

Supplementary Materials for  
**CD4<sup>+</sup> T cells aggravate hemorrhagic brain injury**

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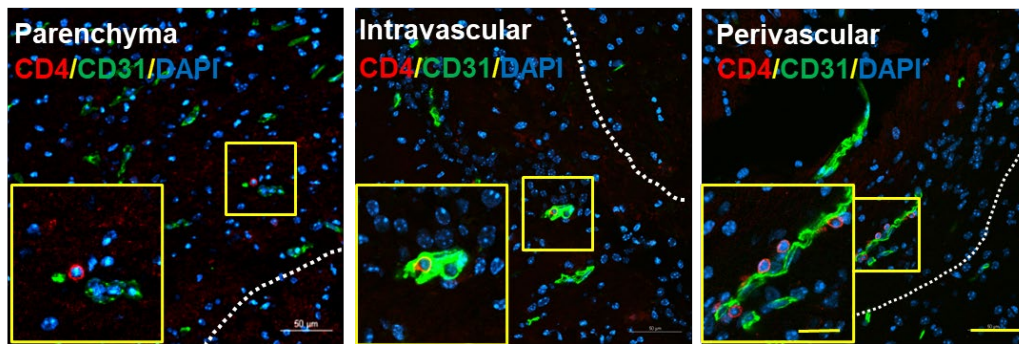
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*Sci. Adv.* **9**, eabq0712 (2023)  
DOI: 10.1126/sciadv.abq0712

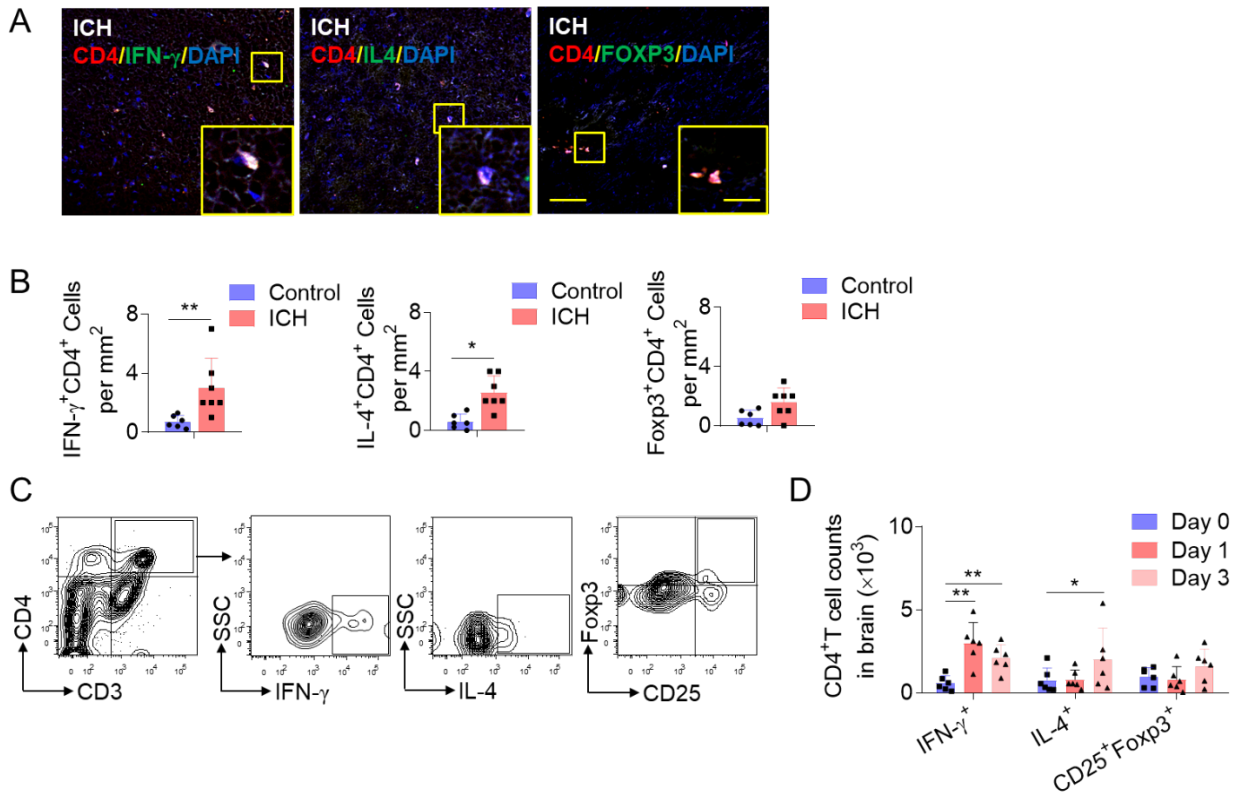
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Figs. S1 to S10  
Table S1

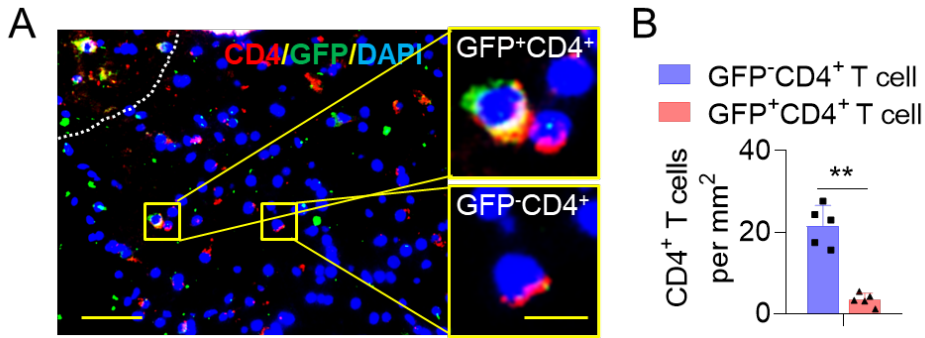
## Supplementary Materials



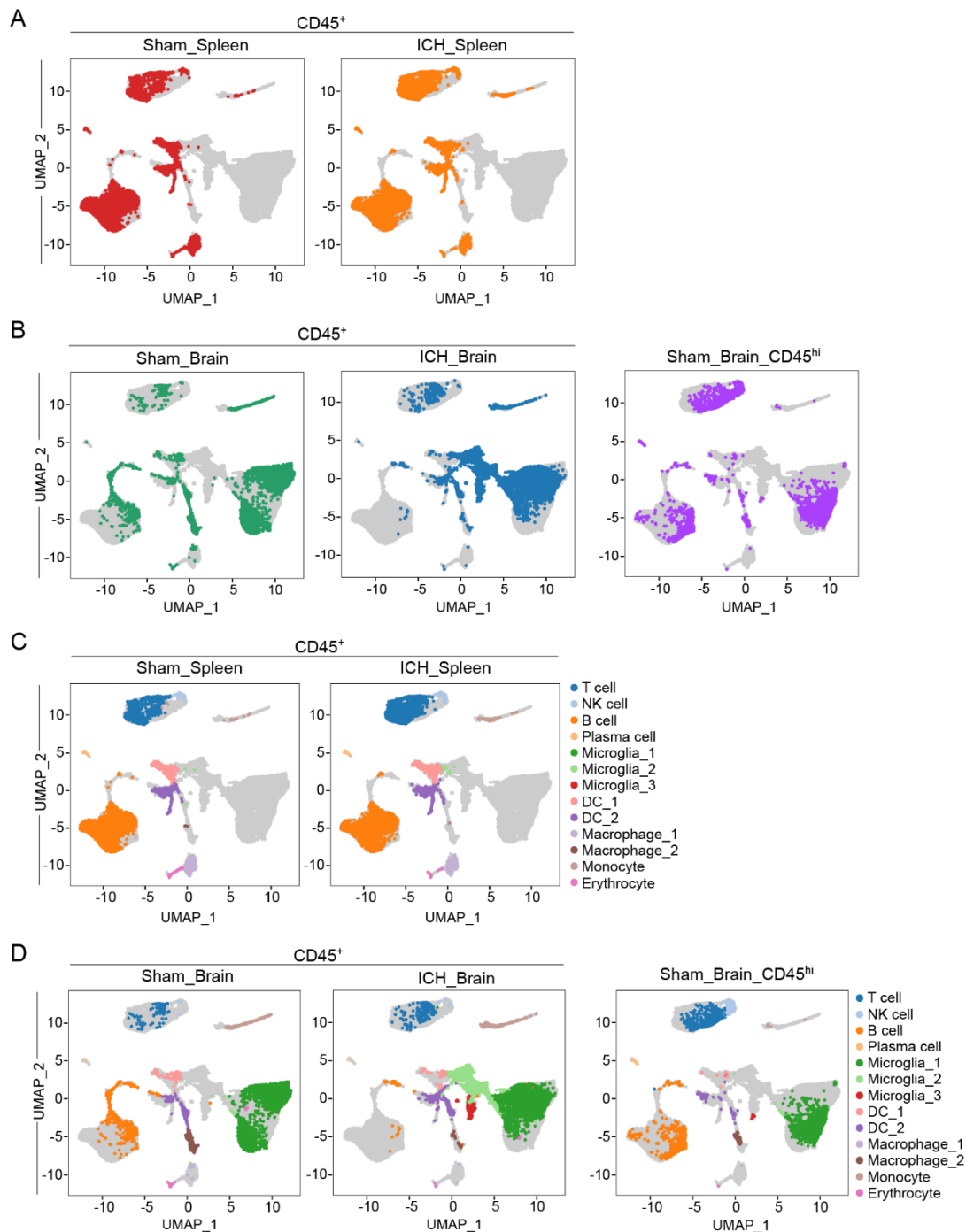
**Fig. S1. The location of CD4<sup>+</sup> T cells in ICH mouse brain.** Immunostaining shows CD4<sup>+</sup> T cells in the perihematomal regions of mice 3 days post ICH surgery. Scale bar: 50 μm (inset: 20 μm).



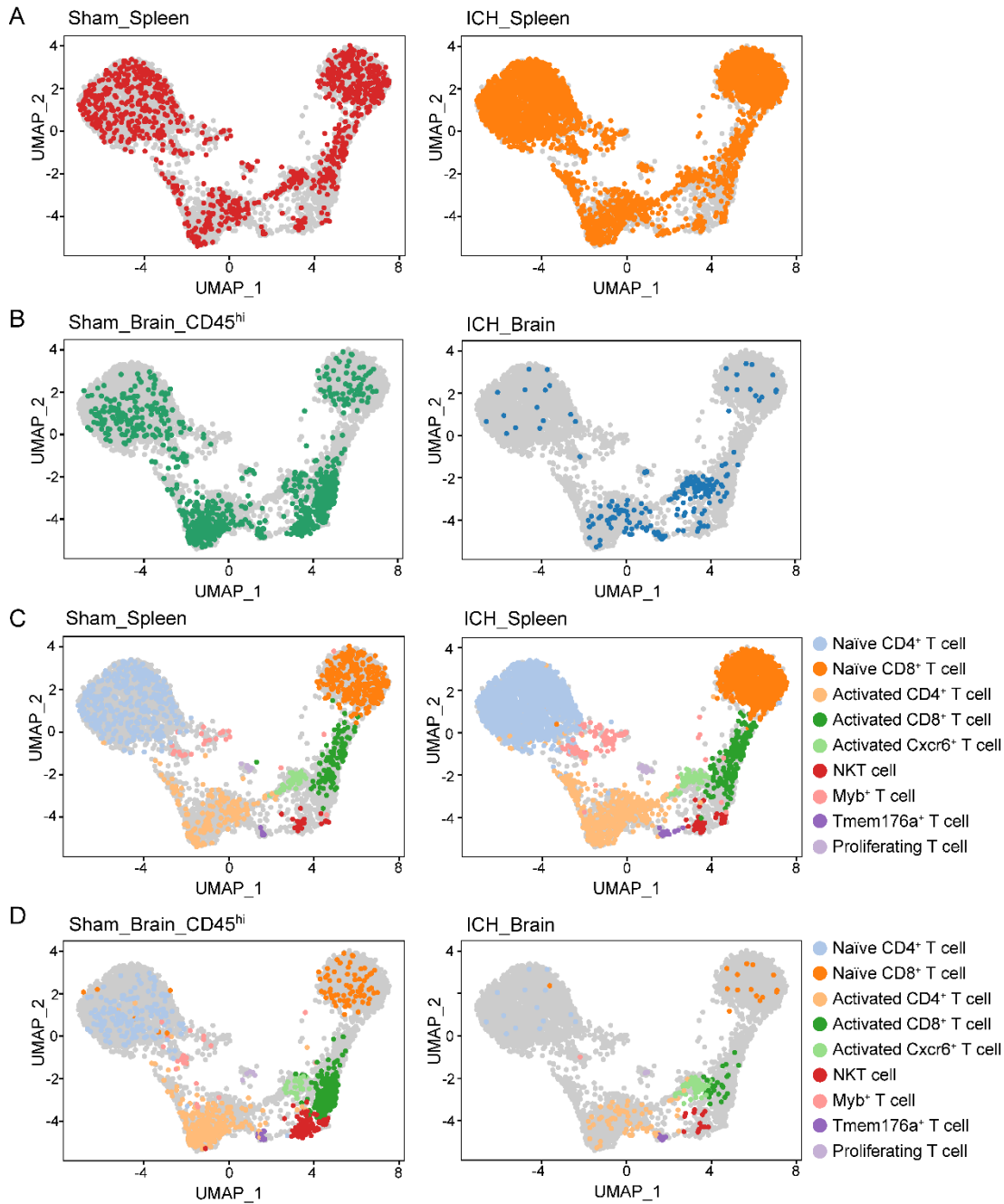
**Fig. S2. T cell subsets in ICH patients and murine models. A-B.** Perihematoma tissues are obtained from brain basal ganglia of ICH patients, who underwent urgent evacuation of hematoma, within 72-hour of onset. Brain tissues for control cases were obtained from individuals that passed from non-neurologic diseases and were without history of neurological or neuropsychiatric conditions, selected tissue sections were region-matched with ICH tissues. Immunostaining (**A**) and quantification (**B**) of Th1 (IFN- $\gamma$ <sup>+</sup>CD4<sup>+</sup>), Th2 (IL-4<sup>+</sup>CD4<sup>+</sup>), and regulatory T (Fcpx3<sup>+</sup>CD4<sup>+</sup>) cells in brain sections from ICH patients in the perihematoma area after ICH and control subjects without neurological disorders. Scale bar: 50  $\mu$ m; inset: 20  $\mu$ m. Quantification was averaged as positive cells per mm<sup>2</sup>. In A and B, ICH patients: n = 7; controls: n = 6. Two-tailed unpaired Student's t-test followed by Mann-Whitney test. Mean  $\pm$  s.e.m. \*p<0.05; \*\*p< 0.01. **C-D.** Flow cytometry plots (**C**) and quantification (**D**) show CD4<sup>+</sup> T cell subsets Th1 (IFN- $\gamma$ <sup>+</sup>CD4<sup>+</sup>), Th2 (IL-4<sup>+</sup>CD4<sup>+</sup>), and regulatory T (CD25<sup>+</sup>Fcpx3<sup>+</sup>CD4<sup>+</sup>) cells in ICH mouse brain from day 0 to day 3. n = 6 mice per group. One-way analysis of variance (ANOVA) followed by Tukey post-hoc test. Mean  $\pm$  SD. \*p<0.05; \*\*p< 0.01.



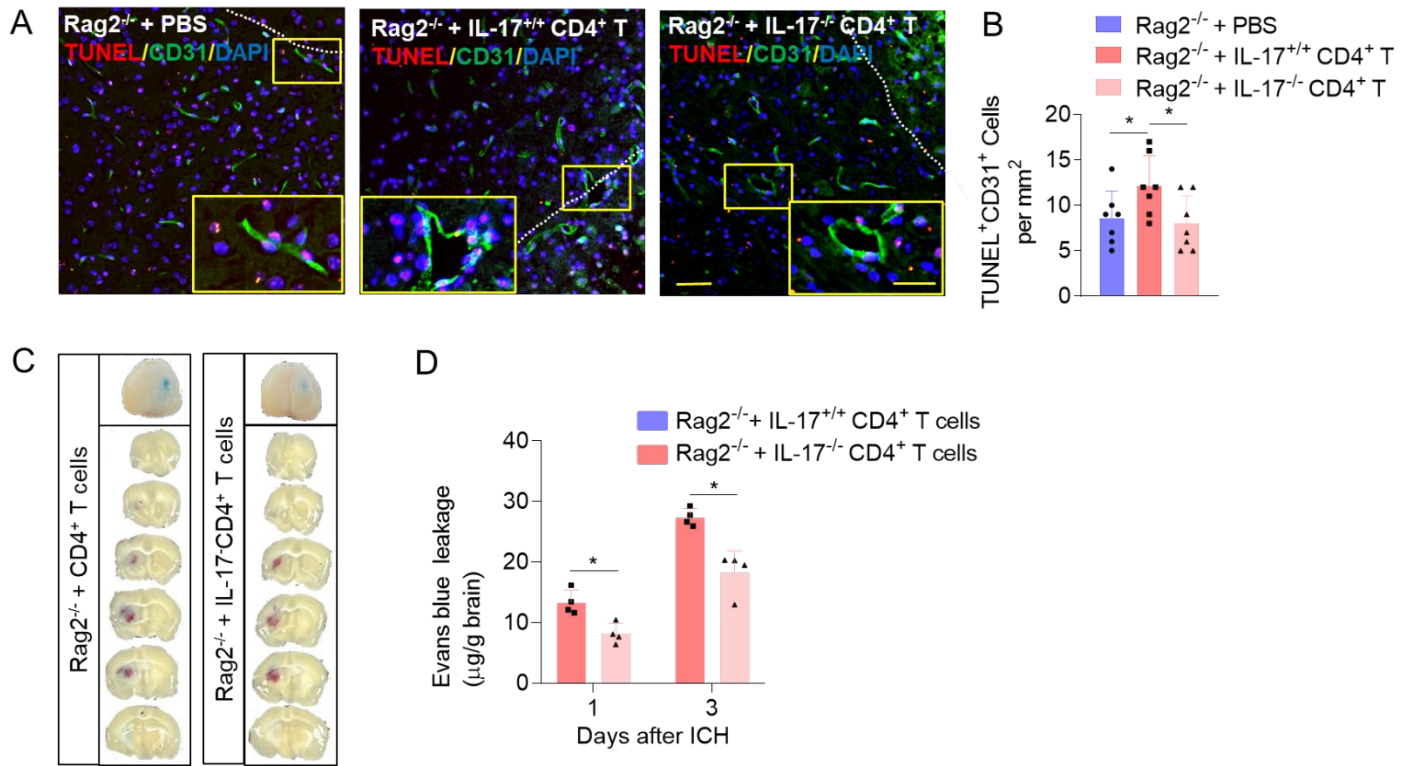
**Fig. S3. Minimal numbers of GFP<sup>+</sup>CD4<sup>+</sup> T cells appeared in mouse PHE areas 72h post ICH surgery.** Blood mononuclear cells from UBC-GFP mice were injected into the brain of wild-type mice with ICH. After 72h, GFP<sup>+</sup>CD4<sup>+</sup> T cells presented in PHE areas were calculated. Immunostaining (**A**) and quantification (**B**) of GFP<sup>-</sup>CD4<sup>+</sup> and GFP<sup>+</sup>CD4<sup>+</sup> T cells in the PHE areas of mice 3 days post ICH surgery. Scale bar: 40  $\mu$ m (inset: 10  $\mu$ m). n=5 mice per group. Two-tailed unpaired Student's t-test followed by Mann-Whitney test. Mean  $\pm$  SD. \*\*p< 0.01.



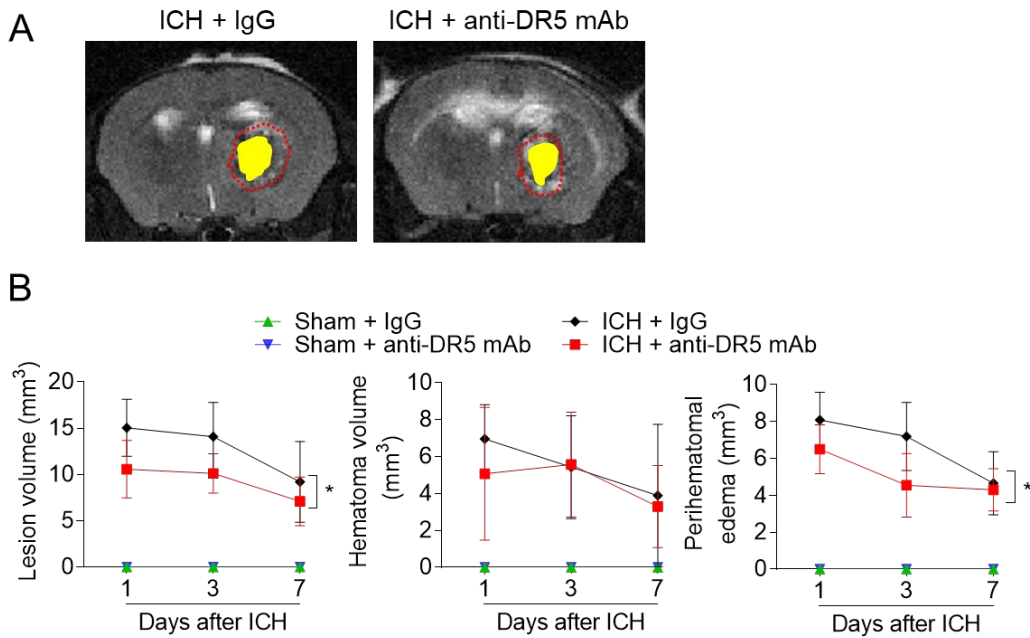
**Fig. S4. The individual UMAP of cell clusters isolated from brain and spleen of sham and ICH mice.** CD45<sup>+</sup> cells were isolated from splenocytes or brain tissues of C57BL/6 mice 72 hours after ICH induction using fluorescence activated cell sorting. CD45<sup>+</sup> cells splenic (Sham spleen) or brain (Sham brain\_CD45<sup>+</sup>) cells from sham-operated mice were used as controls, for unbiasedly single-cell RNA mapping. The optimized FACS-sorting were performed on CD45<sup>hi</sup> cell population, to obtain enough CD4<sup>+</sup> T cells in sham brains for sub-clustering and data processing (Sham brain\_CD45<sup>hi</sup>). The single-cell RNA-sequencing was performed using the 10x Genomics Chromium platform. Uniform Manifold Approximation and Projection (UMAP) plot along components 1 and 2 for all high-quality single cells from ICH brain, ICH spleen, sham brain, sham spleen colored by group (**A-B**) and cell type (**C-D**) were displayed individually.



**Fig. S5.** The individual UMAP of CD3<sup>+</sup> T cells were re-clustered from CD45<sup>+</sup> cell populations based on the single-cell RNA-sequencing data. **A-B.** UMAP plot showing T cell subtypes from ICH brain, ICH spleen, sham brain (CD45<sup>hi</sup>), sham spleen, colored by group (**A-B**) and cell type (**C-D**) were displayed individually.

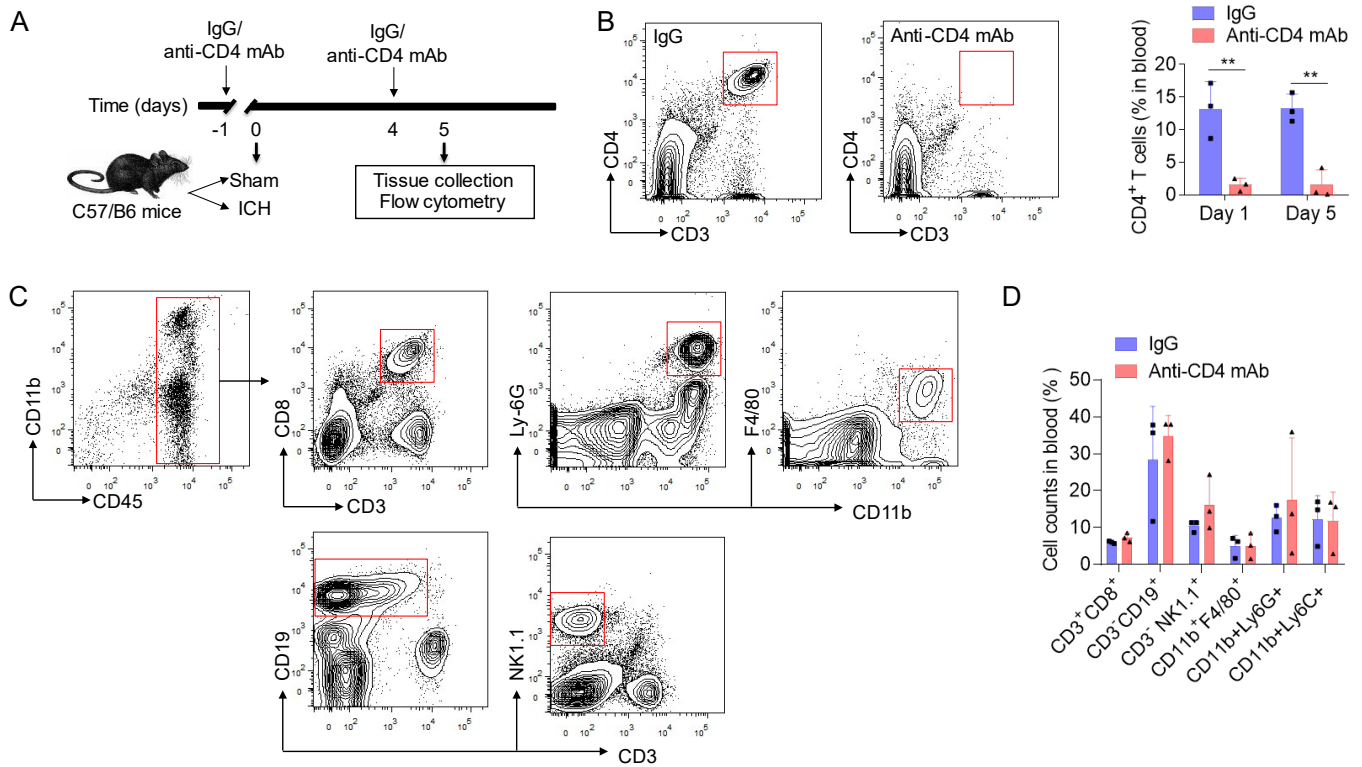


**Fig S6. CD4<sup>+</sup> T cell-induced endothelial apoptosis and BBB disruption were worsened by IL-17 signaling. A-B.** Immunostaining (**A**) and quantification (**B**) of TUNEL in CD31<sup>+</sup> endothelial cells in Rag2<sup>-/-</sup> mice, Rag2<sup>-/-</sup> mice receiving IL-17<sup>+/+</sup> CD4<sup>+</sup> cells and IL-17<sup>-/-</sup> CD4<sup>+</sup> cells at day 3 after ICH. Scale bar: 50 µm; inset: 20 µm. n=7 mice per group. Mann-Whitney test. **C-D.** The concentrations of Evans blue were measured at 1 and 3 days after ICH in Rag2<sup>-/-</sup> mice receiving IL-17<sup>+/+</sup> CD4<sup>+</sup> cells or IL-17<sup>-/-</sup> CD4<sup>+</sup> cells separately. n=4 mice per group. Mann-Whitney test. Mean ± SD. \*p< 0.05.

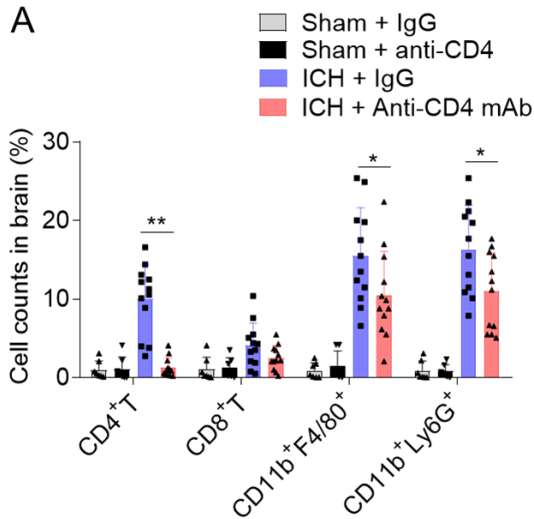


**Fig S7. Disturbed DR5 pathway effectively blocked ICH injury in mouse brain. A.** Mice were injected intraperitoneally with 100  $\mu$ g anti-DR5 mAb (MD5-1) to deplete TRAIL receptor DR5 at 24 hours before ICH induction. **B.** Quantification of lesion volume, hematoma and PHE volume were calculated in the indicated groups. n=5 mice per group. Two-way ANOVA accompanied by Tukey post hoc test. Mean  $\pm$ SD. \*p< 0.05.

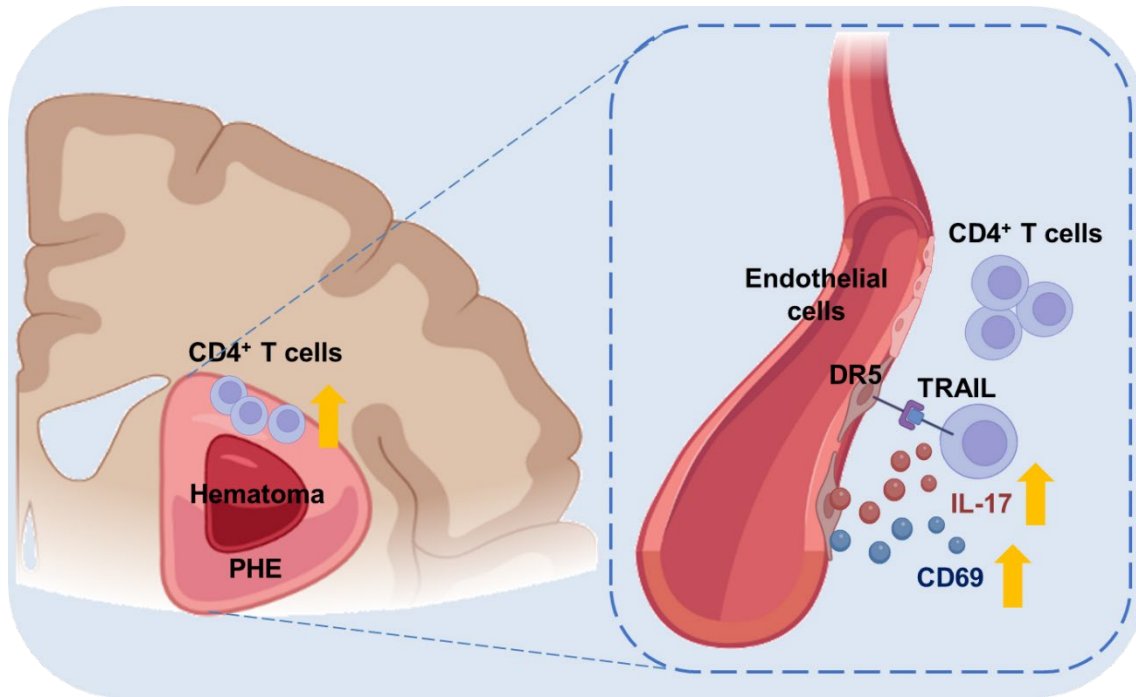




**Fig. S8. Effect of CD4<sup>+</sup> T cells depletion with anti-CD4 mAb treatment.** CD4<sup>+</sup> T cells in peripheral blood were detected from mice receiving IgG control or anti-CD4 mAb, separately. **A-B.** The schematic graph showing the treatment of IgG control or anti-CD4 mAb (**A**). Anti-CD4 monoclonal antibody was intraperitoneally injected at 24 hours before ICH induction, and every five days until the end of experiments. CD4<sup>+</sup> T cells were evaluated by flow cytometry analysis at day 1 and day 5 post model induction (**B**). **C-D.** Flow cytometry gating strategy and quantification of lymphocyte subsets in peripheral blood at day 5 post model induction. n=3 mice per group. Mann-Whitney test. Mean  $\pm$  SD. \*\*p< 0.01.



**Fig. S9. Depletion of CD4<sup>+</sup> T cells reduce leucocyte infiltration in brain of ICH mice as compared to sham control.** Single cell suspensions from brain tissue were collected at day 3 after ICH or sham mice. **A.** Counts of the immune cell populations in brains from sham or ICH mice receiving anti-CD4 mAb or IgG, including brain-infiltrating neutrophils (CD11b<sup>+</sup>CD45<sup>hi</sup>Ly6G<sup>+</sup>), monocytes/macrophages (CD11b<sup>+</sup>CD45<sup>hi</sup>F4/80<sup>+</sup>), CD4<sup>+</sup> T cells (CD45<sup>hi</sup>CD3<sup>+</sup>CD4<sup>+</sup>) and CD8<sup>+</sup> T cells (CD45<sup>hi</sup>CD3<sup>+</sup>CD8<sup>+</sup>). n =12 per group. Two-tailed unpaired Student's t-test. Mean  $\pm$  SD. \*p< 0.05, \*\*p< 0.01.



**Fig S10. CD4<sup>+</sup> T cells are recruited into the brain and commit secondary neural injury involving pro-inflammatory and pro-apoptotic actions following hemorrhagic stroke.** After hemorrhagic stroke, CD4<sup>+</sup> T cells are preferentially recruited to and activated in the ICH brain. CD4<sup>+</sup> T cells accumulated in the peri-hematoma region and drives local inflammation via IL-17 production and engagement of endothelial bearing cell death receptor, leading to diminishing BBB integrity and augmenting brain edema. PHE, perihematomal edema. TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

**Table S1. Information of signature genes in individual cell subtypes based on UMAP cluster.**

gene	avg_logFC	pct.1	pct.2	p_val	p_val_adj	cluster
Trbc2	2.653518658	0.919	0.04	0	0	T cell
Cd3d	2.123214012	0.875	0.024	0	0	T cell
Trbc1	2.074681306	0.556	0.032	0	0	T cell
Ms4a4b	2.071596993	0.868	0.092	0	0	T cell
Cd3g	2.02811362	0.851	0.021	0	0	T cell
Gzma	3.978135255	0.741	0.01	0	0	NK cell
Ccl5	3.359740814	0.991	0.093	0	0	NK cell
Nkg7	2.767603138	0.887	0.08	0	0	NK cell
Klre1	2.28178449	0.655	0.011	0	0	NK cell
Klra4	2.267537558	0.445	0.002	0	0	NK cell
Cd79a	3.049599166	0.983	0.051	0	0	B cell
Ly6d	2.882041749	0.952	0.064	0	0	B cell
Ms4a1	2.808811475	0.959	0.04	0	0	B cell
Ebf1	2.718845957	0.979	0.039	0	0	B cell
Cd79b	2.586438193	0.96	0.163	0	0	B cell
Jchain	5.750721327	0.991	0.084	0	0	Plasma cell
Iglv1	2.715204148	0.797	0.015	0	0	Plasma cell
Derl3	1.617681629	0.915	0.018	0	0	Plasma cell
Eaf2	1.50956576	0.934	0.018	0	0	Plasma cell
Fkbp11	1.415229399	0.788	0.025	0	0	Plasma cell
Cst3	3.054318658	1	0.659	0	0	Microglia_1
Hexb	2.99280809	1	0.439	0	0	Microglia_1
Cx3cr1	2.962822992	0.998	0.164	0	0	Microglia_1
Sparc	2.877620467	0.997	0.116	0	0	Microglia_1
P2ry12	2.648085887	0.987	0.103	0	0	Microglia_1
Spp1	3.062447573	0.9	0.243	0	0	Microglia_2
Fabp5	2.983279124	0.857	0.217	0	0	Microglia_2
Arg1	2.93347129	0.725	0.073	0	0	Microglia_2
Pf4	2.807707945	0.775	0.086	0	0	Microglia_2
Hmox1	2.602153266	0.937	0.338	0	0	Microglia_2
Ttr	5.432057613	1	0.368	0	0	Microglia_3
Enpp2	4.156174472	0.996	0.191	0	0	Microglia_3
Ptgds	2.665228579	0.887	0.045	0	0	Microglia_3
Clu	2.530729453	0.899	0.038	0	0	Microglia_3
Trpm3	2.249992149	0.873	0.02	0	0	Microglia_3
Tbc1d4	2.363980654	0.666	0.083	0	0	DC_1
H2-Eb1	2.085894421	0.997	0.424	0	0	DC_1
H2-Ab1	2.069165752	0.993	0.468	0	0	DC_1
H2-Aa	1.989427664	0.988	0.462	0	0	DC_1
S100a4	1.881347798	0.807	0.151	0	0	DC_1
Lyz2	2.741214797	0.999	0.443	0	0	DC_2
Ifitm3	2.731779222	1	0.248	0	0	DC_2
S100a4	2.251648454	0.936	0.148	0	0	DC_2
Plac8	2.126483677	0.968	0.313	0	0	DC_2
Cybb	2.033165528	0.994	0.352	0	0	DC_2

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Ly6c2	2.514870582	0.99	0.091	0	0	DC_3
Runx2	2.36656234	0.984	0.056	0	0	DC_3
Ccr9	2.356723604	0.927	0.038	0	0	DC_3
Cd209d	2.274470313	0.828	0.006	0	0	DC_3
Cox6a2	1.984111431	0.792	0.029	0	0	DC_3
Vcam1	4.335884502	1	0.076	0	0	Macrophage
Slc40a1	3.579870404	1	0.226	0	0	Macrophage
Axl	2.736382786	0.996	0.066	0	0	Macrophage
Mrc1	2.704581856	0.995	0.067	0	0	Macrophage
Slpi	2.618409231	0.951	0.08	0	0	Macrophage
S100a9	5.762431984	0.91	0.149	0	0	Monocyte
S100a8	5.600455883	0.937	0.134	0	0	Monocyte
Cxcl2	4.38968521	0.803	0.135	0	0	Monocyte
Retnlg	4.281034334	0.579	0.022	0	0	Monocyte
Il1b	4.042037472	0.929	0.098	0	0	Monocyte
Slc4a1	3.285102082	1	0.003	0	0	Erythrocyte
Alas2	3.215354615	1	0.004	0	0	Erythrocyte
Gypa	3.032222711	0.983	0.002	0	0	Erythrocyte
Hemgn	2.907586008	0.883	0.003	0	0	Erythrocyte
Snca	2.794599206	1	0.004	0	0	Erythrocyte
Flt1	3.011654877	0.504	0.021	0	0	Ptprc-
Cldn5	2.939117166	0.408	0.007	0	0	Ptprc-
Igfbp7	2.548746486	0.535	0.02	0	0	Ptprc-
Ptn	2.478432163	0.539	0.01	0	0	Ptprc-
Sparcl1	2.434192829	0.649	0.019	0	0	Ptprc-

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