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Investigation Of Posttranscriptional Regulation After Global Brain Ischemia And Reperfusion Injury

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INVESTIGATION OF POSTTRANSLATIONAL REGULATION AFTER GLOBAL BRAIN ISCHEMIA AND REPERFUSION INJURY

by

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DEDICATION

To my parents, Stephen and Janice, and to all the other family and friends who have given me support and encouragement.

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1.1 Introduction

Ischemia and reperfusion (I/R) injury occurs when a transient reduction in blood flow is followed by restoration of blood flow. In humans, brain I/R injury occurs during the common conditions stroke and cardiac arrest. At present, understanding of the cellular pathology of brain I/R injury is insufficient to provide a foundation on which to develop effective clinical therapies.

When circulation is interrupted prior to entering the brain, a global brain I/R injury affects all neurons. Global brain I/R differentiates neurons into selectively vulnerable and selectively resistance groups. After global brain I/R, both selectively vulnerable and selectively resistant neurons recover from initial disturbances in energy imbalance and ion gradients, and both groups retain the ability to transcribe mRNA. Resistant neurons are differentiated from vulnerable neurons by their ability to translate stress response mRNAs into protein early in reperfusion. It is not understood how resistant neurons translate stress response transcripts into protein, a significant deficit in knowledge which is a barrier to developing neuroprotective therapies.

This thesis work investigates mechanisms of posttranscriptional and translational control in pyramidal cell neurons of the ischemia resistant cornu ammonis 3 (CA3) and ischemia vulnerable CA1 subregions of the rat hippocampus. This work builds on the previous discovery in the DeGracia laboratory of the mRNA granule, a stress-induced messenger ribonucleoprotein complex (mRNP) in reperfused neurons. This work is also based on an extensive literature describing translation control and cellular stress responses in reperfused neurons by the DeGracia laboratory and many others in the field. To

investigate the posttranscriptional regulation of stress response transcripts following global brain I/R injury, the following specific aims were carried out:

1.2 Specific Aims

1.2.1 Specific Aim 1: mRNA Granule Dependence on Polysome-associated mRNA

Upon reperfusion after brain ischemia, cytoplasmic mRNA forms into distinct structures called mRNA granules. mRNA granules form in all injured neurons, but only resolve in neurons which recover from brain I/R injury[1]. While intact during ischemia, polysomes disassemble immediately upon reperfusion, breaking into ribosomal subunits which slowly reassemble as protein synthesis gradually returns during reperfusion[2]. Dissociation of neuronal polysomes is expected to liberate free mRNA into the cytoplasm and therefore may be necessary for mRNA granule formation. In this aim, mRNA was locked onto polysomes with cycloheximide (CHX) to determine if polysome-associated mRNA was required for mRNA granule formation. Because mRNA granules are complex structures under microscopy, texture analysis was used to detect and quantify mRNA granule formation. To further study the relationship between polysomes and mRNA granules, polysome dissociation was induced in uninjured control animals using puromycin, and the effects on mRNA granules were studied under the microscope.

1.2.2 Specific Aim 2: Localization and Interactions of HuR in Reperfused Neurons

Ischemia resistant neurons such as the pyramidal cell neurons of the CA3 region of the hippocampus effectively translate stress-response transcripts into protein early in reperfusion after an ischemic insult[3,4]. Ischemia vulnerable populations such as CA1 pyramidal cell neurons transcribe stress response mRNAs, but they do not translate mRNAs into protein in early reperfusion[3,4]. The mRNA-binding protein (mRBP) Hu antigen R (HuR, also called embryonic lethal, abnormal vision, Drosophila-like 1) localizes to mRNA granules early in reperfusion only in ischemia resistant neurons, but the implications of this finding are unknown. HuR is known to stabilize the mRNA of stress response proteins such as HSP70 by several mechanisms including facilitated nuclear export[5,6], but HuR's effects on *hsp70* mRNA have not been studied in a brain I/R model. To establish a direct interaction between HuR protein and *hsp70* mRNA, RNA immunoprecipitation (RIP) with HuR protein was performed from reperfused neurons. To investigate facilitated nuclear export of *hsp70* mRNA by HuR, relative amounts of HuR protein and *hsp70* mRNA were quantified in nuclear and cytoplasmic fractions from reperfused neurons of CA1 and CA3.

1.2.3 Specific Aim 3: Polysome Characterization and Association with HuR in Reperfused Neurons

Hu proteins can increase translation of their target mRNAs by associating with polysomes[7]. This mechanism may explain why CA3 neurons translate *hsp70* mRNA into protein when CA1 neurons do not. To study the association of HuR and polysomes, polysomes were isolated in linear sucrose gradients (polysome profiles), and HuR was measured in polysome-enriched fractions.

1.2.4 Specific Aim 4: Global Brain Ischemia Translation State Analysis

Previous microarray studies after global brain I/R have had significant limitations. All microarray studies of global brain I/R have use steady-state levels of mRNA which poorly reflect the amount of each transcript being translated on polysomes. Choice of input tissue has also been a limitation. Prior to this study, no expression profiling had compared ischemia resistant CA3 and ischemia vulnerable CA1 neurons after global brain I/R. Here, polysome profiles were used to isolate translating mRNA which was compared to cytoplasmic, non-polysome-bound mRNA. Tissue used in the polysome profiles was microdissected from CA3 and CA1, allowing comparison between resistant and vulnerable neuron populations.

Chapter 2 - Background and Rationale

2.1 Clinical Manifestations of Brain I/R Injury

Ischemia, a restriction of blood flow, causes injury to the brain. Cerebral ischemia can be either global, resulting from reduction of blood flow in vessels entering the brain, or focal, resulting from blockage of blood flow to a specific region within the brain. Clinically, global cerebral ischemia occurs in the context of cardiac arrest. Focal ischemic manifests as ischemic stroke. Blockage of an artery within the brain by an embolus or thrombus in stroke generates a focal ischemic insult with central necrosis and peripheral injury radiating from a point of restricted circulation. Cardiac arrest causes a global ischemia affecting all CNS neurons. Ischemic stroke and cardiac arrest are by far the most common cardiovascular causes of death in the United States. Considered together, cardiovascular disease causes about one-third of all deaths in the United States, more than 2200 deaths per day[8]. Estimated national direct and indirect costs to treat cardiovascular disease total over $280 billion dollars per year[8].

2.1.1 Ischemic Stroke

In 2005, the last year for which data were available, stroke was the fourth leading cause of death in the United States and the leading cause of long-term disability[9]. Annual cost for treatment of stroke in the United States was estimated to be over $18 billion for 2005[9] and lost productivity estimated to cost an additional $15 billion[8]. Although significant advancements have been made in prevention of stroke and in long-term therapy for stroke survivors, acute treatment of the actual I/R injury remains primarily

palliative. While other thrombolytics and surgical approaches may become alternatives, the only approved treatment to directly reverse arterial blockage in stroke is tissue plasminogen activator (tPA). The NINDS recombinant tPA stroke study showed a clear benefit from thrombolysis with tPA given within 3 hours of onset of stroke[10]. tPA also commonly causes hemorrhagic transformation in patients with longer durations of occlusion[4]. The three hour therapeutic window of tPA, along with other common contraindications, limits its use to just 3% of ischemic stroke patients[11]. Most patients will also receive supportive therapy such as oxygen and aspirin and treatment for comorbidities. Despite longstanding intensive research, no new therapies to treat the occlusion and resulting brain damage from stroke have entered clinical practice[12].

2.1.1 Cardiac Arrest and Resuscitation

Advances in the emergency medical system and widespread public education about cardiopulmonary resuscitation currently allow for the return of spontaneous circulation (ROSC) in about 5 to 15% of out-of-hospital cardiac arrest victims[13]. Inpatient cardiac arrest victims fair much better with nearly half having ROSC[14]. Unfortunately, most victims in both the community and inpatient populations do not survive to hospital discharge. In-hospital mortality after cardiac arrest is between 55% and 71%[15] and mortality has not improved since the statistic were first recorded in the 1950s[16]. I/R injury affects the whole body after ROSC, but death is due primarily to brain damage[17] manifesting as seizures, coma, and brain death. Most patients who survive to discharge will continue to have significant neurological sequelae[18].

2.1.4 Development of Neuroprotective Therapies has been Disappointing

Given the immense clinical impact of brain damage after stroke and cardiac arrest, protection of central nervous system (CNS) neurons from I/R injury is an area of intensive research. The short time frame from onset of ischemia to neuron damage makes revascularization therapies unlikely to prevent neuronal injury. However, final death of CNS neurons occurs slowly, often days after the initial ischemia, providing a therapeutic window to protect damaged neurons and promote their recovery. Protracted injury, occurring over days after even a brief ischemic insult, culminates in cell death called delayed neuronal death (DND)[19]. Therapies aimed at salvaging neuron which die by DND are classified as neuroprotection. Unfortunately, all large-scale clinical trials of neuroprotective therapies have failed (reviewed in O'Collins *et al*., 2006)[20]. Neuroprotectants which have failed in clinical trials have targeted a variety of damage mechanisms and shown efficacy in multiple animal models, and their failure in large clinical trials has been a major concern in the field of stroke research[21,22]. Acute treatment with hypothermia may prove to be the exception[23], but robust clinical data measuring long term outcomes after hypothermia are still sparse, and the track record of other all other previous therapies signals caution. Overall, clinical research into neuroprotective therapies indicates a lack of basic understanding of damage pathways and cellular stress responses active in neurons after I/R injury.

2.1.5 Experimental Models of Brain I/R Injury

There are numerous biological models of stroke and brain injury from cardiac arrest ranging from cell culture systems to full animal primate models. Cellular models either deprive cultured neurons of essential nutrients such as the oxygen and glucose, or expose cells to a specific insult such as hydrogen peroxide. Although cellular models benefit from simplicity and reproducibility, they cannot recreate the multifaceted injury seen in whole brains[24]. Whole animal models incorporate the variables of hemodynamics, CNS architecture, support from glial cells, and systemic immune and inflammatory responses. Whole-animal rodent models can replicate the complex pathology of focal and global injuries.

Focal I/R injuries are most often modeled with an occluding filament placed in the middle cerebral artery, the most common site of occlusion in ischemic stroke[25]. Cardiac arrest is modeled by occluding the carotid arteries, usually accompanied in rats and mice by either occlusion of the vertebral arteries (4VO) or hypovolemic hypotension (2VO/HT). Transient global ischemia by 2VO/HT can reproducibly generate a delayed cell death in selectively vulnerable populations of neurons such as the CA1 region of the hippocampus. The adjacent CA3 region of the hippocampus survives the same insult. Because these two groups of cells, both pyramidal neurons in the same structure and receiving the same injury, have dichotomous outcomes, the transient global ischemia model is ideal for the study of the intrinsic ability of neurons to respond to the I/R injury. In this thesis, the 2VO/HT model of Smith *et al.*[26] was used because of its specific relevance to cardiac arrest, and because it allowed for the comparison of vulnerable CA1 and resistant CA3 regions of the hippocampus. Behavioral, histological, biochemical, and electrophysiological studies of this model are all consistent with pathology seen in human brains after cardiac arrest and resuscitation[26]. In the DeGracia laboratory, the 2VO/HT model induces selective cell death of CA1 but not CA3 hippocampal neurons at 3 days reperfusion after 10 minutes of ischemia in adult, male Long Evans rats. More information about the specific methods of the 2VO/HT model and measures of resultant brain injury are explained in detail below.

2.2 Mechanisms of Brain I/R Injury

2.2.1 Introduction

I/R is a complex, protracted injury process. Many damage mechanisms have been shown to be active during brain I/R, but a systematic description of their contribution to DND is lacking. The progression of events in brain I/R begins with loss of glucose and oxygen supplied by the blood. After cardiac arrest, partial pressure of oxygen in the ischemic brain reaches zero after only 2 minutes of ischemia[24]. Without an energy supply, the cell membrane sodium potassium ATPase (NaKATPase) fails,

and concentrations of sodium, potassium, and calcium ions rapidly equilibrate across the membrane. Subsequently, excitatory amino acid neurotransmitters are released from neurons, and mechanisms of lipolysis, proteolysis, and free radical damage are activated. After stroke and in focal ischemia models, inflammatory responses are also important in damage and recovery of injured neurons[27]. Transient global I/R lacks a necrotic focus, so inflammation is less important[28]. The ostensible sequence of damaging processes precipitated by loss of blood flow to neurons is called the ischemic cascade (Figure 1).

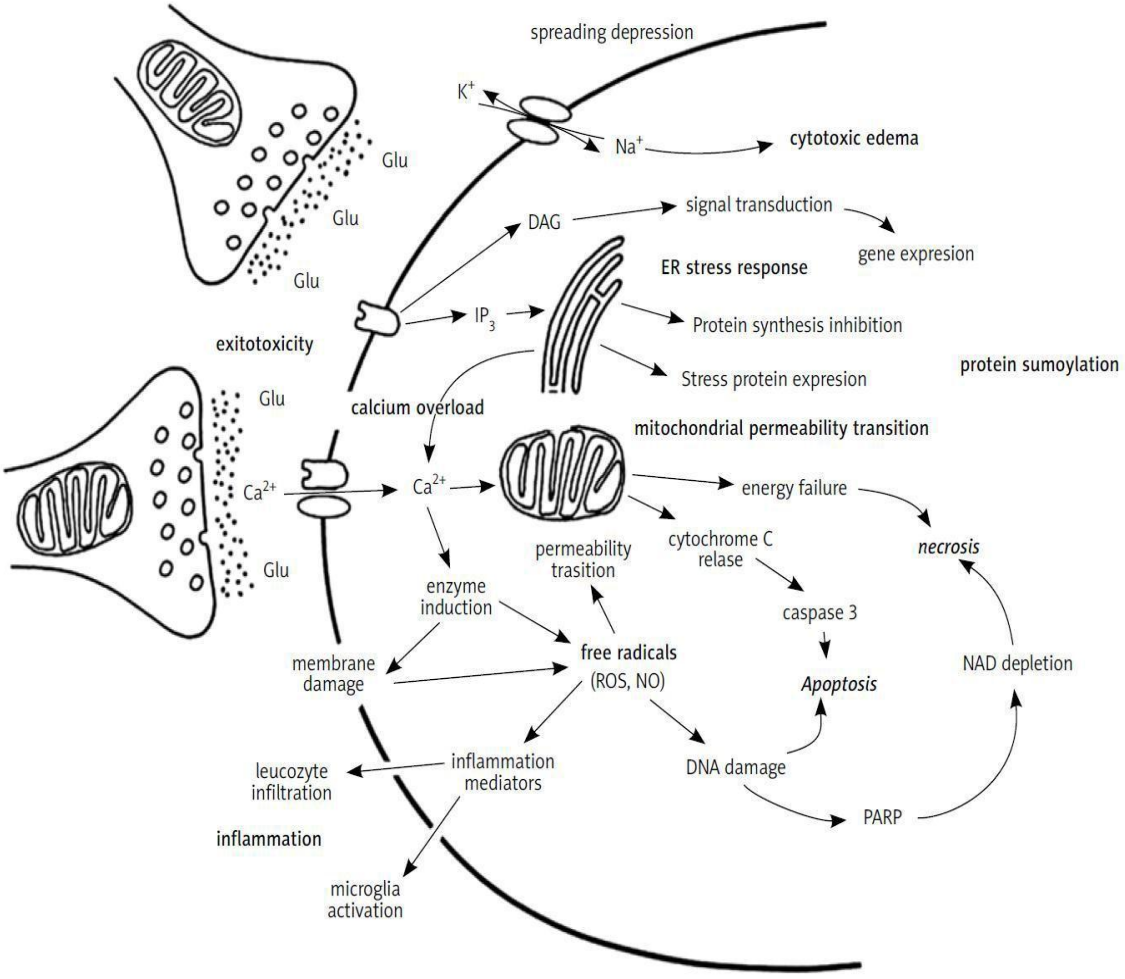

Figure 1: The ischemic cascade. Loss of blood flow rapidly depletes neurons of ATP. NaKATPase fails and sodium and potassium equilibrate across the plasma membrane. Depolarization opens inwardly directed, voltage gated calcium channels, increasing intracellular calcium. Depolarized neurons also release glutamate to the extracellular space, spreading the depolarization to adjacent neurons. Intracellular calcium activates

lipolytic and proteolytic enzymes which, in turn, generate free radicals. A confluence of upstream mechanisms cause damage to DNA and may activate apoptosis. Adapted from Hossmann,2009[29].

2.2.2 Loss of Blood Glucose and Excitotoxicity

In the rat model of global brain ischemia, levels of cellular ATP in the cortex fall rapidly until ATP is undetectable after 30 minutes of ischemia[30,31]. Concurrently lactate produced by anaerobic respiration increases 11-fold[31]. Without blood glucose and resultant ATP, neuronal NaKATPase cannot maintain ion gradients across the cell membrane. Sodium and potassium rapidly equilibrate during ischemia[32]. Ionic imbalances across the plasma membrane have unique consequences for neurons, causing calcium influx and neurotransmitter release. Voltage gated calcium channels open upon depolarization. Influx of calcium drops extracellular calcium concentration from 1.2 mM to less than 100 µM after just one minute of global brain ischemia in rats[33]. Intracellular calcium activates a variety of catabolic enzymes and the downstream effects are discussed below. Depolarization also releases the excitatory neurotransmitter glutamate into the extracellular space, and extracellular glutamate concentration remains elevated for at least 3 hours after global ischemia[34]. Glutamate release in I/R injury opens the 2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl)propanoic acid (AMPA) and N-methyl-D-aspartic acid (NMDA) ionotropic glutamate receptors on neurons, depolarizing them[35]. Depolarization causes further calcium influx and then glutamate efflux, a feed forward phenomenon known as excitotoxicity. However, blood glucose and cellular ATP are both rapidly restored upon reperfusion even in regions of the brain which will eventually die, suggesting that brief loss of energy metabolites is not a direct cause of neuronal death in brain I/R[36].

2.2.3 Increased Intracellular Calcium

Calcium is a powerful signaling molecule within cells and free cytosolic calcium is tightly regulated at 10^{-7} M[37]. Depolarization in brain I/R causes a massive influx of calcium which triggers

damage by calcium-activated enzymes such as calpains[38]. Calpains are a family of cysteine proteases activated by micromolar (μ-calpain) or millimolar (m-calpain) concentrations of intracellular calcium[39]. Calpain activity is increased in neurons after both focal and global ischemia in rat models[38]. In transient global I/R, calpain activity was greatest in CA1 hippocampal neurons at 1 hour reperfusion. Eukaryotic initiation factors 4G (eIF4G)[40] and eIF4E[41] are substrates for calpain, and calpain may damage these complexes during global ischemia as discussed below.

Increased intracellular calcium also triggers lipolysis through activation of phospholipase A_2 (PLA_2)[42]. PLA_2 are a family of esterases that release free fatty acid chains from the second carbon of glycerol[43]. PLA_2 can liberate arachidonic acid from triglycerides, and arachidonic acid is converted by cyclooxygenase-2 (COX-2) and downstream enzymes into pro-inflammatory prostaglandins and leukotrienes[44]. Inducible COX-2 is increased after focal ischemia in humans and localized to necrotic cells near the ischemic focus[45]. Activation of PLA_2 can also generate damaging free radicals and membrane permeability changes as discussed below.

Other calcium-activated enzymes induced by brain I/R include protein kinase C (PKC) and calcium/calmodulin-dependent kinase II-alpha (CaMKII)[46]. PKC is a family of serine/threonine protein kinases with complex signaling functions in eukaryotes. CaMKII is also a family of serine/threonine protein kinases which have been implicated in long term potentiation and mediate glutamate signaling[47]. Both kinases act at the cell membrane, and PKC and CaMKII translocated to cell membranes in neurons near the ischemic focus in a rat focal model I/R model[46]. CaMKII inhibitors have been shown to be protective in a cell culture I/R model[48], but the role of both enzymes remains unclear in brain I/R[49]. Finally, the neuronal nitric oxide synthase (nNOS) is induced by intracellular calcium and generates the highly reactive peroxynitrite free radical[50]. There are many sources of free radicals in brain I/R injury, explained in more detail below.

2.2.4 Free Radicals and Reperfusion

While necessary for survival, restoring blood flow provides a high oxidative potential to brain tissue which is primed by ischemia to produce free radicals. Mitochondria are an important source of free radicals during reperfusion. The mitochondrial inner membrane, which allows the hydrogen ion gradient of the electron transport chain, is permeabilized by mitochondrial calcium accumulation during ischemia[51]. When mitochondrial function is restored upon reperfusion, leakage of the inner mitochondrial generates superoxide. Other sources of free radicals during brain I/R include nNOS (mentioned above), arachidonic acid production by COX-2[52], and xanthine oxidase activity on xanthine to produce superoxide[53].

At fours hours reperfusion after 30 minutes of global ischemia in the rat, nitric oxide, superoxide, and peroxynitrite are all elevated in injured neurons[54]. Superoxide production, prominent in all injured neurons in early reperfusion, was observed only in CA1 pyramidal neurons past 24 hours of reperfusion[55]. Free radicals damage lipid membranes[56] and mitochondria[57] in reperfused neurons and cross-link DNA[58]. Free radical scavengers such Cu/Zn superoxide dismutase and xanthine oxidase inhibitor reduce cell death in models of focal and global brain ischemia (reviewed by Lipton, 1999)[28]. However radical scavengers, like all neuroprotective therapies so far developed, have failed to improve outcome in human patients[20].

All of these damage mechanisms interact in complex pathways. For example, lipid peroxidation by PLA_2 can further inhibit NaKATPase, promoting depolarization[59]. The mitochondrial permeability transition caused by high mitochondrial calcium releases cytochrome c which can trigger apoptosis[60]. These complex interactions make it difficult to quantify the relative contributions of each damage mechanism to overall cell.

2.2.3 Selective Vulnerability and Delayed Neuronal Death

After brain I/R injury, neurons will have one of three outcomes: survival, necrotic cell death, or the protracted DND[19]. Necrotic cell death occurs near the site of blockage in focal brain ischemia. The

extent of necrosis in global I/R depends on the duration of ischemia. Because necrotic cells are not salvageable and because the clinical focus of post-cardiac arrest care is preservation of injured neurons, this dissertation focuses rather on neurons which die by DND in a predictable manner days after ischemia.

It has long been known that progressive brain injury follows even brief ischemic insults. Kirino, *et al.* first showed this phenomenon in the gerbil global I/R model, where 48 hours after a five minute ischemic insult, CA1 pyramidal neurons began to show swelling of organelles followed by plasma membrane clefts and nuclear perikarya[19]. When observed next at 4 days reperfusion, the CA1 neurons were lysed and dead. DND was subsequently observed in the rat global model, also restricted to CA1 pyramidal cells after a ten minute ischemic insult[61]. Isolated hippocampal damage is also consistent with common memory deficits following cardiac arrest[18], and this pattern of cell death was found in humans postmortem after cardiac arrest and resuscitation[62,63] and on MRI[64]. DND restricted to CA1 pyramidal neurons has been shown repeatedly in the DeGracia laboratory using the 2VO/HT model after 10 minutes ischemia[1,65,66] (Figure 2).

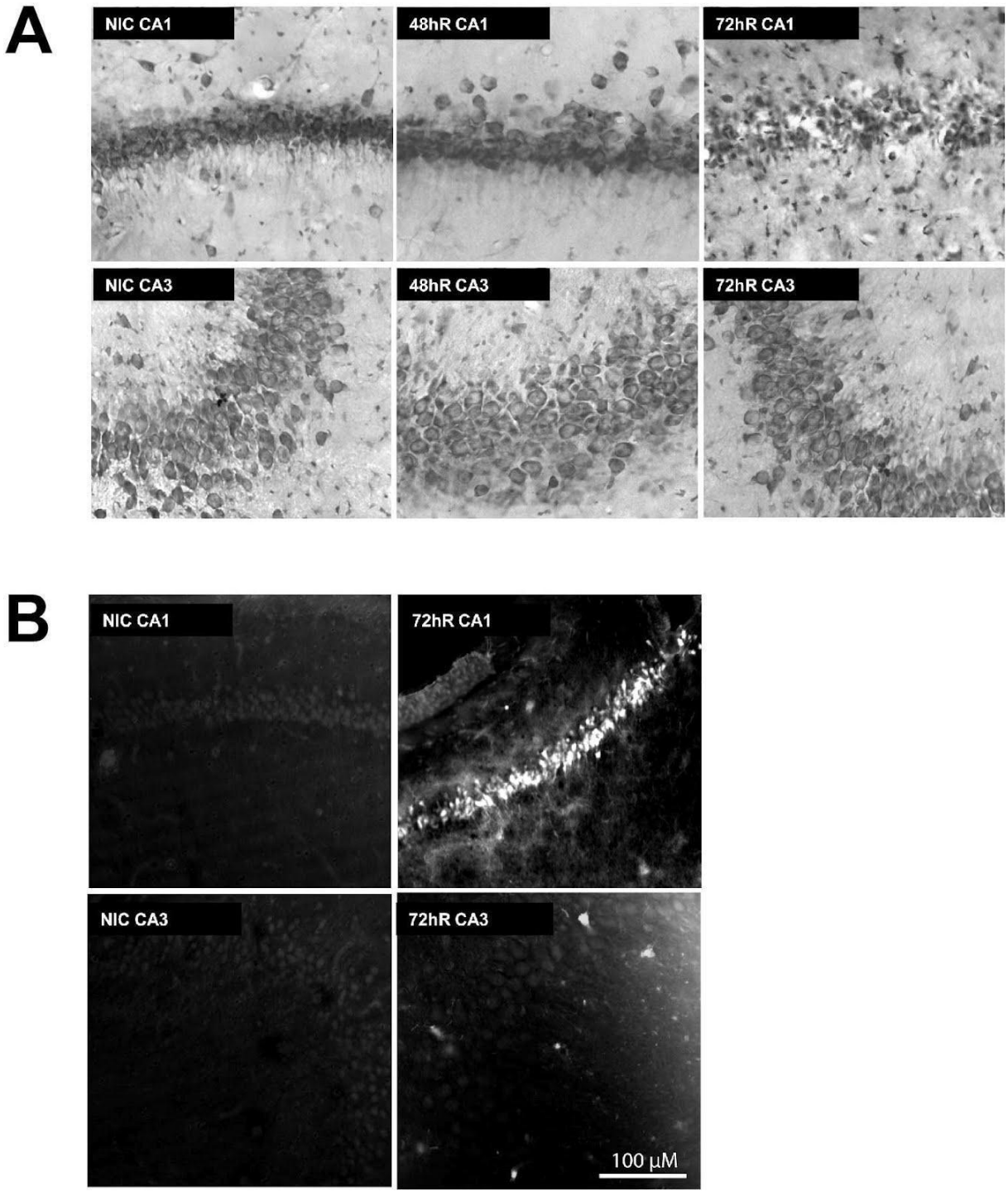

Figure 2: Delayed neuronal death of CA1 hippocampal pyramidal cell neurons. Rat 50 micron dorsal hippocampal sections from non-ischemic controls (NIC), and animals after 15 minutes I/R and reperfusion for 48 hours (48hR) or 72 (72hR) hours. Staining is for A) toluidine blue and B) fluoro-jade. Toluidine blue, a structural stain, shows that CA1 pyramidal cells have died and lysed at 72hR while CA3 cells remain intact. Fluoro-jade labels degenerating neurons and strongly reacts only in the CA1 region at 72hR. Scale bar applies to all panels. Adapted from Jamison, 2008[1].

In human patients and animal models, irreversible brain damage begins after only 4 minutes of cardiac arrest[67]. After 30 minutes of cardiac arrest, the resulting CNS damage is usually too extensive to allow long-term survival[68]. Ischemic durations between 4 and 30 minutes cause DND to neurons depending on the neuron's selective vulnerability to I/R injury. CNS neurons as a whole can be thought of as a selectively vulnerable population relative to surrounding glia. Neurons have a high metabolic demand and consume the majority of glucose supplied to the brain to maintain ion gradients for the propagation action potentials[69].

Considering only neurons, specific brain regions are known to be relatively vulnerable or resistant to I/R injury. As described above, CA1 pyramidal neurons are especially vulnerable to even mild ischemic insults. Consistent with ischemic duration determining magnitude of injury, longer durations of ischemia extended DND into areas of the cortex[70]. With increasing ischemia, DND was found in parts of the hippocampal CA4 region and cortex layers II and V[71] (reviewed in Lipton, 1999)[28].

Because shorter durations of ischemia, 5 minutes in the gerbil and 10 minutes in the rat, cause DND only in CA1, these durations have been used to study intrinsic differences in neurons selectively-vulnerable or selectively-resistant to I/R. At present, there is no coherent explanation of why DND occurs. Classical morphological markers of DND are late-stage changes such as organelle swelling and DNA damage, not visible until at least 24 hours and usually 48 hours after ischemia[72]. These late changes are not merely a delayed necrosis, as selectively-vulnerable neurons show recovery of acute derangements to energy consumption, electrophysiological activity, ion gradients, and physiological pH during early reperfusion. After a CA1-lethal insult, these acute derangements are indistinguishable between CA1 and CA3 in the time preceding DND[73,74] (reviewed in Hossmann, 1993[75] and Dienel, 1980[30]). Cerebral blood flow measurements indicate the metabolic state of human brains is normalized by two hours after ROSC, making acute necrosis an unlikely cause of brain damage after cardiac arrest[76]. Neither are adjacent CA3 neurons simply dying more slowly. Studies out to one year after I/R injury show CA3 neurons are preserved with normal morphology after a CA1-lethal insult[77]. As outlined above, secondary changes in calcium and free radicals are sometimes noted to be restricted to CA1 or more intense in these neurons[78],

but clinical trials of calcium channel blockers and free radical scavengers to treat brain I/R have universally failed[20]. Likewise, while proapoptotic markers are upregulated in reperfusion[66], antiapoptotic treatments fail to reduce injury from brain I/R[20], suggesting DND is not apoptotic cell death.

An important development in the field of brain I/R was the recognition, first made by Kleihues, *et al*., that protein synthesis remained impaired in selectively vulnerable neurons but recovered in resistant neurons[2]. During reperfusion after brief transient ischema, protein synthesis returns slowly over the course of hours in resistant neurons. In ischemia vulnerable neurons, protein synthesis never returns to normal pre-ischemic levels. This variable protein synthesis differentiates CA3 from CA1 much earlier than classic morphological markers. Recovery of protein synthesis is both an absolute requirement for long term survival of the cell and also an immediate requirement for expression stress response proteins which allow the neuron to recover[75,79]. Below, the mechanisms of protein synthesis inhibition and expression of stress response transcripts are reviewed.

2.3 Gene Expression in Reperfused Neurons

2.3.1 Introduction

Expression of protein-coding genes is a complex process regulated by environmental cues. Regulation of gene expression is combinatorial, incorporating multiple levels of control. Typically control of gene expression is divided into transcriptional regulation, posttranscriptional regulation, and regulation of translation. Posttranscriptional regulation of gene expression can be further divided into posttranscriptional modifications, nuclear export, cytoplasmic localization, and mRNA degradation. Posttranscriptional and translational control are particularly important under conditions of acute stress such as brain I/R where cells do not have the time or resources to control gene expression through transcription factors[80]. In reperfused neurons, gene expression is altered in a well-characterized sequence of events: complete translation arrest, upregulation of stress response gene transcription, selective translation of stress response mRNA, and finally return to normal protein synthesis.

2.3.2 Translation Arrest, eIF2α Phosphorylation, and the Integrated Stress Response

2.3.2.1 Normal Translation Initiation

Initiation of normal protein translation requires the formation of the ternary complex composed of initiator tRNA (Met-tRNA$_i$) and eukaryotic initiation factor 2 (eIF2) bound to GTP (Figure 3). eIF2 is a heterotrimer with two catalytic subunits, beta and gamma, and a regulatory subunit, alpha. Formation of the ternary complex is rate-limiting in translation, and therefore a key regulatory point in protein expression[81,82]. After the ternary complex forms, it joins the 40S small ribosomal subunit and other initiation factors to form a 43S preinitiation complex. The 43S preinitation complex is recruited to the 5' untranslated region (UTR) of the mRNA, a process promoted by eIF4F. eIF4F is a heterotrimer of eIF4E, eIF4G, and eIF4A. eIF4E binds the 5' cap structure of the mRNA, eIF4G is a scaffolding protein the links eIF4E to other initiation factors, and eIF4A is a RNA helicase that melts mRNA secondary structure. Together, the subunits of eIF4F facilitate scanning of the 5'UTR for an AUG start codon. Loading of the start codon into the A site of the ribosome causes a conformational change in eIF2 resulting in hydrolysis of eIF2-associated GTP[83]. The small and large ribosomal subunits then join and elongation of the nascent peptide chain proceeds.

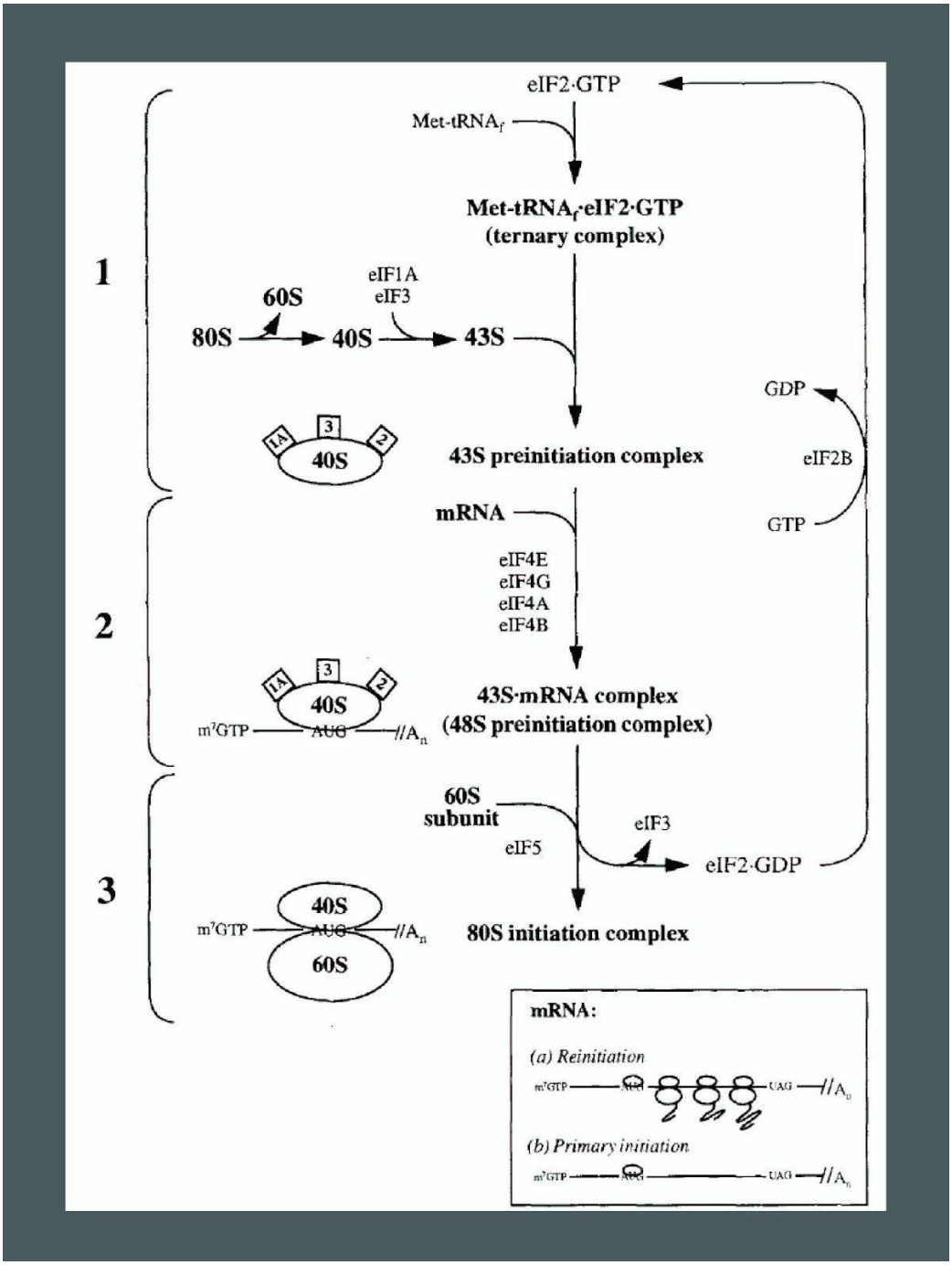

Figure 3: Initiation of protein synthesis. 1) Initiator tRNA (Met-tRNAi) and eukaryotic initiation factor 2 (eIF2) bound to GTP form the ternary complex. Addition of other initiation factors forms a 43S preinitiation complex. 2) The 43S preinitiation complex begins scanning the 5'UTR of the mRNA which is associated with the eIF4F complex. 3) When the preinitiation complex arrives at the start codon, GTP associated with eIF2 is hydrolyzed and the 60S large ribosomal subunit joins the small ribosomal subunit to begin chain elongation. Guanidyl transferase activity of eIF2B recycles GDP back to GTP on eIF2. Adapted from Pain, 1996[81].

2.3.2.2 eIF2αP Causes Transient Translation Arrest in Global Brain I/R

GTP must be restored to eIF2 before a new ternary complex can form and a new round of initiation can begin, a process catalyzed by the eIF2B subunit. Recycling of GTP by eIF2B is blocked by phosphorylation of the regulatory alpha subunit of eIF2 at serine 51[84]. Alpha-phosphorylated eIF2 binds eIF2B with high affinity, sequestering eIF2B and acting as a competitive inhibitor[85]. Inhibition of translation initiation through eIF2(αP) is a common mechanism to regulate many forms of cell stress, a process collectively referred to as the integrated stress response[86].

eIF2 activity is rapidly inhibited upon reperfusion after global brain ischemia. After 15 minutes of transient global brain ischemia and 30 minutes of reperfusion in the rat, the ability of eIF2 to form ternary complexes decreased 50% in both CA1 and CA3[87]. Again in the global rat model, decrease in eIF2 activity was shown to correlate to eIF2(αP) and inhibition of the guanidyl transferase activity of eIF2B[88]. Addition of exogenous, intact eIF2 to homogenates from I/R-injured brain partially reversed inhibition of protein synthesis[89].

In all regions of the brain after transient global I/R, eIF2(αP) gradually remits, returning to control levels by about 4 to 6 hours reperfusion[90,91]. Immunohistochemical mapping and assay of hippocampal subregion microdissections has established that eIF2α phosphorylation persists for the same duration in both ischemia vulnerable CA1 and ischemia resistant neurons CA3[92]. This initial inhibition of protein synthesis is generally considered protective[93], conserving energy and amino acid pools and preventing damage from the failed synthesis of misfolded proteins[94] or the successful synthesis of pro-apoptotic proteins[93]. Identical patterns of eIF2(αP) between CA1 and CA3 suggest that, like energy and ionic disturbances, regulation of eIF2 does not cause DND.

2.3.2.3 eIF2(αP)-induced Translation Arrest Causes Polysome Dissociation

Observations from a variety of whole-animal brain I/R models and post-mortem human tissue show that CNS neuron polysomes remain intact during brain ischemia, a phenomenon Kleihues and Hossmann termed ―ischemic freeze‖[95], but disassemble upon reperfusion. Using polysome profiles,

Kleihues *et al.* first showed this phenomenon in cats subjected to global ischemia for 30 minutes[95]. They made three important observations that would later hold for all animal models investigated: 1) Polysome profiles of ischemic animals which were not reperfused prior to sacrifice resembled those of controls, while animals reperfused after I/R injury lost almost all polysomes. 2) During reperfusion, absence of polysomes correlated to reductions in protein synthesis as measured by *in vivo* radioactive incorporation of amino acids into proteins. 3) Electron microscopy showed well-defined polysome rosettes in CNS neurons of ischemic animals, but not in animals studied during early reperfusion after ischemia.

Kleihues and Hossmann went on to show the same phenomenon in monkeys after 1 hour of global brain ischemia[2,96]. Based on well-defined peaks in absorbance correlating to rRNA concentration at specific densities, this work also showed that rather than separating into individual ribosomes, the polysomes of reperfused neurons dissociated into individual 40S and 60S ribosomal subunits. The same observations were later noted by Cooper *et al.* in a rat model of cerebral compression[97]. The Cooper study also isolated the ―frozen‖ polysomes from ischemic, non-reperfused rat brain and found that they were functional in an *in vitro* reticulocyte lysate system as measured by incorporation of radioactive phenylalanine. The observation that ischemic polysomes remain functional indicates that nonlethal ischemia does not damage the ribosome. The return of polysomes and protein synthesis depends on the duration of the ischemia. *In vivo* radioactive amino acid studies in the rat 4VO global model showed protein synthesis returned to normal 4 hours after ten minutes of ischemia, but protein synthesis was suppressed for 3 days after 30 minutes of ischemia[30].

2.3.2.4 Persistent Translation Arrest in CA1

Although eIF2α phosphorylation remits in all brain regions after about 4 hours in the global brain I/R model, translation arrest persists in CA1 neurons (reviewed in Hossmann, 1993)[75]. This persistent translation arrest differentiates CA1 and CA3 neurons and is the earliest known marker of ischemia vulnerable cells. The cause of persistent translation arrest in CA1 neurons is unknown.

There is evidence that in addition to phosphorylation of the α subunit of eIF2, other initiation factors are altered by ischemia. As mentioned above, eIF4G, a subunit of the mRNA-binding eIF4F complex, is partially degraded in reperfused neurons[38], which may be mediated by μ-calpain activated by increased intracellular calcium[40]. Another eIF4F subunit, eIF4E, was degraded specifically in rat CA1 at 4 hours reperfusion after a 30 minute global ischemia, but eIF4G levels were unchanged[91]. Additional changes in eIF4E-binding proteins and small ribosomal protein subunit S6 have been noted but, in general, changes in protein translational machinery have been poorly characterized relative to other injury mechanisms in I/R[82]. Moreover, damage to initiation factors is not insurmountable, even in CA1 neurons. In the rat, the vulnerable CA1 neurons express stress-response proteins such as HSP70 in amounts comparable to CA3, but only after 30 hours of reperfusion, presumably too late to allow for recovery[65]. Conversely, CA3 makes HSP70 protein as early as 8 hours after ischemia. This time delay is believed to be critical to the difference in outcome between the two regions[79].

The general hypothesis of this thesis is that the exclusive ability of ischemia resistant neurons to effectively translate inducible stress response mRNAs early in reperfusion allows these neurons to recover from I/R injury.

2.3.3 Selective Translation of Stress Response Transcripts

As discussed above, an important focus of brain I/R research has been the development of neuroprotective agents which block damage mechanisms in injured neurons. However neurons, like all stressed cells, exert their own neuroprotection through intrinsic stress responses, some of which require new transcription and translation. Stress responses allow neurons to recover after I/R injury and prevent DND. Understanding neuronal stress responses also offers the potential to manipulate them for therapeutic purposes. In the transient global I/R model, translation of stress response transcripts allow

selectively-vulnerable CA1 neurons to recover as CA3 neurons do, for example, following ischemic preconditioning[90,98].

Kleihues, *et al.* first noted in 1975 that specific proteins were preferentially translated in early reperfusion while global protein synthesis was still suppressed[2]. Later Kiessling *et al.* isolated polysomes from reperfused brain and used them for *in vitro* protein synthesis in a reticulocyte lysate system[99]. Proteins synthesized by the isolated polysomes were systematically studied by 2-D electrophoresis. At 3 hours reperfusion after 30 minutes of 4VO ischemia they found an increase in proteins with molecular weights of 70 kDa, 90 kDa, and 110 kDa. These were later identified as heat shock proteins from the HSP70, HSP90, and HSP110 families[100], respectively, previously known by immunohistochemistry to be expressed in gerbil neurons after global brain I/R[101,102]. HSP70 protein expression localized exclusively to CA3 neurons in the gerbil; gerbil CA1 neurons did not make HSP70 protein after global brain I/R. It is important to note that, in this model of I/R, global protein synthesis was still inhibited at 3 hours reperfusion, and heat shock proteins were preferentially translated in spite of global translation arrest.

Heat shock proteins are a highly conserved superfamily of molecular chaperones that assist in protein folding and prevent damaging protein aggregation[103]. HSP70, the cytoplasmic member of the HSP70 family, is induced in all manner of cellular stress including heavy metal poisoning, hypoxia, glucose deprivation, and many others (reviewed in Lindquist, 1986)[103]. There is strong evidence that HSP70 is neuroprotective in brain I/R. Overexpression of *hsp70* using a herpes simplex vector improved neuron survival after both focal[104] and global ischemia in the rat[105]. Functionally, induction of HSP70 caused reduced aggregation of ubiquitin, a marker for protein misfolding in a focal ischemia model[106].

Other proteins are expressed during the translation inhibition of early reperfusion. c-Fos and c-Jun are immediate early genes (IEGs), a class of genes which are transiently and rapidly upregulated in response to stress. *c-fos* mRNA was present throughout the gerbil hippocampus just fifteen minutes after a five minute global ischemia[107], and c-Fos protein was expressed as early as one hour after transient ischemia[108]. *c-jun* mRNA were found 30 minutes after a 10 minute ischemia in the rat[109]. In the hippocampus, expression of c-Fos and c-Jun proteins was confined to the CA3 and dentate gyrus and

remitted by 8 hours reperfusion[110]. Other proteins expressed while global translation is inhibited in early reperfusion include a caspase-3-like protein[111], brain-derived neurotrophic factor (BDNF)[112], and ornithine decarboxylase[75].

How polysomes in ischemia resistant cells re-assemble and translate specific classes of mRNAs during early reperfusion is not known. After prolonged ischemia, polysomes in both CA1 and CA3 remain intact and able to translate mRNAs in an *in vitro* system[97]. Detailed studies of *hsp70* transcription suggest that transcriptional regulation alone does not allow for increased HSP70 protein expression during early reperfusion. In the DeGracia laboratory, *hsp70* mRNA and HSP70 protein have been measured directly from microdissected, reperfused hippocampal subregions at various time points after 10 minutes of 2VO/HT[65] (Figure 4). Both CA1 and CA3 regions show strong expression of *hsp70* mRNA at 8 hours reperfusion. In fact, *hsp70* mRNA expression in CA1 is four to five-fold greater than in CA3. *hsp70* mRNA expression remains elevated in CA1 until at least 30 hours reperfusion, long after CA3 *hsp70* mRNA expression has returned to baseline. Conversely, HSP70 protein is expressed at 8 hours reperfusion in CA3 but not until 30 hours reperfusion in CA1. Using *in situ* hybridization, other researchers have found *hsp70* mRNA upregulated in the CA1 region in both rat[3] and gerbil[4,107] global ischemia models, but again HSP70 protein was either not synthesized in CA1 or found only late in reperfusion.

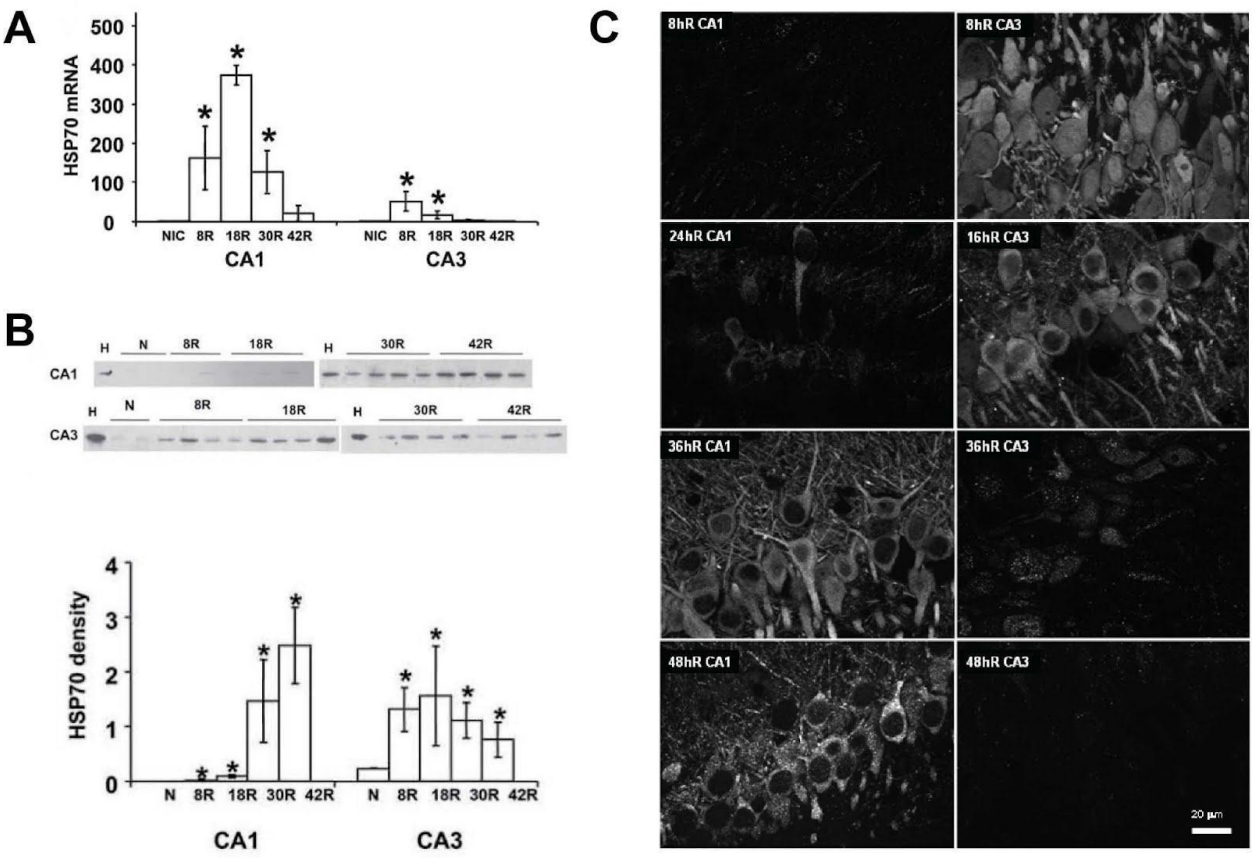

Figure 4: Expression of HSP70 protein correlates to recovery from brain I/R. All data are from rats after 10 minutes 2VO/HT and reperfusion as indicated. A) *hsp70* mRNA is expressed at 8hR in both CA1 and CA3, returns to baseline in CA3, but remains elevated to 30hR in CA1. B) Unlike mRNA expression, HSP70 protein is expressed at 8hR only in CA3 as shown by western blotting and densitometry. C) Immunohistochemistry staining for HSP70 protein confirms expression is early in CA3 and late in CA1. A) and B) adapted from Roberts, *et al.* 2007[65]; C) adapted from Jamison, *et al.* 2008[1].

2.3.3.2 Regulation by 5' UTR elements

Selected stress response transcripts can translated without the formation of a regular 5' cap structure or eIF4E association, called cap-independent translation[113]. Internal ribosome entry sites (IRES) is a cap-independent mechanism present in an estimated 3-5% of all translated mRNAs[114]. A consensus 5'UTR regulatory sequence for IRES has not been found and likely depends on complex secondary or tertiary structure[115]. All eukaryotic instances of IRES regulation have been determined experimentally[116]. Lack of a consensus sequence makes the role of IRES in brain I/R injury uncertain. Human *hsp70*[117] and

grp78[118] 5'UTRs have been reported to contain an IRES[117]. However, the IRES sequence is not necessarily conserved and has not been reported in corresponding rodent transcripts. Transcriptional profiling of mRNA from isolated polysomes after focal ischemia showed that IRES-containing transcripts were not enriched in reperfusion[119].

Another 5'UTR mechanism that may allow translation in early reperfusion is multiple upstream open reading frames (uORFs)[120]. Ribosomes initiate on the first open reading frame encountered in the 5'UTR of a transcript. In unstressed cells, ribosomes will initiate on the uORF and release the transcript before reading the authentic coding region. Under conditions of cell stress, when eIF2(αP) increases, ribosome scanning is prolonged and the likelihood of initiating at the downstream, authentic coding domain increases[41,94]. The best characterized uORF-containing transcripts in brain I/R are CCAAT/-enhancer-binding protein homologous protein (CHOP) and activating transcription factor 4 (ATF), both transcription factors induced by the unfolded protein response (UPR), a stress response pathway in the endoplasmic reticulum. Although the UPR and specifically CHOP are both induced by brain I/R, the resulting amount of CHOP is orders of magnitude less than the amount of HSP70 produced in the heat shock response[65,79]. The low level of CHOP induction relative to HSP70 suggests the UPR and CHOP have minimal influence in gene induction after global I/R.

2.3.3.3 mRBPs and mRNA Operons in Brain I/R

Rapid changes in posttranscriptional and translational regulation of gene expression are a hallmark of the cellular stress response. RNA is regulated by *cis*-acting sequences, usually in the 5' and 3' UTRs and the *trans*-acting mRNA binding proteins (mRBPs) which bind to these regulatory sequences. mRBPs are associated with mRNAs from transcription through degradation, regulating splicing, nuclear export, localization, translation, and degradation[121].

Ribonucleoproteins (mRNPs), groups of mRNAs and associated mRBPs, such as the stress granule (SG) and processing body (PB) change in response to stress. Stress granules are dynamic

cytoplasmic mRNPs which are sites of mRNA –triage,‖ directing mRNAs in stressed cells to storage, degradation, or translation[122]. The number of stress granules increases in cultured cells stressed with arsenate[123] or the protein synthesis inhibitor puromycin[124]. Stress granules also increase with eIF2(αP) in brain ischemia[66], but this increase is transient and does not correlate with cell death.

2.3.3.3.1 mRNA Granules

Previous work by Drs. Jamison and DeGracia has shown that cytoplasmic polyadenylated mRNA undergoes a major rearrangement in reperfused neurons forming mRNA granules[1,125] (Figure 5). mRNA granules are distinct cytoplasmic structures that form rapidly upon reperfusion after brain ischemia and contain mRNA and poly-A binding protein (PABP). mRNA granules form no later than 1 hour into reperfusion in all injured neurons after global brain ischemia. In focal ischemia, mRNA granules do not form neurons of the necrotic lesion core but are abundant in the damaged tissue surround the core (penumbra) (M. Lewis and D. J. DeGracia, unpublished observation). mRNA granules do colocalize with markers for the large[126] or small[1] ribosomal subunit and therefore do not sequester ribosomal subunits during reperfusion. mRNA granules also do not colocalize with markers of SGs or PBs[1].

mRNA granules were present in all neurons known to have repressed protein translation. mRNA granules were present in CA1 neurons from one hour reperfusion out to 48 hours reperfusion, the last time point measured. Conversely, mRNA granules remitted in CA3 neurons before 36 hours reperfusion.

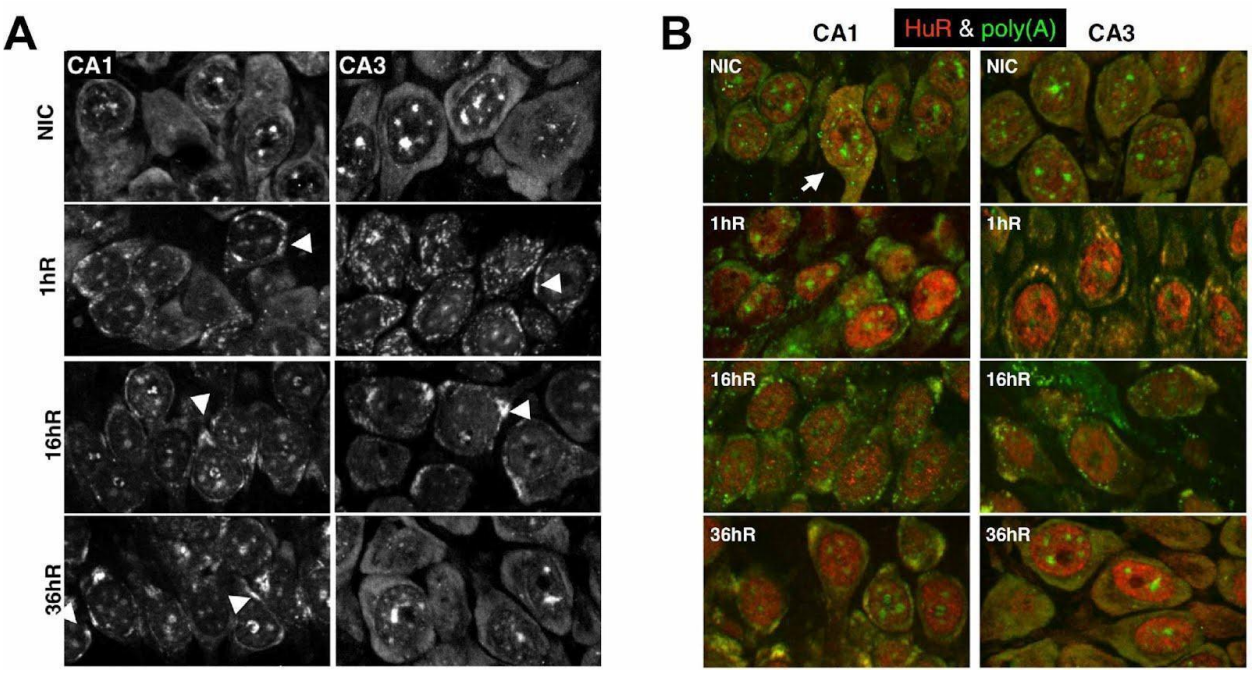

Figure 5: Changes in mRNA granules correlate to survival after brain I/R. A) ***In situ*** **hybridization to polyadenylated RNA in hippocampal pyramidal cells from non-ischemic control animals (NIC) and after 10 minutes I/R for 16 (16hR) or 36 hours (36hR). Arrowheads indicate mRNA granules. B) FISH/ Immunofluorescent microscopy for polyadenylated RNA (green) and HuR protein (red). mRNA granules form in all injured neurons, but only remit in CA3 neurons. HuR is present in CA3 neurons at 16hR, but only enters CA1 neurons at 36hR. Arrow indicates an interneuron. Adapted from Jamison** ***et al*** **[1].**

2.3.3.3.2 HuR

The binding sites in the 5' and 3' untranslated regions (UTRs) of eukaryotic mRNAs allow for the coordinated posttranscriptional regulation of mRNAs through mRBPs. Common UTR binding sites interacting with a mRBP have been called mRNA operons, in reference to prokaryotic operons which allow coordinated expression of genes with a common function behind a single promoter[127,128]. Eukaryotic genomes are not functionally organized like prokaryote genomes, but groups of mRNAs with common UTR binding sites (mRNA operons) can be coordinately regulated by the same mRBPs, generating the same functional effect[128].

The adenylate uridylate-rich element (ARE) is a cis-regulatory sequence found in the 3'UTR of 5%-8% of all mRNAs[129]. Among the proteins encoded by ARE-containing mRNAs are proto-oncogenes,

immediate early genes, transcription factors, and cytokines; proteins that typically exert powerful effects in the cell relative to their steady-state concentrations[130]. *hsp70* mRNA also has a 3'UTR ARE sequence[131] and binds HuR in mammalian cells[132]. In unstressed cells, the ARE identifies transcripts for rapid degradation. Cytoplasmic ARE-containing mRNAs are bound by tristetraprolin (TTP), an mRBP which activates decapping and degradation of these mRNAs at the processing body[133].

HuR is another mRBP which specifically interacts with the ARE. In mammals, HuR is the only ubiquitous member of the embryonic lethal abnormal vision (ELAV)/Hu protein family, which also includes the neuron-specific members HuB, HuC, and HuD[134]. All Hu proteins have three RNA recognition motifs (RRMs) and a hinge region (called the HNS) between RRMs 2 and 3[135]. RRMs 1 and 2 bind specifically to the ARE sequence while motif 3 recognizes the polyadenylated tail of full length mRNA[136].

Under conditions of cell stress, when it is advantageous to increase the steady state levels of stress response transcripts, HuR can stabilize ARE-containing mRNAs[137]. HuR staining can differentiate ischemia resistant CA3 neurons from ischemia vulnerable CA1 neurons after transient global brain ischemia. HuR was present in mRNA granules in CA3 neurons at one hour reperfusion and remains colocalized until the granules dissipate before 36 hours reperfusion (Figure 5B)[1]. In CA1 neurons, HuR was not found in mRNA granules until 36 hours reperfusion[1].

The mechanisms by which HuR influences gene expression are not well characterized and vary between cell types and cellular stresses[138]. Three distinct mechanisms have been shown to independently increase translation of HuR-target transcripts: reduced target degradation[139], facilitated nuclear export of targets[140], and increased association of target mRNAs with polysomes[7]. The most commonly studied mechanism of regulation by HuR is reduced degradation. In stressed cells, cytoplasmic HuR is associated with ARE-containing mRNAs, blocking their transport by TTP to PBs[141]. By preventing the degradation of ARE-containing mRNAs, HuR increases the steady-state concentration of these mRNAs and, therefore, their translation into protein[142]. However, prevention of mRNA degradation is not likely to contribute to regulation of ARE-containing transcripts after brain I/R. As previously described, *hsp70*

mRNA is more abundant in CA1 neurons than CA3 neurons during reperfusion, but is not translated. Even if *hsp70* mRNA is preferentially degraded in reperfusion, this mechanism cannot account for delayed HSP70 protein expression in CA1.

2.3.3.3.3 Facilitated Nuclear Export of ARE-containing mRNAs by HuR

HuR can also increase the translation of its target mRNAs by facilitating their nuclear export. In unstressed cells, HuR is a predominantly nuclear protein, actively shuttled into the nucleus by association with importin B[143]. Under conditions of cell stress, a variety of poorly characterized phosphorylation events cause the HNS region of HuR to associate with the shuttling proteins pp32 and APRIL[144]. pp32 and APRIL both contain nuclear export signals (NES) recognized by nuclear export factor chromosomal region maintenance protein 1 (CRM1). CRM1 actively exports HuR bound to pp32 and APRIL out of the nucleus in response to cell stress. While all proteins exit the nucleus in a GTP-dependent fashion by association with shuttling proteins such as CRM1, most mRNAs use a GTP-independent mechanism associating the TAP/p15 transport receptor. However, there is a subpopulation of mRNAs which shuttle in a CRM1-dependent fashion[145].

In Hela cells stressed with heat shock, HuR specifically colocalized with pp32 and APRIL, and CRM1 immunoprecipitated in complexes with HuR[146]. Inhibition of the CRM1 pathway by leptomycin B blocked nuclear export of HuR after heat shock. Leptomycin B also blocked the export of *hsp70* mRNA, leading Gallouzi *et al*. to speculate that HuR may regulate the nuclear export of *hsp70* mRNA. Subcellular localization of HuR also had functional significance in breast cancer cell lines, where decreased HuR specifically in the cytoplasm increased JNK activity and responsiveness to tamoxifen[6]. HuR regulation of target mRNA nuclear export was later shown in Jurkat T cell culture where APRIL and HuR were necessary for CD83 mRNA nuclear export[5].

2.3.3.3.4 HuR Association with Polysomes

Recently, a third mechanism by which Hu proteins may regulate target mRNAs has been proposed. HuD is a Hu protein family member, highly homologous to HuR, involved in neuronal differentiation[147]. Fukao, *et al*. showed that the HuD directly interacted with eIF4A and that this interaction was necessary for enhanced translation of target mRNAs[7]. The authors hypothesize that because HuD-enhanced translation required target transcripts to have both a 5' m^7G cap and 3' poly(A) tail, that HuD may be promoting circularization of mRNAs. As discussed above, the 5' cap structure of translating mRNAs is bound by eIF4E, and the 3' poly(A) tail is bound by PABP. mRNA circularization on a ribosome occurs when both PABP and eIF4E associate with eIF4G in what is called the closed loop model[148]. Circularization promotes reinitiation of ribosomes on the translating mRNA[148].

Like HuR, HuD binds to both ARE sequences and poly(A). Association of HuD with eIF4A could promote initiation by bringing the polyadenylated 3' tail of ARE-containing mRNAs adjacent to eIF4E. This provides a novel mechanism of selective initiation of ARE-containing mRNAs and may explain how *hsp70* is selectively translated during reperfusion.

2.3.3.4 Measuring Gene Expression in the Reperfused Neuron

Neurons change their transcriptional program after brain I/R injury and express new transcripts which are necessary for recovery[109,149], making reperfused neurons an ideal target for expression profiling studies such as microarray. Reperfused brain tissue has been studied by microarray since the method entered mainstream molecular biology in the early 2000s[150]. Since then nearly 40 microarray studies of brain I/R have published[151]. Most of these studies used the rat focal ischemia model, but global ischemia has also been studied[152].

Despite the large amount of expression profiling work done in brain I/R, present studies have major limitations. Earlier studies were limited by array technology and knowledge of the rat genome. The first global ischemia microarray only contained probes for 750 genes, just 3% of the rat genome. Beyond technical limitations, it is now known that the steady state, whole-cell mRNA levels measured by

microarray do not correlate well to levels of protein expression[153]. This problem has been carefully studied in yeast[154], and recently appreciated in mammals. A recent study in mouse hematopoietic cell lines found that only 40% of protein expression levels could be explained by mRNA abundance[155].

This issue was addressed in the focal brain I/R model in 2004 by MacManus *et al.* who performed microarray analysis on mRNAs extracted from polysome peaks after sucrose density gradient centrifugation, a technique called translation state analysis. Translation state analysis showed only 36% of transcripts upregulated by focal ischemia were bound to polysomes. This finding indicated that previous expression profiling after brain I/R primarily measured transcripts with increased transcription but not increased translation.

Summary

mRNA granules form in all reperfused neurons after I/R injury. Dissociation of mRNA granules correlates with recovery of neurons from I/R injury and differentiates the ischemia vulnerable CA1 neurons from ischemia resistant CA3 neurons. The presence of HuR in mRNA granules correlates to synthesis of HSP70 protein, occurring early reperfusion in CA3 and late in CA1. How HuR may regulate the expression of HSP70 in brain I/R is not known, but several mechanisms of posttranscriptional regulation by HuR have been described in other systems. To investigate the potential mechanisms of HuR regulation of *hsp70* translation in brain I/R, the following hypothesis were proposed:

1. mRNA granule formation is dependent on polysome-associated mRNA. (Chapter 3)
2. HuR binds *hsp70* mRNA and facilitates its export from the nucleus. (Chapter 4)
3. *hsp70* translation in reperfused neurons is regulated by HuR. (Chapter 5)
4. Brain I/R injury causes ARE-containing mRNAs to preferentially associate with polysomes. (Chapter 6)

Chapter 3 - mRNA Granule Dependence on Polysome-associated mRNA

3.1 Introduction

Previous work by Drs. Jamison and DeGracia has identified the mRNA granule as a distinct cytoplasmic structure which forms in all injured neurons during early reperfusion after transient global brain ischemia[1]. The mRNA granule does not colocalize with other mRNPs such as the SG, PB, or polysome[1]. mRNA granules do not contain markers of the small or large ribosomal subunits, but they do contain densities of cytoplasmic polyadenylated mRNA, PABP, and eIF4G. Roughly 2/3 of cytoplasmic mRNA is associated with polysomes in unstressed cells[156]. If translating mRNA on polysomes is required to form mRNA granules, then persistence of mRNA granules could explain the prolonged translation arrest seen in vulnerable neuron populations during reperfusion after brain ischemia. Prior to the present study, the relationship between the polysome and the mRNA granule was not characterized. Here, pharmacological agents that alter polysome function were used to study the relationship between mRNA granules and polysomes.

CHX is a glutarimide antibiotic that prevents the release of tRNA during translation elongation, inhibiting translation in eukaryotes[157]. There is limited evidence that CHX is neuroprotective against brain I/R injury. In a rat 30-minute four-vessel occlusion model of global brain ischemia, 1.5 mg/kg CHX reduced hippocampal DND when given 1 hour prior to ischemia by not 1 hour after ischemia[158]. Another study also found that 2 mg/kg subcutaneous CHX given just prior to reperfusion reduced DND in CA1 neurons after 10 minutes of four-vessel occlusion[159]. However, in a 2-vessel gerbil model, 2 mg/kg CHX given after 10 minutes reperfusion slightly increased DND[160]. Authors finding the CHX reduces brain I/R

injury speculate that the translation arrest induced by CHX conserves cell resources and prevents aberrant protein synthesis, protecting neurons in the same manner as transient eIF2(αP) phosphorylation[158]. Authors finding CHX detrimental in global ischemia models speculate that prolonged protein synthesis inhibition is detrimental and any positive effects likely arise from CHX's ability to lower body temperature[161]. Although the potential of CHX as a neuroprotectant is certainly interesting, here the drug is used because of its proven ability to functionally alter polysomes.

As CHX prevents the release of tRNA, it locks intact polysomes together with translating mRNAs[157]. In cell culture models of cell stress, the formation of PBs[161] and SGs[124] is inhibited by freezing mRNA onto polysomes with CHX. Puromycin is a protein synthesis inhibitor acting by a different mechanism- a nucleoside analog that inhibits translation by causing premature termination, thus destabilizing polysomes and causing their dissociation[162]. By increasing available mRNAs, puromycin increases the number of processing bodies and stress granules formed after stress in cell culture[124].

A robust literature describes the use of CHX and puromycin in mammalian brain establishing effective dosage and method of administration. Using radioactive amino acid incorporation, Jonec *et al.* showed that 1.5 mg/kg IP CHX inhibited 74% of forebrain protein synthesis as early as 15 minutes after injection and protein synthesis inhibition was maintained for at least two hours[163]. Intra-cerebral injection of 90 µg of puromycin per cerebral hemisphere in rat rapidly inhibited 90% of hippocampal protein synthesis, an effect that was maintained from a single dose for over four hours[164].

mRNA granules have not yet been characterized biochemically, but have only been studied under the microscope. mRNA granule formation is a complex process with changes in staining intensity and pattern. Simple methods of measuring fluorescence or colocalization were inadequate to quantitatively measure changes in mRNA granule formation. This problem was addressed with texture analysis. Texture analysis is the calculation of a large, diverse set of image features combined with statistical variable reduction techniques to quantify image features. Texture analysis is commonly used to classify images in the physical sciences[165,166]. In biomedical sciences, texture analysis has been used to classifying brain tumors on MRI[167] and identify cancerous[168] and apoptotic[169] cells under the microscope.

3.1.1 Summary

mRNA granules form in all affected neurons after transient global brain ischemia. mRNA granules abate in the ischemia resistant CA3 region of the hippocampus, but they persist in the ischemia vulnerable CA1. Persistence of mRNA granules correlates to persistent protein synthesis inhibition and selective cell death in CA1. mRNA granules may require translating mRNA from polysomes to form, like other stress-induced mRNPs do. Sequestering mRNA on polysomes with CHX should prevent mRNA granules from forming if they require polysome-associated mRNA. Conversely, liberating polysome-associated mRNA in unstressed cells may cause the formation of mRNA granules as it causes the formation of other stress-induced mRNPs. Since, at present, mRNA granules can only be detected microscopically, texture analysis was used to quantify microscope images.

3.1.2 Hypotheses

1. Preventing polysome dissociation prior to injury will prevent the formation of mRNA granules.
2. mRNA granules will form when polysome dissociation is induced in unstressed cells.

3.2 Methods

3.2.1 Materials

Alexa 488-labeled streptavidin (S32354) was from Invitrogen (Carlsbad, CA). Biotinylated goat anti-streptavidin (BA-0500) was from Vector Laboratories (Burlingame, CA). HuR (sc-5261) antiserum was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). A 5 -biotinylated 50-mer oligo-dT probe was made by Integrated DNA Technologies, Inc. (Coralville, IA). CHX (c1988) and puromycin dihydrochloride (P7255) were purchased from Sigma (St. Lois, MO). Prehybridization buffer was

mRNAlocator *In Situ* Hybridization Kit from Ambion (Austin, TX). All other chemicals were reagent grade.

3.2.2 Animal Model

All animal experiments throughout the thesis were approved by the Wayne State University Animal Investigation Committee and were conducted following the Guide for the Care and Use of Laboratory Animals (National Research Council, revised 2011)[170]. Global forebrain ischemia was induced in adult (275-300 g) male Long Evans rats using the bilateral carotid artery (two-vessel) occlusion and hypovolemic hypotension (2VO/HT) model developed by Smith *et al.*[26] and used commonly in the DeGracia laboratory[65,66,126]. Animals were anesthetized with 5% halothane and anesthesia was maintained at 2% halothane in 100% O_2 using a facemask throughout the duration of the surgery. Throughout the ischemic period and the first hour of reperfusion, body temperature was maintained at 37 ± 0.5 ^{o}C using a homeostatic blanket system and rectal temperature probe. Temperature of the head was also maintained at 37 ± 1 ^{o}C and independently monitored with a thermocouple probe placed in the temporalis muscle. Mean arterial pressure was monitored in real time via tail artery access. Blood gas measurements were maintained at pH = 7.4 ± 0.1, pO_2 = 80 mmHg and pCO_2 = 35 ± 5 mmHg until immediately prior to the initiation of ischemia.

To initiate ischemia, blood was withdrawn from the femoral artery into a 10-mL syringe until a mean arterial pressure of 50 mmHg was reached. Following the withdrawal of blood, the common carotid arteries were clamped using microaneurysm clips. Additional blood was withdrawn to maintain a mean arterial pressure of 40 mmHg for 10 minutes. Following 10 minutes of ischemia, microaneurysm clips were released and blood was reinfused at a rate of 5 mL per minute. All wounds were sutured and anesthesia and temperature control was maintained for one hour following the surgery. Post-surgical animals displaying frank necrosis, weight loss > 15% initial body weight/day, or sustained seizure activity were excluded from the study. Overall survival rate for the reperfusion groups was 75%. Tissue

processing and brain dissection is further described below. As outlined above in Chapter 2, it is well established that transient global brain ischemia can cause DND. DND following 2VO/HT has been shown repeatedly in the DeGracia laboratory[1,65] and independently confirmed in other laboratories[19,70]. In the DeGracia laboratory, multiple, independent lines of evidence have established selective cell death of CA1 pyramidal cell neurons in the 2VO/HT model including fluoro jade staining and morphology of toluidine blue staining (Figure 2).

3.2.3 CHX and Puromycin Treatments

For the CHX study, animals were subjected to 10 minutes ischemia followed by one hour reperfusion. 1.5 mg/kg CHX in saline was administered via intraperitoneal injection at either 15 minutes prior to onset of ten-minute ischemia (C-pre; n = 6) or at 15 minutes into reperfusion after ischemia (C-post; n = 5). One hour reperfused groups were repeated using saline vehicle alone (v-pre; n = 5) (v-post; n = 5), and groups of sham-operated nonischemic animals (NIC; n = 4) and 1 hour reperfused animals (1hR; n = 7) without injection served as controls. The CHX dose and delivery method were chosen because they have previously been shown to rapidly and inhibit protein synthesis in the rat brain[163]. This dose may also be neuroprotective in global ischemia models when given prior to ischemia[158].

For the puromycin study, three non-ischemic animals were injected with puromycin (Pur) and two animals were injected only with saline vehicle (Veh). Sections from two animals previously subjected to 10 minutes of ischemia and one hour of reperfusion (1hR) and and two uninjected non-ischemic animals (NICs) were also stained as controls. Puromycin dose and administration were based on the method of Flexner, *et al.*[164] who showed that intra-cerebral injection 90 µg of puromycin at 9 mg/mL in each cerebral hemisphere in the rat rapidly inhibited 90% of protein synthesis in the hippocampus, an effect that was maintained from a single dose for over four hours.

To administer intra-cerebral injections of puromycin, rats were anesthetized IP with 100mg/kg ketamine and 10mg/kg xylazine and placed in a stereotactic apparatus. Skull was exposed and holes were

drilled tangent to the surface of the skull at the angle formed by the caudal parietal suture and origin of temporalis muscle. In each hemisphere, 90 µg puromycin in 10 µL saline was injected at depth of 3 mm, targeting the hippocampus (Bregma coordinates -2.1 mm caudal, 2.0 mm lateral, and 1.4 mm vertical)[171]. Rats were maintained under anesthesia for two hours after puromycin injection and then sacrificed.

3.2.4 Tissue Fixation and Staining

3.2.4.1 Fixation

One hour after ischemic injury or sham surgery or two hours after puromycin injection, rats were transcardially perfused with 250 mL of ice-cold 0.9% NaCl at a rate of 20.8 mL/min. Following the NaCl solution, 300 ml of 4% paraformaldehyde (PFA) in 0.1 M phosphate buffered saline (PBS) was perfused at a flow rate of 10 mL/min. Once perfusion was complete, brains were excised and post-fixed by immersion in 4% PFA, 0.1M PBS. Duration of the post-fix was 24 hours. Following post-fix, the dorsal hippocampus was sectioned in 50 µm coronal slices using a vibratome. Sections used for staining below were taken near 3.0 mm posterior to Bregma. The tissue was sectioned in 0.1M PBS and resultant sections were stored in cryostat solution at -20 ^{o}C until used for staining.

3.2.4.2 Immunofluorescent Microscopy / Fluorescent *in situ* Hybridization

Immunofluorescent microscopy and fluorescent *in situ* hybridization (IF/FISH) was performed in a two stage procedure. FISH was a modification of the procedure of Bessert and Skoff[172]. The combined procedure was performed exactly as described in Jamison, *et al*[1]. All IF procedures were at room temperature with gentle agitation on an orbital shaker. Brain sections were washed four times for 10 minutes in 0.1 M PBS. After the last wash, sections were pre-blocked for 25 minutes in 0.1M PBS containing 0.3% Triton X-100 and 10% serum of donkey (the host species for the secondary antibody).

After three ten-minute washes in PBS, blocked sections were immersed in 1:25 mouse anti-HuR primary antibody PBS 0.3% Triton X-100 and 1% donkey serum at room temperature overnight. The following day, samples were washed three times for 10 minutes in PBS and incubated with donkey anti-mouse secondary antibody in PBS with 0.3% Triton X-100 and 10% donkey serum for 2 hours. HuR staining provided a useful signal for generating nuclear and cytoplasmic image masks as part of the texture analysis described below.

Primary and secondary antibody concentrations were determined in previous studies in the DeGracia laboratory based on dilution series[1]. HuR was tested with a series of dilutions on control and experimental animals in order to determine the concentration that limits background and provides the best overall signal. Validation of antisera staining included: (1) loss of signal with omission of primary antisera, (2) graded loss of signal with antisera dilution, and (3) agreement with published descriptions of antisera staining patterns were previously reported[1].

The second stage, FISH, was performed under low light illumination or in the dark. At the end of the IF procedure, slices were mounted on lysine-coated slides. IF-stained sections were fixed in 3.6% formaldehyde in PBS for 10 minutes at room temperature and then drained and blotted. Prehybridization was in a box humidified with 50% formamide/4X saline-sodium citrate (SSC) at 32 ^{o}C for 3 hours in prehybridization buffer. Slides were then incubated overnight in the same apparatus in a solution of 50 ng/mL of a 5'- biotinylated 50-mer oligo-dT probe dissolved in hybridization buffer. The next day, all subsequent processing was performed at room temperature. Slides were washed three times in 2X SSC for 10 minutes and then incubated in the dark at 1:500 Alexa 488-labeled streptavidin in 4X SSC/0.1% Triton X-100 for 60 minutes. Slides were then washed once in 4X SSC for 10 minutes followed by incubation in 2X SSC/0.1% Triton X-100 containing 1:667 of biotinylated goat anti-streptavidin for 60 minutes. Slides were again washed once in 4X SSC for 10 minutes and then incubated in 1:667 Alexa 488-labeled streptavidin in 2X SSC/0.1% Triton X-100 for 60 minutes. Slides were then washed sequentially in 4X SSC for 10 minutes and 2X SSC for 10 minutes, and then coverslipped for viewing. Previous studies established that polyadenylated mRNA (poly(A)) and HuR staining patterns were identical on IF/FISH

double-labeled sections and individually stained sections. Negative controls demonstrating the specificity of poly(A) FISH staining including pre-incubating NIC brain slices in DNAse-free RNAse or 0.1 M NaOH were previously reported[1].

3.2.5 Texture Analysis

3.2.5.1 Image Acquisition

Photomicrographs of the CA3 pyramidal cell layer of the dorsal hippocampus were acquired on an Axioplan 2 Imaging System (Carl Zeiss, Oberkochen, Germany) equipped with an ApoTome as previously described[66]. This study focused exclusively on the hippocampal CA3 region because of the large cytoplasmic area of this population of cells. The DeGracia laboratory has previously shown that mRNA granule formation occurs in all post-ischemic neurons[1].

Similar to a laser scanning confocal microscope, the apotome focuses on a specific plane in the tissue and can acquire optical sections moving up or down from the original plane of focus. The microscope is controlled via a computer that is programmed to acquire optical sections of consecutive focal planes marked by a defined distance (the software sets this distance to meet Nyquist sampling requirements). The set of sequential optical sections are referred to as a z-stack. z-Stacks of ten optical sections were acquired under 63× oil immersion lens (1388 × 1040 w × h; pixel spatial dimensions; x = 0.1 m, y = 0.1 m, and z = 0.35 m). Eight-bit per channel maximum intensity orthographic projections were constructed in NIH ImageJ[173] from the 16-bit acquired z-stacks and used as input images for the texture analysis. Excitation at 488 nm and 568 nm, and emission at 518 nm and 600 nm were used for Alexa 488 (green, poly(A)) and Alexa 555 (red, HuR) respectively. Orthographic projections were used because they provided a denser staining pattern than single z-slices[66,125], and were thus more representative of the distribution and density of the mRNA granules in the cell cytoplasm. Each image contained, on average, 24.1 ± 3.8 cells. The ratio of total cytoplasmic area to total cell area was 73.6% ± 5.1%, and the ratio of total nuclear area to total cell area was 23.4% ± 5.1%.

3.2.5.2 Texture Analysis Computations

Texture analysis is a method to quantitatively measure complex changes in images. The data processing elements in the texture analysis pipeline were image masking, feature calculation, feature selection, feature projection, and significance testing.

Image masking divides an image into regions of interest onto which downstream methods are applied. Using distinctive HuR nuclear staining, images were segmented into regions of nucleus, cytoplasm, and background. Semi-automated image segmentation was performed in the EDISON program (Edge Detection and Image Segmentation, v1.1)[174].

Following image masking subsequent texture analysis steps were carried out in MaZda v4.6[175]. Features calculated by MaZda are quantifiable pieces of information which can be measured and compared between images. Calculated features were derived from histogram analysis, absolute gradient methods, run length matrix, co-occurrence matrix, autoregressive modeling, wavelet analysis, and fractal dimension. A brief description of each parameter follows: Histogram analysis quantifies statistical features of the image histogram such as kurtosis. Absolute gradient features compare each pixel to a five-pixel neighborhood matrix. A run length matrix measures how many pixels in any direction maintain the same intensity. A co-occurrence matrix measures the probability two pixels will have the same intensity at a set distance apart. An autoregressive model assumes a pixel is influenced by a weight sum of neighboring pixel intensities. Discrete wavelet transform is a linear transformation of pixel intensity. Fractal dimension measures how image patterns change with the scale at which they are measured. In total, 155 different image features were calculated in MaZda.

Ten features were selected to be compared between sample groups (Table 1). These ten features were the most discriminant between sample groups by Fisher coefficient, a ratio of between-group to within-group variance. The ten most discriminant features were carried forward to feature projection, the transformation of high dimensional feature space into a new, lower dimensional feature. Feature

projection was accomplished by principal component analysis (PCA), a variable reduction technique performed within MaZda. PCA generates a first principal component or most expressive factor (MEF1) which consolidates as much variance as possible from the ten most discriminant original variables. One-way ANOVA was used to test the significance of group differences in MEF1 followed by Tukey post-hoc testing of each possible pair with $p<0.05$ considered significant.

Table 1: Image features selected from the texture analysis. From 155 image features measured, ten each were selected for the pre-treatment and post-treatment experiments by Fisher coefficient (F). For each set, these ten features were then used in PCA, and a first principal component or most expressive factor (MEF1) was calculated for each experimental group.

Input groups	Feature	F	TA method
NIC	Perc.99%	6.84	Histogram
C-pre 1hR	Variance	6.19	Histogram
v-pre 1hR	WavEnHH_s-3	4.86	Wavelet analysis
	Teta1	4.41	Autoregressive
	Teta2	3.92	Autoregressive
	S(2,0)Entropy	3.70	Co-occurrence matrix
	S(2,−2)Entropy	3.66	Co-occurrence matrix
	S(2,2)Entropy	3.52	Co-occurrence matrix
	GrSkewness	3.19	Absolute gradient
	WavEnHH_s-1	3.18	Wavelet analysis
NIC	Horzl_GLevNonU	6.11	Run length matrix
C-post 1hR	Vertl_GLevNonU	6.10	Run length matrix
v-post 1hR	135dr_GLevNonU	6.03	Run length matrix
	45dgr_GLevNonU	6.01	Run length matrix
	S(2,−2)Entropy	5.99	Co-occurrence matrix
	S(2,2)Entropy	5.78	Co-occurrence matrix
	Teta1	5.25	Autoregressive
	WavEnHH_s-1	4.79	Wavelet analysis
	Teta2	4.55	Autoregressive
	S(2,0)Entropy	4.51	Co-occurrence matrix

3.3 Results

3.3.1 Pre-treatment but not Post-treatment with CHX inhibits mRNA granules

Cytoplasmic staining of polyadenylated mRNA was quantified after one hour of reperfusion. Of the six animals pretreated with CHX, only one displayed obvious mRNA granulation (Figure 6A). Following CHX post-treatment, four of the five animals displayed mRNA granules (Figure 6A). Qualitative differences seen in the microscope images were quantified by texture analysis.

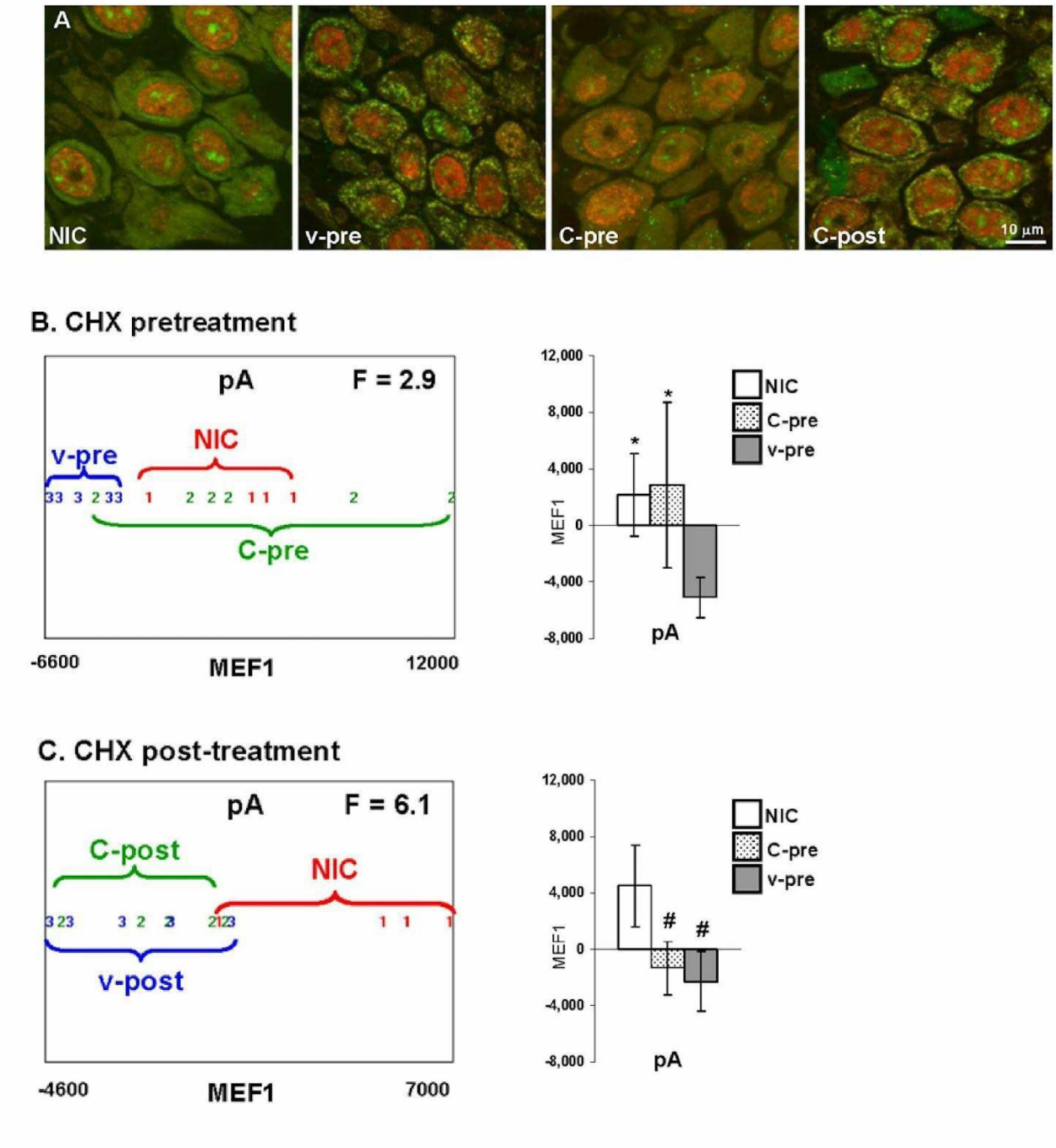

Figure 6: Effect of CHX on mRNA granules at 1 hour reperfusion after global ischemia. (A) Merged images of pA (green) and HuR (red) double-labeling of individual samples as indicated. Scale bar applies to all images. (B) On the left, CHX pretreated TA: PCA scatter plots for pA cytoplasmic textures in experimental groups as indicated with Fisher coefficient in upper right. On the right, average (±standard deviation) of MEF1 for groups as indicated in legend. pA ANOVA p = 0.017, *Tukey HSD post hoc p < 0.05 compared to v-pre group. (C) CHX post-treatment. On the left, TA: PCA scatter plots for pA cytoplasmic textures in experimental groups as indicated. On the right, average (±standard deviation) of MEF1 for groups as

indicated in legend. pA ANOVA p = 0.023, # Tukey post hoc p < 0.01 compared to the NIC group. Modified from Szymanski, *et al.* 2011[176].

Texture analysis was used to quantify these changes and measure the effect of CHX on the C-pre and C-post groups. After quantifying 155 unique image features in the cytoplasm of each image, Fisher coefficient was used to select the ten features most discriminant for all animal groups (Table 1), and these variables were consolidated by principal component analysis. MEF1 consolidated well over half the variance in the ten most discriminant image features. The C-pre animals were indistinguishable from NICs but significantly different from v-pre on Tukey post hoc testing after one-way ANOVA (Figure 6B). Conversely, C-post and v-post groups were indistinguishable, but these groups were significantly different from NICs.

Consistent with my original hypothesis, I found that CHX administered before ischemic and reperfusion injury, when polysomes are intact, inhibited the formation of mRNA granules. CHX administered after brain I/R injury, when polysomes have dissociated, did not inhibit the formation of mRNA granules.

3.3.2 Puromycin induces mRNA granules in neurons of uninjured animals.

Consistent with previous observations[1], NIC animals showed no mRNA granules (Figure 7A), while 1hR animals had robust mRNA granule formation in CA3 neurons (Figure 7B). mRNA granules have never previously been observed outside the context of global brain I/R. Two of three animals injected with puromycin showed mRNA granule formation near the puromycin injection site (Figures 7B and 7C). mRNA granules were not observed over 1 mm away from the injection site. Vehicle-injected animals showed no mRNA granules throughout the entire section, showing that mechanical trauma from the injection did not generate mRNA granules. Thus mRNA granules could be pharmacologically induced in non-ischemic rat brain.

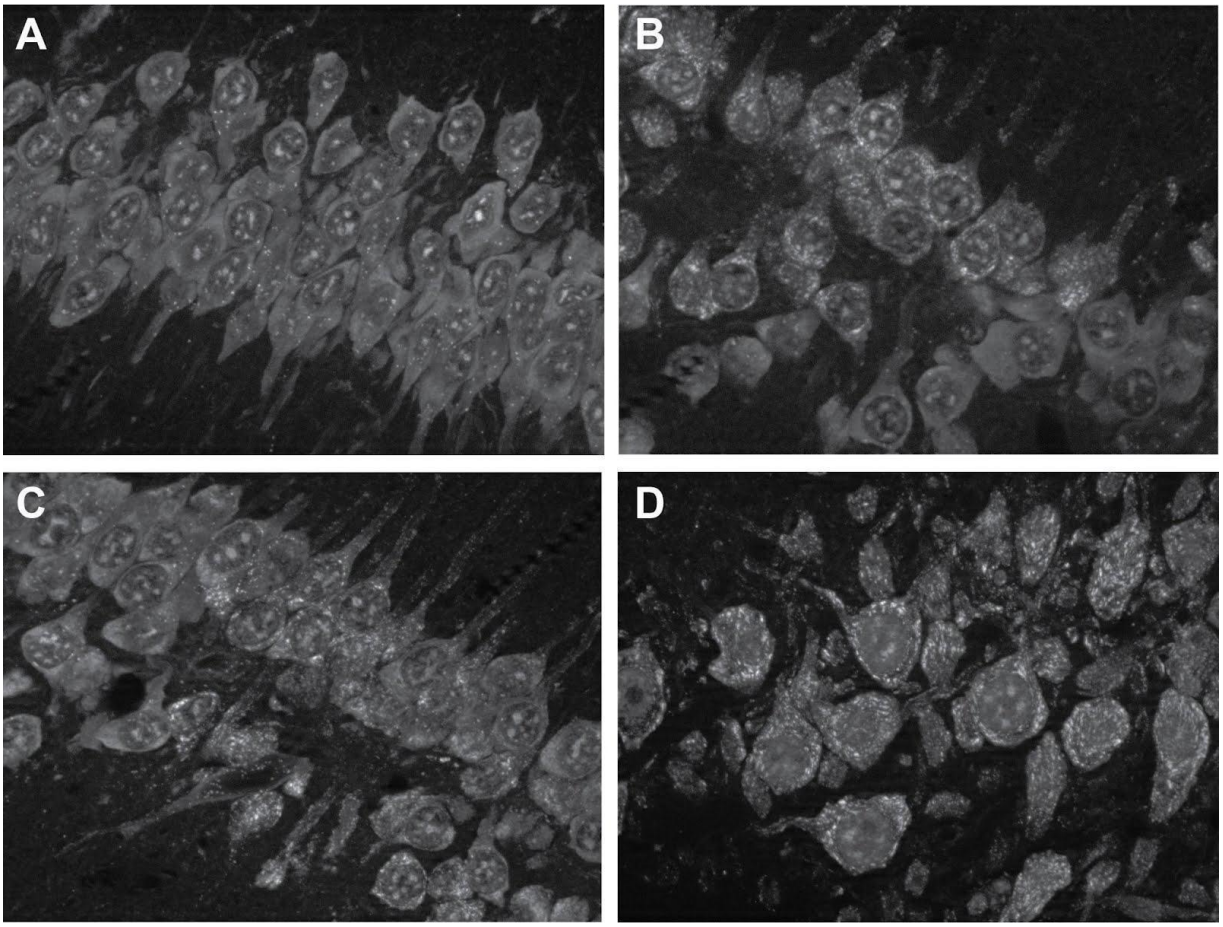

Figure 7: Puromycin induced mRNA granule formation in uninjured neurons. Representative images of pA staining in CA3 hippocampal neurons. A) Vehicle-injected non-ischemic animal without mRNA granules. B) and C) Images from two separate, nonischemic, puromycin-treated animals showing partial mRNA granulation near injection site. D) Animal without puromycin injection after 10 minutes ischemia and 1hR showing diffuse mRNA granulation.

Chapter 4 – HuR Binds *hsp70* mRNA but does not Regulate its Nuclear Export

4.1 Introduction

After transcription, stress-induced mRNAs such as *hsp70* may exit through the CRM1 pathway. Through binding partners APRIL and pp32, HuR exits the nucleus with CRM1 in stressed cells. HuR has been shown to facilitate nuclear export of some target mRNA, increasing their translation[146]. To study the nuclear export of HuR and *hsp70* mRNA, neurons from microdissected CA1 and CA3 hippocampal regions were separated into nuclear and cytoplasmic fractions by differential centrifugation after gentle mechanical homogenization. RNA immunoprecipitation (RIP) detects transcripts specifically bound to a mRBP. RIP was used to show that HuR associates *hsp70* mRNA in reperfused neurons.

4.1.1 Hypotheses

1. If *hsp70* mRNA is associated with HuR in reperfused neurons, RIP will precipitate *hsp70* mRNA with HuR.

2. Facilitated nuclear export of *hsp70* mRNA by HuR will allow translation of *hsp70* mRNA in ischemia resistant CA3 while lack of nuclear export of *hsp70* mRNA in ischemia vulnerable CA1 will prevent translation of *hsp70* mRNA.

4.2 Methods

4.2.1 Animal Model

Animal model was used as described in Chapter 2. RIP used whole cortex tissue, and subcellular localization used microdissection hippocampal subregions as described below. Animal groups were: For RIP- 10 minutes ischemia followed by 8 hours reperfusion (8hR), n=3); for HuR in subcellular fractions- NIC, n=6, and 8hR, n=6. Again for *hsp70* mRNA in subcellular fractions, groups were- NIC, n=6 and 8hR, n=6.

4.2.2 RIP

RIP was based on the method Keene, *et al*[177,178]. All efforts were made to prevent RNase degradation of samples. Consumables were RNase-free and other containers and surfaces were treated with Zap (Ambion). Where possible, procedures were performed under a laminar flow hood and all solutions were prepared with DEPC-treated water.

8hR or NIC animals were sacrificed and brains rapidly removed. Whole forebrain was dissected at 4 ^{o}C and homogenized on ice in 1:5 (w/v) of 50 mM HEPES pH 7.5, 150 mM NaCl, 1% (v/v) Triton X-100, 10% (v/v) glycerol, 1 mM MgCl2, 1 mM EGTA, 80 U/mL RNase inhibitor (Ambion, Austin, TX, USA), 0.2% ribonucleoside vanadyl complexes (Sigma, St. Loius, MO, USA), and 1:85 protease inhibitor cocktail (Sigma). Centrifugation of the 2500 *g* post-nuclear supernatant at 25,000 *g* generated a cytoplasmic supernatant, and 600 µg of supernatant protein was precleared with 1 µL of an unrelated antibody (Lamin AlC, Santa Cruz Biotech, Santa Cruz, CA, USA) plus 20 µL Protein A-Sepharose beads (Invitrogen, Carlsbad, CA, USA). Precleared supernatants were rotated 16 hr, 4°C in 15 µL Protein A-Sepharose prebound with 16 µg HuR antiserum (Santa Cruz) or 10 µg PABP antiserum (Abcam). Beads were washed three times in sterile phosphate buffered saline. RNA was extracted from precipitated protein using TRIzol reagent (Invitrogen). 2 µg total RNA was measured by A260 and reverse transcriptase PCR was performed according to vendor instructions (Roche, Boulder, CO, USA). Primers for PCR were *gapdh* (5'-ACAAGATGGTGAAGGTCGGTGTGA-3', 5'-

TTGTCATTGAGAGCAATGCCAGCC-3'; 1.0 kb product) and *hsp70* (5'-TCTTGGTTGCCAACACCCAAATCC-3', 5'-AAAGGTCACTGCTAGCTCCGTGTT-3'; 0.5 kb product). Amplification products were run on Tris-acetic acid- EDTA-1% agarose gels and visualized by SYBR gold (Invitrogen). For some IP reactions, RNA was not extracted but rather beads were boiled in Laemmli buffer, run on SDS-PAGE gels, and western blotted for HuR or PABP using methods previously described (Jamison, et al., 2008).

4.2.3 Quantification of HuR Protein and *hsp70* mRNA in Nuclear and Cytoplasmic Fractions

4.2.3.1 Hippocampal Subregion Microdissection

Microdissection of dorsal hippocampus CA1 and CA3 subregions was according to the method of Roberts *et al*[65]. Briefly, animals were anesthetized with 5% halothane and quickly decapitated after the appropriate reperfusion duration. For each animal, whole brain was rapidly removed and snap-frozen in a dry ice-ethanol bath for 15 seconds. A semi-frozen coronal section was cut from 2.30 to 3.80 mm posterior to Bregma in a brain blocker. This section, containing the dorsal hippocampus, was further subdivided on an ice-cooled stage under a dissecting microscope (Figure 9A). A vertical cut was made slightly medial to CA3 and surrounding cortex was removed to isolate CA3. CA1 was separated from the dentate gyrus by a horizontal cut through the hippocampal fissure. Corpus callosum and other superior tissues were separated to isolate CA1. Bilateral sections were rapidly weighed and then stored at -80 ^{o}C. Bilateral wet weight for each subregion averaged 25 mg per rat. Microdissection of CA1 and CA3 hippocampal subregions allowed for the comparison of ischemia vulnerable and ischemia resistant neurons in a global ischemic model. Additionally, these regions consist primarily of hippocampal pyramidal cell neurons. While interneurons and glia are still present, pyramidal neurons account for the

majority of the mass of these sections, allowing for study of generally homogenous populations of cells[179].

4.2.3.2 Subcellular Fractionating

Microdissected tissue samples were homogenized at 1:15 (w/v) in a homogenization buffer of 320 mM sucrose, 1 mM $MgCl_2$, 1 mM NaH_2PO_4, pH 6.6. 2.39, 1 mM DTT, 80 units/mL RNase inhibitor (Ambion), 0.5% protease inhibitor cocktail (Sigma). Homogenization used a glass Kontes dounce with 150 µM clearance to preserve the nuclear membrane. Nuclei were pelleted by 850 *g* centrifugation for 10 minutes followed by three washes of the nuclear pellet. Nuclei were then resuspended in 1 mL homogenization buffer and mixed with 5 mL of nuclear purification buffer (2.39 M sucrose, 1 mM $MgCl_2$, 1 mM NaH_2PO_4, 10% (v/v) triton X-100, pH 6.6, 1 mM DTT, 80 units/mL RNase inhibitor, 0.5% protease inhibitor cocktail). Resuspended nuclei were centrifuged 48,000 *g* for 1 hour at 4 ^oC in a Beckman SW50.1 rotor. Pellet of intact nuclei was washed 3 times in homogenization buffer and then resuspended at minimal volume in a lysis buffer of 5 mM EDTA, 50 mM tris, pH 7.5, 2% SDS.

4.2.3.3 Western Blotting

Western blotting was performed exactly according to vendor instructions (Amersham). 2 µL aliquots of each sample were taken in triplicate for Lowry assay to determine protein concentration. All antibody conditions were identical to previous western blotting used in the DeGracia laboratory[1,65]. Prior to loading, all protein samples were boiled for 2 minutes in equal volume of 2x Laemmli buffer. SDS-PAGE gels were 10% total, 0.8% bis acrylamide. Pilot studies determined the total protein from each subcellular fraction necessary to generate good signal in western blotting, and all western blots compared only samples from the same subcellular fractions. After SDS-PAGE, proteins were transferred to nitrocellulose. Gels were silver stained to assess protein loading. Antibody conditions were: mouse anti-

HuR 5261 (Santa Cruz Biotechnology) 1:200 without blocking for 2 hours at room temperature; mouse anti-lamin B1 16048 (Abcam) 1:5000 overnight 4 at ^{o}C without blocking; and rabbit anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) 25778 (Abcam) 1:1000 for one hour at room temperature without blocking. The base buffer for all primary antisera incubations was tris buffered saline with 0.1% tween 20. All peroxidase-linked secondary antibodies were at a concentration of 1:6700 and blocked in 4% serum of the secondary host. Films were scanned at 300 dpi on a Scanmaker 9800XL flatbed scanner (Microtek) and densitometry was performed in BioIamge Intelligent Quantifier v.4.

4.2.3.4 RNA Isolation and Real Time PCR

RNA was extracted from hippocampal subregions by guanidinium-acid-phenol using the TRIzol reagent exactly according to vendor instructions. As described in the method of RIP above, all efforts were made to prevent RNase degradation of samples. Final isolated RNA had an A_{260}:A_{280} ratio >1.7, and RNA integrity was confirmed with denaturing agarose gel electrophoresis. cDNA was generated using the Transcriptor First Strand cDNA Synthesis kit (4896866001) from Roche. cDNA was transcribed using poly-T primers so that only full length transcripts were amplified. This step was taken to ensure that only mature and not nascent transcripts were amplified from the nuclear fraction. The resultant cDNA was input to quantitative PCR reactions using SYBR Green quantitative PCR (qPCR) Master Mix (600548) from Stratagene, on the Mx3000P real time PCR thermocycler. Primers for qPCR were the same as for the RIP method, above measuring differences in *hsp70* and using *gapdh* as a normalizer. After an initial 10 minutes at 95 ^{o}C, all samples were amplified for 40 cycles of one minute annealing at 55 ^{o}C, 30 seconds elongation at 72 ^{o}C, and 30 seconds dissociation at 95 ^{o}C followed by a dissociation curve. Amplification data were analyzed in MxPro v.3.00 from Stratagene using $2^{-\Delta\Delta CT}$ method for relative quantification[180]. Threshold fluorescence was determined using a minimum cycle threshold (Ct) spread algorithm in MxPro which minimizes the overall spread of Ct standard deviation for all replicates in a given run. Differences between hippocampal subregions were measured with a two-tailed t-test.

4.3 Results

4.3.1 RIP

RIP of HuR or PABP was performed on cytoplasmic fractions from forebrain homogenates of NIC and 8hR samples (Figure 8). HuR RIPs selectively brought down *hsp70* mRNA but not *gapdh*. PABP RIPs brought down both *hsp70* and *gapdh* mRNA.

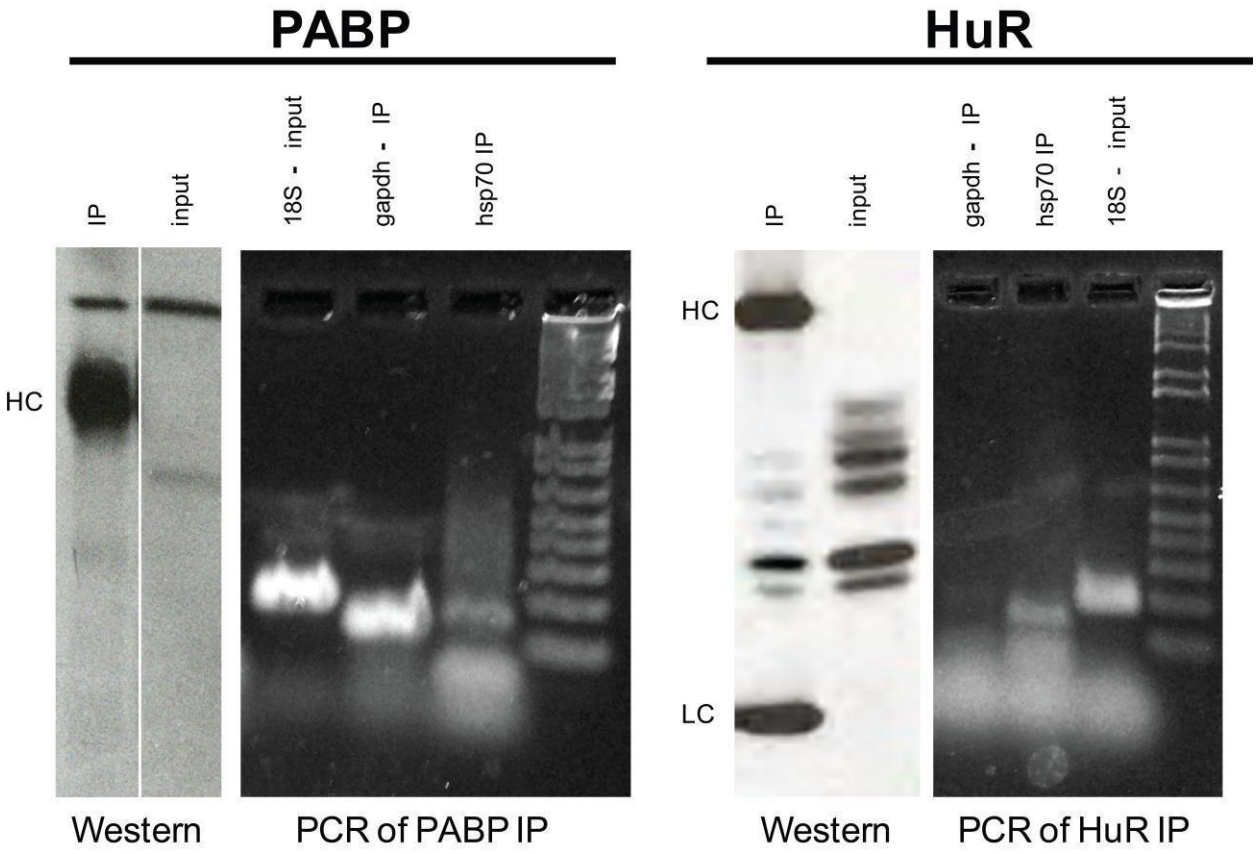

Figure 8: Figure: *hsp70* mRNA co-precipitates specifically with HuR after transient global brain ischemia. Results from PABP (left) and HuR (right) RNA co-immunoprecipitation (RIP) reactions from 8hR forebrain. Left panels for each RIP show enrichment of precipitated protein by western blot. HC and LC are antibody heavy and light chains from the immunoprecipitation. Right panels for each RIP are reverse transcriptase PCR products after 35 cycles. Both *gapdh* mRNA and *hsp70* mRNA co-precipitate with PABP, but only *hsp70* mRNA co-precipitates with HuR. 18S is a loading control.

4.3.2 Nuclear and Cytoplasmic Fractionation

To study whether HuR facilitates the nuclear export of *hsp70* mRNA, nuclear and cytoplasmic fractions of CA1 and CA3 were generated for NIC and 8hR animals. Microdissected regions are shown in Figure 9A. Cortex HSP70 expression was used to validate brain I/R injury for all 8hR samples; NICs did not show appreciable HSP70 expression on western blot.

Subcellular fractionation was validated with GAPDH and Lamin B1, markers for the cytoplasmic and nuclear fractions respectively (Figure 9B). Both markers were present in samples of homogenate prior to fractionation. Nuclear fractions contained only Lamin B1 and cytoplasmic fractions contained only GAPDH. The I/R injury did not affect the fractionation based on these markers.

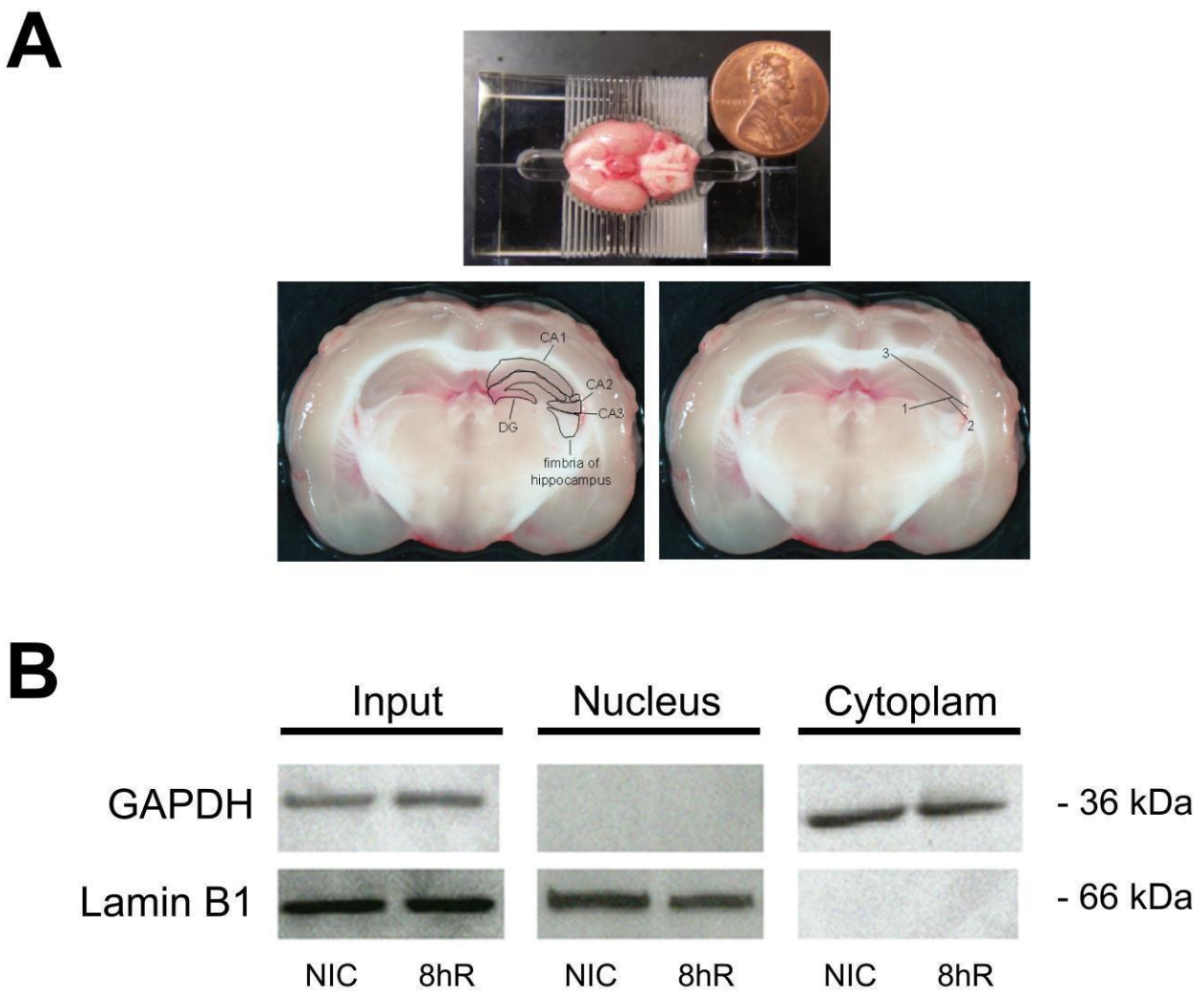

Figure 9: Nucleocytoplasmic fractioning method and controls. A) Example of CA1 and CA3 microdissection from the dorsal hippocampus showing hippocampal subregions and cuts made in the tissue. B) Western blots of NIC and 8hR cortex staining for nuclear and cytoplasmic fraction markers.

4.3.2 HuR Does Not Exit the Nucleus During Global Brain I/R

HuR was quantified in cytoplasmic and nuclear fractions and unfractionated homogenate from microdissected CA1 and CA3 regions. Consistent with previous microscopy work in the DeGracia lab and published reports of other laboratories[144,146], HuR was predominantly nuclear in both NIC and 8hR groups (Figure 10B). Cytoplasmic HuR was 2-3-fold greater in CA1 compared to CA3 ($p<0.05$).

However, the amount cytoplasmic HuR did not change in response to 1/R injury in any region or fraction, indicating that HuR does not exit from the nucleus in response to 1/R injury.

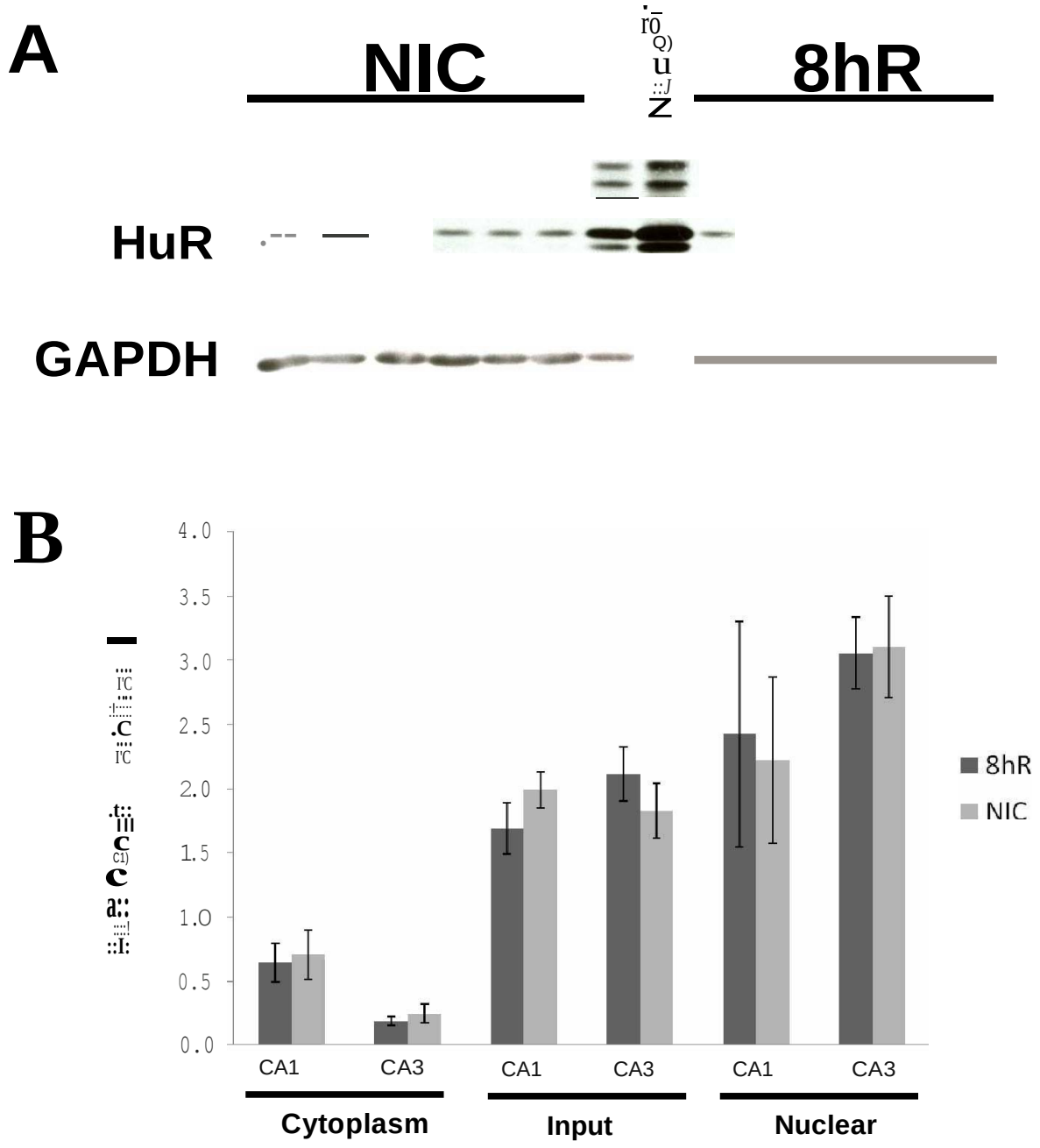

Figure 10: HuR in subcellular fractions. A) Western blot measuring HuR in cytoplasmic fractions from CAl of 6 NIC and 6 8hR animals. NIC unfractionated homogenate and nuclear fmctions were loaded as controls. Similar blots were gene1-ated for unfractionated homogenate and nuclear fractions and for the CA3 region. B) Densitometry for HuR in subcellular fractions. HuR values were normalized to GAPDH for input and

cytoplasmic fractions or silver staining for nuclear fractions. Each column represents 6 samples; error bars are standard deviation, two-tailed t-test p>0.05 between NIC and 8hR for all regions and fractions.

4.3.3 8hR CA1 and CA3 have the same Distribution of Nuclear and Cytoplasmic *hsp70* mRNA

Levels of *hsp70* mRNA were also assessed in each region and fraction by qPCR (Figure 11). *hsp70* in unfractionated homogenate was increased roughly 26-fold in both regions at 8hR with no significant differences between CA1 and CA3. In the cytoplasm, *hsp70* was increased between 200 and 500-fold at 8hR, values consistent with previous cytoplasmic fractions in the 2VO/HT model[65]. Again, there was no significant difference in the amount of *hsp70* mRNA between CA1 and CA3.

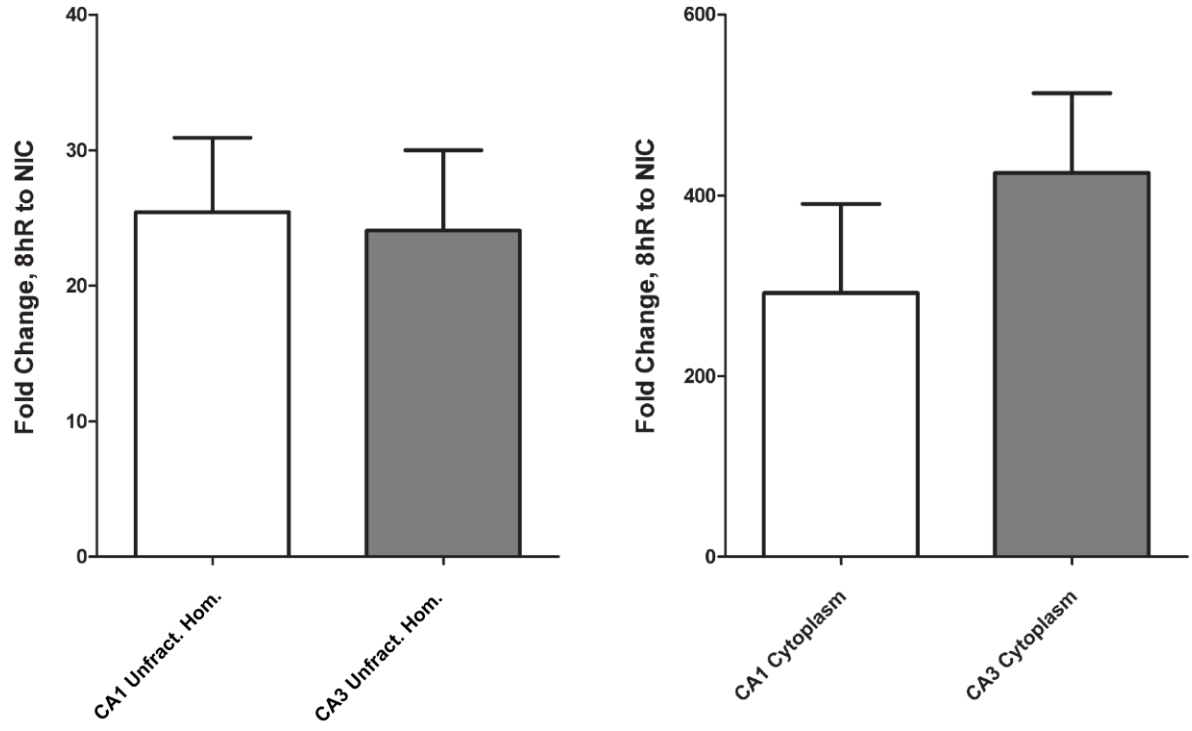

Figure 11: Real time quantitative PCR for *hsp70* in unfractionated homogenate (Unfract. Hom.) and cytoplasmic fractions of CA1 and CA3. Fold change of *hsp70* mRNA from NIC to 8hR in unfractionated homogenate (left) and cytoplasmic (right) fractions from CA1 and CA3. Both unfractionated homogenate and cytoplasmic fold change of *hsp70* were identical for CA1 and CA3 by two-tailed t-test.

Calculation of relative differences by $2^{-\Delta\Delta CT}$ assumes that the increase in amplicon per cycle, the PCR efficiency, is the same for each PCR target. Values for efficiency range from 1 (no efficiency) to 2 (perfect efficiency). Efficiency was quantified using the slope of the log-fold dilutions of each primer from input fraction of a 8hR animal (Figure 12). Efficiencies were 1.8 for both primer sets within error.

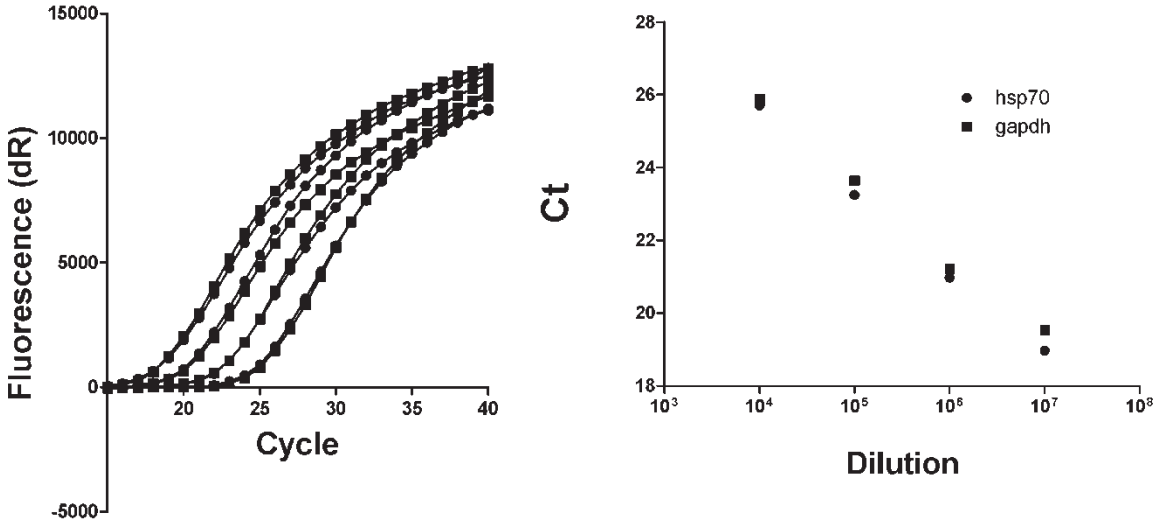

Figure 12: Example of efficiency experiments for *hsp70* and *gapdh*. Amplification curves show linear changes in Ct at logarithmic dilutions (left). Dilution of cDNA (logarithmic) versus CT (right) shows efficiencies of 1.81 (SD 0.08) for *hsp70* and 1.79 (SD 0.07) for *gapdh*.

These studies show that there is no significant difference in the nuclear export of either HuR protein or *hsp70* mRNA between CA1 and CA3 neurons at 8hR after 10 minutes of global brain ischemia.

Chapter 5 – HuR Interactions with the Polysome

5.1 Introduction

The work described in Chapter 4 shows that HuR interacts specifically with *hsp70* mRNA in reperfused neurons, but does not export *hsp70* mRNA out of the nucleus. A second mechanism by which HuR could regulate *hsp70* is by directly enhancing translation through association with eIF4A[7]. Polysome profiles present a method to assess whether HuR increases its association with polysomes during reperfusion. Eight hours reperfusion was again selected as the time point for these studies because rats are known to be actively translating *hsp70* mRNA into protein in CA3 and throughout the cortex at 8hR.

5.1.1 Hypothesis

HuR association with polysomes increases during reperfusion after brain ischemia.

5.2 Methods

5.2.1 Experimental Groups

Animal model was identical to previous chapter. Experimental groups were NIC, n=4 and 8hR, n=4. All tissue was homogenized as whole cortex.

5.2.2 Tissue Processing

Whole cortex from either 8hR animals expressing HSP70 or NICs was homogenized at 1:2 (w/v) in a 7-mL dounce homogenizer in cold lysis buffer (340 mM sucrose, 50 mM tris pH 7.4, 25 mM NaCl, 5 mM $MgCl_2$, 100 µg/mL CHX, 5.2 µL/mL protease inhibitor cocktail, 1 mM DTT, 80 U/mL RNase inhibitor, 1% (v/v) Triton X-100). Methods to control RNase activity were as described in Chapter 4, section 4.2.2.

After 20 strokes with the dounce homogenizer, homogenates were centrifuged 1000 *g* for ten minutes at 4 ^{o}C to pellet nuclei and membranes. Supernatants were then centrifuged 10,000 *g* for 30 minutes at 4 ^{o}C to generate a post-mitochondrial supernatant (PMS).

5.2.3 Polysome Profiles

Polysome profiles were generated based on the methods of MacManus, *et al.*[119] and DeGracia, *et al*[181]. Gradient and homogenate preparation and polysome profile centrifugation were performed on ice or at 4 ^{o}C. Sucrose gradients were prepared by layering sucrose concentrations of 15%, 20%, 25%, 30%, 35%, 40%, and 45% (w/v) sucrose dissolved in gradient buffer (50 mM tris pH 7.4, 25 mM NaCl, 5 mM $MgCl_2$, 100 µg/mL CHX, 5.2 µL/mL protease inhibitor cocktail, 1 mM DTT, 80 U/mL RNase inhibitor) and allowing layers to equilibrate overnight.

Spectrophotometry of PMS was taken at 254 nm and 75 A_{254} units were layered on top of each 11.5-mL sucrose gradient. Sucrose gradient was centrifuged at 240,000 *g* (37500 rpm) for three hours in a Beckman SW41Ti swinging bucket rotor at 4 ^{o}C.

After centrifugation, each gradient was placed in a Beckman fraction recovery stand and tube bottom was pierced. 66% (w/w) sucrose was pumped via peristaltic pump at 1-mL per minute into the bottom of the tube to displace the gradient. Eluent was run through a spectrophotometer photocell measuring A_{254} and then into a fraction collector. Gradients were fractionated into 15 equal volumes of 800 µL and absorbance at 254 nm was continuously recorded with a strip chart recorder advancing at 0.5 mm per second.

For graded input controls, 25, 50, or 75 A_{254} units of NIC cortex were loaded onto gradients identical to above. For CHX control, tissue was homogenized in the presence of 15 mM EDTA. Gradient buffer also had 15 mM EDTA and did not have Mg^{2+}.

5.2.4 Western Blotting

To study protein concentrations of proteins in each fraction of the polysome profile, whole fractions were concentrated by ultrafiltration with a 3 kDa cutoff device (Amicon) and all protein from each fraction was loaded onto one lane of an SDS-PAGE gel. Western blotting and HuR antibody conditions were as described in Chapter 4. Other antibody conditions were: mouse anti-S6 2317S (Cell Signaling) 1:250 in 5% dry milk incubated overnight at 4 ^{o}C, rabbit anti-PABP 21060 (Abcam) 1:500 without blocking incubated overnight at 4 ^{o}C, rabbit anit-L7a 2403 (Cell Signaling) 1:1000 in 5% dry milk incubated overnight at 4 ^{o}C.

For densitometry, raw band densities for each fraction were scaled by average density across the gradient. Scaling allowed comparison of values across gradients. For each fraction, relative density of HuR was compared between NIC and 8hR groups by two tailed t-test with a significance of $p<0.05$.

5.3 Results

5.3.1 Polysome Profile Controls

Traces of A_{254} along the density gradient had similar forms between NIC and 8hR groups (Figure 13). 8hR animals showed peaks similar in height and area to NICs. To detect polysome-containing fractions, all fractions were western blotted for polysome-associated markers (Figure 13). Small ribosomal subunit protein S6 and large ribosomal subunit protein L7a both concentrated in fractions 8-11. PABP concentrated at the low-density end of the gradient, consistent with previous studies[182,183].

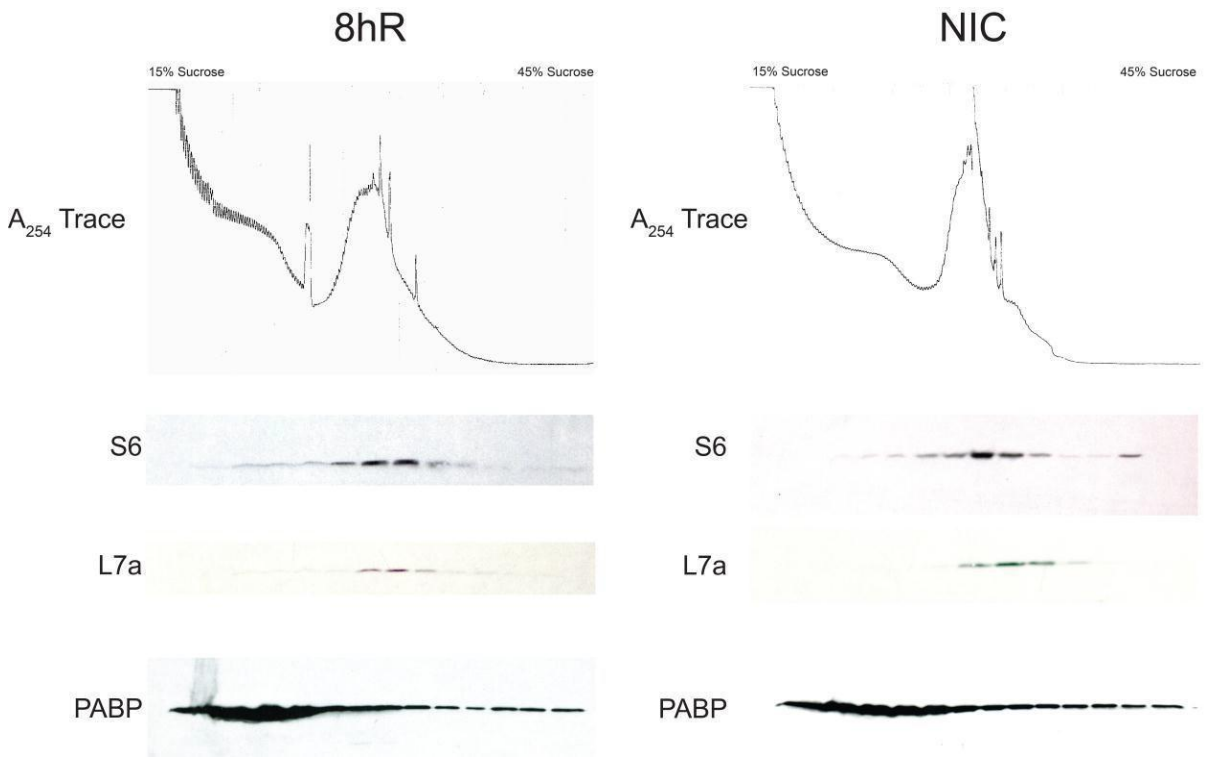

Figure 13: Polysome profile control data from 8hR (left) and NIC (right) cortex. Polysome profile A_{254} traces are similar between conditions. Polysome profiles were divided into 15 fractions for western blotting. S6 and L7a markers of the small and large ribosomal subunit, respectively, concentrate in polysome enriched fractions. PABP concentrated in less dense fractions.

Other controls for polysome profiles included graded increase in polysome peak with sample loaded (Figure 14A), and loss of polysome peak with addition of EDTA (Figure 4B) which chelates Mg^{2+} needed for polysome formation[184].

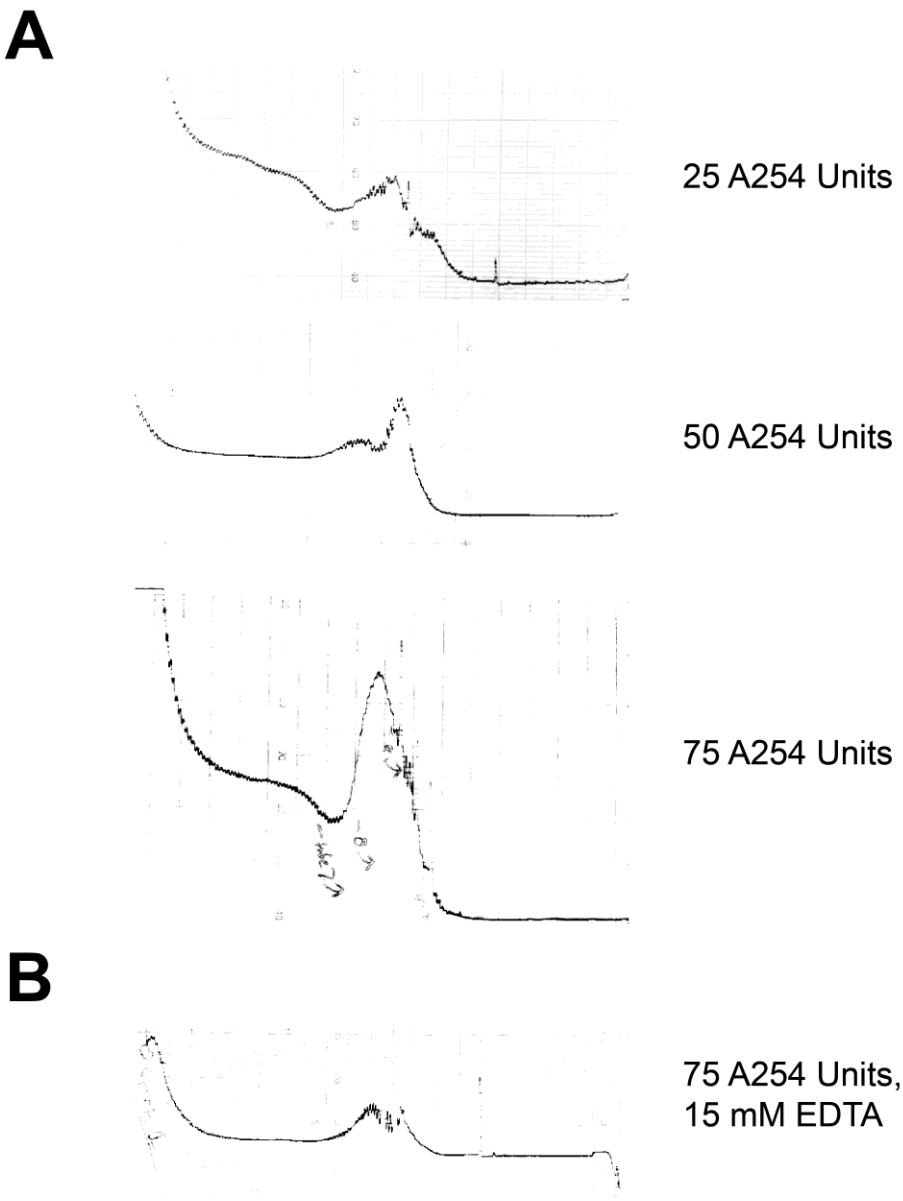

Figure 14: NIC input and EDTA controls. A) Gradients with 25, 50, and 75 A_{254} units showing polysome peak increases with input. B) 15 mM EDTA abolishes polysome peak from 75 NIC A_{254} units.

To assess HuR association with polysomes, densitometry of 8hR and NIC cortex polysome profiles was performed. Densitometry confirmed that HuR distributed evenly across densities relative to polysome-associated or free proteins (Figure 15B). At each fraction, NIC and 8hR HuR density was compared by two-tailed t-test, and there were no significant differences for any fraction.

This finding suggests that HuR does not preferentially associate with polysomes at 8hR.

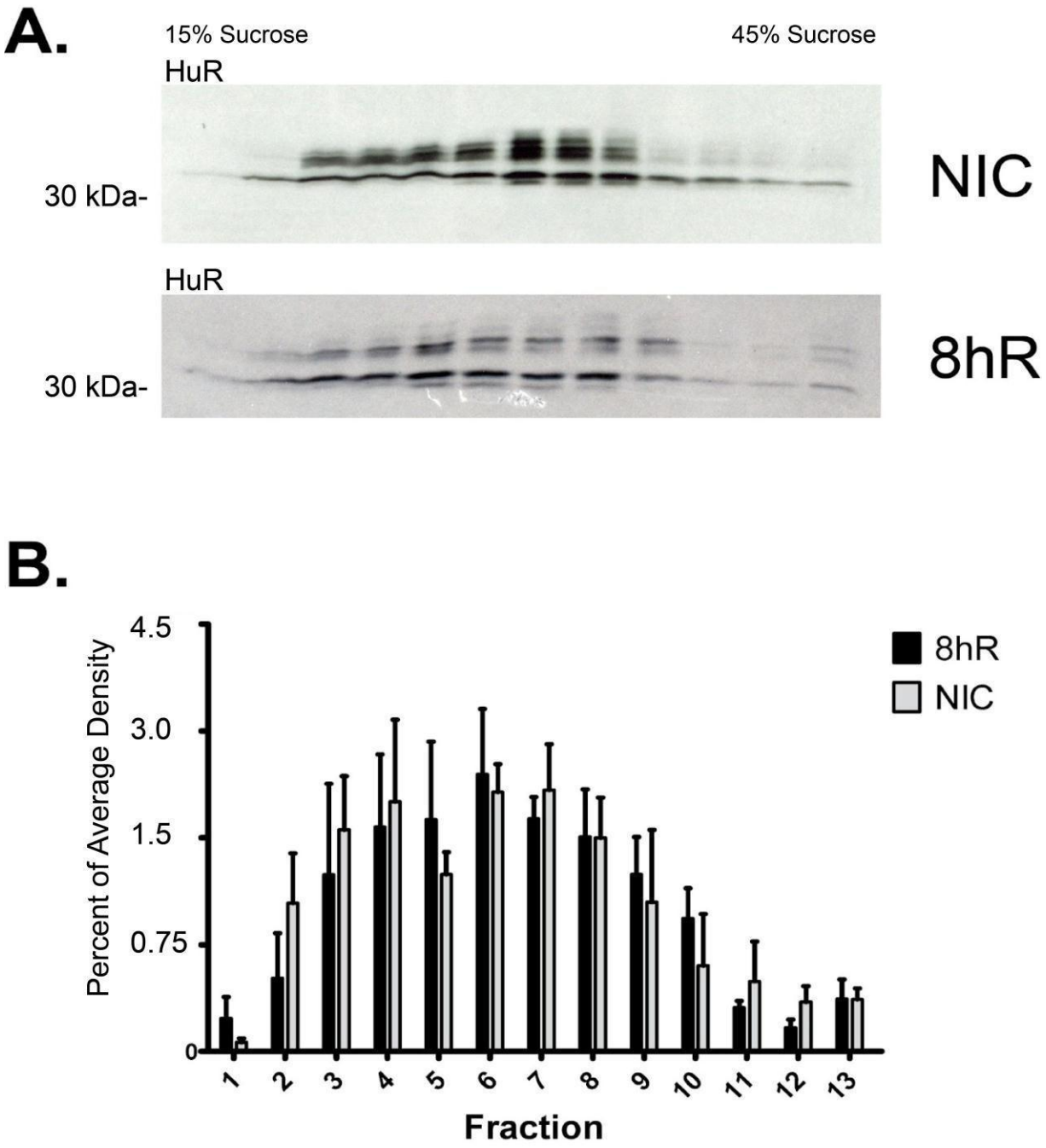

Figure 15: HuR association with polysomes. A) Western blots of HuR (darkest band) in fractions from NIC and 8hR cortex polysomes. B) Densitometry for 4 NIC and 4 8hR cortex polysome profiles expressed as percent of average density of each gradient. Error bars are standard deviation. No differences between NIC and 8hR HuR in any fraction cleared $p<0.05$ t-test.

Chapter 6 - Brain I/R Transcriptomics

6.1 Introduction

In order to recover from I/R injury, neurons need to translate newly-transcribed mRNAs encoding stress response proteins. Although there have been several global[185–187] and many focal[150,152,188–196] microarray studies of reperfused brain, all previous studies suffer from major limitations in measuring the post-ischemic transcriptional program. Many well-designed brain I/R microarray studies were conducted prior to advancements in array technology and genome mapping, making the number of transcripts measured a small fraction of the whole genome. More recently, it has been appreciated that steady-state levels of mRNA and protein are only weakly correlated[153,155], severely limiting the ability of microarrays to measure actively translating transcripts. The loose correlation in mRNA and protein steady states is due, in part, to posttranscriptional RNA regulatory mechanisms. Through specific mRBP interactions, stress response transcript translation can be significantly altered over short time frames in stressed cells[128]. In particular during reperfusion after brain ischemia, stress-response transcripts are able to escape the global suppression of translation[149]. The functional effects of mRBPs and translation suppression are not detectable by microarray of whole-cell RNA.

Translation state analysis can identify upregulated transcripts which localize to the polysome[197]. MacManus, *et al.* performed the only translation state analysis of brain tissue after I/R injury. This study used a mouse focal ischemia model to compare lesioned and unlesioned hemispheres of the brain. While MacManus made an important finding that only 36% of upregulated transcripts were bound to polysomes, use of a focal model limits the interpretation of this result. I/R damage and induction of stress responses are not constant throughout a focal lesion[198]. In fact, genes produced by inflammatory cells in the necrotic core of a focal lesion can be directly antithetical to those expressed in recovering penumbra[199].

Selectively vulnerable CA1 dies by DND while adjacent CA3 neurons survive. This dichotomous outcome is a useful model to study the intrinsic ability of neurons to recover from I/R injury. By studying the CA1 and CA3 regions of the hippocampus in the transient global ischemia model, researchers have shown conclusively that persistent suppression of translation correlates to selective vulnerability. Yet no study has yet compared the CA1 and CA3 regions in global brain ischemia by microarray.

This chapter investigated both the differences between polysome-bound and unbound transcripts and between the CA1 and CA3 regions.

6.1.1 Hypothesis

There are quantifiable differences in the mRNA populations bound to polysomes in CA3 and CA1 neurons after brain I/R.

6.2 Methods

6.2.1 Animal model

The 2VO/HT model was performed exactly as described in Chapter 3. Experimental groups used in this chapter were: NIC (n = 15) and 8hR (n=15). For the purpose of validating the integrity of RNA extraction from polysome profiles, additional NIC (n=5) and 8hR (n=5) animals were performed.

6.2.2 Microdissection

CA1 and CA3 were microdissected exactly as described in Chapter 4.

6.2.3 Tissue processing

Homogenates for microdissected CA1 and CA3 were prepared as described in Chapter 5, section 5.2.2. For rats used to assess integrity of polysome-extracted RNA, whole forebrain homogenates were prepared. Whole forebrain consisted of bisecting the brain coronally at the level of the superior colliculus and taking all structures anterior, including the diencephalon. Whole forebrain was homogenized at 1:2 (w/v) the same buffer used to homogenize CA1 and CA3. Homogenates of CA1, CA3, and whole forebrain were processed exactly as described in Chapter 5, section 5.2.2 to produce PMS fractions that served as input to polysome profiles.

6.2.4 Pooling of microdissected regions

Polysome profiles required loading 75 A_{254} units of PMS to isolate RNA. Microdissection of as single, bilateral CA1 or CA3, in its entirety, provided 20 A_{254} units of PMS. Therefore, to achieve the required quantities for polysome profiles, five homogenates per hippocampal region per experimental group were randomly pooled to generate a single pooled sample. Thus, the four main experimental groups: (1) NIC CA1, (2) NIC CA3, (3) 8hR CA1, and (4) 8hR CA3 each consisted of three pooled samples, where each pooled sample contained 5 pooled homogenates from individual animals (Figure 16A).

Because of the pooling requirements, pilot studies to assess RNA extraction quality were performed on whole forebrain homogenates, where each animal provided enough material to run a single polysome profile (Figure 16B).

6.2.5 Polysome profiles

Polysome profiles of pooled hippocampal regions and forebrain samples were run exactly as described in Chapter 5, section 5.2.3. The data shown in Chapter 5, section 5.3.1 established that gradient fractions 8-11 contain the polysome peaks that concentrated 40S and 60S markers. These fractions were

pooled and constituted the polysome-bound fractions (bound, B). The lower density fractions, 1-6, were also pooled, and these constituted the polysome-unbound fractions (unbound, U) (Figure 16).

6.2.6 RNA extraction.

RNA was extracted from B and U fractions using the same protocol as described in Chapter 4. Ethanol washed RNA pellets, resuspended in sterile water, were shipped overnight on dry ice to Genome Explorations (Memphis, Tennessee) who performed microarray hybridization as described below. An aliquot of resuspended RNA pellets was used to measure RNA concentration by A_{260} and estimate RNA purity by A_{260}/A_{280} ratio.

6.2.7 Summary of Experimental Design

There were a total of 24 samples analyzed by microarray. The overall design of the microarray studies is illustrated in Figure 16. Five samples from forebrain were used for pilot studies on RNA extraction from polysome profiles; the treatments of these samples is shown in Figure 16B.

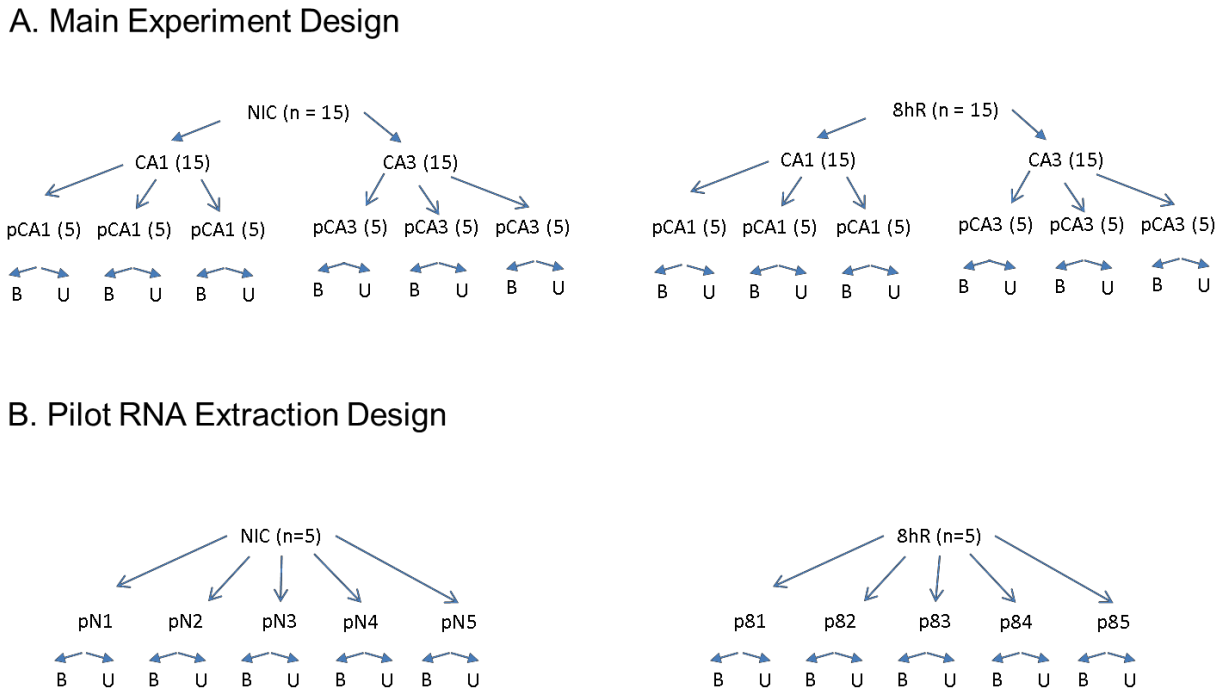

Figure 16: Design of microarray experiments. A) Design of main experiment comparing CA1 and CA3. Each polysome (p) contained tissue from 5 animals, pooled randomly. RNA was isolated from polysome bound (B) and unbound (U) fractions of each polysome profile. B) Design of pilot study for RNA extraction from polysome profiles. Each polysome contained tissue from a single forebrain.

6.2.8 Performance of Microarrays

The following procedures A-C were carried out by Genome Explorations.

A. RNA quality control.

Immediately prior to cDNA synthesis, the purity and concentration of RNA samples were determined from $OD_{260/280}$ readings using a dual beam UV spectrophotometer and RNA integrity was determined by capillary electrophoresis using the RNA 6000 Nano Lab-on-a-Chip kit and the Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) as per the manufacturer's instructions.

B. cDNA synthesis and labeling.

RNA (5-25 ng each sample) was converted to cDNA, amplified, fragmented, and labeled with biotin using the Ovation Pico WTA, Ovation Exon Module, and Encore Biotin Module kits according to the manufacturer's instructions (NuGEN, San Carlos CA).

C. Oligonucleotide array hybridization and analysis.

Fragmented, biotin-labeled cDNA was hybridized for 17hr at 45 ^{o}C to GeneChip® Rat Gene 1.0 ST Arrays (Affymetrix, Santa Clara CA). The Rat Gene 1.0 ST array is a perfect-match-only array comprised of 722,254 unique 25-mer oligonucleotide features representing transcripts from roughly 27,000 rat genes with each gene represented on the array by approximately 27 probes spread across the full length of its transcript[200]. Arrays were washed and stained with phycoerythrin -conjugated streptavidin (Life Technologies, Carlsbad, CA) in a Fluidics Station 450 (Affymetrix) according to the manufacturer's recommended procedures. Fluorescence intensities were determined using a GCS 3000 7G high-resolution confocal laser scanner, and analyzed using programs resident in the Affymetrix GeneChip Operating Software suite, v.3.2. (GCOS; Affymetrix). GCOS quality control outputs included average expression across arrays (ALE) box plots, relative level of expression (RLE) box plots , and positive vs. negative area under curve (AUC) values, and were used to identify potential outlier arrays. Outlier evaluation was also performed by principal component analysis in GeneMaths XT (Applied Maths, Austin TX).

Data Output

Genome Explorations provided the resulting data files generated by the Affymetrix software. These included raw 16-bit pixel intensity image files (.DAT), and probe intensity files (.CEL). All subsequent analyses were performed in the DeGracia laboratory using the data pipeline described next.

Data Analysis

Background correction by the PM-CGBG method and probe set signal summarization using the PLIER algorithm[201,202] was performed in Affymetrix Expression Console[203]. Quantile normalization[204], filtering, hierarchical clustering, and class comparisons to identify probe sets exhibiting significant differential expression were performed using BRB-ArrayTools v.4.2.1 developed by Dr. Richard Simon and the BRB-ArrayTools Development Team. BRB-ArrayTools is a microarray analysis plug-in for Microsoft Excel, freely distributed by the National Cancer Institute at the NIH. The filter criteria used for class comparisons was log intensity variation $\geq 75^{th}$ percentile. Application of this filter reduced the initial 26,309 genes to a set of 6,578 genes. Hierarchical clustering was by Pearson correlation with distances calculated by complete linkage.

The following class comparisons were performed on the 6,578 gene set:

(1) NIC CA1 bound (N1B) vs. 8hR CA1 bound (R1B),

(2) NIC CA3 bound (N3B) vs. 8hR CA3 bound (R3B),

(3) NIC CA1 unbound (N1U) vs. 8hR CA1 unbound (R1U),

(4) NIC CA3 unbound (N3U) vs. 8hR CA3 unbound (R3U),

using an absolute signal fold change ≥ 2, and significance threshold $p < 0.01$ on univariate two-tailed t-test.

Results were expressed as: (1) Tables of significant hits, including p values, false discovery rates (FDR), fold-change, probeset ID, Entrez ID and gene name, and (2) Venn diagrams.

Gene Ontology Analysis

Gene annotation, gene ontology information, and biochemical pathway information were obtained from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov), NetAffx

(ww.affymetrix.com), the Gene Ontology Consortium (http://amigo.geneontology.org), and WebGestalt (http://bioinfo.vanderbilt.edu/webgestalt). Enrichment of gene ontology (GO) categories in each comparison was estimated by hypergeometric distribution using GO TermFinder[205]. A biological process term was considered enriched if the number of genes annotated by the GO term in a list was greater than chance with a significance greater than $p<0.01$, Bonferroni-corrected.

ARE database search

Differentially expressed transcripts were tested for the presence of ARE sites using the AU-Rich element containing database (ARED) (http://brp.kfshrc.edu.sa/ARED/)[206]. ARED is an assembly of all mRNA and 3' expressed sequence tags (ESTs) in the GenBank and EMBL sequences databases, screened for the ARE consensus sequence, WWWU(AUUUA)UUUW, allowing for one mismatch. Gene lists for I/R-upregulated, polysome-bound transcripts were tested in ARED and lists of positive hits recorded.

IRES database search

There is no consensus IRES site for eukaryotic transcripts expressed by cap-independent translation, so these sites are currently determined experimentally. A database of experimentally determined IRES sites (IRESite) is maintained by Charles University in Prague (http://iresite.org/)[207]. Gene lists for I/R-upregulated, polysome-bound transcripts were tested in IRESite and lists of positive hits recorded.

Transcription factor database search

Transcription factor binding site enrichment for was tested in MAtInspector (genomatix)[208]. MatInspector uses a large library of matrix descriptions for transcription factor binding sites to locate matches in DNA sequences. MatInspector assigns a quality rating to matches by aligning them to a weighted probability matrix for each transcription factor in its database. Each position in the matrix is

assigned a score between 0 and 100 for each possible nucleotide. Transcription factor binding is measured as a likelihood of achieving a given score, compared to a random sequence.

6.3 Results

6.3.1 Polysome fractions are enriched in rRNA and translating mRNAs

To assess the integrity of polysome profile RNA, rRNA and transcribing mRNAs were measured across density gradients generated from whole forebrain (Figure 17). Both 8hR and NIC samples showed good RNA integrity for all fractions by rRNA in by denaturing electrophoresis. 18S and 28S rRNA bands concentrated in fractions enriched in polysomes while small nucleotides concentrated at low density. Polysome-enriched fractions of NIC were able to generate *gapdh* product in RT-PCR. 8hR samples are known to be translating large amounts of *hsp70* mRNA. The 8hR polysome-enriched fractions concentrated *hsp70* PCR product, again validating the quality of the isolated RNA.

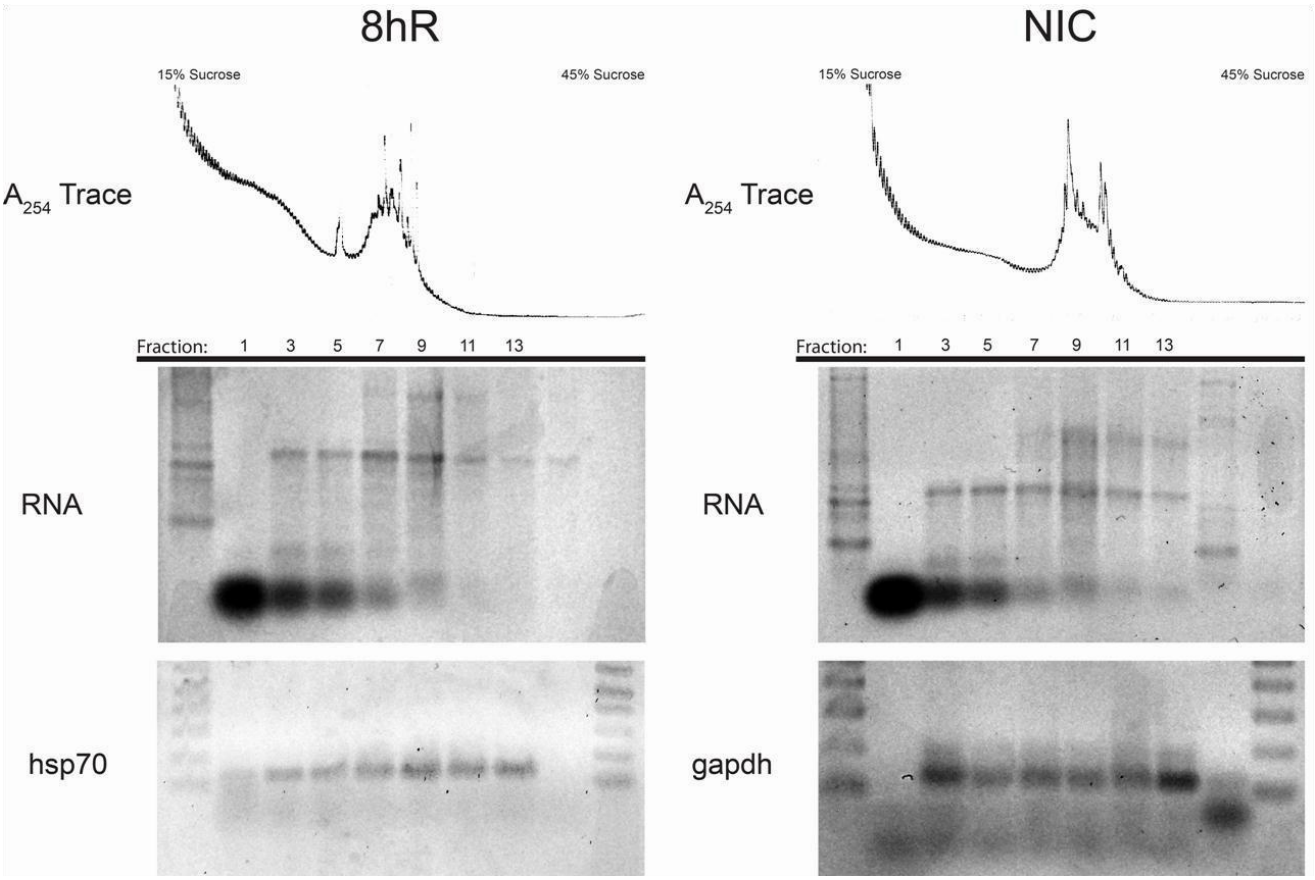

Figure 17: Figure: Polysome profiles for translation state analysis. Representative polysome profile A_{254} traces for 8hR (left) and NIC (right) cortex. Whole RNA from each gradient fraction was isolated by TRIzol. RNA from odd numbered fractions was separated by denaturing agarose electrophoresis. (Agarose gel images inverted for clear viewing.) Samples of 2 µg RNA from odd numbered fractions were input for reverse transcriptase PCR amplifying primers for either *hsp70* (8hR) or *gapdh* (NIC) mRNA. Shown are primer products after 25 cycles on 1.2% agarose.

6.3.2 Microarray Validation

All arrays were normalized by quantile normalization where the values of each array are transformed so that all arrays have the same distribution of transformed probe intensities. Normalization was confirmed by comparing probe intensity box plots before and after quantile normalization (Figure 18).

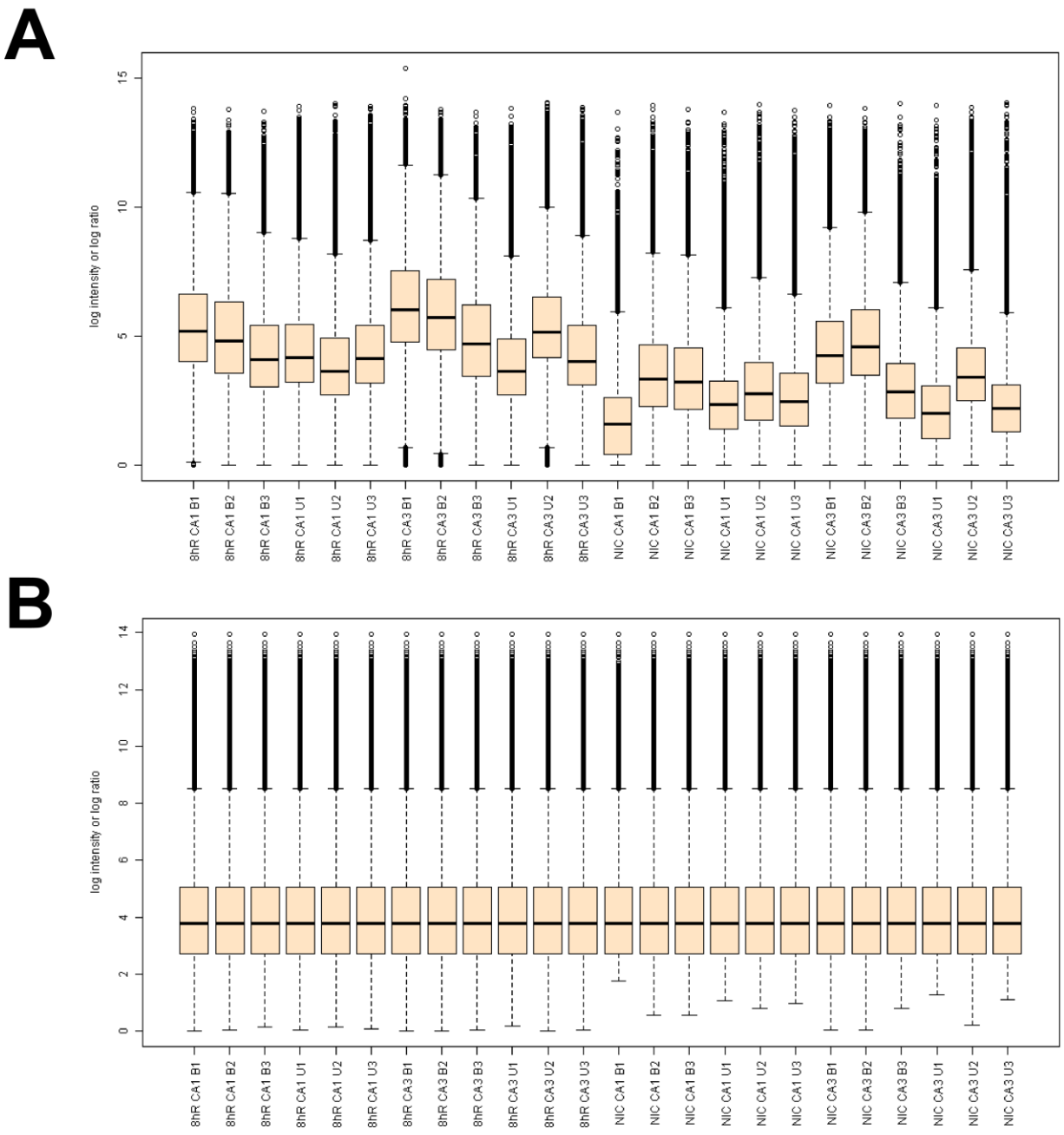

Figure 18: Box plots of probe set intensity values for each microarray expressed as $\log_2$ intensity. A) Probe set intensity values before normalization. B) Probe set intensity values after normalization.

Sample groups were first assessed by hierarchical clustering of genes (Figure 19). Genes with the top ¼ of log intensity variation were used in the clustering analysis. Clustering arranges genes according their similarity of expression by a Pearson correlation. The more closely two genes are arranged in rows

of the heat map, the more similar their expression. Expression was different between bound and unbound fractions for each treatment group and region. Smaller differences are apparent between NIC and 8hR samples from the same fraction.

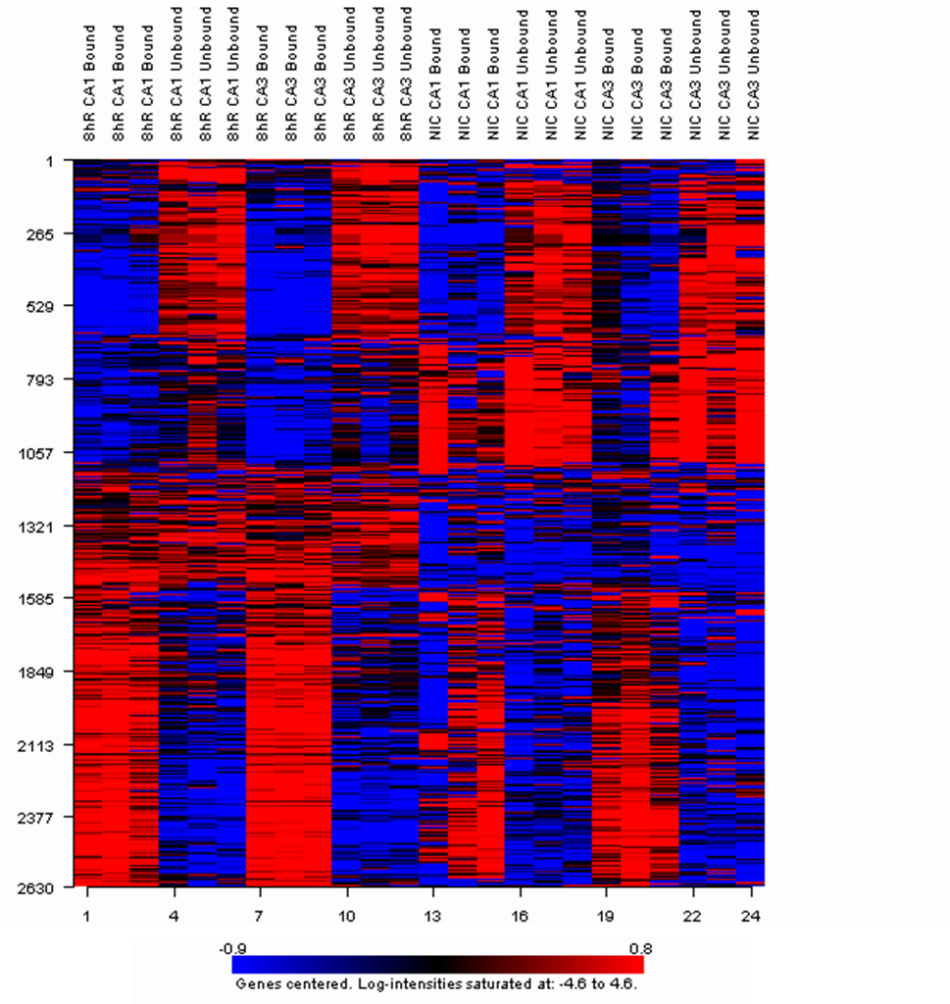

Figure 19: Hierarchical clustering of most differentially expressed 2630 genes. Microarrays in columns are grouped by sample sets. Distances were calculated by complete linkage. Scaled gene expression is shown in blue (downregulated) or red (upregulated) as indicated by the scale bar below the heat map.

Gene clustering in Figure 19 is unsupervised; microarrays were arranged to show the differences between polysome bound and unbound groups. Unsupervised clustering of both genes and samples still grouped all arrays into their respective sample groups (not shown).

A PCA plot of the first three principal components was generated for all samples (Figure 20). Consistent with the hierarchical clustering, microarrays clustered by sample group indicating that no

complete arrays were outliers. There is a sharp divide between polysome-bound (lower left, B) and unbound (upper right, U) transcripts for all groups.

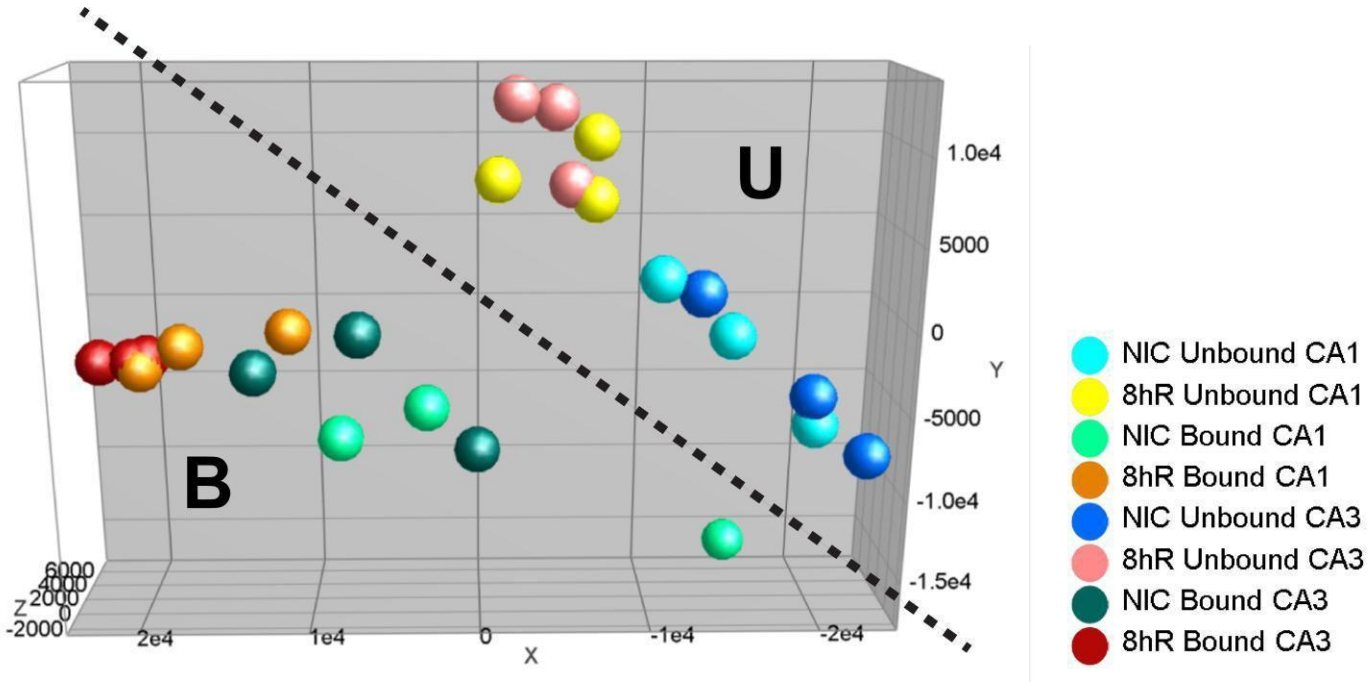

Figure 20: Principal component analysis of all microarrays and genes show the top three principal components as x, y and z axes. Polysome-bound transcripts (B) group to the lower left while unbound transcripts (U) group to the upper right.

6.3.5 Polysome-bound Subpopulations at 8hR

In each hippocampal region transcripts were then compared between the polysome-bound NIC and polysome-bound 8hR groups. Overall, the CA3 region had both more upregulated transcripts at 8hR and higher fold-changes over NIC when compared with CA1 (Figure 21A).

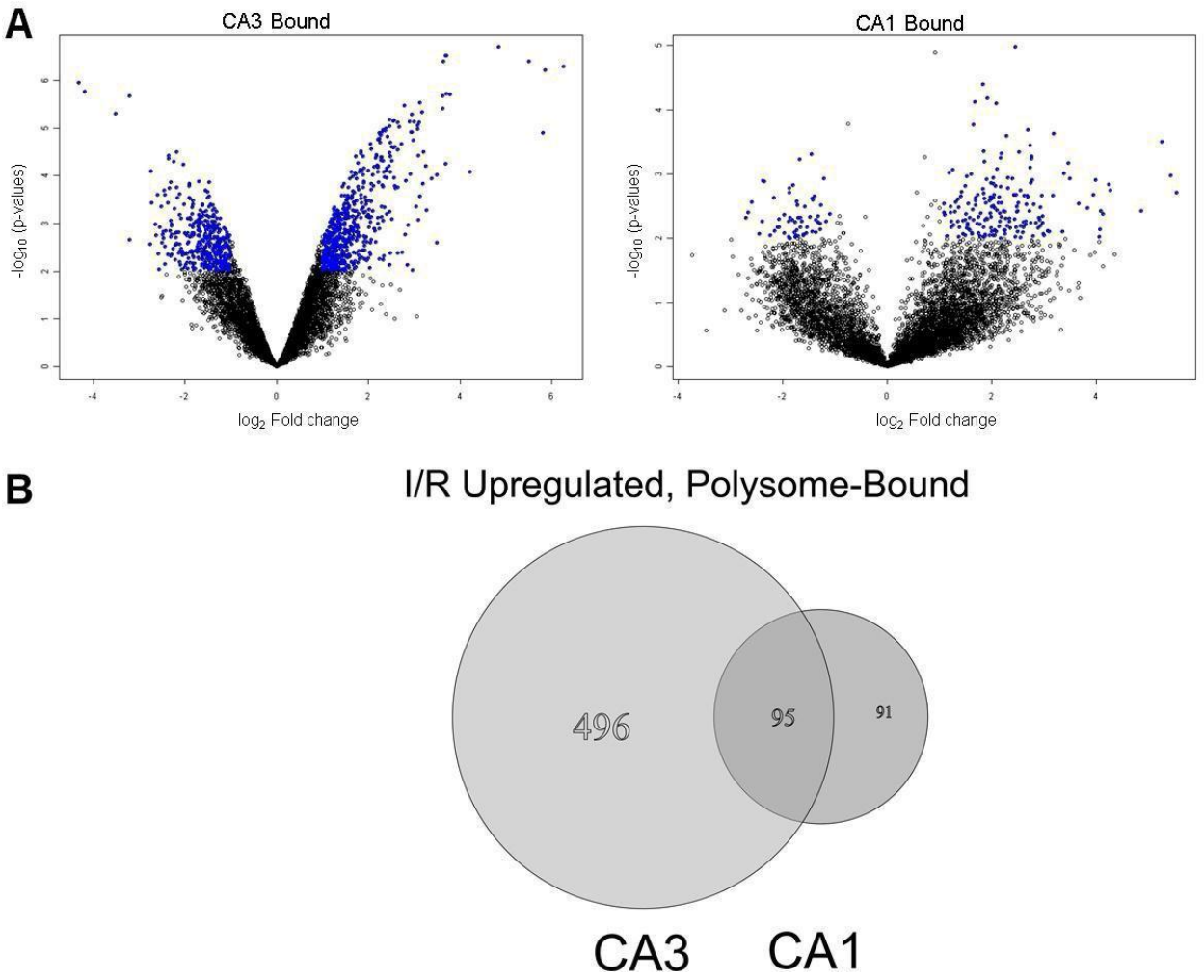

Figure 21: Expression of polysome-bound transcripts. A) Volcano plots of expression changes (Log_2 fold changes) on the x-axis and significance of two-tailed t-test (y-axis) comparing polysome-bound 8hR to NIC samples in CA3 (left) and CA1 (right). Hits above the significance threshold, p<0.01 are in blue. B) Distribution of I/R-induced, polysome-bound transcripts between CA1 and CA3 at p<0.01, >2-fold change. Values in Venn diagrams are for exclusive CA3 hits (left), exclusive CA1 hits (right) and overlapping hits (middle).

1005 transcripts in CA3 region and 247 transcripts in the CA1 region cleared thresholds. Overall, both regions had more upregulated than downregulated transcripts with 75% (185) of differentially expressed CA1 transcripts upregulated and 59% (592) of CA3 transcripts upregulated. Only 95 of the I/R-upregulated, polysome-bound transcripts (R/N-bound) were identical between CA3 and CA1 groups (17% of the CA3 group or 52% of the CA1 group) (Figure 21B).

6.3.6 Unbound Subpopulations at 8hR

Transcripts were then compared between unbound NIC and unbound 8hR groups. As with bound transcripts, the CA3 region had more upregulated transcripts at 8hR, but fold-changes were comparable between CA1 and CA3 (Figure 22A).

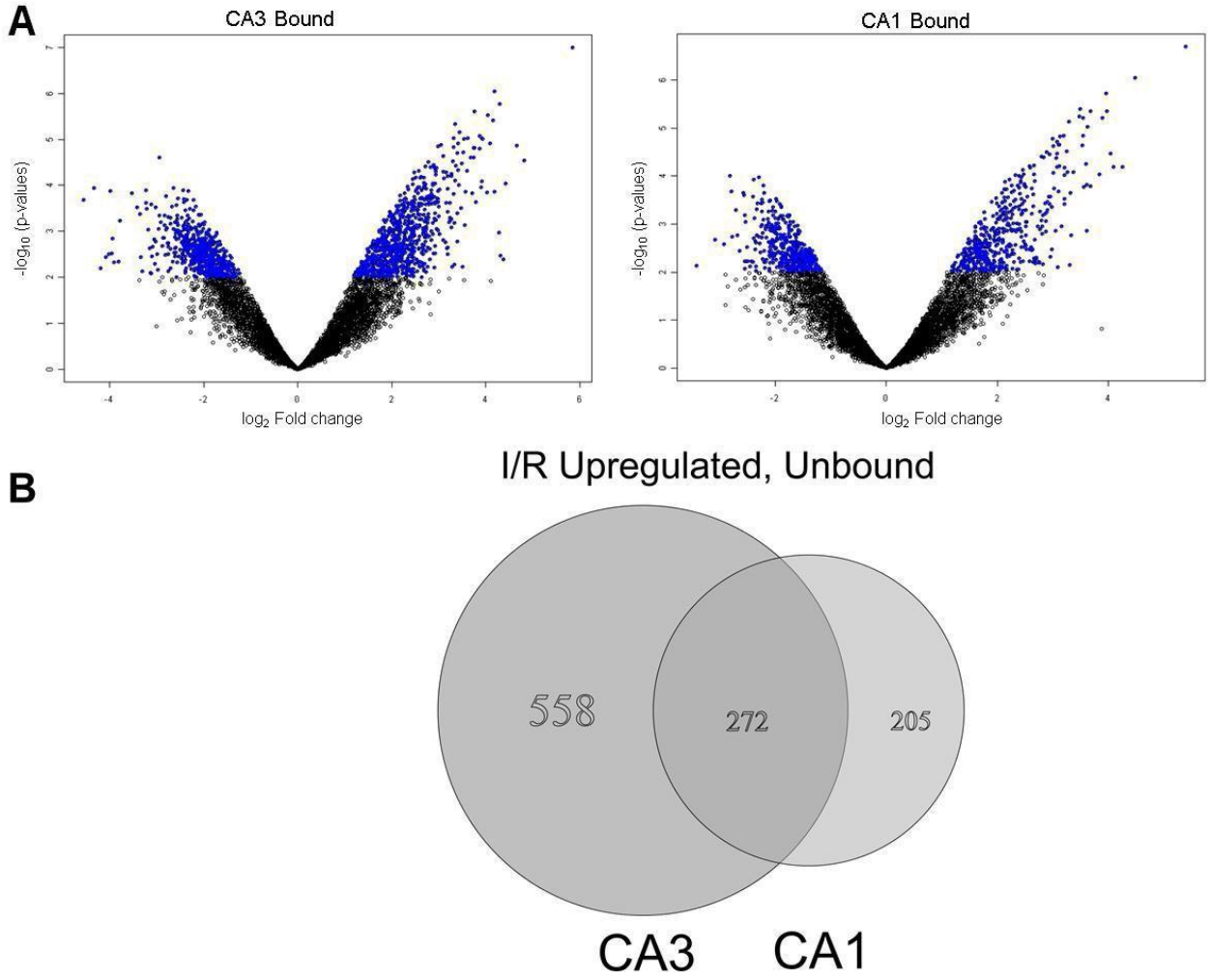

Figure 22: Expression of unbound transcripts. A) Volcano plots of expression changes (Log_2 fold changes) on the x-axis and significance of two-tailed t-test (y-axis) comparing unbound 8hR to NIC samples in CA3 (left) and CA1 (right). Hits above the significance threshold, p<0.01 are in blue. B) Distribution of I/R-induced, unbound transcripts between CA1 and CA3 at p<0.01, >2-fold change. Values in Venn diagrams are for exclusive CA3 hits (left), exclusive CA1 hits (right) and overlapping hits (middle).

1,429 transcripts in CA3 region and 926 transcripts in the CA1 region cleared thresholds. Less differentially regulated transcripts were upregulated relative to the bound groups. 51% (185) of differentially expressed CA1 transcripts upregulated and 58% (830) of CA3 transcripts upregulated. 272 of the I/R-upregulated, unbound transcripts (R/N-unbound) were identical between CA3 and CA1 groups

(32% of the CA3 group or 57% of the CA1 group) (Figure 22B). The percent of CA3 R/N-unbound transcripts shared by CA1 R/N-unbound (32%) is more than double the percent of CA3 R/N-bound transcripts shared by CA1 R/N-bound (17%).

6.3.7 GO for Biological Processes in R/N-bound Groups

Overall, both R/N-bound CA1 and CA3 were enriched in GO groups related to signaling, development, and stress response.

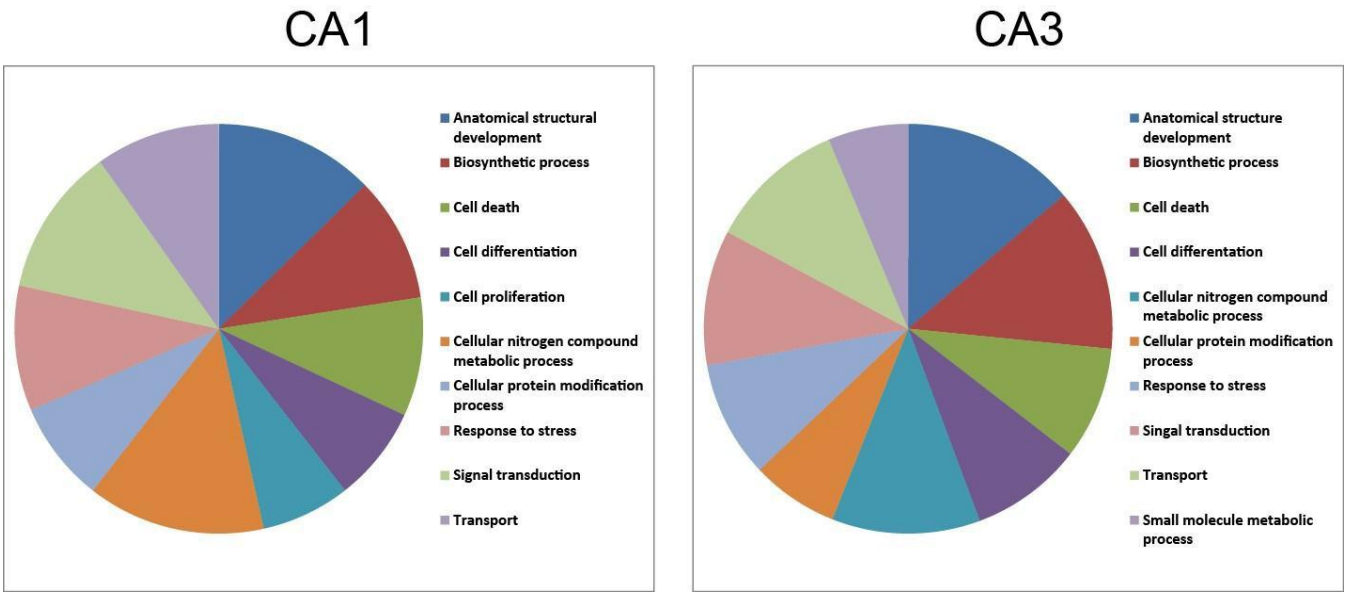

Figure 23: Biological process GO for R/N-bound groups. GO categories were considered upregulated if likelihood if the enrichment of transcripts in the GO category was p<0.01 by chance.

6.3.8 ARE Enrichment in R/N-bound and R/N-unbound Groups

By the relatively stringent standards of ARED, 1.92% of the rat transcriptome contains an ARE sequence. Both R/N-bound CA1 and CA3 were enriched in AREs. In R/N-bound CA1, 16 transcripts contained AREs (3 predicted) and in R/N-bound CA3, 39 transcripts contained AREs (9 predicted). These values correlate to a 5.3- fold enrichment in R/N-bound CA1 AREs and at 4.3- fold enrichment in R/N-bound CA3 AREs. 10 ARE-containing mRNAs were in both CA1 and CA3.

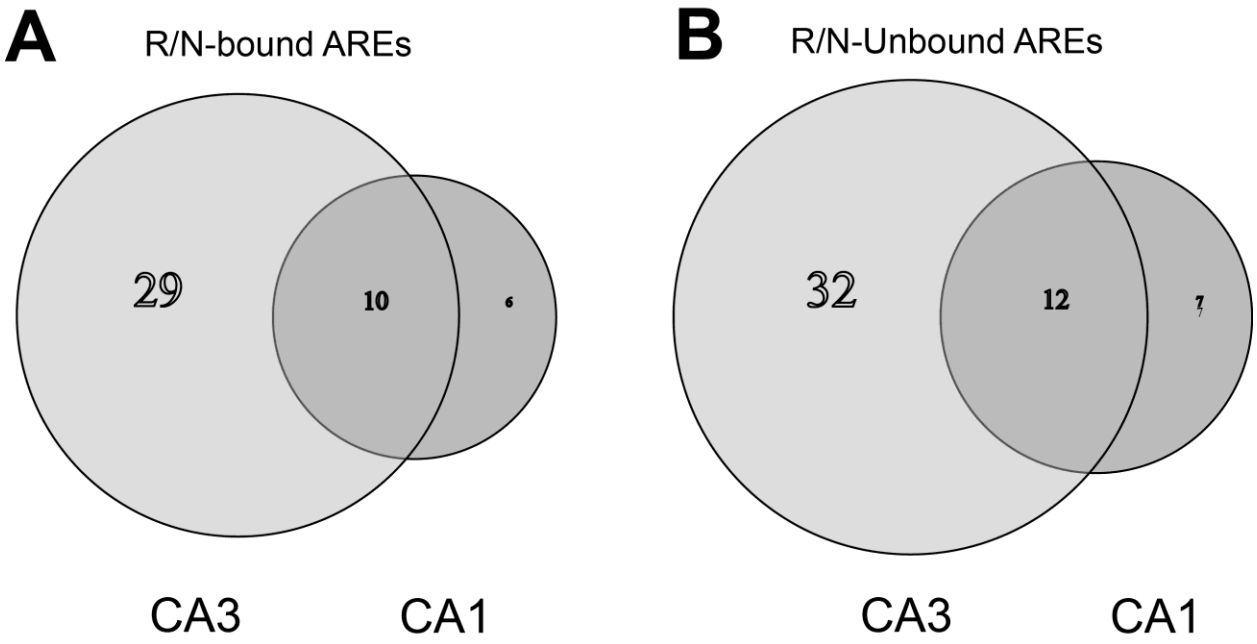

Figure 24: Upregulated R/N-bound (A) and R/N-unbound (B) transcripts were searched in the ARED database to determine number of upregulated transcripts in each list. Values in Venn diagrams are for exclusive CA3 hits (left), exclusive CA1 hits (right) and overlapping hits (middle).

R/N-unbound groups were also enriched in AREs. R/N-unbound CA3 had 44 ARE-containing transcripts (9 predicted, 5-fold increase) and R/N-unbound CA1 had 19 ARE-containing transcripts (4 predicted, 5-fold increase). Both R/N-bound CA1 and CA3 were enriched in AREs. In R/N-bound CA1, 26 transcripts contained AREs (4 predicted) and in R/N-bound CA3, 49 transcripts contained AREs (11 predicted).

6.3.9 Transcription Factor Binding Site Enrichment in R/N-bound Transcripts

Transcription factor binding site enrichment for the R/N-bound transcripts found in both CA3 and CA1 (overlap of Figure 24A) were tested in MAtInspector[208]. The ten most-enriched transcription factors are shown in Table 2.

Table 2: Top ten enriched transcription factors in MAtInspector.

Transcription Factor	*Number of Transcripts Found*	*Score (p<)*

SMTTTTGT motif (uncharacterized)	14	7 e-09
Heat Shock Factor 1	13	6 e-08
Nuclear Factor of Activated T Cells	24	8 e-08
TTCYRGAA motif (uncharacterized)	11	8 e-08
Heat Shock Factor 2	9	1 e-06
MYC	16	1 e-06
Heat Shock Factor 6	8	1 e-06
TATA Binding Protein	18	1 e-06
AACTTT motif (uncharacterized)	22	1 e-06
TTCNRGNNNNTTC motif (uncharacterized)	7	1 e-06

6.3.10 IRES Enrichment in R/N-bound Transcripts

IRESite was screened for the R/N-bound transcripts found in both CA3 and CA1 (overlap of Figure 24A). There was specific evidence from rat for one IRES-containing transcript in the R/N-bound CA1 population, ODC1 encoding the ornithine decarboxylase 1 protein.

Chapter 7 - Discussion

7.1 Summary of Results and Their Implications

In this dissertation, I investigated causal and functional aspects of brain I/R-induced mRNA granule, with emphasis on the role of HuR, whose presence in mRNA granules correlates with translation of *hsp70* mRNA. Specifically, I investigated formation of mRNA granules in brain reperfusion (Chapter 3), the levels of HuR and *hsp70* mRNA in subcellular fractions of control and reperfused samples (Chapter 4), and the distribution of HuR on polysome profiles isolated from control and reperfused samples (Chapter 5), and performed the first microarray studies of (1) the differential transcription between reperfused CA1 and CA3, and (2) translational state analysis of reperfused CA1 and CA3. The main findings are summarized:

Chapter 3

1. Cycloheximide prevented the formation of mRNA granules when given before, but not after global brain ischemia.
2. Puromycin induced mRNA granules in neurons of uninjured animals.

Chapter 4

1. *hsp70* mRNA immunoprecipitated with HuR in cortical homogenates after 8 hours reperfusion.
2. HuR levels in subcellular fractions did not change at 8hR compared to NIC in CA1 and CA3.
3. Increase in *hsp70* mRNA was not different between CA1 and CA3 at 8hR in unfractionated homogenate or cytoplasmic fractions.

Chapter 5

1. HuR distribution on polysome profiles did not change between NIC and 8hR groups.

Chapter 6

1. Differential gene expression was detected between CA1 and CA3 at 8hR as compared to the respective NIC groups.
2. A different and larger population of mRNAs was isolated on polysomes from 8hR CA3 compared to 8hR CA1; there was only fractional overlap in the polysome-bound transcripts between the two regions.
3. Both CA1 and CA3 R/N-bound groups were enriched for similar biological processes by broad categories of gene ontology and express both proliferative and pro-death transcripts.
4. ARE-containing transcripts were enriched in all 8hR compared to NIC groups.
5. Heat shock factor (HSF) binding sites were enriched in the polysome-bound, I/R upregulated transcripts common to CA1 and CA3.
6. There was no evidence of a concentration of mRNA regulation by IRES in the polysome-bound transcripts that increased at 8hR in CA1 and CA3.

Each of these main findings will be discussed in turn. Interspersed through these discussions will be statements of the limitations of each study and suggestions for future directions. The Discussion will conclude with comments about the significance of these findings for the field of brain I/R studies.

7.2 Dependence of mRNA Granules on Polysome State

Pretreatment with CHX blocked the formation of mRNA granules in CA3 neurons after transient global ischemia. The same CHX treatment after ischemia had no effect. These findings are consistent with the hypothesis that polysome-associated mRNA is required to form mRNA granules. Other dynamic mRNPs such as SGs and PBs also increase in response to cell stress[124]. In the 2VO/HT model, SGs were already increased at 10 minutes reperfusion[79]. SGs are known to route mRNAs between other mRNPs, directing mRNAs to translation, storage, or degradation. The rapid increase in SGs in reperfused brain

together with my finding that polysome mRNA is necessary for mRNA granule formation gives rise to the following model:

polysome dissociation → increase SGs to route mRNAs → increase mRNA granules

The present investigation of mRNA granules is limited to the 1hR time point. Therefore, we do not know how suppressing mRNA granule formation may affect cell survival. It is tempting to argue that these findings may explain the neuroprotective effect of CHX, but previous reports in CHX in brain I/R argue against this hypothesis. Some researchers found no effect of CHX of comparable dose and administration in global I/R[209]. Also, some researchers have reported neuroprotection from CHX given after ischemia[159], directly contradicting a mechanism of preventing mRNA granule formation. Despite conflicting reports in the brain I/R field, it is well established that CHX does prevent cell death in cultured cells under stress[210], and this is usually attributed to the drug's ability to exert something like an integrated stress response, suppressing protein synthesis during stress[159]. Reperfused neurons already exert a strong integrated stress response during early reperfusion[87], so it is doubtful that additional protein synthesis inhibition would have much of an effect. Mattson and Furukawa have noted that less than 3% of CHX studies on cellular stress actually measure protein synthesis, and the dose of CHX in many positive studies is known to be too low to block more than a small fraction of protein synthesis[211]. They present the interesting alternative hypothesis that CHX may selectively increase the steady state level or translation of some mRNAs. This has already been shown to be true for several IEGs[211], but the mechanisms are not clear. Given the evidence against CHX neuroprotection through protein synthesis inhibition, the drug's ability to influence behavior of mRNA granules is even more important. More investigation of how CHX could regulate steady state levels of mRNA and translation in the transient global ischemia model are warranted.

Future studies should investigate the effect of a single CHX bolus over a reperfusion time course, to determine whether mRNA granules will eventually form. If CHX can prevent mRNA granules

throughout reperfusion, then outcome studies measuring cell death would indicate the role of the mRNA granule in damage or recovery from I/R. Also, detailed studies of early reperfusion in non-CHX-treated animals should reveal when mRNA granules form relative to SGs.

The novel use of texture analysis allowed me to quantify the changes in cytoplasmic mRNA seen under the microscope. Previously, cellular changes in brain I/R have often been reported descriptively. While still useful, descriptive changes can only be compared nominally; either findings are present, or they are absent. Descriptive findings are typically reported as –representative‖ images. These images may only be a small fraction of the actual cells visible under the microscope, and they cannot express diverse changes in a population. Texture analysis allows quantification of even complex changes among images.

7.3 HuR Does Not Facilitate *hsp70* mRNA Nuclear Export

While it is accepted that HuR can regulate ARE-containing mRNAs in stressed cells, the mechanisms by which HuR exerts posttranscriptional regulation are not well-studied[138]. HuR's best characterized regulatory mechanism is blocking mRNA degradation at the PB[212]. I have not excluded this possibility in reperfused neurons, but there is strong evidence that this mechanism, even if active, is not important in the differential regulation of stress response transcripts between CA1 and CA3. First, reperfused neurons are known to be transcriptionally active and expressing IEG transcripts in response to I/R, even after just minutes of reperfusion[108]. Second, radioactive nucleic acid probes[149] and qPCR[65] have established that *hsp70* mRNA is more abundant in CA1 than CA3 throughout early reperfusion.

The implausibility of differential regulation by blocking mRNA degradation led me to consider alternative mechanisms. In all systems tested, nuclear export of HuR is necessary for the protein to exert posttranscriptional control[146]. Additionally, there is more limited evidence that HuR nuclear export itself can regulate gene expression by exporting associated mRNAs[140]. However, I found that the levels of both nuclear and cytoplasmic HuR were unchanged at 8hR after 10 minutes of 2VO/HT in both CA1 and CA3.

Because unfractionated homogenate was also measured for each region and time point, we may also assert that new HuR is not synthesized at 8hR in response to I/R injury. Together with the main finding that HuR is not exported from the nucleus, this implies that HuR which enters the mRNA granules is present in in the cytoplasm prior to ischemia. Because all known regulatory mechanisms of HuR require nuclear export, HuR may be regulating reperfused neurons by an unknown mechanism.

Increases in *hsp70* mRNA after brain I/R are the same between CA1 and CA3 in cytoplasm and unfractionated homogenate. This is direct evidence that regulation of nuclear export, by HuR or any other means, does not affect the differential translation of *hsp70* after I/R.

I found that HuR binds specifically to *hsp70* mRNA in reperfused neurons at 8hR. HuR did not recognize *gapdh* mRNA, suggesting that its interaction with *hsp70* is not based in the poly(A) tail. Conversely, PABP recognized both *hsp70* and *gapdh* mRNAs at 8hR. This was expected, as PABP associates with all polyadenylated mRNAs as they are being translated. My discovery that HuR specifically binds *hsp70* mRNA in reperfused neurons at 8hR both reinforces previous microscopy work from the DeGracia laboratory and provides justification for future studies of regulation by mRNPs.

An important limitation of this study and of all RIP designs is the possibility of false positives from nonspecific binding or *in vitro* interactions. Despite measures to prevent nonspecific binding, pre-blocking protein A beads and pre-clearing samples, some nonspecific interaction of RNA with protein A sepharose is unavoidable. While PCR is sensitive enough to detect trace amounts of transcripts precipitated by nonspecific interactions, the difference between specifically interacting and nonspecifically bound transcripts should be obvious. Indeed, when limiting PCR to 25 cycles, we found that only *hsp70* mRNA was detectable from HuR immunoprecipitation.

In vitro interactions are more difficult to control. While the majority of cellular mass in cortex tissue comes from neurons, glia and other cell types are also present in the homogenate[179]. It is possible that HuR from other cells in the cortex binds neuronal *hsp70* mRNA once the cells are lysed in homogenate. Mili and Steitz showed an *in vitro* HuR-mRNA interaction in HEK293 cells[213]. They transfected some cells with *c-fos* and transfected different cells with FLAG-tagged HuR. RIP on a mixture

of both cell populations showed FLAG-tagged HuR did pull down c-fos. Presently, the only way to control for *in vitro* interactions in RIP is to add a cross-linker to samples prior to homogenization, but this method introduces the possibility of forming nonspecific interactions within the cell, depending on the strength of the cross-linking[178].

7.4 HuR Distribution on Polysomes

Hu proteins can increase translation of target mRNAs by associating with polysomes through eIF4A[7]. After ruling out the possibility that HuR prevents degradation of *hsp70* mRNA and showing that HuR does not export *hsp70* mRNA out of the nucleus, we decided to investigate the model proposed by Fukao, *et al*. that Hu protein binding to eIF4A could increase translation in the closed loop model. HuR binds 3'UTR ARE and poly(A) sequences. Association of HuR with eIF4A would bring the 3' end of the mRNA adjacent to cap-binding complex of which eIF4A is a subunit.

The distribution of HuR across the polysome profile did not change in response to I/R injury. However, lack of differential association in response to I/R does not necessarily imply that HuR is not regulating translation. Distribution of HuR across the gradient is unlike either polysome-associated proteins or non-polysome-associated PABP. While broadly distributed across densities like PABP, HuR is most concentrated in high-density fractions like ribosomal proteins (Figure15A). With this distribution, HuR is likely constantly associated with the polysomes to some extent. This distribution also suggests that HuR is part of mRNPs of intermediate density between polysomes and free proteins and nucleic acids.

Observations of SGs[214] and PBs[215] indicate that mRNP interactions are complex, and HuR is not likely to be an exception given its diverse role in posttranscriptional regulation. No drugs exist which can specifically suppress HuR in the whole animal. Elucidating HuR's global role in posttranscriptional

regulation will likely require a knockout or knockdown study after which the expression of known HuR targets and their corresponding protein products can be measured.

7.5 Microarray Studies

Comparison to Previous Expression Profiling

While translation state analysis was a novel approach to study global brain I/R, our design was limited by lack of comparisons to total RNA. We have no way to normalize fold changes between the bound and unbound groups (and therefore do not compare them directly). Because we are unable to sum bound and unbound transcripts, we cannot directly compare our quantitative results to those of previous brain I/R microarray studies of total RNA. However, this does not preclude qualitative and order-of-magnitude comparisons with highly-expressed transcripts.

A literature and database search and review article[151] indicated that no microarrays have been performed on brain tissue at 8hR after global ischemia. The most similar study, Büttner, *et al.*, is from a rat transient global ischemia model at 6hR[186]. Still, the Büttner study uses cortex from the entire left hemisphere of post-ischemic brain while our study was of only polysome-bound samples from hippocampus. Differentially regulated transcripts with fold change $\geq 75^{th}$ percentile over all arrays were used to compare upregulated transcripts at 8hR to reported 6hR values. The top ten most differentially expressed transcripts are compared to 6hR transcripts in Table 3.

Table 3: Most differentially expressed transcripts, 8hR polysome-bound (left) and 6hR whole cell RNA (right). Transcripts common to both lists are underlined.

8hR polysome-bound			**Büttner,09 Cortex at 6hR**		
Fold change	Symbol	Name	Fold change	Symbol	Name
54	Hspb1	heat shock protein 1	250	Hspa1b	heat shock 70kD protein 1B (mapped)
50	Atf3	activating transcription factor 3	16	Hspb1	heat shock protein 1
46	Hspa1b	heat shock 70kD protein 1B	13	Fos	FBJ murine osteosarcoma viral oncogene homolog

29	Ptgs2	COX-2	13	Atf3	activating transcription factor 3
18	Cdkn1a	P21 / WAF1	10	Egr2	Early growth response 2
15	Mt1a	metallothionein 1a	9	Ccl2	Chemokine ligand 2
14	Ttr	transthyretin	9	GADD45g	Growth arrest and DNA-damage-inducible 45 gamma
14	Inhba	inhibin beta-A	8	Tnfrsf12a	Tumor necrosis factor receptor superfamily, 12a
14	Hmox1	heme oxygenase (decycling) 1	8	FosB	FBJ murine osteosarcoma viral oncogene homolog B
13	Srxn1	sulfiredoxin 1 homolog	8	Hmox1	heme oxygenase (decycling) 1

Four genes, Hspb1, Atf3, Hspa1b, and Hmox1 are among the top ten most upregulated in both groups, suggesting that these groups of arrays are from similar expression profiles. The proteins encoded by Hspb1, Atf3, Hspa1b, and Hmox1, are HSP27, activating transcription factor-3, HSP70, and heme oxygenase 1, respectively. HSP27, HSP70, and heme oxygenase 1 are all heat shock family member proteins, and ATF3 is a cAMP-responsive transcription factor. All four proteins are known to be expressed in early reperfusion[216–219]. Consistent with the idea that polysome-enriched fractions contain translating mRNA, there is direct evidence for the upregulation of each of the other proteins encoded by the other six genes in the 8hR list- sulfiredoxin 1 homolog[220], transthyretin[221], metallothionein 1a[222], p21[223], and COX-2[224], and inhibin beta-A[220]. It is also noteworthy that *hsp70* and Cdkn1a mRNA are both known targets of HuR[146,225].

Translation State Analysis of 8hR CA1 and CA3

The primary motivation for this study was to see if the mRNAs on the polysomes were concentrated in HuR regulatory sites in their 3'-UTR. That is, this study was the most direct way, without using pharmacological or genetic interventions, to test the role of HuR in mediating selective translation. We could have performed microarray of HuR-precipitated transcripts , but polysome profiles avoid the false positive issues of RIPs discussed above. Whereas it is clearly thermodynamically favorable for mRNA-mRBP bindings to occur *in vitro* after cell disruption[213], it is thermodynamically highly unfavorable that polysomes will spontaneously form in a tissue homogenate that lacks exogenous energy charge. Thus, we had a high degree of confidence of avoiding false positive results and regarding the mRNAs on the isolated polysomes are exactly those that existed *in vivo* before tissue disruption.

We observed an increase in ARE-containing mRNAs, suggesting that HuR and other ARE-binding mRBPs contribute somehow to getting the mRNAs onto the polysomes when global translation itself is suppressed. Limitations of the ARE result are discussed below.

Our design had a number of other advantages over previous global brain I/R expression profiling. This was the first study to compare CA1 and CA3 regions after global brain I/R, and therefore, the first global I/R study of translation state analysis[151]. Previous global brain I/R microarrays necessarily assumed that steady-state levels of total RNA correlated to protein expression. Exclusive comparison of polysome-associated transcripts removes this assumption. The error associated with total RNA microarrays is apparent in our results for unbound transcript comparisons; these changes would be averaged with bound transcript changes in a microarray from total RNA.

Important and unexpected results were 1) R/N-bound CA3 had a more diverse population of mRNAs than R/N-bound CA1 and 2) there was relatively little overlap in the R/N-bound CA1 and CA3 populations. Lack of overlap is due, in part, to differences in steady-state RNA levels between CA1 and CA3 as indicated by comparison of the R/N-unbound populations. However, R/N-unbound did share more transcripts than R/N-bound (32% of R/N-unbound CA3 transcripts versus 17% of R/N-bound transcripts).

A more diverse induction of transcription at the outset of reperfusion may allow CA3 to recovery from I/R injury, while the more limited transcriptional program of CA1 is insufficient to repair the neuron. Alternatively, considering recovery from I/R as dynamic process, CA3 may have a faster rate of recovery than CA1. The larger diversity of transcripts in CA3 may, therefore, reflect recovery of normal translation.

Our microarray results were necessarily limited by the mixed cell population of microdissections. The microdissection procedure minimizes but does not eliminate contamination of other cell types. Interneurons are present in the hippocampus at about a 1:20 ratio with principle neurons[179], as well as glial cells at 1:10. Pyramidal cells account for roughly 90% of cell mass in the pyramidal cell layer. Our design clearly does not distinguish these non-pyramidal cells. There is evidence that some significant

expression differences in the microarray data were the result of non-pyramidal cells. For example, HSP27, one of the biggest fold difference hits, has been previously shown to be exclusively transcribed and translated in astrocytes[218]. That the level of hsp27 mRNA is greater in CA3 than CA1 suggests a functional role for astrocytes in outcome and recovery, and is consistent with work from Rona Giffard who has extensively studied astrocytes after brain I/R[226,227]. Alternative approaches which would prevent mixed cell populations include laser capture microdissection and high throughput *in situ* hybridization, either of which are technically and financial unfeasible.

One obvious future direction will be to repeat this experimental design, but sampling over the entire time course of reperfusion: 1) in CA3 until it fully recovers the control pattern of polysome-bound mRNAs, and 2) in CA1 until the cells die at 72 hr reperfusion. Such a design will reveal the exact time course of all transcript changes, their fates in terms of translation, and shed a completely new light on how stress responses function in post-ischemic neurons, and open up understanding of the diversity of regulatory mechanisms that bear on how individual mRNAs gain access to ribosomes in the reperfusion period.

Database Searches for ARE and IRES-containing mRNAs

Ideally, we would like to screen the 5' and 3'UTRs of all upregulated sequences for enrichment of regulatory sites. Unfortunately, global comparison of RNA regulatory sequences has not matured to the extent of other sequence comparisons such as transcription factor binding sites. RNA regulatory sequences typically depend in secondary if not tertiary structure, making prediction from primary sequences difficult. Some regulatory sequences such as IRES are determined entirely experimentally. Therefore, our database search results pertaining to AREs and IREs concentration in the mRNA populations must be taken only as a first approximation of what is, undoubtedly, a more complex picture.

Given the limits of current mRNA regulatory site databases, empirical measures are absolutely necessary. This could be accomplished by (1) studying individual mRNAs that are high on our hit lists and show, by accepted techniques in the mRNA regulatory field, that they contain functional regulatory

sites. (2) Comparing transcripts upregulated by translational state analysis to transcripts upregulated in RIP for an mRBP of interest such as HuR. Parallel translation state analysis and RIP was used, for example, to identify transcripts regulated synergistically when associated with both the polysome and the fragile X mental retardation mRBP[228].

7.6 Significance of the Work in this Thesis

The discovery of mRNA granules was a significant advancement in the field of brain I/R studies. It offered a new model of translation arrest, and pointed to a causal role for the mRNA granule in post-ischemic outcome. However, prior to this thesis, our understanding of mRNA granules after brain I/R was entirely descriptive, based only on histological and correlative observations. This is the very first study to assess the molecular significance of mRNA granules.

In particular, the studies here have advanced the understanding of how HuR may regulate expression of HSP70. The results here suggest that 2 of the 3 known Hu protein regulatory mechanisms, prevention of transcript degradation and facilitated nuclear export, are unlikely to play a significant role in post-ischemic outcome.

The work here has not ruled out a role of HuR selective translation of stress induced mRNAs. While a change in the binding of HuR to polysomes was not observed during reperfusion, translational state analysis showed a concentration of ARE-containing mRNAs on polysomes in both CA1 and CA3 (the interpretation of which is subject to the constraints listed above).

Perhaps the most important finding, one not anticipated when the studies were conducted, was the greater diversity of transcripts in CA3. This has wide-ranging implications for understanding the response of specific neurons to I/R injury, how stress responses are executed via transcription and translational coupling, and how regulation of stress responses relates to outcome. Much new work is expected to spawn from this observation.

These findings significantly advance the understanding of HuR function, mRNA granules, and the execution of stress responses in post-ischemic neurons beyond the present general understanding in the field.

7.7 Summary and Conclusion

Previous expression profiling studies of the reperfused brain have been almost exclusively focused on hypothesis discovery rather than testing concrete hypotheses. The work described here represents the application of expression profiling technology to answer well-focused questions pertaining to mRNA regulation. To do so, we applied methods commonly used in basic science studies of ribosome and mRNA regulation, where assessing entire populations of mRNAs simultaneously is important for understanding the biology of the system. It is hoped that the present work stands as an example for others in the field as a way to apply –omics technology to address specific, and biologically meaningful questions.

In conclusion, it is hoped that the studies described here will contribute to the development of successful therapies to effectively treat stroke and cardiac arrest brain damage.

APPENDIX A: TRANSCRIPTS DIFFERENTIALLY EXPRESSED IN POLYSOME-BOUND CA3 8HR AND NIC GROUPS

Parametric p-value	FDR	Fold-change	ProbeSet	Name	EntrezID
5E-07	0.000548	76.51	10761128	heat shock protein 1	24471
6E-07	0.000564	57.85	10770710	activating transcription factor 3	25389
1.25E-05	0.00216	56.02	10800426	transthyretin	24856
4E-07	0.000526	45.6	10828154	heat shock 70kD protein 1B (mapped)	294254
2E-07	0.000526	28.85	10764551	prostaglandin-endoperoxide synthase 2	29527
8.21E-05	0.00567	18.54	10828827	cyclin-dependent kinase inhibitor 1A	114851
0.000002	0.00106	13.8	10798702	inhibin beta-A	29200
1.9E-06	0.00106	12.97	10937762	transmembrane protein 27	57395
3E-07	0.000526	12.96	10809392	metallothionein 1a	24567
5.69E-05	0.00492	12.93	10892173	NA	NA
3E-07	0.000526	12.82	10796411	metallothionein 1a	24567
4E-07	0.000526	12.41	10937867	NA	NA
3.9E-06	0.0016	12.36	10831298	heat shock 70kD protein 1B (mapped)	294254
2.1E-06	0.00106	12.34	10900358	growth arrest and DNA-damage-inducible, beta	299626
9.52E-05	0.00609	11.24	10817071	S100 calcium binding protein A8	116547
0.002578	0.0306	11.22	10806122	heme oxygenase (decycling) 1	24451
0.000115	0.00694	10.45	10797566	NA	NA
0.000529	0.0165	9.6	10855701	aquaporin 1	25240
6.17E-05	0.00497	9.51	10840791	sulfiredoxin 1 homolog (S. cerevisiae)	296271
3.21E-05	0.00366	9.21	10909428	membrane frizzled-related protein	315597
0.000148	0.00809	9.13	10929656	potassium inwardly-rectifying channel, subfamily J, member 13	94341
4.7E-06	0.0018	8.98	10899187	glycerol-3-phosphate dehydrogenase 1 (soluble)	60666
0.00027	0.0118	8.74	10830908	ring finger protein 39	171387
2.9E-06	0.00136	8.73	10909892	crystallin, alpha B	25420
7.5E-06	0.0019	8.6	10711401	Bcl2-associated athanogene 3	293524

0.000848	0.0192	8.55	10827686	RT1 class I, locus M6, gene 1	414785
3.74E-05	0.00393	8.52	10727717	neuronal PAS domain protein 4	266734
8.9E-06	0.00211	8.41	10796800	YME1-like 1 (S. cerevisiae)	114217
1.05E-05	0.00216	8.39	10757082	zinc finger, AN1-type domain 2A	360772
0.00045	0.0152	8.22	10759762	Klotho	83504
7.4E-06	0.0019	8	10797527	growth arrest and DNA-damage-inducible, gamma	291005
1.79E-05	0.00262	7.87	10903736	ectonucleotide pyrophosphatase/phosphodiesterase 2	84050
0.009407	0.0581	7.79	10754116	zinc finger and BTB domain containing 20	288105
5.2E-06	0.0018	7.74	10756343	heat shock 105kDa/110kDa protein 1	288444
1.23E-05	0.00216	7.71	10872533	AT rich interactive domain 1A (SWI-like)	297867
1.22E-05	0.00216	7.59	10803991	CD14 molecule	60350
9.39E-05	0.00609	7.52	10896793	tribbles homolog 1 (Drosophila)	78969
7.3E-06	0.0019	7.47	10817552	thioredoxin interacting protein	117514
0.001064	0.0212	7.32	10720215	zinc finger protein 36	79426
0.007462	0.0514	7.17	10779390	Ca++-dependent secretion activator	26989
0.000129	0.00761	7.02	10770022	potassium inwardly-rectifying channel, subfamily J, member 9	116560
0.001709	0.0254	6.94	10765212	coagulation factor V (proaccelerin, labile factor)	304929
3.4E-06	0.00149	6.91	10717331	serum/glucocorticoid regulated kinase 1	29517
0.003999	0.0388	6.68	10862867	growth arrest and DNA-damage-inducible, alpha	25112
2.16E-05	0.0029	6.57	10818687	NA	NA
9.4E-06	0.00213	6.37	10936482	TIMP metallopeptidase inhibitor 1	116510
0.000177	0.00886	6.27	10811126	fatty acid 2-hydroxylase	307855
7.5E-06	0.0019	6.22	10739455	G protein-coupled receptor, family C, group 5, member C	287805
2.89E-05	0.00352	6.22	10768332	regulator of G-protein signaling 2	84583
0.004185	0.0393	6.2	10839579	dual specificity phosphatase 2	311406
0.000184	0.00898	6.02	10927840	NA	NA
2.43E-05	0.00313	5.99	10785461	protocadherin 8	64865
0.000111	0.0069	5.93	10738209	2',3'-cyclic nucleotide 3' phosphodiesterase	25275
0.000164	0.00869	5.93	10757489	VGF nerve growth factor inducible	29461
6.8E-06	0.0019	5.89	1076029	neuronal pentraxin 2	288475

			0		
7.1E-06	0.0019	5.83	1090791 3	matrix metallopeptidase 8	63849
2.59E-05	0.0032 8	5.81	1090774 9	glycosylation dependent cell adhesion molecule 1	25258
0.0008	0.0185	5.73	1092953 6	solute carrier family 16, member 14 (monocarboxylic acid transporter 14)	316578
2.43E-05	0.0031 3	5.7	1086371 0	NA	NA
4.94E-05	0.0044 5	5.68	1074532 3	WD repeat and SOCS box-containing 1	303336
3.89E-05	0.0039 3	5.59	1089167 9	G protein-coupled receptor 68	314386
1.96E-05	0.0027 7	5.58	1081718 3	S100 calcium binding protein A11 (calizzarin)	445415
8.29E-05	0.0056 7	5.58	1082263 1	claudin 11	84588
0.004176	0.0393	5.56	1082074 8	UTP15, U3 small nucleolar ribonucleoprotein, homolog (S. cerevisiae)	310019
6.5E-06	0.0019	5.53	1080450 8	heat shock protein A8	24468
3.23E-05	0.0036 6	5.52	1084346 0	prostaglandin D2 synthase (brain)	25526
0.000571	0.0169	5.52	1084422 3	prostaglandin E synthase	59103
0.000009	0.0021 1	5.49	1093782 7	heat shock protein A8	24468
0.007364	0.0511	5.49	1091493 5	LOC363015	363015
6.16E-05	0.0049 7	5.45	1081029 5	DnaJ (Hsp40) homolog, subfamily B, member 1	361384
1.48E-05	0.0023 7	5.36	1092852 2	NA	NA
7.06E-05	0.0054	5.29	1092097 7	NA	NA
0.000112	0.0069	5.27	1077258 0	NA	NA
0.000628	0.0177	5.27	1092529 1	chemokine (C-X-C motif) receptor 7	84348
0.000078	0.0056 7	5.25	1077551 9	1-acylglycerol-3-phosphate O-acyltransferase 9	305166
0.00001	0.0021 6	5.19	1082975 9	NA	NA
1.11E-05	0.0021 6	5.15	1089218 4	heat shock protein 90, alpha (cytosolic), class A member 1	299331
0.000107	0.0067 2	5.11	1074476 6	aspartoacylase	79251
0.000451	0.0152	5.09	1084970 0	mal, T-cell differentiation protein	25263
8.27E-05	0.0056 7	5.06	1075029 6	chloride intracellular channel 6	304081
0.004832	0.0416	5.05	1081668 8	CDC-like kinase 2	365842
0.00017	0.0087 3	5.01	1091261 4	solute carrier organic anion transporter family, member 2a1	24546
0.005854	0.0458	5.01	1076000 8	ubiquitin specific peptidase like 1	288447
4.88E-05	0.0044 5	4.99	1087811 2	Jun oncogene	24516

0.000861	0.0192	4.99	10856673	solute carrier family 4, sodium bicarbonate cotransporter, member 5	297386
1.09E-05	0.00216	4.97	10800590	heat shock protein A8	24468
0.000156	0.00841	4.92	10808167	NA	NA
4.09E-05	0.00396	4.9	10720813	myelin-associated glycoprotein	29409
0.000449	0.0152	4.89	10935131	ring finger protein 128	315911
1.24E-05	0.00216	4.87	10924230	NA	NA
0.00074	0.0184	4.86	10838823	ChaC, cation transport regulator homolog 1 (E. coli)	362196
0.000047	0.00435	4.85	10853916	NA	NA
1.39E-05	0.00229	4.82	10889219	heat shock protein A8	24468
0.00004	0.00393	4.82	10835692	heat shock protein 5	25617
1.35E-05	0.00228	4.77	10749854	heat shock protein A8	24468
1.98E-05	0.00277	4.75	10732652	dual specificity phosphatase 1	114856
1.24E-05	0.00216	4.74	10868289	DnaJ (Hsp40) homolog, subfamily A, member 1	65028
0.001279	0.0226	4.74	10776667	NA	NA
1.68E-05	0.00257	4.72	10752769	heat shock protein A8	24468
0.005631	0.0449	4.7	10878938	polo-like kinase 3 (Drosophila)	58936
1.66E-05	0.00257	4.66	10909347	heat shock protein A8	24468
0.000134	0.00768	4.65	10869106	solute carrier family 44, member 1	85254
0.000998	0.0206	4.64	10884274	sclerostin domain containing 1	266803
1.75E-05	0.00262	4.62	10881303	heat shock protein A8	24468
6.78E-05	0.00531	4.6	10740869	tumor necrosis factor receptor superfamily, member 12a	302965
0.000062	0.00497	4.59	10815998	heat shock protein A8	24468
8.36E-05	0.00567	4.59	10903459	Kruppel-like factor 10	81813
0.000755	0.0184	4.59	10736697	chemokine (C-C motif) ligand 2	24770
5.96E-05	0.00496	4.55	10749983	coxsackie virus and adenovirus receptor	89843
3.69E-05	0.00393	4.51	10867731	calbindin 1	83839
3.73E-05	0.00393	4.49	10827450	NA	NA
4.27E-05	0.00407	4.48	10868718	DDB1 and CUL4 associated factor 10	313242
0.00246	0.03	4.46	10833031	sphingosine-1-phosphate lyase 1	286896
0.001448	0.0237	4.39	1090558	suppression of tumorigenicity 13	81800

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6.47E-05	0.00513	4.36	10772330	NA	NA
0.001277	0.0226	4.35	10827691	RT1 class I, locus M6, gene 2	365527
0.000165	0.00869	4.3	10812580	NA	NA
9.54E-05	0.00609	4.28	10721865	protein phosphatase 1, regulatory (inhibitor) subunit 15A	171071
0.000538	0.0165	4.25	10734853	aurora kinase B	114592
0.000741	0.0184	4.2	10938209	NA	NA
2.76E-05	0.00343	4.19	10928154	heat shock protein 1 (chaperonin)	63868
0.000349	0.0134	4.18	10931308	prolyl 4-hydroxylase, alpha polypeptide I	64475
0.000194	0.00936	4.17	10739558	solute carrier family 16, member 5 (monocarboxylic acid transporter 6)	690212
0.00222	0.0285	4.17	10880095	serine incorporator 2	313057
0.000148	0.00809	4.15	10801884	solute carrier family 12 (sodium/potassium/chloride transporters), member 2	83629
0.000348	0.0134	4.15	10713583	WD repeat domain 74	690229
2.11E-05	0.00289	4.14	10827989	metallothionein 2A	689415
0.004485	0.0405	4.13	10713602	NA	NA
0.000947	0.0198	4.12	10719977	melanoma inhibitory activity	81510
0.002525	0.0304	4.12	10889719	suppression of tumorigenicity 13	81800
0.000756	0.0184	4.1	10718001	1-acylglycerol-3-phosphate O-acyltransferase 4 (lysophosphatidic acid acyltransferase, delta)	170919
0.004008	0.0388	4.09	10891364	alkB, alkylation repair homolog (E. coli)	362766
0.008592	0.0556	4.08	10760813	NA	NA
0.001191	0.0221	4.04	10799187	DIP2 disco-interacting protein 2 homolog C (Drosophila)	307067
0.000513	0.0162	4.02	10809328	homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1	85430
7.76E-05	0.00567	4.01	10868428	DnaJ (Hsp40) homolog, subfamily B, member 5	313811
0.000328	0.013	4	10781197	stathmin-like 4	79423
0.005452	0.0442	4	10726669	cytochrome c oxidase, subunit VIIIb	25250
0.000648	0.0178	3.99	10788374	mitochondrial tumor suppressor 1	306487
0.000742	0.0184	3.97	10840396	Sec23 homolog B (S. cerevisiae)	362226
0.002432	0.0299	3.92	10753533	myc induced nuclear antigen	266670
0.000699	0.018	3.91	10834241	ATP-binding cassette, subfamily A (ABC1), member 2	79248
0.005957	0.0463	3.91	10847932	DEP domain containing 7	295971

0.001926	0.0267	3.86	10842500	similar to Docking protein 5 (Downstream of tyrosine kinase 5) (Protein dok-5)	502694
0.000332	0.013	3.85	10866819	branched chain aminotransferase 1, cytosolic	29592
0.000398	0.0143	3.84	10891436	general transcription factor IIA, 1	83830
0.000276	0.0118	3.83	10853852	suppression of tumorigenicity 7	296911
0.000577	0.017	3.8	10789059	1-acylglycerol-3-phosphate O-acyltransferase 6 (lysophosphatidic acid acyltransferase, zeta)	290843
0.002031	0.0274	3.8	10826837	cytochrome P450, family 2, subfamily u, polypeptide 1	310848
0.003644	0.0371	3.8	10831802	NA	NA
0.003595	0.0369	3.79	10799291	Kruppel-like factor 6	58954
0.002881	0.0323	3.77	10837520	suppression of tumorigenicity 13	81800
0.005492	0.0444	3.77	10937641	NA	NA
0.000581	0.0171	3.75	10758134	ubiquitin C	50522
0.00013	0.00761	3.74	10825472	ATPase, Na+/K+ transporting, alpha 1 polypeptide	24211
0.001873	0.0265	3.74	10726682	interferon induced transmembrane protein 3	361673
7.06E-05	0.0054	3.73	10906608	solute carrier family 38, member 2	29642
0.002478	0.03	3.73	10762709	coenzyme Q5 homolog, methyltransferase (S. cerevisiae)	304542
0.000142	0.00798	3.72	10761394	phosphoserine phosphatase	304429
3.94E-05	0.00393	3.71	10809399	metallothionein 2A	689415
0.000095	0.00609	3.7	10723383	NA	NA
0.000456	0.0153	3.7	10728028	NA	NA
0.000178	0.00886	3.67	10847761	Cd44 molecule	25406
0.000479	0.0158	3.67	10885959	similar to HESB like domain containing 1	500694
0.003657	0.0372	3.65	10792344	NA	NA
7.87E-05	0.00567	3.61	10812922	NA	NA
0.000364	0.0138	3.61	10737196	NA	NA
0.000502	0.016	3.61	10886846	NA	NA
0.005226	0.0433	3.6	10729970	retinol binding protein 4, plasma	25703
0.000252	0.0114	3.58	10921120	solute carrier family 6 (proline IMINO transporter), member 20	113918
0.00076	0.0184	3.58	10781361	leucine-rich repeat LGI family, member 3	306013
0.000299	0.0122	3.57	10820071	G protein-coupled receptor 150	499486
3.39E-05	0.0037	3.56	1076700	methionine adenosyltransferase II, alpha	171347

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3.06E-05	0.00366	3.55	10899964	methyltransferase like 7B	366792
7.48E-05	0.00566	3.53	10728647	myelin gene regulatory factor	293736
0.001869	0.0265	3.51	10850608	NA	NA
0.005496	0.0444	3.5	10917483	RAB39, member RAS oncogene family	315668
8.31E-05	0.00567	3.48	10725387	cerebellar degeneration-related 2	308958
0.003804	0.038	3.46	10891834	legumain	63865
0.000631	0.0177	3.45	10796941	NA	NA
0.000197	0.00945	3.44	10765090	actin, gamma 1	287876
0.004633	0.0412	3.44	10872626	G protein-coupled receptor 3	266769
0.001714	0.0254	3.43	10808103	telomeric repeat binding factor 2, interacting protein	307861
0.006673	0.0487	3.43	10813392	FYN binding protein (FYB-120/130)	499537
0.001263	0.0225	3.42	10919637	transferrin	24825
0.001544	0.0244	3.42	10767016	cadherin 19, type 2	360835
5.32E-05	0.00467	3.41	10875532	pyruvate dehyrogenase phosphatase catalytic subunit 1	54705
0.000168	0.00869	3.41	10829976	prosaposin	25524
0.001086	0.0213	3.38	10738015	zona pellucida binding protein 2	363676
0.00201	0.0273	3.37	10750489	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 5	288161
0.000549	0.0167	3.35	10935064	proteolipid protein 1	24943
0.000734	0.0184	3.35	10934777	NA	NA
0.002239	0.0286	3.35	10935177	claudin 2	300920
0.000116	0.00694	3.34	10859262	apolipoprotein L domain containing 1	444983
0.000334	0.013	3.34	10858967	tumor necrosis factor receptor superfamily, member 1a	25625
0.0012	0.0222	3.34	10871479	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 1	679532
0.001477	0.0239	3.34	10751945	somatostatin	24797
0.00116	0.0219	3.33	10797657	osteomodulin	83717
9.11E-05	0.00605	3.32	10755013	interleukin 1 receptor accessory protein	25466
3.95E-05	0.00393	3.29	10863221	methionine adenosyltransferase II, alpha	171347
0.004237	0.0394	3.29	10938101	protein kinase, X-linked	501563
0.008187	0.0541	3.29	10853193	round spermatid basic protein 1-like	311987

8.51E-05	0.00571	3.27	10807473	solute carrier family 7 (cationic amino acid transporter, y+ system), member 6	307811
0.001032	0.021	3.26	10826607	UDP glycosyltransferase 8	50555
0.006149	0.0472	3.26	10797811	CD83 molecule	361226
0.000222	0.0103	3.24	10712317	patatin-like phospholipase domain containing 2	361676
0.008799	0.0564	3.23	10872852	similar to RIKEN cDNA 4930555I21	500566
0.000555	0.0167	3.22	10879516	major facilitator superfamily domain containing 2	298504
0.000851	0.0192	3.22	10817696	protein kinase, AMP-activated, beta 2 non-catalytic subunit	64562
0.00036	0.0137	3.21	10895589	carboxypeptidase M	314855
0.000401	0.0143	3.21	10834022	arrestin domain containing 3	309945
0.001245	0.0224	3.21	10739204	survival motor neuron domain containing 1	287768
0.000361	0.0137	3.19	10824059	NA	NA
0.001813	0.026	3.19	10837279	integrin, alpha V	296456
0.004062	0.0389	3.19	10903482	solute carrier family 25, member 32	315023
0.000934	0.0197	3.18	10871511	L-amino acid oxidase 1	298483
0.001513	0.0243	3.18	10869839	NA	NA
0.006605	0.0484	3.18	10891910	interferon, alpha-inducible protein 27 like 2B	299269
0.007375	0.0511	3.18	10840226	similar to Protein C20orf103 precursor	362220
0.001231	0.0224	3.17	10937103	synaptophysin	24804
0.000536	0.0165	3.15	10907324	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 6	683264
0.000697	0.018	3.15	10796149	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	117276
0.001434	0.0236	3.15	10796507	solute carrier family 39 (zinc transporter), member 12	291328
0.002094	0.0277	3.15	10762115	N(alpha)-acetyltransferase 25, NatB auxiliary subunit	360811
0.000104	0.00657	3.14	10902547	lysozyme 2	25211
0.000208	0.00977	3.14	10901231	TIMP metallopeptidase inhibitor 3	25358
0.000283	0.0119	3.13	10774470	NA	NA
0.000257	0.0115	3.12	10860900	pyruvate dehydrogenase kinase, isozyme 4	89813
0.000276	0.0118	3.1	10767605	contactin 2 (axonal)	25356
0.000294	0.0121	3.1	10809269	chemokine (C-X3-C motif) ligand 1	89808
8.23E-05	0.00567	3.09	10775862	prostate androgen-regulated mucin-like protein 1	286894
0.002693	0.0314	3.08	1089558	P55	362855

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0.006351	0.0479	3.08	10726114	cytochrome c oxidase, subunit VIa, polypeptide 2	25278
0.002047	0.0275	3.06	10739353	potassium inwardly-rectifying channel, subfamily J, member 2	29712
0.002054	0.0275	3.06	10730884	survival motor neuron domain containing 1	287768
0.001177	0.0221	3.04	10801260	NA	NA
0.001372	0.0233	3.04	10773235	zinc finger protein 509	305428
0.001756	0.0258	3.03	10728800	transmembrane protein 132A	338474
0.002461	0.03	3.03	10731484	thioredoxin domain containing 11	302899
0.000116	0.00694	3.02	10896992	protein tyrosine phosphatase type IVA, member 3	362930
0.000739	0.0184	3.01	10940380	ATPase, H+ transporting, lysosomal accessory protein 1	83615
0.004756	0.0414	3.01	10852144	prostate transmembrane protein, androgen induced 1	311676
0.000477	0.0158	3	10862473	NA	NA
0.002006	0.0273	3	10769672	regulator of G-protein signaling 4	29480
0.004433	0.0402	3	10826669	NA	NA
0.008454	0.0549	3	10750460	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	304063
0.004946	0.0421	2.99	10763477	Mki67 (FHA domain) interacting nucleolar phosphoprotein	246042
0.00147	0.0239	2.98	10886509	NA	NA
0.001533	0.0244	2.98	10869541	NA	NA
0.000177	0.00886	2.97	10785773	sprouty homolog 2 (Drosophila)	306141
0.000304	0.0123	2.97	10803653	actin, gamma 1	287876
0.00042	0.0146	2.97	10794599	neural precursor cell expressed, developmentally down-regulated 9	291044
0.001445	0.0237	2.97	10850104	solute carrier family 23 (nucleobase transporters), member 2	50622
0.004441	0.0402	2.97	10904511	activity-regulated cytoskeleton-associated protein	54323
0.007542	0.0515	2.96	10823508	cyclin L1	114121
0.000765	0.0184	2.95	10861565	zinc finger, C3HC-type containing 1	296957
0.00142	0.0236	2.95	10877855	NA	NA
0.002355	0.0295	2.95	10890626	glycoprotein hormone beta 5	366668
0.000637	0.0177	2.94	10782986	orthodenticle homeobox 2	305858
0.004579	0.041	2.94	10857314	solute carrier family 6 (neurotransmitter transporter, taurine), member 6	29464
0.000261	0.0115	2.93	10765744	phosphatidylinositol glycan anchor biosynthesis, class M	79112

0.000586	0.0171	2.93	10835757	gelsolin	296654
0.000691	0.018	2.93	10819298	NA	NA
0.002253	0.0287	2.93	10745994	LOC360590	360590
0.000279	0.0118	2.92	10874193	ERBB receptor feedback inhibitor 1	313729
0.000475	0.0158	2.92	10844339	solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 25	246771
0.00039	0.0143	2.91	10724228	Bcl2-like 1	24888
0.000715	0.0183	2.91	10903061	serine hydroxymethyltransferase 2 (mitochondrial)	299857
0.005144	0.0429	2.91	10895585	lysozyme C type 2	688047
0.003122	0.0339	2.9	10765335	cellular repressor of E1A-stimulated genes 1	289185
0.003622	0.0371	2.9	10776419	transmembrane protein 165	364137
0.000553	0.0167	2.89	10723884	serine (or cysteine) peptidase inhibitor, clade H, member 1	29345
0.000604	0.0173	2.89	10763547	NA	NA
0.000319	0.0127	2.88	10770888	actin, gamma 1	287876
0.002224	0.0285	2.88	10749495	lectin, galactoside-binding, soluble, 3 binding protein	245955
0.000796	0.0185	2.87	10882221	transmembrane protein 88B	680723
0.00032	0.0127	2.86	10705872	actin, gamma 1	287876
0.000488	0.0158	2.86	10837537	actin, gamma 1	287876
0.001622	0.0249	2.86	10792441	solute carrier family 20 (phosphate transporter), member 2	29502
0.00855	0.0555	2.86	10732893	cyclin J-like	303059
0.002673	0.0313	2.85	10780170	NA	NA
0.004953	0.0421	2.85	10730148	oligodendrocytic myelin paranodal and inner loop protein	361757
0.000693	0.018	2.84	10914937	NA	NA
0.000702	0.018	2.84	10865531	ubiquitin specific peptidase 5 (isopeptidase T)	297593
0.001369	0.0233	2.83	10816287	ets variant 3	295297
0.001525	0.0244	2.83	10727228	Fas (TNFRSF6)-associated via death domain	266610
0.00259	0.0307	2.83	10714973	HECT domain containing 2	309514
0.00706	0.0498	2.83	10729550	NA	NA
0.009842	0.059	2.83	10832404	minichromosome maintenance complex component 3 associated protein	294339
0.000777	0.0185	2.82	10739399	NA	NA
0.002391	0.0297	2.82	1085934	protein tyrosine phosphatase, receptor type, O	50677

			2		
0.000261	0.0115	2.81	1086466 2	transmembrane protein 111	312640
0.000493	0.0159	2.81	1074961 2	actin, gamma 1	287876
0.002697	0.0314	2.81	1089449 8	P55	362855
0.006393	0.0481	2.8	1087480 2	ubiquitin-conjugating enzyme E2, J2 (UBC6 homolog, yeast)	298689
0.000381	0.0142	2.79	1088420 5	TWIST neighbor	362728
0.000594	0.0172	2.79	1092334 5	heat shock protein 1 (chaperonin 10)	25462
0.001088	0.0213	2.79	1085937 1	serine/threonine kinase receptor associated protein	297699
0.001349	0.0232	2.79	1083181 6	BCL2-antagonist/killer 1	116502
0.001661	0.0253	2.79	1087950 2	CAP, adenylate cyclase-associated protein 1 (yeast)	64185
0.003042	0.0332	2.79	1081738 3	LAG1 homolog, ceramide synthase 2	310667
0.004683	0.0413	2.79	1085265 1	NA	NA
0.000149	0.0080 9	2.78	1086386 6	CCHC-type zinc finger, nucleic acid binding protein	64530
0.000978	0.0204	2.78	1087144 4	solute carrier family 6 (neurotransmitter transporter, glycine), member 9	116509
0.002122	0.0279	2.78	1088861 0	similar to limb-bud and heart	683626
0.002325	0.0294	2.78	1092407 6	ribulose-5-phosphate-3-epimerase	501157
0.004717	0.0414	2.78	1074519 3	protein interacting with cyclin A1	497959
0.000413	0.0145	2.77	1088052 4	NA	NA
0.00424	0.0394	2.77	1092333 8	coenzyme Q10 homolog B (S. cerevisiae)	301416
0.001599	0.0247	2.76	1090973 3	zinc finger protein 259	500989
0.005982	0.0463	2.76	1091114 5	carbonic anyhydrase 12	363085
0.008883	0.0565	2.76	1092822 9	CDC-like kinase 1	301434
0.000624	0.0177	2.75	1075028 2	solute carrier family 5 (sodium/myo-inositol cotransporter), member 3	114507
0.000489	0.0158	2.73	1085544 9	glycoprotein (transmembrane) nmb	113955
0.006079	0.0468	2.73	1088946 2	NA	NA
0.006863	0.0492	2.73	1090810 2	NA	NA
0.001023	0.021	2.72	1086390 4	Sec61 alpha 1 subunit (S. cerevisiae)	80843
0.002315	0.0293	2.72	1072804 6	D4, zinc and double PHD fingers family 2	361711
0.003831	0.0381	2.72	1071900 6	leukocyte receptor cluster (LRC) member 8	361506
0.000166	0.0086 9	2.71	1086585 5	NA	NA

0.006746	0.049	2.71	10797929	glucosaminyl (N-acetyl) transferase 2, I-branching enzyme	306860
0.00082	0.0187	2.7	10936925	NA	NA
0.001968	0.0271	2.7	10864621	jagunal homolog 1 (Drosophila)	502872
0.003727	0.0374	2.7	10786624	5'-nucleotidase domain containing 2	290558
0.00026	0.0115	2.69	10794225	nuclear factor, interleukin 3 regulated	114519
0.002932	0.0325	2.69	10765166	similar to 2810422O20Rik protein	304928
0.00403	0.0388	2.69	10786338	Rho guanine nucleotide exchange factor (GEF) 3	290541
0.005954	0.0463	2.69	10812722	mitochondrial ribosomal protein S27	361883
0.006922	0.0495	2.69	10898654	asparagine-linked glycosylation 10, alpha-1,2-glucosyltransferase homolog (S. pombe)	245960
0.000613	0.0175	2.68	10907165	Fas apoptotic inhibitory molecule 2	246274
0.003125	0.0339	2.68	10833152	cysteine and glycine-rich protein 2	29317
0.009779	0.059	2.68	10833818	NA	NA
0.005087	0.0427	2.67	10762600	GCN1 general control of amino-acid synthesis 1-like 1 (yeast)	690632
0.005239	0.0434	2.67	10932078	NA	NA
0.002822	0.0319	2.65	10793456	splicing factor, arginine/serine-rich 3	361814
0.002991	0.0329	2.65	10878034	NA	NA
0.006759	0.049	2.65	10843749	SEC16 homolog A (S. cerevisiae)	114089
0.009209	0.0575	2.65	10928902	transmembrane BAX inhibitor motif containing 1	316516
0.001024	0.021	2.64	10872291	tyrosyl-tRNA synthetase	313047
0.002003	0.0273	2.63	10895144	dual specificity phosphatase 6	116663
0.004679	0.0413	2.63	10802734	SMAD family member 7	81516
0.000886	0.0194	2.62	10874450	leucine rich repeat containing 47	362672
0.001637	0.0251	2.62	10889263	tribbles homolog 2 (Drosophila)	313974
0.000647	0.0178	2.61	10890650	NA	NA
0.001237	0.0224	2.61	10938217	NA	NA
0.001299	0.0227	2.61	10858686	prohibitin 2	114766
0.001969	0.0271	2.61	10884853	PRP39 pre-mRNA processing factor 39 homolog (S. cerevisiae)	314171
0.003398	0.0355	2.61	10912088	5' nucleotidase, ecto	58813
0.000761	0.0184	2.59	10795194	NA	NA
0.001842	0.0264	2.59	1085410	calumenin	64366

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0.005138	0.0429	2.59	10924326	rcd1 (required for cell differentiation) homolog 1 (S. pombe)	301513
0.000636	0.0177	2.58	10846821	thioredoxin-related transmembrane protein 2	295701
0.001416	0.0236	2.58	10811875	TAF5-like RNA polymerase II, p300/CBP-associated factor (PCAF)-associated factor	307927
0.000973	0.0203	2.57	10747692	dual specificity phosphatase 3	498003
0.002212	0.0285	2.57	10792863	ATPase, class VI, type 11A	306600
0.005119	0.0428	2.57	10790948	jun D proto-oncogene	24518
0.00521	0.0433	2.57	10803602	LIM and senescent cell antigen like domains 2	361303
0.000783	0.0185	2.56	10904169	N-myc downstream regulated 1	299923
0.000809	0.0186	2.56	10805335	solute carrier family 14 (urea transporter), member 1	54301
0.000934	0.0197	2.56	10769131	calcyclin binding protein	289144
0.002887	0.0324	2.56	10835037	GLE1 RNA export mediator homolog (yeast)	362098
0.008268	0.0543	2.56	10795315	NA	NA
0.000916	0.0196	2.55	10752758	BTG family, member 3	54230
0.001275	0.0226	2.55	10789653	insulin receptor substrate 2	29376
0.001392	0.0233	2.55	10763685	NA	NA
0.009211	0.0575	2.55	10908072	G protein-coupled receptor 83	140595
0.000804	0.0186	2.54	10915885	NA	NA
0.001314	0.0228	2.54	10727373	NA	NA
0.005304	0.0436	2.54	10909407	Thy-1 cell surface antigen	24832
0.000891	0.0194	2.53	10744134	eukaryotic translation initiation factor 4A, isoform 1	287436
0.001363	0.0232	2.53	10816405	cellular retinoic acid binding protein 2	29563
0.002122	0.0279	2.53	10934794	interferon stimulated exonuclease gene 20-like 2	361977
0.006409	0.0481	2.53	10830962	NA	NA
0.006409	0.0481	2.53	10875983	NA	NA
0.007034	0.0498	2.53	10787517	growth differentiation factor 15	29455
0.000557	0.0167	2.52	10940364	GDP dissociation inhibitor 1	25183
0.001097	0.0213	2.52	10727643	NA	NA
0.006295	0.0476	2.52	10727806	NA	NA
0.008919	0.0567	2.52	10842517	cleavage stimulation factor, 3' pre-RNA, subunit 1	311670

0.000531	0.0165	2.51	10717941	t-complex 1	24818
0.000596	0.0172	2.51	10796455	signal transducing adaptor molecule (SH3 domain and ITAM motif) 1	498798
0.00188	0.0265	2.51	10929263	secretogranin II (chromogranin C)	24765
0.004657	0.0413	2.51	10931995	A kinase (PRKA) anchor protein 14	60332
0.001387	0.0233	2.5	10705364	SERTA domain containing 1	361526
0.001908	0.0266	2.5	10859108	C-type lectin domain family 2, member g	362447
0.001187	0.0221	2.49	10713160	synovial apoptosis inhibitor 1, synoviolin	361712
0.002607	0.0308	2.49	10865388	vomeronasal 2 receptor, 51	502891
0.00536	0.0437	2.49	10872478	small nuclear ribonucleoprotein 40 (U5)	313056
0.009243	0.0575	2.49	10701924	interferon gamma receptor 1	116465
0.000678	0.018	2.48	10761162	P450 (cytochrome) oxidoreductase	29441
0.003568	0.0368	2.48	10758344	vacuolar protein sorting 37 homolog B (S. cerevisiae)	288659
0.005763	0.0454	2.48	10792859	ATPase, class VI, type 11A	306600
0.007715	0.0523	2.48	10827531	splicing factor, arginine/serine-rich 11	502603
0.007725	0.0523	2.48	10796445	vimentin	81818
0.002337	0.0294	2.47	10836394	membrane-associated ring finger (C3HC4) 7	311059
0.000617	0.0176	2.46	10928275	family with sequence similarity 126, member B	316415
0.002909	0.0324	2.46	10817894	NA	NA
0.006517	0.0484	2.46	10892074	NA	NA
0.000692	0.018	2.45	10784181	zinc finger, DHHC-type containing 20	305923
0.000779	0.0185	2.45	10902564	Mdm2 p53 binding protein homolog (mouse)	314856
0.001267	0.0225	2.45	10721925	glutamate-rich WD repeat containing 1	308592
0.00131	0.0228	2.45	10750080	BTB and CNC homology 1, basic leucine zipper transcription factor 1	304127
0.002726	0.0315	2.45	10709575	sphingomyelin phosphodiesterase 1, acid lysosomal	308909
0.003074	0.0334	2.45	10803947	heparin-binding EGF-like growth factor	25433
0.004957	0.0421	2.45	10865732	potassium voltage-gated channel, shaker-related subfamily, member 1	24520
0.001299	0.0227	2.44	10756597	transmembrane protein 130	304280
0.002054	0.0275	2.44	10932584	chloride channel 5	25749
0.002159	0.0281	2.44	10713626	nuclear RNA export factor 1	59087
0.003316	0.0352	2.44	1081239	NA	NA

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0.003394	0.0355	2.44	1078099 0	cathepsin B	64529
0.003406	0.0355	2.44	1086142 9	RNA binding motif protein 28	312182
0.003768	0.0378	2.44	1082238 6	protein kinase (cAMP-dependent, catalytic) inhibitor alpha	114906
0.001042	0.021	2.43	1072123 2	interferon stimulated exonuclease gene 20-like 2	361977
0.001557	0.0245	2.43	1076763 1	neurofascin	116690
0.003212	0.0344	2.43	1091943 3	NA	NA
0.006647	0.0486	2.43	1073783 8	NA	NA
0.000669	0.018	2.42	1070548 5	fibrillarin	292747
0.008737	0.0563	2.42	1080668 7	coiled-coil domain containing 130	304656
0.009646	0.0583	2.42	1093502 1	NA	NA
0.001346	0.0232	2.41	1091836 4	NA	NA
0.002117	0.0279	2.41	1088541 7	NA	NA
0.005593	0.0448	2.41	1081934 7	NA	NA
0.006942	0.0495	2.41	1074396 4	NA	NA
0.000451	0.0152	2.4	1088500 6	thioredoxin-related transmembrane protein 1	362751
0.003327	0.0352	2.4	1084148 7	RNA-binding region containing protein 2-like	1E+08
0.00693	0.0495	2.4	1088502 9	FERM domain containing 6	257646
0.007187	0.0504	2.4	1077511 3	metal response element binding transcription factor 2	360905
0.009543	0.0583	2.4	1081845 2	solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 24	310791
0.00055	0.0167	2.39	1077427 4	epidermal growth factor receptor	24329
0.000745	0.0184	2.39	1082204 3	cadherin 6	25409
0.001378	0.0233	2.39	1085848 7	mannose-6-phosphate receptor, cation dependent	312689
0.002538	0.0304	2.39	1085195 2	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 5	362275
0.006747	0.049	2.39	1088642 9	solute carrier family 24 (sodium/potassium/calcium exchanger), member 4	314396
0.007716	0.0523	2.39	1079628 0	Sec61 alpha 2 subunit (S. cerevisiae)	361273
0.007873	0.0528	2.39	1093867 1	zinc finger, MYM-type 3	317260
0.008583	0.0556	2.39	1087944 6	defects in morphology 1 homolog (S. cerevisiae)	313563
0.001096	0.0213	2.38	1080664 0	calreticulin	64202
0.001239	0.0224	2.38	1089015 6	F-box protein 33	314157

0.001435	0.0236	2.38	10728507	solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	50567
0.004111	0.0391	2.38	10871711	tRNA isopentenyltransferase 1	362586
0.007052	0.0498	2.38	10871417	NA	NA
0.008775	0.0563	2.38	10813721	glyceraldehyde-3-phosphate dehydrogenase	24383
0.001701	0.0254	2.37	10811531	solute carrier family 7 (cationic amino acid transporter, y+ system), member 5	50719
0.002139	0.0279	2.37	10712706	choline kinase alpha	29194
0.003541	0.0366	2.37	10939570	zinc finger, DHHC-type containing 9	302808
0.005551	0.0446	2.37	10735897	NA	NA
0.000495	0.0159	2.36	10897683	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17	315133
0.000661	0.0179	2.36	10826137	family with sequence similarity 102, member B	365903
0.004612	0.0412	2.36	10827673	myelin oligodendrocyte glycoprotein	24558
0.000859	0.0192	2.34	10909691	beta-site APP cleaving enzyme 1	29392
0.000889	0.0194	2.34	10805895	gene trap locus 3	307642
0.001294	0.0227	2.34	10907793	methyltransferase like 7A	315306
0.002692	0.0314	2.34	10857931	solute carrier family 6 (neurotransmitter transporter, GABA), member 1	79212
0.002777	0.0317	2.34	10923560	basic leucine zipper and W2 domains 1	363232
0.004521	0.0407	2.34	10772478	nuclear transcription factor, X-box binding-like 1	289595
0.005486	0.0444	2.34	10796673	NA	NA
0.006813	0.0492	2.34	10900753	polypyrimidine tract binding protein 1	29497
0.002241	0.0286	2.33	10714788	cleavage stimulation factor, 3' pre-RNA subunit 2, tau	309338
0.002766	0.0316	2.33	10760397	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2	304290
0.002994	0.0329	2.33	10803238	NA	NA
0.007809	0.0526	2.33	10845306	calcium channel, voltage-dependent, beta 4 subunit	58942
0.00463	0.0412	2.32	10722818	solute carrier organic anion transporter family, member 3a1	140915
0.005483	0.0444	2.32	10787630	neurocan	58982
0.005642	0.0449	2.32	10923270	oligonucleotide/oligosaccharide-binding fold containing 2A	363227
0.00775	0.0523	2.32	10817759	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)	24450
0.001024	0.021	2.31	10889772	NA	NA
0.008759	0.0563	2.31	10840272	glyceraldehyde-3-phosphate dehydrogenase	24383
0.009427	0.0581	2.31	1085985	NA	NA

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0.001575	0.0245	2.3	10767067	NA	NA
0.001805	0.026	2.3	10808362	ubiquitin specific peptidase 10	307905
0.004146	0.0392	2.3	10800200	RIO kinase 3 (yeast)	361293
0.006437	0.0482	2.3	10830561	sestrin 1	294518
0.002181	0.0283	2.29	10738576	granulin	29143
0.00238	0.0297	2.29	10823049	NA	NA
0.002909	0.0324	2.29	10752050	transformer 2 beta homolog (Drosophila)	117259
0.003988	0.0388	2.29	10931919	lysosomal-associated membrane protein 2	24944
0.004294	0.0397	2.29	10720572	amyloid beta (A4) precursor-like protein 1	502317
0.004749	0.0414	2.29	10917361	hypothetical protein LOC100125362	1E+08
0.004957	0.0421	2.29	10747147	NA	NA
0.004966	0.0421	2.29	10799133	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5	307766
0.006854	0.0492	2.29	10709020	phosphoglucomutase 2-like 1	685076
0.008205	0.0541	2.29	10843125	protein-L-isoaspartate (D-aspartate) O-methyltransferase domain containing 2	311726
0.00088	0.0194	2.28	10707824	selenoprotein S	286900
0.000902	0.0194	2.28	10899248	activating transcription factor 1	315305
0.004157	0.0393	2.28	10928207	similar to hypothetical protein FLJ37953	301419
0.000824	0.0188	2.27	10939837	membrane magnesium transporter 1	302864
0.008293	0.0544	2.27	10766253	transmembrane protein 63a	289318
0.009554	0.0583	2.27	10906323	choline kinase beta	29367
0.00177	0.0258	2.26	10776968	transformer 2 beta homolog (Drosophila)	117259
0.004013	0.0388	2.26	10797681	inositol 1,3,4,5,6-pentakisphosphate 2-kinase	306808
0.00823	0.0541	2.26	10888758	brain and reproductive organ-expressed protein	362704
0.00322	0.0344	2.25	10867486	NA	NA
0.003827	0.0381	2.25	10707121	general transcription factor IIH, polypeptide 1	361580
0.004937	0.0421	2.25	10860951	asparagine synthetase	25612
0.000924	0.0197	2.23	10918368	anterior pharynx defective 1 homolog B (C. elegans)	300802
0.001154	0.0218	2.23	10917183	neural cell adhesion molecule 1	24586
0.001208	0.0222	2.22	10726979	cathepsin D	171293

0.002021	0.0274	2.22	10873303	dolichyl-diphosphooligosaccharide-protein glycosyltransferase	313648
0.00363	0.0371	2.22	10757599	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide	56010
0.00478	0.0415	2.22	10781191	tripartite motif-containing 35	498538
0.006344	0.0479	2.22	10734382	peripheral myelin protein 22	24660
0.006685	0.0487	2.22	10887981	splicing factor, arginine/serine-rich 7, 35kDa	362687
0.007457	0.0514	2.22	10890463	tripartite motif-containing 9	155812
0.001519	0.0243	2.21	10911765	ELOVL family member 5, elongation of long chain fatty acids (yeast)	171400
0.001684	0.0253	2.21	10921527	NA	NA
0.002198	0.0283	2.21	10936827	ATPase, H+ transporting, lysosomal accessory protein 2	302526
0.002387	0.0297	2.21	10779093	ubiquitin specific peptidase 54	408223
0.002469	0.03	2.21	10884200	EF-hand calcium binding domain 10	362727
0.003356	0.0353	2.21	10853515	similar to RIKEN cDNA C030048B08	296840
0.006051	0.0466	2.21	10894999	transmembrane and coiled-coil domain family 3	314751
0.009593	0.0583	2.21	10886109	similar to FLJ20689	314325
0.002387	0.0297	2.2	10702309	TATA box binding protein-like 1	689030
0.006005	0.0465	2.2	10722718	NA	NA
0.002273	0.0289	2.19	10872266	ring finger protein 19B	313806
0.00179	0.0259	2.18	10756692	aminoacyl tRNA synthetase complex-interacting multifunctional protein 2	288480
0.002565	0.0305	2.18	10913866	NA	NA
0.0034	0.0355	2.18	10864874	Ras association (RalGDS/AF-6) domain family member 4	362423
0.003975	0.0387	2.18	10824926	phosphatidylinositol-4-phosphate 5-kinase, type 1, alpha	365865
0.005251	0.0434	2.18	10760760	similar to Leukosialin precursor (Leucocyte sialoglycoprotein) (Sialophorin) (CD43) (W3/13 antigen)	288521
0.001801	0.026	2.17	10803577	synaptotagmin IV	64440
0.001751	0.0258	2.16	10814521	arylacetamide deacetylase-like 1	294930
0.003663	0.0372	2.16	10719728	ATPase, Na+/K+ transporting, alpha 3 polypeptide	24213
0.003152	0.0339	2.15	10756393	solute carrier family 7 (cationic amino acid transporter, y+ system), member 1	25648
0.005094	0.0427	2.15	10720430	proteasome (prosome, macropain) 26S subunit, non-ATPase, 8	292766
0.005735	0.0454	2.15	10724529	tripeptidyl peptidase I	83534
0.006139	0.0472	2.15	10931930	family with sequence similarity 70, member A	313453
0.006975	0.0495	2.15	1081547	doublecortin-like kinase 1	83825

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0.008344	0.0545	2.15	10751769	NA	NA
0.002474	0.03	2.14	10874866	nucleolar complex associated 2 homolog (S. cerevisiae)	313777
0.003339	0.0353	2.14	10854446	caldesmon 1	25687
0.004037	0.0388	2.14	10746014	NA	NA
0.005367	0.0437	2.14	10774375	pellino 1	305549
0.001434	0.0236	2.13	10776699	transmembrane protein 33	59303
0.00205	0.0275	2.13	10919034	NA	NA
0.003778	0.0378	2.13	10886816	similar to GTL2, imprinted maternally expressed untranslated	500717
0.00441	0.0402	2.13	10726244	similar to RIKEN cDNA 2310057M21	309029
0.005017	0.0425	2.12	10798856	cullin 2	361258
0.005302	0.0436	2.12	10773613	macrophage erythroblast attacher	298982
0.006169	0.0473	2.12	10861117	similar to CG3570-PA	500034
0.004709	0.0414	2.1	10929054	hypothetical protein MGC:72616	316530
0.00644	0.0482	2.1	10936993	pim-2 oncogene	317366
0.008767	0.0563	2.1	10877573	deleted in bladder cancer 1 (human)	140610
0.009061	0.0571	2.1	10881740	NA	NA
0.002897	0.0324	2.09	10814119	sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5A	310207
0.003513	0.0364	2.09	10803843	eukaryotic translation termination factor 1	307503
0.003884	0.0382	2.09	10851502	serine incorporator 3	296350
0.004029	0.0388	2.09	10843039	DnaJ (Hsp40) homolog, subfamily C, member 5	79130
0.004537	0.0408	2.09	10788053	NA	NA
0.004683	0.0413	2.09	10702996	insulin-like growth factor 2 receptor	25151
0.007203	0.0505	2.09	10792268	phosphatidic acid phosphatase type 2 domain containing 1B	680466
0.001993	0.0273	2.08	10776064	UTP3, small subunit (SSU) processome component, homolog (S. cerevisiae)	305258
0.004749	0.0414	2.08	10826039	seryl-tRNA synthetase	266975
0.005667	0.045	2.08	10749352	jumonji domain containing 6	360665
0.006276	0.0476	2.08	10757940	Williams-Beuren syndrome chromosome region 17 homolog (human)	288611
0.007047	0.0498	2.08	10758727	protein tyrosine phosphatase, non-receptor type 11	25622
0.008981	0.0568	2.08	10823462	solute carrier family 33 (acetyl-CoA transporter), member 1	64018

0.00922	0.0575	2.08	10857296	transmembrane protein 43	362401
0.001691	0.0253	2.07	10804391	transmembrane emp24 protein transport domain containing 7	252889
0.002134	0.0279	2.07	10889064	selenoprotein I	362713
0.00265	0.0312	2.07	10928220	potassium channel tetramerisation domain containing 18	301436
0.005039	0.0425	2.07	10914923	centrosomal protein 57kDa	315423
0.006676	0.0487	2.07	10827517	cystathionase (cystathionine gamma-lyase)	24962
0.001775	0.0258	2.06	10817898	tetraspanin 2	64521
0.003633	0.0371	2.06	10802579	mex-3 homolog C (C. elegans)	307271
0.004878	0.0419	2.06	10713706	glucosidase, alpha; neutral AB	293721
0.005057	0.0425	2.06	10748347	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	287765
0.005365	0.0437	2.06	10812954	polo-like kinase 2 (Drosophila)	83722
0.007814	0.0526	2.06	10745271	proteasome (prosome, macropain) subunit, alpha type 3	29670
0.002426	0.0299	2.05	10871597	forkhead box J3	313554
0.005058	0.0425	2.05	10927588	NA	NA
0.006205	0.0475	2.05	10883726	Rho-associated coiled-coil containing protein kinase 2	25537
0.006278	0.0476	2.05	10935935	solute carrier family 6 (neurotransmitter transporter, creatine), member 8	50690
0.006746	0.049	2.05	10877630	multiple EGF-like-domains 9	313270
0.007461	0.0514	2.05	10761329	similar to 0610007L01Rik protein	288616
0.003869	0.0382	2.04	10727303	immunoglobulin mu binding protein 2	29532
0.006816	0.0492	2.04	10782284	NA	NA
0.008811	0.0564	2.04	10792035	dual specificity phosphatase 4	60587
0.00409	0.039	2.03	10832299	pituitary tumor-transforming 1 interacting protein	365548
0.004707	0.0414	2.03	10856050	NA	NA
0.008821	0.0564	2.03	10872062	proteasome (prosome, macropain) subunit, beta type 2	29675
0.009631	0.0583	2.03	10918620	transcription factor 12	25720
0.005803	0.0455	2.02	10834812	mitochondrial ribosomal protein S2	362094
0.008451	0.0549	2.02	10864441	RING1 and YY1 binding protein	312603
0.005082	0.0427	2.01	10714576	very low density lipoprotein receptor	25696
0.005884	0.0459	2.01	10912003	trophoblast glycoprotein	83684
0.00648	0.0483	2.01	1093658	ubiquitin-like modifier activating enzyme 1	314432

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0.006609	0.0484	2.01	1090059 2	NA	NA
0.008183	0.0541	2.01	1086167 8	plexin A4, A	312213
0.009811	0.059	2.01	1070382 0	hippocampus abundant gene transcript 1	1E+08
0.002466	0.03	2	1091800 4	leucine rich repeat containing 49	300763
0.004969	0.0421	2	1088860 8	similar to limb-bud and heart	683626
0.003838	0.0381	0.5	1072270 6	NA	NA
0.004362	0.0402	0.5	1077014 0	NA	NA
0.004616	0.0412	0.5	1092992 9	myeloma overexpressed 2	681389
0.004805	0.0415	0.5	1090795 8	NA	NA
0.009841	0.059	0.5	1093512 0	NA	NA
0.001761	0.0258	0.49	1094002 0	NA	NA
0.00233	0.0294	0.49	1086734 5	NA	NA
0.003152	0.0339	0.49	1085616 8	NA	NA
0.003254	0.0348	0.49	1092328 5	NA	NA
0.003448	0.0358	0.49	1076360 9	NA	NA
0.007265	0.0506	0.49	1082174 6	NA	NA
0.007589	0.0517	0.49	1088342 3	hypothetical protein LOC500625	500625
0.008866	0.0565	0.49	1086537 2	NA	NA
0.008926	0.0567	0.49	1075804 8	NA	NA
0.009538	0.0583	0.49	1073281 9	NA	NA
0.002069	0.0275	0.48	1092662 7	NA	NA
0.002831	0.0319	0.48	1093561 1	NA	NA
0.004724	0.0414	0.48	1092038 8	NA	NA
0.005399	0.0439	0.48	1089905 3	NA	NA
0.007249	0.0506	0.48	1092216 9	NA	NA
0.007348	0.051	0.48	1093575 9	NA	NA
0.007933	0.0531	0.48	1076675 6	NA	NA
0.009218	0.0575	0.48	1073119 3	NA	NA
0.001567	0.0245	0.47	1083097 2	NA	NA

0.001685	0.0253	0.47	10708466	NA	NA
0.001764	0.0258	0.47	10708338	SH3-domain GRB2-like 3	81921
0.002702	0.0314	0.47	10917645	NA	NA
0.006021	0.0465	0.47	10909307	NA	NA
0.00629	0.0476	0.47	10725245	coenzyme Q7 homolog, ubiquinone (yeast)	25249
0.007618	0.0518	0.47	10925572	NA	NA
0.008962	0.0568	0.47	10764972	hypothetical protein LOC100302372	1E+08
0.001344	0.0232	0.46	10740502	NA	NA
0.00206	0.0275	0.46	10751969	NA	NA
0.002195	0.0283	0.46	10731416	NA	NA
0.002535	0.0304	0.46	10860806	NA	NA
0.002535	0.0304	0.46	10860809	NA	NA
0.002535	0.0304	0.46	10860812	NA	NA
0.002808	0.0318	0.46	10824790	NA	NA
0.003149	0.0339	0.46	10898467	pannexin 2	362979
0.004129	0.0391	0.46	10930299	NA	NA
0.004213	0.0393	0.46	10826416	NA	NA
0.004591	0.0411	0.46	10727854	NA	NA
0.004762	0.0414	0.46	10918480	NA	NA
0.005504	0.0444	0.46	10755670	NA	NA
0.005809	0.0455	0.46	10767032	NA	NA
0.005965	0.0463	0.46	10720144	selenoprotein V	499113
0.006865	0.0492	0.46	10874936	ribosomal protein S8-like	297756
0.007338	0.051	0.46	10787549	similar to RIKEN cDNA 2810428I15	306348
0.008981	0.0568	0.46	10831148	NA	NA
0.009058	0.0571	0.46	10867033	solute carrier organic anion transporter family, member 1a4	170698
0.00126	0.0225	0.45	10836145	NA	NA
0.002907	0.0324	0.45	10823425	NA	NA
0.004859	0.0418	0.45	10812779	NA	NA
0.005611	0.0449	0.45	1090005	olfactory receptor 1007	288887

			9		
0.006034	0.0466	0.45	10710089	phosphodiesterase 3B, cGMP-inhibited	29516
0.006583	0.0484	0.45	10837554	olfactory receptor 590	404858
0.00677	0.049	0.45	10812834	ADAM metallopeptidase with thrombospondin type 1 motif, 6	361886
0.009228	0.0575	0.45	10853710	glucocorticoid induced transcript 1	500026
0.001993	0.0273	0.44	10884599	neuronal PAS domain protein 3	299016
0.002278	0.0289	0.44	10863570	NA	NA
0.003046	0.0332	0.44	10851650	troponin C type 2 (fast)	296369
0.003144	0.0339	0.44	10903187	NA	NA
0.00331	0.0352	0.44	10909384	tripartite motif-containing 29	300656
0.003543	0.0366	0.44	10701636	NA	NA
0.004784	0.0415	0.44	10856070	NA	NA
0.006284	0.0476	0.44	10939180	NA	NA
0.00659	0.0484	0.44	10813833	NA	NA
0.00763	0.0518	0.44	10751209	NA	NA
0.007733	0.0523	0.44	10764365	NA	NA
0.008315	0.0545	0.44	10788542	NA	NA
0.008374	0.0546	0.44	10909196	olfactory receptor 1247	405093
0.008778	0.0563	0.44	10767039	NA	NA
0.009278	0.0576	0.44	10904414	NA	NA
0.00136	0.0232	0.43	10825111	NA	NA
0.002135	0.0279	0.43	10881917	similar to KIAA0833 protein	362665
0.004037	0.0388	0.43	10715697	deleted in primary ciliary dyskinesia	294004
0.005027	0.0425	0.43	10881341	NA	NA
0.0059	0.0459	0.43	10785005	protein phosphatase 3, catalytic subunit, gamma isoform	171378
0.006378	0.048	0.43	10915190	NA	NA
0.007511	0.0514	0.43	10756268	NA	NA
0.007511	0.0514	0.43	10756270	NA	NA
0.007511	0.0514	0.43	10756272	NA	NA
0.007511	0.0514	0.43	10759435	NA	NA

0.007511	0.0514	0.43	10759445	NA	NA
0.008004	0.0533	0.43	10910805	NA	NA
0.00918	0.0575	0.43	10915287	olfactory receptor 1155	300414
0.009632	0.0583	0.43	10918861	similar to RIKEN cDNA 2900055D03	367117
0.00079	0.0185	0.42	10779636	NA	NA
0.001034	0.021	0.42	10896284	regulating synaptic membrane exocytosis 2	116839
0.001353	0.0232	0.42	10883799	NA	NA
0.002543	0.0304	0.42	10934113	NA	NA
0.002728	0.0315	0.42	10811879	cytochrome c oxidase subunit VIc-1	286962
0.002985	0.0329	0.42	10780801	NA	NA
0.003404	0.0355	0.42	10813146	hypothetical protein LOC499530	499530
0.00419	0.0393	0.42	10766035	NA	NA
0.004214	0.0393	0.42	10798551	olfactory receptor 1662	291361
0.005048	0.0425	0.42	10882532	NA	NA
0.006568	0.0484	0.42	10763342	serpin peptidase inhibitor, clade B (ovalbumin), member 13	304690
0.00079	0.0185	0.41	10862457	NA	NA
0.001247	0.0224	0.41	10703424	NA	NA
0.001566	0.0245	0.41	10867795	NA	NA
0.001906	0.0266	0.41	10933574	NA	NA
0.002088	0.0277	0.41	10889223	NA	NA
0.002418	0.0298	0.41	10746000	glycerophosphodiester phosphodiesterase domain containing 1	303407
0.00371	0.0374	0.41	10754642	NA	NA
0.004246	0.0394	0.41	10847963	NA	NA
0.004378	0.0402	0.41	10824010	NA	NA
0.006247	0.0476	0.41	10863285	NA	NA
0.006847	0.0492	0.41	10714017	NA	NA
0.009221	0.0575	0.41	10864425	eukaryotic translation initiation factor 4E family member 3	297481
0.009424	0.0581	0.41	10776751	NOL1/NOP2/Sun domain family, member 7	305339
0.009588	0.0583	0.41	10702592	NA	NA
0.009967	0.0596	0.41	1088836	neurexin 1	60391

			8		
0.000561	0.0167	0.4	10889232	NA	NA
0.001071	0.0212	0.4	10804402	NA	NA
0.001144	0.0217	0.4	10875309	NA	NA
0.001231	0.0224	0.4	10804947	NA	NA
0.002865	0.0322	0.4	10773047	NA	NA
0.002934	0.0325	0.4	10905188	NA	NA
0.003268	0.0348	0.4	10774466	NA	NA
0.004181	0.0393	0.4	10883969	NA	NA
0.00483	0.0416	0.4	10783080	RNA component of mitochondrial RNA processing endoribonuclease	29536
0.005764	0.0454	0.4	10808194	NA	NA
0.005881	0.0459	0.4	10803194	establishment of cohesion 1 homolog 1 (S. cerevisiae)	680014
0.006236	0.0476	0.4	10723656	ankyrin repeat domain 42	293117
0.006267	0.0476	0.4	10809852	NA	NA
0.006533	0.0484	0.4	10813027	NA	NA
0.006593	0.0484	0.4	10800323	NA	NA
0.006955	0.0495	0.4	10832326	NA	NA
0.008192	0.0541	0.4	10836414	NA	NA
0.008192	0.0541	0.4	10845626	NA	NA
0.008877	0.0565	0.4	10879797	NA	NA
0.009382	0.058	0.4	10833744	NA	NA
0.000489	0.0158	0.39	10936161	NA	NA
0.000662	0.0179	0.39	10829828	NA	NA
0.000701	0.018	0.39	10886868	NA	NA
0.000896	0.0194	0.39	10796925	NA	NA
0.000933	0.0197	0.39	10799983	NA	NA
0.001268	0.0225	0.39	10734475	NA	NA
0.001433	0.0236	0.39	10867142	NA	NA
0.001913	0.0267	0.39	10923000	KDEL (Lys-Asp-Glu-Leu) containing 1	316370
0.002031	0.0274	0.39	10717801	NA	NA

0.002797	0.0318	0.39	10833823	sine oculis-binding protein homolog-like (Drosophila)	309860
0.002811	0.0318	0.39	10903212	NA	NA
0.003938	0.0385	0.39	10930606	NA	NA
0.003939	0.0385	0.39	10901999	mitochondrial ribosomal protein L42	299743
0.004022	0.0388	0.39	10827289	NA	NA
0.004322	0.0399	0.39	10791509	NA	NA
0.006768	0.049	0.39	10932339	NA	NA
0.009853	0.059	0.39	10938319	NA	NA
0.00041	0.0145	0.38	10723623	NA	NA
0.000523	0.0165	0.38	10745039	NA	NA
0.000699	0.018	0.38	10793493	NA	NA
0.000894	0.0194	0.38	10769016	Ral GEF with PH domain and SH3 binding motif 2	304887
0.00107	0.0212	0.38	10888422	NA	NA
0.001135	0.0217	0.38	10793052	NA	NA
0.001174	0.0221	0.38	10817040	NA	NA
0.001238	0.0224	0.38	10812464	NA	NA
0.001542	0.0244	0.38	10870010	NA	NA
0.003297	0.0351	0.38	10856175	NA	NA
0.003508	0.0364	0.38	10823819	relaxin/insulin-like family peptide receptor 1	295144
0.003848	0.0381	0.38	10940013	NA	NA
0.005541	0.0446	0.38	10889370	NA	NA
0.005781	0.0454	0.38	10793988	family with sequence similarity 193, member B	498703
0.007144	0.0502	0.38	10822420	NA	NA
0.007263	0.0506	0.38	10832137	NA	NA
0.007486	0.0514	0.38	10813029	solute carrier family 38, member 9	310091
0.000405	0.0144	0.37	10886888	NA	NA
0.001507	0.0242	0.37	10839174	NA	NA
0.002124	0.0279	0.37	10869614	NA	NA
0.002672	0.0313	0.37	10887400	NA	NA
0.004962	0.0421	0.37	1086613	immunoreceptor Ly49si2	494207

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0.005865	0.0459	0.37	10864968	NA	NA
0.007829	0.0526	0.37	10835985	NA	NA
0.008085	0.0538	0.37	10753185	crystallin, zeta (quinone reductase)-like 1	288256
0.008321	0.0545	0.37	10859935	chaperonin containing TCP1, subunit 8 (theta)-like 1	499967
0.008788	0.0563	0.37	10823348	NA	NA
0.000135	0.00768	0.36	10930588	NA	NA
0.000273	0.0118	0.36	10753257	phosphatidylinositol glycan anchor biosynthesis, class P	288238
0.00045	0.0152	0.36	10925692	NA	NA
0.000726	0.0184	0.36	10835652	NA	NA
0.001106	0.0215	0.36	10710886	NA	NA
0.001127	0.0217	0.36	10733298	olfactory receptor 1406	405064
0.001145	0.0217	0.36	10790346	NA	NA
0.001212	0.0222	0.36	10839968	NA	NA
0.001327	0.023	0.36	10714903	interferon-induced protein with tetratricopeptide repeats 3	309526
0.001687	0.0253	0.36	10761309	NA	NA
0.001896	0.0266	0.36	10893586	mitochondrial ribosomal protein L54	299628
0.001938	0.0268	0.36	10897891	NA	NA
0.002409	0.0298	0.36	10723231	NA	NA
0.002978	0.0329	0.36	10798467	NA	NA
0.004243	0.0394	0.36	10845947	serine/threonine kinase 39, STE20/SPS1 homolog (yeast)	54348
0.004427	0.0402	0.36	10714346	NA	NA
0.004427	0.0402	0.36	10813854	NA	NA
0.004427	0.0402	0.36	10815804	NA	NA
0.004427	0.0402	0.36	10905668	NA	NA
0.004427	0.0402	0.36	10921286	NA	NA
0.004902	0.042	0.36	10764376	NA	NA
0.005295	0.0436	0.36	10927668	NA	NA
0.006054	0.0466	0.36	10776262	NA	NA
0.007074	0.0499	0.36	10839296	NA	NA

0.007878	0.0528	0.36	10870108	NA	NA
0.008205	0.0541	0.36	10855090	olfactory receptor 808	405140
0.008982	0.0568	0.36	10935506	NA	NA
0.009077	0.0571	0.36	10903965	NA	NA
0.009616	0.0583	0.36	10888269	NA	NA
0.000215	0.01	0.35	10782599	similar to HT021	289928
0.000288	0.0119	0.35	10724307	olfactory receptor 127	405911
0.000549	0.0167	0.35	10797584	NA	NA
0.000686	0.018	0.35	10865105	ELKS/RAB6-interacting/CAST family member 1	266806
0.000898	0.0194	0.35	10858275	NA	NA
0.001046	0.021	0.35	10915492	ferredoxin 1-like	313786
0.00108	0.0213	0.35	10860848	microRNA mir-653	1E+08
0.001919	0.0267	0.35	10782166	propionyl-coenzyme A carboxylase, alpha polypeptide	687008
0.001931	0.0267	0.35	10838278	NA	NA
0.002146	0.028	0.35	10876206	NA	NA
0.002738	0.0316	0.35	10767034	NA	NA
0.00388	0.0382	0.35	10912581	anaphase promoting complex subunit 13	685029
0.004668	0.0413	0.35	10910722	NA	NA
0.005367	0.0437	0.35	10779724	NA	NA
0.008133	0.0541	0.35	10886958	NA	NA
0.000203	0.00962	0.34	10741918	RAN binding protein 17	303029
0.00086	0.0192	0.34	10841718	NA	NA
0.001181	0.0221	0.34	10896941	NA	NA
0.001383	0.0233	0.34	10809730	NA	NA
0.00167	0.0253	0.34	10765557	prefoldin subunit 2	685607
0.002089	0.0277	0.34	10733300	olfactory receptor 1407	287262
0.003157	0.0339	0.34	10740325	NA	NA
0.004004	0.0388	0.34	10887336	NA	NA
0.006444	0.0482	0.34	10728494	solute carrier family 22, member 25	192273
0.006451	0.0482	0.34	1076449	NA	NA

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0.006951	0.0495	0.34	1093884 7	NA	NA
0.007317	0.0509	0.34	1093922 3	NA	NA
0.000399	0.0143	0.33	1075721 1	COP9 constitutive photomorphogenic homolog subunit 6 (Arabidopsis)	304343
0.00059	0.0172	0.33	1080528 5	NA	NA
0.000678	0.018	0.33	1077279 9	NA	NA
0.000683	0.018	0.33	1073922 3	NA	NA
0.00107	0.0212	0.33	1084581 4	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (GalNAc-T3)	366061
0.001867	0.0265	0.33	1087873 2	NA	NA
0.002223	0.0285	0.33	1078839 9	myotubularin related protein 7	306490
0.002354	0.0295	0.33	1080059 2	similar to AW554918 protein	498832
0.003167	0.034	0.33	1077774 4	NA	NA
0.003613	0.037	0.33	1079854 7	NA	NA
0.004175	0.0393	0.33	1075291 9	glutamate receptor, ionotropic, kainate 1	29559
0.004687	0.0413	0.33	1080350 5	similar to 4930429A08Rik protein	500551
0.004772	0.0415	0.33	1070360 3	vomeronasal 2 receptor, 17	690716
0.005731	0.0454	0.33	1081227 0	NA	NA
0.007025	0.0498	0.33	1092869 8	NA	NA
0.008659	0.056	0.33	1084042 9	similar to RIKEN cDNA 1700010M22	311491
0.000395	0.0143	0.32	1092938 1	NA	NA
0.000419	0.0146	0.32	1090118 2	olfactory receptor 1096	302027
0.000526	0.0165	0.32	1079953 2	UPF2 regulator of nonsense transcripts homolog (yeast)	361271
0.000732	0.0184	0.32	1093816 9	NA	NA
0.000733	0.0184	0.32	1073913 5	proteasome (prosome, macropain) 26S subunit, ATPase, 5	81827
0.000753	0.0184	0.32	1079592 1	NA	NA
0.00093	0.0197	0.32	1072831 2	FK506 binding protein 2	293702
0.002378	0.0297	0.32	1071121 9	syntaxin 4	81803
0.002788	0.0318	0.32	1077141 2	lin-54 homolog (C. elegans)	305171
0.003328	0.0352	0.32	1093392 2	NA	NA
0.003834	0.0381	0.32	1074081 0	olfactory receptor 1382	287093

0.004423	0.0402	0.32	10750651	olfactory receptor 1561	288186
0.00563	0.0449	0.32	10771891	NA	NA
0.007103	0.05	0.32	10930576	NA	NA
0.007108	0.05	0.32	10793046	NA	NA
0.007565	0.0516	0.32	10709405	olfactory receptor 78	293221
0.000131	0.00761	0.31	10857435	NA	NA
0.000661	0.0179	0.31	10860272	membrane associated guanylate kinase, WW and PDZ domain containing 2	113970
0.000766	0.0184	0.31	10926054	NA	NA
0.000906	0.0195	0.31	10764314	mitochondrial ribosomal protein L42	299743
0.001045	0.021	0.31	10930409	NA	NA
0.001143	0.0217	0.31	10814678	NA	NA
0.001312	0.0228	0.31	10702201	A kinase (PRKA) anchor protein 7	361458
0.001396	0.0233	0.31	10901176	NA	NA
0.001397	0.0233	0.31	10767075	NA	NA
0.001487	0.024	0.31	10755890	similar to RIKEN cDNA 2310008H04	498119
0.001761	0.0258	0.31	10877876	NA	NA
0.001784	0.0259	0.31	10737680	SNF8, ESCRT-II complex subunit, homolog (S. cerevisiae)	287645
0.00181	0.026	0.31	10722419	NA	NA
0.00245	0.03	0.31	10860691	KRIT1, ankyrin repeat containing	362317
0.002513	0.0304	0.31	10760047	NA	NA
0.00276	0.0316	0.31	10742192	NA	NA
0.005523	0.0445	0.31	10845708	interferon induced with helicase C domain 1	499801
0.009765	0.059	0.31	10808563	cadherin 15	361432
0.000842	0.0191	0.3	10925761	fer (fms/fps related) protein kinase, testis specific 2	301737
0.00094	0.0198	0.3	10875087	NA	NA
0.00113	0.0217	0.3	10858871	sodium channel, nonvoltage-gated 1 alpha	25122
0.003067	0.0334	0.3	10886874	NA	NA
0.003388	0.0355	0.3	10794534	ataxin 1	25049
0.003587	0.0369	0.3	10777639	G protein-coupled receptor kinase 4	59077
0.004101	0.039	0.3	1075204	NA	NA

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0.005147	0.0429	0.3	10894808	NA	NA
0.000894	0.0194	0.29	10921290	TBC1 domain family, member 5	501088
0.000989	0.0205	0.29	10882071	similar to RIKEN cDNA 2810405K02	362676
0.003718	0.0374	0.29	10803025	F-box protein 15	361354
0.006846	0.0492	0.29	10776144	odontogenic, ameloblast asssociated	641555
0.008713	0.0562	0.29	10707352	NA	NA
0.009317	0.0577	0.29	10772160	NA	NA
0.000335	0.013	0.28	10875091	NA	NA
0.000394	0.0143	0.28	10791318	transmembrane protein 192	361137
0.00046	0.0153	0.28	10791461	similar to 2700029M09Rik protein	290706
0.000634	0.0177	0.28	10760762	NA	NA
0.001109	0.0215	0.28	10895024	NA	NA
0.001193	0.0221	0.28	10900122	NA	NA
0.001571	0.0245	0.28	10886894	NA	NA
0.001594	0.0247	0.28	10703568	NA	NA
0.001684	0.0253	0.28	10866205	NA	NA
0.001689	0.0253	0.28	10815150	La ribonucleoprotein domain family, member 1B	310348
0.001872	0.0265	0.28	10741831	NA	NA
0.003936	0.0385	0.28	10875656	NA	NA
0.004078	0.039	0.28	10722483	NA	NA
0.004789	0.0415	0.28	10871127	MOB1, Mps One Binder kinase activator-like 2C (yeast)	313511
0.005223	0.0433	0.28	10701648	vomeronasal 2 receptor, 5	679691
0.006897	0.0494	0.28	10768294	NA	NA
0.006939	0.0495	0.28	10821955	NA	NA
0.008355	0.0545	0.28	10755135	kininogen 1-like 1	288001
0.000145	0.00808	0.27	10768130	NA	NA
0.000164	0.00869	0.27	10845645	NA	NA
0.000285	0.0119	0.27	10875483	epithelial splicing regulatory protein 1	500409
0.000302	0.0123	0.27	10742866	NA	NA

0.000749	0.0184	0.27	10730591	ADP-ribosylation factor-like 3	64664
0.000795	0.0185	0.27	10900085	olfactory receptor 1059	288831
0.00081	0.0186	0.27	10774892	NA	NA
0.001074	0.0212	0.27	10937599	NA	NA
0.00185	0.0265	0.27	10879969	similar to 4930429A08Rik protein	500551
0.001874	0.0265	0.27	10796170	NA	NA
0.003691	0.0374	0.27	10709759	NA	NA
0.003869	0.0382	0.27	10770197	v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma)	29414
0.00396	0.0386	0.27	10818684	NA	NA
0.00653	0.0484	0.27	10714632	NA	NA
0.000778	0.0185	0.26	10775857	NA	NA
0.001582	0.0245	0.26	10739864	NA	NA
0.001776	0.0258	0.26	10854338	muskelin 1, intracellular mediator containing kelch motifs	83536
0.002466	0.03	0.26	10880381	eyes absent homolog 3 (Drosophila)	313027
0.002618	0.0309	0.26	10807881	NA	NA
0.004286	0.0397	0.26	10886858	NA	NA
0.005335	0.0437	0.26	10878314	NA	NA
0.006614	0.0484	0.26	10803824	cell division cycle 25 homolog C (S. pombe)	307511
0.008862	0.0565	0.26	10724260	olfactory receptor 92	293233
0.000398	0.0143	0.25	10795346	glutathione peroxidase 5	113919
0.001608	0.0248	0.25	10869664	NA	NA
0.001703	0.0254	0.25	10729629	NA	NA
0.004217	0.0393	0.25	10714879	NA	NA
0.004384	0.0402	0.25	10725078	pleckstrin homology domain containing, family A member 7	499249
0.007482	0.0514	0.25	10799896	NA	NA
0.008345	0.0545	0.25	10801580	NA	NA
5.84E-05	0.00493	0.24	10926769	Rh-associated glycoprotein	65207
5.84E-05	0.00493	0.24	10926806	Rh-associated glycoprotein	65207
0.003407	0.0355	0.24	10843142	GTPase activating protein testicular GAP1	294892
0.00393	0.0385	0.24	1092078	NA	NA

			7		
0.004068	0.0389	0.24	1090861 7	NA	NA
0.004693	0.0413	0.24	1088700 8	NA	NA
0.009161	0.0574	0.24	1079254 7	defensin NP-4 precursor	286958
0.000242	0.0111	0.23	1077847 2	zona pellucida binding protein	498415
0.000646	0.0178	0.23	1087508 9	NA	NA
0.000788	0.0185	0.23	1083566 6	mitogen-activated protein kinase associated protein 1	296648
0.001544	0.0244	0.23	1088686 2	NA	NA
0.002745	0.0316	0.23	1079152 0	NA	NA
0.002761	0.0316	0.23	1083404 6	NA	NA
0.003845	0.0381	0.23	1082766 9	olfactory receptor 1749	294203
0.004115	0.0391	0.23	1081577 8	similar to Restin	361953
0.006298	0.0476	0.23	1092202 7	cysteine-rich secretory protein 2	360445
0.007265	0.0506	0.23	1088688 0	NA	NA
3.19E-05	0.0036 6	0.22	1073243 9	guanine nucleotide binding protein (G protein), gamma 13	685451
0.000178	0.0088 6	0.22	1071199 5	NA	NA
0.000286	0.0119	0.22	1079984 8	NA	NA
0.001538	0.0244	0.22	1085348 1	ankyrin repeat and IBR domain containing 1	368062
0.001985	0.0273	0.22	1088932 8	NA	NA
0.002591	0.0307	0.22	1088689 6	NA	NA
0.00276	0.0316	0.22	1080257 4	NA	NA
0.002804	0.0318	0.22	1076200 9	anaphase promoting complex subunit 7	304490
0.005746	0.0454	0.22	1076002 4	similar to mKIAA0774 protein	498136
5.04E-05	0.0044 8	0.21	1075042 6	tetratricopeptide repeat domain 3	360702
0.000496	0.0159	0.21	1090814 5	NA	NA
0.001572	0.0245	0.21	1080032 8	NA	NA
0.003348	0.0353	0.21	1077732 8	Ly1 antibody reactive homolog (mouse)	289707
0.000135	0.0076 8	0.2	1080271 6	dymeclin	291433
0.000184	0.0089 8	0.2	1081857 1	NA	NA
0.000184	0.0089 8	0.2	1090809 6	NA	NA

0.000879	0.0194	0.2	10936847	NA	NA
0.001034	0.021	0.2	10767380	NA	NA
0.001127	0.0217	0.2	10756334	NA	NA
0.001454	0.0237	0.2	10750308	NA	NA
0.001687	0.0253	0.2	10886866	NA	NA
0.001873	0.0265	0.2	10852636	sulfide quinone reductase-like (yeast)	691966
0.004522	0.0407	0.2	10859762	NA	NA
0.004797	0.0415	0.2	10742927	olfactory receptor 1418	287313
3.85E-05	0.00393	0.19	10939168	SNF related kinase	170837
4.35E-05	0.00409	0.19	10907231	NA	NA
0.000373	0.014	0.19	10850440	NA	NA
0.001453	0.0237	0.19	10939795	NA	NA
0.004483	0.0405	0.19	10844681	ABO-family member 5	652927
0.000203	0.00962	0.18	10874166	arginine-glutamic acid dipeptide (RE) repeats	116665
0.000253	0.0114	0.18	10930555	NA	NA
0.001506	0.0242	0.18	10919074	NA	NA
0.001686	0.0253	0.18	10756530	NA	NA
0.002057	0.0275	0.18	10710674	NA	NA
0.002558	0.0305	0.18	10933974	NA	NA
0.002988	0.0329	0.18	10836241	NA	NA
0.007145	0.0502	0.18	10756519	NA	NA
0.000446	0.0152	0.17	10894814	NA	NA
0.001203	0.0222	0.17	10838538	NA	NA
0.004341	0.04	0.17	10774825	similar to hypothetical protein FLJ40298	498431
0.009157	0.0574	0.17	10830640	NA	NA
0.000253	0.0114	0.16	10894812	NA	NA
0.001056	0.0212	0.16	10887010	NA	NA
0.001748	0.0258	0.16	10787814	NA	NA
7.89E-05	0.00567	0.15	10856024	NA	NA
0.000375	0.014	0.15	1093026	ankyrin repeat domain 12	316775

			8		
0.002711	0.0315	0.15	10729065	NA	NA
2.1E-06	0.00106	0.11	10932269	NA	NA
0.002182	0.0283	0.11	10911797	glutathione S-transferase alpha 4	300850
0.000005	0.0018	0.088	10703224	NA	NA
1.7E-06	0.00106	0.055	10855946	NA	NA
1.1E-06	0.000904	0.05	10862359	NA	NA

APPENDIX B: TRANSCRIPTS DIFFERENTIALLY EXPRESSED IN POLYSOME-BOUND CA1 8HR AND NIC GROUPS

Parametricp-value	FDR	Fold-change	ProbeSet	Name	EntrezID
0.001923	0.197	46.41	10828154	heat shock 70kD protein 1B (mapped)	294254
0.001064	0.197	43.13	10770710	activating transcription factor 3	25389
0.000316	0.173	38.24	10761128	heat shock protein 1	24471
0.003758	0.211	28.93	10764551	prostaglandin-endoperoxide synthase 2	29527
0.001802	0.197	19.34	10785461	protocadherin 8	64865
0.001452	0.197	18.84	10831298	heat shock 70kD protein 1B (mapped)	294254
0.004214	0.212	17.54	10757082	zinc finger, AN1-type domain 2A	360772
0.003745	0.211	17.06	10806122	heme oxygenase (decycling) 1	24451
0.007367	0.234	16.89	10828827	cyclin-dependent kinase inhibitor 1A	114851
0.0096	0.243	16.69	10909892	crystallin, alpha B	25420
0.001245	0.197	15.88	10756343	heat shock 105kDa/110kDa protein 1	288444
0.002119	0.197	15.41	10797566	NA	NA
0.003376	0.202	14.25	10798702	inhibin beta-A	29200
0.002923	0.199	12.66	10711401	Bcl2-associated athanogene 3	293524
0.00119	0.197	11.22	10810295	DnaJ (Hsp40) homolog, subfamily B, member 1	361384
0.000675	0.185	11	10758344	vacuolar protein sorting 37 homolog B (S. cerevisiae)	288659
0.000981	0.197	10.44	10857314	solute carrier family 6 (neurotransmitter transporter, taurine), member 6	29464
0.007906	0.238	10.19	10768332	regulator of G-protein signaling 2	84583
0.000235	0.15	9.05	10767001	methionine adenosyltransferase II, alpha	171347
0.002147	0.197	9.04	10928154	heat shock protein 1 (chaperonin)	63868
0.007808	0.238	8.6	10925291	chemokine (C-X-C motif) receptor 7	84348
0.007796	0.238	8.44	10711053	NA	NA
0.009041	0.241	8.02	10803947	heparin-binding EGF-like growth factor	25433
0.008613	0.241	7.94	10837279	integrin, alpha V	296456
0.004553	0.212	7.86	10842500	similar to Docking protein 5 (Downstream of tyrosine kinase 5) (Protein dok-5)	502694
0.005689	0.213	7.83	10799291	Kruppel-like factor 6	58954
0.002146	0.197	7.76	10901367	NA	NA
0.003304	0.202	7.63	10802734	SMAD family member 7	81516
0.006426	0.221	7.6	10906608	solute carrier family 38, member 2	29642
0.005114	0.212	7.54	10890354	cyclin-dependent kinase-like 1 (CDC2-related kinase)	314198
0.004777	0.212	7.49	10834022	arrestin domain containing 3	309945
0.006866	0.221	7.42	10845725	potassium voltage-gated channel, subfamily H (eag-related), member 7	170739
0.00574	0.213	7.2	10903210	NA	NA
0.002998	0.199	6.95	10796673	NA	NA

0.009195	0.241	6.89	10860900	pyruvate dehydrogenase kinase, isozyme 4	89813
0.00123	0.197	6.85	10931308	prolyl 4-hydroxylase, alpha polypeptide I	64475
0.00057	0.177	6.77	10855701	aquaporin 1	25240
0.000535	0.177	6.76	10708672	prolylcarboxypeptidase (angiotensinase C)	293118
0.005812	0.214	6.71	10869772	NA	NA
0.001372	0.197	6.7	10863221	methionine adenosyltransferase II, alpha	171347
0.000792	0.197	6.64	10785773	sprouty homolog 2 (Drosophila)	306141
0.000358	0.177	6.62	10787005	NA	NA
0.003777	0.211	6.6	10865349	solute carrier family 2 (facilitated glucose transporter), member 3	25551
0.000202	0.148	6.48	10862461	NA	NA
0.002298	0.197	6.43	10847761	Cd44 molecule	25406
0.002167	0.197	6.39	10806981	SMAD family member 1	25671
0.002964	0.199	6.3	10936753	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked	317335
0.006058	0.215	6.27	10874866	nucleolar complex associated 2 homolog (S. cerevisiae)	313777
0.005408	0.212	6.24	10880095	serine incorporator 2	313057
0.00216	0.197	6.13	10827686	RT1 class I, locus M6, gene 1	414785
0.006813	0.221	6.11	10772580	NA	NA
0.005599	0.212	6.08	10836394	membrane-associated ring finger (C3HC4) 7	311059
0.008056	0.239	6.04	10821900	DnaJ (Hsp40) homolog, subfamily C, member 21	192210
0.001789	0.197	5.84	10845095	origin recognition complex, subunit 4-like (yeast)	295596
0.00645	0.221	5.83	10935131	ring finger protein 128	315911
0.009705	0.244	5.83	10742464	zinc finger protein 2	497897
0.000454	0.177	5.73	10749983	coxsackie virus and adenovirus receptor	89843
0.002841	0.199	5.67	10772330	NA	NA
0.007999	0.238	5.65	10928229	CDC-like kinase 1	301434
0.003723	0.211	5.61	10922962	NA	NA
0.00325	0.202	5.59	10713102	mitogen-activated protein kinase kinase kinase 11	309168
0.003883	0.211	5.56	10853202	putative homeodomain transcription factor 2	296762
0.002112	0.197	5.54	10867163	recombination signal binding protein for immunoglobulin kappa J region	679028
0.006698	0.221	5.52	10884205	TWIST neighbor	362728
0.009411	0.243	5.48	10799158	DIP2 disco-interacting protein 2 homolog C (Drosophila)	307067
1.05E-05	0.0414	5.45	10933345	toll-like receptor 7	317468
0.00498	0.212	5.43	10868289	DnaJ (Hsp40) homolog, subfamily A, member 1	65028
0.002099	0.197	5.37	10809328	homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1	85430
0.00118	0.197	5.24	10752754	nuclear receptor interacting protein 1	304157
0.006869	0.221	5.1	10864481	contactin 3 (plasmacytoma associated)	54279
0.004942	0.212	5.08	10854446	caldesmon 1	25687
0.005529	0.212	5.05	10927055	family with sequence similarity 135, member A	367235
0.00166	0.197	4.95	10776667	NA	NA
0.001137	0.197	4.94	10924230	NA	NA
0.006348	0.221	4.92	10812580	NA	NA

0.000251	0.15	4.85	10796507	solute carrier family 39 (zinc transporter), member 12	291328
0.004938	0.212	4.8	10729999	nucleolar complex associated 3 homolog (S. cerevisiae)	361753
0.007583	0.234	4.79	10721232	interferon stimulated exonuclease gene 20-like 2	361977
0.003831	0.211	4.78	10871133	MAP kinase-interacting serine/threonine kinase 1	500526
0.00865	0.241	4.78	10810951	NA	NA
0.007569	0.234	4.73	10702792	claudin 20	680178
0.003756	0.211	4.68	10924076	ribulose-5-phosphate-3-epimerase	501157
0.002779	0.199	4.56	10895152	KIT ligand	60427
0.000476	0.177	4.54	10701717	katanin p60 (ATPase-containing) subunit A1	292464
0.004681	0.212	4.54	10701663	similar to hypothetical protein	292449
0.001584	0.197	4.51	10730884	survival motor neuron domain containing 1	287768
0.000554	0.177	4.48	10789709	NA	NA
0.009907	0.247	4.48	10880404	platelet-activating factor receptor	58949
0.004091	0.212	4.47	10867731	calbindin 1	83839
0.005509	0.212	4.47	10872867	NA	NA
0.006653	0.221	4.43	10739204	survival motor neuron domain containing 1	287768
0.002266	0.197	4.4	10862473	NA	NA
0.002847	0.199	4.35	10732113	NA	NA
0.00084	0.197	4.3	10805100	similar to chromosome 18 open reading frame 54	361346
0.003715	0.211	4.29	10931717	complement component 3	24232
0.000078	0.0855	4.26	10884853	PRP39 pre-mRNA processing factor 39 homolog (S. cerevisiae)	314171
0.007931	0.238	4.26	10844801	zinc finger and BTB domain containing 6	366029
0.001537	0.197	4.21	10875939	zinc finger protein 292	50552
0.003356	0.202	4.17	10787212	myosin IXb	25486
0.007579	0.234	4.17	10938101	protein kinase, X-linked	501563
0.00912	0.241	4.17	10872291	tyrosyl-tRNA synthetase	313047
0.002103	0.197	4.11	10875936	zinc finger protein 292	50552
0.002328	0.197	4.09	10763913	retinoblastoma binding protein 5	304794
0.001405	0.197	4.07	10903482	solute carrier family 25, member 32	315023
0.001143	0.197	4.06	10781197	stathmin-like 4	79423
0.003168	0.201	4.06	10864874	Ras association (RalGDS/AF-6) domain family member 4	362423
0.008933	0.241	4.06	10733321	CDC like kinase 4	287269
0.007991	0.238	4.01	10935229	NA	NA
0.002398	0.197	3.99	10832478	pre-B lymphocyte 3	365550
0.003175	0.201	3.99	10854558	tripartite motif-containing 24	500084
0.00701	0.225	3.97	10890626	glycoprotein hormone beta 5	366668
0.001371	0.197	3.95	10751769	NA	NA
0.004692	0.212	3.95	10899964	methyltransferase like 7B	366792
0.002268	0.197	3.91	10884921	hypothetical protein LOC502894	502894
0.004128	0.212	3.91	10712340	CD151 molecule (Raph blood group)	64315
0.002617	0.199	3.87	10768339	NA	NA

0.009065	0.241	3.87	10752771	protease, serine, 7 (enterokinase)	288291
6.54E-05	0.0855	3.78	10772795	NA	NA
0.005039	0.212	3.76	10934631	lysophosphatidic acid receptor 4	302378
0.004954	0.212	3.74	10811531	solute carrier family 7 (cationic amino acid transporter, y+ system), member 5	50719
0.001208	0.197	3.73	10703508	NA	NA
0.002793	0.199	3.71	10774274	epidermal growth factor receptor	24329
0.005124	0.212	3.66	10844033	general transcription factor IIIC, polypeptide 5	362095
0.008903	0.241	3.66	10833337	golgi associated PDZ and coiled-coil motif containing	309774
0.009227	0.241	3.66	10886928	NA	NA
0.009227	0.241	3.66	10886960	NA	NA
0.000647	0.185	3.64	10778834	NA	NA
0.002373	0.197	3.61	10923345	heat shock protein 1 (chaperonin 10)	25462
0.006794	0.221	3.61	10918848	solute carrier family 17 (anion/sugar transporter), member 5	363103
0.000436	0.177	3.6	10907913	matrix metallopeptidase 8	63849
0.002017	0.197	3.59	10762115	N(alpha)-acetyltransferase 25, NatB auxiliary subunit	360811
0.009542	0.243	3.59	10827989	metallothionein 2A	689415
0.001701	0.197	3.57	10824051	dual endothelin 1, angiotensin II receptor	446170
3.91E-05	0.0855	3.55	10840396	Sec23 homolog B (S. cerevisiae)	362226
0.00409	0.212	3.44	10877431	haloacid dehalogenase-like hydrolase domain containing 3	688746
0.006029	0.215	3.43	10822099	sertolin	64038
0.009166	0.241	3.41	10934794	interferon stimulated exonuclease gene 20-like 2	361977
0.009219	0.241	3.41	10746014	NA	NA
0.004603	0.212	3.37	10767763	proline/arginine-rich end leucine-rich repeat protein	84400
0.008401	0.241	3.37	10903736	ectonucleotide pyrophosphatase/phosphodiesterase 2	84050
0.002121	0.197	3.31	10727493	NA	NA
0.00598	0.215	3.28	10765335	cellular repressor of E1A-stimulated genes 1	289185
0.002362	0.197	3.24	10883785	ornithine decarboxylase 1	24609
0.003032	0.199	3.22	10901783	apoptotic peptidase activating factor 1	78963
7.43E-05	0.0855	3.21	10856673	solute carrier family 4, sodium bicarbonate cotransporter, member 5	297386
0.002621	0.199	3.21	10705485	fibrillarin	292747
0.005184	0.212	3.15	10827531	splicing factor, arginine/serine-rich 11	502603
0.000168	0.138	3.14	10886280	spermatogenesis associated 7	192225
0.003544	0.208	3.09	10820213	NA	NA
0.008239	0.241	3.08	10886868	NA	NA
0.0086	0.241	3.06	10889657	NA	NA
0.001	0.197	3.03	10811875	TAF5-like RNA polymerase II, p300/CBP-associated factor (PCAF)-associated factor	307927
0.004773	0.212	2.99	10757175	cytochrome P450, family 3, subfamily a, polypeptide 9	171352
0.007494	0.234	2.99	10762704	splicing factor, arginine/serine-rich 9	288701
0.007241	0.231	2.95	10885417	NA	NA
0.002891	0.199	2.92	10763477	Mki67 (FHA domain) interacting nucleolar phosphoprotein	246042
0.005905	0.215	2.9	10844779	ring finger and CCCH-type zinc finger domains 2	311909

0.006437	0.221	2.9	10833366	NA	NA
0.006865	0.221	2.87	10885006	thioredoxin-related transmembrane protein 1	362751
0.00109	0.197	2.86	10727373	NA	NA
0.004217	0.212	2.84	10789246	NA	NA
0.001522	0.197	2.81	10818687	NA	NA
0.005184	0.212	2.8	10736914	NA	NA
0.009134	0.241	2.8	10747564	family with sequence similarity 134, member C	360632
0.008617	0.241	2.74	10788374	mitochondrial tumor suppressor 1	306487
0.005375	0.212	2.73	10839579	dual specificity phosphatase 2	311406
0.001907	0.197	2.67	10763685	NA	NA
0.002194	0.197	2.65	10890210	MAM domain containing glycosylphosphatidylinositol anchor 2	314180
0.006677	0.221	2.59	10859774	NA	NA
0.003331	0.202	2.58	10934118	androgen receptor	24208
0.009515	0.243	2.57	10713089	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	309165
0.005511	0.212	2.55	10897203	NA	NA
0.002463	0.199	2.54	10798471	TAF5-like RNA polymerase II, p300/CBP-associated factor (PCAF)-associated factor	307927
0.002768	0.199	2.48	10909646	FXYD domain-containing ion transport regulator 2	29639
0.003884	0.211	2.45	10937688	NA	NA
0.006289	0.221	2.4	10766565	similar to probable ATP-dependent RNA helicase - mouse	364073
0.000847	0.197	2.38	10826137	family with sequence similarity 102, member B	365903
0.00543	0.212	2.34	10937558	TSR2, 20S rRNA accumulation, homolog (S. cerevisiae)	317418
0.006825	0.221	2.34	10853916	NA	NA
0.003177	0.201	2.31	10794721	signal sequence receptor, alpha	361233
0.003989	0.212	2.31	10917361	hypothetical protein LOC100125362	1E+08
0.000938	0.197	2.26	10739558	solute carrier family 16, member 5 (monocarboxylic acid transporter 6)	690212
0.009148	0.241	2.2	10770252	transcription factor B2, mitochondrial	289307
0.004338	0.212	2.15	10889522	dihydrolipoamide dehydrogenase	298942
0.002859	0.199	2.13	10802579	mex-3 homolog C (C. elegans)	307271
0.003938	0.211	2.11	10824091	doublecortin-like kinase 2	310698
0.004174	0.212	0.47	10837528	olfactory receptor 566	404867
0.005454	0.212	0.45	10918485	NA	NA
0.001171	0.197	0.43	10915988	NA	NA
0.003008	0.199	0.41	10908175	olfactory receptor 1148	405173
0.005869	0.214	0.41	10710886	NA	NA
0.005969	0.215	0.41	10859838	NA	NA
0.007391	0.234	0.41	10846948	olfactory receptor 480	404890
0.004592	0.212	0.4	10805285	NA	NA
0.005541	0.212	0.4	10756225	NA	NA
0.005613	0.212	0.4	10716498	NA	NA
0.005159	0.212	0.39	10909105	olfactory receptor 1197	406021
0.008218	0.241	0.39	10885235	protein kinase C, eta	81749

0.002221	0.197	0.38	10836352	NA	NA
0.000494	0.177	0.37	10708466	NA	NA
0.002389	0.197	0.37	10853137	F-box and leucine-rich repeat protein 13	1E+08
0.002555	0.199	0.37	10866120	immunoreceptor Ly49si1	494206
0.007982	0.238	0.37	10932617	NA	NA
0.00467	0.212	0.36	10871038	NA	NA
0.006763	0.221	0.35	10939186	NA	NA
0.0084	0.241	0.34	10779766	NA	NA
0.003208	0.201	0.33	10856168	NA	NA
0.008892	0.241	0.33	10894810	NA	NA
0.008945	0.241	0.33	10791964	NA	NA
0.004723	0.212	0.32	10763351	serine (or cysteine) peptidase inhibitor, clade B (ovalbumin), member 11	304689
0.005441	0.212	0.32	10742866	NA	NA
0.000591	0.177	0.31	10775134	NA	NA
0.004816	0.212	0.31	10901713	NA	NA
0.006715	0.221	0.31	10889328	NA	NA
0.009367	0.243	0.31	10798284	Prolactin family 3, subfamily b, member 1	24283
0.002696	0.199	0.3	10721315	tonin	24841
0.003073	0.2	0.3	10860226	leucine rich repeat containing 17	502715
0.005556	0.212	0.3	10933559	protein phosphatase, EF-hand calcium binding domain 1	317498
0.00752	0.234	0.3	10836065	NA	NA
0.009138	0.241	0.3	10722483	NA	NA
0.009353	0.243	0.3	10756749	zinc finger protein 316	304293
0.001484	0.197	0.29	10909196	olfactory receptor 1247	405093
0.00811	0.239	0.29	10839970	NA	NA
0.00949	0.243	0.29	10927264	NA	NA
0.00518	0.212	0.28	10714610	NA	NA
0.009929	0.247	0.28	10939202	NA	NA
0.001637	0.197	0.27	10710089	phosphodiesterase 3B, cGMP-inhibited	29516
0.001949	0.197	0.27	10752253	NA	NA
0.004765	0.212	0.27	10862947	NA	NA
0.005437	0.212	0.27	10813253	complement component 6	24237
0.009573	0.243	0.27	10834600	NA	NA
0.009993	0.248	0.27	10847229	olfactory receptor 720	405955
0.004335	0.212	0.25	10814176	NA	NA
0.008403	0.241	0.25	10765169	NA	NA
0.005076	0.212	0.24	10750655	olfactory receptor 1563	288184
0.006799	0.221	0.24	10771206	hypothetical protein LOC100188984	1E+08
0.006799	0.221	0.23	10920741	NA	NA
0.002365	0.197	0.22	10781525	NA	NA
0.007718	0.237	0.22	10938824	NA	NA

0.009026	0.241	0.21	10873885	NA	NA
0.001304	0.197	0.2	10732439	guanine nucleotide binding protein (G protein), gamma 13	685451
0.001262	0.197	0.19	10724047	NA	NA
0.005518	0.212	0.19	10932269	NA	NA
0.008487	0.241	0.18	10760760	similar to Leukosialin precursor (Leucocyte sialoglycoprotein) (Sialophorin) (CD43) (W3/13 antigen)	288521
0.002766	0.199	0.17	10704376	NA	NA
0.003945	0.211	0.16	10900041	NA	NA
0.00484	0.212	0.15	10830824	olfactory receptor 1722	406016

APPENDIX C: TRANSCRIPTS DIFFERENTIALLY EXPRESSED IN UNBOUND CA3 8HR AND NIC GROUPS

Parametric p-value	FDR	Fold-change	ProbeSet	Name	EntrezID
1E-07	0.000658	57.55	10719977	melanoma inhibitory activity	81510
9E-07	0.00296	18.15	10761128	heat shock protein 1	24471
1.7E-06	0.00373	19.57	10725387	cerebellar degeneration-related 2	308958
2.5E-06	0.00395	13.54	10821914	brix domain containing 2	294799
0.000003	0.00395	16.43	10770710	activating transcription factor 3	25389
3.8E-06	0.00417	17.84	10737196	NA	NA
4.7E-06	0.00442	10.25	10779825	NA	NA
6.9E-06	0.0046	10.94	10732652	dual specificity phosphatase 1	114856
8.4E-06	0.0046	14.61	10797527	growth arrest and DNA-damage-inducible, gamma	291005
9.3E-06	0.0046	12.24	10864662	transmembrane protein 111	312640
9.4E-06	0.0046	10.01	10773722	NA	NA
9.5E-06	0.0046	15.13	10868662	zinc finger, CCHC domain containing 7	298086
9.9E-06	0.0046	11.62	10703128	NA	NA
9.9E-06	0.0046	15.27	10809392	metallothionein 1a	24567
1.23E-05	0.0046	17	10862867	growth arrest and DNA-damage-inducible, alpha	25112
1.23E-05	0.0046	10.69	10809909	NA	NA
1.31E-05	0.0046	8.26	10773180	heparan sulfate (glucosamine) 3-O-sulfotransferase 1	84406
1.37E-05	0.0046	25.39	10831077	immediate early response 3	294235
1.41E-05	0.0046	7.92	10864621	jagunal homolog 1 (Drosophila)	502872
1.48E-05	0.0046	9.94	10734828	NA	NA
1.54E-05	0.0046	13.39	10796411	metallothionein 1a	24567
1.54E-05	0.0046	13.52	10858686	prohibitin 2	114766
1.61E-05	0.0046	14.66	10937867	NA	NA
0.000019	0.00521	11.44	10703104	Park2 co-regulated	499021

2.01E-05	0.00529	10.53	10937523	NA	NA
0.000023	0.00574	8.52	10827686	RT1 class I, locus M6, gene 1	414785
2.45E-05	0.00574	13.44	10767001	methionine adenosyltransferase II, alpha	171347
2.46E-05	0.00574	0.13	10737647	NA	NA
2.53E-05	0.00574	12.82	10800426	transthyretin	24856
2.85E-05	0.00625	28.11	10748336	NA	NA
3.03E-05	0.00643	9.92	10845624	NA	NA
3.13E-05	0.00643	6.84	10923580	CASP8 and FADD-like apoptosis regulator	117279
0.000033	0.00658	11.26	10828827	cyclin-dependent kinase inhibitor 1A	114851
0.000036	0.00676	7.42	10839579	dual specificity phosphatase 2	311406
3.71E-05	0.00676	10.29	10914935	LOC363015	363015
3.83E-05	0.00676	7.44	10899187	glycerol-3-phosphate dehydrogenase 1 (soluble)	60666
3.84E-05	0.00676	7.34	10838823	ChaC, cation transport regulator homolog 1 (E. coli)	362196
3.95E-05	0.00676	6.57	10823733	programmed cell death 10	494345
4.04E-05	0.00676	7.09	10918820	NA	NA
4.11E-05	0.00676	7.79	10870837	thioredoxin domain containing 12 (endoplasmic reticulum)	298370
4.31E-05	0.00691	7.6	10809893	NA	NA
4.71E-05	0.00721	10.64	10711894	protein phosphatase 2, regulatory subunit B, delta isoform	246255
4.94E-05	0.00721	5.97	10872062	proteasome (prosome, macropain) subunit, beta type 2	29675
4.94E-05	0.00721	12.14	10790002	guanine nucleotide binding protein-like 3 (nucleolar)	290556
5.03E-05	0.00721	7.65	10710611	ubiquitin family domain containing 1	293454
5.04E-05	0.00721	8.55	10721865	protein phosphatase 1, regulatory (inhibitor) subunit 15A	171071
5.25E-05	0.00729	5.6	10730895	NA	NA
5.32E-05	0.00729	8.91	10914074	5-azacytidine induced 2	316051
5.54E-05	0.00744	9.6	10744766	aspartoacylase	79251
5.78E-05	0.0076	7.69	10728538	polymerase (RNA) II (DNA directed) polypeptide G	117017
6.26E-05	0.00799	9.16	10868935	Sec61 beta subunit	298068
6.32E-05	0.00799	9.67	10858487	mannose-6-phosphate receptor, cation dependent	312689
6.53E-05	0.00806	6.16	10939816	motile sperm domain containing 1	317312
6.78E-05	0.008	6.4	1076298	replication protein A3	296883

	06		1		
6.86E-05	0.00806	5.68	10807722	thioredoxin-like 4B	292008
6.87E-05	0.00806	6.09	10778399	motile sperm domain containing 1	317312
6.98E-05	0.00806	8.6	10809899	NA	NA
7.23E-05	0.0082	5.45	10850160	tRNA methyltransferase 6 homolog (S. cerevisiae)	311441
7.76E-05	0.00865	10.23	10803953	steroid receptor RNA activator 1	252891
8.05E-05	0.00883	15.2	10736697	chemokine (C-C motif) ligand 2	24770
8.89E-05	0.00933	6.44	10732805	NA	NA
9.03E-05	0.00933	7.37	10725289	glycoprotein 2 (zymogen granule membrane)	171459
9.06E-05	0.00933	21.48	10900358	growth arrest and DNA-damage-inducible, beta	299626
9.24E-05	0.00933	7.56	10879545	NA	NA
9.49E-05	0.00933	5.43	10871415	NA	NA
0.000095	0.00933	6.6	10799291	Kruppel-like factor 6	58954
0.000095	0.00933	6.54	10811589	ring finger protein 166	365022
9.84E-05	0.00947	10.72	10888608	similar to limb-bud and heart	683626
9.93E-05	0.00947	7.35	10890003	protein phosphatase 2, regulatory subunit B", gamma	362739
0.000106	0.00963	6.42	10792268	phosphatidic acid phosphatase type 2 domain containing 1B	680466
0.000107	0.00963	5.08	10806122	heme oxygenase (decycling) 1	24451
0.00011	0.00963	14.88	10792859	ATPase, class VI, type 11A	306600
0.000111	0.00963	6.43	10709875	adrenomedullin	25026
0.000111	0.00963	5.92	10834022	arrestin domain containing 3	309945
0.000112	0.00963	6	10737518	leucine rich repeat containing 59	287633
0.000114	0.00963	0.05	10771222	NA	NA
0.000115	0.00963	0.16	10884574	NA	NA
0.000115	0.00963	6.59	10829703	transcription factor A, mitochondrial	83474
0.000117	0.00963	9.11	10772330	NA	NA
0.000118	0.00963	6.62	10795422	v-ral simian leukemia viral oncogene homolog A (ras related)	81757
0.000121	0.00963	8.07	10890156	F-box protein 33	314157
0.000123	0.00963	5.11	10740869	tumor necrosis factor receptor superfamily, member 12a	302965
0.000124	0.00963	5.79	10829759	NA	NA

0.000126	0.00963	7.17	10734242	microfibrillar-associated protein 4	287382
0.000127	0.00963	6.19	10937241	cysteine-rich protein 2	338401
0.000127	0.00963	0.11	10813833	NA	NA
0.000127	0.00963	0.18	10847166	olfactory receptor 678	295884
0.000131	0.00967	5.72	10732113	NA	NA
0.000133	0.00967	7.95	10834159	transmembrane protein 203	311800
0.000134	0.00967	0.063	10742192	NA	NA
0.000134	0.00967	0.2	10722862	NA	NA
0.000138	0.00982	11.9	10710620	dynactin 5	308961
0.000139	0.00982	18.14	10764551	prostaglandin-endoperoxide synthase 2	29527
0.000141	0.00987	16.36	10768332	regulator of G-protein signaling 2	84583
0.000146	0.0101	5.9	10750371	NA	NA
0.000149	0.0101	0.087	10703603	vomeronasal 2 receptor, 17	690716
0.000149	0.0101	13.16	10878112	Jun oncogene	24516
0.00015	0.0101	4.86	10891364	alkB, alkylation repair homolog (E. coli)	362766
0.000152	0.0101	9.97	10763477	Mki67 (FHA domain) interacting nucleolar phosphoprotein	246042
0.000154	0.0101	7.19	10804145	Yip1 domain family, member 5	361315
0.000164	0.0106	7.28	10887939	CDC42 effector protein (Rho GTPase binding) 3	313838
0.000166	0.0107	4.55	10780908	NA	NA
0.000169	0.0107	7.37	10898947	NA	NA
0.000171	0.0107	6.86	10928902	transmembrane BAX inhibitor motif containing 1	316516
0.000174	0.0107	0.14	10798126	NA	NA
0.000176	0.0107	7.5	10704413	N-ethylmaleimide-sensitive factor attachment protein, alpha	140673
0.000178	0.0107	6.75	10891026	zinc finger, FYVE domain containing 1	299188
0.000179	0.0107	7.03	10924438	NA	NA
0.00018	0.0107	5.58	10927051	NA	NA
0.000181	0.0107	5.56	10907793	methyltransferase like 7A	315306
0.000183	0.0107	6.22	10701797	splicing factor 3b, subunit 5	680891
0.00019	0.0107	0.17	10702023	NA	NA
0.000191	0.010	7.86	1084608	methyltransferase like 5	502632

	7		7		
0.000193	0.0107	6.12	10876270	stomatin (Epb7.2)-like 2	298203
0.000194	0.0107	6.25	10878009	NA	NA
0.000195	0.0107	4.89	10890354	cyclin-dependent kinase-like 1 (CDC2-related kinase)	314198
0.000197	0.0107	7.17	10820393	family with sequence similarity 151, member B	499507
0.000199	0.0107	6.81	10752897	RWD domain containing 2B	304132
0.000199	0.0107	6.45	10830908	ring finger protein 39	171387
0.000202	0.0107	7.89	10890484	NA	NA
0.000202	0.0107	0.19	10820611	NA	NA
0.000203	0.0107	4.94	10850744	proteasome inhibitor subunit 1	689852
0.000205	0.0107	0.19	10778137	neurofilament, heavy polypeptide	24587
0.000206	0.0107	6.9	10873258	similar to RIKEN cDNA 1110008F13	296315
0.000206	0.0107	6.53	10757082	zinc finger, AN1-type domain 2A	360772
0.000206	0.0107	0.21	10703224	NA	NA
0.000206	0.0107	5.64	10842500	similar to Docking protein 5 (Downstream of tyrosine kinase 5) (Protein dok-5)	502694
0.000208	0.0107	4.77	10907749	glycosylation dependent cell adhesion molecule 1	25258
0.00021	0.0107	0.042	10729065	NA	NA
0.000213	0.0108	4.54	10874450	leucine rich repeat containing 47	362672
0.000226	0.0109	5.39	10772243	NA	NA
0.00023	0.0109	7.59	10819653	B-cell CLL/lymphoma 10	83477
0.000231	0.0109	13.86	10871413	NA	NA
0.000231	0.0109	7.95	10819555	general transcription factor IIB	81673
0.000234	0.0109	7.73	10822356	NA	NA
0.000236	0.0109	0.19	10866180	killer cell lectin-like receptor subfamily A, member 22	24933
0.000236	0.0109	5.03	10936067	ubiquitin-like 4	293864
0.000238	0.0109	4.84	10769131	calcyclin binding protein	289144
0.000238	0.0109	5.53	10910666	NA	NA
0.000238	0.0109	0.11	10774825	similar to hypothetical protein FLJ40298	498431
0.000238	0.0109	9.46	10769672	regulator of G-protein signaling 4	29480
0.000239	0.0109	6.92	10808836	ARV1 homolog (S. cerevisiae)	292097

0.000243	0.0109	8.91	10796134	RNA binding motif protein 17	291295
0.000246	0.0109	5.59	10915333	zinc finger protein 426-like	367034
0.000247	0.0109	5.09	10709261	similar to RIKEN cDNA 3200002M19	293155
0.000248	0.0109	7.24	10808103	telomeric repeat binding factor 2, interacting protein	307861
0.000248	0.0109	5.31	10765115	vesicle-associated membrane protein 4	364033
0.000248	0.0109	6.14	10841637	neuronatin	94270
0.000249	0.0109	5.93	10792344	NA	NA
0.000249	0.0109	7.57	10828154	heat shock 70kD protein 1B (mapped)	294254
0.000255	0.011	4.7	10868289	DnaJ (Hsp40) homolog, subfamily A, member 1	65028
0.000257	0.011	6.27	10932310	NA	NA
0.000257	0.011	0.12	10724047	NA	NA
0.000261	0.0111	6.62	10859371	serine/threonine kinase receptor associated protein	297699
0.000262	0.0111	6.93	10756671	similar to CG14980-PB	360768
0.000264	0.0111	0.17	10886840	NA	NA
0.000265	0.0111	4.79	10767016	cadherin 19, type 2	360835
0.000266	0.0111	5.41	10761394	phosphoserine phosphatase	304429
0.000271	0.0112	4.72	10800200	RIO kinase 3 (yeast)	361293
0.000275	0.0113	4.49	10805254	peroxisomal biogenesis factor 19	289233
0.000277	0.0113	4.3	10773716	selenoprotein M	498398
0.000279	0.0113	4.33	10807625	nuclear import 7 homolog (S. cerevisiae)	192180
0.00028	0.0113	5.98	10776968	transformer 2 beta homolog (Drosophila)	117259
0.000281	0.0113	6.1	10912003	trophoblast glycoprotein	83684
0.000285	0.0114	5.54	10935177	claudin 2	300920
0.000291	0.0115	0.16	10893364	olfactory receptor 990	298724
0.000291	0.0115	8.04	10901231	TIMP metallopeptidase inhibitor 3	25358
0.000294	0.0115	0.22	10855946	NA	NA
0.000296	0.0115	5.62	10785620	COMM domain containing 6	498559
0.000297	0.0115	4.37	10911993	mitochondrial ribosomal protein L41	296551
0.000301	0.0115	4.81	10867882	splicing factor, arginine/serine-rich 18	297942
0.000301	0.011	0.09	1072458	NA	NA

	5	3	2		
0.000303	0.0115	5.05	10922173	NA	NA
0.000305	0.0115	0.12	10722373	NA	NA
0.00031	0.0116	5.39	10801996	RNA binding motif protein 22	307410
0.000314	0.0117	11.16	10810295	DnaJ (Hsp40) homolog, subfamily B, member 1	361384
0.000321	0.0119	4.97	10868007	caspase 8 associated protein 2	313128
0.000324	0.0119	0.2	10810236	latrophilin 1	65096
0.000324	0.0119	0.11	10847047	olfactory receptor 545	405941
0.000338	0.0123	5.86	10780478	guanosine monophosphate reductase 2	192357
0.000341	0.0123	5.59	10918368	anterior pharynx defective 1 homolog B (C. elegans)	300802
0.000341	0.0123	6.48	10798702	inhibin beta-A	29200
0.000342	0.0123	5.88	10864874	Ras association (RalGDS/AF-6) domain family member 4	362423
0.000344	0.0123	0.19	10782434	NA	NA
0.00035	0.0124	4.62	10853916	NA	NA
0.000352	0.0124	0.22	10903518	NA	NA
0.000355	0.0124	5.85	10910836	similar to 2010321M09Rik protein	300782
0.000355	0.0124	4.32	10840321	small nuclear ribonucleoprotein polypeptide B"	362223
0.000356	0.0124	4.85	10853676	ACN9 homolog (S. cerevisiae)	362323
0.000358	0.0124	4.49	10755426	ATP-binding cassette, subfamily F (GCN20), member 3	287982
0.000359	0.0124	5.32	10737770	NA	NA
0.000363	0.0124	4.69	10773160	similar to HESB like domain containing 1	500694
0.000369	0.0126	9.13	10853515	similar to RIKEN cDNA C030048B08	296840
0.000375	0.0127	4.81	10885959	similar to HESB like domain containing 1	500694
0.000375	0.0127	3.88	10812580	NA	NA
0.000397	0.0133	4.4	10843854	mitochondrial ribosomal protein L41	296551
0.000405	0.0135	4.74	10780003	NA	NA
0.000406	0.0135	7.62	10871133	MAP kinase-interacting serine/threonine kinase 1	500526
0.000409	0.0135	4.24	10926155	similar to RIKEN cDNA 1700001E04	302192
0.000411	0.0135	10.59	10912076	similar to Selenoprotein H	502642
0.000417	0.0135	0.098	10729312	NA	NA

0.000424	0.0135	3.98	10880233	YTH domain family, member 2	313053
0.000427	0.0135	5.92	10927259	coiled-coil domain containing 115	363213
0.000427	0.0135	6.84	10718089	proline rich region 18	361481
0.000427	0.0135	3.93	10901239	F-box protein 7	366854
0.000433	0.0135	6.11	10924230	NA	NA
0.000433	0.0135	6.42	10752758	BTG family, member 3	54230
0.000433	0.0135	0.24	10862359	NA	NA
0.000438	0.0135	5.54	10890397	salvador homolog 1 (Drosophila)	299116
0.000439	0.0135	5.97	10733321	CDC like kinase 4	287269
0.000439	0.0135	0.23	10765836	olfactory receptor 1589	289251
0.000442	0.0135	4.54	10821105	NA	NA
0.000446	0.0135	4.68	10785509	protocadherin 20	306081
0.000447	0.0135	0.2	10850555	cystatin 11	245916
0.000447	0.0135	0.17	10858077	H1 histone family, member O, oocyte-specific	502875
0.000447	0.0135	0.13	10899693	NA	NA
0.000449	0.0135	0.14	10933974	NA	NA
0.000449	0.0135	3.99	10775761	cyclin G2	29157
0.000456	0.0137	4.53	10721205	similar to CG15432-PA	365238
0.000458	0.0137	5.1	10817705	SEC22 vesicle trafficking protein homolog B (S. cerevisiae)	310710
0.000459	0.0137	4.07	10923345	heat shock protein 1 (chaperonin 10)	25462
0.000462	0.0137	7.97	10832927	NA	NA
0.000467	0.0138	4.67	10743964	NA	NA
0.00047	0.0138	3.85	10928154	heat shock protein 1 (chaperonin)	63868
0.000473	0.0138	5.27	10815425	asparagine-linked glycosylation 5, dolichyl-phosphate beta-glucosyltransferase homolog (S. cerevisiae)	295051
0.000481	0.0139	4.79	10861986	insulin induced gene 1	64194
0.000484	0.0139	0.15	10847901	NA	NA
0.000485	0.0139	5.04	10712171	interferon induced transmembrane protein 1	293618
0.000486	0.0139	4.79	10857430	ADP-ribosylation-like factor 6 interacting protein 5	66028
0.000492	0.014	3.99	10846821	thioredoxin-related transmembrane protein 2	295701
0.000493	0.014	5.04	1092333	coenzyme Q10 homolog B (S. cerevisiae)	301416

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0.000498	0.014	0.15	1078114 6	PDZ binding kinase	290326
0.0005	0.014	4.16	1072668 7	NA	NA
0.000502	0.014	6.68	1092615 3	NA	NA
0.000502	0.014	5.7	1086250 5	similar to RIKEN cDNA 4921507P07	500124
0.000504	0.014	6.88	1086953 3	NA	NA
0.000504	0.014	0.18	1088962 2	NA	NA
0.00051	0.014	9.42	1070293 6	Wilms tumor 1 associated protein	499020
0.000513	0.014	4.58	1083005 0	SAR1 homolog A (S. cerevisiae)	361842
0.000514	0.014	5.14	1084849 9	coiled-coil domain containing 32	296081
0.000514	0.014	6.85	1084776 1	Cd44 molecule	25406
0.000517	0.014	0.2	1093226 9	NA	NA
0.00052	0.014	4.12	1092407 6	ribulose-5-phosphate-3-epimerase	501157
0.000521	0.014	4.04	1075501 3	interleukin 1 receptor accessory protein	25466
0.00053	0.014 2	7.46	1083063 0	reticulon 4 interacting protein 1	309912
0.000537	0.014 3	0.17	1086907 6	NA	NA
0.000537	0.014 3	9.69	1078258 3	small nuclear ribonucleoprotein 27kDa (U4/U6.U5)	362392
0.000542	0.014 4	6.02	1085926 2	apolipoprotein L domain containing 1	444983
0.000546	0.014 4	0.17	1086430 0	NA	NA
0.000548	0.014 4	4.01	1070781 2	NA	NA
0.000548	0.014 4	6.23	1082154 6	mitochondrial ribosomal protein S30	294767
0.000557	0.014 5	7.69	1072105 0	CCAAT/enhancer binding protein (C/EBP), gamma	25301
0.000557	0.014 5	3.71	1088485 3	PRP39 pre-mRNA processing factor 39 homolog (S. cerevisiae)	314171
0.000565	0.014 5	6.83	1079602 7	aldo-keto reductase family 1, member C-like 2	307091
0.000571	0.014 5	7.58	1088342 0	similar to CG14903-PA	298861
0.000575	0.014 5	3.56	1073616 3	flotillin 2	83764
0.00058	0.014 5	4.91	1089062 6	glycoprotein hormone beta 5	366668
0.000581	0.014 5	0.2	1093979 5	NA	NA
0.000585	0.014 5	4.26	1089937 8	GRP1 (general receptor for phosphoinositides 1)-associated scaffold protein	192254
0.000588	0.014 5	9.44	1088701 6	NA	NA

0.000588	0.0145	9.44	10887020	NA	NA
0.000588	0.0145	9.44	10887024	NA	NA
0.000588	0.0145	9.44	10887028	NA	NA
0.000588	0.0145	9.44	10887032	NA	NA
0.000588	0.0145	9.44	10887036	NA	NA
0.000588	0.0145	9.44	10887040	NA	NA
0.00059	0.0145	0.073	10900122	NA	NA
0.00059	0.0145	4.5	10853918	inhibitor of growth family, member 3	312154
0.000591	0.0145	6.55	10859655	mediator complex subunit 21	312849
0.000595	0.0145	3.97	10850578	NA	NA
0.000603	0.0145	0.21	10891977	serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 9	299274
0.000606	0.0145	4.39	10863866	CCHC-type zinc finger, nucleic acid binding protein	64530
0.000607	0.0145	6.49	10701846	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	114490
0.000608	0.0145	5.19	10711401	Bcl2-associated athanogene 3	293524
0.000608	0.0145	4.25	10756638	tectonin beta-propeller repeat containing 1	304285
0.000609	0.0145	5.38	10778375	NA	NA
0.000618	0.0147	5.1	10763820	Rab7b, member RAS oncogene family	501854
0.000625	0.0147	3.62	10782658	potassium channel tetramerisation domain containing 6	305792
0.000625	0.0147	0.19	10708462	olfactory receptor 23	293085
0.000625	0.0147	0.13	10722483	NA	NA
0.000634	0.0148	4.52	10748861	NA	NA
0.000635	0.0148	0.25	10821047	NA	NA
0.000637	0.0148	0.16	10863660	NA	NA
0.00064	0.0148	9.38	10780237	similar to 1700123O20Rik protein	361038
0.000649	0.0149	0.17	10901607	Spi-C transcription factor (Spi-1/PU.1 related)	314711
0.00065	0.0149	5.44	10841961	intraflagellar transport 52 homolog (Chlamydomonas)	362265
0.000654	0.0149	5.3	10794225	nuclear factor, interleukin 3 regulated	114519
0.000655	0.0149	10.12	10782901	cornichon homolog (Drosophila)	289994
0.000656	0.0149	6.78	10858497	NA	NA
0.000666	0.015	4.08	1076572	peroxisomal biogenesis factor 19	289233

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0.000669	0.0151	7.32	10726669	cytochrome c oxidase, subunit VIIIb	25250
0.000679	0.0152	0.15	10848140	NA	NA
0.00068	0.0152	4.83	10922182	NA	NA
0.000681	0.0152	4.51	10831892	TAF11 RNA polymerase II, TATA box binding protein (TBP)-associated factor	309638
0.000683	0.0152	4.24	10767373	chemokine (C-X-C motif) receptor 4	60628
0.000683	0.0152	0.25	10860914	NA	NA
0.000707	0.0156	0.2	10781525	NA	NA
0.000709	0.0156	0.25	10869644	NA	NA
0.00071	0.0156	0.23	10812148	NA	NA
0.00073	0.0159	4.35	10833239	cell division cycle and apoptosis regulator 1	361849
0.000731	0.0159	4.15	10938729	Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1	64466
0.000732	0.0159	5.05	10805996	plasma membrane proteolipid (plasmolipin)	64364
0.000734	0.0159	6.05	10745285	family with sequence similarity 58, member B	303321
0.000734	0.0159	0.19	10833744	NA	NA
0.00074	0.0159	5.78	10750672	ST3 beta-galactoside alpha-2,3-sialyltransferase 6	304023
0.00074	0.0159	0.24	10820010	NA	NA
0.000743	0.0159	0.17	10936915	NA	NA
0.000745	0.0159	0.14	10866142	killer cell lectin-like receptor, subfamily A, member 7	502905
0.000748	0.0159	11.76	10934005	NA	NA
0.000753	0.016	0.19	10840663	NA	NA
0.000756	0.016	0.23	10812150	NA	NA
0.000769	0.0162	0.19	10720533	NA	NA
0.000769	0.0162	4.38	10863710	NA	NA
0.000774	0.0162	4.36	10890650	NA	NA
0.000784	0.0164	4.44	10935229	NA	NA
0.000791	0.0164	5.6	10894238	zinc finger protein 563	314584
0.000793	0.0164	4.6	10856040	RSA-14-44 protein	297173
0.000793	0.0164	6	10767280	cyclin T2	304758
0.000798	0.0165	6.67	10805895	gene trap locus 3	307642

0.000815	0.0168	3.58	10710958	potassium channel tetramerisation domain containing 13	293497
0.000818	0.0168	9.68	10738140	insulin-like growth factor binding protein 4	360622
0.00083	0.0168	0.21	10858142	olfactory receptor 824	405215
0.000831	0.0168	0.2	10709759	NA	NA
0.000832	0.0168	6.64	10757636	chemokine (C-C motif) ligand 26	685958
0.000833	0.0168	0.22	10826451	solute carrier family 44, member 3	295417
0.000835	0.0168	0.22	10870039	NA	NA
0.000836	0.0168	5.13	10882829	NA	NA
0.000845	0.0169	5.84	10889579	HMG-box transcription factor 1	27080
0.000845	0.0169	5.49	10804478	NA	NA
0.000855	0.0169	3.89	10850131	proliferating cell nuclear antigen	25737
0.000857	0.0169	3.49	10895303	lin-7 homolog a (C. elegans)	85327
0.000858	0.0169	5.12	10754642	NA	NA
0.000858	0.0169	3.52	10736914	NA	NA
0.000859	0.0169	0.14	10878314	NA	NA
0.000863	0.0169	6.36	10908089	TATA box binding protein (TBP)-associated factor, RNA polymerase I, D, 41kDa	363017
0.000864	0.0169	5.11	10849863	small nuclear ribonucleoprotein polypeptides B and B1	171365
0.000866	0.0169	5.38	10780813	fibroblast growth factor 9	25444
0.00087	0.0169	4.24	10712706	choline kinase alpha	29194
0.000872	0.0169	0.19	10938584	NA	NA
0.000878	0.0169	0.25	10828152	NA	NA
0.000881	0.0169	5.54	10808929	leucine rich repeat containing 57	311346
0.000885	0.0169	4.99	10891297	similar to HSPC288	299207
0.000886	0.0169	5.39	10852144	prostate transmembrane protein, androgen induced 1	311676
0.00089	0.0169	0.13	10826002	NA	NA
0.000892	0.0169	0.21	10755035	tumor protein p63	246334
0.000893	0.0169	0.19	10719265	NA	NA
0.000899	0.0169	0.16	10852191	NA	NA
0.000902	0.0169	7.73	10868428	DnaJ (Hsp40) homolog, subfamily B, member 5	313811
0.00091	0.016	0.14	1090816	olfactory receptor 1128	405345

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0.000915	0.0169	6.37	10753279	v-ets erythroblastosis virus E26 oncogene homolog (avian)	170909
0.000921	0.0169	4.92	10747891	hypothetical protein LOC685233	685233
0.000922	0.0169	0.11	10722419	NA	NA
0.000922	0.0169	0.27	10798551	olfactory receptor 1662	291361
0.000923	0.0169	6.66	10708760	asparagine-linked glycosylation 8, alpha-1,3-glucosyltransferase homolog (S. cerevisiae)	293129
0.000926	0.0169	0.19	10886894	NA	NA
0.000927	0.0169	3.48	10761463	RAN, member RAS oncogene family	84509
0.000932	0.0169	4.35	10863221	methionine adenosyltransferase II, alpha	171347
0.000933	0.0169	0.19	10776260	NA	NA
0.000933	0.0169	4.06	10867596	similar to RIKEN cDNA 2610301B20; EST AI428449	362483
0.000933	0.0169	4.54	10758383	CAP-GLY domain containing linker protein 1	65201
0.000935	0.0169	3.85	10902696	NA	NA
0.000935	0.0169	5.3	10802710	NA	NA
0.000936	0.0169	4.4	10722005	potassium inwardly rectifying channel, subfamily J, member 11	83535
0.000938	0.0169	0.2	10787447	microtubule associated serine/threonine kinase 3	688540
0.00094	0.0169	3.4	10917095	REX2, RNA exonuclease 2 homolog (S. cerevisiae)	300689
0.000942	0.0169	3.34	10724967	dickkopf homolog 3 (Xenopus laevis)	171548
0.000945	0.0169	7.95	10778311	transmembrane emp24 protein transport domain containing 4	305502
0.000948	0.0169	0.22	10754176	Cd80 molecule	25408
0.000949	0.0169	0.17	10800328	NA	NA
0.00095	0.0169	4.88	10756251	insulin receptor	24954
0.000952	0.0169	0.17	10848118	olfactory receptor 775	296028
0.000976	0.0173	6.46	10877411	cell division cycle 26	366381
0.00098	0.0173	7.21	10855701	aquaporin 1	25240
0.000991	0.0174	0.27	10930555	NA	NA
0.000995	0.0175	4.88	10754201	G protein-coupled receptor 156	260430
0.000999	0.0175	3.83	10832879	TAF9 RNA polymerase II, TATA box binding protein (TBP)-associated factor	373541
0.001002	0.0175	5.52	10852600	regulator of G-protein signaling 19	59293
0.001006	0.0175	3.63	10803315	carbohydrate (N-acetylgalactosamine 4-0) sulfotransferase 9	291770

0.001008	0.0175	0.17	10792660	kelch repeat and BTB (POZ) domain containing 11	306617
0.001013	0.0175	0.17	10769436	NA	NA
0.001018	0.0175	3.88	10797918	PAK1 interacting protein 1	361232
0.001019	0.0175	0.18	10795346	glutathione peroxidase 5	113919
0.001021	0.0175	0.17	10739477	NA	NA
0.001021	0.0175	4.52	10901403	NA	NA
0.001023	0.0175	0.24	10798547	NA	NA
0.001033	0.0176	4.13	10826039	seryl-tRNA synthetase	266975
0.001038	0.0176	6.6	10749656	family with sequence similarity 195, member B	360677
0.00104	0.0176	0.24	10932430	WD repeat domain 13	317370
0.001043	0.0176	3.32	10903061	serine hydroxymethyltransferase 2 (mitochondrial)	299857
0.001048	0.0176	0.26	10807883	NA	NA
0.00105	0.0176	5.51	10888341	NA	NA
0.001052	0.0176	19.41	10932422	RNA binding motif (RNP1, RRM) protein 3	114488
0.001057	0.0177	4.95	10884961	ADP-ribosylation factor 6	79121
0.001064	0.0177	0.3	10750865	NA	NA
0.001068	0.0177	4.47	10921190	programmed cell death 6 interacting protein	501083
0.001069	0.0177	5.42	10892962	prostaglandin E synthase 3 (cytosolic)	362809
0.001071	0.0177	3.86	10844268	NA	NA
0.001075	0.0178	0.23	10813730	NA	NA
0.001078	0.0178	6.38	10726394	hypothetical LOC100302465	1E+08
0.001082	0.0178	4.78	10785773	sprouty homolog 2 (Drosophila)	306141
0.001083	0.0178	0.2	10729284	aldehyde dehydrogenase family 1, subfamily A7	29651
0.001097	0.018	0.23	10722435	NA	NA
0.001105	0.018	0.17	10852195	NA	NA
0.001139	0.0185	0.27	10893332	olfactory receptor 931	404943
0.00114	0.0185	3.85	10842517	cleavage stimulation factor, 3' pre-RNA, subunit 1	311670
0.00114	0.0185	4.09	10805335	solute carrier family 14 (urea transporter), member 1	54301
0.001145	0.0185	0.3	10802026	calcium/calmodulin-dependent protein kinase II alpha	25400
0.00115	0.018	6.39	1082469	S100 calcium binding protein A9	94195

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0.001154	0.0185	7.34	10778850	non-protein coding RNA 117	289864
0.001155	0.0185	8.08	10737815	mitochondrial ribosomal protein L45	287656
0.001155	0.0185	4.1	10823427	NA	NA
0.00116	0.0185	3.47	10739204	survival motor neuron domain containing 1	287768
0.001163	0.0185	0.26	10776359	NA	NA
0.001171	0.0185	3.59	10798490	histone cluster 1, H4b	64627
0.001172	0.0185	0.19	10909590	myelin protein zero-like 2	300679
0.001173	0.0185	6.71	10896748	ring finger protein 139	315000
0.001186	0.0187	5.33	10867731	calbindin 1	83839
0.00119	0.0187	4.47	10809906	NA	NA
0.001198	0.0187	0.23	10889328	NA	NA
0.001198	0.0187	5.57	10739541	H3 histone, family 3B	117056
0.001198	0.0187	5.57	10888125	H3 histone, family 3B	117056
0.001211	0.0188	0.22	10841624	v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)	83805
0.001212	0.0188	0.18	10750661	NA	NA
0.001216	0.0188	3.54	10795297	histone cluster 1, H4b	64627
0.001222	0.0188	3.53	10798499	histone cluster 1, H4b	64627
0.001224	0.0188	3.7	10783330	methyltransferase-like 3	361035
0.001225	0.0188	4.08	10752109	chloride channel 2	29232
0.001229	0.0188	0.18	10913722	myosin, light chain 3, alkali; ventricular, skeletal, slow	24585
0.00123	0.0188	4.28	10819338	mitogen-activated protein kinase kinase 1 interacting protein 1	362045
0.001232	0.0188	5.6	10928522	NA	NA
0.001252	0.0191	0.27	10747236	keratin complex 1, acidic, gene 5	287698
0.001254	0.0191	0.2	10771951	UDP glycosyltransferase 2 family, polypeptide B	24862
0.001263	0.0192	5.48	10916075	prostate and testis expressed 4	363041
0.001281	0.0194	0.3	10860584	NA	NA
0.001291	0.0195	3.72	10795280	NA	NA
0.001296	0.0195	3.56	10874918	lymphocyte antigen 96	448830
0.001296	0.0195	4.84	10907913	matrix metallopeptidase 8	63849

0.001297	0.0195	5.99	10756692	aminoacyl tRNA synthetase complex-interacting multifunctional protein 2	288480
0.001299	0.0195	6.95	10871417	NA	NA
0.001315	0.0196	0.19	10765818	olfactory receptor 1583	289241
0.001317	0.0196	0.28	10805014	NA	NA
0.00132	0.0196	5.22	10723576	protease, serine, 23	308807
0.001332	0.0196	5.2	10921120	solute carrier family 6 (proline IMINO transporter), member 20	113918
0.001333	0.0196	0.19	10883397	adenylate cyclase 3	64508
0.001333	0.0196	0.22	10906362	NA	NA
0.001337	0.0196	6.74	10761519	NA	NA
0.001341	0.0196	0.19	10788713	glutamic-oxaloacetic transaminase 1-like 1	306540
0.001342	0.0196	5.35	10924659	monoacylglycerol O-acyltransferase 1	363261
0.001342	0.0196	0.16	10767380	NA	NA
0.001346	0.0196	6.5	10895585	lysozyme C type 2	688047
0.001346	0.0196	4.84	10805029	protein tyrosine phosphatase, non-receptor type 2	117063
0.001364	0.0198	0.14	10776144	odontogenic, ameloblast asssociated	641555
0.001365	0.0198	4.24	10722897	hyaluronan and proteoglycan link protein 3	308773
0.001369	0.0198	3.59	10736863	chemokine (C-C motif) ligand 4	116637
0.00137	0.0198	3.46	10735870	reticulon 4 receptor-like 1	303311
0.00137	0.0198	0.22	10871588	zinc finger, MYND-type containing 12	313552
0.001379	0.0198	0.29	10756105	NA	NA
0.001381	0.0198	4.83	10732636	biorientation of chromosomes in cell division 1	287173
0.001388	0.0199	0.25	10909373	NA	NA
0.001389	0.0199	4.28	10773235	zinc finger protein 509	305428
0.001403	0.02	4.41	10816791	SHC (Src homology 2 domain containing) transforming protein 1	85385
0.001405	0.02	5.37	10829163	NA	NA
0.001406	0.02	3.64	10841066	microtubule-associated protein, RP/EB family, member 1	114764
0.001409	0.02	0.14	10756519	NA	NA
0.001418	0.0201	4.46	10749983	coxsackie virus and adenovirus receptor	89843
0.001425	0.0201	4.29	10788374	mitochondrial tumor suppressor 1	306487
0.001426	0.020	0.19	1071705	peroxisomal biogenesis factor 7	308718

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0.001435	0.0202	0.24	10752046	NA	NA
0.00144	0.0202	0.21	10750308	NA	NA
0.001446	0.0202	0.23	10820000	NA	NA
0.001449	0.0202	0.17	10714017	NA	NA
0.001451	0.0202	0.26	10846850	NA	NA
0.001461	0.0203	0.065	10722407	NA	NA
0.001464	0.0203	3.26	10833468	NA	NA
0.001466	0.0203	0.24	10886858	NA	NA
0.00147	0.0203	4.36	10746916	ORM1-like 3 (S. cerevisiae)	360618
0.00147	0.0203	0.12	10768294	NA	NA
0.001481	0.0204	4.08	10923670	nucleolar protein NOP58	60373
0.0015	0.0206	3.58	10798461	histone cluster 1, H4b	64627
0.001503	0.0206	0.17	10814831	NA	NA
0.001506	0.0206	4.93	10736417	NA	NA
0.001508	0.0206	4.11	10895152	KIT ligand	60427
0.00153	0.0208	7.19	10712134	proteasome (prosome, macropain) 26S subunit, non-ATPase, 13	365388
0.001533	0.0208	3.65	10840565	somatostatin receptor 4	25555
0.001543	0.0208	3.26	10766795	NA	NA
0.001546	0.0208	8.59	10935036	similar to Microsomal signal peptidase 23 kDa subunit (SPase 22 kDa subunit) (SPC22/23)	317409
0.001554	0.0208	3.43	10891679	G protein-coupled receptor 68	314386
0.001557	0.0208	0.24	10856189	NA	NA
0.001558	0.0208	4.01	10702792	claudin 20	680178
0.001559	0.0208	5.13	10876011	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 6	297990
0.001561	0.0208	4.76	10926384	tubulin folding cofactor C	316221
0.001562	0.0208	0.28	10830804	olfactory receptor 1699	406011
0.001571	0.0208	0.19	10793046	NA	NA
0.001572	0.0208	5.97	10895990	retinol dehydrogenase 2	299511
0.001572	0.0208	0.2	10894417	NA	NA
0.001573	0.0208	0.2	10702306	transcription factor 21	252856

0.001577	0.0208	4.41	10899248	activating transcription factor 1	315305
0.001578	0.0208	0.24	10750524	myxovirus (influenza virus) resistance 2	286918
0.001578	0.0208	3.06	10787556	transmembrane protein 59-like	306349
0.001582	0.0208	0.2	10859935	chaperonin containing TCP1, subunit 8 (theta)-like 1	499967
0.001584	0.0208	4.2	10802734	SMAD family member 7	81516
0.001592	0.0208	0.25	10863028	NA	NA
0.001592	0.0208	3.48	10774171	uridine phosphorylase 1	289801
0.001594	0.0208	0.23	10894295	vomeronasal 1 receptor 107	494260
0.0016	0.0208	0.27	10834598	NA	NA
0.001602	0.0208	3.48	10938952	TAF9B RNA polymerase II, TATA box binding protein (TBP)-associated factor	171152
0.001604	0.0208	0.18	10844681	ABO-family member 5	652927
0.001609	0.0208	3.27	10730884	survival motor neuron domain containing 1	287768
0.001609	0.0208	0.22	10725302	uromodulin	25128
0.001613	0.0208	0.25	10765042	NA	NA
0.001622	0.0208	3.5	10938019	eukaryotic translation initiation factor 1A, Y-linked	302697
0.001627	0.0208	5.84	10726682	interferon induced transmembrane protein 3	361673
0.001629	0.0208	4.86	10891878	NA	NA
0.00163	0.0208	3.69	10728812	transmembrane protein 109	361732
0.001636	0.0208	3.26	10844779	ring finger and CCCH-type zinc finger domains 2	311909
0.001639	0.0208	6.59	10817071	S100 calcium binding protein A8	116547
0.001641	0.0208	0.25	10935120	NA	NA
0.001641	0.0208	5.57	10752050	transformer 2 beta homolog (Drosophila)	117259
0.001644	0.0208	3.33	10875880	origin recognition complex, subunit 3-like (yeast)	313138
0.001647	0.0208	0.25	10750027	NA	NA
0.001651	0.0208	10.51	10733389	SAR1 homolog B (S. cerevisiae)	287276
0.001662	0.0209	3.28	10872018	trafficking protein particle complex 3	362599
0.001673	0.021	3.21	10814098	death-associated protein	64322
0.001679	0.0211	0.32	10929937	NA	NA
0.001684	0.0211	0.12	10770896	vomeronasal 2 receptor, 70	364086
0.001689	0.021	5.36	1092277	NA	NA

	1		9		
0.001694	0.0211	0.29	10932699	NA	NA
0.0017	0.0211	3.44	10774375	pellino 1	305549
0.001701	0.0211	0.25	10745470	transmembrane and immunoglobulin domain containing 1	363654
0.001716	0.0212	3.76	10721232	interferon stimulated exonuclease gene 20-like 2	361977
0.001721	0.0212	0.24	10779766	NA	NA
0.001735	0.0212	0.26	10718782	NA	NA
0.001735	0.0212	3.49	10702485	programmed cell death 6	308061
0.001736	0.0212	0.26	10855909	NA	NA
0.001736	0.0212	3.37	10918326	sorting nexin 1	84471
0.001737	0.0212	3.63	10932795	acyl-CoA synthetase long-chain family member 4	113976
0.001743	0.0212	0.27	10858618	vomeronasal 2 receptor, 50	500312
0.001744	0.0212	0.31	10722692	NA	NA
0.00175	0.0212	4.73	10810940	similar to RIKEN cDNA 2400003C14	307833
0.001753	0.0212	0.18	10835624	peptidyl-tRNA hydrolase 1 homolog (S. cerevisiae)	362113
0.001755	0.0212	0.27	10862942	NA	NA
0.001757	0.0212	8.85	10877431	haloacid dehalogenase-like hydrolase domain containing 3	688746
0.001762	0.0212	0.25	10741902	LOC360508	360508
0.001765	0.0212	0.15	10791509	NA	NA
0.001766	0.0212	3.45	10771649	chemokine (C-X-C motif) ligand 11	305236
0.001766	0.0212	0.2	10847223	olfactory receptor 716	404814
0.00177	0.0212	4.25	10786338	Rho guanine nucleotide exchange factor (GEF) 3	290541
0.001772	0.0212	3.03	10823508	cyclin L1	114121
0.001772	0.0212	3.86	10841033	NA	NA
0.001797	0.0215	0.18	10830574	Ab2-018	499469
0.001802	0.0215	3.46	10717882	dynein light chain Tctex-type 1	83462
0.001815	0.0216	4.75	10934631	lysophosphatidic acid receptor 4	302378
0.00182	0.0216	0.15	10830763	olfactory receptor 1675	405196
0.001831	0.0217	0.26	10752253	NA	NA
0.001836	0.0218	3.2	10755890	similar to RIKEN cDNA 2310008H04	498119

0.001848	0.0219	4.23	10919699	ubiquitin-like modifier activating enzyme 5	300968
0.001859	0.0219	5.75	10776064	UTP3, small subunit (SSU) processome component, homolog (S. cerevisiae)	305258
0.001859	0.0219	0.21	10718482	NA	NA
0.001875	0.0219	3.03	10809399	metallothionein 2A	689415
0.001875	0.0219	3.23	10837744	C1q and tumor necrosis factor related protein 4	311184
0.001875	0.0219	0.22	10887048	NA	NA
0.00188	0.0219	0.26	10764972	hypothetical protein LOC100302372	1E+08
0.001882	0.0219	0.27	10734475	NA	NA
0.001883	0.0219	3.28	10844658	RAB14, member RAS oncogene family	94197
0.001888	0.0219	0.29	10739211	NA	NA
0.001888	0.0219	4.53	10885145	JNK1/MAPK8-associated membrane protein	299127
0.00189	0.0219	0.19	10831041	mediator of DNA damage checkpoint 1	309595
0.001895	0.0219	3.59	10821542	NA	NA
0.001917	0.022	5.23	10884292	transmembrane protein 195	362732
0.00192	0.022	5.4	10879047	ATPase, H+ transporting, lysosomal 21kDa, V0 subunit b	298451
0.001925	0.022	6.27	10883785	ornithine decarboxylase 1	24609
0.001929	0.022	3.71	10898158	NA	NA
0.001929	0.022	3.71	10910752	NA	NA
0.00193	0.022	0.32	10712791	phosphatidylinositol transfer protein, membrane-associated 1	361694
0.001936	0.022	4.54	10767067	NA	NA
0.001937	0.022	3.63	10881293	podoplanin	54320
0.001942	0.022	0.31	10719445	NA	NA
0.001943	0.022	0.2	10897048	NA	NA
0.001943	0.022	4.4	10882397	NA	NA
0.001944	0.022	0.19	10792547	defensin NP-4 precursor	286958
0.001949	0.0221	0.25	10704376	NA	NA
0.001952	0.0221	0.24	10808407	NA	NA
0.001967	0.0222	0.29	10774420	NA	NA
0.001971	0.0222	0.19	10906666	NA	NA
0.001973	0.022	0.27	1074114	tuberous sclerosis 2	24855

	2		3		
0.001982	0.0222	0.23	10756530	NA	NA
0.001987	0.0222	4.58	10770890	hypothetical LOC498316	498316
0.001989	0.0222	0.23	10725078	pleckstrin homology domain containing, family A member 7	499249
0.001997	0.0223	3.57	10781197	stathmin-like 4	79423
0.002	0.0223	0.26	10703800	vomeronasal 2 receptor, pseudogene 45	286982
0.002007	0.0223	0.24	10764491	NA	NA
0.002011	0.0223	6.47	10754337	IQ motif containing B1	303915
0.002019	0.0224	4.96	10931010	similar to lymphocyte antigen 6 complex, locus E ligand	501282
0.002024	0.0224	0.23	10715117	G protein-coupled receptor 120	294075
0.002029	0.0224	4.08	10885006	thioredoxin-related transmembrane protein 1	362751
0.002034	0.0224	0.21	10893338	NA	NA
0.002035	0.0224	0.21	10899975	olfactory receptor 881	288792
0.002041	0.0224	3.7	10701990	BCL2-associated transcription factor 1	293017
0.002043	0.0224	4.5	10928207	similar to hypothetical protein FLJ37953	301419
0.002051	0.0224	0.26	10892643	NA	NA
0.002054	0.0224	0.21	10791199	NA	NA
0.002059	0.0224	7.59	10869666	small nuclear RNA activating complex, polypeptide 3	362537
0.002062	0.0224	0.31	10907231	NA	NA
0.002068	0.0224	4.45	10833818	NA	NA
0.002069	0.0224	4.8	10902957	phosphatidylinositol-5-phosphate 4-kinase, type II, gamma	140607
0.00207	0.0224	0.22	10908214	olfactory receptor 1174	405390
0.002072	0.0224	0.22	10887078	NA	NA
0.002075	0.0224	0.21	10916876	tetratricopeptide repeat domain 36	300676
0.002083	0.0224	0.24	10819467	NA	NA
0.002085	0.0224	0.27	10821955	NA	NA
0.002085	0.0224	0.19	10917145	NA	NA
0.002092	0.0224	5.42	10746789	NA	NA
0.002095	0.0224	6.39	10750047	NA	NA
0.002099	0.0224	0.23	10808563	cadherin 15	361432

0.002108	0.0224	0.24	10706515	SH3 and multiple ankyrin repeat domains 1	78957
0.002109	0.0224	4.61	10719464	NA	NA
0.00211	0.0224	0.22	10865531	ubiquitin specific peptidase 5 (isopeptidase T)	297593
0.002111	0.0224	3.9	10840684	ectonucleoside triphosphate diphosphohydrolase 6	85260
0.002111	0.0224	0.25	10856070	NA	NA
0.002115	0.0224	0.14	10774892	NA	NA
0.002116	0.0224	0.27	10735773	NA	NA
0.002117	0.0224	0.2	10939202	NA	NA
0.002126	0.0224	0.23	10791497	high mobility group box 2	29395
0.002126	0.0224	5.02	10761805	similar to density-regulated protein	687565
0.002153	0.0226	0.26	10873327	phospholipase A2, group IIC	29387
0.002157	0.0226	3.07	10711686	budding uninhibited by benzimidazoles 3 homolog (S. cerevisiae)	361662
0.002158	0.0226	3.26	10723224	SEC11 homolog A (S. cerevisiae)	65166
0.002159	0.0226	3.47	10799241	isopentenyl-diphosphate delta isomerase 1	89784
0.002161	0.0226	0.22	10937496	NA	NA
0.002164	0.0226	0.12	10939533	NA	NA
0.002167	0.0226	3.92	10819298	NA	NA
0.002168	0.0226	0.15	10822352	LRRGT00056	499573
0.002173	0.0226	3.49	10906470	zinc finger CCHC-type and RNA binding motif 1	362990
0.002179	0.0226	4.12	10817438	HORMA domain containing 1	365868
0.002188	0.0226	4.25	10872867	NA	NA
0.002189	0.0226	4.63	10851497	fat storage-inducing transmembrane protein 2	311617
0.002192	0.0226	3.28	10894115	NA	NA
0.002198	0.0226	2.91	10769509	TIP41, TOR signaling pathway regulator-like (S. cerevisiae)	360869
0.002204	0.0226	0.23	10930576	NA	NA
0.002205	0.0226	0.2	10721308	glandular kallikrein 11	408242
0.002213	0.0226	9.14	10925373	ubiquitin-conjugating enzyme E2F (putative)	363284
0.002213	0.0226	0.21	10865289	murinoglobulin 1	497794
0.002214	0.0226	6.27	10896992	protein tyrosine phosphatase type IVA, member 3	362930
0.002215	0.022	4.65	1091748	RAB39, member RAS oncogene family	315668

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0.002225	0.0226	0.19	10842704	NA	NA
0.002225	0.0226	5.53	10926577	mitochondrial ribosomal protein L14	301250
0.002227	0.0226	3.83	10864623	calcium/calmodulin-dependent protein kinase I	171503
0.00223	0.0226	6.79	10860217	armadillo repeat containing 10	296758
0.002232	0.0226	3.18	10729662	NA	NA
0.002232	0.0226	0.19	10909149	olfactory receptor 1223	300549
0.002236	0.0226	0.2	10787726	NA	NA
0.002243	0.0226	0.24	10753348	high-mobility group nucleosome binding domain 1	360704
0.002244	0.0226	3.98	10823605	beta-1,3-N-acetylgalactosaminyltransferase 1	310508
0.00225	0.0226	4.01	10866512	matrix Gla protein	25333
0.002272	0.0228	0.27	10907369	type II keratin Kb23	407759
0.002276	0.0228	0.17	10903187	NA	NA
0.002289	0.0229	0.11	10882875	NA	NA
0.002291	0.0229	4.28	10785377	NA	NA
0.002297	0.0229	5.95	10762709	coenzyme Q5 homolog, methyltransferase (S. cerevisiae)	304542
0.002315	0.023	0.25	10870012	NA	NA
0.002316	0.023	0.21	10782271	plasminogen activator, urokinase	25619
0.002324	0.0231	9.09	10840791	sulfiredoxin 1 homolog (S. cerevisiae)	296271
0.002332	0.0231	3.88	10891864	hypothetical protein LOC100233176	1E+08
0.002336	0.0231	3.66	10835618	torsin family 2, member A	362112
0.002336	0.0231	4.96	10887665	brix domain containing 1	294436
0.002339	0.0231	0.2	10803136	centrin, EF-hand protein, 1	84592
0.002343	0.0231	3.86	10737380	NA	NA
0.002354	0.0232	0.16	10729019	NA	NA
0.002364	0.0232	3.02	10839857	NOP56 ribonucleoprotein homolog (yeast)	362214
0.002365	0.0232	4.52	10780922	NA	NA
0.002375	0.0232	5.58	10709844	wee 1 homolog (S. pombe)	308937
0.002376	0.0232	3.52	10831802	NA	NA
0.002378	0.0232	0.33	10719134	NA	NA

0.002378	0.0232	5.99	10878034	NA	NA
0.002379	0.0232	4	10834225	neural proliferation, differentiation and control, 1	296562
0.002384	0.0232	7	10860612	NA	NA
0.002385	0.0232	4.82	10844747	RNA binding motif protein 18	311902
0.002389	0.0232	0.15	10767034	NA	NA
0.00239	0.0232	0.26	10909251	olfactory receptor 1266	405088
0.002397	0.0232	0.3	10722473	NA	NA
0.0024	0.0232	5.95	10845095	origin recognition complex, subunit 4-like (yeast)	295596
0.002402	0.0232	4.22	10871792	Ras-related GTP binding C	298514
0.002407	0.0232	0.2	10859164	killer cell lectin-like receptor, subfamily D, member 1	25110
0.00241	0.0232	0.25	10780360	phosphoenolpyruvate carboxykinase 2 (mitochondrial)	361042
0.002412	0.0232	2.8	10909407	Thy-1 cell surface antigen	24832
0.002419	0.0232	3.15	10778361	transforming growth factor beta regulator 4	360977
0.002423	0.0232	5.95	10887966	NA	NA
0.002428	0.0232	6.34	10872626	G protein-coupled receptor 3	266769
0.002438	0.0232	0.25	10847174	olfactory receptor 687	295892
0.002441	0.0232	5.45	10889728	ADP-ribosylation factor-like 4A	29308
0.002444	0.0232	0.23	10722425	NA	NA
0.00245	0.0232	3.39	10935882	zinc finger protein 275	293849
0.00245	0.0232	6.99	10914517	NA	NA
0.002452	0.0232	0.25	10839674	c-mer proto-oncogene tyrosine kinase	65037
0.002453	0.0232	0.19	10806016	Bardet-Biedl syndrome 2	113948
0.002453	0.0232	0.28	10797541	SECIS binding protein 2	79049
0.002456	0.0232	4.14	10781773	NA	NA
0.002462	0.0232	5.1	10834812	mitochondrial ribosomal protein S2	362094
0.002487	0.0234	3.82	10711852	protein tyrosine phosphatase, receptor type, E	114767
0.002494	0.0234	0.19	10707887	NA	NA
0.002503	0.0234	5.22	10927840	NA	NA
0.002503	0.0234	0.23	10733843	similar to Robo-1	691277
0.002506	0.023	0.28	1084697	olfactory receptor 495	295759

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0.00251	0.0234	3.61	10839208	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase like 2	311368
0.002519	0.0234	3.98	10790523	NA	NA
0.002524	0.0234	3.44	10761329	similar to 0610007L01Rik protein	288616
0.002525	0.0234	0.26	10894414	similar to hypothetical protein 4930509O22	300308
0.002526	0.0234	0.19	10939206	NA	NA
0.00253	0.0234	0.22	10788365	fibrinogen-like 1	246186
0.00253	0.0234	3.39	10863153	mitochondrial ribosomal protein L35	297334
0.002535	0.0235	3.62	10816540	NA	NA
0.002545	0.0235	0.28	10718381	vomeronasal 1 receptor 15	494305
0.002547	0.0235	0.18	10871127	MOB1, Mps One Binder kinase activator-like 2C (yeast)	313511
0.00255	0.0235	3.37	10773331	cappuccino homolog (mouse)	364183
0.00256	0.0235	0.22	10878741	forkhead box E3	171302
0.002568	0.0235	3.13	10895144	dual specificity phosphatase 6	116663
0.00257	0.0235	0.32	10708980	olfactory receptor 36	405257
0.002571	0.0235	0.24	10855371	similar to Zinc finger and SCAN domain containing protein 2 (Zinc finger protein 29)	312310
0.002573	0.0235	3.34	10880824	PTEN induced putative kinase 1	298575
0.002583	0.0236	0.28	10940022	NA	NA
0.002584	0.0236	4.79	10799483	antigenic determinant of rec-A protein homolog (mouse)	689197
0.002588	0.0236	3.19	10812737	survival motor neuron 1	64301
0.002595	0.0236	3.2	10865790	NA	NA
0.002595	0.0236	4.32	10872671	sphingomyelin phosphodiesterase, acid-like 3B	362619
0.00262	0.0238	4.58	10758219	eukaryotic translation initiation factor 2B, subunit 1 alpha	64514
0.002637	0.0239	3.93	10803991	CD14 molecule	60350
0.002638	0.0239	0.25	10870200	NA	NA
0.002654	0.0239	3.68	10937327	NA	NA
0.002661	0.0239	6.62	10775862	prostate androgen-regulated mucin-like protein 1	286894
0.002664	0.0239	3.15	10756899	guanine nucleotide binding protein (G protein) alpha 12	81663
0.002664	0.0239	0.27	10803824	cell division cycle 25 homolog C (S. pombe)	307511
0.002669	0.0239	2.86	10770070	cell adhesion molecule 3	360882

0.002674	0.0239	0.32	10902970	kinesin family member 5A	314906
0.00268	0.0239	0.35	10870269	DnaJ (Hsp40) homolog, subfamily C, member 6	313409
0.002682	0.0239	0.26	10744995	family with sequence similarity 101, member B	287534
0.002683	0.0239	3.99	10872000	LSM10, U7 small nuclear RNA associated	366468
0.002685	0.0239	3.17	10752650	similar to 4930453N24Rik protein	304176
0.00269	0.0239	5.57	10878032	CDC28 protein kinase regulatory subunit 1B	499655
0.002697	0.0239	0.28	10829824	NA	NA
0.0027	0.0239	0.2	10755670	NA	NA
0.002701	0.0239	0.31	10739160	NA	NA
0.002707	0.0239	0.29	10718377	vomeronasal 1 receptor 12	494306
0.00271	0.0239	0.3	10923385	NA	NA
0.002715	0.0239	0.24	10797007	NA	NA
0.002716	0.0239	4.21	10750795	RNA (guanine-9-) methyltransferase domain containing 1	304012
0.002717	0.0239	4.48	10842577	RAE1 RNA export 1 homolog (S. pombe)	362281
0.002721	0.0239	6.36	10835396	exosome component 2	366017
0.002722	0.0239	0.19	10753870	resistin like beta	498074
0.002751	0.0241	5.41	10840318	NA	NA
0.002755	0.0241	3.18	10746156	NA	NA
0.002756	0.0241	0.28	10816884	NA	NA
0.002764	0.0242	3.87	10771765	NA	NA
0.002778	0.0243	0.34	10848393	RAS guanyl releasing protein 1 (calcium and DAG-regulated)	29434
0.002792	0.0244	4.62	10745323	WD repeat and SOCS box-containing 1	303336
0.002797	0.0244	0.23	10874116	NA	NA
0.002801	0.0244	2.83	10879963	hippocalcin	29177
0.002805	0.0244	4.6	10890210	MAM domain containing glycosylphosphatidylinositol anchor 2	314180
0.00282	0.0245	3.95	10846982	NA	NA
0.002829	0.0245	4.18	10843132	polymerase (RNA) III (DNA directed) polypeptide K	366277
0.002833	0.0245	0.22	10724219	tripartite motif-containing 21	308901
0.002837	0.0245	0.23	10847207	olfactory receptor 707	405953
0.002845	0.024	3.6	1093502	NA	NA

	6		1		
0.002849	0.0246	0.29	10896945	NA	NA
0.002854	0.0246	2.87	10795194	NA	NA
0.002876	0.0247	4.31	10810811	solute carrier family 7, member 6 opposite strand	246187
0.002879	0.0247	5.49	10866265	NA	NA
0.002898	0.0248	0.17	10772013	transmembrane protease, serine 11b	365265
0.002902	0.0248	0.16	10720508	NA	NA
0.002902	0.0248	4.91	10818687	NA	NA
0.002907	0.0248	3.98	10825153	Fc fragment of IgG, high affinity Ia, receptor (CD64)	295279
0.00291	0.0248	0.25	10910764	NA	NA
0.002925	0.0249	0.22	10886773	NA	NA
0.002928	0.0249	6.24	10865970	chromobox homolog 3-like	1E+08
0.002936	0.025	4.72	10903482	solute carrier family 25, member 32	315023
0.002953	0.0251	0.24	10909192	olfactory receptor 1245	405095
0.002962	0.0251	0.2	10898556	adrenomedullin 2	399475
0.002976	0.0252	4.61	10850068	NA	NA
0.002992	0.0253	7.55	10729120	NA	NA
0.002998	0.0253	0.21	10803655	protein C	25268
0.002999	0.0253	0.26	10866120	immunoreceptor Ly49si1	494206
0.003001	0.0253	7.42	10775260	similar to RIKEN cDNA 1700081O22	363337
0.003007	0.0253	0.18	10869043	NA	NA
0.00302	0.0254	0.25	10730765	NA	NA
0.003031	0.0254	0.24	10856740	similar to hypothetical protein FLJ12056	500230
0.003036	0.0254	0.3	10890645	WD repeat domain 89	314243
0.003038	0.0254	0.29	10915887	beta-galactosidase-like protein	494244
0.003055	0.0255	3.56	10731738	similar to chromosome 16 open reading frame 5	360480
0.003067	0.0256	4.46	10829639	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1	361825
0.003082	0.0257	0.064	10722383	NA	NA
0.003082	0.0257	0.064	10722385	NA	NA
0.003088	0.0257	3.53	10933408	motile sperm domain containing 2	363463

0.003089	0.0257	4.29	10766057	NA	NA
0.003094	0.0257	3.88	10703864	zinc finger protein 583	499068
0.003105	0.0257	0.15	10788086	odz, odd Oz/ten-m homolog 3 (Drosophila)	306451
0.003106	0.0257	0.13	10772799	NA	NA
0.003107	0.0257	0.2	10934197	NA	NA
0.00311	0.0257	0.22	10750603	NA	NA
0.003119	0.0257	9.12	10934772	mannose-6-phosphate receptor, cation dependent	312689
0.003132	0.0257	5.3	10871479	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 1	679532
0.003137	0.0257	0.28	10793650	cathepsin Q	246147
0.003138	0.0257	0.23	10706974	NA	NA
0.003139	0.0257	3.02	10842130	phosphatidylinositol glycan anchor biosynthesis, class T	296360
0.003152	0.0258	0.22	10719159	NA	NA
0.003166	0.0259	0.062	10722379	NA	NA
0.003183	0.026	6.38	10827531	splicing factor, arginine/serine-rich 11	502603
0.003199	0.0261	0.18	10852437	potassium voltage-gated channel, KQT-like subfamily, member 2	170848
0.003202	0.0261	3.54	10820071	G protein-coupled receptor 150	499486
0.003202	0.0261	4.69	10712648	mitochondrial ribosomal protein L21	309140
0.003206	0.0261	0.26	10856152	NA	NA
0.003218	0.0261	3.98	10880647	lysophospholipase 2	83510
0.003222	0.0261	0.14	10899979	olfactory receptor 886	302254
0.003227	0.0261	0.29	10877675	NA	NA
0.003243	0.0262	0.24	10858871	sodium channel, nonvoltage-gated 1 alpha	25122
0.003253	0.0263	3.44	10798856	cullin 2	361258
0.003256	0.0263	0.25	10894140	NA	NA
0.003266	0.0263	0.29	10859197	taste receptor, type 2, member 120	690448
0.003276	0.0264	0.25	10823498	NA	NA
0.00328	0.0264	0.22	10940025	NA	NA
0.003284	0.0264	0.34	10712606	LRRG00136-like	1E+08
0.003296	0.0264	3.75	10791618	RWD domain containing 4A	502084
0.003314	0.026	5.39	1079746	NA	NA

	5		0		
0.003337	0.0266	3.81	10813392	FYN binding protein (FYB-120/130)	499537
0.003342	0.0266	0.28	10802272	NA	NA
0.003343	0.0266	3.72	10908149	NA	NA
0.003346	0.0266	0.31	10718766	NLR family, pyrin domain containing 9	292577
0.00335	0.0266	3.25	10767243	Ly6/Plaur domain containing 1	360838
0.003353	0.0266	0.28	10718725	NLR family, pyrin domain containing 5	308325
0.003353	0.0266	3.25	10817845	V-set domain containing T cell activation inhibitor 1	295322
0.003356	0.0266	0.26	10894796	NA	NA
0.003363	0.0266	0.37	10739595	NA	NA
0.003369	0.0266	0.2	10920711	NA	NA
0.003371	0.0266	0.29	10796917	zinc finger protein 107-like	1E+08
0.003372	0.0266	0.34	10909307	NA	NA
0.00338	0.0267	3.44	10764773	regulator of G-protein signaling 16	360857
0.003398	0.0268	5.19	10745683	chaperonin containing Tcp1, subunit 6B (zeta 2)	363658
0.003406	0.0268	19.9	10720215	zinc finger protein 36	79426
0.003414	0.0268	4.23	10791921	vacuolar protein sorting 37 homolog A (S. cerevisiae)	290775
0.003418	0.0268	2.77	10894919	NA	NA
0.003425	0.0268	4.62	10774625	NA	NA
0.003434	0.0268	0.21	10935662	NA	NA
0.003435	0.0268	0.24	10897384	NA	NA
0.003438	0.0268	0.25	10837351	solute carrier family 43, member 1	311168
0.00344	0.0268	0.29	10936265	NA	NA
0.003446	0.0268	0.28	10823348	NA	NA
0.003446	0.0268	3.11	10929319	RAB18, member RAS oncogene family	307039
0.003459	0.0269	0.16	10720950	NA	NA
0.003466	0.0269	0.17	10813005	NA	NA
0.00347	0.0269	0.28	10889370	NA	NA
0.003472	0.0269	0.24	10782080	FERM, RhoGEF (Arhgef) and pleckstrin domain protein 1 (chondrocyte-derived)	306183
0.003479	0.0269	0.21	10887010	NA	NA

0.003486	0.0269	0.27	10820280	NA	NA
0.003503	0.027	0.28	10796436	NA	NA
0.003524	0.0272	0.34	10739148	NA	NA
0.003533	0.0272	0.31	10920741	NA	NA
0.003534	0.0272	0.25	10725045	cytochrome P450, family 2, subfamily r, polypeptide 1	361631
0.003535	0.0272	0.23	10847205	olfactory receptor 707	405953
0.003542	0.0272	3.62	10890936	enhancer of rudimentary homolog (Drosophila)	681415
0.003544	0.0272	4.03	10916867	transmembrane protein 25	689172
0.003551	0.0272	0.19	10862281	olfactory receptor 801	405372
0.003553	0.0272	0.12	10867380	NA	NA
0.003564	0.0272	3.42	10801290	Nedd4 family interacting protein 1	291609
0.003568	0.0272	0.27	10819946	NA	NA
0.003571	0.0272	4.36	10790966	inositol-3-phosphate synthase 1	290651
0.003586	0.0273	0.27	10859128	C-type lectin domain family 9, member a	502901
0.003587	0.0273	4.61	10905462	Josephin domain containing 1	315134
0.003589	0.0273	2.89	10794257	NA	NA
0.003599	0.0273	2.99	10739455	G protein-coupled receptor, family C, group 5, member C	287805
0.003619	0.0274	5.39	10848279	NA	NA
0.00362	0.0274	3.03	10898229	parvin, beta	362973
0.00362	0.0274	3.13	10927692	four and a half LIM domains 2	63839
0.003633	0.0274	0.059	10707641	NA	NA
0.003643	0.0275	0.098	10857641	NA	NA
0.00365	0.0275	0.23	10903212	NA	NA
0.00365	0.0275	0.23	10770728	NA	NA
0.003653	0.0275	4.13	10913174	NA	NA
0.003663	0.0275	0.33	10912119	RAS protein-specific guanine nucleotide-releasing factor 1	192213
0.003682	0.0276	0.27	10877637	NA	NA
0.003687	0.0276	0.29	10801603	NA	NA
0.003706	0.0277	3.6	10728046	D4, zinc and double PHD fingers family 2	361711
0.003707	0.027	0.28	1081124	NA	NA

	7		8		
0.003708	0.0277	4.12	10874802	ubiquitin-conjugating enzyme E2, J2 (UBC6 homolog, yeast)	298689
0.003709	0.0277	4.11	10727634	RNA binding motif protein 4	293663
0.003732	0.0278	5.51	10705485	fibrillarin	292747
0.003744	0.0278	4.04	10734853	aurora kinase B	114592
0.003746	0.0278	0.27	10806882	TBC1 domain family, member 9	304645
0.003757	0.0279	2.98	10855449	glycoprotein (transmembrane) nmb	113955
0.003772	0.028	0.31	10837097	NA	NA
0.003774	0.028	0.33	10704200	vomeronasal 1 receptor 51	494281
0.003778	0.028	3.43	10775193	epoxide hydrolase 4	289440
0.003791	0.028	3.17	10917597	cholinergic receptor, nicotinic, alpha 3	25101
0.003807	0.0281	0.27	10933559	protein phosphatase, EF-hand calcium binding domain 1	317498
0.003808	0.0281	4.31	10742466	NA	NA
0.003816	0.0281	0.38	10797351	unc-5 homolog A (C. elegans)	60629
0.003817	0.0281	0.27	10884912	NA	NA
0.003825	0.0281	0.26	10836065	NA	NA
0.003828	0.0281	0.24	10764365	NA	NA
0.003852	0.0282	0.18	10753561	olfactory receptor 1553	288192
0.003852	0.0282	2.81	10753533	myc induced nuclear antigen	266670
0.003855	0.0282	0.21	10877689	NA	NA
0.003855	0.0282	0.31	10776287	NA	NA
0.003863	0.0282	0.25	10820686	NA	NA
0.003875	0.0282	0.24	10929590	NA	NA
0.003877	0.0282	4.38	10921527	NA	NA
0.003877	0.0282	4.39	10928229	CDC-like kinase 1	301434
0.003884	0.0282	2.84	10713089	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	309165
0.003895	0.0283	0.25	10811135	NA	NA
0.003901	0.0283	0.18	10794972	NA	NA
0.003926	0.0284	0.31	10847004	olfactory receptor 516	295769
0.003931	0.0284	3.28	10705874	Tyro protein tyrosine kinase binding protein	361537

0.003934	0.0284	2.83	10934128	NA	NA
0.003935	0.0284	0.23	10792456	transmembrane phosphatase with tensin homology	364629
0.003938	0.0284	0.33	10873341	phospholipase A2, group IIA (platelets, synovial fluid)	29692
0.003952	0.0285	0.25	10923799	Cd28 molecule	25660
0.003989	0.0287	5.06	10874866	nucleolar complex associated 2 homolog (S. cerevisiae)	313777
0.004031	0.0289	7.03	10787113	hypothetical protein LOC688495	688495
0.004032	0.0289	5.78	10873336	phospholipase A2, group IID	298579
0.004033	0.0289	0.22	10776185	theobromine induced protein	689604
0.004041	0.029	0.28	10906142	mitogen-activated protein kinase 12	60352
0.004058	0.029	2.81	10909892	crystallin, alpha B	25420
0.004065	0.0291	0.23	10829751	NA	NA
0.004083	0.0292	4.4	10727717	neuronal PAS domain protein 4	266734
0.004091	0.0292	20.76	10702716	NA	NA
0.004091	0.0292	20.76	10915103	NA	NA
0.004095	0.0292	4.6	10838497	hypothetical protein LOC691543	691543
0.004101	0.0292	4.13	10721904	cytohesin 2	116692
0.004107	0.0292	0.27	10813253	complement component 6	24237
0.004128	0.0292	3	10931308	prolyl 4-hydroxylase, alpha polypeptide I	64475
0.004129	0.0292	2.94	10817894	NA	NA
0.004131	0.0292	4.65	10911309	general transcription factor IIA, 2	83828
0.004133	0.0292	2.9	10833523	LIM and senescent cell antigen-like domains 1	499443
0.004134	0.0292	3.19	10884738	NA	NA
0.004134	0.0292	2.97	10721188	pleckstrin homology domain containing, family F (with FYVE domain) member 1	308543
0.004137	0.0292	4.16	10802405	chromatin modifying protein 1B	689364
0.004141	0.0292	0.31	10709200	phosphodiesterase 2A, cGMP-stimulated	81743
0.004149	0.0292	4.5	10709582	fractured callus expressed transcript 1	84384
0.004153	0.0292	0.24	10922743	NA	NA
0.004167	0.0293	3.63	10837974	autophagy/beclin 1 regulator 1	59319
0.004188	0.0293	2.93	10757489	VGF nerve growth factor inducible	29461
0.004189	0.029	3.12	1089768	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17	315133

	3		3		
0.004191	0.0293	3.4	10860900	pyruvate dehydrogenase kinase, isozyme 4	89813
0.004191	0.0293	0.27	10909196	olfactory receptor 1247	405093
0.004201	0.0293	0.24	10854988	prolactin induced protein	64673
0.004202	0.0293	0.3	10837504	olfactory receptor 554	295797
0.004209	0.0293	0.32	10705358	biliverdin reductase B (flavin reductase (NADPH))	292737
0.004212	0.0293	5.06	10820151	NA	NA
0.004224	0.0293	3.18	10844531	pre-B-cell leukemia homeobox 3	311876
0.004225	0.0293	0.29	10916330	olfactory receptor pseudogene 1290	300597
0.004241	0.0293	0.3	10841020	NA	NA
0.004244	0.0293	0.34	10847012	olfactory receptor 527	404878
0.004245	0.0293	4.53	10703905	RAS-like, family 2, locus 9	751812
0.004245	0.0293	0.19	10838741	NA	NA
0.004252	0.0293	0.22	10837559	olfactory receptor 594	404856
0.004256	0.0293	0.24	10707927	NA	NA
0.004256	0.0293	0.29	10739994	NA	NA
0.00426	0.0293	3.92	10878047	phospholipase A2, activating protein	116645
0.004265	0.0293	0.22	10752586	NA	NA
0.004267	0.0293	3.62	10857538	tRNA nucleotidyl transferase, CCA-adding, 1	312616
0.004271	0.0293	4.12	10705192	ATP5S-like	361520
0.004308	0.0295	0.29	10775134	NA	NA
0.00431	0.0295	0.26	10729282	NA	NA
0.004324	0.0296	0.25	10931285	engrailed homeobox 1	685360
0.004324	0.0296	0.22	10909006	NA	NA
0.004329	0.0296	0.23	10709464	hypothetical protein LOC499219	499219
0.004333	0.0296	4	10926342	mitochondrial ribosomal protein S10	363187
0.004364	0.0297	2.82	10739886	septin 9	83788
0.004372	0.0298	0.29	10795313	NA	NA
0.004388	0.0298	0.19	10871432	NA	NA
0.004402	0.0299	3.36	10927213	primase, DNA, polypeptide 2	301323

0.004406	0.0299	3.16	10875532	pyruvate dehyrogenase phosphatase catalytic subunit 1	54705
0.004413	0.0299	3.43	10882221	transmembrane protein 88B	680723
0.004432	0.03	3.05	10845767	Cobl-like 1	311088
0.004445	0.03	3.04	10707121	general transcription factor IIH, polypeptide 1	361580
0.004452	0.03	0.21	10914019	NA	NA
0.004459	0.03	0.24	10916338	olfactory receptor 1294	367064
0.004469	0.03	0.21	10706240	NA	NA
0.004474	0.03	3.46	10822386	protein kinase (cAMP-dependent, catalytic) inhibitor alpha	114906
0.004474	0.03	4.65	10776667	NA	NA
0.004488	0.03	0.16	10755775	NA	NA
0.00449	0.03	2.98	10797388	NA	NA
0.004506	0.03	6.06	10757420	hypothetical protein LOC684993	684993
0.004509	0.03	4.21	10908100	NA	NA
0.00451	0.03	5.3	10758344	vacuolar protein sorting 37 homolog B (S. cerevisiae)	288659
0.004519	0.03	5.06	10757175	cytochrome P450, family 3, subfamily a, polypeptide 9	171352
0.004533	0.03	0.27	10896524	NA	NA
0.004534	0.03	3.44	10741416	tryptase alpha/beta 1	54271
0.004535	0.03	0.071	10707645	NA	NA
0.004535	0.03	0.071	10707647	NA	NA
0.004535	0.03	0.071	10722371	NA	NA
0.004535	0.03	0.071	10722389	NA	NA
0.004535	0.03	0.071	10722393	NA	NA
0.004535	0.03	0.071	10722395	NA	NA
0.004535	0.03	0.071	10722397	NA	NA
0.004535	0.03	0.071	10722399	NA	NA
0.004535	0.03	0.071	10722403	NA	NA
0.004535	0.03	0.071	10722411	NA	NA
0.004535	0.03	5.87	10869310	solute carrier family 31 (copper transporters), member 1	171135
0.004544	0.03	2.56	10756343	heat shock 105kDa/110kDa protein 1	288444
0.004548	0.03	4.14	1080175	sorting nexin 24	361328

			2		
0.004559	0.03	0.28	1084696 8	olfactory receptor 491	405252
0.004565	0.03	0.25	1086955 7	NA	NA
0.004565	0.03	0.25	1090108 7	NA	NA
0.004581	0.03	0.32	1076545 2	hydroxysteroid (17-beta) dehydrogenase 7	29540
0.004591	0.03	0.25	1089427 0	NA	NA
0.004592	0.03	0.35	1076473 6	nicotinamide nucleotide adenylyltransferase 2	289095
0.004599	0.03	4.3	1079667 3	NA	NA
0.0046	0.03	5.43	1088952 2	dihydrolipoamide dehydrogenase	298942
0.004604	0.03	4.25	1087097 4	ATP/GTP binding protein-like 4	362562
0.004604	0.03	0.23	1084250 7	melanocortin 3 receptor	29310
0.004607	0.03	4.89	1082330 9	stress-associated endoplasmic reticulum protein 1	80881
0.004608	0.03	2.7	1074567 7	chemokine (C-C motif) ligand 3	25542
0.004617	0.03	0.36	1093060 6	NA	NA
0.004623	0.03	3.71	1087564 2	NA	NA
0.004627	0.03	0.29	1079595 2	NA	NA
0.004628	0.03	3.46	1082462 4	NA	NA
0.004636	0.03	4.38	1086372 2	NA	NA
0.004657	0.030 2	0.22	1071478 2	mannose-binding lectin (protein C) 2	64668
0.004698	0.030 4	0.21	1074993 1	NA	NA
0.0047	0.030 4	0.22	1071670 4	sterile alpha motif domain containing 5	365038
0.004713	0.030 4	0.17	1093550 6	NA	NA
0.004727	0.030 5	0.28	1074218 3	gamma-aminobutyric acid (GABA) A receptor, alpha 6	29708
0.004727	0.030 5	3.48	1076029 0	neuronal pentraxin 2	288475
0.004734	0.030 5	0.34	1080113 5	NA	NA
0.004736	0.030 5	0.29	1079922 9	NA	NA
0.004742	0.030 5	0.31	1082760 5	olfactory receptor 1687	309574
0.004746	0.030 5	0.19	1082302 4	NA	NA
0.004781	0.030 7	0.26	1085734 1	similar to chromosome 3 open reading frame 20	500258
0.004807	0.030 7	6.13	1075360 2	NA	NA

0.004809	0.0307	0.067	10722375	NA	NA
0.004809	0.0307	0.067	10722377	NA	NA
0.004814	0.0307	5	10842781	oxysterol binding protein-like 2	296461
0.004835	0.0309	2.56	10902547	lysozyme 2	25211
0.004843	0.0309	0.24	10881622	NA	NA
0.004856	0.0309	0.22	10832083	MAM domain containing glycosylphosphatidylinositol anchor 1	309659
0.004864	0.0309	0.18	10847229	olfactory receptor 720	405955
0.004876	0.031	3.04	10871582	NA	NA
0.004889	0.031	0.34	10769657	NUF2, NDC80 kinetochore complex component, homolog (S. cerevisiae)	304951
0.004905	0.0311	0.34	10703459	NA	NA
0.004918	0.0312	0.21	10861038	NA	NA
0.00493	0.0312	4.3	10925291	chemokine (C-X-C motif) receptor 7	84348
0.004933	0.0312	0.28	10939789	NA	NA
0.004946	0.0312	3.7	10916052	family with sequence similarity 118, member B	315549
0.00495	0.0312	0.32	10883071	NA	NA
0.004951	0.0312	0.29	10844648	NA	NA
0.004955	0.0312	6.3	10843028	tumor protein D52-like 2	296480
0.004957	0.0312	5.99	10872478	small nuclear ribonucleoprotein 40 (U5)	313056
0.004978	0.0313	3.89	10936145	NA	NA
0.004979	0.0313	3.1	10876759	olfactory receptor 845	298046
0.004991	0.0313	2.78	10899943	ORM1-like 2 (S. cerevisiae)	288783
0.005004	0.0314	0.37	10704792	NA	NA
0.005008	0.0314	3.52	10760813	NA	NA
0.005017	0.0314	6.52	10816558	signal sequence receptor, beta	295235
0.005038	0.0315	2.83	10714788	cleavage stimulation factor, 3' pre-RNA subunit 2, tau	309338
0.005057	0.0316	3.69	10805767	cadherin 8	84408
0.005071	0.0316	5.65	10875085	NA	NA
0.005084	0.0317	0.32	10733730	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 10 (GalNAc-T10)	170501
0.005102	0.0318	5.19	10745492	similar to hypothetical protein	287554
0.005126	0.031	2.75	1071463	cell division cycle 37 homolog (S. cerevisiae)-like 1	293886

	9		6		
0.005129	0.0319	0.26	10824812	RAF domain and POZ/BTB containing protein T2-like	1E+08
0.005138	0.0319	2.66	10773613	macrophage erythroblast attacher	298982
0.005169	0.032	0.12	10866182	NA	NA
0.005174	0.032	0.38	10893325	olfactory receptor 905	288797
0.005178	0.032	3.24	10901409	carbohydrate (chondroitin 4) sulfotransferase 11	314694
0.005179	0.032	3.33	10900050	NA	NA
0.005188	0.0321	0.21	10804303	NA	NA
0.005232	0.0323	3.29	10823591	tripartite motif-containing 59	365813
0.005239	0.0323	3.21	10894268	NA	NA
0.005239	0.0323	3.21	10918270	NA	NA
0.00524	0.0323	0.28	10730345	NA	NA
0.005253	0.0323	4.07	10707580	NA	NA
0.00527	0.0324	0.27	10886866	NA	NA
0.005297	0.0325	0.24	10938312	NA	NA
0.005313	0.0326	10.11	10899633	coatomer protein complex, subunit zeta 1	315345
0.005314	0.0326	0.35	10747692	dual specificity phosphatase 3	498003
0.005325	0.0326	0.39	10793838	NA	NA
0.005357	0.0328	0.3	10898408	NA	NA
0.005372	0.0328	2.92	10836394	membrane-associated ring finger (C3HC4) 7	311059
0.005383	0.0329	5.1	10751973	replication factor C (activator 1) 4	288003
0.005403	0.033	3.69	10763137	NA	NA
0.005409	0.033	0.27	10892674	NA	NA
0.005431	0.0331	0.26	10935622	melanoma antigen family A, 11	302845
0.005441	0.0331	4.73	10850603	DnaJ (Hsp40) homolog, subfamily B, member 6	362293
0.005457	0.0331	0.36	10835987	NA	NA
0.005462	0.0331	2.7	10861117	similar to CG3570-PA	500034
0.005464	0.0331	6.38	10884205	TWIST neighbor	362728
0.005467	0.0331	5.04	10812331	Fas apoptotic inhibitory molecule	140930
0.005467	0.0331	5.04	10912456	Fas apoptotic inhibitory molecule	140930

0.005486	0.0332	0.33	10828340	butyrophilin-like 4	294268
0.005505	0.0333	3.27	10716969	Vps20-associated 1 homolog (S. cerevisiae)	292640
0.005513	0.0333	0.2	10715541	carboxypeptidase N, polypeptide 1	365466
0.005523	0.0333	3.6	10916855	archain 1	300674
0.005564	0.0335	2.86	10806314	sal-like 1 (Drosophila)	307740
0.005579	0.0336	0.26	10939779	NA	NA
0.005581	0.0336	0.38	10732494	F-box and leucine-rich repeat protein 16	494223
0.005592	0.0336	0.3	10922650	inositol polyphosphate-4-phosphatase, type 1	80849
0.005598	0.0336	3.39	10901039	rCG29233-like	1E+08
0.005609	0.0337	2.57	10841710	small nucleolar RNA host gene 11	362256
0.005622	0.0337	4.12	10765166	similar to 2810422O20Rik protein	304928
0.005627	0.0337	0.24	10825655	NA	NA
0.00563	0.0337	0.24	10709432	olfactory receptor 98	293240
0.005662	0.0339	3.55	10798455	histone cluster 1, H2ai-like	291159
0.005674	0.0339	4.25	10868134	ubiquitin-conjugating enzyme E2, J1 (UBC6 homolog, yeast)	297961
0.005677	0.0339	0.25	10767503	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3	304775
0.005685	0.0339	0.36	10853467	NA	NA
0.005714	0.034	4.64	10808362	ubiquitin specific peptidase 10	307905
0.005725	0.0341	6	10703139	ribonuclease T2	292306
0.005727	0.0341	0.25	10738015	zona pellucida binding protein 2	363676
0.005742	0.0341	5.1	10720430	proteasome (prosome, macropain) 26S subunit, non-ATPase, 8	292766
0.005795	0.0344	3.53	10806960	ATP-binding cassette, subfamily E (OABP), member 1	361390
0.005797	0.0344	0.28	10782447	NA	NA
0.005801	0.0344	0.27	10805285	NA	NA
0.005823	0.0345	2.67	10791421	NIMA (never in mitosis gene a)-related kinase 1	290705
0.005832	0.0345	4.32	10910490	hexosaminidase A	300757
0.005842	0.0345	0.23	10772160	NA	NA
0.005849	0.0345	0.37	10734368	NA	NA
0.005861	0.0346	9.8	10878386	NA	NA
0.005886	0.034	3.04	1071484	NA	NA

	7		2		
0.005893	0.0347	0.28	10786774	oxoglutarate dehydrogenase-like	290566
0.005901	0.0347	2.78	10875420	pro-histogranin	502940
0.005903	0.0347	6.77	10920797	COX assembly mitochondrial protein homolog (S. cerevisiae)	363162
0.005928	0.0348	2.69	10927603	carbohydrate sulfotransferase 10	140568
0.005943	0.0348	2.54	10756040	ubiquitin fusion degradation 1 like (yeast)	84478
0.005947	0.0348	2.77	10795200	pro-histogranin	502940
0.00595	0.0348	3.84	10934526	NA	NA
0.005972	0.035	0.27	10864968	NA	NA
0.005987	0.035	2.6	10751945	somatostatin	24797
0.005994	0.035	0.33	10775737	NA	NA
0.005996	0.035	6.07	10734025	COP9 constitutive photomorphogenic homolog subunit 3 (Arabidopsis)	287367
0.006008	0.035	11.35	10872155	NA	NA
0.006014	0.035	0.34	10703626	vomeronasal 2 receptor, 22	690758
0.00603	0.0351	4.82	10871975	mitochondrial ribosomal protein S15	298517
0.00603	0.0351	7.48	10749681	THO complex 4	690585
0.006036	0.0351	0.32	10859877	engrailed homeobox 2	499964
0.006056	0.0352	0.26	10818428	similar to solute carrier family 25 member 24 isoform 1	691448
0.006063	0.0352	4.8	10912439	retinol binding protein 1, cellular	25056
0.006068	0.0352	0.23	10866306	taste receptor, type 2, member 106	1E+08
0.006074	0.0352	0.28	10938319	NA	NA
0.006106	0.0353	0.27	10709665	olfactory receptor 234	293379
0.006119	0.0354	2.78	10796280	Sec61 alpha 2 subunit (S. cerevisiae)	361273
0.006133	0.0354	0.24	10724260	olfactory receptor 92	293233
0.006136	0.0354	0.31	10915810	similar to hypothetical protein FLJ32949	300461
0.006144	0.0354	0.33	10709546	olfactory receptor 198	405919
0.00615	0.0354	3.81	10885029	FERM domain containing 6	257646
0.006167	0.0355	4.43	10879667	akirin 1	595134
0.006176	0.0355	3.86	10906963	protein kinase, AMP-activated, gamma 1 non-catalytic subunit	25520
0.006179	0.0355	0.29	10792348	NA	NA

0.006203	0.0356	0.32	10927039	NA	NA
0.006221	0.0357	4.15	10738716	ADP-ribosylation factor 2	79119
0.006229	0.0357	3.78	10909328	sodium channel, voltage-gated, type III, beta	245956
0.006235	0.0357	0.24	10897411	similar to apolipoprotein L2; apolipoprotein L-II	362951
0.006263	0.0358	2.7	10765411	NA	NA
0.006263	0.0358	2.99	10745266	transmembrane protein 97	303330
0.006267	0.0358	0.29	10837247	NA	NA
0.006269	0.0358	0.29	10938810	NA	NA
0.006284	0.0358	0.15	10820963	NA	NA
0.0063	0.0359	0.33	10788070	NA	NA
0.006311	0.0359	0.32	10862581	NA	NA
0.006319	0.0359	0.055	10722401	NA	NA
0.006328	0.0359	0.24	10858566	C-type lectin domain family 4, member a2	297584
0.006378	0.0362	0.3	10803438	NA	NA
0.006395	0.0362	3.46	10726655	blocked early in transport 1 homolog (S. cerevisiae) like	54400
0.006399	0.0362	0.39	10938790	NA	NA
0.006407	0.0362	0.19	10701648	vomeronasal 2 receptor, 5	679691
0.006411	0.0362	2.55	10934888	transmembrane protein 35	308134
0.006416	0.0362	0.35	10893416	olfactory receptor 1065	304633
0.006417	0.0362	0.31	10756620	NA	NA
0.006437	0.0363	4.21	10899756	coenzyme Q10 homolog A (S. cerevisiae)	362810
0.00648	0.0365	0.22	10873868	NA	NA
0.00648	0.0365	0.22	10881454	NA	NA
0.00649	0.0365	0.32	10755580	armadillo repeat gene deleted in velo-cardio-facial syndrome	303798
0.006494	0.0365	0.36	10820458	NA	NA
0.006495	0.0365	0.32	10935402	NA	NA
0.0065	0.0365	3.02	10936827	ATPase, H+ transporting, lysosomal accessory protein 2	302526
0.006501	0.0365	3.78	10863502	WD repeat domain 54	500226
0.006509	0.0365	4.23	10875363	thymocyte selection-associated high mobility group box	362481
0.006521	0.036	3.52	1092611	p300/CBP-associated factor	301164

	5		3		
0.006522	0.0365	5.02	10890024	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	25493
0.006522	0.0365	4.13	10815706	guanine monphosphate synthetase	295088
0.006558	0.0366	5.52	10739826	similar to RIKEN cDNA 1110005A03	287918
0.006573	0.0366	0.28	10923031	NA	NA
0.006573	0.0366	2.96	10790651	transmembrane protein 38a	306327
0.006584	0.0367	6.4	10734250	B9 protein domain 1	287383
0.006587	0.0367	3.04	10793467	similar to UPF0308 protein C9orf21	498685
0.006591	0.0367	0.21	10813856	NA	NA
0.00662	0.0367	5.4	10827691	RT1 class I, locus M6, gene 2	365527
0.00662	0.0367	0.24	10815913	NA	NA
0.006623	0.0367	0.23	10916377	olfactory receptor 1307	315573
0.00663	0.0367	0.35	10934976	NA	NA
0.006658	0.0369	3.48	10932029	similar to hypothetical protein FLJ22965	313433
0.00667	0.0369	0.29	10896811	NA	NA
0.006672	0.0369	2.41	10795384	ependymin related protein 1 (zebrafish)	291180
0.00669	0.0369	5.19	10939016	NA	NA
0.006691	0.0369	0.24	10916946	CD3 molecule, gamma	300678
0.006696	0.0369	0.28	10765478	NA	NA
0.006703	0.0369	2.43	10880685	leucine zipper protein 1	79428
0.006727	0.037	0.31	10859149	killer cell lectin-like receptor, family E, member 1	297645
0.006733	0.037	3.95	10938077	acyl-CoA thioesterase 9	302640
0.006758	0.0371	4.66	10851650	troponin C type 2 (fast)	296369
0.006759	0.0371	3.38	10828823	splicing factor, arginine/serine-rich 3	361814
0.006778	0.0372	3.77	10838155	catalase	24248
0.006782	0.0372	4.98	10812903	mCG51409-like	1E+08
0.006803	0.0373	0.22	10703216	NA	NA
0.006827	0.0374	4.05	10895581	P55	362855
0.006853	0.0375	3.13	10736486	carboxypeptidase D	25306
0.006868	0.0375	2.76	10720572	amyloid beta (A4) precursor-like protein 1	502317

0.006897	0.0376	2.9	10876015	NA	NA
0.006903	0.0376	0.27	10829218	trafficking protein particle complex 10	309678
0.006913	0.0376	2.67	10882896	family with sequence similarity 98, member A	313873
0.006917	0.0376	0.31	10759497	NA	NA
0.006927	0.0376	2.76	10873805	NA	NA
0.006931	0.0376	3.08	10766378	signal recognition particle 9	690345
0.006933	0.0376	2.53	10815998	heat shock protein A8	24468
0.006934	0.0376	0.33	10774752	similar to hypothetical protein FLJ31438	289865
0.006944	0.0377	4.26	10878082	NA	NA
0.006959	0.0377	0.13	10863363	NA	NA
0.006976	0.0378	3.63	10937762	transmembrane protein 27	57395
0.006982	0.0378	0.41	10879867	neurochondrin	89791
0.00699	0.0378	3.5	10886406	NA	NA
0.007009	0.0378	0.3	10836241	NA	NA
0.007012	0.0378	0.27	10903967	LOC500876	500876
0.007021	0.0378	0.19	10787814	NA	NA
0.007021	0.0378	3.52	10771004	transmembrane emp24 protein transport domain containing 5	289883
0.007042	0.0379	6.45	10885287	NA	NA
0.007043	0.0379	0.36	10929383	NA	NA
0.007064	0.038	0.27	10893373	olfactory receptor 995	405265
0.007075	0.038	0.33	10931123	chromobox homolog 2 (Pc class homolog, Drosophila)	303730
0.007099	0.0381	0.27	10939805	family with sequence similarity 122B	501647
0.007106	0.0381	0.38	10848113	olfactory receptor 769	296022
0.00715	0.0383	3.31	10825226	similar to 5930416I19Rik protein	297627
0.007153	0.0383	0.39	10924084	NA	NA
0.007187	0.0384	2.62	10817830	ganglioside-induced differentiation-associated-protein 2	362004
0.007189	0.0384	3.26	10806640	calreticulin	64202
0.007207	0.0385	0.22	10907676	NA	NA
0.007228	0.0385	3.57	10895406	pleckstrin homology-like domain, family A, member 1	29380
0.007231	0.038	0.36	1073269	F-box and WD repeat domain containing 11	303024

	5		7		
0.007235	0.0385	0.26	10775573	bone morphogenetic protein 3	25667
0.007241	0.0385	0.31	10783796	neural retina leucine zipper	290221
0.007244	0.0385	2.43	10916534	sterol-C5-desaturase (ERG3 delta-5-desaturase homolog, S. cerevisiae)-like	114100
0.007271	0.0386	0.1	10859796	NA	NA
0.007279	0.0386	2.68	10847693	apoptosis inhibitor 5	362170
0.007298	0.0386	4.27	10758212	Rab interacting lysosomal protein-like 2	288652
0.0073	0.0386	4.39	10730572	NA	NA
0.007307	0.0386	2.5	10827989	metallothionein 2A	689415
0.007307	0.0386	0.29	10813027	NA	NA
0.007313	0.0386	0.27	10781353	NA	NA
0.007317	0.0386	0.31	10750489	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 5	288161
0.007321	0.0386	5.82	10750572	ADP-ribosylation factor-like 6	363760
0.007324	0.0386	0.27	10793516	hydroxysteroid (17-beta) dehydrogenase 3	117182
0.007326	0.0386	0.29	10894244	NA	NA
0.007327	0.0386	2.91	10840226	similar to Protein C20orf103 precursor	362220
0.007333	0.0386	3.75	10876032	smu-1 suppressor of mec-8 and unc-52 homolog (C. elegans)	117541
0.007342	0.0386	2.9	10718591	myeloid-associated differentiation marker	369016
0.00735	0.0386	0.27	10845382	NA	NA
0.007355	0.0386	3.05	10807386	nuclear transport factor 2	291981
0.007361	0.0386	0.15	10804339	NA	NA
0.007362	0.0386	0.28	10751209	NA	NA
0.007381	0.0387	0.28	10866186	immunoreceptor Ly49i3	494208
0.007391	0.0387	0.24	10752974	NA	NA
0.007395	0.0387	2.99	10885808	presenilin 1	29192
0.007415	0.0387	0.3	10835892	NA	NA
0.007425	0.0388	0.32	10930201	NA	NA
0.007453	0.0389	5.24	10921131	leucine zipper transcription factor-like 1	316102
0.007461	0.0389	0.23	10779636	NA	NA
0.007466	0.0389	5.34	10707824	selenoprotein S	286900

0.007466	0.0389	2.67	10729715	sphingomyelin synthase 1	353229
0.007493	0.039	0.28	10768254	similar to complement factor H-related protein	498241
0.007517	0.0391	3.55	10789059	1-acylglycerol-3-phosphate O-acyltransferase 6 (lysophosphatidic acid acyltransferase, zeta)	290843
0.007536	0.0391	0.3	10707113	potassium voltage gated channel, Shaw-related subfamily, member 1	25327
0.007564	0.0392	3.24	10897203	NA	NA
0.007566	0.0392	2.99	10774310	protein phosphatase 3, regulatory subunit B, alpha isoform	29748
0.007611	0.0394	4.01	10724682	NA	NA
0.007625	0.0394	2.77	10719659	similar to RIKEN cDNA 1700008P20	292706
0.007626	0.0394	3.9	10867398	mitochondrial ribosomal protein L15	297799
0.007626	0.0394	0.3	10827936	general transcription factor II H, polypeptide 4	294236
0.007627	0.0394	2.41	10831425	activating transcription factor 6 beta	406169
0.007648	0.0395	4.33	10736476	coiled-coil domain containing 55	303346
0.007673	0.0395	2.84	10752771	protease, serine, 7 (enterokinase)	288291
0.007683	0.0395	2.36	10729999	nucleolar complex associated 3 homolog (S. cerevisiae)	361753
0.007684	0.0395	3.05	10831298	heat shock 70kD protein 1B (mapped)	294254
0.007702	0.0395	6.65	10917106	RNA binding motif protein 7	315634
0.007706	0.0395	0.36	10938847	NA	NA
0.007708	0.0395	0.3	10869037	NA	NA
0.007711	0.0395	0.29	10754390	stefin A2-like 1	408214
0.007719	0.0395	0.25	10915283	olfactory receptor 1151	300411
0.007725	0.0395	0.23	10930132	solute carrier organic anion transporter family, member 6c1	287006
0.007727	0.0395	3.32	10902885	tetraspanin 31	362890
0.007727	0.0395	0.25	10812061	coiled-coil domain containing 7	497041
0.007732	0.0395	0.29	10791964	NA	NA
0.007755	0.0396	4.17	10879516	major facilitator superfamily domain containing 2	298504
0.007765	0.0396	2.73	10747482	hypocretin	25723
0.007781	0.0396	3.05	10933015	similar to 4930453N24Rik protein	304176
0.007789	0.0396	0.28	10925572	NA	NA
0.007789	0.0396	2.39	10906872	cyclin T1	315291
0.007789	0.039	4.35	1077247	nuclear transcription factor, X-box binding-like 1	289595

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0.007818	0.0397	0.27	10939223	NA	NA
0.007819	0.0397	0.3	10747011	tensin 4	303517
0.007821	0.0397	0.11	10744789	olfactory receptor 1475	287487
0.007826	0.0397	4.75	10818690	asparagine-linked glycosylation 14 homolog (S. cerevisiae)	362031
0.007849	0.0397	2.63	10717793	regulator of G-protein signaling 17	308118
0.00785	0.0397	3.01	10890609	potassium voltage-gated channel, subfamily H (eag-related), member 5	171146
0.00786	0.0398	0.23	10703461	NA	NA
0.007923	0.0401	0.29	10901176	NA	NA
0.007933	0.0401	0.31	10800838	NA	NA
0.007942	0.0401	0.28	10878147	NA	NA
0.007945	0.0401	3.46	10711657	NA	NA
0.007964	0.0401	0.29	10770391	NA	NA
0.007979	0.0402	0.19	10859762	NA	NA
0.007999	0.0402	5.53	10867165	lactamase, beta 2	297768
0.008002	0.0402	3.48	10766998	NA	NA
0.008014	0.0403	3.55	10779673	lectin, galactoside-binding, soluble, 3	83781
0.008047	0.0404	0.23	10934209	diacylglycerol O-acyltransferase 2-like 6	678749
0.008053	0.0404	0.27	10849125	elongation factor RNA polymerase II-like 3	296102
0.008058	0.0404	4.95	10851991	activity-dependent neuroprotector homeobox	64622
0.008071	0.0404	0.24	10737252	ring finger protein 43	303412
0.008079	0.0404	0.28	10719680	similar to BC049730 protein	292711
0.008084	0.0404	3.46	10825931	glutathione S-transferase mu 4	499689
0.008091	0.0404	0.32	10905335	caspase recruitment domain family, member 10	315120
0.008094	0.0404	0.31	10847100	NA	NA
0.008101	0.0404	3.32	10773247	neuron specific gene family member 1	25247
0.008121	0.0405	0.3	10863035	NA	NA
0.008126	0.0405	0.34	10829378	collagen, type XVIII, alpha 1	85251
0.00814	0.0405	5.41	10798465	NA	NA
0.00815	0.0406	2.56	10839154	protein disulfide isomerase family A, member 3	29468

0.008198	0.0408	4.67	10861226	family with sequence similarity 3, member C	312159
0.008209	0.0408	2.65	10806687	coiled-coil domain containing 130	304656
0.008222	0.0408	0.32	10835841	olfactory receptor 398	405119
0.008231	0.0408	0.24	10891010	NA	NA
0.008236	0.0408	4.87	10771049	glomulin, FKBP associated protein	289437
0.008248	0.0408	0.33	10820163	phosphoinositide-3-kinase, catalytic, beta polypeptide	85243
0.008256	0.0408	2.62	10773452	low density lipoprotein receptor-related protein associated protein 1	116565
0.008258	0.0408	0.33	10935179	similar to hypothetical protein E230019M04	315915
0.008259	0.0408	0.38	10776165	casein alpha s2-like B	286759
0.008267	0.0408	0.34	10905362	ankyrin repeat domain 54	362957
0.008291	0.0409	2.5	10703364	TATA box binding protein	117526
0.0083	0.0409	0.34	10837613	olfactory receptor 659	295865
0.008308	0.0409	3.75	10877984	kelch-like 9 (Drosophila)	313348
0.008311	0.0409	0.31	10714913	kinesin family member 20B	309523
0.008324	0.0409	2.68	10785176	dolichyl-phosphate mannosyltransferase polypeptide 3	502017
0.008324	0.0409	2.68	10816766	dolichyl-phosphate mannosyltransferase polypeptide 3	502017
0.008345	0.041	0.4	10817512	synaptic vesicle glycoprotein 2a	117559
0.008349	0.041	4.31	10759585	NA	NA
0.008371	0.041	3.1	10763913	retinoblastoma binding protein 5	304794
0.008372	0.041	0.43	10748459	calcium channel, voltage-dependent, gamma subunit 4	140725
0.00838	0.041	3.61	10796912	similar to Zinc finger protein 208	680290
0.008396	0.0411	0.12	10862830	NA	NA
0.008498	0.0416	0.33	10821180	NA	NA
0.008516	0.0416	0.33	10732819	NA	NA
0.008551	0.0418	2.75	10777034	similar to 1810013D10Rik protein	501923
0.008588	0.0419	0.25	10846912	olfactory receptor 441	295712
0.008636	0.0421	0.26	10882461	similar to Glutaminyl-peptide cyclotransferase precursor (QC)	313837
0.008719	0.0425	0.27	10934517	LRRGT00193	680227
0.008745	0.0426	0.36	10875309	NA	NA
0.008762	0.042	5.01	1081055	NA	NA

	6		1		
0.00878	0.0427	3.95	10849905	family with sequence similarity 113, member A	296158
0.008788	0.0427	3.95	10832287	ubiquitin-conjugating enzyme E2G 2 (UBC7 homolog, yeast)	294331
0.008822	0.0428	0.27	10850812	defensin beta 24	641632
0.00883	0.0428	0.35	10712112	secretoglobin, family 1C, member 1	309100
0.008845	0.0428	3.51	10772795	NA	NA
0.008851	0.0428	3.91	10822269	leucine rich repeat and coiled-coil domain containing 1	266808
0.008853	0.0428	2.3	10934049	NA	NA
0.008881	0.043	0.35	10908123	LRRGT00142	689840
0.008963	0.0433	2.99	10791511	NA	NA
0.008967	0.0433	0.32	10922615	von Willebrand factor A domain containing 3B	501126
0.008975	0.0433	0.31	10901182	olfactory receptor 1096	302027
0.008989	0.0434	0.39	10900228	phosphatidylinositol-4-phosphate 5-kinase, type I, gamma	314641
0.008998	0.0434	0.35	10909237	NA	NA
0.009022	0.0434	0.29	10704299	vomeronasal 1 receptor 58	494226
0.009028	0.0434	0.31	10814176	NA	NA
0.009041	0.0435	3.34	10836560	NA	NA
0.009058	0.0435	0.35	10779681	NA	NA
0.00906	0.0435	2.78	10851502	serine incorporator 3	296350
0.009078	0.0436	0.31	10881301	NA	NA
0.009106	0.0437	3.75	10833152	cysteine and glycine-rich protein 2	29317
0.009114	0.0437	0.22	10930588	NA	NA
0.009138	0.0437	3.56	10817552	thioredoxin interacting protein	117514
0.009164	0.0438	3.12	10820213	NA	NA
0.009165	0.0438	2.81	10880658	NA	NA
0.00917	0.0438	2.57	10861196	NA	NA
0.009185	0.0438	2.78	10730391	mitochondrial ribosomal protein L43	309440
0.009194	0.0439	0.31	10877876	NA	NA
0.009208	0.0439	2.85	10909358	NA	NA
0.009227	0.0439	3.13	10884274	sclerostin domain containing 1	266803

0.009234	0.044	2.85	10782028	DnaJ (Hsp40) homolog, subfamily C, member 3	63880
0.009246	0.044	0.35	10782041	NA	NA
0.009264	0.044	0.33	10844960	low density lipoprotein-related protein 1B (deleted in tumors)	311926
0.009277	0.044	5.05	10781568	nuclear fragile X mental retardation protein interacting protein 1	364430
0.009278	0.044	2.49	10763740	YOD1 OTU deubiquinating enzyme 1 homolog (S. cerevisiae)	363982
0.009301	0.0441	3.42	10835845	olfactory receptor 400	296661
0.009321	0.0442	0.26	10936701	NA	NA
0.009325	0.0442	5.28	10841487	RNA-binding region containing protein 2-like	1E+08
0.009331	0.0442	0.33	10846948	olfactory receptor 480	404890
0.009342	0.0442	3.18	10886854	NA	NA
0.009347	0.0442	0.3	10890166	NA	NA
0.009357	0.0442	0.36	10784212	NA	NA
0.009418	0.0444	2.42	10795174	histone cluster 1, H2ba	24829
0.009427	0.0444	0.29	10723553	NA	NA
0.009431	0.0444	2.49	10797493	similar to RIKEN cDNA 2900010M23	361805
0.009443	0.0445	2.74	10832478	pre-B lymphocyte 3	365550
0.009448	0.0445	5.55	10794752	NA	NA
0.009455	0.0445	2.47	10868193	akirin 2	297968
0.009472	0.0445	0.27	10904418	NA	NA
0.009497	0.0446	3.25	10860749	NA	NA
0.00955	0.0448	4.05	10903290	similar to DKFZP434I092 protein	500853
0.009554	0.0448	2.35	10894857	PCTAIRE protein kinase 2	314743
0.00956	0.0448	0.32	10787801	NA	NA
0.009588	0.0449	3.87	10813126	biliverdin reductase A	116599
0.009602	0.0449	0.41	10875656	NA	NA
0.009614	0.0449	4.35	10810631	tubulin polymerization-promoting protein family member 3	291966
0.009627	0.045	0.34	10909384	tripartite motif-containing 29	300656
0.009629	0.045	3.34	10814752	mitofusin 1	192647
0.009642	0.045	3.51	10766463	dual specificity phosphatase 10	63995
0.009644	0.045	0.34	1072904	olfactory receptor 349	405393

			5		
0.009659	0.045	0.3	10883971	NA	NA
0.009678	0.0451	0.29	10908154	NA	NA
0.009749	0.0454	2.42	10776671	NA	NA
0.009767	0.0454	0.29	10827577	NA	NA
0.009773	0.0454	0.29	10859772	NA	NA
0.009786	0.0454	2.98	10882888	NA	NA
0.009794	0.0454	6.23	10713583	WD repeat domain 74	690229
0.009825	0.0455	0.26	10745702	NA	NA
0.009826	0.0455	0.17	10722405	NA	NA
0.009872	0.0457	0.39	10828477	synaptic Ras GTPase activating protein 1 homolog (rat)	192117
0.009883	0.0457	0.22	10740810	olfactory receptor 1382	287093
0.00989	0.0457	3.67	10926967	similar to Glutathione S-transferase A1 (GTH1) (HA subunit 1) (GST-epsilon) (GSTA1-1) (GST class-alpha)	501110
0.009914	0.0458	0.34	10935811	gamma-aminobutyric acid (GABA) A receptor, epsilon	65191
0.009938	0.0459	0.31	10824796	NA	NA
0.009968	0.046	0.28	10932721	lipoma HMGIC fusion partner-like 1	300286
0.009976	0.046	0.37	10936925	NA	NA
0.009983	0.046	0.33	10838439	olfactory receptor 773	296026
0.009997	0.046	0.39	10910722	NA	NA

APPENDIX D: TRANCRIPTS DIFFERENTIALLY EXPRESSED IN UNBOUND CA1 8HR AND NIC GROUPS

Parametricp-value	FDR	Fold-change	ProbeSet	Name	EntrezID
2E-07	0.00132	42.06	10719977	melanoma inhibitory activity	81510
9E-07	0.00296	22.48	10756606	NA	NA
1.9E-06	0.00408	15.53	10871413	NA	NA
0.000004	0.00408	11.29	10898158	NA	NA
0.000004	0.00408	11.29	10910752	NA	NA
4.4E-06	0.00408	15.77	10761128	heat shock protein 1	24471
4.4E-06	0.00408	12.86	10828827	cyclin-dependent kinase inhibitor 1A	114851
5.7E-06	0.00408	11.13	10840791	sulfiredoxin 1 homolog (S. cerevisiae)	296271
6.1E-06	0.00408	14.83	10757082	zinc finger, AN1-type domain 2A	360772
6.2E-06	0.00408	11.67	10770710	activating transcription factor 3	25389
7.3E-06	0.00437	9.8	10736697	chemokine (C-C motif) ligand 2	24770
9.6E-06	0.00526	12.37	10844268	NA	NA
1.43E-05	0.00649	11.67	10835845	olfactory receptor 400	296661
1.46E-05	0.00649	9.19	10764551	prostaglandin-endoperoxide synthase 2	29527
1.48E-05	0.00649	8.73	10795280	NA	NA
1.68E-05	0.00691	7.99	10790966	inositol-3-phosphate synthase 1	290651
1.95E-05	0.00755	8.46	10795297	histone cluster 1, H4b	64627
2.21E-05	0.00755	8.7	10798490	histone cluster 1, H4b	64627
2.23E-05	0.00755	8.62	10798461	histone cluster 1, H4b	64627
2.32E-05	0.00755	11.57	10703104	Park2 co-regulated	499021
2.41E-05	0.00755	8.02	10798499	histone cluster 1, H4b	64627
3.11E-05	0.00886	9.54	10928522	NA	NA
0.000032	0.00886	7.38	10933947	NA	NA
3.36E-05	0.00886	8.43	10809392	metallothionein 1a	24567
0.000034	0.00886	16.52	10727717	neuronal PAS domain protein 4	266734
0.000035	0.00886	7.67	10796411	metallothionein 1a	24567
3.95E-05	0.00956	6.93	10736914	NA	NA
4.07E-05	0.00956	7.14	10839579	dual specificity phosphatase 2	311406
4.22E-05	0.00957	8.33	10761394	phosphoserine phosphatase	304429

5.63E-05	0.0117	12.17	10797527	growth arrest and DNA-damage-inducible, gamma	291005
6.09E-05	0.0117	6.31	10825151	histone cluster 2, H4	295277
6.16E-05	0.0117	8.67	10702428	ADP-ribosylation factor 1	64310
0.000062	0.0117	5.99	10795335	zinc finger with KRAB and SCAN domains 3	306977
6.42E-05	0.0117	19.1	10862867	growth arrest and DNA-damage-inducible, alpha	25112
6.45E-05	0.0117	17.07	10732652	dual specificity phosphatase 1	114856
6.49E-05	0.0117	8.4	10891026	zinc finger, FYVE domain containing 1	299188
6.66E-05	0.0117	6.02	10878009	NA	NA
6.76E-05	0.0117	9.22	10931010	similar to lymphocyte antigen 6 complex, locus E ligand	501282
7.53E-05	0.0127	5.53	10872062	proteasome (prosome, macropain) subunit, beta type 2	29675
8.34E-05	0.0133	9.65	10937867	NA	NA
8.37E-05	0.0133	10.39	10746789	NA	NA
8.67E-05	0.0133	8.04	10820151	NA	NA
9.11E-05	0.0133	6.3	10720215	zinc finger protein 36	79426
9.31E-05	0.0133	14.3	10923338	coenzyme Q10 homolog B (S. cerevisiae)	301416
9.37E-05	0.0133	5.57	10869533	NA	NA
9.41E-05	0.0133	5.03	10910666	NA	NA
9.48E-05	0.0133	9.29	10703139	ribonuclease T2	292306
9.81E-05	0.0134	0.14	10798547	NA	NA
9.98E-05	0.0134	5.27	10875420	pro-histogranin	502940
0.000105	0.0138	5.4	10795200	pro-histogranin	502940
0.000107	0.0138	0.2	10938312	NA	NA
0.000119	0.0149	4.66	10913174	NA	NA
0.00012	0.0149	0.19	10722483	NA	NA
0.000125	0.015	5.34	10851484	NA	NA
0.000125	0.015	6.37	10934772	mannose-6-phosphate receptor, cation dependent	312689
0.000132	0.0156	4.38	10923345	heat shock protein 1 (chaperonin 10)	25462
0.000138	0.016	5.33	10707824	selenoprotein S	286900
0.000144	0.0164	5.6	10768332	regulator of G-protein signaling 2	84583
0.000154	0.0166	12.18	10792268	phosphatidic acid phosphatase type 2 domain containing 1B	680466
0.000156	0.0166	5.15	10709875	adrenomedullin	25026
0.000156	0.0166	5.59	10758930	heat shock protein B8	113906
0.000157	0.0166	0.21	10703420	NA	NA
0.000159	0.0166	4.77	10873336	phospholipase A2, group IID	298579

0.000162	0.0167	12.77	10733047	NA	NA
0.000168	0.0169	11.76	10733045	NA	NA
0.00017	0.0169	5.7	10872654	protein phosphatase 1, regulatory (inhibitor) subunit 8	313030
0.000177	0.0173	5.26	10909733	zinc finger protein 259	500989
0.000181	0.0175	8.1	10897045	mesenchymal stem cell protein DSCD75	300015
0.000184	0.0175	6.13	10711401	Bcl2-associated athanogene 3	293524
0.000186	0.0175	4.36	10719464	NA	NA
0.00019	0.0176	4.47	10897543	H1 histone family, member 0	24437
0.000204	0.0186	4.93	10773235	zinc finger protein 509	305428
0.000206	0.0186	8.56	10900358	growth arrest and DNA-damage-inducible, beta	299626
0.000213	0.0189	0.14	10723064	mannosidase 2, alpha 2	308757
0.000219	0.0189	4.78	10832287	ubiquitin-conjugating enzyme E2G 2 (UBC7 homolog, yeast)	294331
0.000222	0.0189	0.22	10703640	vomeronasal 2 receptor, 25	502286
0.000224	0.0189	0.17	10932699	NA	NA
0.000224	0.0189	9.93	10769672	regulator of G-protein signaling 4	29480
0.000227	0.0189	4.25	10799914	NA	NA
0.000234	0.0193	5.28	10778375	NA	NA
0.00024	0.0194	6.25	10757636	chemokine (C-C motif) ligand 26	685958
0.000254	0.0204	0.17	10852437	potassium voltage-gated channel, KQT-like subfamily, member 2	170848
0.000266	0.0208	5.8	10846740	frizzled-related protein	295691
0.000269	0.0208	0.19	10866134	immunoreceptor Ly49si2	494207
0.00027	0.0208	7.6	10759585	NA	NA
0.000272	0.0208	7.76	10831077	immediate early response 3	294235
0.000277	0.0208	4.34	10909892	crystallin, alpha B	25420
0.000279	0.0208	4.57	10868289	DnaJ (Hsp40) homolog, subfamily A, member 1	65028
0.000286	0.0212	3.95	10899378	GRP1 (general receptor for phosphoinositides 1)-associated scaffold protein	192254
0.000297	0.0215	9.27	10878034	NA	NA
0.000298	0.0215	5.38	10720430	proteasome (prosome, macropain) 26S subunit, non-ATPase, 8	292766
0.0003	0.0215	0.25	10772799	NA	NA
0.000305	0.0216	5.03	10853916	NA	NA
0.000314	0.022	0.21	10938176	NA	NA
0.000324	0.0222	0.23	10722435	NA	NA
0.000331	0.0222	0.23	10728494	solute carrier family 22, member 25	192273
0.000333	0.0222	0.25	10803438	NA	NA

0.000335	0.0222	4.39	10838823	ChaC, cation transport regulator homolog 1 (E. coli)	362196
0.000336	0.0222	4.94	10769131	calcyclin binding protein	289144
0.000338	0.0222	5.62	10740869	tumor necrosis factor receptor superfamily, member 12a	302965
0.00034	0.0222	3.74	10890271	NA	NA
0.000345	0.0222	4.27	10805895	gene trap locus 3	307642
0.000351	0.0223	7.51	10750371	NA	NA
0.000352	0.0223	0.21	10887336	NA	NA
0.000364	0.0226	4.24	10820504	betaine-homocysteine methyltransferase 2	365972
0.000366	0.0226	0.2	10934209	diacylglycerol O-acyltransferase 2-like 6	678749
0.00037	0.0226	7.28	10871417	NA	NA
0.000375	0.0226	3.97	10772795	NA	NA
0.000377	0.0226	0.21	10823425	NA	NA
0.000379	0.0226	0.26	10907676	NA	NA
0.000382	0.0226	7.7	10765411	NA	NA
0.000385	0.0226	5.45	10922182	NA	NA
0.000388	0.0226	9.29	10845624	NA	NA
0.000393	0.0227	4.7	10811452	coactosin-like 1 (Dictyostelium)	361422
0.000422	0.0238	5.23	10776667	NA	NA
0.000423	0.0238	6.24	10766760	lysophosphatidylglycerol acyltransferase 1	679692
0.000423	0.0238	4.08	10912076	similar to Selenoprotein H	502642
0.000428	0.0239	0.27	10774420	NA	NA
0.000437	0.0239	0.27	10724047	NA	NA
0.000437	0.0239	5.02	10924230	NA	NA
0.000441	0.0239	0.24	10910872	immunoglobulin superfamily, DCC subclass, member 4	363081
0.000446	0.024	7.9	10893242	DnaJ (Hsp40) homolog, subfamily C, member 14	114481
0.000448	0.024	4.39	10801584	COMM domain containing 10	361323
0.000469	0.0249	4.9	10711657	NA	NA
0.000482	0.0252	4.48	10737770	NA	NA
0.000486	0.0252	4.25	10866672	RecQ protein-like (DNA helicase Q1-like)	312824
0.000487	0.0252	5.27	10868428	DnaJ (Hsp40) homolog, subfamily B, member 5	313811
0.0005	0.0254	0.2	10918867	NA	NA
0.000501	0.0254	7.49	10858497	NA	NA
0.000503	0.0254	0.15	10846980	olfactory receptor 500	404884
0.000506	0.0254	5.69	10918820	NA	NA

0.000522	0.026	4.17	10829703	transcription factor A, mitochondrial	83474
0.000528	0.0261	5.52	10858487	mannose-6-phosphate receptor, cation dependent	312689
0.000536	0.0261	0.24	10751086	NA	NA
0.000536	0.0261	4.77	10936063	solute carrier family 10 (sodium/bile acid cotransporter family), member 3	501665
0.000543	0.0261	0.23	10799370	tubulin, alpha-like 3	291287
0.000544	0.0261	0.25	10866306	taste receptor, type 2, member 106	1E+08
0.000552	0.0262	6.82	10934700	apolipoprotein O-like	317191
0.000555	0.0262	0.21	10701630	NA	NA
0.000559	0.0262	7.43	10896992	protein tyrosine phosphatase type IVA, member 3	362930
0.000562	0.0262	4.4	10734873	NA	NA
0.000575	0.0264	3.41	10928154	heat shock protein 1 (chaperonin)	63868
0.000577	0.0264	4.32	10774964	NA	NA
0.000578	0.0264	4.6	10901231	TIMP metallopeptidase inhibitor 3	25358
0.000583	0.0265	5.45	10917106	RNA binding motif protein 7	315634
0.0006	0.027	3.97	10780003	NA	NA
0.00061	0.0272	7.26	10919960	testis expressed 264	300988
0.000613	0.0272	6.13	10896628	NA	NA
0.000621	0.0273	5.39	10906323	choline kinase beta	29367
0.000623	0.0273	4.82	10802336	NA	NA
0.000647	0.0277	0.24	10811248	NA	NA
0.00065	0.0277	0.17	10761287	GATS protein-like 2	304410
0.00065	0.0277	0.3	10909251	olfactory receptor 1266	405088
0.000652	0.0277	7.09	10909639	FXYD domain-containing ion transport regulator 6	63847
0.000657	0.0277	4.25	10892962	prostaglandin E synthase 3 (cytosolic)	362809
0.000657	0.0277	0.29	10755035	tumor protein p63	246334
0.000664	0.0278	4.46	10770045	NA	NA
0.000669	0.0279	3.79	10869541	NA	NA
0.000675	0.0279	0.27	10908229	NA	NA
0.000685	0.0281	0.27	10838416	olfactory receptor 757	404802
0.00069	0.0281	0.29	10770109	NA	NA
0.000693	0.0281	4.11	10879963	hippocalcin	29177
0.000711	0.0287	0.3	10874116	NA	NA
0.000748	0.0298	4.75	10860580	NA	NA
0.000751	0.0298	4.54	10865855	NA	NA

0.000758	0.0298	0.19	10731291	limkain b1	170946
0.000759	0.0298	0.25	10893325	olfactory receptor 905	288797
0.000762	0.0298	0.26	10765820	NA	NA
0.000772	0.0299	4.01	10910490	hexosaminidase A	300757
0.000774	0.0299	0.23	10765904	olfactory receptor 1598	405221
0.00078	0.0299	3.43	10900112	NA	NA
0.000787	0.0299	4.4	10782901	cornichon homolog (Drosophila)	289994
0.000791	0.0299	0.27	10812061	coiled-coil domain containing 7	497041
0.000795	0.0299	4.14	10925373	ubiquitin-conjugating enzyme E2F (putative)	363284
0.000796	0.0299	0.26	10916867	transmembrane protein 25	689172
0.000817	0.0303	0.28	10837554	olfactory receptor 590	404858
0.000818	0.0303	4.46	10844229	dystonia 1	266606
0.000825	0.0303	0.22	10933753	NA	NA
0.000827	0.0303	4.32	10846982	NA	NA
0.000828	0.0303	6.97	10859655	mediator complex subunit 21	312849
0.000859	0.0312	7.32	10896748	ring finger protein 139	315000
0.000899	0.0322	3.23	10809399	metallothionein 2A	689415
0.000911	0.0322	0.31	10902129	NA	NA
0.000912	0.0322	5.79	10855462	similar to chromosome 7 open reading frame 30	297082
0.000913	0.0322	5.69	10918272	U5 small nuclear RNA	113834
0.000916	0.0322	0.3	10884614	NA	NA
0.000916	0.0322	0.17	10837504	olfactory receptor 554	295797
0.000921	0.0322	0.28	10908660	neuropeptide S receptor 1	300458
0.000927	0.0322	3.92	10812580	NA	NA
0.000931	0.0322	3.17	10823309	stress-associated endoplasmic reticulum protein 1	80881
0.000947	0.0326	0.23	10719159	NA	NA
0.000954	0.0327	0.25	10888617	similar to 14-3-3 protein sigma	298795
0.000966	0.0329	4.89	10810831	CTF8, chromosome transmission fidelity factor 8 homolog (S. cerevisiae)	364996
0.000971	0.0329	4.59	10774171	uridine phosphorylase 1	289801
0.000981	0.033	5.12	10776064	UTP3, small subunit (SSU) processome component, homolog (S. cerevisiae)	305258
0.000984	0.033	0.26	10768754	NA	NA
0.000999	0.0333	0.23	10884820	NA	NA
0.001014	0.0336	5.23	10763477	Mki67 (FHA domain) interacting nucleolar phosphoprotein	246042
0.001016	0.0336	0.25	10877307	major urinary protein 5	298107

0.001022	0.0336	3.29	10795194	NA	NA
0.001035	0.0337	0.24	10878314	NA	NA
0.001037	0.0337	0.21	10703216	NA	NA
0.001039	0.0337	4.51	10897203	NA	NA
0.001046	0.0337	0.3	10855090	olfactory receptor 808	405140
0.00106	0.034	4.64	10829759	NA	NA
0.001069	0.0341	0.33	10791964	NA	NA
0.00109	0.0343	0.3	10711087	NA	NA
0.001093	0.0343	0.22	10718152	NA	NA
0.001098	0.0343	7	10911309	general transcription factor IIA, 2	83828
0.001106	0.0343	0.21	10811999	NA	NA
0.001109	0.0343	0.32	10909196	olfactory receptor 1247	405093
0.001113	0.0343	0.25	10714610	NA	NA
0.001116	0.0343	5.71	10838873	inositol 1,4,5-trisphosphate 3-kinase A	81677
0.001118	0.0343	0.3	10820221	NA	NA
0.00112	0.0343	3.03	10742975	olfactory receptor 1457	363608
0.001126	0.0343	6.6	10738140	insulin-like growth factor binding protein 4	360622
0.001136	0.0343	8.93	10748336	NA	NA
0.001139	0.0343	0.22	10765818	olfactory receptor 1583	289241
0.001145	0.0343	0.25	10837489	olfactory receptor 527	404878
0.001146	0.0343	3.38	10865420	calsyntenin 3	171393
0.001151	0.0343	0.24	10759459	NA	NA
0.001157	0.0343	0.26	10934197	NA	NA
0.001168	0.0343	0.22	10865681	tubulin, alpha 3A	500319
0.001168	0.0343	3.04	10827989	metallothionein 2A	689415
0.001196	0.0347	0.3	10902970	kinesin family member 5A	314906
0.001202	0.0347	0.26	10932339	NA	NA
0.001203	0.0347	3.8	10818317	proteasome (prosome, macropain) subunit, alpha type 5	29672
0.001205	0.0347	0.23	10745782	phosphatidylinositol glycan anchor biosynthesis, class W	378774
0.00121	0.0347	0.25	10936923	histone variant H2al2-like	1E+08
0.001219	0.0347	3.48	10879047	ATPase, H+ transporting, lysosomal 21kDa, V0 subunit b	298451
0.001222	0.0347	3.26	10884853	PRP39 pre-mRNA processing factor 39 homolog (S. cerevisiae)	314171
0.001225	0.0347	0.24	10863430	hexokinase 2	25059
0.001229	0.0347	4.28	10811126	fatty acid 2-hydroxylase	307855

0.001233	0.0347	0.26	10900010	olfactory receptor 921	366798
0.001248	0.0347	0.25	10814392	NA	NA
0.001257	0.0347	3.75	10796455	signal transducing adaptor molecule (SH3 domain and ITAM motif) 1	498798
0.001264	0.0347	4.09	10936302	ubiquitin-conjugating enzyme E2A (RAD6 homolog)	298317
0.001265	0.0347	0.26	10797541	SECIS binding protein 2	79049
0.001266	0.0347	5.33	10779825	NA	NA
0.001274	0.0347	4.37	10796134	RNA binding motif protein 17	291295
0.001282	0.0347	5.17	10804856	thioredoxin-like 1	140922
0.001282	0.0347	5.2	10799558	phytanoyl-CoA 2-hydroxylase	114209
0.001283	0.0347	6.84	10725387	cerebellar degeneration-related 2	308958
0.001292	0.0348	0.22	10858612	vomeronasal 2 receptor, 49	688392
0.001298	0.0348	4.73	10827686	RT1 class I, locus M6, gene 1	414785
0.001303	0.0348	0.3	10781982	NA	NA
0.00131	0.0348	3.41	10938209	NA	NA
0.001322	0.0348	4.78	10774625	NA	NA
0.001327	0.0348	0.33	10725078	pleckstrin homology domain containing, family A member 7	499249
0.001329	0.0348	0.31	10776185	theobromine induced protein	689604
0.001334	0.0348	3.97	10876213	rCG55084-like	1E+08
0.001338	0.0348	4.94	10907913	matrix metallopeptidase 8	63849
0.001342	0.0348	3.68	10890024	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	25493
0.001345	0.0348	0.23	10723553	NA	NA
0.001374	0.0349	0.24	10894295	vomeronasal 1 receptor 107	494260
0.001377	0.0349	4.81	10834035	endosulfine alpha	60334
0.001379	0.0349	4.52	10883094	protein phosphatase 1G (formerly 2C), magnesium-dependent, gamma isoform	259229
0.001381	0.0349	12.24	10738703	reprimo-like	685826
0.001389	0.0349	8.31	10820843	CART prepropeptide	29131
0.001391	0.0349	0.33	10805285	NA	NA
0.0014	0.0349	0.25	10800744	excision repair cross-complementing rodent repair deficiency, complementation group 3	291703
0.001409	0.0349	7.32	10805882	casein kinase 2, alpha prime polypeptide	307641
0.00141	0.0349	0.25	10807889	NA	NA
0.001411	0.0349	3.05	10747075	keratin 28	360623
0.001411	0.0349	5.38	10835618	torsin family 2, member A	362112
0.001413	0.0349	0.34	10916365	olfactory receptor 1301	300602
0.001423	0.0351	3.87	10876011	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 6	297990

0.00143	0.0351	5.07	10762709	coenzyme Q5 homolog, methyltransferase (S. cerevisiae)	304542
0.001448	0.0354	0.3	10845708	interferon induced with helicase C domain 1	499801
0.001461	0.0356	4.08	10832224	NA	NA
0.001468	0.0356	3.49	10917483	RAB39, member RAS oncogene family	315668
0.001512	0.0365	4.31	10863722	NA	NA
0.001515	0.0365	0.21	10939533	NA	NA
0.00156	0.0372	0.33	10813253	complement component 6	24237
0.00156	0.0372	0.25	10729019	NA	NA
0.001563	0.0372	0.25	10891991	serine (or cysteine) peptidase inhibitor, clade A, member 3K	24794
0.001568	0.0372	7.01	10919034	NA	NA
0.001586	0.0374	0.29	10889723	NA	NA
0.001609	0.0374	4.03	10749789	hypothetical protein LOC619574	619574
0.001613	0.0374	3.95	10934794	interferon stimulated exonuclease gene 20-like 2	361977
0.001616	0.0374	0.33	10902418	NA	NA
0.001629	0.0374	3.26	10713583	WD repeat domain 74	690229
0.001631	0.0374	3.9	10924326	rcd1 (required for cell differentiation) homolog 1 (S. pombe)	301513
0.001633	0.0374	0.32	10939779	NA	NA
0.00164	0.0374	0.28	10815913	NA	NA
0.001643	0.0374	0.27	10885482	NA	NA
0.001647	0.0374	0.32	10826451	solute carrier family 44, member 3	295417
0.001674	0.0374	0.28	10908167	olfactory receptor 1128	405345
0.001679	0.0374	0.26	10934683	NA	NA
0.00169	0.0374	0.2	10756105	NA	NA
0.001691	0.0374	10.09	10887016	NA	NA
0.001691	0.0374	10.09	10887020	NA	NA
0.001691	0.0374	10.09	10887024	NA	NA
0.001691	0.0374	10.09	10887028	NA	NA
0.001691	0.0374	10.09	10887032	NA	NA
0.001691	0.0374	10.09	10887036	NA	NA
0.001691	0.0374	10.09	10887040	NA	NA
0.001693	0.0374	0.27	10826616	NA	NA
0.001713	0.0377	5.73	10927840	NA	NA
0.001728	0.0379	0.16	10814286	fatty acid binding protein 4, adipocyte	79451
0.001738	0.0379	3.59	10874450	leucine rich repeat containing 47	362672

0.00174	0.0379	3.37	10745266	transmembrane protein 97	303330
0.001751	0.038	3.61	10807722	thioredoxin-like 4B	292008
0.001764	0.0382	3.04	10910252	snurportin 1	316108
0.001774	0.0383	3.85	10787209	CCR4-NOT transcription complex, subunit 7	306492
0.001779	0.0383	3.38	10829639	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1	361825
0.001811	0.0386	0.2	10706240	NA	NA
0.001811	0.0386	0.15	10837881	low density lipoprotein receptor-related protein 4	83469
0.001815	0.0386	0.27	10791227	NA	NA
0.001829	0.0388	5.4	10928207	similar to hypothetical protein FLJ37953	301419
0.001836	0.0388	3.08	10758344	vacuolar protein sorting 37 homolog B (S. cerevisiae)	288659
0.001842	0.0388	0.28	10776260	NA	NA
0.001856	0.0388	0.3	10867318	NA	NA
0.001858	0.0388	0.3	10796679	NA	NA
0.001864	0.0388	3.05	10879516	major facilitator superfamily domain containing 2	298504
0.001864	0.0388	0.27	10774892	NA	NA
0.001877	0.0389	0.21	10866205	NA	NA
0.001886	0.039	0.32	10767503	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3	304775
0.001896	0.0391	0.22	10848118	olfactory receptor 775	296028
0.001902	0.0391	0.29	10729762	NA	NA
0.001926	0.0395	0.28	10938671	zinc finger, MYM-type 3	317260
0.001945	0.0397	0.25	10887050	NA	NA
0.001966	0.04	4.08	10912439	retinol binding protein 1, cellular	25056
0.001985	0.0402	0.26	10795719	NA	NA
0.001986	0.0402	0.19	10884912	NA	NA
0.002007	0.0405	3.65	10780237	similar to 1700123O20Rik protein	361038
0.002027	0.0408	2.78	10889657	NA	NA
0.002044	0.041	0.28	10881685	kinesin family member 1B	117548
0.002081	0.0414	0.27	10738097	Rap guanine nucleotide exchange factor (GEF)-like 1	303515
0.002088	0.0414	0.24	10837097	NA	NA
0.002089	0.0414	6.4	10775260	similar to RIKEN cDNA 1700081O22	363337
0.002098	0.0414	3	10827238	NA	NA
0.002098	0.0414	4.27	10797918	PAK1 interacting protein 1	361232
0.002105	0.0415	3.84	10702485	programmed cell death 6	308061
0.002118	0.0415	0.28	10828046	HLA-B associated transcript 2	294250

0.002122	0.0415	0.29	10715255	NA	NA
0.002129	0.0415	0.29	10863035	NA	NA
0.002134	0.0415	0.12	10864300	NA	NA
0.002143	0.0416	4.03	10729120	NA	NA
0.00215	0.0416	3.17	10860801	similar to OEF2	500011
0.002158	0.0416	3.45	10791250	lipoprotein lipase	24539
0.002166	0.0417	3.47	10850666	NA	NA
0.002175	0.0417	0.28	10858142	olfactory receptor 824	405215
0.00219	0.0418	0.28	10934517	LRRGT00193	680227
0.002197	0.0418	4.14	10773722	NA	NA
0.002198	0.0418	0.25	10881350	NA	NA
0.002216	0.042	3.43	10935131	ring finger protein 128	315911
0.002225	0.0421	0.29	10730750	NA	NA
0.002259	0.0425	0.19	10877943	protein tyrosine phosphatase-like A domain containing 2	362540
0.002261	0.0425	3.87	10891850	NA	NA
0.002266	0.0425	2.87	10761463	RAN, member RAS oncogene family	84509
0.002287	0.0426	5.54	10828154	heat shock 70kD protein 1B (mapped)	294254
0.002294	0.0426	4.27	10914935	LOC363015	363015
0.002295	0.0426	0.32	10893338	NA	NA
0.002312	0.0428	3.21	10890354	cyclin-dependent kinase-like 1 (CDC2-related kinase)	314198
0.002315	0.0428	0.25	10854998	seminal vesicle antigen-like 1	680174
0.002325	0.0428	0.31	10764491	NA	NA
0.002344	0.0431	3.02	10909407	Thy-1 cell surface antigen	24832
0.002361	0.0432	0.25	10848111	olfactory receptor 768	405234
0.002366	0.0432	0.3	10812495	NA	NA
0.002386	0.0434	0.29	10901043	NA	NA
0.00239	0.0434	2.99	10809899	NA	NA
0.002394	0.0434	0.23	10922247	NA	NA
0.002404	0.0434	0.24	10833906	NA	NA
0.002419	0.0435	0.31	10707887	NA	NA
0.002434	0.0435	2.77	10743117	ADP-ribosylation factor 1	64310
0.002437	0.0435	3.38	10867165	lactamase, beta 2	297768
0.002438	0.0435	4.27	10866265	NA	NA
0.002441	0.0435	3.04	10824300	solute carrier family 25, member 44	365841

0.002463	0.0438	0.2	10881301	NA	NA
0.002471	0.0438	0.33	10913722	myosin, light chain 3, alkali; ventricular, skeletal, slow	24585
0.002501	0.0439	3.18	10751945	somatostatin	24797
0.002505	0.0439	0.2	10716056	NA	NA
0.002507	0.0439	0.28	10846944	NA	NA
0.00253	0.0439	3.05	10827517	cystathionase (cystathionine gamma-lyase)	24962
0.00253	0.0439	0.27	10903182	retinol dehydrogenase 7	360420
0.002531	0.0439	0.34	10925572	NA	NA
0.002536	0.0439	3.6	10798465	NA	NA
0.00254	0.0439	3.27	10752050	transformer 2 beta homolog (Drosophila)	117259
0.002547	0.0439	3.64	10843169	NA	NA
0.00255	0.0439	0.31	10857265	NA	NA
0.002559	0.0439	2.82	10867731	calbindin 1	83839
0.002567	0.0439	2.78	10822902	NA	NA
0.00257	0.0439	0.24	10714175	NA	NA
0.002579	0.0439	0.35	10715541	carboxypeptidase N, polypeptide 1	365466
0.002579	0.0439	3.49	10884205	TWIST neighbor	362728
0.00259	0.044	4.03	10889728	ADP-ribosylation factor-like 4A	29308
0.002596	0.044	0.35	10737838	NA	NA
0.002601	0.044	5.67	10794016	PDZ and LIM domain 7	286908
0.002609	0.044	0.32	10923877	ADAM metallopeptidase domain 23	301460
0.00263	0.0441	4.67	10729970	retinol binding protein 4, plasma	25703
0.00263	0.0441	0.27	10733843	similar to Robo-1	691277
0.002659	0.0444	3.13	10846286	nuclear factor, erythroid derived 2, like 2	83619
0.002661	0.0444	0.13	10729065	NA	NA
0.002677	0.0446	0.36	10774782	spectrin, beta, non-erythrocytic 1	305614
0.002691	0.0447	5	10832927	NA	NA
0.002702	0.0447	0.32	10833744	NA	NA
0.002704	0.0447	0.31	10774825	similar to hypothetical protein FLJ40298	498431
0.002708	0.0447	0.27	10908214	olfactory receptor 1174	405390
0.002722	0.0447	0.28	10795346	glutathione peroxidase 5	113919
0.002732	0.0447	3.3	10845110	NS5A (hepatitis C virus) transactivated protein 4	311934
0.002734	0.0447	3	10704652	NA	NA
0.002753	0.0449	0.24	10703461	NA	NA

0.002774	0.0452	4.76	10837053	NA	NA
0.002783	0.0452	0.27	10724575	NA	NA
0.002801	0.0454	3.28	10895128	NA	NA
0.002831	0.0457	5.71	10752650	similar to 4930453N24Rik protein	304176
0.002836	0.0457	0.27	10856166	NA	NA
0.002858	0.0458	3.61	10794225	nuclear factor, interleukin 3 regulated	114519
0.002859	0.0458	4.56	10923155	asparagine synthetase domain containing 1	299507
0.002862	0.0458	3.92	10746532	NA	NA
0.002866	0.0458	0.34	10891780	fibulin 5	29158
0.002882	0.0459	3.6	10822433	NA	NA
0.002918	0.0463	0.24	10724819	DENN/MADD domain containing 5A	308942
0.002923	0.0463	3.48	10702792	claudin 20	680178
0.002959	0.0468	0.32	10915299	olfactory receptor 1177	405160
0.002971	0.0469	4.64	10903061	serine hydroxymethyltransferase 2 (mitochondrial)	299857
0.002986	0.0469	0.18	10759497	NA	NA
0.002988	0.0469	4.5	10890484	NA	NA
0.003001	0.047	5.38	10883865	NA	NA
0.003009	0.047	3.38	10851739	solute carrier family 35, member C2	311637
0.003017	0.047	3.06	10934777	NA	NA
0.003026	0.0471	3.5	10871415	NA	NA
0.003042	0.0472	5.62	10739455	G protein-coupled receptor, family C, group 5, member C	287805
0.003063	0.0473	0.23	10760762	NA	NA
0.003067	0.0473	6.1	10849863	small nuclear ribonucleoprotein polypeptides B and B1	171365
0.003079	0.0473	3.11	10931308	prolyl 4-hydroxylase, alpha polypeptide I	64475
0.00308	0.0473	3.56	10725289	glycoprotein 2 (zymogen granule membrane)	171459
0.003107	0.0475	0.3	10706974	NA	NA
0.003119	0.0475	0.25	10845890	sodium channel, voltage-gated, type IX, alpha	78956
0.003128	0.0475	3.2	10726682	interferon induced transmembrane protein 3	361673
0.003134	0.0475	0.27	10750898	NA	NA
0.003135	0.0475	0.31	10867380	NA	NA
0.00314	0.0475	0.33	10738469	RAD52 motif 1	287726
0.003143	0.0475	2.87	10869666	small nuclear RNA activating complex, polypeptide 3	362537
0.00316	0.0477	3.17	10915885	NA	NA
0.003172	0.0477	0.28	10702306	transcription factor 21	252856

0.003174	0.0477	0.3	10846962	NA	NA
0.003181	0.0477	3.25	10834225	neural proliferation, differentiation and control, 1	296562
0.003221	0.048	0.35	10776109	NA	NA
0.003227	0.048	0.38	10922577	NA	NA
0.003233	0.048	2.98	10890626	glycoprotein hormone beta 5	366668
0.003233	0.048	3.06	10863866	CCHC-type zinc finger, nucleic acid binding protein	64530
0.003244	0.048	2.69	10871711	tRNA isopentenyltransferase 1	362586
0.003249	0.048	0.33	10871127	MOB1, Mps One Binder kinase activator-like 2C (yeast)	313511
0.003282	0.0483	2.94	10795384	ependymin related protein 1 (zebrafish)	291180
0.003287	0.0483	2.82	10714973	HECT domain containing 2	309514
0.00329	0.0483	0.25	10862921	NA	NA
0.003325	0.0486	3	10937558	TSR2, 20S rRNA accumulation, homolog (S. cerevisiae)	317418
0.003333	0.0486	0.36	10869137	fukutin	362520
0.003337	0.0486	5.97	10795174	histone cluster 1, H2ba	24829
0.003346	0.0486	2.64	10721050	CCAAT/enhancer binding protein (C/EBP), gamma	25301
0.003349	0.0486	6.24	10910089	cellular retinoic acid binding protein 1	25061
0.003353	0.0486	0.29	10810236	latrophilin 1	65096
0.003362	0.0486	5.76	10769509	TIP41, TOR signaling pathway regulator-like (S. cerevisiae)	360869
0.003369	0.0486	4.22	10912567	kyphoscoliosis peptidase	315962
0.00338	0.0487	0.19	10856086	NA	NA
0.003402	0.0489	3.06	10749656	family with sequence similarity 195, member B	360677
0.003425	0.049	7.89	10879697	NA	NA
0.003435	0.049	3.21	10704413	N-ethylmaleimide-sensitive factor attachment protein, alpha	140673
0.003442	0.049	0.37	10892832	NA	NA
0.003448	0.049	6.12	10899756	coenzyme Q10 homolog A (S. cerevisiae)	362810
0.003448	0.049	4.85	10933015	similar to 4930453N24Rik protein	304176
0.00347	0.0492	0.3	10857408	NA	NA
0.003495	0.0493	0.29	10783048	olfactory receptor 1629	405389
0.003504	0.0493	0.3	10707113	potassium voltage gated channel, Shaw-related subfamily, member 1	25327
0.003508	0.0493	0.38	10729055	olfactory receptor 378	405366
0.003511	0.0493	3.12	10721315	tonin	24841
0.003523	0.0493	4.5	10726924	HCCA2 protein	499288
0.003531	0.0493	0.32	10906666	NA	NA
0.003534	0.0493	3.65	10824695	S100 calcium binding protein A9	94195

0.003535	0.0493	0.24	10798255	NA	NA
0.003573	0.0495	2.68	10781197	stathmin-like 4	79423
0.003573	0.0495	0.4	10762142	NA	NA
0.003579	0.0495	2.82	10796768	NA	NA
0.003585	0.0495	0.37	10884574	NA	NA
0.003587	0.0495	0.16	10801135	NA	NA
0.003612	0.0496	2.65	10765115	vesicle-associated membrane protein 4	364033
0.003615	0.0496	3.14	10906470	zinc finger CCHC-type and RNA binding motif 1	362990
0.00364	0.0499	0.36	10844681	ABO-family member 5	652927
0.003686	0.0502	0.22	10894260	vomeronasal 2 receptor, 55	691442
0.003697	0.0502	0.31	10763342	serpin peptidase inhibitor, clade B (ovalbumin), member 13	304690
0.003701	0.0502	0.26	10914019	NA	NA
0.003717	0.0502	3.47	10874193	ERBB receptor feedback inhibitor 1	313729
0.003719	0.0502	0.34	10815915	NA	NA
0.003721	0.0502	0.35	10756225	NA	NA
0.003724	0.0502	0.23	10852195	NA	NA
0.003728	0.0502	0.32	10737647	NA	NA
0.003733	0.0502	4.48	10888398	protein serine-threonine phosphatase catalytic subunit PP-1c	316717
0.003755	0.0503	3.77	10745285	family with sequence similarity 58, member B	303321
0.003761	0.0503	3.26	10747292	keratin 16	303530
0.003772	0.0503	3.36	10791902	solute carrier family 7 (cationic amino acid transporter, y+ system), member 2	64554
0.003773	0.0503	3.03	10724507	ADP-ribosylation factor interacting protein 2	293344
0.003779	0.0503	4.36	10768814	immediate early response 5	498256
0.0038	0.0504	0.31	10813856	NA	NA
0.003821	0.0504	0.27	10837638	olfactory receptor 727	404811
0.003824	0.0504	0.31	10858645	NA	NA
0.003836	0.0504	0.32	10739211	NA	NA
0.003837	0.0504	0.31	10739994	NA	NA
0.003844	0.0504	0.34	10806016	Bardet-Biedl syndrome 2	113948
0.003844	0.0504	2.64	10802405	chromatin modifying protein 1B	689364
0.003855	0.0504	3.04	10809893	NA	NA
0.003857	0.0504	3.36	10745323	WD repeat and SOCS box-containing 1	303336
0.003859	0.0504	0.39	10704376	NA	NA
0.003871	0.0504	0.31	10803824	cell division cycle 25 homolog C (S. pombe)	307511

0.003894	0.0506	3.9	10756671	similar to CG14980-PB	360768
0.003905	0.0507	0.36	10801580	NA	NA
0.003923	0.0507	3.45	10859371	serine/threonine kinase receptor associated protein	297699
0.003927	0.0507	3.4	10818090	solute carrier family 16, member 1 (monocarboxylic acid transporter 1)	25027
0.003945	0.0509	0.21	10736606	NA	NA
0.00396	0.051	2.86	10756343	heat shock 105kDa/110kDa protein 1	288444
0.003979	0.0511	0.27	10748440	NA	NA
0.003995	0.0512	0.28	10803721	NA	NA
0.004006	0.0513	2.96	10722897	hyaluronan and proteoglycan link protein 3	308773
0.004019	0.0513	0.24	10778438	NA	NA
0.004024	0.0513	0.24	10830957	RT1 class I, locus M1, gene 2	414786
0.004042	0.0513	0.37	10822390	NA	NA
0.004048	0.0513	0.32	10919788	NA	NA
0.004057	0.0513	0.27	10864684	ATPase, Ca++ transporting, plasma membrane 2	24215
0.004058	0.0513	3.19	10766463	dual specificity phosphatase 10	63995
0.004072	0.0514	0.33	10703102	NA	NA
0.004081	0.0514	0.34	10764942	NA	NA
0.0041	0.0515	0.25	10934115	NA	NA
0.004102	0.0515	4.08	10729604	similar to RIKEN cDNA 5033414D02	293888
0.004113	0.0515	0.36	10839238	tripartite motif-containing 69	311373
0.004129	0.0516	2.71	10701797	splicing factor 3b, subunit 5	680891
0.004135	0.0516	3.08	10809909	NA	NA
0.004167	0.0519	0.39	10917984	NA	NA
0.004187	0.0519	2.56	10756040	ubiquitin fusion degradation 1 like (yeast)	84478
0.004189	0.0519	0.36	10782271	plasminogen activator, urokinase	25619
0.004192	0.0519	3.53	10823733	programmed cell death 10	494345
0.004212	0.0521	0.29	10793071	NA	NA
0.004219	0.0521	4.77	10794257	NA	NA
0.004227	0.0521	3.42	10792187	proline synthetase co-transcribed homolog (bacterial)	306544
0.00424	0.0521	4.94	10757175	cytochrome P450, family 3, subfamily a, polypeptide 9	171352
0.004259	0.0523	3.12	10794206	serine palmitoyltransferase, long chain base subunit 1	361213
0.004273	0.0523	0.29	10867497	family with sequence similarity 110, member B	500400
0.004274	0.0523	0.35	10886840	NA	NA
0.0043	0.0525	3.89	10855599	NA	NA

0.004329	0.0527	4.52	10736863	chemokine (C-C motif) ligand 4	116637
0.004346	0.0528	0.37	10832715	ankyrin 3, node of Ranvier	361833
0.004395	0.0528	0.31	10742254	cytoplasmic FMR1 interacting protein 2	303073
0.004408	0.0528	0.4	10940025	NA	NA
0.004409	0.0528	3.47	10801996	RNA binding motif protein 22	307410
0.00441	0.0528	0.25	10856189	NA	NA
0.004416	0.0528	2.44	10804508	heat shock protein A8	24468
0.004418	0.0528	0.31	10771951	UDP glycosyltransferase 2 family, polypeptide B	24862
0.004429	0.0528	0.36	10930132	solute carrier organic anion transporter family, member 6c1	287006
0.004445	0.0528	0.31	10820000	NA	NA
0.004455	0.0528	0.24	10873887	NA	NA
0.004455	0.0528	0.23	10756519	NA	NA
0.004459	0.0528	0.35	10782080	FERM, RhoGEF (Arhgef) and pleckstrin domain protein 1 (chondrocyte-derived)	306183
0.004459	0.0528	0.39	10808563	cadherin 15	361432
0.004462	0.0528	0.31	10922487	NA	NA
0.004471	0.0528	0.33	10881624	NA	NA
0.004475	0.0528	0.28	10887088	NA	NA
0.004486	0.0528	0.37	10810556	TNFRSF1A-associated via death domain	246756
0.004488	0.0528	3.24	10847693	apoptosis inhibitor 5	362170
0.004491	0.0528	0.34	10865531	ubiquitin specific peptidase 5 (isopeptidase T)	297593
0.004544	0.0534	0.27	10749931	NA	NA
0.004578	0.0535	3.09	10924076	ribulose-5-phosphate-3-epimerase	501157
0.004584	0.0535	0.33	10780281	NA	NA
0.0046	0.0535	4.67	10791921	vacuolar protein sorting 37 homolog A (S. cerevisiae)	290775
0.004601	0.0535	0.32	10848956	NA	NA
0.004602	0.0535	3.34	10754201	G protein-coupled receptor 156	260430
0.004622	0.0535	0.32	10733884	olfactory receptor 1443	287331
0.004639	0.0535	0.24	10899997	olfactory receptor 906	404951
0.004639	0.0535	0.41	10718377	vomeronasal 1 receptor 12	494306
0.004644	0.0535	0.3	10935402	NA	NA
0.004645	0.0535	2.84	10907749	glycosylation dependent cell adhesion molecule 1	25258
0.004645	0.0535	0.37	10846912	olfactory receptor 441	295712
0.004649	0.0535	2.76	10823049	NA	NA
0.004675	0.0536	0.38	10837506	olfactory receptor 555	295798

0.004684	0.0536	0.28	10878741	forkhead box E3	171302
0.004688	0.0536	3.83	10891910	interferon, alpha-inducible protein 27 like 2B	299269
0.004691	0.0536	0.28	10728644	flap structure-specific endonuclease 1	84490
0.00471	0.0537	3.46	10821914	brix domain containing 2	294799
0.004737	0.0539	0.37	10792547	defensin NP-4 precursor	286958
0.004765	0.0539	3.3	10937660	trafficking protein particle complex 2	501550
0.004768	0.0539	0.33	10709405	olfactory receptor 78	293221
0.004773	0.0539	0.28	10922105	NA	NA
0.004776	0.0539	4.51	10873258	similar to RIKEN cDNA 1110008F13	296315
0.004779	0.0539	0.27	10873327	phospholipase A2, group IIC	29387
0.004793	0.054	0.35	10924044	NA	NA
0.004802	0.054	0.38	10783445	NA	NA
0.00484	0.0542	2.65	10939480	MORC family CW-type zinc finger 4	315914
0.004856	0.0542	3.07	10741841	ATPase, H+ transporting, lysosomal 9kDa, V0 subunit e1	94170
0.004865	0.0542	0.37	10733195	TBC1 domain family, member 9B	360520
0.004873	0.0542	0.36	10724268	NA	NA
0.004875	0.0542	2.43	10937827	heat shock protein A8	24468
0.004879	0.0542	7.73	10819442	tetraspanin 5	362048
0.004883	0.0542	0.28	10787117	mediator complex subunit 26	306328
0.004887	0.0542	0.23	10729771	NA	NA
0.004893	0.0542	0.33	10901713	NA	NA
0.004926	0.0545	0.35	10730765	NA	NA
0.004938	0.0545	3.3	10704655	NA	NA
0.004953	0.0545	0.38	10702076	NA	NA
0.004954	0.0545	0.34	10847187	olfactory receptor 696	405951
0.004963	0.0545	3.23	10885417	NA	NA
0.005022	0.0551	0.33	10938203	NA	NA
0.005042	0.0552	0.31	10860765	NA	NA
0.005072	0.0552	0.4	10760307	lemur tyrosine kinase 2	304286
0.005074	0.0552	0.24	10858618	vomeronasal 2 receptor, 50	500312
0.005075	0.0552	0.27	10722862	NA	NA
0.005078	0.0552	6.5	10731941	NA	NA
0.005093	0.0552	0.38	10803767	NA	NA
0.005097	0.0552	0.37	10901607	Spi-C transcription factor (Spi-1/PU.1 related)	314711

0.005101	0.0552	0.39	10870269	DnaJ (Hsp40) homolog, subfamily C, member 6	313409
0.005127	0.0552	3.39	10887966	NA	NA
0.005132	0.0552	0.31	10722479	NA	NA
0.005141	0.0552	6.31	10852144	prostate transmembrane protein, androgen induced 1	311676
0.005142	0.0552	0.27	10837247	NA	NA
0.005143	0.0552	2.94	10880233	YTH domain family, member 2	313053
0.005162	0.0553	0.4	10859201	NA	NA
0.005178	0.0554	0.28	10718725	NLR family, pyrin domain containing 5	308325
0.005191	0.0554	3.52	10773180	heparan sulfate (glucosamine) 3-O-sulfotransferase 1	84406
0.0052	0.0554	0.32	10735492	ankyrin repeat and FYVE domain containing 1	303292
0.005205	0.0554	5.27	10879402	CTP synthase	313560
0.005224	0.0555	3.37	10821900	DnaJ (Hsp40) homolog, subfamily C, member 21	192210
0.00525	0.0557	2.65	10716303	NA	NA
0.00528	0.0558	3.15	10852600	regulator of G-protein signaling 19	59293
0.005285	0.0558	0.39	10719935	NA	NA
0.005291	0.0558	3.43	10777034	similar to 1810013D10Rik protein	501923
0.005297	0.0558	2.79	10756253	NA	NA
0.005297	0.0558	2.79	10756255	NA	NA
0.005318	0.0558	0.41	10869076	NA	NA
0.005319	0.0558	0.36	10715117	G protein-coupled receptor 120	294075
0.005329	0.0558	3.49	10902564	Mdm2 p53 binding protein homolog (mouse)	314856
0.005343	0.0558	0.21	10894244	NA	NA
0.005346	0.0558	0.35	10883971	NA	NA
0.005375	0.056	2.93	10782028	DnaJ (Hsp40) homolog, subfamily C, member 3	63880
0.005389	0.056	4.2	10924669	NA	NA
0.005392	0.056	0.39	10849125	elongation factor RNA polymerase II-like 3	296102
0.005398	0.056	0.33	10935622	melanoma antigen family A, 11	302845
0.005441	0.0563	0.31	10886880	NA	NA
0.005457	0.0563	0.38	10751868	similar to hypothetical protein A430031N04	498100
0.005458	0.0563	0.27	10857177	NA	NA
0.005467	0.0563	3.47	10748861	NA	NA
0.005472	0.0563	0.29	10900041	NA	NA
0.005492	0.0564	0.24	10848105	olfactory receptor 760	405957
0.00552	0.0565	3.27	10708167	NA	NA

0.005522	0.0565	0.38	10863028	NA	NA
0.005526	0.0565	3.26	10883785	ornithine decarboxylase 1	24609
0.005578	0.0569	0.22	10807883	NA	NA
0.005587	0.0569	0.42	10935023	NA	NA
0.00559	0.0569	3.4	10888341	NA	NA
0.005607	0.057	0.24	10845382	NA	NA
0.005617	0.057	0.28	10829713	bicaudal C homolog 1 (Drosophila)	361832
0.005631	0.057	0.37	10769436	NA	NA
0.005645	0.057	0.25	10722425	NA	NA
0.005648	0.057	6.48	10922415	IMP4, U3 small nucleolar ribonucleoprotein, homolog (yeast)	316317
0.005655	0.057	0.37	10891977	serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 9	299274
0.005664	0.057	0.39	10890166	NA	NA
0.005666	0.057	0.3	10814528	tumor necrosis factor (ligand) superfamily, member 10	246775
0.005693	0.057	0.37	10829751	NA	NA
0.005705	0.057	2.67	10928626	LanC lantibiotic synthetase component C-like 1 (bacterial)	114515
0.005715	0.057	2.68	10718486	NA	NA
0.00573	0.057	4.18	10794464	thiopurine S-methyltransferase	690050
0.00574	0.057	0.29	10823498	NA	NA
0.005754	0.057	3.83	10938094	NA	NA
0.005757	0.057	0.38	10916876	tetratricopeptide repeat domain 36	300676
0.005765	0.057	0.28	10890570	NA	NA
0.005777	0.057	4.12	10728723	transmembrane protein 216	361727
0.005777	0.057	0.38	10709464	hypothetical protein LOC499219	499219
0.005796	0.057	0.37	10840429	similar to RIKEN cDNA 1700010M22	311491
0.005801	0.057	0.36	10820280	NA	NA
0.005802	0.057	0.29	10813005	NA	NA
0.005839	0.057	3.59	10821546	mitochondrial ribosomal protein S30	294767
0.005865	0.057	0.17	10915287	olfactory receptor 1155	300414
0.005874	0.057	5.51	10815205	NA	NA
0.005875	0.057	2.85	10903459	Kruppel-like factor 10	81813
0.005878	0.057	0.18	10775303	NA	NA
0.005891	0.057	3.54	10723253	adaptor-related protein complex 3, beta 2 subunit	308777
0.005948	0.057	0.34	10922743	NA	NA
0.005957	0.057	5.98	10923606	STE20-related kinase adaptor beta	501146

0.005957	0.057	0.17	10933168	NA	NA
0.005962	0.057	0.33	10857341	similar to chromosome 3 open reading frame 20	500258
0.005963	0.057	0.3	10882702	NA	NA
0.005964	0.057	0.34	10863363	NA	NA
0.005967	0.057	0.35	10750325	NA	NA
0.005968	0.057	0.36	10734475	NA	NA
0.005971	0.057	3.13	10701697	NA	NA
0.005992	0.057	0.19	10882892	NA	NA
0.005997	0.057	0.41	10918485	NA	NA
0.005998	0.057	0.32	10814096	NA	NA
0.005999	0.057	2.83	10836244	ADP-ribosylation-like factor 6 interacting protein 6	499798
0.006002	0.057	0.4	10729282	NA	NA
0.006005	0.057	2.58	10811289	NA	NA
0.006007	0.057	0.29	10763421	NA	NA
0.006015	0.057	0.32	10793493	NA	NA
0.006016	0.057	0.32	10890645	WD repeat domain 89	314243
0.006046	0.057	0.4	10864968	NA	NA
0.00606	0.057	2.96	10923270	oligonucleotide/oligosaccharide-binding fold containing 2A	363227
0.006062	0.057	0.29	10932721	lipoma HMGIC fusion partner-like 1	300286
0.006068	0.057	0.39	10753348	high-mobility group nucleosome binding domain 1	360704
0.006083	0.057	0.33	10840502	NA	NA
0.006084	0.057	6.75	10891864	hypothetical protein LOC100233176	1E+08
0.006098	0.057	4.93	10865956	C-type lectin domain family 2 member D2	362445
0.006103	0.057	0.27	10911797	glutathione S-transferase alpha 4	300850
0.006105	0.057	4.25	10842500	similar to Docking protein 5 (Downstream of tyrosine kinase 5) (Protein dok-5)	502694
0.006109	0.057	0.34	10863000	rCG64263-like	502812
0.006121	0.057	5.35	10760605	carbohydrate (chondroitin 4) sulfotransferase 12	304322
0.006128	0.057	3.15	10870837	thioredoxin domain containing 12 (endoplasmic reticulum)	298370
0.006143	0.057	6.25	10746916	ORM1-like 3 (S. cerevisiae)	360618
0.006144	0.057	3.44	10804164	NA	NA
0.006154	0.057	0.19	10766277	NA	NA
0.006168	0.057	0.27	10764365	NA	NA
0.00617	0.057	0.36	10712606	LRRG00136-like	1E+08
0.006171	0.057	0.3	10865397	LOC362433	362433

0.006173	0.057	0.29	10704299	vomeronasal 1 receptor 58	494226
0.006178	0.057	3.87	10859108	C-type lectin domain family 2, member g	362447
0.006181	0.057	0.29	10873885	NA	NA
0.006183	0.057	0.19	10705225	cytochrome P450, family 2, subfamily b, polypeptide 1	24300
0.006197	0.0571	0.36	10716498	NA	NA
0.006231	0.0573	0.32	10756749	zinc finger protein 316	304293
0.006247	0.0573	0.22	10779529	NA	NA
0.00625	0.0573	0.22	10755135	kininogen 1-like 1	288001
0.006259	0.0573	0.4	10869551	NA	NA
0.006265	0.0573	0.38	10755670	NA	NA
0.006271	0.0573	3.56	10805577	NA	NA
0.006277	0.0573	3.13	10740191	neuropeptide B	259222
0.006288	0.0573	2.82	10900753	polypyrimidine tract binding protein 1	29497
0.006311	0.0574	3.59	10888610	similar to limb-bud and heart	683626
0.006318	0.0574	6.76	10940413	emerin	25437
0.006343	0.0575	3.47	10788766	farnesyltransferase, CAAX box, alpha	25318
0.006355	0.0576	2.54	10731493	lipopolysaccharide-induced TNF factor	65161
0.006364	0.0576	0.26	10837613	olfactory receptor 659	295865
0.006412	0.0579	2.35	10749854	heat shock protein A8	24468
0.006415	0.0579	0.31	10703706	paired-Ig-like receptor A2	361492
0.006422	0.0579	0.23	10832083	MAM domain containing glycosylphosphatidylinositol anchor 1	309659
0.006473	0.0582	0.24	10706750	TEA domain family member 2	308582
0.00648	0.0582	0.27	10871588	zinc finger, MYND-type containing 12	313552
0.006497	0.0582	2.66	10927413	ARP1 actin-related protein 1 homolog B (yeast)	316333
0.006498	0.0582	0.33	10890622	NA	NA
0.006504	0.0582	4.32	10887981	splicing factor, arginine/serine-rich 7, 35kDa	362687
0.006524	0.0583	0.29	10936701	NA	NA
0.006544	0.0584	0.29	10766880	microRNA mir-29b-2	1E+08
0.006559	0.0585	0.4	10759479	NA	NA
0.006587	0.0586	4.12	10807625	nuclear import 7 homolog (S. cerevisiae)	192180
0.006589	0.0586	3.7	10845124	Rho family GTPase 3	295588
0.006619	0.0588	3.58	10806640	calreticulin	64202
0.006642	0.0589	0.37	10731247	myosin, heavy chain 11, smooth muscle	24582
0.006665	0.059	2.97	10863710	NA	NA

0.006696	0.0592	0.39	10900767	hypothetical LOC500797	500797
0.006748	0.0596	6.44	10827691	RT1 class I, locus M6, gene 2	365527
0.00676	0.0596	0.29	10750606	olfactory receptor 1528	405995
0.006772	0.0596	0.35	10939186	NA	NA
0.006773	0.0596	3.52	10719659	similar to RIKEN cDNA 1700008P20	292706
0.00679	0.0596	0.41	10815222	NA	NA
0.006798	0.0596	0.31	10850555	cystatin 11	245916
0.006801	0.0596	2.61	10855449	glycoprotein (transmembrane) nmb	113955
0.006827	0.0597	3.75	10926577	mitochondrial ribosomal protein L14	301250
0.006843	0.0597	3.27	10760290	neuronal pentraxin 2	288475
0.006848	0.0597	3.06	10723032	calcium and integrin binding 1 (calmyrin)	81823
0.006859	0.0598	0.36	10730272	cyclin M1	309387
0.006886	0.0598	0.36	10883452	NA	NA
0.006887	0.0598	0.39	10877675	NA	NA
0.006938	0.0602	0.34	10830828	NA	NA
0.00696	0.0602	7.06	10914517	NA	NA
0.006961	0.0602	0.28	10800328	NA	NA
0.007025	0.0607	0.27	10789727	zinc finger, CCHC domain containing 24	361104
0.00703	0.0607	2.32	10889219	heat shock protein A8	24468
0.007068	0.0609	3	10826300	coiled-coil domain containing 76	499697
0.0071	0.0611	9.9	10772330	NA	NA
0.007128	0.0612	0.26	10722373	NA	NA
0.007128	0.0612	0.36	10847105	olfactory receptor 621	404849
0.007179	0.0616	2.32	10909347	heat shock protein A8	24468
0.007257	0.0622	0.36	10859772	NA	NA
0.007268	0.0622	0.093	10857641	NA	NA
0.007281	0.0622	0.27	10828340	butyrophilin-like 4	294268
0.007329	0.0625	2.76	10758212	Rab interacting lysosomal protein-like 2	288652
0.00737	0.0627	0.41	10857546	inositol 1,4,5-triphosphate receptor, type 1	25262
0.007376	0.0627	0.32	10792894	NA	NA
0.007378	0.0627	0.36	10894417	NA	NA
0.007388	0.0627	2.53	10810295	DnaJ (Hsp40) homolog, subfamily B, member 1	361384
0.007401	0.0627	3.49	10904511	activity-regulated cytoskeleton-associated protein	54323
0.007403	0.0627	0.35	10939206	NA	NA

0.007425	0.0627	0.42	10835624	peptidyl-tRNA hydrolase 1 homolog (S. cerevisiae)	362113
0.007425	0.0627	0.35	10829378	collagen, type XVIII, alpha 1	85251
0.007475	0.0629	0.42	10745470	transmembrane and immunoglobulin domain containing 1	363654
0.007482	0.0629	0.39	10742921	olfactory receptor 1415	405059
0.007492	0.0629	4.49	10834159	transmembrane protein 203	311800
0.007519	0.0629	0.43	10846850	NA	NA
0.00752	0.0629	0.36	10774752	similar to hypothetical protein FLJ31438	289865
0.007523	0.0629	0.18	10753561	olfactory receptor 1553	288192
0.007524	0.0629	2.28	10800590	heat shock protein A8	24468
0.007531	0.0629	4.23	10834999	ubiquitin related modifier 1 homolog (S. cerevisiae)	311840
0.007557	0.0631	2.64	10822356	NA	NA
0.007572	0.0631	0.33	10830640	NA	NA
0.007627	0.0633	2.3	10881303	heat shock protein A8	24468
0.007636	0.0633	3.85	10720126	NA	NA
0.007637	0.0633	0.32	10932693	NA	NA
0.007643	0.0633	0.3	10807965	NA	NA
0.007643	0.0633	2.29	10752769	heat shock protein A8	24468
0.007661	0.0633	0.27	10899693	NA	NA
0.007667	0.0633	0.33	10866056	killer cell lectin-like receptor subfamily C, member 2	29684
0.007676	0.0633	2.7	10813752	myotubularin related protein 12	310155
0.007686	0.0633	3.95	10719967	RAB4B, member RAS oncogene family	50866
0.007687	0.0633	0.37	10725045	cytochrome P450, family 2, subfamily r, polypeptide 1	361631
0.007704	0.0633	3.75	10850958	peroxisomal membrane protein 4	282634
0.007727	0.0635	3.03	10797657	osteomodulin	83717
0.007744	0.0635	0.2	10768294	NA	NA
0.007811	0.0639	3.66	10937347	asparagine-linked glycosylation 13 homolog (S. cerevisiae)	300284
0.007824	0.0639	3.01	10860749	NA	NA
0.007825	0.0639	0.29	10863285	NA	NA
0.00783	0.0639	3.85	10894919	NA	NA
0.007838	0.0639	0.25	10796436	NA	NA
0.007868	0.0641	2.71	10935798	NA	NA
0.007902	0.0641	0.36	10878617	NA	NA
0.007906	0.0641	0.31	10752586	NA	NA
0.007911	0.0641	0.29	10863615	exocyst complex component 6B	500233

0.007917	0.0641	0.31	10930201	NA	NA
0.007918	0.0641	0.41	10775737	NA	NA
0.007939	0.0641	4.08	10875532	pyruvate dehyrogenase phosphatase catalytic subunit 1	54705
0.007947	0.0641	0.38	10877689	NA	NA
0.007961	0.0642	0.38	10852191	NA	NA
0.008007	0.0645	4.27	10785895	NA	NA
0.008028	0.0645	0.26	10938584	NA	NA
0.008032	0.0645	0.37	10929937	NA	NA
0.00805	0.0645	0.43	10811980	NA	NA
0.008054	0.0645	0.35	10935662	NA	NA
0.00807	0.0646	8.5	10817183	S100 calcium binding protein A11 (calizzarin)	445415
0.008094	0.0647	3.46	10874802	ubiquitin-conjugating enzyme E2, J2 (UBC6 homolog, yeast)	298689
0.008107	0.0647	0.33	10894140	NA	NA
0.008111	0.0647	3.83	10717793	regulator of G-protein signaling 17	308118
0.008119	0.0647	0.32	10886896	NA	NA
0.008149	0.0648	2.66	10872018	trafficking protein particle complex 3	362599
0.008159	0.0648	3.28	10861196	NA	NA
0.008173	0.0648	0.3	10909237	NA	NA
0.008184	0.0649	0.22	10767034	NA	NA
0.008221	0.0651	2.45	10829678	similar to RIKEN cDNA 1700049L16	502418
0.008231	0.0651	0.31	10853467	NA	NA
0.008245	0.0651	3.33	10789246	NA	NA
0.008275	0.0652	0.37	10838439	olfactory receptor 773	296026
0.008279	0.0652	0.33	10851380	NA	NA
0.008286	0.0652	2.75	10730884	survival motor neuron domain containing 1	287768
0.008291	0.0652	4.42	10724228	Bcl2-like 1	24888
0.008317	0.0653	2.61	10737636	solute carrier family 35, member B1	287642
0.008347	0.0653	2.79	10762600	GCN1 general control of amino-acid synthesis 1-like 1 (yeast)	690632
0.008356	0.0653	0.39	10739160	NA	NA
0.008361	0.0653	0.29	10896524	NA	NA
0.008363	0.0653	0.31	10933974	NA	NA
0.008381	0.0654	4.82	10850125	similar to chromosome 20 open reading frame 30; HSPC274 protein	681315
0.008401	0.0655	0.17	10847221	olfactory receptor 715	404815
0.008429	0.0655	3.22	10830908	ring finger protein 39	171387

0.008446	0.0655	3.68	10884738	NA	NA
0.008447	0.0655	4.47	10901920	small nuclear ribonucleoprotein polypeptide F	680737
0.008452	0.0655	0.43	10909616	sodium channel, voltage-gated, type II, beta	25349
0.008452	0.0655	0.23	10707254	NA	NA
0.008467	0.0655	0.32	10737736	NA	NA
0.008484	0.0655	2.98	10863502	WD repeat domain 54	500226
0.008492	0.0655	0.34	10747371	ATP citrate lyase	24159
0.00851	0.0655	0.36	10862281	olfactory receptor 801	405372
0.008516	0.0655	0.32	10736792	NA	NA
0.00853	0.0655	4.08	10864662	transmembrane protein 111	312640
0.008532	0.0655	0.32	10887108	NA	NA
0.008546	0.0655	0.44	10909590	myelin protein zero-like 2	300679
0.008553	0.0655	0.44	10886773	NA	NA
0.008569	0.0655	2.8	10721232	interferon stimulated exonuclease gene 20-like 2	361977
0.008603	0.0655	0.33	10927175	NA	NA
0.008603	0.0655	2.97	10841637	neuronatin	94270
0.008605	0.0655	0.36	10795313	NA	NA
0.008609	0.0655	3.09	10832905	sirtuin (silent mating type information regulation 2 homolog) 1 (S. cerevisiae)	309757
0.008612	0.0655	0.38	10737587	protein phosphatase 1, regulatory subunit 9B	84686
0.00862	0.0655	0.35	10703459	NA	NA
0.008627	0.0655	0.29	10764493	NA	NA
0.008649	0.0656	3.29	10704452	Meis homeobox 3	361514
0.008652	0.0656	0.36	10869301	NA	NA
0.008678	0.0657	5.11	10809007	carbonic anhydrase 7	291819
0.008703	0.0657	4.15	10927692	four and a half LIM domains 2	63839
0.008706	0.0657	0.36	10752974	NA	NA
0.008734	0.0659	4.47	10929600	phosphodiesterase 6D, cGMP-specific, rod, delta	363272
0.008759	0.066	0.31	10903965	NA	NA
0.00881	0.0663	0.4	10720510	NA	NA
0.008818	0.0663	3.32	10909338	adipocyte-specific adhesion molecule	286939
0.008824	0.0663	0.42	10836019	kynureninase (L-kynurenine hydrolase)	116682
0.008833	0.0663	5.2	10938077	acyl-CoA thioesterase 9	302640
0.008855	0.0663	3.5	10910167	NA	NA
0.008908	0.0666	0.36	10719824	cornifelin	365217

0.008914	0.0666	2.34	10937523	NA	NA
0.00895	0.0668	3.21	10899387	nuclear receptor subfamily 4, group A, member 1	79240
0.008998	0.067	3.49	10934548	similar to RIKEN cDNA 2610029G23	363485
0.009014	0.067	3.43	10729331	similar to RIKEN cDNA 1110059E24	361740
0.00902	0.067	3.65	10876520	exosome component 3	313243
0.00902	0.067	3.39	10790002	guanine nucleotide binding protein-like 3 (nucleolar)	290556
0.009054	0.0671	3.48	10760514	NA	NA
0.00906	0.0671	0.4	10872361	brain-specific angiogenesis inhibitor 2	313058
0.009062	0.0671	3.46	10894268	NA	NA
0.009062	0.0671	3.46	10918270	NA	NA
0.009079	0.0671	0.35	10746602	nuclear factor, erythroid derived 2,-like 1	360610
0.009102	0.0671	0.37	10887078	NA	NA
0.009105	0.0671	3.37	10701846	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	114490
0.009106	0.0671	3.42	10767001	methionine adenosyltransferase II, alpha	171347
0.009142	0.0672	0.3	10917568	acyl-CoA synthetase bubblegum family member 1	171410
0.009148	0.0672	0.42	10891436	general transcription factor IIA, 1	83830
0.009165	0.0673	2.86	10799657	N-myristoyltransferase 2	291318
0.009226	0.0677	3.29	10902547	lysozyme 2	25211
0.009261	0.0678	0.45	10734368	NA	NA
0.009302	0.068	0.36	10720945	NA	NA
0.009304	0.068	0.38	10791563	NA	NA
0.009314	0.068	3.23	10730297	zinc finger, FYVE domain containing 27	309376
0.009325	0.068	2.26	10737125	peptidyl-tRNA hydrolase 2	287593
0.009341	0.068	0.31	10754176	Cd80 molecule	25408
0.009345	0.068	2.53	10811589	ring finger protein 166	365022
0.009385	0.0682	3.53	10864893	NA	NA
0.009442	0.0685	2.44	10908414	queuine tRNA-ribosyltransferase 1	64016
0.009473	0.0686	0.2	10772013	transmembrane protease, serine 11b	365265
0.009479	0.0686	4.09	10927680	transforming growth factor, beta receptor associated protein 1	301373
0.009488	0.0686	0.31	10788707	adrenergic, beta-3-, receptor	25645
0.0095	0.0686	3.4	10809882	NA	NA
0.009507	0.0686	3.79	10707121	general transcription factor IIH, polypeptide 1	361580
0.009526	0.0687	0.41	10861563	NA	NA
0.00954	0.0687	0.29	10785867	NA	NA

0.009556	0.0687	0.28	10724280	NA	NA
0.00957	0.0687	0.27	10848138	olfactory receptor 785	404792
0.00957	0.0687	0.23	10871773	NA	NA
0.009591	0.0688	2.78	10916456	NA	NA
0.00967	0.0693	0.35	10927264	NA	NA
0.009691	0.0694	0.32	10889328	NA	NA
0.009702	0.0694	2.73	10796991	aminopeptidase O	290963
0.009814	0.0701	0.3	10898849	NA	NA
0.00983	0.0701	3.44	10895406	pleckstrin homology-like domain, family A, member 1	29380
0.009862	0.0703	0.33	10915283	olfactory receptor 1151	300411
0.00988	0.0703	0.44	10772160	NA	NA
0.009956	0.0708	2.27	10806884	NA	NA
0.009962	0.0708	3.86	10792035	dual specificity phosphatase 4	60587

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and Genetics12. Risk Factor: Smoking/Tobacco Use13. Risk Factor: High Blood Cholesterol and Other Lipids14. Risk Factor: Physical Inactivity15. Risk Factor: Overweight and Obesity16. Risk Factor: Diabetes Mellitus17. End-Stage Renal Disease and Chronic Kidney Disease18. Metabolic Syndrome19. Nutrition20. Quality of Care21. Medical Procedures22. Economic Cost of Cardiovascular Disease23. At-a-Glance Summary Tables24. Glossary A Report From the American Heart Association. *Circulation* **123**, e18–e209 (2011).

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ABSTRACT

INVESTIGATION OF POSTTRANSLATIONAL REGULATION AFTER GLOBAL BRAIN ISCHEMIA AND REPERFUSION INJURY

by

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December, 2012

Advisor: Dr. Donald J. DeGracia

Major: Physiology

Degree: Doctor of Philosophy

The final cause of death in most patients revived after cardiac arrest is ischemia and reperfusion (I/R) injury in the brain. Survival after brain I/R injury depends on the expression of new stress response proteins such as heat shock protein 70 (HSP70). Little is known about why recovering neurons are able to express new stress response proteins while neurons that will die can transcribe RNA but do not translated protein in early reperfusion. Previous studies suggested that the mRNA-binding protein HuR may regulate hsp70 mRNA in reperfused neurons through a novel cytoplasmic structure, the mRNA granule. To determine the roles of HuR and the mRNA granule in reperfused neurons and to characterize global translation regulation, we 1) prevented mRNA granule formation in reperfused neurons or induced mRNA granules in uninjured neurons by pharmacological manipulation polysomes, 2. studied potential HuR posttranscriptional regulation mechanisms of facilitated nuclear export and polysome association, and 3. performed translation state analysis of reperfused neurons from hippocampal subregions CA1 and CA3.

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