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By

Bright A. Test

A Thesis Submitted to the

Graduate School

In Partial Fulfillment of the

Requirements for the Degree of

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2020

By

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By

Bright A. Test

DEDICATION

This thesis is dedicated to my loving family. Thank you for your unconditional love and support. I will forever be especially grateful to you, mama and papa.

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This thesis would not have been possible without the mentorship and support from the Vierra lab. Everyone contributed to a healthy and positive environment that allowed me to work at my best. Not a stale day went by as I chugged away to complete my thesis.

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Abstract

By Bright A. Test

University of the Pacific 2020

The fungus, Hericium erinaceus, has outstanding chemical properties, displaying health benefits in digestive, hepatic, and nervous tissues. Its ease of accessibility and use makes it one of the most common substances used for treatment in Eastern medicine. More and more recent research is confirming the incredible health benefits of this fungus, especially the impact that is seen on nervous tissue growth and recovery post-treatment. Such neurite outgrowth and myelin sheath regeneration could illustrate the beginning of the cure to lifelong neurodegenerative diseases such as Multiple Sclerosis. In this first-of-its-kind study, we cultured and differentiated fetal rat neural stem cells while treating the samples with varying concentrations of aqueous extract of Hericium erinaceus mycelium. The cells were then harvested and lysed at various time points as the proteins were isolated and purified prior to analysis by LC-ESI mass spectrometry. A proteomic analysis was conducted where statistically significant changes in protein expression were observed between the control groups and the treated trials of both time points. While our initial targets of interest were not found, an up to 4-fold increase in protein expression was seen in a group of Histone H1 variants following treatment with *Hericium* erinaceus. These Histone H1 variants are known to be linker histones which interact with the core histone bead and play a role in chromatin remodeling. It is clear that *Hericium erinaceus*

plays a role in increasing the protein expression of Histone H1 variants which could lead to downstream effects yet to be revealed. This exploratory research should serve as a helpful launching point for those determined to understand the underlying mechanisms behind this phenomenon and the results it may have on the nervous system.

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LIST OF ABBREVIATIONS

cDNA	complementary DNA
CNS	Central Nervous System
DDT	dithiothreitol
DNA	deoxyribonucleic acid
IAA	indole-3-acetic acid
LC-ESI	liquid chromatography - electrospray ionization
μg	microgram
μL	microliter
MBP	myelin basic protein
mg	milligram
mL	milliliter
mm	millimeter
MS	Multiple Sclerosis
MS/MS	tandem mass spectrometry
ng	nanogram
NGF	nerve growth factor
NSC	neural stem cells
PNS	Peripheral Nervous System
RNA	ribonucleic acid
RT-qPCR	reverse transcription quantitative polymerase chain reaction

CHAPTER 1: INTRODUCTION

The nervous system is arguably the most complex biological system in our body. There are many different types of cells and molecules that play vital roles in maintaining the health and wellbeing of the system. In vertebrates, the nervous system is divided into two categories: the central nervous system (CNS) and peripheral nervous system (PNS). The CNS is composed of the brain and spinal cord, whereas the PNS consists of the peripheral nerves and ganglia that stem from the brain and spinal cord. There are two different cell categories in the nervous system, which include neurons and neuroglia (glia). Neurons are the nerve cells that send electrical signals, known as action potentials, throughout the human body which control muscle cell contraction and relaxation. Glial cells are supporting cells that play auxiliary roles and keep the overall environment healthy and proper for the neurons. Neurons can also be supported by neuropeptides known as neurotrophic factors. There are many neurotrophic factors that are involved in the regulation of growth, maintenance, proliferation, and survival of nerve cells, but the most common one that supports the growth of certain target neurons is known as nerve growth factor (NGF). NGF plays a crucial role in a lot of nervous tissue development and is a subject of interest in many studies. After many years of research, various protein markers have been visualized and have been used to determine the developmental state of nervous tissue. During development, all nervous tissue begins as neural stem cells which then differentiate into a variety of different neuronal cell types. Nestin is the most widely known of these protein markers and is mostly only found in undifferentiated neural stem cells. Other markers include neuron-specific enolase and fibrillary acidic protein, which is commonly observed inside glia. These markers are typically visualized during the quantitative analysis stages of research¹⁹.

While there are multiple kinds of neurons and glial cells, oligodendrocytes are the largest glial cells and play an irreplaceable role. Oligodendrocytes are glial cells in the central nervous system that generate myelin, an important sheath that is responsible for enveloping neurons and ensuring proper action potential propagation. They are aligned in rows between the nerve fibers of white matter and are close to the somata of neurons in gray matter. The cytoplasmic processes of oligodendrocytes typically extend to multiple axons at once and wrap themselves around the length of the axon until multiple lamellas are formed. These cells and the myelin they generate are presumed to be majorly affected in patients suffering from Multiple Sclerosis (MS).

Overview of Multiple Sclerosis

There are many neurological diseases in today's world that are currently not curable, one of which is Multiple Sclerosis. MS is a demyelinating disorder in which the immune cells invade the CNS to remove myelin debris, but overdo it and cause neuron and oligodendrocyte death which leads to physical, mental, and psychiatric impairment problems¹. White matter infiltration by our nervous system's immune cells is the focal point of the pathology of MS¹⁷. The immune cells most responsible for the inflammatory processes that occur during the onset and progression of MS are CD4-positive T lymphocytes; however, monocytes, macrophages, neutrophils, and B lymphocytes are also involved. While it was previously thought that the human brain does not change much after the first stages of development, it is now common to come across research showing the brain demonstrating structural and functional plasticity throughout the course of human life. This brain plasticity is affected when these infiltrating immune cells secrete various factors that control and regulate neuronal function and signal formation in neuronal synapses¹⁸. While Multiple Sclerosis is not an inherently deadly disease, patients suffering from MS typically have a reduced life expectancy. Initially, non-durable

remyelination occurs which typically leads to neurological function recovery in the short-term. As time progresses, however, the pathological changes become dominated by widespread microglial activation associated with extensive and chronic neurodegeneration, the clinical correlate being progressive accumulation of disability¹⁵. Paraclinical investigations show abnormalities that indicate the distribution of inflammatory lesions and axonal loss, interference of conduction in previously myelinated pathways, and intrathecal synthesis of oligoclonal antibody¹⁵. Cellular and secretory activity of infiltrating leukocytes contributes to the creation of these inflammatory demyelinated lesions in the white matter of the brain. The gray matter of patients with MS is also affected, leading to motor, sensory, visual, and cognitive impairment with the ability to memorize and learn being severely impacted¹⁸. MS is a chronic condition that cycles between relapses and remissions. The remission periods can last up to years, but symptoms flare up again eventually. Multiple Sclerosis is sometimes more specifically referred to as Relapsing-remitting MS because of this.

The current immunotherapies inhibit further demyelination, but do not act to enhance remyelination¹. Licensed disease-modifying agents reduce the frequency of new episodes but do not reverse fixed deficits and have questionable effects on the long-term accumulation of disability and disease progression¹⁵. These treatments are also very expensive. The cost-effectiveness of a few disease-modifying drugs from a US societal perspective was analyzed and the results illustrated that dimethyl fumarate was the most preferred therapy to manage relapsing-remitting multiple sclerosis³. In 2014, the average annual medication cost of dimethyl fumarate was about \$47,718¹⁶. Between 2011 and 2015, the annual disease-modifying therapy (DMT) cost per MS patient increased from \$26,772 to \$43,606, a 13.0% average annual growth rate¹⁶. When comparing DMT users to non-DMT users, the annual health care cost per DMT user was

74% higher in 2011 (\$50,352 vs \$28,881), increasing to more than double in 2015 (\$70,683 vs \$29,821¹⁶. In the United States, the prevalence of MS has been presumed to be approximately 100/100,000 people². Baldassari et al. describes in great length the therapeutic strategies being developed to promote myelin repair²². Almost all cells in the nervous system are potential targets as drug manufacturers try to modulate cellular activity and environment to promote myelination and to inhibit demyelination. There have been several laboratories that identified compounds which promote endogenous oligodendrocyte progenitor cell (OPC) function. Mesenchymal stem cell transplantation, high-dose biotin treatment, and protein pathway blocking are treatment methods that have been tested and are currently being explored some more due to the difficulty of successful implementation and monitoring in vivo. Other drugs such as dopamine antagonists, atypical antipsychotics, thyroid hormone inducers, and testosterone stimulators are also currently being investigated in clinical trials for remyelination potential in MS. Baldassari's group also describes multiple methods of screening and assessing myelin integrity: positron emission tomography, magnetization transfer imaging, myelin water imaging, and diffusion tensor imaging. There are also indirect ways of measuring myelin integrity: neurite orientation dispersion and density imaging, functional MRI, and magnetic resonance spectroscopy. These forms of measurement will become more accurate as the field focuses more on repairing myelin instead of simply trying to halt demyelination, but as of right now, the major difficulties involve lack of accurate biomarkers and lack of specificity when tracking affected myelin. It is important to fully understand the mechanism of action of remyelinating agents and their long-term safety and reliability before applying such advances to clinical care²².

Chemical Properties and Effects of Hericium erinaceus

There may be a cheaper and more effective alternative on the horizon hiding inside a fungus. *Hericium erinaceus* (HE), also known as Lion's Mane, is a medicinal mushroom that contains neurotrophic and neuroprotective properties and has been widely consumed in Asian countries such as China and Japan⁴. The first account of this mushroom being consumed dates back to 264 A.D. on Taiwan Island where the natives ate *houtougeng* (translated to monkey head thick soup) and considered the soup to be beneficial in neutralizing the ill-effects of alcoholic beverages⁵. This mushroom is widely found in Asia, Europe, and northern temperate latitudes where beech and oak trees grow⁶. Even with modern technology and transportation methods, there is an unfortunate lack of *H. erinaceus* consumption and utilization in North America. The American species, *H. americanum* is also not commonly consumed.

As any fungus, HE is composed of mycelium and fruiting bodies. The powder of this crushed mushroom must be boiled in hot water to successfully extract the active compounds responsible for the health benefits experienced when consumed. These active compounds come in many forms including hericenones, erinacines, and polysaccharides, to name a few. Hericenones are a group of aromatic compounds that have previously been found in the fruiting bodies of HE. There were multiple aromatic compounds in this category that were isolated and purified by multiple researchers and the studies also revealed some anticancer properties tied to these compounds²¹. Hericinones, however, failed to stimulate NGF gene expression in primary cultured rat astroglial cells and 1231N1 human astrocytoma cells²³. Li et al. describes erinacines as groups of cyathin diterpenoids that show biological activities as stimulators of NGF synthesis²⁴. To date, 15 erinacines (erinacines A-K and P-S) have been identified and further investigations have demonstrated that eight of them have various neuroprotective properties,

such as enhancing NGF release (erinacines A-I), reducing amyloid-β deposition, increasing insulin-degrading enzyme (IDE) expression (erinacines A and S), or managing neuropathic pain (erinacine E), while others are either being currently discovered or have different pharmacological activities²⁴. Li's group concludes by stating that erinacine A is effective in reducing neurodegenerative disease-induced cell death, but because there have been no studies illustrating erinacine A's crossing of the blood-brain barrier, it is hard to say how effective this compound will prove to be when consumed orally. Beneficial polysaccharides have also been discovered and analyzed. A polysaccharide EP-1 isolated from HE mycelia culture demonstrated antioxidant activity and prevented oxidative stress induced by H₂O₂ through mitochondrial dependent apoptotic pathways in gastric mucosa epithelial cells²⁰. A heteropolysaccharide (HEP-S) was isolated from the fruiting bodies of *Hericium erinaceus* and was observed to function as an immunostimulator to stimulate both the innate and adaptive immune responses in mouse cells²⁵.

H. erinaceus has been reported to illustrate incredible health benefits for the body's digestive, immune, circulatory, and nervous systems. Helicobacter pylori is a bacteria found in the stomach and is the main pathogenic factor of chronic gastritis, peptic ulcers, and adenocarcinoma of the distal stomach⁷, but its growth can be inhibited by using ethanol extracts and ethyl acetate fractions of HE⁸. The exopolymer produced in submerged mycelial culture of HE has been shown to significantly reduce the plasma triglyceride, total cholesterol, low-density lipoprotein cholesterol, phospholipid, and liver total cholesterol level in rats, implying that it has hypolipidemic effects^{9,10}. There is a plethora of benefits to the nervous system such as, but not limited to, peroneal nerve recovery after crush injury¹¹, coordination of neuron functions associated with complex neurodegenerative diseases⁶, and enhanced myelination in mature

myelinating fibers¹². Another study has also found that HE can activate the synthesis of nerve growth factor $(NGF)^{13}$. The stimulation of this neurotrophic factor can be beneficial in increasing the neurite outgrowth in a nervous system trying to combat a neurodegenerative disease. Most importantly and according to a study cited by many, aqueous extract of this mushroom has been shown to improve and expedite the process of myelination in nerve cells¹⁴. This commonly cited study by Kolotushkina et. al. examined the effects of applying *H. erinaceus* extract to cultures of newborn WISTAR rat cerebellums. When this group added the extract to their cells, they noticed no drastic changes in the development of nerve and glial cells, but the number of lamellae in the myelin sheaths did increase at a faster rate during development than untreated groups¹⁴. This activity, in theory, can directly counter the demyelination of nerves as seen in patients with Multiple Sclerosis. There are not many studies that cover this subject which make it difficult to find other studies that concur with the findings of this one. To our knowledge, there were no studies completed beforehand analyzing the proteomic contents of nerve cells in vitro being treated with Hericium erinaceus. Proteome analysis of the mushroom itself was conducted recently where mass spectrometry was used to identify a total of 2543 unique proteins in the *H. erinaceus* genome²⁶. We can use the information from this study to help in our proteomic analysis, but the change in protein levels of rat fetal neural cells after treatment of *H. erinaceus* remains to be analyzed for the first time. Therefore, analyzing the proteomic contents of these neural cells treated with this mushroom can lead to uncovering more details about the proteins involved in the aforementioned myelinating process.

Proteomics

Proteomics is the large-scale study of proteins and requires the use of a variety of techniques stemming from fields such as molecular biology, biochemistry, and genetics. One vital technique, known as mass spectrometry, is utilized when analyzing a large amount of proteins at once. After isolating, purifying, and breaking down the proteins of interest into smaller peptides, the sample is fed into a mass spectrometer where the now-accessible peptides are charged and turned into precursor ions through techniques like Electrospray ionization (ESI). Mass spectrometry is used to measure the mass-to-charge ratio of these ions that are pulled through the mass spectrometer machine by an oppositely charged current. These are measured by a mass analyzer to generate what is known as a MS1 spectrum. As the ions are analyzed, certain ones are pulled through the machine where they collide with inert gas and fragment into charged amino acids through a process called Higher-energy Collisional Dissociation (HCD). These fragments are analyzed a second time by either a different mass analyzer or the same one from before, generating a MS2 spectrum which we can further investigate using specific computer software. This information allows investigators to see exactly what peptides were recognized by the mass spectrometry, making it possible to match discovered peptides to large databases in an effort to map back to proteins of interest. The parameters for which precursor ions are sent to further dissociate are set on the machine's software before the run begins. The machine we used for our studies is known as the Thermo Scientific[™] Orbitrap Fusion[™] TribridTM mass spectrometer. This machine's mode of function and interior is further explained in a thorough paper published by Hebert and colleagues²⁷.

Purpose and Goal

This study aimed to analyze the proteome of neural stem cells after *H. erinaceus* extract treatment. The primary objective was to explore whether treatment of neuronal stem cells with *H. erinaceus* extract resulted in changes in gene expression profiles for proteins involved in the myelination synthesis pathway. In our studies, we demonstrated that treatment of rat neuronal stem cells with *H. erinaceus* extract did not upregulate the expression of myelin basic protein or myelin expression factor 2, two known markers associated with myelin biosynthesis. However, they did reveal increased expression of histone 1 variants, suggesting treatment of neuronal stem cells with *H. erinaceus* extract leads to alteration in chromatin remodeling and gene silencing, which may suggest associations with controlling the neuronal differentiation program.

CHAPTER 2: MATERIALS AND METHODS

Neural Stem Cell Differentiation and Sample Preparation

Rat Fetal Neural Stem Cells (Invitrogen[™] N744-100) were expanded using T75 flasks. The T75 flasks were coated with a matrix consisting of CELLStartTM (GibcoTM A10142001) diluted 1:100 in D-PBS with calcium and magnesium. 560 mL of this matrix was made in total. 14 mL of this matrix was used to coat each flask which were then incubated at 37°C in a humidified atmosphere of 5% CO₂ in air for 1 hour before being stored until use. Two different solutions were made: one for expanding the cells and one for differentiating the cells. Cells were passaged using StemPro® NSC SFM complete medium consisted of KnockOutTM D-MEM/F-12 with StemPro® NSC SFM Supplement, EGF, bFGF, and GlutaMAXTM-I (all from GibcoTM and ThermoFisher). A mixture of penicillin and streptomycin was added to prevent bacterial and fungal contamination. See Table 1 for the breakdown of concentrations. The same medium lacking EGF and bFGF was used to differentiate the cells at P2. The volume remained unchanged after removing these small amounts of growth factor. To begin with, 4 of the previously matrix-primed T75 flasks were coated with cells and 20 mL of complete medium in each. Each flask contained about 5×10^5 cells at this point. After letting the cells grow for 24 hours, the complete media was siphoned and replaced with fresh complete media to minimize the accumulation of cellular debris. The cells expanded for a total of 3 days in Passage 0. Photos were taken every day for three days using light microscopy (Leica DMI3000 B). After three days, two of the four T75 flasks were further expanded while the other two flasks were frozen and stored in liquid nitrogen. Cells were passaged according to the manufacturer's instructions, except for the centrifugation step where $400 \times g$ was used instead of $300 \times g$ (MAN0001642).

The cells were then distributed evenly and plated into 8 T75 flasks to begin P1. The same procedure was followed and progress was recorded every day by light microscopy. The P1 cells were allowed to approach confluency (2 days of growth) and these cells were split (P2). P2 cells were split into smaller 100mm \times 20mm plates and these cells were used as separate trials during the analysis. 6 mL of complete medium was used per plate which contained about 0.8 mL of cells. Cells were expanded for 3 days while progress was recorded. Three plates were harvested prior to the addition of the differentiation media. These cells belonged to an undifferentiated neural stem cell group of trials. The rest of the plates underwent an exchange of media where the complete medium was exchanged for one lacking EGF and bFGF (other concentrations remained the same as these small amounts did not affect volume significantly). Different versions of the incomplete medium were made containing different concentrations of dissolved *H. erinaceus* mycelia (supplied by Real Mushrooms) to be used for the cultivation of the trials. The mushroom powder was weighed out and dissolved in the media lacking the growth factors. The amounts used and concentrations generated are listed in Table 2. All proportions remained the same. Every remaining plate was coated with 10 mL of the differentiation medium. The remaining plates were grouped into different trials with different timepoints. Three replicates of the negative control, low concentration, medium concentration, and high concentration groups were harvested after 3 days of differentiation. At this time, the media was exchanged for media containing the mushroom extract. 5 mL was siphoned off to prevent the cells from being exposed to air and 10 mL of fresh differentiation media was added. The remaining three replicates of each group were harvested after 7 days of differentiation. Light microscopy was used to record the progress of cell differentiation. All cells were harvested and washed using the same procedure per plate. 7 mL of media was transferred to a conical tube using a sterile pipette and cells were washed with 5 mL of D-PBS lacking Ca^{2+} and Mg^{2+} . The D-PBS was aspirated off and 1 mL of accutase was added to each plate. Cells were rinsed with 4 mL of matching media which was then transferred to the same conical tube. Cells were centrifuged at 400 × g for 4 minutes and the supernatant was discarded. The pellet was resuspended with 1 mL of cold D-PBS and spun again. This step was repeated once more and the resulting supernatant was removed. The cell pellet was frozen at -80°C. All cell subculturing protocols were carried out aseptically under a Laminar flow hood.

Table 1					
Component Distribution for	100 mL S	<i>StemPro</i> ®	NSC SFM	Complete .	Medium

Component	Concentration	Amount
KnockOut TM D-MEM/F-12	1X	97 mL
GlutaMAX TM -I Supplement	2 mM	1 mL
bFGF	20 ng/mL	2 µg
EGF	20 ng/mL	2 µg
StemPro® NSC SFM Supplement	2%	2 mL
Penicillin + Streptomycin	0.5%	0.5 mL

Note. Table adapted from InvitrogenTM manual MAN0001642.

H. erinaceus Trial Name	Concentration	Amount
Low	0.1 mg/mL	12.5 mg
Medium	0.25 mg/mL	31.25 mg
High	0.5 mg/mL	62.5 mg

Table 2Concentrations of H. erinaceus in 125 mL StemPro® NSC SFM Incomplete Medium

Peptide Purification

In-solution tryptic digestion was performed to prepare samples for mass spectrometer analysis. A total of 200 μ L of 6 M urea/100 mM Tris buffer (pH 7.8) was used to lyse the cells. Sonication was also conducted as an additional measure to ensure complete cell lysis. To help denature proteins, 5 μ L of reducing reagent, 200 mM DTT, was added and the sample was mixed by gentle vortex. After letting sit for 10 minutes in room temperature, 20 μ L of alkylating reagent, 200 mM IAA, was mixed into the tube and vortexed. After another 10 minutes, 20 μ L of 200 mM DTT was mixed in and allowed to sit for 10 minutes at room temperature. 775 μ L of sterile MilliQ water was added to each sample to dilute the urea concentration and create an environment where trypsin can retain its activity. Initially, 3 μ L of Trypsin Gold Solution (0.5 mg/mL) was added and the digestion was carried out overnight at 37°C. The next day, another 1.5 μ L of Trypsin was added and allowed to digest for another 2 hours. The reaction was then stopped by adding 5.125 μ L of 10% TFA.

PierceTM C18 Spin Columns (Thermo ScientificTM No. 89873) were used to purify the samples. The columns were activated with 50% ACN, equilibrated with 5% ACN + 0.5% TFA solution, and used according to the manufacturer's instructions. About 1 mL of sample was bound, washed, and eluted. 20 μ L of elution buffer was run through the columns twice to

guarantee a thorough product. This resulted in a 40 μ L solution that would be stored and later used for analysis.

Colorimetric Peptide Assay and Normalization

Eighteen samples were analyzed by use of the Pierce Quantitative Colorimetric Peptide Assay (Thermo Scientific[™] No. 23275). Prior to this analysis, these samples were lyophilized and then resuspended with 15 μ L of 5% ACN + HPLC grade water + 0.1% Formic Acid solution. All reagents were made as per the manufacturer's instructions and the protocol was followed stringently. 2 μ L of each sample was mixed with 18 μ L of autoclaved water before being mixed with the 180 μ L of working reagent. After running the software analysis, some discrepancies were visible between the biological replicates. In order to maximize the amount of peptide binding to the mass spectrometer analytical column, the biological replicates with the higher peptide concentration were selected. These samples were normalized with HPLC grade water + 0.1% Formic Acid to match the limiting concentration of one of the samples (the control for Day 3). The concentrations and samples selected are illustrated in Table 3. After randomizing the order in which they would be processed, the samples were inserted into the mass spectrometer to begin the proteomic analysis. Every sample was injected twice at a volume of 25 µL per injection, creating technical replicates. The second injections were treated as separate samples when it came to randomizing the order.

Sample	Initial Concentration (µg/mL)	Concentration Needed (µg/mL)	Volume of Sample (μL)	Volume of Water Mixture (μL)
3C2	174.495	1.74495	10	40
7L1	200.901	1.74495	8.68	41.32
7H2	213.293	1.74495	8.18	41.82
3M2	236.433	1.74495	7.38	42.62
NSC2	250.005	1.74495	6.98	43.02
7M1	262.165	1.74495	6.65	43.35
3L2	324.988	1.74495	5.37	44.63
7C1	365.978	1.74495	4.77	45.23
3H1	438.012	1.74495	3.98	46.02
HeLa	155.022	-	20	80

Table 3Observed Initial Concentrations of Chosen Samples and Post-Normalization Values

Note. Samples are notated by the format of [Trial Day][Trial Group][Replicate Number]. For example, 3C2 is Day 3 Control no. 2. NSC2 refers to the undifferentiated Nerve Stem Cells sample used. HeLa cells were included to check for consistency during the Mass Spectrometry run every 24 hours. Water mixture consists of HPLC grade water + 0.1% Formic Acid. All samples except for the HeLa cells were injected twice (25 μ L per injection). The HeLa cells were injected four times with the same amount of protein per injection.

Proteomic Analysis

Peptides were eluted from the C18 column and subject to electrospray ionization and the samples were analyzed using our Thermo ScientificTM Orbitrap FusionTM TribridTM mass spectrometer. Individual samples were subject to approximately a 2 hr chromatographic run using a previously established X-calibur program. The entire running time on the mass spectrometer was about 72 hours. A sample of HeLa cells was injected every 24 hours to confirm spray stability of the needle during the run. The total volume of HeLa cells was divided into four injections for a total of 25 μ L per injection. Once completed, chromatography graphs,

MS and MS/MS data were generated. Data were further analyzed through quantitative analytical software, such as Peaks Studio and Protein Scaffold 4.

RT-qPCR Analysis

In order to complete reverse transcription and quantitative polymerase chain reaction analysis (RT-qPCR), total RNA was isolated, washed, and solubilized. A standard Trizol RNA isolation procedure was done according to Invitrogen's manual (Pub.No. MAN0001271 B.0). All samples were lysed with Trizol containing 60 μ L of 100% chloroform and centrifuged to generate separate phases within tubes. Each sample's aqueous phase was transferred to a new tube and precipitated using 150 μ L of 100% isopropanol along with centrifugation at 4°C. The gel-like pellets that resulted were then washed three times 3 with 300 μ L of 70% ethanol. After vacuum-drying the pellets for about 20 minutes, the pellets were resuspended in 20 μ L of RNase-free water. The RNA yield for each sample was determined by use of the ThermoScientificTM NanoDrop 2000c Spectrophotometer and the appropriate absorbance values were recorded.

The purified RNA was then prepared for RT-qPCR analysis by mixing with the proper reagents from the PowerUpTM SYBRTM Green Master Mix (Applied BiosystemsTM). The RNA was first diluted to a concentration of 12.5 ng/µL. Then, the following mixture was produced: 4 µL of 12.5 ng/µL RNA; 10 µL of 2x SYBR green dye; 0.2 µL QN SYBR green Reverse Transcriptase mix; 1 µL of 20x primer mix; 4.8 µL RNase free water. Samples were analyzed for each of the five primers and technical duplicates were also generated. Forward and reverse primers were mixed (see Table 4) and checked for their ability to amplify their specific amplicon beforehand using 2% agarose gel electrophoresis. A 96-well plate was used to load 18 µL of each mixture and was then processed by the continuous fluorescence detector.

Forward 5' to 3' Forward MT (°C) Reverse MT (°C) Histone Name Reverse 5' to 3' H1.1 gaagcctgcgaaagct gaaactgcaggcttctt 64 66 gctgt gggc H1.2 gtcggaaactgctcctg ggcttggcctcgccag 68 70 ctgc aagct 64 H1.3 atgcggctgttgttcttc 68 ttgaaacatgtctgaaac agetee tcc ccgccttcttgttgagtt 68 62 H1.4 aagaagaaggcccgc aaggcc tga H1.5 aagaagaagacaaaaa cttagccttgggcttgg 66 68 aagctggc cttc

Table 4Primer Sequences for Histone H1 Family

Note. Melting Temperatures (MT) for both forward and reverse primers are shown. Primers are shown as 5' to 3'.

CHAPTER 3: RESULTS

Neural Stem Cell Differentiation

Light microscopy was used to examine the process of growth and differentiation of the nerve stem cells. Photos were taken every day in greyscale and monitored for healthy progression. The Day 3 trial groups were compared to the Day 3 control and undifferentiated stem cells at Passage 2 (Figure 1). A visible difference between the morphology of the stem cells and the morphology of the differentiated cells was seen (Figure 1). The differentiated cells in both the control group and the trials seemed to successfully form neural connections typically seen in nervous tissue. The morphology of the cells from the trials did not seem to significantly deviate from the control group, illustrating that the addition of the *H. erinaceus* extract did not cause any significant changes to the physical appearance of the cells.



Figure 1. Comparison of morphology between undifferentiated stem cells and Day 3 Control and treatment groups using light microscopy. The yellow measurement bar is set to 100 μ m for reference. The treated groups and undifferentiated stem cells are portrayed in 200x magnification while the Control group is portrayed in 100x magnification.

The same could not be said about the Day 7 group. In comparison to the Day 3 group, the Day 7 group illustrated more solid groupings of nerve cells and glia with more distinct crater-like low-density spaces in between the formations (Figure 2) illustrating a progression in maturation. A uniquely dense cluster of cells, similar to controls, was found when analyzing the Low Concentration trial for Day 7 as seen in Figure 2. When comparing the Day 7 trials, the ones that were treated with the medium and high concentrations of *H. erinaceus* exhibited what seemed to be less specific condensed grouping of cells which resulted in less crater-like low-density space compared to the low concentration and control trials, and which appeared to mimic the cells at Day 3 of treatment as opposed to untreated controls and low-dose treatments.



Figure 2. Comparison of morphology between undifferentiated stem cells and Day 7 control and treatment groups using light microscopy. The yellow measurement bar is set to 100 μ m for reference. The treated groups and Control are portrayed in 100x magnification while the undifferentiated stem cells are portrayed in 200x magnification.

Proteomic Analysis and Mass Spectrometry

Prior to use for proteomic analysis, the grown cells from all samples were washed with D-PBS lacking Ca²⁺ and Mg²⁺ and detached at their respective time points with acutase, followed by cell lysis in urea. Following crude lysate collection and tryptic digestion, samples were purified using C18 spin columns and checked for peptide concentration using Colorimetric Peptide Assay. The initial concentrations were recorded (Table 3). After normalizing the concentrations to the baseline concentration of sample 3C2, samples were inserted into the mass spectrometer and analyzed over a 4 day period. Each individual sample was analyzed for about 2 hours. Chromatographic graphs were generated to illustrate the relative abundance of peptides eluting off of the column at every point in time. Similarities between retention time and relative abundance are best illustrated between the control and trial groups of the same day (Figure 3).

There are certain notable differences in the graphs, but for the most part, there is not much variation to be seen in the overall shape. This implies that the samples were handled and processed properly with little error. For the Day 3 group, there was a peak missing in 3C at roughly the 22.70 minute retention time that was visible in all the other trials in varying abundances (Figure 3). The Day 7 control group seemed to differ from the Day 7 treatment groups, most notably in the end of the chromatographic run around 91-94 minutes. There were a couple of peaks showing more abundance in the treated groups compared to the control, signifying that there may be proteins that are uniquely expressed and representative peptides are eluted off the column at that point that are not found in the control group. It may also be the case that those same peptides might have simply been eluted at a different point in a spread out way. This is unlikely, however, since by looking at the overall curve of the 7C graph, it is hard to spot where these peaks could have shifted to. Overall, these figures confirm our assertion that the samples analyzed display similar quantities of peptides, allowing us to carry on with further analysis using additional software.



Figure 3. Raw chromatography files from the Thermo ScientificTM Orbitrap FusionTM TribridTM mass spectrometer after completing the sample processing. These graphs compare the relative abundance of peptide (y-axis) to the retention time (x-axis). A: Day 3 Group color-coded and labeled. B: Day 7 Group color-coded and labeled. Note the difference in order from top to bottom between A and B.

Quantitative Proteomic Analysis

Mass spectrometry data were analyzed using Protein Scaffold (Proteome Software, Inc.[™]), a software algorithm designed for quantitative proteomics. Within this software algorithm, MS/MS spectra were tallied up and normalized in preparation for quantitative comparison and analysis. Protein sequences, statistical correlation, and probability percentages were illustrated among many other useful metrics. Prior to optimization, a total of 365,537 spectra were obtained from the combined 8 sets of data (Day 3 Control, Day 7 Control, Day 3 Low, Day 3 Medium, Day 3 High, Day 7 Low, Day 7 Medium, Day 7 High). The protein threshold value, minimum number of peptides value, and peptide threshold value were modified. As the software conducts the internal statistical analysis, it checks for the probability of a protein or peptide actually existing in the sample according to the registered and recognized spectra. The protein and peptide thresholds are in reference to these probabilities. For our analysis, protein and peptide thresholds were set to 95%, indicating that Scaffold would only display proteins and peptides that the algorithm calculated of having at least a 95% probability of being present in the samples. The minimum number of unique peptides used for protein identification was set to 2, which is commonly accepted by the proteomic community to confirm the presence of proteins. These parameters narrowed the results and allowed for a more stringent and accurate observation of proteins that were most likely to be "real". These parameters also minimized the false discovery rate and dramatically lowered the chances of encountering false positives in the data. After setting such parameters, there were a total of 648 unique proteins and 21,602 spectra found when comparing the Day 3 Control to the Day 3 Trial groups. A statistical two-tailed T-test was conducted and found that 61 of these proteins were statistically significant with a p-value of less than 0.05. Of these 61 proteins, 26 were expressed in higher quantities, up to a 4-fold difference,
in the Day 3 Control compared to the trials. The rest of the 35 proteins were expressed in lower quantities, up to a 4-fold difference, in the Day 3 Control compared to the trials (Appendix A). The same statistical analysis was conducted for the Day 7 groups where a total of 1042 unique proteins and 37,541 spectra were found following analysis using the same parameters. After finding 193 statistically significant proteins, 64 of them were expressed in higher amounts, up to a 4-fold difference, in the Day 7 Control compared to the trials. The other 129 proteins were expressed in lower quantities, up to a 4-fold difference, in the Day 7 Control compared to the trials (Appendix B). A final statistical analysis, ANOVA (p-value < 0.05), was conducted to illustrate significant variance between all three groups of trials (Controls vs Day 3 trials vs Day 7 trials). A total of 129 unique proteins across all samples were found to be statistically significant according to this analysis with respect to changes in protein expression (Appendix C). Of these proteins, 36 were unanimously expressed in higher amounts, up to a 4-fold difference, in the Day 3 and Day 7 trials when compared to the Control groups. On the other hand, 17 proteins were expressed in lower amounts, up to a 4-fold difference, in the Day 3 and Day 7 trials when compared to the Control groups. These proteins were researched one-by-one to find any correlation to previous studies about H. erinaceus or Multiple Sclerosis. While there were no statistically significant proteins found that have a direct role in either of these subjects, there was a profound change seen in the expression of certain histones between the control groups and the trial groups. There were multiple notable histories such as H2A, H1.1, H1.2, H1.3, H1.4, and H1.5. Most of these histories were variants of the H1 family. Differences in expression are visualized in Figure 4 where there was at least a 4-fold difference in normalized spectral counts between the control and trial groups. The largest differences are visualized in Figures 4B, 4C, and 4D. Simultaneously, the undifferentiated cells were compared to the controls to confirm that differentiation was successful on a genotypic level. Nestin, a protein expressed at high levels in neural stem cells and a widely employed marker²⁸, was shown to be expressed less in the differentiated controls as illustrated in Figure 5.



B



(Figure 4 Continued)



(Figure 4 Continued)



Figure 4. Normalized total spectra vs biosample. These graphs were exported as raw data from Protein Scaffold. The raw number of Total Spectra is not as important as the comparison in values and changes between the groups of samples. All histones significantly increased in expression when treated with *H. erinaceus*, regardless of elapsed days. A: histone H2A Type 1-C. B: histone H1.4. C: histone cluster 1 H1 family member d (histone H1.3). D: histone H1.5. E: histone H1.1. F: histone cluster 1 H1 family member c (histone H1.2).



Figure 5. Normalized total spectra of nestin in controls vs nerve stem cells. The lower amount of spectral counts in the controls implies that less of the marker protein is expressed in these samples. Based on this evidence, differentiation did successfully occur.

The Protein Scaffold software also generated scatterplots after the ANOVA test was conducted (Figure 6). The control group (Day 3 Control and Day 7 Control) was compared to the Day 3 trials separately from the Day 7 trials. Both graphs illustrated a similar pattern when comparing averaged normalized total spectra. This demonstrated that there was little variation in the trials due to the different time points. As a result, however; there was a clear significant difference shown between the controls and the trials.



Figure 6. ANOVA test results of histones in controls vs trials. The highlighted green box in both A and B refer to histone H1.4 simply for reference. The numbers on both axes represent Total Normalized Spectra as an average in those groups. The green boxes represent a protein that significantly shows more normalized spectra in a trial group than a control group and vice-versa. In this case, the six green boxes refer to the histones in Figure 4.

The amino acid sequences of these proteins were also examined to see if there were any substantial differences in the order of amino acids that could further explain the specific function of these histone variants. As illustrated in Figure 7, all of the six examined histone variants were rich in both arginine and lysine with minor variation in overall charge. This was to be expected, as histones must use an overall positive charge to tightly bind to DNA when condensing it into chromatin.



Figure 7. Recognized peptide sequences matched with a proteome database and mapped back to the histones of interest. The amino acids highlighted in yellow are confirmed to match by available spectra data. All peptides from each protein were examined, but only the best-matched peptide from each protein was chosen to be compared.

Since we selected a neural cell line based on previous studies of myelin sheath regeneration being affected by *H. erinaceus*¹⁴, it was useful to look for any proteins that could correlate our results to these previous studies. In our analysis, only one myelin related protein was found in every sample. This protein, known as Myelin expression factor 2, did not show any significant variation (ANOVA) in expression between the control group, the Day 3 trials, and the Day 7 trials (p-value = 0.34). The control for day 3 showed a drastically different result from the control for day 7 whereas the other groups did not show much variance within their respective groups. Based on this data, we cannot make any solid conclusions, but it was interesting to see the decrease in normalized total spectra when going from the Day 3 group to the Day 7 group. If it were true that Myelin expression factor 2 was being expressed less as time went on, then expression levels of myelin basic protein (MBP) would increase. This is because Myelin

expression factor 2 is a transcriptional repressor for MBP. MBP is one of the main components of myelin sheath so seeing an increase in expression of this protein would illustrate an increase in total myelination occurring. The normalized total spectra data is illustrated in Figure 8.



Figure 8. Normalized total spectra of myelin expression factor 2 in the analyzed samples. This data was not deemed statistically significant by the ANOVA test, but was still worth looking at as it could present a lead for future studies.

Post-Translational Modifications

Post-translational modifications (PTMs) have always been a talking point when it comes to histones. Since many different studies have addressed specific PTMs, it was important to analyze the possible PTMs that were found in our samples to see if the affected charge and structure in the peptide sequence would affect histone binding and function. Using a different program now in the form of Peaks Studio X, we compared our samples using a similar algorithm. In this software; however, we were capable of visualizing the proper PTMs at certain sites within the amino acid sequences of each peptide. When comparing the controls to the trials, multiple amino acid sequences were found to be linked with the histone peptides. Some of these sequences had PTMs that would occur more often than others. For example, when the five Histone H1 variants were processed, four of them showed sequences that were acetylated at the first serine in the amino acid chain (Figure 9). This does not mean that all of these peptides were acetylated, but it does show that some were. Further comprehension of Figure 9 shows that there were other modifications such as carbamylation and ubiquitination. There was little PTM variation seen between the histones for the first 65 amino acids with Histone H1.1 and Histone H1.5 being the most different. De novo peptides were fully matched and the confident modification sites were shown with a minimal ion intensity of 5%. This means that a pair of major fragment ions (b and y ions) must be found showing fragmentation before and after the modified amino acid with at least the given minimum intensity of 5%.



Figure 9. Amino acid sequences of the five variants of histone H1 with possible PTMs labeled at certain sites. Not all copies of these peptides are modified, but there are some that were modified in the samples. A: Histone H1.1. B: Histone cluster 1 family member C. C: Histone cluster 1 family member D. D: Histone H1.4. E: Histone H1.5. Little variation is illustrated between B, C, and D for the first 65 amino acids. Histone H1.1 and Histone H1.5 peptides were capable of being modified a bit differently.

RT-qPCR Analysis

Before the RT-qPCR analysis was conducted, the prepared RNA of every sample was measured for purity by calculating absorbance values through use of the NanoDrop[™] 2000c Spectrophotometer (Thermo Scientific[™]). These values are illustrated below in Table 5.

Sample	Concentration ($\mu g/\mu L$)	A260/A280	A260/A230
C3	1.9282	1.78	2.21
L3	1.2177	1.75	2.25
M3	0.8436	1.78	2.24
Н3	1.2680	1.86	2.08
C7	1.1582	1.80	2.23
L7	1.1262	1.81	2.20
M7	1.0629	1.81	2.09
H7	1.3195	1.84	2.01

Table 5*RNA Absorbance Values*

Since the samples were confirmed to be pure enough by this data, the RT-qPCR analysis carried on with the use of a continuous fluorescence detector. This machine generated curves that illustrated the relative fluorescence as the cycles carried on. Forty cycles were completed according to the standard PCR specifications listed in the user guide (MAN0013511). After the curves were generated, a cycle time threshold of 0.180 was set to compare how quickly the contents amplified between the different histone H1 variants. Most of the variants exhibited the same basic trend where the curves of the treated groups would cross the threshold sooner than the curves of the control groups for both time points. The (c)T values (cycle time values) were

typically lower for the treatment groups and a bit higher for the control groups, illustrating that generally there was more histone H1 variant RNA in the cells post-treatment with *H*.*erinaceus* as shown in Figure 10.





Histone H1.2 - Day 3 | Day 7



(Figure 10 Continued)



Histone H1.3 - Day 3 | Day 7

Histone H1.4 - Day 3 | Day 7



(Figure 10 Continued)



Histone H1.5 - Day 3 | Day 7

Figure 10. The difference in cycle time compared across the histone H1 variants. The equation is Δ Ct = Cycle time of Trial - Cycle time of Control. The day 3 trials were compared to the day 3 control and the day 7 trials were compared to the day 7 control. The threshold of 0.180 was crossed during the RT-qPCR analysis of each sample for every histone H1 variant. Both time points are illustrated in each graph. The more negative differences indicate more initial RNA in the sample for the depicted histone H1 variant as compared to the more positive differences which indicate less. Similar trends are visualized across all histone H1 variants analyzed.

CHAPTER 4: DISCUSSION

Neural Stem Cell Differentiation

Nervous tissue is notorious for being very delicate and although we performed every step meticulously, there was always a chance for contamination or inefficient growth. We kept this in mind as the results were processed. We initially wanted to treat the neural stem cells with larger concentrations of *H. erinaceus* extract to rival studies published previously; however, this proved to be difficult as the *H. erinaceus* extract powder failed to completely dissolve when added to the incomplete medium. After decreasing the concentrations through multiple iterations, the strongest concentration that could be completely dissolved in the medium (defined by no particles visible) was set to 0.5 mg/mL. The other two weaker concentrations were chosen for relative ease of calculation. After confirming that there were no particles to be seen in the mixture following addition of the powder, the mixtures were poured over a microfilter as another safety measure to ensure no contamination during the mixing process. Unfortunately, some of the material that was filtered out of the solution seemed to be particles of *H. erinaceus*. We were not able to measure the exact amount that did not pass through the microfilter and we did not know how this would affect our results. We were unable to restart the procedure as the cells were already in the process of growing and our supply of media was running low. It would have been ideal to run this experiment once more using different conditions, but due to financial and time restraints, we were limited to this path that we already started on. Despite this hiccup, we decided to continue with the experiment as it was. When observing the morphology of the differentiated cells under the electron microscope, little variation between the different concentrations of treatment groups was seen within the same day of trials. The data analyzed

during the later stages of the proteomic analysis reinforces the lack of variation except for when it came to the dense clustering of the cells seen in the Low Day 7 trials. The medium and high concentration counterparts did not exhibit such specific clustering and showed a broader distribution of cells with less clusters. According to these signs, one would assume that these higher concentrations are affecting the differentiation in an impactful way. However, even with the unique clusters seen in the Low Day 7 trials, the proteomic data bolsters the fact that this interesting morphology does not significantly impact the expression of proteins. It is unclear if this is due to insufficient concentration of *H. erinaceus* affecting the cells or if more time has to pass to visualize these differences more distinctly. It may also be possible that *H. erinaceus* has no effect whatsoever on the morphology of the cells and that it may only affect gene and protein expression unrelated to external physical appearance.

Normalization and Mass Spectrometry

Prior to running our samples through the mass spectrometer, we decided to lyophilize the samples in order to increase the concentration of protein. This allows the mass spectrometer to register more effective scans. During the colorimetric peptide assay, peptide concentration was recorded; however, one of the two biological replicates of each sample was more concentrated than the other and was chosen to be used for the mass spectrometry. Using the sample with the lowest concentration of total peptides (3C2), we normalized the other samples so the total peptide concentration would be closely similar across the board (Table 3). This was important to maintain consistency during the experiment. Randomizing the order of the samples during the run was key in preventing the occurrence of unwanted bias and variables. The samples were double-injected in order to create technical replicates. The technical replicates of the samples were also randomized and did not go in the same order as the original samples. A sample of

HeLa cells was injected multiple times in equal time intervals in between our samples to provide evidence for the machine's consistency in spray pattern. In essence, this functioned as a technical control to see if there were any issues with the mass spectrometer's run itself. No significant variation in spray pattern pattern was found, illustrating that the machine executed the procedure without issues. The mass spectrometer generated chromatographs (Figure 3) that were used to validate our data and vet it before moving on with the software-based quantitative proteomic analysis. The technical replicates generated identical chromatographs as those shown in Figure 3 and were not listed to avoid redundancy.

Quantitative Proteomic Analysis

To ensure a False Discovery Rate (FDR) of less than 0.1%, certain parameters had to be set during the Scaffold analysis. After setting the thresholds to the defined values mentioned in the Results section, we were able to achieve a point where we could analyze every peptide with extremely high confidence. For measuring protein abundance, spectral counting is one of the most effective methods to use. There is a very strong correlation between protein abundance and spectral counts³⁰. While another method of protein quantification, measuring ion peak intensity, is available for use, it typically has practical constraints when used for complex biological samples³¹. Because of this, we used spectral counting for our analysis. However, it was vital to normalize the spectral counts in order to measure the variation between samples accurately. The Scaffold program normalized protein spectrum counts by completing multiple calculations in a stepwise manner. First, the total number of spectra in each biosample was calculated. Then, the average number of spectra across all biosamples was calculated. Finally, the spectral count of each protein in every sample was multiplied by the average count over the biosample's total spectral count. An example of this calculation is illustrated by Table 6.

Biosample A	Spectral Count	Biosample B	Spectral Count	
Protein 1	12	Protein 1	8	
Protein 2	6	Protein 2	3	
Protein 3	4	Protein 3	3	
Total	22	Total	14	Total Average: 18
Biosample A	Normalized	Biosample B	Normalized	
Protein 1	10	Protein 1	10	
Protein 2	5	Protein 2	4	
Protein 3	3	Protein 3	4	

Table 6Example of Spectral Count Normalization in Scaffold

Note. Biosample A protein spectral counts are multiplied by 18/22. Biosample B protein spectral counts are multiplied by 18/14.

The histones illustrated in Figure 6 had overall high normalized spectral counts. Pairing this information with the significance resulting from the statistical analysis, these peptides made for our best targets in the final stages of our research. It seems as though these linker histones could play a role in neural stem cell differentiation. Histone H1 is the most variable of the histones and has multiple variants as seen in this study, but it also has strongly conserved regions which could explain the similar function between the variants³². If one Histone H1 variant is being expressed at lower amounts, other variants show a compensatory increase in expression controlled by an unknown mechanism³³. However, if enough H1 subtypes are lost, embryonic stem cell differentiation is impaired and the silencing of pluripotency factors during DNA methylation mediation is interrupted. This shows that modulating the levels of these linker histones and chromatin compaction may help in regulating stem cell pluripotency³⁴. Based on this information from previous studies, we initially believed that the increase in Histone H1

protein expression that is witnessed in Figure 6 correlated to an increase in embryonic stem cell differentiation and was resulting in a faster differentiation process. To verify this, we sought out potential downstream targets that might be regulated by these changes in the histories. These targets were markers that were previously discussed in other studies relating to the nervous system and differentiation. If our initial theory was correct, then the markers for increased differentiation should increase in expression. Lower levels would be illustrated in the control groups and higher levels would be illustrated in the treated samples. We examined standard markers such as neuron-specific enolase (for neuron growth) and glial fibrillary acidic protein (for glial growth), but the results did not support our theory. Levels of these markers were actually higher in the control group, demonstrating that differentiation was not occurring at a faster rate in the treatment groups since neuronal and glial synthesis was not accelerating according to these keystone markers. There is still room for improvement on this end as not even a major proteomic marker of myelin sheath synthesis, MBP, was found during the proteomic analysis. Considering that it is capable of being found by mass spectrometry³⁷, our protocol could be refined to better isolate this protein from within the lipid membrane of myelin³⁸. The lack of this protein in the analyzed sample is odd considering that the transcriptional repressor for it was found and changes in protein expression levels were seen as the days passed. This may be due to the time limits implemented during this study being too short and the protein itself not forming in enough abundance yet. Although there was glial growth as illustrated by the darker spots during light microscopy, perhaps not enough mature oligodendrocyte formation occurred.

Post-Translational Modifications

Another software, Peaks Studio X (Proteome Software, Inc.[™]) was used to confirm the data found in Scaffold. Peaks Studio X uses a slightly different algorithm to calculate the False Discovery Rate and other metrics; however, all the results still pointed towards the previously discussed histones. More importantly, this software allowed us to analyze the effect of posttranslational modifications on the Histone H1 family. It is known that Histone H1 variants are filled with many lysine and arginine residues and are therefore positively charged. This strong positive charge will allow for tight binding to DNA during chromatin compaction. These linker histones can act as transcriptional repressors or promoters when this binding occurs. However, more studies link these histories to the function of local repression³⁵. Post translational modifications such as methylation and acetylation make it more difficult for these linker histones to tightly bind to the wound chromatin due to the decrease in positive charge and the structural change of the histories. These changes particularly impact the terminal parts of the protein sequence which are thought to be involved in binding of chromatin proteins regulating transcriptional activity³⁶. Our results are illustrated in Figure 9, showing post-translational modifications that have occurred at certain parts in the sequence for a certain number of Histone H1 peptides. Not all H1 peptides experienced post-translational modifications, but the ones that did experienced them in various regions. The most similar ones being Histone H1.2 (family member C), H1.3 (family member D), and H1.4 with a lysine residue at position 64 and/or 65 experiencing ubiquitination. Histone H1.1 is most likely experiencing significantly different modifications because it is considered a specific variant for thymus, testis, spleen, lymphocytic, and neuronal cells³⁶. Although the Histone H1 proteins act as regulators of individual gene transcription through chromatin remodeling, nucleosome spacing, and DNA methylation, not

much methylation was witnessed in our data. The charge of the proteins would remain overwhelmingly positive and there would not be a large enough shift in molecular structure seen to affect the tight binding of these linker histones significantly. Still, this is worth exploring in the future to calculate the actual difference in binding between modified histones and nonmodified histones.

Conclusion and Future Directions

This exploratory research should serve as a gateway for future projects in the field of proteomics, *H. erinaceus*, and alternative medicine. There are still many unknowns about the pathways within the nervous system and how it is affected when treated with *H. erinaceus*. Based on previous studies, it is clear that there are benefits to consuming this mushroom, but to what extent these treatments provide benefits is still left to be seen. The relationship between the histones observed in this study and the fungal extract provides a small, yet novel benefit to the differentiation process in stem cells. With protocol refinement, *H. erinaceus* might be used in the future as a supplement to growth media when differentiating cells.

It is worth noting that we did have limitations that future studies can avoid when attempting to build upon this one. To our knowledge, we were the first to attempt a quantitative and label-free proteomic analysis on rat neural stem cells after being treated with aqueous extract of *H. erinaceus*. We did not find any previous studies that covered this exact topic; therefore, we did not have a specific guide on how to proceed flawlessly. We were forced to pull information from many facets of the groups currently analyzing *H. erinaceus* and its effects. We were also limited due to the sensitive nature of the neural stem cells. We would have preferred to work with human tissue to analyze the medicinal benefits in a better way, but due to costs, our lab was limited to animal cells such as those from rats. These cells were cultured from a primary cell line

and, as a result, were very difficult to work with. Having an immortal cell line would allow us more time and resources and we would not have to worry about running out of products. It would have been less expensive and would have also allowed us to run the procedure for a longer period of time to witness the effects on a deeper level. Running the experiment again for a longer period of time would be very useful in specifically seeing if MBP was indeed in the sample, but did not have enough time to build up to significant protein levels. Previously successful studies have demonstrated progress when utilizing nerve growth factor in their experiments involving neural stem cells¹⁹.

Although having a larger group working on analyzing and researching every protein in our data would have allowed us to deliver more insight on this topic, the results presented in this paper are of the best quality that was allowed due to our restrictions. We are confident that this is a good first step through the door and that others will find this information helpful when proceeding with more sharpened procedures for analyzing the proteomic contents of rat neural tissue post-treatment with *H. erinaceus* extract.

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APPENDIX A: LIST OF T-TEST SIGNIFICANT PROTEINS AND FOLD CHANGES IN DAY 3 GROUPS



25	A high parties OC-Pather percentage OV-10116 CN-16m PE-1 SV-2	021000	Minn	54 kDa	-	0.026	
35	▼ ▼ Willelium US-Rattus indrvegicus UX-10110 GH-Vill FE-1 SY-2	F31000	Winth 26k	14 kDa	1	0.025	
27	▼ ■ Installe nzb 05-katus liotvegicus 0x-1010 dii-installizuk rt-3 5V-1	GSV9C7	C-12	E710a		0.025	
3/	▼ 1-complex protein 1 subunit beta 05=Rattus norvegicus 0X=10116 GR=Cct2 PE=1 SV=3	QSXIM9	CCC2	57 KDa		0.027	Y.
38	Peptidyl-prolyl cis-trans isomerase FKBP1A 05=Rattus norvegicus 0X=10116 GN=FKbp1a PE=1 SV=3	Q62658	FKDp1a	12 KDa		0.031	Y Y
39	✓ ★ Calmodulin-3 05=Rattus norvegicus 0X=10116 GN=Calm3 PE=1 SV=1	PODP31	Calm3	17 kDa		0.031	U
40	✓ ★ Metastasis-associated protein MTA1 05=Rattus norvegicus 0X=10116 GN=Mta1 PE=1 5V=1	A0A140TA9	Mta1	83 kDa		0.033	1 · · ·
41	🗹 🖈 Dihydropyrimidinase-related protein 2 OS=Rattus norvegicus OX=10116 GN=Dpysl2 PE=1 5V=1	P47942	Dpysl2	62 kDa	*	0.033	- 10 - 10 - 10 - 10 - 10 - 10 - 10 - 10
42	✓ ★ Histone H2B OS=Rattus norvegicus OX=10116 GN=Hist1h2bl PE=3 SV=1	MOR4L7	Hist1h2bl	14 kDa	*1	0.033	
43	🗹 🜟 Cleavage and polyadenylation specificity factor subunit 5 05=Rattus norvegicus 0X=10116 GN=Nudt21 PE=2 SV=1	B4F764	Nudt21	26 kDa	_	0.034	
44	✓ ★ RCG55135, isoform CRA_b 05=Rattus norvegicus 0X=10116 GN=TIn1 PE=1 SV=1	G3V852	Tin1	270 kDa	*	0.035	₽↑
45	key key statistics initiation initiation factor 4H OS=Rattus norvegicus 0X=10116 GN=Eif4h PE=1 SV=1	Q5X172	Eif4h	27 kDa		0.036	10₽
46	✓ ★ Brain acid soluble protein 1 05=Rattus norvegicus 0X=10116 GN=Basp1 PE=1 SV=2	005175	Basp1	22 kDa	*	0.036	₽ ♠
47	Peptidyl-prolyl cis-trans isomerase 05=Rattus norvegicus 0X=10116 GN=Pin1 PE=1 5V=1	BOBNL2	Pin1	18 kDa		0.036	☆↓
-18	✓ ★ 10 kDa heat shock protein, mitochondrial 05=Rattus norvegicus 0X=10116 GN=11spe1 PE=1 SV=1	A0A0G2JTG	lispe1	9 kDa		0.037	
49	In the second	D4A9A3	Cenpv	28 kDa		0.038	₽
50	🗹 📩 Alpha-enolase OS=Rattus norvegicus OX=10116 GN=Eno1 PE=1 SV=4	P04764 (+1)	Eno1	47 kDa	*	0.038	\$₽
51	🗹 ★ Matrin-3 05=Rattus norvegicus 0X=10116 GN=Matr3 PE=1 5V=2	P43244	Matr3	94 kDa		0.040	
52	🗹 📩 Transient receptor potential cation channel subfamily V member 4 05=Rattus norvegicus 0X=10116 GN=Trpv4 PE=1 SV=1	Q9ERZ8	Trpv4	98 kDa		0.041	☆ ₩
53	🗹 📩 ATP synthase membrane subunit DAPIT, mitochondrial OS=Rattus norvegicus OX=10116 GN=Atp5md PE=1 5V=1	Q9JJW3	Atp5md	6 kDa		0.041	
54	✓ ★ Aly/REF export factor 05=Rattus norvegicus 0X=10116 GN=Alyref PE=1 5V=1	D3ZXH7	Alyref	20 kDa		0.042	₽ ↑
55	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial OS=Rattus norvegicus OX=10116 GN=Cox4i1 PE=1 5V=1	P10888	Cox4i1	20 kDa		0.042	☆ ₩
56	🗹 📩 605 acidic ribosomal protein P1 05=Rattus norvegicus 0X=10116 GN=Rplp1 PE=3 SV=1	P19944	Rplp1	11 kDa		0.043	.
57	✓ ★ Isoaspartyl peptidase/L-asparaginase 05=Rattus norvegicus 0X=10116 GN=Asrgl1 PE=1 SV=1	Q8VI04	Asrgl1	34 kDa		0.043	☆
58	A calponin-3 0S=Rattus norvegicus 0X=10116 GN=Cnn3 PE=1 SV=1	P37397	Cnn3	36 kDa	* 1	0.043	☆ ₿
59	Polypyrimidine tract-binding protein 1 05=Rattus norvegicus 0X=10116 GN=Ptbp1 PE=1 SV=1	A0A0G2JTV.	Ptbp1	59 kDa		0.045	
60	Macrophage migration inhibitory factor OS=Rattus norvegicus OX=10116 GN=Mif PE=1 5V=4	P30904	Mif	12 kDa		0.046	
61		04FZY0	Efhd2	27 kDa		0.046	A
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B4F764 Nudt21	26 kDa 0.034 unknown		(0) (4) (3) 3
G3V852 Tln1	270 kDa \star 0.035 unknown		(0) 6 8 7
Q5XI72 Eif4h	27 kDa 0.036 Rattus norvegicus		e 18 (4) 7 (2)
Q05175 Basp1	22 kDa \star 0.036 Rattus norvegicus		e (0) 24 24 18
BOBNL2 Pin1	18 kDa 0.036 unknown		6 (0) (2) (0)
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P43244 Matr3	94 kDa 0.040 Rattus norvegicus	• • • • • • •	e 24 37 34 34
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P37397 Cnn3	36 kDa 🔺 0.043 Rattus norvegicus		18 12 11 9
A0A0G2JTVPtbp1	59 kDa 0.045 unknown		(0) 10 13 14
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APPENDIX B: LIST OF T-TEST SIGNIFICANT PROTEINS AND FOLD CHANGES IN DAY 7 GROUPS

# 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 20 30 31 22 25 26 27 28 30 31 33 33 34	Ibio View: Bio View: <t< th=""><th>2) subunit DAPIT, mitochondrial OS=Rattu orvegicus OX=10116 GH=SIMT PE=15V- thonucleoprotein D0 OS=Rattus norvegicus SUS=2011016 GH=Call SHE SUS=20 SUS=2011016 GH=SIMT SUS=20 SUS=2011010000000000000000000</th><th>80% to 94% 50% to 79% 20% to 79% 20% to 49% 0% to 19% 0% to 19% 10% to 1</th><th>Atp5md PE=1 SV=1 =1 SV=2 1 SV=1 2 c6a11 PE=1 SV=1 =2 L SV=2 =1 =1 SV=1 E=1 SV=2</th><th></th><th>4 4 4 4 4 4 4 4 4 4 4 4 4 4</th><th>4 2 3 0</th><th>(x) (x) (x) (x) (x) (x) (x) (x)</th><th>all the set of the se</th></t<>	2) subunit DAPIT, mitochondrial OS=Rattu orvegicus OX=10116 GH=SIMT PE=15V- thonucleoprotein D0 OS=Rattus norvegicus SUS=2011016 GH=Call SHE SUS=20 SUS=2011016 GH=SIMT SUS=20 SUS=2011010000000000000000000	80% to 94% 50% to 79% 20% to 79% 20% to 49% 0% to 19% 0% to 19% 10% to 1	Atp5md PE=1 SV=1 =1 SV=2 1 SV=1 2 c6a11 PE=1 SV=1 =2 L SV=2 =1 =1 SV=1 E=1 SV=2		4 4 4 4 4 4 4 4 4 4 4 4 4 4	4 2 3 0	(x) (x) (x) (x) (x) (x) (x) (x)	all the set of the se
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35	▼ ★ 605 ribosomal protein L8 05=Rattus norvegicus 0X=10116 GN=Rpl8 PE=2 SV=2	P62919	Rol8	28 kDa		0.0030	₽
36	✓ ★ T-complex protein 1 subunit eta 05=Rattus norvegicus 0X=10116 GN=Cct7 PE=1 5V=1	D4AC23	Cct7	60 kDa		0.0034	A
37	▼ ★ Serum albumin 05=Bos taurus GN=ALB PE=1 5V=4	P02769	ALB	69 kDa	*	0.0037	- V
38	Pcbp2 protein OS=Rattus norvegicus OX=10116 GN=Pcbp2 PE=1 SV=1	04V8F6 (+2)	Pcbp2	35 kDa	*	0.0041	
39	🗹 🛨 Glutamine synthetase OS=Rattus norvegicus OX=10116 GN=Glul PE=1 SV=3	P09606	Glul	42 kDa		0.0043	- V
40	🗹 🗇 Proteasome subunit alpha type-1 05=Rattus norvegicus 0X=10116 GN=Psma1 PE=1 5V=2	P18420	Psma1	30 kDa		0.0044	.
41	🗹 ★ Heterogeneous nuclear ribonucleoprotein M 05=Rattus norvegicus 0X=10116 GN=Hnrnpm PE=1 SV=1	F1LV13 (+1)	Hnrnpm	74 kDa		0.0044	
42	✓ ★ ATP synthase-coupling factor 6, mitochondrial OS=Rattus norvegicus OX=10116 GN=Atp5pf PE=1 SV=1	P21571	Atp5pf	12 kDa		0.0046	.
43	🗹 🜟 Dynein light chain 1, cytoplasmic 05=Rattus norvegicus 0X=10116 GN=Dynll1 PE=1 5V=1	P63170	Dynll1	10 kDa	*	0.0046	
44	🗹 🜟 10 kDa heat shock protein, mitochondrial OS=Rattus norvegicus OX=10116 GN=Hspe1 PE=1 SV=3	P26772	Hspe1	11 kDa	*	0.0048	
45	✓ ★ Pyruvate kinase PKM 05=Rattus norvegicus 0X=10116 GN=Pkm PE=1 SV=3	P11980	Pkm	58 kDa	*	0.0049	 ∲
46	🗹 🜟 Far upstream element-binding protein 1 05=Rattus norvegicus 0X=10116 GN=Fubp1 PE=1 5V=1	AOA140TAJ.	.Fubp1	68 kDa	*	0.0049	
47	🗹 🜟 Ribosome-binding protein 1 05=Rattus norvegicus 0X=10116 GN=Rrbp1 PE=1 SV=3	F1M853	Rrbp1	158 kDa		0.0049	- 4
48	🗹 🖈 Mitochondrial import receptor subunit TOM22 homolog OS=Rattus norvegicus OX=10116 GN=Tomm22 PE=1 SV=1	Q75Q41	Tomm	15 kDa		0.0051	
49	🗹 🌟 Heat shock protein HSP 90-alpha OS=Rattus norvegicus OX=10116 GN=Hsp90aa1 PE=1 SV=3	P82995	Hsp90	85 kDa	*	0.0053	
50	✓ ★ Fatty acid synthase OS=Rattus norvegicus OX=10116 GN=Fasn PE=1 5V=3	P12785	Fasn	273 kDa		0.0056	
51	✓ ★ Splicing factor proline and glutamine-rich OS=Rattus norvegicus OX=10116 GN=Sfpq PE=1 SV=1	A0A0G2K8	Sfpq	75 kDa	*	0.0059	₽
52	✓ ★ Vinculin OS=Rattus norvegicus OX=10116 GN=Vcl PE=1 5V=1	A0A0G2K8	Vcl	124 kDa		0.0061	1 €
53	🗹 🜟 Fructose-bisphosphate aldolase C OS=Rattus norvegicus OX=10116 GN=Aldoc PE=1 SV=3	P09117	Aldoc	39 kDa	*	0.0063	₽
54	🗹 ★ Sodium/potassium-transporting ATPase subunit alpha-2 05=Rattus norvegicus 0X=10116 GN=Atp1a2 PE=1 5V=1	P06686	Atp1a2	112 kDa	*	0.0064	₽
55	🗹 🜟 G3BP stress granule assembly factor 1 OS=Rattus norvegicus OX=10116 GN=G3bp1 PE=1 SV=1	D3ZY57	G3bp1	52 kDa	*	0.0065	
56	🗹 🜟 265 proteasome regulatory subunit 7 05=Rattus norvegicus 0X=10116 GN=Psmc2 PE=1 5V=1	G3V7L6 (+1)	Psmc2	49 kDa		0.0068	☆ ₽
57	🗹 🜟 605 acidic ribosomal protein P1 05=Rattus norvegicus 0X=10116 GN=Rplp1 PE=3 SV=1	P19944	Rplp1	11 kDa		0.0069	
58	🗹 🜟 Regulatory factor X, 5 (Influences HLA class II expression) (Predicted) 05=Rattus norvegicus 0X=10116 GN=Rfx5 PE=1 5V=1	D3ZHD7	Rfx5	72 kDa		0.0073	
59	🗹 ★ Alpha-1,4 glucan phosphorylase OS=Rattus norvegicus OX=10116 GN=Pygb PE=1 SV=2	G3V6Y6	Pygb	97 kDa	*	0.0073	
60	🗹 🖈 Transaldolase OS=Rattus norvegicus OX=10116 GN=Taldo1 PE=1 5V=2	Q9EQS0	Taldo1	37 kDa	1	0.0075	₽
61	🗹 🖄 Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 05=Rattus norvegicus 0X=10116 GN=Gnb1 PE=1 SV=4	P54311	Gnb1	37 kDa		0.0076	- V
62	🗹 🌟 605 ribosomal protein L6 OS=Rattus norvegicus OX=10116 GN=Rpl6-ps1 PE=3 SV=1	F1LQ53 (+2)	Rpl6-p	34 kDa		0.0076	- -
63	🗹 🜟 265 proteasome non-ATPase regulatory subunit 2 05=Rattus norvegicus 0X=10116 GN=Psmd2 PE=1 5V=1	Q4FZT9	Psmd2	100 kDa		0.0079	∂ ,
64	🗹 🜟 Macrophage migration inhibitory factor OS=Rattus norvegicus OX=10116 GN=Mif PE=1 SV=4	P30904	Mif	12 kDa		0.0084	
65	🗹 🜟 COP9 signalosome complex subunit 3 OS=Rattus norvegicus OX=10116 GN=Cops3 PE=1 SV=1	Q68FW9	Cops3	48 kDa		0.0085	₽
66	🗹 🜟 Phospholipid phosphatase 3 05=Rattus norvegicus 0X=10116 GN=Plpp3 PE=1 SV=1	P97544 (+1)	Plpp3	35 kDa		0.0087	
67	🗹 👚 Enoyl-CoA delta isomerase 1, mitochondrial OS=Rattus norvegicus OX=10116 GN=Eci1 PE=1 SV=1	A0A0G2K2R	2Eci1	31 kDa	*	0.0095	₽
68	🗹 🜟 Mitochondrial 2-oxoglutarate/malate carrier protein OS=Rattus norvegicus OX=10116 GN=Slc25a11 PE=1 SV=1	G3V6H5	Slc25a	34 kDa		0.0095	₽ ₽

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norvegicus OX=10116 GN=Atp5pf PE=1 SV=1	P21571 Atp5pf	12 kDa 0.0046	- ÷	Rattus	•••	•	• •	•	•		• • •					6	19	18	18
X=10116 GN=Dynll1 PE=1 SV=1	P63170 Dynll1	10 kDa \star 0.0046		Rattus	•••		••		••	•	• •	• •	•	• •		(0)	9	10	9
egicus OX=10116 GN=Hspe1 PE=1 SV=3	P26772 Hspe1	11 kDa \star 0.0048	- *°	Rattus	•						• •	•				(0)	30	27	31
t=Pkm PE=1 5V=3	P11980 Pkm	58 kDa + 0.0049		Rattus	•••		•		•			•••				36	9	9	12
ISICUS OX-10110 GN-FUDD1 PE-1 SV-1	FIMPE2 Debet	159 kDa 0.0049		unkno												40	42	46	42
-Pattus nonuoisus 0X-10116 CN-Tomm33 PE-1 SV-1	075041 Tomm	15/0a 0.0051	i i i i i i i i i i i i i i i i i i i	Dattur												11	26	24	26
X=10116 GN=Hsp90aa1 PF=1 SV=3	P82995 Hsp90	85 kDa + 0.0053		Rattus												61	23	19	19
=Fasn PE=1 SV=3	P12785 Fasn	273 kDa 0.0056		Rattus												64	10	7	(3)
egicus OX=10116 GN=Sfpg PE=1 SV=1	A0A0G2K8 Sfpg	75 kDa 🔺 0.0059	- 4	unkno												14	35	34	32
/=1	A0A0G2K8 Vcl	124 kDa 0.0061	💼 🔂	unkno												19	(2)	(4)	(4)
DX=10116 GN=Aldoc PE=1 SV=3	P09117 Aldoc	39 kDa ★ 0.0063		Rattus			• •	•				•		•		38	63	65	67
DS=Rattus norvegicus OX=10116 GN=Atp1a2 PE=1 SV=1	P06686 Atp1a2	112 kDa \star 0.0064		Rattus	•••		• •	•	•		•			•		40	88	88	94
icus 0X=10116 GN=G3bp1 PE=1 SV=1	D3ZYS7 G3bp1	52 kDa 🔺 0.0065	💼 🔂 🗣	unkno												(5)	(2)	(2)	(3)
cus OX=10116 GN=Psmc2 PE=1 SV=1	G3V7L6 (+1) Psmc2	49 kDa 0.0068		unkno												18	(4)	4	(3)
=10116 GN=Rplp1 PE=3 SV=1	P19944 Rplp1	11 kDa 0.0069	- * 0	Rattus	••			•	•	•		• •	•		•	(0)	20	18	18
) (Predicted) OS=Rattus norvegicus OX=10116 GN=Rfx5 PE=1 SV=1	D3ZHD7 Rfx5	72 kDa 0.0073	- * ¥	unkno												(0)	(2)	(2)	(3)
(=10116 GN=Pygb PE=1 5V=2	G3V6Y6 Pygb	97kDa * 0.0073	11	unkno							10 M					28	(3)	(0)	(0)
	Q9EQ50 Taido1	37 KDa 0.0075		Rattus				1.0		•						(0)	28	30	32
t beta-1 US=Rattus norvegicus UX=10116 GN=GnD1 Pt=1 SV=4	P54311 GnD1	37 104 0.0076	1×	Rattus	•••		•										11		12
Hus nonvenicus OV=10116 GN=Denvd2 DE=1 SV=1	OAEZT9 Pamd2	100 kDa 0.0070	- XX	Pattus												20	(2)	(0)	(3)
nicus OX=10116 GN=Mif PE=1 SV=4	P30904 Mif	12 kDa 0.007 5		Rattus												(0)	16	14	17
us 0X=10116 GN=Cops3 PE=1 SV=1	068FW9 Cops3	48 kDa 0.0085	4	Rattus												(0)	(2)	(2)	(3)
0116 GN=Plpp3 PE=1 SV=1	P97544 (+1) Plpp3	35 kDa 0.0087	- ÷÷	Rattus												(0)	20	23	23
rvegicus OX=10116 GN=Eci1 PE=1 SV=1	A0A0G2K2R2Eci1	31 kDa \star 0.0095	📕 🐺	unkno												(0)	(2)	(2)	(3)
Rattus norvegicus OX=10116 GN=Slc25a11 PE=1 SV=1	G3V6H5 Slc25a	. 34 kDa 0.0095	📕 🐺 🐺	unkno												(0)	(2)	(2)	(3)

9	🗹 🔆 Metallothionein OS=Rattus norvegicus OX=10116 GN=Mt1m PE=3 SV=1	D3ZHV3 (+1)	Mt1m	6 kDa	-	0.0095
D	🗹 🜟 Acetyl-CoA acetyltransferase, cytosolic 05=Rattus norvegicus 0X=10116 GN=Acat2 PE=1 5V=1	Q5X122	Acat2	41 kDa		0.0096
L	🗹 🜟 Heterogeneous nuclear ribonucleoprotein A1 OS=Rattus norvegicus OX=10116 GN=Hnrnpa1 PE=1 SV=3	P04256 (+1)	Hnrnpa1	34 kDa	*	0.0099
2	🗹 🜟 Microtubule-associated protein 05=Rattus norvegicus 0X=10116 GN=Map2 PE=1 5V=3	F1LNK0 (+1)	Map2	211 kDa		0.010
3	🗹 🕼 RCG39700, isoform CRA_d OS=Rattus norvegicus OX=10116 GN=Rab6a PE=1 SV=1	AOAOH2UH	Rab6a	24 kDa		0.010
4	🗹 📩 Peptidylprolyl isomerase OS=Rattus norvegicus OX=10116 GN=Fkbp3 PE=1 SV=1	G3V6L9	Fkbp3	25 kDa		0.011
5	🗹 🜟 Thioredoxin domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	A0A0G2K3		22 kDa		0.011
	🗹 🛧 Acyl-CoA-binding protein OS=Rattus norvegicus OX=10116 GN=Dbi PE=1 SV=3	P11030	Dbi	10 kDa	*	0.011
	🗹 ★ Bifunctional purine biosynthesis protein PURH OS=Rattus norvegicus OX=10116 GN=Atic PE=1 SV=2	035567	Atic	64 kDa		0.011
	🗹 🜟 T-complex protein 1 subunit delta OS=Rattus norvegicus OX=10116 GN=Cct4 PE=1 5V=3	Q7TPB1	Cct4	58 kDa		0.011
	🗹 📩 Parathymosin 05=Rattus norvegicus 0X=10116 GN=Ptms PE=1 5V=1	B3DM95	Ptms	12 kDa		0.011
	🗹 🜟 Actin, alpha skeletal muscle 05=Rattus norvegicus 0X=10116 GN=Acta1 PE=1 5V=1	P68136	Acta1	42 kDa	* 1	0.011
	🗹 🜟 Rho GDP-dissociation inhibitor 1 OS=Rattus norvegicus OX=10116 GN=Arhgdia PE=1 SV=1	Q5X173	Arhgdia	23 kDa	1	0.011
	🗹 🜟 3-ketoacyl-CoA thiolase, mitochondrial O5=Rattus norvegicus OX=10116 GN=Acaa2 PE=1 5V=1	P13437	Acaa2	42 kDa		0.011
	🗹 🜟 Cytochrome c oxidase subunit 4 isoform 1, mitochondrial OS=Rattus norvegicus OX=10116 GN=Cox4i1 PE=1 5V=1	P10888	Cox4i1	20 kDa	1	0.011
	🗹 📩 NIF3-like protein 1 OS=Rattus norvegicus OX=10116 GN=Nif3l1 PE=1 SV=1	Q4V7D6 (+1)	Nif3l1	42 kDa		0.012
	🗹 📩 Histone H2B OS=Rattus norvegicus OX=10116 GN=LOC102549061 PE=3 SV=1	A0A0G2JXE0	LOC10	14 kDa	*	0.012
	🗹 📩 Protein phosphatase 2 (Formerly 2A), regulatory subunit A (PR 65), alpha isoform, isoform CRA_a OS=Rattus norvegicus OX=10116 GN=Ppp2r1a	Q5XI34	Ppp2r1a	65 kDa	*	0.012
	🗹 📩 Cortactin, isoform CRA_c 05=Rattus norvegicus 0X=10116 GN=Cttn PE=1 SV=3	D3ZGE6 (+1)	Cttn	61 kDa	1	0.012
	🖌 📩 Nucleolin 05=Rattus norvegicus 0X=10116 GN=Ncl PE=1 5V=3	P13383 (+1)	Ncl	77 kDa	1	0.013
	🗹 🜟 605 ribosomal protein L28 05=Rattus norvegicus 0X=10116 GN=Rpl28 PE=2 5V=1	Q642E2	Rpl28	16 kDa		0.013
	🗹 📩 Reticulon 05=Rattus norvegicus 0X=10116 GN=Rtn3 PE=1 5V=1	AOAOH2UH	Rtn3	28 kDa	- 1	0.014
	🗹 🜟 Ras-related protein Rab-1A OS=Rattus norvegicus OX=10116 GN=Rab1A PE=1 SV=3	Q6NYB7	Rab1A	23 kDa	*	0.014
	🗹 📩 Spectrin alpha chain, non-erythrocytic 1 OS=Rattus norvegicus OX=10116 GN=Sptan1 PE=1 SV=1	A0A0G2JZ69	Sptan1	287 kDa	1	0.014
	🗹 🜟 Glial fibrillary acidic protein 05=Rattus norvegicus 0X=10116 GN=Gfap PE=1 5V=2	P47819	Gfap	50 kDa	*	0.014
	🗹 🜟 L-lactate dehydrogenase B chain OS=Rattus norvegicus OX=10116 GN=Ldhb PE=1 SV=2	P42123	Ldhb	37 kDa		0.014
	🗹 🜟 Core histone macro-H2A 05=Rattus norvegicus 0X=10116 GN=H2afy PE=1 SV=1	A0A140TA	H2afy	39 kDa	*	0.014
	🗹 📩 ADP/ATP translocase 2 05=Rattus norvegicus 0X=10116 GN=Slc25a5 PE=1 SV=3	Q09073	SIc25a5	33 kDa	*	0.014
	🗹 🜟 Proteasome subunit alpha type-5 OS=Rattus norvegicus OX=10116 GN=Psma5 PE=1 SV=1	P34064 (+1)	Psma5	26 kDa	1	0.015
	🗹 📩 Ubiquitin-like modifier-activating enzyme 1 05=Rattus norvegicus 0X=10116 GN=Uba1 PE=1 SV=1	Q5U300	Uba1	118 kDa		0.015
	🗹 🜟 L-lactate dehydrogenase A chain 05=Rattus norvegicus 0X=10116 GN=Ldha PE=1 SV=1	P04642	Ldha	36 kDa	1	0.015
D	🗹 🜟 ATP synthase subunit beta, mitochondrial O5=Rattus norvegicus OX=10116 GN=Atp5f1b PE=1 SV=2	P10719	Atp5f1b	56 kDa		0.015
1	🗹 🜟 Uncharacterized protein 05=Rattus norvegicus 0X=10116 PE=4 5V=2	D3Z525 (+1)		24 kDa	*	0.015
12	📝 ★ RNA-binding motif protein, X chromosome retrogene-like OS=Rattus norvegicus OX=10116 GN=Rbmxrtl PE=1 SV=1	P84586	Rbmxrtl	42 kDa	*	0.016

	1							Biologica	Process		6	ellular Compon	ent	Moleci	ılar Fu	nction	Contr	Day 7	7
Probability Legend: over 95% 80% to 84% 50% to 75% 20% to 45% 6% to 19%	Accession Nurber	Alternate ID Molecular Weight	Protein Grouping Ambiguity	T-Test (p-value) (p < 0.05)	Quantitative Profile	Taxonomy	biological adhesion biological regulation cellular process development of localization establishment of localization month.	in mune system process localization locanotion matabut morese	meraconc.process multi-organism process multicelluter organismal process pigment/ation	reproduction reproductive process rhydraic process	m al process Golgi apparatus cytoskaleton endoplasmic reticulum	endosome extracellular region intracellular coganelle membrane mutochondrion	organelle menterane organelle part plassma mentbrane ribosome antioxidant activity	binding catalytic activity electron carrier activity enzyme regulator activity metallischesenne activity	molecular function molecular transducer activity	motor activity protein tag structural molecula activity transporter activity transporter activity	0	200	DH.
Lm PE=3 SV=1	D3ZHV3 (+1) M	tim 6kD		0.0095		unkno											(0) (2)) (2)	(3)
gicus OX=10116 GN=Acat2 PE=1 SV=1	Q5X122 Ac	cat2 91kL	a 🔸	0.0096	<u>*1</u>	Rattus									:		6 37	33	32
=10116 GN=Map2 PE=1 5V=3	F1LNK0 (+1) Ma	ap2 211 kd	Da a	0.010	₩Å.	unkno						a a					5 26	27	30
16 GN=Rab6a PE=1 SV=1	A0A0H2UH Ra	ab6a 24kD	a 📃	0.010	**	unkno											(0) 4	4	5
6 GN=Fkbp3 PE=1 5V=1	G3V6L9 Fk	25 kD 22 kD	a 🗧	0.011	**	unkno				10.1							(0) 8	16	10
6 GN=Dbi PE=1 SV=3	P11030 Db	10 kD	a \star 🗖	0.011	40	Rattus		T+ 1	•				• •	•	•		55 124	4 125	136
norvegicus OX=10116 GN=Atic PE=1 SV=2	035567 At	tic 64 kD	a 📒	0.011	û	Rattus	••		•	•		•		• •	•		21 6	(3)	(3)
0X=10116 GN=Cct4 PE=1 SV=3	Q7TPB1 Cc	t4 58 k0	3	0.011		Rattus	••••	•	•	• •	••	• •		•	•		14 (2)	1 (0)	(0)
116 GN=Acta1 PE=1 SV=1	P68136 Ac	tal 42 kD	a \star 🗖	0.011	ě.	Rattus											125 260	0 238	254
(=10116 GN=Arhgdia PE=1 SV=1	Q5XI73 Ar	nhgdia 23 kC	a 📒	0.011	û.	Rattus				•			•	• •	•		29 14	12	12
picus OX=10116 GN=Acaa2 PE=1 SV=1	P13437 Ac	caa2 42 kD	a	0.011		Rattus	••••	•							2		(0) 6	7	6
Nif311 PE=1 SV=1	04V7D6 (+1) Nit	f3l1 42 kD	a a	0.012	Å Å	Rattus			•						•		(0) (5)) (4)	(4)
2549061 PE=3 SV=1	ADAOG2JXED LO	C10 14kD	a \star 🗖	0.012	**	Rattus	•							•	•		49 388	8 451	395
A (PR 65), alpha isoform, isoform CRA_a OS=Rattus norvegicus OX=10116 GN=Ppp2r1	Q5XI34 Pp	p2r1a 65kD	a \star 🗖	0.012		unkno											27 (4)	(0)	(0)
W=3	P13383 (+1) No	1 77 kD	a 🗖	0.013	A	Rattus											28 8	(3)	(5)
16 GN=Rpl28 PE=2 SV=1	Q642E2 Rp	16 kD	a 📒	0.013	**	unkno											(0) 7	(6)	(6)
1 SV=1	A0A0H2UH Rt	tn3 28 kD	a 📕	0.014		unkno											(0) 13	11	12
gicus 0X=10116 GN=Sptan1 PE=1 SV=1	A0A0G2JZ69 50	otan1 287 kd	a a	0.014	Å.	unknom											57 20	23	15
116 GN=Gfap PE=1 SV=2	P47819 Gf	ap 50 kC	• *	0.014	û 🐺	Rattus			•	•	• •	• •		•	•		92 40	33	44
X=10116 GN=Ldhb PE=1 SV=2	P42123 Ld	1hb 37 kD	a 🔒	0.014		Rattus	•							••	•		28 12	9	12
GN=R28y PE=1 SV=1 GN=Sk25a5 PE=1 SV=3	009073 Sk	c25a5 33 kD	a *	0.014	¥.	Rattus											3 70	57	63
DX=10116 GN=Psma5 PE=1 SV=1	P34064 (+1) Ps	ma5 26 kD	a 📃	0.015	**	Rattus	•					• •		•	•		(0) 12	10	12
rvegicus OX=10116 GN=Uba1 PE=1 SV=1	Q5U300 Ub	ba1 118 k	Da la	0.015		Rattus							••		:		35 15	11	14
vegicus OX=10116 GN=Atp5f1b PE=1 SV=2	P10719 At	pSf1b 56 kD	a	0.015	44	Rattus			•						••		71 17	13	24
5 PE=4 SV=2	D3Z525 (+1)	24 kD	a \star 🗖	0.015	₽ ₩	unkno											6 22	20	23
OS=Rattus norvegicus OX=10116 GN=Rbmxrtl PE=1 SV=1	P84586 Rb	bmxrtl 42 kC	ə \star 🗖	0.016	₽ ₽	Rattus			•	•		•••••	•	•			(1) 28	29	33
103 🗹 \star Glutathione S-transferase alpha 4 05=Rattus	orvegicus OX	=10116 (SN=Gst	a4 PE=2 SV=	=1							A9UMW1	Gsta4	26 kDa	*	0.0	16	÷	· 🔂
104 🖌 🖈 Elongation factor 1-alpha OS=Rattus norvegic	s OX=10116	GN=LOC1	003604	13 PE=3 SV:	=1							M0R757	LOC10	. 50 kDa		0.0	16	4	· 🏠
105 🖌 🖈 605 ribosomal protein L24 05=Rattus norvegi	us OX=10116	GN=Rpl2	4 PE=1	SV=1								AOAOH2UH	Rpl24	18 kDa		0.0	16	•	· fr
106 📝 🔺 Histone H1.4 OS=Rattus norvegicus OX=10116	GN=H1-4 PE=	=1 SV=3										P15865	H1-4	22 kDa	*	0.0	16	÷	· 🏠
107 📝 🔺 Histidine triad nucleotide-binding protein 1 OS	Rattus norve	egicus OX	=10116	6 GN=Hint1 F	PE=1 SV	=5						P62959	Hint1	14 kDa		0.0	16	÷	· fr
108 🖌 🛧 Heterogeneous nuclear ribonucleoproteins A2	B1 OS=Rattu	s norvegi	cus OX:	=10116 GN=	Hnrnpa	2b1 PE=	1 SV=1					F1LNF1	Hnrnp	. 37 kDa	*	0.0	17	-	1
109 🗹 🛉 Protein phosphatase 1 regulatory subunit 14B	DS=Rattus no	orvegicus	OX=10	116 GN=Ppp	olr14b P	E=2 SV	=1				,	Q8K3F3	Ppp1r1.	16 kDa		0.0	17	-	· fr
110 🖌 📩 Isoaspartyl peptidase/L-asparaginase OS=Ra	tus norvegicu	us OX=10	116 GN:	=Asrgl1 PE=	1 SV=1							Q8VI04	Asrgl1	34 kDa		0.0	17		1
111 🗹 📩 Sodium/potassium-transporting ATPase subu	it beta OS=R	attus nor	vegicus	s OX=10116	GN=Atp	1b2 PE=	=1 SV=1					Q5M9H4	Atp1b2	33 kDa		0.0	17	÷	· 1
112 🖌 🖈 Far upstream element-binding protein 3 05=R	ttus norvegi	cus OX=1	0116 G	N=Fubp3 PE	=1 SV=1	L						G3V829	Fubp3	61 kDa	*	0.0	18		· îr
113 🖌 🛧 405 ribosomal protein S12 05=Rattus norvegi	us OX=10116	GN=Rps	12 PE=1	1 SV=2								P63324	Rps12	15 kDa	*	0.0	18		•
114 🖌 🖈 Microtubule-associated protein OS=Rattus no	vegicus OX=1	10116 GN	=Map4	PE=1 SV=1								A0A0G2JW	Map4	233 kDa	*	0.0	18		
115 🖌 🖈 Heterogeneous nuclear ribonucleoprotein U 09	=Rattus norv	regicus 0	(=1011	L6 GN=Hnrnp	DU PE=1	SV=1						A0A0G2JZ	5Hnrnpu	88 kDa		0.0	18	-	· fr
116 🖌 🖈 ATP synthase subunit f, mitochondrial OS=Rat	us norvegicu:	s OX=101	16 GN=	Atp5mf PE=	1 SV=1							D3ZAF6	Atp5mf	10 kDa		0.0	18	÷	· û
117 🗹 \star RCG61099, isoform CRA_b OS=Rattus norvegi	us OX=10116	GN=Srsf	3 PE=1	SV=1								A0A0U1RR	Srsf3	14 kDa	*	0.0	19	į.	· û
118 🖌 🛉 Rat glutathione S-transferase OS=Rattus nor	egicus OX=10	116 PE=2	SV=1									Q6LDP3		26 kDa	*	0.0	21	Į.	· fr
119 Acidic leucine-rich nuclear phosphoprotein 32	amily membe	r A OS=R	attus n	norvegicus O	X=1011	6 GN=A	np32a PE=:	1 SV=1				AOAOG2K7	Anp32a	26 kDa		0.0	22		
120 🖌 🖈 Reticulon OS=Rattus norvegicus OX=10116 G	=Rtn4 PE=1 S	5V=1					22					F1LQN3 (+:	1) Rtn4	126 kDa		0.0	23		
121 RCG45615, isoform CRA a OS=Rattus norvegi	us OX=10116	GN=Rol1	2 PE=2	2 SV=1								B2RYU2	Rpl12	18 kDa		0.0	23		r îr
122 ATP synthase protein 8 05=Rattus porvegicus	OX=10116 GM	ATP8 P	E=3 5V	=1								05UA35	ATP8	8 kDa		0.0	23	į,	4
123 Histone H2A type 1-C 05=Rattus norvegicus 0	=10116 PF=	1 SV=2										P0C169		14 kDa	*	0.0	23	Å	- Ā-
124 Cell division control protein 42 homolog 05=R:	thus norvegic	US 0X=10	116 6	N=Cdc42 PE=	1 SV=2							ORCEN2	Cdc42	21 kDa		0.0	24	Å	
125 Myelin expression factor 2 05=Battus porveni	us 0X=10116	GN=MV	f2 PF=	1 SV=1								4040G2K4	Myef2	63 kDa		0.0	24	, i	- A
126 Splicing factor 3b subunit 1 OS=Pattus porver	CUS 0X=1011	6 GN=SF	h1 PF=	1 SV=1								G3V7T6	Sf3h1	146 kDa		0.0	24	Å	
127 A Sodium/patacsium-transporting ATPase subu	it aloba-1 05	-Dattue	onveni	icus 0Y-101	16 CN-	Atolal	DE-1 SV-1					006685	Atolal	113 kDa	+	0.0	24	, i	Ă
128 V + Neural cell adhesion molecule 1.05=Dattus por	venicus OX=1	0116 CN	Ncam	1 PF=1 SV=1	1 0 0 0 0	chia1						P13596	Ncam1	95 kDa	-	0.0	25	, i	~~
129 Flongation factor 2 OS=Battus nonvenieur OV-	10116 GN=E	f2 PF=1 G	V=4	111-1 34-1								P05197	Fef2	95 kDa	+	0.0	25		
130 Heterogeneous nuclear ribonucleanatain A2	C-Dattue nor	ne ru-13	W-101	16 CN-How	1033 DE-	-1 SV-1						OGUDKA	Hornes	3 40 40=	2	0.0	26	-	ň
131 A phosphate carrier protein mitochondrial OC-T	attus nonioni	icus OV-1	0116	N=Slc25-2	DE-1 CU	-1						C3V741 /+	1) 5625-3	40 kDa	~	0.0	26	, j	Ă
132 A Eathy acid-binding protein 5 05-Dathy acid-	icus OV-1011	IG CN-E-	hos Dr-	-1 51-2	-1 5V	-1						DEE0E2	Eabor	15 60-		0.0	26	ž	~
132 Fatty accoming protein 5 05=Rattus norvey	Cus UA-1011	V-10110	CH-PE=	-1 54-3	V-2							DAAAC2 (.	1)Henno	71 40-	+	0.0.	20		5
124 reat snock cognate /1 kba protein OS=Rattus	norvegicus 0	A=10116	GN=ris	Spat PE=3 5	14 DF. 1	CV-7						U4A453 (+	1) nspa8	210 40-	-	0.0.	27	×	H
125 M Chromodomain helicase DNA-binding protein 4	OV-1011	orvegicus	UA=10	ULIO GN=Cho	u4 Pt=1	3V=3						C3PU01	Cild4	47 KDa	*	0.0.	27	Y	X
135 V Ustone alusten 111 for the second second	SOX=10116 (GN=Kpi4	10.00	V-1	-1.01							QOP3V9	Kpi4	4/ KDa		0.0.	21		Y.
130 Mistone cluster 1 H1 family member c 05=Rational State (State State)	us norvegicus	5 UX=101	10 GN=	mistinic PE	=1 5V=1							AUAUG2K6	54 Hist1h1	C ZIKDa	*	0.0	28		° 1

uspiay uptions: Quantitative Value (Normalized Total Spectra) V Reg Mods: N	o Filter 🗸 Search:	1 4]										
Probability Legend: over 95% 80% to 94%		2			Biological Proces	ss	Ш	Cellular Comp	onent	Mol	ecular Fu	nction Contr	Day 7
50% to 79%		higue		ess alzati	Cess ess mail pr	2	6	2		vity chiulty	ctivity ir acti	activit r activ	
20% to 49%	dh hber	eng An	Profile	alation lation of loc	m proc	proces timulu	us deticate	egion	nbrane rane	thy the sector and th	ction soluce	ecule . gulato ctivity	
	ccession Nur ternate ID decular Wei	otein Group Test (p-val) o < 0.05)	uantitative i sconomy	ological adhu ological regu sitular proces svelopmenta trablishment	owth mune syste calization comotion etabolic proi ulti-organism ulticelfular or	gmentation production sponse to s ythmic proc	ral process olgi apparati toplasm toskeleton dodlocmir n	idosome tracellular r tracellular or embrane Rochondrion	ucleus ganelle men ganelle part asma membr oosome	ntiovidant ac inding stalytic activ ectron carrie	etallochaper decular fune decular trar	otor activity otein tag ructural mol anslation re- ansporter a	
DX=10116 GN=Gsta4 PE=2 5V=1	A9UMW1 Gsta4 26 kDa	★ 0.016	₩ unkno	-	9/2/0/0/6/6/6/6/	9151515151 - 11	510101018	181819181818	5 8 8 8 8 8	8121011818	1212121	(3)	14 15 13
5 GN=LOC100360413 PE=3 SV=1 6 GN=Rpi24 PE=1 SV=1	A0A0H2UH Rpl24 18 kDa	0.016	♦ 1 unkno	-								(0)	93 104 10 13 16 14
E=1 SV=3 vegicus 0X=10116 GN=Hint1 PE=1 SV=5	P15865 H1-4 22 kDa P62959 Hint1 14 kDa	* 0.016 0.016	Rattus Rattus					:	::	:.	12	13 (0)	268 312 25 27 33 28
tus norvegicus 0X=10116 GN=Hnrnpa2b1 PE=1 SV=1	F1LNF1 Hnrnp 37kDa OBK3F3 Pop1r1 16kDa	* 0.017	♣☆ unkno			1.0						53	124 119 13 6 4 5
cus 0X=10116 GN=Asrgl1 PE=1 5V=1	Q8VI04 Asrgl1 34kDa	0.017	Rattus				•			•		(0)	12 10 10
gicus OX=10116 GN=Fubp3 PE=1 SV=1	G3V829 Fubp3 61kDa	* 0.018	♦ 1 unkno	-								4	14 14 17
16 GN=Rps12 PE=1 SV=2 =10116 GN=Map4 PE=1 SV=1	P63324 Rps12 15 kDa A0A0G2JW Map4 233 kDa	* 0.018 * 0.018	☆ Rattus	- •		•	•	•				- 18 60	4 (3) (6 33 29 35
rvegicus OX=10116 GN=Hnrnpu PE=1 SV=1 cus OX=10116 GN=Atp5mf PE=1 SV=1	A0A0G2JZ5Hnmpu 88 kDa D3ZAF6 Atp5mf 10 kDa	0.018	♣☆ unkno ♣☆ Rattus									17 (0)	31 35 33 12 14 15
16 CN=Srsf3 PE=1 SV=1 10116 PE=2 SV=1	A0A0U1RR 5rsf3 14kDa 06LDP3 26kDa	+ 0.019 + 0.021	Unkno	-								8	20 21 23
ber A OS=Rattus norvegicus OX=10116 GN=Anp32a PE=1 SV=1	A0A0G2K7 Anp32a 26kDa	0.022	unkno	-								12	(0) (0) (3
16 GN=Rpl12 PE=2 5V=1	B2RYU2 Rpl12 18kDa	0.023	unkno ↓ 1 unkno	-								(0)	9 11 12
GN=ATP8 PE=3 SV=1 =1 SV=2	Q5UAJ5 ATP8 8 kDa P0C169 14 kDa	0.023 0.023	♦ 1 unkno ♦ 1 Ratture	•					• •			(0)	45 41 53 96 87 81
picus 0X=10116 GN=Cdc42 PE=1 SV=2 16 GN=Mvef2 PE=1 SV=1	Q8CFN2 Cdc42 21kDa A0A0G2K4 Myef2 63kDa	0.024	合导 Rattus		••••	• • •	••••	• •	•••	••		16	(3) 5 6 23 24 23
116 GN=Sf3b1 PE=1 SV=1	G3V7T6 Sf3b1 146 kDa	0.024	unkno			1.1						10	(0) (2) (0
=10116 GN=Ncam1 PE=1 SV=1	P13596 Ncam1 95kDa	0.025	Rattus						- -			27	42 44 40
Eet2 PE=1 SV=4 iorvegicus OX=10116 GN=Hnrnpa3 PE=1 SV=1	Q6URK4 Hnrnpa3 40 kDa	* 0.025 * 0.026	☆ ↓ Rattus Rattus		- -				••••			• 41	17 14 19 12 14 19
egicus OX=10116 GN=Slc25a3 PE=1 SV=1 116 GN=Fabp5 PE=1 SV=3	G3V741 (+1) Slc25a3 40 kDa P55053 Fabp5 15 kDa	0.026	♣☆ unkno ♣☆ Rattus	2								(0)	23 18 22 35 30 32
0X=10116 GN=Hspa8 PE=3 5V=3 porcepticus 0X=10116 GN=Chd4 PE=1 5V=3	D4A453 (+1) Hspa8 71 kDa F9PU01 Cbd4 218 kDa	* 0.027 * 0.027	Unkno	-								37	99 97 11 (2) (0) (0
6 GN=Rpl4 PE=1 SV=1	Q6P3V9 Rpl4 47kDa	0.027	unkno									(4)	20 16 17
137 Sector and the sector of t	attus norvegicus OX=1011	6 GN=Gmps PE=	=1 SV=1					04776	Gmps	77 kDa		0.028	
138 🗹 🖈 Astrocytic phosphoprotein PEA-15 05=Rattus	norvegicus OX=10116 GN	=Pea15 PE=1 SV	/=1					Q5U318	Pea15	15 kDa	a	0.029	
139 🗹 🛨 Lamin-B1 05=Rattus norvegicus 0X=10116 GM	I=Lmnb1 PE=1 SV=1		cu-cl-z-r pr	1 01-2				G3V7U4	Lmnb1	67 kDa	*	0.029	
 140 Transitional endoplasmic reticulum ATPase OS 141 Transitional endoplasmic reticulum ATPase OS 	=Rattus norvegicus OX=1	0116 GN=Vcp P	GN=SIC/a5 PE	=1 5V=2				Q63016 P46462	SIC/a5	89 kDa		0.030	A L
142 📝 🔺 605 ribosomal protein L13 05=Rattus norvegi	cus OX=10116 GN=Rpl13 F	E=1 5V=2						P41123	Rpl13	24 kDa		0.030	4
143 🗹 🛨 14-3-3 protein beta/alpha OS=Rattus norvegi	cus OX=10116 GN=Ywhab	PE=1 SV=3			1 05 - 2 51			P35213	Ywhab	28 kDa	*	0.030	
 144 V Topicnyl-dipnosphooligosaccharideprotein gr 145 V topicnyl-dipnosphooligosaccharideprotein gr 	-RF1 OS=Rattus norvegici	1 05=Rattus no	=Slc9a3r1 PF:	10116 GN=R	pn1 PE=2 SV=	1		091119	Slc9a3	1 39 kDa		0.030	Ϋ́Υ Α
146 🗹 🖄 Activated RNA polymerase II transcriptional o	oactivator p15 05=Rattus	norvegicus OX:	=10116 GN=5u	b1 PE=1 SV:	=3			Q63396	Sub1	14 kDa	3	0.031	- ÷÷
147 🗹 🖈 Protein-L-isoaspartate O-methyltransferase O	S=Rattus norvegicus OX=	10116 GN=Pcm	t1 PE=1 SV=1					A0A140TA	A Pcmt1	21 kDa	3	0.031	
148 MICOS complex subunit MIC60 OS=Rattus nor 149 Micos chromosomal protein HMG-17 OS	=Rattus porvegicus OX=10	mt PE=1 SV=1	2 PF=1 SV=2					A0A0G2JV	/H4 Immt Hman2	9 kDa	•	0.031	
150 🗹 🖈 Protein disulfide-isomerase OS=Rattus norveg	jicus OX=10116 GN=Pdia3	PE=1 SV=1						A0A0H2U	H Pdia3	57 kDa		0.031	
151 V Phytanoyl-CoA hydroxylase-interacting protei	in-like OS=Rattus norvegi	cus OX=10116 0	N=Phyhipl PE=	1 5V=2				Q6AYN4	Phyhip	42 kDa		0.031	- *
152 ✓ ★ S-phase kinase-associated protein 1 05=Rattic 153 ✓ ★ Filamin A 05=Pattus populaticus 0Y=10116 CN	us norvegicus OX=10116 G	SN=Skp1 PE=1 S	SV=1					A0A0G2K4	L Skp1	19 kDa 280 kD	3	0.031	
154 V * Splicing factor 3b, subunit 2 OS=Rattus norveg	icus OX=10116 GN=Sf3b2	PE=1 5V=3						D3ZMS1	Sf3b2	98 kDa	3	0.032	₩.
155 😿 🖈 Tropomodulin-2 OS=Rattus norvegicus OX=10	116 GN=Tmod2 PE=1 SV=	1						P70566	Tmod2	39 kDa	3	0.032	
156 ✓ ★ Calponin-3 05=Rattus norvegicus 0X=10116 G	SN=Cnn3 PE=1 SV=1	E=1 6V=1						P37397	Cnn3	36 kDa	*	0.032	
158 V ± 605 ribosomal protein L14 05=Rattus norvegi	cus OX=10116 GN=Rpl34 F	E=1 SV=1						B2RZD4	Rpl34	13 kDa	3	0.033	
159 🗹 🗇 Phosphoglycerate kinase 1 OS=Rattus norveg	icus OX=10116 GN=Pgk1 F	E=1 SV=2						P16617	Pgk1	45 kDa	*	0.033	- ÷÷
160 🗹 🖈 Tubulin beta-5 chain OS=Rattus norvegicus OX	(=10116 GN=Tubb5 PE=1 9	5V=1						P69897	Tubb5	50 kDa	*	0.034	
161 V 405 ribosomal protein 525 05=Rattus norvegi 162 V Peroviredoxin-2 05=Rattus norvegicus 0X=10	cus 0X=10116 GN=Rps25	PE=2 5V=1						P62853	Rps25	22 kDa		0.035	
163 V * Dynactin subunit 2 OS=Rattus norvegicus OX=	10116 GN=Dctn2 PE=1 SV	=1						A0A0G2JU	J Dctn2	46 kDa		0.035	↓
164 🕑 🛧 Protein/nucleic acid deglycase DJ-1 05=Rattu	s norvegicus OX=10116 G	N=Park7 PE=1 9	V=1					088767	Park7	20 kDa		0.035	
 Vesicle-associated membrane protein-associa Vesicle-associated membrane protein-associa Vesicle-associated membrane protein-associated Vesicle-associated membrane protein-associated 	OX=10116 GN=Syne1 PF=	Orvegicus OX=	10116 GN=Vap	a PE=1 SV=	3			A0A5P804	Vapa K4Svne1	28 KD	a	0.035	
167 ATP synthase subunit e, mitochondrial OS=Rat	ttus norvegicus OX=10116	GN=Atp5me Pl	=1 SV=3					P29419	Atp5m	e 8 kDa	ē i	0.036	↓
								007562	Tracht	5 kDa		0.036	₽ ♠
168 Thymosin beta OS=Rattus norvegicus OX=101	16 GN=Tmsb15b2 PE=2 5	V=1						F97303	THISDI			0.030	
168 ✓ □ Thymosin beta OS=Rattus norvegicus OX=101 169 ✓ ★ Gap junction alpha-1 protein OS=Rattus norve 170 ✓ ♠ BCG34610 isoform CPA c OS=Pattus norve	16 GN=Tmsb15b2 PE=2 S gicus OX=10116 GN=Gja1 cus OX=10116 GN=Greft Di	PE=1 SV=2						P08050	Gja1	43 kDa		0.037	

				1																					
Probability Legend: over 95% 80% to 84% 90% to 79% 20% to 45% 0% to 19%	ccession Number	kernate ID	Iolecular Weight	rotein Grouping Ambiguity	Test (p+value) p < 0.05)	Justititative Profile	axonomy	iological adhesion iological regulation ellular process	evelopmental process stablishment of localization rowth	mune system process icalization icomotion	etabolic process uulti-organism process uulticellular organismal process	igneritation sproduction sproduction	ssponse to stimulus vythmic process iral process	org apparatus ytoplasm otoplasmic reticulum	ndosome crosome crosome contraction contractico contractico contractico contractico contra	itochondrion ucleus roanelle membrane	rganelle part lasma membrane bosome	ntioxidant activity inding statvitic activity	lectron carrier activity nzyme regulator activity at-all-chanerone activity	net transformer activity of the second activi	rotein tag tructural molecule activity anslation regulator activity	Contr		Day 7	0
views OV=10116 CHaComes DE=1 6V=1	OMUTCE	Course	7710	10.1	0.029	AR	Datter	וסומומו	01010	1212121	51515	1012121	ETEISIC	01010101	0101212	151010	101012		101015	1515151	DIWIDI1		(0)	(2)	(D)
Jcus 0x-10110 un-unips PE-1 5V-1	Querres	Gimps	1510-		0.020		Rattus								-								(0)	141	(0)
0X=10116 GN=Pea15 PE=1 SV=1	Q50318	Peals	15 KDa		0.029	ř.	Rattus															21	11	12	9
	G3V/04	Lmnb1	67 KDa	*	0.029	H.	unkno															40	21	21	20
=Rattus norvegicus 0X=10116 GN=Sic7a5 PE=1 SV=2	Q63016	Sic7a5	56 KDa		0.030	*¥	Rattus												1.1.1			(0)	10	10	13
rvegicus OX=10116 GN=Vcp PE=1 SV=3	P46462	Vcp	89 KDa		0.030	¥.	Rattus	•••	•	•	••		••	• •	••	•	•	••	•			59	37	41	42
16 GN=RpH3 PE=1 SV=2	P41123	Rph13	20 100		0.030	*¥	Rattus		· .		•						•					(0)	30	23	31
16 GN=TWINAD PE=1 SV=3	P35213	Twnab	20 KD8	*	0.030	2Y	Rattus							- T - 2								20	40	43	45
Terase subunit 1 05=Rattus norvegicus 0X=10116 GN=Rpn1 Pt=2 SV=1	P0/153(+1)	Rpn1	03 KU3		0.030	*¥	Rattus															4	8	9	8
sttus norvegicus OX=10116 GN=5k9a3r1 Pt=1 SV=3	Q91119	Slc9a3r1	39 KDa		0.030	*¥¥	Rattus		••	•	•						•••					(0)	8	9	6
)15 OS=Rattus norvegicus OX=10116 GN=Sub1 PE=1 SV=3	Q63396	Sub1	14 KDa		0.031	**	Rattus	••					•			•	•	••				(3)	-	1	8
orvegicus OX=10116 GN=Pcmt1 Pt=1 SV=1	A0A140TA	Pcmt1	21 KDB		0.031	*¥	unkno															(0)	1	8	9
:10116 GN=Immt PE=1 SV=1	A0A0G2JVH4	4 Immt	86 KDa		0.031	¥.	unkno									120						- /	(3)	(4)	(4)
vegicus OX=10116 GN=Hmgn2 PE=1 SV=2	P18437	Hmgn2	9 kDa	*	0.031	4 B	Rattus							•	•	•	•	•		•		(4)	(8)	(8)	(9)
116 GN=Pdia3 PE=1 SV=1	A0A0H2UH	. Pdia3	57 kDa		0.031	4 B	unkno															25	43	48	50
tattus norvegicus OX=10116 GN=Phyhipi PE=1 SV=2	Q6AYN4	Phyhipl	42 kDa		0.031	**	Rattus							•								(3)	7	7	8
us OX=10116 GN=Skp1 PE=1 SV=1	A0A0G2K4	Skp1	19 kDa		0.031	₽ ₩	unkno															18	(5)	(0)	(0)
SV=1	COJPT7	Fina	280 kDa	*	0.032	₽ ₽	unkno															22	57	63	69
116 GN=Sf3b2 PE=1 SV=3	D3ZMS1	Sf3b2	98 kDa		0.032	₽ ₽	unkno															4	7	8	8
iod2 PE=1 SV=1	P70566	Tmod2	39 kDa		0.032		Rattus		•		•			• •	•		•	•		•		9	(2)	(2)	(0)
=1 SV=1	P37397	Cnn3	36 kDa	*	0.032	₽ ₽	Rattus	••	•						•		•	٠		•		11	28	29	33
16 GN=Rpl14 PE=1 SV=1	BSDEM5 (+2)Rpl14	24 kDa		0.033	₽ ₽	unkno															(0)	(5)	(3)	(4)
16 GN=Rpl34 PE=1 SV=1	B2RZD4	Rpl34	13 kDa		0.033	₽ ₽	unkno															(0)	(5)	(3)	(4)
116 GN=Pgk1 PE=1 SV=2	P16617	Pgk1	45 kDa	*	0.033		Rattus							•					6 1	•		19	32	34	37
=Tubb5 PE=1 SV=1	P69897	Tubb5	50 kDa	*	0.034	☆ -	Rattus									•	•			•		65	26	15	10
16 GN=Rps25 PE=2 SV=1	P62853	Rps25	14 kDa		0.035	₽ ₽	Rattus	•					•	•	•	•	• •			•	•	(0)	17	12	14
ix2 PE=1 SV=1	A0A0G2JSH	9 Prdx2	22 kDa		0.035	₽ ₽	unkno															20	40	38	45
Dctn2 PE=1 SV=1	A0A0G2JU	Dctn2	46 kDa		0.035	4	unkno															4	21	16	21
s OX=10116 GN=Park7 PE=1 5V=1	088767	Park7	20 kDa		0.035	÷.	Rattus		••				•				• •		i	•		18	30	33	35
A OS=Rattus norvegicus OX=10116 GN=Vapa PE=1 SV=3	Q9Z270	Vapa	28 kDa		0.035	44	Rattus		•											•		10	(0)	(2)	(3)
GN=Syne1 PE=2 SV=1	A0A5P8DHK	45yne1	998 kDa		0.036	₽ ♠	unkno															(0)	(9)	11	13
icus 0X=10116 GN=Atp5me PE=1 SV=3	P29419	Atp5me	8 kDa		0.036	₽	Rattus	•	•	•							•			•		4	18	22	23
:b15b2 PE=2 5V=1	P97563	Tmsb1	5 kDa		0.036	40	unkno															(2)	11	9	9
0116 GN=Gja1 PE=1 SV=2	P08050	Gja1	43 kDa		0.037	₽	Rattus						• •					•		•		(1)	30	42	36
16 GN=Srsf1 PE=1 SV=1	D4A9L2	Srsf1	28 kDa		0.037	₽ ♠	unkno															6	25	21	19

171 ✓ ★ Serine/threonine-protein kinase PAK 2 05=F 172 ✓ △ Protein quaking 05=Rattus norvegicus 0X=1 173 ✓ ★ Endoplasmin 05=Rattus norvegicus 0X=101						
172 ✓ △ Protein quaking OS=Rattus norvegicus OX=1 173 ✓ ★ Endoplasmin OS=Rattus norvegicus OX=101	Cattus norvegicus UX=10116 GN=Pakz Pt=1 5V=1	Q64303	Pak2	58 kDa	0.038	10₽
173 🗹 \star Endoplasmin OS=Rattus norvegicus OX=101	10116 GN=Oki PE=1 5V=2	091XU1	Oki	38 kDa	0.038	₽ ♠
	16 GN=Hsp90b1 PE=1 SV=1	ADADAOMY	Hsp90b1	93 kDa 🔸	0.038	A
174 605 acidic ribosomal protein P2 05=Rattus m	torvegicus OX=10116 GN=Rplp2 PE=1 SV=2	P02401	Rolp2	12 kDa	0.038	4
175 V theat shock protein HSP 90-beta OS=Rattus	norvegicus OX=10116 GN=Hsp90ab1 PE=1 SV=4	P34058	Hsn90	83 kDa 🔸	0.038	Å .
176 A the second	OS=Rattus porvenicus OX=10116 GN=Rac1 PF=1 SV=1	A0A062K0	Ract	24 kDa	0.039	I.A
177 Fatty acid-binding protein brain OS=Pattus	nonvenicus OX=10116 GN=Fabr 7 DE=1 SV=2	P55051	Fabn7	15 kDa	0.039	ÅÅ.
178 Aby/PEE export factor OS=Pattus populariou	OV-10116 CN-Abref PE-1 SV-1	037787	Abreaf	20 kDa	0.040	ĂĂ.
170 Ally/Rel export actor 05-Rattas norvegicus	vic nonvogicus (V=10116 CN=Honacam DE=1 GV=3	032514	Honor	47 kDa	0.040	ĂÅ.
190 A cullin accepted MEDD& discontrated protein	1 05 = Dathus paperaisus 0Y=10116 CN=Cand1 PE=1 CV=1	007526	Candt	176 400	0.040	
100 V Cullin-associated NEDD8-dissociated protein	105-Rattus norvegicus 0x-10116 GN-Cand1 PE-1 5V-1	P97330	Callur	42402	0.040	
101 V Actin, cytoplasmic 1 05=Rattus norvegicus (JX=10116 GN=ACCD PE=1 SV=1	P60/11 (+) ACCD	15 400	0.040	The second secon
102 Uncharacterized protein US=Rattus norvegi	cus 0x=10116 PE=4 SV=1	D4A412		13 KDa	0.041	× Y
183 V 405 ribosomal protein 520 05=Rattus norve	gicus 0X=10116 GN=Rps20 PE=3 SV=1	AUAUHZUH	Rps20	13 KDa	0.041	T T
104 V D-3-phosphoglycerate denydrogenase US=R	tattus norvegicus 0X=10116 GN=Phgdh Pt=1 SV=3	008651	Pngan	SO KDa	0.041	*T
185 Yructose-bisphosphate aldolase A 05=Rattu	s norvegicus OX=10116 GN=Aldoa PE=1 SV=2	P05065	Aldoa	39 KDa 🙀	0.042	* <u>*</u> *
186 Matrin-3 OS=Rattus norvegicus OX=10116 G	IN=Matr3 PE=1 SV=2	P43244	Matr3	94 KDa	0.042	* **
187 🛃 🖈 Microtubule-associated protein RP/EB family	rember 1 05=Rattus norvegicus 0X=10116 GN=Mapre1 PE=1 SV=3	Q66HR2	Mapre1	30 kDa 🔺	0.043	**
188 🗹 🖈 Rab GDP dissociation inhibitor alpha OS=Rat	tus norvegicus OX=10116 GN=Gdi1 PE=1 SV=1	P50398	Gdi1	51 kDa 🔺	0.043	P
189 405 ribosomal protein S14 05=Rattus norver	gicus OX=10116 GN=Rps14 PE=2 SV=3	P13471 (+) Rps14	16 kDa	0.046	*P
190 🗹 🖈 Protein disulfide-isomerase A6 OS=Rattus no	orvegicus OX=10116 GN=Pdia6 PE=1 SV=1	A0A0G2JS	Pdia6	49 kDa	0.047	₽ ₽
191 🗹 🖈 Catalase OS=Rattus norvegicus OX=10116 G	N=Cat PE=1 SV=3	P04762	Cat	60 kDa 🔺	0.047	10 V
192 FUS RNA-binding protein OS=Rattus norvegia	cus OX=10116 GN=Fus PE=1 SV=1	Q5PQK2	Fus	53 kDa 🤺	0.050	₩1
193 🖌 🖈 4F2 cell-surface antigen heavy chain OS=Rat	ttus norvegicus OX=10116 GN=Slc3a2 PE=1 SV=1	Q794F9	Slc3a2	58 kDa	0.050	₽.
80% to 94% 50% to 79% 20% to 49%	k k let be	r nocess crocess contrained contr		ty svity svity	activity bhity activity	
0% to 19%	e ID Frveigi Groupin My Prvalue S) alexis S) alexi	system process in process in process abion abion close close pro- close paratus abion abio abio abio abio ab	e membran e part nembrane e ant activity	activity carrier activ regulator act haperone ac hunction	in transducer thirty tag al molecule ac on regulator - ther activity	
0% to 19%	Constant of the second se	minuura system (coalesation) coalesation coalesation coalesation multi-coalesation systemic process signal systemic process signal systemic process signal systemic coales try coalesatic co try coalesatic co strate and a coalesation systemic process signal systemic coalesation try coalesatic co strate and a coalesation strate and a coalesation	organelle membran organelle part plasma membrane fibosome antiooidant activity	catalytic activity electron carrier activity enzyme regulator act metallochaperone ad molecular function	melectuler transducer melectuler activity protein tag structural molecule ac transporter activity Cr	
0% to 19% con 0X=10116 GH=Pak2 PE=1 SV=1 PE=1 SV=2	CH-1000 CH-1000 <t< td=""><td>minume systems, personal control control multi-systems multi-systems personal control</td><td>organelle membran organelle part plasma membrane ribosome intoxidant activity intrino</td><td> statytic activity electron carrier activity insyme regulator act metallochaperore act molecular function </td><td>and the second s</td><td>) (3) (0) 5 19 18</td></t<>	minume systems, personal control control multi-systems multi-systems personal control	organelle membran organelle part plasma membrane ribosome intoxidant activity intrino	 statytic activity electron carrier activity insyme regulator act metallochaperore act molecular function 	and the second s) (3) (0) 5 19 18
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0% to 19% % % % % % % % % % % % % % % % % % %	1 1 0	телия з орбано состановановано состановановано состановано состановано состановано состановано состановано состановано состановано состановано состановано состановановано состановановановано состановановановановановановановано состановановановановано состановановановановано состановановановановано состановановановановано состановановановановановановановано состановановановановано состановано состановановановановано состанованованованованованованованованованов	organelle membran organelle part blassma membrane fibosome inchooldant activity	atalytic activity example activity intrymer activity intrymer activity interallichterator interallichterator interation	C 22 C 2000 C	r: (2) (2) (3) (0) 5 19 18 1 (4) (5) 2 64 75 6 11 13
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APPENDIX C: LIST OF ANOVA SIGNIFICANT PROTEINS AND FOLD CHANGES IN DAY 3 AND 7 GROUPS

************************************	Image: Second	Arthogene State		
egend: Note: N	Big Control Control <thcontrol< th=""> <thcontrol< th=""> <thcontr< td=""><td>Biological Process Cabladr Component union union union union union union autoria union <td< td=""><td>Hotecodar Function</td><td>Controls Day 3 Triak Day 2 Triak (1)</td></td<></td></thcontr<></thcontrol<></thcontrol<>	Biological Process Cabladr Component union union union union union union autoria union <td< td=""><td>Hotecodar Function</td><td>Controls Day 3 Triak Day 2 Triak (1)</td></td<>	Hotecodar Function	Controls Day 3 Triak Day 2 Triak (1)

35	2	Venentin 05-Rattus norvegicus 0X-10116 GH-Ven PE=1 SV-2	P31000	Vara	541Ce	٠	0.025	**
36	×.	Histone HQB 05=Rattus norvegicus 0X=30116 GR=Hist3h2bk PE=3 SF=1	GWKZ	Hist3h2bk	141Ca	٠	0.025	**
37	8	T-complex protein 1 subunit beta 05=Rattus norvegicus 0X=30116 GN=Cct2 PE=1 SV=3	Q50(3P49	602	57 kDa		0.027	44
38	æ	Peptidyli prolyl cis trans isomerase PKBP1A 05=Rattus norvegicus 0K=10116 GN=Fkbp1a PE=1 5V=3	Q62658	Fkhpla	12 KDa		6.831	40
39	æ	Calmodulm-3 05=Rattus norvegicus 0K=10116 GN=Calm3 PE=1 SV=1	P00#31	Calm3	17 iOe		0.031	**
-0	×	Hetastasis associated protein MTA1 Q5::Rattas norvegicus QX::10116 GR::Mta1 PE:1 SY::1	ADA340TA9	FRa1	83 kDa		6.833	**
41	8	Dihydropyrinidinase-related protein 2 05=Rattus norvegicus 00=18116 GR=Dpysi2 PE=1 SH=1	P47942	David	62 kDe		6.633	**
42	×	Histone H28 05=Rattus norvegicus 0X=38116 GR=Hist3h2bl PE=3 SY=1	P108.4L7	Histshah	14 i De	٠	6.633	**
43	8	Cleavage and polyademylation specificity factor subunit 5 05::Rattus norvegicus 0X:10116 GR=Budt21 PE=2 5V=1	847764	Mudt21	25 kDe		6.034	4.0
-44	×	RCG35135, isoform CRA_b 05+Rattus norvegicus 0K+10116 G8+Tin1 PE=1 5V+1	G3V852	Tint	270 kCe	٠	0.035	4.
45	æ	Eukaryotic translation initiation factor 4H 05=Rattus norvegicus OE=10136 GH=EI4h PE=1 SV=1	Q5X372	Eddh	27 kDa		6.036	**
-	S	Brain acid soluble protein 1 05=Rattus norvegicus OK=10116 GN=Basp1 PE=1 5V=2	Q05175	Basp1	22 KD#	٠	8.636	4.0
47	æ	Peptidyl-prolyl cis-trans isomerase OS=Rattus norvegicus OX=30116 GR=Pin1 PE=1 SV=1	806NE.2	Pin1	18 kDe		6.036	**
-48	×	# 30 kDa heat shock protein, mitochondrial 05=Rattan norvegicus 0X=30116 GR=Hspc1 PE=1 SV=1	ANANG23TG	1. Huger 1	9 kDa		0.037	4.
-9	æ	Centromere protein V OS=Rattus norvegicus OX=38116 GR=Cenpv PE=3 SY=3	D4ASA3	Cengw	28 kDa		6.038	4.0
50	×	Alpha-enolase OS=Rattus sorvegicus OK=10116 GN=Eno1 PE=1 SV=4	P04764 (+1)	fmo1	471Oa	٠	6.038	**
51	×	Hatrin 3 05=Rattus norvegicus 0K=30336 G8=Hatr3 PE=1 5V=2	P43244	Hefr3	941Ca		6.040	**
52	S	Transient receptor potential cation channel subfamily V member 4 05=Rattus norvegicus 0X=38116 GN=Trpv4 PE=1 SV=1	Q9ER.ZB	Trput	58 kDa		0.041	**
53	×	ATP synthase membrane subunit DAPET, mitochondrial OS=Rattus norvegicus OK=10136 G8=AIp5md PE=1 SV=1	Q937W3	Alg5md	6 kDa		6.641	40
54	æ	Av/REF export factor 05=Rattas norvegicas 0X=38116 GR=Alyref PE=1 SV=1	032007	Adynet	20 kQre		0.042	40
55	×	Cytochrome c exidase subunit 4 isoform 1, mitochondrial 05=Rattas norvegicus 0X=38116 GR=Cex461 PC=1 SY=1	P10688	Cautio	20 kOa		6.642	**
56	×	605 acidic ribosomal protein P1 05=Rattus norvegicus 0X=10116 GN=Ralp1 PE=3 5V=1	P19944	Rplp1	11 kDe		6.643	**
57	æ	Isoaspartyl peptidase/L-asparaginase 05=Rattus norvegicus 0X=10116 GR=Asrgl1 Pt=1 SV=1	Q8V304	Asrgil	34 KDa		6.643	**
58	×	Calponin-3 05=ikattus norvegicus 0X=10116 GR=Cnn3 PE=1 SV=1	833397	Can3	36 kDe	٠	6.643	**
59	×	Polypyrinidine tract-binding protein 1 05=Rattas norvegicus 0X=38116 GN=Ptbp1 Pt=1 5V=1	ABABG2JTV	.Pthpi	19 iCa		6.045	4.
60	×	Hacrophage migration inhibitory factor OS=Rattus norvegicus OX=30116 GR=PHF PE=1 SV=4	P30904	Pod	12 kDe		6.546	40
61	×	EF-band domain-containing protein D2 05=Rattus norvegicus OK=10116 GN=Efbd2 PE=1 5V=1	Q4FZYB	Ell-42	27 kDa		0.046	**
Display Options:	uantit	ative Value (Normalized Total Spectra) v Req Mods: No Filter v Search:						

		-		Biological Process					-	Cellular Component					Molecul	ar Euroction	60	strole	0	Day 3 Trials			Day 7 Trials			
egeno: 4% 4% 9% 9%	Alternate ID	Molecular Weight	Protein Grouping Ambiguity ANOVA Test (p-value) (p < 0.05)	Quantitative Profile	biological adhesion biological regulation	cellular process developmental process establishment of localization	growth mmune system process ocalization	ocomotion metabolic process multi-organism process multi-elluter provintenal process	bigmentation reproduction reproductive process response to stimulus	viral process Golgi apparatus	cytophasm cytoskaleton endoplasmic reticulum	extracellular region intracellular organelle membrane	nucleus organelle membrane	organelle part plasma membrane nbosome	antioxidant activity binding catalotic activity	electron carrier activity enzyme regulator activity metallochaperone activity	morecular function molecular transducer activity motor activity pructural molecule activity translation regulator activity	transporter activity C3	0	5	E.	13	0	214	14	
I1 PE=1 SV=1 P63170	Dynll1	10 kDa	* 0.00	Ratt	US 0	• •			00			• •	•	•		•	• •	(0)	(0)	(0)	(2)	(3)	7	8	7	
3 PE=1 SV=2 P08009	Gstm3	26 kDa	* 0.00	Ratt	US	•					•				•			(0)	(0)	(0)	(0)	(0)	8	5	5	
=Usp9x PE=1 SV=1 A0A0G28	2 Usp9x	290 kDa	0.00	unkr	10													(4)	3	(0)	(0)	(0)	(0)	(0)	(0)	
Q7ТРКО	Fdps	98 kDa	* 0.00	unkr	10													(5)	3	(0)	(0)	(0)	(0)	(0)	(0)	
Nasp PE=1 SV=1 Q66HD3	Nasp	84kDa	0.00	Ratt	us	•••	•	•	•••				•	•				11	11	10	13	15	(3)	(3)	(2)	
Hist1h1c PE=1 SV=1 A0A0G2	654 Hist1h1c	21 KDa	* 0.00	Ratt	US •								•					68	15	191	1/9	201	220	242	184	
=1 SV=1 P9/544 (+1) Pipp3	35 KD8	0.00	Ratt	us	•					•	••		••				(0)	(0)	1/	13	9	16	18	18	
-150-1 GJ076	Ymbag	28 kDa	+ 0.00	Datt	10													(24)	17	40	33	36	20	20	30	
V-2 P01903	Nedds	9 kDa	0.00	Ratt											- C -			(0)	(0)	(0)	(2)	(2)	5	5	5	
OPVIIS	Oga	103kDa	+ 0.00	Patt														(7)	4	(0)	(0)	(0)	(0)	(0)	(0)	
N=Fubo1 PE=1 SV=1 A0A140T	Al. Fubpl	68 kDa	* 0.00	unkr	0													(0)	3	25	21	16	13	12	13	
OX=10116 GN=Hibadh PE=1 SV=3 P29266	Hibadh	35 kDa	0.00	Ratt	us	•						•						(0)	(0)	(0)	(0)	(0)	(3)	5	5	
norvegicus 0X=10116 GN=Atp6v1e1 PE=1 SV=1 G3V7L8 (+1) Atp6y	26 kDa	0.00	unkr	0													(0)	(2)	(0)	(0)	(0)	3	3	4	
1 4040623	Z13 Cttn	53 kDa	0.00	unkr	0													(0)	(0)	(0)	(2)	(2)	5	4	5	
D4A3K5	H1-1	22 kDa	* 0.00	Ratt	us •	•						•	•	•				(0)	(0)	(30)	50	57	47	50	48	
X=10116 GN=Entpd2 PE=1 SV=1 F1M7N2	+1)Entpd2	60 kDa	0.00	unkr	IO													(0)	(0)	(0)	(0)	(0)	6	4	(3)	
Rps27a PE=1 SV=2 P62982	Rps27a	18 kDa	* 0.00	Ratt	US	•				1.1		•	•	• •				(0)	(0)	(0)	(0)	(2)	3	5	5	
pacam PE=1 SV=2 D3ZEI4	Hepac	47 kDa	0.00	unkr	io													(4)	(0)	17	9	10	24	19	27	
P0DP31	Calm3	17 kDa	0.00	Ratt	US 0	• •	•		•					• •		•		22	38	(0)	(3)	(6)	(6)	(5)	(6)	
1 SV=1 A0A0H2U	H Rab6a	24 kDa	* 0.00	unkr	IO													(0)	(0)	(0)	(0)	(2)	3	3	4	
ah11 PE=4 SV=3 E9PU24	Dnah11	516 kDa	0.00	unkr	0													(0)	(0)	(0)	(0)	(0)	4	3	(2)	
P02769	ALB	69 kDa	* 0.00	Bost	a •	•			•		•	•						49	18	79	86	80	91	86	83	
I=Rrm1 PE=1 SV=1 Q5U2Q5	Rrm1	90 kDa	0.00	unkr	10													(4)	2	(0)	(0)	(0)	(0)	(0)	(0)	
1 SV=2 P16617	Pgk1	45 kDa	* 0.00	Ratt	us •	•						100						18	15	30	33	26	25	27	29	
D116 GN=Idh1 PE=1 SV=1 P41562	Idh1	47 kDa	0.00	Ratt	US •		100				1.25							(4)	7	(0)	(2)	(2)	(0)	(0)	(0)	
6 GN=Fkbp1a PE=1 SV=3 Q62658	Fkbp1a	12 kDa	0.00	Ratt	US •	•••	••	•	•		•	••	•	••				(0)	4	13	11	10	4	6	7	
na3 PE=1 SV=3 P18422 (+1) Psma3	28 KDa	0.00	Ratt	US •	•							•					(0)	(0)	(0)	(0)	(0)	5	1	(3)	
10 GR=ECI1 PE=1 SV=1 P23965 (+1) tol	SZ KUB		Ratt	US									-				(0)	(0)	(0)	(2)	(3)	0	7	7	
P9/563	Chr1	31 10a	+ 0.00	Unkr	0													(0)	(2)	(3)	6	7	(0)	(0)	(0)	
-13-2 P4/2/	Dhal	7240+	0.00	Ratt										-				(0)	6	17	14	14	10	0	12	
C6L8E0 P25886	Pol29	17k0a	+ 0.00	Datt	10													(0)	(0)	(0)	(0)	(0)	(2)	4	12	
116 GN=Hnmpa1 PE=1 SV=3 P04256 (+1) Hnrnpal	34kDa	* 0.00	Ratt	us •													29	37	30	24	27	18	17	20	
69	🗹 🜟 Lamina-associated polypeptide 2, isoform beta OS=Rattus norvegicus OX=10116 GN=Tmpo PE=1 SV=3	Q62733	Tmpo	50 kDa	0.00	₽₽																				
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70	🗹 ★ Sodium/potassium-transporting ATPase subunit alpha-2 05=Rattus norvegicus 0X=10116 GN=Atp1a2 PE=1 5V=1	P06686	Atp1a2	112 kDa	* 0.00	₽₽																				
71	🗹 ★ ADP/ATP translocase 1 05=Rattus norvegicus 0X=10116 GN=5lc25a4 PE=1 SV=1	Q6P9Y4	SIc25a4	33 kDa	* 0.00	₽₽																				
72	🗹 \star Reticulon 05=Rattus norvegicus 0X=10116 GN=Rtn3 PE=1 SV=1	A0A0H2UH	. Rtn3	28 kDa	0.00	₽11																				
73	🗹 ★ UTP20 small subunit processome component 05=Rattus norvegicus 0X=10116 GN=Utp20 PE=1 5V=3	F1LXT3	Utp20	317 kDa	0.012	₽₽																				
74	🗹 🜟 Microtubule-associated protein 4 05=Rattus norvegicus 0X=10116 GN=Map4 PE=1 5V=1	Q5M7W5	Map4	110 kDa	* 0.012	₽ ₽ ↑																				
75	🗹 ★ Breast carcinoma-amplified sequence 1 homolog 05=Rattus norvegicus 0X=10116 GN=Bcas1 PE=1 SV=1	A0A0G2K07	9 Bcas1	38 kDa	* 0.013	₽₽0																				
76	🗹 ★ Thymosin beta-10 05=Rattus norvegicus 0X=10116 GN=Tmsb10 PE=1 5V=2	P63312	Tmsb10	5 kDa	* 0.013	₽₽₽																				
77	🗹 ★ Erythrocyte membrane protein band 4.1-like 2 05=Rattus norvegicus 0X=10116 GN=Epb41l2 PE=1 SV=3	D3ZDT1	Epb41l2	103 kDa	* 0.013	₽₽																				
78	🗹 🜟 605 ribosomal protein L24 OS=Rattus norvegicus OX=10116 GN=Rpl24 PE=2 SV=1	P83732	Rpl24	18 kDa	0.013	₽₽																				
79	🗹 🖈 Splicing factor 1 OS=Rattus norvegicus OX=10116 GN=Sf1 PE=1 SV=2	F1LSC3	Sf1	68 kDa	0.013	₽₽ ₽																				
80	🗹 ★ N-myc downstream regulated gene 2, isoform CRA_b 05=Rattus norvegicus 0X=10116 GN=Ndrg2 PE=1 SV=1	A0A0G2J5	Ndrg2	39 kDa	0.015	☆₽☆																				
81	🗹 🖈 Brain acid soluble protein 1 05=Rattus norvegicus 0X=10116 GN=Basp1 PE=1 5V=2	Q05175	Basp1	22 kDa	* 0.015	₽ ₩																				
82	🗹 🜟 Complexin-2 OS=Rattus norvegicus OX=10116 GN=Cplx2 PE=1 SV=1	P84087	Cplx2	15 kDa	0.015	₽₽₽																				
83	🗹 🜟 RCG34610, isoform CRA_c OS=Rattus norvegicus OX=10116 GN=Srsf1 PE=1 SV=1	D4A9L2	Srsf1	28 kDa	0.015	₽₽																				
84	🗹 ★ Heterogeneous nuclear ribonucleoprotein H OS=Rattus norvegicus OX=10116 GN=Hnrnph1 PE=1 SV=1	A0A0G2JTG	7 Hnrnph1	49 kDa	* 0.016	☆ ₩																				
85	🗹 🖈 Heterogeneous nuclear ribonucleoprotein A3 05=Rattus norvegicus 0X=10116 GN=Hnrnpa3 PE=1 5V=1	Q6URK4	Hnrnpa3	40 kDa	* 0.016	☆₽₽																				
86	🗹 🖈 Glutamine synthetase 05=Rattus norvegicus 0X=10116 GN=Glul PE=1 SV=3	P09606	Glul	42 kDa	0.016	₽₽₽																				
87	🗹 ★ ATP synthase protein 8 05=Rattus norvegicus 0X=10116 GN=ATP8 PE=3 5V=1	Q5UAJ5	ATP8	8 kDa	0.017	₽00																				
88	🗹 🖈 RALY heterogeneous nuclear ribonucleoprotein OS=Rattus norvegicus OX=10116 GN=Raly PE=1 5V=2	E9PTI6 (+1)	Raly	33 kDa	0.018	₽₽ ₽																				
89	🗹 🖈 Vimentin OS=Rattus norvegicus OX=10116 GN=Vim PE=1 SV=2	P31000	Vim	54 kDa	* 0.018	☆₽₽																				
90	🗹 🜟 Proteasome subunit beta type-1 05=Rattus norvegicus 0X=10116 GN=Psmb1 PE=1 5V=3	P18421 (+1)) Psmb1	26 kDa	0.018	₽₽																				
91	🗹 🖈 Matrin-3 OS=Rattus norvegicus OX=10116 GN=Matr3 PE=1 5V=2	P43244	Matr3	94 kDa	0.019	₽₽ ₽																				
92	🗹 🖈 Neural cell adhesion molecule 1 05=Rattus norvegicus 0X=10116 GN=Ncam1 PE=1 SV=1	P13596	Ncam1	95 kDa	0.019	₽₽																				
93	🗹 🜟 Heat shock cognate 71 kDa protein OS=Rattus norvegicus OX=10116 GN=Hspa8 PE=3 SV=3	D4A453 (+1)Hspa8	71 kDa	* 0.023	₽ ₩																				
94	🗹 🜟 605 ribosomal protein L19 05=Rattus norvegicus 0X=10116 GN=Rpl19 PE=1 SV=1	P84100	Rpl19	23 kDa	0.023	₽₽																				
95	🗹 ★ 605 ribosomal protein L7a OS=Rattus norvegicus OX=10116 GN=Rpl7a PE=4 SV=2	F1M013	Rpl7a	30 kDa	* 0.023	₽₽																				
96	🗹 🜟 Sodium/potassium-transporting ATPase subunit alpha-1 05=Rattus norvegicus 0X=10116 GN=Atp1a1 PE=1 SV=1	P06685	Atp1a1	113 kDa	* 0.024	₽₽																				
97	🗹 🖈 Plectin OS=Rattus norvegicus OX=10116 GN=Plec PE=1 SV=1	Q653A0	Plec	534 kDa	* 0.024	☆₽☆																				
98	🗹 ★ Syntaxin-12 OS=Rattus norvegicus OX=10116 GN=Stx12 PE=1 SV=1	A0A0G2JVB	5 Stx12	32 kDa	0.025	₽₽																				
99	🗹 🚖 Isoaspartyl peptidase/L-asparaginase 05=Rattus norvegicus 0X=10116 GN=Asrgl1 PE=1 5V=1	Q8VI04	Asrgl1	34 kDa	0.027	₽₽																				
100	🗹 🜟 Transcription intermediary factor 1-beta 05=Rattus norvegicus 0X=10116 GN=Trim28 PE=1 SV=2	008629	Trim28	89 kDa	0.027	₽₽																				
101	🗹 🚖 Cleavage and polyadenylation specificity factor subunit 5 05=Rattus norvegicus 0X=10116 GN=Nudt21 PE=2 5V=1	B4F764	Nudt21	26 kDa	0.027	₽û																				
102	🗹 🛧 Proteasome subunit alpha type-1 OS=Rattus norvegicus OX=10116 GN=Psma1 PE=1 5V=2	P18420	Psma1	30 kDa	0.027	₽ ��																				
Display Option	s: Quantitative Value (Normalized Total Spectra) v Req Mods: No Filter v Search:																									
ogond:	Biological Process Cellular Component	Molecular Function	Con	trols	Day 3 Trials	Day 7 Trials																				
eyenu:																										

75 14% 19% 19%	Accession Number	Atemste ID	Molecular Weight	Protein Grouping Anbiguity ANOVA Test (p-value) (p < 0.05)	Quantitative Profile Taxonomy	biological adhesion biological regulation	developmental process establishment of localization	growth mmune system process ocalization	ocomotion metabolic process sudti-organism process sudti-adhidar process	bigmentation reproduction reproductive process	response to stimulus hiythmic process viral process	uoogi apperatus cytoplesin cytoblesinic reticulum endoplasmic reticulum	extracellular region ntracellular organelle wentrane witochondrion	nucleus organelle membrane organelle pært	plasma membrane fibosome antioxidant activity	onding catalytic activity electron carrier activity enzyme regulator activity	metallochaperone activity molecular function molecular transducer activity motor activity	protein tag structural molecule activity translation regulator activity resonanter activity	ranspurer scurr,r C3	0	5	N3	5	2	20	24
0116 GN=Tmpo PE=1 SV=3	Q62733	Tmpo	50 kDa	0.00	Rattu	5 C											· · · · · · · · · · · · · · · · · · ·		(4)	12	23	27	22	14	20	15
icus OX=10116 GN=Atp1a2 PE=1 SV=1	P06686	Atp1a2	112 kDa	* 0.00	Rattu	5 • •	٠	•	• •	6 B	•	• •			•	• •	•		(7)	31	18	35	45	69	69	73
SV=1	Q6P9Y4	Slc25a4	33 kDa	* 0.00	unkno)													(16)	7	(0)	(9)	(11)	33	24	32
	A0A0H2UH	L., Rtn3	28 kDa	0.00	unkno														(0)	(0)	(8)	(6)	12	10	9	9
116 GN=Utp20 PE=1 SV=3	F1LXT3	Utp20	317 kDa	0.012	unkno)													(0)	(0)	(0)	(0)	(0)	(2)	4	(3)
04 PE=1 SV=1	Q5M7W5	Map4	110 kDa	* 0.012	Rattu	5			•				•				•		(0)	(0)	(0)	(9)	(0)	(15)	27	32
=10116 GN=Bcas1 PE=1 SV=1	A0A0G2K0	79 Bcas1	38 kDa	* 0.013	unkno)													(0)	9	(5)	6	6	15	17	18
2	P63312	Tmsb10	5 kDa	* 0.013	Rattu	5 • •	•						•				•		(7)	7	20	14	14	5	8	9
0116 GN=Epb41l2 PE=1 SV=3	D3ZDT1	Epb41l2	103 kDa	* 0.013	unkno														11	14	8	11	12	20	16	17
2 SV=1	P83732	Rpl24	18 kDa	0.013	Rattu	s 🔍							•		•	•	•		(0)	(0)	(0)	(2)	8	10	13	11
	F1LSC3	SH	68 kDa	0.013	unkno)													(7)	(1)	18	15	12	6	9	7
OX=10116 GN=Ndrg2 PE=1 SV=1	A0A0G235	- Ndrg2	39 kDa	0.015	unkno)													25	17	(7)	9	6	15	12	16
1 SV=2	Q05175	Basp1	22 kDa	* 0.015	Rattu	S •						•	••	• •	•		•		(0)	14	33	33	24	29	30	38
	P84087	Cpbx2	15 kDa	0.015	Rattu	5 • •	•	••			•	•	• •	•		•			(0)	(0)	8	7	(4)	(0)	(3)	(2)
SV=1	D4A9L2	Srsf1	28 kDa	0.015	unkno)													(9)	5	(7)	12	11	19	16	15
16 GN=Hnrnph1 PE=1 SV=1	A0A0G2JT	G7 Hnrnph1	49 kDa	* 0.016	unkno														11	19	(0)	6	8	(0)	(0)	(0)
116 GN=Hnrnpa3 PE=1 SV=1	Q6URK4	Hnrnpa3	40 kDa	* 0.016	Rattu	5	•	•					•	• •					25	32	15	20	23	10	11	15
	P09606	Glul	42 kDa	0.016	Rattu	s • •	•		•		•	• •	•••		•	••			(0)	(0)	(0)	13	10	17	17	19
=1	Q5UAJ5	ATP8	8 kDa	0.017	unkno														(0)	(0)	(0)	20	25	35	32	41
10116 GN=Raly PE=1 5V=2	E9PTI6 (+:	1) Raly	33 kDa	0.018	unkno)													(0)	(0)	- 7	12	9	7	3	(2)
	P31000	Vim	54 kDa	* 0.018	Rattu	5	•	•	••		•	••	•	• •					232	432	104	75	63	107	114	127
b1 PE=1 SV=3	P18421 (+	1) Psmb1	26 kDa	0.018	Rattu	5 ·							•	•		•			(0)	(0)	(0)	(0)	(0)	(3)	7	(3)
	P43244	Matr3	94 kDa	0.019	Rattu	S		•			•		•	•••		2 C			34	12	52	47	48	29	29	24
1 PE=1 SV=1	P13596	Ncam1	95 kDa	0.019	Rattu	5 • • • •	•		••		•		•		•	•	•		(0)	21	12	11	11	33	34	31
spa8 PE=3 SV=3	D4A453 (+	1)Hspa8	/1 kDa	* 0.023	unkno				1.1				-	1.1	12				16	29	33	64	76	78	76	89
SV=1	P84100	Rpl19	23 kDa	0.023	Rattu	5	•		•				•		•				(0)	(2)	(0)	5	12	15	15	15
SV=2	F1M013	Rpl7a	30 808	* 0.023	unkno						a 1								(0)	2	(0)	(2)	12	22	1/	14
KUS 0X=10116 GN=Atp1a1 PE=1 SV=1	P06685	Atp1a1	113 KD8	* 0.024	Rattu	S • •	•	•	•		•		••		•			•	(0)	19	(0)	(0)	20	35	28	- 39
	Q653A0	Plec	534 KDa	* 0.024	unkno)													(0)	49	(U)	(2)	(0)	61	4/	56
	AUA0G2JV	86 Stx12	SZ KDa	0.025	unkno)													(0)	(0)	(0)	(0)	(U)			12)
=Asrgil Pt=1 SV=1	Q8VI04	Asrgl1	on kDa	0.027	Rattu	5													(7)	(0)	(0)	(0)	(2)	10	8	8
5N=Trim28 PE=1 5V=2	008629	Trim28	ay kDa	0.027	Rattu	S • •	•	••	•••		•		•	• •		•			18	10	25	35	38	17	21	23
picus 0X=10116 GN=Nudt21 PE=2 SV=1	841764	nudt21	20 kDa	0.027	unkno									10 NO					(0)	(0)	(5)	(4)		12)	3	(0)
na1 Pt=1 5V=2	P18420	Psma1	30 kDa	0.027	Rattu	S • •		•					•	• •		•			(5)	(0)	10	1	11	9	8	8

| | 103 | V . | t Cla | athrin light chain OS=Rattus
 | norvegic | us OX= | 10116 | GN=Clta
 | PE=2 SV=1 | | |
 | | | |
 | | 05 | PPP1 | Clta
 | 24 kDa
 | | 0.027 | ₽₽₽ | |
|--|---|---|--
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| | 104 | | Alc | do-keto reductase family 1
 | member B | 31 OS=F | Rattus | norvegi
 | cus 0X=10116 | GN=Ak | r1b1 P | E=1 5
 | V=3 | | |
 | | PO | 7943 | Akr1b1
 | 36 kDa
 | | 0.028 | i i A | |
| | 105 | | Ac | tin, alpha skeletal muscle O
 | S=Rattus | norven | icus 0 | =1011
 | 6 GN=Acta1 PE | =1 SV= | 1 |
 | - | | |
 | | P6 | 8136 | Acta1
 | 42 kDa
 | + | 0.029 | ÅÅÅ | |
| | 106 | | Nu | cleophosmin 05=Rattus nor
 | venicus (| DX=101 | 16 GN= | Nom1 F
 | F=1 SV=1 | | - |
 | | | |
 | | P1 | 3084 | Nom1
 | 33 kDa
 | | 0.029 | Å Å Å | |
| | 107 | | He | eterogeneous nuclear ribonu
 | cleonrote | in F OS | =Rattu | s norve
 | aicus OX=101 | 16 GN=1 | Hornof | FPF=1
 | SV=3 | | |
 | | 07 | 94F4 | Hornof
 | 46 kDa
 | | 0.029 | ÅĨĨ | |
| | 108 | | Su | ineroxide dismutase [Cu-7n]
 | OS=Ratt | tus norv | renicus | OX=10
 | 116 GN=Sod1 | PE=1 SV | /=2 |
 | 51-5 | | |
 | | PO | 7632 | Sod1
 | 16 kDa
 | | 0.029 | Å ÅÅ | |
| | 109 | | Vin | nculin 05=Rattus norvegicus
 | OX=101 | 16 GN= | Vcl PF= | 1 SV=1
 | | | |
 | | | |
 | | P8 | 5972 | Vd
 | 117 kDa
 | | 0.030 | Å ÅÅ | |
| | 110 | | Pre | otein-I-isoaspartate 0-met
 | hyltransfe | erase O | S=Rat | US DOD
 | enicus OX=10 | 116 GN= | Pent | 1 PF=1
 | SV=1 | | |
 | | 40 | A140TAB | 9 Pcmt1
 | 21 kDa
 | | 0.030 | Ĩ A A | |
| | 111 | | 60 | S ribosomal protein 111 05
 | -Pattus n | onvenic | UE OY= | 10116
 | GN=Rol11 PF= | 1 SV=2 | i cinc |
 | | | |
 | | PG | 2014 | Pol11
 | 20 kDa
 | | 0.031 | ÅII | |
| | 112 | | Le | ucine-rich repeat-containing
 | 47.05=6 | attus | onveni | CUS OX=
 | =10116 GN=1 m | rc47 PE: | =1 SV= | -2
 | | | |
 | | FI | 1749 | Irrc47
 | 64 kDa
 | | 0.031 | ÅÅÅ | |
| | 113 | | De | otain disulfida-isomarase A
 | ROS-Datt | tue non | renicue | OY-10
 | 116 CN-Rdia3 | DE-1 C | V-2 |
 | | | |
 | | D1 | 1508 | Ddia3
 | 57kDa
 | | 0.032 | i à à | |
| | 114 | | | WCE delta catenin family m
 | ambar OF | -Datte | regicus
c nom | Daicus (
 | V-10116 CN- | Amerf D | -1 -1 | 1-1
 | | | |
 | | PA | 5752 | Amof
 | 105 kDa
 | | 0.032 | ÅÅÅ | |
| | 115 | | Ha | aterochromatin protein 1-bir
 | ding prot | tain 3 Of | C-DaH | cylcus o
 | agicus OV-10 | 116 CN- | -Hoth | 03 DE-
 | 1 SV-1 | | |
 | | 04 | 0747 | Hotho?
 | 61 kDa
 | | 0.034 | ĂĂĂ | |
| | 116 | | Ac | idis (Lousing rich) pustors a
 | hosphore | etain 2 | 2 famil |
 | han A OC-Datt | ITO GH- | - npion | OV-1
 | 01160 | - Ann 22- | DE-2 C | 1-1
 | | 05 | DDHO | App222
 | 20 40a
 | | 0.035 | ĂĂĂ. | |
| | 117 | | AC | duc (Leucine-rich) huciear p
 | lospilopr | in D lile | | y, mem
 | Der A US-Katt | -1011C | CN-U |
 | 0110 0 | -Anpoza | 172-23 | -1
 | | Q3 | CWIIZ | Hipsza
 | 25 400
 | 1 | 0.035 | | |
| | 110 | | ne | eterogeneous nuclear Hoonu
 | cleoprote | OV-IR | e 05-1 | accus i
 | orvegicus UX- | -10110 | GN-HI | nrnpai
 | PC-15 | V-1 | |
 | | 45 | SWUS | nimpai
 | 25 kDa
 | ~ | 0.030 | XXX | |
| | 110 | | Pe | roxiredoxin-6 US=Rattus no
 | orvegicus | UX=10. | 116 GN | =Prax6
 | PE=1 SV=3 | | |
 | | | |
 | | 03 | 5244 | Praxo
 | 25 KDd
 | | 0.038 | Y YY | |
| | 120 | | AC | yi-CoA-binding protein US=
 | kattus no | rvegicu | SUX= | 10110 G
 | N=DDIPE=1 St | V=3 | |
 | | | |
 | | PI | 1030 | DDI
 | 2410
 | | 0.040 | XXX | |
| | 120 | | Ra | as-related C3 botulinum toxi
 | n substra | te 1 05 | =Rattu | is norve
 | gicus OX=101 | 16 GN=1 | Raci P | E=1 5
 | /=1 | | |
 | | AU | AUG2KU | Raci
 | 24 KDa
 | | 0.040 | YYY | |
| | 121 | | r Sin | milar to RIKEN CDNA 231002
 | 2805 05= | Rattus | norveg | ICUS OX
 | =10116 GN=R | GD1559 | 896 Pt | E=1 5V
 | =1 | Dr-1 CV- | |
 | | 03 | 244/ | RGD15
 | 36 KDa
 | | 0.040 | | |
| | 122 | | * Pin | tochondrial import receptor
 | Subunit I | 1011221 | nomolo | g 05=R
 | attus norvegi | cus OX= | 10116 | GN=1
 | omm24 | PE=1 SV= | =1 |
 | | Q | 5Q41 | Iomm
 | 15 KDa
 | | 0.041 | Y | |
| | 123 | | Pro | oteasome subunit alpha typ
 | e OS=Rat | ttus nor | vegicu | s 0X=10
 | D116 GN=Psma | a5 PE=1 | SV=1 |
 | | | |
 | | Qe | P9V6 | Psma5
 | 26 KDa
 | | 0.042 | | |
| | 124 | | * Pro | otein disulfide-isomerase 05
 | s=Rattus | norveg | icus OX | =10116
 | GN=P4hb PE= | =1 SV=2 | |
 | | | |
 | | PO | 4/85 | P4hb
 | 57 KDa
 | | 0.043 | **v | |
| | 125 | | Ph | osphoglycerate mutase 1 0
 | S=Rattus | norveg | picus O | x=1011
 | 6 GN=Pgam1 F | PE=1 SV | =4 |
 | | | |
 | | P2 | 5113 | Pgam1
 | 29 KDa
 | * | 0.043 | 🔶 ជួ ជួ | |
| | 126 | | r Pro | othymosin alpha OS=Rattus
 | norvegic | us OX= | 10116 | GN=Ptn
 | na PE=1 SV=2 | | |
 | | | |
 | | PO | 6302 | Ptma
 | 12 kDa
 | | 0.045 | ប់ប់🕂 | |
| | 127 | v | Pe | eptidylprolyl isomerase 05=1
 | Rattus no | rvegicu | s OX=1 | 0116 G
 | N=Fkbp3 PE=1 | SV=1 | |
 | | | |
 | | G3 | V6L9 | Fkbp3
 | 25 kDa
 | | 0.046 | ₩ûû | |
| | 128 | - | k He | eterogeneous nuclear ribonu
 | cleoprote | ein D0 O | S=Rat | tus norv
 | regicus OX=10 | 116 GN | =Hnrn | pd PE=
 | 1 SV=2 | | |
 | | Q9 | 3354 | Hnrnpd
 | 38 kDa
 | | 0.048 | ╈╈₽ | |
| | 129 | ~ | 🖈 Mie | icrotubule-associated protei
 | in OS=Rat | ttus nor | vegicu | s OX=10
 | 0116 GN=Map | 2 PE=1 9 | 5V=3 |
 | | | |
 | | F1 | LNKO | Map2
 | 211 kDa
 | * | 0.049 | ♥✿✿ | |
| | | on a little birt | in Mallin. | An and the set of the | Concernant and a start of the s | And and the second
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 | 0.0 | | | |
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 | Accession Number | Alternate ID | Molecular Weight | Protein Grouping Ambiguity
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