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TITLE: Glial Cell Dysfunction in Neurodegenerative Sequelae of Repetitive Mild Traumatic Brain Injury

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14. ABSTRACT: The precise nature of how TBI leads to or precipitates ADRD pathogenesis is not understood. To address this problem we have, generated molecular profiles of AD and TBI pathogenesis in preclinical 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 and to correlate these with longitudinal changes in cognition and in the neuropathological landscape. Our analyses have highlighted the critical role of neuroinflammation after TBI, the particular significance of microglial responses. Understanding TBI neurodegeneration, and the triggers that encourage the pathogenic sequelae of TBI to follow paths toward ADRD, will be critical to the identification of effective therapeutic approaches. We have established and characterized a preclinical model of mTBI which has been validated by our clinical collaborators and which demonstrates lifelong neuroinflammatory responses and cognitive dysfunction following repetitive mTBI at a young age. We consider that repetitive mTBI induces significant and persistent changes in the microglial population over time after injury with lifelong consequences on the neuroinflammatory milieu. These microglial responses are critical to TBI neurodegeneration and in the context of other potentially pathogenic proteins such as tau or amyloid, ADRD pathobiology can be triggered.

This proposal will focus on the details of microglia pathobiology in mouse models and human cases of r-mTBI and ADRD proteinopathies. We will utilize a novel single cell array approach to interrogate the gene expression and transcript profiles in a homogenous population of resting and/or activated microglia. We will address how microglia pathobiology contributes to the relationship between TBI neurodegeneration and ADRD proteinopathies, and what role young vs aged microglia play in precipitating sequelae subsequent to TBI.

15. SUBJECT TERMS: Glial cells, pathobiology, TBI, AD, Animal Models

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1. INTRODUCTION:

A considerable lack of understanding still exists concerning the molecular mechanisms responsible for the pathogenic interrelationship between TBI and ADRD. Our preliminary data strongly implicate microglia pathobiology as a common denominator, and early event, and could represent an important molecular trigger for TBI mediated neurodegeneration and its increased risk for ADRD pathogenesis. Microglia are vital players of several physiological functions in the brain, and dysfunction of their supportive role can lead to the surrender of a host of these functions. This proposal will focus on the details of microglia pathobiology in mouse models and human cases of r-mTBI and ADRD proteinopathies. We will utilize a novel single cell array approach to interrogate the gene expression and transcript profiles in a homogenous population of resting and/or activated microglia. We will address how microglia pathobiology contributes to the relationship between TBI neurodegeneration and ADRD proteinopathies, and what role young vs aged microglia play in precipitating sequelae subsequent to TBI.

2. KEYWORDS:

Microglial cells, proteinopathy, amyloid, tau, TBI, AD, Animal Models.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Objective/Hypothesis: Repetitive mTBI induces significant and persistent changes in the microglial population over time after injury with lifelong consequences on the neuroinflammatory milieu. These microglial responses are critical to TBI neurodegeneration and in the context of other potentially pathogenic proteins such as tau or amyloid, ADRD pathobiology can be triggered.

Major Task 1: Part One: Delineation of the effects of chronic repetitive mTBI on microglia pathobiology and related proteinopathy in young and aged wild type, humanized tau and humanized amyloid mouse models at 14days, 3 and 6 months post-injury**. Part two:** Investigation of microglial pathological lesions in autopsied brains from human AD/CTE cases.

Description: Histopathological assessment of microglia pathobiology in WT, hTau and APP^{NLF} mouse models exposed to r-mTBI, or sham injury (r-sham) when young (3 months – Young cohort) or aged (12 months – Aged cohort) and analyzed at 14days, 3 and 6 months post-injury. Histopathological assessment of microglia pathobiology and in human AD/TBI cases.

Major Task 2: Part One: Generation of single cell genomic profiles in different populations of microglia obtained from the same mouse models described in Aim 1. **Part two:** Single cell genomic profiling of different population of microglia in autopsied brains from human AD/CTE cases.

Description: Single cell genomic profiles in different populations of microglia obtained from young and aged WT, hTau, and APP^{NLF} mice exposed to r-mTBI or r-sham and analyzed at 14days, 3 and 6 months post-injury. Validation of distinct microglial cell population of interest and identified gene profiles using flow cytometry and cell sorting. Single cell genomic profiles of microglia from autopsied human AD/CTE brains.

What was accomplished under these goals?

Summary of accomplishments Aims 1 and 2.

Aim 1: Mouse and Human IHC analyses - All sections have been stained for IBA1, CD68, CD45, MHCII and are undergoing quantitative image analyses.

Aim 2: Our subcontractor has finished conducting single cell gene analyses of microdissected microglia from tissue sections in our healthy controls and TBI/CTE groups.

We have also completed microglial cell RNAseq analyses of the Young/Aged cohort of C57BL6, Tau and APP-KI models. Validation of differentially regulated transcripts is currently ongoing.

SUBTASK DESCRIPTIONS FOR MAJOR TASK 1

Subtask 1: Obtaining ACURO approval and submitting ORP Cadaver form.
We have obtained regulatory approval for animal and autopsy tissue studies (100% complete)
Substask 2a: Breeding of cohorts for Aged mice TBI studies (36 mice per genotype - mixed gender).
Completed breeding of aged mice (100% complete)
Subtask 2b: Breeding of cohorts for Young mice TBI studies (36 mice per genotype – mixed gender).
Completed breeding of young mice (100% complete)
Subtask 3: Histopathological analyses of microglia pathobiology in human AD/TBI cases.
Completed staining of human tissue, analyzed all images (100% complete)
Subtask 4: Administering injuries to Young cohort (36 per genotype).
Mice used will be mixed gender WT, hTau, and APP^{NLF} transgenic mice on the C57BL/6 background, aged 3 months at injury. Animals will receive 5 closed head injuries/week with a CCI device over a 1-month period (Ojo et al., 2016). For each of the three genotypes there will be two groups - r-mTBI young and r-sham young - each with 18 mice per group. Of these 18 mice, 6 will be euthanized for analysis at each of the three timepoints.
Completed all cohorts (100% complete)

Subtask 5: Euthanasia of Young cohort; for each of the three genotypes (WT, hTau, and APP^{NLF}) there will be 36 mice, of which 18 will have received r-mTBI and 18 received r-sham. For each genotype, 6 r-mTBI and 6 r-sham mice will be euthanized at 1 day post-injury, 3 mos post-injury and 9 mos post-injury.

Completed all cohorts (100% complete)

Subtask 6: Sectioning and histopathological staining of Young cohort tissues with microglia, tau and amyloid antibodies; and TUNEL, BrdU, EM preps.

Completed for all cohorts (100% complete)

Subtask 7: Stereological analyses and Image quantitation of brain sections from Young cohort (staggered over time)

Analyzing young C57BL/6J, TauKI and APPKI cohorts (50% completed)

Subtask 8: Administering injuries to Aged cohort (36 per genotype).

Mice used will be mixed gender WT, hTau, and APP^{NLF} transgenic mice on the C57BL/6 background, aged 12 months at injury.

Completed for all cohorts (100% complete)

Subtask 9: Euthanasia of Aged cohort; for each of the three genotypes (WT, hTau, and APP^{NLF}) there will be 36 mice, of which 18 will have received r-mTBI and 18 received r-sham. For each genotype, 6 r-mTBI and 6 r-sham mice will be euthanized at 1 day post-injury, 3 mos post-injury and 9 mos post-injury.

Completed for all cohorts (100% complete)

Subtask 10: Sectioning and histopathological staining of Aged cohort tissues with microglia, tau and amyloid antibodies; and TUNEL, BrdU, EM preps.

Completed for all cohorts (100% complete)

Subtask 11: Stereological analyses and Image quantitation of brain sections from Aged cohort

Analyzing aged C57BL/6J cohort (50% completed)

Subtask 12: Interpretation of data and consultation with clinical neuropathologists

Ongoing.

SUBTASK DESCRIPTIONS FOR MAJOR TASK 2

Subtask 1: Laser capture microdissection of microglia from human AD/CTE cases for single cell array for gene expression profiling.

Barrow (Dr Mufson) – 100% completed

Subtask 2: Single cell analyses of reactive microglia from Young Cohort WT, hTau, and APP^{NLF} mice for gene expression profiling (staggered overtime).

Completed for all cohorts (100% complete)

Subtask 3: Single cell analyses of reactive microglia from Aged Cohort WT, hTau, and APP^{NLF} mice for gene expression profiling (staggered overtime).

Completed for all cohorts (100% complete)

Subtask 4: Validation of select gene transcript profiles that are altered in microglia in both Young and Aged cohorts.

Ongoing

Subtask 5: Data analysis and interpretation and correlation studies

Ongoing

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

METHODOLOGY

Mouse transcriptomics

Microglia cells were isolated using enzymatic degradation and percoll gradient centrifugation followed by magnetic assisted cell sorting (MACS). **HiSeq sequencing:** RNA samples were prepared using the Qiagen RNeasy Kit, quantified and checked for integrity. RNA library preparation with PolyA and sequencing reactions was conducted at Genewiz (South Plainfield, NJ). The NEBNext Ultra RNA Library Prep Kit for Illumina was used for RNAseq library preparations. Briefly, mRNA was first enriched with Oligod(T) beads and fragmented, with the 1st and 2nd strand cDNA subsequently synthesized. cDNA fragments were end repaired and adenylated, and universal adaptor ligated to fragments, followed by index addition and library enrichment with limited cycle PCR. Validated and quantified sequencing libraries were multiplexed and clustered on one lane of a Flow Cell. After clustering, the flow cell was loaded on the Illumina instrument, with samples sequenced using a 2×150 Paired End configuration. Raw sequence data generated from Illumina HiSeq was converted into Fastq files and de-multiplexed. **Bioinformatic analyses**: After quality checking of Fastq files, sequence reads were trimmed, and mapped to the Mus musculus GRCm38 reference genome. Data were inspected for outlying samples using unsupervised hierarchical clustering and principal component analysis. Combat batch correction was applied to combine the datasets and reduce systematic sources of variability. Differential gene expression analysis: This was conducted to determine relationships between gene expression levels and injury status. The covariates were included in all models to adjust for any potential confounding influence on gene expression between main group effects. This was conducted using the Wald test (in DESeq2Genes with FDR adj $p<0.05$ and log2 fold changes >1 were classified as differentially expressed genes (DEG).). Gene ontology/ pathway analyses: This was used to identify pathways to which significantly regulated genes belong, including their cell-type origin, cellular components, molecular functions and canonical signaling pathways. We conducted enrichment tests for DEGs using hypergeometric statistic, controlling for background set enrichment and multiple testing, or a leading-edge rank-based method. Upstream regulator analysis: This was performed in the Ingenuity Pathway Analysis software to predict upstream regulators driving the observed DEG's.

Human microglia gene array

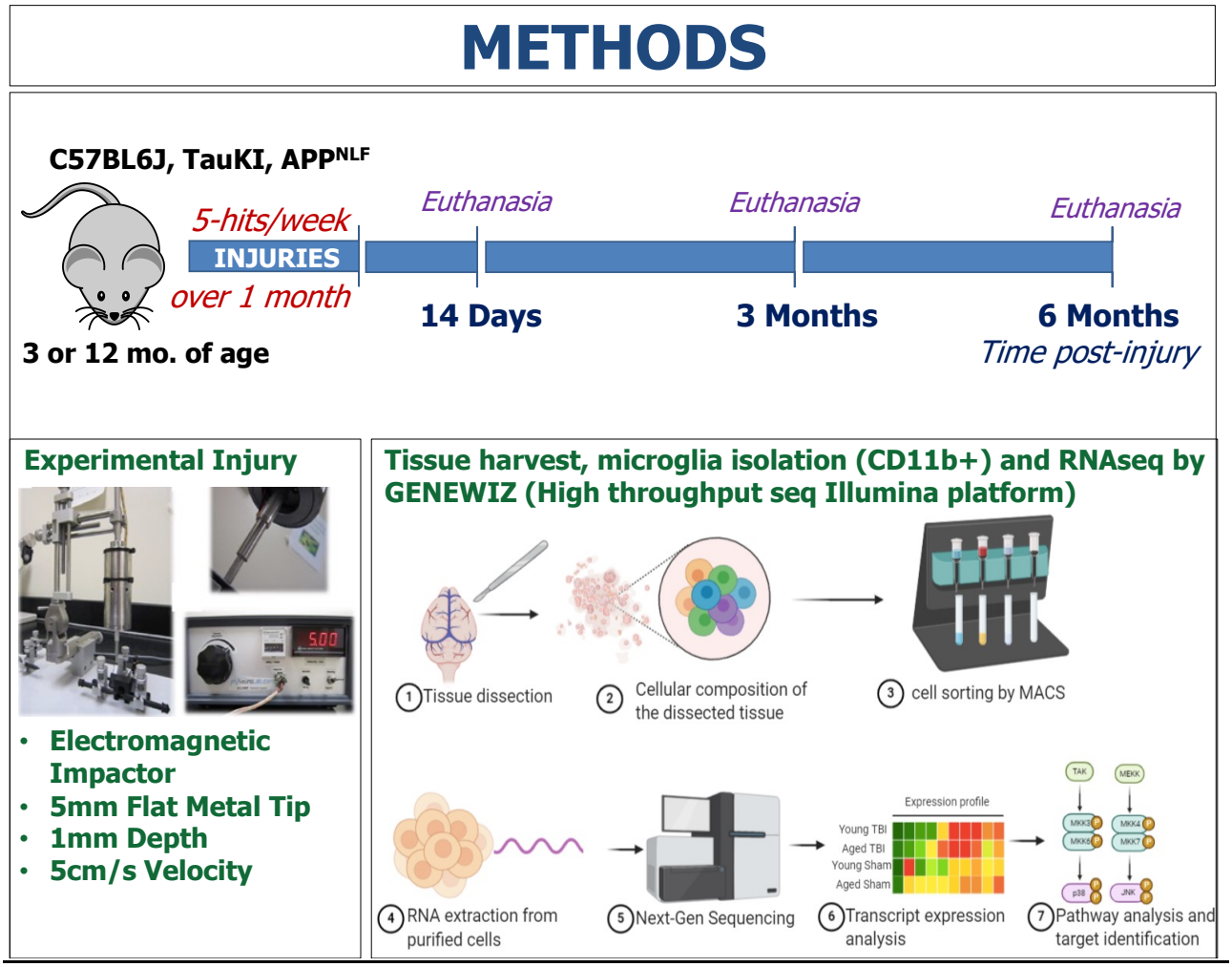
IBA1 Immunostaining: Briefly, sections were deparaffinized in xylene and rehydrated in a decreasing gradient of ethanol before the IHC procedure. Sections were rinsed in distilled water and subsequently incubated at room temperature in a solution of endogenous peroxidase blocking solution containing 3% hydrogen peroxide diluted in PBS for 15 minutes. For primary antibodies requiring antigen retrieval, sections were treated with either (I) DAKO target retrieval citrate buffer solution (pH 6) (Dako) for 8 minutes in the microwave. Following antigen retrieval, sections were blocked for 1 hour in Dako protein serum-free protein block (Dako). Sections were immunostained in batches with IBA1 primary antibodies made up in supersensitive wash buffer and antibody diluent background-reducing agent. After overnight incubation, sections were rinsed with PBS and transferred to a solution containing the appropriate conjugated (peroxidase) secondary antibody (Vectastain Elite ABC Kit, Vector Laboratories) for 1 hour, depending on the specific requirement of the antibody protocol. Immunoreactivity was visualized with a compatible chromogen. Development with the chromogen was timed and applied as a constant across batches to limit technical variability before progressing to quantitative image analysis. Mounted sections were progress through a graded series of alcohols (dehydrated), cleared in xylene, and cover-slipped with permanent mounting medium.

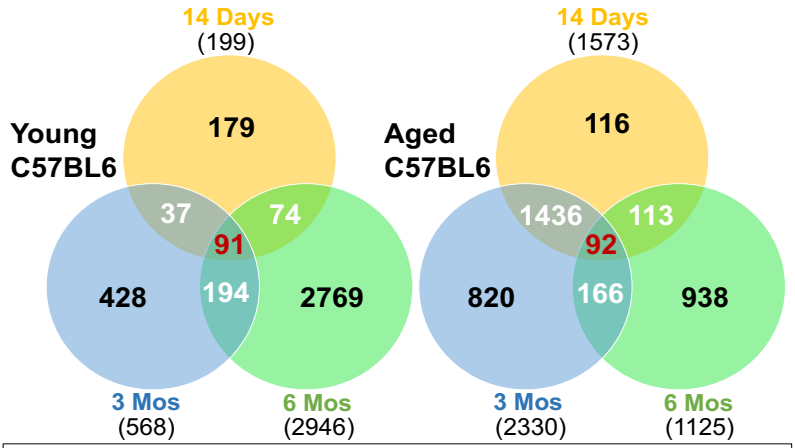
Laser capture microdissection (LCM) and gene array: A LCM instrument will be used for these studies. LCM methodology results in accurate dissection of the microglial cells of interest with minimal disruption of the surrounding tissue. For the proposed studies, 2500 IBA1+ microglia will be captured per reaction, per condition for subsequent linear terminal continuation (TC) RNA amplification and microarray analysis. TC RNA amplification was developed in Dr. Ginsberg's laboratory. The method entails

synthesizing first strand cDNA complementary to the RNA template, generating second strand cDNA complementary to the first strand cDNA, and finally *in vitro* transcription using the double stranded cDNA as template. This process displays high fidelity, reproducibility and increased signal sensitivity (4-fold) and flexibility compared to other RNA amplification protocols. Radiolabeled RNA probes will be hybridized to custom-designed microarrays. The array platforms consist of 1 µg of linearized cDNA purified from plasmid preparations adhered to high-density nitrocellulose using an arrayer robot (VersArray, Bio-Rad). Each cDNA and/or expressed sequence-tagged cDNA(EST) is verified by sequence analysis and restriction digestion. Mouse and human clones are employed on the custom-designed array. Approximately 864 cDNAs/ESTs organized into 22gene ontology groups are present on the current array platform.

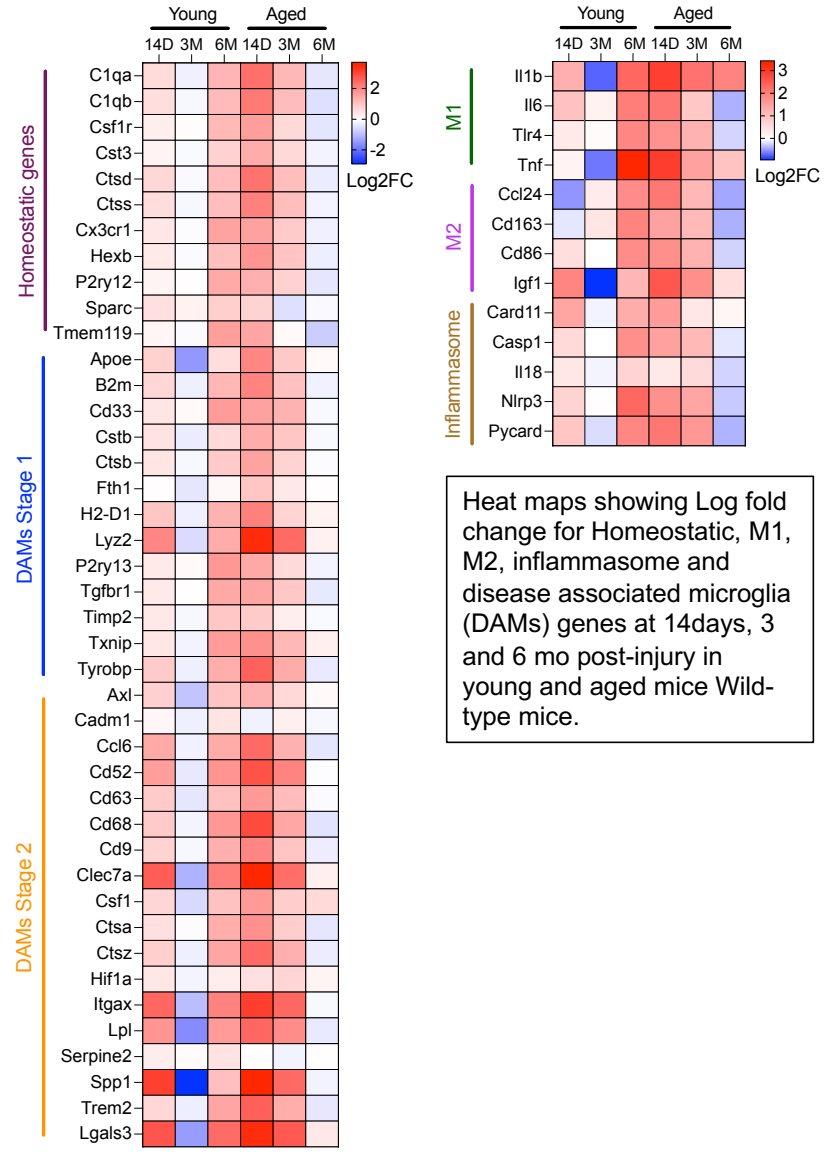
Statistical procedures (using GeneLinker Gold**)** for custom-designed microarray analysis have been described in detail previously. Gene expression differences due to genotype or TBI condition will be assessed with respect to the hybridization signal intensity ratio of the total signal of all array genes. For each gene the signal intensity ratio is modeled as a function of mouse study group, using mixed effects models with random mouse effect to account for the correlation between repeated assays on the same mouse. Between subject versus within-subject variation will be analyzed by random intercept, fixed effect covariate, Kenward-Roger denominator degrees of freedom, unstructured covariance structure, and log-transformed expression levels. Significance will be judged at the level α=0.01, two-sided; false discovery rate (FDR) based on an empirical null distribution due to strong correlation between genes is controlled at level α=0.1.

Mouse Transcriptomics data summary

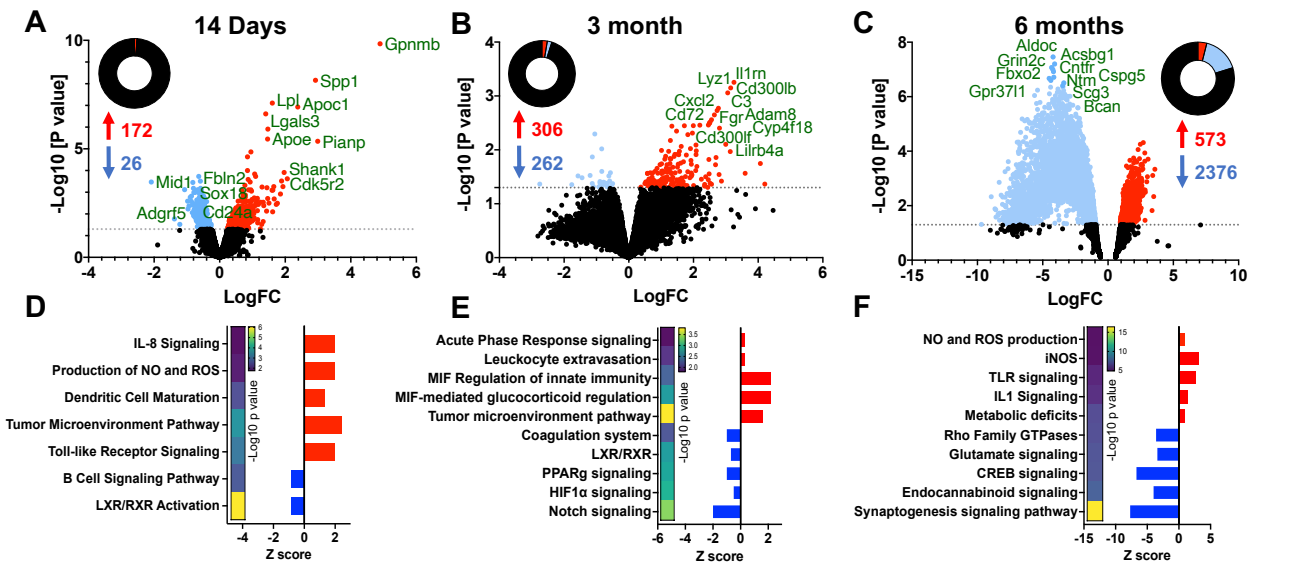




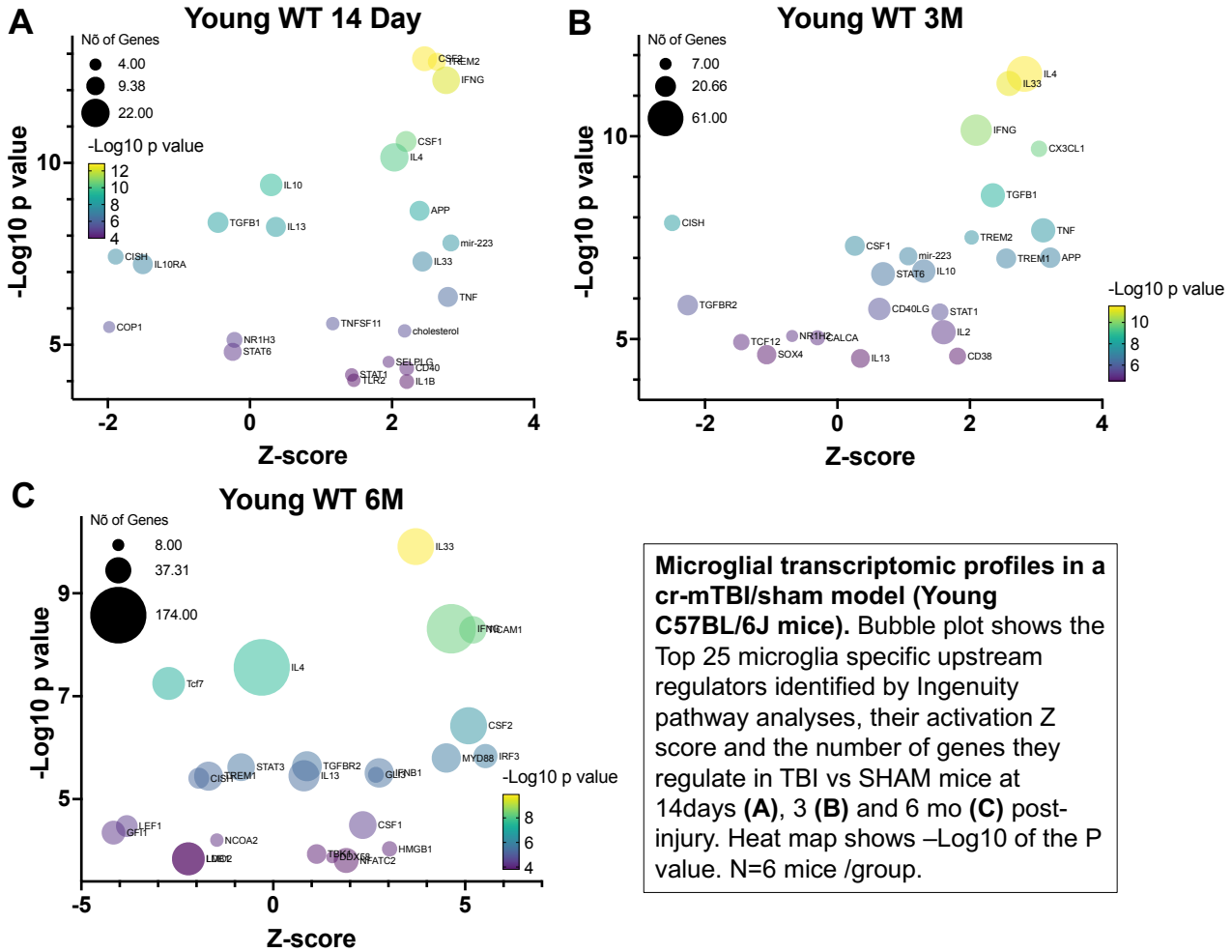
Venn diagram showing overlap in differentially regulated microglia genes in r-mTBI/r-sham C57BL6 mice - Young [3M] vs Aged [12M]) at 14 days, 3 & 6M post-injury.



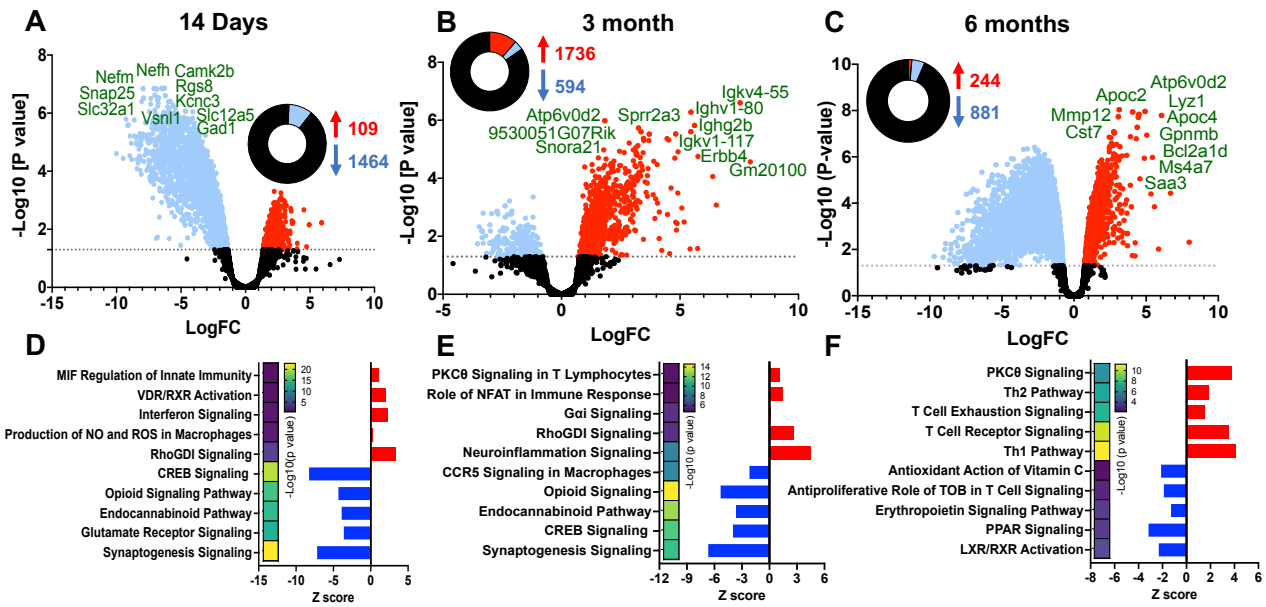
Heat maps showing Log fold change for Homeostatic, M1, M2, inflammasome and disease associated microglia (DAMs) genes at 14days, 3 and 6 mo post-injury in young and aged mice Wild-type mice.



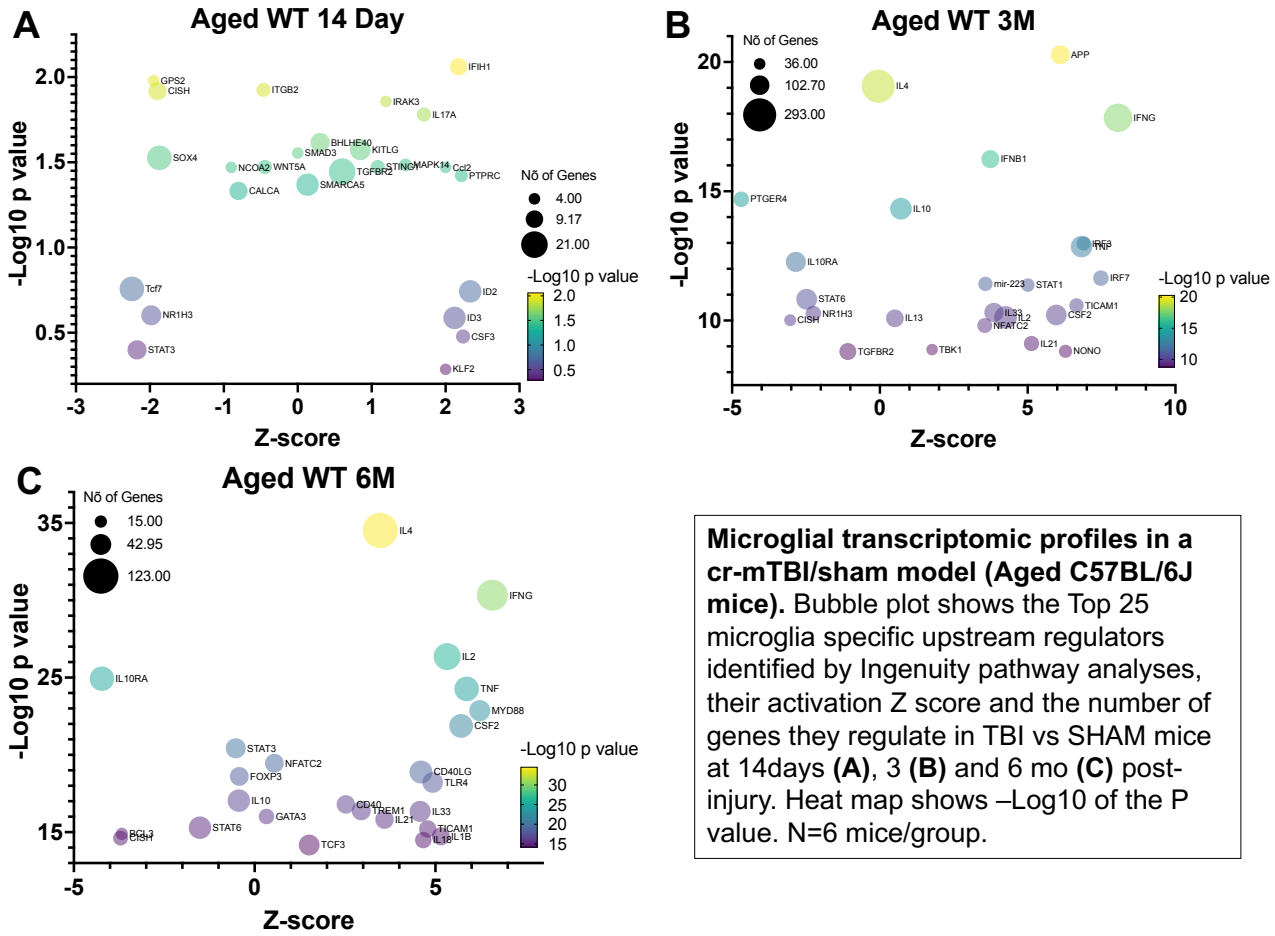
Microglial transcriptomic profiles in a cr-mTBI/sham model (Young C57BL/6J mice). Volcano plot shows microglia specific genes and the corresponding Top pathways identified by Ingenuity pathway analyses in TBI vs SHAM mice at 14days **(A, D)**, 3 **(B, E)** and 6 mo **(C, F)** post-TBI. Top 10 genes are in green. Pie chart shows directionality of significant genes. N=6 mice /group.



Microglial transcriptomic profiles in a cr-mTBI/sham model (Young C57BL/6J mice). Bubble plot shows the Top 25 microglia specific upstream regulators identified by Ingenuity pathway analyses, their activation Z score and the number of genes they regulate in TBI vs SHAM mice at 14days **(A)**, 3 **(B)** and 6 mo **(C)** post-injury. Heat map shows –Log10 of the P value. N=6 mice /group.



Microglial transcriptomic profiles in a cr-mTBI/sham model (Aged C57BL/6J mice). Volcano plot shows microglia specific genes and the corresponding Top pathways identified by Ingenuity pathway analyses in TBI vs SHAM mice at 14days **(A, D)**, 3 **(B, E)** and 6 mo **(C, F)** post-TBI. Top 10 genes are in green. Pie chart shows directionality of significant genes. N=6 mice/group.



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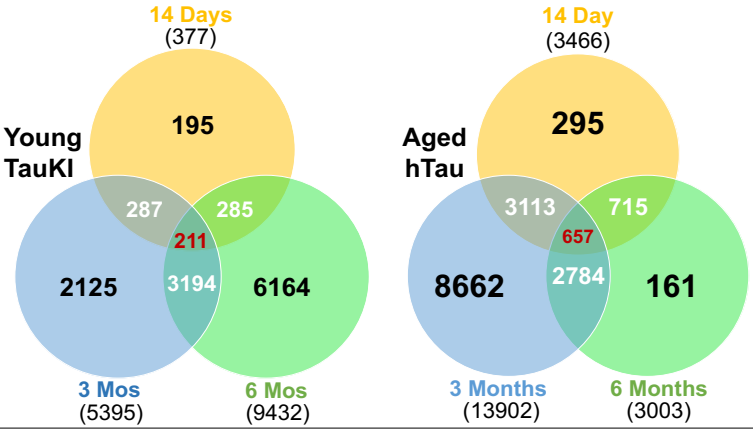
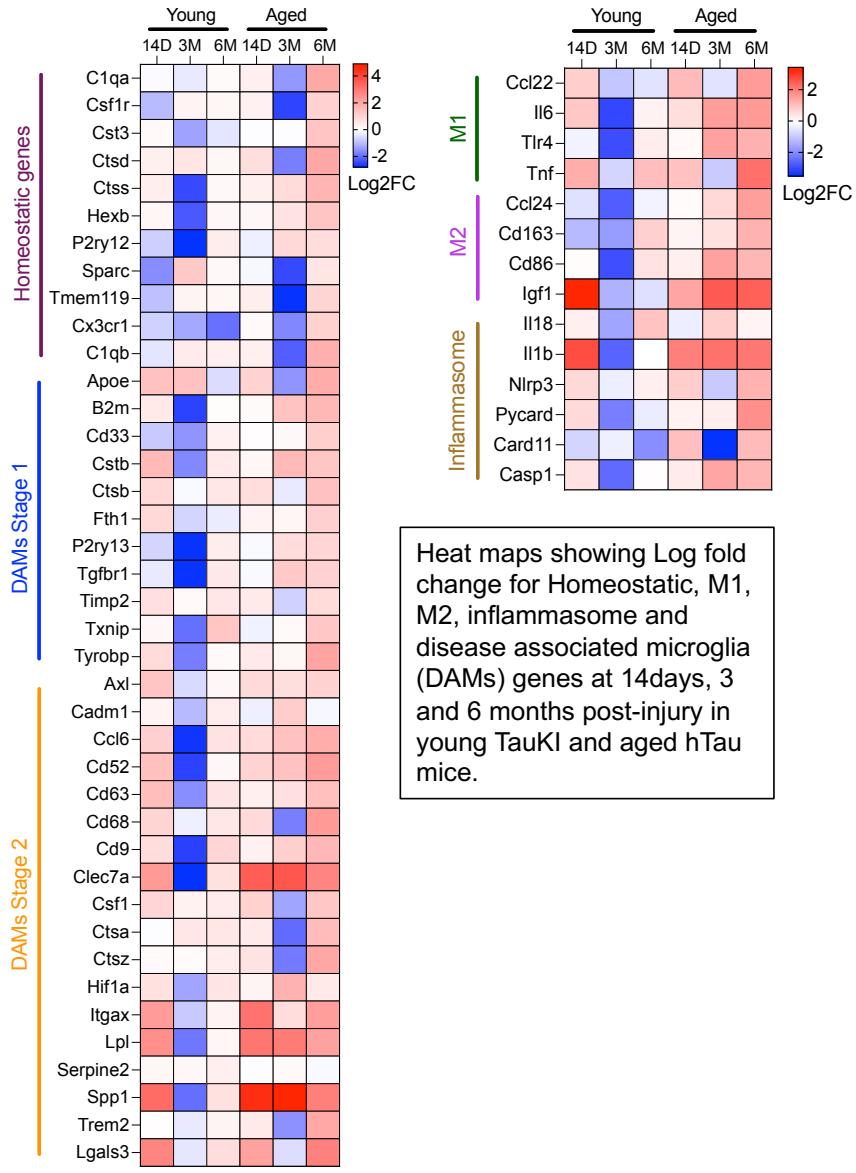
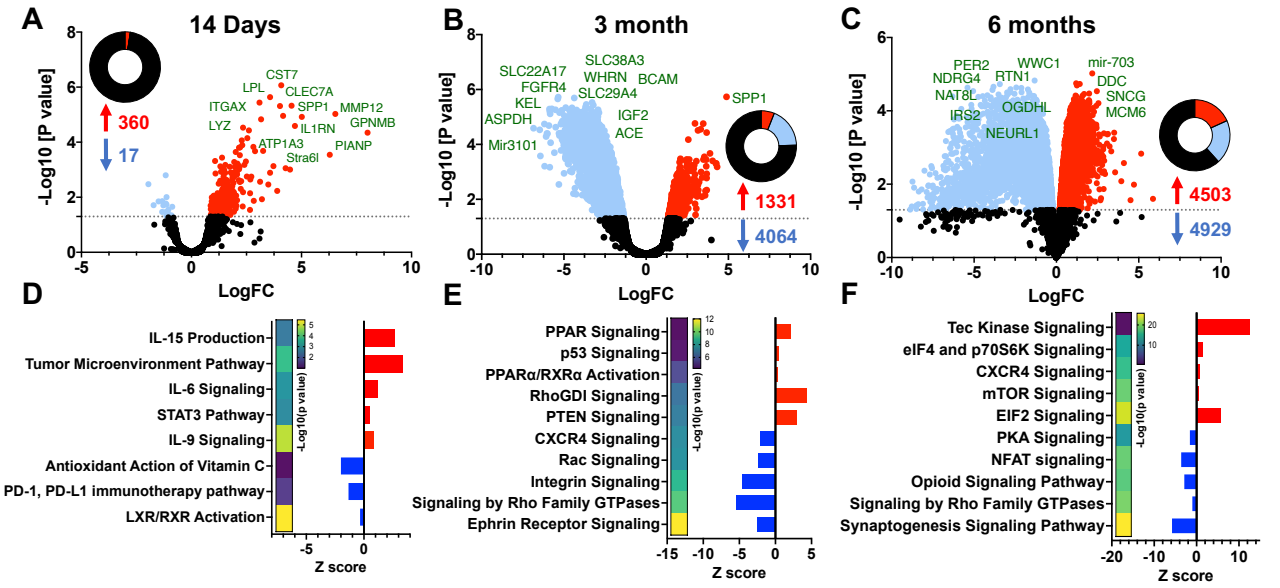


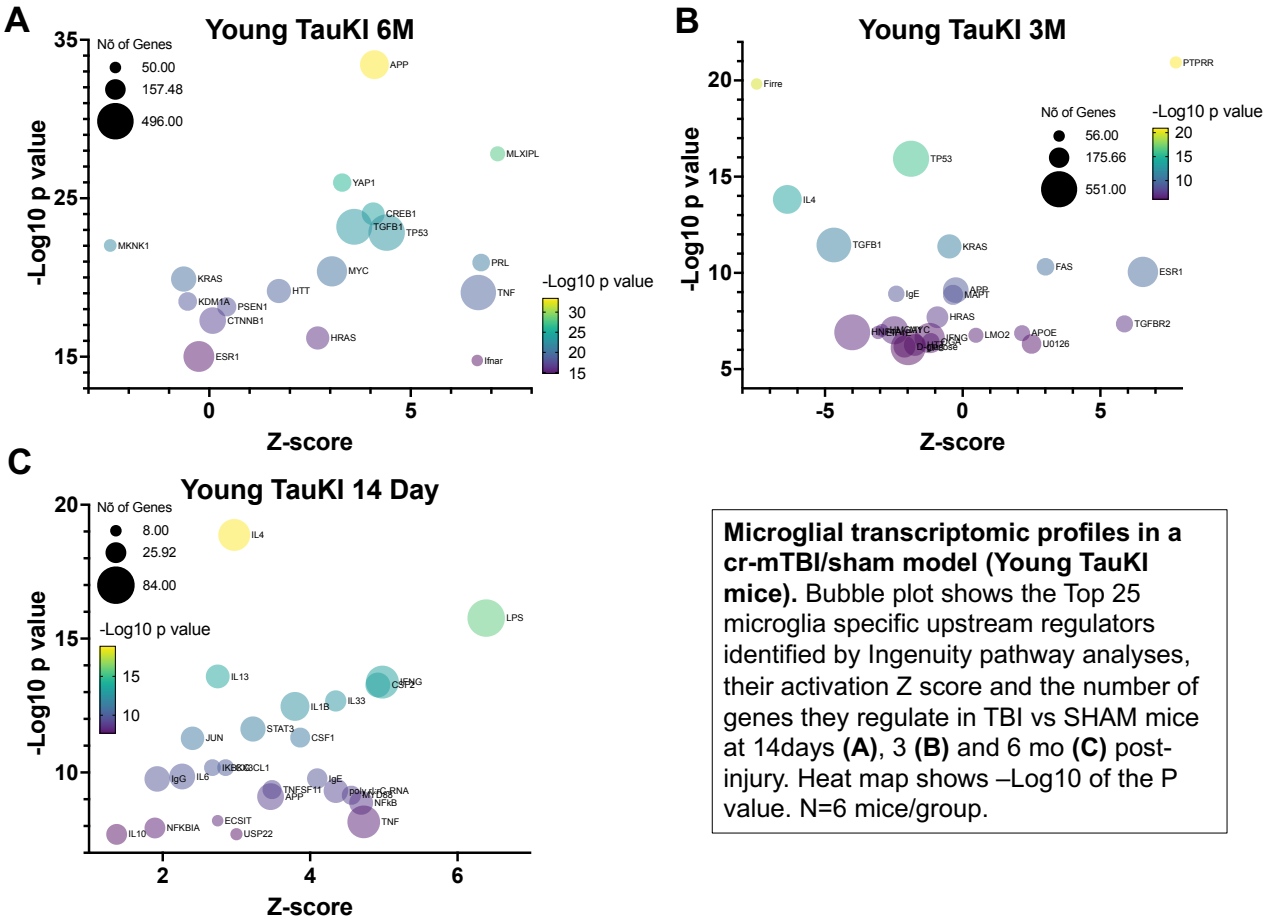
Venn diagram showing overlap in differentially regulated microglia genes in r-mTBI/ r-sham TauKI/hTau mice - Young [3M] vs Aged [12M]) at 14 days, 3 & 6M post-injury.



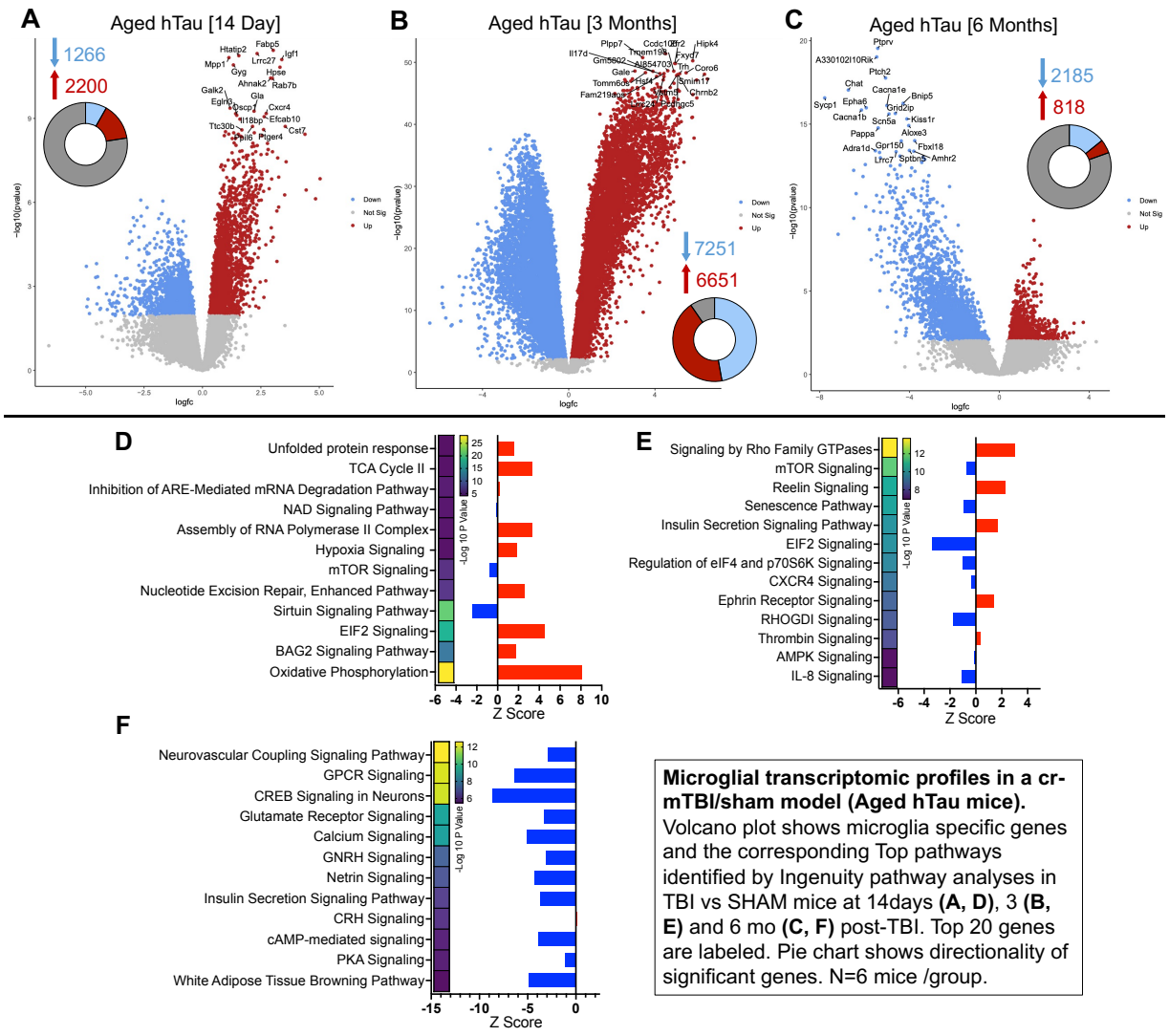
Heat maps showing Log fold change for Homeostatic, M1, M2, inflammasome and disease associated microglia (DAMs) genes at 14days, 3 and 6 months post-injury in young TauKI and aged hTau mice.



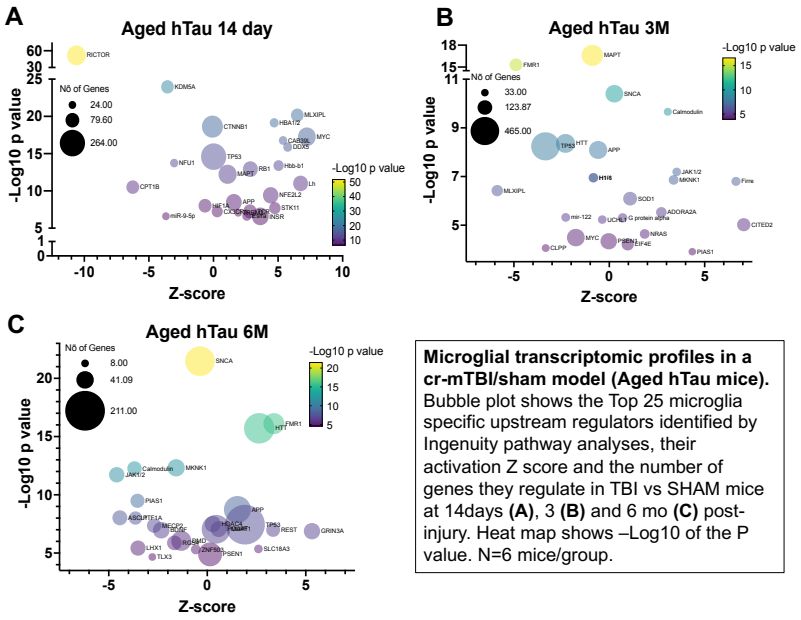
Microglial transcriptomic profiles in a cr-mTBI/sham model (Young TauKI mice). Volcano plot shows microglia specific genes and the corresponding Top pathways identified by Ingenuity pathway analyses in TBI vs SHAM mice at 14days **(A, D)**, 3 **(B, E)** and 6 mo **(C, F)** post-TBI. Top 12 genes are in green. Pie chart shows directionality of significant genes. N=6 mice /group.



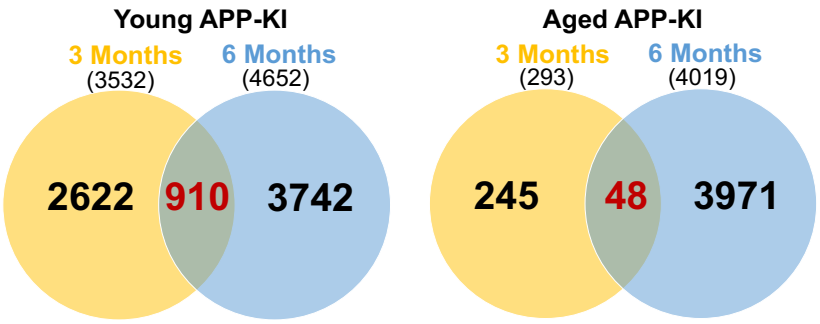
Microglial transcriptomic profiles in a cr-mTBI/sham model (Young TauKI mice). Bubble plot shows the Top 25 microglia specific upstream regulators identified by Ingenuity pathway analyses, their activation Z score and the number of genes they regulate in TBI vs SHAM mice at 14days **(A)**, 3 **(B)** and 6 mo **(C)** post-injury. Heat map shows –Log10 of the P value. N=6 mice/group.



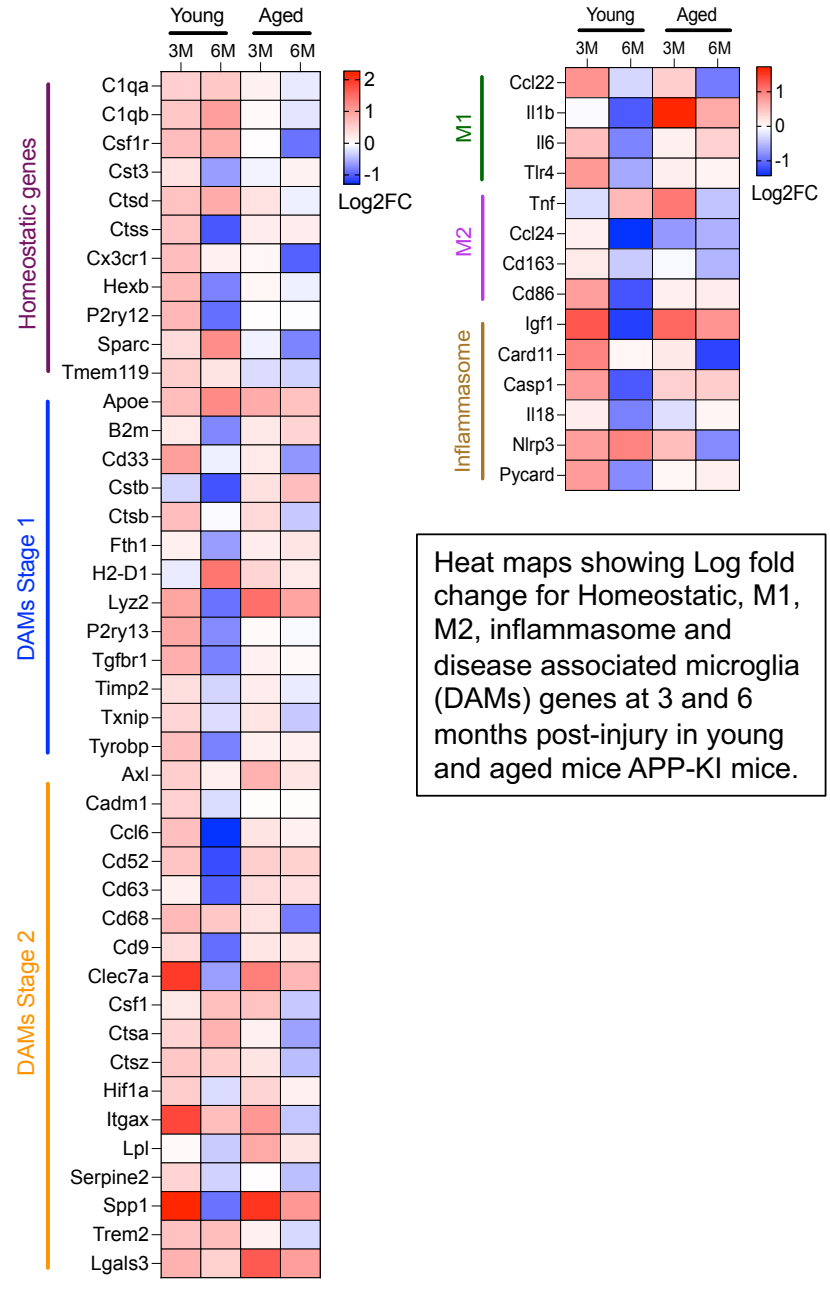
Microglial transcriptomic profiles in a cr-mTBI/sham model (Aged hTau mice). Volcano plot shows microglia specific genes and the corresponding Top pathways identified by Ingenuity pathway analyses in TBI vs SHAM mice at 14days **(A, D)**, 3 **(B, E)** and 6 mo **(C, F)** post-TBI. Top 20 genes are labeled. Pie chart shows directionality of significant genes. N=6 mice /group.



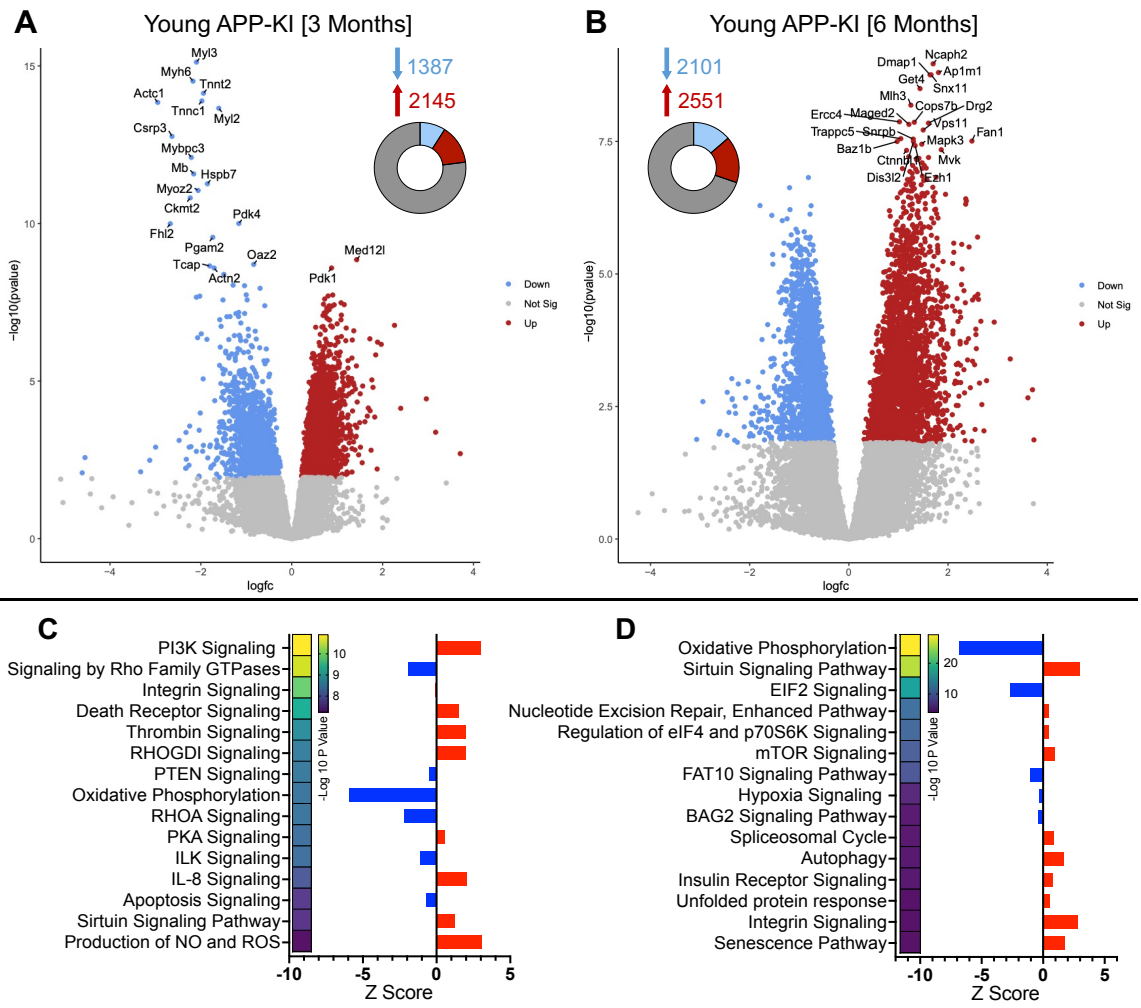
Microglial transcriptomic profiles in a cr-mTBI/sham model (Aged hTau mice). Bubble plot shows the Top 25 microglia specific upstream regulators identified by Ingenuity pathway analyses, their activation Z score and the number of genes they regulate in TBI vs SHAM mice at 14days **(A)**, 3 **(B)** and 6 mo **(C)** post-injury. Heat map shows –Log10 of the P value. N=6 mice/group.



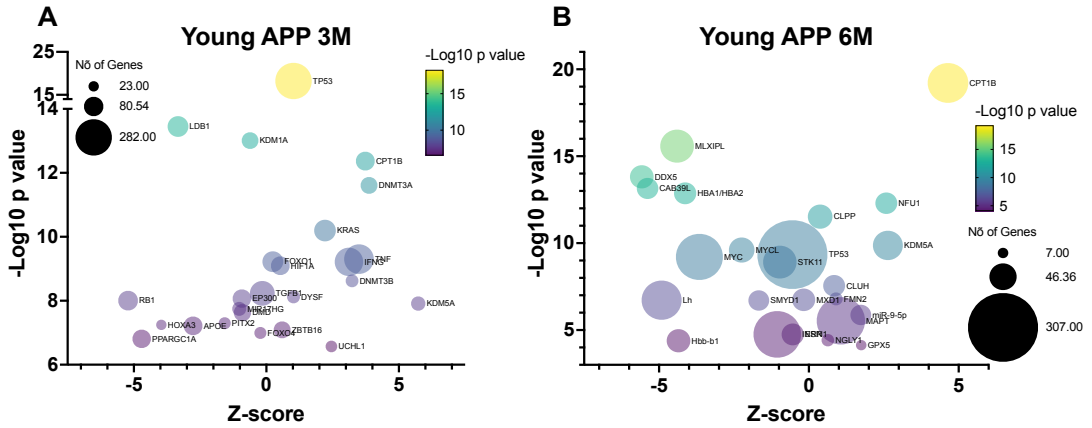
Venn diagram showing overlap in differentially regulated microglia genes in r-mTBI/r-sham APP-KI mice - Young [3M] vs Aged [12M]) at 3M & 6M post-injury.



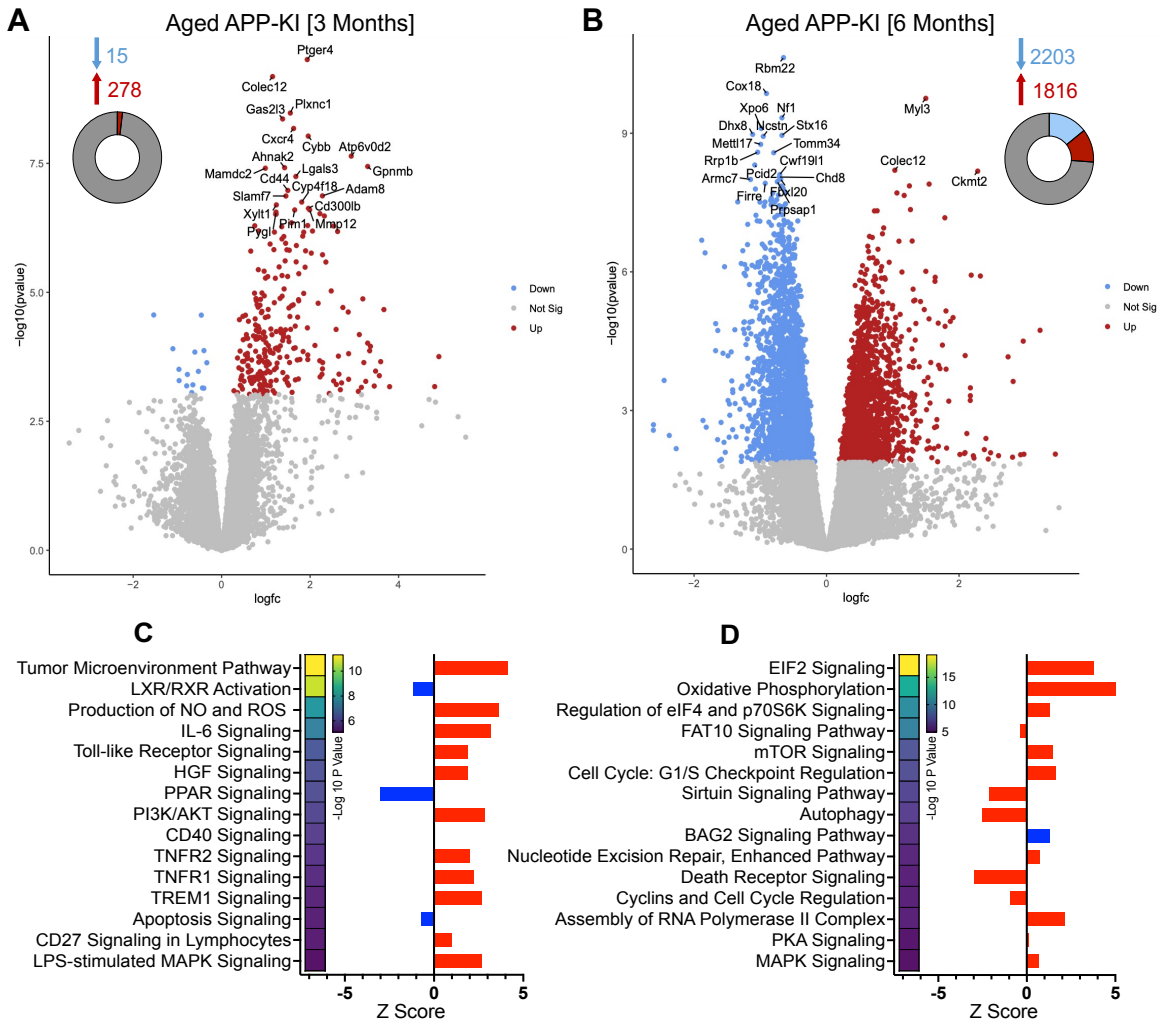
Heat maps showing Log fold change for Homeostatic, M1, M2, inflammasome and disease associated microglia (DAMs) genes at 3 and 6 months post-injury in young and aged mice APP-KI mice.



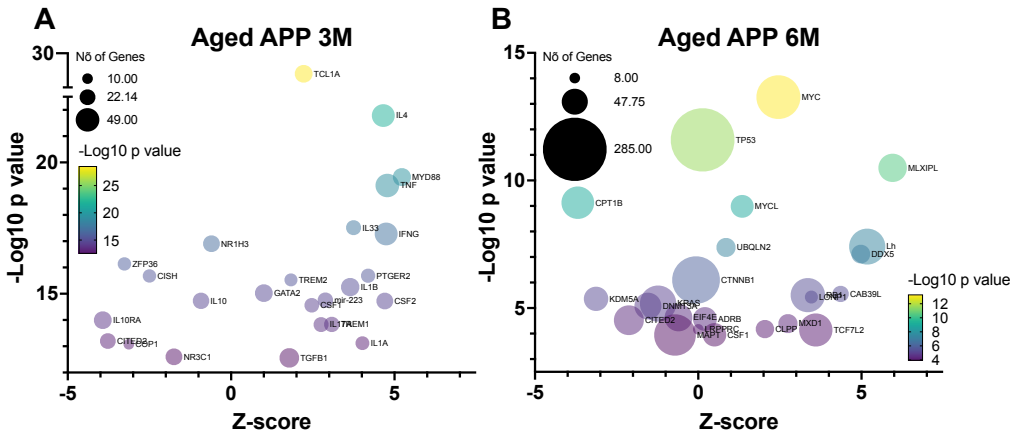
Microglial transcriptomic profiles in a cr-mTBI/sham model (Young APP-KI mice). Volcano plot shows microglia specific genes and the corresponding Top pathways identified by Ingenuity pathway analyses in TBI vs SHAM mice at 3 **(A, C)** and 6 months **(B, D)** post-TBI. Top 20 genes are labeled. Pie chart shows directionality of significant genes. N=6 mice /group.



Microglial transcriptomic profiles in a cr-mTBI/sham model (Young APP-KI mice). Bubble plot shows the Top 25 microglia specific upstream regulators identified by Ingenuity pathway analyses, their activation Z score and the number of genes they regulate in TBI vs SHAM mice at 3 **(A)** and 6 months **(B)** post-injury. Heat map shows –Log10 of the P value. N=6 mice/group.

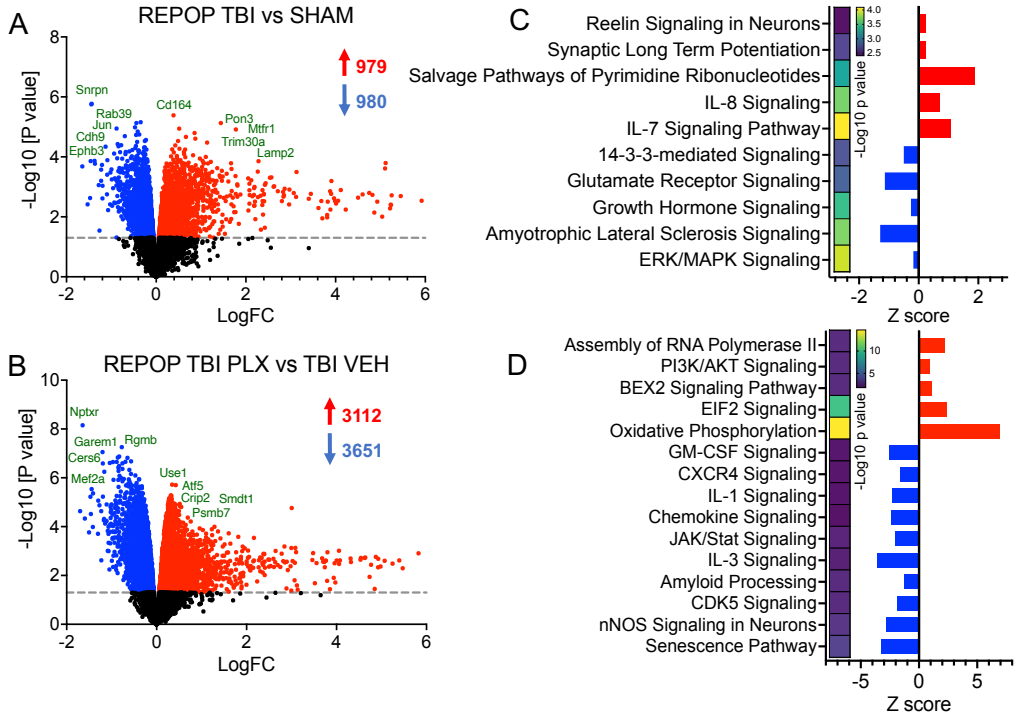


Microglial transcriptomic profiles in a cr-mTBI/sham model (Aged APP-KI mice). Volcano plot shows microglia specific genes and the corresponding Top pathways identified by Ingenuity pathway analyses in TBI vs SHAM mice at 3 **(A, C)** and 6 months **(B, D)** post-TBI. Top 20 genes are labeled. Pie chart shows directionality of significant genes. N=6 mice /group.

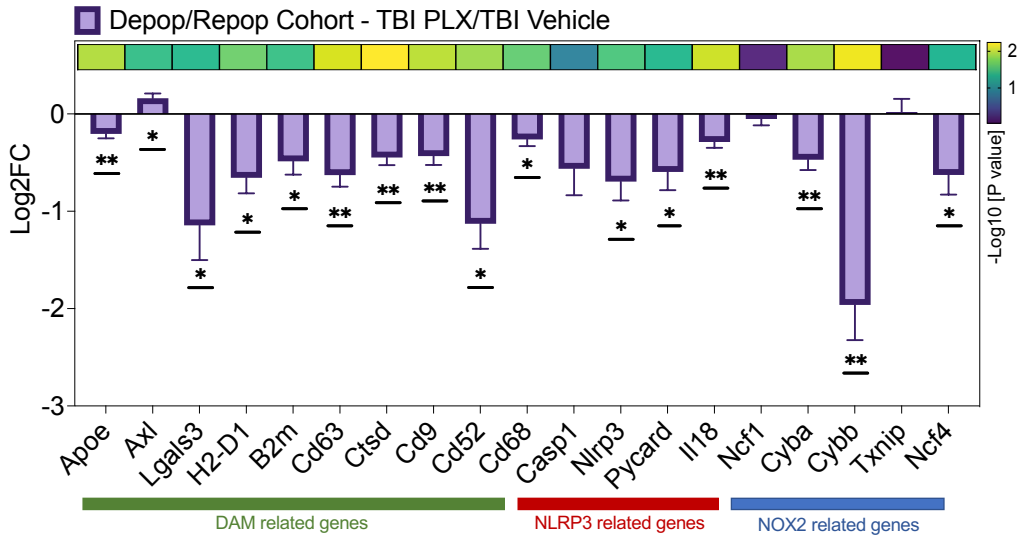


Microglial transcriptomic profiles in a cr-mTBI/sham model (Aged APP-KI mice). Bubble plot shows the Top 25 microglia specific upstream regulators identified by Ingenuity pathway analyses, their activation Z score and the number of genes they regulate in TBI vs SHAM mice at 3 **(A)** and 6 months **(B)** post-injury. Heat map shows –Log10 of the P value. N=6 mice/group.

Additional dataset of microglia ablation study



Hippocampal transcriptomic profiles in a r-mTBI/sham model after depopulation and repopulation of microglia. Volcano plot shows significant genes at 6 mo post-injury in TBI vs sham vehicle treated mice (A) and in PLX5622 vs vehicle treated TBI mice (B). Number/ arrows in A and B shows up- & down-regulated significant genes. Histogram shows top up- & down-regulated pathways and their activation Z score in TBI vs sham vehicle treated mice (C) and in PLX5622 vs vehicle treated TBI mice (D) (corresponding –Log 10 P value is shown in the heat map). Data generated using Ilumina next generation sequencing platform.



DAM, NLRP3 and NOX2-related gene expression levels in hippocampal tissue of r-mTBI mice following microglia depopulation and repopulation paradigm. Histogram reveal Log2 Fold change between TBI mice treated with PLX5662 vs Vehicle. PLX5622 treatment was administered for 14 days leading to the 3 months timepoint and withdrawn until euthanasia at 6 months post-injury. Heatmap represents –Log 10 of the P value for each transcript. Data generated using Ilumina next generation sequencing platform. Asterisk denote: *P<0.05, **P<0.01

Human microglia gene array

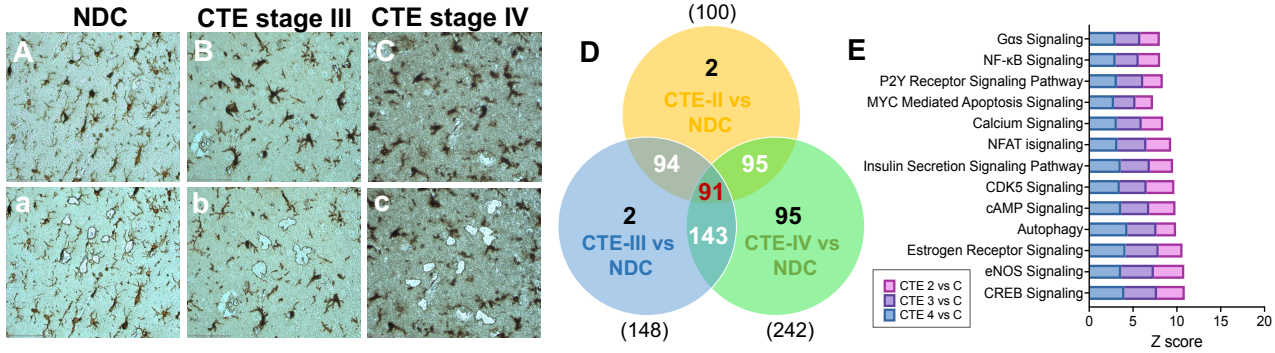
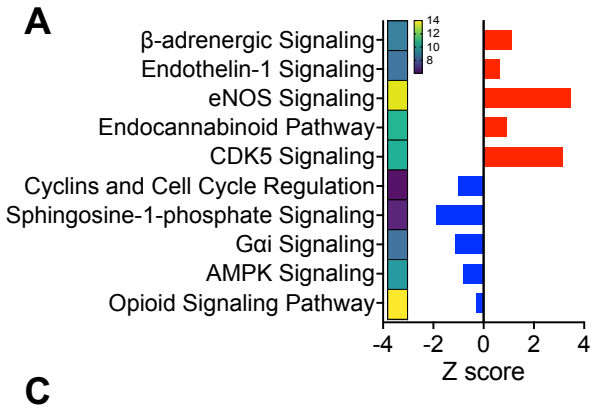
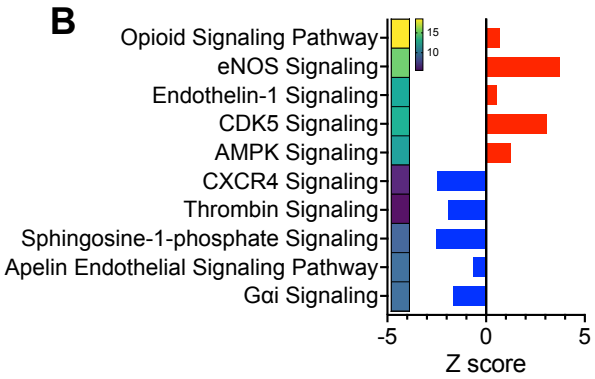
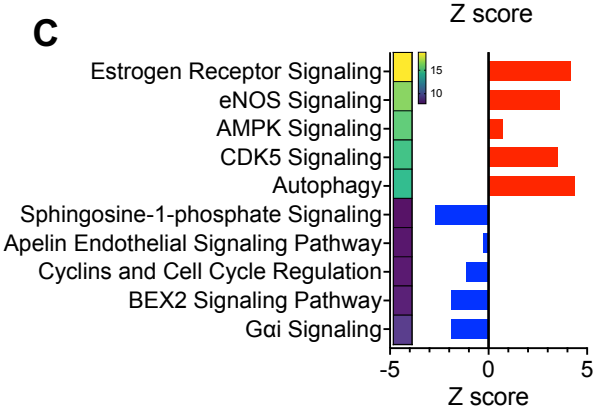


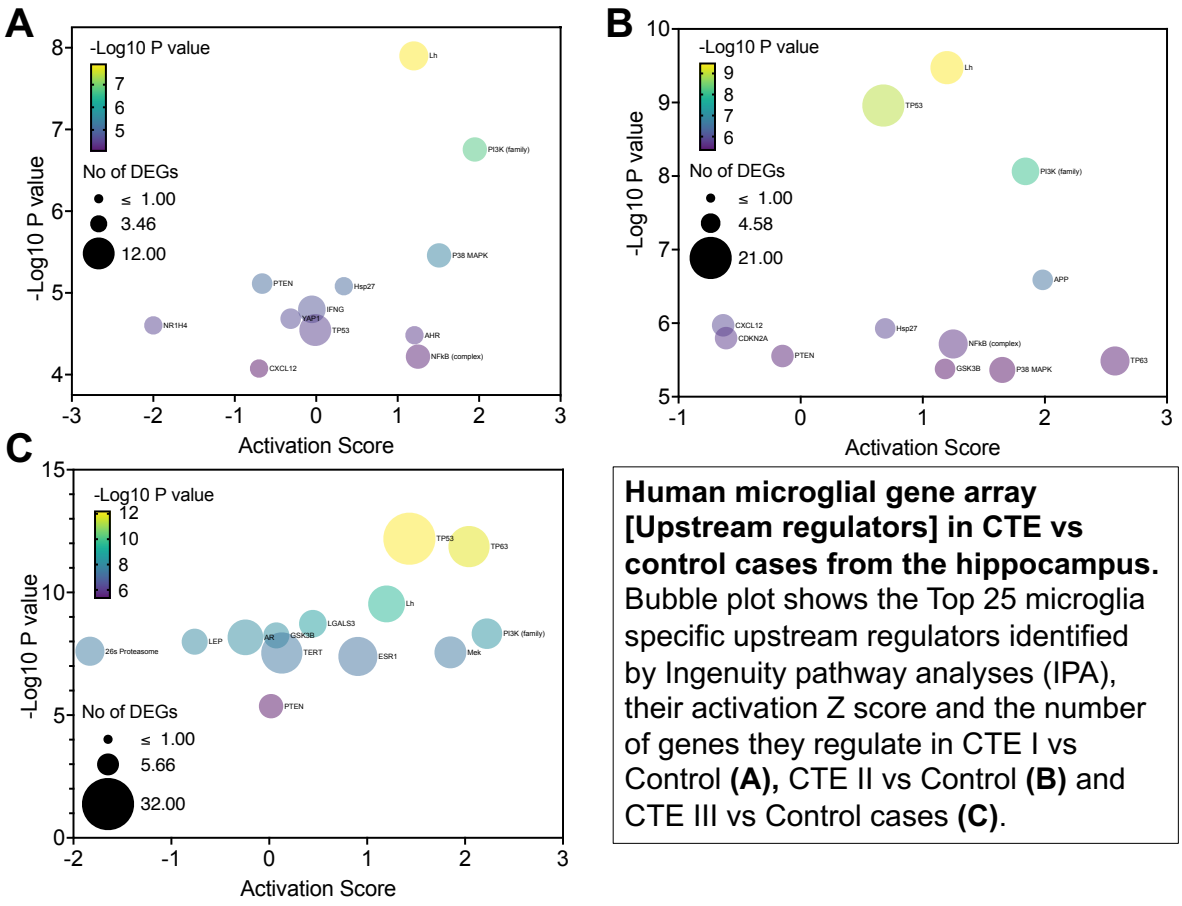
Figure [Aa, Bb, Cc] shows IBA1 stained control/CTE tissue, pre [A,B,C] and post microglia LCM extraction [a,b,c] (white circles show micro-dissected cells). **[D]** shows Venn diagram with overlapping significant microglia genes across CTE staging II-IV vs non demented control (NDC). Graph in **[E]** shows activation Z score of the top significant pathways identified from gene microarray in IBA1 bearing microglia from different stages of CTE vs NDC.







Human microglial gene array [dysregulated pathways] in CTE vs control cases from the hippocampus. Top upregulated and downregulated pathways were identified by Ingenuity pathway analyses (IPA) in CTE I vs Control **(A),** CTE II vs Control **(B)** and CTE III vs Control **(C)**. N=6 mice /group.



Human microglial gene array [Upstream regulators] in CTE vs control cases from the hippocampus. Bubble plot shows the Top 25 microglia specific upstream regulators identified by Ingenuity pathway analyses (IPA), their activation Z score and the number of genes they regulate in CTE I vs Control **(A),** CTE II vs Control **(B)** and CTE III vs Control cases **(C)**.

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

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

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

We will continue and/or complete studies in subtask 7, and 11 of Major Task 1, and subtask 2-4 of Major Task 2. We aim to have completed human/mouse histopathological analyses and single cell gene analyses of mouse and human autopsy tissue.

MAJOR TASK 1
Subtask 7: Complete stereological analyses and Image quantitation of brain sections from young TauKI and APP-KI mice (staggered over time).
Subtask 11: Complete stereological analyses and Image quantitation of brain sections from aged C57BL/6J, TauKI and APP-KI mice (staggered over time).

MAJOR TASK 2
Subtask 2-4: Finish bio-informatic analyses of microglia RNAseq profiles and validation of transcripts from ALL Young and Aged C57BL6, Tau and APP-KI cohorts.

ALL plans are consistent with the original approved SOW.

4. **IMPACT:**

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

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Our histological and microscopy (mouse) work (i.e. sectioning, image analyses etc.) has also been setback by approx. 6 months due to the COVID-19 lock-down and slow pace in activities in general, however, we have began maximizing our efforts and hope to make significant grounds in the next quarter.

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

As mentioned above, due to COVID-19, there has been a small reduction in work output which has significantly setback our histological and imaging analyses. We plan to request for an additional extra 6-8 months (no-cost extension) to enable completion of our studies for this award. 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 year.

Changes that had a significant impact on expenditures

Nothing to report

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

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:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to report (Two manuscripts planned for summer of 2022)

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Nothing to report (planned NNS conference presentation in June/July 2022)

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

Nothing to report

- **Technologies or techniques**

Nothing to report

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

Nothing to report

- **Other Products**

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. 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:

Please find the quad chart attached on the next page.

9. APPENDICES:

Nothing to report

Glial cell dysfunction in the pathobiological sequelae of repetitive mild traumatic injury

Log Number AZ170115

W81XWH-18-1-0811

PI: Drs Fiona Crawford / Joseph Ojo **Org:** Roskamp Institute, Sarasota, FL **Award Amount:** $720,000

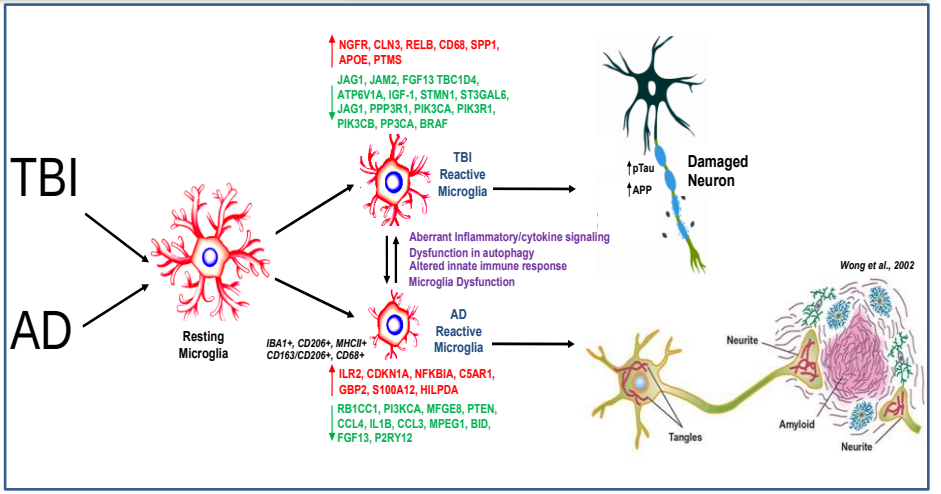
Study Aim 1: Delineation of the effects of chronic repetitive mTBI on microglia pathobiology and related proteinopathy in tau and amyloid bearing preclinical models at multiple time points post-injury, and validation in autopsied brains from human AD/CTE cases.

Study Aim 2: Generation of single cell genomic profiles in different populations of microglia obtained from preclinical models and validation in autopsied brains from human AD/CTE cases.

Approach

i) Histopathological assessment of microglia pathobiology in WT, hTau and APPNLF mouse models exposed to r-mTBI, or sham injury, when young (3 months) or aged (12 months) and analyzed at 14days, 3 and 6 months post-injury. Histopathological assessment of microglia pathobiology and in human AD/TBI cases.

ii) Single cell genomic profiles in different populations of microglia obtained from young and aged WT, hTau, and APPNLF mice exposed to r-mTBI or r-sham and analyzed at 14days, 3 and 6 months post-injury. Validation of distinct microglial cell population of interest and identified gene profiles using flow cytometry and cell sorting. Single cell genomic profiles of microglia from autopsied human AD/CTE brains.



Accomplishment

Completed analyses of mouse microglial cell RNAseq data from both young and aged models and human LCM/microglial gene array analyses. Continuing with histopathological and electron microscopy analyses of mouse brains.

Timeline and Cost

Activities CY	19	20	21
MAJOR TASK ONE OR AIM 1			
MAJOR TASK TWO OR AIM 2			
Estimated Direct Budget (500K)	**$130K**	**$205K**	**$165K**

Last updated: (September 2018)

Goals/Milestones

CY18 Goal

☑ Obtain regulatory approval to begin animal and human specimen studies

☑ Histological assessment of human control/TBI/AD brains tissue

CY19 Goals

☑ Initiate breeding and administer injuries to different mouse models

☑ Continue histological assessment of human control/TBI/AD brains tissue

☑ Gene analyses of microdissected microglia from autopsy brain tissue

CY20 Goals

☑ Histological assessment of microglia and proteinopathy in mouse models

☑ Complete human histological and gene array analyses

CY21 Goal

☑ Gene analyses of microdissected microglia from mouse models

☑ Validation experiments of gene array data in mouse models

☑ Histological assessment of microglia and proteinopathy in mouse models

Comments/Challenges/Issues/Concerns

- Histopathology and microscopy has been held back due to COVID-19

Projected Expenditure: $703,488.80 **Actual Expenditure**: 703,488.80