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Original Article

Retinal proteomic changes under different ischemic conditions – implication of an epigenetic regulatory mechanism

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Abstract: In retina, an ischemic injury-resistant condition (ischemic tolerance) can be induced by a sub-lethal ischemic treatment (preconditioning) prior to an otherwise injurious ischemic insult. In this work, we compared retinal proteomic changes under three different ischemic conditions, as a means to identify the effector mechanisms that underlie retinal ischemic tolerance. Transient retinal ischemia was induced by elevating the intraocular pressure (IOP) in three groups of adult rats as follows: Group 1, ischemic-preconditioned, 110 mmHg for 8 minutes followed by 48 hours reperfusion; Group 2, ischemic-injured, 110 mmHg for 60 minutes followed by 24 hours reperfusion; Group 3, ischemic-tolerant, preconditioning treatment followed by another 60 minutes of 110 mmHg and 24 hours reperfusion. Protein quantities in retinas from each of the afore-mentioned retinal ischemic conditions, as determined by quantitative mass spectrometry, were compared with that of the contralateral control eyes (sham-treated). As a result, a total of 328 proteins were identified and quantified; among them, 30-60% of proteins showed a change in abundance under one or more retinal ischemic conditions. In particular, in ischemic-tolerant retinas, histone proteins H2B, H3 and H4 demonstrated an increase in abundance, whereas histone H2A showed a decrease in abundance. Further immunohistochemical analyses confirmed the results of proteomic analyses, and detected an up regulation of tri-methylated histone H3, mono-ubiquitinated histone H2A and Polycomb group protein RING2. Together, these results suggest a role of epigenetic regulation in the induction of retinal ischemic tolerance that involves histone and polycomb proteins.

Keywords: Neuroprotection, ischemia, epigenetics, proteomics, retina, high intraocular pressure

Introduction

Retinal and optic nerve head ischemia, a condition that can be experimentally modeled by elevating the intraocular pressure (IOP), may contribute to the onset of multiple disorders in the visual system including glaucomatous damage. Studies have shown that retinal injury caused by acute high IOP (HIOP) can be prevented by exposing the retina to a brief preconditioning ischemia or other forms of non-injurious ischemic or hypoxic insults, prior to an otherwise injurious ischemia - a condition termed ischemic tolerance [1-3] (for simplicity, HIOP conditions are referred as ischemic conditions in this work). Hence, a preconditioning ischemia in the retina produces an endogenous protection against ischemic injury. The effectors of this inducible neuroprotective mechanism in the retina are unknown. Work by Kamphuis et al. [4, 5] and Thiersch et al. [6] have shown that preconditioning ischemia in the retina results in increased expression of genes involved in amino acid transport, transcription regulation, antioxidative pathways and cell death regulation. In none of these studies, however, was the ischemic-tolerant retina, the condition in which the effectors of tolerance are at play, included.

In a recent study on ischemic-tolerant rodent brains, we have found that a group of epigenetic regulator proteins including several histone and Polycomb group (PcG) proteins are up regulated, and an alteration in the PcG protein level has a profound impact on the outcome of ischemic stroke [7]. PcG proteins are epigenetic gene repressor proteins; they exert their roles in epigenetic regulation by modifying histone proteins. Accordingly, in brain, a PcG protein-mediated epigenetic mechanism that underlies

preconditioning-induced neuroprotection against ischemic brain injury has been elucidated [7]. As the first step in understanding the molecular mechanisms that underlie retinal ischemic tolerance, we conducted an unbiased, quantitative proteomic study on rat retinas under different ischemic conditions including ischemic-tolerant retinas. The proteomic results revealed differential and condition-specific changes of histone proteins, including changes that are either similar to or different from those found in brain. Results of follow-up immunohistochemical analyses demonstrated increased abundance of PcG protein RING2 in the ischemic-tolerant retina. Thus an involvement of histone and PcG proteins in the induction of ischemic tolerance in retina is implicated by the results of this study.

Matherials and methods

Retinal ischemia in rats

All animals were treated in accordance with the National Institutes of Health Guide for the use of animals in research, and all protocols were approved by the local Institutional Animal Care and Use Committee. Adult Sprague-Dawley rats (250 g–300 g) were purchased from Charles River Laboratories (Wilmington, MA). The animals were housed in a temperature- and humidity-controlled room with a 12-hour light:12-hour dark cycle and provided with food and water *ad libitum*.

Retinal ischemia was induced by transiently and manometrically increasing the IOP. Briefly, rats were anesthetized with ketamine/xylazine (55/5 mg/kg). A HIOP condition was achieved by inserting a 30-gauge needle into the anterior chamber. The needle was connected to a saline -filled reservoir, which was positioned at a corresponding height above the eye to achieve a sustained IOP of 110 mmHg. Three groups of animals (n=4 each) were subjected to different durations and levels of HIOP and reperfusion as follows: (1) preconditioning - 8 minutes IOP at 110 mm Hg, 48 hours reperfusion; (2) injurious - 60 minutes IOP at 110 mm Hg, 24 hours reperfusion; (3) tolerant - 8 minutes IOP at 110 mm Hg, 48 hours reperfusion, followed by another 60 minutes of IOP at 110 mm Hg and 24 hours reperfusion. All contralateral eyes were treated as sham controls by setting the IOP at 20 mmHg for corresponding durations. At the termination of reperfusions, animals were anesthetized with ketamine/xylazine and euthanized by an intracardiac injection of Euthasol® (pentobarbital, 100 mg/kg). The eyes were enucleated, and the entire retinas were collected and kept at –80°C until further analyses.

Protein extraction and tryptic digestion

Retinal specimen were thawed on ice, boiled directly into 250 µL pre-heated ddH_2O for 10 minutes, chilled on ice, and then homogenized with a hand-held homogenizer. After homogenization, an additional 250 µL of pre-chilled ddH_2O was added, and samples were centrifuged at 16,000 g for 30 minutes at 4°C. Protein concentrations in the cleared supernatants were determined by Bradford Assay. Next, for each IOP treatment group, samples from 4 animals were pooled. Twenty micrograms of protein from each pool were lyophilized and resuspended with 100 mM ammonium bicarbonate, pH 8.0, to a final protein concentration of 1 µg/µL. Proteins were denatured by incubation at 95°C for 10 minutes in the presence of 0.05% RapiGest SF (Waters, Milford, MA) followed by incubation at 60°C for 30 minutes with 20 mM dithiothreitol, and a final incubation with 20 mM iodoacetamide for 10 minutes in the dark at the room temperature. The proteins were then digested with sequencing grade trypsin ($2.25x10^6$ unit/µL; Promega, Madison, WI) at 37°C overnight. The RapiGest SF in the digestion mixture was precipitated by the addition of trifluoroacetic acid to pH 2-2.5 and incubation at 37°C for 10 minutes.

Mass spectrometry (MS) and bioinformatic analyses

The tryptic digests from each pooled sample were analyzed by a non-labeling, quantitative MS method, as previously described [7], with 3 technical replications. Briefly, the MS system consisted of a nanoflow ultra performance liquid chromatography (UPLC) machine coupled inline to a Micromass Global Ultima Quadrupole-Time-of-light mass spectrometer (Waters). The UPLC included a 20 cm x 75 µm bridged-ethyl hybrid C_{18} (1.7 µm) analytical column. The separation of tryptic peptides by UPLC and the subsequent dual-energy MS identification and quantification of detected peptides, as managed by ProteinLynx Global Server version 2.3 (Waters) and with the use of a custom database

of annotated, non-redundant rat proteins from The Universal Protein Resource (Unitprot, www.UniProt.org) were performed as previously described [7]. The fmol amounts for each identified protein in a sample were determined by comparison to an internal standard. For each sample (treatment group), protein quantities determined in each individual MS run were normalized using the total fmol numbers for each run. Proteins that were found in at least two of the three runs for each pool of retina sample were accepted as valid entries. For each accepted protein, a fmol ratio was established between the ischemic eye and the contralateral control eye, and a ratio of ≥ 1.5 (increased) or ≤ -1.5 (decreased) was defined as a change in ischemic eyes.

For all regulated proteins, their known Gene Ontology (GO) terms were retrieved with the assistance of the batch retrieval tool provided by UniProt. An enriched presence of these proteins in particular biological processes, metabolic and signaling pathways were analyzed with the assistance of the MetaCore program (GeneGo, Inc. West Lothian, UK).

Antibodies

The following primary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA): histone H2A – rabbit polyclonal; histone H3 – rabbit polyclonal; histone H4 - mouse monoclonal; RING2 (a.k.a Ring1B) – mouse monoclonal. Monoclonal antibody against mono -ubiquitinated histone H2A was purchased from Millipore (Billerica, MA), and monoclonal antibody against tri-methylated histone H3 at lysine 27 was from Abcam (Cambridge, MA). Fluorescein isothiocyanate (FITC)- or Cy3-conjugated secondary antibodies were from Jackson ImmunoResearch (West Grove, PA).

Fluorojade-B staining and fluorescent immunohistochemistry (IHC)

Whole eye globes from rats that underwent the HIOP and reperfusion procedures described earlier were removed immediately after euthanization, fixed in 4% paraformaldehyde in phosphate balanced saline (PBS) for 24 hours, and then frozen in 2-methylbutane. Sections at 12-µm thickness were prepared. Fluorojade-B staining was performed following standard protocols to reveal injury [8]. For immunohistochemical analyses, retinal sections were incubated with appropriate primary antibodies (dilutions are specified in figure legends) at 4°C overnight. The next day, the sections were washed three times with PBS, incubated for 1 hour with an appropriate secondary antibody, washed with ddH_2O, dried and mounted with a 4',6-diamidino-2-phenylindole (DAPI)-containing mounting media to counterstain nuclei. The fluorescent images were examined and documented with an epifluorescence microscope (Leica Microsystems, Inc. Bannockburn, IL) attached to a Magnifire digital color camera (ChipCoolers, Warwick, RI), with the assistance of the BIOQUANT program (Bioquant Image Analysis, Nashville, TN).

Results and discussion

Modeling retinal ischemic tolerance in rats

Preconditioning treatment with a brief or mild insult has been shown to have a protective effect against a subsequent, more severe insult in both the brain and in the retina. In retina, a decrease in ischemia-induced injury of multiple retinal cell layers has been reported for chemical-, HIOP (ischemic)- or hypoxic-preconditioned conditions [1-5, 9-15]. For ischemic preconditioning in rats, 130 - 170 mmHg IOPs have been used to produce tolerance in published studies [4, 5, 16] . Though effective in producing retinal insults, such IOP levels are very high relative to what is observed under various pathophysiological conditions in human eyes. We attempted to apply an IOP level that is lower than 130 mmHg, but would still produce detectable retinal injuries by neuroanatomical means and at a relatively early time point, and would still be effective at inducing ischemic tolerance within a controlled period of time. **Figure 1** and **Table 1** present the experimental paradigm and HIOP conditions used in the present work. As demonstrated by the results of fluorojade-B staining shown in **Figure 2**, a 60-minute 110 mmHg HIOP produced injuries across multiple cell layers in the retina, when examined 24 hours after the HIOP treatment, and such injuries were greatly reduced in the eyes treated with a preconditioning HIOP (8 minutes of 110 mmHg at 48 hours prior to the injurious 110 mmHg). Therefore, these results verified the establishment of a HIOP (ischemic)-tolerant paradigm in rats using 110 mmHg. The exact retinal cell types that were protected in this experimental

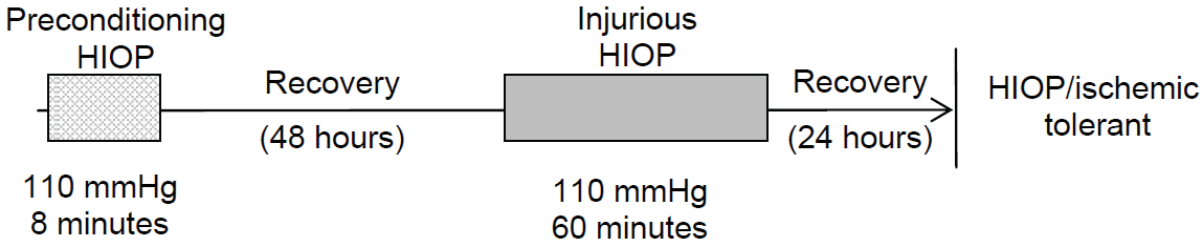

Figure 1. Experimental paradigm of HIOP treatments. Ipsilateral eyes of rats were subjected to the following HIOP conditions with reperfusions: Preconditioning HIOP - 110 mmHg for 8 minutes followed by 24 hours reperfusion; Injurious HIOP - 110 mmHg for 60 minutes followed by 24 hours reperfusion; For the tolerant condition: – 8 minutes preconditioning HIOP with 48 hours of reperfusion, followed by another 60 minutes of HIOP and 24 hours reperfusion. Retina samples were collected at the end of reperfusions (for the tolerant conditions, at the end of the second reperfusion). In each group, the IOP of contralateral eyes was sustained at 20 mmHg for the same duration as that of the HIOP treatment of the ipsilateral eyes followed by the same duration of reperfusions.

Table 1. Retinal HIOP conditions (IOP (mmHg)/duration (minutes))

Conditions	n	Day 1	Day 2	Day 3	Day 4
Preconditioned	4	110/8		Harvest	
Contralateral to Preconditioned	4	20/8		Harvest	
Injured	4	110/60	Harvest		
Contralateral to Injured	4	20/60	Harvest		
Tolerant	4	110/8		110/60	Harvest
Contralateral to Tolerant	4	20/8		20/60	Harvest

paradigm remain to be further defined with detailed IHC analyses for appropriate cell markers. It is apparent that the retinal ganglion cell (RGC) layer and the outer nuclear layer (ONL) are protected.

Next, we proceeded to analyzing proteomes of ischemic-preconditioned, ischemic-injured and ischemic-tolerant retinas, as a means to identify potential effector proteins of retinal ischemic tolerance.

Retinal proteomic changes under different ischemic conditions

Table 2 provides the numbers of proteins that were identified and quantified in each group of eyes, and the numbers of proteins that were increased or decreased in abundance by at least 1.5 fold in ischemia-treated eyes when compared to that in their contralateral control eyes. A complete list of the proteins that were detected and quantified in each sample is provided in Supplemental **Table 1**.

Using a simple, one-step protein extraction protocol in this study, a total of 328 retinal proteins were identified and quantified; this number includes proteins that were detected only in one or few groups of eyes but not in others. While this number is far less than the predicted numbers of translated proteins in eukaryotic cells, it is comparable to the numbers reported in a limited number of published proteomic studies on retinas of different species, in which similar or even more comprehensive pre-MS preparation steps were involved [17-25]. Obviously, these proteins cannot possibly provide us a thorough description of the retinal proteome, which can only be achieved through more comprehensive

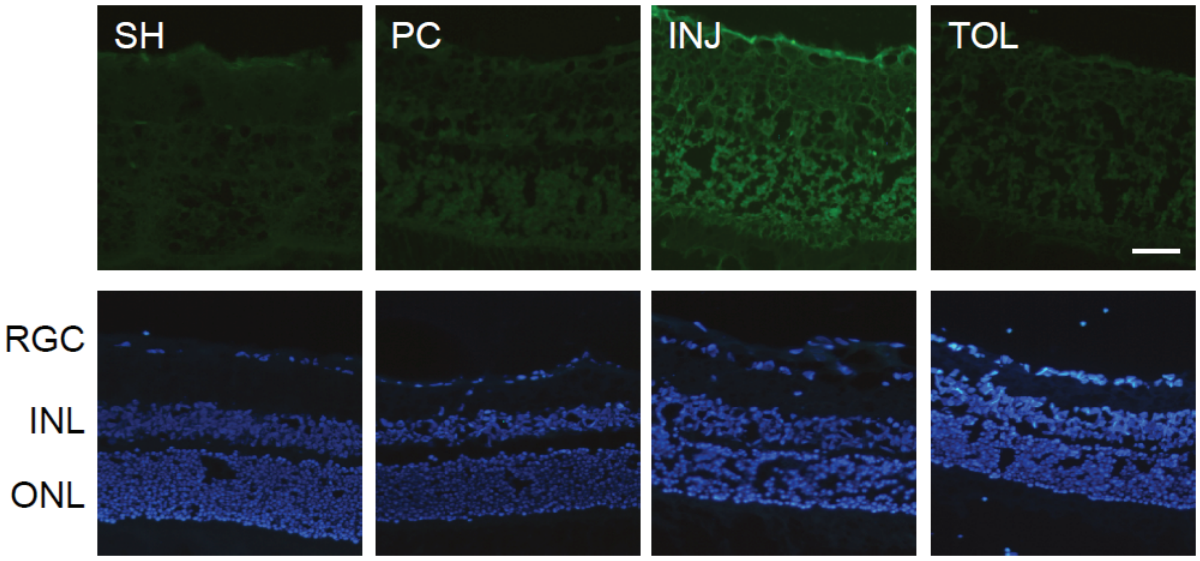

Figure 2. Examination of HIOP-induced retinal injury by fluorojade B staining. Top: fluorojade B staining of retina sections. Bottom: DAPI staining of consecutive retinal sections to reveal nuclei. The scale bar represents 50 μm. SH: sham-treated (contralateral to HIOP-treated); PC: HIOP-preconditioned; INJ: HIOP-injured; TOL: HIOP-tolerant; RGC: retina ganglion cell layer; INL: inner nuclear layer; ONL: outer nuclear layer. Images in this figure and figures below are representative results of analyses of at least three animals under each HIOP treatment condition, and at least two sections from each eye.

Table 2. Numbers of identified and quantified retinal proteins under each HIOP condition

	Preconditioned	Injured	Tolerant
HIOP-treated	176	181	165
Contralateral control	200	168	161
Total for both*	239	240	210
≥ 1.5 fold change in abundance**			
Increased	83 (47.2%)	92 (50.8%)	99 (60.0%)
Decreased	80 (40.0%)	52 (31.0%)	74 (46.0%)

*The numbers include proteins that were detected either in both eyes or only in one eye. **Proteins that were detected only in the HIOP-treated eyes are considered increased in abundance relevant to contralateral control eyes; likewise, proteins that were detected only in the control eyes are considered decreased in the HIOP-treated eyes.

proteomic studies in the future by employing additional protein enrichment protocols and MS procedures. Rather, the present proteomic data, as described below, provide an initial view of the most readily detectable proteomic changes in rat retinas under different ischemic conditions.

First, in the present study, relatively high percentages of retinal proteins showed a change in abundance in ischemia-treated eyes (**Table 2**). In the literature for retina and other tissues, the extent of ischemia-induced changes or changes induced by other forms of insults in gene transcripts or proteins, as determined by high throughput approaches such as cDNA microarrays and quantitative MS, respectively, varies greatly, from just a few to several tens of per-

Table 3. Biological processes associated with up-regulated proteins*

	Preconditioned	Injured	Tolerant
	Up-regulated		
1	organelle organization	glycolysis	glycolysis
2	cellular macromolecular complex assembly	glucose catabolic process	glucose catabolic process
3	cellular component organization	hexose catabolic process	hexose catabolic process
4	cellular macromolecular complex subunit organization	monosaccharide catabolic process	monosaccharide catabolic process
5	cellular component assembly	cellular carbohydrate catabolic process	cellular carbohydrate catabolic process
6	cellular component biogenesis	alcohol catabolic process	alcohol catabolic process
7	anatomical structure formation	carbohydrate catabolic process	carbohydrate catabolic process
8	macromolecular complex assembly	glucose metabolic process	glucose metabolic process
9	macromolecular complex subunit organization	generation of precursor metabolites and energy	hexose metabolic process
10	nucleosome assembly	hexose metabolic process	monosaccharide metabolic process
	Up-regulated only under specific conditions		
1	muscle thin filament assembly	gluconeogenesis	anti-apoptosis
2	skeletal myofibril assembly	hexose biosynthetic process	respiratory burst during acute inflammatory response
3	cardiac myofibril assembly	response to misfolded protein	regulation of protein folding in endoplasmic reticulum
4	protein polymerization	monosaccharide biosynthetic process	production of molecular mediator of acute inflammatory response
5	cardiac cell development	glycolysis	negative regulation of apoptosis
6	cardiac muscle cell development	pyruvate metabolic process	negative regulation of programmed cell death
7	cytoskeleton organization	acute inflammatory response	negative regulation of cell death
8	cellular component assembly	alcohol biosynthetic process	oxygen transport
9	microtubule-based process	glucose catabolic process	regulation of apoptosis
10	cellular component organization	hexose catabolic process	gas transport

* All listed processes were significantly regulated with p values ≤ 0.01, as a result of bioinformatic analyses of up-regulated proteins with the MetaCore program. Please see Table S2 for down-regulated biological processes.

centages [7, 26-31]. Besides variations in ischemic models and post-ischemia time points at which samples are harvested, the choice of controls also differs (for example, contralateral tissue of the same animal versus ipsilateral tissue of a different animal) [27]. Another important but often overlooked issue in high throughput proteomic studies is how to report proteins detected only in one or more but not all conditions, since no ratio numbers could be established for these proteins. The protein lists that we report in **Table 2** include such proteins. This may explain, at least partially, the relatively high percentages of regulated proteins that we report here.

Table 4. HIOP condition-specific changes in the abundance of select nuclear proteins

Protein Name	Gene Name	Preconditioned	Injured	Tolerant
COP9 signalosome complex subunit 2	Cops2	ND	ND	Up (1)
Heterogeneous nuclear ribonucleoprotein D0	Hnrnpd	Up (1)	UC (1)	UC (1)
Histone H1	Hist1h1t/1c	Up (2)	UC (2)	UC (1), Up (1)
Histone H2A	H2afz, H2afj	Down (8)	UC (1), Up (7)	Down (8)
Histone H2B	Hist1h2ba	UC (1), Up (1)	UC (2)	Up (2)
Histone H3	H3f3b	Up (2)	UC (2)	Up (2)
Histone H4	Hist1h4b	UC (1)	Up (1)	Up (1)
Host cell factor 2	Hcfc2	Up (1)	ND	ND
Methyl CpG binding protein 2	Mecp2	UC (1)	ND	ND
Non-histone chromosomal protein HMG-17	Hmgn2	Up (1)	ND	ND
Nuclear protein Hcc-1	Hcc1	UC (1)	Up (1)	Down (1)
Nucleosome assembly protein 1-like 4	Nap1l4	Down (1)	Up (1)	ND

The numbers in the parentheses designate the numbers of isoforms that were detected under each HIOP condition. UC: unchanged; ND: not detected.

Table 3 reports the most significant biological processes (by GO terms) that are associated with up-regulated retinal proteins under different retinal ischemic conditions. In both ischemic-injured and ischemic-tolerant retinas, the most significantly up-regulated biological processes are those of glucose or hexose metabolism, with little difference between the two conditions, whereas in ischemic-preconditioned retinas, the top ten up-regulated biological processes are those of macromolecule and organelle organization. A possible increase in glucose and carbohydrate metabolism processes in ischemic-tolerant retinas, as noted above, is somehow a surprising result that is different from what is observed in ischemic tolerant brains, in which decreased energy metabolism processes has been suggested [7, 32]. At this time, the exact metabolic condition in the ischemic-tolerant retina is unknown. It is an important issue to be addressed in future studies using both biochemical and physiological approaches, and at different reperfusion time points following retinal ischemia.

When proteins that were uniquely up-regulated under each of the three retinal ischemic conditions (that is, after excluding proteins that also changed in other conditions) were analyzed for their bioinformatics, an up regulation of anti-cell death processes was recognized in the ischemic-tolerant retina (**Table 3**). In light of our recent description of a gene repressor protein-mediated mechanism for ischemic tolerance in brain [7], and to consider the mechanism(s) that underlie the increased anti-cell death processes in ischemic-tolerant retinas, we paid attention to changes of histone proteins that were detected in our present proteomic datasets. We found an increase of variants of histone proteins H1, H2B, H3 and H4, and a decrease of histone H2A in the ischemic-tolerant retina (**Table 4**). The abundance of a post-translationally modified form of histone H2A (an epigenetic mark), however, as demonstrated next by results of immunohistochemical analyses, showed an increase in the ischemic-tolerant retina.

Little is known about how expression levels and modifications of histone proteins are regulated in ischemic retinas. Recently, Crosson et al have reported that inhibition of histone deacetylase (HDAC) protects retinas from ischemia (HIOP)-induced injury in rats [33], whereas work by Chen and Cepko shows that, in mice, HDAC4 activity is beneficial in retinal neuronal survival with the involvement of hypoxia-inducible factor

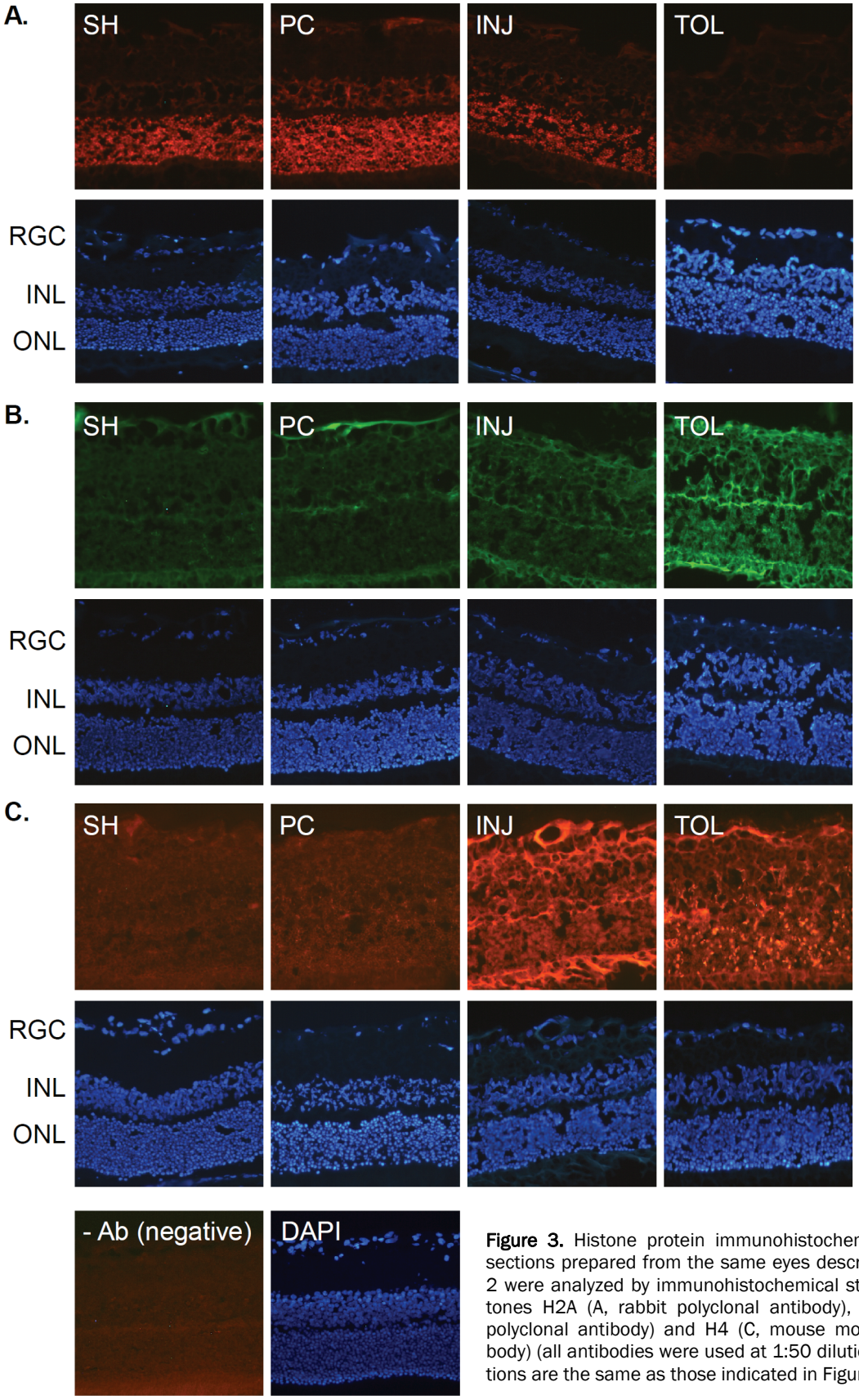

Figure 3. Histone protein immunohistochemistry. Retinal sections prepared from the same eyes described in Figure 2 were analyzed by immunohistochemical staining for histones H2A (A, rabbit polyclonal antibody), H3 (B, rabbit polyclonal antibody) and H4 (C, mouse monoclonal antibody) (all antibodies were used at 1:50 dilution). Abbreviations are the same as those indicated in Figure 2.

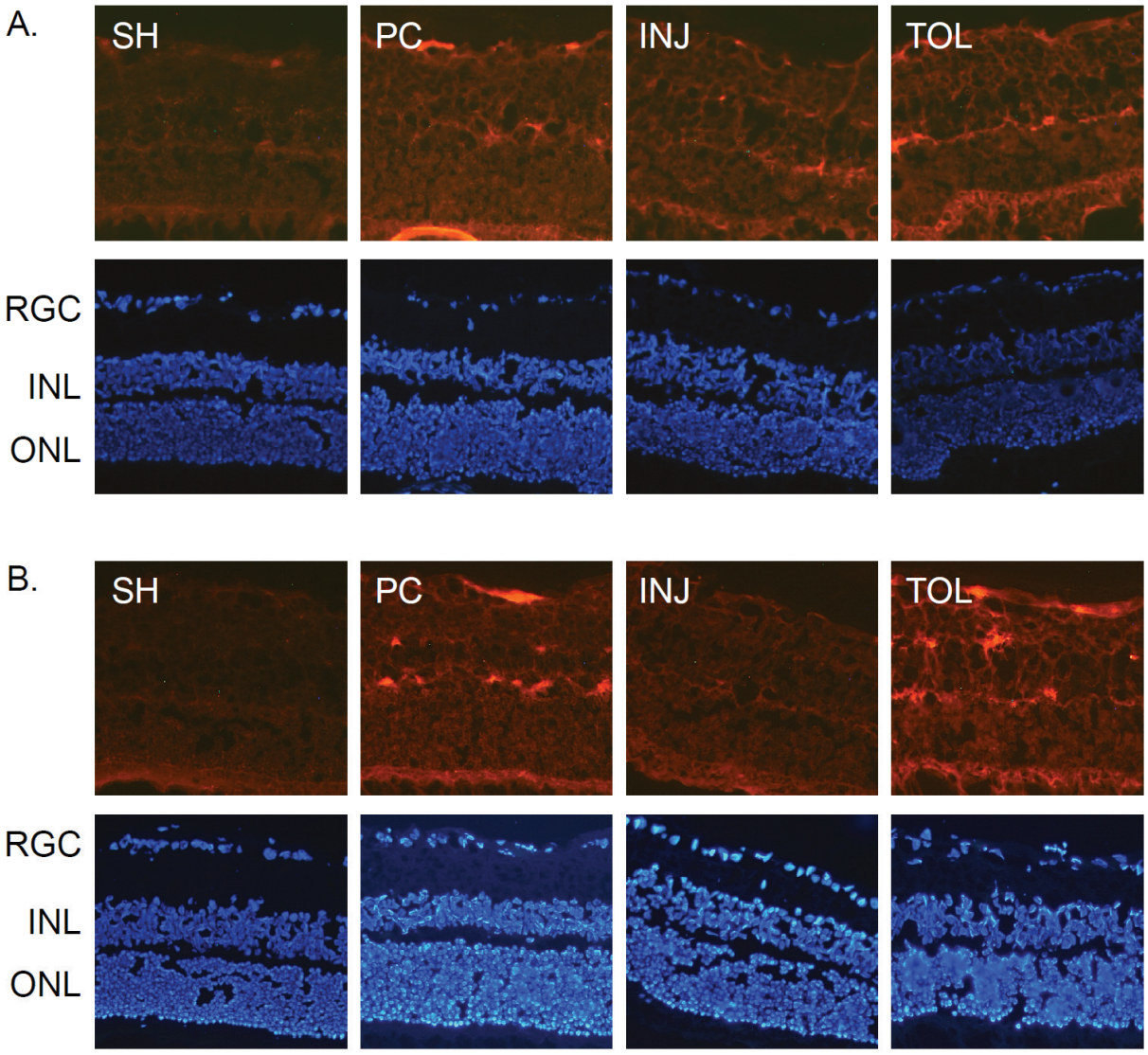

Figure 4. Immunohistochemical analyses of modified histone H2A and H3 proteins. The analyses were performed using mouse monoclonal antibodies against mono-uniquitinated H2A (A, 1:100) and tri-methylated H3 at lysine 27 (B, 1:50), respectively, and with appropriate Cy3-conjugated secondary antibodies (1:760).

1a [34]. Histones H2A, H2B, H3 and H4 are all subject to acetylation. While the results of these studies do not include direct analyses of histone protein levels, they support a critical role of histones in retinal disorders through an epigenetic mechanism. Our current proteomic finding - the increased levels of several histone proteins in the ischemic-tolerant retina, points in the direction that a preconditioning ischemia in retina may induce an endogenous neuroprotective mechanism that includes an epigenetic component.

Enriched presence of epigenetic gene repressor proteins in ischemic-tolerant retinas

The results of the above-introduced proteomic characterization of ischemic-tolerant retinas prompted us to further examine changes of selected histone proteins and PcG proteins under different ischemic conditions by IHC, as a means to determine whether or not an epigenetic mechanism that is revealed in our recent studies on ischemic-tolerant brains [7] may also be at play in retinal ischemic tolerance.

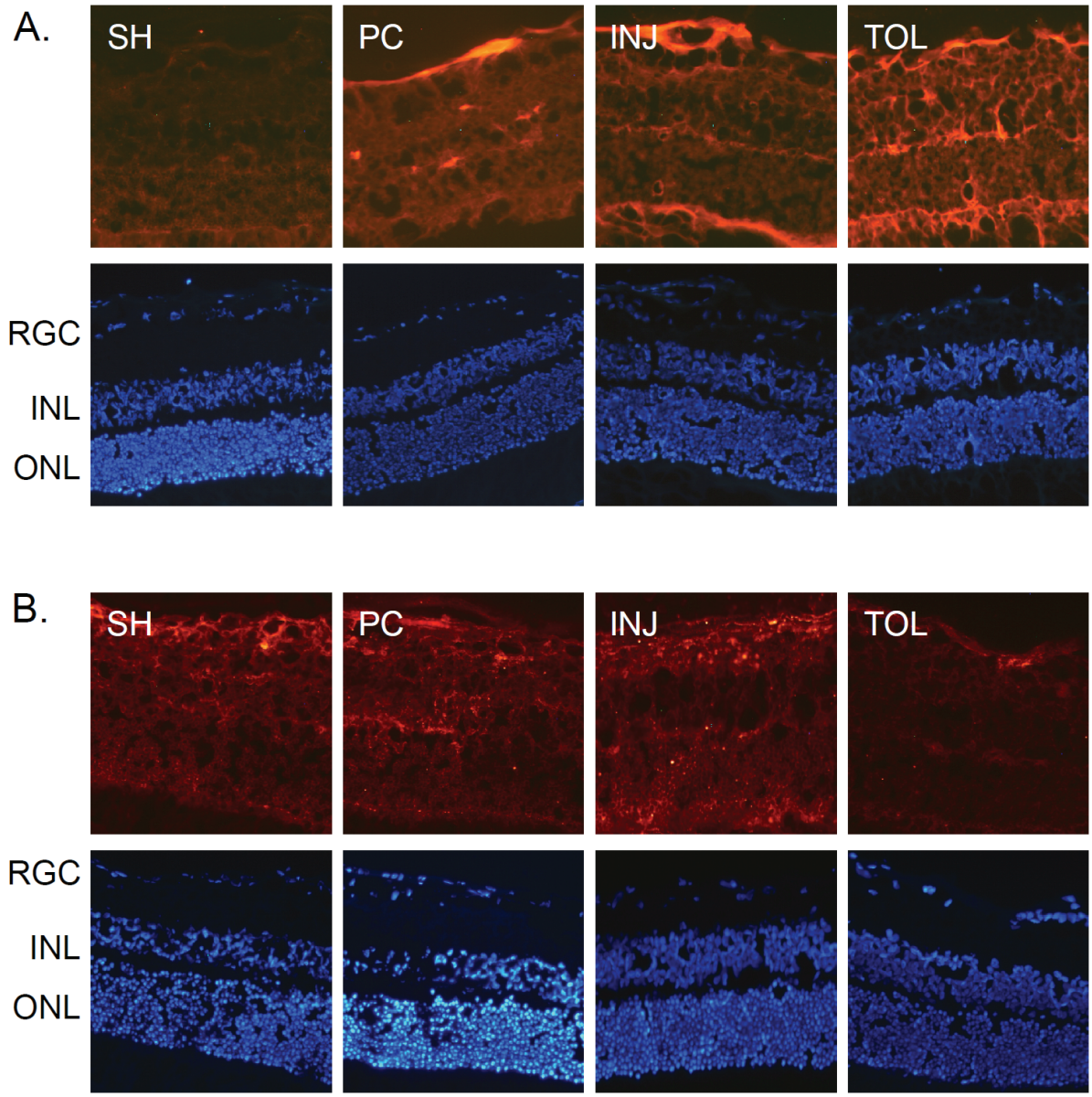

Figure 5. Immunohistochemical analyses of PcG proteins. The analyses were performed using a mouse monoclonal antibody against RING2 (A, 1:100) or a rabbit polyclonal antibody against EZH1 (B, 1:500) and with appropriate Cy3-conjugated secondary antibodies, respectively, at 1:760.

Figure 3 shows the changes in immunoreactivity for histone proteins H2A, H3 and H4; the results were essentially in agreement with the results of MS analyses (**Table 4**), hence validating our proteomic results. Specifically, in ischemic - tolerant retinas, the immunoreactivity of histone H2A was greatly diminished, whereas the immunoreactivity of histone H3 and H4 was robustly up regulated.

As introduced earlier, histone H2A and H3 are subject to mono-ubiquitination (at lysine 119) and tri-methylation (at lysine 27), respectively, by the action of PcG proteins. A concerted H2A mono-ubiquitination and H3 methylation are critical for epigenetic transcriptional suppression (for review, see Bantignies and Cavalli, 2006 [35]). While a decreased level of H2A seen in the ischemic-tolerant retina, as revealed

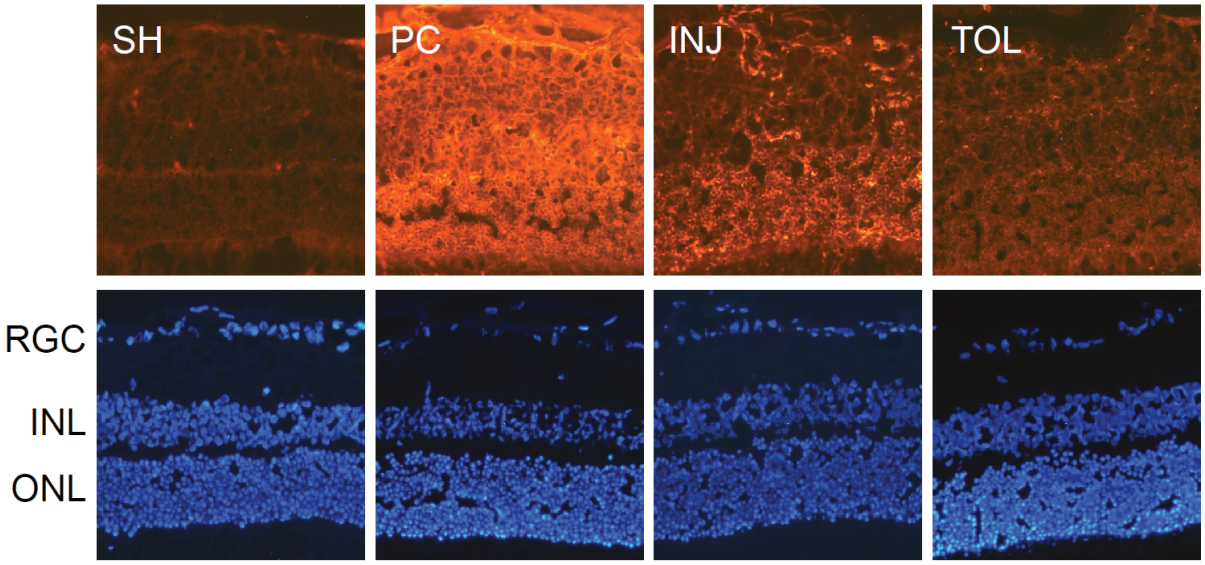

Figure 6. Immunohistochemical analyses of CSN2/TRIP15. The analyses were performed on retinal sections prepared from the same eyes used in Figures. 2-5. A goat polyclonal antibody against CSN2/TRIP15 was used (1:50).

by IHC using an antibody that does not distinguish modified forms of H2A, does not seem to support a role of neuroprotection against ischemic retinal injury for H2A, it is possible that there may be an increase in the abundance of mono-ubiquitinated H2A, especially if in the ischemic-tolerant retina there is an increase of PcG proteins. Indeed, results of IHC analyses for mono-ubiquitinated H2A demonstrate an increase of its abundance in the tolerant retina (**Figure 4A**). The mechanism that underlies an overall decrease in the level of histone H2A in the ischemic-tolerant retina is unknown. One possible explanation is that there is an increased rate of conversion of H2A to its mono-ubiquitinated form and/or a change in H2A metabolic rate. This will be an issue to be addressed in future studies. Interestingly, the abundance of tri-methylated H3 was also increased in the ischemic-tolerant retina (**Figure 4B**). In other words, in ischemic-tolerant retinas, the abundance of both mono-ubiquitinated H2A and tri-methylated H3 was increased. This suggests a possible involvement of PcG proteins in the induction of the retinal ischemic-tolerance.

Figure 5 presents the results of IHC analyses for PcG proteins RING2 (a.k.a. Ring1B), an E3 ligase that mediates H2A mono-ubiquitination, and EZH1, a PcG protein mediating tri-methylation for histone H3 at lysine 27. RING2 has been shown to play a role in retinal development [36]. Similar to the results of our previous studies on ischemic-tolerant brains, the immunoreactivity of RING2 was increased in the ischemic-tolerant retina (**Figure 5A**). Unlike the changes in tri-methylated H3, the abundance of EZH1 did not show an increase in the ischemic-tolerant retina. Instead, an overall decrease across the retina was observed (**Figure 5B**). This observation does not seem to correspond to the increase of tri-methylated H3 in the ischemic-tolerant retina. In literature, elevated levels of EZH1 has been reported for cancer cells, and an anti-apoptotic role has been proposed for it [37]. It remains to be examined whether or not there may be a change in EZH1 biosynthesis or metabolism. Ischemia-induced changes in retinal levels of EZH1 and tri-methylated H3 may also exhibit different temporal orders.

A histone and PcG protein-mediated mechanism in retinal ischemic tolerance was further implicated by an up regulation of COPS9 signalosome complex subunit 2 (CSN2, a.k.a. TRIP15 in humans and Alien in drosophilia) that was detected in ischemic-tolerant (**Table 4**) and ischemic-preconditioned retinas (**Figure 6**) in the present study. Recently, CSN2 has been shown to interact with E3 ubiquitin ligases, and Alien was shown to be a chromatin-associated protein that binds specifically to histones H3 and H4 and participates in gene repression [38, 39], although it is not known at this time whether or not CSN2/TRIP15 may interact with

PcG proteins directly.

Taken together, the results of the present study revealed ischemic condition-specific changes of the retinal proteome, with marked increase in anti-cell death processes and the abundance of several histone proteins and a PcG protein in the ischemic-tolerant retina. Future studies are needed to establish the retinal cell populations in which histone and PcG proteins are endogenously expressed and regulated by ischemic stress. A possible essential role of these gene repressor proteins in the retinal neuroprotection against ischemic insults remains to be demonstrated by approaches such as gene knockdown or over-expression.

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Abbreviations: IOP – intraocular pressure; HIOP – high IOP; PcG – Polycomb group; MS – mass spectrometry; UPLC – ultra performance liquid chromatography; GO – gene ontology; FITC – fluorescein isothiocyanate; IHC – immunohistochemistry; PBS – phosphate balance saline; DAPI – 4',6-diamidino-2-phenylindole; HDAC – histone deacetylase; CSN2 – COPS9 signalosome complex subunit; RGC – retina ganglion cells; INL – inner nuclear layer; ONL – outer nuclear layer.

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Table S1 All identified and quantified retinal proteins.

Ratios between HIOP-treated eyes and their contralateral control (sham) eyes are averages of triplicate MS determinations.
Each sample for MS analyses was a pool of retinal protein extracts from 4 animals.

Protein ID	Gene Name	Protein Name	PC:SH	INJ:SH	TOL:SH
CH10_RAT	Hspe1	10 kDa heat shock protein	-1.29	-1.18	1.00
1433B_RAT	Ywhab	14-3-3 protein beta/alpha	1.70	-1.44	1.23
1433E_RAT	Ywhae	14-3-3 protein epsilon	-1.08	1.08	1.70
1433F_RAT	Ywhah	14-3-3 protein eta	treated only	treated only	2.46
1433G_RAT	Ywhag	14-3-3 protein gamma	treated only	-1.37	1.91
1433T_RAT	Ywhaq	14-3-3 protein theta	2.90	-1.08	1.82
1433Z_RAT	Ywhaz	14-3-3 protein zeta/delta	1.67	-1.26	1.46
PLCD_RAT 1	Agpat4	1-acyl-sn-glycerol-3-phosphate acyltransferase delta			treated only
RS19_RAT	Rps19	40S ribosomal protein S19	1.11	-1.09	-1.31
RSSA_RAT 4	Rpsa	40S ribosomal protein SA			treated only
HEM0_RAT	Alas2	5-aminolevulinate synthase			sham only
CH60_RAT	Hspd1	60 kDa heat shock protein		treated only	
RLA1_RAT	Rplp1	60S acidic ribosomal protein P1		sham only	
RLA2_RAT	Rplp2	60S acidic ribosomal protein P2	1.27	-1.50	treated only
F263_RAT	Pfkfb3	6-phosphofructo-2-kinase/fructose-2	sham only		
GRP78_RAT	Hspa5	78 kDa glucose-regulated protein	1.34	1.09	1.54
AN32A_RAT	Anp32a	Acidic leucine-rich nuclear phosphoprotein 32 family member A	1.29	-1.84	-1.05
AN32B_RAT	Anp32b	Acidic leucine-rich nuclear phosphoprotein 32 family member B	treated only		
AN32E_RAT	Anp32e	Acidic leucine-rich nuclear phosphoprotein 32 family member E	1.43	-2.30	sham only
ACTB_RAT	Actb	Actin	1.51	-1.01	1.56
ACTA_RAT	Acta2	Actin	1.57	-1.35	1.37
ACTG_RAT	Actg1	Actin	1.51	-1.01	1.56
ACTH_RAT	Actg2	Actin	1.57	-1.35	1.51
ACTC_RAT	Actc1	Actin	1.57	-1.35	1.36
ACTS_RAT	Acta1	Actin	1.48	-1.35	1.37
ACBP_RAT	Dbi	Acyl-CoA-binding protein	treated only		1.85
KAD1_RAT	Ak1	Adenylate kinase isoenzyme 1	-1.01	treated only	sham only
KAD2_RAT	Ak2	Adenylate kinase isoenzyme 2	sham only		
AFAD_RAT	Mllt4	Afadin	sham only	sham only	
A1AT_RAT	Serpina1	Alpha-1-antiproteinase		treated only	treated only
A1I3_RAT	A1i3	Alpha-1-inhibitor 3		treated only	
FETUA_RAT	Ahsg	Alpha-2-HS-glycoprotein		treated only	
ENOA_RAT	Eno1	Alpha-enolase	1.55	1.61	1.50
SYUA_RAT	Snca	Alpha-synuclein	1.22	-2.97	-1.21

SCNNA_RAT	Scnn1a	Amiloride-sensitive sodium channel subunit alpha			sham only
AMPH_RAT	Amph	Amphiphysin	1.33	treated only	sham only
TRY1_RAT	Prss1	Anionic trypsin-1	1.21	-1.78	sham only
APOE_RAT	Apoe	Apolipoprotein E		sham only	sham only
ARAF_RAT	Araf	A-Raf proto-oncogene serine/threonine-protein kinase	sham only		
AATC_RAT	Got1	Aspartate aminotransferase	1.34	1.14	treated only
PEA15_RAT	Pea15	Astrocytic phosphoprotein PEA-15	sham only	treated only	
ATPA_RAT	Atp5a1	ATP synthase subunit alpha	-1.26	5.60	2.06
ATPB_RAT	Atp5b	ATP synthase subunit beta	2.08	1.56	1.85
ATP5H_RAT	Atp5h	ATP synthase subunit d	-1.55	treated only	1.00
ATPD_RAT	Atp5d	ATP synthase subunit delta	1.09	-1.35	1.23
ATP5J_RAT	Atp5j	ATP synthase-coupling factor 6	1.24	-1.69	1.22
BASI_RAT	Bsg	Basigin	1.09	-1.20	-2.63
CRBB2_RAT	Crybb2	Beta-crystallin B2	sham only		
ENOB_RAT	Eno3	Beta-enolase	1.71	1.91	1.98
SYUB_RAT	Sncb	Beta-synuclein	1.84	-2.51	sham only
BASP_RAT	Basp1	Brain acid soluble protein 1	-1.18	-1.07	-2.52
CALB1_RAT	Calb1	Calbindin		sham only	
CALM_RAT	Calm1	Calmodulin	-1.63	-3.12	-1.03
CALL3_RAT	Calml3	Calmodulin-like protein 3	-1.31	sham only	-1.41
CALX_RAT	Canx	Calnexin	treated only		sham only
CALR_RAT	Calr	Calreticulin	1.52	1.00	3.11
CALB2_RAT	Calb2	Calretinin	treated only	-1.51	2.11
CALU_RAT	Calu	Calumenin	sham only		1.73
CAH2_RAT	Ca2	Carbonic anhydrase 2	sham only	sham only	
CADM2_RAT	Cadm2	Cell adhesion molecule 2	2.74	sham only	-3.39
JIP1_RAT	Mapk8ip1	C-jun-amino-terminal kinase-interacting protein 1	treated only		
CLCA_RAT	Clta	Clathrin light chain A	1.17	-1.61	-1.19
CLCB_RAT	Cltb	Clathrin light chain B		sham only	
CLUS_RAT	Clu	Clusterin		treated only	
COF1_RAT	Cfl1	Cofilin-1	sham only	sham only	sham only
CCD45_RAT	Ccdc45	Coiled-coil domain-containing protein 45		treated only	
CCD51_RAT	Ccdc51	Coiled-coil domain-containing protein 51			sham only
CO1A1_RAT	Col1a1	Collagen alpha-1(I) chain		treated only	
C1S_RAT	C1s	Complement C1s subcomponent		treated only	
CNKR2_RAT	Cnksr2	Connector enhancer of kinase suppressor of ras 2		treated only	
CSN2_RAT C	Cops2	COP9 signalosome complex subunit 2			treated only
CFDP1_RAT	Cfdp1	Craniofacial development protein 1		sham only	
KCRB_RAT	Ckb	Creatine kinase B-type	1.46	1.36	-1.07
QCR2_RAT	Uqcrc2	Cytochrome b-c1 complex subunit 2		treated only	

COX5A_RAT	Cox5a	Cytochrome c oxidase subunit 5A	sham only	treated only	
DUT_RAT	Dut	Deoxyuridine 5'-triphosphate nucleotidohydrolase			treated only
DESM_RAT	Des	Desmin	2.03	1.19	treated only
DPYL2_RAT	Dpysl2	Dihydropyrimidinase-related protein 2 GN	treated only		treated only
DPYL3_RAT	Dpysl3	Dihydropyrimidinase-related protein 3			treated only
RAD50_RAT	Rad50	DNA repair protein RAD50	sham only		
DBPA_RAT	Csda	DNA-binding protein A	sham only		
STAU2_RAT	Stau2	Double-stranded RNA-binding protein Staufen homolog 2	sham only		
DUOX1_RAT	Duox1	Dual oxidase 1			sham only
DCTN2_RAT	Dctn2	Dynactin subunit 2	sham only	sham only	treated only
DNM1L_RAT	Dnm1l	Dynamin-1-like protein			treated only
DNAI1_RAT	Dnai1	Dynein intermediate chain 1	sham only		
ENC2_RAT	Klhl25	Ectoderm-neural cortex protein 2	sham only		
EF1A1_RAT	Eef1a1	Elongation factor 1-alpha 1			treated only
EF1A2_RAT	Eef1a2	Elongation factor 1-alpha 2			treated only
EMD_RAT	Emd	Emerin	treated only		
ENPL_RAT	Hsp90b1	Endoplasmin	treated only	treated only	sham only
NHERF_RAT	Slc9a3r1	Ezrin-radixin-moesin-binding phosphoprotein 50	-1.04	-1.45	-1.20
FUBP2_RAT	Khsrp	Far upstream element-binding protein 2	1.08	sham only	-1.58
FXL20_RAT	Fbxl20	F-box/LRR-repeat protein 20		treated only	
FNDC5_RAT	Fndc5	Fibronectin type III domain-containing protein 5		treated only	
ALDOA_RAT	Aldoa	Fructose-bisphosphate aldolase A	1.13	10.58	1.51
ALDOB_RAT	Aldob	Fructose-bisphosphate aldolase B			treated only
ALDOC_RAT	Aldoc	Fructose-bisphosphate aldolase C	-1.04	10.62	2.67
ENOG_RAT	Eno2	Gamma-enolase	1.57	1.19	1.53
GFAP_RAT	Gfap	Glial fibrillary acidic protein		sham only	
G6PI_RAT	Gpi	Glucose-6-phosphate isomerase	1.51	treated only	treated only
NMDE2_RAT	Grin2b	Glutamate [NMDA] receptor subunit epsilon-2		sham only	
GLNA_RAT	Glul	Glutamine synthetase	1.70	treated only	1.95
G3P_RAT	Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	1.45	1.92	-1.05
C1GLT_RAT	C1galt1	Glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase 1	sham only		
GRAP1_RAT	Gripap1	GRIP1-associated protein 1	treated only		
GNA12_RAT	Gna12	Guanine nucleotide-binding protein alpha-12 subunit	sham only	sham only	treated only
GNAI2_RAT	Gnai2	Guanine nucleotide-binding protein G(i)	treated only	treated only	-1.35
GNAI1_RAT	Gnai1	Guanine nucleotide-binding protein G(i)	1.00	treated only	sham only
GBG11_RAT	Gng11	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-11	sham only		
GBB1_RAT	Gnb1	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	1.59	1.58	1.07
GBB2_RAT	Gnb2	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	5.37	1.84	1.07
GBB3_RAT	Gnb3	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-3		1.00	
GNAI3_RAT	Gnai3	Guanine nucleotide-binding protein G(k) subunit alpha	1.00	treated only	2.22

GNAO_RAT	Gnao1	Guanine nucleotide-binding protein G(o) subunit alpha	1.98	treated only	1.16
GNAL_RAT	Gnal	Guanine nucleotide-binding protein G(olf) subunit alpha	treated only	treated only	1.30
GNAS2_RAT	Gnas	Guanine nucleotide-binding protein G(s) subunit alpha isoforms short		treated only	sham only
GNAS1_RAT	Gnas	Guanine nucleotide-binding protein G(s) subunit alpha isoforms XLas	treated only	treated only	treated only
GNAT3_RAT	Gnat3	Guanine nucleotide-binding protein G(t) subunit alpha-3	2.14	-2.70	-1.15
GBB4_RAT	Gnb4	Guanine nucleotide-binding protein subunit beta-4	11.91	1.76	-1.03
HSP71_RAT	Hspa1a	Heat shock 70 kDa protein 1A/1B	1.71	2.41	1.04
HS71L_RAT	Hspa1l	Heat shock 70 kDa protein 1L	1.52	1.84	1.11
HSP7C_RAT	Hspa8	Heat shock cognate 71 kDa protein	1.82	1.89	1.33
TRAP1_RAT	Trap1	Heat shock protein 75 kDa	sham only		
HS90A_RAT	Hsp90aa1	Heat shock protein HSP 90-alpha	1.86	1.76	-1.12
HS90B_RAT	Hsp90ab1	Heat shock protein HSP 90-beta	2.79	2.18	-1.11
HSP72_RAT	Hspa2	Heat shock-related 70 kDa protein 2	1.37	1.27	1.35
HEMGN_RAT	Hemgn	Hemogen		treated only	
HBA_RAT	Hba1	Hemoglobin subunit alpha-1/2	1.31	-1.39	treated only
HBB1_RAT	Hbb	Hemoglobin subunit beta-1	-1.18	-1.17	1.68
HBB2_RAT	N/A	Hemoglobin subunit beta-2	-1.28	-1.28	
HEMO_RAT	Hpx	Hemopexin		treated only	
HDGF_RAT	Hdgf	Hepatoma-derived growth factor		sham only	sham only
HDGR2_RAT	Hdgfrp2	Hepatoma-derived growth factor-related protein 2	treated only	sham only	
HDGR3_RAT	Hdgfrp3	Hepatoma-derived growth factor-related protein 3	1.21	sham only	-1.11
ROA1_RAT	Hnrnpa1	Heterogeneous nuclear ribonucleoprotein A1	sham only	treated only	1.22
ROA3_RAT	Hnrnpa3	Heterogeneous nuclear ribonucleoprotein A3	1.46	1.04	1.20
HNRPC_RAT	Hnrnpc	Heterogeneous nuclear ribonucleoprotein C (Fragment)	1.10	-1.02	treated only
HNRPD_RAT	Hnrnpd	Heterogeneous nuclear ribonucleoprotein D0	1.88	-1.43	1.12
HNRDL_RAT	Hnrpdl	Heterogeneous nuclear ribonucleoprotein D-like	1.02	treated only	sham only
HNRPF_RAT	Hnrnpf	Heterogeneous nuclear ribonucleoprotein F			treated only
HNRPG_RAT	Rbmx	Heterogeneous nuclear ribonucleoprotein G	1.42	treated only	1.90
HNRH1_RAT	Hnrnph1	Heterogeneous nuclear ribonucleoprotein H	sham only		1.30
HNRPK_RAT	Hnrnpk	Heterogeneous nuclear ribonucleoprotein K	1.04	-1.09	1.86
HNRPM_RAT	Hnrnpm	Heterogeneous nuclear ribonucleoprotein M		treated only	
HMGB1_RAT	Hmgb1	High mobility group protein B1	1.12	-1.04	-1.40
HMGB2_RAT	Hmgb2	High mobility group protein B2	1.08	sham only	1.30
H12_RAT	Hist1h1c	Histone H1.2	1.82	-1.33	1.10
H1T_RAT	Hist1h1t	Histone H1t	1.60	-1.02	1.67
H2A1_RAT	N/A	Histone H2A type 1	-1.85	1.54	sham only
H2A1C_RAT	N/A	Histone H2A type 1-C	-1.85	1.54	sham only
H2A1E_RAT	N/A	Histone H2A type 1-E	-1.85	1.54	sham only
H2A1F_RAT	N/A	Histone H2A type 1-F	-1.85	1.54	-1.61
H2A3_RAT	N/A	Histone H2A type 3	-1.85	1.54	sham only

H2A4_RAT	N/A	Histone H2A type 4	-1.85	1.54	sham only
H2AJ_RAT	H2afj	Histone H2A.J	-1.85	1.54	sham only
H2AZ_RAT	H2afz	Histone H2A.Z	-1.92	1.07	sham only
H2B1_RAT	N/A	Histone H2B type 1	1.74	1.02	2.12
H2B1A_RAT	Hist1h2ba	Histone H2B type 1-A	1.35	-1.46	1.56
H31_RAT	N/A	Histone H3.1	2.61	-1.46	2.00
H33_RAT	H3f3b	Histone H3.3	2.61	-1.46	2.01
H4_RAT	Hist1h4b	Histone H4	1.30	treated only	2.01
HOME3_RAT	Homer3	Homer protein homolog 3			sham only
HCFC2_RAT	Hcfc2	Host cell factor 2	treated only		
F10A1_RAT	St13	Hsc70-interacting protein		sham only	
HMCS1_RAT	Hmgcs1	Hydroxymethylglutaryl-CoA synthase	sham only		
IGSF1_RAT	Igsf1	Immunoglobulin superfamily member 1		treated only	
IMPG1_RAT	Impg1	Interphotoreceptor matrix proteoglycan 1	sham only	-3.09	sham only
IMPG2_RAT	Impg2	Interphotoreceptor matrix proteoglycan 2	-2.06		
ITSN1_RAT	Itsn1	Intersectin-1			treated only
JKIP1_RAT	Jakmip1	Janus kinase and microtubule-interacting protein 1		treated only	
JPH2_RAT	Jph2	Junctophilin-2	sham only		
K1C20_RAT	Krt20	Keratin		sham only	
K2C8_RAT	Krt8	Keratin	sham only		sham only
K2C6A_RAT	Krt6a	Keratin	sham only	sham only	2.92
K1C18_RAT	Krt18	Keratin	sham only	sham only	
K1C19_RAT	Krt19	Keratin	sham only	sham only	treated only
K1C42_RAT	Krt42	Keratin	sham only	sham only	treated only
K1C17_RAT	Krt17	Keratin	sham only	sham only	treated only
K1C14_RAT	Krt14	Keratin	sham only	sham only	
K1C15_RAT	Krt15	Keratin	sham only	sham only	
K1C13_RAT	Krt13	Keratin	sham only	sham only	
K1C40_RAT	Krt40	Keratin		sham only	
K1C12_RAT	Krt12	Keratin		sham only	
K1C10_RAT	Krt10	Keratin	sham only	sham only	2.09
K1C24_RAT	Krt24	Keratin	sham only		
K2C4_RAT	Krt4	Keratin	sham only	sham only	
K2C1B_RAT	Krt77	Keratin	-10.68	sham only	2.03
K22E_RAT	Krt2	Keratin	sham only	sham only	
K2C73_RAT	Krt73	Keratin	-15.76	sham only	2.46
K2C75_RAT	Krt75	Keratin	sham only	sham only	
K2C1_RAT	Krt1	Keratin	-9.58	sham only	2.31
K2C5_RAT	Krt5	Keratin	sham only	sham only	
LAP2_RAT	Tmpo	Lamina-associated polypeptide 2 isoform beta	treated only	treated only	1.30

LMNB1_RAT	Lmnb1	Lamin-B1	1.39	1.07	1.16
LR16B_RAT	Lrrc16b	Leucine-rich repeat-containing protein 16B	treated only		
LASP1_RAT	Lasp1	LIM and SH3 domain protein 1	sham only		treated only
LDHA_RAT	Ldha	L-lactate dehydrogenase A chain	1.45	1.97	2.57
LDHB_RAT	Ldhb	L-lactate dehydrogenase B chain	treated only	sham only	treated only
LDHC_RAT	Ldhc	L-lactate dehydrogenase C chain			treated only
MDHC_RAT	Mdh1	Malate dehydrogenase	1.38	1.13	4.00
MDHM_RAT	Mdh2	Malate dehydrogenase	treated only	treated only	treated only
MRP_RAT	Marcksl1	MARCKS-related protein		treated only	
MMP24_RAT	Mmp24	Matrix metalloproteinase-24			sham only
MAGI2_RAT	Magi2	Membrane-associated guanylate kinase		treated only	
PGRC1_RAT	Pgrmc1	Membrane-associated progesterone receptor component 1	treated only		sham only
MECP2_RAT	Mecp2	Methyl-CpG-binding protein 2	1.00		
MAP2_RAT	Map2	Microtubule-associated protein 2	1.17	treated only	treated only
MAP4_RAT	Map4	Microtubule-associated protein 4	1.40	sham only	sham only
MAP6_RAT	Map6	Microtubule-associated protein 6	-1.98		treated only
TAU_RAT	Mapt	Microtubule-associated protein tau	-1.07	-1.09	sham only
MPIP1_RAT	Cdc25a	M-phase inducer phosphatase 1			sham only
MPIP2_RAT	Cdc25b	M-phase inducer phosphatase 2	sham only		
MUCDL_RAT	Mupcdh	Mucin and cadherin-like protein	sham only		
MUG1_RAT	Mug1	Murinoglobulin-1		treated only	
MUG2_RAT	Mug2	Murinoglobulin-2		treated only	
BIN1_RAT	Bin1	Myc box-dependent-interacting protein 1	1.19		
MTM1_RAT	Mtm1	Myotubularin	treated only		
MARCS_RAT	Marcks	Myristoylated alanine-rich C-kinase substrate	-1.17	-1.37	-2.16
DDAH2_RAT	Ddah2	N(G)	treated only		2.04
NCAM1_RAT	Ncam1	Neural cell adhesion molecule 1	1.39	-1.29	-1.01
NFH_RAT	Nefh	Neurofilament heavy polypeptide	sham only	sham only	
HMGN2_RAT	Hmgn2	Non-histone chromosomal protein HMG-17	treated only		
NLTP_RAT	Scp2	Non-specific lipid-transfer protein	1.09		
NSF1C_RAT	Nsfl1c	NSFL1 cofactor p47	sham only	treated only	treated only
HCC1_RAT	Hcc1	Nuclear protein Hcc-1	-1.03	treated only	sham only
NUCKS_RAT	Nucks1	Nuclear ubiquitous casein and cyclin-dependent kinases substrate	treated only		treated only
YBOX1_RAT	Ybx1	Nuclease-sensitive element-binding protein 1	-1.90	treated only	sham only
NUCL_RAT	Ncl	Nucleolin	1.37	sham only	1.83
NPM_RAT	Npm1	Nucleophosmin	2.16	sham only	sham only
NP1L4_RAT	Nap1l4	Nucleosome assembly protein 1-like 4	sham only	treated only	
PSPC1_RAT	Pspc1	Paraspeckle component 1		treated only	
PRVA_RAT P	Pvalb	Parvalbumin alpha		sham only	treated only
PSIP1_RAT	Psip1	PC4 and SFRS1-interacting protein		sham only	

PPIA_RAT	Ppia	Peptidyl-prolyl cis-trans isomerase A	sham only		sham only
PERF_RAT	Prf1	Perforin-1	treated only		
PERI_RAT	Prph	Peripherin	sham only	1.61	1.19
PRDX2_RAT	Prdx2	Peroxiredoxin-2			treated only
ECHP_RAT	Ehhadh	Peroxisomal bifunctional enzyme	sham only		
PHOS_RAT	Pdc	Phosducin	1.87	-1.27	1.27
PEBP1_RAT	Pebp1	Phosphatidylethanolamine-binding protein 1	1.29	-1.19	1.11
PI5PA_RAT	Inpp5j	Phosphatidylinositol 4	treated only		
SHIP2_RAT	Inppl1	Phosphatidylinositol-3		sham only	
PGK1_RAT	Pgk1	Phosphoglycerate kinase 1	-1.01	treated only	1.14
PGAM1_RAT	Pgam1	Phosphoglycerate mutase 1	treated only	treated only	1.22
PAIRB_RAT	Serbp1	Plasminogen activator inhibitor 1 RNA-binding protein	1.16	treated only	sham only
PGFRL_RAT	Pdgfrl	Platelet-derived growth factor receptor-like protein	treated only		
ATP4A_RAT	Atp4a	Potassium-transporting ATPase alpha chain 1	treated only	treated only	treated only
AT12A_RAT	Atp12a	Potassium-transporting ATPase alpha chain 2	sham only	sham only	
P4HA1_RAT	P4ha1	Prolyl 4-hydroxylase subunit alpha-1	treated only		
PSB9_RAT	Psmb9	Proteasome subunit beta type-9		treated only	
CDV3_RAT	Cdv3	Protein CDV3 homolog		treated only	
DEK_RAT	Dek	Protein DEK	-1.34	treated only	
PDIA1_RAT	P4hb	Protein disulfide-isomerase		treated only	
PDIA3_RAT	Pdia3	Protein disulfide-isomerase A3	sham only	1.00	1.00
PARK7_RAT	Park7	Protein DJ-1	treated only	1.00	sham only
F113B_RAT	Fam113b	Protein FAM113B		treated only	
PACN1_RAT	Pacsin1	Protein kinase C and casein kinase substrate in neurons protein 1	treated only	sham only	
SET_RAT	Set	Protein SET	1.12	-1.63	-1.25
PTMA_RAT	Ptma	Prothymosin alpha	1.10	-1.21	-1.15
KPYM_RAT	Pkm2	Pyruvate kinase isozymes M1/M2	1.37	2.32	2.29
KPYR_RAT	Pklr	Pyruvate kinase isozymes R/L	1.38	treated only	2.39
GDIA_RAT	Gdi1	Rab GDP dissociation inhibitor alpha	sham only		
GDIB_RAT	Gdi2	Rab GDP dissociation inhibitor beta	sham only		
RABE2_RAT	Rabep2	Rab GTPase-binding effector protein 2	sham only		
AKT2_RAT	Akt2	RAC-beta serine/threonine-protein kinase			sham only
RAB12_RAT	Rab12	Ras-related protein Rab-12 (Fragment)		sham only	
RAB14_RAT	Rab14	Ras-related protein Rab-14		sham only	
RAB1B_RAT	Rab1b	Ras-related protein Rab-1B		sham only	
RAB35_RAT	Rab35	Ras-related protein Rab-35		sham only	
RAB8A_RAT	Rab8a	Ras-related protein Rab-8A		sham only	
RAB8B_RAT	Rab8b	Ras-related protein Rab-8B		sham only	
ARRS_RAT	Sag	S-arrestin	1.60	1.78	1.54
SEP12_RAT	Sept12	Septin-12			sham only

SPA3L_RAT	Serpina3l	Serine protease inhibitor A3L		treated only	
STK3_RAT	Stk3	Serine/threonine-protein kinase 3			treated only
MARK2_RAT	Mark2	Serine/threonine-protein kinase MARK2	treated only		
TRFE_RAT	Tf	Serotransferrin		treated only	treated only
ALBU_RAT	Alb	Serum albumin	treated only	13.74	5.04
3BP5_RAT	Sh3bp5	SH3 domain-binding protein 5		treated only	
NAC2_RAT	Slc8a2	Sodium/calcium exchanger 2			treated only
AT1A1_RAT	Atp1a1	Sodium/potassium-transporting ATPase subunit alpha-1	-1.09	treated only	treated only
AT1A2_RAT	Atp1a2	Sodium/potassium-transporting ATPase subunit alpha-2	1.04	treated only	1.49
AT1A3_RAT	Atp1a3	Sodium/potassium-transporting ATPase subunit alpha-3	1.04	treated only	1.20
AT1A4_RAT	Atp1a4	Sodium/potassium-transporting ATPase subunit alpha-4	sham only		1.43
SFRS5_RAT	Sfrs5	Splicing factor	sham only	sham only	
SFRS9_RAT	Sfrs9	Splicing factor		treated only	
STMN1_RAT	Stmn1	Stathmin	1.07	-3.69	sham only
STML2_RAT	Stoml2	Stomatin-like protein 2	treated only		
STIP1_RAT	Stip1	Stress-induced-phosphoprotein 1		treated only	
SMC1A_RAT	Smc1a	Structural maintenance of chromosomes protein 1A	treated only		
SAP_RAT	Psap	Sulfated glycoprotein 1			sham only
SODC_RAT	Sod1	Superoxide dismutase [Cu-Zn]	1.01	-1.48	-1.09
SYCP1_RAT	Sycp1	Synaptonemal complex protein 1	treated only		
SNP25_RAT	Snap25	Synaptosomal-associated protein 25	-1.30	1.01	-2.35
STX1B_RAT	Stx1b	Syntaxin-1B	6.71		sham only
TLR4_RAT	Tlr4	Toll-like receptor 4	sham only		
TALDO_RAT	Taldo1	Transaldolase		sham only	
PURB_RAT	Purb	Transcriptional activator protein Pur-beta	-3.00	treated only	treated only
TERA_RAT T	Vcp	Transitional endoplasmic reticulum ATPase			treated only
TPIS_RAT	Tpi1	Triosephosphate isomerase	1.38	-1.19	1.15
TRUA_RAT	Pus1	tRNA pseudouridine synthase A			treated only
TPM3_RAT	Tpm3	Tropomyosin alpha-3 chain	sham only	sham only	treated only
TPM4_RAT	Tpm4	Tropomyosin alpha-4 chain		-1.38	
TPM2_RAT	Tpm2	Tropomyosin beta chain		treated only	
TBA1A_RAT	Tuba1a	Tubulin alpha-1A chain	1.51	-1.13	1.14
TBA1B_RAT	Tuba1b	Tubulin alpha-1B chain	1.51	-1.13	1.14
TBA1C_RAT	Tuba1c	Tubulin alpha-1C chain	1.37	-1.15	1.08
TBA3_RAT	Tuba3a	Tubulin alpha-3 chain	1.40	-1.11	1.14
TBA4A_RAT	Tuba4a	Tubulin alpha-4A chain	1.51	1.01	1.18
TBA8_RAT	Tuba8	Tubulin alpha-8 chain	1.67	1.10	1.14
TBB2A_RAT	Tubb2a	Tubulin beta-2A chain	1.40	1.10	1.07
TBB2B_RAT	Tubb2b	Tubulin beta-2B chain	1.45	1.43	1.07
TBB2C_RAT	Tubb2c	Tubulin beta-2C chain	1.32	1.08	-1.08

TBB3_RAT	Tubb3	Tubulin beta-3 chain	1.63	2.58	1.34
TBB5_RAT	Tubb5	Tubulin beta-5 chain	1.39	1.04	1.07
UBIQ_RAT	Rps27a	Ubiquitin	1.32	-1.78	1.39
UCHL1_RAT	Uchl1	Ubiquitin carboxyl-terminal hydrolase isozyme L1		-2.52	treated only
CB063_RAT		Uncharacterized protein C2orf63 homolog		treated only	
CP062_RAT	N/A	UPF0505 protein C16orf62 homolog	treated only		
U639_RAT	N/A	UPF0639 protein	sham only		
VAMP2_RAT	Vamp2	Vesicle-associated membrane protein 2	-1.05	-1.33	treated only
VAMP3_RAT	Vamp3	Vesicle-associated membrane protein 3	-1.08	-1.33	treated only
VIGLN_RAT	Hdlbp	Vigilin	1.00		
VIME_RAT	Vim	Vimentin	11.32	-1.19	1.35
VINC_RAT	Vcl	Vinculin			sham only
XYLB_RAT X	Xylb	Xylulose kinase		treated only	treated only