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Identification of miRNA/mRNA-Negative Regulation Pairs in Nasopharyngeal Carcinoma

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Background: Nasopharyngeal carcinoma (NPC) is a common malignancy in South-East Asia. NPC is characterized by distant metastasis and poor prognosis. The pathophysiological mechanism of nasopharyngeal carcinoma is unknown. This study aimed to identify the crucial miRNAs in nasopharyngeal carcinoma and their target genes, and to discover the potential mechanism of nasopharyngeal carcinoma development.


Material/Methods: Microarray expression profiling of miRNA and mRNA from the Gene Expression Omnibus database was downloaded, and we performed a significance analysis of differential expression. An interaction network of miRNAs and target genes was constructed. The underlying function of differentially expressed genes was predicted through Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses. To validate the microarray analysis data, significantly different expression levels of miRNAs and target genes were validated by quantitative real-time polymerase chain reaction.

Results: We identified 27 differentially expressed miRNAs and 982 differentially expressed mRNAs between NPC and normal control tissues. 12 miRNAs and 547 mRNAs were up-regulated and 15 miRNAs and 435 mRNAs were down-regulated in NPC samples. We found a total of 1185 negative correlation pairs between miRNA and mRNA. Differentially expressed target genes were significantly enriched in pathways in cancer, cell cycle, and cytokine-cytokine receptor interaction signaling pathways. Significantly differentially expressed miRNAs and genes, such as hsa-miR-205, hsa-miR-18b, hsa-miR-632, hsa-miR-130a, hsa-miR-34b, PIGR, SMPD3, CD22, DTX4, and CDC6, may play essential roles in the development of nasopharyngeal carcinoma.

Conclusions: hsa-miR-205, hsa-miR-18b, hsa-miR-632, hsa-miR-130a, and hsa-miR-34b may be related to the development of nasopharyngeal carcinoma by regulating the genes involved in pathways in cancer and cell cycle signaling pathways.

MeSH Keywords: **Gene Regulatory Networks • MicroRNAs • Nasopharyngeal Neoplasms • Real-Time Polymerase Chain Reaction**

Full-text PDF: <http://www.medscimonit.com/abstract/index/idArt/896047>

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Background

Nasopharyngeal carcinoma is a head and neck cancer characterized as highly malignancy and regional selection [1,2]. NPC is a rare cancer in Western countries, but it is common in Asian countries [3]. NPC frequently occurs in southern China, including Guangdong, Fujian, Hong Kong and Southeast Asia, including Malaysia, Indonesia, and Singapore.

The incidence rate of NPC is 2 per 100 000 worldwide [4]. The NPC incidence in southern China is 20–50 times higher than in Western countries [5]. Radiotherapy is the main curative treatment for NPC to extend patient survival time [6,7]. NPC presents highly malignant recurrence with local tissue invasion and distant metastasis, which is the dominant reason for radiotherapy failure [8].

Currently, the major etiological factors of NPC are reported to be genetic susceptibility, environmental factors, and Epstein-Barr virus (EBV) infection. Familial and large-scale case-control studies report that HLA class I genes in the MHC locus at chromosome 6p21 are notably associated with high risk of NPC. In addition, CDK5, TEL2, CELF2, and IKKB [9–12] are also reported to be associated with NPC pathogenesis. Environmental risk factors include eating salt-preserved food [13], insufficient intake of fresh vegetables and fruits [14], alcohol consumption [15], and tobacco smoking [13,16]. Epstein-Barr virus (EBV) infection is an extensively researched etiological factor for NPC. EBV belongs to the gamma herpes virus family, persistently infects B lymphocytes in more than 90% of adults, and is related to NPC tumorigenesis [17].

In addition to the above-mentioned etiological factors of NPC, mounting evidence shows that microRNAs (miRNAs) may play essential roles in NPC tumorigenesis by regulating target genes. miRNAs are small (20–25 nucleotides) non-coding RNAs that negatively regulate expression level of target gene [18]. Numerous studies have reported that miRNAs are associated with NPC cell proliferation, migration, invasion, metastasis, and irradiation sensitivity by suppressing their target genes. miR-142-3p promotes NPC cell proliferation via suppressing SOCS6 expression [19]. miR-4649-3p inhibits NPC cell proliferation by targeting protein tyrosine phosphatase SHP-1 [20]. miR-29a/b regulates SPARC and COL3A1 gene expression to promote NPC cell migration and invasion [21]. miR-23a targets IL-8/Stat3 pathway results in radio-sensitivity in NPC [22], while miR-504 down-regulates nuclear respiratory factor 1 result in radio-resistance in NPC [23]. However, the mechanism of pathogenesis in NPC remains unclear.

In this study we used bioinformatics methods to integrate miRNA and mRNA expression data, which are available in the GEO database, to identify differentially expressed miRNAs and

target genes between NPC and normal control tissues, aiming to provide valuable information for use in defining the mechanism of pathogenesis in NPC.

Material and Methods

Gene expression datasets

We searched the Gene Expression Omnibus database (GEO, <http://www.ncbi.nlm.nih.gov/geo>) for mRNA and miRNA expression profiling of NPC, and downloaded the raw expression data. GEO is a public repository for high-throughput gene expression data [24]. We only retained the microarray studies between tumor and normal tissues. The following information was extracted from each identified study: GEO accession number, platform, number of cases and controls, time, and author.

Data processing

Different sequencing platforms and clinical samples commonly cause the heterogeneity among different microarray datasets, and make it difficult to compare microarray datasets directly. We downloaded the raw expression dataset and preprocessed it by log₂ transformation and Z-score normalization.

Analysis of differentially expressed miRNA and mRNA

The miRNAs and mRNA differentially expressed between the NPC and normal control samples were identified using the limma method, which is a linear model for microarray data analysis [25]. We selected differentially expressed miRNA as false discovery rate (FDR) <0.05, and selected differentially expressed mRNA as FDR <0.001.

Identification of miRNA target genes

To obtain the target genes of miRNAs, the selected miRNAs were integrated into the miRWalk database (<http://www.umh.uni-heidelberg.de/apps/zmf/mirwalk/>) [26], in which the correlation between target genes and miRNAs have been confirmed. miRWalk is a comprehensive database that provides predicted and experimentally validated miRNA-target interactions for humans, mice, and rats. It combines the predicted and validated information with a comparison of binding sites resulting from 12 existing miRNA-target prediction programs [26,27]. In our study, we used 6 algorithms: DIANAmt, miRanda, miRDB, miRWalk, PICTAR, and TargetScan to predict target genes of miRNA; if more than 4 of 6 algorithms predicted the same gene of miRNA, the gene was considered as a target gene of the miRNA [26]. Reverse correlations of miRNA-target gene interacting pairs were subject to construct miRNA-RNA interaction network analysis.

Table 1. Genes and primers for qRT-PCR.

miRNA/mRNA	Primer sequence (5'to3')
SMPD3	Forward-CCAACAAGTGTAAACGACGATGCC
	Reverse-CGCTGGACGAGGAGGTAGATTTTC
CD22	Forward-ATGCCGATTTCGAGAAGGAGACAC
	Reverse-CCACGAGCACCAACTATTACAAGC
DTX4	Forward-AGAAAGGTAAAACCCAGAGGAAGT
	Reverse-ATGGCAACCAAGCAGTAGATGTG
CDC6	Forward-TTGAAGCAAGAAGGAGCACAAGATT
	Reverse-CTTCAAGAGCCCTGAAAGTGACA
PIGR	Forward-AGGTGCTAGACTCTGGTTTTCGG
	Reverse-TCTGCTCCCATCGGCTTGA
β-actin	Forward-ACTTAGTTGCGTTACACCTT
	Reverse-GTCACCTTCACCGTTCCA
hsa-Mir-205	Forward-TCCTTCATCCACCGGAGTCTG
hsa-Mir-18b	Forward-TAAGGTGCATCTAGTGCAGTTAGAA
hsa-Mir-632	Forward-GTGCTGCTTCCTGTGGG
hsa-Mir-130a	Forward-CAGTGCAATGTAAAAGGGC
hsa-Mir-34b	Forward-CAATCACTAACTCCACTGCCAT
U6	Forward-CTCGCTTCGGCAGCACA
	Reverse-AACGCTTCACGAATTGCGT

miRNA-target gene network

Differentially expressed miRNA and differentially expressed target genes were used to construct the interaction network by using Cytoscape software (<http://cytoscape.org>) [28]. In the miRNA-gene network, a circular node represented the mRNA and a diamond node represented the miRNA, and their association was represented by a line.

Functional enrichment analysis

The underlying function of differentially expressed target genes was predicted by the Gene Ontology (GO) [29] function and Kyoto Encyclopedia of Genes and Genomes (KEGG) [30] pathway enrichment analysis using the DAVID tool (Database for Annotation, Visualization, and Integrated Discovery) (<http://www.david.niaid.nih.gov>) [31]. We set $p < 0.05$ and FDR < 0.05 as the cut-off for selecting significantly enriched functional GO terms and KEGG pathway, respectively.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA of fresh frozen tissues was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The SuperScript III Reverse Transcription Kit (Invitrogen, Carlsbad, CA, USA) was used to synthesize the

cDNA according to the manufacturer's instructions. qRT-PCR reactions were performed using Power SYBR Green PCR Master Mix [32] (Applied Biosystems, Foster City, CA) on the Applied Biosystems 7500 (Applied Biosystems, Foster City, CA). The miRcute miRNA First-Strand cDNA Kit (Tiangen, Beijing, China) and miRcute miRNA qPCR Detection Kit (Tiangen, Beijing, China) were used for miRNA expression level detection. U6 and β-actin were used as internal control for miRNA and mRNA detected. The relative expression of target genes was calculated using the $2^{-\Delta\Delta CT}$ equation. The PCR primers were used as shown in Table 1.

Statistical analysis

At least 3 independent experiments were performed for statistical evaluation. qRT-PCR experimental data were expressed as means \pm SD. The statistical significance was evaluated using the Student's t-test and $p < 0.05$ was considered as a significant difference.

Results

Differentially expressed miRNAs and mRNAs in the NPC

In this work, we collected a total of 3 mRNA expression profiles including 74 NPC and 31 normal control (NC) samples and

Table 2. Characteristics of mRNA and miRNA expression profiling of the nasopharyngeal carcinoma.

GEO ID	Platform	Samples (N:P)	Time	Author
mRNA expression profiling				
GSE53819	GPL6480Agilent-014850 Whole Human Genome Microarray 4x44K G4112F (Probe Name version)	18:18	2014	Qian CN
GSE13597	GPL96[HG-U133A] Affymetrix Human Genome U133A Array	3:25	2009	Wei W
GSE12452	GPL570[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	10:31	2008	Ahlquist P
miRNA expression profiling				
GSE32906	GPL11350 Illumina Custom Prostate Cancer DASL Panel miRNA	6:16	2014	Luo Z
GSE46172	GPL16770Agilent-031181 Unrestricted_Human_miRNA_V16.0_Microarray (miRBase release 16.0 miRNA ID version)	4:4	2013	Bethony JM
GSE22587	GPL8933Illumina Human Beta-version microRNA expression BeadChip	4:8	2012	YANG S
GSE32960	GPL14722microRNA array	18:312	2012	Ma J
GSE36682	Human miRNA 1K	6:62	2012	Wei R

N – normal samples; P – patients' samples.

5 miRNA expression profiling including 402 NPC and 38 NC samples, as shown in Table 2. After normalization of the raw microarray data, significantly differentially expressed genes including 27 miRNA and 982 mRNA were identified in NPC compared to normal nasopharyngeal tissues; 27 miRNAs consisted of 12 up-regulated and 15 down-regulated miRNAs; 982 mRNA consisted of 547 up-regulated and 435 down-regulated mRNAs (Supplementary Table 1). hsa-miR-205, hsa-miR-196b, and hsa-miR-632 was the most significantly up-regulated miRNAs, while hsa-miR-130a, hsa-let-7a, and hsa-miR-34b were the most significantly down-regulated miRNAs in NPC compared with the normal control (Table 3).

The interaction network of miRNAs and target genes

Based on the identified miRNA-target gene interaction pairs of reverse association, we compared the interaction network between miRNAs and target genes in NPC and visualized them with Cytoscape software. We used 1185 miRNA-target gene pairs of reverse correlation, including 316 pairs of up-regulated miRNA and 735 pairs of down-regulated miRNA, to construct the miRNA-target genes interaction network. The target predictions of hsa-miR-376a* and hsa-miR-18a* are not available in miRWalk databases.

In this network, the significantly differentially expressed hsa-miR-632, hsa-miR-205, hsa-miR-18b, hsa-miR-34b, and hsa-miR-130a were targeted in significantly differentially expressed PIGR, CDC6, CD22, SMPD3 and DTX4, respectively, as shown in Figure 1.

GO classification of miRNA target genes

To obtain insights into the biological roles of differentially expressed miRNA target genes, we analyzed the predicted target gene of miRNAs using GO annotation. The threshold of GO terms was $p\text{-value} < 0.05$. Nuclear division (GO: 0000280, $p=3.05E-05$) and cell cycle G2/M phase transition (GO: 0044839, $p=4.02E-05$) were the most significant enrichments of targets genes biological process. Intracellular organelle part (GO: 0044446, $p=1.95E-05$) and organelle part (GO: 0044422, $p=3.62E-05$) were the highest enrichments of cellular component. Catalytic activity (GO: 0003824, $p=1.09E-04$) and hydrolase activity (GO: 0016787, $p=1.75E-04$) were the highest enrichments of molecular function, as shown in Table 4.

Pathway analysis of miRNA target genes

We performed the KEGG pathway enrichment analysis for differentially expressed miRNA-target genes. $FDR < 0.05$ was used as the criteria for pathway detection. The highest enrichment of pathways in our analysis was the pathway in cancer ($FDR=1.78E-11$) and cell cycle ($FDR=1.48E-07$) (Table 5).

qRT-PCR validation of differentially expressed miRNAs and target genes

To validate the microarray analysis data, the levels of significantly differentially expressed miRNAs (hsa-miR-632, hsa-miR-34b, hsa-miR-130a, hsa-miR-205, and hsa-miR-18b) and target genes (CD22, CDC6, DTX4, PIGR, and SMPD3) were quantified

Table 3. Significantly dysregulated miRNAs.

miRNAs	p-value	FDR
Up-regulated miRNAs		
hsa-miR-205	0.00E+00	0.00E+00
hsa-miR-196b	1.58E-05	1.85E-03
hsa-miR-632	8.13E-05	3.56E-03
hsa-miR-18b	7.37E-05	3.56E-03
hsa-miR-93	3.81E-04	9.52E-03
hsa-miR-326	3.78E-04	9.52E-03
hsa-miR-210	3.18E-04	9.52E-03
hsa-miR-376a*	1.19E-03	2.46E-02
hsa-miR-200c	1.64E-03	3.02E-02
hsa-miR-18a*	2.26E-03	3.77E-02
hsa-miR-542-3p	2.60E-03	4.09E-02
hsa-miR-9*	3.47E-03	4.49E-02
Down-regulated miRNAs		
hsa-miR-130a	2.02E-11	3.54E-09
hsa-let-7a	3.18E-05	2.78E-03
hsa-miR-34b	4.60E-05	3.22E-03
hsa-let-7e	7.38E-05	3.56E-03
hsa-let-7d	1.46E-04	5.67E-03
hsa-miR-30d	2.24E-04	7.83E-03
hsa-miR-146a	3.31E-04	9.52E-03
hsa-miR-98	1.09E-03	2.46E-02
hsa-miR-10b	1.18E-03	2.46E-02
hsa-miR-138	1.45E-03	2.83E-02
hsa-miR-31	1.75E-03	3.05E-02
hsa-miR-363	2.69E-03	4.09E-02
hsa-miR-564	2.90E-03	4.23E-02
hsa-let-7g	3.09E-03	4.33E-02
hsa-miR-29a	3.31E-03	4.45E-02

FDR – false discovery rate.

by qRT-PCR in 2 NPC samples and 2 normal control samples. As shown in Figure 2A–2C, the expression levels of hsa-miR-632, hsa-miR-205, and hsa-miR-18b were significantly up-regulated and the respective target genes CD22, PIGR, and SMPD3 were significantly down-regulated in NPC samples ($p < 0.01$). The expressions of hsa-miR-34b and hsa-miR-130a were significantly

down-regulated and the respective target genes CDC6 and DTX4 were significantly up-regulated in NPC samples ($p < 0.01$), as shown in Figure 2D, 2E. In conclusion, the qRT-PCR validation of differentially expressed miRNAs and target genes between NPC and normal control samples was in accordance with results of our microarray data bioinformatics analysis.

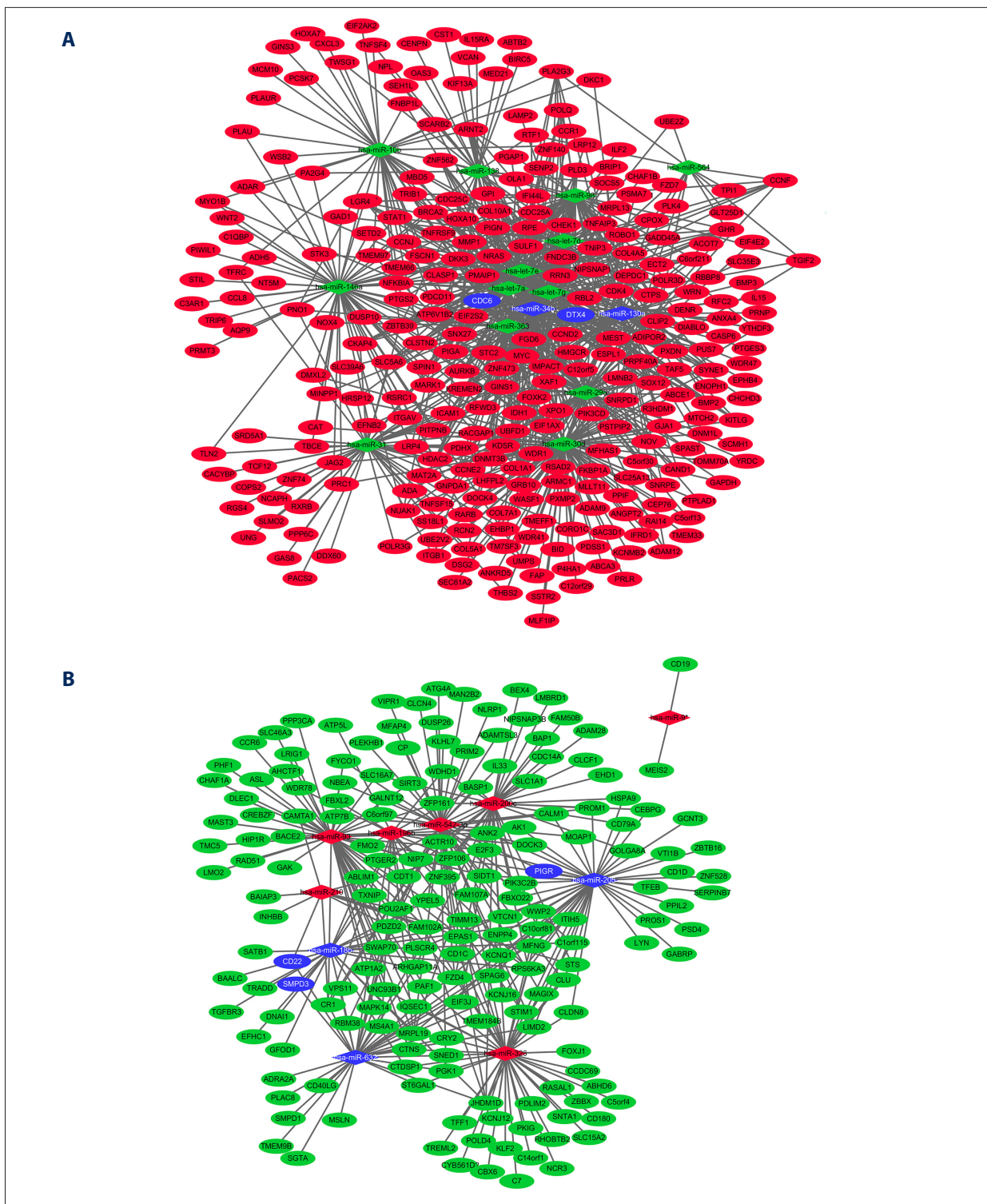


Figure 1. miRNA-mRNA interaction network of NPC. **(A)** Down-regulation miRNA and up-regulation mRNA interaction network. The green and blue diamond nodes represent down-regulation, and red and blue circular nodes represent up-regulation. **(B)** Up-regulation miRNA and down-regulation mRNA interaction network. The green and blue circular nodes represent down-regulation, and the red and blue diamond nodes represent up-regulation. Circular nodes represent mRNAs and diamond nodes represent miRNAs. Solid lines indicate interaction associations between the miRNAs and mRNAs. The blue diamond nodes and blue circular nodes represent verified miRNA and mRNA through qRT-PCR.

Table 4. GO function enrichment analysis of differentially expressed miRNA target genes (top 15).

GO ID	GO Term	Count	P-value	FDR
Biological process				
GO: 0000280	Nuclear division	7	3.05E-05	1.48E-01
GO: 0044839	Cell cycle G2/M phase transition	6	4.02E-05	9.76E-02
GO: 0000086	G2/M transition of mitotic cell cycle	6	4.02E-05	6.50E-02
GO: 0007067	Mitotic nuclear division	6	7.71E-05	9.37E-02
GO: 0022617	Extracellular matrix disassembly	6	8.75E-05	8.50E-02
GO: 0030574	Collagen catabolic process	6	8.75E-05	7.08E-02
GO: 0044243	Multicellular organismal catabolic process	6	8.75E-05	6.07E-02
GO: 0048285	Organelle fission	7	9.46E-05	5.74E-02
GO: 0051301	Cell division	9	1.45E-04	7.83E-02
GO: 0044763	Single-organism cellular process	191	1.87E-04	9.08E-02
GO: 1903047	Mitotic cell cycle process	12	2.80E-04	1.24E-01
GO: 0044772	Mitotic cell cycle phase transition	9	2.84E-04	1.15E-01
GO: 0044770	Cell cycle phase transition	9	2.84E-04	1.06E-01
GO: 0051726	Regulation of cell cycle	41	2.92E-04	1.01E-01
GO: 0032963	Collagen metabolic process	6	3.49E-04	1.13E-01
Cellular component				
GO: 0044446	Intracellular organelle part	159	1.95E-05	1.09E-02
GO: 0044422	Organelle part	162	3.62E-05	1.01E-02
GO: 0005581	Collagen trimer	5	6.87E-05	1.28E-02
GO: 0005829	Cytosol	42	4.30E-04	6.01E-02
GO: 0044424	Intracellular part	222	7.32E-04	8.19E-02
Molecular function				
GO: 0003824	Catalytic activity	88	1.09E-04	1.06E-01
GO: 0016787	Hydrolase activity	7	1.75E-04	8.55E-02
GO: 0004000	Adenosine deaminase activity	2	5.17E-04	1.68E-01
GO: 0016814	Hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in cyclic amidines	2	5.17E-04	1.26E-01

FDR – false discovery rate.

Discussion

miRNAs play essential roles in many fundamental biological processes, including cell proliferation, migration, invasion, and metastasis. miRNAs can function as oncogenes or oncosuppressor, depending on the targets suppressed.

In our study, we found that hsa-miR-205, hsa-miR-632, hsa-miR-196b, hsa-miR-18b, and hsa-miR-93 are the top 5 up-regulated miRNAs and we found that hsa-miR-130a, hsa-let-7a,

hsa-miR-34b, hsa-let-7e, and hsa-let-7d are the top 5 down-regulated miRNAs in NPC patients. hsa-miR-205 and hsa-miR-18a are significantly up-regulated, and hsa-miR-34b is significantly down-regulated in NPC biopsy tissues [33,34], which is in accordance with our bioinformatics analysis and further was validated through qRT-PCR (Table 3, Figure 2D). Moreover, hsa-miR-205 and hsa-miR-34b expression level influence the development of NPC [33]. Functions of hsa-miR-632 up-regulation and hsa-miR-130a down-regulation in NPC are not reported.

Table 5. KEGG pathway enrichment analysis of differentially expressed miRNA target genes (top 15).

KEGG ID	KEGG term	Count	FDR	Genes
hsa05200	Pathways in cancer	27	1.78E-11	CCNE2, STAT1, KITLG, RARB, BIRC5, E2F3, EPAS1, FZD7, BMP2, NRAS, COL4A5, CDK4, NFKBIA, RAD51, WNT2, BID, FZD4, MYC, PIK3CD, ITGAV, BRCA2, ITGB1, ARNT2, ZBTB16, HDAC2, PTGS2, MMP1
hsa04110	Cell cycle	14	1.48E-07	CCNE2, ESPL1, CDC14A, GADD45A, E2F3, CHEK1, CCND2, CDK4, CDC25C, CDC6, RBL2, MYC, CDC25A, HDAC2
hsa05222	Small cell lung cancer	11	9.81E-07	CCNE2, RARB, E2F3, COL4A5, CDK4, NFKBIA, MYC, PIK3CD, ITGAV, ITGB1, PTGS2
hsa04060	Cytokine-cytokine receptor interaction	16	3.08E-05	INHBB, IL15RA, KITLG, IL15, BMP2, CXCL3, CLCF1, TNFSF15, PRLR, CCR1, GHR, CD40LG, CCL8, TNFRSF9, TNFSF4, CCR6
hsa04662	B cell receptor signaling pathway	8	3.15E-04	CD22, CD79A, NRAS, NFKBIA, LYN, PIK3CD, PPP3CA, CD19
hsa05162	Measles	10	3.87E-04	CCNE2, STAT1, EIF2AK2, TNFAIP3, CCND2, CDK4, NFKBIA, PIK3CD, OAS3, ADAR
hsa04115	p53 signaling pathway	5	4.14E-04	CCNE2, GADD45A, CHEK1, CCND2, CDK4
hsa04640	Hematopoietic cell lineage	8	4.18E-04	CD22, KITLG, CD1D, TFRC, CD1C, CD19, MS4A1, CR1
hsa05220	Chronic myeloid leukemia	7	6.01E-04	E2F3, NRAS, CDK4, NFKBIA, MYC, PIK3CD, HDAC2
hsa04630	Jak-STAT signaling pathway	10	7.27E-04	IL15RA, STAT1, IL15, CCND2, CLCF1, SOCS5, PRLR, MYC, PIK3CD, GHR
hsa05140	Leishmaniasis	4	8.58E-04	STAT1, NFKBIA, ITGB1, PTGS2
hsa05160	Hepatitis C	9	8.98E-04	STAT1, EIF2AK2, CLDN8, NRAS, NFKBIA, TRADD, PIK3CD, MAPK14, OAS3
hsa05340	Primary immunodeficiency	5	9.13E-04	CD79A, ADA, CD40LG, CD19, UNG
hsa05219	Bladder cancer	3	1.22E-03	E2F3, CDK4, MYC
hsa04610	Complement and coagulation cascades	6	1.77E-03	PLAUR, PROS1, C3AR1, C7, CR1, PLAU

FDR – false discovery rate.

Tang et al. reported that miR-205-5p has significant diagnostic value as a novel candidate biomarker in NPC. They performed a comprehensive analysis of microRNA expression patterns of 3 NPC biopsies and 3 normal nasopharyngeal epithelium specimens, then validated the differentially expressed miRNAs in 67 NPC and 25 normal tissues with qRT-PCR, finding that miR-205-5p is 1 of 5 significantly differentially expressed miRNAs in NPC [35]. In addition, miR-205 is related to radio-resistance of NPC, and miR-205 up-regulation results in radio-resistance of NPC through suppressing the PTEN pathway [36,37]. In our work, PIGR was predicted as target gene of miR-205, and this was validated in NPC tissues by qRT-PCR (Figure 2B). PIGR had significantly lower expression in NPC specimens, but was frequently expressed in non-tumor controls [38], which is consistent with our results (Figure 2B).

Target CDC6 was up-regulated by hsa-miR-34b in NPC tissues, as Figure 2D shows. miR-34b/c and TP-53 polymorphisms may contribute to the risk of NPC, and gene-gene interaction of miR-34b/c rs4938723 and TP-53 Arg72-Pro increases the risk of NPC [39]. It is reported that CDC6 is associated with cancer prognosis and proliferation, while CDC6 functions in NPC are not reported. CDC6 is significantly up-regulated by miR-26a/b in lung cancer specimens compared with the adjacent normal tissues, suggesting that CDC6 is associated with poorer prognosis of lung cancer [40]. Knockdown of CDC 6 effectively inhibits proliferation of tongue squamous cell carcinoma Tca8113 cells [41]. In our work, CDC6 was enriched in cell cycle, which is the top 2 KEGG enrichment pathway of differentially expressed miRNA target genes (Table 5).

hsa-miR-18b was up-regulated and consequentially caused significant down-regulation of target genes ABLIM1 and SMPD3

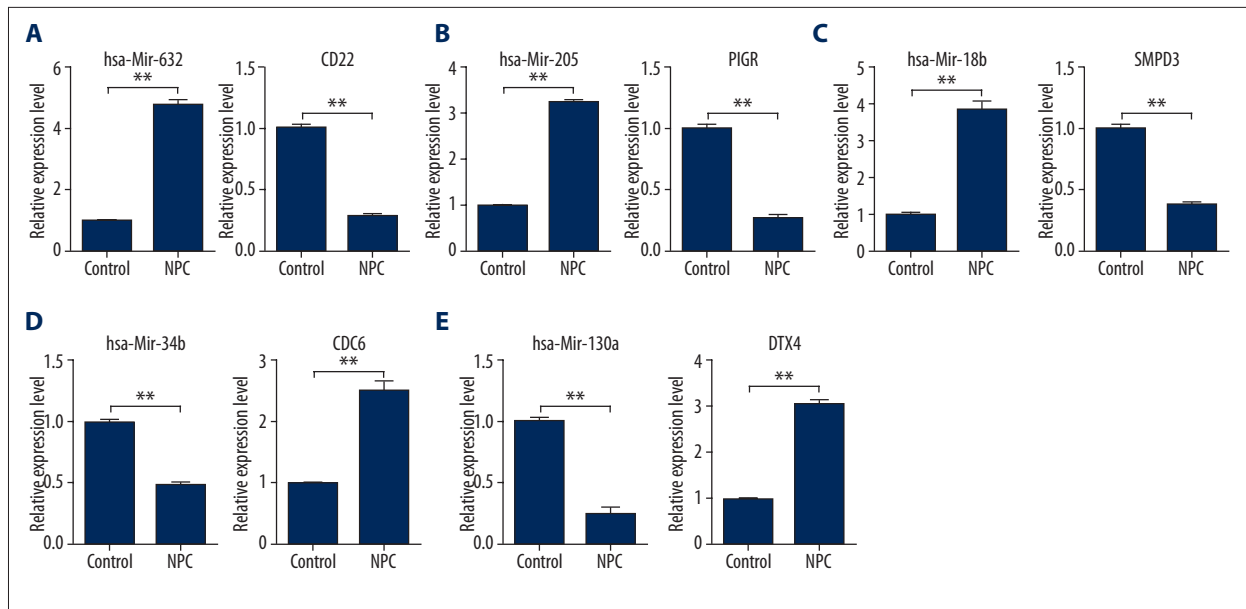


Figure 2. miRNA and mRNA expression level in NPC and control tissues by qRT-PCR. (A) hsa-miR-632 and CD22; (B) hsa-miR-205 and PIGR; (C) hsa-miR-18b and SMPD3; (D) hsa-miR-34b and CDC6; (E) hsa-miR-130a and DTX4. Control tissues mean adjacent cancer tissues of NPC.

(NSMASE2) in NPC (Table 3, Figure 2C). SMPD3 was down-regulated by both of hsa-miR-18b and hsa-miR-632 in our study. hsa-miR-18b influences cancer cell proliferation, growth, and metastasis, while hsa-miR-18b up-regulation is mediated by loss of connective tissue growth factor through PI3K/AKT/C-Jun and C-Myc signaling to promote cell growth and cell proliferation of NPC [42]. Oxidative stress modulates NSMASE2 sub-cellular localization in plasma membranes to generate ceramide and induces apoptosis of lung carcinoma A549 cell line [43]. NSMASE2 regulates exosomal miRNA secretion and promotes angiogenesis within the tumor microenvironment, as well as in metastasis [44].

Deltex 4, E3 ubiquitin ligase is the official name of DTX4; it functions as a negative regulator of Notch signaling [45], which is crucial for the T-cell development in early stages and angiogenesis during carcinogenesis [46,47]. Dysregulation of Notch and Wnt promotes cell differentiation and tumorigenesis. In our study, DTX4 was up-regulated by hsa-miR-130a, hsa-miR-7a, hsa-miR-7g, hsa-miR-7d, and hsa-miR-7e in NPC patients.

CD22 was down-regulated by both of hsa-miR-632 and hsa-miR-210. CD22 was enriched in B cell receptor and hematopoietic cell lineage signaling pathway via KEGG pathway analysis (Table 5). It is reported that pan-B lymphocytes have scant peri-tumoral areas and are absent in 29 out of 50 NPC biopsies through using immunohistological detection [48]. hsa-miR-632 is reportedly associated with breast cancer tumorigenesis, and high expression level of hsa-miR-632 down-regulates

DNAJB6, leading to significantly increased invasive and metastatic ability of breast cancer cells compared to mammary epithelial cells [49].

Pathway in cancer was identified as the most significantly enriched pathway in NPC (Table 5). Pathway in cancer is related to pathogenesis of various cancer types, such as colorectal, pancreatic, thyroid, and lung cancer [50–54], indicating that pathways in cancer may play an important role in NPC pathogenesis.

There are limitations in our work. We have constructed the regulatory network of miRNAs and mRNA inverse correlations pairs, and the pathogenesis of key miRNAs and mRNAs in NPC need to be elucidated through *in vivo* and *in vitro* experiments.

Conclusions

We identified 27 differentially expressed miRNAs and 982 differentially expressed mRNAs between NPC and normal tissues. We used 1185 miRNA-target gene pairs of inverse correlations to construct an interaction network. In this network, we found several miRNAs and genes that may play important roles in NPC, such as hsa-miR-205, hsa-miR-34b, hsa-miR-18b, hsa-miR-632, hsa-miR-130a, PIGR, CDC6, CD22, SMPD3, and DTX4. The pathway in cancer may be involved in the pathogenesis mechanism of NPC. Our findings may provide an important contribution to further elucidate the pathogenesis mechanisms of NPC.

Supplementary Table 1. Full list of differentially expressed mRNA in nasopharyngeal carcinoma.

Genes	FDR	Up/down regulation	Genes	FDR	Up/down regulation
LMNB2	1.00E-11	Up	SAC3D1	2.45E-07	Up
HDGFRP3	2.32E-11	Up	VCAN	2.52E-07	Up
FJX1	2.89E-11	Up	FGF1	2.78E-07	Up
RBBP8	4.91E-11	Up	ESM1	2.78E-07	Up
LHX2	5.18E-11	Up	C12orf11	2.86E-07	Up
TNFAIP6	8.16E-11	Up	P4HA1	3.59E-07	Up
ECT2	1.02E-10	Up	PBK	3.83E-07	Up
C12orf48	1.74E-10	Up	RNASEH2A	3.91E-07	Up
CHAF1B	2.93E-10	Up	DOCK4	3.91E-07	Up
VRK2	4.51E-10	Up	CNIH4	3.91E-07	Up
NFE2L3	8.20E-10	Up	NUAK1	3.91E-07	Up
TFRC	1.08E-09	Up	AHCY	3.99E-07	Up
NPL	1.08E-09	Up	TBCE	4.26E-07	Up
FAM64A	1.42E-09	Up	UBE2S	4.33E-07	Up
GALNT11	2.59E-09	Up	COL5A1	4.39E-07	Up
TNFSF4	2.98E-09	Up	NOX4	4.49E-07	Up
GPSM2	3.06E-09	Up	GAPDH	4.52E-07	Up
MAD2L1	4.03E-09	Up	EIF4E2	4.52E-07	Up
GAD1	4.59E-09	Up	DTL	4.52E-07	Up
CDC6	5.29E-09	Up	STK3	4.94E-07	Up
PFDN4	5.31E-09	Up	PSMD14	4.94E-07	Up
ATF5	6.91E-09	Up	DSG2	4.94E-07	Up
MRPL42	7.85E-09	Up	CENPF	5.08E-07	Up
ZWILCH	8.93E-09	Up	PTTG3P	5.15E-07	Up
FOXM1	9.23E-09	Up	FANCL	5.16E-07	Up
RAN	1.00E-08	Up	PPIF	5.26E-07	Up
OLA1	1.31E-08	Up	PSMA4	5.52E-07	Up
OIP5	1.99E-08	Up	HDAC2	6.06E-07	Up
PSRC1	2.17E-08	Up	MCM4	6.38E-07	Up
FAP	2.17E-08	Up	CKS1B	6.38E-07	Up
MINPP1	2.86E-08	Up	UCK2	6.89E-07	Up
SEC61A2	3.30E-08	Up	EIF2S2	6.90E-07	Up
CCNF	3.30E-08	Up	TIPIN	7.68E-07	Up
C12orf5	4.54E-08	Up	FSCN1	7.68E-07	Up
ARNT2	4.54E-08	Up	HSPE1	7.86E-07	Up
RIF1	4.86E-08	Up	MMP12	8.05E-07	Up
RCN2	5.33E-08	Up	TMEM194A	8.19E-07	Up
DTX4	6.03E-08	Up	KIF18A	8.22E-07	Up
RAD54B	7.31E-08	Up	INSM1	8.24E-07	Up
CLASP1	7.31E-08	Up	RAI14	8.77E-07	Up
KIF14	7.46E-08	Up	UNG	9.44E-07	Up
GNPDA1	7.46E-08	Up	PIK3CB	1.12E-06	Up
EXO1	7.46E-08	Up	TRIP6	1.15E-06	Up
PMAIP1	8.23E-08	Up	MTX2	1.21E-06	Up
KCTD3	1.10E-07	Up	CEP152	1.27E-06	Up
GRB10	1.31E-07	Up	STAR	1.30E-06	Up
GINS3	1.63E-07	Up	PLAU	1.37E-06	Up
PTGS2	1.69E-07	Up	HOMER3	1.38E-06	Up
PUS7	1.82E-07	Up	ME3	1.39E-06	Up
XPOT	2.00E-07	Up	RIC8B	1.40E-06	Up
PLA2G3	2.04E-07	Up	ZFP64	1.56E-06	Up
PALB2	2.20E-07	Up	NUP155	1.58E-06	Up
PRMT3	2.36E-07	Up	TOMM40	1.65E-06	Up

Genes	FDR	Up/down regulation
PGAP1	1.67E-06	Up
ITGAV	1.88E-06	Up
KIF4A	1.90E-06	Up
SRD5A1	2.03E-06	Up
ZNF124	2.17E-06	Up
RAD51AP1	2.17E-06	Up
PTTG1	2.26E-06	Up
BRCA2	2.31E-06	Up
GGCT	2.35E-06	Up
ANXA4	2.41E-06	Up
ADA	2.74E-06	Up
TMEM14A	2.77E-06	Up
PAK1IP1	3.08E-06	Up
TMEM33	3.10E-06	Up
HSPD1	3.11E-06	Up
GORASP1	3.11E-06	Up
USP18	3.12E-06	Up
MARK1	3.14E-06	Up
SLC39A6	3.17E-06	Up
ZNF562	3.39E-06	Up
CDC25C	3.48E-06	Up
RPE	3.50E-06	Up
POLQ	3.50E-06	Up
SNRPF	3.79E-06	Up
SSX2IP	3.81E-06	Up
STAP2	3.84E-06	Up
CDC45	3.84E-06	Up
POMGNT1	3.86E-06	Up
BID	3.88E-06	Up
STC2	4.00E-06	Up
BRIP1	4.00E-06	Up
TM7SF3	4.21E-06	Up
DUSP10	4.50E-06	Up
PSMG1	4.62E-06	Up
CCL4	4.74E-06	Up
MED21	4.88E-06	Up
NOV	5.14E-06	Up
SUMO1	5.21E-06	Up
CPOX	5.30E-06	Up
COL7A1	5.30E-06	Up
CAT	5.39E-06	Up
STIL	5.84E-06	Up
CCT2	5.93E-06	Up
DNM1L	5.95E-06	Up
SLC25A13	6.29E-06	Up
IMPACT	6.52E-06	Up
CACYBP	6.61E-06	Up
RGS4	6.63E-06	Up
PHF14	7.10E-06	Up
ILF2	7.10E-06	Up
ENO1	7.13E-06	Up
PAIP1	7.30E-06	Up
TNFSF15	7.34E-06	Up
EPHB4	7.34E-06	Up
ATP6V1B2	7.34E-06	Up

Genes	FDR	Up/down regulation
OAS3	7.83E-06	Up
GSTO1	7.93E-06	Up
TPI1	7.99E-06	Up
IL15RA	8.23E-06	Up
HOXA7	8.40E-06	Up
SETD2	8.66E-06	Up
C1QB	8.95E-06	Up
IFRD1	9.15E-06	Up
NT5M	9.92E-06	Up
PITPNB	1.01E-05	Up
CENPN	1.02E-05	Up
GPR137B	1.03E-05	Up
TK1	1.05E-05	Up
TMEFF1	1.09E-05	Up
SULF1	1.12E-05	Up
ERCC6L	1.13E-05	Up
DENR	1.21E-05	Up
C5orf13	1.21E-05	Up
ATP2C1	1.22E-05	Up
STAT1	1.27E-05	Up
TTK	1.30E-05	Up
SS18L1	1.30E-05	Up
GABPB1	1.30E-05	Up
BIRC5	1.33E-05	Up
WNT2	1.38E-05	Up
HRSP12	1.42E-05	Up
SNRPG	1.46E-05	Up
PFDN2	1.48E-05	Up
POLR3D	1.54E-05	Up
GJB1	1.57E-05	Up
POGLUT1	1.58E-05	Up
TNIP3	1.63E-05	Up
PSMB3	1.64E-05	Up
YWHAQ	1.69E-05	Up
NCBP2	1.80E-05	Up
PLD3	1.80E-05	Up
TWSG1	1.87E-05	Up
SKP2	2.00E-05	Up
YWHAH	2.03E-05	Up
CASP6	2.08E-05	Up
IRF6	2.10E-05	Up
CKAP4	2.12E-05	Up
SLC5A6	2.20E-05	Up
GPI	2.27E-05	Up
C1orf112	2.29E-05	Up
MYC	2.32E-05	Up
NIT2	2.40E-05	Up
LGR4	2.49E-05	Up
HOXC6	2.53E-05	Up
FPR3	2.53E-05	Up
GLT25D1	2.56E-05	Up
RACGAP1	2.62E-05	Up
MIF	2.65E-05	Up
CXCL3	2.69E-05	Up
CTPS	2.73E-05	Up

Genes	FDR	Up/down regulation
FGD6	2.75E-05	Up
FNDC3B	2.76E-05	Up
LHFPL2	2.81E-05	Up
CDC25A	2.90E-05	Up
CD70	2.97E-05	Up
TOMM70A	3.03E-05	Up
COPS2	3.12E-05	Up
CCND2	3.12E-05	Up
MARCO	3.17E-05	Up
WDR41	3.40E-05	Up
TAF5	3.40E-05	Up
AP3M2	3.49E-05	Up
TMEM39A	3.55E-05	Up
TMEM185B	3.55E-05	Up
PLK4	3.64E-05	Up
COL10A1	3.64E-05	Up
C11orf41	3.68E-05	Up
KITLG	3.70E-05	Up
PLOD1	3.79E-05	Up
UBFD1	3.79E-05	Up
NFKBIA	3.79E-05	Up
CORO1C	3.85E-05	Up
MORC4	3.95E-05	Up
HOXA10	3.98E-05	Up
MRPL13	4.11E-05	Up
GINS1	4.17E-05	Up
ZFP112	4.20E-05	Up
EXT1	4.23E-05	Up
KIF13A	4.25E-05	Up
TMEM97	4.31E-05	Up
PIK3CD	4.44E-05	Up
IFIT3	4.59E-05	Up
ETV7	4.76E-05	Up
NCAPD3	4.77E-05	Up
ANGPT2	4.83E-05	Up
SNRPD1	5.10E-05	Up
NDUFA9	5.24E-05	Up
TMPO	5.33E-05	Up
CD14	5.38E-05	Up
CDK5	5.44E-05	Up
DKC1	5.55E-05	Up
ASB9	5.76E-05	Up
TRMT61B	5.82E-05	Up
C1QBP	5.91E-05	Up
WRN	5.97E-05	Up
TMEM106C	5.97E-05	Up
SYNE1	6.22E-05	Up
ICAM1	6.27E-05	Up
FAM60A	6.41E-05	Up
SRSF9	6.72E-05	Up
PRR5L	6.72E-05	Up
MLF1IP	6.78E-05	Up
GNL2	6.80E-05	Up
PSMB2	6.94E-05	Up
ASPN	7.03E-05	Up

Genes	FDR	Up/down regulation
YRDC	7.17E-05	Up
KPNA2	7.25E-05	Up
TYROBP	7.30E-05	Up
EXOSC5	7.33E-05	Up
TNFRSF9	7.34E-05	Up
GAS8	7.37E-05	Up
TBCA	7.38E-05	Up
FAM162A	7.38E-05	Up
MAPKAPK5	7.41E-05	Up
POSTN	7.82E-05	Up
SSTR2	7.89E-05	Up
SUPV3L1	8.01E-05	Up
RSAD2	8.05E-05	Up
RFC2	8.05E-05	Up
H2AFZ	8.05E-05	Up
UBE2V2	8.11E-05	Up
MFHAS1	8.29E-05	Up
SYNCRIP	8.60E-05	Up
C17orf75	8.72E-05	Up
WDR47	8.75E-05	Up
FASTKD3	8.81E-05	Up
NAA50	8.85E-05	Up
ITGB1	8.94E-05	Up
CAND1	9.04E-05	Up
PTPN12	9.22E-05	Up
MAT2A	9.40E-05	Up
DERA	9.71E-05	Up
KIAA0146	9.90E-05	Up
EPCAM	9.97E-05	Up
C12orf29	1.05E-04	Up
PYCARD	1.06E-04	Up
MDK	1.07E-04	Up
BMP3	1.07E-04	Up
RAB17	1.08E-04	Up
PLAUR	1.08E-04	Up
FASTKD2	1.09E-04	Up
AIMP2	1.12E-04	Up
WDR12	1.13E-04	Up
ABCE1	1.13E-04	Up
SERPINH1	1.15E-04	Up
DEPDC1	1.16E-04	Up
CYC1	1.18E-04	Up
ITGB6	1.22E-04	Up
PSMA7	1.23E-04	Up
ZNF74	1.24E-04	Up
SLMO2	1.24E-04	Up
SCO2	1.24E-04	Up
DMXL2	1.26E-04	Up
BST2	1.26E-04	Up
XPO1	1.27E-04	Up
ENOPH1	1.27E-04	Up
SCG5	1.28E-04	Up
PAICS	1.29E-04	Up
WASF1	1.31E-04	Up
BCL2A1	1.32E-04	Up

Genes	FDR	Up/down regulation
LGALS1	1.32E-04	Up
RXR	1.32E-04	Up
RRM1	1.34E-04	Up
PXD	1.37E-04	Up
PTRH2	1.38E-04	Up
JMJD4	1.38E-04	Up
MBD5	1.39E-04	Up
RNF19B	1.40E-04	Up
PRC1	1.43E-04	Up
PRDM4	1.46E-04	Up
NPM3	1.48E-04	Up
UBE2Z	1.48E-04	Up
KIF23	1.53E-04	Up
RRN3	1.55E-04	Up
ZNF532	1.58E-04	Up
PIGA	1.58E-04	Up
WBP5	1.59E-04	Up
PIGN	1.59E-04	Up
IL13RA2	1.60E-04	Up
GTF3C3	1.60E-04	Up
ARMC1	1.63E-04	Up
NOL10	1.64E-04	Up
COL4A5	1.65E-04	Up
GADD45A	1.65E-04	Up
FOXK2	1.71E-04	Up
CCT3	1.71E-04	Up
IDH1	1.72E-04	Up
ADIPOR2	1.72E-04	Up
PSMC4	1.72E-04	Up
FCGR1B	1.72E-04	Up
ROBO1	1.72E-04	Up
CST1	1.74E-04	Up
MEST	1.78E-04	Up
CLSTN2	1.79E-04	Up
UPF3B	1.81E-04	Up
URB2	1.81E-04	Up
TPT1	1.81E-04	Up
FAH	1.81E-04	Up
B4GALT2	1.82E-04	Up
PPIA	1.82E-04	Up
NME1	1.89E-04	Up
AQP9	1.95E-04	Up
UQCRH	1.96E-04	Up
UBE2C	1.96E-04	Up
MRPS35	1.97E-04	Up
ADAM9	1.97E-04	Up
DKK3	2.00E-04	Up
RBL2	2.00E-04	Up
DDX60	2.07E-04	Up
ZBTB39	2.09E-04	Up
MCTP2	2.13E-04	Up
LOC100499177	2.13E-04	Up
SCMH1	2.15E-04	Up
CUEDC2	2.17E-04	Up
ZNF140	2.17E-04	Up

Genes	FDR	Up/down regulation
TAP1	2.18E-04	Up
E2F6	2.20E-04	Up
PA2G4	2.26E-04	Up
BMP2	2.29E-04	Up
DIABLO	2.35E-04	Up
PSMD1	2.37E-04	Up
CKAP5	2.41E-04	Up
PRLR	2.43E-04	Up
ERO1LB	2.44E-04	Up
SPIN1	2.45E-04	Up
PRNP	2.45E-04	Up
PIWIL1	2.45E-04	Up
TLN2	2.47E-04	Up
SRP9	2.49E-04	Up
SOCS5	2.52E-04	Up
CCR1	2.55E-04	Up
CEP76	2.58E-04	Up
SCARB2	2.60E-04	Up
MRPL12	2.65E-04	Up
ISYNA1	2.66E-04	Up
RPL32	2.70E-04	Up
PKD2	2.70E-04	Up
KAT2B	2.78E-04	Up
TRIB1	2.80E-04	Up
KDSR	2.84E-04	Up
IFI44L	2.84E-04	Up
UGCG	2.86E-04	Up
POLR3G	2.89E-04	Up
THBS2	2.97E-04	Up
IGSF6	2.97E-04	Up
STRAP	3.12E-04	Up
EIF2AK2	3.14E-04	Up
ADAM12	3.14E-04	Up
SNX27	3.15E-04	Up
KREMEN2	3.15E-04	Up
CEP192	3.15E-04	Up
PPP6C	3.21E-04	Up
LOC730101	3.23E-04	Up
PSMA6	3.25E-04	Up
TGFBI	3.25E-04	Up
PSTPIP2	3.25E-04	Up
CCL8	3.28E-04	Up
RPS29	3.29E-04	Up
PLA2G7	3.30E-04	Up
PNO1	3.32E-04	Up
RARB	3.45E-04	Up
CLIP2	3.45E-04	Up
LAMP2	3.45E-04	Up
CCT7	3.45E-04	Up
ABTB2	3.45E-04	Up
ABCA3	3.47E-04	Up
NCK1	3.53E-04	Up
CDK8	3.59E-04	Up
WSB2	3.62E-04	Up
DNMT3B	3.63E-04	Up

Genes	FDR	Up/down regulation
EVL	3.69E-04	Up
PTPLAD1	3.74E-04	Up
GLA	3.81E-04	Up
ADH5	3.88E-04	Up
CDK4	3.94E-04	Up
UMPS	3.99E-04	Up
UCP2	3.99E-04	Up
PTGES3	4.01E-04	Up
LOC100127972	4.01E-04	Up
FZD7	4.01E-04	Up
TUBGCP4	4.04E-04	Up
PPA1	4.07E-04	Up
ARPC1A	4.08E-04	Up
PDSS1	4.12E-04	Up
IFIT1	4.17E-04	Up
INPP1	4.17E-04	Up
KCNE1	4.22E-04	Up
PXMP2	4.24E-04	Up
IL15	4.28E-04	Up
PCSK7	4.33E-04	Up
PRPF40A	4.34E-04	Up
MLLT11	4.34E-04	Up
MKKS	4.52E-04	Up
NIPSNAP1	4.57E-04	Up
TSR2	4.59E-04	Up
LDHA	4.62E-04	Up
CHCHD3	4.76E-04	Up
NCAPH	4.82E-04	Up
R3HDM1	4.98E-04	Up
SPAST	4.98E-04	Up
PDHX	5.01E-04	Up
C2orf47	5.01E-04	Up
CBX1	5.02E-04	Up
PACS2	5.10E-04	Up
C3AR1	5.23E-04	Up
ANAPC10	5.24E-04	Up
CCNJ	5.25E-04	Up
TARS	5.41E-04	Up
ATP5G3	5.49E-04	Up
HSPA13	5.52E-04	Up
TCF12	5.54E-04	Up
EIF1AX	5.59E-04	Up
CBX5	5.75E-04	Up
YBX1	5.79E-04	Up
CTSL1	5.82E-04	Up
RFWD3	5.83E-04	Up
ZNF473	5.84E-04	Up
PDCD11	5.85E-04	Up
TGIF2	5.86E-04	Up
GSTK1	5.90E-04	Up
GPR143	5.94E-04	Up
NMD3	5.96E-04	Up
JAG2	5.97E-04	Up
DIAPH1	5.99E-04	Up
GJA1	6.03E-04	Up

Genes	FDR	Up/down regulation
KCNMB2	6.13E-04	Up
ACOT7	6.15E-04	Up
TMEM66	6.15E-04	Up
REPIN1	6.18E-04	Up
TRAP1	6.28E-04	Up
SPDEF	6.32E-04	Up
YTHDF3	6.46E-04	Up
TNFAIP3	6.63E-04	Up
SNRPE	6.63E-04	Up
WDYHV1	6.64E-04	Up
FNBP1L	6.64E-04	Up
C6orf211	6.65E-04	Up
ATP5B	6.65E-04	Up
SEH1L	6.81E-04	Up
EFNB2	6.81E-04	Up
C1orf109	6.81E-04	Up
SOX12	6.84E-04	Up
SCAI	7.20E-04	Up
ISG15	7.29E-04	Up
RTF1	7.30E-04	Up
ANKRD5	7.35E-04	Up
AURKB	7.40E-04	Up
DARS	7.52E-04	Up
SUMO2	7.58E-04	Up
WDR1	7.68E-04	Up
LRP4	7.68E-04	Up
MYO1B	7.73E-04	Up
MCM10	7.73E-04	Up
BYSL	7.73E-04	Up
MMP1	7.81E-04	Up
CCNE2	7.91E-04	Up
ADAR	7.96E-04	Up
MTCH2	7.97E-04	Up
CHEK1	8.17E-04	Up
XAF1	8.22E-04	Up
LRP12	8.42E-04	Up
PAFAH1B3	8.43E-04	Up
STAMPB	8.44E-04	Up
NRAS	8.54E-04	Up
GHR	8.54E-04	Up
ATP5J2	8.68E-04	Up
HMGR	8.75E-04	Up
SENP2	8.78E-04	Up
SLC35E3	9.08E-04	Up
ORC1	9.19E-04	Up
C5orf30	9.42E-04	Up
GSTM2	9.49E-04	Up
RSRC1	9.61E-04	Up
DEGS1	9.70E-04	Up
RPS6KC1	9.88E-04	Up
RFC3	9.89E-04	Up
ESPL1	9.93E-04	Up
FKBP1A	9.96E-04	Up
FBXO11	9.98E-04	Up
EHBP1	9.98E-04	Up

Genes	FDR	Up/down regulation
COL1A1	9.98E-04	Up
DNAL1	0.00E+00	Down
ALDH1L1	1.36E-12	Down
SPAG8	4.00E-11	Down
SCRN1	4.64E-11	Down
FOXJ1	4.64E-11	Down
CLU	4.64E-11	Down
KLF2	1.91E-10	Down
SCGB1A1	2.34E-10	Down
SCGB2A1	2.93E-10	Down
VPS11	6.74E-10	Down
SYBU	8.20E-10	Down
PDCD5	9.56E-10	Down
GPR162	2.24E-09	Down
STIM1	2.40E-09	Down
SLC44A4	3.44E-09	Down
PCYT2	3.97E-09	Down
EPAS1	3.97E-09	Down
ABLIM1	4.29E-09	Down
MT3	4.59E-09	Down
ATP2C2	5.67E-09	Down
CRYL1	7.85E-09	Down
C6orf97	7.93E-09	Down
PROM1	8.64E-09	Down
BLK	8.81E-09	Down
B3GALT4	1.31E-08	Down
CD22	1.88E-08	Down
MSLN	1.99E-08	Down
CIRBP	2.02E-08	Down
WDR78	2.08E-08	Down
MSRA	2.08E-08	Down
PTGDS	2.47E-08	Down
SLC16A7	3.25E-08	Down
SMPD3	3.41E-08	Down
CD1C	3.97E-08	Down
VIPR1	4.54E-08	Down
GNMT	6.09E-08	Down
TCTA	7.46E-08	Down
C10orf81	1.06E-07	Down
PIGR	1.23E-07	Down
IGHD	1.23E-07	Down
FAM107A	1.23E-07	Down
ASL	1.26E-07	Down
NBEA	1.28E-07	Down
AQP5	1.61E-07	Down
SGSM3	1.66E-07	Down
MSMB	1.76E-07	Down
IK	1.78E-07	Down
PDLIM2	2.21E-07	Down
DDAH2	2.22E-07	Down
FBXO22	2.29E-07	Down
PIK3C2B	2.77E-07	Down
VPREB3	3.67E-07	Down
PDCD6IP	4.01E-07	Down
TREML2	4.33E-07	Down

Genes	FDR	Up/down regulation
FCRL2	4.33E-07	Down
FAM174B	4.33E-07	Down
DPEP2	4.52E-07	Down
CLMN	4.87E-07	Down
RNASE4	5.08E-07	Down
PPP3CA	5.08E-07	Down
GNG7	5.18E-07	Down
AGR2	5.39E-07	Down
GLTSCR2	5.44E-07	Down
GALNT12	5.54E-07	Down
ABHD14A	6.19E-07	Down
KLHDC2	6.55E-07	Down
C6orf103	7.80E-07	Down
CD72	7.96E-07	Down
AK1	8.20E-07	Down
LXN	8.22E-07	Down
FAM102A	8.56E-07	Down
CR1	8.77E-07	Down
CD1D	8.77E-07	Down
MEIS3P1	8.84E-07	Down
LMO2	1.01E-06	Down
CYP2F1	1.04E-06	Down
BAIAP3	1.05E-06	Down
RPS6KA3	1.21E-06	Down
WFDC2	1.24E-06	Down
CD19	1.35E-06	Down
BACE2	1.40E-06	Down
DALRD3	1.41E-06	Down
SIDT2	1.44E-06	Down
PIP	1.61E-06	Down
ABCB1	1.88E-06	Down
FAM65B	1.90E-06	Down
RRAGA	1.92E-06	Down
AGBL2	2.06E-06	Down
C5orf45	2.19E-06	Down
E2F3	2.55E-06	Down
SFMBT1	2.56E-06	Down
TUBA4A	2.58E-06	Down
BANK1	2.58E-06	Down
TNFRSF13B	2.64E-06	Down
HMGCL	2.67E-06	Down
TTC12	2.84E-06	Down
GCNT3	2.87E-06	Down
VTCN1	2.88E-06	Down
C10orf116	3.00E-06	Down
MFNG	3.01E-06	Down
CD180	3.10E-06	Down
AHCTF1	3.12E-06	Down
TFF3	3.13E-06	Down
IQSEC1	3.17E-06	Down
POU2AF1	3.24E-06	Down
ENPP4	3.24E-06	Down
BEND5	3.51E-06	Down
CLDN10	3.59E-06	Down
CD40LG	3.79E-06	Down

Genes	FDR	Up/down regulation
ANG	4.04E-06	Down
GPR110	4.04E-06	Down
SERHL2	4.17E-06	Down
ACAA1	4.21E-06	Down
KAT5	4.62E-06	Down
TBC1D22A	4.74E-06	Down
FZD4	5.30E-06	Down
ATP1A2	5.30E-06	Down
KCNJ16	5.30E-06	Down
HSPB8	5.99E-06	Down
CCDC69	6.02E-06	Down
CYB561D2	6.59E-06	Down
TINF2	7.58E-06	Down
ST6GAL1	7.74E-06	Down
QARS	7.89E-06	Down
SELENBP1	9.03E-06	Down
CRY2	9.09E-06	Down
DUSP26	9.93E-06	Down
MPG	1.01E-05	Down
COX7A1	1.02E-05	Down
SWAP70	1.05E-05	Down
MS4A1	1.05E-05	Down
IFT88	1.09E-05	Down
DND1	1.12E-05	Down
KCNQ1	1.16E-05	Down
CH25H	1.21E-05	Down
LYL1	1.22E-05	Down
SRPX	1.24E-05	Down
NME5	1.30E-05	Down
CEBPG	1.30E-05	Down
PLA2G16	1.32E-05	Down
ZNF395	1.41E-05	Down
PLEKHB1	1.47E-05	Down
C11orf71	1.49E-05	Down
RAD51	1.54E-05	Down
BEX4	1.63E-05	Down
C3	1.71E-05	Down
SMPD1	1.75E-05	Down
CLCF1	1.76E-05	Down
GPRIN2	1.77E-05	Down
SGSM2	1.79E-05	Down
CAND2	2.00E-05	Down
WDHD1	2.10E-05	Down
ARMCX6	2.10E-05	Down
FGD2	2.18E-05	Down
FMO2	2.21E-05	Down
C6	2.35E-05	Down
LMBRD1	2.53E-05	Down
AK2	2.53E-05	Down
ABCA7	2.71E-05	Down
NXF2	2.75E-05	Down
GABRP	2.82E-05	Down
DNAH6	2.84E-05	Down
CTDSP1	2.86E-05	Down
PLAC8	2.97E-05	Down

Genes	FDR	Up/down regulation
BCAS4	3.03E-05	Down
BAALC	3.03E-05	Down
GTF2F2	3.05E-05	Down
VILL	3.12E-05	Down
POLD4	3.17E-05	Down
ITIH5	3.17E-05	Down
DAZAP2	3.17E-05	Down
SIGIRR	3.22E-05	Down
EPHB6	3.30E-05	Down
MEIS2	3.35E-05	Down
ICAM3	3.35E-05	Down
CCR6	3.35E-05	Down
PSD4	3.49E-05	Down
EFCAB1	3.64E-05	Down
TGFBR3	3.78E-05	Down
TMEM184B	3.84E-05	Down
SPATA6	3.85E-05	Down
CRLF1	3.95E-05	Down
CLDN8	4.17E-05	Down
ADCY2	4.30E-05	Down
PIGH	4.32E-05	Down
TFF1	4.33E-05	Down
TSPAN1	4.55E-05	Down
SIDT1	4.63E-05	Down
PHF1	4.65E-05	Down
ARHGAP44	4.76E-05	Down
TMEM121	4.97E-05	Down
CRIP2	4.99E-05	Down
APOM	5.07E-05	Down
FUCA1	5.18E-05	Down
NEIL1	5.19E-05	Down
LPAR1	5.20E-05	Down
PSMA2	5.25E-05	Down
LRIG1	5.32E-05	Down
SRSF5	5.44E-05	Down
ZFP106	5.54E-05	Down
CRIP1	5.64E-05	Down
MAST3	5.82E-05	Down
DHX38	5.82E-05	Down
PRKCB	6.01E-05	Down
MRPL19	6.11E-05	Down
ABCD4	6.37E-05	Down
HSD17B8	6.72E-05	Down
GPR18	6.72E-05	Down
DNAH3	6.72E-05	Down
ADAMTSL3	6.72E-05	Down
INHBB	6.77E-05	Down
NCR3	6.79E-05	Down
CLDN3	6.79E-05	Down
RNASE1	6.80E-05	Down
CCNA1	6.86E-05	Down
GOLGA8A	7.00E-05	Down
PDZD2	7.03E-05	Down
CCDC19	7.03E-05	Down
EIF1B	7.25E-05	Down

Genes	FDR	Up/down regulation
DLEC1	7.32E-05	Down
EIF3J	7.41E-05	Down
S1PR4	7.82E-05	Down
ACTR10	7.96E-05	Down
CHL1	7.99E-05	Down
PPRC1	8.05E-05	Down
TSPAN32	8.06E-05	Down
LRRC23	8.06E-05	Down
CGRRF1	8.29E-05	Down
EFCAB6	8.32E-05	Down
LAMB2	8.40E-05	Down
CAP2	8.51E-05	Down
DUSP22	8.73E-05	Down
ADAM28	8.76E-05	Down
LMAN1	8.77E-05	Down
TDG	8.81E-05	Down
AES	9.04E-05	Down
VNN2	9.22E-05	Down
NUCB2	9.22E-05	Down
TIMP4	9.25E-05	Down
TLE2	9.39E-05	Down
PROS1	9.40E-05	Down
EPHX1	9.74E-05	Down
PGK1	9.76E-05	Down
CLCN4	9.78E-05	Down
YPEL5	9.90E-05	Down
ERCC1	1.01E-04	Down
TMC6	1.01E-04	Down
RASGRP2	1.05E-04	Down
SERPINB7	1.05E-04	Down
ZFYVE21	1.05E-04	Down
GAK	1.06E-04	Down
GFOD1	1.07E-04	Down
ARHGAP11A	1.07E-04	Down
MAGIX	1.08E-04	Down
SPAG6	1.16E-04	Down
LRMP	1.17E-04	Down
FLII	1.18E-04	Down
SNED1	1.21E-04	Down
PPOX	1.24E-04	Down
UBA7	1.24E-04	Down
BASP1	1.24E-04	Down
IGJ	1.27E-04	Down
C5orf4	1.32E-04	Down
CTNS	1.34E-04	Down
TEX264	1.35E-04	Down
ABHD6	1.36E-04	Down
TIMM13	1.39E-04	Down
CCDC28A	1.40E-04	Down
P4HTM	1.40E-04	Down
IFIH1	1.43E-04	Down
HIP1R	1.44E-04	Down
STS	1.46E-04	Down
ATP7B	1.46E-04	Down
STAG3	1.54E-04	Down

Genes	FDR	Up/down regulation
ZNF137P	1.56E-04	Down
ZNF528	1.57E-04	Down
TMEM9B	1.57E-04	Down
PACRG	1.57E-04	Down
DEPDC5	1.63E-04	Down
CCDC81	1.65E-04	Down
PRIM2	1.70E-04	Down
RPS6KA1	1.72E-04	Down
C14orf1	1.73E-04	Down
CDT1	1.74E-04	Down
NLRP1	1.76E-04	Down
LPPR3	1.77E-04	Down
ANKHD1	1.77E-04	Down
CD52	1.79E-04	Down
TCL1A	1.79E-04	Down
ERCC5	1.79E-04	Down
RNASET2	1.81E-04	Down
KRT7	1.81E-04	Down
FAM184A	1.81E-04	Down
TMEM132A	1.81E-04	Down
C11orf16	1.82E-04	Down
CAMTA1	1.82E-04	Down
HDC	1.93E-04	Down
NIPSNAP3B	1.96E-04	Down
LIMD2	1.98E-04	Down
BAP1	2.02E-04	Down
ZNF821	2.06E-04	Down
ZBBX	2.09E-04	Down
ZCWPW1	2.18E-04	Down
TNNC2	2.18E-04	Down
CALM1	2.22E-04	Down
MAPK14	2.23E-04	Down
SEL1L3	2.26E-04	Down
VT11B	2.28E-04	Down
P2RX5	2.28E-04	Down
CHAF1A	2.29E-04	Down
SGTA	2.30E-04	Down
MNS1	2.35E-04	Down
FYCO1	2.35E-04	Down
IL33	2.35E-04	Down
SCUBE2	2.38E-04	Down
TRIM24	2.41E-04	Down
HSD17B2	2.42E-04	Down
RASAL1	2.42E-04	Down
ANXA11	2.44E-04	Down
B3GNT3	2.44E-04	Down
GLT8D1	2.45E-04	Down
BCAR3	2.45E-04	Down
ZBTB16	2.47E-04	Down
ADRA2A	2.49E-04	Down
USP19	2.51E-04	Down
RARRES2	2.51E-04	Down
SLC46A3	2.60E-04	Down
MAGOHB	2.62E-04	Down
SIRT3	2.63E-04	Down

Genes	FDR	Up/down regulation
STAP1	2.64E-04	Down
INPP4B	2.66E-04	Down
PEPD	2.66E-04	Down
JHDM1D	2.80E-04	Down
COBL	2.80E-04	Down
ITIH4	2.81E-04	Down
DOCK3	2.84E-04	Down
ADH1C	2.86E-04	Down
C11orf2	2.87E-04	Down
RHOBTB2	3.00E-04	Down
SNW1	3.01E-04	Down
CD79A	3.02E-04	Down
TMC5	3.06E-04	Down
ALDH3B1	3.14E-04	Down
CDC14A	3.22E-04	Down
SNTA1	3.23E-04	Down
LYN	3.32E-04	Down
LTF	3.33E-04	Down
MLYCD	3.41E-04	Down
DNAI1	3.45E-04	Down
C1orf115	3.45E-04	Down
CBX6	3.46E-04	Down
KCNJ12	3.63E-04	Down
ZBTB25	3.65E-04	Down
UNC93B1	3.69E-04	Down
C7orf44	3.72E-04	Down
KIAA0125	3.80E-04	Down
PARP3	3.94E-04	Down
LOC284244	3.99E-04	Down
FOLR1	3.99E-04	Down
PPFIA4	4.13E-04	Down
PKIG	4.15E-04	Down
MOAP1	4.17E-04	Down
C17orf59	4.24E-04	Down
EFHC1	4.26E-04	Down
CRISP2	4.26E-04	Down
ANK2	4.29E-04	Down
RHOH	4.40E-04	Down
APOD	4.45E-04	Down
TFEB	4.49E-04	Down
PAIP2B	4.49E-04	Down
CREBZF	4.52E-04	Down
OCEL1	4.64E-04	Down
SNX3	4.72E-04	Down
TFB2M	4.82E-04	Down
ALDH6A1	4.90E-04	Down
RPGRIP1	4.93E-04	Down
PNMA1	5.02E-04	Down
TCEB1	5.18E-04	Down
ATP5L	5.25E-04	Down
LOC728855	5.28E-04	Down
RPL36AL	5.52E-04	Down
XRCC4	5.69E-04	Down
TMEM63A	5.74E-04	Down

Genes	FDR	Up/down regulation
C9orf9	5.92E-04	Down
SLC15A2	6.00E-04	Down
FAIM3	6.20E-04	Down
VAV1	6.20E-04	Down
CHKB	6.24E-04	Down
CP	6.41E-04	Down
PAF1	6.53E-04	Down
MAGED2	6.58E-04	Down
RRAD	6.58E-04	Down
TXNIP	6.64E-04	Down
ZFP161	6.79E-04	Down
DIO1	6.79E-04	Down
PTGER2	6.81E-04	Down
SATB1	6.82E-04	Down
PRR15L	6.82E-04	Down
TRADD	6.87E-04	Down
SLC1A1	6.88E-04	Down
CHD7	7.19E-04	Down
CRYM	7.20E-04	Down
C7	7.20E-04	Down
RBM38	7.34E-04	Down
WWP2	7.40E-04	Down
DTYMK	7.48E-04	Down
OR7E47P	7.52E-04	Down
NIP7	7.54E-04	Down
FAM50B	7.57E-04	Down
XPA	7.58E-04	Down
KLHL7	7.73E-04	Down
ZNF839	7.81E-04	Down
SLC34A1	7.81E-04	Down
FBXL2	7.85E-04	Down
PLSCR4	7.92E-04	Down
PBXIP1	7.93E-04	Down
NAT6	7.94E-04	Down
PPIL2	7.97E-04	Down
KLHL36	8.19E-04	Down
MAN2B2	8.24E-04	Down
EHD1	8.42E-04	Down
GPR183	8.75E-04	Down
MFAP4	8.84E-04	Down
HCLS1	8.99E-04	Down
APEH	9.01E-04	Down
LOC100129973	9.17E-04	Down
DPAGT1	9.23E-04	Down
TREX1	9.39E-04	Down
PPP3CC	9.39E-04	Down
ATG4A	9.44E-04	Down
LY9	9.49E-04	Down
TERF2IP	9.53E-04	Down
HSPA9	9.53E-04	Down
ITGA7	9.61E-04	Down
CAPNS1	9.63E-04	Down

FDR: false discovery rate

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