Supplementary Information for

Intranasal delivery of interleukin-4 attenuates chronic cognitive deficits via beneficial microglial responses in experimental traumatic brain injury

Hongjian Pu*1,2, Cheng Ma*2, Yongfang Zhao*2, Yangfan Wang2, Wenting Zhang1,2, Wanying Miao2, Fang Yu2, Xiaoming Hu1,2, Yejie Shi1,2, Rehana K. Leak3, T. Kevin Hitchens4, C. Edward Dixon1,5, Michael V.L. Bennett6, and Jun Chen1,2

1Geriatric Research, Education and Clinical Center, Veterans Affairs Pittsburgh Health Care System, Pittsburgh, PA 15240, USA; 2Pittsburgh Institute of Brain Disorders & Recovery and Department of Neurology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA; 3Graduate School of Pharmaceutical Sciences, School of Pharmacy, Duquesne University, Pittsburgh, PA 15282, USA; 4Animal Imaging Center, University of Pittsburgh School of Medicine, Pittsburgh, PA 15203, USA; 5Department of Neurosurgery, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA; 6Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, USA.

Address correspondence to:
Dr. Jun Chen
Geriatric Research, Education and Clinical Center
Veterans Affairs Pittsburgh Health Care System
University Drive C
Pittsburgh, PA 15240, USA
Tel: +1 (412) 624-4258
Email: chenj2@upmc.edu

This PDF file includes:
- Supplementary Methods
- References for SI reference citations
- Figs. S1 to S8
- Tables S1 to S2
Supplementary Methods

Construction of PPARγ conditional knockout mice
C57BL/6J WT, PPARγ\textsuperscript{flox/flox}, PDGFR\textsuperscript{α}CreER, and CX3CR1\textsuperscript{CreER} mice were purchased from the Jackson Laboratory (Bar Harbor, ME, United States). Oligodendrocyte progenitor cell-specific knockout (OPC-cKO) mice were obtained by crossing the PPARγ\textsuperscript{flox/flox} mice and PDGFR\textsuperscript{α}CreER mice for two generations. Microglia/macrophage-specific PPARγ knockout (PPARγ mKO) mice were generated by crossing the PPARγ\textsuperscript{flox/flox} mice and CX3CR1\textsuperscript{CreER} mice for two generations. The PPARγ OPC-cKO mice (genotype: PDGFR\textsuperscript{α}CreER\textsuperscript{(+/-)}; PPARγ\textsuperscript{flox/flox}) and PPARγ mKO mice (genotype: CX3CX1\textsuperscript{CreER\textsuperscript{(+/-)}; PPARγ\textsuperscript{flox/flox}) were viable, fertile, and normal in weight and did not exhibit any gross physical or behavioral abnormalities. The deletion of PPARγ in OPCs and microglia/microphages was induced by intraperitoneal injection of tamoxifen (Sigma, St. Louis, MO, USA, 75 mg/kg daily for 5 consecutive days). WT control mice received the same tamoxifen treatment. Mice were subjected to traumatic brain injury (TBI) 10 days after the last tamoxifen injection. Only male mice were used in this study.

Murine model of traumatic brain injury
TBI was performed by unilateral controlled cortical impact (CCI) as previously described.\textsuperscript{1} Animals were randomly assigned to sham and TBI groups, and anesthetized with 1.5% isoflurane in a mixture of 68.5% N\textsubscript{2}O/30% O\textsubscript{2} through a nose cone. Mouse heads were stabilized in a stereotaxic frame and a skin incision was made under aseptic conditions to expose the skull and bregma. A parietal craniotomy (3.5 mm diameter; centered 0.5 mm anterior and 2.0 mm lateral to bregma) was prepared with a drill to expose the dura and cerebral cortex. CCI was performed using a pneumatically driven CCI device (Precision Systems and Instrumentation, Fairfax Station, VA, USA) with a 3-mm (diameter) flat-tipped impactor to compress the exposed dura mater and underlying brain to a depth of 1.5 mm at a peak velocity of 3.75 m/s for a dwell time of 150 ms. Rectal temperatures of mice were maintained at 37.5 ± 0.5°C during the surgery with a heating pad. After surgery, the skin was sealed, and mice were placed in a clean cage. For the sham group, only anesthesia and skin incisions were performed. Ketoprofen (1-3 mg/kg) was administered via subcutaneous injections as analgesic prior to surgery and once a day for 3 days following surgery.

Preparation and intranasal administration of IL-4 liposome nanoparticles
IL-4 liposome nanoparticles were prepared as described.\textsuperscript{1} Briefly, lecithin (150 mg) and cholesterol (15 mg) were dissolved in a chloroform-methanol mixed solution (3:1, volume: volume; total volume: 5 mL), and then evaporated under vacuum distillation to form a lipid bilayer. Recombinant endotoxin-free IL-4 protein (1 mg, Peprotech, Rocky Hill, NJ, USA; endotoxin level less than 0.01 ng/μg of protein) was added for 30 min to the lipid bilayer with 0.01M PBS for hydration. The mixture was homogenized with ultrasonic probes for 15 min in an ice bath and filtered through a 0.45-μm membrane, followed by ultra-filtration with centrifugal filter units (100-KDa cutoff) to remove free IL-4 protein. Particle size, polydispersity index, and zeta potential of
IL-4 protein-loaded liposomes were evaluated by using dynamic light scattering. The concentration of IL-4 protein in the liposomes was examined by the bicinchoninic protein determination kit (Thermo Fisher Scientific, Pittsburgh, PA, USA).

Mice were randomly assigned to receive intranasal administration of vehicle or IL-4 (50 μg/kg body weight) nanoparticles under anesthesia, starting at 6 h and then once daily on days 1-7 and once weekly on day 14, 21, and 28 after CCI, as described. Nanoparticle-packed IL-4 was diluted to a concentration of 0.1 μg/μl, and five drops (~ 2 μl/drop) of the vehicle or diluted IL-4 nanoparticles were pipetted into each nostril with an interval of 2 min between drops. Intranasal administration at this dose is known to result in significant elevation in IL-4 concentration in various brain regions, including the cortex, striatum, corpus callosum, and external capsule, for up to 12-24 h. This intranasal IL-4 treatment regimen has been shown improve long-term outcomes in mouse models of focal cerebral ischemia and TBI.

**Behavior tests**

**Morris water maze:** The Morris water maze test was carried out daily for three days immediately before CCI and then daily from days 22 to 27 after CCI. Briefly, a square Plexiglas platform (11 cm × 11 cm) was submerged 1.5 cm under the water’s surface in the center of the north-west quadrant of a circular pool (diameter: 109 cm; depth: 33±0.5 cm). The water was maintained at 20±1°C and non-toxic white tempera paint was added in. Prominent spatial cues were arranged around the pool. The mouse was placed in one of the quadrants without the platform and allowed to swim 60 seconds to find the platform, and then placed on the platform for 30 seconds to reinforce learning at the end of each trail. This test was performed 3 days before CCI to habituate the mice to swimming. Mice that failed to swim or had a bias towards a particular quadrant were excluded from the experiment. The average time spent finding the platform during three trials was quantified as a measure of spatial learning from days 22 to 26 after CCI. On the 27th day after CCI, the platform was removed, and the mouse was placed in the pool for a single 60-second-long probe trail. The time spent swimming in the target quadrant where the platform had been located previously was recorded as ‘spatial memory’ and expressed as a percentage of total testing time. Average swim speeds were recorded every day to assess gross motor skills, and mice were excluded if unable to swim the three days before CCI.

**Novel object recognition:** The novel object recognition test was performed 15 and 29 days after CCI. Mice were placed in the center of the arena for 10 minutes to gain familiarity with two identical, non-appetitive objects placed in two corners (8 cm from adjacent corner walls). Following the pre-test phase, the mouse was placed in the home cage for one hour. For the novel object test phase, one original object was replaced by a novel object (both objects were consistent in height and volume but different in shape), and the mouse was placed back into the arena for 3 minutes. Novel object exploration was defined when the mouse approached an object with its snout (snout within a 2-cm perimeter around the object). The time spent exploring new objects was recorded. The exploration time (%) [(exploration time with novel object / total exploration time)]
and the discrimination index [(exploration time with novel object − exploration time with familiar object)/ total exploration time] were calculated, respectively.

**Passive avoidance test:** The passive avoidance test was performed 35 days after CCI.\(^5\) The chamber was divided into a lightroom and a darkroom, with a gate between the two. Stainless steel grids were placed on the floor of the darkroom to produce a mild foot shock. In the training phase, the gate was opened as the mouse was placed in the lightroom, and the mouse was allowed to enter the darkroom. Once the mouse was entirely in the darkroom, the gate was closed, and a foot shock (0.25 mA, 2s) was delivered. The latency until the animal crossed into the darkroom was recorded. One day after training, retention tests were performed to evaluate fear memory. Each mouse was placed in the lightroom with the gate opened, and the latency until entry into the darkroom from the lightroom was recorded (up to 300s). No electrical stimulation was applied in the test period.

**Immunofluorescence and image analysis**

At 1, 3, 7 and 35 days after TBI, mice were euthanized in a CO\(_2\) chamber and transcardially perfused with cold normal saline followed by 4% paraformaldehyde (PFA, Sigma-Aldrich; St. Louis, MO) in PBS. The harvested brains were cryoprotected in 30% sucrose in PBS for two days. Coronal brain sections (25 μm) were cut on a freezing microtome (model CM3050S; Leica Biosystems, Wetzlar, Germany). Brain sections were blocked in 5% donkey serum (Jackson ImmunoResearch Laboratories, West Grove, PA) in 0.3% PBST for 1 hour, followed by primary antibody overnight incubation at 4°C. The following primary antibodies were used: rabbit anti-NeuN (Millipore, 1:500, St. Louis, MO), rabbit anti-neurofilament heavy chain subunit 200 (NF200; Abcam, 1:500; Cambridge, MA); rat anti-CD16/32 (BD Biosciences, 1:200, Franklin Lakes, NJ); goat anti-arginase-1 (Arg1, Santa Cruz Biotechnology, 1:200, Dallas, TX); rabbit anti-Iba1 (Wako, 1:500, Richmond, VA); rabbit anti-Transmembrane Protein 119 (TMEM119, Abcam, 1:500, Cambridge, MA); rabbit anti-peroxisome proliferator-activated receptor gamma (PPAR\(_\gamma\), Santa Cruz Biotechnology, 1:100). Detailed sources and concentrations of primary antibodies can be found in Supplemental Table 1. After washing, brain sections were incubated for 1 hour at room temperature with donkey secondary antibodies conjugated to AlexaFluor 647, 488 or Cy3 (1:1000, Jackson Immuno Research Laboratories). After washing, brain sections were counterstained with Fluoromount-G containing 4’, 6-diamidino-2-phenylindole (DAPI; Southern Biotech, Birmingham, AL).

Fluorescence images were taken on a confocal microscope (Fluoview FV1000, Olympus). Images were randomly captured in each mouse in the perilesional CA1, CA3, and DG. The number of neurons, microglia and myelin debris were manually counted in ImageJ software (NIH) by an investigator blinded to experimental grouping.

**Diffusion tensor imaging (DTI)**

DTI was used to evaluate hippocampal white matter integrity after TBI.\(^1\) At 35 days after TBI, mice were euthanized in a CO\(_2\) chamber and perfused with normal saline, followed by 4% PFA.
Ex vivo brains within intact skulls were post-fixed in 4% PFA solution overnight at 4°C and then transferred to PBS before scanning. Magnetic resonance imaging (MRI) was performed using a Bruker AV3HD 11.7 Tesla/89mm vertical-bore microimaging system equipped with a Micro2.5 gradient set capable of 1500mT/m, ParaVision 6.0.1, and a 20 mm quadrature RF resonator (Bruker Biospin, Billerica, MA). Images of 25 contiguous coronal 0.5-mm slices were obtained with a 16 x 15 mm field of view (FOV), 160 ×160 acquisition matrices, 22 ms echo time (TE), 2800 ms repetition time (TR), 3000 s/mm² b-value, and 11.0/5.0 ms Δ/δ. Every slice was scanned twice, and the mean of two scans was collected and further calculated. This setup has been established for the acquisition of high-resolution DTI scanning in small animals.¹

DTI data were analyzed by using DSI Studio software (http://dsi-studio.labsolver.org/). ROIs encompassing the hippocampus were drawn manually in a blinded manner to outline the fiber tracts between CA1 and CA3. Fractional anisotropy (FA) reflects overall microstructural integrity; mean diffusivity (MD) is an inverse measure of membrane density (generally sensitive to cellularity, edema, and necrosis); radial diffusivity (RD) is generally sensitive to myelin integrity. ROIs in the hippocampus were manually drawn by investigators blinded to experimental grouping, where FA, AD, MD, and RD were determined. Directionally-encoded color (DEC), FA, RD, and 3D-reconstruction maps were generated by DSI Studio software.

Proteomic array analysis

Hippocampi harvested from the TBI hemisphere were used for proteomic arrays according to manufacturer’s instructions (RayBiotech, Cat No. AAM-INF-1). Briefly, total protein was extracted from hippocampi isolated from TBI or sham-operated animals five days post-surgery, when neuroinflammation is reaching peak in this model. Protein concentrations were quantified by the Bradford method (Bio-Rad, Hercules, USA). A total of 250 µg protein per sample was then loaded to the membrane for detection of inflammatory factors. The optical density of immunoreactive signal was measured using ImageJ software and the expression of inflammatory factors was normalized to the corresponding sham-injured group. Data are presented as fold changes.
References

1. Pu H, Zheng X, Jiang X, et al. Interleukin-4 improves white matter integrity and functional recovery after murine traumatic brain injury via oligodendroglial PPARγ. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* 2021; 41: 511-529.

2. Zhang Q, Zhu W, Xu F, et al. The interleukin-4/PPARγ signaling axis promotes oligodendrocyte differentiation and remyelination after brain injury. *PLoS biology* 2019; 17: e3000330.

3. Xia Y, Pu H, Leak RK, et al. Tissue plasminogen activator promotes white matter integrity and functional recovery in a murine model of traumatic brain injury. *Proceedings of the National Academy of Sciences of the United States of America* 2018; 115: E9230-e9238.

4. Iliff JJ, Chen MJ, Plog BA, et al. Impairment of glymphatic pathway function promotes tau pathology after traumatic brain injury. *Journal of Neuroscience* 2014; 34: 16180-16193.

5. Choi T-Y, Lee S-H, Kim S-J, et al. BK channel blocker paxilline attenuates thalidomide-caused synaptic and cognitive dysfunctions in mice. *Scientific Reports* 2018; 8: 1-9.
S Figure 1. IL-4 treatment does not change cognitive functions after sham surgery. Mice received intranasal administration of IL-4 (50 μg/kg) or vehicle starting at 6 h after sham surgery and repeated daily at 1-7 days and then weekly at 2, 3, and 4 weeks. (a-c) Morris water maze test. The escape latency during the cued learning test on days 22-26 (a), the number of platform crossings and the time spent in target quadrant on day 27 (b), and swim speed (c) are presented. n=5 per group. (d) Novel object recognition test. Exploration times and discrimination indices are presented. n=5 per group. (e) Passive avoidance test. The step-through latency on day-35 is presented. n=6 per group. Statistical analyses: Two-way repeated-measures ANOVA with Bonferroni post hoc test for a and d. Unpaired t-test for b and c. Mann-Whitney test for e. Shown are the mean ± SD or box plots.
S Figure 2. IL-4 treatment improves long-term cognitive functions in PPARγ OPC-cKO mice after TBI. (a-d) Morris water maze test. Swim speed (a), the escape latency during cued learning test on days 22-26 (b), representative track plot for learning and memory tests (c), the number of platform crossings and the time spent in target quadrant in the memory test on day 27 (d) after TBI or sham surgery. n=7, OPC-cKO sham group; n=10 per OPC-cKO TBI group. (e) Passive avoidance test. Step-through latency on day 35. n=8, OPC-cKO sham group; n=11, OPC-cKO TBI Veh group; n=12, OPC-cKO TBI IL-4 group. Statistical analyses: One-way ANOVA followed by Bonferroni post hoc test for a, d (target quadrant time), and e. Kruskal–Wallis test with Dunn post hoc test for d (platform crossings). Two-way repeated-measures ANOVA with Bonferroni post hoc test for b. Shown are mean ± SD or box plots. *p< 0.05, **p< 0.01, ***p< 0.001 as indicated, ns: no significance.
S Figure 3. Diffusion tensor imaging (DTI) of the hippocampus after TBI: gross alterations. (a) Representative images of FA and RD maps (upper 2 panels; at the coronal level of AP -2.18mm), and 3D reconstruction of DTI images based on 4 scanning planes that encompass the hippocampus of mouse brain (bottom panel; at the coronal levels of Bregma -1.06mm, -1.58mm, -2.18mm, -2.54 mm) at 35 days after TBI or sham surgery. (b) Quantification of hippocampal volume based on 3D reconstructions of DTI. (c) Hippocampal volume presented as a percentage of the contralateral hippocampus. (d) Quantification of fractional anisotropy (FA), axial diffusivity (AD), mean diffusivity (MD), and radial diffusivity (RD) based on the above 4 scanning planes. n=6, sham group; n=8, TBI Veh group; n=6, TBI IL-4 group. **Statistical analyses**: One-way ANOVA followed by Bonferroni post hoc test for b, c, and d (FA). Kruskal–Wallis test with Dunn post hoc test for d (MD, AD, and RD). Shown are the mean ± SD or box plots. *p< 0.05 as indicated.
S Figure 4. The majority of Iba1+ cells in post-TBI hippocampi are microglia. Double-label immunofluorescent staining for Iba1 and TMEM119 was performed at 3 days after TBI. (a) Representative images of TMEM119 (red) and Iba1 (green) immunofluorescence in the peri-lesion cortex (CTX), and hippocampal CA1, CA3, and DG regions in the hemisphere ipsilateral to TBI. The corresponding regions in the contralateral hemisphere serve as controls. The regions of interest depicted by white squares in the fourth column are enlarged and presented in the fifth column. Scale bar=50 µm. (b) Quantification of TMEM119+Iba1+ cells (percentage of total Iba1+ cells) in CTX, CA1, CA3, and DG of both hemispheres. n=6 per group. Mann-Whitney test. Shown are box plots. **p<0.01 as indicated, ns: no significance.
S Figure 5. IL-4 treatment reduces inflammatory burden in the hippocampus after TBI (see main figure 4). (a) A panel of 40 inflammatory makers was measured in hippocampal extracts 5 days after TBI or sham surgery and treatment with IL-4 or vehicle. The heatmap shows fold changes over the sham group. n=3 per sham group; n=6 per TBI group. Statistical analyses: Two-way ANOVA with Bonferroni post hoc test was used. #p< 0.05, ##p< 0.01, ###p < 0.001 vehicle-treated TBI group vs. sham group. *p< 0.05, **p< 0.01, ***p< 0.001 vehicle-treated TBI group vs. IL-4 treated TBI group. (b) Representative images of protein array membranes on all 4 experimental groups. The 10 inflammatory markers that exhibited significant changes by IL-4
treatment after TBI are labeled in red boxes. The array map in the lower panel shows the identifications of all 40 inflammatory markers and their corresponding positions in the protein array membrane.
S Figure 6. Temporal profile and cellular distribution of PPARγ immunofluorescence after TBI. (a) Representative double-label immunofluorescence for PPARγ (red) with Iba1 (green), NeuN (green), GFAP (green), or APC (green) at 1, 3, and 7 days after TBI. Scale bar=50 μm. The white squares indicate where the enlarged images were captured. (b) Quantification of PPARγ-positive cells double-labeled with Iba1, NeuN, GFAP, and APC at 1, 3, and 7 days after TBI or sham surgery. n=5, sham group; n=5, 1d post-TBI group; n=6, 3d post-TBI group; n=6, 7d post-TBI group. Statistical analyses: One-way ANOVA followed by Bonferroni post hoc test for b (microglia, astrocyte, and oligodendrocyte). Kruskal–Wallis test with Dunn post hoc for b (neuron). Shown are the mean ± SD or box plots. *p< 0.05, **p< 0.01, ***p< 0.001 as indicated.
S Figure 7. IL-4 polarizes microglia in the hippocampus after TBI (full-scale figure for Figure 5d). Representative images of triple-label immunofluorescence for Iba1 (white), CD16 (red), and Arg1 (green) in CA1, CA3, and DG of PPARγ mKO mice at 7 days after TBI or sham surgery. Scale bar=50 µm.
S Figure 8. IL-4 does not alter cognitive functions in PPARγ mKO mice after sham surgery. (a-d) Morris water maze test. The escape latency in the cued learning test on days 22-26 (a), the number of platform crossings and time spent in the target quadrant in the memory test on day 27 (b), and swim speed (c) of PPARγ mKO mice treated with vehicle or IL4 after sham surgery. (d) Passive avoidance test. Step-through latency on day 35 after sham surgery. (e) Novel object recognition test. Exploration times and discrimination indices measured before and 15 and 29 days after sham surgery. n=4, vehicle-treated mKO sham group; n=5, IL-4-treated mKO sham group. **Statistical analyses**: Two-way repeated-measures ANOVA with Bonferroni post hoc test for a and e. Student’s t-test for b and c. Kruskal–Wallis test with Dunn post hoc for d. Shown are mean ± SD or box plots.
S Table 1. Sources and concentrations of primary antibodies used in this study.

| Primary antibody target | Source | Catalog# (Host) | Application (dilution) |
|-------------------------|--------|-----------------|------------------------|
| NeuN                   | Millipore | ABN78 (Rabbit) | 1:500                  |
| Neurofilament 200      | Abcam  | Ab8135 (Rabbit) | 1:500                  |
| CD16/32                | Biosciences | 553142 (Rat) | 1:200                  |
| Arg1                   | Santa Cruz Biotechnology | sc-18351 (Goat) | 1:200                  |
| Iba1                   | Wako | 019-19741 (Rabbit) | 1:500                  |
|                        | Abcam | Ab5076 (Goat) | 1:500                  |
| Transmembrane Protein 119 | Abcam | ab209064 (Rabbit) | 1:500                  |
| Peroxisome proliferator-activated receptor gamma | Santa Cruz Biotechnology | sc-271392 (Mouse) | 1:100                  |
| MBP                    | Abcam | ab40390 (Rabbit) | 1:500                  |
### S Table 2. Statistics reporting

| Figure | N (mouse number) | Data Structure | Test Used | Statistic s | p Value |
|--------|------------------|----------------|-----------|-------------|---------|
| Figure 1(c) Escape latency | Sham n=10 TBI Veh n=10 TBI IL-4 n=10 | Normal distribution | Two-way Repeated-Measures ANOVA; Bonferroni post hoc | $F(2, 27) = 7.084$ | $p = 0.0034$; Sham vs. TBI Veh $**p = 0.0012$ TBI Veh vs. TBI IL-4 $p = 0.1087$ Sham vs. TBI IL-4 $*p = 0.04366$ |
|        |                  |                |           |             |         |
|        |                  |                |           | Pre: Sham vs. TBI Veh $p = 0.8724$; TBI Veh vs. TBI IL-4 $p = 0.7701$; Sham vs. TBI IL-4 $p = 0.9800$; 22d: Sham vs. TBI Veh $p = 0.1829$; TBI Veh vs. TBI IL-4 $p = 0.9523$; Sham vs. TBI IL-4 $p = 0.3068$; 23d: Sham vs. TBI Veh $***p < 0.0001$; TBI Veh vs. TBI IL-4 $p = 0.3391$; Sham vs. TBI IL-4 $p = 0.0122$; 24d: Sham vs. TBI Veh $**p = 0.0023$; TBI Veh vs. TBI IL-4 $p = 0.5748$; Sham vs. TBI IL-4 $p = 0.0441$; 25d: Sham vs. TBI Veh $***p < 0.0001$; TBI Veh vs. TBI IL-4 $**p = 0.0083$; Sham vs. TBI IL-4 $p = 0.0993$; 26d: Sham vs. TBI Veh $*p = 0.0299$; TBI Veh vs. TBI IL-4 $p = 0.1311$; Sham vs. TBI IL-4 $p = 0.8054$. |
| Figure 1(d) Platform crossing Count | Sham n=10 TBI Veh n=10 TBI IL-4 n=10 | Non-normal distribution | Kruskal-Wallis test; Dunn post hoc | Kruskal-Wallis statistic $= 8.896$ | $p = 0.0117$; Sham vs. TBI $**p = 0.0086$; TBI Veh vs. TBI IL-4 $p = 0.3772$; Sham vs. TBI Veh $p = 0.4402$. |
| Figure 1(d) Target quadrant time% | Sham n=10 TBI Veh n=10 TBI IL-4 n=10 | Normal distribution | One-way ANOVA; Bonferroni post hoc | $F(2, 27) = 10.62$ | $p = 0.0004$; Sham vs. TBI Veh $***p = 0.0008$; TBI Veh vs. TBI IL-4 $**p = 0.0024$; Sham vs. TBI IL-4 $p > 0.9999$. |
| Figure 1(c) | Swimming Speed |
|-------------|----------------|
| Sham n=10  | TBI Veh n=10   |
| TBI IL-4 n=10 | Non-normal distribution | Kruskal-Wallis test; Dunn post hoc |
| p = 0.9101; | Sham vs. TBI Veh \( p > 0.9999; \) |
| TBI Veh vs. TBI IL-4 \( p > 0.9999; \) |
| Sham vs. TBI IL-4 \( p > 0.9999. \) |

| Figure 1(f) | NOR Exploration time % |
|-------------|------------------------|
| Sham n=10  | TBI Veh n=11           |
| TBI IL-4 n=11 | Normal distribution | Two-way Repeated-Measures ANOVA; Bonferroni post hoc |
| ANOVA \( p = 0.023 \) | Pre: |
| Sham vs. TBI Veh \( p = 0.4671; \) |
| TBI Veh vs. TBI IL-4 \( p > 0.9999; \) |
| Sham vs. TBI IL-4 \( p > 0.9999. \) |
| 15d: |
| Sham vs. TBI Veh \( p = 0.2690; \) |
| TBI Veh vs. TBI IL-4 \( p > 0.9999; \) |
| Sham TBI IL-4. \( p = 0.1904. \) |
| 29d: |
| Sham vs. TBI Veh \( **p = 0.0025; \) |
| TBI Veh vs. TBI IL-4 \( *p = 0.0374; \) |
| Sham vs. TBI IL-4 \( p = 0.9114. \) |

| Figure 1(f) | NOR Discrimination index |
|-------------|-------------------------|
| Sham n=10  | TBI Veh n=11            |
| TBI IL-4 n=11 | Normal distribution | Two-way Repeated-Measures ANOVA; Bonferroni post hoc |
| ANOVA \( p = 0.023 \) | Pre: |
| Sham vs. TBI Veh \( p = 0.4671; \) |
| TBI Veh vs. TBI IL-4 \( p > 0.9999; \) |
| Sham vs. TBI IL-4 \( p > 0.9999. \) |
| 15d: |
| Sham vs. TBI Veh \( p = 0.2690; \) |
| TBI Veh vs. TBI IL-4 \( p > 0.9999; \) |
| Sham TBI IL-4. \( p = 0.1904. \) |
| 29d: |
| Sham vs. TBI Veh \( **p = 0.0025; \) |
| TBI Veh vs. TBI IL-4 \( *p = 0.0374; \) |
| Sham vs. TBI IL-4 \( p = 0.9114. \) |

| Figure 1(g) | Passive avoidance |
|-------------|-------------------|
| Sham n=12  | TBI Veh n=14      |
| TBI IL-4 n=16 | Normal distribution | Welch ANOVA; Dunnett T3 post hoc |
| \( p < 0.0001. \) | Sham vs. TBI Veh \( ***p = 0.0002 \) |
| TBI Veh vs. TBI IL-4 \( ***p < 0.0001 \) |
| Sham vs. TBI IL-4 \( p = 0.6110. \) |

| Figure 2(a) | I-O fEPSP-current intensity curve |
|-------------|----------------------------------|
| Sham n=6   | TBI Veh n=5                     |
| TBI IL-4 n=5 | Normal distribution | Two-way Repeated-Measures ANOVA; Bonferroni post hoc |
| \( p < 0.0001 \) | Sham vs. TBI Veh \( ***p < 0.0001; \) |
| TBI Veh vs. TBI IL-4 \( p > 0.9999; \) |
| Sham vs. TBI IL-4 \( ***p < 0.0001. \) |

| Figure 2(b) | fEPSP slope % |
|-------------|---------------|
| Sham n=6   | TBI Veh n=5   |
| Normal distribution | Two-way Repeated-Measures |
| \( p < 0.0001 \) | Sham vs. TBI Veh \( ***p = 0.0004; \) |
| Figure | Description | Sham | TBI Veh | TBI IL-4 | Analysis | Pearson R | p-value |
|--------|-------------|-------|---------|----------|----------|-----------|----------|
| 2(c)   | Learning (MWM)-fEPSP slope correlation | n=6   | n=5     | n=5      | ANOVA; Bonferroni post hoc | r = -0.5958 | p = 0.0149 |
| 2(c)   | Memory (MWM)-fEPSP slope correlation | n=6   | n=5     | n=5      | ANOVA; Bonferroni post hoc | r = 0.6018 | p = 0.0136 |
| 2(d)   | Passive avoidance-fEPSP slope correlation | n=6   | n=5     | n=5      | ANOVA; Bonferroni post hoc | r = 0.4999 | p = 0.0487 |
| 2(e)   | NOR-fEPSP slope correlation | n=6   | n=5     | n=5      | ANOVA; Bonferroni post hoc | r = 0.0641 | p = 0.8136 |
| 2(h)   | Density of fiber tracts | n=6   | n=8     | n=6      | One-way ANOVA; Bonferroni post hoc test | F (2,17) = 12.74 | p = 0.0004; Sham vs. TBI Veh ***p = 0.0003; TBI Veh vs. TBI IL-4 *p = 0.0369; Sham vs. TBI IL-4 p = 0.1675. |
| 2(i)   | Learning (MWM)-DTI correlation | n=6   | n=8     | n=6      | ANOVA; Bonferroni post hoc | r = -0.4921 | p = 0.0275 |
| 2(i)   | Memory (MWM)-DTI correlation | n=6   | n=8     | n=6      | ANOVA; Bonferroni post hoc | r = -0.0074 | p = 0.9753 |
| 2(j)   | Passive | n=6   | n=8     | n=6      | ANOVA; Bonferroni post hoc | r = 0.5236 | p = 0.0178 |
| Table Title | Legend | Statistical Test | Parameter 1 | Parameter 2 | Parameter 3 | p-value |
|-------------|--------|------------------|-------------|-------------|-------------|---------|
| avoidance-DTI correlation | TBI IL-4 n=6 | regression analysis | | | | |
| Figure 2(k) NOR-DTI correlation | Sham n=6; TBI Veh n=8; TBI IL-4 n=6 | N/A | Pearson product linear regression analysis | r = 0.4770 | p = 0.0334 |
| Figure 3(b) CA1 | Sham n=8; TBI Veh n=10; TBI IL-4 n=10 | Normal distribution | One-way ANOVA; Bonferroni post hoc | F (2, 25) = 2.576 | p = 0.0961  
Sham vs. TBI Veh p = 0.2370;  
TBI Veh vs. TBI IL-4 p = 0.1543;  
Sham vs. TBI IL-4 p > 0.9999. |
| Figure 3(b) CA3 | Sham n=8; TBI Veh n=10; TBI IL-4 n=10 | Normal distribution | One-way ANOVA; Bonferroni post hoc | F (2, 25) = 6.300 | ANOVA p = 0.0061  
Sham vs. TBI Veh **p = 0.0093;  
TBI Veh vs. TBI IL-4 *p = 0.0326;  
Sham vs. TBI IL-4 p > 0.9999. |
| Figure 3(b) DG | Sham n=8; TBI Veh n=10; TBI IL-4 n=10 | Normal distribution | One-way ANOVA; Bonferroni post hoc | F (2, 25) = 0.0257 | p = 0.9747  
Sham vs. TBI Veh p > 0.9999;  
TBI Veh vs. TBI IL-4 p > 0.9999;  
Sham vs. TBI IL-4 p > 0.9999. |
| Figure 3(c) CA3 NeuN+- Learning (MWM) correlation | Sham n=8; TBI Veh n=10; TBI IL-4 n=10 | N/A | Pearson product linear regression analysis | r = -0.2010 | p = 0.3050 |
| Figure 3(c) CA3 NeuN+- Memory (MWM) correlation | Sham n=8; TBI Veh n=10; TBI IL-4 n=10 | N/A | Pearson product linear regression analysis | r = 0.3234 | p = 0.0932 |
| Figure 3(d) CA3 NeuN+- Passive avoidance correlation | Sham n=8; TBI Veh n=10; TBI IL-4 n=10 | N/A | Pearson product linear regression analysis | r = 0.5182 | p = 0.0047 |
| Figure 3(e) CA3 NeuN+- Passive avoidance correlation | Sham n=8; TBI Veh n=10; TBI IL-4 n=10 | N/A | Pearson product linear regression analysis | r = 0.7058 | p < 0.0001 |
| Figure 3(g) NF200 intensity | Sham n=6; TBI Veh n=6; TBI IL-4 n=6 | Normal distribution | One-way ANOVA; Bonferroni post hoc test | F (2,15) = 39.26 | p < 0.0001  
Sham vs. TBI Veh ***p < 0.0001;  
TBI Veh vs. TBI IL-4 *p = 0.0205;  
Sham vs. TBI IL-4 ***p = 0.0001. |
| Figure 3(h) NF200-Learning | Sham n=6 | N/A | Pearson product | r = -0.6 | p = 0.0085 |
| (MWM) correlation | TBI Veh n=6 | linear regression analysis | N/A | Pearson product linear regression analysis | r = 0.2605 | p = 0.2965 |
|---|---|---|---|---|---|---|
| Figure 3(h) NF200-Memory (MWM) correlation | Sham n=6 TBI Veh n=6 TBI IL-4 n=6 | | N/A | Pearson product linear regression analysis | r = 0.7548 | p = 0.0003 |
| Figure 3(i) NF200-Passive avoidance correlation | Sham n=6 TBI Veh n=6 TBI IL-4 n=6 | | N/A | Pearson product linear regression analysis | r = 0.1018 | p = 0.6877 |
| Figure 3(j) NF200-Passive avoidance correlation | Sham n=6 TBI Veh n=6 TBI IL-4 n=6 | Normal distribution | CA1: F(2,15)= 264.5 CA3: W = 62.30 DG: W = 101.0 | CA1: p < 0.0001 Sham vs. TBI Veh ***p < 0.0001; TBI Veh vs. TBI IL-4 ***p < 0.0001; Sham vs TBI IL-4 *p = 0.0280. CA3: p < 0.0001 Sham vs. TBI Veh **p = 0.0002; TBI Veh vs. TBI IL-4 *p = 0.0675. DG: p < 0.0001 Sham vs. TBI Veh ***p = 0.0001; TBI Veh vs. TBI IL-4 ***p = 0.0002; Sham vs. TBI IL-4 ***p = 0.0001. | |
| Figure 4(b) CD16^+Iba1^+ cells | Sham n=6 TBI Veh n=6 TBI IL-4 n=6 | Normal distribution | Brown-Forsythe ANOVA test; Dunnett T3 post hoc test | CA1: F = 43.52 CA3: F = 28.89 DG: F = 37.09 | CA1: p < 0.0001 Sham vs. TBI Veh p = 0.2166 TBI Veh vs. TBI IL-4 ***p = 0.0003 Sham vs. TBI IL-4 ***p < 0.0001 CA3: p = 0.0006 Sham vs. TBI Veh *p = 0.0107; TBI Veh vs. TBI IL-4 *p = 0.0109; Sham vs. TBI IL-4 **p = 0.0040. DG: p = 0.0007 Sham vs. TBI p = 0.2438; | |
| Figure 4(d) Protein array | Sham Veh n=3 | Sham IL-4 n=3 | TBI Veh n=6 | TBI IL-4 n=6 | Normal distribution | Two-way ANOVA; Bonferroni post hoc | TNFSF8 | Sham vs. TBI ##p = 0.0011 | TBI Veh vs. TBI IL-4 ***p < 0.0001 | CCL11 | Sham vs. TBI #p = 0.0482 | TBI Veh vs. TBI IL-4 **p = 0.0097 | Fas Ligand | Sham vs. TBI ###p = 0.0006 | TBI Veh vs. TBI IL-4 ***p < 0.0001 | IFN-γ | Sham vs. TBI ##p = 0.0011 | TBI Veh vs. TBI IL-4 *p = 0.0495 | IL-1β | Sham vs. TBI ###p < 0.0001 | TBI Veh vs. TBI IL-4 ***p < 0.0001 | IL-2 | Sham vs. TBI #p = 0.0124 | TBI Veh vs. TBI IL-4 *p = 0.0112 | IL-3 | Sham vs. TBI ###p = 0.0066 | TBI Veh vs. TBI IL-4 **p = 0.0027 | CXCL9 | Sham vs. TBI ###p < 0.0001 | TBI Veh vs. TBI IL-4 ***p < 0.0001 | CCL3 | Sham vs. TBI #p = 0.0359 | TBI Veh vs. TBI IL-4 ***p = 0.0009 | CCL25 | Sham vs. TBI ###p = 0.0049 | TBI Veh vs. TBI IL-4 ***p = 0.0008 |
|--------------------------|------------|------------|-------------|-------------|---------------------|---------------------------------|--------|-------------------------|--------------------------|--------|-------------------------|--------------------------|-------------------------|-------------------------|--------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Figure 4(f) PPARγ^Iba1^ cells | TBI Veh n=6 | TBI IL-4 n=6 | Non-normal distribution | Mann-Whitney test | Mann-Whitney test U = 1 | **p = 0.0065 |
| Figure 4(h) Phagocytosis | TBI Veh n=6 | TBI IL-4 n=6 | Normal distribution | Welch’s t test | t = 5.889; df = 6.197 | ***p = 0.0009 |
| Figure 5(c) PPARγ intensity | Control n=6 mKO n=9 | Non-normal distribution | Mann-Whitney test | Mann-Whitney test U = 0 | ***p = 0.0004 |
| Figure 5(e) CD16⁺Iba1⁺ cells | mKO Sham n=6 | Normal distribution | Brown-Forsythe ANOVA test; Dunnett T3 post hoc test | CA1: F = 36.10 | CA1: p = 0.0005  
 mKO Sham vs. mKO TBI  **p = 0.0088;  
 mKO TBI Veh vs. mKO TBI IL-4  p = 0.9960;  
 mKO Sham vs. mKO TBI IL-4  ***p = 0.0001.  
 CA3: F = 21.78  
 DG: F = 58.24  
 mKO Sham vs. mKO TBI Veh  *p = 0.0244;  
 mKO TBI Veh vs. mKO TBI IL-4  p = 0.9912;  
 mKO Sham vs. mKO TBI IL-4  ***p = 0.0006.  
 DG: p < 0.0001  
 mKO Sham vs. mKO TBI Veh  **p = 0.0048;  
 mKO TBI Veh vs. mKO TBI IL-4  p = 0.4656;  
 mKO Sham vs. mKO TBI IL-4  ***p < 0.0001. |
| Figure 5(f) Arg1⁺/Iba1⁺ cells | mKO Sham n=6 | Non-Normal distribution | Kruskal-Wallis test | CA1: Kruskal-Wallis statistic = 11.16  
 CA3: Kruskal-Wallis statistic = 8.255  
 DG: Kruskal-Wallis statistic = 11.66  
 mKO Sham vs. mKO TBI Veh  *p = 0.0457;  
 mKO TBI Veh vs. mKO TBI IL-4  p > 0.9999;  
 mKO Sham vs. mKO TBI IL-4  **p = 0.0049.  
 CA3: p = 0.0077  
 mKO Sham vs. mKO TBI Veh  *p = 0.0186;  
 mKO TBI Veh vs. mKO TBI IL-4  p > 0.9999;  
 mKO Sham vs. mKO TBI IL-4  p = 0.1292.  
 DG: p = 0.0001  
 mKO Sham vs. mKO TBI  **p = 0.0052;  
 mKO TBI Veh vs. mKO TBI IL-4  p > 0.9999;  
 mKO Sham vs. mKO TBI IL-4  *p = 0.0256. |
| Figure 6(b) DTI Fiber density | mKO TBI Veh n=6 | Normal distribution | Student t test | t = 0.1281  
 df = 8  
 p = 0.9012 |
| Figure 6(d) NF200 intensity | mKO Sham n=6  
mKO TBI Veh n=5  
mKO TBI IL-4 n=5 | Non-normal distribution | Kruskal-Wallis test | Kruskal-Wallis statistic =10.63 | $p = 0.0006$  

mKO Sham vs mKO TBI Veh. *$p = 0.0227$;  
mKO TBI Veh vs mKO TBI IL-4 $p > 0.9999$;  
mKO Sham vs mKO TBI IL-4 *$p = 0.0120$. |
| Figure 6(f) CA1 NeuN$^+$ cells | mKO Sham n=9  
mKO TBI Veh n=10  
mKO TBI IL-4 n=12 | Normal distribution | One-way ANOVA;  
Bonferroni post hoc | F (2, 28) = 2.797 | $p = 0.0781$  

mKO Sham vs mKO TBI Veh $p = 0.0759$;  
mKO TBI Veh vs mKO TBI IL-4 $p = 0.6273$;  
mKO Sham vs mKO TBI IL-4 $p = 0.7050$. |
| Figure 6(f) CA3 NeuN$^+$ cells | mKO Sham n=9  
mKO TBI Veh n=10  
mKO TBI IL-4 n=12 | Normal distribution | One-way ANOVA;  
Bonferroni post hoc | F (2, 28) = 26.76 | $p < 0.0001$  

mKO Sham vs mKO TBI Veh ***$p < 0.0001$;  
mKO TBI Veh vs mKO TBI IL-4 $p > 0.9999$;  
mKO Sham vs mKO TBI IL-4 ***$p < 0.0001$. |
| Figure 6(f) DG NeuN$^+$ cells | mKO Sham n=9  
mKO TBI Veh n=10  
mKO TBI IL-4 n=12 | Non-normal distribution | Kruskal-Wallis test | Kruskal-Wallis statistic = 9.379 | $p = 0.0092$  

mKO Sham vs mKO TBI Veh **$p = 0.0096$;  
mKO TBI Veh vs mKO TBI IL-4 $p > 0.9999$;  
mKO Sham vs mKO TBI IL-4 $p = 0.0623$. |
| Figure 6(g) CA3 NeuN$^+$ cells-Learning (MWM) correlation | mKO TBI Veh n=10  
mKO TBI IL-4 n=12 | N/A | Pearson product linear regression analysis | $r = -0.7686$ | $p < 0.0001$ |
| Figure 6(g) CA3 NeuN$^+$ cells-Memory (MWM) correlation | mKO TBI Veh n=10  
mKO TBI IL-4 n=12 | N/A | Pearson product linear regression analysis | $r = 0.8813$ | $p < 0.0001$ |
| Figure 6(h) CA3 NeuN$^+$ cells-Passive avoidance correlation | mKO TBI Veh n=10  
mKO TBI IL-4 n=12 | N/A | Pearson product linear regression analysis | $r = 0.4883$ | $p = 0.0211$ |
| Figure 6(i) CA3 NeuN$^+$ cells-NOR correlation | mKO TBI Veh n=10  
mKO TBI IL-4 n=12 | N/A | Pearson product linear regression analysis | $r = 0.9384$ | $p < 0.0001$ |
| Figure 7(a) Escape latency | mKO Sham n=12  
mKO TBI Veh n=13 | Normal distribution | Two-way Repeated-Measures ANOVA; | F (2, 35) = 7.860 | $p = 0.0015$  

mKO Sham vs. mKO TBI Veh **$p = 0.0077$;  
mKO TBI Veh vs. mKO TBI IL-4 $p = 0.7808$. |
mKO TBI IL-4 n=13

Bonferroni post hoc

mKO Sham vs. mKO TBI IL-4
**p = 0.0031

Pre:
mKO Sham vs. mKO TBI Veh  p > 0.9999;
mKO TBI Veh vs. mKO TBI IL-4  p > 0.9999;
mKO Sham vs. mKO TBI IL-4  p > 0.9999

22d:
mKO Sham vs. mKO TBI Veh  p > 0.9999;
mKO TBI Veh vs. mKO TBI IL-4  p > 0.9999;
mKO Sham vs. mKO TBI IL-4  p > 0.9999

23d:
mKO Sham vs. mKO TBI Veh  p = 0.6421;
mKO TBI Veh vs. mKO TBI IL-4  p > 0.9999;
mKO Sham vs. mKO TBI IL-4  p = 0.1117

24d:
mKO Sham vs. mKO TBI Veh  p = 0.1069;
mKO TBI Veh vs. mKO TBI IL-4  p > 0.9999;
mKO Sham vs. mKO TBI IL-4  p = 0.0847

25d:
mKO Sham vs. mKO TBI Veh  *p  = 0.0132;
mKO TBI Veh vs. mKO TBI IL-4  p > 0.9999;
mKO Sham vs. mKO TBI IL-4  *p =0.0128

26d:
mKO Sham vs. mKO TBI Veh  ***p = 0.0003;
mKO TBI Veh vs. mKO TBI IL-4  p > 0.9999;
mKO Sham vs. mKO TBI IL-4  ***p = 0.0007.
| Figure 7(b) Platform crossing count | mKO Sham n=12  
mKO TBI Veh n=13  
mKO TBI IL-4 n=13 | Non-normal distribution | Kruskal-Wallis test | Kruskal-Wallis statistic = 22.43 | $p < 0.0001$  
mKO Sham vs. mKO TBI Veh  
***$p = 0.0001$;  
mKO TBI Veh vs. mKO TBI IL-4  
$p > 0.9999$  
mKO Sham vs. mKO TBI Veh  
***$p < 0.0001$ |
| Figure 7(b) Target quadrant time% | mKO Sham n=12  
mKO TBI Veh n=13  
mKO TBI IL-4 n=13 | Normal distribution | One-way ANOVA; Bonferroni post hoc | $F (2, 35) = 6.684$ | **$p = 0.0035$  
mKO Sham vs. mKO TBI Veh  
**$p = 0.0099$;  
mKO TBI Veh vs. mKO TBI IL-4  
$p > 0.9999$  
mKO Sham vs. mKO TBI Veh  
**$p = 0.0083$ |
| Figure 7(c) Swimming Speed | mKO Sham n=12  
mKO TBI Veh n=13  
mKO TBI IL-4 n=13 | Normal distribution | One-way ANOVA; Bonferroni post hoc | $F (2, 35) = 0.2230$ | $p = 0.8012$  
mKO Sham vs. mKO TBI Veh  
$p > 0.9999$;  
mKO TBI Veh vs. mKO TBI IL-4  
$p > 0.9999$  
mKO Sham vs. mKO TBI Veh  
$p > 0.9999$ |
| Figure 7(d) Passive avoidance | mKO Sham n=12  
mKO TBI Veh n=13  
mKO TBI IL-4 n=13 | Normal distribution | Two-way ANOVA; Bonferroni post hoc | $F (2, 32) = 3.780$ | $p = 0.0336$  
Pre:  
mKO Sham vs. mKO TBI Veh  
$p > 0.9999$;  
mKO+ Vehicle vs. mKO TBI IL-4  
$p > 0.9999$;  
mKO Sham vs. mKO TBI IL-4  
$p > 0.9999$  
35d:  
mKO Sham vs. mKO TBI Veh  
**$p = 0.0081$  
mKO+ Vehicle vs. mKO TBI IL-4  
$p > 0.9999$;  
mKO Sham vs. mKO TBI IL-4  
**$p = 0.0053$ |
| Figure 7(e) NOR Exploration time% | mKO Sham n=9  
mKO TBI Veh n=13  
mKO TBI IL-4 n=13 | Normal distribution | Two-way ANOVA; Bonferroni post hoc | $F (2, 32) = 1.441$ | $p = 0.2516$  
Pre:  
mKO Sham vs. mKO TBI Veh  
$p = 0.6354$;  
mKO Veh vs. mKO TBI IL-4  
$p = 0.8213$;  
mKO Sham vs. mKO TBI IL-4  
$p > 0.9999$  
15d:  
mKO Sham vs. mKO TBI Veh  
$p = 0.4757$;  
mKO TBI Veh vs. mKO TBI IL-4  
$p > 0.9999$  
mKO Sham vs. mKO TBI IL-4  
$p > 0.9999$ |
27

| Figure 7(e) NOR Discrimination index | mKO Sham n=9 | mKO TBI Veh n=13 | mKO TBI IL-4 n=13 | Normal distribution | Two-way ANOVA; Bonferroni post hoc | F (2, 32) = 1.441 | p = 0.2516 |
|----------------------------------------|--------------|------------------|-------------------|---------------------|---------------------------------|-----------------|----------|
|                                       |              |                  |                   |                     | Pre:                             |                 |          |
|                                       |              |                  |                   |                     | mKO Sham vs. mKO TBI Veh p = 0.6354; |                 |          |
|                                       |              |                  |                   |                     | mKO TBI Veh vs. mKO TBI IL-4 p = 0.8213; |                 |          |
|                                       |              |                  |                   |                     | mKO Sham vs. mKO TBI IL-4 p > 0.9999. |                 |          |

15d:
- mKO Sham vs. mKO TBI Veh  p = 0.4757;
- mKO TBI Veh vs. mKO TBI IL-4  p > 0.9999;
- mKO Sham vs. mKO TBI IL-4  p > 0.9999.

29d:
- mKO Sham vs. mKO TBI Veh  p > 0.9999;
- mKO TBI Veh vs. mKO TBI IL-4  p > 0.9999;
- mKO Sham vs. mKO TBI IL-4  p > 0.9999.

2 Figure 1(a) Escape latency
- Sham Veh n=5
- Sham IL-4 n=5
| Normal distribution | Two-way Repeated-Measures ANOVA; Bonferroni post hoc | F (1, 8) = 0.0612 | p = 0.8108 |
|----------------------|---------------------------------------------------|------------------|------------|
|                      |                                                   |                  |            |
|                      |                                                   |                  |            |

22d: Sham Veh vs Sham IL-4  p > 0.9999;
23d: Sham Veh vs Sham IL-4  p > 0.9999;
24d: Sham Veh vs Sham IL-4  p > 0.9999;
25d: Sham Veh vs Sham IL-4  p > 0.9999;
26d: Sham Veh vs Sham IL-4  p > 0.9999.

3 Figure 1(b) Platform crossing count
- Sham Veh n=5
- Sham IL-4 n=5
| Normal distribution | Unpaired Student t test | t = 0.8179; df = 8 | p = 0.4371 |
|----------------------|-------------------------|-------------------|------------|
|                      |                         |                   |            |
|                      |                         |                   |            |

4 Figure 1(b) Target quadrant time%
- Sham Veh n=5
- Sham IL-4 n=5
| Normal distribution | Unpaired Student t test | t = 0.6147; df = 8 | p = 0.5558 |
|----------------------|-------------------------|-------------------|------------|
|                      |                         |                   |            |
|                      |                         |                   |            |

5 Figure 1(c) Sham Veh n=5
| Normal distribution | Unpaired Student t test | t = 0.122; df = 8 | p = 0.9057 |
|----------------------|-------------------------|------------------|------------|
|                      |                         |                   |            |
|                      |                         |                   |            |

27
|                                    | Sham IL-4  | Welch’s correction | df = 8 |                                |
|------------------------------------|------------|--------------------|--------|--------------------------------|
| **Swimming speed**                 | Sham Veh n=5 | Normal distribution |        |                                |
| S Figure 1(d)                      |            | Two-way ANOVA;     |        | $F (1, 8) = 1.104$ $p = 0.3241$ |
| NOR Exploration time%              | Sham IL-4 n=5 |                  |        | Pre: $p > 0.9999$ (Sham Vehicle vs Sham IL-4); |
|                                    |            |                     |        | 15d: $p = 0.3400$ (Sham Vehicle vs Sham IL-4); |
|                                    |            |                     |        | 29d: $p > 0.9999$ (Sham Vehicle vs Sham IL-4). |
| **NOR Discrimination index**       | Sham Veh n=5 | Normal distribution |        | $F (1, 8) = 1.104$ $p = 0.3241$ |
|                                    | Sham IL-4 n=5 |                  |        | Pre: $p > 0.9999$ (Sham Vehicle vs Sham IL-4); |
|                                    |            |                     |        | 15d: $p = 0.3400$ (Sham Vehicle vs Sham IL-4); |
|                                    |            |                     |        | 29d: $p > 0.9999$ (Sham Vehicle vs Sham IL-4). |
| **Passive avoidance**              | Sham Veh n=5 | Non-normal distribution | | $p = 0.6991$ |
|                                    | Sham IL-4 n=5 |                  |        |                                |
| **Swimming speed**                 | OPC-cKO Sham n=7 | Normal distribution |        |                                |
| S Figure 2(a)                      | OPC-cKO TBI Veh n=10 |                  |        |                                |
|                                    | OPC-cKO TBI IL-4 n=10 |             |        |                                |
|                                    |            | Welch ANOVA;       |        | $W = 1.133$ $p = 0.3518$. |
|                                    |            | Dunnett T3 post    |        | OPC-cKO Sham vs. OPC-cKO TBI Veh $p = 0.9100$; |
|                                    |            | hoc                |        | OPC-cKO TBI Veh vs. OPC-cKO TBI IL-4 $p = 0.9423$; |
|                                    |            |                    |        | OPC-cKO Sham vs. OPC-cKO TBI IL-4 $p = 0.3865$. |
| **Escape Latency**                 | OPC-cKO Sham n=7 | Normal distribution |        |                                |
| S Figure 2(b)                      | OPC-cKO TBI Veh n=10 |                  |        |                                |
|                                    | OPC-cKO TBI IL-4 n=10 |             |        |                                |
|                                    |            | Two-way Repeated-  |        | $F (2, 24) = 20.99$ $p < 0.0001$ |
|                                    |            | Measures ANOVA;    |        | OPC-cKO Sham vs. OPC-cKO TBI Veh ***$p < 0.0001$ |
|                                    |            | Bonferron i post hoc|        | OPC-cKO TBI Veh vs. OPC-cKO TBI IL-4 ***$p < 0.0001$; |
|                                    |            |                    |        | OPC-cKO Sham vs. OPC-cKO TBI IL-4 ***$p < 0.0001$. |
|                                    |            |                    |        | 22d: OPC-cKO Sham vs. OPC-cKO TBI Veh $p = 0.0554$; |
|                                    |            |                    |        | OPC-cKO TBI Veh vs. OPC-cKO TBI IL-4 $p > 0.9999$; |
|                                    |            |                    |        | 23d: OPC-cKO Sham vs. OPC-cKO TBI Veh **$p = 0.0054$; |
|                                    |            |                    |        | OPC-cKO TBI Veh vs. OPC-cKO TBI IL-4 $p = 0.0802$; |
|                                    |            |                    |        | 24d: OPC-cKO Sham vs. OPC-cKO TBI Veh ***$p < 0.0001$; |
| S Figure 2(d) | Platform crossing count | OPC-cKO Sham n=7 | OPC-cKO TBI Veh n=10 | OPC-cKO TBI IL-4 n=10 | Non-normal distribution | Kruskal-Wallis test | Kruskal-Wallis statistic | p = 0.0002. | OPC-cKO Sham vs. OPC-cKO TBI Veh **p = 0.0001; OPC-cKO TBI Veh vs. OPC-cKO TBI IL-4 *p = 0.0590; OPC-cKO Sham vs. OPC-cKO TBI IL-4 p = 0.1393 |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| S Figure 2(d) | Target quadrant time% | OPC-cKO Sham n=7 | OPC-cKO TBI Veh n=10 | OPC-cKO TBI IL-4 n=10 | Normal distribution | Welch ANOVA; Dunnett T3 post hoc | W = 15.38 | p = 0.0005. | OPC-cKO Sham vs. OPC-cKO TBI Veh **p = 0.0025; OPC-cKO TBI Veh vs. OPC-cKO TBI IL-4 *p = 0.0321; OPC-cKO Sham vs. OPC-cKO TBI IL-4 p = 0.6927. |
| S Figure 2(e) | Passive avoidance | OPC-cKO Sham n=8 | OPC-cKO TBI Veh n=11 | OPC-cKO TBI IL-4 n=12 | Normal distribution | One-way ANOVA; Bonferroni post hoc | F (2,28) = 51.26 | p < 0.0001 | OPC-cKO Sham vs. OPC-cKO TBI Veh ***p < 0.0001; OPC-cKO TBI Veh vs. OPC-cKO TBI IL-4 ***p < 0.0001; OPC-cKO Sham vs. OPC-cKO TBI IL-4 **p = 0.0002. |
| S Figure 3(b) | Volume of hippocampus | Sham n=6 | TBI Veh n=8 | TBI IL-4 n=6 | Normal distribution | One-way ANOVA; Bonferroni post hoc | F (2, 17) = 4.072 | p = 0.0359 | Sham vs. TBI Veh p = 0.0778 TBI Veh vs. TBI IL-4 p = 0.0874 Sham vs. TBI IL-4 p > 0.9999 |
| S Figure 3(c) | DTI volume of hippocampus (% of contralateral) | Sham n=6 | TBI Veh n=8 | TBI IL-4 n=6 | Normal distribution | Welch ANOVA; Dunnett T3 post hoc | W = 0.4228 | p = 0.6658 | Sham vs. TBI Veh p > 0.9999; TBI Veh vs. TBI IL-4 p = 0.9472; Sham vs. TBI IL-4 p = 0.7318. |
| Figure | Group | n  | Distribution | Test | Statistic | p     | Significance |
|--------|-------|----|--------------|------|-----------|-------|--------------|
| 3(d)   | Sham  | 6  | Normal       | One-way ANOVA; Bonferroni post hoc | F (2,17) = 0.4706 | p = 0.6326 | Sham vs. TBI Veh p > 0.9999; TBI Veh vs. TBI IL-4 p > 0.9999; Sham vs. TBI IL-4 p > 0.9999. |
|        | TBI Veh | 8  | Non-normal  | Kruskal-Wallis test | Kruskal-Wallis statistic = 7.446 | p = 0.0177 | Sham vs. TBI Veh *p = 0.0330; TBI Veh vs. TBI IL-4 p > 0.9999; Sham vs. TBI IL-4 p = 0.0843. |
|        | TBI IL-4 | 6  | Non-normal  |              |              |       |              |
| 4(b)   | CTX    | 6  | Non-normal  | Mann-Whitney test | Mann-Whitney U = 0 | **p = 0.0022 |              |
|        | CA1    | 6  | Non-normal  | Mann-Whitney test | Mann-Whitney U = 16 | p = 0.7619 |              |
|        | CA3    | 6  | Non-normal  | Mann-Whitney test | Mann-Whitney U = 15 | p = 0.6970 |              |
|        | DG     | 6  | Non-normal  | Mann-Whitney test | Mann-Whitney U = 15 | p = 0.6970 |              |
| 5      | Protein array | | Normal distribution | Two-way ANOVA; Bonferroni post hoc | F (3, 560) = 97.12 | CXCL13  | Sham vs. TBI p = 0.0801; TBI Veh vs. TBI IL-4 p = 0.0431; TNFSF8 p = 0.0011; TBI Veh vs. TBI IL-4 p < 0.0001; CCL11 p = 0.0082; TBI Veh vs. TBI IL-4 p = 0.0097; CCL24 p = 0.0570; TBI Veh vs. TBI IL-4 p = 0.120; Fas Ligand p = 0.0006; TBI Veh vs. TBI IL-4 p < 0.0001; CXCL1 p > 0.9999; TBI Veh vs. TBI IL-4 p > 0.9999; GCSF p = 0.9281 |
| Condition          | Sham vs. TBI p Value | TBI Veh vs. TBI IL-4 p Value |
|--------------------|----------------------|-----------------------------|
| GM-CSF             | 0.1984               | 0.2560                      |
| IFN-γ              | 0.0011               | 0.0495                      |
| IL-1α              | 0.1246               | 0.5528                      |
| IL-1β              | < 0.0001             | < 0.0001                    |
| IL-2               | 0.0124               | 0.0112                      |
| IL-3               | 0.0066               | 0.0027                      |
| IL-4               | > 0.9999             | > 0.9999                    |
| IL-6               | > 0.9999             | > 0.9999                    |
| IL-9               | > 0.9999             | > 0.9999                    |
| IL-10              | 0.5234               | 0.1653                      |
| IL-12 p40          | > 0.9999             | > 0.9999                    |
| IL-12 p70          | 0.2741               | 0.0542                      |
| IL-13              | 0.1430               | 0.0078                      |
| IL-17A             | 0.4744               | 0.8759                      |
| CXCL11             | 0.4851               | > 0.9999                    |
| CXCL1              | 0.2501               | 0.5181                      |
| Leptin             | 0.8832               | > 0.9999                    |
|                |        |        |        |        |
|----------------|--------|--------|--------|--------|
| CXCL5          | Sham n=5 | Normal distribution | Welch ANOVA | W = 20.38 | p = 0.0002. |
|                | TBI 1d    |        |        |        |        |
|                | n=5       |        |        |        |        |
| CXCL5          | Sham vs. TBI | p > 0.9999 | | | |
| TBI Veh vs. TBI IL-4 | p > 0.9999 | | | | |
| XCL1           | Sham vs. TBI | p > 0.9999 | | | |
| TBI Veh vs. TBI IL-4 | p > 0.9999 | | | | |
| MCP-1          | Sham vs. TBI | p > 0.9999 | | | |
| TBI Veh vs. TBI IL-4 | p > 0.9999 | | | | |
| M-CSF          | Sham vs. TBI | p > 0.9999 | | | |
| TBI Veh vs. TBI IL-4 | p > 0.9999 | | | | |
| CXCL9          | Sham vs. TBI | p < 0.0001 | | | |
| TBI Veh vs. TBI IL-4 | p < 0.0001 | | | | |
| CCL3           | Sham vs. TBI | p = 0.0359 | | | |
| TBI Veh vs. TBI IL-4 | p = 0.0009 | | | | |
| CCL9           | Sham vs. TBI | p = 0.8437 | | | |
| TBI Veh vs. TBI IL-4 | p = 0.4841 | | | | |
| CCL5           | Sham vs. TBI | p > 0.9999 | | | |
| TBI Veh vs. TBI IL-4 | p > 0.9999 | | | | |
| CXCL12a        | Sham vs. TBI | p > 0.9999 | | | |
| TBI Veh vs. TBI IL-4 | p > 0.9999 | | | | |
| CCL1           | Sham vs. TBI | p > 0.9999 | | | |
| TBI Veh vs. TBI IL-4 | p > 0.9999 | | | | |
| CCL25          | Sham vs. TBI | p = 0.0049 | | | |
| TBI Veh vs. TBI IL-4 | p = 0.0008 | | | | |
| TIMP-1         | Sham vs. TBI | p > 0.9999 | | | |
| TBI Veh vs. TBI IL-4 | p = 0.1360 | | | | |
| TIMP-2         | Sham vs. TBI | p = 0.4209 | | | |
| TBI Veh vs. TBI IL-4 | p = 0.2476 | | | | |
| TNF-α          | Sham vs. TBI | p > 0.9999 | | | |
| TBI Veh vs. TBI IL-4 | p = 0.1254 | | | | |
| TNF RI         | Sham vs. TBI | p = 0.4726 | | | |
| TBI Veh vs. TBI IL-4 | p = 0.0239 | | | | |
| TNF RII        | Sham vs. TBI | p > 0.9999 | | | |
| TBI Veh vs. TBI IL-4 | p = 0.4112 | | | |
| Figure | Condition | Group 1 | Group 2 | Statistical Test | Kruskal-Wallis Statistic | p Value |
|--------|-----------|---------|---------|------------------|--------------------------|---------|
| 6(b)   | Neuron    | Sham n=5 TBI 1d n=5 TBI 3d n=6 TBI 7d n=6 | Non-normal distribution | Kruskal-Wallis test | 16.99 | p = 0.0007. Sham vs. 1d \( p = 0.5389 \); Sham vs. 3d \( **p = 0.0088 \); Sham vs. 7d \( ***p = 0.0004 \). |
| 6(b)   | Astrocyte | Sham n=5 TBI 1d n=5 TBI 3d n=6 TBI 7d n=6 | Normal distribution | Welch ANOVA; Dunnett T3 post hoc | W = 42.85 | \( p < 0.0001 \). Sham vs. 1d \( p = 0.3957 \); Sham vs. 3d \( ***p < 0.0001 \); Sham vs. 7d \( **p = 0.0068 \). |
| 6(b)   | Oligodendrocyte | Sham n=5 TBI 1d n=5 TBI 3d n=6 TBI 7d n=6 | Normal distribution | Welch ANOVA; Dunnett T3 post hoc | W = 145.8 | \( p < 0.0001 \). Sham vs. 1d \( *p = 0.01621 \); Sham vs. 3d \( **p = 0.0048 \); Sham vs. 7d \( ***p < 0.0001 \). |
| 8(a)   | Escape latency | mKO Sham Veh n=4 mKO Sham IL-4 n=5 | Normal distribution | Two-way Repeated-Measures ANOVA; Bonferroni post hoc | \( F (1, 7) = 1.681e-005 \) | \( p = 0.9968 \). mKO Sham Veh vs. mKO Sham IL-4 22d: \( p > 0.9999 \); 23d: \( p > 0.9999 \); 24d: \( p > 0.9999 \); 25d: \( p = 0.9999 \); 26d: \( p > 0.9999 \). |
| 8(b)   | Platform crossing count | mKO Sham Veh n=4 mKO Sham IL-4 n=5 | Normal distribution | Student t test | \( t = 0.9249 \); \( df = 7 \) | \( p = 0.3858 \). |
| 8(b)   | Target quadrant time% | mKO Sham Veh n=4 mKO Sham IL-4 n=5 | Normal distribution | Student t test | \( t = 0.4801 \); \( df = 7 \) | \( p = 0.6458 \). |
| 8(c)   | Swimming speed | mKO Sham Veh n=4 mKO Sham IL-4 n=5 | Normal distribution | Student t test | \( t = 1.698 \); \( df = 7 \) | \( p = 0.1334 \). |
| S Figure 8(d) | mKO Sham Veh n=4 | mKO Sham IL-4 n=5 | Normal distribution | Unpaired t test with Welch's correction | t = 1.000; df = 3.000 | p = 0.3910 |
| S Figure 8(e) | mKO Sham Veh n=4 | mKO Sham IL-4 n=5 | Normal distribution | Two-way Repeated-Measures ANOVA; Bonferroni post hoc | F (1, 7) = 0.1498 | p = 0.7102 |
| NOR Exploration time % | mKO Sham Veh n=4 | mKO Sham IL-4 n=5 | Normal distribution | Two-way Repeated-Measures ANOVA; Bonferroni post hoc | F (1, 7) = 0.1498 | p = 0.7102 |
| NOR Discrimination index | mKO Sham Veh n=4 | mKO Sham IL-4 n=5 | Normal distribution | Two-way Repeated-Measures ANOVA; Bonferroni post hoc | F (1, 7) = 0.1498 | p = 0.7102 |