Identification of Gene Targets of Transcription Factor NeuroD2

by

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This is to certify that I have examined this copy of a master's thesis by

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ABSTRACT

For an organism to examine its environment and develop a behavior is fundamental for its survival. Nervous system plays a central role to gather information and to create a response for fight or flight. In higher organisms, brain is the core of this system. It has unique complexity due to numerous cell types and their communications among themselves. This complexity enables us to learn, to remember, and to perform the simplest movements.

Development of nervous system is under the control of various transcription factors, basic helix-loop-helix transcription factors constituting the biggest family among them. Neurogenic Differentiation factor 2 (NeuroD2) belonging to basic helix-loop-helix transcription factor family has been characterized as a key player in neurogenesis.

Genome-wide identification of direct target gene of transcription factors gives comprehensive insight to complexity of gene regulatory network. Although NeuroD2 emerges as an important transcription factor in neurogenesis, its genome-wide target genes are poorly understood.

Chromatin immunoprecipitation followed by sequencing (ChIP-Seq) is one of the powerful tools to investigate transcription factor binding profiles genome-wide. In this study, I have identified the direct target genes of basic helix-loop-helix transcription factor, NeuroD2 by performing ChIP experiment followed by high throughput sequencing in postnatal cerebral cortical tissues from mice under physiological conditions.

ÖZET

Bir organizmanın hayatta kalabilmesi için, etrafını gözlemleyebilmesi ve herhangi bir tehlikeye karşı bir davranış geliştirebilmesi temeldir. Sinir sistemi bilgi toplanması ve kaç ya da savaş cevabını oluşturması için merkezi rol oynamaktadır. Yüksek organizmalarda, beyin bu organizmanın merkezidir. Beynin eşsiz karışıklığının sebebi çok fazla hücre tipinin olması ve bu hücrelerin birbirleri arasındaki iletişimleridir. Bu karışıklık bizim öğrenmemizi, hatırlamamızı ve en basit hareketleri yapmamızı sağlamaktadır.

Sinir sisteminin gelişimi, birçok transkripsiyon faktörünün kontrolü altındadır. Bazik sarmal-ilmek-sarmal transkripsiyon faktörleri bunlar içerisindeki en büyüğüdür. Nöron Farklılaştırma faktörü 2 (NeuroD2), bazik sarmal-ilmik-sarmal transkripsiyon faktör ailesine ait nörojenez için anahtar oyuncudur.

Transkripsiyon faktörlerinin direkt hedef genlerinin genom kapsamlı belirlenmesi gen yönetme ağının karışıklığına geniş kapsamlı bakış açısı sunmaktadır. Her ne kadar NeuroD2 nörojenez için önemli bir transkripsiyon faktörü olsa da direkt hedef genleri genom bazında çok az bilinmektedir.

Kromatin immunopresipitasyonu takiben sekanslama, transkripsiyon faktörlerinin genom bazında belirlenmesi için güçlü bir yöntemdir. Bu çalışmada, doğar doğmaz alınan farelerin beyin serebral kortikal düzlem dokusunda bazik sarmal-ilmik-sarmal transkripsiyon faktörü NeuroD2'nin genom bazında direkt hedef genleri kromatin immunopresipitasyonu takiben yüksek verimli sekanslama ile fizyolojik koşullar altında belirlenmiştir.

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To my family and

my little sweet niece, Deniz…

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NOMENCLATURE

APS	Ammonium persulfate
BSA	Bovine Serum Albumin
BME	Basal Medium Eagle
DAPI	4’,6-diamidino-2-phenylindole
DMEM	Dulbecco’s Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethyleneglycoltetraacetic acid
FBS	Fetal Bovine Serum
GFP	Green Fluorescent Protein
HBSS	Hank’s Balanced Salt Solution
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid
HRP	Horseradish peroxidase
kDa	Kilodalton
MEM	Minimum Essential Media
PBS	Phosphate buffered saline
PEG	Polyethylene glycol

PCR	Polymerase chain reaction
P/S	Penicillin/Streptomycin
RIPA	Radioimmunoprecipitation assay buffer
shRNA	short hairpin RNA
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TBS-T	Tris buffered saline-Tween
TEMED	Tetramethylethylenediamine

Chapter 1

INTRODUCTION

Nervous system is fundamental for an organism to observe it's surrounding, to detect danger and to create a response for fight or flight; in other words nervous system is required to survive. In higher organisms, this system has central nervous system component in which brain is the core. We still do not know most of the mechanisms which form the brain and its unique complexity which enables us to think, to dream, to remember, and to do simplest movements. There are two basic reasons for this complexity; numerous unique cell types and their communications among themselves.

Development of the brain starts with the proliferation of adequate amount of cells. At the exact time point, these cells exit cell cycle and migrate to their final destination in the brain while initiating their own differentiation programs. Different types of neurons, which are the electrically excitable cells that transmit information, gain their characteristics at their destination. Neurites of neurons, in other words axon and dendrites, outgrow in a highly dynamic state with many branch additions and eliminations to establish connections with other neurons. Various molecular players mediate each of these different mechanisms to form proper functional brain.

Transcription factors being the basis of gene expression networks direct the differentiation programs in each tissue of an organism. Basic helix-loop-helix transcription factors are found to be important in regulation of differentiation in many cell types such as muscle cells, neuronal cells [1] programming myogenesis, and neurogenesis.

NeuroD (Neurogenic Differentiation) family of bHLH transcription factors are one of the protein families orchestrating neurogenesis. Although, some of the members of this family are well studied such as NeuroD1, for many of them, we still do not have comprehensive data about how they manage differentiation and which mechanisms they regulate.

Genome-wide identification of direct target gene of transcription factors enables us to resolve complexity of gene regulatory network. Chromatin immunoprecipitation followed by sequencing (ChIP-Seq) (Figure 1.1) is one of the powerful tools to investigate transcription factor binding profiles genome-wide [2]. Although there are many ways to perform ChIP-Seq experiments such as overexpressing the protein of interest in cell lines, the ones performed in tissue provides us with the closest insight to what really happens in the cells. Here, in this study, I have identified the direct target genes of basic helix-loop-helix transcription factor, NeuroD2 by performing ChIP-Seq in postnatal cerebral cortical tissues which reveals physiologically the most relevant results.

In Chapter 2, the review of the current literature about NeuroD2 protein and its importance for proper development in the brain is explained. The materials and methods used in this study are described in Chapter 3. The results of my project and their importance and implications for development of brain are presented in Chapter 4 and Chapter 5, respectively.

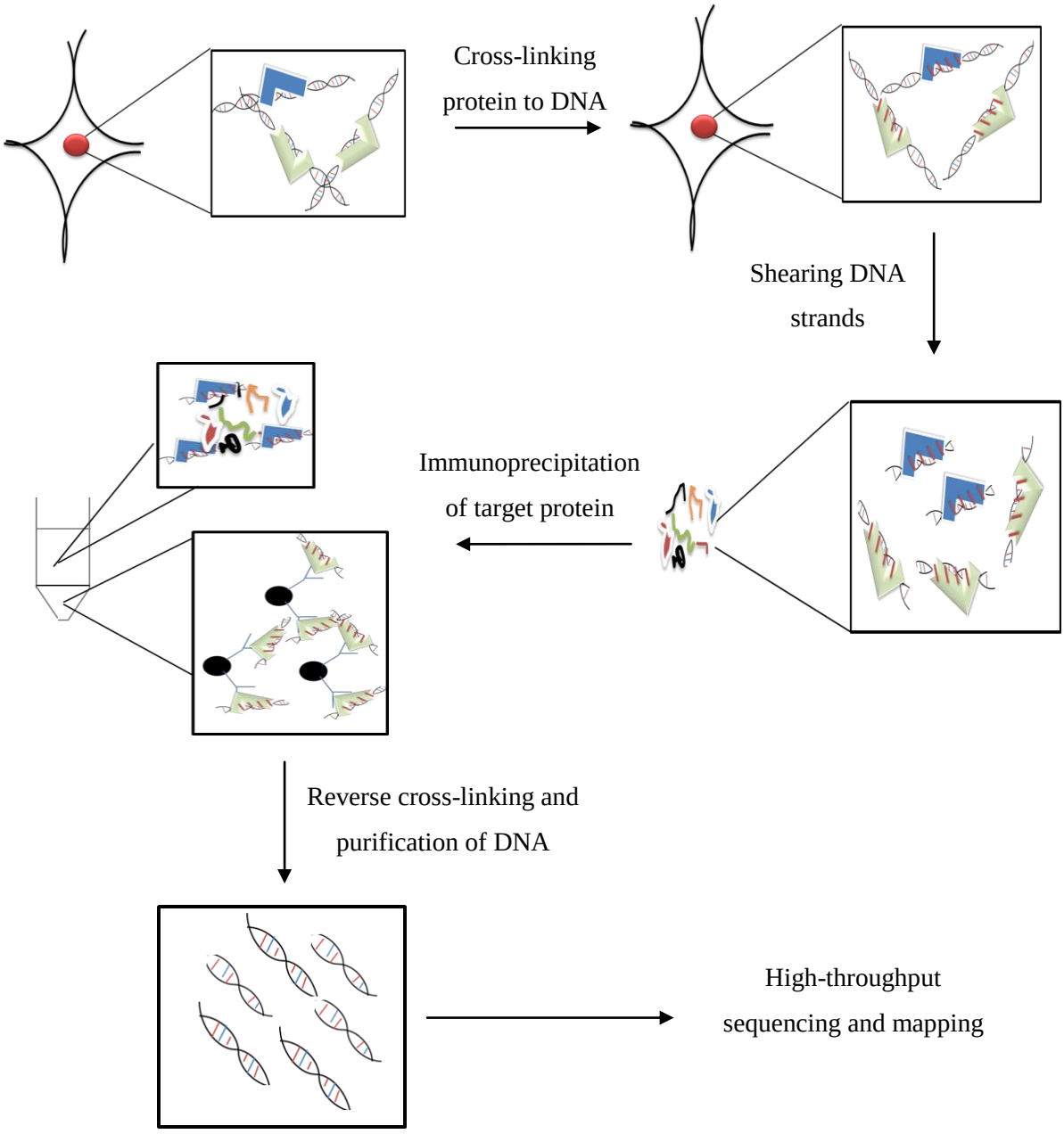


Figure 1.1: Schematic outline of chromatin immunoprecipitation followed by sequencing.

Chapter 2

LITERATURE REVIEW

2.1 Overview

For an organism to be able to examine its environment, gather information and process a behavior, proper development of brain's complicated architecture is fundamental.

Embryonic neurodevelopment starts with the formation of the neuronal tube from the ectoderm layer of embryo as a thin sheet of neuroepithelial cells [3]. Neurons and glial cells that form the nervous system are generated from the ventricular zone of the telencephalon [4], the embryonic structure that matures to the cerebrum. Before starting neurogenesis, neuroepithelial cells increase their number by dividing vertically, hence symmetrically giving rise to two identical daughter cells, both contacting the ventricular zone [4]. When cortical neurogenesis is initiated, neuroepithelial cells transform gradually to elongated radial glial cells [3], [5]. Radial glial cells, retaining their cell bodies in the ventricular zone, expand through developing cerebrum [3]–[5]. Radial glial cells are defined as the neuronal progenitors [6], [7]. Asymmetric division through apical-basal polarity of radial glial cells give rise to one radial glial cell and one immature neuron or intermediate progenitor [3], [8], [9]. Immature neurons exit the ventricular zone and migrate on the radial glial cells to the top of previously formed cerebral cortex layer giving the inside-out characteristic development feature of the cerebrum [4]. At their final destination, they mature and establish connections with other neurons. Intermediate progenitor cells, on the other hand, migrate to subventricular zone [5], [10], [11], superficially located to ventricular zone and divide

symmetrically to give rise to two neurons and expand the neuronal population [9]. After giving rise to neurons and helping them migrate to their final locations, radial glial cells detach from ventricular zone, migrate through cortical layers and differentiate to supportive glial cells such as astrocytes, oligodendrocytes [5] while some of them keep neural progenitor cell identity in the adult brain [3] (Figure 2.1).

Both maintenance of the progenitor character of the radial glial cells and differentiation of glial cells and various types of neurons are guided by transcription factors [5]. Basic helix-loop-helix transcription factors, approximately 125 members present in human genome [12], direct the many mechanisms during development involving the conservation of the progenitor identity, differentiation of neurons as well as supportive glial cells in the central nervous system [3], [13]. Oligodendrocyte differentiation of glial cells is induced by Olig1/2 bHLH factors [14], [15] and Hes and Id family of bHLH factors maintains the neuronal progenitor cell identity [13]. While Hes family transcription factors repress the neuronal differentiation by physically interacting with neuronal differentiation transcription factors and blocking them [16], Id family inhibits neurogenesis by sequestering E proteins required for the activity of proneural bHLH transcription factors [17]. The proneural bHLH transcription factors including Neurogenin family and NeuroD family, are the key regulators of neurogenesis guiding neuronal fate determination and subtype identity [3], [13]. Neurogenin transcription factors initiate the neurogenesis while NeuroD family members have roles in terminal neurogenesis [13]. Both family members can induce differentiation of neural progenitor cells to neurons when expressed ectopically [18] and decrease proliferation of precursor cells by promoting cell cycle arrest [19].

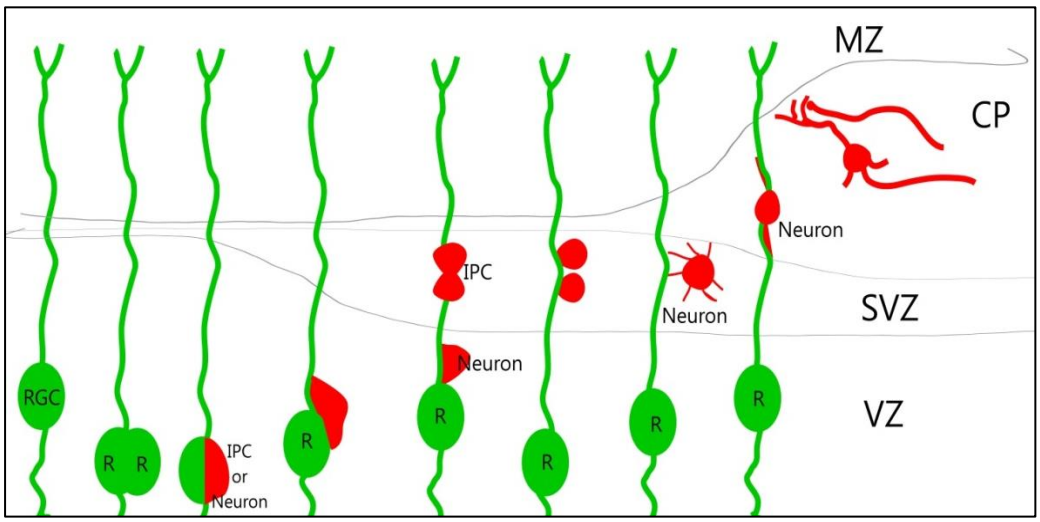


Figure 2.1: Schematic presentation of neuronal migration and differentiation during neurogenesis. Abbreviations: RGC and R, radial glial cells; IPC, intermediate progenitor cells; VZ, ventricular zone; SVZ, subventricular zone; CP, cortical plate; MZ, marginal zone [20].

2.2 NeuroD Family

NeuroD family members are basic-helix-loop-helix transcription factors that emerge as one of the important regulators of neurogenesis [21], [22]. This family members are orthologous to the *Drosophila melonogaster* gene *atonal* [1]. The first family member identified was NeuroD1. When *neuroD1* is constituvely expressed in *Xenopus* embryos, ectopic neurogenesis in ectodermal cells is observed [23]. By using sequence similarity to *neuroD1* gene, two new genes in NeuroD family were isolated, namely, *neuroD2* and *neuroD3* [1], [23].

NeuroD2 (NDRF) contains 383 amino acids and it has 53.4% sequence identity to NeuroD1 [24]. NeuroD2 was shown to be capable of inducing ectopic neurogenesis

and premature conversion of precursor cells into neurons in *Xenopus* embryos as did NeuroD1 [1]. Both NeuroD1 and NeuroD2 transcription factors form heterodimers with ubiquitously expressed E proteins and they both activate simple reporter construct composed of multimerized E-boxes composed of CANNTG nucleotide sequence [1], [24]. It is suggested that NeuroD bHLH transcription factor family members have redundant overlapping functions in regulating neurogenesis [13], [22].

2.3 NeuroD2 is necessary for neuronal differentiation and maintenance

NeuroD2 is expressed in postmitotic neurons and is shown to induce differentiation in neuronal precursor cells [1], [24], [25]. Farah *et al.* reported that transient expression of *neuroD2* bHLH transcription factor in combination with a bHLH dimerization partner, E12 protein, is sufficient to generate neurons from pluripotent mouse P19 cells. They showed that P19 cells undergo alterations upon *neuroD2* expression such as neurite extensions, expression of neuron-specific proteins, cell cycle withdrawal and ability for electrical signaling [25]. Besides inducing differentiation, NeuroD2 is also necessary for the survival of the neurons in the cerebellum and for the proper development of brain [26]. Olson *et al.* studied brain development in *neuroD2*-null mice and they showed that at a gross histological level the cerebella of *neuroD2*-null mice were smaller compared to wild-type. Although, all of the neuron types in cerebellum were present in *neuroD2*-null mice, apoptosis rate after postnatal day 14 increased significantly compared to wild-type mice [26]. Thereafter, *neuroD2*-null mice started to die between P14 and P35. They also showed that expression of brain-derived neurotrophic factor (BDNF), which plays role in cerebellar neuron survival, was diminished in *neuroD2*-null mice brain [26] suggesting BDNF might be a target gene of NeuroD2.

2.4 Expression Pattern of *neuroD2* in Brain

McCormick *et al.* showed that in mice *neuroD2* expression was first detected in embryonic day 11 (E11), its abundance increased through E12.5, and persisted in postmitotic neurons throughout adulthood. During development, *neuroD2* expression was detected in the neocortex, cerebellum, spinal cord, optic tectum and brain stem [26], [27]. In adulthood, *neuroD2* expression was restricted to the region of Purkinje cells and hippocampus [1], [26].

2.5 Regulation of NeuroD2 Expression and Activation

Neurogenin family is specifically expressed in neural progenitors that will differentiate into neurons [18]. Exogenous expression of Neurogenin1 protein in dividing precursor cells cultured from rat E14 cortices is sufficient to increase number of precursor cells that differentiate into neurons [18]. The importance of Neurogenin1 to activate *neuroD2* gene was first claimed in P19 embryonal carcinoma cells. H. Oda *et al.* created *neuroD2* gene promoter driven luciferase reporter and co-transfected it along with expression vectors of different candidate bHLH proteins to P19 cells. Only Neurogenin1 among these proteins was able to transactivate the reporter construct significantly [28]. Lin *et al*. also confirmed that Neurogenin1 regulates expression of *neuroD2* [29]. Activation of *neuroD2* by Neurogenin1 was confirmed by breeding *neurogenin1*-null mice with *neuroD2*+/- mice which expressed *lacZ* gene under the control of the *neuroD2* promoter. In these mice, *lacZ* expression was missing from the cortical plate in neurons where *neuroD2* is expressed normally [29].

2.6 Regulation of NeuroD2 Transcriptional Activity

Following the migration and differentiation of neurons during development of cerebral cortex, neurons start to connect with each other. During early development, spontaneous neuronal activity establishes the connections between neurons. As the organism develops, specific input and experience-dependent neuronal activity shapes the more well-defined and permanent connections [30]. Calcium ion influx to neurons is stimulated by neuronal activity [31] and calcium-activated gene expression is key for maturation of the nervous system. In other words, neuronal activity, hence calcium influx is required to establish proper connections between peripheral stations and cortex through intermediate structures.

NeuroD2 is identified as one of the calcium-activated transcription factors [27] by using the "transactivator trap" strategy [32]. Briefly, Ince-Dunn *et al.* used a GAL4-UAS system [33] in which NeuroD2 is fused to Gal4 DNA binding domain to mediate calcium-dependent transcription of a UAS driven reporter gene. After transfection of these vectors to cortical neurons, reporter gene expression was detected upon induction of calcium influx [27].

2.7 NeuroD2 functions to regulate formations of connections in the brain

NeuroD2 bHLH transcription factor is a calcium-activated transcription factor [27] and plays key roles in neurogenesis [1], [25]. Much of the information about the function of NeuroD2 comes from studies of *neuroD2* knockout mice. NeuroD2 is shown to involve maturation of thalamocortical and hippocampal synapses, development of amygdala which plays role in emotional learning, and formation of cortical commissural fiber tracts [27], [34]–[36].

Sensory input from the peripheral systems is relayed to cortex through intermediate stations with the axonal fiber tracts. These tract formations are guided by neuronal activity. Ince-Dunn *et al.* studied thalamocortical connections that convey tactile input from the whiskers in the rodent to the cortex through brainstem and thalamus [27]. Thalamocortical axons reaching to cortex form synapses with neurons in the cortex and creates structures that represent individual whisker follicles and are called "barrels". Each barrel receives input from one whisker creating a topographic map of the whisker field (Figure 2.2) [27], [37].

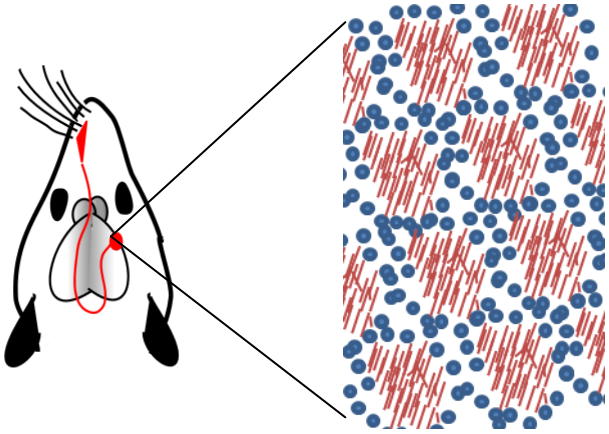


Figure 2.2: Schematic overview of the topographic map of the whisker field to barrel cortex through thalamocortical patterning in the mouse [37].

Ince-Dunn *et al.* reported that NeuroD2 expression correlated with the establishment of thalamocortical connections both in time and in place [27]. Since thalamocortical connections require neuronal activity [38] and NeuroD2 is a transcription factor activated by neuronal activity [27], they examined the thalamocortical connections in *neuroD2* knockout mice. They reported that organization of barrel field was completely disrupted in *neuroD2*-null mice, meaning

that NeuroD2 is required for the formation of proper thalamocortical axon segregation [27].

Neuronal activity in excitatory pyramidal neurons is transmitted through glutamatergic synapses. There are two main types of neurons in the cerebral cortex; inhibitory interneurons and excitatory pyramidal neurons [39]. Inhibitory neurons release GABA neurotransmitter and lead to inhibition of postsynaptic neurons therefore they are called GABAergic neurons and excitatory neurons release glutamate neurotransmitters leading to activation postsynaptic neurons, therefore called glutamatergic neurons. Metabotropic and ionotropic glutamate receptors located at postsynaptic membrane are both gated by glutamate neurotransmitter. Subtypes of ionotropic glutamate receptors are NMDA, AMPA and kainate receptors. In early development, mostly NMDA type of glutamate receptors mediates thalamocortical synaptic activity [38]. Later, AMPA receptors relay synaptic currents more than NMDA receptors [40] indicating an increased ratio of AMPA receptors to NMDA receptors as a marker for postnatal maturation of glutamatergic synapses [41]. Lu *et al.* showed that the mouse having impaired barrel formation has also reduced AMPAR/NMDAR current ratio meaning that development of AMPA currents is related to barrel cortex formation [42]. Ince-Dunn *et al.* supported this finding that in *neuroD2* knockout mice, contribution of AMPA receptors to synaptic currents are significantly less than NMDA receptors [27] suggesting that NeuroD2 has a crucial role in the maturation of synaptic currents.

In accordance with the establishment of neuronal connections in the brain, neuronal activity induces morphological changes in synapses. Excitatory synaptic neurotransmitters, such as glutamate, bind to postsynaptic protrusions called dendritic spines [34]. NeuroD2 transcription factor being activated by calcium-signaling and expressed in excitatory post-mitotic neurons [1], [27] is shown to regulate morphological differentiation of excitatory synapses [34]. Wilke *et al.* studied

hippocampal synapse formation [34] in NeuroD2 knockout mice. They studied mossy fibers connecting the dentate gyrus to CA3 regions in the hippocampus [43], [44].

Wilke *et al.* used *neuroD2* null mice as well as targeted knockdown of NeuroD2 *in vivo*. They have reported that genomic *neuroD2* deletion leads to significant reduction in thorny excresences (TE) head numbers which are the characteristics of mossy fiber dendritic sites in P14 and P21 compared to wild type littermates [34]. Moreover, they found that NeuroD2 is required for the functional maturation of mossy fiber synapses. As in the case of thalamocortical synapses [27], they showed that *neuroD2* null mice have reduced AMPA receptor to NMDA receptor ratio in glutamatergic synapse maturation [34]. They reported that NeuroD2 exerts its effects for synapse maturation through post-synaptic scaffolding protein, PSD95 (postsynaptic density protein 95). In the absence of NeuroD2, PSD95 expression is reduced and targeted knockdown of PSD95 leads to similar reduction in TE spine head numbers as *neuroD2* null mice [34]. Since PSD95 functions to modulate AMPA receptor contents of developing synapses, they suggested that NeuroD2 regulates maturation of glutamatergic MF synapses through PSD95 protein [34].

2.8 NeuroD2 cooperates with NeuroD6 to regulate formation of commissural fiber tracts

Commissural fiber tracts, namely hippocampal commissure, anterior commissure, and corpus callosum are axonal fiber bundles connecting two cerebral hemispheres [45]. These tracts are formed by neocortical pyramidal projecting neurons which are glutamatergic neurons from the neocortex and hippocampus [46] at the same developmental time with NeuroD2 expression [22].

Bormuth *et al.* demonstrated double mutant mice of NeuroD2 and NeuroD6 display lack of both corpus collosum and anterior commissural fiber tracts [36]. However in the background of *neuroD2*+/- genotype, *neuroD6* mutants are able to develop both corpus collosum and anterior commissural fiber tracts which is also true vice versa [36]. They claimed that initial generation of pyramidal projecting neurons and initial axon outgrowth are normal in double mutant mice [36]. Consistent with the studies by Ince-Dunn *et al.* and Wilke *et al.*, *neuroD2/6* double mutants had significantly reduced GAP-43 positive axon bundles and they displayed reduced glutamatergic synaptic activity, hence neuronal activity [36]. This study confirms that NeuroD2 and NeuroD6 are coexpressed in postmitotic projecting neurons and their guidance is required for the establishment of connections between two hemispheres, i.e. formation of commissural fiber bundles [36], [46].

2.9 Absence of NeuroD2 causes defects in amygdala functions

The amygdala incorporates emotional learning and emotional responses [47]. Formation of emotional memory involves synaptic strengthening and remodeling which again requires calcium-dependent second messenger pathways leading to permanent synaptic change by transcription-dependent mechanisms [35]. Lin *et al.* especially chose to study role of NeuroD2 in amygdala region formation since heterozygote *neuroD2* mice exhibit aggressive behaviors [35]. They have reported that compared to wild type littermates, *neuroD2*+/- mice have significantly reduced number of neuronal cells in lateral and basolateral amygdala and *neuroD2* null mice completely lack of these regions [35].

Since amygdala is important in responding an unconditioned risk in the environment, they performed standard unconditioned anxiety tests on heterozygote

neuroD2 mice. Elevated plus-maze test is composed of plus shaped maze with two open and two closed arms and is based on aversion of mice from open-ended places [48]. These tests revealed that *neurod2*+/- mice did not avoid unprotected areas failing to respond appropriately to risk [35] suggesting that NeuroD2 transcription factor is important for the proper function of amygdala. Glutamatergic receptors involved in excitatory neuron synaptic transmission are fundamental for fear conditioning in the lateral amygdala [49]. They reported at the molecular level, AMPA receptors are significantly diminished in *neuroD2* heterozygotes compared to wild type littermates [35] consistent with the fact that AMPA receptors are synthesized and inserted to postsynaptic membrane during fear conditioning [50]. They concluded that haploinsufficiency of *neuroD2* gene results in an amygdala defect.

2.10 Gene targets of NeuroD2

Various studies revealed some of the putative gene targets of NeuroD2 transcription factor. For example, GAP-43 protein highly expressed in presynaptic axonal sites during development is reported to be activated by NeuroD2 [1]. Ince-Dunn *et al.* showed that expression level of GAP-43 is reduced in *neuroD2* knockout mice. Moreover, in another study, GAP-43 mutant mouse is shown to display similar disrupted barrel cortex formation [51] further suggesting that GAP-43 is a possible direct target gene of NeuroD2 and its transcription is regulated in activity-dependent manner [27]. Similarly, expression level of PSD-95 protein which is a postsynaptic scaffolding protein highly concentrated in glutamatergic synaptic sites is shown to be reduced in *neuroD2* knockout mice [34]. Konishi *et al.* showed that type 1 inositol 1,4,5-triphosphate receptor, which is a Ca^{+2} channel protein expressed in central nervous system in the Purkinje neurons and hippocampus, is activated upon binding of NeuroD2 to E-box consensus sequence [52].

Olson *et al.* studied changes in gene expression by expression array analysis in *neuroD2*-null mice. Only 27 of approximately 6000 mRNAs were diminished in *neuroD2*-null mice cerebellum at P21 [26]. Some mRNAs that are decreased in the absence of NeuroD2 were BDNF, tyro3/sky, ras-p21, MAPK, c-fos, c-jun. These genes play a role in cerebellar neuron survival [26].

Later, Tapscott *et al.* compared genome-wide transcription profiles of NeuroD2-mediated neurogenesis and MyoD-mediated myogenesis. They used the pluripotent mouse cell line P19 which were converted to neurons by transduction of NeuroD2-expressing lentivirus as described by Farah *et al.* [25], [53]. They first performed expression array analysis and found that 532 genes were upregulated and 278 genes were downregulated with the expression of *neuroD2*. They reported that upregulated genes are related to neuron development and differentiation categories according to Gene Ontology Analysis. Next, to identify binding sites of NeuroD2, they performed chromatin immunoprecipitation followed by high-throughput sequencing in the cell line with two different antibodies [53]. They reported that majority of the peaks were located in introns and intergenic regions, and which were not further analyzed [53]. As in the expression array analysis, they found that all annotated genes whose transcription start site was ±2 kb in NeuroD2 binding regions enriched for neurogenesis, neuronal differentiation and development Gene Ontology categories [53].

Chapter 3

MATERIALS AND METHODS

3.1 Chromatin Immunoprecipitation followed by High Throughput Sequencing

Five cerebral cortices of postnatal day 0 (zero) mice were dissected in 1X HBSS containing 100mM HEPES. Tissues were triturated in 10ml 1X HBSS containing 100mM Hepes one time and transferred to petri plates.

A stock of 37% formaldehyde was added onto tissue such that the final concentration of formaldehyde became 1%. Crosslinking was carried out for 10 minutes at room temperature on slow shaker. To quench crosslinking reaction, glycine dissolved in ddH_2O was added to a concentration of glycine 125mM. Glycine incubation was performed for 10 minutes at room temperature on slow shaker. Tissue was washed with 1X HBSS containing 10mM HEPES (PH) twice at 4000 rpm at 4˚C for 4 minutes each. 1ml of 1X RIPA (0.05 M Tris-HCl pH 7.5, 0.15M NaCl, 1% Triton-X 100, 1% Na-DOC, 0.1% SDS) containing 1 tablet of protease inhibitor cocktail (Roche cOmplete Mini, EDTA-free) was added onto tissue and transferred to an eppendorf tube.

Sonication was carried out in cold room (Bandelin Sonopuls HD2070). A power of 93 and 70% output for 20 seconds was applied 30 times. The samples were placed onto ice for at least 1 minute to cool down between each cycle. After sonication was complete, the samples were centrifuged at 14000 rpm at 4˚C for 20 minutes, supernatants were collected and pellets were discarded.

100µl of lysates were separated as input and rest of the lysates were transferred to new eppendorf tubes containing Protein A/G Magnetic Beads (Thermo Scientific, Cat. #88802) for pre-clearing. Sample pre-clearing was done with 75µl of Protein A/G magnetic beads which were washed with 1X RIPA lysis buffer three times. Pre-clearing was done in cold room for 1 hour while continuously rotating. After 1 hour, lysates were transferred into new eppendorf tubes and antibodies were added. Lysate-antibody incubation was done in cold room, on a rotator from 2 hours to overnight. Again, 50µl of Protein A/G magnetic beads are washed with 1X RIPA lysis buffer three times. Lysate-antibody samples were added onto washed magnetic beads. The Protein A/G magnetic beads-lysate-antibody incubation was done in cold room, on the rotator for 1 to 1.5 hours. After immunoprecipitation, supernatants of samples were separated from the beads on a magnetic stand. Post-immunoprecipitation supernatants were kept at -20 ˚C. Protein A/G beads bearing antibody-protein complexes were washed with 7 different washing buffers, twice for each buffer, 10 minutes in cold room on a rotator (Wash Buffer 1: 1X PBS, 0.1% SDS, 0.5% Na-DOC, 0.5% NP-40. Wash Buffer 2: 5X PBS, 0.1% SDS, 0.5% Na-DOC, 0.5% NP-40. Wash Buffer 3: 15mM Tris-HCl, pH 7.5, 5mM EDTA, 2.5mM EGTA, 1% Triton X-100, 1% Na-DOC, 0.1% SDS, 120mM NaCl, 25mM KCl. Wash Buffer 4: 15mM Tris-HCl, pH 7.5, 5mM EDTA, 2.5mM EGTA, 1% Triton X-100, 1% Na-DOC, 0.1% SDS, 1M NaCl. Wash Buffer 5: 15mM Tris-HCl, pH 7.5, 5mM EDTA. Wash Buffer 6: 50mM Tris-HCl, pH 7.5, 150mM NaCl, 1mM $MgCl_2$, 0.05% NP-40. Wash Buffer 7: 50mM Tris-HCl, pH 7.5, 10mM $MgCl_2$, 0.5% NP-40). After the last wash buffer, Elution Buffer 1 (sterile 1% SDS, 0.1M $NaHCO_3$) was added onto Protein A/G magnetic beads and input sample in the amount of 200µl and 300µl, respectively. The samples were incubated at 65˚C in a shaking heat block for 1 hour to reverse the crosslinking. The eluate was separated from beads and was incubated at 65˚C heat block overnight (not more than 15 hours). The following day, Elution Buffer 2 (sterile 100mM Tris-HCl, 20mM EDTA pH 8.0) was added onto ChIP samples and input sample in the amount of 200µl and 300µl, respectively. 1µl of

RNaseA (Thermo Scientific, Cat. #R1253) was added and they were incubated at 37˚C for 1 hour. Then, 4µl of Proteinase K, concentration of 20mg/ml (Thermo Scientific, Cat. #EO0491) was added and they were incubated at 50˚C for 2 hours.

DNA samples were purified by phenol-chloroform extraction. First, 500µl of phenol-chloroform solution was added onto samples and mixed slowly for 10 minutes. After centrifugation at 14000 rpm for 5 minutes, upper layers were transferred into new eppendorf tubes. The extraction step was repeated once more. Na-acetate, pH 5.2, was added in the amount of 1/3 volume of samples and 100% isopropanol in the amount of 10X volume of samples. These were mixed gently once and incubated at -20˚C for 2 hours. After incubation, samples were centrifuged at 14000 rpm at 4˚C for 20 minutes. Supernatants were removed and the pellets were washed with 70% ethanol, twice. The pellets were air dried under the hood. When all the ethanol was evaporated, 30µl of ultrapure water (RNase and DNase free) was added and pellets were dissolved.

PCR was done for the verification of ChIP experiment. Forward and reverse primers (Appendix G) for one of the candidate genes, Nhlh2 [53], were designed. PCR experiment was done in 50µl mixture containing 5ng DNA templates, 1X *Taq* Buffer with KCl, 0.5µM of forward and 0.5µM of reverse primers, 200µM dNTP's, 2mM $MgCl_2$ and 2.5 Unit *Taq* DNA Polymerase (Thermo Scientific, Cat. #EP0402). PCR conditions for denaturing, annealing and extension were set to 95°C for 30 seconds, 58°C for 30 seconds and 72˚C for 30 seconds respectively and repeated 35 cycles. The samples were run in the 1.4% agarose gel by electrophoresis and visualized under UV-light box.

Table 3.1: Antibodies used in immunoprecipitation

	Antibody	**Host**	**Company**	**Catalogue Number**	**Dilution**
NeuroD2 Ab(1)	Anti-NeuroD2	Mouse	Abcam	ab168932	1:100
NeuroD2 Ab(2)	Anti-NeuroD2	Rabbit	Abcam	ab104430	1:100
NeuroD2 Ab(3)	RabMAbs Anti-NeuroD2 [EPR5135]	Rabbit	Abcam	ab109406	1:100
	Anti-GFP	Rabbit	Santa Cruz Biotechnology	H0612	1:100

3.2 Transfection of Neuro2A cell line

Neuro2A cell line was grown in MEM (Gibco Life Technologies, Cat. #31095029) containing 10% FBS (HyClone), 1% P/S (Gibco Life Technologies, Cat. #15140122), 0.5% L-Glutamine (HyClone). Before transfection, the confluency of the cells was expected to be 60-70%. Transfection of Neuro2A cell line was done with TurboFect Transfection Reagent (Thermo Scientific, Cat. #R0531). Following the product protocol, desired amount of DNA was diluted in DMEM medium (Sigma Life Sciences, Cat. #D5546) in 1:100 (w/v) ratio and TurboFect reagent was added slowly to the mixture in the recommended amount by manufacturer. Typically, 3µg of DNA was diluted in 300µl DMEM medium and 6µl TurboFect reagent was used for 60-mm plates. The mixture was incubated at room temperature for 20 minutes and added into plates dropwise while constant slow shaking. Transfected Neuro2A cells were incubated in incubator (37 ˚C, 5% CO_2) for 24-48 hr.

3.3 SDS-PAGE

A 5% stacking SDS-PAGE (5% Acrylamide-Biasacrylamide (29:1), 126mM Tris-HCl, pH 6.8, 0.1% APS, 0.1% SDS, 0.1% TEMED) gel was added on top of a 10% separating SDS-PAGE (10% Acrylamide-Bisacrylamide (29:1), 375mM Tris-HCl, pH 8.8, 0.2% APS, 0.2% SDS, 2.5% TEMED) gel. Appropriate amounts of proteins were mixed with 2X SDS-Loading Buffer (100mM Tris-HCl pH 6.8, 2% β-mercaptoethanol, 4% SDS and 0.02% bromophenol blue, 20% glycerol) and boiled for 5 minutes and loaded to wells. Samples were run at 100V for 1.5-2 hours in SDS running buffer (10X, pH 8.3; 30g Tris Base, 187.6g Glycine, 1% SDS in total volume of 1L). After gel electrophoresis was completed, gels were further processed for western blot analysis.

3.4 Western Blotting

SDS gels that were run with proteins transferred to a polyvinyl difluoride membrane, PVDF, (Thermo Scientific, Cat. #88518) using wet-transfer method (1.45 g Tris Base, 7.25 g Glycine, 150 ml Methanol in total volume of 1L). Membrane was blocked in 5% Nonfat Dried Milk Powder (AppliChem, Cat. #A0830) in TBS-T (10mM Tris-HCl pH7.5, 150mM NaCl, 0.05% Tween) for 1 hour. Incubation with primary antibody, diluted in blocking buffer, was performed for 2 hours at room temperature or overnight at cold room. Membrane was washed with TBS-T for 15 minutes, three times. Membrane was incubated with secondary antibody for 1 hour at room temperature. Membrane was washed with TBS-T for 15 minutes, three times. Membrane was incubated in SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific, Cat. #34080) and visualized under Gel Logic 2200 Imaging System (Molecular Imaging System CareStream Health Inc.).

Table 3.2: Antibodies used in western blotting

Antibody	Host	Company	Catalogue Number	Dilution Ratio
Anti-NeuroD2	Rabbit	Abcam	ab104430	1:5000
Anti-NeuroD2	Mouse	Abcam	ab168932	1:1000
Anti-beta Actin	Rabbit	Thermo Scientific	PA5-16914	1:5000
Anti-TurboGFP(d)	Rabbit	Evrogen	AB513	1:1000
Anti-myc	Mouse	Santa Cruz Technology	J2512	1:1000
Anti-rabbit IgG, HRP-linked	Goat	Cell Signaling Technology	7074	1:2500
Anti-mouse IgG, HRP-linked	Horse	Cell Signaling Technology	7076	1:2500

3.5 Bioinformatics Analysis of ChIP-Seq

Next generation sequencing was purchased from Genewiz, Inc. (www.genewiz.com). An Illumina HiSeq 2500 platform was used for single-end 50bp sequencing. Fastq files were received for input DNA, ChIP with NeuroD2 Ab1, 2, 3 and GFP. Galaxy online server (usegalaxy.org) was used for the bioinformatic analysis of ChIP sequenced data.

The first step was to upload the five files to server which are Input, ChIP_ND2_M, ChIP_ND2_R1, ChIP_ND2_R2 and, ChIP_GFP.

Steps of bioinformatic analyses are described below:

1. FASTQ file formats were converted to Solexa quality scores type by using following parameters:

NGS Toolbox Beta

NGS: QC and manipulation

FASTQ Groomer

Selection of files to groom

Input FASTQ quality scores type: Solexa

EXECUTE

2. Multiplexing was performed during sequencing therefore six nucleotide barcodes were present at the 5’ end of each read. These barcodes were removed from each read using the “trim” menu option in galaxy. Following parameters were used.

NGS Toolbox Beta

NGS: QC and manipulation

Trim Sequences

Choose library to trim

First base to keep: 8th

Last base to keep: 57th

EXECUTE

3. 50 bp reads were mapped to the mouse genome using Bowtie. Bowtie is a short read aligner designed to be ultrafast and memory-efficient. Mouse (Mus musculus) mm10 was used as reference genome. Since the sequencing was done from the single-end, single-end option was selected for the library. All alignments for a read were suppressed if more than one reportable alignment exists except the alignment that matched the best. Following parameters were used:

NGS Toolbox Beta

NGS: Mapping

Map with Bowtie for Illumina

Use a built-in index

Reference genome: Mouse (Mus musculus): mm10

Is this library mate-paired: Single-end

Trimmed sequences will be used.

Bowtie settings to use: Full-parameter list

Skip the first n reads (-s): 0

Only align the first n reads (-u): -1, meaning “off”

Trim n bases from high-quality (left) end of each read before alignment (-5): 0

Trim n bases from low-quality (right) end of each read before alignment (-3): 0

Maximum number of mismatches permitted in the seed (-n): 2

Maximum permitted total of quality values at mismatched read positions (-e): 70

Seed length (-l): 28

Whether or not to round to the nearest 10 and saturating at 30 (--nomaqround): Round to nearest 10

Number of mismatches for SOAP-like alignment policy (-v): -1

Whether or not to try as hard as possible to find valid alignments when they exist (-y): Do not try hard

Report up to n valid alignments per read (-k): 1

Whether or not to report all valid alignments per read (-a): Do not report all valid alignments

Suppress all alignments for a read if more than n reportable alignments exist (-m): 1

Write all reads with a number of valid alignments exceeding the limit set with the –m option to a file (--max): Yes

Write all reads that could not be aligned to a file (--un): Yes

Whether or not to make Bowtie guarantee that reported singleton alignments are ‘best’ in terms of stratum and in terms of the quality values at the mismatched positions (--best): Use best

Maximum number of backtracks permitted when aligning a read (--maxbts): 800

Whether or not to report only those alignments that fall in the best stratum if many valid alignments exist and are reportable (--strata): Use strata option

Override the offrate of the index to n (-o): -1

Seed for pseudo-random number generator (--seed): -1

Suppress the header in the output SAM file: No

EXECUTE

4. Since sequencing procedure contains PCR amplification step, duplicates caused by PCR were removed by using “rmdup” option in galaxy. Following parameters were used:

NGS Tool Box BETA

NGS: SAM Tools

Rmdup remove PCR duplicates

BAM File: Mapped reads

Is data paired-end: BAM is single-end

EXECUTE

5. All of the applications on the data were done in BAM format which is a binary format for storing sequence data. Although BAM format is designed to compress well, in order to view the data it is converted into SAM format. Conversion from BAM to SAM was applied on duplicate removed mapped reads, using the "BAM-to-SAM" menu item. Following parameters were used:

NGS Tool Box BETA

NGS: SAM Tools

BAM-to-SAM converts BAM format to SAM format

BAM file to convert: rmdup on data

Include header in output: Yes

EXECUTE

6. Although the unmapped reads were written in the separate files from the mapped reads, the mapped reads still contained the unmapped ones. Unmapped reads were filtered by using the "Filter SAM" menu item on galaxy. Following parameters were used:

NGS Tool Box BETA

NGS: Filter SAM

Dataset to filter was selected

Add new flag

Flag 1

Type: The read is unmapped

Set the states for this flag: No

EXECUTE

7. Peak calling was performed to identify regions in the genome that are enriched by aligned reads. Model-based analysis of ChIP-Seq (MACS) was used [54]. Following parameters were used:

NGS Tool Box BETA

NGS: Peak Calling

MACS Model-Based Analysis of ChIP-Seq

Experiment Name: NeuroD2 Chip

Paired End Sequencing: Single End

ChIP-Seq Tag File: ChIP Data

ChIP-Seq Control File: Input Data

Effective genome size: 1870000000.0

Tag Size: 50

Band Width: 200

Pvalue cutoff for peak detection: 1e-50

Select the regions with MFOLD high-confidence enrichment ratio against background to build model: 20

Parse xls files into distinct interval files: No

Save shifted raw tag count at every bp into a wiggle file: Save

Extend tag from its middle point to a wigextend size fragment.: -1

Resolution for saving wiggle files: 10

Use fixed background lambda as local lambda for every peak region: Yes

3 levels of regions around the peak region to calculate the maximum lambda as local lambda: 1000, 5000, 10000

Build model: Build the shifting model

Diagnosis report: Do not produce report

Perform the new peak detection method (futurefdr): No

EXECUTE

Chapter 4

RESULTS

4.1 Characterization of Antibodies

Neuro2A cells do not express NeuroD2 protein endogenously. 1.5μg of *neuroD2* gene in pcDNA4A backbone vector or 1.5μg of pcDNA4A vector were transfected to Neuro2A cells with TurboFect reagent. *neuroD2* gene was expressed as a fusion protein with myc-tag. After 24 hours, lysates from transfected Neuro2A cells were collected. SDS-PAGE and western blotting were performed subsequently with anti-myc antibody, demonstrating that Neuro2A cell line was successfully expressed NeuroD2-myc fusion protein (Figure 4.1).

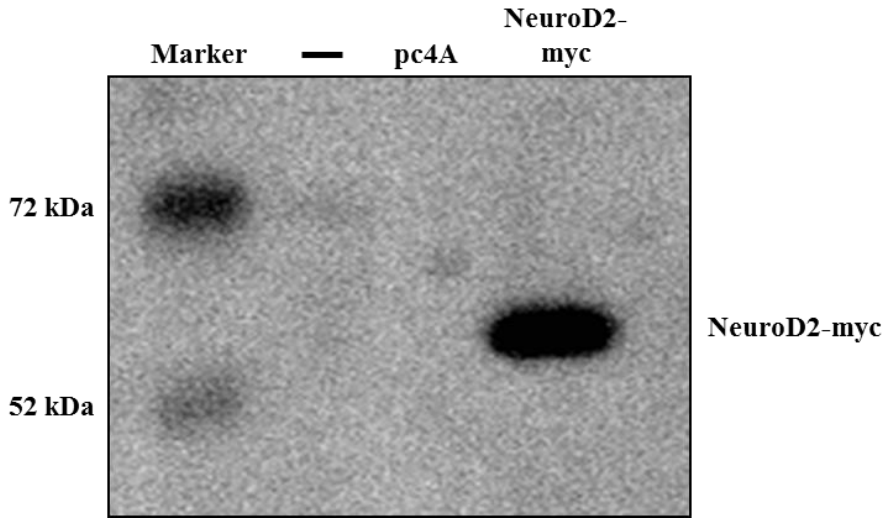


Figure 4.1: NeuroD2-myc fusion protein was expressed in Neuro2A cells.

The primary characterization of antibodies for NeuroD2 transcription factor used in chromatin immunoprecipitation experiment was carried out with immunoblot analysis. NeuroD2-myc fusion protein was overexpressed in Neuro2A cells. Also, endogenously expressed NeuroD2 protein was obtained via homogenization of rom P0 mice cortices tissue using 1X RIPA lysis buffer. 10µl of Neuro2A cell lysate overexpressing NeuroD2-myc and 8µl of P0 cortex sample were loaded onto SDS-Page and subsequent western blotting was done with anti-NeuroD2 antibodies. All of the three antibodies were able to recognize NeuroD2 protein (Figure 4.2).

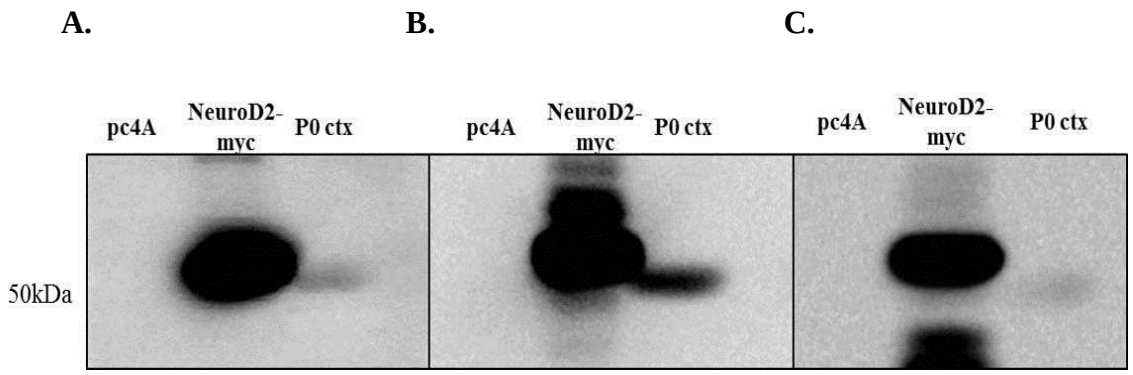


Figure 4.2: Expressions of NeuroD2-myc fusion protein in Neuro2A cells and endogenous NeuroD2 protein from P0 mice cortices were detected. 10µl of pcDNA4A and NeuroD2-myc transfected Neuro2A cell lysates and 8µl of P0 mice cortices lysates were loaded on SDS-Page for each three antibodies. Western blotting was done with **A.** anti-NeuroD2 Ab(1), **B.** anti-NeuroD2 Ab(2) and **C.** anti-NeuroD2 Ab(3).

Secondary characterization of antibodies was carried out by immunoprecipitation of NeuroD2-myc fusion protein overexpressed in Neuro2A cells. In these experiments, immunoprecipitation of samples of pcDNA4A backbone vector transfected Neuro2A cells was used as negative control to evaluate background of antibodies in immunoprecipitation. Also, immunoprecipitation with anti-GFP antibody was used as another negative control. Both pcDNA4A transfected Neuro2A cell lysates and NeuroD2-myc transfected Neuro2A cell lysates were immunoprecipitated with both anti-NeuroD2 and anti-GFP antibodies.

At the last step of immunoprecipitation, the last wash buffer of the ChIP-Seq protocol was removed and 10µl of 2X SDS-Loading Buffer was added onto the beads. 10µl input samples from both pcDNA4A and NeuroD2-myc transfected Neuro2A cells and 10µl supernatant samples after immunoprecipitations were loaded onto SDS-Page. Supernatant samples represented all proteins present in the lysates after immunoprecipitation.

Molecular weight of NeuroD2 protein is around 52 kDa. Boiling of the IP'ed samples lead to the cleavage of heavy chain and light chain of antibodies from the beads and molecular weight of heavy chain is around 50 kDa. If antibodies used for both immunoprecipitation and western blotting were taken from same species, heavy chain of antibody from immunoprecipitation was seen at western blotting masking the NeuroD2 protein. Therefore, western blotting followed by SDS-Page was evaluated with rabbit anti-NeuroD2 antibody if the immunoprecipitation was done with mouse anti-NeuroD2 antibody and vice versa (Figure 4.3).

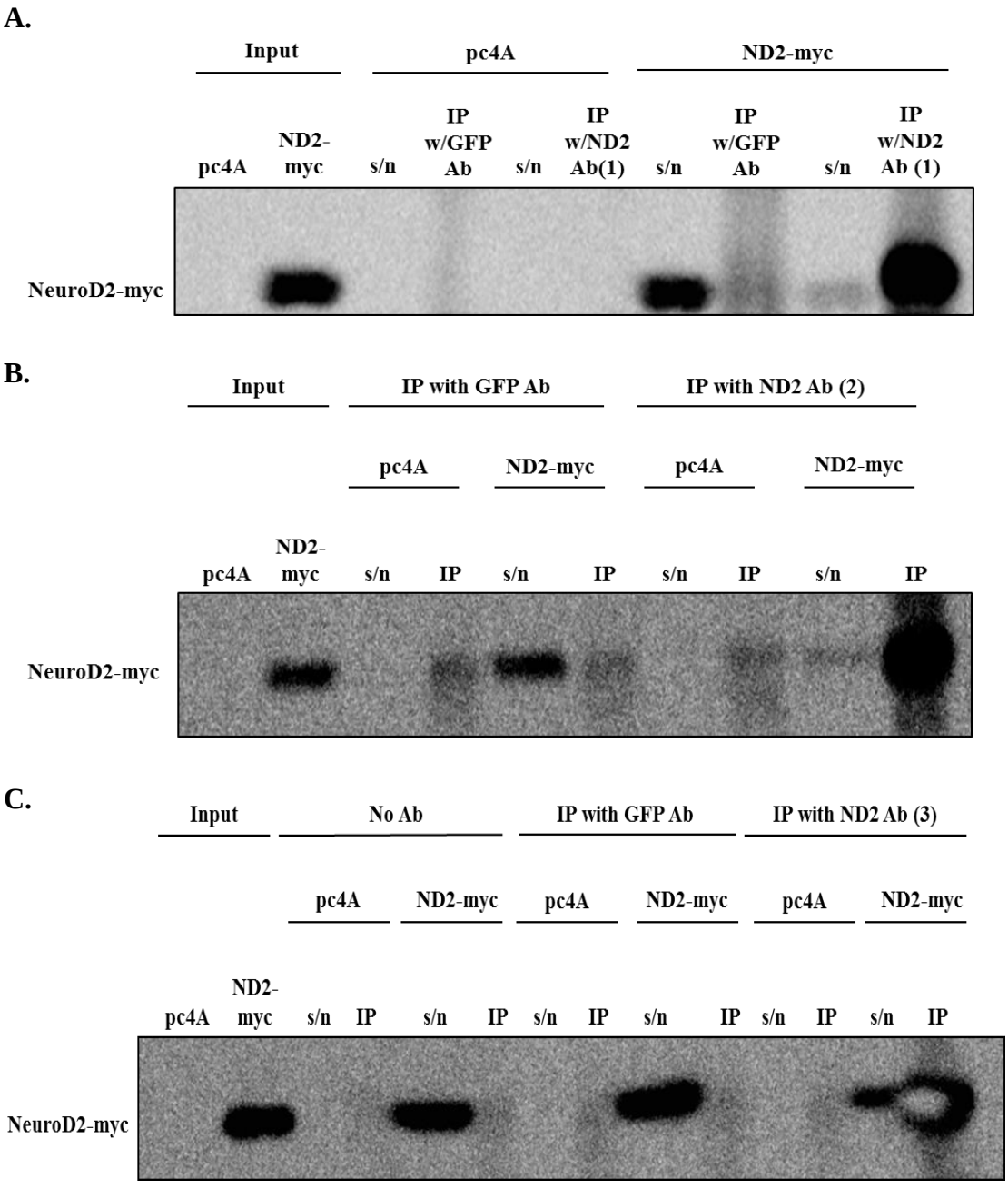


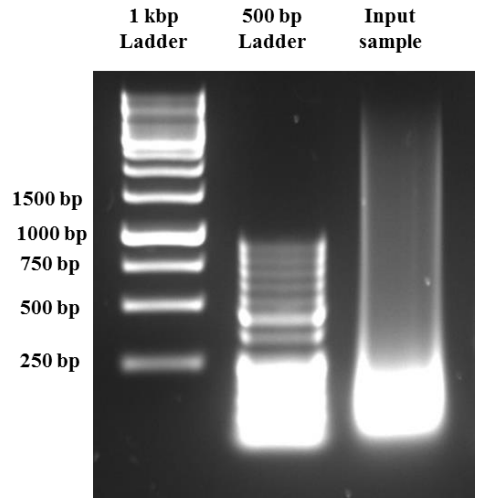
Figure 4.3: Immunoprecipitation by three different antibodies were verified in Neuro2A cells. **A.** Immunoprecipitation with ND2 Ab(1) followed by western blotting with ND2 Ab(2) **B.** Immunoprecipitation with ND2 Ab(2) followed by western blotting with ND2 Ab(1) **C.** Immunoprecipitation with ND2 Ab(3) followed by western blotting with ND2 Ab(1).

4.2 Verification of Chromatin Immunoprecipitation

Chromatin immunoprecipitation followed by sequencing experiments require an optimum size range of DNA fragments between 100bp to 500bp for sequencing efficiency. Before the samples were sent for high-throughput sequencing, 500ng of input sample was run on 1.5% agarose gel to verify the success of sonication. Genomic DNA was sheared between 250bp and 100bp (Figure 4.4.A).

Moreover, PCR was done using primers designed against promoter region of one of the candidate genes, *nhlh2* [53], to show the success of chromatin immunoprecipitation specificity. *nhlh2* gene promoter was enriched in ChIP samples from the three anti-NeuroD2 antibodies but not from the anti-GFP antibody. One PCR was set up with no DNA template for the negative control (Figure 4.4.B).

A.



B.

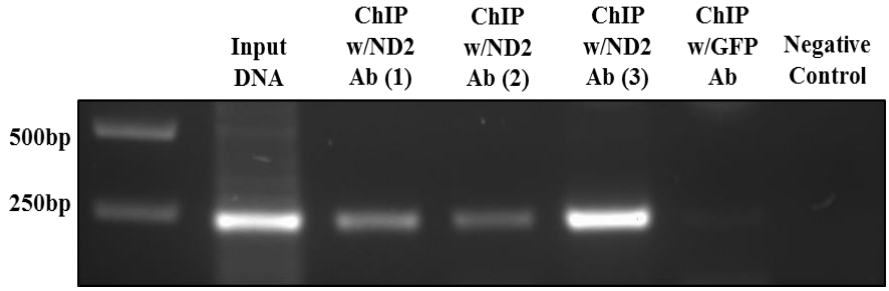


Figure 4.4: A. Sonication of 5 P0 mice cortices was verified. **B.** Success of chromatin immunoprecipitation was verified with PCR which was set with primers designed for promoter region of *nhlh2* gene. Negative control did not contain any DNA template.

Next generation sequencing was purchased from Genewiz, Inc. (www.genewiz.com). An Illumina HiSeq 2500 platform was used for single-end 50bp sequencing. Fastq files were received for input DNA, ChIP with NeuroD2 Ab1, 2, 3 and GFP performed on cerebral and hippocampal tissue of P0 mice. Galaxy online server (usegalaxy.org) was used to analyze ChIP sequenced data.

4.3 Overview of Bioinformatics Analysis of High Throughput Sequencing

Next generation sequencing service provided fastq files for five samples and galaxy online server was used to identify which regions in the genome were enriched in NeuroD2 binding. Following pipeline briefly summarizes the processes done on the raw ChIP data to evaluate the target genes of NeuroD2 transcription factor.

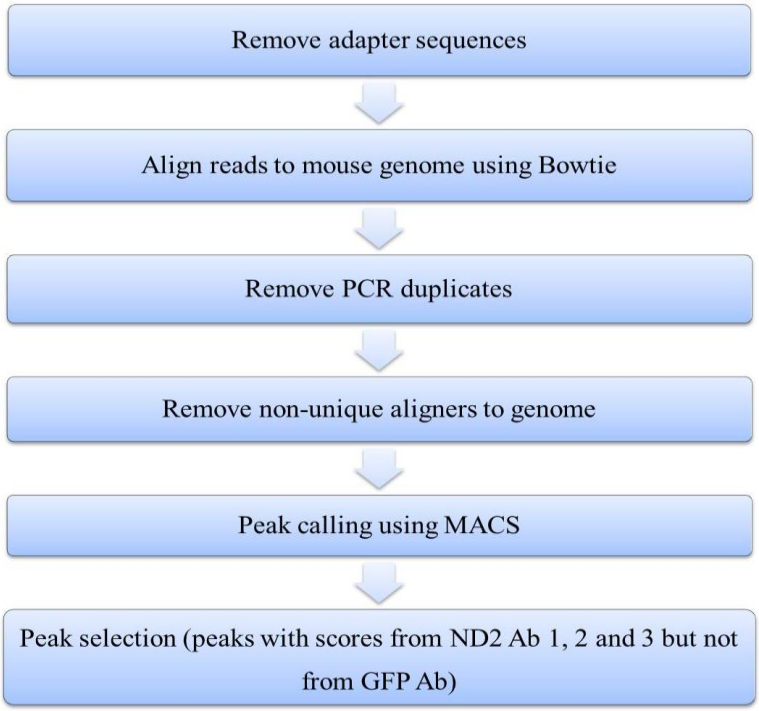


Figure 4.5: Flowchart of bioinformatics analysis.

4.4 Quality Scores of Sequencing and Correlation Coefficients among Antibodies

It is important to read each base of ChIP-Seq data with high accuracy. Q score is a metric to evaluate the quality of base calling of that particular run. Q score is logarithmically related to base calling error probabilities. For instance, if Q score is 30; incorrect base calling probability is 1 out of 1000 calling. Table 4.1 shows that mean Q score of each ChIP-Seq data is above 38 indicating that base calling was performed in %99.99 accuracy (Table 4.1).

In this study, three different NeuroD2 antibodies were used for ChIP experiments. It is very crucial and also expected that reads obtained from those antibodies are correlated among themselves. Correlation coefficient is a widely used statistical analysis to evaluate how two variables correlate with each other, correlation coefficient of 1 showing a perfect linear correlation [55].

Correlation coefficients were calculated using $-10 \times \log_{10}$ (p-value) for each peak identified by MACS analysis for each IP sample. ChIP data from anti-GFP antibodies showed no correlation with any of anti-NeuroD2 antibodies. On the other hand, anti-NeuroD2 antibodies displayed higher correlation suggesting that ChIP data from each anti-NeuroD2 antibodies are not random but rather specific for NeuroD2 transcription factor (Table 4.2).

Table 4.1: Quality of ChIP samples

Sample ID	Yield (Mbases)	# Reads	% of Q Scores>=30	Mean Q Score
Input	1516	29726454	96.51	37.94
ChIP with ND2 Ab(1)	2159	42340321	97.6	38.45
ChIP with ND2 Ab(2)	2071	40604773	97.55	38.43
ChIP with ND2 Ab(3)	1934	37921677	97.56	38.43
ChIP with GFP	2320	45482014	97.48	38.39

Table 4.2: Correlation coefficients of antibodies

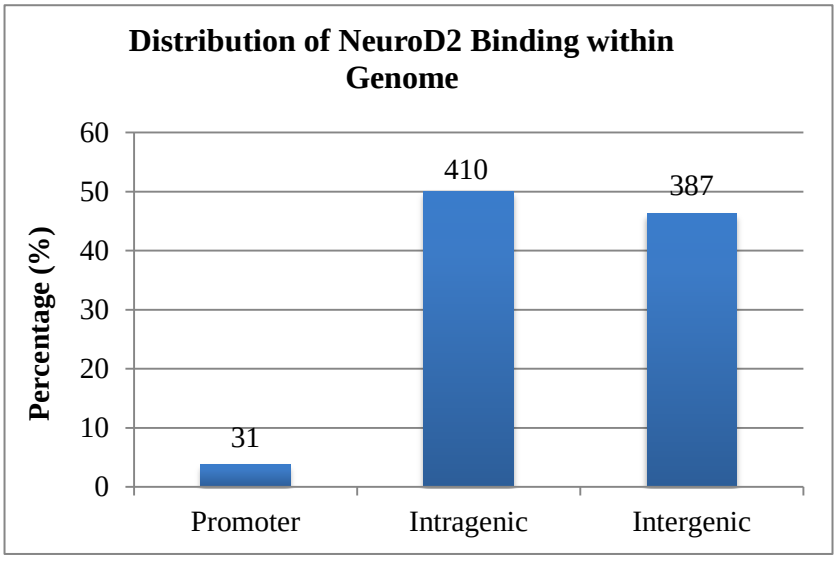
	ND2 Ab(1)	ND2 Ab(2)	ND2 Ab(3)	GFP Ab
ND2 Ab(1)	1	0.87	0.6	-0.08
ND2 Ab(2)	0.87	1	0.64	-0.14
ND2 Ab(3)	0.6	0.64	1	-0.02
GFP Ab	-0.08	-0.14	-0.02	1

4.5 Distribution of peaks among mouse genome

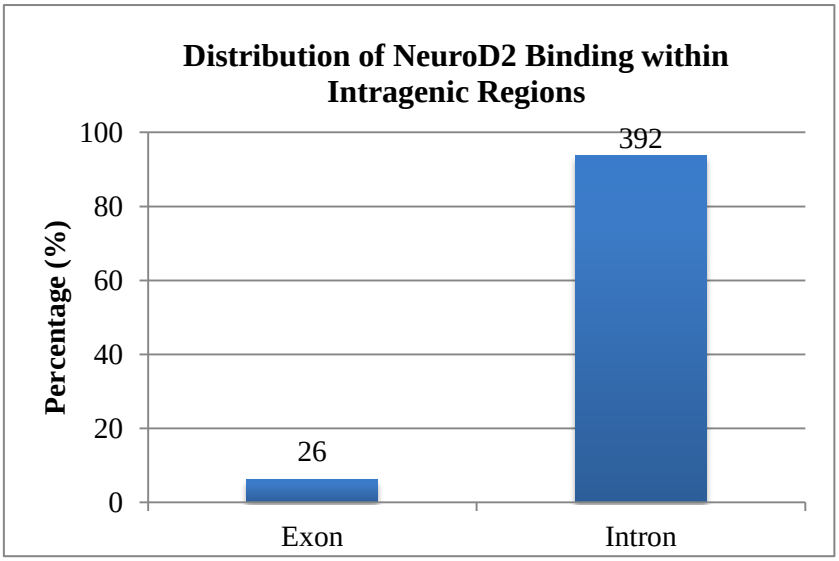
Peaks obtained from three NeuroD2 ChIPed samples intersecting at least 1 bp were identified using “join” menu tool on galaxy. Once these peaks were identified, they were collapsed at single peak, and called as merge peaks. Then, these merged peaks were located in whole genome again using “join” menu tool on galaxy.

Intragenic was defined as regions with known transcript units while intergenic was defined as regions not represented by any known transcript (intragenic, 49.5; intergenic, 46.7%). 2kb upstream of transcription start sites (TSSs) in mouse genome were obtained. Merged peaks were joined with this dataset and were defined as promoter (3.7%). Within intragenic regions, peaks located in exons and introns were identified (introns, 94%; exons, 6%) and those identified in exons were further located to 5’UTR, coding regions and 3’UTR regions (5’UTR, 15%; coding region, 41%; 3’UTR, 44%).

A.



B.



C.

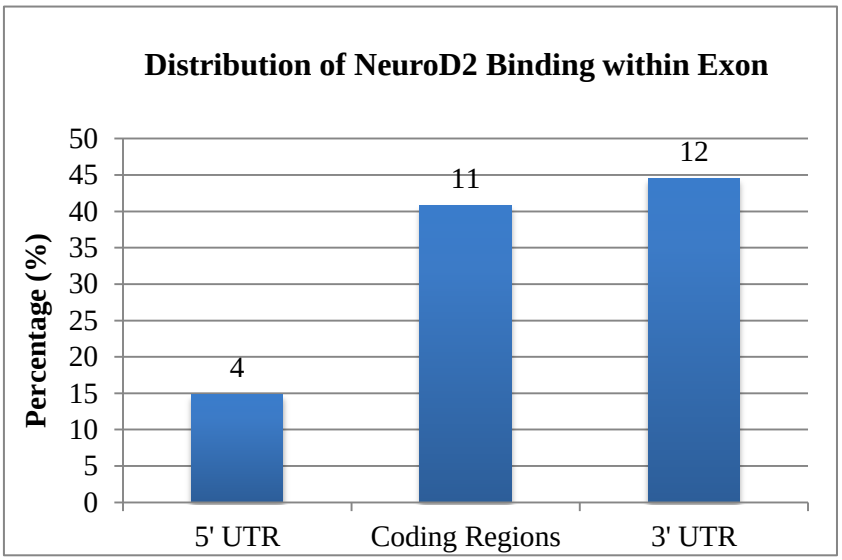


Figure 4.6: Distribution of merged peaks were identified among genome. **A.** 387 peaks were located in intergenic regions, while 410 and 31 peaks were located to transcript and promoter region of a transcript, respectively. **B.** 392 peaks were located in introns and 26 of them in exons within intragenic regions. **C.** 12, 11 and 4 peaks were located to 3'UTR, coding regions and 5'UTR within exons.

4.6 Top target genes of NeuroD2 transcription factor

To determine top target genes of NeuroD2 transcription factor, significance scores of each of ChIP experiment (NeuroD2 Ab1, 2 and 3) were added to assign significance values to the merge peaks. The merged peaks got score from ChIP experiment with GFP Ab as well, were discarded. The significant scores were calculated by the following formula:

$$\text{Score} = -10 \times \log_{10}(\text{p-value})$$

$$\text{Score}_{\text{total}} = \text{Score}_{\text{Ab1}} + \text{Score}_{\text{Ab2}} + \text{Score}_{\text{Ab3}}$$

Ensemble ID's of the genes corresponding to merge peaks were assigned using both whole transcript location and 2000bp upstream of whole transcripts from mus musculus, mm10 genome. 441 peaks of NeuroD2 transcription factor mapping to known transcripts were identified (Appendix I). Stim1 was identified as the top target gene of NeuroD2 and top 20 target genes were listed in the Table 4.3.

Table 4.3: Top 20 target genes of NeuroD2 transcription factor

Gene ID	Ensemble ID	Peak ID	Chromosome #	Merge Midpoint	Total score -10*log10(p-value)
Stim1	ENSMUST00000033289	Merge_461	chr7	102369912	4886.93
Rps6kc1	ENSMUST00000162500	Merge_834	chr1	190872237	3590.04
Erg	ENSMUST00000122199	Merge_278	chr16	95390955	3230.41
Tecr	ENSMUST00000165740	Merge_914	chr8	83584978	3058.53
Ephb1	ENSMUST00000149800	Merge_879	chr9	102039407	2830.58
Mcm9	ENSMUST00000075540	Merge_177	chr10	53612380	2762.02
Col15a1	ENSMUST00000102917	Merge_613	chr4	47291222	2620.75
Myt1l	ENSMUST00000049784	Merge_85	chr12	29663508	2601.01
Nfasc	ENSMUST00000094569	Merge_800	chr1	132591658	2526.01
Ccdc60	ENSMUST00000050178	Merge_569	chr5	116280701	2499.57
Cdkal1	ENSMUST00000006353	Merge_47	chr13	29332826	2497.11
Btbd17	ENSMUST00000141481	Merge_158	chr11	114795453	2459.12
Rps6ka2	ENSMUST00000024575	Merge_217	chr17	7292185	2278.32
Kcnn3	ENSMUST00000000811	Merge_687	chr3	89540118	2130.26
Rabgap1l	ENSMUST00000028049	Merge_814	chr1	160278483	2123.63
Slc9a9	ENSMUST00000033463	Merge_874	chr9	95168636	1993.82
Zdhhc14	ENSMUST00000089185	Merge_215	chr17	5621978	1934.33
Glis3	ENSMUST00000065113	Merge_352	chr19	28436699	1926.15
Fam211a	ENSMUST00000143262	Merge_136	chr11	62625000	1891.26
Sdccag8	ENSMUST00000027785	Merge_821	chr1	176994898	1854.75

4.7 Gene Ontology Analysis of Target Genes of NeuroD2

The Database for Annotation, Visualization and Integrated Discovery (DAVID) gene functional classification was used to categorize target genes of NeuroD2 protein by molecular function (MF), biological pathway (BP) and cellular component (CC) [56], [57].

Enrichment score is a measurement for gene ontology categories calculated based on the biological importance of this particular group in target gene list. 66 annotation clusters were identified with the highest enrichment score 4.8 (Appendix J). For each gene ontology term, p-value was indicated.

Table 4.4a: Annotation cluster 1 with enrichment score 4.8

Category	Term	Count	p-value	Fold Enrichment	FDR
GOTERM_CC_FAT:0045202	synapse	22	1.29E-07	4.01091	0.00017
GOTERM_CC_FAT:0044456	synapse part	15	1.68E-05	4.11496	0.02184
GOTERM_CC_FAT:0045211	postsynaptic membrane	9	1.44E-03	4.15415	1.86139

Table 4.4b: Annotation cluster 2 with enrichment score 3.0

Category	Term	Count	p-value	Fold Enrichment	FDR
GOTERM_BP_FAT:0048667	cell morphogenesis involved in neuron differentiation	13	7.12E-05	4.14774	0.11571
GOTERM_BP_FAT:0000904	cell morphogenesis involved in differentiation	13	2.99E-04	3.56080	0.48460
GOTERM_BP_FAT:0048858	cell projection morphogenesis	12	7.41E-04	3.44961	1.19709
GOTERM_BP_FAT:0000902	cell morphogenesis	15	8.98E-04	2.81885	1.44888
GOTERM_BP_FAT:0048812	neuron projection morphogenesis	11	9.19E-04	3.62927	1.48339
GOTERM_BP_FAT:0032989	cellular component morphogenesis	16	1.08E-03	2.64699	1.74257
GOTERM_BP_FAT:0032990	cell part morphogenesis	12	1.09E-03	3.28689	1.75660
GOTERM_BP_FAT:0030030	cell projection organization	15	1.23E-03	2.73049	1.98478
GOTERM_BP_FAT:0007411	axon guidance	8	1.47E-03	4.74028	2.35966
GOTERM_BP_FAT:0048666	neuron development	14	1.60E-03	2.78410	2.57617
GOTERM_BP_FAT:0006928	cell motion	16	1.69E-03	2.53159	2.70746
GOTERM_BP_FAT:0007409	axonogenesis	10	2.00E-03	3.56248	3.20425
GOTERM_BP_FAT:0030182	neuron differentiation	16	3.74E-03	2.32856	5.90242
GOTERM_BP_FAT:0031175	neuron projection development	11	4.43E-03	2.93006	6.95393

Table 4.4c: Annotation cluster 3 with enrichment score 2.3

Category	Term	Count	p-value	Fold Enrichment	FDR
GOTERM_CC_FAT:0042734	presynaptic membrane	5	1.24E-03	10.38538	1.59937
GOTERM_MF_FAT:0016247	channel regulator activity	4	7.83E-03	9.62899	10.39470
GOTERM_MF_FAT:0005246	calcium channel regulator activity	3	1.74E-02	14.44348	21.76382

Table 4.4d: Annotation cluster 4 with enrichment 2.04

Category	Term	Count	p-value	Fold Enrichment	FDR
GOTERM_MF_FAT:0043167	ion binding	92	6.05E-04	1.35109	0.84202
GOTERM_MF_FAT:0043169	cation binding	90	1.02E-03	1.33839	1.41239
GOTERM_MF_FAT:0046872	metal ion binding	89	1.20E-03	1.33555	1.65971
GOTERM_MF_FAT:0046914	transition metal ion binding	51	2.20E-01	1.12978	96.90511
GOTERM_MF_FAT:0008270	zinc ion binding	39	4.04E-01	1.07040	99.92792

Table 4.4e: Annotation cluster 5 with enrichment score 1.98

Category	Term	Count	p-value	Fold Enrichment	FDR
GOTERM_MF_FAT:0017016	Ras GTPase binding	6	2.94E-03	6.08146	4.02987
GOTERM_MF_FAT:0031267	small GTPase binding	6	3.42E-03	5.87531	4.67208
GOTERM_MF_FAT:0051020	GTPase binding	6	4.24E-03	5.59102	5.76247
GOTERM_MF_FAT:0019899	enzyme binding	9	4.53E-02	2.27059	47.66880
GOTERM_MF_FAT:0017048	Rho GTPase binding	3	6.37E-02	7.22174	60.10829

4.8 Consensus Sequence of NeuroD2

MEME-ChIP online service (http://meme.nbcr.net/meme/cgi-bin/meme-chip.cgi) was designed to discover motif by analyzing ChIP-Seq peak regions. MEME and DREME were the two algorithms the web service used to identify motifs [58]. Basic helix-loop-helix transcription factors bind to E box motifs which is a DNA sequence of CANNTG. My motif analysis identified that NeuroD2 protein binds to consensus sequence "CAGATGG".

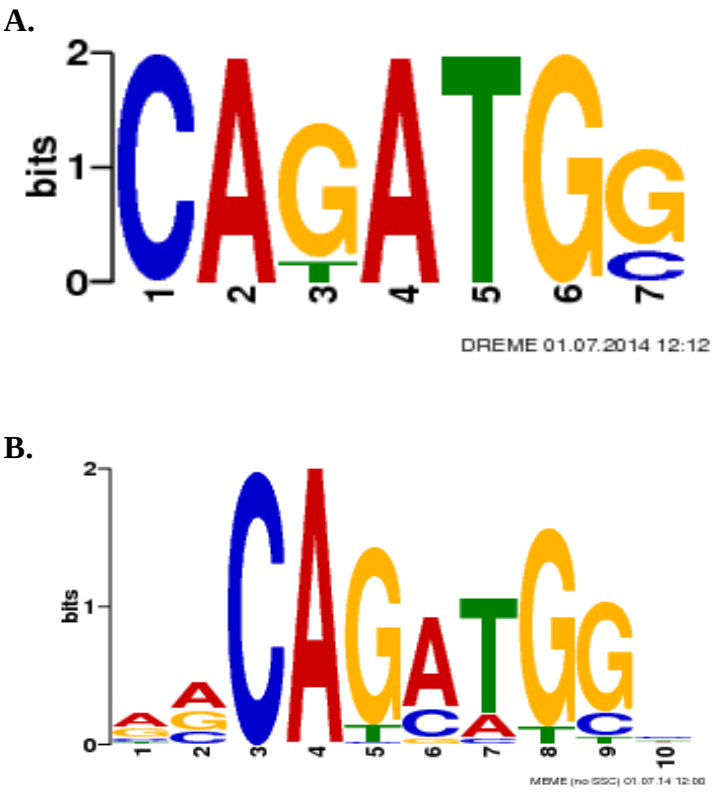


Figure 4.7: NeuroD2 transcription factor binds to "CAGATGG" consensus sequence. Motif analysis was done with **A.** DREME enrichment program (E-value: 7.8e-083) **B.** MEME-ChIP enrichment program (E-value: 3.5e-422).

Chapter 5

DISCUSSION

This study reveals comprehensive direct target gene list of one of the key players in neurogenesis, NeuroD2 basic helix-loop-helix transcription factor under physiological conditions. The findings are important because chromatin immunoprecipitation followed by high throughput sequencing was performed on the brain tissue.

441 binding sites of NeuroD2 transcription factor mapping to transcriptional units and 2000bp upstream regions of TSS with a p-value cutoff of 10^{-5} are identified. 387 binding sites are located in intergenic regions meaning that they have no corresponding transcript present in mouse genome. 12 binding regions represent riken genes those of no information are known except their sequence. NeuroD2 peaks map to 377 unique genes.

Most of the peaks are located in introns rather than promoter regions which were defined as 2 kb upstream of any known transcript in this study. It is not unusual for transcription factors to regulate transcription by binding to introns. Although introns have been defined as “junk DNA” for a long time, many studies point their importance in regulation of transcription [59], [60]. Especially, first introns were shown to be longer and enriched in motifs like TATA box that general transcription factors bind [59]. Also, for some genes such as TNF-α, other than first introns were reported to be required as an enhancer site [61]. Additionally, Fong *et al.* who identified the target genes of NeuroD2 using lentiviral expression of NeuroD2 in P19 cells reported that majority of NeuroD2 peaks were mapped to introns and intergenic regions which is consistent.

Atlas of Developing Human Brain is a web service to visualize expression of genes in human brain development in sixteen structures across the full time course (http://www.brainspan.org/). Web service creates a heatmap showing the genes correlated with a selected transcript with respect to expression time among cortical and subcortical structures. Many genes correlated spatiotemporally with NeuroD2 are found to be direct target genes of NeuroD2. *zbtb18* and *neuroD6* are the most strongly correlated two genes with *neuroD2* gene expression and both of them are found to be direct target genes of NeuroD2. It is interesting that Zbtb18 is important for survival of postmitotic neurons and it is required for neuronal differentiation [62]. Also, Zbtb18 is reported to reprogram fibroblasts into neurons [63]. Since overexpression of NeuroD2 causes P19 cells to adapt neuronal characteristics [25], Zbtb18 can be further suggested as downstream effector of NeuroD2. Xiang *et al.* reported one more protein, Myt1l involving in this reprogramming [63] which is also identified a direct target gene of NeuroD2 in this study.

Moreover, GAP-43 suggested to be activated by NeuroD2 transcription factor [1], [27], is confirmed as direct target. Another study reported that the levels of 27 mRNAs including Mapk were decreased in *neuroD2* knockout mice at the stage of P21 [26]. This study reveals that NeuroD2 protein regulates transcription of *Mapk10* directly at the stage of P0, as well.

To have better understanding of function of NeuroD2 transcription factor through its direct target genes, gene ontology database DAVID is used. Based on enrichment score and p-values, NeuroD2 regulates genes most significantly involved in synapse formation. This finding is well correlated with the fact that *neuroD2* null mice displayed reduced number of dendritic spines in development of hippocampal neuronal connections [34] as well as impaired thalamocortical connections during development due to functional defects in synaptic sites [27].

Further supporting the information obtained from gene ontology, Stim1 protein is identified as the top target gene according to total significance scores from three different antibodies. STIM1 which is a type-I transmembrane protein located on endoplasmic reticulum membrane, is firstly identified in nonexcitable cells [64] and very recently, it is shown to be expressed in high levels in Purkinje neurons [65]. Although the expression of NeuroD2 in Purkinje neurons contradicts with some studies [26], [29], several studies suggest that it is expressed in these neurons [1].

Briefly, STIM1 functions to mediate Ca^{+2} influx through store-operated Ca^{+2} channels by sensing decreased Ca^{+2} level in the ER [64]. Calcium influx is essential to regulate synaptic transmittance. Synapses have the ability to change termed as synaptic plasticity, and synaptic activity regulates this change in terms of synaptic strength between two neurons. Calcium mediates the synaptic plasticity both in brief, short-term by transient signal and in long-term by regulating gene transcription. Early and late long-term potentiation (LTP), and long-term depression (LTD) are the two main features of synaptic plasticity involved in learning and memory and both of them are mediated by calcium signaling [66]–[68]. *stim1* knockout mice displayed defects in synaptic transmission dependent on metabotropic glutamatergic receptor type 1 [65] which is a key player which mediates activity-dependent synaptic plasticity [69].

Moreover, among the target genes, *dlgap1, nrxn1*, *nrxn3* and *znrf1* are important for calcium channel regulation, hence synaptic transmission [70] and exclusively expressed in Purkinje neurons. Also, supporting the previous findings that NeuroD2 is crucial for synaptic maturation [27], [34], eight target genes (*grm5, prkca, camk4, rps6ka2, camk2d, ppp1r12a, grm1, itpr2*) are categorized in long-term potentiation and depression with gene ontology analysis.

It is really interesting that NeuroD2 activated by calcium signal directly regulates many players involved in calcium signaling pathways important for learning

and memory. These findings suggest that NeuroD2 creates a positive feedback loop for maturation of synapses.

Consistent with the gene ontology analyses of Fong *et al.* [53], target genes of NeuroD2 significantly take parts in cell morphogenesis involved in neuronal differentiation including axonogenesis, cell part organization, and cell motion under physiological conditions, as well. Molecular function of 92 genes out of total 332 genes (rest of them were not recognized by DAVID tool) is ion binding including cations like Na^+, K^+ which are important in excitability of neurons and metals like zinc. Some house-keeping genes are also identified as the targets of NeuroD2 protein such as genes involved in glycoprotein biosynthesis processes.

In this study, NeuroD2 comes out to be crucial for neurodevelopmental disorders like autism spectrum disorder, intellectual disability and schizophrenia. These disorders were generally characterized by pathogenic copy number variants caused by deletion or duplication of thousands of bases and also nucleotide substitutions in many genes [71], [72]. Fromer *et al.* conducted the largest exome sequencing to date and they have reported hundreds of genes with *de novo* mutations in three neurodevelopmental disorders [71]. Many genes reported to be mutated in schizophrenia are direct target genes of NeuroD2 including *abca13*, *dnah9*, *nfasc* and *lingo2* [71]. Fromer *et al*. reported mutated genes in autism spectrum disorder as well and among those, *disc1*, *dusp14*, *nrxn1*, *robo1* are direct target genes of NeuroD2 [71]. Moreover, many genes including *abca13*, *lrp1*, *stxbp1* were claimed to be involving in intellectual disability [71] which is a generalized disorder with significantly impaired cognitive functioning and these three genes are identified as target genes of NeuroD2. These genes implicated to be crucial for neurodevelopmental disorders further illustrate that NeuroD2 is a key factor for proper development of human brain. These target genes in the perspective of their regulation mechanisms by NeuroD2 require more detailed research.

NeuroD2 is a basic helix-loop-helix transcription factor and those transcription factors bind a motif, called E-box, a generic "CANNTG" sequence. Motif analysis for the enriched nucleotide sequence shows that NeuroD2 prefers to bind a consensus sequence of "CAGATGG" consistent with the previous findings [53]. Although, Fong *et al.* reported the consensus E-box for NeuroD2 as "CAGATG", both of the used motif analysis program added G nucleotide at the end of this 6-mer as it is highly enriched for this data set.

In summary, direct target genes of NeuroD2 transcription factor at the stage postnatal day 0 are reported in this study. Experiments that will give information about functional relationship between those targets genes and NeuroD2 protein should be the following steps. Moreover, transcription regulation by NeuroD2 is known to be activated by calcium signaling and its target genes should be identified upon neuronal activity in primary neuronal cultures, for instance and compared to those identified *in vivo.* Since the expression of *neuroD2* starts as early as embryonic day 11 [1], ChIP-Seq experiment can be performed with younger animals so that NeuroD2 dependent mechanisms can be analyzed further in the aspect of developmental time.

Future Aspect

To verify and analyze functional relationship between NeuroD2 and its target genes, mRNA levels of targets genes in the presence and in the absence of NeuroD2 can be compared. In this study, these experiments are initiated with the characterization of potent shRNA's to knockdown NeuroD2 and their specificity (Appendix A and B). Lentiviruses containing shRNA's are generated to transduce primary neuron cultures (Appendix C and D). To compare mRNA levels of target genes by quantitative PCR (Q-PCR), these initiated experiments should be further studied to reach high transduction efficiencies in primary neuron cultures.

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VITA

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Appendix A: Determination of NeuroD2 shRNA knockdown efficiency

There were three candidate shRNA vectors that were designed to knock down NeuroD2. 1μg of each candidate shRNA's were transfected to Neuro2A cell line along with 3μg NeuroD2-myc vector using TurboFect reagent. After 24 hours, cells were collected with freshly prepared 1X RIPA lysis buffer containing protease inhibitor tablet.

Knock down of NeuroD2-myc protein was analyzed with western blotting done against myc protein. TurboGFP protein was expressed by shRNA vectors which enable to verify success of transfection efficiency. Non-silencing shRNA was designed such that it does not have target transcript. Therefore, non-silencing shRNA was expected not to knock down NeuroD2 protein. Two of the three shRNAs were able to knock down NeuroD2 protein. Lower beta actin levels were probably due to the toxic effect of TurboFect reagent or the fewer amounts of cells plated for these conditions.

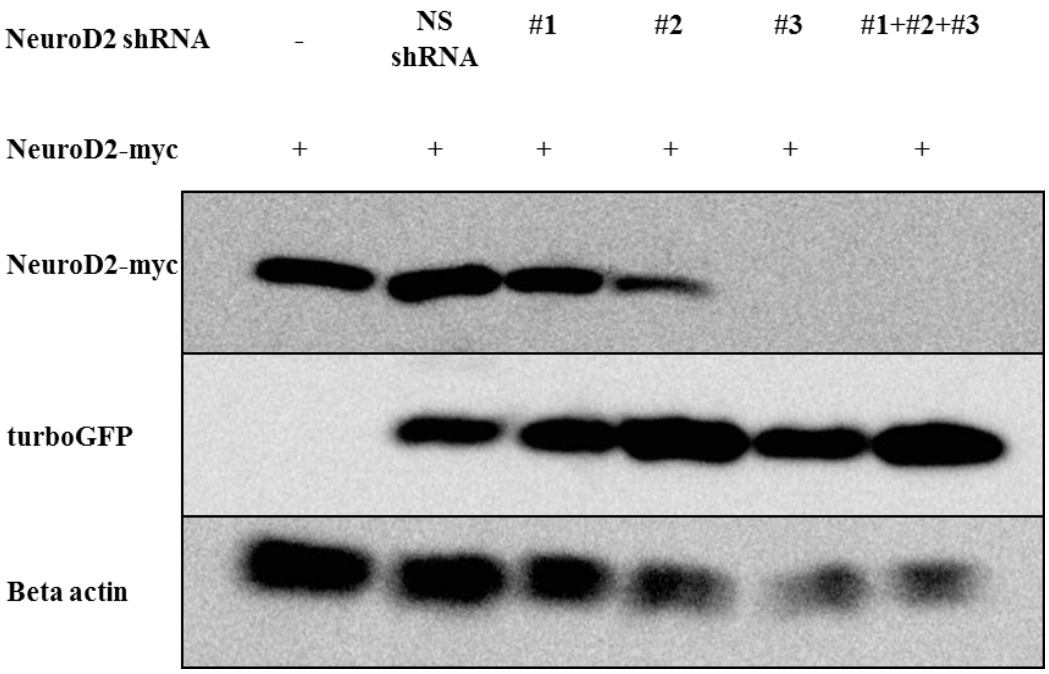


Figure A.1: Neuro2A cells were transfected with NeuroD2-myc vector and candidate shRNA's. 10µl from each sample were loaded on SDS-Page and western blotting was done against myc protein.

Appendix B: Determination of NeuroD2 shRNA specificity

The closest homolog of NeuroD2 protein is NeuroD1 [1], [24]. To verify that NeuroD2 shRNA targets only NeuroD2 but not NeuroD1, NeuroD1 was cloned.

neuroD1 gene consists of one exon and PCR was set up from genomic DNA. PCR was done in 50µl reaction mixture containing 70ng genomic DNA template, 1X Phusion High-Fidelity Buffer, 200µM dNTP's, 0.5µM of both forward and reverse NeuroD1 primers (Appendix G), and 1 Unit Phusion Hot Start Flex DNA Polymerase (New England BioLabs, Cat. #M0535L) as DNA polymerase. PCR conditions for denaturing, annealing and extension were set to 98°C for 10 seconds, 56°C for 25 seconds and 72˚C for 30 seconds respectively and repeated for 30 cycles.

PCR product was purified with EZ-10 Spin Column PCR Purification Kit (Bio Basic Inc. Cat. #BS363). Forward primer for NeuroD1 cloning was designed to contain BamHI restriction enzyme site and reverse primer was to contain EcoRI restriction enzyme site. Digestion was set up for 100ng pcDNA4A and 100ng NeuroD1 in 20µl reaction mixture containing 1X Red Buffer, 1X BSA, 5 Unit EcoRI (Thermo Scientific, Cat. #ER0271), 5 Unit BamHI (Thermo Scientific, Cat. #ER0051). Samples were double-digested at 37°C, overnight. They were again purified with PCR purification kit. Ligation to pcDNA4A backbone vector was set up in 10µl reaction mixture containing 1X T4 Ligase Buffer and 5 Unit T4 DNA Ligase and incubated at room temperature for 2 hours.

Half of the ligation product was transformed to chemically competent DH5α *E.coli* cells. Transformation was achieved using heat shock; cell-plasmid mixture was incubated on ice for 10 minutes and transferred to a water bath at 42°C, incubated there for 60 seconds and then added onto 500ml of LB medium with no selection was added and incubated at 37°C for 60 minutes. Cells were plated on an agar medium plate with ampicillin selection since the vector pcDNA4A had an ampicillin resistant gene.

Mini-prep cultures were incubated at 37°C overnight with constant shaking and plasmid DNA's were purified by GeneJET Plasmid Miniprep Kit (Thermo Scientific, Cat. #K0503). After diagnostic digestion, 500µl of one of the NeuroD1 containing plasmid cultures was picked; maxi-prep culture was started and plasmid DNA containing NeuroD1 was purified with GeneJet Plasmid Maxiprep Kit (Thermo Scientific, Cat. #K0491). Generated construct was validated by direct sequencing.

In order to verify that NeuroD1 transcription factor was not knocked down by NeuroD2 shRNA, 1.5µg non-silencing and NeuroD2 shRNA were transfected along with 1.5µg NeuroD1-myc plasmid to Neuro2A cells. After 48 hours, cells were collected with freshly prepared 1X RIPA lysis buffer containing protease inhibitor tablet.

Knock down of NeuroD1-myc protein was analyzed with western blotting done against myc protein. Success of transfection was verified with the western blotting done against the turboGFP protein. Non-silencing shRNA was expected not to knock down NeuroD1 protein and shRNA #2 was not able to knock down NeuroD1, showing the specificity of shRNA for NeuroD2 protein.

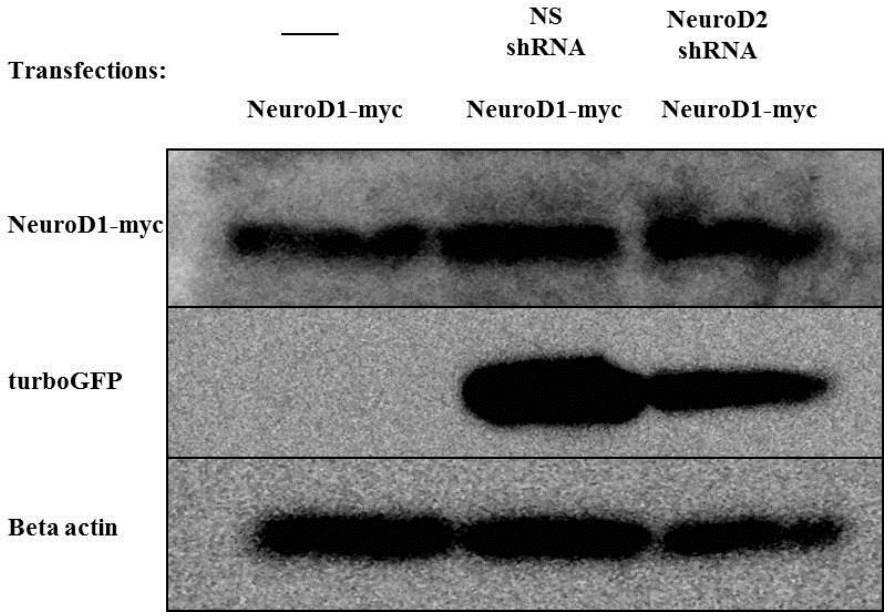


Figure A.2: Neuro2A cells were transfected with NeuroD1-myc and shRNA #2. 10µl of samples were loaded onto SDS-Page and western blotting was done against myc protein.

Appendix C: Generation of lentiviruses

Candidate NeuroD2 shRNA's were purchased in pGIPZ lentivrial constructs (Figure A.3, Thermo Scientific Open Biosystems). To transfect primary cortical neurons in high efficiency as desired, lentiviruses were packaged using HEK293T cells. HEK293T cells were split in proliferating state up to 11^{th} or 12^{th} passages. HEK293T cells were grown in DMEM (Sigma Life Sciences, Cat. #D5671) containing 10% FBS, 1% P/S, 0.5% L-glutamine and 1mM Sodium-pyruvate. Between 2.5 and 3.0 million HEK293T cells were plated in 8ml medium per 100-mm plates. Next day, 225ng of VSV-G, 2250ng of GaqPol viral vectors and 2500ng lentiviral DNA of interest were transfected to HEK293T cells using TurboFect reagent.

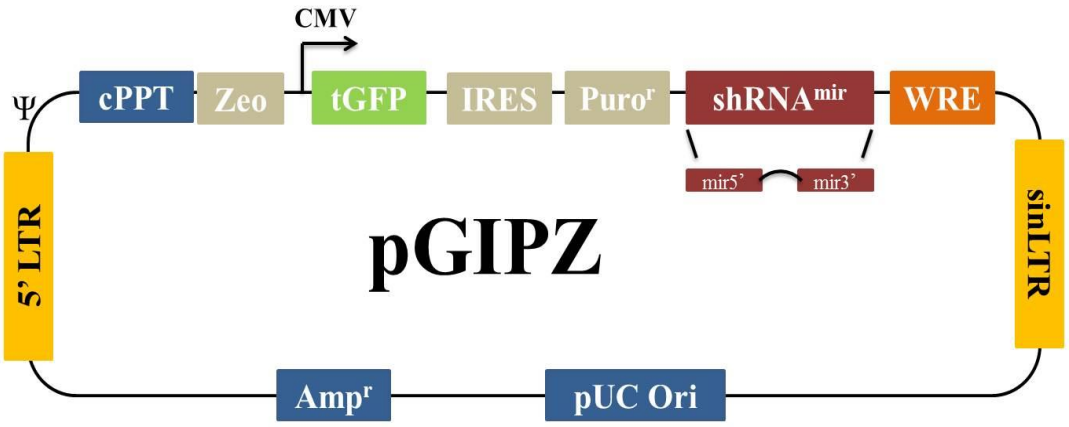


Figure A.3: pGIPZ lentiviral vector

After 48-hours, cells were checked under fluorescence microscope; the vector of shRNAs contains TurboGFP gene, transfected HEK293T cells were expected to be TurboGFP positive. The medium of the plates were transferred into 50-ml falcon tubes, this was called first harvest. New 8ml of medium was added onto them and incubated for additional 24-hours. Second harvests were collected and the HEK293T cells were bleached and disposed. Harvested media were centrifuged at 1400 rpm for 5 minutes at 4°C and the media were filtered through 0.45µm filters to new 50-ml falcon tubes. 50% (w/v) PEG in 1X PBS were added onto media to 20% (v/v) final concentration. These virus containing media were left at 4°C for two days. Precipitates were observed at the bottom of the 50-ml falcon tubes. They were centrifuged at 2500 rpm for 20 minutes at 4°C. Most of the media were removed and they were centrifuged again at 1500 rpm for 5 minutes at 4°C. Rest of the media was removed as much as possible. Onto the pellets, cold 1X PBS was added to make concentrated viruses. They were aliquot in desired volume for further use and stored at -80°C immediately.

Appendix D: Transduction of primary neuron cultures

Coating of Plates

1mg of Poly-D-lysine Hydrobromide (Sigma Life Sciences, Cat. #P9155) and 0.1mg Mouse Laminin (Millipore, Cat. #CC905) were added to 30ml sterile ddH_2O. For 24-well plates, 500µl of the coating solution for one well and for 60-mm plates, 5ml of the coating solution was used. Plates were coated for 12-16 hours in the tissue culture incubator. Next day, plates were washed twice with sterile ddH_2O. They were stored in 4°C for 2-3 weeks or used for culturing immediately.

Primary Neuronal Culture Preparation

When the pregnancy of female mouse reached to 18 days, female was euthanized with CO_2 gas and embryos were removed immediately. Cortices of embryonic mice were dissected in cold 1X HBSS containing 10mM HEPES. Dissected cortices were incubated in the enzyme solution (6.4mg cysteine and 400 units of papain in dissociation media (82mM Na_2SO_4, 30mM K_2SO_4, 5.8mM $MgCl_2$, 0.252mM $CaCl_2$, 1mM HEPES (pH 7.4), 20mM glucose, 0.001% phenol red, 0.2mN NaOH in ddH_2O)) for 15 minutes at 37˚C. Then, the tissues were transferred into the light inhibitor (10mg BSA and 10mg trypsin inhibitor in dissociation media, adjusting the pH with 0.1N NaOH) and incubated for 1 minute at room temperature. Tissues were transferred to heavy inhibitor (100mg BSA and 100mg trypsin inhibitor in dissociation media, adjusting pH with 0.1N NaOH) for another 1 minute. Tissues were washed twice with BME (Lonza, Cat. #BE12-105F) containing 1% P/S, 0.5% L-glutamine, and 5% FBS (BF). Tissues were triturated three times using serological pipette and let tissue clumps to settle down for 1-2 minutes. Upper cell suspension was collected. Cells were counted and 200.000 cells were plated to one well of 24-well plate in 500µl volume and 2 million cells were plated to 60-mm plates in 2.5ml volume in BF. After 3-4 hours, N-2 (Invitrogen, Cat. #17502) supplemented BF media was added in the same volume making one well of 24-well plates 1ml in total and 60-mm plates 5ml in total. The day after, half of the media were sucked and freshly prepared media were added onto plates. This procedure was continued until cells were collected for following analyses.

Viral Transduction of Primary Cortical Cultures

Two hours after neurons were plated onto coated plates, desired amounts of viruses along with 8µg/ml Polybrene was added onto cultures. The following day, virus containing media were removed and N2-supplemented BF media were added. The media of culture were replenished in 1:1 ratios in every two days until they were collected for further analyses.

Appendix E: Determination of primer efficiencies

Total RNA Isolation

Total RNA isolation procedure was performed in clean environment to avoid any contamination. One P0 cortex was used. First, 500µl of Qiazol reagent (Qiagen, Cat. #79306) was added onto tissue and homogenized by grinding, and then 500µl of reagent was added. Tissue was incubated with Qiazol reagent for 5 minutes at room temperature, then, it was transferred to eppendorf tubes. 200µl of chloroform was added onto samples. Samples were mixed vigorously for 15 seconds and left at room temperature for 3 minutes for incubation. Samples were centrifuged at 12000 g for 15 minutes at 4°C. Upper aqueous phase was transferred to new eppendorf tubes and 100µl of isopropanol was added and mixed by vortexing. Later, samples were incubated at room temperature for 10 minutes and centrifuged again at 12000 g for 10 minutes at 4°C. Visible RNA pellet was formed at the bottom of eppendorf tubes. Supernatant was removed and pellet was washed with RNase free 75% ethanol. Samples centrifuged

briefly at 7500 g for 5 minutes at 4°C. Supernatant was discarded and RNA pellet was dissolved in appropriate amount of DNase and RNase free ultrapure water.

cDNA synthesis

Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Cat. #05081955001) was used to synthesize cDNA from 250ng total RNA. Random primers were used to synthesize cDNA according to manufacturer's protocol.

Real Time PCR (Q-PCR)

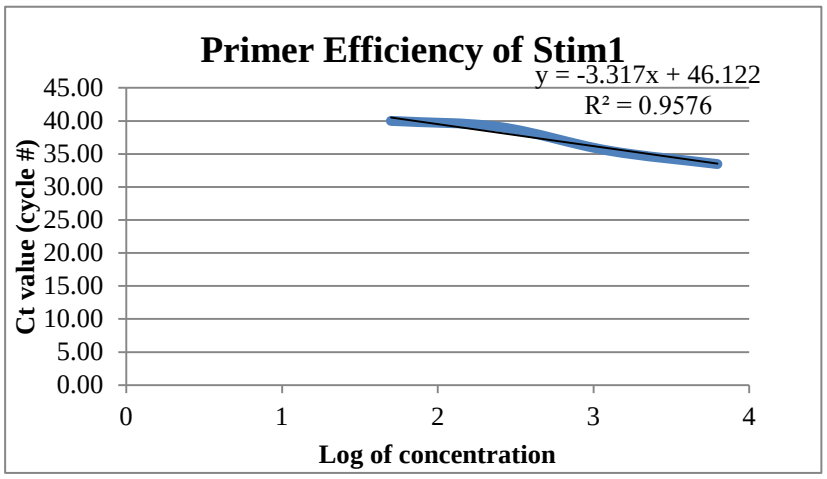
To determine primer efficiency designed for some of the top target genes (Appendix G) of NeuroD2, quantitative Real Time PCR was performed using Luminaris HiGreen qPCR Master Mix (Thermo Scientific, Cat. #K0992) according to manufacturer's recommendations.

Primer efficiencies were calculated by the following formula:

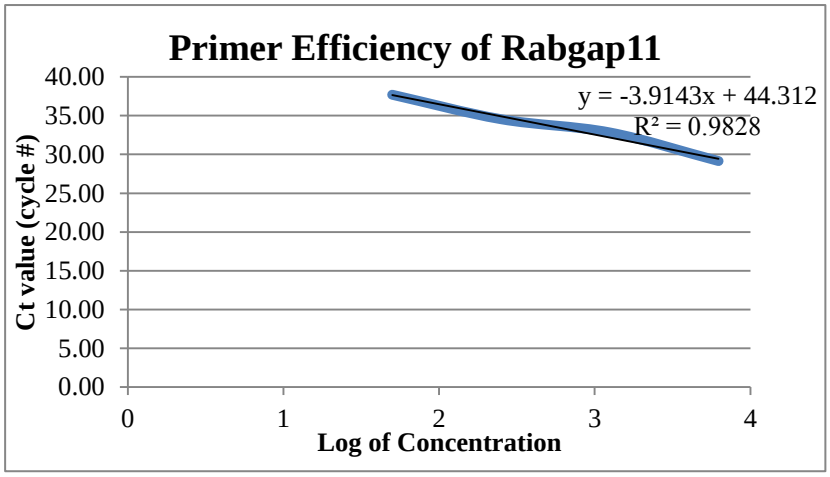
(10^ (-1/m)-1)*100 where;

y=mx+b

A.


Primer Efficiency of Stim1
y = -3.317x + 46.122
R² = 0.9576
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Ct value (cycle #)
0
1
2
3
4
Log of concentration

B.


Primer Efficiency of Rabgap11
y = -3.9143x + 44.312
R² = 0.9828
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Ct value (cycle #)
0
1
2
3
4
Log of Concentration

C.

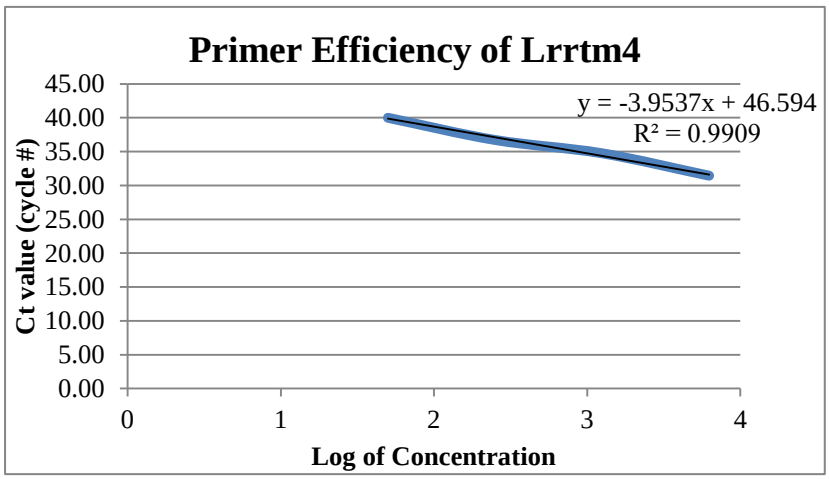


Figure A.4: Primer efficiencies of some of the target genes of NeuroD2 were determined using luminaris HiGreen qPCR master mix. For each primer, cDNA concentrations of 6250pg, 1250 pg, 250pg and 50pg reactions were set up in duplicates. For negative control, water was added instead of cDNA. **A.** Efficiency of primer designed against Stim1 is 100% **B.** Efficiency of primer designed against Rabgap11 is 81% **C.** Efficiency of primer designed against Lrrtm4 is 79%.

Appendix F: Site-Directed Mutagenesis of *neuroD2*

Three point silent mutations were introduced to generate shRNA resistant *neuroD2*. Site-directed mutagenesis PCR was done in 50µl reaction mixture containing 1X Phusion HF Reaction Buffer, 200µM dNTP's, 0.5µM forward and 0.5µM reverse primers (Appendix G), 3% DMSO, 10ng DNA template which is NeuroD2-pcDNA4A plasmid and 1 Unit Phusion Hot Start Flex DNA Polymerase (New England BioLabs, Cat. #M0535L). PCR conditions for denaturing, annealing and extension were set to 98°C for 45 seconds, 60°C for 2 minutes, 72°C for 7 minutes respectively and repeated 20 times. PCR product was purified with PCR purification kit. Primers for the site-directed mutagenesis did not have phosphate group. Therefore phosphorylation reaction was set up in 50µl mixture containing 36ng purified PCR product as DNA template, 1X Polynucleotide Kinase Buffer, 1mM ATP, and 10 Unit Polynucleotide Kinase (Thermo Scientific, Cat. #EK0032). Reaction was incubated at 37°C for 60 minutes and kinase was deactivated at 65°C for 25 minutes. Ligation reaction was set up in 20µl mixture containing 1X T4 Ligation Buffer, 1.4µl of phosphorylation reaction product, and 2.5 Unit T4 DNA Ligase (Thermo Scientific, Cat. #EL0011) and was incubated at room temperature for 2 hours.

Ligation product was transferred to chemically competent DH5α *E.coli* cells by heat-shock method. Plasmid DNA's were purified from the mini-prep cultures and success of site-directed mutagenesis was validated by direct sequencing.

To verify that shRNA resistant *neuroD2* was not knocked, 1.5µg non-silencing shRNA and NeuroD2 shRNA were transfected along with either 1.5µg NeuroD2-myc or resistant NeuroD2-myc plasmid to Neuro2A cells. After 48 hours, cells were collected with freshly prepared 1X RIPA lysis buffer and knocked down of NeuroD2-myc protein and resistant NeuroD2-myc was analyzed with western blotting done against myc protein.

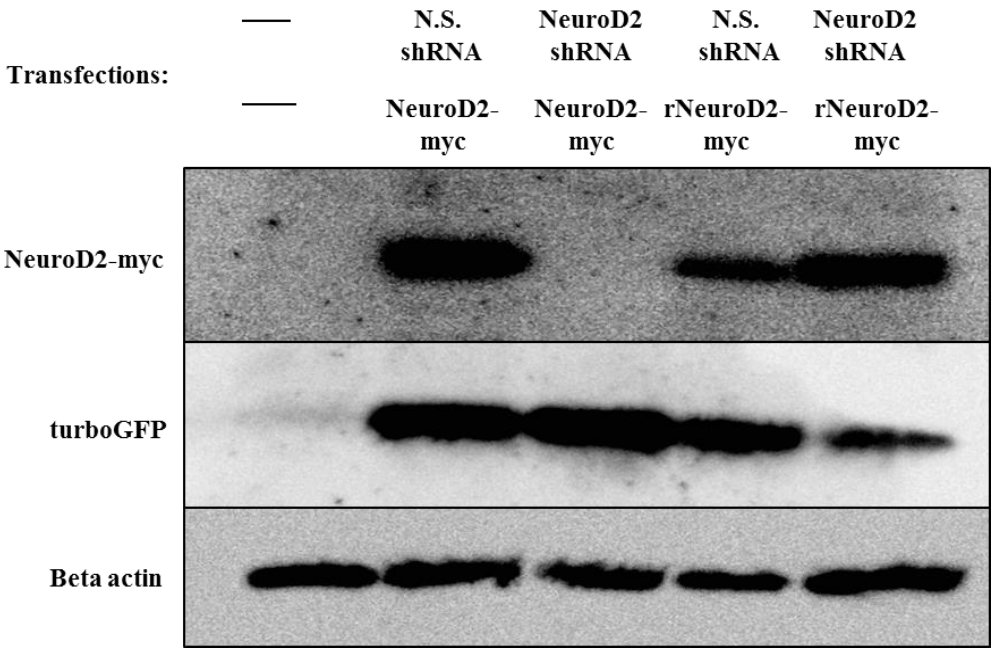


Figure A.5: Neuro2A cells were transfected with NeuroD2-myc or resistant NeuroD2-myc along with non-silencing shRNA and shRNA#2. 10µl of samples were loaded onto SDS-Page and western blotting was done against myc protein.

Appendix G: Primer List

Primer Name	Primer Sequence
NeuroD1-Forward Primer	5’-TTTGGATCCATGACCAAATCATACAGCGA-3’
NeuroD1-Reverse Primer	5’-TTTGAATTCATCGTGAAAGATGGCATTAA-5’
Nhlh2-Forward Primer	5’-CTCACGAACTTCACCCGCAC-3’
Nhlh2-Reverse Primer	5’-AAATTGACTCCTCGGCCCTC-3’
NeuroD2-shRNA resistant-Forward Primer	5’-TTCACCACGATCGGGGCCCCATGTAC-3’
NeuroD2-shRNA resistant-Reverse Primer	5’-GTGGACGCCCCCGCGCACG-3’
Stim1-Forward Primer	5’-CCTCTCTTGACTCGGCATAATC-3’
Stim1-Reverse Primer	5’-GACCTTCTCTACTTCCACAGTTC-3’
Rabgap11-Forward Primer	5’-GGAAGAGAAGAGGAAGCAAGAG-3’
Rabgap11-Reverse Primer	5’-GCTCCTTCCTTGCTGAAGAT-3’
Lrrtm4-Forward Primer	5’-TGCCAGGTTTCCGTCTAATC-3’
Lrrtm4-Reverse Primer	5’-CATCTTCGTCCACTGAGCTAAT-3’

Appendix H: Peak Module used by MACS

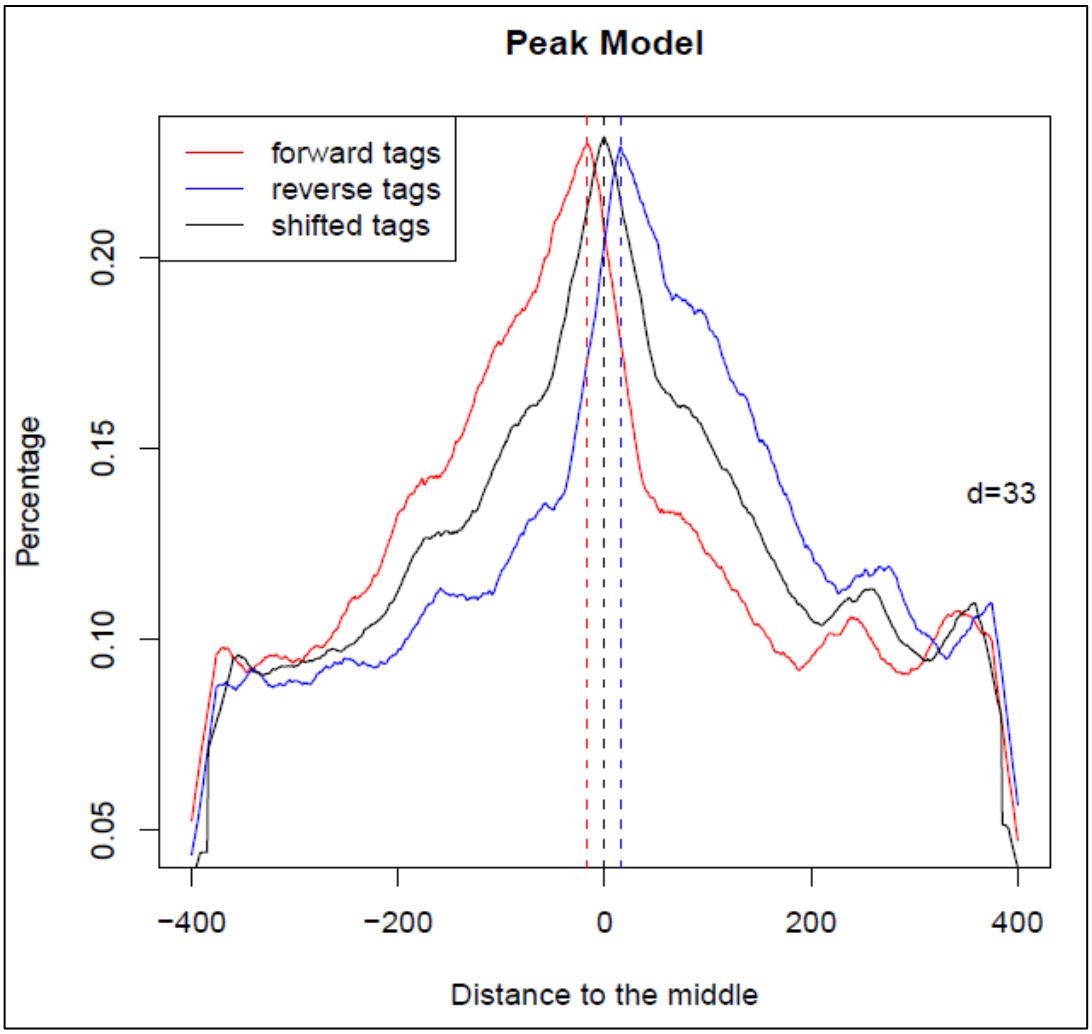


Figure A.6: Peak calling model used by MACS analysis.

Appendix I: Direct target genes of NeuroD2

Promoter Binding					
Gene ID	**Ensemble ID**	**Peak ID**	**Chromosome**	**Merge Midpoint**	**Total score -10*log10(p-value)**
Gm26801	ENSMUST00000180591	Merge_823	chr1	177444580	1830.1
Syt3	ENSMUST00000132399	Merge_438	chr7	44382688	1823.13
Gm11731	ENSMUST00000139704	Merge_159	chr11	117057626	1709.7
Slc35c2	ENSMUST00000156134	Merge_751	chr2	165288643	1566.38
Gm23278	ENSMUST00000157911	Merge_343	chr14	87345468	1315.01
Gm12861	ENSMUST00000145395	Merge_641	chr4	118969854	906.38
Mir876	ENSMUST00000104659	Merge_608	chr4	36647025	867.31
Cmtr1	ENSMUST00000172516	Merge_227	chr17	29680389	843.26
Nsg2	ENSMUST00000109409	Merge_123	chr11	32000452	814.55
Gm15423	ENSMUST00000150600	Merge_820	chr1	176933562	723.01
Prss36	ENSMUST00000156152	Merge_480	chr7	127935948	644.74
Nfia	ENSMUST00000133011	Merge_632	chr4	97911019	634.77
Mlip	ENSMUST00000184322	Merge_870	chr9	77252087	588.31
Lgals3	ENSMUST00000151405	Merge_331	chr14	47367132	576.78
4930529M08Rik	ENSMUST00000144066	Merge_744	chr2	146045563	539.29
Gm16220	ENSMUST00000131874	Merge_189	chr10	75994703	534.6
Mir876	ENSMUST00000104659	Merge_609	chr4	36647244	519.32
Clasp2	ENSMUST00000166734	Merge_889	chr9	113812118	506.95
4931408C20Rik	ENSMUST00000097801	Merge_772	chr1	26687660	445.1
3300002A11Rik	ENSMUST00000126476	Merge_103	chr12	99340205	434.43
Gm16280	ENSMUST00000162458	Merge_197	chr10	86907307	408.84
Slc28a3	ENSMUST00000140760	Merge_57	chr13	58558429	401.9
Mta3	ENSMUST00000176054	Merge_242	chr17	83782929	346.77
Hlcs	ENSMUST00000099512	Merge_277	chr16	94289345	341.54
Sez6	ENSMUST00000138346	Merge_142	chr11	77951025	340.7
Npsr1	ENSMUST00000154644	Merge_846	chr9	24283305	335.33
Gm16220	ENSMUST00000131874	Merge_190	chr10	75995032	319.88
Stxbp1	ENSMUST00000113222	Merge_708	chr2	32817411	315.03
Cd96	ENSMUST00000023336	Merge_265	chr16	46121588	312.6
Gm25614	ENSMUST00000104013	Merge_334	chr14	58651425	233.51

Alg8	ENSMUST00000154107	Merge_458	chr7	97387173	192.19
Intragenic Binding					
Gene ID	**Ensemble ID**	**Peak ID**	**Chromosome #**	**Merge Midpoint**	**Total score= -10*log10(p-value)**
Stim1	ENSMUST00000033289	Merge_461	chr7	102369912	4886.93
Rps6kc1	ENSMUST00000162500	Merge_834	chr1	190872237	3590.04
Erg	ENSMUST00000122199	Merge_278	chr16	95390955	3230.41
Tecr	ENSMUST00000165740	Merge_914	chr8	83584978	3058.53
Ephb1	ENSMUST00000149800	Merge_879	chr9	102039407	2830.58
Mcm9	ENSMUST00000075540	Merge_177	chr10	53612380	2762.02
Col15a1	ENSMUST00000102917	Merge_613	chr4	47291222	2620.75
Myt1l	ENSMUST00000049784	Merge_85	chr12	29663508	2601.01
Nfasc	ENSMUST00000094569	Merge_800	chr1	132591658	2526.01
Ccdc60	ENSMUST00000050178	Merge_569	chr5	116280701	2499.57
Cdkal1	ENSMUST00000006353	Merge_47	chr13	29332826	2497.11
Btbd17	ENSMUST00000141481	Merge_158	chr11	114795453	2459.12
Rps6ka2	ENSMUST00000024575	Merge_217	chr17	7292185	2278.32
Kcnn3	ENSMUST00000000811	Merge_687	chr3	89540118	2130.26
Rabgap1l	ENSMUST00000028049	Merge_814	chr1	160278483	2123.63
Slc9a9	ENSMUST00000033463	Merge_874	chr9	95168636	1993.82
Zdhhc14	ENSMUST00000089185	Merge_215	chr17	5621978	1934.33
Glis3	ENSMUST00000065113	Merge_352	chr19	28436699	1926.15
Fam211a	ENSMUST00000143262	Merge_136	chr11	62625000	1891.26
Sdccag8	ENSMUST00000027785	Merge_821	chr1	176994898	1854.75
Hivep3	ENSMUST00000106307	Merge_642	chr4	119986775	1832.63
1700008O03Rik	ENSMUST00000134535	Merge_438	chr7	44382688	1823.13
Sh3rf3	ENSMUST00000135526	Merge_179	chr10	58924898	1807.03
Nfasc	ENSMUST00000094569	Merge_801	chr1	132650930	1802.55
Auts2	ENSMUST00000182575	Merge_586	chr5	132161487	1701.09
Lrrtm4	ENSMUST00000147663	Merge_516	chr6	80622888	1683.28
Gm3764	ENSMUST00000180635	Merge_686	chr3	88226105	1659.76
Brca1	ENSMUST00000017290	Merge_152	chr11	101526237	1658.31
Grm1	ENSMUST00000105560	Merge_165	chr10	11061881	1626.02
Auts2	ENSMUST00000182575	Merge_584	chr5	132012038	1606.4
Elmo2	ENSMUST00000103091	Merge_751	chr2	165288643	1566.38
Phf20	ENSMUST00000132129	Merge_747	chr2	156233854	1547.96

Camk2d	ENSMUST00000169051	Merge_698	chr3	126764947	1547.45
Cog7	ENSMUST00000057576	Merge_474	chr7	121939843	1530.46
Dgki	ENSMUST00000143046	Merge_497	chr6	36926707	1529.21
Aff3	ENSMUST00000027250	Merge_779	chr1	38438647	1520.96
Krtap16-1	ENSMUST00000105050	Merge_150	chr11	99985836	1497.46
Rere	ENSMUST00000105680	Merge_657	chr4	150550235	1473.15
Ttc28	ENSMUST00000156290	Merge_564	chr5	111058658	1468.1
Macrod1	ENSMUST00000040261	Merge_349	chr19	7148689	1435.04
Pde10a	ENSMUST00000115724	Merge_219	chr17	8702793	1408.03
Pde9a	ENSMUST00000137927	Merge_228	chr17	31418836	1348.95
Myo1d	ENSMUST00000041065	Merge_143	chr11	80551883	1345.47
B3galt5	ENSMUST00000113800	Merge_279	chr16	96249877	1311.46
Diap3	ENSMUST00000022599	Merge_342	chr14	86748995	1291.99
Prex1	ENSMUST00000140624	Merge_754	chr2	166653691	1286.54
Grm7	ENSMUST00000174018	Merge_527	chr6	111373203	1286.05
Rasal1	ENSMUST00000156722	Merge_574	chr5	120656526	1277.54
Arfip1	ENSMUST00000154148	Merge_685	chr3	85160002	1256.95
Tbc1d31	ENSMUST00000110175	Merge_298	chr15	57949485	1240.83
Itpr2	ENSMUST00000053273	Merge_533	chr6	146282253	1231
Prex1	ENSMUST00000036719	Merge_753	chr2	166637005	1204.04
Nfix	ENSMUST00000126806	Merge_915	chr8	84787732	1203.14
Thrb	ENSMUST00000022303	Merge_321	chr14	17900634	1194.55
Myt1l	ENSMUST00000049784	Merge_84	chr12	29639916	1176.86
Pard3b	ENSMUST00000094906	Merge_787	chr1	62216032	1161.79
Ebf1	ENSMUST00000138452	Merge_131	chr11	44626233	1153.2
Glt8d2	ENSMUST00000065815	Merge_193	chr10	82662658	1115.37
Prkca	ENSMUST00000059595	Merge_154	chr11	108081131	1109.9
E330009J07Rik	ENSMUST00000039008	Merge_502	chr6	40432531	1103.72
Sema6d	ENSMUST00000103241	Merge_734	chr2	124524436	1102.94
Smyd3	ENSMUST00000131684	Merge_825	chr1	179184961	1101.74
Poc1a	ENSMUST00000072206	Merge_883	chr9	106327752	1099.67
Slc39a11	ENSMUST00000071539	Merge_157	chr11	113360380	1097.1
Nkain3	ENSMUST00000102998	Merge_605	chr4	20460507	1081.38
Nrxn1	ENSMUST00000173917	Merge_245	chr17	90087006	1078.68
2610035D17Rik	ENSMUST00000127263	Merge_155	chr11	113098600	1072.47
Plxna4	ENSMUST00000115096	Merge_495	chr6	32224449	1055.42

Tenm4	ENSMUST00000107166	Merge_456	chr7	96832777	1040.59
Cacna2d2	ENSMUST00000164988	Merge_884	chr9	107456867	1039.82
Diap1	ENSMUST00000080033	Merge_366	chr18	37880198	1027.38
Glg1	ENSMUST00000169020	Merge_923	chr8	111218287	1006.79
Nfia	ENSMUST00000148930	Merge_631	chr4	97880506	1006.42
Dscam	ENSMUST00000056102	Merge_281	chr16	96886029	998.89
Sema5a	ENSMUST00000067458	Merge_286	chr15	32324311	994.96
Rbfox3	ENSMUST00000103023	Merge_160	chr11	118760711	987.7
Zfp462	ENSMUST00000098070	Merge_615	chr4	55073147	974.9
Auts2	ENSMUST00000182575	Merge_583	chr5	131794726	974.81
Eml6	ENSMUST00000058902	Merge_121	chr11	29774536	967.41
Auts2	ENSMUST00000161108	Merge_585	chr5	132029810	965.51
Clstn2	ENSMUST00000162295	Merge_876	chr9	97638965	964.53
Leprel1	ENSMUST00000039990	Merge_258	chr16	25960526	941.46
Man1c1	ENSMUST00000176606	Merge_648	chr4	134580618	929.57
Pcsk5	ENSMUST00000025618	Merge_350	chr19	17603490	921.67
Gm14066	ENSMUST00000134801	Merge_739	chr2	139311582	918.99
Ppp1r12a	ENSMUST00000070663	Merge_201	chr10	108180538	917.27
Dhrs7	ENSMUST00000021512	Merge_92	chr12	72654833	902.08
Hydin	ENSMUST00000043141	Merge_922	chr8	110319880	890.11
Sh3rf3	ENSMUST00000135526	Merge_182	chr10	58936628	884.73
Gm12068	ENSMUST00000153153	Merge_119	chr11	24631811	884.54
Msra	ENSMUST00000067927	Merge_337	chr14	64173546	877.41
Cntnap2	ENSMUST00000114641	Merge_506	chr6	47231678	872.28
Lingo2	ENSMUST00000108122	Merge_610	chr4	36647475	872.05
Lingo2	ENSMUST00000124999	Merge_608	chr4	36647025	867.31
Dscam	ENSMUST00000056102	Merge_282	chr16	97008415	859.15
Hs3st4	ENSMUST00000106437	Merge_475	chr7	124131466	858.35
Gm15800	ENSMUST00000042614	Merge_575	chr5	121240053	845.92
Cmtr1	ENSMUST00000024816	Merge_227	chr17	29680389	843.26
Vcl	ENSMUST00000022369	Merge_324	chr14	20971161	840.17
Pard3	ENSMUST00000162536	Merge_938	chr8	127108093	838.64
Mad1l1	ENSMUST00000128579	Merge_591	chr5	140098906	835.93
Pitpnm3	ENSMUST00000075258	Merge_140	chr11	72067481	824.44
Wwox	ENSMUST00000160862	Merge_928	chr8	114591435	822.94
Dlgap2	ENSMUST00000150247	Merge_898	chr8	14473018	815.87

B4galt5	ENSMUST00000109221	Merge_755	chr2	167317110	815.51
Myrip	ENSMUST00000048121	Merge_892	chr9	120444850	811.69
Slc2a9	ENSMUST00000129099	Merge_548	chr5	38388601	805.4
Grid1	ENSMUST00000043349	Merge_328	chr14	34944803	799.23
Nek10	ENSMUST00000136826	Merge_320	chr14	14984312	797.43
Dock1	ENSMUST00000084488	Merge_484	chr7	134682866	797.14
Hs3st4	ENSMUST00000106437	Merge_476	chr7	124358259	793.37
Syt17	ENSMUST00000081574	Merge_471	chr7	118429166	787.51
Tcte2	ENSMUST00000135850	Merge_220	chr17	13584058	785.08
Ccbe1	ENSMUST00000061103	Merge_379	chr18	66082416	778
Fat3	ENSMUST00000082170	Merge_845	chr9	16345423	776.28
Nrxn3	ENSMUST00000057634	Merge_99	chr12	89377484	767.24
Dgki	ENSMUST00000146656	Merge_498	chr6	37038671	765.42
Slc26a9	ENSMUST00000049027	Merge_798	chr1	131770774	762.76
Erc2	ENSMUST00000090302	Merge_326	chr14	28387289	760.51
Mapk10	ENSMUST00000112847	Merge_561	chr5	102953635	756.94
Cachd1	ENSMUST00000030257	Merge_633	chr4	100945507	754.85
Mcc	ENSMUST00000089874	Merge_370	chr18	44580536	738.3
Cdkn3	ENSMUST00000067426	Merge_330	chr14	46767305	735.79
Nckap5	ENSMUST00000094609	Merge_796	chr1	126154011	733.59
Sntb1	ENSMUST00000039769	Merge_297	chr15	55652252	732.36
Npas3	ENSMUST00000101432	Merge_87	chr12	53802013	732.11
Plb1	ENSMUST00000101376	Merge_540	chr5	32335319	731.31
Gm26561	ENSMUST00000180743	Merge_236	chr17	70932097	727.83
Sdccag8	ENSMUST00000133305	Merge_820	chr1	176933562	723.01
Kcnq3	ENSMUST00000070256	Merge_303	chr15	66239364	721.55
Gm26861	ENSMUST00000180807	Merge_43	chr13	10527461	720.16
Slc15a2	ENSMUST00000164579	Merge_261	chr16	36775833	716.17
Stx6	ENSMUST00000027743	Merge_811	chr1	155193191	709.49
Tnik	ENSMUST00000161964	Merge_671	chr3	28457628	706.9
Csmd1	ENSMUST00000131778	Merge_900	chr8	17465607	703.96
Dst	ENSMUST00000182697	Merge_778	chr1	34041575	698.95
Mpp7	ENSMUST00000115869	Merge_357	chr18	7608953	696.98
Dpysl5	ENSMUST00000114729	Merge_539	chr5	30728952	696.45
Trak1	ENSMUST00000045903	Merge_893	chr9	121398737	695.23
Gadl1	ENSMUST00000121770	Merge_890	chr9	115943298	694.25

Rgs6	ENSMUST00000161801	Merge_97	chr12	82921549	692.16
Atp9a	ENSMUST00000140188	Merge_758	chr2	168678877	689.25
Neurl1a	ENSMUST00000111808	Merge_353	chr19	47211878	688.35
Scaper	ENSMUST00000037408	Merge_861	chr9	55710812	688.24
Ctbp2	ENSMUST00000166439	Merge_483	chr7	133003616	685.48
Creb3l2	ENSMUST00000041093	Merge_500	chr6	37339158	672.31
Tph1	ENSMUST00000049298	Merge_439	chr7	46648635	671.14
Adam18	ENSMUST00000173833	Merge_901	chr8	24627022	667.09
Dmgdh	ENSMUST00000048001	Merge_65	chr13	93722034	666.32
Nupl1	ENSMUST00000041905	Merge_335	chr14	60220076	665.83
Sorcs2	ENSMUST00000037370	Merge_547	chr5	36313616	660.63
Ncor2	ENSMUST00000111393	Merge_576	chr5	125129156	657.06
Disc1	ENSMUST00000121953	Merge_934	chr8	125081206	654.25
Frmd4b	ENSMUST00000032146	Merge_521	chr6	97462532	653.67
Abat	ENSMUST00000115838	Merge_252	chr16	8595580	653.1
2810471M01Rik	ENSMUST00000146554	Merge_120	chr11	28692989	648.35
Gap43	ENSMUST00000102817	Merge_263	chr16	42255796	646.12
Prss36	ENSMUST00000150591	Merge_480	chr7	127935948	644.74
Psd3	ENSMUST00000150169	Merge_911	chr8	68044977	644.53
9630028H03Rik	ENSMUST00000153402	Merge_737	chr2	135541241	643.71
Atf7	ENSMUST00000184772	Merge_317	chr15	102584273	642.12
Nav3	ENSMUST00000032719	Merge_202	chr10	109684713	641.87
Gm26739	ENSMUST00000181041	Merge_931	chr8	120598705	637.19
Pex5l	ENSMUST00000108219	Merge_672	chr3	33000524	636.74
Sh3rf3	ENSMUST00000135526	Merge_180	chr10	58925268	635.9
Tbc1d5	ENSMUST00000024717	Merge_232	chr17	50763563	635.43
Nfia	ENSMUST00000107057	Merge_632	chr4	97911019	634.77
Rad23a	ENSMUST00000144675	Merge_916	chr8	84835211	633.59
Stat1	ENSMUST00000070968	Merge_782	chr1	52150200	632.37
Dnah9	ENSMUST00000080665	Merge_138	chr11	65905671	630.25
Lonp2	ENSMUST00000121673	Merge_917	chr8	86713971	629.9
Disp1	ENSMUST00000003035	Merge_826	chr1	183181194	626.78
Arpp21	ENSMUST00000160240	Merge_886	chr9	112102636	626.41
Pde5a	ENSMUST00000066728	Merge_696	chr3	122814709	623.75
Col14a1	ENSMUST00000110221	Merge_296	chr15	55418052	620.81
Hs6st3	ENSMUST00000065904	Merge_347	chr14	119789790	619.82

Atp9a	ENSMUST00000109176	Merge_757	chr2	168677887	618.76
Pkib	ENSMUST00000177299	Merge_178	chr10	57675889	617.02
Nrg3	ENSMUST00000166968	Merge_329	chr14	39044021	616.81
Rph3al	ENSMUST00000066504	Merge_141	chr11	75869991	613.7
E130114P18Rik	ENSMUST00000126721	Merge_630	chr4	97575686	613.07
Abca13	ENSMUST00000042740	Merge_116	chr11	9269268	612.59
Itgb2l	ENSMUST00000131567	Merge_280	chr16	96439143	611.19
Tmem178b	ENSMUST00000180886	Merge_501	chr6	40071195	609.92
Gse1	ENSMUST00000034279	Merge_930	chr8	120557686	608.84
Mpp7	ENSMUST00000115869	Merge_358	chr18	7609315	604.84
Lama2	ENSMUST00000092639	Merge_170	chr10	27104161	603.67
Rtn1	ENSMUST00000078505	Merge_91	chr12	72285661	599.54
Cux1	ENSMUST00000176745	Merge_589	chr5	136486651	598.71
Grip1	ENSMUST00000147356	Merge_207	chr10	120038445	597.97
Tmem241	ENSMUST00000092075	Merge_359	chr18	12120618	590.89
Nek10	ENSMUST00000136826	Merge_319	chr14	14984064	590.07
Robo1	ENSMUST00000023600	Merge_273	chr16	72795182	589.76
Mlip	ENSMUST00000184848	Merge_870	chr9	77252087	588.31
Dock1	ENSMUST00000084488	Merge_485	chr7	134683265	587.37
Tfap4	ENSMUST00000005862	Merge_247	chr16	4559662	586.21
Asic2	ENSMUST00000066197	Merge_144	chr11	81767858	585.06
Ppp1r16b	ENSMUST00000045503	Merge_748	chr2	158673784	580.65
Tnrc18	ENSMUST00000152247	Merge_595	chr5	142761578	580.15
Tenm4	ENSMUST00000107165	Merge_455	chr7	96474855	578.54
Cacng4	ENSMUST00000021066	Merge_153	chr11	107784310	576.83
Rps6ka5	ENSMUST00000043599	Merge_104	chr12	100665466	576.51
Alk	ENSMUST00000086639	Merge_238	chr17	72015506	575.07
Grxcr1	ENSMUST00000094715	Merge_553	chr5	68072065	570.82
Heg1	ENSMUST00000126532	Merge_260	chr16	33733155	569.25
Deptor	ENSMUST00000100660	Merge_295	chr15	55137565	561.82
Cd40	ENSMUST00000017799	Merge_750	chr2	165069086	560.39
A330043C09Rik	ENSMUST00000139823	Merge_725	chr2	75151206	556.68
Clstn2	ENSMUST00000162295	Merge_875	chr9	97534364	552.22
Kcnq3	ENSMUST00000070256	Merge_302	chr15	66115350	551.49
Dock1	ENSMUST00000084488	Merge_486	chr7	134799794	549.69
Dnm3	ENSMUST00000161155	Merge_815	chr1	162334977	546.94

Etv5	ENSMUST00000079601	Merge_255	chr16	22430461	545.8
Khdrbs2	ENSMUST00000027226	Merge_774	chr1	32645168	543.87
Ush2a	ENSMUST00000124358	Merge_829	chr1	188385117	543.04
Grm1	ENSMUST00000105561	Merge_164	chr10	10942465	541.29
4930529M08Rik	ENSMUST00000125223	Merge_744	chr2	146045563	539.29
Camk2d	ENSMUST00000149311	Merge_697	chr3	126725825	535.03
Gm5134	ENSMUST00000134234	Merge_189	chr10	75994703	534.6
Dpp6	ENSMUST00000071500	Merge_538	chr5	27203298	532.95
Glt8d2	ENSMUST00000020485	Merge_194	chr10	82666628	530.81
Mrvi1	ENSMUST00000154466	Merge_463	chr7	110953028	527.54
Gm16308	ENSMUST00000162171	Merge_307	chr15	71810527	524.82
Fam192a	ENSMUST00000034226	Merge_920	chr8	94584514	524.44
Cbfa2t2	ENSMUST00000099178	Merge_745	chr2	154460188	523.89
Rps6kc1	ENSMUST00000159367	Merge_835	chr1	190872866	523.61
Eif3h	ENSMUST00000022925	Merge_294	chr15	51834332	519.82
Msra	ENSMUST00000067927	Merge_336	chr14	64140304	519.46
Lingo2	ENSMUST00000124999	Merge_609	chr4	36647244	519.32
Dusp14	ENSMUST00000108101	Merge_145	chr11	84048588	516.25
Ccdc33	ENSMUST00000098682	Merge_862	chr9	58035750	515.81
Tcte2	ENSMUST00000135850	Merge_224	chr17	13655005	514.88
Susd1	ENSMUST00000040166	Merge_617	chr4	59383986	508.13
Gm20388	ENSMUST00000127664	Merge_932	chr8	122931942	502.96
Gm973	ENSMUST00000114243	Merge_786	chr1	59535524	501.8
Ebf3	ENSMUST00000033378	Merge_487	chr7	137272390	499.65
Afap1	ENSMUST00000141824	Merge_546	chr5	35925384	496.41
Vat1l	ENSMUST00000049509	Merge_927	chr8	114306452	495.85
Exoc4	ENSMUST00000052266	Merge_496	chr6	33499936	492.93
Ncald	ENSMUST00000153775	Merge_291	chr15	37534342	491.78
Atp2b2	ENSMUST00000135199	Merge_528	chr6	113857070	491.21
Disc1	ENSMUST00000117658	Merge_933	chr8	125080629	490.17
Kcnip1	ENSMUST00000101368	Merge_124	chr11	33772877	488.37
Zfyve28	ENSMUST00000094868	Merge_541	chr5	34197404	485.33
Csmd2	ENSMUST00000144298	Merge_645	chr4	128399121	484.53
Dusp14	ENSMUST00000100705	Merge_146	chr11	84053915	483.13
L3mbtl4	ENSMUST00000093007	Merge_234	chr17	68422870	476.88
Afap1	ENSMUST00000141824	Merge_545	chr5	35918584	470.77

Osbpl5	ENSMUST00000134056	Merge_489	chr7	143748598	466.99
Ttc25	ENSMUST00000132143	Merge_151	chr11	100564445	465.46
Enox1	ENSMUST00000022589	Merge_340	chr14	77639902	461.9
Fmn1	ENSMUST00000102547	Merge_732	chr2	113682310	459.92
Camk4	ENSMUST00000042868	Merge_363	chr18	32955161	457.91
Lars2	ENSMUST00000038863	Merge_894	chr9	123384825	456.51
Slc39a11	ENSMUST00000106633	Merge_156	chr11	113266957	455.45
2010107G23Rik	ENSMUST00000027719	Merge_183	chr10	62128517	453.7
Rspo1	ENSMUST00000030687	Merge_643	chr4	125008893	452.42
B3galt1	ENSMUST00000180887	Merge_723	chr2	68020339	451.77
Ush2a	ENSMUST00000142159	Merge_830	chr1	188616806	448.84
Grk4	ENSMUST00000001112	Merge_542	chr5	34729328	448.46
Fat3	ENSMUST00000082170	Merge_844	chr9	16036573	446.82
Nek11	ENSMUST00000038648	Merge_881	chr9	105349291	443.11
Robo1	ENSMUST00000023600	Merge_274	chr16	72888389	442.85
Atxn10	ENSMUST00000163242	Merge_312	chr15	85403523	441.39
Phlpp1	ENSMUST00000061047	Merge_793	chr1	106202147	438.74
Nuak1	ENSMUST00000020220	Merge_196	chr10	84387560	436.66
Prim2	ENSMUST00000027312	Merge_775	chr1	33522074	435.91
Ak4	ENSMUST00000131397	Merge_634	chr4	101450536	434.98
Foxn3	ENSMUST00000046859	Merge_103	chr12	99340205	434.43
Kif21b	ENSMUST00000130864	Merge_806	chr1	136137937	432.55
Gpatch2l	ENSMUST00000071106	Merge_98	chr12	86277058	427
Add2	ENSMUST00000032069	Merge_517	chr6	86103119	426.78
Chrna7	ENSMUST00000032738	Merge_445	chr7	63170596	426.65
Kcnab1	ENSMUST00000159525	Merge_683	chr3	65322678	423.63
Nek10	ENSMUST00000112630	Merge_318	chr14	14983818	419.83
Dner	ENSMUST00000049126	Merge_791	chr1	84523106	415.98
Chd7	ENSMUST00000170391	Merge_601	chr4	8850685	414.31
Kcnh8	ENSMUST00000039366	Merge_233	chr17	52920919	413.58
Stab2	ENSMUST00000035288	Merge_197	chr10	86907307	408.84
Kctd21	ENSMUST00000054107	Merge_457	chr7	97339848	405.9
Chrna7	ENSMUST00000032738	Merge_444	chr7	63164802	405.89
Lamc1	ENSMUST00000027752	Merge_809	chr1	153309103	404.8
Col27a1	ENSMUST00000183913	Merge_618	chr4	63291267	403.46
Slc28a3	ENSMUST00000022036	Merge_57	chr13	58558429	401.9

Arf1	ENSMUST00000061242	Merge_135	chr11	59217144	401.2
Nav2	ENSMUST00000183659	Merge_441	chr7	49272628	400.8
Snx8	ENSMUST00000031539	Merge_592	chr5	140371813	395.2
Gabra2	ENSMUST00000000572	Merge_554	chr5	70995788	394.32
Ttc28	ENSMUST00000156290	Merge_563	chr5	111015108	393.82
Dlgap1	ENSMUST00000148486	Merge_235	chr17	70630113	387.27
Neurod6	ENSMUST00000044767	Merge_513	chr6	55681229	386.14
Etv6	ENSMUST00000164648	Merge_531	chr6	134219540	386.03
Elf2	ENSMUST00000091144	Merge_678	chr3	51289299	384.66
Sclt1	ENSMUST00000026866	Merge_675	chr3	41692675	383.71
Kif5c	ENSMUST00000028102	Merge_715	chr2	49672629	383.13
Garnl3	ENSMUST00000139778	Merge_709	chr2	33015037	381.75
Wbscr17	ENSMUST00000086023	Merge_582	chr5	131222916	381.66
Elmod1	ENSMUST00000048409	Merge_860	chr9	53916198	379.26
Dstyk	ENSMUST00000045110	Merge_799	chr1	132430981	376.6
Sfswap	ENSMUST00000053737	Merge_581	chr5	129534725	374.37
Med13l	ENSMUST00000100816	Merge_572	chr5	118576951	372.52
Ric3	ENSMUST00000147580	Merge_462	chr7	109039636	369.84
Upp2	ENSMUST00000071543	Merge_720	chr2	58586494	369.61
Gm17566	ENSMUST00000110279	Merge_832	chr1	190213365	368.75
Pcdh15	ENSMUST00000155701	Merge_187	chr10	73930986	368.5
Ebf1	ENSMUST00000109268	Merge_132	chr11	44978017	368.46
Pacsin2	ENSMUST00000056177	Merge_309	chr15	83447918	367.55
Cblb	ENSMUST00000114471	Merge_269	chr16	52134750	366.59
Uqcrc2	ENSMUST00000148958	Merge_473	chr7	120648037	363.72
Tcte2	ENSMUST00000135850	Merge_225	chr17	13681311	363.02
Ush2a	ENSMUST00000060479	Merge_831	chr1	188682104	358.45
Ppp2r1b	ENSMUST00000175645	Merge_857	chr9	50861447	356.29
Dst	ENSMUST00000183034	Merge_777	chr1	33947260	355.32
Tg	ENSMUST00000163495	Merge_304	chr15	66843064	354.45
Spon1	ENSMUST00000046687	Merge_465	chr7	113972786	354.21
Znrf1	ENSMUST00000173922	Merge_924	chr8	111615019	353.89
Gm17333	ENSMUST00000169531	Merge_276	chr16	77851764	353.37
Coro2b	ENSMUST00000048043	Merge_867	chr9	62477252	350.3
Cntnap2	ENSMUST00000114641	Merge_505	chr6	46704703	350
Polr1d	ENSMUST00000110557	Merge_597	chr5	147085481	349.41

Gm15958	ENSMUST00000143568	Merge_380	chr18	66299011	349.18
Nfib	ENSMUST00000107248	Merge_626	chr4	82416121	348.54
Rufy2	ENSMUST00000143726	Merge_184	chr10	63004780	348.46
Ptprn2	ENSMUST00000070733	Merge_108	chr12	116623195	347.19
Mta3	ENSMUST00000112350	Merge_242	chr17	83782929	346.77
Plekha8	ENSMUST00000119706	Merge_510	chr6	54642170	344.93
Ston2	ENSMUST00000164713	Merge_100	chr12	91709818	344.72
Nlrc3	ENSMUST00000180200	Merge_246	chr16	3974768	344.33
Arhgap15	ENSMUST00000112824	Merge_713	chr2	44029545	342.96
Hlcs	ENSMUST00000163193	Merge_277	chr16	94289345	341.54
Dok5	ENSMUST00000029075	Merge_760	chr2	170855328	340.77
Sez6	ENSMUST00000093995	Merge_142	chr11	77951025	340.7
Sipa1l3	ENSMUST00000182011	Merge_435	chr7	29425498	340.42
Chia	ENSMUST00000079132	Merge_693	chr3	106131919	339.84
Arhgap26	ENSMUST00000149112	Merge_367	chr18	38641741	335.54
Ablim3	ENSMUST00000049378	Merge_375	chr18	61845391	335.53
Npsr1	ENSMUST00000059650	Merge_846	chr9	24283305	335.33
Prex1	ENSMUST00000099080	Merge_752	chr2	166633146	332.44
Sox6	ENSMUST00000166877	Merge_468	chr7	115966359	332.17
Cpa6	ENSMUST00000035577	Merge_768	chr1	10452303	331.44
41699	ENSMUST00000110258	Merge_910	chr8	66203919	329.54
Laptm4b	ENSMUST00000022867	Merge_287	chr15	34281524	329.11
Eml6	ENSMUST00000058902	Merge_122	chr11	29924546	326.66
Gm5134	ENSMUST00000134234	Merge_190	chr10	75995032	319.88
Fgfr2	ENSMUST00000124096	Merge_482	chr7	132281970	318.08
Lrp1	ENSMUST00000049149	Merge_211	chr10	127558026	317.99
Pvt1	ENSMUST00000181416	Merge_300	chr15	62295577	317.65
Stxbp1	ENSMUST00000050000	Merge_708	chr2	32817411	315.03
Efcab6	ENSMUST00000156187	Merge_310	chr15	83997708	314.17
Dab1	ENSMUST00000106827	Merge_637	chr4	104439852	312.87
Cntfr	ENSMUST00000084701	Merge_611	chr4	41682079	312.4
Frmd4a	ENSMUST00000091497	Merge_704	chr2	4267630	312.19
Magi1	ENSMUST00000055224	Merge_520	chr6	94150021	307.13
Nfasc	ENSMUST00000163770	Merge_802	chr1	132659776	306.94
Krtap4-7	ENSMUST00000055121	Merge_148	chr11	99643811	305.84
Dgki	ENSMUST00000090314	Merge_499	chr6	37119444	302.95

Tubb2b	ENSMUST00000075774	Merge_48	chr13	34129606	302.19
Sh3rf3	ENSMUST00000153031	Merge_181	chr10	58925454	297.26
Col23a1	ENSMUST00000102765	Merge_133	chr11	51391634	296.08
Bnc2	ENSMUST00000176418	Merge_628	chr4	84633569	295.99
Fgf1	ENSMUST00000131348	Merge_368	chr18	38839016	291.43
Rhobtb1	ENSMUST00000167286	Merge_185	chr10	69155988	290.09
Gm26561	ENSMUST00000180743	Merge_237	chr17	70932813	288.53
Slc26a9	ENSMUST00000147800	Merge_797	chr1	131751987	288.03
Npl	ENSMUST00000041874	Merge_810	chr1	153507255	287.74
Gm17202	ENSMUST00000171723	Merge_562	chr5	107415240	287.59
Dlgap2	ENSMUST00000133298	Merge_899	chr8	14583769	284.94
Crtc3	ENSMUST00000122255	Merge_453	chr7	80688003	283.06
Tbc1d16	ENSMUST00000036113	Merge_162	chr11	119209600	282.96
Dock9	ENSMUST00000100299	Merge_348	chr14	121739269	282.72
Tiam2	ENSMUST00000169838	Merge_214	chr17	3331747	277.46
Slc4a10	ENSMUST00000054484	Merge_721	chr2	62294171	276.89
Htra1	ENSMUST00000153290	Merge_481	chr7	130954494	271.83
Ralgps1	ENSMUST00000042615	Merge_710	chr2	33347751	269.05
Clic4	ENSMUST00000037099	Merge_649	chr4	135240484	265.15
Slc25a13	ENSMUST00000169197	Merge_490	chr6	6167876	264.89
Col27a1	ENSMUST00000036300	Merge_619	chr4	63295818	261.19
Negr1	ENSMUST00000041425	Merge_701	chr3	156690434	259.98
Ccdc112	ENSMUST00000072835	Merge_372	chr18	46284530	257.66
Tbc1d9	ENSMUST00000034145	Merge_913	chr8	83177007	252.16
Prim2	ENSMUST00000027312	Merge_776	chr1	33522615	249.56
Frmd4a	ENSMUST00000175669	Merge_705	chr2	4437089	247.41
Fat3	ENSMUST00000082170	Merge_843	chr9	16029250	247.32
Apbb2	ENSMUST00000160870	Merge_552	chr5	66437044	246.95
Lypd6	ENSMUST00000053208	Merge_716	chr2	50179296	243.03
Sorbs2	ENSMUST00000149752	Merge_908	chr8	45715472	241.8
Schip1	ENSMUST00000029346	Merge_684	chr3	68246343	234
Mapkap1	ENSMUST00000113126	Merge_711	chr2	34531881	233.05
Ablim2	ENSMUST00000114206	Merge_544	chr5	35879413	232.44
Pdss2	ENSMUST00000162008	Merge_173	chr10	43420879	232.35
Gm19402	ENSMUST00000180297	Merge_191	chr10	77690550	230.22
Sv2b	ENSMUST00000085164	Merge_449	chr7	75252422	229.28

Tcte2	ENSMUST00000135850	Merge_221	chr17	13585591	221.33
Rgs6	ENSMUST00000161801	Merge_96	chr12	82687195	219.35
2810055G20Rik	ENSMUST00000183333	Merge_275	chr16	77237100	217.26
Bai3	ENSMUST00000146592	Merge_769	chr1	25166160	214.61
Grm5	ENSMUST00000107263	Merge_454	chr7	87787781	211.9
Snx9	ENSMUST00000002436	Merge_216	chr17	5886201	209.61
Enox1	ENSMUST00000022589	Merge_339	chr14	77569778	209.03
Fli1	ENSMUST00000016231	Merge_850	chr9	32491676	208.38
Fam135b	ENSMUST00000022953	Merge_306	chr15	71622977	199.66
Ncald	ENSMUST00000148652	Merge_290	chr15	37397953	198.37
Alg8	ENSMUST00000147297	Merge_458	chr7	97387173	192.19
Fmn1	ENSMUST00000081349	Merge_731	chr2	113662439	191.95
Wwc1	ENSMUST00000018993	Merge_127	chr11	35857630	189.29
1700091E21Rik	ENSMUST00000127716	Merge_377	chr18	64220462	188.48
Ptprt	ENSMUST00000109441	Merge_749	chr2	161662157	186.73
Cd244	ENSMUST00000004829	Merge_819	chr1	171568697	185.42
Magi1	ENSMUST00000093769	Merge_519	chr6	93782778	178.75
Stam2	ENSMUST00000127316	Merge_718	chr2	52731016	176.91
Intergenic Binding					
Gene ID	**Ensemble ID**	**Peak ID**	**Chromosome #**	**Merge Midpoint**	**Total score - 10*log10(p-value)**
-	-	Merge_101	chr12	93981515	198.88
-	-	Merge_102	chr12	97156934	342.62
-	-	Merge_105	chr12	112437088	1027.18
-	-	Merge_106	chr12	112440183	602.78
-	-	Merge_117	chr11	12503865	484.61
-	-	Merge_118	chr11	19899831	630.3
-	-	Merge_123	chr11	32000452	814.55
-	-	Merge_128	chr11	35992093	568.05
-	-	Merge_129	chr11	40491765	260.23
-	-	Merge_130	chr11	42650404	377.47
-	-	Merge_134	chr11	56762884	535.92
-	-	Merge_137	chr11	65403061	1149.05
-	-	Merge_139	chr11	70293999	1531.76
-	-	Merge_147	chr11	91426886	2541.24
-	-	Merge_149	chr11	99730387	413.78

-	-	Merge_159	chr11	117057626	1709.7
-	-	Merge_161	chr11	118973097	477.65
-	-	Merge_163	chr10	9005758	264.91
-	-	Merge_166	chr10	11515289	465.89
-	-	Merge_167	chr10	19277333	2302.99
-	-	Merge_168	chr10	19471813	735.11
-	-	Merge_169	chr10	20381052	1040.03
-	-	Merge_171	chr10	33733410	697.56
-	-	Merge_172	chr10	39321358	887.52
-	-	Merge_174	chr10	44378100	287.87
-	-	Merge_175	chr10	45983857	1219.72
-	-	Merge_176	chr10	53464811	519.06
-	-	Merge_186	chr10	71667298	943.81
-	-	Merge_188	chr10	74933670	1590.29
-	-	Merge_192	chr10	77742097	383.84
-	-	Merge_195	chr10	84358301	4146.39
-	-	Merge_198	chr10	89824206	551.92
-	-	Merge_199	chr10	89825608	486.74
-	-	Merge_200	chr10	95035714	430.63
-	-	Merge_203	chr10	115776782	478.09
-	-	Merge_204	chr10	118640389	688.52
-	-	Merge_205	chr10	118641204	396.17
-	-	Merge_206	chr10	118825018	509.22
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-	-	Merge_209	chr10	125121944	699.64
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-	-	Merge_212	chr17	3251346	447.45
-	-	Merge_213	chr17	3254031	1081.21
-	-	Merge_218	chr17	8205329	889.2
-	-	Merge_226	chr17	28388751	1231.68
-	-	Merge_229	chr17	31777395	923.86
-	-	Merge_230	chr17	45169482	842.99
-	-	Merge_231	chr17	48052866	249.53
-	-	Merge_239	chr17	73882337	395
-	-	Merge_240	chr17	74984862	366.33
-	-	Merge_241	chr17	80801674	649.54

-	-	Merge_243	chr17	88316788	733.38
-	-	Merge_244	chr17	89654603	301.79
-	-	Merge_248	chr16	5316538	311.49
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-	-	Merge_250	chr16	6736759	884.54
-	-	Merge_251	chr16	7788375	897.06
-	-	Merge_253	chr16	10468330	299.47
-	-	Merge_254	chr16	13025634	308.1
-	-	Merge_256	chr16	25040033	679.24
-	-	Merge_257	chr16	25255828	1091.38
-	-	Merge_259	chr16	33673728	380.51
-	-	Merge_262	chr16	41173042	934.71
-	-	Merge_264	chr16	44680862	168.4
-	-	Merge_265	chr16	46121588	312.6
-	-	Merge_266	chr16	49336176	885.46
-	-	Merge_267	chr16	51910252	629.95
-	-	Merge_268	chr16	51937505	416.77
-	-	Merge_270	chr16	62433336	196.55
-	-	Merge_271	chr16	63091575	983.21
-	-	Merge_272	chr16	63116614	2637.18
-	-	Merge_283	chr16	97673780	259.36
-	-	Merge_285	chr15	31634807	1482.37
-	-	Merge_288	chr15	34685581	677.32
-	-	Merge_289	chr15	36621994	538.32
-	-	Merge_292	chr15	40280821	644.6
-	-	Merge_293	chr15	43764793	667.82
-	-	Merge_299	chr15	60310151	692.02
-	-	Merge_301	chr15	64429771	605.45
-	-	Merge_305	chr15	69924008	475.67
-	-	Merge_308	chr15	73438163	430.94
-	-	Merge_311	chr15	84352456	414.63
-	-	Merge_313	chr15	86815059	607.65
-	-	Merge_314	chr15	86963110	647.41
-	-	Merge_315	chr15	88451434	518.8
-	-	Merge_316	chr15	92830551	254.89
-	-	Merge_322	chr14	18500403	482.62

-	-	Merge_325	chr14	24578739	1108.12
-	-	Merge_327	chr14	32405604	271.74
-	-	Merge_331	chr14	47367132	576.78
-	-	Merge_332	chr14	47915728	818.3
-	-	Merge_333	chr14	54060060	420.54
-	-	Merge_334	chr14	58651425	233.51
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-	-	Merge_341	chr14	80233137	429.14
-	-	Merge_343	chr14	87345468	1315.01
-	-	Merge_344	chr14	102903125	233.29
-	-	Merge_345	chr14	114959903	1247.46
-	-	Merge_346	chr14	117981700	617.88
-	-	Merge_351	chr19	27598593	819.88
-	-	Merge_354	chr19	57521915	615.62
-	-	Merge_360	chr18	14261452	808.56
-	-	Merge_361	chr18	19604210	532.92
-	-	Merge_362	chr18	29516357	571.88
-	-	Merge_364	chr18	33751289	170.46
-	-	Merge_365	chr18	37217396	315.92
-	-	Merge_371	chr18	46112923	463
-	-	Merge_373	chr18	50769347	337.9
-	-	Merge_376	chr18	62761673	248.15
-	-	Merge_378	chr18	65855722	592.43
-	-	Merge_381	chr18	73318704	1262.01
-	-	Merge_382	chr18	76354789	458.8
-	-	Merge_383	chr18	82284404	2912.06
-	-	Merge_436	chr7	37388972	583.95
-	-	Merge_44	chr13	13546985	548.21
-	-	Merge_440	chr7	46718770	389.88
-	-	Merge_442	chr7	54340733	454.45
-	-	Merge_443	chr7	55744746	730.13
-	-	Merge_446	chr7	68852233	377.93
-	-	Merge_447	chr7	73186660	396.26
-	-	Merge_448	chr7	73918229	904.38
-	-	Merge_45	chr13	16645517	287.26
-	-	Merge_450	chr7	75880768	776.51

-	-	Merge_451	chr7	77573145	409.1
-	-	Merge_452	chr7	77994003	780.24
-	-	Merge_459	chr7	99374416	772.75
-	-	Merge_46	chr13	16646253	760.52
-	-	Merge_460	chr7	101101365	264.81
-	-	Merge_464	chr7	111785706	733.04
-	-	Merge_466	chr7	114290139	406.87
-	-	Merge_467	chr7	114874374	508.89
-	-	Merge_469	chr7	117186083	661.33
-	-	Merge_470	chr7	117710134	293.68
-	-	Merge_472	chr7	119047533	1538.56
-	-	Merge_477	chr7	124855190	595.28
-	-	Merge_478	chr7	125102619	687.97
-	-	Merge_479	chr7	126266088	548.75
-	-	Merge_488	chr7	138640854	315.26
-	-	Merge_49	chr13	46363737	336.19
-	-	Merge_491	chr6	14142283	1648.79
-	-	Merge_492	chr6	18688967	861.36
-	-	Merge_493	chr6	22390812	408.15
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-	-	Merge_50	chr13	47414053	461.25
-	-	Merge_503	chr6	42231407	733.46
-	-	Merge_504	chr6	45034303	289
-	-	Merge_507	chr6	49590512	357.65
-	-	Merge_508	chr6	50890762	786.98
-	-	Merge_509	chr6	50937313	416.38
-	-	Merge_51	chr13	47892785	402.94
-	-	Merge_511	chr6	54802570	622.33
-	-	Merge_512	chr6	55543541	497.26
-	-	Merge_514	chr6	59489169	398.28
-	-	Merge_515	chr6	78460361	405.23
-	-	Merge_518	chr6	91360049	722.3
-	-	Merge_52	chr13	47896825	354.25
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-	-	Merge_523	chr6	99904954	364.85
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-	-	Merge_526	chr6	104313198	906.74
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-	-	Merge_530	chr6	119070995	276.09
-	-	Merge_532	chr6	140776548	575.17
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-	-	Merge_536	chr5	24307474	316.38
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-	-	Merge_54	chr13	57099736	266.14
-	-	Merge_543	chr5	35138152	1515.93
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-	-	Merge_556	chr5	79856056	516.21
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-	-	Merge_560	chr5	100190225	228.76
-	-	Merge_565	chr5	111720820	339.11
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-	-	Merge_567	chr5	112092099	533.87
-	-	Merge_570	chr5	116886404	439.62
-	-	Merge_571	chr5	117032350	469
-	-	Merge_573	chr5	119492090	415.81
-	-	Merge_577	chr5	125244068	1614.23
-	-	Merge_578	chr5	126526092	423.72
-	-	Merge_579	chr5	129044666	355.97
-	-	Merge_58	chr13	69862505	919.03
-	-	Merge_580	chr5	129391210	369.87
-	-	Merge_587	chr5	133156629	888.54
-	-	Merge_588	chr5	133889938	3394.47
-	-	Merge_59	chr13	69862895	226.23

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-	-	Merge_593	chr5	141084228	573.49
-	-	Merge_594	chr5	141116048	622.83
-	-	Merge_596	chr5	144704256	691.56
-	-	Merge_598	chr5	148453914	856.37
-	-	Merge_60	chr13	76189414	281.34
-	-	Merge_602	chr4	13147002	2266.01
-	-	Merge_603	chr4	13300365	605.13
-	-	Merge_604	chr4	13600967	511.16
-	-	Merge_607	chr4	33697160	517.01
-	-	Merge_612	chr4	43720690	247.55
-	-	Merge_614	chr4	54073764	309.98
-	-	Merge_616	chr4	56088904	1629.76
-	-	Merge_62	chr13	83987932	493.8
-	-	Merge_620	chr4	64497910	239.9
-	-	Merge_621	chr4	69538381	820.74
-	-	Merge_622	chr4	70058963	1153.16
-	-	Merge_624	chr4	70805277	798.26
-	-	Merge_625	chr4	80048147	1069.02
-	-	Merge_627	chr4	82891131	860.41
-	-	Merge_629	chr4	97236235	853.38
-	-	Merge_63	chr13	88554692	1656.35
-	-	Merge_635	chr4	103576544	2249.74
-	-	Merge_636	chr4	103577576	568.28
-	-	Merge_638	chr4	105820242	370.16
-	-	Merge_639	chr4	106474497	842.1
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-	-	Merge_640	chr4	111866887	591.59
-	-	Merge_641	chr4	118969854	906.38
-	-	Merge_644	chr4	125946202	560.26
-	-	Merge_646	chr4	133509073	854.69
-	-	Merge_647	chr4	134430696	1180.66
-	-	Merge_650	chr4	138689452	401.03
-	-	Merge_651	chr4	138883850	404.29
-	-	Merge_652	chr4	145208012	590.17
-	-	Merge_656	chr4	148239805	712.13

-	-	Merge_658	chr4	152572507	647.68
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-	-	Merge_66	chr13	96952056	270.77
-	-	Merge_660	chr4	153930167	774.16
-	-	Merge_661	chr3	4834674	420.92
-	-	Merge_662	chr3	5754915	912.5
-	-	Merge_663	chr3	9960183	371.22
-	-	Merge_664	chr3	15194547	731.12
-	-	Merge_665	chr3	18625796	892.24
-	-	Merge_666	chr3	18640468	373.84
-	-	Merge_667	chr3	18669083	257.6
-	-	Merge_668	chr3	18683566	222.77
-	-	Merge_669	chr3	18715926	1011.57
-	-	Merge_67	chr13	97292151	1948.73
-	-	Merge_670	chr3	20776604	731.65
-	-	Merge_673	chr3	39746165	656.67
-	-	Merge_674	chr3	40283111	629.92
-	-	Merge_676	chr3	42023291	407.4
-	-	Merge_677	chr3	49945064	302.38
-	-	Merge_679	chr3	53416253	616.09
-	-	Merge_68	chr13	97537483	499.14
-	-	Merge_680	chr3	54064224	929.14
-	-	Merge_681	chr3	55540172	498.02
-	-	Merge_688	chr3	95027880	612.33
-	-	Merge_69	chr13	102352641	369.05
-	-	Merge_690	chr3	102071207	680.7
-	-	Merge_691	chr3	102644526	588.22
-	-	Merge_692	chr3	102677332	547.1
-	-	Merge_694	chr3	107431559	473.43
-	-	Merge_695	chr3	120571012	483.21
-	-	Merge_699	chr3	137110632	224.75
-	-	Merge_70	chr13	110673688	194.7
-	-	Merge_700	chr3	137728869	984.53
-	-	Merge_706	chr2	8112568	555.33
-	-	Merge_707	chr2	26040102	698.18
-	-	Merge_71	chr13	111905027	666.7

-	-	Merge_712	chr2	35477508	557.26
-	-	Merge_714	chr2	44979528	292.07
-	-	Merge_717	chr2	50655101	309.28
-	-	Merge_719	chr2	53437526	1031.43
-	-	Merge_72	chr12	5744249	377.95
-	-	Merge_722	chr2	63453029	422.76
-	-	Merge_724	chr2	71652503	348.19
-	-	Merge_726	chr2	75750534	590.32
-	-	Merge_727	chr2	85213919	562.74
-	-	Merge_728	chr2	92772854	227.11
-	-	Merge_729	chr2	95136825	846.2
-	-	Merge_73	chr12	5787532	403.96
-	-	Merge_733	chr2	118262897	889.11
-	-	Merge_735	chr2	125032084	1271.32
-	-	Merge_736	chr2	129998655	608.06
-	-	Merge_738	chr2	136803280	751.82
-	-	Merge_74	chr12	6639071	3387.59
-	-	Merge_740	chr2	143217904	598.11
-	-	Merge_741	chr2	144019402	206.83
-	-	Merge_742	chr2	144019682	264.98
-	-	Merge_743	chr2	144435891	219.45
-	-	Merge_746	chr2	154584230	988.32
-	-	Merge_75	chr12	8662948	1154.68
-	-	Merge_756	chr2	168441009	691.01
-	-	Merge_759	chr2	169421641	689.54
-	-	Merge_76	chr12	13769212	686.08
-	-	Merge_761	chr2	171713381	640.74
-	-	Merge_762	chr2	172227211	254.71
-	-	Merge_763	chr2	178841049	1569.44
-	-	Merge_764	chr2	179391965	705.63
-	-	Merge_765	chr2	179392279	403.48
-	-	Merge_766	chr2	180545576	663.37
-	-	Merge_767	chr1	6483521	199.12
-	-	Merge_77	chr12	17558117	772.63
-	-	Merge_772	chr1	26687660	445.1
-	-	Merge_78	chr12	25871380	3045

-	-	Merge_781	chr1	51671553	465.74
-	-	Merge_783	chr1	52578530	375.04
-	-	Merge_784	chr1	57224969	372.57
-	-	Merge_785	chr1	57529548	307.34
-	-	Merge_788	chr1	75653951	594.57
-	-	Merge_789	chr1	75729024	726.35
-	-	Merge_79	chr12	26191918	422.22
-	-	Merge_790	chr1	82794199	333.19
-	-	Merge_792	chr1	89237857	430.41
-	-	Merge_794	chr1	119899793	748.67
-	-	Merge_795	chr1	122464132	286.35
-	-	Merge_80	chr12	26781726	1160.86
-	-	Merge_803	chr1	133820289	357.34
-	-	Merge_804	chr1	134024796	707.33
-	-	Merge_805	chr1	135604895	613.57
-	-	Merge_807	chr1	136386174	514.18
-	-	Merge_808	chr1	147343735	1337.06
-	-	Merge_81	chr12	26802748	652.29
-	-	Merge_812	chr1	156997204	318.85
-	-	Merge_813	chr1	159565438	392.93
-	-	Merge_816	chr1	169060806	481.14
-	-	Merge_817	chr1	169624567	313.89
-	-	Merge_818	chr1	171540495	906.74
-	-	Merge_82	chr12	27054593	900.83
-	-	Merge_822	chr1	177364510	706.87
-	-	Merge_823	chr1	177444580	1830.1
-	-	Merge_824	chr1	177573509	1018.79
-	-	Merge_828	chr1	183794140	411.59
-	-	Merge_83	chr12	27417862	599.44
-	-	Merge_833	chr1	190298085	343.82
-	-	Merge_836	chr1	193600040	334.41
-	-	Merge_837	chr1	194161432	1754.49
-	-	Merge_838	chr1	194303079	396.02
-	-	Merge_847	chr9	27192635	288.85
-	-	Merge_848	chr9	27454328	624.6
-	-	Merge_849	chr9	27541857	446.11

-	-	Merge_851	chr9	33822788	253.72
-	-	Merge_852	chr9	34411921	196.37
-	-	Merge_853	chr9	41665493	741.65
-	-	Merge_854	chr9	43700091	1257.78
-	-	Merge_855	chr9	49489121	343.27
-	-	Merge_856	chr9	50452153	1525.54
-	-	Merge_858	chr9	51517858	501.11
-	-	Merge_859	chr9	53900016	377.92
-	-	Merge_86	chr12	52675238	543.62
-	-	Merge_863	chr9	58176270	283.29
-	-	Merge_864	chr9	59197743	588.52
-	-	Merge_865	chr9	60957645	831.82
-	-	Merge_866	chr9	62222634	396.85
-	-	Merge_868	chr9	75306684	323.81
-	-	Merge_869	chr9	76331283	308.1
-	-	Merge_871	chr9	78880509	691.7
-	-	Merge_872	chr9	79004867	2331.08
-	-	Merge_873	chr9	84936875	315.77
-	-	Merge_877	chr9	98131094	451.29
-	-	Merge_878	chr9	101581243	184.07
-	-	Merge_882	chr9	106112957	298.18
-	-	Merge_885	chr9	111805083	247.7
-	-	Merge_887	chr9	112454848	427.02
-	-	Merge_888	chr9	113793284	400.18
-	-	Merge_889	chr9	113812118	506.95
-	-	Merge_891	chr9	116077170	1273.19
-	-	Merge_895	chr8	9124570	420.77
-	-	Merge_896	chr8	12126696	959.68
-	-	Merge_90	chr12	70657137	584.03
-	-	Merge_902	chr8	26731313	362.6
-	-	Merge_903	chr8	27000306	362.5
-	-	Merge_904	chr8	28299249	292.31
-	-	Merge_905	chr8	32793050	852.87
-	-	Merge_906	chr8	35180238	231.92
-	-	Merge_907	chr8	35708733	314.36
-	-	Merge_909	chr8	52571423	482.26

-	-	Merge_912	chr8	68830292	637.34
-	-	Merge_918	chr8	89514468	809.25
-	-	Merge_919	chr8	91677084	1043.32
-	-	Merge_921	chr8	109416648	672.9
-	-	Merge_925	chr8	113873967	553.12
-	-	Merge_926	chr8	113886714	243.53
-	-	Merge_929	chr8	115429112	1055.58
-	-	Merge_935	chr8	125959583	738.87
-	-	Merge_936	chr8	126045931	283.1
-	-	Merge_937	chr8	126064151	1067.22
-	-	Merge_94	chr12	78001990	2019.01
-	-	Merge_95	chr12	80736818	605.05

Appendix J: Gene ontology analyses of target genes of NeuroD2

Annotation Cluster 1	Enrichment Score: 4.83507018535234					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_CC_FAT:0045202	synapse	22	1.29E-07	DLGAP1, GABRA2, CTBP2, CLSTN2, DLGAP2, GRIP1, NRXN3, PSD3, NRXN1, ZNRF1, GRM1, MYRIP, GRM7, SNTB1, CAMK2D, SV2B, CHRNA7, ERC2, SEZ6, SYT17, GAP43, GRID1	4.011	1.68E-04
GOTERM_CC_FAT:0044456	synapse part	15	1.68E-05	DLGAP1, GABRA2, CLSTN2, GRIP1, NRXN3, DLGAP2, PSD3, NRXN1, GRM1, GRM7, CHRNA7, SV2B, ERC2, SYT17, GRID1	4.115	2.18E-02
GOTERM_CC_FAT:0045211	postsynaptic membrane	9	1.44E-03	DLGAP1, GABRA2, CLSTN2, DLGAP2, GRIP1, PSD3, CHRNA7, GRM1, GRID1	4.154	1.86E+00
Annotation Cluster 2	**Enrichment Score: 2.9716960807554362**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_BP_FAT:0048667	cell morphogenesis involved in neuron differentiation	13	7.12E-05	KIF5C, DPYSL5, STXBP1, NFASC, PCDH15, EPHB1, SEMA5A, ATP2B2, ROBO1, APBB2, DST, GAP43, DSCAM	4.148	1.16E-01
GOTERM_BP_FAT:0000904	cell morphogenesis involved in differentiation	13	2.99E-04	KIF5C, DPYSL5, STXBP1, NFASC, PCDH15, EPHB1, SEMA5A, ATP2B2, ROBO1, APBB2, DST, GAP43, DSCAM	3.561	4.85E-01
GOTERM_BP_FAT:0048858	cell projection morphogenesis	12	7.41E-04	SEMA5A, ROBO1, KIF5C, NFASC, DPYSL5, STXBP1, PCDH15, APBB2, DST, EPHB1, GAP43, DSCAM	3.450	1.20E+00
GOTERM_BP_FAT:0000902	cell morphogenesis	15	8.98E-04	PARD3, KIF5C, NFASC, DPYSL5, STXBP1, PCDH15, SOX6, EPHB1, SEMA5A, ATP2B2, ROBO1, APBB2, DST, GAP43, DSCAM	2.819	1.45E+00
GOTERM_BP_FAT:0048812	neuron projection morphogenesis	11	9.19E-04	SEMA5A, ROBO1, KIF5C, NFASC, DPYSL5, STXBP1, APBB2, DST, EPHB1, GAP43, DSCAM	3.629	1.48E+00
GOTERM_BP_FAT:0032989	cellular component morphogenesis	16	1.08E-03	PARD3, KIF5C, NFASC, DPYSL5, STXBP1, PCDH15, SOX6, EPHB1, SEMA5A, ATP2B2, ROBO1, NEURL1A, APBB2, DST, GAP43, DSCAM	2.647	1.74E+00
GOTERM_BP_FAT:0032990	cell part morphogenesis	12	1.09E-03	SEMA5A, ROBO1, KIF5C, NFASC, DPYSL5, STXBP1, PCDH15, APBB2, DST, EPHB1, GAP43, DSCAM	3.287	1.76E+00

GOTERM_BP_FAT:0030030	cell projection organization	15	1.23E-03	KIF5C, DPYSL5, STXBP1, NFASC, PCDH15, EPHB1, VCL, SEMA5A, ATP2B2, ROBO1, NEURL1A, APBB2, DST, GAP43, DSCAM	2.730	1.98E+00
GOTERM_BP_FAT:0007411	axon guidance	8	1.47E-03	SEMA5A, ROBO1, KIF5C, NFASC, DPYSL5, APBB2, EPHB1, GAP43	4.740	2.36E+00
GOTERM_BP_FAT:0048666	neuron development	14	1.60E-03	SCLT1, KIF5C, DPYSL5, STXBP1, NFASC, PCDH15, EPHB1, SEMA5A, ATP2B2, ROBO1, APBB2, DST, GAP43, DSCAM	2.784	2.58E+00
GOTERM_BP_FAT:0006928	cell motion	16	1.69E-03	PRKCA, SCHIP1, KIF5C, NFASC, DPYSL5, PEX5L, EPHB1, SEMA5A, DAB1, DOCK1, ROBO1, CHRNA7, LAMC1, NEURL1A, APBB2, GAP43	2.532	2.71E+00
GOTERM_BP_FAT:0007409	axonogenesis	10	2.00E-03	SEMA5A, ROBO1, KIF5C, NFASC, DPYSL5, STXBP1, APBB2, DST, EPHB1, GAP43	3.562	3.20E+00
GOTERM_BP_FAT:0030182	neuron differentiation	16	3.74E-03	SCLT1, KIF5C, NFASC, DPYSL5, STXBP1, PCDH15, EPHB1, SEMA5A, ATP2B2, ROBO1, CUX1, APBB2, DST, GAP43, USH2A, DSCAM	2.329	5.90E+00
GOTERM_BP_FAT:0031175	neuron projection development	11	4.43E-03	SEMA5A, ROBO1, KIF5C, NFASC, DPYSL5, STXBP1, APBB2, DST, EPHB1, GAP43, DSCAM	2.930	6.95E+00
Annotation Cluster 3	**Enrichment Score: 2.25765917065514**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_CC_FAT:0042734	presynaptic membrane	5	1.24E-03	NRXN3, GRM7, CHRNA7, ERC2, NRXN1	10.385	1.60E+00
GOTERM_MF_FAT:0016247	channel regulator activity	4	7.83E-03	SCLT1, NRXN3, GRM7, NRXN1	9.629	1.04E+01
GOTERM_MF_FAT:0005246	calcium channel regulator activity	3	1.74E-02	NRXN3, GRM7, NRXN1	14.443	2.18E+01
Annotation Cluster 4	**Enrichment Score: 2.036510262530812**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_MF_FAT:0043167	ion binding	92	6.05E-04	SLC9A9, UQCRC2, CHIA, THRB, CLSTN2, CACHD1, KCNAB1, SUSD1, PHF20, ZNRF1, CBFA2T2, KCNIP1, ATP2B2, KCNQ3, CCBE1, PRIM2, SNTB1, NEURL1A, PRKCA, L3MBTL4, WBSCR17, NCALD, MTA3, RPH3AL, CACNG4, STIM1, NEK10, PDE10A, NRXN1, TECR, NEK11, MYT1L, IGSF5, ZDHHC14, MYRIP, PITPNM3, CAMK4, ZFP462, CLIC4, ATP9A, PDE5A, ZFYVE28, PDE9A, ADAM18, MCC, KCNH8, LEPREL1, TPH1, DST, ADD2, ENOX1, PHLPP1, GLIS3, ABLIM2, EFCAB6, SLC39A11, SCAPER, TBC1D9, ABLIM3, CDKAL1, PEX5L, RASAL1, FAT3,	1.351	8.42E-01

				DNER, HEG1, B4GALT5, GABRA2, CPA6, B3GALT1, SMYD3, PCDH15, STAT1, CACNA2D2, MAN1C1, BRCA1, ITPR2, RUFY2, RNF8, RPS6KA5, VAT1L, CBLB, SLC4A10, LRP1, SLC25A13, EBF3, RPS6KA2, BNC2, ATF7, EBF1, HIVEP3, SH3RF3, RERE		
GOTERM_MF_FAT:0043169	cation binding	90	1.02E-03	SLC9A9, UQCRC2, CHIA, THRB, CLSTN2, CACHD1, KCNAB1, SUSD1, PHF20, ZNRF1, CBFA2T2, KCNIP1, ATP2B2, KCNQ3, CCBE1, PRIM2, SNTB1, NEURL1A, PRKCA, L3MBTL4, WBSCR17, NCALD, MTA3, RPH3AL, CACNG4, STIM1, NEK10, PDE10A, NRXN1, TECR, NEK11, MYT1L, IGSF5, ZDHHC14, MYRIP, PITPNM3, CAMK4, ZFP462, ATP9A, PDE5A, ZFYVE28, PDE9A, ADAM18, MCC, KCNH8, LEPREL1, TPH1, DST, ADD2, ENOX1, PHLPP1, GLIS3, ABLIM2, EFCAB6, SLC39A11, SCAPER, TBC1D9, ABLIM3, CDKAL1, PEX5L, RASAL1, FAT3, DNER, HEG1, B4GALT5, CPA6, B3GALT1, SMYD3, PCDH15, STAT1, CACNA2D2, MAN1C1, BRCA1, ITPR2, RUFY2, RNF8, RPS6KA5, VAT1L, CBLB, SLC4A10, LRP1, SLC25A13, EBF3, RPS6KA2, BNC2, ATF7, EBF1, HIVEP3, SH3RF3, RERE	1.338	1.41E+00
GOTERM_MF_FAT:0046872	metal ion binding	89	1.20E-03	SLC9A9, UQCRC2, THRB, CLSTN2, CACHD1, KCNAB1, SUSD1, PHF20, ZNRF1, CBFA2T2, KCNIP1, ATP2B2, KCNQ3, CCBE1, PRIM2, SNTB1, NEURL1A, PRKCA, L3MBTL4, WBSCR17, NCALD, MTA3, RPH3AL, CACNG4, STIM1, NEK10, PDE10A, NRXN1, TECR, NEK11, MYT1L, IGSF5, ZDHHC14, MYRIP, PITPNM3, CAMK4, ZFP462, ATP9A, PDE5A, ZFYVE28, PDE9A, ADAM18, KCNH8, MCC, LEPREL1, TPH1, DST, ADD2, ENOX1, PHLPP1, GLIS3, ABLIM2, EFCAB6, SLC39A11, SCAPER, TBC1D9, ABLIM3, CDKAL1, PEX5L, RASAL1, FAT3,	1.336	1.66E+00

				DNER, HEG1, B4GALT5, CPA6, B3GALT1, SMYD3, PCDH15, STAT1, CACNA2D2, MAN1C1, BRCA1, ITPR2, RUFY2, RNF8, RPS6KA5, VAT1L, CBLB, SLC4A10, LRP1, SLC25A13, RPS6KA2, EBF3, BNC2, ATF7, EBF1, HIVEP3, SH3RF3, RERE		
GOTERM_MF_FAT:0046914	transition metal ion binding	51	2.20E-01	UQCRC2, THRB, PHF20, ZNRF1, CBFA2T2, PRIM2, NEURL1A, PRKCA, L3MBTL4, WBSCR17, MTA3, RPH3AL, PDE10A, TECR, NEK11, ZDHHC14, MYT1L, MYRIP, ZFP462, ZFYVE28, PDE5A, ADAM18, PDE9A, LEPREL1, TPH1, ENOX1, PHLPP1, ABLIM2, GLIS3, SLC39A11, SCAPER, ABLIM3, CDKAL1, PEX5L, RASAL1, B4GALT5, CPA6, B3GALT1, SMYD3, BRCA1, RUFY2, RNF8, VAT1L, CBLB, EBF3, BNC2, EBF1, ATF7, SH3RF3, HIVEP3, RERE	1.130	9.69E+01
GOTERM_MF_FAT:0008270	zinc ion binding	39	4.04E-01	UQCRC2, ABLIM2, GLIS3, SLC39A11, THRB, SCAPER, ABLIM3, PHF20, ZNRF1, CBFA2T2, PEX5L, RASAL1, NEURL1A, PRKCA, L3MBTL4, CPA6, MTA3, RPH3AL, SMYD3, PDE10A, BRCA1, RUFY2, RNF8, VAT1L, ZDHHC14, MYT1L, MYRIP, CBLB, ZFP462, EBF3, BNC2, ZFYVE28, ATF7, EBF1, PDE5A, SH3RF3, HIVEP3, ADAM18, RERE	1.070	9.99E+01
Annotation Cluster 5	**Enrichment Score: 1.982032146104171**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_MF_FAT:0017016	Ras GTPase binding	6	2.94E-03	MYRIP, RPH3AL, DOCK9, DGKI, DIAP3, DIAP1	6.081	4.03E+00
GOTERM_MF_FAT:0031267	small GTPase binding	6	3.42E-03	MYRIP, RPH3AL, DOCK9, DGKI, DIAP3, DIAP1	5.875	4.67E+00
GOTERM_MF_FAT:0051020	GTPase binding	6	4.24E-03	MYRIP, RPH3AL, DOCK9, DGKI, DIAP3, DIAP1	5.591	5.76E+00
GOTERM_MF_FAT:0019899	enzyme binding	9	4.53E-02	MYRIP, RPH3AL, DOCK9, ABAT, CHRNA7, DGKI, APBB2, DIAP3, DIAP1	2.271	4.77E+01
GOTERM_MF_FAT:0017048	Rho GTPase binding	3	6.37E-02	DOCK9, DIAP3, DIAP1	7.222	6.01E+01
Annotation Cluster 6	**Enrichment Score: 1.9711275716746413**					

Category	Term	Count	PValue	Genes	Fold Enrichment	FDR
GOTERM_MF_FAT:0030695	GTPase regulator activity	19	5.42E-05	RALGPS1, TNIK, TBC1D9, PREX1, RPH3AL, PSD3, DOCK9, RABGAP1L, DGKI, ARHGAP15, 9630014M24RIK, ARHGAP26, TBC1D16, RASAL1, GARNL3, MYRIP, DOCK1, TIAM2, TBC1D5, RGS6	3.041	7.56E-02
GOTERM_MF_FAT:0060589	nucleoside-triphosphatase regulator activity	19	6.69E-05	RALGPS1, TNIK, TBC1D9, PREX1, RPH3AL, PSD3, DOCK9, RABGAP1L, DGKI, ARHGAP15, 9630014M24RIK, ARHGAP26, TBC1D16, RASAL1, GARNL3, MYRIP, DOCK1, TIAM2, TBC1D5, RGS6	2.991	9.34E-02
GOTERM_MF_FAT:0005083	small GTPase regulator activity	13	4.95E-04	TNIK, TBC1D9, PREX1, PSD3, DOCK9, RPH3AL, RABGAP1L, ARHGAP15, TBC1D16, GARNL3, MYRIP, TIAM2, TBC1D5	3.368	6.90E-01
GOTERM_MF_FAT:0005096	GTPase activator activity	10	6.13E-03	TBC1D16, GARNL3, RASAL1, TIAM2, TBC1D9, TBC1D5, RGS6, RABGAP1L, ARHGAP15, 9630014M24RIK, ARHGAP26	3.009	8.22E+00
GOTERM_BP_FAT:0051056	regulation of small GTPase mediated signal transduction	10	1.70E-02	TBC1D16, GARNL3, RASAL1, TIAM2, TBC1D9, PREX1, TBC1D5, PSD3, RABGAP1L, DGKI	2.547	2.43E+01
GOTERM_MF_FAT:0005099	Ras GTPase activator activity	5	2.57E-02	TBC1D16, TBC1D9, TBC1D5, RABGAP1L, ARHGAP15	4.444	3.05E+01
GOTERM_MF_FAT:0008047	enzyme activator activity	10	2.88E-02	TBC1D16, GARNL3, RASAL1, TIAM2, TBC1D9, TBC1D5, RGS6, RABGAP1L, ARHGAP15, 9630014M24RIK, ARHGAP26	2.320	3.35E+01
GOTERM_BP_FAT:0032313	regulation of Rab GTPase activity	4	2.89E-02	TBC1D16, TBC1D9, TBC1D5, RABGAP1L	5.956	3.79E+01
GOTERM_BP_FAT:0032483	regulation of Rab protein signal transduction	4	2.89E-02	TBC1D16, TBC1D9, TBC1D5, RABGAP1L	5.956	3.79E+01
GOTERM_BP_FAT:0046578	regulation of Ras protein signal transduction	8	3.66E-02	TBC1D16, TIAM2, TBC1D9, PREX1, TBC1D5, PSD3, RABGAP1L, DGKI	2.567	4.54E+01
GOTERM_MF_FAT:0005097	Rab GTPase activator activity	4	3.76E-02	TBC1D16, TBC1D9, TBC1D5, RABGAP1L	5.374	4.15E+01
GOTERM_BP_FAT:0032318	regulation of Ras GTPase activity	5	4.85E-02	TBC1D16, TBC1D9, TBC1D5, RABGAP1L, DGKI	3.629	5.54E+01
GOTERM_BP_FAT:0043087	regulation of GTPase activity	5	7.57E-02	TBC1D16, TBC1D9, TBC1D5, RABGAP1L, DGKI	3.122	7.22E+01
GOTERM_BP_FAT:0051336	regulation of hydrolase activity	5	4.36E-01	TBC1D16, TBC1D9, TBC1D5, RABGAP1L, DGKI	1.481	1.00E+02
Annotation Cluster 7	**Enrichment Score: 1.937393305654709**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
KEGG_PATHWAY:mmu04720	mmu04720:Long-term potentiation	8	1.81E-04	GRM5, PRKCA, CAMK4, RPS6KA2, CAMK2D, PPP1R12A, GRM1, ITPR2	6.558	2.02E-01
KEGG_PATHWAY:mmu04730	mmu04730:Long-term depression	5	3.51E-02	GRM5, PRKCA, PPP2R1B, GRM1, ITPR2	3.985	3.30E+01

KEGG_PATHWAY:mmu04020	mmu04020:Calcium signaling pathway	8	4.61E-02	GRM5, PRKCA, ATP2B2, CAMK4, CAMK2D, CHRNA7, GRM1, ITPR2	2.403	4.10E+01
KEGG_PATHWAY:mmu04540	mmu04540:Gap junction	5	6.06E-02	GRM5, PRKCA, TUBB2B, GRM1, ITPR2	3.336	5.03E+01
Annotation Cluster 8	**Enrichment Score: 1.8843580145259702**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_BP_FAT:0010324	membrane invagination	10	5.17E-03	STON2, DNM3, LRP1, DOCK1, PACSIN2, STXBP1, CHRNA7, STAB2, ELMO2, ELMOD1	3.089	8.08E+00
GOTERM_BP_FAT:0006897	endocytosis	10	5.17E-03	STON2, DNM3, LRP1, DOCK1, PACSIN2, STXBP1, CHRNA7, STAB2, ELMO2, ELMOD1	3.089	8.08E+00
GOTERM_BP_FAT:0016192	vesicle-mediated transport	16	1.44E-02	STX6, STON2, DNM3, RPH3AL, STXBP1, STAB2, ELMO2, LRP1, DOCK1, ARF1, PACSIN2, EXOC4, CHRNA7, SV2B, CUX1, ELMOD1	1.994	2.10E+01
GOTERM_BP_FAT:0016044	membrane organization	11	1.91E-02	STON2, SCLT1, DNM3, LRP1, DOCK1, PACSIN2, STXBP1, CHRNA7, STAB2, ELMO2, ELMOD1	2.348	2.69E+01
GOTERM_BP_FAT:0006909	phagocytosis	4	5.15E-02	LRP1, DOCK1, ELMO2, ELMOD1	4.740	5.77E+01
Annotation Cluster 9	**Enrichment Score: 1.7403715233395785**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_CC_FAT:0005856	cytoskeleton	36	3.50E-04	MAD1L1, ABLIM2, PARD3, SDCCAG8, TUBB2B, KRTAP16-1, UPP2, 9630014M24RIK, VCL, CORO2B, SNTB1, CHRNA7, AFAP1, KIF21B, DISC1, ELMOD1, DNM3, DLGAP1, DLGAP2, MYO1D, KIF5C, PSD3, EML6, GRM1, BRCA1, ELMO2, ARHGAP26, FMN1, MYRIP, RPS6KA2, FRMD4A, KRTAP4-7, FRMD4B, ERC2, DST, ADD2, DIAP1	1.866	4.55E-01
GOTERM_CC_FAT:0043232	intracellular non-membrane-bounded organelle	43	5.22E-02	MAD1L1, ABLIM2, PARD3, SDCCAG8, TUBB2B, KRTAP16-1, UPP2, 9630014M24RIK, VCL, CORO2B, CHD7, PRIM2, SNTB1, CHRNA7, AFAP1, KIF21B, USH2A, DISC1, ELMOD1, DNM3, DLGAP1, POLR1D, DLGAP2, MYO1D, KIF5C, PSD3, PCDH15, EML6, GRM1, BRCA1, NEK11, ELMO2, ARHGAP26, FMN1, RNF8, MYRIP, RPS6KA2, FRMD4A, KRTAP4-7, FRMD4B, ERC2, DST, ADD2, DIAP1	1.303	5.03E+01

GOTERM_CC_FAT:0043228	non-membrane-bounded organelle	43	5.22E-02	MAD1L1, ABLIM2, PARD3, SDCCAG8, TUBB2B, KRTAP16-1, UPP2, 9630014M24RIK, VCL, CORO2B, CHD7, PRIM2, SNTB1, CHRNA7, AFAP1, KIF21B, USH2A, DISC1, ELMOD1, DNM3, DLGAP1, POLR1D, DLGAP2, MYO1D, KIF5C, PSD3, PCDH15, EML6, GRM1, BRCA1, NEK11, ELMO2, ARHGAP26, FMN1, RNF8, MYRIP, RPS6KA2, FRMD4A, KRTAP4-7, FRMD4B, ERC2, DST, ADD2, DIAP1	1.303	5.03E+01
GOTERM_CC_FAT:0044430	cytoskeletal part	19	1.15E-01	DNM3, DLGAP1, SDCCAG8, PARD3, TUBB2B, KRTAP16-1, DLGAP2, KIF5C, MYO1D, PSD3, UPP2, GRM1, EML6, BRCA1, RPS6KA2, KRTAP4-7, CHRNA7, KIF21B, DISC1	1.428	7.95E+01
Annotation Cluster 10	**Enrichment Score: 1.731961979961568**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_MF_FAT:0003779	actin binding	13	4.25E-03	ABLIM2, ABLIM3, MYO1D, VCL, FMN1, CORO2B, MYRIP, SNTB1, DST, DIAP3, AFAP1, ADD2, DIAP1	2.608	5.77E+00
GOTERM_MF_FAT:0008092	cytoskeletal protein binding	16	5.47E-03	ABLIM2, ABLIM3, MYO1D, PTPRT, VCL, FMN1, CORO2B, MYRIP, PACSIN2, SNTB1, DST, DIAP3, AFAP1, ADD2, USH2A, DIAP1	2.233	7.37E+00
GOTERM_CC_FAT:0015629	actin cytoskeleton	6	2.74E-01	FMN1, ABLIM2, MYRIP, MYO1D, DST, VCL	1.702	9.85E+01
Annotation Cluster 11	**Enrichment Score: 1.7110691238922635**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_BP_FAT:0030001	metal ion transport	19	6.17E-04	SLC9A9, GM5134, SLC39A11, CACHD1, KCNAB1, KCTD21, CACNG4, STIM1, KCNIP1, CACNA2D2, TECR, ITPR2, ATP2B2, SLC4A10, KCNQ3, KCNN3, CAMK2D, KCNH8, CHRNA7	2.496	9.98E-01
GOTERM_MF_FAT:0022836	gated channel activity	14	1.19E-03	GABRA2, KCNAB1, KCTD21, CACNG4, CACNA2D2, KCNIP1, ITPR2, KCNQ3, CLIC4, GRM7, KCNN3, CHRNA7, KCNH8, GRID1	2.878	1.65E+00
GOTERM_BP_FAT:0006811	ion transport	24	2.63E-03	SLC9A9, GM5134, GABRA2, SLC39A11, CACHD1, KCNAB1, KCTD21, CACNG4, STIM1, KCNIP1, CACNA2D2, TECR, ITPR2, ATP2B2, SLC4A10, SLC25A13, KCNQ3, CLIC4, KCNN3, SLC26A9, CAMK2D, KCNH8, CHRNA7, GRID1	1.957	4.20E+00
GOTERM_BP_FAT:0006812	cation transport	19	3.38E-03	SLC9A9, GM5134, SLC39A11, CACHD1, KCNAB1, KCTD21, CACNG4, STIM1, KCNIP1, CACNA2D2,	2.142	5.35E+00

				TECR, ITPR2, ATP2B2, SLC4A10, KCNQ3, KCNN3, CAMK2D, KCNH8, CHRNA7		
GOTERM_MF_FAT:0046873	metal ion transmembrane transporter activity	13	4.54E-03	SLC39A11, KCNAB1, KCTD21, CACNG4, CACNA2D2, KCNIP1, ITPR2, ATP2B2, KCNQ3, GRM7, KCNN3, CHRNA7, KCNH8	2.590	6.16E+00
GOTERM_BP_FAT:0006816	calcium ion transport	8	4.82E-03	ATP2B2, CACHD1, CAMK2D, CACNG4, STIM1, CHRNA7, CACNA2D2, ITPR2	3.839	7.55E+00
GOTERM_BP_FAT:0015674	di-, tri-valent inorganic cation transport	9	6.60E-03	ATP2B2, CACHD1, CAMK2D, CACNG4, STIM1, CHRNA7, TECR, CACNA2D2, ITPR2	3.246	1.02E+01
GOTERM_MF_FAT:0005216	ion channel activity	14	7.62E-03	GABRA2, KCNAB1, KCTD21, CACNG4, CACNA2D2, KCNIP1, ITPR2, KCNQ3, CLIC4, GRM7, KCNN3, CHRNA7, KCNH8, GRID1	2.318	1.01E+01
GOTERM_MF_FAT:0022838	substrate specific channel activity	14	9.83E-03	GABRA2, KCNAB1, KCTD21, CACNG4, CACNA2D2, KCNIP1, ITPR2, KCNQ3, CLIC4, GRM7, KCNN3, CHRNA7, KCNH8, GRID1	2.247	1.29E+01
GOTERM_MF_FAT:0022803	passive transmembrane transporter activity	14	1.08E-02	GABRA2, KCNAB1, KCTD21, CACNG4, CACNA2D2, KCNIP1, ITPR2, KCNQ3, CLIC4, GRM7, KCNN3, CHRNA7, KCNH8, GRID1	2.216	1.40E+01
GOTERM_MF_FAT:0015267	channel activity	14	1.08E-02	GABRA2, KCNAB1, KCTD21, CACNG4, CACNA2D2, KCNIP1, ITPR2, KCNQ3, CLIC4, GRM7, KCNN3, CHRNA7, KCNH8, GRID1	2.216	1.40E+01
GOTERM_MF_FAT:0005261	cation channel activity	11	1.09E-02	KCNQ3, KCNAB1, GRM7, KCNN3, KCTD21, CACNG4, KCNH8, CHRNA7, CACNA2D2, KCNIP1, ITPR2	2.563	1.42E+01
GOTERM_MF_FAT:0022832	voltage-gated channel activity	9	1.25E-02	KCNQ3, KCNAB1, CLIC4, GRM7, KCTD21, CACNG4, KCNH8, CACNA2D2, KCNIP1	2.905	1.61E+01
GOTERM_MF_FAT:0005244	voltage-gated ion channel activity	9	1.25E-02	KCNQ3, KCNAB1, CLIC4, GRM7, KCTD21, CACNG4, KCNH8, CACNA2D2, KCNIP1	2.905	1.61E+01
GOTERM_MF_FAT:0022843	voltage-gated cation channel activity	7	2.34E-02	KCNQ3, KCNAB1, GRM7, KCTD21, CACNG4, KCNH8, CACNA2D2	3.160	2.82E+01
GOTERM_MF_FAT:0005245	voltage-gated calcium channel activity	3	5.01E-02	GRM7, CACNG4, CACNA2D2	8.253	5.12E+01
GOTERM_MF_FAT:0005267	potassium channel activity	6	7.24E-02	KCNQ3, KCNAB1, KCNN3, KCTD21, KCNH8, KCNIP1	2.687	6.50E+01
GOTERM_MF_FAT:0005262	calcium channel activity	4	1.12E-01	GRM7, CACNG4, CACNA2D2, ITPR2	3.398	8.11E+01
GOTERM_CC_FAT:0034702	ion channel complex	6	1.35E-01	GABRA2, KCNQ3, CLIC4, KCTD21, CHRNA7, CACNA2D2	2.209	8.48E+01
GOTERM_BP_FAT:0006813	potassium ion transport	6	1.41E-01	KCNQ3, KCNAB1, KCNN3, KCTD21, KCNH8, KCNIP1	2.178	9.15E+01
GOTERM_BP_FAT:0015672	monovalent inorganic cation transport	9	1.48E-01	SLC9A9, SLC4A10, GM5134, KCNQ3, KCNAB1, KCNN3, KCTD21, KCNH8, KCNIP1	1.725	9.26E+01
GOTERM_MF_FAT:0005249	voltage-gated potassium channel activity	4	2.43E-01	KCNQ3, KCNAB1, KCTD21, KCNH8	2.334	9.80E+01

GOTERM_MF_FAT:0031420	alkali metal ion binding	6	2.85E-01	SLC9A9, SLC4A10, KCNQ3, KCNAB1, KCNH8, KCNIP1	1.683	9.91E+01
GOTERM_MF_FAT:0030955	potassium ion binding	4	3.32E-01	KCNQ3, KCNAB1, KCNH8, KCNIP1	1.958	9.96E+01
Annotation Cluster 12	**Enrichment Score: 1.4399889351321193**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_BP_FAT:0031644	regulation of neurological system process	7	1.32E-02	LAMA2, GRM5, ATP2B2, STXBP1, CHRNA7, CACNA2D2, GRM1	3.597	1.94E+01
GOTERM_BP_FAT:0044057	regulation of system process	9	2.27E-02	LAMA2, GRM5, PRKCA, ATP2B2, THRB, STXBP1, CHRNA7, CACNA2D2, GRM1	2.600	3.12E+01
GOTERM_BP_FAT:0050804	regulation of synaptic transmission	6	2.88E-02	LAMA2, GRM5, ATP2B2, STXBP1, CHRNA7, CACNA2D2	3.484	3.78E+01
GOTERM_BP_FAT:0051969	regulation of transmission of nerve impulse	6	3.69E-02	LAMA2, GRM5, ATP2B2, STXBP1, CHRNA7, CACNA2D2	3.256	4.57E+01
GOTERM_BP_FAT:0048167	regulation of synaptic plasticity	3	1.99E-01	GRM5, ATP2B2, STXBP1	3.629	9.73E+01
Annotation Cluster 13	**Enrichment Score: 1.3419729867208743**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_BP_FAT:0007267	cell-cell signaling	12	1.15E-02	FGFR2, DLGAP1, CAMK4, NRXN3, DLGAP2, GRM7, STXBP1, ABAT, SV2B, CHRNA7, NRXN1, FGF1	2.403	1.72E+01
GOTERM_BP_FAT:0007268	synaptic transmission	9	1.17E-02	DLGAP1, CAMK4, NRXN3, GRM7, STXBP1, ABAT, SV2B, CHRNA7, NRXN1	2.936	1.75E+01
GOTERM_BP_FAT:0019226	transmission of nerve impulse	10	1.60E-02	DLGAP1, CAMK4, NRXN3, GRM7, STXBP1, CACNG4, ABAT, SV2B, CHRNA7, NRXN1	2.569	2.31E+01
GOTERM_BP_FAT:0001505	regulation of neurotransmitter levels	4	9.03E-02	NRXN3, ABAT, SV2B, NRXN1	3.746	7.85E+01
GOTERM_BP_FAT:0050877	neurological system process	18	9.96E-01	DLGAP1, THRB, NRXN3, STXBP1, CACNG4, PCDH15, NRXN1, GRM1, GRM5, ATP2B2, CHD7, CAMK4, GRM7, ABAT, CHRNA7, SV2B, USH2A, DIAP1	0.622	1.00E+02
Annotation Cluster 14	**Enrichment Score: 1.3202718910564186**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_BP_FAT:0006928	cell motion	16	1.69E-03	PRKCA, SCHIP1, KIF5C, NFASC, DPYSL5, PEX5L, EPHB1, SEMA5A, DAB1, DOCK1, ROBO1, CHRNA7, LAMC1, NEURL1A, APBB2, GAP43	2.532	2.71E+00
GOTERM_BP_FAT:0051674	localization of cell	9	1.16E-01	PRKCA, SCHIP1, DOCK1, DAB1, CHRNA7, LAMC1, NEURL1A, APBB2, PEX5L	1.840	8.65E+01
GOTERM_BP_FAT:0048870	cell motility	9	1.16E-01	PRKCA, SCHIP1, DOCK1, DAB1, CHRNA7, LAMC1, NEURL1A, APBB2, PEX5L	1.840	8.65E+01
GOTERM_BP_FAT:0016477	cell migration	7	2.31E-01	PRKCA, SCHIP1, DOCK1, DAB1, LAMC1, APBB2, PEX5L	1.694	9.86E+01
Annotation Cluster 15	**Enrichment Score: 1.1401029994109855**					

Category	Term	Count	PValue	Genes	Fold Enrichment	FDR
GOTERM_BP_FAT:0009101	glycoprotein biosynthetic process	6	5.09E-02	SLC4A10, B3GALT1, B3GALT5, TRAK1, ALG8, MAN1C1	2.978	5.72E+01
GOTERM_BP_FAT:0043413	biopolymer glycosylation	5	7.11E-02	SLC4A10, B3GALT1, B3GALT5, TRAK1, ALG8	3.191	6.99E+01
GOTERM_BP_FAT:0070085	glycosylation	5	7.11E-02	SLC4A10, B3GALT1, B3GALT5, TRAK1, ALG8	3.191	6.99E+01
GOTERM_BP_FAT:0006486	protein amino acid glycosylation	5	7.11E-02	SLC4A10, B3GALT1, B3GALT5, TRAK1, ALG8	3.191	6.99E+01
GOTERM_BP_FAT:0009100	glycoprotein metabolic process	6	1.09E-01	SLC4A10, B3GALT1, B3GALT5, TRAK1, ALG8, MAN1C1	2.370	8.46E+01
Annotation Cluster 16	**Enrichment Score: 1.1045726081106997**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_CC_FAT:0070160	occluding junction	5	4.12E-02	IGSF5, PARD3, MAGI1, MPP7, PARD3B	3.826	4.22E+01
GOTERM_CC_FAT:0005923	tight junction	5	4.12E-02	IGSF5, PARD3, MAGI1, MPP7, PARD3B	3.826	4.22E+01
KEGG_PATHWAY:mmu04530	Tight junction	6	8.31E-02	PRKCA, PPP2R1B, IGSF5, PARD3, MAGI1, EXOC4	2.550	6.21E+01
GOTERM_CC_FAT:0043296	apical junction complex	5	9.50E-02	IGSF5, PARD3, MAGI1, MPP7, PARD3B	2.879	7.28E+01
GOTERM_CC_FAT:0016327	apicolateral plasma membrane	5	1.00E-01	IGSF5, PARD3, MAGI1, MPP7, PARD3B	2.823	7.48E+01
GOTERM_CC_FAT:0005911	cell-cell junction	6	1.76E-01	IGSF5, PARD3, MAGI1, MPP7, PARD3B, VCL	2.017	9.19E+01
Annotation Cluster 17	**Enrichment Score: 1.0885215952903593**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_MF_FAT:0035091	phosphoinositide binding	5	4.04E-02	SNX9, SNX8, ZFYVE28, RPS6KC1, ITPR2	3.852	4.38E+01
GOTERM_MF_FAT:0005543	phospholipid binding	6	5.49E-02	SNX9, DAB1, SNX8, ZFYVE28, RPS6KC1, ITPR2	2.913	5.46E+01
GOTERM_MF_FAT:0008289	lipid binding	9	2.44E-01	PRKCA, SNX9, PITPNM3, DAB1, PLEKHA8, SNX8, ZFYVE28, RPS6KC1, ITPR2	1.507	9.80E+01
Annotation Cluster 18	**Enrichment Score: 1.03786065126072**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_MF_FAT:0047555	3',5'-cyclic-GMP phosphodiesterase activity	3	1.47E-02	PDE5A, PDE10A, PDE9A	15.757	1.87E+01
GOTERM_MF_FAT:0004114	3',5'-cyclic-nucleotide phosphodiesterase activity	3	6.37E-02	PDE5A, PDE10A, PDE9A	7.222	6.01E+01
GOTERM_MF_FAT:0004112	cyclic-nucleotide phosphodiesterase activity	3	6.84E-02	PDE5A, PDE10A, PDE9A	6.933	6.29E+01
KEGG_PATHWAY:mmu00230	Purine metabolism	5	2.86E-01	POLR1D, PRIM2, PDE5A, PDE10A, PDE9A	1.827	9.77E+01
GOTERM_MF_FAT:0008081	phosphoric diester hydrolase activity	3	3.53E-01	PDE5A, PDE10A, PDE9A	2.407	9.98E+01
Annotation Cluster 19	**Enrichment Score: 1.0311708783505216**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_MF_FAT:0003700	transcription factor activity	23	1.49E-02	GLIS3, L3MBTL4, ERG, ELF2, THRB, MTA3, AFF3, NFIX, SOX6, STAT1, CBFA2T2, FOXN3, MYT1L, FLI1, EBF1, ATF7, CREB3L2,	1.712	1.89E+01

				CUX1, ETV6, ETV5, NFIA, RERE, NFIB		
GOTERM_MF_FAT:0030528	transcription regulator activity	31	2.59E-02	GLIS3, ELF2, THRB, NFIX, SOX6, CBFA2T2, FLI1, NPAS3, CREB3L2, ETV6, ETV5, ERG, L3MBTL4, CTBP2, MTA3, AFF3, STAT1, MED13L, FOXN3, MYT1L, CAMK4, EBF3, EBF1, ATF7, HIVEP3, CUX1, NEUROD6, NFIA, RERE, NCOR2, NFIB	1.485	3.07E+01
GOTERM_BP_FAT:0006355	regulation of transcription, DNA-dependent	33	9.01E-02	ABLIM2, GLIS3, ELF2, THRB, ABLIM3, NFIX, SOX6, CBFA2T2, FLI1, NPAS3, TRAK1, CREB3L2, ETV6, ETV5, ERG, L3MBTL4, CTBP2, MTA3, STAT1, MED13L, FOXN3, RPS6KA5, MYT1L, ZFP462, EBF3, ATF7, EBF1, KCNH8, CUX1, NFIA, RERE, NCOR2, NFIB	1.308	7.85E+01
GOTERM_BP_FAT:0051252	regulation of RNA metabolic process	33	1.05E-01	ABLIM2, GLIS3, ELF2, THRB, ABLIM3, NFIX, SOX6, CBFA2T2, FLI1, NPAS3, TRAK1, CREB3L2, ETV6, ETV5, ERG, L3MBTL4, CTBP2, MTA3, STAT1, MED13L, FOXN3, RPS6KA5, MYT1L, ZFP462, EBF3, ATF7, EBF1, KCNH8, CUX1, NFIA, RERE, NCOR2, NFIB	1.288	8.35E+01
GOTERM_MF_FAT:0003677	DNA binding	38	1.29E-01	GLIS3, ELF2, TNRC18, THRB, RAD23A, NFIX, PHF20, SOX6, CBFA2T2, MCM9, CHD7, FLI1, NPAS3, PRIM2, CREB3L2, ETV6, ETV5, ERG, L3MBTL4, POLR1D, MTA3, AFF3, STAT1, FOXN3, BRCA1, FMN1, MYT1L, EBF3, BNC2, ATF7, EBF1, HIVEP3, CUX1, NEUROD6, NFIA, RERE, NCOR2, NFIB	1.233	8.55E+01
GOTERM_MF_FAT:0043565	sequence-specific DNA binding	14	1.65E-01	ERG, ELF2, THRB, MTA3, SOX6, FOXN3, FLI1, ATF7, CREB3L2, ETV6, CUX1, ETV5, NCOR2, RERE	1.455	9.19E+01
GOTERM_BP_FAT:0045449	regulation of transcription	44	2.18E-01	CRTC3, GLIS3, ABLIM2, ELF2, EFCAB6, THRB, ABLIM3, NFIX, PHF20, SOX6, CBFA2T2, NLRC3, CHD7, FLI1, NPAS3, TRAK1, CREB3L2, ETV6, ETV5, L3MBTL4, ERG, CTBP2, KHDRBS2, MTA3, AFF3, STAT1, MED13L, FOXN3, RPS6KA5, MYT1L, ZFP462, EBF3, BNC2, ATF7, EBF1, HIVEP3, KCNH8, CUX1, NEUROD6, APBB2, NFIA, RERE, NCOR2, NFIB	1.147	9.82E+01

GOTERM_BP_FAT:0006350	transcription	34	3.32E-01	CRTC3, GLIS3, ELF2, EFCAB6, THRB, NFIX, PHF20, SOX6, CHD7, FLI1, NPAS3, PRIM2, CREB3L2, ETV6, ERG, KHDRBS2, CTBP2, POLR1D, AFF3, STAT1, MED13L, FOXN3, MYT1L, EBF3, BNC2, ATF7, EBF1, HIVEP3, CUX1, NEUROD6, NFIA, RERE, NCOR2, NFIB	1.114	9.99E+01
Annotation Cluster 20	**Enrichment Score: 1.0129088435747287**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_MF_FAT:0004672	protein kinase activity	18	2.39E-02	PRKCA, FGFR2, TNIK, NUAK1, DSTYK, RPS6KC1, NEK10, MAPK10, CLK1, ALK, NEK11, EPHB1, RPS6KA5, CAMK4, RPS6KA2, CAMK2D, KCNH8, GRK4	1.784	2.87E+01
GOTERM_MF_FAT:0004674	protein serine/threonine kinase activity	14	3.11E-02	PRKCA, TNIK, NUAK1, DSTYK, RPS6KC1, NEK10, MAPK10, CLK1, NEK11, RPS6KA5, CAMK4, RPS6KA2, CAMK2D, GRK4	1.921	3.57E+01
GOTERM_BP_FAT:0006796	phosphate metabolic process	23	4.18E-02	FGFR2, PRKCA, TNIK, NUAK1, PTPRN2, DSTYK, RPS6KC1, NEK10, PTPRT, MAPK10, CLK1, ALK, CDKN3, GRM1, NEK11, EPHB1, RPS6KA5, CAMK4, DUSP14, RPS6KA2, PPP1R12A, CAMK2D, GRK4	1.542	5.00E+01
GOTERM_BP_FAT:0006793	phosphorus metabolic process	23	4.18E-02	FGFR2, PRKCA, TNIK, NUAK1, PTPRN2, DSTYK, RPS6KC1, NEK10, PTPRT, MAPK10, CLK1, ALK, CDKN3, GRM1, NEK11, EPHB1, RPS6KA5, CAMK4, DUSP14, RPS6KA2, PPP1R12A, CAMK2D, GRK4	1.542	5.00E+01
GOTERM_BP_FAT:0006468	protein amino acid phosphorylation	18	4.92E-02	PRKCA, FGFR2, TNIK, NUAK1, DSTYK, RPS6KC1, NEK10, MAPK10, CLK1, ALK, GRM1, NEK11, EPHB1, RPS6KA5, CAMK4, RPS6KA2, CAMK2D, GRK4	1.633	5.59E+01
GOTERM_MF_FAT:0001882	nucleoside binding	35	8.72E-02	FGFR2, NUAK1, DSTYK, HLCS, CLK1, EPHB1, MCM9, ATP2B2, NLRC3, CHD7, LONP2, CAMK2D, DMGDH, CHST15, KIF21B, ABCA13, SLC28A3, PRKCA, TNIK, MAGI1, MYO1D, KIF5C, RPS6KC1, NEK10, PDE10A, LARS2, MAPK10, ALK, NEK11, RPS6KA5, CAMK4, RPS6KA2, ATP9A, PDE5A, GRK4	1.298	7.20E+01
GOTERM_BP_FAT:00016310	phosphorylation	18	1.11E-01	PRKCA, FGFR2, TNIK, NUAK1, DSTYK, RPS6KC1, NEK10, MAPK10, CLK1, ALK, GRM1, NEK11, EPHB1, RPS6KA5, CAMK4, RPS6KA2, CAMK2D, GRK4	1.456	8.53E+01

GOTERM_MF_FAT:0001883	purine nucleoside binding	34	1.15E-01	FGFR2, NUAK1, DSTYK, HLCS, CLK1, EPHB1, MCM9, ATP2B2, NLRC3, CHD7, LONP2, CAMK2D, DMGDH, CHST15, KIF21B, ABCA13, PRKCA, TNIK, MAGI1, MYO1D, KIF5C, RPS6KC1, NEK10, PDE10A, LARS2, MAPK10, ALK, NEK11, RPS6KA5, CAMK4, RPS6KA2, ATP9A, PDE5A, GRK4	1.269	8.19E+01
GOTERM_MF_FAT:0030554	adenyl nucleotide binding	33	1.47E-01	FGFR2, NUAK1, DSTYK, HLCS, CLK1, EPHB1, MCM9, ATP2B2, NLRC3, CHD7, LONP2, CAMK2D, DMGDH, CHST15, KIF21B, ABCA13, PRKCA, TNIK, MAGI1, KIF5C, MYO1D, RPS6KC1, NEK10, PDE10A, LARS2, MAPK10, ALK, NEK11, RPS6KA5, CAMK4, RPS6KA2, ATP9A, GRK4	1.242	8.91E+01
GOTERM_MF_FAT:0017076	purine nucleotide binding	39	1.57E-01	FGFR2, TUBB2B, NUAK1, DSTYK, HLCS, CLK1, EPHB1, MCM9, ATP2B2, NLRC3, CHD7, LONP2, CAMK2D, RHOBTB1, DMGDH, CHST15, KIF21B, ABCA13, PRKCA, DNM3, TNIK, MAGI1, MYO1D, KIF5C, DOCK9, RPS6KC1, NEK10, PDE10A, LARS2, MAPK10, ALK, NEK11, RPS6KA5, CAMK4, ARF1, RPS6KA2, ATP9A, PDE5A, GRK4	1.204	9.08E+01
GOTERM_MF_FAT:0032559	adenyl ribonucleotide binding	31	1.76E-01	FGFR2, NUAK1, DSTYK, HLCS, CLK1, EPHB1, MCM9, ATP2B2, NLRC3, LONP2, CHD7, CAMK2D, KIF21B, ABCA13, PRKCA, TNIK, MAGI1, KIF5C, MYO1D, RPS6KC1, NEK10, PDE10A, LARS2, MAPK10, ALK, NEK11, RPS6KA5, CAMK4, RPS6KA2, ATP9A, GRK4	1.227	9.33E+01
GOTERM_MF_FAT:0032555	purine ribonucleotide binding	37	1.86E-01	FGFR2, TUBB2B, NUAK1, DSTYK, HLCS, CLK1, EPHB1, MCM9, ATP2B2, NLRC3, CHD7, LONP2, CAMK2D, RHOBTB1, KIF21B, ABCA13, PRKCA, DNM3, TNIK, MAGI1, MYO1D, KIF5C, DOCK9, RPS6KC1, NEK10, PDE10A, LARS2, MAPK10, ALK, NEK11, RPS6KA5, ARF1, CAMK4, RPS6KA2, ATP9A, PDE5A, GRK4	1.190	9.44E+01
GOTERM_MF_FAT:0032553	ribonucleotide binding	37	1.86E-01	FGFR2, TUBB2B, NUAK1, DSTYK, HLCS, CLK1, EPHB1, MCM9, ATP2B2, NLRC3, CHD7, LONP2, CAMK2D, RHOBTB1, KIF21B, ABCA13, PRKCA, DNM3, TNIK, MAGI1, MYO1D, KIF5C, DOCK9, RPS6KC1, NEK10, PDE10A, LARS2, MAPK10, ALK, NEK11, RPS6KA5, ARF1,	1.190	9.44E+01

				CAMK4, RPS6KA2, ATP9A, PDE5A, GRK4		
GOTERM_MF_FAT:0005524	ATP binding	30	2.15E-01	FGFR2, NUAK1, DSTYK, HLCS, CLK1, EPHB1, MCM9, ATP2B2, NLRC3, LONP2, CHD7, CAMK2D, KIF21B, ABCA13, PRKCA, TNIK, MAGI1, KIF5C, MYO1D, NEK10, RPS6KC1, MAPK10, LARS2, ALK, NEK11, RPS6KA5, CAMK4, RPS6KA2, ATP9A, GRK4	1.201	9.66E+01
GOTERM_MF_FAT:0000166	nucleotide binding	42	2.97E-01	FGFR2, TUBB2B, NUAK1, DSTYK, HLCS, CLK1, EPHB1, MCM9, ATP2B2, NLRC3, CHD7, LONP2, CAMK2D, RHOBTB1, DMGDH, CHST15, KIF21B, ABCA13, PRKCA, DNM3, CTBP2, TNIK, MAGI1, MYO1D, KIF5C, DOCK9, RPS6KC1, NEK10, PDE10A, LARS2, MAPK10, NAV3, ALK, NEK11, RPS6KA5, CAMK4, ARF1, RPS6KA2, ATP9A, PDE5A, GRK4, ENOX1	1.112	9.93E+01
Annotation Cluster 21	**Enrichment Score: 0.9763052381384759**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_BP_FAT:0060113	inner ear receptor cell differentiation	4	1.44E-02	ATP2B2, PCDH15, CUX1, USH2A	7.742	2.10E+01
GOTERM_BP_FAT:0007605	sensory perception of sound	6	1.47E-02	ATP2B2, CHD7, THRB, PCDH15, USH2A, DIAP1	4.148	2.13E+01
GOTERM_BP_FAT:0048839	inner ear development	6	1.76E-02	FGFR2, ATP2B2, CHD7, PCDH15, CUX1, USH2A	3.959	2.51E+01
GOTERM_BP_FAT:0050954	sensory perception of mechanical stimulus	6	2.09E-02	ATP2B2, CHD7, THRB, PCDH15, USH2A, DIAP1	3.787	2.91E+01
GOTERM_BP_FAT:0042490	mechanoreceptor differentiation	4	2.34E-02	ATP2B2, PCDH15, CUX1, USH2A	6.452	3.20E+01
GOTERM_BP_FAT:0043583	ear development	6	3.33E-02	FGFR2, ATP2B2, CHD7, PCDH15, CUX1, USH2A	3.350	4.23E+01
GOTERM_BP_FAT:0042491	auditory receptor cell differentiation	3	5.40E-02	ATP2B2, PCDH15, CUX1	7.918	5.94E+01
GOTERM_BP_FAT:0042472	inner ear morphogenesis	4	1.08E-01	FGFR2, ATP2B2, CHD7, PCDH15	3.467	8.43E+01
GOTERM_BP_FAT:0042471	ear morphogenesis	4	1.42E-01	FGFR2, ATP2B2, CHD7, PCDH15	3.056	9.17E+01
GOTERM_BP_FAT:0007423	sensory organ development	8	1.54E-01	FGFR2, ATP2B2, CHD7, PCDH15, CUX1, EPHB1, USH2A, DSCAM	1.808	9.33E+01
GOTERM_BP_FAT:0048562	embryonic organ morphogenesis	4	5.24E-01	FGFR2, ATP2B2, CHD7, PCDH15	1.443	1.00E+02
GOTERM_BP_FAT:0048568	embryonic organ development	5	5.96E-01	FGFR2, ATP2B2, CHD7, PCDH15, PCSK5	1.205	1.00E+02
GOTERM_BP_FAT:0048598	embryonic morphogenesis	4	9.48E-01	FGFR2, ATP2B2, CHD7, PCDH15	0.647	1.00E+02
GOTERM_BP_FAT:0050890	cognition	10	1.00E+00	GRM5, ATP2B2, CHD7, THRB, GRM7, CHRNA7, PCDH15, GRM1, USH2A, DIAP1	0.392	1.00E+02
GOTERM_BP_FAT:0007600	sensory perception	8	1.00E+00	ATP2B2, CHD7, THRB, GRM7, PCDH15, GRM1, USH2A, DIAP1	0.331	1.00E+02
Annotation Cluster 22	**Enrichment Score: 0.8904818759490303**					

Category	Term	Count	PValue	Genes	Fold Enrichment	FDR
GOTERM_MF_FAT:0001871	pattern binding	6	7.05E-02	FGFR2, CHIA, RSPO1, NAV2, STAB2, FGF1	2.708	6.40E+01
GOTERM_MF_FAT:0030247	polysaccharide binding	6	7.05E-02	FGFR2, CHIA, RSPO1, NAV2, STAB2, FGF1	2.708	6.40E+01
GOTERM_MF_FAT:0005539	glycosaminoglycan binding	5	1.34E-01	FGFR2, RSPO1, NAV2, STAB2, FGF1	2.534	8.66E+01
GOTERM_MF_FAT:0008201	heparin binding	4	1.72E-01	FGFR2, RSPO1, NAV2, FGF1	2.784	9.29E+01
GOTERM_MF_FAT:0030246	carbohydrate binding	8	3.07E-01	GLG1, FGFR2, CHIA, RSPO1, NAV2, WBSCR17, STAB2, FGF1	1.458	9.94E+01
Annotation Cluster 23	**Enrichment Score: 0.8707298789805075**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_BP_FAT:0046907	intracellular transport	13	6.83E-02	STX6, STON2, SNX9, STAM2, RPH3AL, ARFIP1, MYRIP, SLC25A13, CAMK4, TRAK1, APBB2, CUX1, DST	1.751	6.83E+01
GOTERM_BP_FAT:0015031	protein transport	17	9.44E-02	STX6, STON2, SNX9, SLC15A2, SNX8, STAM2, RPH3AL, STXBP1, ARFIP1, NUPL1, MYRIP, COG7, PLEKHA8, ARF1, TRAK1, EXOC4, SV2B	1.516	8.00E+01
GOTERM_BP_FAT:0008104	protein localization	19	9.67E-02	STX6, STON2, SNX9, SLC15A2, GRIP1, SNX8, STAM2, RPH3AL, STXBP1, ARFIP1, NUPL1, PEX5L, MYRIP, COG7, PLEKHA8, ARF1, TRAK1, EXOC4, SV2B	1.465	8.08E+01
GOTERM_BP_FAT:0045184	establishment of protein localization	17	9.89E-02	STX6, STON2, SNX9, SLC15A2, SNX8, STAM2, RPH3AL, STXBP1, ARFIP1, NUPL1, MYRIP, COG7, PLEKHA8, ARF1, TRAK1, EXOC4, SV2B	1.505	8.16E+01
GOTERM_BP_FAT:0006886	intracellular protein transport	8	1.96E-01	STX6, STON2, SNX9, MYRIP, STAM2, TRAK1, RPH3AL, ARFIP1	1.683	9.71E+01
GOTERM_BP_FAT:0034613	cellular protein localization	8	2.56E-01	STX6, STON2, SNX9, MYRIP, STAM2, TRAK1, RPH3AL, ARFIP1	1.554	9.92E+01
GOTERM_BP_FAT:0070727	cellular macromolecule localization	8	2.60E-01	STX6, STON2, SNX9, MYRIP, STAM2, TRAK1, RPH3AL, ARFIP1	1.543	9.92E+01
Annotation Cluster 24	**Enrichment Score: 0.8587888296544002**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_BP_FAT:0007626	locomotory behavior	9	5.41E-02	GRM5, PRKCA, ATP2B2, CHD7, NPAS3, ROBO1, PCDH15, GRM1, DSCAM	2.187	5.95E+01
GOTERM_BP_FAT:0030534	adult behavior	5	7.57E-02	CHD7, GRM7, ABAT, CHRNA7, PCDH15	3.122	7.22E+01
GOTERM_BP_FAT:0007610	behavior	12	8.95E-02	GRM5, PRKCA, ATP2B2, CHD7, NPAS3, ROBO1, GRM7, ABAT, CHRNA7, PCDH15, GRM1, DSCAM	1.721	7.82E+01
GOTERM_BP_FAT:0050890	cognition	10	1.00E+00	GRM5, ATP2B2, CHD7, THRB, GRM7, CHRNA7, PCDH15, GRM1, USH2A, DIAP1	0.392	1.00E+02
Annotation Cluster 25	**Enrichment Score: 0.8387275158346306**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**

GOTERM_CC_FAT:0005912	adherens junction	5	1.08E-01	FMN1, PARD3, MPP7, 9630014M24RIK, ARHGAP26, VCL	2.743	7.76E+01
GOTERM_CC_FAT:0070161	anchoring junction	5	1.60E-01	FMN1, PARD3, MPP7, 9630014M24RIK, ARHGAP26, VCL	2.364	8.97E+01
GOTERM_CC_FAT:0005911	cell-cell junction	6	1.76E-01	IGSF5, PARD3, MAGI1, MPP7, PARD3B, VCL	2.017	9.19E+01
Annotation Cluster 26	**Enrichment Score: 0.8085425781717052**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
KEGG_PATHWAY:mmu04012	ErbB signaling pathway	5	6.27E-02	PRKCA, CBLB, NRG3, CAMK2D, MAPK10	3.298	5.15E+01
KEGG_PATHWAY:mmu04912	GnRH signaling pathway	4	2.34E-01	PRKCA, CAMK2D, MAPK10, ITPR2	2.366	9.49E+01
KEGG_PATHWAY:mmu04310	Wnt signaling pathway	5	2.56E-01	PRKCA, PPP2R1B, CTBP2, CAMK2D, MAPK10	1.926	9.63E+01
Annotation Cluster 27	**Enrichment Score: 0.7906846535896617**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_BP_FAT:0007268	synaptic transmission	9	1.17E-02	DLGAP1, CAMK4, NRXN3, GRM7, STXBP1, ABAT, SV2B, CHRNA7, NRXN1	2.936	1.75E+01
GOTERM_BP_FAT:0001505	regulation of neurotransmitter levels	4	9.03E-02	NRXN3, ABAT, SV2B, NRXN1	3.746	7.85E+01
GOTERM_BP_FAT:0007269	neurotransmitter secretion	3	1.26E-01	NRXN3, SV2B, NRXN1	4.839	8.89E+01
GOTERM_BP_FAT:0032940	secretion by cell	6	2.16E-01	NRXN3, RPH3AL, STXBP1, EXOC4, SV2B, NRXN1	1.873	9.81E+01
GOTERM_BP_FAT:0006887	exocytosis	4	2.92E-01	RPH3AL, STXBP1, EXOC4, SV2B	2.112	9.96E+01
GOTERM_BP_FAT:0046903	secretion	6	3.30E-01	NRXN3, RPH3AL, STXBP1, EXOC4, SV2B, NRXN1	1.577	9.99E+01
GOTERM_BP_FAT:0006836	neurotransmitter transport	3	4.00E-01	NRXN3, SV2B, NRXN1	2.178	1.00E+02
GOTERM_BP_FAT:0003001	generation of a signal involved in cell-cell signaling	3	4.24E-01	NRXN3, SV2B, NRXN1	2.074	1.00E+02
Annotation Cluster 28	**Enrichment Score: 0.7804732313404785**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_BP_FAT:0010604	positive regulation of macromolecule metabolic process	18	4.52E-02	PRKCA, ABLIM2, GLIS3, THRB, ABLIM3, NFIX, CD40, SOX6, RNF8, CBLB, ZFP462, EBF3, EBF1, HIVEP3, FGF1, ETV5, NFIA, NFIB	1.651	5.28E+01
GOTERM_BP_FAT:0045935	positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	15	5.72E-02	GLIS3, ABLIM2, THRB, ABLIM3, NFIX, CD40, SOX6, RNF8, ZFP462, EBF3, EBF1, HIVEP3, ETV5, NFIA, NFIB	1.708	6.16E+01
GOTERM_BP_FAT:0051173	positive regulation of nitrogen compound metabolic process	15	6.94E-02	GLIS3, ABLIM2, THRB, ABLIM3, NFIX, CD40, SOX6, RNF8, ZFP462, EBF3, EBF1, HIVEP3, ETV5, NFIA, NFIB	1.656	6.89E+01
GOTERM_BP_FAT:0045941	positive regulation of transcription	13	1.17E-01	GLIS3, ABLIM2, THRB, ABLIM3, NFIX, SOX6, ZFP462, EBF3, EBF1, HIVEP3, ETV5, NFIA, NFIB	1.589	8.68E+01
GOTERM_BP_FAT:0010628	positive regulation of gene expression	13	1.35E-01	GLIS3, ABLIM2, THRB, ABLIM3, NFIX, SOX6, ZFP462, EBF3, EBF1, HIVEP3, ETV5, NFIA,	1.547	9.05E+01

				NFIB		
GOTERM_BP_FAT:0010557	positive regulation of macromolecule biosynthetic process	13	2.00E-01	GLIS3, ABLIM2, THRB, ABLIM3, NFIX, SOX6, ZFP462, EBF3, EBF1, HIVEP3, ETV5, NFIA, NFIB	1.424	9.74E+01
GOTERM_BP_FAT:0031328	positive regulation of cellular biosynthetic process	13	2.39E-01	GLIS3, ABLIM2, THRB, ABLIM3, NFIX, SOX6, ZFP462, EBF3, EBF1, HIVEP3, ETV5, NFIA, NFIB	1.368	9.88E+01
GOTERM_BP_FAT:0009891	positive regulation of biosynthetic process	13	2.50E-01	GLIS3, ABLIM2, THRB, ABLIM3, NFIX, SOX6, ZFP462, EBF3, EBF1, HIVEP3, ETV5, NFIA, NFIB	1.355	9.91E+01
GOTERM_BP_FAT:0006357	regulation of transcription from RNA polymerase II promoter	14	2.60E-01	GLIS3, ABLIM2, ELF2, THRB, ABLIM3, NFIX, SOX6, MED13L, ZFP462, TRAK1, CUX1, NCOR2, NFIA, NFIB	1.320	9.93E+01
GOTERM_BP_FAT:0045944	positive regulation of transcription from RNA polymerase II promoter	9	2.73E-01	ABLIM2, GLIS3, ZFP462, THRB, ABLIM3, NFIX, SOX6, NFIA, NFIB	1.460	9.94E+01
GOTERM_BP_FAT:0045893	positive regulation of transcription, DNA-dependent	9	4.16E-01	ABLIM2, GLIS3, ZFP462, THRB, ABLIM3, NFIX, SOX6, NFIA, NFIB	1.256	1.00E+02
GOTERM_BP_FAT:0051254	positive regulation of RNA metabolic process	9	4.30E-01	ABLIM2, GLIS3, ZFP462, THRB, ABLIM3, NFIX, SOX6, NFIA, NFIB	1.247	1.00E+02
Annotation Cluster 29	**Enrichment Score: 0.7583791989729805**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_BP_FAT:0006885	regulation of pH	3	9.85E-02	SLC9A9, SLC4A10, SLC26A9	5.620	8.14E+01
GOTERM_BP_FAT:0055067	monovalent inorganic cation homeostasis	3	1.38E-01	SLC9A9, SLC4A10, SLC26A9	4.584	9.10E+01
GOTERM_MF_FAT:0015300	solute:solute antiporter activity	3	1.69E-01	SLC9A9, SLC4A10, SLC26A9	4.031	9.25E+01
GOTERM_BP_FAT:0055080	cation homeostasis	6	2.09E-01	SLC9A9, PRKCA, ATP2B2, SLC4A10, SLC26A9, TECR	1.894	9.78E+01
GOTERM_BP_FAT:0050801	ion homeostasis	8	2.39E-01	SLC9A9, PRKCA, ATP2B2, SLC4A10, SLC26A9, CHRNA7, TECR, PEX5L	1.585	9.88E+01
GOTERM_MF_FAT:0015297	antiporter activity	3	2.45E-01	SLC9A9, SLC4A10, SLC26A9	3.151	9.80E+01
Annotation Cluster 30	**Enrichment Score: 0.7454241139914374**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_CC_FAT:0005578	proteinaceous extracellular matrix	10	6.95E-02	LAMA2, COL14A1, NAV2, COL27A1, COL15A1, LAMC1, PRSS36, FGF1, USH2A, SPON1	1.958	6.09E+01
GOTERM_CC_FAT:0044420	extracellular matrix part	5	7.30E-02	LAMA2, COL27A1, COL15A1, LAMC1, USH2A	3.161	6.27E+01
GOTERM_CC_FAT:0031012	extracellular matrix	10	8.38E-02	LAMA2, COL14A1, NAV2, COL27A1, COL15A1, LAMC1, PRSS36, FGF1, USH2A, SPON1	1.882	6.80E+01
GOTERM_CC_FAT:0005604	basement membrane	4	1.29E-01	LAMA2, COL15A1, LAMC1, USH2A	3.187	8.36E+01
GOTERM_CC_FAT:0044421	extracellular region part	13	6.80E-01	COL15A1, LAMA2, COL14A1, RSPO1, NAV2, COL27A1, LAMC1, FGF1, PRSS36, PCSK5, USH2A, ENOX1, SPON1	0.977	1.00E+02

GOTERM_CC_FAT:0005576	extracellular region	24	9.00E-01	FGFR2, TG, CHIA, NRG3, CPA6, LYPD6, COL15A1, CD40, LAMA2, BTBD17, COL14A1, RSPO1, NAV2, HTRA1, COL27A1, CCBE1, LAMC1, PRSS36, FGF1, PCSK5, SEZ6, USH2A, ENOX1, SPON1	0.831	1.00E+02
Annotation Cluster 31	**Enrichment Score: 0.6992655944112344**					
Category	**Term**	**Count**	**PValue**	**Genes**	**Fold Enrichment**	**FDR**
GOTERM_MF_FAT:0008066	glutamate receptor activity	4	2.74E-02	GRM5, GRM7, GRM1, GRID1	6.081	3.21E+01
KEGG_PATHWAY:mmu05080	Neuroactive ligand-receptor interaction	7	2.92E-01	GRM5, PARD3, GABRA2, THRB, GRM7, GRM1, GRID1	1.545	9.79E+01
GOTERM_BP_FAT:0007186	G-protein coupled receptor protein signaling pathway	7	1.00E+00	GRM5, GABRA2, GRM7, RGS6, BAI3, NPSR1, GRM1	0.217	1.00E+02