

Transcriptomic analysis reveals that prolactin modulates the  $\beta$ -casein synthesis mechanism in bovine mammary epithelial cells

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SUPPLEMENTARY FILE

1

## **Materials and Methods**

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### **Ethical Compliance**

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The Northeast Agricultural University's Animal Experimentation Committee (approval number: 2019-2; Harbin, China) gave its approval to all animal experiments, which were conducted in accordance with the approved policies and procedures.

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### **Cell culture and treatments**

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The primary BMECs were isolated from the mammary gland tissues of three female lactating Holstein cows, as referred to in the previous study. (Lin et al., 2018). All of the cows were in their third parity and 90 DIM. The three cows were healthy and had SCCs of 50,000 cells/mL. Primary BMECs were cultured in DMEM/F 12 (Life Technologies, Carlsbad, CA) medium supplemented with 10% FBS (Life Technologies), 100 U/mL penicillin, 100 mg/mL streptomycin, 5  $\mu$ g/mL insulin, and 1  $\mu$ g/mL hydrocortisone (Sigma-Aldrich). After three passages, pure mammary epithelial cells were obtained. The expression of endogenous cytokeratin 18 (CK18) was measured in cells by immunofluorescence to detect the cell purity. Cells were grown on glass cover-slips in six-well plates until they reached 40%-60% confluence and then fastened with 4% formaldehyde at 4°C for 10 min. Then it was incubated in blocking buffer for 1 h at 37°C. After that, it was incubated with the anti-CK18 primary antibody (Abbiotec, San Diego, USA) for overnight at 4°C. Following that, they were each incubated with FITC-conjugated secondary antibodies (ABclonal, Wuhan, China) for 1 h at 37°C and DAPI for 15 min at 37°C in the dark room. Finally, the fluorescence signal of the cover-slips with cells was observed by a confocal laser scanning microscope. The above experimental results were repeated three times. To observe the effects of PRL on purified cells, the culture medium was changed to serum-free medium and stimulated with

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23 5µg/mL PRL, as previously reported (Zhou et al., 2020) for 24 h. Then cells were used for further  
24 detection.

### 25 **Western blot analysis**

26 The mammary epithelial cells were collected in lysis buffer (Beyotime, Shanghai, China) by  
27 ultrasonication. Use the BCA assay kit (Beyotime, Shanghai, China) to assess the total protein  
28 concentration according to the manufacturer's instructions. Then the protein samples with the  
29 loading buffer were boiled for 10 min at 100°C. Also, 8–10% SDS polyacrylamide separation gel  
30 electrophoresis was used to separate equal amounts of lysates in each lane. The proteins were  
31 transferred onto nitrocellulose membranes and incubated with the anti-β-casein primary antibodies  
32 (Bioss, Beijing, China), followed by incubation with HRP-conjugated secondary antibodies  
33 (Biosharp, Hefei, China). Finally, the protein bands were observed through the ECL reagent and  
34 quantified by Image-Pro Plus, with β-actin as a loading control. The effect of PRL on β-casein  
35 expression was studied using a two-tailed unpaired t-test. A significant difference was defined as *P*  
36 < 0.05.

### 37 **RNA isolation and analysis**

38 The Magen HiPure Total RNA Mini Kit (Magen, Guangzhou, China) was used to extract  
39 total RNA from BMECs. A NanoDrop 2000 (Thermo Scientific, Wilmington, DE) was used to  
40 evaluate the RNA samples, and a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) was  
41 used to determine the RNA quality. In this investigation, all of the RNA samples had an integrity  
42 value greater than 8.

### 43 **Sequencing and data analysis**

44 According to the manufacturer's instructions, total RNA (5 µg) was prepared for RNA-seq  
45 transcriptome libraries by the TruSeq RNA Sample Preparation Kit (Illumina, San Diego, CA).  
46 mRNA was isolated according to the standard poly (A) selection method by oligo (dT) beads and  
47 the fragmentation was performed in fragmentation buffer. Then use a SuperScript Double-stranded  
48 cDNA Synthesis Kit (Invitrogen, California, USA) with random hexamer primers (Illumina, San  
49 Diego, CA) to synthesize cDNA. It is necessary for the obtained cDNA to undergo end-repair,  
50 phosphorylation, and A-base addition at the 3' end. The cDNA target fragments (200-300 bp) were  
51 selected in libraries using 2% ultra agarose, followed by 15 cycles of PCR amplification. Finally,  
52 quantitative analysis was carried out using a TBS380 fluorometer (American Instrument Exchange,  
53 Inc., Haverhill, MA), and the paired-end RNA-seq libraries were sequenced on an Illumina HiSeq  
54 4000 (2 • 150 read lengths).

55 The sequenced data were compared to the bovine reference genome (UMD3.1), and the  
56 FPKM principle was used to analyze the expression of each transcript (Dewey and Li, 2011). The  
57 R language software EdgeR was used to select genes with differential expression plicity above  
58 2-fold (Robinson et al., 2009). Also, Goatools (<https://github.com/tanghaibao/Goatools>) and  
59 KOBAS 3.0 (<http://kobas.cbi.pku.edu.cn/index.php>) were used to perform Gene Ontology (GO)  
60 enrichment analysis of differentially expressed genes as well as KEGG signaling pathway  
61 enrichment analysis.

### 62 **The validation of RNA-seq results**

63 Eight genes (four up-regulated genes and four down-regulated genes) were randomly selected  
64 from the obtained differentially expressed genes for qRT-PCR to verify the accuracy of the  
65 sequencing results. Total RNA (1 µg) was used to synthesize the cDNA by the reverse

66 transcription system (TaKaRa, Tokyo, Japan). The final volumes of 20  $\mu$ l were subjected to  
67 qRT-PCR using an ABI PRISM 7300 Real-Time PCR System (Applied Biosystems, Foster City,  
68 CA, USA). The qPCR data were normalized using the  $2^{-\Delta\Delta C_t}$  method with  *$\beta$ -actin* as loading  
69 controls.  
70

71 **Supplementary Table S1 The up-regulated genes in PRL-stimulated cells compared to**  
72 **control cells**

Gene name	Gene description	Log2FC (PRL/Control)	P-value	FDR
	-	8.23	3.12194E-32	1.86034E-30
<i>bta-mir-2904-3</i>	bta-mir-2904-1	6.94	0.002861182	0.005508666
<i>bta-mir-374b</i>	bta-mir-374b	5.72	1.0109E-07	6.82074E-07
<i>BCL6B</i>	B-cell CLL/lymphoma 6B	5.48	6.51443E-33	3.96033E-31
<i>PRND</i>	prion like protein doppel	4.83	0.000116349	0.000360395
<i>SCUBE3</i>	signal peptide, CUB domain and EGF like domain containing 3	4.20	0.002738663	0.00531015
<i>NME5</i>	NME/NM23 family member 5	4.03	0.005255601	0.009084519
<i>APBA1</i>	amyloid beta precursor protein binding family A member 1	4.03	0.005255601	0.009084519
<i>FBXO24</i>	F-box protein 24	3.91	0.008008863	0.012829788
<i>EGFL8</i>	EGF like domain multiple 8	3.83	0.01016534	0.015599267
<i>IL17REL</i>	interleukin 17 receptor E like	3.83	0.01016534	0.015599267
<i>FGF9</i>	fibroblast growth factor 9	3.83	0.01016534	0.015599267
<i>IDO1</i>	indoleamine 2,3-dioxygenase 1	3.73	0.000523333	0.001323152
<i>PARK2</i>	E3 ubiquitin-protein ligase parkin	3.61	0.019830803	0.026641098
<i>LAPTM5</i>	lysosomal protein transmembrane 5	3.49	0.001861289	0.003818067
<i>FGFR4</i>	fibroblast growth factor receptor 4	3.49	0.001861289	0.003818067
<i>PRKG1</i>	protein kinase, cGMP-dependent, type I	3.49	0.001861289	0.003818067
<i>ZC2HC1C</i>	zinc finger C2HC-type containing 1C	3.35	0.003518276	0.006489372
	-	3.35	0.003518276	0.006489372
<i>ADAMTSL2</i>	ADAMTS-like protein 2 precursor	3.35	0.003518276	0.006489372
<i>LOC100848478</i>	60S ribosomal protein L7a pseudogene	3.35	0.039053421	0.045480624
<i>ANKRD34A</i>	ankyrin repeat domain 34A	3.35	0.039053421	0.045480624
<i>PDE6G</i>	phosphodiesterase 6G	3.35	0.039053421	0.045480624
<i>MCOLN2</i>	mucolipin 2	3.20	0.000124313	0.000380947
<i>NOBOX</i>	NOBOX oogenesis homeobox	3.20	0.006657768	0.010975053
	-	3.14	0.008091622	0.012934822
<i>ATP12A</i>	ATPase H+/K+ transporting non-gastric alpha2 subunit	3.03	0.00041868	0.001091782
<i>PTPN7</i>	tyrosine-protein phosphatase non-receptor type 7	3.03	0.012606824	0.018578463
<i>PLCLI</i>	phospholipase C like 1	3.03	0.012606824	0.018578463
<i>C14orf37</i>	chromosome 14 open reading frame 37	3.03	0.012606824	0.018578463
<i>PSTPIP1</i>	proline-serine-threonine phosphatase interacting protein 1	3.03	0.012606824	0.018578463
<i>AMY2B</i>	amylase, alpha 2B	2.95	1.63234E-06	8.3398E-06
<i>GHR</i>	growth hormone receptor	2.83	0.001398378	0.003016547
<i>HOGA1</i>	4-hydroxy-2-oxoglutarate aldolase 1	2.83	0.001398378	0.003016547

<i>LOC782061</i>	aldo-keto reductase family 1, member C1-like	2.83	0.023871199	0.030936549
<i>SBSN</i>	suprabasin	2.83	0.023871199	0.030936549
<i>ARTN</i>	artemin	2.83	0.023871199	0.030936549
<i>KCNQ5</i>	potassium voltage-gated channel subfamily Q member 5	2.83	0.023871199	0.030936549
<i>TEX14</i>	testis expressed 14, intercellular bridge forming factor	2.83	0.023871199	0.030936549
<i>STYXL1</i>	serine/threonine/tyrosine interacting like 1	2.61	0.000521185	0.001319077
<i>CDH16</i>	cadherin 16	2.49	0.008321842	0.013232547
<i>ZBTB7C</i>	zinc finger and BTB domain containing 7C	2.49	0.008321842	0.013232547
<i>CACNA1A</i>	voltage-dependent P/Q-type calcium channel subunit alpha-1A	2.49	0.008321842	0.013232547
<i>LIFR</i>	LIF receptor alpha	2.44	0.001634241	0.003416365
<i>GNAZ</i>	G protein subunit alpha z	2.44	0.001634241	0.003416365
<i>CD300LG</i>	CD300 molecule like family member g	2.43	0.013249101	0.019326224
<i>MTX3</i>	metaxin 3	2.35	0.000118794	0.000367213
<i>C8H9orf152</i>	uncharacterized protein C9orf152 homolog	2.35	0.002872022	0.005525999
	-	2.35	0.002872022	0.005525999
<i>ASIC1</i>	acid sensing ion channel subunit 1	2.35	0.014930149	0.021227035
<i>WDR27</i>	WD repeat domain 27	2.35	0.014930149	0.021227035
<i>SYT17</i>	synaptotagmin 17	2.35	0.014930149	0.021227035
	-	2.27	0.000997108	0.002284398
<i>CHI3L2</i>	chitinase 3 like 2	2.25	0.005017023	0.008742056
<i>CYB5D1</i>	cytochrome b5 domain-containing protein 1	2.25	0.005017023	0.008742056
<i>IQCG</i>	IQ motif containing G	2.20	0.000122766	0.000377092
<i>U6</i>	U6 spliceosomal RNA	2.20	0.001713953	0.003560739
	-	2.20	0.001713953	0.003560739
<i>ABAT</i>	4-aminobutyrate aminotransferase	2.20	0.02660027	0.033573571
<i>HIST2H2AB</i>	histone cluster 2 H2A family member b	2.20	0.02660027	0.033573571
<i>JAZF1</i>	JAZF zinc finger 1	2.20	0.02660027	0.033573571
<i>ROBO1</i>	roundabout guidance receptor 1	2.20	0.02660027	0.033573571
	-	2.20	0.02660027	0.033573571
<i>STAR</i>	steroidogenic acute regulatory protein	2.16	0.000592974	0.001469862
<i>SLC25A40</i>	solute carrier family 25 member 40	2.14	0.000207132	0.000592786
<i>HOXB7</i>	homeobox B7	2.14	0.008703763	0.013752635
<i>GPD1</i>	glycerol-3-phosphate dehydrogenase 1	2.05	9.11784E-07	4.95717E-06
	-	2.03	0.01498013	0.021278791
	Complement factor D	1.95	0.009723765	0.01502894
<i>TIGD7</i>	tigger transposable element derived 7	1.93	0.008358173	0.01327979

<i>BCL11B</i>	B-cell CLL/lymphoma 11B	1.92	0.000549149	0.001374819
<i>RAB39B</i>	RAB39B, member RAS oncogene family	1.90	0.001588574	0.00334296
<i>LCA5</i>	LCA5, lebercilin	1.90	0.025546266	0.032643474
<i>TXK</i>	tyrosine-protein kinase TXK	1.90	0.025546266	0.032643474
	-	1.85	0.007403506	0.011982644
<i>TBC1D4</i>	TBC1 domain family member 4	1.83	0.013944425	0.020127053
<i>ARHGEF9</i>	Cdc42 guanine nucleotide exchange factor 9	1.83	0.013944425	0.020127053
<i>DCHS2</i>	dachsous cadherin-related 2	1.83	0.013944425	0.020127053
<i>PRRT3</i>	proline rich transmembrane protein 3	1.76	0.043100803	0.0490829
<i>HHATL</i>	hedgehog acyltransferase-like	1.76	0.043100803	0.0490829
<i>AOX4</i>	Bos taurus aldehyde oxidase 1-like (AOX4), mRNA.	1.76	0.043100803	0.0490829
<i>TNNC1</i>	troponin C1, slow skeletal and cardiac type	1.76	0.043100803	0.0490829
<i>ZBTB20</i>	zinc finger and BTB domain containing 20	1.70	0.012472314	0.018434405
	-	1.67	0.001952837	0.003978737
	-	1.67	0.001347021	0.002929916
	-	1.67	0.002079618	0.004204308
<i>GPR155</i>	G protein-coupled receptor 155	1.66	0.001157358	0.002583674
	-	1.61	0.010895403	0.016509119
	-	1.61	0.020114674	0.026934575
<i>COL9A2</i>	collagen type IX alpha 2 chain	1.61	0.020114674	0.026934575
<i>RFTN2</i>	raftlin family member 2	1.61	0.037633041	0.044246
<i>SERPINB13</i>	serpin family B member 13	1.61	0.037633041	0.044246
<i>KDM7A</i>	lysine demethylase 7A	1.57	0.000491787	0.001252043
<i>TRERF1</i>	transcriptional regulating factor 1	1.56	0.001576329	0.003320676
<i>TNFSF15</i>	TNF superfamily member 15	1.55	7.59266E-05	0.000247947
<i>KCNJ10</i>	potassium voltage-gated channel subfamily J member 10	1.55	0.005142735	0.008927505
<i>SPAG4</i>	sperm associated antigen 4	1.54	0.009376349	0.014566795
<i>ZC4H2</i>	zinc finger C4H2-type containing	1.54	3.55812E-07	2.14146E-06
<i>CAP2</i>	CAP, adenylate cyclase-associated protein, 2 (yeast)	1.51	0.032085613	0.03900383
<i>SGTB</i>	small glutamine rich tetratricopeptide repeat containing beta	1.49	0.007988752	0.0128078
<i>NUDT13</i>	nudix hydrolase 13	1.47	0.014616582	0.020885617
<i>CYP7B1</i>	cytochrome P450 family 7 subfamily B member 1	1.44	0.006759415	0.011109144
<i>PIH1D2</i>	PIH1 domain containing 2	1.44	0.006759415	0.011109144
<i>RGS14</i>	regulator of G protein signaling 14	1.43	0.000837074	0.001968717
	-	1.43	0.000703474	0.001701035

	-	1.42	0.003161015	0.005980709
<i>SLC37A2</i>	solute carrier family 37 member 2	1.42	0.003161015	0.005980709
	-	1.42	0.012299098	0.018227626
<i>TEK</i>	TEK receptor tyrosine kinase	1.42	0.012299098	0.018227626
<i>ADAMTSL3</i>	ADAMTS like 3	1.39	0.022556261	0.029544146
<i>PRKAR2B</i>	protein kinase cAMP-dependent type II regulatory subunit beta	1.38	0.010298179	0.015750906
<i>LPO</i>	lactoperoxidase	1.37	0.001057202	0.002392927
<i>ARFGEF3</i>	ARFGEF family member 3	1.37	8.63388E-07	4.72441E-06
<i>ADAM1B</i>	disintegrin and metalloproteinase domain-containing protein 1b	1.35	0.018753715	0.025524568
<i>INTS6L</i>	integrator complex subunit 6 like	1.34	8.16755E-05	0.000265281
	Protein FAM127	1.33	0.000151577	0.00045274
<i>COL11A1</i>	collagen alpha-1(XI) chain	1.33	0.000743878	0.001780137
	-	1.31	0.001311256	0.00286871
<i>DMXL2</i>	Dmx like 2	1.31	0.034428821	0.041209868
<i>ABCC11</i>	ATP binding cassette subfamily C member 11	1.31	0.034428821	0.041209868
<i>ZKSCAN2</i>	zinc finger with KRAB and SCAN domains 2	1.31	0.034428821	0.041209868
<i>BCL7A</i>	BCL tumor suppressor 7A	1.31	0.034428821	0.041209868
<i>C1S</i>	complement C1s	1.30	9.13759E-17	2.04442E-15
<i>SLC3A1</i>	solute carrier family 3 member 1	1.29	0.000208327	0.000595073
	-	1.29	0.012863773	0.018888033
<i>ARL5B</i>	ADP ribosylation factor like GTPase 5B	1.29	0.012863773	0.018888033
	-	1.29	0.012863773	0.018888033
<i>TCN1</i>	transcobalamin 1	1.27	0.028301012	0.035301608
<i>STRC</i>	stereocilin	1.24	1.16019E-05	4.76629E-05
<i>SP4</i>	Sp4 transcription factor	1.24	0.002809303	0.0054244
<i>DNALI1</i>	dynein axonemal light chain 1	1.23	0.000916734	0.002124522
<i>ZNF268</i>	zinc finger protein 268	1.22	1.65969E-05	6.55868E-05
<i>PDE3A</i>	phosphodiesterase 3A	1.21	0.000631353	0.001553479
<i>C3orf62</i>	chromosome 3 open reading frame 62	1.20	0.004409931	0.007848351
<i>TMSB4X</i>	Thymosin beta-4 Hematopoietic system regulatory peptide	1.20	0.002168331	0.004357312
<i>CCL17</i>	C-C motif chemokine ligand 17	1.20	0.000434565	0.001126393
<i>POU2F1</i>	POU domain, class 2, transcription factor 1	1.20	0.001319901	0.002882384
<i>KREMEN2</i>	kringle containing transmembrane protein 2	1.20	0.042234269	0.048327399
<i>JCHAIN</i>	joining chain of multimeric IgA and IgM	1.20	0.042234269	0.048327399
	-	1.19	0.001092554	0.002455707



	Serum amyloid A protein	Amyloid protein A	1.19	2.93622E-12	4.15805E-11
<i>FAM199X</i>	family with sequence similarity 199, X-linked		1.18	0.010558173	0.016054723
	Tctex1 domain-containing protein 2		1.18	0.002772859	0.005364357
<i>ANKRD37</i>	ankyrin repeat domain 37		1.18	0.034382083	0.041188161
	-		1.18	0.034382083	0.041188161
<i>ZC3H12C</i>	zinc finger CCCH-type containing 12C		1.18	7.18547E-07	4.0379E-06
<i>SATB1</i>	SATB homeobox 1		1.17	0.00867347	0.013722784
<i>FRS2</i>	fibroblast growth factor receptor substrate 2		1.17	1.07834E-06	5.74335E-06
<i>PCDHGB2</i>	protocadherin gamma subfamily B, 2		1.13	0.003631857	0.006662119
<i>ETV1</i>	ETS variant 1		1.13	0.015254801	0.021572216
<i>STAC</i>	SH3 and cysteine rich domain		1.11	0.006867449	0.011249834
	-		1.10	0.040716651	0.04708051
<i>USP49</i>	ubiquitin specific peptidase 49		1.10	0.040716651	0.04708051
<i>OLR1</i>	oxidized low-density lipoprotein receptor 1		1.10	0.040716651	0.04708051
	-		1.09	0.026841671	0.033810628
<i>BLNK</i>	B-cell linker		1.08	2.58464E-06	1.26778E-05
<i>SDHAF3</i>	succinate dehydrogenase complex assembly factor 3		1.08	0.017808408	0.024491883
<i>ZNF570</i>	zinc finger protein 570		1.07	9.04806E-06	3.83223E-05
	-		1.06	7.79278E-08	5.42835E-07
<i>ANXA13</i>	annexin A13		1.06	0.004386513	0.007822319
<i>RASEF</i>	RAS and EF-hand domain containing		1.05	0.000922187	0.002136336
<i>CEP162</i>	Bos taurus centrosomal protein 162kDa (CEP162), mRNA.		1.04	2.48535E-05	9.34883E-05
<i>SLC7A11</i>	solute carrier family 7 member 11		1.03	0.031002343	0.037995211
<i>SLPI</i>	secretory leukocyte peptidase inhibitor		1.02	1.63914E-12	2.36575E-11

73 FDR, false discovery rate.

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76 **Supplementary Table S2 The down-regulated genes in PRL-stimulated cells compared to**  
77 **control cells**

Gene name	Gene description	Log2FC (PRL/Con)	P-value	FDR
<i>TNNI3</i>	troponin I3, cardiac type	-1.00	0.002793733	0.005397798
	-	-1.01	0.005895812	0.009925615
<i>PCDHB7</i>	protocadherin beta 7	-1.01	0.009776932	0.015099481
<i>SOD3</i>	superoxide dismutase 3	-1.01	0.011646905	0.017441442
	-	-1.01	0.013951192	0.020130797
<i>bta-mir-2904-3</i>	bta-mir-2904-1	-1.02	2.14561E-10	2.35646E-09
<i>PCDHB16</i>	protocadherin beta 16	-1.02	0.04033117	0.046706566
	-	-1.02	0.001208289	0.002677076
<i>LIPT1</i>	lipoyltransferase 1	-1.02	0.001438042	0.003083322
<i>CA11</i>	carbonic anhydrase 11	-1.02	0.024228843	0.031298953
<i>SLC16A13</i>	solute carrier family 16 member 13	-1.02	7.64837E-14	1.30143E-12
<i>CST7</i>	cystatin F	-1.03	0.029179359	0.036224548
<i>RPS13</i>	ribosomal protein S13	-1.04	0.021923337	0.028828072
<i>bta-mir-19b</i>	bta-mir-19b	-1.04	0.0425016	0.048547505
	tubulin polymerization promoting protein	-1.04		
<i>TPPP3</i>	family member 3		0.0425016	0.048547505
<i>GJC2</i>	gap junction protein gamma 2	-1.04	0.0425016	0.048547505
<i>LMO1</i>	LIM domain only 1	-1.04	0.0425016	0.048547505
<i>TBX2</i>	T-box 2	-1.04	0.005872539	0.009905832
<i>PHEX</i>	phosphate-regulating neutral endopeptidase	-1.08	0.001913233	0.003904657
	-	-1.09	0.00150992	0.003216092
<i>PGP</i>	Glycerol-3-phosphate phosphatase	-1.10	5.20977E-38	3.77773E-36
<i>PARD6A</i>	partitioning defective 6 homolog alpha	-1.10	0.002535611	0.004961193
<i>NPNT</i>	nephronectin	-1.11	0.03595609	0.042649292
<i>MYL6B</i>	myosin light chain 6B	-1.11	3.22127E-06	1.54112E-05
<i>bta-mir-16a</i>	bta-mir-16a	-1.12	2.49433E-05	9.36649E-05
<i>bta-mir-141</i>	bta-mir-141	-1.12	0.012364941	0.018298173
<i>TLN2</i>	talin 2	-1.13	0.04329761	0.049260418
<i>PGF</i>	placental growth factor	-1.14	0.001804991	0.003724161
<i>CALHM2</i>	calcium homeostasis modulator 2	-1.14	0.000771089	0.001832867
<i>GPR19</i>	G protein-coupled receptor 19	-1.14	0.000771089	0.001832867
<i>LSMEM2</i>	leucine rich single-pass membrane protein 2	-1.14	0.006035395	0.010109701
<i>ALKBH6</i>	alkB homolog 6	-1.15	2.35441E-06	1.16722E-05
	protein phosphatase, Mg <sup>2+</sup> /Mn <sup>2+</sup>	-1.15		
<i>PPMIN</i>	dependent 1N (putative)		4.4911E-05	0.000156924
<i>GAS1</i>	growth arrest specific 1	-1.16	3.1254E-10	3.31168E-09
	-	-1.16	0.020810311	0.027715714
<i>UI</i>	U1 spliceosomal RNA	-1.17	0.000279411	0.000768224
<i>ABCG4</i>	ATP binding cassette subfamily G member	-1.17	0.008541635	0.013539114

	4			
	YdjC chitoooligosaccharide deacetylase			
<i>YDJC</i>	homolog	-1.17	0.008541635	0.013539114
	T-cell immunoglobulin and mucin domain			
<i>TIMD4</i>	containing 4	-1.17	0.000239174	0.000672019
<i>RBP4</i>	retinol binding protein 4	-1.18	0.000645854	0.00158398
<i>RGS16</i>	regulator of G protein signaling 16	-1.20	0.005015448	0.008742056
	-	-1.20	0.005015448	0.008742056
<i>PLAC8L1</i>	PLAC8 like 1	-1.20	0.000318977	0.000857807
<i>TMIE</i>	transmembrane inner ear [	-1.21	0.002496781	0.004894766
	-	-1.22	0.035529194	0.042259532
	-	-1.22	0.035529194	0.042259532
<i>SLC52A3</i>	solute carrier family 52 member 3	-1.22	0.035529194	0.042259532
	Histone H2B type 1	-1.23	0.034680262	0.041438141
	-	-1.24	0.017045724	0.023632818
<i>SAP25</i>	Sin3A associated protein 25	-1.24	0.004986388	0.008703796
	atypical chemokine receptor 1 (Duffy blood			
<i>ACKR1</i>	group)	-1.25	0.008312966	0.013232417
	-	-1.26	0.021100419	0.028021477
<i>GNAT1</i>	G protein subunit alpha transducin 1	-1.26	0.000833839	0.001962644
<i>SLC45A2</i>	solute carrier family 45 member 2	-1.27	0.004828413	0.008479694
<i>CEP57L1</i>	centrosomal protein 57 like 1	-1.27	0.004828413	0.008479694
<i>ZNF331</i>	zinc finger protein 331	-1.28	1.75813E-05	6.90239E-05
	-	-1.29	0	0
	ATP binding cassette subfamily G member			
<i>ABCG2</i>	2 (Junior blood group)	-1.30	0.005675364	0.009648955
	ST8 alpha-N-acetyl-neuraminide			
<i>ST8SIA1</i>	alpha-2,8-sialyltransferase 1	-1.30	0.011570684	0.017344561
<i>TMEM150B</i>	transmembrane protein 150B	-1.30	0.023920404	0.030973638
	scavenger receptor cysteine rich family			
<i>SSC5D</i>	member with 5 domains	-1.30	0.023920404	0.030973638
<i>SLC4A11</i>	solute carrier family 4 member 11	-1.34	1.1417E-05	4.70151E-05
	murine retrovirus integration site 1			
<i>MRVII</i>	homolog	-1.34	0.028219931	0.03522237
<i>5_8S_rRNA</i>	5.8S ribosomal RNA	-1.36	5.06997E-11	5.98308E-10
	leucine rich repeat and Ig domain			
<i>LINGO1</i>	containing 1	-1.37	0.015938089	0.022333815
<i>PRSS35</i>	protease, serine 35	-1.37	0.004478569	0.007957405
<i>LRRC66</i>	leucine rich repeat containing 66	-1.37	1.76547E-05	6.91847E-05
<i>C3H1orf87</i>	uncharacterized protein C1orf87 homolog	-1.38	0.034870302	0.041632151
	-	-1.39	6.19933E-06	2.74344E-05
<i>APC2</i>	APC2, WNT signaling pathway regulator	-1.39	6.72529E-05	0.000223379
<i>RBBP8NL</i>	RBBP8 N-terminal like	-1.39	0.009072285	0.01422008
<i>FN3K</i>	fructosamine 3 kinase	-1.39	0.009072285	0.01422008

	colony stimulating factor 2 receptor alpha			
<i>CSF2RA</i>	subunit	-1.40	0.01383092	0.020000407
<i>SERPINA1</i>	serpin family A member 1	-1.41	0.00519598	0.008999172
<i>CLEC11A</i>	C-type lectin domain containing 11A	-1.41	0.000871795	0.002039663
	tartrate-resistant acid phosphatase type 5			
<i>ACP5</i>	precursor	-1.41	0.018577092	0.025328713
<i>TMEM240</i>	transmembrane protein 240	-1.41	0.018577092	0.025328713
<i>5_8S_rRNA</i>	5.8S ribosomal RNA	-1.42	2.83644E-05	0.000104411
	-	-1.43	3.79346E-10	3.94318E-09
	-	-1.43	0.001728434	0.003582441
<i>STC1</i>	stanniocalcin 1	-1.46	0.038590047	0.045129695
	Bos taurus chondroitin sulfate proteoglycan			
<i>CSPG5</i>	5 (neuroglycan C) (CSPG5), mRNA.	-1.47	0.001983007	0.004033381
<i>SEZ6L2</i>	seizure related 6 homolog like 2	-1.48	0.021489517	0.028437803
<i>AATK</i>	apoptosis associated tyrosine kinase	-1.48	0.000665991	0.001624102
	-	-1.49	0.012105534	0.018007272
	spermidine/spermine N1-acetyltransferase			
<i>SAT2</i>	family member 2	-1.52	0.000756866	0.001806908
<i>AVIL</i>	advillin [	-1.52	0.000439955	0.001136916
<i>ZNF580</i>	zinc finger protein 580	-1.57	1.6647E-13	2.75249E-12
	-	-1.59	0.02063585	0.027519902
<i>SSPO</i>	SCO-spondin	-1.63	0.005000935	0.008726657
<i>DFNB59</i>	deafness, autosomal recessive 59	-1.65	0.015471863	0.021807435
	src-related kinase lacking C-terminal			
	regulatory tyrosine and N-terminal	-1.66		
<i>SRMS</i>	myristylation sites		0.001061881	0.00240186
	Density-regulated protein	-1.67	0.010967288	0.0166055
<i>NRTN</i>	neurturin	-1.67	0.001832866	0.003775204
<i>PITX1</i>	paired like homeodomain 1	-1.69	3.01452E-05	0.000110358
<i>RHPN1</i>	rhophilin Rho GTPase binding protein 1	-1.70	0.00039531	0.001039853
<i>ALX3</i>	ALX homeobox 3	-1.74	0.009645596	0.014928633
<i>TNFRSF18</i>	TNF receptor superfamily member 18	-1.78	0.003448794	0.006397929
<i>DUSP27</i>	dual specificity phosphatase 27 (putative)	-1.80	0.000270616	0.000747114
<i>EPOR</i>	erythropoietin receptor	-1.81	0.001251379	0.002755745
<i>MAP6D1</i>	MAP6 domain containing 1	-1.85	0.00213822	0.004305429
<i>S100A3</i>	S100 calcium binding protein A3	-1.85	0.029927301	0.036902903
<i>PPP4R4</i>	protein phosphatase 4 regulatory subunit 4	-1.85	0.029927301	0.036902903
<i>SEC14L5</i>	SEC14 like lipid binding 5	-1.85	0.029927301	0.036902903
	leucine zipper and EF-hand containing			
<i>LETM2</i>	transmembrane protein 2	-1.87	0.000777833	0.001847438
	cytochrome P450 family 2 subfamily W			
<i>CYP2W1</i>	member 1	-1.88	0.010309691	0.015756501
<i>CCIN</i>	calicin	-1.88	0.010309691	0.015756501
<i>FAM78A</i>	protein FAM78A	-1.93	0.000177041	0.000519009

<i>DMRTA2</i>	DMRT like family A2	-1.97	0.00621777	0.010358216
	-	-1.97	0.017811844	0.024491883
	-	-2.04	0.001341268	0.002920309
<i>BSN</i>	bassoon presynaptic cytomatrix protein family with sequence similarity 129	-2.08	3.74261E-09	3.38213E-08
<i>FAM129C</i>	member C	-2.09	0.000296029	0.000806544
<i>SNORA70</i>	Small nucleolar RNA SNORA70	-2.10	3.06148E-12	4.30504E-11
<i>HPDL</i>	4-hydroxyphenylpyruvate dioxygenase like potassium calcium-activated channel	-2.11	0.000805063	0.00190086
<i>KCNMA1</i>	subfamily M alpha 1	-2.14	0.002210085	0.004430774
<i>DTX1</i>	E3 ubiquitin-protein ligase DTX1 recombination signal binding protein for	-2.14	0.030459661	0.037450895
<i>RBPJL</i>	immunoglobulin kappa J region like	-2.20	0.006142105	0.010259892
<i>ANPEP</i>	alanyl aminopeptidase, membrane	-2.20	0.006142105	0.010259892
<i>TPPP</i>	tubulin polymerization promoting protein Tubulin alpha-1D chain Detyrosinated	-2.20	0.006142105	0.010259892
	tubulin alpha-1D chain	-2.20	0.005822086	0.009848329
<i>HESX1</i>	HESX homeobox 1	-2.30	0.000765593	0.001822682
	cytochrome P450 family 17 subfamily A member 1	-2.30	0.003566854	0.00655687
<i>CYP17A1</i>	USH1 protein network component	-2.30	0.003566854	0.00655687
<i>USH1C</i>	harmonin	-2.30	0.003566854	0.00655687
<i>RCSD1</i>	RCSD domain containing 1	-2.30	0.017342064	0.023970698
<i>RNF180</i>	E3 ubiquitin-protein ligase RNF180	-2.30	0.017342064	0.023970698
	relaxin/insulin like family peptide receptor 4	-2.30	0.017342064	0.023970698
<i>RXFP4</i>	-	-2.34	0.005831511	0.009855961
<i>ASB12</i>	ankyrin repeat and SOCS box containing 12	-2.35	9.90781E-05	0.000314009
<i>TTC12</i>	tetratricopeptide repeat domain 12	-2.39	0.002058578	0.004167373
<i>GIMAP8</i>	GTPase, IMAP family member 8	-2.39	0.002058578	0.004167373
	tyrosine kinase with immunoglobulin like and EGF like domains 1	-2.41	5.80234E-05	0.000196519
<i>TIE1</i>	lactate dehydrogenase D	-2.43	0.009802729	0.015131556
<i>LDHD</i>	-	-2.56	0.000675058	0.001638907
	MFNG O-fucosylpeptide	-2.56	0.000675058	0.001638907
<i>MFNG</i>	3-beta-N-acetylglucosaminyltransferase	-2.56	0.000675058	0.001638907
<i>ZBPB</i>	zona pellucida-binding protein 1	-2.56	0.005508449	0.00942642
	cAMP-dependent protein kinase inhibitor alpha	-2.56	0.005508449	0.00942642
<i>PKIA</i>	transcription factor HES-7	-2.56	0.005508449	0.00942642
<i>HES7</i>	nuclear receptor interacting protein 3	-2.56	0.005508449	0.00942642
<i>NRIP3</i>	-	-2.56	0.005508449	0.00942642
	potassium voltage-gated channel subfamily J member 15	-2.62	4.97136E-05	0.000171659
<i>KCNJ15</i>				

<i>GPR143</i>	G protein-coupled receptor 143	-2.67	0.003080362	0.005855725
<i>ADH6</i>	alcohol dehydrogenase 6 (class V)	-2.67	0.003080362	0.005855725
	transient receptor potential cation channel			
<i>TRPM3</i>	subfamily M member 3	-2.67	0.003080362	0.005855725
<i>CABP2</i>	calcium binding protein 2	-2.67	0.003080362	0.005855725
<i>BARX2</i>	BARX homeobox 2	-2.71	0.000217648	0.000618761
<i>RASAL3</i>	RAS protein activator like 3	-2.78	0.000122946	0.000377334
<i>STX1B</i>	syntaxin 1B	-2.78	0.026614426	0.033573571
	-	-2.78	0.026614426	0.033573571
<i>RUNX3</i>	runt related transcription factor 3	-2.78	0.026614426	0.033573571
<i>PHOX2A</i>	paired mesoderm homeobox protein 2A	-2.78	0.026614426	0.033573571
<i>ASB9</i>	ankyrin repeat and SOCS box containing 9	-2.78	0.026614426	0.033573571
<i>SLC13A3</i>	solute carrier family 13 member 3	-2.88	0.000952415	0.002199589
	-	-2.96	0.012549754	0.01851922
	-	-2.97	0.002099962	0.004241163
<i>DQX1</i>	DEAQ-box RNA dependent ATPase 1	-2.97	0.014238481	0.020457056
<i>GUCA1A</i>	guanylate cyclase activator 1A	-2.97	0.014238481	0.020457056
	-	-2.97	0.014238481	0.020457056
<i>Metazoa_SRP</i>	Metazoan signal recognition particle RNA	-3.02	5.33116E-07	3.11512E-06
<i>PLCD4</i>	phospholipase C delta 4	-3.06	0.000291248	0.000795319
<i>VWCE</i>	von Willebrand factor C and EGF domains	-3.06	0.000291248	0.000795319
<i>LRRC14B</i>	leucine rich repeat containing 14B	-3.14	0.007614601	0.012270052
<i>FSCN2</i>	fascin actin-bundling protein 2, retinal	-3.30	0.004073517	0.007340285
	ATPase plasma membrane Ca <sup>2+</sup>			
<i>ATP2B3</i>	transporting 3	-3.30	0.042244667	0.048327399
	potassium calcium-activated channel			
	subfamily N member 1	-3.30	0.042244667	0.048327399
<i>KCNN1</i>	subfamily N member 1			
<i>OGN</i>	osteoglycin	-3.30	0.042244667	0.048327399
<i>GP5</i>	glycoprotein V platelet	-3.30	0.042244667	0.048327399
	-	-3.30	0.042244667	0.048327399
<i>GDF9</i>	Growth/differentiation factor 9	-3.30	0.042244667	0.048327399
<i>GNG4</i>	G protein subunit gamma 4	-3.30	0.042244667	0.048327399
	-	-3.39	4.55917E-06	2.08069E-05
<i>5_8S_rRNA</i>	5.8S ribosomal RNA	-3.41	0.009113746	0.014254491
<i>5_8S_rRNA</i>	5.8S ribosomal RNA	-3.41	0.009113746	0.014254491
<i>5_8S_rRNA</i>	5.8S ribosomal RNA	-3.41	0.009113746	0.014254491
<i>5_8S_rRNA</i>	5.8S ribosomal RNA	-3.41	0.009113746	0.014254491
<i>SARDH</i>	sarcosine dehydrogenase	-3.43	0.002180961	0.004379768
<i>Metazoa_SRP</i>	Metazoan signal recognition particle RNA	-3.45	0.000150915	0.000451434
	pleckstrin homology and RhoGEF domain			
<i>PLEKHG4</i>	containing G4	-3.56	4.41146E-06	2.01905E-05
<i>CSF2</i>	colony stimulating factor 2	-3.56	0.021704748	0.028617366
<i>CAPN3</i>	calpain 3	-3.56	0.021704748	0.028617366
<i>ZNF365</i>	zinc finger protein 365	-3.56	0.021704748	0.028617366

<i>MYH3</i>	myosin-3	-3.56	0.021704748	0.028617366
	family with sequence similarity 167			
<i>FAM167A</i>	member A	-3.56	0.021704748	0.028617366
<i>PTAFR</i>	platelet activating factor receptor	-3.56	0.021704748	0.028617366
	estradiol 17-beta-dehydrogenase 12-like	-3.56	0.021704748	0.028617366
<i>MMP19</i>	matrix metalloproteinase 19	-3.67	0.000627571	0.001544806
<i>MB</i>	myoglobin	-3.78	0.011251863	0.016968074
	-	-3.85	0.009075206	0.01422008
	-	-3.97	0.005880732	0.009908542
<i>REN</i>	renin precursor	-3.97	0.005880732	0.009908542
	family with sequence similarity 71 member			
<i>FAM71F2</i>	F2	-3.97	0.005880732	0.009908542
	-	-3.97	0.005880732	0.009908542
<i>Metazoa_SRP</i>	Metazoan signal recognition particle RNA	-3.98	4.74723E-09	4.18934E-08
<i>Metazoa_SRP</i>	Metazoan signal recognition particle RNA	-3.98	4.74723E-09	4.18934E-08
<i>VAV1</i>	vav guanine nucleotide exchange factor 1	-4.14	2.87425E-05	0.000105545
	endoplasmic reticulum to nucleus signaling			
<i>ERN2</i>	2	-4.14	2.87425E-05	0.000105545
	family with sequence similarity 170			
<i>FAM170A</i>	member A	-4.14	0.003096683	0.005875617
	ELKS/RAB6-interacting/CAST family			
<i>ERC2</i>	member 2	-4.14	0.003096683	0.005875617
	mab-21 like 1 [Source:HGNC			
<i>MAB21L1</i>	Symbol;Acc:HGNC:6757]	-4.14	0.003096683	0.005875617
	translin associated factor X interacting			
<i>TSNAXIP1</i>	protein 1	-4.30	0.001642062	0.003424388
<i>TSSK6</i>	testis specific serine kinase 6	-4.30	0.001642062	0.003424388
<i>CIQTNF6</i>	C1q and TNF related 6	-4.30	0.001642062	0.003424388
<i>GOLGA7B</i>	golgin A7 family member B	-4.30	0.001642062	0.003424388
	-	-4.54	0.000516457	0.001308762
	-	-4.56	0.000464859	0.001193076
	-	-4.93	5.57898E-05	0.000189809
	-	-5.06	2.27918E-05	8.67633E-05
	interstitial collagenase precursor	-5.31	3.40982E-06	1.61974E-05
<i>RNaseP_nuc</i>	Nuclear RNase P	-5.43	1.23673E-06	6.51779E-06
<i>SNRPG</i>	Small nuclear ribonucleoprotein G	-7.03	0.008801209	0.013873827

78 FDR, false discovery rate.

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