

#### Supplementary Figure 1. Positive and negative probes for RNAscope

Representative RNAscope images of positive RNA (red) and negative RNA in the hemorrhagic perifocal area at day 3 of ICH. Scale bar =  $50 \mu m$ . Arrows indicated the colocalization of positive RNA with DPAI.

Supplementary Figure 2. The construction of Aldh1I1<sup>CreERT2+/-</sup>: CCL5<sup>f/f</sup> mice and verification of CCL5 knockout efficiency



**A.** Schematic representation of Aldh111<sup>CreERT2+/-</sup>: CCL5<sup>f/f</sup> mice construction. **B.** RT-PCR analysis of CCL5 mRNA expression levels in the hemorrhagic perifocal area at day 3 of ICH in Oil ICH and Tam ICH groups. n = 4 mice in Oil ICH group (3 females, 1 male), n = 5 mice in Tam ICH group (2 females, 3 male). GAPDH was used as an internal control. Two-sided, unpaired Student's test. **C.** Representative immunoblots of CCL5

protein expression levels in the hemorrhagic perifocal area at day 3 of ICH in Oil ICH and Tam ICH groups. **D.** Quantification of CCL5 protein expression levels in the hemorrhagic perifocal area at day 3 of ICH in Oil ICH and Tam ICH groups. n = 4 mice in Oil ICH group (3 female, 1 male), n = 5 mice in Tam ICH group (2 female, 3 male).  $\beta$ -actin was used as an internal control. Two-sided, unpaired Student's test. All data are presented as mean ± SD. \**p* < 0.05, \*\**p* < 0.01.

#### Supplementary Figure 3. Knockout of CCL5 in astrocytes did not alter myeloid



#### cell infiltration

**A**, **B**. Representative flow cytometry pseudocolor dot plots showed gating strategy of dendritic cells (CD11c<sup>+</sup>) (**A**), monocytes (Ly6C<sup>high</sup>, Ly6C<sup>int</sup>) (**B**), and neutrophils (Ly6G<sup>+</sup>) (**B**) in Oil Sham, Tam Sham, Oil ICH and Tam ICH groups. **C**, **D**, **E**, **F**. The number of dendritic cells (CD11c<sup>+</sup>) (**C**), monocytes (Ly6C<sup>high</sup>, Ly6C<sup>int</sup>) (**D**, **E**), and neutrophils (Ly6G<sup>+</sup>) per 10000 cells in Oil Sham, Tam Sham, Oil ICH and Tam ICH and Tam ICH groups. n = 3 mice in Oil Sham and Tam Sham groups (1 female, 2 male), n = 4 mice in Oil ICH and Tam ICH groups (1 female, 3 male). (**C**, **E**, **F**) One-way ANOVA with Tukey's multiple

comparisons test. (**D**) Brown-Forsythe and Welch ANOVA tests with Dunnett T3 test. All data are presented as mean  $\pm$  SD. ns, no significance.



#### Supplementary Figure 4. Schematic diagram of the flow cytometry gating

strategy for infiltrating peripheral immune cells

**A**, **B**. Representative flow cytometry pseudocolor dot plots showed the gating strategy of lymphocytes (**A**) and myeloid cells (**B**).

#### Supplementary Figure 5. tSNE analysis of infiltrating peripheral immune cells



## in hemorrhagic stroke brain

A. Gating strategy of Flowjo tSNE analysis of lymphocytes. B. tSNE analysis of
CD3+ T cells. C. Bar graph showed that number of CD3+/CD4+ T cells and
CD3+/CD8+ T cells. D. Gating strategy of Flowjo tSNE analysis of myeloid cells. E.
tSNE analysis of CD45+CD11b+ cells. F. Bar graph showed that number of

neutrophil, monocyte/macrophage, microglia and DC cells.

## Supplementary Figure 6. Expression of CCR1, CCR3, CCR4 and CCR5 in CD4<sup>+</sup> T



and CD 8<sup>+</sup> T cells

RT-PCR analysis of CCR1, CCR3, CCR4 and CCR5 in different immune cells at 3 days after ICH. n = 6 mice per group (all male). GAPDH was used as an internal control. Two-way ANOVA with Bonferroni multiple comparisons test. All data are presented as mean  $\pm$  SD. \*\*\*p < 0.001.

## Supplementary Figure 7. Schematic representation of the location of ICH



Location of the peri-hemorrhagic zone.

# Supplementary Figure 8. Specific knockout of CCL5 in astrocytes reduced neuronal apoptosis



**A.** Representative immunostaining images of NeuN (green) and TUNEL (red) in the hemorrhagic perifocal area at day 3 of ICH in Oil ICH and Tam ICH groups. Scale bar = 50  $\mu$ m. Arrows indicated the colocalization of NeuN with TUNEL. **B.** Number of apoptotic neurons in the hemorrhagic perifocal area at day 3 of ICH in Oil ICH and Tam ICH groups. n = 3 mice per group (all male). Two-sided, unpaired Student's test. All data are presented as mean ± SD. \**p* < 0.05.

Supplementary Figure 9. Viability of bEnd.3 cells that treated with EGTA and cocultured with CD8<sup>+</sup> T cells.



**A.** Viability of bEnd.3 cells treated with different concentration of EGTA for 24 h. n = 3 biologically independent bEnd.3 cell cultures. One-way ANOVA with Tukey's multiple comparisons test. **B.** Viability of bEnd.3 cells co-cultured with CD8<sup>+</sup> T cells at cell ratios of 1:2, 1:5, 1:10 and 1:20 for 24h and 48h. Two-way ANOVA with Bonferroni multiple comparisons test. n = 3 biologically independent bEnd.3 cell cultures. All data are presented as mean  $\pm$  SD. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001, ns, no significance.

Supplementary Figure 10. Raw data of western blot

![](_page_12_Figure_0.jpeg)

![](_page_12_Figure_1.jpeg)