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**TITLE:** Novel Therapeutic Target Identification through analysis of Convergent AD and TBI Pathogenic Mechanisms

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**CONTRACTING ORGANIZATION:** The Roskamp Institute, Inc., Sarasota, FL

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| <b>13. SUPPLEMENTARY NOTES:</b> N/A                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                    |                               |                                   |                                                  |                                                   |
| <b>14. ABSTRACT</b><br>Traumatic Brain Injury (TBI), in particular mild TBI (mTBI) is a major cause of disability in military and in civilian populations, and for many years has been known to be an epigenetic risk factor for Alzheimer's Disease (AD) and other neurodegenerative conditions. However, the precise nature of how TBI leads to or precipitates AD pathogenesis is not understood. To address this problem, we have generated molecular profiles of AD and TBI pathogenesis in mouse models at a range of ages/timepoints post-injury respectively, in order to identify molecules and pathways that are common to both AD and TBI. Extensive datasets have been generated, and our comparison and integration of omic profiles clearly points to overlapping disruption of brain lipids and related protein signaling pathways in AD mice with age and TBI mice with time post injury. These include activation of lipid related leukotriene signaling, deficiencies in the eicosanoid signaling, dysfunctional PI3-kinase/Akt/mTOR/insulin and RXR/PPAR pathway. Lipids, particularly phospholipids are essential components of neuronal and glial cell membranes and axonal myelin, they modulate protein-protein interaction and cell signal transduction. Thus the TBI-induced pathobiology we have observed may precipitate development of AD and lower the threshold for onset of pathogenic mechanisms. We therefore now propose to validate these convergent AD and TBI mechanisms as novel targets to block the negative sequelae of TBI that lead to AD. |                    |                               |                                   |                                                  |                                                   |
| <b>15. SUBJECT TERMS:</b> AD, TBI, Pathology, Animal Models, Novel Therapeutic Targets                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                    |                               |                                   |                                                  |                                                   |
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| Unclassified                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Unclassified       | Unclassified                  | Unclassified                      | 43                                               |                                                   |

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## 1. INTRODUCTION:

In this project we plan to identify and provide preliminary validation of novel targets (eicosanoid signaling, dysfunctional PI3-kinase/Akt/mTOR/insulin and RXR/PPAR pathway) for the TBI-AD interrelationship discovered through our molecular level profiling. The goal here is target validation and provision of these therapeutic targets for future studies in drug discovery and preclinical efficacy; i.e. IND enabling studies of new therapeutic approaches to the AD sequelae of TBI.

## 2. KEYWORDS:

AD, TBI, Pathology, Animal Models, Novel Therapeutic Targets

## 3. ACCOMPLISHMENTS:

### What were the major goals of the project?

**Objective/Hypothesis:** Molecular pathways that are triggered in response to both TBI sequelae and AD pathogenesis represent novel molecular targets to mitigate the AD-neurodegenerative sequelae of TBI. From our interrogation of omic datasets of AD pathogenesis and TBI sequelae we have identified coincident lipid disturbances and related pathways which are potential targets for therapeutic intervention. Therefore we propose to first: **Aim 1:** Evaluate target engagement and efficacy in recovering normal molecular profiles in TBI mice using compounds that modulate the TBI and AD coincident molecular changes in a short-term treatment paradigm. **Aim 2:** In vivo validation of therapeutic targets in mice with TBI using chronic intervention with the top two performing compounds for two different targets from Aim 1 to evaluate the long term neurobehavioral, neuropathological and biochemical consequences.

**Major Task 1:** Validation of potential targets for therapeutic intervention in the pathogenic TBI-AD interrelationship - eicosanoid signaling, PI3-kinase/Akt/PTEN/mTOR/insulin signaling and RXR/PPAR pathway. [100% Complete]

**Subtask 1:** Obtaining ACURO approval (Roskamp Institute approval for TBI procedures is already in place, and will be amended to include the treatment paradigms). **Subtask 2:** Evaluation of bioactive lipids and downstream signaling in stored TBI and AD mouse model samples to identify targets from Leukotriene and Sphingomyelin signaling. **Subtask 3:** Administration of r-mTBI or r-sham injury to 180-220 male hTau mice (C57BL/6 humanized tau transgenics) at 2-3 months of age. **Subtask 4:** Administration of therapeutic compounds to mice for 2 weeks immediately prior to 3 month timepoint post TBI/sham. **Subtask 5:** Euthanasia of hTau mice at 3 months post-TBI/sham, followed by brain omic and antibody based analyses. **Subtask 6:** Validation of

identified targets in human TBI brains with history of repetitive concussions. **Subtask 7:** Evaluation and selection of therapeutics for each target for use in Task 2.

**Task One Deliverable:** Acute study validation of 6 coincident TBI and AD molecular targets and verification of up to eleven compounds as engaging those targets *in vivo*. *In task one, up to 110 hTau mice will receive the r-mTBI procedure, and up to 110 hTau mice will receive the r-sham procedure.*

**Major Task 2:** Chronic evaluation of the efficacy of three potential therapeutics against the pathogenic TBI-AD interrelationship. [40% Complete]

**Milestone Two:** Chronic evaluation of the efficacy of three potential therapeutics against the pathogenic TBI-AD interrelationship.

**Subtask 1:** Administration of r-mTBI or r-sham injury to 180 male hTau mice at 2-3 months of age (60 mice per group). **Subtask 2:** Administration of therapeutic compounds to hTau mice for 6 months beginning at 3 months post TBI/sham. **Subtask 3:** Administration of therapeutic compounds to hTau mice for 6 months beginning at 3 months post TBI/sham. **Subtask 4:** Neurobehavioral testing of hTau mice in the two weeks prior to 9 months post injury timepoint. **Subtask 5:** Euthanasia of hTau mice at 9 months post-TBI/sham, followed by further evaluation of outcomes.

**Subtask 5:** Data analysis and interpretation to identify most potent therapeutic approach.

**Task Two Deliverable:** Chronic study validation of top three candidate treatments that rescue TBI-dependent sequelae from convergence with AD pathogenic mechanisms. *In task two, 90 hTau mice will receive the r-mTBI procedure, and 90 hTau mice will receive the r-sham procedure.*

### **What was accomplished under these goals?**

*For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

### **Summary of accomplishments for each Aim.**

**Aim 1:** Validation of 6 potential targets for therapeutic intervention in the pathogenic TBI-AD interrelationship.

*We have completed target engagement and investigation of the therapeutic efficacy (i.e. ability to ameliorate glial reactivity, axonal and white matter changes in our TBI model) of 5 of our potential molecular targets.*

*Given the important role of neuronal and glial cells in TBI pathophysiology, we also included as part of this Aim an additional in vitro screening platform for our compounds in injured astrocytes, and microglia cell lines and primary cultures. We developed a timeline of pathological profiles of inflammatory markers in these cell culture models. We used this platform to screen the efficacy of our top performing compounds, utilizing this as an additional validation step in the selection of*

*some of our lower tier lists of top performing compounds prior to initiating our long term treatment studies in Aim 2. These studies have been conducted with some of our top performing compounds, using two cell lines to detail drug induced effects following injury to the cell lines. The top two drug candidates have now progressed to Aim two for the long-term treatment study.*

**Aim 2:** Chronic evaluation of the efficacy of two top performing therapeutics against the pathogenic TBI-AD interrelationship.

For the second aim of this project, we have initiated a chronic treatment and post-injury timepoint study using our top two performing compounds, we have selected Pioglitazone and Zileuton as the best performing compounds based on our in vivo/in vitro work.

120 mice for the chronic pioglitazone/Zileuton study (using two doses) have been aged until 3 months old, and received their injuries. Half of the cohort have begun to receive their 3 month daily treatment (beginning at 3 months post-injury to 6 month post-injury). This cohort is half way through the treatment regimen. The remaining half of the cohort will begin their treatment in two months time. Prior to euthanasia we will conduct behavioral analyses for cognitive function, sensori-motor behavior, and tests for dis-inhibitory behavior. After euthanasia brain tissue will be collected for molecular and pathological studies. 6 Groups [r-mTBI treated dose 1 and 2; r-mTBI vehicle; r-sham treated dose 1 and 2; r-sham control] each with 10 mice per group. We anticipate that these studies will be completed in May 2020.

#### SUBTASK DESCRIPTIONS FOR MAJOR TASK 1

**Subtask 1: Obtained ACURO approval (completed in March 20<sup>th</sup>, 2018)**

**Subtask 2: Evaluation of bioactive lipids and downstream signaling in stored TBI and AD mouse model samples to identify proposed targets from Leukotriene and Sphingomyelin signaling. [Completed]**

**Subtask 3: Administration of r-mTBI or r-sham injury to 180-220 male C57BL6/J mice at 2-3 months of age. [Completed]**

**Subtask 4: Administration of therapeutic compounds to C57BL6/J mice for 2 weeks immediately prior to 2 month timepoint post-TBI/sham. [Completed]**

Subtask 4a-d: Administration of therapeutic compounds to target (eicosanoid signaling, PI3-kinase/Akt/PTEN/mTOR/insulin signaling and RXR/PPAR pathway)

Subtask 4e: Target exploration of downstream eicosanoid pathway and administration of therapeutic compounds to target leukotriene signaling

Subtask 4f: Target exploration of downstream sphingomyelin pathway and administration of therapeutic compounds to target sphingomyelin signaling

**Subtask 5: Euthanasia of hTau mice at 3 months post-TBI/sham, followed by brain omic and antibody based analyses. [Completed]**

**Subtask 6: Validation of identified targets in human TBI brains with history of repetitive concussions. [Completed]**

**Subtask 7: Evaluation and selection of therapeutics for each target for use in Task 2. [Completed]**

**MAJOR TASK 1 DELIVERABLE:** Acute study validation of coincident TBI and AD molecular targets and verification of up to 8 compounds as engaging those targets *in vivo*.  
Selection of Top 3 performing compounds for study in MAJOR TASK 2.

#### SUBTASK DESCRIPTIONS FOR MAJOR TASK 2

***Subtask 1: Administration of r-mTBI or r-sham injury to 180 male hTau mice at 2-3 months of age (60 mice per group) - [Completed]***

Animals will receive closed head injuries over a 1 month period.

For each of 3 potential therapeutics there will be 6 groups– [r-mTBI treated low dose; r-mTBI treated high dose; r-mTBI vehicle; r-sham treated low dose; r-sham treated high dose; r-sham vehicle] each with 10 hTau mice

***Subtask 2: Administration of therapeutic compounds to hTau mice for 3 months beginning at 3 months post TBI/sham - (50% Completion in March 2021)***

Subtask 2a: Administration of Top performing therapeutic compound I

Subtask 2b: Administration of Top performing therapeutic compound II

Subtask 2c: Administration of Top performing therapeutic compound III

***Subtask 3: Neurobehavioral testing of hTau mice in the two weeks prior to 6 months post injury timepoint - (0% Completion in March 2021)***

At the last two weeks prior to the 6 month post-injury time-point, animals will undergo neurobehavioral testing in the Barnes maze for 7 days, followed by rotarod test , open field and elevated plus maze tests.

***Subtask 4: Euthanasia of hTau mice at 6 months post-TBI/sham, followed by further evaluation of outcomes - (0% Completion in May-June 2021)***

Subtask 4a: Brain neuropathological analyses

Subtask 4b: Molecular Omics and antibody based analyses

***Subtask 5: Data analysis and interpretation to identify most potent therapeutic approach (Completion in June 2020)***

**MAJOR TASK 2 DELIVERABLE:** Chronic study validation of top three candidate treatments that rescue TBI-dependent sequelae from convergence with AD pathogenic mechanisms.

## **BELOW IS A SUMMARY OF THE MAIN FINDINGS FROM OUR STUDIES THIS YEAR**

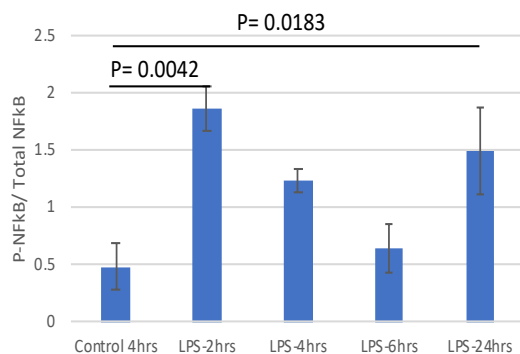
### **Summary – first quarter**

1. Cell culture exposure to various LPS concentrations over a 24 hour timeframe
  - IMG microglia
  - SH-SY-5Y Neurons
  - C8-D1A Astrocytes
2. In vitro investigation of the effect of PTEN and mTOR inhibition following LPS exposure at 6 & 24 hours
3. Behavioural data analysis of Cre-lox PTEN sham vs TBI mice
  - Open Field test
  - Elevated plus maze

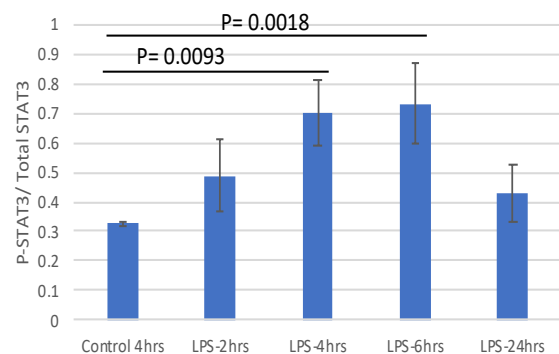
### **Methodology**

- Cells were grown to 85-90% confluency in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) (C8-D1A astrocytes grown in just DMEM), cells cultured at 37C and 5%CO2
- Cells were exposed to Various concentrations of LPS for 2/4/6 & 24 hours (IMG-microglia 10ng/ml) (C8-D1A-Astrocytes 50ng/ml) (SH-SY-5Y neurons 50ng/ml).
- Cells were Lysed using Mammalian protein extraction reagent (MPER) supplemented with Protease and Phosphatase inhibitors.
- Protein concentration was assessed by (BCA) assay, 15ug of protein was then loaded onto a Bis-tris polyacrylamide gel electrophoresis (PAGE) using a 4-20% gradient gel. Protein was then transferred to a PVDF membrane overnight at 90mA.

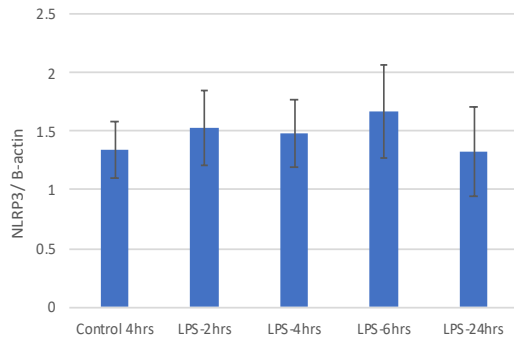
### **IMG microglia- In vitro Inflammatory characteristics and response to LPS data**



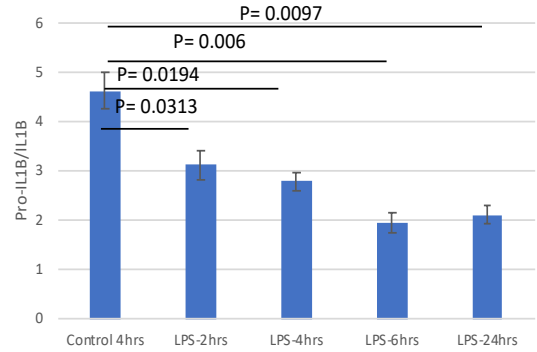
A graph showing the protein expression of Phospho- NFkB (Serine 536) in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to Total NFkB and to B-actin. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @10ng/ml for 2 hours (n=3) P= 0.0042. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @10ng/ml for 24 hours (n=3) P= 0.0183. Error bars represent SD.



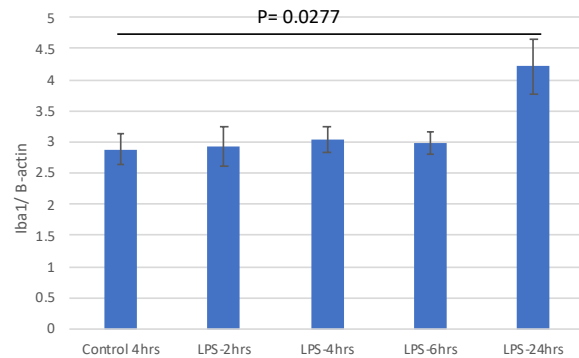
A graph showing the protein expression of Phospho- STAT3 (Tyrosine 705) in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to Total NFkB and to B-actin. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @10ng/ml for 4 hours (n=3) P= 0.00932. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @10ng/ml for 24 hours (n=3) P= 0.0018. Error bars represent SD.



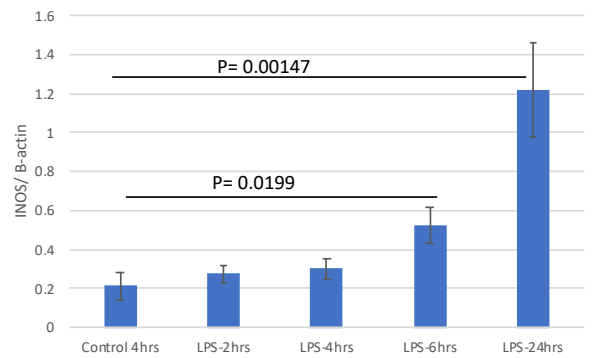
A graph showing the protein expression of NLRP3 in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to B-actin. Error bars represent SD.



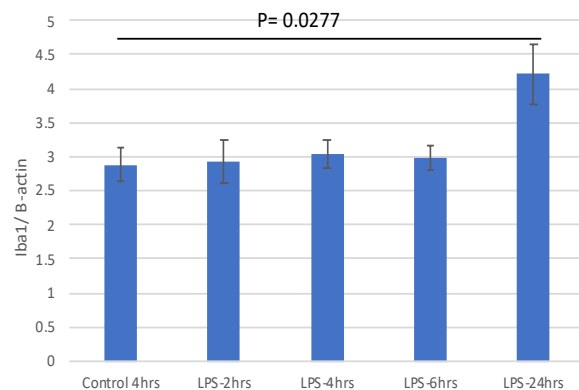
A graph showing the protein expression ratio of Pro-IL1B to mature IL1B in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to B-actin. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @10ng/ml for 2 hours (n=3) P= 0.0313. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @10ng/ml for 4 hours (n=3) P= 0.0194. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @10ng/ml for 6 hours (n=3) P= 0.006. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @10ng/ml for 24 hours (n=3) P= 0.0097. Error bars represent SD.



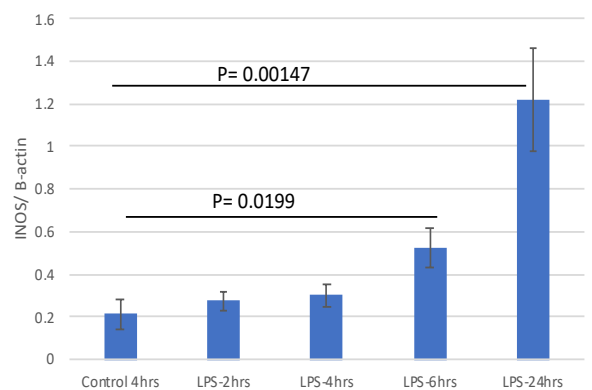
A graph showing the protein expression of Iba1 in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to B-actin. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @10ng/ml for 24 hours (n=3) P= 0.0277. Error bars represent SD.



A graph showing the protein expression of Inducible Nitric oxide synthase (iNOS) in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to B-actin. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @10ng/ml for 2 hours (n=3) P= 0.0042. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @10ng/ml for 24 hours (n=3) P= 0.0183. Error bars represent SD.

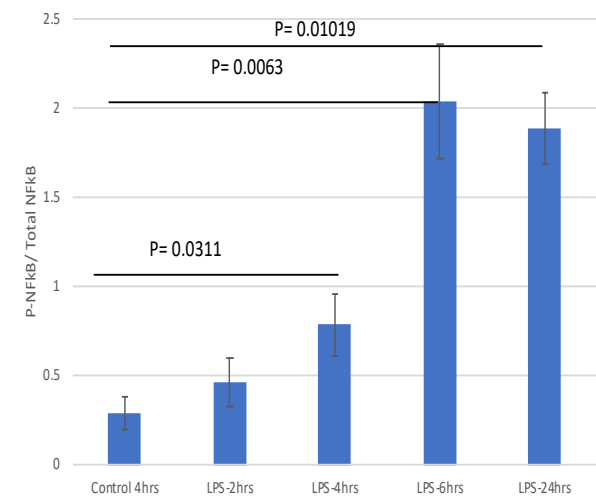


A graph showing the protein expression of Iba1 in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to B-actin. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @10ng/ml for 24 hours (n=3) P= 0.0277. Error bars represent SD.

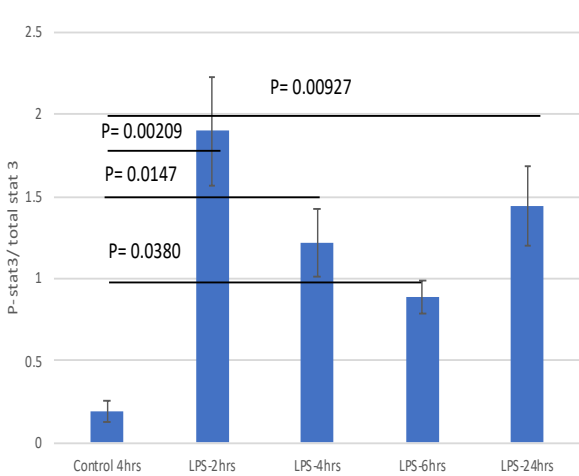


A graph showing the protein expression of Inducible Nitric oxide synthase (iNOS) in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to B-actin. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @10ng/ml for 2 hours (n=3) P= 0.0042. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @10ng/ml for 24 hours (n=3) P= 0.0183. Error bars represent SD.

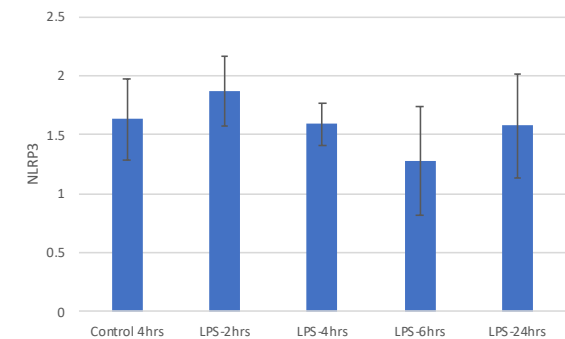
SH-SY-5Y neuroblastoma cells- In vitro Inflammatory characteristics and response to LPS data



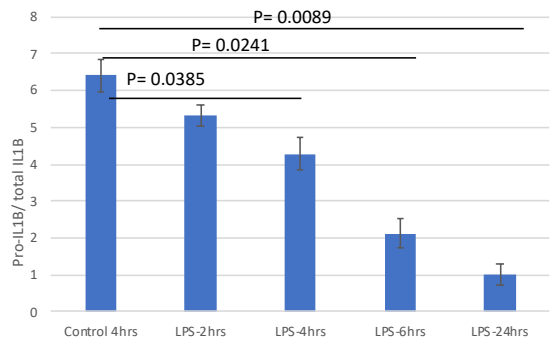
A graph showing the protein expression of Phospho- NfκB (Serine 536) in the cytoplasmic/ nuclear fraction of cell lysate from SH-SY-5Y neuroblastoma cells (n=3/group), data were normalized to Total NfκB and to B-actin. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 4 hours (n=3) P= 0.0311. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 6 hours (n=3) P= 0.0063. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 24 hours (n=3) P= 0.01019. Error bars represent SD.



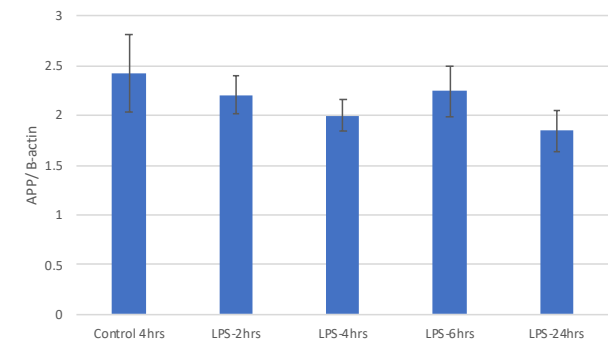
A graph showing the protein expression of Phospho- STAT3 (Tyrosine 705) in the cytoplasmic/ nuclear fraction of cell lysate from SH-SY-5Y neuroblastoma cells (n=3/group), data were normalized to Total STAT3 and to B-actin. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 2 hours (n=3) P= 0.00209. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 4 hours (n=3) P= 0.0147. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 6 hours (n=3) P= 0.0380. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 24 hours (n=3) P= 0.00927. Error bars represent SD.



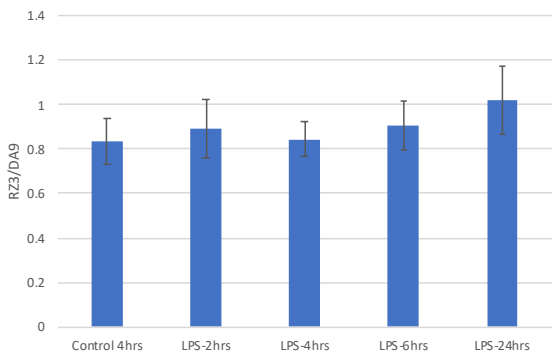
A graph showing the protein expression of NLRP3 in the cytoplasmic/ nuclear fraction of cell lysate from SH-SY-5Y neuroblastoma cells (n=3/group), data were normalized to B-actin. Error bars represent SD.



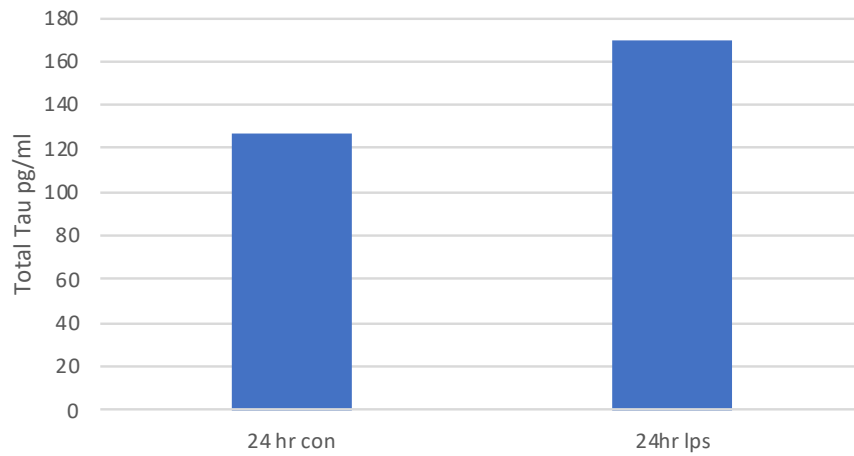
A graph showing the protein expression ratio of Pro-IL18 to mature IL18 in the cytoplasmic/ nuclear fraction of cell lysate from SH-SY-5Y neuroblastoma cells (n=3/group), data were normalized to B-actin. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 4 hours (n=3) P= 0.0385. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 6 hours (n=3) P= 0.0241. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 24 hours (n=3) P= 0.0089. Error bars represent SD.



A graph showing the protein expression of Amyloid Precursor Protein (APP) in the cytoplasmic/ nuclear fraction of cell lysate from SH-SY-5Y neuroblastoma cells (n=3/group), data were normalized to B-actin. Error bars represent SD.

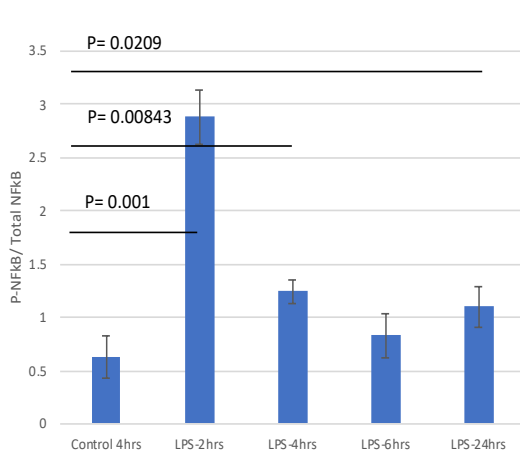


A graph showing the protein expression of R23 phospho Tau (Thr231) in the cytoplasmic/ nuclear fraction of cell lysate from SH-SY-5Y neuroblastoma cells (n=3/group), data were normalized to Total tau (DA9) and to B-actin. Error bars represent SD.

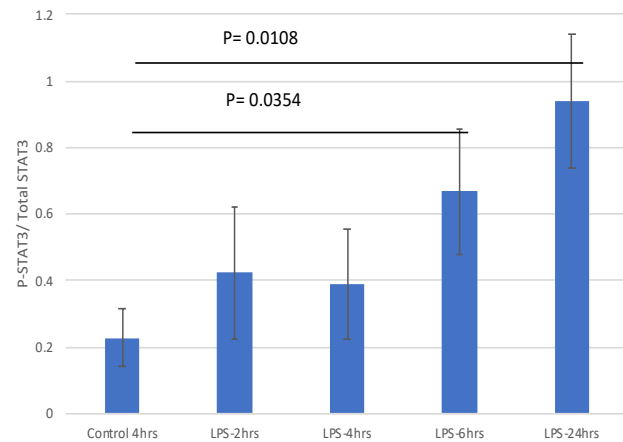


A graph showing the amount of tau secreted into cell culture media by SH-SY-5Y neuroblastoma cells after 24 hours of exposure to 50ng/ml of LPS.

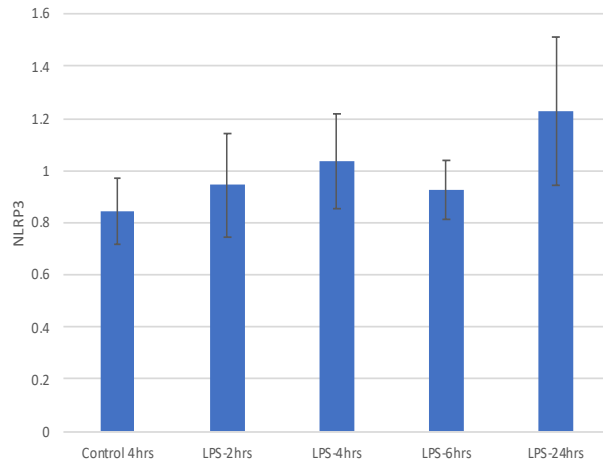
#### Astrocytes C8-D1A- In vitro Inflammatory characteristics and response to LPS data



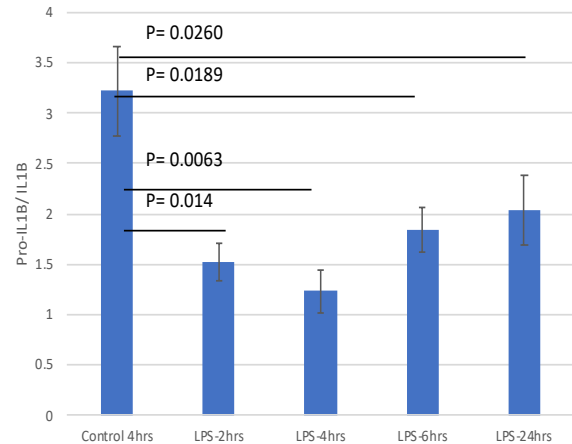
A graph showing the protein expression of Phospho- NFkB (Serine 536) in the cytoplasmic/ nuclear fraction of cell lysate from C8-D1A immortalised astrocyte cells (n=3/group), data were normalized to Total NFkB and to B-actin. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 2 hours (n=3) P= 0.001. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 4 hours (n=3) P= 0.00843. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 24 hours (n=3) P= 0.0209. Error bars represent SD.



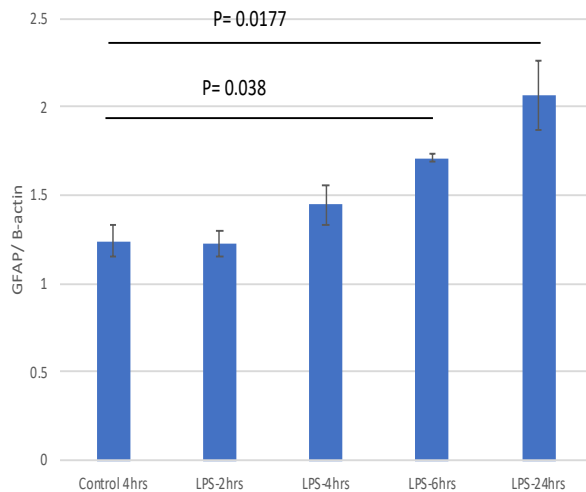
A graph showing the protein expression of Phospho- STAT3 (Tyrosine 705) in the cytoplasmic/ nuclear fraction of cell lysate from C8-D1A immortalised astrocyte cells (n=3/group), data were normalized to Total STAT3 and to B-actin. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 6 hours (n=3) P= 0.0354. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 24 hours (n=3) P= 0.0108. Error bars represent SD.



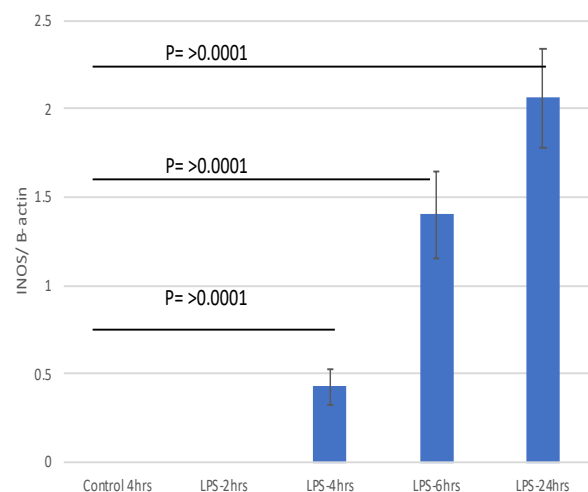
A graph showing the protein expression of NLRP3 in the cytoplasmic/ nuclear fraction of cell lysate from C8-D1A immortalised astrocyte cells (n=3/group), data were normalized to B-actin. Error bars represent SD.



A graph showing the protein expression ratio of Pro-IL1B to mature IL1B in the cytoplasmic/ nuclear fraction of cell lysate from C8-D1A immortalised astrocyte cells (n=3/group), data were normalized to B-actin. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 2 hours (n=3) P= 0.014. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 4 hours (n=3) P= 0.0063. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 6 hours (n=3) P= 0.0189. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 24 hours (n=3) P= 0.0260. Error bars represent SD.



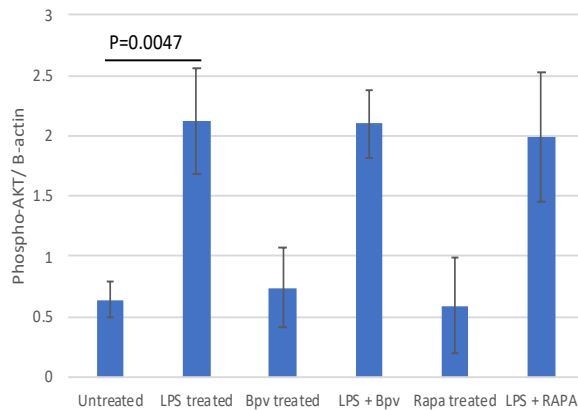
A graph showing the protein expression of GFAP in the cytoplasmic/ nuclear fraction of cell lysate from C8-D1A immortalised astrocyte cells (n=3/group), data were normalized to B-actin. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 6 hours (n=3) P= 0.038. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 24 hours (n=3) P= 0.0177. Error bars represent SD.



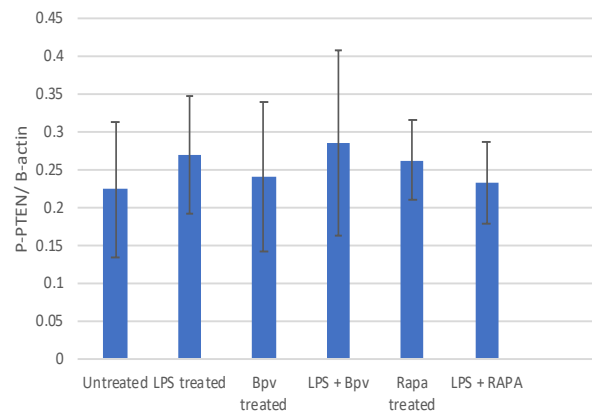
A graph showing the protein expression of Inducible Nitric oxide synthase (iNOS) in the cytoplasmic/ nuclear fraction of cell lysate from C8-D1A immortalised astrocyte cells (n=3/group), data were normalized to B-actin. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @10ng/ml for 4 hours (n=3) P= >0.0001. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 6 hours (n=3) P= >0.0001. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS @50ng/ml for 24 hours (n=3) P= >0.0001. Error bars represent SD.

## **PTEN and mTOR inhibition study**

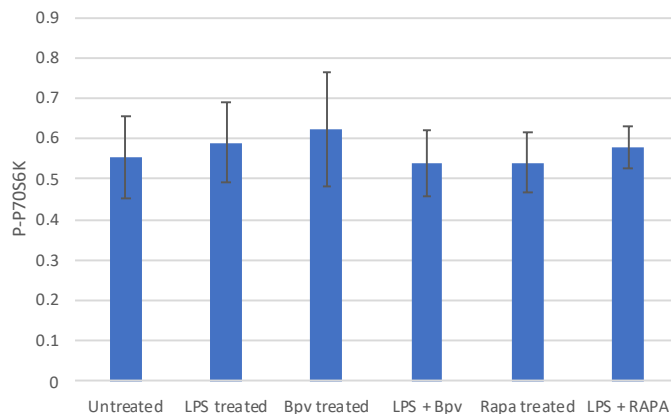
- IMG - Immortalized microglial cells were grown to 85-90% confluency in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) cells cultured at 37C and 5%CO<sub>2</sub>.
- Cells were exposed to 10ng/ml LPS & alone or in combination with 100nm Bis-Peroxyvanadium or 250nm Rapamycin for 6 or 24 hours
- Cells were Lysed using Mammalian protein extraction reagent (MPER) supplemented with Protease and Phosphatase inhibitors.
- Protein concentration was assessed by (BCA) assay, 15ug of protein was then loaded onto a Bis-tris polyacrylamide gel electrophoresis (PAGE) using a 4-20% gradient gel. Protein was then transferred to a PVDF membrane overnight at 90mA.



A graph showing the protein expression of Phospho-Akt (Serine 473) in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to Total Akt and to B-actin. Cells treated with standard media @ 4 hours (n=3) vs Cells treated with LPS alone @10ng/ml for 6 hours (n=3) P= 0.0047. Error bars represent SD.



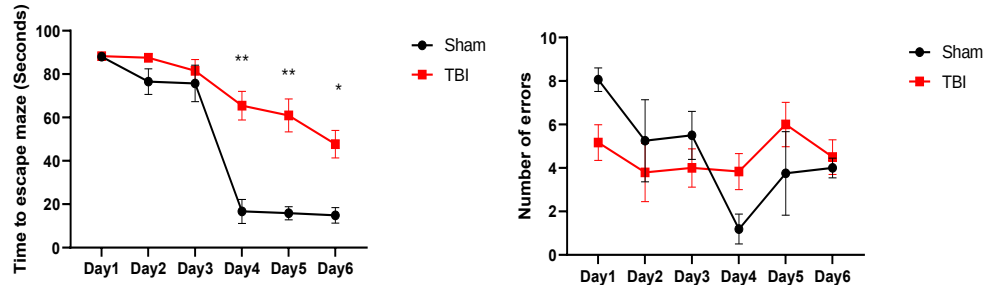
A graph showing the protein expression of Phospho-PTEN (Serine 380) in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to Total PTEN and to B-actin.. Error bars represent SD.



A graph showing the protein expression of Phospho-P70 S6 kinase (Threonine 389) in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to Total PTEN and to B-actin. Error bars represent SD.

Cre\_CX3CR1-PTEN\_Flox (K.O hetero) mice – exposed to injuries for one month (5/week 20 total) and administered tetracycline analogue beginning immediately after injury for 2 weeks post last injury.

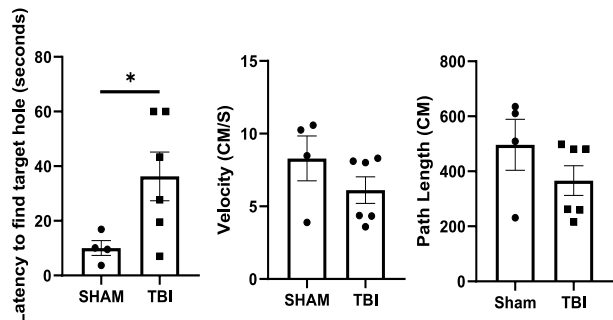
#### Cre-PTEN behavior data- Barnes maze - Acquisition



Left graph - Injured PTEN-Cx3cr1-KO (hetero) mice perform worse than shams in latency to platform

Right graph – No major changes between injured vs sham PTEN-Cx3cr1-KO (hetero) mice in no of error (except on day 4 of the acquisition training)

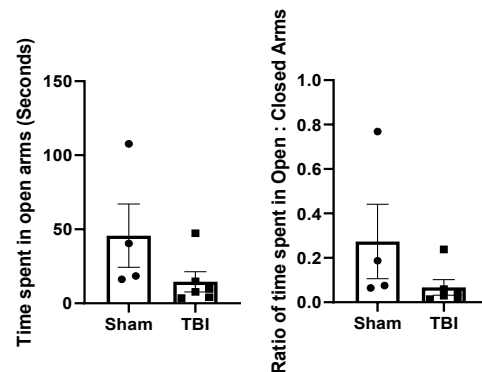
#### Cre-PTEN behavior data- Barnes maze – Probe



Path length and velocity of Injured vs sham PTEN-Cx3cr1-KO (hetero) mice showed no significant change on the probe trial on day 7.

Latency to platform was significantly higher for injured PTEN-Cx3cr1-KO (hetero) mice compared to their sham counterparts in the probe trial on day 7.

#### Cre-PTEN behavior data-Elevated plus maze



Elevated plus maze – 5 minutes testing  
Data depict the time spent in the open arm, and the ratio of time spent in the open/closed arm showing a trend towards decrease in injured PTEN-Cx3cr1-KO (hetero) mice compared to their sham counterparts.

### Conclusion

We have shown a comprehensive timeframe of inflammatory and neurodegenerative markers in three cell lines for our planned *in vitro* compound screening.

In the next series of experiments we expanded our profiling to 15 – 4hrs timepoint where we anticipate most changes are occurring. From these studies our goal was screen our compounds at the most relevant optimal timepoint.

Our PTEN study *in vitro* and *in vivo* showed very minimal effect on rescuing inflammatory *in vitro* toxicity or TBI response *in vivo*.

## Summary – Second quarter

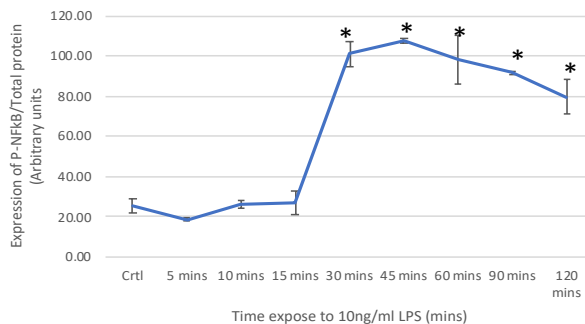
Cell culture exposure to various LPS concentrations over a **15mins to 4hrs**

- IMG microglia and Primary sham/TBI microglia
- C8-D1A Astrocytes and Primary sham/TBI microglia

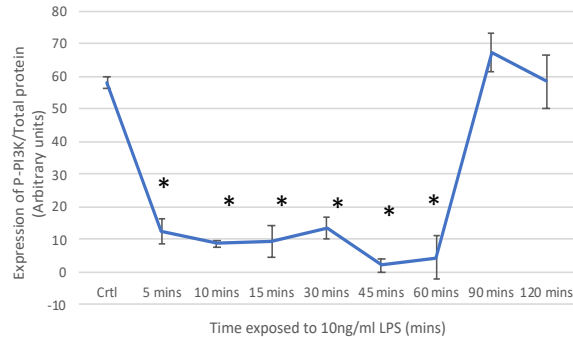
## Methodology

- Cells were grown to 85-90% confluency in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) (C8-D1A astrocytes grown in just DMEM), cells cultured at 37C and 5%CO2
- Cells were exposed to LPS for 15mins to 4hrs (IMG-microglia 10ng/ml) (C8-D1A- Astrocytes 50ng/ml).
- Cells were Lysed using Mammalian protein extraction reagent (MPER) supplemented with Protease and Phosphatase inhibitors.
- Protein concentration was assessed by (BCA) assay, 15ug of protein was then loaded onto a Bis-tris polyacrylamide gel electrophoresis (PAGE) using a 4-20% gradient gel. Protein was then transferred to a PVDF membrane overnight at 90mA.

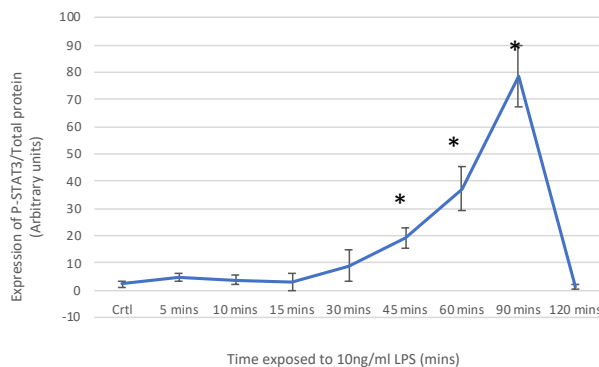
## IMG microglia- In vitro Inflammatory characteristics and response to LPS data



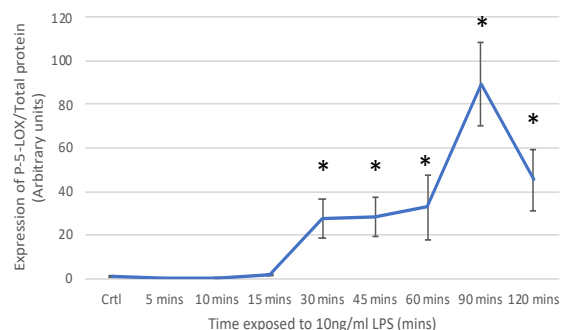
A graph showing the protein expression of Phospho- NFκB (Serine 536) in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to total Protein. Cells treated with either control DMEM/F12 media or media containing LPS @10ng/ml for varying lengths of time. Data were analyzed via students T- test significance of treated time point vs control is shown by \*. Control vs 30 mins P=<0.001. Control vs 45 mins P=<0.001. Control vs 60 mins P=0.0044. Control vs 90 mins P=0.0051. Control vs 120 mins P=0.0079. Error bars represent SEM.



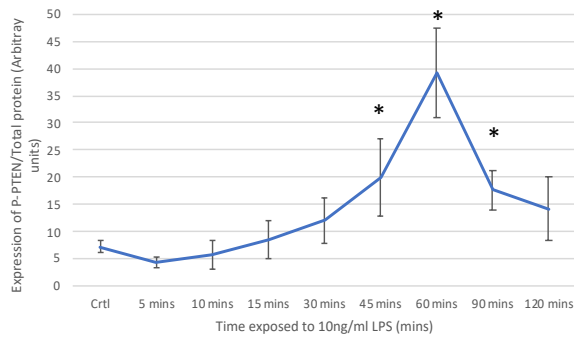
A graph showing the protein expression of Phospho-PI3K (Tyrosine 199) P55 in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to total Protein. Cells treated with either control DMEM/F12 media or media containing LPS @10ng/ml for varying lengths of time. Data were analyzed via students T- test significance of treated time point vs control is shown by \*. Control vs 5 mins P=0.0067. Control vs 10 mins P=<0.001. Control vs 15 mins P=0.0048. Control vs 30 mins P=0.0053. Control vs 45 mins P=<0.001. Control vs 60 mins P=0.0049. Error bars represent SEM.



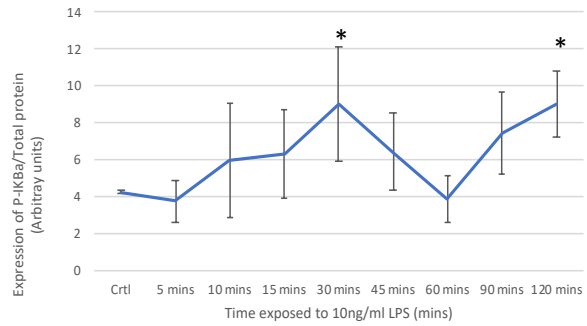
A graph showing the protein expression of Phospho-STAT3 (Tyrosine 705) in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to total Protein. Cells treated with either control DMEM/F12 media or media containing LPS @10ng/ml for varying lengths of time. Data were analyzed via two-tailed students T- test significance of treated time point vs control is shown by \*. Control vs 45 mins P=0.02114. Control vs 60 mins P=0.0093. Control vs 90 mins P=0.004. Error bars represent SEM.



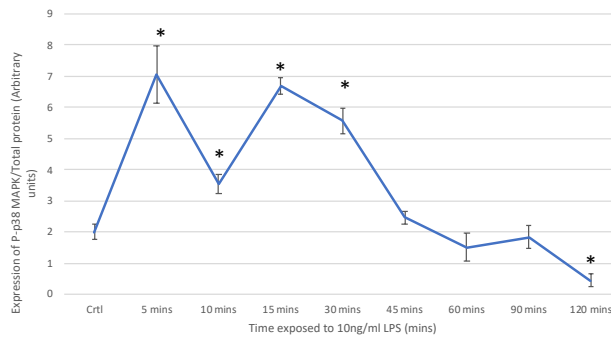
A graph showing the protein expression of Phospho-5-LOX (Serine 663) in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to total Protein. Cells treated with either control DMEM/F12 media or media containing LPS @10ng/ml for varying lengths of time. Data were analyzed via students T-test significance of treated time point vs control is shown by \*. Control vs 30 mins P=<0.001. Control vs 45 mins P=<0.001. Control vs 60 mins P=0.017. Control vs 90 mins P=<0.001. Control vs 120 mins P=<0.001. Error bars represent SEM.



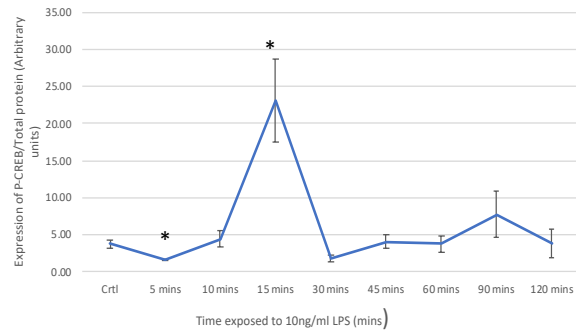
A graph showing the protein expression of Phospho-P-TEN (Serine 380) in the cytoplasmic/nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to total Protein. Cells treated with either control DMEM/F12 media or media containing LPS @10ng/ml for varying lengths of time. Data were analyzed via students T-test significance of treated time point vs control is shown by \*. Control vs 45 mins P=0.0411. Control vs 60 mins P=0.010. Control vs 90 mins P=0.0239. Error bars represent SEM.



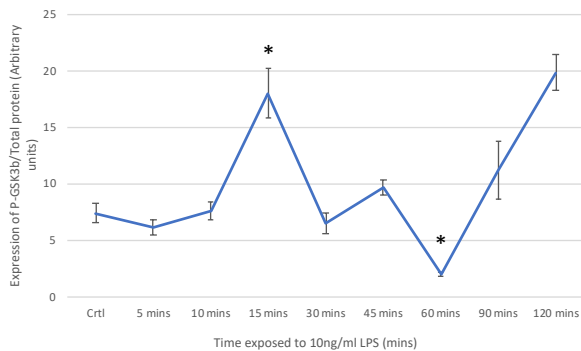
A graph showing the protein expression of Phospho-IKBa (Serine 32) in the cytoplasmic/nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to total Protein. Cells treated with either control DMEM/F12 media or media containing LPS @10ng/ml for varying lengths of time. Data were analyzed via students T-test significance of treated time point vs control is shown by \*. Control vs 30 mins P=0.0373. Control vs 120 mins P=0.0211. Error bars represent SEM.



A graph showing the protein expression of Phospho-p38 MAPK (Threonine 180/Tyrosine 182) in the cytoplasmic/nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to total Protein. Cells treated with either control DMEM/F12 media or media containing LPS @10ng/ml for varying lengths of time. Data were analyzed via students T-test significance of treated time point vs control is shown by \*. Control vs 5 mins P=0.0062. Control vs 10 mins P=0.0154. Control vs 15 mins P<0.001. Control vs 30 mins P=0.0017. Control vs 120 mins P=0.008. Error bars represent SEM.

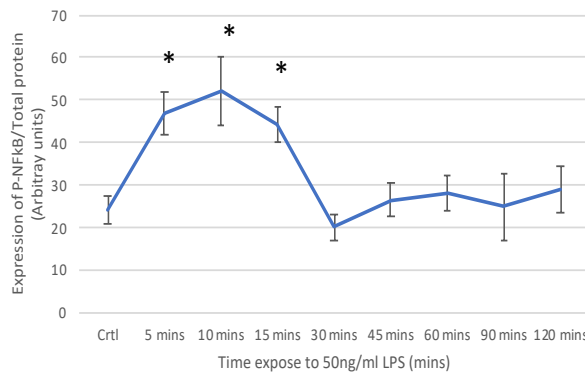


A graph showing the protein expression of Phospho-CREB (Serine 133) in the cytoplasmic/nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to total Protein. Cells treated with either control DMEM/F12 media or media containing LPS @10ng/ml for varying lengths of time. Data were analyzed via students T-test significance of treated time point vs control is shown by \*. Control vs 5 mins P=0.0215. Control vs 15 mins P=0.0266. Error bars represent SEM.

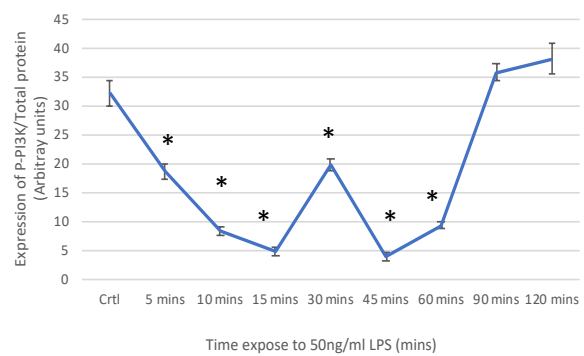


A graph showing the protein expression of Phospho-GSK3b (Serine 9) in the cytoplasmic/nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to total Protein. Cells treated with either control DMEM/F12 media or media containing LPS @10ng/ml for varying lengths of time. Data were analyzed via students T-test significance of treated time point vs control is shown by \*. Control vs 15 mins P=0.0112. Control vs 60 mins P=0.00296. Error bars represent SEM.

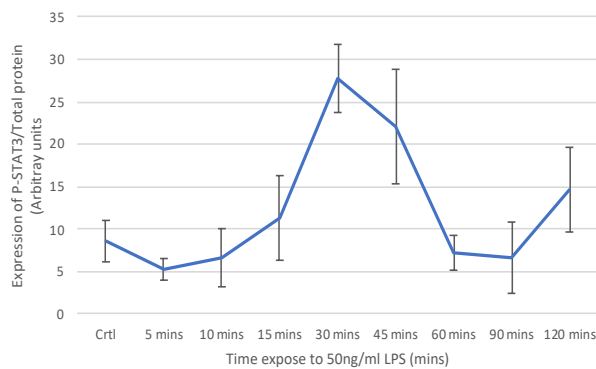
## C8-D1A- In vitro Inflammatory characteristics and response to LPS data



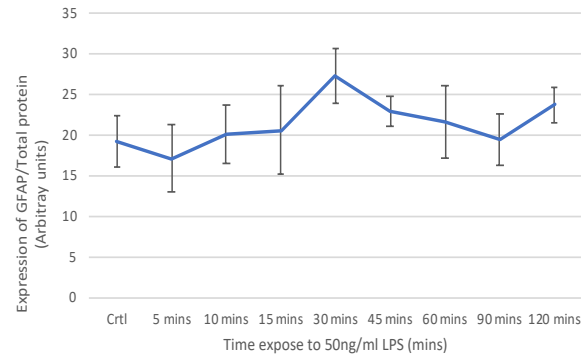
A graph showing the protein expression of Phospho- NFkB (Serine 536) in the cytoplasmic/ nuclear fraction of cell lysate from C8D1-A immortalized Astrocytes cells (n=3/group), data were normalized to total Protein. Cells treated with either control DMEM/F12 media or media containing LPS @50ng/ml for varying lengths of time. Data were analyzed via students T- test significance of treated time point vs control is shown by \*. Control vs 5 mins P=0.0182. Control vs 10 mins P=0.0049. Control vs 15 mins P=0.0020. Error bars represent SEM.



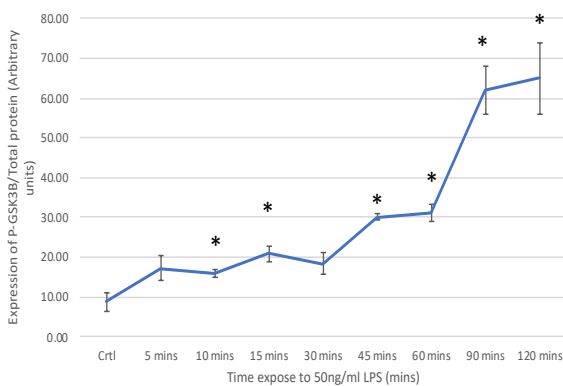
A graph showing the protein expression of Phospho-PI3K (Tyrosine 199) P55 in the cytoplasmic/ nuclear fraction of cell lysate from C8D1-A immortalized Astrocytes cells (n=3/group), data were normalized to total Protein. Cells treated with either control DMEM/F12 media or media containing LPS @50ng/ml for varying lengths of time. Data were analyzed via students T- test significance of treated time point vs control is shown by \*. Control vs 5 mins P=0.0043. Control vs 10 mins P<0.001. Control vs 15 mins P<0.001. Control vs 30 mins P<0.001. Control vs 45 mins P<0.001. Control vs 60 mins P<0.001. Error bars represent SEM.



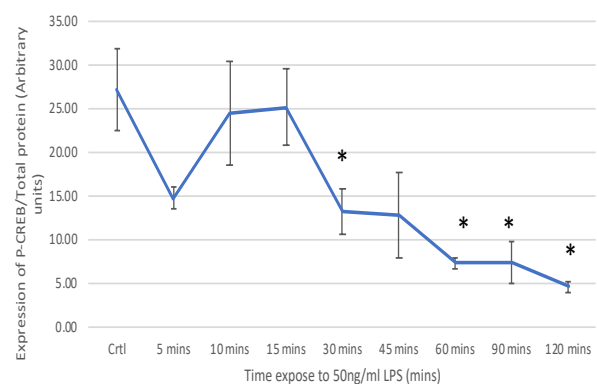
A graph showing the protein expression of Phospho-STAT3 (Tyrosine 705) in the cytoplasmic/ nuclear fraction of cell lysate from C8D1-A immortalized Astrocytes cells (n=3/group), data were normalized to total Protein. Cells treated with either control DMEM/F12 media or media containing LPS @50ng/ml for varying lengths of time. Data were analyzed via two-tailed students T- test significance of treated time point vs control is shown by \*. Control vs 30 mins P=0.0198. Control vs 45 mins P=0.0305. Error bars represent SEM.



A graph showing the protein expression of GFAP in the cytoplasmic/ nuclear fraction of cell lysate from C8D1-A immortalized Astrocytes cells (n=3/group), data were normalized to total Protein. Cells treated with either control DMEM/F12 media or media containing LPS @50ng/ml for varying lengths of time. Data were analyzed via students T- test significance of treated time point vs control is shown by \*. Control vs 30 mins P=0.0392. Error bars represent SEM.



A graph showing the protein expression of Phospho-GSK3b (Serine 9) in the cytoplasmic/ nuclear fraction of cell lysate from C8D1-A immortalized Astrocytes cells (n=3/group), data were normalized to total Protein. Cells treated with either control DMEM/F12 media or media containing LPS @50ng/ml for varying lengths of time. Data were analyzed via two-tailed students T- test significance of treated time point vs control is shown by \*. Control vs 10 mins P=0.042. Control vs 15 mins P=0.0172. Control vs 45 mins P<0.001. Control vs 60 mins P=0.0017. Control vs 90 mins P=0.0012. Control vs 120 mins P=0.0037. Error bars represent SEM.

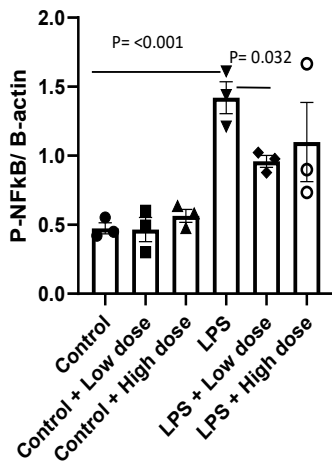


A graph showing the protein expression of Phospho-CREB (Serine 133) in the cytoplasmic/ nuclear fraction of cell lysate from C8D1-A immortalized Astrocytes cells (n=3/group), data were normalized to total Protein. Cells treated with either control DMEM/F12 media or media containing LPS @50ng/ml for varying lengths of time. Data were analyzed via two-tailed students T- test significance of treated time point vs control is shown by \*. Control vs 30 mins P=0.05. Control vs 60 mins P=0.01322. Control vs 90 mins P=0.0197. Control vs 120 mins P=0.0085. Error bars represent SEM.

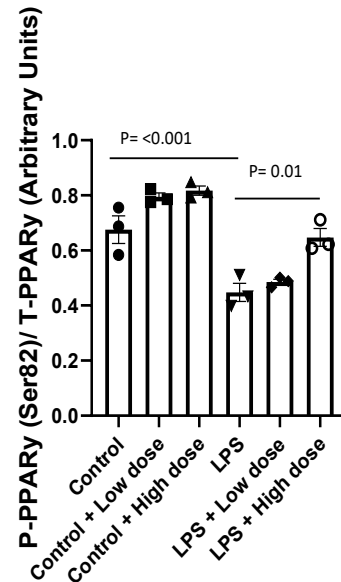
### Compound screening study at optimal timepoint

IMG - Immortalised microglial cells and C8-D1A astrocytes were grown to 85-90% confluency in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) or DMEM (for astrocytes), with cells cultured at 37°C and 5%CO<sub>2</sub>.

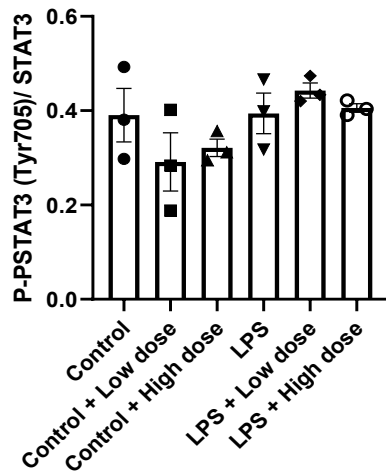
- Cells were treated with several compounds for 24hrs with/without LPS (long-term treatment) or for 30 mins prior to LPS exposure which lasted for 15mins to 45hrs (short-term treatment) (IMG-microglia 10ng/ml) (C8-D1A-Astrocytes 50ng/ml). *Here we first present the long-term treatment study.*
- Cells were Lysed using Mammalian protein extraction reagent (MPER) supplemented with Protease and Phosphatase inhibitors.
- Protein concentration was assessed by (BCA) assay, 15ug of protein was then loaded onto a Bis-tris polyacrylamide gel electrophoresis (PAGE) using a 4-20% gradient gel. Protein was then transferred to a PVDF membrane overnight at 90mA.



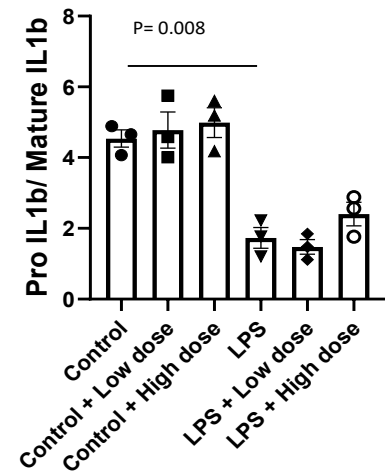
A graph showing the protein expression of Phospho-NFkB (Serine 536) in the cytoplasmic/nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to Total NFkB and to B-actin. Cells treated with either control DMEM/F12 media or media containing LPS @10ng/ml and Pioglitazone @ 2.5uM (Low dose) or 10uM (High dose) for 24 hours. Data were analysed via students T-test. Control vs LPS treated P<0.001. LPS vs LPS + 2.5uM Pioglitazone treated P=0.32. Error bars represent SEM.



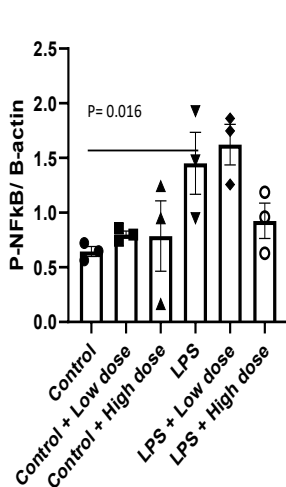
A graph showing the protein expression of Phospho-PPARy (Serine 82) in the cytoplasmic/nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to Total NFkB and to B-actin. Cells treated with either control DMEM/F12 media or media containing LPS @10ng/ml and Pioglitazone @ 2.5uM (Low dose) or 10uM (High dose) for 24 hours. Data were analysed via students T-test. Control vs LPS treated P<0.001. LPS vs LPS + 10uM Pioglitazone treated P=0.01. Error bars represent SEM.



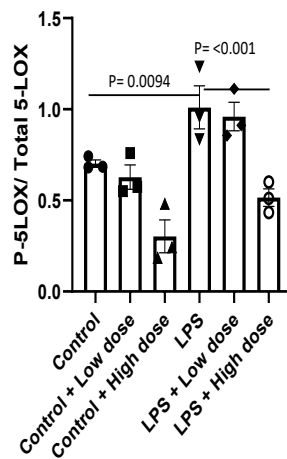
A graph showing the protein expression of Phospho- STAT3 (Tyrosine 705) in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to Total NFkB and to B-actin. Cells treated with either control DMEM/F12 media or media containing LPS @10ng/ml and Pioglitazone @ 2.5uM (Low dose) or 10uM (High dose) for 24 hours. Data were analysed via students T-test, no significance was found. Error bars represent SEM.



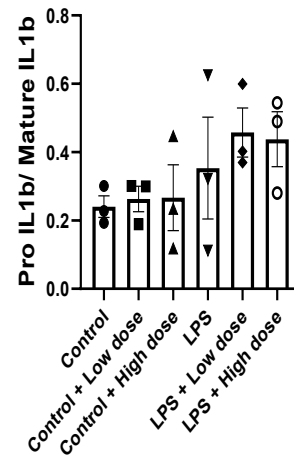
A graph showing the ratio of protein expression of Pro-IL1b against Mature IL1b in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to Total NFkB and to B-actin. Cells treated with either control DMEM/F12 media or media containing LPS @10ng/ml and Pioglitazone @ 2.5uM (Low dose) or 10uM (High dose) for 24 hours. Data were analysed via students T-test Control vs LPS treated P=0.008. Error bars represent SEM.



A graph showing the protein expression of Phospho- NFkB (Serine 536) in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to Total NFkB and to B-actin. Cells treated with either control DMEM/F12 media or media containing LPS @10ng/ml and Zileuton @ 25uM (Low dose) or 100uM (High dose) for 24 hours. Data were analysed via students T-test. Control vs LPS treated P=0.016. Error bars represent SEM.



A graph showing the protein expression of Phospho- 5-LOX (Serine 663) in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to Total NFkB and to B-actin. Cells treated with either control DMEM/F12 media or media containing LPS @10ng/ml and Zileuton @ 25uM (Low dose) or 100uM (High dose) for 24 hours. Data were analysed via students T-test. LPS vs LPS + 100um Zileuton P<0.001. Control vs LPS treated P=0.0094 Error bars represent SEM.



A graph showing the ratio of protein expression of Pro-IL1b against Mature IL1b in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglial cells (n=3/group), data were normalized to Total NFkB and to B-actin. Cells treated with either control DMEM/F12 media or media containing LPS @10ng/ml and Zileuton @ 25uM (Low dose) or 100uM (High dose) for 24 hours Data were analysed via students T-test. Error bars represent SEM.

## Conclusion

Both Pioglitazone and Zileuton showed target engagement *in vitro* (see pPPARg and p-5-LOX, respectively).

Pioglitazone showed very potent effects in our *in vitro* platform with some of the inflammatory markers analyzed (phospho-NFkB and proIL1 $\beta$ /mature IL1 $\beta$ ).

## Summary – Third Quarter

Cell culture exposure to various LPS concentrations over a 15mins to 4hrs

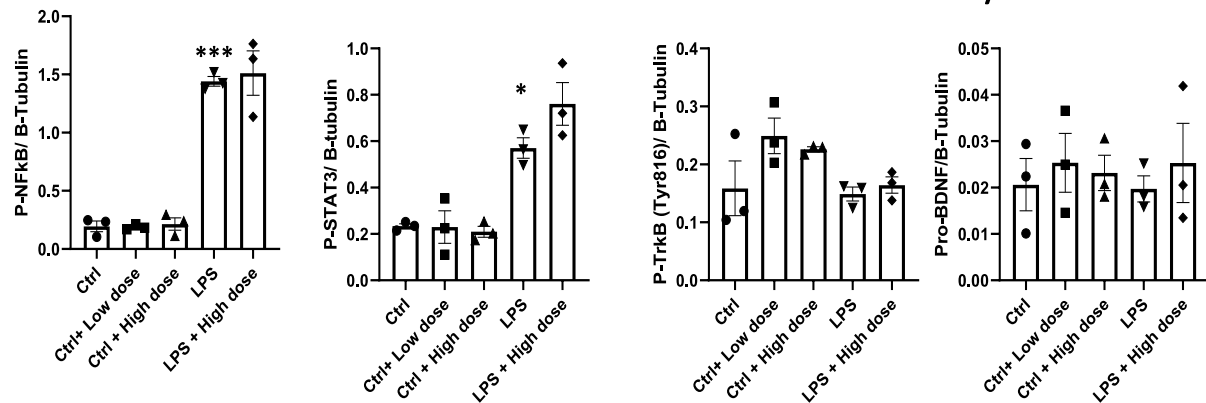
- IMG microglia and Primary sham/TBI microglia
- C8-D1A Astrocytes and Primary sham/TBI microglia

## Methodology

- IMG - Immortalized microglial cells and C8-D1A astrocytes were grown to 85-90% confluency in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) or DMEM (for astrocytes), with cells cultured at 37°C and 5%CO<sub>2</sub>.
- Cells were treated with several compounds for 30 mins prior to LPS exposure which lasted for 15mins to 45hrs (short-term treatment) (IMG-microglia 10ng/ml) (C8-D1A- Astrocytes 50ng/ml).
- Cells were Lysed using Mammalian protein extraction reagent (MPER) supplemented with Protease and Phosphatase inhibitors.
- Protein concentration was assessed by (BCA) assay, 15ug of protein was then loaded onto a Bis-tris polyacrylamide gel electrophoresis (PAGE) using a 4-20% gradient gel. Protein was then transferred to a PVDF membrane overnight at 90mA.

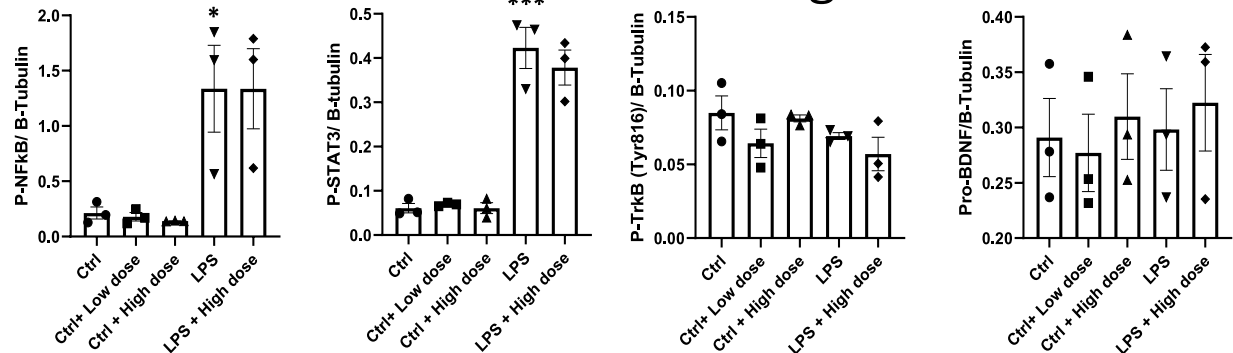
## More compound screening study

### 7'8 DHF effect on C8D1a Astrocytes



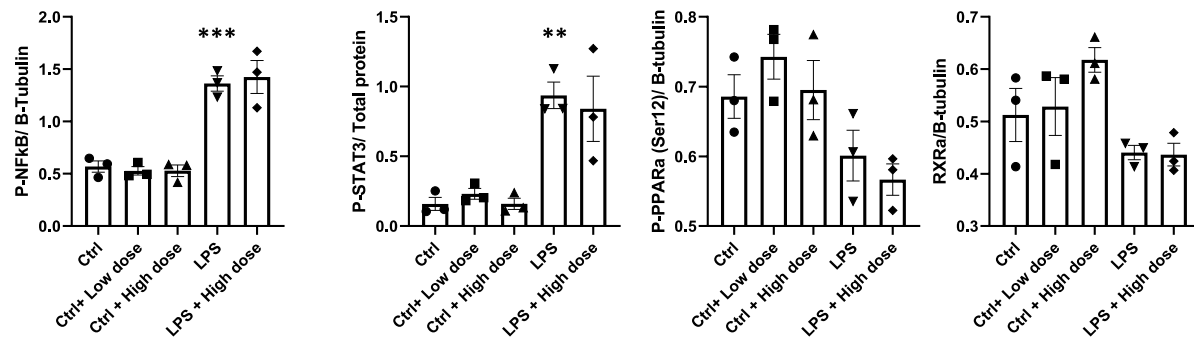
A graph showing the protein expression of Phospho-NFkB (Serine536), Phospho-STAT3(Tyrosine705), Phospho-TrkB(Tyrosine816) and Pro-BDNF in the cytoplasmic/ nuclear fraction of cell lysate from C8D1a immortalized astrocyte cells following a 30 minute pre-treatment with 7'8-Dihydroxyflavone (7'8-DHF) at either a low (15uM) or high dose (60uM), the cell culture media was then replaced with fresh media containing 50ng/ml Lipopolysaccharide (LPS) for 45 minutes (n=3/group), data were normalized to B-tubulin. Data were assessed for normal distribution and significance was assessed using a one-way ANOVA following by post hoc testing with a two-stage linear setup by Benjamini-Hochberg and Yekutieli to control for false discovery rate. Significance vs control is indicated on the graph (\*=P<0.05) (\*\*=P<0.01) (\*\*\*=P<0.001). Significance vs LPS treated is indicated on the graph (#=P<0.05) (##=P<0.01) (###=P<0.001)

### 7'8-DHF effect on IMG microglia



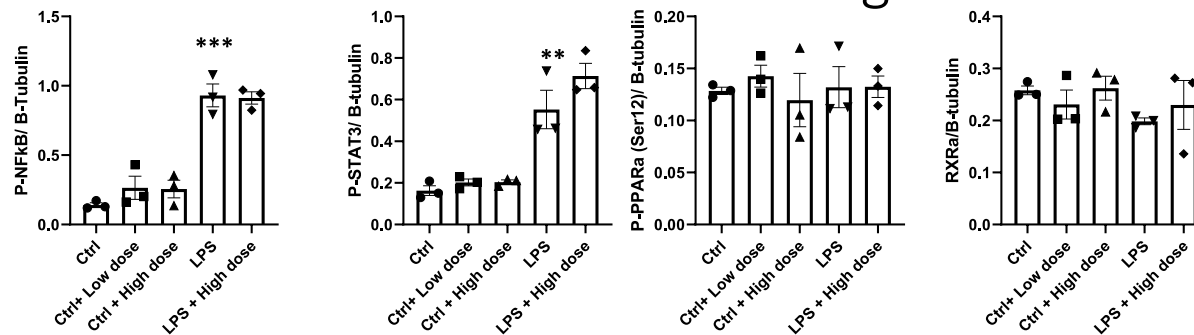
A graph showing the protein expression of Phospho-NFkB (Serine536), Phospho-STAT3(Tyrosine705), Phospho-TrkB(Tyrosine816) and Pro-BDNF in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglia cells following a 30 minute pre-treatment with 7'8-Dihydroxyflavone (7'8-DHF) at either a low (15uM) or high dose (60uM), the cell culture media was then replaced with fresh media containing 10ng/ml Lipopolysaccharide (LPS) for 45 minutes (n=3/group), data were normalized to B-tubulin. Data were assessed for normal distribution and significance was assessed using a one-way ANOVA following by post hoc testing with a two-stage linear setup by Benjamini-Hochberg and Yekutieli to control for false discovery rate. Significance vs control is indicated on the graph (\*=P<0.05) (\*\*=P<0.01) (\*\*\*=P<0.001). Significance vs LPS treated is indicated on the graph (#=P<0.05) (##=P<0.01) (###=P<0.001)

## OEA effects on C8D1a Astrocytes



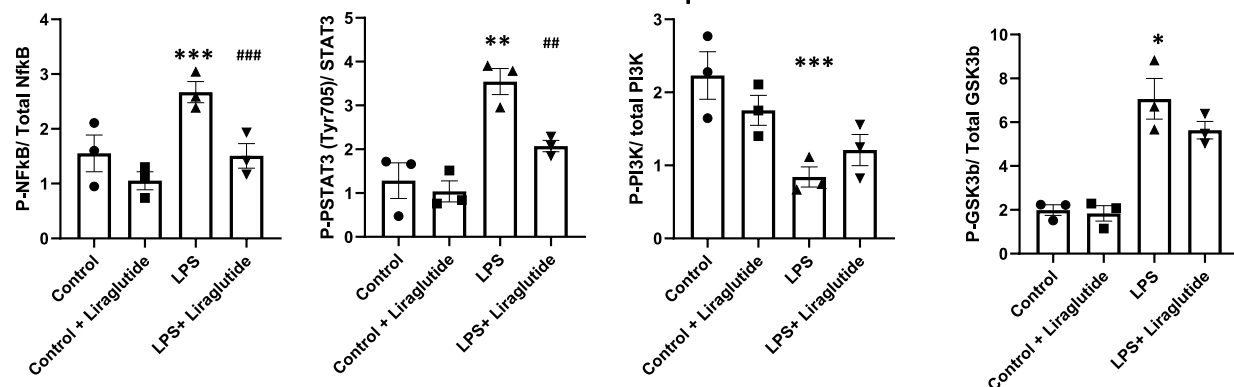
A graph showing the protein expression of Phospho-NFkB (Serine536), Phospho-STAT3(Tyrosine705), Phospho-PPARα(Serine12) and RXRa in the cytoplasmic/ nuclear fraction of cell lysate from C8D1a immortalized astrocyte cells following a 30 minute pre-treatment with Oleoylethanolamide (OEA) at either a low (25uM) or high dose (100uM), the cell culture media was then replaced with fresh media containing 50ng/ml Lipopolysaccharide (LPS) for 90 minutes (n=3/group), data were normalized to B-tubulin. Data were assessed for normal distribution and significance was assessed using a one-way ANOVA following by post hoc testing with a two-stage linear setup by Benjamini-Hochberg and Yekutieli to control for false discovery rate. Significance vs control is indicated on the graph (\*=P<0.05) (\*\*=P<0.01) (\*\*\*=P<0.001). Significance vs LPS treated is indicated on the graph (#=P<0.05) (##=P<0.01) (###=P<0.001)

## OEA effects on IMG microglia



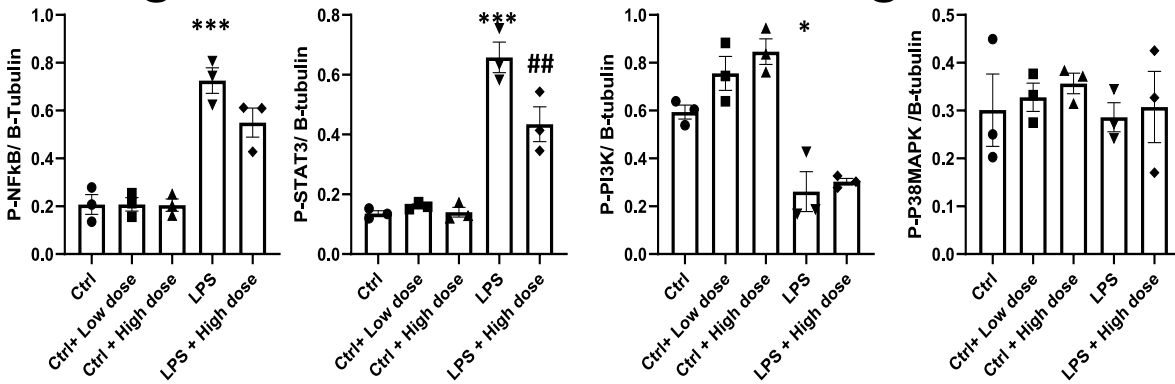
A graph showing the protein expression of Phospho-NFkB (Serine536), Phospho-STAT3(Tyrosine705), Phospho-PPARα(Serine12) and RXRa in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglia cells following a 30 minute pre-treatment with Oleoylethanolamide (OEA) at either a low (25uM) or high dose (100uM), the cell culture media was then replaced with fresh media containing 10ng/ml Lipopolysaccharide (LPS) for 90 minutes (n=3/group), data were normalized to B-tubulin. Data were assessed for normal distribution and significance was assessed using a one-way ANOVA following by post hoc testing with a two-stage linear setup by Benjamini-Hochberg and Yekutieli to control for false discovery rate. Significance vs control is indicated on the graph (\*=P<0.05) (\*\*=P<0.01) (\*\*\*=P<0.001). Significance vs LPS treated is indicated on the graph (#=P<0.05) (##=P<0.01) (###=P<0.001)

## Astrocyte treatment with Liraglutide @ 200nM at 45 mins after a 30min pre treatment



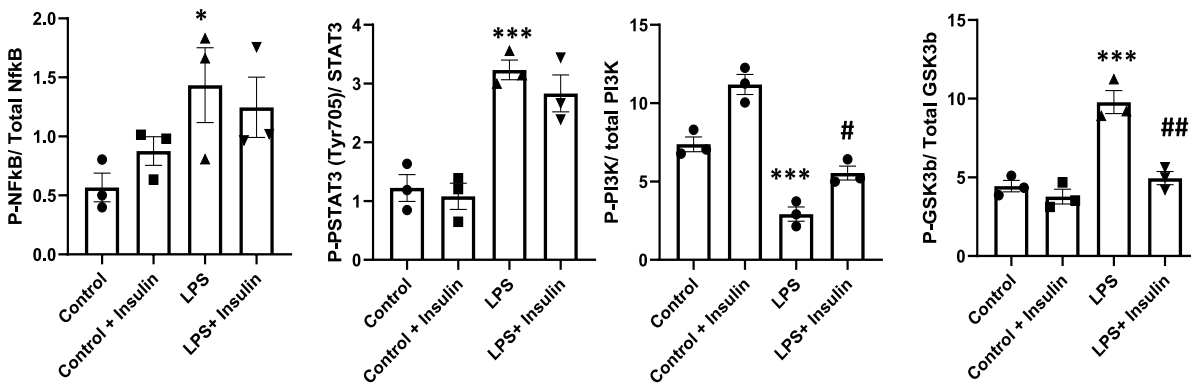
A graph showing the protein expression of Phospho-NFkB (Serine536), Phospho-STAT3(Tyrosine705), Phospho-PI3K-P55 fragment (Tyr199) and Phospho-GSK3b(Serine9) in the cytoplasmic/ nuclear fraction of cell lysate from C8D1a immortalized astrocyte cells following a 30 minute pre-treatment with Liraglutide at either a low (50nM) or high dose (200nM), the cell culture media was then replaced with fresh media containing 50ng/ml Lipopolysaccharide (LPS) for 45 minutes (n=3/group), data were normalized to B-tubulin. Data were assessed for normal distribution and significance was assessed using a one-way ANOVA following by post hoc testing with a two-stage linear setup by Benjamini-Hochberg and Yekutieli to control for false discovery rate. Significance vs control is indicated on the graph (\*=P<0.05) (\*\*=P<0.01) (\*\*\*=P<0.001). Significance vs LPS treated is indicated on the graph (#=P<0.05) (##=P<0.01) (###=P<0.001)

## Liraglutide effect on IMG microglia



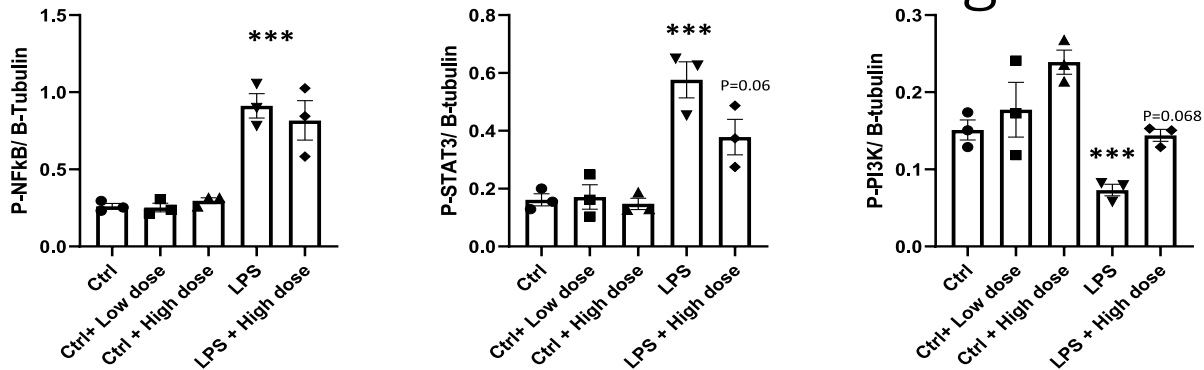
A graph showing the protein expression of Phospho-NFkB (Serine536), Phospho-STAT3(Tyrosine705), Phospho-PI3K-P55 fragment (Tyr199) and Phospho-GSK3b(Serine9) in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglia cells following a 30 minute pre-treatment with Liraglutide at either a low (50nM) or high dose (200nM), the cell culture media was then replaced with fresh media containing 10ng/ml Lipopolysaccharide (LPS) for 45 minutes (n=3/group), data were normalized to B-tubulin. Data were assessed for normal distribution and significance was assessed using a one-way ANOVA following by post hoc testing with a two-stage linear setup by Benjamini-Hochberg and Yekutieli to control for false discovery rate. Significance vs control is indicated on the graph (\*=P<0.05) (\*\*=P<0.01) (\*\*\*=P<0.001). Significance vs LPS treated is indicated on the graph (#=P<0.05) (##=P<0.01) (###=P<0.001)

## Astrocyte treatment with Insulin @ 200nM at 45 mins after a 30min pre treatment



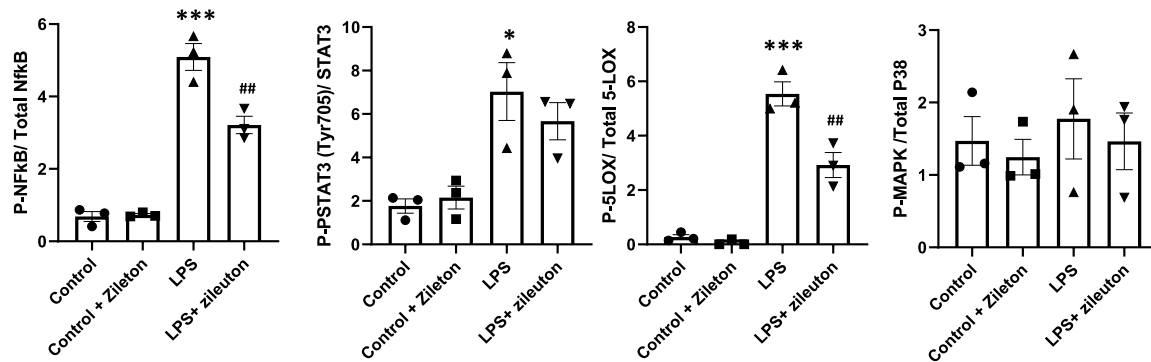
A graph showing the protein expression of Phospho-NFkB (Serine536), Phospho-STAT3(Tyrosine705), Phospho-PI3K-P55 fragment (Tyr199) and Phospho-GSK3b(Serine9) in the cytoplasmic/ nuclear fraction of cell lysate from C8D1a immortalized astrocyte cells following a 30 minute pre-treatment with Insulin at either a low (50nM) or high dose (200nM), the cell culture media was then replaced with fresh media containing 50ng/ml Lipopolysaccharide (LPS) for 45 minutes (n=3/group), data were normalized to B-tubulin. Data were assessed for normal distribution and significance was assessed using a one-way ANOVA following by post hoc testing with a two-stage linear setup by Benjamini-Hochberg and Yekutieli to control for false discovery rate. Significance vs control is indicated on the graph (\*=P<0.05) (\*\*=P<0.01) (\*\*\*=P<0.001). Significance vs LPS treated is indicated on the graph (#=P<0.05) (##=P<0.01) (###=P<0.001)

# Insulin effect on IMG microglia



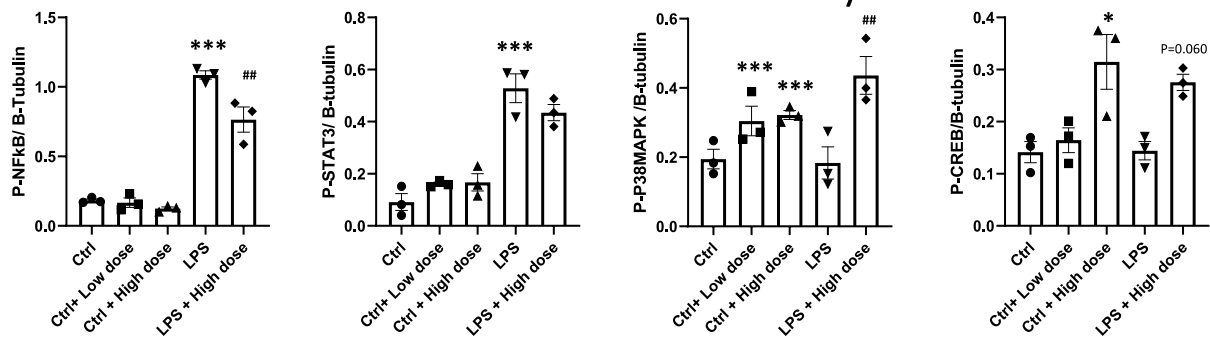
A graph showing the protein expression of Phospho-NFkB (Serine536), Phospho-STAT3(Tyrosine705), Phospho-PI3K-P55 fragment (Tyr199) in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglia cells following a 30 minute pre-treatment with Insulin at either a low (50nM) or high dose (200nM), the cell culture media was then replaced with fresh media containing 10ng/ml Lipopolysaccharide (LPS) for 45 minutes (n=3/group), data were normalized to B-tubulin. Data were assessed for normal distribution and significance was assessed using a one-way ANOVA following by post hoc testing with a two-stage linear setup by Benjamini-Hochberg and Yekutieli to control for false discovery rate. Significance vs control is indicated on the graph (\*=P<0.05) (\*\*=P<0.01) (\*\*#=P<0.001). Significance vs LPS treated is indicated on the graph (##=P<0.05) (###=P<0.01) (####=P<0.001)

# Microglia- treatment with Zileuton @ 100uM at 90 mins after a 30min pre treatment



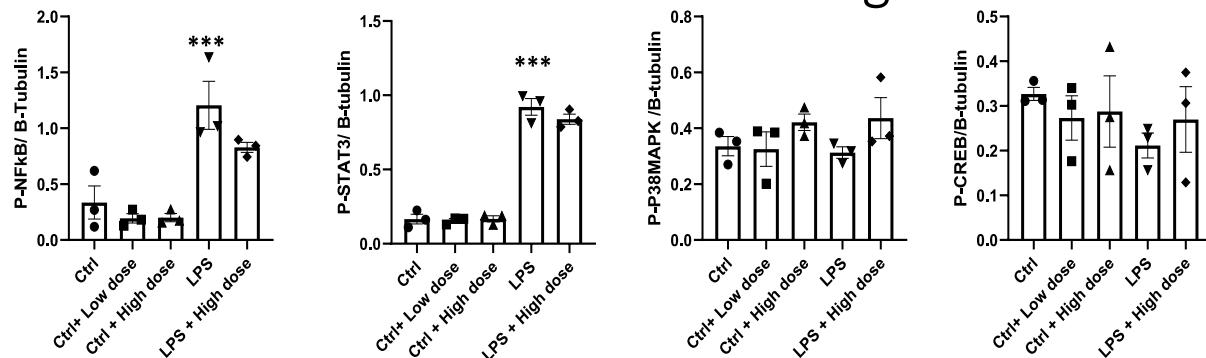
A graph showing the protein expression of Phospho-NFkB (Serine536), Phospho-STAT3(Tyrosine705), Phospho-5-Lipoxygenase (Serine663) (5-LOX) and Phospho-P38 (Thr180/Tyr182) MAPK in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglia cells following a 30 minute pre-treatment with Insulin at either a low (50nM) or high dose (200nM), the cell culture media was then replaced with fresh media containing 10ng/ml Lipopolysaccharide (LPS) for 45 minutes (n=3/group), data were normalized to B-tubulin. Data were assessed for normal distribution and significance was assessed using a one-way ANOVA following by post hoc testing with a two-stage linear setup by Benjamini-Hochberg and Yekutieli to control for false discovery rate. Significance vs control is indicated on the graph (\*=P<0.05) (\*\*=P<0.01) (\*\*#=P<0.001). Significance vs LPS treated is indicated on the graph (##=P<0.05) (###=P<0.01) (####=P<0.001)

# Effect of montelukast on Astrocytes



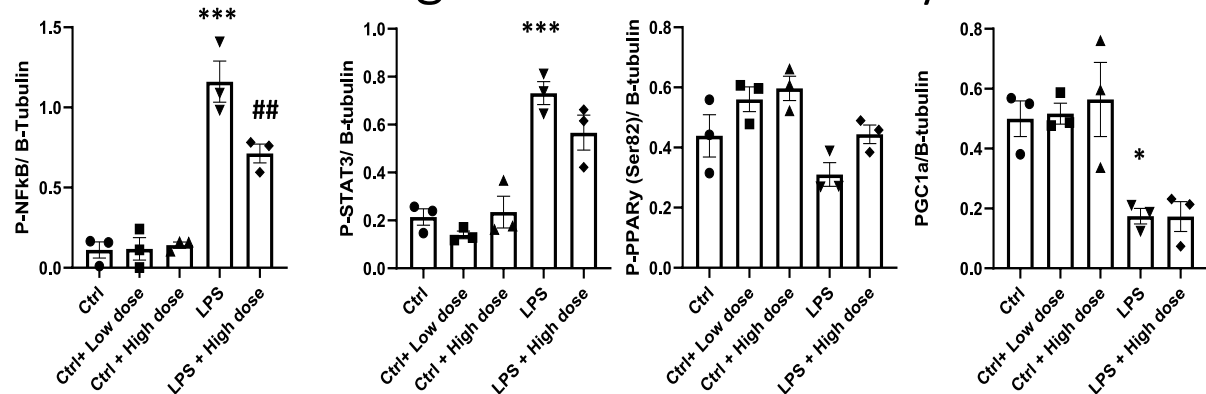
A graph showing the protein expression of Phospho-NFkB (Serine536), Phospho-STAT3(Tyrosine705), Phospho-P38 (Thr180/Tyr182) MAPK and Phospho-CREB(Serine133) in the cytoplasmic/ nuclear fraction of cell lysate from C8D1a immortalized astrocyte cells following a 30 minute pre-treatment with Montelukast at either a low (2.5uM) or high dose (10uM), the cell culture media was then replaced with fresh media containing 50ng/ml Lipopolysaccharide (LPS) for 45 minutes (n=3/group), data were normalized to B-tubulin. Data were assessed for normal distribution and significance was assessed using a one-way ANOVA following by post hoc testing with a two-stage linear setup by Benjamini-Hochberg and Yekutieli to control for false discovery rate. Significance vs control is indicated on the graph (\*=P<0.05) (\*\*=P<0.01) (\*\*#=P<0.001). Significance vs LPS treated is indicated on the graph (##=P<0.05) (###=P<0.01) (####=P<0.001)

## Effect of montelukast on microglia



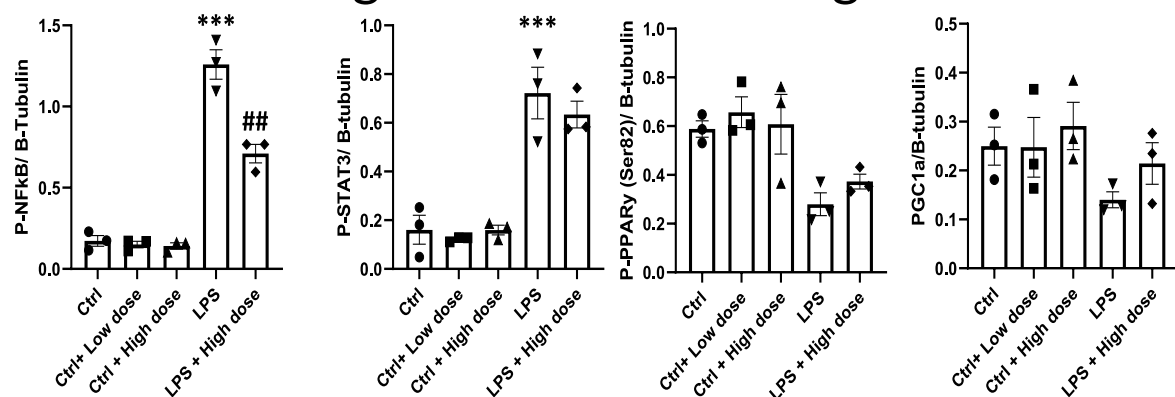
A graph showing the protein expression of Phospho-NFkB (Serine536), Phospho-STAT3(Tyrosine705), Phospho-P38 (Thr180/Tyr182) MAPK and Phospho-CREB(Serine133) in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglia cells following a 30 minute pre-treatment with Insulin at either a low (50nM) or high dose (200nM), the cell culture media was then replaced with fresh media containing 10ng/ml Lipopolysaccharide (LPS) for 45 minutes (n=3/group), data were normalized to B-tubulin. Data were assessed for normal distribution and significance was assessed using a one-way ANOVA following by post hoc testing with a two-stage linear setup by Benjamini-Hochberg and Yekutieli to control for false discovery rate. Significance vs control is indicated on the graph (\*=P<0.05) (\*\*=P<0.01) (\*\*\*=P<0.001). Significance vs LPS treated is indicated on the graph (#=P<0.05) (##=P<0.01) (###=P<0.001)

## Effect of Pioglitazone on Astrocytes



A graph showing the protein expression of Phospho-NFkB (Serine536), Phospho-STAT3(Tyrosine705), Phospho-PPARy and PGC1a in the cytoplasmic/ nuclear fraction of cell lysate from C8D1a immortalized astrocyte cells following a 30 minute pre-treatment with Pioglitazone at either a low (2.5uM) or high dose (10uM), the cell culture media was then replaced with fresh media containing 50ng/ml Lipopolysaccharide (LPS) for 60 minutes (n=3/group), data were normalized to B-tubulin. Data were assessed for normal distribution and significance was assessed using a one-way ANOVA following by post hoc testing with a two-stage linear setup by Benjamini-Hochberg and Yekutieli to control for false discovery rate. Significance vs control is indicated on the graph (\*=P<0.05) (\*\*=P<0.01) (\*\*\*=P<0.001). Significance vs LPS treated is indicated on the graph (#=P<0.05) (##=P<0.01) (###=P<0.001)

## Effect of Pioglitazone on Microglia



A graph showing the protein expression of Phospho-NFkB (Serine536), Phospho-STAT3(Tyrosine705), Phospho-PPARy and PGC1a in the cytoplasmic/ nuclear fraction of cell lysate from IMG immortalized microglia cells following a 30 minute pre-treatment with Insulin at either a low (50nM) or high dose (200nM), the cell culture media was then replaced with fresh media containing 10ng/ml Lipopolysaccharide (LPS) for 60 minutes (n=3/group), data were normalized to B-tubulin. Data were assessed for normal distribution and significance was assessed using a one-way ANOVA following by post hoc testing with a two-stage linear setup by Benjamini-Hochberg and Yekutieli to control for false discovery rate. Significance vs control is indicated on the graph (\*=P<0.05) (\*\*=P<0.01) (\*\*\*=P<0.001). Significance vs LPS treated is indicated on the graph (#=P<0.05) (##=P<0.01) (###=P<0.001)

**Data below shows proteomic studies (list of differentially expressed proteins) conducted on cell lysates of LPS exposed astrocytes and microglial cells (same methods as above) treated with Pioglitazone and Zileuton for 24hrs. Proteomic analyses was conducted using 10-plex TMT labeling and LC/MC-MS approach.**

| <b>List of significant genes C8-D1A LPS VS LPS Zileuton</b>                                                                  | <b>Difference</b> | <b>P- Value</b> |
|------------------------------------------------------------------------------------------------------------------------------|-------------------|-----------------|
| Replication protein A subunit OS=Mus musculus OX=10090 GN=Rpa1 PE=2 SV=1                                                     | 0.817807          | 0.0048          |
| Heterogeneous nuclear ribonucleoprotein D0 OS=Mus musculus OX=10090 GN=Hnrnpd PE=1 SV=1                                      | 0.368586          | 0.0067          |
| Luc7-like protein 3 OS=Mus musculus OX=10090 GN=Luc7l3 PE=1 SV=1                                                             | 0.460955          | 0.0099          |
| L-lactate dehydrogenase OS=Mus musculus OX=10090 GN=Ldha PE=2 SV=1                                                           | 0.588747          | 0.0132          |
| Nuclear pore complex protein Nup93 OS=Mus musculus OX=10090 GN=Nup93 PE=1 SV=1                                               | 0.371547          | 0.0145          |
| Translationally-controlled tumor protein OS=Mus musculus OX=10090 GN=Tpt1 PE=1 SV=1                                          | 0.381682          | 0.0152          |
| High mobility group protein B2 OS=Mus musculus OX=10090 GN=Hmgb2 PE=1 SV=3                                                   | 0.417482          | 0.0166          |
| Dipeptidyl peptidase 3 OS=Mus musculus OX=10090 GN=Dpp3 PE=2 SV=1                                                            | 0.639895          | 0.0179          |
| Methylthioribose-1-phosphate isomerase OS=Mus musculus OX=10090 GN=Mri1 PE=1 SV=1                                            | 0.521281          | 0.018           |
| Casein kinase II subunit beta OS=Mus musculus OX=10090 GN=Csnk2b PE=1 SV=1                                                   | 0.510334          | 0.0235          |
| Proteasome subunit alpha type-3 OS=Mus musculus OX=10090 GN=Psm3 PE=1 SV=3                                                   | 0.302727          | 0.0241          |
| Ribosomal protein L22 OS=Mus musculus OX=10090 GN=Rpl22 PE=1 SV=1                                                            | 0.33259           | 0.0281          |
| Glutathione S-transferase Mu 2 OS=Mus musculus OX=10090 GN=Gstm2 PE=1 SV=2                                                   | 0.506697          | 0.0322          |
| MKIAA4025 protein (Fragment) OS=Mus musculus OX=10090 GN=Hspa4 PE=2 SV=1                                                     | 0.400671          | 0.0328          |
| 26S proteasome regulatory subunit 6B OS=Mus musculus OX=10090 GN=Psmc4 PE=1 SV=2                                             | 1.227374          | 0.0359          |
| Rps19 protein (Fragment) OS=Mus musculus OX=10090 GN=Rps19 PE=2 SV=1                                                         | 0.354629          | 0.037           |
| Heme oxygenase OS=Mus musculus OX=10090 GN=Hmox1 PE=1 SV=1                                                                   | 0.848878          | 0.0371          |
| Vesicular integral-membrane protein VIP36 OS=Mus musculus OX=10090 GN=Lman2 PE=1 SV=2                                        | 0.465516          | 0.0389          |
| ADP-ribosylation factor 4 OS=Mus musculus OX=10090 GN=Arf4 PE=1 SV=2                                                         | 0.470296          | 0.0463          |
| Vinculin OS=Mus musculus OX=10090 GN=Vcl PE=1 SV=4                                                                           | 0.656746          | 0.048           |
| 60S ribosomal protein L8 OS=Mus musculus OX=10090 GN=Rpl8 PE=1 SV=2                                                          | 0.351675          | 0.0494          |
| Phosphoglycerate mutase OS=Mus musculus OX=10090 GN=Pgam1 PE=1 SV=1                                                          | 0.272223          | 0.0495          |
| High mobility group protein HMG-I/HMG-Y OS=Mus musculus OX=10090 GN=Hmga1 PE=1 SV=4                                          | 0.418861          | 0.0025          |
| Structural maintenance of chromosomes protein 4 OS=Mus musculus OX=10090 GN=Smc4 PE=1 SV=1                                   | 0.785415          | 0.0033          |
| V-type proton ATPase subunit C OS=Mus musculus OX=10090 GN=Atp6v1c1 PE=2 SV=1                                                | 0.212107          | 0.0087          |
| AP complex subunit beta OS=Mus musculus OX=10090 GN=Ap2b1 PE=2 SV=1                                                          | 0.356374          | 0.0089          |
| Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B (Fragment) OS=Mus musculus OX=10090 GN=Ppp2r2a PE=2 SV=1 | 1.488101          | 0.0125          |
| Glutamate-cysteine ligase regulatory subunit OS=Mus musculus OX=10090 GN=Gclm PE=1 SV=1                                      | 0.418333          | 0.0207          |
| Serrate RNA effector molecule homolog OS=Mus musculus OX=10090 GN=Srrt PE=1 SV=1                                             | 0.367382          | 0.0211          |
| Transferrin receptor protein 1 OS=Mus musculus OX=10090 GN=Tfrc PE=1 SV=1                                                    | 1.064567          | 0.0228          |
| Ribosomal protein L26 OS=Mus musculus OX=10090 GN=Rpl26 PE=1 SV=1                                                            | 1.021256          | 0.0249          |
| Hepatoma-derived growth factor OS=Mus musculus OX=10090 GN=Hdgf PE=1 SV=2                                                    | 0.204808          | 0.0286          |
| Hexokinase-2 OS=Mus musculus OX=10090 GN=Hk2 PE=1 SV=1                                                                       | 0.794619          | 0.0377          |
| Cathepsin B OS=Mus musculus OX=10090 GN=Ctsb PE=1 SV=2                                                                       | 0.635821          | 0.0421          |
| Peptidyl-prolyl cis-trans isomerase D OS=Mus musculus OX=10090 GN=Ppid PE=1 SV=3                                             | 0.399331          | 0.0443          |
| Uncharacterized protein OS=Mus musculus OX=10090 GN=Ddx21 PE=2 SV=1                                                          | 0.789396          | 0.046           |
| Pyruvate dehydrogenase E1 component subunit alpha OS=Mus musculus OX=10090 GN=Pdha1 PE=1 SV=1                                | 0.273721          | 0.0482          |
| Ras-related protein Rab-2A OS=Mus musculus OX=10090 GN=Rab2a PE=1 SV=1                                                       | 0.249916          | 0.0497          |

| <b>List of significant genes C8-D1A LPS vs LPS Pioglitazone</b>                                             | <b>Difference</b> | <b>P- Value</b> |
|-------------------------------------------------------------------------------------------------------------|-------------------|-----------------|
| Enolase 1, alpha non-neuron OS=Mus musculus OX=10090 GN=EG433182 PE=1 SV=1                                  | 0.42359           | 0.0123          |
| Vesicular integral-membrane protein VIP36 OS=Mus musculus OX=10090 GN=Lman2 PE=1 SV=2                       | 0.496119          | 0.0285          |
| Protein disulfide-isomerase A4 OS=Mus musculus OX=10090 GN=Pdia4 PE=1 SV=1                                  | 0.22782           | 0.0301          |
| Replication protein A subunit OS=Mus musculus OX=10090 GN=Rpa1 PE=2 SV=1                                    | 0.582559          | 0.0309          |
| Hepatoma-derived growth factor OS=Mus musculus OX=10090 GN=Hdgf PE=1 SV=2                                   | 0.220368          | 0.0197          |
| Structural maintenance of chromosomes protein 4 OS=Mus musculus OX=10090 GN=Smc4 PE=1 SV=1                  | 0.547713          | 0.0254          |
| Transferrin receptor protein 1 OS=Mus musculus OX=10090 GN=Tfrc PE=1 SV=1                                   | 0.974813          | 0.0354          |
| Pyruvate dehydrogenase E1 component subunit beta, mitochondrial OS=Mus musculus OX=10090 GN=Pdhfb PE=1 SV=1 | 0.764578          | 0.0401          |

| List of significant genes C8-D1A Control vs LPS                                                    | Difference | P- Value |
|----------------------------------------------------------------------------------------------------|------------|----------|
| High mobility group protein HMG-I/HMG-Y OS=Mus musculus OX=10090 GN=Hmga1 PE=1 SV=4                | 0.408834   | 0.0029   |
| Adenine phosphoribosyltransferase OS=Mus musculus OX=10090 GN=Aprt PE=1 SV=2                       | 0.787485   | 0.0396   |
| Isochorismatase domain-containing protein 1 OS=Mus musculus OX=10090 GN=Isoc1 PE=1 SV=1            | 0.719686   | 0.0038   |
| Hepatoma-derived growth factor OS=Mus musculus OX=10090 GN=Hdgf PE=1 SV=2                          | 0.263058   | 0.0073   |
| Replication protein A subunit OS=Mus musculus OX=10090 GN=Rpa1 PE=2 SV=1                           | 0.713555   | 0.0106   |
| Enolase 1, alpha non-neuron OS=Mus musculus OX=10090 GN=EG433182 PE=1 SV=1                         | 0.369089   | 0.0256   |
| Uncharacterized protein OS=Mus musculus OX=10090 GN=Dctn2 PE=1 SV=1                                | 0.919161   | 0.0269   |
| Casein kinase II subunit beta OS=Mus musculus OX=10090 GN=Csnk2b PE=1 SV=1                         | 0.495245   | 0.0273   |
| 26S proteasome regulatory subunit 6B OS=Mus musculus OX=10090 GN=Psmc4 PE=1 SV=2                   | 1.255704   | 0.0321   |
| Aminopeptidase N OS=Mus musculus OX=10090 GN=Anpep PE=1 SV=4                                       | 0.449673   | 0.0358   |
| Paxillin OS=Mus musculus OX=10090 GN=Pxn PE=1 SV=2                                                 | 1.044688   | 0.0398   |
| Microtubule-associated protein 1B OS=Mus musculus OX=10090 GN=Map1b PE=2 SV=1                      | 0.238778   | 0.0421   |
| Proteasome subunit alpha type OS=Mus musculus OX=10090 GN=Pma4 PE=2 SV=1                           | 0.33362    | 0.0491   |
| DNA replication licensing factor MCM6 OS=Mus musculus OX=10090 GN=Mcm6 PE=1 SV=1                   | 0.396324   | 0.0068   |
| AHNAK nucleoprotein (desmoyokin) OS=Mus musculus OX=10090 GN=Ahnak PE=1 SV=1                       | 0.680657   | 0.0336   |
| Lpxn protein OS=Mus musculus OX=10090 GN=Lpxn PE=2 SV=1                                            | 0.506994   | 0.0342   |
| STE20/SPS1-related proline-alanine-rich protein kinase OS=Mus musculus OX=10090 GN=Stk39 PE=1 SV=1 | 0.337727   | 0.0351   |
| Proteasome subunit alpha type OS=Mus musculus OX=10090 GN=Pma7 PE=2 SV=1                           | 0.184316   | 0.0353   |

| List of significant genes IMG - Microglia Control vs LPS                                                          | Difference  | P-Value |
|-------------------------------------------------------------------------------------------------------------------|-------------|---------|
| Acidic leucine-rich nuclear phosphoprotein 32 family member B OS=Mus musculus OX=10090 GN=Anp32b PE=1 SV=1        | 0.420653635 | 0.0002  |
| Uncharacterized protein (Fragment) OS=Mus musculus OX=10090 GN=Ptma PE=2 SV=1                                     | 0.52386314  | 0.0008  |
| AHNAK nucleoprotein 2 (Fragment) OS=Mus musculus OX=10090 GN=Ahnak2 PE=1 SV=1                                     | 0.385292393 | 0.0009  |
| 40S ribosomal protein S13 OS=Mus musculus OX=10090 GN=Rps13 PE=1 SV=2                                             | 0.50615876  | 0.001   |
| Glutathione S-transferase P 1 OS=Mus musculus OX=10090 GN=Gstp1 PE=1 SV=2                                         | 0.220600387 | 0.0017  |
| Glutamate-cysteine ligase regulatory subunit OS=Mus musculus OX=10090 GN=Gclm PE=1 SV=1                           | 0.427616827 | 0.0019  |
| Septin-9 OS=Mus musculus OX=10090 GN=Septin9 PE=1 SV=1                                                            | 0.3251181   | 0.0026  |
| Sorting nexin-6 OS=Mus musculus OX=10090 GN=Snx6 PE=1 SV=2                                                        | 0.420535426 | 0.0034  |
| Uncharacterized protein (Fragment) OS=Mus musculus OX=10090 GN=Nono PE=2 SV=1                                     | 0.25557207  | 0.0065  |
| Isopentenyl-diphosphate Delta-isomerase 1 OS=Mus musculus OX=10090 GN=Idi1 PE=1 SV=1                              | 0.238600737 | 0.0073  |
| T-complex protein 1 subunit gamma OS=Mus musculus OX=10090 GN=Cct3 PE=1 SV=1                                      | 0.415549267 | 0.0076  |
| E3 SUMO-protein ligase RanBP2 OS=Mus musculus OX=10090 GN=Ranbp2 PE=1 SV=2                                        | 0.641712602 | 0.0077  |
| Myosin light polypeptide 6 OS=Mus musculus OX=10090 GN=Myl6 PE=1 SV=1                                             | 0.45052321  | 0.0077  |
| Annexin A3 OS=Mus musculus OX=10090 GN=Anxa3 PE=1 SV=4                                                            | 0.348047823 | 0.0081  |
| Flavin reductase (NADPH) OS=Mus musculus OX=10090 GN=Blvrb PE=1 SV=3                                              | 0.452370827 | 0.0082  |
| Staphylococcal nuclease domain-containing protein OS=Mus musculus OX=10090 GN=Snd1 PE=2 SV=1                      | 0.228532607 | 0.0084  |
| Histone H2A type 2-B OS=Mus musculus OX=10090 GN=Hist2h2ab PE=1 SV=3                                              | 0.64050611  | 0.0107  |
| Glycogen phosphorylase, muscle form OS=Mus musculus OX=10090 GN=Pygm PE=1 SV=3                                    | 0.273404347 | 0.0108  |
| Alkylglycerone-phosphate synthase OS=Mus musculus OX=10090 GN=Agps PE=1 SV=1                                      | 0.698148273 | 0.0112  |
| Adenylosuccinate synthetase isozyme 2 OS=Mus musculus OX=10090 GN=Adss PE=1 SV=1                                  | 0.463697477 | 0.0121  |
| ATP-binding cassette sub-family F member 1 OS=Mus musculus OX=10090 GN=Abcf1 PE=1 SV=1                            | 0.627894274 | 0.0125  |
| Mitogen-activated protein kinase OS=Mus musculus OX=10090 GN=Mapk3 PE=1 SV=1                                      | 0.319948313 | 0.0128  |
| Calcium-binding protein 39 OS=Mus musculus OX=10090 GN=Cab39 PE=1 SV=2                                            | 0.332419648 | 0.013   |
| RNA-binding protein Raly OS=Mus musculus OX=10090 GN=Raly PE=1 SV=3                                               | 1.021039346 | 0.0169  |
| Rab5B OS=Mus musculus OX=10090 GN=Rab5b PE=2 SV=1                                                                 | 0.418035505 | 0.0194  |
| Switch-associated protein 70 OS=Mus musculus OX=10090 GN=Swap70 PE=1 SV=2                                         | 0.413094173 | 0.0204  |
| Structural maintenance of chromosomes protein 3 OS=Mus musculus OX=10090 GN=Smc3 PE=1 SV=2                        | 0.2144749   | 0.0206  |
| Lpxn protein OS=Mus musculus OX=10090 GN=Lpxn PE=2 SV=1                                                           | 0.434647672 | 0.0232  |
| Serrate RNA effector molecule homolog OS=Mus musculus OX=10090 GN=Srrt PE=1 SV=1                                  | 0.576671768 | 0.0237  |
| ATP-dependent 6-phosphofructokinase, liver type OS=Mus musculus OX=10090 GN=Pfk1 PE=1 SV=4                        | 0.55782502  | 0.0257  |
| Proliferating cell nuclear antigen OS=Mus musculus OX=10090 GN=Pcna PE=1 SV=2                                     | 0.538223113 | 0.0293  |
| Aconitate hydratase, mitochondrial OS=Mus musculus OX=10090 GN=Aco2 PE=1 SV=1                                     | 0.402378953 | 0.0321  |
| BTB/POZ domain-containing protein KCTD12 OS=Mus musculus OX=10090 GN=Kctd12 PE=1 SV=1                             | 0.707254372 | 0.0325  |
| Histone H1.0 OS=Mus musculus OX=10090 GN=H1-0 PE=2 SV=4                                                           | 0.580041581 | 0.0336  |
| Acyl-protein thioesterase 2 OS=Mus musculus OX=10090 GN=Lypla2 PE=1 SV=1                                          | 0.46205051  | 0.0346  |
| Hypoxanthine-guanine phosphoribosyltransferase OS=Mus musculus OX=10090 GN=Hprt1 PE=1 SV=3                        | 0.434994133 | 0.0382  |
| Heterogeneous nuclear ribonucleoprotein A0 OS=Mus musculus OX=10090 GN=Hnrnpa0 PE=1 SV=1                          | 0.400475607 | 0.0389  |
| RNA-splicing ligase RtcB homolog OS=Mus musculus OX=10090 GN=RtcB PE=1 SV=1                                       | 0.77844984  | 0.0393  |
| 60S ribosomal protein L13 OS=Mus musculus OX=10090 GN=Rpl13 PE=1 SV=3                                             | 0.561949606 | 0.0404  |
| Heterogeneous nuclear ribonucleoprotein D-like OS=Mus musculus OX=10090 GN=Hnrnpdl PE=1 SV=1                      | 0.4278286   | 0.0408  |
| Transmembrane emp24 domain-containing protein 2 OS=Mus musculus OX=10090 GN=Tmed2 PE=1 SV=1                       | 0.33789412  | 0.0411  |
| Tubulin-specific chaperone D OS=Mus musculus OX=10090 GN=Tbcd PE=1 SV=1                                           | 0.972116768 | 0.0419  |
| Uncharacterized protein OS=Mus musculus OX=10090 GN=Nap114 PE=2 SV=1                                              | 0.218431347 | 0.0441  |
| Heat shock protein 1-like protein OS=Mus musculus OX=10090 PE=3 SV=1                                              | 0.18238734  | 0.0462  |
| Very-long-chain 3-oxoacyl-CoA reductase OS=Mus musculus OX=10090 GN=Hsd17b12 PE=1 SV=1                            | 0.44096239  | 0.0474  |
| Adenylate kinase 2, mitochondrial OS=Mus musculus OX=10090 GN=Ak2 PE=1 SV=5                                       | 0.291037413 | 0.048   |
| Adenine phosphoribosyltransferase OS=Mus musculus OX=10090 GN=Aprt PE=1 SV=2                                      | 0.504837673 | 0.049   |
| Insulin-like growth factor 2 mRNA-binding protein 3 OS=Mus musculus OX=10090 GN=Igf2bp3 PE=1 SV=1                 | 0.677334171 | 0.0492  |
| U2 small nuclear ribonucleoprotein A' OS=Mus musculus OX=10090 GN=Snrpa1 PE=1 SV=2                                | 0.774280467 | 0.0062  |
| Vesicular integral-membrane protein VIP36 OS=Mus musculus OX=10090 GN=Lman2 PE=1 SV=2                             | 0.405303907 | 0.0109  |
| EH-domain containing 1 (Fragment) OS=Mus musculus OX=10090 GN=Ehd1 PE=2 SV=2                                      | 0.242018027 | 0.0114  |
| Env polyprotein OS=Mus musculus OX=10090 PE=4 SV=1                                                                | 0.630857473 | 0.0139  |
| B5 domain-containing protein OS=Mus musculus OX=10090 GN=Farsb PE=2 SV=1                                          | 0.469147424 | 0.0141  |
| Structural maintenance of chromosomes protein 1A OS=Mus musculus OX=10090 GN=Smc1a PE=1 SV=4                      | 0.27968841  | 0.015   |
| Small glutamine-rich tetratricopeptide repeat-containing protein alpha OS=Mus musculus OX=10090 GN=Sgta PE=1 SV=2 | 1.117266225 | 0.0202  |
| Phosphoserine aminotransferase OS=Mus musculus OX=10090 GN=Psat1 PE=2 SV=1                                        | 0.751296283 | 0.0213  |
| Enoyl-CoA delta isomerase 1, mitochondrial OS=Mus musculus OX=10090 GN=Eci1 PE=1 SV=2                             | 0.554318462 | 0.0227  |
| Ribonucleoside-diphosphate reductase large subunit OS=Mus musculus OX=10090 GN=Rrm1 PE=1 SV=2                     | 0.381797519 | 0.0244  |
| CDW13/WDR61 OS=Mus musculus OX=10090 GN=Wdr61 PE=1 SV=1                                                           | 0.316355384 | 0.0266  |
| Importin-4 OS=Mus musculus OX=10090 GN=Ipo4 PE=1 SV=1                                                             | 0.611204168 | 0.0267  |
| 26S proteasome regulatory subunit 6B OS=Mus musculus OX=10090 GN=Psmc4 PE=1 SV=2                                  | 1.345970607 | 0.0298  |
| Tripeptidyl-peptidase 2 OS=Mus musculus OX=10090 GN=Tpp2 PE=1 SV=3                                                | 0.239552943 | 0.0315  |
| Astrocytic phosphoprotein PEA-15 OS=Mus musculus OX=10090 GN=Pea15 PE=1 SV=1                                      | 0.786248901 | 0.0315  |
| T-complex protein 1 subunit eta OS=Mus musculus OX=10090 GN=Cct7 PE=1 SV=1                                        | 0.452980576 | 0.0336  |
| T-complex protein 1 subunit delta OS=Mus musculus OX=10090 GN=Cct4 PE=1 SV=3                                      | 0.43642477  | 0.0397  |
| Protein transport protein Sec31A OS=Mus musculus OX=10090 GN=Sec31a PE=1 SV=2                                     | 1.022692983 | 0.0429  |
| S-formylglutathione hydrolase OS=Mus musculus OX=10090 GN=Esd PE=1 SV=1                                           | 0.692714057 | 0.0434  |
| THO complex subunit 4 OS=Mus musculus OX=10090 GN=Alyref PE=1 SV=3                                                | 0.370603297 | 0.0438  |
| Uncharacterized protein OS=Mus musculus OX=10090 GN=Lcp1 PE=2 SV=1                                                | 0.155936433 | 0.0464  |

| List of significant genes IMG - Microglia LPS vs LPS+Zileuton                                                    | Difference | P-Value |
|------------------------------------------------------------------------------------------------------------------|------------|---------|
| 60S ribosomal protein L8 OS=Mus musculus OX=10090 GN=Rpl8 PE=1 SV=2                                              | 0.5016301  | 0.0002  |
| Vesicle-traffic protein SEC22b OS=Mus musculus OX=10090 GN=Sec22b PE=1 SV=3                                      | 0.966868   | 0.0004  |
| Uncharacterized protein OS=Mus musculus OX=10090 GN=Lcp1 PE=2 SV=1                                               | 0.3070355  | 0.0009  |
| Serine/arginine repetitive matrix protein 1 OS=Mus musculus OX=10090 GN=Srrm1 PE=1 SV=2                          | 0.551859   | 0.001   |
| Voltage-dependent anion-selective channel protein 3 OS=Mus musculus OX=10090 GN=Vdac3 PE=1 SV=1                  | 0.8046569  | 0.0011  |
| X-ray repair cross-complementing protein 6 OS=Mus musculus OX=10090 GN=Xrcc6 PE=1 SV=1                           | 0.3534343  | 0.0012  |
| Transmembrane emp24 domain-containing protein 2 OS=Mus musculus OX=10090 GN=Tmed2 PE=1 SV=1                      | 0.6088626  | 0.0014  |
| Fibronectin OS=Mus musculus OX=10090 GN=Fn1 PE=1 SV=4                                                            | 0.7869987  | 0.0016  |
| Proteasome (Prosome, macropain) 28 subunit, 3 OS=Mus musculus OX=10090 GN=Psme3 PE=1 SV=1                        | 0.9906757  | 0.0021  |
| Sorting nexin-6 OS=Mus musculus OX=10090 GN=Snx6 PE=1 SV=2                                                       | 0.4053267  | 0.0042  |
| Inter alpha-trypsin inhibitor, heavy chain 4 OS=Mus musculus OX=10090 GN=Itih4 PE=1 SV=2                         | 0.6587772  | 0.0046  |
| PH domain-containing protein OS=Mus musculus OX=10090 GN=Fermt3 PE=1 SV=1                                        | 0.5713915  | 0.008   |
| Dynactin subunit 1 OS=Mus musculus OX=10090 GN=Dctn1 PE=1 SV=3                                                   | 0.9134321  | 0.0092  |
| Ribosomal protein L12 OS=Mus musculus OX=10090 GN=Rpl12 PE=1 SV=1                                                | 0.3475889  | 0.0118  |
| Isoleucine--tRNA ligase, mitochondrial OS=Mus musculus OX=10090 GN=lars2 PE=1 SV=1                               | 0.7028074  | 0.0134  |
| 60 kDa heat shock protein, mitochondrial OS=Mus musculus OX=10090 GN=Hspd1 PE=1 SV=1                             | 0.349204   | 0.0142  |
| MKIAA0031 protein (Fragment) OS=Mus musculus OX=10090 GN=Efud2 PE=2 SV=1                                         | 0.1911965  | 0.0148  |
| U2 small nuclear ribonucleoprotein A' OS=Mus musculus OX=10090 GN=Snrpa1 PE=1 SV=2                               | 0.6611579  | 0.0153  |
| Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial | 0.5248387  | 0.0199  |
| Actin-related protein 2/3 complex subunit 5 OS=Mus musculus OX=10090 GN=Arpc5 PE=2 SV=1                          | 0.5318125  | 0.0205  |
| V-type proton ATPase subunit d1 OS=Mus musculus OX=10090 GN=Atp6v0d1 PE=1 SV=2                                   | 0.8293243  | 0.0235  |
| Dihydropyrimidine-related protein 3 OS=Mus musculus OX=10090 GN=Dpysl3 PE=1 SV=1                                 | 1.056176   | 0.0254  |
| Uncharacterized protein OS=Mus musculus OX=10090 GN=Hnnpa1 PE=2 SV=1                                             | 0.249095   | 0.026   |
| Calcium-binding protein 39 OS=Mus musculus OX=10090 GN=Cab39 PE=1 SV=2                                           | 0.2883492  | 0.0274  |
| 60S ribosomal protein L21 OS=Mus musculus OX=10090 GN=Rpl21 PE=1 SV=3                                            | 0.6686273  | 0.0277  |
| Superoxide dismutase [Mn], mitochondrial OS=Mus musculus OX=10090 GN=Sod2 PE=1 SV=3                              | 0.5347019  | 0.0283  |
| U2 snRNP-associated SURP motif-containing protein OS=Mus musculus OX=10090 GN=U2surp PE=1 SV=3                   | 0.6827661  | 0.0307  |
| PUA domain-containing protein OS=Mus musculus OX=10090 GN=Dkc1 PE=2 SV=1                                         | 0.5155536  | 0.0319  |
| B5 domain-containing protein OS=Mus musculus OX=10090 GN=Farsh PE=2 SV=1                                         | 0.3998789  | 0.0322  |
| Signal recognition particle receptor subunit beta OS=Mus musculus OX=10090 GN=Sprpb PE=1 SV=1                    | 0.5996106  | 0.0331  |
| CTP synthase 1 OS=Mus musculus OX=10090 GN=Ctsp1 PE=1 SV=2                                                       | 0.4946502  | 0.0358  |
| Acidic leucine-rich nuclear phosphoprotein 32 family member A OS=Mus musculus OX=10090 GN=Anp32a PE=1 SV=1       | 0.6922235  | 0.0368  |
| Adenylosuccinate synthetase isozyme 1 OS=Mus musculus OX=10090 GN=Adssl1 PE=1 SV=1                               | 0.4157413  | 0.037   |
| Protein transport protein Sec31A OS=Mus musculus OX=10090 GN=Sec31a PE=1 SV=2                                    | 1.0520077  | 0.0375  |
| Short-chain specific acyl-CoA dehydrogenase, mitochondrial OS=Mus musculus OX=10090 GN=Acads PE=1 SV=2           | 0.4885551  | 0.038   |
| Ubiquitin carboxyl-terminal hydrolase isozyme L3 OS=Mus musculus OX=10090 GN=Uchl3 PE=1 SV=2                     | 0.5599831  | 0.039   |
| Charged multivesicular body protein 5 OS=Mus musculus OX=10090 GN=Chmp5 PE=1 SV=1                                | 0.52434    | 0.0401  |
| Beta-enolase OS=Mus musculus OX=10090 GN=Eno3 PE=1 SV=3                                                          | 0.8719059  | 0.0404  |
| Eukaryotic translation initiation factor 3 subunit F OS=Mus musculus OX=10090 GN=Elf3f PE=2 SV=1                 | 0.4865929  | 0.0408  |
| AA_TRNA_LIGASE_II domain-containing protein OS=Mus musculus OX=10090 GN=Dars PE=2 SV=1                           | 0.5710718  | 0.0411  |
| ATP-dependent 6-phosphofructokinase, muscle type OS=Mus musculus OX=10090 GN=Pfkf PE=1 SV=3                      | 0.1612043  | 0.043   |
| FERM domain-containing protein (Fragment) OS=Mus musculus OX=10090 GN=Rdx PE=2 SV=1                              | 0.4890448  | 0.0438  |
| Proteasome subunit alpha type-3 OS=Mus musculus OX=10090 GN=Pma3 PE=1 SV=3                                       | 0.2973517  | 0.0439  |
| Acidic leucine-rich nuclear phosphoprotein 32 family member B OS=Mus musculus OX=10090 GN=Anp32b PE=1 SV=1       | 1.068505   | <.0001  |
| ADP/ATP translocase 1 OS=Mus musculus OX=10090 GN=Slc25a4 PE=1 SV=4                                              | 0.6159321  | 0.0007  |
| Annexin A3 OS=Mus musculus OX=10090 GN=Anxa3 PE=1 SV=4                                                           | 0.4695238  | 0.0012  |
| CS domain-containing protein OS=Mus musculus OX=10090 GN=Ptges3 PE=2 SV=1                                        | 1.299711   | 0.0013  |
| SUMO-conjugating enzyme UBC9 OS=Mus musculus OX=10090 GN=Ube2l PE=1 SV=1                                         | 1.0482371  | 0.0013  |
| Mitochondrial-processing peptidase subunit beta OS=Mus musculus OX=10090 GN=Pmpcb PE=1 SV=1                      | 1.1265736  | 0.0015  |
| Uncharacterized protein OS=Mus musculus OX=10090 GN=Ctnna1 PE=2 SV=1                                             | 2.4221269  | 0.0025  |
| MKIAA4075 protein (Fragment) OS=Mus musculus OX=10090 GN=Chd4 PE=2 SV=1                                          | 0.5678949  | 0.0036  |
| Cytochrome c oxidase subunit 5A, mitochondrial OS=Mus musculus OX=10090 GN=Cox5a PE=1 SV=2                       | 0.7179645  | 0.0039  |
| Calmodulin-1 OS=Mus musculus OX=10090 GN=Calm1 PE=1 SV=1                                                         | 0.2818568  | 0.0041  |
| Aldehdh domain-containing protein OS=Mus musculus OX=10090 GN=Aldh9a1 PE=2 SV=1                                  | 0.2379044  | 0.0043  |
| Glutathione S-transferase A3 OS=Mus musculus OX=10090 GN=Gsta3 PE=1 SV=2                                         | 0.867562   | 0.0048  |
| 3'[2],5'-bisphosphate nucleotidase 1 OS=Mus musculus OX=10090 GN=Bpnt1 PE=1 SV=1                                 | 1.1150049  | 0.005   |
| 60S ribosomal protein L3 OS=Mus musculus OX=10090 GN=Rpl3 PE=1 SV=3                                              | 0.171673   | 0.0057  |
| KH domain-containing, RNA-binding, signal transduction-associated protein 1                                      | 0.444941   | 0.0068  |
| W2 domain-containing protein OS=Mus musculus OX=10090 GN=Elf5 PE=1 SV=1                                          | 0.2096931  | 0.0094  |
| Heterogeneous nuclear ribonucleoprotein H3 OS=Mus musculus OX=10090 GN=Hnnp3 PE=1 SV=1                           | 0.3374609  | 0.0114  |
| Vesicle-associated membrane protein-associated protein A OS=Mus musculus OX=10090 GN=Vapa PE=1 SV=2              | 0.5409939  | 0.0129  |
| Uncharacterized protein OS=Mus musculus OX=10090 GN=Hdlbp PE=2 SV=1                                              | 0.3301349  | 0.0132  |
| Very-long-chain 3-oxoacyl-CoA reductase OS=Mus musculus OX=10090 GN=Hsd17b12 PE=1 SV=1                           | 0.5683522  | 0.0132  |
| High mobility group box 3 OS=Mus musculus OX=10090 GN=Hmgb3 PE=1 SV=1                                            | 1.2426177  | 0.0138  |
| Serine hydroxymethyltransferase, mitochondrial OS=Mus musculus OX=10090 GN=Shmt2 PE=1 SV=1                       | 0.1439534  | 0.0145  |
| Spermidine synthase OS=Mus musculus OX=10090 GN=Srm PE=1 SV=1                                                    | 0.6607884  | 0.0155  |
| Drebrin-like protein OS=Mus musculus OX=10090 GN=Dbrl PE=1 SV=2                                                  | 0.7851284  | 0.0159  |
| Malate dehydrogenase, mitochondrial OS=Mus musculus OX=10090 GN=Mdh2 PE=1 SV=3                                   | 0.390868   | 0.0165  |
| Biliverdin reductase A OS=Mus musculus OX=10090 GN=Blvra PE=1 SV=1                                               | 0.5551517  | 0.0168  |
| Glutamine-fructose-6-phosphate aminotransferase [isomerizing] 1 OS=Mus musculus OX=10090 GN=Gfpt1 PE=1 SV=3      | 0.4344016  | 0.0172  |
| Electron transfer flavoprotein subunit beta OS=Mus musculus OX=10090 GN=Etfb PE=1 SV=3                           | 0.7499795  | 0.0186  |
| Sister chromatid cohesion protein PD55 homolog B OS=Mus musculus OX=10090 GN=Pds5b PE=1 SV=1                     | 1.9540072  | 0.0186  |
| Heat shock protein HSP 90-alpha OS=Mus musculus OX=10090 GN=Hsp90aa1 PE=1 SV=4                                   | 0.254302   | 0.0197  |
| DNA replication licensing factor MCM5 OS=Mus musculus OX=10090 GN=Mcm5 PE=1 SV=2                                 | 0.8396487  | 0.0202  |
| Uncharacterized protein OS=Mus musculus OX=10090 GN=Prep PE=2 SV=1                                               | 0.49785    | 0.0209  |
| Methionine adenosyltransferase 2 subunit beta OS=Mus musculus OX=10090 GN=Mat2b PE=1 SV=1                        | 0.5322213  | 0.0221  |
| Ferritin light chain 1 OS=Mus musculus OX=10090 GN=Ft11 PE=1 SV=2                                                | 0.3320784  | 0.0248  |
| RNA-binding protein 39 OS=Mus musculus OX=10090 GN=Rbm39 PE=1 SV=2                                               | 0.6278944  | 0.0256  |
| NHP2-like protein 1 OS=Mus musculus OX=10090 GN=Snru13 PE=1 SV=4                                                 | 0.3252988  | 0.0276  |
| Apoptosis-associated speck-like protein containing CARD OS=Mus musculus OX=10090 GN=Pycard PE=1 SV=1             | 0.6675936  | 0.0276  |
| Condensin complex subunit 1 OS=Mus musculus OX=10090 GN=Ncapd2 PE=1 SV=1                                         | 0.971441   | 0.0276  |
| Dihydropyrimidine-related protein 2 OS=Mus musculus OX=10090 GN=Dpysl2 PE=1 SV=2                                 | 0.4137595  | 0.0282  |
| Leucine-rich repeat flightless-interacting protein 1 (Fragment) OS=Mus musculus OX=10090 GN=Lrrfip1 PE=1 SV=1    | 0.5790959  | 0.0294  |
| NAD-dependent malic enzyme, mitochondrial OS=Mus musculus OX=10090 GN=Me2 PE=1 SV=1                              | 0.6671194  | 0.0294  |
| Heterogeneous nuclear ribonucleoprotein A0 OS=Mus musculus OX=10090 GN=Hnnpa0 PE=1 SV=1                          | 0.4221518  | 0.0302  |
| Hepatoma-derived growth factor OS=Mus musculus OX=10090 GN=Hdgf PE=1 SV=2                                        | 0.5918044  | 0.0313  |
| BTB/P02 domain-containing protein KCTD12 OS=Mus musculus OX=10090 GN=Kctd12 PE=1 SV=1                            | 0.7074584  | 0.0325  |
| Ribose-phosphate pyrophosphokinase 2 OS=Mus musculus OX=10090 GN=Prps2 PE=1 SV=4                                 | 0.6720932  | 0.0363  |
| Proteasome subunit beta type-5 OS=Mus musculus OX=10090 GN=Psb5 PE=1 SV=3                                        | 0.4788725  | 0.0366  |
| Histone-binding protein RBBP7 OS=Mus musculus OX=10090 GN=Rbbp7 PE=1 SV=1                                        | 0.4970637  | 0.0388  |
| Transformer-2 protein homolog beta OS=Mus musculus OX=10090 GN=Tra2b PE=1 SV=1                                   | 1.8589901  | 0.0403  |
| H-2 class II histocompatibility antigen, D-K alpha chain OS=Mus musculus OX=10090 GN=H2-D1 PE=4 SV=2             | 1.400861   | 0.0459  |
| Proteasome subunit alpha type OS=Mus musculus OX=10090 GN=Pma1 PE=1 SV=1                                         | 0.3798642  | 0.0464  |

| List of significant genes IMG - Microglia LPS vs LPS+Pioglitazone                                                 | Difference | P- Value |
|-------------------------------------------------------------------------------------------------------------------|------------|----------|
| Tripeptidyl-peptidase 2 OS=Mus musculus OX=10090 GN=Thpp2 PE=1 SV=3                                               | 0.3889367  | 0.0019   |
| B5 domain-containing protein OS=Mus musculus OX=10090 GN=Far5b PE=2 SV=1                                          | 0.5957108  | 0.0035   |
| L-lactate dehydrogenase OS=Mus musculus OX=10090 GN=Ldha PE=2 SV=1                                                | 0.4702607  | 0.0039   |
| Small glutamine-rich tetratricopeptide repeat-containing protein alpha OS=Mus musculus OX=10090 GN=Sgta PE=1 SV=2 | 1.3850286  | 0.006    |
| Uncharacterized protein OS=Mus musculus OX=10090 GN=Api5 PE=2 SV=1                                                | 0.7025573  | 0.0061   |
| Protein transport protein Sec31A OS=Mus musculus OX=10090 GN=Sec31a PE=1 SV=2                                     | 1.4117442  | 0.0079   |
| Rps6ka3 protein (Fragment) OS=Mus musculus OX=10090 GN=Rps6ka3 PE=2 SV=1                                          | 0.9473473  | 0.0091   |
| Alpha-actinin-1 OS=Mus musculus OX=10090 GN=Actn1 PE=1 SV=1                                                       | 0.4603555  | 0.0102   |
| Calmodulin-1 OS=Mus musculus OX=10090 GN=Calm1 PE=1 SV=1                                                          | 0.2364897  | 0.0116   |
| Ptms protein OS=Mus musculus OX=10090 GN=Ptms PE=2 SV=1                                                           | 1.4465407  | 0.0155   |
| Env polypeptide OS=Mus musculus OX=10090 PE=4 SV=1                                                                | 0.6166565  | 0.0157   |
| S-formylglutathione hydrolase OS=Mus musculus OX=10090 GN=Esd PE=1 SV=1                                           | 0.838518   | 0.0167   |
| Structural maintenance of chromosomes protein 1A OS=Mus musculus OX=10090 GN=Smc1a PE=1 SV=4                      | 0.2711534  | 0.0177   |
| Enoyl-CoA delta isomerase 1, mitochondrial OS=Mus musculus OX=10090 GN=Eci1 PE=1 SV=2                             | 0.5809214  | 0.0178   |
| Uncharacterized protein OS=Mus musculus OX=10090 GN=Lcp1 PE=2 SV=1                                                | 0.1872979  | 0.0188   |
| 60 kDa heat shock protein, mitochondrial OS=Mus musculus OX=10090 GN=Hspd1 PE=1 SV=1                              | 0.3177963  | 0.0234   |
| D-3-phosphoglycerate dehydrogenase OS=Mus musculus OX=10090 GN=Phgdh PE=1 SV=3                                    | 0.7465025  | 0.0277   |
| Proteasome subunit alpha type OS=Mus musculus OX=10090 GN=Psm5 PE=2 SV=1                                          | 0.2220701  | 0.0326   |
| EH-domain containing 1 (Fragment) OS=Mus musculus OX=10090 GN=Ehd1 PE=2 SV=2                                      | 0.1837231  | 0.0465   |
| Septin-9 OS=Mus musculus OX=10090 GN=Septin9 PE=1 SV=1                                                            | 0.4459096  | 0.0003   |
| Adenylosuccinate synthetase isozyme 2 OS=Mus musculus OX=10090 GN=Adss PE=1 SV=1                                  | 0.7347295  | 0.0007   |
| Isopentenyl-diphosphate Delta-isomerase 1 OS=Mus musculus OX=10090 GN=Idi1 PE=1 SV=1                              | 0.3162207  | 0.0012   |
| Heat shock protein 1-like protein OS=Mus musculus OX=10090 PE=3 SV=1                                              | 0.3319991  | 0.0016   |
| Insulin degrading enzyme OS=Mus musculus OX=10090 GN=Ide PE=2 SV=1                                                | 0.6335591  | 0.0024   |
| Flavin reductase (NADPH) OS=Mus musculus OX=10090 GN=Blvrb PE=1 SV=3                                              | 0.5303363  | 0.0031   |
| Heterogeneous nuclear ribonucleoprotein H3 OS=Mus musculus OX=10090 GN=Hnrrnp3 PE=1 SV=1                          | 0.4089122  | 0.0037   |
| F-actin-capping protein subunit alpha OS=Mus musculus OX=10090 GN=Capza1 PE=2 SV=1                                | 0.4577389  | 0.0038   |
| Mitogen-activated protein kinase OS=Mus musculus OX=10090 GN=Mapk3 PE=1 SV=1                                      | 0.3804405  | 0.0047   |
| Tyrosine-protein kinase BAZ1B OS=Mus musculus OX=10090 GN=Baz1b PE=1 SV=2                                         | 0.5728954  | 0.0049   |
| Histone H1.0 OS=Mus musculus OX=10090 GN=H1-0 PE=2 SV=4                                                           | 0.823047   | 0.0049   |
| Annexin A3 OS=Mus musculus OX=10090 GN=Anxa3 PE=1 SV=4                                                            | 0.3780426  | 0.005    |
| Calpain-2 catalytic subunit OS=Mus musculus OX=10090 GN=Capn2 PE=1 SV=4                                           | 0.3159963  | 0.0058   |
| RNA-binding protein Raly OS=Mus musculus OX=10090 GN=Raly PE=1 SV=3                                               | 1.2245949  | 0.006    |
| Uncharacterized protein (Fragment) OS=Mus musculus OX=10090 GN=Ptma PE=2 SV=1                                     | 0.3713224  | 0.0068   |
| X-ray repair cross-complementing protein 6 OS=Mus musculus OX=10090 GN=Xrcc6 PE=1 SV=1                            | 0.2668957  | 0.0071   |
| Delta-1-pyrroline-5-carboxylate synthase OS=Mus musculus OX=10090 GN=Aldh18a1 PE=2 SV=1                           | 0.4856325  | 0.0093   |
| Myosin light polypeptide 6 OS=Mus musculus OX=10090 GN=Myl6 PE=1 SV=1                                             | 0.4300461  | 0.0101   |
| 40S ribosomal protein S13 OS=Mus musculus OX=10090 GN=Rps13 PE=1 SV=2                                             | 0.3488249  | 0.0101   |
| Trifunctional enzyme subunit beta, mitochondrial OS=Mus musculus OX=10090 GN=Hadhb PE=1 SV=1                      | 0.6677157  | 0.0107   |
| Fibronectin OS=Mus musculus OX=10090 GN=Fn1 PE=1 SV=4                                                             | 0.5742499  | 0.011    |
| BTB/POZ domain-containing protein KCTD12 OS=Mus musculus OX=10090 GN=Kctd12 PE=1 SV=1                             | 0.8481502  | 0.0126   |
| Importin subunit alpha-3 OS=Mus musculus OX=10090 GN=Kpna4 PE=1 SV=1                                              | 0.4327818  | 0.0129   |
| ATP-dependent RNA helicase DDX18 OS=Mus musculus OX=10090 GN=Ddx18 PE=1 SV=1                                      | 0.7026569  | 0.0135   |
| Serine hydroxymethyltransferase, mitochondrial OS=Mus musculus OX=10090 GN=Shmt2 PE=1 SV=1                        | 0.143302   | 0.0148   |
| Calcium-binding protein 39 OS=Mus musculus OX=10090 GN=Cab39 PE=1 SV=2                                            | 0.3129251  | 0.018    |
| Heat shock 70 kDa protein 1B OS=Mus musculus OX=10090 GN=Hspa1b PE=1 SV=3                                         | 0.4866949  | 0.0185   |
| MKIAA0225 protein (Fragment) OS=Mus musculus OX=10090 GN=Nup205 PE=2 SV=1                                         | 0.4751356  | 0.0188   |
| Nucleoside diphosphate kinase OS=Mus musculus OX=10090 GN=Gm20390 PE=3 SV=1                                       | 0.4789956  | 0.0201   |
| Isocitrate dehydrogenase [NAD] subunit, mitochondrial OS=Mus musculus OX=10090 GN=Idh3b PE=1 SV=1                 | 0.4864369  | 0.0216   |
| Ras-related protein Rab-18 OS=Mus musculus OX=10090 GN=Rab18 PE=1 SV=1                                            | 0.7439698  | 0.0221   |
| Deoxyuridine triphosphatase OS=Mus musculus OX=10090 GN=Dut PE=1 SV=1                                             | 0.4564682  | 0.0255   |
| Acidic leucine-rich nuclear phosphoprotein 32 family member B OS=Mus musculus OX=10090 GN=Anp32b PE=1 SV=1        | 0.1960181  | 0.0263   |
| Heterogeneous nuclear ribonucleoprotein U-like protein 2 OS=Mus musculus OX=10090 GN=Hnrrnpul2 PE=1 SV=2          | 0.7402061  | 0.0265   |
| Glutathione S-transferase A3 OS=Mus musculus OX=10090 GN=Gsta3 PE=1 SV=2                                          | 0.6264115  | 0.0289   |
| Histone H2A.Z OS=Mus musculus OX=10090 GN=H2az1 PE=1 SV=2                                                         | 0.4656709  | 0.0295   |
| W2 domain-containing protein OS=Mus musculus OX=10090 GN=Eif5 PE=1 SV=1                                           | 0.1690029  | 0.0296   |
| Sorting nexin-6 OS=Mus musculus OX=10090 GN=Snx6 PE=1 SV=2                                                        | 0.2854351  | 0.0297   |
| Signal transducer and activator of transcription OS=Mus musculus OX=10090 GN=Stat1 PE=1 SV=1                      | 0.2592257  | 0.0305   |
| Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial OS=Mus musculus OX=10090 GN=Hadh PE=1 SV=2                    | 0.5676971  | 0.0323   |
| 26S proteasome regulatory subunit 4 OS=Mus musculus OX=10090 GN=Psmc1 PE=1 SV=1                                   | 0.1911803  | 0.0357   |
| Staphylococcal nuclease domain-containing protein OS=Mus musculus OX=10090 GN=Snd1 PE=2 SV=1                      | 0.1737097  | 0.0358   |
| Pinin OS=Mus musculus OX=10090 GN=Pnn PE=1 SV=1                                                                   | 0.3047657  | 0.0369   |
| Fatty acid-binding protein, adipocyte OS=Mus musculus OX=10090 GN=Fabp4 PE=1 SV=3                                 | 0.6618099  | 0.0381   |
| Osteoclast-stimulating factor 1 OS=Mus musculus OX=10090 GN=Ostf1 PE=1 SV=2                                       | 0.3775627  | 0.0385   |
| ADP/ATP translocase 1 OS=Mus musculus OX=10090 GN=Slc25a4 PE=1 SV=4                                               | 0.3058736  | 0.0407   |
| Uncharacterized protein OS=Mus musculus OX=10090 GN=Nap114 PE=2 SV=1                                              | 0.2178495  | 0.0446   |
| Vesicle-trafficking protein SEC22b OS=Mus musculus OX=10090 GN=Sec22b PE=1 SV=3                                   | 0.4329626  | 0.045    |
| Heterogeneous nuclear ribonucleoprotein D-like OS=Mus musculus OX=10090 GN=Hnrrnpdl PE=1 SV=1                     | 0.4128299  | 0.0481   |
| Barrier-to-autointegration factor OS=Mus musculus OX=10090 GN=Banf1 PE=1 SV=1                                     | 0.3693948  | 0.0481   |
| ELAV-like protein 1 OS=Mus musculus OX=10090 GN=Elavl1 PE=1 SV=2                                                  | 0.6690994  | 0.0482   |
| Alcohol dehydrogenase class-3 OS=Mus musculus OX=10090 GN=Adh5 PE=1 SV=3                                          | 0.4458014  | 0.0488   |

## Conclusion

Target engagement was demonstrated with our top performing candidate compounds.

We noted that Zileuton, Pioglitazone and Liraglutide showed some of the most potent effect *in vitro*, ameliorating some of the inflammatory effects of LPS in astrocytes and microglia.

Our proteomic study showed greater effect of Zileuton and Pioglitazone on microglia proteome response.

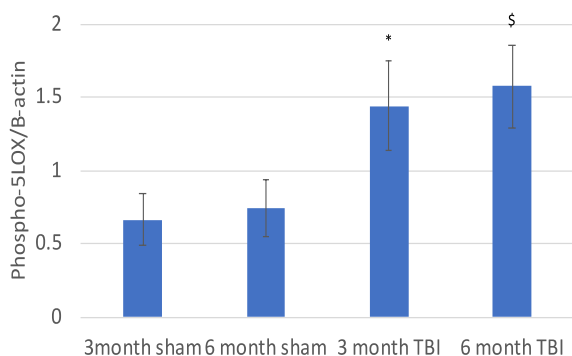
## Summary – Fourth Quarter.

Confirming **target engagement markers** p5-LOX, pPPARgamma, pPI3K, p-p38 MAPK and p-AKT levels in our new 20hit model.

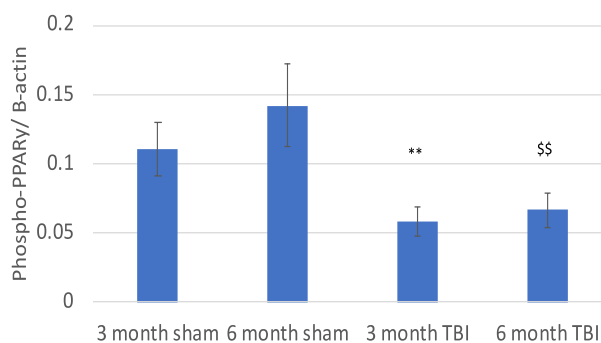
# 5 to20 hit translation work

Aim 1- To see if the targets identified in the 5 model faithfully translate across to the 20 hit model.

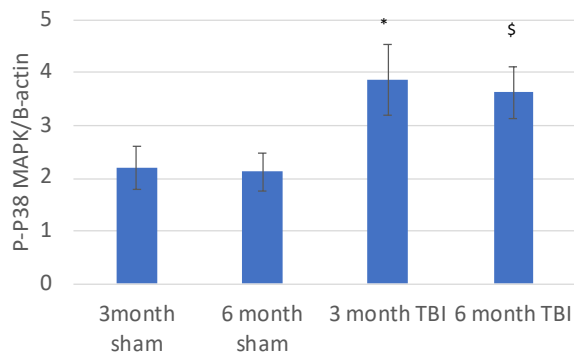
Aim 2- To determine if the targets identified at the 3 month time point would further persist if therapeutic intervention strategies were not applied.



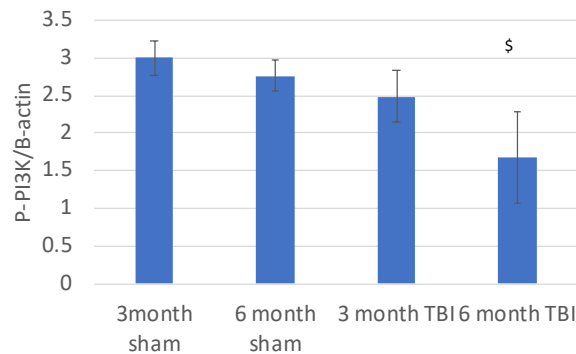
A graph showing the protein expression of Phospho- 5-Lipoxygenase (P-5-LOX) (Serine 663) in the cytoplasmic/ nuclear fraction of cortex homogenate derived from sham and injured wild type mice at 3 and 6 months post injury (N=4 group). Data were normalized to total 5-LOX and to B-actin. Data were assessed for normality using the Shapiro-Wilk test and then analyzed using a one way Anova and then corrected for family wise error rate using the Benjamini Hochberg and Yekutieli correction for false discovery rate. 3 month Sham Vs 3 month TBI  $P=0.0207$  (Indicated by \*). 6 month Sham vs 6 month TBI  $P=0.0114$  (indicated by \$). Error bars represent SEM.



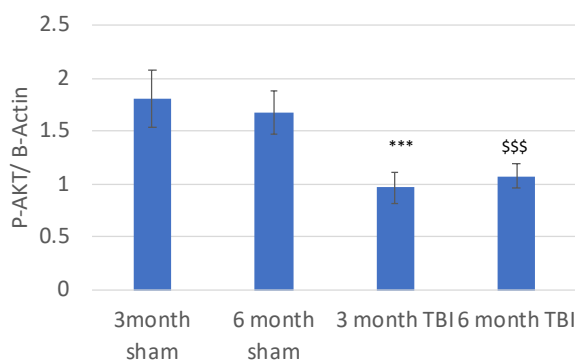
A graph showing the protein expression of Phospho-Peroxisome proliferator activated receptor gamma (PPARγ) (Serine 112) in the cytoplasmic/ nuclear fraction of cortex homogenate derived from sham and injured wild type mice at 3 and 6 months post injury (N=4 group). Data were normalized to B-actin. Data were assessed for normality using the Shapiro-Wilk test and then analyzed using a one way Anova and then corrected for family wise error rate using the Benjamini Hochberg and Yekutieli correction for false discovery rate. 3 month Sham Vs 3 month TBI  $P=0.00461$  (Indicated by \*\*). 6 month Sham vs 6 month TBI  $P=0.0083$  (indicated by \$\$). Error bars represent SEM.



A graph showing the protein expression of Phospho-P38 Mitogen associated protein kinase (P-P38 MAPK) (Threonine 180/tyrosine 182) in the cytoplasmic/ nuclear fraction of cortex homogenate derived from sham and injured wild type mice at 3 and 6 months post injury (N=4 group). Data were normalized to B-actin. Data were assessed for normality using the Shapiro-Wilk test and then analyzed using a one way Anova and then corrected for family wise error rate using the Benjamini Hochberg and Yekutieli correction for false discovery rate. 3 month Sham Vs 3 month TBI  $P=0.0194$  (Indicated by \*). 6 month Sham vs 6 month TBI  $P=0.0083$  (indicated by \$). Error bars represent SEM.



A graph showing the protein expression of Phospho-phosphoinositide 3-kinase (P-PI3K) (Tyrosine458/ Tyrosine199) in the cytoplasmic/ nuclear fraction of cortex homogenate derived from sham and injured wild type mice at 3 and 6 months post injury (N=4 group). Data were normalized to B-actin. Data were assessed for normality using the Shapiro-Wilk test and then analyzed using a one way Anova and then corrected for family wise error rate using the Benjamini Hochberg and Yekutieli correction for false discovery rate. 6 month Sham vs 6 month TBI  $P=0.0178$  (indicated by \$). Error bars represent SEM.



A graph showing the protein expression of Phospho-AKT(P-AKT) (Serine 473) in the cytoplasmic/ nuclear fraction of cortex homogenate derived from sham and injured wild type mice at 3 and 6 months post injury (N=4 group). Data were normalized to B-actin. Data were assessed for normality using the Shapiro-Wilk test and then analyzed using a one way Anova and then corrected for family wise error rate using the Benjamini Hochberg and Yekutieli correction for false discovery rate. 3 month Sham Vs 3 month TBI  $P<0.001$  (Indicated by \*\*\*). 6 month Sham vs 6 month TBI  $P<0.001$  (indicated by \$\$\$). . Error bars represent SEM.

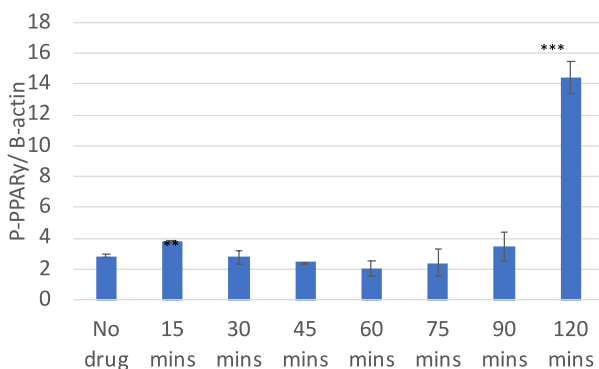
## Conclusion

We observed significant changes in our target engagement markers ‘p5-LOX, pPPARgamma, pPI3K, p-p38 MAPK and p-AKT’ levels in our 20 hit model at chronic timepoints post exposure (at 3 and/or 6 months post-exposure).

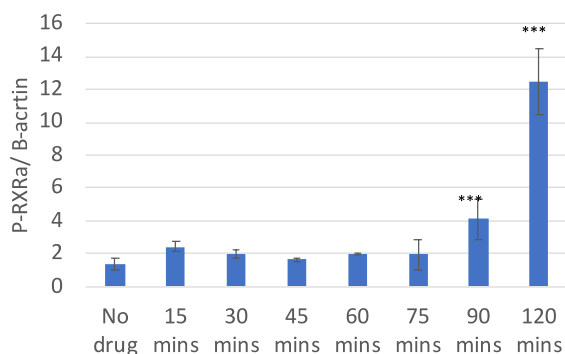
Determining **optimal pretreatment** timepoint to observe beneficial effect with our top four compounds (Zileuton is omitted from this presentation).

## In vitro work – Drug timing optimization

Aim- Investigate drug timing with response of primary target of drug to optimize in vitro drug treatment paradigm

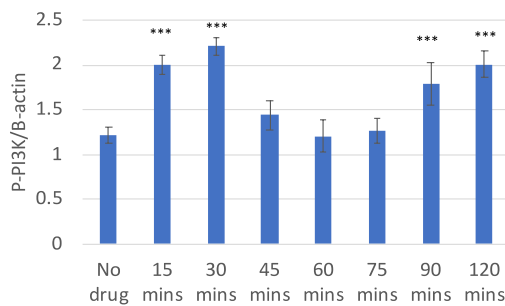


A graph showing the protein expression of Phospho-PPARγ (Serine 112) expression in the cytoplasmic/nuclear fraction of IMG immortalized microglial cells (N=3/group). Data were normalized to B-actin. Cells were treated with DMEM/F12 media containing Pioglitazone @10uM for 15- 120 minutes to identify the peak of temporal target engagement. Data were assessed for normality using the Shapiro Wilk test and significance was assessed by a students T-test of a treated time point Vs the untreated control. No drug control Vs 15 minutes treated samples  $P=0.001$  (Indicated by \*\*) No drug control Vs 120 minutes treated  $P<0.001$  (Indicated by \*\*\*). Error bars represent SEM.

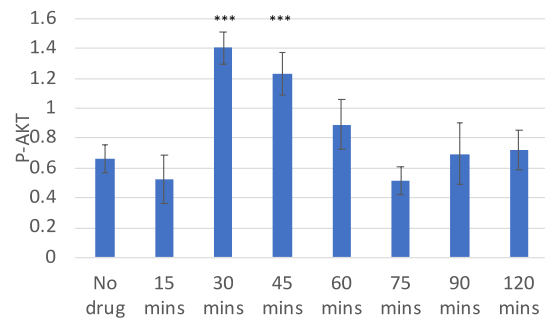


A graph showing the protein expression of Phospho-RXRα (Serine 112) expression in the cytoplasmic/nuclear fraction of IMG immortalized microglial cells (N=3/group). Data were normalized to B-actin. Cells were treated with DMEM/F12 media containing Pioglitazone @10uM for 15- 120 minutes to identify the peak of temporal target engagement. Data were assessed for normality using the Shapiro Wilk test and significance was assessed by a students T-test of a treated time point Vs the untreated control. No drug control Vs 120 minutes treated  $P<0.001$  (Indicated by \*\*\*). No drug control Vs 90 minutes treated  $P<0.001$  (Indicated by \*\*\*). Error bars represent SEM.

## In vitro work – Drug timing optimization-Insulin

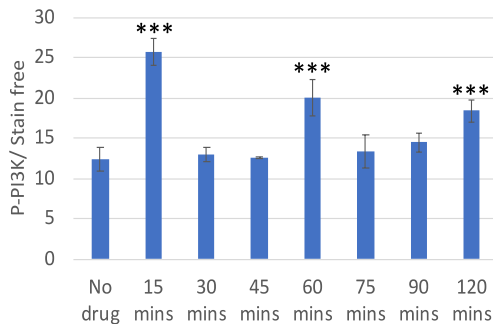


A graph showing the protein expression of Phospho- PI3K (Tyrosine458/ Tyrosine199) expression in the cytoplasmic/ nuclear fraction of IMG immortalized microglial cells (N=3/group). Data were normalized to B-actin. Cells were treated with DMEM/F12 media containing Insulin @25nM for 15- 120 minutes to identify the peak of temporal target engagement. Data were assessed for normality using the Shapiro Wilk test and significance was assessed by a students T-test of a treated time point Vs the untreated control. No drug control Vs 15 minutes treated samples  $P<0.001$  (Indicated by \*\*\*). No drug control Vs 30 minutes treated samples  $P<0.001$  (Indicated by \*\*\*). No drug control Vs 90 minutes treated samples  $P<0.001$  (Indicated by \*\*\*). No drug control Vs 120 minutes treated samples  $P<0.001$  (Indicated by \*\*\*). Error bars represent SEM.

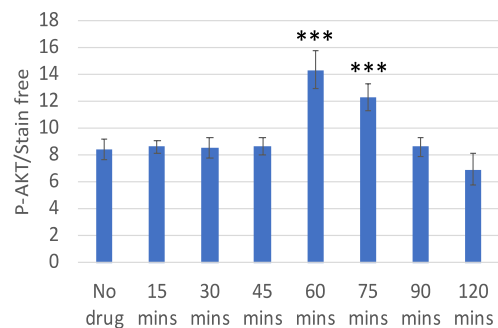


A graph showing the protein expression of Phospho-AKT (Serine 473) expression in the cytoplasmic/ nuclear fraction of IMG immortalized microglial cells (N=3/group). Data were normalized to B-actin. Cells were treated with DMEM/F12 media containing Insulin @25nM for 15- 120 minutes to identify the peak of temporal target engagement. Data were assessed for normality using the Shapiro Wilk test and significance was assessed by a students T-test of a treated time point Vs the untreated control. No drug control Vs 30 minutes treated samples  $P<0.001$  (Indicated by \*\*\*). No drug control Vs 45 minutes treated samples  $P<0.001$  (Indicated by \*\*\*). Error bars represent SEM.

## In vitro work – Drug timing optimization-Liraglutide



A graph showing the protein expression of Phospho- PI3K (Tyrosine458/ Tyrosine199) expression in the cytoplasmic/ nuclear fraction of IMG immortalized microglial cells (N=3/group). Data were normalized to B-actin. Cells were treated with DMEM/F12 media containing Insulin @25nM for 15- 120 minutes to identify the peak of temporal target engagement. Data were assessed for normality using the Shapiro Wilk test and significance was assessed by a students T-test of a treated time point Vs the untreated control. No drug control Vs 15 minutes treated samples  $P<0.001$  (Indicated by \*\*\*). No drug control Vs 60 minutes treated samples  $P<0.001$  (Indicated by \*\*\*). No drug control Vs 120 minutes treated samples  $P<0.001$  (Indicated by \*\*\*). Error bars represent SEM.



A graph showing the protein expression of Phospho-AKT (Serine 473) expression in the cytoplasmic/ nuclear fraction of IMG immortalized microglial cells (N=3/group). Data were normalized to B-actin. Cells were treated with DMEM/F12 media containing Liraglutide @25nM for 15- 120 minutes to identify the peak of temporal target engagement. Data were assessed for normality using the Shapiro Wilk test and significance was assessed by a students T-test of a treated time point Vs the untreated control. No drug control Vs 60 minutes treated samples  $P<0.001$  (Indicated by \*\*\*). No drug control Vs 75 minutes treated samples  $P<0.001$  (Indicated by \*\*\*). Error bars represent SEM.

## **Conclusion**

Pre-treatment timepoint can significantly impact on the outcome of target engagement depending on the site of action of the drug compound.

For example, effects of Pioglitazone are not observed until after 2hrs post treatment, suggesting that intracellular transport mechanisms may slow down the time to observe phospho-proteomic changes. Liraglutide which acts on the cell surface (GLP-1 receptors) elicits an immediate effect within 15 mins of incubation.

These pre-treatment timepoints need to be considered for future in vitro screening studies.

We have now begun the *in vivo* long-term treatment with our top two compounds Pioglitazone and Zileuton selected from our in vitro and short-term in vivo study. Data on all cohorts will be available in March 2021.

## **What opportunities for training and professional development has the project provided?**

*If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.*

Nothing to Report

## **How were the results disseminated to communities of interest?**

*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.*

Nothing to Report

**What do you plan to do during the next reporting period to accomplish the goals?**

*If this is the final report, state "Nothing to Report."*

*Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

We are currently ageing injured mice or administering drug compounds to injured mice for the planned behavioral and neuropathological/molecular analyses below.

**OVER THE NEXT QUARTER WE PLAN TO CONTINUE OR BEGIN:**

**MAJOR TASK TWO**

***Subtask 2: Administration of therapeutic compounds to mice for 3 months beginning at 3 months to 6 months post TBI/sham -***

Subtask 2a: Administration of Top performing therapeutic compound I - ***(Completion in the next quarter, November 2020)***

Subtask 2b: Administration of Top performing therapeutic compound II- ***(Completion in the next two quarters, March 2020)***

***Subtask 3: Neurobehavioral testing of mice in the two weeks prior to 6 months post injury timepoint - (100% completed for the first cohort of mice in Nov-Dec 2020, remaining half cohort will be completed in the next two quarter March 2021)***

At the last two weeks prior to the 6 month post-injury time-point, animals will undergo neurobehavioral testing in the Barnes maze for 7 days, followed by rotarod test , open field and elevated plus maze tests.

***Subtask 4: Euthanasia of mice at 6 months post-TBI/sham, followed by further evaluation of outcomes -***

Subtask 4a: Brain neuropathological analyses (sectioning, immunostaining, quantitative analyses)

Subtask 4b: Molecular Omics and antibody based analyses (RNAseq, western blotting, ELISA) ***(50% completed for the first cohort of mice in the next quarter Dec-Jan 2021, remaining half cohort will be completed in the next two quarter April 2021)***

**ALL plans are consistent with the original approved SOW.**

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

**What was the impact on the development of the principal discipline(s) of the project?**

*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).*

*Nothing to Report*

**What was the impact on other disciplines?**

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.*

*Nothing to Report*

**What was the impact on technology transfer?**

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:*

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

*Nothing to Report*

**What was the impact on society beyond science and technology?**

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:*

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

*Nothing to Report*

- 5. CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:*

Due to COVID-19, there has been a small reduction in work output at the Roskamp Institute. This has set us back by around 6 months. However, work is now progressing as normal and we aim to gain grounds in the work out over the next few quarters, and finally complete the project in the first half of 2021.

**Actual or anticipated problems or delays and actions or plans to resolve them**

*Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

As mentioned above, due to COVID-19, there has been a small reduction in work output. This set us back by around 6 months, we already requested for an 8 month extension for the project, with no budgetary changes. Apart from this set back we do not anticipate any new problems going forward. Work is now progressing as normal and we aim to gain grounds over the next two quarters.

**Changes that had a significant impact on expenditures**

*Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.*

Nothing to report

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

*Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.*

**Significant changes in use or care of human subjects**

N/A

**Significant changes in use or care of vertebrate animals**

Nothing to report

**Significant changes in use of biohazards and/or select agents**

Nothing to report

**6. PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- Publications, conference papers, and presentations**

*Report only the major publication(s) resulting from the work under this award.*

**Journal publications.** List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume; year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report (Two manuscripts planned for July/Aug 2021)

**Books or other non-periodical, one-time publications.** Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report

**Other publications, conference papers and presentations.** Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (\*) if presentation produced a manuscript.

Nothing to report (planned NNS conference presentation in July 2021)

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**

*Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.*

*Nothing to report*

- **Other Products**

*Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:*

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

*Nothing to report*

## **7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

**What individuals have worked on the project?**

*Name: Fiona Crawford (no change)*  
*Project Role: PI*  
*Researcher Identifier (e.g. ORCID ID): N/A*  
*Nearest person month worked: 1.2*  
*Contribution to Project: Dr Crawford directs all aspects of this project and provides supervision on the overall approach and data interpretation for the experiments outlined in this application. In particular, she will interact with all of the team members listed on this application and provide full oversight as they implement the work proposed in this application, including regular communication with the expert consultants.*

*Name: Joseph Ojo (no change)*  
*Project Role: Co-PI*  
*Researcher Identifier (e.g. ORCID ID): N/A*  
*Nearest person month worked: 2.4*  
*Contribution to Project: Dr. Ojo works alongside Dr. Crawford in directing all aspects of this project and providing supervision on mTBI animal modeling, histopathological analyses and data interpretation. He is responsible for overseeing all aspects of animal manipulation and ensuring that the projects are executed in a timely fashion. He will also perform histopathological assessments in both humans and animal models as described in the proposal.*

*Name: Andrew Pearson (no change)*  
*Project Role: Graduate Student*  
*Researcher Identifier (e.g. ORCID ID): N/A*  
*Nearest person month worked: 12*  
*Contribution to Project: Mr. Pearson will be involved in animal handling and surgical procedures, as well as histopathological characterization and molecular analyses, such as western blotting and ELISA. He will assist in all surgical procedures (primarily years 1 and 2) and conduct histopathological analyses with the supervision of Dr. Ojo (primarily Years 2 & 3).*

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

*Nothing to Report*

**What other organizations were involved as partners?**

## **8. SPECIAL REPORTING REQUIREMENTS**

**COLLABORATIVE AWARDS:**

**QUAD CHARTS:**

**APPENDICES:**

# Novel Therapeutic Target Identification through analysis of Convergent AD and TBI

## Pathogenic Mechanisms

Log Number: AZ160110

W81XWH-17-1-0638

PI: Drs Fiona Crawford / Joseph Ojo

Org: Roskamp Institute, Sarasota, FL

Award Amount:



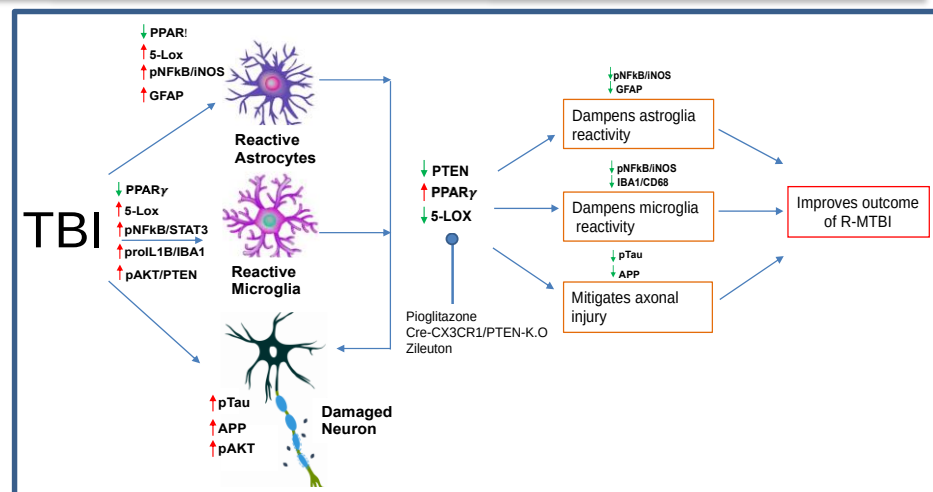
### Study Aim(s)

**Aim 1:** Validation of potential targets for therapeutic intervention in the pathogenic TBI-AD interrelationship - eicosanoid signaling, PI3-kinase/Akt/Pten/mTOR/insulin signaling and RXR/PPAR pathway.

**Aim 2:** Chronic evaluation of the efficacy of three potential therapeutics against the pathogenic TBI-AD interrelationship

### Approach

- Evaluate target engagement and efficacy in recovering normal molecular profiles in TBI mice using compounds that modulate the TBI and AD coincident molecular changes in a short-term (2wks) treatment Paradigm
- In vivo validation of therapeutic targets in mice with TBI using chronic intervention (6-month treatment regimen) with the top three performing compounds for three different targets from Aim 1 to evaluate the long-term neurobehavioral, neuropathological and biochemical outcomes
- Selection of downstream convergent tractable targets between the three different compounds for further exploration.



**Accomplishment:** i) Screening of Top compounds in an *in vitro* platform as an additional platform to validate candidates in microglia and astrocyte cultures. ii) Began chronic treatment studies, animals have been exposed to injuries and are either receiving treatment or ageing prior to initiation of treatment from 3 to 6 months post-injury.

## Timeline and Cost

| Activities              | CY | 18 | 19 | 20/21 |
|-------------------------|----|----|----|-------|
| MAJOR TASK ONE OR AIM 1 |    |    |    |       |
| MAJOR TASK TWO OR AIM 2 |    |    |    |       |
| Estimated Direct Budget |    |    |    |       |

Last updated: (September 2017)

### Goals/Milestones

#### CY17 Goal

- ✓ Obtain regulatory approval to begin animal studies
- ✓ Validate identified targets in mouse and human TBI/AD brains

#### CY18 Goals

- ✓ Initiate short treatment study to evaluate target engagement and efficacy

#### CY19 Goals

- ✓ Evaluate and select Top performing therapeutics for use in Aim 2
- ✓ Initiate chronic treatment study with the Top 2 performing compounds

#### CY20 Goal

- ✓ Complete chronic treatment study and evaluate the neurobehavioral, neuropathological and biochemical outcomes (*ongoing - finish May 2021*)

### Comments/Challenges/Issues/Concerns

- 6 Months behind schedule due to lag in time for ACURO review/COVID-19
- An eight month extension has been requested and granted.

### Budget Expenditure to Date

Projected Expenditure: Actual Expenditure: