Figure S1: FACS gating strategy used to isolate naïve $CD4^+$ T cells ($CD45^+CD4^+CD25^-CD44^{low}CD62L^{high}$).

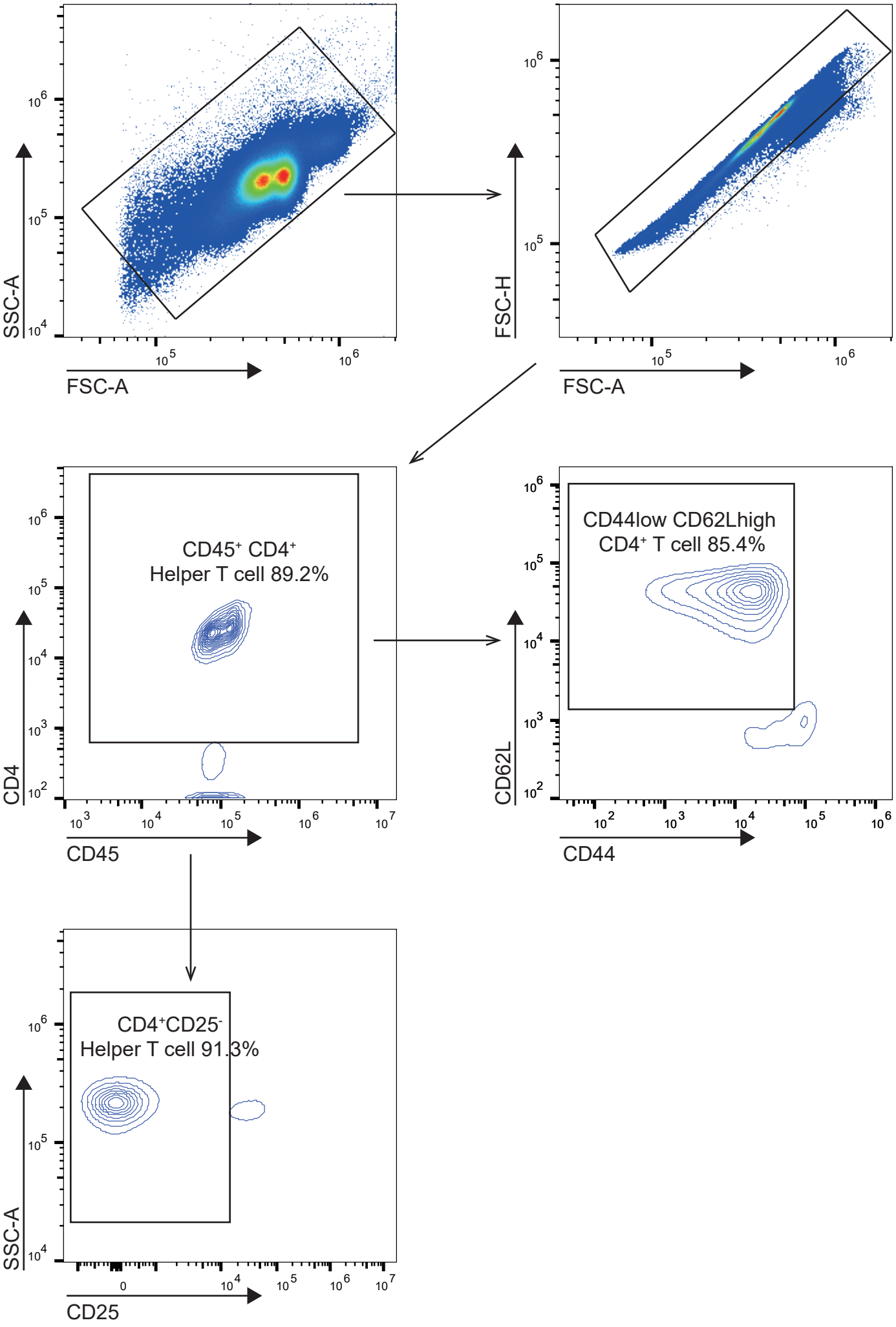
Figure S2: (A) Linear discriminant analysis effect size (LEfSe) showing statistically significant differences in gut microbiota composition between SPF and ABX groups. Taxa with LDA threshold > 4 are shown (n = 5 per group). (B) Bacterial taxonomic composition at the genus level in SPF and ABX mice on day 3 post-ICH (n = 5 per group).

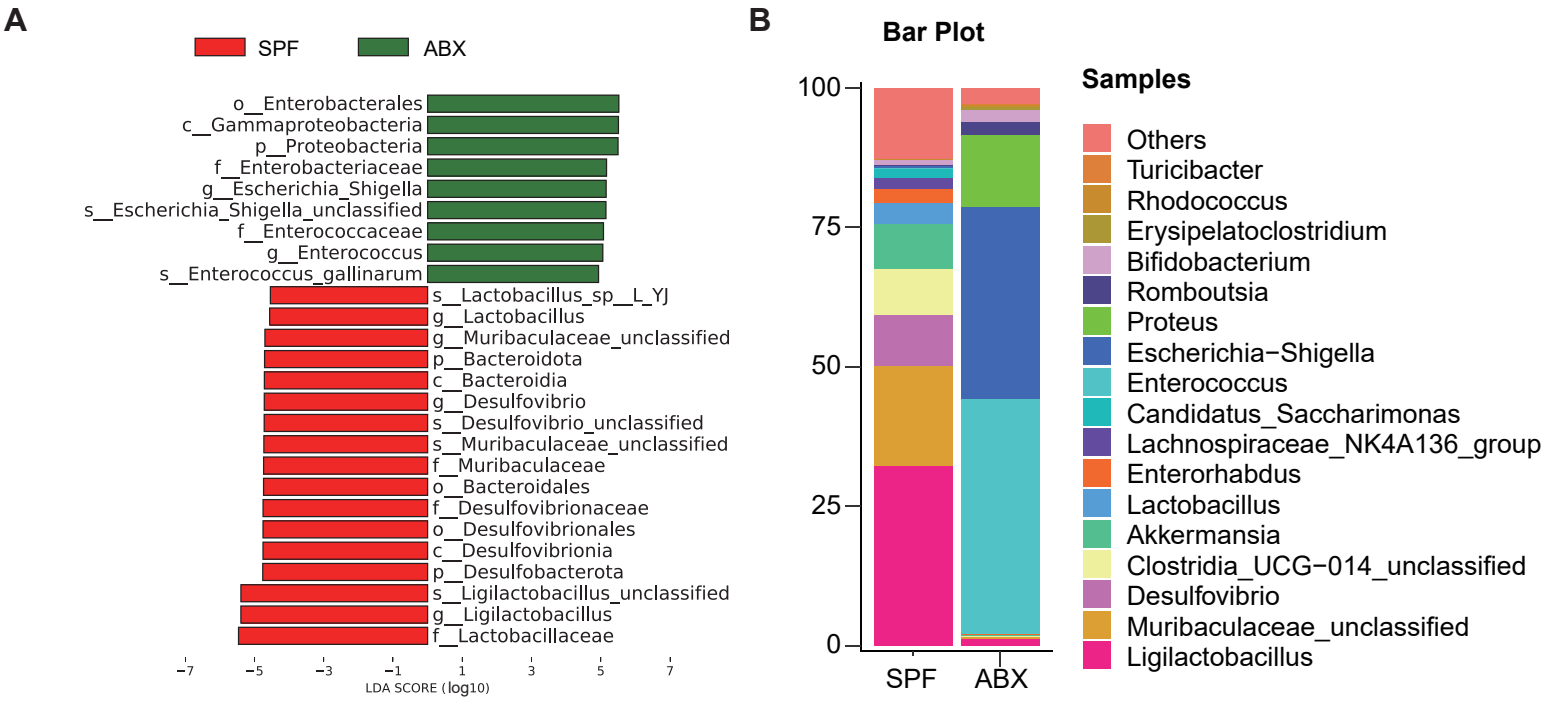
Figure S3: Treg proliferation and tissue distribution following after ICH. (A) FACS gating strategy to isolate proliferating $Ki67^+$ Tregs ($LIVE/DEAD^-CD45^{int/hi}CD4^+CD25^+Foxp3^+Ki67^+$). Percentages of $Ki67^+$ Tregs in the brain on day 3 post-ICH, assessed in SPF, ABX, and FMT groups. Data are presented as mean ± SD (n = 6 per group). (B) Percentages of Tregs in the thymus (top) and blood (bottom) of SPF, ABX, and FMT groups, determined by flow cytometry. Data are presented as mean ± SD (n = 6 per group). (C) Percentages of $Ki67^+$ Tregs in the lamina propria of the large intestine (left), small intestine (middle), and spleen (right) on day 3 post-ICH, assessed in SPF, ABX, and FMT groups. Data are presented as mean ± SD (n = 6 per group). ns = not significant.

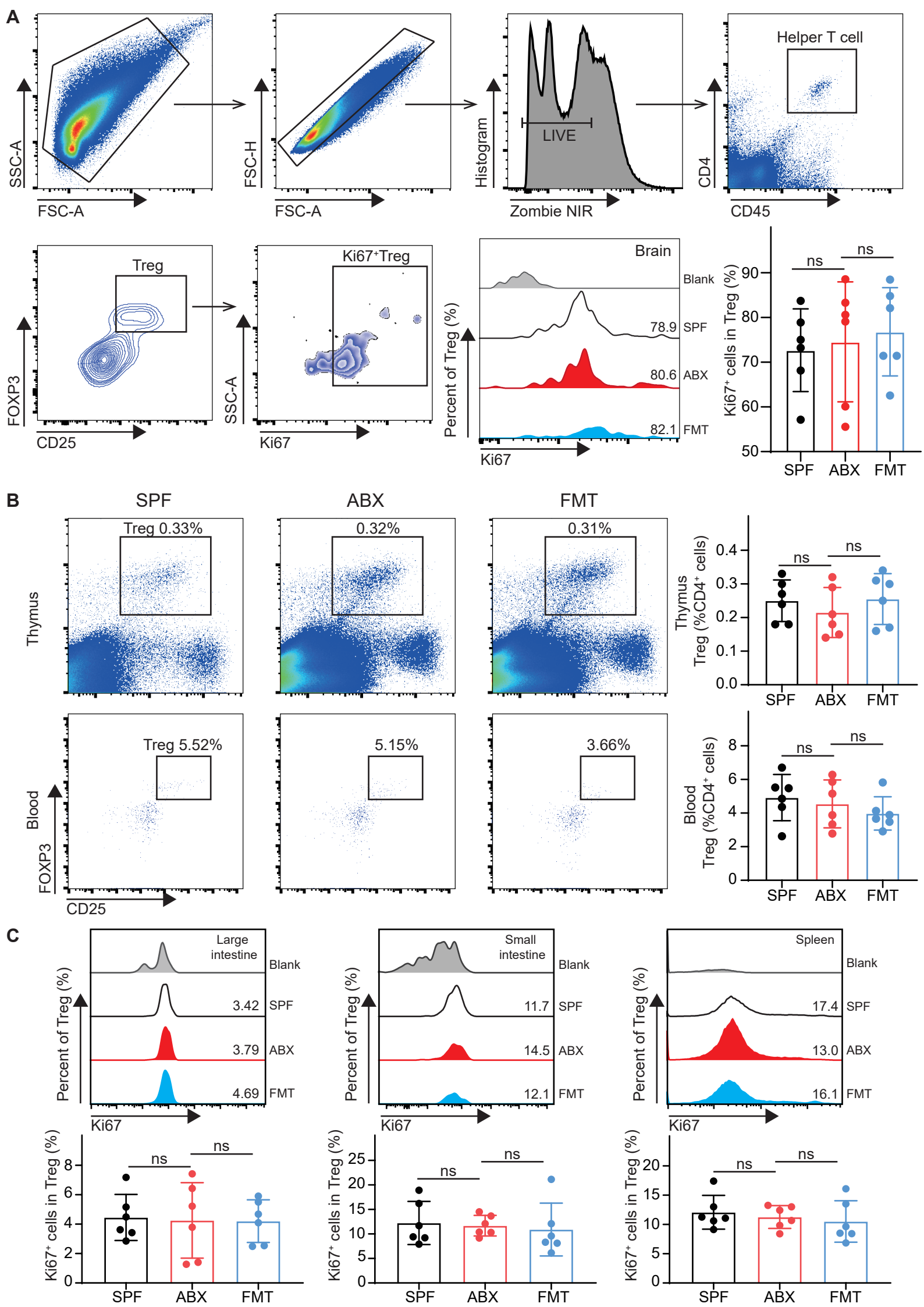
Figure S4: Principal component analysis of serum metabolites in SPF + Sham, SPF + ICH, ABX + Sham, and ABX + ICH mice (n = 8 per group), showing distinct separation among groups. ANOVA followed by Bonferroni post hoc test was used to determine significance. Fold-change scale was adjusted to 4; Uni $p < 0.05$ was considered statistically significant.

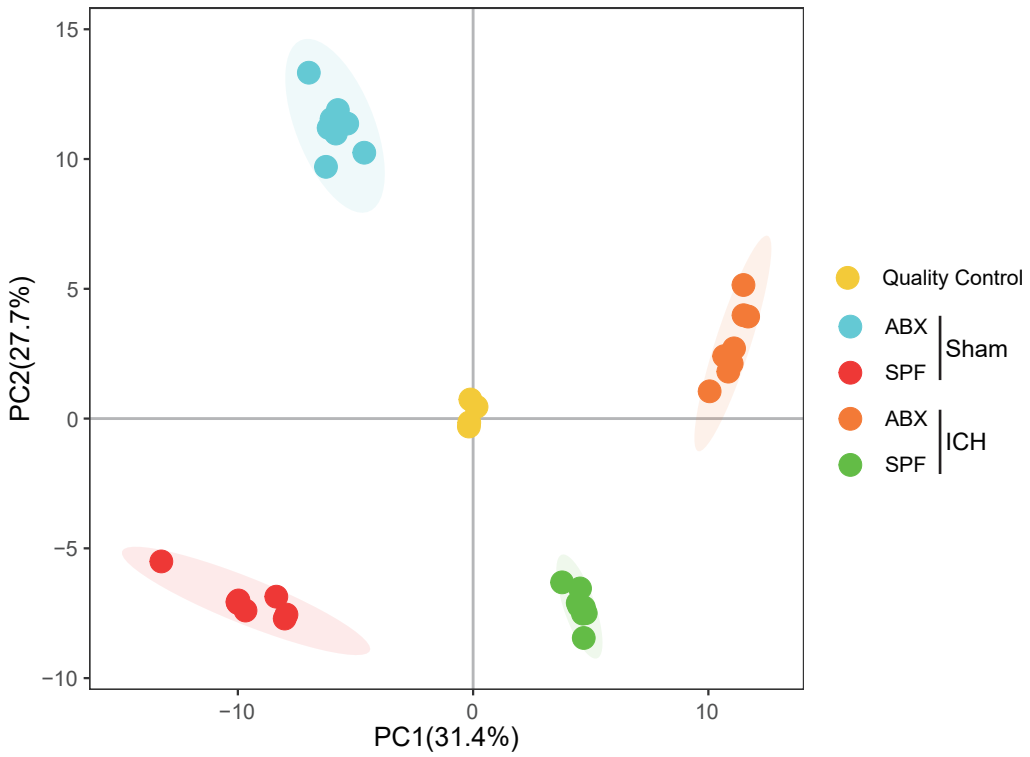
Figure S5: Schematic overview of the experimental design and downstream analytical methods. Abbreviations: ICH, intracerebral hemorrhage; MRI, Magnetic resonance imaging; WB, Western blotting; ELISA, Enzyme-linked immunosorbent assay; TβMCA, Tauro-β-muricholic acid; PCA, p-coumaric acid; TCA, taurocholic acid; PMA, phorbol-12-myristate-13-acetate.

Table S1. Sample sizes used in each experiment. Abbreviations: ICH, intracerebral hemorrhage; MRI, Magnetic resonance imaging; WB, Western blotting; ELISA, Enzyme-linked immunosorbent assay; TβMCA, Tauro-β-muricholic acid; PCA, p-coumaric acid; TCA, taurocholic acid; PMA, phorbol-12-myristate-13-acetate.

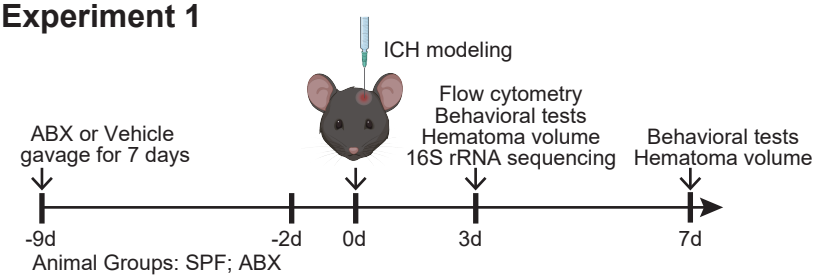

SSC-A
FSC-A
FSC-H
FSC-A
CD45⁺ CD4⁺
Helper T cell 89.2%
CD4
CD45
CD44low CD62Lhigh
CD4⁺ T cell 85.4%
CD62L
CD44
CD4⁺CD25⁻
Helper T cell 91.3%
SSC-A
CD25


A
SPF
ABX
o__Enterobacterales
c__Gammaproteobacteria
p__Proteobacteria
f__Enterobacteriaceae
g__Escherichia_Shigella
s__Escherichia_Shigella_unclassified
f__Enterococcaceae
g__Enterococcus
s__Enterococcus_gallinarum
s__Lactobacillus_sp__L_YJ
g__Lactobacillus
g__Muribaculaceae_unclassified
p__Bacteroidota
c__Bacteroidia
g__Desulfovibrio
s__Desulfovibrio_unclassified
s__Muribaculaceae_unclassified
f__Muribaculaceae
o__Bacteroidales
f__Desulfovibrionaceae
o__Desulfovibrionales
c__Desulfovibrionia
p__Desulfobacterota
s__Ligilactobacillus_unclassified
g__Ligilactobacillus
f__Lactobacillaceae
−7
−5
−3
−1
1
3
5
7
LDA SCORE (log10)
B
Bar Plot
100
75
50
25
0
SPF
ABX
Samples
Others
Turicibacter
Rhodococcus
Erysipelatoclostridium
Bifidobacterium
Romboutsia
Proteus
Escherichia−Shigella
Enterococcus
Candidatus_Saccharimonas
Lachnospiraceae_NK4A136_group
Enterorhabdus
Lactobacillus
Akkermansia
Clostridia_UCG−014_unclassified
Desulfovibrio
Muribaculaceae_unclassified
Ligilactobacillus

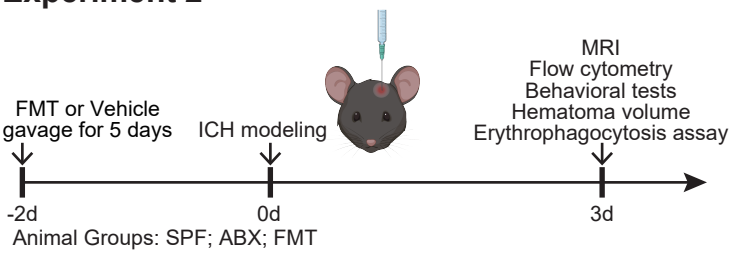

A
SSC-A
FSC-A
FSC-H
FSC-A
Histogram
LIVE
Zombie NIR
Helper T cell
CD4
CD45
Treg
FOXP3
CD25
Ki67⁺Treg
SSC-A
Ki67
Brain
Blank
78.9
SPF
80.6
ABX
82.1
FMT
Percent of Treg (%)
Ki67
Ki67⁺ cells in Treg (%)
ns
ns
SPF
ABX
FMT
B
SPF
ABX
FMT
Thymus
Treg 0.33%
0.32%
0.31%
Thymus Treg (%CD4⁺ cells)
Blood
Treg 5.52%
5.15%
3.66%
Blood Treg (%CD4⁺ cells)
FOXP3
CD25
C
Large intestine
Small intestine
Spleen
Blank
3.42
SPF
3.79
ABX
4.69
FMT
11.7
14.5
12.1
17.4
13.0
16.1
Percent of Treg (%)
Ki67
Ki67⁺ cells in Treg (%)


15
10
5
0
−5
−10
PC2(27.7%)
−10
0
10
PC1(31.4%)
Quality Control
ABX
SPF
Sham
ABX
SPF
ICH

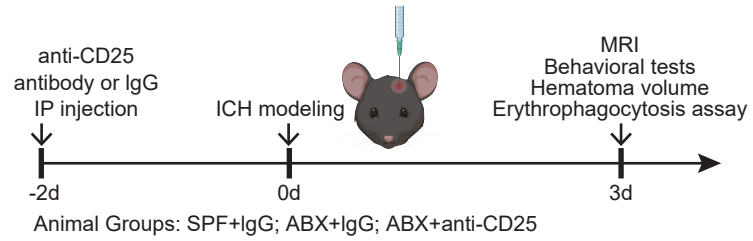
Experiment 1



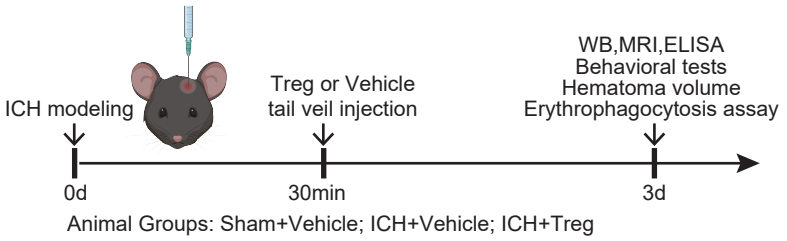
Experiment 2



Experiment 3



Experiment 4



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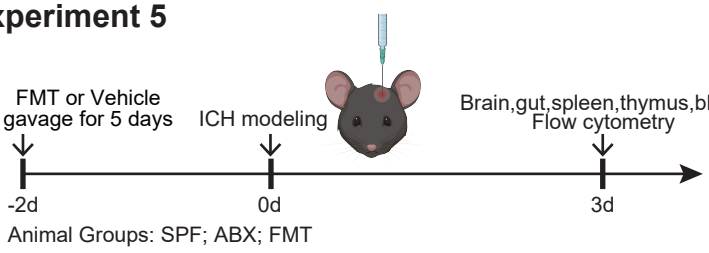

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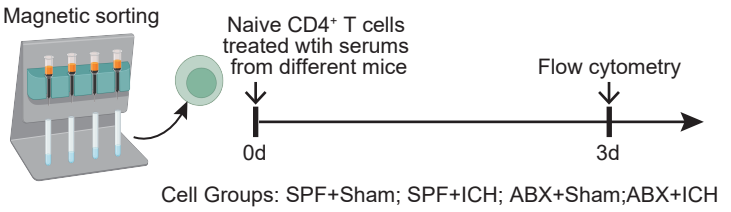
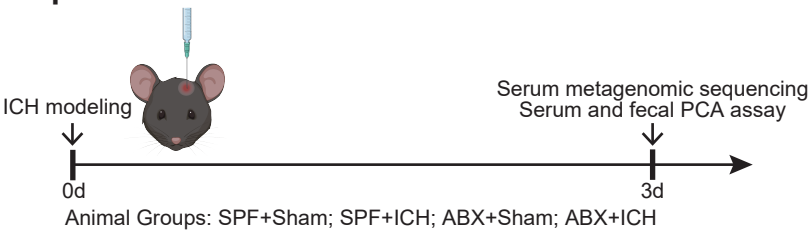
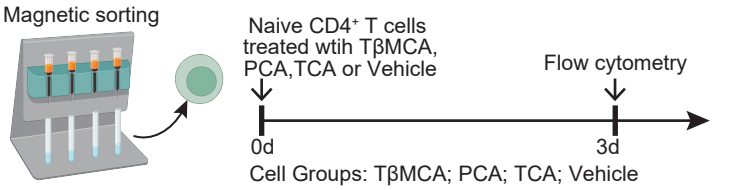


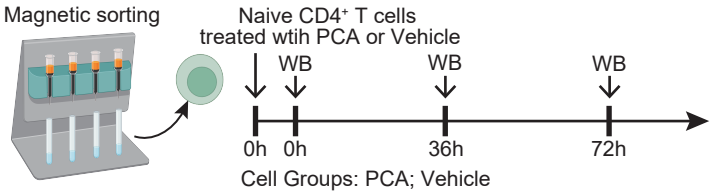
Experiment 7



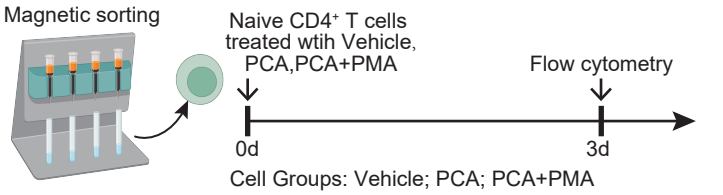
Experiment 8



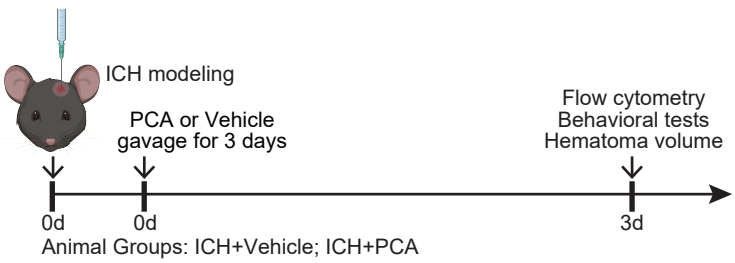
Experiment 9



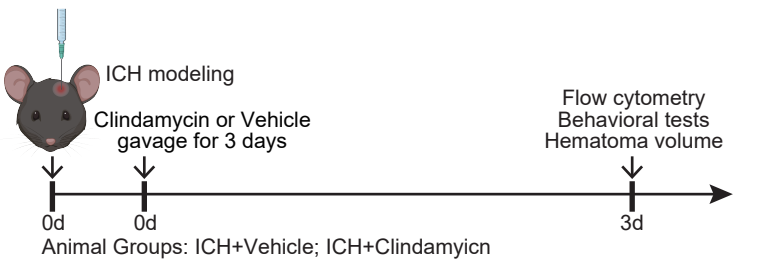
Experiment 10



Experiment 11



Experiment 12



Group	Survived	Dead	Excluded	Total	Usage
Experiment 1					1. 9 mice in each group in ICH 3d and ICH 7d were used for behavioral tests and hematoma volume measurement 2. 5 mice in each group in ICH 3d were used for 16S rRNA sequencing 3. 6 mice in each group in ICH 3d were used for flow cytometry
SPF	24	0	1	25	
ABX	24	0	0	24	
Experiment 2					1. 6 mice in each group were used for MRI 2. 9 mice in each group were used for flow cytometry 2. 9 mice in each group were used for behavioral tests and hematoma volume measurement 3. 6 mice in each group were used for erythrophagocytosis assay
SPF	30	1	1	32	
ABX	30	0	1	31	
FMT	30	1	2	33	
Experiment 3					1. 6 mice in each group were used for MRI 2. 9 mice in each group were used for behavioral tests and hematoma volume measurement 3. 6 mice in each group were used for erythrophagocytosis assay
SPF+IgG	21	1	0	22	
ABX+IgG	21	0	0	21	
ABX+anti-CD25	21	1	1	23	
Experiment 4					1. 6 mice in each group were used for WB 2. 9 mice in each group were used for MRI 3. 6 mice in each group were used for ELISA 4. 9 mice in each group were used for behavioral tests and hematoma volume measurement 5. 6 mice in each group were used for erythrophagocytosis assay
Sham+Vehicle	36	0	0	36	
ICH+Vehicle	36	2	2	40	
ICH+Treg	36	1	1	38	
Experiment 5					1. 6 mice in each group were used for brain, gut, spleen, thymus, blood Treg flow cytometry 2. 6 mice in each group were used for brain, gut, spleen, thymus $Ki67^+$ Treg flow cytometry
SPF	12	1	0	13	
ABX	12	0	1	13	
FMT	12	0	1	13	
Experiment 6					1. 6 wells of cells in each group were used for flow cytometry
SPF+Sham	6	0	0	6	
SPF+ICH	6	0	0	6	
ABX+Sham	6	0	0	6	
ABX+ICH	6	0	0	6	
Experiment 7					1. 8 mice in each group were used for serum metagenomic sequencing 2. 8 mice in SPF + Sham, SPF + ICH, and ABX + ICH group were used for serum and fecal PCA assay
SPF+Sham	16	0	0	16	
SPF+ICH	16	2	1	19	
ABX+Sham	8	0	0	8	
ABX+ICH	16	1	1	18	
Experiment 8					1. 6 wells of cells in each group were used for flow cytometry
TβMCA	6	0	0	6	
PCA	6	0	0	6	
TCA	6	0	0	6	
Vehicle	6	0	0	6	
Experiment 9					1. 6 wells of cells in each group at 0h, 36h, 72h were used for WB
Vehicle	18	0	0	18	
PCA	18	0	0	18	
Experiment 10					1. 6 wells of cells in each group were used for flow cytometry
Vehicle	6	0	0	6	
PCA	6	0	0	6	
PCA+PMA	6	0	0	6	
Experiment 11					1. 6 mice in each group were used for flow cytometry 2. 12 mice in each group were used for behavior tests 3. 9 mice in each group were used for hematoma volume measurement
ICH+Vehicle	27	2	0	29	
ICH+PCA	27	1	0	28	
Experiment 12					1. 6 mice in each group were used for flow cytometry 2. 12 mice in each group were used for behavior tests 3. 10 mice in each group were used for hematoma volume measurement
ICH+Vehicle	28	1	1	30	
ICH+Clindamycin	28	1	0	29	