SUPPLEMENTARY MATERIAL

TITLE : Enhancing endocannabinoid signaling in astrocytes promotes recovery from traumatic brain injury

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SUPPLEMENTARY MATERIAL & METHODS

Animals

Mgllflox/flox animals were generated by the Texas A&M Institute for Genomic Medicine. Knockout mouse production was performed according to the scheme shown in Supplemental Figs. 1 and 2A. Briefly, the mutant allele carries the LoxP sites flanking Exon 2 of the gene. The LoxP sites were introduced by homologous recombination with a targeting vector in the C57BL/6N ES cell line JM8. The TIGM proprietary vector carried Neomycin transferase cassette for selection of correctly targeted clones; this cassette was flanked by Frt recombination sites that were later removed by breeding with the "Flpe deleter" mouse line to produce conditional-ready knockout ($Mgll^{flox/flox}$). Deletion of the targeted exons was confirmed by crossing $Mgll^{flox/flox}$ mice with Tg(Sox2-cre)1Amc/J (JAX Stock No: 004783) Sox2-Cre, resulting in a total/global MAGL knockout. The correct targeting and recombination were confirmed by Long Distance PCR and sequencing. Neuronal and astrocytic MAGL KO mice (nKO and aKO) were generated by crossing *mgll* $^{flox/flox}$ mice with Syn1-cre mice (JAX Stock No: 003966) and GFAP-cre mice (JAX Stock No: 024098), respectively. Animals were randomly assigned to groups (WT vs KO; sham vs TBI) from different genotypes. The number of animals per experimental group were calculated by power analysis using power=80%, α=0.05, and variables based on our previously published results of similar experiments. Both male and female mice at ages of 8 to 12 weeks were used in the present study. CB1R knockout mice were intraperitoneally injected with 4-Nitrophenyl-4-[bis (1,3-benzodioxol-5-yl)(hydroxy)methyl]piperidine-1-carboxylate (JZL184, 10 mg/kg) 30 minutes after each TBI and then once a day for 4 consecutive days (total 7 injections). JZL184 was prepared and dissolved in a vehicle containing Tween 80 (10%), dimethylsulfoxide (10%) and saline (80%) as described previously[1-4]. The experiments were performed in a blinded fashion.

All animal studies were performed in compliance with the US Department of Health and Human Services Guide for the Care and Use of Laboratory Animals, and the care and use of the animals reported in this study were approved by the Institutional Animal Care and Use Committee of Louisiana State University Health Sciences Center and the Institutional Animal Care and Use Committee of University of Texas Health San Antonio.

Cell culture

Primary hippocampal neurons (astroglial cells < 2%) from embryos (E18) and astrocytes (astrocytes > 95%) from postnatal P4-5 pups of WT, tKO, nKO and aKO were cultured as described previously [2,5-8]. The extent of neurons and astrocytes in cultures was controlled by different treatments and estimated by using immunostaining with NeuN or GFAP in conjunction with staining with 4',6-diamidino-2-phenylindole (DAPI) as well as with other markers (*e.g.*, CD11b), as described previously [6,8]. Briefly, for relatively pure neuronal culture, the hippocampus from embryos was dissected out under microscope and triturated in serum-free culture medium after meninges were removed. Tissue was incubated in oxygenated trypsin for 10 minutes at 37°C and then mechanically triturated. Cells were spun down and resuspended in Neurobasal/B27 medium supplemented with 0.5 mM L-glutamine, penicillin/ streptomycin and 25 μM glutamate. Cells (1×10^6) were loaded into 6-well plates for immunoblot analysis. the proliferation of astroglial cells was inhibited by treatment of cultures with 5-10 μM cytosine arabinoside. The treatment of AraC led to a reduction of astroglial cells in culture to ~2 %. For astrocyte-enriched cultures, cerebral hemispheres from P4~5 pups were dissected out and cleaned of meninges. The tissue was incubated in oxygenated trypsin for 10 minutes at 37 °C and then mechanically triturated in MEM containing 10% FBS. The medium was modified with extra substances to produce a final composition of 7.5 mM glucose, double concentrations of amino acids, quadruple concentrations of vitamins, double concentrations of $NaHCO_3$, 2 mM L-glutamine, penicillin (250,000 IU/L) and 0.5% streptomycin. Cells were grown on 6-well plates for immunoblot analysis. The medium was changed after 3 days of culturing and thereafter three times a week. The cultures were grown at 37°C in a humidified atmosphere of 5% CO_2 until use. As shown in Supplementary Fig. 3A, no signal for MAGL was detected in astrocytes cultured from tKO and aKO mice, but detected from WT or nKO mice. Concurrently, a strong signal for GFAP was detected in astrocytes cultured from each genotype (Supplementary Figure 3A). NG108-15 cells were cultured for PPARγ luciferase activity as described previously [2].

Repeated mild closed head injury

In the present study, we used a mouse model of repeated mCHI with three impacts at an interval of 24 hours, as described previously [3]. Briefly, repeated mCHI were induced using an electromagnetic controlled stereotaxic impact device (Impact One™ Stereotaxic Impactor, Leica

Biosystem, IL). Mice were placed in a stereotaxic frame after anesthesia with isoflurane. The skull was exposed by a midline skin incision. A 3 mm blunt metal impactor tip was positioned at 1.8 mm caudal to bregma and 2.0 mm left of midline. The injury was triggered by the electromagnetic device driving the tip to the exposed skull at a strike velocity of 3.0 m/s, depth 2.2 mm with a dwell time of 100 msec. After impact, the skin was sutured and the mice were allowed to recover from anesthesia on a warming pad and then returned to their home cages. A second and third identical closed-skull TBI procedure was performed at days 2 and 3 after the original injury. For sham injuries, the same procedures and anesthesia were performed except that no hits were delivered.

Hippocampal slice preparation

Hippocampal slices were prepared from mice as described previously[1,2,5,7]. Briefly, after decapitation, brains were rapidly removed and placed in cold oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF) containing: 125.0 NaCl, 2.5 KCl, 1.0 $MgCl_2$, 25.0 $NaHCO_3$, 1.25 NaH_2PO_4, 2.0 $CaCl_2$, 25.0 glucose, 3 pyruvic acid, and 1 ascorbic acid. Slices were cut at a thickness of 350-400 μm and transferred to a holding chamber in an incubator containing ACSF at 36 °C for 0.5 to 1 hour, and maintained in an incubator containing oxygenated ACSF at room temperature (~22-24 °C) for >1.5 h before recordings. Slices were then transferred to a recording chamber where they were continuously perfused with 95% O_2, 5% CO_2-saturated standard ACSF at ~32-34 °C.

Electrophysiological recordings

Field EPSP (fEPSP) recordings at hippocampal Schaffer-collateral synapses in response to stimuli at a frequency of 0.05 Hz were made using an Axoclamp-2B patch-clamp amplifier in bridge mode, as described previously [1-3,5]. Recording pipettes were pulled from borosilicate glass with a micropipette puller (Sutter Instrument), filled with artificial ACSF (~4 MΩ). As described previously [1-3,5,7], long-term potentiation (LTP) at CA3-CA1 synapses was induced by a high-frequency stimulation (HFS) consisting of three trains of 100Hz stimulation (1 sec duration and a 20 sec inter-train interval).

Western blots

Western blot assay was conducted to determine expression of glutamate receptor subunits GluA1, GluA2, GluN1, GluN2A, and GluN2B, TDP-43, and p-tau (p-tau-T181), p-tau-S202, p-tau-T231, p-tau-S396, p-tau-S404, PPARγ, p-NF-kB, MAGL, and FLAG tag in cortical or hippocampal tissues from WT and MAGL KO mice, including tKO, nKO, and aKO mice, that received three impacts. In addition, expression of NeuN, GFAP, and MAGL in primary cultures was also assessed. Cortical or hippocampal tissues were extracted and immediately homogenized in RIPA lysis buffer and protease inhibitors, and incubated on ice for 30 min, then centrifuged for 10 min at 10,000 rpm at 4ºC. Supernatants were fractionated on 4-15% SDS-PAGE gels (Bio-Rad) and transferred onto PVDF membranes (Bio-Rad). The antibodies used to detect the expression of proteins are as listed in Supplemental Table 1. The membrane was incubated with specific antibodies at 4ºC overnight. The blots were washed and incubated with a secondary antibody (goat anti-rabbit 1:2,000, Cell Signaling) at room temperature for 1 hr. Proteins were visualized by enhanced [2,3] chemiluminescence (ECL, Amersham Biosciences, UK). The densities of specific bands were quantified by densitometry using GE/Amersham Imager 680 UV. Band densities were normalized to the total amount of protein loaded in each well as determined by mouse anti β-actin, as described previously [1-3,5,8].

Reverse transcription and real-time PCR

Total RNAs were prepared from harvested tissue with the RNeasy Mini Kit (Qiagen) and treated with RNase-free DNase (Qiagen) according to the manufacturer's instructions. The RNA concentration was measured by spectrophotometer (DU 640; BECKMAN). RNA integrity was verified by electrophoresis in a 1% agarose gel.

The iScript cDNA synthesis kit (BioRad) was used for the reverse transcription reaction. We used 1 µg total RNA, with 4 µl 5× iscript reaction mix and 1 µl iscript reverse transcriptase. The total volume was 20 µl. Samples were incubated for 5 min at 25 °C. All samples were then heated to 42 °C for 30 min, and reactions were stopped by heating to 85 °C for 5 min. Real-time RT-PCR specific primers for MAGL, CB1R, IL-1β, IL-6, TNFα, vimentin (Vim), and GAPDH were selected using Beacon Designer Software (BioRad) and synthesized by IDT (Coralville, IA). The primers used in the present study are listed in Supplemental Table 2. The PCR amplification

of each product was further assessed using 10-fold dilutions of mouse brain cDNA library as a template and was found to be linear over five orders of magnitude and at greater than 95% efficiency. All the PCR products were verified by sequencing. The reactions were set up in duplicate in total volumes of 25 µl containing 12.5 µl 2× iQSYBR green Supermix (BioRad) and 5 µl template (1:10 dilution from RT product) with a final concentration of 400 nM of the primer. The PCR cycle was as follows: 95 °C/3 min, 45 cycles of 95 °C/30 sec, 58 °C/45 sec and 95 °C/1 min, and the melt-curve analysis was performed at the end of each experiment to verify that a single product per primer pair was amplified. Furthermore, the sizes of the amplified DNA fragments were verified by gel electrophoresis on a 3% agarose gel. The amplification and analysis were performed using an iCycler iQ Multicolor Real-Time PCR Detection System (BioRad). Samples were compared using the relative CT method. The fold increase or decrease were determined relative to naïve or sham controls after normalizing to a housekeeping gene using $2^{-\Delta\Delta CT}$, where ΔCT is (gene of interest CT) - (GAPDH CT), and ΔΔCT is (ΔCT treated) - (ΔCT control), as described previously [2,3].

Immunohistochemistry

Immunohistochemical analyses were performed to assess amyloid precursor protein (APP), MAGL, TDP-43, p-tau, Iba1, and GFAP in coronal brain sections as described previously [1-3,5]. Animals were anesthetized with ketamine/xylazine (200/10 mg/kg) and subsequently transcardially perfused with PBS followed by 4% paraformaldehyde in phosphate buffer. The brains were quickly removed from the skulls and fixed in 4% paraformaldehyde overnight, and then transferred into the PBS containing 30% sucrose until sinking to the bottom of the small glass jars. Cryostat sectioning was made on a freezing Vibratome at 30 µm thickness and a series of five equally spaced (every 10th section) sections spanning the brain were collected in 0.1M phosphate buffer. Free floating sections were immunostained using specific antibodies as listed in Supplemental Table 1 and followed by incubation with the corresponding fluorescent-labeled secondary antibody. 4′-6-Diamidino-2-phenylindole (DAPI), a fluorescent stain that binds strongly to DNA, was used it to detect cell nuclei in the sections. The sections were then mounted on slides for immunofluorescence imaging. A Zeiss deconvolution microscope and the Slidebook software (Intelligent Imaging Innovations.com, Denver, Colorado) were used for immunostaining

data acquisition. The immunofluorescence intensity (in arbitrary densitometric units, ADU) of the channel detected in the region of interest as immunoreactivity of a specific antibody was imaged in each section. All the images were acquired with the same exposure time. The thresholds were determined from channel histograms. The immunoreactivity of APP, MAGL, TDP-43, phosphorylated tau, Iba1, and GFAP antibodies in each image was analyzed and quantified using SlideBook 6.0, and normalized to the sham controls, as described previously [1-3,5].

Histochemistry

Degenerated neurons were detected using Fluoro-Jade C (FJC), which is an anionic dye that specifically stains the soma and neurites of degenerating neurons and thus is unique as a neurodegenerative marker. Cryostat cut sections were incubated in the solution with FJC (0.0001% solution, EMD Millipore) and DAPI (0.5 μg/ml) for 10 min, followed by 3× 1-min wash with distilled water. Slices were dried naturally at room temperature without light. The FJC positive cells in the cortex and hippocampus were imaged and analyzed using a Zeiss deconvolution microscope with SlideBook 6.0 software, as described previously [1,3].

Luciferase activity assay

PPARγ activity stimulated by 2-AG or 15d-PGJ2 in the presence and absence of Rimonabant (RIM) or GW9662 (GW) was assessed in NG108-15 cells transfected with pCMX-Gal-LBD-mPPARγ and TK-MH100x4-luc vectors, as described previously [2]. Luciferase values were normalized to the level of β-galactosidase activity.

Single-cell sample Preparation

Single-cell suspensions from *mgll*$^{lox/lox}$-non cre (WT), nKO, and aKO mice that received repeated mCHI (once a day for three days) were made using an Adult Brain Dissociation Kit (MACS Miltenyi Biotec, Cat# 130-107-677) according to the instructions provided by the manufacturer with some modification. Briefly, mice were anesthetized with ketamine/xylazine (200/10 mg/kg) 24 hours after the last impact and the brains were immediately removed and washed in ice-cold D-

PBS. The ipsilateral side of the brains from two animals per group was dissected out and cut as 200 mm slices in Enzyme mix 1. The slices were transferred into T25 flasks containing Enzyme mix 2 and incubated at 37ºC for 25 min. The enzymatically digested cells were transferred into 15 ml tubes with cold D-PBS and centrifuged briefly. The samples at the bottom of the tubes were resuspended with D-PBS and the supernatants were transferred to MACS SmartStrainer (70 m) on 50 ml tubes. After repeated twice, suspensions were centrifuged at 300x for 10 min at 4ºC. The pellets were carefully resuspended in cold D-PBS and final cell suspensions were obtained following removal of dead cells and debris with the cold Debris Removal Solution and removal of blood cells with the cold Red Blood Cell Removal Solution according to the procedures instructed by the manufacture.

Single-cell RNA sequencing library preparation

Single cell suspensions were loaded into the 10x Genomics Chromium microfluidic chips with the intention of capturing 10,000 cells within individual Gel Beads-in-emulsion (GEM). Inside the GEMs the cells were lysed and their RNA was reverse transcribed using a poly(dT) priming. During reverse transcription Cell Barcodes and Unique Molecular Identifiers (UMI) were added to each of the cDNA transcripts. The libraries were prepared for sequencing following the manufacturer's recommendations for the 10x Genomics 3' Gene Expression v3 chemistry, and sequenced at the North Texas Genome Center located at UT Arlington on an Illumina NovaSeq S4 150 PE flow cell.

Single-cell RNA-seq data analysis

The filtered unique molecular identifiers (UMI) feature-barcode matrices were produced by CellRanger Mkfatsq/Count Analysis (10xGenomics.com), and Seurat R package (v3) was used for subsequent analysis [9]. For quality control, cells with mitochondrial content > 30% and with < 400 or > 6000 genes per cell were removed; and genes with a count of 1 in at least 20 cells were retained. LogNormalize", a global-scaling normalization method, was used to normalize the data by a scale factor of 10000. Principal component analysis (PCA) was performed using the top 3000 variable genes prior to clustering. To visualize profiles in two-dimensional space, t-distributed

stochastic neighbor embedding (t-SNE) was performed with the top 20 principal components based on the ElbowPlot. Clustering was performed using the FindClusters function (working on the K-nearest neighbor graph model) in Seurat R package, and the resolution is 0.3. For identifying the cell type, 'aqp4', 'gja1', 'slc1a2' and 'gpr37l1' were used as specific cell markers for astrocytes, while 'aif1', 'itgam', 'csf1r', and 'tmem119' were used as specific cell markers for microglia. These markers were used to assign cell-type annotations manually for each cell cluster. Differentially expressed genes (DEG) between Sham and TBI group were identified using FindMarkers function of Seurat package in R, which works on the Wilcox method. Averaged log2 (fold change) of gene expression, the percentage of cells expressing the genes in each group (pct.1 and pct.2), *p* value and adjusted p value were generated. Min.pct = 0 and logfc. threshold = 0 were used in finding DEGs. DEGs lists were produced by filtering all genes for log2 fold changes > 0.1 and adjusted $P < 0.05$.

Morris water Maze

The classic Morris water maze (MWM) test was used to determine spatial learning and memory, as described previously [1-3,5,10]. A circular water tank (diameter 120 cm and 75 cm in high) was filled with water and the water was made opaque with non-toxic white paint. A round platform (diameter 15 cm) was hidden 1 cm beneath the surface of the water at the center of a given quadrant of the water tank. WT and MAGL KO mice that were treated with sham or TBI received learning acquisition training in the Morris water maze for 7 days and each session was consisted of 4 trials. For each trial, the mouse was released from the wall of the tank and allowed to search, find, and stand on the platform for 10 seconds within the 60-second trial period. For each training session, the starting quadrant and sequence of the four quadrants from where the mouse was released into the water tank were randomly chosen so that it was different among the separate sessions for each animal and was different for individual animals. The mouse movement in the water pool was recorded by a video-camera and the task performances, including swimming paths, speed, and time spent in each quadrant, were recorded using an EthoVision video tracking system (Noldus, version 14). A probe trial test was conducted 24 hours after the completion of the learning acquisition training. During the probe test, the platform was removed from the pool, and the task performances were recorded for 60 seconds.

AAV injection

WT or aKO mice at 2 months of age were anesthetized with ketamine/Xylazine (200/10 mg/kg) and placed in a stereotaxic frame. AAV5-GFAP-eGFP-m-PPARγ-shRNAmir(2) vectors, AAV5-GFAP-h-PPARγ-FLAG-WPRE vectors, or AAV5-GFAP-eGFP control vectors were provided by Vector Biolabs (Malvern, Pennsylvania). AAV Vectors were stereotaxically injected into the left side of the hippocampus in WT or aKO mice at the coordinate: AP, -2.3, ML, 2, and DV, -2 (2.0 μl at 0.2 μl /min), as described previously [2,7]. Repeated mCHI were induced 30 days following AAV injections and all the assessments, including immunoblot and the Morris water maze test, were carried out 30 days after the first impact.

Liquid Chromatography/Mass Spectrometry

The content of 2-AG in brain tissues from WT, tKO, nKO, and aKO mice were detected, as described previously [11]. Briefly, animals were sacrificed by decapitation after suffocation with CO2 and brains were rapidly frozen in liquid nitrogen. Tissue samples were weighed and placed into borosilicate glass tubes containing acetonitrile solution with 4.5 nmol/mL of 2-AG-d5 internal standard during tissue extracting. Tissue was homogenized with a glass rod and sonicated in ice for 10 min. Samples were stored overnight at -20°C to precipitate proteins. Samples were centrifuged at 3,500 rpm at 4ºC for 10~15 min, and supernatants were removed to a new glass tube and evaporated to dryness under N2 gas. The samples were resuspended in 70 μl of methanol, vortexed well, and then added 40 μl H_2O. Finally, Samples were centrifuged at 3,500 rpm at 4ºC for 10~15 min. All analytes were detected using a SCIEX 6500 QTrap via selected reaction monitoring. Analytes were quantitated by stable isotope dilution against their deuterated internal standard. Data were normalized to tissue mass and are presented as normalization to the 2-AG level of WT mice.

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Supplementary Table 1. List of antibodies used in the present study.

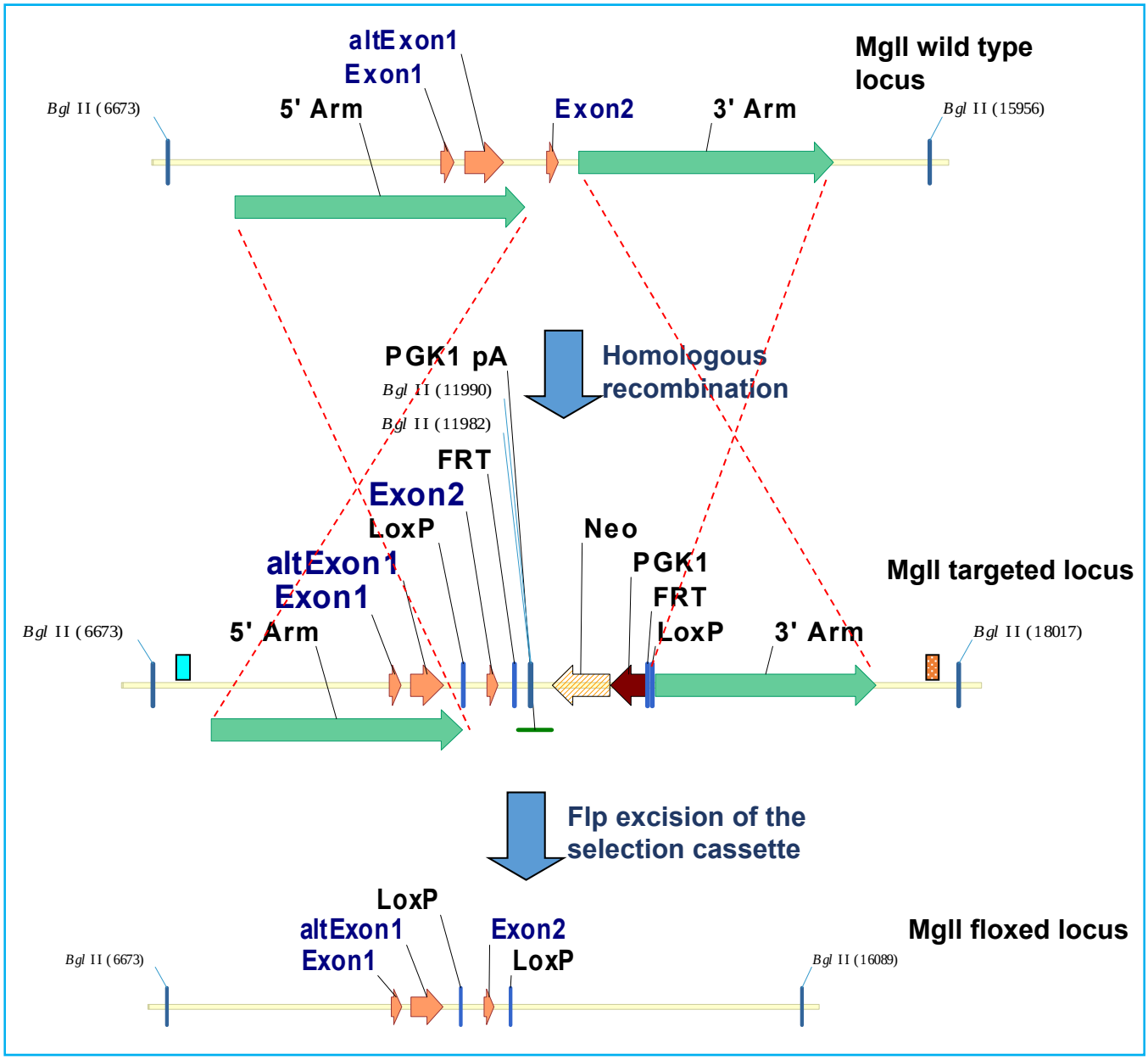
Antibody	Ratio (WB)	Ratio IHC)	Manufacture	Cat#	RRID
BACE1	1:1,000		Covance	PRB-617C-100 617C17C-100	AB_10063987
APP	1:1,000	1:200	MilliporeSigma	A8717	AB_258409
NCT	1:1,000		Boster Biological	PA2250	
ADAM10	1:1,000		MilliporeSigma	AB19026	AB_2242320
CB1	1:1,000		Cayman Chem	10006590	AB_10098690
CB2	1:500		Thermos Fisher	PA-1746	AB_2082908
GluA1	1:1,000		Abcam	ab31232	AB_2113447
GluA2	1:1,000		Abcam	ab133477	AB_2620181
GluN1	1:500		Abcam	ab109182	AB_10862307
GluN2A	1:1,000		MilliporeSigma	07-632	AB_310837
GluN2B	1:1,000		Abcam	ab65783	AB_1658870
Synaptophysin	1:1,000		Abcam	ab8049	AB_2198854
PSD-95	1:1,000		Abcam	ab99009	AB_10676078
MAGL	1:1,000	1:500	Frontier Institute	MGL-Rb-Af200	
MAGL	1:1,000		Dr. Ken Mackie	MAGL-m	
NeuN	1:1,000	1:200	MilliporeSigma	MAB377	AB_2298772
PPARγ	1:1,000		Abcam	ab59256	AB_944767
PPARγ	1:1.000		Abcam	ab27649	AB_777390
PPARγ		1:200	ABclonal	NBP2-22106	
TDP-43	1:1,000	1:500	Cell Signaling	3449	AB_2200511
p-tau-T181	1:1,000	1:500	Genscript	A00403	
p-tau-S202	1:5,000		Abcam	AB108387	AB_10860874
p-tau-T231	1:1,000		Cell Signaling	71429	AB_2888624
p-tau-S396	1:1,500		ABclonal	AP1028	AB_2863912
p-tau-S404	1:1,500		ABclonal	AP0170	AB_2771600
p-NF-kB	1:1,000		Cell Signaling	3039	AB_330579
GFAP	1:1,000	1:500	MilliporeSigma	G3893	AB_477010
Iba-1		1:200	MilliporeSigma	MABN92	AB_10917271
CD11b		1:500	Abcam	ab1211	AB_442947
Flag tag	1:400		MilliporeSigma	F7425	AB_439687
β-Actin	1:2,000		Santa Cruz	Sc-47778	AB_626632

Supplementary Table 2. List of primers used in the present study.

Gene	Accession number	Forward (5'~3')	Reverse (5'~3')
GAPDHH	M32599	ACCACAGTCCATGCCATCAC	ACCTTGCCCACAGCCTTG
IL-1β	NM_008361.33	TGGAGAGTGTGGATCCCAAGCAAT	TGTCCTGACCACTGTTGTTTCCCA
IL-6	NM_031168.1	TCTCTGGGAAATCGTGGAAATG	ACTCCAGGTAGCTATGGTACTC
TNFα	NM_013693	GTCTACTGAACTTCGGGGTGA	CACTTGGTGGTTTGCTACGAC
Vim	NM_011701	AGATGGCTCGTCACCTTCGTGAAT	TTGAGTGGGTGTCAACCAGAGGAA
CB1R	BC079564	TGCTGGTGCTATGTGTCATCC	GCTGTGAAGGAGGCGGTAAC
MAGL	NM_011844.3	AACTCCACAGAATGTTCCCTAC	CAACATGGTCATGGGCAAATAC

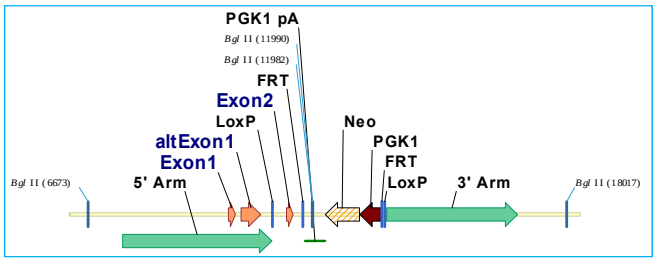
Supplementary Table 3. Total number of astrocytes and microglia assessed.

Genotype	Sham		TBI	
	Microglia	**Astrocytes**	**Microglia**	**Astrocytes**
WT	2319	1372	2542	1352
nKO	2173	1724	2367	627
aKO	1536	1797	2580	1449

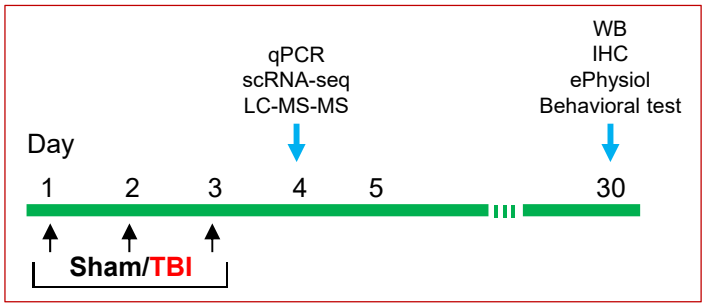


Supplementary Fig. 1. Mgll targeting strategy. The mutant allele carries the LoxP sites flanking Exon 2 of the gene. The LoxP sites were introduced by homologous recombination with a targeting vector in the C57BL/6N ES cell line JM8. TIGM proprietary vector carried Neomycin transferase cassette for selection of correctly targeted clones; this cassette was flanked by Frt recombination sites that were later removed by breeding with the "Flpe deleter" mouse line FLP to produce conditional-ready knockout (Mgll$^{flox/flox}$). The correct targeting and recombination was confirmed by Long Distance PCR and sequencing.

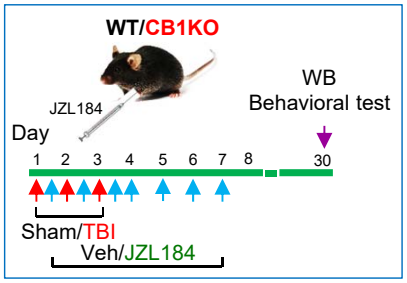
A Generation of MAGL conditional knockout mice



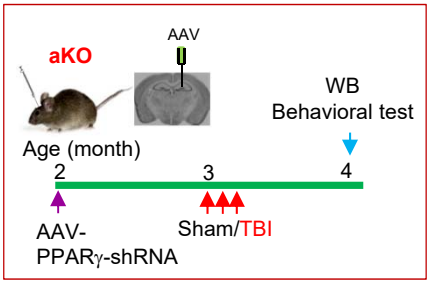
B The experimental protocol for TBI and assessments

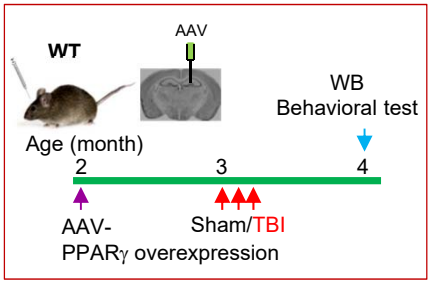


C The experimental protocol for assessment of CB1R in MAGL inactivation-produced neuroprotection in TBI

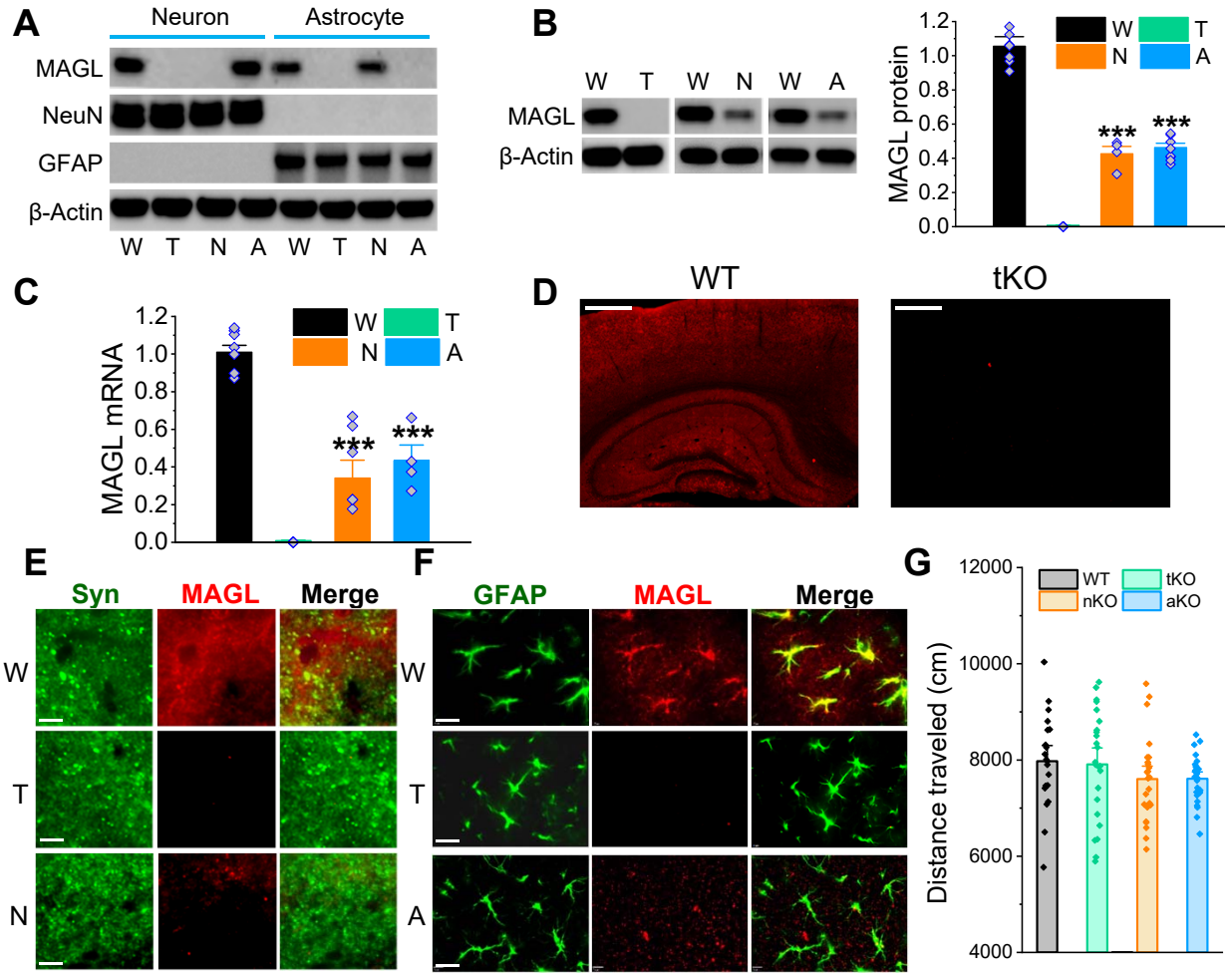


D The experimental procedures/protocols for assessment of astrocytic PPARγ in MAGL inactivation-produced neuroprotection in TBI

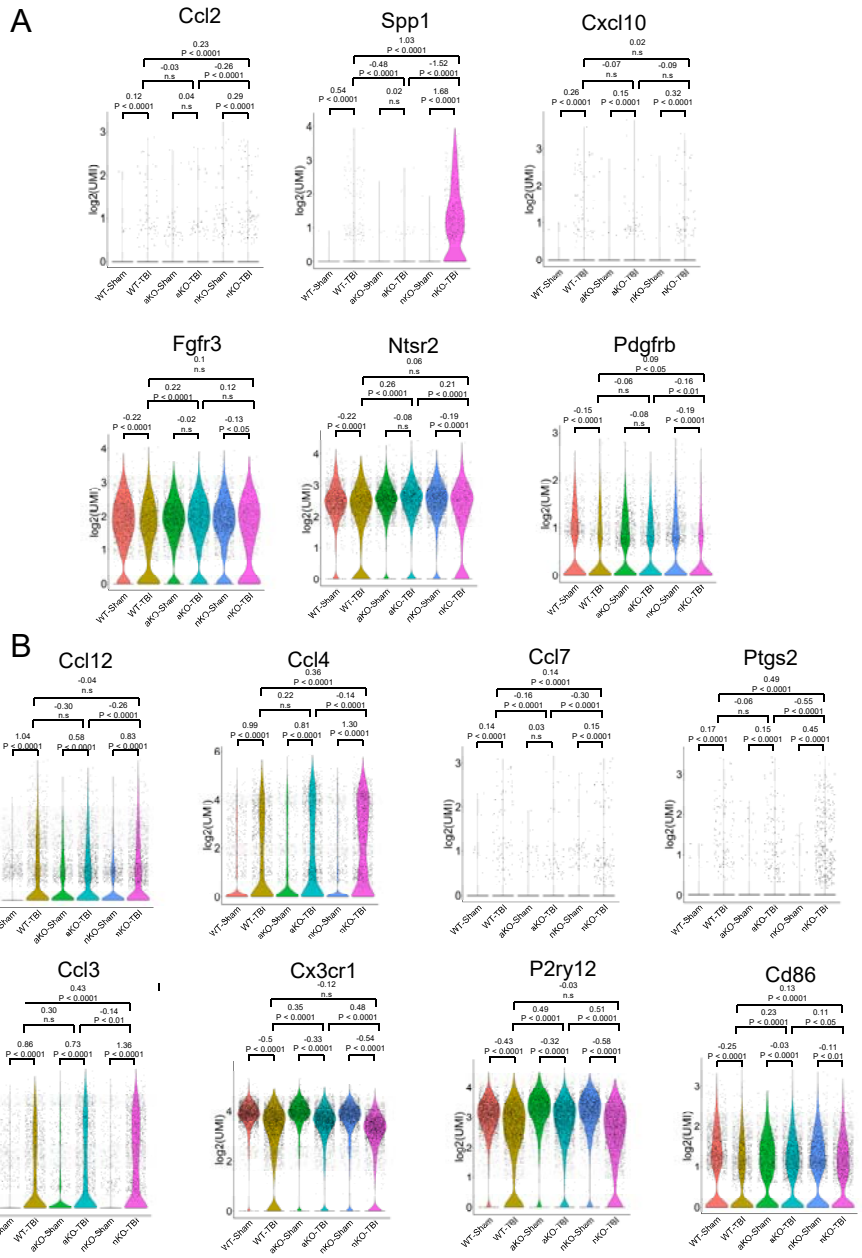




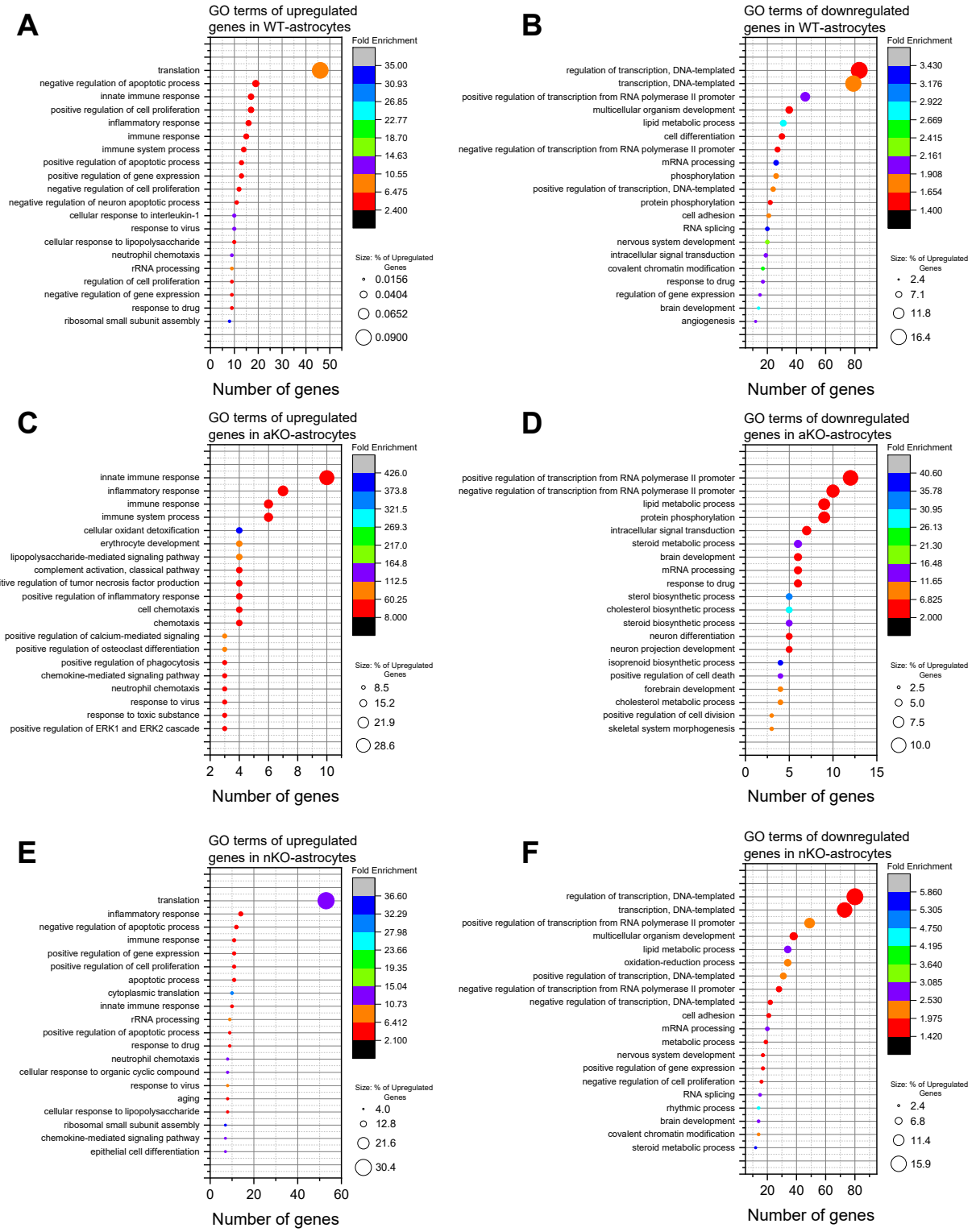
Supplementary Fig 2. Study design and experimental protocols. (**A**) Scheme of mgll targeting strategy for creating conditional mgll knockout mice. (**B**) Schematic illustration of the experimental protocol and assessments. (**C**) The dosing regimen of JZL184 injection and the experimental protocol in CB1R knockout mice. (**D**) The experimental protocols for AAV-mediated silencing of PPARγ in aKO mice and AAV-mediated overexpression of PPARγ in WT mice.



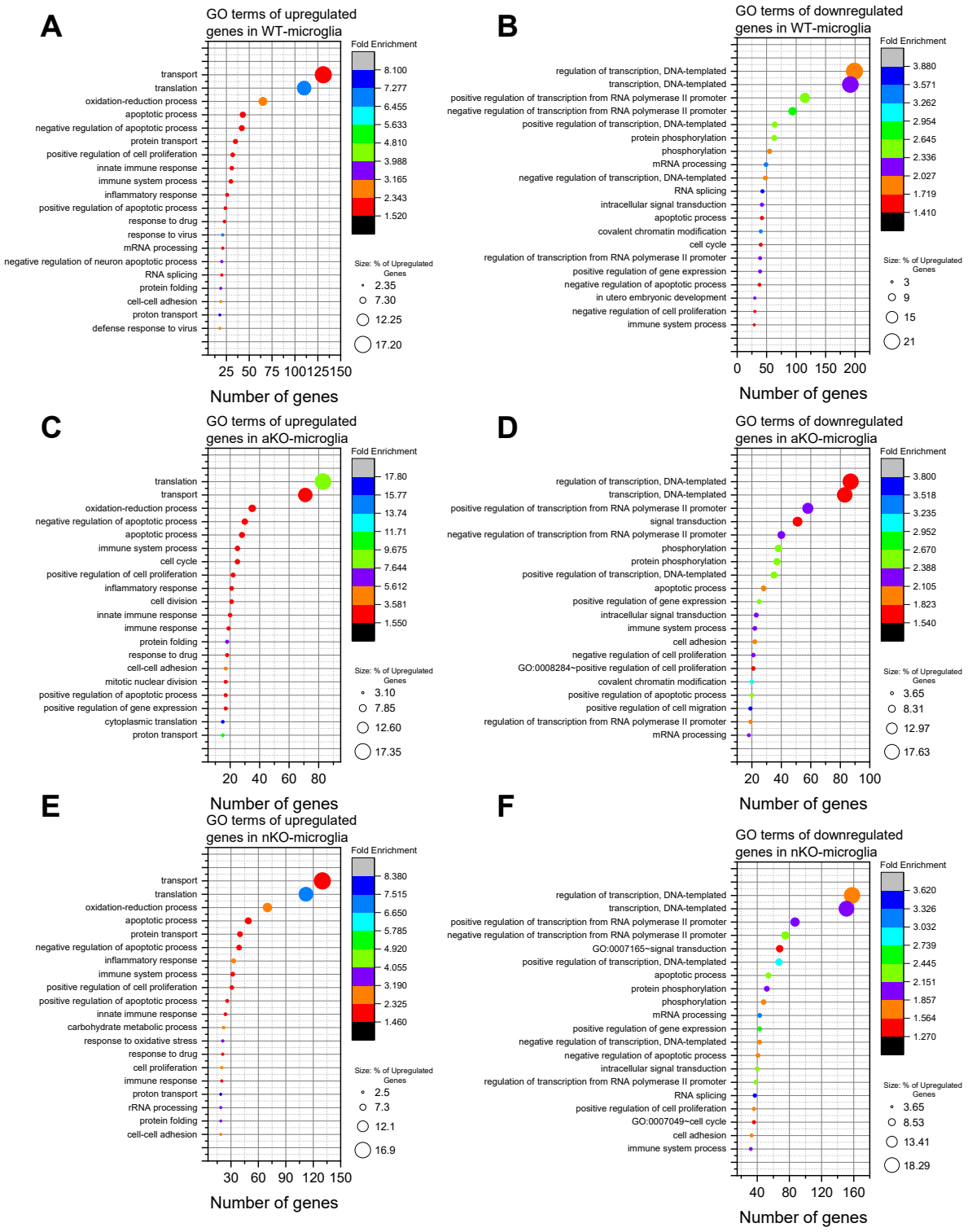
Supplementary Fig. 3. Cell type-specific knockout of MAGL in the mouse brain. (**A**) Immunoblot analysis of MAGL in neuronal and astrocytic cultures derived from WT (W), tKO (T), nKO (N), and aKO (A) mice. NeuN (neuronal marker) and GFAP (astrocytic marker) were used to confirm neuron-specific or astrocyte-specific deletion of MAGL. (**B**) Immunoblot analysis of MAGL in hippocampal tissues from WT, tKO, nKO, and aKO mice. The data are means ±SEM. ***P<0.001 compared with WT (ANOVA with Fisher's PLSD post-hoc test, n=4~7/group). (**C**) qPCR analysis of MAGL expression in the hippocampus from WT, tKO, nKO, and aKO mice. The data are means ±SEM. ***P<0.001 compared with WT (ANOVA with Fisher's PLSD post-hoc test, n=4~7/group). (**D**) Immunostaining of MAGL in the brains of WT and tKO mice. Scale bar: 400 μm. (**E**) Immunostaining of MAGL in the hippocampal region of WT, tKO, and nKO mice. Synaptophysin was used as a synaptic/neuronal marker colocalized with MAGL. Scale bar: 10 μm. (**F**) Immunostaining of MAGL in the cortical region of WT, tKO, and aKO mice. GFAP was used as an astrocytic marker colocalized with MAGL. Scale bar: 20 μm. (**G**) Distance traveled of WT, tKO, nKO, and aKO mice in the open-field test. The data are means ±SEM (n=20~26/group).



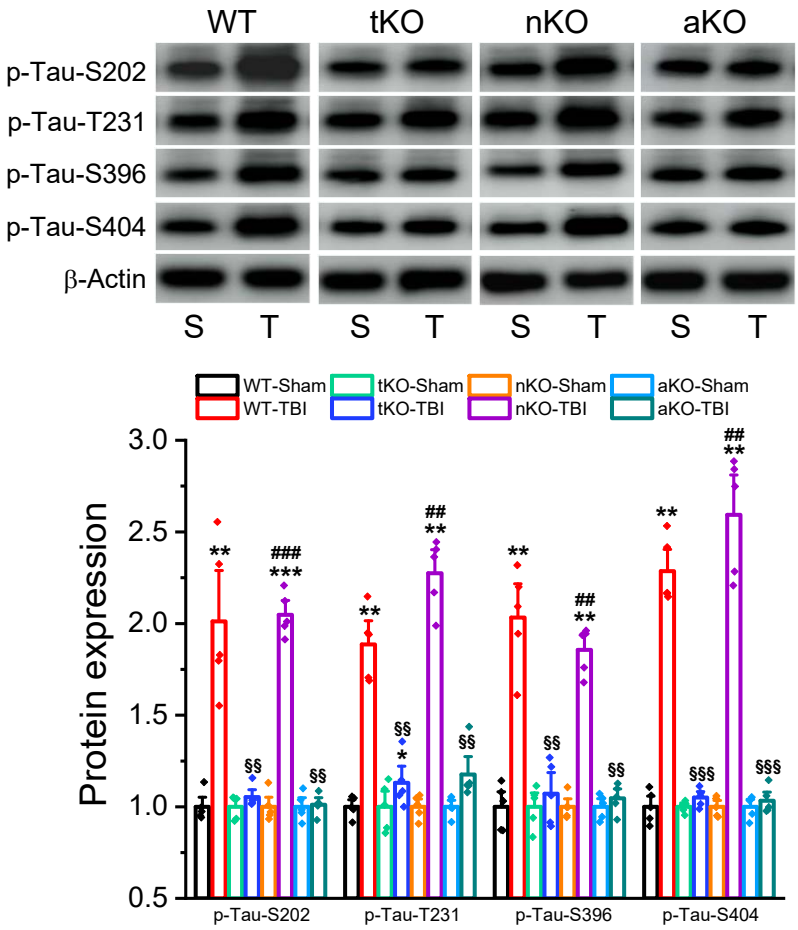
Supplementary Fig. 4. Violin plots for genes expressed in astrocytes and microglia in WT, aKO, and nKO mice that received Sham and TBI. (**A**) Expressions of Ccl2, Spp1, Cxcl10, Fgfr3, Ntsr2, and Pdgfrb in astrocytes from WT, aKO, and nKO mice in sham and TBI. (**B**) Expressions of Ccl12, Ccl4, Ccl7, Ptgs2, Ccl3, Cx3cr1, P2ry12, and Cd86 in microglia from WT, aKO, and nKO mice in sham and TBI. Wilcoxon Rank Sum test was used to determine statistical significance.



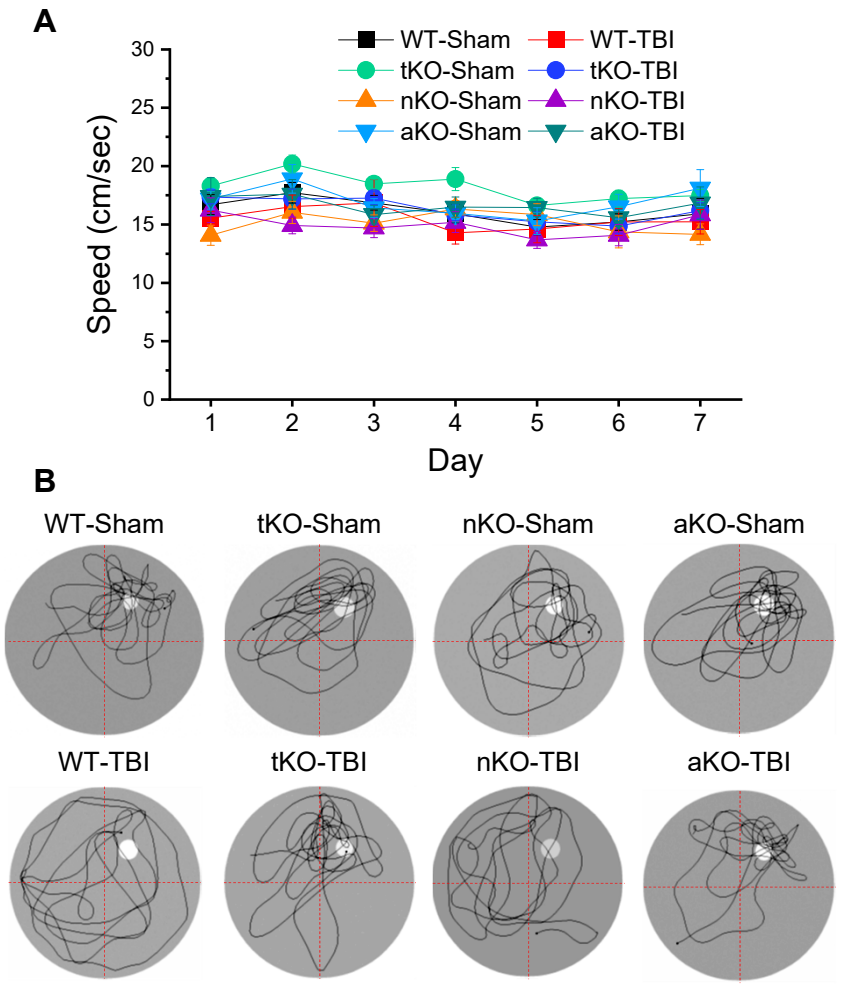
Supplementary Fig. 5. Gene ontology analysis of DEGs in astrocytes in WT, aKO and nKO mice exposed to TBI. (**A** and **B**) Top 20 biological pathways enriched for upregulated or downregulated genes in astrocytes in WT mice. (**C** and **D**) Top 20 biological pathways enriched for upregulated or downregulated genes in astrocytes in aKO mice. (**E** and **F**) Top 20 biological pathways enriched for upregulated or downregulated genes in astrocytes in nKO mice.



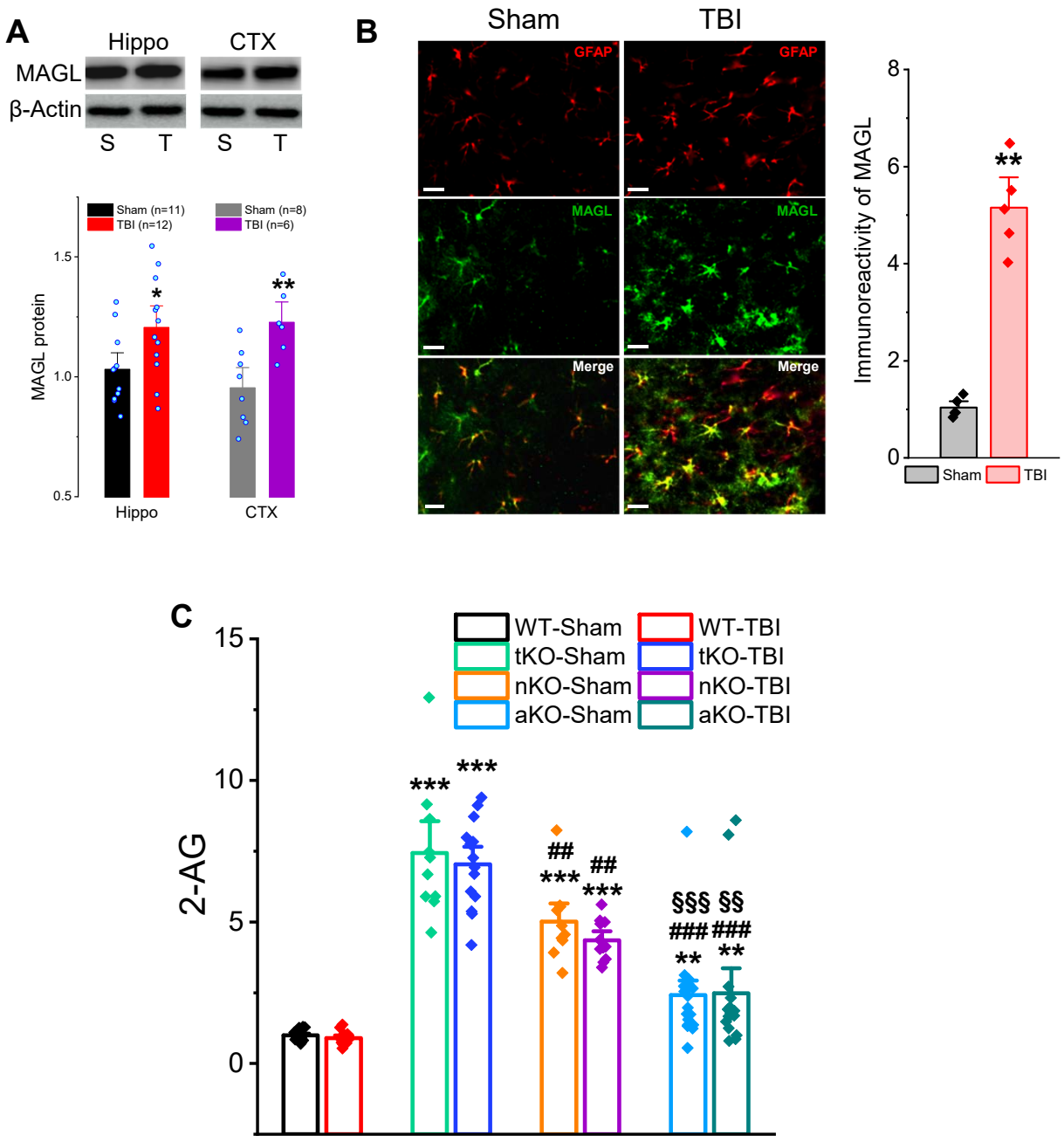
Supplemental Fig. 6. Gene ontology analysis of DEGs in microglia in WT, aKO and nKO mice that received TBI. (**A** and **B**) Top 20 biological pathways enriched for upregulated or downregulated genes in microglia in WT mice. (**C** and **D**) Top 20 biological pathways enriched for upregulated or downregulated genes in microglia in aKO mice. (**E** and **F**) Top 20 biological pathways enriched for upregulated or downregulated genes in microglia in nKO mice.



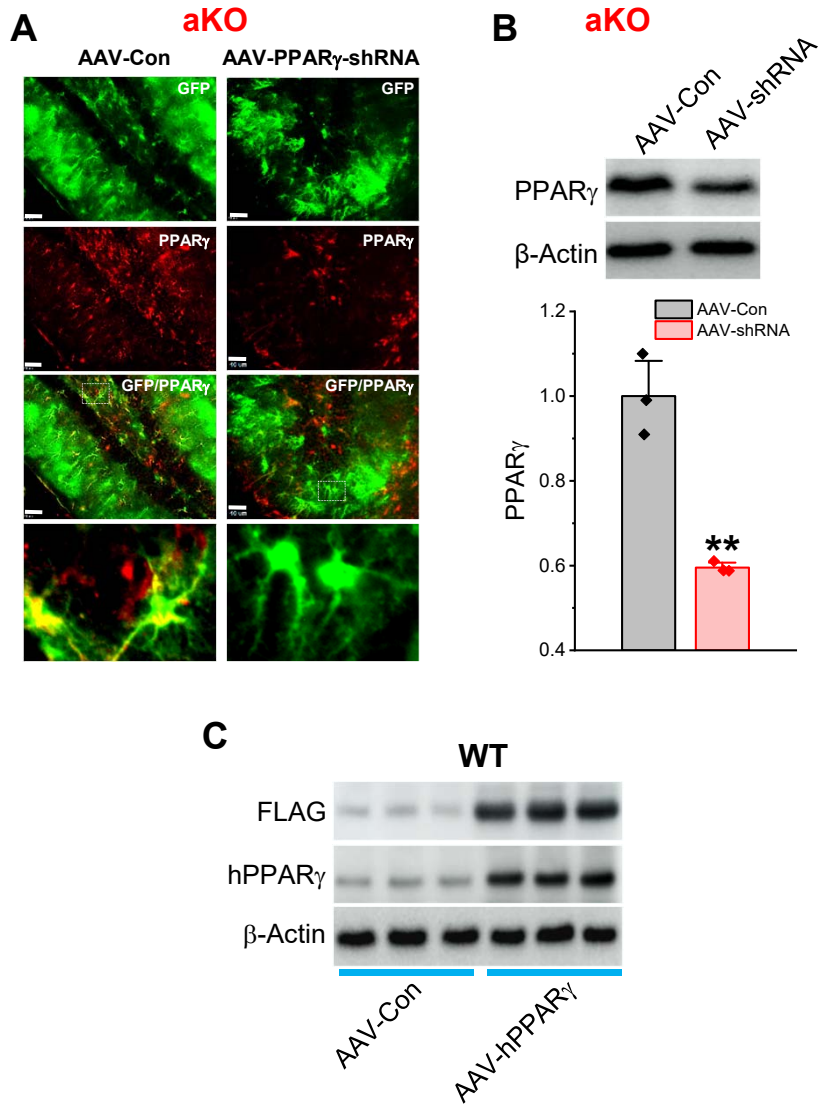
Supplementary Fig. 7. Repeated mild closed head injury induces tau phosphorylation at multiple sites in the hippocampus of WT and nKO mice, but not in tKO and aKO mice. Western blot analysis of phosphorylated tau (p-tau), including p-tau-Ser202, p-tau-Thr231, p-tau-Ser396, and p-tau-Ser404, in the hippocampus of WT, tKO, nKO, and aKO mice that received repeated mCHI. The data are means ± SEM. ***P*<0.01, ****P*<0.001, compared with WT-Sham; §§*P*<0.01, §§§*P*<0.001 compared with WT-TBI; ##*P*<0.01, ###*P*<0.001 compared with nKO-Sham (ANOVA with Fisher's PLSD post-hoc test, n=5 animals/group).



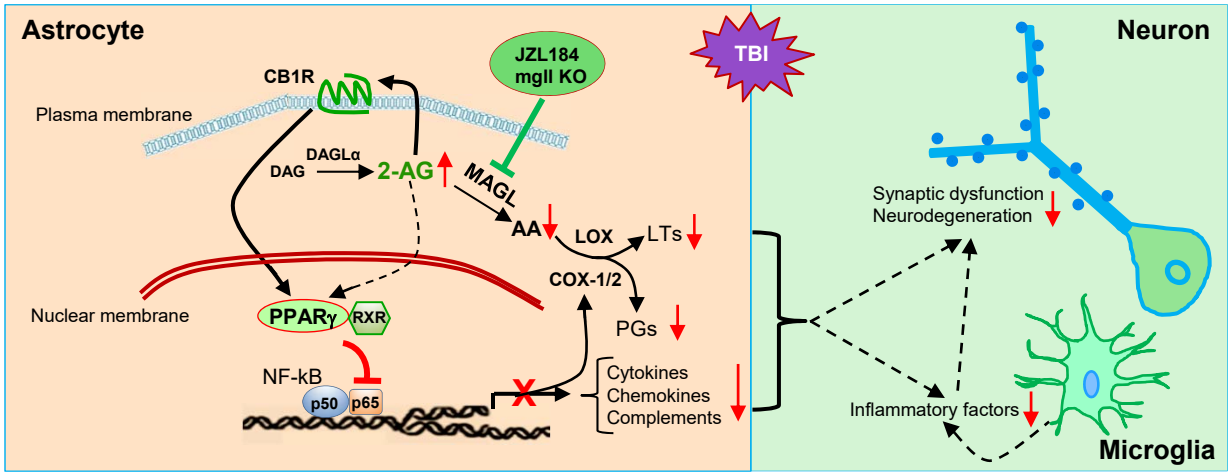
Supplementary Fig. 8. (**A**) Swim speed during learning acquisition in the Morris water maze test. (**B**) The representative traces on the probe trial at day 8



Supplementary Fig. 9. TBI evokes expression of MAGL in astrocytes. (**A**) Immunoblot analysis of brain MAGL expression in WT mice exposed to repetitive mCHI. The data are means ±SEM. *P<0.05, **P<0.01 (Unpaired t-test). (**B**) Double immunostaining of MAGL with GFAP in WT mice that received 3 impacts. The data are means ±SEM **P<0.01 (ANOVA with Bonferroni post-hoc test, n=5 animals/group). Scale bar: 20 µm. (**C**) LC-MS-MS spectrometry analysis of 2-AG content in the brain of WT, tKO, nKO, and aKO mice that received sham or TBI. All the data are normalized to WT-Sham. The data are means ±SEM. **P<0.01, ***P<0.001 compared with WT-Sham; ##P<0.01, ###P<0.001 compared with tKO-Sham; §§<P0.01, §§§P<0.001 compared with nKO-Sham (ANOVA with Bonferroni post-hoc test, n= WT-Sham: 18, WT-TBI: 14; tKO-Sham: 10, tKO-TBI: 14; nKO-Sham: 10, nKO-TBI: 11; aKO-Sham: 18, aKO-TBI: 16).



Supplementary Fig. 10. AAV vector-mediated silencing or overexpression of PPARγ in astrocytes of aKO or WT mice. (A) Immunostaining analysis of PPARγ and GFP expressions in the hippocampus from aKO mice that were stereotaxically injected with AAV-GFAP-eGFP-m-PPARγ-shRNA or AAV-GFAP-eGFP vectors. Scale bars: 40 μm. (**B**) Expression of PPARγ is reduced in the hippocampus from aKO mice that received AAV-GFAP-eGFP-m-PPARγ-shRNA or AAV-GFAP-eGFP vectors. **P<0.01 compared with AAV-Con (Unpaired t-teste, n=3/group). (**C**) Expression of human PPARγ is elevated in astrocytes of the hippocampus from WT mice that were stereotaxically injected with AAV-GFAP-hPPARγ-FLAG-WPRE or AAV-GFAP-eGFP vectors.



Supplementary Fig. 11. Cartoon illustrating signaling pathways that mediate astrocytic MAGL inactivation-produced neuroprotection in TBI.
Pharmacological or genetic inactivation of MAGL in astrocytes augments 2-AG signaling and reduces its immediate metabolite arachidonic acid (AA), leading to reduction of prostaglandins (PGs) catalyzed by cyclooxygenase 1 and 2 (COX-1/2) and leukotrienes (LTs) catalyzed by lipoxygenase (LOX). Enhanced 2-AG signaling acts on CB1R, which in turn activates peroxisome proliferator-activated receptor γ(PPARγ). PPAR γand retinoic x receptor (RXR) complex interacts with NF-kB p50/p65, limiting its transcription and expression of genes involved in inflammatory responses. Reduced inflammatory factors, including cytokines, chemokines, complements, PGs and LTs, released from astrocytes, alleviate TBI-induced neuropathology and microglial reactivity, which in turn, prevent TBI-induced synaptic and cognitive declines.

Uncut gels

Figure 3A

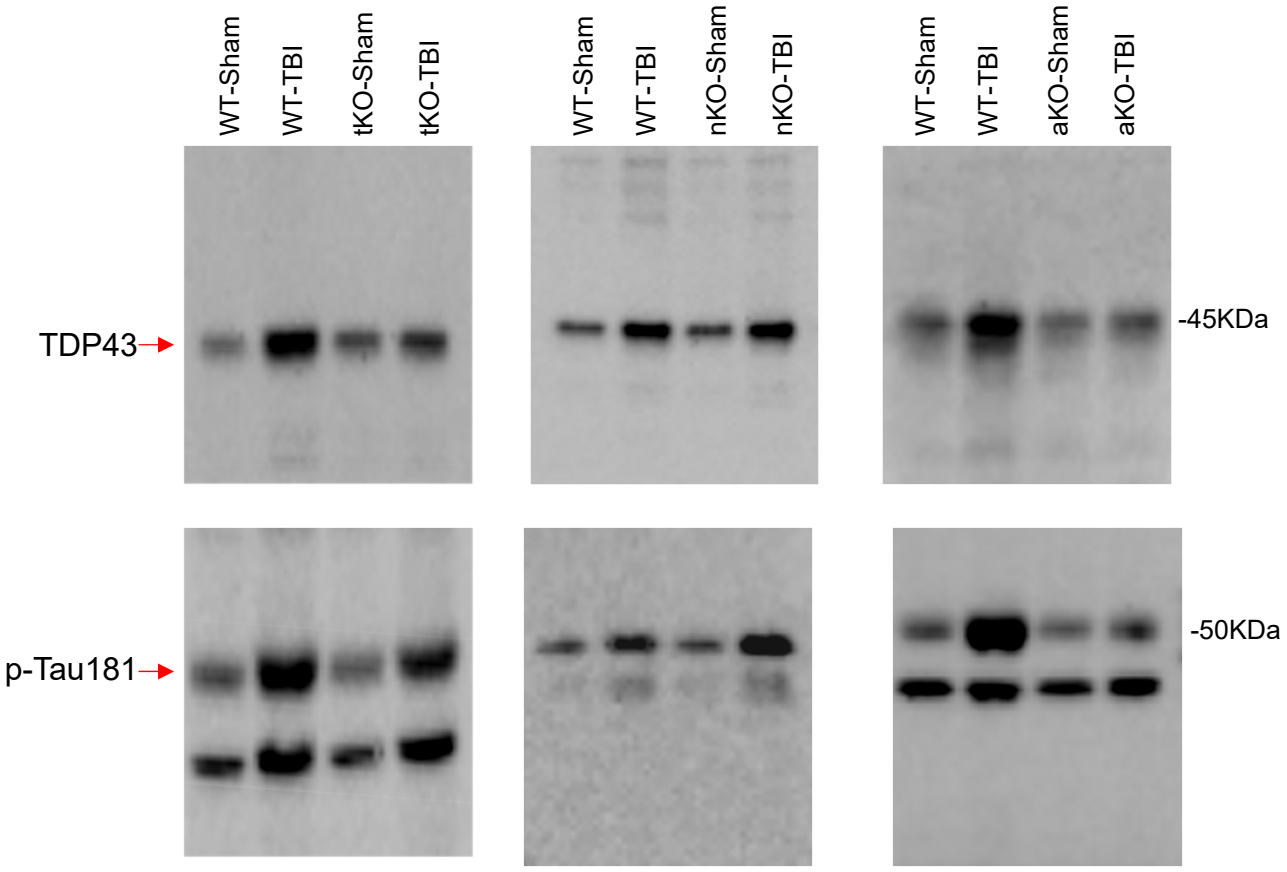


Figure 5E~G

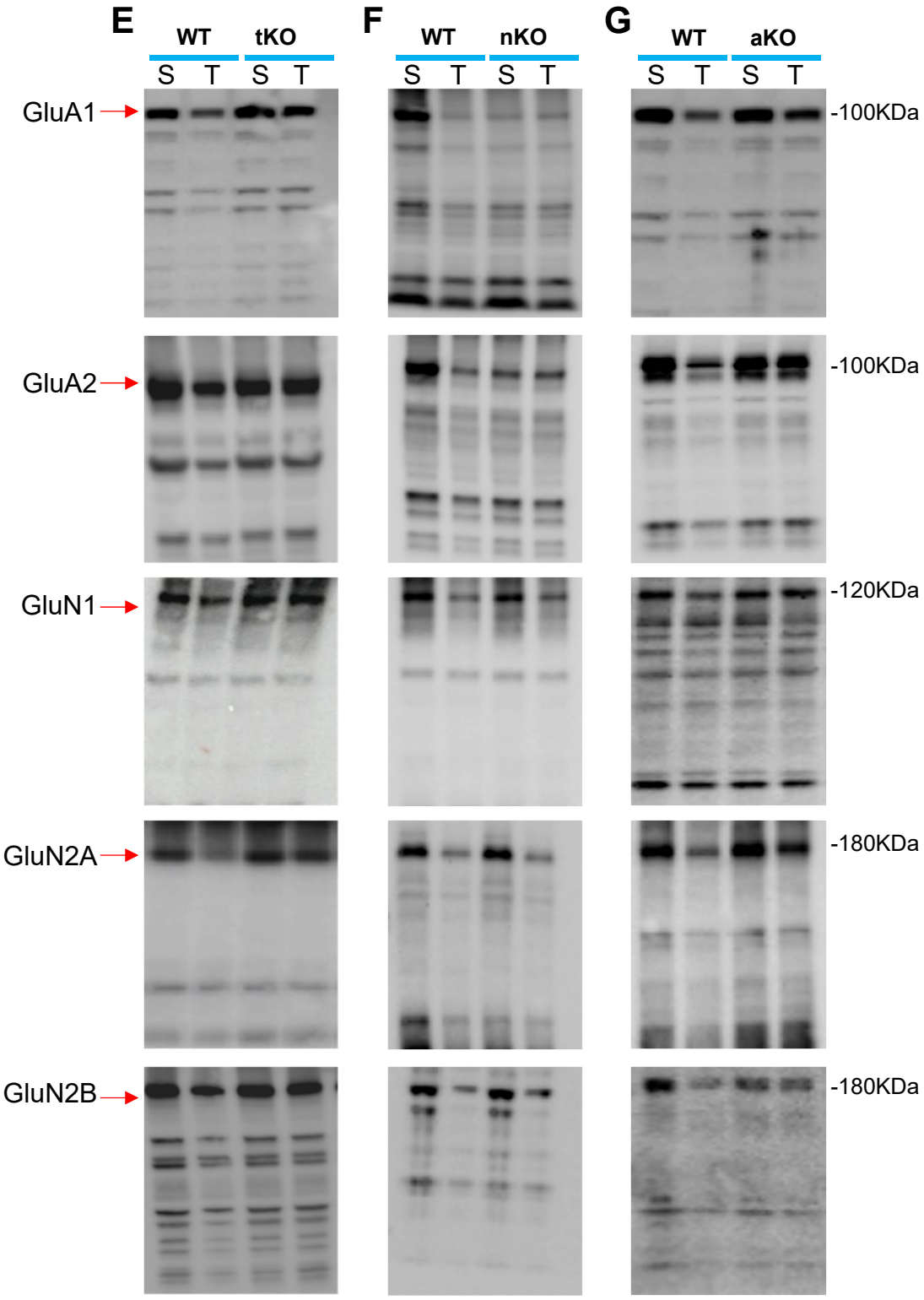

E
WT
tKO
S T S T
F
WT
nKO
S T S T
G
WT
aKO
S T S T
GluA1
GluA2
GluN1
GluN2A
GluN2B
-100KDa
-100KDa
-120KDa
-180KDa
-180KDa

Figure 6B&D

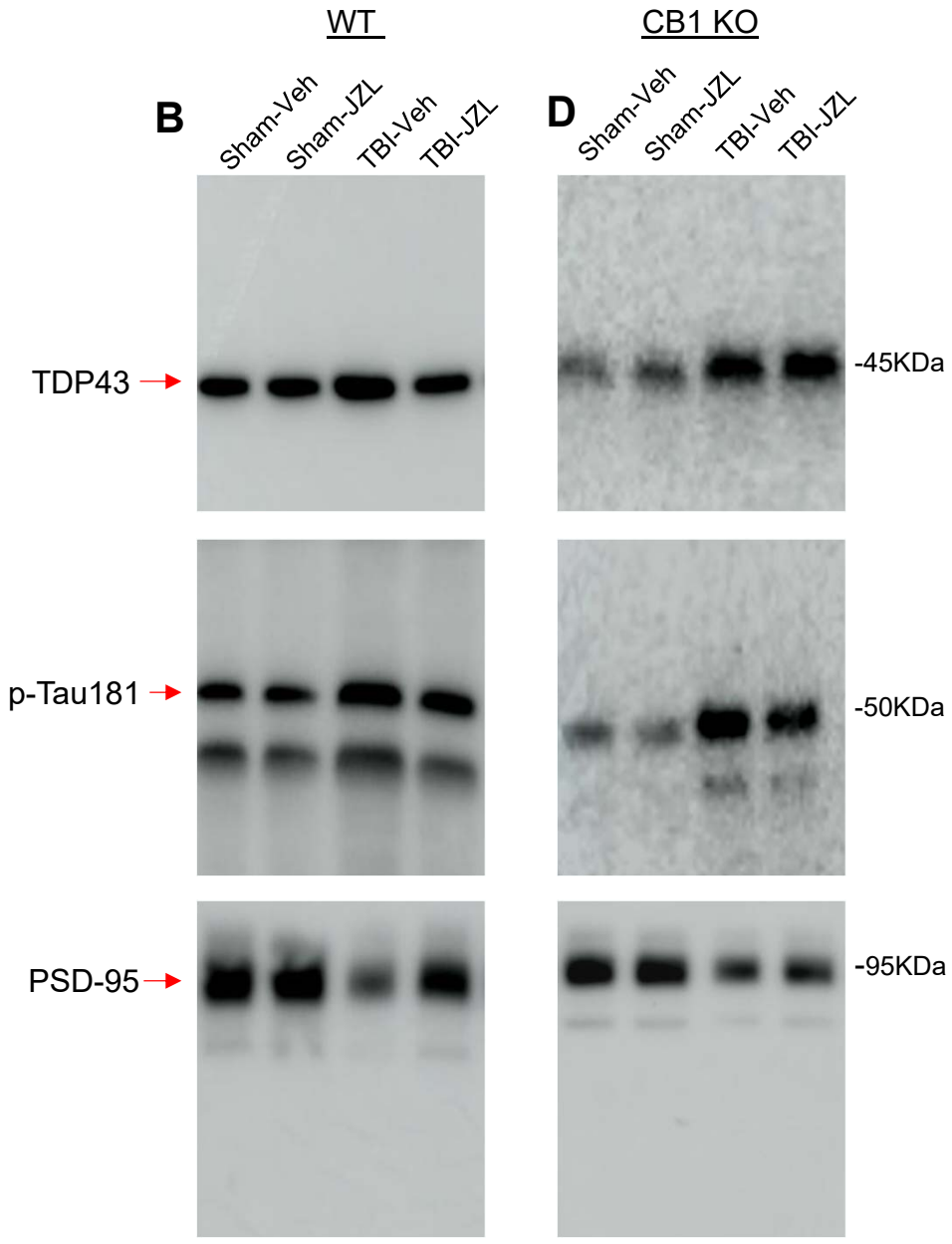

WT
CB1 KO
B
D
Sham-Veh
Sham-JZL
TBI-Veh
TBI-JZL
TDP43
p-Tau181
PSD-95
-45KDa
-50KDa
-95KDa

Figure 7 B~D

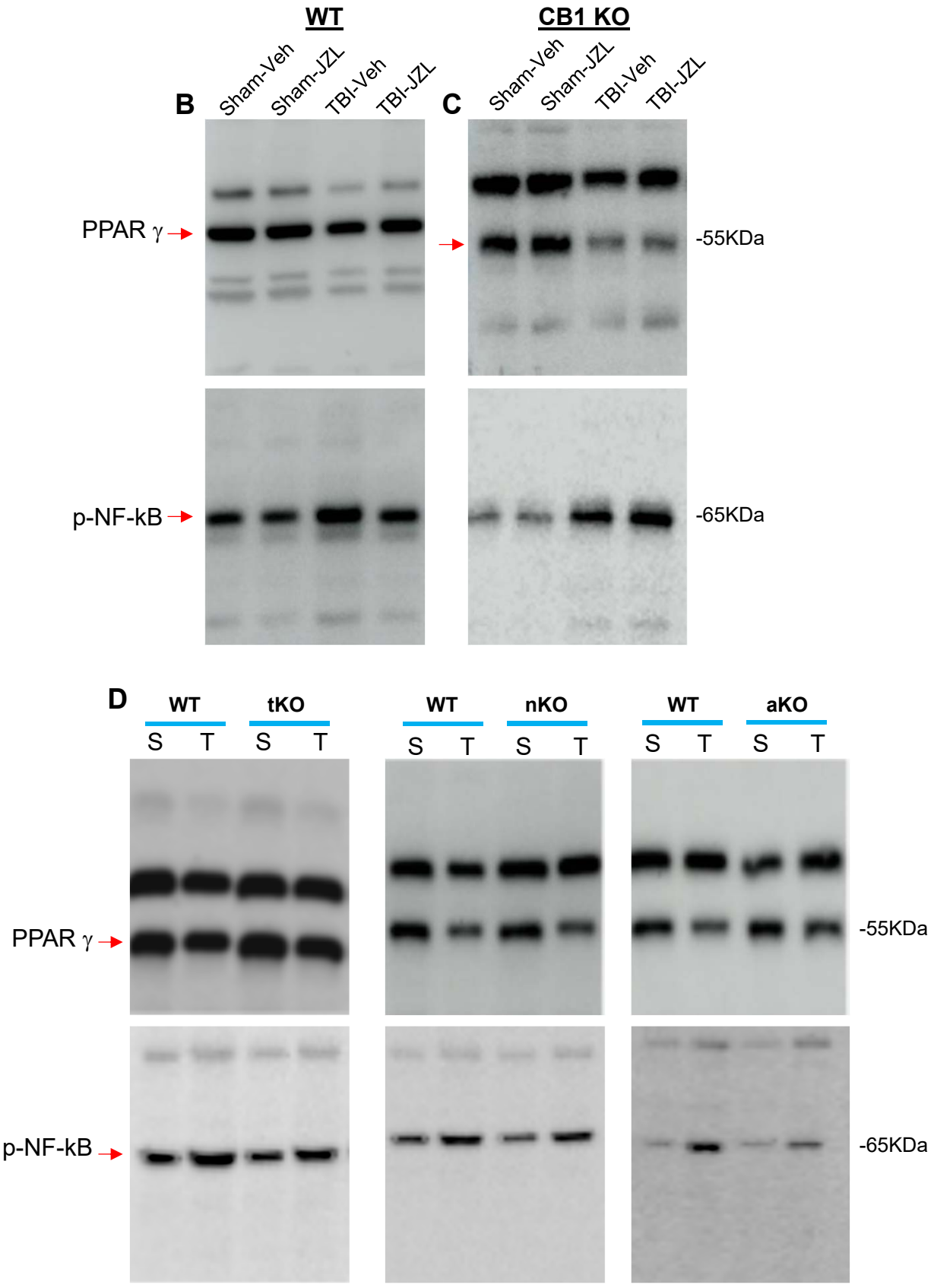
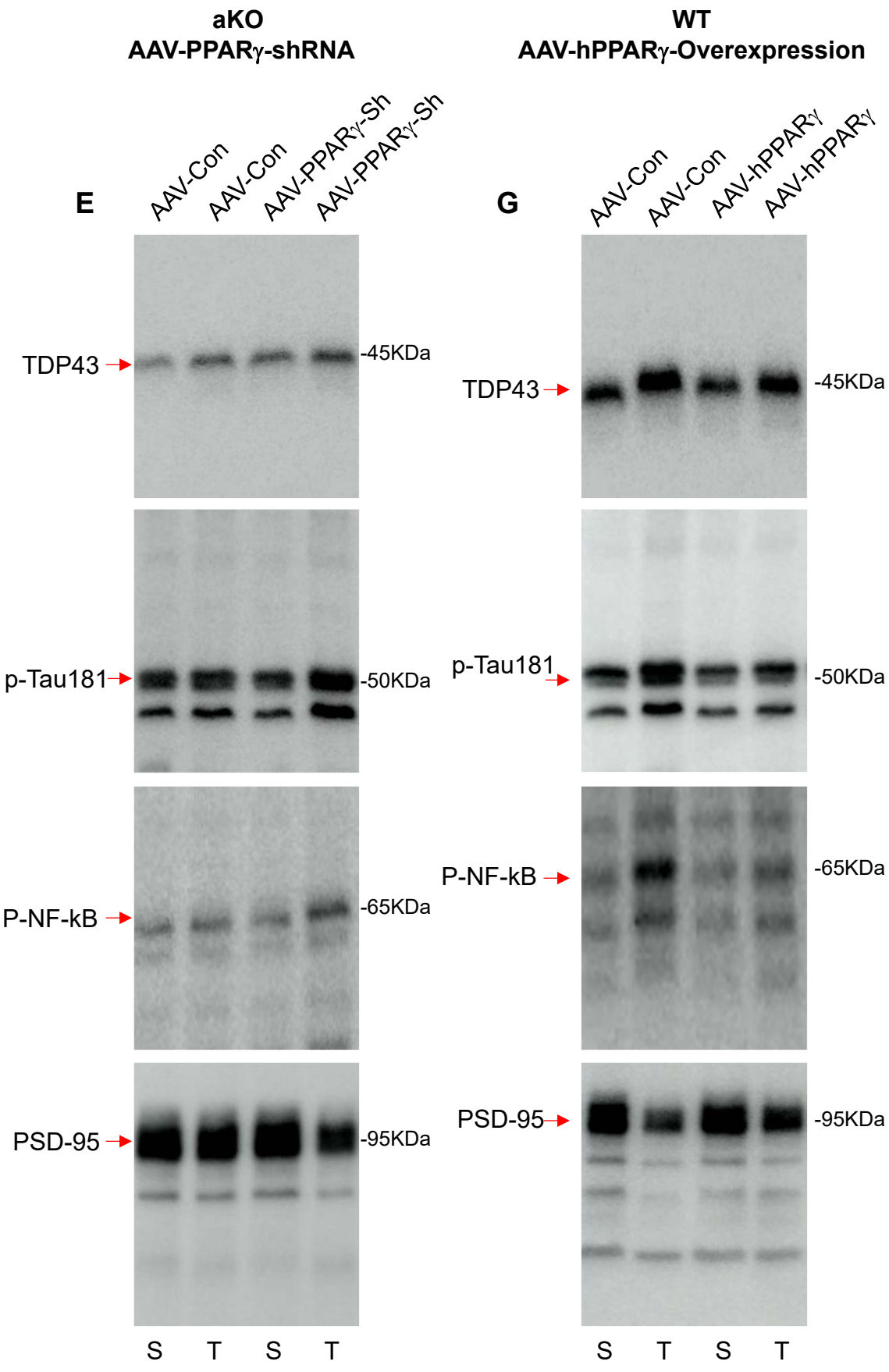
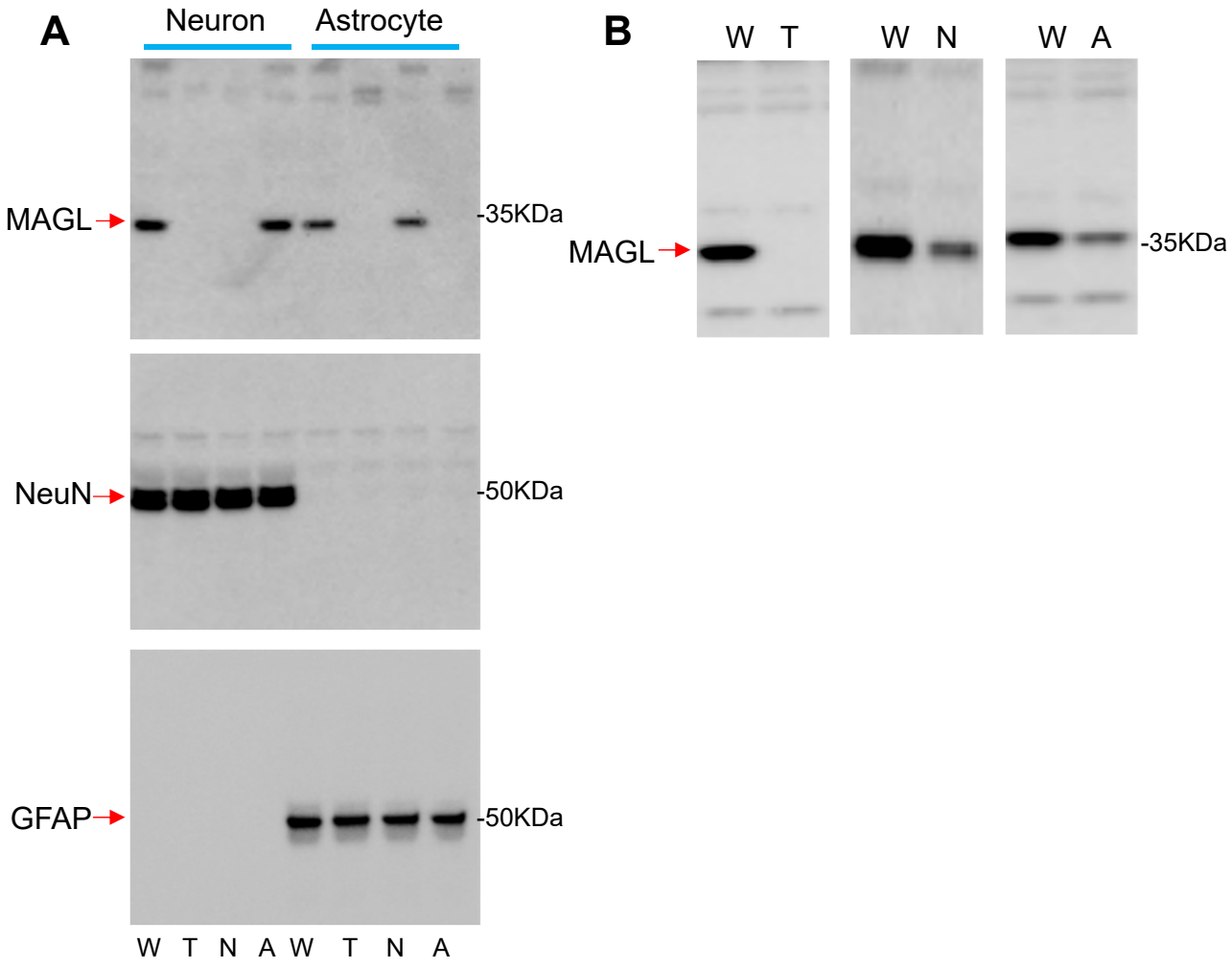

WT
CB1 KO
B
C
Sham-Veh
Sham-JZL
TBI-Veh
TBI-JZL
PPAR γ
-55KDa
p-NF-kB
-65KDa
D
WT
tKO
nKO
aKO
S
T

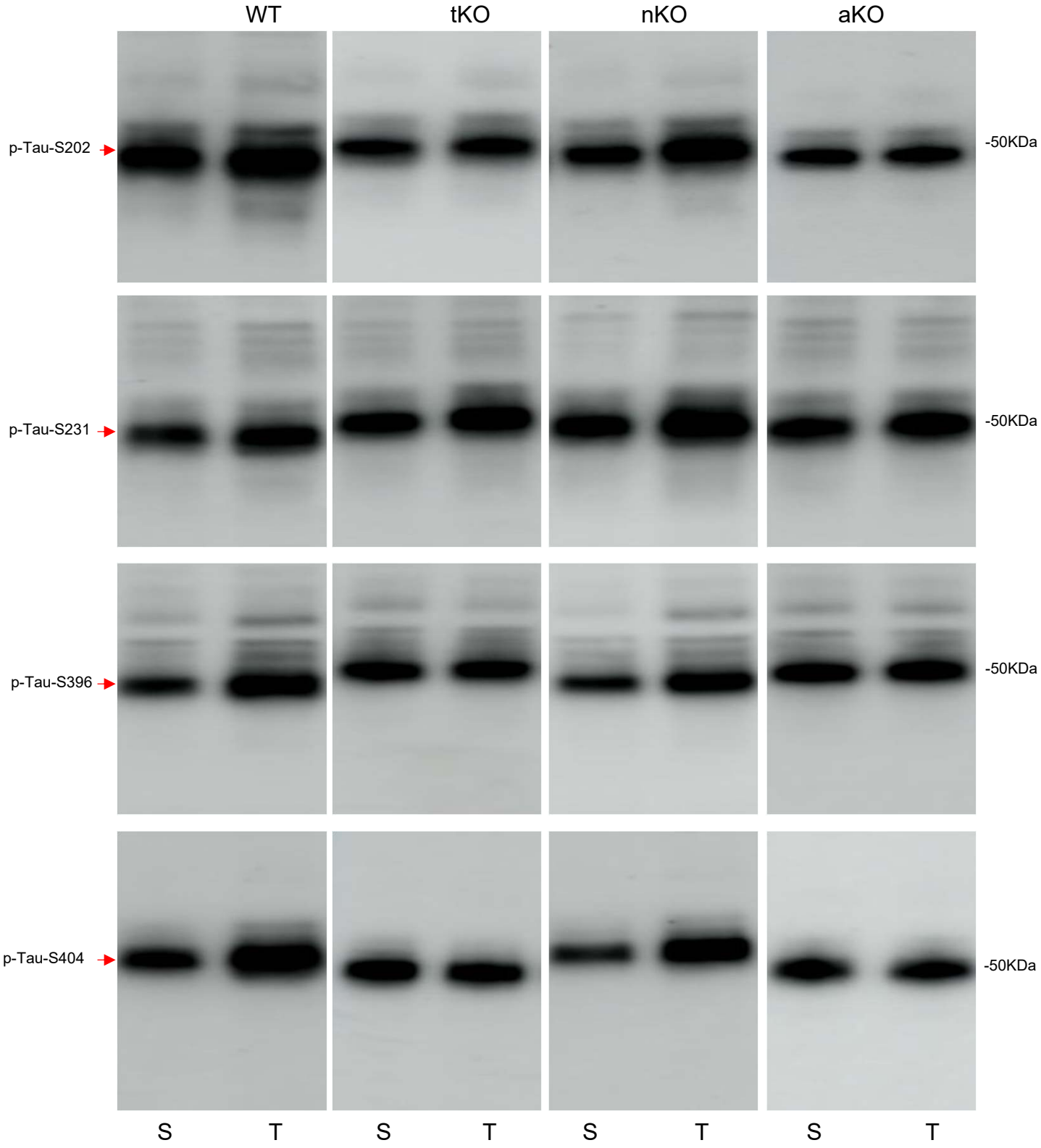
Figure 7E&G


aKO
AAV-PPARγ-shRNA
WT
AAV-hPPARγ-Overexpression
E
G
AAV-Con
AAV-Con
AAV-PPARγ-Sh
AAV-PPARγ-Sh
AAV-Con
AAV-Con
AAV-hPPARγ
AAV-hPPARγ
TDP43
-45KDa
p-Tau181
-50KDa
P-NF-kB
-65KDa
PSD-95
-95KDa
S T S T
S T S T

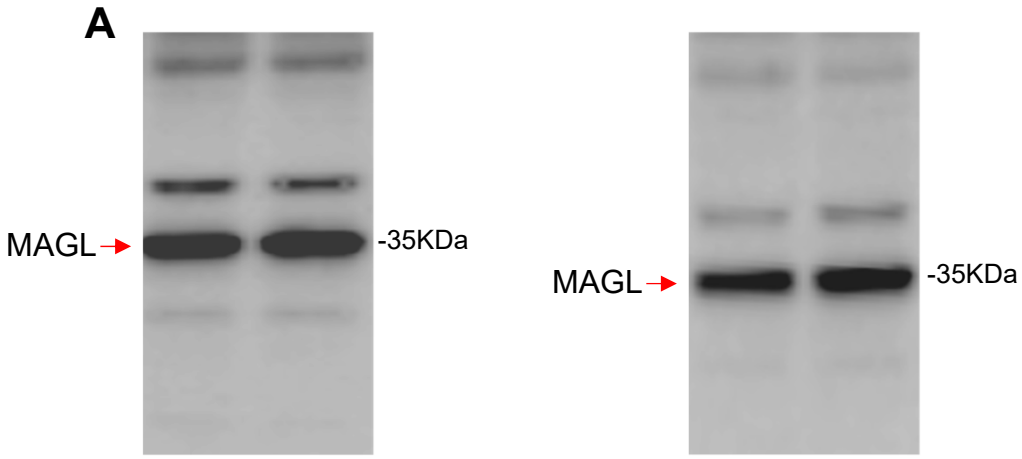
Supplementary Figure 3A



Supplementary Figure 7



Supplementary Figure 9A



Supplementary Figure 10 B&C