

Supplemental Materials & Methods

Animals: The procedures for animal use were approved by the Institutional Animal Care and Use Committee (IACUC) of Weill Cornell Medical College. They were carried out in accordance with guidelines from the National Institutes of Health and Animal Research: Reporting of In Vivo Experiments (ARRIVE). The animals were maintained under controlled conditions with regulated temperature and humidity and on a 12 h light/dark cycle. Each cage contained a maximum of 5 mice and was equipped with ventilation and irradiated bedding (1/8-inch Bed O's Cobs, The Anderson, Maumee, OH). Sterilized food (PicoLab Rodent diet 5053, LabDiet, St. Louis, MO) and water were readily available *ad libitum* in the cages. Mice with CD36 conditionally knocked out in monocyte/M ϕ (cKO^{MM ϕ}) were generated by crossing mice containing loxP flanked CD36 in C57 background (CD36^{fl/fl}; from Maria Febbraio)⁸¹ with LyzM Cre mice (B6.129P2-Lyz2tm1(cre)Ifo; Jackson Laboratory). The cKO^{MM ϕ} mice have a CD36 specifically deleted in macrophages without disrupting CD36 expression in other tissue (Figure S8). All experiments used male and female mice aged 3-4-month. TH-GFP⁺ mice (from Kazuto Kobayashi) expressed GFP in TH⁺ dopaminergic neurons⁸² were used to investigate for transneuronal degeneration. These genetically modified mice are C57BL/6 congenic. All experiments used male and female mice aged 3-4-month. Randomization was performed using a computer-generated scheme to assign mice to control (sham) or experimental (RIC) groups. To ensure objectivity, all investigators involved in surgery, treatment administration, and outcome assessments were blinded to group assignments. Post-randomization, any mice meeting exclusion criteria were removed to preserve the integrity of the experimental groups.

Transient middle cerebral artery occlusion (MCAO): The procedure for 30-minute transient MCAO and post-stroke care has been described previously⁸³. Briefly, mice were anesthetized with a mixture of isoflurane, oxygen, and nitrogen. A 6-0 Teflon-coated black monofilament surgical suture (Doccol, Redland, CA) was inserted into the exposed external carotid artery and advanced into the internal carotid artery until it was wedged into the Circle of Willis, obstructing the origin of the middle cerebral artery (MCA). The filament was left in place for 30 minutes before being removed. Cerebral blood flow (CBF) was measured before, during, and after the stroke using Laser-Doppler flowmetry (Periflux System 5010; Perimed, Järfälla, Sweden). Only animals that had both >80% reduction of pre-ischemic baseline CBF during MCAO and CBF >80% of baseline after 10 min of reperfusion were included in the study. Body temperature was maintained at $37 \pm 0.5^{\circ}\text{C}$ during, and for 30 minutes after, the MCA procedure using a rectal probe connected to a thermocouple-regulated heating water coil on the surgical board. Mice were placed in a recovery cage, and their body temperatures were maintained at $37 \pm 0.5^{\circ}\text{C}$ until they regained consciousness and resumed activity, after which they were returned to their home cages. During the acute phase, the mice were administered warm saline subcutaneously to prevent dehydration. The animals were given softened food and hydrogel (Clear H₂O) during the first week of recovery. Typically, the mice began to regain body weight at 3-5 days post-stroke and continued to recover.

Remote limb ischemic conditioning (RIC): RIC was performed on the left hindlimb of the isoflurane-anesthetized mice. This involved applying a total of 5 cycles of inflation and deflation (200 mmHg, 5 minutes each, with a 5-minute interval between cycles) using a small blood pressure cuff (Hokanson, Bellevue, WA). In a poststroke application, RIC was applied 2 h after

1 reperfusion. Blood flow in the left hindlimb was measured during RIC and monitored by Laser-
2 Doppler flowmetry (Periflux System 5010; Perimed, Järfälla, Sweden) (Figure S1). The Sham
3 group, exposed to the same duration of isoflurane, served as a control.

4
5 **Assessment of monocyte subsets *in vitro*:** Sera were collected from Sham or RIC mice 24 h
6 after MCAO surgery. The sera from each group (Sham: n=3; RIC: n=4) were combined and
7 stored at -80°C until use. Naive splenocytes from WT or cKO^{MMφ} were collected and prepared
8 for single-cell suspensions. After the splenocyte collection, CD11b⁺ splenocytes were isolated
9 using CD11b Microbeads (Miltenyi Biotec, San Diego, CA) following the manufacturer's
10 instructions. The CD11b⁺ splenocytes (2x10⁵/well) were then incubated without serum, with
11 Sham serum, or with RLC serum for 4 h at 37°C with 5% CO₂. After the 4h incubation, the cells
12 were collected and incubated with antibody for flow cytometry. A total of 20,000 cells were
13 analyzed. Each sample was analyzed in triplicate to confirm the reliability of the data.

14
15 **Brain immune cell isolation:** Mice were anesthetized with isoflurane and ketamine/xylazine
16 before being perfused with ice-cold phosphate-buffered saline (PBS) containing heparin. Brains
17 were removed, and the hemispheres were separated before being placed into ice-cold Hanks'
18 balanced salt solution without Ca²⁺ and Mg²⁺ (HBSS, Life Technologies, Grand Island, NY).
19 Tissue was enzymatically and mechanically dissociated using a MACS Neural Tissue
20 Dissociation Kit (Miltenyi Biotec, San Diego, CA) and then treated with a myelin debris removal
21 solution (Miltenyi Biotec, San Diego, CA). Isolated brain immune cells were used for either flow
22 cytometer analyses (at least 20,000 cells/analysis) or cultured for *in vitro* efferocytosis assays
23 (2x10⁵/well).

Flow cytometry analysis: From single-cell preparations, we determined the total cells by multiplying the dilution factors to the cell event reads. Initial gating is conducted using forward and side scatter to exclude debris and non-viable cells. Furthermore, doublets are discriminated to isolate individual cells. For the flow cytometry gating to identify mononuclear phagocytes (monocyte/M ϕ , microglia), we gated out for all lineage markers [Lin⁺]. The lineage [Lin⁺] markers included anti-CD90.2 (T cells), anti-CD45R/B220 (B cells), anti-NK-1.1 (natural killer cells), and anti-Ly6G (neutrophils/granulocytes). The [CD45⁺/CD11b⁺] cells in the resulting [Lin⁻] population were considered to be microglia and M ϕ . CD45 subset analyses identified CD45^{Low} as microglia in the contralateral hemisphere. In the ipsilateral hemisphere, the CD45^{High} population is M ϕ and the CD45^{Low} subset is the mixture of microglia and M ϕ that undergo a phenotype change to “microglia-like cells. The primary antibodies used for cell staining were as follows: CD11b (PE-Vio770 anti-mouse CD11b REA, 1:50); CD45 (Vio-blue anti-mouse CD45 REA, 1:50); CD36 (Vio Bright B515 anti-mouse CD36 REA, 1:50) or (APC anti-mouse CD36 REA, 1:50); Ly6C (FITC anti-mouse Ly6C REA, 1:50) or (APC anti-mouse Ly6C REA, 1:50); and a mixture of lineage markers (Lin) conjugated with APC (APC-Lin) against T cells (APC anti-mouse CD90.2 REA), B cells (APC anti-mouse CD45R/B220 REA), natural killer cells (APC anti-mouse NK-1.1 REA, and APC anti-mouse CD49b REA), and neutrophils/granulocytes (APC anti-mouse Ly-6G REA) [29]. After a 20-minute incubation at room temperature in the dark, the cells were washed with PBS and then analyzed with a MACS Quant VYB flow cytometer (Miltenyi Biotec, San Diego, CA). The antibodies used for flow cytometry were purchased from Miltenyi Biotec. Antibody specificity was confirmed by analyzing cells only and using single and double antibody controls for validation. We determined

the optimal compensation for fluorescence spillover from fluorochrome-conjugated antibodies using REA compensation beads (Miltenyi Biotec, San Diego, CA). Each sample was analyzed in triplicate to confirm the reliability of the data.

Efferocytosis assay: For the *in vitro* assays, single immune cells isolated from the brain (2×10^5 /well) were cultured in 24-well plates in Macrophage-SFM medium (Thermo Fisher Scientific, Waltham, MA) containing 10% FBS for 2h at 37°C incubator. To simulate stroke milieu and to probe efferocytosis activity of the isolated brain immune cells, apoptotic cells (ACs) were co-cultured with the isolated immune cells. ACs were generated by placing splenocytes (1×10^7 cells/100mm dish) under UV light for 30 min, which induces apoptosis (see Figure S3). The splenocytes were further labeled with GFP Fluorescent Cell Linker to label the lipid region of the cell membrane (MINI67/126, Sigma-Aldrich, St. Louis, MO), which allows >99% of cells to be labeled with the GFP, and ~70% of GFP+ splenocytes are ACs, based on propidium iodide staining. GFP+ apoptotic cells (1×10^6) are added to the cultured immune cells (brain cells: APCs, 1:5) for 1h, washed with PBS. Co-cultures are harvested and analyzed by flow cytometry for efferocytosis of GFP+ cells in [CD45+ /CD11b+/Lin-] populations. For *in vivo* assay, efferocytosis activity was determined by infusion of fluorescent microspheres (beads580/605; F-13083, Fisher Scientific, Waltham Thermo, MA) at 2d after MCAO via the retro-orbital venous sinus. Brain tissue was harvested one day later (3d post-MCAO) and determined bead+ cells in [CD45+ /CD11b+/Lin-] populations by flow cytometry for the assessment of *in vivo* efferocytosis. Each sample was analyzed in triplicate to confirm the reliability of the data from *in vitro* or *in vivo* assays.

Western blotting: Brain tissue, heart, and peritoneal macrophages were homogenized and lysed in lysis buffer (C3228, Sigma-Aldrich, St. Louis, MO) with added complete Mini protease

inhibitor (11836170001, Sigma-Aldrich). Lysates were centrifuged to obtain supernatants for protein analysis. Protein concentrations were determined, and 30 µg of protein were separated on NuPAGE 4–12% Bis-Tris gels (NP0322box, Thermo Fisher Scientific, Waltham, MA). After electrophoresis, proteins were transferred to PVDF membranes (#1620255, Bio-Rad, Hercules, CA). The membranes were blocked with Licor blocking buffer (#927-60001, Licorbio, Lincoln, NE) for 1 hour and then incubated overnight at 4°C with anti-mouse CD36 (1:2000, MAB1258, Sigma-Aldrich) or anti-rabbit GAPDH (1:5000, sc-25778, Santa Cruz Biotechnology, Dallas, TX) antibodies. After washing with Tris-buffered saline containing 0.05% Tween 20, membranes were incubated with IRDye 800CW donkey anti-mouse (1:2000, #926-32212, Licorbio) or IRDye 680CW goat anti-rabbit (1:2000, #926-68071, Licorbio) secondary antibodies in blocking buffer for 1 hour at room temperature. Protein bands were visualized using the Odyssey imaging system (Licorbio).

Brain acute infarct size measurement: Three days following MCAO, the brains were extracted, cryosectioned at a thickness of 20 µm, and collected at intervals of 600 µm. Infarct size and brain swelling were assessed using a total of 13 brain sections with Axiovision software (Carl Zeiss, Thornwood, NY), following the method outlined in a previous study⁸³. The collected sections were examined under phase-contrast microscopy to identify the infarcted regions. The infarcted area was delineated in each section, and the infarct volume was calculated by multiplying the infarct area (mm²) in each section by the distance to the next section (0.6 mm), then summing the values from all sections. The infarct volume was adjusted for edema by subtracting the difference between the hemispheric volumes. To determine the percentage of

hemispheric swelling, the difference in volume between the ipsilateral and contralateral hemispheres was divided by the contralateral hemisphere volume and multiplied by 100.

Whole brain imaging: Fixed adult mouse brains from TH-GFP+ animals were delipidated using a modified Adipo-Clear protocol⁸⁴. Briefly, brains were first washed with B1n buffer (H₂O/0.1% Triton X-100/0.3 M glycine, pH 7), then transferred to a methanol gradient series (20%, 40%, 60%, 80% methanol in B1n buffer) using 4 mL for each brain and 1 hour for each step. This gradient series was followed by incubation in 100% methanol for 1 hour, then an overnight incubation in a 2:1 mixture of DCM: methanol and an 1.5-hour incubation in 100% dichloromethane (DCM) the following day. After which the brains were placed in 100% methanol for 1 hour three times, before treatment with a reverse methanol gradient series (80%, 60%, 40%, 20% methanol in B1n buffer) with 30 minutes for each step. Finally, brains were washed in B1n buffer for 1 hour, and then overnight in fresh B1n buffer. All these procedures were conducted at room temperature with rocking to ensure complete delipidation. The delipidated brain tissue was then blocked in PTxwH buffer (PBS/0.1% Triton X-100/0.05% Tween 20 containing 5% DMSO and 0.3M glycine) for 3 hours at 37°C before an overnight wash in fresh PTxwH buffer at 37°C. Following this, tissue was successively washed at room temperature with fresh PTxwH buffer for 1 hour, 2 hours, and then overnight.

For staining, brain samples were immersed in a mixture of primary antibodies TH (Rabbit anti-TH, Thermo Fisher Scientific 701949, 1:100) and GFP (Mouse anti-GFP, Addgene 180084-rAb, 1:300) in PTxwH buffer for 14 days at 37°C. After which, samples were washed in PTxwH buffer for 1 h, 2 h, 4 h, and overnight, before three consecutive 1-day washes. Secondary antibodies used were AlexaFluor-594 (Jackson ImmunoResearch 711-587-003, donkey-anti-

1 rabbit, 1:50) and AlexaFluor-647 (Jackson ImmunoResearch 115-607-186, goat-anti-mouse
2 IgG2a, 1:250). Tissue was incubated with secondary antibodies were diluted in PTxwH for 10
3 days before being washed with PTxwH for 1 h, 2 h, 4 h, overnight, then three consecutive 1-day
4 washes. The final wash was with PBS for one day. Tissues samples were then cleared with the
5 iDISCO+ protocol ⁸⁵. Briefly, samples were dehydrated with a methanol gradient that started
6 with water an concluded with 100% methanol, and followed by incubation with a
7 DCM/methanol mixture overnight, and 100% DCM for 1 h twice the next day. Brain tissue was
8 cleared for 4 h in dibenzyl ether and stored in a fresh dibenzyl ether before imaging.

9 Cleared brain samples were imaged horizontally in tiled sections with a LifeCanvas
10 SmartSPIM lightsheet microscope. Imaging used 488/561/647 nm lasers with 3.6×/0.2 detection
11 lens were used for green/red/far-red channels. Lightsheet illumination was focused with an NA
12 0.2 lense and axially scanned with an electrically tunable lense coupled to the camera
13 (Hamamatsu Orca Fusion) in slit mode. The camera exposure was set at fast mode (2 ms) with
14 16-bit imaging with an X/Y sampling rate of 1.8 μm and Z step of 2 μm. Stitched lightsheet
15 images were converted to a hierarchical chunked data organization (Neuroglancer Precomputed
16 format) for visualization and processing for downstream analyses of regions of interest. Format
17 conversion and hierarchical down sampling were performed using the Python library
18 CloudVolume (version 8.24.2, <https://github.com/seung-lab/cloud-volume/>) and the Igneous
19 software ⁸⁶(version 4.20.3, <https://github.com/seung-lab/igneous>). Whole-brain 3D visualization
20 was created with the ORS Dragonfly software using a downsampled version of the full image
21 volume of the appropriate brain sample. Neuroglancer (<https://github.com/google/neuroglancer>)
22 provides convenient 2D visualization of arbitrary orthogonal virtual cross-sections.

Quantification of SNc volume and TH⁺ neuron count in the SNc region:

3D image analysis used Imaris software version 10.1.1 (Bitplane AG, Zurich, Switzerland). Prior to analysis, the original whole brain stacks were flipped and cropped to provide smaller stacks that preserved the original voxel size, but only included regions encompassing the SNc. The same pre-processing procedure was used for each channel. Processed 3D image stacks were imported in Imaris at a voxel size of 1.8 x 1.8 x 2 μm and rendered using maximum intensity projections. Imported images were used to create a composite Imaris image object consisting of two channels with the first channel (pseudo-colored in green) containing the signal produced by TH⁺ neurons and the second channel (pseudo-colored in red) containing the signal produced by GFP expressing-TH⁺ neurons.

To isolate and quantify the neurons specifically within the SNc, freehand 3D regions of interest (ROI) around the left and right SNc volumes were created for each image object using manually drawn contours around the left and right SNc on every 55th 2D plane of the image object (corresponding to z-plane intervals of 100 μm). Autofluorescence signal in the green channel was used as an anatomical guide. Separate left and right SNc surfaces were created for each brain to enable independent quantification of the volume and the number of TH⁺ cells in the SNc of each hemisphere. The volume of each SNc region was calculated and provided by Imaris.

To count SNc TH⁺ cells, we developed a machine learning (ML)-based cell quantification procedure that utilized a pixel classifier provided by the ML Segmentation option in the automatic surface creation feature of Imaris. The pixel classifier was trained to segment TH⁺ cells using input from both channels and user's visual recognition. Training with both channels or with a cell having both bright cytoplasm and bright nuclei, rather than with only the

green channel with a cell having only a bright cytoplasm, allowed for more accurate cell segmentation. The training set was 9 volumes consisting of three samples sets containing three 90- μ m thick sub-volumes (sampling the rostral, middle, and caudal regions of the left SNc). For each sub-volume, training involved manually selecting and marking 20-30 cells with both sparse and dense distribution throughout the volume. After classifying the pixels, a quality filter of -4 and a number-of-voxels filter of 14 were applied before calculating the cell count per volume. After training, we tested the entire machine learning-based procedure on a new sample set of SNc regions expected to have an opposite cell count trend. To determine the actual cell count per SNc region, the output of the machine learning-based procedure was filtered to include only cells within the volume enclosed by the surface contours. Separate cell count values were performed for the left and right SNc regions of each brain.

Immunohistochemistry: Mice were transcardially perfused with 4% paraformaldehyde in 0.1 mol/L phosphate buffer. Brains were collected and post-fixed overnight in a 30% sucrose solution before being cut into 30 μ m-thick sections via a cryostat. All sections were washed in PBS, incubated with 1% bovine serum albumin or 5% normal goat serum for 1 hour at room temperature. After which sections incubated with anti-sheep Tyrosine Hydroxylase (TH, 1:1000, Novus Biologicals, Centennial, CO, NB300-110), anti-mouse NeuN (1:1000, Sigma-Aldrich, St. Louis, MO), anti-goat Myeloperoxidase (MPO, 1:200, Bio-Techne, Minneapolis, MN) or anti-rabbit Iba-1 (1:1000, Wako, Richmond, VA) overnight at 4°C. This was followed by incubation with either Alexa Fluor 488 goat anti-sheep IgG (1:2000, Life Technologies, A11015) for anti-TH, Alexa Fluor 488 goat anti-mouse IgG (1:1000, Life Technologies, A11029) for anti-NeuN, Alexa Fluor 594 donkey anti-goat IgG (1:1000, Life Technologies, A11058) for anti-MPO or

Alexa Fluor 647 goat anti-rabbit IgG (1:1000, Life Technologies, A21245) for anti-Iba-1 secondary antibodies for 1 hour. After washing with PBS, the sections were mounted using Vectashield (Vector Laboratories, Newark, CA). Negative controls were stained with either the secondary antibody or the IgG isotype antibody to validate the specificity of the antibodies and distinguish genuine target staining from non-specific binding background. Confocal image stacks were generated with an inverted A1R-HD25 confocal microscope (Nikon Instruments Inc., Melville, NY) using a 40x (NA 1.3) oil objective in 0.3 μm z-steps and at 0.43 μm /pixel.

Neurological assessment

Behavior assessments were performed as described previously^{53,87,88}. Stroke-induced behavior outcomes were reported against individual pre-ischemic baseline. Analyses of behavior with the inclusion of deceased mice by assigning lowest registered scores within the cohort.

Open field test: To assess motor function, animals were placed in a 0.4m x 0.4m square field surrounded by a 0.4m high wall and allowed to freely explore the platform for 10 minutes^{53,88}. The total distance traversed was measured, as well as central travel distance to assess anxiety-related behavior, using ANYmaze software (Stoelting Co. in Wood Dale, IL).

Rotarod: The mice were placed on a rotating rod (indented rod, 3.5 cm diameter) that accelerated from 4 revolutions per minute (r/min) to 80 r/min over the course of 5 minutes. The latency to fall was used to assess motor performance and was evaluated as described previously⁸⁷. To achieve peak performance, animals were trained on the rotarod for five days with five daily trials and compared with pre-stroke baseline. The reported values are the mean of five consecutive trials.

Grip strength: Grip strength in the hindlimbs of the mice was measured using a grip strength meter (Columbus Instruments, Columbus, OH). The basal strength was measured the week prior to MCAO. Grip strength was assessed three times, and the average of these measurements was used as the final value.

Statistics: The data from the *in vivo* studies were presented using scatter dot plots to show the distribution and mean \pm SD. For *in vitro* studies, the data were displayed with bar with scattered dot plots as mean \pm SD. Multigroup analyses were performed using two-way ANOVA for the effect of stroke (Contralateral vs. Ipsilateral) and effect of conditioning (Sham conditioning vs. RIC), followed by *post hoc* Fisher's Least Significant Difference (LSD) test. The student's t-test was used to compare the differences between the sham and RIC. For sample sizes $n \geq 6$, we used the Shapiro-Wilk test or Kolmogorov-Smirnov test to assess the normality of data distributions prior to performing parametric analyses. For non-normally distributed dataset, we used Mann-Whitney U test for two group comparisons (Fig 3B-C) and the Scheirer-Ray-Hare test followed by *post hoc* the Conover-Iman test for multiple comparisons (Fig 1D, 1H; Fig 2B, 2H; Fig S6A-B; Fig S7C; Fig S10B). For sample sizes $n < 6$, we applied non-parametric analyses using Mann-Whitney U test for two group comparisons (Fig 8A-B; Fig S10G; Fig S11B) and the Scheirer-Ray-Hare test followed by *post hoc* the Conover-Iman test for multiple comparisons (Fig 1I-L; Fig 5B-H; Fig 6A-L; Fig 7C-D; Fig S4A-B; Fig S9A-D; Fig S10G; Fig S11B). All analyses were carried out using Prism software (GraphPad Software Inc., La Jolla, CA) and RStudio software (Posit Software, Boston, MA), and differences were considered statistically significant at $p < 0.05$. The corresponding figure legends present the detailed statistical analysis for each

- 1 experiment. All representative images were selected to represent the group average across all
- 2 available data.

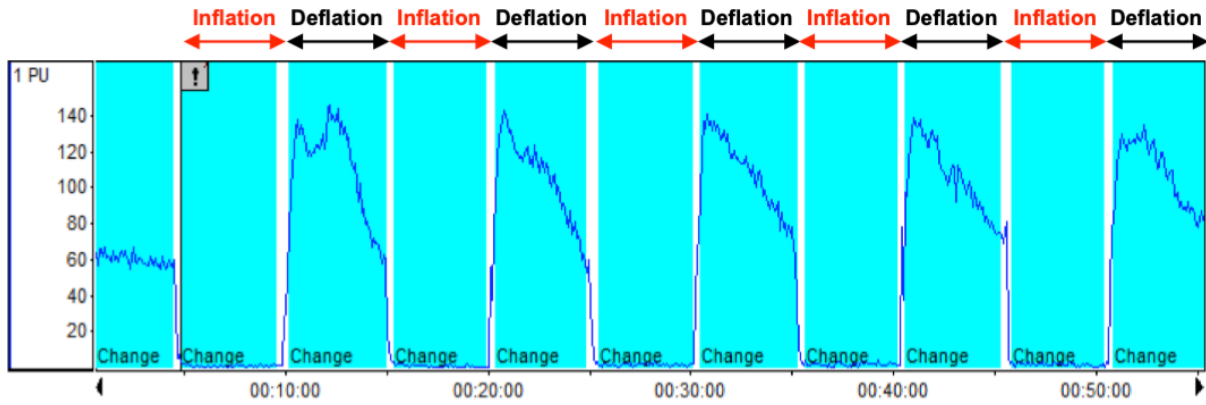


Figure S1. Remote limb ischemic conditioning (RIC)

RIC was performed on the left hindlimb of the mice by applying a total of 5 cycles of inflation and deflation (5 min X 5 min intervals between cycles). The hindlimb blood flow was measured using Laser-Doppler flowmetry.

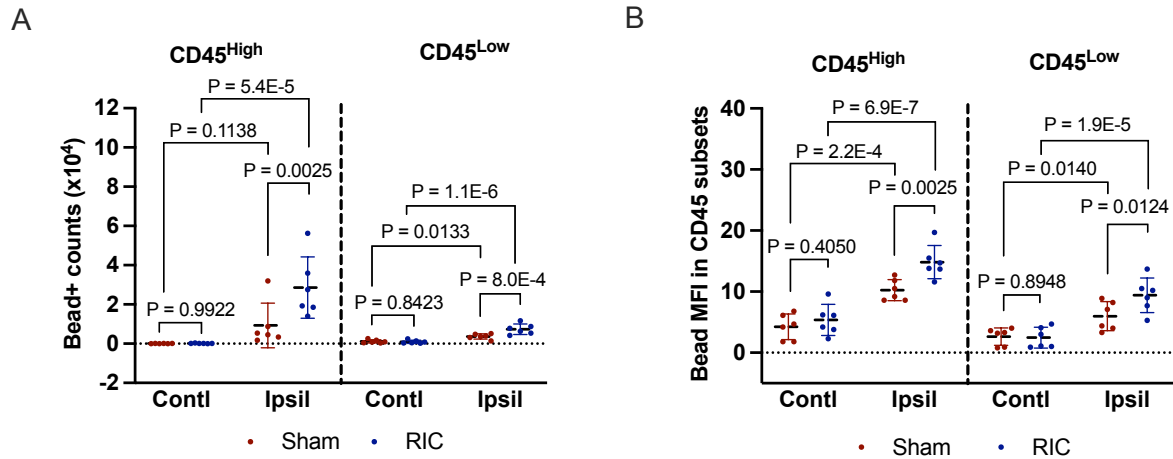


Figure S2. *In vivo* phagocytosis assay.

The effect of RIC on phagocytosis in CD45 subsets in the brain immune cells isolated from 3d post-ischemia.

Brain immune cells were gated for [CD45+/CD11b+/Lin-] Mφ/microglia and assessed for number (A) and

mean fluorescent intensity (MFI) (B) of bead+ cells in CD45^{High} and CD45^{Low} subsets. N=6/group. Analyses

were conducted using the average of triplicate measurements for each animal. Statistical significance was

assessed with two-way ANOVA followed by post hoc Fisher's LSD test. Contl vs. Ipsil (Effect of stroke);

Sham vs. RIC (Effect of RIC).

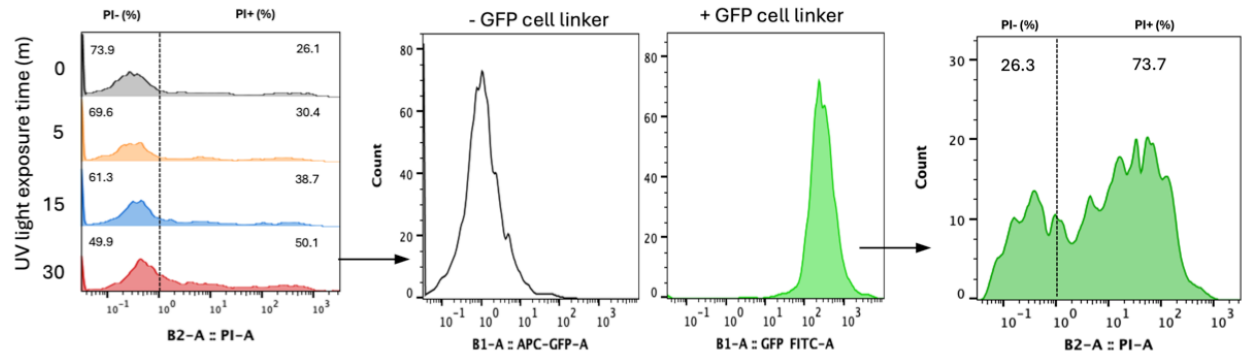


Figure S3. Generation of apoptotic cells (ACs).

Isolated splenocytes incubated under UV light for 30 min cause the half of the cells to become apoptotic (propidium Iodide PI+). Incubation of cells with GFP Fluorescent Cell Linker labels the lipid region of the cell membrane (>99% efficiency). Approximately 70% GFP+ cells are PI+ (AC^{GFP}).

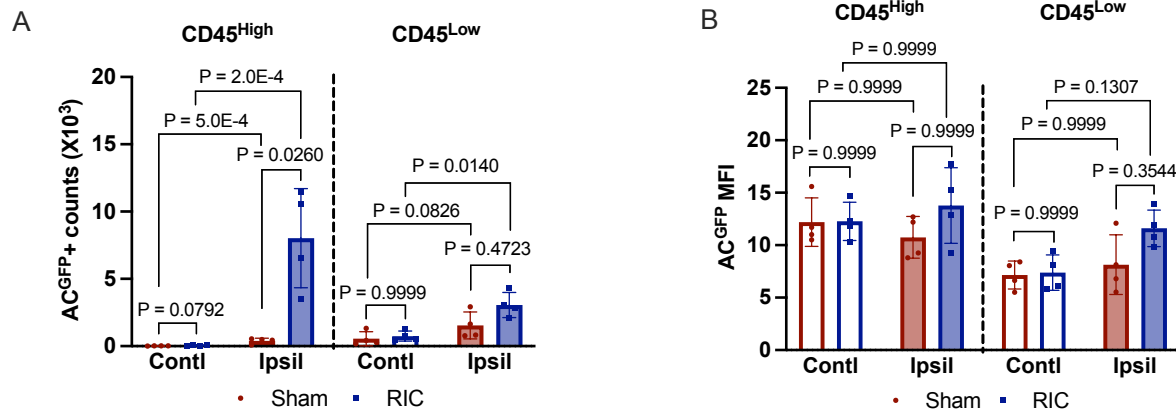


Figure S4. *In vitro* efferocytosis assay.

The effect of RIC on efferocytosis in CD45 subsets assessed in the brain immune cells isolated from 3d post-ischemia. Brain immune cells were gated for Mφ/microglia [CD45⁺/CD11b⁺/Lin⁻] and assessed for number (A) and mean fluorescent intensity (MFI) (B) of AC^{GFP}⁺ cells in CD45^{High} and CD45^{Low} subsets. N=4/group.

Analyses were conducted using the average of triplicate measurements for each animal. Statistical significance was assessed with Scheirer–Ray–Hare (SRH) test followed by the Conover-Iman test for post hoc analyses. ~~with two-way ANOVA followed by post hoc Fisher's LSD test.~~ Contl vs. Ipsil (Effect of stroke); Sham vs. RIC (Effect of RIC).

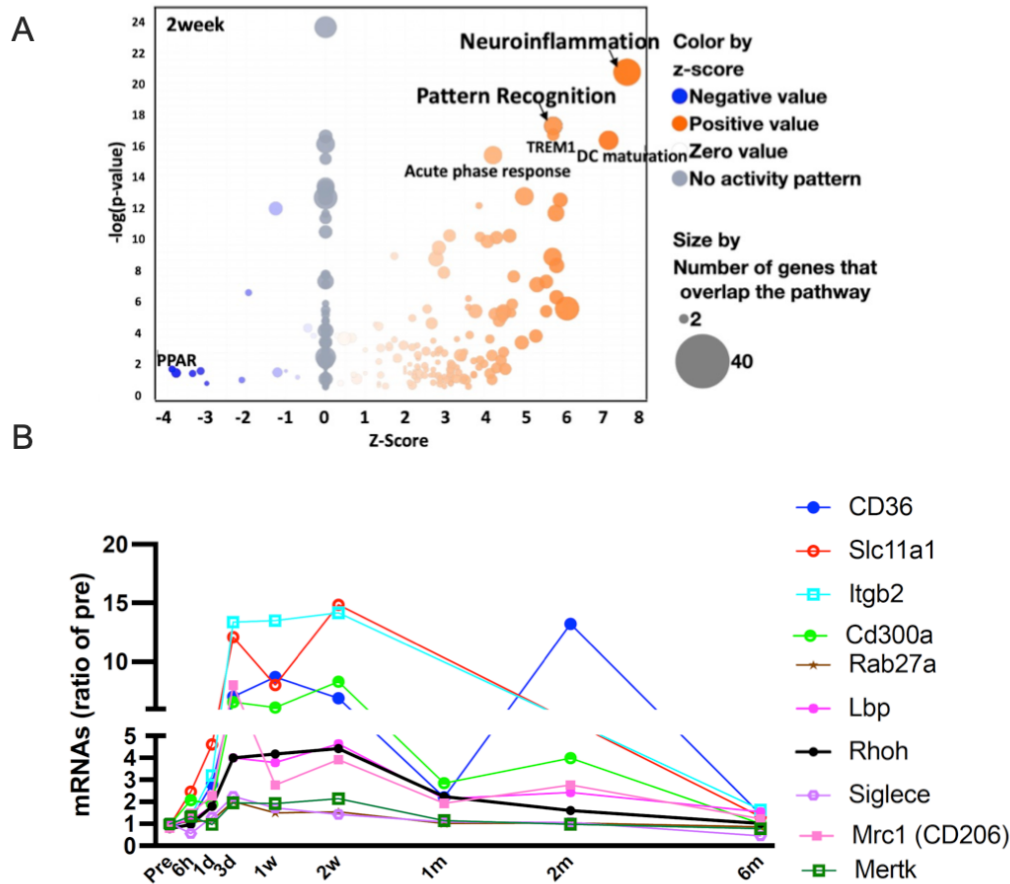
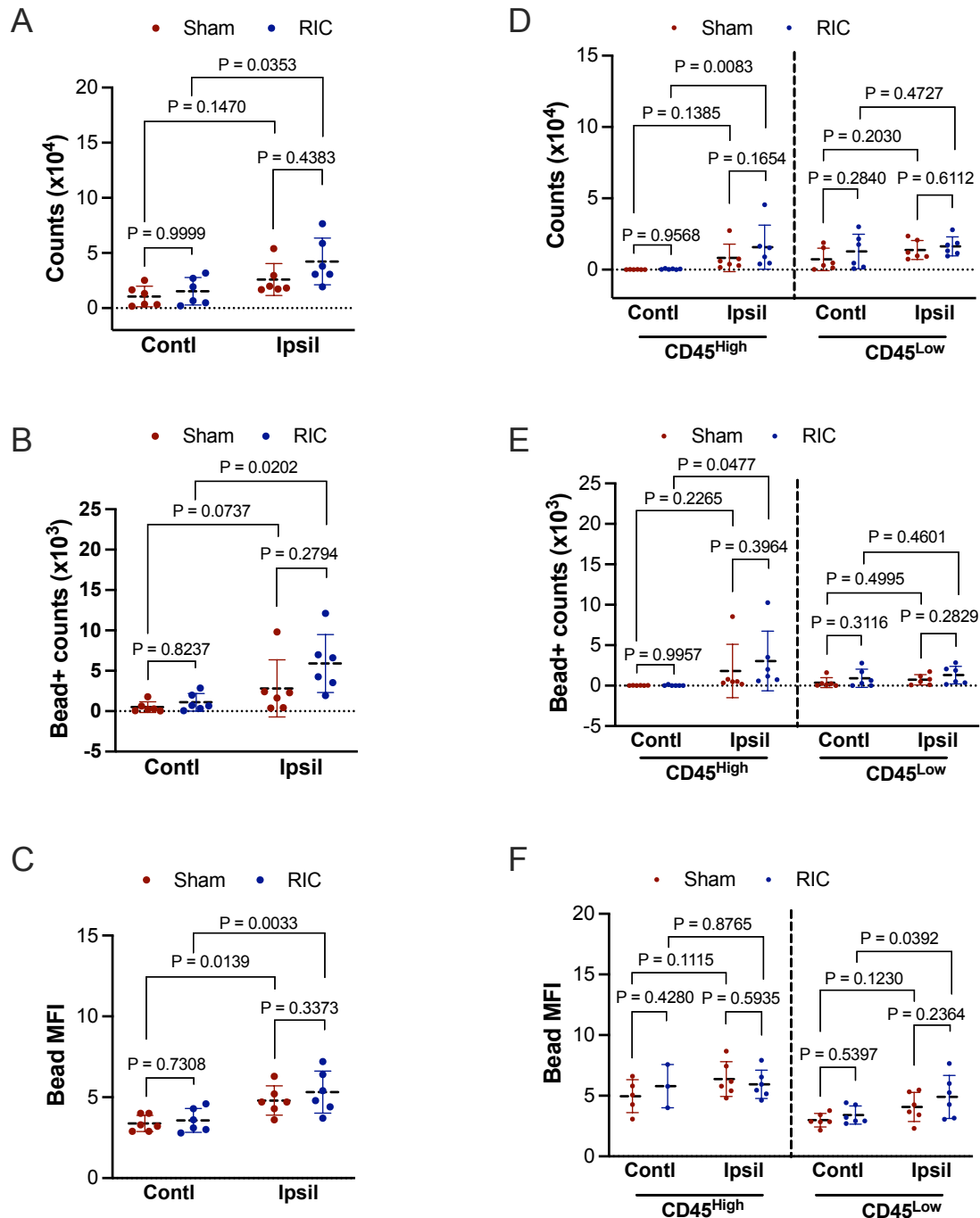


Figure S5. Stroke persistently upregulates the genes pertaining to pattern recognition.

A, Ingenuity canonical pathway analysis. Volcano plot of RNA-seq data at 2w after stroke showed the most upregulated gene signatures are neuroinflammation and pattern recognition. NCBI SRS [National Center for Biotechnology Information Short Read Archive], accession number: PRJNA525413)²⁷ **B**, Sustained upregulation of genes in the ipsilateral hemisphere from an acute phase out to the 6m recovery phase of stroke are related to pattern recognitions.



1

2 **Figure S6. RIC-enhanced phagocytosis does not occur in cells that do not express CD36.**

3 All assessments were performed in [CD45⁺/CD11b⁺/Lin⁻] populations **A**, Quantification of the number of

4 CD36-negative cells. **B**, the number of bead+/CD36- cells, **C**, Bead MFI in CD36- cells, **D**, Quantification of

5 the number of CD36-negative cells in CD45^{High} and CD45^{Low} subsets. **E**, the number of bead+/CD36- cells in

1 CD45 subsets, F, Bead MFI in CD36- cells. N=6/group. Analyses were conducted using the average of
2 triplicate measurements for each animal. Statistical significance was assessed with Scheirer–Ray–Hare
3 (SRH) test followed by the Conover-Iman test for post hoc analyses (A, B) and two-way ANOVA followed by
4 *post hoc* Fisher’s LSD test (C-F). Contl vs. Ipsil (Effect of stroke); no RIC effects are observed. Abbreviations:
5 Contl (contralateral); Ipsil (ipsilateral); Sham (sham conditioning); RIC (remote ischemic conditioning).

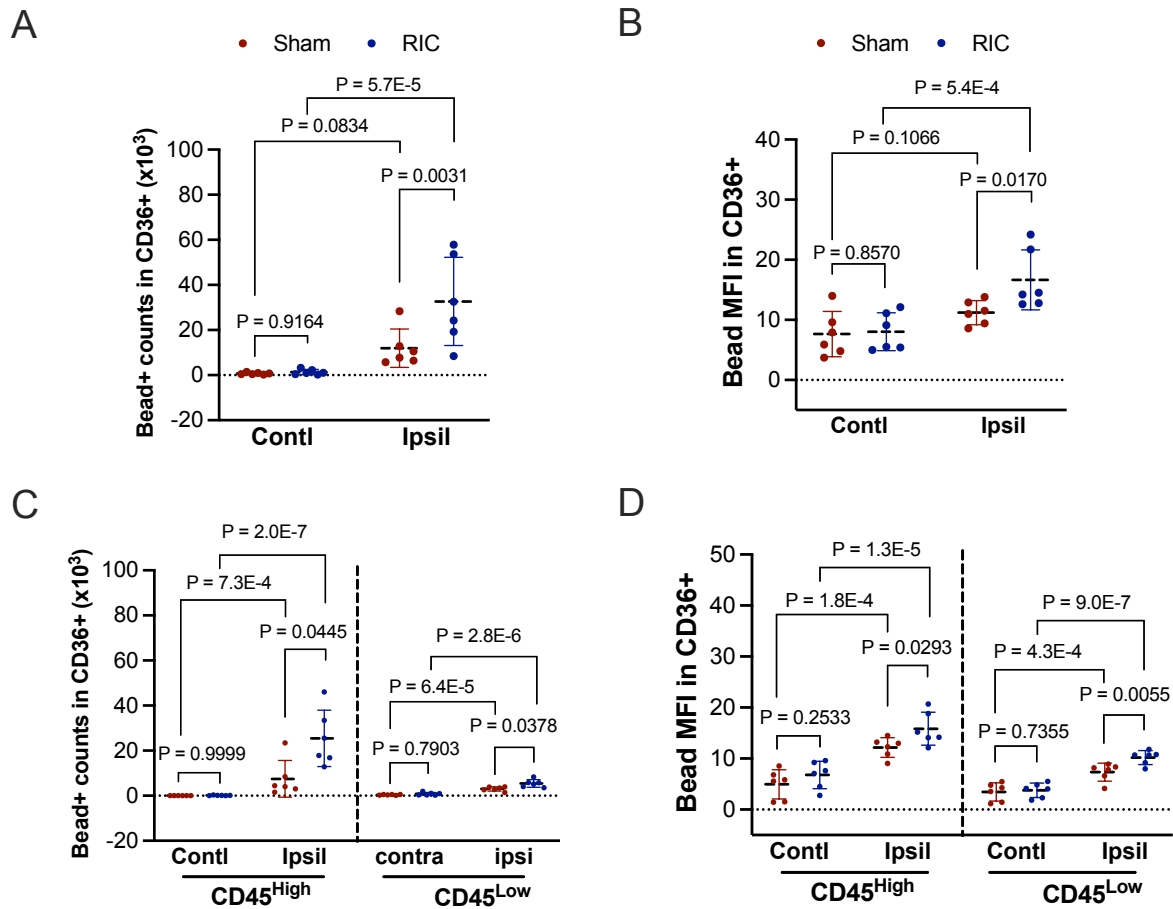


Figure S7. RIC increased the number of beads+ cells and bead intensity in cells that express CD36.

Phagocytosis assays were performed in [CD45⁺/CD11b⁺/Lin⁻] populations. **A, B**, Quantifications of the number of bead+ (A) and bead intensity (B) in CD36⁺ cells. **C, D**, Quantifications of the number of bead+ cells (C) and bead intensity (D) in CD45^{High} and CD45^{Low} subsets. n=6/group. Analyses were conducted using the average of triplicate measurements for each animal. Contl, Contralateral; Ipsil, Ipsilateral; Sham, Sham conditioning; RIC, Remote ischemic conditioning. Statistical significance was assessed with two-way ANOVA followed by *post hoc* Fisher's LSD test (A, B, D) and Scheirer-Ray-Hare (SRH) test followed by the Conover-Iman test for post hoc analyses (C).; Sham vs. RIC (Effect of RIC).

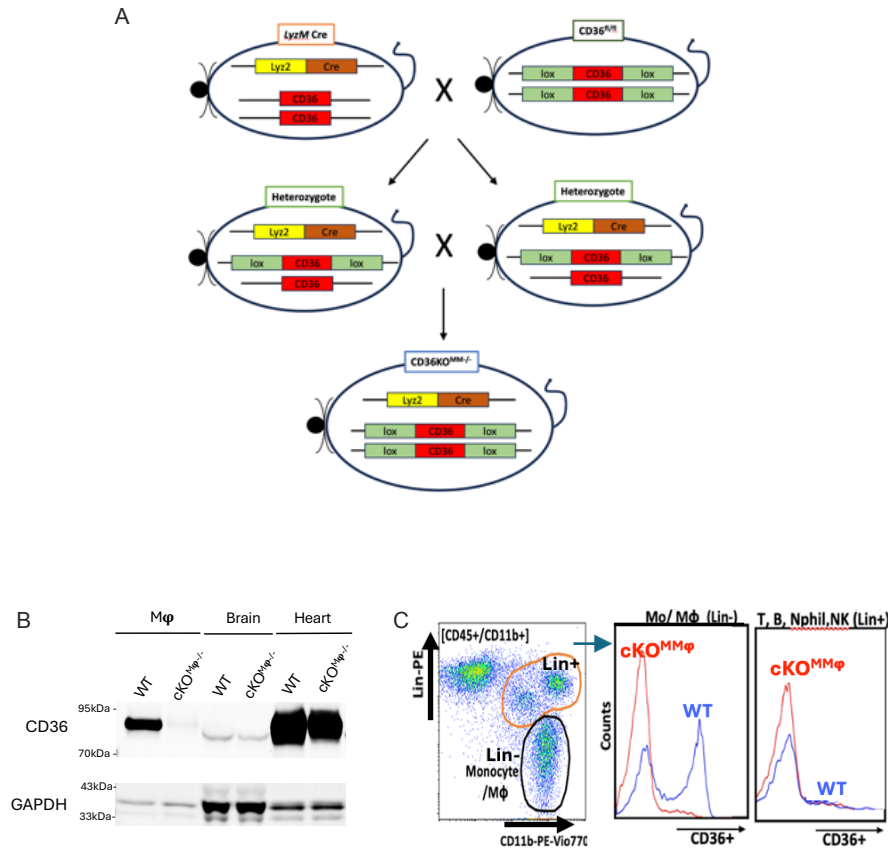


Figure S8. Generation and characterization of cKO^{MMφ} mice.

A, Generation of cKO^{MMφ} mice. LoxP flanked CD36 in C57 background (CD36^{fl/fl}, WT)⁷⁹ were crossed with *LyzM* Cre mice (B6.129P2-*Lyz2*^{tm1(cre)If0}, Jackson Lab) to generate mice with a conditional deletion of CD36 in monocytes/Mφ. **B**, Characterization of cKO^{MMφ} mice. CD36 Western blots in CD36^{fl/fl} (WT) and cKO^{MMφ} mice. Note that cKO^{MMφ} showed a selective CD36 deficiency in Mφ while retaining the expression in the brain (e.g., microvascular endothelial cells) and heart (cardiomyocytes). **C**, Flow cytometry analysis for CD36 expression in the blood. [Lin-] cells in the [CD45+/CD11b+] population for monocyte gating and [Lin+] for T- and B-cells, neutrophils, NK cells. Note that both WT (CD36^{fl/fl}) and cKO^{MMφ} mice display extremely low levels of CD36 expression in T and B lymphocytes, NK cells, and neutrophils with no detectable differences between the genotypes.

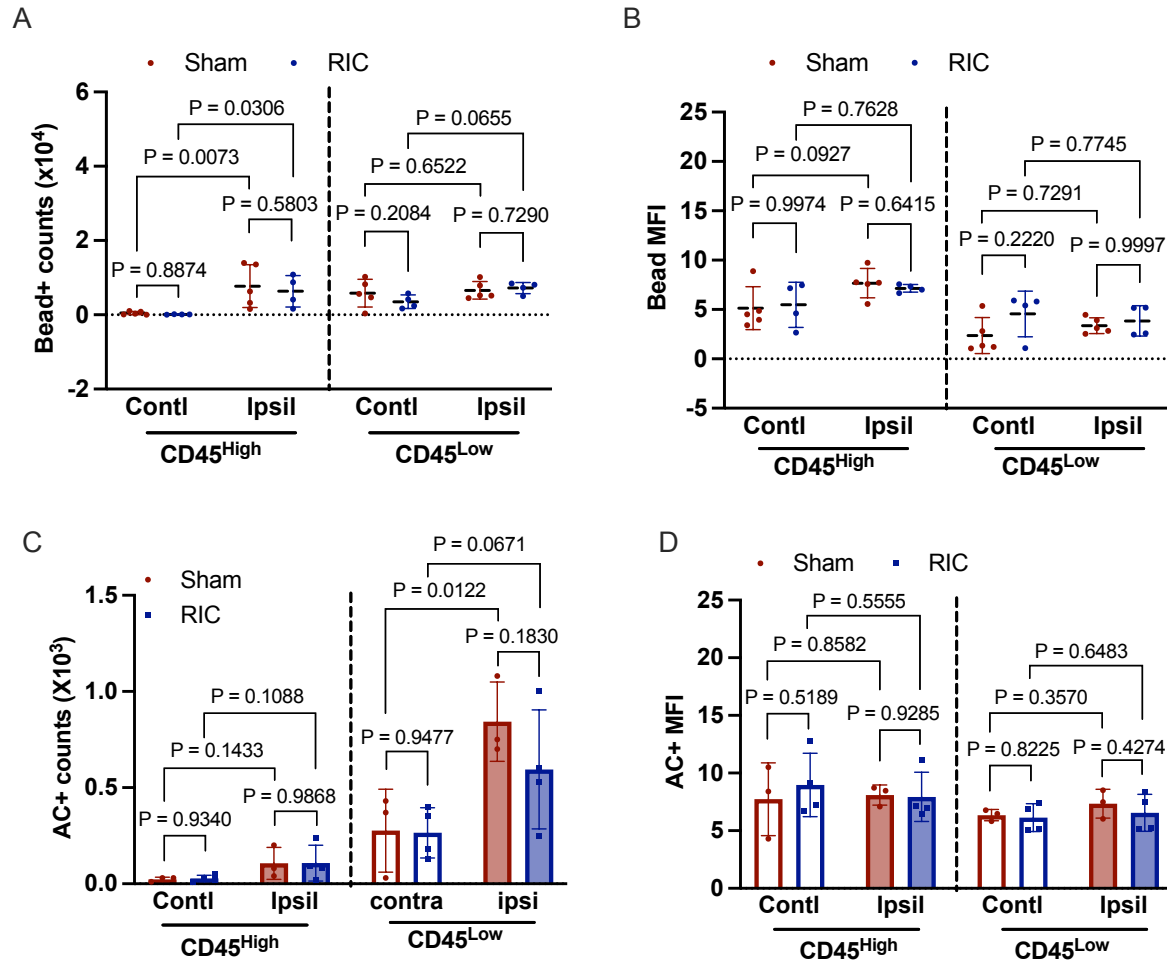
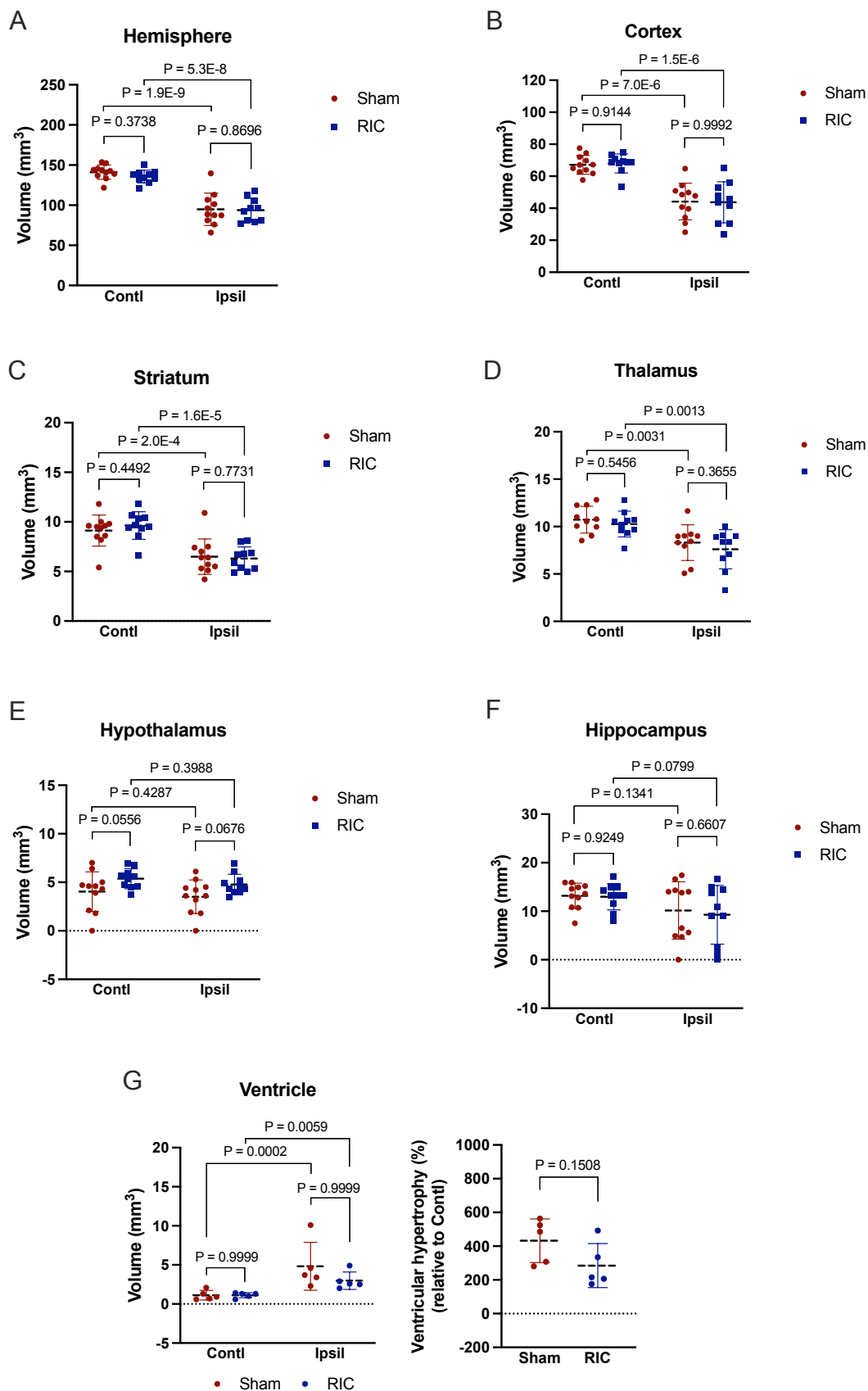


Figure S9. CD36 mediates RIC-enhanced phagocytosis/efferocytosis in the post-ischemic brain

cKO^{MMφ} mice with 30 min MCAO were subjected to Sham or RIC at 2h post-MCAO. **A, B** *In vivo* phagocytosis assay, Number of Beads^{580/605}+ cells (A) and MFI (B) in CD45^{High} and CD45^{Low} Mφs/microglia. (Sham: n=5; RIC: n=4). Each sample was analyzed in triplicate. **C, D**, *In vitro* efferocytosis assay, Number of apoptotic cell-containing (AC+) cells (C) and MFI of AC+ cells (D) in CD45^{High} and CD45^{Low} Mφs/microglia in cKO^{MMφ} mice. (Sham: n=3; RIC: n=4). Analyses were conducted using the average of triplicate measurements for each animal. Contralateral; Ipsil, Ipsilateral; Sham, Sham conditioning; RIC, Remote ischemic conditioning. Statistical significance was assessed with ~~two-way ANOVA followed by post hoc Fisher's LSD test (A, C, D) and~~ Scheirer-Ray-Hare (SRH) test followed by the Conover-Iman test for post hoc analyses ~~(B)~~. Contl vs. Ipsil (Effect of stroke); Sham vs. RIC (Effect of RIC).



1 **Figure S10. Effect of RIC on atrophy of brain sub-regions at 2m post-stroke.** C57 mice were subjected to
2 MCAO and received sham or RIC 2h after MCAO. Volume assessment in the hemisphere (A), primary injury
3 sites (cortex (B) and striatum (C)), and remote areas (thalamus (D) hypothalamus (E) and hippocampus (F)).
4 Sham: n=11; RIC: n=10. The assessment of ventricle volume and percentage of ventricle hypertrophy
5 (Ipsilateral/Contralateral) (G). n=5/group. Statistical significance was assessed with two-way ANOVA
6 followed by *post hoc* Fisher's LSD test (A, C-~~FG~~)~~and~~, Scheirer-Ray-Hare (SRH) test followed by the
7 Conover-Iman test for post hoc analyses (B, G left), and Mann-Whitney U test (G right): Contl vs. Ipsil
8 (Effect of stroke); Sham vs. RIC (Effect of RIC).

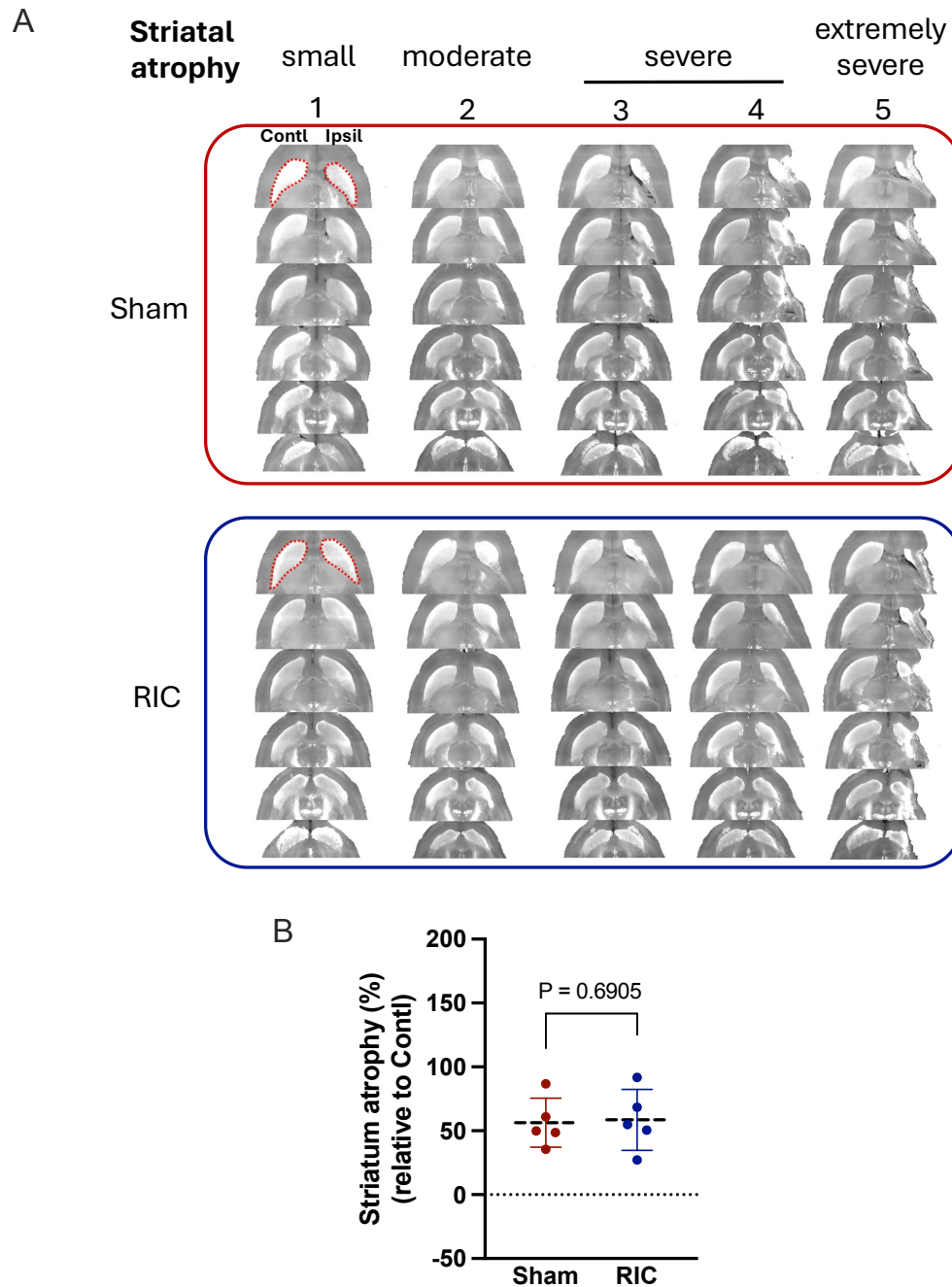
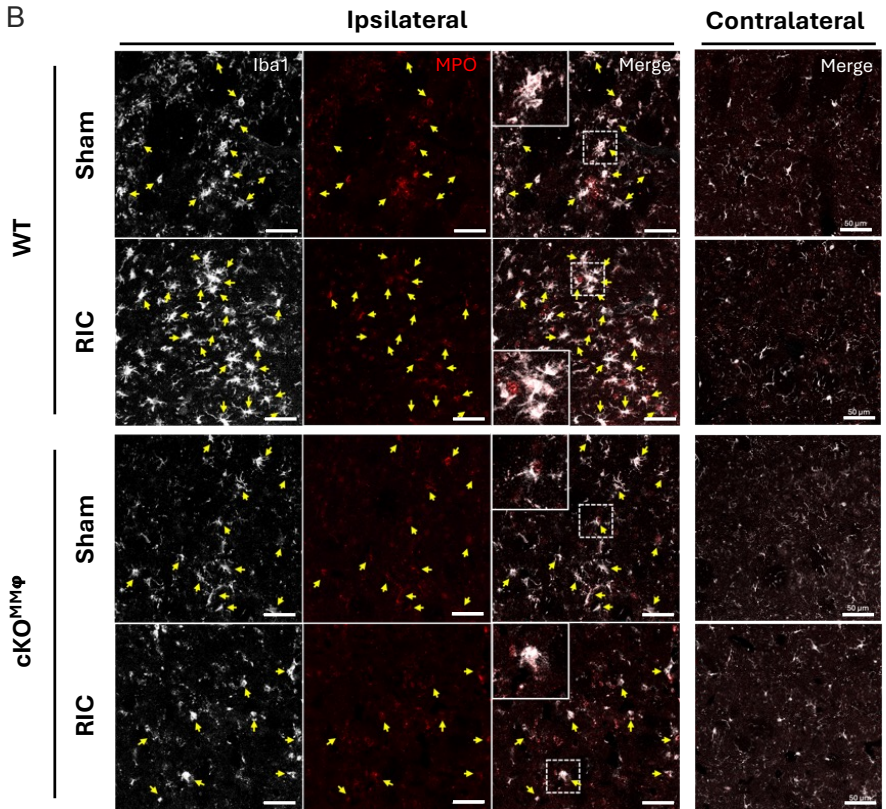
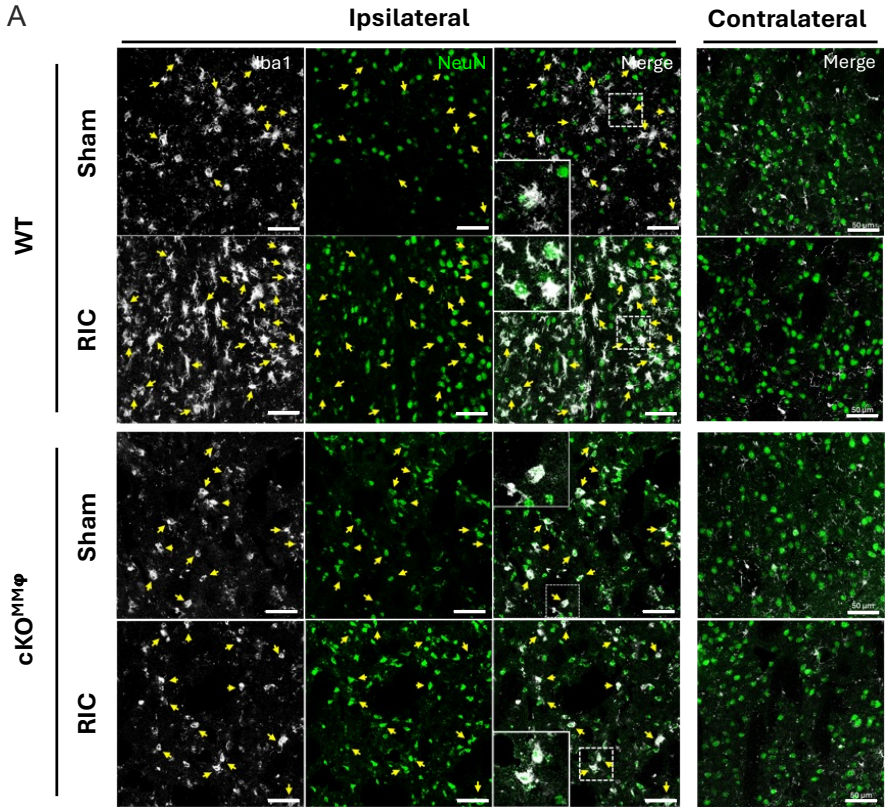


Figure S11. Effect of RIC on striatal injury size. **A**, Horizontal view of serial section images containing the striatum at 2 month post-stroke. Images were captured at 400 um intervals. **B**, Tissue atrophy in the ipsilateral striatum was expressed as percentages of the contralateral striatum. N=5/group. Statistical significance was assessed with Mann-Whitney U test. ~~student's t-test.~~



1 **Figure S12. Effect of RIC on M-Mφ efferocytosis in the post-ischemic brain in WT and cKO^{MMφ} mice.**

2 Representative immunofluorescence images of Iba1+ cells engulfing neurons and neutrophils in the striatum at
3 3d post-MCAO. n=3/group **A**, Efferocytosis of neurons by M-Mφ [NeuN⁺/Iba1⁺] indicated by yellow arrows.
4 **B**, Efferocytosis of neutrophils by M-Mφ [MPO⁺/Iba1⁺] indicated by yellow arrows. Note that RIC increases
5 the number of Iba1+ cells and Iba1+ cells colocalized with NeuN and MPO+ cells in WT mice, not in cKO^{MMφ}
6 mice, Scale bar = 50 μm. All representative images were selected to represent the group average across all
7 available data.