] < -1.5\) for down regulated genes. All results of DEGs were downloaded in text format, hierarchical clustering analysis being conducted.

GO and pathway enrichment of DEGs in PCOS
To reflect gene functions, GO (http://geneontology.org/) [13] has been used in three terms: biological processes (BP), cellular component (CC) and molecular function (MF). ToppGene (ToppFun) (https://toppgene.cchmc.org/enrichment.jsp) [14] is an online database offering a comprehensive collection of resources for functional annotation to recognize the biological significance behind a broad list of genes. The functional enrichment analyses of DEGs, including GO analysis and REACTOME (https://reactome.org/) [15] pathway enrichment analysis, were performed using ToppGene in the present study, using the cut-off criterion P-value<0.05 and gene enrichment count>2.

PPI networks construction and module analysis
The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING: https://string-db.org/) is online biological database and website designed to evaluate PPI information [16] Proteins associated with DEGs were selected based on information in the STRING database (PPI score >0.7), and then PPI networks were constructed using Cytoscape software (http://cytoscape.org/) [17]. In this investigation, node degree [18], betweenness centrality [19], stress centrality [20] and closeness centrality [21], these constitutes a fundamental parameters in network theory, were adopted to calculate the nodes in a network. The topological properties of hub genes were calculated using Cytoscape plugin Network
Analyzer. The PEWCC1 (http://apps.cytoscape.org/apps/PEWCC1) [22], a plugin for Cytoscape, was used to screen the modules of the PPI network. The criteria were set as follows: degree cutoff=2, node score cutoff=0.2, k-core=2 and maximum depth=100. Moreover, the GO and pathway enrichment analysis were performed for DEGs in these modules.

**Construction of miRNA - target regulatory network**

Furthermore, the target genes of the significant target genes were predicted by using miRNet database (https://www.mirnet.ca/) [23], when the miRNAs shared a common target gene. Finally, the miRNA - target genes regulatory network depicted interactions between miRNAs and their potential targets in PCOS were visualized by using Cytoscape.

**Construction of TF - target regulatory network**

Furthermore, the target genes of the significant target genes were predicted by using TF database (https://www.mirnet.ca/) [23], when the TFs shared common target genes. Finally, the TF - target genes regulatory network depicted interactions between TFs and their potential targets in PCOS were visualized by using Cytoscape.

**Receiver operating characteristic (ROC) curve analysis**

The ROC curve was used to evaluate classifiers in bioinformatics applications. To further assess the predictive accuracy of the hub genes, ROC analysis was performed to discriminate PCOS from normal control. ROC curves for hub genes were generated using pROC in R [24] based on the obtained hub genes and their expression profiling by high throughput sequencing data. The area under the ROC curve (AUC) was determined and used to compare the diagnostic value of hub genes.

**Validation of the expression levels of candidate genes by RT-PCR**

Total RNA was extracted from PCOS (UWB1.289 (ATCC® CRL-2945™)) and normal ovarian cell line (MES-OV (ATCC® CRL-3272™)) using TRI Reagent™ (Sigma, USA). The Reverse transcription cDNA kit

Table 1 Primers used for quantitative PCR

| Primer sequence (5’→3’) | Gene | Forward | Reverse |
|-------------------------|------|---------|---------|
| TCGTCCCCTTGGGAGGCTTTTG | SAA1 | AGGTCCCCTTTGGGACGC | ATCA |
| CTCCTGTTCCCTAAAGTGGAT | ADCY6 | GGAGGACAGCTCATATAGGCGG | |
| GGAGAGTGTCACACAGAA | POLR2K | TCGAGGCTAAACAGAACC | AT |
| CCCCCGATGATCGGCCACTA | RPS15 | CCATGCCTTTACGGGCTTGTAG | |
| CTCAAAAGTCATCGTGCGGT | RPS15A | TGAATGGCAGCTCAATCTGG | |
| GTGACAAACGGACAGTGACAC | CTNN | TTCTCGGCAAATCCGACCC | |
| CCCACTCAACAGGCTTC | ESR1 | CGTGATTATCTGAATTGCGC | T |
| GACATGGACGATGGATGGA | NEDD4L | TTTCGGCCTAAATTGCACT | |
| ACCTGAGTGTGTCGTTACAG | KNTC1 | CACTGTGTTGGCGCTAC | AATAA |
| CCTACGGCTACTACCAGGATG | NGFR | CACACGGTGTTCTGCTTGT | |

Fig. 1 Volcano plot of differentially expressed genes. Genes with a significant change of more than two-fold were selected. Green dot represented up regulated significant genes and red dot represented down regulated significant genes
| Gene Symbol | logFC | p Value | adj. P.Val | t value | Regulation | Gene Name |
|-------------|-------|---------|------------|---------|------------|-----------|
| SAA2        | 4.381364 | 3.5E-07 | 0.00095   | 5.718643 | Up         | serum amyloid A2 |
| PLOD2       | 4.201209 | 5.64E-07 | 0.001025  | 5.393469 | Up         | procollagen-lysine,2-oxoglutarate 5-dioxygenase 2 |
| ABI3BP      | 4.13442  | 2.07E-07 | 0.000779  | 5.856015 | Up         | ABI family member 3 binding protein |
| RPPH1       | 4.09338  | 7.43E-06 | 0.002243  | 4.901502 | Up         | ribonuclease P RNA component H1 |
| C7orf50     | 4.074966 | 4.01E-07 | 0.00095   | 5.683289 | Up         | chromosome 7 open reading frame 50 |
| RMRP        | 3.989952 | 7.74E-06 | 0.002243  | 4.890252 | Up         | RNA component of mitochondrial RNA processing endoribonuclease |
| BNIP3       | 3.899781 | 2.99E-07 | 0.00095   | 5.760151 | Up         | BCL2 interacting protein 3 |
| POLE4       | 3.874065 | 6.35E-07 | 0.001025  | 5.562056 | Up         | DNA polymerase epsilon 4, accessory subunit |
| RHBDD2      | 3.859497 | 4.02E-06 | 0.001884  | 5.068914 | Up         | rhomboid domain containing 2 |
| F1A1        | 3.806046 | 1.82E-06 | 0.001545  | 5.283097 | Up         | coagulation factor XIII A chain |
| SIVA1       | 3.784719 | 7.02E-06 | 0.001025  | 5.368773 | Up         | SIVA1 apoptosis inducing factor |
| TMEM54      | 3.721    | 1.5E-09  | 0.002243  | 4.75139  | Up         | transmembrane protein 54 |
| SBDS        | 3.630263 | 1.28E-05 | 0.002885  | 4.75139  | Up         | SBDS ribosome maturation factor |
| CERS2       | 3.600241 | 1.32E-06 | 0.001336  | 5.368773 | Up         | ceramide synthase 2 |
| PLA2G2A     | 3.575964 | 6.92E-07 | 0.001025  | 5.539611 | Up         | phospholipase A2 group II |
| SAA1        | 3.551756 | 8.00E-07 | 0.001025  | 5.539611 | Up         | serum amyloid A1 |
| PHB2        | 3.526406 | 1.39E-05 | 0.003049  | 4.728928 | Up         | prohibitin 2 |
| LSM14A      | 3.519088 | 2.94E-06 | 0.001776  | 5.153832 | Up         | LSM14A mRNA processing body assembly factor |
| MRPL20      | 3.513535 | 4.93E-05 | 0.005042  | 4.370518 | Up         | mitochondrial ribosomal protein L20 |
| NEDD8       | 3.493441 | 7.9E-06  | 0.002264  | 4.884608 | Up         | NEDD8 ubiquitin like modifier |
| LRRCS9      | 3.487927 | 5.07E-08 | 0.003344  | 6.219575 | Up         | leucine rich repeat containing 59 |
| NAA38       | 3.481411 | 0.000119 | 0.008042  | 4.113375 | Up         | N (alpha)-acyetyltransferase 38, NatC auxiliary subunit |
| R3HDM4      | 3.472879 | 0.000181 | 0.009987  | 3.989599 | Up         | R3H domain containing 4 |
| P4KB        | 3.463829 | 2.36E-05 | 0.003497  | 4.579687 | Up         | phosphatidylinositol 4-kinase beta |
| RPS27L      | 3.462436 | 6.2E-05  | 0.005556  | 4.304481 | Up         | ribosomal protein S27 like |
| UBL5        | 3.458582 | 4.83E-05 | 0.005015  | 4.376605 | Up         | ubiquitin like 5 |
| S100A1      | 3.431536 | 0.000323 | 0.013388  | 3.813673 | Up         | S100 calcium binding protein A1 |
| TEAD3       | 3.414631 | 1.1E-08  | 0.061182  | 4.661182 | Up         | TEA domain transcription factor 3 |
| PREL1D1     | 3.395554 | 1.51E-08 | 0.003137  | 4.706077 | Up         | PREL1 domain containing 1 |
| SNCG        | 3.381526 | 0.001426 | 0.0285    | 3.432577 | Up         | synuclein gamma |
| NDUFAF3      | 3.380114 | 3.84E-05 | 0.00453   | 3.440243 | Up         | NADH-ubiquinoneoxidoreductase complex assembly factor 3 |
| LOC101926960| 3.379084 | 8.99E-05 | 0.006783  | 4.196554 | Up         | uncharacterized LOC101926960 |
| MRAP        | 3.376232 | 0.000186 | 0.011077  | 3.981504 | Up         | melanocortin 2 receptor accessory protein |
| EIF1AX      | 3.363678 | 3.15E-05 | 0.004025  | 4.498903 | Up         | eukaryotic translation initiation factor 1A X-linked |
| FIS1        | 3.36153  | 0.000316 | 0.013190  | 3.819875 | Up         | fission, mitochondrial 1 |
| LSM4        | 3.342924 | 2.22E-06 | 0.001669  | 5.229869 | Up         | LSM4 homolog, U6 small nuclear RNA and mRNA degradation associated |
| MRPL24      | 3.341155 | 8.03E-05 | 0.006322  | 4.229412 | Up         | mitochondrial ribosomal protein L24 |
| ZNF32       | 3.328313 | 2.03E-06 | 0.001623  | 5.253128 | Up         | zinc finger protein 32 |
| SAP1B       | 3.320223 | 3.41E-05 | 0.004196  | 4.47629  | Up         | Sin3A associated protein 18 |
| ZFAN6D       | 3.310728 | 6.93E-05 | 0.005946  | 4.272145 | Up         | zinc finger AN1-type containing 6 |
| PLCG2       | 3.309309 | 2.45E-05 | 0.003542  | 4.570246 | Up         | phospholipase C gamma 2 |
| COA4        | 3.297112 | 1.12E-06 | 0.001179  | 5.412446 | Up         | cytochrome c oxidase assembly factor 4 homolog |
| GTF3A       | 3.27873 | 0.000407 | 0.015016  | 3.742231 | Up         | general transcription factor IIIA |
| Gene Symbol | logFC    | p Value   | adj. P.Val | t value    | Regulation | Gene Name                                           |
|-------------|----------|-----------|------------|------------|------------|-----------------------------------------------------|
| SMIM19      | 3.278578 | 1.23E-05  | 0.002829   | 4.763305   | Up         | small integral membrane protein 19                 |
| DYN1L2      | 3.274028 | 1.21E-05  | 0.002818   | 4.76751    | Up         | dynein light chain LCB-type 2                       |
| WSAB2       | 3.272902 | 0.000138  | 0.008726   | 4.070129   | Up         | WD repeat and SOCS box containing 2                |
| MMAB        | 3.272634 | 2.33E-05  | 0.003497   | 4.583501   | Up         | metabolism of cobalamin associated B               |
| COXSB       | 3.250497 | 8.68E-05  | 0.006676   | 4.206576   | Up         | cytochrome c oxidase subunit 5B                     |
| ZNF706      | 3.247455 | 1.57E-05  | 0.003137   | 4.695098   | Up         | zinc finger protein 706                            |
| SUMO3       | 3.244703 | 1.07E-05  | 0.002689   | 4.799887   | Up         | small ubiquitin like modifier 3                    |
| ZHX1        | 3.24233  | 2.98E-05  | 0.003952   | 4.514129   | Up         | zinc fingers and homeoboxes 1                      |
| DCAF6       | 3.218358 | 7.16E-05  | 0.005946   | 4.263031   | Up         | NADH-ubiquinone oxidoreductase subunit A2           |
| COMT        | 3.19553  | 7.49E-06  | 0.002243   | 4.899155   | Up         | catechol-O-methyltransferase                        |
| USP24       | 3.193685 | 7.7E-06   | 0.002243   | 4.891689   | Up         | ubiquitin specific peptidase 24                    |
| CLEC3B      | 3.192924 | 0.000932  | 0.023147   | 3.480824   | Up         | C-type lectin domain family 3 member B             |
| S100A16     | 3.181908 | 2.08E-05  | 0.00341    | 4.615343   | Up         | S100 calcium binding protein A16                   |
| PIH1D1      | 3.168542 | 3.25E-06  | 0.007611   | 4.136503   | Up         | PIH1 domain containing 1                           |
| PEX2        | 3.15767  | 6.91E-05  | 0.005946   | 4.273176   | Up         | peroxisomal biogenesis factor 2                    |
| BCCIP       | 3.156384 | 5.08E-06  | 0.002061   | 5.005401   | Up         | BRCA2 and CDKN1A interacting protein               |
| UCHL1       | 3.144433 | 0.000603  | 0.018411   | 3.61943    | Up         | ubiquitin C-terminal hydrolase L1                  |
| CCND2       | 3.137527 | 0.000795  | 0.021152   | 3.531905   | Up         | cyclin D2                                          |
| MTDH        | 3.134009 | 8.58E-05  | 0.006622   | 4.210031   | Up         | metadherin                                         |
| ATP6V1D     | 3.1239   | 0.001     | 0.007145   | 4.164159   | Up         | ATPase H+ transporting V1 subunit D                |
| BOD1        | 3.11664  | 6.28E-06  | 0.002168   | 4.947675   | Up         | biorientation of chromosomes in cell division 1    |
| MRPL12      | 3.114756 | 7.59E-05  | 0.006143   | 4.245866   | Up         | mitochondrial ribosomal protein L12                |
| FOXN3       | 3.113552 | 0.000114  | 0.007817   | 4.126313   | Up         | forkhead box N3                                   |
| POLR3GL     | 3.098647 | 0.000131  | 0.008485   | 4.084894   | Up         | RNA polymerase III subunit G like                  |
| ALKBH7      | 3.098442 | 0.000306  | 0.012934   | 3.830063   | Up         | ak2 homolog 7                                      |
| CDV3        | 3.096327 | 0.000108  | 0.007553   | 4.141317   | Up         | CDV3 homolog                                       |
| ZSCAN16-AS1 | 3.095847 | 7.34E-07  | 0.001025   | 5.523754   | Up         | ZSCAN16 antisense RNA 1                            |
| S100A13     | 3.091633 | 5.32E-05  | 0.005263   | 4.348914   | Up         | S100 calcium binding protein A13                   |
| GFPT1       | 3.085405 | 5.33E-05  | 0.005263   | 4.348282   | Up         | glutamine--fructose-6-phosphate transaminase 1     |
| CC3ER2      | 3.079066 | 3.98E-05  | 0.004532   | 4.431771   | Up         | coiled-coil serine rich protein 2                  |
| PET100      | 3.05957  | 0.000152  | 0.009259   | 4.041067   | Up         | PET100 cytochrome c oxidase chaperone              |
| POLR2J1     | 3.05183  | 0.000358  | 0.014077   | 3.781554   | Up         | RNA polymerase II subunit J                        |
| SF3B6       | 3.047526 | 0.000179  | 0.009929   | 3.992753   | Up         | splicing factor 3b subunit 6                       |
| TSPAN17     | 3.044512 | 7.59E-06  | 0.002243   | 4.895829   | Up         | tetraspanin 17                                     |
| ECSIT       | 3.043372 | 1.61E-05  | 0.003137   | 4.688241   | Up         | ECSIT signaling integrator                         |
| TMED4       | 3.041199 | 2.05E-05  | 0.00341    | 4.620554   | Up         | transmembrane p24 trafficking protein 4            |
| ROMO1       | 3.039801 | 0.000339  | 0.013645   | 3.798806   | Up         | reactive oxygen species modulator 1               |
| SCARN2A     | 3.037947 | 0.000602  | 0.018408   | 3.619837   | Up         | small Cajal body-specific RNA 2                    |
| RDX         | 3.036218 | 1.11E-05  | 0.002709   | 4.790401   | Up         | radixin                                            |
| ATP6AP2     | 3.032219 | 0.000537  | 0.01724    | 3.656205   | Up         | ATPase H+ transporting accessory protein 2         |
| MRPS6       | 3.031793 | 0.001246  | 0.026775   | 3.386766   | Up         | mitochondrial ribosomal protein S6                 |
| MAD2L1BP    | 3.030044 | 1.83E-05  | 0.003308   | 4.651088   | Up         | MAD2L1 binding protein                             |
| Gene Symbol | logFC  | p Value  | adj. P.Val | t value  | Regulation | Gene Name                                           |
|-------------|--------|----------|------------|----------|------------|-----------------------------------------------------|
| NNAT        | 3.01612| 0.001284 | 0.027046   | 3.376953 | Up         | neuronatin                                         |
| SNX8        | 3.011952| 0.00341  | 4.611584   | Up       | sorting nexin 8                                   |
| ITGAV       | 3.006812| 0.009643 | 4.011701   | Up       | integrin subunit alpha V                           |
| TBCA        | 3.005083| 0.006772 | 4.200708   | Up       | tubulin folding cofactor A                         |
| SNAPIN      | 3.00314 | 0.005556 | 4.30272    | Up       | SNAP associated protein                             |
| TIMM8B      | 2.999367| 0.007409 | 4.149862   | Up       | translocase of inner mitochondrial membrane 8 homolog B |
| TADA3       | 2.994876| 0.005    | 4.38301    | Up       | transcriptional adaptor 3                          |
| HLA-DPB1    | 2.992346| 0.014705 | 3.754948   | Up       | major histocompatibility complex, class II, DP beta 1 |
| MGP         | 2.98607 | 0.019612 | 3.583067   | Up       | matrix Gla protein                                 |
| LAMTOR4     | 2.985466| 0.022949 | 3.486633   | Up       | late endosomal/lysosomal adaptor, MAPK and MTOR activator 4 |
| BTF3L4      | 2.981702| 0.006776 | 4.199218   | Up       | basic transcription factor 3 like 4                |
| TMX2        | 2.979038| 0.002595 | 4.040171   | Up       | thioredoxin related transmembrane protein 2        |
| CFL2        | 2.977258| 0.004025 | 4.497382   | Up       | coillin 2                                          |
| FAM149A     | 2.975549| 0.005946 | 4.276377   | Up       | family with sequence similarity 149 member A       |
| DTYMK       | 2.966972| 0.012546 | 3.84771    | Up       | deoxythymidylate kinase                            |
| MT1X        | 2.96256 | 0.015758 | 3.716594   | Up       | metallothionein 1X                                 |
| TSG101      | 2.95913 | 0.012156 | 3.868591   | Up       | tumor susceptibility 101                           |
| CPED1       | 2.957683| 0.016679 | 3.680123   | Up       | cadherin like and PC-esterase domain containing 1  |
| EXOCS       | 2.949442| 0.012156 | 3.867668   | Up       | exocyst complex component 5                        |
| BCYRN1      | 2.947993| 0.003137 | 4.695075   | Up       | brain cytoplasmic RNA 1                            |
| ANX4A       | 2.947244| 0.00341  | 4.602361   | Up       | annexin A4                                         |
| DPP7        | 2.946214| 0.006776 | 4.198665   | Up       | dipeptidyl peptidase 7                             |
| SYVNI       | 2.942727| 0.005556 | 4.302587   | Up       | synoviolin 1                                       |
| NDUFA13     | 2.941729| 0.020483 | 3.556961   | Up       | NADH-ubiquinoneoxidoreductase subunit A13           |
| C9orf16     | 2.940481| 0.014994 | 3.743119   | Up       | chromosome 9 open reading frame 16                  |
| AMDHD2      | 2.940188| 0.00358  | 4.5612     | Up       | amidohydrolase domain containing 2                  |
| KLHL12      | 2.939522| 0.003867 | 4.527463   | Up       | kelch like family member 12                        |
| EIF2D       | 2.938722| 0.011982 | 3.878331   | Up       | eukaryotic translation initiation factor 2D        |
| NDUFB2      | 2.935453| 0.006191 | 4.239855   | Up       | NADH-ubiquinoneoxidoreductase subunit B2            |
| GLRX3       | 2.933185| 0.024195 | 3.452028   | Up       | glutaredoxin 3                                     |
| THRSP       | 2.927803| 0.04933  | 2.990471   | Up       | thyroid hormone responsive                         |
| SLC25A11    | 2.925157| 0.017445 | 3.649379   | Up       | solute carrier family 25 member 11                 |
| NDFIP1      | 2.924945| 0.006317 | 4.230473   | Up       | Nedd4 family interacting protein 1                  |
| UBA1        | 2.919604| 0.008978 | 4.058832   | Up       | UBA domain containing 1                             |
| SETD3       | 2.915915| 0.011326 | 3.913355   | Up       | SET domain containing 3, actin histidinemethyltransferase |
| UQCRH       | 2.914264| 0.010586 | 3.950821   | Up       | ubiquinol-cytochrome c reductase hinge protein      |
| LMBRD1      | 2.91352 | 0.009259 | 4.039227   | Up       | LMBR1 domain containing 1                          |
| C1S         | 2.913138| 0.007707 | 4.175345   | Up       | complement C1 s                                    |
| YIPF6       | 2.909794| 0.014783 | 3.74923    | Up       | Yip1 family member 6                                |
| COX41       | 2.908785| 0.014772 | 3.749898   | Up       | cytochrome c oxidase subunit 41                     |
| NBN         | 2.90833 | 0.018884 | 5.076794   | Up       | nibrin                                             |
| TAF7        | 2.908109| 0.013777 | 3.793503   | Up       | TATA-box binding protein associated factor 7        |
| ANAPC13     | 2.904023| 0.038877 | 3.147704   | Up       | anaphase promoting complex subunit 13               |
Table 2 The statistical metrics for key differentially expressed genes (DEGs) (Continued)

| Gene Symbol | logFC | p value | adj. P.Val | t value | Regulation | Gene Name |
|-------------|-------|---------|------------|---------|------------|-----------|
| LYVE1       | 2.902347 | 0.001203 | 0.026382 | 3.398322 | Up | lymphatic vessel endothelial hyaluronan receptor 1 |
| PSMD14      | 2.901936 | 0.000247 | 0.016999 | 3.89503 | Up | proteasome 26S subunit, non-ATPase 14 |
| FAM32A      | 2.900891 | 0.000535 | 0.01724 | 3.657406 | Up | family with sequence similarity 32 member A |
| UROS        | 2.900315 | 0.00024 | 0.011528 | 3.904656 | Up | uroporphyrinogen III synthase |
| OST4        | 2.898201 | 0.002022 | 0.034115 | 3.226353 | Up | oligosaccharyltransferase complex subunit 4, non-catalytic |
| RBBP7       | 2.897646 | 0.000946 | 0.023286 | 3.476066 | Up | RB binding protein 7, chromatin remodeling factor |
| PRG4        | 2.897614 | 4.83E-05 | 0.005015 | 4.376505 | Up | proteoglycan 4 |
| COX7A2      | 2.896609 | 0.000818 | 0.021597 | 3.522952 | Up | cytochrome c oxidase subunit 7A2 |
| HMGCL       | 2.895335 | 0.000179 | 0.009929 | 3.993321 | Up | 3-hydroxy-3-methylglutaryl-CoA lyase |
| FAM3A       | 2.894614 | 8.47E-05 | 0.006573 | 4.213715 | Up | family with sequence similarity 3 member A |
| BAK1        | 2.892595 | 2.69E-05 | 0.00371 | 4.543614 | Up | BCL2 antagonist/killer 1 |
| ELOVL6      | 2.8913 | 7.93E-05 | 0.006283 | 4.232941 | Up | ELOVL fatty acid elongase 6 |
| CHCHD2      | 2.888344 | 0.000193 | 0.010317 | 3.970255 | Up | coiled-coil-helix-coiled-helix domain containing 2 |
| PRMT1       | 2.886326 | 0.000236 | 0.01142 | 3.907222 | Up | protein arginine methyltransferase 1 |
| HCF1C1R1    | 2.88509 | 0.000187 | 0.010126 | 3.979988 | Up | host cell factor C1 regulator 1 |
| RPS8        | 2.88263 | 0.000763 | 0.02086 | 3.545152 | Up | ribosomal protein S8 |
| JMJD8       | 2.880095 | 0.000466 | 0.016117 | 3.700249 | Up | jumonji domain containing 8 |
| VPS28       | 2.868412 | 0.00038 | 0.014306 | 3.72157 | Up | VPS28 subunit of ESCRT-I |
| EIF5        | 2.867934 | 0.00037 | 0.014306 | 3.72157 | Up | eukaryotic translation initiation factor 5 |
| ADCY6       | 2.866913 | 0.000145 | 0.009003 | 4.055897 | Up | adenylatecyclase 6 |
| NDUFC2      | 2.865496 | 0.001531 | 0.029477 | 3.319099 | Up | NADH:ubiquinoneoxidoreductase subunit C2 |
| PIGS        | 2.862467 | 0.002273 | 0.036573 | 3.186856 | Up | phosphatidylinositol glycan anchor biosynthesis class S |
| C1QA        | 2.861687 | 0.000816 | 0.021594 | 3.546607 | Up | complement C1q A chain |
| FDX1        | 2.861442 | 0.00394 | 0.014749 | 3.752127 | Up | ferredoxin 1 |
| RBX1        | 2.85968 | 0.000974 | 0.023505 | 3.466607 | Up | ring-box 1 |
| TRIB1       | 2.8594 | 3.17E-05 | 0.004025 | 4.496888 | Up | tribbles pseudokinase 1 |
| COX6B1      | 2.858458 | 0.000469 | 0.01616 | 3.698433 | Up | cytochrome c oxidase subunit 6B1 |
| MDFI        | 2.857869 | 0.000277 | 0.021363 | 3.860877 | Up | MyoD family inhibitor |
| RASD1       | 2.855883 | 0.00079 | 0.021079 | 3.533796 | Up | ras related dexamethasone induced 1 |
| SLC40A1     | 2.854864 | 0.003422 | 0.045502 | 3.046747 | Up | solute carrier family 40 member 1 |
| POLR2K      | 2.854222 | 0.001838 | 0.032468 | 3.253847 | Up | RNA polymerase II subunit K |
| CYB5B       | 2.852742 | 9.79E-05 | 0.00709 | 4.171566 | Up | cytochrome b5 type B |
| C1orf115    | 2.850887 | 0.001855 | 0.03266 | 3.251582 | Up | chromosome 1 open reading frame 115 |
| EIF3L       | 2.846772 | 0.000651 | 0.019135 | 3.595545 | Up | eukaryotic translation initiation factor 3 subunit L |
| TMEM219     | 2.834679 | 0.001098 | 0.025262 | 3.427919 | Up | transmembrane protein 219 |
| UQCR11      | 2.833847 | 0.000542 | 0.017312 | 3.652923 | Up | ubiquinol-cytochrome c reductase, complex III subunit XI |
| AGFG1       | 2.83177 | 8.83E-05 | 0.006711 | 4.201612 | Up | ArfGAP with FG repeats 1 |
| MRPS15      | 2.831483 | 0.000629 | 0.018848 | 3.606346 | Up | mitochondrial ribosomal protein S15 |
| UBE2E1      | 2.82743 | 0.00062 | 0.018805 | 3.610605 | Up | ubiquitin conjugating enzyme E2 E1 |
| NQO1        | 2.827398 | 3.12E-05 | 0.004025 | 4.50132 | Up | NAD(P) H quinone dehydrogenase 1 |
| MORF4L1     | 2.825223 | 0.000145 | 0.009003 | 4.055546 | Up | mortality factor 4 like 1 |
| TM7SF3      | 2.823384 | 0.0001 | 0.007145 | 4.165181 | Up | transmembrane 7 superfamily member 3 |
| RPL35A      | 2.822157 | 0.000266 | 0.012089 | 3.87247 | Up | ribosomal protein L35a |
| Gene Symbol | logFC  | p value | adj. P.Val | t value  | Regulation | Gene Name                                                                 |
|-------------|--------|---------|------------|----------|------------|----------------------------------------------------------------------------|
| TMEM160     | 2.812975 | 0.000317 | 0.01319    | 3.819678 | Up         | transmembrane protein 160                                                 |
| LSM3        | 2.809963 | 0.000282 | 0.012404   | 3.854606 | Up         | LSM3 homolog, U6 small nuclear RNA and mRNA degradation associated         |
| PHB         | 2.808084 | 0.000258 | 0.011974   | 3.881731 | Up         | prohibitin                                                                |
| MRPS21      | 2.805911 | 0.000295 | 0.012696   | 3.841189 | Up         | mitochondrial ribosomal protein S21                                      |
| TMEM256     | 2.804853 | 0.003453 | 0.045785   | 3.04363  | Up         | transmembrane protein 256                                                |
| MRPS12      | 2.804789 | 0.000223 | 0.011092   | 3.925752 | Up         | mitochondrial ribosomal protein S12                                      |
| PLTP        | 2.803513 | 0.000258 | 0.011965   | 3.882486 | Up         | phospholipid transfer protein                                             |
| TNPO2       | 2.794461 | 0.000852 | 0.022167   | 3.509567 | Up         | transportin 2                                                             |
| SKIL        | 2.793991 | 1.01E-05 | 0.002625   | 4.815964 | Up         | SKI like proto-oncogene                                                    |
| SEC11A      | 2.784473 | 0.001174 | 0.026198   | 3.406284 | Up         | SEC11 homolog A, signal peptide complex subunit                           |
| RPS10       | 2.783754 | 0.000749 | 0.020638   | 3.550811 | Up         | ribosomal protein S10                                                     |
| APOD        | 2.777795 | 0.00264  | 0.039615   | 3.136112 | Up         | apolipoprotein D                                                          |
| RAB4A       | 2.777311 | 0.000182 | 0.01001    | 3.864784 | Up         | RAB4A, member RAS oncogene family                                         |
| RBMX        | 2.77453  | 6.17E-05 | 0.005556   | 4.306198 | Up         | RNA binding motif protein X-linked                                        |
| ARFIP2      | 2.772975 | 0.000459 | 0.016062   | 3.705244 | Up         | ADP ribosylation factor interacting protein 2                             |
| CPE         | 2.770836 | 0.001392 | 0.028193   | 3.350337 | Up         | carboxypeptidase E                                                        |
| TCTN3       | 2.770597 | 8.94E-05 | 0.006776   | 4.198026 | Up         | tectonic family member 3                                                  |
| YWHAH       | 2.77017  | 0.000866 | 0.02234    | 3.504563 | Up         | tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein eta   |
| PLXNA1      | 2.769986 | 1.66E-05 | 0.003137   | 4.67951  | Up         | plexin A1                                                                 |
| AK1         | 2.767903 | 0.000273 | 0.012251   | 3.864784 | Up         | adenylate kinase 1                                                         |
| ORMDL1      | 2.767548 | 7.27E-05 | 0.005968   | 4.258602 | Up         | ORMDL sphingolipid biosynthesis regulator 1                               |
| SLTM        | 2.767084 | 0.000706 | 0.020072   | 3.569499 | Up         | SAFB like transcription modulator                                         |
| PSCM5       | 2.760402 | 0.00097  | 0.023505   | 3.468114 | Up         | proteasome 26S subunit, ATPase 5                                          |
| UHMK1       | 2.759638 | 0.000128 | 0.008394   | 4.09178  | Up         | U2AF homology motif kinase 1                                              |
| AIFM1       | 2.757223 | 7.05E-05 | 0.005956   | 4.267206 | Up         | apoptosis inducing factor mitochondria associated 1                       |
| TATDN1      | 2.756526 | 0.000672 | 0.019534   | 3.853379 | Up         | TatDDNase domain containing 1                                             |
| COX6C       | 2.753983 | 0.000372 | 0.014337   | 3.769771 | Up         | cytochrome c oxidase subunit 6C                                           |
| GIPC1       | 2.753721 | 0.000335 | 0.013596   | 3.802772 | Up         | GIPC PDZ domain containing family member 1                                |
| RPS21       | 2.753031 | 0.000158 | 0.009439   | 4.030076 | Up         | ribosomal protein S21                                                     |
| CCDC85B     | 2.752819 | 0.001517 | 0.029361   | 3.22182  | Up         | coiled-coil domain containing 85B                                         |
| UAP1        | 2.74977  | 0.001158 | 0.026017   | 3.410666 | Up         | UDP-N-acetylglucosaminepyrophosphorylase 1                                |
| POFUT1      | 2.742386 | 0.000309 | 0.012965   | 3.827384 | Up         | protein O-fucosyltransferase 1                                            |
| C3AR1       | 2.736533 | 2.1E-05  | 0.00341    | 4.613724 | Up         | complement C3a receptor 1                                                  |
| SRP72       | 2.734833 | 5.43E-05 | 0.005283   | 4.342922 | Up         | signal recognition particle 72                                            |
| ABHD17A     | 2.734708 | 0.001052 | 0.024558   | 3.441677 | Up         | abhydrolase domain containing 17A                                         |
| TOMM7       | 2.730177 | 0.001231 | 0.026634   | 3.390625 | Up         | translocase of outer mitochondrial membrane 7                             |
| ANP32B      | 2.728749 | 0.00161  | 0.030287   | 3.302334 | Up         | acidic nuclear phosphoprotein 32 family member B                          |
| TMEM230     | 2.728085 | 0.001136 | 0.025784   | 3.416807 | Up         | transmembrane protein 230                                                |
| NABP2       | 2.7271   | 0.000369 | 0.014306   | 3.772639 | Up         | nucleic acid binding protein 2                                             |
| CDC27       | 2.726993 | 0.001334 | 0.027538   | 3.646495 | Up         | cell division cycle 27                                                    |
| FAM20B      | 2.723814 | 0.000841 | 0.021962   | 3.513811 | Up         | FAM20B glycosaminoglycan xylosylkinase                                    |
| ETF1        | 2.7213   | 0.000538 | 0.01724    | 3.653315 | Up         | eukaryotic translation termination factor 1                               |
| GRHPR       | 2.720938 | 0.000857 | 0.022226   | 3.507777 | Up         | glyoxylate and hydroxypyruvate reductase                                  |
Table 2 The statistical metrics for key differentially expressed genes (DEGs) (Continued)

| Gene Symbol | logFC   | p Value   | adj. P.Val | t value | Regulation | Gene Name                                                                 |
|-------------|---------|-----------|------------|---------|------------|---------------------------------------------------------------------------|
| DERL2       | 2.720492| 0.000154  | 0.009266   | 4.038321| Up         | derlin 2                                                                 |
| RPL36A      | 2.720487| 0.000898  | 0.02272    | 3.492922| Up         | ribosomal protein L36a                                                   |
| C19orf53    | 2.718168| 0.000499  | 0.016701   | 3.678807| Up         | chromosome 19 open reading frame S3                                      |
| GTF2A2      | 2.716967| 0.000389  | 0.014658   | 3.756692| Up         | general transcription factor IIA subunit 2                               |
| GADD45B     | 2.716143| 0.000261  | 0.011982   | 3.878655| Up         | growth arrest and DNA damage inducible beta                              |
| CDIPT       | 2.714923| 0.00013   | 0.00844    | 4.088275| Up         | CDP-diacylglycerol--inositol 3-phosphatidytransferase                     |
| COX6A1      | 2.712855| 0.000535  | 0.01724    | 3.67442 | Up         | cytochrome c oxidase subunit 6A1                                         |
| SRSF6       | 2.711655| 0.000694  | 0.01986    | 3.57514 | Up         | serine and arginine rich splicing factor 6                               |
| SCARB1      | 2.709938| 0.00054   | 0.017244   | 3.654526| Up         | scavenger receptor class B member 1                                      |
| ASAP3       | 2.709641| 3.15E-05  | 0.004025   | 4.498522| Up         | ArfGAP with SH3 domain, ankyrin repeat and PH domain 3                   |
| RNASE1      | 2.702048| 0.00234   | 0.037096   | 3.177048| Up         | ribonuclease A family member 1, pancreatic                               |
| NAPRT       | 2.698312| 0.000786  | 0.021056   | 3.536522| Up         | nicotinamidephosphoribosyltransferase                                    |
| COX7C       | 2.697196| 0.000888  | 0.02598    | 3.49607 | Up         | cytochrome c oxidase subunit 7C                                          |
| PSMB1       | 2.694767| 0.000391  | 0.014705   | 3.754384| Up         | proteasome 20S subunit beta 1                                            |
| ECH1        | 2.694774| 0.00021   | 0.034115   | 3.226513| Up         | enoyl-CoA hydratase 1                                                   |
| RPS15A      | 2.694313| 0.000873  | 0.022405   | 3.52067 | Up         | ribosomal protein S15a                                                  |
| ERH         | 2.693982| 9.3E-05   | 0.006902   | 4.18654 | Up         | ERH mRNA splicing and mitosis factor                                     |
| PSMD3       | 2.69342 | 0.000362  | 0.014114   | 3.77823 | Up         | proteasome 26S subunit, non-ATPase 3                                    |
| MRPS24      | 2.689672| 2.97E-06  | 0.001776   | 5.15088 | Up         | mitochondrial ribosomal protein S24                                      |
| HSD17B10    | 2.689486| 0.000184  | 0.010093   | 3.98458 | Up         | hydroxysteroid 17-beta dehydrogenase 10                                  |
| ICMT        | 2.68742 | 0.001623  | 0.030348   | 3.29977 | Up         | isoprenylcysteine carboxyl methyltransferase                             |
| COXA8       | 2.68611 | 0.00082   | 0.021597   | 3.52181 | Up         | cytochrome c oxidase subunit 8A                                          |
| RPS23       | 2.683611| 0.001311  | 0.027304   | 3.37012 | Up         | ribosomal protein S23                                                   |
| TMEM165     | 2.683585| 0.000309  | 0.012965   | 3.82763 | Up         | transmembrane protein 165                                               |
| TMEM97      | 2.683426| 1.54E-06  | 0.001401   | 5.32708 | Up         | transmembrane protein 97                                                |
| COX7A2L     | 2.680847| 0.0015    | 0.029177   | 3.32536 | Up         | cytochrome c oxidase subunit 7A2 like                                     |
| FAM89B      | 2.67751 | 0.003528  | 0.046326   | 3.03615 | Up         | family with sequence similarity 89 member 1                              |
| SMAD5       | 2.676775| 0.0005   | 0.016701   | 3.67851 | Up         | SMAD family member 5                                                    |
| CCNC        | 2.675154| 0.002737  | 0.040448   | 3.12378 | Up         | cyclin C                                                                |
| CDC42EP2    | 2.672348| 0.000165  | 0.009593   | 4.01692 | Up         | CDC42 effector protein 2                                                 |
| UQCRC1      | 2.67139 | 3.96E-05  | 0.004552   | 4.43344 | Up         | ubiquinol-cytochrome c reductase complex protein 1                       |
| COP57A      | 2.671388| 0.001825  | 0.032315   | 3.26074 | Up         | COP9 signalosome subunit 7A                                             |
| CCDC80      | 2.670347| 0.003064  | 0.042867   | 3.08501 | Up         | coiled-coil domain containing 80                                       |
| PSMB4       | 2.669349| 0.000572  | 0.017831   | 3.63605 | Up         | proteasome 20S subunit beta 4                                            |
| EIF6        | 2.664726| 0.000227  | 0.011159   | 3.92065 | Up         | eukaryotic translation initiation factor 6                               |
| PFDN5       | 2.657659| 0.001117  | 0.025474   | 3.42221 | Up         | prefoldin subunit 5                                                    |
| AFF1        | 2.657404| 0.00036   | 0.014077   | 3.78051 | Up         | AF4/FMR2 family member 1                                                |
| UQCRF51     | 2.656808| 0.000434  | 0.01563    | 3.72249 | Up         | ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1       |
| TYROBP      | 2.652926| 0.0038    | 0.047966   | 3.01017 | Up         | TYRO protein tyrosine kinase binding protein                              |
| ANAPC5      | 2.650988| 0.000463  | 0.016691   | 3.70254 | Up         | anaphase promoting complex subunit 5                                    |
| TRMT112     | 2.643049| 0.001145  | 0.025902   | 3.41420 | Up         | tRNAmethyltransferase subunit 11-2                                       |
| ARF5        | 2.641389| 0.002168  | 0.035481   | 3.20295 | Up         | ADP ribosylation factor 5                                               |
| Gene Symbol | logFC  | p Value | adj. P.Val | t value | Regulation | Gene Name                                      |
|-------------|--------|---------|------------|---------|------------|-----------------------------------------------|
| POLR1D      | 2.64106| 0.000197| 0.010419   | 3.963486| Up         | RNA polymerase I and III subunit D            |
| SNX9        | 2.63958| 0.000559| 0.017629   | 3.643451| Up         | sorting nexin 9                              |
| NDUFS5      | 2.63493| 0.000625| 0.018808   | 3.608112| Up         | NADH-tubiquinoneoxidoreductase subunit S5      |
| KDSR        | 2.63460| 0.000894| 0.022598   | 3.495893| Up         | 3-ketohydrophosphogine reductase              |
| KRTCAP2     | 2.63419| 0.001854| 0.032651   | 3.255495| Up         | keratinocyte associated protein 2             |
| RPAIN       | 2.63292| 0.00075 | 0.020638   | 3.55055 | Up         | RPA interacting protein                       |
| OXCT1       | 2.63204| 0.003122| 0.043255   | 3.078538| Up         | 3-oxoacid CoA-transferase 1                   |
| CAPNS1      | 2.63169| 0.000733| 0.020454   | 3.557742| Up         | calpain small subunit 1                       |
| SERPINH1    | 2.63103| 0.000261| 0.011982   | 3.878996| Up         | serpin family H member 1                      |
| TNIP2       | 2.63086| 0.000242| 0.016164   | 3.901038| Up         | TNFAIP3 interacting protein 2                 |
| RASSF3      | 2.62826| 0.004015| 0.049293   | 2.990897| Up         | Ras association domain family member 3        |
| RPL27       | 2.62685| 0.00108  | 0.024036   | 3.455675| Up         | ribosomal protein L27                         |
| ARHGAP12    | 2.62407| 0.00007  | 0.019959   | 3.572316| Up         | Rho GTPase activating protein 12              |
| PRPF31      | 2.62367| 0.000229| 0.018399   | 4.090841| Up         | pre-mRNA processing factor 31                 |
| KLB         | 2.62323| 0.001365| 0.027884   | 3.536946| Up         | klotho beta                                  |
| TMX4        | 2.61862| 0.00108  | 0.024945   | 3.433183| Up         | thioredoxin related transmembrane protein 4   |
| NQQ2        | 2.61727| 0.002855| 0.041527   | 3.109292| Up         | N-ribosylhydroxycineamidequonereductase 2     |
| NME2        | 2.61749| 0.002462| 0.038092   | 3.159908| Up         | NME/NM23 nucleoside diphosphate kinase 2      |
| SLC25A26    | 2.61679| 0.00270  | 0.028881   | 4.754142| Up         | solute carrier family 25 member 26           |
| TOMM5       | 2.61540| 0.000222| 0.043977   | 3.067588| Up         | translocase of outer mitochondrial membrane 5  |
| ETFB        | 2.61472| 0.001976| 0.033674   | 3.234172| Up         | electron transfer flavoprotein subunit beta   |
| MOAP1       | 2.61423| 0.000133| 0.008495   | 4.081606| Up         | modulator of apoptosis 1                      |
| NACC1       | 2.61313| 0.002801| 0.022829   | 4.761618| Up         | nucleus accumens associated 1                 |
| NAGLU        | 2.61249| 0.000258| 0.011965   | 3.882494| Up         | N-acetyl-alpha-glucosaminidase                |
| XRCC5       | 2.61143| 0.000462| 0.016091   | 3.703217| Up         | X-ray repair cross complementing 5            |
| RPL35       | 2.61072| 0.000229| 0.011194   | 3.918588| Up         | ribosomal protein L35                        |
| KRCC1       | 2.60894| 0.00226  | 0.068664   | 4.189205| Up         | lysine rich coiled-coil 1                     |
| PSMB7       | 2.60892| 0.001141| 0.025828   | 3.641541| Up         | proteasome 20S subunit beta 7                |
| RPS29       | 2.60891| 0.001176| 0.026198   | 3.405623| Up         | ribosomal protein S29                        |
| SNRPD2      | 2.60428| 0.000499| 0.016701   | 3.679267| Up         | small nuclear ribonucleoprotein D2 polypeptide|
| RSL24D1     | 2.60123| 0.001206| 0.026399   | 3.397372| Up         | ribosomal L24 domain containing 1            |
| RBM3        | 2.60246| 0.000751| 0.020661   | 3.549857| Up         | RNA binding motif protein 3                   |
| RPL14       | 2.59832| 0.002801| 0.041085   | 3.115761| Up         | ribosomal protein L14                        |
| DBI         | 2.59376| 0.001225| 0.026608   | 3.392185| Up         | diazepam binding inhibitor, acyl-CoA binding protein |
| RPL13A      | 2.59297| 0.000949| 0.023287   | 3.474966| Up         | ribosomal protein L13a                       |
| NAB1        | 2.59168| 0.000791| 0.021082   | 3.533288| Up         | NGFI-A binding protein 1                      |
| STX10       | 2.59027| 0.003718| 0.047358   | 3.01781 | Up         | syntaxin 10                                  |
| SLC35E1     | 2.58977| 0.000244| 0.011642   | 3.809816| Up         | solute carrier family 35 member E1           |
| GCHFR       | 2.58635| 0.00154  | 0.029477   | 3.37136 | Up         | GTP cyclohydrolase I feedback regulator       |
| WIP11       | 2.58604| 0.001049| 0.024512   | 3.442577| Up         | WD repeat domain, phosphoinositide interacting 1 |
| TMEM50A     | 2.58603| 0.001551| 0.029566   | 3.314827| Up         | transmembrane protein 50A                    |
| NELFB        | 2.58591| 0.000623| 0.018805   | 3.609156| Up         | negative elongation factor complex member B  |
| MRPL9        | 2.58269| 0.000637| 0.019003   | 3.60222 | Up         | mitochondrial ribosomal protein L9           |
Table 2 The statistical metrics for key differentially expressed genes (DEGs) (Continued)

| Gene Symbol | logFC  | p Value | adj. P.Val | t value | Regulation | Gene Name |
|-------------|--------|---------|------------|---------|------------|-----------|
| DIP2C       | 2.577846 | 1.51E-06 | 0.001401  | 5.333124 | Up         | disco interacting protein 2 homolog C |
| PFDN1       | 2.577843 | 0.001923 | 0.033201  | 3.488378 | Up         | prefoldin subunit 1 |
| PCYT2       | 2.574996 | 0.000343 | 0.013741  | 3.795229 | Up         | phosphate cytidylyltransferase 2, ethanolamine |
| RPL29       | 2.574629 | 0.000917 | 0.022969  | 3.486043 | Up         | ribosomal protein L29 |
| RPL30       | 2.572361 | 0.00205  | 0.034257  | 3.221746 | Up         | ER membrane protein complex subunit 4 |
| EMC4        | 2.571676 | 0.002479 | 0.038185  | 3.157525 | Up         | ribosomal protein L24 |
| RPS15       | 2.570156 | 0.001711 | 0.031119  | 3.282153 | Up         | ribosomal protein S15 |
| SMARCD2     | 2.569052 | 0.000525 | 0.017152  | 3.66321  | Up         | SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 2 |
| C9orf78     | 2.568679 | 9.48E-05 | 0.007003  | 4.180864 | Up         | chromosome 9 open reading frame 78 |
| BLOC1S1     | 2.568295 | 0.001664 | 0.03072   | 3.291398 | Up         | biogenesis of lysosomal organelles complex 1 subunit 1 |
| ATP9A       | 2.567757 | 0.000243 | 0.011614  | 3.900357 | Up         | ATPase phospholipid transporting 9A (putative) |
| MS4A4A      | 2.566919 | 0.000549 | 0.018752  | 4.136129 | Up         | membrane spanning 4-domains A4A |
| AFAP1L1     | 2.565076 | 0.002187 | 0.035677  | 3.199902 | Up         | actin filament associated protein 1 like 1 |
| CDKN2B      | 2.565157 | 0.000599 | 0.018321  | 3.621709 | Up         | cyclin dependent kinase inhibitor 2B |
| IFI27       | 2.563118 | 0.00371  | 0.047358  | 3.01863  | Up         | interferon alpha inducible protein 27 |
| MED4        | 2.556663 | 0.001188 | 0.026311  | 3.402174 | Up         | mediator complex subunit 4 |
| DPH5        | 2.553328 | 3.71E-05 | 0.004462  | 4.452413 | Up         | diphthamide biosynthesis 5 |
| WIZ         | 2.550803 | 0.000617 | 0.018752  | 3.612543 | Up         | WIZ zinc finger |
| PEX19       | 2.549485 | 0.003066 | 0.042867  | 3.084727 | Up         | peroxisomal biogenesis factor 19 |
| RABGEF1     | 2.547097 | 0.001793 | 0.032062  | 3.266518 | Up         | RAB guanine nucleotide exchange factor 1 |
| SLCA3R1     | 2.544376 | 0.000906 | 0.022852  | 3.490141 | Up         | SLCA3 regulator 1 |
| ANO6        | 2.541247 | 0.00097  | 0.023505  | 3.468073 | Up         | anoctamin 6 |
| RPLP0       | 2.539879 | 0.000147 | 0.009101  | 4.051259 | Up         | ribosomal protein lateral stalk subunit P0 |
| EZH1        | 2.538446 | 0.000787 | 0.021056  | 3.532593 | Up         | enhancer of zeste 1 polycomb repressive complex 2 subunit |
| PSKH1       | 2.53776  | 0.001027 | 0.024288  | 3.449585 | Up         | protein serine kinase H1 |
| RPL34       | 2.53733  | 0.000646 | 0.0191    | 3.597964 | Up         | ribosomal protein L34 |
| NDUFS4      | 2.53691  | 0.000267 | 0.012096  | 3.871781 | Up         | NADH:ubiquinoneoxidoreductase subunit S4 |
| BANF1       | 2.535865 | 0.000572 | 0.017831  | 3.636063 | Up         | barrier to autointegration factor 1 |
| MRPL54      | 2.533785 | 0.000952 | 0.023287  | 3.473973 | Up         | mitochondrial ribosomal protein L54 |
| CKS1B       | 2.533771 | 0.000911 | 0.022881  | 3.488195 | Up         | CDC28 protein kinase regulatory subunit 1B |
| TEMEM184B   | 2.532583 | 1.67E-05 | 0.003137  | 4.676649 | Up         | transmembrane protein 184B |
| PSM10D1     | 2.530765 | 0.00053  | 0.01724   | 3.660242 | Up         | proteasome 26S subunit, non-ATPase 10 |
| RPS5        | 2.529965 | 0.000597 | 0.018297  | 3.622831 | Up         | ribosomal protein S5 |
| CUL4A       | 2.529656 | 0.001663 | 0.03072   | 3.291653 | Up         | cullin 4A |
| MFAP5       | 2.527961 | 0.000821 | 0.021597  | 3.521542 | Up         | microfibril associated protein 5 |
| SNTA1       | 2.526907 | 0.000372 | 0.014337  | 3.770146 | Up         | syntrophin alpha 1 |
| RPS20       | 2.52651  | 0.002839 | 0.01407   | 3.111181 | Up         | ribosomal protein S20 |
| RSL1D1      | 2.524784 | 0.000405 | 0.014978  | 3.743879 | Up         | ribosomal L1 domain containing 1 |
| RPS24       | 2.523665 | 0.002187 | 0.035677  | 3.200019 | Up         | ribosomal protein S24 |
| ICAM2       | 2.52328  | 0.00175  | 0.031637  | 3.274701 | Up         | intercellular adhesion molecule 2 |
| B4GALT2     | 2.52208  | 0.001703 | 0.031013  | 3.283844 | Up         | beta-1,4-galactosyltransferase 2 |
| NUDT16      | 2.521704 | 0.001545 | 0.029527  | 3.315981 | Up         | nudix hydrolase 16 |

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### Table 2  The statistical metrics for key differentially expressed genes (DEGs) (Continued)

| Gene Symbol | logFC | $p$ Value | adj. P.Val | t value | Regulation | Gene Name |
|-------------|-------|-----------|------------|---------|------------|-----------|
| PFN2        | 2.519815 | 0.001062 | 0.024764 | 3.438594 | Up | profilin 2 |
| RAB10       | 2.519714 | 0.003057 | 0.04281 | 3.040293 | Up | RAB10, member RAS oncogene family |
| SF3B5       | 2.519177 | 0.003604 | 0.046771 | 3.028686 | Up | splicing factor 3b subunit 5 |
| TCEAL8      | 2.516685 | 0.003486 | 0.04603 | 3.040293 | Up | transcription elongation factor A like 8 |
| CD99L2      | 2.51621 | 0.001957 | 0.03358 | 3.23728 | Up | CD99 molecule like 2 |
| SUCLG1      | 2.514761 | 0.001177 | 0.026198 | 3.405243 | Up | succinate-CoA ligase alpha subunit |
| LAMA2       | 2.514019 | 0.000535 | 0.01724 | 3.657403 | Up | laminin subunit alpha 2 |
| EIF3E       | 2.511931 | 0.000766 | 0.020887 | 3.543592 | Up | eukaryotic translation initiation factor 3 subunit E |
| BCL7B       | 2.511858 | 0.003631 | 0.041048 | 3.779795 | Up | BAF chromatin remodeling complex subunit BCL7B |
| SF3B5       | 2.511732 | 0.00202 | 0.010561 | 3.955986 | Up | splicing factor 3b subunit 5 |
| TCEAL8      | 2.511606 | 0.000883 | 0.022598 | 3.498175 | Up | transcription elongation factor A like 8 |
| PMM1        | 2.511461 | 0.00143 | 0.032855 | 3.341501 | Up | phosphomannomutase 1 |
| DNPH1       | 2.50873 | 0.002905 | 0.041811 | 3.103319 | Up | 2’-deoxynucleoside 5’-phosphate N-hydrolase 1 |
| RARRES2     | 2.504265 | 0.00202 | 0.010561 | 3.955986 | Up | selenophosphatesynthetase 2 |
| COA3        | 2.50271 | 0.001102 | 0.022981 | 3.485574 | Up | mitochondrial ribosomal protein S34 |
| MRPS34      | 2.502001 | 0.000919 | 0.022981 | 3.485574 | Up | mitochondrial ribosomal protein S34 |
| SLC30A7     | -3.56691 | 4.14E-07 | 0.00095 | -5.67461 | Down | solute carrier family 30 member 7 |
| VT11A       | -3.31043 | 1.05E-07 | 0.000478 | -6.03155 | Down | vesicle transport through interaction with t-SNAREs 1A |
| ERG         | -3.30133 | 3.23E-05 | 0.001776 | -5.17359 | Down | AP2 associated kinase 1 |
| COA3        | -3.3173 | 2.73E-06 | 0.00108 | -5.49481 | Down | URB2 ribosome biogenesis homolog |
| IGIP        | -3.12032 | 5.55E-05 | 0.005363 | -4.36463 | Down | IgA inducing protein |
| ITIH4       | -2.9168 | 3.51E-06 | 0.001852 | -5.10571 | Down | inter-alpha-trypsin inhibitor heavy chain 4 |
| LOC646214   | -2.85088 | 0.000346 | 0.013805 | -3.79234 | Down | p21 protein (Cdc42/Rac)-activated kinase 2 pseudogene |
| ZNF486      | -2.84793 | 1.14E-05 | 0.001776 | -5.12426 | Down | leupaxin |
| HIVEP1      | -2.79883 | 1.73E-05 | 0.00174 | -4.66687 | Down | golgi SNAP receptor complex member 1 |
| PDP1        | -2.78381 | 4.99E-05 | 0.005058 | -4.43763 | Down | pyruvate dehydrogenase phosphatase regulatory subunit |
| TNKS        | -2.74917 | 9.17E-05 | 0.006848 | -4.19071 | Down | tankyrase |
| ECD         | -2.73445 | 0.000348 | 0.013823 | -3.79107 | Down | ecdysoneless cell cycle regulator |
| ABCC5       | -2.72387 | 1.16E-05 | 0.002752 | -4.77862 | Down | ATP binding cassette subfamily C member 5 |
| STXB5       | -2.71632 | 0.00176 | 0.009858 | -3.99744 | Down | syntaxin binding protein 5 |
| WDR19       | -2.69989 | 0.000615 | 0.018721 | -3.61344 | Down | WD repeat domain 19 |
| SARM1       | -2.69717 | 1.19E-09 | 0.018721 | -7.17529 | Down | sterile alpha and TIR motif containing 1 |
| TRAF5       | -2.66677 | 0.00107 | 0.007492 | -4.14579 | Down | TNF receptor associated factor 5 |
| SYNE2       | -2.663 | 5.87E-05 | 0.005493 | -4.32047 | Down | spectrin repeat containing nuclear envelope protein 2 |
| CFP         | -2.6567 | 0.000675 | 0.019585 | -3.58386 | Down | complement factor properdin |
| ANKR120A9P  | -2.64322 | 0.001714 | 0.031148 | -3.28161 | Down | ankyrin repeat domain 20 family member A9, pseudogene |
| FAT4        | -2.63099 | 0.000494 | 0.016631 | -3.68187 | Down | FAT atypical cadherin 4 |
| HIVEP1      | -2.62581 | 8.18E-05 | 0.006387 | -4.22422 | Down | HIVEP zinc finger 1 |
| Gene Symbol | logFC  | p Value  | adj. P.Val | t value  | Regulation | Gene Name |
|------------|--------|----------|------------|----------|------------|-----------|
| SNAP47     | -2.61436 | 0.000216 | 0.010911 | -3.93599 | Down       | synaptosome associated protein 47 |
| DMRT2      | -2.61357 | 0.000161 | 0.009544 | -4.02349 | Down       | doublesex and mab-3 related transcription factor 2 |
| GEMINS     | -2.60553 | 0.000465 | 0.004269 | -4.6757  | Down       | gem nuclear organelle associated protein 5 |
| LOC643406  | -2.59315 | 0.000435 | 0.015643 | -3.72139 | Down       | uncharacterized LOC643406 |
| OBSCN      | -2.58615 | 0.000237 | 0.011453 | -3.90744 | Down       | obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF |
| ATP13A1    | -2.57902 | 0.000556 | 0.013019 | -4.03019 | Down       | ATPase 13A1 |
| LOC10537279S | -2.56184 | 0.000177 | 0.005205 | -3.92158 | Down       | uncharacterized LOC10537279S |
| PXMP4      | -2.55289 | 0.000508 | 0.016883 | -3.67316 | Down       | peroxisomal membrane protein 4 |
| FAS        | -2.51889 | 0.000221 | 0.011049 | -3.92874 | Down       | Fas cell surface death receptor |
| MGAM       | -2.5179  | 0.000126 | 0.026608 | -3.9213  | Down       | maltase-glucoamylase |
| ERCC6L2    | -2.49433 | 0.000462 | 0.016091 | -3.70269 | Down       | ERCC excision repair 6 like 2 |
| MFA3P3     | -2.48427 | 0.000709 | 0.017482 | -4.17183 | Down       | microfibril associated protein 3 |
| TPD52      | -2.48206 | 0.0001319| 0.027418 | -3.6809  | Down       | tumor protein D52 |
| PPP1R3B    | -2.47428 | 0.000536 | 0.029477 | -3.1797  | Down       | protein phosphatase 1 regulatory subunit 3B |
| PTGDR      | -2.46965 | 0.000995 | 0.025915 | -5.66312 | Down       | prostaglandin D2 receptor |
| AHSPP      | -2.45099 | 0.000995 | 0.025915 | -5.66312 | Down       | alpha hemoglobin stabilizing protein |
| ODF2       | -2.44884 | 0.000531 | 0.01724  | -3.65942 | Down       | outer dense fiber of sperm tails 2 |
| MAU2       | -2.44852 | 0.000267 | 0.039958 | -3.1315  | Down       | MAU2 sister chromatid cohesion factor |
| PTOV1-AS2  | -2.44504 | 0.000242 | 0.048521 | -4.85271 | Down       | PTOV1 antisense RNA 2 |
| SLC04A1    | -2.42489 | 0.000679 | 0.019486 | -4.1986  | Down       | solute carrier organic anion transporter family member 4A1 |
| RAP1GAP2   | -2.41727 | 0.000428 | 0.015491 | -3.72656 | Down       | RAP1 GTPase activating protein 2 |
| GUSBP11    | -2.41706 | 0.000316 | 0.046966 | -4.6966  | Down       | GUSB pseudogene 11 |
| CHST14     | -2.40289 | 0.000217 | 0.049093 | -4.94093 | Down       | carbohydrate sulfotransferase 14 |
| SMYD4      | -2.3923  | 0.000898 | 0.022598 | -3.49612 | Down       | SET and MYND domain containing 4 |
| CACNA2D4   | -2.38689 | 0.0003137| 0.048521 | -4.6856  | Down       | calcium voltage-gated channel auxiliary subunit alpha2delta4 |
| ERO1A      | -2.3862  | 0.0001237| 0.026966 | -3.7975  | Down       | endoplasmic reticulum oxidoreductase 1 alpha |
| ATRNL1     | -2.37103 | 0.000338 | 0.046947 | -4.6947  | Down       | attractin like 1 |
| ATL1       | -2.37076 | 0.000548 | 0.017445 | -3.64947 | Down       | atlastinGTPase 1 |
| EPB4       | -2.36058 | 0.000451 | 0.015882 | -3.71047 | Down       | EP receptor B4 |
| AOC4P      | -2.35367 | 0.000884 | 0.057705 | -5.07705 | Down       | amine oxidase copper containing 4, pseudogene |
| SLC25A13   | -2.34818 | 0.000815 | 0.047881 | -4.07881 | Down       | solute carrier family 25 member 13 |
| FAM13B     | -2.34187 | 0.000485 | 0.035577 | -3.05577 | Down       | family with sequence similarity 13 member B |
| TRAK1      | -2.33457 | 0.000883 | 0.022598 | -3.49344 | Down       | trafficking kinesin protein 1 |
| ABI3       | -2.3343  | 0.000274 | 0.012278 | -3.8636  | Down       | ABI family member 3 |
| SEMA6B     | -2.33392 | 0.000495 | 0.039958 | -4.39248 | Down       | semaphorin 6B |
| METTL21A   | -2.33362 | 0.000279 | 0.040954 | -3.11724 | Down       | methyltransferase like 21A |
| ZNF778     | -2.33087 | 0.001884 | 0.057521 | -5.07521 | Down       | zinc finger protein 778 |
| PDF4B      | -2.32078 | 0.001056 | 0.035577 | -3.05577 | Down       | phosphodiesterase 4B |
| NDC1       | -2.31958 | 0.000208 | 0.010631 | -3.94732 | Down       | NDC1 transmembrane nucleoporin |
| SLC9A7     | -2.31311 | 0.001151 | 0.025956 | -3.41266 | Down       | solute carrier family 9 member A7 |
| NLG2       | -2.31264 | 0.000627 | 0.042344 | -4.23441 | Down       | neuropilin 2 |
| IKZF4      | -2.30922 | 0.000113 | 0.007788 | -4.12818 | Down       | IKAROS family zinc finger 4 |
| MAST3      | -2.3067  | 0.000142 | 0.024428 | -3.44474 | Down       | microtubule associated serine/threonine kinase 3 |
| Gene Symbol | logFC | p Value | adj. P.Val | t value | Regulation | Gene Name |
|-------------|-------|---------|------------|---------|------------|-----------|
| SAP25       | -2.30632 | 0.000324 | 0.013394 | -3.81298 | Down | Sin3A associated protein 25 |
| ZNF213-AS1  | -2.30592 | 3.03E-06 | 0.001776 | -5.14592 | Down | ZNF213 antisense RNA 1 (head to head) |
| ADAMTS13    | -2.30408 | 2.03E-06 | 0.001623 | -5.25359 | Down | ADAM metallopeptidase with thrombospondin type 1 motif 13 |
| CDH19       | -2.30007 | 9.85E-06 | 0.002623 | -4.82394 | Down | cadherin 19 |
| DOCK9       | -2.28015 | 0.002517 | 0.038469 | -3.15239 | Down | dedicator of cytokinesis 9 |
| PARP10      | -2.27632 | 0.003654 | 0.047012 | -3.0239 | Down | poly (ADP-ribose) polymerase family member 10 |
| LOC648987   | -2.27592 | 2.87E-06 | 0.001776 | -5.16 | Down | uncharacterized LOC648987 |
| GIMAP8      | -2.27461 | 0.001512 | 0.029333 | -3.3209 | Down | GTPase, IMAP family member 8 |
| COL8A1      | -2.27126 | 0.00307 | 0.042867 | -3.08427 | Down | collagen type VIII alpha 1 chain |
| MYO7A       | -2.26997 | 5.89E-06 | 0.002131 | -4.96524 | Down | myosin VIIa |
| SLC25A35    | -2.26394 | 4.18E-05 | 0.004645 | -4.41821 | Down | solute carrier family 25 member 35 |
| ESRI        | -2.25478 | 0.000772 | 0.020954 | -3.54124 | Down | estrogen receptor 1 |
| FAM71F2     | -2.25074 | 5.55E-06 | 0.002118 | -4.98153 | Down | family with sequence similarity 71 member F2 |
| ZNF493      | -2.24829 | 0.000671 | 0.019525 | -3.58587 | Down | zinc finger protein 493 |
| CEP135      | -2.24319 | 1.89E-05 | 0.003342 | -4.64292 | Down | centrosomal protein 135 |
| CEP126      | -2.24051 | 0.001032 | 0.024341 | -3.44789 | Down | centrosomal protein 126 |
| ASTN2       | -2.23592 | 0.001156 | 0.026012 | -3.41115 | Down | astrotactin 2 |
| IPP         | -2.22612 | 0.000101 | 0.007163 | -4.16214 | Down | intracisternal A particle-promoted polypeptide |
| TANGO6      | -2.2248 | 0.000418 | 0.015262 | -3.73417 | Down | transport and golgi organization 6 homolog |
| ZXD         | -2.22107 | 0.001926 | 0.033211 | -3.24265 | Down | zinc finger protein C |
| TMCO4       | -2.21386 | 0.000912 | 0.022891 | -3.48775 | Down | transmembrane and coiled-coil domains 4 |
| WSCD1       | -2.20938 | 1.36E-05 | 0.003004 | -4.73545 | Down | WSC domain containing 1 |
| PLXNB3      | -2.20331 | 0.001904 | 0.010359 | -3.96765 | Down | plexin B3 |
| SPA5T       | -2.19723 | 0.003713 | 0.047358 | -3.01835 | Down | spastin |
| LRSAM1      | -2.19647 | 0.00028 | 0.012403 | -3.85725 | Down | leucine rich repeat and sterile alpha motif containing 1 |
| BANP        | -2.19578 | 0.002678 | 0.039958 | -3.13116 | Down | BTG3 associated nuclear protein |
| SCAF4       | -2.1951 | 0.001471 | 0.028954 | -3.33217 | Down | SR-related CTD associated factor 4 |
| SDCCAG8     | -2.19392 | 0.001272 | 0.026966 | -3.38007 | Down | serologically defined colon cancer antigen 8 |
| KCNQ1OT1    | -2.19178 | 0.002417 | 0.037819 | -3.16609 | Down | KCNQ1 opposite strand/antisense transcript 1 |
| CD82        | -2.18167 | 0.001254 | 0.026814 | -3.38461 | Down | CD82 molecule |
| KIAA0754    | -2.17697 | 0.000994 | 0.023761 | -3.46018 | Down | KIAA0754 |
| PTPRN2      | -2.17646 | 0.000999 | 0.023704 | -3.46132 | Down | protein tyrosine phosphatase receptor type N2 |
| MYLK-AS1    | -2.16901 | 0.000153 | 0.002925 | -4.03959 | Down | MYLK antisense RNA 1 |
| PLXDC1      | -2.16377 | 0.001307 | 0.027262 | -3.37099 | Down | plexin domain containing 1 |
| TDRD5       | -2.15738 | 4.87E-05 | 0.005015 | -4.37413 | Down | tudor domain containing 5 |
| LCAT        | -2.15173 | 0.000278 | 0.012383 | -3.85932 | Down | lecithin-cholesterol acyltransferase |
| PRDM11      | -2.15109 | 6.78E-07 | 0.001025 | -5.54474 | Down | PR/SET domain 11 |
| RASIP1      | -2.14799 | 0.000745 | 0.02059 | -3.55262 | Down | Ras interacting protein 1 |
| SN1         | -2.14456 | 0.002682 | 0.039958 | -3.13065 | Down | sialophorin |
| SHISA9      | -2.14372 | 0.00386 | 0.048313 | -3.0047 | Down | shisa family member 9 |
| ANKR6D      | -2.14267 | 0.003628 | 0.046875 | -3.0264 | Down | ankyrin repeat domain 6 |
| MAK         | -2.13779 | 2.41E-05 | 0.003515 | -4.57405 | Down | male germ cell associated kinase |
| EPHA4       | -2.13565 | 0.000848 | 0.022087 | -3.51135 | Down | EPH receptor A4 |
| Gene Symbol | logFC  | p Value | adj. P.Val | t value | Regulation | Gene Name                                           |
|------------|--------|---------|-----------|---------|-----------|-----------------------------------------------------|
| UBR5-AS1   | -2.12673 | 2.81E-05 | 0.003842  | -4.53078 | Down      | UBR5 antisense RNA 1                                |
| CTC1       | -2.12661 | 0.003144 | 0.043426  | -3.07609 | Down      | CST telomere replication complex component 1        |
| NPHP4      | -2.12045 | 7.65E-05 | 0.006152  | -4.24347 | Down      | nephrocystin 4                                      |
| RFX3       | -2.11961 | 0.000286  | 0.012464  | -3.85121 | Down      | regulatory factor X3                               |
| PLD1       | -2.10857 | 0.000478  | 0.016343  | -3.69227 | Down      | phospholipase D1                                   |
| ZNF474     | -2.10767 | 3.19E-06  | 0.001776  | -5.13193 | Down      | zinc finger protein 474                            |
| LCK        | -2.10338 | 0.000224  | 0.011092  | -3.92537 | Down      | LCK proto-oncogene, Src family tyrosine kinase     |
| ZCCHC4     | -2.10229 | 0.002042  | 0.034193  | -3.22302 | Down      | zinc finger CCHC-type containing 4                  |
| PRPS2      | -2.09872 | 0.001319  | 0.027418  | -3.3681  | Down      | phosphoribosyl pyrophosphate synthetase 2          |
| TAF4B      | -2.09126 | 0.000171  | 0.001692  | -3.92537 | Down      | TATA-box binding protein associated factor 4b       |
| CDC42BPA   | -2.08947 | 0.001389  | 0.028155  | -3.3511  | Down      | CDC42 binding protein kinase alpha                   |
| LIN12120   | -2.0891  | 0.000333  | 0.013567  | -3.80731 | Down      | long intergenic non-protein coding RNA 1230         |
| CPAMD8     | -2.08813 | 0.000302  | 0.012829  | -3.83385 | Down      | C3 and PZP like alpha-2-macroglubulin domain containing 8 |
| PARP9      | -2.08698 | 0.001921  | 0.033188  | -3.24363 | Down      | poly (ADP-ribose) polymerase family member 9       |
| ZFYVE27    | -2.08654 | 0.000479  | 0.016343  | -3.69181 | Down      | zinc finger FYVE-type containing 27                 |
| IWS1       | -2.08516 | 0.000281  | 0.011092  | -3.10619 | Down      | interacts with SUPT6H, CTD assembly factor 1        |
| MMRN1      | -2.08143 | 0.000451  | 0.015882  | -3.7104  | Down      | multimerin 1                                       |
| ST20-AS1   | -2.07521 | 6.76E-06  | 0.00223  | -4.92723 | Down      | ST20 antisense RNA 1                                |
| CALCR      | -2.07464 | 5.03E-05  | 0.005083  | -4.36486 | Down      | calcitonin receptor                                 |
| KAT7       | -2.06821 | 0.003226  | 0.043983  | -3.06718 | Down      | lysine acetyltransferase 7                          |
| ARHGAP22   | -2.05873 | 4.78E-06  | 0.002061  | -5.02181 | Down      | Rho GTPase activating protein 22                    |
| TMEM199    | -2.05777 | 0.002257  | 0.036467  | -3.18933 | Down      | transmembrane protein 199                          |
| NTN1       | -2.05766 | 0.000168  | 0.009643  | -4.01109 | Down      | netrin 1                                           |
| LAMA5      | -2.05607 | 0.002141  | 0.035221  | -3.20706 | Down      | laminin subunit alpha 5                            |
| SPNS3      | -2.05195 | 2.18E-05  | 0.00341   | -4.60239 | Down      | sphingolipid transporter 3 (putative)              |
| NEDD4L     | -2.04969 | 0.001542  | 0.029477  | -3.16178 | Down      | NEDD4 like E3 ubiquitin protein ligase              |
| BSN        | -2.04855 | 2.9E-05   | 0.003871  | -4.52186 | Down      | bassoon presynaptic cytomatrix protein             |
| TMED8      | -2.04722 | 7.63E-06  | 0.002243  | -4.89431 | Down      | transmembrane p24 trafficking protein family member 8 |
| STRBP      | -2.04479 | 0.003521  | 0.046281  | -3.0684  | Down      | spermatid perinuclear RNA binding protein          |
| ATP13A4    | -2.04282 | 5.91E-05  | 0.005493  | -4.3185  | Down      | ATPase 13A4                                        |
| KLC2       | -2.03984 | 0.001017  | 0.024181  | -3.45276 | Down      | kinesin light chain 2                               |
| RLF        | -2.03665 | 0.001899  | 0.032833  | -3.2492  | Down      | rearranged L-rc my fusion                          |
| TTF1       | -2.03264 | 0.002608  | 0.03937   | -3.14019 | Down      | transcription termination factor 1                 |
| TMEM79     | -2.0311  | 5.41E-05  | 0.005279  | -4.34419 | Down      | transmembrane protein 79                           |
| ZCCHC2     | -2.02905 | 0.002297  | 0.036735  | -3.18334 | Down      | zinc finger CCHC-type containing 2                 |
| ANKR1D13B  | -2.02722 | 0.001008  | 0.024036  | -3.45558 | Down      | ankyrin repeat domain 13B                          |
| IFT122     | -2.02015 | 0.002868  | 0.041571  | -3.10772 | Down      | intraflagellar transport 122                       |
| MAP3K14    | -2.01534 | 0.003484  | 0.046026  | -3.0405  | Down      | mitogen-activated protein kinase kinase 14          |
| SPATA6L    | -2.01191 | 3.29E-05  | 0.004116  | -4.48594 | Down      | spermatogenesis associated 6 like                  |
| SCOC-AS1   | -2.01128 | 0.000933  | 0.023147  | -3.48171 | Down      | SCOC antisense RNA 1                                |
| LMO7       | -2.00579 | 0.000573  | 0.017831  | -3.63573 | Down      | LIM domain 7                                       |
| MEX3B      | -1.99674 | 2.01E-05  | 0.00341   | -4.6259  | Down      | mex-3 RNA binding family member B                  |
| IQCK       | -1.99382 | 0.00025   | 0.011782  | -3.89149 | Down      | IQ motif containing K                              |
| Gene Symbol | logFC  | p Value  | adj. P.Val | t value  | Regulation | Gene Name |
|-------------|--------|----------|------------|----------|------------|-----------|
| GPATCH1     | -1.9934 | 0.000126 | 0.0008296  | -4.09746 | Down       | G-patch domain containing 1 |
| HPS4        | -1.98942 | 0.002434 | 0.037382   | -3.16378 | Down       | HPS4 biogenesis of lysosomal organelles complex 3 subunit 2 |
| FGF8        | -1.98469 | 1.56E-05 | 0.003137   | -4.6955  | Down       | fibroblast growth factor receptor 3 |
| GABRA4      | -1.98306 | 0.000116 | 0.007921   | -4.1216  | Down       | gamma-aminobutyric acid type A receptor alpha4 subunit |
| TMEM116     | -1.97917 | 0.000648 | 0.0191     | -3.5967  | Down       | transmembrane protein 116 |
| CTU1        | -1.97326 | 4.92E-06 | 0.002061   | -5.01408 | Down       | cytosolic thiouridylase subunit 1 |
| PPIEL       | -1.9687  | 0.002144 | 0.035221   | -3.20669 | Down       | peptidylprolyl isomerase E like pseudogene |
| FUT11       | -1.965   | 0.000688 | 0.019867   | -3.57787 | Down       | fucosyltransferase 11 |
| TOP3A       | -1.96066 | 0.00295  | 0.04205    | -3.098   | Down       | DNA topoisomerase III alpha |
| C2orf34     | -1.95954 | 0.000251 | 0.011782   | -3.89083 | Down       | chromosome 22 open reading frame 34 |
| ASIC3       | -1.9537  | 4.65E-05 | 0.005      | -4.38764 | Down       | acid sensing ion channel subunit 3 |
| SNPH        | -1.95235 | 0.002152 | 0.035284   | -3.20546 | Down       | syntaphilin |
| ZNFS47      | -1.95217 | 0.000487 | 0.016474   | -3.68677 | Down       | zinc finger protein 547 |
| FEZ1        | -1.94468 | 0.000873 | 0.022405   | -3.50206 | Down       | fasciculation and elongation protein zeta 1 |
| NHE2DS      | -1.94339 | 0.000264 | 0.012058   | -3.87463 | Down       | ectonucleoside triphosphate diphosphohydrolase 5 (inactive) |
| LOC297683   | -1.94235 | 2.6E-05  | 0.003635   | -4.55269 | Down       | uncharacterized LOC297683 |
| S1PRS       | -1.94099 | 8.19E-05 | 0.006387   | -4.22381 | Down       | sphingosine-1-phosphate receptor 5 |
| ZNF300P1    | -1.9392  | 0.000171 | 0.009664   | -4.00596 | Down       | zinc finger protein 300 pseudogene 1 |
| UNC13C      | -1.93485 | 0.000438 | 0.015649   | -3.7196  | Down       | unc-13 homolog C |
| LOC101928370| -1.93447 | 2.11E-06 | 0.001634   | -5.24334 | Down       | uncharacterized LOC101928370 |
| ZNFS55      | -1.92846 | 0.000376 | 0.014411   | -3.76684 | Down       | zinc finger protein 555 |
| GADD45G     | -1.92479 | 0.001994 | 0.03385    | -3.23101 | Down       | growth arrest and DNA damage inducible gamma |
| KCNC1       | -1.92247 | 2.19E-05 | 0.003495   | -4.60996 | Down       | potassium voltage-gated channel subfamily C member 1 |
| CARD14      | -1.91822 | 6.28E-06 | 0.002168   | -4.9474  | Down       | caspase recruitment domain family member 14 |
| STK36       | -1.91359 | 0.003625 | 0.046875   | -3.0267  | Down       | serine/threonine kinase 36 |
| TRIB3       | -1.91164 | 0.000301 | 0.012992   | -3.82577 | Down       | tribbles pseudokinase 3 |
| ZNF124      | -1.9092  | 0.000271 | 0.012156   | -3.86767 | Down       | zinc finger protein 124 |
| MPZL3       | -1.90884 | 0.000298 | 0.012703   | -3.83854 | Down       | myelin protein zero like 3 |
| TOR4A       | -1.90324 | 0.000292 | 0.012546   | -3.84663 | Down       | torsin family 4 member A |
| MIR3916     | -1.90072 | 4.48E-06 | 0.002039   | -5.09398 | Down       | microRNA 3916 |
| ZNF334      | -1.89919 | 6.18E-06 | 0.002168   | -4.9521  | Down       | zinc finger protein 334 |
| TITL3       | -1.89535 | 0.000105 | 0.007409   | -4.1499  | Down       | tubulin tyrosine ligase like 3 |
| ZKSCAN3     | -1.89411 | 0.001595 | 0.030071   | -3.30542 | Down       | zinc finger with KRAB and SCAN domains 3 |
| PAPLN       | -1.88842 | 0.001527 | 0.029477   | -3.31986 | Down       | papilin, proteoglycan like sulfated glycoprotein |
| KNTC1       | -1.88703 | 0.000207 | 0.010161   | -3.94852 | Down       | kinetochore associated 1 |
| SLC1A7      | -1.88316 | 0.000163 | 0.009559   | -4.01961 | Down       | solute carrier family 1 member 7 |
| BCL9        | -1.87682 | 0.00158  | 0.029935   | -3.30857 | Down       | BCL9 transcription coactivator |
| ZNF496      | -1.87502 | 0.00041  | 0.015068   | -3.74029 | Down       | zinc finger protein 496 |
| LNX2        | -1.87272 | 0.003479 | 0.045998   | -3.04098 | Down       | ligand of numb-protein X 2 |
| DNAJC18     | -1.8714  | 0.000203 | 0.010561   | -3.95515 | Down       | DNA J heat shock protein family (Hsp40) member C18 |
| ZNF114      | -1.86888 | 6.34E-05 | 0.0056     | -4.29807 | Down       | zinc finger protein 114 |
| VWA3B       | -1.86543 | 3.01E-05 | 0.003964   | -4.51187 | Down       | von Willebrand factor A domain containing 3B |
| ARNTL2      | -1.86522 | 0.002029 | 0.034127   | -3.22522 | Down       | aryl hydrocarbon receptor nuclear translocator like 2 |
| Gene Symbol | logFC   | p Value | adj. P.Val | t value | Regulation | Gene Name                                |
|-------------|---------|---------|------------|---------|------------|------------------------------------------|
| NGFR        | -1.85434| 0.000295| 0.012696   | -3.84106| Down       | nerve growth factor receptor             |
| CLEC1A      | -1.85306| 0.001166| 0.026095   | -3.40829| Down       | C-type lectin domain family 1 member A   |
| ZNF687      | -1.85305| 0.003658| 0.047012   | -3.02347| Down       | zinc finger protein 687                  |
| ZNF69       | -1.85271| 2.47E-05| 0.003545   | -4.56704| Down       | zinc finger protein 69                   |
| MTF1        | -1.85038| 0.000974| 0.023505   | -3.46684| Down       | metal regulatory transcription factor 1   |
| ZNF154      | -1.84509| 0.00012  | 0.008053   | -4.11197| Down       | zinc finger protein 154                  |
| SLAMF6      | -1.84139| 0.00019  | 0.010241   | -3.9745 | Down       | SLAM family member 6                      |
| TMEM255B    | -1.8379 | 5.28E-05| 0.005263   | -4.35097| Down       | transmembrane protein 255B               |
| HLA-H       | -1.83482| 0.002733| 0.040755   | -3.11929| Down       | major histocompatibility complex, class I, H (pseudogene) |
| KBTBD7      | -1.82918| 0.000254| 0.011836   | -3.88686| Down       | kelch repeat and BTB domain containing 7 |
| CDC20B      | -1.82896| 6.91E-05| 0.005946   | -4.2731 | Down       | cell division cycle 20B                  |
| SLC44A5     | -1.82893| 1.48E-05| 0.003137   | -4.71193| Down       | solute carrier family 44 member 5         |
| MEN1        | -1.8227 | 0.001593| 0.030071   | -3.30582| Down       | menin 1                                  |
| SCNN1D      | -1.82156| 0.003912| 0.048656   | -3.0003 | Down       | sodium channel epithelial 1 delta subunit|
| PFA5        | -1.81549| 0.002896| 0.017776   | -3.10436| Down       | phosphoribosylformylglycinamidine synthase|
| EVA1C       | -1.81455| 0.001419| 0.028438   | -3.34407| Down       | evo-1 homolog C                          |
| TIAM2       | -1.80764| 6.94E-05| 0.005946   | -4.27171| Down       | TIAM Rac1 associated GEF 2               |
| HLA-F-AS1   | -1.80482| 0.002916| 0.01853    | -3.10204| Down       | HLA-F antisense RNA 1                     |
| TRAPPCo9    | -1.80093| 0.000991| 0.022881   | -3.48851| Down       | trafficking protein particle complex 9    |
| APCDD1L     | -1.80071| 1.64E-05| 0.003137   | -4.68254| Down       | APC down-regulated 1 like                 |
| NUTM1       | -1.79716| 2.14E-05| 0.00341    | -4.60821| Down       | NUT midline carcinoma family member 1     |
| ARHGAP39    | -1.79263| 0.000319| 0.013245   | -3.81744| Down       | Rho GTPase activating protein 39          |
| SPTB        | -1.79073| 0.001482| 0.028982   | -3.32988| Down       | spectrin beta, erythrocytic               |
| ZNF469      | -1.78919| 7.59E-05| 0.006143   | -4.24571| Down       | zinc finger protein 469                   |
| AZIN1-A51   | -1.78541| 1.92E-05| 0.003347   | -4.63776| Down       | AZIN1 antisense RNA 1                     |
| BCL2L11     | -1.78395| 0.001862| 0.032689   | -3.25392| Down       | BCL2 like 11                             |
| CLDN1       | -1.78076| 0.000331| 0.013573   | -3.80619| Down       | claudin 1                                |
| ZNF836      | -1.77956| 6.7E-06  | 0.00223    | -4.92984| Down       | zinc finger protein 836                   |
| APBA1       | -1.77749| 0.002859| 0.041537   | -3.10878| Down       | amyloid beta precursor protein binding family A member 1 |
| C8orf37     | -1.77616| 0.000155| 0.009321   | -4.03521| Down       | chromosome 8 open reading frame 37       |
| LOC391322   | -1.77531| 9.16E-06| 0.00249    | -4.84394| Down       | D-pachymetamerase-like                    |
| SPATA2L     | -1.77449| 0.002137| 0.035219   | -3.2077 | Down       | spermatogenesis associated 2 like         |
| GLIS1       | -1.77119| 2.07E-05| 0.00341    | -4.61666| Down       | GLIS family zinc finger 1                 |
| LOC389765   | -1.76734| 7.38E-07| 0.001025   | -5.5224 | Down       | kinesin family member 27 pseudogene      |
| DENND2C     | -1.76558| 1.69E-05| 0.003137   | -4.67437| Down       | DENN domain containing 2C                |
| MIFAP3L     | -1.76481| 0.003083| 0.042962   | -3.08279| Down       | microfibril associated protein 3 like     |
| TREML1      | -1.76402| 0.005493| 0.043165   | -4.31615| Down       | triggering receptor expressed on myeloid cells like 1 |
| DNHD1       | -1.76398| 0.001285| 0.027046   | -3.37662| Down       | dynein heavy chain domain 1              |
| SETD6       | -1.75933| 5.78E-05| 0.005462   | -4.32489| Down       | SET domain containing 6, protein lysine methyltransferase |
| TTC34       | -1.75712| 4.74E-05| 0.005     | -4.38193| Down       | tetratricopeptide repeat domain 34       |
| SAR DH       | -1.74727| 0.000444| 0.015767   | -3.71564| Down       | sarcosine dehydrogenase                  |
| ZNF385C     | -1.74659| 5.06E-06| 0.002061   | -5.00645| Down       | zinc finger protein 385C                 |
| Gene Symbol | logFC  | p Value  | adj. P.Val | t value | Regulation | Gene Name                                |
|------------|-------|----------|------------|---------|------------|------------------------------------------|
| NEXN-AS1   | -1.74174 | 0.001791 | 0.03205 | -3.26688 | Down       | NEXN antisense RNA 1                        |
| CDHR3      | -1.741  | 5.08E-05 | 0.00511 | -4.36224 | Down       | cadherin related family member 3            |
| SPRYD4     | -1.7362 | 0.00381 | 0.048018 | -3.09934 | Down       | SPRY domain containing 4                   |
| ZSCAN25    | -1.73514 | 0.000224 | 0.011092 | -3.92529 | Down       | zinc finger and SCAN domain containing 25  |
| FAM157C    | -1.73405 | 3.95E-05 | 0.004532 | -4.43393 | Down       | family with sequence similarity 157 member C|
| ARSG       | -1.73192 | 0.000264 | 0.012045 | -3.8757  | Down       | arylsulfatase G                             |
| GLI2       | -1.72398 | 8.75E-06 | 0.002428 | -4.85662 | Down       | GLI family zinc finger 2                   |
| NSUN7      | -1.72112 | 0.000383 | 0.014539 | -3.76087 | Down       | NOP2/Sun RNA methyltransferase family member 7|
| BMP3       | -1.71974 | 0.00051 | 0.016932 | -3.67185 | Down       | bone morphogenetic protein 3               |
| PPT2       | -1.71813 | 1.01E-05 | 0.002625 | -4.81647 | Down       | palmitoyl-protein thioesterase 2           |
| CCNJL      | -1.7168  | 0.00297 | 0.042241 | -3.0957  | Down       | cyclin J like                              |
| C3orf70    | -1.71506 | 1.17E-05 | 0.002752 | -4.77652 | Down       | chromosome 3 open reading frame 70         |
| FFB1       | -1.71426 | 0.002996 | 0.042479 | -3.09265 | Down       | Fas binding factor 1                        |
| SEC14L2    | -1.70866 | 7.5E-05  | 0.006105 | -4.24929 | Down       | SEC14 like lipid binding 2                 |
| YPEL4      | -1.70708 | 0.000495 | 0.016631 | -3.68141 | Down       | yippee like 4                              |
| PCDH11Y    | -1.70054 | 0.00111 | 0.025344 | -3.42438 | Down       | protocadherin 11 Y-linked                  |
| AFAP1L2    | -1.68648 | 4.19E-05 | 0.004645 | -4.41718 | Down       | actin filament associated protein 1 like 2 |
| ZNF674-A51 | -1.6796  | 0.001198 | 0.026347 | -3.39666 | Down       | ZNF674 antisense RNA 1 (head to head)      |
| KCNQ4      | -1.67351 | 1.12E-05 | 0.002709 | -4.78912 | Down       | potassium voltage-gated channel subfamily Q member 4 |
| SULT1B1    | -1.67126 | 9.73E-05 | 0.00709  | -4.17312 | Down       | sulfotransferase family 1B member 1        |
| MORN1      | -1.66843 | 0.000538 | 0.01724  | -3.6552  | Down       | MORN repeat containing 1                   |
| PCDH17     | -1.66757 | 7.41E-05 | 0.006068 | -4.25287 | Down       | protocadherin 17                           |
| FOXP2      | -1.66727 | 0.000941 | 0.023272 | -3.47788 | Down       | forkhead box P2                            |
| XYLB       | -1.66722 | 0.001703 | 0.031013 | -3.28371 | Down       | xylulokinase                               |
| CTNNA3     | -1.66694 | 0.000163 | 0.009544 | -4.02144 | Down       | catenin alpha 3                            |
| NLRP6      | -1.66633 | 0.000524 | 0.017152 | -3.66377 | Down       | NLR family pyrin domain containing 6       |
| SLC16A13   | -1.66401 | 0.000624 | 0.018805 | -3.60875 | Down       | solute carrier family 16 member 13         |
| GLI3       | -1.6638  | 0.003245 | 0.044179 | -3.06511 | Down       | GLI family zinc finger 3                   |
| SYNJ2      | -1.66358 | 0.000209 | 0.010651 | -3.94618 | Down       | synaptojanin 2                             |
| C2orf15    | -1.6617  | 0.000283 | 0.012404 | -3.85419 | Down       | chromosome 2 open reading frame 15         |
| SCIMP      | -1.65716 | 0.002226 | 0.036099 | -3.19403 | Down       | SLP adaptor and CSK interacting membrane protein |
| KIR2DL4    | -1.6566  | 0.000227 | 0.01115  | -3.92146 | Down       | killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 4 |
| FHL3       | -1.65441 | 0.00305 | 0.042759 | -3.08659 | Down       | four and a half LIM domains 3              |
| OVGP1      | -1.65215 | 1.12E-06 | 0.001179 | -5.41243 | Down       | oviductal glycoprotein 1                   |
| N4BP3      | -1.65201 | 0.000581 | 0.017987 | -3.63113 | Down       | NEDD4 binding protein 3                    |
| ELOVL7     | -1.64755 | 3.88E-05 | 0.00453 | -4.43907 | Down       | ELOVL fatty acid elongase 7                |
| ESRG       | -1.64744 | 0.001428 | 0.028535 | -3.34192 | Down       | embryonic stem cell related                |
| ACTA2-AS1  | -1.64552 | 0.000128 | 0.008394 | -4.09176 | Down       | ACTA2 antisense RNA 1                      |
| CNKSR3     | -1.64218 | 7.19E-05 | 0.005962 | -4.26146 | Down       | CNKSR family member 3                      |
| ZNF347     | -1.63729 | 0.002754 | 0.040644 | -3.12164 | Down       | zinc finger protein 347                    |
| SGMS1-AS1  | -1.6362  | 3.3E-06  | 0.001776 | -5.12249 | Down       | SGMS1 antisense RNA 1                      |
| ZKSCAN2    | -1.63535 | 4.67E-05 | 0.005  | -4.38616 | Down       | zinc finger with KRAB and SCAN domains 2    |
| AMIGO1      | -1.63486 | 0.000936 | 0.023198 | -3.47947 | Down       | adhesion molecule with Ig like domain 1     |
Table 2: The statistical metrics for key differentially expressed genes (DEGs) (Continued)

| Gene Symbol | logFC  | p Value  | adj. P.Val | t value | Regulation | Gene Name |
|-------------|--------|----------|------------|---------|------------|-----------|
| ZC3H10      | -1.63262 | 0.001463 | 0.028876  | -3.33409 | Down       | zinc finger CCCH-type containing 10 |
| MUC17       | -1.62593 | 0.000109 | 0.007553  | -4.14108 | Down       | mucin 17, cell surface associated |
| ZNF559-ZNF177 | -1.62399 | 6.21E-05 | 0.005556  | -4.30427 | Down       | ZNF559-ZNF177 readthrough |
| NUTM2D      | -1.62355 | 0.000228 | 0.011159  | -3.92008 | Down       | NUT family member 2D |
| ABCB4       | -1.62247 | 3.99E-06 | 0.001884  | -5.07099 | Down       | ATP binding cassette subfamily B member 4 |
| LOC100652768 | -1.61749 | 0.000307 | 0.007513  | -3.82956 | Down       | uncharacterized LOC100652768 |
| C8orf58     | -1.61509 | 5.62E-05 | 0.002118  | -4.97786 | Down       | chromosome 8 open reading frame 58 |
| WRAP73      | -1.61325 | 4.07E-06 | 0.001884  | -5.06554 | Down       | WD repeat containing, antisense to TP73 |
| SLC24A4     | -1.61174 | 3.03E-05 | 0.00713   | -4.61911 | Down       | solute carrier family 24 member 4 |
| LENG8-AS1   | -1.61071 | 2.39E-05 | 0.003497  | -4.57709 | Down       | LENG8 antisense RNA 1 |
| C2orf66     | -1.60563 | 1.03E-05 | 0.002643  | -4.81096 | Down       | chromosome 2 open reading frame 66 |
| SMIM10L2A   | -1.60523 | 0.000107 | 0.007513  | -4.14119 | Down       | small integral membrane protein 10 like 2A |
| CYP1B1-AS1  | -1.60433 | 4.19E-05 | 0.004645  | -4.4176  | Down       | CYP1B1 antisense RNA 1 |
| CTNND1      | -1.60088 | 3.03E-05 | 0.003497  | -4.57709 | Down       | catenin delta 1 |
| ADCY10P1    | -1.60028 | 4.19E-05 | 0.004645  | -4.4176  | Down       | ADCY10 pseudogene 1 |
| LOC100130298| -1.59936 | 3.39E-05 | 0.004196  | -4.77779 | Down       | hCG1B16373-like |
| FLJ2627     | -1.59816 | 0.001285 | 0.027046  | -3.37677 | Down       | uncharacterized LOC645644 |
| CRISPLD1    | -1.59815 | 0.000698 | 0.019931  | -3.57312 | Down       | cysteine rich secretory protein LCCL domain containing 1 |
| MESTIT1     | -1.59755 | 1.51E-06 | 0.001401  | -5.32225 | Down       | MEST intronic transcript 1, antisense RNA |
| ASPG        | -1.59743 | 0.001369 | 0.027894  | -3.5594  | Down       | asparaginase |
| PRICKLE2-AS1| -1.59631 | 0.000336 | 0.013596  | -3.80182 | Down       | PRICKLE2 antisense RNA 1 |
| LINCO0865   | -1.59557 | 2.66E-05 | 0.00371   | -4.54615 | Down       | long intergenic non-protein coding RNA 865 |
| LINCO1001   | -1.59556 | 1.77E-06 | 0.001545  | -5.29009 | Down       | long intergenic non-protein coding RNA 1001 |
| GRK4        | -1.59417 | 1.69E-05 | 0.003137  | -4.67411 | Down       | G protein-coupled receptor kinase 4 |
| ZNF300      | -1.59305 | 5.6E-05  | 0.005391  | -4.33388 | Down       | zinc finger protein 300 |
| CADM3-AS1   | -1.59265 | 7.19E-05 | 0.005962  | -4.26163 | Down       | CADM3 antisense RNA 1 |
| SIT1        | -1.59069 | 0.000397 | 0.014772  | -3.75003 | Down       | signaling threshold regulating transmembrane adaptor 1 |
| LINCO1138   | -1.59045 | 0.001479 | 0.028971  | -3.33042 | Down       | long intergenic non-protein coding RNA 1138 |
| DNAH5       | -1.58778 | 0.000268 | 0.012109  | -3.87093 | Down       | dynein axonemal heavy chain 5 |
| FAM155B     | -1.58584 | 3.27E-06 | 0.003871  | -4.52525 | Down       | family with sequence similarity 155 member B |
| SLCSA4      | -1.58234 | 0.001776 | 0.012479  | -5.12479 | Down       | solute carrier family 5 member 4 |
| ZNF573      | -1.58059 | 6.33E-06 | 0.002168  | -4.94541 | Down       | zinc finger protein 573 |
| RCAN3       | -1.5793 | 3.95E-05 | 0.005391  | -4.33388 | Down       | RCAN family member 3 |
| DNAH3       | -1.57822 | 0.00296  | 0.012696  | -3.83989 | Down       | dynein axonemal heavy chain 3 |
| UBXN10      | -1.57771 | 1.9E-05  | 0.003343  | -4.64092 | Down       | UBX domain protein 10 |
| CLEC4A      | -1.57555 | 0.003924 | 0.048741  | -2.99895 | Down       | C-type lectin domain family 4 member A |
| RFFL        | -1.57423 | 0.00171  | 0.009664  | -4.06626 | Down       | ring finger and FYVE like domain containing E3 ubiquitin protein ligase |
| TMEM130     | -1.57294 | 0.000437 | 0.015643  | -3.72015 | Down       | transmembrane protein 130 |
| KCNB4       | -1.57256 | 0.003907 | 0.048665  | -3.0005  | Down       | potassium calcium-activated channel subfamily M regulatory beta subunit 4 |
| LINCO0924   | -1.57057 | 0.000165 | 0.009593  | -4.0716  | Down       | long intergenic non-protein coding RNA 924 |
| EREG        | -1.57034 | 0.000125 | 0.008283  | -4.0939  | Down       | epiregulin |
| URAD        | -1.56639 | 0.000704 | 0.020012  | -3.5708  | Down       | ureidomimidazoline (2-oxo-4-hydroxy-4-carboxy-5-) decarboxylase |
| SLC22A15    | -1.56367 | 0.000564 | 0.017736  | -3.64079 | Down       | solute carrier family 22 member 15 |
| Gene Symbol | logFC  | p Value | adj. P.Val | t value | Regulation | Gene Name                                      |
|------------|--------|---------|------------|---------|------------|-----------------------------------------------|
| ADRA2B     | -1.56339 | 0.000592 | 0.018192 | -3.62512 | Down       | adrenoceptor alpha 2B                         |
| CDH26      | -1.56317 | 0.00033  | 0.013567 | -3.80719 | Down       | cadherin 26                                   |
| ZNF596     | -1.5628  | 0.000227 | 0.01115  | -3.92153 | Down       | zinc finger protein 596                       |
| NYAP1      | -1.55572 | 3.9E-05  | 0.004533 | -4.43763 | Down       | neuronal tyrosine phosphorylated phosphoinositide-3-kinase adaptor 1 |
| SRPS      | -1.5537  | 0.000468 | 0.016151 | -3.699   | Down       | secreted frizzled related protein 5           |
| LOC101929574 | -1.55461 | 0.001368 | 0.027894 | -3.35602 | Down       | uncharacterized LOC101929574                 |
| RNF207     | -1.54999 | 7.3E-06  | 0.002243 | -4.90599 | Down       | ring finger protein 207                      |
| NRL        | -1.54936 | 2.58E-05 | 0.003634 | -4.55549 | Down       | neural retina leucine zipper                 |
| ANGPTL6    | -1.54763 | 0.00025  | 0.011782 | -3.89133 | Down       | angiopoietin like 6                          |
| ALOX15     | -1.54749 | 0.000145 | 0.009003 | -4.0563  | Down       | arachidonate 15-lipoxygenase                |
| CHEK2      | -1.54682 | 0.001992 | 0.03385  | -3.23132 | Down       | checkpoint kinase 2                          |
| ZNF543     | -1.54508 | 0.00303  | 0.02657  | -3.08876 | Down       | zinc finger protein 543                      |
| ZNF717     | -1.54439 | 0.000156 | 0.009363 | -4.03319 | Down       | zinc finger protein 717                      |
| FASLG      | -1.5428  | 0.001265 | 0.026877 | -3.38181 | Down       | Fas ligand                                   |
| LILRA3     | -1.5435  | 1.84E-05 | 0.003308 | -4.65038 | Down       | leukocyte immunoglobulin like receptor A3    |
| NEK10      | -1.54346 | 0.00012  | 0.008053 | -4.11146 | Down       | NIMA related kinase 10                       |
| MIR1914    | -1.543   | 0.000489 | 0.016519 | -3.68532 | Down       | microRNA 1914                                |
| BATF3      | -1.54097 | 0.000518 | 0.017057 | -3.667   | Down       | basic leucine zipper ATF-like transcription factor 3 |
| AVIL       | -1.54065 | 0.000532 | 0.013573 | -3.80487 | Down       | advillin                                     |
| KLK10      | -1.54034 | 7.07E-05 | 0.005956 | -4.26664 | Down       | kallikrein related peptidase 10              |
| ZNF689     | -1.53954 | 0.003253 | 0.04422  | -3.06429 | Down       | zinc finger protein 689                      |
| MKLN1-AS   | -1.53911 | 0.00209  | 0.0347   | -3.2153  | Down       | MKLN1 antisense RNA                          |
| LOC100128398 | -1.53652 | 0.002092 | 0.034722 | -3.21487 | Down       | uncharacterized LOC100128398                 |
| LRPI-A5    | -1.53646 | 4.89E-05 | 0.005015 | -4.37323 | Down       | LRPI antisense RNA                           |
| NPIPA1     | -1.5363  | 0.003113 | 0.043191 | -3.07949 | Down       | nuclear pore complex interacting protein family member A1 |
| NLRP2      | -1.53625 | 0.000332 | 0.013573 | -3.82661 | Down       | NLR family pyrin domain containing 2         |
| MME1       | -1.5346  | 0.000336 | 0.013596 | -3.80163 | Down       | membrane metalloendopeptidase like 1         |
| SPDY5      | -1.53457 | 0.000116 | 0.070921 | -4.12051 | Down       | speedy/RINGO cell cycle regulator family member E5 |
| CCL24      | -1.5342  | 5.69E-05 | 0.005435 | -4.32942 | Down       | C-C motif chemokine ligand 28               |
| KLHDC1     | -1.53297 | 0.000501 | 0.016732 | -3.67754 | Down       | kelch domain containing 1                    |
| FRMPD1     | -1.53279 | 4.28E-05 | 0.004701 | -4.41134 | Down       | FERM and PDZ domain containing 1             |
| LINC00211  | -1.53271 | 0.00019  | 0.010241 | -3.97414 | Down       | long intergenic non-protein coding RNA 211    |
| ATP6V1G2   | -1.53264 | 0.000799 | 0.021227 | -3.53045 | Down       | ATPase H+ transporting V1 subunit G2         |
| PRIMA1     | -1.53113 | 0.000786 | 0.021056 | -3.53546 | Down       | proline rich membrane anchor 1              |
| NPTX1      | -1.52859 | 0.000253 | 0.011808 | -3.88811 | Down       | neuronal pentraxin 1                         |
| LOC101928107 | -1.5272  | 0.0003  | 0.012783 | -3.83612 | Down       | uncharacterized LOC101928107                |
| GFRA3      | -1.5245  | 8.59E-06 | 0.002428 | -4.86161 | Down       | GDNF family receptor alpha 3                 |
| LOC10192959 | -1.51972 | 1.12E-05 | 0.020709 | -4.78841 | Down       | uncharacterized LOC10192959                 |
| FLJ37453   | -1.51859 | 7.55E-06 | 0.002243 | -4.8971  | Down       | uncharacterized LOC729614                   |
| MACC1      | -1.51799 | 0.000573 | 0.017831 | -3.6354  | Down       | MET transcriptional regulator MACC1          |
| LINC00607  | -1.51637 | 2.68E-05 | 0.00371  | -4.5447  | Down       | long intergenic non-protein coding RNA 607   |
| FAM8681    | -1.5153  | 0.000332 | 0.013573 | -3.80528 | Down       | family with sequence similarity 86 member B1 |
| MIR4477B   | -1.51191 | 2.05E-05 | 0.00341  | -4.62019 | Down       | microRNA 4477b                               |
(Thermo Fisher Scientific, Waltham, MA, USA) and 7 Flex real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA) were used for reverse transcription and real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assay. Polymerase chain reaction primer sequences are listed in Table 1. β-actin was used as an internal control for quantification. The relative expression levels of target transcripts were calculated using the \(2^{-\Delta\Delta C_T}\) method [25]. The thermocycling conditions used for RT-PCR were as follows: initial denaturation at 95°C for 15 min, followed by 40 cycles at 95°C for 10 sec, 60°C for 20 sec and 72°C for 20 sec.

### Molecular docking studies

Surflex-docking studies of the standard drug molecule used in polycystic ovary syndrome were used on over expressed genes and were collected from PDB data bank using perpetual SYBYL-X 2.0 software. Using ChemDraw Software, all the drug molecules were illustrated, imported and saved in sdf template using open babel free software. The protein structures of POLR2K, RPS15, RPS15 alpha and SAA1 of their co-crystallised protein of PDB code 1LE9, 3OW2, 1G1X and 4IP8 respectively were extracted from Protein Data Bank [26–28]. Gasteiger Huckel (GH) charges were applied along with the TRIPOS force field to all the drug molecules and is standard for the structure optimization process. In addition, energy minimization was achieved using MMFF94s and MMFF94 algorithm methods. The protein preparation was carried out after incorporation of protein. The co-crystallized ligand was extracted from the crystal structure and all water molecules; more hydrogen was added and the side chain was set. For energy minimisation, the TRIPOS force field was used. The interaction efficiency of the compounds with the receptor was represented in kcal / mol units by the

### Table 2 The statistical metrics for key differentially expressed genes (DEGs) (Continued)

| Gene Symbol | logFC | p Value | adj. P.Val | t value | Regulation | Gene Name |
|-------------|-------|---------|------------|---------|------------|-----------|
| CNNM2       | -1.50798 | 0.001668 | 0.03072 | -3.29062 | Down | cyclin and CBS domain divalent metal cation transport mediator 2 |
| SKA1        | -1.50659 | 0.00086 | 0.02226 | -3.50665 | Down | spindle and kinetochore associated complex subunit 1 |
| ABRACL      | -1.50603 | 0.001136 | 0.025784 | -3.41701 | Down | ABRA C-terminal like |
| LINC00954   | -1.50484 | 0.000652 | 0.019135 | -3.59472 | Down | long intergenic non-protein coding RNA 954 |
| ZBP1        | -1.50412 | 0.002141 | 0.035221 | -3.20719 | Down | Z-DNA binding protein 1 |
| ZNF528      | -1.50042 | 0.000464 | 0.016097 | -3.70168 | Down | zinc finger protein 528 |
| ACTG1P4     | -1.50023 | 0.000252 | 0.011803 | -3.88932 | Down | actin gamma 1 pseudogene 4 |
| MYO10       | -1.50014 | 0.003103 | 0.043113 | -3.08058 | Down | myosin X |

Fig. 2 Heat map of differentially expressed genes. Legend on the top left indicate log fold change of genes. (A1 – A2 = normal control samples; B1 – B30 = PCOS samples)
### Table 3 The enriched GO terms of the up and down regulated differentially expressed genes

| GO ID       | CATEG ORY | GO Name                          | P Value | FDR B&H | FDR B&Y | Bonferroni Gene Count | Gene                                                                 |
|------------|-----------|----------------------------------|---------|---------|---------|-----------------------|----------------------------------------------------------------------|
| Up regulated genes |
| GO: 0006518 | BP        | peptide metabolic process        | 1.51E-13| 2.66E-10| 2.37E-10| 6.37E-10              | 52 RPL24, RPL27, RPL30, RPL36A, RPL30, RPLP0, RMRP12, RMRP51, RPS5, RPS8, EFTF1, RPS15, EFTI5, RPS15, RSR72, RPS20, RPS23, RPS24, RPS29, EFTI6, EFTI3, RMRP51, RMRP24, RMRP20, COA3, RMR, TRMT112, RMRP54, CPE, RPL14, RPS27, EFTI2D, DPH5, EFTI1AX, ATP6AP2, FN706E, SEC11A, RMRP24, UH-HMK1, EFTI1, RMRP5, RMRP6, RMRP34, LSM14A, RPL35, RPL33, RMRP9, RSL24D1 |
| GO: 0006886 | BP        | intracellular protein transport   | 6.75E-12| 8.16E-10| 7.28E-09| 2.86E-08              | 66 GIPC1, SNAPIN, RPL24, TCTN3, RPL27, RPL30, ROMO1, RPL35A, RPL36A, RPLP0, RMRP55, RPS58, EFTI10, RPS15, RSR72, RPS20, HMCG1, RPS21, RPS23, RPS24, RPS29, APEF1, TOMM5, EFTI6AB1, ATP6V1D0, SNX8, PEX19, AGF1, PEX2, ZF AND6, RABGEF1, SNX9, RABAB, PMM1, TOMM7, SRSF6, RPA1B2, RPAIN, TPNO2, DER1, FIS1, RPL14, APOD, ECH1, JF127, WYHAH, TMED4, ARFS5, NDUF8, NDUF21, EXOC5, EFT2D, UBL5, TMMIB, CMT, UH-HMK1, DYNL2, VPS28, RPL35, SYVNI, RPL13, STX10 |
| GO: 0031967 | CC        | organelle envelope                | 2.36E-19| 1.71E-16| 1.47E-16| 72 ROMO1, PRELID1, MRPL12, RMRP12, PET101, BAK1, PHB2, LRRCS9, HMGC1, GHFR, COA4, NDUF21, SL2C25A26, BLOC51, ECT1, SLUG1, NDUF28, TOMM5, NDUF2C, BANF1, MOAP1, NDUF34, NDUF35, PHB, BNP3, PHR8, DFX1, MRPM21, AGF1G1, SL2C25A11, TMEM97, UQRC1, UQRC2F1, UQRC2Y, UQRC1, NM2E2, MRPL24, CERS2, MRPL20, TOMM70, COA3, COX41I, COX5B, DTYMK, ANXA4, COX6A1, NDUF2A3, COX6B1, MRPL54, COX6D, COX7A2, COX7C, COX7A2F1, IFI27, MAD2L1, BP, CCNDO2, NDUF21, CYB5B, TMEM256, TIMM8B, AIM1, RMRP2A, RMRP15, CHCH2, DMRP34, RMRP34, COX7A2L, UQCRQ, HSD17B10, MTD3R, MRPL9 |
| GO: 1902494 | CC        | catalytic complex                 | 1.31E-12| 5.84E-10| 4.09E-10| 8.17E-09              | 67 PRPF31, DCAF6, ERH, SAP18, O54, PMS1B1, PMS1B2, EFTF7, PMSB7, PSMC5, PSM5D, PSM5D10, NDUF2A2, E2H1, SLUG1, NDUF2B2, NDUF2C2, NDUF34, NDUF35, LSM3, CK18B, TPSA, N17, SLCA3R1, LUBE261, BMX, MORP4L1, LPEX2, AK1, PRMT1, UQRC1, UQRC2F1, UQRC2Y, TAF7, ANAPC5S, TADA3, ANAPC13, RBX1, CUL4A, POLR3L, RBP7F1, PG5, POLR2K, DERL2, BCCP1, POLE4, NA338, CCN1, CCN2, NM2R, NDUF2A3, POL13D1, GFIA2, GRMLD1, SEC11A1, PSEN1D6, DYNL2, SMARC2, UQCRQ, HSD17B10, KHLH1, LS1YNI, CDC27, PSM1D-1, SNRPD2, RPHH1, JRTCP2 |
| GO: 0003723 | MF        | RNA binding                      | 1.66E-10| 4.49E-07| 3.30E-07| 1.45E-07              | 76 RPL24, PFRF31, RPL27, RPL30, RPL29, RPL34, RPL36A, EFTI10, RPL10, S1P15, RPS15, S1P15, RPS15, RSR72, RPS20, LRRCS9, RPS21, RPS23, RPS24, UQRC1, UQRC2F1, UQRC2Y, TAF7, ANAPC5, TADA3, ANAPC13, RBX1, CUL4A, POLR3L, RBP7F1, PG5, POLR2K, DERL2, BCCP1, POLE4, NA338, CCN1, CCN2, NM2R, NDUF2A3, POL13D1, GFIA2, GRMLD1, SEC11A1, PSEN1D6, DYNL2, SMARC2, UQCRQ, HSD17B10, KHLH1, LS1YNI, CDC27, PSM1D-1, SNRPD2, RPHH1, JRTCP2 |
| GO: 0008134 | MF        | transcription factor binding      | 1.05E-02| 1.83E-01| 1.00E+00| 1.00E+00              | 23 NAB1, PHB2, PSMC5S, AMA98B, NDUF2M, MDP10D1, MDP10E17, SETD3, RBX1, WIP1, ANX4A4, TEAD3, IFI27, WYHAH, GTF2A2, MED4, CMT, CHCH2, HSD17B10, MTD3R, TRIB1 |
| Down regulated genes |
| GO: 0120036 | BP        | plasma membrane bounded cell projection organization | 7.28E-05| 2.19E-02| 1.94E-01| 2.91E-01              | 49 EPHB4, STX3, MAKPAST, ZFYVE27, TLL1, MYO7A, MYO10, SDCCA8G5, ST8TA, SARM1, RAPI1, GAD2P, KHRF4, GFRAM3, AMIGOI1, HECW2, GLI2, GLI3, SYN2E, WRAP73, NGFRPNT1, NLNQ2, FGF13, PLD1, CEP112G, NYAP1, DNLH8, UBXN10, PLXN3B, Sema6A, FAT4-NPT1X1, NED4D, STK85B, FBF1, LAMA5, RFLL, COF2, TAF3, RPS3, AT1L, TRAK1, WDR19, FE21, AVIL, CEP135, JFT122, PHA4 |
| GO: 000902  | BP        | cell morphogenesis                | 3.10E-03| 1.93E-01| 1.00E+00| 1.00E+00              | 31 EPHB4, CDH26, PAST, ZFYVE27, MYO7A, MYO10, CDHR3, SPTB, SARM1, GFRAM3, AMIGOI1, HECW2, GLI2, GLI3, NGFR,
Surflex-Dock score. The interaction between the protein and the ligand, the best pose was incorporated into the molecular area. The visualization of ligand interaction with receptor is done by using discovery studio visualizer.

Results

Identification of DEGs

Expression profiling by high throughput sequencing dataset was obtained from the National Center for Biotechnology Information GEO database containing PCOS samples and normal control samples: GSE84958. Then, the R package named "limma" was processed for analysis with adjusted $P < 0.05$, and $|\text{logFC}| > 2.5$ for up regulated genes and $|\text{logFC}| < -1.5$ for down regulated genes.

All DEGs were displayed in volcano maps (Fig. 1). A total of 739 DEGs including 360 up regulated and 379 down regulated genes (Table 2) were identified in PCOS samples compared to normal control samples. The results are shown in the heatmap (Fig. 2).

GO and pathway enrichment of DEGs in PCOS

The top 739 DEGs were chosen to perform GO and REACTOME pathway enrichment analyses. Gene Ontology (GO) analysis identified that the DEGs were significantly enriched in BP, including the peptide metabolic process, intracellular protein transport, plasma membrane bounded cell projection organization and cell morphogenesis (Table 3). In terms of CC, DEGs were mainly enriched in organelle envelope, catalytic complex, neuron projection and cell junction were the most significantly enriched GO term (Table 3). In addition, MF demonstrated that the DEGs were enriched in the RNA binding, transcription factor binding, DNA-binding transcription factor activity, RNA polymerase II-specific and ATP binding (Table 3). REACTOME pathway enrichment analysis was used to screen the signaling pathways for differential genes. These results were mainly involved in the translation, respiratory electron transport, generic transcription pathway and transmembrane transport of small molecules (Table 4).

PPI networks construction and module Analysis

Following the analysis based on the PPI networks, 4141 nodes and 14853 edges were identified in Cytoscape (Fig. 3a). The genes with higher scores were the hub genes, as the genes of node degree, betweenness centrality, stress centrality, closeness centrality may be linked with PCOS. The top 10 hub genes were SAC1, ADCY6, POLR2K, RPS15, RPS15A, ESR1, LCK, S1PR5 and CTNND1 and are listed in Table 5. Enrichment analysis demonstrated that module 1 (Fig. 3b) and module 2 (Fig. 3c) might be associated with respiratory electron transport, organelle envelope, catalytic complex, gene expression, signaling by NGF and neuron projection.
After combining the results of miRNA-target genes with the interactive network of miRNAs, 281 hub genes were selected and 2138 were miRNAs. The genes and miRNAs are shown in Fig. 4a. Specifically, 97 miRNAs (ex, hsa-mir-8067) that regulate RPL13A, 95 miRNAs (ex, hsa-mir-4518) that regulate RPS15A, 71 miRNAs (ex, hsa-mir-3685) that regulate RPLP0, 65 miRNAs (ex, hsa-mir-1202) that regulates ADCY6, 48 miRNAs (ex, hsa-mir-4461) that regulate RPS29, 129 miRNAs (ex, hsa-mir-8082) that regulate CTNNB1, 98 miRNAs (ex, hsa-mir-4422) that regulate ESR1, 76 miRNAs (ex, hsa-mir-548am-5p) that regulate NEDD4L, 62 miRNAs (ex, hsa-mir-6868-3p) that regulate NGFR were detected (Table 6).
Construction of TF - target regulatory network

After combining the results of TF-target genes with the interactive network of TFs, 455 hub genes were selected and 274 were TFs. The genes and TFs are shown in Fig. 4b. Specifically, 15 TFs (ex, PER3) that regulate RBX1, 13 TFs (ex, CTCF) that regulate RPS15, 12 TFs (ex, E2F7) that regulate RPS20, 11 TFs (ex, LMO2) that regulate ADCY6, 9 TFs (ex, POLR2H) that regulate POLR2K, 122 TFs (ex, NCOA2) that regulate ESR1, 21 miRNAs (ex, EBF1) that regulate LCK, 18 TFs (ex, SMAD2) that regulate GLI3, 17 TFs (ex, JUND) that regulate NEDD4L, and 15 TFs (ex, FOXO3) that regulate CALCR were detected (Table 6).

Receiver operating characteristic (ROC) curve analysis

Moreover, ROC curve analysis using “pROC” packages was performed to calculate the capacity of ten hub genes to distinguish PCOS from normal control. SAA1, ADCY6, POLR2K, RPS15, RPS15A, CTNND1, ESR1, NEDD4L, KNTC1 and NGFR all exhibited excellent diagnostic efficiency (AUC > 0.7) (Fig. 5).

Validation of the expression levels of hub genes by RT-PCR

Aiming to further verify the expression patterns of selected hub genes, real-time PCR, which allows quantitative analysis of hub gene expression, was applied. The results showed that the relative expression levels of 10 hub genes including SAA1, ADCY6, POLR2K, RPS15, RPS15A, CTNND1, ESR1, NEDD4L, KNTC1 and NGFR were consistent with the expression profiling by high throughput sequencing (Fig. 6).

Molecular docking studies

In the present analysis, the docking simulations are performed to classify the active site conformation and significant interactions with the receptor binding sites responsible for complex stability. The over expressed genes is recognized in polycystic ovary syndrome and their x-ray crystallographic proteins structure are selected from PDB for docking studies. The standard drugs containing steroid nucleus are most commonly used either alone or in combination with other drugs. The docking studies of standard molecules containing the steroid ring have been carried out using Sybyl X 2.1 drug design software. The docking studies were performed to know the binding interaction of standard molecules on identified overexpressed genes of protein. The X-RAY crystallographic structure of one protein in each of four over expressed genes of POLR2K, RPS15, RPS15 and SAA1 of their co-crystallised protein of PDB code 1LE9, 3OW2, 1G1X and 4IP8 respectively were selected for the docking (Fig. 7). A total of three drug molecules of ethinylestradiol (ETE), levonorgestril (LNG) and desogestril (DSG) were docked with over expressed proteins to assess the binding affinity with proteins. The binding score greater than six are said to be good, all three drug molecules obtained binding score greater than 7 respectively. The molecules ETE obtained with a high binding score of 9.943 with SAA1 of PDB code 4IP8 and 8.260, 8.223 and 8.019 with 1G1X, 3OW2 and 1LE9. The LNG obtained highest binding score of 8.535 with SAA1 of PDB code 4IP8 and 8.351, 7.973 and 7.854 with RPS15, POLR2K and RPS15 alpha of PDB code 3OW2, 1LE9 and 1G1X respectively. DSG: highest with POLR2K of 8.273 with PDB code 1LE9, 8.158 with SAA1 of PDB code 4IP8, 7.745 with RPS15 alpha of PDB code 1G1X and obtained least binding score of 5.674 with RPS15 of
| Regulation | Node   | Degree | Betweenness | Stress   | Closeness |
|------------|--------|--------|-------------|----------|-----------|
| Up         | SAA1   | 315    | 0.10107     | 69407430 | 0.321603  |
| Up         | ADCY6  | 312    | 0.072777    | 44726506 | 0.302897  |
| Up         | POLR2K | 236    | 0.037471    | 28379862 | 0.302013  |
| Up         | RPS15  | 212    | 0.008152    | 26431684 | 0.30863   |
| Up         | RPS15A | 211    | 0.007809    | 26602340 | 0.309741  |
| Up         | RPS5   | 209    | 0.007859    | 26492428 | 0.309463  |
| Up         | RPL13A | 207    | 0.007846    | 22124842 | 0.309533  |
| Up         | RPS23  | 205    | 0.005989    | 23951504 | 0.308794  |
| Up         | RPLP0  | 205    | 0.00818     | 22450450 | 0.310438  |
| Up         | RPL35  | 203    | 0.006165    | 21837168 | 0.309174  |
| Up         | RPS20  | 202    | 0.005355    | 24073032 | 0.308564  |
| Up         | RPS29  | 198    | 0.004498    | 20672292 | 0.308426  |
| Up         | RBX1   | 197    | 0.044386    | 26711280 | 0.318217  |
| Up         | POLR2J | 192    | 0.020679    | 20252592 | 0.293659  |
| Up         | RPL30  | 187    | 0.006891    | 19563996 | 0.308265  |
| Up         | RPS8   | 177    | 0.004042    | 18164736 | 0.30678   |
| Up         | RPL35A | 174    | 0.003759    | 16445648 | 0.30783   |
| Up         | RPS24  | 174    | 0.003192    | 15939464 | 0.304838  |
| Up         | RPL24  | 173    | 0.003533    | 16772430 | 0.306803  |
| Up         | C3AR1  | 172    | 0.003177    | 5121210  | 0.274955  |
| Up         | RPL34  | 172    | 0.004039    | 16061864 | 0.30719   |
| Up         | RPL29  | 171    | 0.00494     | 18453558 | 0.307647  |
| Up         | RPS21  | 170    | 0.002685    | 15196960 | 0.304636  |
| Up         | SNRPD2 | 168    | 0.03814     | 33998368 | 0.309486  |
| Up         | RPS10  | 164    | 0.002949    | 14807414 | 0.306055  |
| Up         | RPL14  | 163    | 0.00328     | 15070824 | 0.306826  |
| Up         | RPL27  | 160    | 0.002277    | 14404254 | 0.305716  |
| Up         | PSMC5  | 145    | 0.016771    | 20051716 | 0.327066  |
| Up         | PSMD14 | 138    | 0.008797    | 20693344 | 0.312311  |
| Up         | RBBP7  | 136    | 0.039699    | 19307334 | 0.314733  |
| Up         | PSMD3  | 132    | 0.006138    | 19507196 | 0.312264  |
| Up         | SRSF6  | 130    | 0.01551     | 18603044 | 0.305242  |
| Up         | PSMB4  | 128    | 0.006024    | 19460760 | 0.31217   |
| Up         | PSMB1  | 126    | 0.005935    | 19159108 | 0.312736  |
| Up         | PSMD10 | 125    | 0.006564    | 19293068 | 0.313162  |
| Up         | PSMB7  | 121    | 0.004879    | 18406276 | 0.311911  |
| Up         | CDC27  | 121    | 0.014082    | 17205180 | 0.314709  |
| Up         | PLCG2  | 119    | 0.031441    | 10514426 | 0.284595  |
| Up         | UBE2E1 | 111    | 0.01698     | 18575766 | 0.315597  |
| Up         | MRPL24 | 111    | 0.004006    | 3985902  | 0.27419   |
| Up         | SEC11A | 107    | 0.005316    | 8457436  | 0.302168  |
| Up         | EIF3E  | 105    | 0.00271     | 6755804  | 0.298099  |
| Up         | EIF1AX | 105    | 0.00175     | 6248608  | 0.298099  |
| Up         | ETF1   | 104    | 0.003209    | 7817310  | 0.300348  |
| Regulation | Node   | Degree | Betweenness | Stress  | Closeness |
|------------|--------|--------|-------------|---------|-----------|
| Up         | SF3B5  | 104    | 0.003133    | 6307886 | 0.271298  |
| Up         | EIF5   | 103    | 0.002157    | 5951468 | 0.298056  |
| Up         | MRPS12 | 103    | 0.001566    | 3803642 | 0.27381   |
| Up         | RPS27L | 103    | 0.003987    | 8037762 | 0.3043    |
| Up         | RBMX   | 102    | 0.006533    | 8629778 | 0.302543  |
| Up         | SF3B6  | 101    | 0.007747    | 10034416| 0.30272   |
| Up         | RPL36A | 101    | 0.001189    | 3109720 | 0.271102  |
| Up         | RSL24D1| 100    | 0.010421    | 6175826 | 0.267684  |
| Up         | ANAPC5 | 100    | 0.005802    | 14180882| 0.312854  |
| Up         | ITGAV  | 98     | 0.047765    | 36204220| 0.313518  |
| Up         | SRP72  | 98     | 0.001539    | 6042806 | 0.299696  |
| Up         | NME2   | 94     | 0.034644    | 15645818| 0.312029  |
| Up         | YWHAH  | 93     | 0.030402    | 22230232| 0.316369  |
| Up         | MRPS15 | 86     | 0.001926    | 2385992 | 0.260394  |
| Up         | UQCRQ  | 83     | 0.003421    | 1615130 | 0.240866  |
| Up         | CCNC   | 83     | 0.010303    | 8511008 | 0.281231  |
| Up         | UQCRC1 | 82     | 0.02328     | 14231790| 0.289875  |
| Up         | MED4   | 80     | 0.018252    | 17775072| 0.301683  |
| Up         | UQCRF1 | 78     | 0.001699    | 1157234 | 0.244233  |
| Up         | CCND2  | 77     | 0.023553    | 14830028| 0.314399  |
| Up         | MRPL12 | 77     | 0.002782    | 2194820 | 0.259789  |
| Up         | POLR1D | 75     | 0.0061      | 4678140 | 0.274081  |
| Up         | UQCRH  | 72     | 0.001221    | 959168  | 0.244147  |
| Up         | KLHL12 | 68     | 0.003828    | 3608276 | 0.303719  |
| Up         | SLC9A3R1| 63     | 0.021981    | 10906810| 0.303987  |
| Up         | XRCCS  | 63     | 0.016826    | 12450448| 0.298895  |
| Up         | EIF6   | 62     | 0.005109    | 3168676 | 0.251672  |
| Up         | GTF3A  | 61     | 0.016181    | 6249890 | 0.308426  |
| Up         | MRPS6  | 60     | 6.2E-04     | 932952  | 0.249563  |
| Up         | PLA2G2A| 59     | 0.016536    | 3353206 | 0.267442  |
| Up         | NDUF2C | 58     | 0.009137    | 6243750 | 0.288562  |
| Up         | TAF7   | 58     | 0.008456    | 6200048 | 0.248053  |
| Up         | NDUF1A3| 58     | 0.010438    | 6851160 | 0.289086  |
| Up         | TSG101 | 57     | 0.016521    | 11031600| 0.306145  |
| Up         | SAP18  | 57     | 0.012307    | 5315482 | 0.302256  |
| Up         | NDUF54 | 56     | 0.001242    | 656204  | 0.239889  |
| Up         | SMAD5  | 56     | 0.020424    | 14462992| 0.296201  |
| Up         | RSL1D1 | 56     | 0.011475    | 16770568| 0.229134  |
| Up         | NDUF2A | 54     | 7.42E-04    | 556554  | 0.239861  |
| Up         | NBN    | 54     | 0.015436    | 11207188| 0.30323   |
| Up         | NEDD8  | 53     | 0.011483    | 3600034 | 0.320831  |
| Up         | PRMT1  | 52     | 0.012592    | 15575898| 0.301332  |
| Up         | MORF4L1| 51     | 0.010667    | 4279006 | 0.296795  |
| Up         | CUL4A  | 50     | 0.007675    | 2678820 | 0.299544  |
Table 5 Topology table for up and down regulated genes (Continued)

| Regulation | Node  | Degree | Betweenness | Stress  | Closeness |
|------------|-------|--------|-------------|---------|-----------|
| Up         | LAMA2 | 49     | 0.007709    | 857026  | 0.278844  |
| Up         | COX6A1| 48     | 0.001525    | 801088  | 0.240334  |
| Up         | AK1   | 46     | 0.003174    | 1615960 | 0.250894  |
| Up         | COX5B | 42     | 0.001215    | 595708  | 0.239861  |
| Up         | TADA3 | 40     | 0.010349    | 2368830 | 0.281307  |
| Up         | SUMO3 | 40     | 0.011044    | 5466788 | 0.300348  |
| Up         | CDKN2B| 36     | 0.006521    | 2033654 | 0.280983  |
| Up         | SERPINH1| 36   | 0.01212     | 7196482 | 0.29909   |
| Up         | MRPL20| 34     | 0.002069    | 1471944 | 0.293201  |
| Up         | COMT  | 34     | 0.015004    | 4152910 | 0.286109  |
| Up         | LSM4  | 34     | 0.007332    | 2056240 | 0.254456  |
| Up         | EXOC5 | 33     | 0.004443    | 1785404 | 0.263041  |
| Up         | SVN1  | 32     | 0.012143    | 5434366 | 0.291078  |
| Up         | BAK1  | 31     | 0.006508    | 1183542 | 0.267425  |
| Up         | RAB10 | 30     | 0.008921    | 6109230 | 0.296328  |
| Up         | FDN5  | 29     | 0.010966    | 3969486 | 0.290567  |
| Up         | SCARB1| 28     | 0.010079    | 5204710 | 0.299544  |
| Up         | COX6C | 27     | 4.36E-04    | 219006  | 0.232011  |
| Up         | COX4I1| 26     | 6.32E-04    | 186792  | 0.232493  |
| Up         | EZH1  | 26     | 0.001591    | 543666  | 0.245377  |
| Up         | SUCLG1| 26     | 0.008369    | 8540110 | 0.200727  |
| Up         | OXCT1 | 25     | 0.007475    | 2818178 | 0.199778  |
| Up         | PHB   | 24     | 0.005122    | 2747910 | 0.293388  |
| Up         | BANF1 | 20     | 0.005143    | 1975740 | 0.295651  |
| Up         | BCCIP | 20     | 0.003654    | 1838164 | 0.269444  |
| Up         | RDX   | 18     | 0.004798    | 4656272 | 0.29563   |
| Up         | CFL2  | 15     | 0.005519    | 1602836 | 0.286624  |
| Up         | ARF5  | 12     | 0.004972    | 1234824 | 0.284419  |
| Up         | UCHL1 | 12     | 0.001629    | 954606  | 0.29618   |
| Up         | PFN2  | 11     | 0.003745    | 1598036 | 0.28734   |
| Up         | NDUFB2| 8      | 0           | 0       | 0.225040  |
| Up         | COX6B1| 8      | 0           | 0       | 0.225073  |
| Up         | COX7A2L| 8     | 0           | 0       | 0.225073  |
| Up         | COX7C | 8      | 0           | 0       | 0.225073  |
| Up         | PRPF31| 8      | 1.73E-04    | 119394  | 0.213127  |
| Up         | COX8A | 8      | 0           | 0       | 0.225073  |
| Up         | NDUFS5| 8      | 0           | 0       | 0.225040  |
| Up         | PHB2  | 8      | 0.001363    | 712242  | 0.308449  |
| Up         | ETFB  | 4      | 0           | 0       | 0.224963  |
| Up         | NELFB | 3      | 2.95E-04    | 570978  | 0.27419   |
| Up         | CKS1B | 3      | 6.28E-06    | 1544    | 0.254284  |
| Up         | ANAPC13| 3     | 0           | 0       | 0.245741  |
| Up         | GTF2A2| 3      | 0           | 0       | 0.236342  |
| Up         | POLE4 | 3      | 2.39E-04    | 114402  | 0.254863  |
| Regulation | Node    | Degree | Betweenness | Stress     | Closeness |
|------------|---------|--------|-------------|------------|-----------|
| Up         | TYROBP  | 2      | 0           | 0          | 0.248948  |
| Up         | DCAF6   | 2      | 0           | 0          | 0.241724  |
| Up         | SKIL    | 2      | 0           | 0          | 0.239764  |
| Up         | S100A16 | 2      | 0           | 0          | 0.172622  |
| Up         | HMGCL   | 2      | 0           | 0          | 0.172622  |
| Up         | ARHGAP12| 2      | 7.30E-07    | 10130      | 0.210056  |
| Up         | STX10   | 2      | 0           | 0          | 0.222748  |
| Up         | UQCR11  | 2      | 0           | 0          | 0.194129  |
| Up         | GADD45B | 1      | 0           | 0          | 0.188748  |
| Up         | PFDN1   | 1      | 0           | 0          | 0.225159  |
| Up         | CDIPT   | 1      | 0           | 0          | 0.221556  |
| Up         | TOMM7   | 1      | 0           | 0          | 0.211009  |
| Up         | MRPS24  | 1      | 0           | 0          | 0.206607  |
| Up         | SBDS    | 1      | 0           | 0          | 0.201078  |
| Up         | COPS7A  | 1      | 0           | 0          | 0.230512  |
| Up         | DERL2   | 1      | 0           | 0          | 0.225466  |
| Up         | DTYMK   | 1      | 0           | 0          | 0.237835  |
| Up         | NDFIP1  | 1      | 0           | 0          | 0.237726  |
| Up         | BTF3L4  | 1      | 0           | 0          | 0.213292  |
| Up         | AFAP1L1 | 1      | 0           | 0          | 0.195053  |
| Up         | ATP6V1D | 1      | 0           | 0          | 0.235992  |
| Up         | LSM3    | 1      | 0           | 0          | 0.202852  |
| Up         | ARFIP2  | 1      | 0           | 0          | 0.22145   |
| Up         | F13A1   | 1      | 0           | 0          | 0.201558  |
| Up         | ABI3BP  | 1      | 0           | 0          | 0.18668   |
| Up         | NDUFAF3 | 1      | 0           | 0          | 0.193485  |
| Up         | APOD    | 1      | 0           | 0          | 0.201401  |
| Up         | UBL5    | 1      | 0           | 0          | 0.242915  |
| Up         | MFAP5   | 1      | 0           | 0          | 0.160727  |
| Up         | RAB4A   | 1      | 0           | 0          | 0.234402  |
| Up         | SNAPIN  | 1      | 0           | 0          | 0.232493  |
| Up         | UROS    | 1      | 0           | 0          | 0.212264  |
| Up         | PLTP    | 1      | 0           | 0          | 0.201401  |
| Up         | VPS28   | 1      | 0           | 0          | 0.234402  |
| Up         | EIF3L   | 1      | 0           | 0          | 0.229656  |
| Up         | NUDT16  | 1      | 0           | 0          | 0.237835  |
| Up         | BLOC1S1 | 1      | 0           | 0          | 0.256619  |
| Down       | ESR1    | 250    | 0.13227     | 1.07E+08   | 0.34572   |
| Down       | LCK     | 209    | 0.099202    | 9.97999E+4 | 0.328259  |
| Down       | STIP5   | 174    | 0.006652    | 8.092966   | 0.28169   |
| Down       | CCL28   | 174    | 0.003314    | 5.208440   | 0.274992  |
| Down       | CTNNB1  | 106    | 0.042469    | 4.2968374  | 0.310275  |
| Down       | KNTC1   | 101    | 0.02909     | 8.0024314  | 0.25565   |
| Down       | NGFR    | 100    | 0.036602    | 6.3182380  | 0.318388  |
| Regulation | Node | Degree | Betweenness | Stress   | Closeness |
|-----------|------|--------|-------------|----------|-----------|
| Down      | TIAM2| 95     | 0.021154    | 62011932 | 0.265317  |
| Down      | OBSCN| 86     | 0.010365    | 36096562 | 0.247534  |
| Down      | PLD1 | 73     | 0.033394    | 17246708 | 0.30421   |
| Down      | CALCR| 71     | 0.002592    | 2172836  | 0.25466   |
| Down      | PTGDR| 70     | 0.002067    | 1966748  | 0.25524   |
| Down      | FASLG| 69     | 0.019813    | 7248566  | 0.301244  |
| Down      | NEDD4L| 68    | 0.023391    | 18526624 | 0.311841  |
| Down      | GLI3 | 68     | 0.017183    | 7308126  | 0.2879    |
| Down      | CEP135| 65    | 0.013666    | 21907982 | 0.267857  |
| Down      | SDCCAG8| 65   | 0.013666    | 21907982 | 0.267857  |
| Down      | ARHGAP39| 61   | 0.006186    | 28702190 | 0.252655  |
| Down      | TNKS | 60     | 0.004206    | 3754360  | 0.297842  |
| Down      | CHEK2| 60     | 0.016237    | 7289936  | 0.307601  |
| Down      | GLI2 | 58     | 0.012509    | 5073890  | 0.282922  |
| Down      | ENTPD5| 57    | 0.005964    | 2743528  | 0.250015  |
| Down      | FAM13B| 56    | 0.004818    | 23506158 | 0.251122  |
| Down      | ARHGAP22| 56   | 0.004818    | 23506158 | 0.251122  |
| Down      | LAMAS| 52     | 0.00723     | 12112106 | 0.258896  |
| Down      | SYNJ2| 52     | 0.010084    | 13175450 | 0.253879  |
| Down      | BCL2L11| 52   | 0.016836    | 10636772 | 0.313969  |
| Down      | ALOX15| 49    | 0.008753    | 5227944  | 0.244161  |
| Down      | FGFR3| 49     | 0.011331    | 25238840 | 0.255587  |
| Down      | GEMIN5| 47    | 0.001525    | 2623902  | 0.254973  |
| Down      | FAS  | 47     | 0.009545    | 4674560  | 0.293347  |
| Down      | PDE4B| 46     | 0.005717    | 3857072  | 0.252024  |
| Down      | KCNC1| 45     | 0.011352    | 17732982 | 0.215233  |
| Down      | TAF4B| 45     | 0.004747    | 2685668  | 0.266101  |
| Down      | TOP3A| 45     | 0.011616    | 46078906 | 0.231272  |
| Down      | TFF1 | 43     | 0.001327    | 799808   | 0.270889  |
| Down      | KCNQ4| 42     | 0.027528    | 31515762 | 0.226874  |
| Down      | MUC17| 42     | 0.019711    | 25257640 | 0.157086  |
| Down      | NTN1 | 40     | 0.012007    | 10110832 | 0.266152  |
| Down      | COL8A1| 38    | 0.013121    | 17046748 | 0.212558  |
| Down      | MMRN1| 38     | 0.011827    | 11815884 | 0.252424  |
| Down      | EPHA4| 38     | 0.003668    | 1364992  | 0.273358  |
| Down      | MEN1 | 35     | 0.006804    | 6211974  | 0.272297  |
| Down      | GOSR1| 34     | 0.008526    | 393382   | 0.285714  |
| Down      | SKA1 | 32     | 0.002897    | 9913724  | 0.243129  |
| Down      | VTI1A| 31     | 0.007925    | 4181998  | 0.286386  |
| Down      | SYNE2| 30     | 0.007519    | 9249888  | 0.228212  |
| Down      | ITH4 | 26     | 0.007262    | 1264158  | 0.272297  |
| Down      | LCAT | 26     | 0.005782    | 971078   | 0.252178  |
| Down      | TRIB3| 24     | 0.002492    | 6242350  | 0.248261  |
| Down      | TRAF5| 24     | 0.00684     | 5476244  | 0.212395  |
| Regulation | Node    | Degree | Betweenness | Stress     | Closeness |
|------------|---------|--------|-------------|------------|-----------|
| Down       | DNAH3   | 24     | 0.007222    | 8565654    | 0.215524  |
| Down       | PFA5    | 24     | 0.014988    | 8957870    | 0.288321  |
| Down       | SNPH    | 24     | 0.00801     | 1545944    | 0.224536  |
| Down       | GADD45G | 20     | 0.003326    | 13947820   | 0.23265   |
| Down       | SFRP5   | 20     | 0.006774    | 5015824    | 0.211927  |
| Down       | SPTB    | 20     | 0.0063      | 11636704   | 0.233978  |
| Down       | KAT7    | 18     | 0.003392    | 3121342    | 0.235683  |
| Down       | EPHB4   | 18     | 0.001434    | 905566     | 0.260034  |
| Down       | DNAH5   | 17     | 0.002849    | 3119232    | 0.205745  |
| Down       | HIVEP1  | 17     | 0.005211    | 11367638   | 0.232859  |
| Down       | DNAJC18 | 16     | 0.004758    | 4513144    | 0.228489  |
| Down       | SCNN1D  | 16     | 0.001683    | 759732     | 0.291057  |
| Down       | ZBP1    | 16     | 0.0032      | 6672792    | 0.233555  |
| Down       | RAP1GAP2| 15     | 0.003133    | 3581154    | 0.225872  |
| Down       | SARDH   | 15     | 0.005309    | 5233702    | 0.183926  |
| Down       | PPRI2   | 15     | 0.006081    | 1607396    | 0.285281  |
| Down       | MYO10   | 15     | 0.004221    | 1310316    | 0.235723  |
| Down       | ABCB4   | 14     | 5.9E-04     | 1308566    | 0.22748   |
| Down       | SPN     | 13     | 0.003074    | 2890384    | 0.244522  |
| Down       | ERO1A   | 13     | 0.004612    | 1425588    | 0.287161  |
| Down       | LPXN    | 13     | 0.002728    | 4156340    | 0.242304  |
| Down       | CD82    | 12     | 0.001605    | 269660     | 0.279107  |
| Down       | CFP     | 12     | 0.004063    | 2388686    | 0.220588  |
| Down       | CDC20B  | 12     | 7.24E-04    | 204270     | 0.243429  |
| Down       | EREG    | 11     | 0.00292     | 4060738    | 0.221568  |
| Down       | GABRA4  | 11     | 0.004826    | 844010     | 0.188362  |
| Down       | STK36   | 10     | 5.16E-06    | 1410       | 0.231582  |
| Down       | CLDN1   | 10     | 0.002949    | 1746194    | 0.196311  |
| Down       | SCAF4   | 10     | 0.003013    | 2202372    | 0.198039  |
| Down       | MFAP3   | 9      | 0.003861    | 3290616    | 0.191498  |
| Down       | PPT2    | 9      | 0.003141    | 1767236    | 0.210548  |
| Down       | NPHP4   | 9      | 0.003191    | 1398268    | 0.215199  |
| Down       | ABI3    | 8      | 0.001847    | 2267982    | 0.229515  |
| Down       | BATF3   | 8      | 6.07E-04    | 393104     | 0.226291  |
| Down       | KLC2    | 8      | 0.00245     | 1875364    | 0.222485  |
| Down       | GRK4    | 8      | 5.59E-04    | 417560     | 0.222533  |
| Down       | ARNTL2  | 7      | 0.001968    | 1639758    | 0.209684  |
| Down       | PPP1R3B | 7      | 0.002897    | 149078    | 0.211732  |
| Down       | IFT122  | 7      | 0.001127    | 614274     | 0.187492  |
| Down       | AHSPP   | 7      | 0.002414    | 2877402    | 0.186226  |
| Down       | ASIC3   | 7      | 0.001895    | 501964     | 0.235361  |
| Down       | WDR19   | 7      | 0.001287    | 982818     | 0.187492  |
| Down       | MGAM    | 7      | 0.002455    | 406020     | 0.181388  |
| Down       | STXBP5  | 6      | 0.00108     | 832104     | 0.196993  |
PDB code 3OW2 respectively (Table 7). The molecule ETE and LNG has highest binding score its interaction with protein 4IP8 and hydrogen bonding and other bonding interactions with amino acids are depicted by 3D (Fig. 8) and 2D (Fig. 9).

**Table 5** Topology table for up and down regulated genes (Continued)

| Regulation | Node | Degree | Betweenness | Stress | Closeness |
|------------|------|--------|-------------|--------|-----------|
| Down       | SLC30A7 | 6      | 0.002041   | 1162028 | 0.211776  |
| Down       | NRL    | 6      | 0.001496   | 1056416 | 0.226267  |
| Down       | KCNMB4 | 5      | 0.001932   | 644596  | 0.189223  |
| Down       | SPAST  | 5      | 0.001932   | 3015012 | 0.17458   |
| Down       | MTF1   | 5      | 8.87E-04   | 1194192 | 0.226862  |
| Down       | FOXP2  | 5      | 0.001059   | 361276  | 0.219804  |
| Down       | IWS1   | 4      | 4.91E-04   | 486888  | 0.21999   |
| Down       | LRSAM1 | 4      | 4.66E-04   | 327264  | 0.292456  |
| Down       | ECD    | 4      | 3.00E-08   | 6       | 0.246517  |
| Down       | RFX3   | 3      | 9.66E-04   | 260498  | 0.235696  |
| Down       | IPP    | 3      | 4.83E-04   | 255296  | 0.199412  |
| Down       | ERG    | 3      | 4.001157   | 1993680 | 0.288401  |
| Down       | SLC9A7 | 2      | 4.83E-04   | 102030  | 0.189205  |
| Down       | KBTBD7 | 2      | 4.83E-04   | 116108  | 0.284029  |
| Down       | CARD14 | 2      | 2.67E-05   | 3564    | 0.219501  |
| Down       | ANKRD6 | 2      | 3.60E-07   | 366     | 0.212744  |
| Down       | ZFYVE1 | 2      | 4.83E-04   | 123952  | 0.234428  |
| Down       | LNX2   | 1      | 0          | 0       | 0.215132  |
| Down       | ATL1   | 1      | 0          | 0       | 0.148637  |
| Down       | LMO7   | 1      | 0          | 0       | 0.195467  |

**Discussion**

PCOS is a most prevalent endocrine disorder with hyperandrogenism and chronic anovulation [29]. If not treated promptly and effectively, PCOS can seriously reduce the quality of life. There is no doubt that considerate syndrome at the molecular level will help to develop their diagnosis and treatment [30]. Up to now, various biomarkers have been identified to be linked with PCOS and might be selected as therapeutic targets, but the detailed mechanism of gene regulation leading to syndrome advancement remains elusive [31].

In our investigation, we aimed to identify biomarkers of PCOS and uncover their biological functions through bioinformatics analysis. Dataset GSE84958 was selected as expression profiling by high throughput sequencing dataset in our analysis. As a result, 360 up regulated and 379 down regulated genes were identified.
genes at least 4-fold change between PCOS and normal control samples were screened out. ABI3BP protein expression in heart tissue was significantly related with cardiovascular disease [32], but this gene might be liable for progression of PCOS. Romo-Yáñez et al [33] have revealed the expression of BNIP3 was linked with diabetic in pregnancies, but this gene might be responsible for progression of PCOS. F13A1 is an essential regulatory factor to be associated in PCOS development [34]. An investigation has reported that the ITIH4 can promote non-alcoholic fatty liver disease [35], but this gene might be important for progression of PCOS. Da et al [36] have suggested that the TET3 is an important role in controlling type 2 diabetes progressions, but this gene might be key role in PCOS.

The GO and pathway enrichment analysis was of great importance for interpreting the molecular mechanisms of the key cellular activities in PCOS. RP55 [37], RBM3 [38], BAK1 [39], NDUFC2 [40], NDUFS4 [41], NDUFS5 [42], UQCRFS1 [43], COX6B1 [44], NDUFA13 [45], PRMT1 [46], RDX (radixin) [47], EPHB4 [48], SYNE2 [49], DNAH5 [50], NEDD4L [51], PDE4B [52] and CTNND1 [53] plays a critical role in the process of cardiovascular disease, but these genes might be linked with development of PCOS. Ostergaard et al [54], Zi et al [55], Kunej et al [56], Van der Schueren et al [57], Jin et al [58], Emdad et al [59], Liu et al [60], Scherag et al [61], Shi and Long [62], Sharma et al [63], Parente et al [64], Saint-Laurent et al [65] and Lee [66] demonstrated that over expression of COA3, PHB (prohibitin),
### Table 6 miRNA - target gene and TF - target gene interaction

| Regulation | Target Genes | Degree | MicroRNA | Regulation | Target Genes | Degree | TF |
|------------|--------------|--------|----------|------------|--------------|--------|----|
| Up         | RPL13A       | 97     | hsa-mir-8067 | Up         | RBX1         | 15     | PER3 |
| Up         | RPS15A       | 95     | hsa-mir-4518 | Up         | RPS15        | 13     | CTCF |
| Up         | RPLP0        | 71     | hsa-mir-3685 | Up         | RPS20        | 12     | E2F7 |
| Up         | ADCY6        | 65     | hsa-mir-1202 | Up         | ADCY6        | 11     | LMO2 |
| Up         | RPS29        | 48     | hsa-mir-4461 | Up         | POLR2K       | 9      | POLR2H |
| Up         | RPL30        | 47     | hsa-mir-6811-5p | Up      | RPS29        | 5      | JUN  |
| Up         | RPL35        | 44     | hsa-mir-2278 | Up         | RPL35        | 8      | MYB  |
| Up         | RPS23        | 40     | hsa-mir-4282 | Up         | RPS23        | 5      | USF1 |
| Up         | SAA1         | 33     | hsa-mir-4701-3p | Up     | RPL13A       | 5      | NFIY |
| Up         | RPSS         | 29     | hsa-mir-1301-3p | Up     | RPS29        | 5      | JUN  |
| Up         | RBX1         | 29     | hsa-mir-5187-3p | Up     | POLR2J       | 5      | POLR2C |
| Up         | RPS20        | 26     | hsa-mir-708-5p | Up         | RPL30        | 4      | IRF7 |
| Up         | RPS15        | 23     | hsa-mir-1260b | Up         | RPLP0        | 3      | SMAD3 |
| Up         | POLR2K       | 13     | hsa-mir-5680 | Up         | RPSS         | 3      | GABPA |
| Up         | POLR2J       | 9      | hsa-mir-129-2-3p | Up     | SAA1         | 2      | CEBP8 |
| Down       | CTNNND1      | 129    | hsa-mir-8082 | Up         | PHB2         | 1      | PHB2 |
| Down       | ESR1         | 98     | hsa-mir-4422 | Down       | ESR1         | 122    | NCOA2 |
| Down       | NEDD4L       | 76     | hsa-mir-548am-5p | Down | LCK          | 21     | EBF1 |
| Down       | KNTC1        | 62     | hsa-mir-6886-3p | Down     | GLI3         | 18     | SMAD2 |
| Down       | NGFR         | 56     | hsa-mir-9500 | Down       | NEDD4L       | 17     | JUND |
| Down       | TIAM2        | 49     | hsa-mir-3679-5p | Down     | CALCR        | 15     | FOXO3 |
| Down       | GLI3         | 33     | hsa-mir-1913 | Down       | NGFR         | 10     | POU2F1 |
| Down       | FASLG        | 25     | hsa-mir-7849-3p | Down     | FASLG        | 8      | DAXX |
| Down       | PLD1         | 22     | hsa-mir-3200-3p | Down     | CTNNND1      | 7      | GATA1 |
| Down       | OBSCN        | 19     | hsa-mir-3657 | Down       | KNTC1        | 5      | SP1  |
| Down       | CALCR        | 12     | hsa-mir-4735-5p | Down     | PLD1         | 4      | E2F1 |
| Down       | PTGDR        | 11     | hsa-mir-4477b | Down       | TIAM2        | 4      | FOXD1 |
| Down       | CCL28        | 8      | hsa-mir-770-5p | Down       | GLI2         | 1      | GLI3 |
| Down       | LCK          | 6      | hsa-mir-520c-3p | Down     | ERG          | 1      | ESR1 |
| Down       | S1PRS        | 4      | hsa-mir-31-5p | Down       | PTGDR        | 1      | RORA |
|            |              |        |           | Down       | CCL28        | 1      | FOS  |
|            |              |        |           | Down       | OBSCN        | 1      | CUX1 |
UQCRC1, COX4I1, IFI27, MTDH (metadherin), S100A16, SDCCAG8, GLI2, NTN1, NLGN2, FGFR3 and PTPRN2 could cause obesity, but these genes might be involved in progression of PCOS. Alsters et al [67], Lee et al [68], Shiffman et al [69], Yaghootkar et al [70], Rotroff et al [71], Cheng et al [72], Baig et al [73], Zhang et al [74], Lebaillly et al [75], Ferris et al [76], Lempainen et al [77] and McCallum et al [78] presented that high expression of CPE (carboxypeptidase E), RPL13A, CERS2, CCND2, PRPF31, SARM1, PLD1, EPHA4, ARNTL2, BATF3, IKZF4 and MEN1 were associated with diabetes, but these genes might be linked with advancement of PCOS. Wang et al [79], Tian et al [80], Zhang et al [81] and Carr et al [82] demonstrated that over expression of ATP6AP2, FIS1, GRK4 and KCNQ4 were found to be substantially related to hypertension, but these genes might be essential for PCOS progression. Atiomo et al [83], Lara et al [84] and Douma et al [85] were reported that NQO1, NGFR (nerve growth factor receptor) and ESR1 could be an index for PCOS. Jin et al [86] presented that GLI3 was associated with non-alcoholic fatty liver disease, but this gene might be linked with development of PCOS.
In the present investigation, PPI network and its modules has been shown that significant amount of hub gene might be associated with progression of PCOS. Zhang et al [87] proposed that SAA1 was linked with progression of obesity, but this gene might be important for progression of PCOS. Deng et al [88] indicated that ADCY6 was responsible for development of cardiovascular disease, but this gene might be associated with advancement of PCOS. POLR2K, RPS15, RPS15A, ESR1, LCK (LCK proto-oncogene, Src family tyrosine kinase), S1PR5, CCL28, CTNND11, UQCRQ (ubiquinol-cytochrome c reductase complex III subunit VII), UQCRH (ubiquinol-cytochrome c reductase hinge protein), COX7C, COX6C, COX8A, COX5B, COX6A1, COX7A2L, ARHGAP39, OBSCN (obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF) and TIAM2 might be novel biomarkers for PCOS.

MiRNA-target genes and TF-target genes regulatory networks revealed that the miRNAs, TF and target genes were might be involved in PCOS. Hsa-mir-6886-3p was liable for progression of hypertension [89], but this gene might be involved in progression of PCOS. Some investigations determined that expression of PER3 [90] and SMAD2 [91] were associated with diabetes, but these genes might be linked with advancement of PCOS. NCOA2 was found to be associated with advancement of obesity [92], but this gene might be involved in progression of PCOS. Recently, increasing evidence demonstrated that EBF1 was expressed in coronary artery disease [93], but this gene might be responsible for progression of PCOS. FOXO3 was involved in progression of PCOS [94]. RPLP0, RPS29, KNTC1, hsa-mir-8067, hsa-mir-4518, hsa-mir-3685, hsa-mir-1202, hsa-mir-4461, hsa-mir-8082, hsa-mir-4422, hsa-mir-4518, hsa-mir-9500, RBX1, RPS20, CALCR (calcitonin receptor), CTCF (CCCTC-binding factor), E2F7, LMO2, POLR2H and JUND (jun D proto-oncogene) might be novel biomarkers for PCOS.

Among all three of molecules of ethinylestradiol, levo-norgestrel and desogestrel respectively, ethinylestradiol has obtained highest binding score (c-score) of 9.943 with protein of PDB code 1G1X and obtained 8.260, 8.223 and 8.019 with protein of PDB 1G1X, 3OW2 and 1LE9 respectively. The phenolic -OH group in ring A of ethinylestradiol formed favourable bonding interactions with ALA-14 of Chain A and pi-pi bonding interactions of alicyclic ring B TRP-18. Ethinylestradiol also formed alkyl and pi-alkyl interaction of ring B, C and D with TRP-18, ARG-62, TYR-21, PHE-69, ILE-65 and ILE-58. Ethinylestradiol also formed Van der Waals interactions with ACA-61, MET-17, MET-24 and GLN-66 respectively. It is assumed that the highest binding score (c-score) of ethinylestradiol is due to the presence of aromatic ring and the phenolic – OH group.

In conclusion, we used a series of bioinformatics analysis methods to find the crucial genes and pathways associated in PCOS initiation and development from expression profiling by high throughput sequencing containing PCOS samples and normal control samples. Our investigations provide a more specific molecular mechanism for the advancement of PCOS, detail information on the potential biomarkers and therapeutic targets. However, the interacting mechanism and function of genes need to be confirmed in further experiments.

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Informed consent
No informed consent because this study does not contain human or animals participants.

Authors’ contributions
Praveenkumar Devarbhai - Investigation and resources. Lata Telang - Writing original draft and investigation. Basavaraj Vastrad - Writing original draft, and review and editing. Anandkumar Tengli - Investigation, and review and editing. Chanabasayya Vastrad - Software and investigation. Iranna Kotturshetti - Supervision and resources. The authors read and approved the final manuscript.

Availability of data and materials
The datasets supporting the conclusions of this article are available in the GEO (http://www.ncbi.nlm.nih.gov/geo) repository. ([GSE84958] (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE84958))
Ethics approval and consent to participate
This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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