

REPORT DOCUMENTATION PAGE

Form Approved
OMB NO. 0704-0188

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1. AGENCY USE ONLY (Leave Blank)		2. REPORT DATE 31 Jul 07	3. REPORT TYPE AND DATES COVERED Final Progress Report (Jul 05 - Apr 07)	
4. TITLE AND SUBTITLE Rat Models and Identification of Candidate Early Serum Biomarkers of Battlefield Traumatic Brain Injury		5. FUNDING NUMBERS Contract # W911NF-05-1-038		
6. AUTHOR(S) Fabio Leonessa, MD		8. PERFORMING ORGANIZATION REPORT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc 1401 Rockville Pike, Suite 600; Rockville, MD 20852-1402		10. SPONSORING / MONITORING AGENCY REPORT NUMBER 49021-LS-DRP		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U. S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211		11. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.		
12 a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited.		12 b. DISTRIBUTION CODE		
13. ABSTRACT (Maximum 200 words) This study is part of a larger multi-year study that has two main specific aims: a. the characterization of a "closed head" (lateral fluid percussion, FPI) vs a penetrating ballistic rat model of traumatic brain injury (PBI) and b. the identification of specific plasma proteomic biomarkers of TBI. Funding has covered year 1 of this study, and the following related objectives: 1. Application of TBI, neurobehavioral testing and collection of biofluids and tissues; 2. Optimization of proteomic protocols for the identification of differentially expressed proteins in the plasma of brain-traumatized rats; 3. Preliminary identification of proteins differentially expressed in plasma from rat models of TBI. All the objectives set for this part of the larger study have been met. One hundred thirty nine Sprague-Dawley rats have been used to achieve Objective 1. Results show a significant neurobehavioral effect of both kinds of TBI, more drastic for the PBI model. Optimization of proteomic technology (Objective 2) has allowed the analysis of replicate samples collected at the different time points following injury. We have thus met Objective 3 by identifying several potential time-dependent plasma protein biomarkers of TBI, including many proteins known to be normally expressed in the central nervous system. Future evaluations will need to include the verification of some of the most statistically and biologically significant plasma proteome changes observed in this study and evaluation of their dependence on trauma intensity.				
14. SUBJECT TERMS Biomarker, TBI, Rat		15. NUMBER OF PAGES 54		16. PRICE CODE
17. SECURITY CLASSIFICATION OR REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION ON THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL	

NSN 7540-01-280-5500

Standard Form 298 (Rev.2-89)
Prescribed by ANSI Std. Z39-18
298-102

Enclosure 1

FINAL PROGRESS REPORT

Project: Rat Models and Identification of Candidate Early Serum Biomarkers of Battlefield Traumatic Brain Injury

Principal Investigator: Fabio Leonessa, M.D.

Contract /Grant number: W911NF-05-1-038

ARO proposal number: 49021-LS-DRP

Report date: July 31, 2007

Period covered by report: July 2005-date

ORIGINAL STATEMENT OF WORK AND OBJECTIVES FOR YEAR 1

The two major goals of this study are: 1. the characterization of a penetrating ballistic vs a closed head model (lateral fluid percussion) of traumatic brain injury (TBI); 2. the identification of biomarkers of traumatic brain injury

Operationally, these goals translate into the following Specific Aims, as described in the original proposal for this study:

Aim 1. Characterization and validation of animal models of traumatic brain injury;

Aim 2. Optimization of proteomic protocols for the identification of differentially expressed proteins in serum* and brain tissues of TBI rat models;

Aim 3. Identification of proteins differentially expressed in the serum* from rat models of traumatic brain injury;

Aim 4. Optimization of antibody-based assays for the detection of the proteins preliminarily identified by MDLC/MS-MS approach;

Aim 5. Validation and characterization of proteins differentially expressed in the serum* of brain traumatized rats as candidate serum biomarkers of traumatic brain injury.

Achievement of all aims was projected over three years. As part of the overall Statement of Work, the following were the *Objectives* for year 1 of this study (which was the only part covered by the grant in question) :

Objective 1: Application of trauma (two traumatic brain injury models), functional

evaluations, collection of serum* and brain specimens. Objective 1 is aimed to support specific Aims 1 (years 1 through 3), 3 (year 1) and 5 (year 3);

Objective 2: Optimization of proteomic protocols for the identification of differentially expressed proteins in the serum* of brain-traumatized rats. This objective addresses Specific Aim 2 (year 1).

Objective 3: Preliminary identification of proteins differentially expressed in serum* from rat models of traumatic brain injury. Objective 3 coincides with Specific Aim 3 of this study (year 1).

*Note: This has subsequently been changed to plasma (see below)

EXPERIMENTAL DESIGN (year 1 objectives)

Objective 1: Application of traumatic brain injury, neurobehavioral testing and collection of biofluids and tissues.

This objective is fundamental to the achievement of the two main goals of this study. The objective involves the application of the *penetrating ballistic injury (PBI)* and the *lateral fluid percussion injury (FPI)* models of TBI, at different intensities, on male Sprague-Dawley rats. Following injury (or non-injury equivalent for control groups), rats undergo neurological evaluations, except for those cases where animals are euthanized before full recovery from anesthesia is achieved. Biosamples (primarily plasma and brains, and secondarily blood mononuclear cells and erythrocytes) are collected at the time of euthanasia, at different time points following injury time (or equivalent, in non-brain injured animals).

The plan for the full study (years 1 to 3), as outlined in the sections “Research Design” and “Timelines for Completion of Work” of the original application calls for the application of the two TBI models at three different levels of intensity over three years. All animals are to be sacrificed and biosamples collected and stored frozen at different times following injury (up to 3 days). As described in the “Timelines for Completion of Work” section, the original plan calls for only part of this objective to be achieved during the course of year 1 (covered by this grant).

Objective 2 Optimization of proteomic protocols for the identification of differentially expressed proteins in the serum of brain-traumatized rats.

We have made two modifications to the original experimental design: 1. we changed our target for biomarker discovery from serum to plasma; 2. we adopted a higher throughput approach to the initial proteomic identification of potential biomarkers.

At the time the initial statement of work was written we were considering using serum as our target. Technically, serum and plasma are generally considered equivalent in terms of ease of handling for liquid chromatography/mass spectrometry analyses, though a few studies may have shown the relative advantage of one with respect to the other. However, different than serum, separation of plasma allows for the collection and preservation of blood cells, another potential source for proteomic, as well as transcriptomic biomarkers. For this reason, we have since changed our target to plasma and decided to preserve the mononuclear and erythrocyte fraction from the rat blood samples for possible separate future studies.

Initially, we foresaw the use of metabolic labeling and/or isotope coded affinity tagging (ICAT) for the initial identification and quantitation of TBI-related proteomic changes (objective 3 for year 1). This methodology allows for a more accurate quantitation of relative changes in protein concentrations already at the time of the initial liquid chromatography/mass spectrometry analyses. The disadvantage of these approaches is that they are highly time-consuming and extremely low throughput. Consequently, in the original design, we planned to carry the initial identification of TBI-induced plasma proteome changes only on samples collected at 60 min after injury. Moreover, because of the low throughput, plasma samples would have been pooled from different animals in the same experimental group. We would have then used antibody-based assays to confirm and evaluate the correlation of these changes with trauma intensity and time of collection after injury. Potential biomarkers detectable only at times different than 60 min post-injury would have been missed.

As an alternative to metabolic labeling and ICAT, the “subtractive proteomics” approach does not involve pre-labeling or tagging samples. Relative differences in expression are quantitated in terms of differences in the number of tryptic peptides that can be identified for a specific protein following tandem mass spectrometry analysis. Changes in this number appear to roughly correlate with changes in concentrations of the related protein [Sanders *et al*, Mol Cell Biol.

2002 Jul;22(13):4723-38, Rappsilber *et al*, Genome Res. 2002 Aug;12(8):1231-45]. Though less accurately quantitative than comparison of tagged peptides, the subtractive proteomics approach appears to be adequate for studies like this, where the scope of initial mass spectrometry-based proteomic evaluations is to provide a preliminary “hit-list” of potential predictors that will then need to be confirmed and more accurately quantitated by antibody-based assays. Most importantly, the differential proteomics approach allows for a much higher throughput than that allowed by the other approaches. Adoption of this approach has allowed us to plan the preliminary proteomic analyses to be carried out on individual plasma samples (four from each involved experimental group) collected not just at 60 min but at different times after injury, as described in the following section.

Objective 3 Preliminary identification of proteins differentially expressed in plasma from rat models of traumatic brain injury.

The original plan called for the sera collected at 60 min after either form of TBI to be used for the identification of serum proteomic changes following TBI. In order to allow for proteomic analysis, as originally envisioned, the different samples from each experimental group needed to be pooled. The achievement of Objective 2 and adoption of a proteomic technology with higher throughput has allowed us to modify the experimental design. The new “Subtractive Proteomics” approach has allowed us to test single plasma samples (not pooled) from 104 rats, i.e. from 4 rats in each of 26 experimental groups (as defined by TBI treatment and collection time and described in Table 1).

MATERIAL AND METHODS

Animals

One hundred seventy nine Sprague-Dawley male rats (Taconic, Germantown, NY) weighing 250-300 g have been used. Rats were housed in the facilities of the Laboratory Animal Medicine (LAM), at the Uniformed Services University of the Health Sciences (USUHS), with *ad libitum* access to food and water. Excluding the ones used for technical refinement, 139 rats have been used for actual experiments. Experimental group allocation of these rats is summarized in Table 1. Twenty-six experimental conditions were tested as defined by TBI treatment and time of collection: (1) naïve, n = 5; (2) anesthesia only (ANS) at 15 min, n = 5; (3) ANS at 60 min, n = 5; (4) ANS at 6 hours, n = 5; (5) ANS at 24 hours, n = 5; (6) ANS at 72 hours, n = 5; (7) lateral fluid percussion injury at 2.5 atm (FPI-III) at 15 min, n = 5; (8) FPI-III at 60 min, n = 6; (9) FPI-III at 6 hours, n = 5; (10) FPI-III at 24 hours, n = 6; (11) FPI-III at 72 hours, n = 5; (12) Surgical

preparation for FPI without TBI (FPI-0), at 15 min, n = 5; (13) FPI-0 at 60 min, n = 5; (14) FPI-0 at 6 hr, n = 5; (15) FPI-0 at 24 hour, n = 5; (16) FPI-0 at 72 hour, n = 6; (17) Penetrating Ballistic Injury at 7.5% volume (FPI-III) at 15 min, n = 6; (18) FPI-III at 60 min, n = 5; (19) FPI-III at 6 hour, n = 7; (20) FPI-III at 24 hour, n = 6; (21) FPI-III at 72 hour, n = 6; (22) Surgical preparation for PBI without TBI (PBI-0) at 15 min, n = 5; (23) PBI-0 at 1 hour, n = 5; (24) PBI-0 at 6 hour, n = 5; (25) PBI-0 at 24 hour, n = 5; (26) PBI-0 at 72 hour, n = 5. At least 3 days were given to allow each animal to equilibrate to their new surroundings after delivery.

Surgery (application of traumatic brain injury)

All surgeries were performed using aseptic technique. Animals were checked for pain/distress immediately prior to anesthesia/surgery. Naïve animals were not exposed to anesthesia or surgery until time of biosample collection. All other animals were anesthetized with 3.5% isoflurane in oxygen and maintained with 1-3% isoflurane. Body temperature was maintained with a heating pad. For the anesthesia control groups, animals were maintained under anesthesia in the induction chamber for approximately 1 hour (average duration of the surgical procedure). Anesthesia was discontinued and the animal transferred to a recovery area. Once animal was ambulatory, it was transferred to its home cage, where it was allowed to behave freely with *ad libitum* access to food and water. All animals in the 24 hour and 72 hour groups were returned to LAM within 12 hours of leaving. Pain/distress checks were performed at 3, 6, 12, 24, 36, 48, 60, and 72 hours post-injury.

Fluid Percussion Injury (FPI)

For animals in the surgical control and injury groups, a midline incision was made to expose the dorsal surface of the skull, and a 5 mm burr hole drilled over the right cerebral cortex at 2.5 mm caudal and 2.5 mm right of bregma. A modified cannula hub was fitted to the burr hole and glued in place. After carefully attaching the fluid percussion device (MCV Bioengineering, Richmond, VA), a fluid percussion pulse was either not given (for the surgical controls) or administered at 0.5, 1.5, or 2.5 atm. The hub was immediately removed and the incision closed with wound clips. The animal was recovered as above.

Penetrating Ballistic Injury (PBI)

For animals in the surgical control and injury groups, a midline incision was made to expose the dorsal surface of the skull and the occipital protuberance. A 3 mm burr hole was drilled through the occipital plate at 4 mm ventral and 2 mm right of midline. The animals in the surgical control group remained on anesthesia for the remainder of the hour prior to wound closure and recovery. Animals in the PBI-I, -II, and -III groups were injured using the PBI device (Dragonfly R&D, Ridgeley, WV). An inflatable probe (Dragonfly R&D, Ridgeley, WV) was inserted 15 mm through the right hemisphere and either not inflated (PBI-0) or inflated to create a 5% or 7.5% brain volume injury. The probe was immediately removed and the wound closed with wound clips. The animal was recovered as above.

Neurobehavioral evaluations

Prior to biosample collection, animals in the 24 hour and 72 hour groups were evaluated using the Neurologic Severity Score (NSS), NIH Stroke Severity Score (NIH), and Neurobehavioral Scale (NBS). The criteria used to obtain the scores are detailed in Tables 2 and 3. As an additional endpoint, we also recorded the time needed for each animal to “right up” following the interruption of anesthesia (“righting reflex”).

Biosample collection and processing

At the time of terminal biosample collection, animals were anesthetized with 3.5% isoflurane in oxygen and maintained at 1-3% isoflurane.

CSF collection and processing

After placement in the stereotactic apparatus using blunt-tip ear bars only, a midline incision was made and the muscle dissected away to expose the atlanto-occipital membrane. The area was kept free of blood until puncture of the membrane to collect CSF. Ten minutes were maintained between time of induction and time of CSF collection. CSF was collected using a 25G permanently attached needle on a 1cc insulin syringe (Monoject, lot # 524416) into sterile 0.6 ml tubes (FisherBrand, lot # 451706) and kept at room temperature. CSF was spun at 15 minutes post-collection at 1300 x g for 10 min at 22°C. The supernatant was spun at 35 minutes at 12,000 x g for 15 min at 22°C in sterile 0.6 ml tubes (FisherBrand, lot # 451706). The supernatant was placed in a liquid nitrogen storage tank at 55 minutes post-collection in 1 ml cryogenic vials (Nalgene System-100, lot # 554872).

After collection of CSF, animals were removed from the stereotax, placed supine, and anesthesia increased to 3-5% isoflurane. A midline incision was made over the ribs and abdomen, and the abdominal muscles were cut laterally below the diaphragm.

Blood collection and processing

At 5 minutes post-CSF collection, blood was collected transcardially after cutting through the diaphragm/ribs to expose the heart. Blood was collected in a 4ml EDTA vacutainer (BD, lot # 5157524) using a 20G blood collection needle (Monoject, lot # 216025) and kept at room temperature. At 10 minutes post-collection, the blood was spun at 1321 x g for 10 min at 22°C. Plasma was transferred into two 2 ml tubes (FisherBrand, lot # 534388) and spun at 12,000 x g for 15 min at 22°C. The buffy coat was transferred into a 15 ml tube (Phenix, lot # 3218P) containing 6 ml of RPMI 1640 (Biosource, lot # 604085) and resuspended. The buffy coat mixture was then carefully transferred into a 15 ml tube (Phenix, lot # 3218P) containing 5 ml of Histopaque-1077 (Sigma, lot # 065K6208) and spun at 800 x g for 15 minutes at room temperature. Of the original blood separation, 10 µl of the red blood cell (RBC) pellet was transferred into a 15 ml tube (Phenix, lot # 3218P) containing 10 ml of RPMI 1640 (Biosource, lot # 604085), resuspended, and kept at room temperature. Plasma supernatant was transferred into 1 ml cryogenic vials (Nalgene System-100, lot # 554872) and stored in a liquid nitrogen storage tank at 50 minutes post-blood collection. At 60 minutes after blood collection, the mononuclear cell (MNC) layer from the Histopaque preparation is transferred into a 15 ml tube (Phenix, lot # 3218P) containing 10 ml of RPMI 1640 (Biosource, lot # 604085), resuspended, and spun along with the RBC suspension at 460 x g for 10 min at 22°C. The pellets were resuspended in 2 ml freezing medium (10% DMSO; 10% calf serum; 80% RPMI 1640, Biosource, lot # 604085). The RBC and MNC suspensions were split into 1 ml cryogenic vials (Nalgene System-100, lot # 554872). By 80 minutes after blood collection, the RBC and MNC suspensions were placed in a styrofoam “jacket” at -80°C overnight before being transferred into the liquid nitrogen storage tank.

Brain collection and processing

Immediately after blood collection, animals were either fixed or the brains collected fresh. For fixation, animals were transcardially perfused with PBS until the fluid ran clear from the cut right atrium, then perfused with 10% formalin until the body became stiff. Brains were dissected out and kept at 4°C in 10% formalin overnight, in 10% sucrose overnight, in 20% sucrose for 2-3 days, in 30% sucrose for 2-3 days, then frozen in OCT (Optimum Cutting temperature) embedding compound using isopentane/dry ice. Frozen brains are stored at -80°C. Brains collected fresh began the freezing process in OCT Compound using isopentane/dry ice 2-3 minutes after blood collection.

Proteomic analysis of plasma samples

Phase I: High abundant protein depletion

High-abundant proteins were first *depleted* from rat plasma samples using Agilent's MARS (Multiple Affinity Removal System) mouse cartridges. This system utilizes an immunoaffinity cartridge packed with antibody-modified resin. The cartridge is specifically designed to remove three highly-abundant proteins (albumin, IgG and transferrin) simultaneously from serum or plasma. An enriched pool of low-abundant proteins was then collected and pooled from several flow-through fractions. Agilent's 5000 Da molecular weight cut off (MWCO) spin concentrators were then used for desalting and concentrating the low-abundant proteins.

Quality control for this phase was achieved by using every fifth rat plasma sample to test the efficiency of the MARS cartridges. An aliquot of each flow-through fraction and an aliquot of fractions containing high abundant proteins is removed and run on 4-12% Bis-Tris NuPAGE gels.

Phase II: protein digestion

Protein digestion was accomplished through the use of sequencing grade modified trypsin (Promega). Resultant peptides were subjected to solid phase extraction (SPE) using 3MTM EmporeTM High Performance Extraction Disk Cartridges (3M Bioanalytical Technologies, St. Paul, MN). This procedure not only concentrates resultant peptides, but also removes or minimizes substances that may interfere with downstream sample processing.

For *quality control* purposes, complete digestion was verified by removing an aliquot of each trypsin-digested plasma sample and running it on a 4-12% Bis-Tris NuPAGE gel. BCA protein assays (Pierce, Rockford, IL) were performed on all rat plasma samples both prior to trypsin digestion and prior to SCX processing.

Phase III: Strong Cation Exchange (SCX) Fractionation

SCX chromatography was used to reduce sample complexity prior to tandem mass spectrometry. Briefly, strong cation exchange chromatography was performed using a HP 1100 LC system (Agilent Technologies, Palo Alto, CA) equipped with a polysulfoethyl A column (1.0 x 150mm, 5µm, 300Å pore size, PolyLC, Inc.). An ammonium formate, pH 3.0/acetonitrile multi-step gradient was used to elute peptides from the column at a flow rate of 50 µl/min. Peptide separation was monitored by fluorescence (266/340 nm). A total of 96 fractions are collected which were subsequently pooled into 10 fractions, lyophilized and re-suspended in 0.1% trifluoroacetic acid (TFA) for tandem mass spectrometry.

Phase IV: Tandem Mass Spectrometry

A total of 1040 samples were been prepared and analyzed by tandem mass spectrometry (104 plasma samples x 10 fractions/sample). μ RPLC-MS/MS was performed using an Agilent 1100 capillary LC system (Agilent Technologies, Palo Alto, CA) coupled online to a linear ion trap (LIT) mass spectrometer (LTQ, Thermo Electron, Waltham, MA, USA). Separations were performed using 75 μ m i.d. x 360 μ m o.d. x 10 cm long fused silica capillary columns (Polymicro Technologies, Phoenix, AZ) slurry-packed in house with 3 μ m, 300Å pore size C-18 silica-bonded stationary phase (Vydac, Hysperia, CA).

The mass spectrometer was operated in a data-dependent MS/MS mode with each full MS scan being followed by seven MS/MS scans where the seven most abundant molecular ions were dynamically selected for collision-induced dissociation (CID) using a normalized collision energy of 35%.

Phase V: Bioinformatics

Tandem mass spectra were searched against the UniProt rat proteomic database (10-26-05 release) from the European Bioinformatics Institute (www.ebi.ac.uk/integr8) with SEQUEST[®] (Thermo Electron). For a fully tryptic peptide to be considered legitimately identified, it had to achieve stringent charge state and proteolytic cleavage-dependent cross correlation (X_{corr}) scores of 1.9 for $[M+H]^+$, 2.2 for $[M+2H]^{2+}$, and 3.1 for $[M+3H]^{3+}$, and a minimum delta correlation (ΔC_n) of 0.08. SEQUEST results were further filtered and analyzed using software developed in-house.

Analysis of proteomic data

To identify proteins differentially represented in the plasma of TBI and control rats, we compared the mean number of unique peptide sequences detected per each protein. Prioritization of protein “hits” was based on the statistical significance of observed differences. This was a two-step process with a first t test carried out on the differences in number of unique peptides between each TBI experimental condition (as defined kind of TBI and time of collection after injury) and its own surgical sham (e.g., FPI-III vs FPI-0), for all proteins detected (overall, 10217 proteins were identified by at least two unique peptides). This first statistical test compared the results in plasma from 4 rats in each of the two experimental groups. Only proteins for which the level of statistical significance for the observed difference is $p \leq 0.2$ were selected for the second statistical test. The second t test compared the mean number of unique peptides for each of the selected proteins between each TBI conditions (as defined by kind of TBI and time of collection – corresponding to the mean of 4 samples) and all control conditions (including all the different control conditions at the different collection times – corresponding to the mean of 64 samples). The result of this two-step analysis is represented by tables, one for each TBI conditions, in which plasma proteins are ranked according to the statistical significance of the observed differences (*i.e.*, in order of ascending p value).

RESULTS

As described in the application for this grant, we designed this as a 3-year study, but we applied and obtained funding only for year 1, with the idea to apply additional funding based on year 1 results. What follows describes these results.

Objective 1

Application of traumatic brain injury, neurobehavioral testing and collection of biofluids (plasma, CSF, mononuclear blood cells and erythrocytes) and brains have been carried out on 138 rats, including at least 5 rats for each of 26 experimental conditions, as defined by kind of injury, related control conditions and time tissue/plasma collection after injury. Experimental conditions and the number of animals in each experimental group are summarized in Table 1. The experimental conditions include all 3 control conditions (surgical preparation without actual application of TBI, anesthesia only, and baseline control), and the lateral fluid percussion (FPI) and Penetrating Ballistic (PBI) forms of TBI at one of 3 planned intensities (2.5 atm and 7.5% volume, respectively). Application of two additional intensities is part of the work proposed for year 2 and 3 of the study.

Results of the procedures are summarized in Table 4. For each rat that was used, the table details the study identifier, experimental group, amount of plasma and CSF collected, whether red blood cells (RBC) and mononuclear cells (MNC) were collected, whether the brain was collected fresh or fixed, the Neuroscores (NSS, NBS and NIH) and righting reflex time (time needed for the animal to right itself onto all four paws, following interruption of anesthesia).

We observed that CSF collection is achievable at 6 and more hours after brain injury, but not 1 hour or at shorter times after injury. Consequently, this medium could not be used as a source of early biomarkers of TBI (1 hour or less after injury).

Brains will be used in year 2 and 3 of this study for characterization of the lateral fluid percussion *vs* penetrating ballistic penetrating models of traumatic brain injury by histochemistry and immunohistochemistry.

CSF and mononuclear and red blood cells may be used in further and separate studies on proteomic and/or transcriptomic biomarkers from these sources.

Functional scores have been collected both for characterization purposes and to evaluate their correlation with possible plasma proteomic changes. The righting reflex times and the three neurobehavioral scores demonstrate a significant effect of both kinds of TBI. The scores also suggest a more intense effect of PBI as compared to FPI.

Overall, we believe Objective 1 for the first year of this study has been fully met.

Objective 2

As described in the Experimental Design section of this report, Objective 2 has been met by the adoption of a “differential proteomics approach” to the identification of proteins differentially expressed in plasma of TBI rats. The higher throughput of this approach has allowed us to greatly expand the number of plasma samples that can be subjected to proteomic analysis as part of Objective 3 for year 1.

Objective 3

Plasma samples from 104 animals (4 rats for each of 26 treatment groups described in the Experimental Design section of this report and in Table 1) have been delivered to the proteomic lab directed by Dr. Veenstra.

Proteomic analysis has been completed. This is a large study and, even with the greatly increased throughput afforded by the differential proteomics approach to data analysis, processing and analyzing these many samples and the resulting data has represented a formidable task (as detailed below), that has taken considerable more time than originally planned.

As described in the *Material and Methods* section of this report, proteins differentially represented in plasma of TBI rats (representing potential biomarkers of TBI) have been identified by a subtractive proteomic approach, whereas the number of unique peptides detected for each protein represents a measure of its abundance. Overall, 10217 proteins were identified by at least two unique peptides across the 104 plasma samples that were tested. On average, between 1500 and 2500 proteins were identified within each plasma sample. To identify the best

potential biomarkers of TBI, proteins were ranked according to the statistical significance of observed differences (see *Material and Methods* section).

Fig. 1a (FPI) and Fig 1b (PBI) provide a graphical representation of the number of differentially represented plasma proteins in relation to time of collection after injury, using different cut-offs for statistical significance of observed differences. The most striking aspect of these results is the difference between the FPI and PBI model. The results suggest a much more dramatic impact of the penetrating ballistic injury as compared to the lateral fluid percussion injury. This is consistent with the results of neurobehavioral evaluations showing a more profound effect of PBI. These results also show a correlation between protein changes and time after injury. This correlation is particularly evident for PBI, where a first peak is observed at 15 min after injury, followed by a bigger peak at 6 hours. It could be speculated that the first peak may correspond to the immediate release of tissue factors due to the primary injury, while the second wave may represent secondary injury.

Given the amount of data involved (including more than 10,000 proteins overall), only the proteins for which the most significant differences were observed are reported here. Tables 5a-e report proteins, for which the statistical significance of FPI-related differences corresponds to $p < 10^{-3}$. Table a-e report the equivalent results for PBI, but with a lower cut-off ($p < 10^{-4}$), given the much greater depth of differences observed. In each table, the mean number of unique peptides being compared is color coded in blue, the p value in red, and the proteins known to be associated with nervous tissue are marked bold.

The results represented in Tables 5 and 6 show most of the proteins were identified at the lowest level of detection (2-4 unique peptides across 4 plasma samples). This stresses the need for more sensitive, antibody-based assays to verify and better the observed results. Given the amount of proteins involved, only a small fraction of these can be carried on to further verification and validation studies. Tables 7a-j represent a broad selection from the proteins listed in Tables 5 and 6, based on a combination of criteria: biological significance (e.g., known expression in the central nervous), statistical significance of observed differences, identification of significant differences across different times of collection, availability of antibody based assays. We selected 3-6 proteins for each TBI model/collection time though there are differences in statistical significance for proteins identified in different experimental groups. Some of these proteins (butyrophilin-like protein 2 and testicular cell adhesion molecule 1) are among the most

significant across different TBI collection times. Consequently, they have been selected even if their association with the central nervous tissue is presently unknown. Interestingly, testicular cell adhesion molecule 1 is member of a family of cell adhesion molecules including at least one (ICAM-1) whose intestinal levels are known to increase in response to TBI [Hang *et al.*, World J Gastroenterol. 2005; 11(8): 1149-54]. In tables 7a-j, a graph is associated to each of the selected proteins representing the observed behavior through time.

Some of the selected proteins are of particular interest for their potentially high biological significance. For example, *syntaxin-1A* ($p = 2.3 \times 10^{-9}$) appears to be specifically expressed in neurons [Yi *et al.*, J Neurotrauma 2006, 23(1): 86-96]. *Ischemia related factor vof-16* ($p = 4.3 \times 10^{-11}$) is a scarcely known factor which has been first identified as upregulated in hippocampus and brain cortex following brain ischemia [Todha *et al.*, Biol.Pharm.Bull. 2004, 27(8): 1228-35]. Proenkephalin B ($p = 4.3 \times 10^{-11}$) is a known precursor of several opioid peptides with important functions in the brain [Henry *et al.*, Exp Neurol 2003, 183(2):458-68; Arendt *et al.* Brain Res. 1985, 345(2):230-7]

CONCLUSIONS

Objectives 1-3 for year 1 of this multi-year Project have been fully met. TBI has been applied and functional outcome data and biosamples collected from more than half of the animals minimally required for this 3-year study. We have adopted an approach to proteomic analysis that has allowed us to vastly expand the scope of the third Objective for year 1, **allowing us to screen not only for potential early, but also for later biomarkers of TBI.** We now have a list of potential biomarkers on closed-head (FPI model) and penetrating ballistic injury (PBI) at different times following injury. Further funding will be needed both to expand the study to include both a verification and better quantitation, and the evaluation of the trauma intensity-dependence of the observed plasma proteome changes.

TABLE 1 Number of rats used for each experimental conditions tested in the course of this study

		<i>Naive^a</i>	<i>ANS only^b</i>	<i>Lateral Fluid Percussion Model</i>		<i>Ballistic Penetrating Model</i>	
				0 sham	III 2.5atm	0 sham	III 7.5%
Biosample collection time (time since injury)	No time ref ^a	5	--	--	--	--	--
	15 min	--	5	5	5	5	6
	60 min	--	5	5	6	5	5
	6 hours	--	5	5	5	5	7
	24 hours	--	5	5	6	5	6
	72 hours	--	5	6	5	5	6

^a Baseline control group (animals receive no injury, surgery, nor anesthesia). ^b Animals treated with anesthesia only

TABLE 2 Neurologic Severity Score (NSS)

Neurologic Severity Score (NSS)	Points
Inability to exit from a circle (50 cm in diameter) when left in its center	
Within 30 min	1
Within 60 min	1
At >60 min	1
Loss of righting reflex	
For 20 min	1
For 40 min	1
For 60 min	1
Hemiplegia-inability of the rat to resist forced changes in position	1
Flexion of hindlimb when raised by the tail	1
Inability to walk straight when placed on the floor	1
Inability to move	1
Reflexes	
Startle reflex	1
Pinna reflex	1
Clinical grade	
Loss of seeking behavior	1
Prostration	1
Limb Reflexes	
Loss of placing reflexes	
Forelimbs left	1
Forelimbs right	1
Hindlimbs left	1
Hindlimbs right	1
Functional tests	
Failure in beam balancing task (1 .5 cm wide)	
For 20 s	1
For 40 s	1
For 60 s	1
Failure in beam-walking task	
8.5 cm wide	1
5.0 cm wide	1
2.5 cm wide	1
TOTAL POINTS	24

TABLE 3 Neurobehavioral Scale and NIH Stroke Severity Score

Neurobehavioral Scale

Each category is scored on a scale of 0-4 with 0 being non-functional and 4 being normal.

Forelimb Flexion upon suspension by tail
Decrease in resistance to lateral pulsion
Circling behavior upon spontaneous ambulation
Ability to stand on an inclined plane
Open Field Activity/ Exploratory Behavior

TOTAL POINTS 20

NIH Stroke Severity Score

Based on observed deficits, a functional grade is assessed:

Grade 0: Normal rat

Grade 1: Lethargy

Grade 2: Clear signs of paresis in at least one limb but able to walk

Grade 3: Severe paresis/paralysis, unable to walk

Grade 4: Dead

Table 4: Biosamples and neurobehavioral scores (Part 1 of 5)

Sample ID	Expt Group	Total Amount of Plasma (ml)	Total Amount of CSF (µl)	brain	RBC in storage	MNC in storage	NSS	NIH	NBS	righting time (min)	
AA123	naïve	2.04	140	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AA148	naïve	1.9	50	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AA164	naïve	2.05	120	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AA268	naïve	1.97	120	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AB027	naïve	2	120	fresh frozen	+	+	n/a	n/a	n/a	n/a	
AA084	A 15min	1.94	0	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AA194	A 15min	2	90	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AA260	A 15min	2	80	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AB017	A 15min	*	80	fresh frozen	+	+	n/a	n/a	n/a	n/a	
AB018	A 15min	1.75	90	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AA131	A 60min	2	80	fixed frozen	+	+	n/a	n/a	n/a	4	
AA173	A 60min	1.85	60	fixed frozen	+	+	n/a	n/a	n/a	*	
AA206	A 60min	1.9	110	fixed frozen	+	+	n/a	n/a	n/a	*	
AA269	A 60min	2	110	fixed frozen	+	+	n/a	n/a	n/a	2	
AB035	A 60min	2.1	160	fresh frozen	+	+	n/a	n/a	n/a	3	
							Mean =	n/a	n/a	n/a	3
AA129	A 6hr	1.8	140	fixed frozen	+	+	0	0	20	NR	
AA161	A 6hr	1.8	140	fixed frozen	+	+	0	0	20	3	
AA215	A 6hr	1.9	140	fixed frozen	+	+	0	0	20	3	
AA259	A 6hr	1.92	120	fixed frozen	+	+	0	0	20	2	
AA290	A 6hr	1.8	120	fresh frozen	+	+	0	0	20	2	
							Mean =	0	0	20	2.5
AA070	A 24hr	1.86	120	fixed frozen	+	+	0	0	20	2	
AA163	A 24hr	1.94	80	fixed frozen	+	+	0	0	20	2	
AA192	A 24hr	1.92	140	fixed frozen	+	+	0	0	20	*	
AA233	A 24hr	2	100	fixed frozen	+	+	0	0	20	5	
AB005	A 24hr	1.98	75	fresh frozen	+	+	0	0	20	1	
							Mean =	0	0	20	2.5
AA072	A 72hr	2	110	fixed frozen	+	+	0	0	20	2	
AA190	A 72hr	1.9	130	fixed frozen	+	+	0	0	20	*	
AA234	A 72hr	2	110	fixed frozen	+	+	0	0	20	*	
AA266	A 72hr	1.9	100	fixed frozen	+	+	0	0	20	NR	
AB003	A 72hr	1.75	100	fresh frozen	+	+	0	0	20	*	
							Mean =	0	0	20	2
							Mean for All ANS Animals =	0	0	20	2.6

(continued)

Table 4: Biosamples and neurobehavioral scores (Part 2 of 5)

Sample ID	Expt Group	Total Amount of Plasma (ml)	Total Amount of CSF (µl)	brain	RBC in storage	MNC in storage	NSS	NIH	NBS	righting time (min)	
AA036	FPI 0 15min	1.76	120	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AA130	FPI 0 15min	2	80	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AA168	FPI 0 15min	1.94	0	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AA196	FPI 0 15min	2	100	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AA267	FPI 0 15min	1.92	120	fresh frozen	+	+	n/a	n/a	n/a	n/a	
							Mean =	n/a	n/a	n/a	3.4
AA124	FPI 0 60min	2	140	fixed frozen	+	+	n/a	n/a	n/a	2	
AA154	FPI 0 60min	1.1	90	fixed frozen	+	+	n/a	n/a	n/a	4	
AA174	FPI 0 60min	2	100	fixed frozen	+	+	n/a	n/a	n/a	2	
AA236	FPI 0 60min	2.04	76	fixed frozen	+	+	n/a	n/a	n/a	7	
AB034	FPI 0 60min	2	96	fresh frozen	+	+	n/a	n/a	n/a	2	
							Mean =	0	0	20	3.2
AA122	FPI 0 6hr	1.95	140	fixed frozen	+	+	0	0	20	4	
AA155	FPI 0 6hr	2	100	fixed frozen	+	+	0	0	20	3	
AA195	FPI 0 6hr	2	180	fixed frozen	+	+	0	0	20	4	
AA258	FPI 0 6hr	2	140	fixed frozen	+	+	0	0	20	3	
AA281	FPI 0 6hr	2.1	90	fresh frozen	+	+	0	0	20	2	
							Mean =	0	0	20	3.2
AA035	FPI 0 24hr	1.96	110	fixed frozen	+	+	0	0	20	5	
AA045	FPI 0 24hr	1.6	150	fixed frozen	+	+	0	0	20	NR	
AA076	FPI 0 24hr	1.88	120	fixed frozen	+	+	0	0	20	NR	
AA273	FPI 0 24hr	2.1	160	fixed frozen	+	+	1	0	20	2	
AB051	FPI 0 24hr	2	0	fresh frozen	+	+	0	0	20	4	
							Mean =	0.2	0	20	3.7
AA019	FPI 0 72hr	1.66	85	fixed frozen	+	+	0	0	18	4	
AA068	FPI 0 72hr	1.76	80	fixed frozen	+	+	0	0	20	3	
AA193	FPI 0 72hr	2	140	fixed frozen	+	+	3	0	16	6	
AB011	FPI 0 72hr	1.9	60	fixed frozen	+	+	0	0	20	3	
AB004	FPI 0 72hr*	2.1	70	fresh frozen	+	+	0	0	20	3	
							Mean =	0.6	0	18.8	3.8
Mean for All FPI-0 Animals =								0.3	0	19.6	3.5

(continued)

Table 4: Biosamples and neurobehavioral scores (Part 3 of 5)

Sample ID	Expt Group	Total Amount of Plasma (ml)	Total Amount of CSF (µl)	brain	RBC in storage	MNC in storage	NSS	NIH	NBS	righting time (min)	
AA024	FPI III 15min	1.72	0	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AA082	FPI III 15min	1.8	0	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AA113	FPI III 15min	1.8	0	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AA208	FPI III 15min	1.95	0	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AA292	FPI III 15min	2	0	fresh frozen	+	+	n/a	n/a	n/a	n/a	
							Mean =	n/a	n/a	n/a	9.6
							p-value (T test FPI-0 vs FPI-III at 1 hour) =	#DIV/0!	#DIV/0!	#DIV/0!	0.001
AA149	FPI III 60min	0.19	50	fixed frozen	+	+	n/a	n/a	n/a	10	
AA162	FPI III 60min	1.9	0	fixed frozen	+	+	n/a	n/a	n/a	11	
AA169	FPI III 60min	1.94	0	fixed frozen	+	+	n/a	n/a	n/a	7	
AA282	FPI III 60min	1.88	0	fixed frozen	+	+	n/a	n/a	n/a	10	
AB050	FPI III 60min	1.94	0	fresh frozen	+	+	n/a	n/a	n/a	10	
							Mean =	3.6	0.8	17.2	8
							p-value (T test FPI-0 vs FPI-III at 6 hours) =	0.0265	0.0039	0.0081	0.0008
AA112	FPI III 6hr	1.8	80	fixed frozen	+	+	0	0	20	5	
AA147	FPI III 6hr	1.95	0	fixed frozen	+	+	2	1	17	10	
AA172	FPI III 6hr	1.92	90	fixed frozen	+	+	4	1	17	9	
AA207	FPI III 6hr	2	0	fixed frozen	+	+	8	1	15	8	
AA271	FPI III 6hr	1.84	50	fresh frozen	+	+	4	1	17	8	
							Mean =	1.2	0.3	17.7	11.7
							p-value (T test FPI-0 vs FPI-III at 24 hours) =	0.157	0.186	0.047	0.066
AA020	FPI III 24hr	1.8	80	fixed frozen	+	+	0	0	20	6	
AA071	FPI III 24hr	unk	0	fixed frozen	+	+	0	0	18	8	
AA167	FPI III 24hr	2	25	fixed frozen	+	+	2	1	15	13	
AA235	FPI III 24hr	2	40	fixed frozen	+	+	3	1	15	21	
AA272	FPI III 24hr	1.9	40	fresh frozen	+	+	2	0	18	6	
AC-010	FPI III 24hr*	1.9	130	fresh frozen	+	+	0	0	20	16	
							Mean =	0.2	0	19.2	10.4
							p-value (T test FPI-0 vs FPI-III at 72 hours) =	0.545	#DIV/0!	0.663	0.017
AA034	FPI III 72hr	unk	0	fixed frozen	+	+	0	0	20	7	
AA069	FPI III 72hr	1.96	80	fixed frozen	+	+	0	0	20	6	
AA191	FPI III 72hr	2	100	fixed frozen	+	+	0	0	19	18	
AA232	FPI III 72hr	2.1	50	fixed frozen	+	+	1	0	18	10	
AB006	FPI III 72hr*	1.8	120	fresh frozen	+	+	0	0	19	11	
							Mean for All FPI-III Animals =	1.6	0.4	18	10
							p-value (T test All FPI-0 vs All FPI-III) =	0.035	0.007	0.008	1.84E-07

(continued)

Table 4: Biosamples and neurobehavioral scores (Part 4 of 5)

Sample ID	Expt Group	Total Amount of Plasma (ml)	Total Amount of CSF (µl)	brain	RBC in storage	MNC in storage	NSS	NIH	NBS	righting time (min)	
AB060	PBI 0 15min	1.9	80	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AB095	PBI 0 15min	1.85	80	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AB167	PBI 0 15min	2	70	fixed frozen	-	-	n/a	n/a	n/a	n/a	
AB168	PBI 0 15min	1.95	60	fixed frozen	+	+	n/a	n/a	n/a	n/a	
							Mean =	n/a	n/a	n/a	6
AB098	PBI 0 60min	2	100	fixed frozen	+	+	n/a	n/a	n/a	2	
AB149	PBI 0 60min	2.05	124	fixed frozen	+	+	n/a	n/a	n/a	5	
AB182	PBI 0 60min	1.95	55	fixed frozen	+	+	n/a	n/a	n/a	6	
AB231	PBI 0 60min	2	110	fresh frozen	+	+	n/a	n/a	n/a	3	
AB253	PBI 0 60min	2	57	fixed frozen	+	+	n/a	n/a	n/a	14	
							Mean =	0	0	19.6	5.2
AB185	PBI 0 6hr	1.97	140	fixed frozen	+	+	n/a	n/a	n/a		
AB094	PBI 0 6hr	1.95	70	fixed frozen	+	+	0	0	20	4	
AB116	PBI 0 6hr	2.1	140	fixed frozen	+	+	0	0	20	5	
AB143	PBI 0 6hr	2	70	fixed frozen	+	+	0	0	18	5	
AB184	PBI 0 6hr	2	85	fixed frozen	+	+	0	0	20	3	
AB230	PBI 0 6hr	2.05	115	fresh frozen	+	+	0	0	20	9	
							Mean =	0	0	20	5.2
AB090	PBI 0 24hr	2.15	90	fixed frozen	+	+	0	0	20	4	
AB117	PBI 0 24hr	2.1	60	fixed frozen	+	+	0	0	20	3	
AB180	PBI 0 24hr	1.98	75	fixed frozen	+	+	0	0	20	7	
AB181	PBI 0 24hr	2.1	117	fixed frozen	+	+	0	0	20	6	
AB197	PBI 0 24hr	2.05	115	fixed frozen	+	+	0	0	20	6	
							Mean =	0	0	20	5.2
AB066	PBI 0 72hr	2.1	80	fixed frozen	+	+	0	0	20	6	
AB105	PBI 0 72hr	2.3	100	fixed frozen	+	+	1	0	20	5	
AB133	PBI 0 72hr	1.97	60	fixed frozen	+	+	0	0	20	3	
AB191	PBI 0 72hr	1.9	95	fixed frozen	+	+	0	0	20	3	
AB194	PBI 0 72hr	2	65	fresh frozen	+	+	0	0	20	2	
							Mean =	0.2	0	20	3.8
Mean for All PBI-0 Animals =								0.1	0.0	19.9	5.1

(continued)

Table 4: Biosamples and neurobehavioral scores (Part 5 of 5)

Sample ID	Expt Group	Total Amount of Plasma (ml)	Total Amount of CSF (µl)	brain	RBC in storage	MNC in storage	NSS	NIH	NBS	righting time (min)	
AB079	PBI III 15min	1.85	0	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AB099	PBI III 15min	2.1	100	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AB148	PBI III 15min	2	0	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AB219	PBI III 15min	2	0	fresh frozen	+	+	n/a	n/a	n/a	n/a	
AB237	PBI III 15min	1.85	0	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AB252	PBI III 15min	1.85	15	fixed frozen	+	+	n/a	n/a	n/a	n/a	
AB074	PBI III 60min	2	0	fixed frozen	+	+	n/a	n/a	n/a	19	
AB121	PBI III 60min	2.1	0	fixed frozen	+	+	n/a	n/a	n/a	10	
AB207	PBI III 60min	1.97	0	fixed frozen	+	+	n/a	n/a	n/a	9	
AB220	PBI III 60min	1.98	60	fresh frozen	+	+	n/a	n/a	n/a	5	
AB248	PBI III 60min	1.9	35	fixed frozen	+	+	n/a	n/a	n/a	12	
							Mean =	n/a	n/a	n/a	11
p-value (T test PBI-0 vs PBI-III at 1 hour) =							#DIV/0!	#DIV/0!	#DIV/0!	0.149	
AB120*	PBI III 6hr	1.8	64	fixed frozen	+	+	6	0	11	17	
AB147	PBI III 6hr	1.9	60	fixed frozen	+	+	10	2	8	35	
AB201	PBI III 6hr	1.95	0	fixed frozen	+	+	10	2	8	17	
AB218	PBI III 6hr	1.85	70	fresh frozen	+	+	13	2	8	*	
AB236	PBI III 6hr	2	15	fixed frozen	+	+	4	0	16	7	
AB251	PBI III 6hr	1.9	30	fixed frozen	+	+	8	2	12	6	
AB268	PBI III 6hr	2	0	fresh frozen	+	+	9	1	10	36	
							Mean =	8.6	1.3	10.4	19.7
p-value (T test PBI-0 vs PBI-III at 6 hours) =							7.47E-05	0.014	5.47E-05	0.039	
AB068	PBI III 24hr	1.85	0	fixed frozen	+	+	7	2	13	30	
AB138	PBI III 24hr	2	0	fixed frozen	+	+	6	0	16	9	
AB246	PBI III 24hr	1.95	0	fixed frozen	+	+	10	2	13	6	
AB247	PBI III 24hr	1.87	10	fixed frozen	+	+	10	2	12	8	
AB265	PBI III 24hr	1.95	70	fixed frozen	+	+	7	1	15	21	
AB266	PBI III 24hr	1.95	0	fresh frozen	+	+	7	0	16	9	
							Mean =	7.8	1.2	14.2	13.8
p-value (T test PBI-0 vs PBI-III at 24 hours) =							3.36E-06	0.027	3.68E-05	0.079	
AB067*	PBI III 72hr	2.2	0	fixed frozen	+	+	12	0	11	17	
AB106	PBI III 72hr	2.15	100	fixed frozen	+	+	5	0	16	33	
AB156	PBI III 72hr	2.2	30	fixed frozen	+	+	4	0	18	11	
AB160	PBI III 72hr	2.17	60	fixed frozen	+	+	3	0	18	7	
AB195*	PBI III 72hr	2.05	0	fresh frozen	+	+	5	0	18	7	
AB227	PBI III 72hr	2	90	fixed frozen	+	+	7	0	12	14	
							Mean =	6	0	15.5	14.8
p-value (T test PBI-0 vs PBI-III at 72 hours) =							0.003	#DIV/0!	0.013	0.035	
							Mean for All PBI-III Animals =	7.5	0.8	13.2	15
p-value (T test All PBI-0 vs All PBI-III) =							1.19E-11	0.002	1.67E-08	7.96E-05	

Table 5a: List of FPI-III 15 Min proteins (p≤ 10-3)

Protein	Accession Number	p value	Controls	FPI							PBI				
				15m	1 h	6 h	24h	72 h	15m	1 h	6 h	24h	72 h		
				0.5	0	0	0	0	0.25	0	0	0	0		
Voltage-dependent P/Q-type calcium channel alpha-1A subunit	Q2YSS37	4.315E-11	0	0.5	0	0	0	0	0	0.25	0	0	0	0	
Na+ channel (Fragment)	Q63360	9.039E-07	0.015625	0.5	0	0	0	0	0	0	0	0	0	0	
Putative integral membrane transport protein	O70609	2.158E-05	0.078125	0.75	0	0	0	0	0	0	0	0.25	0.75	0	
Probable saccharopine dehydrogenase (EC 1.5.1.9)	Q6AY30	3.929E-05	0.265625	1.5	0.5	0.25	0.25	0.5	0	0.25	0.5	0	0	0	
Fucosyltransferase II	Q68FV3	4.97E-05	0.140625	1	0	0	0	0	1	0.5	0.25	0	0	0	
UDP-glucuronosyltransferase I-3	Q64637	5.538E-05	0.03125	0.5	0	0	0	0	0	0	0	0	0	0	
Feline leukemia virus subgroup C receptor-related protein 2	P60815	5.538E-05	0.03125	0.5	0.25	0	0	0	0	0	0	0.25	0	0	
Putative taste receptor T2R20 (T2R16)	Q67ET0	5.538E-05	0.03125	0.5	0	0.5	0	0	0	0	0	0	0.25	0	
Putative taste receptor T2R14	Q67ET6	6.85E-05	0.0625	0.75	0	0	0	0.5	0	0	0	0.25	0.25	0	
ORF protein	Q62693	9.437E-05	0.09375	0.75	0	0	0	0	0.25	0	0.25	0	0	0	
Orphan nuclear receptor HZF-2	Q62702	0.0002249	0.078125	0.75	0	0	0	0	0	0.25	0.25	0.5	0	0	
Terf protein	Q5BIL9	0.0002249	0.078125	0.75	0	0	0.25	0	0	0	0	0	0	0.25	
Vomerinal Vir-type receptor Virg11	Q5J3G0	0.0002259	0.203125	1	0.25	0.25	0.5	0.25	0.25	0.25	0	0	0	0.5	
Acetyl-Coenzyme A carboxylase 2	Q1HEC0	0.0003921	0.1875	1	0.75	0	0.25	0	0.5	0	0.25	0.25	0.25	0.25	
Beta-1,3-N-acetylglicosaminyltransferase radical fringe	Q9R1U9	0.0005106	0.140625	1	0	0	0	0	0	1	0.25	0.25	0.25	0	
Progestin membrane receptor alpha	Q4PU88	0.0005437	0.046875	0.5	0	0	0	0	0	0	0	0	0	0.5	
Plasmaligin (Plasma membrane proteolipid)	P47987	0.0005437	0.046875	0.5	0	0	0	0	0.25	0	0.25	0	0.25	0	
LRRGT00159	Q6QI49	0.0005437	0.046875	0.5	0	0	0	0	0.25	0	0.25	0	0	0	
Carbohydrate kinase-like	Q3MDD4	0.0005437	0.046875	0.5	0	0	0	0	0.25	0	0.25	0	0.25	0.5	
Vascular chymase	O70500	0.0005437	0.046875	0.5	0	0	0	0	0	0	0	0	0.25	0.25	
NADP-dependent leukotriene B4 12-hydroxydehydrogenase	P97584	0.000581	0.09375	0.75	0	0	0	0	0.25	0.25	0.25	0.25	0	0	
GDP-mannose pyrophosphorylase A	Q5XIC1	0.0005998	0.171875	1	0.25	0.25	0	0	0	0.75	0.5	0.75	0.25	0.25	
Neural F box protein NFB42	Q971X8	0.0007416	0.125	0.75	0	0.25	0	0.25	0	0	0.25	0.25	0.25	0	
Mannosidase 2, alpha B1	Q6P762	0.0007616	0.296875	1.25	0.5	0	0.25	0	0.25	0.25	1	0	0	1	
Succinyl-CoA ligase [GDP-forming] alpha-chain, mitochondrial	P13086	0.0008381	0.15625	1	0.25	0.25	0.5	0	0.5	0.25	0	0.25	0	0.25	

Table 5b: List of FPI-III 1 Hour proteins (p≤ 10-3)

Protein	Accession Number	p value	Controls	FPI					PBI					
				15m	1 h	6 h	24h	72 h	15m	1 h	6 h	24h	72 h	
<i>Ischemia related factor vof-16</i>	Q8K4N0	4.315E-11	0	0.25	0.5	0	0	0	0	0	0	0.25	0	0
<i>GTP cyclohydrolase I</i>	P22288	2.331E-09	0.03125	0.25	0.75	0.25	0	0.25	0	0	0.25	0	0.25	0
<i>Cytochrome c oxidase polypeptide VIc-2</i>	P11951	9.039E-07	0.015625	0.25	0.5	0	0	0	0	0.25	0	0	0	0
CL3BB	Q4LDM5	9.039E-07	0.015625	0.25	0.5	0	0	0	0	0	0	0	0	0
<i>Hypothetical protein LOC312102</i>	Q5BK23	2.979E-06	0.0625	0	0.75	0	0	0.25	0	0	0	0	0	0
<i>40S ribosomal protein S10</i>	P63326	5.538E-05	0.03125	0	0.5	0	0	0	0	0	0	0	0	0
<i>3-mercaptopyruvate sulfurtransferase (MST)</i>	P97532	5.538E-05	0.03125	0	0.5	0	0	0	0	0	0.25	0	0	0
<i>Rps2r1</i>	Q35805	5.538E-05	0.03125	0	0.5	0	0	0	0.25	0	0	0	0	0
<i>Vomerousal Vir-type receptor Vir20</i>	Q5J3F4	0.0001091	0.15625	0	1	0	0	0	0.25	0.25	0.25	0.5	0	0.75
<i>Junction adhesion molecule 2</i>	Q3MHC0	0.0005437	0.046875	0	0.5	0	0	0.5	0	0	0.5	0	0	0
<i>Cell surface glycoprotein gp42 precursor</i>	P23505	0.0005437	0.046875	0	0.5	0	0.25	0	0.25	0	0.25	0	0.75	0
<i>Ly49 stimulatory receptor 5</i>	Q5DLU5	0.0005437	0.046875	0	0.5	0	0	0	0	0	0	0.25	0.25	0.25
<i>Glia maturation factor beta (GMF-beta)</i>	Q63228	0.0005437	0.046875	0	0.5	0	0	0	0	0	0	0	0	0
<i>Kinesin heavy chain isoform 5C (Kinesin heavy chain neuron-specific 2)</i>	P56536	0.0005437	0.046875	0.25	0.5	0	0	0	0	0	0	0	0.25	0
<i>40S ribosomal protein S7 (S8)</i>	P62083	0.0005437	0.046875	0.25	0.5	0	0.25	0	0	0	0.25	0	0	0.25
<i>G protein-coupled receptor kinase</i>	P97548	0.0005437	0.046875	0.25	0.5	0	0	0	0	0	0	0.25	0	0.25
<i>Actin-like protein 2 (Actin-related protein 2)</i>	Q5M7U6	0.000581	0.09375	0	0.75	0	0	0.25	0	0.25	0	0.25	0.25	0
<i>Potassium channel modulatory factor I (Predicted)</i>	Q5XIQ2	0.000581	0.09375	0	0.75	0	0	0.25	0.25	0.25	0.25	0.75	0.5	0.25

Table 5d: List of FPI-III 24 Hour proteins (p_≤ 10⁻³)

Protein	Accession Number	p value	Controls	FPI						PBI					
				15m	1 h	6 h	24h	72 h	15m	1 h	6 h	24h	72 h		
<i>Testicular cell adhesion molecule 1 (TCAM1)</i>	Q9Z133	5.538E-05	0.03125	0	0	0	0.5	0	0.75	0	0.25	0.75	0		
<i>T-cell ecto-ADP-ribosyltransferase 2 precursor</i>	P20974	5.538E-05	0.03125	0	0	0	0.5	0	0	0.25	0	0	0		
<i>Neurexin 1 receptor 2 (NMU-R2)</i>	Q9ESQ4	5.538E-05	0.03125	0	0	0	0.5	0	0	0	0.25	0	0		
<i>Rat bone protein 1 (BP-1) (Fragment)</i>	Q63069	5.538E-05	0.03125	0	0	0	0.5	0	0	0	0	0.25	0		
<i>ARF-related protein (ARP)</i>	Q63055	5.538E-05	0.03125	0.25	0.25	0.25	0.5	0.25	0.25	0.25	0.25	0	0		
<i>Synaptonemal complex protein 3 (SCP-3)</i>	Q63520	5.538E-05	0.03125	0.25	0.25	0.25	0.5	0.25	0.5	0	0	0.25	0		
<i>60 kDa lysophospholipase</i>	O88202	5.538E-05	0.03125	0.25	0.25	0	0.5	0.25	0.25	0.25	0	0.25	0.25		
<i>Hypothetical protein RGD1308517</i>	Q3B7T8	0.000497	0.21875	0.25	0.25	0.5	1.25	0.25	0.25	0.25	0.5	0.25	0.25		
<i>Testicular ecto-ATPase</i>	Q5RJP4	0.0005437	0.046875	0	0	0	0.5	0	0	0	0	0	0		
<i>Glutathione peroxidase 2</i>	P83645	0.0005437	0.046875	0	0	0	0.5	0.25	0.25	0	0	0	0		
<i>Protease, serine, 16 (Thymus)</i>	Q3MHS0	0.0005437	0.046875	0.25	0	0	0.5	0	0.25	0	0	0	0.5		
<i>Hypothetical protein LOC619440</i>	Q5X197	0.000581	0.09375	0	0.25	0.25	0.75	0.25	0	0	0.5	0.5	0		
<i>Protein AATF (Apoptosis-antagonizing transcription factor)</i>	Q9QYW0	0.000581	0.09375	0	0	0	0.75	0.25	0.5	0.5	1	1	0.75		
<i>Rab proteins geranylgeranyltransferase component A 1</i>	P37727	0.0006956	0.234375	0	0.5	0	1.25	0.25	0	0	0.75	0.25	0.5		

Table 5e: List of FPI-III 72 Hour proteins (p≤ 10-3)

Protein	Accession Number	p value	Controls	FPI						PBI					
				15m	1 h	6 h	24h	72 h	15m	1 h	6 h	24h	72 h		
				0.25	0	0	0	0.75	0	0	0.25	0	0		
Syntaxin-1A (Neuron-specific antigen HPC-1)	P32851	2.331E-09	0.03125	0.25	0	0	0	0.75	0	0	0.25 <td>0</td> <td>0</td> <td>0</td>	0	0	0	
Nucleic acid binding protein	Q4JFV5	9.039E-07	0.015625	0	0	0	0	0.5	0	0	0	0	0	0	
Muellerian-inhibiting factor precursor (MIS)	P49000	5.432E-06	0.140625	0	0.5	0.25	0	1.25	1	0	0.5	0.25	0	0	
Butyrophilin-like protein 2 precursor	Q6MG97	2.158E-05	0.078125	0	0.25	0	0.75	0.75	0	0.25	0	0	0	0.25	
Vomerolasal VI r-type receptor Virc39	Q5J3I1	5.538E-05	0.03125	0	0	0	0	0.5	0	0	0	0	0	0	
Hypothetical protein (Frgment)	Q6GX83	5.538E-05	0.03125	0	0.25	0	0	0.5	0.25	0	0.25	0	0.25	0	
Bms11 protein [Frgment]	Q561Z9	5.538E-05	0.03125	0	0	0	0.25	0.5	0.25	0	0.25	0	0.25	0	
Receptor-type tyrosine-protein phosphatase alpha	Q03348	5.538E-05	0.03125	0	0.25	0	0	0.5	0	0	0	0	0	0.25	
Similar to cDNA sequence BC021608	Q66H45	6.85E-05	0.0625	0	0	0	0.25	0.75	0.25	0	0.25	0	0	0	
Withns' tumor protein homolog	P49952	0.0003496	0.15625	0.25	0.5	0	0	1	0.25	0	0.5	0	0	0.25	
Junction adhesion molecule 2	Q3MHC0	0.0005437	0.046875	0	0.5	0	0	0.5	0	0	0.5	0	0	0	
Ac2-086	Q7TPK4	0.0005437	0.046875	0	0	0	0	0.5	0.25	0	0.25	0	0.25	0	
Cysteine-rich secretory protein 1 precursor	P12020	0.0005437	0.046875	0	0	0	0	0.5	0	0	0.25	0.5	0.25	0	

Table 6a: List of PBI-III 15 Min proteins (p_≤ 10⁻⁴) Part 1 of 3

Protein	Accession Number	p value	Controls	FPI							PBI				
				15m	1 h	6 h	24h	72 h	15m	1 h	6 h	24h	72 h		
Trace amine-associated receptor 8a	Q923X9	4.315E-11	0	0	0	0	0	0	0	0.5	0	0	0	0.25	
PDZ domain-containing protein 1	Q9JH40	1.265E-09	0.21875	0	0.25	0	0.25	0	0.25	1.75	1	1	0.5	0.5	
Testicular cell adhesion molecule 1	Q9ZL33	2.331E-09	0.03125	0	0	0	0.5	0	0.75	0	0.25	0.75	0	0	
Serine hydroxymethyl transferase 2 (Mitochondrial)	Q5U3Z7	2.49E-09	0.15625	0.25	0	0	0.25	0.5	1.5	0	0.5	0.5	0	0	
Thyrotropin-releasing hormone receptor	Q01717	3.444E-09	0.046875	0.25	0	0	0	0.25	1	0	0.25	0	0	0	
ATP-sensitive inward rectifier potassium channel 1	P35560	4.172E-09	0.078125	0	0	0	0	0.25	1	0	0.25	0	0.25	0	
2'-5' oligoadenylate synthetase 2-like protein	Q5MYT9	2.101E-08	0.125	0.25	0.5	0.25	0	0	1.25	0	0.5	0	0	0	
Cathepsin L precursor	P07154	1.511E-07	0.328125	0	0.25	0.25	0	0	2	0.25	1	0.5	0	1	
Low mol. weight phosphotyrosine protein phosphatase	P41498	1.791E-07	0.046875	0	0.25	0	0	0	0.75	0.5	0	0	0	0	
Branched-chain-amino-acid aminotransferase, mitochondrial	Q35854	2.63E-07	0.046875	0	0	0	0	0	1	0	0.25	0	0.25	0.5	
Tubulin tyrosine ligase-like 1 (Predicted)	Q5PP19	3.294E-07	0.125	0	0.25	0	0	0	1.25	0.25	0.25	0.25	0.25	0.5	
Carnitine deficiency-associated protein expressed in ventricle 1	P83829	3.595E-07	0.28125	0.5	0.25	0	0.25	0.25	2	0.5	1.25	0.25	0.25	0.5	
Ribonuclease 12	Q5GAL8	3.659E-07	0.078125	0	0	0	0	0.25	1	0	0.25	0	0	0	
Caspase recruitment domain-containing protein 9 (rCARD9)	Q9EPY0	4.56E-07	0.484375	0.25	0.5	0	0	0.5	2.25	0	1.25	0.5	0.75	0.75	
Hypothetical protein LOC361016	Q4V7B1	4.584E-07	0.234375	0.25	0.25	0	0.25	0.25	1.75	0	0	0.5	0.75	0.75	
PH domain leucine-rich repeat protein phosphatase	Q9WTR8	5.646E-07	0.65625	0.5	0.75	0.75	0.25	0.25	3.5	0.75	1.75	0.75	1.25	1.25	
Cd5 protein	Q4KLZ9	9.039E-07	0.015625	0	0	0	0	0	0.5	0	0	0	0	0	
Similar to RIKEN cDNA 2810037C03	Q499W2	9.039E-07	0.015625	0	0	0	0	0	0.5	0	0	0	0	0	
Axl receptor tyrosine kinase	O55059	9.039E-07	0.015625	0	0	0	0.25	0	0.5	0	0	0	0	0	
Mysin light polypeptide 6	Q64119	9.039E-07	0.015625	0	0.25	0	0	0	0.5	0.25	0	0	0	0	
Olfactory receptor 1	P70526	9.039E-07	0.015625	0	0	0	0	0	0.5	0	0	0	0	0	
Src family associated phosphoprotein 1	Q4V7G1	9.039E-07	0.015625	0	0	0	0	0	0.5	0	0.25	0	0	0	
Putative pheromone receptor V2R2a (Fragment)	Q99PC1	9.039E-07	0.015625	0	0	0	0	0	0.5	0	0	0	0	0.25	
BRCA1-associated RING domain protein 1 beta isoform	Q61724	9.039E-07	0.015625	0.25	0	0	0	0	0.5	0	0	0	0	0	
Placental prolactin-like protein K precursor	Q91KL9	9.039E-07	0.015625	0.25	0.25	0.25	0.25	0.25	0.5	0	0	0	0	0.25	
Drebrin-like protein	Q9JHL4	9.746E-07	0.140625	0	0	0.25	0.25	0.25	1.25	0.75	0.5	0.25	0.25	0.25	
Hypothetical protein RGD1305287	Q510F1	1.391E-06	0.0625	0	0	0	0	0.25	1	0	0.25	0.25	0.25	0	
Beta-1,3-N-acetylglucosaminyltransferase lunatic fringe	Q924T4	1.737E-06	0.53125	0.25	0.25	0.25	0	0.5	2.5	0.75	2.5	0.75	0.25	0.25	
Hypothetical protein RGD1307084	Q510J4	1.967E-06	0.28125	0.25	0	0	0.5	0	1.75	0	0.25	0	0	0	
Neurexophilin-4 precursor	Q9Z2N4	2.33E-06	0.125	0	0	0	0.25	0	1.25	0.5	0.75	0	0.25	0.25	

Table 6a: List of PBI-III 15 Min proteins (p_≤ 10⁻⁴) Part 2 of 3

Protein	Accession Number	p value	Controls	FPI						PBI					
				15m	1 h	6 h	24h	72 h	15m	1 h	6 h	24h	72 h		
Killer cell lectin-like receptor subfamily H, member 1	Q8K4F1	2.503E-06	0.15625	0	0	0	0	0	1.25	0	0.25	0.5	0.25		
Charged multivesicular body protein 5	Q4QQV8	2.979E-06	0.0625	0	0	0	0	0	0.75	0	0.25	0	0		
Fln29 protein	Q5M806	6.35E-06	0.328125	0	0	0.5	0.25	0	1.75	0.75	1	0.25	0		
Gonadotropin-regulated long chain acyl-CoA synthetase	Q924N5	7.544E-06	0.390625	0.25	0.25	0	0.5	0	2	0.25	0.25	0.25	0		
Calcium/calmodulin-dependent protein kinase type II alpha chain	P11275	1.193E-05	0.1875	0	0	0	0	0	1.25	0.25	0.5	0	0.25		
Gut-enriched kruppel-like factor (Kruppel-like factor 4) (Gut)	Q923V7	1.446E-05	0.8125	0.25	0.75	1.5	0.25	1.25	2.75	1	1.5	0.5	1		
Ab2-060	Q7TP64	1.473E-05	0.046875	0	0	0	0	0.25	0.75	0.25	0.25	0.25	0		
Follistatin-related protein 3	Q99PW7	1.473E-05	0.046875	0	0.5	0.25	0	0	0.75	0.25	0	0	0.25		
GA repeat binding protein, beta 1	Q4KM28	1.473E-05	0.046875	0	0	0	0	0	0.75	0	0	0	0		
Epithelium-specific RNase-like (Ribonuclease 9)	Q5QJV4	1.473E-05	0.046875	0.5	0	0	0	0	0.75	0.25	0.25	0	0		
Hypothetical protein RGD1307598	Q32PZ9	1.946E-05	0.84375	1.25	1	0.75	1	0.5	2.25	0.75	1	0.5	1		
TNFAIP3 interacting protein 2	Q4V7E7	1.987E-05	0.125	0	0.25	0	0	0.25	1	0	0.5	0.25	0.25		
Aa114	Q7TQ12	2.007E-05	0.140625	0	0	0.25	0.25	0.25	1.25	0	0.75	0.75	0		
Thyrotberin	P01150	2.158E-05	0.078125	0.5	0.25	0	0	0.25	0.75	0	0.5	0.25	0		
Suppressor of cytokine signaling 2 (SOCS-2)	Q88582	2.601E-05	0.21875	0.25	0	0.25	0	0.25	1.5	0.5	0.5	0.25	0.5		
Inhibin beta C chain precursor (Activin beta-C chain)	Q9WUK5	2.627E-05	0.25	0.5	0	1	0.25	0.25	1.5	0	0.5	0.25	0.25		
Hypothetical protein LOC300963	Q4KLY0	3.237E-05	0.453125	0.75	0.5	0.25	0.5	0.75	2	0.75	0.75	0.25	0.75		
Nuclear receptor coactivator p120	Q5TLG7	4.133E-05	0.21875	0.25	0.75	0.25	0	0	1.25	0.25	1.25	0.25	0.5		
Estradiol 17-beta-dehydrogenase 1	P51657	4.133E-05	0.21875	0	0.25	0	0.25	0	1.25	0	0.25	0	0		
Hypothetical protein LOC361399	Q3ZAV8	4.481E-05	0.4375	0.25	0.25	0.75	0.25	0.5	2	0.75	1.25	0.75	0.25		
Beta-2-glycoprotein 1 (Apolipoprotein H)	P26644	4.512E-05	7.53125	9.5	6.5	8.75	7.5	6.5	12	8.25	8.75	7.5	9		
Zinc finger CCH type domain containing 8	Q6AYB0	4.73E-05	0.53125	0.25	0.25	0.25	0.75	0.5	2.25	0.5	1.25	0.5	1.5		
Hypothetical protein RGD1307787	Q5BK61	4.922E-05	0.40625	0.5	1	0.5	0.5	1.25	2	0	0.75	0.25	0.25		
Muellerian-inhibiting factor	P49000	4.97E-05	0.140625	0	0.5	0.25	0	1.25	1	0	0.5	0.25	0		
Fucosyltransferase 11	Q68FV3	4.97E-05	0.140625	1	0	0	0	0	1	0.5	0.25	0	0		
Hypothetical protein RGD1309552	Q566C8	4.97E-05	0.140625	0.25	0.25	0	0	0.25	1	0.25	0.5	0	0.25		
Synaptonemal complex protein 3 (SCP-3)	Q63520	5.538E-05	0.03125	0.25	0.25	0.25	0.5	0.25	0.5	0	0	0.25	0		
Leucine-rich repeat-containing protein 59 (Protein p34)	Q5RJR8	5.538E-05	0.03125	0	0.25	0	0	0	0.5	0	0	0	0		
Hypothetical protein	Q498D3	5.538E-05	0.03125	0	0	0	0	0	0.5	0	0	0	0		

Table 6a: List of PBI-III 15 Min proteins ($p \leq 10^{-4}$) Part 3 of 3

Protein	Accession Number	P value	Controls	FPI							PBI				
				15m	1 h	6 h	24h	72 h	15m	1 h	6 h	24h	72 h		
<i>Tdrd7</i> protein	Q5FVVD0	5.538E-05	0.03125	0	0	0	0	0.25	0.5	0	0	0	0		
<i>RAB30</i> , member RAS oncogene family (Predicted)	Q5BK72	5.538E-05	0.03125	0	0.5	0.25	0.25	0.25	0.5	0	0.25	0	0		
Ab2-379	Q7TP46	5.538E-05	0.03125	0	0	0	0	0	0.5	0.5	0	0.25	0		
HES-related repressor protein 2 HERP2 (Fragment)	Q8VIH4	5.538E-05	0.03125	0	0	0.25	0	0	0.5	0.25	0	0.5	0		
Hypothetical protein LOC498176	Q5U3Z5	5.538E-05	0.03125	0.25	0	0	0	0	0.5	0	0.25	0.25	0		
Ectodermal-neurid cortex 1	Q2V9T0	5.622E-05	0.40625	0.25	0.75	0	0.5	0.25	1.75	0.75	1.5	0.25	0.5		
<i>Neurexin-1-beta</i> precursor (<i>Neurexin 1-beta</i>)	Q63373	6.85E-05	0.0625	0	0.25	0.75	0	0.25	0.75	0.25	0.5	0.25	0		
<i>Disabled-1</i>	Q8CJH2	6.85E-05	0.0625	0	0.25	0.25	0	0	0.75	0	0.5	0	0		
Four and a half LIM domains protein 2 (FHL-2)	Q35115	7.055E-05	1.140625	1.5	0.75	1	1	0.5	3	1.25	2.75	1.5	0.5		
<i>Spnb4</i> protein (Fragment)	Q5BJU8	8.087E-05	0.296875	0.5	0.5	0.5	0.25	0.75	1.5	0.25	1.5	0.25	0.25		
78 kDa glucose-regulated protein precursor (GRP 78)	P06761	8.285E-05	2.0625	1.75	1	0.5	2.5	0.75	5.5	1.75	4.25	2.75	3		
<i>Glutathione s-transferase M5</i> (EC 2.5.1.18)	Q9Z1B2	9.437E-05	0.09375	0	0	0	0.5	0	0.75	0.25	0.25	0	0.5		
LRRGT00162	Q6Q146	9.437E-05	0.125	0.25	0	0.25	0	0.25	1	0.25	0	0.25	0		
<i>Plectin 1</i>	Q6S3A5	9.437E-05	0.09375	0	0	0.5	0.25	0	0.75	0	0.5	0.25	0		
Hypothetical protein	Q2MHH0	9.437E-05	0.125	0.75	0.5	0	0	0	1	0	0	0	0.5		
Mitochondrial 2-oxodicarboxylate carrier	Q99JID3	9.437E-05	0.09375	0	0	0	0	0	0.75	0.5	0.5	0	0		
Tissue factor pathway inhibitor-2 precursor	Q8CF99	9.437E-05	0.09375	0	0	0	0.5	0	0.75	0.25	0.5	0	0.25		
Similar to Dner protein	Q5U215	9.437E-05	0.125	0	0	0	0	0	1	0	0	0	0.5		
Serine/threonine/prosine interacting-like 1	Q4G076	9.437E-05	0.09375	0.25	0	0	0	0	0.75	0	0	0	0		
Aminopeptidase O (AP-O)	P69527	9.548E-05	0.359375	1	0.25	0.25	0	0	2	0.25	1	0.25	0.75		

Table 6b: List of PBI-III 1 Hour proteins (p≤ 10-4)

Protein	Accession Number	p value	Controls	FPI						PBI					
				15m	1 h	6 h	24h	72 h	15m	1 h	6 h	24h	72 h		
Tazarotene-induced protein 1	Q58NB7	4.315E-11	0	0	0.25	0	0	0	0	0	0	0.5	0	0	0
Pulmonary surfactant-associated protein C	P11685	7.961E-08	0.078125	0.25	0.5	0	0	0	0	0	0	1.25	0	0.25	0
Olfactory receptor (Fragment)	Q9QWX1	1.791E-07	0.046875	0	0	0	0.25	0	0	0	0	0.75	0	0	0
Sodium/calcium exchanger 1 splice variant NaCa10	Q9Z0T8	9.039E-07	0.015625	0	0	0	0	0	0	0	0	0.5	0	0	0
40S ribosomal protein S9	P29314	9.039E-07	0.015625	0	0	0	0	0	0	0	0	0.5	0.25	0	0
50 kD glycoprotein (Rh50)	O88300	9.039E-07	0.015625	0	0	0	0	0	0	0	0	0.5	0.25	0	0
Hypothetical protein LOC500909	Q5M862	9.039E-07	0.015625	0	0	0	0	0	0	0	0	0.5	0	0.25	0
Phosphoglycerate dehydrogenase like 1	Q3KR85	9.039E-07	0.015625	0	0	0	0	0.25	0	0	0	0.5	0	0	0
Nerve growth factor-regulated G-protein-coupled receptor 1	Q9JKM5	9.746E-07	0.140625	0	0	0.25	0	0	0	0	0	1.25	0.5	0	0
Apoptosis-antagonizing transcription factor	Q9QYW0	1.820E-06	0.09375	0	0	0	0.75	0.25	0.5	0.5	1	1	1	0.75	0
Aa2-296	Q7TP76	2.979E-06	0.0625	0	0	0	0	0	0	0	0	0.75	0.5	0	0
Putative taste receptor T2R33	Q67ER8	2.979E-06	0.0625	0.5	0	0	0	0	0.25	0.75	0.25	0.25	0	0	0
Dnaj-like protein	Q9QZW8	6.716E-06	0.109375	0	0.25	0	0	0	0	0	0	1	0.25	0.25	0.75
Proteasome subunit beta type 9	P28077	1.010E-05	0.125	0.5	0	0	0	0.25	0.5	1.25	0.25	0.75	0.75	0.25	0.25
Glutamate--cysteine ligase regulatory subunit	P48508	1.473E-05	0.046875	0.5	0	0	0	0	0	0.75	0.75	0.75	0.5	0.25	0.25
Mago-nashi-like proliferation-associated protein	Q27W02	1.473E-05	0.046875	0	0	0	0	0	0	0.25	0.75	0	0	0.25	0.25
Mas-related G-protein coupled receptor member D	Q7TN41	2.158E-05	0.078125	0	0	0	0	0.5	0	0.75	0.75	0.25	0	0	0
Ankyrin repeat domain 13 (Predicted)	Q5U313	2.158E-05	0.078125	0	0.5	0	0.5	0	0.5	0.75	0.75	0.75	0.25	0.25	0.5
Serine protease inhibitor Kazal-type 6 precursor	Q6IE47	2.158E-05	0.078125	0	0	0	0	0	0	0.75	0.75	0.25	0	0	0
Hypothetical protein RGD1311362	Q5B1V9	2.158E-05	0.078125	0.25	0	0	0	0	0	0.25	0.75	0.25	0	0.25	0.25
Ab2-379	Q7TP46	5.538E-05	0.03125	0	0	0	0	0	0	0.5	0.5	0	0.25	0	0
Ras-related protein Rab-3C	P62824	5.538E-05	0.03125	0	0	0	0	0	0.25	0	0.25	0.5	0	0	0
Annexin A6 (Annexin VI)	P48037	5.538E-05	0.03125	0	0	0	0	0	0	0	0	0.5	0	0	0
Hypothetical protein	Q32PX1	5.538E-05	0.03125	0.25	0	0.25	0	0	0	0	0	0.5	0.25	0	0
Protein kinase C delta-binding protein	Q9Z1H9	6.850E-05	0.0625	0	0	0	0	0	0	0.5	0.75	0.5	0	0.25	0.25
Achaete-scute homolog 2	P19360	8.725E-05	0.359375	0.5	0.25	0	0.75	0	0.5	1.75	0	0	0	0.75	0.75
Hypothetical protein RGD1307700	Q510I5	9.437E-05	0.09375	0	0.5	0	0	0	0	0.5	0.75	0.25	0	0.25	0.25

Table 6c: List of PBI-III 6 Hour proteins (p \leq 10-4) Part 1 of 6

Protein	Accession Number	p value	Controls	FPI						PBI					
				15m	1 h	6 h	24h	72 h	15m	1 h	6 h	24h	72 h		
<i>Prostaglandin G/H synthase 1</i>	PGHI_RAT	1.369E-14	0.125	0	0.25	0	0.25	0.5	0	0.25	0	0.25	2	0	0.5
<i>WD repeat domain 78</i>	Q4V8G4	4.681E-13	0.015625	0	0	0	0	0	0.25	0	0	0.25	1	0	0
<i>Proteasome subunit alpha type 4</i>	PSA4_RAT	1.279E-12	0.09375	0	0	0.25	0	0	0.25	0	0.25	0	1.5	0	0.5
<i>Beta-neoendorphin-dynorphin</i>	PDDYN_RAT	4.315E-11	0	0	0	0	0	0	0	0	0	0	0.5	0	0
<i>Olfactory receptor</i>	O35184	4.315E-11	0	0	0	0	0	0	0	0	0	0	0.5	0	0
<i>Rat Ly6-C antigen</i>	Q63318	4.315E-11	0	0	0	0	0	0	0	0	0	0.25	0.5	0	0
<i>LOC502872 protein</i>	Q4KM64	4.315E-11	0	0.25	0	0	0	0	0	0	0	0	0.5	0	0
<i>Protein FAM26B</i>	FA26B_RAT	9.286E-11	0.03125	0	0.25	0	0	0	0.25	0	0.25	0	1	0.25	0
<i>Neuronal pentraxin-1</i>	NPTX1_RAT	1.372E-10	0.1875	0.25	0.5	0	0.25	0.5	1	0.25	0.25	0.75	1.75	0.5	0.75
<i>Glucocorticoid receptor (GR)</i>	GCR_RAT	1.663E-09	0.828125	0.75	0.5	0	0.75	0.5	1.5	0.75	4	1.75	0.25	0.25	0.25
<i>CRAMP (Fragment)</i>	Q71KM5	2.331E-09	0.03125	0	0	0	0	0	0.25	0.25	0.75	0	0.75	0	0.25
<i>Sdceag1 protein (Fragment)</i>	Q5D022	2.943E-09	0.125	0.25	0.25	0.25	0.25	0	0.25	0.25	1.5	0.25	1.5	0.25	0
<i>Skeletal muscle and kidney enriched inositol phosphatase</i>	Q5XIU8	3.444E-09	0.046875	0	0	0.25	0	0	0	0	0	0	1	0	0.25
<i>RGD1306356 protein</i>	Q4G035	4.172E-09	0.078125	0	0	0	0	0	0	0.25	0	0.25	1	0.5	0
<i>Neurexin-3 receptor (NMB-R)</i>	NMBR_RAT	8.823E-09	0.171875	0	0.25	0	0	0	0	0	0	0	1.5	0	0.5
<i>NUAK family, SNF1-like kinase, 2</i>	Q66HE5	1.267E-08	0.671875	0	0.25	0	0.25	0.25	0.75	1	3.5	0.25	0.5	0.25	0.5
<i>Insulin responsive sequence DNA binding protein-1</i>	Q5ZOU0	1.3E-08	0.34375	0.5	0	0.25	0.25	0.75	1	0.5	2.5	0	0	0	0
<i>Myosin-9 (Myosin heavy chain, nonmuscle IIa)</i>	MYH9_RAT	1.412E-08	0.40625	0	0.25	0	0	0.25	0.5	0.5	2.25	0.25	0.25	0.75	0.75
<i>LOC290150 protein</i>	Q562C1	2.104E-08	0.09375	0	0	0	0	0.25	0.5	0	1.25	0	0	0	0
<i>Kinesin heavy chain (Ubiquitous kinesin heavy chain) (UKHC)</i>	KINH_RAT	4.34E-08	0.78125	0.25	0	0.5	0.25	1	1	0.75	3.5	1.75	1.75	0.5	0.5
<i>LOC500180 protein</i>	Q5M838	4.694E-08	0.3125	0.5	0	0	0.25	0.25	0.5	0.75	2.25	0.25	0.25	0.25	0.25
<i>Aldose reductase-like protein</i>	Q91W30	4.835E-08	0.0625	0	0	0	0	0.25	0	0	1	0.25	0	0.25	0
<i>Fatty acid-binding protein, epidermal (E-FABP)</i>	FABPE_RAT	4.835E-08	0.0625	0	0	0	0	0	0.25	0.25	1	0.5	0.25	0.25	0
<i>Type-II keratin Kb20 (Type-II keratin Kb2)</i>	Q6IMF1	5.115E-08	0.640625	0.75	0.25	0.25	0.5	0	0.25	0.75	3.5	1.75	1.75	1	1
<i>Heterogeneous nuclear ribonucleoprotein A1</i>	ROA1_RAT	9.135E-08	0.140625	0.25	0	0	0.5	0	0.25	0.25	1.25	0.5	0.5	0.25	0.25
<i>Hypothetical protein LOC502479</i>	Q32PX9	9.495E-08	0.265625	0.25	0.5	0.5	0.5	0	1	0.25	2.25	0	0	0	0
<i>Similar to enthoprofin; epsin 4</i>	Q6DGF2	1.407E-07	0.203125	0.25	0	0	0	0.25	0.5	0	1.75	0.25	0.25	0.25	0.25
<i>Exocyst complex component 3</i>	EXOC3_RAT	1.511E-07	0.328125	0.25	0.25	0	0	0.25	0.25	0.25	2	0.25	0.25	0.25	0.25
<i>Cell surface glycoprotein gp42 precursor</i>	GP42_RAT	1.791E-07	0.046875	0	0.5	0	0.25	0	0.25	0	0.75	0	0.75	0	0.25
<i>Mitochondrial import inner membrane translocase subunitTIM16</i>	TIM16_RAT	1.791E-07	0.046875	0	0	0	0	0	0	0.25	0.75	0	0.75	0	0.25

Table 6c: List of PBI-III 6 Hour proteins (p \leq 10-4) Part 2 of 6

Protein	Accession Number	p value	Controls	FPI						PBI					
				15m	1 h	6 h	24h	72 h	15m	1 h	6 h	24h	72 h		
Hypothetical protein LOC500705	Q6AXQ2	1.791E-07	0.046875	0	0	0	0.5	0	0.5	0	0.5	0	0.75	0	0.25
BING4 protein	Q6MGC3	2.24E-07	0.25	0	0	0.5	0	0.75	0.5	0.25	0.5	0	1.75	0.25	0
Hypothetical protein	Q4V7C3	2.475E-07	0.625	1	0.75	0.5	0.5	0	2.25	0.25	0.25	0.25	3.25	0.25	1.25
Hypothetical protein LOC361571	Q3TIL0	2.631E-07	0.421875	0.5	0.5	0	0.25	0.25	0.5	0.5	0	0	2.5	0.75	0.5
Pannexin-2	PANX2_RAT	2.923E-07	0.453125	0	0.5	0	1	0.75	0.75	0.75	0.5	1	2.25	0.75	0.5
Gene corresponding to Riken cDNA clone 3930402F13	Q6MGD2	2.992E-07	0.3125	0.25	0	1	0.25	0.5	0.5	0.5	1	1	2	0.5	0.75
Antisense RNA overlapping MCH protein	Q9EQI0	3.225E-07	0.359375	0.25	0.25	0	0.25	0	0.25	0	0.5	0.25	2.25	0.25	0
Amyloid beta A4 precursor protein-binding family A member 3 (Neuron-specific XIII2 protein)	APBA3_RAT	3.659E-07	0.078125	0.25	0.5	0	0	0.25	0.25	0.5	0	0	1	0.25	0
Alpha-2,6-sialyltransferase ST6GalNAc V (Fragment)	Q6ZXY9	3.659E-07	0.078125	0	0	0.25	0	0.25	0	0.25	0	0	1	0.25	0
Neurokinin-B precursor (NKB) (Neuromedin-K)	TKNK_RAT	3.899E-07	0.265625	0.25	0.25	0	0	0.25	0.25	0.75	0.5	0.5	1.75	0	0.25
Armadillo repeat-containing X-linked protein 1	ARMX1_RAT	3.982E-07	0.25	0.5	0	0	0.25	0.25	1	0.25	1	0.25	2	0.75	0.25
Bone specific CMF608	Q6WRH9	4.004E-07	2.90625	2.5	1.25	1.25	2.25	3	5.75	4.75	8	3.25	3.75	0.25	0.25
Alcohol dehydrogenase [NADP+]	AKIA1_RAT	4.112E-07	0.109375	0.25	0.25	0	0	0	0	0	0	0	1	0	0.5
Immunoreceptor Ly49i5 (Ly49 inhibitory receptor 5)	Q5MPV0	4.112E-07	0.109375	0.25	0.25	0.25	0	0.25	0.25	0.25	0	0	1	0.5	0
Tudor domain containing 3 (Predicted)	Q66HC1	4.689E-07	0.546875	0	0.5	0.5	1.25	0.5	1	1.5	2.75	0.25	1.25	0.25	1.25
Phosphatidylinositol 4,5-bisphosphate 5-phosphatase A	PI5PA_RAT	5.042E-07	1.109375	1	0.25	0.5	0.75	0.25	2.25	2	4.25	1	2	1	2
Ankyrin repeat and SAM domain-containing protein 3	ANKS3_RAT	5.362E-07	0.296875	0.25	0	0	0.25	0.5	0.5	0.25	0.25	0.75	2	0.75	0
PIPAS	Q80WX6	7.732E-07	0.25	0.5	0.25	0.5	0.25	0.25	0.25	0.25	1	1.75	0	0.25	0.25
ATP-binding cassette transporter sub-family A member 7	Q7TNJ2	8.277E-07	0.953125	0.25	1	0.25	0.75	0.5	1	1.5	3.75	1	2.25	1	2.25
RT1 class II, DM beta (Hla-dmb protein)	Q6MGA7	8.872E-07	0.109375	0	0	0	0	0	0.5	0	0	0	1.25	0	0.25
Zeta-chain (TCR) associated protein kinase 70kDa	Q5FVN9	8.872E-07	0.109375	0.25	0.25	0.75	0	0.25	0.25	0.25	0.5	0.5	1.25	0.25	0.25
Heat sensitive channel TRPV3	Q4QYD9	8.91E-07	0.359375	0	0	0.5	0	0.25	0	0	0	0	2	0	0.75
Tropomyosin T, slow skeletal muscle (TnTs)	TNNT1_RAT	9.039E-07	0.015625	0	0	0	0	0	0	0.25	0	0.25	0.5	0	0.5
LOC367586 protein	Q5M7V3	9.039E-07	0.015625	0	0	0	0	0	0	0	0	0	0.5	0	0
Cytochrome c oxidase subunit Vlb isoform 2	CX6B2_RAT	9.039E-07	0.015625	0	0	0	0	0	0.25	0	0.25	0	0.5	0	0
Tube33 protein (Fragment)	Q5U3X6	9.039E-07	0.015625	0	0	0	0	0	0.25	0	0.25	0	0.5	0	0
Hypothetical protein (Fragment)	Q9QXL5	9.039E-07	0.015625	0	0	0.25	0	0	0	0	0	0	0.5	0	0
Bcl-2-binding component 3	BBC3_RAT	9.039E-07	0.015625	0	0	0	0.25	0	0	0	0	0	0.5	0	0
Insulin-induced gene 1 protein (INSG-1)	INSI1_RAT	9.039E-07	0.015625	0	0	0	0	0	0.25	0	0.25	0	0.5	0	0

Table 6c: List of PBI-III 6 Hour proteins (p \leq 10⁻⁴) Part 3 of 6

Protein	Accession Number	P value	Controls	FPI					PBI						
				15m	1h	6h	24h	72h	15m	1h	6h	24h	72h		
Hypothetical protein MGCI12688	Q501W2	9.039E-07	0.015625	0	0	0	0	0	0	0	0.25	0.25	0.5	0	0
Prostacyclin receptor (Prostanoid IP receptor) (PGI receptor) (Prostaglandin I2 receptor)	PI2R_RAT	9.039E-07	0.015625	0	0	0	0	0	0	0	0.25	0.25	0.5	0	0
Reproductive homeobox on X chromosome 4	Q4TU78	9.039E-07	0.015625	0	0	0	0.25	0	0	0	0	0	0.5	0	0
Hypothetical LOC364620	Q6AXY1	9.039E-07	0.015625	0	0	0	0	0	0	0	0	0	0.5	0	0.25
Cysteine and glycine-rich protein 3	CSRP3_RAT	9.316E-07	0.171875	0.25	0	0	0.25	0.25	0.25	0.25	0.25	0.25	1.25	0	0.25
LOC499781 protein (Fragment)	Q4V7B0	1.076E-06	0.640625	1	0.25	0	0	0.75	0	0.75	0	0.5	2.5	0.5	0.5
Kelch-like protein 17 (Actinfilin)	KLH17_RAT	1.199E-06	0.34375	0.25	0.25	0.25	0.25	0.75	0.75	1.25	0.25	0.25	1.75	1	0.25
F-box only protein 17	FBX17_RAT	1.82E-06	0.09375	0	0	0.25	0.25	0.25	0.25	0.25	0.25	0.5	1	0.25	0
Prostate overexpressed G protein coupled receptor	Q5MD65	1.856E-06	0.03125	0	0.25	0	0	0	0	0	0	0	0.75	0	0
LRRT100008	Q6TXJ1	1.967E-06	0.28125	0.5	0.25	0	0.5	0.25	0.25	0.25	0.5	0.5	1.75	0.5	1
Vigilin (High density lipoprotein-binding protein)	VIGLN_RAT	2.11E-06	0.78125	0.75	0.75	0.25	0.25	0.25	0.25	1.5	0.25	0.25	3.5	1	0.75
Golph4 protein (Fragment)	Q5BJK8	2.979E-06	0.0625	0	0	0	0	0	0	0	0	0	0.75	0.5	0.75
60S ribosomal protein L27a	RL27A_RAT	2.979E-06	0.0625	0	0	0	0	0	0	0.25	0.25	0.25	0.75	0.25	0.5
Torsin-2A precursor (Torsin family 2 member A)	TOR2A_RAT	2.979E-06	0.0625	0	0	0	0.25	0	0	0.5	0	0	0.75	0.25	0.5
Abhydrolase domain-containing protein 5	ABHD5_RAT	2.979E-06	0.0625	0	0	0	0	0.25	0	0	0.25	0	0.75	0	0
LRRT100003	Q6TXJ6	2.979E-06	0.0625	0	0	0	0	0	0	0	0	0	0.75	0	0
Transmembrane 9 superfamily protein member 4	Q4KLL4	2.979E-06	0.0625	0	0	0	0	0	0	0.25	0	0	0.75	0.25	0
Thymidine kinase, cytosolic (EC 2.7.1.21) (Fragment)	KITH_RAT	2.979E-06	0.0625	0	0.25	0	0	0	0	0.25	0.25	0.25	0.75	0.5	0
Wt47 protein (Fragment)	Q5BJR0	2.988E-06	0.296875	0.5	0.25	0	0.25	0.5	0.5	0.75	0	0.75	1.75	0.75	0.75
Microtubule-associated protein 1B (MAP 1B) (Neuraxin)	MAP1B_RAT	3.265E-06	2.484375	1.25	1.5	1	2.25	0.75	0.75	4.75	2.75	2.75	7	3.25	2.25
Galainin receptor type 3 (GAL3-R) (GALR3)	GALR3_RAT	4.667E-06	0.109375	0	0	0.25	0.25	0	0	0.5	0.75	1.25	0	0	0
2D6 glycoprotein (Beta-defensin 22)	Q99JD1	6.716E-06	0.109375	0	0	0	0	0	0	0.25	0.5	1	1	0	0
LRRT100169	Q6Q139	6.716E-06	0.109375	0	0	0	0	0	0	0	0.25	0.25	1	0	0
3-2-trans-enoyl-CoA isomerase, mitochondrial precursor	D3D2_RAT	6.716E-06	0.109375	0	0	0	0	0	0	0.25	0.25	0	1	0	0
Regenerating islet-derived protein 3 gamma precursor	REG3G_RAT	6.716E-06	0.109375	0.25	0.25	0.25	0	0	0	0	0	0	1	0	0
Joubertin (Abelson helper integration site 1 protein homolog)	AHI1_RAT	6.963E-06	0.9375	1.25	0.75	1.5	0.5	0.25	0.25	1.75	1	4	0.5	0.5	0.5
Syntaxin binding protein Munc18-3	Q99PV2	7.46E-06	0.4375	0.75	0.25	0.5	0.5	0	0	0.25	0	2.25	0.25	0.25	0.25
Synaptopodin	SYNPO_RAT	1.075E-05	0.71875	0.5	0.75	0.25	0.75	1.25	1	1	1	3	0	0	0
FYVE, RhoGEF and PH domain containing 1	Q2YDU5	1.109E-05	0.28125	0	0	0	0	0	0	0.25	0.75	0.75	1.75	0.75	1

Table 6c: List of PBI-III 6 Hour proteins (p \leq 10⁻⁴) Part 4 of 6

Protein	Accession Number	p value	Controls	FPI						PBI					
				15m	1 h	6 h	24h	72 h	15m	1 h	6 h	24h	72 h		
<i>Catenin (Cadherin-associated protein), alpha 1, 102kDa</i>	Q5U302	1.131E-05	0.703125	0.25	0.5	0.25	0.25	0.5	0.75	0.75	2.75	0.75	0.75		
<i>Basic transcription factor 3</i>	Q5U3Y8	1.148E-05	0.15625	0.25	0	0	0.25	0	0	0	1.25	0.25	0		
<i>Autogenous vein graft remodelling associated protein 1, AVGRI</i>	Q2MCS9	1.343E-05	0.59375	0.75	0.25	0.75	0.75	0.75	0.5	0.25	2.75	0.75	0.5		
<i>Nucleolar protein 5A</i>	Q4KIK7	1.465E-05	0.453125	0.25	0.25	0.25	0.5	0.75	0.75	0.75	2.25	1	1		
<i>Glutamate--cysteine ligase regulatory subunit</i>	GSH0_RAT	1.473E-05	0.046875	0.5	0	0	0	0	0	0.75	0.75	0.5	0.25		
<i>Hypothetical protein</i>	Q3B8N7	1.6E-05	0.75	1.25	0.75	0.25	1.5	0.5	1.25	0.5	3	1	0.5		
<i>Ribonucleoside-diphosphate reductase M2 subunit</i>	RIR2_RAT	1.622E-05	0.265625	0	0	0.25	0	0.25	0.5	1	1.5	0	0.5		
<i>Breast cancer type 2 susceptibility protein homolog</i>	BRCA2_RAT	1.641E-05	0.09375	0	0.25	0.25	0	0	0	0.5	1	0	0		
<i>Dsr-1B</i>	Q99PU3	1.641E-05	0.09375	0	0	0	0	0	0.25	0.25	1	0.25	0.25		
<i>Interleukin-10 receptor, alpha chain precursor</i>	Q99ND6	1.701E-05	0.234375	0.25	0	0	0	0.5	0.5	1	1.5	0.25	0.5		
<i>60S ribosomal protein L18</i>	RL18_RAT	1.701E-05	0.234375	0	0.25	0.25	0	0	0.25	0.25	1.5	0.25	0		
<i>Neurexin alpha</i>	Q9RIK2	1.912E-05	1.125	0.75	0.5	0.25	0.75	1.5	2	1.25	3.5	1.25	0.75		
<i>4-trimethylaminobutyraldehyde dehydrogenase</i>	AL9A1_RAT	1.987E-05	0.125	0	0	0	0.25	0	0.25	0.75	1	0	0.25		
<i>GDP--fucose protein O-fucosyltransferase I precursor</i>	OFUT1_RAT	1.987E-05	0.125	0	0	0	0	0	0	0.25	1	0	0.5		
<i>Olfactomedin 2 (Predicted)</i>	Q568Y7	2.127E-05	0.15625	0	0	0	0	0	0.5	0	1	0.5	0.5		
<i>Splicing factor, arginine/serine-rich 2 (Splicing factor SC35)</i>	SFRS2_RAT	2.134E-05	0.3125	0	0	0	0	0.25	0.25	0.25	1.75	0.75	0.25		
<i>Ribonuclease 11</i>	Q5GAL9	2.158E-05	0.078125	0	0	0	0	0	0	0	0.75	0	0		
<i>Cyclic nucleotide-gated cation channel beta 3 (Fragment)</i>	Q6Q215	2.158E-05	0.078125	0	0	0	0	0	0.5	0	0.75	0.25	0		
<i>RING finger protein 141</i>	RN141_RAT	2.158E-05	0.078125	0	0	0	0.25	0.25	0	0	0.75	0	0.25		
<i>Echinoderm microtubule associated protein like 1</i>	Q4V8C3	2.249E-05	0.546875	0.5	1	0.5	0.5	1.5	1.5	0.5	2.25	0.5	0.75		
<i>Neuronal acetylcholine receptor protein subunit alpha-4</i>	ACHA4_RAT	2.62E-05	0.640625	0.75	0.75	0.5	0.75	0.75	1.25	1	2.25	0.75	1.25		
<i>Peptidylprolyl isomerase (Cyclophilin)-like 2 (Predicted)</i>	Q5RKH8	2.68E-05	0.65625	0.5	0.25	0	0.5	0.5	1	0.5	2.5	0	1.25		
<i>Calcium/calmodulin-dependent protein kinase kinase 2</i>	KKCC2_RAT	2.686E-05	0.46875	0	0.25	0.5	0.25	0.25	0.75	1	2.25	0.75	1.5		
<i>DEAH (Asp-Glu-Ala-His) box polypeptide 30 (Predicted)</i>	Q5BJS0	2.771E-05	0.671875	0.5	0.75	0.25	0.25	0	1.5	1.25	2.75	0.5	0.5		
<i>Phosphatidylinositol-4-phosphate 3-kinase C2 domain-containing gamma polypeptide</i>	P3CG2_RAT	2.873E-05	0.6875	0.25	0	0.25	0.5	0.25	1	0.5	3	0.5	1.25		
<i>XK-related protein 7</i>	XKR7_RAT	2.875E-05	0.328125	0	0.75	0	0.5	0.75	0.5	0.25	1.75	0.5	0		
<i>CD96 antigen</i>	Q5BK49	2.936E-05	0.4375	0	0.25	0	1	0.25	1.25	0.25	2.25	0.25	0.5		
<i>Alkylthiohydroxacetonephosphate synthase, peroxisomal</i>	ADAS_RAT	3.152E-05	0.703125	0.5	0.75	0.25	1.5	0.75	1.5	0.5	2.75	0.25	0.5		
<i>Zinc finger protein 289</i>	Q3MID3	3.808E-05	0.34375	0.5	1	0.25	0.5	0.25	0.5	0	1.75	0.5	0.25		
<i>P66 alpha</i>	Q5EB93	3.905E-05	0.65625	0	0.25	0.75	0.75	0.5	1.25	1	2.5	0.75	0.5		

Table 6c: List of PBI-III 6 Hour proteins (p \leq 10-4) Part 5 of 6

Protein	Accession Number	p value	Controls	FPI						PBI					
				15m	1 h	6 h	24h	72 h	15m	1 h	6 h	24h	72 h		
Potassium voltage-gated channel subfamily H member 2	KCNH2_RAT	4.062E-05	0.78125	0	0.5	0.5	0.75	0.75	1.25	0.25	2.5	1.25	0.75		
Hypothetical protein MGCI08951	Q5BK36	4.067E-05	0.1875	0	0.25	0	0	0.25	0.5	0.25	1.25	0	0.25		
S-arrestin (Retinal S-antigen) (S-AG)	ARRS_RAT	4.067E-05	0.1875	0	0	0.5	0.5	0	0.75	0	1.25	0.25	0		
Hydroxyacylglutathione hydrolase (EC 3.1.2.6) (Glyoxalase II)	GLO2_RAT	4.213E-05	0.109375	0	0.25	0	0	0	0.25	0.5	1	0.25	0		
Vascular endothelial growth factor C precursor (VEGF-C)	VEGFC_RAT	4.213E-05	0.109375	0.25	0	0.25	0.25	0	0	0.5	1	0.75	0		
Annexin V-binding protein (ABP-10) (Fragment)	P70489	4.219E-05	0.359375	0.5	0.25	1	0	0	1.5	0.75	2	0.25	0		
Titin protein homolog (Fragment)	P97850	5.273E-05	0.171875	0.5	0.25	0.25	0	0	0	0.25	1	0	0		
Potassium-transporting ATPase subunit beta	ATP4B_RAT	5.538E-05	0.03125	0	0	0	0	0	0	0	0.5	0	0		
Sodium-dependent proline transporter	SC6A7_RAT	5.538E-05	0.03125	0	0	0	0	0	0	0	0.5	0	0		
Hypothetical protein	Q3KQW9	5.538E-05	0.03125	0	0	0	0	0	0	0	0.5	0	0		
Vomer nasal V1r-type receptor V1re5	Q51310	5.538E-05	0.03125	0	0	0	0	0	0	0	0.5	0	0		
Phosphatidylcholine:ceramide cholinephosphotransferase 1	SMS1_RAT	5.538E-05	0.03125	0	0	0	0	0	0	0.25	0.5	0	0		
Defensin alpha-related sequence 1	Q4JEI2	5.538E-05	0.03125	0	0	0	0	0	0	0	0.5	0	0		
Anchor attachment protein 1	Q6AYM8	5.538E-05	0.03125	0	0.25	0.25	0	0	0	0	0.5	0	0.25		
RT1 class I, M10, gene 1	Q6MFEZ4	5.538E-05	0.03125	0	0.25	0	0	0	0	0	0.5	0	0		
Prolactin-like protein C-related protein	Q62654	5.538E-05	0.03125	0	0	0	0	0	0.25	0	0.5	0	0		
SUP (Homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1)	Q9ESS9	5.538E-05	0.03125	0	0.25	0	0	0	0	0	0.5	0	0.25		
Ezrin (p81) (Cytovillin) (Villin-2)	EZRI_RAT	5.538E-05	0.03125	0	0	0	0.25	0.5	0	0	0.5	0.25	0		
Cathepsin R	Q4V894	5.538E-05	0.03125	0	0	0	0	0	0	0	0.5	0	0.5		
C-type lectin domain family 4 member D	CLC4D_RAT	5.538E-05	0.03125	0	0	0	0	0	0	0	0.5	0	0		
Hemojuvelin	RGMC_RAT	5.538E-05	0.03125	0	0.25	0	0	0	0	0	0.5	0.25	0		
Cyclic nucleotide-gated channel beta subunit	Q35788	5.538E-05	0.03125	0.5	0	0	0.25	0	0	0	0.5	0.25	0		
FXYD domain-containing ion transport regulator 4	FXYD4_RAT	5.538E-05	0.03125	0	0	0	0	0	0.25	0	0.5	0.25	0		
Early growth response protein 3 (EGR-3)	EGR3_RAT	6.85E-05	0.0625	0	0	0	0	0.25	0	0	0.75	0	0		
Zfp protein	Q562A2	6.85E-05	0.0625	0	0	0	0	0	0	0	0.25	0	0		
Transmembrane protein 100	TM100_RAT	6.85E-05	0.0625	0.25	0	0	0	0	0	0	0.75	0	0		
Coroin-1B (Coroin-2)	COR1B_RAT	6.85E-05	0.0625	0.25	0	0.25	0	0	0	0	0.75	0	0		
Interleukin adhesion molecule 1 precursor (ICAM-1)	ICAM1_RAT	6.979E-05	0.203125	0	0.25	0	0.25	0	0.25	0	1.25	0	0.5		
Glypican-1 (HSPG M2)	GPCI_RAT	6.979E-05	0.203125	0	0	0	0	0	0.25	0.25	0.5	1.25	0.5		

Table 6c: List of PBI-III 6 Hour proteins (p \leq 10-4) Part 6 of 6

Protein	Accession Number	P value	Controls	FPI							PBI				
				15m	1 h	6 h	24h	72 h	15m	1 h	6 h	24h	72 h		
<i>Dock2 protein</i>	Q3MIE5	7.315E-05	0.59375	0.75	0.5	0.5	0.75	1	1.25	0.25	2	0.5	0.75		
<i>Hypothetical protein RGD1308637</i>	Q4V897	7.328E-05	0.53125	0.25	0.25	0.75	0.25	0	0.25	0.5	2	1.25	0.25		
<i>Axotrophin</i>	Q5XI50	7.498E-05	0.59375	0.5	1	0.5	1	1	2.25	1	2.25	2	1		
<i>Spergen-1 (Spermatogenic specific-gene1)</i>	Q920Q3	7.519E-05	0.09375	0.25	0.25	0.25	0	0	0	0	1	0	0		
<i>End/VASP-like protein</i>	EVL_RAT	7.651E-05	0.5	0	0	0.25	0.5	0.5	1	0.75	2.25	0	1		
<i>Hypothetical protein RGD1311946</i>	Q4V7A5	7.818E-05	0.546875	0.25	0.25	0	0.25	0	1	0.75	2.25	0.25	0.75		
<i>Ubiquitin specific peptidase 18</i>	Q6AYC7	8.087E-05	0.296875	0.5	0	0	0	0.25	0.25	0.25	1.5	0.25	0		
<i>Four and a half LIM domains protein 2 (FHL-2)</i>	FHL2_RAT	8.176E-05	1.140625	1.5	0.75	1	1	0.5	3	1.25	2.75	1.5	0.5		
<i>Linkain b1</i>	Q8VIG2	8.179E-05	1.1875	1.75	1	0	1	0.75	1.25	1.75	3.75	1	1.25		
<i>Activin receptor type 1C precursor</i>	ACVIC_RAT	8.237E-05	0.265625	0.25	0.25	0.25	0.25	0.75	0.75	0.25	1.5	0	0.75		
<i>Vacuolar sorting protein SNF8</i>	SNF8_RAT	8.769E-05	0.375	0.5	0	0.5	0.25	0.5	0.25	0.25	1.5	0.25	0.5		
<i>Timeless homolog (rTIM) (Timeless-like protein) (rTLP)</i>	TIM_RAT	8.769E-05	0.375	0.5	0.25	0.25	0.25	0.75	0.75	0.5	1.5	0.5	0.5		
<i>LRRG100150</i>	Q6QI58	9.114E-05	0.421875	0	0.5	0.25	0.5	1	0.75	0.75	2	0.5	1		
<i>Putative taste receptor T2R25</i>	Q67E55	9.437E-05	0.09375	0.25	0.25	0	0	0	0	0	0.75	0.25	0		
<i>Protein farnesyltransferase/geranylgeranyltransferase type I alpha subunit</i>	PFTA_RAT	9.437E-05	0.09375	0	0	0.5	0	0	0.75	0	0.75	0	0		
<i>UDP-glucose 4-epimerase</i>	GALE_RAT	9.437E-05	0.09375	0	0.25	0	0.5	0	0	0	0.75	0	0.25		
<i>Dlg 3 (Fragment)</i>	O88954	9.437E-05	0.09375	0	0	0.25	0.25	0	0	0.25	0.75	0	0.25		

Table 6d: List of PBI-III 24 Hour proteins (p≤ 10⁻⁴)

Protein	Accession Number	p value	Controls	FPI					PBI					
				15m	1 h	6 h	24h	72 h	15m	1 h	6 h	24h	72 h	
Family with sequence similarity 96, member A	Q5RIS3	1.791E-07	0.046875	0	0	0	0	0	0	0	0.25	0	0.75	0
60S ribosomal protein L23a	P62752	4.112E-07	0.109375	0	0	0	0.25	0	0.25	0.5	0.5	1	0.5	0
Psmc4 protein	Q5EAP2	9.039E-07	0.015625	0	0	0	0	0	0	0	0	0	0.5	0
Tropomodulin 1	Q6IMZ5	9.039E-07	0.015625	0	0	0	0	0	0	0	0	0	0.5	0
Testicular cell adhesion molecule 1 (TCAM1)	Q9Z133	1.856E-06	0.03125	0	0	0	0.5	0	0.75	0	0.25	0.75	0.75	0
CD40 ligand (CD40-L)	Q9Z2V2	2.979E-06	0.0625	0	0	0	0	0.25	0.25	0.5	0.5	0.5	0.75	0
Interleukin-1 receptor-like 1	Q62611	4.462E-06	0.359375	0.25	0	0.25	0.25	0.5	1	0.75	1.25	2	2	0.5
TSPY-like 4 (Predicted)	Q66H46	4.82E-06	0.40625	0.25	0	0.25	0.25	0.25	0.25	0	0.75	2	0.75	0
Histidine decarboxylase	P16453	7.261E-06	0.296875	0	0.25	0	0	0	0	0.5	0	1.75	0.25	0
Translocon-associated protein gamma subunit (TRAP-gamma)	Q08013	1.987E-05	0.125	0	0	0	0	0	0.75	0	0.5	1	0.25	0
Putative integral membrane transport protein	O70609	2.158E-05	0.078125	0.75	0	0	0	0	0	0	0.25	0.75	0	0
Urotensin II receptor (UR-II-R) (G-protein coupled receptor 14)	P49684	4.213E-05	0.109375	0	0	0	0	0	0	0	0	1	0.75	0
ULF-250 protein	Q9Z212	4.97E-05	0.140625	0.25	0.25	0.25	0	0.5	0	0.25	0	1	0.5	0
Phospholipase A2, group IID (Predicted)	Q5BK35	5.538E-05	0.03125	0	0	0	0	0	0	0	0	0.5	0	0
BAK protein (Bak1 protein)	Q91K59	5.538E-05	0.03125	0	0.25	0	0	0	0.25	0	0	0.5	0	0
Peptidoglycan recognition protein precursor	Q9JLN4	5.538E-05	0.03125	0	0	0	0	0	0.25	0.25	0	0.5	0	0
Serine protease	Q63546	5.538E-05	0.03125	0	0	0	0	0	0	0	0	0.25	0.5	0
Protein yippe-like 4	Q5XID5	5.538E-05	0.03125	0	0.25	0.25	0.5	0	0	0	0	0.5	0.25	0
GPR3 protein	Q8K1Q3	5.538E-05	0.03125	0	0	0	0	0	0.25	0	0	0.5	0.25	0
Solute carrier family 19 (Thiamine transporter), member 2	Q499Q0	5.538E-05	0.03125	0	0.25	0	0	0.25	0	0	0	0.5	0.25	0
Long palate, lung and nasal epithelium carcinoma-associated protein 4	Q05704	6.85E-05	0.0625	0	0	0	0.25	0.25	0	0	0	0	0.75	0
Hypothetical protein RGD1306477	Q4V8F3	9.437E-05	0.09375	0	0.25	0	0	0	0	0.25	0.25	0.75	0.25	0

Table 6e: List of PBI-III 72 Hour proteins (p_≤ 10⁻⁴)

Protein	Accession Number	p value	Controls	FPI						PBI					
				15m	1 h	6 h	24h	72 h	15m	1 h	6 h	24h	72 h		
Mitochondrial 28S ribosomal protein S10 (S10m) (MRP-S10)	Q7TQ82	8.092E-17	0	0.25	0	0	0	0	0	0	0	0	0.25	0.25	1
Hyaluronan proteoglycan link protein 3	Q5R1X6	4.315E-11	0	0	0	0	0	0	0	0.25	0	0	0.25	0	0.5
Ankrd10 protein (Fragment)	Q4G039	4.315E-11	0	0	0	0	0.25	0	0.25	0	0.25	0	0.25	0	0.5
Follistatin precursor (FS) (Activin-binding protein)	P21674	3.981E-10	0.0625	0.25	0	0.25	0	0	0	0	0	0.25	0.25	0.25	1.25
Four and a half LIM domains protein 5 (FHL-5)	Q6AXT1	7.138E-08	0.203125	0.25	0	0	0	0	0	0.5	0	0	0.25	0.25	1.5
Chemokine receptor 7-like protein	Q6U2D6	9.63E-08	0.015625	0	0	0	0.25	0	0	0	0.25	0	0	0	0.75
Tropomitin T, slow skeletal muscle (TnTs)	Q7TNB2	9.039E-07	0.015625	0	0	0	0	0	0	0	0.25	0.5	0	0	0.5
Col6a2 protein (Fragment)	Q5EB88	1.82E-06	0.09375	0.25	0	0	0	0	0	0.75	0	0.5	0.25	0.25	1
Golp4 protein (Fragment)	Q5BJK8	2.979E-06	0.0625	0	0	0	0	0	0	0	0	0.75	0.5	0.5	0.75
Neurexophilin-3 precursor	Q9Z2N5	7.275E-06	0.140625	0	0	0.25	0	0	0	0.25	0.75	0	0	0	1
Threonyl-tRNA synthetase-like 2	Q5X117	1.148E-05	0.15625	0	0	0.25	0	0	0	0	0.25	0.75	0.5	0.5	1.25
G patch domain containing 2 (Predicted)	Q6AY15	1.473E-05	0.046875	0	0.25	0.5	0.25	0.25	0	0	0	0.5	0	0	0.75
GABA theta subunit	Q91ZM7	1.641E-05	0.09375	0	0	0.25	0	0	0	0	0	0.25	0	0	1
Transcription factor AP-2 alpha (AP2-alpha)	P58197	1.641E-05	0.09375	0	0	0.25	0	0	0	0.25	0	0	0	0.25	1
17-beta hydroxysteroid dehydrogenase 13 precursor	Q5M875	1.987E-05	0.125	0.25	0	0	0.25	0	0	0	0	0.5	0.25	0.25	1
Zinc finger protein 57	Q6MFPY0	2.127E-05	0.15625	0.25	0	0	0.25	0	0	1	1	1	0.75	0.75	1
Similar to RIKEN cDNA 2400010D15 (Predicted)	Q5PPI8	4.133E-05	0.21875	0	0.25	0	0.25	0.25	0	0	0	0.25	0	0	1.25
Collagen prolyl 4-hydroxylase alpha III subunit	Q6W3E9	4.97E-05	0.140625	0.25	0	0.5	0	0	0	0.5	0	0	0	0	1
Hypothetical protein LOC498065	Q66H14	5.273E-05	1.015625	0.25	0.25	1	1	0.25	1	1	1	2	0.5	0.5	3.5
Cathepsin R	Q4V894	5.538E-05	0.03125	0	0	0	0	0	0	0	0	0.5	0	0	0.5
TTF-2 protein	O08771	5.538E-05	0.03125	0	0	0	0	0	0	0	0	0.25	0	0	0.5
Hypothetical LOC316275 (Progesterin membrane receptor beta)	Q6AY03	5.538E-05	0.03125	0	0	0	0	0	0	0.25	0	0.25	0	0	0.5
Chemokine (C-C motif) receptor 6 (Predicted)	Q5BK58	5.538E-05	0.03125	0	0	0	0	0	0	0.75	0	0.75	0	0	0.5
Beta-defensin 29	Q3Z7G3	5.538E-05	0.03125	0	0	0	0	0.25	0	0	0	0.25	0	0	0.5
Syntaxin-8	Q9Z2Q7	5.538E-05	0.03125	0.25	0	0	0.25	0	0.25	0	0.25	0	0.25	0	0.5
Guanine nucleotide-binding protein alpha-12 subunit (G alpha-12)	Q63210	5.538E-05	0.03125	0.25	0	0.75	0.25	0	0.25	0	0.25	0	0.25	0.25	0.5
D(2) dopamine receptor (Dopamine D2 receptor)	P61169	9.437E-05	0.125	0	0.25	0	0	0	0.5	0.75	0.75	0.75	0.25	0.25	1

Table 7a: Protein Selection for FPI 15 Min

Protein	p (T test)	Tissues Referenced	Available Antibodies	Graph of the Average # of Unique Peptides vs Experimental Group
Voltage-dependent P/Q-type calcium channel alpha-1A subunit	4.31547E-11	Brain	Yes	<p>Average # of Unique Peptides</p> <p>Control = 0</p>
Calcium-chelate transporter (CCT)	5.54E-05	Hypophysis	Yes (human, rat reactivity unknown)	<p>Average # of Unique Peptides</p> <p>Control = 0.03125</p>
Beta-1,3-N-acetylglucosaminyltransferase radical fringe	0.000510571	Brain	Yes (human, rat reactivity unknown)	<p>Average # of Unique Peptides</p> <p>Control = 0.140625</p>
Plasmalogen (Plasma membrane proteolipid)	0.000543725	Oligodendrocytes	Yes (from investigator?)	<p>Average # of Unique Peptides</p> <p>Control = 0.048975</p>

Table 7b: Protein Selection for FPI 1 Hour

Protein	p (T test)	Tissues Referenced	Available Antibodies	Graph of the Average # of Unique Peptides vs Experimental Group
Ischemia related factor vof-16	4.31547E-11	Brain	No	<p>Average # of Unique Peptides</p> <p>Control = 0</p>
GTP cyclohydrolase I (GTP-CH-I)	2.3313E-09	Liver, Brain	Yes (rat reactivity unknown)	<p>Average # of Unique Peptides</p> <p>Control = 0.03125</p>
3-mercaptopyruvate sulfurtransferase (MST)	5.53838E-05	Liver, Spinal Cord, Other	Yes (rat reactivity unknown)	<p>Average # of Unique Peptides</p> <p>Control = 0.03125</p>
Glia maturation factor beta (GMF-beta)	0.000543725	CNS (predominant)	Yes	<p>Average # of Unique Peptides</p> <p>Control = 0.046975</p>

Table 7b (cont) : Protein Selection for FPI 1 Hour

Protein	p (T test)	Tissues Referenced	Available Antibodies	Graph of the Average # of Unique Peptides vs Experimental Group																						
Kinesin heavy chain isoform 5C (Kinesin heavy chain neuron-specific 2)	0.000543725	Brain, Testis	Yes (rat reactivity unknown)	<p>Averaged SPIKEs</p> <table border="1"> <caption>Data from the Averaged SPIKEs Graph</caption> <thead> <tr> <th>Experimental Group</th> <th>Averaged SPIKEs (Approximate)</th> </tr> </thead> <tbody> <tr> <td>FPI-3 15m</td> <td>0.15</td> </tr> <tr> <td>FPI-3 60m</td> <td>0.45*</td> </tr> <tr> <td>FPI-3 6h</td> <td>0.05</td> </tr> <tr> <td>FPI-3 24h</td> <td>0.05</td> </tr> <tr> <td>FPI-3 72h</td> <td>0.05</td> </tr> <tr> <td>PBI-3 15m</td> <td>0.05</td> </tr> <tr> <td>PBI-3 60m</td> <td>0.05</td> </tr> <tr> <td>PBI-3 6h</td> <td>0.05</td> </tr> <tr> <td>PBI-3 24h</td> <td>0.15</td> </tr> <tr> <td>PBI-3 72h</td> <td>0.05</td> </tr> </tbody> </table> <p>Control = 0.046875</p>	Experimental Group	Averaged SPIKEs (Approximate)	FPI-3 15m	0.15	FPI-3 60m	0.45*	FPI-3 6h	0.05	FPI-3 24h	0.05	FPI-3 72h	0.05	PBI-3 15m	0.05	PBI-3 60m	0.05	PBI-3 6h	0.05	PBI-3 24h	0.15	PBI-3 72h	0.05
Experimental Group	Averaged SPIKEs (Approximate)																									
FPI-3 15m	0.15																									
FPI-3 60m	0.45*																									
FPI-3 6h	0.05																									
FPI-3 24h	0.05																									
FPI-3 72h	0.05																									
PBI-3 15m	0.05																									
PBI-3 60m	0.05																									
PBI-3 6h	0.05																									
PBI-3 24h	0.15																									
PBI-3 72h	0.05																									

Table 7c: Protein Selection for FPI 6 Hours

Protein	p (T test)	Tissues Referenced	Available Antibodies	Graph of the Average # of Unique Peptides vs Experimental Group
Neurexin-1-beta	6.84971E-05	Brain	Yes	<p>Average # of Unique Peptides</p> <p>Control = 0.0625</p>
GPI-anchored ceruloplasmin	0.003753308	Brain	Yes	<p>Average # of Unique Peptides</p> <p>Control = 37.96975</p>
TGF-beta receptor type-2	0.006158223	Pituitary	Yes	<p>Average # of Unique Peptides</p> <p>Control = 0.375</p>

Table 7d: Protein Selection for FPI 24 Hours

Protein	p (T test)	Tissues Referenced	Available Antibodies	Graph of the Average # of Unique Peptides vs Experimental Group																								
Bone morphogenetic protein 3b precursor (BMP-3b)	2.15811E-05	Costa, Costicartilage, Femur, Calvaria, Trachea, Aorta and Brain	Yes (rat reactivity unknown)	<p>Average # of Unique Peptides</p> <table border="1"> <tr><th>Group</th><th>Average # of Unique Peptides</th></tr> <tr><td>FPI-3 15m</td><td>~0.45</td></tr> <tr><td>FPI-3 60m</td><td>~0.25</td></tr> <tr><td>FPI-3 6h</td><td>~0.75*</td></tr> <tr><td>FPI-3 24h</td><td>~0.75*</td></tr> <tr><td>FPI-3 72h</td><td>~0.75*</td></tr> <tr><td>PBI-3 15m</td><td>~0.15</td></tr> <tr><td>PBI-3 60m</td><td>~0.15</td></tr> <tr><td>PBI-3 6h</td><td>~0.15</td></tr> <tr><td>PBI-3 24h</td><td>~0.15</td></tr> <tr><td>PBI-3 72h</td><td>~0.15</td></tr> <tr><td>Control</td><td>0.078125</td></tr> </table>	Group	Average # of Unique Peptides	FPI-3 15m	~0.45	FPI-3 60m	~0.25	FPI-3 6h	~0.75*	FPI-3 24h	~0.75*	FPI-3 72h	~0.75*	PBI-3 15m	~0.15	PBI-3 60m	~0.15	PBI-3 6h	~0.15	PBI-3 24h	~0.15	PBI-3 72h	~0.15	Control	0.078125
Group	Average # of Unique Peptides																											
FPI-3 15m	~0.45																											
FPI-3 60m	~0.25																											
FPI-3 6h	~0.75*																											
FPI-3 24h	~0.75*																											
FPI-3 72h	~0.75*																											
PBI-3 15m	~0.15																											
PBI-3 60m	~0.15																											
PBI-3 6h	~0.15																											
PBI-3 24h	~0.15																											
PBI-3 72h	~0.15																											
Control	0.078125																											
Butyrophilin-like protein 2	2.15811E-05	Brain, other tissues	No	<p>Average # of Unique Peptides</p> <table border="1"> <tr><th>Group</th><th>Average # of Unique Peptides</th></tr> <tr><td>FPI-3 15m</td><td>~0.15</td></tr> <tr><td>FPI-3 60m</td><td>~0.25</td></tr> <tr><td>FPI-3 6h</td><td>~0.75*</td></tr> <tr><td>FPI-3 24h</td><td>~0.75*</td></tr> <tr><td>FPI-3 72h</td><td>~0.75*</td></tr> <tr><td>PBI-3 15m</td><td>~0.15</td></tr> <tr><td>PBI-3 60m</td><td>~0.15</td></tr> <tr><td>PBI-3 6h</td><td>~0.15</td></tr> <tr><td>PBI-3 24h</td><td>~0.15</td></tr> <tr><td>PBI-3 72h</td><td>~0.15</td></tr> <tr><td>Control</td><td>0.078125</td></tr> </table>	Group	Average # of Unique Peptides	FPI-3 15m	~0.15	FPI-3 60m	~0.25	FPI-3 6h	~0.75*	FPI-3 24h	~0.75*	FPI-3 72h	~0.75*	PBI-3 15m	~0.15	PBI-3 60m	~0.15	PBI-3 6h	~0.15	PBI-3 24h	~0.15	PBI-3 72h	~0.15	Control	0.078125
Group	Average # of Unique Peptides																											
FPI-3 15m	~0.15																											
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FPI-3 6h	~0.75*																											
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FPI-3 72h	~0.75*																											
PBI-3 15m	~0.15																											
PBI-3 60m	~0.15																											
PBI-3 6h	~0.15																											
PBI-3 24h	~0.15																											
PBI-3 72h	~0.15																											
Control	0.078125																											
Testicular cell adhesion molecule 1 (TCAM1)	5.53838E-05	Testis	No	<p>Average # of Unique Peptides</p> <table border="1"> <tr><th>Group</th><th>Average # of Unique Peptides</th></tr> <tr><td>FPI-3 15m</td><td>~0.15</td></tr> <tr><td>FPI-3 60m</td><td>~0.25</td></tr> <tr><td>FPI-3 6h</td><td>~0.75*</td></tr> <tr><td>FPI-3 24h</td><td>~0.75*</td></tr> <tr><td>FPI-3 72h</td><td>~0.75*</td></tr> <tr><td>PBI-3 15m</td><td>~0.15</td></tr> <tr><td>PBI-3 60m</td><td>~0.15</td></tr> <tr><td>PBI-3 6h</td><td>~0.15</td></tr> <tr><td>PBI-3 24h</td><td>~0.15</td></tr> <tr><td>PBI-3 72h</td><td>~0.15</td></tr> <tr><td>Control</td><td>0.03125</td></tr> </table>	Group	Average # of Unique Peptides	FPI-3 15m	~0.15	FPI-3 60m	~0.25	FPI-3 6h	~0.75*	FPI-3 24h	~0.75*	FPI-3 72h	~0.75*	PBI-3 15m	~0.15	PBI-3 60m	~0.15	PBI-3 6h	~0.15	PBI-3 24h	~0.15	PBI-3 72h	~0.15	Control	0.03125
Group	Average # of Unique Peptides																											
FPI-3 15m	~0.15																											
FPI-3 60m	~0.25																											
FPI-3 6h	~0.75*																											
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PBI-3 15m	~0.15																											
PBI-3 60m	~0.15																											
PBI-3 6h	~0.15																											
PBI-3 24h	~0.15																											
PBI-3 72h	~0.15																											
Control	0.03125																											
Neuromedin U receptor 2 (NMR2)	5.53838E-05	Uterus, Hypothalamus, Medulla Oblongata, Spinal Cord, Lung, Ovary, Gastrointestinal Tract	Yes	<p>Average # of Unique Peptides</p> <table border="1"> <tr><th>Group</th><th>Average # of Unique Peptides</th></tr> <tr><td>FPI-3 15m</td><td>~0.15</td></tr> <tr><td>FPI-3 60m</td><td>~0.25</td></tr> <tr><td>FPI-3 6h</td><td>~0.75*</td></tr> <tr><td>FPI-3 24h</td><td>~0.75*</td></tr> <tr><td>FPI-3 72h</td><td>~0.75*</td></tr> <tr><td>PBI-3 15m</td><td>~0.15</td></tr> <tr><td>PBI-3 60m</td><td>~0.15</td></tr> <tr><td>PBI-3 6h</td><td>~0.15</td></tr> <tr><td>PBI-3 24h</td><td>~0.15</td></tr> <tr><td>PBI-3 72h</td><td>~0.15</td></tr> <tr><td>Control</td><td>0.03125</td></tr> </table>	Group	Average # of Unique Peptides	FPI-3 15m	~0.15	FPI-3 60m	~0.25	FPI-3 6h	~0.75*	FPI-3 24h	~0.75*	FPI-3 72h	~0.75*	PBI-3 15m	~0.15	PBI-3 60m	~0.15	PBI-3 6h	~0.15	PBI-3 24h	~0.15	PBI-3 72h	~0.15	Control	0.03125
Group	Average # of Unique Peptides																											
FPI-3 15m	~0.15																											
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FPI-3 6h	~0.75*																											
FPI-3 24h	~0.75*																											
FPI-3 72h	~0.75*																											
PBI-3 15m	~0.15																											
PBI-3 60m	~0.15																											
PBI-3 6h	~0.15																											
PBI-3 24h	~0.15																											
PBI-3 72h	~0.15																											
Control	0.03125																											

Table 7d (cont): Protein Selection for FPI 24 Hours

Protein	p (T test)	Tissues Referenced	Available Antibodies	Graph of the Average # of Unique Peptides vs Experimental Group																						
Synaptonemal complex protein 3 (SCP-3)	5.53838E-05	Testis, Brain	Yes (rat reactivity unknown)	<p>Average # of Unique Peptides</p> <table border="1"> <caption>Data for Average # of Unique Peptides vs Experimental Group</caption> <thead> <tr> <th>Experimental Group</th> <th>Average # of Unique Peptides (approx.)</th> </tr> </thead> <tbody> <tr> <td>FPI-3 15m</td> <td>0.18</td> </tr> <tr> <td>FPI-3 60m</td> <td>0.18</td> </tr> <tr> <td>FPI-3 6h</td> <td>0.22</td> </tr> <tr> <td>FPI-3 24h</td> <td>0.48*</td> </tr> <tr> <td>FPI-3 72h</td> <td>0.25</td> </tr> <tr> <td>PBI-3 15m</td> <td>0.52*</td> </tr> <tr> <td>PBI-3 60m</td> <td>0.18</td> </tr> <tr> <td>PBI-3 6h</td> <td>0.22</td> </tr> <tr> <td>PBI-3 24h</td> <td>0.25</td> </tr> <tr> <td>PBI-3 72h</td> <td>0.25</td> </tr> </tbody> </table> <p>Control = 0.03125</p>	Experimental Group	Average # of Unique Peptides (approx.)	FPI-3 15m	0.18	FPI-3 60m	0.18	FPI-3 6h	0.22	FPI-3 24h	0.48*	FPI-3 72h	0.25	PBI-3 15m	0.52*	PBI-3 60m	0.18	PBI-3 6h	0.22	PBI-3 24h	0.25	PBI-3 72h	0.25
Experimental Group	Average # of Unique Peptides (approx.)																									
FPI-3 15m	0.18																									
FPI-3 60m	0.18																									
FPI-3 6h	0.22																									
FPI-3 24h	0.48*																									
FPI-3 72h	0.25																									
PBI-3 15m	0.52*																									
PBI-3 60m	0.18																									
PBI-3 6h	0.22																									
PBI-3 24h	0.25																									
PBI-3 72h	0.25																									

Table 7e: Protein Selection for FPI 72 Hours

Protein	p (T test)	Tissues Referenced	Available Antibodies	Graph of the Average # of Unique Peptides vs Experimental Group
Syntaxin-1A (Neuron-specific antigen HPC-1)	2.3313E-09	Cerebral Cortex, Hippocampus, Cerebellum, Adrenal Medulla and Retina minor: Non-Neuronal Tissues.	Yes	<p>Average # of Unique Peptides</p> <p>Control = 0.03125</p>
Muellerian-inhibiting factor (Mullerian-inhibiting substance) (MIS)	5.43183E-06	Testis, Motor Neuron Survival Factor	Yes	<p>Average # of Unique Peptides</p> <p>Control = 0.140625</p>
Butyrophilin-like protein 2	2.15811E-05	Brain, other tissues	No	<p>Average # of Unique Peptides</p> <p>Control = 0.076125</p>

Table 7i: Protein Selection for PBI 15 Minutes

Protein	p (T test)	Tissues Referenced	Available Antibodies	Graph of the Average # of Unique Peptides vs Experimental Group
Testicular cell adhesion molecule 1 (TCAM1)	2.3313E-09	Testis	No	<p>Average # of Unique Peptides</p> <p>Control = 0.03125</p>
Thyrotropin-releasing hormone receptor (TRH-R)	3.44368E-09	Pituitary	Yes (human, rat reactivity unknown)	<p>Average # of Unique Peptides</p> <p>Control = 0.046875</p>
Tubulin tyrosine ligase-like 1 (Predicted)	3.29351E-07	Brain	No	<p>Average # of Unique Peptides</p> <p>Control = 0.125</p>
Suprachiasmatic nucleus circadian oscillatory protein	5.64589E-07	Brain	Yes	<p>Average # of Unique Peptides</p> <p>Control = 0.65625</p>

Table 7f (cont): Protein Selection for FPI 24 Hours

Protein	p (T test)	Tissues Referenced	Available Antibodies	Graph of the Average # of Unique Peptides vs Experimental Group																						
Drebrin-like protein (SH3 domain-containing protein 7)	9.74558E-07	Hippocampus, Heart	Yes (expect Rat)	<p>Average # of Unique Peptides</p> <table border="1"> <thead> <tr> <th>Experimental Group</th> <th>Average # of Unique Peptides</th> </tr> </thead> <tbody> <tr><td>FPI-3 15m</td><td>~0.1</td></tr> <tr><td>FPI-3 60m</td><td>~0.1</td></tr> <tr><td>FPI-3 6h</td><td>~0.2</td></tr> <tr><td>FPI-3 24h</td><td>~0.3</td></tr> <tr><td>FPI-3 72h</td><td>~0.15</td></tr> <tr><td>PBL-3 15m</td><td>~1.25*</td></tr> <tr><td>PBL-3 60m</td><td>~0.7</td></tr> <tr><td>PBL-3 6h</td><td>~0.5</td></tr> <tr><td>PBL-3 24h</td><td>~0.2</td></tr> <tr><td>PBL-3 72h</td><td>~0.2</td></tr> </tbody> </table> <p>Control = 0.140625</p>	Experimental Group	Average # of Unique Peptides	FPI-3 15m	~0.1	FPI-3 60m	~0.1	FPI-3 6h	~0.2	FPI-3 24h	~0.3	FPI-3 72h	~0.15	PBL-3 15m	~1.25*	PBL-3 60m	~0.7	PBL-3 6h	~0.5	PBL-3 24h	~0.2	PBL-3 72h	~0.2
Experimental Group	Average # of Unique Peptides																									
FPI-3 15m	~0.1																									
FPI-3 60m	~0.1																									
FPI-3 6h	~0.2																									
FPI-3 24h	~0.3																									
FPI-3 72h	~0.15																									
PBL-3 15m	~1.25*																									
PBL-3 60m	~0.7																									
PBL-3 6h	~0.5																									
PBL-3 24h	~0.2																									
PBL-3 72h	~0.2																									
Neurexophilin-4 precursor	2.3302E-06	Brain, Kidney	Yes (rat reactivity unknown)	<p>Average # of Unique Peptides</p> <table border="1"> <thead> <tr> <th>Experimental Group</th> <th>Average # of Unique Peptides</th> </tr> </thead> <tbody> <tr><td>FPI-3 15m</td><td>~0.1</td></tr> <tr><td>FPI-3 60m</td><td>~0.1</td></tr> <tr><td>FPI-3 6h</td><td>~0.2</td></tr> <tr><td>FPI-3 24h</td><td>~0.2</td></tr> <tr><td>FPI-3 72h</td><td>~0.1</td></tr> <tr><td>PBL-3 15m</td><td>~1.25*</td></tr> <tr><td>PBL-3 60m</td><td>~0.6</td></tr> <tr><td>PBL-3 6h</td><td>~0.7</td></tr> <tr><td>PBL-3 24h</td><td>~0.1</td></tr> <tr><td>PBL-3 72h</td><td>~0.1</td></tr> </tbody> </table> <p>Control = 0.21875</p>	Experimental Group	Average # of Unique Peptides	FPI-3 15m	~0.1	FPI-3 60m	~0.1	FPI-3 6h	~0.2	FPI-3 24h	~0.2	FPI-3 72h	~0.1	PBL-3 15m	~1.25*	PBL-3 60m	~0.6	PBL-3 6h	~0.7	PBL-3 24h	~0.1	PBL-3 72h	~0.1
Experimental Group	Average # of Unique Peptides																									
FPI-3 15m	~0.1																									
FPI-3 60m	~0.1																									
FPI-3 6h	~0.2																									
FPI-3 24h	~0.2																									
FPI-3 72h	~0.1																									
PBL-3 15m	~1.25*																									
PBL-3 60m	~0.6																									
PBL-3 6h	~0.7																									
PBL-3 24h	~0.1																									
PBL-3 72h	~0.1																									

Table 7g: Protein Selection for PBI 1 Hour

Protein	p (T test)	Tissues Referenced	Available Antibodies	Graph of the Average # of Unique Peptides vs Experimental Group
Nerve growth factor-regulated G-protein-coupled receptor 1	9.74558E-07	Brain, Skin minor: Spleen, Lung	Yes	<p>Average # of Unique Peptides vs Experimental Group</p> <p>Control = 0.140625</p>
Mago-nashi-like proliferation-associated protein (Fragment)	1.47316E-05	Hippocampus	Yes (rat reactivity unknown)	<p>Average # of Unique Peptides vs Experimental Group</p> <p>Control = 0.046875</p>
Mas-related G-protein coupled receptor member D	2.15811E-05	Cerebellum	Yes (human, rat reactivity unknown)	<p>Average # of Unique Peptides vs Experimental Group</p> <p>Control = 0.078125</p>
Achaete-scute homolog 2	8.72527E-05	Neuronal Precursor Cells	No (Mouse only)	<p>Average # of Unique Peptides vs Experimental Group</p> <p>Control = 0.359375</p>

Table 7g (cont): Protein Selection for FPI 24 Hours

Protein	p (T test)	Tissues Referenced	Available Antibodies	Graph of the Average # of Unique Peptides vs Experimental Group																						
Beta-1,3-N-acetylglucosaminyltransferase radical fringe	0.000189726	Brain minor: Lung, Kidney	Yes (human, rat reactivity unknown)	<p>Average # of Unique Peptides</p> <table border="1"> <caption>Data from Graph of the Average # of Unique Peptides vs Experimental Group</caption> <thead> <tr> <th>Experimental Group</th> <th>Average # of Unique Peptides (approx.)</th> </tr> </thead> <tbody> <tr> <td>FPI-3 15m</td> <td>1.0*</td> </tr> <tr> <td>FPI-3 60m</td> <td>0.0</td> </tr> <tr> <td>FPI-3 6h</td> <td>0.0</td> </tr> <tr> <td>FPI-3 24h</td> <td>0.0</td> </tr> <tr> <td>FPI-3 72h</td> <td>0.0</td> </tr> <tr> <td>PBI-3 15m</td> <td>0.0</td> </tr> <tr> <td>PBI-3 60m</td> <td>1.0*</td> </tr> <tr> <td>PBI-3 6h</td> <td>0.1</td> </tr> <tr> <td>PBI-3 24h</td> <td>0.1</td> </tr> <tr> <td>PBI-3 72h</td> <td>0.0</td> </tr> </tbody> </table> <p>Control = 0.140825</p>	Experimental Group	Average # of Unique Peptides (approx.)	FPI-3 15m	1.0*	FPI-3 60m	0.0	FPI-3 6h	0.0	FPI-3 24h	0.0	FPI-3 72h	0.0	PBI-3 15m	0.0	PBI-3 60m	1.0*	PBI-3 6h	0.1	PBI-3 24h	0.1	PBI-3 72h	0.0
Experimental Group	Average # of Unique Peptides (approx.)																									
FPI-3 15m	1.0*																									
FPI-3 60m	0.0																									
FPI-3 6h	0.0																									
FPI-3 24h	0.0																									
FPI-3 72h	0.0																									
PBI-3 15m	0.0																									
PBI-3 60m	1.0*																									
PBI-3 6h	0.1																									
PBI-3 24h	0.1																									
PBI-3 72h	0.0																									

Table 7h: Protein Selection for PBI 6 Hours

Protein	p (T test)	Tissues Referenced	Available Antibodies	Graph of the Average # of Unique Peptides vs Experimental Group
Beta-neoendorphin-dynorphin precursor (Proenkephalin B)	4.31547E-11	Hypothalamus	Yes	<p>Average # of Unique Peptides</p> <p>Control = 0</p>
Neuronal pentraxin-1 precursor (NP1)	1.37162E-10	Cerebellum, Hippocampus and Cerebral Cortex	Yes (human, rat reactivity unknown)	<p>Average # of Unique Peptides</p> <p>Control = 0.1875</p>
Sdcag1 protein (Fragment)	2.94347E-09	Brain	Yes (human, rat reactivity unknown)	<p>Average # of Unique Peptides</p> <p>Control = 0.125</p>
Neuromedin-B receptor (NMB-R)	8.82256E-09	Olfactory Bulb, Central Thalamic Regions, and Esophagus.	Yes (human, rat reactivity unknown)	<p>Average # of Unique Peptides</p> <p>Control = 0.171875</p>

Table 7h (cont): Protein Selection for FPI 24 Hours

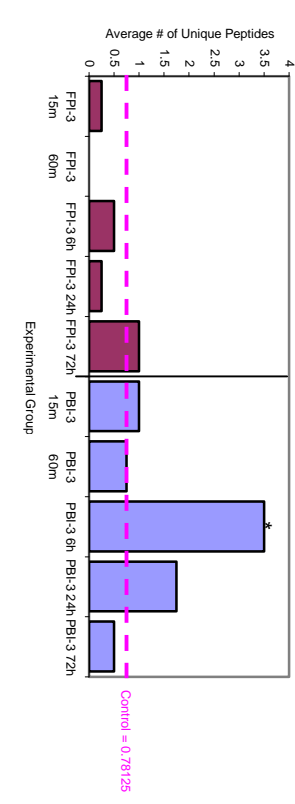
Protein	p (T test)	Tissues Referenced	Available Antibodies	Graph of the Average # of Unique Peptides vs Experimental Group																						
Kinesin heavy chain (Ubiquitous kinesin heavy chain) (UKHC)	4.34013E-08	Brain, Liver, Didey, Spleen, Heart, Lung and Sciatic Nerve.	Yes	 <p>Average # of Unique Peptides</p> <table border="1"> <caption>Data from Graph of the Average # of Unique Peptides vs Experimental Group</caption> <thead> <tr> <th>Experimental Group</th> <th>Average # of Unique Peptides (approx.)</th> </tr> </thead> <tbody> <tr> <td>FPI-3 15m</td> <td>0.5</td> </tr> <tr> <td>FPI-3 60m</td> <td>0.5</td> </tr> <tr> <td>FPI-3 6h</td> <td>0.8</td> </tr> <tr> <td>FPI-3 24h</td> <td>0.8</td> </tr> <tr> <td>FPI-3 72h</td> <td>1.2</td> </tr> <tr> <td>PBI-3 15m</td> <td>1.2</td> </tr> <tr> <td>PBI-3 60m</td> <td>1.2</td> </tr> <tr> <td>PBI-3 6h</td> <td>3.5*</td> </tr> <tr> <td>PBI-3 24h</td> <td>2.2</td> </tr> <tr> <td>PBI-3 72h</td> <td>0.8</td> </tr> </tbody> </table> <p>Control = 0.78125</p>	Experimental Group	Average # of Unique Peptides (approx.)	FPI-3 15m	0.5	FPI-3 60m	0.5	FPI-3 6h	0.8	FPI-3 24h	0.8	FPI-3 72h	1.2	PBI-3 15m	1.2	PBI-3 60m	1.2	PBI-3 6h	3.5*	PBI-3 24h	2.2	PBI-3 72h	0.8
Experimental Group	Average # of Unique Peptides (approx.)																									
FPI-3 15m	0.5																									
FPI-3 60m	0.5																									
FPI-3 6h	0.8																									
FPI-3 24h	0.8																									
FPI-3 72h	1.2																									
PBI-3 15m	1.2																									
PBI-3 60m	1.2																									
PBI-3 6h	3.5*																									
PBI-3 24h	2.2																									
PBI-3 72h	0.8																									

Table 71: Protein Selection for PBI 24 Hours

Protein	p (T test)	Tissues Referenced	Available Antibodies	Graph of the Average # of Unique Peptides vs Experimental Group
Testicular cell adhesion molecule 1 (TCAM1)	1.85648E-06	Testis	No	<p>Average # of Unique Peptides</p> <p>Control = 0.03125</p>
Histidine decarboxylase (HDC)	7.26131E-06	Brain, glandular regions of the stomach, mast cells and fetal liver.	Yes	<p>Average # of Unique Peptides</p> <p>Control = 0.296875</p>
G-protein coupled sensory epithelial neuropeptide-like receptor	4.21344E-05	Circumvallate papilla, urinary bladder, Pheochromocytoma. Preferentially expressed in neural and sensory tissues.	Yes	<p>Average # of Unique Peptides</p> <p>Control = 0.109375</p>
Peptidoglycan recognition protein	5.53838E-05	Brain, Spleen	Yes (Human & Mouse, rat reactivity unknown)	<p>Average # of Unique Peptides</p> <p>Control = 0.03125</p>

Table 7j: Protein Selection for PBI 72 Hours

Protein	p (T test)	Tissues Referenced	Available Antibodies	Graph of the Average # of Unique Peptides vs Experimental Group
Neurexophilin-3	7.27484E-06	Brain, other	No	<p>Average # of Unique Peptides</p> <p>Control = 0.140625</p>
Transcription factor AP-2 alpha (AP2-alpha)	1.64141E-05	Hippocampus, Hypothalamus and Cerebral Cortex.	Yes	<p>Average # of Unique Peptides</p> <p>Control = 0.06375</p>
Guanine nucleotide-binding protein alpha-12 subunit	5.53838E-05	Brain	Yes (Human, expect Rat)	<p>Average # of Unique Peptides</p> <p>Control = 0.03125</p>
D(2) dopamine receptor (Dopamine D2 receptor)	9.4373E-05	Brain	Yes	<p>Average # of Unique Peptides</p> <p>Control = 0.125</p>

Figure 1a: Number of proteins detected as differentially represented in plasma vs FPI, time of collection and p cut-off

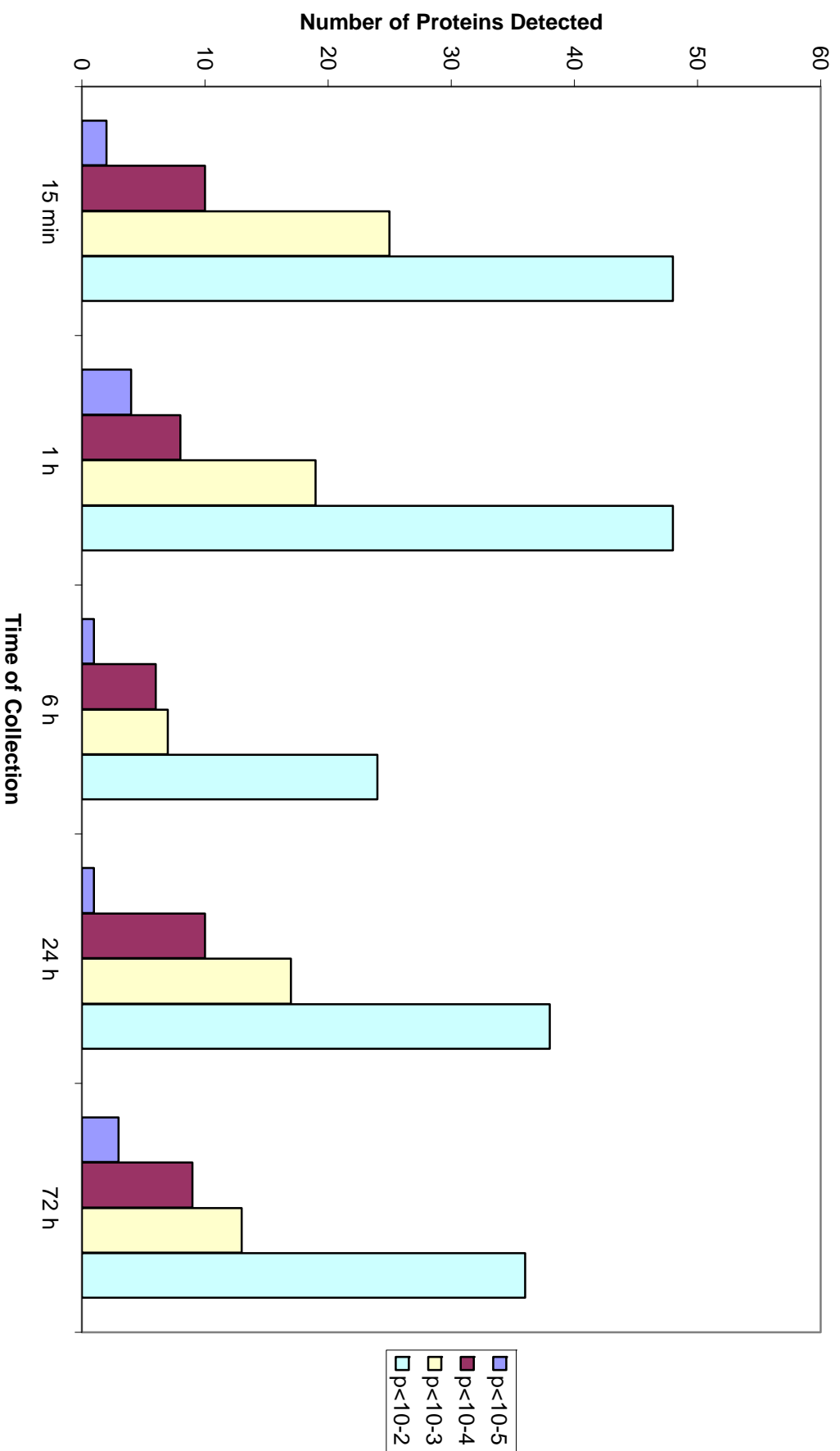


Figure 1b: Number of proteins detected as differentially represented in plasma vs PBI, time of collection and p cut-off

