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Global transcriptome study of Dip2B-deficient mouse embryonic lung fibroblast reveals its important roles in cell proliferation and development



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ABSTRACT

Disco-interacting protein 2 homolog B (Dip2B) is a member of Dip2 family encoded by Dip2b gene. Dip2B has been reported to regulate murine epithelial KIT⁺ progenitor cell expansion and differentiation epigenetically via exosomal miRNA targeting during salivary gland organogenesis. However, its molecular functions, cellular activities and biological process remain unstudied. Here, we investigated the transcriptome of Dip2B-deficient mouse embryonic lung fibroblasts (MELFs) isolated from E14.5 embryos by RNA-Seq. Expression profiling identified 1369 and 1104 differentially expressed genes (DEGs) from Dip2b^{-/-} and $Dip2b^{+/-}$ MELFs in comparisons to wild-type ($Dip2b^{+/+}$). Functional clustering of DEGs revealed that many gene ontology terms belong to membrane activities such as 'integral component of plasma membrane', and 'ion channel activity', suggesting possible roles of Dip2B in membrane integrity and membrane function. KEGG pathway analysis revealed that multiple metabolic pathways are affected in $Dip2b^{-/-}$ and $Dip2b^{+/-}$ when compared to $Dip2b^{+/+}$ MELFs. These include 'protein digestion and absorption', 'pancreatic secretion' and 'steroid hormone synthesis pathway'. These results suggest that Dip2B may play important roles in metabolism. Molecular function analysis shows transcription factors including Hox-genes, bHLH-genes, and Forkhead-genes are significantly down-regulated in Dip2b^{-/-} MELFs. These genes are critical in embryo development and cell differentiation. In addition, Dip2B-deficient MELFs demonstrated a reduction in cell proliferation and migration, and an increase in apoptosis. All results indicate that Dip2B plays multiple roles in cell proliferation, migration and apoptosis during embryogenesis and may participate in control of metabolism. This study provides valuable information for further understanding of the function and regulatory mechanisms of Dip2B.

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1. Introduction

Disco gene was identified as a transcription factor with two C2H2 type zinc finger domains that involve neuronal connection in visual system of Drosophila melanogaster [1-2]. By using yeast two-hybrid system, Mukhopadhyay et al. [3] identified a protein that interacts with Disco and named disco-interacting protein 2 (Dip2). Dip2 is highly conserved from insects to mammals and

evolved into three different proteins, DIP2A, DIP2B and DIP2C in mammals [3]. Based on amino acid sequences, Dip2B has three putative functional domains, a binding domain for the transcriptional regulator DMAP1 (DNMT1-associated protein 1), an AMPbinding domain and an adenylate-forming domain [4]. Study suggests that Dip2B may play important roles in DNA methylation and metabolism. Dip2B may epigenetically control cell proliferation and differentiation through DNA methylation [4]. Dip2B deficiency has been associated with mental retardation and developmental delay [4,5]. A small RNA miR-133b-3p can down-regulate Dip2B and epigenetically repress genes for KIT⁺K5 progenitor cell expansion [6]. A correlation in methylation status between colorectal cancer and aberrant miR-133b expression was also reported [7].

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However, the mechanism underlining most of Dip2B's roles including on cell proliferation, cell differentiation are still not clear.

RNA sequencing (RNA-Seq) takes advantage of the secondgeneration sequencing technology and is a powerful approach for studying global gene expression profile in a particular cell or tissue. It helps us to understand the regulatory network of genes and pathways [8,9]. Fibroblasts cells were identified as ubiquitous mesenchymal cells that play many essential roles including tissue repair and wound healing [10]. Embryonic fibroblasts are highly diversified and have potential to differentiate into different cell types. Mouse embryonic lung fibroblasts (MELFs) cells from genetically manipulated mouse models have been used to study the molecular mechanism of genes and their regulatory networks, especially those genes that their ablations resulted in mouse prenatal lethality. To explore the potential role of Dip2B, MELFs were isolated from embryos of homozygous knockout ($Dip2b^{-/-}$). heterozygous $(Dip2b^{+/-})$ and wild type $(Dip2b^{+/+})$ at E14.5. Genes and pathways under Dip2B regulation were investigated.

2. Materials and methods

2.1. Animals

Mouse study has been approved by Institutional Animal Care and Use Committee for Animal Experimental Ethics Committee of Northeast Normal University with approval number of (NENU/ IACUC, AP2018011) and carried out in accordance with the *Guide for Care and Use of Laboratory Animals of National Institutes of Health* as well. Mice were housed in a pathogen-free facility in Northeast Normal University with temperature at 21 ± 1 °C, humidity 30– 60%, 12:12 light/dark cycles and free access to water and food.

2.2. Isolation and culture of lung MELFs

MELFs were isolated from mouse embryos obtained by intercrossing of $Dip2b^{+/-}$ transgenic mice. $Dip2b^{+/-}$ pregnant mouse at E14.5 day post-coitus (p.c.) was euthanized by cervical dislocation and soaked in 70% ethanol for 5 min, then the uterine horns were dissected out and rinsed in 70% EtOH. Uterine horns were then transferred into petri dish and the embryos were separated individually. Visceral organs of each embryo were removed except lungs. Lungs were washed in $1 \times PBS$ and placed in a new Petri dish. Lung tissues were gently minced using a sterile razor blade till possible to pipette. The tissues were then incubated with 0.25% trypsin (Sigma Aldrich) at 37 °C. Trypsin was inactivated 15 min later by adding Dulbecco's modified Eagle's medium (DMEM) (Gibco, Shanghai, China) containing 10% fetal bovine serum (Sigma, St Louis, USA) and 1% penicillin/streptomycin (Invitrogen Life Technologies, USA). Plates were coated with 0.2% gelatin (Gelatin from bovine skin, Type B, Sigma) for 2 h. The cells were then cultured at 37 °C in a humidified incubator with 5% CO₂.

2.3. Hematoxylin and eosin staining (H&E)

Mice embryos from E19.5 were dissected out from uterus and fixed with 4% paraformaldehyde (PFA) (Sigma-Aldrich; EMD Millipore) for overnight at 4 °C. Fixed tissues were then washed three times in 1x PBS and processed in 70% ethanol. Tissues were dehydrated with a series of graded alcohols, cleared in xylene, and embedded in paraffin. Six- μ m thick sections were obtained, mounted on glass slides and stained with Hematoxylin and eosin using standard protocol. Images were taken using microscope (Olympus, Tokyo, Japan).

2.4. Cell proliferation assay by MTT

Methylthiazolyldiphenyl-tetrazolium (MTT, Sigma) test was performed on $Dip2b^{-/-}$, $Dip2b^{+/-}$ and $Dip2b^{+/+}$ MELFs to measure the proliferation and cell viability. MELFs were detached in 0.25% trypsin/EDTA and seeded into 96-well plates with 5×10^3 cells per well in 200 µl DMEM containing 10% FBS. Cells were incubated at 37 °C and 5% CO₂ for 24 h and allowed to grow to 70–80% confluence. Then 20 µl of MTT (5 mg/ml in 1× PBS) was added to each well and incubated for 4hrs. The medium was discarded and 100 µl of dimethyl sulfoxide (DMSO) added to each well. Optical density was analyzed at 490 nm using (BioTek Instruments, Winooski, VT). A total of 4 replicate for each sample was prepared and experiment repeated 3 times.

2.5. Cell apoptosis analysis by FITC-Annexin-V and PI

Apoptosis was detected using FITC-Annexin-V and PI staining kit (Cat #630109, Takara, Japan) according to manufacturer's instructions. Briefly, $Dip2b^{-/-}$, $Dip2b^{-/+}$ and WT MELFs were seeded in 6-well plates at a concentration of 3×10^5 cells/well. MELFs were trypsinized and washed twice with 1X PBS. A 5 µl Annexin V-FITC and 10 µl propidium iodide (PI) at 50 µg/ml in $1 \times$ binding buffer (10 mM HEPES/pH 7.4, 140 mM NaOH, 2.5 mM CaCl₂) were added for 15 min at room temperature in the dark. Apoptotic cells were analyzed using a Becton-Dickinson FACScan cytofluorometer (Mansfield, MA). Both early (Annexin V-positive, PI negative) and late (Annexin V-positive and PI-positive) were considered as apoptotic cells.

2.6. Cell cycle analysis

 $Dip2b^{+/+}$, $Dip2b^{+/-}$ and $Dip2b^{-/-}$ MELFs were seeded in 6-well plates at a density of 2 \times 10⁵ cells/well. Cells were trypsinized and washed twice with 1 \times PBS. Then cells were fixed with 70% ice-cold EtOH overnight. Fixed cells were treated with RNase (200 µg/ml) for 2hrs at RT, followed by staining with 500 µl PI (50 µg/ml) (Cat#630109, Takara, Japan). Cells were transferred to FACS tubes and incubated for 30 min at RT in dark. Afterwards, cells were subjected to FACS analysis using Caliber flow cytometer (Becton Dickinson, USA). Dead cells and cell debris were excluded.

2.7. Cell migration assay

Cells were grown in 6-well culture plates. Confluent monolayer was scratched using a 200 μ l pipette tips and medium replaced with fresh medium to remove cell debris. Cells were allowed to grow for 48 h. Photographs were taken with an inverted microscope (Olympus, Tokyo, Japan). Digital straight lines were drawn on the borders of scratches and distance of cell growth with time was recorded.

2.8. Preparation of RNA-Seq libraries

Total RNA was purified from MELFs of *Dip2b^{+/+}*, *Dip2b^{+/-}* and *Dip2b^{-/-}* using RNAiso plus reagent (Takara, Dalian, China) according to manufacturer's protocol. RNA concentration and quality were determined using NanoDrop 2000 (Thermo Fisher Scientific, USA) and Bioanalyzer (Agilent Technologies, USA). Messenger RNA was purified using oligo (dT)-attached magnetic beads, fragmented before cDNA Synthesis.



Fig. 1. MELF cell isolation and *Dip2b* mRNA expression analysis by qPCR (A) Cell images of MELF cultures at low (Top penal) and high (Bottom panel) density. (B) Relative expression levels of *Dip2b* mRNA in MELFs by qPCR. (C) Gel electrophoresis image showing PCR products.

Table	1
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Summary of sequencing data processing.

Sample	Total Mapping (%)	Uniquely Mapping (%)
Dip2b ^{+/+}	97.06	79.67
Dip2b ^{+/–}	97.08	80.82
Dip2b ^{–/–}	96.98	81.42

2.9. RNA-Seq data processing and identification of differentially expressed genes (DEGs)

Low quality reads, adaptor-only and reads with more than one unknown base (N) were removed through SOAPnuke software [11] to obtain clean reads. Q20 (%), Q30 (%) and GC content (%) were calculated. More than 1.14 Gigabyte clean reads were obtained as FASTQ format from each library. Reads were assembled into longer transcripts and mapped to reference genome using HISAT (Hierarchical Indexing for Spliced Alignment of Transcripts) [12] and Bowtie2 tool [13].

The level of transcripts was quantified and presented as pairedend RNA-Seq FPKM (Fragments Per Kilobase per Million mapped reads) normalized reads. Differentially expressed genes (DEGs) were identified by comparison of two different libraries using Possion distribution [14] and Expectation-Maximization (RSEM) softwares [15].

2.10. Gene ontology and Kyoto Encyclopedia of genes and genome pathways analysis

DEGs with FDR of \leq 0.01 and absolute value of FC \geq 2 (Two-fold change) were considered significant for further analysis. Gene ontology (GO) annotation enrichment analysis of DEGs was implemented using GOseq R package software and estimated by hypergeometric test. DEGs were also used to identify the enriched Kyoto Encyclopedia of Genes and Genome (KEGG) pathways [16].

2.11. Validation of RNA-Seq results by quantitative real-time PCR (qPCR)

One μg of total RNA was reverse-transcribed into first-strand complementary DNA (cDNA) with Prime Script RT Reagent Kit

Table 2Statistical summary of filtered reads.

(Perfect Real Time, TaKaRa, Dalian, China) according to the manufacturer's instructions. Quantitative real-time PCR (qPCR) was performed with 50 ng of cDNA using One-Step SYBR PrimeScript[™] RT-PCR kit (Takara, Dalian, China). All reactions were performed in triplicate. All primers were initially evaluated for efficiency using relative standard curve and electrophoresis on gel.

2.12. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.01 (GraphPad Software Inc). Significant differences between groups were evaluated using Student's *t* test. *P*-values were two-sided and *P*-values <0.05 was considered statistically significant.

3. Results and discussion

3.1. Dip2b mRNA expression level of Dip2B-deficient MELFs

Since $Dip2b^{-/-}$ mice dies postnatally and lung development seems the major cause (Supplementary Fig. 1), intercrosses of $Dip2b^{+/-}$ mice were used to prepare MELFs and total RNAs from all three genotypes, $Dip2b^{-/-}$, $Dip2b^{+/-}$ and $Dip2b^{+/+}$ at E14.5 (Fig. 1A). Dip2b mRNA expression in MELFs was confirmed by quantitative real-time PCR (qPCR) (Fig. 1B, C). Results show expected decrease of Dip2b mRNA expression. The mRNAs from MELFs were prepared for RNA-Seq.

3.2. Gene expression profiling of MELFs under Dip2B

To study the potential biological role of Dip2B, three libraries were generated from *Dip2b^{-/-}*, *Dip2b^{+/-}* and *Dip2b^{+/+}*MELFs and analyzed on BGISEQ-500 platform. The raw data of RNA-Seq is accessible at the Sequence Read Archive (SRA) database in NCBI with the following link (https://www.ncbi.nlm.nih.gov/bioproject/ PRJNA647133/) under the accession number PRJNA647133. Clean reads were mapped to *Mus musculus* reference genome (GRCm38. p6/NCBI, GCF_000001635.26). As shown in Table 1, ~97% of total mapping was acquired and >80% was uniquely mapped, indicating the reliability of sequencing data. Table 2 is summarizing the sequencing reads among samples. Normalized FPKM was calculated and expression level distribution shown in (Supplementary

Sample	Total Raw Reads (M)	Total Clean Reads (M)	Total Clean Bases (Gb)	Clean Reads Q20	Clean Reads Q30	Clean Reads Ratio
Dip2b ^{+/+}	23.38	22.7	1.14	99.12	96.3	97.1
Dip2b ^{+/–}	23.51	22.92	1.15	99.19	96.75	97.5
Dip2b ^{-/–}	23.44	22.81	1.14	99.1	96.55	97.3



Fig. 2. Overview of gene expression profiling. (A) Venn diagram showing unique and overlapping DEGs between heterozygous and homozygous MELFs (FC ≥ 1 and FDR ≤ 0.001). (B) Number of up- and down-regulated DEGs in *Dip2b^{+/-}* and *Dip2b^{+/-}* vs to *Dip2b^{+/+}* (C) Volcano plots highlighting significant DEG among three comparative samples. Each dot in plot corresponds to one differentially expressed gene, the y-axis represents $-\log_{10}$ (FDR) and the x-axis displays the differences of FC values in samples. Blue and red dots represent up- and down-regulated differentially expressed genes, whereas gray dots indicate genes with no change. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 2A). The overlapped genes were identified 1677 and 118 genes differentially expressed in both homozygous and heterozygous MELFs. 269 unique DEGs were expressed in $Dip2b^{+/-}$, 740 unique DEGs were expressed in $Dip2b^{-/-}$ vs to $Dip2b^{+/+}$ and 73 unique DEGs were expressed in $Dip2b^{+/-}$ vs to $Dip2b^{-/-}$ MELFs respectively (Fig. 2A).

Comparisons between $Dip2b^{-/-}$ vs $Dip2b^{+/+}$ and $Dip2b^{+/-}$ vs $Dip2b^{+/+}$ identified 1369 and 1104 differentially expressed genes (DEGs) based on fold change ≥ 2 and adjusted FDR ≤ 0.01 . Among them, 839 and 549 are significantly up- and 530 and 555 significantly down-regulated (Fig. 2B). Volcano plot and heatmap are used to show the significant DEGs (Fig. 2C and Supplementary Fig. 2B). Fifty of the most up- and down-regulated DEGs are listed in Table 3 and Supplementary Table 1. Among them, six genes *Ear2*, *Entpd4*, *Fpr2*, *Hist2h3c1*, *Zfp967* and *Gm14296* are the most significantly up-regulated while 6 genes *Hmga1b*, *Havcr1*, *Pax2*, *Pax8*, *Hoxa10*, *Hoxa11* and *Hoxac10* are the most significantly down-regulated.

3.3. Gene ontology (GO) analysis of Dip2b-regulated DEGs.

In order to identify the potential biological roles of Dip2B in embryonic stages, DEGs identified from comparison of Dip2Bdeficient and WT MELFs were used for GO analysis. GO is classified into three independent categories, biological process (BP), molecular function (MF) and cellular component (CC). Each DEG could be assigned to one or more GO terms. A total of 1369 and 1104 DEGs with FC > 2 and FDR < 0.01 were identified from comparisons of $Dip2b^{+/-}$ vs $Dip2b^{+/+}$ and $Dip2b^{+/-}$ vs $Dip2b^{+/+}$ MELFs. GO analysis found 28 BP. 17 MF and 12 CC significantly annotated (Fig. 3, Supplementary Fig. 3 and Supplementary Fig. 4). Annotated BP categories include 'cell adhesion', 'cell differentiation', 'regulation of signaling receptor activity', 'multicellular organism development'. MF categories include 'ion channel activity', 'ligand-gated sodium channel activity', 'voltage-gated potassium channel activity', 'extracellular matrix structural constituent', 'calcium ion binding' and 'DNA binding transcription activity'. DEGs-annotated CC categories are 'extracellular region', 'integral component of plasma membrane', 'anchored component of plasma membrane', 'basolateral plasma membrane' 'cornified envelop', 'cell surface' and 'apical plasma membrane' (Supplementary Table 2). Based on DAVID (Database for Annotation, Visualization and Integrated Discovery [17,18]), the functional annotation clustering tool revealed that a list of cluster terms from BP, MF, and CC categories were mainly enriched with DEGs related to membrane structure and its activities (Supplementary Table 3). All the results suggest that Dip2B may play important roles in cell to cell interactions, membrane integrity and membrane activities which are critical for cell differentiation and function.

Dip2B-deficient MELFs resulted in upregulation of 60 (*Dip2b^{-/-}* vs *Dip2b^{+/+}*) and 52 (*Dip2b^{+/-}* vs *Dip2b^{+/+}*) DEGs that are involved in immune system and inflammatory responses respectively. In immune system process, 43 genes were enriched in innate immune response, 14 genes in defense response to virus and 11 genes in adaptive immune response (Supplementary Table 4, Supplementary Table 5). In inflammatory response category, most of the genes are chemotaxis genes including (*Fpr2, Fpr1, Ccr2, Cxcl15, Ccl9, Ccl6, Ccl11, Cxcl2, Ccl22, S100a8, Ccl12, Ccr5, Ccl7, Pik3cg, Ccl2, Cxcl3*), 12 genes in neutrophil chemotaxis, 7 genes in Eosinophil chemotaxis, 8 genes in monocyte chemotaxis and 8 genes in lymphocyte chemotaxis (Supplementary Table 6), indicating the importance of Dip2B in regulation of cell migration in immune responses.

3.4. KEGG pathway analysis on Dip2B-regulated DEGs

To elucidate the potential biological pathways under Dip2B regulation, KEGG pathway analysis was performed for Dip2B-regulated DEGs. KEGG classification revealed that 'signal transduction' and 'immune system' terms have the highest number of DEGs (Supplementary Fig. 5). The Q value <0.05 was considered significantly represented and KEGG pathways were enriched based on FC \geq 2 and FDR < 0.01. Top 20 most enriched pathways between Dip2b^{-/-} vs Dip2b^{+/+} and Dip2b^{+/+} vs Dip2b^{+/-} are shown in Fig. 4

and Supplementary Fig. 6. Among them, 'ko04974 protein digestion and absorption', 'ko04924 renin secretion', 'ko04972 pancreatic secretion', 'ko04060 cytokines-cytokines receptor interaction' and 'ko00140 steroid hormone synthesis' metabolism pathways are the most significantly down-regulated pathways, whereas 'ko04380 Osteoclast differentiation', 'ko04621 NOD-like receptor signaling pathway' and 'ko04145 Phagosome' were the most upregulated pathways (Supplementary Table 7a and 7b). Results demonstrate that multiple pathways are under Dip2B regulation and confirms that Dip2B is important in regulating membrane activities and be involved in regulation of metabolism.

3.5. Hox gene family are regulated by Dip2B

Homeodomain proteins encoded by *Hox* genes are responsible for regulating expression of many target genes involved in cell proliferation and differentiation [19–22]. These genes are also known to be involved in cancers and controlled by DNA methylation

Computational and Structural Biotechnology Journal 18 (2020) 2381–2390

[23,24]. Among differentially expressed transcription factors (TFs), homeobox (*Hox*) gene family was the most dysregulated genes in Dip2B-deficient MELFs (38 down- and 6 up-regulated genes in both heterozygous and homozygous MELFs). Results strongly suggest that Dip2B may regulate embryo development and differentiation through *Hox* gene expression. Dip2B contains DMAP1 binding domain and may regulate *Hox* gene transcription by DNA methylation [4]. Several TFs that belong to zinc finger (zf-C2H2), basic helix-loop-helix (bHLH) and Fox proteins are also significantly dysregulated. TFs with fold change \geq 2 and FDR < 0.01 are listed in Table 4 and Supplementary Tables 8 and 9.

3.6. Dip2B regulates cell proliferation

Cell proliferation is an important process during development and tissue maintenance [25]. GO analysis shows that DEGs were significantly enriched in 'GO:0008285 ~negative regulation of cell proliferation' and 'GO:0030308 ~negative regulation of cell

Table 3

The 50 most differentially expressed genes between $Dip2b^{+/+}$ vs $Dip2b^{-/-}$ (Fold change \geq 2, FDR < 0.01).

far1 13.11374217 0 0 Pack -10.82815648 2.238-12 4.84E-13 Ear10 10.37424254 0 0 Haver1 -10.82815688 2.238-12 4.84E-13 Ear2 10.37424254 0 0 Haver1 -10.4850955 1.70E-104 6.57E-164 Enol b 9.370433927 0 0 Hava10 -10.24317398 1.20E-302 1.60E-344 Enol b 8.37957214 7.78E-15 1.50E-15 Hava10 -9.84073724 5.05E-141 Endd 8.258E1808 2.50E-53 2.43E-33 c.cdr198 -9.84073724 5.05E-161 3.81E-52 Zip667 8.184875343 6.81E-36 6.67E-37 Hmc10 -9.564784619 1.18E-245 1.93E-247 Middl1 8.05698797 7.06E-6 2.34E-66 Hava70 -9.518245602 2.19E-17 3.80E-18 Gril 4250 7.768184252 6.93E-404 1.70E-14 Clar -9.54874562 2.19E-14 Middr21 8.6665331 1.34E-15	Up-regulated genes	$\text{FC} \geq 2$	FDR	P value	Down-regulated genes	FC < 2	FDR	P value
Earlo 11.05934445 5.87E-53 4.20E-54 Pac2 -10.03150268 6.36E-13 1.34E-13 Earlo 0.35574956 7.82E-95 3.25E-96 Spr2f -10.4817938 1.20E-43 8.77E-104 6.77E-106 Enrol 9.370433277 0 0 Haxa10 -10.2431738 1.20E-138 1.20E-138 1.20E-138 2.20E-104 8.77E-104 6.77E-106 9.372 1.94E-139 5.62E-141 Entpd4 8.5985508 2.20E-65 1.47E-66 Haxa10 -9.50328505 2.22E-108 8.23E-110 Stell 8.23881869 3.05E-86 2.37E-76 7.05E-06 1.93E-247 1.93E-247 3.81E-62 5.98E-07 7.011 -9.584784612 1.18E-45 1.93E-247 7.44E-59 7.44E-59 7.44E-59 7.44E-59 7.44E-59 7.44E-57 7.47E-59 7.44E-59 7.44E-59 <th< td=""><td>Ear1</td><td>13.11374217</td><td>0</td><td>0</td><td>Pax8</td><td>-10.82813648</td><td>2.23E-12</td><td>4.84E-13</td></th<>	Ear1	13.11374217	0	0	Pax8	-10.82813648	2.23E-12	4.84E-13
far2 10.37424254 0 0 Harr -10.48850865 1.70E-104 6.57E-105 pr2 205.9745 7.32E-95 3.25E-96 Spr21 -10.2431738 1.20E-302 1.60E-34 bit 8.359550214 7.79E-15 1.50E-15 Hxx010 -10.2431738 1.20E-302 5.62E-141 Engl 8.56855608 2.50E-65 1.47E-66 Hxx010 -9.54828566 0 0 Sell 8.258F1331 0.56E-38 2.38E-37 Hmg1b -9.53828566 0 0 Vigic1 8.06608919 1.88E-06 5.88E-07 TjT -9.53874568 2.19E-17 3.80E-18 Gif1 8.056905 7.498506 5.28E-07 TjT -9.398745892 2.19E-17 3.80E-18 Gif1 8.056905 7.398506 5.28E-07 TjT -9.398745892 2.19E-17 3.80E-18 Gif1 8.056905 7.798140 1.39E-047 JJBE JBE JBE JBE JBE JBE JBE JBE	Ear10	11.05934446	5.87E-53	4.20E-54	Pax2	-10.60316268	6.36E-13	1.34E-13
pp2 10.35974956 7.82E-95 3.25E-96 Spr2/1 -10.46147948 1.11E-46 8.74E-48 Nulqi11b 8.393579214 7.79E-15 1.50E-15 Hox11 -9.03578214 2.0E-302 1.60E-304 Nulqi11b 8.393579214 7.79E-15 Hox11 -9.03578255 2.22E-108 8.23E-110 Self 8.22881869 3.05E-38 2.83E-39 ccdc198 -9.84077724 6.00E-61 3.81E-62 Zip667 8.14875343 6.81E-36 6.67E-37 Hmc10 -9.35474619 1.18E-245 1.93E-74 Mifdl21 8.0608919 1.38E-66 5.38E-07 TJT -9.32475124 1.08E-80 5.25E-82 Sig1 7.06E-96 5.32E-40 4.70E-61 Hace3 -9.22357813 2.75E-18 4.06E-19 Mid22b 7.49185306 5.35E-04 1.41E-04 Clas1 -8.85480331 1.9E-77 5.97E-79 Adora3 6.345490051 2.05E-15 3.83E-16 Reg1 -8.764871891 1.38E-13 7.6E-15 Gim	Ear2	10.37424254	0	0	Havcr1	-10.48850965	1.70E-104	6.57E-106
Enolb 9.370435927 0 0 Provide -10.24317398 1.20E-302 1.60E-304 Mulgit Ib 8.569855608 2.50E-65 1.47E-66 How110 -9.920352855 2.22E-108 8.23E-110 Sell 8.258E1869 3.05E-38 2.38E-39 cdc198 -9.940352855 0.22E 0.96E 8.31E-62 Zipbor 8.184875343 6.51E-36 6.67E-37 Himgalb -9.63828506 0 0 Misch 8.06608919 1.88E-06 5.98E-07 Tffi -9.398748692 1.98E-247 3.80E-18 Vigla 8.06608919 1.88E-06 5.98E-07 Tffi -9.398748692 1.98E-78 8.74E-59 Gin1 2.076E-06 2.34E-06 Haxch -9.20457114 1.08E-85 5.25E-82 Misch2ac 7.20818425 6.35E-40 1.47E-04 Cat -8.85480833 1.9E-75 5.97E-79 Misch2ac 5.32E-400 1.41E-04 Cat<1 -8.364902013 1.48E-14 1.55E-17 Misch2ac 5	Fpr2	10.35974956	7.82E-95	3.25E-96	Sprr2f	-10.46147945	1.11E-46	8.74E-48
Ndugir ib 8.939579214 7.79E-15 Hoxr11 -10.05799172 1.94E-139 5.62E-141 brind 8.22881869 3.05E-38 2.83E-39 cdc198 -9.84077724 6.00E-61 3.81E-62 2fg067 8.1487334 6.81E-36 6.67E-37 Hmmalb -9.648784619 1.18E-245 1.93E-347 Viffc21 8.0608191 1.89E-06 5.08E-07 TM -9.564784619 1.18E-245 1.93E-347 Viffc21 8.0608191 1.89E-06 5.08E-07 TM -9.36874802 2.0E-17 3.00E-18 Viffc21 8.027905997 7.06E-06 2.34E-06 Hace3 -9.221587121 1.08E-58 2.25E-58 Gm14296 7.4918425 6.55F-08 1.07E-08 Hace3 -9.02357813 2.75E-18 4.06E-19 Hist2h2co 7 3.55E-04 1.81E-16 Reg1 -8.574697133 1.95E-77 Adma's 6.584962501 7.79E-15 1.06E-15 Kade3 -9.85420038 8.74E-17 7.5E-14 Katn 6.584962501	Eno1b	9.370435927	0	0	Hoxa10	-10.24317398	1.20E-302	1.60E-304
Engind4 8.569855608 2.50E-55 1.47E-66 How/10 -9.92032855 2.22E-108 8.28E-110 Sele 2.8184869 3.05E-38 2.83E-39 ccdc198 -9.84077792 6.09E-61 3.81E-62 Zipo67 8.184875343 6.81E-36 6.67E-37 Hom/10 -9.5628866 0.00E-06 Mid2L1 8.05608919 1.89E-66 5.8E-07 TJI -9.398743602 2.19E-17 3.8DE-18 Sigh1 8.0760997 7.06E-06 2.34E-06 Hoxe -9.204571144 1.28E-57 8.74E-59 Mis2Daca 7 8.38640511 0.35E-14 1.41E-04 Clca1 -8.85480383 1.19E-77 5.97E-79 Adora 6.584905101 7.35E-14 2.37E-12 2.77E-18 7.66329915193 3.48E-13 7.18E-14 Msrn 6.58496501 7.79E-15 1.36E-16 Regin -8.51209513 2.48E-17 5.5E-17 Ang5 6.53915811 0.58E-60 1.37E-14 How/10 -8.532991519 3.4E-17 5.5E-17 <	Ndufa11b	8.939579214	7.79E-15	1.50E-15	Hoxa11	-10.05799172	1.94E-139	5.62E-141
self 8.228.81869 3.05E-38 2.83E-39 ccdc198 -9.84077924 6.09E-61 3.81E-62 Jippor 8.159871337 9.74E-07 3.02E-17 Hmach -9.564784619 1.18E-245 1.93E-247 Vigldz1 8.05668191 1.88E-06 5.58E-07 T/ff -9.398743602 2.19E-17 3.80E-18 Stp1 8.027905997 7.06E-06 2.34E-06 Haxe -9.221637121 1.08E-80 5.25E-82 Mit2Di2C 7.401853006 5.23E-04 4.07E-41 Hist2Di2C -0.022367813 2.27E-18 4.06E-19 Hist2Di3C 7.401853006 5.23E-10 4.07E-1 Baye 2.07E-13 3.33E-16 Regt -8.54867833 1.19E-77 5.97E-73 Hist2Di3C 7.40185306 0.53E-13 3.33E-16 Regt -8.54867833 1.38E-18 2.28E-19 Kinn 6.584902501 7.79E-15 3.33E-16 Regt -8.5487033 3.4E-17 1.55E-17 Mitan 6.53490350 3.55E-04 1.41E-04 Gat1	Entpd4	8.569855608	2.50E-65	1.47E-66	Hoxd10	-9.920352855	2.22E-108	8.23E-110
ZipoR7 8.14487333 6.81E-36 6.67E-37 Hmgal b -9.63828506 0 0 Hamp2 8.1687133 9.74E-07 3.02E-07 Hor(10) -9.368748612 1.18E-245 1.93E-247 WiftC1 8.06608819 1.88E-066 5.86E-07 Tfl -9.368748612 1.18E-265 5.82E-82 Hist2h3c1 7.768184225 6.95E-08 1.97E-06 Hacc3 -9.202367B13 1.32E-57 8.74E-59 Gm14296 7.491853096 5.23E-04 1.41E-04 Cla1 -8.85480583 1.15F-77 5.97E-79 Adora3 6.845490051 2.05E-15 3.83E-16 Reg1 -8.7187674423 3.48E-18 7.19E-14 Mstn 6.584965201 7.79E-15 1.50E-15 Cypda12b -8.632905197 2.87E-54 2.01E-55 Gm39743 6.539158811 6.80E-04 2.79E-04 Hoxd11 -8.581200528 8.74E-17 1.55E-17 Ang5 6.539158811 1.75E-10 A2ZE-16 Pkp1 -8.361943774 3.32E-07 7.27E-78 </td <td>Sell</td> <td>8.22881869</td> <td>3.05E-38</td> <td>2.83E-39</td> <td>ccdc198</td> <td>-9.840777924</td> <td>6.09E-61</td> <td>3.81E-62</td>	Sell	8.22881869	3.05E-38	2.83E-39	ccdc198	-9.840777924	6.09E-61	3.81E-62
Hamp2 8.159871337 9.74E-07 3.02E-07 Hax:00 -9.564784619 1.18E-245 1.93E-47 Wiftc21 8.0608919 1.89E-06 5.8E-07 Tff -9.39874562 2.19E-17 3.80E-18 Stfi1 8.027905997 7.06E-06 2.34E-06 Hax:B -9.204571144 1.32E-57 8.74E-59 Gm14290 7.481853096 5.23E-40 1.41E-04 Clcal -8.85486383 1.19E-77 5.97E-79 Adora 6.584905501 1.53E-12 2.27E-22 Tf2 -8.764871591 1.38E-18 2.28E-19 Ev2 6.584905501 1.53E-11 5.0E-15 Cydal2b -8.372987191 3.48E-13 7.19E-14 Adora 6.53915811 6.70E-10 4.22E-11 Spaca7 -8.558420731 4.44E-56 6.78E-87 Lydk 6.491853066 3.55E-04 1.41E-04 Gata1 -8.438791853 2.42E-17 7.67E-18 Bmx 6.49431619 3.45E-07 7.58E-16 Rp1 -8.347879353 2.82E-01 1.21E-92	Zfp967	8.184875343	6.81E-36	6.67E-37	Hmga1b	-9.63828506	0	0
Up/Ed/L 8.06600819 1.880-66 5.98L-07 Tf1 -9.398743692 2.19E-17 3.80E-18 Stip1 0.06E-06 2.34E-06 HoxcS -9.2215871144 1.32E-57 8.74E-59 Hist2h2ac1 7.768184325 6.95E-08 1.97E-08 HoxcS -9.204571144 1.32E-57 8.74E-59 Gm14206 7.491853096 5.23E-40 4.70E-41 Hist2h2ac2 -9.202357113 2.75E-18 4.60E-19 Adoma 6.845462501 1.53E-21 2.27E-22 Tf2 -8.7487131 1.38E-18 2.28E-19 Kv1 6.584462501 7.79E-15 1.50E-15 Cyplat12b -8.632095197 2.87E-54 2.21E-57 Amp5 6.53915811 6.80E-04 2.79E-04 Hoxd11 -8.58120052 8.74E-17 1.55E-17 Amp5 6.53915811 0.80E-04 2.79E-04 Hoxd11 -8.58120052 8.74E-57 7.57E-18 Bmx 6.459431619 3.99E-15 7.58E-16 Pkp1 -8.361943774 3.32E-01 1.21E-92 <	Hamp2	8.159871337	9.74E-07	3.02E-07	Hoxc10	-9.564784619	1.18E-245	1.93E-247
strict 8.027905997 7.06E-06 2.34E-06 Face: -9.21587121 1.06E-80 5.25E-82 Mit4206 7.491853096 5.38-04 1.97E-08 Hox:02 -9.02457114 1.32E-57 8.74E-59 Mit4206 7.491853096 5.35E-04 1.41E-04 Clca1 -8.85486333 1.19E-77 5.97E-79 Adora3 6.645490051 2.05E-15 3.83E-16 Reg1 -8.76487151 1.38E-18 2.28E-19 Ev2 6.834902501 1.53E-11 2.27E-22 T/// -8.61299197 2.87E-54 2.01E-55 Gm39743 6.539158811 6.50E-04 2.79E-04 Hox11 -8.5581200582 8.74E-17 1.55E-17 Ly6k 6.491853096 3.55E-04 1.41E-04 Gata1 -8.43271653214 4.37E-17 7.67E-18 Bmx 6.459431619 1.14E-13 2.31E-14 Hst1h4m -8.361943774 3.12E-07 9.29E-08 Cl177 6.459431619 3.65E-06 1.18E-06 Cln4343 -8.2021084713 1.48E-68 6.162837 <td>Wfdc21</td> <td>8.06608919</td> <td>1.89E-06</td> <td>5.98E-07</td> <td>Tff1</td> <td>-9.398743692</td> <td>2.19E-17</td> <td>3.80E-18</td>	Wfdc21	8.06608919	1.89E-06	5.98E-07	Tff1	-9.398743692	2.19E-17	3.80E-18
Hiszb2aci 7,68184325 6,55E-08 197E-08 HoxeO -9.024571144 1,32E-57 8,74E-59 Gm14206 7,41853096 5,23E-40 4,70E-41 Hiszb2ac2 -9.022367813 2,75E-18 4,60E-19 Hiszb2aac2 7 3,55E-04 1,41E-04 Clca1 -8.85486333 1,19E-77 5,97E-79 Adora3 6,84962501 1,53E-21 2,27E-22 TJZ -8.717676423 3,48E-13 7,19E-14 Msrn 6,584962501 7,79E-15 1,50E-15 Cyp4a12b -8.532995197 2,87E-54 2,01E-55 Gm39743 6,339158811 6,80E-04 2,79E-04 Hoxd11 -8.5512042713 1,49E-85 6,78E-87 Jyck 6,491853096 3,55E-04 1,41E-04 Gra1 -8.438791853 2,82E-91 1,21E-92 Cd177 6,459431619 3,99E-15 7,58E-16 Pkp1 -8.438791853 2,32E-91 1,21E-92 Cd1477 6,459431619 3,1EE-75 4,75E-76 Hoxd9 -8.162391329 1,74E-66 1,01E-67	Stfa1	8.027905997	7.06E-06	2.34E-06	Hoxc8	-9.221587121	1.08E-80	5.25E-82
Gm14296 7.491853066 5.28-40 4.70E-41 Hist2h2a2 -9.022367813 2.75E-18 4.60E-19 Hist2h2aa2 7 3.55E-04 1.41E-04 Clan -8.85486383 1.19E-77 5.97E-79 Adora3 6.844962501 2.05E-15 3.88E-16 Reg1 -8.717676423 3.48E-13 7.19E-14 Mstm 6.584962501 7.79E-15 1.50E-15 Cyp412b -8.531200582 8.74E-17 1.55E-17 Amp5 6.539158811 6.80E-04 2.79E-04 Mod11 -8.55420713 1.44E-85 6.78E-87 Lypk 6.49931619 3.99E-15 7.58E-16 Pkp1 -8.43671853 2.82E-91 1.21E-92 Cd177 6.459431619 3.14E-13 2.31E-14 Hist1h4m -8.361943774 3.12E-07 9.29E-68 Lipf 6.303780748 3.65E-06 1.18E-06 Cm1434 -8.290018847 1.33E-81 6.36E-83 Cm14548 6.12894530 3.09E-12 2.58E-64 1.35E-65 1.00E-05 1.01E-05 1.02E-05	Hist2h3c1	7.768184325	6.95E-08	1.97E-08	Нохс9	-9.204571144	1.32E-57	8.74E-59
Hiscilzan2 7 358:-04 1.41E-04 Clcn1 -8.854868383 1.19E.77 5.97:-79 Adora3 6.854902501 2.05E:15 3.85:16 Reg1 -8.764871591 1.38E:13 2.23E:14 Mstn 6.584962501 7.79E:15 1.50E:15 Cyphal2b -8.632995197 2.87E:54 2.01E:55 Gm39743 6.539158811 1.75E:10 4.22E:11 Spaca7 -8.632905197 2.87E:54 2.01E:55 Ang5 6.39158811 6.80E-04 2.79E:04 Hoxd11 -8.558420713 1.49E:85 6.78E:87 Lyck 6.49931619 3.99E:15 7.58E:16 Pkp1 -8.438791853 2.82E:01 1.21E:02 Cd177 6.459431619 3.99E:15 7.58E:16 Pkp1 -8.438791853 2.82E:01 1.21E:02 Ligf 6.303780748 3.65E:06 1.18E:06 Cm14344 -8.20018847 1.33E:81 6.36E:83 Cm14548 6.169922501 1.22E:11 2.35E:12 Cm420 -8.142107057 6.44E:10 2.47E:105 </td <td>Gm14296</td> <td>7.491853096</td> <td>5.23E-40</td> <td>4.70E-41</td> <td>Hist2h3c2</td> <td>-9.022367813</td> <td>2.75E-18</td> <td>4.60E-19</td>	Gm14296	7.491853096	5.23E-40	4.70E-41	Hist2h3c2	-9.022367813	2.75E-18	4.60E-19
Adora36.8454900512.05E-153.83E-16Regt-8.7464715911.38E-182.28E-19Evi26.5849625011.53E-212.27E-22Tff2-8.7176764233.48E-137.19E-14Mstm6.5849625017.79E-151.50E-15Cyp4a12b-8.6329951972.87E-542.10E-55Gm397436.5391588111.75E-104.22E-11Spcar0-8.5584207131.49E-856.78E-87Lyök6.4918530963.55E-041.41E-04Gtal-8.4716752144.37E-177.67E-18Lyök6.4594316193.99E-157.58E-16Pkp1-8.487918532.82E-911.21E-92Cd1776.4594316193.95E-061.18E-06Cm14434-8.2900188471.33E-816.36E-83Lipf6.3037807483.65E-061.18E-06Cm1434-8.2900188471.33E-816.36E-83Loc1000410576.2479275134.88E-091.28E-09Klh1-8.1548181092.26E-641.35E-55Loc1004040576.249453963.30E-215.85E-22Spink8-8.066089193.44E-108.17E-11Cm462236.168876825.48E-208.53E-21Cm210-7.99082754700Cm448056.1699250011.22E-112.75E-12Cdec18a-8.066089193.45E-141.76E-14Semib106.1258439332.30E-681.40E-69Mald1-7.990827547000Cm448056.1618876825.48E-208.53E-21Cm22-7.77487068.75E-141.76E-14	Hist2h2aa2	7	3.55E-04	1.41E-04	Clca1	-8.854868383	1.19E-77	5.97E-79
Évi2 6.584962201 1.53E-21 2.27E-22 T/Z -8.717676423 3.48E-13 7.19E-14 Mstm 6.539158811 1.50E-10 4.22E-11 Spaca7 -8.581200582 8.74E-17 1.55E-17 Ang5 6.539158811 6.500-04 2.79E-04 Hox11 -8.558420713 1.49E-85 6.73E-87 Lyck 6.459431619 3.99E-15 7.58E-16 Pkp1 -8.438791853 2.82E-91 1.21E-92 Cd177 6.459431619 1.14E-13 2.31E-14 HistIh4m -8.20018847 1.33E-81 6.36E-83 Gm14548 6.28128611 9.11E-75 Hox40 -8.20018847 1.33E-81 6.36E-83 Lpf 6.303780748 3.65E-06 1.18E-06 Clarb4 -8.162391329 1.74E-66 1.01E-67 Cm40223 6.247927513 4.88E-09 1.28E-09 Khl1 -8.162391329 1.74E-64 1.35E-65 LOC100041057 6.209453366 3.65E-06 1.18E-06 Clarb -8.142107057 6.44E-105 2.47E-106	Adora3	6.845490051	2.05E-15	3.83E-16	Reg1	-8.764871591	1.38E-18	2.28E-19
Msm 6.584962501 7.79E-15 1.50E-15 Op412b -8.632995197 2.87E-54 2.01E-55 Gm39743 6.539158811 1.75E-10 4.22E-11 Spaca7 -8.581200582 8.74E-17 1.55E-17 Ang5 6.539158811 6.80E-04 2.79E-04 Hoxd11 -8.558420713 1.49E-85 6.78E-87 Lyök 6.499431619 3.95E-04 1.41E-04 Gx1a1 -8.438791853 2.82E-91 1.21E-92 Cd177 6.459431619 1.14E-13 2.31E-14 HistIh4m -8.361943774 3.12E-07 9.29E-08 Lipf 6.303780748 3.65E-06 1.18E-06 Cm14434 -8.20018847 1.33E-81 6.36E-83 Lipf 6.303780748 3.65E-06 1.18E-06 Cla3b -8.162391329 1.74E-66 1.01E-67 Cm46223 6.247927513 4.88E-09 1.28E-09 Khl1 -8.162391329 1.74E-66 1.01E-67 Msda8 6.1618876455 3.90E-21 5.85E-22 Spink8 -8.06608919 3.34E-10 8.17E-11 <td>Evi2</td> <td>6.584962501</td> <td>1.53E-21</td> <td>2.27E-22</td> <td>Tff2</td> <td>-8.717676423</td> <td>3.48E-13</td> <td>7.19E-14</td>	Evi2	6.584962501	1.53E-21	2.27E-22	Tff2	-8.717676423	3.48E-13	7.19E-14
Gm39743 6.539158811 1.75E-10 4.22E-11 Spaca7 -8.581200582 8.74E-17 1.55E-17 Ang5 6.539158811 6.80E-04 2.79E-04 Hoxd11 -8.558420713 1.49E-85 6.78E-87 Lyck 6.494183090 3.55E-04 1.41E-04 Gata1 -8.4371675214 4.37E-17 7.67E-18 Bmx 6.459431619 3.99E-15 7.58E-16 Pkp1 -8.361943774 3.12E-07 9.29E-08 Lipf 6.303780748 3.65E-06 1.18E-06 Gm14324 -8.209018847 1.33E-81 6.36E-83 Cm14524 6.2417927513 4.88E-09 1.28E-09 Klh11 -8.154818109 2.26E-64 1.31E-65 LOC100041057 6.209453366 3.65E-02 Spink8 -8.06608919 3.34E-10 8.17E-11 DX8ay18 6.161887682 5.48E-20 8.53E-22 Spink8 -8.066089199 3.34E-10 8.15E-65 Ms4a8a 6.161887682 5.48E-20 8.53E-21 Gm24 -7.948367232 1.39E-15 2.59E-16	Mstn	6.584962501	7.79E-15	1.50E-15	Cyp4a12b	-8.632995197	2.87E-54	2.01E-55
Ang5 6.539158811 6.80E-04 2.79E-04 Hoxd11 -8.558420713 1.40E-85 6.78E-87 Ly6k 6.491853096 3.55E-04 1.41E-04 Gra1 -8.43871853 2.82E-91 1.21E-92 Cd177 6.459431619 1.14E-13 2.31E-14 Hirthm -8.361943774 3.12E-07 9.29E-08 Lipf 6.303780748 3.65E-06 1.18E-06 Gm14434 -8.162391329 1.74E-66 1.01E-67 Cm46223 6.247927513 4.88E-09 1.28E-09 Khl1 -8.154818109 2.26E-64 1.35E-65 LOC100041057 6.209453366 3.65E-05 1.18E-06 Clca2b -8.142107057 6.44E-105 2.47E-105 M54080 6.168925001 1.22E-11 2.75E-12 Clc18a -8.03342302 7.99E-54 5.65E-55 M54080 6.161887682 5.48E-20 8.3E-21 Gm8210 -7.9948367232 1.39E-15 2.59E-16 Tex45 6.13271392 3.28E-34 1.40E-69 Malrd1 -7.694836657 6.56E-85 2.7	Gm39743	6.539158811	1.75E-10	4.22E-11	Spaca7	-8.581200582	8.74E-17	1.55E-17
Lyök 6.491853096 3.55E-04 1.41E-04 Gsta1 -8.471675214 4.37E-17 7.67E-18 Bmx 6.459431619 3.99E-15 7.58E-16 Pkp1 -8.438791853 2.82E-91 1.21E-92 Lipf 6.459431619 1.14E-13 2.31E-14 Hist1h4m -8.36194374 3.12E-07 9.29E-08 Lipf 6.303780748 3.65E-06 1.18E-06 Gm14434 -8.20018847 1.33E-81 6.36E-83 Gm14528 6.247927513 4.88E-09 1.28E-09 Khl1 -8.1542107057 6.44E-105 2.47F-106 Ear6 6.189824559 3.90E-21 5.85E-22 Spink8 -8.06608919 3.34E-10 8.17E-11 DXB0y18 6.169925001 1.22E-11 2.75E-12 Clec180 -7.949367232 1.39E-15 2.59E-16 Cm44805 6.11887682 5.48E-20 8.33E-21 Gm210 -7.94837232 1.39E-15 2.59E-16 Cm44805 6.132713922 3.28E-33 3.39E-34 Pr32 -7.7478706 8.75E-14 1.76E-14	Ang5	6.539158811	6.80E-04	2.79E-04	Hoxd11	-8.558420713	1.49E-85	6.78E-87
Bmx6.4594316193.99E-157.58E-16Pkp1-8.4387918532.82E-911.21E-92Cd1776.4594316191.14E-132.31E-14HistIhdm-8.3619437743.12E-079.29E-08Lipf6.3037807483.65E-061.18E-06Cm14434-8.200188473.12E-079.29E-08Cm145486.2479275134.88E-091.28E-09Klhl1-8.1548181092.26E-641.35E-65LOC1000410576.2094333663.65E-061.18E-06Clca3b-8.1421070576.44E-1052.47FE-106Ear66.1898245593.90E-215.85E-22Spink8-8.066089193.34E-108.17E-11DXBay186.1618876825.48E-208.53E-21Cm8210-7.99082754700Cm448056.149747120.0089474640.00428538II24-7.9483672321.39E-152.59E-16Lex456.1327139223.28E-333.39E-34Prr32-7.774787068.75E-141.76E-14Serpinb106.1327139223.28E-333.39E-34Prr32-7.774787068.75E-141.76E-14Serpinb106.1327139223.28E-333.39E-34Prr32-7.7631843256.05E-852.77E-86Cyp4f186.0648155977.19E-259.49E-26C-7.707330148.46E-112.00E-21Sod36.0443941193.55E-041.41E-04Dmb11-7.6934869576.44E-753.35E-76I.0C6775256.0443941193.55E-041.41E-04Dmb11-7.6934869576.44E-74 <td< td=""><td>Lv6k</td><td>6.491853096</td><td>3.55E-04</td><td>1.41E-04</td><td>Gsta1</td><td>-8.471675214</td><td>4.37E-17</td><td>7.67E-18</td></td<>	Lv6k	6.491853096	3.55E-04	1.41E-04	Gsta1	-8.471675214	4.37E-17	7.67E-18
Cd177 6.459431619 1.14E-13 2.31E-14 HistIhdm -8.361943774 3.12E-07 9.29E-08 Lipf 6.303780748 3.65E-06 1.18E-06 Cm14434 -8.290018847 1.33E-81 6.36E-83 Cm14548 6.28128611 9.11E-75 A75E-76 Hoxd9 -8.154818109 2.26E-64 1.35E-65 L0C100041057 6.209453366 3.65E-06 1.18E-06 Clca3b -8.142107057 6.44E-105 2.47E-106 DXB0y18 6.169925001 1.22E-11 2.75E-12 Clce18a -8.033423002 7.99E-54 5.63E-55 Ms4a8a 6.161887682 5.48E-20 8.53E-21 Gm8210 -7.990827547 0 0 0 Gm44505 6.14974712 0.008947464 0.00428538 II24 -7.948367232 1.39E-15 2.59E-16 Tex45 6.13258333 2.50E-68 1.40E-69 Malrd1 -7.763184325 6.05E-85 2.77E-86 Cypdf18 6.076815597 7.19E-25 9.49E-26 Gc -7.707359132 1.36E-21	Bmx	6.459431619	3.99E-15	7.58E-16	Pkp1	-8.438791853	2.82E-91	1.21E-92
Lipf6.3037807483.65E-061.18E-06Gm1434-8.2900188471.33E-816.36E-83Gm145486.281286119.11E-754.75E-76Hoxd9-8.1623913291.74E-661.01E-67Gm462236.247927514.88E-09I.28E-09Klh11-8.1548181092.26E-641.35E-65LOC1000410576.2094533663.65E-061.18E-06Clca3b-8.1421070576.44E-1052.47E-106Ear66.1698245593.90E-215.85E-22Spink8-8.066089193.34E-108.17E-11DXBay186.1618876825.48E-208.53E-21Gm8210-7.99082754700Gm448056.149747120.0089474640.00428538II24-7.9483672321.39E-152.59E-16Serpinb106.1258439332.50E-681.40E-69Malrd1-7.7681843256.05E-852.77E-86Cyp4f186.0768155977.19E-259.49E-26Cc-7.7073591321.36E-212.00E-22A530032D15Rik6.0443941193.55E-041.41E-04Apela-7.6073303148.46E-112.00E-11Sod36.04285633800Hist1h3c-7.546894667.07E-052.58E-05F13a16.0056245900Hist1h3c-7.4346282281.75E-133.56E-14Awat5.9307373381.34E-073.89E-08Pmp2-7.4346282281.75E-133.56E-14Awat5.9307373381.34E-073.89E-08Pmp2-7.4346282281.75E-133.56E-14 <t< td=""><td>Cd177</td><td>6.459431619</td><td>1.14E-13</td><td>2.31E-14</td><td>Hist1h4m</td><td>-8.361943774</td><td>3.12E-07</td><td>9.29E-08</td></t<>	Cd177	6.459431619	1.14E-13	2.31E-14	Hist1h4m	-8.361943774	3.12E-07	9.29E-08
Gm14548 6.28128611 9.11E-75 4.75E-76 Hoxd9 -8.162391329 1.74E-66 1.01E-67 Gm46223 6.247927513 4.88E-09 1.28E-09 Klhl1 -8.154818109 2.26E-64 1.35E-65 LOC100041057 6.209453366 3.65E-06 1.18E-06 Clca3b -8.142107057 6.44E-105 2.47E-106 Ear6 6.189824559 3.90E-21 5.85E-22 Spink8 -8.06608919 3.34E-10 8.17E-11 DXBay18 6.169925001 1.22E-11 2.75E-12 Clce18a -8.033423002 7.990827547 0 0 Gm44805 6.14974712 0.008947464 0.00428538 II24 -7.948367232 1.39E-15 2.59E-16 Tex45 6.132514393 2.50E-68 1.40E-69 Malrd1 -7.768184325 6.05E-85 2.77E-86 Cyp4f18 6.076815597 7.19E-25 9.49E-26 Cc -7.707359132 1.36E-21 2.00E-21 A53032D15Rik 6.044394119 3.55E-04 1.41E-04 Dmb11 -7.693836957 <td< td=""><td>Lipf</td><td>6.303780748</td><td>3.65E-06</td><td>1.18E-06</td><td>Gm14434</td><td>-8.290018847</td><td>1.33E-81</td><td>6.36E-83</td></td<>	Lipf	6.303780748	3.65E-06	1.18E-06	Gm14434	-8.290018847	1.33E-81	6.36E-83
Gm46223 6.247927513 4.88E-09 1.28E-09 Klhl1 -8.154818109 2.26E-64 1.35E-65 LOC100041057 6.209453366 3.65E-06 1.18E-06 Clca3b -8.142107057 6.44E-105 2.47E-106 DXBay18 6.169925001 1.22E-11 2.75E-12 Clce18a -8.033423002 7.99E-54 5.63E-55 Ms4aba 6.161887682 5.48E-20 8.53E-21 Gm8210 -7.990827547 0 0 Gm44805 6.14974712 0.008947464 0.00428538 II24 -7.948367232 1.39E-15 2.59E-16 Tex45 6.132713922 3.28E-33 3.39E-34 Pr32 -7.77478706 8.75E-14 1.76E-14 Serpinb10 6.125843933 2.50E-68 1.40E-69 Mahd1 -7.768184325 6.05E-85 2.77E-86 Cyp4f18 6.076815597 7.19E-25 9.49E-26 C -7.707359132 1.36E-21 2.00E-21 A530032D15Rik 6.04428638 0 0 Hibt1 -7.669330314 8.46E-11 2.00E-21	Gm14548	6.28128611	9.11E-75	4.75E-76	Hoxd9	-8.162391329	1.74E-66	1.01E-67
LOC1000410576.2094533663.65E-061.18E-06Clca3b-8.1421070576.44E-1052.47E-106Ear66.1898245593.90E-215.85E-22Spink8-8.060608193.34E-108.17E-11DXBay186.1699250011.22E-112.75E-12Clcc18a-8.0334230027.99E-545.63E-55Ms4a8a6.1618876825.48E-208.53E-21Clm210-7.99082754700Gm448056.149747120.0089474640.00428538ll24-7.9483672321.39E-152.59E-16Tex456.1327139223.28E-333.39E-34Pr32-7.774787068.75E-141.76E-14Gyp4f186.0768155977.19E-259.49E-26Gc-7.7073591321.36E-212.00E-22A530032D15Rik6.0443941193.55E-041.41E-04Dmbt1-7.6934869576.44E-753.35E-76LOC6775256.0443941193.55E-041.41E-04Apela-7.607303148.46E-112.00E-11Sod36.042853800Hixth3c-7.54689446.7.07E-052.58E-05F13a16.00562454900Hoxd4-7.4594316198.27E-261.06E-26Prss165.9307373381.34E-073.89E-08Pmp2-7.4346282281.75E-133.56E-14Gm365045.8826430499.74E-073.02E-07Pitx2-7.3098552632.07E-432.26E-44Rmase2b5.8328900140.0089422330.00428538Kcnj16-7.2352164622.60E-1835.72E-185 </td <td>Gm46223</td> <td>6.247927513</td> <td>4.88E-09</td> <td>1.28E-09</td> <td>Klhl1</td> <td>-8.154818109</td> <td>2.26E-64</td> <td>1.35E-65</td>	Gm46223	6.247927513	4.88E-09	1.28E-09	Klhl1	-8.154818109	2.26E-64	1.35E-65
Ear66.1898245593.90E-215.85E-22Spink8-8.066089193.34E-108.17E-11DXBay186.1699250011.22E-112.75E-12Clce18a-8.0334230027.99E-545.63E-55Ms4a8a6.1618876825.48E-208.53E-21Gm8210-7.99082754700Gm448056.149747120.0089474640.00428538II24-7.9483672321.39E-152.59E-16Tex456.1327139223.28E-333.39E-34Pr32-7.77487068.75E-141.76E-14Serpinb106.1258439332.50E-681.40E-69Malrd1-7.6934869576.44E-753.35E-76Cyp4f186.0768155977.19E-259.49E-26Gc-7.7073591321.36E-112.00E-22A530032D15Rik6.0443941193.55E-041.41E-04Dmbt1-7.6934869576.44E-753.35E-76I/OC6775256.0443941193.55E-041.41E-04Apela-7.6073303148.46E-112.00E-11Sod36.0425833800Hoxtd4-7.4594316198.27E-261.06E-26F13a16.00562454900Hoxtd4-7.4594316198.27E-261.06E-26Prss165.9307373381.34E-073.89E-08Pmp2-7.4408794364.25E-235.94E-24Rmase2b5.8328900140.008942230.00428538Kcnj16-7.2352164622.60E-1835.72E-1852010005H15Rik5.8176232581.43E-222.04E-23Scin-7.140687785.49E-2439.12E-245	LOC100041057	6.209453366	3.65E-06	1.18E-06	Clca3b	-8.142107057	6.44E-105	2.47E-106
DXBay186.1699250011.22E-112.75E-12Clec18a-8.0334230027.99E-545.63E-55Ms4aba6.1618876825.48E-208.53E-21Gm8210-7.99082754700Gm448056.149747120.0089474640.00428538II24-7.9483672321.39E-152.59E-16Tex456.1327139223.28E-333.39E-34Pr32-7.774787068.75E-141.76E-14Serpinb106.1258439332.50E-681.40E-69Malrd1-7.681843256.05E-852.77E-86Cyp4f186.0768155977.19E-259.49E-26Gc-7.7073591321.36E-212.00E-22A530032D15Rik6.0443941193.55E-041.41E-04Dmbt1-7.6934869576.44E-753.35E-76LOC6775256.0443941193.55E-041.41E-04Apela-7.6073303148.46E-112.00E-11Sod36.04285633800Hist1h3c-7.546894467.07E-052.58E-05F13a16.00562454900Hoxd4-7.4594316198.27E-261.06E-26Prss165.9307373381.34E-073.09E-07Pitz2-7.3098552632.70E-432.26E-44Rmase2b5.8328900140.0089422230.00428538Krnj16-7.2352164622.60E-1835.72E-1852010005H15Rik5.8176232581.36E-054.64E-06Dcdc2a-7.14426604600Gm57965.7548875021.36E-054.64E-06Dcdc2a-7.140687785.49E-2439.12E-245 <t< td=""><td>Ear6</td><td>6.189824559</td><td>3.90E-21</td><td>5.85E-22</td><td>Spink8</td><td>-8.06608919</td><td>3.34E-10</td><td>8.17E-11</td></t<>	Ear6	6.189824559	3.90E-21	5.85E-22	Spink8	-8.06608919	3.34E-10	8.17E-11
Ms4a8a6.1618876825.48E-208.53E-21Gm8210-7.99082754700Gm448056.149747120.0089474640.00428538ll24-7.9483672321.39E-152.59E-16Tex456.1327139223.28E-333.39E-34Pr32-7.774787068.75E-141.76E-14Serpinb106.1258439332.50E-681.40E-69Malrd1-7.681843256.05E-852.77E-86Cyp4f186.0768155977.19E-259.49E-26Gc-7.7073591321.36E-212.00E-22A530032D15Rik6.0443941193.55E-041.41E-04Dmbt1-7.6934869576.44E-753.35E-76LOC6775256.0443941193.55E-041.41E-04Apela-7.5073303148.46E-112.00E-11Sod36.04285633800Hist1h3c-7.454894467.07E-052.58E-05F13a16.00562454900Hox44-7.4594316198.27E-261.06E-26Prss165.9307373381.34E-073.89E-08Pmp2-7.4346282281.75E-133.56E-14Awat15.9068905965.07E-051.82E-05Gsdmc2-7.4008794364.25E-235.94E-24Gm365045.8826430499.74E-073.02E-07Pitx2-7.309852632.70E-432.26E-44Gm365045.8176232581.43E-222.04E-23Scin-7.1922928146.79E-1931.41E-194Gm37305.7548875021.36E-054.64E-06Dcdc2a-7.144266046000Gm77	DXBav18	6.169925001	1.22E-11	2.75E-12	Clec18a	-8.033423002	7.99E-54	5.63E-55
Gm448056.149747120.0089474640.00428538II24-7.9483672321.39E-152.59E-16Tex456.1327139223.28E-333.39E-34Pr32-7.774787068.75E-141.76E-14Serpinb106.1258439332.50E-681.40E-69Malrd1-7.7681843256.05E-852.77E-86Cyp4f186.0768155977.19E-259.49E-26Gc-7.7073591321.36E-122.00E-22A530032D15Rik6.0443941193.55E-041.41E-04Dmbt1-7.6934869576.44E-753.35E-76LOC6775256.0443941193.55E-041.41E-04Apela-7.6073303148.46E-112.00E-11Sod36.04285033800Hist1h3c-7.546894467.07E-052.58E-05F13a16.00562454900Hoxd4-7.4594316198.27E-261.06E-26Prs165.9307373381.34E-073.89E-08Pmp2-7.4308526332.70E-432.26E-44Awat15.9068905965.07E-051.82E-05Gsdmc2-7.4008794364.25E-235.94E-24Gm365045.8826430499.74E-073.02E-07Pitz2-7.3098552632.70E-432.26E-44Rnase2b5.8328900140.0089422230.00428538Kcnj16-7.2352164622.60E-1835.72E-185201005H15Rik5.8176232581.43E-222.04E-23Scin-7.104267785.49E-2439.12E-245Gm75965.7548875021.36E-054.64E-06Dcdc2a-7.140687785.49E-243 <td< td=""><td>Ms4a8a</td><td>6.161887682</td><td>5.48E-20</td><td>8.53E-21</td><td>Gm8210</td><td>-7.990827547</td><td>0</td><td>0</td></td<>	Ms4a8a	6.161887682	5.48E-20	8.53E-21	Gm8210	-7.990827547	0	0
Tex456.1327139223.28E-333.39E-34Pr32-7.774787068.75E-141.76E-14Serpinb106.1258439332.50E-681.40E-69Malrd1-7.7681843256.05E-852.77E-86Cyp4/186.0768155977.19E-259.49E-26Gc-7.7073591321.36E-212.00E-22A530032D15Rik6.0443941193.55E-041.41E-04Dmbt1-7.6934869576.44E-753.35ELOC6775256.0443941193.55E-041.41E-04Apela-7.6073303148.46E-112.00E-11Sod36.04285633800Hist1h3c-7.546894467.07E-052.58E-05F13a16.00562454900Hoxd4-7.4594316198.27E-261.06E-26Prss165.9307373381.34E-073.89E-08Pmp2-7.4346282281.75E-133.56E-14Awat15.9068905965.07E-051.82E-05Gsdmc2-7.3098552632.70E-432.26E-44Rnase2b5.8326430499.74E-073.02E-07Pitx2-7.3098552632.70E-432.26E-44Rnase2b5.8328900140.0089422230.00428538Kcnj16-7.2352164622.60E-1835.72E-1852010005H15Rik5.8176232581.43E-222.04E-23Scin-7.1402604600Gm77335.7548875021.36E-054.64E-06Dcdc2a-7.140687785.49E-2439.12E-455Gpr155.7279204553.55E-041.41E-04Spns3-7.140687783.44E-108.17E-11 <tr< td=""><td>Gm44805</td><td>6.14974712</td><td>0.008947464</td><td>0.00428538</td><td>Il24</td><td>-7.948367232</td><td>1.39E-15</td><td>2.59E-16</td></tr<>	Gm44805	6.14974712	0.008947464	0.00428538	Il24	-7.948367232	1.39E-15	2.59E-16
Serpinb106.1258439332.50E-681.40E-69Malrd1-7.7681843256.05E-852.77E-86Cyp4f186.0768155977.19E-259.49E-26Gc-7.7073591321.36E-212.00E-22A530032D15Rik6.0443941193.55E-041.41E-04Dmbt1-7.6934869576.44E-753.35E-76LOC6775256.0443941193.55E-041.41E-04Apela-7.6073303148.46E-112.00E-11Sod36.04285633800Hist1h3c-7.546894467.07E-052.58E-05F13a16.00562454900Hoxd4-7.4594316198.27E-261.06E-26Prss165.9307373381.34E-073.89E-08Pmp2-7.4346282281.75E-133.56E-14Awut15.9068905965.07E-051.82E-05Gsdmc2-7.4008794364.25E-235.94E-24Gm365045.8826430499.74E-073.02E-07Pitx2-7.3098552632.70E-432.26E-44Rnase2b5.8328900140.0089422230.00428538Kcnj16-7.2352164622.60E-1835.72E-1852010005H15Rik5.8176232581.43E-222.04E-23Scin-7.1402604600Gm77335.7548875021.36E-054.64E-06Dcdc2a-7.140687785.49E-2439.12E-245Gpr155.7279204553.55E-041.41E-04Spns3-7.1085244573.34E-108.17E-11Fhl55.7004397180.0024779770.001093626Dnajc19-ps-7.0980320832.70E-041.06E	Tex45	6.132713922	3.28E-33	3.39E-34	Prr32	-7.77478706	8.75E-14	1.76E-14
Cyp4f186.0768155977.19E-259.49E-26Gc-7.7073591321.36E-212.00E-22A530032D15Rik6.0443941193.55E-041.41E-04Dmbt1-7.6934869576.44E-753.35E-76LOC6775256.0443941193.55E-041.41E-04Apela-7.6073303148.46E-112.00E-11Sod36.04285633800Hist1h3c-7.546894467.07E-052.58E-05F13a16.00562454900Hoxd4-7.4594316198.75E-261.06E-26Prss165.9307373381.34E-073.89E-08Pmp2-7.4346282281.75E-133.56E-14Awat15.9068905965.07E-051.82E-05Gsdmc2-7.4008794364.25E-235.94E-24Gm365045.8826430499.74E-073.02E-07Pitx2-7.3098552632.70E-432.26E-44Rnase2b5.8328900140.0089422230.00428538Kcnj16-7.2352164622.60E-1835.72E-1852010005H15Rik5.8176232581.43E-222.04E-23Scin-7.14226014600Gm57965.7548875020.0089495610.00428538Dclk3-7.140687785.49E-2439.12E-245Gpr155.7209204553.55E-041.41E-04Spns3-7.1085244573.34E-108.17E-11Fhl55.7004397180.0024779770.001093626Dnajc19-ps-7.0980320832.70E-041.06E-04Klrb1b5.7004397189.74E-073.02E-07Hoxd8-7.0962759111.84E-828.73	Serpinb10	6.125843933	2.50E-68	1.40E-69	Malrd1	-7.768184325	6.05E-85	2.77E-86
A30032D15Rik6.0443941193.55E-041.41E-04Dmbt1-7.6934869576.44E-753.35E-76LOC6775256.0443941193.55E-041.41E-04Apela-7.6073303148.46E-112.00E-11Sod36.04285633800Hist1h3c-7.546894467.07E-052.58E-05F13a16.00562454900Hoxd4-7.4594316198.27E-261.06E-26Prss165.9307373381.34E-073.89E-08Pmp2-7.4346282281.75E-133.56E-14Awat15.9068905965.07E-051.82E-05Gsdmc2-7.4008794364.25E-235.94E-24Gm365045.8826430499.74E-073.02E-07Pitx2-7.3098552632.70E-432.26E-44Rnase2b5.8328900140.0089422230.00428538Kcnj16-7.2352164622.60E-1835.72E-1852010005H15Rik5.8176232581.43E-222.04E-23Scin-7.14026046600Gm97335.7548875021.36E-054.64E-06Dcdc2a-7.140687785.49E-2439.12E-245Gpr155.7279204553.55E-041.41E-04Spns3-7.1085244573.34E-108.17E-11Fhl55.7004397180.0024779770.001093626Dnajc19-ps-7.0980320832.70E-041.06E-04Klrb1b5.7004397189.74E-073.02E-07Hoxd8-7.0962759111.84E-828.73E-84Mccmp15.6982477271.99E-1107.28E-112Lhx1-7.052824365.42E-218.18E-2	Cvp4f18	6.076815597	7.19E-25	9.49E-26	Gc	-7.707359132	1.36E-21	2.00E-22
LOC6775256.0443941193.55E-041.41E-04Apela-7.6073303148.46E-112.00E-11Sod36.04285633800Hist1h3c-7.546894467.07E-052.58E-05F13a16.00562454900Hoxd4-7.4594316198.27E-261.06E-26Prss165.9307373381.34E-073.89E-08Pmp2-7.4346282281.75E-133.56E-14Awat15.9068905965.07E-051.82E-05Gsdmc2-7.4008794364.25E-235.94E-24Gm365045.8826430499.74E-073.02E-07Pitx2-7.3098552632.70E-432.26E-44Rnase2b5.8328900140.0089422230.00428538Kcnj16-7.2352164622.60E-1835.72E-1852010005H15Rik5.8176232581.43E-222.04E-23Scin-7.1426604600Gm77965.7548875021.36E-054.64E-06Dclc2a-7.140687785.49E-2439.12E-245Gpr155.7279204553.55E-041.41E-04Spns3-7.1068748573.34E-108.17E-11Fhl55.7004397180.0024779770.001093626Dnajc19-ps-7.0980320832.70E-041.06E-04Klrb1b5.7004397189.74E-073.02E-07Hoxd8-7.0962759111.84E-828.73E-84Mccmp15.6982477271.99E-1107.28E-112Lhx1-7.052824365.42E-218.18E-22Sfma15.6972475246.79E-007.24E-12Lhx1-7.052824365.42E-218.18E-22 <td>A530032D15Rik</td> <td>6.044394119</td> <td>3.55E-04</td> <td>1.41E-04</td> <td>Dmbt1</td> <td>-7.693486957</td> <td>6.44E-75</td> <td>3.35E-76</td>	A530032D15Rik	6.044394119	3.55E-04	1.41E-04	Dmbt1	-7.693486957	6.44E-75	3.35E-76
Sod36.04285633800Hist1h3c-7.546894467.07E-052.58E-05F13a16.00562454900Hoxd4-7.4594316198.27E-261.06E-26Prss165.9307373381.34E-073.89E-08Pmp2-7.4346282281.75E-133.56E-14Awat15.9068905965.07E-051.82E-05Gsdmc2-7.4008794364.25E-235.94E-24Gm365045.8826430499.74E-073.02E-07Pitx2-7.3098552632.70E-432.26E-44Rnase2b5.8328900140.0089422230.00428538Kcnj16-7.2352164622.60E-1835.72E-1852010005H15Rik5.8176232581.43E-222.04E-23Scin-7.1922928146.79E-1931.41E-194Gm57965.7548875021.36E-054.64E-06Dcdc2a-7.14426604600Gm97335.7548875020.0089495610.00428538Dclk3-7.140687785.49E-2439.12E-245Gpr155.7279204553.55E-041.41E-04Spns3-7.1085244573.34E-108.17E-11Fhl55.7004397180.0024779770.001093626Dnajc19-ps-7.0980320832.70E-041.06E-04Klrb1b5.7004397189.74E-073.02E-07Hoxd8-7.052824365.42E-218.18E-22Klrb1b5.6982477271.99E-1107.28E-112Lhx1-7.0552824365.42E-218.18E-22Sfma15.6972475246.37E-007.41E-20Clrm7.0042041101.27E-002.34E-10 <td>LOC677525</td> <td>6.044394119</td> <td>3.55E-04</td> <td>1.41E-04</td> <td>Apela</td> <td>-7.607330314</td> <td>8.46E-11</td> <td>2.00E-11</td>	LOC677525	6.044394119	3.55E-04	1.41E-04	Apela	-7.607330314	8.46E-11	2.00E-11
F13a16.00562454900Hoxd4-7.4594316198.27E-261.06E-26Prss165.9307373381.34E-073.89E-08Pmp2-7.4346282281.75E-133.56E-14Awat15.9068905965.07E-051.82E-05Gsdmc2-7.4008794364.25E-235.94E-24Gm365045.8826430499.74E-073.02E-07Pitx2-7.3098552632.70E-432.26E-44Rnase2b5.8328900140.0089422230.00428538Kcnj16-7.2352164622.60E-1835.72E-1852010005H15Rik5.8176232581.43E-222.04E-23Scin-7.1922928146.79E-1931.41E-194Gm57965.7548875021.36E-054.64E-06Dcdc2a-7.14426604600Gm97335.7548875020.0089495610.00428538Dclk3-7.140687785.49E-2439.12E-245Gpr155.7279204553.55E-041.41E-04Spns3-7.1085244573.34E-108.17E-11Fhl55.7004397180.0024779770.001093626Dnajc19-ps-7.0980320832.70E-041.06E-04Klrb1b5.7004397189.74E-073.02E-07Hoxd8-7.0962759111.84E-828.73E-84Mccmp15.6982477271.99E-1107.28E-112Lhx1-7.0552824365.42E-218.18E-22Sfma15.67245246.72E-207.44E-20Clrm7.0042041101.27E-002.34E-12	Sod3	6.042856338	0	0	Hist1h3c	-7.54689446	7.07E-05	2.58E-05
Prss165.9307373381.34E-073.89E-08Pmp2-7.4346282281.75E-133.56E-14Awat15.9068905965.07E-051.82E-05Gsdmc2-7.4008794364.25E-235.94E-24Gm365045.8826430499.74E-073.02E-07Pitx2-7.3098552632.70E-432.26E-44Rnase2b5.8328900140.0089422230.00428538Kcnj16-7.2352164622.60E-1835.72E-1852010005H15Rik5.817632581.43E-222.04E-23Scin-7.1922928146.79E-1931.41E-194Gm57965.7548875021.36E-054.64E-06Dcdc2a-7.14426604600Gm97335.7548875020.0089495610.00428538Dclk3-7.140687785.49E-2439.12E-245Gpr155.7279204553.55E-041.41E-04Spns3-7.1085244573.34E-108.17E-11Fhl55.7004397180.0024779770.001093626Dnajc19-ps-7.0980320832.70E-041.06E-04Klrb1b5.7004397189.74E-073.02E-07Hoxd8-7.0962759111.84E-828.73E-84Mcemp15.6982477271.99E-1107.28E-112Lhx1-7.0552824365.42E-218.18E-22Sfma15.67245246.73E-207.41E-20Clrm7.0042041101.27E-002.32E-12	F13a1	6.005624549	0	0	Hoxd4	-7.459431619	8.27E-26	1.06E-26
Awat15.9068905965.07E-051.82E-05Gsdmc2-7.4008794364.25E-235.94E-24Gm365045.8826430499.74E-073.02E-07Pitx2-7.3098552632.70E-432.26E-44Rnase2b5.8328900140.0089422230.00428538Kcnj16-7.2352164622.60E-1835.72E-1852010005H15Rik5.8176232581.43E-222.04E-23Scin-7.1922928146.79E-1931.41E-194Gm57965.7548875021.36E-054.64E-06Dcdc2a-7.14426604600Gm97335.7548875020.0089495610.00428538Dclk3-7.140687785.49E-2439.12E-245Gpr155.7279204553.55E-041.41E-04Spns3-7.1085244573.34E-108.17E-11Fhl55.7004397180.0024779770.001093626Dnajc19-ps-7.0980320832.70E-041.06E-04Klrb1b5.7004397189.74E-073.02E-07Hoxd8-7.0962759111.84E-828.73E-84Mccmp15.6982477271.99E-1107.28E-112Lhx1-7.0552824365.42E-218.18E-22Sfma15.672435246.72E-207.24E-12Lhx1-7.0552824365.42E-218.18E-22	Prss16	5.930737338	1.34E-07	3.89E-08	Pmp2	-7.434628228	1.75E-13	3.56E-14
Gm365045.8826430499.74E-073.02E-07Pitx2-7.3098552632.70E-432.26E-44Rnase2b5.8328900140.0089422230.00428538Kcnj16-7.2352164622.60E-1835.72E-1852010005H15Rik5.8176232581.43E-222.04E-23Scin-7.1922928146.79E-1931.41E-194Gm57965.7548875021.36E-054.64E-06Dcdc2a-7.1402604600Gm97335.7548875020.0089495610.00428538Dclk3-7.140687785.49E-2439.12E-245Gpr155.7279204553.55E-041.41E-04Spns3-7.1085244573.34E-108.17E-11Fhl55.7004397180.0024779770.001093626Dnajc19-ps-7.0980320832.70E-041.06E-04Klrb1b5.7004397189.74E-073.02E-07Hoxd8-7.0962759111.84E-828.73E-84Mcemp15.6982477271.99E-1107.28E-112Lhx1-7.0552824365.42E-218.18E-22Sfma15.672452426.72E-207.44E-20Clrm7.0042041101.27E-002.34E-12	Awat1	5.906890596	5.07E-05	1.82E-05	Gsdmc2	-7.400879436	4.25E-23	5.94E-24
Rnase2b5.8328900140.0089422230.00428538Kcnj16-7.2352164622.60E-1835.72E-1852010005H15Rik5.8176232581.43E-222.04E-23Scin-7.1922928146.79E-1931.41E-194Gm57965.7548875021.36E-054.64E-06Dcdc2a-7.14426604600Gm97335.7548875020.0089495610.00428538Dclk3-7.140687785.49E-2439.12E-245Gpr155.7279204553.55E-041.41E-04Spns3-7.1085244573.34E-108.17E-11Fh155.7004397180.0024779770.001093626Dnajc19-ps-7.0980320832.70E-041.06E-04Klrb1b5.7004397189.74E-073.02E-07Hoxd8-7.0962759111.84E-828.73E-84Mcemp15.6982477271.99E-1107.28E-112Lhx1-7.0552824365.42E-218.18E-22Sfma15.672435246.72E-207.41E-20Clrm-7.0402041101.27E-002.32E-10	Gm36504	5.882643049	9.74E-07	3.02E-07	Pitx2	-7.309855263	2.70E-43	2.26E-44
2010005H15Rik5.8176232581.43E-222.04E-23Scin-7.1922928146.79E-1931.41E-194Gm57965.7548875021.36E-054.64E-06Dcdc2a-7.14426604600Gm97335.7548875020.0089495610.00428538Dclk3-7.140687785.49E-2439.12E-245Gpr155.7279204553.55E-041.41E-04Spns3-7.1085244573.34E-108.17E-11Fh155.7004397180.0024779770.001093626Dnajc19-ps-7.0980320832.70E-041.06E-04Klrb1b5.7004397189.74E-073.02E-07Hoxd8-7.0962759111.84E-828.73E-84Mcemp15.6982477271.99E-1107.28E-112Lhx1-7.0552824365.42E-218.18E-22Sfma15.672435246.72E-207.41E-20Clrm7.042041101.27E-002.32E-10	Rnase2b	5.832890014	0.008942223	0.00428538	Kcni16	-7.235216462	2.60E-183	5.72E-185
Gm57965.7548875021.36E-054.64E-06Dcdc2a-7.14426604600Gm97335.7548875020.0089495610.00428538Dclk3-7.140687785.49E-2439.12E-245Gpr155.7279204553.55E-041.41E-04Spns3-7.1085244573.34E-108.17E-11Fhl55.7004397180.0024779770.001093626Dnajc19-ps-7.0980320832.70E-041.06E-04Klrb1b5.7004397189.74E-073.02E-07Hoxd8-7.0962759111.84E-828.73E-84Mccmp15.6982477271.99E-1107.28E-112Lhx1-7.0552824365.42E-218.18E-22Sfma15.672435246.72E-207.41E-20Clum7.0402041101.23E-002.32E-10	2010005H15Rik	5.817623258	1.43E-22	2.04E-23	Scin	-7.192292814	6.79E-193	1.41E-194
Gm97335.7548875020.0089495610.00428538Dclk3-7.140687785.49E-2439.12E-245Gpr155.7279204553.55E-041.41E-04Spns3-7.1085244573.34E-108.17E-11Fhl55.7004397180.0024779770.001093626Dnajc19-ps-7.0980320832.70E-041.06E-04Klrb1b5.7004397189.74E-073.02E-07Hoxd8-7.0962759111.84E-828.73E-84Mcemp15.6982477271.99E-1107.28E-112Lhx1-7.0552824365.42E-218.18E-22Sfma15.672435426.72E-207.41E-20Clum7.0402041101.27E-002.32E-10	Gm5796	5.754887502	1.36E-05	4.64E-06	Dcdc2a	-7.144266046	0	0
Gpr15 5.727920455 3.55E-04 1.41E-04 Spns3 -7.108524457 3.34E-10 8.17E-11 Fhl5 5.700439718 0.002477977 0.001093626 Dnajc19-ps -7.098032083 2.70E-04 1.06E-04 Klrb1b 5.700439718 9.74E-07 3.02E-07 Hoxd8 -7.096275911 1.84E-82 8.73E-84 Mcemp1 5.698247727 1.99E-110 7.28E-112 Lhx1 -7.055282436 5.4E-21 8.18E-22 Sfma1 5.672435242 6.73E-20 7.04E-20 Clrm 7.04204110 1.27E-00 2.34E-10	Gm9733	5.754887502	0.008949561	0.00428538	Dclk3	-7.14068778	5.49E-243	9.12E-245
FhI5 5.700439718 0.002477977 0.001093626 Dnajc19-ps -7.098032083 2.70E-04 1.06E-04 Klrb1b 5.700439718 9.74E-07 3.02E-07 Hoxd8 -7.096275911 1.84E-82 8.73E-84 Mcemp1 5.698247727 1.99E-110 7.28E-112 Lhx1 -7.055282436 5.42E-21 8.18E-22 Sfma1 5.697435242 6.37E-20 7.41E-20 Chrm 7.044204110 1.32E-00 2.34E-10	Gpr15	5.727920455	3.55E-04	1.41E-04	Spns3	-7.108524457	3.34E-10	8.17E-11
Klrb1b 5.700439718 9.74E-07 3.02E-07 Hoxd8 -7.096275911 1.84E-82 8.73E-84 Mcemp1 5.698247727 1.99E-110 7.28E-112 Lhx1 -7.055282436 5.42E-21 8.18E-22 Sfma1 5.697425242 6.37E-20 7.41E-20 Chrm 7.04204110 1.32E-00 2.32E-10	Fhl5	5.700439718	0.002477977	0.001093626	Dnajc19-ps	-7.098032083	2.70E-04	1.06E-04
Mcemp1 5.698247727 1.99E-110 7.28E-112 Lhx1 -7.055282436 5.42E-21 8.18E-22 Sfma1 5.677435242 6.37E-20 7.04204110 1.32E-00 2.34E-10	Klrb1b	5.700439718	9.74E-07	3.02E-07	Hoxd8	-7.096275911	1.84E-82	8.73E-84
Sfra1 5 672/4252/42 6 27E 20 7 /1E 20 Cltm 7 0//20/110 1 22E 00 2 2//E 10	Mcemp1	5.698247727	1.99E-110	7.28E-112	Lhx1	-7.055282436	5.42E-21	8.18E-22
3jipui 3.07242.3342 0.37E-29 7.41E-30 Cilili — 7.044.394119 1.32E-09 3.34E-10	Sftpa1	5.672425342	6.37E-29	7.41E-30	Cltrn	-7.044394119	1.32E-09	3.34E-10



Fig. 3. GO analysis. Top seven GO terms of up- and down-regulated DEGs of $Dip2b^{+/+}$ vs $Dip2b^{-/-}$ (FC ≥ 2 , FDR < 0.01).





Fig. 4. KEGG pathway analysis of DEGs from Dip2b^{+/+} vs Dip2b^{-/-} MELFs. Adjusted p-values (Q-values) depicting significant enrichment (q-value < 0.05).

growth', indicating Dip2B knockout reduces cell proliferation and growth (Fig. 5A, B). Genes including *Adora3*, *Il1b*, *Slfn1* and *Nrk* [26–30] responsible for inhibition of cell proliferation were significantly up-regulated. To validate the role of Dip2B in cell proliferation, isolated MELFs were subjected to MTT viability assay. As shown in Fig. 5C, growth rates of MELFs from $Dip2b^{-/-}$ and $Dip2b^{+/-}$ are significantly slower than cells from $Dip2b^{+/+}$ (p < 0.01), and

Up-regulated KEGG enriched terms

more obvious in $Dip2b^{-/-}$ MELFs (p < 0.001). Result suggests that Dip2B may be a critical factor in regulation of cell proliferation.

3.7. Dip2B regulates apoptosis and cell cycle

GO analysis shows that apoptosis and cell cycle are regulated by Dip2B. Several DEGs were found significantly enriched in 'positive

Table 4

List of differentially expressed TFs between	n Dip2b ^{-/-} and Dip2b ^{+/+}	$(FC \ge 2, FDR < 0.01).$
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TF symbol	Number of dysregulated genes	Rich Ratio	P value	Q value
Homeobox	44	0.195556	9.21E-10	2.67E-08
zf-C2H2	15	0.026978	1	1
bhlh	11	0.102804	0.235915	0.484594
Fork_head	10	0.227273	0.001856	0.026911
HMG	5	0.098039	0.39226	0.531165
TF_bZIP	4	0.076923	0.614627	0.742674
Tub	3	0.6	0.004512	0.043619
ETS	3	0.111111	0.372	0.531165
AP-2	2	0.4	0.054668	0.396346
ESR-like	2	0.222222	0.159449	0.420366
PAX	2	0.222222	0.159449	0.420366
SAND	2	0.25	0.130642	0.420366



Fig. 5. Effect of Dip2B on cell proliferation and growth. Heatmap for DEGs associated with GO biological process terms of (A) 'negative regulation of cell proliferation' and (B) 'negative regulation of cell growth'. (C) MTT viability assay of MELFs derived from $Dip2b^{-/-}$, $Dip2b^{+/-}$ and $Dip2b^{+/+}$.

regulation of apoptotic process', such as up-regulation of *Wnt11*, *Pycard*, *ll1b*, *Trp73*, *Clu* and *Fas* [27,31–35]. To verify whether Dip2B affects apoptosis, cells were analyzed using Annexin V and Pl staining and cell cycle by flow cytometry (Fig. 6A). Results indicate that percentage of later apoptotic MELFs is significantly increased in $Dip2b^{-/-}$ in comparison to $Dip2b^{+/-}$ heterozygous or WT MELFs. The early apoptotic proportions in $Dip2b^{-/+}$ MELFs are similar to $Dip2b^{+/+}$ MELFs but increased later (Fig. 6B). $Dip2b^{-/-}$ MELFs exhib-

ited an increase in G2/M population in cell-cycle profiles (Fig. 6C). Results confirm that Dip2B can significantly inhibit MELF proliferation and induce apoptosis and cell cycle arrest.

3.8. Dip2B regulates cell migration

Transcriptomic data revealed that Dip2B is involved in promotion of cell migration. Knockout of Dip2B results in significantly S. Adlat et al.



Fig. 6. Apoptosis and cell cycle analysis. (A) Heatmap for DEGs associated with GO terms 'positive regulation of apoptotic process'. (B) Apoptotic cells measured using a Becton-Dickinson FACScan cytofluorometer. (C) Histogram of flow cytometric analysis of cell cycle showing the distribution of cell phase.

Table 5 Differentially expressed genes involved in cell migration.

Gene symbol	Dip2b ^{+/+} vs Dip2b ^{+/-}		Dip2b ^{+/+} vs Dip2b ^{-/-}			
	$FC \geq 2$	FDR	P value	$FC \geq 2$	FDR	P value
Cdh2	-2.212266596	0	0	-2.974162444	0	0
Podxl	-1.835315003	5.00E-51	2.81E-52	-2.297219108	2.88E-67	1.65E-68
L1cam	-1.975442896	1.38E-97	4.16E-99	-2.256855831	1.74E-115	6.12E-117
Bcar1	-1.223105744	0	0	-1.223105744	2.66E-05	3.28E-06
Tdgf1	-6.794415866	2.73E-12	4.78E-13	-6.794415866	2.73E-12	5.94E-13
Vil1	-7.658211483	7.92E-33	2.30E-37	-6.073248982	7.92E-33	8.27E-34
Adra2a	-3.195256291	3.47E-108	3.99E-85	-4.445799753	3.47E-108	1.29E-109
Erbb4	-3.841302254	7.09E-24	5.32E-24	-4.426264755	7.09E-24	9.66E-25
Tfap2a	-3.145677455	6.38E-24	3.12E-21	-3.660250628	6.38E-24	8.68E-25
Lgr6	-2.220729372	1.16E-139	9.02E-90	-3.42003818	1.16E-139	3.33E-141
Sema5b	-1.247927513	5.76E-04	0.0794798	-3.247927513	5.76E-04	2.35E-04
Hbegf	-2.279689532	0	0	-2.713977039	0	0
Cav1	-2.302638712	0	0	-2.604238401	0	0
Gper1	-4.812498225	1.93E-18	1.58E-34	-2.379538818	1.93E-18	3.20E-19
Sema3g	-2.212993723	2.63E-06	1.97E-06	-2.350497247	2.63E-06	8.44E-07
Podxl	-1.835315003	2.88E-67	2.81E-52	-2.297219108	2.88E-67	1.65E-68
Pdgfd	-2.460097222	6.32E-90	4.99E-93	-2.242945253	6.32E-90	2.74E-91
Sema6a	-1.808903709	2.14E-71	3.83E-57	-2.191760803	2.14E-71	1.16E-72
Col18a1	-1.61403128	0	0	-2.074086318	0	0
Sema7a	-1.018378529	4.99E-47	1.49E-18	-2.025796001	4.99E-47	3.92E-48
F2rl1	-2.152928449	8.34E-270	1.25E-299	-2.003572503	8.34E-270	1.23E-271
Fam107a	-3	1.56E-04	4.89E-05	-1.192645078	0.038533376	0.0208544
Ntrk3	-2.237039197	5.97E-04	2.03E-04	-1.137503524	0.025580458	0.01329282
Podn	2.162271429	3.41E-12	5.72E-13	3.402098444	3.81E-44	3.14E-45
Ptprf	0.567122422	2.89E-54	1.55E-55	1.018699606	1.21E-204	2.37E-206
Adora3	6.906890596	4.77E-16	6.58E-17	6.845490051	2.05E-15	3.83E-16

down-regulation of *Cdh2*, *Podxl*, *L1cam*, *Bcar1*, *Tdgf1* and *Hbegf* [36–41] that are known for promoting cell migration (Table 5). Genes including *Podn*, *Ptprf* and *Adora3* [26,42,43] responsible for inhibition of cell migration were up-regulated. To confirm the effect of Dip2B in cell migration, MELFs were allowed to grow to confluence and scratch wounds were made. Gap closing were recorded at different time points. Results show that *Dip2b^{-/-}* MELFs was signifi-

cantly slower in migration than that of $Dip2b^{+/-}$ and $Dip2b^{+/+}$ MELFs (Fig. 7).

3.9. Verification of RNA-Seq results

Ten important DEGs were validated by qPCR. Overall, qPCR results are highly consistent with the RNA-Seq results (Fig. 8). Primer sequences are listed in Supplementary Table 10.



Fig. 7. Scratching assay for cell migration. Images were taken at 0, 24, 48hrs post-scratching.



Fig. 8. Gene expression validation of DEGs from RNA-Seq by qPCR.

4. Conclusions

In this study, MELFs were isolated from *Dip2b* knockout mouse embryos at E14.5. *Dip2b^{+/+}*, *Dip2b^{+/-}* and *Dip2b^{-/-}* MELFs were examined for genome-wide gene expression. DEGs were identified and analyzed by GO and KEGG for Dip2B-regulated bioprocesses and pathways. The most enriched bioprocesses and pathways were confirmed that include cell proliferation, cell cycle, cell apoptosis and cell migration.

GO analysis showed that DEGs were mostly annotated to membrane-related GO terms including 'integral component of plasma membrane', 'anchored component of plasma membrane', 'basolateral plasma membrane', 'ion channel activity', and 'voltagegated potassium channel activity, whereas KEGG pathways enriched DEGs are related to metabolism including protein digestion and absorption', 'renin secretion', 'pancreatic secretion', and 'steroid hormone synthesis'. Results demonstrate that Dip2B promotes cell proliferation, increases cell migration and inhibits cell apoptosis. Knockout Dip2B leads to cell arrest at G2/M phase. Results indicate that Dip2B is involved in multiple biological processes and pathways. Dip2B may regulate many important biologic functions that are highly correlated with development, differentiation and morphogenesis. Dip2B caused upregulation of multiple genes involves in innate and adaptive immune response and inflammatory response. Our analysis has identified upregulation of potential leukocyte chemotaxis genes (*Ccl9, Ccl6, Ccl11, Ccl22, Ccl12, Ccr5, Ccl7, Ccl2, Cxcl2, Cxcl3, Cxcl15*), highlighting the potential role of Dip2B in regulation of the immune cell mobilization and inflammatory responses. A number

S. Adlat et al.

of transcription factors such as homeobox (*Hox*) gene family, zinc finger (zf-C2H2), basic helix-loop-helix (bHLH) and Fox proteins were found to be significantly dysregulated. The most dysregulated TFs was Homeobox (*Hox*) genes suggesting that Dip2B may regulate cell differentiation and embryo development. Together, these information are valuable for further deciphering Dip2B roles in development and disease.

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Conflict of Interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csbj.2020.08.030.

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