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Resubmission of #####: TIMP1: An Emerging Translational Biomarker of Chronic Neuropathic Pain

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Abstract

Chronic neuropathic pain (CNP) is one of the most significant unmet clinical needs in modern medicine. Alongside the lack of effective treatments, there is a great deficit in the availability of objective diagnostic methods to reliably facilitate an accurate diagnosis. We therefore aimed to determine the feasibility of a simple diagnostic test by analyzing differentially expressed genes in the blood of CNP patients and healthy human controls. Refinement of microarray expression data was performed using correlation analysis with 3,900 human 2-color microarray experiments. Selected genes were analysed in the dorsal horn of Sprague Dawley rats after L5 spinal nerve ligation (SNL), using qRT-PCR and ddPCR, to determine if they may be associated with the mechanisms underpinning CNP and may therefore represent translational biomarkers of CNP. We found that of the 15 potential biomarkers identified, tissue inhibitor of matrix metalloproteinase-1 (*TIMP1*) gene expression was upregulated in CNP patients ($p = 0.0049$) which positively correlated ($R = 0.68$, $p = \leq 0.05$) with increased plasma TIMP1 levels in CNP ($p = 0.0433$). Moreover, plasma TIMP1 was also significantly higher in CNP patients than in those with chronic inflammatory back pain ($p = 0.0272$). In the SNL model, upregulation of the *Timp1* gene was also observed ($p = 0.0058$) alongside a strong trend for the upregulation of *Mc1r* ($p = 0.0847$). Our data therefore highlights several genes that warrant further investigation, and of these, *TIMP1* shows the greatest potential as an accessible and translational CNP biomarker.

Keywords: Neuropathic pain, TIMP1, biomarker, Plasma, Dorsal Horn, Chronic Lower Back Pain, Inflammatory pain

Introduction

Chronic neuropathic pain (CNP) is a physically debilitating and pathologically complex disorder featuring maladaptive cellular responses and the subsequent development of ectopic discharge and neuronal hyperexcitability [43]. This manifests as allodynia and hyperalgesia in up to 50% of patients with CNP [26]. CNP is often a consequence of traumatic nerve injury but is also associated with numerous peripheral and centrally mediated factors, such as diabetes, multiple sclerosis and stroke [51] [11]. CNP diagnosis is inherently complex and remains subjective, with the primary methods for diagnosis being the painDETECT [17], Neuropathic Pain Questionnaire (NPQ) [31] and the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) screening tools [7], alongside clinical assessment. The stark deficit in **effective treatments** and objective diagnostic tools therefore provides the impetus to determine novel **and translational** diagnostic biomarkers to promote early intervention in CNP.

The majority of gene expression studies have focused solely on animal models of neuropathic pain (NP) [32], ranging from chronic constriction injury (CCI) [6] and spinal nerve ligation (SNL) [30] to the spared nerve injury [13] and drug-induced neuropathy [24] models. Such studies have identified extensive groups of genes whose expression is altered after nerve injury [62]. There is a growing body of evidence which suggests an increasing involvement of non-neuronal mechanisms in the pathophysiology of CNP. These often pertain to the immune system to such a degree that CNP has been considered a neuro-immune disorder with glial cell and cytokine involvement [3; 41]. The diversity, complexity and involvement of these non-neuronal mechanisms associated with CNP may therefore herald the opportunity to determine an accessible biomarker in blood. Much of the research searching for CNP biomarkers in humans to date has considered cerebrospinal fluid [4] and the brain [63] with functional magnetic resonance imaging (fMRI) being proposed as a way of determining CNS biomarkers to guide clinical practice [9]. However, such methodology is likely to be impractical as acceptable and cost-effective method of biomarker detection in clinical practice.

In animal studies, gene expression analysis has provided valuable insights into the mechanisms underpinning the development and maintenance of CNP. Little though, has been translated to advances in diagnostic biomarkers in human CNP subjects. A previous study sought to determine biomarkers by determining correlations in gene expression changes between rat blood and ipsilateral lumbar dorsal quadrant after CCI, using bioinformatics in conjunction with microarray analysis [19], but did not include human cohorts. In order to bridge this gap, we sought to determine the feasibility of a minimally invasive method of sample collection by performing microarray analysis to establish a panel of genes differentially expressed in blood from a cohort of CNP patients and healthy human controls. After further analysis, several of these genes were then analysed for expression changes in the dorsal horn of sham and L5 SNL Sprague-Dawley rats. This strategy therefore prioritized the search for viable biomarkers in human blood whilst determining if these biomarkers are translational across species. Using the translational approach to biomarker discovery also highlights whether these molecules may be perturbed in the mechanisms underpinning CNP and therefore, whether they may hold potential as disease indicators and/or pharmacological targets in preclinical analgesic drug development.

Methods

Human Clinical Samples

PAXgene Blood RNA Tubes (IVD; PreAnalytiX GmbH, Hombrechtikon, Switzerland) from 10 individuals with CNP of the back or lower back lasting for more than 3 months was obtained through ProteoGenex tissue procurement services (Culver City, CA), alongside a further 10 age-gender matched controls also acquired through ProteoGenex tissue procurement services. Patients were recruited after clinical assessment of their pain symptoms, including CT and MRI scans, electroneuromyography, microneurography and assessment of the nociceptive flexion reflex. Pain intensities were determined using the verbal rating scale (VRS). All patients were non-responsive to non-narcotic and anti-inflammatory analgesics. Plasma was obtained using BD Vacutainer K2-EDTA tubes with centrifugation at 1,000xg for 10 minutes and immediate storage of the plasma at -80°C. Patients with major psychiatric disorders, cancer or diabetes were excluded from this study. Donor consent was obtained

through ProteoGenex under Protocol PG-ONG2003/1, titled: Collection of Tissue, Blood and Bone Marrow. Plasma from a total of 12 patients with chronic inflammatory back pain (CIBP) was also obtained to delineate between a potential translational biomarker of CNP and one of CIBP. Ethics approval was obtained from Galway University Hospitals (Ref: C.A. 1037), Galway, Ireland. The absence of CNP was determined by clinical assessment, and an S-LANSS score of less than 12. Pain severity was determined using the Chronic Pain Grade questionnaire. Demographic data on the patient and control groups is presented in Supplementary Table 1.

RNA Isolation

Total RNA was isolated from the PAXgene Blood RNA Tubes using the Preserved Blood RNA Purification Kit II (Norgen, Biotek, ON, Canada) according to the manufacturer's instructions. In brief, the RNA was treated with DNase and purified on columns. RNA concentration was measured on a NanoDrop ND2000 ultraviolet-visible spectrophotometer (Labtech International Ltd, UK), and RNA integrity was checked on an Agilent 2100 Bioanalyzer (Agilent Technologies, Amsterdam, The Netherlands). RNA was judged as suitable for gene expression analysis only if samples showed intact bands of 28S and 18S ribosomal RNA subunits, displayed no chromosomal peaks or RNA degradation products, and had a RNA integrity number (RIN) above 7.0.

Affymetrix Microarray and Data Analysis

Total RNA was labeled using an Ambion WT Expression kit (Life Technologies, Bleiswijk, The Netherlands) and hybridized to Affymetrix Human Gene 1.0 ST expression arrays (Affymetrix, Santa Clara, CA, USA). Sample labeling, hybridization to chips, and image scanning were performed according to the manufacturer's instructions on an Affymetrix GeneTitan instrument. Quality control was performed using Affymetrix Expression Console and interpretation of data was facilitated by Affymetrix Transcriptome Analysis Console 2.0 (TAC2.0). Transcripts exhibiting a fold change of ≥ 1.2 and a p -value of ≤ 0.05 (ANOVA) were considered differentially expressed and suitable for further correlation analysis and refinement.

Analysis of General Gene-Gene Correlations

A total of 3,900 human 2-color microarray experiments were downloaded from NCBI's Gene Expression Omnibus (GEO) and normalized as described previously [64]. Two-color arrays were chosen because they reflect how gene expression differs between two conditions, usually experimental and control, which emphasizes how genes are correlated in their response. Gene-gene Pearson's correlation coefficients were calculated using only the experiments where the two genes were present on the same microarray.

Further Refinement of Expression Data

In order to determine the genes with the greatest evidence for involvement in CNP, refinement of genes was undertaken with specified criteria, which includes a greater statistical stringency (Table 1), the presence of a gene within our correlation analysis output, and finally, whether there is a body of literature pertaining to the role of the molecule in pain pathways. Literature was searched to include all publications available up to, and including, February 2016, using both PubMed and general electronic information databases with the gene name or symbol, along with the terms 'pain', 'neuropathic' or 'neuropathic pain'.

Plasma TIMP1 Quantification

In order to determine if circulating levels of tissue inhibitor of matrix metalloproteinase-1 (TIMP1) varied between patients with CNP, CIBP and healthy controls, a total of 32 plasma samples were subject to a TIMP1 enzyme-linked immunosorbent assay (ELISA) (Invitrogen, UK). The assay was performed according to manufacturer's instructions. In total 10µl of plasma was diluted to 200µl prior to the procedure, each sample was analysed in duplicate and absorbance data was obtained using an Infinite F50 microplate reader (Tecan, UK). Absorbance data were converted into plasma TIMP1 levels using the standards provided and the data were analysed using GraphPad Prism 4.0 using an Mann-Whitney test and Kruskal-Wallis test ($p = \leq 0.05$ considered statistically significant).

Animal Husbandry, L5 SNL Surgery & Tissue Harvest

Adult male Sprague-Dawley rats ($n = 18$) (matched at 7-8 weeks of age upon delivery and 250-350 g at time of experimentation; Harlan, UK) were housed singly, with food and water available *ad libitum* and maintained at constant temperature ($21 \pm 2^\circ\text{C}$) under 12 hour cycling of light-dark exposure (lights on at 07.00 h). The experimental procedures were approved by the Animal Care and Research Ethics Committee, National University of Ireland, Galway, and carried out under license from the Department of Health in the Republic of Ireland and in accordance with EU Directive 2010/63. One week following delivery and acclimatization to the animal unit, animals underwent surgery after allocation into either L5 SNL ($n = 10$) or sham ($n = 8$) groups. In brief, the rats were anaesthetized under isoflurane anaesthesia (3% induction, 1.5-2% maintenance in 0.5 L/min O_2) and upon exposure of the left L5 spinal nerve, a ligature was applied. Sham rats were treated identically, aside from ligation of the L5 nerve. Animals were maintained until 35 days post-surgery at which point euthanasia was performed by decapitation and tissue was harvested from the spinal cord dorsal horn ipsilateral to the side of nerve injury, snap-frozen on dry ice and stored at -80°C . RNA was extracted from tissue using the NucleoSpin® RNA kit (Machery–Nagel) with on-column DNase treatment followed by storage at -80°C .

Quantitative Real Time PCR

A total of 25ng of RNA from each dorsal horn sample was used for reverse transcription and subsequent amplification using the QuantiTect Whole Transcriptome Kit (Qiagen, X). This was performed according to manufacturer's instructions and included an 8 hour incubation stage for high yield cDNA synthesis. After serial dilution of the amplification product, quantitative real time polymerase chain reaction (qRT-PCR) was performed using a CFX96 instrument (Bio-Rad Laboratories, UK). Analysis of samples was performed in triplicate with each 12 μl reaction containing 6 μl of iTaq™ Universal SYBR® Green Supermix (Bio-Rad Laboratories), 300nM of each forward and reverse primer (Supplementary Table 2) and 5 μl of diluted cDNA. Incubation consisted of polymerase activation and DNA denaturation at 95°C for 2 minutes followed by 40 cycles of denaturation at 95°C for 5 seconds with annealing and extension at 60°C (unless otherwise stated in supplementary table 2) for 30

seconds followed by fluorescence detection. Upon completion of thermal cycling, melt-curve analysis was performed to confirm reaction specificity. Baseline subtraction and determination of the threshold cycle (C_q) was performed using Bio-Rad CFX Manager 3.1 (Bio-Rad Laboratories). Data was subsequently analysed with qbase+ software (Biogazelle, Belgium) using an unpaired t-test. Normalization of expression data was performed using both *Atp5b* and *Ubc*. Of 12 reference genes analysed with the rat geNorm kit (Primerdesign, UK), both were found to be comparably highly stable (M=0.419, CV=0.145).

Digital Droplet PCR

A total of 20ng of RNA was reverse transcribed using the Verso cDNA synthesis kit (Thermo Fisher Scientific, UK). This was performed according to manufacturer's instructions. Random hexamers and anchored oligo-dT were both included at a ratio of 3:1 (v/v), 0.5µl of RT enhancer per 10µl reaction was also included to remove of contaminating gDNA, followed by incubation at 42°C for 60 minutes and 95°C for 2 minutes. The cDNA was subsequently diluted to 100µl. Further dilutions were performed for the reference genes *Atp5b* and *Ubc* to avoid saturation of the digital droplet PCR (ddPCR) system) (All reagents and equipment used for ddPCR were from Bio-Rad Laboratories). Each 20µl PCR reaction consisted of 10µl of QX200™ ddPCR™ Evagreen Supermix, 250nM of forward and reverse primer, 5µl of diluted cDNA and nuclease free water. This was loaded in to a DG8™ Cartridge with accompanying DG8™ Gasket and 70µl of QX200™ Droplet Generation Oil for Evagreen for droplet generation using a QX200™ Droplet Generator. 96-well plates were then sealed using pierceable foil plate seals with a PX1™ PCR plate sealer. A T100™ Thermal Cycler was used with the following cycling conditions; enzyme activation for 5 minutes at 95°C, followed by 40 cycles of denaturation at 95°C for 30 seconds and annealing/extension at 60°C for 1 minute. Signal stabilisation was achieved by cooling to 4°C for 5 minutes, heating to 90°C for 5 minutes. A ramp rate of 2°C per second was required for each step in the PCR. Data was then obtained using a QX200™ Droplet Reader with ddPCR™ Droplet Reader Oil and QuantaSoft™ Software, version 1.7. Normalisation of data was performed by dividing the total number of transcript copies per 20 µl

reaction, by the geometric mean of *Atp5b* and *Ubc*. Data was analysed using GraphPad Prism 4.0 using an unpaired t-test ($p = \leq 0.05$ considered statistically significant).

Results

Human Blood Transcriptome Analysis

In order to determine differentially regulated genes, we used gene microarray analysis to determine the expression of intracellular RNA from whole blood in patients with CNP and in healthy controls. Our analysis highlighted a diverse range of genes that may be perturbed in the development or maintenance of CNP, which in-turn may function as potential biomarkers of CNP. These include genes pertaining to immune function, inflammatory response and extracellular matrix turnover (Supplementary Table 3).

Correlation Analysis

We also sought to evaluate how the differentially expressed genes in this study were correlated with each other in prior microarray experiments, by analysis of 3,900 human 2-colour arrays obtained from NCBI's GEO database. Global correlations (i.e., correlations not dependent upon the experimental condition being studied) between genes suggest their involvement in a common transcriptional response network. Similarly, negatively correlated gene sets may suggest how the response under the conditions being studied here (CNP) differs from the general trends.

We also analyzed prior correlations among differentially regulated genes from the 2-colour microarrays, with high up- and downregulation (Figure 1). Within the most strongly upregulated genes, a subset of 3 – *TIMP1*, *ORM2* and *PROX1* were highly correlated in other experiments. *TIMP1* [16; 20-22; 44], *ORM2* [16; 20] and *PROX1* [27; 58] have all been found in proteomic studies in plasma, suggesting they may have potential as circulating biomarkers. Literature-mining analysis of the three genes [65] identifies ANG-1 (angiopoietin, a regulator of postnatal angiogenesis) as their strongest commonality.

Expression Analysis Refinement

TIMP1, which was highlighted by our correlation analysis, dipeptidyl peptidase III (*DPP3*) and melanocortin 1 receptor (*MC1R*) all exhibited a strong basis of literature supporting the role of these genes in pain pathways [5; 8; 46; 47]. Similarly, both orosomucoid 2 (*ORM2*) and prospero homeobox 1 (*PROX1*) were present in the correlation analysis, though *PROX1* did not meet any of our other refinement criteria. Caspase 5 (*CASP5*) and NLR family CARD domain-containing protein 4 (*NLRC4*) were also upregulated in patients with CNP, alongside other genes such as toll-like receptor 5 (*TLR5*) and clusterin (*CLU*) (Table 1). We also observed several downregulated transcripts in CNP patients, including ring finger protein 185 (*RNF185*) and lin-28 homolog A (*LIN28A*).

Plasma TIMP1 Quantification

The mean (\pm SD) level of plasma TIMP1 in healthy control subjects was 157.3 (\pm 33.2) ng/ml (range: 100.9-233.6 ng/ml), in contrast the mean level in CNP patients (\pm SD) was 278.4 (\pm 131.4) ng/ml (range: 130.3-546.8 ng/ml). In patients with CIBP, the mean (\pm SD) was 147.8 (\pm 75.55) ng/ml (range: 82.56-316.9 ng/ml). Plasma TIMP1 concentrations were therefore significantly elevated in patients with CNP when compared to controls ($p = 0.0433$) and between patients with CNP and CIBP ($p = 0.0272$) (Figure 2). There was no significant change between controls and CIBP patients ($p = 0.6682$) When analysing controls, CNP and CIBP patients together, significance of elevated TIMP1 was similarly observed ($p = 0.0434$). Plasma TIMP1 levels for controls and CNP patients were moderately positive correlated to *TIMP1* mRNA levels isolated from whole blood (Pearson's Correlation, $R=0.68$, $p \leq 0.05$). Age ($p = 0.4980$) and gender ($p = 0.9948$) did not significantly influence TIMP1 levels, as determined by ANOVA and unpaired t-test, respectively.

SNL Dorsal Horn Expression Analysis

Genes found to be differentially regulated in human blood were subsequently analysed in the dorsal horn of Sprague Dawley rats that had undergone L5 SNL, in conjunction with their sham counterparts (Table 2). *Timp1* expression was notably upregulated ($p = 0.0058$) 35 days post SNL. This was accompanied by a strong trend for upregulation of *Mc1r* ($p = 0.0847$).

Discussion

In the search for biomarkers of pain in the blood, research has focused on proteomic analysis. It has been reported that the levels of serum biomarkers correlate with lower back pain and subsequent functional impairment [52] and that the severity of polyneuropathy is associated with elevated tumor necrosis factor-alpha and interleukin-6 (IL-6) [38]. Moreover, increased IL-6 in has been postulated to be a predictive blood biomarker in herpes zoster, indicating propensity to develop post-herpetic neuralgia [67]. The potential of blood as a source of biomarkers based on the transcriptome has already been considered for numerous diseases of the central nervous system, including Alzheimer's disease (AD) [18] [33] and major depressive disorder [15]. These studies sought to detect microRNAs, which perform post-transcriptional modification of gene expression and can be found in blood within exosomes. The potential of these molecules has also been considered as sources of biomarkers for both neuropathic and inflammatory pain [1]. A similar study using only rats found multiple changes in gene expression which correlated between blood and ipsilateral lumbar dorsal quadrant, including *Icoslg* [19], which showed increased expression in our CNP patients, but not our rodent model of NP. This may however, be may explained by the contrasting animal pain model used.

In order to determine the viability of blood as a source of CNP biomarkers, based on the results obtained, we initially established a list of genes differentially regulated in human blood in patients with CNP. After analysis and refinement of these genes using our predetermined criteria, 10 of the 15 differentially regulated genes had existing associations with CNP, pain-related conditions or mechanisms underpinning pain. TIMP1, an inducible, soluble and secreted protein with cytokine-like properties [45], was significantly upregulated in the blood of CNP

patients, highlighted in our correlation analysis and was supported by a plethora of publications (Table 1). TIMPs function to inhibit the matrix metalloproteinases (MMPs), a group of zinc-dependent endopeptidases. Such inhibition has been shown to reverse allodynia post SNL [29]. As a circulating prognostic marker, TIMP-1 has been scrutinized in gastric cancer [61], breast cancer [57] and as a marker of pre-diagnosis pancreatic ductal adenocarcinoma [25]. The potential of TIMP1 as a biomarker of CNP was further emphasized by our determination that plasma TIMP1 levels were also elevated, which both provides a degree of validation to our microarray data and our methodological approach to CNP biomarker discovery.

We also showed that *Timp1* is significantly upregulated in Sprague Dawley ipsilateral dorsal horn, which supports its potential role in the mechanisms underpinning the maintenance or development of CNP. Such upregulation has also been observed in a rat spinal cord injury model 28 days after surgery, which again led to the suggestion that *Timp1* may be involved in pain persistence [47]. This was observed in the absence of significantly upregulated MMP9, which supports growing evidence that TIMPs may have MMP-independent functions [53] [10]. Similar upregulation after sciatic nerve transection (SNT) was also observed in the L4 and L5 DRG 28 days post-surgery [23]. In addition, *Icam-1* and *Timp1* were found, alongside a cluster of secretion-related genes, to be upregulated in the spinal cord after CCI [46]. Interestingly, this upregulation was not observed in rats with complete Freund's adjuvant induced inflammatory pain, which suggests *Timp1* expression may be discriminatory neuropathic and inflammatory pain, thereby lending further support to our determination that TIMP1 was significantly higher in the plasma of CNP patients, than those with CIBP.

DPP3, an enkephalinase and single member of the M49 family of metallopeptidases, exhibits a strong association with the mechanisms that underpin nociception and was also notably upregulated in patients with CNP. DPP3 plays a critical role in the degradation of enkephalin within the pain modulatory system [8]. Within the rodent CNS, high densities of endomorphin 2-like fibres were found to be localized to the superficial laminae of the DH [40]. DPP3 has also been highlighted as a potential target for the pharmacological management of pain [56] with

inhibition of DPP3 by the endogenous opioid peptide spinorphin demonstrating analgesia in mice [59]. Interestingly, DPP3 activity in human cerebrospinal fluid was also reduced in subjects with acute pain when compared to pain free subjects [48]. The potential of DPP3 as a pharmacological target, and the potential role of DPP3 as a biomarker for CNP, is therefore highly supportive and certainly warrants further investigation.

Our results also demonstrate an upregulation of genes involved in inflammation, in particular *CASP5* and *NLRC4*. *CASP5*, alongside the caspases -1, -4 and -12, is a mediator of the innate immune response and upon activation by cellular stimulation, becomes integrated within the inflammasome, a multiprotein oligomer which facilitates the activation of inflammatory cytokines. *CASP5* has been determined to be upregulated in peripheral blood of fibromyalgia patients reporting high pain, in contrast to those with low pain [39]. It has also been shown that mice deficient in *NLRC4* inflammasomes showed attenuated carrageenan induced mechanical and thermal acute inflammatory hyperalgesia which coincided with reduced levels of interleukin-1 β (p17) and caspase-1 [37]. Moreover, after rat cervical spinal cord injury, *CASP11* (*CASP4*), the ortholog of human *CASP5*, was upregulated and activation of the *NALP1* inflammasome, which incorporates *CASP11*, was observed. Antibody mediated neutralisation of a component of this multiprotein complex, apoptosis-associated speck-like protein containing a caspase recruitment domain (*ASC*), also resulted in notable tissue sparing and functional improvement [12]. Taken together with our expression analysis, these findings suggest that upregulation of *CASP5* and *NLRC4* may be useful indicators of injury and inflammatory processes, but further clarification is required to determine their specificity to CNP.

MC1R was significantly upregulated in patients with CNP, and trended strongly towards upregulation in the SNL model of NP. Point mutations in *MC1R* are often cited as responsible for the red-haired phenotype [49]. Individuals with this phenotype have been shown to exhibit greater anesthetic requirement [35] and increased sensitivity to thermal pain [34]. It has also been determined that females with two variant *MC1R* alleles experience enhanced analgesia with pentazocine [42]. Antagonism of the melanocortin system has been associated with a

reduction in mechanical and cold allodynia and the reversal of morphine-induced hyperalgesia [2; 28; 60]. Conversely, a role for MC1R in sex-specific variation in inflammatory pain but not CNP, has also been shown [14]. Although there is a lack of a defined mechanism for MC1R in pain, with multiple studies highlighting varying associations between the melanocortin system, pain and sex-specificity, this clearly does not discredit it as a potential translational biomarker or therapeutic target.

We also observed differential regulation of a number of other genes in the blood of patients with CNP. The extracellular chaperone and complement inhibitor, CLU, has been researched in the realms of several diseases, in particular AD [50] [55]. After SNL, activation of the complement cascade has been observed alongside increased dorsal horn mRNA expression of the *Clu* [36]. We did not, however, observe a similar upregulation of *Clu* in the SNL model of NP. Upregulation of toll-like receptor 5 (*TLR5*) was also observed in CNP patients. Recent studies have highlighted the potential of TLR5 in CNP, with *tlr5^{-/-}* mice exhibiting reduced tactile allodynia after L5 SNL [54] whilst TLR5 has also been utilized, due to its expression in DRG A-fibre neurons, to facilitate the blockage of sodium currents through co-administration of the TLR5 ligand flagellin, and a membrane impermeable lidocaine derivative [66]. Although CLU and TLR5 are examples of differentially regulated genes in human blood that did not translate to the SNL animal model, with such variability in the animal pain models available, further analysis using central and peripheral models of NP would be required to exclude these genes as potential translational biomarkers.

We have therefore identified a range of genes differentially regulated in the blood of patients with CNP and of these, *TIMP1*, *DPP3* and *MC1R* possess a relatively strong literature basis supporting their role in CNP. Further scrutiny of these genes has facilitated the development of the list of candidate genes that warrants further investigation. The presence of differential regulation of *Timp1*, and to a lesser degree, *Mclr*, in the rat dorsal horn following L5 SNL suggests they may function as translational biomarkers and may be perturbed in the mechanisms underpinning CNP in the dorsal horn. We therefore present *TIMP1* as potential CNP translational biomarker

which is able to differentiate between patients with CNP and CIBP. Further analysis is now required to validate these findings with the aim of deciphering a molecular signature which, alongside traditional diagnostic methods, has the ability to vastly improve the diagnosis of CNP.

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Figure legends

Figure 1: Prior transcriptional correlations between a subset of highly downregulated (A) and upregulated (B) genes in CNP patients using 3,900 human 2-colour microarrays.

Using a matrix of transcriptional correlations derived from the analysis of 3,900 human 2-colour microarrays from NCBI's Gene Expression Omnibus (GEO), which includes data from a variety of control and experimental samples, gene-gene Pearson's correlation coefficients were determined. In the 3,900 microarrays used to perform gene-gene correlations, *PROX1*, *ORM2* and *TIMP1* we found to positively correlate with each other, which was supported by our CNP data analysis. In the 2-colour microarray analysis, other upregulated genes, including *CST1*, *SLC12A9*, *CDK17*, *ARMCX6*, were usually negatively correlated (green) to *PROX1*, *ORM2* and *TIMP1* (the brightest red squares are the self-self comparisons along the diagonal). However, our analysis highlighted that both groups of genes were upregulated, thus providing evidence that *PROX1*, *ORM2* and *TIMP1*, which are highly correlated in previous experiments, may be associated with the pathophysiology of CNP and may function as CNP biomarkers.

(*ARHGAP11B* - rho GTPase activating protein 11B; *ARMCX6* - armadillo repeat containing X-linked 6; *C10orf62* - chromosome 10 open reading frame 62; *C1orf189* - chromosome 1 open reading frame 189; *CDK17* - cyclin-dependent kinase 17; *CETN2* - centrin, EF-hand protein, 2; *CST1* - cystatin SN; *CYP4Z2P* - cytochrome P450, family 4, subfamily Z, polypeptide 2, pseudogene; *DPP3* - dipeptidyl-peptidase 3; *DRG1* - developmentally regulated GTP binding protein 1; *FAM129C* - family with Sequence Similarity 129, member C; *GSTM2* - glutathione S-transferase mu 2 (muscle); *HIST1H2BK* - histone cluster 1, H2bk; *JAZF1* - juxtaposed with another zinc finger protein 1; *LRRN3* - leucine rich repeat neuronal 3; *NPRL3* - nitrogen permease regulator-like 3; *OR5M3* - olfactory receptor, family 5, subfamily M, member 3; *ORM2* - orosomucoid 2; *PASK* - PAS domain containing serine/threonine kinase; *PHOSPHO1* - phosphatase, orphan 1; *PROX1* - prospero homeobox 1; *RNF185* - ring finger protein 185; *PID1* - phosphotyrosine interaction domain containing 1; *PSG4* - pregnancy specific beta-1-glycoprotein 4; *ROM1* - retinal outer segment membrane protein 1; *SDHD* - succinate dehydrogenase complex, subunit D, integral membrane protein; *SLC12A9* - solute carrier family 12 (potassium/chloride transporters), member 9; *TIMP1* - tissue inhibitor of metalloproteinases 1; *TRAPPC6B* - trafficking protein particle complex 6B; *XAGE5* - X antigen family, member 5; *YOD1* - YOD1 deubiquitinase; *ZNF285* - zinc finger protein 285).

Figure 2: Plasma TIMP1 concentrations in healthy controls and patients with CNP or CIBP

Analysis of plasma TIMP1 concentrations in healthy controls ($n = 10$), CNP patients ($n = 10$) and CIBP patients ($n = 12$) was carried out using an ELISA. Diluted plasma samples were exposed to human TIMP1 monoclonal antibody coated wells and treated with human TIMP1 antibody conjugated to biotin. After Streptavidin-Peroxidase treatment, addition of substrate allows for colourimetric detection at 450nm. Greater absorbance recordings correlate to higher plasma TIMP1 levels. $*p = \leq 0.05$ (Mann-Whitney).

Table 1 – CNP biomarker panel of transcripts differentially regulated in human whole blood

Array ID	Accession Number	Gene Name	Gene Symbol	<i>p</i> -value	FC in CNP	CA	Literature
7951385	NM_004347	Caspase 5	<i>CASP5</i>	0.0449	↑2.23	No	[5; 17]
8149927	NM_001831	Clusterin	<i>CLU</i>	0.0489	↑1.85	No	[15]
7941621	NM_005700	Dipeptidyl-Peptidase 3	<i>DPP3</i>	0.0028	↑1.50	No	[3; 4; 12; 25]
7908793	NM_004433	E74-Like Factor 3	<i>ELF3</i>	0.0095	↑1.62	No	[13; 21]
7937707	NR_026643	Family with Sequence Similarity 99, Member A	<i>FAM99A</i>	0.0017	↑1.64	No	-
8070720	NM_015259	Inducible T-Cell Co-Stimulator Ligand	<i>ICOSLG</i>	0.0007	↑1.20	No	[7]
8065011	NM_024674	Lin-28 Homolog A (C. elegans)	<i>LIN28A</i>	0.0183	↓1.50	No	[27]
7998055	NM_002386	Melanocortin 1 Receptor	<i>MC1R</i>	0.0005	↑1.40	No	[1; 2; 6; 9; 14; 19]
8051396	NM_021209	NLR Family CARD Domain-Containing Protein 4	<i>NLRC4</i>	0.0437	↑1.99	No	[16]
8157450	NM_000608	Orosomucoid 2	<i>ORM2</i>	0.0225	↑1.97	Yes	-
7982287	NM_001039841	Rho GTPase Activating Protein 11B	<i>ARHGAP11B</i>	0.0025	↑1.57	No	-
8075477	NM_152267	Ring Finger Protein 185	<i>RNF185</i>	0.0032	↓1.68	No	-
7967972	NG_043316	RNA, U6 Small Nuclear 76, Pseudogene	<i>RNU6-76P</i>	0.0049	↓1.54	No	-
8167185	NM_003254	TIMP metalloproteinase Inhibitor 1	<i>TIMP1</i>	0.0049	↑1.50	Yes	[8; 10; 11; 18; 20; 22-24]
7924499	NM_003268	Toll-Like Receptor 5	<i>TLR5</i>	0.0428	↑1.75	No	[26]

Genes documented here and subsequently analysed in the SNL model either exhibited a p value of ≤ 0.005 and a fold change (FC) of ≥ 1.5 , or were present in our correlation analysis (CA)/literature search with a p value of 0.005-0.05 and a FC of ≥ 1.5 or a p value of ≤ 0.005 and a FC of 1.2-1.5.

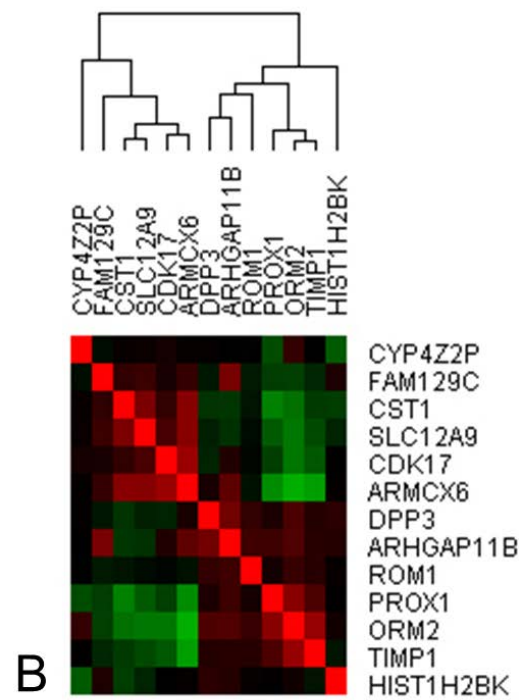
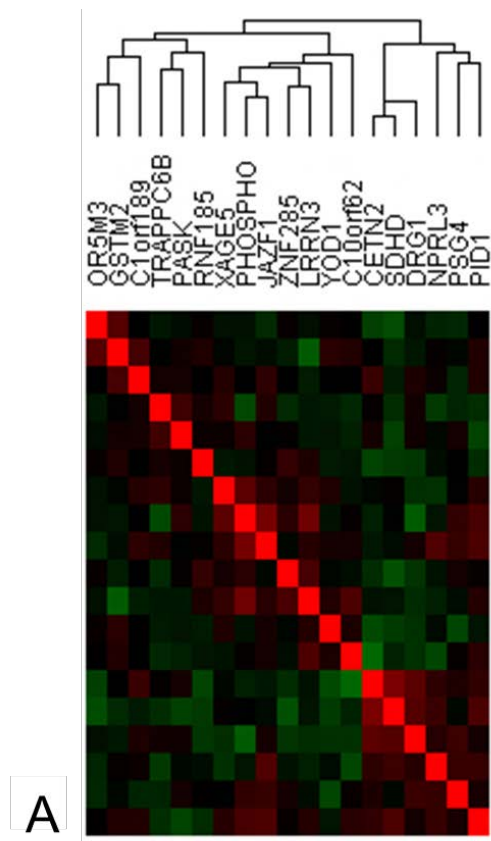
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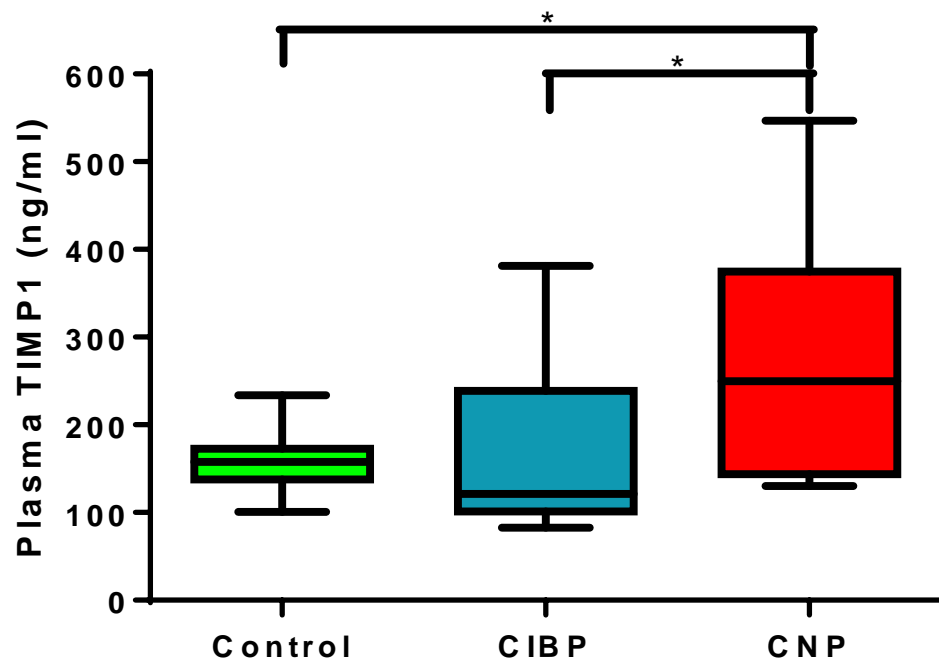
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Table 2 – qRT-PCR and ddPCR analysis of gene expression changes after SNL in Sprague Dawley rats

Accession Number	Gene Name	Gene Symbol	p-value	FC in SNL
NM_053736	Caspase 4*	<i>Casp4</i>	0.1951	↑1.22
NM_053021	Clusterin	<i>Clu</i>	0.9990	1.00
NM_053748	Dipeptidyl-Peptidase 3	<i>Dpp3</i>	0.4670	↓1.16
XM_006256260	Inducible T-Cell Co-Stimulator Ligand	<i>Icoslg</i>	0.7920	↓1.16
NM_001109269	Lin-28 Homolog A	<i>Lin28a</i>	0.7860	↑1.30
XM_006255795	Melanocortin 1 Receptor*	<i>Mcl1r</i>	0.0847	↑2.72
NM_001309432	NLR Family CARD Domain-Containing Protein 4*	<i>Nlrc4</i>	0.5242	↑1.15
NM_001168524	Rho GTPase Activating Protein 11A*	<i>Arhgap11a</i>	0.8516	↓1.03
NM_001024271	Ring Finger Protein 185	<i>Rnf185</i>	0.3900	↓2.18
NM_053819	TIMP Metalloproteinase Inhibitor 1	<i>Timp1</i>	0.0058	↑2.19
NM_001145828	Toll-Like Receptor 5	<i>Tlr5</i>	0.6820	↑1.51

*Denotes analysis by ddPCR. The ortholog, or closely related gene with high sequence similarity was selected as appropriate comparison to represent human gene. Genes not described here are either not present within the rat genome with no apparent ortholog, or were not reliably detected for robust statistical analysis.





Group/Diagnosis	Sex	Age	Ethnic background	Location
Control	M	70	Caucasian	-
Control	M	69	Caucasian	-
Control	M	71	Caucasian	-
Control	F	76	Caucasian	-
Control	F	67	Caucasian	-
Control	M	80	Caucasian	-
Control	F	80	Caucasian	-
Control	M	74	Caucasian	-
Control	M	61	Caucasian	-
Control	F	36	Caucasian	-

CNP#	M	72	Caucasian	Lower back
CNP	M	71	Caucasian	Back
CNP	F	68	Caucasian	Back
CNP	M	80	Caucasian	Lower back
CNP	M	75	Caucasian	Lower back
CNP	M	73	Caucasian	Lower back
CNP	F	75	Caucasian	Back
CNP	F	75	Caucasian	Lower back
CNP	F	78	Caucasian	Lower back
CNP	F	81	Caucasian	Back

CIBP*	F	27	Caucasian	Lower back
CIBP	M	62	Caucasian	Lower back
CIBP	F	40	Caucasian	Lower back
CIBP	F	54	Caucasian	Lower back
CIBP	M	74	Caucasian	Lower Back
CIBP	F	72	Caucasian	Lower Back
CIBP	F	57	Caucasian	Lower back
CIBP	F	69	Caucasian	Lower back
CIBP	F	50	Caucasian	Middle-upper back
CIBP	F	88	Caucasian	Low back
CIBP	F	85	Caucasian	Lower back
CIBP	F	63	Caucasian	Lower-mid back

CNP = Chronic Neuropathic Pain (Lower back pain) and were measured using the Verbal Ratio

* CIBP = Chronic Inflammatory Back Pain and were measured using the Chronic Pain Grade Qu

Duration	Severity
-	-
-	-
-	-
-	-
-	-
-	-
-	-
-	-
-	-
-	-

> 6 months	Grade 2/3
> 6 months	Grade 2/3
> 6 months	Grade 3
> 6 months	Grade 3
> 6 months	Grade 3
> 6 months	Grade 2/3
> 6 months	Grade 2/3
> 6 months	Grade 3
> 6 months	Grade 2/3
> 6 months	Grade 2/3

> 6 months	Grade 2
> 6 months	Grade 1
> 6 months	Grade 3
> 6 months	Grade 2
> 6 months	Grade 1
> 6 months	Grade 4
> 6 months	Grade 1
> 6 months	Grade 4
> 6 months	Grade 4
> 6 months	Grade 3
> 6 months	Grade 2
> 6 months	Grade 4

ng Scale (0=no pain, 1=mild |
 uestionnaire (0=no pain, 1=lc

Comorbidities/Medical History
migraine
no apparent comorbidities
no apparent comorbidities
no apparent comorbidities
cystitis
hypertension
no apparent comorbidities
cataract
no apparent comorbidities
no apparent comorbidities

hypertension
migraine, depression
hemorrhoid
iliary arrhythmia
anxiety
major depression
cataract
no comorbidities
chronic gastritis
no comorbidities

hip dislocation, previous osteotomy
degenerative disc disease
degenerative disc disease
osteoarthritis
degenerative disc disease
inflammatory back
degenerative disc disease
degenerative disc disease
degenerative disc disease
severe osteoarthritis, L2 fracture, degenerative disc disease, r/s sacroiliac joint disease
degenerative disc disease
scoliosis, osteomyelitis, previous arthroplasty

pain, 2=moderate pain & 3 =severe pain).

ow intensity pain, 2=high intensity pain, 3=moderate pain & 4=severe pain).

Medication
Dipyron
No current medication
No current medication
No current medication
Antibiotics
Captopril
No current medication
Aspirin
No current medication
No current medication

Duloxetine, Tramadol
Dipyron
Tramadol
Tramadol
Tramadol
Dipyron
Reboxetine
Tramadol
Morphine
Dipyron

Paracetamol, Diclofenac
Diclofenac
Diclofenac
Ibuprofen, Diclofenac
Methylprednisolone
Tramadol, Paracetamol
Lidocaine patch
Diclofenac, paracetamol, Capsaicin cream, Tapentadol
Codeine, Paracetamol, Tramadol, Oxycodone, Diazepam, trigger point injections
Paracetamol, steroid injections
Paracetamol
Tramadol, Paracetamol

Supplementary Table 2 – Primer sequences for gene expression analysis in the SNL model of CNP

Gene Symbol	Accession Number	Primer	Sequence
<i>Arhgap11a</i>	NM_001168524	Forward Primer	5' TGTTCGCTGTCGTGCCTTATG 3'
		Reverse Primer	5' CAAGATTGCTGCTGTCCATTT 3'
<i>Clu</i>	NM_053021	Forward Primer	5' GGGCGATGACCAGTACCTT 3'
		Reverse Primer	5' TCAAACAGCTTCACCACCAC 3'
<i>Dpp3</i>	NM_053748	Forward Primer	5' GATCCGGTCACTGGGCAA 3'
		Reverse Primer	5' CCTGCAACCACATCCCCT 3'
<i>Elf3</i>	NM_001024768	Forward Primer	5' TCTTCGTTTCAGAGGCTGTGG 3'
		Reverse Primer	5' CGTTCAGGATCTCCCGTTT 3'
<i>Icoslg</i>	XM_006256260	Forward Primer	5' TCAGTACGCCTGTCATCAGC 3'
		Reverse Primer	5' ACGTCATACAGGCCCAACTC 3'
<i>Lin28a</i>	NM_001109269	Forward Primer	5' GTATTGGGAGTGAGCGACGG 3'
		Reverse Primer	5' ACAGTTGTAGCACCTGTCTCC 3'
<i>Rnf185</i>	NM_001024271	Forward Primer	5' TGGCTTTCAGATGTCTTTTGG 3'
		Reverse Primer	5' CAAACAGGAAGAGGCGTGAC 3'
<i>Timp1</i>	NM_053819	Forward Primer	5' CCTGGCATAATCTGAGCCCT 3'
		Reverse Primer	5' TTTGCAAGGGATGGCTGAAC 3'
<i>Tlr5</i>	NM_001145828	Forward Primer (Ann: 62.4°C)	5' CTGTCTGACCTCAAGCGTGT 3'
		Reverse Primer (Ann: 62.4°C)	5' GGGCCACCTCAAATACTGCT 3'

Sequences were not available for *Casp4*, *Mc1r*, *Nlrc4*, *Orml* (BioRad PrimePCR Assays for ddPCR) and for *Atp5b* and *Ubc* (Primer Design geNORM Assays)

Transcript Cluster ID	Neuropathic Pain Bi-weight Avg Signal (log2)
8175583	2.22
8164428	4.69
8067981	3.48
8102560	2.04
8151572	2.37
7998055	5.15
8113701	5.58
8083061	2.65
7954926	2.48
8070720	5.6
8109926	4.16
7912303	3.98
8167742	2.5
8147479	2.8
7922137	2.89
8106816	4.65
8097780	2.48
7937707	4.26
7985898	3.79
7945740	5.8
8170257	2.36
7982287	3.47
8138527	2.73
8107857	9.54
8059387	4.38
7928967	1.87
7941621	4.78
8104580	4.95
8139721	1.97
8075477	3.85
8056250	2.54
7956668	5.68
8164698	5.42
7904959	5.69
7968128	5.28
8092763	2.17
7916629	3.24
8038839	4.82
8106512	3.45
8168391	3.73
7962367	5.74
8081431	5.17
7963157	3.51
7953211	2.7
8107720	3.8
7967872	2.67
8167185	9.3
8049071	5.34
8060205	4.28

7981714	5.69
7967898	4.94
8109637	3.65
7978739	3.41
7976146	1.58
8157668	1.66
8003056	5.38
8002057	5.8
8021301	5.18
8051422	5.01
8151745	2.46
7923967	5.81
8138706	2.12
7944931	4.85
7905365	5.34
7907349	3.8
7943314	2.3
7940660	4.8
8053030	5.22
8088047	6.7
8016607	3.85
8045583	1.63
7914334	4.27
7983381	2.37
8120717	4.62
8108981	3.94
7994730	4.47
7991186	3.63
8059712	2.03
7975995	5.74
8025990	1.72
7939988	3.17
7908793	3.67
8121144	3.48
8105267	2.95
8125731	6.25
8026787	5.49
8081298	2.26
8135488	3.07
8124457	2.01
7947221	3.82
8074856	3.16
8106929	1.97
7924773	2.42
7916582	3.63
7998157	6.99
7900639	3.42
8037459	3.12
7903803	4.43
7907960	2.02

8138789	7.11
8113664	3.51
8136832	4.82
8066027	4.17
7975971	3.71
8006081	3.3
8127079	2.28
8013307	7.18
8128712	5.34
8145085	4.89
8091101	2.7
7978570	5.89
7940147	2.94
8088772	4.11
8171493	3.72
7998959	3.14
8112971	2.28
8039771	4.27
8089478	4.95
8059565	4.28
8113232	1.87
7976512	4.68
8039635	2.25
8038792	6.13
8131844	2.64
7952129	10.89
8066482	4.55
8023708	4.37
8146120	2.57
8107646	4.78
8015280	4.91
8147877	2.82
8150846	2.52
7965652	4.31
7914324	3.38
7914322	3.38
8058591	2.48
8151898	2.12
8139820	2.51
7940662	4.64
8072488	5.09
8028281	1.62
7979800	1.71
7943158	6.76
8048205	5.41
7912250	6.32
7983661	3.29
8023382	7.87
8131703	2.25
7899018	4.72

8063156	4.87
8124160	4.57
8134965	6.5
7955419	5.57
8165723	4.03
8176297	4.03
8016540	7.67
8123678	4.37
8155508	2.72
8127362	2.33
8070538	4.56
7946027	2.72
8044295	5.38
7980574	2.18
7921868	6.98
8160431	2.45
8094625	3.91
8051427	4.84
8116829	1.85
8155547	5.86
7923189	2.2
7909681	3.32
7959232	4.63
7915284	4.76
7971075	4.69
8018646	4.71
8039642	2.32
7910030	1.88
7918465	2.12
8063074	4.24
8060698	5.14
8070867	5.1
8075918	4.04
8070930	5.26
7937959	2.12
7943853	6.43
8176986	4.8
7930398	6.62
8121363	1.65
7897964	2.45
8075009	6.39
7898167	4.64
8001587	5.19
8001387	4.93
8159965	6.09
8067901	2.74
8065011	2.57
7963375	3.85
8036318	2.79
8081073	4.39

8105189	4.63
8171087	4.63
8002379	5.97
8178586	4.91
7902365	2.85
7912839	5.74
8001402	4.06
8174887	2.19
8175250	1.78
7977125	2.19
8144719	1.56
8121152	2.14
8142403	1.65
7929674	3.55
8045205	3.06
8055236	3.06
7992891	1.93
8091385	2.66
8045343	1.74
8110463	4.1
7926708	2.14
7898357	4.48
8116906	1.94
8157300	4.31
7920799	6.78
7911233	2.83
8083777	4.97
8103755	4.62
8167895	2.64
8096663	6.82
7910369	3.89
7914809	6.12
7946328	1.7
8179814	5.01
7911657	4.86
8021043	3.8
7968931	3.83
7981525	4.71
8157450	3.82
8149318	2.06
7899273	5.96
7906863	3.94
7943036	3.49
8098439	2.1
8117630	3.39
8126916	3.16
8013804	6.11
8171879	4.32
8115838	3.01
7900585	8.73

8084788	3.31
8085060	2.21
8096002	5.01
8097013	3.46
7922462	4.52
8097126	3.25
7923027	4.72
7915896	2.93
8108706	2.76
8103892	3.33
7903753	4.89
8059710	2.22
7950699	1.85
8124492	6.3
8116831	3.63
8087119	4.88
8041383	4.24
8007607	4.78
7961569	2.79
8123606	5.73
8002571	4.23
7902495	4.69
8110668	6.32
8145529	7.05
8054465	4.15
8054354	3.11
8089029	4.68
7938070	2.46
8037197	4.29
7963134	4.12
7939102	3.11
8074972	3.19
7900876	1.92
8132725	6.06
8175755	5.47
8129315	2.41
7983377	1.36
7968883	2.18
7932635	5.91
7977567	5.28
7944751	3.78
8044743	1.7
8022174	2.67
8043487	2.19
8088371	2.86
8063351	6.31
7977596	3.15
7915468	5.46
8148640	5.51
8148932	5.72

7969341	4.11
8081838	4.46
8041061	5.45
8117594	5.65
8038505	4.61
7909866	3.65
7914178	6.03
8051571	2.22
8168873	5.23
7897955	3.27
8042283	6.06
8085052	2.2
8037283	4.67
7947548	2.33
8137582	2.58
8023727	2.49
8123959	2.75
7900508	2.73
8088337	2.3
8118100	5.17
8080445	3.87
8082406	4.01
7949371	6.05
7904429	3.54
8123340	4.57
8031344	5.6
7922416	3.07
8096688	2.92
7933088	2.57
8152512	3.18
8024708	6.59
8174132	1.56
8087116	6.58
8179472	5.41
8118535	5.41
8160392	1.82
7912196	1.6
8053427	3.89
8128001	3.14
8020508	6.79
8020491	3.6
8137352	5.71
8165711	4.91
7902396	2.25
8127201	3.43
7957467	3.87
7984631	3.4
8123728	4.33
7916986	2.55
8055666	1.62

8162645	7
8168387	3.9
8048108	5.35
8166525	3.62
8054611	9.44
8160487	4.94
8152640	2.04
7980720	5.16
8054769	5.95
7910054	2.23
7970691	4.65
7996160	4.45
8065412	3.87
8092275	2.19
7903777	2.85
8123726	3.8
8058203	5.03
8125042	4.76
7897849	6.68
7947027	3.8
8113871	1.86
7908730	2.43
8027519	2.16
8005755	2.06
8020421	2.12
8054943	1.98
7988625	2.72
8060893	1.81
7967089	3.51
8157214	4.23
8072610	7.74
7920487	3.21
8055600	1.53
8119096	3.6
7901299	5.53
7950895	4.24
8089232	1.79
8001325	1.72
7965842	6.1
7980390	3.42
8077635	5.21
8043833	1.86
8084206	4.21
8097690	2.08
7950810	3.58
8008967	2.86
7996391	2.32
7929497	2.67
8042827	3.19
8161646	1.56

7929990	4.6
8141560	5.18
8066051	4.77
7903214	3.01
8036430	2.15
8054041	2.29
7948910	2.16
7953518	2.62
8052372	1.85
8018439	8.32
8015095	2.29
8123579	3.41
8041982	4.61
8081069	3.18
8168580	3.8
8058535	3.39
7960143	3.72
8154476	4.49
8176336	5.66
8105311	3.87
8128726	2.27
8133000	1.5
7898371	4.81
7912861	4.81
7905058	5.26
8132913	3.39
7964660	3.86
8090936	5.98
8179238	4.36
8030092	5.63
7950442	3.24
7906128	5.41
8005689	3.88
8167897	3.92
7993833	7.7
7957041	2.34
7953697	3.95
7924499	6.28
8060080	2.63
7994572	5.72
7925174	4.36
8130807	2.62
8130980	2.62
8081214	3.39
8084478	4.46
8091491	3.36
8051396	5.83
7942503	4.3
7989216	2.42
8167013	2.92

7927708	3.9
7937975	2.26
8015681	4.65
8104490	3.5
8097790	1.57
7915592	1.93
8070396	2.58
7904303	4.56
7918379	2.84
7951385	6.01
8084923	3.88
8129608	2.64
8043363	8.96
8020971	2.24
7983143	3.61
8052945	2.56
8104070	3.21
7927097	2.19
8057797	5.61
8084016	5.08
8176286	5.12
8124129	3.49
8152582	2.08
8117408	4.74
7933228	6.26
7951091	7.99
8058201	4.98
8083779	2.26
8178399	5.08
8179683	5.08
8023133	4.3
7982388	1.69
7982152	4.9
7986943	4.9
7986661	4.9
7984016	5.4
8098242	3.08
8149955	2.02
8168841	1.82
7963590	6.36
8005265	2.19
7938195	1.91
8123437	4.39
8143747	1.44
8000411	4.15
7991323	3.77
8157922	4.52
8175574	3.39
7955987	2.71
7993179	2.42

8114205	1.68
8141361	4.06
7991465	6.33
8115865	5.19
7942594	9.7
8139786	1.83
8099797	6.66
8149927	6.48
8138043	2.57
8046279	4.44
8112994	4.18
8042519	3.91
8091954	4.13
8115464	3.85
7919193	6.37
7961275	2.51

Control Bi-weight Avg Signal (log2)	Fold Change (linear) (Neuropathic Pain vs. Control)
1.83	1.31
5	-1.24
3.78	-1.24
2.53	-1.4
2.79	-1.33
4.66	1.4
5.14	1.36
3.06	-1.33
2.93	-1.36
5.34	1.2
4.46	-1.24
4.26	-1.21
2.93	-1.35
2.23	1.49
3.15	-1.2
4.13	1.44
3.03	-1.46
3.55	1.64
4.07	-1.22
6.17	-1.29
1.86	1.42
2.82	1.57
3.18	-1.36
9.07	1.38
4.65	-1.2
2.27	-1.32
4.19	1.5
4.68	1.2
2.6	-1.55
4.6	-1.68
2.85	-1.24
5.12	1.47
5.13	1.22
5.05	1.56
5.59	-1.24
2.43	-1.2
2.92	1.25
5.21	-1.31
2.71	1.68
3.99	-1.2
6.03	-1.22
5.49	-1.25
3.84	-1.26
3.03	-1.26
3.05	1.69
3.29	-1.54
8.72	1.5
4.95	1.31
4.75	-1.39

5.01	1.6
4.38	1.48
3.28	1.29
3.94	-1.45
2.09	-1.42
1.98	-1.25
5.12	1.2
5.42	1.31
4.61	1.49
4.73	1.21
2.95	-1.41
6.6	-1.73
2.52	-1.32
5.14	-1.22
4.9	1.36
3.2	1.52
2.82	-1.44
4.48	1.25
4.85	1.29
6.32	1.3
4.41	-1.48
1.89	-1.2
4.72	-1.36
2.68	-1.24
4.36	1.2
3.68	1.2
4.75	-1.21
3.31	1.25
2.71	-1.61
5.48	1.2
2.07	-1.27
3.99	-1.77
2.98	1.62
3.08	1.32
3.27	-1.25
5.99	1.2
5.1	1.31
2.61	-1.28
3.84	-1.71
2.41	-1.32
4.21	-1.31
3.56	-1.31
2.24	-1.2
2.78	-1.28
4.1	-1.39
7.7	-1.63
3.68	-1.2
3.8	-1.6
4.97	-1.45
2.5	-1.39

7.57	-1.37
3.04	1.39
4.38	1.35
2.9	2.41
3.33	1.3
4.07	-1.7
2.57	-1.22
8.14	-1.95
5.71	-1.3
5.19	-1.23
2.13	1.48
5.49	1.31
3.28	-1.27
4.5	-1.31
3.38	1.27
3.55	-1.33
2.54	-1.2
3.99	1.21
4.59	1.28
4.82	-1.45
2.2	-1.26
4.38	1.24
2.65	-1.32
5.85	1.21
3.17	-1.44
11.38	-1.41
4.23	1.25
4.09	1.22
2.89	-1.25
5.1	-1.25
4.62	1.23
3.22	-1.32
2.85	-1.26
3.85	1.38
3.71	-1.25
3.71	-1.25
2.82	-1.27
2.72	-1.51
2.82	-1.24
4.18	1.38
5.69	-1.51
1.9	-1.22
2.07	-1.28
7.27	-1.42
5.1	1.24
6.04	1.22
3.94	-1.57
7.61	1.2
2.79	-1.46
4.98	-1.2

4.55	1.25
3.76	1.75
6.18	1.24
5.09	1.4
4.38	-1.27
4.38	-1.27
8.13	-1.37
3.95	1.34
3.75	-2.05
2.04	1.22
4.92	-1.28
3.28	-1.48
5.72	-1.27
2.65	-1.38
6.5	1.39
2.71	-1.2
3.53	1.3
4.58	1.2
2.33	-1.39
6.19	-1.26
2.57	-1.29
2.85	1.39
4.32	1.24
4	1.69
2.84	3.62
4.45	1.2
2.67	-1.28
2.16	-1.21
1.65	1.38
4.56	-1.25
5.4	-1.2
4.8	1.24
4.3	-1.2
5.59	-1.25
2.46	-1.26
6.83	-1.32
5.06	-1.2
7.17	-1.47
2	-1.27
2.76	-1.24
6.69	-1.23
4.31	1.26
4.78	1.33
4.68	1.2
5.35	1.67
3.15	-1.33
3.15	-1.5
4.21	-1.28
3.15	-1.29
4.68	-1.22

4.05	1.49
4.91	-1.21
6.68	-1.63
4.4	1.42
3.37	-1.44
5.39	1.28
3.78	1.22
2.52	-1.26
2.06	-1.21
2.65	-1.37
1.93	-1.29
2.55	-1.33
1.97	-1.24
4	-1.37
2.44	1.53
2.44	1.53
2.25	-1.25
3.17	-1.42
2.22	-1.39
3.81	1.22
2.45	-1.24
4.78	-1.23
2.24	-1.23
3.79	1.43
7.13	-1.27
3.44	-1.53
4.67	1.24
5	-1.31
2.93	-1.22
7.13	-1.24
4.28	-1.31
5.72	1.32
2.99	-2.44
4.55	1.37
4.53	1.26
4.08	-1.22
4.17	-1.27
5.09	-1.3
2.84	1.97
2.42	-1.29
5.57	1.31
3.64	1.23
3.91	-1.34
2.89	-1.73
3.69	-1.23
2.84	1.25
5.67	1.36
3.96	1.29
3.47	-1.38
9.11	-1.31

3.62	-1.23
2.58	-1.29
4.37	1.56
3.2	1.2
4.89	-1.29
3.62	-1.29
4.36	1.29
2.37	1.48
3.25	-1.4
4.15	-1.76
5.45	-1.47
2.58	-1.28
2.16	-1.24
5.71	1.51
4.04	-1.32
5.22	-1.27
3.8	1.36
5.41	-1.55
3.32	-1.44
5.12	1.53
4.58	-1.27
4.24	1.37
5.69	1.55
6.66	1.31
3.7	1.36
3.8	-1.62
4.95	-1.21
2.84	-1.3
3.99	1.24
4.38	-1.2
3.63	-1.43
2.83	1.29
2.31	-1.31
5.53	1.44
5.77	-1.23
2.73	-1.24
1.7	-1.27
2.56	-1.3
5.32	1.5
4.93	1.27
4.24	-1.37
2.17	-1.39
3.09	-1.34
2.45	-1.2
3.27	-1.33
5.94	1.29
2.87	1.21
5.08	1.3
4.94	1.48
5.45	1.2

4.37	-1.2
4.2	1.2
5	1.36
6.27	-1.54
4.27	1.26
3.32	1.26
5.73	1.23
2.54	-1.25
4.53	1.62
2.97	1.24
5.49	1.49
2.9	-1.63
5.06	-1.31
2.71	-1.3
3.11	-1.45
2.76	-1.2
3.22	-1.39
3.09	-1.28
2.8	-1.41
4.78	1.31
4.17	-1.23
4.41	-1.32
5.26	1.73
4.16	-1.54
4.24	1.26
6.34	-1.67
3.39	-1.25
3.3	-1.3
2.12	1.36
3.57	-1.31
6.2	1.31
1.86	-1.23
6.31	1.21
5.75	-1.26
5.75	-1.26
2.29	-1.38
1.95	-1.28
5.07	-2.27
3.44	-1.23
7.42	-1.54
3.27	1.26
5.42	1.22
4.61	1.23
2.63	-1.3
3.7	-1.21
4.15	-1.22
3.02	1.3
4.63	-1.23
2.87	-1.25
2.25	-1.54

6.55	1.36
4.22	-1.25
4.92	1.34
3.9	-1.21
8.87	1.48
5.32	-1.29
2.35	-1.24
4.86	1.23
5.42	1.44
2.56	-1.25
4.28	1.29
4.16	1.22
3.45	1.34
2.61	-1.34
3.2	-1.28
3.51	1.22
5.4	-1.29
4.22	1.46
6.4	1.21
4.08	-1.22
2.19	-1.25
2.96	-1.44
1.67	1.41
2.33	-1.21
2.4	-1.21
2.24	-1.2
2.36	1.28
2.55	-1.67
3.1	1.32
3.63	1.52
8.64	-1.86
3.78	-1.49
1.81	-1.21
3.93	-1.26
5.91	-1.29
3.94	1.23
2.31	-1.43
2.16	-1.35
6.67	-1.49
3.87	-1.36
4.94	1.21
2.24	-1.3
3.93	1.21
2.46	-1.3
3.88	-1.23
3.18	-1.26
2.61	-1.22
3.1	-1.35
3.59	-1.32
1.93	-1.29

4.92	-1.25
4.75	1.35
5.07	-1.23
2.68	1.25
2.56	-1.33
2.65	-1.28
2.86	-1.63
3.04	-1.34
2.13	-1.22
7.91	1.33
2.59	-1.23
3.73	-1.25
4.92	-1.24
3.54	-1.28
4.26	-1.38
3.8	-1.33
4.08	-1.28
4.76	-1.21
5.33	1.26
4.19	-1.24
2.54	-1.2
1.84	-1.26
4.56	1.2
4.56	1.2
4.85	1.33
3.76	-1.29
3.61	1.2
5.64	1.26
3.97	1.32
5.29	1.27
3.53	-1.23
5.03	1.3
3.37	1.42
4.22	-1.23
7.28	1.34
2.64	-1.24
4.21	-1.2
5.47	1.75
3.11	-1.39
5.12	1.51
4.66	-1.23
2.88	-1.2
2.88	-1.2
4.16	-1.7
4.12	1.27
3.04	1.25
4.84	1.99
4.59	-1.22
2.13	1.23
3.18	-1.2

4.35	-1.37
2.54	-1.22
4.92	-1.21
3.81	-1.24
1.86	-1.23
2.51	-1.5
3.08	-1.42
4.91	-1.28
3.36	-1.43
4.85	2.23
3.62	1.2
3.4	-1.69
8.45	1.43
2.79	-1.46
3.2	1.33
2.29	1.21
3.58	-1.29
2.46	-1.2
5.07	1.46
4.73	1.28
4.78	1.26
3.83	-1.27
2.57	-1.4
5.01	-1.21
6.89	-1.54
7.42	1.48
5.55	-1.49
2.54	-1.22
4.76	1.25
4.76	1.25
4.69	-1.31
1.96	-1.21
5.51	-1.52
5.51	-1.52
5.51	-1.52
4.92	1.39
3.35	-1.21
2.48	-1.37
2.14	-1.25
6.06	1.23
2.5	-1.24
2.23	-1.25
4.68	-1.22
2.3	-1.81
3.75	1.33
3.49	1.22
4.15	1.29
3.74	-1.28
1.91	1.74
3	-1.5

2.02	-1.26
3.79	1.21
5.51	1.77
5.48	-1.23
10.32	-1.54
2.12	-1.22
6.99	-1.25
5.59	1.85
3	-1.35
4.84	-1.32
3.67	1.42
4.28	-1.3
3.83	1.23
3.58	1.21
6.84	-1.39
2.99	-1.39

ANOVA p-value (Neuropathic Pain vs. Control)	FDR p-value (Neuropathic Pain vs. Control)
0.000272	0.999991
0.000333	0.999991
0.000402	0.999991
0.000438	0.999991
0.000448	0.999991
0.000482	0.999991
0.000525	0.999991
0.000647	0.999991
0.000677	0.999991
0.000733	0.999991
0.000955	0.999991
0.00097	0.999991
0.001178	0.999991
0.001217	0.999991
0.00137	0.999991
0.001525	0.999991
0.001657	0.999991
0.001667	0.999991
0.00186	0.999991
0.002053	0.999991
0.002395	0.999991
0.002475	0.999991
0.002544	0.999991
0.002564	0.999991
0.00264	0.999991
0.002722	0.999991
0.002831	0.999991
0.002864	0.999991
0.002884	0.999991
0.003184	0.999991
0.003323	0.999991
0.003325	0.999991
0.003603	0.999991
0.003741	0.999991
0.003799	0.999991
0.003815	0.999991
0.003841	0.999991
0.004034	0.999991
0.004072	0.999991
0.004203	0.999991
0.004215	0.999991
0.004386	0.999991
0.004392	0.999991
0.004504	0.999991
0.004648	0.999991
0.004877	0.999991
0.004922	0.999991
0.004949	0.999991
0.004991	0.999991

0.005348	0.999991
0.005375	0.999991
0.005468	0.999991
0.005471	0.999991
0.00548	0.999991
0.005581	0.999991
0.0056	0.999991
0.005604	0.999991
0.005723	0.999991
0.00573	0.999991
0.005854	0.999991
0.005898	0.999991
0.006079	0.999991
0.006316	0.999991
0.00647	0.999991
0.00655	0.999991
0.006803	0.999991
0.007009	0.999991
0.007123	0.999991
0.007264	0.999991
0.007338	0.999991
0.007438	0.999991
0.007635	0.999991
0.007931	0.999991
0.008132	0.999991
0.008147	0.999991
0.008158	0.999991
0.008468	0.999991
0.008774	0.999991
0.008803	0.999991
0.008935	0.999991
0.008977	0.999991
0.009465	0.999991
0.009521	0.999991
0.009542	0.999991
0.009631	0.999991
0.009642	0.999991
0.009651	0.999991
0.009744	0.999991
0.009754	0.999991
0.00996	0.999991
0.009992	0.999991
0.010009	0.999991
0.010028	0.999991
0.010038	0.999991
0.010414	0.999991
0.010448	0.999991
0.010547	0.999991
0.010677	0.999991
0.010805	0.999991

0.010863	0.999991
0.010897	0.999991
0.010983	0.999991
0.011006	0.999991
0.011127	0.999991
0.01121	0.999991
0.011338	0.999991
0.011339	0.999991
0.011589	0.999991
0.011765	0.999991
0.011774	0.999991
0.011841	0.999991
0.01198	0.999991
0.012018	0.999991
0.012189	0.999991
0.012259	0.999991
0.01229	0.999991
0.012377	0.999991
0.01239	0.999991
0.012448	0.999991
0.012532	0.999991
0.012572	0.999991
0.012698	0.999991
0.012862	0.999991
0.012886	0.999991
0.012959	0.999991
0.01305	0.999991
0.013055	0.999991
0.013063	0.999991
0.013181	0.999991
0.013302	0.999991
0.013555	0.999991
0.013638	0.999991
0.013729	0.999991
0.013874	0.999991
0.013874	0.999991
0.014069	0.999991
0.014146	0.999991
0.014285	0.999991
0.014285	0.999991
0.014299	0.999991
0.014301	0.999991
0.014301	0.999991
0.014305	0.999991
0.014358	0.999991
0.014388	0.999991
0.014483	0.999991
0.014566	0.999991
0.014576	0.999991
0.014728	0.999991

0.014744	0.999991
0.01485	0.999991
0.014862	0.999991
0.014886	0.999991
0.015274	0.999991
0.015274	0.999991
0.015567	0.999991
0.015624	0.999991
0.015709	0.999991
0.015764	0.999991
0.015862	0.999991
0.015881	0.999991
0.015927	0.999991
0.016049	0.999991
0.016069	0.999991
0.016076	0.999991
0.016243	0.999991
0.016292	0.999991
0.016296	0.999991
0.016345	0.999991
0.01638	0.999991
0.016436	0.999991
0.01644	0.999991
0.016455	0.999991
0.016615	0.999991
0.016879	0.999991
0.016882	0.999991
0.016915	0.999991
0.016919	0.999991
0.016962	0.999991
0.016983	0.999991
0.017003	0.999991
0.017156	0.999991
0.01721	0.999991
0.01726	0.999991
0.017302	0.999991
0.017314	0.999991
0.017474	0.999991
0.017961	0.999991
0.017975	0.999991
0.017979	0.999991
0.017987	0.999991
0.01802	0.999991
0.018284	0.999991
0.018285	0.999991
0.018303	0.999991
0.018345	0.999991
0.018348	0.999991
0.018349	0.999991
0.018354	0.999991

0.018676	0.999991
0.018728	0.999991
0.018773	0.999991
0.018832	0.999991
0.019	0.999991
0.019139	0.999991
0.019485	0.999991
0.019599	0.999991
0.019612	0.999991
0.019633	0.999991
0.019641	0.999991
0.01971	0.999991
0.019714	0.999991
0.019869	0.999991
0.019957	0.999991
0.019957	0.999991
0.020001	0.999991
0.020138	0.999991
0.020209	0.999991
0.020223	0.999991
0.02038	0.999991
0.020414	0.999991
0.020445	0.999991
0.020468	0.999991
0.020482	0.999991
0.020581	0.999991
0.020595	0.999991
0.020629	0.999991
0.021013	0.999991
0.021018	0.999991
0.021132	0.999991
0.021515	0.999991
0.021583	0.999991
0.021831	0.999991
0.022069	0.999991
0.022305	0.999991
0.022351	0.999991
0.022412	0.999991
0.022544	0.999991
0.022643	0.999991
0.022665	0.999991
0.02267	0.999991
0.022784	0.999991
0.023324	0.999991
0.023337	0.999991
0.023406	0.999991
0.023428	0.999991
0.02344	0.999991
0.023492	0.999991
0.023685	0.999991

0.023782	0.999991
0.023818	0.999991
0.024026	0.999991
0.024047	0.999991
0.02422	0.999991
0.024303	0.999991
0.024384	0.999991
0.024562	0.999991
0.024564	0.999991
0.024617	0.999991
0.024726	0.999991
0.024771	0.999991
0.024908	0.999991
0.025044	0.999991
0.025167	0.999991
0.025212	0.999991
0.025217	0.999991
0.025238	0.999991
0.025298	0.999991
0.025321	0.999991
0.025383	0.999991
0.025573	0.999991
0.025594	0.999991
0.025655	0.999991
0.025784	0.999991
0.025911	0.999991
0.026156	0.999991
0.026276	0.999991
0.026293	0.999991
0.026377	0.999991
0.02656	0.999991
0.026576	0.999991
0.026578	0.999991
0.026817	0.999991
0.026843	0.999991
0.026922	0.999991
0.027001	0.999991
0.027044	0.999991
0.027202	0.999991
0.027409	0.999991
0.027435	0.999991
0.027497	0.999991
0.027724	0.999991
0.027883	0.999991
0.028004	0.999991
0.02809	0.999991
0.028129	0.999991
0.028134	0.999991
0.028176	0.999991
0.028197	0.999991

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0.02824	0.999991
0.028474	0.999991
0.028646	0.999991
0.028696	0.999991
0.028877	0.999991
0.028911	0.999991
0.028992	0.999991
0.029013	0.999991
0.029017	0.999991
0.029044	0.999991
0.029083	0.999991
0.029089	0.999991
0.029311	0.999991
0.02934	0.999991
0.029391	0.999991
0.029413	0.999991
0.02945	0.999991
0.02975	0.999991
0.030024	0.999991
0.030127	0.999991
0.030497	0.999991
0.030743	0.999991
0.030818	0.999991
0.030884	0.999991
0.030974	0.999991
0.03101	0.999991
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0.031396	0.999991
0.031458	0.999991
0.031482	0.999991
0.03157	0.999991
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0.032407	0.999991
0.032447	0.999991
0.03249	0.999991
0.032512	0.999991
0.032562	0.999991
0.032575	0.999991
0.032616	0.999991
0.032687	0.999991
0.032936	0.999991
0.033143	0.999991

0.033186	0.999991
0.033339	0.999991
0.033489	0.999991
0.033522	0.999991
0.033562	0.999991
0.033675	0.999991
0.03379	0.999991
0.03383	0.999991
0.0339	0.999991
0.033961	0.999991
0.03413	0.999991
0.034186	0.999991
0.034245	0.999991
0.034317	0.999991
0.034415	0.999991
0.034456	0.999991
0.034549	0.999991
0.034612	0.999991
0.034676	0.999991
0.03471	0.999991
0.034721	0.999991
0.034852	0.999991
0.034872	0.999991
0.035828	0.999991
0.036053	0.999991
0.036589	0.999991
0.036684	0.999991
0.036718	0.999991
0.036747	0.999991
0.036884	0.999991
0.03689	0.999991
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0.037284	0.999991
0.037287	0.999991
0.037357	0.999991
0.037697	0.999991
0.037784	0.999991
0.037958	0.999991
0.038089	0.999991
0.038377	0.999991
0.039099	0.999991
0.039571	0.999991
0.039615	0.999991
0.039627	0.999991
0.039786	0.999991
0.039855	0.999991
0.039944	0.999991
0.039979	0.999991
0.040156	0.999991
0.04021	0.999991

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0.040375	0.999991
0.040425	0.999991
0.040515	0.999991
0.040786	0.999991
0.040805	0.999991
0.041013	0.999991
0.041024	0.999991
0.041187	0.999991
0.041207	0.999991
0.041214	0.999991
0.041273	0.999991
0.041323	0.999991
0.04138	0.999991
0.041393	0.999991
0.04142	0.999991
0.04156	0.999991
0.04157	0.999991
0.041587	0.999991
0.041853	0.999991
0.041927	0.999991
0.041971	0.999991
0.042038	0.999991
0.042038	0.999991
0.042057	0.999991
0.042062	0.999991
0.042074	0.999991
0.042146	0.999991
0.04216	0.999991
0.042184	0.999991
0.04221	0.999991
0.04247	0.999991
0.042522	0.999991
0.042531	0.999991
0.042679	0.999991
0.042737	0.999991
0.042806	0.999991
0.042819	0.999991
0.042879	0.999991
0.042958	0.999991
0.043107	0.999991
0.043295	0.999991
0.043295	0.999991
0.043364	0.999991
0.043452	0.999991
0.043571	0.999991
0.043656	0.999991
0.043911	0.999991
0.043918	0.999991
0.043969	0.999991

0.044418	0.999991
0.044503	0.999991
0.044542	0.999991
0.044551	0.999991
0.044559	0.999991
0.044625	0.999991
0.044642	0.999991
0.044712	0.999991
0.044775	0.999991
0.044898	0.999991
0.045003	0.999991
0.045144	0.999991
0.045181	0.999991
0.045242	0.999991
0.045417	0.999991
0.045508	0.999991
0.045632	0.999991
0.045899	0.999991
0.045964	0.999991
0.046183	0.999991
0.046203	0.999991
0.046374	0.999991
0.046384	0.999991
0.046507	0.999991
0.046534	0.999991
0.046581	0.999991
0.046601	0.999991
0.046601	0.999991
0.046624	0.999991
0.046624	0.999991
0.046785	0.999991
0.046832	0.999991
0.047032	0.999991
0.047032	0.999991
0.047032	0.999991
0.047071	0.999991
0.047228	0.999991
0.047389	0.999991
0.047453	0.999991
0.047563	0.999991
0.04761	0.999991
0.047828	0.999991
0.047844	0.999991
0.048011	0.999991
0.048021	0.999991
0.048038	0.999991
0.048231	0.999991
0.04833	0.999991
0.048405	0.999991
0.048408	0.999991

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0.048587	0.999991
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0.048612	0.999991
0.048639	0.999991
0.048745	0.999991
0.048753	0.999991
0.048756	0.999991
0.048917	0.999991
0.049035	0.999991
0.049438	0.999991
0.049465	0.999991
0.049582	0.999991
0.0496	0.999991
0.049761	0.999991
0.049976	0.999991

Gene Symbol

RNA5SP517

TRUB2

MAD2L1

SNX16

MC1R

RP11-4H14.1

PDZRN4

ICOSLG

GABRP

DFFA

XAGE5

NME7

ZBTB8OSP1

FAM99A

WDR93

RPL26P30

LOC645188

ARHGAP11B

STEAP1B

RNA5SP191

FAM124B

DPP3

FAM105B

TUBBP6

RNF185

GCG

RP11-813P10.2

MED27

PABPC3

RNU1-20P

C1orf87

SIGLEC8

FAM226B

ZCRB1

ALCAM

RACGAP1

C12orf5

LOC100131792

RNU6-76P

TIMP1

PASK

TRAPPC6B

OR1J2

GAFA2

ACD

RAB27B

MYADML

YOD1

SLC37A2

PIP5K1A

JRKL

NAT8B

TMEM110-MUSTN1

SNRNP40

C15orf43

C16orf92

NTRK3

SNORD82

C14orf178

RNA5SP466

OR5M3

MANEA

ITGA2

ZBTB22

FAM129C

GPR128

LRRN3

LIN7C

PRAME

NPRL3

WDR65

ZNF285

JAZF1
HMGN2P27
C7orf34

SAMD15

GSTA5
FOXO3

POLR3D

SNX6
FAM111B

CTPS2
RP11-433P17.1
HAPLN1
ZNF132
GCSAM
PID1

DICER1-AS1
DUXA
ETFB
GPNMB
RPS25
WFDC5

PRDM6
KRT37
TRHR

CDK17
SNORD103B
SNORD103B
ACADL
C8orf37
ZNF680
ROM1
DRG1

SCARNA9
IGFBP2
LOC727721

TMEM57

RNF144B
SLC12A9

SHOX
SHOX
PHOSPHO1
PXDC1

C2CD2
OR51A1P
SH3RF3

FCGR3A
MIR31HG
KLHL5
FEZ2

PTGER4P2-CDK2AP2P2
KIF14
PROX1
CLIC1P1

RN7SKP1
FOXJ1
VN1R1
DNAH14

ZSWIM1
AP5S1
PRED57
LOC100506271
PRED62

SDHD
PPP2R3B
MXI1

GUCD1
CTRC
KIFC3
SALL1

KRT84
ZNF566
C3orf38

RNU6-23P
LY6G6C

CROCCP2
CHD9
RP13-192B19.1
MIR19B2

FUT9

C10orf62
TISP43
TISP43

CP

ZNF354B
THNSL1
MST1

BSPRY
YY1AP1
OR2T8
RP11-440K22.1
FBXO8
FOXR2
CISD2
HIST3H2BB
KIAA0319L

LY6G6C
LOC728690
RNA5SP454
COG3
CDCA4
ORM2
LINC00529
NUDC
UAP1
TRIM49
EPCAM
ZNF165
PGK2
DHRS13
RP11-298C3.2

YBX1

CLDN16

MRPS14
PP12613
GLRX2
CYP4Z2P
PCDHB17

GSTM2
SNORD20

HIST1H2BK
LOC100130275

LTBP1
RUNDC3A

MGC39372
PHLPP2
NEXN

LOC729164

DHFRL1
RNA5SP329
CXCL17
NCKAP5L
ELP4
GSTTP1

UPP1
CETN2

LACC1

KLHL33

ACTR3BP2
DNASE1L3
SLC9A8
RNASE9
CCDC23
RP11-661A12.5
ZNF517

TDRD3
ARHGAP31

HIST1H2BM
SIGLEC11

Mar-02

ARMCX6
AADA4L4
LGALS1

PSG4
RAG2

DSEL

MICA
ITIH1
RNA5SP139
LOC399904
HSD3BP4

LOC100653052
SNORD75

TNFRSF11B
MRPL54

TMEM89
RNF5
RNF5
IFNA16

SLIT3
CGA
RIOK3

SLC4A2
PLCXD1
RABGGTB
COL21A1
C12orf29
HEXA-AS1
LYRM4
NEGR1

HIATL2
FAM226B

POLA1
LOC541471
PLAA
FAM83A
TTC7B
LOC100506748
DNAH14
GPR12
LOC388282
CST1

GSTM5

ALS2CR12
LY6G6C
MIIP
UEVLD

RPL11P5

C1orf189

RAB44
CMPK1

NOXRED1
OGG1

B3GNT5
RP11-292D4.4

C10orf129

PPRC1
TFR2
NDRG3
LPPR4
ZNF781
TRIM43
SNHG1
DSTNP2
MIR217
H3F3B
KRT28
C6orf195
ACYP2
ZNF654
APOOL
CRYGA
LOC100996464
SH3GL2
ASMTL-AS1
NDUFS4
PPIL6

LOC729574
LOC729574
LOC729574
SUMO2P3
AVPR1A
RNA5SP142
MICA
SEC1P
RP11-864N7.4
SLC25A44
LOC101060794
RRAGB
METTL9

RP11-69M1.3
TLR5
OR6B2
SNX29P2
TOMM20
LOC441178
LOC441178
GPR15
FAM131A

NLRC4
PPME1

PHF16

ARL4P
OR51A8P
CCR10

RNU5D-1

CD101
GSTM3
CASP5
LINC00885
TAAR3

STARD9
ZNF638-IT1

ACTR3BP5
SDPR
PIK3CA
PLCXD1
NHLRC1
DSCC1
HIST1H2AE

Mar-08

SERPINI1
NRM
NRM

C2CD4A

PBK

CSAD

NLRP14
KIF25

KDM8
PEX11A
LMX1B
SLITRK4
OR6C72P

OR2AE1

BOD1
SNORD15B

RELL1
CLU

EIF2S2P4

PCYOX1
GOLIM4
HAVCR2
LOC101060261
TAS2R13

Description

RNA, 5S ribosomal pseudogene 517

TruB pseudouridine (psi) synthase homolog 2 (E. coli)

MAD2 mitotic arrest deficient-like 1 (yeast)

sorting nexin 16

melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor)

PDZ domain containing ring finger 4

inducible T-cell co-stimulator ligand

gamma-aminobutyric acid (GABA) A receptor, pi

DNA fragmentation factor, 45kDa, alpha polypeptide

X antigen family, member 5

NME/NM23 family member 7

zinc finger and BTB domain containing 8 opposite strand pseudogene 1

family with sequence similarity 99, member A (non-protein coding)

WD repeat domain 93

ribosomal protein L26 pseudogene 30

uncharacterized LOC645188

Rho GTPase activating protein 11B

STEAP family member 1B

RNA, 5S ribosomal pseudogene 191

family with sequence similarity 124B

dipeptidyl-peptidase 3

family with sequence similarity 105, member B

tubulin, beta class I pseudogene 6

ring finger protein 185; RNF185 antisense RNA 1

glucagon

mediator complex subunit 27

poly(A) binding protein, cytoplasmic 3

RNA, U1 small nuclear 20, pseudogene

chromosome 1 open reading frame 87

sialic acid binding Ig-like lectin 8

family with sequence similarity 226, member B (non-protein coding); family with sequence similarity 226, men

zinc finger CCHC-type and RNA binding motif 1

activated leukocyte cell adhesion molecule

Rac GTPase activating protein 1

chromosome 12 open reading frame 5

uncharacterized LOC100131792

RNA, U6 small nuclear 76, pseudogene

TIMP metalloproteinase inhibitor 1

PAS domain containing serine/threonine kinase

trafficking protein particle complex 6B

olfactory receptor, family 1, subfamily J, member 2

FGF-2 activity-associated protein 2

adrenocortical dysplasia homolog (mouse)

RAB27B, member RAS oncogene family

myeloid-associated differentiation marker-like (pseudogene)

YOD1 deubiquitinase

solute carrier family 37 (glycerol-3-phosphate transporter), member 2

phosphatidylinositol-4-phosphate 5-kinase, type I, alpha

jerky homolog-like (mouse)

N-acetyltransferase 8B (GCN5-related, putative, gene/pseudogene)

TMEM110-MUSTN1 readthrough; musculoskeletal, embryonic nuclear protein 1

small nuclear ribonucleoprotein 40kDa (U5)

chromosome 15 open reading frame 43

chromosome 16 open reading frame 92

neurotrophic tyrosine kinase, receptor, type 3

small nucleolar RNA, C/D box 82

chromosome 14 open reading frame 178

RNA, 5S ribosomal pseudogene 466

olfactory receptor, family 5, subfamily M, member 3; olfactory receptor, family 5, subfamily M, member 4 pse

mannosidase, endo-alpha

integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)

zinc finger and BTB domain containing 22

family with sequence similarity 129, member C

G protein-coupled receptor 128

leucine rich repeat neuronal 3

lin-7 homolog C (C. elegans)

preferentially expressed antigen in melanoma

nitrogen permease regulator-like 3 (S. cerevisiae)

WD repeat domain 65

zinc finger protein 285

JAZF zinc finger 1
high mobility group nucleosomal binding domain 2 pseudogene 27
chromosome 7 open reading frame 34

sterile alpha motif domain containing 15

glutathione S-transferase alpha 5
forkhead box O3; forkhead box O3B pseudogene

polymerase (RNA) III (DNA directed) polypeptide D, 44kDa

sorting nexin 6
family with sequence similarity 111, member B

CTP synthase 2

hyaluronan and proteoglycan link protein 1
zinc finger protein 132
germinal center-associated, signaling and motility
phosphotyrosine interaction domain containing 1

DICER1 antisense RNA 1
double homeobox A
electron-transfer-flavoprotein, beta polypeptide
glycoprotein (transmembrane) nmb
ribosomal protein S25
WAP four-disulfide core domain 5

PR domain containing 6
keratin 37
thyrotropin-releasing hormone receptor

cyclin-dependent kinase 17
small nucleolar RNA, C/D box 103B; small nucleolar RNA, C/D box 103A
small nucleolar RNA, C/D box 103B; small nucleolar RNA, C/D box 103A
acyl-CoA dehydrogenase, long chain
chromosome 8 open reading frame 37
zinc finger protein 680
retinal outer segment membrane protein 1
developmentally regulated GTP binding protein 1

small Cajal body-specific RNA 9; small Cajal body-specific RNA 9-like
insulin-like growth factor binding protein 2, 36kDa
uncharacterized LOC727721; microRNA 34a

transmembrane protein 57

ring finger protein 144B
solute carrier family 12 (potassium/chloride transporters), member 9

short stature homeobox
short stature homeobox
phosphatase, orphan 1
PX domain containing 1

C2 calcium-dependent domain containing 2
olfactory receptor, family 51, subfamily A, member 1 pseudogene
SH3 domain containing ring finger 3

Fc fragment of IgG, low affinity IIIa, receptor (CD16a)
MIR31 host gene (non-protein coding)
kelch-like family member 5
fasciculation and elongation protein zeta 2 (zygin II)

PTGER4P2-CDK2AP2P2 readthrough transcribed pseudogene
kinesin family member 14
prospero homeobox 1
chloride intracellular channel 1 pseudogene 1

RNA, 7SK small nuclear pseudogene 1
forkhead box J1
vomeronasal 1 receptor 1
dynein, axonemal, heavy chain 14

zinc finger, SWIM-type containing 1
adaptor-related protein complex 5, sigma 1 subunit

uncharacterized LOC100506271

succinate dehydrogenase complex, subunit D, integral membrane protein
protein phosphatase 2, regulatory subunit B, beta
MAX interactor 1, dimerization protein

guanylyl cyclase domain containing 1
chymotrypsin C (caldecrin)
kinesin family member C3
sal-like 1 (Drosophila)

keratin 84
zinc finger protein 566
chromosome 3 open reading frame 38

RNA, U6 small nuclear 23, pseudogene
lymphocyte antigen 6 complex, locus G6C

ciliary rootlet coiled-coil, rootletin pseudogene 2
chromodomain helicase DNA binding protein 9

microRNA 19b-2

fucosyltransferase 9 (alpha (1,3) fucosyltransferase)

chromosome 10 open reading frame 62
uncharacterized LOC150527; uncharacterized LOC646743
uncharacterized LOC150527; uncharacterized LOC646743

ceruloplasmin (ferroxidase)

zinc finger protein 354B
threonine synthase-like 1 (*S. cerevisiae*)
macrophage stimulating 1 (hepatocyte growth factor-like)

B-box and SPRY domain containing
YY1 associated protein 1
olfactory receptor, family 2, subfamily T, member 8

F-box protein 8
forkhead box R2
CDGSH iron sulfur domain 2
histone cluster 3, H2bb
KIAA0319-like

lymphocyte antigen 6 complex, locus G6C
uncharacterized LOC728690
RNA, 5S ribosomal pseudogene 454
component of oligomeric golgi complex 3
cell division cycle associated 4
orosomuroid 2
long intergenic non-protein coding RNA 529
nuclear distribution C homolog (*A. nidulans*)
UDP-N-acetylglucosamine pyrophosphorylase 1
tripartite motif containing 49; tripartite motif containing 49C; tripartite motif-containing protein 49-like protein
epithelial cell adhesion molecule
zinc finger protein 165
phosphoglycerate kinase 2
dehydrogenase/reductase (SDR family) member 13

Y box binding protein 1

claudin 16

mitochondrial ribosomal protein S14

uncharacterized LOC100192379

glutaredoxin 2

cytochrome P450, family 4, subfamily Z, polypeptide 2, pseudogene

protocadherin beta 17 pseudogene

glutathione S-transferase mu 2 (muscle)

small nucleolar RNA, C/D box 20

histone cluster 1, H2bk

uncharacterized LOC100130275

latent transforming growth factor beta binding protein 1

RUN domain containing 3A

serpin peptidase inhibitor, clade B (ovalbumin), member 9 pseudogene

PH domain and leucine rich repeat protein phosphatase 2

nexilin (F actin binding protein)

hCG1732469

dihydrofolate reductase-like 1

RNA, 5S ribosomal pseudogene 329

chemokine (C-X-C motif) ligand 17

NCK-associated protein 5-like

elongator acetyltransferase complex subunit 4

glutathione S-transferase theta pseudogene 1

uridine phosphorylase 1

centrin, EF-hand protein, 2

laccase (multicopper oxidoreductase) domain containing 1

kelch-like family member 33

ARP3 actin-related protein 3 homolog B (yeast) pseudogene 2

deoxyribonuclease I-like 3

solute carrier family 9, subfamily A (NHE8, cation proton antiporter 8), member 8

ribonuclease, RNase A family, 9 (non-active)

coiled-coil domain containing 23

zinc finger protein 517

tudor domain containing 3
Rho GTPase activating protein 31

histone cluster 1, H2bm
sialic acid binding Ig-like lectin 11
mitochondrial amidoxime reducing component 2

armadillo repeat containing, X-linked 6
arylacetamide deacetylase-like 4
lectin, galactoside-binding-like

pregnancy specific beta-1-glycoprotein 4
recombination activating gene 2

dermatan sulfate epimerase-like

MHC class I polypeptide-related sequence A
inter-alpha-trypsin inhibitor heavy chain 1
RNA, 5S ribosomal pseudogene 139
uncharacterized LOC399904; uncharacterized LOC100130348
hydroxy-delta-5-steroid dehydrogenase, 3 beta, pseudogene 4

putative killer cell immunoglobulin-like receptor like protein KIR3DP1-like; killer cell immunoglobulin-like re
small nucleolar RNA, C/D box 75

tumor necrosis factor receptor superfamily, member 11b
mitochondrial ribosomal protein L54

transmembrane protein 89
ring finger protein 5, E3 ubiquitin protein ligase
ring finger protein 5, E3 ubiquitin protein ligase
interferon, alpha 16

slit homolog 3 (Drosophila)
glycoprotein hormones, alpha polypeptide
RIO kinase 3

solute carrier family 4, anion exchanger, member 2 (erythrocyte membrane protein band 3-like 1)
phosphatidylinositol-specific phospholipase C, X domain containing 1
Rab geranylgeranyltransferase, beta subunit; small nucleolar RNA, C/D box 45C; small nucleolar RNA, C/D bo
collagen, type XXI, alpha 1
chromosome 12 open reading frame 29
HEXA antisense RNA 1
LYR motif containing 4
neuronal growth regulator 1

hippocampus abundant transcript-like 2
family with sequence similarity 226, member B (non-protein coding)

polymerase (DNA directed), alpha 1, catalytic subunit
uncharacterized LOC541471; long intergenic non-protein coding RNA 152
phospholipase A2-activating protein
family with sequence similarity 83, member A
tetratricopeptide repeat domain 7B
uncharacterized LOC100506748; HBV preS1-transactivated protein 4
dynein, axonemal, heavy chain 14
G protein-coupled receptor 12
uncharacterized LOC388282
cystatin SN

glutathione S-transferase mu 5

amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 12
lymphocyte antigen 6 complex, locus G6C
migration and invasion inhibitory protein
UEV and lactate/malate dehydrogenase domains

ribosomal protein L11 pseudogene 5

chromosome 1 open reading frame 189

RAB44, member RAS oncogene family
cytidine monophosphate (UMP-CMP) kinase 1, cytosolic

NADP-dependent oxidoreductase domain containing 1
8-oxoguanine DNA glycosylase

UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 5

chromosome 10 open reading frame 129

peroxisome proliferator-activated receptor gamma, coactivator-related 1
transferrin receptor 2
NDRG family member 3
lipid phosphate phosphatase-related protein type 4
zinc finger protein 781
tripartite motif containing 43; tripartite motif containing 43B; tripartite motif containing 43C, pseudogene
small nucleolar RNA host gene 1 (non-protein coding); small nucleolar RNA, C/D box 25; small nucleolar RNA
destrin (actin depolymerizing factor) pseudogene 2
microRNA 217
H3 histone, family 3B (H3.3B); microRNA 4738; H3 histone, family 3A
keratin 28
chromosome 6 open reading frame 195
acylphosphatase 2, muscle type
zinc finger protein 654
apolipoprotein O-like
crystallin, gamma A
zinc finger protein 84-like; zinc finger protein 84
SH3-domain GRB2-like 2
ASMTL antisense RNA 1
NADH dehydrogenase (ubiquinone) Fe-S protein 4, 18kDa (NADH-coenzyme Q reductase)
peptidylprolyl isomerase (cyclophilin)-like 6

UPF0627 protein ENSP00000341061/ENSP00000339743; UPF0627 protein ENSP00000341061/ENSP000003
UPF0627 protein ENSP00000341061/ENSP00000339743; UPF0627 protein ENSP00000341061/ENSP000003
UPF0627 protein ENSP00000341061/ENSP00000339743; uncharacterized LOC388692; UPF0627 protein EN
SMT3 suppressor of mif two 3 homolog 2 (*S. cerevisiae*) pseudogene 3
arginine vasopressin receptor 1A
RNA, 5S ribosomal pseudogene 142
MHC class I polypeptide-related sequence A
secretory blood group 1, pseudogene

solute carrier family 25, member 44
protein USP6-like 2-like
Ras-related GTP binding B
methyltransferase like 9

toll-like receptor 5
olfactory receptor, family 6, subfamily B, member 2
sorting nexin 29 pseudogene 2
translocase of outer mitochondrial membrane 20 homolog (yeast)
uncharacterized LOC441178
uncharacterized LOC441178
G protein-coupled receptor 15
family with sequence similarity 131, member A

NLR family, CARD domain containing 4
protein phosphatase methylesterase 1

PHD finger protein 16

ADP-ribosylation factor-like 4 pseudogene
olfactory receptor, family 51, subfamily A, member 8 pseudogene
chemokine (C-C motif) receptor 10

RNA, U5D small nuclear 1

CD101 molecule
glutathione S-transferase mu 3 (brain)
caspase 5, apoptosis-related cysteine peptidase
long intergenic non-protein coding RNA 885
trace amine associated receptor 3 (gene/pseudogene)

StAR-related lipid transfer (START) domain containing 9
ZNF638 intronic transcript 1 (non-protein coding); zinc finger protein 638

ARP3 actin-related protein 3 homolog B (yeast) pseudogene 5
serum deprivation response
phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha
phosphatidylinositol-specific phospholipase C, X domain containing 1
NHL repeat containing 1
DNA replication and sister chromatid cohesion 1
histone cluster 1, H2ae
membrane-associated ring finger (C3HC4) 8, E3 ubiquitin protein ligase

serpin peptidase inhibitor, clade I (neuroserpin), member 1
nurim (nuclear envelope membrane protein)
nurim (nuclear envelope membrane protein)

C2 calcium-dependent domain containing 4A

PDZ binding kinase

cysteine sulfinic acid decarboxylase

NLR family, pyrin domain containing 14
kinesin family member 25

lysine (K)-specific demethylase 8
peroxisomal biogenesis factor 11 alpha
LIM homeobox transcription factor 1, beta
SLIT and NTRK-like family, member 4
olfactory receptor, family 6, subfamily C, member 72 pseudogene

Comment

number A (non-protein coding)

ndogene

.1-like; tripartite motif containing 49D2, pseudogene; tripartite motif containing 49D1

ceptor, three domains, long cytoplasmic tail, 1; killer cell immunoglobulin-like receptor, two domains, long c

ox 45A; small nucleolar RNA, C/D box 45B

A, C/D box 22; small nucleolar RNA, C/D box 26; small nucleolar RNA, C/D box 27; small nucleolar RNA,

39743-like

39743-like

SP00000358171-like; UPF0627 protein ENSP00000341061/ENSP00000339743-like

ide diphosphate linked moiety X)-type motif 4 pseudogene 1; uncharacterized LOC100996752

cytoplasmic tail, 2; killer cell immunoglobulin-like receptor, three domains, short cytoplasmic tail, 1; killer cell

C/D box 28; small nucleolar RNA, C/D box 31; small nucleolar RNA, C/D box 29

immunoglobulin-like receptor, two domains, long cytoplasmic tail, 3; killer cell immunoglobulin-like receptor

; two domains, long cytoplasmic tail, 5A; killer cell immunoglobulin-like receptor, two domains, short cytopla:

smic tail, 5; killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail, 3; killer cell immun

oglobulin-like receptor, two domains, short cytoplasmic tail, 2; killer cell immunoglobulin-like receptor three c

1 domains long cytoplasmic tail 3; killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 4

5B; killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 4; killer cell immunoglobulin

-like receptor, thre