mRNA and long non-coding RNA expression profiles in rats reveal inflammatory features in sepsis-associated encephalopathy

Running title: Inflammatory rodent transcriptome

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Abstract

Background: Sepsis-associated encephalopathy (SAE) is related to cognitive sequelae in patients in the intensive care unit (ICU) and can have serious impacts on quality of life after recovery. Although various pathogenic pathways are involved in SAE development, little is known concerning the global role of long non-coding RNAs (lncRNAs) in SAE.

Methods: Herein, we employed transcriptome sequencing approaches to characterize the effects of lipopolysaccharide (LPS) on lncRNA expression patterns in brain tissue isolated from Sprague-Dawley (SD) rats with and without SAE. We performed high-throughput transcriptome sequencing after LPS was intraperitoneally injected and predicted targets and functions using bioinformatics tools. Subsequently, we explored the results in detail according to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses.

Results: lncRNAs were differentially expressed in brain tissue after LPS treatment. After 6 h of LPS exposure, expression of 400 lncRNAs were significantly changed, including an increase in 316 lncRNAs and a decrease in 84 lncRNAs. In addition, 155 mRNAs were differentially expressed, with 84 up-regulated and 71 down-regulated. At 24 h post-treatment, expression of 117 lncRNAs and 57 mRNAs was consistently elevated, while expression of 79 lncRNAs and 21 mRNAs was decreased (change > 1.5-fold; $p < 0.05$). We demonstrated for the first time that differentially expressed lncRNAs were predicted to be enriched in a post-chaperonin tubulin folding pathway (GO : 007023), which is closely related to the key step in the tubulin folding process.

Interestingly, the predicted pathway (KEGG 04360: axon guidance) was significantly changed under the same conditions. These results reveal that LPS might influence the construction and polarization of microtubules, which exert predominant roles in synaptogenesis and related biofunctions in the rodent central nervous system (CNS).

Conclusions: An inventory of LPS-modulated expression profiles from the rodent CNS is an important step toward understanding the function of mRNAs, including lncRNAs, and suggests that microtubule malformation and dysfunction may be involved in SAE pathogenesis.

Keywords

lipopolysaccharide, long non-coding RNA, sepsis-associated encephalopathy, transcriptome, tubulin

Summary:

We explored the expression profiles of long non-coding RNAs (lncRNAs) and mRNAs in rodent brain tissues by RNA-sequencing, and identified aberrant expression of 596 lncRNAs and 195 mRNAs (fold change > 1.5, $P < 0.05$), suggesting lncRNAs were affected by sepsis-associated encephalopathy (SAE). When applying gene ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, we discovered that these dysregulated genes were mainly involved in cell shape and synaptic plasticity, such as changes in post-chaperonin tubulin folding. We demonstrated for the first time that NONRATT020317 was differentially expressed in SAE.

Background

Sepsis is a severe disease with high mortality and morbidity. Although there has been a focus on transcription of the genome, the mechanisms still need to be further addressed, especially when the central nervous system (CNS) is involved. Sepsis-associated encephalopathy (SAE) is the most common complication in septic patients, which manifests mental abnormality and motor dysfunction. This type of diffuse brain dysfunction that occurs secondary to sepsis has limited therapeutic options with poor prognosis. As much as 70% survived septic patients in intensive care unit (ICU) had neurocognitive impairment, such as confusion or coma, even never fully recovered [1]. To improve the quality of life, alleviate burdens of patients' families and promote the therapeutic effect on sepsis, we urgently need to clarify the pathogenesis of SAE.

Long non-coding RNAs (lncRNAs) are transcripts >200 nucleotides (nt) that actively participate in genomic regulatory pathways via diverse mechanisms (epigenetic regulation, transcription modulation and post-transcription modulation) [2-4]. lncRNAs were previously viewed as "transcriptional noise" without protein-coding capability [5, 6]. Recently, lncRNAs have been extensively investigated and accumulating evidence indicates that they are emerging as key and essential transcriptional and post-transcriptional mediators in diverse physiological and pathological processes in a tissue-specific manner [7-9]. Nevertheless, lncRNAs are highly expressed in developing and adult mammalian brains [10, 11]. lncRNAs have already been implicated to play key roles in brain development, neural plasticity, cognitive function, and neural organization [10]. In the CNS, their emerging roles in

diverse neurodegenerative and neurological disorders have been verified [12-14].

It has been shown recently that aberrant lncRNAs are involved in CNS disturbance in rodents [15], and establishing their functions might help to eliminate the neurological consequences. Consequently, it is reasonable to speculate that lncRNA-directed epigenetic regulation in neural communication is linked to the pathophysiology of cognitive dysfunction. However, despite the abundance and pivotal roles of lncRNAs in brain function, their specific expression pattern and underlying functions in SAE are still poorly understood. To explore the lncRNA landscape in rodent models of CNS endotoxemia, we used RNA-seq to provide insights into the genomic roles of lncRNAs under acute and chronic inflammatory conditions in LPS-induced SAE.

Methods

Animals

Eight-week-old Sprague–Dawley rats weighing 150–200 g were approved for use by the Ethics Review Committee for Animal Experimentation of the China Medical University, in compliance with the *Guide for the Care and Use of Laboratory Animals*. The rats were housed under a constant temperature ($\sim 23 \pm 1^\circ\text{C}$) with adequate food and water. Rats were given lipopolysaccharide (LPS) to create septic model. Animals were divided into three groups (n=20): normal saline (control); L6, rats were killed 6 h after a single intraperitoneal injection of 5 mg/kg LPS; and L24, rats were euthanized 24 h after a single intraperitoneal injection of 5 mg/kg LPS. Brains were removed and

dissected on ice.

RNA extraction

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA quantity and purity were measured using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and RNA 6000 Nano LabChip Kit (Agilent Technologies). The integrity of the RNA was assessed through agarose gel electrophoresis. RNA samples with RNA integrity number >7.0 , OD 260/280 ≥ 1.8 and OD 260/230 ≥ 1.5 were viewed as high purity and used in the following experiment.

Library construction and sequencing

rRNA was removed from 5 μ g total RNA (including mRNAs and lncRNAs) using the Ribo-Zero rRNA Removal Kit (Epidemiology version) (Epicenter, Madison, WI, USA). For accuracy and reliability, the raw data were preprocessed by trimming the adaptors and low-quality reads. Following purification, mRNA was fragmented into small pieces using divalent cations under elevated temperature. The cleaved RNA fragments were amplified and reverse-transcribed into the final cDNA library using the mRNA-Seq Sample Preparation Kit (Illumina, San Diego, CA, USA). The average insert size for the paired-end libraries was 300 ± 50 bp. Cluster generation and sequencing were performed on a HiSeq2500 sequencer (Illumina) at LC-BIO (Hangzhou, China) using the paired-end sequencing (100 bp).

Data analysis

Reads were aligned to the rat genome (from UCSC Genome Browser) using Bowtie 2(2.1.0). The expression level of each transcript in each sample was evaluated by reads per kilobase per million mapped reads (RPKM). $RPKM = \frac{\text{total exon reads}}{[\text{mapped reads (millions)} \times \text{exon length (kb)}]}$. All the lncRNAs were annotated with the corresponding target genes and potential regulatory mechanisms.

GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) analyses

RNA-seq provided a new perspective on the coordinated correlation of lncRNA expression in the rodent transcriptome under specific stresses. We investigated the function of all the unknown lncRNAs by data curation and reprocessing. Predicted gene targets were separately analyzed using GO (<http://www.geneontology.org/>) and KEGG (<http://www.kegg.jp/kegg/pathway.html>). GO terms were used to depict aberrantly expressed RNAs from three aspects: cellular component, molecular function and biological process. KEGG terms were applied to annotate RNAs in biological pathways. Sequencing analysis was performed by LC-BIO.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance followed by Student's *t* test using SPSS version 13.0 (SPSS, Chicago, IL, USA). Quantitative data

were expressed as mean $\pm$ standard deviation. Statistical significance was noted with a threshold of fold change >1.5 and $p < 0.05$ for analysis of sequencing data, and the false discovery rate was calculated to correct the p value. Two-sided Fisher's exact test was used to designate GO category and KEGG results.

Results

Alignment of RNA-seq reads

There were 24,954,481 clean reads and an average of 8,318,160 per sample, with a valid ratio of 99.82%. The unique clean reads were 14,123,311, with a valid ratio of 99.73%. The workflow of sequencing in this study is shown in Fig. 1. The valid clean data were analyzed with Bowtie software, which mapped these data to reference values in the mRNA/lncRNA database for subsequent analysis of differential expression and function of the obtained transcripts (Table 1). The characteristic expression tendency of mRNAs and lncRNAs is shown in Fig. 2.

Differential expression profile of mRNAs

To reveal the differential expression profile of the RNA transcriptome, the 8-week-old rodent brain mRNAs exposed to 5 mg/kg LPS were identified by sequencing. In addition to the significantly altered genes, Fig. 3A and B shows the changes in mRNA, including the non-significantly expressed genes in the adult rodent brain transcriptome. After short-term exposure to LPS (L6 group), 155 mRNAs were significantly changed, whereas the number decreased to 78 after long duration of

LPS exposure (L24 group). Thirty-eight genes were changed in common between the two groups (Fig. 3C). The genes that were significantly up- and down-regulated after LPS treatment are shown in Fig. 3D, as compared with the genes in the control groups. The Hierarchical cluster analysis of the differentially expressed mRNAs in response to the two durations of LPS exposure is shown by the heatmaps in Fig. 3E and F. Integrative analysis of mRNAs, including the fold changes (experimental vs. control groups) and p values calculated from the normalized expression data, was performed as outlined in Tables 2 and 3. Table 4 gives details of differential expression of mRNAs commonly altered in response to LPS exposure ($p < 0.05$).

GO and KEGG analysis

To elucidate further the alterations of cellular component, molecular function, biological process and biological pathways induced by LPS, GO annotations and KEGG analysis were subsequently applied to the differentially expressed genes.

In the L6/C (L6 vs. control) group, the up-regulated mRNAs were involved in 96 biological processes, 16 cellular components, and 29 molecular functions. The down-regulated mRNAs were involved in 85 biological processes, 16 cellular components, and 31 molecular functions. In the L24/C (L24 vs. control) group, the up-regulated mRNAs were involved in 64 biological processes, 8 cellular components, and 35 molecular functions. The down-regulated mRNAs were involved in 22 biological processes, 6 cellular components, and 11 molecular functions. The most frequently represented biological process items were “response to hyperoxia

(GO:0055093)” in the L6/C group and “post-chaperonin tubulin folding pathway (GO:007023)” in the L24/C group. The most significant cellular component terms were “respiratory chain (GO:0070469)”, “respiratory chain complex IV (GO:0045277)”, and “mitochondrial respiratory chain complex I (GO:0005747)” in the L6/C group, and “gap junction (GO:0005921)” and “extracellular exosome (GO:0070062)” in the L24/C group. The most frequent molecular function terms were “NADH dehydrogenase (ubiquinone) activity (GO:0008137)”, “GTPase activity (GO:0003924)” and “receptor signaling complex scaffold activity (GO:0030159)” in the L6/C, and “antigen binding (GO:0003823)” in the L24/C groups (Fig. 4A–D).

KEGG analysis revealed the substantial enriched pathways of the significantly aberrantly expressed genes (Fig. 4E–G). Compared with the control groups, 6 pathways were significantly up-regulated, and the highest enrichment score in the pathways was “axon guidance” in the L6/C group. The other up-regulated pathways were “arachidonic acid metabolism”, “sphingolipid metabolism”, “antigen processing and presentation”, “renin–angiotensin system” and “protein processing in endoplasmic reticulum” in the L6/C group. Similarly, five pathways were significantly up-regulated in the L24/C group; the top three were “sulfur relay system”, “other glycan degradation” and “fructose and mannose metabolism”.

Differential expression profile of lncRNAs

Figure 5A and B shows the changes in lncRNAs in the mature rodent brain transcriptome after LPS exposure. Compared with the control groups, we identified

316 significantly differentially expressed up-regulated and 84 significantly differentially expressed down-regulated lncRNAs after short-term exposure to LPS (L6 group) (fold change >1.5 , $p < 0.05$). The numbers decreased to 117 and 79 after long-term exposure to LPS (L24 group) (Fig. 5C). Altogether, it revealed that lncRNAs, both up-regulated and down-regulated in L6 group, changed more than with long-term duration. Hierarchical cluster analysis of the differentially expressed lncRNAs is shown by the heatmaps in Fig. 5D and E. The integrative analysis of significantly expressed down-regulated lncRNAs is shown in Table 5.

Characterization of the lncRNAs from RNA-seq data

The single-exon and multi-exon numbers occupied 10,012 (38.89%) and 15,732 (61.11%) of 25,745 lncRNA transcripts. There were 1,743 (8.23%) single exons and 19,439 (91.77%) multi-exons in the 21,183 mRNA transcripts (Fig. 6A). The length distribution analysis demonstrated that the lncRNAs and mRNAs mainly changed in the range $>1,000$ bp (Fig. 6B). Opening reading frame (ORF) analysis was based on the principle of six-frame translation, which aimed to provide evidence for gene prediction. From this prediction algorithm, it was clear that the ORFs coded by mRNA were longer than those coded by lncRNAs. It is possible that lncRNAs can code 500 amino acids, which is the classical hallmark of protein-coding genes (both from ncRNAs and mRNAs) (Fig. 6C and D).

Discussion

The mammalian brain expresses an abundant number of lncRNAs that have gradually become novel modulators for intervening in neuronal disease [16]. Using a rodent model of LPS-induced SAE and deep RNA sequencing (RNA-seq), we profiled LPS-modulated transcriptomes in adult rodent brains to determine the mechanism involved in neurological dysfunction after sepsis. In the present study, RNA-seq analysis of rodent brains under two conditions of LPS treatment revealed 195 responsive genes, among which, 38 mRNAs were changed in both groups. These results suggest that lncRNAs play critical roles in regulating gene expression, neurogenesis, function and disease in the CNS, which is in accordance with previous reports [17-20].

Various lncRNAs are highly expressed in the nervous system, which modulate gene expression via diverse mechanisms, including genetic imprinting, mRNA decay, chromatin remodeling, gene network reprogramming, and translational regulation [16, 21]. lncRNA dysfunction is related to the onset and progress of nervous system disorders [16, 22]. The growing evidence evokes awareness of the importance of lncRNAs in the CNS. However, because these molecules are novel and poorly understood, their role in defense against infection is largely unexplored. The prevalence of high-throughput RNA-seq provides an opportunity to map the lncRNA transcriptome. Comprehensive transcriptomic analysis helps us to understand the gene regulatory mechanisms in the LPS stress response in rats.

Recently, the pathogenesis of neural dysfunction and intellectual disorder is increasingly focused on defects in the microtubulin system [23]. Microtubules are an

essential component of the cytoskeleton of almost all eukaryotic cells, and they are also responsible for the structural backbone of axons that participates in a diverse array of indispensable neural functions throughout life. They are enriched in the cell body and in the axonal initial and distal segments, such stable distribution of microtubules is vital to normal function of neurons [24]. If the process of microtubule production and polymerization in the CNS were changed, it would be reasonable to believe that there would also be morphogenetic changes in axons and many cellular processes, such as growth, division, polarity, migration, organelle positioning and cell-wall deposition [25-28]. Furthermore, these highly dynamic structures are intracellular transport trackers for signal transduction, organelle positioning and nutrient supplementation between cell bodies and processes [29, 30]. Their adaptive plasticity is strong, which can quickly respond to changes in the environment [24].

LPS is toxic to cultured eukaryotic cells and inhibites in vitro microtubule formation[31]. LPS targeted to intracellular microtubule architecture results in microtubule disassembly[32]. LPS can also bind to tubulin to cause microtubule fragmentation and shortening[33]. In the present study, after 6 or 24 h exposure to LPS, the comprehensive profiling suggested that protein-coding genes were frequently enriched in GO:007023 (post-chaperonin tubulin folding change). It is acknowledged that tubulins are the most highly conserved proteins across species [34]. The correct and complex folding of α - and β -tubulin is assisted by chaperonins, then tubulins are further processed to reach their final functional heterodimers, mediated by a set of five different tubulin binding cofactors, TBCA, TBCB, TBCC, TBCD

and TBCE [35, 36]. In the present study, the most preferentially affected tubulin gene, *Tbcc*, which encodes one of the post-chaperonins, was significantly altered after 6 or 24 h exposure to LPS ($p < 0.01$). *Tbcc* is present at the centrosome, which is the organization center during the early phase of microtubule formation [37]. It is also involved in mitosis, which points to its vital role in eukaryotic genomic stability [37]. *Tbcc* is associated with the assembly of polarized α - and β -tubulin heterodimers in a head-to-tail fashion [38]. The ability to polymerize microtubules is a key cytoskeletal process in axonal growth and maintenance [37, 39].

Based on BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>) analysis, our newly identified lncRNAs (NONRATT020317) in rodent brains shared many characteristics with *BC1*, which implied that both possess analogous function in rodents. Rat *BC1* is a highly conserved ncRNA exclusively expressed in the rodent nervous system [40, 41], and its counterpart *BC200* is expressed in human brains [42, 43]. Both of them are located in postsynaptic domains of neurons. Furthermore, *BC1* and *BC200* are expressed in the same subpopulation of neurons in rats and primates, respectively [42]. Thus, the two kinds of RNA are probably functional analogs. *BC1* is formed in neurons and transferred through microtubules to synapses. It controls microtubule construction through inhibiting protein synthesis in dendrites, to modulate signal transduction [44, 45]. The mislocalization and overexpression of *BC1* result in microtubular malformation, and microtubules are closely associated with synaptic plasticity [46]. It is reported that *BC1* knockout mice have lower survival rates than control mice, which is manifested as more anxiety and lower exploratory behavior

[47]. Dysfunction of primate-specific *BC200* impairs the delivery of RNA to the synapses, which is the main pathogenetic process of neurodegenerative diseases, such as Alzheimer's disease [42]. Similarly, NONRATT020317 expressed in adult rat brains is probably responsible for modulating synaptic plasticity.

We found that the functional pathway KEGG 04360: axon guidance was significantly changed, which provides further evidence that microtubules are affected by inflammatory sequelae. Axon guidance guides the growth and development of axons in immature brains, whereas in developed brains, it controls synaptic plasticity [48, 49]. The aforementioned results verify that, after the inflammatory response, CNS dysfunction is partly associated with microtubular dysfunction and axon guidance. However, the exact mechanism of involvement of *BC1* in SAE remains to be elucidated. Elucidation of unknown expression patterns would be useful to explore the specific potential for this lncRNA in clinical translation.

The results of RNA-seq and the predictions of bioinformatics are preliminary, so evidence for lncRNAs in CNS dysfunction in sepsis is still needed. Although detailed functional validation was beyond the scope of this study, accurate verification of novel lncRNAs warrants further investigation through biological experiments. Additionally, with the help of biotechnological developments, the function of more lncRNAs in different types of cell stress will be established. Further studies could be conducted to determine the complex noncoding transcriptional networks in SAE. There is an urgency to develop genetic model systems and identify their functions *in vivo*. Finally, future research should be based on the prioritized lncRNAs to examine

how the related noncoding transcriptional pathways intersect.

Conclusions

This study is believed to be the first identification of LPS-responsive lncRNAs and mRNAs in adult rodent brains, with the potential to discover an unknown lncRNA-based molecular mechanism in SAE. The predicted lncRNA-mediated effects on microtubules could represent a mechanism for neural damage by LPS. For the preliminary characterization of the bioinformatics tool, the molecular and functional contributions are still warranted for further study by the integrational neural model. Undoubtedly, these data may also provide a basis for the new biomarkers, such as lncRNAs/mRNAs, with the aim of achieving early diagnosis or attenuating LPS-induced CNS sequelae.

Abbreviations

CNS: Central nervous system; GO: Gene Ontology; ICU: Intensive care unit; KEGG: Kyoto Encyclopedia of Genes and Genomes; LPS: lipopolysaccharide; lncRNAs: Long non-coding RNAs; NT: nucleotides; RPKM: Reads per kilobase per million mapped reads; SAE: Sepsis-associated encephalopathy; SD: Sprague-Dawley.

Competing interests

The authors report no competing interests.

Author's contributions

Wenchong Sun was a major contributor in manuscript writing and data analysis. Ling Pei gave an important direction for study design. Zuodi Liang participated in animal care and RNA extraction. All authors read and approved the final manuscript.

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Table 1

Table 1 Statistics of mapped references of mRNA and lncRNA on valid data

	control		L6		L24	
Total unique gene						
mRNA	30493	100%	30493	100%	30493	100%
lncRNA	30832	100%	30832	100%	30832	100%
Mapped gene						
mRNA	24743	81.14%	25037	82.11%	25046	82.14%
lncRNA	25730	83.45%	25758	83.54%	25845	83.83%

Table 2

Table 2 Differentially expressed mRNAs after 6 h exposure

Accession	Gene Symbol	Locus	Regulation	log2fold change	P value	FDR
ENSRNOT00000047593	AABR07011951.1	chr1:131584306-131585731	up	1.04	4.13E-14	0.00
ENSRNOT00000033372	AABR07032261.1	chr2:177651241-177653288	up	1.48	7.66E-03	0.43
ENSRNOT00000089762	AABR07033324.1	chr2:220817080-220838905	up	1.04	1.05E-11	0.00
ENSRNOT00000071591	AABR07053533.1	chr14:46523589-46523999	up	1.01	4.59E-02	0.98
ENSRNOT00000072794	AABR07067037.1	chr14:46523594-46529375	up	1.43	1.96E-03	0.16
ENSRNOT00000082300	AABR07067760.1	chr14:46523658-46524476	up	1.53	8.80E-14	0.00
ENSRNOT00000044608	AABR07070032.1	chr14:46588406-46588816	up	1.03	2.63E-14	0.00
ENSRNOT00000084870	Abrac1	chr14:46649971-46650381	up	1.20	2.54E-02	0.94

ENSRNOT00000060436	AC121204.1	chr14:46649976-46650359	up	1.09	9.93E-04	0.10
ENSRNOT00000010056	Acer2	chr14:46653035-46653445	up	1.22	3.90E-02	0.98
ENSRNOT00000012478	Anp32b	chr18:57444533-57445045	up	1.70	1.27E-02	0.60
ENSRNOT00000057347	Arglu1	chr11:17336444-17340373	up	1.07	2.73E-02	0.98
ENSRNOT00000064068	Arpc5	chr3:112605768-112614503	up	1.10	1.29E-06	0.00
ENSRNOT00000079861	Arpc5	chr4:142453013-142453531	up	1.33	4.69E-05	0.01
ENSRNOT00000060006	Ccdc711	chr6:30638733-30639143	up	1.34	4.87E-02	0.98
ENSRNOT00000026249	Cfdp1	chr6:30638738-30643594	up	1.87	8.65E-04	0.09
ENSRNOT00000089873	Cfdp1	chr9:27343853-27345070	up	2.26	5.99E-03	0.36
ENSRNOT00000028892	Chgb	chr9:61692154-61694599	up	1.30	3.49E-14	0.00
ENSRNOT00000078599	Chi3l1	chr8:44990014-44993179	up	1.42	3.60E-04	0.05
ENSRNOT00000014997	Cmc2	chr1:13165572-13175876	up	1.12	2.96E-02	0.98
ENSRNOT00000016749	Cpne4	chr16:68986868-68987147	up	1.23	1.27E-05	0.00
ENSRNOT00000044346	Csnk1a1	chr5:58967418-58968209	up	1.07	2.28E-04	0.03
ENSRNOT00000078783	Csnk1a1	chr7:127081426-127081704	up	1.21	6.31E-04	0.07
ENSRNOT00000027727	Cst6	chr5:105230580-105279731	up	1.03	3.16E-04	0.04
ENSRNOT00000087560	Dio2	chr5:62071346-62094633	up	1.67	2.34E-02	0.90
ENSRNOT00000010926	Dpf3	chr16:86600877-86625083	up	1.50	2.34E-02	0.90
ENSRNOT00000047310	Epha6	chr13:70174970-70183874	up	1.05	1.26E-04	0.02
ENSRNOT00000009899	Epha7	chr13:70175032-70182646	up	1.29	2.25E-05	0.00
ENSRNOT00000018252	Gadd45g	chr8:120439994-120446507	up	1.65	4.98E-02	0.98
ENSRNOT00000087662	Gpd1	chr13:50103189-50149839	up	1.27	2.23E-05	0.00
ENSRNOT00000051483	Hba1	chr13:50091430-50153823	up	2.59	3.57E-03	0.25
ENSRNOT00000048977	Hba2	chr2:230901264-231132039	up	2.55	2.14E-03	0.17
ENSRNOT00000025559	Herpud1	chr6:51662224-51662874	up	1.02	2.96E-02	0.98
ENSRNOT00000015152	Hnrnpa2b1	chr19:43989596-44078320	up	1.20	2.85E-06	0.00

ENSRNOT00000011047	Hnrnpm	chr19:43971474-44078336	up	1.05	2.73E-02	0.98
ENSRNOT00000036172	Hnrnpm	chr3:125428260-125441594	up	1.01	1.92E-02	0.80
ENSRNOT00000009153	Hpca	chr13:51022681-51030802	up	1.67	6.75E-65	0.00
ENSRNOT00000025067	Hspa5	chr19:49433590-49448072	up	1.09	6.10E-14	0.00
ENSRNOT00000041306	Hspa8	chr6:72359791-72373695	up	1.04	9.65E-15	0.00
ENSRNOT00000032015	Ifi2712b	chr8:113105814-113586418	up	3.45	2.25E-02	0.88
ENSRNOT00000035017	Iqgap2	chr18:56887354-56917711	up	1.18	3.90E-02	0.98
ENSRNOT00000072294	LOC100910689	chr18:56887722-56910901	up	1.82	1.46E-02	0.66
ENSRNOT00000013159	LOC680121	chr1:220727292-220729000	up	1.04	1.74E-15	0.00
ENSRNOT00000006169	LOC682905	chr12:40018937-40219291	up	1.01	2.00E-02	0.82
ENSRNOT00000050716	Lrrc10b	chr6:114478293-114488880	up	1.60	2.11E-08	0.00
ENSRNOT00000020721	Lsm1	chr1:198284473-198288113	up	1.25	4.87E-02	0.98
ENSRNOT00000020100	Mas1	chr6:106721349-106971250	up	1.08	2.96E-02	0.98
ENSRNOT00000004223	Mip	chr13:39434217-39643361	up	1.01	7.68E-50	0.00
ENSRNOT00000080421	Morf411	chr16:64798766-64806044	up	1.78	9.93E-15	0.00
ENSRNOT00000008566	Mtmr2	chr11:42259761-42843865	up	1.32	3.63E-03	0.25
ENSRNOT00000017479	Nkain3	chr19:11046909-11057254	up	1.48	3.75E-02	0.98
ENSRNOT00000014639	Nmb	chr5:172364421-172365188	up	5.20	2.25E-02	0.88
ENSRNOT00000065288	Nrip3	chr4:81237496-81241282	up	1.35	2.22E-16	0.00
ENSRNOT00000005706	Nts	chr7:18516512-18554227	up	1.92	3.50E-04	0.04
ENSRNOT00000019399	Paip2b	chr7:18516512-18554227	up	1.53	1.28E-04	0.02
ENSRNOT00000052202	Pitpnb	chr5:147294820-147303346	up	1.02	4.23E-02	0.98
ENSRNOT00000003567	Ptgs2	chr3:13838304-13842762	up	1.31	3.97E-02	0.98
ENSRNOT00000051368	Ptma	chr5:99031013-99033107	up	1.12	2.23E-13	0.00
ENSRNOT00000004475	Rasd1	chr6:127336305-127337791	up	1.73	1.54E-04	0.02
ENSRNOT00000016020	Rem2	chr1:213750192-213751405	up	1.90	4.40E-02	0.98

ENSRNOT00000030850	Rerg	chr9:80118029-80144789	up	1.48	4.21E-03	0.28
ENSRNOT00000075725	RGD1566359	chr15:37790141-37790286	up	1.07	6.21E-04	0.07
ENSRNOT00000021596	Rgs14	chr2:26167199-26438790	up	1.43	1.64E-03	0.14
ENSRNOT00000090536	Rgs14	chr16:6992171-7007051	up	1.40	1.64E-03	0.14
ENSRNOT00000092145	Rhou	chr16:6992062-7007287	up	1.34	1.68E-03	0.15
ENSRNOT00000028097	Rnaseh2c	chr6:42471066-42473738	up	1.51	2.00E-02	0.82
ENSRNOT00000033276	Rprml	chr3:120024876-120076788	up	1.61	7.02E-07	0.00
ENSRNOT00000068028	Rragd	chr3:120023917-120087136	up	1.02	1.16E-02	0.57
ENSRNOT00000042965	Rtn4	chr1:222183276-222189604	up	1.01	1.04E-06	0.00
ENSRNOT00000009132	Sema3c	chr1:11963836-11968939	up	1.39	1.68E-02	0.73
ENSRNOT00000014827	Smco4	chr10:78168715-78173610	up	2.16	4.98E-02	0.98
ENSRNOT00000000336	Smpd2	chr14:46588411-46593828	up	1.27	9.62E-03	0.50
ENSRNOT00000085027	Strip2	chr3:71437241-71450807	up	2.20	8.24E-06	0.00
ENSRNOT00000073079	Tbcc	chr11:13499164-13501263	up	1.50	7.66E-03	0.43
ENSRNOT00000001490	Tesc	chr18:86279179-86279680	up	1.13	6.08E-08	0.00
ENSRNOT00000036140	Tmed9	chr1:226500692-226501920	up	1.14	2.36E-04	0.03
ENSRNOT00000037489	Tmem14a	chr16:71046475-71057883	up	1.12	8.84E-03	0.48
ENSRNOT00000033285	Tmem54	chr1:48077033-48108216	up	1.69	1.17E-22	0.00
ENSRNOT00000016145	Trappc4	chr7:2635743-2642848	up	1.25	3.23E-02	0.98
ENSRNOT00000067618	Trim2	chr8:97474092-97494834	up	1.01	1.33E-05	0.00
ENSRNOT00000074096	Tuba8	chrMT:7919-8599	up	1.08	6.70E-03	0.39
ENSRNOT00000058116	Ufm1	chrMT:7758-7961	up	1.09	2.05E-02	0.83
ENSRNOT00000012755	Wipf3	chrMT:7006-7689	up	1.11	1.05E-03	0.11
ENSRNOT00000083039	Wnt2	chrMT:8599-9382	up	1.04	2.20E-02	0.87
ENSRNOT00000075481	AABR07004232.1	chrMT:14136-15278	down	-1.05	1.13E-02	0.56
ENSRNOT00000078914	AABR07013065.1	chr8:12284528-12333316	down	-1.04	2.22E-03	0.18

ENSRNOT00000088111	AABR07015055.1	chrMT:3904-4942	down	-1.24	8.04E-79	0.00
ENSRNOT00000078682	AABR07015057.1	chrMT:10160-11537	down	-1.26	3.75E-68	0.00
ENSRNOT00000079005	AABR07015057.1	chrMT:9870-10166	down	-2.45	0	0
ENSRNOT00000072398	AABR07015066.1	chr6:34553367-34555306	down	-1.24	8.04E-79	0.00
ENSRNOT00000085875	AABR07015078.1	chr1:228724900-228727160	down	-1.24	8.04E-79	0.00
ENSRNOT00000080984	AABR07015078.2	chr13:93560364-93561301	down	-1.27	9.27E-81	0.00
ENSRNOT00000079916	AABR07015080.2	chr10:70100661-70101169	down	-1.24	8.04E-79	0.00
ENSRNOT00000056573	AABR07061755.1	chrMT:2740-3694	down	-1.03	3.11E-02	0.98
ENSRNOT00000082885	AABR07063424.1	chr1:35526239-35527033	down	-1.24	8.04E-79	0.00
ENSRNOT00000087960	AABR07063425.3	chr2:210299509-210299770	down	-1.26	3.75E-68	0.00
ENSRNOT00000079622	AC113785.2	chr10:70412352-70417724	down	-1.29	2.71E-03	0.20
ENSRNOT00000055893	AC135400.1	chr12:24744579-24745035	down	-1.03	1.87E-02	0.79
ENSRNOT00000037199	Arpp21	chr11:26720424-26721540	down	-1.19	3.85E-03	0.26
ENSRNOT00000004078	Atp2b4	chr1:167672812-167673926	down	-1.54	1.65E-02	0.73
ENSRNOT00000046273	Atp2b4	chr17:78835332-78835594	down	-1.56	1.65E-02	0.73
ENSRNOT00000015564	Camk2d	chr6:7035966-7036226	down	-1.53	4.80E-02	0.98
ENSRNOT00000007365	Coch	chr9:12406343-12409575	down	-2.72	2.51E-02	0.94
ENSRNOT00000001697	Cux2	chr14:7500401-7501512	down	-1.82	3.91E-02	0.98
ENSRNOT00000027022	Doc2a	chr17:46322995-46324104	down	-1.18	4.41E-02	0.98
ENSRNOT00000003527	Dpp10	chr2:143404985-143406099	down	-1.24	4.41E-02	0.98
ENSRNOT00000015725	Dusp26	chr1:6278881-6279793	down	-1.36	1.37E-03	0.13
ENSRNOT00000022032	Fxyd6	chr5:34174411-34813116	down	-1.80	3.52E-03	0.25
ENSRNOT00000082205	Fxyd6	chr1:142721002-142724511	down	-1.76	8.20E-03	0.45
ENSRNOT00000082696	Garnl3	chr3:154043873-154046330	down	-1.44	4.15E-02	0.98
ENSRNOT00000018769	Hes5	chr3:154043873-154046330	down	-1.22	5.82E-04	0.07
ENSRNOT00000020216	Ifitm2	chr9:45901741-46081880	down	-1.60	3.18E-02	0.98

ENSRNOT00000023068	Igfbp2	chr1:174385424-174411141	down	-1.84	3.26E-03	0.24
ENSRNOT00000076392	Il17d	chr7:44111151-44121130	down	-2.21	2.51E-02	0.94
ENSRNOT00000023984	Itih3	chr10:83328410-83332851	down	-1.13	4.32E-04	0.05
ENSRNOT00000041216	Itih3	chr17:14607442-14627937	down	-1.12	5.89E-04	0.07
ENSRNOT00000033327	Kcnf1	chr4:115487313-115516296	down	-1.89	8.92E-03	0.48
ENSRNOT00000047158	Kcnip3	chr3:110442637-110485702	down	-1.24	1.18E-02	0.57
ENSRNOT00000078994	Kcnip3	chr3:92403582-92502089	down	-1.29	1.93E-03	0.16
ENSRNOT00000028704	Kcnk4	chr12:51280150-51341716	down	-1.71	3.91E-02	0.98
ENSRNOT00000074325	LOC100909555	chr3:2686123-2689084	down	-1.15	0.00E+00	0
ENSRNOT00000071572	LOC257642	chr13:67351087-67359335	down	-1.26	3.75E-68	0.00
ENSRNOT00000046112	LOC499823	chr13:89873243-89874008	down	-3.82	0	0
ENSRNOT00000046108	Mt-atp6	chr9:98313632-98364205	down	-1.44	0	0
ENSRNOT00000046201	Mt-atp8	chr10:46330368-46332172	down	-1.42	0	0
ENSRNOT00000043693	Mt-co2	chr15:33121273-33125752	down	-1.03	0	0
ENSRNOT00000049683	Mt-cox3	chr4:171069191-171176581	down	-1.11	0	0
ENSRNOT00000042098	Mt-cytb	chr17:9457719-9458288	down	-1.32	0	0
ENSRNOT00000040993	Mt-nd2	chr17:9777925-9792007	down	-1.59	5.91E-88	0.00
ENSRNOT00000042928	Mt-nd4	chr17:9777842-9791781	down	-1.34	1.47E-73	0.00
ENSRNOT00000044582	Mt-nd4l	chr13:88054817-88061108	down	-1.02	4.55E-20	0.00
ENSRNOT00000034166	Nnat	chr1:100299635-100343385	down	-1.38	1.82E-11	0.00
ENSRNOT00000072502	Nnat	chr3:162084315-162147393	down	-1.38	5.39E-10	0.00
ENSRNOT00000059571	Npas2	chr8:14060394-14079972	down	-1.60	4.80E-02	0.98
ENSRNOT00000007133	Nxph3	chr20:46211892-46215120	down	-2.26	2.43E-03	0.19
ENSRNOT00000020532	Ogn	chr4:57204944-57246675	down	-2.25	3.89E-02	0.98
ENSRNOT00000010471	Pak6	chr9:16405185-16406338	down	-1.22	3.74E-02	0.98
ENSRNOT00000064282	Pamr1	chr12:44141422-44174583	down	-1.31	3.74E-02	0.98

ENSRNOT00000020926	Ptgds	chr1:222179436-222182721	down	-1.33	2.05E-27	0.00
ENSRNOT00000027012	Ramp1	chr17:8400146-8429338	down	-1.30	5.99E-03	0.36
ENSRNOT00000003774	Rgs4	chr4:147156948-147163467	down	-1.52	2.30E-04	0.03
ENSRNOT00000017556	Satb1	chr17:9560761-9565249	down	-1.31	9.40E-03	0.49
ENSRNOT00000077417	Satb1	chr9:27333956-27351295	down	-1.36	3.57E-02	0.98
ENSRNOT00000000313	Scarf2	chr5:147288723-147296702	down	-2.66	3.89E-02	0.98
ENSRNOT00000026100	Shank1	chr1:28455051-28576553	down	-1.05	1.37E-02	0.64
ENSRNOT00000044257	Shank1	chr1:28454966-28576261	down	-1.04	2.53E-02	0.94
ENSRNOT00000025843	Slc13a3	chr8:48723755-48726963	down	-1.97	4.09E-02	0.98
ENSRNOT00000078008	Tex40	chr2:183149174-183213228	down	-2.36	3.89E-02	0.98
ENSRNOT00000016390	Tgfb1	chr3:166743544-166746597	down	-2.30	9.72E-03	0.50
ENSRNOT00000010748	Timp4	chr4:153774486-153791328	down	-1.03	4.39E-02	0.98
ENSRNOT00000030327	Tpd5211	chr17:77252100-77261731	down	-2.41	4.09E-02	0.98
ENSRNOT00000078841	Tpd5211	chr2:143096271-143104412	down	-2.23	4.09E-02	0.98
ENSRNOT00000073788	Tshz2	chr11:3119885-3164751	down	-2.37	2.01E-06	0.00
ENSRNOT00000066850	Ucma	chr4:84597323-84676775	down	-Inf	5.00E-02	0.98
ENSRNOT00000042316	Vgll3	chr4:45292437-45332420	down	-4.21	1.64E-02	0.72

Table 3

Table 3 Differentially expressed mRNAs after 24 h exposure

Accession	Gene Symbol	Locus	Regulation	log2fold change	P value	FDR
ENSRNOT00000085321	AABR07046866.1	chr2:41467064-41467252	up	1.71	3.06E-02	0.99
ENSRNOT00000072794	AABR07067037.1	chr14:46523658-46524476	up	1.22	1.22E-02	0.99
ENSRNOT00000082300	AABR07067760.1	chr5:10125070-10125252	up	1.51	8.38E-13	0.00

ENSRNOT00000060436	AC121204.1	chr4:142453013-142453531	up	1.30	9.53E-05	0.03
ENSRNOT00000012478	Anp32b	chr9:27343853-27345070	up	1.94	3.85E-03	0.56
ENSRNOT00000022943	Calb2	chr9:61692154-61694599	up	1.45	2.55E-02	0.99
ENSRNOT00000023869	Cartpt	chr5:58967418-58968209	up	2.38	1.97E-02	0.99
ENSRNOT00000026249	Cfdp1	chr5:62071346-62094633	up	1.81	1.77E-03	0.33
ENSRNOT00000089873	Cfdp1	chr4:168752133-168755023	up	2.23	6.99E-03	0.76
ENSRNOT00000078783	Csnk1a1	chr8:120439994-120446507	up	1.14	1.88E-03	0.35
ENSRNOT00000012455	Fuca1	chr19:41482728-41509658	up	1.04	2.26E-02	0.99
ENSRNOT00000058362	Gjc2	chr2:30125249-30127269	up	1.18	2.14E-02	0.99
ENSRNOT00000015152	Hnrnpa2b1	chr19:43989596-44078320	up	1.23	2.99E-06	0.00
ENSRNOT00000015325	Hnrnpa2b1	chr19:43971474-44078336	up	1.02	8.85E-05	0.03
ENSRNOT00000009153	Hpca	chr6:72359791-72373695	up	1.14	9.06E-26	0.00
ENSRNOT00000032015	Ifi27l2b	chr19:55929609-55946250	up	4.45	1.46E-03	0.28
ENSRNOT00000045874	Igh-1a	chr19:55929664-55946250	up	Inf	3.97E-06	0.00
ENSRNOT00000031991	Igkc	chr18:56887722-56910901	up	4.59	4.41E-03	0.59
ENSRNOT00000042165	Igkc	chr10:16970626-16973418	up	4.41	1.46E-03	0.28
ENSRNOT00000074175	Igkc	chr5:154269118-154286544	up	4.43	2.11E-03	0.38
ENSRNOT00000086911	Igkc	chr8:49676540-49703419	up	4.59	2.53E-03	0.43
ENSRNOT00000042900	LOC100361105	chr8:49676616-49703410	up	Inf	2.53E-03	0.43
ENSRNOT00000035257	LOC680217	chr10:45526745-45534570	up	1.09	4.61E-02	0.99
ENSRNOT00000006169	LOC682905	chr4:81237496-81241282	up	1.19	6.77E-03	0.75
ENSRNOT00000059159	LOC685203	chr4:81237496-81241152	up	1.23	1.46E-02	0.99
ENSRNOT00000050716	Lrrc10b	chr5:147294820-147303346	up	1.38	6.75E-06	0.00
ENSRNOT00000025279	Mobp	chr6:127336305-127337791	up	1.18	6.02E-17	0.00
ENSRNOT00000025343	Mobp	chr6:138092131-138093643	up	1.02	6.22E-10	0.00
ENSRNOT00000080421	Morf4l1	chr4:98370797-98523273	up	1.87	9.67E-16	0.00

ENSRNOT00000000201	Mpst	chr4:98337367-98523473	up	1.28	2.59E-02	0.99
ENSRNOT00000008566	Mtmr2	chr4:98457810-98523473	up	1.28	6.99E-03	0.76
ENSRNOT00000005706	Nts	chr9:53440272-53440595	up	2.27	1.26E-05	0.00
ENSRNOT00000019399	Paip2b	chr10:85573369-85574889	up	1.40	9.17E-04	0.19
ENSRNOT00000066892	Pitpnb	chr1:226500692-226501920	up	1.06	3.38E-02	0.99
ENSRNOT00000025245	Plekfb1	chr2:164628566-164634434	up	1.04	1.82E-06	0.00
ENSRNOT00000082697	Plekfb1	chr8:128829680-128842739	up	1.04	8.87E-07	0.00
ENSRNOT00000025093	Ptma	chr8:128824508-128850358	up	1.10	4.78E-11	0.00
ENSRNOT00000051368	Ptma	chr8:97474092-97494834	up	1.23	4.36E-15	0.00
ENSRNOT00000004475	Rasd1	chr7:119626637-119631425	up	1.41	3.88E-03	0.56
ENSRNOT00000003177	RGD1310587	chr8:12284528-12333316	up	1.59	3.63E-02	0.99
ENSRNOT00000066715	RGD1560608	chr15:4130808-4132467	up	Inf	4.41E-03	0.59
ENSRNOT00000021596	Rgs14	chr13:93560364-93561301	up	1.09	2.94E-02	0.99
ENSRNOT00000090536	Rgs14	chr10:70100661-70101169	up	1.05	2.94E-02	0.99
ENSRNOT00000092145	Rhou	chr2:121129199-121130675	up	1.18	9.92E-03	0.92
ENSRNOT00000033276	Rprml	chr17:46322995-46324104	up	1.26	3.93E-04	0.10
ENSRNOT00000015958	S100a4	chr10:17048251-17075139	up	1.27	3.81E-02	0.99
ENSRNOT00000023350	Sord	chr3:154043873-154046330	up	1.23	3.81E-02	0.99
ENSRNOT00000085027	Strip2	chr7:44111151-44121130	up	1.88	4.78E-04	0.11
ENSRNOT00000073079	Tbcc	chr4:115487313-115516296	up	1.66	2.72E-03	0.44
ENSRNOT00000036140	Tmed9	chr12:51281987-51341663	up	1.03	1.42E-03	0.28
ENSRNOT00000033285	Tmem54	chr1:165665911-165680176	up	1.10	7.32E-09	0.00
ENSRNOT00000022863	Tppp3	chr1:165665909-165680206	up	1.03	1.98E-02	0.99
ENSRNOT00000022113	Ttr	chr9:93545396-93549436	up	1.57	8.46E-03	0.85
ENSRNOT00000074081	U2af1	chr13:89873243-89874008	up	1.10	5.36E-03	0.67
ENSRNOT00000058116	Ufm1	chr10:46330368-46332172	up	1.12	2.53E-02	0.99

ENSRNOT00000012755	Wipf3	chr13:102780877-102790639	up	1.26	2.76E-04	0.07
ENSRNOT00000019860	Zic1	chr6:135856218-135861059	up	2.09	1.07E-02	0.97
ENSRNOT00000073231	AABR07008066.2	chr17:9777925-9792007	down	-1.84	3.67E-02	0.99
ENSRNOT00000079005	AABR07015057.1	chr17:9777842-9791781	down	-1.84	0	0
ENSRNOT00000056573	AABR07061755.1	chr13:88054817-88061108	down	-1.06	2.48E-02	0.99
ENSRNOT00000010289	Apold1	chr19:56435577-56441733	down	-1.75	3.46E-02	0.99
ENSRNOT00000037199	Arpp21	chr10:91710495-91711499	down	-1.01	6.99E-03	0.76
ENSRNOT00000007365	Coch	chr2:189997129-189999604	down	-2.17	2.22E-02	0.99
ENSRNOT00000068231	Cpne7	chr11:87722350-87733734	down	-1.01	1.72E-02	0.99
ENSRNOT00000077701	Cpne7	chr3:114176309-114207366	down	-1.04	6.10E-03	0.72
ENSRNOT00000005383	Dusp1	chr4:57204944-57246675	down	-1.66	5.96E-03	0.71
ENSRNOT00000022032	Fxyd6	chr12:24682041-24710019	down	-1.46	1.17E-02	0.99
ENSRNOT00000082205	Fxyd6	chr9:16405185-16406338	down	-1.38	2.56E-02	0.99
ENSRNOT00000046112	LOC499823	chr17:9560761-9565249	down	-2.79	0	0
ENSRNOT00000072274	LOC691153	chr5:147288723-147296702	down	-2.03	3.46E-02	0.99
ENSRNOT00000018402	Lxn	chr19:37424324-37427989	down	-1.02	5.56E-03	0.68
ENSRNOT00000039398	Neurl1b	chr3:166743544-166746597	down	-1.38	3.02E-02	0.99
ENSRNOT00000034166	Nnat	chr18:15532963-15540177	down	-1.02	5.30E-08	0.00
ENSRNOT00000003774	Rgs4	chr20:10396621-10407554	down	-1.00	5.56E-03	0.68
ENSRNOT00000000313	Scarf2	chr17:77252100-77261731	down	-2.37	3.55E-02	0.99
ENSRNOT00000049601	Stx1a	chr2:143096271-143104412	down	-1.01	1.21E-03	0.25
ENSRNOT00000073788	Tshz2	chr4:84597323-84676775	down	-1.11	3.72E-03	0.55
ENSRNOT00000066850	Ucma	chr8:98734295-98738446	down	-5.02	4.69E-02	0.99

Table 4

Table 4 Common differentially expressed mRNAs after 6 h and 24 h exposure

mRNA	Log2 fold change		P value		Locus		Regulation
	L6/C	L24/C	L6/C	L24/C	L6/C	L24/C	
AABR07067037.1	-1.26	-1.84	3.75E-68	0.00	chr14:46523594-46529375	chr14:46523658-46524476	Up-regulation
AABR07067760.1	-2.45	1.71	0.00	3.06E-02	chr14:46523658-46524476	chr5:10125070-10125252	Up-regulation
AC121204.1	-1.27	-1.06	9.27E-81	2.48E-02	chr14:46649976-46650359	chr4:142453013-142453531	Up-regulation
Anp32b	1.48	1.22	7.66E-03	1.22E-02	chr18:57444533-57445045	chr9:27343853-27345070	Up-regulation
Cfdp1	-1.26	1.94	3.75E-68	3.85E-03	chr6:30638738-30643594	chr5:62071346-62094633	Up-regulation
Csnk1a1	1.09	-1.01	9.93E-04	6.99E-03	chr5:58967418-58968209	chr8:120439994-120446507	Up-regulation
Hnrnpa2b1	1.87	1.81	8.65E-04	1.77E-03	chr19:43989596-44078320	chr19:43989596-44078320	Up-regulation
Hpca	1.42	-2.17	3.60E-04	2.22E-02	chr13:51022681-51030802	chr6:72359791-72373695	Up-regulation
Ifi271l2b	1.23	-1.01	1.27E-05	1.72E-02	chr8:113105814-113586418	chr19:55929609-55946250	Up-regulation
LOC682905	-1.82	1.23	3.91E-02	2.99E-06	chr12:40018937-40219291	chr4:81237496-81241282	Up-regulation
Lrrc10b	1.67	1.14	2.34E-02	9.06E-26	chr6:114478293-114488880	chr5:147294820-147303346	Up-regulation
Morf4l1	-1.36	4.59	1.37E-03	4.41E-03	chr16:64798766-64806044	chr4:98370797-98523273	Up-regulation
Mtmr2	1.05	4.43	1.26E-04	2.11E-03	chr11:42259761-42843865	chr4:98457810-98523473	Up-regulation
Nts	1.05	1.23	2.73E-02	1.46E-02	chr7:18516512-18554227	chr9:53440272-53440595	Up-regulation
Paip2b	1.01	-2.03	1.92E-02	3.46E-02	chr7:18516512-18554227	chr10:85573369-85574889	Up-regulation
Pitpnb	1.67	1.38	6.75E-65	6.75E-06	chr5:147294820-147303346	chr1:226500692-226501920	Up-regulation
Ptma	1.04	1.02	9.65E-15	6.22E-10	chr5:99031013-99033107	chr8:128824508-128850358	Up-regulation
Rasd1	3.45	1.28	2.25E-02	2.59E-02	chr6:127336305-127337791	chr7:119626637-119631425	Up-regulation
Rgs14	1.18	2.02	3.90E-02	7.07E-13	chr2:26167199-26438790	chr13:93560364-93561301	Up-regulation
Rprml	-1.24	1.18	1.18E-02	2.53E-02	chr3:120024876-120076788	chr17:46322995-46324104	Up-regulation
Strip2	-3.82	2.27	0.00	1.26E-05	chr3:71437241-71450807	chr7:441111151-44121130	Up-regulation
Tbcc	1.04	1.40	1.74E-15	9.17E-04	chr11:13499164-13501263	chr4:115487313-115516296	Up-regulation
Tmed9	1.60	1.06	2.11E-08	3.38E-02	chr1:226500692-226501920	chr12:51281987-51341663	Up-regulation

Tmem54	1.08	1.04	2.96E-02	1.82E-06	chr1:48077033-48108216	chr1:165665911-165680176	Up-regulation
Ufm1	-1.42	1.41	0.00	3.88E-03	chrMT:7758-7961	chr10:46330368-46332172	Up-regulation
AABR07015057.1	-1.34	1.05	1.47E-73	2.94E-02	chrMT:10160-11537	chr17:9777842-9791781	Down-regulation
AABR07061755.1	-1.45	-1.00	2.59E-66	5.56E-03	chrMT:2740-3694	chr13:88054817-88061108	Down-regulation
Arpp21	-1.00	1.26	2.62E-02	3.93E-04	chr11:26720424-26721540	chr10:91710495-91711499	Down-regulation
Coch	-1.84	1.27	5.78E-03	3.81E-02	chr9:12406343-12409575	chr2:189997129-189999604	Down-regulation
Fxyd6	1.48	1.66	3.75E-02	2.72E-03	chr5:34174411-34813116	chr9:16405185-16406338	Down-regulation
LOC499823	1.12	1.03	2.23E-13	1.42E-03	chr13:89873243-89874008	chr17:9560761-9565249	Down-regulation
Nnat	-1.97	1.57	4.09E-02	8.46E-03	chr3:162084315-162147393	chr18:15532963-15540177	Down-regulation
Rgs4	-1.03	1.10	4.39E-02	5.36E-03	chr4:147156948-147163467	chr20:10396621-10407554	Down-regulation
Scarf2	1.69	-5.02	1.17E-22	4.69E-02	chr5:147288723-147296702	chr17:77252100-77261731	Down-regulation
Tshz2	-4.21	1.26	1.64E-02	2.76E-04	chr11:3119885-3164751	chr4:84597323-84676775	Down-regulation
Ucma	1.11	2.09	1.05E-03	1.07E-02	chr4:84597323-84676775	chr8:98734295-98738446	Down-regulation

Table 5

Table 5 Significantly changed down-regulated lncRNAs					
Transcript id	Log2fold change	P value	FDR	regulation	Transcript type
L6/C					
NONRATT000107	-3.26	4.46E-03	0.20	down	antisense
NONRATT000108	-2.96	1.43E-02	0.44	down	antisense
NONRATT000532	-1.13	4.61E-02	0.85	down	sense_overlapping
NONRATT001122	-1.79	4.33E-02	0.82	down	sense_overlapping
NONRATT001483	-2.13	1.45E-02	0.44	down	antisense

NONRATT002002	-1.24	2.56E-02	0.63	down	lincRNA
NONRATT002509	-1.23	1.29E-02	0.41	down	sense_overlapping
NONRATT002726	-Inf	3.77E-02	0.77	down	lincRNA
NONRATT003070	-1.06	5.16E-19	0.00	down	sense_overlapping
NONRATT003337	-1.32	2.56E-02	0.63	down	lincRNA
NONRATT004080	-1.54	3.73E-02	0.77	down	lincRNA
NONRATT004414	-2.29	4.62E-02	0.85	down	sense_intronic
NONRATT005352	-4.94	1.09E-02	0.37	down	sense_overlapping
NONRATT005957	-1.44	4.03E-02	0.80	down	sense_overlapping
NONRATT006395	-4.74	1.01E-03	0.07	down	lincRNA
NONRATT006508	-1.59	1.91E-02	0.52	down	lincRNA
NONRATT006743	-2.12	2.51E-02	0.62	down	lincRNA
NONRATT006758	-2.59	1.43E-02	0.44	down	sense_overlapping
NONRATT006823	-1.25	3.91E-02	0.79	down	sense_overlapping
NONRATT006926	-1.96	4.65E-04	0.04	down	sense_overlapping
NONRATT007622	-3.36	3.77E-02	0.77	down	antisense
NONRATT008210	-1.10	1.52E-02	0.45	down	lincRNA
NONRATT008590	-3.04	3.77E-02	0.77	down	sense_intronic
NONRATT009224	-2.24	4.33E-02	0.82	down	lincRNA
NONRATT009376	-1.35	0.00E+00	0	down	lincRNA
NONRATT009377	-3.48	0.00E+00	0	down	lincRNA
NONRATT009710	-2.94	4.62E-02	0.85	down	antisense
NONRATT010002	-1.36	3.22E-02	0.71	down	antisense
NONRATT010669	-1.01	2.84E-02	0.67	down	antisense
NONRATT011133	-1.19	4.97E-02	0.88	down	sense_intronic
NONRATT011680	-2.66	4.62E-02	0.85	down	sense_overlapping

NONRATT012653	-1.22	2.54E-05	0.00	down	sense_intronic
NONRATT012759	-2.17	4.27E-03	0.19	down	sense_overlapping
NONRATT013156	-1.81	4.33E-02	0.82	down	antisense
NONRATT015238	-1.01	4.37E-03	0.19	down	lincRNA
NONRATT015617	-1.06	1.92E-02	0.52	down	lincRNA
NONRATT015764	-1.47	1.91E-03	0.11	down	lincRNA
NONRATT015765	-1.25	2.56E-02	0.63	down	lincRNA
NONRATT015907	-1.15	3.91E-02	0.79	down	sense_overlapping
NONRATT017287	-2.21	4.62E-02	0.85	down	sense_overlapping
NONRATT017495	-1.46	2.55E-02	0.63	down	sense_overlapping
NONRATT018202	-1.02	2.23E-02	0.58	down	lincRNA
NONRATT018321	-3.49	0.00E+00	0	down	sense_overlapping
NONRATT018471	-1.02	3.61E-02	0.76	down	sense_overlapping
NONRATT018581	-1.09	1.28E-04	0.01	down	sense_overlapping
NONRATT018620	-2.91	2.56E-02	0.63	down	lincRNA
NONRATT018926	-1.70	1.07E-03	0.07	down	sense_overlapping
NONRATT019181	-2.84	1.43E-02	0.44	down	sense_overlapping
NONRATT019524	-1.15	2.64E-03	0.13	down	lincRNA
NONRATT019894	-1.05	1.22E-12	0.00	down	lincRNA
NONRATT019895	-1.15	8.08E-12	0.00	down	lincRNA
NONRATT019912	-1.48	3.11E-02	0.70	down	lincRNA
NONRATT020004	-1.39	3.22E-02	0.71	down	antisense
NONRATT020688	-1.52	1.07E-02	0.37	down	sense_overlapping
NONRATT020689	-1.53	6.53E-04	0.05	down	lincRNA
NONRATT021678	-2.94	3.77E-02	0.77	down	sense_overlapping
NONRATT022314	-3.84	2.01E-02	0.54	down	sense_overlapping

NONRATT022337	-3.48	0.00E+00	0	down	sense_intronic
NONRATT022363	-Inf	1.09E-02	0.37	down	lincRNA
NONRATT022382	-1.99	1.33E-02	0.42	down	sense_overlapping
NONRATT022384	-1.45	1.99E-03	0.11	down	lincRNA
NONRATT023434	-4.21	2.01E-02	0.54	down	sense_overlapping
NONRATT023949	-2.04	4.33E-02	0.82	down	lincRNA
NONRATT024620	-1.19	1.23E-03	0.08	down	lincRNA
NONRATT024621	-1.05	6.93E-03	0.27	down	lincRNA
NONRATT024624	-1.25	1.07E-03	0.07	down	lincRNA
NONRATT024627	-1.14	1.45E-03	0.08	down	lincRNA
NONRATT024629	-1.12	2.64E-03	0.13	down	lincRNA
NONRATT024635	-1.14	2.23E-02	0.58	down	lincRNA
NONRATT024933	-1.24	2.56E-02	0.63	down	sense_overlapping
NONRATT024934	-1.05	2.23E-02	0.58	down	antisense
NONRATT025697	-3.21	3.77E-02	0.77	down	antisense
NONRATT025936	-1.04	8.73E-03	0.31	down	sense_intronic
NONRATT026602	-1.11	4.61E-02	0.85	down	sense_overlapping
NONRATT027353	-3.49	3.77E-02	0.77	down	sense_overlapping
NONRATT027644	-1.51	1.74E-05	0.00	down	sense_overlapping
NONRATT028105	-3.84	2.01E-02	0.54	down	sense_overlapping
NONRATT029020	-4.43	2.01E-02	0.54	down	sense_intronic
NONRATT029046	-1.09	6.65E-05	0.01	down	antisense
NONRATT029047	-1.14	2.26E-03	0.12	down	antisense
NONRATT029125	-1.05	6.24E-03	0.25	down	lincRNA
NONRATT029127	-1.02	4.37E-03	0.19	down	lincRNA
NONRATT029758	-3.94	3.77E-02	0.77	down	lincRNA

NONRATT031254	-1.72	3.73E-02	0.77	down	sense_overlapping
L24/C					
NONRATT000533	-1.12	4.01E-02	0.98	down	sense_overlapping
NONRATT000963	-1.28	9.59E-04	0.11	down	lincRNA
NONRATT002754	-1.43	4.51E-02	0.98	down	lincRNA
NONRATT003494	-3.71	7.11E-03	0.46	down	sense_overlapping
NONRATT003737	-1.45	2.76E-02	0.98	down	sense_overlapping
NONRATT004080	-2.57	1.29E-02	0.66	down	lincRNA
NONRATT004138	-2.13	1.29E-02	0.66	down	sense_overlapping
NONRATT004410	-5.58	1.87E-02	0.83	down	sense_overlapping
NONRATT004414	-2.95	4.29E-02	0.98	down	sense_intronic
NONRATT004750	-1.35	3.57E-02	0.98	down	sense_overlapping
NONRATT005334	-2.09	4.00E-03	0.31	down	sense_overlapping
NONRATT005335	-1.03	3.55E-02	0.98	down	sense_overlapping
NONRATT005867	-1.12	2.51E-03	0.22	down	sense_intronic
NONRATT005952	-1.05	1.65E-02	0.76	down	antisense
NONRATT005954	-1.17	3.10E-02	0.98	down	sense_overlapping
NONRATT005957	-1.76	1.67E-02	0.77	down	sense_overlapping
NONRATT006395	-3.77	3.94E-03	0.31	down	lincRNA
NONRATT006743	-1.84	2.26E-02	0.90	down	lincRNA
NONRATT006758	-2.07	3.96E-02	0.98	down	sense_overlapping
NONRATT007528	-1.23	6.54E-10	0.00	down	sense_intronic
NONRATT007549	-1.81	2.26E-02	0.90	down	lincRNA
NONRATT007569	-1.09	4.61E-02	0.98	down	sense_overlapping
NONRATT007622	-3.39	3.55E-02	0.98	down	antisense
NONRATT007762	-1.41	4.51E-02	0.98	down	sense_overlapping

NONRATT008174	-1.08	7.67E-03	0.48	down	sense_intronic
NONRATT008234	-2.39	4.29E-02	0.98	down	antisense
NONRATT008642	-1.78	1.99E-02	0.86	down	lincRNA
NONRATT009350	-2.65	4.29E-02	0.98	down	sense_overlapping
NONRATT009377	-1.94	0.00E+00	0	down	lincRNA
NONRATT009510	-1.31	4.51E-02	0.98	down	sense_overlapping
NONRATT009710	-2.39	4.29E-02	0.98	down	antisense
NONRATT009762	-1.03	2.74E-02	0.98	down	sense_overlapping
NONRATT009800	-1.02	2.74E-02	0.98	down	sense_overlapping
NONRATT009823	-1.54	3.34E-04	0.05	down	lincRNA
NONRATT009835	-1.79	1.99E-02	0.86	down	lincRNA
NONRATT009895	-3.39	3.55E-02	0.98	down	sense_overlapping
NONRATT010410	-1.04	4.89E-04	0.07	down	sense_intronic
NONRATT011167	-1.37	4.51E-02	0.98	down	lincRNA
NONRATT011531	-1.94	4.29E-02	0.98	down	lincRNA
NONRATT011680	-2.96	4.29E-02	0.98	down	sense_overlapping
NONRATT011688	-1.68	3.36E-02	0.98	down	other
NONRATT012335	-3.34	7.11E-03	0.46	down	sense_overlapping
NONRATT012611	-3.48	1.87E-02	0.83	down	sense_overlapping
NONRATT012754	-1.01	3.70E-05	0.01	down	sense_overlapping
NONRATT012877	-Inf	3.55E-02	0.98	down	lincRNA
NONRATT013954	-1.23	1.36E-02	0.69	down	sense_overlapping
NONRATT014771	-1.53	4.51E-02	0.98	down	sense_intronic
NONRATT016456	-1.46	1.43E-04	0.03	down	sense_overlapping
NONRATT017349	-1.41	3.25E-12	0.00	down	sense_overlapping
NONRATT017357	-3.77	3.55E-02	0.98	down	sense_overlapping

NONRATT017495	-1.50	2.23E-02	0.90	down	sense_overlapping
NONRATT017739	-1.40	4.51E-02	0.98	down	lincRNA
NONRATT018201	-2.29	3.96E-02	0.98	down	lincRNA
NONRATT018321	-2.67	0.00E+00	0	down	sense_overlapping
NONRATT018831	-1.96	2.26E-02	0.90	down	lincRNA
NONRATT019200	-1.72	4.45E-04	0.07	down	lincRNA
NONRATT019524	-1.04	3.32E-03	0.28	down	lincRNA
NONRATT020009	-2.98	3.55E-02	0.98	down	sense_overlapping
NONRATT020047	-2.07	4.29E-02	0.98	down	sense_overlapping
NONRATT021154	-1.94	9.92E-04	0.11	down	sense_intronic
NONRATT021330	-4.07	3.55E-02	0.98	down	sense_overlapping
NONRATT022337	-1.94	0.00E+00	0	down	sense_intronic
NONRATT022476	-1.04	1.45E-02	0.71	down	sense_overlapping
NONRATT022934	-1.23	7.01E-03	0.46	down	lincRNA
NONRATT023115	-1.27	2.21E-02	0.89	down	sense_overlapping
NONRATT024196	-1.15	1.07E-02	0.60	down	lincRNA
NONRATT025659	-1.01	4.61E-02	0.98	down	lincRNA
NONRATT025697	-3.24	3.55E-02	0.98	down	antisense
NONRATT027511	-1.24	1.36E-02	0.69	down	sense_overlapping
NONRATT027644	-1.22	1.30E-04	0.03	down	sense_overlapping
NONRATT027777	-1.77	3.96E-02	0.98	down	lincRNA
NONRATT029125	-1.33	5.21E-04	0.07	down	lincRNA
NONRATT029288	-1.03	4.61E-02	0.98	down	sense_overlapping
NONRATT029334	-1.65	3.36E-02	0.98	down	sense_overlapping
NONRATT029335	-1.55	2.76E-02	0.98	down	sense_overlapping
NONRATT029952	-1.18	1.06E-03	0.11	down	lincRNA

NONRATT030187	-1.44	4.51E-02	0.98	down	sense_overlapping
NONRATT031090	-1.27	3.41E-02	0.98	down	sense_overlapping
NONRATT031134	-2.18	4.29E-02	0.98	down	lincRNA

Figure legends

Fig 1: Flowchart of RNA-seq workflow. Schematic representation of analytical procedures for biomarkers of SAE and healthy controls using Hiseq2500 sequencing technology.

Fig 2: Expression of mRNA and lncRNA in each sample. (A) Distribution of mRNA expression calculated in $\log_{10}$ RPKM among three different groups. (B) Distribution of lncRNA expression calculated in $\log_{10}$ RPKM among three different groups. (C) Boxplots (showing the 15th, 25th, 50th, 75th, and 95th percentiles) show the characteristics of mRNA and lncRNA expression.

Fig 3: Analysis of differentially expressed mRNAs. (A) Volcano figure showing overall distribution of differentially expressed mRNA. Red indicates the significantly differentially expressed genes (change >1.5-fold and $p < 0.05$) and blue the non-significant ones. (B) Scatter plots give a direct picture of the tendency of data flow, where red indicates the significantly down-regulated genes green the significantly up-regulated genes, and blue plots the non-significant genes. (C) Pie charts illustrate the significantly differentially expressed genes when compared between the L6/L24 and control groups. (D) 141 up-regulated genes (green) and 92 down-regulated genes (red) after different duration of exposure to 5 mg/kg LPS ($p < 0.05$). (E) Hierarchical cluster analysis of mRNA expression in response to 6 h exposure to 5 mg/kg LPS compared to control groups. (F) Hierarchical cluster analysis of mRNA expression in response to 24 h exposure to 5 mg/kg LPS compared to control groups ($p < 0.05$). The clustering tree is listed on the left. Each row represents an individual gene, while colors correspond to different expression variance: green represents lower level, red means higher level, and black means median value (log scale 2, from -2.0 to +2.5).

Fig 4: GO and KEGG analysis. (A) Significantly enriched GOs of up-regulated mRNAs in L6/C group. (B) Significantly enriched GOs of down-regulated mRNAs in L6/C group. (C) Significantly up-regulated GOs of up-regulated mRNAs in L24/C group. (D) Significantly down-regulated GOs of down-regulated mRNAs in L24/C group. Results in A–D were all analyzed from the following three aspects of GO analysis: biological process, cellular component, and molecular function. (E) Significantly enriched pathways of up-regulated mRNAs in L6/C group. (F) Significantly enriched pathways of down-regulated mRNAs in L6/C group. (G) Significantly up-regulated pathways of up-regulated mRNAs in L24/C group.

Fig 5: Analysis of differentially expressed lncRNAs. (A), Volcano showing the overall distribution of differentially expressed lncRNAs. Red indicates the significantly differentially expressed lncRNAs (change >1.5-fold and $p < 0.05$) and blue the non-significant ones. (B) Scatter plots give a direct picture of the tendency of data flow, where red indicates the significantly down-regulated lncRNAs, green the significantly up-regulated lncRNAs, and blue means median value. (C) 433 up-regulated lncRNAs (green) and 163 down-regulated lncRNAs (red) after different duration of exposure to 5 mg/kg LPS. (D) Cluster analysis of lncRNA expression in response to 6 h exposure to 5 mg/kg LPS compared to control groups. (E) Cluster analysis of lncRNA expression in response to 24 h exposure to 5 mg/kg LPS compared to control groups ($p < 0.05$). The clustering tree is listed on the left. Each row represents an individual gene, while colors corresponds to different expression variance: green means lower level, red means higher level, and black means the median value (log scale 2, from -2.0 to +2.5).

Fig 6: Detailed characteristics of significantly altered lncRNAs induced by LPS under different conditions in rodent brains. (A) Exon number of the transcripts of lncRNAs and mRNAs. (B) Length distribution of lncRNAs and mRNAs. (C) and (D) represent the length distribution of ORFs of lncRNAs and mRNAs. The x axis shows the length of the ORF (expressed by number of amino acids) and the y axis shows the number of ORFs in

the lncRNA or mRNA sequence. (E) and (F) represent the regulation mode of expression of lncRNAs and mRNAs.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6