Figure supplement legends

Figure S1. 50% withdrawal threshold to von Frey filament stimulation in the ipsilesional hind paw was unaffected by thalamic hemorrhage.

(A) No significant difference was found in the withdrawal threshold of the ipsilesional hind paw between the Control and thalamic hemorrhage (TH) groups ($p > 0.05$, group effect by two-way repeated measures ANOVA). (B) There was no significant difference in the motor function of the ipsilesional hind limb assessed by the ladder walk test ($p > 0.05$, group effect by two-way repeated measures ANOVA).

Figure S2. Microglia-related genes were upregulated in the ipsilesional thalamus and S1 but not in the spinal cord on day 4 after thalamic hemorrhage.

(A, B) Microglial-related genes were upregulated in the ipsilesional thalamus and in the ipsilesional S1 in the TH group, compared with the contralesional side in the TH group or with the ipsilesional side in the Control group (**$p < 0.01$, *$p < 0.05$, one-way measures ANOVA followed by Tukey's multiple comparisons test). (C) There were no significant differences in spinal microglia-related genes between the groups ($p > 0.05$, group effect by one-way repeated measures ANOVA).

Figure S3. Microglial depletion prevented the development of TH-induced allodynia in

796 female mice.

797 (A) Experimental design. The von Frey test was performed on female mice for three
798 groups; Control group (n = 6), TH group (n = 8), and TH+PLX group 1 (n = 10) as in
799 Figure 3. (B) The 50% withdrawal threshold in the TH group was significantly reduced
800 after hemorrhage compared with the Control group (**$p < 0.01$, *$p < 0.05$, two-way
801 repeated measures ANOVA followed by Tukey's multiple comparisons test). TH+PLX
802 group 1, which started PLX treatment before lesion induction, exhibited a higher
803 withdrawal threshold than the TH group ($^{\#\#}p < 0.01$, $^{\#}p < 0.05$, two-way repeated
804 measures ANOVA followed by Tukey's multiple comparisons test).

805

806 Figure S4. Long-term administration of CSF1R inhibitor (PLX3397) eliminated almost
807 all microglia in the brain but had little effect on macrophages and neutrophils.
808 The quantities of microglia, macrophages and neutrophils in the brain were analyzed by
809 flow cytometry with expression of CD11b, CD45, Ly6C, Ly6G and CX3CR1. (A) Dot
810 plots showing $CX3CR1^{high}$ positive cells as microglial population in $CD11b^{+}CD45^{low}$
811 $Ly6C^{low}Ly6G^{-}$ gated cells, $CX3CR1^{low}$ positive cells as macrophages in $CD11b^{+}$
812 $CD45^{high}Ly6C^{high}Ly6G^{-}$gated cells, and CX3CR1 negative cells as neutrophils in
813 $CD11b^{+}$ $CD45^{high}Ly6C^{high}Ly6G^{+}$gated cells. (B) The number of immune cells in the

814 brain tended to increase on day 4 after hemorrhage (the left three figures). Comparison
815 of the cell population between groups on day 4 post hemorrhage (far right figure). The
816 proportion of neutrophils and macrophages was unaffected among the three groups ($p >$
817 0.05, group effect by one-way repeated measures ANOVA). More than 99.5% of
818 microglia were eliminated (*$p < 0.01$, one-way measures ANOVA followed by Tukey's
819 multiple comparisons test).
820
821 Figure S5. Microglial depletion by the oral administration of PLX3397 exerted no effect
822 on lesion volume after thalamic hemorrhage.
823 (A) Lesion volume was calculated 4 days after hemorrhage induction. There was no
824 difference between the thalamic hemorrhage (TH) group and TH+PLX group 1
825 (corresponding to Figure 3). The PLX treatment was initiated from 21 days before
826 hemorrhage until day 4 after hemorrhage ($p > 0.05$, unpaired t test). (B) No difference
827 was detected between the TH group and TH+PLX group 1 (PLX treatment was given
828 from 21 days before hemorrhage until day 7 after hemorrhage) on post-lesion day 7 ($p >$
829 0.05, unpaired t test). (C) Lesion volume at 21 days post-hemorrhage. No difference
830 was detected between the TH group and PLX-treated groups ($p > 0.05$, group effect by
831 one-way measures ANOVA).

Figure S1

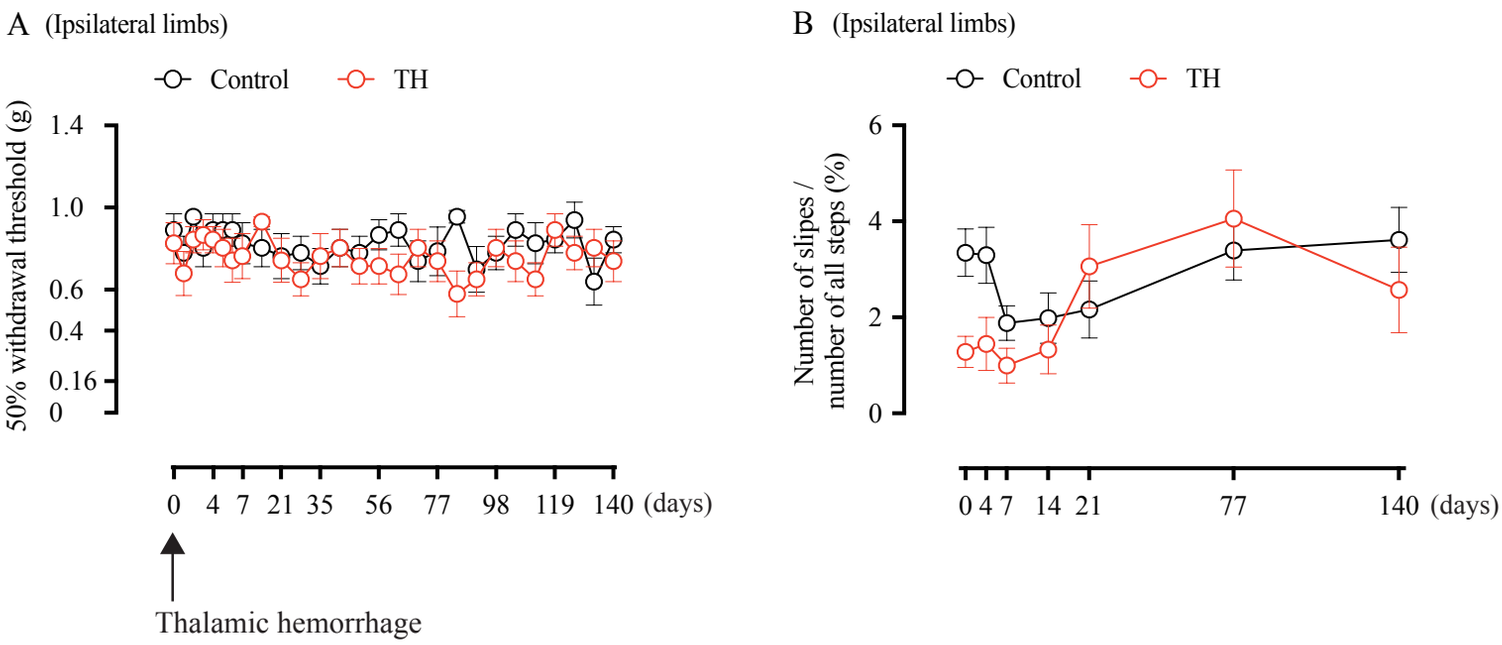

A (Ipsilateral limbs)
Control
TH
50% withdrawal threshold (g)
1.4
1.0
0.6
0.4
0.16
0
0 4 7 21 35 56 77 98 119 140 (days)
Thalamic hemorrhage
B (Ipsilateral limbs)
Control
TH
Number of slipes / number of all steps (%)
6
4
2
0
0 4 7 14 21 77 140 (days)

Figure S2

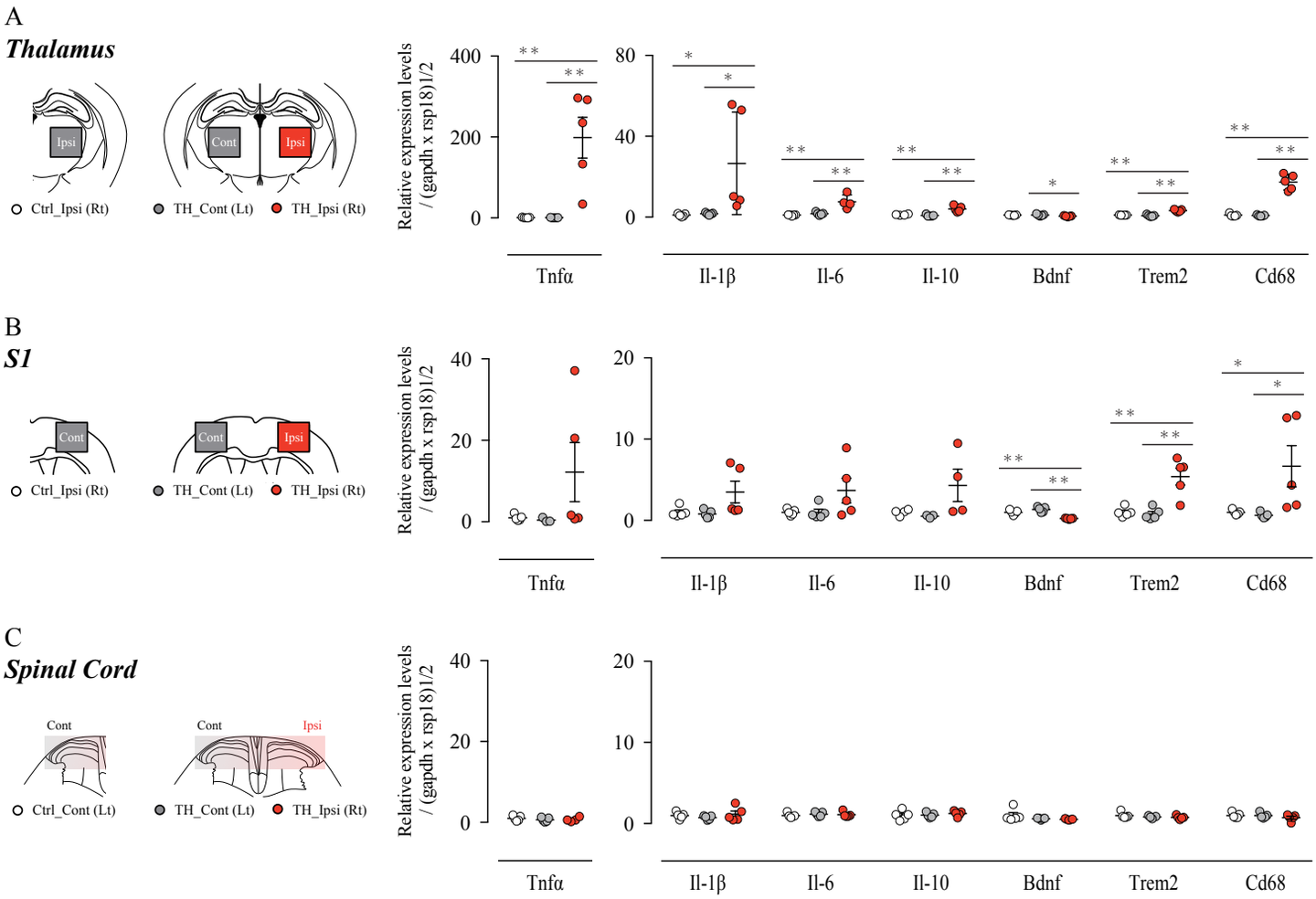
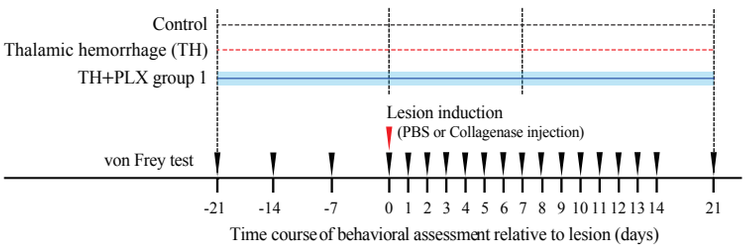

A
Thalamus
Ipsi
Cont
Ipsi
Ctrl_Ipsi (Rt)
TH_Cont (Lt)
TH_Ipsi (Rt)
Relative expression levels / (gapdh x rsp18)1/2
400
200
0
80
40
0
Tnfα
Il-1β
Il-6
Il-10
Bdnf
Trem2
Cd68
B
S1
Cont
Cont
Ipsi
Ctrl_Ipsi (Rt)
TH_Cont (Lt)
TH_Ipsi (Rt)
40
20
0
20
10
0
C
Spinal Cord
Cont
Cont
Ipsi
Ctrl_Cont (Lt)
TH_Cont (Lt)
TH_Ipsi (Rt)

Figure S3

A


Control
Thalamic hemorrhage (TH)
TH+PLX group 1
Lesion induction
(PBS or Collagenase injection)
von Frey test
-21 -14 -7 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 21
Time course of behavioral assessment relative to lesion (days)

B

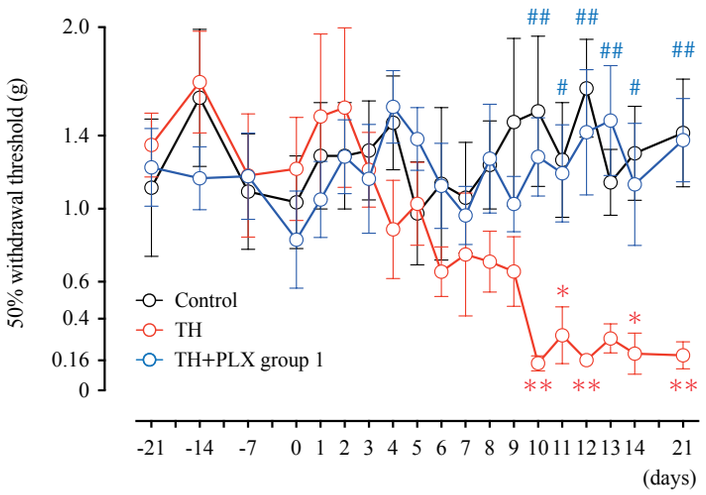
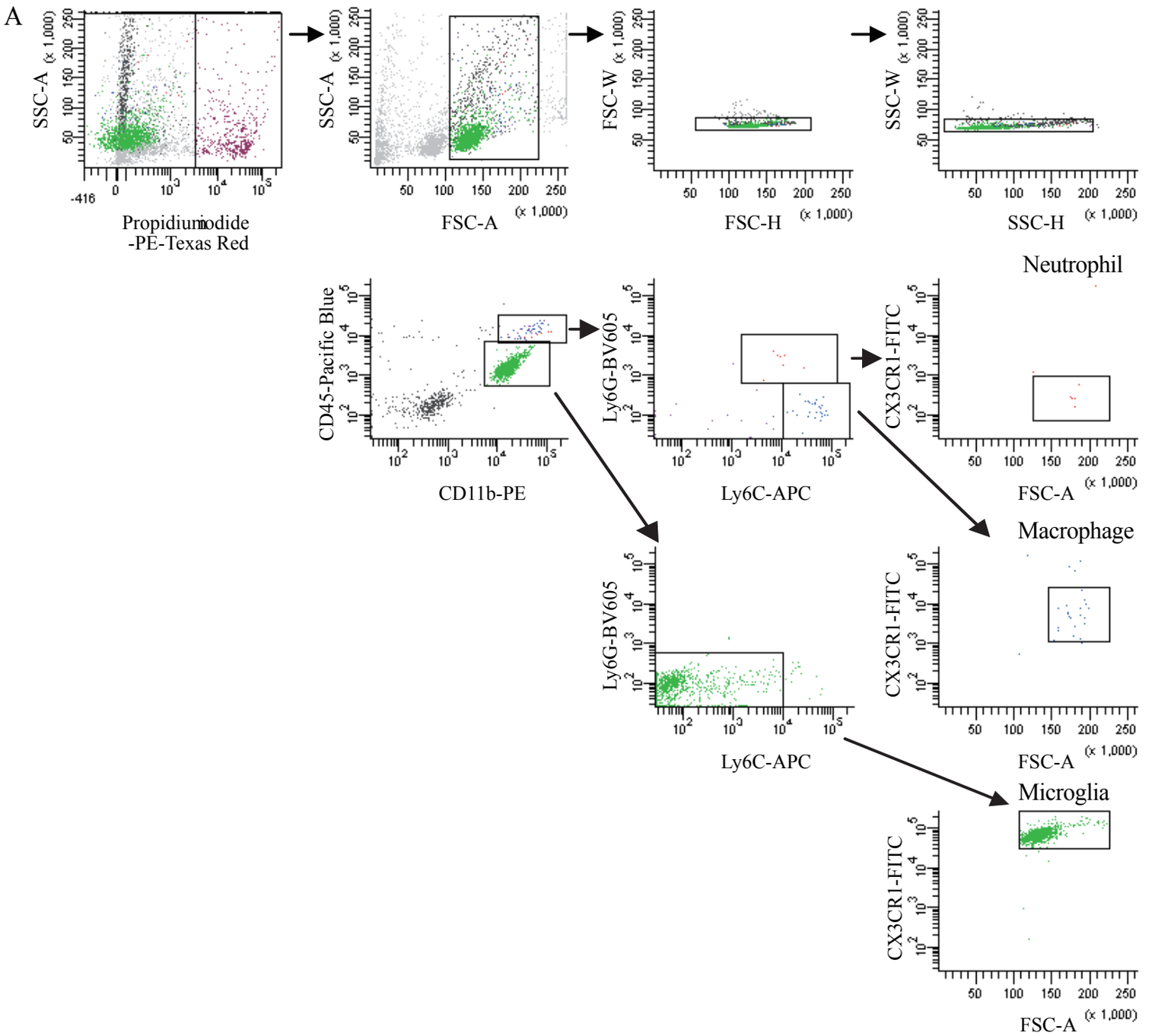

50% withdrawal threshold (g)
2.0
1.4
1.0
0.6
0.4
0.16
0
Control
TH
TH+PLX group 1
-21 -14 -7 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 21
(days)

Figure S4


A
SSC-A
Propidium iodide -PE-Texas Red
FSC-A
FSC-W
FSC-H
SSC-W
SSC-H
CD45-Pacific Blue
CD11b-PE
Ly6G-BV605
Ly6C-APC
CX3CR1-FITC
Neutrophil
Macrophage
Microglia
(x 1,000)

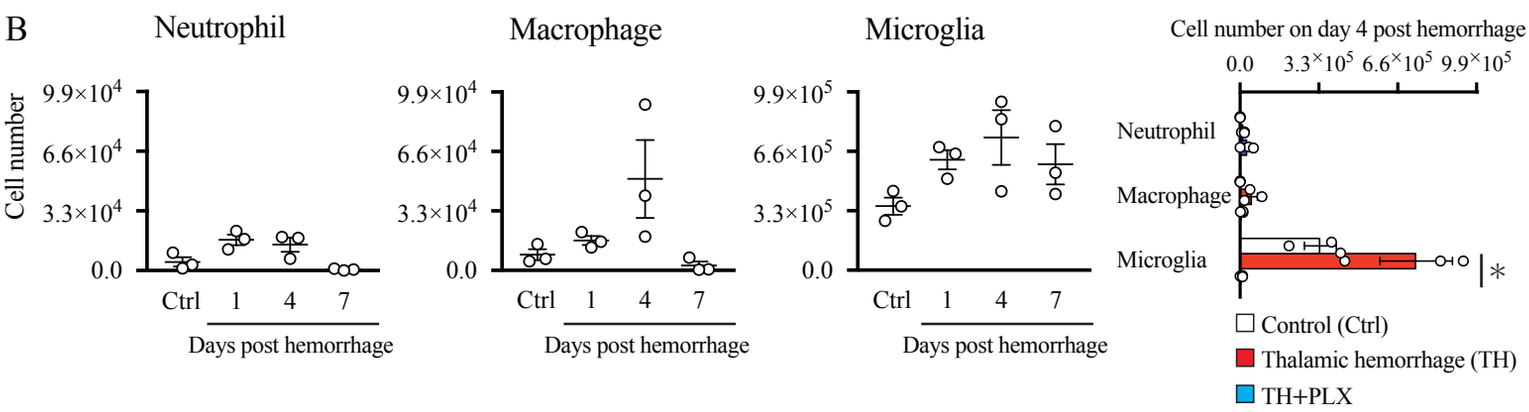
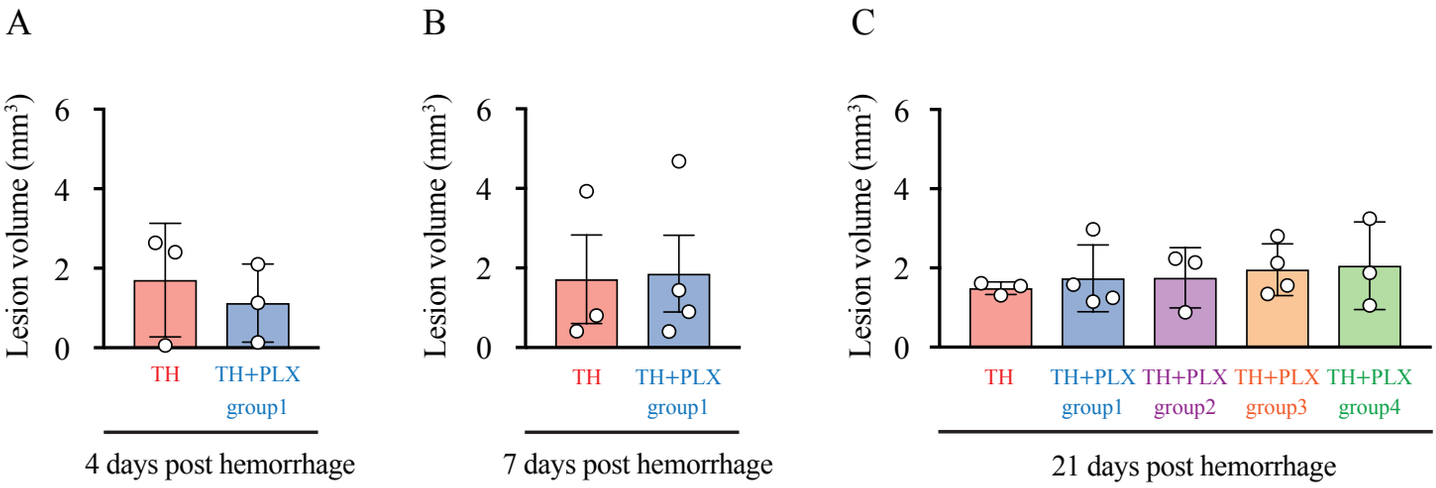

B
Neutrophil
Macrophage
Microglia
Cell number
Ctrl
1
4
7
Days post hemorrhage
Cell number on day 4 post hemorrhage
Control (Ctrl)
Thalamic hemorrhage (TH)
TH+PLX
*

Figure S5


A
B
C
Lesion volume (mm3)
6
4
2
0
TH
TH+PLX
group1
4 days post hemorrhage
7 days post hemorrhage
TH+PLX
group2
TH+PLX
group3
TH+PLX
group4
21 days post hemorrhage