

Transcription Factor CUX1-Regulated Gene Expression in the Developing Cortex

by

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ABSTRACT

Nervous system contains a large variety of cells with different dendritic morphologies, axonal connections, neurotransmitters and gene expression patterns. Diverse intercellular signaling mechanisms, temporally regulated factors and intrinsic cues play important roles in the generation of this complex system.

The cerebral cortex, which instructs higher order sensory, cognitive and motor functions, is organized into six functional layers. Certain transcription programs intrinsically regulate the positioning and layer identity of cortical neurons. *Cux1* (Cut-like homeobox 1) gene encodes a homeodomain transcription factor which regulates the differentiation and physiology of layer II-III cortical neurons by inducing dendritic branching, increasing the number and the density of dendritic spines and modulating synaptogenesis. It also regulates the formation of corpus callosum axon tract which facilitates the connection between two hemispheres.

There is a lack of comprehensive data on the genome-wide targets of CUX1 through which it maintains layer identity in cortical neurons. Studies aimed to identify these gene targets are currently limited to in vitro experiments on non-neuronal cell lines.

In this study, I have identified the genome-wide binding profiles and target genes of CUX1 homeodomain transcription factor by chromatin immunoprecipitation followed by sequencing (ChIP-Seq) experiment in the developing mouse cerebral cortex.

My results revealed that CUX1 transcription factor regulates excitatory synapses by direct binding to a set of genes that regulate glutamatergic signaling. CUX1 also targets several genes regulating axon guidance, a portion of which are responsible for formation and regulation of corpus callosum axon tract.

ÖZET

Sinir sisteminde bulunan hücreler birbirinden çok farklı dendritik morfolojiler, akson bağlantıları, nörotransmitterler ve gen ifade modelleri taşırlar. Bu karmaşık sistemin oluşmasında çeşitli hücreler arası sinyal mekanizmaları, geçici olarak düzenlenen faktörler ve hücre içi bilgiler önemli rol oynar. Üst seviye duysal, bilişsel ve motor fonksiyonları yöneten serebral korteks, altı fonksiyonel tabakadan oluşur. Belirli yazılım programları serebral sinir hücrelerinin pozisyonlanmasını ve hangi tabaka kimliklerini düzenlerler.

Cux1 (*Cux-like homeobox 1*) geni, dendrit dallanmasını arttırarak ve sinaps gelişimini düzenleyerek korteksin II. ve III. tabakalarındaki nöronların farklılaşmasını ve fizyolojisini düzenleyen bir ‘homeodomain’ transkripsiyon faktörü kodlar. Bu gen ayrıca korpus kallozum akson siteminin oluşumunu da kontrol eder.

CUX1 proteininin kortikal sinir hücrelerine kortikal tabaka kimliği kazandırabilmek için genom üzerindeki hangi genleri hedef aldığı ve onlara bağlandığı ile ilgili kapsamlı bir çalışma bulunmamaktadır. Şimdiye kadar bu transkripsiyon faktörünün hedef genlerini bulmayı amaçlayan çalışmalar nöron harici hücrelerde yapılmış *in vitro* deneyleri içerir.

Bu çalışmada, embriyonik dönemdeki farelerin beyin serebral kortikal düzlem dokusunda CUX1 transkripsiyon faktörünün genom bazında bağlanma profilini ve direk hedef genlerini kromatin imünopresipitasyonunu takiben sekanslama yöntemini kullanarak belirledim.

Sonuçlarım CUX1 proteininin glutamaterjik sinaps yolağındaki genlere direk olarak bağlanarak bu yolağın işlevini düzenlediğini gösterir. Ayrıca CUX1’in korpus kallozum oluşumunu akson yönelimi yolağını düzenleyen bir gizi gene bağlanarak düzenlediğini işaret etmektedir

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To my family....

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NOMENCLATURE

APS	Ammonium persulfate
BSA	Bovine Serum Albumin
BME	Basal Medium Eagle
BF	BME-FBS
bp	Basepair
cDNA	Complementary Deoxyribonucleic acid
ChIP	Chromatin Immunoprecipitation
CP	Cortical plate
DAPI	4',6-diamidino-2-phenylindole
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethyleneglycoltetraacetic acid
FA	Formaldehyde
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
IZ	Intermediate zone
kDa	Kilodalton
LB	Luria Broth
MEM	Minimum Essential Media
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PFA	Paraformaldehyde
P/S	Penicillin/Streptomycin
Q-PCR	Quantitative Polymerase Chain Reaction
RIPA	Radioimmunoprecipitation Assay Buffer
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
shRNA	short hairpin RNA
SVZ	Subventricular Zone
TBS-T	Tris Buffered Saline-Tween
TEMED	Tetramethylethylenediamine
VZ	Ventricular Zone

Chapter 1

INTRODUCTION

The nervous system contains a large variety of cells possessing different properties in terms of their dendritic morphologies, axonal connections, neurotransmitters and gene expression patterns. This highly complex system is generated by the mitotically active progenitor cells using diverse intercellular signaling mechanisms, temporally regulated factors and intrinsic cues [1]. The cerebral cortex, which enables performing higher order sensory, cognitive and motor functions, is organized into six horizontal layers, each containing neurons with unique molecular and physiological properties. Development of this structure starts with symmetrical divisions of the radial glial cells during early development and continues with their asymmetrical divisions which give rise to intermediate progenitor cells and immature neurons. Neurons migrate towards the cortical surface in an inside-out fashion while at the same time exiting the cell cycle, and make up the six-layered structure of the cortex.

Transcription factors have important roles in regulating the development of the cerebral cortex. Cell-specific basic helix-loop-helix (bHLH) transcription factors instruct differentiation of progenitor cells to specific cell types. Hes and Id family of transcription factors are required for maintaining progenitor cell identity [2], whereas Olig1/2 transcription factors direct differentiation of oligodendrocytes [3] or Neurogenin and NeuroD family transcription factors initiate and control neurogenesis, respectively [2], [4]. There are also certain transcription programs that instruct neuronal positioning and layer identity [5]–[7].

Sox5, Tbr1, Fezf2 and Bcl11b transcription factors are expressed by neurons in deep layers of the cortex whereas Satb2, Cux1, Cux2, Pou3f2 and Pou3f3 are expressed by upper-layer neurons.

NeuroD2 is a member of NeuroD family transcription factors and play roles in controlling neurogenesis [8], inducing electrical excitability of the cells and promoting neurite growth [9]. This calcium-regulated bHLH transcription factor starts to be expressed in the developing mouse brain at embryonic day 10 and its expression by postmitotic neurons persists through adulthood. Importantly, together with NeuroD6, it is required for the formation of corpus callosum and anterior commissural fiber tracts [10]. A previous genome-wide chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) study of NEUROD2 revealed many target genes having role in layer identity determination of cortical projection neurons. One of such target gene was *Cux1*, of which the promoter and enhancer regions strongly associated with NEUROD2 and the protein expression levels decreased significantly when NEUROD2 was silenced. Therefore, *Cux1* is a direct target gene of NEUROD2 [11].

Cut like homeobox protein 1 (Cux1) is a member of Cux family transcription factors which are conserved in all metazoans and are involved in the control of cell proliferation and differentiation. Members of this family contain a Cut homeodomain and different numbers of Cut repeat domains for DNA binding in their structure. Expression of different isoforms of CUX1 are regulated in a cell-cycle dependent manner. Full-length CUX1 protein (p200) contains three Cut repeats and one Cut homeodomain and is expressed in early G1 phase of the cell cycle. It plays role in DNA repair mechanisms and transcriptional repression. In late G1 phase, it is proteolytically processed into smaller isoforms (p150, p110 and p90) which have distinct DNA binding properties and act as transcriptional activators or repressors.

Positioning and layer identity of the cortical neurons are also intrinsically regulated by certain transcription factors. *Cux1* is one such transcription factor which is specifically expressed by layer II-III and IV excitatory cortical neurons in adult brain. Besides being a marker for the superficial neurons in the cerebral cortex, *Cux1* regulates the dendritic morphology of these neurons. It significantly induces dendritic branching, by primarily affecting basal dendrites of upper layer cortical neurons. Moreover, it has role in increasing both the number and the density of dendritic spines formed by these neurons. Inducing dendritic growth, thus enabling the cells to integrate information from multiple axonal inputs, *Cux1* functions in modulating synaptogenesis.

Callosal projections are mainly formed by the myelinated axons of the neurons in layers II-III which project their axons contralaterally, i.e, to the other hemisphere. *Cux1* enables these interhemispheric connections of these upper layer neurons by transcriptionally regulating Kv1 voltage-gated potassium channels [12].

Although the roles of *Cux1* on regulation of dendritogenesis and corpus callosum (CC) formation have previously been identified, there is a lack of comprehensive data on how it manages these roles, i.e, which genes it regulates to maintain these functions. Studies of CUX1 transcription factor aiming to identify its target genes are limited to the experiments on non-neuronal cell lines. The complexity of gene regulatory networks can be resolved by genome-wide identification of the direct genes of transcription factors. Chromatin immunoprecipitation followed by sequencing (ChIP-seq) is a powerful high-throughput method to identify genome-wide transcription factor binding profiles. In order to analyze *in vivo* gene targets of CUX1 in the cerebral cortex and to have the closest insight to physiological conditions in the organism, I have performed the ChIP-seq experiment using embryonic mouse cerebral cortical tissues and revealed the genes that are regulated by CUX1 **(Figure 1.1)**.

Chapter 2 includes the review of the current literature on CUX1 protein and the explanation of its roles in cerebral cortical neurons. Chapter 3 contains the materials and methods used in the study. The results of the study are demonstrated in Chapter 4 and the implications of the results are discussed in Chapter 5.



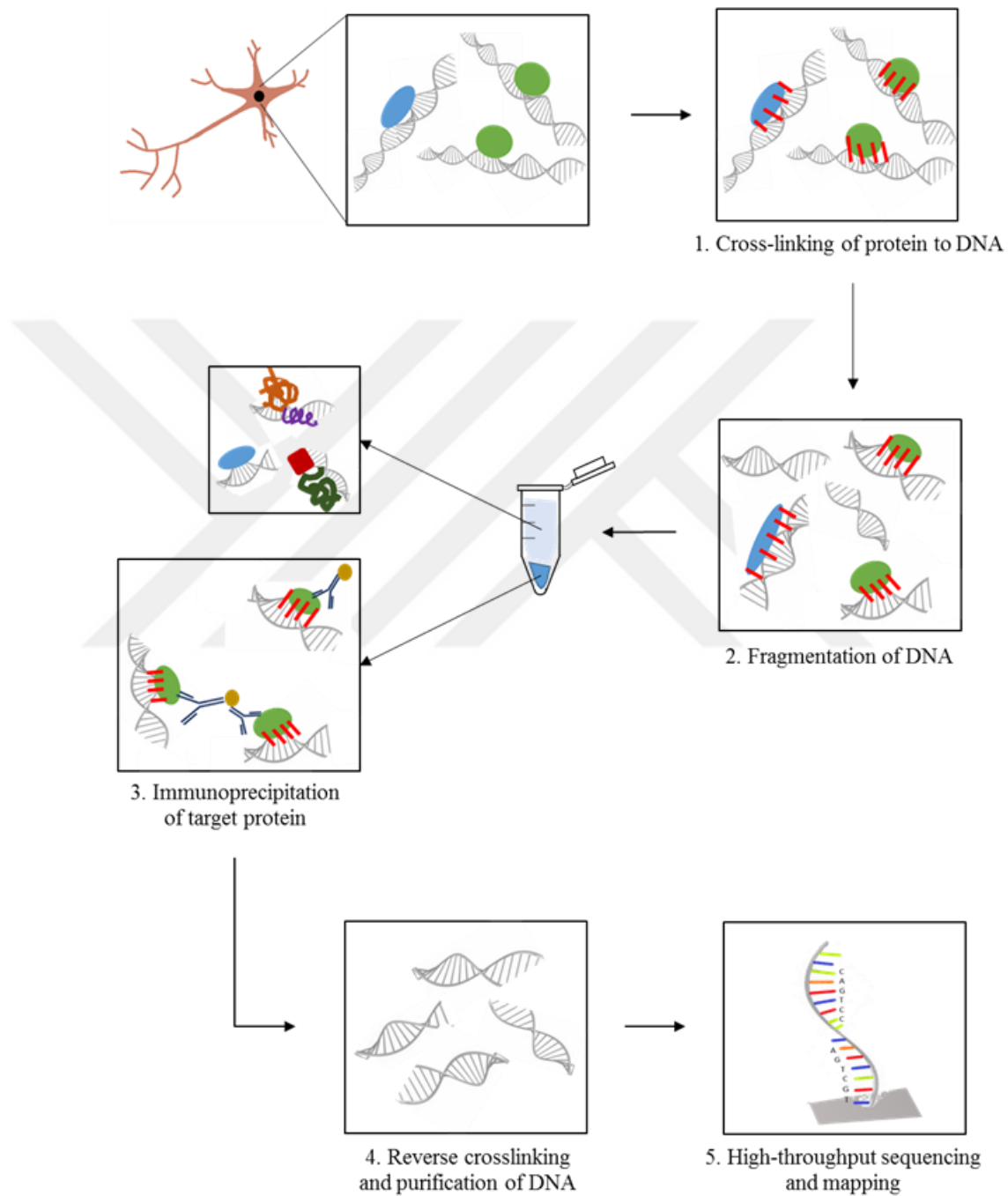


Figure 1.1: Chromatin Immunoprecipitation followed by Sequencing (ChIP-seq)

Chapter 2

LITERATURE REVIEW

2.1 Overview

The remarkably complex and highly organized mammalian central nervous system is fundamental for many sensory, cognitive and motor functions as well as for the proper development of the brain.

The ectodermal germ layer of the embryo derives three major domains: the externally positioned surface ectoderm (epidermis), the internally positioned neural plate and neural crest cells (peripheral neurons, pigment, facial cartilage) which connect the neural plate and the surface ectoderm [13]. Neural plate is the structure that eventually gives rise to the central nervous system. During the neurulation process it folds, the two dorsolateral apical surfaces fuse at the dorsal midline, and this way the neural tube is formed [13].

Telencephalon is the rostral-most region of the neural tube and it matures to the cerebrum. The mammalian dorsolateral telencephalon matures to the neocortex, the evolutionarily youngest part of the cerebral cortex. The cerebral cortex, which is an extensive sheet of neural tissue at the outermost layer of the brain, is responsible for many higher order cognitive, sensory and motor functions: sensation, voluntary movement, self-awareness, communication, language and information processing. The mammalian neocortex is organized into six horizontal layers parallel to the cortical surface. Neurons in each layer, including glutamatergic excitatory projecting neurons and GABAergic inhibitory interneurons, have unique molecular and physiological properties [14], [15].

There are two decisive processes that take place during early development and regulate the formation of laminar-dependent patterns of functional neocortical connectivity: the first is neuronal migration which indicates the movement of neurons from the ventricular zone, their birthplace, to their final position within the cortex, and the second is the ability of neurons to gain distinct neuronal identities and form connections [16].

Development of cerebral cortex starts with the formation of neural tube as a thin sheet of neuroepithelial cells. Cortical neurogenesis is initiated in mouse brain at embryonic day (E) 10.5, when the neuroepithelial cells change their division from symmetrical to asymmetrical and transform gradually to highly polarized radial glial cells (RGC) [17], [18]. Radial glial cells are the primary neuronal progenitor cells and are generated in the ventricular zone of the telencephalon such that their cell bodies reside within the VZ but their fibers span through the developing cerebrum [19]. During early development, RGCs undergo proliferative symmetric divisions to increase the number of progenitors, later at mid-neurogenesis (E13.5) they undergo asymmetric cell divisions each of which gives rise to one progenitor cell and one immature neuron or intermediate (basal) progenitor (BP) [20], [21]. During the development, RGCs have the capacity to differentiate into various types of cells. They can produce astrocytes and oligodendrocytes in the cortical plate, post-mitotic ependymal cells in the ventricle or neurons by keeping their neurogenic characteristics [22]. Immature neurons formed by the RGCs migrate radially towards the cortical surface by using the fibers of radial glial cells as a scaffold, and simultaneously differentiate into cortical projection neurons after migrating from VZ to outer cortical layers, whereas BP cells migrate to the subventricular zone (SVZ) and form more neurons by divisions [23]. Inhibitory cerebral cortical neurons are generated from the progenitors that reside in the ventral telencephalon and they reach to the cerebral cortex via different routes of migration [24]. Excitatory cerebral cortical neurons, on the other hand, migrate radially to reach the cortical plate. Early-born excitatory neurons dominate deep layers of the cortex whereas late-born

cells bypass the already existing layers and produce superficial layers, resulting with the inside-out characteristic organization of the cerebral cortex [25], [26]. As soon as neurons reach their final destination on the cortex they mature and establish connections with other neurons, giving the six-layered structure of the neocortex (**Figure 2.1**).

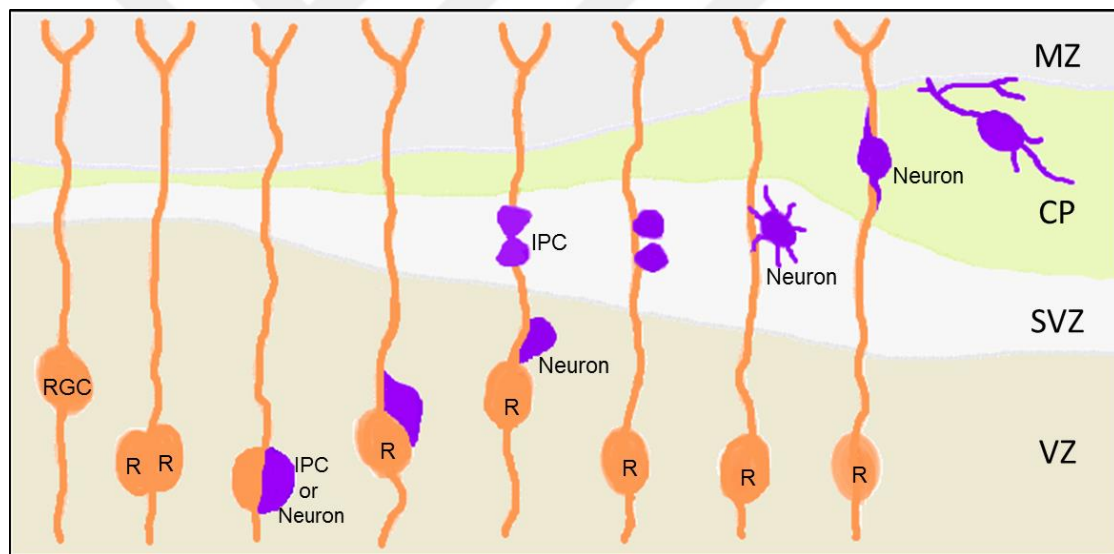


Figure 2.1: Neuronal migration and development of cerebral cortex

The projection neurons in each layer of the cerebral cortex differ from each other in their layer-specific and subtype-specific molecular expression and axon projection patterns [15]. Layer I, which is named the Marginal Zone (MZ), is the most superficial layer of the cortex and contains Cajal-Retzius neurons and the apical dendrites of pyramidal cells from lower layers. Layers II and III, termed the supragranular layers, contain excitatory projection neurons which project intracortically, either ipsilaterally (within the same hemisphere) or contralaterally (to the other hemisphere). These neurons contribute significantly to formation of the corpus callosum (CC), a bundle of fibers made of intracortical axonal projections and facilitating the connection between the two hemispheres [16]. Layer IV is termed the granule layer of the cortex as it contains pyramidal neurons and spiny stellate (granule) excitatory interneurons. Neurons in this layer connect to the axons of thalamocortical neurons from which they receive sensory information. Layer V projection neurons project their axons to brainstem, spinal cord, thalamus, striatum or cortex and most of the layer VI projection neurons contribute to the corticothalamic tract. Layer V and layer VI contain the largest pyramidal neurons and together make up the deep layers of the neocortex [16], [27].

Development of the cerebral cortex is intrinsically regulated by transcription factors. While some transcription factors guide the progenitor characters or differentiation of radial glial cells, some of them regulate the differentiation into specific neuronal or non-neuronal types.

Cell-specific basic helix-loop-helix transcription factors may direct both the progenitor character of precursor cells and differentiation of specific cell types. Hes and Id family of transcription factors are required for maintaining the progenitor cell identity [2]. Olig1/2 transcription factors direct the differentiation of oligodendrocytes from progenitor cells [3]. Furthermore, the proneural Neurogenin transcription factors direct the initiation of

neurogenesis [2], [4] and NeuroD family transcription factors, which are the final effectors of Neurog2, play role in controlling neurogenesis [28].

Gene expression studies have identified certain transcription programs which instruct neuronal positioning and layer identity [5], [6]. Expression of Sex determining region Y-box 5 (Sox5) is specific to layer V and layer VI projection neurons, from E14.5 to P7 [29], [30]. Other two transcription factors which are expressed by deep layer neurons are family zinc finger 2 (Fezf2, formerly Fezl or Zfp312) and B cell leukemia/lymphoma 11B (Bcl11b, formerly Ctip2), both of which are specifically enriched in layer V and are required for the subcortical axon development of these neurons [5], [31]. T-box brain factor 1 (TBR1) is selectively expressed by layer VI neurons starting from E12.5 and has role in many cortical developmental processes [32]. Expression of late-born cortical projection neurons, i.e the neurons which are positioned in the upper layers of the cortex are also controlled by certain transcription factors. Special AT-rich sequence binding protein 2 (SATB2), which regulates the development of upper layer neurons, is expressed by the cells in layer II-V [33]. SATB2 positively regulates the transcription of cut-like homeobox 2 (Cux2), cadherin 10 (Cdh10) and RAR related orphan receptor beta (Rorb), which are also layer specific markers of cortico-cortical projection neurons [33]. Cut homeobox proteins CUX1 and CUX2, which start to be expressed in the VZ and SVZ of the embryonic brain, are also known markers for layer II-III and IV neurons in the postnatal brain [34]. POU3F2 and POU3F3 are two other transcription factors controlling neuronal migration and neuronal identity of superficial neurons [35].

2.2 Glutamatergic Synapse Pathway

Cerebral cortical neurons can be categorized into two main groups according to the neurotransmitters they release: GABAergic inhibitory neurons and glutamatergic excitatory neurons. GABAergic neurons result with the inhibition of postsynaptic neurons whereas glutamatergic neurons activate the postsynaptic neurons by releasing glutamate: the major excitatory neurotransmitter in the nervous system. Glutamatergic synapses are crucial for maintaining or modulating the excitability of pyramidal neurons.

Glutamate receptors which are numerous and complex, are divided into two main categories: metabotropic or ionotropic. Ionotropic glutamate receptors are voltage-sensitive, fast-acting and once opened, can produce a large current change. When glutamate binds to an ionotropic glutamate receptor, the receptor undergoes a conformational change so that a sodium influx and potassium efflux are created and membrane depolarization is triggered in the postsynaptic cell, which is followed by the signal transmission. There are three types of ionotropic glutamate receptors: NMDA, AMPA and kainate receptors. NMDA receptors are mostly responsible for the early development of synapse whereas AMPA receptors have role in regulating the mature synapses [36]. Metabotropic glutamate receptors are ligand-gated receptors which act slower and generally exert their effects indirectly through gene expression and protein synthesis. These receptors have role in rendering the cells more excitable, regulating the neurotransmission process, and contributing to synaptic plasticity [37]. When glutamate binds to a metabotropic receptor, a post-synaptic membrane-bound G-protein becomes activated and triggers a second messenger system which then opens a membrane channel (different from the receptor itself) to enable signal transmission. Simultaneously, the activated G-protein also triggers functional changes in the cytoplasm which result in gene expression and protein synthesis. A summary of the glutamate receptor pathway is demonstrated in **Figure 2.2**.

Glutamate transporters are important players in the glutamate synapse pathway since they regulate glutamate concentrations at both the synapse and in extra-synaptic locations and this way ensure normal synaptic transmission and to prevent excitotoxicity [38]. Dendritic spines are post-synaptic protrusions which also help mediating glutamatergic synapses [39].

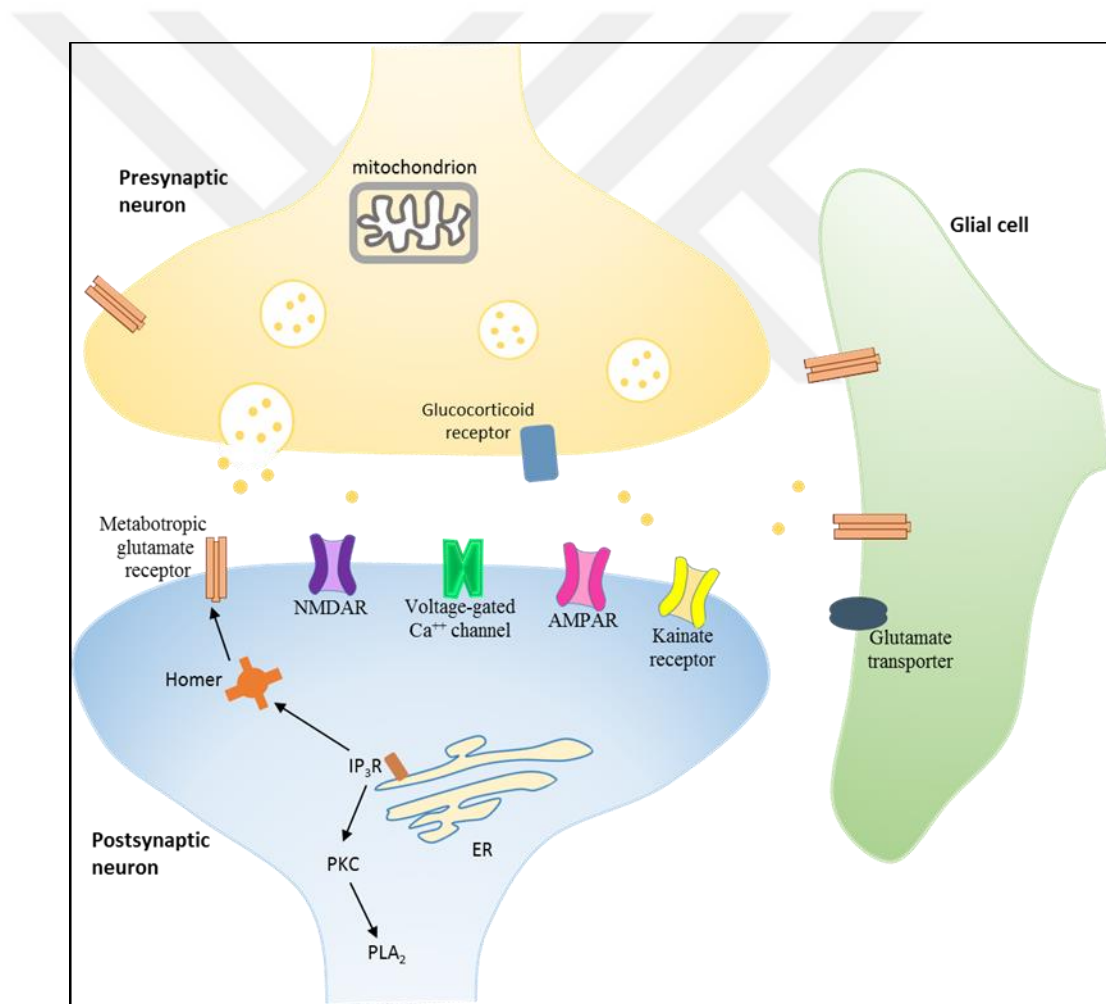


Figure 2.2: Schematic representation of glutamatergic synapse pathway

2.3 NEUROD2 Transcription Factor

NEUROD2 is a calcium-regulated basic helix-loop-helix transcription factor and is a member of NeuroD family which have significant roles in controlling neurogenesis [8], [40]. It has been shown that the proneural transcription factors Neurog1 and Neurog2 are upstream proteins promoting the expression of NeuroD2 [41], [42], which starts to be expressed at E10 and is expressed by the postmitotic neurons through adulthood. In the developing brain, NeuroD2 is expressed in the neocortex, cerebellum, amygdala, spinal cord and the brainstem whereas in the adult brain it is expressed in hippocampus and the layer where Purkinje cells reside [40], [43]. Multipotent mouse P19 embryonal carcinoma cells ectopically expressing NeuroD2 have been shown to exit cell cycle, induce electrical excitability and promote neurite growth [9].

Besides inducing neurogenesis in multipotent cells, NeuroD2 has a regulatory role in thalamocortical axon segregation [43]. NeuroD2 was also shown to regulate the maturation of glutamatergic synapses through the protein PSD95 [44]. Importantly, it was shown that both corpus callosum and anterior commissural fiber tracts were deficient in *NeuroD2-NeuroD6* double-mutant mice, because of dysregulation of axonal signaling receptors Cntn2 and Robo1 [10]. Thus, it is suggested that neuronal bHLH proteins like NeuroD2 provide transcriptional specification to the cortical projection neurons and may serve as regulators of cortical commissure formation [10].

2.4 *Cux1* is a direct target gene of NEUROD2

In a previous study, genome-wide targets of NEUROD2 at embryonic day 14 in mouse cortex and the major pathways regulated by NEUROD2 were identified [11]. Many of the target genes were found to have role in layer identity determination of cortical projection neurons. One of the major findings was that NEUROD2 associated strongly with the promoter and enhancer regions of *Cux1*, regulating its transcriptional activity. Furthermore, the expression of *Cux1* mRNA significantly decreased upon *Neurod2* knockdown in primary cortical neurons, concluding that *Cux1* is dependent on NEUROD2 [11].

2.5 Cux Family of Transcription Factors

CDP/Cux/Cut (CCAAT-displacement protein/cut homeobox) proteins are a family of transcription factors present in all metazoans and involved in the control of proliferation and differentiation. The term “cut” is derived from the “cut wing” phenotype observed in a mutant of *D. melanogaster* [45], [46]. They contain a Cut homeodomain and one or more ‘Cut repeat’ domains which enable DNA binding. Both full-length *Cux1* and *Cux2* contain three Cut repeats and a Cut homeodomain.

CUX1 is expressed as several isoforms which are named by their molecular weight: p150, p110, p90, p80 and p75, and have distinct DNA binding and transcriptional properties [47], [48]. p75 isoform is the product of a shorter mRNA that is initiated at downstream transcription start site within intron 20, whereas p90 and p110 isoforms are generated by proteolytic processing by cathepsin L and p150 isoform by cysteine protease. Interaction of the isoforms with DNA is determined by the DNA binding domains that are present on the proteins.

The expression and functions of CUX1 are regulated in a cell cycle-dependent manner [49], [50]. The full-length CUX1 protein (p200) is proteolytically processed by cathepsin L in late G1 phase, generating a short-lived N-terminal peptide and a more stable C-terminal peptide which can be of 110 or 90 kDa depending on the cell type [51], [52]. p200 CUX1 functions as a transcriptional repressor [53] and displays fast “on” and “off” rates of DNA binding in vitro, while the shorter isoforms p75, p90 and p110 can either repress or activate transcription depending on promoter context, exhibit significantly slower DNA binding kinetics and binding preference for ATCRAT sequences [51], [54], [55]. Moreover, these isoforms exhibit distinct biochemical activities that enable them to accelerate entry into S phase and stimulate cell proliferation [52], [55]. Post-translational modifications of CUX1 include phosphorylation, dephosphorylation, acetylation and proteolytic processing [50].

2.6 Expression Pattern of *Cux1* in Brain

Cux1 is widely expressed in several tissues in the embryonic and adult mouse [56]. In the embryonic mouse brain, it is expressed in the proliferative regions of the developing cerebral cortex, as well as in the emerging population of postmitotic upper layer neurons. At E14, the majority of cells in the cortical plate represent cells that are destined to form the deep cortical layers, most of which do not express *Cux1*. On the other hand, expression of *Cux1* is abundant in the VZ and SVZ [34]. At embryonic day 16, when a great proportion of newly born upper layer neurons undergo migration towards the cortical plate, *Cux1* is expressed by the cells migrating through the intermediate zone (IZ). Consequently, *Cux1* marks an early step in the acquisition of the neuronal identity of upper layer neurons. Because, it is expressed both by the precursor cell population and by migrating immature neurons which later form upper layer neurons [34] (**Figure 2.3**).

Expression of *Cux1* in the adult cortex is found in the majority of pyramidal neurons of layers II–IV but only in a few cells of the deep layers. Cells of the marginal zone (MZ) do not express *Cux1* [34]. Being almost exclusively expressed in layer II–III and IV neurons makes *Cux1* an ideal candidate as the regulator of their neuronal identity.



Figure 2.3: Expression pattern of *Cux1* in developing mouse brain at a) embryonic day 15.5, b) embryonic day 18.5, and in c) adult brain (P56) (Allen Brain Atlas).

2.7 *Cux1* regulates dendritic morphology of upper layer cortical neurons

CUX1 homeodomain transcription factor plays key roles in dendritogenesis in the postmitotic neuronal populations where it is expressed. *Cubelos et. al.* showed that cortical pyramidal neurons of *Cux1* knockout mice contained significantly shorter dendrites and less number of branches [57]. The same study also demonstrated that *Cux1* controls the number of dendritic spines and their morphological characteristics. *Cux1* induces dendritic growth and thus facilitates the ability of layer II–III neurons to integrate information from multiple axonal inputs. Therefore, it also has role in modulating synaptogenesis of superficial excitatory cortical neurons.

Although the changes in the expression of Cux proteins (CUX1 and CUX2) affect both apical and basal dendritic branching, modifying only CUX1 levels cause alterations mainly on basal dendrites, and likewise, modifying only CUX2 levels have a more profound effect on apical dendrites. Thus, two Cux paralogs have complementary functions on the dendritic morphology of upper layer neurons [58].

2.8 Cux1-regulated mechanisms are necessary for proper corpus callosum development

Corpus callosum (CC) is a wide bundle of neural fibers that connects the two hemispheres and facilitates communication between them. It is formed by myelinated axons projected by layer II-III neurons (80%) and layer V neurons (20%) [59].

Rodriguez-Tornos et. al. showed that, in the developing brain Cux1 transcription factor controls the formation of CC projections by transcriptionally regulating Kv1 voltage-gated potassium channels [12]. *Cux1* loss-of-function experiments resulted with decreased Kv1 transcript levels, atypical neuronal firing responses and impaired CC contralateral innervation [12].

2.9 Absence of *Cux1* causes reduction in dendritic branching and total dendrite length

Cux1 knockout mouse was created via homologous recombination, by introducing premature translational termination [60]. Although *Cux1* heterozygous animals were normal and fertile, homozygous ones were significantly smaller than their littermates in size, and most of them died after their first week. Additionally, the animals that survived to adulthood had normal life-span but were severely growth retarded, and they were more susceptible to bacterial infections [60].

The nervous system of *Cux1* knockout mice showed no specific phenotype. Their cortical morphology, the expression of the upper layer markers and that of *Cux2* homolog also remained unchanged [57]. On the other hand, *Cux1-Cux2* double KO mice showed early embryonic lethality where only a few animals could survive until birth. These KO mice did not show defects in neuronal migration or in the expression of layer-specific markers. In both of the KO models, morphologies of upper layer cortical pyramidal neurons were altered: they showed simpler morphology, decreased dendritic length, synaptic defects and, significantly reduced density and number of dendritic spines [57], [61]. Loss-of-function experiments of *Cux1* also resulted in dramatic reduction in dendritic branching and total dendrite length [57]. Therefore, *Cux1* modulates the formation of functional synapses.

2.10 Gene targets of CUX1

Various studies previously revealed some of the gene targets of Cux1 transcription factor. A genome-wide location analysis for p110 CUX1 using chromatin affinity purification (ChAP) identified cell cycle targets by ChIP and cell synchronization experiments and demonstrated various targets of CUX1, including Cyclin A2 (CCNA2), Cell division cycle 25A, B and C (CDC25A, CDC25B, CDC25C), Anaphase-promoting complex subunit 4 and 10 (ANAPC4, APC10), Kinesin family member 11 (KIF11) and Kinesin like 7 (KNSL7) [47]. Another study demonstrated two direct target genes of CUX1: p27^{kip1} (Cdkn1b) gene, showing binding at two separate sites in the promoter of the gene, and Grg4 (Groucho 4) gene, a known effector of Notch signaling, in newborn kidney tissue [62].

Xlr3b and Xlr4b genes, chromatin remodeling genes which cause cognitive defects [63], were shown as targets of both CUX1 and CUX2 transcription factors via an in silico analysis and ChIP assays in adult cortices and embryonic primary cortical cell culture. *Cux1* controls dendritic branching and spines partly by directly regulating the expression of these two genes [57].

A recent study has demonstrated two other genes that are transcriptionally regulated by Cux1 in cortical neuronal cells: voltage-gated potassium channels Kv1.1 and Kv1.3 [12]. Opening at voltages close to AP threshold and having role in limiting cell excitability and regulating distinct firing modes, Kv1 channels are crucial for the strong adapting behavior of P10–P12 upper layer neurons [64], [65]. Binding to Kv1.1 and Kv1.3, Cux1 instructs the switch to a Kv1-dependent firing mode which is crucial for interhemispheric innervation and thus regulates axonal connectivity by regulating the firing response of upper layer projecting neurons [12].

Chapter 3

MATERIAL AND METHODS

3.1 Chromatin Immunoprecipitation Followed by High Throughput Sequencing

Cerebral cortices from six Balb/c mouse embryos at embryonic day 18 were dissected in 1X ice-cold HBSS containing 100mM HEPES. Tissues were triturated three times in 10ml of 1X HBSS containing 10mM HEPES and transferred to petri dishes. A stock of 37% formaldehyde was added onto tissues drop by drop such that the final concentration of formaldehyde would be 1%. Tissues were crosslinked for 10 minutes at room temperature on slow shaker. 1M glycine was added onto the samples drop by drop such that the final concentration would be 125mM, in order to quench crosslinking reaction. Samples were incubated with glycine for 10 minutes at room temperature, on a slow shaker. Then, samples were taken into falcon tubes and centrifuged at 4000rpm at +4°C for 4 minutes. Supernatants were discarded and pellets were washed twice with ice-cold HBSS containing 100mM HEPES. At the last wash step, all samples are mixed together and then separated again, to achieve homogenously crosslinked samples in each tube. Then, samples were centrifuged last time at the same conditions, and the pellets were dissolved in 800µl of 1X RIPA-M (10mM Tris-HCl pH 8, 1mM EDTA, 0.5mM EGTA, 150mM NaCl, 1% Triton-X, 0.5% Na-DOC, 0.1% SDS, ddH₂O) containing 1 tablet of protease inhibitor cocktail (Roche Complete Mini, EDTA-free) and transferred into a 1.5ml Eppendorf tube.

Sonication was carried out in cold room (Bandelin Sonoplus HD2070) with the following conditions: power: 93%, output: 70%, time: 20 seconds. This method was applied

for 40 times while the samples were placed on ice for one minute between each sample to prevent over-heating of the samples and denaturation of the proteins. After the sonication part, samples were centrifuged at 14000rpm at 4°C for 20 minutes. Supernatants were collected into clean tubes and pellets were discarded.

100µl of the sonicated lysates were separated as the input sample and kept at +4°C. The rest of the samples were mixed together and separated once more in order to obtain homogenously sonicated lysates. 100µl of Protein A/G Magnetic Beads (Thermo Scientific, Cat. #88802) were washed with 1ml of 1X RIPA-M lysis buffer three times. Then, sonicated lysates were added onto the beads for pre-clearing. Pre-clearing of samples was carried out in cold room for one hour while continuously rotating. Next, lysates were transferred into clean tubes using a magnetic stand and antibodies were added. Lysate-antibody incubation was done in cold room, on a rotator from 2 hours to overnight. 75µl of magnetic beads were washed with 1X RIPA-M lysis buffer three times. Lysate-antibody samples were added onto these washed magnetic beads and the mixture was incubated in cold room for 1 to 1.5 hours. After the immunoprecipitation, supernatants 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 three different washing buffers for 15 minutes, three times with each buffer, in the cold room on a rotator. Then, the samples were washed once with the fourth wash buffer. (Wash Buffer 1: 200mM Tris-HCl pH 8, 2mM EDTA, 2mM EGTA, 150mM NaCl, 1% NP-40, 0.5% DOC, 0.2% SDS. Wash Buffer 2: 200mM Tris-HCl pH 9, 2mM EDTA, 2mM EGTA, 500mM NaCl, 1% NP-40, 0.5% DOC, 0.1% SDS. Wash Buffer 3: 50mM Tris-HCl pH 7.5, 2mM EDTA, 1mM EGTA, 0.5M LiCl, 1% NP-40, 0.7% DOC. Wash Buffer 4: 10mM Tris-HCl pH 8, 1mM EDTA). After the last wash, 200µl of elution buffer (10mM Tris-HCl pH 8, 10mM EDTA, 1% SDS) was added onto the samples and 300µl of elution buffer was added onto the input sample. The samples were incubated at 65°C on a shaking heat block at 700rpm for one hour to reverse the

crosslinking. The eluate was separated from the beads on a magnetic stand and was incubated at 65°C heat block by shaking for 12-15 hours. After the incubation, 2µl of RNaseA (Thermo Scientific, Cat. #R1253) was added onto the samples and they were again incubated at 37°C for one hour. 5µl of Proteinase K in 20mg/ml concentration (Thermo Scientific, Cat. #EO0941) was added and samples were incubated at 50°C on heat block for two hours.

Phenol-chloroform extraction was performed for the purification of ChIP DNA samples. 250µl of phenol-chloroform solution was added onto the samples and mixed slowly for 10 minutes on a rotator. Then, the samples were centrifuged at 14000rpm for 5 minutes at 4°C, followed by the transfer of upper layers (aqueous phase) into clean eppendorf tubes. The extraction step was repeated. Then, Na-acetate pH 5.2 was added to the tubes in the amount of 1/10 volume and 100% isopropanol was added in the amount of 3X volume of samples. The samples were then gently mixed and incubated at -20°C from 2 hours to overnight. Next, samples were centrifuged at 14000rpm for 20 minutes at 4°C and supernatants were discarded. Pellets were first washed with 70% ethanol, twice, and then air-dried under the hood. Upon ethanol evaporation, pellets were dissolved in 30µl of ultrapure (RNase and DNase free) water and the obtained ChIP-DNA was kept at -20°C.

ChIP-PCR was performed for verification of the ChIP experiment. For two of the previously published candidate genes, *Kcna1* and *Cdkn1b*, forward and reverse primers (APPENDIX) were designed. PCR experiments were carried out in 50µl mixture containing 5ng of ChIP-DNAs as templates, 1X *Taq* Buffer with KCl, 0.5µM of forward and 0.5µM of reverse primers, 200µM dNTPs, 2mM MgCl₂ and 2.5 Unit *Taq* DNA Polymerase enzyme (Thermo Scientific, Cat. #EP0402). PCR conditions for denaturation, annealing and extension were as follows: 95°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds and repeated for 33 cycles for *Kcna1* primers and for 37 cycles for *Cdkn1b* primers. The

samples were run on 2% agarose gel at 110V for 50 minutes by electrophoresis and visualized under UV-light box.

Table 3.1: Antibodies used in Immunoprecipitation

	Antibody	Host	Company	Cat. #	Dilution Ratio
Ab (1)	CDP	Rabbit	SantaCruz	SC-13024	1:75
Ab (2)	CUTL1	Mouse	Abcam	ab54583	1:250
	tGFP	Mouse	Origene	TA150041	1:125

3.2 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 and two sets of ChIP DNA with Cux1 antibody 1 and GFP. The bioinformatics analysis of ChIP sequenced data was performed using Galaxy online server (usegalaxy.org).

The five files (Set1 Cux1_ChIP DNA, Set1 GFP_ChIP DNA, Set2 Cux1_ChIP DNA, Set2 GFP_ChIP DNA and input DNA) were first uploaded to Galaxy server. Then, they were converted to Sanger quality scores type using FASTQ GROOMER with the following parameters:

NGS Toolbox Beta

NGS: QC and manipulation

FASTQ Groomer

Selection of files to groom

Input FASTQ quality scores type: Sanger & Illumina 1.8+

EXECUTE

Then, 50bp reads were mapped to the mouse genome using Bowtie, an ultrafast and memory-efficient short read aligner. Mouse (*Mus Musculus*) mm10 was selected as the reference genome. Single-end option was selected for the library. All alignments were suppressed if more than one reportable alignment existed except the alignment that matched the best:

NGS Toolbox Beta

NGS: Mapping

Map with Bowtie Illumina

Use a built-in index

Reference genome: Mouse (*Mus Musculus*) mm10

Is this library mate-paired: single-end

Selection of Fastq files

Bowtie settings to use: Commonly used (default)

Suppress the header in the output SAM file: No

EXECUTE

In order to identify the regions in the genome which are enriched by aligned reads, peak calling was performed using ‘MACS2 callpeak: Call peaks from alignment results’ using the following parameters:

NGS Toolbox Beta

NGS: Peak calling

MACS2 callpeak: Call peaks from alignment results

Selection of ChIP-seq treatment (set1 and set2 Cux1) and control (set1 and set2 GFP) files

Are your inputs Paired-end BAM files? No

Effective genome size: M. musculus (2,150,570,000)

Band width for picking regions to compute fragment size: 200

M-fold settings: Lower M-fold bound: 5, Upper M-fold bound: 50

Peak detection based on: p-value

p-value cutoff for peak detection: 0.001

Build Model: Build the shifting model

Outputs: peaks as tabular file, peak summits, scores in bedGraph files, summary page (html)

Advanced options: hide advanced options

EXECUTE

3.3 Quantitative PCR (qPCR) with ChIP DNA template

Equal volumes of ChIP and input DNA were used in ChIP-qPCR experiments. 1µl of 1:5 diluted ChIP DNA sample was used for quantitative PCR experiments. Primers were designed to amplify 200-400 base pair regions of the targeted part of the genome were used in final concentration of 3µM. Luminaris HiGreen qPCR Master Mix (Thermo scientific, Cat. #K0991) was added to reaction solution, and dH₂O was added to reach final concentration of reactants. Reaction mixtures were prepared on ice and kept away from the light. Log₂5(~2.32) was subtracted from the Ct value read from input sample to get the adjusted input Ct value, as correction for 1:5 dilution. Normalization of ChIP DNA data was performed by the following equation: $100 \times 2^{(\text{Adjusted input Ct} - \text{ChIP Ct})}$.

3.4 Cloning of *Cux1*-targeting shRNAs into pSUPER vector

pSUPER vector system (OligoEngine, Cat. #VEC-PBS-0006) was used for the preparation of the shRNA expressing plasmids. Oligonucleotides were designed as 60mers where each oligonucleotide contained 19 base-pair mRNA target sequence and the perfect complement sequence of these oligomers formed a hairpin RNA. The loop regions, which allowed the formation of a hairpin by the RNA, was centered in the oligomer sequence between the complementing target sequences. Oligonucleotides contained sequences that were complementary to the sequences of restriction enzyme cut sites to allow the cloning

into the pSUPER-neo-GFP vector. Oligonucleotides were complementing with their pairs perfectly except the flanking sequences that are left as sticky ends to allow for the ligation into the digested pSUPER-neo-GFP vector.

In the cloning of shRNAs into pSUPER-neo-GFP vector, 300µg of the vector was first digested with 10 units of BglII (New England Biolabs, Cat. #R0144S) and 10 units of HindIII (New England Biolabs, Cat. #R0104S) enzymes, in 1X NEBuffer 3 (New England Biolabs, Cat. #B7003S). Digestion was performed for 3 hours at 37°C. The reaction was then incubated at 80°C for 20 minutes to allow the inactivation of the restriction enzymes. Simultaneously, oligonucleotide annealing was performed by mixing 1.6µl of oligonucleotide pair (100µM) in 46.8µl oligonucleotide annealing buffer (100Mm NaCl and 50mM HEPES pH 7.4 in ddH₂O). The oligonucleotide mixture was incubated at 94°C for 4 minutes, and gradually cooled to 90°C and incubated for 4 minutes again. This slow-cooling process was repeated by decreasing the temperature 5°C at each step and incubating the sample at each temperature for 4 minutes, until the temperature reached to 40°C. After this last 4-minute-incubation, samples were incubated at 37°C for 20 minutes. Next, both digested vector and annealed oligonucleotides were immediately used for ligation which was done by mixing 1µl of digested pSUPER-neo-GFP vector, 2µl of annealed oligonucleotides in 5µl of distilled water, and 5 units of T4 DNA ligase (Thermo Scientific, Cat. #EL0014) added together with 1X T4 DNA ligase buffer (Thermo Scientific, Cat. #B69). Non-annealed oligonucleotide sample was added as the negative control sample. The ligation reactions were incubated either at room temperature for 2 hours, or overnight at 16°C.

Ligation product was transformed to into chemically competent DH5a *E.coli* cells via heat-shock method. Briefly, 50 µl of competent bacteria was taken from -80°C store and melted on ice until the frozen cells thawed. 1µl of ligation product added onto cells and cells were incubated for 10 minutes on ice. Cells were then heat-shocked for 1 minute at 42°C

using the heat block. After the heat shock, 500 μ l LB was added on the cells and the samples were incubated at 37°C for 1 hour. Cells were plated on LB-Agar plate containing the antibiotic that the plasmid have resistant gene for. Plates were incubated overnight at 37°C. The next day, several of the colonies formed on the plate were picked up and grown in 5ml of LB media containing their respective antibiotics. These single colony cultures were used for purposes including plasmid DNA isolation, maxiprep culture start or glycerol stock preparation.

The plasmid DNA of the bacteria was then isolated by using GeneJET Plasmid Miniprep Kit (Thermo Scientific, Cat. #K0503). Diagnostic digestion was performed to determine the positive miniprep samples using 6 units of EcoRI-HF (New England Biolabs, Cat. #R3101S) and 6 units of HindIII-HF (New England Biolabs, Cat. #R3104S) enzyme with 1x CutSmart (New England Biolabs, Cat. #B7204S) and 5 μ l isolated plasmid DNA. Reactions were incubated for one hour at 37°C. After the incubation, 6x gel loading dye (New England Biolabs, Cat. # B7021S) was added to reach 1x final dilution. Samples were loaded on 1.5% agarose gel stained with ethidium bromide. The loaded gel was run at 120V for 40 minutes, and imaged under UV light. The negative results, i.e samples which do not contain the oligonucleotide pair in the vector, would give a band with 240bp size whereas the positive samples, i.e samples where the oligonucleotide pair was ligated to the vector, would result with a band with 281bp size. One of the LB culture samples having the correct size of insert was selected and the presence of the designed oligonucleotides was confirmed by Sanger sequencing. After the confirmation, maxiprep culture was started in 150ml LB containing 150 μ l ampicillin (1000X) by the addition of 1.5 ml of sample culture, so that a high concentration and transfection-quality plasmid would be obtained. Following the overnight incubation of the maxiprep culture at 37°C, PureLink Maxiprep kit (Invitrogen, Cat. # K210006) was used for the maxiprep protocol and the isolated plasmid DNA was stored at -20°C.

3.5 Cloning of Cux1 cDNA into pcDNA4a vector using TOPO vector

To amplify the Cux1 cDNA, a PCR reaction was set up using the cDNA which was previously synthesized from E18 Balb/c brains by isolating their total RNA. The reaction mixture contained 5X Phusion HF buffer, 2.5mM dNTPs, 10mM of forward and reverse primers, cDNA template, Phusion polymerase enzyme, and ddH₂O. The PCR method was 2 minutes at 98°C followed by 15 seconds at 98°C, 30 seconds at 60°C and 2 minutes at 72°C, which were repeated for 40 cycles.

PCR-amplified Cux1 cDNA was first cloned into Topo vector to ease the cloning into the final pcDNA4a vector. The first step was generation of the 3' Adenine overhangs post-amplification, since the PCR that was set by Phusion polymerase enzyme generated blunt-end products. The reaction mixture was prepared with 18µl of purified PCR product, 2mM dNTPs, 10X Taq Buffer containing KCl, 25mM MgCl₂, Taq DNA polymerase enzyme and ddH₂O, and the mixture was incubated for 2 minutes at 95°C followed by an incubation for 5 minutes at 50°C. The ligation reaction was immediately set up since 3' Adenine overhangs were not stable. Accordingly, TOPO cloning reaction was carried out using 1µl of PCR product, 1µl salt solution, 1µl Topo vector and 2µl ddH₂O which was used from the kit. The ligation reaction was incubated 30 minutes at room temperature and then transformed into competent cells. The next day, colonies were selected and incubated in 5ml LB with ampicillin, which is also incubated overnight at 37°C. Then, miniprep and diagnostic digestion (with 10 units of EcoRI enzyme) were performed. Samples were run on 1.5% agarose gel and the ones showing the expected bands were used for subcloning of Cux1 cDNA into pcDNA4a vector.

A new PCR reaction was designed for Cux1 sub-cloning, in which, the reaction mixture contained 5X Phusion HF buffer, 2mM dNTPs, 10mM of forward and reverse primers, 0.5µl of Phusion polymerase enzyme, 10ng of Topo-cloned cDNA and ddH₂O. The

PCR method was as follows: 2 minutes at 98°C, followed by 15 seconds at 98°C, 30 seconds at 62°C and 2 minutes at 72°C, which were repeated for 40 cycles. 5µl of Cux1 PCR product was digested using 5 units of XbaI (New England Biolabs, Cat. #R0145S) and BamHI-HF (New England Biolabs, Cat. #R3136S) enzymes in the presence of 2µl of CutSmart buffer (New England Biolabs, Cat. #B7204S), incubating the mixture at 37°C for one hour. Then, the digested PCR product was ligated into pcDNA4a vector by preparing a ligation reaction which contains 5ng of Cux1, 5ng of pcDNA4a vector, 5 units of T4 DNA ligase (Thermo Scientific, Cat. #EL0014) added together with 1X T4 DNA ligase buffer (Thermo Scientific, Cat. #B69), incubated at room temperature for 2 hours. After transformation, miniprep and diagnostic digestion experiments, the sequence of Cux1_cDNA was confirmed. Then, the maxiprep of the cDNA is prepared and the DNA is stored at -20°C.

3.6 Transfection of Neuro2A and HEK293T cell lines using Lipofectamine® 2000 reagent

Neuro2A cell line (N2A) was grown in MEM (Gibco Life Technologies, Cat. #31095029) that contained 5% FBS (HyClone), 1% P/S (Gibco Life Technologies, Cat. #15140122) and 0.5% L-Glutamine (HyClone). At the confluency of 70%, transfection was performed using Lipofectamine® 2000 reagent. 5µl of Lipofectamine® 2000 reagent was added onto 150µl OPTI-MEM®I and a total of 5µg DNA was added onto 150µl OPTI-MEM®I for each well of the 6-well plate. (12µl of Lipofectamine® 2000 reagent, 300µl OPTI-MEM®I and a total of 10µg DNA was added for 60mm plates.) After 5 minutes of incubation at room temperature, the two solutions were mixed together and the solution is incubated 20 minutes at room temperature. 300µl of the mixture was added onto each well of the plate drop by drop. The plate was then incubated in the tissue culture incubator.

HEK293T cell line was grown in DMEM (Gibco Life Technologies, Cat. #11960044) containing 5% FBS (HyClone), 1% P/S (Gibco Life Technologies, Cat. #15140122) and 0.5% L-Glutamine (HyClone). The same transfection protocol was applied to HEK293T cells.

3.7 Plate coating for primary neuronal culture

The coating solution was prepared with 0.6 μ M Poly-L- lysine Hydrobromide (Sigma Life Sciences, Cat. #P9155), 15 nM Mouse Laminin (Millipore, Cat. #CC095) and 1X PBS. For immunofluorescence experiments, autoclaved and ethanol-washed cover glasses (Heinz Herenz Medizinalbedarf GmbH, Cat. #1051204) were placed into the wells of 24-well plates before coating solution was applied. For other experiments, coating solution was directly added to each well of the plate. For 24-well plates, 500 μ l of coating solution was added to each well. Plates were incubated for 12-16 hours at 37°C, 5% CO₂ in the tissue culture incubator. After 16 hours, coated plates were washed three times with ddH₂O, and air dried in the tissue culture hood. If not used immediately, plates were kept at +4°C for later use up to 2 weeks.

3.8 Primary Neuronal Culture Preparation

All equipment used in this experiment was ethanol-sterilized before the operation. Embryos were removed from time-pregnant (between embryonic day 14 to 18 (E14-18)) mice which is euthanized by cervical dislocation. Heads of the embryos were removed immediately and kept in ice-cold 1X Hanks Balanced Salt Solution (HBSS, Gibco, Cat. #14185-045) with 10mM HEPES (pH 7.4). Brains of the embryos were taken out of the skull,

and telencephalic hemispheres and meninges were separated from the brain, respectively. Hippocampus and olfactory bulbs were taken out, and cortex was separated. The dissected cortices were taken using a glass pipette and incubated in the enzyme solution containing cysteine (3.2mg) and 200 unit papain (Worthington, Cat. # LS003126) 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 ddH₂O) for 10 minutes at 37°C. The enzyme solution was gently mixed at the 5th minute of the incubation time. Cortices were then transferred into the light inhibitor (10mg BSA and 10mg trypsin inhibitor in 10 ml dissociation media, pH-adjusted with 0.1N NaOH and filtered) and incubated for 1 minute at room temperature. Then, they were transferred into the heavy inhibitor (50mg BSA and 50mg trypsin inhibitor in 5 ml dissociation media, pH-adjusted with 0.1N NaOH and filtered) and were also incubated for 1 minute. Tissues were gently washed twice in freshly prepared BF solution (5%FBS added to BME (Lonza, Cat. #BE12-105F) containing 1% P/S and 1% Ultra-glut (Lonzo, Cat. #BE17-605E/U1)). Washed cortices were taken into another falcon containing BF, and triturated using a serological pipette several times for mechanical dissociation of the cells from the cortex tissue. After trituration, sample was let for settling down of large tissue fragments. The upper part of the sample was taken and passed through cell strainer. Cells were placed on a hemocytometer and counted. 300.000 cells were seeded in 0.5 ml BF for one well of a coated 24-well plate. All the washing, trituration and counting steps of the cortices were performed in BME without FBS only for the nucleofection experiment. Cells were put into the cell culture incubator for 2 hours. After 2 hours, BF media was removed and 0.5 ml BNBF (BF media containing 1x N2 (Gibco Life Technologies, Cat. #17502-048) and 1x B-27 (Gibco Life Technologies, Cat. #12587-010)), was added to each well. Two days after, the culture media was changed by removing half of the media from the wells and replacing it with freshly prepared BNBF. This procedure was repeated until cells were collected at certain DIV for following experiments.

3.9 Transfection of primary neuronal culture by using Lipofectamine® 2000 reagent

Cells were plated into 24-well plates on Poly-D-lysine-treated support. Transfection of cortical neurons was performed at 2 days in vitro (2DIV) with 1µg of DNA, using 2µl Lipofectamine 2000 (Invitrogen). Following the transfection protocol, the media in the wells was replaced with 500µl Opti-MEM®I and collected into a new falcon tube for incubation at 37°C. The plate with 500µl Opti-MEM®I was put into the tissue culture incubator. Another solution was prepared by dissolving 1µg of DNA in 250µl Opti-MEM®I for each well of a 24-well plate. This solution is incubated for 10 minutes at room temperature. Then, these two solutions were mixed with each other and incubated for 20 minutes at room temperature. After this incubation, 500µl of the mixture was added dropwise into each well. The plate was incubated in the 37°C incubator for 2 hours. Then, the Opti-MEM®I was aspirated and 1 ml of fresh media (50% BDNF + 50% conditional media) was added into the well. The plate was incubated for 2 days in the tissue culture incubator (37°C/5%CO₂) before analysis.

3.10 Immunofluorescence of primary neuronal cultures and Neuro2A cell line

Both in primary neuronal culture and Neuro2A cell line, cells were fixed two days after transfection with 500µl of 4% Paraformaldehyde (dissolved in ddH₂O with heating and stirring, adjusted pH to 7.2) being added to wells containing cells in media very slowly under hood and incubated at room temperature for 20 minutes. After fixation, PFA was aspirated and wells were washed three times with 1X cold PBS.

Coverslips were placed onto the parafilm laid inside a staining chamber. Each coverslip was blocked with blocking solution (3% BSA, 0.5% TritonX-100 in 1X PBS) for 45 minutes

at RT. Coverslips were then incubated with 50 μ l of primary antibody diluted in blocking solution for 2 hours at RT. Dilution ratios are indicated in Table 3.2. After the primary antibody incubation, coverslips were washed with 50 μ l of 1X cold PBS 3 times, each wash being 5 minutes. Coverslips were incubated with 50 μ l of secondary antibody for 1 hour at room temperature in dark. Coverslips were again washed with 50 μ l of 1X PBS for 5 minutes in dark once and then incubated with 50 μ l of DAPI (diluted 1:1000 in 1X PBS) for 10 minutes in dark. Coverslips were lastly washed with 50 μ l of 1X cold PBS for 5 minutes in dark. Then they were mounted using mounting media (Thermo Scientific, Cat. #TA-060-UG). Immunofluorescent images were captured using Nikon Eclipse 90i confocal microscope and NIS-Elements AR software.

Table 3.2: Primary antibodies used in Immunofluorescence

Antibody	Host	Company	Cat. #	Dilution Ratio
CDP	Rabbit	Santa Cruz	7076S	1:200
CUTL1	Mouse	Abcam	7074S	1:100
GFP	Chick	Aves Labs	1020	1:200
ND2	Mouse	Abcam	168932	1:500
ND2	Rabbit	Abcam	109406	1:1000

Table 3.3: Secondary antibodies used in Immunofluorescence

Antibody	Host	Company	Cat. #	Dilution Ratio
Donkey Anti-Mouse IgG (H+L) Alexa Fluor®488	Mouse	Invitrogen	A21202	1:1000
Donkey Anti-Rabbit IgG (H+L) Alexa Fluor®488	Rabbit	Invitrogen	A21206	1:1000
Goat Anti-Chick IgG (H+L) Alexa Fluor®488	Chick	Life Technologies	A11039	1:1000
Donkey Anti-Rabbit IgG (H+L) Alexa Fluor®594	Rabbit	Invitrogen	A21207	1:1000
Anti-Mouse Alexa Fluor®594	Donkey	Invitrogen	A21203	1:1000

3.11 Cell lysate collection from primary neuronal culture, Neuro2A and HEK293T Cell Lines

Cells were taken from the incubator and kept on ice during the experiment. Cell media was removed, and cells were washed once with 1X ice-cold PBS solution. 1X RIPA lysis buffer containing protease inhibitor was added to the cells and let for 10 minutes shaking on 4°C. 200µl of lysis buffer was used for lysate collection from each well in 12-well plate. Cell lysate was collected from the plate and transferred into an eppendorf tube. Lysates were centrifuged at 14000 rpm for 20 minutes, at 4°C. The supernatant was transferred into a clean eppendorf tube kept at -20°C for storage.

3.12 SDS-PAGE and Western Blot Analysis

5% stacking SDS-PAGE gel (5% Acrylamide-Bisacrylamide (29:1), 126mM Tris-HCl pH 6.8, 0.1% SDS, 0.1% APS, 0.1% TEMED) was prepared and added on top of 7.5% separating gel (7.5% Acrylamide-Bisacrylamide (29:1), 375mM Tris-HCl pH 8.8, 0.2% SDS, 0.2% APS, 2.5% TEMED) for CUX1 western blot analysis. Proteins of 7.5µg – 10µg were mixed in 1:1 volume with 2X SDS Sample Buffer (100mM Tris-HCl pH 6.8, 20% Glycerol, 4% SDS, 0.02% bromophenol blue, 2% β-mercaptoethanol). The mixture was boiled at 95°C for 5 minutes and then loaded into the wells of the gel. GangNam-STAIN™ Prestained Protein Ladder (Intron Biotechnology, Cat. #24052) was used as the protein ladder. Samples were run at 85V for 30 minutes and at 105V for 2 hours in 1L of 1X SDS Running Buffer pH 8.3 (10X buffer contained 30g Tris base, 187.6g Glycine, 1% SDS in a total volume of 1L). Gels were used for western blot analysis.

For western blot analysis, wet-transfer method which includes the transfer of proteins on the gel onto a PVDF – polyvinyl difluoride membrane – (Thermo Scientific, Cat. #88518) was performed using 1X transfer buffer (1.45g Tris Base, 7.25g Glycine, 150ml methanol in a total volume of 1L). Wet transfer was done in cold room either at 55mA overnight or at 110mA for 2 hours. After this step, membrane containing the proteins was incubated for one hour in blocking buffer (5% Nonfat Dried Milk Powder (AppliChem, Cat. #A0830) in TBS-T (10mM Tris-HCl, 150mM NaCl, 0.05% Tween)) for one hour. After blocking, the membrane was incubated with primary antibody diluted in blocking buffer either for 2 hours at room temperature, or overnight in cold room. Membrane was then washed three times with TBS-T, each wash taking 15 minutes, at room temperature. Next, the membrane was incubated with HRP-coupled secondary antibody which was selected against the primary antibody and diluted in TBS-T containing 5% non-fat dried milk powder for one hour at room temperature. Three time TBS-T wash were repeated at room temperature, for 15

minutes for each wash. Then, HRP reagent SuperSignal West Femto Maximum Sensitivity Substrate (Cat. #34096) was applied onto membrane and incubated for 3 minutes at room temperature. The blot was visualized under Gel Logic 2200 Imaging System (Molecular Imaging System CareStream Health Inc.) The image quantifications were done using ImageJ software.

Table 3.4: List of primary antibodies used in Western Blotting

Antibody	Host	Company	Cat. #	Dilution Ratio
CUTL1	Mouse	Abcam	ab54583	1:2000
CDP	Rabbit	SantaCruz	sc-13024	1:2000
Myc	Mouse	Millipore	05-724	1:1000
B-Actin	Rabbit	Thermo Scientific	PA5-16914	1:2000

Table 3.5: List of secondary antibodies used in Western Blotting

Antibody	Host	Company	Cat. #	Dilution Ratio
Anti-mouse IgG HRP-linked	Mouse	Cell signalling	7076S	1:2000
Anti-rabbit IgG HRP-linked	Rabbit	Cell signalling	7074S	1:2000

Chapter 4

RESULTS

4.1 CUX1 protein is expressed in mouse cortex at different developmental time points and by cortical neurons at different days in vitro

To validate the expression of CUX1 in the cerebral cortex and in primary cortical neurons, primary neuronal culture was performed using E14 mouse brains and neuronal lysates were collected at various days in vitro (DIV). Neuronal lysates were collected starting at 3DIV. After 8DIV, Ara-C was added into neuronal media to inhibit proliferation of glial cells. Then the expression levels of CUX1 were compared in each DIV (**Figure 4.1.a**).

The expression of CUX1 in primary cortical neurons was confirmed. The protein was expressed highest at 3DIV, which corresponded to the embryonic day 17 (E17). Its expression persisted through 15 DIV, the longest period at which lysates were collected.

CUX1 expression levels in mouse brains were also determined. Embryonic and postnatal brains at various developmental time points (E14, P0, P7, P14, P28, adult) were collected and homogenized. As a results of the SDS gel electrophoresis and western blot analysis, all tissues were shown to express various CUX1 isoforms. The highest CUX1 expression was detected at P7 and P14 brains (**Figure 4.1.b**).

Since neuronal migration is almost complete and CUX1-expressing cortical pyramidal neurons position at the superficial layers of the cortex towards the end stages of

embryonic development, I started collecting cortex tissues for ChIP experiment at embryonic day 18 (E18).

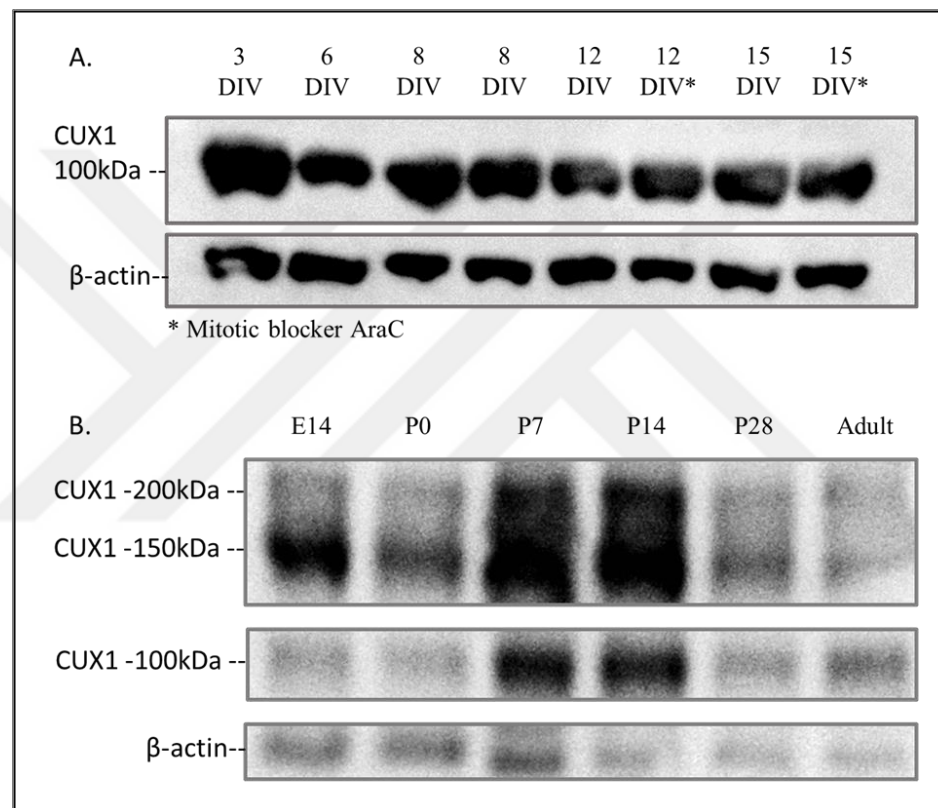


Figure 4.1: Endogenous CUX1 protein isoforms are expressed by cortical neurons in vitro and by cortex tissues during development. **A.** Primary cortical neuronal lysates express endogenous CUX1 protein at all DIVs. **B.** Various isoforms of endogenous CUX1 are expressed by Balb/c mouse brain tissues at all developmental time points (E14, P0, P7, P14, P28, adult). Beta-actin is used as the loading control.

4.2 CUX1- NEUROD2 immunostaining in primary cortical neurons and E18 brains

Primary cortical neurons were cultured at E14.5, fixed and stained with CUX1 and NEUROD2 antibodies and DAPI at 4DIV. Although most cells co-expressed CUX1 and NEUROD2, approximately 15% of the cells counted expressed only CUX1 but not NEUROD2 (**Figure 4.2.a, Table 4.1**).

In order to determine the expression pattern of CUX1 and NEUROD2 within the developing cerebral cortex, brain sections taken from E18 Balb/c embryos were co-stained against NEUROD2 and CUX1. It was observed that a population of neurons co-expressed CUX1 and NEUROD2. However, CUX1 mainly localized to the upper layers of the cortical surface and NEUROD2 positive neurons localized both in the cortical plate and within the intermediate zone constituting migratory population (**Figure 4.2.b**).

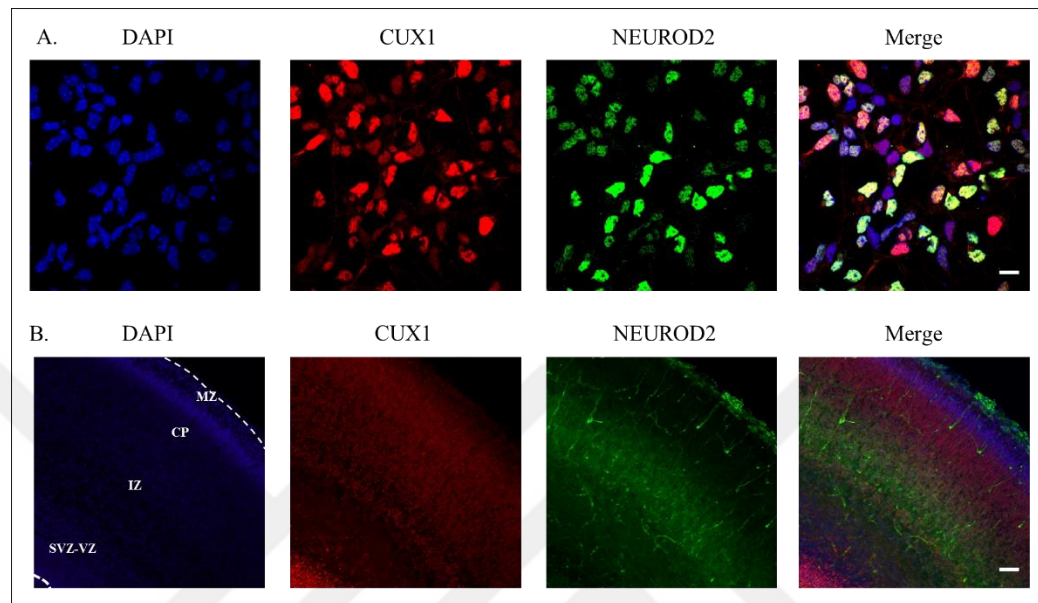


Figure 4.2: **A.** Immunofluorescent staining against CUX1 and NEUROD2, and DAPI staining on E14.5 primary cortical neurons. Most NEUROD2- and CUX1- expressing cells were overlapped. Scale bar, 50 μ m. **B.** Immunofluorescent staining against NEUROD2 and CUX1, and DAPI staining on coronal sections prepared from E18 mouse brain. NEUROD2 positive neurons are observed in the CP and IZ whereas CUX1 positive neurons are localized within the upper layers of the cortex. Scale bar, 50 μ m.

Table 4.1: Quantification of CUX1 and NEUROD2 expression in primary neurons. Quantification was done using ImageJ software. Total of 221 cells were counted from three images. 185 of all cells co-expressed CUX1 and NEUROD2, 33 cells expressed only CUX1 and 3 cells expressed only NEUROD2.

	Only Cux1(+)	Only ND2(+)	Cux1(+) ND2 (+)
1	24	-	82
2	8	-	40
3	1	3	63
Total	33	3	185

4.3 Chromatin Immunoprecipitation is verified via ChIP-PCR

The optimal DNA fragment size for high efficiency of sequencing in chromatin immunoprecipitation followed by sequencing experiments is in the range of 100-500bp. Thus, it is crucial to ensure that the input sample is successfully sonicated before proceeding with the immunoprecipitation steps.

To verify the efficient sonication, approximately 1000 ng of input sample was loaded on 1.5% agarose gel. Initial results showed that the genomic DNA could not be sheared into fragments that are short enough to continue the experiment (**Figure 4.3.a**). Experimental protocol was improved by increasing the number of sonication cycles from 30 to 40, using fresh phenol in the DNA extraction step, and using fresh formaldehyde for the crosslinking of DNA.

Following the improvements in the procedure, the genomic DNA was successfully sheared into fragments between the lengths of 100 – 250 bp (**Figure 4.3.b**).

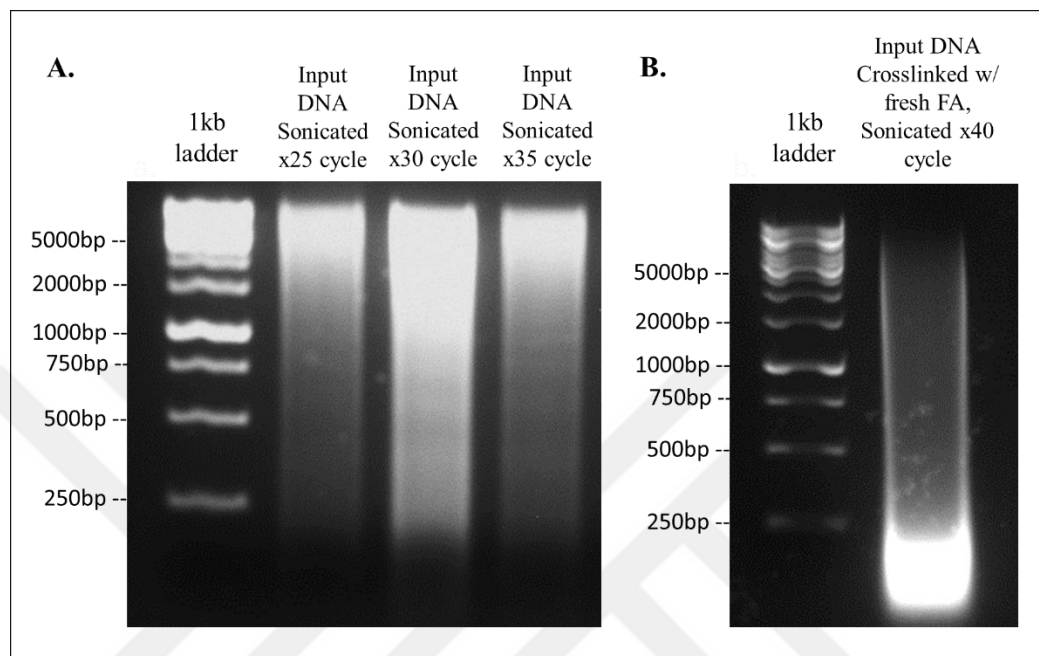


Figure 4.3: Verification of efficient fragmentation of the genomic DNA. **A.** Samples are sonicated for i: 25, ii: 30 and iii: 35 cycles. **B.** Sample is sonicated for 40 cycles using fresh formaldehyde for crosslinking of DNA.

In order to test the success of chromatin immunoprecipitation, ChIP-PCR was performed aiming to amplify the promoter regions of two of the previously known target genes of *Cux1*; *Kcna3* and *Cdkn1b* [12], [62]. Both promoters were enriched in ChIP samples from CUX1 antibody #2 (Abcam, Cat. #ab54583), but neither of them were amplified from CUX1 antibody #1 (SantaCruz, Cat. #SC-13024) or from anti-GFP antibody. Input sample was included in the PCR reaction as the positive control since it included whole genomic DNA (**Figure 4.4**).

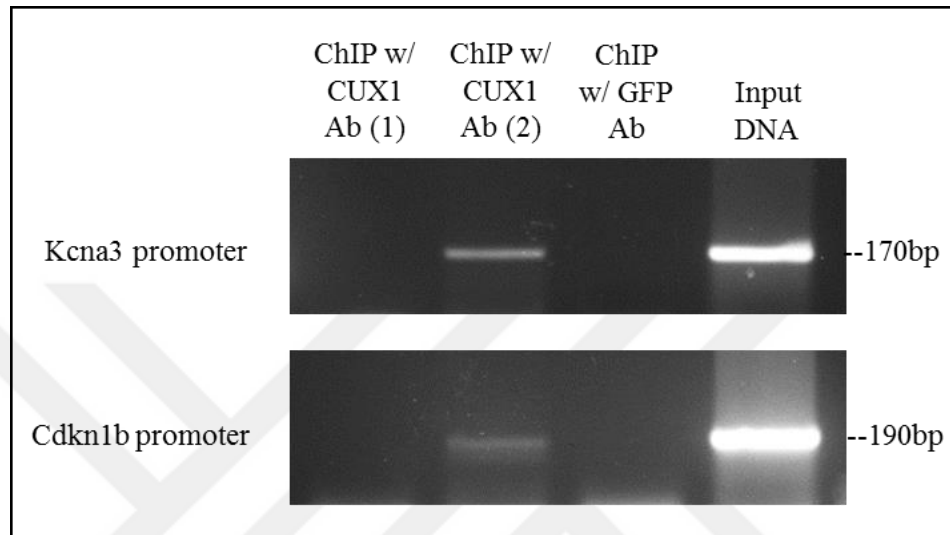


Figure 4.4: Verification of chromatin immunoprecipitation with two known targets of CUX1. **A.** Kcna3 promoter was amplified using the template DNA immunoprecipitated with Cux1 ab#2 but not using GFP ChIP-DNA. **B.** Cdkn1b promoter was amplified using the Cux1 ab#2-ChIP template DNA but not GFP ChIP-DNA. (Ab#1: CDP rabbit polyclonal antibody (SantaCruz, Cat# sc-13024), Ab#2: CUTL1 mouse monoclonal antibody (Abcam, Cat # ab54583)).

4.4 Quality scores of ChIP Sequencing were determined

Next generation sequencing was purchased from Genewiz, Inc. (www.genewiz.com). An Illumina HiSeq platform was used for single-end 50 bp sequencing. The total of five Fastq files received were as follows: input DNA, Set1: ChIP DNA with Cux1 and GFP, Set2: ChIP DNA with Cux1 and GFP.

Q score is a metric which is used for evaluation of the quality base calling of one particular run. Since high accuracy reading for each base is required in ChIP-seq, Q score is used for determination of the error probabilities in a logarithmical manner. Each ChIP-seq data shown in **Table 4.2** has a QC value greater than 35, meaning that the incorrect base calling probability is 1 out of 3200 calling, thus, base calling was performed with greater than 99.97% accuracy.

Table 4.2: Quality Analysis of CUX1 ChIP samples

Sample	Index	# Reads	Yield (Mbases)	% of Q scores ≥ 30	Mean Q Score
All samples	-	291.286.890	14,856	93.96	35.48
Set-1-Cux1	TTAGGC	59.741.910	3,047	94.07	35.5
Set-1-GFP	CAGATC	58.461.002	2,982	94.41	35.56
Input	CGATGT	52.749.215	2,690	92.85	35.27
Set-2-Cux1	TGACCA	60.013.726	3,061	94.29	35.54
Set-2-GFP	CCGTCC	60.321.037	3,076	94.2	35.53

4.5 Overview of Bioinformatics Analysis of High Throughput Sequencing

Sequenced ChIP data was analyzed using Galaxy online server (usegalaxy.org) in order to determine which regions in the genome were enriched in CUX1 binding. The summary of the bioinformatics analyses processed on the raw ChIP data to determine the target genes of CUX1 transcription factor is demonstrated in **Figure 4.5**.

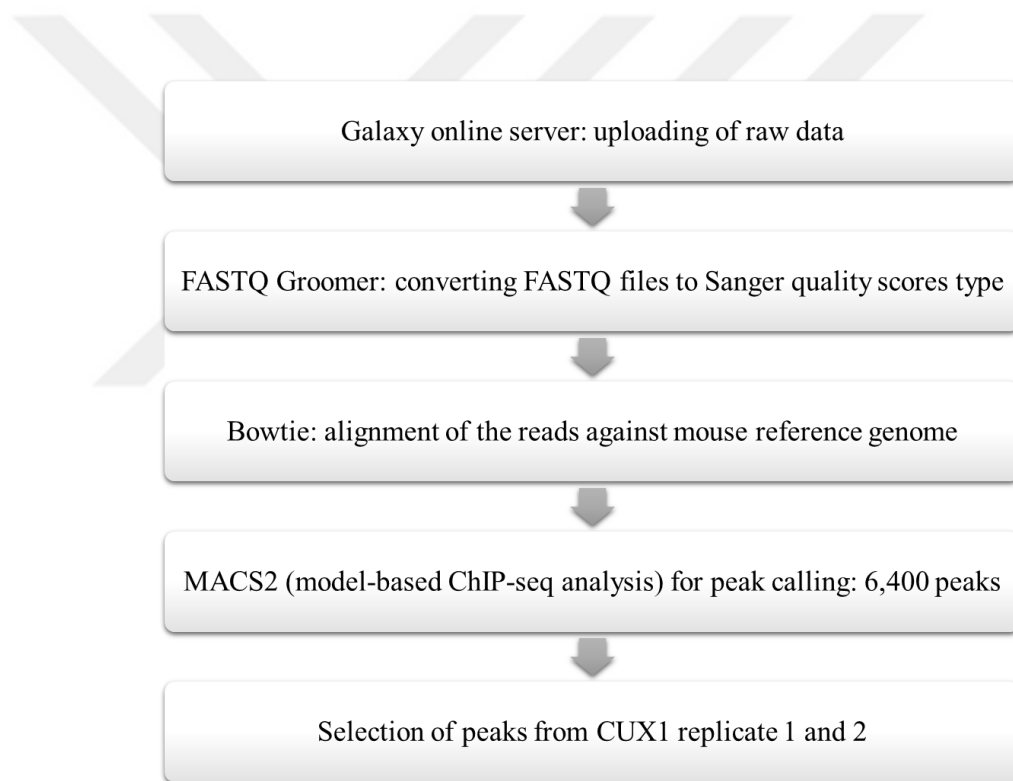


Figure 4.5: Flowchart of bioinformatics analysis of CUX1 ChIP-seq

4.6 Top targets genes of CUX1 transcription factor were identified

To determine the top target genes of CUX1 TF, FastQ samples which were uploaded to the Galaxy online server were converted to Sanger quality scores type using FastQ Groover program, and mapped against the reference genome (*Mus musculus*, mm10) using Bowtie program [66]. Then the peaks were called using Model-based ChIP-seq analysis 2 (MACS2) both for set1 and set2 data [67]. Ensemble IDs and gene IDs corresponding to the peak locations were identified using both whole transcript location and 1000 bp upstream of whole transcripts from mouse genome. The gene ID list was then sorted according to the p-values of the target genes. At $p=0,001$, replicate 1 data gave 544 peaks corresponding to 249 known transcripts whereas replicate 2 data gave 6,402 peaks corresponding to 3,368 transcripts in mouse genome. The difference in the number of genes obtained from two sets was large, and the two sets included very few, only 93, overlapping target genes (**Figure 4.6**). It was observed that a high amount of data coming from set2 data was absent from the overlapping genes. Therefore, in order not to miss any meaningful data from our analysis, we excluded the results coming from set1 and continued our analysis and confirmation experiments with only the data from set2 in the rest of the ChIP analysis procedure. Top 25 targets of CUX1 are listed in **Table 4.3**.

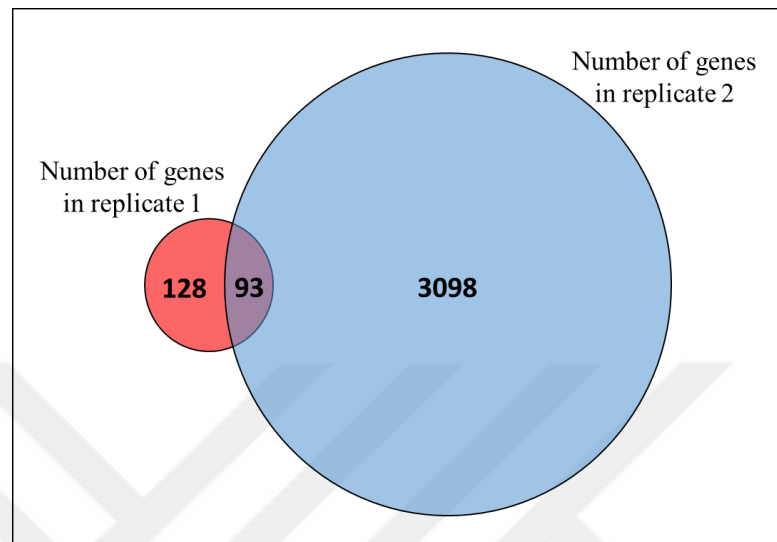


Figure 4.6: Venn diagram demonstrates the number of known transcripts obtained from each replicate. The number of transcripts overlapped from both replicates are shown as the intersection of two diagrams.

Table 4.3: Top 25 targets of CUX1 revealed by ChIP-seq

Peak ID	Chr.	start	end	p-value	Gene ID	Ensemble ID
484	chr10	22258137	22258205	0,000483	Raet1e	ENSMUST00000178026
2669	chr17	67741384	67741522	0,001132	Lama1	ENSMUST00000035471
2022	chr15	9016144	9016338	0,014993	Ranbp3l	ENSMUST00000053308
4742	chr5	146261248	146261295	0,0172	Cdk8	ENSMUST00000161652
4045	chr4	45193510	45193634	0,028321	Frmpd1	ENSMUST00000044773
4595	chr5	113233453	113233500	0,037232	Tmem211	ENSMUST00000086615
2021	chr15	9015687	9016033	0,037355	Ranbp3l	ENSMUST00000053308
4821	chr6	29748377	29748527	0,042406	Smo	ENSMUST00000001812
3366	chr2	105257923	105258310	0,047736	Them7	ENSMUST00000131060
132	chr1	63630456	63630688	0,05322	Dytn	ENSMUST00000090313
3382	chr2	110293651	110293698	0,053701	Bbox1	ENSMUST00000046233
4548	chr5	99643706	99643774	0,05546	A930011G23Rik	ENSMUST00000142639
5593	chr8	14307283	14307337	0,056742	Dlgap2	ENSMUST00000043279
3368	chr2	105258913	105259041	0,078442	Them7	ENSMUST00000131060
2506	chr17	7956197	7956257	0,080367	Rsph3a	ENSMUST00000097423
2403	chr16	57391594	57391641	0,081812	Filip1l	ENSMUST00000099667
2532	chr17	13552789	13552955	0,087058	Tcte2	ENSMUST00000137459
5497	chr7	128188419	128188476	0,092442	Itgad	ENSMUST00000177111
2529	chr17	13552123	13552243	0,095805	Tcte2	ENSMUST00000137459
3801	chr3	96844959	96845132	0,106557	Pdzk1	ENSMUST00000128789
2526	chr17	13550727	13550818	0,110143	Tcte2	ENSMUST00000137459
1716	chr13	113807010	113807084	0,116549	Arl15	ENSMUST00000091201
3137	chr2	20899883	20900003	0,117579	Arhgap21	ENSMUST00000140230
2491	chr17	3353294	3353342	0,118272	Tiam2	ENSMUST00000169838
3367	chr2	105258402	105258531	0,118272	Them7	ENSMUST00000131060

4.7 Distribution of CUX1 peaks among mouse genome

After the ChIP peaks obtained from Set2 data were identified, they were located in mouse genome using 'Join' tool on the Galaxy server. This tool serves for the identification of peaks that are intersecting at least 1 bp.

Intragenic regions were defined as the regions containing known transcripts whereas intergenic regions meant the regions which were not represented by any known transcripts (intragenic, 37%; intergenic, 62%). 1kb upstream of transcription start sites (TSSs) were included in intragenic regions as it contained the promoter regions (promoter, 1%). The peaks were categorized with respect to their positions on exons or introns (introns, 93%; exons, 7%) (**Figure 4.7.a**). Moreover, the peaks located on exons were further categorized by their positioning to 5'UTR, coding regions and 3'UTR regions (coding regions, 58%; 5' UTR, 9%; 3' UTR, 32%) (**Figure 4.7.b**).

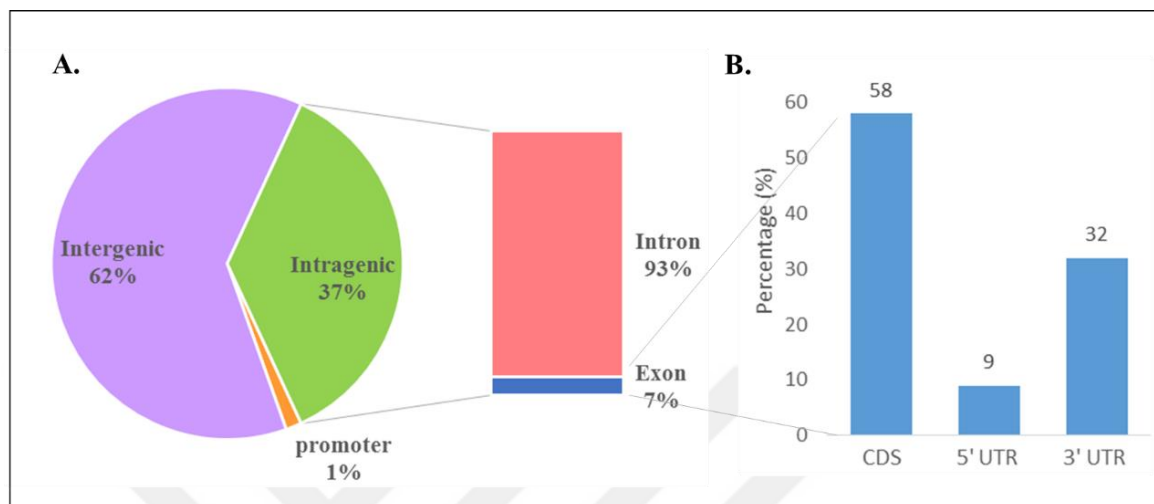


Figure 4.7: Distribution of peaks were identified among mouse genome. **A.** 4051 peaks were located in the intergenic regions, 95 peaks were located in promoter regions of transcripts and 2351 peaks were located in the intragenic regions of transcripts. Bar graph shows that 166 of the intragenic peaks belonged to exons whereas 2185 of those belonged to intronic sequences. **B.** Distribution of CUX1 binding within exons is demonstrated on the bar graph. 97, 15 and 54 peaks were located to coding sequences (CDS), 5' UTR and 3' UTR, respectively.

4.8 Consensus sequence prediction of Cux1

MEME-ChIP enrichment program, a motif analyzer of large nucleotide datasets (www.meme-suite.org/tools/meme-chip), was used to predict the consensus motif of Cux1 [Machanick and Bailey, Bioinformatics, 2011]. Although homeodomain-binding motif was previously identified as 'ATTA', CUX binding motif was not well-identified [Andres, 1993]. Two previous studies defined preferential binding sites of CUX proteins as 'ATCGAT'

consensus motif [Nepveu, 1994], [Andres, 1993]. However, since CUX family proteins contain more than one DNA-binding domain, studies showed broad and overlapping DNA-binding preferences. In this study, consensus motif analysis was made using all 6,402 peak regions, only promoter regions, intragenic regions and intergenic regions as input for the MEME-ChIP suite, separately. All of these analyses which were carried out using different parameters resulted with the same consensus motif sequence, 'CCCTAA', as shown in **Figure 4.8** and **Figure 4.9**.

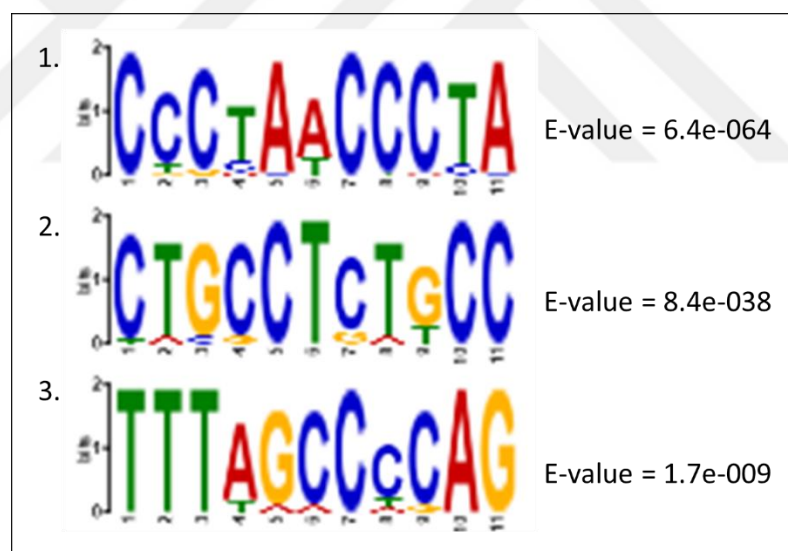


Figure 4.8: The three most significant motif sequences of CUX1, predicted by MEME-ChIP suite. All peaks were used as the template file.

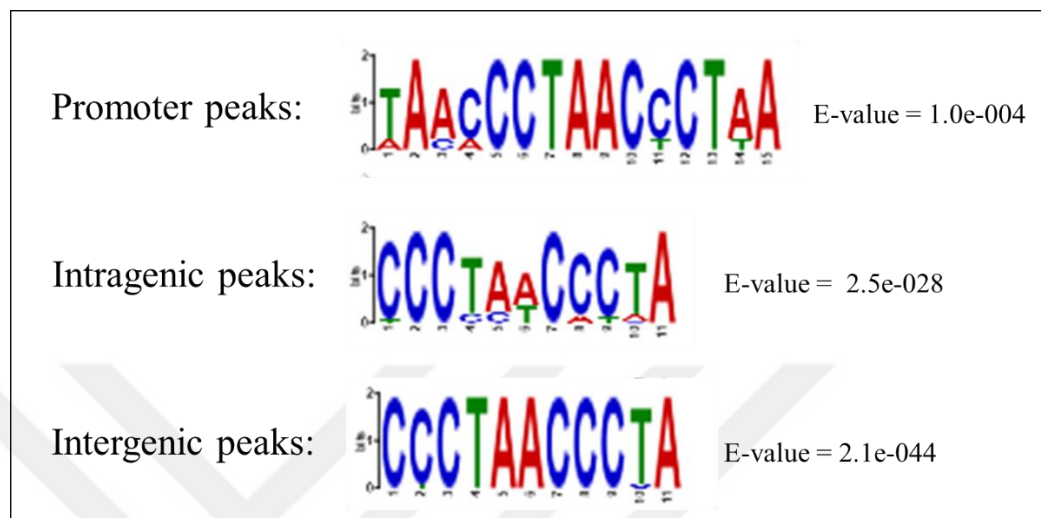


Figure 4.9: The most significant motif sequences of CUX1, predicted by MEME-ChIP suite. Promoter, intragenic and intergenic peaks were used as the template file, respectively.

4.9 ChIP-seq reveals Glutamatergic Synapse and Axon Guidance pathway genes targeted by CUX1

4.9.1 Functional classification of CUX1 target genes

To functionally classify the target genes of CUX1 transcription factor, we used the Database for Annotation, Visualization and Integrated Discovery (DAVID) 6.8 [68], [69]. Using DAVID database, we categorized target genes of CUX1 with respect to their molecular function (MF), biological pathway (BP) and cellular component (CC). The database calculated an enrichment score for each characterization based on the biological importance of each particular group in target gene list.

Table 4.4.a: Annotation cluster 1 based on Biological Pathway with 83% enrichment

Term	Count	p-value	Benjamini-Hochberg corrected P-Value
cell adhesion	97	9.5E-12	4.6E-8
protein phosphorylation	105	3.6E-10	8.6E-7
nervous system development	74	7.9E-9	1.3E-5
phosphorylation	102	7.8E-8	9.4E-5
transcription, DNA-templated	247	5.4E-7	5.2E-4
regulation of Rho protein signal transduction	24	6.5E-7	5.2E-4
calcium ion transport	34	1.5E-6	1.0E-3
ion transport	93	2.4E-6	1.4E-3
positive regulation of transcription from RNA polymerase II promoter	141	4.4E-6	2.3E-3
transport	234	5.1E-6	2.5E-3
axon guidance	34	5.4E-6	2.3E-3
intracellular signal transduction	67	1.4E-5	5.6E-3
regulation of membrane potential	25	1.4E-5	5.3E-3
cell differentiation	113	1.7E-5	5.8E-3
positive regulation of transcription, DNA-templated	88	2.3E-5	7.4E-3
calcium ion transmembrane transport	23	2.7E-5	7.6E-3
learning	19	4.1E-5	1.0E-2
actin cytoskeleton organization	30	9.3E-5	2.0E-2
neuromuscular process controlling balance	17	9.4E-5	1.9E-2
excitatory postsynaptic potential	13	9.6E-5	1.9E-2

Table 4.4.b.: Annotation Cluster 2 based on Cellular Compartments with 89% enrichment

Term	Count	P-value	Benjamini-Hochberg corrected P-value
membrane	907	1.7E-31	1.4E-28
synapse	130	5.0E-26	2.0E-23
cytoplasm	828	4.3E-22	1.1E-19
cell junction	151	1.1E-20	2.1E-18
postsynaptic density	70	1.7E-17	2.7E-15
neuronal cell body	111	7.4E-15	9.8E-13
neuron projection	92	7.9E-14	8.9E-12
dendrite	99	1.5E-12	1.4E-10
postsynaptic membrane	56	3.3E-11	2.9E-9
axon	74	2.0E-9	1.6E-7

Table 4.4.c: Annotation cluster 3 based on Molecular Function with 81% enrichment

Term	Count	p-value	Benjamini-Hochberg corrected P-Value
protein binding	595	1.5E-28	2.0E-25
metal ion binding	438	2.1E-11	1.4E-8
nucleotide binding	269	1.6E-9	7.3E-7
ATP binding	218	2.8E-9	9.6E-7
protein kinase activity	94	2.5E-8	6.9E-6
zinc ion binding	160	7.9E-8	1.8E-5
Rho GEF activity	25	8.1E-8	1.6E-5
GTPase activator activity	51	1.2E-7	2.2E-5
kinase activity	109	2.2E-7	3.4E-5
guanyl-nucleotide exchange factor activity	37	9.4E-7	1.3E-4
transferase activity	200	1.4E-6	1.8E-4
protein serine/threonine kinase activity	74	2.2E-6	2.5E-4
actin binding	62	2.3E-6	2.5E-4
calcium ion binding	105	1.1E-5	1.1E-3
protein complex binding	62	1.5E-5	1.4E-3
ion channel binding	28	3.4E-5	2.9E-3

4.9.2 Gene Ontology Analysis of CUX1 target genes

The gene ontology analysis of the identified target genes was carried out using KEGG and database, which identified the glutamatergic synapse pathway as the most significant CUX1 target, followed by the axon guidance pathway (**Table 4.5**).

Table 4.5: Pathways targeted by CUX1 in KEGG database

Term	Count	P-value	Benjamini-Hochberg corrected P-value
Glutamatergic synapse	31	4.3E-8	1.2E-5
Axon guidance	33	5.6E-8	7.6E-6
Pathways in cancer	68	1.4E-7	1.3E-5
FoxO signaling pathway	32	4.8E-7	3.3E-5
MAPK signaling pathway	48	7.1E-7	3.9E-5
Renal cell carcinoma	21	7.7E-7	3.5E-5
ErbB signaling pathway	24	1.3E-6	5.0E-5
Cholinergic synapse	28	1.4E-6	4.7E-5
Rap1 signaling pathway	42	1.6E-6	5.0E-5
Focal adhesion	41	1.7E-6	4.8E-5
Chronic myeloid leukemia	21	2.7E-6	6.8E-5
cAMP signaling pathway	39	3.3E-6	7.0E-5

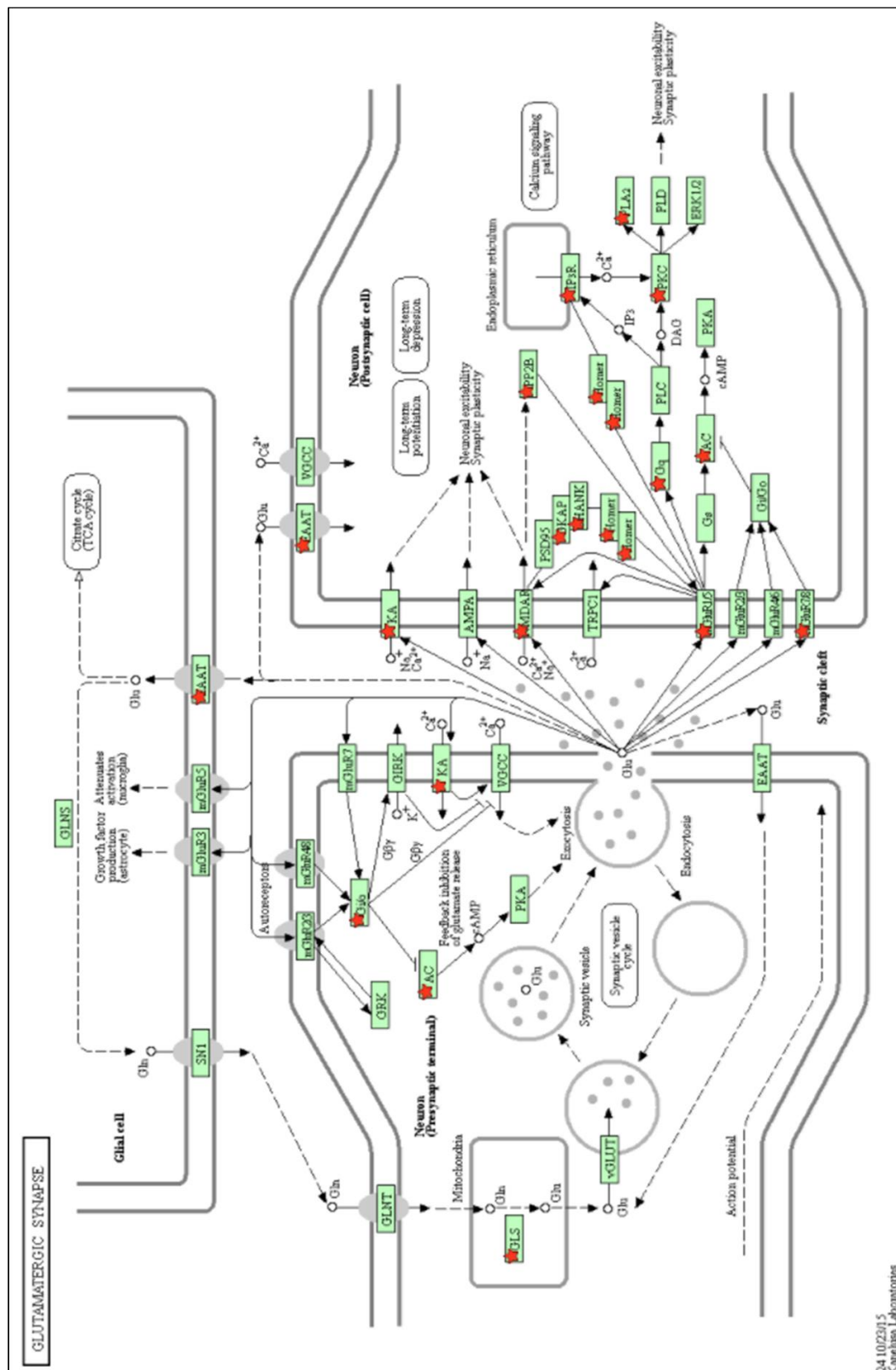
4.9.3 CUX1 targets genes involved in Glutamatergic Synapse pathway

Out of a total of 115 genes which are members of glutamatergic synapse pathway (**Appendix B**), 30 were identified in the KEGG database as targets of Cux1 (**Table4.6**). These members included glutamate ionotropic receptor subunits (Grik1), G protein subunits (Gnb1), Adenylyl cyclase subunits (Adcy2 and Adcy9), proteins regulating glutamate receptor function (Homer1 and Homer2), Protein Kinase C subunit (Prkca) among others, all of which play key roles in glutamatergic synapse pathway (**Figure 4.10**).

Table 4.6: CUX1 target genes involved in glutamatergic synapse pathway

Target #	peak ID	p-value	Fold enrichment	Gene ID
46	MACS2_peak_1604	0,174	525.159	Adcy2
273	MACS2_peak_2259	0,273	429.676	Adcy9
285	MACS2_peak_2455	0,273	429.676	Grik1
392	MACS2_peak_455	0,273	429.676	Grm1
384	MACS2_peak_4336	0,273	429.676	Gnb1
550	MACS2_peak_2674	0,275	428.347	Dlgap1
1150	MACS2_peak_5279	0,338	381.934	Shank1
1372	MACS2_peak_5279	0,338	381934	Shank1
705	MACS2_peak_1603	0,338	381.934	Adcy2
776	MACS2_peak_2278	0,338	381.934	Grin2a
1171	MACS2_peak_538	0,338	381.934	Grik2
1016	MACS2_peak_4209	0,338	381.934	Grik3
1131	MACS2_peak_5051	0,338	381.934	Grm7
1033	MACS2_peak_4337	0,338	381.934	Gnb1
708	MACS2_peak_1654	0,338	381.934	Homer1

640	MACS2_peak_1064	0,338	381.934	Prkca
966	MACS2_peak_3891	0,338	381.934	Ppp3ca
1456	MACS2_peak_2673	0,350	372.270	Dlgap1
1571	MACS2_peak_1131	0,413	334.192	Adcy3
1721	MACS2_peak_2121	0,413	334.192	Adcy8
2026	MACS2_peak_4058	0,413	334.192	Grin3a
2235	MACS2_peak_5119	0,413	334.192	Grin2b
2117	MACS2_peak_456	0,413	334.192	Grm1
2276	MACS2_peak_5381	0,413	334.192	Homer2
2277	MACS2_peak_5382	0,413	334.192	Homer2
2241	MACS2_peak_5150	0,413	334.192	Itpr2
2242	MACS2_peak_5151	0,413	334.192	Itpr2
2215	MACS2_peak_5046	0,413	334.192	Itpr1
1876	MACS2_peak_320	0,413	334.192	Pla2g4a
2297	MACS2_peak_5476	0,413	334.192	Prkcb
1709	MACS2_peak_2019	0,413	334.192	Slc1a3
2667	MACS2_peak_6025	0,415	333.159	Grik4
2673	MACS2_peak_749	0,415	333.159	Gls2
2639	MACS2_peak_3021	0,415	333.159	Slc1a1
2706	MACS2_peak_5550	0,425	325.737	Shank2
3165	MACS2_peak_537	0,498	286.450	Grik2
3072	MACS2_peak_4353	0,498	286.450	Grm3
2943	MACS2_peak_2991	0,498	286.450	Gnaq
2944	MACS2_peak_2992	0,498	286.450	Gnaq
2997	MACS2_peak_3402	0,498	286.450	Plcb2
3006	MACS2_peak_3446	0,498	286.450	Plcb4
2813	MACS2_peak_1065	0,498	286.450	Prkca
3342	MACS2_peak_5050	0,500	285.565	Grm7



4.9.4 CUX1 target genes involved in Axon Guidance pathway

Axon Guidance pathway was determined as the second most significant pathway containing CUX1 target genes by KEGG database. CUX1 target genes involved in this pathway include *Ntgn2*, *Lrrc4c*, *Ppp3ca*, *Dcc*, *Ptk2 (FAK)*, *Cdc42*, *Ablim1*, *Ablim3*, *Unc5d*, *Efna5*, *Nras*, *Ngef*, *Slit1*, *Slit3*, *Robo1*, *Srgap1-2-3*, *Plxna2-4*, *Plxnc1*, *Limk2*, *Rnd1*, *Sema4b*, *Sema4c*, *Sema6a*, and *Sema6d*. A schematic representation of the pathway adapted from KEGG database is shown in **Figure 4.11**.

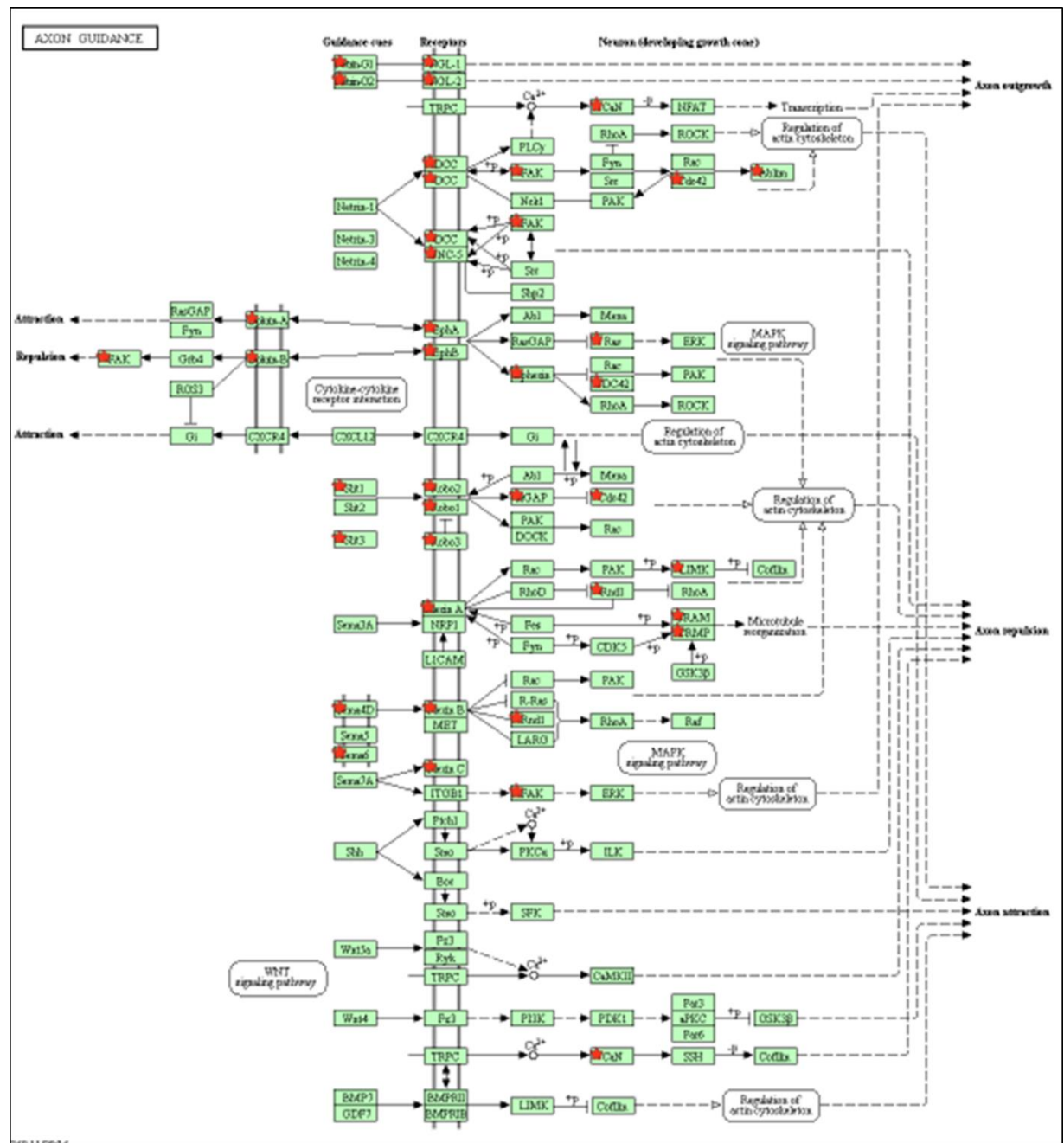


Figure 4.11: Axon Guidance Pathway. Red stars demonstrate the target genes of CUX1.

4.10 CUX1 associates with the binding regions of its target genes that are in the glutamatergic synapse pathway

To validate the binding of CUX1 on the genes involved in the glutamatergic synapse pathway, I performed ChIP-qPCR using CUX1 ChIP DNA. Six members of the pathway were selected: *Adcy9*, *Dlgap1*, *Grik1*, *Gnb1*, *Homer1* and *Prkca*. In ChIP-qPCR experiment, GFP ChIP DNA was used as negative control in order to exclude the possibility that the CUX1 antibody pulled the DNA fragment due to nonspecific interactions coming from the antibody chain. The reads of the qPCR were plotted in ratio to input DNA and calculated as percent input. All of the selected genes were shown to be associated with CUX1, thus, they are indeed targets of CUX1 (**Figure 4.12**).

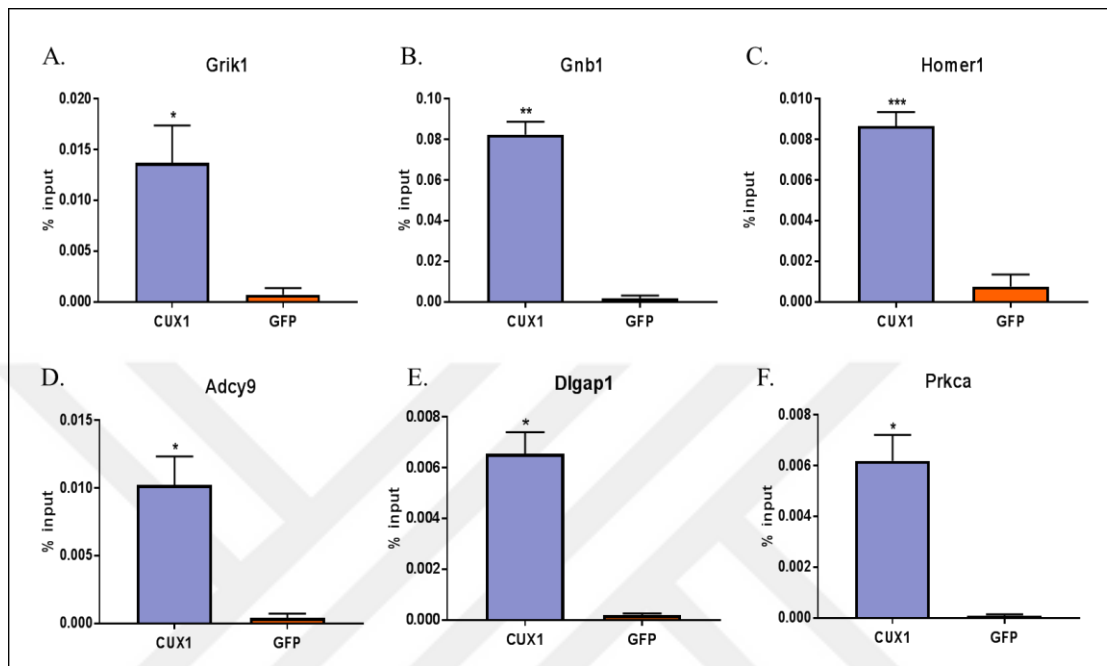


Figure 4.12: CUX1 ChIP followed by qPCR confirms CUX1 binding to the promoter regions of all six members of glutamatergic synapse pathway relative to a negative control (GFP ChIP) (p -value < 0.05). Data is normalized to the amount of input DNA as described in the Methods section. Bars represent standard error of mean.

4.11 Cux1 shRNA is cloned and knockdown efficiency is shown in HEK293T and Neuro2A cell lines

To knock down CUX1 expression, three shRNA oligos were designed and shRNAs were cloned into pSUPER vector system as explained in Methods part (**Figure 4.13**). Then, knockdown efficiency of each shRNA was determined by western blot.

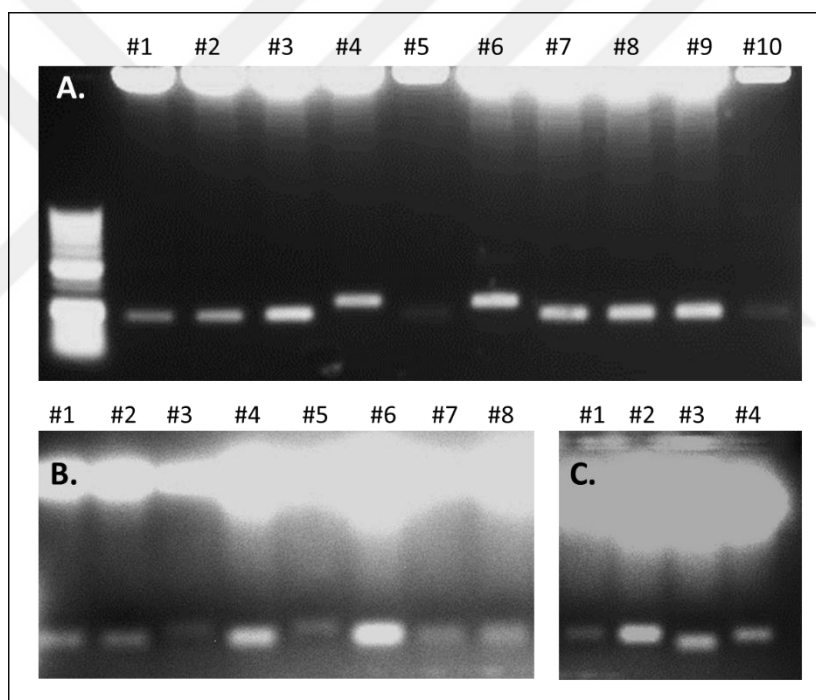


Figure 4.13: Three shRNAs were cloned into the pSUPER vector system. **A.** shRNA#1 samples #4 and #6 contain shRNA oligos. **B.** shRNA#2 samples #3 and #5 contain shRNA oligos. **C.** shRNA#3 samples #1, #2 and #4 contain shRNA oligos.

In order to observe the expression of exogenous Cux1 and the knockdown efficiency of the cloned shRNAs in Neuro2A and HEK293T cells, cells were transfected with different combinations of vectors. (1) pcDNA4a vector together with pSUPER-non-silencing (NS)-GFP vector, (2) Cux1 cDNA in pCDNA4A vector together with pSUPER-NS-GFP vector, (3) Cux1 cDNA in pCDNA4a vector together with Cux1 shRNA#1, (4) Cux1 cDNA in pCDNA4a vector together with Cux1 shRNA#2, (5) Cux1 cDNA in pCDNA4a vector together with shRNA#3, were transfected with Lipofectamine2000 reagent. 48 hours after the transfection, cell lysates were collected, SDS-PAGE and western blotting were performed using anti-myc tag antibody (**Figure 4.14.a**). The cells transfected with shRNA3 did not survive, implicating that it was toxic to cells. The experiments showed that CUX1-myc fusion protein was successfully expressed and CUX1 shRNA#1 successfully silenced the expression of exogenous CUX1 protein both in N2A cells (**Figure 4.14.b**) and HEK cells (**Appendix C**).

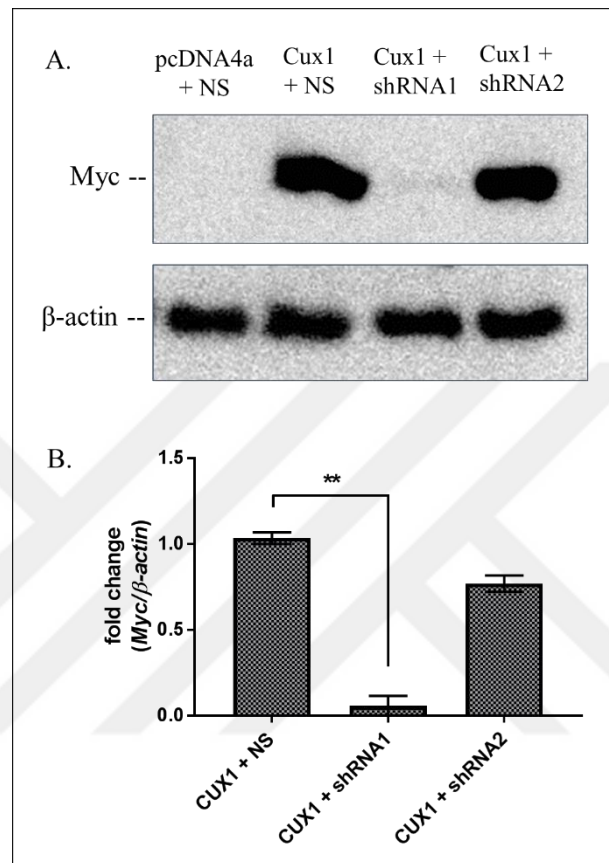


Figure 4.14: shRNA#1 against CUX1 silenced the expression of exogenous CUX1 protein. **A.** Western blot with Myc antibody demonstrated exogenous CUX1 expression in N2A cells. Transfection conditions: (i) pcDNA4a vector co-transfected with pSUPER-NS-GFP vector, (ii) Cux1-pCDNA4a vector co-transfected with pSUPER-NS-GFP vector, (iii) Cux1-pCDNA4a vector co-transfected with pSUPER-shRNA1-GFP vector, (iv) Cux1-pCDNA4a vector co-transfected with pSUPER-shRNA2-GFP vector. Beta-actin was used as the loading control. **B.** Quantification of the western blot showed efficient knockdown by Cux1 shRNA1. (One-way Anova, $p < 0.01$)

Chapter 5

DISCUSSION

CUX1 homeodomain transcription factor regulates the differentiation and physiology upper-layer cortical pyramidal neurons and controls dendritic branching, dendritic spine density, corpus callosum formation and synaptogenesis. This study reveals comprehensive direct target genes of this transcription factor in the developing cortex tissue under physiological conditions. Although some of the targets of CUX1 were previously identified using *in silico* or *in vitro* analyses, the findings presented in this study are of importance as it is the first study revealing *in vivo* genome-wide targets of CUX1 transcription factor.

Our ChIP-Seq analysis has identified a total of 6,402 binding sites of CUX1 transcription factor. 2,351 of these sites corresponded to intragenic regions that contained known transcripts. Additionally, 95 peaks were mapped to promoter regions, which were described as the peaks within 1,000 bp upstream and downstream regions of TSSs. Finally, 4,051 peaks were mapped to intergenic regions that did not correspond to any known transcript present in the reference genome.

Promoter sequences were defined as 1 kb upstream and downstream of known transcripts. CUX1 showed a low preference for binding on promoter regions, with only 2% of the peaks located on these sites. Additionally, 94% of the intragenic peaks were located on intronic elements whereas only 6% of them were located on exons. Although transcription factors were conventionally expected to regulate transcription of their target genes by binding on their promoter sites and exons, transcriptional regulation by introns was argued in various

previous studies [70], [71]. Introns are known to act as distal enhancer elements, silencers or other elements with the ability of modulating the function of the main upstream promoter [72]. Moreover, some introns are especially rich for DNA-binding motifs to which certain transcription factors have a binding preference [70].

Although the general binding motif for homeodomain proteins was validated as the ‘ATTA’ sequence, it was argued that CUX1 protein did not preferably bind to this motif site [Nepveu 1994]. Instead, CUX1-binding motif was stated as ‘ATCGAT’ core consensus in two independent studies [73], [74]. A more recent study showed that CUX1 preferentially binds to the sequences containing not just one but two ‘CAAT’ or ‘CGAT’ motifs [47]. In our study, MEME-ChIP motif analyzer program identified an unexpected motif prediction: ‘CCCTAA’. This motif sequence had a significant e-value and remained the most significant motif when the analysis was repeated using different templates (all CUX1 peaks, only promoter peaks, only intragenic peaks or only intergenic peaks). However, the sites where the motif were not centrally enriched. Only 37 sites contained the motif sequence within 6,402 peaks, and all of these sites were intergenic. Repetition of the analysis after discarding the binding sites which contained the ‘CCCTAA’ motif did not result with a consistent motif. CCCTAA sequence has been mentioned as a telomere-binding motif in the literature [75]. Given that CUX1 has functions related to apoptosis and cell senescence [76], its binding on telomeres could be relevant. However, since telomeric sequences were discarded in our analysis, this possibility could be investigated in another study.

Bioinformatics analysis of CUX1 peaks revealed many top target genes which are important in regulating neurite outgrowth and neuronal cell formation or involved in Sonic Hedgehog or Wnt signaling pathways.

Lama1 (laminin alpha 1) gene encodes a protein that is one of the three chains of laminin basement membrane protein. LAMA1 protein has been associated to neurite

outgrowth and regulation of radial glial survival [77]. Several studies reported impaired cerebellum development in *Lama1* conditional knockout mice, in addition to defects in meninges formation, significantly reduced proliferation and migration of granule cell precursors and significantly fewer number of dendritic processes of Purkinje cell precursors [77], [78].

Dlgap2 (Discs Large Homolog Associated Protein 2) gene encodes PSD-95/SAP90-associated protein-2 (DLGAP2/SAPAP2/GKAP2) which is a postsynaptic scaffolding protein regulating synaptogenesis. This protein interacts with DLG proteins and SHANKs, which are also members of glutamatergic synapse pathway [79] and has been identified as CUX1 targets in our analysis. Mutations in synaptic scaffold proteins have previously been associated to many psychiatric and neurodevelopmental disorders [80]. Furthermore, *Dlgap2* was shown to be a candidate gene for autism and suggested as a susceptibility gene for schizophrenia [81], [82]. *Cux1* regulates dendritic branching, dendritic spine formation and synaptogenesis in cerebral cortical neurons and has been associated with autism in many studies [83], [84]. Thus, it would be interesting to investigate CUX1-DLGAP2 interaction in the context of synapse formation and cognitive circuitry in the future.

Smo (smoothened, frizzled class receptor) came up as another top target gene of CUX1. SMO membrane-bound protein is a key signal transducer that interacts with the PATCHED protein, the major receptor for Hedgehog proteins, which also appeared as a target gene of CUX1 in our study. Hedgehog signaling is an important regulator of cell proliferation, polarity differentiation, stem cell population and carcinogenesis [85]. In the absence of ligand, SMO is inhibited by PATCHED. However, upon ligand binding, SMO is allowed to signal downstream with two important events, conformational change on the protein [86] and ciliary translocation of SMO protein, the latter was linked to neuropilin 1/2 [87], which has also been identified as a CUX1 target in our study. Sonic hedgehog signaling

was shown to promote the proliferation of neuronal precursors in cerebellum [88]. Our results suggest that CUX1 is a regulator of a portion of the genes involved in this pathway, thus, it might be an indirect regulator of Sonic Hedgehog pathway.

Functional classification analysis was carried out using DAVID gene ontology database in order to better understand the functions of CUX1 transcription factor through its gene targets. The analysis resulted that CUX1-regulated genes most significantly involved in cell adhesion, protein phosphorylation and nervous system development. It was known that CUX1 had important roles on nervous system development such as regulation of dendritic branching, dendritic spine formation and synapse formation [57]. Apart from that, the function of CUX1 in cell adhesion also correlated with the literature, since previous studies showed that CUX1 had several gene targets that coded for proteins involved in cell-cell and cell-matrix adhesion [89]. Additionally, functional classification based on cellular components resulted in many neuron-specific compartments such as synapse, post-synaptic density, neuronal cell body, dendrite and axon. These results supported our findings on the roles of CUX1 in the nervous system.

In order to identify the biological pathways significantly regulated by CUX1 transcription factor, gene ontology analysis was carried out using the gene ontology database DAVID. KEGG database identified glutamatergic synapse pathway and axon guidance pathway respectively, as the most significantly regulated pathways by CUX1, based on the enrichment score and p-values. A summary of CUX1 target genes based on their biological pathways is demonstrated in **Figure 5.1**.

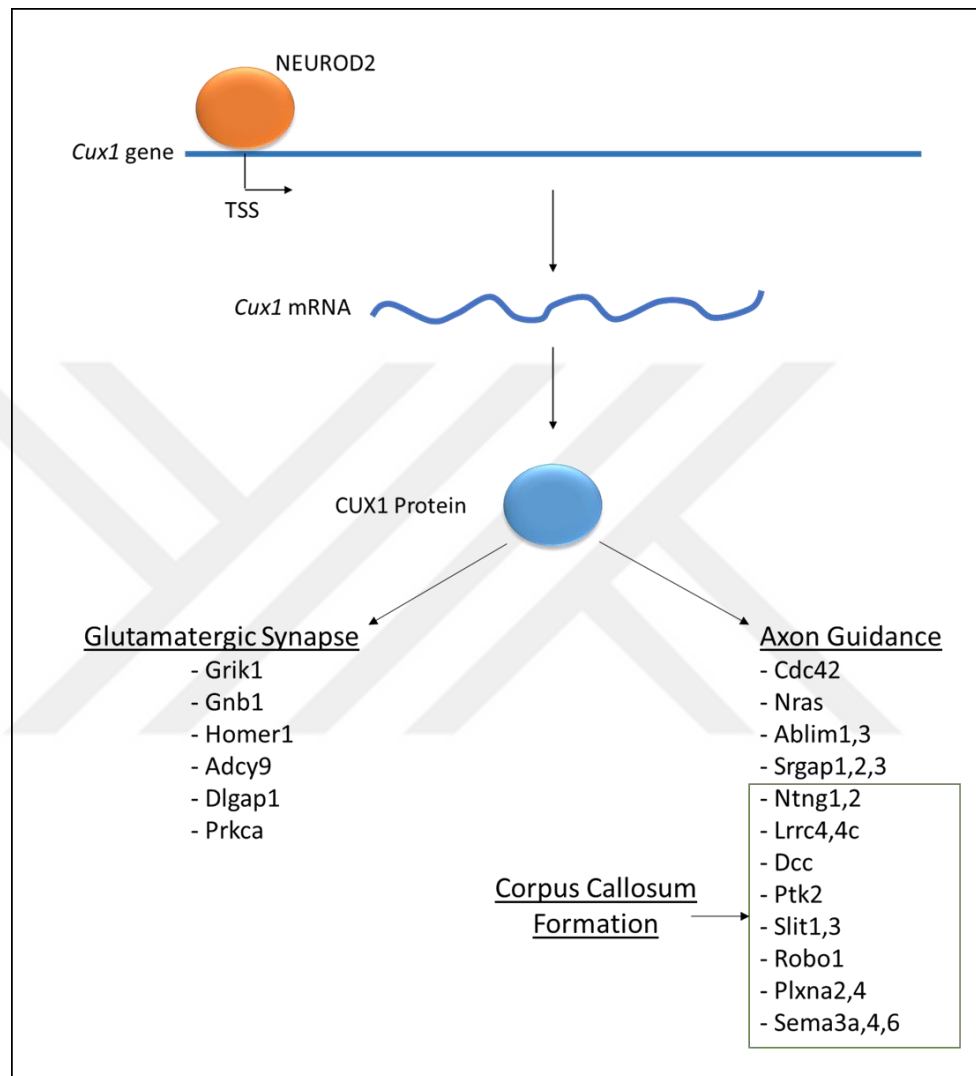


Figure 5.1: Summary of CUX1 target genes. NEUROD2 transcription factor binds to the promoter region of Cux1 gene and regulates its activity. CUX1 targets several genes in glutamatergic synapse and axon guidance pathways, some of which have role in the formation and regulation of corpus callosum axon tract.

Regulation of glutamatergic synapses by CUX1 specifically caught our interest since CUX had not been linked to this pathway before. A total of 43 genes involved in the glutamatergic pathway were targets of CUX1. These included ionotropic and metabotropic glutamate receptor subunits, subunits of adenylyl cyclase and protein kinase C, scaffolding proteins which bind to and regulate glutamate receptor functions. The strong association between CUX1 and six genes (*Grik1*, *Gnb1*, *Homer1*, *Dlgap1*, *Adcy9* and *Prkca*) involved in this pathway was confirmed with ChIP-qPCR. My results revealed that CUX1 targets key components that play roles in the glutamatergic system, a pathway that uses the major excitatory neurotransmitter glutamate and is tightly regulated by a complex network of mechanisms.

Grik1 is a glutamate ionotropic receptor kainate type subunit which has important roles in synaptic plasticity and long-term potentiation (LTP) [90], [91]. The protein encoded by this gene was shown to be interacting with a protein named Normin, and mice lacking either the gene or the protein showed a behavioural phenotype associated with schizophrenia. *Gnb1* (heterotrimeric GTP-binding protein $\beta 1$ subunit gene) is a member of the N-methyl-D-aspartic acid (NMDAR) synaptic transmission gene set [92] and mutations on this gene have been associated with cognitive disability [93] and severe neurodevelopmental diseases [94]. *Dlgap1* (Disk like guanylate-associated protein 1) gene encodes a postsynaptic scaffolding protein which has been associated with obsessive compulsive disorder [95] and mentioned as a possible susceptibility gene for schizophrenia and autism spectrum disorders (ASD) [96], [97]. *Homer1* gene encodes a Homer scaffolding protein that is one of the most abundant scaffolding proteins in postsynaptic density especially in the developing brain, and its expression is regulated by synaptic activity [98]. This protein works synergistically with another scaffolding protein Shank1, and regulate maturation of dendritic spines [99]. *Homer1* and 2 knock out mice exhibited increased levels of fear associated behavior or anxiety and showed significant defects in motor learning [100], [101]. All of the mentioned genes are

shown to be targets of CUX1, thus, CUX1 may regulate spine formation and synaptogenesis through its targets on glutamatergic synapse pathway.

Gene ontology analysis of CUX1 ChIP-seq revealed 27 genes involved in the axon guidance pathway. These included *Ntng2* (Netrin G2), *Lrrc4c* (Netrin G1 ligand) and *Dcc* (Deleted in Colorectal Carcinoma / Netrin 1 receptor) that encode for important molecules in netrin signaling. DCC receptor is a transmembrane protein whose cytoplasmic tail interacts with PTK2 (Protein Tyrosine Kinase 2 / Focal Adhesion Kinase / FAK), which is also a member of axon guidance pathway and a target of CUX1 transcription factor. FAK was shown to be required for radial migration of cortical pyramidal cells [102] and for axonal growth of neurons in different species [103]. Other CUX1 target genes involved in axon guidance are *Cdc42* and *Nras* which have intrinsic GTPase activity, and *Ngef* (Neuronal Guanine Nucleotide Exchange Factor) which is a Guanine Exchange Factor and activates Rho, Rac1 and Cdc42. Additionally, some targets of CUX1 having role in axon guidance, *Ntng2*, *Lrrc4c*, *Dcc*, *Plxn* family genes, *Sema* family genes, *Slit1-3* and *Robo1* have also been implicated in formation and regulation of corpus callosum, and will be further discussed in this context.

In a previous study, *Rodrigues-Tornos et al.* have shown that Cux1-regulated mechanisms are necessary for corpus callosum development [12]. CUX1 is expressed abundantly by superficial cortical neurons, 80% of which project their axons to contralateral side of the cortex passing through corpus callosum (CC). Although *Kcna3* gene was shown *in silico* to be transcriptionally regulated by CUX1 to enable interhemispheric connections, we demonstrate here some novel targets of CUX1 which have been implicated in CC formation. One of CUX1 target genes, the chromatin remodeling gene *Arid1b* has been recently implicated as a cause of CC anomalies [104]. Furthermore, Fgf8 signaling to downstream Nfi transcription factors has been considered as a key regulatory step in CC

formation [105]. Mutations on *Nfia* and *Nfib* genes, both of which are targets of CUX1, are associated with developmental defects in interhemisphery remodeling [105]. Another target of CUX1, *Disc1* (Disrupted in Schizophrenia 1) gene which inhibits phosphorylation of beta catenin and proliferation of neural progenitor population has also been associated with agenesis of CC [106]. Importantly, axon guidance molecules Slit and Netrin1 and axon guidance receptor Robo also have crucial roles in the formation of CC. Netrin1 functions in the initial attraction of callosal pioneering axons derived from the cingulate cortex, and DCC-Netrin signaling further attracts commissural axons to the midline. Simultaneously, Slit-Robo repels post-crossing axons away from this region. As a result of the interaction of DCC and Robo1, the repulsive effects of Slit-Robo signaling are modulated [107]. We have identified *Slit1*, *Slit3*, *Robo1* and *Dcc* as gene targets of CUX1. Another CUX1 targets identified by our ChIP-seq analysis is *Npn1* gene, encoding Neuropilin1, a receptor for the class 3 semaphorins (Semas), and members of *Plxna* subfamily. Neuropilin1 has shown to be expressed on the axons of cingulate pioneers as they reach the CC, and interact with *Plxna* proteins, regulating axon guidance along this tract [108]. Our results suggest that CUX1 does not only regulates callosal axonal tracts by regulating a limited number of voltage-gated potassium channels such as *Kcna3*, but may rather regulate a group of ion channels, signaling molecules and other proteins together

In summary, direct target genes of CUX1 transcription factor at the embryonic day 18 stage are reported in this study. Furthermore, confirmation of direct targets of CUX1 transcription factor is reported. Binding of CUX1 on selected genes were confirmed by ChIP-qPCR. To better understand the functional relationships between these target genes and CUX1 protein, investigation of target gene expression regulation by CUX1 by RT-qPCR should be the following steps. The results demonstrated that CUX1 controls glutamatergic synapse and axon guidance pathways and regulates several genes which play role in formation and regulation of corpus callosum axon tract.

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Appendix A: Target genes of CUX1 Transcription Factor

Table 1: List of Promoter Binding Target Genes

peak id	chr	peak start	peak end	gene name	Ensembl gene id	p-value
MACS2_peak_297	chr1	135719676	135719726	Csrp1	ENSMUST00000097561	2,49E-08
MACS2_peak_2219	chr15	91908541	91908594	Muc19	ENSMUST00000180213	1,09E-07
MACS2_peak_6144	chr9	78588363	78588410	Slc17a5	ENSMUST00000119213	7,98E-07
MACS2_peak_4886	chr6	47773141	47773191	Gm24975	ENSMUST00000178492	9,45E-07
MACS2_peak_5312	chr7	59731688	59731747	Gm25944	ENSMUST00000178758	1,33E-06
MACS2_peak_5411	chr7	97739111	97739159	Aqp11	ENSMUST00000084986	1,33E-06
MACS2_peak_2218	chr15	91908185	91908279	Muc19	ENSMUST00000180213	1,47E-06
MACS2_peak_1483	chr13	23756156	23756209	Hist1h4b	ENSMUST00000102965	2,33E-06
MACS2_peak_3378	chr2	108578982	108579031	Gm13913	ENSMUST00000121126	2,33E-06
MACS2_peak_3472	chr2	146512808	146512865	Ralgapa2	ENSMUST00000135974	2,33E-06
MACS2_peak_3789	chr3	96415435	96415487	Terc	ENSMUST00000082862	2,33E-06
MACS2_peak_3853	chr3	122232989	122233036	Gm22903	ENSMUST00000175193	2,33E-06
MACS2_peak_4539	chr5	95866539	95866586	Gm25937	ENSMUST00000083912	2,33E-06
MACS2_peak_3257	chr2	57629540	57629697	Gm13532	ENSMUST00000120373	2,58E-06
MACS2_peak_3793	chr3	96696334	96696381	Pias3	ENSMUST00000176302	3,44E-06
MACS2_peak_4134	chr4	88775210	88775257	Gm10580	ENSMUST00000097985	8,11E-06
MACS2_peak_5313	chr7	59887296	59887385	Gm24658	ENSMUST00000179927	8,11E-06
MACS2_peak_2220	chr15	91908662	91908904	Muc19	ENSMUST00000180213	1,40E-05
MACS2_peak_115	chr1	55364291	55364342	Boll	ENSMUST00000159564	1,94E-05
MACS2_peak_1317	chr12	86650503	86650560	uc_338	ENSMUST00000184362	1,94E-05
MACS2_peak_1959	chr14	110161460	110161508	n-R5s50	ENSMUST00000083168	1,94E-05
MACS2_peak_2585	chr17	34600964	34601014	Gm20463	ENSMUST00000174756	1,94E-05
MACS2_peak_2592	chr17	36707786	36707833	Gm20523	ENSMUST00000174340	1,94E-05
MACS2_peak_2962	chr19	6045372	6045419	Gm8034	ENSMUST00000178853	1,94E-05
MACS2_peak_3015	chr19	25454078	25454199	Gm26207	ENSMUST00000082909	1,94E-05
MACS2_peak_3759	chr3	86986082	86986129	Cd1d2	ENSMUST00000041920	1,94E-05
MACS2_peak_3924	chr3	151837897	151837944	Ptgfr	ENSMUST00000029670	1,94E-05
MACS2_peak_4241	chr4	132303836	132303891	Rab42	ENSMUST00000040411	1,94E-05

MACS2_peak_5375	chr7	80371457	80371504	Man2a2	ENSMUST00000098346	1,94E-05
MACS2_peak_5588	chr8	13404465	13404526	Grk1	ENSMUST00000033827	1,94E-05
MACS2_peak_5774	chr8	84663606	84663653	Ier2	ENSMUST00000060427	1,94E-05
MACS2_peak_595	chr10	75934928	75935006	Chchd10	ENSMUST00000058906	1,94E-05
MACS2_peak_6027	chr9	43829402	43829449	Gm3898	ENSMUST00000180221	1,94E-05
MACS2_peak_611	chr10	80388404	80388451	Mex3d	ENSMUST00000105350	1,94E-05
MACS2_peak_739	chr10	125966069	125966126	Lrig3	ENSMUST00000074807	1,94E-05
MACS2_peak_748	chr10	127523310	127523357	Shmt2	ENSMUST00000026470	1,94E-05
MACS2_peak_925	chr11	68502384	68502431	Pik3r6	ENSMUST00000153671	1,94E-05
MACS2_peak_942	chr11	73046808	73046883	Ncbp3	ENSMUST00000021135	1,94E-05
MACS2_peak_4172	chr4	107889520	107889567	0610037L13Rik	ENSMUST00000153593	2,56E-05
MACS2_peak_1568	chr13	55622007	55622054	Caml	ENSMUST00000021963	2,73E-05
MACS2_peak_2601	chr17	39846895	39846993	Gm26924	ENSMUST00000182010	5,39E-05
MACS2_peak_5092	chr6	122874127	122874174	Necap1	ENSMUST00000032477	0,000100675
MACS2_peak_5491	chr7	127043220	127043267	Kif22	ENSMUST00000032915	0,000100675
MACS2_peak_2598	chr17	39844802	39844869	Gm26917	ENSMUST00000182520	0,000122744
MACS2_peak_1010	chr11	96354892	96354946	Hoxb3os	ENSMUST00000147410	0,000144461
MACS2_peak_1041	chr11	101599802	101599849	Gm11634	ENSMUST00000154147	0,000144461
MACS2_peak_1082	chr11	114090769	114090816	1700025D23Rik	ENSMUST00000129365	0,000144461
MACS2_peak_1176	chr12	22157395	22157450	Gm4425	ENSMUST00000172838	0,000144461
MACS2_peak_1313	chr12	85271504	85271551	Mlh3	ENSMUST00000166821	0,000144461
MACS2_peak_139	chr1	66864025	66864126	Acadl	ENSMUST00000027153	0,000144461
MACS2_peak_1843	chr14	55617565	55617612	Rec8	ENSMUST00000140335	0,000144461
MACS2_peak_2667	chr17	67355131	67355183	Ptpm	ENSMUST00000037974	0,000144461
MACS2_peak_2761	chr18	4812200	4812260	Gm10556	ENSMUST00000180904	0,000144461
MACS2_peak_2951	chr19	3985884	3985969	Acy3	ENSMUST00000054030	0,000144461
MACS2_peak_3003	chr19	23076125	23076204	C330002G04Rik	ENSMUST00000181623	0,000144461
MACS2_peak_3167	chr2	28477131	28477178	Rpsa-ps9	ENSMUST00000120702	0,000144461
MACS2_peak_3279	chr2	71211287	71211334	Dync1i2	ENSMUST00000149735	0,000144461
MACS2_peak_3403	chr2	118997011	118997058	Gm14089	ENSMUST00000130025	0,000144461
MACS2_peak_3491	chr2	154612489	154612536	Zfp341	ENSMUST00000126421	0,000144461
MACS2_peak_3493	chr2	155236215	155236262	Dynlrb1	ENSMUST00000150602	0,000144461
MACS2_peak_4298	chr4	148627829	148627876	Tardbp	ENSMUST00000172073	0,000144461

MACS2_peak_4315	chr4	152071347	152071499	Plekhhg5	ENSMUST00000105662	0,000144461
MACS2_peak_4351	chr5	8623404	8623451	Rundc3b	ENSMUST00000115378	0,000144461
MACS2_peak_4979	chr6	83837358	83837405	Gm23014	ENSMUST00000104144	0,000144461
MACS2_peak_5148	chr6	145614513	145614560	Ifldt1	ENSMUST00000148739	0,000144461
MACS2_peak_5230	chr7	27675525	27675572	Map3k10	ENSMUST00000138243	0,000144461
MACS2_peak_5283	chr7	45239464	45239522	Cd37	ENSMUST00000146293	0,000144461
MACS2_peak_5420	chr7	99654396	99654443	Gm25409	ENSMUST00000083764	0,000144461
MACS2_peak_5490	chr7	127021941	127021988	Prrt2	ENSMUST00000161728	0,000144461
MACS2_peak_5631	chr8	27041632	27041679	Prosc	ENSMUST00000098851	0,000144461
MACS2_peak_5843	chr8	111093835	111093882	Pdpr	ENSMUST00000144377	0,000144461
MACS2_peak_5963	chr9	21150214	21150263	Cdc37	ENSMUST00000019615	0,000144461
MACS2_peak_6117	chr9	65628965	65629026	Rbpms2	ENSMUST00000169003	0,000144461
MACS2_peak_731	chr10	123077098	123077145	Mon2	ENSMUST00000170935	0,000144461
MACS2_peak_774	chr11	6066738	6066787	Camk2b	ENSMUST00000123391	0,000144461
MACS2_peak_939	chr11	72313919	72313976	Fbxo39	ENSMUST00000108504	0,000144461
MACS2_peak_969	chr11	79905868	79905938	Gm11204	ENSMUST00000122438	0,000144461
MACS2_peak_2980	chr19	11912067	11912143	Patl1	ENSMUST00000061618	0,000149734
MACS2_peak_4958	chr6	73222069	73222116	Dnah6	ENSMUST00000114040	0,000149734
MACS2_peak_3537	chr2	166714716	166714783	Prex1	ENSMUST00000127553	0,000157877
MACS2_peak_3547	chr2	168334963	168335025	Gm22704	ENSMUST00000083027	0,000193562
MACS2_peak_1404	chr12	109673586	109673633	Gm23347	ENSMUST00000083823	0,00023297
MACS2_peak_4135	chr4	88779625	88779680	Gm13272	ENSMUST00000179425	0,00023297
MACS2_peak_746	chr10	127264172	127264219	Kif5a	ENSMUST00000099172	0,000247201
MACS2_peak_5268	chr7	38177232	38177286	Gm22203	ENSMUST00000158605	0,000495222
MACS2_peak_2597	chr17	39844611	39844706	Gm26917	ENSMUST00000182520	0,000929865
MACS2_peak_1600	chr13	67114439	67114523	Rslcan18	ENSMUST00000091526	0,000943974
MACS2_peak_1876	chr14	66969719	66969766	Gm24258	ENSMUST00000083739	0,000943974
MACS2_peak_2828	chr18	36454678	36454769	Pfdn1	ENSMUST00000025204	0,000943974
MACS2_peak_3046	chr19	42035314	42035361	Ankrd2	ENSMUST00000026172	0,000943974
MACS2_peak_3204	chr2	34965715	34965774	Gm13450	ENSMUST00000121107	0,000943974
MACS2_peak_3602	chr3	7502367	7502419	Gm23592	ENSMUST00000157859	0,000943974
MACS2_peak_397	chr1	182282861	182282986	Degs1	ENSMUST00000133052	0,000943974
MACS2_peak_5773	chr8	84663453	84663500	Ier2	ENSMUST00000060427	0,000943974

Table 2: List of Intragenic Binding Target Genes

peak id	chr	peak start	peak end	gene name	Ensembl gene id	p-value
MACS2_peak_484	chr10	22258137	22258205	Raet1e	ENSMUST00000178026	6,94E-34
MACS2_peak_2669	chr17	67741384	67741522	Lama1	ENSMUST00000035471	3,46E-30
MACS2_peak_2022	chr15	9016144	9016338	Ranbp3l	ENSMUST00000053308	5,74E-19
MACS2_peak_4742	chr5	146261248	146261295	Cdk8	ENSMUST00000031640	2,27E-18
MACS2_peak_4045	chr4	45193510	45193634	Frmpd1	ENSMUST00000134280	3,32E-16
MACS2_peak_4595	chr5	113233453	113233500	Tmem211	ENSMUST00000086615	5,12E-15
MACS2_peak_2021	chr15	9015687	9016033	Ranbp3l	ENSMUST00000053308	5,29E-15
MACS2_peak_4821	chr6	29748377	29748527	Smo	ENSMUST00000001812	1,88E-14
MACS2_peak_3366	chr2	105257923	105258310	Them7	ENSMUST00000068813	6,14E-14
MACS2_peak_132	chr1	63630456	63630688	Dytn	ENSMUST00000090313	1,82E-13
MACS2_peak_3382	chr2	110293651	110293698	Bbox1	ENSMUST00000133608	1,99E-13
MACS2_peak_4548	chr5	99643706	99643774	A930011G23Rik	ENSMUST00000161516	2,75E-13
MACS2_peak_5593	chr8	14307283	14307337	Dlgap2	ENSMUST00000150247	3,46E-13
MACS2_peak_3368	chr2	105258913	105259041	Them7	ENSMUST00000068813	8,82E-12
MACS2_peak_2506	chr17	7956197	7956257	Rsph3a	ENSMUST00000097423	1,12E-11
MACS2_peak_2403	chr16	57391594	57391641	Filip1l	ENSMUST00000159816	1,34E-11
MACS2_peak_2532	chr17	13552789	13552955	Tcte2	ENSMUST00000137459	2,50E-11
MACS2_peak_4741	chr5	146261013	146261079	Cdk8	ENSMUST00000031640	4,14E-11
MACS2_peak_5497	chr7	128188419	128188476	Itgad	ENSMUST00000177111	4,56E-11
MACS2_peak_2529	chr17	13552123	13552243	Tcte2	ENSMUST00000137459	6,51E-11
MACS2_peak_3801	chr3	96844959	96845132	Pdzk1	ENSMUST00000128789	1,89E-10
MACS2_peak_2526	chr17	13550727	13550818	Tcte2	ENSMUST00000137459	2,63E-10
MACS2_peak_1716	chr13	113807010	113807084	Arl15	ENSMUST00000091201	4,62E-10
MACS2_peak_3137	chr2	20899883	20900003	Arhgap21	ENSMUST00000140230	5,05E-10
MACS2_peak_2491	chr17	3353294	3353342	Tiam2	ENSMUST00000169838	5,36E-10
MACS2_peak_3367	chr2	105258402	105258531	Them7	ENSMUST00000068813	5,36E-10
MACS2_peak_4661	chr5	129125738	129125799	Gpr133	ENSMUST00000056617	7,67E-10
MACS2_peak_1728	chr13	119600240	119600287	Gm7120	ENSMUST00000099147	1,54E-09
MACS2_peak_5592	chr8	14307113	14307208	Dlgap2	ENSMUST00000150247	1,71E-09
MACS2_peak_4820	chr6	29748119	29748168	Smo	ENSMUST00000001812	2,03E-09
MACS2_peak_4187	chr4	118102332	118102405	St3gal3	ENSMUST00000097912	2,26E-09
MACS2_peak_4137	chr4	88794720	88794767	Gm13275	ENSMUST00000178347	3,74E-09
MACS2_peak_1693	chr13	107859379	107859560	Zswim6	ENSMUST00000105097	8,49E-09
MACS2_peak_5386	chr7	82771126	82771194	Eftud1	ENSMUST00000039881	9,97E-09
MACS2_peak_3697	chr3	51766641	51766772	Maml3	ENSMUST00000121440	1,74E-08
MACS2_peak_1158	chr12	16747858	16747910	Greb1	ENSMUST00000161998	2,38E-08
MACS2_peak_2528	chr17	13551205	13551275	Tcte2	ENSMUST00000137459	2,38E-08
MACS2_peak_1192	chr12	28537619	28537669	Dcdc2c	ENSMUST00000020963	2,49E-08
MACS2_peak_1559	chr13	54554777	54554829	4833439L19Rik	ENSMUST00000153065	2,49E-08

MACS2_peak_1604	chr13	68718317	68718381	Adcy2	ENSMUST00000022013	2,49E-08
MACS2_peak_2142	chr15	72969026	72969119	Trappc9	ENSMUST00000168191	2,49E-08
MACS2_peak_2306	chr16	17638271	17638329	Smpd4	ENSMUST00000170273	2,49E-08
MACS2_peak_2889	chr18	65622742	65622801	Zfp532	ENSMUST00000182973	2,49E-08
MACS2_peak_3090	chr19	57926576	57926629	Atrml1	ENSMUST00000077282	2,49E-08
MACS2_peak_3341	chr2	92989741	92989806	Prdm11	ENSMUST00000178666	2,49E-08
MACS2_peak_3779	chr3	93211402	93211538	Flg2	ENSMUST00000098884	2,49E-08
MACS2_peak_382	chr1	178212637	178212690	Desi2	ENSMUST00000159284	2,49E-08
MACS2_peak_64	chr1	34274318	34274368	Dst	ENSMUST00000097786	2,49E-08
MACS2_peak_2958	chr19	5475799	5475847	Efemp2	ENSMUST00000164204	2,64E-08
MACS2_peak_4201	chr4	123655711	123655762	Macf1	ENSMUST00000147030	2,64E-08
MACS2_peak_3226	chr2	41772789	41772836	Lrp1b	ENSMUST00000167270	2,84E-08
MACS2_peak_1464	chr13	13752981	13753035	Lyst	ENSMUST00000110559	4,96E-08
MACS2_peak_3695	chr3	51766137	51766192	Maml3	ENSMUST00000121440	4,96E-08
MACS2_peak_133	chr1	63630847	63630906	Dytn	ENSMUST00000090313	5,28E-08
MACS2_peak_2701	chr17	80356689	80356739	Arhgef33	ENSMUST00000068175	6,06E-08
MACS2_peak_2009	chr15	6316908	6316955	Dab2	ENSMUST00000162140	6,19E-08
MACS2_peak_3138	chr2	20900068	20900194	Arhgap21	ENSMUST00000140230	8,85E-08
MACS2_peak_1689	chr13	107858381	107858641	Zswim6	ENSMUST00000105097	9,86E-08
MACS2_peak_2570	chr17	29286567	29286649	BC004004	ENSMUST00000149405	2,01E-07
MACS2_peak_3330	chr2	90373412	90373476	Gm13767	ENSMUST00000124074	2,01E-07
MACS2_peak_5146	chr6	145440268	145440354	Ifltd1	ENSMUST00000111706	2,01E-07
MACS2_peak_4136	chr4	88792465	88792543	Gm26525	ENSMUST00000180666	2,39E-07
MACS2_peak_4291	chr4	147275177	147275235	Gm26624	ENSMUST00000181486	2,52E-07
MACS2_peak_1040	chr11	101413335	101413395	Gm27029	ENSMUST00000107257	2,53E-07
MACS2_peak_1118	chr11	119369876	119369930	Slc26a11	ENSMUST00000151612	2,53E-07
MACS2_peak_1294	chr12	79289220	79289267	Zfyve26	ENSMUST00000021547	2,53E-07
MACS2_peak_1743	chr14	9931369	9931420	Fhit	ENSMUST00000162817	2,53E-07
MACS2_peak_1783	chr14	29135988	29136048	Cacna2d3	ENSMUST00000022567	2,53E-07
MACS2_peak_1812	chr14	41985858	41985927	1700024B05Rik	ENSMUST00000168972	2,53E-07
MACS2_peak_1849	chr14	56796647	56796696	Zmym5	ENSMUST00000173954	2,53E-07
MACS2_peak_1868	chr14	64720331	64720378	Kif13b	ENSMUST00000100473	2,53E-07
MACS2_peak_1895	chr14	75966001	75966048	Gtf2f2	ENSMUST00000088922	2,53E-07
MACS2_peak_2002	chr14	124491596	124491645	Fgf14	ENSMUST00000095529	2,53E-07
MACS2_peak_2012	chr15	7284826	7284875	Egflam	ENSMUST00000160207	2,53E-07
MACS2_peak_211	chr1	93444785	93444876	Hdlbp	ENSMUST00000042498	2,53E-07
MACS2_peak_2351	chr16	31156565	31156640	Acap2	ENSMUST00000058033	2,53E-07
MACS2_peak_2516	chr17	10664790	10664851	Pacrg	ENSMUST00000041463	2,53E-07
MACS2_peak_2584	chr17	34159899	34159953	H2-DMb1	ENSMUST00000174765	2,53E-07
MACS2_peak_2716	chr17	85498817	85498865	Rpl31-ps16	ENSMUST00000181130	2,53E-07
MACS2_peak_2723	chr17	86427195	86427270	Prkce	ENSMUST00000097274	2,53E-07
MACS2_peak_2730	chr17	87362752	87362805	Ttc7	ENSMUST00000154255	2,53E-07
MACS2_peak_2769	chr18	9717900	9717947	Colec12	ENSMUST00000040069	2,53E-07
MACS2_peak_285	chr1	133258080	133258127	Plekha6	ENSMUST00000105082	2,53E-07
MACS2_peak_2952	chr19	3997711	3997760	Tbx10	ENSMUST00000041871	2,53E-07
MACS2_peak_3018	chr19	27772735	27772782	Rfx3	ENSMUST00000173863	2,53E-07

MACS2_peak_3026	chr19	32306635	32306682	Sgms1	ENSMUST00000141655	2,53E-07
MACS2_peak_3110	chr2	5637165	5637212	Camk1d	ENSMUST00000155927	2,53E-07
MACS2_peak_3123	chr2	10114018	10114073	Itih2	ENSMUST00000155809	2,53E-07
MACS2_peak_3162	chr2	28029313	28029360	Gm13372	ENSMUST00000123129	2,53E-07
MACS2_peak_3287	chr2	72747790	72747841	6430710C18Rik	ENSMUST00000125382	2,53E-07
MACS2_peak_3410	chr2	121242683	121242740	Trp53bp1	ENSMUST00000129752	2,53E-07
MACS2_peak_3435	chr2	130452863	130452926	Ptpra	ENSMUST00000077303	2,53E-07
MACS2_peak_3492	chr2	155143504	155143551	Itch	ENSMUST00000109685	2,53E-07
MACS2_peak_3501	chr2	156991318	156991378	Ndr3	ENSMUST00000132748	2,53E-07
MACS2_peak_3544	chr2	167726190	167726249	A530013C23Rik	ENSMUST00000141923	2,53E-07
MACS2_peak_374	chr1	174784719	174784767	Fmn2	ENSMUST00000030039	2,53E-07
MACS2_peak_3781	chr3	93216142	93216189	Flg2	ENSMUST00000098884	2,53E-07
MACS2_peak_3795	chr3	96841199	96841351	Pdzk1	ENSMUST00000128789	2,53E-07
MACS2_peak_3832	chr3	113623952	113624028	Rnpc3	ENSMUST00000106535	2,53E-07
MACS2_peak_3867	chr3	127098584	127098631	Ank2	ENSMUST00000182050	2,53E-07
MACS2_peak_3885	chr3	133353157	133353204	Ppa2	ENSMUST00000106315	2,53E-07
MACS2_peak_3958	chr4	3661527	3661577	2210414B05Rik	ENSMUST00000139600	2,53E-07
MACS2_peak_4270	chr4	138316056	138316123	Pink1	ENSMUST00000105816	2,53E-07
MACS2_peak_4317	chr4	152109792	152109855	Plekhg5	ENSMUST00000140085	2,53E-07
MACS2_peak_4403	chr5	30841483	30841542	Mapre3	ENSMUST00000123334	2,53E-07
MACS2_peak_4432	chr5	41640244	41640296	Rab28	ENSMUST00000031011	2,53E-07
MACS2_peak_4612	chr5	115453776	115453823	Msi1	ENSMUST00000150779	2,53E-07
MACS2_peak_4622	chr5	117632493	117632540	Ksr2	ENSMUST00000180430	2,53E-07
MACS2_peak_4969	chr6	79956277	79956329	Gm4409	ENSMUST00000130734	2,53E-07
MACS2_peak_5069	chr6	115923250	115923299	Ift122	ENSMUST00000155565	2,53E-07
MACS2_peak_5086	chr6	120879972	120880024	Bcl2l13	ENSMUST00000009256	2,53E-07
MACS2_peak_5112	chr6	129537586	129537633	Gabarapl1	ENSMUST00000032264	2,53E-07
MACS2_peak_5123	chr6	137489844	137489891	Eps8	ENSMUST00000111878	2,53E-07
MACS2_peak_5235	chr7	28891630	28891677	Capn12	ENSMUST00000066880	2,53E-07
MACS2_peak_5253	chr7	35752190	35752247	Dpy19l3	ENSMUST00000124116	2,53E-07
MACS2_peak_5276	chr7	41336859	41336906	Gm9246	ENSMUST00000117587	2,53E-07
MACS2_peak_5379	chr7	80643522	80643569	Gm15880	ENSMUST00000142678	2,53E-07
MACS2_peak_5394	chr7	84348275	84348323	Arnt2	ENSMUST00000085077	2,53E-07
MACS2_peak_5423	chr7	100303564	100303611	P4ha3	ENSMUST00000138465	2,53E-07
MACS2_peak_5425	chr7	101397687	101397737	Arap1	ENSMUST00000098243	2,53E-07
MACS2_peak_5484	chr7	126041867	126041919	Gsg1l	ENSMUST00000073935	2,53E-07
MACS2_peak_5503	chr7	130726308	130726391	Tacc2	ENSMUST00000033141	2,53E-07
MACS2_peak_5624	chr8	25991247	25991294	Pomk	ENSMUST00000061850	2,53E-07
MACS2_peak_5666	chr8	46839256	46839308	Irf2	ENSMUST00000034041	2,53E-07
MACS2_peak_585	chr10	70269535	70269584	Slc16a9	ENSMUST00000155933	2,53E-07
MACS2_peak_5909	chr8	125182657	125182711	Disc1	ENSMUST00000121953	2,53E-07
MACS2_peak_5938	chr9	10740620	10740672	Cntn5	ENSMUST00000160358	2,53E-07
MACS2_peak_5944	chr9	13697299	13697356	Maml2	ENSMUST00000177755	2,53E-07
MACS2_peak_5992	chr9	31310701	31310748	Prdm10	ENSMUST00000117389	2,53E-07
MACS2_peak_6023	chr9	42462193	42462246	Tbcel	ENSMUST00000134374	2,53E-07
MACS2_peak_622	chr10	81195317	81195364	Atcayos	ENSMUST00000129834	2,53E-07

MACS2_peak_626	chr10	81265728	81265775	Mrpl54	ENSMUST00000145452	2,53E-07
MACS2_peak_635	chr10	83563369	83563416	Washc4	ENSMUST00000038388	2,53E-07
MACS2_peak_742	chr10	127019222	127019292	Tsfm	ENSMUST00000152054	2,53E-07
MACS2_peak_760	chr11	3337961	3338107	Pik3ip1	ENSMUST00000130747	2,53E-07
MACS2_peak_781	chr11	9259858	9259916	Abca13	ENSMUST00000042740	2,53E-07
MACS2_peak_920	chr11	66357196	66357244	Shisa6	ENSMUST00000123454	2,53E-07
MACS2_peak_926	chr11	68584709	68584756	4930535C22Rik	ENSMUST00000148019	2,53E-07
MACS2_peak_970	chr11	80531056	80531112	Myo1d	ENSMUST00000041065	2,53E-07
MACS2_peak_126	chr1	59943778	59943848	Fam117b	ENSMUST00000036540	2,66E-07
MACS2_peak_1754	chr14	13439485	13439540	Synpr	ENSMUST00000070323	2,66E-07
MACS2_peak_4234	chr4	130691219	130691266	Pum1	ENSMUST00000097862	2,66E-07
MACS2_peak_5625	chr8	26160894	26160951	Thap1	ENSMUST00000036807	2,66E-07
MACS2_peak_5663	chr8	45734811	45734858	Sorbs2	ENSMUST00000150102	2,66E-07
MACS2_peak_806	chr11	19934262	19934333	Spred2	ENSMUST00000093299	2,66E-07
MACS2_peak_845	chr11	36161860	36161907	Tenm2	ENSMUST00000149787	2,66E-07
MACS2_peak_1691	chr13	107859024	107859137	Zswim6	ENSMUST00000105097	2,90E-07
MACS2_peak_5190	chr7	15462378	15462436	Obox1	ENSMUST00000181001	2,98E-07
MACS2_peak_5143	chr6	145169022	145169098	Lrmp	ENSMUST00000132948	3,32E-07
MACS2_peak_2151	chr15	74537152	74537199	Bai1	ENSMUST00000042035	3,91E-07
MACS2_peak_4887	chr6	47892094	47892145	Zfp282	ENSMUST00000125957	3,91E-07
MACS2_peak_6122	chr9	71559581	71559628	Myzap	ENSMUST00000169573	3,91E-07
MACS2_peak_4779	chr6	4924070	4924189	Ppp1r9a	ENSMUST00000177456	4,21E-07
MACS2_peak_757	chr11	3126278	3126341	Pisd-ps1	ENSMUST00000119128	4,27E-07
MACS2_peak_2291	chr16	11988210	11988272	Shisa9	ENSMUST00000182154	5,43E-07
MACS2_peak_355	chr1	166281339	166281386	Ildr2	ENSMUST00000111416	5,63E-07
MACS2_peak_2675	chr17	70963701	70963761	Gm26561	ENSMUST00000180743	6,70E-07
MACS2_peak_5121	chr6	136851171	136851233	Art4	ENSMUST00000032341	6,75E-07
MACS2_peak_1419	chr12	112776973	112777046	Ahnak2	ENSMUST00000128258	7,98E-07
MACS2_peak_3883	chr3	133351864	133351964	Ppa2	ENSMUST00000106315	7,98E-07
MACS2_peak_5821	chr8	104509674	104509738	Ccdc79	ENSMUST00000159713	7,98E-07
MACS2_peak_6095	chr9	60600758	60600816	Lrrc49	ENSMUST00000166168	7,98E-07
MACS2_peak_2284	chr16	11144228	11144288	Zc3h7a	ENSMUST00000140755	1,03E-06
MACS2_peak_1712	chr13	113803989	113804074	Arl15	ENSMUST00000091201	1,11E-06
MACS2_peak_260	chr1	126135100	126135147	Nckap5	ENSMUST00000162877	1,11E-06
MACS2_peak_2797	chr18	20621348	20621395	Gm16090	ENSMUST00000130296	1,33E-06
MACS2_peak_4319	chr4	152289115	152289193	Hes3	ENSMUST00000094438	1,33E-06
MACS2_peak_6138	chr9	77532926	77532973	Lrrc1	ENSMUST00000184811	1,33E-06
MACS2_peak_6162	chr9	89110393	89110441	Gm26567	ENSMUST00000181441	1,33E-06
MACS2_peak_6216	chr9	110444189	110444320	Klhl18	ENSMUST00000150173	1,33E-06
MACS2_peak_6244	chr9	118112447	118112611	Cmc1	ENSMUST00000044220	1,33E-06
MACS2_peak_2294	chr16	13312568	13312686	Mkl2	ENSMUST00000056715	1,35E-06
MACS2_peak_4408	chr5	32738075	32738126	Pisd	ENSMUST00000071829	1,35E-06
MACS2_peak_6074	chr9	56223822	56223933	C230081A13Rik	ENSMUST00000061552	1,52E-06
MACS2_peak_5782	chr8	86514424	86514471	Abcc12	ENSMUST00000156066	1,54E-06
MACS2_peak_2223	chr15	91910172	91910371	Muc19	ENSMUST00000088547	1,60E-06
MACS2_peak_1690	chr13	107858856	107858951	Zswim6	ENSMUST00000105097	1,75E-06

MACS2_peak_5368	chr7	79829096	79829151	Anpep	ENSMUST00000049004	2,08E-06
MACS2_peak_137	chr1	64800394	64800486	Plekhn3	ENSMUST00000097713	2,09E-06
MACS2_peak_1834	chr14	52312724	52312771	Sall2	ENSMUST00000058326	2,09E-06
MACS2_peak_3669	chr3	40650993	40651040	Intu	ENSMUST00000061590	2,09E-06
MACS2_peak_3799	chr3	96844162	96844222	Pdzk1	ENSMUST00000128789	2,09E-06
MACS2_peak_1004	chr11	95047310	95047361	Itga3	ENSMUST00000120375	2,33E-06
MACS2_peak_1025	chr11	98692964	98693012	Psmc3	ENSMUST00000123676	2,33E-06
MACS2_peak_1039	chr11	101410212	101410268	Gm27029	ENSMUST00000107257	2,33E-06
MACS2_peak_1099	chr11	116326597	116326649	n-R5s74	ENSMUST00000082654	2,33E-06
MACS2_peak_1104	chr11	117228949	117229003	42987	ENSMUST00000093907	2,33E-06
MACS2_peak_1120	chr11	119895014	119895061	Rptor	ENSMUST00000136662	2,33E-06
MACS2_peak_1124	chr11	120288750	120288797	Bahcc1	ENSMUST00000118987	2,33E-06
MACS2_peak_1164	chr12	18515133	18515183	5730507C01Rik	ENSMUST00000177778	2,33E-06
MACS2_peak_1197	chr12	29589202	29589258	Myt1l	ENSMUST00000049784	2,33E-06
MACS2_peak_1198	chr12	29607224	29607281	Myt1l	ENSMUST00000049784	2,33E-06
MACS2_peak_125	chr1	59935393	59935440	Fam117b	ENSMUST00000036540	2,33E-06
MACS2_peak_1281	chr12	72448332	72448379	Lrrc9	ENSMUST00000161957	2,33E-06
MACS2_peak_13	chr1	9156528	9156596	Sntg1	ENSMUST00000134075	2,33E-06
MACS2_peak_130	chr1	63507535	63507582	Adam23	ENSMUST00000146403	2,33E-06
MACS2_peak_1301	chr12	81729045	81729092	Map3k9	ENSMUST00000035987	2,33E-06
MACS2_peak_1316	chr12	86370864	86370911	Esrrb	ENSMUST00000116402	2,33E-06
MACS2_peak_1363	chr12	100303358	100303405	Ttc7b	ENSMUST00000062957	2,33E-06
MACS2_peak_1364	chr12	101004671	101004740	Ccdc88c	ENSMUST00000085096	2,33E-06
MACS2_peak_1397	chr12	107776833	107776898	Gm15208	ENSMUST00000134448	2,33E-06
MACS2_peak_1406	chr12	110765401	110765448	Wdr20a	ENSMUST00000095410	2,33E-06
MACS2_peak_1417	chr12	112767264	112767311	Pld4	ENSMUST00000063888	2,33E-06
MACS2_peak_1422	chr12	112788581	112788636	Ahnak2	ENSMUST00000124526	2,33E-06
MACS2_peak_1429	chr12	113366212	113366312	Gm17421	ENSMUST00000167845	2,33E-06
MACS2_peak_1433	chr12	115386915	115387002	Ighv1-62-1	ENSMUST00000171270	2,33E-06
MACS2_peak_1439	chr12	118104304	118104351	Dnah11	ENSMUST00000084806	2,33E-06
MACS2_peak_144	chr1	69105183	69105230	Gm16076	ENSMUST00000140116	2,33E-06
MACS2_peak_1462	chr13	13637515	13637568	Lyst	ENSMUST00000110559	2,33E-06
MACS2_peak_147	chr1	71090252	71090299	Bard1	ENSMUST00000027393	2,33E-06
MACS2_peak_1499	chr13	29438999	29439061	Cdkal1	ENSMUST00000006353	2,33E-06
MACS2_peak_1501	chr13	29735514	29735562	Cdkal1	ENSMUST00000123641	2,33E-06
MACS2_peak_1506	chr13	30894199	30894260	Exoc2	ENSMUST00000102946	2,33E-06
MACS2_peak_1521	chr13	41194684	41194731	Elovl2	ENSMUST00000117096	2,33E-06
MACS2_peak_1556	chr13	53184622	53184695	Ror2	ENSMUST00000149299	2,33E-06
MACS2_peak_1560	chr13	54682887	54682963	Rnf44	ENSMUST00000145215	2,33E-06
MACS2_peak_1578	chr13	57897227	57897277	Spock1	ENSMUST00000172326	2,33E-06
MACS2_peak_158	chr1	75253485	75253610	Ptpm	ENSMUST00000027404	2,33E-06
MACS2_peak_1580	chr13	58056136	58056242	Klhl3	ENSMUST00000091583	2,33E-06
MACS2_peak_1621	chr13	76724145	76724192	Mctp1	ENSMUST00000137052	2,33E-06
MACS2_peak_1627	chr13	78249383	78249445	A830082K12Rik	ENSMUST00000183171	2,33E-06
MACS2_peak_1656	chr13	94111984	94112032	Lhfp12	ENSMUST00000156071	2,33E-06
MACS2_peak_1685	chr13	104348988	104349035	Adamts6	ENSMUST00000065766	2,33E-06

MACS2_peak_1699	chr13	108854978	108855047	Pde4d	ENSMUST00000151111	2,33E-06
MACS2_peak_1705	chr13	109748698	109748745	Pde4d	ENSMUST00000135275	2,33E-06
MACS2_peak_1772	chr14	22824237	22824284	1700112E06Rik	ENSMUST00000075639	2,33E-06
MACS2_peak_1790	chr14	30952374	30952428	Nek4	ENSMUST00000050171	2,33E-06
MACS2_peak_1826	chr14	49329628	49329680	Slc35f4	ENSMUST00000146164	2,33E-06
MACS2_peak_1847	chr14	56681352	56681411	Mphosph8	ENSMUST00000116468	2,33E-06
MACS2_peak_1857	chr14	59992745	59992798	Atp8a2	ENSMUST00000080368	2,33E-06
MACS2_peak_1890	chr14	75505402	75505449	Siah3	ENSMUST00000164848	2,33E-06
MACS2_peak_1896	chr14	76089202	76089280	Gpalpp1	ENSMUST00000022585	2,33E-06
MACS2_peak_1971	chr14	117354332	117354379	Gpc6	ENSMUST00000125435	2,33E-06
MACS2_peak_1972	chr14	117462726	117462773	Gpc6	ENSMUST00000125435	2,33E-06
MACS2_peak_200	chr1	89468804	89468864	Agap1	ENSMUST00000074945	2,33E-06
MACS2_peak_2035	chr15	18918703	18918778	Cdh10	ENSMUST00000176146	2,33E-06
MACS2_peak_2053	chr15	30326823	30326870	Ctnnd2	ENSMUST00000081728	2,33E-06
MACS2_peak_2073	chr15	41789294	41789341	Oxr1	ENSMUST00000170127	2,33E-06
MACS2_peak_2108	chr15	58997752	58997799	Mtss1	ENSMUST00000080371	2,33E-06
MACS2_peak_2180	chr15	81821826	81821873	Tef	ENSMUST00000168632	2,33E-06
MACS2_peak_2192	chr15	84671515	84671562	Prr5	ENSMUST00000171460	2,33E-06
MACS2_peak_2212	chr15	91391379	91391430	Slc2a13	ENSMUST00000109283	2,33E-06
MACS2_peak_2233	chr15	98668415	98668481	Rnd1	ENSMUST00000109149	2,33E-06
MACS2_peak_2238	chr15	99695590	99695719	Asic1	ENSMUST00000023758	2,33E-06
MACS2_peak_2259	chr16	4390584	4390631	Adcy9	ENSMUST00000117801	2,33E-06
MACS2_peak_2286	chr16	11325207	11325254	Snx29	ENSMUST00000115814	2,33E-06
MACS2_peak_2289	chr16	11725375	11725422	Snx29	ENSMUST00000096273	2,33E-06
MACS2_peak_2296	chr16	13682608	13682683	Bfar	ENSMUST00000132349	2,33E-06
MACS2_peak_2302	chr16	16460113	16460162	Fgd4	ENSMUST00000162124	2,33E-06
MACS2_peak_2303	chr16	17177515	17177578	Ube2l3	ENSMUST00000115700	2,33E-06
MACS2_peak_2325	chr16	22108166	22108213	Igf2bp2	ENSMUST00000151087	2,33E-06
MACS2_peak_2355	chr16	32622249	32622300	Tfrc	ENSMUST00000120680	2,33E-06
MACS2_peak_2363	chr16	33461084	33461142	Zfp148	ENSMUST00000165418	2,33E-06
MACS2_peak_2372	chr16	36860319	36860366	Iqcb1	ENSMUST00000114819	2,33E-06
MACS2_peak_2404	chr16	57568549	57568597	Filip1l	ENSMUST00000159414	2,33E-06
MACS2_peak_2421	chr16	67463008	67463055	Cadm2	ENSMUST00000123266	2,33E-06
MACS2_peak_2455	chr16	88010010	88010057	Grik1	ENSMUST00000114137	2,33E-06
MACS2_peak_2488	chr16	97502422	97502469	Fam3b	ENSMUST00000049721	2,33E-06
MACS2_peak_2514	chr17	10437987	10438046	Pacrg	ENSMUST00000041463	2,33E-06
MACS2_peak_256	chr1	124017950	124017997	Dpp10	ENSMUST00000140361	2,33E-06
MACS2_peak_2641	chr17	55760569	55760624	Emr4	ENSMUST00000025004	2,33E-06
MACS2_peak_2646	chr17	56643470	56643517	Catsperd	ENSMUST00000112979	2,33E-06
MACS2_peak_2692	chr17	78601624	78601678	Vit	ENSMUST00000024880	2,33E-06
MACS2_peak_2694	chr17	79079177	79079235	Qpct	ENSMUST00000040789	2,33E-06
MACS2_peak_270	chr1	130134872	130134919	Thsd7b	ENSMUST00000151700	2,33E-06
MACS2_peak_271	chr1	130196286	130196335	Thsd7b	ENSMUST00000140834	2,33E-06
MACS2_peak_2729	chr17	87264112	87264167	Mcf2d	ENSMUST00000155904	2,33E-06
MACS2_peak_273	chr1	130833295	130833354	Pigr	ENSMUST00000137782	2,33E-06
MACS2_peak_2733	chr17	88445411	88445458	Foxn2	ENSMUST00000134107	2,33E-06

MACS2_peak_2762	chr18	5221236	5221304	Zfp438	ENSMUST00000063989	2,33E-06
MACS2_peak_2774	chr18	12732053	12732100	Ttc39c	ENSMUST00000025294	2,33E-06
MACS2_peak_278	chr1	132325771	132325819	Nuak2	ENSMUST00000072177	2,33E-06
MACS2_peak_2780	chr18	14970270	14970337	Kctd1	ENSMUST00000168989	2,33E-06
MACS2_peak_28	chr1	16085783	16085830	4930444P10Rik	ENSMUST00000145070	2,33E-06
MACS2_peak_2810	chr18	24997967	24998025	Fhod3	ENSMUST00000037097	2,33E-06
MACS2_peak_2874	chr18	60528511	60528558	Dctn4	ENSMUST00000025505	2,33E-06
MACS2_peak_2887	chr18	65003928	65003975	Nedd4l	ENSMUST00000163516	2,33E-06
MACS2_peak_2893	chr18	67642838	67642899	Psmg2	ENSMUST00000025418	2,33E-06
MACS2_peak_2914	chr18	76983317	76983366	Katnal2	ENSMUST00000137354	2,33E-06
MACS2_peak_2954	chr19	4124325	4124372	Aip	ENSMUST00000127767	2,33E-06
MACS2_peak_2965	chr19	6509152	6509200	Nrxn2	ENSMUST00000113459	2,33E-06
MACS2_peak_298	chr1	136884681	136884747	Nr5a2	ENSMUST00000027649	2,33E-06
MACS2_peak_3011	chr19	24272140	24272240	Fxn	ENSMUST00000132688	2,33E-06
MACS2_peak_3023	chr19	29768300	29768347	9930021J03Rik	ENSMUST00000059484	2,33E-06
MACS2_peak_3045	chr19	41718428	41718480	Slit1	ENSMUST00000171586	2,33E-06
MACS2_peak_3057	chr19	47293322	47293369	Sh3pxd2a	ENSMUST00000081619	2,33E-06
MACS2_peak_3079	chr19	55474655	55474713	Vti1a	ENSMUST00000095950	2,33E-06
MACS2_peak_3080	chr19	55770489	55770536	Tcf7l2	ENSMUST00000133008	2,33E-06
MACS2_peak_3086	chr19	56389459	56389508	Nrap	ENSMUST00000166203	2,33E-06
MACS2_peak_3088	chr19	57124130	57124178	Ablim1	ENSMUST00000133782	2,33E-06
MACS2_peak_3098	chr19	61106353	61106400	Zfp950	ENSMUST00000122927	2,33E-06
MACS2_peak_3103	chr2	4111304	4111354	Frmd4a	ENSMUST00000177457	2,33E-06
MACS2_peak_3104	chr2	4165772	4165838	Frmd4a	ENSMUST00000132710	2,33E-06
MACS2_peak_3127	chr2	12390945	12390997	Fam188a	ENSMUST00000129348	2,33E-06
MACS2_peak_313	chr1	146533602	146533649	Brinp3	ENSMUST00000128345	2,33E-06
MACS2_peak_3130	chr2	14185260	14185307	Tmem236	ENSMUST00000077517	2,33E-06
MACS2_peak_3147	chr2	24096200	24096247	Tbpl2	ENSMUST00000153338	2,33E-06
MACS2_peak_3155	chr2	26637729	26637784	Snhg7	ENSMUST00000180659	2,33E-06
MACS2_peak_3160	chr2	27459467	27459549	Brd3	ENSMUST00000130932	2,33E-06
MACS2_peak_3178	chr2	30112060	30112107	Zer1	ENSMUST00000133354	2,33E-06
MACS2_peak_3211	chr2	37504912	37504976	Strbp	ENSMUST00000183690	2,33E-06
MACS2_peak_3260	chr2	58425970	58426119	Acvr1	ENSMUST00000145495	2,33E-06
MACS2_peak_3272	chr2	65987534	65987581	Csrnp3	ENSMUST00000112394	2,33E-06
MACS2_peak_3275	chr2	68214795	68214845	Stk39	ENSMUST00000139407	2,33E-06
MACS2_peak_3293	chr2	73860790	73860860	Atf2	ENSMUST00000128531	2,33E-06
MACS2_peak_3326	chr2	85195786	85195833	Lrrc55	ENSMUST00000111601	2,33E-06
MACS2_peak_3338	chr2	92883084	92883134	Gm13791	ENSMUST00000123263	2,33E-06
MACS2_peak_3371	chr2	105957010	105957057	Immp1l	ENSMUST00000037499	2,33E-06
MACS2_peak_34	chr1	20311582	20311634	Pkhd1	ENSMUST00000088448	2,33E-06
MACS2_peak_3430	chr2	127953753	127953804	Acox1	ENSMUST00000110344	2,33E-06
MACS2_peak_3436	chr2	130557827	130557878	Ptpra	ENSMUST00000079964	2,33E-06
MACS2_peak_3440	chr2	132063903	132063960	Slc23a2	ENSMUST00000137910	2,33E-06
MACS2_peak_3460	chr2	142795676	142795731	Kif16b	ENSMUST00000043589	2,33E-06
MACS2_peak_3486	chr2	152268316	152268363	Csnk2a1	ENSMUST00000136063	2,33E-06
MACS2_peak_3489	chr2	153311024	153311125	Kif3b	ENSMUST00000028977	2,33E-06

MACS2_peak_3499	chr2	156698224	156698272	Dlgap4	ENSMUST00000109567	2,33E-06
MACS2_peak_3510	chr2	162500798	162500845	Ptprt	ENSMUST00000109441	2,33E-06
MACS2_peak_3525	chr2	166110220	166110269	Sulf2	ENSMUST00000146497	2,33E-06
MACS2_peak_3535	chr2	166669611	166669665	Prex1	ENSMUST00000140624	2,33E-06
MACS2_peak_3557	chr2	170057676	170057724	Tshz2	ENSMUST00000123300	2,33E-06
MACS2_peak_3605	chr3	8837966	8838013	Mrps28	ENSMUST00000042148	2,33E-06
MACS2_peak_3606	chr3	8946705	8946763	Tpd52	ENSMUST00000124956	2,33E-06
MACS2_peak_3608	chr3	9419083	9419131	C030034L19Rik	ENSMUST00000180874	2,33E-06
MACS2_peak_3620	chr3	14070381	14070449	Raly1	ENSMUST00000171075	2,33E-06
MACS2_peak_369	chr1	169929323	169929370	Ccdc190	ENSMUST00000094348	2,33E-06
MACS2_peak_3714	chr3	59167723	59167770	Med12l	ENSMUST00000040325	2,33E-06
MACS2_peak_3730	chr3	68957823	68957875	Ift80	ENSMUST00000176754	2,33E-06
MACS2_peak_3741	chr3	79570952	79570999	4930589L23Rik	ENSMUST00000091023	2,33E-06
MACS2_peak_3775	chr3	90611270	90611317	S100a5	ENSMUST00000001049	2,33E-06
MACS2_peak_3782	chr3	93216247	93216337	Flg2	ENSMUST00000098884	2,33E-06
MACS2_peak_3792	chr3	96638363	96638410	Pex11b	ENSMUST00000165842	2,33E-06
MACS2_peak_3794	chr3	96840805	96841143	Pdzk1	ENSMUST00000128789	2,33E-06
MACS2_peak_3804	chr3	97700975	97701023	Pde4dip	ENSMUST00000090750	2,33E-06
MACS2_peak_3880	chr3	133145105	133145152	Arhgef38	ENSMUST00000147041	2,33E-06
MACS2_peak_3889	chr3	135840921	135840968	Slc39a8	ENSMUST00000029810	2,33E-06
MACS2_peak_3935	chr3	153481153	153481243	St6galnac3	ENSMUST00000064460	2,33E-06
MACS2_peak_3966	chr4	5737482	5737530	Fam110b	ENSMUST00000141479	2,33E-06
MACS2_peak_3974	chr4	10711273	10711320	1700123O12Rik	ENSMUST00000146783	2,33E-06
MACS2_peak_401	chr1	183202798	183202855	Disp1	ENSMUST00000003035	2,33E-06
MACS2_peak_4080	chr4	59509416	59509479	Ptbp3	ENSMUST00000134879	2,33E-06
MACS2_peak_4101	chr4	70440699	70440746	Megf9	ENSMUST00000107359	2,33E-06
MACS2_peak_4120	chr4	82521302	82521375	Nfib	ENSMUST00000155821	2,33E-06
MACS2_peak_414	chr1	189883421	189883472	Smyd2	ENSMUST00000130804	2,33E-06
MACS2_peak_4144	chr4	95889904	95889951	Fggy	ENSMUST00000156223	2,33E-06
MACS2_peak_4152	chr4	100842923	100842970	Cachd1	ENSMUST00000097955	2,33E-06
MACS2_peak_4159	chr4	104019120	104019167	Dab1	ENSMUST00000106827	2,33E-06
MACS2_peak_4163	chr4	105015707	105015754	1700024P16Rik	ENSMUST00000106803	2,33E-06
MACS2_peak_4180	chr4	113590627	113590674	Skint5	ENSMUST00000170105	2,33E-06
MACS2_peak_4192	chr4	120118644	120118699	Hivep3	ENSMUST00000166542	2,33E-06
MACS2_peak_421	chr1	192670972	192671042	Hhat	ENSMUST00000154755	2,33E-06
MACS2_peak_4235	chr4	130820709	130820758	Sdc3	ENSMUST00000140623	2,33E-06
MACS2_peak_4269	chr4	138163264	138163311	Eif4g3	ENSMUST00000140796	2,33E-06
MACS2_peak_428	chr1	195037664	195037711	A330023F24Rik	ENSMUST00000181273	2,33E-06
MACS2_peak_4318	chr4	152261064	152261111	Acot7	ENSMUST00000105652	2,33E-06
MACS2_peak_4334	chr4	155143088	155143148	Morn1	ENSMUST00000030915	2,33E-06
MACS2_peak_4336	chr4	155528866	155528923	Gnb1	ENSMUST00000176637	2,33E-06
MACS2_peak_4405	chr5	31468656	31468703	Zfp512	ENSMUST00000076264	2,33E-06
MACS2_peak_4422	chr5	38044569	38044616	Stx18	ENSMUST00000042146	2,33E-06
MACS2_peak_446	chr10	6870286	6870333	Oprm1	ENSMUST00000141897	2,33E-06
MACS2_peak_4484	chr5	71924660	71924720	Gabrb1	ENSMUST00000031122	2,33E-06
MACS2_peak_4493	chr5	74696731	74696790	Lnx1	ENSMUST00000117388	2,33E-06

MACS2_peak_4523	chr5	89752927	89752985	Adams3	ENSMUST00000061427	2,33E-06
MACS2_peak_4537	chr5	92864269	92864326	Shroom3	ENSMUST00000168878	2,33E-06
MACS2_peak_455	chr10	11024289	11024388	Grm1	ENSMUST00000105561	2,33E-06
MACS2_peak_4578	chr5	111160862	111160909	Ttc28	ENSMUST00000040111	2,33E-06
MACS2_peak_4579	chr5	111241631	111241678	Ttc28	ENSMUST00000040111	2,33E-06
MACS2_peak_4635	chr5	120619703	120619750	Ddx54	ENSMUST00000031598	2,33E-06
MACS2_peak_4659	chr5	127693578	127693639	Glt1d1	ENSMUST00000118139	2,33E-06
MACS2_peak_467	chr10	18114545	18114592	Reps1	ENSMUST00000144287	2,33E-06
MACS2_peak_4683	chr5	134620955	134621002	Eif4h	ENSMUST00000036125	2,33E-06
MACS2_peak_4709	chr5	139707808	139707863	Mical2	ENSMUST00000170773	2,33E-06
MACS2_peak_4713	chr5	140082463	140082510	Mad1l1	ENSMUST00000110829	2,33E-06
MACS2_peak_4749	chr5	149140328	149140409	5730422E09Rik	ENSMUST00000180733	2,33E-06
MACS2_peak_4807	chr6	23462800	23462855	Cadps2	ENSMUST00000115356	2,33E-06
MACS2_peak_4832	chr6	33851219	33851266	Exoc4	ENSMUST00000143190	2,33E-06
MACS2_peak_4853	chr6	40122780	40122827	Tmem178b	ENSMUST00000061740	2,33E-06
MACS2_peak_4859	chr6	41557735	41557790	Trbv31	ENSMUST00000103300	2,33E-06
MACS2_peak_4868	chr6	45737018	45737085	Cntnap2	ENSMUST00000114641	2,33E-06
MACS2_peak_4891	chr6	48556781	48556840	Lrrc61	ENSMUST00000146245	2,33E-06
MACS2_peak_493	chr10	25475285	25475332	Epb4.1l2	ENSMUST00000092645	2,33E-06
MACS2_peak_4935	chr6	64558018	64558076	Grid2	ENSMUST00000095852	2,33E-06
MACS2_peak_4955	chr6	72787450	72787497	Tcf7l1	ENSMUST00000149446	2,33E-06
MACS2_peak_496	chr10	26366810	26366857	L3mbtl3	ENSMUST00000174766	2,33E-06
MACS2_peak_4990	chr6	86551021	86551068	1600020E01Rik	ENSMUST00000181928	2,33E-06
MACS2_peak_4992	chr6	86932621	86932695	Aak1	ENSMUST00000113668	2,33E-06
MACS2_peak_5014	chr6	93041157	93041226	9530026P05Rik	ENSMUST00000181121	2,33E-06
MACS2_peak_5018	chr6	94119148	94119195	Magi1	ENSMUST00000089317	2,33E-06
MACS2_peak_5027	chr6	97250892	97250939	Lmod3	ENSMUST00000095655	2,33E-06
MACS2_peak_5030	chr6	97911765	97911812	Mitf	ENSMUST00000043637	2,33E-06
MACS2_peak_5031	chr6	99028623	99028670	Foxp1	ENSMUST00000177507	2,33E-06
MACS2_peak_506	chr10	33097960	33098007	Trdn	ENSMUST00000095762	2,33E-06
MACS2_peak_5088	chr6	121212489	121212539	Tuba8	ENSMUST00000032233	2,33E-06
MACS2_peak_5091	chr6	122560641	122560769	Gm16556	ENSMUST00000162608	2,33E-06
MACS2_peak_5098	chr6	125364006	125364053	Plekhg6	ENSMUST00000042647	2,33E-06
MACS2_peak_5131	chr6	141750681	141750728	Gm5724	ENSMUST00000148411	2,33E-06
MACS2_peak_5132	chr6	142095076	142095126	Slco1a6	ENSMUST00000111827	2,33E-06
MACS2_peak_5133	chr6	142702090	142702144	Abcc9	ENSMUST00000073173	2,33E-06
MACS2_peak_5137	chr6	144083316	144083363	Sox5	ENSMUST00000129050	2,33E-06
MACS2_peak_5138	chr6	144501092	144501197	Sox5	ENSMUST00000149451	2,33E-06
MACS2_peak_514	chr10	36712401	36712454	Hs3st5	ENSMUST00000171521	2,33E-06
MACS2_peak_5187	chr7	12673492	12673550	Vmn2r55	ENSMUST00000167771	2,33E-06
MACS2_peak_5192	chr7	16206528	16206575	Dhx34	ENSMUST00000133518	2,33E-06
MACS2_peak_5193	chr7	16454328	16454408	Tmem160	ENSMUST00000019302	2,33E-06
MACS2_peak_5196	chr7	16850892	16850940	Prkd2	ENSMUST00000086104	2,33E-06
MACS2_peak_5229	chr7	27635273	27635346	Akt2	ENSMUST00000085917	2,33E-06
MACS2_peak_5236	chr7	29034759	29034812	Ryr1	ENSMUST00000032813	2,33E-06
MACS2_peak_5238	chr7	29341629	29341681	Sipa1l3	ENSMUST00000182829	2,33E-06

MACS2_peak_5250	chr7	35014157	35014210	Pepd	ENSMUST00000161900	2,33E-06
MACS2_peak_5282	chr7	45193964	45194011	Ccdc155	ENSMUST00000121017	2,33E-06
MACS2_peak_5329	chr7	65920247	65920301	Pcsk6	ENSMUST00000176209	2,33E-06
MACS2_peak_5333	chr7	67063549	67063596	Adamts17	ENSMUST00000107478	2,33E-06
MACS2_peak_5356	chr7	75328341	75328389	1500012K07Rik	ENSMUST00000181846	2,33E-06
MACS2_peak_5401	chr7	91163331	91163415	Dlg2	ENSMUST00000146563	2,33E-06
MACS2_peak_5409	chr7	96964337	96964397	Nars2	ENSMUST00000149204	2,33E-06
MACS2_peak_542	chr10	52922265	52922320	Slc35f1	ENSMUST00000105473	2,33E-06
MACS2_peak_543	chr10	52998785	52998859	Slc35f1	ENSMUST00000105473	2,33E-06
MACS2_peak_5469	chr7	119888643	119888690	Dcun1d3	ENSMUST00000098080	2,33E-06
MACS2_peak_5475	chr7	122162790	122162847	Plk1	ENSMUST00000033154	2,33E-06
MACS2_peak_5537	chr7	139090016	139090063	Dpysl4	ENSMUST00000121184	2,33E-06
MACS2_peak_5590	chr8	13918898	13918961	Fbxo25	ENSMUST00000043520	2,33E-06
MACS2_peak_5615	chr8	24065356	24065403	Zmat4	ENSMUST00000123412	2,33E-06
MACS2_peak_5616	chr8	24568444	24568491	Ido2	ENSMUST00000121992	2,33E-06
MACS2_peak_5619	chr8	25690799	25690886	Whsc1l1	ENSMUST00000084026	2,33E-06
MACS2_peak_5652	chr8	40448758	40448805	Zdhhc2	ENSMUST00000168799	2,33E-06
MACS2_peak_567	chr10	60283283	60283332	Psap	ENSMUST00000004316	2,33E-06
MACS2_peak_5670	chr8	48232882	48232935	Tenm3	ENSMUST00000129821	2,33E-06
MACS2_peak_5685	chr8	54710606	54710663	Wdr17	ENSMUST00000148806	2,33E-06
MACS2_peak_5690	chr8	57571617	57571664	Galnt7	ENSMUST00000156907	2,33E-06
MACS2_peak_5691	chr8	57871488	57871539	Galntl6	ENSMUST00000098757	2,33E-06
MACS2_peak_570	chr10	61780526	61780574	H2afy2	ENSMUST00000020283	2,33E-06
MACS2_peak_5704	chr8	63164207	63164265	Spock3	ENSMUST00000119068	2,33E-06
MACS2_peak_571	chr10	61862120	61862202	Col13a1	ENSMUST00000051826	2,33E-06
MACS2_peak_5710	chr8	66131263	66131310	42795	ENSMUST00000152320	2,33E-06
MACS2_peak_5715	chr8	68901735	68901782	Lpl	ENSMUST00000015712	2,33E-06
MACS2_peak_5721	chr8	70321996	70322043	Gdf1	ENSMUST00000165819	2,33E-06
MACS2_peak_5726	chr8	70502752	70502800	Crlf1	ENSMUST00000127983	2,33E-06
MACS2_peak_5728	chr8	70505346	70505393	2810428I15Rik	ENSMUST00000135773	2,33E-06
MACS2_peak_5730	chr8	70532304	70532351	Fkbp8	ENSMUST00000126613	2,33E-06
MACS2_peak_5731	chr8	70600829	70600876	Ssbp4	ENSMUST00000049908	2,33E-06
MACS2_peak_5737	chr8	71392981	71393035	Ushbp1	ENSMUST00000049184	2,33E-06
MACS2_peak_5775	chr8	84861304	84861360	Farsa	ENSMUST00000156970	2,33E-06
MACS2_peak_5829	chr8	107094191	107094238	Terf2	ENSMUST00000068388	2,33E-06
MACS2_peak_5836	chr8	108905681	108905731	Zfhx3	ENSMUST00000043896	2,33E-06
MACS2_peak_5854	chr8	114465484	114465543	Wwox	ENSMUST00000161753	2,33E-06
MACS2_peak_5855	chr8	114580701	114580748	Wwox	ENSMUST00000160862	2,33E-06
MACS2_peak_5865	chr8	115286902	115286949	Wwox	ENSMUST00000162052	2,33E-06
MACS2_peak_5885	chr8	119937057	119937115	Usp10	ENSMUST00000143615	2,33E-06
MACS2_peak_5900	chr8	123087559	123087606	Spq7	ENSMUST00000152972	2,33E-06
MACS2_peak_5904	chr8	123983108	123983160	Gm20388	ENSMUST00000127664	2,33E-06
MACS2_peak_5906	chr8	124191324	124191386	Gm20388	ENSMUST00000127664	2,33E-06
MACS2_peak_5908	chr8	125159888	125159935	Disc1	ENSMUST00000121953	2,33E-06
MACS2_peak_5914	chr8	127088204	127088251	Pard3	ENSMUST00000161277	2,33E-06
MACS2_peak_1248	chr12	53919201	53919252	Npas3	ENSMUST00000101432	3,44E-06

MACS2_peak_1315	chr12	86261275	86261322	Gpatch2l	ENSMUST00000071106	3,44E-06
MACS2_peak_135	chr1	64798145	64798202	Plekhn3	ENSMUST00000097713	3,44E-06
MACS2_peak_1788	chr14	30661611	30661658	Rft1	ENSMUST00000143534	3,44E-06
MACS2_peak_2140	chr15	72659493	72659546	Trappc9	ENSMUST00000089770	3,44E-06
MACS2_peak_2971	chr19	7182062	7182109	Macrodl	ENSMUST00000040261	3,44E-06
MACS2_peak_4183	chr4	117216097	117216144	Gm1661	ENSMUST00000077500	3,44E-06
MACS2_peak_4692	chr5	136187148	136187204	Prkrip1	ENSMUST00000149151	3,44E-06
MACS2_peak_5174	chr7	9935830	9935877	Vmn2r48	ENSMUST00000165611	3,44E-06
MACS2_peak_5890	chr8	120678315	120678362	Gm20388	ENSMUST00000127664	3,44E-06
MACS2_peak_4744	chr5	147611444	147611536	Flt1	ENSMUST00000031653	3,57E-06
MACS2_peak_5068	chr6	115807763	115807814	Rpl32	ENSMUST00000081840	3,74E-06
MACS2_peak_5571	chr8	9297315	9297378	Fam155a	ENSMUST00000110969	3,74E-06
MACS2_peak_6243	chr9	118111807	118111883	Cmc1	ENSMUST00000044220	3,74E-06
MACS2_peak_1267	chr12	69654111	69654225	Sos2	ENSMUST00000182396	3,91E-06
MACS2_peak_2915	chr18	77095871	77095942	Pias2	ENSMUST00000114776	3,92E-06
MACS2_peak_4035	chr4	41615396	41615494	Dnaic1	ENSMUST00000102963	3,92E-06
MACS2_peak_1695	chr13	107859842	107860004	Zswim6	ENSMUST00000105097	4,35E-06
MACS2_peak_1229	chr12	46708291	46708404	Nova1	ENSMUST00000021438	4,74E-06
MACS2_peak_1030	chr11	98943137	98943184	Rara	ENSMUST00000147985	4,76E-06
MACS2_peak_1407	chr12	111065384	111065431	Rcor1	ENSMUST00000116388	4,76E-06
MACS2_peak_145	chr1	70178502	70178550	Spag16	ENSMUST00000161310	4,76E-06
MACS2_peak_1715	chr13	113806442	113806559	Arl15	ENSMUST00000091201	5,56E-06
MACS2_peak_4320	chr4	152291050	152291097	Hes3	ENSMUST00000094438	5,74E-06
MACS2_peak_5366	chr7	79278868	79278915	Abhd2	ENSMUST00000135333	6,11E-06
MACS2_peak_1113	chr11	117873319	117873371	Tha1	ENSMUST00000033230	6,49E-06
MACS2_peak_1952	chr14	105893988	105894035	Spry2	ENSMUST00000022709	6,49E-06
MACS2_peak_2509	chr17	8888426	8888473	Pde10a	ENSMUST00000024647	6,49E-06
MACS2_peak_3030	chr19	33647737	33647784	Lip1	ENSMUST00000025694	6,49E-06
MACS2_peak_2602	chr17	39847301	39847358	Gm26924	ENSMUST00000182010	6,77E-06
MACS2_peak_2604	chr17	39848478	39848583	Gm26924	ENSMUST00000182010	7,68E-06
MACS2_peak_2507	chr17	7956533	7956585	Rsph3a	ENSMUST00000097423	8,11E-06
MACS2_peak_2798	chr18	20622128	20622228	Gm16090	ENSMUST00000130296	8,11E-06
MACS2_peak_3721	chr3	64105291	64105401	Vmn2r1	ENSMUST00000029406	8,11E-06
MACS2_peak_3800	chr3	96844722	96844906	Pdzk1	ENSMUST00000128789	8,11E-06
MACS2_peak_389	chr1	180244330	180244392	Psen2	ENSMUST00000111104	8,11E-06
MACS2_peak_4267	chr4	137795841	137795891	Alpl	ENSMUST00000138463	8,11E-06
MACS2_peak_4652	chr5	124564502	124564600	Ddx55	ENSMUST00000142451	8,11E-06
MACS2_peak_192	chr1	87544009	87544056	Ngef	ENSMUST00000068681	8,74E-06
MACS2_peak_261	chr1	127419496	127419543	Mgat5	ENSMUST00000038361	8,74E-06
MACS2_peak_4046	chr4	45193711	45193906	Frmpd1	ENSMUST00000134280	8,74E-06
MACS2_peak_495	chr10	26001545	26001592	Tmem200a	ENSMUST00000066049	8,74E-06
MACS2_peak_758	chr11	3148722	3148786	Sfi1	ENSMUST00000138126	1,09E-05
MACS2_peak_4343	chr5	3962669	3962740	Akap9	ENSMUST00000044492	1,16E-05
MACS2_peak_4899	chr6	50112954	50113001	Mpp6	ENSMUST00000171601	1,16E-05
MACS2_peak_6245	chr9	118113716	118113807	Cmc1	ENSMUST00000044220	1,16E-05
MACS2_peak_2966	chr19	6527295	6527342	Nrxn2	ENSMUST00000113459	1,94E-05

MACS2_peak_2969	chr19	6848366	6848429	Ccdc88b	ENSMUST00000113440	1,94E-05
MACS2_peak_2974	chr19	8603400	8603447	Slc22a8	ENSMUST00000170817	1,94E-05
MACS2_peak_2979	chr19	10636066	10636129	Vwce	ENSMUST00000055115	1,94E-05
MACS2_peak_2995	chr19	16750670	16750720	Vps13a	ENSMUST00000068156	1,94E-05
MACS2_peak_2998	chr19	19030331	19030378	Rorb	ENSMUST00000112832	1,94E-05
MACS2_peak_3007	chr19	23876023	23876130	Apba1	ENSMUST00000025830	1,94E-05
MACS2_peak_301	chr1	139063132	139063179	Dennd1b	ENSMUST00000094505	1,94E-05
MACS2_peak_3017	chr19	27405559	27405648	D19Bwg1357e	ENSMUST00000076219	1,94E-05
MACS2_peak_302	chr1	139317177	139317285	Crb1	ENSMUST00000132845	1,94E-05
MACS2_peak_3024	chr19	30074621	30074668	Uhrf2	ENSMUST00000129420	1,94E-05
MACS2_peak_3037	chr19	37279271	37279326	Ide	ENSMUST00000154339	1,94E-05
MACS2_peak_3041	chr19	39555664	39555711	Cyp2c39	ENSMUST00000025968	1,94E-05
MACS2_peak_3056	chr19	46314572	46314619	Psd	ENSMUST00000096029	1,94E-05
MACS2_peak_3061	chr19	48471089	48471136	Sorcs3	ENSMUST00000078880	1,94E-05
MACS2_peak_3066	chr19	50525005	50525066	Sorcs1	ENSMUST00000164039	1,94E-05
MACS2_peak_3070	chr19	52998356	52998403	Xpnp1	ENSMUST00000182877	1,94E-05
MACS2_peak_3074	chr19	53977142	53977189	Shoc2	ENSMUST00000169861	1,94E-05
MACS2_peak_3075	chr19	54045245	54045319	Adra2a	ENSMUST00000036700	1,94E-05
MACS2_peak_3092	chr19	58348448	58348496	Gfra1	ENSMUST00000140141	1,94E-05
MACS2_peak_3093	chr19	58810904	58810963	Hspa12a	ENSMUST00000066285	1,94E-05
MACS2_peak_3116	chr2	7764739	7764786	Gm13211	ENSMUST00000141649	1,94E-05
MACS2_peak_3129	chr2	13436527	13436574	Cubn	ENSMUST00000091436	1,94E-05
MACS2_peak_3136	chr2	20142964	20143011	Etl4	ENSMUST00000114610	1,94E-05
MACS2_peak_3143	chr2	22240547	22240594	Myo3a	ENSMUST00000138850	1,94E-05
MACS2_peak_3170	chr2	29019875	29019922	Cfap77	ENSMUST00000157048	1,94E-05
MACS2_peak_3171	chr2	29223296	29223360	Ntng2	ENSMUST00000071201	1,94E-05
MACS2_peak_3179	chr2	30134724	30134781	Tbc1d13	ENSMUST00000134013	1,94E-05
MACS2_peak_3185	chr2	31166835	31166898	Gpr107	ENSMUST00000172422	1,94E-05
MACS2_peak_3189	chr2	32054308	32054367	Gm16534	ENSMUST00000149300	1,94E-05
MACS2_peak_3202	chr2	34734075	34734122	Gapvd1	ENSMUST00000142436	1,94E-05
MACS2_peak_3206	chr2	35648446	35648493	Dab2ip	ENSMUST00000112992	1,94E-05
MACS2_peak_3221	chr2	39078648	39078695	Scal	ENSMUST00000038874	1,94E-05
MACS2_peak_3234	chr2	44240784	44240833	Arhgap15	ENSMUST00000055776	1,94E-05
MACS2_peak_3238	chr2	44638658	44638729	Gtdc1	ENSMUST00000148279	1,94E-05
MACS2_peak_3244	chr2	49235678	49235725	Mbd5	ENSMUST00000112745	1,94E-05
MACS2_peak_3249	chr2	52732826	52732873	Stam2	ENSMUST00000155516	1,94E-05
MACS2_peak_3252	chr2	53199281	53199328	Arl6ip6	ENSMUST00000028336	1,94E-05
MACS2_peak_3264	chr2	60270545	60270594	Cd302	ENSMUST00000028356	1,94E-05
MACS2_peak_3268	chr2	62570827	62570874	Fap	ENSMUST00000173745	1,94E-05
MACS2_peak_3271	chr2	65943530	65943577	Csrp3	ENSMUST00000145598	1,94E-05
MACS2_peak_3291	chr2	73585967	73586071	Gm13705	ENSMUST00000147235	1,94E-05
MACS2_peak_3294	chr2	73899285	73899332	Gm10822	ENSMUST00000181596	1,94E-05
MACS2_peak_3309	chr2	79314068	79314115	Itga4	ENSMUST00000099972	1,94E-05
MACS2_peak_331	chr1	155171289	155171336	Stx6	ENSMUST00000027743	1,94E-05
MACS2_peak_3311	chr2	79937378	79937455	Pde1a	ENSMUST00000146076	1,94E-05
MACS2_peak_3325	chr2	85014502	85014601	P2rx3	ENSMUST00000111616	1,94E-05

MACS2_peak_3333	chr2	90909845	90909907	Ptpmt1	ENSMUST00000125001	1,94E-05
MACS2_peak_3334	chr2	91662106	91662157	Arhgap1	ENSMUST00000111330	1,94E-05
MACS2_peak_3340	chr2	92899760	92899807	Gm13791	ENSMUST00000123263	1,94E-05
MACS2_peak_3343	chr2	93758630	93758677	Ext2	ENSMUST00000123649	1,94E-05
MACS2_peak_3350	chr2	97197342	97197389	Lrrc4c	ENSMUST00000162807	1,94E-05
MACS2_peak_3351	chr2	97418891	97418938	Lrrc4c	ENSMUST00000162807	1,94E-05
MACS2_peak_3363	chr2	103717218	103717267	Abtb2	ENSMUST00000131122	1,94E-05
MACS2_peak_3364	chr2	103743855	103743912	Nat10	ENSMUST00000145189	1,94E-05
MACS2_peak_3370	chr2	105807725	105807775	Elp4	ENSMUST00000152272	1,94E-05
MACS2_peak_3386	chr2	111165381	111165441	4930430A15Rik	ENSMUST00000142636	1,94E-05
MACS2_peak_3392	chr2	113695421	113695468	Fmn1	ENSMUST00000152255	1,94E-05
MACS2_peak_3409	chr2	120864738	120864811	Ubr1	ENSMUST00000153868	1,94E-05
MACS2_peak_3413	chr2	122241424	122241471	Sord	ENSMUST00000110551	1,94E-05
MACS2_peak_3424	chr2	126036363	126036410	Fgf7	ENSMUST00000110442	1,94E-05
MACS2_peak_3429	chr2	127775842	127775947	Nphp1	ENSMUST00000110357	1,94E-05
MACS2_peak_3433	chr2	129166288	129166344	A1847159	ENSMUST00000148997	1,94E-05
MACS2_peak_3441	chr2	132077684	132077732	Slc23a2	ENSMUST00000127724	1,94E-05
MACS2_peak_3442	chr2	132792827	132792874	Chgb	ENSMUST00000028826	1,94E-05
MACS2_peak_3443	chr2	133445238	133445286	A430048G15Rik	ENSMUST00000149965	1,94E-05
MACS2_peak_345	chr1	162338627	162338674	Dnm3	ENSMUST00000161155	1,94E-05
MACS2_peak_3452	chr2	140174226	140174274	Ndufaf5	ENSMUST00000125913	1,94E-05
MACS2_peak_3456	chr2	142111623	142111670	MacroD2	ENSMUST00000138786	1,94E-05
MACS2_peak_3462	chr2	143830335	143830382	Bfsp1	ENSMUST00000099296	1,94E-05
MACS2_peak_3466	chr2	144687516	144687563	Dtd1	ENSMUST00000137406	1,94E-05
MACS2_peak_3498	chr2	156566426	156566473	Aar2	ENSMUST00000109570	1,94E-05
MACS2_peak_3504	chr2	158001118	158001165	Tti1	ENSMUST00000126541	1,94E-05
MACS2_peak_3506	chr2	158522669	158522716	Arhgap40	ENSMUST00000165398	1,94E-05
MACS2_peak_3513	chr2	163533971	163534034	Hnf4a	ENSMUST00000143911	1,94E-05
MACS2_peak_3518	chr2	165072312	165072359	Cd40	ENSMUST00000017799	1,94E-05
MACS2_peak_353	chr1	165705807	165705870	Rcsd1	ENSMUST00000097474	1,94E-05
MACS2_peak_3536	chr2	166692342	166692397	Prex1	ENSMUST00000140624	1,94E-05
MACS2_peak_354	chr1	165852243	165852290	Cd247	ENSMUST00000086002	1,94E-05
MACS2_peak_3583	chr2	179775066	179775153	Cdh4	ENSMUST00000108911	1,94E-05
MACS2_peak_3584	chr2	179871855	179871927	Cdh4	ENSMUST00000000314	1,94E-05
MACS2_peak_3589	chr2	181530678	181530725	Dnajc5	ENSMUST00000108796	1,94E-05
MACS2_peak_36	chr1	21486388	21486474	Kcnq5	ENSMUST00000115299	1,94E-05
MACS2_peak_3609	chr3	9440814	9440861	Zfp704	ENSMUST00000041124	1,94E-05
MACS2_peak_3642	chr3	26715798	26715850	Spata16	ENSMUST00000047005	1,94E-05
MACS2_peak_3647	chr3	27681788	27681835	Fndc3b	ENSMUST00000046157	1,94E-05
MACS2_peak_3649	chr3	30398723	30398770	Mecom	ENSMUST00000173411	1,94E-05
MACS2_peak_3650	chr3	30927298	30927345	Phc3	ENSMUST00000124472	1,94E-05
MACS2_peak_3662	chr3	37349789	37349837	Fgf2	ENSMUST00000038885	1,94E-05
MACS2_peak_3699	chr3	52095143	52095234	Maml3	ENSMUST00000121440	1,94E-05
MACS2_peak_37	chr1	21731871	21731918	Kcnq5	ENSMUST00000115299	1,94E-05
MACS2_peak_372	chr1	173802200	173802250	Gm16340	ENSMUST00000155076	1,94E-05
MACS2_peak_3725	chr3	66171335	66171388	Veph1	ENSMUST00000029419	1,94E-05

MACS2_peak_373	chr1	174608082	174608129	Fmn2	ENSMUST00000030039	1,94E-05
MACS2_peak_375	chr1	176054779	176054826	Pld5	ENSMUST00000156184	1,94E-05
MACS2_peak_3758	chr3	86556589	86556649	Lrba	ENSMUST00000107635	1,94E-05
MACS2_peak_376	chr1	176238824	176238871	Pld5	ENSMUST00000065967	1,94E-05
MACS2_peak_3765	chr3	87930779	87930832	Isg2012	ENSMUST00000055984	1,94E-05
MACS2_peak_3768	chr3	88936747	88936794	Dap3	ENSMUST00000172942	1,94E-05
MACS2_peak_3772	chr3	89537858	89537905	Kcnn3	ENSMUST00000000811	1,94E-05
MACS2_peak_3780	chr3	93215653	93215700	Flg2	ENSMUST00000098884	1,94E-05
MACS2_peak_38	chr1	22602181	22602228	Rims1	ENSMUST00000097808	1,94E-05
MACS2_peak_3813	chr3	103052483	103052552	Csde1	ENSMUST00000029446	1,94E-05
MACS2_peak_3821	chr3	105752374	105752421	Rap1a	ENSMUST00000090678	1,94E-05
MACS2_peak_3825	chr3	108560063	108560110	Tmem167b	ENSMUST00000090546	1,94E-05
MACS2_peak_3843	chr3	116350537	116350584	Cdc14a	ENSMUST00000127704	1,94E-05
MACS2_peak_3854	chr3	122473765	122473870	Bcar3	ENSMUST00000029766	1,94E-05
MACS2_peak_3866	chr3	126693361	126693408	Camk2d	ENSMUST00000066452	1,94E-05
MACS2_peak_3872	chr3	129625656	129625703	Elovl6	ENSMUST00000071402	1,94E-05
MACS2_peak_3878	chr3	132806691	132806738	Tbck	ENSMUST00000169172	1,94E-05
MACS2_peak_3891	chr3	136696497	136696544	Ppp3ca	ENSMUST00000056758	1,94E-05
MACS2_peak_3893	chr3	137884970	137885017	Dnajb14	ENSMUST00000125226	1,94E-05
MACS2_peak_3897	chr3	139004015	139004062	Rap1gds1	ENSMUST00000029796	1,94E-05
MACS2_peak_3901	chr3	142632770	142632817	Gbp2	ENSMUST00000165774	1,94E-05
MACS2_peak_3908	chr3	144527040	144527087	Hs2st1	ENSMUST00000043325	1,94E-05
MACS2_peak_391	chr1	181383463	181383548	Cnih3	ENSMUST00000162685	1,94E-05
MACS2_peak_3929	chr3	152915687	152915734	St6galnac5	ENSMUST00000044278	1,94E-05
MACS2_peak_3936	chr3	153672534	153672581	St6galnac3	ENSMUST00000064460	1,94E-05
MACS2_peak_3969	chr4	8216335	8216438	Car8	ENSMUST00000066674	1,94E-05
MACS2_peak_3982	chr4	15006214	15006265	Necab1	ENSMUST00000108273	1,94E-05
MACS2_peak_3989	chr4	19897509	19897556	Atp6v0d2	ENSMUST00000029900	1,94E-05
MACS2_peak_3991	chr4	20325721	20325768	Nkain3	ENSMUST00000102998	1,94E-05
MACS2_peak_3992	chr4	20702844	20702915	Nkain3	ENSMUST00000102998	1,94E-05
MACS2_peak_4	chr1	4858590	4858656	Tcea1	ENSMUST00000081551	1,94E-05
MACS2_peak_4018	chr4	36899048	36899095	Lingo2	ENSMUST00000145615	1,94E-05
MACS2_peak_4021	chr4	38815389	38815453	Gm12381	ENSMUST00000126314	1,94E-05
MACS2_peak_4030	chr4	41200468	41200531	Ubap2	ENSMUST00000108068	1,94E-05
MACS2_peak_4031	chr4	41206671	41206718	Ubap2	ENSMUST00000108068	1,94E-05
MACS2_peak_4033	chr4	41386180	41386231	Ubap1	ENSMUST00000132235	1,94E-05
MACS2_peak_4036	chr4	42941930	42941986	N28178	ENSMUST00000069184	1,94E-05
MACS2_peak_4038	chr4	43032059	43032106	Gm26643	ENSMUST00000180854	1,94E-05
MACS2_peak_4039	chr4	43398275	43398352	Rusc2	ENSMUST00000136360	1,94E-05
MACS2_peak_4040	chr4	43564056	43564103	Creb3	ENSMUST00000159923	1,94E-05
MACS2_peak_4048	chr4	45502745	45502799	Shb	ENSMUST00000147448	1,94E-05
MACS2_peak_4053	chr4	48425034	48425081	Invs	ENSMUST00000143433	1,94E-05
MACS2_peak_4054	chr4	48450275	48450338	Tex10	ENSMUST00000030030	1,94E-05
MACS2_peak_4076	chr4	57754768	57754815	Gm20459	ENSMUST00000150412	1,94E-05
MACS2_peak_4078	chr4	58897008	58897135	AI314180	ENSMUST00000055822	1,94E-05
MACS2_peak_4081	chr4	59839769	59839816	Snx30	ENSMUST00000030080	1,94E-05

MACS2_peak_4082	chr4	59994709	59994756	Mup6	ENSMUST00000107521	1,94E-05
MACS2_peak_4089	chr4	63108384	63108469	Zfp618	ENSMUST00000064814	1,94E-05
MACS2_peak_4090	chr4	63148501	63148565	Ambp	ENSMUST00000030041	1,94E-05
MACS2_peak_411	chr1	188087696	188087781	Esrrg	ENSMUST00000110938	1,94E-05
MACS2_peak_4112	chr4	76220012	76220088	Ptprd	ENSMUST00000107287	1,94E-05
MACS2_peak_4124	chr4	83787950	83788045	Ccdc171	ENSMUST00000183729	1,94E-05
MACS2_peak_4129	chr4	86551078	86551125	Fam154a	ENSMUST00000133793	1,94E-05
MACS2_peak_4130	chr4	86666420	86666467	Plin2	ENSMUST00000140382	1,94E-05
MACS2_peak_4148	chr4	97797721	97797808	Nfia	ENSMUST00000107057	1,94E-05
MACS2_peak_4160	chr4	104394531	104394578	Dab1	ENSMUST00000146078	1,94E-05
MACS2_peak_4171	chr4	107740140	107740187	1700047F07Rik	ENSMUST00000143064	1,94E-05
MACS2_peak_4176	chr4	111077845	111077973	Agbl4	ENSMUST00000080744	1,94E-05
MACS2_peak_4177	chr4	111370081	111370130	Agbl4	ENSMUST00000148038	1,94E-05
MACS2_peak_4181	chr4	116444272	116444319	Mast2	ENSMUST00000106486	1,94E-05
MACS2_peak_4184	chr4	117650907	117650954	Eri3	ENSMUST00000134379	1,94E-05
MACS2_peak_4190	chr4	120031428	120031482	Hivep3	ENSMUST00000106307	1,94E-05
MACS2_peak_4196	chr4	120716906	120716964	Kcnq4	ENSMUST00000129478	1,94E-05
MACS2_peak_4198	chr4	123437089	123437136	Macf1	ENSMUST00000106224	1,94E-05
MACS2_peak_4199	chr4	123468427	123468474	Macf1	ENSMUST00000147030	1,94E-05
MACS2_peak_4200	chr4	123545995	123546042	Macf1	ENSMUST00000146000	1,94E-05
MACS2_peak_4203	chr4	124572195	124572242	RP23-230F2.8	ENSMUST00000184062	1,94E-05
MACS2_peak_4209	chr4	125570238	125570285	Grik3	ENSMUST00000030676	1,94E-05
MACS2_peak_4210	chr4	126090229	126090289	Oscp1	ENSMUST00000035497	1,94E-05
MACS2_peak_4228	chr4	129616855	129616902	Tmem234	ENSMUST00000174073	1,94E-05
MACS2_peak_4238	chr4	131863537	131863584	Mecr	ENSMUST00000143067	1,94E-05
MACS2_peak_4252	chr4	134591216	134591289	Man1c1	ENSMUST00000176606	1,94E-05
MACS2_peak_4254	chr4	134837644	134837772	Tmem57	ENSMUST00000136113	1,94E-05
MACS2_peak_4256	chr4	135246599	135246646	Clic4	ENSMUST00000037099	1,94E-05
MACS2_peak_4258	chr4	135761828	135761896	Myom3	ENSMUST00000105854	1,94E-05
MACS2_peak_4263	chr4	137001073	137001120	Zbtb40	ENSMUST00000049583	1,94E-05
MACS2_peak_4264	chr4	137339997	137340059	Cdc42	ENSMUST00000030417	1,94E-05
MACS2_peak_4279	chr4	140065344	140065391	Igsf21	ENSMUST00000039331	1,94E-05
MACS2_peak_4290	chr4	147104257	147104307	C230088H06Rik	ENSMUST00000181084	1,94E-05
MACS2_peak_4302	chr4	149035344	149035401	Pex14	ENSMUST00000148203	1,94E-05
MACS2_peak_4305	chr4	150289843	150289928	Rere	ENSMUST00000105682	1,94E-05
MACS2_peak_4307	chr4	151048839	151048886	Vamp3	ENSMUST00000030797	1,94E-05
MACS2_peak_4308	chr4	151190253	151190303	Camta1	ENSMUST00000049790	1,94E-05
MACS2_peak_4331	chr4	154174763	154174810	Megf6	ENSMUST00000030897	1,94E-05
MACS2_peak_4337	chr4	155556508	155556559	Gnb1	ENSMUST00000176308	1,94E-05
MACS2_peak_4352	chr5	9342781	9342839	9330182L06Rik	ENSMUST00000154662	1,94E-05
MACS2_peak_4365	chr5	16229699	16229777	Cacna2d1	ENSMUST00000115281	1,94E-05
MACS2_peak_4372	chr5	20694775	20694864	Magi2	ENSMUST00000101558	1,94E-05
MACS2_peak_4375	chr5	21530797	21530845	Fbxl13	ENSMUST00000115234	1,94E-05
MACS2_peak_4380	chr5	24485101	24485184	Agap3	ENSMUST00000024123	1,94E-05
MACS2_peak_4384	chr5	25107243	25107301	2900005J15Rik	ENSMUST00000128569	1,94E-05
MACS2_peak_4394	chr5	28539120	28539168	9530036O11Rik	ENSMUST00000180878	1,94E-05

MACS2_peak_44	chr1	25826953	25827006	Bai3	ENSMUST00000135518	1,94E-05
MACS2_peak_4402	chr5	30791696	30791743	Dpysl5	ENSMUST00000114729	1,94E-05
MACS2_peak_441	chr10	4529274	4529327	Ccdc170	ENSMUST00000138112	1,94E-05
MACS2_peak_442	chr10	4535643	4535690	Ccdc170	ENSMUST00000157064	1,94E-05
MACS2_peak_4439	chr5	44489471	44489522	Ldb2	ENSMUST00000070748	1,94E-05
MACS2_peak_4458	chr5	53650880	53650927	Rbpj	ENSMUST00000037618	1,94E-05
MACS2_peak_4467	chr5	64970450	64970513	Fam114a1	ENSMUST00000031080	1,94E-05
MACS2_peak_4471	chr5	66449480	66449527	Apb2	ENSMUST00000162401	1,94E-05
MACS2_peak_4472	chr5	66585117	66585230	Apb2	ENSMUST00000159847	1,94E-05
MACS2_peak_4476	chr5	67835298	67835362	Atp8a1	ENSMUST00000143013	1,94E-05
MACS2_peak_4477	chr5	68060340	68060404	Grxcr1	ENSMUST00000094715	1,94E-05
MACS2_peak_4478	chr5	69123625	69123672	Kctd8	ENSMUST00000054095	1,94E-05
MACS2_peak_448	chr10	7166992	7167039	Cnksr3	ENSMUST00000015346	1,94E-05
MACS2_peak_4489	chr5	73610684	73610738	Lrrc66	ENSMUST00000087177	1,94E-05
MACS2_peak_450	chr10	7733472	7733590	Katna1	ENSMUST00000173400	1,94E-05
MACS2_peak_452	chr10	8781958	8782005	Sash1	ENSMUST00000015449	1,94E-05
MACS2_peak_4520	chr5	89583240	89583287	Npffr2	ENSMUST00000048557	1,94E-05
MACS2_peak_4529	chr5	91391148	91391195	Btc	ENSMUST00000121044	1,94E-05
MACS2_peak_454	chr10	9876816	9876870	Stxbp5	ENSMUST00000125200	1,94E-05
MACS2_peak_4542	chr5	97468624	97468675	4930467D21Rik	ENSMUST00000181724	1,94E-05
MACS2_peak_4544	chr5	98026946	98026999	Antxr2	ENSMUST00000031281	1,94E-05
MACS2_peak_4549	chr5	99644300	99644347	A930011G23Rik	ENSMUST00000161516	1,94E-05
MACS2_peak_4550	chr5	100127343	100127390	Tmem150c	ENSMUST00000165548	1,94E-05
MACS2_peak_4557	chr5	103029586	103029643	Mapk10	ENSMUST00000112846	1,94E-05
MACS2_peak_4558	chr5	103546726	103546773	Ptpn13	ENSMUST00000048957	1,94E-05
MACS2_peak_4559	chr5	103715199	103715246	Aff1	ENSMUST00000126335	1,94E-05
MACS2_peak_4562	chr5	104954530	104954607	Abcg3	ENSMUST00000031239	1,94E-05
MACS2_peak_4568	chr5	107456596	107456643	Btd8	ENSMUST00000094541	1,94E-05
MACS2_peak_4589	chr5	112589518	112589565	1700034G24Rik	ENSMUST00000183186	1,94E-05
MACS2_peak_4598	chr5	113503611	113503662	Wscd2	ENSMUST00000094452	1,94E-05
MACS2_peak_4600	chr5	113613601	113613648	Cmklr1	ENSMUST00000047936	1,94E-05
MACS2_peak_4602	chr5	114082190	114082272	Svop	ENSMUST00000150106	1,94E-05
MACS2_peak_4603	chr5	114260649	114260705	Foxn4	ENSMUST00000144050	1,94E-05
MACS2_peak_4613	chr5	115574298	115574345	Gcn11	ENSMUST00000129424	1,94E-05
MACS2_peak_4624	chr5	118054772	118054819	Tesc	ENSMUST00000151792	1,94E-05
MACS2_peak_4632	chr5	120160297	120160344	Rbm19	ENSMUST00000031590	1,94E-05
MACS2_peak_4641	chr5	122135222	122135282	Ccdc63	ENSMUST00000146235	1,94E-05
MACS2_peak_4646	chr5	122881206	122881253	Kdm2b	ENSMUST00000132419	1,94E-05
MACS2_peak_4647	chr5	123228769	123228816	Psmd9	ENSMUST00000133386	1,94E-05
MACS2_peak_4650	chr5	123519430	123519516	Diablo	ENSMUST00000031385	1,94E-05
MACS2_peak_4651	chr5	123920748	123920798	Denr	ENSMUST00000023869	1,94E-05
MACS2_peak_4654	chr5	125088819	125088866	Ncor2	ENSMUST00000086083	1,94E-05
MACS2_peak_4658	chr5	127562539	127562606	Tmem132c	ENSMUST00000132592	1,94E-05
MACS2_peak_4666	chr5	131165588	131165641	Wbscr17	ENSMUST00000161228	1,94E-05
MACS2_peak_4669	chr5	131762754	131762801	Auts2	ENSMUST00000182575	1,94E-05
MACS2_peak_4680	chr5	134197662	134197709	Gtf2ird2	ENSMUST0000016086	1,94E-05

MACS2_peak_4681	chr5	134443724	134443771	Gtf2ird1	ENSMUST00000167084	1,94E-05
MACS2_peak_4693	chr5	136254238	136254285	Cux1	ENSMUST00000177297	1,94E-05
MACS2_peak_4696	chr5	136518315	136518362	Cux1	ENSMUST00000177305	1,94E-05
MACS2_peak_4698	chr5	136555151	136555212	Cux1	ENSMUST00000177305	1,94E-05
MACS2_peak_4703	chr5	137636126	137636173	Lrch4	ENSMUST00000175968	1,94E-05
MACS2_peak_4704	chr5	137660354	137660401	Agfg2	ENSMUST00000151839	1,94E-05
MACS2_peak_4707	chr5	138181411	138181529	Taf6	ENSMUST00000130473	1,94E-05
MACS2_peak_4715	chr5	140099215	140099263	Mad1l1	ENSMUST00000128579	1,94E-05
MACS2_peak_4719	chr5	140962108	140962155	Card11	ENSMUST00000085786	1,94E-05
MACS2_peak_4720	chr5	140980191	140980238	Card11	ENSMUST00000085786	1,94E-05
MACS2_peak_4734	chr5	143697489	143697561	Cyth3	ENSMUST00000110727	1,94E-05
MACS2_peak_4743	chr5	146300897	146300944	Cdk8	ENSMUST00000031640	1,94E-05
MACS2_peak_4790	chr6	12436850	12436897	Thsd7a	ENSMUST00000119581	1,94E-05
MACS2_peak_4791	chr6	12685624	12685671	Thsd7a	ENSMUST00000119581	1,94E-05
MACS2_peak_4793	chr6	13585501	13585551	Tmem168	ENSMUST00000149123	1,94E-05
MACS2_peak_4811	chr6	24510383	24510430	Iqub	ENSMUST00000052277	1,94E-05
MACS2_peak_4812	chr6	24637368	24637429	Wasl	ENSMUST00000031695	1,94E-05
MACS2_peak_4825	chr6	30389564	30389611	Zc3hc1	ENSMUST00000129796	1,94E-05
MACS2_peak_4835	chr6	34420168	34420236	Gm13857	ENSMUST00000150561	1,94E-05
MACS2_peak_4862	chr6	42587355	42587402	Fam115e	ENSMUST00000069023	1,94E-05
MACS2_peak_4888	chr6	47922736	47922792	Zfp212	ENSMUST00000009411	1,94E-05
MACS2_peak_4889	chr6	48076930	48077003	Zfp746	ENSMUST00000073124	1,94E-05
MACS2_peak_4894	chr6	49138818	49138870	Igf2bp3	ENSMUST00000031838	1,94E-05
MACS2_peak_4895	chr6	49184160	49184218	Igf2bp3	ENSMUST00000031838	1,94E-05
MACS2_peak_4896	chr6	49196393	49196441	Igf2bp3	ENSMUST00000031838	1,94E-05
MACS2_peak_4906	chr6	52715276	52715323	Tax1bp1	ENSMUST00000129660	1,94E-05
MACS2_peak_4912	chr6	54043272	54043319	Chn2	ENSMUST00000127323	1,94E-05
MACS2_peak_4913	chr6	54060089	54060209	Chn2	ENSMUST00000127323	1,94E-05
MACS2_peak_4914	chr6	54554438	54554505	Scrn1	ENSMUST00000019268	1,94E-05
MACS2_peak_492	chr10	25381764	25381826	Epb4.1l2	ENSMUST00000053748	1,94E-05
MACS2_peak_4932	chr6	63971403	63971475	Grid2	ENSMUST00000161105	1,94E-05
MACS2_peak_4934	chr6	64480549	64480596	Grid2	ENSMUST00000095852	1,94E-05
MACS2_peak_4936	chr6	64604581	64604646	Grid2	ENSMUST00000095852	1,94E-05
MACS2_peak_4954	chr6	72590602	72590661	Elmod3	ENSMUST00000152705	1,94E-05
MACS2_peak_4984	chr6	85482036	85482083	Fbxo41	ENSMUST00000161078	1,94E-05
MACS2_peak_4985	chr6	85485187	85485236	Fbxo41	ENSMUST00000161078	1,94E-05
MACS2_peak_499	chr10	28154318	28154365	Ptprk	ENSMUST00000166468	1,94E-05
MACS2_peak_4996	chr6	87632302	87632369	Ap1f	ENSMUST00000032130	1,94E-05
MACS2_peak_5000	chr6	88767578	88767625	Mgll	ENSMUST00000163271	1,94E-05
MACS2_peak_5002	chr6	88785087	88785134	Mgll	ENSMUST00000163271	1,94E-05
MACS2_peak_5003	chr6	88983292	88983372	4933427D06Rik	ENSMUST00000168837	1,94E-05
MACS2_peak_5012	chr6	92168382	92168429	Nr2c2	ENSMUST00000146175	1,94E-05
MACS2_peak_5017	chr6	94097091	94097139	Magi1	ENSMUST00000089317	1,94E-05
MACS2_peak_5029	chr6	97414315	97414366	Frmd4b	ENSMUST00000124050	1,94E-05
MACS2_peak_5051	chr6	111225532	111225579	Grm7	ENSMUST00000174018	1,94E-05
MACS2_peak_901	chr11	58966348	58966421	Trim17	ENSMUST00000075141	1,94E-05

MACS2_peak_907	chr11	61156628	61156730	Usp22	ENSMUST00000173525	1,94E-05
MACS2_peak_910	chr11	62009002	62009060	Specc1	ENSMUST00000136971	1,94E-05
MACS2_peak_911	chr11	62088877	62088977	Specc1	ENSMUST00000092415	1,94E-05
MACS2_peak_916	chr11	63984073	63984120	Cox10	ENSMUST00000049091	1,94E-05
MACS2_peak_917	chr11	64060657	64060704	Cox10	ENSMUST00000049091	1,94E-05
MACS2_peak_924	chr11	68186320	68186373	Stx8	ENSMUST00000021285	1,94E-05
MACS2_peak_927	chr11	68706993	68707040	Myh10	ENSMUST00000155765	1,94E-05
MACS2_peak_930	chr11	69791066	69791113	Chmb1	ENSMUST00000045971	1,94E-05
MACS2_peak_940	chr11	72357443	72357490	Tekt1	ENSMUST00000021155	1,94E-05
MACS2_peak_961	chr11	77781811	77781874	Myo18a	ENSMUST00000169105	1,94E-05
MACS2_peak_963	chr11	77975679	77975726	Sez6	ENSMUST00000151982	1,94E-05
MACS2_peak_987	chr11	88040418	88040471	Gm11508	ENSMUST00000129337	1,94E-05
MACS2_peak_989	chr11	88685375	88685439	Msi2	ENSMUST00000144699	1,94E-05
MACS2_peak_996	chr11	94340400	94340503	Ankrd40	ENSMUST00000107818	1,94E-05
MACS2_peak_997	chr11	94411141	94411245	Cacna1g	ENSMUST00000107785	1,94E-05
MACS2_peak_1500	chr13	29640923	29640970	Cdkal1	ENSMUST00000137225	1,97E-05
MACS2_peak_2195	chr15	85043541	85043588	Fam118a	ENSMUST00000023069	1,97E-05
MACS2_peak_2282	chr16	11137115	11137162	Zc3h7a	ENSMUST00000138282	1,97E-05
MACS2_peak_3191	chr2	32579587	32579647	Pip5kl1	ENSMUST00000100188	1,97E-05
MACS2_peak_4418	chr5	36106657	36106704	Sorcs2	ENSMUST00000135324	1,97E-05
MACS2_peak_4423	chr5	38149004	38149063	Nsg1	ENSMUST00000031009	1,97E-05
MACS2_peak_4831	chr6	33825793	33825840	Exoc4	ENSMUST00000143190	1,97E-05
MACS2_peak_5175	chr7	9937538	9937585	Vmn2r48	ENSMUST00000165611	1,97E-05
MACS2_peak_558	chr10	58946804	58946906	Sh3rf3	ENSMUST00000133151	1,97E-05
MACS2_peak_5888	chr8	120493485	120493545	Gse1	ENSMUST00000034279	1,97E-05
MACS2_peak_6257	chr9	120953359	120953407	Cttnb1	ENSMUST00000170729	1,97E-05
MACS2_peak_1421	chr12	112782271	112782334	Ahnak2	ENSMUST00000124526	1,99E-05
MACS2_peak_1729	chr13	119602908	119603004	Gm7120	ENSMUST00000099147	2,00E-05
MACS2_peak_1038	chr11	101108186	101108233	Fam134c	ENSMUST00000147201	2,03E-05
MACS2_peak_1170	chr12	20920769	20920816	3110053B16Rik	ENSMUST00000140360	2,03E-05
MACS2_peak_1493	chr13	27297634	27297681	4930511O05Rik	ENSMUST00000155912	2,03E-05
MACS2_peak_1572	chr13	56857642	56857758	Trpc7	ENSMUST00000173817	2,03E-05
MACS2_peak_1648	chr13	90094243	90094290	Tmem167	ENSMUST00000012566	2,03E-05
MACS2_peak_186	chr1	84391388	84391435	Dner	ENSMUST00000049126	2,03E-05
MACS2_peak_1861	chr14	61013966	61014031	Tnfrsf19	ENSMUST00000111234	2,03E-05
MACS2_peak_1904	chr14	77667994	77668042	Enox1	ENSMUST00000022589	2,03E-05
MACS2_peak_1978	chr14	117948926	117948973	Gpc6	ENSMUST00000125435	2,03E-05
MACS2_peak_2060	chr15	33144181	33144228	Cpq	ENSMUST00000042167	2,03E-05
MACS2_peak_2105	chr15	57830791	57830838	Zhx2	ENSMUST00000096430	2,03E-05
MACS2_peak_2197	chr15	85414519	85414581	Atxn10	ENSMUST00000163242	2,03E-05
MACS2_peak_264	chr1	128013457	128013529	Zranb3	ENSMUST00000097597	2,03E-05
MACS2_peak_3022	chr19	29578400	29578447	C030046E11Rik	ENSMUST00000162492	2,03E-05
MACS2_peak_3048	chr19	43147960	43148023	Hpse2	ENSMUST00000099428	2,03E-05
MACS2_peak_3158	chr2	27233750	27233802	Sardh	ENSMUST00000102886	2,03E-05
MACS2_peak_3223	chr2	40682675	40682722	Lrp1b	ENSMUST00000134032	2,03E-05
MACS2_peak_3232	chr2	44189720	44189784	Arhgap15	ENSMUST00000055776	2,03E-05

MACS2_peak_3337	chr2	92385418	92385472	Mapk8ip1	ENSMUST00000111279	2,03E-05
MACS2_peak_339	chr1	158547875	158547922	Astn1	ENSMUST00000046110	2,03E-05
MACS2_peak_3487	chr2	152271020	152271083	Csnk2a1	ENSMUST00000136063	2,03E-05
MACS2_peak_3522	chr2	165650478	165650525	Eya2	ENSMUST00000088132	2,03E-05
MACS2_peak_3523	chr2	165867374	165867421	Zmynd8	ENSMUST00000150871	2,03E-05
MACS2_peak_3651	chr3	31151635	31151709	Cldn11	ENSMUST00000046174	2,03E-05
MACS2_peak_3778	chr3	93202903	93202989	Flg2	ENSMUST00000098884	2,03E-05
MACS2_peak_4247	chr4	133730067	133730114	Arid1a	ENSMUST00000145664	2,03E-05
MACS2_peak_4383	chr5	25011529	25011608	Prkag2	ENSMUST00000030784	2,03E-05
MACS2_peak_4547	chr5	99612033	99612080	A930011G23Rik	ENSMUST00000161516	2,03E-05
MACS2_peak_4567	chr5	106597679	106597726	RP24-421H18.2	ENSMUST00000149128	2,03E-05
MACS2_peak_4752	chr5	149558883	149558959	Wdr95	ENSMUST00000121108	2,03E-05
MACS2_peak_4980	chr6	83935121	83935168	Zfml	ENSMUST00000113836	2,03E-05
MACS2_peak_4983	chr6	85440426	85440538	Smyd5	ENSMUST00000045693	2,03E-05
MACS2_peak_5102	chr6	127147641	127147688	Ccnd2	ENSMUST00000000188	2,03E-05
MACS2_peak_5324	chr7	64503700	64503747	Apba2	ENSMUST00000137732	2,03E-05
MACS2_peak_5371	chr7	80092300	80092372	Zfp710	ENSMUST00000049680	2,03E-05
MACS2_peak_5427	chr7	102110350	102110415	Art1	ENSMUST00000033300	2,03E-05
MACS2_peak_561	chr10	59767878	59767925	Micu1	ENSMUST00000172034	2,03E-05
MACS2_peak_5732	chr8	70757515	70757562	Rab3a	ENSMUST00000110092	2,03E-05
MACS2_peak_5793	chr8	90937568	90937615	Chd9	ENSMUST00000109614	2,03E-05
MACS2_peak_6015	chr9	40506487	40506534	Gramd1b	ENSMUST00000119373	2,03E-05
MACS2_peak_682	chr10	105368155	105368218	Tmtc2	ENSMUST00000143691	2,03E-05
MACS2_peak_699	chr10	111262041	111262088	Osbpl8	ENSMUST00000095310	2,03E-05
MACS2_peak_726	chr10	120827295	120827342	Msrb3	ENSMUST00000130950	2,03E-05
MACS2_peak_906	chr11	61052047	61052094	Kcnj12	ENSMUST00000089184	2,03E-05
MACS2_peak_973	chr11	82830362	82830436	Rffl	ENSMUST00000126660	2,03E-05
MACS2_peak_2239	chr15	99944296	99944391	Gm21917	ENSMUST00000177831	2,09E-05
MACS2_peak_609	chr10	80258432	80258479	Gamt	ENSMUST00000020359	2,09E-05
MACS2_peak_6210	chr9	108852687	108852734	Celsr3	ENSMUST00000024238	2,09E-05
MACS2_peak_3112	chr2	6439421	6439468	Usp6nl	ENSMUST00000042503	2,15E-05
MACS2_peak_1023	chr11	98368927	98368999	Stard3	ENSMUST00000152559	2,73E-05
MACS2_peak_2521	chr17	12742944	12742991	Igf2r	ENSMUST00000024599	2,73E-05
MACS2_peak_2553	chr17	26722569	26722625	Crebrf	ENSMUST00000151681	2,73E-05
MACS2_peak_2673	chr17	70308921	70308968	Dlgap1	ENSMUST00000130971	2,73E-05
MACS2_peak_3567	chr2	173582602	173582688	Ppp4r1l-ps	ENSMUST00000132669	2,73E-05
MACS2_peak_3588	chr2	181334882	181334929	Rtel1	ENSMUST00000148252	2,73E-05
MACS2_peak_4342	chr5	3891473	3891520	Mterf	ENSMUST00000044746	2,73E-05
MACS2_peak_4688	chr5	135469964	135470011	Hip1	ENSMUST00000060311	2,73E-05
MACS2_peak_4691	chr5	136135699	136135774	Lrwd1	ENSMUST00000150406	2,73E-05
MACS2_peak_5743	chr8	72374536	72374617	Eps15l1	ENSMUST00000163643	2,73E-05
MACS2_peak_5881	chr8	119304761	119304808	Cdh13	ENSMUST00000151842	2,73E-05
MACS2_peak_2152	chr15	74563498	74563545	Bai1	ENSMUST00000042035	2,89E-05
MACS2_peak_1694	chr13	107859672	107859741	Zswim6	ENSMUST00000105097	3,13E-05
MACS2_peak_2713	chr17	84292898	84292954	Thada	ENSMUST00000047524	3,25E-05

Table 3: List of Intergenic Binding (top 50 peaks)

peak id	chr	peak start	peak end	p-value
MACS2_peak_430	chr10	3109442	3109796	3,36E-39
MACS2_peak_432	chr10	3111254	3112237	3,57E-28
MACS2_peak_433	chr10	3112290	3114004	4,78E-28
MACS2_peak_431	chr10	3109898	3110418	5,43E-27
MACS2_peak_434	chr10	3114139	3114364	5,72E-27
MACS2_peak_438	chr10	3115991	3117154	3,24E-25
MACS2_peak_3957	chr4	3180932	3181012	4,9E-25
MACS2_peak_4765	chr6	3137963	3138020	1,12E-24
MACS2_peak_435	chr10	3114469	3114689	9,72E-21
MACS2_peak_2348	chr16	30829571	30829622	9,31E-20
MACS2_peak_2464	chr16	91454788	91454918	2,98E-19
MACS2_peak_2260	chr16	5709193	5709247	2,37E-18
MACS2_peak_4143	chr4	93844439	93844493	3,19E-17
MACS2_peak_1888	chr14	74065957	74066007	1,14E-16
MACS2_peak_3953	chr4	3176871	3176974	2,53E-16
MACS2_peak_2490	chr17	3029931	3029984	3,32E-16
MACS2_peak_4710	chr5	1,4E+08	1,4E+08	3,32E-16
MACS2_peak_6236	chr9	1,15E+08	1,15E+08	5,88E-16
MACS2_peak_2170	chr15	78753116	78753207	1,39E-15
MACS2_peak_436	chr10	3114830	3115338	1,4E-15
MACS2_peak_4325	chr4	1,53E+08	1,53E+08	1,47E-15
MACS2_peak_2548	chr17	25451796	25451869	2,48E-15
MACS2_peak_3920	chr3	1,49E+08	1,49E+08	3,14E-15
MACS2_peak_19	chr1	12657598	12657661	2,43E-14
MACS2_peak_2610	chr17	42744253	42744417	3,32E-14
MACS2_peak_5185	chr7	12017353	12017407	6,14E-14
MACS2_peak_1068	chr11	1,09E+08	1,09E+08	7,17E-14
MACS2_peak_1508	chr13	34103646	34104007	1,94E-13
MACS2_peak_485	chr10	22448394	22448453	2,26E-13
MACS2_peak_5973	chr9	24542025	24542131	3,81E-13
MACS2_peak_5180	chr7	12011636	12011752	7,45E-13
MACS2_peak_2753	chr18	3117863	3118211	7,91E-13
MACS2_peak_5181	chr7	12012772	12012905	9,52E-13
MACS2_peak_2524	chr17	13305971	13306084	4,45E-12

MACS2_peak_6205	chr9	1,08E+08	1,08E+08	4,54E-12
MACS2_peak_2390	chr16	45353012	45353090	7,88E-12
MACS2_peak_3921	chr3	1,49E+08	1,49E+08	9,18E-12
MACS2_peak_2609	chr17	42744070	42744139	1,12E-11
MACS2_peak_5644	chr8	34512269	34512342	1,12E-11
MACS2_peak_4778	chr6	4762452	4762533	1,47E-11
MACS2_peak_4217	chr4	1,29E+08	1,29E+08	1,5E-11
MACS2_peak_3749	chr3	83124134	83124185	2,1E-11
MACS2_peak_1201	chr12	30578598	30578741	2,21E-11
MACS2_peak_2608	chr17	42743658	42743747	2,52E-11
MACS2_peak_4772	chr6	3201186	3201267	5,19E-11
MACS2_peak_5184	chr7	12015748	12015803	5,68E-11
MACS2_peak_2897	chr18	68692100	68692193	6,16E-11
MACS2_peak_5768	chr8	83756926	83756976	6,69E-11
MACS2_peak_5598	chr8	15142350	15142402	7,13E-11
MACS2_peak_2607	chr17	42743262	42743358	7,95E-11

Appendix B: Genes involved in the Glutamatergic Synapse Pathway (Adapted from KEGG Database)

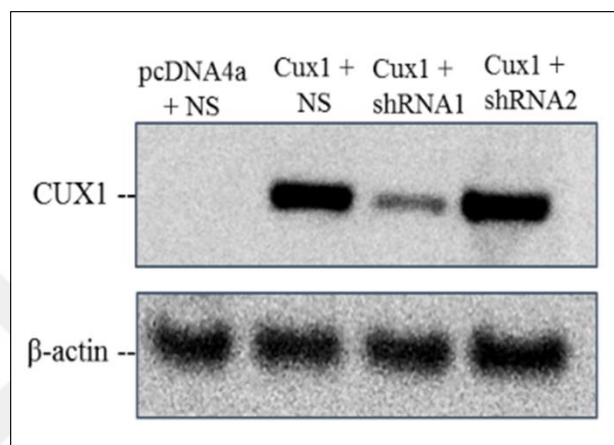
G protein-coupled receptor kinase 2(Grk2)
G protein-coupled receptor kinase 3(Grk3)
GNAS (guanine nucleotide binding protein, alpha stimulating) complex locus(Gnas)
SH3/ankyrin domain gene 1(Shank1)
SH3/ankyrin domain gene 2(Shank2)
SH3/ankyrin domain gene 3(Shank3)
adenylate cyclase 1(Adcy1)
adenylate cyclase 2(Adcy2)
adenylate cyclase 3(Adcy3)
adenylate cyclase 4(Adcy4)
adenylate cyclase 5(Adcy5)
adenylate cyclase 6(Adcy6)
adenylate cyclase 7(Adcy7)
adenylate cyclase 8(Adcy8)

adenylate cyclase 9(Adcy9)
calcium channel, voltage-dependent, L type, alpha 1C subunit(Cacna1c)
calcium channel, voltage-dependent, L type, alpha 1D subunit(Cacna1d)
calcium channel, voltage-dependent, P/Q type, alpha 1A subunit(Cacna1a)
discs, large (Drosophila) homolog-associated protein 1(Dlgap1)
discs, large homolog 4 (Drosophila)(Dlg4)
glutamate receptor ionotropic, NMDA3A(Grin3a)
glutamate receptor, ionotropic, AMPA1 (alpha 1)(Gria1)
glutamate receptor, ionotropic, AMPA2 (alpha 2)(Gria2)
glutamate receptor, ionotropic, AMPA3 (alpha 3)(Gria3)
glutamate receptor, ionotropic, AMPA4 (alpha 4)(Gria4)
glutamate receptor, ionotropic, NMDA1 (zeta 1)(Grin1)
glutamate receptor, ionotropic, NMDA2A (epsilon 1)(Grin2a)
glutamate receptor, ionotropic, NMDA2B (epsilon 2)(Grin2b)
glutamate receptor, ionotropic, NMDA2C (epsilon 3)(Grin2c)
glutamate receptor, ionotropic, NMDA2D (epsilon 4)(Grin2d)
glutamate receptor, ionotropic, NMDA3B(Grin3b)
glutamate receptor, ionotropic, kainate 1(Grik1)
glutamate receptor, ionotropic, kainate 2 (beta 2)(Grik2)
glutamate receptor, ionotropic, kainate 3(Grik3)
glutamate receptor, ionotropic, kainate 4(Grik4)
glutamate receptor, ionotropic, kainate 5 (gamma 2)(Grik5)
glutamate receptor, metabotropic 1(Grm1)
glutamate receptor, metabotropic 2(Grm2)
glutamate receptor, metabotropic 3(Grm3)
glutamate receptor, metabotropic 4(Grm4)
glutamate receptor, metabotropic 5(Grm5)
glutamate receptor, metabotropic 6(Grm6)
glutamate receptor, metabotropic 7(Grm7)
glutamate receptor, metabotropic 8(Grm8)
glutamate-ammonia ligase (glutamine synthetase)(Glu1)
glutaminase 2 (liver, mitochondrial)(Gls2)
glutaminase(Gls)
guanine nucleotide binding protein (G protein), alpha inhibiting 1(Gnai1)
guanine nucleotide binding protein (G protein), alpha inhibiting 2(Gnai2)
guanine nucleotide binding protein (G protein), alpha inhibiting 3(Gnai3)
guanine nucleotide binding protein (G protein), beta 1(Gnb1)

guanine nucleotide binding protein (G protein), beta 2(Gnb2)
guanine nucleotide binding protein (G protein), beta 3(Gnb3)
guanine nucleotide binding protein (G protein), beta 4(Gnb4)
guanine nucleotide binding protein (G protein), beta 5(Gnb5)
guanine nucleotide binding protein (G protein), gamma 10(Gng10)
guanine nucleotide binding protein (G protein), gamma 11(Gng11)
guanine nucleotide binding protein (G protein), gamma 12(Gng12)
guanine nucleotide binding protein (G protein), gamma 13(Gng13)
guanine nucleotide binding protein (G protein), gamma 2(Gng2)
guanine nucleotide binding protein (G protein), gamma 3(Gng3)
guanine nucleotide binding protein (G protein), gamma 4(Gng4)
guanine nucleotide binding protein (G protein), gamma 5(Gng5)
guanine nucleotide binding protein (G protein), gamma 7(Gng7)
guanine nucleotide binding protein (G protein), gamma 8(Gng8)
guanine nucleotide binding protein (G protein), gamma transducing activity polypeptide 1(Gngt1)
guanine nucleotide binding protein (G protein), gamma transducing activity polypeptide 2(Gngt2)
guanine nucleotide binding protein, alpha O(Gnao1)
guanine nucleotide binding protein, alpha q polypeptide(Gnaq)
guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-5(LOC102641276)
homer scaffolding protein 1(Homer1)
homer scaffolding protein 2(Homer2)
homer scaffolding protein 3(Homer3)
inositol 1,4,5-triphosphate receptor 2(Itpr2)
inositol 1,4,5-triphosphate receptor 3(Itpr3)
inositol 1,4,5-trisphosphate receptor 1(Itpr1)
mitogen-activated protein kinase 1(Mapk1)
mitogen-activated protein kinase 3(Mapk3)
phospholipase A2, group IVA (cytosolic, calcium-dependent)(Pla2g4a)
phospholipase A2, group IVB (cytosolic)(Pla2g4b)
phospholipase A2, group IVC (cytosolic, calcium-independent)(Pla2g4c)
phospholipase A2, group IVD(Pla2g4d)
phospholipase A2, group IVE(Pla2g4e)
phospholipase A2, group IVF(Pla2g4f)
phospholipase C, beta 1(Plcb1)
phospholipase C, beta 2(Plcb2)
phospholipase C, beta 3(Plcb3)
phospholipase C, beta 4(Plcb4)

phospholipase D1(Pld1)
phospholipase D2(Pld2)
potassium inwardly-rectifying channel, subfamily J, member 3(Kcnj3)
predicted gene 15776(Gm15776)
predicted gene 5741(Gm5741)
protein kinase C, alpha(Prkca)
protein kinase C, beta(Prkcb)
protein kinase C, gamma(Prkcg)
protein kinase, cAMP dependent, catalytic, alpha(Prkaca)
protein kinase, cAMP dependent, catalytic, beta(Prkacb)
protein phosphatase 3, catalytic subunit, alpha isoform(Ppp3ca)
protein phosphatase 3, catalytic subunit, beta isoform(Ppp3cb)
protein phosphatase 3, catalytic subunit, gamma isoform(Ppp3cc)
protein phosphatase 3, regulatory subunit B, alpha isoform (calcineurin B, type I)(Ppp3r1)
protein phosphatase 3, regulatory subunit B, alpha isoform (calcineurin B, type II)(Ppp3r2)
solute carrier family 1 (glial high affinity glutamate transporter), member 2(Slc1a2)
solute carrier family 1 (glial high affinity glutamate transporter), member 3(Slc1a3)
solute carrier family 1 (glutamate transporter), member 7(Slc1a7)
solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6(Slc1a6)
solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1(Slc1a1)
solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6(Slc17a6)
solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 7(Slc17a7)
solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 8(Slc17a8)
solute carrier family 38, member 1(Slc38a1)
solute carrier family 38, member 2(Slc38a2)
solute carrier family 38, member 3(Slc38a3)
transient receptor potential cation channel, subfamily C, member 1(Trpc1)

Appendix C: Knockdown of exogenous CUX1 protein by Cux1-shRNA#1 in HEK293T cells



Supplementary Figure 1: shRNA#1 against CUX1 silenced the expression of exogenous CUX1 protein in HEK cells. Transfection conditions: (i) pcDNA4a vector co-transfected with pSUPER-NS-GFP vector, (ii) Cux1-pCDNA4a vector co-transfected with pSUPER-NS-GFP vector, (iii) Cux1-pCDNA4a vector co-transfected with pSUPER-shRNA1-GFP vector, (iv) Cux1-pCDNA4a vector co-transfected with pSUPER-shRNA2-GFP vector. Beta-actin was used as loading control.

Appendix D: Determination of CUX1 expression at Different Developmental Time Points

Lysate preparation from mouse cortex tissue

Cortices from mice at different time points (embryonic day 14, postnatal day 0, 7, 14, 28 and adult) were dissected in ice-cold 1X PBS. Cortices were homogenized in 500 μ l of cold 1X PBS using a glass homogenizer. Homogenized tissues were transferred into 1.5 ml centrifuge tubes and samples were spin down for 10 seconds. 100 μ l of 1X RIPA Buffer containing 1X protease and phosphatase inhibitor (Thermo Scientific, Cat. #78420) was used

to dissolve samples by pipetting. Samples were sonicated for 7 seconds, 10 cycles at cold room. Samples were centrifuged at 14000 rpm for 10 minutes at 4°C and pellets were discarded. Supernatants were transferred into fresh 1.5 ml centrifuge tubes and stored at -20°C for western blotting.

Appendix E: Primer List

Primer name	Primer sequence
Cux1_BamHI_Forward	AAAGGATCCATGGCGGCAATGTGGGATC
Cux1_XbaI_Reverse	AAATCTAGAAACTGCCATAAGTCACCACTGC
Cux_SiteDirected_F	ATGGCCCGCACCATCAGCTTCTTTTA
Cux_SiteDirected_R	CTTGTTGGAGAGAATCAGACGGCCCATG
Cux1_pSUPER_shRNA1_F	GATCCCCAACTGAATGCCAAGAATAGTTCAAGAGACTATTCTTGGCATTCACTTTTTTA
Cux1_pSUPER_shRNA1_R	AGCTTAAAAAACTGAATGCCAAGAATAGTCTCTTGAAGTATTCTTGGCATTCACTTTGGG
Cux1_pSUPER_shRNA2_F	GATCCCCATAGTCAATACCCACAGAGTTCAAGAGACTCTGTGGGTATTGACTATTTTTTA
Cux1_pSUPER_shRNA2_R	AGCTTAAAAAATAGTCAATACCCACAGAGTCTCTTGAAGTCTGTGGGTATTGACTATGGG
Cux1_pSUPER_shRNA3_F	GATCCCCAATGTCCGAAGATGCGCTGTTCAGAGACAGCGCATCTTCGGACATTTTTTA
Cux1_pSUPER_shRNA3_R	AGCTTAAAAAATGTCCGAAGATGCGCTGTCTCTTGAACAGCGCATCTTCGGACATTGGG
Kcna3_F1	CCCAGAGCAGACAGAAATAAATAC
Kcna3_r1	GTAAGAGTGATCTGTCGTCATGT
Cdkn1b_promoter_F	GCCTGTCCTACATAGCAGAGACTT
Cdkn1b_promoter_R	TATTAGGAGCTTCGGTGGCTAGT
Cdkn1b_Exon1_F	CGGTCAATCATGAAGAACTAACC
Cdkn1b_Exon1_R	GTAGTAGAACTCGGGCAAGCTG
Adcy9_ChIPqPCR_F1	CACACAGGCACACACAAGATGCAT
Adcy9_ChIPqPCR_R1	GAAGTAGATGCTGTGAGTGAATGGGAG
Dlgap1_ChIPqPCR_F1	GTTCTTGGCTTCTACAAATGC
Dlgap1_ChIPqPCR_R1	GAATGCCTGCAATTAGGACAAACC
Grik1_ChIPqPCR_F1	GGGATTATGGTTCATGACGTGAG
Grik1_ChIPqPCR_R1	GAAGTACTGATGCCAGCTAATTCTGAG
Grm1_ChIPqPCR_F1	CAGTCAGGAGGATACTCCTCC
Grm1_ChIPqPCR_R1	GTATCCTCCTGACTGCAGCAG
Gnb1_ChIPqPCR_F1	GGAAGTGGGGCATCAAGTTGGGT
Gnb1_ChIPqPCR_R1	GTCTCGTGGTGAGCCTTGAGCT
Homer1_ChIPqPCR_F1	ACCCCTTCACATCCTCATT
Homer1_ChIPqPCR_R1	GCAGAGACAGAGAGGATTAGTCAAG
Prkca_ChIPqPCR_F1	GGGTCAAGAGATCTAGGATCC
Prkca_ChIPqPCR_R1	TTTGGAGCCCACTAGGAG