Materials and Methods

Animals and drug administration. All the animals were raised in a standard environment with controlled temperature on a 12-h light/dark cycle, and maintained in the Institutional Animal Care and Use Committee of China Medical University. An equal number of male and female mice 8 to 12 weeks old were used for each experiment.

The tremor rat was supplied by the Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University (Kyoto, Japan). TRPM7-CKO conditional knockout mice were generated by crossing the floxed TRPM7 allele ($TRPM7^{f/f}$) mice with B6.Cg-Tg(Camk2a-cre)T29-1Stl/J (JAX Stock No: 005359) (*CaMKII*-Cre) transgenic mice (Shanghai Southern Model Biological Corporation Construct). $TRPM7^{f/f}$ mice were obtained by crossing between $TRPM7^{f/w}$ mice, which were generated by GemPharmatech. All wild-type, $TRPM7^{f/w}$, $TRPM7^{f/f}$, *CaMKII*-Cre and TRPM7-CKO mice were maintained in C57BL/6J strain background.

Compounds were dissolved in normal saline (0.9%). Status epilepticus (SE) was induced by pilocarpine (PILO, 300 mg/kg i.p.). All mice were given scopolamine methyl bromide (2 mg/kg i.p.) to eliminate peripheral cholinergic effects, and 30 minutes later, they were intraperitoneally injected with PILO or saline to induce seizures. Then, diazepam (6mg/kg i.p.) was administered to terminate seizures after two hours. After 24h, mice that triggered seizures (scores IV and V on the Racine scale) were used in subsequent experiments.

Experiment 1: To investigate whether the up-regulation of pyroptosis associated factors and the abnormal accumulation of intracellular Zn^{2+} exist in PILO-induced epileptic models, the mice were randomly assigned to one of two groups (6 mice per group): (a) control group (control) and (b) PILO group (PILO). Mice in the PILO group received intraperitoneal injection of PILO (300 mg/kg i.p.). Mice in the control group received saline injection. All these mice were applied by western blot, immunofluorescence, and Zn^{2+} staining analysis.

Experiment 2: To examine the effects of recombinant TRPM7 knockdown

adeno-associated virus (AAV-sh-TRPM7) in PILO-induced epileptic models, the mice were randomly assigned to one of two groups (6 mice per group): (a) PILO+AAV-sh-NC group (PILO+AAV-sh-NC) and (b) PILO+AAV-sh-TRPM7 group (PILO+AAV-sh-TRPM7). One month after adeno-associated virus (AAV) injection, the expression of vector-transduced green fluorescent protein (EGFP) in the hippocampus was analyzed with a confocal fluorescence microscope (Nikon A1R). PILO induction was initiated 4 weeks after AAV injection and these mice were used for western blot, immunofluorescence, and electrophysiological analysis.

Experiment 3: To explore the pharmacological effect of TRPM7 inhibitor NS8593, the mice were randomly assigned to one of three groups (6 mice per group): (a) control group (control), (b) PILO group (PILO), and (c) PILO+NS8593 group (PILO+NS8593). Mice in the PILO group received saline injection, and 30 minutes later, they were intraperitoneally injected with PILO (300 mg/kg i.p.). Mice in the PILO+NS8593 group received NS8593 (5 mg/kg i.v.) intravenous injection, and 30 minutes later, they were intraperitoneally injected with PILO (300 mg/kg i.p.). Mice in the control group received saline injection. All these mice were used for western blot and immunofluorescence analysis.

Experiment 4: To explore the pharmacological effect of NS8593 and NLRP3 inflammasome inhibitor MCC950, the mice were randomly assigned to one of six groups (6 mice per group): (a) control group (control), (b) PILO group (PILO), (c) PILO+NS8593 low-dosage group (PILO+NS8593 5 mg/kg), (d) PILO+NS8593 high-dosage group (PILO+NS8593 10 mg/kg), (e) PILO+MCC950 group (PILO+MCC950 50 mg/kg), and (f) PILO+NS8593 (10 mg/kg) combined with MCC950 (50 mg/kg) group (PILO+NS8593+MCC950). Mice in the PILO group received saline injection, and 30 minutes later, they were intraperitoneally injected with PILO (300 mg/kg i.p.). Mice in the PILO+NS8593 low-dosage group, PILO+NS8593 high-dosage group, PILO+MCC950 group, and PILO+NS8593+MCC950 group received NS8593 (5 mg/kg i.v.), NS8593 (10 mg/kg i.v.), MCC950 (50 mg/kg i.p.), and NS8593 (10 mg/kg i.v.) combined with MCC950 (50 mg/kg i.p.) injection, respectively, and 30 minutes later, they were

intraperitoneally injected with PILO (300 mg/kg i.p.). Mice in the control group received saline injection. All these mice were used for electrophysiological analysis.

Experiment 5: To investigate whether the up-regulation of pyroptosis associated factors exist in PILO-treated TRPM7-CKO mice, the mice were randomly assigned to one of two groups (6 mice per group): (a) PILO+TRPM7$^{f/f}$ mice group and (b) PILO+TRPM7-CKO group. All these mice were used for western blot, immunofluorescence, and electrophysiological analysis.

Experiment 6: To explore the pharmacological effect of SDUY-225, the mice were randomly assigned to one of five groups (6 mice per group): (a) control group (control), (b) PILO group (PILO), (c) PILO+225 low-dosage group (PILO+225 2.5 mg/kg), (d) PILO+SDUY-225 high-dosage (PILO+225 5 mg/kg), and (e) PILO+NS8593 group (PILO+NS8593 5 mg/kg). Mice in the PILO group received saline injection, and 30 minutes later, they were intraperitoneally injected with PILO (300 mg/kg i.p.). Mice in the PILO+225 low-dosage, PILO+225 high-dosage, and PILO+NS8593 groups received SDUY-225 (2.5 mg/kg i.v. and 5 mg/kg i.v.) and NS8593 (5 mg/kg i.v.) intravenous injection, respectively, and 30 minutes later, they were intraperitoneally injected with PILO (300 mg/kg i.p.). Mice in the control group received saline injection. All these mice were used for western blot, immunofluorescence, and electrophysiological analysis.

Cell culture and drug administration. The mouse neuroblastoma neuro-2a (N2a) cells were purchased from Fenghbio (CL0243). The murine microglial cell line BV2 cells were purchased from Procell Co., Ltd. (CL-0493). The N2a cells and BV2 cells were maintained in DMEM medium (Gibco, C119955500BT) supplemented with 10% fetal bovine serum (FBS) (Procell, 164210-50) and 1% penicillin-streptomycin antibiotic mixture (MRC, CCS30032.01) at 37 °C in 95% air and 5% CO_2 atmosphere. The human embryonic kidney 293 (HEK293) cells stably expressing tetracycline-inducible human TRPM7 protein were cultured in DMEM medium supplemented with 10% fetal bovine serum, blasticidin (5 μg ml−1) and zeocin (0.4 mg ml−1) in a 5% CO_2-humidified atmosphere at 37 °C. Protein expression was induced the day preceding the experiments by adding 1 μg ml−1 tetracycline to the

culture medium. Patch-clamp measurements were performed 18-22 h post-induction

PILO (purity = 98.64%) was dissolved in 10% FBS DMEM with 1% penicillin-streptomycin antibiotic mixture medium. Inhibitors, including Nigericin (Sigma-Aldrich, 28380-24-7), (N,N,N',N'-Tetrakis (2-pyridylmethyl) ethylenediamine (TPEN, MACKLIN, 16858-02-9), deferoxamine (DFX, MACKLIN, 138-14-7), tetrathiomolybdate (TTM, Sigma-Aldrich, 15060-55-6), 6-nitrobenzo[b]thiophene-1,1-dioxide (Stattic, Sigma-Aldrich, 19983-44-9), N-acetylcysteine (NAC, Sigma-Aldrich, 616-91-1), and tyrphostin AG490 (AG490, Sigma-Aldrich, 133550-30-8) were dissolved in dimethyl sulfoxide (DMSO, Solarbio, D8371). Lipopolysaccharide (LPS, Solarbio, L8880) was dissolved in PBS. Zinc chloride ($ZnCl_2$, MACKLIN, 7646-85-7) was dissolved in double distilled water. The cells were treated with PILO alone or combined with the pretreatment of inhibitors including TPEN (1 μM), Stattic (0.5 μM), DFX (2 μM), TTM (1 μM), NAC (5 mM), and AG490 (50 μM) for 30 minutes, respectively. To checked whether Zn^{2+} can induce pyroptosis *in vitro*, N2a cells were treated with 30 μM and 100 μM $ZnCl_2$ for 24 h, respectively.

Cell viability assay. The cell viability was measured by the Cell Counting Kit-8 assay (CCK-8, Abbkine, BMU106-CN). The N2a cells were seeded in 96-well plates and treated with a range of concentrations of PILO including 2, 4, 8, 16, 32, 64, 128, and 256 mM for 48 h, and then analyzed the cell viability according to the manufacturer’ s instructions. The half-maximal inhibitory concentration (IC_{50}) was analyzed using GraphPad Prism v. 9.0 (GraphPad Software, CA, USA).

Small interfering RNA (siRNA) and overexpression plasmid transfection. Knockdown of TRPM7 was conducted by using specific siRNA synthesized by GenePharma, and the siRNA against signal transducer and activator of transcription 3 (STAT3) was obtained from JTS scientific. TRPM7 (ion channel segment) overexpression plasmid was a gift from Professor Jingjing Duan’ s lab in Nanchang university in China. The ion channel segment of TRPM7 was cloned into PEG-Backman vector to prepare overexpression plasmids. PEG-Backman vector alone or scrambled siRNA were transfected into PILO-treated or normal N2a cells

separately as negative controls (NC), respectively. Before transfection, N2a cells were plated in 6-well plate and allowed to grow approximately to 70-80%. The siRNA and DNA plasmids were transfected with the Lipo8000™ transfection reagent (Beyotime Institute of Biotechnology) according to the manufacturer's instructions. All overexpression and siRNA target sequences were provided in Table 1.

RNA isolation and quantitative real-time qRT-PCR. Total RNA was extracted and purified with the RNAiso Plus (TaKaRa) according to the manufacturer's instructions. Then 500 ng RNA from each sample was reversely transcribed into complementary DNA (cDNA) with PrimeScript RT Master Mix (TaKaRa). The cDNAs were amplified by qRT-PCR using 2×SYBR Green qPCR Master Mix (Bimake) according to the manufacturer's instructions. The relative mRNA quantification was normalized against GAPDH using the ΔΔCt-method. All primers used were listed in Table 2.

RNA sequencing (RNA-seq) and bioinformatic analysis. Samples (n = 5 in each group) were prepared by a mixture of the RNA from PILO-treated or control C57BL/6 mice. The preparation of the cDNA library from each sample and the sequencing were performed by SEQHEALTH (Wuhan, China). All acquired data from two datasets were screened with "Limma" package in "R" language (Version X64 3.6.1) to identify the differentially expressed (DE) mRNAs. |Log2FoldChange (FC)|> 1 and p value < 0.05 were set as the cutoff criteria. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes pathway (KEGG, http://www.genome.jp/kegg/pathway.html) analyses were carried out for determining enriched metabolic or signal transduction pathways associated with the up-regulated differentially expressed genes (DEGs). The GO terms of up-regulated DEGs from two datasets underwent Venn diagram analysis (https://jvenn.toulouse.inrae.fr/app/) to discover the GO terms which appeared simultaneously in two datasets.

Chromatin immunoprecipitation (ChIP) assay. After 24 h pretreatment with or without PILO (10 μM), N2a cells were harvested for ChIP. Chromatin was crosslinked for 10 min at 37 °C with 1% formaldehyde directly added to cell culture medium, followed by sonicated, diluted, and immunoprecipitated with anti-STAT3

(CST, #12640), anti- phospho-STAT3 (CST, #9145) or normal mouse IgG (Santa Cruz, sc-2025) antibody at 4 °C overnight. Protein A-Sepharose beads were added and then washed successively with low-salt buffer, high-salt buffer, LiCl buffer, and TE buffer. The protein-DNA complexes were eluted and the crosslinking was reversed at 65 °C. DNA fragments were purified and analyzed by qRT-PCR. For qRT-PCR, the 2x SYBR Green qPCR Master Mix (Bimake) was used and the samples were amplified via the QuantStudio1 (Applied Biosystems). The primers used in qRT-PCR were listed in Table 3.

Primary neuron cultures and drug administration. Hippocampal neurons were obtained from Wistar rat embryonic brains and plated on 6 well glass bottom plates coated with 0.01% poly-L-Lysine (Sigma-Aldrich) and 0.1 mg/mL laminin (ThermoFisher Scientific). The neurons were maintained in Neurobasal^{TM} medium (ThermoFisher Scientific) and DMEM / F-12 (1:1) (1x) (GibcoTM) supplemented with B-27 Supplement (50X) (ThermoFisher Scientific), MEM Mon-essential Amino Acid Solution (100x) (Sigma-Aldrich), N-2 Supplement (100X) (ThermoFisher Scientific), glutaMAX^{TM} (ThermoFisher Scientific), and penicillin/streptomycin/amphotericin. On day 7-9, hippocampal neurons were prepared for drug administrations. For western blot and immunofluorescence analysis, hippocampal neurons were treated with PILO (10 μM) for 24 h, then the culture was exposed to physiological recording solution for the further study. As the epileptic discharge model group, hippocampal neurons were cultured in Mg^{2+}-free extracellular fluid for 3 h before cultured in the original medium for 21 h, and neurons untreated with Mg^{2+}-free medium were taken as the control group. For patch clamp experiments, hippocampal neurons were treated with PILO (10 μM), PILO (10 μM) combined with NS8593 (10 μM and 30 μM), and PILO (10 μM) combined with MCC950 (10 μM and 30 μM) for 24 h, then the culture was exposed to physiological recording solution for the further study.

Chemical Synthesis. General information. All reagents and solvents were purchased from commercial sources and used without further purification. Unless noted otherwise, reactions were performed under air. Reactions were monitored by

thin-layer chromatography (TLC) on 0.25 mm silica gel plates, and the spots were visualized with UV light, and iodine vapor. 1H and 13C NMR spectra were obtained on a Brucker DRX spectrometer at 400 MHz. Chemical shifts are shown in parts per million (ppm) relative to tetramethylsilane (TMS) as the internal standard and are significant. 1H NMR data are reported in the following order: multiplicity (m, multiplet; q, quartet; t, triplet; d, doublet; s, singlet) number of protons. Mass spectra were obtained using ESI-MS spectrometry instrument model API4000 manufactured by Thermo Fisher. (R)-N-(1,2,3,4-tetrahydronaphthalen-1-yl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-amine (SDUY-225): To the solution of TCDI (242 mg, 1.36 mmol) in acetonitrile (10 mL) was added (R)-1-amino-1,2,3,4-tetrahydronaphthalene (200 mg, 1.36 mmol) stirred at room temperature (RT) for 3 h. Then, trifluoromethylbenzene (240 mg, 1.36 mmol) was added to the reaction solution and stirred at 50 ℃ for 3 hours. TLC showed a complete conversion of starting material. Then, the organic phase was concentrated in vacuo to give the crude product followed by purification using silica gel column chromatography with petroleum ether/ethyl acetate (5:1) to obtain the desired product SDUY-225-a 298 mg, yield: 79%. To a semi-solution of thiourea SDUY-225-a (310 mg, 0.85 mmol) in acetonitrile (5 mL) was added BOP (752 mg, 1.70 mmol) and DBU (388 mg, 2.55 mmol) and stirred at RT for 3 hours. TLC showed a complete conversion of starting material. Then, the organic phase was concentrated in vacuo to generate the crude product followed by purification using silica gel column chromatography with petroleum ether/ethyl acetate (1:1) to obtain the desired product SDUY-225 180mg, yield: 64%. MS (ESI) calcd for C18H16F3N3:332.1355; found: 332.1354. 1H NMR (400 MHz, DMSO-d6) δ 10.89 (s, 1H), 7.43 (s, 1H), 7.40 (d, J = 8.7 Hz, 1H), 7.33 (d, J = 7.3 Hz, 1H), 7.29 (d, J = 8.1 Hz, 1H), 7.26 - 7.10 (m, 4H), 5.09 (dd, J = 13.8, 6.5 Hz, 1H), 2.88 - 2.67 (m, 2H), 2.12 - 1.99 (m, 1H), 1.98 - 1.84 (m, 2H), 1.80 (dd, J = 14.0, 6.8 Hz, 1H). 13C NMR (151 MHz, DMSO) δ 157.61, 157.16, 138.28, 137.49, 134.10, 129.21, 128.80, 127.30, 126.27, 115.63, 114.66, 111.34, 109.20, 105.97, 50.56, 30.29, 29.25, 20.16.

Patch clamp. Action potential studies were performed in the whole-cell

current-clamp mode. The extracellular bath solution containing (in mM): 145 NaCl, 4 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 HEPES, and 10 Glucose; pH 7.35 (~310 mOsm). The pipettes solution containing (in mM): 140 K-gluconate, 3 KCl, 10 HEPES, 0.2 EGTA, 2 $MgCl_2$, and 2 Na_2ATP_3; pH 7.25 (280-290 mOsm). Recordings were low-pass Bessel filtered at 5 kHz and digitized at 50 kHz. Action potentials were evoked by a 1-s depolarizing current injection with a maximum of 200 pA in a ramp mode. Membrane potential was clamped to −80 mV for both spontaneous and evoked action potential firing measurements. The patch-clamp electrodes were obtained with capillary tubes pulled using a P-1000 puller, which had a resistance of approximately 3-5 MΩ. Patch clamping was carried out using an EPC-10 amplifier and a PatchMaster software (HEKA, Lambrecht, Germany). All experiments were carried out at room temperature (22±2 °C), and data were analyzed via Igor Pro 6.12A software.

TRPM7 currents were performed in the tight-seal whole-cell configuration at 21-25 °C. Voltage ramps of 50 ms duration spanning the voltage range of -100 to +100 mV were delivered from a holding potential of 0 mV at a rate of 0.5 Hz. All voltages were corrected for liquid junction potential. Currents were filtered at 2.9 kHz and digitized at 500 μs intervals. The low-resolution temporal development of currents at +80 mV was extracted from individual ramp current records by measuring the current amplitudes at voltages of +80 mV. For patch-clamp experiments, human embryonic kidney 293 (HEK293) cells were kept in a bath solution containing (in mM): 140 NaCl, 1 $CaCl_2$, 2 $MgCl_2$, 2.8 KCl, 10 HEPES, and 11 Glucose; pH 7.2 (~300 mOsm). Pipette solutions contained (in mM): 130 Cs-glutamate, 8 NaCl, 1 mM $MgCl_2$, 10 mM EGTA, 10 HEPES; pH 7.2 was adjusted with CsOH (~300 mOsm). SDUY-225 was dissolved in external solution from a stock solution of 50 mM in DMSO. DMSO at 0.1‰ in the external solution was used as control application.

Stereotaxic injection. Mice were anesthetized with isoflurane, and then fixed into a stereotaxic instrument (ZS-FD Zhongshi Dichuan). The scalp was sterilized with iodophors and 75% ethanol, in turn, and incised along the skull midline. A total volume of 1μL virus: (a) Recombinant TRPM7 knockdown adeno-associated virus

(pAAV-U6-shRNA (Trpm7)-CMV-EGFP-WPRE) with titer 7.39E+12 vg/mL, (b) NC (pAAV-U6-shRNA (NC2)-CMV-EGFP-WPRE-spolyA) with titer 1.18E+13 vg/mL, obtained from OBIO Technology, were injected into the location within the ventral hippocampus (AP: −3.28 mm, ML: ±2.75 mm, DV: −2.5 mm). The needle syringe was left in place for 5 minutes before withdrawal. The skin was sutured, and then sterilized with iodophors and 75% ethanol. Stereotaxic injection was operated with syringe pump R462 (RWD Life Science Co., Ltd). 1μL of virus was injected at each DV location in 1μL increments at a ow rate of 1μL /min.

Electroencephalogram (EEG) recordings. Both stainless steel screws (1.0 mm diameter) and single tungsten LFP electrodes (0.08 mm diameter, 1.5mm length) serving as EEG electrodes were placed over the bilateral somatosensory cortex (AP: −1.0 mm, ML: ±1.5 mm) under 1–1.5% isoflurane anesthesia. A reference screw electrode was implanted on the cerebellum. EEG electrodes were attached to a head device (Bio-Signal Technologies, Cat No. 2625）by silver wires (100 μm), then the assembly was secured with dental cement. Following surgery, mice were individually housed in clear acrylic cages under temperature-, humidity-, and light-controlled conditions (12-h light–dark schedule) with ad libitum food and water.

One week after mice surgery, cortical EEG signals were amplified, filtered, and digitalized with a resolution of 1000 Hz using a tethered data acquisition system (Bio-Signal Technologies) and continuously synchronized with infrared video for at least one hour and then analyzed off-line in the custom code and brainstorm based on Matlab 2021.

Zn^{2+} staining. The N-(6-methoxy-8-quinolyl)-para-toluenesulfonamide (TSQ; AAT BIOQUEST, 21254) is a membrane-permeant Zn^{2+} indicator. For TSQ staining, the mice were deeply anesthetized before sacrificed at the designated time points. The brains were embedded in optimum cutting temperature compound (OCT, SAKURA) and frozen on dry ice before coronally sectioned with a 20μm thickness using a freezing microtome (Leica CM1950). Under the light proof condition, the brain slices were immersed in a solution of 4.5 μM TSQ, 140 mM sodium barbital, and 140 mM sodium acetate (pH 10.5–11) for five to ten minutes and then rinsed once or twice

with PBS. There were three mice in each group and three coronal sections from each animal were observed and photographed under fluorescent microscopy (Olympus BX61) with 360 nm UV light. The mean gray value of TSQ-stained area was expressed using ImageJ software.

Immunofluorescence. The hippocampal neurons were seeded on cell climbing sheets. The mice were deeply anesthetized before transcardially perfused with saline. The whole brains were isolated and postfixed in 4% paraformaldehyde (PFA) for 24 h at 4 ℃ followed by cryoprotection using 30% sucrose. Brains were embedded in optimum cutting temperature compound (OCT, SAKURA) and coronally sectioned with a 20μm thickness. Neurons and sections were fixed by 4% PFA for 10 min and then permeated membrane using 0.3% Triton X-100 (Solarbio) for 10 min at room temperature before blocked (at room temperature for 1 h) using 5% BSA blocking buffer (Solarbio). Next, the neurons were incubated at 4 ℃ for 1 h with primary antibodies. And the sections were incubated overnight at 4 ℃ with primary antibodies. The following primary antibodies were used at 1:100: anti-TRPM7 (Abcam, ab729), anti-NLRP3 (Proteintech, 19771-1-AP), neuronal marker anti-NeuN (Abcam, ab104224), anti-IBA1 (Proteintech, 66827-1-Ig), and anti-GFAP (Bioawamp, PAB32097). Then neurons and sections were incubated with secondary antibody at room temperature for 2 h. Finally, the nuclei were stained with DAPI (Solarbio, C0060). The coronal sections from each animal were observed and photographed under confocal fluorescence microscopy (Nikon A1R). Images were analyzed using ImageJ software.

Measurement of intracellular reactive oxygen species (ROS). Intracellular ROS production was measured by using the reactive oxygen species assay kit (Beyotime) according to the manufacturer' s instructions. DCF fluorescence signals were determined by confocal fluorescence microscopy (Nikon A1R). Relative ROS was quantified from mean intensity of DCF fluorescence and normalized to control conditions. Images were analyzed using ImageJ software.

Measurement of mitochondrial membrane potential (MMP). The inner membrane electrical potential across mitochondrial membrane (MMP) in N2a cells

was measured using JC-1 dye (Beyotime) according to the manufacturer' s instructions. JC-1 fluorescence signals were captured and recorded using confocal fluorescence microscopy (Nikon A1R). MMP fluorescence intensity was calculated as the red/green fluorescence ratio. Images were analyzed using ImageJ software.

Western blot. After treatment or transfection, the N2a cells and BV2 cells were harvested and lysed using radioimmunoprecipitation assay (RIPA) lysis buffer (Solarbio), and the cytoplasmic and nuclear protein fractions were isolated using nuclear and cytoplasmic protein extraction kit (Beyotime). 30 μg protein were separated by 12 % SDS-PAGE and transferred to polyvinylidene fluoride membrane. Then the membranes were blocked (for 1.5 h) in 5% skim milk and incubated (at 4 °C overnight) with the corresponding primary antibodies. The following primary antibodies were used at 1:1,000: anti-NLRP3 (ABclonal, A5652); anti-phospho-STAT3 (Tyr705) (Bioss, bs-1658R); anti-caspase 1 (Proteintech, 22915-1-AP); anti-GFAP (Bioawamp, PAB32097); anti-NeuN (Abcam, ab104224); anti-STAT3 (Proteintech, 10253-2-AP); anti-GSDMD (Abcam, ab219800); anti-phospho-JAK2 (Y1007+Y1008) (Bioawamp, PAB30711); anti-JAK2 (Abcam, ab32101); and anti-Tubulin (Santa Cruz, sc-8035). The following primary antibodies were used at 1:5,000: anti-beta actin (Proteintech, 20536-1-AP); anti-TRPM7 (Abcam, ab245408); and anti-GAPDH (Proteintech, 10494-1-AP). The membranes were then incubated with HRP-labeled anti-rabbit, anti-mouse, or anti-goat (Proteintech) secondary antibodies. The images were quantitated with Image J software.

Enzyme-linked immunosorbent assay (ELISA). After collection of the human whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifugated again. Human IL-1β and IL-18 were measured using ELISA kits (Wuhan ColorfulGene Biological Technology) as instructed by the manufacturer.

After PILO treatment for 24 h, the hippocampus tissue was homogenized in RIPA lysis buffer containing protease inhibitors and centrifuged for 20 min at 16,000×g at 4 °C. The supernatants were harvested and measured for inflammatory

cytokines (IL-1β and IL-18) release using ELISA kits (Bioswamp) following the manufacturer' s instructions.

Patient samples. All human serum samples were obtained from the the First Affiliated Hospital of China Medical University. Serum samples were obtained from 11 patients who diagnosed as idiopathic epilepsy and 9 healthy volunteers recruited from advertisements as the control group. Detailed information on the patients and volunteers were shown in Supplemental Table 1 and Table 2, respectively. Epilepsy was diagnosed according to criteria proposed by the International League Against Epilepsy. All patients with epilepsy had typical symptoms and EEG features, and their seizures were refractory to combination therapy with the maximal tolerable doses of at least three antiepileptic drugs (AEDs) for more than 2 years. All the patients underwent detailed medical evaluations, including assessment of epilepsy manifestations, a neurological examination, neuroimaging (e.g., positron emission tomographycomputed tomography or high-resolution magnetic resonance imaging), and electrophysiological examination (24 h video EEG). Patients who were on hormonal drugs during admission should also be screened or excluded to reduce the fluctuation of inflammatory factor levels caused by the medication.

Statistics. All statistical analyses were performed by GraphPad Prism 9.0 software. Data were presented as mean ± SEM. P values < 0.05 were regarded statistically significant. Normal distribution and homogeneity of variances were evaluated using the Shapiro-Wilk test and Brown-Forsythe test, respectively. For normally distributed data, Student's t-test (± Welch correction) or one-way analysis of variance (ANOVA) was performed to determine significance. When there were two groups and one or more normality are rejected, Mann Whitney U test was performed. For three or more groups, when the main issue was whether a significant difference between groups and whether there are interactions between factors, one-way ANOVA or Kruskal Wallis ANOVA was performed depending on normality. When the ANOVA result was significant different ($P < 0.05$), the Tukey's multiple comparisons test was used.

Study approval. All animal experiments were conducted in accordance with the

guidelines and the protocols approved by the Institutional Animal Care and Use Committee of China Medical University (IACUC-CMU2021474). The human study complied with the Declaration of Helsinki and the ethical principles of the National Institutes of Health and was approved by the Committee on Human Research of the First Affiliated Hospital of China Medical University.