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Original Paper

Nmnat 1: a Security Guard of Retinal Ganglion Cells (RGCs) in Response to High **Glucose Stress**

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Key Words

Diabetic retinopathy • Nmnat1 • Retinal ganglion cell • MAPK signaling

Abstract

Background/Aims: Retinal neurodegeneration is an early event in the pathological process of diabetic retinopathy (DR). Retinal ganglion cell (RGC) injury is an important pathological feature during neurodegenerative process. Protecting RGCs from high glucose-induced injury is a promising strategy for delaying or hindering diabetes mellitus-related retinal neuropathy. This study aims to investigate the role of Nmnat1, an enzyme which catalyzes a key step in the biosynthesis of nicotinamide adenine dinucleotide (NAD), in high glucose-induced RGC injury. Methods: Western blot and immunofluorescence analysis was conducted to detect Nmnat1 expression pattern in the retina and RGC-5 cell. MTT assay, Hoechst staining, trypan blue staining, and calcein-AM/ propidium iodide (PI) staining was conducted to determine the effect of Nmnat1 knockdown on RGC-5 cell function. Microarray and bioinformatics analysis was conducted to identify potential signaling pathways affected by Nmnat1 knockdown. Pharmacological intervention, molecular intervention, and in vitro experiments were conducted to reveal molecular mechanism of Nmnat1-mediated protective effect on RGC-5 cell function. **Results:** Nmnat1 is constitutively expressed in retina and RGC-5 cells. Nmnat1 knockdown aggravates RGC injury, and accelerates the development of RGC-5 cell apoptosis upon high glucose stress. MAPK signaling is the primary signaling pathway affected by Nmnat1 knockdown. Under high glucose stress, Nmnat1 knockdown leads to p38-MAPK signaling inactivation. p38-MAPK pathway inhibitor strongly blocks Nmnat1-mediated protective effect on RGC-5 cell function. Conclusion: Nmnat1 protects RGC against high glucose-induced injury via p38-MAPK signaling pathway. Nmnat1 may serve as a neuroprotective target for © 2016 The Author(s) diabetes mellitus-related retinal neuropathy. Published by S. Karger AG, Basel

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Introduction

Diabetic retinopathy (DR) is the leading cause of vision impairment and blindness in both developing and developed countries [1]. Microvascular dysfunction is an important hallmark of DR. Thus, DR is often considered as a microcirculatory disease of retina [2]. Recently, increasing studies have revealed that retinal neurodegeneration is also involved in the pathogenesis of DR, which contributes to the development of microvascular abnormalities [3-5]. Understanding the mechanism of retinal neurodegeneration contributes to the development of new prevention and treatment strategies for DR.

Neural apoptosis and reactive gliosis are the important features of retinal neurodegeneration during DR [6]. Retinal ganglions cells (RGCs) located in the inner retina are the retinal neurons in which apoptotic process related to diabetes is first detected [7, 8]. RGC loss leads to a marked reduction in the thickness of retinal nerve fiber layer, which has been reported in rats with streptozotocin (STZ)-induced diabetes, and diabetic patients without or with only minimal DR [8, 9]. Thus, protecting RGCs from high glucose-induced injury could delay or hinder DR-related retinal neurodegeneration.

Nicotinamide mononucleotide adenylyltransferase (Nmnat) constitutes an NAD⁺ salvage/recycling pathway using NAM as the precursor in mammalian cells. To date, three different Nmnat enzymes have been identified, including Nmnat1, Nmnat2, and Nmnat3. The function of Nmnat1 was first identified in Wallerian degeneration slow (Wld^S) mouse model. Overexpression of a chimeric nuclear protein Wld^S with Nmnat1 activity leads to axonal protective phenotype in these mice [10, 11]. Both mammalian Nmnat1 and Drosophila Nmnat1 show neuronal protective activity, although in some studies with reduced efficacy compared to Wld^S protein [12-14]. In some experimental systems, Nmnat enzymatic activity is critical for neuronal protection phenotype [12, 15]. However, the role of Nmnat1 in DR-related retinal neurodegeneration is still unclear.

In this study, we show that Nmnat1 is constitutively expressed in retina and RGC-5 cell. Nmnat1 knockdown could aggravate RGC damage, and accelerate the development of RGC apoptosis. Under high glucose stress, Nmnat1-mediated protective effect on RGC function is mainly mediated through p38-MAPK signaling pathway. Nmnat1 may become a promising therapeutic target for the treatment of DR-related retinal neurodegeneration.

Materials and Methods

Cell culture and transfection

Retinal ganglion cell line, RGC-5 cells, were maintained in Dulbecco's modified Eagle's medium (DMEM; Life Technologies) containing 2 mM glutamine, 10 IU/mL penicillin, 10 μ g/mL streptomycin, and 10% fetal bovine serum (FBS, Gibco). They were incubated with a humidified atmosphere of 5% CO₂-95% air at 37°C. Nmnat1 siRNAs were designed and synthesized by Shanghai GenePharma Co. Ltd (Shanghai, China). Lipofectamine 2000 (Invitrogen) was used to transfect siRNAs into RGC-5 cells according to the manufacturer's instruction.

Pharmacological intervention

RGC-5 cells were transfected with Nmnat1 siRNA or Nmnat1 plasmid to change Nmnat1 expression levels. After that, RGC-5 cells were pretreated with MAPK inhibitors (U0126, 10 μ M; SB203580, 10 μ M; SP600125, 10 μ M) for 1 h to inhibit MAPK signaling, and then exposed to high glucose (30 mM) for 48 h. U0126 (ERK inhibitor), SB203580 (p38 inhibitor), and SP600125 (JNK inhibitor) were purchased from Sigma Chemical.

Western blot analysis

RGC-5 cells were incubated with high glucose for 48 h (30 mM) with or without Nmnat1 siRNA transfection, and then washed with ice-cold PBS. These cells were homogenized in lysis buffer, and centrifuged at $12,000 \times g$ for 15 min. Protein concentration was detected using BCA assay. Protein (30 µg)



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was separated by 8-10% SDS-PAGE, and then transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% non-fat dry milk for 1 h at room temperature. The membranes were subsequently incubated with primary antibody and horseradish-conjugated secondary antibody. The blots were detected using the enhanced chemiluminescence (ECL) kit.

Cell viability assay

Cell viability was determined using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Briefly, MTT solution (Sigma, 0.5 mg/ml) was added into each well of a 96-well plate, and incubated at 37°C for 3 h. The plates were then removed. Dimethyl sulfoxide (DMSO) was added to dissolve the purple formazan product. The absorbance at the wavelength of 570 nm was detected using a Multi-Mode microplate reader (Molecular Device).

Hoechst staining assay

Hoechst 33258 fluorescent dye was used to detect DNA condensation and nuclear fragmentation. In brief, RGC-5 cells were (2×10^5 cells/well) were seeded onto 6-well plates for the required treatment. These cells were washed with PBS, and fixed with 4% paraformaldehyde for 15 min. They were then washed with PBS, and stained with Hoechst 33258 (mg/ml) for 15 min. Finally, these cells were observed using an Olympus IX-73 microscope.

Trypan blue staining

RGC-5 cells were seeded onto 12-well plates at the density of 5×10^4 cells/well. After the required treatment, these cells were trypsinized. 4% trypan blue solution (Gibico) was added for 10 min staining. The stained cells were observed using an Olympus IX-73 microscope.

Calcein-AM/propidium iodide (PI) staining

Calcein-AM and PI double staining was used to discriminate viable and dead cells. Briefly, RGC-5 cells were fixed with 4% paraformaldehyde for 15 min after the required treatment, and then stained with Calcein-AM solution (Molecular Probes, 10 μ mol/L) for additional 15 min. After washing with PBS for three times, these cells were then stained with PI (Molecular Probes, 10 μ mol/L) for 10 min. Viable cells were observed using a 490 nm excitation filter, while dead cells were observed using a 545 nm excitation filter.

Immunofluorescence analysis

Retina tissue was fixed in 4% paraformaldehyde for 1 h, immersed in 30% sucrose for 4 h, embedded in optimal cutting temperature (OCT) compound (Tissue-Tek; Sakura Finetek, Torrance, CA), and sectioned at 30 μ m. Retinal slices were then incubated in 1% Triton X-100 in 0.1% citrate for 1 h and stained with Nmnat1 antibody conjugated with Cy-3 at 4°C overnight. Immunofluorescence labeling was then observed using an Olympus IX-73 microscope.

Microarray analysis

Total RNAs were isolated from Nmnat1-konckdown RGC-5 cells and scrambled siRNA-transfected RGC-5 cells. The cDNAs were labeled using the Superscript Plus Direct cDNA labeling system (Invitrogen), and then hybridized to the chip. Hybridization images were collected using an Agilent Microarray Scanner G2565BA. Data was first analyzed using the Agilent Feature Extraction software. Further analysis was performed using the GeneSpring GX v11.5.1 software package (Agilent Technologies). Differentially expressed mRNAs were input into the Database for Annotation, Visualization and Integrated Discovery (DAVID, http://david.abcc. ncifcrf.gov) for the annotation and functional analysis, including gene set enrichment analysis and mapping gene sets to KEGG pathway.

Statistical analysis

Statistical analysis was performed using SPSS 13.0 software. Data was shown as mean \pm SEM. Differences between different groups were analyzed by Student's *t* test or one-way ANOVA. *P* <0.05 was considered statistically significant.



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Results

Detection of Nmnat1 expression pattern in retina and RGC-5 cell

Nmnat1 is a protein involved in mammalian NAD⁺ salvage/recycling signaling. Its expression is regulated by nutrient and stress in a number of human cell lines and primary rat tissues [16, 17]. Although several studies have shown that the Nmnat1 is expressed in many tissues [18-21], it is still unknown whether it is expressed in retina and RGC-5 cell. Western blot analysis showed that Nmnat1was expressed in RGC-5 cell and in the retina of mouse and rat (Fig. 1A). Immunofluorescence experiments revealed that Nmnat1 was expressed in the inner plexiform layer, outer plexiform layer, and ganglion cell layer of mouse retina mouse (Fig. 1B). We also found that Nmnat1 was localized in both the nucleus and cytoplasm of RGC-5 cell (Fig. 1C).

Nmnat1 knockdown affects RGC-5 cell function in vitro

To reveal the functional relevance of Nmnat1 in response to high glucose stress, we determined the effect of Nmnat1 knockdown on RGC function *in vitro*. Western blot analysis showed that Nmnat1 expression was significantly reduced after Nmnat1 siRNA transfection (Fig. 2A). High glucose significantly decreased the number of viable cells (Fig. 2B) and reduced RGC-5 cell viability (Fig. 2C) as detected by trypan blue staining and MTT assay. Nmnat1 knockdown could further reduce the viability of RGC-5 cells (Fig. 2B and 2C).



Fig. 1. Detection of Nmnat1 expression pattern in retina and RGC-5 cell. (A) Total proteins were extracted from mouse retinas, rat retinas, and RGC-5 cells. Western blots were performed to detect Nmnat1 expression. Tubulin was detected as the internal control. A representative immunoblot was shown. (B) Immunofluorescence experiments were conducted to detect Nmnat1 expression in mouse retina. Scale bar, 100 μm. (C) Immunofluorescence experiments were conducted to detect Nmnat1 expression in RGC-5 cells. Scale bar,



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Fig. 2. Nmnat1 k n o c k d o w n affects RGC-5 cell function in vitro. (A) RGC-5 cells were transfected with Nmnat1 siRNA, scrambled (Scr) siR-NA, or left untreated (Ctrl) for 48 h. Western blot was conducted to detect Nmnat1 expression. Tubulin was detected as the interal control. A representative immunoblot was shown along with the densitometric quantitative result. (B-E) RGC-5 cells were transwith fected s c r a m b l e d siRNA (Scr), Nmnat1 siR-NA, or left untreated (Ctrl), and then exposed with or without high glucose (30)mM) for 48 h. Viable cells were assessed

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by cell counting after trypan blue exclusion. Scale bar: 50 μ m. (B). Cell viability was detected using MTT method (C). The data was expressed as the relative change compared with Ctrl group without high glucose treatment. (D) Apoptotic cells were analyzed using Hoechst staining and quantitated. The data was shown as means±S.E.M. and represented four independent experiments in which >300 cells were counted. Scale bar: 20 μ m. (E) Apoptotic cells were analyzed using PI/calcein-AM double staining. Scale bar: 50 μ m. "" indicated a significant difference compared with the corresponding control group. "#" indicated a significant difference between the marked groups.

To determine whether Nmnat1 regulates the development of high glucose-induced apoptosis, RGC-5 cells were treated with Nmnat1 siRNA, scrambled siRNA, or left untreated, followed by high glucose treatment. Compared with high glucose-treated group, the combination of Nmnat1 knockdown and high glucose treatment resulted in higher apoptotic

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Table 1. Differentially expressed genes between Nmnat1 knockdown group and wild-type group

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| 3.89246 Carnin 3.732 Serginaria -3.835 Charghe - 2.2213 Grange - 4.3354 Nuit2 -6.39854 Nit2 3.8764 Hart 3.737 Serginci -5.8381 Respect -4.3344 Nuit2 -6.39854 Nit2 3.8764 Myata -3.737 Serginci -5.8381 Respect -2.2080 BIL -4.3344 Nuit2 -6.43885 Hit 3.87654 Spats115 -3.7705 Endu2 -5.8481 Nuit2 -2.2384 Ulpin2 -4.3347 App2. -6.43885 Hit 3.46978 Spats115 -3.7706 Endu2 -5.8494 Trem2.298 -2.20311 Entric6 -4.337 Pap2. -6.44928 Scic.33 3.410791 Libypin -3.8387 Spin4 -5.8227 Opt -2.30381 Ulpika -4.3385 Cap5 -6.5047 Proc 3.410791 Libypin -3.8387 Nit1 -5.2314 Fint -2.32407 Nith -4.3485 Main <t< td=""><td>3.904084</td><td>Pax4</td><td>-3.7355</td><td>Casp2</td><td>-5.81425</td><td>Ritz</td><td>-2.21566</td><td>Sycn</td><td>-4.3257</td><td>Gata4</td><td>-6.39518</td><td>lgsf1</td></t<> | 3.904084 | Pax4 | -3.7355 | Casp2 | -5.81425 | Ritz | -2.21566 | Sycn | -4.3257 | Gata4 | -6.39518 | lgsf1 |
| 3.888.26 Alg211 3.73.2 Myn 5.888.88 Magez 2.22648 Guap13 4.334 Mynlik -6.6822 Park 3.87654 Heig 3.757 Merd 3.8897 2.22648 Guap13 4.3548 Kallob -6.1161 GUG0500 3.75945 Heig 3.7594 Mag23 3.7611 Mar2 -5.8442 Degit1 -2.23848 Bull -4.3648 Mc10000221 -6.43885 Grif 3.67564 Spatiala5 3.7776 Hurla -5.8737 Rirg -2.29971 Dit1600 -4.364 Stocias -6.43895 Mc121 3.44613 China -3.7857 Chira -5.8737 Thira -2.29971 Dit1600 -4.364 Mc221 -6.94626 Mc121 3.44104 1.4114 -3.8537 Form -5.9879 Thira -3.3834 Mpica -4.3648 Mc121 -4.4478 Mar22 -6.9574 Optica -5.9574 Mur23 3.27647 Optica -4.393 | 3.892462 | Ccrn4l | -3.7521 | Serpina10 | -5.83567 | Chmp4b | -2.22132 | Grm8 | -4.3291 | Sult1c3 | -6.39854 | Nr1i2 |
| 388849 Heis -3.737 Pieka -3.8899 Cdc2 -4.347 Relub -6.41161 DCG6050 375451 Dayl 3.775 Serpici -5.8899 Galiti -2.2884 MAYR -4.3472 Rynol -6.41561 DCG6050 3.697548 Spatsal -3.775 Hurla -5.8613 Mageloi -2.23961 DCI00054562 -4.3615 RC010054562 -4.3615 RC01005452 -6.49564 RU1121 -5.4914 Mater -3.2017 Parabas -4.3615 RC010941055 -6.50162 Rupat -3.3017 Parabas -4.3127 Rupat -5.3128 Rupat -3.3016 Hurla -4.3128 Rupat -5.3128 Rupat -3.3016 Hurla -4.3128 Rupat -5.3264 Rupat -3.3016 Hurla -4.3128 Rupat | 3.886235 | Atp2b1 | -3.7522 | Myo7b | -5.83868 | Magec2 | -2.22643 | Dusp13 | -4.3334 | Myo18a | -6.40327 | Pax8 |
| 347654 App2 3-757 Sepnel 5-84145 Senel 2-2486 MAPK 4-337 App2 6-42561 Swa6 377594 App23 3-761 Htria 5-86139 Mageb16 2-28048 Eu 4-3374 App22 00271 6-44374 Hini 3670544 Spinstall 3-7776 Htria 5-86734 Kmrg 2-28091 Diction255462 4-3134 App23 6-649462 Siccias 344674 TrpC 3-7807 Rmrg 5-87941 Tmem229 -6.50192 Arbigap28 344094 Lähypel 3-3837 Ppy -58820 Spr37 -3338 Gmichoa 4-3354 Kong2 -6.50192 Arbigap28 344094 Lähypel -33337 Ppy -588070 Ntrk3 -2.33261 Spr37 -4.4395 Admrg23 -6.50192 Arbigap28 323156 Chrin -38857 Npr1 -38877 Apr23 -4.44108 Reg18 -6.52287 Spr37 323139< | 3.884896 | Hes5 | -3.7577 | Pde4a | -5.83881 | Rasgrp1 | -2.23894 | Cdca2 | -4.3454 | Rasl10b | -6.41161 | LOC680590 |
| 373594 App23 3.761 Mu2 5.8445 Phot2 -2.25048 Eliz -4.396 LOCI0036227 -6.4885 fu72 3.67958 Spats11s 3.7715 Htr1a -5.8442 Phot21 -2.25066 LOCI0036242 -3.6485 Hu72 3.67958 Spats11s 3.7715 Htr1a -5.87941 Furm229a -2.2011 Hu764 -4.3575 Furd20a -6.65192 Arhapp28 3.416543 Mot1 -3.8237 Spints -5.88704 Nucla -2.3031 Furd60a +3.385 Corp76 -6.50192 Arhapp28 3.407061 Nucl11 -3.8251 Macm -5.92161 Furd -3.23267 Syrt +3.99 Accop76 -6.50192 Arhapp28 3.270616 Nucl11 -3.8351 Corp7 -5.9167 Furd -2.32211 Furd +3.993 Accop76 -6.51128 Styr4 3.270457 Turd -3.8057 Kur12 -2.3211 Furd +4.412 Reg3b -6.52285 Furd 3.27047 Turd -3.8047 Kur12 -2 | 3.876654 | Dsg1 | -3.7577 | Serpinc1 | -5.83991 | Galnt1 | -2.2486 | MAPK6 | -4.3472 | Rspo3 | -6.42561 | Svs6 |
| 3.8695.00 Systal.31.3 3.7715 Findu2 5.84429 Opagt1 -2.2384 Ubeln2 -4.360 LOC100362421 -6.4434 Htrlz 3.4697.46 Systal.31.3 3.7716 Finu2 -5.8714 Tm 2.2396 LOC10036542 -4.5364 Kinzl 3.4497 Tmz -3.7804 Tm 2.2391 IOI10005642 -4.5364 Kinzl 3.4494 Lähypith -3.7804 Tm 2.2391 IOI10056462 -4.5364 Kinzl 3.44094 Lähypith -3.8355 Rypr -5.8822 Gelat -3.3855 Cergat -5.5566 Wir7le 3.376647 Tina3 -3.8525 Gahademin -5.0121 Kinzle -2.32811 Fyrth -4.393 Adam22 -5.5566 Wir7le 3.376447 Cini3 -3.8574 Maral -2.32811 Fyrth -4.393 Adam2 -5.5158 Wir7le 3.37647 Cini3 -3.8574 Maral -2.32812 Cahna -4.4418 <t< td=""><td>3.735942</td><td>Atp2a3</td><td>-3.7631</td><td>Mau2</td><td>-5.84145</td><td>Setd5</td><td>-2.25043</td><td>Ell2</td><td>-4.3547</td><td>Atp2b2</td><td>-6.43885</td><td>Gif</td></t<> | 3.735942 | Atp2a3 | -3.7631 | Mau2 | -5.84145 | Setd5 | -2.25043 | Ell2 | -4.3547 | Atp2b2 | -6.43885 | Gif |
| 3,67954 Spatialis -3,710 Htrla -5,86139 Magehlo -2,2096 LOC10035452 -4,3613 Derfi -4,4447 Petci 3,446137 TrpC -3,7856 Trnz -5,87741 Tmem229 -2,29371 Dirls -4,4956 KIC11 SIC2aL -4,4956 KIC2 KIC2 -4,4956 KIC2 -4,4956 KIC2 -4,4976 KIC2 -5,5074 KIC2 KIC2 KIC2 KIC2 -4,4978 KIC2 K | 3.689584 | Svs5 | -3.7705 | Eftud2 | -5.84429 | Dpagt1 | -2.25384 | Ubqln2 | -4.3606 | LOC100362271 | -6.44385 | Hrh2 |
| 3.4613 Obin1 -3.757 Chrma7 -5.8778 Kurg -2.2991 Nir165 -4.364 Sit221 -6.498.66 Kit21 3.41647 Tyrc -3.7864 Timen229 -2.3031 Fun165 -4.367.4 Fam20 -6.50126 Arbapa2 3.41647 Tyrc -3.7864 Fin165 -4.377.4 Fam20 -6.50142 Arbapa2 3.370647 Fin13 -3.8821 Madeam1 -5.9111 Fin12 -2.33242 Version -4.495.6 CoL00910392 -6.50142 Fin12 3.370647 Fin13 -3.8861 Upk301 -5.926.6 Str74 -2.33242 Version -4.5123 Str74 3.203052 Adopa1 -3.877 App3 -5.9265 Europa -5.2285 Europa -5.2285 Europa -5.2285 Europa -5.2285 Europa -5.2285 Europa -5.2285 Fin4 -3.3324 Kea2 -4.4218 Kea1 -5.2285 Fin4 -3.3324 Kea2 -4.4218 <t< td=""><td>3.679548</td><td>Spata31a5</td><td>-3.7716</td><td>Htr1a</td><td>-5.86139</td><td>Mageb16</td><td>-2.29086</td><td>LOC100365462</td><td>-4.3613</td><td>Dcaf6</td><td>-6.44474</td><td>Pde1c</td></t<> | 3.679548 | Spata31a5 | -3.7716 | Htr1a | -5.86139 | Mageb16 | -2.29086 | LOC100365462 | -4.3613 | Dcaf6 | -6.44474 | Pde1c |
| 3.4467 TrpC2 -3.7606 Tin2 -5.87941 Timen2290 -2.29931 Rn11639 -4.3676 R0D1309422 -6.48926 Sizča3 3.410944 Libypah -3.8817 Pipr -5.8827 Cpc3 -2.3031 Run11639 -4.378 Ren239 -6.50124 Athaga26 3.410944 Libypah -3.88158 RUn12 -5.8122 Netra -3.23256 Gib -4.389 Adam220355 -6.50124 Netra -5.0126 Netra | 3.465139 | Cbln1 | -3.7757 | Chrna7 | -5.87578 | Rxrg | -2.29371 | Olr1500 | -4.364 | Slc22a1 | -6.49546 | Klhl21 |
| 341654 Mtol -3.8227 Spink5 -5.88227 GpC3 -2.3031 Pam169a -4.3764 Fam29a -6.50476 Photopace 341094 Lightycha -3.8377 Pipy -5.88709 Nirk3 -2.33284 Upkka -4.3855 Cap76 -6.50476 Photopace 341094 Lightycha -3.8313 ROD155/747 -5.91016 Rhow -2.32267 Six Adamaz -6.50476 Opr11 -3.8477 Agang -6.50476 Opr11 -3.8477 Marka -4.3376 Nirk3 -6.5172 Sirk4 Ups42 -5.50740 Ups42 -5.50746 Nirk12 -2.32901 Nirk3 -4.4240 Regab -6.51273 Ups42 Lmm -5.50879 Nirk1 -5.50879 Nirk1 -5.50879 Nirk1 -5.50879 Nirk1 -2.32921 Nirk3 -4.4101 Nirk4 -6.53873 Nirk1 -5.50879 Nirk4 -2.398669 Adam2 -4.4076 Nirk4 -6.55979 Nirk4 -2.398669 Adam2 -4.3976 Robirk4 Nirk4 -6.55973 Nirk1 -3.3927 Grirk4 | 3.44877 | Trpc2 | -3.7806 | Tln2 | -5.87941 | Tmem229a | -2.29931 | Rnf165 | -4.3657 | RGD1305422 | -6.49826 | Slc26a3 |
| 3.410994 L3hypdh -3.8379 Phy -5.88709 NtrA -2.32326 Cisk -4.383 Cep76 -6.50476 Puoc 3.370645 Nutl1 -3.8313 RDL577447 F.1016 Habra -2.32267 Syrt -4.393 Adm22 -6.50742 Opr11 3.370647 Grinha -3.8321 Madcan1 -5.91811 Igf18 -2.322811 Prph -4.393 LOCL00910359 -6.51078 Usp42 3.213899 Pip1 -3.867 Hyskin -5.5226 Sard7 -2.33911 Mall -4.4478 Rontal -6.52397 Syrt7 3.172565 Cabp4 -3.877 Agr37 Syrt7 -5.95226 Asrl 1 -2.34675 Masp2 -4.448 Rontal -6.55827 Teps13 3.07497 Cul9 -3.8842 Hbb -5.9648 Tan11 -2.31511 Kdm6a -4.447 ROL55827 Gris6 Cabp14 -4.467 RoL5262 Gris76 Gris75 Asrl 2 -5.9739 Pos1 Cabp14 -4.468 Knat -6.58737 Rol32 Cabp2 Sta14 <td>3.416543</td> <td>Mto1</td> <td>-3.8257</td> <td>Spink5</td> <td>-5.88227</td> <td>Gpc3</td> <td>-2.3031</td> <td>Fam169a</td> <td>-4.3764</td> <td>Fam29a</td> <td>-6.50192</td> <td>Arhgap28</td> | 3.416543 | Mto1 | -3.8257 | Spink5 | -5.88227 | Gpc3 | -2.3031 | Fam169a | -4.3764 | Fam29a | -6.50192 | Arhgap28 |
| 3.40706 Nudtl -3.8513 Rolp159747 -5.91016 Rhos? -2.32687 Syr7 -4.389 Acm23 -6.50742 Opril 3.270646 Nudtl -3.8512 Madama -5.9181 Igf -2.32687 Syr7 -4.393 Mall -4.1919 -6.50742 Opril 3.271646 Nutcl -5.9246 Start -2.32811 Ptp -4.4212 Reg3b -6.5172 Usp42 3.211839 Tp1 -3.8667 Upxbl -5.9268 Start -2.32407 Mall -4.4410 Metal -6.51278 Luppt 3.173265 Cabp4 -3.877 Agp3 -5.96227 Asti -2.34017 Mata -4.4401 Metal -6.55277 Grid -5.9979 Tp4 2.982124 Ubcd4 -3.8976 Ptper4 -5.98758 Tm13 -5.98758 Tm3 -5.98759 Tp4 -5.99179 Mult -2.41642 Haft Tb4 -5.58979 Tp4 -5.99179 Mult -2.41247 Mult3 -6.4711 Tmental -6.58973 Tm13 -5.99179 Mu | 3.410994 | L3hypdh | -3.8357 | Рру | -5.88709 | Ntrk3 | -2.30384 | Upk3a | -4.3835 | Cep76 | -6.50476 | Pnoc |
| 3.370610 Nudt11 -3.8521 Madeum1 -5.91811 Igf3 -2.32867 Syrt -4.39 Adam22 -6.50742 Oprl1 3.327647 Grinsa -3.852 Gabra -5.9240 Fab -2.32817 Pyrh -4.396 LCU0010305 -6.51678 Ups42 3.218397 Tpi -3.8071 Kvr1 -5.93267 Arr1c12 -2.32401 Saa4 -4.4408 Retnla -6.51278 Ups42 3.170525 Cabp4 -3.8787 Agp3 -5.90222 Ast1 -2.34075 Mage -4.4618 Kcrig -6.53293 Tyrt 3.17525 Cabp4 -3.8787 Agp3 -5.90222 Ast1 -2.34075 Mage -4.664 Mccab -6.53293 Tyrt 3.998069 Gabra1 -3.9076 Pigre4 -5.9046 Hult -2.3142 LOGG876 -4.4671 Tyrtme122 -6.52753 Tyrt3 2.995086 Cabl2 -3.9076 Pigre4 -5.90495 Hult -2.40267 Hyrt3 -4.4671 Tyrtam122 -6.59758 Tyrt3 Tyrt3 Tyrt3< | 3.407081 | Sit1 | -3.8513 | RGD1559747 | -5.91016 | Rhox9 | -2.32526 | Gls | -4.3859 | Kcng3 | -6.50586 | Wdr78 |
| 3.27647 Grin3a -3.8552 Gabra 4 -5.92361 TrC20 -2.32811 Ptyph -4.3936 LOC10910359 -6.51123 Strp4 3.218356 Ghrmi -3.8647 Upk3b -5.9266 Stard7 -2.32342 Vesa -4.4126 Reg3b -6.55285 Lrmp 3.193372 Defal1 -3.8734 Ctrl -5.94529 BinB -2.34432 Galt2 -4.4408 Retlan -6.52837 Syl7 3.17265 Cabp4 -3.8774 App3 -5.96429 BinB -2.34472 Galt2 -4.4076 Opr1 -6.55827 Grik6 2908669 Gabrat -3.9076 Piger4 -5.9648 Tnc1 -2.3477 Mac3 -4.4676 Gpr2 -6.55827 Grik6 290869 Gabrat -3.9137 Bug4 -5.9646 Inlc1 -2.340267 Hgrat -4.467 Hgrat -6.56935 Tnd2 280858 Hgrat -3.9137 Bug4 -5.96494 Inlc1 -2.40267 Hgrat -4.418 Tub4a -6.56935 Tdd2 280858 | 3.370616 | Nudt11 | -3.8521 | Madcam1 | -5.91811 | Igfl3 | -2.32687 | Syt7 | -4.39 | Adam22 | -6.50742 | Oprl1 |
| 3.235165 Chrm1 -3.8064 Nml2 -5.92404 Fabb -2.32907 Mail -4.4128 Bass -6.51678 Ups42 3.218390 Tipl -3.8071 Nx17 -5.93667 Air121 -2.32424 Visa2 -4.4128 Regin -6.52304 ZipBr0 3.109372 Defil -3.8775 Agp3 -5.9622 As11 -2.342475 Mag2 -4.4618 Kcrij 4 -6.55297 Trik 2.90869 Gabry 4 -3.8967 Tmoo5a -5.97198 Vili -2.34142 LOG84871 -4.4664 Necab2 -6.55297 Trik 2.90869 Gabry 4 -3.8967 Propert -5.9897 Nuil -2.31727 Mac3 -4.4677 ROIJ562726 -6.57038 Pron 2.90869 Gabry 4 -3.9137 Big4 -5.94950 Idri -2.34902 Adam6 -4.4711 Tmem162 -6.55978 Trik 2.80763 Krik -3.9137 Big4 -5.99295 Idrid -2.424567 Zik -4.4990 Gabry 4.4990 Gabry 4.4990 Gabry 4.4990 Grap 4.49 | 3.276847 | Grin3a | -3.8552 | Gabra4 | -5.92361 | Tcf20 | -2.32811 | Ptprh | -4.3936 | LOC100910359 | -6.51123 | Sfrp4 |
| 3.218399 Tp1 -3.3667 Upk3bi -5.9266 Stard7 -2.33242 Veca -4.4218 Reg3b -6.52284 Limp 3.193327 Defal1 -3.8715 NK7 -5.93667 Akric 12 -2.3442 Gand2 -4.4408 Retnla -6.52942 Styl 7 3.175265 Gabd -3.877 Agap3 -5.9622 Asin -2.34677 Masp2 -4.4616 Kenla -6.53992 Tiga13 3.07497 Cul9 -3.8442 Hub -5.9664 Nunl -2.3511 Kum6a -4.4616 Necab2 -6.55892 Gick3 Poil 2.986196 Galca -3.9079 Figh4 -5.90866 Calc1 -2.3077 Mus -4.6671 ROI1562726 -6.5734 Poil 2.986196 Iolar1 -2.4066 Dex -4.448 Tube44 -6.59833 Tid2 2.881855 Higha -3.9139 Bigh4 -5.99869 Iolar1 -2.4066 Dex -4.4190 Poil2h -6.59483 Cars2 2.881855 Higha -3.918 Mylk - | 3.235156 | Chrm1 | -3.8604 | Nmt2 | -5.92404 | Fshb | -2.32907 | Mall | -4.4196 | Dnase1 | -6.51678 | Usp42 |
| 3.20902 Adyap1 -3.8715 NM7 -5.93667 Akr1c12 -2.3401 Sam -4.4408 Refnla -6.5293 Zyp70 3.175265 Cabp4 -3.8774 Agap3 -5.96222 Ass1 -2.34675 Mag2 -4.4616 Kcn[3] -6.53923 Trga13 2.998649 Gabrg1 -3.896 Tmco5a -5.97198 Vill -2.34612 UcMas -4.4661 Kcn[3] -6.53929 Tmcs 2.998124 Ubc24 -3.907 Piter -5.98278 Clic3 -2.32965 Adam -4.4671 Tmen182 -6.5893 Tmc3 2.898768 Man12 -3.917 Ryk -5.98665 Nolc1 -2.40460 Decx -4.4931 Grasp -6.59938 LoC298139 2.881555 Hgfac -3.9167 Pity -5.99805 LoC1298039 Catc40 -2.4148 Pits2 -4.4318 Grasp -6.59938 LoC298139 2.881523 Hgfac -3.916 Pity -5.99810 LoC298139 Silla -6.62634 Figlica -6.62764 Figlica -6.63183 | 3.218399 | Tjp1 | -3.8687 | Upk3bl | -5.9266 | Stard7 | -2.33242 | Vcsa2 | -4.4212 | Reg3b | -6.52285 | Lrmp |
| 3.19332 Defal1 -3.873 Agar -5.9422 Asta Asta -4.4616 Keni3 -6.5392 Tspa13 3.07497 Cul9 -3.842 Hbb -5.9622 Asta -2.4675 Masp2 -4.4616 Keni3 -6.53992 Tspa13 3.07497 Cul9 -3.8442 Hbb -5.9648 Txnl1 -2.3511 Kumas -4.4616 Necal2 -6.55892 Greb 2.985080 Gal2 -3.907 Piger - 5.9866 Cidn1 -2.3727 Muc8 -4.467 ROL562726 -6.5733 Pon3 2.985080 Alm12 -3.9137 Big4 -5.9866 Nol1 -2.4067 Harm -4.418 Tube14a -6.59458 Cars 2.881855 Hgfac -3.9167 Big4 -5.9986 Iolr1 -2.4067 Harm -4.5106 Lump1 -6.60495 Fxxa2 2.8421 Ioc63313 -3.9375 Fog101 -5.99789 Lin1 -2.4323 Amma -4.516 Mas14 -6.6234 Fgb13 2.714815 Big2 -3.974 Fog11 | 3.209052 | Adcyap1 | -3.8715 | Nxf7 | -5.93667 | Akr1c12 | -2.3401 | Saa4 | -4.4408 | Retnla | -6.52304 | Zfp870 |
| 3.17525 Cabp4 -3.8767 Agep3 -5.96222 Ass1 -2.34675 Masp2 -4.4616 Ken3 -6.55827 Grik 2.98680 Gabrg1 -3.896 Tmco5a -5.57198 Vill -2.36142 LOG684871 -4.4676 Gpr20 -5.55827 Price 2.998026 Cab2 -3.9076 Piter -5.90276 Cicla -2.38965 Adam6 -4.4171 Tmen182 -5.5783 Tmia 2.898768 Manl2 -3.9137 Bitq -5.9981 Cicle -2.38965 Adam6 -4.418 Tubb4a -5.59835 Tub2 2.881763 Manl2 -3.9137 Bitq -5.9919 Ogde40 -2.4143 Prila -4.491 Frab -5.59585 TuC2 -2.53735 TuD2 2.815423 Ar33 -3.9327 Graf -5.5937 DiC299139 DiC299139 DiC299139 Adam5 -4.516 Larap1 -6.62567 Serpina3m 2.713915 Bitg2 -3.9338 Fegbp1 -6.02567 Serpina3m -4.5151 Mida5 -6.62567 Serpina3m | 3.193372 | Defal1 | -3.8734 | Ctrl | -5.94529 | Btn18 | -2.34432 | Galnt2 | -4.4478 | Oprd1 | -6.52937 | Syt7 |
| 3.07497 Cul9 -3.8842 Hbb -5.96848 Sml1 -2.3511 Kumás -4.6464 Necko -5.58927 Griko 2.986806 Gab2 -3.8967 Piger4 -5.8046 Cla1 -2.3727 Mu33 -4.6477 RCD1562726 -6.58935 Tmail 2.887263 Mam12 -3.9137 Big4 -5.89459 Icl1 -2.40267 Hpca -4.4390 Pick1 -5.59458 Tmail -6.59458 Tmail -6.59458 Tmail -6.59458 Cars 2.86558 Krd18 -3.9163 Piger -5.99458 Icl2 Frika -4.4990 Pick2 -6.59458 Cars 2.81542 Arr3 -3.9358 Fegbp11 -5.99268 Ithi -2.4257 Dik3 -4.516 Gra1 -6.62435 Sic231 2.713815 Big2 -3.9374 Frit -6.02478 Piper -2.43876 Dik4 -6.6333 Sic2476 Sic341 -6.63835 Sic2471 -6.64183 Sic2475 | 3.175265 | Cabp4 | -3.8787 | Agap3 | -5.96222 | Ass1 | -2.34675 | Masp2 | -4.4616 | Kcnj3 | -6.53992 | Tsga13 |
| 298809 Gabrg1 -3.896 Tmco5a -5.97190 Vill -2.36142 UC6684971 -4.4676 Gpr20 -6.58999 Tmc4 2992124 Ubc244 -3.9072 III.0ra -5.98278 Cilc3 -2.37277 Muc3 -4.4711 Tmcm182 -6.58753 Tmc32 2887263 Manl2 -3.9137 Big -5.98278 Cilc3 -2.38965 Adam6 -4.4709 Polt2h -6.59458 Carrs 2881583 Hgfac -3.9187 Myik -5.99191 Cilc40 -2.4143 Prila -4.5106 Lurap1 -6.60405 Fbro32 2.815423 Arr3 -3.9388 Fold10 -5.99278 Lilh4 -2.42567 Dr33 -4.5136 Gra1 -6.63235 Sic231 2.713815 Big2 -3.9374 Fyl -6.0737 Tac121 -2.44287 Alp2 -4.5336 Gra1 -6.64133 Sic341 2.645633 Ug2b17 -3.9797 Dg1 -6.07207 Tac2121 -2.44587 | 3.07497 | Cul9 | -3.8842 | Hbb | -5.96848 | Txnl1 | -2.3511 | Kdm6a | -4.4664 | Necab2 | -6.55827 | Grk6 |
| 2.982142 Ubc244 -3.8976 Piger4 -5.99046 Cidn1 -2.3727 Muc3 -4.4677 RoL1562/226 -6.57034 Pon3 2.995006 Calb2 -3.9029 IIIona -5.98246 IIIca -2.40266 Pica -4.48 Tubb4a -6.58935 Tudo2 2.881855 Hgfac -3.9163 Fy -5.98466 NIC1 -2.40266 Dec -4.480 Tubb4a -6.59588 Carrs 2.84521 OC663313 -3.9123 Fight -5.9919 Dight -2.4126 Hfa -4.5115 Prilan -6.62567 Serpin33m 2.794833 Klr13 -3.9354 IOC110255039 Loca171 -6.01731 Mc11 -2.43876 Witdc6 -6.3333 Git2 -6.63255 Siz2121 2.710513 Zecan20 -3.9343 Fyl -6.01731 Mc11 -2.43876 Witdc6 -6.3333 Git2 -6.6332 Irr 2.664581 Sic341 -3.9433 Sico210 -6.0472 Ar | 2.988689 | Gabrg1 | -3.896 | Tmco5a | -5.97198 | Vill | -2.36142 | LOC684871 | -4.4676 | Gpr20 | -6.55899 | Tbx4 |
| 295900 Cab2 -3.902 H10ra -5.98275 Clic3 -2.39265 Adam6 -4.4711 Tmen182 -6.58753 Tmai3 2.881763 Manl2 -3.9137 Big4 -5.98495 Indi1 -2.40267 Hyoe -4.48 Tubba -6.59458 Cars 2.881582 Hgfa -3.9187 Mylk -5.98931 Cdc400 -2.4143 Prilsa2 -4.4901 Grap -6.59458 Cars 2.815423 Arr3 -3.9338 Fcphl1 -5.99266 Inth4 -2.42567 Nr3 -4.5115 Prila7 -6.62875 Fpina3m 2.719131 Big2 -3.9391 Coll -6.00477 Ppilr1 -2.44387 Muba -4.533 Grm2 -6.63325 Hir 2.666531 Sic411 -3.9734 Pril -6.02498 Pipl21 -2.44023 Appl2 -4.533 Grm1 -6.64134 Sic141 -6.63020 Hir -6.63020 Hir -6.63024 Hir -6.63024 Hir -6.63034 Hir -6.64114 Ats712 -6.64134 Ats712 -6.64134 | 2.982124 | Ube2d4 | -3.8976 | Ptger4 | -5.98046 | Cldn1 | -2.37277 | Muc3 | -4.4677 | RGD1562726 | -6.57034 | Pon3 |
| 2.887263 Maml2 -3.9133 Fty -5.98666 Noll -2.40267 Hpca -4.48 Tubb4a -6.58935 Tub 2.881655 Hgfac -3.9163 Fty -5.98666 Noll -2.40466 Dec. -4.4991 Grasp -6.59458 LOC298139 2.84521 LOC683313 -3.9327 Grash -5.9919 Diglob -2.41276 Nt3 -4.5116 Msfa1 -6.62567 Serpina3m 2.714331 Big2 -3.9391 Cox11 -6.00374 Ppp1rlt -2.43237 Mulc6a -4.533 Gru2 -6.63302 Hr 2.667522 Kifab -3.9743 Ftyd -6.01731 Mchr1 -2.4397 Mulc5a -4.533 Gru2 -6.63302 Hr 2.667522 Kifab -3.9747 Yon2731 -6.02763 Ta221-21 -2.47963 Lop2 -4.5437 Gru2 -6.6313 Ser141 2.664681 Kifab -3.983 Sincolu -6.01793 Ta21-21 -2.47864 | 2.959008 | Calb2 | -3.9029 | Il10ra | -5.98278 | Clic3 | -2.38965 | Adam6 | -4.4711 | Tmem182 | -6.58753 | Tnni3 |
| 2881855 Hghc -39183 Fr -598666 Nolc1 -240466 Dcx -44990 Polr2h -659498 Corresponde 28658 Krdtl8 -39187 Mylk -59991 Cdc400 -24143 PriBa2 -44931 Grapp -65998 LOC298139 2815423 Arra -3338 Fogbpl1 -59926 Ith4 -242567 Zhx3 -45115 PriBa7 -66283 Fgb 2713815 Staca -33936 LoC10250394 -59997 Lama1 -243237 Ambn +5116 Mrs41 -66284 Fgb 2713815 Staca -39745 Vom2r31 -602768 Tas2r121 -245999 MA2855 -4552 Krdap -664184 Atxn72 2666953 Ug2b17 -39793 Sac241 -602763 Tas2r121 -247631 Fgf21 -45536 Gem1 -664184 Atxn72 2646405 Sltktk1 -39845 Smd6 -60573 Art2b -248671 Fgf21 | 2.887263 | Maml2 | -3.9137 | Btg4 | -5.98495 | Ildr1 | -2.40267 | Hpca | -4.48 | Tubb4a | -6.58935 | Tdo2 |
| 28658 Kctula -3.917 Mylk -5.9910 Cdc40 -2.443 PriBa2 -4.4931 Grasp -6.5996 LOC298139 2.813421 LOC683313 -3.922 Gra4 -5.9910 Dgbb -2.42176 Nt73 -4.5105 PriBa7 -6.6205 Serplina3m 2.718415 Btg2 -3.9336 LOC10255034 -5.9997 Lama1 -2.43233 Ambn -4.5135 Git2 -6.63235 Sit231 2.710513 Zscan20 -3.9743 Fryl -6.0134 Prpli71 -2.44923 Aplp2 -4.5136 Gram -6.64113 Sct231 2.666583 Ugt2b17 -3.9797 Dgg1 -6.02763 Tas2r121 -2.4999 MAP3K5 -4.533 Graf1 -6.68118 Sct131 2.646581 Sitc341 -3.983 Snodb -6.0513 Art2b -2.47763 Up40 -4.539 Kc11 -6.68043 Adora1 2.639705 MAP3K13 -4.004 Krab5 -6.0553 Art2b -2. | 2.881855 | Hgfac | -3.9163 | Fry | -5.98666 | Nolc1 | -2.40466 | Dcx | -4.4909 | Polr2h | -6.59458 | Cars |
| 283421 L0C683313 -3.9227 Grn4 -5.9919 Dgbb -2.42167 Ntfa -4.5106 Lurnp1 -6.60405 Fbwa32 2815423 Arra -3.9338 Fogbpl -5.99266 Linh -2.42567 Zhx3 -4.5116 Msfa1 -6.6233 Sigla 2.713815 Btg2 -3.9374 Foyl -6.0171 Mchr1 -2.43276 Mtfa6a -4.533 Git2 -6.63235 Sigla1 2.667522 Kifab -3.974 Foyl -6.01741 Mchr1 -2.43976 Dna2 -4.543 Grn1 -6.64113 Secl413 2.666953 Ug2lp17 -3.9797 Von271 -6.04209 Fup21 -2.47033 Ug404 -4.5537 Eaf1 -6.68018 Cmhh1 2.664905 Silrk1 -3.9843 Smado -6.0513 Artp21 -2.47763 Ug404 -4.5639 Galt1 -6.69474 Artp3 Apt2 -4.5637 Galt3 -6.69474 Artp3 Apt2 -4.5637 Galt3 -6.69473 Apt3 -6.70533 Sta14 -2.48014 Hafsa -4.5637 | 2.8658 | Kctd18 | -3.9187 | Mylk | -5.98931 | Ccdc40 | -2.4143 | Prl8a2 | -4.4931 | Grasp | -6.59598 | LOC298139 |
| 2.815423 Arr3 -3.938 Fcgbpli -5.9926 tlith -2.42567 Znx -4.5115 Prlån7 -6.62567 Serpina3m 2.794838 Kilca -3.9356 LoCiOl2550394 -5.99972 Lamal -2.43233 Ambn -4.5115 Ms4a1 -6.62343 Sic23a1 2.710315 Staca -3.9743 Fyd -6.0171 Mchr1 -2.43976 Dna2 -4.543 Grm1 -6.664138 Sic12a1 2.666525 Ugt2h1 -3.9777 Dsg1 -6.02478 Prpla7 -2.44923 Aplp2 -4.5516 Gcm1 -6.664134 Axr712 2.664581 Sit241 -3.9977 Dsg1 -6.04972 Arp21 -2.47631 Ug40 -4.5539 Eaf1 -6.66434 Axr712 2.646581 Sit241 -3.9835 Smad6 -6.05215 Arhap10 -2.47631 Ug40 -4.5639 Eaf1 -6.69434 Acar1 2.629276 Ctr -3.9845 Smad6 -6.0513 Arhap10 -2.48504 Kcn42 -4.587 Staf3 -6.70138 Calcb <t< td=""><td>2.83421</td><td>LOC683313</td><td>-3.9227</td><td>Gfra4</td><td>-5.9919</td><td>Dgkb</td><td>-2.42176</td><td>Ntf3</td><td>-4.5106</td><td>Lurap1</td><td>-6.60405</td><td>Fbxo32</td></t<> | 2.83421 | LOC683313 | -3.9227 | Gfra4 | -5.9919 | Dgkb | -2.42176 | Ntf3 | -4.5106 | Lurap1 | -6.60405 | Fbxo32 |
| 2.794833 Kll1c3 -9.936 LOČ10250394 -5.99972 Lamalt -2.42233 Ambn -4.5116 M4alt -6.62834 Fgb 2.718815 Btg2 -3.9374 Fyl -601731 Mchr1 -2.43876 Wfdc6a -4.5353 Git2 -6.63235 Sic23a1 2.667552 Kilb -3.9734 Fyl -601731 Mchr1 -2.43876 Dia2 -4.543 Grm2 -6.6302 Hr 2.666953 Ug12b17 -3.9775 Yom271 -602498 Pipla7 -2.45989 MAP3K5 -4.5526 Crrd1 -6.66113 Sec1413 2.666953 Ug12b17 -3.9797 Arp21 -2.47683 Ug2b17 -4.5537 Eaf1 -6.66918 Atora1 2.64905 Siltrk1 -3.9853 Smad6 -6.05215 Arhgap10 -2.47846 Cd244 -4.5786 Apcs -6.669843 Adora1 2.59108 MAP3K13 +40024 Knh5 -60573 Stra10 -2.48016 Atsg +5858 Galn13 -6.7038 Calcb 2.59737 Knh6 +4024 <td>2.815423</td> <td>Arr3</td> <td>-3.9338</td> <td>Fcgbpl1</td> <td>-5.99286</td> <td>Itih4</td> <td>-2.42567</td> <td>Zhx3</td> <td>-4.5115</td> <td>Prl8a7</td> <td>-6.62567</td> <td>Serpina3m</td> | 2.815423 | Arr3 | -3.9338 | Fcgbpl1 | -5.99286 | Itih4 | -2.42567 | Zhx3 | -4.5115 | Prl8a7 | -6.62567 | Serpina3m |
| 2.713815 Btg2 -9.3931 Cox11 -6.00347 Pp1rt -2.43876 Wideóa -4.533 Git2 -6.63235 Šic23a1 2.710513 Zscan20 -3.9743 Fryl -6.01731 Mchr1 -2.44976 Dna2 -4.543 Grm2 -6.63103 Feld 2.665525 Ugt2h17 -3.977 Dgt1 -6.02763 TaS21121 -2.449989 MP3KS -4.552 Krdap -6.64184 Atxn712 2.664581 Sic341 -3.9983 Sto2b1 -6.04972 Arpp1 -2.47763 Usp40 -4.5639 Eci1 -6.66843 Adora1 2.639267 Ctsr -3.9853 Smad6 -6.05215 Arhgap10 -2.47846 Cd244 -4.5639 Eci1 -6.66843 Adora1 2.591693 MAPSK13 -4.0204 Knh5 -6.05513 Start10 -2.48016 Ans2 -4.5879 Stada1 -6.70535 Ctsq12 2.591693 MAPSK13 -4.024 Knh5 -6.07868 RD131595 -2.48014 Stac3 -4.5976 Sic8a1 -6.7151 Tgg1 | 2.794833 | Klk1c3 | -3.9356 | LOC102550394 | -5.99972 | Lama1 | -2.43233 | Ambn | -4.5116 | Ms4a1 | -6.62834 | Fgb |
| 2710513 Zscan20 -3.9743 Fryl -6.01731 Mchr1 -2.43976 Dna2 +5.43 Grm2 -6.63302 Hr 2.667522 Ki73b -3.9779 Dsg1 -6.02763 Tas2r121 -2.43998 MAP3K5 -4.552 Krtdap -6.64113 Stec1413 2.664581 Sic3411 -3.983 Sico2b1 -6.04209 Fut2 -2.45871 Fg21 -4.553 Eaf1 -6.6818 Ktm71 2.664581 Sici441 -3.983 Sico2b1 -6.04209 Put2 -2.47866 U24 -4.553 Eaf1 -6.6817 Ncan 2.634905 Sitrk1 -3.9953 Smad6 -6.05215 Arhgap10 -2.47866 Act24 +4.5786 Apcs -6.69843 Adora1 2.591693 MAP3K13 -4.0024 Kcn15 -6.05613 Stard10 -2.48016 Arkg2 +4.587 Staf3 -6.7153 Ctsg12 2.591752 Cmah -4.0214 Spint5 -6.0637 Lydgfe -2.48504 Krd2 +4.587 Staf3 -6.7151 Tgps1 2.52931 </td <td>2.713815</td> <td>Btg2</td> <td>-3.9391</td> <td>Cox11</td> <td>-6.00347</td> <td>Ppp1r1c</td> <td>-2.43876</td> <td>Wfdc6a</td> <td>-4.5353</td> <td>Git2</td> <td>-6.63235</td> <td>Slc23a1</td> | 2.713815 | Btg2 | -3.9391 | Cox11 | -6.00347 | Ppp1r1c | -2.43876 | Wfdc6a | -4.5353 | Git2 | -6.63235 | Slc23a1 |
| 2.667522 Kir3b -3.9754 Vom2r31 -6.02408 Pnpla7 -2.44023 Aplp2 -4.5516 Gcm1 -6.64113 Sec14l3 2.6656915 Ugt2b17 -3.977 Dsq1 -6.02763 Tas2r121 -2.45989 MAP3K5 -4.552 Krtdap -6.64113 Sec14l3 2.646581 Sic341 -3.983 Slco2b1 -6.04209 Fut2 -2.48917 Fg21 -4.5537 Eci1 -6.68918 Aton712 2.649581 Sic341 -3.9853 Smad6 -6.05215 Arhgap10 -2.47763 Usp40 -4.5638 Eci1 -6.68918 Adora1 2.629276 Ctsr -3.9853 Smad6 -6.0553 Art2b -2.48016 Ahsg -4.5874 Stra3 -6.7033 Catcb 2.554066 Serpinb2 -4.0214 Km55 -6.0579 Jv5g6e -2.48044 Ktad2 +3.587 Stra3 -6.7153 JccC679475 2.554086 Serpinb2 -4.0214 Prh -6.07668 RD1311595 -2.48904 Hta4 -4.5874 Stra3 -6.72835 LocC679475 | 2.710513 | Zscan20 | -3.9743 | Fryl | -6.01731 | Mchr1 | -2.43976 | Dna2 | -4.543 | Grm2 | -6.63302 | Hr |
| 265693 Ugt2b17 -3.9797 Dsg1 -6.02763 Tas2r121 -2.45999 MAP3K5 -4.552 Krtdap -6.64184 Atxn712 2646581 Sk1341 -3.983 Sko2b1 -6.04209 Fut2 -2.47876 Usp0 -4.5537 Eaf1 -6.68167 Ncan 2.629276 Ctsr -3.9853 Smad6 -6.05215 Arhgap10 -2.47846 Cd244 -4.5786 Apcs -6.69843 Adora1 2.591693 MAP3K13 -4.004 Kenl5 -6.05513 Stard10 -2.48804 Krda2 -4.8574 Stfa3 -6.70138 Calcb 2.57408 Serpinb2 -4.0214 Kenl5 -6.05613 Stard10 -2.48504 Krda2 -4.8574 Stfa3 -6.70593 L0C679475 2.529371 ROD1565494 -4.0234 Prihr -6.07868 ROD1311595 -2.48614 Stac3 -4.5956 Sicea3 -6.7151 Tpgs1 2.48139 Fdx11 -4.0234 Prihr -6.09886 RAP3K1 -2.49327 Hdgf11 +4.6213 Acem2 -6.72836 Lmym3 | 2.667522 | Kif3b | -3.9754 | Vom2r31 | -6.02498 | Pnpla7 | -2.44023 | Aplp2 | -4.5516 | Gcm1 | -6.64113 | Sec1413 |
| 2646581 Sic341 -3.983 Sic2b1 -6.04209 Fut2 -2.46871 Fg21 -4.5537 Eaf1 -6.68018 Cmnb1 2.634905 Slitk1 -3.9853 Smade -6.05215 Arhgap10 -2.47763 Usp40 -4.5689 Eci1 -6.68967 Ncan 2.591693 MAP3K13 -4.0064 KenJ5 -6.05513 Art2b -2.48016 Ahsg -4.5786 Apcs -6.69843 Adoral 2.551046 Serpinb2 -4.0214 Spink5 -6.05513 Str2b -2.48040 Knda -4.5878 Galant -6.70335 Ctsql2 2.5520371 ROD1565349 -4.0224 Point -6.09868 RO1311595 -2.48614 Stac3 -4.5975 Sica3 -6.7151 Tpg51 2.46332 Pcp2 -4.0234 Prihr -6.09868 MAP3K1 -2.49802 Htr4 -4.6034 Utrm -6.71873 Dgcr8 2.46332 Pcp2 -4.0323 Adrb3 -6.1169 Dmah1 -2.49323 Tox -4.634 Htra -6.72978 Sic9a1 2 | 2.656953 | Ugt2b17 | -3.9797 | Dsg1 | -6.02763 | Tas2r121 | -2.45989 | MAP3K5 | -4.552 | Krtdap | -6.64184 | Atxn712 |
| 2634905 Slitrk1 -3.9842 Kb15 -6.04972 Arpp21 -2.47763 Usp40 -4.5639 Ecil -6.68567 Ncan 2629276 Ctsr -3.9833 Smad6 -6.05215 Arhgap10 -2.47846 Cd244 -4.5786 Apcs -6.68567 Ncan 2.591693 MAP3K13 -4.004 Kcn5 -6.0553 Art2b -2.48016 Ahsg -5.858 Gaint13 -6.70138 Calcb 2.577025 Cmah -4.0214 Kpink5 -6.05613 Stard10 -2.48004 Ktn2 -4.5874 Stra3 -6.70533 CocoG79475 2.53326 Tshz1 -4.0234 Prihr -6.07686 KD1311595 -2.48614 Stac3 -4.5784 Kc13 -6.71873 Dgcr8 2.46332 Pcp2 -4.0234 Prihr -6.07866 Col091 Dnm1 -2.49327 Hdgf1 -4.6234 Acm2 -6.72836 Zmym3 2.46323 Pcp2 -4.0325 Sp3 -6.11459 Dcm5 | 2.646581 | Slc34a1 | -3.983 | Slco2b1 | -6.04209 | Fut2 | -2.46871 | Fgf21 | -4.5537 | Eaf1 | -6.68018 | Ctnnb1 |
| 2.629276 Ctsr -3.9853 Smad6 -6.05215 Arhgap10 -2.47846 Cd244 -4.5786 Apcs -6.69843 Adora1 2.591603 MAP3K13 -4.0064 Kenb5 -6.05513 Ard20 -2.48016 Ahsg -4.5874 Sta3 -6.70338 Calcb 2.577252 Cmah -4.0214 Spink5 -6.06379 Lyógéce -2.48504 Kncl2 -4.5874 Sta3 -6.70539 LCG79475 2.529371 RO1156349 +0.0234 Poll11 -6.07868 RO1311595 -2.48614 Sta3 -4.5975 Sta3 -6.7151 Tpgs1 2.513752 Tsh21 -4.0234 Poll1 -6.09886 RAP3X1 -2.48902 Htr4 -4.6034 Utrn -6.7183 Dgcr8 2.46332 Pc2 -4.032 Sp3 -6.1169 Rab19 -2.51774 Nel1 -4.6218 Acs218 -6.72978 Sic931 2.46332 Pc2 -4.032 Sp3 -6.1169 Rab19 -2.5177 | 2.634905 | Slitrk1 | -3.9842 | Kb15 | -6.04972 | Arpp21 | -2.47763 | Usp40 | -4.5639 | Eci1 | -6.68567 | Ncan |
| 2.591093 MAP3R13 -4.0064 Kenj5 -6.0553 Mr2b -2.48016 Ahag -4.5858 Gaint13 -6.70138 Calcb 2.577252 Cmah -4.0214 Spink5 -6.05613 Stard10 -2.48504 Kcnd2 -4.5874 Stad3 -6.70535 Ctsql2 2.554086 Serpinb2 -4.0214 Spink5 -6.06379 Lyóg6e -2.48504 Ptlat -4.5975 Stad3 -6.7151 Tpgs1 2.513526 Tsh1 -4.0234 Pelhr -6.07686 MCD1311595 -2.48614 Stad3 -4.5183 -6.71873 Dgcr8 2.46139 Fak11 -4.0234 Pelhr -6.0866 MAP3K1 -2.4902 Htd -4.6181 Ube3a -6.72978 Slc9a1 2.462072 Rib2 -4.0324 Adrb3 -6.11459 Dcm5 -2.49327 Hdgf1 -4.6213 Acsm2a -6.72978 Slc9a1 2.36407 Tmi3k -4.0421 Naba -6.11459 Dcm5 -2.5232 Tox +4.53 Acsm2a -6.73551 LDGc81005 2.36407 <t< td=""><td>2.629276</td><td>Ctsr</td><td>-3.9853</td><td>Smad6</td><td>-6.05215</td><td>Arhgap10</td><td>-2.47846</td><td>Cd244</td><td>-4.5786</td><td>Apcs</td><td>-6.69843</td><td>Adora1</td></t<> | 2.629276 | Ctsr | -3.9853 | Smad6 | -6.05215 | Arhgap10 | -2.47846 | Cd244 | -4.5786 | Apcs | -6.69843 | Adora1 |
| 2.57252 Cmah 4.0204 Km5 -6.05613 Stard10 -2.48504 Kcnd2 -4.5874 Stfa3 -6.70532 Ctsq12 2.554086 Serpinb2 -4.0214 Spink5 -6.06379 Ljógége -2.48504 Prlåa4 -4.5897 Smad9 -6.70532 Ctsq12 2.529371 ROD1565349 -4.0234 Prlhr -6.07868 ROD1311595 -2.48614 Stac3 -4.5956 Site3a -6.7151 Tpgs1 2.513526 Tshz1 -4.0234 Prlhr -6.09886 RAD1311 -2.44902 Htr4 -4.6034 Utrn -6.71837 Dgcr8 2.46327 Rib2 -4.032 Prlhr -6.09886 RAD19 -2.47932 Hdgf11 -4.6218 dcs2 -6.72395 Lico81005 2.371643 Syp12 -4.031 Nrap -6.12092 Shd -2.5247 Kcn2 -4.632 RD1309651 -6.73535 Lico810051 2.367445 Syp12 -4.0451 Nst2 -6.14719 Fu4 | 2.591693 | MAP3K13 | -4.0064 | Kcnj5 | -6.0553 | Art2b | -2.48016 | Ahsg | -4.5858 | Galnt13 | -6.70138 | Calcb |
| 2.554086 Serpinb2 -4.0214 Spink5 -6.06379 Lyg6gee -2.48504 Pril8a4 -4.5897 Smad9 -6.7053 LOC679475 2.523937 RCD1565349 -4.0234 Prihr -6.0986 RDA15155 -2.48614 Stac3 -4.5956 Sic8a3 -6.7151 Tpgs1 2.513526 Tshz1 -4.0234 Prihr -6.0986 RDA15155 -2.48612 Yura -4.6034 Utrn -6.71873 Dgcr8 2.48139 Fdx1 -4.0259 RGD1565166 -6.10091 Dnah1 -2.49134 Tnfrsf9 -4.6181 Ube3a -6.72978 Sic9a1 2.46632 Pcp2 -4.0322 Sp3 -6.1168 Rab19 -2.51774 Nell -4.6232 Pbsn -6.73551 Hmgcs2 2.367445 Cyp11b1 -4.0421 Csde1 -6.12105 Hk1 -2.55236 Thz2 -4.6612 Agtr2 -6.78185 Gpd1 2.307372 Scy13 -4.0531 Mx2 -6.16471 Yur3 <t< td=""><td>2.577252</td><td>Cmah</td><td>-4.0204</td><td>Kcnh5</td><td>-6.05613</td><td>Stard10</td><td>-2.48504</td><td>Kcnd2</td><td>-4.5874</td><td>Stfa3</td><td>-6.70535</td><td>Ctsql2</td></t<> | 2.577252 | Cmah | -4.0204 | Kcnh5 | -6.05613 | Stard10 | -2.48504 | Kcnd2 | -4.5874 | Stfa3 | -6.70535 | Ctsql2 |
| 2.529371 RCD1565349 4.0228 Poul1 -6.07868 RCD1311595 -2.48614 Stac3 -4.5956 Slc8a3 -6.7151 Tpgs1 2.513526 Tshz1 -4.0234 Prihr -6.09886 MAP3K1 -2.48902 Htr4 -4.6034 Utrn -6.71873 Dgcr8 2.48139 Fdx11 -4.0234 Adrb3 -6.11459 Dcm5 -2.49327 Hdgf1 -4.6213 Acsm2a -6.72836 Zmym3 2.460272 Rib2 -4.0321 Sp3 -6.11648 Rab19 -2.5174 Nefl1 -4.6218 Acsm2a -6.72836 Zmym3 2.46072 Rib2 -4.0361 Nrap -6.12005 Hk1 -2.5232 Tox -4.63 Accr2 -6.7355 LOC681005 2.36447 Styl116 -4.0451 Acus1 -2.524 Kcnc3 -4.632 RG1309651 -6.7847 Hadc10 2.367445 Styl116 -4.0451 Acus1 -2.524 Kcnc3 -4.657 Lof290876 -6.776 <td>2.554086</td> <td>Serpinb2</td> <td>-4.0214</td> <td>Spink5</td> <td>-6.06379</td> <td>Ly6g6e</td> <td>-2.48504</td> <td>Prl8a4</td> <td>-4.5897</td> <td>Smad9</td> <td>-6,70593</td> <td>LOC679475</td> | 2.554086 | Serpinb2 | -4.0214 | Spink5 | -6.06379 | Ly6g6e | -2.48504 | Prl8a4 | -4.5897 | Smad9 | -6,70593 | LOC679475 |
| 2.513526 Tshz1 4.0234 Prihr -6.09886 MAP3K1 -2.49902 Htr4 -4.6034 Utrn -6.71873 Dgcr9 2.48139 Fdx11 -4.0239 RGD1565166 -6.10091 Dnah1 -2.49134 Tnfrsf9 -4.6181 Ubra -6.72978 Sic9a1 2.46332 Pcp2 -4.0322 Sp3 -6.1168 Rab19 -2.51774 Nell -4.6282 Pbsn -6.73535 LOC681005 2.371603 Sypl2 -4.0321 Csd1 -6.12092 Shd -2.5232 Tox -4.633 RCD1309651 -6.73651 Hmgcs2 2.367445 Cyp11b1 -4.0421 Csde1 -6.12105 Hk1 -2.5248 Kcnc3 -4.632 AG720 -6.78614 Hdac10 2.367445 Cyp11b1 -4.0464 Tbx2 -6.16449 You33 -2.5603 Hth3 -4.6612 Agtr2 -6.78056 Tmem79 2.307372 Scyl3 -4.051 Mx2 -6.16499 Syce3 -2.560 Hth3 -4.6612 Agtr2 -6.78055 Tmem79 2.297349 <td>2.529371</td> <td>RGD1565349</td> <td>-4.0228</td> <td>Pou1f1</td> <td>-6.07868</td> <td>RGD1311595</td> <td>-2.48614</td> <td>Stac3</td> <td>-4.5956</td> <td>Slc8a3</td> <td>-6.7151</td> <td>Tpgs1</td> | 2.529371 | RGD1565349 | -4.0228 | Pou1f1 | -6.07868 | RGD1311595 | -2.48614 | Stac3 | -4.5956 | Slc8a3 | -6.7151 | Tpgs1 |
| 2.48139 Fdx11 -4.0259 RGD1565166 -6.10091 Dnah1 -2.49134 Tnfrsf9 -4.6181 Ube3a -6.72836 Zmym3 2.46332 Pcp2 -4.0323 Adrb3 -6.11459 Dcm5 -2.49327 Hdgf1 -4.6213 Acsm2a -6.72878 Sle9a1 2.462072 Ribc2 -4.0325 Sp3 -6.11459 Dcm5 -2.49327 Hdgf1 -4.6213 Acsm2a -6.72878 Sle9a1 2.36407 Tnni3k -4.0431 Nrap -6.12092 Shd -2.5174 Nell -4.632 Recr2 -6.7355 LOC681005 2.36407 Tnni3k -4.0441 Tbx2 -6.14719 Fut9 -2.547 Carl12 -4.6567 LOC290876 -6.7766 Chrna10 2.307445 Cyp11b1 -4.0464 Tbx2 -6.16361 Vac14 -2.55236 Tlx2 -4.6612 Agtr2 -6.78165 Gpd1 2.30748 Robb -4.0051 Mx14 -6.15331 -2.5603 Hrh1 -4.6657 Lofc290876 -6.78165 Flme79 2.30748 K | 2.513526 | Tshz1 | -4.0234 | Prlhr | -6.09886 | MAP3K1 | -2.48902 | Htr4 | -4.6034 | Utrn | -6.71873 | Døcr8 |
| 24632 Pcp2 -4.0293 Adrb3 -6.11459 Dcm5 -2.49327 Hdgfl1 -4.6213 Acsm2a -6.7297 SIc91 2.462072 Ribc2 -4.0322 Sp3 -6.1168 Rab19 -2.1774 Nell -4.6213 Acsm2a -6.7297 SIc91 2.371683 Syp12 -4.0361 Nrap -6.12092 Shd -2.5232 Tox -4.63 Acer2 -6.73651 Hmgrs2 2.367445 Cyp11b1 -4.0421 Csde1 -6.12105 Hkl -2.524 Kcnc3 -4.6322 Agcr2 -6.73651 Hmgrs2 2.367445 Cyp11b1 -4.0461 Tbx -2.5424 Kcnc3 -4.632 Agtr2 -6.786 Chrma10 2.30748 Fosb -4.0511 Mx14 -2.5526 Tbx2 -4.6612 Agtr2 -6.7865 Tmem79 2.297349 Nr0b2 -4.008 RGD1306233 -6.16449 Syce3 -2.5607 Fh11 -4.6612 Kcf71 Dkk2 -6.78665 <td>2.48139</td> <td>Fdx1l</td> <td>-4.0259</td> <td>RGD1565166</td> <td>-6.10091</td> <td>Dnah1</td> <td>-2.49134</td> <td>Tnfrsf9</td> <td>-4.6181</td> <td>Ube3a</td> <td>-6.72836</td> <td>Zmym3</td> | 2.48139 | Fdx1l | -4.0259 | RGD1565166 | -6.10091 | Dnah1 | -2.49134 | Tnfrsf9 | -4.6181 | Ube3a | -6.72836 | Zmym3 |
| 2.462072 Ribc2 4.0322 Sp3 -6.1168 Rab19 -2.51774 Nell1 -4.6282 Pbsn -6.73535 LOC681005 2.371683 Sypl2 -4.0361 Nrap -6.12092 Shd -2.52332 Tox -4.63 Acer2 -6.73551 Hmges2 2.36847 Tnni3k -4.0421 Csde1 -6.12095 Hk1 -2.52332 Tox -4.63 Acer2 -6.73651 Hmges2 2.36847 Tnni3k -4.0444 Tbx2 -6.14719 Fut9 -2.5457 Carl 2 +4.657 LOC290876 -6.776 Chrna10 2.307372 Scyl3 -4.0571 Mx2 -6.16442 Pou373 -2.5603 Hrh3 -4.6647 Lepr -6.7866 Rimbr92 2.394848 Fosb -4.0914 Nxp4 -6.1649 Syce3 -2.5603 Hh1 -4.6647 Lepr -6.78668 Strpc 2.1339 Kcnh7 -4.0914 Nxph4 -6.1777 LOC685527 Cnr1 -4.6458 </td <td>2.46332</td> <td>Pcp2</td> <td>-4.0293</td> <td>Adrb3</td> <td>-6.11459</td> <td>Dcm5</td> <td>-2.49327</td> <td>Hdgfl1</td> <td>-4.6213</td> <td>Acsm2a</td> <td>-6.72978</td> <td>Slc9a1</td> | 2.46332 | Pcp2 | -4.0293 | Adrb3 | -6.11459 | Dcm5 | -2.49327 | Hdgfl1 | -4.6213 | Acsm2a | -6.72978 | Slc9a1 |
| 2.371683 Sypl2 -4.0361 Nrap -6.12092 Shd -2.52332 Tox -4.63 Acer2 -6.73651 Hmgcs2 2.367445 Cyp11b1 -4.0421 Csde1 -6.12105 Hk1 -2.524 Kcnc3 -4.633 Acer2 -6.73651 Hmgcs2 2.367445 Cyp11b1 -4.0464 Tbx -6.14719 Fut9 -2.547 Kcnc3 -4.6332 RGD1309651 -6.7364 Hdac10 2.307475 Cyp11b1 -4.0464 Tbx -6.16361 Vac14 -2.5526 Tbx2 -4.6612 Agtr2 -6.78165 Gpd1 2.304848 Fosb -4.0571 Mx2 -6.16442 Pou33 -2.560 Fhl1 -4.6617 Lepr -6.78055 Tmem79 2.297349 Nr0b2 -4.0808 RGD1306233 -6.16499 Syce3 -2.5607 Cd163 -4.6713 Dkk2 -6.78658 Tmem79 2.1339 Kch7 -4.094 Nryth -6.79656 Hr1 -4.6655 Kcn | 2.462072 | Ribc2 | -4.0322 | Sp3 | -6.1168 | Rab19 | -2 51774 | Nell1 | -4.6282 | Phsn | -673535 | LOC681005 |
| 2.36847 Tmil3k -4.0421 Csde1 -6.12105 Hk1 -2.524 Knnc3 -4.6332 RGD1309651 -6.75874 Hdac10 2.36747 Scyl1 -4.0464 Tbx2 -6.14719 Fut9 -2.5457 Carl2 -4.6332 RGD1309651 -6.75874 Hdac10 2.307372 Scyl3 -4.0552 Lrfn4 -56.361 Vac14 -2.5526 Tbx2 -4.61210 Fut9 -2.5457 Carl2 -4.6567 LOC290876 -6.7760 Chrna10 2.307372 Scyl3 -4.0552 Lrfn4 -6.1649 Put9 -2.5503 Hrh3 -4.6612 Agtr2 -6.78605 Rimb92 2.1963 Pof1b -4.0914 Nzph4 -6.1649 Syce3 -2.5607 Fh11 -4.6645 Kcn2 -6.78605 Rimb92 2.1963 Pof1b -4.0914 Nzph4 -6.1718 Isl1 -2.5607 Cnr1 -4.6945 Ryr1 -6.78668 Strpc 2.090766 Bcl212 -4.0969 | 2.371683 | Svpl2 | -4.0361 | Nrap | -6.12092 | Shd | -2 52332 | Tox | -4.63 | Acer2 | -6.73651 | Hmgcs2 |
| 2.367445 Cyp11b1 -4.0464 Tbx2 -6.14719 Fut9 -2.5457 Car12 -4.6567 LOC290876 -6.776 Chrna10 2.307372 Scyl3 -4.0552 Lrfn4 -6.16361 Vac14 -2.5523 Tix2 -4.6567 LOC290876 -6.77815 Gpd1 2.307372 Scyl3 -4.0552 Lrfn4 -6.16361 Vac14 -2.55236 Tix2 -4.6612 Agtr2 -6.78185 Gpd1 2.304848 Fosb -4.0671 Mx2 -6.16449 Pou33 -2.5608 Hrh1 -4.6657 Kcne2 -6.78605 Rimbp2 2.1963 Pof1b -4.0914 Nxph4 -6.1718 Isl -2.59567 Cnr1 -4.6945 Ryr1 -6.79656 Hrle2 2.09766 Bcl212 -4.0969 P2ry4 -6.1777 LOC685527 -2.60507 Ptptk -4.6943 Ryr1 -6.81041 Aplnr 2.09766 MaPRE1 -4.1076 Msc156664 -2.62233 Gpr13 -4.703 | 2.36847 | Tnni3k | -4.0421 | Csde1 | -6.12105 | Hk1 | -2.524 | Kenc3 | -4.6332 | RGD1309651 | -6.75874 | Hdac10 |
| 2.307372 Scyl3 -4.0552 Lrfn4 -6.16361 Vac14 -2.55236 Tix2 -4.6612 Agtr2 -6.78185 Gpd1 2.304848 Fosb -4.0571 Mx2 -6.16442 Pou3f3 -2.5503 Tix2 -4.6612 Agtr2 -6.78185 Gpd1 2.297349 Nrbb -4.0611 B3gat1 -6.16442 Pou3f3 -2.5609 Fhl1 -4.6651 Cherce -6.78605 Timem79 2.297349 Nrbb -4.0911 B3gat1 -6.16499 Syce3 -2.5609 Fhl1 -4.6651 Cherce -6.78605 Timem79 2.1393 Kenh7 -4.0911 B3gat1 -6.16498 Mycbp2 -2.58007 Cd163 -4.6713 Dkk2 -6.78605 Fhlre 2.039766 Bcl12 -4.0969 Pzyt4 -6.1777 I/DC685527 -2.60507 Ptprk -4.6945 Mprc1 -6.81041 Aplar 2.05056 Meg6 -4.1224 Atp1a2 -6.19799 RGD1565959 -2.62233 | 2.367445 | Cyp11b1 | -4.0464 | Tbx2 | -6.14719 | Fut9 | -2.5457 | Car12 | -4.6567 | LOC290876 | -6.776 | Chrna10 |
| 2.304848 Fosb -4.0571 Mx2 -6.16442 Pou3f3 -2.5603 Hrbs -4.6647 Lepr -6.78265 Timem79 2.297349 Nr0b2 -4.0808 RGD1306233 -6.16499 Syce3 -2.560 Hrb1 -4.6647 Lepr -6.78265 Rimbp2 2.1963 Pof1b -4.0914 Mxph4 -6.17898 Strpc -2.5609 Fhl1 -4.6647 Lepr -6.78265 Rimbp2 2.1933 Pof1b -4.0914 Nxph4 -6.1718 Isl1 -2.59567 Cnr1 -4.6945 Ryr1 -6.79666 Hfc2 2.090766 Bcl212 -4.0969 P2ry4 -6.17777 L0C685527 -2.60507 Ptprk -4.6945 Ryr1 -6.79666 Hfc2 2.07969 MAPRE1 -4.1076 Mas1 -2.6237 Gpr135 -4.703 Apex1 -6.81041 Apln 2.05056 Megf6 -4.1224 Atpla2 -6.19769 RCD1565959 -2.62301 Stg14 -4.762 | 2.307372 | Scy13 | -4.0552 | Lrfn4 | -6.16361 | Vac14 | -2.55236 | TIx2 | -4.6612 | Agtr2 | -6.78185 | Gpd1 |
| 2.297349 Nr0b2 -4.0808 RGD1306233 -6.16499 Syce3 -2.569 Fhill -4.665 Kcne2 -6.78605 Rimby2 2.1963 Pof1b -4.0911 B3gat1 -6.1698 Mycbp2 -2.58007 Cd163 -4.6713 Dkk2 -6.78605 Rimby2 2.1393 Kcnh7 -4.0911 B3gat1 -6.1698 Mycbp2 -2.58007 Cd163 -4.6713 Dkk2 -6.78605 Rimby2 2.03076 Bcl12 -4.0969 Pzyt4 -6.1777 L0C685527 -2.60507 Ptptk -4.6945 Ryr1 -6.79656 Hfe2 2.07969 MAPREI -4.1076 Mas1 -6.17879 R0D156559 -2.62253 Gpr135 -4.703 Apex1 -6.81041 Aplnr 2.056056 Meg6 -4.1224 Atp1a2 -6.19769 R0D1565959 -2.62253 Gpr13 -4.7162 Pzry14 -6.81048 Samd12 -2.01157 Vom2r18 Lgmn -6.19664 P2rx14 -2.62874 < | 2.304848 | Fosb | -4.0571 | Mx2 | -6.16442 | Pou3f3 | -2.5603 | Hrh3 | -4.6647 | Lepr | -6.78265 | Tmem79 |
| 2.1963 Pof1b -4.0911 B3gat1 -6.1698 Mycbp2 -2.58007 Cd163 -4.6713 Dkk2 -6.78868 Strpc 2.1339 Kcnh7 -4.0914 Nxph4 -6.17188 Isl1 -2.58007 Cd163 -4.6713 Dkk2 -6.78868 Strpc 2.090766 Bcl212 -4.0969 P2ry4 -6.17777 IOC685527 -2.60507 Ptprk -4.6945 Ryr1 -6.79656 H/e2 2.09766 Bcl212 -4.0969 P2ry4 -6.18709 RGD1564664 -2.62253 Gpr135 -4.703 Apex1 -6.81091 Samd12 2.05056 Meg6 -4.1224 Atp1a2 -6.19789 RGD1565595 -2.62301 Stg14 -4.7162 P2ry14 -6.81808 Samd12 -2.01044 Lgmn -4.1322 Lepr -6.19769 P2ry14 -2.62874 Rimbp2 -4.7261 Tcerg11 -6.81836 Klk1c10 -2.01157 Vom2r18 -4.1499 Gzmf -6.22244 Slc2a24 | 2.297349 | Nr0b2 | -4.0808 | RGD1306233 | -6.16499 | Syce3 | -2.569 | Fhl1 | -4.665 | Kcne2 | -6.78605 | Rimbp2 |
| 2.1339 Kcnh7 -4.0914 Nxph4 -6.17188 Isl1 -2.5956 Cnr1 -4.6945 Ryr1 -6.79656 Hfe2 2.09766 Bcl2l2 -4.0969 P2ry4 -6.1777 LOC685527 -2.60507 Ptprk -4.6945 Ryr1 -6.79656 Hfe2 2.09766 MAPRE1 -4.1076 Mast -6.17778 LOC685527 -2.60507 Ptprk -4.6945 Ryr1 -6.79656 Hfe2 2.07969 MAPRE1 -4.1076 Mast -6.19709 ROD1565959 -2.62301 Stpg1 -4.7162 P2ry14 -6.81041 Applor 2.01084 Lgmn -4.132 Lepr -6.19964 P2rx1 -2.62874 Rimbp2 -4.7261 Tcerg11 -6.81836 Klk1c10 -2.01157 Vom2r18 -4.1862 Fabp7 -6.23217 Sall1 -2.67364 Tmem229a -4.7372 Cdc45 -6.88736 Cnmah -2.03276 Cd2 -4.1862 Fabp7 -6.23217 Sall1 -2.673 | 2.1963 | Pof1b | -4.0911 | B3gat1 | -6.1698 | Mycbp2 | -2 58007 | Cd163 | -4 6713 | Dkk2 | -6 78869 | Sftnc |
| 2.090766 Bcl2l2 -4.0969 P2ry4 -6.17777 L0C685527 -2.60507 Ptprk -4.0976 Ptprk -6.81009 Sult=1 2.090766 Bcl2l2 -4.0969 P2ry4 -6.17777 L0C685527 -2.60507 Ptprk -4.0930 Phrs7c -6.81009 Sult=1 2.07605 MaPRE1 -4.1076 Mas1 -6.19709 RGD1564664 -2.62253 Gpr135 -4.703 Apex1 -6.81009 Sult=1 2.056056 Megf6 -4.122 Atp1a2 -6.19789 RGD156599 -2.62301 Stpg1 -4.7162 P2ry14 -6.81036 Samd12 -2.01084 Lgmn -4.132 Lepr -6.2244 Slc2a24 -2.64423 Ihh -4.7261 Tecrg11 -6.81836 Klk1c10 -2.01157 Vom2r18 -4.1862 Fabp7 -6.23217 Sall1 -2.67306 Tmem229a -4.7357 Cdc45 -6.8873 Cmah -2.03276 Cd2 -4.1866 Ftb17 -6.23617 Sall1 <td>2.1339</td> <td>Kcnh7</td> <td>-4.0914</td> <td>Nxph4</td> <td>-6.17188</td> <td>Isl1</td> <td>-2 59567</td> <td>Cnr1</td> <td>-4 6945</td> <td>Ryr1</td> <td>-6.79656</td> <td>Hfe2</td> | 2.1339 | Kcnh7 | -4.0914 | Nxph4 | -6.17188 | Isl1 | -2 59567 | Cnr1 | -4 6945 | Ryr1 | -6.79656 | Hfe2 |
| 2.07969 MAPRE1 -4.1076 Mas1 -6.18709 RGD1564664 -2.62353 Gpr135 -4.703 Apex1 -6.681041 Aplnr 2.05056 Megf6 -4.124 Atp1a2 -6.19789 RGD1565959 -2.62301 Stgp1 -4.7162 P2ry14 -6.81804 Aplnr 2.01084 Lgmn -4.132 Lepr -6.19769 P2ry1 -2.62301 Stgp1 -4.7162 P2ry14 -6.81806 Kilk1c10 -2.01084 Lgmn -4.139 Gzmf -6.22244 Slc2a24 -2.64287 Rimbp2 -4.7261 Tcerg11 -6.81836 Kilk1c10 -2.01157 Vom2r18 -4.1862 Fabp7 -6.23217 Sall1 -2.67306 Tmem229a -4.7357 Cdc45 -6.68873 Cmah -2.03276 Cd2 -4.1862 Fabp7 -6.23217 Sall1 -2.67306 Tmem229a -4.7379 Fabl 200076 Fabp1200767 -2.03276 Cd2 -4.1866 Fb1200766 Fabp1200767 Fabl 200767 | 2.090766 | Bcl2l2 | -4.0969 | P2rv4 | -6.17777 | LOC685527 | -2.60507 | Ptork | -4.6993 | Dhrs7c | -6.81000 | Sult1e1 |
| 2056056 Megf6 -4.1224 Atpla2 -6.19789 RGD1565959 -2.6220 Opt 1/2 -0.001/2 -0.001/2 Applie -2.01084 Lgmn -4.132 Lepr -6.19789 RGD1565959 -2.62874 Rimbp2 -4.7261 Tcerg11 -6.8108 Samit2 -2.01084 Lgmn -4.132 Lepr -6.1964 P2xx1 -2.62874 Rimbp2 -4.7261 Tcerg11 -6.81808 Samit2 -2.01157 Vom2r18 -4.1496 Gzm37 -6.22244 Sic22a24 -2.64874 Rimbp2 -4.7344 Apbec2 -6.88536 Licam -2.03276 Cd2 +4.1862 Fabp7 -6.23217 Sall1 -2.67306 Tmem229a -4.7372 Cdc45 -6.88736 Cmah -2.03276 Cd2 -4.1866 Fsti2 -6.59366 Fbi1 -2.67306 Tmem229a -4.7372 Cdc45 -6.88736 Cmah | 2.07969 | MAPRE1 | -4.1076 | Mas1 | -6.18709 | RGD1564664 | -2 62252 | Gpr135 | -4 703 | Apex1 | -6.81041 | AnInr |
| -2.01084 Lgmn -4.132 Lepr -6.1964 P2rx1 -2.62505 opg -1.7261 Terg11 -6.81836 Klk1c10 -2.01157 Vom2r18 -4.1499 Gzmf -6.22244 Slc22a24 -2.64423 lhh -4.7344 Apobec2 -6.88536 L1cam -2.01891 Dock8 -4.1862 Fabp7 -6.23217 Sall1 -2.67306 Tmem229a -4.7357 Cdc45 -6.8873 Cmah -2.03276 Cd2 -4.1866 Fts12 -6.5956 Pth1 -2.67306 Tmem229a -4.7357 Cdc45 -6.8873 Cmah -2.03276 Cd2 -4.1866 Fts12 -6.5956 Pth1 -2.67306 Pth1 -2. | 2.056056 | Megf6 | -4.1224 | Atp1a2 | -6.19789 | RGD1565959 | -2 62301 | Stng1 | -4 7162 | P2rv14 | -6.8108 | Samd12 |
| -2.01157 Vom2r18 -4.1499 Gzmf -6.22244 Slc22a24 -2.64423 lhh -4.7344 Apobec2 -6.88536 Licam -2.01891 Dock8 -4.1862 Fabp7 -6.23217 Sall1 -2.67306 Tmem229a -4.7357 Cdc45 -6.8873 Cmah -2.03276 Cd2 -4.1866 Fts12 -6.59536 Pth1 -2.67306 Tmem229a -4.7357 Cdc45 -6.8873 Cmah | -2.01084 | Lgmn | -4.132 | Lepr | -6.19964 | P2rx1 | -2 62874 | Rimbn2 | -4 7261 | Tcerg1 | -681836 | Klk1c10 |
| -2.01891 Dock8 -4.1862 Fabp7 -6.23217 Sall1 -2.67306 Tmem229a -4.7357 Cdc45 -6.8873 Cmah | -2.01157 | Vom2r18 | -4.1499 | Gzmf | -6.22244 | Slc22a24 | -2 64422 | lbb | -4 7244 | Anobec? | -6 88536 | L1cam |
| -2.03276 Cd2 +4.1866 Ftsi2 -6.25936 Pth1 -7.6740 Thiology -7.7477 CdC43 -0.0675 Cliffal | -2.01891 | Dock8 | -4.1862 | Fabp7 | -6.23217 | Sall1 | -2 67306 | Tmem229a | 4 7357 | Cdc45 | -6.8873 | Cmah |
| *0.888 KGD1309036 | -2.03276 | Cd2 | -4.1866 | Ftsj2 | -6.25936 | Pth1r | -2.67433 | Srv | -4.7473 | Ecel1 | -6.888 | RGD1309036 |

continued

percentage than high glucose treatment alone, as shown by increased apoptotic nuclei (condensed or fragmented) (Fig. 2D) and more PI-positive cells (dying or dead cells) (Fig. 2E).

Genome-wide analysis of differentially expressed genes between Nmnat1 knockdown cells and wild-type RGC-5 cells

To reveal the potential mechanism by which Nmnat1 regulates RGC-5 cell function, we performed microarray experiments using total RNAs from Nmnat1 siRNA-transfected RGCs-5 cells and scrambled siRNA-transfected RGC-5 cells. We found that 794 genes



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| logFC | Gene.symbol | logFC | Gene.symbol | logFC | Gene.symbol | logFC | Gene.symbol | logFC | Gene.symbol | logFC | Gene.symbol |
|----------|--------------|---------|------------------|-------------|--------------------|----------|----------------------|-------------------|-----------------|-----------|---------------|
| -2 67582 | Prdm11 | -4 7555 | Mto1 | -6.89654 | Wdr95 | -3.06222 | Ucn3 | -5 2299 | Nr1i3 | -5.92361 | Tef20 |
| -2.6767 | Nr4a3 | -4 7595 | Defa24 | -6.90895 | Giman1 | -3.06998 | Dapp1 | -5 2345 | Rnf125 | -592404 | Fshh |
| -2 67742 | Vdr | -4 7909 | 100680406 | -690972 | Avor2 | -3.07142 | Bean | -5 2368 | Camky | -5.9266 | Stard7 |
| -2 69287 | Kynu | -4 7974 | Glyatl2 | -691395 | Wnt10a | -3.07801 | Cn5 | -5 2389 | Vom1r101 | -5.92667 | Akr1c12 |
| -2.6932 | Synl2 | -4.8165 | Amn1 | -691414 | Pacef10 | -3 10995 | The 2r12 | -5 2561 | PCD1562126 | -5.94529 | Ptol9 |
| -2.69381 | Cap | -4.8265 | Nnffr1 | -6.91451 | Semina1 | -3.11196 | Sut6 | -5.2565 | Acan | -5.94329 | Acel |
| -2.09301 | Clen4 | -4.934 | Cnibbn1 | -6.02166 | MAP2K10 | -2 1121 | Cdk11b | -5 2712 | Ctdepl | -5.96222 | Typl1 |
| -2.71203 | Evtl1 | -4.9342 | Incenn | -6.92100 | Trhr | -2 11025 | Mdan1 | -5.2052 | Dui2 | -5.90040 | Vill |
| -2.72208 | Nr2c1 | -4.0342 | Mkv2-5 | -6.93735 | Slc5o7 | -3.11623 | Muga1 Dog2g | -3.2032 E 2002 | Drib Cub2 | -3.97196 | Cldm1 |
| -2.72704 | Nr2C1 | -4.0304 | NKX2-5 | -0.94/93 | MADVAD1 | -3.14509 | Reg3g | -3.2882 | CDD2 | -5.98046 | Clani |
| -2.73008 | Nmnat2 | -4.8407 | Nubp1 Kenk15 | -0.93339 | MAPKAPI | -3.15143 | SITPA1 PCD1560794 | -5.2886 | Lt04r Three | -5.98278 | Ulic3 Ude1 |
| -2./3921 | LUC100911437 | -4.8432 | KCHK15 | -0.904/9 | Palul | 2 15075 | Eaf14 | 5 2020 | 100670551 | 5 00666 | Nole1 |
| -2.74895 | Fgd4 | -4.8512 | Dnall1 | -6.99117 | Raiyi | -3.138/3 | rgi 14 | -5.3029 | LUC0/9551 | -5.98000 | NOICI |
| -2.75019 | Sctr | -4.8535 | Krt19 | -7.01164 | Spata19 | -3.16501 | PIKID4 | -5.306/ | Prkagz | -5.98931 | Ccac40 |
| -2.76183 | Mbd4 | -4.8651 | SIc34a1 | -7.03968 | Nf2 | -3.16/59 | Aldhoal | -5.30/4 | RGD1310495 | -5.9919 | Dgkb |
| -2.76598 | Reep6 | -4.8739 | Notch4 | -7.05073 | Cpne5 | -3.1/135 | Reps1 | -5.3079 | Zswim8 | -5.99286 | Itin4 |
| -2.77191 | Lrig3 | -4.887 | Mybphl | -7.06281 | Prrt4 | -3.17473 | LOC100364488 | -5.3089 | Kcna2 | -5.99972 | Lama1 |
| -2.78409 | Grxcr1 | -4.8897 | Dip2c | -7.09496 | Spata9 | -3.18643 | Irx5 | -5.3163 | Adipoq | -6.00347 | Ppp1r1c |
| -2.79331 | Ptger1 | -4.8932 | Kcnmb1 | -7.11434 | Crisp2 | -3.19983 | Ripply1 | -5.3209 | Asic4 | -6.01731 | Mchr1 |
| -2.79599 | Gp9 | -4.8988 | Arhgap20 | -7.11747 | Cnnm1 | -3.20094 | Ccr4 | -5.3231 | MAPK7 | -6.02498 | Pnpla7 |
| -2.79651 | LOC681335 | -4.901 | RGD1306750 | -7.14927 | Them5 | -3.22925 | MAP3K12 | -5.3373 | Dnajc2 | -6.02763 | Tas2r121 |
| -2.82588 | Pde7a | -4.9018 | Rbm20 | -7.16217 | Flvcr2 | -3.23557 | Hoxa1 | -5.3403 | RGD1564614 | -6.04209 | Fut2 |
| -2.8297 | RGD1308195 | -4.9031 | Olr1078 | -7.16788 | Scnn1a | -3.24943 | RGD1624210 | -5.3419 | Cel | -6.04972 | Arpp21 |
| -2.83118 | Gsg1 | -4.9194 | Abcc6 | -7.18342 | Asphd2 | -3.2642 | Adra2c | -5.3497 | Cd8b | -6.05215 | Arhgap10 |
| -2.83244 | Arhgef7 | -4.9328 | Vstm5 | -7.18546 | Klra17 | -3.26422 | Atp1b4 | -5.3499 | Zg16 | -6.0553 | Art2b |
| -2.83312 | Lrfn2 | -4.9363 | Zfp709 | -7.20407 | Mroh4 | -3.28484 | Pnp | -5.356 | Tal1 | -6.05613 | Stard10 |
| -2.83486 | Ccdc25 | -4.9545 | P4ha3 | -7.24113 | Myrfl | -3.28695 | Ucn2 | -5.357 | Drn2 | -6.06379 | Ly6g6e |
| -2.83576 | Grm4 | -4 9557 | Cabn1 | -7 2926 | Slco1c1 | -3 28786 | Tceal6 | -5.3667 | LOC685577 | -6.07868 | RGD1311595 |
| -2.84505 | Guev2e | -4.9592 | FIF3 | -7 294 | Gnl2 | -3 29042 | Fød5 | -5 3725 | 100246267 | -6.09886 | MAP3K1 |
| -2.84817 | Taffar3 | -4.9594 | Hed3h6 | -733032 | Nrvn3 | -3 20954 | 01:957 | -5.20 | Dtf1a | -610001 | Dash1 |
| -2.04017 | Nr4a2 | 4.9615 | Phome | 7 20671 | Carbr | 2 21677 | 01-297 | 5 2021 | Dultar | 6 11450 | DamE |
| -2.05202 | Trine 66 | 4.9615 | Cuput | -7.39071 | Clumt2 | -3.310/7 | Maurith | -3.3031 E 2010 | FINCE Sin 2n | 6 1 1 6 0 | Deh10 |
| 2.03070 | Clo2EoE2 | 4.9070 | Cyyri Cym2o10 | 7 5 2 7 4 2 | Tubb6 | -3.32307 | Neurito Domiti | -5.3918 | 51038 | -0.1108 | KaD19 |
| -2.8617 | SIC25a55 | -4.9702 | Cyp3a18 | -7.53743 | Tubbo | -3.33347 | Ppm11 | -5.3933 | LOC100365339 | -6.12092 | Shd |
| -2.86597 | Mir144 | -4.973 | LOC100912109 | -7.56261 | Ugt2b15 | -3.33557 | Spsb3 | -5.3936 | Smyd1 | -6.12105 | HK1 |
| -2.8/15/ | Gpr173 | -4.9737 | Oprk1 | -8.33459 | LOC100360606 | -3.33772 | Fam167b | -5.4213 | Gabra5 | -6.14719 | Fut9 |
| -2.87196 | Cebpa | -4.9848 | Lgi4 | -8.4148 | Letm2 | -3.34391 | Isl1 | -5.4274 | Aunip | -6.16361 | Vac14 |
| -2.87812 | Nrarp | -4.9885 | Gab2 | -8.53611 | Lgals9 | -3.3481 | Mep1a | -5.4281 | MAP3K3 | -6.16442 | Pou3f3 |
| -2.8788 | Mobp | -4.9893 | Gad2 | -5.67572 | Chat | -3.34958 | Dnali1 | -5.4323 | RGD1565959 | -6.16499 | Syce3 |
| -2.88566 | Rapgef1 | -4.9928 | Arhgap27 | -5.68078 | MAP3K13 | -3.3609 | Dmrt2 | -5.4399 | MAP2K1 | -6.1698 | Mycbp2 |
| -2.8879 | Cysltr1 | -4.9963 | Cldn14 | -5.69721 | Capn3 | -3.36777 | S100a8 | -5.4675 | Itgb5 | -6.17188 | Isl1 |
| -2.88902 | Rerg | -4.9978 | Pam | -5.70424 | Mapk4 | -3.37193 | MAP3K5 | -5.4727 | En2 | -6.17777 | LOC685527 |
| -2.89028 | Vpreb3 | -5.0218 | LOC100363236 | -5.72018 | Tcf23 | -3.3742 | Tnnt2 | -5.4751 | Nkx2-2 | -6.18709 | RGD1564664 |
| -2.8939 | Hmgn5b | -5.0422 | U2af2 | -5.72126 | Cldn16 | -3.38842 | Crb3 | -5.4878 | Mro | -6.19789 | RGD1565959 |
| -2.89969 | Ajap1 | -5.0432 | Znf750 | -5.73199 | Mb | -3.38854 | Ppp3ca | -5.5056 | Igf2 | -6.19964 | P2rx1 |
| -2.91 | Hoxc4 | -5.0439 | Mybpc2 | -5.73413 | Nppa | -3.39656 | Zfp90 | -5.5164 | DIx5 | -6.22244 | Slc22a24 |
| -2.9134 | Calm15 | -5.0461 | Tmcc1 | -5.74844 | Hhip | -3,40028 | Rvr2 | -5.5195 | Tmem179 | -6.23217 | Sall1 |
| -2.92262 | Galr2 | -5.0576 | Ly49s7 | -5.76047 | Galnt2 | -3.40872 | Tas2r118 | -5.5237 | LOC680988 | -6.25936 | Pth1r |
| -2.93986 | Gper1 | -5.0727 | Sstr2 | -5.76518 | Cvct | -3 42454 | II2ra | -5 5246 | Padi4 | -62667 | 0lr59 |
| -2.95224 | Pde11a | -5.0895 | Slc35d3 | -5.77755 | Kcnh1 | -3.42901 | Htr7 | -5 5373 | Hsd17b6 | -6.26681 | Lenr |
| -2.95413 | LOC688090 | -5.0938 | ll2rb | -5.7785 | Bpifb2 | -3 43339 | Gad1 | -5 538 | Gpr158 | -627064 | Ohn3 |
| -2.96535 | Dos | -5.0948 | Clca1 | -5.7864 | Prss45 | -3 43673 | Pchn3 | -5 5454 | 0lr442 | -627078 | LOC100125362 |
| -2.98072 | МАРКАРКЗ | -5.0952 | Gap43 | -5.79176 | Cldn5 | -3 45162 | Rnase? | -5 5456 | Cldn6 | -627122 | Cvn2a2 |
| -2.98885 | Olr1082 | -5.1091 | Pou3f1 | -5.79627 | LOC100365150 | -3 45567 | Su2h | -5 5504 | 100696124 | -6 27402 | 7an70 |
| -2.9899 | Magi3 | -5 1131 | Innn5a | -5.79696 | Cma1 | -3.45507 | 3V20 | 5.5504 | Cdk2op1 | -6.2000 | Zap70 |
| -2 99153 | Csf3r | -5 1136 | Myh7 | -5 79734 | Tmcc1 | -3.43077 | DrdE | -3.3310 | Nada | -0.2000 | rgi1 |
| -2.00045 | PCD1200104 | -5 1120 | Kenk10 | -5 90502 | Rmnr2 | *3.45805 | Drus | -5.5613 | Ngan | -0.30369 | Acer2 |
| -2.0026 | Sult1d1 | -5.1136 | Runk10 | -5.00392 | Cno1 | -3.4/398 | Kmo | -5.5688 | Prss42 | -6.3193 | SHIFK3 |
| -3.0030 | SlaZeO | 5.1210 | Tatawi di | 5.01425 | Diaz | -3.47667 | MICUZ | -5.5699 | RGD1560559 | -6.3196 | Акар13 |
| -3.01483 | 5107.89 | -5.1219 | Ictexial | -5.81425 | KILZ Churrenth | -3.47957 | Ky | -5.5742 | Cldn1 | -6.32843 | Tubb4a |
| -3.01332 | Nofe | -5.1415 | Haver1 | -3.8330/ | Chinp40 Magaar2 | -3.49577 | Trim63 | -5.5854 | Best2 | -6.34084 | Obscn |
| -3.0222 | Nem | -3.142 | KCHCI | -3.83808 | Magec2 | -3.49717 | Slc30a2 | -5.5904 | Ccdc117 | -6.35925 | Acmsd |
| -3.02331 | Acapi | -0.1486 | 1 FRF | -3.83881 | Rasgrpi | -3.50048 | Asic1 | -5.5986 | Rem2 | -6.36611 | Zdhhc22 |
| -3.02543 | Sulli | -5.1569 | Pri3d1 | -5.83991 | Gainti | -3.5037 | Trim9 | -5.6037 | Ahr | -6.36618 | Fam195a |
| -3.03369 | wipt3 | -5.1697 | Cachd1 | -5.84145 | Setd5 | -3.50555 | Greb1 | -5.6038 | Cacng2 | -6.37235 | Pdk4 |
| -3.03469 | Arpp21 | -5.1852 | Lhx5 | -5.84429 | Dpagt1 | -3.50587 | Scn4a | -5.608 | Dmrtc1c1 | -6.3868 | Tmem200c |
| -3.04157 | Psx1 | -5.1884 | RGD1307603 | -5.86139 | Mageb16 | -3.50762 | Gkn2 | -5.6104 | Fam155a | -6.38955 | Inpp4b |
| -3.04299 | Epb41l4b | -5.1996 | Cacng4 | -5.87578 | Rxrg | -3.51757 | Pfkfb2 | -5.625 | RGD1310209 | -6.38985 | Sez612 |
| -3.04519 | LOC680928 | -5.209 | Oacyl | -5.87941 | Tmem229a | -3.52576 | Cxcr5 | -5.6483 | Dpysl4 | -6.39283 | Nat8 |
| -3.04526 | Ush1c | -5.2129 | Klk1c7 | -5.88227 | Gpc3 | -3.52672 | Oaz3 | -5.6595 | Cldn22 | -6.39518 | lgsf1 |
| -3.04714 | Chd6 | -5.2189 | Prkag3 | -5.88709 | Ntrk3 | -3.52782 | Gnaz | -5.6658 | Fam181b | -6.39854 | Nr1i2 |
| -3.0544 | Cd96 | -5.2199 | Mecom | -5.91016 | Rhox9 | -3.53681 | Eri2 | -5.667 | Muc13 | -6.40327 | Pax8 |
| -3.06132 | Rap1b | -5.2241 | Cnpy1 | -5.91811 | lgfl3 | | | -3.5478 | Ccdc91 | -6.41161 | LOC680590 |

were differentially expressed between Nmnat1 knockdown group and scrambled siRNAtransfected group, including 641 down-regulated genes and 76 up-regulated genes (Table 1). We next conducted GO analysis, which covers the following 3 domains, including cellular component, biological process, and molecular function. The highest enriched GO terms targeted by differentially expressed transcripts included the regulation of transcription (biological process), transcription regulator activity (molecular function), and plasma membrane (cellular component) (Fig. 3). KEGG pathway analysis showed that MAPK signaling was the top pathway enriched among these differentially expressed mRNAs (Table 2). These results suggest that MAPK signaling pathway may harbor significance and/or contribute to the pathogenesis of Nmnat1 knockdown-mediated RGC dysfunction.

Nmnat1 protects RGC-5 cells against high glucose-induced injury through p38-MAPK signaling

The above-mentioned results suggest that MAPK signaling pathway seems to emerge as a major signaling pathway involved in regulating RGC-5 cell function. We then conducted western blots to verify MAPK signaling activation *in vitro*. We showed that high glucose treatment resulted in an obvious increase in phosphorylated p38 and phosphorylated



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Fig. 3. Genome-wide analysis of differentially expressed genes between Nmnat1 knockdown cells and wild-type RGC-5 cells. Microarray analysis was conducted to compare gene expression difference between Nmnat1 siR-NA-transfected RGC-5 cells and scrambled siRNA-transfected RGC-5 cells. Gene enrichment (GO) analysis was used to reveal the biological modules of differentially expressed mRNAs, including biological process, cellular component, and molecular function.



Table 2. Top 10 signaling pathways affected by Nmnat1 knockdown

| Term | P Value | Fold Enrichment |
|---|----------|-----------------|
| MAPK signaling pathway | 3.48E-13 | 3.137629097 |
| Neuroactive ligand-receptor interaction | 1.15E-06 | 2.710084034 |
| Calcium signaling pathway | 0.002031 | 2.276470588 |
| Tight junction | 0.028798 | 1.814198783 |
| Wnt signaling pathway | 0.029715 | 4.110294118 |
| Leukocyte transendothelial migration | 0.044901 | 1.858567775 |
| Endocytosis | 0.060841 | 1.554141059 |
| Cell adhesion molecules (CAMs) | 0.063781 | 1.666335453 |
| rno04930:Type II diabetes mellitus | 0.07389 | 2.348739496 |
| rno04060:Cytokine-cytokine receptor interaction | 0.087847 | 1.502239474 |

ERK1/2 levels. Nmnat1 knockdown significantly reduced phosphorylated p38 levels, but had no effect on the levels of phosphorylated ERK1/2 or JNK1/2 (Fig. 4A and 4B), suggesting that Nmnat1 knockdown leads to inaction of p38-MAPK signaling inaction. To further examine whether p38 is involved in Nmnat1-mediated protective effect on RGC-5 cell function, Nmnat1 was overexpressed in RGC-5 cells. These cells were pretreated with SB203580, U0126, or SP600125, and then exposed to high glucose for 48 h. MTT assays showed that SB203580, a p38-MAPK signaling inhibitor, strongly blocked Nmnat1-mediated protective effect on RGC-5 cell function, whereas U0126 (ERK inhibitor) or SP600125 (JNK inhibitor) treatment did not affect Nmnat1-mediated protective effect on RGC-5 cell function (Fig. 4C). Hoechst 33342 staining showed that Nmnat1 overexpression decreased high glucose-



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Fig. 4. Nmnat1 protect RGC-5 cells against high glucose-induced injury through p38-MAPK signaling. (A, B) RGC-5 cells were transfected with Nmnat1 siRNA, scrambled siRNA (Scr), or left untreated, and then exposed to high glucose (30 mM) for 48 h. The untreated group was taken as the control group (Ctrl). Representative immunoblots (A) of total p38, total ERK, total JNK, p-p38, p-ERK, p-JNK, and tubulin were shown along with the densitometric quantitative results (B). (C) RGC-5 cells were transfected with Nmnat1 plasmid or vector (pcDNA3.0), then pretreated with SB203580, U0126, or SP600125 for 1 h, and finally exposed to high glucose (30 mM) for additional 48 h. Cell viability was detected by MTT assay (n = 4). (D, E) RGC-5 cells were treated as shown in Fig. 4C. Apoptotic cells were analyzed using Hoechst staining and quantitated. The data was shown as means ± S.E.M. and represented four independent experiments in which > 300 cells were counted. Statistical result was shown in Fig. 4D. A representative image for Hoechst staining was shown Fig. 4E. Scale bar: 20 μm. *indicated a significant difference compared with wild-type (WT) group. #indicated a significant difference between the marked groups. NS: no significant difference.

induced RGC-5 cell apoptosis. SB203580 treatment could interrupt this protective effect, whereas U0126 or SP600125 treatment had no effect on this effect (Fig. 4D). Collectively, these results suggest that p38-MAPK signaling is involved in Nmnat1-mediated protective effect on RGC-5 cell function.



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Discussion

Nmnat1 encodes an enzyme which catalyzes a key step in the biosynthesis of nicotinamide adenine dinucleotide (NAD), which is highly conserved from archaebacteria to human [22]. Studies in Drosophila and mice have revealed a neuroprotective role of Nmnat1 in neurodegenerative conditions including tauopathies, Charcot-Marie-Tooth (CMT) disease, Parkinson's disease, and glaucoma. Loss of Nmnat1 could lead to rapid and severe neurodegeneration [23-26]. In this study, we reveal a role of Nmnat1 in diabetes mellitus-related retinal neurodegeneration. Nmnat1 could protect RGCs against high glucose-induced injury *in vitro*.

DR is not only a microvascular disease but also a neurodegenerative disease [27]. Hyperglycemia could lead to the apoptosis of both retinal neurons and vascular cells [28, 29]. RGCs are located in the inner retina, and are easily injured in response to high glucose stress. Nmnat1 knockdown could aggravate RGC injury, and accelerate the development of RGC apoptosis, suggesting a protective role of Nmnat1. A recent study has revealed that cytoplasmic overexpression of Nmnat1 could protect mouse retinal ganglion cell axons and soma against from glaucomatous and ischemic-induced injury [30], also suggesting a protective role of Nmnat1 is emerged as a security guard of RGCs in response to stress.

To understand the mechanism of Nmnat1-mediated protective effect on RGC function, we conducted genome-wide analysis of gene expression through microarray, which can simultaneously detect the expression of thousands of transcripts. We found that the highest enriched GOs targeted by differentially expressed mRNAs were regulation of transcription (ontology: biological process), plasma membrane (ontology: cellular component), and transcription regulator activity (ontology: molecular function), suggesting that Nmnat1 knockdown may affect gene transcription, which in turn affects the expression of genes associated with RGC function. Signaling pathway analysis provides the knowledge about genomes and the relationship to biological system. We show that these differentially expressed mRNAs are most enriched in MAPK signaling pathway. MAPK family is a common participant in mediating growth factor receptor as well as adhesive and tensional signals from the cell surface to the nucleus. They are activated in response to physiological angiogenic stimuli, such as elevated shear stress and mechanical stretch [31, 32]. The activation of MAPK signaling in RGCs may be responsible for diverse downstream actions such as cell proliferation, cell apoptosis, and electrical signal transduction.

Microarray and bioinformatics analysis suggests that Nmnat1 knockdown affects the activity of MAPK signaling. Thus, we investigated the involvement of MAPK signaling in Nmnat1-mediated protective effect on RGC function. MAPKs are a family of serine/threonine kinases, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal protein kinase (JNK), and p38 MAPK [31, 33]. Nmnat1 knockdown leads to the inaction of p38 MAPK signaling. SB203580, a p38 MAPK pathway inhibitor, strongly blocks the effect of Nmnat1-mediated protective effect on RGC function, suggesting that p38-MAPK signaling is mainly involved in Nmnat1-mediated protective effect in RGC function. MAPK signaling activation has been reported to contribute to neuroprotection, and be involved in the development of diabetic retinopathy [34-37]. MAPK signaling could regulate a variety of cellular activities, including proliferation, differentiation, survival, and death. These biological processes may co-regulate to cope with diverse extracellular and intracellular stimuli [31]. In this study, we show novel evidence that MAPK signaling is involved in neuroprotection.

In conclusion, we show that Nmnat1 knockdown could aggravate RGC injury, and accelerate the development of RGC apoptosis in response to high glucose stress. Nmnat1 protects RGC against high glucose-induced injury via p38-MAPK signaling pathway. Nmnat1 may serve as a neuroprotective target for diabetes mellitus-related retinal neuropathy.

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Disclosure Statement

None declared.

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In the article by Zhou et al., entitled "Nmnat 1: a Security Guard of Retinal Ganglion Cells (RGCs) in Response to High Glucose Stress" [Cell Physiol Biochem 2016;38:2207-2218 (DOI: 10.1159/000445576)] there is an error in Figure 2. The correct figure and the legend is reproduced correctly here. The authors sincerely regret this error due to wrong submission during the review process.

Fig. 2. Nmnat1 knockdown affects RGC function in vitro. (A) RGCs were transfected with Nmnat1 siRNA, scrambled (Scr) siR-NA, or left untreated (Ctrl) for 48 h. Western blots were performed to detect Nmnat1 expression. Tubulin was detected as the internal control. A representative immunoblot was shown along with quantitative result. (B-E) RGCs were transfected with Scr siRNA, Nmnat1 siRNA, or left untreated (Ctrl), and then exposed with or without high glucose (30 mM) for 48 h. Viable cells were detected by cell counting after trypan blue exclusion. Scale bar: 50 µm. (B). Cell viability was detected using MTT method (C). The data was expressed as relative change compared with Ctrl group without high glucose treatment. (D) Apoptotic cells were analyzed using Hoechst staining and quantitated. The



data was shown as means±S.E.M. and represented four independent experiments in which >300 cells were counted. (E) Apoptotic cells were analyzed using PI/Calcein-AM double staining. Scale bar: 50 μ m. "" indicated significant difference compared with the corresponding control group. "#" indicated significant difference between the marked groups.