## SUPPLEMENTAL INFORMATION

"Characterization of circRNA-associated ceRNA networks in folate deficiency-induced neural tube defects" by Shan Wang et al.

Supplemental Experimental Procedures

Circrna Identification and Quantification

CircRNA identification: Unmapped Reads were aligned with BWA mem (bwa mem-t 1-k 16-t 20): Partial alignments of segments within a single read that mapped to satisfy three conditions, first, reads have to be on the same chromosome regions and less than 1Mb distance in between; second, they are on the same strand; third, they have to be in reverse order, and were retained as candidates supporting head-to-tail junction. The strength of possible splicing sites supported by these candidate head-to-tail junction reads was then calculated by MaxEntScan33. The actual site was decided by the highest splicing strength score between acceptor and

Supplementary Figure S1. Gene Ontology (GO) enrichment of biological process molecular function and cell component of mRNA. The -$\log_{10}$ P value of enrichment is shown on X axis; the numbers represent the number of associated proteins for each term.
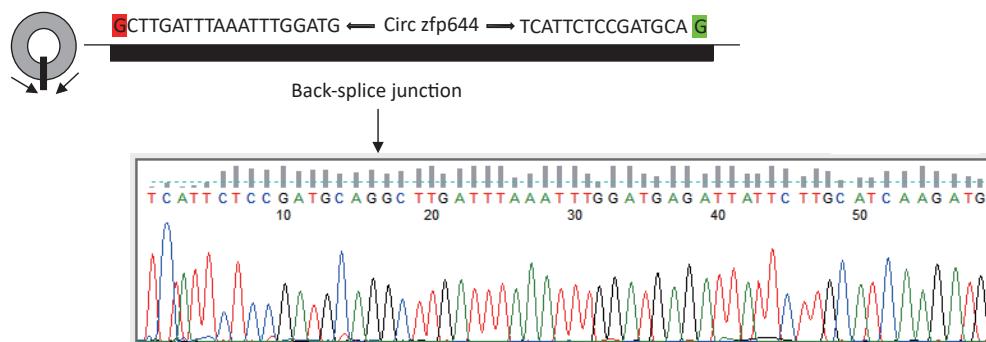
donor sites. Candidate circRNAs were selected if the head-to-tail junction was sustained by equal or more than two reads and the splicing score was greater than or equal to 10. All unmapped reads are rearranged using the following parameters (bWA mem-t 1-K 16-t 20) to achieve circRNA expression. There is no direct evidence for the exact sequence of most circrnas, and we populate the sequence with exon annotations. A ring connection is formed at the 5' end and connected with the 3' end. Counted each candidate map reads (with an overhang of at least 6 nt).

Antibodies

Antibodies used in immunoblotting: Gas7 (santa cruz), GAPDH antibody (proteintech).

Rna Immunoprecipitation Assays

RNA immunoprecipitation was performed by according the Magna RIP RNA-binding protein immunoprecipitation kit's protocol (Sigma-Aldrich, USA). For the RIP assay of Ago2, anti-Ago2 antibody (Millipore) was applied to RIP assay after the transfection for 48 h. Coprecipitated RNA was detected by fluorescence RT-qPCR.

Supplementary Figure S2. Characterization of circzfp644 in F9 cells. Genomic locus of circzfp644 gene. Circzfp644 is produced at the zfp644 gene (NM_0023982.4) locus containing exons 8–9. The back-splice junction of circzfp644 was identified by Sanger sequencing.
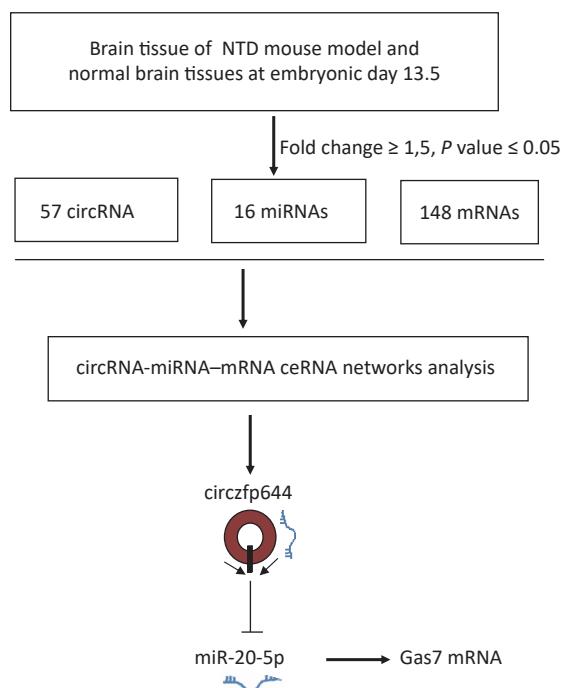
A

	Folate	
	Con (*n* = 8)	NTDs (*n* = 8)
Mean	0.216843625	0.05752525
SD	0.041416157	0.017349674
Mix	0.257867	0.0746
Min	0.158632	0.01867
IQR	0.238202	0.063000
Q1	0.18119575	0.055083
Q3	0.2441975	0.066083
Mann-Whitney *U* test *P* < 0.001		

B

	circZFP644		GAS 7		miR-20-5p	
	Con (*n* = 8)	NTDs (*n* = 8)	Con (*n* = 8)	NTDs (*n* = 8)	Con (*n* = 8)	NTDs (*n* = 8)
Mean	11.20909	10.71571	9.335455	8.568571	13.00273	13.33308
SD	0.148489	0.341979	0.338094	0.442508	0.129545	0.27759
Mix	11.42	11.33	9.74	9.47	13.22	13.71
Min	10.95	10.08	8.81	7.73	12.75	12.82
IQR	11.25	10.665	9.44	8.565	12.98	13.41
Q1	11.115	10.5275	9.065	8.3675	12.95	13.2
Q3	11.29	10.9025	9.575	8.7925	13.045	13.51
Mann-Whitney *U* test	*P* = 0.001		*P* = 0.001		*P* = 0.004	

Supplementary Figure S3. (A) Statistical analysis of f maternal serum folate concentrations between normal fetus and NTDs fetus. (B) Statistical analysis of Cirnzfp644, *Gas7* and miRNA-20-5p expression between human con and NTDs tissues.

Supplementary Figure S4. The flowchart delineates the steps for identifying and validating circRNAs in brain tissue of mouse NTDs.

1.Report gene sequence		
Position 5227-5233 of Gas7 3'UTR	5' ...GUAACUUUUGCUAAACACUUUAU...	
Mmu-miR-	3' GAUGGACGUGAUACUCGUGAAAC	||||||
Position 476-482 of circzfp644	5' ...GCAGCA GAG UCUUGCUC CACUUUG...	
Mmu-miR-20b-5p	3'. GAUGGACGUGAUACUC GUGAAAC	
2.miRNA-mimics and SiRNA		
Mmu-miR-20b-5p mimics	5'CAAAGUGCUCAUAGUGCAGGUAG '3	
SiRNA-circZfp644-1	5'CUCCGAUGCAGGCUUGAUUTT'3	
SiRNA-circZfp644-2	5'GAUGCAGGCUUGAUUUAAATT'3	

3.Primer		
Name	Primer sequence Forward	Primer sequence Reverse
CircRNA-zfp644	5'CATTCTCCGATGCAGGCTTG '3	3'ACCAGTAATGTCGGTGTTTATCTTC '5
Zfp644	5'CACTCCTGCTGTTCCTACTGA'3	3'CCAGGGGTTAATGAAACACTACC'5
Gas7	5'GGAGATCAAGTTGAGCAACAAG '3	3'CACCATCTCTTCAAACCACTTG '5
GAPDH	5'AAGGTCATCCCAGAGCTGAA '3	3'CTGCTTCACCACCTTCTTGA '5
miRNA-20b-5p	5'GACGGCAAAGTGCTCATAGTG '3	3'TATGGTTGTTCACGACTCCTTCAC'5
U6	5'CGCTTCGGCAGCACATATAC'3	3'TTCACGAATTTGCGTGTCATC'5

AccID	NTD	Control	Log2FC
chr11_62438515_62419598_-18917-Ncor1	7.611199314	0.69192721	3.459431619
chr14_87474840_87472076_+2764-Tdrd3	11.07083537	0.553541768	4.321928095
chr14_97916548_97901301_-15247-Dach1	0.69192721	7.472813872	-3.432959407
chr15_58106911_58104431_-2480-Atad2	5.535417683	0.553541768	3.321928095
chr16_33468183_33421321_+46862-Zfp148	6.227344893	0.69192721	3.169925001
chr17_5053617_5040580_+13037-Arid1b	8.995053735	0.69192721	3.700439718
chr17_63490887_63471408_-19479-Fbxl17	0.69192721	7.957162919	-3.523561956
chr17_65816050_65803211_+12839-Ppp4r1	12.45468979	1.383854421	3.169925001
chr19_24957990_24938396_-19594-Cbwd1	1.383854421	6.780886662	-2.292781749
chr19_43875020_43866589_-8431-Dnmbp	0.69192721	10.93244992	-3.981852653
chr2_106783858_106744700_+39158-Mpped2	0.69192721	8.02635564	-3.5360529
chr2_119451450_119448205_-3245-Ino80	8.303126524	0.69192721	3.584962501
chr2_32183300_32182458_+842-Prrc2b	1.383854421	22.83359794	-4.044394119
chr3_94871325_94862422_+8903-Pogz	0.69192721	6.227344893	-3.169925001
chr4_34848035_34839332_-8703-Zfp292	7.611199314	0.69192721	3.459431619
chr4_83696436_83693747_+2689-Ccdc171	0.69192721	6.365730335	-3.201633861
chr5_128269377_128268492_-885-Tmem132d	0.69192721	10.79406448	-3.963474124
chr5_32645107_32640331_+4776-Yes1	0.69192721	6.780886662	-3.292781749
chr6_83404824_83402667_-2157-Tet3	0.69192721	7.472813872	-3.432959407
chr7_126552338_126551975_-363-Eif3c	0.69192721	9.963751829	-3.847996907
chr7_134737726_134724330_+13396-Dock1	0.69192721	7.33442843	-3.40599236
chr7_99955540_99924296_-31244-Rnf169	0.69192721	8.441511967	-3.608809243
chr8_124305668_124295358_+10310-Galnt2	8.303126524	1.383854421	2.584962501
chr8_84864552_84864058_+494-Farsa	1.037890816	9.133439177	-3.137503524
chr9_22514093_22490818_+23275-Bbs9	6.227344893	0.69192721	3.169925001
chry_1158634_1129870_-28764-Uty	11.76276258	0.553541768	4.409390936
chr1_5098133_5089009_+9124-Atp6v1h	6.227344893	40.68531997	-2.707819249
chr1_66802168_66762048_-40120-Kansl1l	1.383854421	9.410210061	-2.765534746
chr10_116549105_116517317_-31788-Cnot2	13.146617	3.32125061	1.984893108
chr10_75700095_75684292_-15803-Cabin1	2.075781631	11.76276258	-2.502500341
chr13_91564694_91524585_+40109-Ssbp2	2.421745236	11.62437713	-2.263034406
chr15_8141359_8139851_+1508-Nup155	15.91432584	1.799010747	3.145050333
chr17_86103000_86098512_-4488-Srbd1	11.07083537	2.767708841	2
chr3_9474602_9470892_-3710-Zfp704	3.459636052	15.63755495	-2.176322773
chr4_91308854_91260761_-48093-Elavl2	7.611199314	1.383854421	2.459431619

chr5_106666845_106619540_-47305-Zfp644	2.075781631	9.271824619	-2.159198595
chr7_132973623_132949585_+24038-Zranb1	13.146617	1.245468979	3.399930607
chr8_26013555_26007187_-6368-Fnta	8.303126524	1.799010747	2.206450877
chr9_65066496_65034875_+31621-Dpp8	6.227344893	25.04776502	-2.007992791
chr9_80103764_80087430_+16334-Senp6	5.535417683	17.99010747	-1.700439718
chr1_165608604_165604610_-3994-Mpzl1	38.05599657	13.97692965	1.445076326
chr1_181961919_181956394_-5525-Enah	25.60130678	9.271824619	1.46529227
chr10_121931509_121925816_-5693-Srgap1	15.22239863	35.98021494	-1.2410081
chr11_70887171_70874746_+12425-Rabep1	5.535417683	19.78911822	-1.837943242
chr13_9735466_9710146_-25320-Zmynd11	22.83359794	8.856668293	1.366322214
chr14_45343727_45334653_+9074-Psmc6	15.91432584	5.397032241	1.560087832
chr15_79543856_79541034_-2822-Ddx17	2.767708841	19.23557645	-2.797012978
chr19_57642628_57629085_+13543-Atrnl1	29.06094284	13.70015877	1.084888898
chr2_131282768_131273911_+8857-Pank2	11.76276258	3.32125061	1.824428435
chr3_148859533_148846849_-12684-Lphn2	14.53047142	3.32125061	2.129283017
chr4_85006524_84971131_+35393-Cntln	22.83359794	47.88136296	-1.068306013
chr5_111349574_111346483_+3091-Pitpnb	31.13672447	10.65567904	1.546994651
chr7_46635556_46590607_-44949-Sergef	16.60625305	5.535417683	1.584962501
chr7_61925420_61813262_-112158-A230057D06Rik	3.459636052	13.28500244	-1.941106311
chr8_72380306_72367904_-12402-Eps15l1	28.36901563	69.74626281	-1.297799824
chr8_84772315_84771784_-531-Nfix	16.60625305	37.64084024	-1.180572246
chrx_143716148_143709648_+6500-Pak3	7.611199314	22.6952125	-1.576192291

FDR	Style
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0.00052	up
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0.019211818	up
0.02659815	up
0.000202	up
0.04354519	down
0.000185	up
0.043778985	down
0.00583657	down
0.004240865	down
0.022965547	up
0.013052234	down
0.014734487	down
0.008188802	up
0.029847992	down
0.00000427	down
0.016353624	down
0.040482875	down
0.022043254	down
0.00513538	down
0.011293211	down
0.005690964	up
0.025230293	down
0.010610862	up
0.0002	up
0.000308	down
0.02859832	down
0.013673812	up
0.003203746	down
0.035422624	down
0.018318162	up
0.048149834	up
0.000211	down
0.023618127	up

0.026774927	down
0.00032	up
0.040657695	up
0.000344	down
0.018530163	down
0.026249203	up
0.027666092	up
0.002657487	down
0.006301986	down
0.023635329	up
0.046069617	up
0.0000018	down
0.014548841	up
0.00973166	up
0.000911	up
0.002449394	down
0.000963	up
0.025577109	up
0.037616864	down
0.000000186	down
0.009376534	down
0.033924772	down

GOID	GOTerm	DifGene	AllDifGene
GO:0016568	chromatin modification	5	53
GO:0046329	negative regulation of JNK cascade	2	53
GO:0051225	spindle assembly	2	53
GO:0006493	protein O-linked glycosylation	2	53
GO:0006897	endocytosis	3	53
GO:0006351	transcription, DNA-templated	10	53
GO:0045944	positive regulation of transcription from RNA polymerase II promoter	6	53
GO:0000122	negative regulation of transcription from RNA polymerase II promoter	5	53
GO:0007275	multicellular organismal development	6	53
GO:0006355	regulation of transcription, DNA-templated	10	53
GO:0010629	negative regulation of gene expression	2	53
GO:0030036	actin cytoskeleton organization	2	53

GeneInGO	AllGene	P-Value	FDR	Enrichment	(-log10P)
259	22483	0.000356687	0.041574846	8.189334887	3.447712445
20	22483	0.001008156	0.041574846	42.42075472	2.996472387
23	22483	0.001336375	0.041574846	36.8876128	2.874071546
25	22483	0.001579861	0.041574846	33.93660377	2.801381013
155	22483	0.005850354	0.047199966	8.210468655	2.232817852
1814	22483	0.009021803	0.050637672	2.338520106	2.044706659
786	22483	0.010172957	0.053339287	3.238225551	1.992552797
566	22483	0.010552997	0.053875826	3.747416494	1.976624193
989	22483	0.028228245	0.083762563	2.573554381	1.549316123
2235	22483	0.034075543	0.088814056	1.898020345	1.467557221
138	22483	0.042046428	0.099475697	6.147935466	1.376270889
141	22483	0.043706736	0.101316294	6.017128329	1.359451626

GeneInGO	AllGene	P-Value	FDR	Enrichment	(-log10P)
259	22483	0.000356687	0.041574846	8.189334887	3.447712445

AccID	NTD	Control
Sfrp5	3.474084259	12.42271183
Islr	475.0066179	745.8794463
Col27a1	356.0242026	659.8821964
Doc2a	49.29105476	24.27888302
Sbspon	2.976440708	11.12365956
Hcrtr2	25.22427536	10.76964537
Sorbs3	302.5402606	479.0002785
Fam107a	56.04723271	141.4312581
Kcnip2	15.27379713	31.11004897
Pdyn	3.852633948	14.78061987
Tmem160	17.56458702	37.94912505
Cldn4	91.14840141	153.7689553
Prokr2	59.22675154	103.014609
Gas7	340.6914938	547.2787243
Robo4	101.4619209	153.0903997
Pcdhb22	75.87663121	121.4563951
Pcdhb21	46.69776463	82.12163289
Atp6v1c2	1.522821758	8.870324362
Pear1	127.9103599	217.5769035
P4ha3	247.0549122	439.4104542
Dmc1	83.70472568	47.6009998
Col5a1	1926.327774	2919.791132
Il3ra	27.33756673	55.08682778
Mon1a	138.5763412	211.9585205
Nlrx1	19.31903925	41.24352982
C330018D20Rik	311.0071194	205.207752
Grb7	42.05169236	81.60322575
Galnt15	6.288206702	21.4397568
Papss2	150.738773	347.7841014
Ubtd1	122.2148685	189.1218279
Rnf126	402.67599	611.7494265
LOC102639422	43.73856992	83.53718011
Ifitm1	31.52552597	54.41653947
Tmprss13	2.976440708	9.913044277
F2rl2	58.40262451	24.60790442
Creb3l1	44.80070468	95.32003104
Eln	163.8132287	441.6861123
Col14a1	193.6314395	367.3849807
Igfbp6	1.488220354	32.40865619
Ttll3	589.3012034	1042.765352
Prr24	37.44811748	62.8670438
LOC102631834	169.0595912	102.4877328
Cbr2	5.375455707	15.21503927
Iqsec2	155.2307512	232.8547558
Ano3	99.41732409	63.41713298
Mmp11	278.7840245	424.5835451
Adam12	229.6942339	373.0353613
Myzap	59.17048323	95.05794846
Gm10651	22.34917431	58.19790485
Krt15	76.04315157	171.4642616
LOC102635842	179.427165	388.2439317

Tcf23	0.382861189	23.8525599
Matn4	16.22143893	54.55625522
Gdf10	94.60070935	200.6918367
Ptger3	32.36505205	69.96698157
Nfic	470.0561537	795.1802131
Extl1	9.95254398	28.99725469
Afp	1.139960569	14.64231894
Padi2	27.40061109	53.11615247
Lmcd1	22.08993729	43.809241
Inha	50.34692167	90.13854298
Lin28a	119.6059288	49.99664792
Hist2h3c2	2252.403949	5891.504007
Xdh	1.144272068	20.70621826
Pex26	192.6613333	293.8196796
LOC102635707	5.496505918	20.44434523
Esr1	0.761410879	29.90957601
Gsn	426.1341456	663.8141727
Ly6a	0.757099379	19.65877505
Reps2	162.5051138	269.7576662
Ptrf	508.0498894	927.8333417
1700024P16Rik	5.260673345	16.04964043
Muc16	0.765722379	30.66408757
Il6ra	14.42582804	33.51431852
Ssh3	53.01637064	92.1845846
Naaa	30.53886186	54.78031587
Mmp23	9.848739768	27.0435404
Ikbke	34.55616923	60.5111818
Hist1h2ah	833.7959294	1492.626366
Efcab1	194.0264765	110.2971554
Otud1	50.88778962	84.60189995
Usp44	238.3310951	158.4643802
Ltbp3	287.9882015	469.1820033
Zfp703	279.0908363	448.2995761
Tgfb3	251.6322899	439.2118388
Actg2	12.17188531	172.5669874
Btbd6	165.8822793	262.2667193
Tnfsf12	14.70068292	37.5836983
Dnali1	3.768141491	12.5445613
Myh7	18.10987588	275.4323197
Bcl9l	818.5980582	1312.550456
Pgf	50.57855214	79.43603819
Serpina3n	0.37854969	30.78613099
Gpx3	360.4490767	560.7126632
Hist1h2ae	324.3313469	558.0675815
Hist1h2ad	574.1251944	1151.501156
Col9a3	85.23903379	194.9922957
Col9a2	146.8084226	269.5556393
LOC102639600	0.757099379	5.904431426
Epn3	16.82495245	38.24473396
Mafb	163.3492796	283.6421989
Myh11	15.37122409	233.8374607
Bcl6	29.37343109	57.73479626
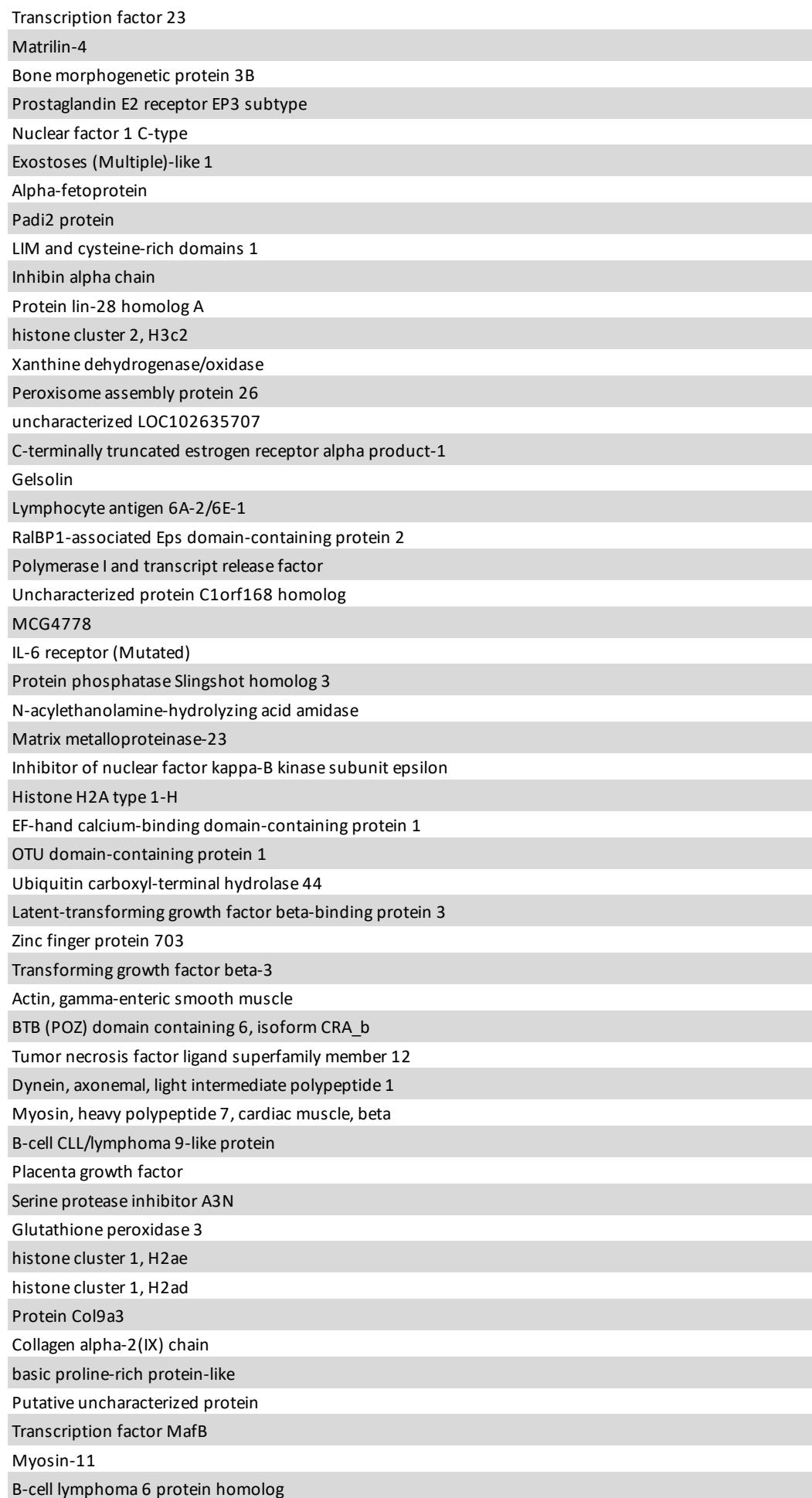
Sema4f	80.85589242	126.5945868
Stra6	282.8181861	467.7633108
Wdfy4	34.48914158	60.51149741
Apobec3	61.41786872	93.81360509
Hopx	17.23368263	35.96857366
Mfap2	596.0857985	916.6527055
Ahdc1	878.4490843	1329.688356
Hspa1a	15.94669345	35.55512727
Cebpa	16.75132873	34.46604584
Tenc1	365.7828714	616.7694881
Tbx5	8.300049011	30.88362667
Tnip1	198.2103042	317.9857411
Blnk	6.275272202	20.73759249
Chmp4c	1.148583568	7.087498308
LOC102640935	118.2799878	70.27955151
Myl3	0.37854969	26.71388
Myl2	0.37854969	33.97360127
Ifi27	4.244118138	14.37138721
Map3k15	49.91427883	27.25351125
Slc9a3r2	170.2000211	259.1936485
Myl9	521.0507215	783.2369964
Cngb3	1.101047665	6.087047822
Rab39	88.73141339	53.61051363
Pitx3	3.311765994	13.00098063
Tmem200c	41.16861366	66.61130031
Plac9b	6.958747868	24.97569281
Gm5820	7.536282982	22.82189154
Ticam1	101.9573187	154.0817192
Svopl	7.677043748	19.94494515
Mx2	17.31150844	5.58650697
Eva1b	111.9317095	173.8878538
Atoh1	88.31159565	49.55081597
B4galnt2	11.35649069	0.635848911
B4galnt3	30.72335524	70.30722157
LOC102634126	5.340854302	0.265383256
Scara5	70.9830212	123.8212664
Slc38a11	17.17741433	5.962836704
Lmod3	0.343948285	7.828745227
Cygb	40.54796355	71.29185181
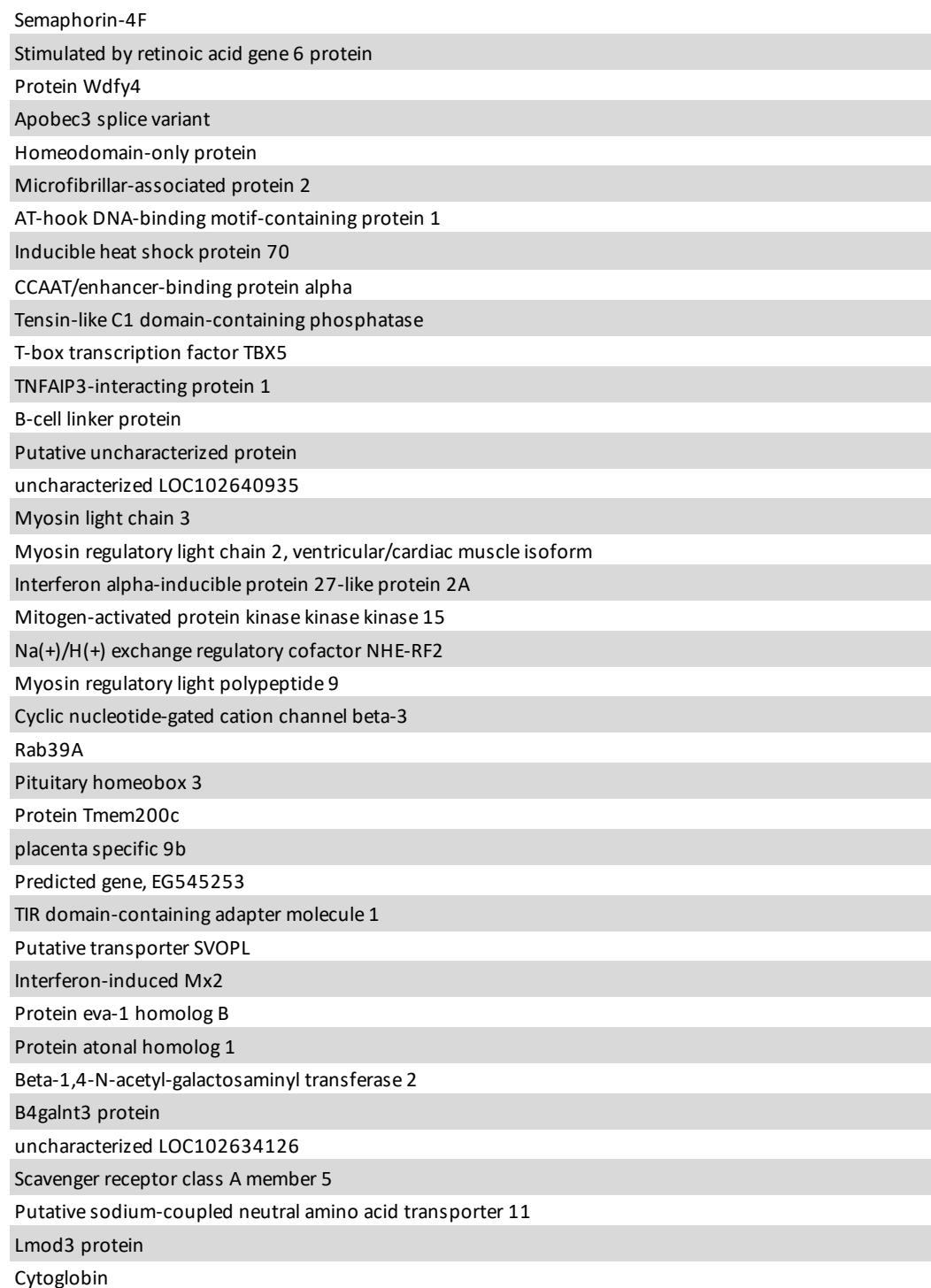
Log2FC	FDR	Style
-1.838275493	0.022462354	down
-0.650994859	0.00553012	down
-0.890233175	0.025455093	down
1.021623803	0.00189774	up
-1.901971436	0.024735634	down
1.22784208	0.003171262	up
-0.662899353	1.11022E-16	down
-1.335385961	0.000797425	down
-1.026321899	0.010384877	down
-1.939789752	0.002580655	down
-1.111396967	0.010441017	down
-0.754475005	0.035210826	down
-0.798528083	1.21605E-05	down
-0.683809845	0.016186251	down
-0.593445435	0.000106808	down
-0.678710923	0.011448964	down
-0.814408823	0.00118645	down
-2.542239772	0.041394619	down
-0.766392301	3.31011E-09	down
-0.830737452	0.00018371	down
0.814317199	0.001980428	up
-0.600011964	0.021925206	down
-1.01082255	0.004690815	down
-0.613100992	0.002545969	down
-1.094144461	0.022821686	down
0.599862374	7.69369E-06	up
-0.95646232	0.02454073	down
-1.769567994	0.030969164	down
-1.206141429	0.001904923	down
-0.629896072	3.07392E-05	down
-0.603321391	1.25191E-05	down
-0.933512394	0.000119727	down
-0.787524773	0.025463735	down
-1.735740019	0.044726982	down
1.246911398	0.001454896	up
-1.089257996	0.037176076	down
-1.430969605	0.002218268	down
-0.923979429	0.012687096	down
-4.44471914	0.016965498	down
-0.823337435	0.017960347	down
-0.747410729	0.008983216	down
0.722080628	9.40097E-08	up
-1.501039088	0.047781548	down
-0.585015964	1.53241E-07	down
0.648624616	0.012095657	up
-0.606900569	0.048067984	down
-0.699597755	3.71095E-06	down
-0.683929589	0.037664003	down
-1.380745684	0.004134115	down
-1.173017676	0.000196453	down
-1.113565047	0.002722426	down

-5.961178875	0.003295379	down
-1.74984282	0.004021454	down
-1.085059029	3.33067E-16	down
-1.11223743	0.00892477	down
-0.758448744	4.84107E-06	down
-1.542779073	0.008725306	down
-3.683088228	0.000373352	down
-0.954942578	0.003223383	down
-0.987845897	0.040978611	down
-0.840240559	0.0008564	down
1.258385628	0.002177885	up
-1.387170393	0.018281724	down
-4.177562066	0.029346622	down
-0.608863974	5.54634E-06	down
-1.895115152	0.003156342	down
-5.295788466	0.005074378	down
-0.639471774	0.025751812	down
-4.698546934	0.01776303	down
-0.731178842	0.027690389	down
-0.868895517	0.000197118	down
-1.609221601	0.034238116	down
-5.323584791	0.00290845	down
-1.216123466	0.024021529	down
-0.798087608	3.75767E-05	down
-0.843011289	0.002154339	down
-1.457272998	0.039980298	down
-0.808258467	0.044193514	down
-0.840086842	0.028572589	down
0.81485795	3.59611E-09	up
-0.733370536	0.020719612	down
0.588808606	4.19238E-05	up
-0.704137968	0.002179058	down
-0.68372838	0.012203243	down
-0.803599879	0.011067497	down
-3.825531948	0.044279071	down
-0.660874971	7.75153E-08	down
-1.35422386	0.001554319	down
-1.735136975	0.026609579	down
-3.926849295	0.04454509	down
-0.681145733	6.14175E-12	down
-0.651267932	0.010266198	down
-6.345654074	0.001533203	down
-0.637466199	0.025838473	down
-0.782971374	0.038333077	down
-1.004078587	0.003434013	down
-1.193830978	0.002883793	down
-0.876648352	2.63153E-05	down
-2.96324355	0.02881554	down
-1.184658685	0.006091739	down
-0.796112094	1.47258E-11	down
-3.927202104	0.045012011	down
-0.974929285	0.01365468	down

-0.646790896	0.005695205	down
-0.725903815	0.046839158	down
-0.811067065	0.018413637	down
-0.611138712	0.003267034	down
-1.061505929	0.012379994	down
-0.620855238	0.001292512	down
-0.598057581	6.63826E-08	down
-1.156800309	0.002000233	down
-1.040900257	0.007015654	down
-0.753743877	2.2715E-07	down
-1.895650418	0.004462394	down
-0.68193011	0.00630856	down
-1.724498471	0.014377369	down
-2.625420658	0.021931454	down
0.75102911	0.000130781	up
-6.140963037	0.002354558	down
-6.48778766	0.001100325	down
-1.759662606	0.040232544	down
0.873010468	0.043591599	up
-0.60679915	0.003410758	down
-0.588025095	1.64168E-07	down
-2.466865775	0.042954301	down
0.726928992	0.000395659	up
-1.972947804	0.0320763	down
-0.694222078	0.019519672	down
-1.843625057	0.015570667	down
-1.598493328	0.000324097	down
-0.595730367	0.001632349	down
-1.377400382	0.040589833	down
1.631713032	0.039086766	up
-0.635538363	0.00083252	down
0.833694067	0.024828012	up
4.158689284	0.029424293	up
-1.194337104	0.000992277	down
4.330921274	0.026342661	up
-0.802713235	0.037717729	down
1.526442154	0.008039884	up
-4.508517525	0.000766938	down
-0.814107736	0.001127931	down

Description
Secreted frizzled-related protein 5
Immunoglobulin superfamily containing leucine-rich repeat protein
Collagen alpha-1(XXVII) chain
Double C2-like domain-containing protein alpha
Somatomedin-B and thrombospondin type-1 domain-containing protein
Hypocretin (Orexin) receptor 2
Vinexin-g
Protein Fam107a
Kv channel-interacting protein 2
Proenkephalin-B
Transmembrane protein 160
Claudin-4
Prokineticin receptor 2
Growth arrest-specific protein 7
Roundabout homolog 4
Pcdhb22 protein
Putative uncharacterized protein
V-type proton ATPase subunit C 2
Platelet endothelial aggregation receptor 1
Prolyl 4-hydroxylase subunit alpha-3
Disrupted meiotic cDNA 1 homolog, isoform CRA_a
Collagen alpha-1(V) chain
Interleukin-3 receptor subunit alpha
Vacuolar fusion protein MON1 homolog A
NLR family member X1
Glutaredoxin-like protein C5orf63 homolog
Growth factor receptor-bound protein 7
UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 2
Bifunctional 3'-phosphoadenosine 5'-phosphosulfate synthase 2
Ubiquitin domain-containing protein 1
Putative uncharacterized protein
basic proline-rich protein-like
Interferon-induced transmembrane protein 1
Transmembrane protease serine 13
Proteinase-activated receptor 3
Cyclic AMP-responsive element-binding protein 3-like protein 1
Elastin
Procollagen, type XIV, alpha 1, isoform CRA_a
Insulin-like growth factor-binding protein 6
Tubulin monoglycylase TTLL3
Protein Inafm1
zinc finger protein 709-like
Carbonyl reductase [NADPH] 2
IQ motif and SEC7 domain-containing protein 2
Anoctamin-3
Stromelysin-3
Disintegrin and metalloproteinase domain-containing protein 12
Myocardial zonula adherens protein
predicted pseudogene 10651
Keratin, type I cytoskeletal 15
serine/arginine repetitive matrix protein 2-like

Transcription factor 23

Matrilin-4

Bone morphogenetic protein 3B

Prostaglandin E2 receptor EP3 subtype

Nuclear factor 1 C-type

Exostoses (Multiple)-like 1

Alpha-fetoprotein

Padi2 protein

LIM and cysteine-rich domains 1

Inhibin alpha chain

Protein lin-28 homolog A

histone cluster 2, H3c2

Xanthine dehydrogenase/oxidase

Peroxisome assembly protein 26

uncharacterized LOC102635707

C-terminally truncated estrogen receptor alpha product-1

Gelsolin

Lymphocyte antigen 6A-2/6E-1

RalBP1-associated Eps domain-containing protein 2

Polymerase I and transcript release factor

Uncharacterized protein C1orf168 homolog

MCG4778

IL-6 receptor (Mutated)

Protein phosphatase Slingshot homolog 3

N-acylethanolamine-hydrolyzing acid amidase

Matrix metalloproteinase-23

Inhibitor of nuclear factor kappa-B kinase subunit epsilon

Histone H2A type 1-H

EF-hand calcium-binding domain-containing protein 1

OTU domain-containing protein 1

Ubiquitin carboxyl-terminal hydrolase 44

Latent-transforming growth factor beta-binding protein 3

Zinc finger protein 703

Transforming growth factor beta-3

Actin, gamma-enteric smooth muscle

BTB (POZ) domain containing 6, isoform CRA_b

Tumor necrosis factor ligand superfamily member 12

Dynein, axonemal, light intermediate polypeptide 1

Myosin, heavy polypeptide 7, cardiac muscle, beta

B-cell CLL/lymphoma 9-like protein

Placenta growth factor

Serine protease inhibitor A3N

Glutathione peroxidase 3

histone cluster 1, H2ae

histone cluster 1, H2ad

Protein Col9a3

Collagen alpha-2(IX) chain

basic proline-rich protein-like

Putative uncharacterized protein

Transcription factor MafB

Myosin-11

B-cell lymphoma 6 protein homolog

Semaphorin-4F

Stimulated by retinoic acid gene 6 protein

Protein Wdfy4

Apobec3 splice variant

Homeodomain-only protein

Microfibrillar-associated protein 2

AT-hook DNA-binding motif-containing protein 1

Inducible heat shock protein 70

CCAAT/enhancer-binding protein alpha

Tensin-like C1 domain-containing phosphatase

T-box transcription factor TBX5

TNFAIP3-interacting protein 1

B-cell linker protein

Putative uncharacterized protein

uncharacterized LOC102640935

Myosin light chain 3

Myosin regulatory light chain 2, ventricular/cardiac muscle isoform

Interferon alpha-inducible protein 27-like protein 2A

Mitogen-activated protein kinase kinase kinase 15

Na(+)/H(+) exchange regulatory cofactor NHE-RF2

Myosin regulatory light polypeptide 9

Cyclic nucleotide-gated cation channel beta-3

Rab39A

Pituitary homeobox 3

Protein Tmem200c

placenta specific 9b

Predicted gene, EG545253

TIR domain-containing adapter molecule 1

Putative transporter SVOPL

Interferon-induced Mx2

Protein eva-1 homolog B

Protein atonal homolog 1

Beta-1,4-N-acetyl-galactosaminyl transferase 2

B4galnt3 protein

uncharacterized LOC102634126

Scavenger receptor class A member 5

Putative sodium-coupled neutral amino acid transporter 11

Lmod3 protein

Cytoglobin

GOID	GO Term (BP)	DifGene	AllDifGene
GO:0055010	ventricular cardiac muscle tissue morphogenesis	4	143
GO:0030199	collagen fibril organization	4	143
GO:0003229	ventricular cardiac muscle tissue development	2	143
GO:0008285	negative regulation of cell proliferation	9	143
GO:0010718	positive regulation of epithelial to mesenchymal transition	3	143
GO:0045071	negative regulation of viral genome replication	3	143
GO:0008016	regulation of heart contraction	3	143
GO:0043124	negative regulation of I-kappaB kinase/NF-kappaB signaling	3	143
GO:0007200	phospholipase C-activating G-protein coupled receptor signaling pathway	3	143
GO:0045087	innate immune response	6	143
GO:0048286	lung alveolus development	3	143
GO:0060048	cardiac muscle contraction	3	143
GO:0016049	cell growth	3	143
GO:0071392	cellular response to estradiol stimulus	2	143
GO:0030041	actin filament polymerization	2	143

GOID	GO Term (CC)	DifGene	AllDifGene
GO:0005578	proteinaceous extracellular matrix	10	148
GO:0016459	myosin complex	5	148
GO:0005576	extracellular region	27	148
GO:0032982	myosin filament	3	148
GO:0031012	extracellular matrix	8	148
GO:0030016	myofibril	4	148
GO:0005594	collagen type IX trimer	2	148
GO:0005581	collagen trimer	5	148
GO:0005615	extracellular space	16	148
GO:0005859	muscle myosin complex	2	148
GO:0031672	A band	2	148
GO:0000932	cytoplasmic mRNA processing body	3	148
GO:0001725	stress fiber	3	148
GO:0090534	calcium ion-transporting ATPase complex	1	148
GO:0031674	I band	2	148

GOID	GO Term(MF)	DifGene	AllDifGene
GO:0008307	structural constituent of muscle	3	141
GO:0005201	extracellular matrix structural constituent	3	141
GO:0008376	acetylgalactosaminyltransferase activity	2	141
GO:0003774	motor activity	4	141
GO:0005509	calcium ion binding	11	141
GO:0005044	scavenger receptor activity	3	141
GO:0003785	actin monomer binding	2	141
GO:0004843	ubiquitin-specific protease activity	3	141
GO:0016758	transferase activity, transferring hexosyl groups	3	141

GO:0070287	ferritin receptor activity	1	141
GO:0047844	deoxycytidine deaminase activity	1	141
GO:0070119	ciliary neurotrophic factor binding	1	141
GO:0045504	dynein heavy chain binding	1	141
GO:1990175	EH domain binding	1	141
GO:0004854	xanthine dehydrogenase activity	1	141

GeneInGO	AllGene	P-Value	FDR	Enrichment	(-log10P)
29	22483	3.29322E-05	0.02058263	21.6860381	4.482379149
39	22483	0.000108556	0.03392374	16.12551551	3.964346302
4	22483	0.000239028	0.041303776	78.61188811	3.621552128
329	22483	0.000264344	0.041303776	4.300954365	3.577830271
24	22483	0.000462349	0.057386844	19.65297203	3.335029634
27	22483	0.000658906	0.057386844	17.46930847	3.181176223
27	22483	0.000658906	0.057386844	17.46930847	3.181176223
28	22483	0.000734552	0.057386844	16.8454046	3.133977685
34	22483	0.001304864	0.086944212	13.87268614	2.88443462
201	22483	0.001836435	0.086944212	4.693247051	2.73602447
39	22483	0.001947159	0.086944212	12.09413663	2.710598542
40	22483	0.002095305	0.086944212	11.79178322	2.678752704
42	22483	0.002412177	0.086944212	11.23026973	2.617590839
15	22483	0.003995197	0.086944212	20.96317016	2.398461777
15	22483	0.003995197	0.086944212	20.96317016	2.398461777

GeneInGO	AllGene	P-Value	FDR	Enrichment	(-log10P)
268	22331	1.24886E-05	0.001357104	5.630042356	4.903487203
53	22331	2.65396E-05	0.001357104	14.23444671	4.576105451
1744	22331	2.8273E-05	0.001357104	2.33595106	4.548627975
12	22331	6.00657E-05	0.002162367	37.72128378	4.221373129
218	22331	0.000106053	0.002680838	5.537069179	3.974476484
38	22331	0.000114755	0.002680838	15.8826458	3.940226731
3	22331	0.000130319	0.002680838	100.5900901	3.884993841
87	22331	0.000285278	0.005134996	8.671559491	3.544732409
943	22331	0.000535083	0.008561329	2.560087128	3.271578777
6	22331	0.000643117	0.009260891	50.29504505	3.191709726
13	22331	0.003243851	0.042464958	23.21309771	2.488939107
54	22331	0.005526513	0.066318154	8.382507508	2.25754882
57	22331	0.006426024	0.068169169	7.941322902	2.192057689
1	22331	0.006627558	0.068169169	150.8851351	2.178646456
20	22331	0.007665413	0.073587964	15.08851351	2.115464447

GeneInGO	AllGene	P-Value	FDR	Enrichment	(-log10P)
20	22058	0.000269139	0.060556303	23.46595745	3.570023168
28	22058	0.00074502	0.062572237	16.76139818	3.127832341
7	22058	0.000834296	0.062572237	44.6970618	3.078679582
71	22058	0.001115519	0.062747948	8.813505144	2.952523001
665	22058	0.003683266	0.075697563	2.587724631	2.433766916
49	22058	0.003799586	0.075697563	9.577941815	2.420263676
15	22058	0.004033891	0.075697563	20.85862884	2.39427583
54	22058	0.004998054	0.075697563	8.691095351	2.301199061
58	22058	0.006103392	0.075697563	8.091709464	2.214428747

1	22058	0.006392239	0.075697563	156.4397163	2.19434702
1	22058	0.006392239	0.075697563	156.4397163	2.19434702
1	22058	0.006392239	0.075697563	156.4397163	2.19434702
1	22058	0.006392239	0.075697563	156.4397163	2.19434702
1	22058	0.006392239	0.075697563	156.4397163	2.19434702
1	22058	0.006392239	0.075697563	156.4397163	2.19434702

PathwayID	PathwayTerm	DifGene	AllDifGene	GeneInPathway	AllGene
PATH:04974	Protein digestion and absorption	6	63	89	7605
PATH:05414	Dilated cardiomyopathy	6	63	89	7605
PATH:05410	Hypertrophic cardiomyopathy (HCM)	5	63	84	7605
PATH:05164	Influenza A	6	63	170	7605
PATH:04530	Tight junction	5	63	137	7605
PATH:05034	Alcoholism	6	63	199	7605
PATH:05322	Systemic lupus erythematosus	5	63	148	7605
PATH:04261	Adrenergic signaling in cardiomyocytes	5	63	151	7605
PATH:04260	Cardiac muscle contraction	3	63	79	7605
PATH:04510	Focal adhesion	5	63	208	7605
PATH:05161	Hepatitis B	4	63	145	7605
PATH:05323	Rheumatoid arthritis	3	63	84	7605
PATH:04144	Endocytosis	5	63	222	7605
PATH:04915	Estrogen signaling pathway	3	63	98	7605

P-Value	FDR	Enrichment	(-log10P)
8.62487E-05	0.004916175	8.138041734	4.064247499
8.62487E-05	0.004916175	8.138041734	4.064247499
0.000620136	0.023565168	7.18537415	3.207513052
0.002705112	0.077095684	4.260504202	2.567814792
0.005367865	0.111281924	4.405630865	2.27019845
0.005856943	0.111281924	3.639626705	2.232328975
0.007410153	0.114713512	4.078185328	2.13017283
0.008050071	0.114713512	3.997161779	2.094200289
0.027435811	0.305091799	4.584086799	1.561682198
0.02845067	0.305091799	2.901785714	1.545907497
0.031733356	0.305091799	3.330049261	1.498483993
0.032114926	0.305091799	4.31122449	1.493293072
0.036220941	0.31762979	2.718790219	1.441040271
0.047285814	0.385041627	3.695335277	1.325269132

AccID	NTD	Control	Log2FC	FDR	Style
mmu-miR-20b-5p	8191.983754	5456.711072	0.586181198	3.77476E-15	up
tsRNA-Leu-5017b	1129.257017	1716.455868	-0.604058887	2.88696E-08	down
tsRNA-Asp-5023a	277.1950161	418.3485806	-0.593804222	1.34627E-07	down
mmu-let-7j	20.08727523	60.97890098	-1.602028268	6.61172E-07	down
mmu-miR-28c	27.4291865	61.30658382	-1.160330181	4.10822E-05	down
mmu-let-7i-5p	1557.887436	3108.44925	-0.996604029	0.000133266	down
mmu-miR-196b-3p	0	7.885101775	-20	0.000314683	down
mmu-miR-455-5p	123.4196635	214.2879894	-0.795978725	0.000463397	down
mmu-miR-16-2-3p	240.3757027	158.1880381	0.603650565	0.000503813	up
tsRNA-Phe-1010	432.8364466	239.9938403	0.850824607	0.000653671	up
mmu-miR-98-5p	198.8501912	369.9477591	-0.895639611	0.00067637	down
mmu-miR-302c-5p	35.08176147	12.90107952	1.443229397	0.000687283	up
mmu-miR-25-3p	2975.162448	4738.705087	-0.671524434	0.000828325	down
mmu-miR-196b-5p	14.74199426	338.054066	-4.519250393	0.006776443	down
mmu-miR-10b-5p	43.22997054	697.4580537	-4.012002695	0.010951645	down
mmu-miR-181d-3p	180.2844772	97.17387398	0.891634793	0.015038969	up
mmu-miR-500-5p	16.17401803	35.61201439	-1.138685919	0.025633637	down
mmu-miR-367-3p	30.64401459	13.01637462	1.235277635	0.032119063	up
mmu-miR-302d-5p	27.16531811	13.62071946	0.995963031	0.049081375	up

CircRNA_miRNA			
QueryID	miRNA_Style	SubjectID	CircRNA_Style
mmu-miR-196b-5p	down	chr4_91308854_91260761_-48093-Elavl2	up
mmu-miR-196b-5p	down	chr4_91308854_91260761_-48093-Elavl2	up
mmu-miR-20b-5p	up	chr1_66802168_66762048_-40120-Kansl1l	down
mmu-miR-20b-5p	up	chr1_66802168_66762048_-40120-Kansl1l	down
mmu-miR-20b-5p	up	chr1_66802168_66762048_-40120-Kansl1l	down
mmu-miR-20b-5p	up	chr1_66802168_66762048_-40120-Kansl1l	down
mmu-miR-20b-5p	up	chr1_66802168_66762048_-40120-Kansl1l	down
mmu-miR-20b-5p	up	chr1_66802168_66762048_-40120-Kansl1l	down
mmu-miR-20b-5p	up	chr7_61925420_61813262_-112158-A230057D06Ri	down
mmu-miR-20b-5p	up	chr7_61925420_61813262_-112158-A230057D06Ri	down
mmu-miR-20b-5p	up	chr7_61925420_61813262_-112158-A230057D06Ri	down
mmu-miR-20b-5p	up	chr7_61925420_61813262_-112158-A230057D06Ri	down
mmu-miR-20b-5p	up	chr7_61925420_61813262_-112158-A230057D06Ri	down
mmu-miR-20b-5p	up	chr7_61925420_61813262_-112158-A230057D06Ri	down
mmu-miR-20b-5p	up	chr5_106666845_106619540_-47305-Zfp644	down
mmu-miR-20b-5p	up	chr5_106666845_106619540_-47305-Zfp644	down
mmu-miR-20b-5p	up	chr5_106666845_106619540_-47305-Zfp644	down
mmu-miR-20b-5p	up	chr5_106666845_106619540_-47305-Zfp644	down
mmu-miR-20b-5p	up	chr5_106666845_106619540_-47305-Zfp644	down
mmu-miR-20b-5p	up	chr5_106666845_106619540_-47305-Zfp644	down
mmu-miR-181d-3p	up	chr7_61925420_61813262_-112158-A230057D06Ri	down
mmu-miR-181d-3p	up	chr7_61925420_61813262_-112158-A230057D06Ri	down
mmu-miR-181d-3p	up	chr7_61925420_61813262_-112158-A230057D06Ri	down
mmu-miR-181d-3p	up	chr7_61925420_61813262_-112158-A230057D06Ri	down
mmu-miR-181d-3p	up	chr7_61925420_61813262_-112158-A230057D06Ri	down
mmu-miR-181d-3p	up	chr7_61925420_61813262_-112158-A230057D06Ri	down
mmu-miR-181d-3p	up	chr10_75700095_75684292_-15803-Cabin1	down
mmu-miR-181d-3p	up	chr10_75700095_75684292_-15803-Cabin1	down
mmu-miR-181d-3p	up	chr10_75700095_75684292_-15803-Cabin1	down
mmu-miR-181d-3p	up	chr10_75700095_75684292_-15803-Cabin1	down
mmu-miR-181d-3p	up	chr10_75700095_75684292_-15803-Cabin1	down
mmu-miR-181d-3p	up	chr10_75700095_75684292_-15803-Cabin1	down

mmu-miR-181d-3p	up	chr11_70887171_70874746_+12425-Rabep1	down
mmu-miR-181d-3p	up	chr11_70887171_70874746_+12425-Rabep1	down
mmu-miR-181d-3p	up	chr11_70887171_70874746_+12425-Rabep1	down
mmu-miR-181d-3p	up	chr11_70887171_70874746_+12425-Rabep1	down
mmu-miR-181d-3p	up	chr11_70887171_70874746_+12425-Rabep1	down
mmu-miR-181d-3p	up	chr11_70887171_70874746_+12425-Rabep1	down
mmu-let-7i-5p	down	chry_1158634_1129870_-28764-Uty	up
mmu-let-7i-5p	down	chry_1158634_1129870_-28764-Uty	up
mmu-miR-367-3p	up	chr7_61925420_61813262_-112158-A230057D06Ri	down
mmu-miR-367-3p	up	chr5_106666845_106619540_-47305-Zfp644	down
mmu-miR-302d-5p	up	chr1_66802168_66762048_-40120-Kansl1l	down
mmu-miR-302d-5p	up	chr1_66802168_66762048_-40120-Kansl1l	down
mmu-miR-302d-5p	up	chr7_61925420_61813262_-112158-A230057D06Ri	down
mmu-miR-302d-5p	up	chr7_61925420_61813262_-112158-A230057D06Ri	down
mmu-miR-98-5p	down	chry_1158634_1129870_-28764-Uty	up
mmu-miR-28c	down	chr17_5053617_5040580_+13037-Arid1b	up
mmu-miR-28c	down	chr3_148859533_148846849_-12684-Lphn2	up
mmu-miR-28c	down	chr10_116549105_116517317_-31788-Cnot2	up

miRNA_mRNA			
QueryID	miRNA_Style	SubjectID	mRNA_Style
mmu-miR-196b-5p	down	Doc2a	up
mmu-miR-196b-5p	down	Lin28a	up
mmu-miR-20b-5p	up	Cngb3	down
mmu-miR-20b-5p	up	Gas7	down
mmu-miR-20b-5p	up	Nfic	down
mmu-miR-20b-5p	up	Chmp4c	down
mmu-miR-20b-5p	up	Scara5	down
mmu-miR-20b-5p	up	Cygb	down
mmu-miR-20b-5p	up	Cngb3	down
mmu-miR-20b-5p	up	Gas7	down
mmu-miR-20b-5p	up	Nfic	down
mmu-miR-20b-5p	up	Chmp4c	down
mmu-miR-20b-5p	up	Scara5	down
mmu-miR-20b-5p	up	Cygb	down
mmu-miR-20b-5p	up	Cngb3	down
mmu-miR-20b-5p	up	Gas7	down
mmu-miR-20b-5p	up	Nfic	down
mmu-miR-20b-5p	up	Chmp4c	down
mmu-miR-20b-5p	up	Scara5	down
mmu-miR-20b-5p	up	Cygb	down
mmu-miR-181d-3p	up	Cldn4	down
mmu-miR-181d-3p	up	Ubtd1	down
mmu-miR-181d-3p	up	Il6ra	down
mmu-miR-181d-3p	up	Nfic	down
mmu-miR-181d-3p	up	Padi2	down
mmu-miR-181d-3p	up	Cygb	down
mmu-miR-181d-3p	up	Cldn4	down
mmu-miR-181d-3p	up	Ubtd1	down
mmu-miR-181d-3p	up	Il6ra	down
mmu-miR-181d-3p	up	Nfic	down
mmu-miR-181d-3p	up	Padi2	down
mmu-miR-181d-3p	up	Cygb	down

mmu-miR-181d-3p	up	Cldn4	down
mmu-miR-181d-3p	up	Ubtd1	down
mmu-miR-181d-3p	up	Il6ra	down
mmu-miR-181d-3p	up	Nfic	down
mmu-miR-181d-3p	up	Padi2	down
mmu-miR-181d-3p	up	Cygb	down
mmu-let-7i-5p	down	Doc2a	up
mmu-let-7i-5p	down	Lin28a	up
mmu-miR-367-3p	up	Cebpa	down
mmu-miR-367-3p	up	Cebpa	down
mmu-miR-302d-5p	up	Sema4f	down
mmu-miR-302d-5p	up	Padi2	down
mmu-miR-302d-5p	up	Sema4f	down
mmu-miR-302d-5p	up	Padi2	down
mmu-miR-98-5p	down	Lin28a	up
mmu-miR-28c	down	LOC102634120	up
mmu-miR-28c	down	LOC102634120	up
mmu-miR-28c	down	LOC102634120	up