**Supplementary Table 1. PCR primers.**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer-forward</th>
<th>Primer-reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd301</td>
<td>AGATGAAGCTGGCAAGGAC</td>
<td>GAGGAGTCCAAACTCCAGGC</td>
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<tr>
<td>Arg1</td>
<td>GGACATCGTGTACATCGGCT</td>
<td>CTTCCTTCCCAGCTAGCT</td>
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<tr>
<td>Mrc1</td>
<td>AACTTCATCTGCCAGGACA</td>
<td>CGTGCTCTTTCCAGGTCTT</td>
</tr>
<tr>
<td>Actb</td>
<td>GCAGGAGTACGATGAGTCCG</td>
<td>ACGCAGCTCAGTAACAGTCC</td>
</tr>
</tbody>
</table>
Methods

Animals and Drug administration
Adult male Sprague-Dawley rats (10-week-old, 300-320g) were housed in a temperature- and humidity-controlled environment with *ad libitum* access to food and water. The light in the room was controlled in a 12-h light/dark cycle. All experimental protocols were approved by the Institutional Animal Care and Use Committee and in accordance with the National Institutes of Health guide for the care and use of laboratory animals.

AZD2098 and JR-AB2-011 were dissolved in 10% DMSO and administered via i.p. injection. The rCCL17 was dissolved in sterile deionized water and administered via i.n. administration. Three different dosages of rCCL17 (10, 30, and 60 μg/kg) were given to rats at 1 h after SAH.

Modified Garcia Score and Beam balance test
The modified Garcia Score and Beam balance test were used to assess short-term neurological deficits at 24 h and 72 h post-SAH as previously described. Briefly, the modified Garcia test with a 21-point score was conducted to evaluate spontaneous activity, axial sensation, symmetry of limb movement, vibrissae proprioception, and forelimb walking. Beam balance test is used to assess the rat’s ability to walk on the wooden beam for 1 min. The evaluation was as follows: 0 = not walk and fall; 1 = not walk but remain on beam; 2 = walk but fall; 3 = walk < 20 cm; and 4 = walk beyond 20 cm.

Brain water content measurement
The wet/dry method was used to measure BWC according to previous study. The rats were sacrificed and the brains were collected immediately at 24 h after surgery and cut into four sections: left cerebral hemisphere, right cerebral hemisphere, cerebellum and brain stem. Each section was weighed immediately after removal to obtain the wet weight (WW). Subsequently, each part of the brain was baked at 100 °C for 48 h and record the dry weight (DW). The BWC was measured using the following formula: (WW-DW)/WW×100%.

Western blotting analysis
At each post-SAH time point, the brain samples were collected and prepared for western blot
analysis. The left hemisphere sample was prepared using RIPA lysis buffer. 40 ng of protein sample was loaded onto an SDS-PAGE gel and transferred to a nitrocellulose membrane. The membrane was blocked with 5% non-fat milk and incubated with the following primary antibodies: rabbit anti-CCR4 (1:1000, GTX53474, Gene Tex); rabbit anti-CCL17 (1:1000, ab182793, Abcam); rabbit anti-Akt (1:1000, 9272S, Cell Signaling Technology); rabbit anti-phosphorylated AKT ser473 (1:1000, 4060S, Cell Signaling Technology); rabbit anti-PKCα (1:1000, 2056S, Cell Signaling Technology); rabbit anti-phosphorylated PKCα (1:1000, ab180848, Abcam); rabbit anti-PKC (1:1000, ab179522, Abcam); rabbit anti-phosphorylated 4EBP1 (1:1000, 2855S, Cell Signaling Technology), rabbit anti-4EBP1 (1:1000, 9644S, Cell Signaling Technology), rabbit anti-phosphorylated STAT6 (1:1000, 5397S, Cell Signaling Technology); rabbit anti-STAT6 (1:1000, 56554S, Cell Signaling Technology); and rabbit anti-β-actin (1:1000, Santa Cruz; 1:5000, Abcam) for 2 h at room temperature.

**RNA isolation and quantitative PCR**

Preparation of mRNA and real-time quantitative PCR was previously described. Total RNA was extracted from left hemisphere samples and cells using TRIzol reagent (Invitrogen) after washing with PBS. cDNA was synthesized from purified RNA using a SuperScript III First-Strand cDNA synthesis system (18080051, Life Technologies) according to the manuscript’s instructions. SYBR Green PCR Master Mix (Applied Biosystem, CA, USA) was used for PCR amplification and a real-time PCR machine (iQ5, Bio-Rad Laboratories) was used to quantify the expression of mRNAs. β-actin was used as an endogenous control and the expression levels were quantified using 2^{-ΔΔCt} method. All primer sequences are listed in Supplementary Table 1, and detection of each primer was performed in the triplicate.

**SAH Model and SAH Grading**

The endovascular perforation SAH model was conducted as previously described. Briefly, isoflurane-anesthetized rats were intubated with mechanical ventilation. A sharp 4–0 monofilament was inserted from the left external carotid artery to the internal carotid artery and perforated the bifurcation of the anterior and middle cerebral arteries. Rats in the sham group underwent the same
procedures without vessel puncture. Measurements of respiration, heart rate, skin pigmentation, and pedal reflex were recorded intraoperatively every 5 min to confirm the anesthetic status and prevent distress.

The SAH grading was blindly evaluated by two independent investigators according to the previous study. Briefly, the basal cistern was divided into six segments and scored from 0 to 3 based on the subarachnoid blood clot. The final score combined all segments and ranged from 0 to 18. If there was no blood present, a score of 0 was assigned. Rats with a score under 9 were excluded from this study.

**Primary hippocampal neuron, microglia and astrocytes cultures**

Within two days after birth, newborn Wistar rat pups were anesthetized and decapitated. The brain was quick removed, soaked in Hank’s solution, and stored at 4 °C. The hippocampus was dissected rapidly under a microscope ice bath, and the tissue was cleaned three times with 5mL refrigerated DMEM/F12 medium. The hippocampal tissue was digested with 2 mL pectinase and gently agitated twice. The cell suspension was removed to repeat the process with pectinase until complete digestion was achieved. After digestion, the cells were evenly mixed with the culture medium from hippocampal neurons and counted for lamination. Half-volume of medium was replaced every other day 24 h, the neuronal state was observed for 9 days and subsequent experiments were carried out. Simultaneously, cerebral cortices were enzymatically and mechanically dissociated and cells were seeded in DMEM/F12 medium supplemented with 10% FBS, 1% penicillin-streptomycin, 1 mM sodium pyruvate. The medium was replaced every 4 days. On day 14, the medium was removed, and cultures were trypsinized to isolate the monolayer of astrocytes and adherent microglia. Oxygenated hemoglobin (OxyHb, 10 μM, Sigma-Aldrich, USA) was introduced into the medium for 24 h to simulate SAH insult in vitro as previously described.

**Elisa assay for CCL17**

The rat primary neuron was treated with or without OxyHb (10 μM, Sigma-Aldrich, USA) to simulate SAH for 24 h. The supernatant was collected for quantitative analysis of endogenous CCL17 using ELISA kit (LifeSpan BioSciences, LS-F4911) following the manufacturer’s instruction.
shRictor adeno-associated virus vector construction

To knockdown the expression of Rictor (a composition of mTORC2) in microglia in vivo, we constructed adeno-associated virus (AAV)-CD68 mediated Rictor interference virus vector pHBAAV2/9-CD68-shRictor (AAV-CD68-shRictor) (Hanbio Technology). The sequences were GGCTGTGATATTCTAAAGT (sense) and ACTTTAGAATATCACAGCC (anti-sense). The verification of the inhibitory effects was conducted 3 weeks later after stereotaxic injection to the cerebral ventricle.

Immunofluorescence staining

Animals were anesthetized and perfused with 200 ml PBS followed by 50 mL10% formalin transcardially at 24 h after SAH. Brain samples were fixed in 10% formalin for 2 days. 30% sucrose was used to dehydrate the brain for a further 3 days. Brain sections were prepared as 10 μm slices. Samples were co-incubated with primary antibodies at 4 ℃ overnight anti-CCL17 (1:100, LS-C198166, LifeSpan BioSciences), anti-CCR4 (1:100, ab59550, Abcam), anti-CD206 (1:100, sc58986, Santa cruz biotechnology), anti-Iba-1 (1:100, ab178847, Abcam), anti-GFAP (1:200, ab53554, Abcam), and anti-NeuN (1:200, ab177487, Abcam). Slides were incubated with the appropriate secondary antibodies and observed using a fluorescence microscope (Leica Microsystems, USA).

TUNEL staining

According to the manufacturer’s instruction, staining of TUNEL was applied to quantify cell apoptosis with in situ Apoptosis Detection Kit (Roche, Indianapolis, USA) at 24 h post-SAH. The number of TUNEL-positive cells was analyzed in the hemorrhagic region. Four random visual fields per slide were observed by a blinded observer using a microscope at 200x magnification.

FJC staining

The number of degenerating neurons was assessed by FJC staining using a modified FJC Ready-to-Dilute Staining Kit (Millipore, Billerica, MA, USA) at 24 h post-SAH. In accordance with the manufacturer’s instructions, slides were washed with PBS incubated with the FJC working solution
for 20 min, and then visualized using a fluorescence microscope. The FJC-positive neurons in four parts of the hippocampus of each brain were manually counted using a microscope at 200x magnification and the ImageJ software (ImageJ 1.5, NIH, USA).

**Stereotaxic cerebroventricular AAV-Rictor injection**

Rats were anesthetized and then mounted onto a stereotaxic apparatus (Stoelting Instruments, Wood Dale, IL, USA). The AAV-CD68-shRictor (1.3×10^{12} TU/ml) was injected into both of the lateral cerebral ventricles (0.5 μl each; AP: -1.5 mm, ML:+/-1.1 mm, DV:-4.5 mm, relative to Bregma). The injection speed was 0.1 μl/min using an injection pump (Harvard Apparatus, Holliston, MA, USA) equipped with a 1-μl Hamilton syringe. Before withdrawing the syringe, it remained in place for at least five minutes. SAH induction was performed 3 weeks after injection.
Supplementary Figure 1. Experimental design and animal groups.
SAH, subarachnoid hemorrhage; rCCL17, recombination C-C chemokine ligand17; CCR4, C-C chemokine receptor 4; WB, western blot; IF, immunofluorescence; BWC, brain water content; DMSO, dimethyl sulfoxide; i.n., intranasally; intracerebroventricularly.

Supplementary Figure

Experiment 1: Time course of endogenous and cellular localization
CCL17 and CCR4 after SAH.

Experiment 2: The effects of rCCL17 treatment on short-term neurobehavior, brain edema, cell apoptosis and neuronal degeneration after SAH.

Experiment 3: The role of CCR4/mTORC2 signal pathways post-SAHA
with the administration of rCCL17.

Experiment 4: The role of CCL17/CCR4/mTORC2 axis in microglia post-SAHA

Group (WB, IF) Mortality
(1) Sham (n = 3, 3) 0
(2) SAH 3 h (n = 3, 3) 1
(3) SAH 6 h (n = 3, 3) 2
(4) SAH 12 h (n = 3, 3) 2
(5) SAH 24 h (n = 3, 3) 3
(6) SAH 72 h (n = 3, 3) 2

Group (Short-term neurobehavior, BWC, IF; pQPCR) Mortality
(1) Sham (n = 6/24 h, 6/72 h, 3, 4) 6
(2) SAH+Vehicle (n = 6/24 h, 6/72 h, 3, 4) 2
(3) SAH+30 µg rCCL17 (n = 6/24 h, 6/72 h, 3, 4) 2
(4) SAH+60 µg rCCL17 (n = 6/24 h, 6/72 h, 3, 4) 1

Group (Short-term neurobehavior, BWC, IF; pQPCR) Mortality
(1) Sham (n = 5/24 h, 5/72 h, 3, 4) 10
(2) SAH+Vehicle (n = 5/24 h, 5/72 h, 3, 4) 3
(3) SAH+CCL17 (n = 5/24 h, 5/72 h, 3, 4) 3
(4) SAH+CCL17+DMSO (n = 5/24 h, 5/72 h, 3, 4) 2
(5) SAH+CCL17+AZD2014 (n = 5/24 h, 5/72 h, 3, 4) 3
(6) SAH+CCL17+JNK-201 (n = 5/24 h, 5/72 h, 3, 4) 3

Group (Short-term neurobehavior, BWC, IF; pQPCR) Mortality
(1) Sham+AAV-Control (n = 5/24 h, 5/72 h, 3, 4) 0
(2) SAH+Vehicle+AAV-Control (n = 5/24 h, 5/72 h, 3, 4) 0
(3) SAH+CCL17+AAV-Control (n = 5/24 h, 5/72 h, 3, 4) 0
(4) Sham+AAV-rRas (n = 5/24 h, 5/72 h, 3, 4) 1
(5) SAH+Vehicle+AAV-rRas (n = 5/24 h, 5/72 h, 3, 4) 2
(6) SAH+CCL17+AAV-rRas (n = 5/24 h, 5/72 h, 3, 4) 3

Total ratio 523
Mortality 14.0% (77/523)

Supplementary Figure 2. The expression of CCL17 in CA1, CA3, and DG region at 24 post-SAH.

CCL17 (green) co-localized with NeuN (red) in sham animals and animals in CA1, CA3, and DG region at 24 post-SAH. Nuclei were stained with DAPI (blue), scale bar = 20 μm. n=3/group. All t-tests were two-tailed. Mean ± SEM. ***P < 0.001.
Supplementary Figure 3. CCL17 secreted from neuron after OxyHb stimulation.

(A) With stimulation of OxyHb (10 μM) for 24 h, the expression of CCL17 in rat primary neurons was examined by Western blot. n=3/group. (B) The level of CCL17 in the supernatant after stimulation was tested with ELISA assay. n=8/group. All t-tests were two-tailed. Mean ± SEM. ***P < 0.001.
Supplementary Figure 4. SAH grade of rats SAH model.