

Supplementary information

Supplementary Materials and Methods

Animals

Healthy C57BL/6J wild-type male (8–10 weeks old, 23–25g) and female (8–10 weeks old, 20–24g) mice, *Trdc*^{CreERT2} mice, *Cd8a*^{flx/flx} mice were purchased from Vital River Laboratory Animal Technology Co Ltd., Beijing, China. *Trdc*^{CreERT2} mice were crossed with *Cd8a*^{flx/flx} mice to generate *Trdc*^{CreERT2} *Cd8a*^{flx/flx} offspring. Tamoxifen (150 mg/kg) was injected intraperitoneally to *Trdc*^{CreERT2} *Cd8a*^{flx/flx} mice for 5 consecutive days before CRS modeling to induce Cre activity in *Trdc*-marked $\gamma\delta$ T cells. Kaede-transgenic (Kaede-Tg) male and female mice (B6. Cg-Gt (ROSA)26Sor<tm1.1(CAG-kikGR) Kgwa>) were generously provided by Dr. Michio Tomura (Osaka Ohtani University, Osaka, Japan). Animals were housed and maintained in standard specific pathogen-free conditions with a 12 hours light/dark cycles in ambient temperature and humidity-controlled, as well as available access to food and water. All the rodent experiments were approved by the committee of experimental animals of Tongji Medical College (Permission number: 4628) and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All experiments were carried out in accordance with the ARRIVE guidelines (Animal Research: Reporting in Vivo Experiments).

Animal model

Chronic restraint stress (CRS) was conducted as previously described [1]. Briefly, mice were individually housed in ventilated 50 mL conical tubes, which were perforated on the sides and bottom to ensure adequate airflow. The animals were restrained for 4 hours daily, from 14:00 to 18:00 (Fig. 1A). During this period, the mice were unable to turn around or move freely, thereby inducing psychological stress without causing pain or physical injury. Control mice remained in their home cages under identical conditions but without physical restraint. Notably, both CRS-exposed and control groups were deprived of food and water during the 4-hour restraint period to minimize potential confounding factors. After each session, all animals were returned to standard housing conditions. Following 4 weeks of CRS exposure, depressive-like behaviors were assessed using a series of validated behavioral tests.

Stereotaxic surgery and injection of AAV

Stereotaxic surgery was performed as we previously described [2]. One day before the CRS procedure, mice were randomly assigned to the CRS + AAV-IL-17A group or the CRS + AAV-control group and received a single hippocampal injection of virus at the specific coordinates. Briefly, mice were anesthetized with a xylazine-ketamine mixture and placed in a stereotaxic apparatus (High Speed Drill & Microinjection Robot, NeuroStar, Germany). Microinjection into the bilateral hippocampus cornu ammonis (CA) 1 region (anteroposterior (AP): ± 2.15 mm; mediolateral (ML): -2.5 mm; dorsoventral (DV): -2.25 mm) was performed using a Hamilton Gastight syringe with a 33-gauge needle (Hamilton, Reno, NV) and a microinjection pump

system. The IL-17A overexpressing adeno-associated virus (AAVs, OBiO Technology, #H443864) were diluted with enzyme-free water to 5×10^{12} v.g./mL, and a volume of 500 nanoliters (nL) AAV mixture was injected into each side of hippocampus at a rate of 100 nL/min. The needle was left in place for additional 5 min before withdrawal to ensure proper diffusion and prevent backflow.

Sucrose preference test (SPT)

After 28 days of CRS or control conditions, mice were individually housed and habituated to two bottles containing 1% sucrose solution for 24 hours to minimize taste neophobia [3]. They were then food- and water-deprived for 8 hours, followed by a 16-hour test period during which they had free access to one bottle of 1% sucrose and one bottle of tap water. Bottle positions were swapped at the 8-hour mark to prevent side bias. Both bottles were weighed before and after the testing period, and sucrose preference for each mouse was calculated as the percentage of sucrose consumption relative to the total fluid intake (sum of water and sucrose intakes).

Open field test (OFT)

Mice were acclimated to the testing room for 2 hours in a quiet and noise-free environment. Immediately prior to each trial, the arena was cleaned with 75% ethanol to remove residual odors. Each mouse was placed gently in the center of the open field and allowed to explore freely for 5 min. An overhead camera recorded locomotor activity, and movement trajectories and total distance traveled were analyzed using TopScan Lite (Clever Sys. Inc.).

Tail suspension test (TST)

Depressive-like behavior was assessed by suspending each mouse by the tail at a height of 50 cm above the floor using adhesive tape. Sessions were recorded for 6 min with an overhead camera. Immobility time during the last 4 min was quantified using TopScan Lite (Clever Sys., Inc.), with immobility defined as movement amplitude < 0.08 .

Forced swimming test (FST)

The FST was carried out in a transparent cylinder specially for mice, which was filled with 23 ± 2 °C of clean water to a depth of about 25 cm. Each mouse was gently placed into the water, and behavior was recorded for 6 min. Immobility during the final 4 min was quantified using TopScan Lite (Clever Sys., Inc.), with immobility defined as a movement amplitude < 0.08 .

16S rRNA gene sequence analysis

DNA extraction: Fresh stool pellets (approximately 100 mg per sample) were collected, and genomic DNA was extracted using the CTAB method following the manufacturer's protocol. DNA quality and concentration were assessed by agarose gel electrophoresis and NanoDrop spectrophotometry, respectively [4].

PCR amplification and sequencing: The V3–V4 regions of the bacterial 16S rRNA gene were amplified using universal primers 341F and 805R. PCR products were verified on a 2% agarose gel, purified with AMPure XT beads (Beckman Coulter Genomics), and quantified using a Qubit fluorometer (Invitrogen). Purified amplicons were pooled, and library size and concentration were measured on an Agilent 2100 Bioanalyzer and with the Kapa Library Quantification Kit for Illumina (Kapa Biosciences), respectively. Libraries were sequenced on an Illumina NovaSeq PE250 platform according to the manufacturer's instructions.

Data analysis: Raw sequencing reads were quality-filtered using fqtrim (v0.94) to generate high-quality clean tags. Chimeric sequences were removed with VSEARCH (v2.3.4), and DADA2 was used for dereplication to produce amplicon sequence variants (ASVs) and an ASV feature table. To control for sequencing depth, each sample was rarefied by random subsampling to the same number of reads. Taxonomic assignment of ASVs was performed against the SILVA database (release 138), and feature abundances were converted to relative abundances per sample. Alpha diversity metrics (Chao1, observed species, ACE, Shannon, Simpson, and Goods coverage) were calculated in QIIME2 to assess community richness and evenness. Beta diversity was evaluated by principal coordinate analysis (PCoA) based on Bray–Curtis distance, with plots generated in R. Representative ASV sequences were aligned using BLAST and annotated against SILVA. Additional visualizations and statistical analyses were performed in R (v3.5.2).

Western blot

Total protein was extracted from tissue samples by homogenization in radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime, #P0013B) supplemented with protease inhibitors (MCE, #HY-K0010). Protein concentration was determined using a BCA protein colorimetric assay kit (Elabscience, #E-BC-K318-M). Equal amounts of protein (based on molecular weight) were separated on 8%, 10%, or 15% SDS-PAGE gels and transferred to 0.45 μ m PVDF membranes (Millipore). Membranes were blocked with 5% skim milk for 2 hours at room temperature, then incubated overnight at 4 °C with primary antibodies: PSD95 (1:1000, Cell Signaling Technology, #3450), synaptophysin (SYN, 1:1000, Proteintech, #17785-1-AP), IL-17A (1:1000, Santa Cruz, #sc-374218), occludin (1:1000, Proteintech, #27260-1-AP), ZO-1 (1:1000, Proteintech, #21773-1-AP), PINK1 (1:1000, Proteintech, #23274-1-AP), parkin (1:1000, Proteintech, #14060-1-AP), P62 (1:1000, Abclonal, #A19700), LC3B (1:1000, Abcam, #T55992), beclin1 (1:1000, Abclonal, #A7353), GAPDH (1:1000, Proteintech, #60004-1-Ig), and β -actin (1:1000, Proteintech, #66009-1-Ig). Membranes were then incubated overnight at 4 °C with goat anti-rabbit IgG (1:5000, Abcam, #ab150077) or goat anti-mouse IgG (1:5000, Abcam, #ab150116), selected according to the host species of the primary antibody. After washing with TBST, bands were visualized using ECL Plus Western Blotting Detection Reagents (Beyotime, #P0018S) and imaged on a UVP gel documentation system (UVP, LLC, Phoenix). Densitometry was performed with ImageJ software (1.8.0, NIH, USA).

Immunofluorescence staining

Mice were anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and perfused transcardially first with 0.9% saline, then with 4% paraformaldehyde (PFA). Brains were carefully harvested and promptly fixed in 4% PFA for 24 hours and cryoprotected in 30% sucrose at 4 °C for 72 hours. Tissues were completely precipitated, the coronal sections of the brain were sliced into 20 µm sections. Wash three times with PBS and incubate in 3% bovine serum albumin (BSA) and 0.3% Triton X-100 at room temperature for 1 hour. Sections were then incubated with primary antibody (LC3B, 1:200, Abcam, #ab192890) overnight at 4 °C. The following day, sections were washed three times in PBS and incubated with another primary antibody (TOMM20, 1:100, Abcam, #ab56783) overnight at 4 °C. After incubation of at least 12 hours, sections were washed three times in PBS as well and incubated with diluted mixed secondary antibody (Alexa Fluor 488-conjugated goat anti-rabbit IgG, 1:500, Abcam, #ab150077; Alexa Fluor 647-conjugated goat anti-mouse IgG, 1:500, Abcam, #ab150115;) at room temperature for 2 hours. Finally, sections were washed, counterstained with DAPI (Fluoromount-G, SouthernBiotech), and coverslipped.

Immediately after perfusion with saline, the small intestine was excised, gently flushed with PBS to remove luminal contents, and fixed in 4% PFA for at least 24 hours. Specimens were embedded in paraffin and sectioned at 5 µm. The sections were deparaffinized in xylene, rehydrated through graded ethanol, and antigen-retrieved in citrate buffer (pH 6.0) at 95 °C for 20 min. After cooling to room temperature, the sections were blocked with 5% BSA and 0.3% Triton X-100 for 1 hour at room temperature, then incubated overnight at 4 °C with primary antibody (MUC2, 1:500, Proteintech, #27675-1-AP). Detection and counterstaining were performed as described above for brain sections.

Enzyme-linked immunosorbent assay (ELISA)

Hippocampal tissue was homogenized in ice-cold PBS containing protease inhibitors, and centrifuged at 3000 × g for 10 min at 4 °C. Supernatants were carefully collected and stored at –80 °C until analysis. The levels of IL-17A in the hippocampus were quantified using a commercial ELISA kit (NeoBioscience, #EMC 008.96) following the manufacturer's protocol.

Golgi staining

Golgi impregnation and analysis were performed as previously described [5]. Mice were deeply anesthetized, and brains were removed and rinsed briefly in distilled water. Golgi staining was performed using the FD Rapid Golgi StainTM Kit (FD Neurotechnology, #PK401) according to instructions of manufacturer. Brains were first immersed in an equal-volume mixture of Solution A and B overnight, then transferred to fresh impregnation solution for 15 days at 4 °C. Samples were subsequently placed in Solution C for 7 days. Coronal sections (100 µm) were cut on a cryomicrotome, air-dried, and processed according to the kit protocol. Dendritic images in the hippocampus were captured by light microscopy, and spine density and number were quantified using ImageJ (v1.8.0, NIH).

Transmission electron microscopy (TEM)

Mice were deeply anesthetized, and brains were removed. Hippocampal tissue was dissected into 1 mm³ dices and fixed in 2.5 % glutaraldehyde solution overnight at 4 °C. Samples were then post-fixed in 1% osmium tetroxide solution for 2 hours, dehydrated through a graded ethanol series, and infiltrated and embedded in epoxy resin. Resin blocks were polymerized at 60 °C for 48 hours. Ultrathin sections (60–70 nm) were cut on an ultramicrotome, mounted on copper grids, and stained with uranyl acetate followed by lead citrate. Sections were examined with a Hitachi transmission electron microscope (Tokyo, Japan), and representative micrographs were captured.

Evans blue dye permeability assay

Mice received an intravenous injection of 2% Evans Blue dye (4 mL/kg; Biofroxx #2277GR-001) via the tail vein. After 3 h, animals were deeply anesthetized and perfused transcardially with 0.9% saline to remove intravascular dye. Brains and small intestines were harvested, blotted to remove surface moisture, and weighed. Tissues were then homogenized in 1 mL of *N, N*-dimethylformamide (Sigma-Aldrich #D4551), incubated at 60 °C overnight, and centrifuged at 12000 × *g* for 20 min. The absorbance of the supernatant was measured at 620 nm. Evans Blue dye extravasation was calculated as follows: Evans Blue extravasation (μg/g) = (Evans Blue concentration (μg/ml) × 1 ml)/tissue wet weight (g) [6]. The concentration of Evans blue dye within samples were obtained from the standard curve.

Flow cytometry analysis

Flow cytometry analysis was performed following an established protocol [7, 8]. Mice were anesthetized and perfused with at least 20 mL of cold PBS through the heart. Brains, meninges and small intestines were collected and kept on ice. For the small intestines, after removing the mesangial and adipose tissues, it were longitudinally opened and carefully washed in PBS, cut into 1 mm sections, added to 20 ml of pre-digestion solution (containing HBSS with 10 mM HEPES, 8% fetal bovine serum (FBS), 4 mM EDTA, and 0.5 mM dithiothreitol), and incubated in a constant temperature shaker at 37 °C at 200 rpm/min for 10 min. Repeat once, and washed in PBS to remove the residual EDTA, then discard the supernatant, and retain the tissue sediment. Thereafter, the tissue pieces were finely chopped and digested in RPMI-1640 medium containing 10% FBS, collagenase IV (1 mg/mL), and DNase I (0.1 mg/mL) in a constant temperature shaker at 37 °C for 40 min. After digestion, the tissue suspensions were filtered through a 100 μm cell strainer to obtain the single-cell suspension and followed by a centrifugation at 2000 rpm for 10 min, which was washed once with PBS. Then the cell pellet was resuspended in 8 ml of 40% Percoll solution, and carefully spread on the upper layer of 4 ml of 80% Percoll solution. The sample was centrifuged at 2000 rpm for 5 minutes, and the middle layer of immune cells was aspirated and washed once with PBS. For the meninges, place it on a 70 μm filter, add 4 mL of digestion solution (with the same components as for the small intestine) and use the tip of a 1 mL syringe plunger to thoroughly grind the tissue. For the brain tissue, it was cut into pieces

thoroughly using sharp scissors, and added to 5 mL digestion solution (DMEM medium containing 10% FBS, 1 mg/mL collagenase IV, and 0.5 mg/mL DNase I). Next, the digestion solution containing tissues were incubated in a constant temperature shaker at 37 °C at 200 rpm/min for 40 min. After digestion, the tissue suspensions were filtered through a 100 µm cell strainer to obtain the single-cell suspension and followed by a centrifugation at 2000 rpm for 10 min, which was washed once with PBS. Then the cerebral cell pellet was resuspended in 7 ml of 40% Percoll solution, and centrifuged at 2000 rpm/min for 20 min. Then remove all the upper cells and the supernatant. The bottom cell pellet is washed once with cold PBS.

The cell precipitates were collected and resuspended in 100 µL PBS for subsequent antibody labeling. For the samples that require IL-17A or IFN-γ staining, the cell precipitate was counted as 1×10^6 and resuspended with 500 µl of complete medium that containing 10% FBS and 1% Penicillin-Streptomycin solution (Procell, #PB180120) and added to a 48-well cell culture plate, with 1 µl of cell activation cocktail (Biolegend, #423304) added to each well. Collect the cells after 6 hours of incubation for subsequent staining. The following antibodies were used for extracellular staining: fixable viability dye (Biolegend, #423102), CD45 (Biolegend, #103132), CD3e (BD Biosciences, #552774), γδ TCR (Biolegend, #118116), CD8a (Biolegend, #100765), IL-7R (Biolegend, #135025), CD11b (BD Biosciences, #557960), CD4 (BD Biosciences, #557307). After surface staining with fluorescently labeled antibodies, the cells were subsequently permeabilized using the Transcription Factor Buffer Set (Biolegend, #562574) adhering to guidelines provided by the manufacturer and stained for IL-17A (Biolegend, #506926) and IFN-γ (BD Biosciences, #554411). Samples were analyzed on a CytoFLEX flow cytometer (Beckman Coulter, USA). Data were analyzed using FlowJo 10.0 software (FlowJo, Oregon, USA).

Tandem mass tag labeling (TMT) quantitative proteomics

Protein extraction and TMT labeling: Three hippocampal samples per group were processed by Shanghai Biotree Biotech Co., Ltd. (Shanghai, China). Tissue was lysed in RIPA buffer with sonication, and protein concentration was determined by BCA assay. Aliquots of 100 µg protein were diluted to 1 mg/mL in water, precipitated overnight at -20 °C with five volumes of cold acetone, and pelleted at 12000 rpm for 10 min. Pellets were dissolved in buffer (100 mM HEPES, 1% SDS), reduced with 5 mM DTT at 55 °C for 20 min, and alkylated with 15 mM iodoacetamide in the dark for 30 min. Samples were digested with trypsin (1:50 enzyme: protein), and resulting peptides were labeled using a TMT kit according to the manufacturer's instructions.

LC-MS/MS acquisition: For each TMT-labeled sample, 200 ng of peptide was separated on a PePSep C18 reversed-phase column (1.9 µm, 75 µm × 15 cm) using a nanoElute² UPLC coupled to a timsTOF Pro2 instrument (Bruker). Data-dependent acquisition was performed in PASEF mode with a nano-electrospray source.

Data processing and bioinformatics: Raw spectra were analyzed in SpectroMine (4.1.230421.52329) using Sequest HT against the reviewed *Mus musculus* UniProt database

(2022_11 release). The false discovery rate (FDR) was set to 0.01 for both PSM and peptide levels. Peptide identification was performed with an initial precursor mass deviation of up to 10 ppm and a fragment mass deviation of 0.02 Da. Unique peptide and Razor peptide were used for protein quantification and total peptide amount for normalization. Proteins exhibiting a fold change (FC) ≥ 1.2 or ≤ 0.83 , and with a *P* value < 0.05 were identified as differentially expressed proteins (DEP). Functional annotation and pathway enrichment were conducted using Gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Clusters of Orthologous Groups of proteins (COG) databases. Gene Set Enrichment Analysis (GSEA) was performed in R packages to identify biological processes impacted by CRS.

Adenosine triphosphate (ATP) assay

Hippocampal ATP levels were measured using the ATP assay kit (Beyotime, #S0026) according to the manufacturer's instructions. Tissue was lysed in the kit's lysis buffer and centrifuged at 12000 g for 5 min at 4 °C. Supernatant were collected, and protein concentration was determined by BCA assay. For each sample, an aliquot containing equal protein (as determined by BCA) was mixed with 100 μ L of ATP detection reagent and incubated briefly to ensure complete reaction. Luminescence was measured on a plate luminometer, and ATP concentration was calculated from a standard curve, then normalized to protein content and expressed as nmol ATP per mg protein.

Micro-PET/CT imaging

Mice were fasted for 6 hours to stabilize glucose levels before receiving an intravenous injection of [^{18} F]-fluorodeoxyglucose (FDG: 15.6 ± 1.7 MBq in 0.5 mL saline). After injection, animals rested in a warm, quiet environment for 45 min to promote tracer uptake and minimize muscle activity. Mice were then anesthetized with isoflurane and scanned on a high-resolution micro-PET/CT system covering the entire brain. A 10 min CT scan provided anatomical reference, followed by a PET acquisition to assess glucose metabolism. Images were processed in Inveon Research Workplace software to calculate the standardized uptake value (SUV)—the mean [^{18} F]-FDG concentration normalized to injected dose and body weight—for quantitative analysis of brain metabolic activity [2].

Statistical analysis

Data were expressed as mean $\pm$ standard deviation (SD) and analyzed using GraphPad Prism 8 (La Jolla, California, USA), with *P* < 0.05 considered statistically significant. Heatmap in Fig. 2F and 4B were constructed and visualized using ChiPlot (<https://www.chiplot.online/>). Normality distribution of the data was determined by D'Agostino & Pearson test (used when *n* ≥ 8) or Shapiro-Wilk test (used when *n* < 8). For comparisons between two groups, unpaired two-tailed Student's t-test was used for data satisfying normality and homogeneity of variance; otherwise, the Mann-Whitney U test was applied. For comparisons among three or more groups, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for parametric data.

280 For data that did not meet the assumptions of parametric testing, the Kruskal-Wallis test
281 followed by Dunn's post hoc test was employed. Correlations were analyzed by Pearson
282 correlation. Animals unable to complete behavioral assays (e.g., risk of drowning in the FST or
283 escape during the TST) were excluded from analysis.

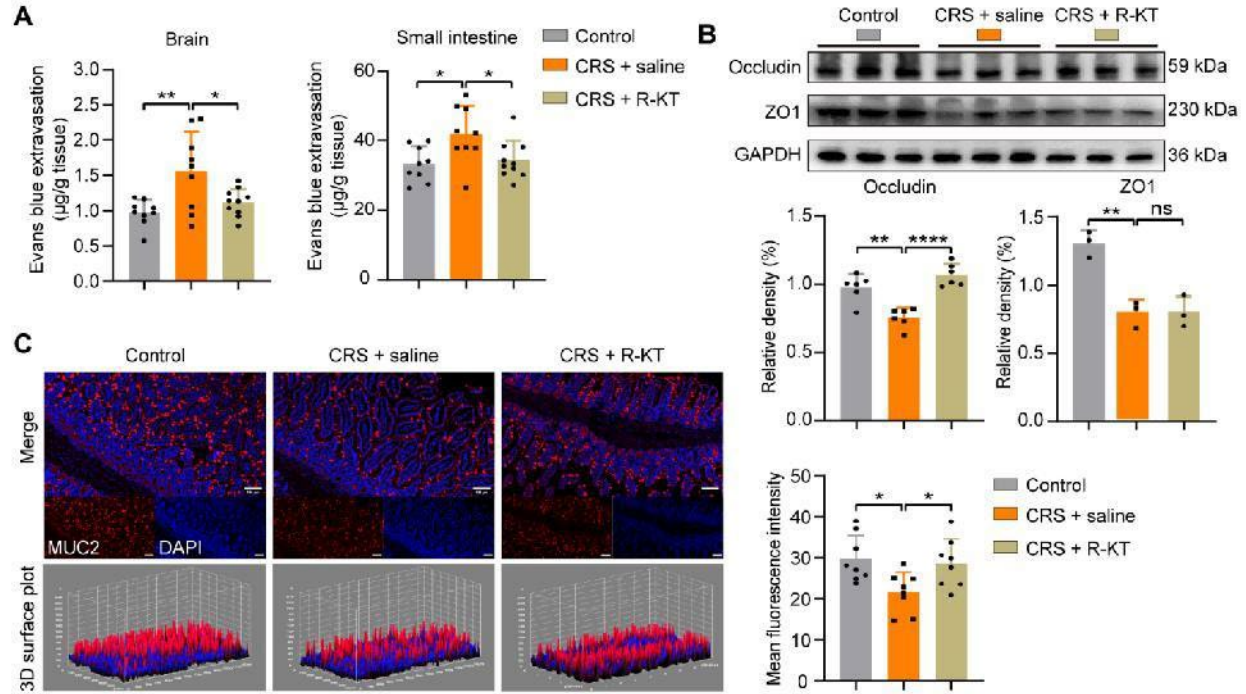


Fig. S1. Arketamine alleviates CRS-induced intestinal barrier disruption.

A Quantification of Evans blue extravasation in the small intestine and brain (n = 9–10). **B** Western blot analysis and quantification of intestinal tight junction proteins, including occludin and zonula occludens-1 (ZO-1) (n = 3–6). **C** Immunofluorescence staining images and 3D surface plots for small intestinal mucin-2 (MUC2) and nuclear counterstaining with 4',6-diamidino-2-phenylindole (DAPI) (n = 8). Scale bars: 100 μm. Data are presented as individual values plus mean ± SD. **P* < 0.05, ***P* < 0.01, *****P* < 0.0001; ns, not significant. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison tests.

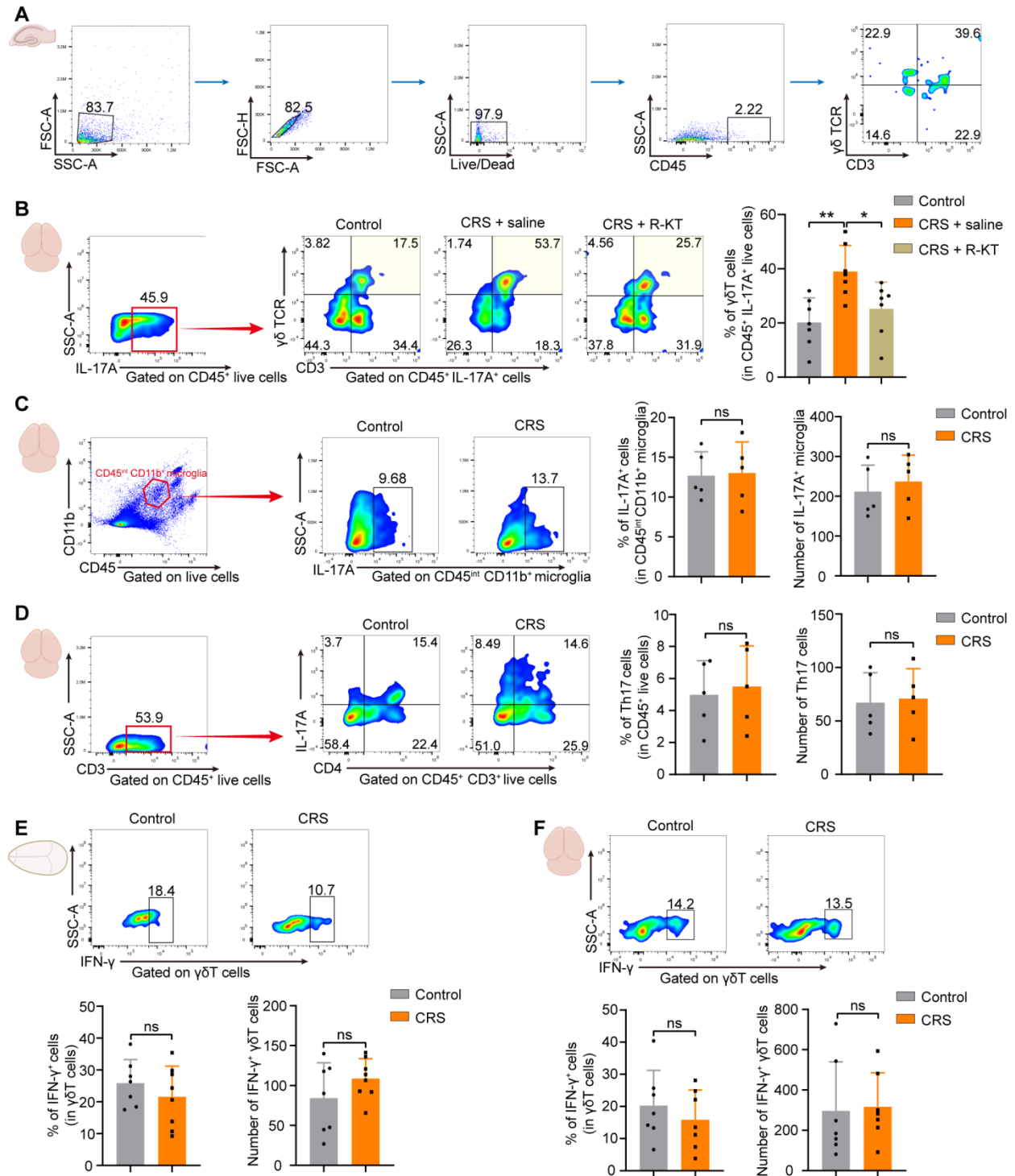


Fig. S2. The predominant increase in IL-17A secretion within the brain was observed in $\gamma\delta$ T cells following CRS treatment.

A FCM gating strategy for $\gamma\delta$ T cells and representative FCM plots of $\gamma\delta$ T cells in the hippocampus ($n = 3$). The bilateral hippocampi from three mice were pooled into one sample for flow cytometry analysis; however, the number of $CD45^+$ cells remained insufficient for detailed $\gamma\delta$ T cell analysis. **B** Representative FCM plots and statistical graphs illustrating the proportion

of $\gamma\delta$ T cells among IL-17A-secreting cells in the brain ($n = 6-7$). **C** Representative FCM plots, percentages, and absolute numbers of IL-17A expression in CD45^{int} CD11b⁺ microglia in the brain ($n = 5$). **D** Representative FCM plots, percentages, and absolute numbers of Th17 cells in the brain ($n = 5$). **E** Representative FCM plots depicting the expression level of interferon (IFN)- γ in meningeal $\gamma\delta$ T cells, along with statistical graphs showing the proportion of IFN- γ -expressing $\gamma\delta$ T cells and the number of IFN- γ ⁺ $\gamma\delta$ T cells in the meninges ($n = 7-8$). **F** Representative FCM plots depicting the expression level of IFN- γ in cerebral $\gamma\delta$ T cells, along with statistical graphs showing the proportion of IFN- γ -expressing $\gamma\delta$ T cells and the number of IFN- γ ⁺ $\gamma\delta$ T cells in the brain ($n = 7$). Data are presented as individual values plus mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; ns, not significant. Statistical analysis using one way ANOVA followed by Tukey's multiple comparison tests (B), unpaired t tests (C-F).

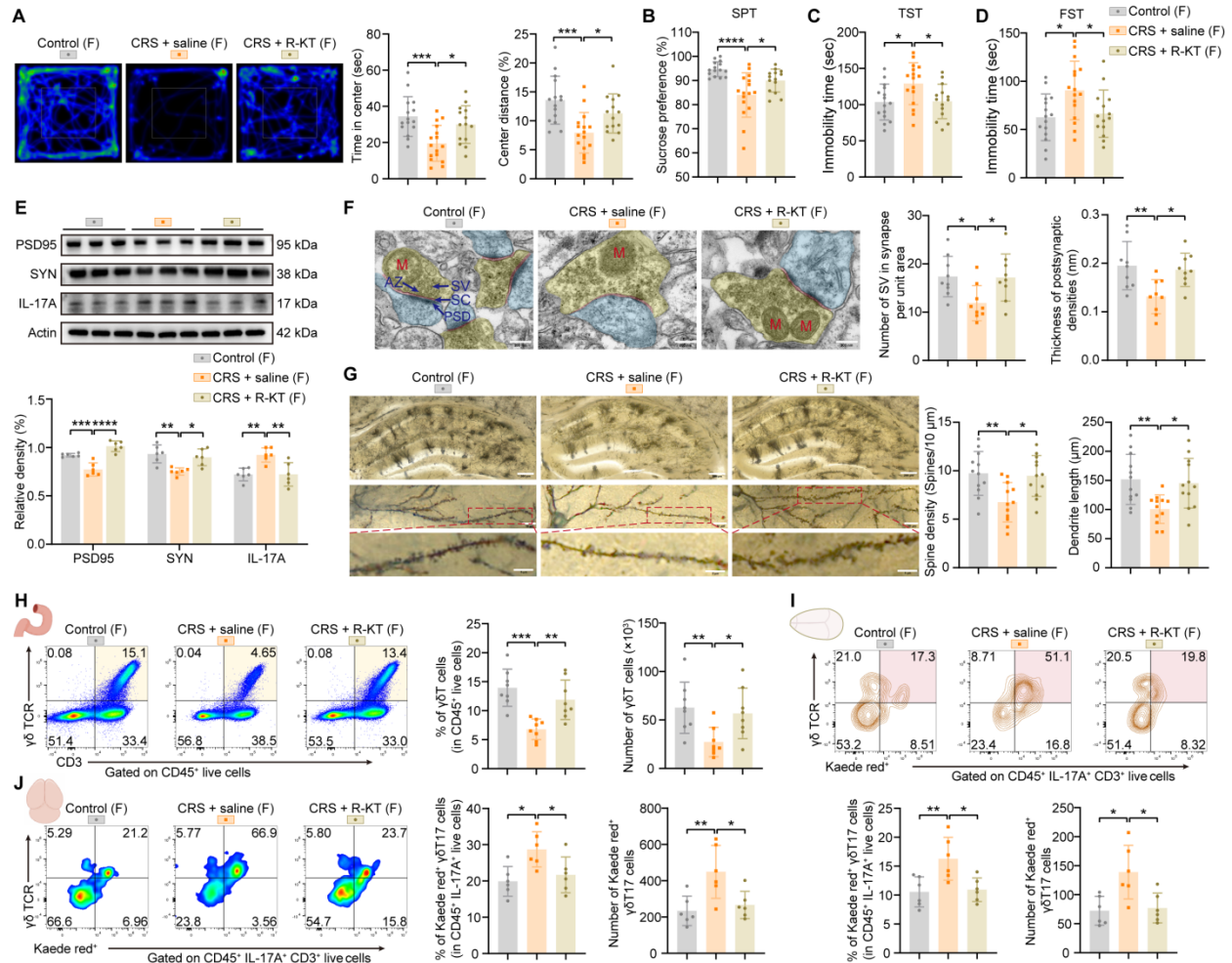


Fig. S3. Arketamine alleviates CRS-induced depressive-like behaviors, synaptic damage and infiltration of gut-derived γδ T17 cells in female mice.

A Open field test (OFT, n = 14–16). **B** Sucrose preference test (SPT, n = 14–16). **C** Tail suspension test (TST, n = 14–16). **D** Forced swimming test (FST, n = 14–16). **E** Western blot analysis and quantification of postsynaptic density protein 95 (PSD95), Synaptophysin (SYN) and IL-17A levels in the hippocampus of female mice (n = 6). **F** Transmission electron microscopy (TEM) images of synapses in the hippocampus and quantification of synaptic vesicle number and postsynaptic density thickness (n = 9). The red "M" indicates mitochondria. Scale bar = 200 nm. **G** Representative images and summary statistics of spine density and dendrite length analyses of hippocampal neurons (n = 12). Scale bar: 200 μm (top), 10 μm (middle), 5 μm (bottom). **H** Representative FCM plots, percentages, and quantification of γδ T cells in the small intestine (n = 8). **I** Representative FCM plots show Kaede red⁺ γδ T17 cells in the meninges of Kaede female mice. Statistical graphs represent the percentage of Kaede red⁺ γδ T17 cells among CD45⁺ IL-17A⁺ immune cells (calculated as (CD3⁺/CD45⁺ IL-17A⁺) × (Kaede red⁺ γδ TCR⁺/CD45⁺ IL-17A⁺ CD3⁺) × 0.01) and the absolute number of Kaede red⁺ γδ T17 cells in the meninges (n = 6). **J** Representative FCM plots show Kaede red⁺ γδ T17 cells in the brain of Kaede female mice. Statistical graphs depict the percentage and number of Kaede red⁺ γδ T17

329 cells in the brain ($n = 6$). Data are presented as individual values with mean $\pm$ SD. $^*P < 0.05$, $^{**}P$
330 < 0.01 , $^{***}P < 0.001$, $^{****}P < 0.0001$; ns, not significant. Statistical analyses were conducted using
331 one-way ANOVA followed by Tukey's multiple comparisons tests. SV, synaptic vesicle; SC,
332 synaptic cleft; AZ, active zone.

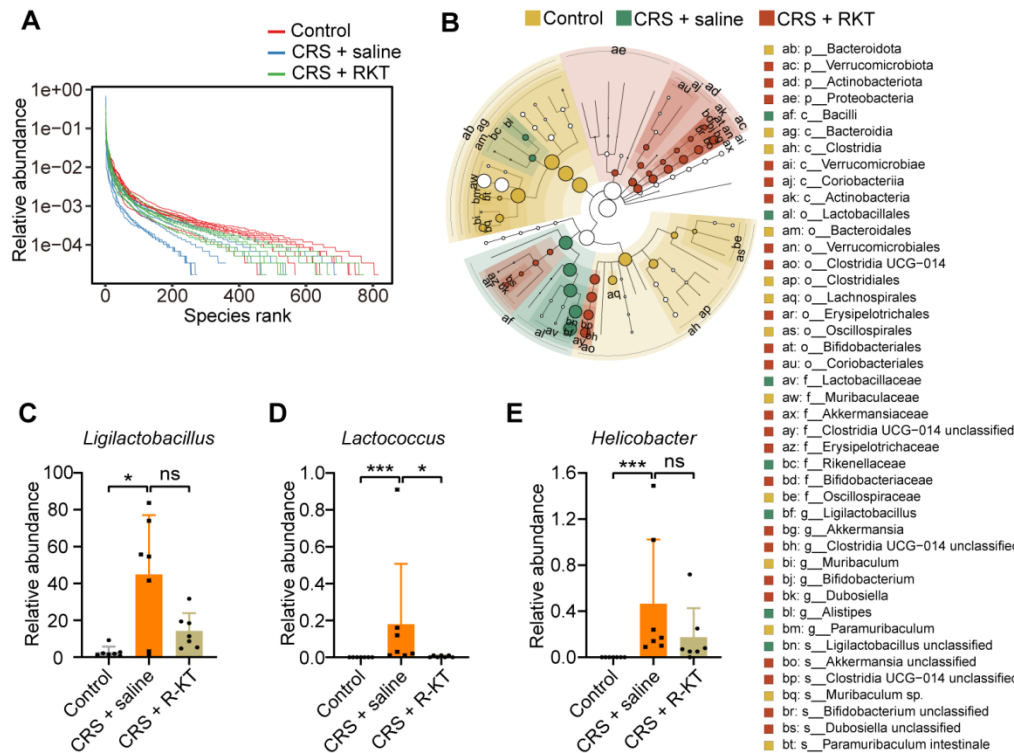


Fig. S4. Arketamine reverses CRS-induced gut microbiota alterations.

A Rank-abundance curves for individual fecal samples, illustrating microbial richness and evenness across groups (n = 7). **B** Functional branching diagram generated using the linear discriminant analysis effect size (LEfSe) algorithm, highlighting differences among the three groups at various taxonomic levels. Each successive circle represents differentially abundant taxonomic clades from the inner to outer rings, corresponding to the phylum, class, order, family, genus, and species levels. Relative abundance at the genus level: *Ligilactobacillus* (**C**), *Lactococcus* (**D**), and *Helicobacter* (**E**) among the three groups (n = 7). Data are presented as individual values plus mean $\pm$ SD. * P < 0.05, *** P < 0.001; ns, not significant. Statistical analysis was conducted using Kruskal-Wallis with Dunn's multiple-comparison test (C, D, E).

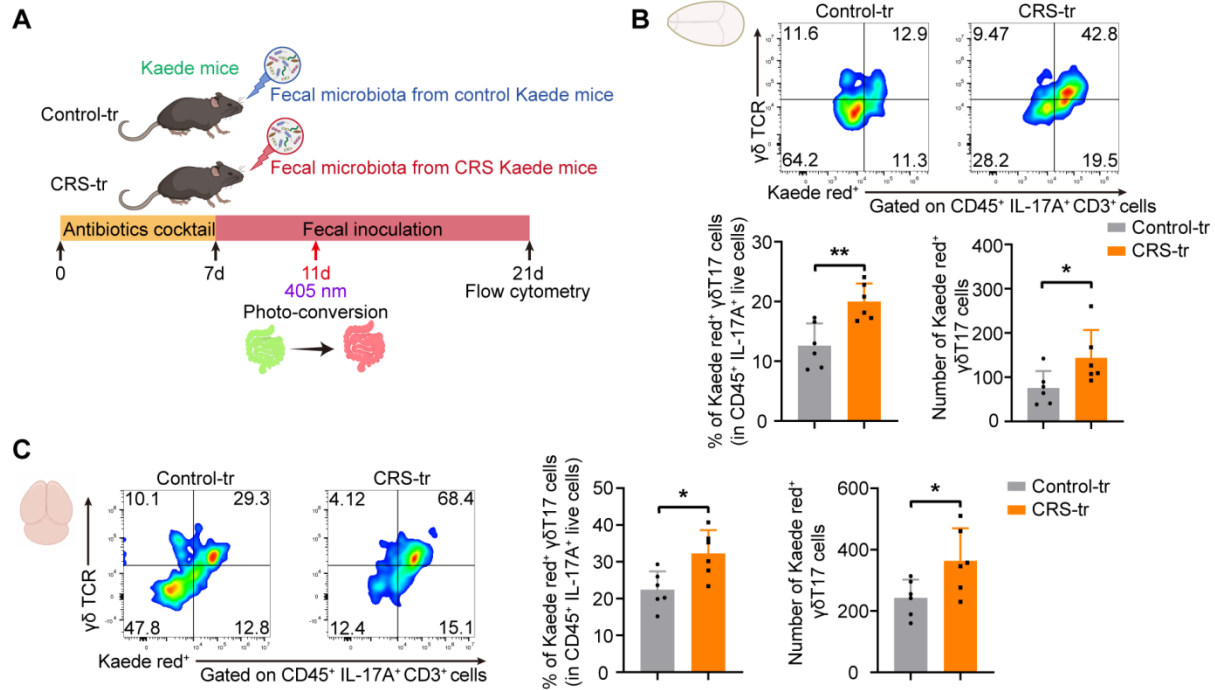


Fig. S5. Gut microbiota from CRS mice drive migration of small intestinal $\gamma\delta$ T cells to the meninges and brain.

A Experimental schematic. Kaede-transgenic mice were treated with an antibiotic cocktail in drinking water to deplete resident microbiota. Mice then received oral gavage of fecal suspensions from CRS or control donors (200 μ L every other day for 14 days). Ten days prior to tissue collection, the small intestine was exposed to 405 nm UV light to photoconvert Kaede-expressing cells. **B** Representative FCM plots of meningeal Kaede red⁺ $\gamma\delta$ T17 cells and statistical graphs showing the frequency (calculated as $(CD3^+/CD45^+ IL-17A^+) \times (Kaede\ red^+ \gamma\delta\ TCR^+/CD45^+ IL-17A^+ CD3^+) \times 0.01$) and absolute number of Kaede red⁺ $\gamma\delta$ T17 cells in the meninges of Kaede mice (n = 6). **C** Representative FCM plots, frequency, and absolute number of Kaede red⁺ $\gamma\delta$ T17 cells in the brain of Kaede mice (n = 6). Data are presented as individual values plus mean $\pm$ SD. * P < 0.05, *** P < 0.001; ns, not significant. Statistical analysis was conducted using unpaired t test (B, C).

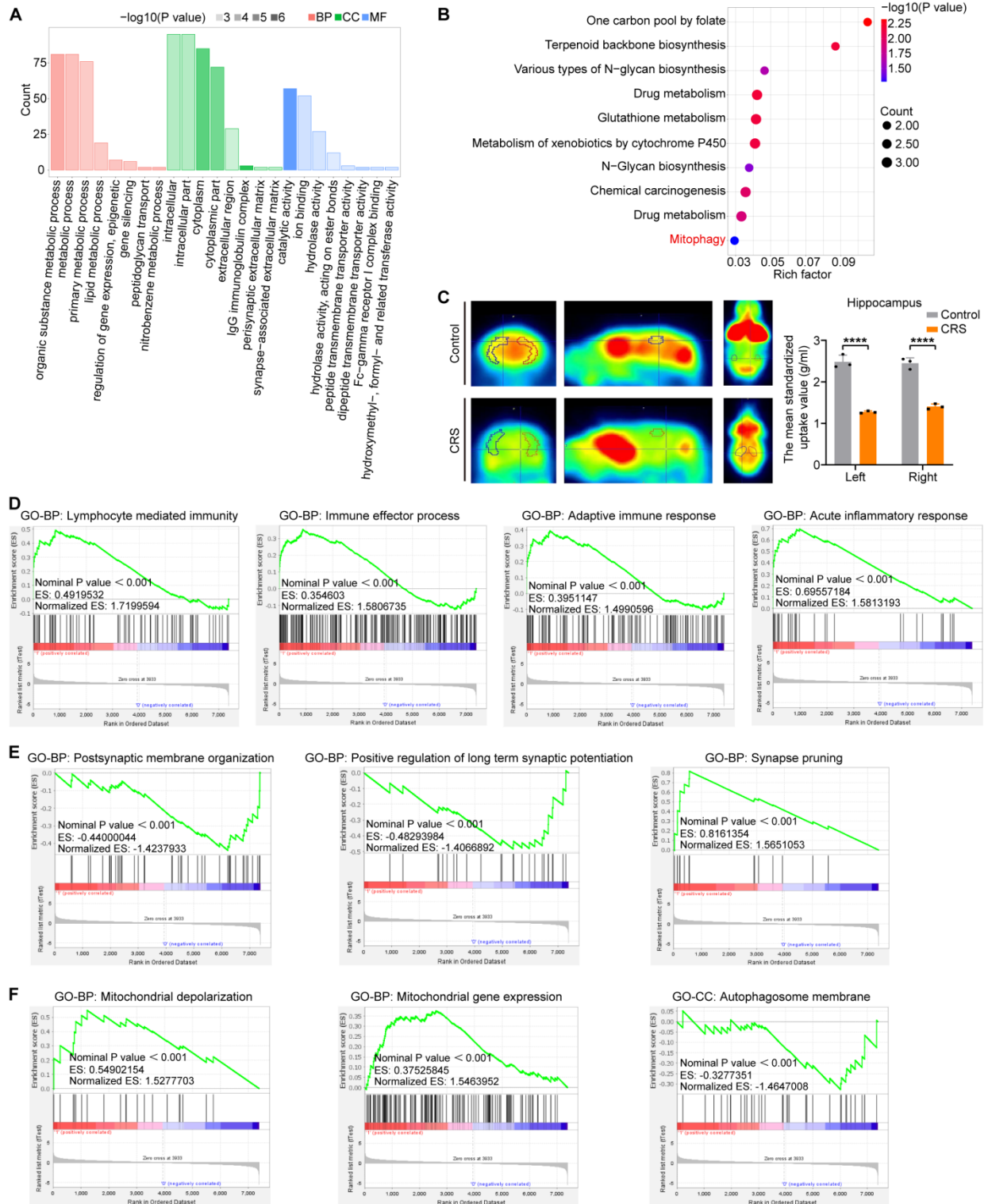


Fig. S6. Proteomic and metabolic alterations in CRS versus control mice.

A Gene Ontology (GO) enrichment of all differentially expressed proteins (DEPs), showing top enriched biological processes, molecular functions, and cellular components. **B** Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of DEPs, highlighting

364 affected signaling and metabolic pathways. **C** Representative micro-positron emission
365 tomography (PET) images and analysis of brain glucose metabolism in mice ($n = 3$). Gene set
366 enrichment analysis (GSEA) plots for **(D)** inflammatory and immune-related pathways, **(E)**
367 synaptic function pathways, and **(F)** mitochondria and autophagosome-related pathways. Data
368 are presented as individual values plus mean $\pm$ SD. $*P < 0.05$, $***P < 0.001$; ns, not significant.
369 Statistical analysis was conducted using two-way ANOVA with Sidak's multiple-comparison
370 test (B).

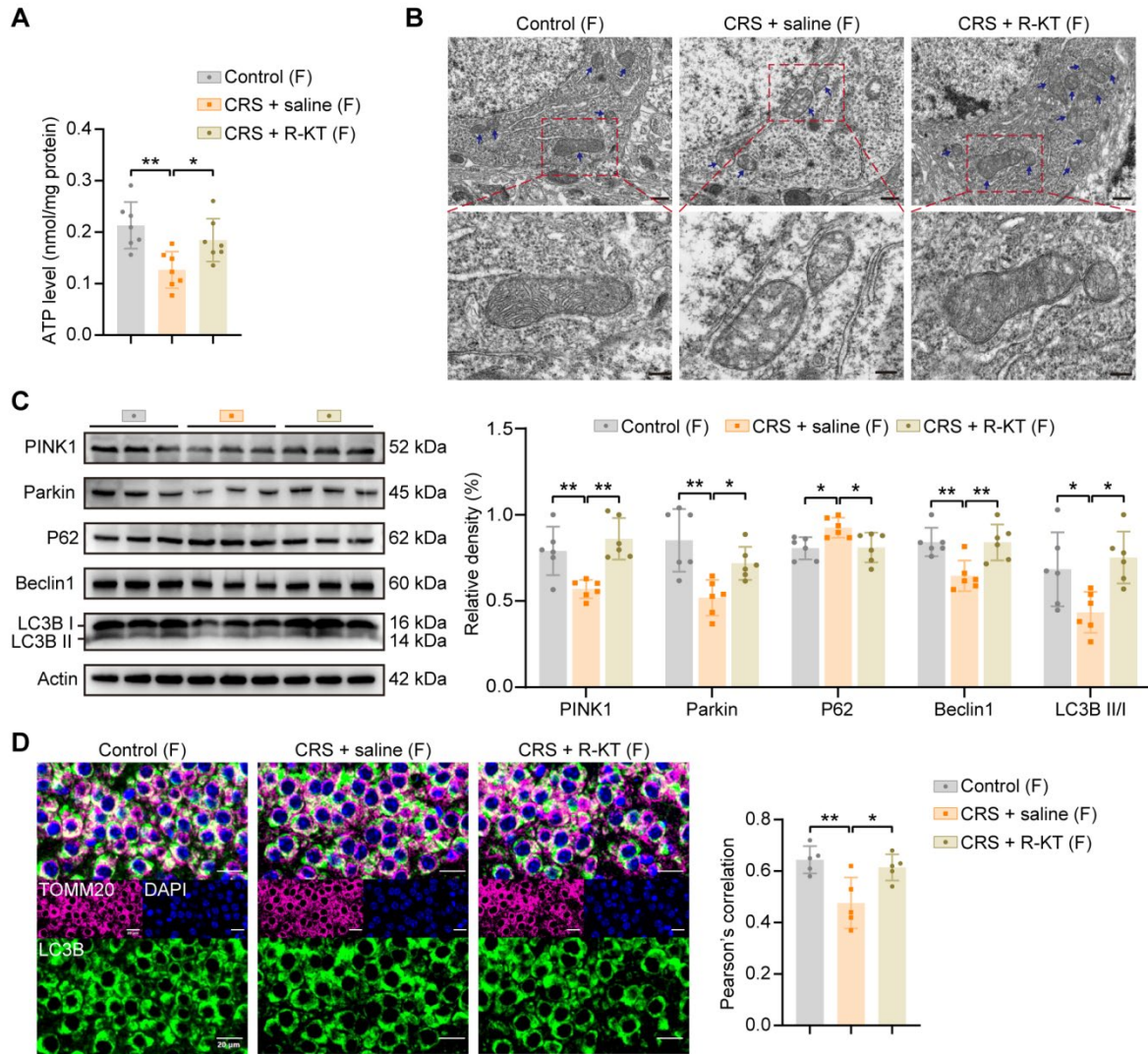


Fig. S7. Arketamine ameliorates CRS-induced defects in PINK1/Parkin-mediated mitophagy in female mice.

A Measurement of ATP levels in the hippocampus of female mice ($n = 7$). **B** TEM images showing mitochondrial morphology in three groups within the hippocampus of female mice ($n = 3$). The blue and orange arrows represent mitochondria and mitophagosomes, respectively. Scale bar (upper) = 500 nm, Scale bar (bottom) = 200 nm. **C** Western blot analysis and quantification of mitophagy-related proteins, including PINK1, Parkin, P62, Beclin1, and LC3B, in the hippocampus of mice ($n = 6$). **D** Immunofluorescence staining and semi-quantitative analysis of the co-localization of LC3B and TOMM20 ($n = 5$). Scale bar = 20 μm . Data are presented as individual values with mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; ns, not significant. Significant differences were determined using one-way ANOVA followed by Tukey's multiple comparisons tests.

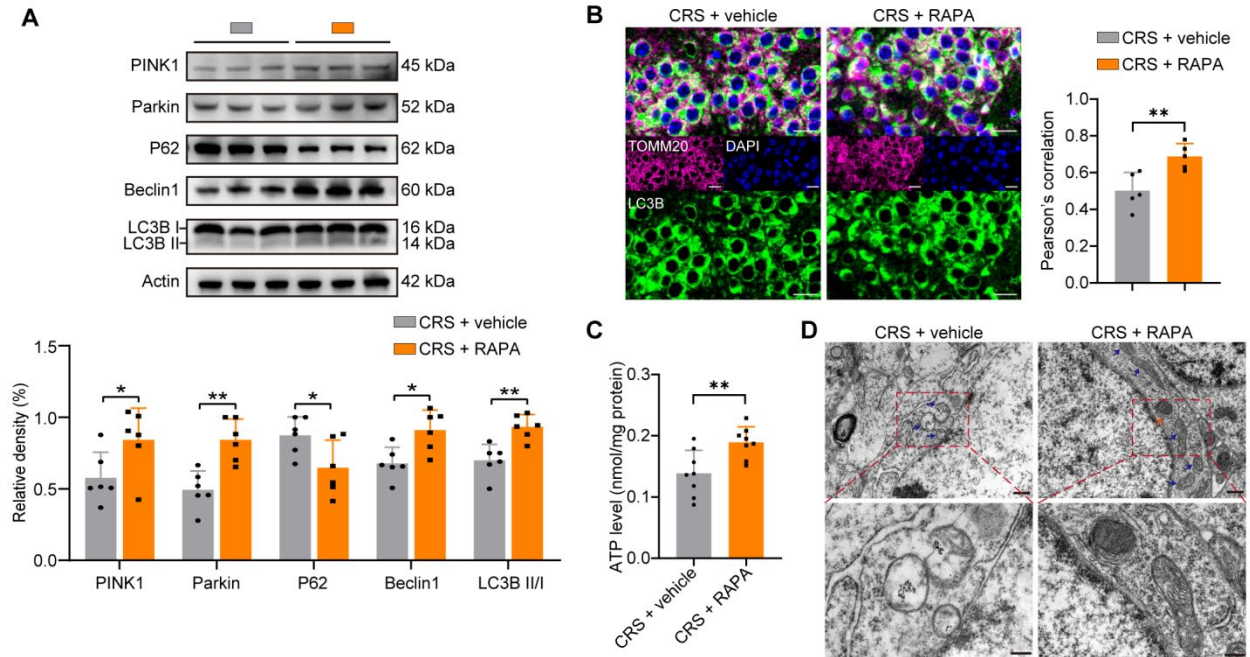


Fig. S8. Rapamycin promotes PINK1/Parkin-mediated mitophagy in the hippocampus.
A Western blot analysis and quantification of mitophagy-related proteins, including PINK1, Parkin, P62, Beclin1, and LC3B, in the hippocampus of mice (n = 6). **B** Immunofluorescence staining and semi-quantitative analysis of the co-localization of LC3B and TOMM20 (n = 5). Scale bar = 20 μ m. **C** Measurement of ATP levels in the hippocampus (n = 8). **D** TEM images showing mitochondrial morphology in three groups within the hippocampus of female mice (n = 3). The blue and orange arrows represent mitochondria and mitophagosomes, respectively. Scale bar (upper) = 500 nm, Scale bar (bottom) = 200 nm. Data are presented as individual values with mean $\pm$ SD. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001; ns, not significant. Significant differences were determined using unpaired t-tests.

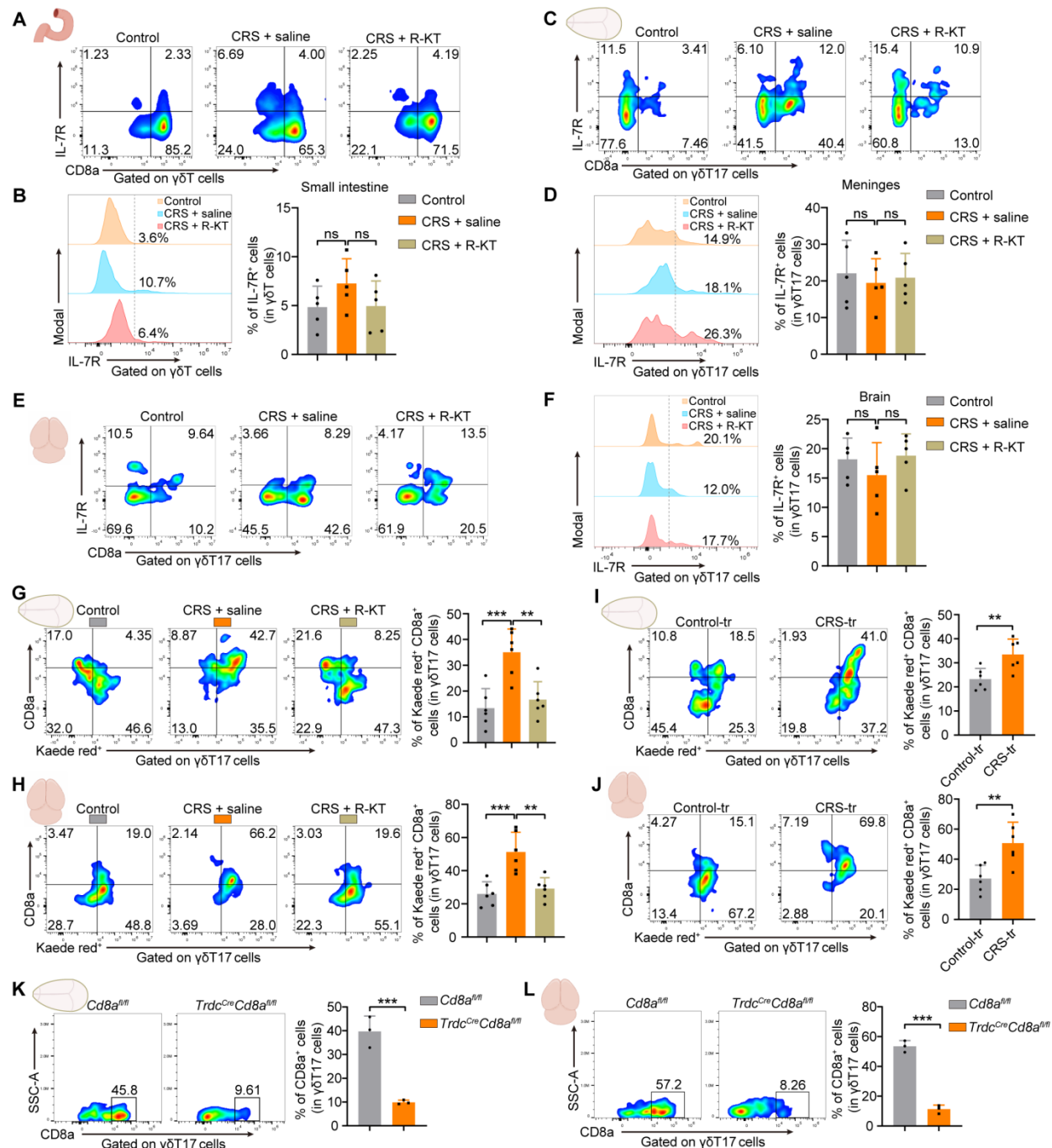


Fig. S9. CRS induces migration of CD8α⁺ γδ T17 cells from small intestine to meninges and brain.

A Representative FCM plots showing frequencies of CD8α⁺ IL-7R⁻ cells (right lower quadrant) and IL-7R⁺ CD8α⁻ cells (left upper quadrant) in γδ T cells in the small intestine. **B** Representative FCM histogram and proportions of IL-7R⁺ cells in γδ T cells in the small intestine (n = 5). **C** Representative FCM plots of expression of CD8α and IL-7R in γδ T17 cells in the meninges. **D** Representative FCM histogram and proportions of IL-7R⁺ cells in γδ T17 cells in the meninges (n = 5). **E** Representative FCM plots of the expression of CD8α and IL-7R in γδ T17 cells in the brain. **F** Representative FCM histogram and proportions of IL-7R⁺ cells in γδ T17 cells in the brain (n = 5). **G** Representative FCM plots of expression of CD8α and Kaede red in γδ T17 cells in the small intestine. **H** Representative FCM histogram and proportions of Kaede red⁺ CD8α⁺ cells in γδ T17 cells in the small intestine (n = 5). **I** Representative FCM plots of expression of CD8α and Kaede red in γδ T17 cells in the meninges. **J** Representative FCM histogram and proportions of Kaede red⁺ CD8α⁺ cells in γδ T17 cells in the meninges (n = 5). **K** Representative FCM plots of expression of CD8α and SSC-A in γδ T17 cells in the small intestine. **L** Representative FCM histogram and proportions of CD8α⁺ cells in γδ T17 cells in the small intestine (n = 5).

in $\gamma\delta$ T17 cells in the brain. **F** Representative FCM histogram and proportions of IL-7R⁺ cells in $\gamma\delta$ T17 cells in the brain (n = 5). Representative FCM plots and proportions of Kaede red⁺ CD8 α ⁺ cells in $\gamma\delta$ T17 cells in the meninges (**G**) and brain (**H**) between three groups (n = 6). Representative FCM plots and proportions of Kaede red⁺ CD8 α ⁺ cells in $\gamma\delta$ T17 cells in the meninges (**I**) and brain (**J**) in control-tr mice and CRS-tr mice (n = 6). Representative FCM plots and statistical proportions of CD8 α ⁺ cells in $\gamma\delta$ T17 cells in the meninges (**K**) and brain (**L**), which identified the effectiveness of knockout of CD8 α ⁺ $\gamma\delta$ T cells (n = 3). Data are presented as individual values plus mean $\pm$ SD. * P < 0.05, *** P < 0.001; ns, not significant. Statistical analysis was conducted using one way ANOVA followed by Tukey's multiple comparison tests (B, D, F, G, H) and unpaired t test (I–L).

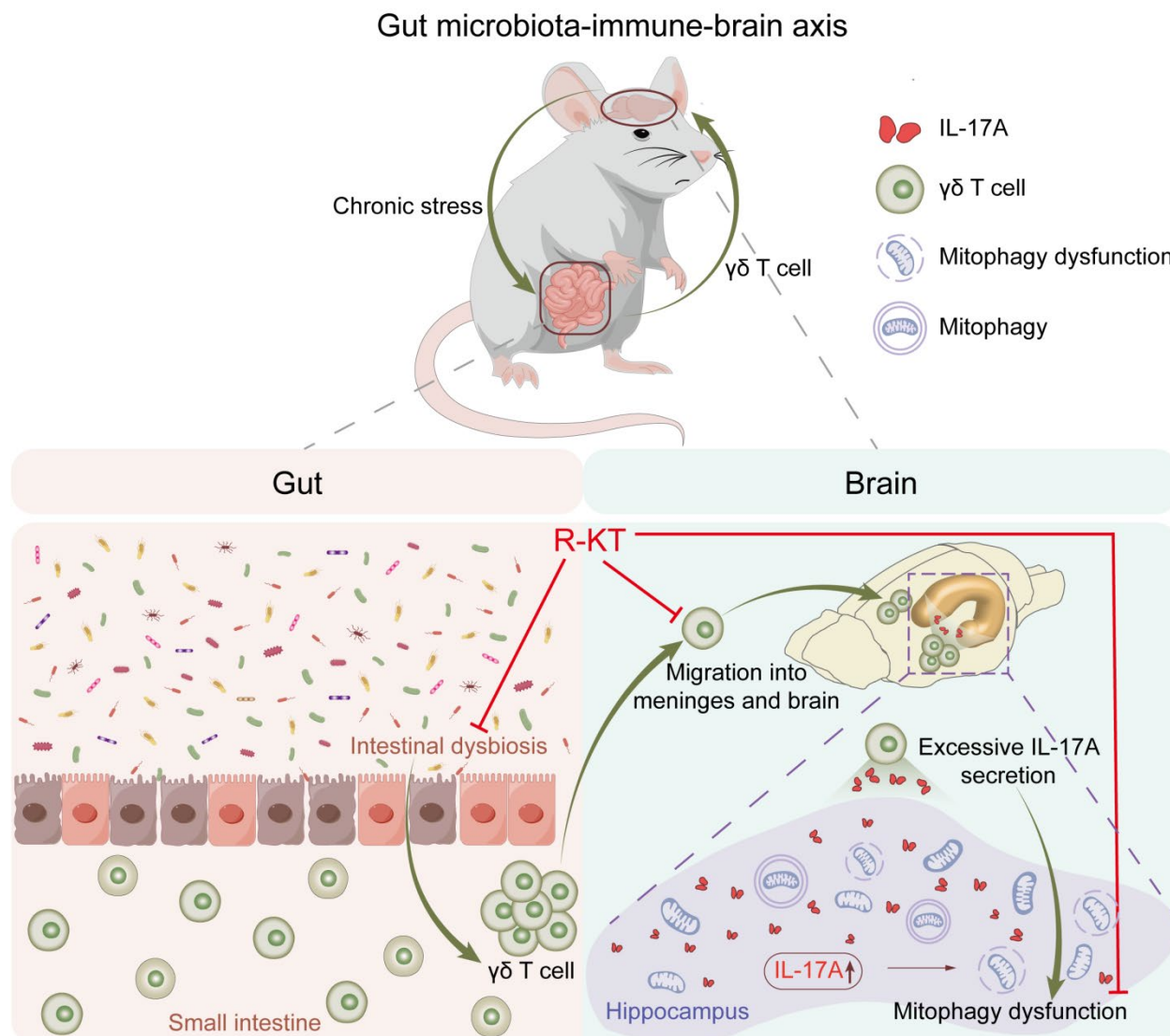


Fig. S10. Gut-derived $\gamma\delta$ T cells impair hippocampal mitophagy to exacerbate depressive-like behaviors. Chronic restraint stress–induced gut dysbiosis drives small intestinal $\gamma\delta$ T cells to migrate into the meninges and brain parenchyma, where their IL-17A secretion impairs PINK1/Parkin–mediated mitophagy in the hippocampus, exacerbating depressive-like behaviors. R-ketamine restores microbial balance, prevents $\gamma\delta$ T cell infiltration, reduces hippocampal IL-17A, rescues mitophagy and synaptic integrity, and reverses depressive-like phenotypes.

References

1. Wang Y, Cui P, Cao M, Ai L, Zeng L, Li X et al. Chronic restraint stress affects the diurnal rhythms of gut microbial composition and metabolism in a mouse model of depression. *BMC Microbiol* 2025; 25: 38.
2. Wu Y, Zhang Y, Yu Y, Wang X, Zhen Z, Yuan Y et al. Small intestinal $\gamma\delta$ T17 cells promote SAE through STING/C1q-induced microglial synaptic pruning in male mice. *Nat Commun* 2025; 16: 6779.
3. Zhang X, He T, Wu Z, Wang Y, Liu H, Zhang B et al. The role of CD38 in inflammation-induced depression-like behavior and the antidepressant effect of (R)-ketamine. *Brain Behav Immun* 2024; 115: 64-79.
4. Zhang Y, Xie B, Chen X, Zhang J, Yuan S. A key role of gut microbiota-vagus nerve/spleen axis in sleep deprivation-mediated aggravation of systemic inflammation after LPS administration. *Life Sci* 2021; 265: 118736.
5. Liu Q, Zhao JN, Fang ZT, Wang X, Zhang BG, He Y et al. BGP-15 alleviates LPS-induced depression-like behavior by promoting mitophagy. *Brain Behav Immun* 2024; 119: 648-664.
6. Xie B, Zhang Y, Han M, Wang M, Yu Y, Chen X et al. Reversal of the detrimental effects of social isolation on ischemic cerebral injury and stroke-associated pneumonia by inhibiting small intestinal $\gamma\delta$ T-cell migration into the brain and lung. *J Cereb Blood Flow Metab* 2023; 43: 1267-1284.
7. Xie B, Wang M, Zhang X, Zhang Y, Qi H, Liu H et al. Gut-derived memory $\gamma\delta$ T17 cells exacerbate sepsis-induced acute lung injury in mice. *Nat Commun* 2024; 15: 6737.
8. Zhu X, Sakamoto S, Ishii C, Smith MD, Ito K, Obayashi M et al. Dectin-1 signaling on colonic $\gamma\delta$ T cells promotes psychosocial stress responses. *Nat Immunol* 2023; 24: 625-636.