**Supplemental material**

**Table S1. Primers used in the study.**

| Target gene | Forward (5' – 3') | Reverse (5' – 3') |
|---|---|---|
| *P. yoelii 18S* | TGAACGAGGAATGCCTAGTAAG | TTCATCATACTTTTCAATCGGTAGG |
| *ß-actin* | GCTCTGGCTCCTAGCACCAT | GCCACCGATCCACACAGAGT |
| *GAPDH* | CGTCGCGAAGGATACTCT | GGCAGCAGATTTCACTGTGAAG |
| *18S* | CGGCTACCACATCCAAGGAA | GCTGGAATTACCGCGGCT |
| *ICAM1* | GGCTTGGAGACTCAGTGGCT | CCAGTTGGGTCCACTCTCGA |
| *VCAM1* | AAAACGATCGCTCAAATCGG | CGTAGTGCTGCAAGTGAGGG |
| *PECAM1* | CGTGTTAGTGTTTCGCTGCC | TGCAATTTGAATCCGGACAG |
| *P-selectin* | TTCCGGTTCCCAGTAAAGCC | AGGTACCGAAGGGATCCGAG |
| *E-selectin* | TGGAACACGACATGCACGTT | TCCAACTCGCCTGTACCCTT |
| *vWFA* | ACCACGAGGTCATCAACGC | GAATCCGCTGTAGCTGCACA |
| *TF* | GAAGGATGTGACCTGGGCCT | CTCCGTGGGACAGAGAGGAC |
| *Fn* | TTCCCATTACGCCATTGGA | GCTTAAAGCCAGCGTCAGACA |
| *VEGF* | TTTCGTCCAACTTCTGGGCT | GCGCAGACCACGGCTACTAC |
| *PF4* | GTTTCTGCCAGCGGTGGTT | TCGCTTTCTTCGGGACCAG |

**Table S2. Hematological analysis of blood samples.**

| | RBC ($\times 10^4$) | | HCT (%) | | PLT ($\times 10^2$) | | Mouse number | |
|---|---|---|---|---|---|---|---|---|
| **dpi** | **PL** | **CLL** | **PL** | **CLL** | **PL** | **CLL** | **PL** | **CLL** |
| **0** | 801.8±95.6 | 819.6±97.4 | 36.9±4.6 | 37.7±4.6 | 69.5±8.1 | 69.5±9.1 | 10 | 10 |
| **3** | 725.3±99.2 | 774.0±108.4 | 33.5±4.4 | 35.7±5.4 | 35.4±8.8$^{*}$ | 39.7±8.5$^{*}$ | 10 | 10 |
| **6** | 532.5±97.3$^{*}$ | 485.2±113.5$^{*}$ | 21.5±3.1$^{*}$ | 24.6±5.2$^{*}$ | 21.1±3.7$^{*}$ | 22.7±7.8$^{*}$ | 10 | 10 |
| **7** | 466.5±85.2$^{*}$ | 516.8±135.3$^{*}$ | 22.0±3.7$^{*}$ | 23.7±5.4$^{*}$ | 17.1±5.2$^{*}$ | 56.8±33.4$^{\dagger}$ | 10 | 10 |
| **8** | 292±31.2$^{*}$ | 501.2±90.2$^{*,\dagger}$ | 15.6±0.6$^{*}$ | 24.6±2.9$^{\dagger,*}$ | 14.1±1.5$^{*}$ | 33.5±2.2$^{\dagger,*}$ | 10 | 3 |
| **11** | 199.8±9.4$^{*}$ | 549.0±0 | 13.5±0.6$^{*}$ | 26.2±0 | 17.4±7.5$^{*}$ | 23.7 | 10 | 1 |

Samples were from *P. yoelii*-infected mice treated with either PBS liposomes (PL) or clodronate liposomes (CLL) on day 6 p.i. The results represent the average values± SEM for the mice. Data from two independent experiments were combined. Significant differences as analyzed by student t test are denoted by superscript symbols: † compared with values on the same day p.i. between mice infected with PL or CLL; * compared with values on day 0 in the same experimental group. RBC, red blood cell; HCT, hematocrit; PLT, platelets.

## Materials and methods

### Flow cytometric analysis and antibodies

The cell populations of spleen were examined by flow cytometry. Single-cell suspensions prepared from the spleens were suspended in cold PBS containing 0.5% bovine serum albumin. The cells were treated with FcBlock™ to avoid the non-specific adherence of monoclonal antibodies to Fc receptors, and then incubated with their respective monoclonal antibodies for 30 min at 4°C. The stained cells ($CD11b^+$ $F4/80^+$, $CD11c^+$, $Gr.1^+$, $NK.1^+CD3^+$, $NK.1^+CD3^-$, $CD4^+CD3^+$ and $CD8^+CD3^+$) were washed with cold PBS, fixed with 0.5% paraformaldehyde in PBS, and examined with an EPICS XL flow cytometer (Beckman Coulter, Hialeah, USA).

## Figure legends

**Figure S1.** Population of macrophages, dendritic cells, granulocytes, natural killer cells NKT cells and T cells in spleen. (A) Samples from naïve mice treated with PBS liposome (CPL) or clodronate liposome (CCLL) were collected one day after treatment, and then examined by flow cytometry. (B) Samples from *P. yoelii*-infected mice treated with either PBS liposomes (PL) or clodronate liposome (CLL) on day 6 p.i. were collected on day 7 p.i., and then examined by flow cytometry. * Indicates the significant difference between the groups as analyzed by Student's *t* test.

**Figure S2.** Biochemical analysis of urine samples. Samples from *P. yoelii*-infected mice treated with either control liposome (PL) or clodronate liposome (CLL) on day 6 p.i. were collected on day 7 p.i. The results represent the average values (± SEM) for the mice (PL, n

= 10; CLL, n = 7). Data from two independent experiments were combined. * Indicates the significant difference between the groups as analyzed by Student's t test. TP, total protein; CRE, creatinine; BUN, blood urea nitrogen.

**Figure S3.** Histopathological observations of hepatic and renal tissues of uninfected mice treated with clodronate liposome. Tissues were sampled for histopathological studies one day after treatment with clodronate liposome. Hepatic sections (A) and renal sections (B and C; similar region to Fig. 2B and D, respectively).

**Figure S4.** Gene expression of endothelial-cell adhesion molecules in hepatic (A) and renal tissues (B). Samples from *P. yoeli*i-infected mice treated with either PBS liposomes (PL) or clodronate liposome (CLL) on day 6 p.i. were collected on day 7 p.i. Uninfected mice treated with PBS liposome (CPL) or clodronate liposome (CCLL) were sampled one day after treatment. The results represent the $2^{-\Delta\Delta}Ct$ average of relative expression (± SEM) of each targeted gene in the mice (uninfected, n = 5; infected, n = 15) relative to that of the corresponding gene in uninfected mice treated with PBS liposomes (CPL). * Indicates the significant difference between the groups as analyzed by one-way ANOVA analysis of variance, followed by Tukey's multiple-comparison test.

**Figure S5.** Relative gene expression of cytokine in spleen. Samples from *P. yoelii*-infected mice treated with either PBS liposomes (PL) or clodronate liposome (CLL) on day 6 p.i. were collected on day 7 p.i. Samples from uninfected mice treated with PBS liposome (CPL) or clodronate liposome (CCLL) were collected one day after treatment. The results represent the $2^{-\Delta\Delta}Ct$ average of relative expression (± SEM)

for each experimental group (uninfected, n = 5; infected, n = 15). * Indicates the significant difference between the groups as analyzed by one-way ANOVA analysis of variance, followed by Tukey's multiple-comparison test.

**Figure S6.** Histopathological observations of hepatic and renal tissues of aspirin-treated mice. Samples from *P. yoelii*-infected mice treated with clodronate liposome (CLL) on day 6 p.i. and infected mice treated with PBS (CLL (PBS)) or aspirin (CLL (Aspirin)) on days 5 and 6 p.i., were collected on day 7 p.i. and then examined. (A) Hepatic sections and (B, C) renal sections. Macrophage-depleted mice showed focal hepatic necrosis (A), tubular necrosis (B), blood stasis (C). HE staining (A–C). Both groups of mice showed similar lesions in liver and kidney. However, the frequency of these lesions was lower in the aspirin-treated mice than in the PBS-treated mice after infection with *P. yoelii.* Arrowheads indicate lesions. Scale bars are indicated.

**Figure S7. Analysis of the expression of genes encoding endothelial adhesion- and coagulation-related molecules in renal tissues.** Samples were from *P. yoelii*-infected mice collected on day 7 p.i. after the injection of either control liposomes (PL) or clodronate liposomes (CLL) on day 6 p.i. The mice were also treated with aspirin (gray column) or PBS (black column) from day 5 to day 8 p.i. The results represent the $2^{-\Delta\Delta}Ct$ average of relative expression (± SEM) of each targeted gene in the mice (n = 10) relative to that of the corresponding gene in infected mice treated with PBS liposomes (PL). * Indicates the significant difference between the groups as analyzed by one-way ANOVA analysis of variance, followed by Tukey's multiple-comparison test.

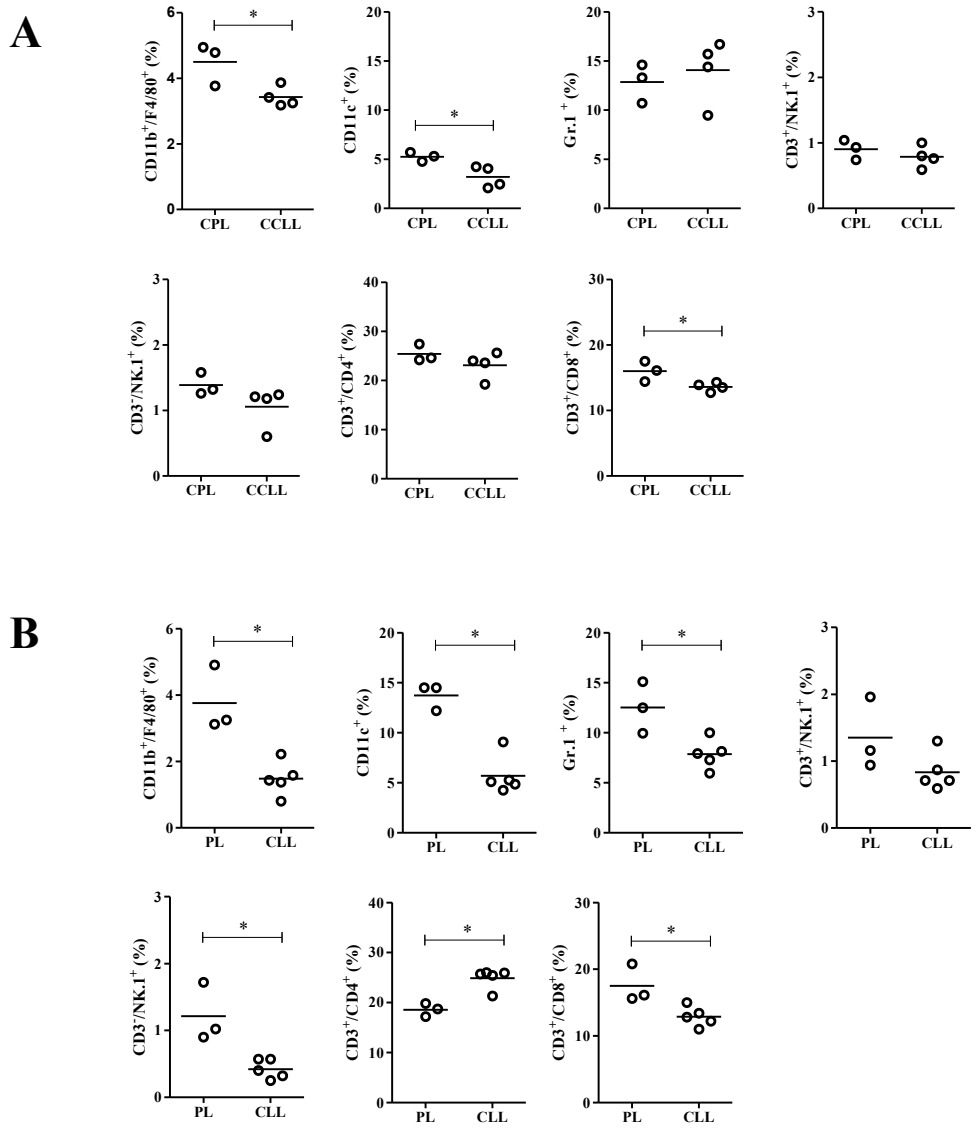
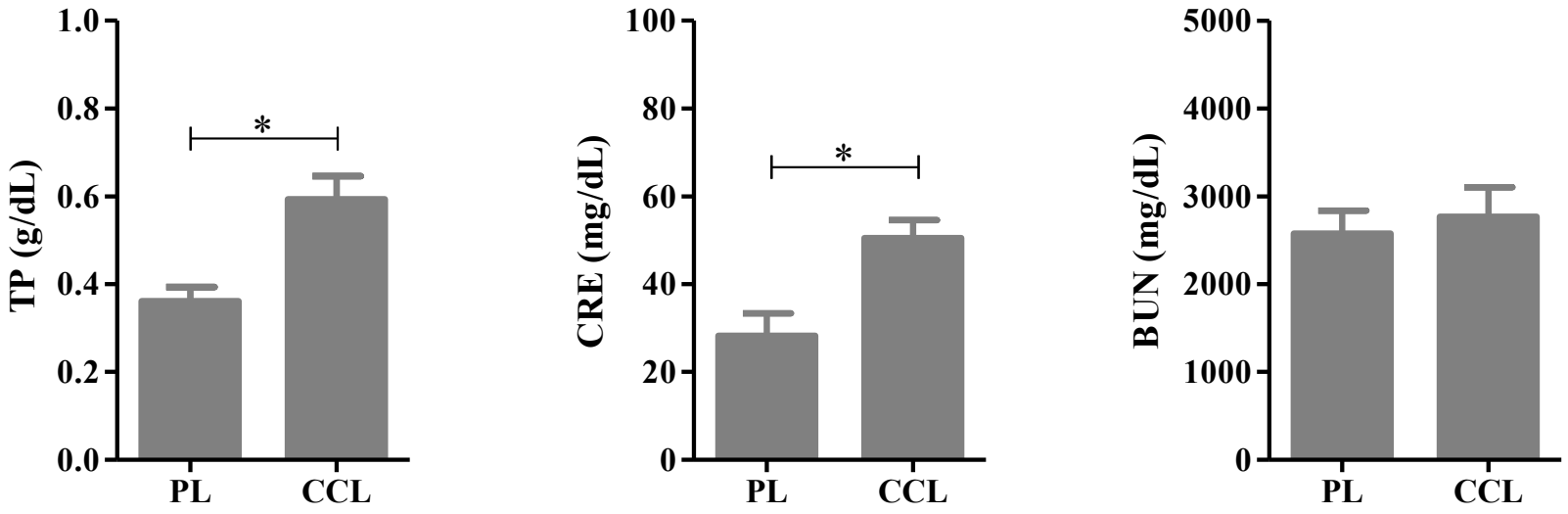

A
CD11b+/F4/80+ (%)
CD11c+ (%)
Gr.1+ (%)
CD3+/NK.1+ (%)
CD3-/NK.1+ (%)
CD3+/CD4+ (%)
CD3+/CD8+ (%)
CPL
CCLL
B
CD11b+/F4/80+ (%)
CD11c+ (%)
Gr.1+ (%)
CD3+/NK.1+ (%)
CD3-/NK.1+ (%)
CD3+/CD4+ (%)
CD3+/CD8+ (%)
PL
CLL

**Figure S1.**


TP (g/dL)
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0.8
0.6
0.4
0.2
0.0
*
PL
CCL
CRE (mg/dL)
100
80
60
40
20
0
*
PL
CCL
BUN (mg/dL)
5000
4000
3000
2000
1000
0
PL
CCL

**Figure S2.**

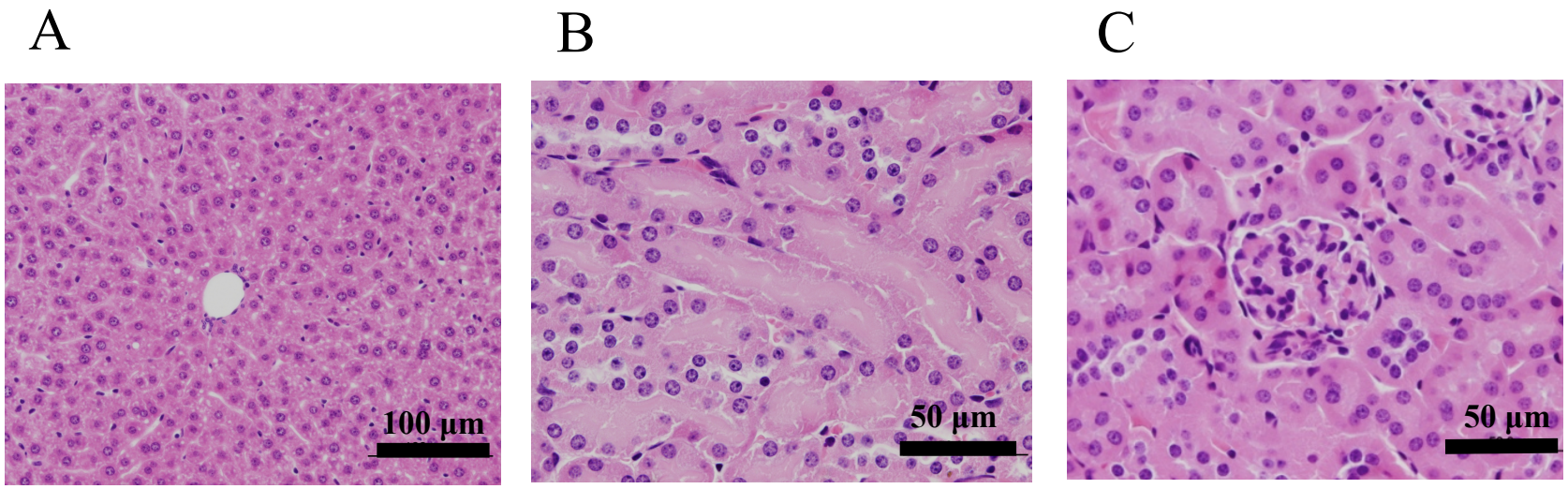


**Figure S3.**

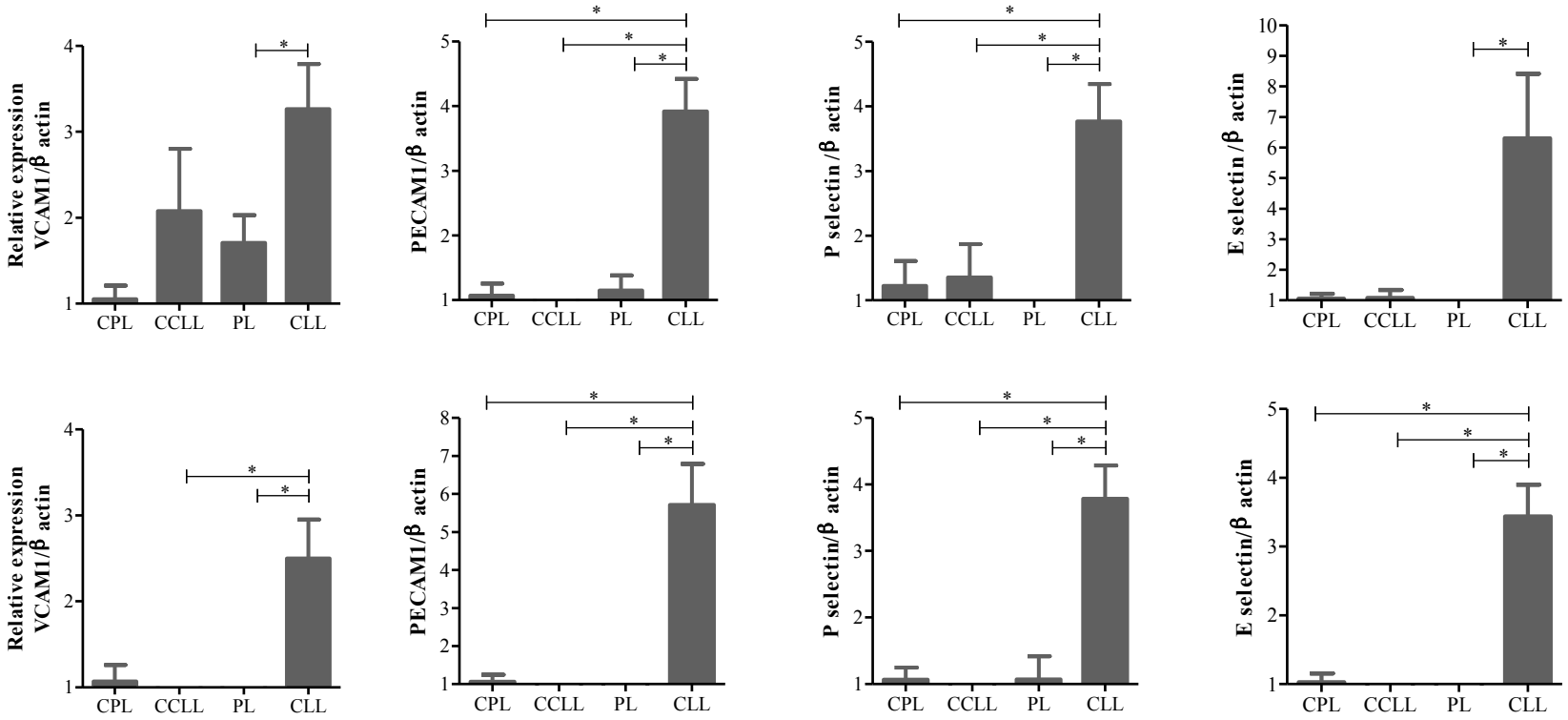
Relative expression VCAM1/β actin
PECAM1/β actin
P selectin /β actin
E selectin /β actin
Relative expression VCAM1/β actin
PECAM1/β actin
P selectin/β actin
E selectin/β actin
CPL
CCLL
PL
CLL

**Figure S4.**

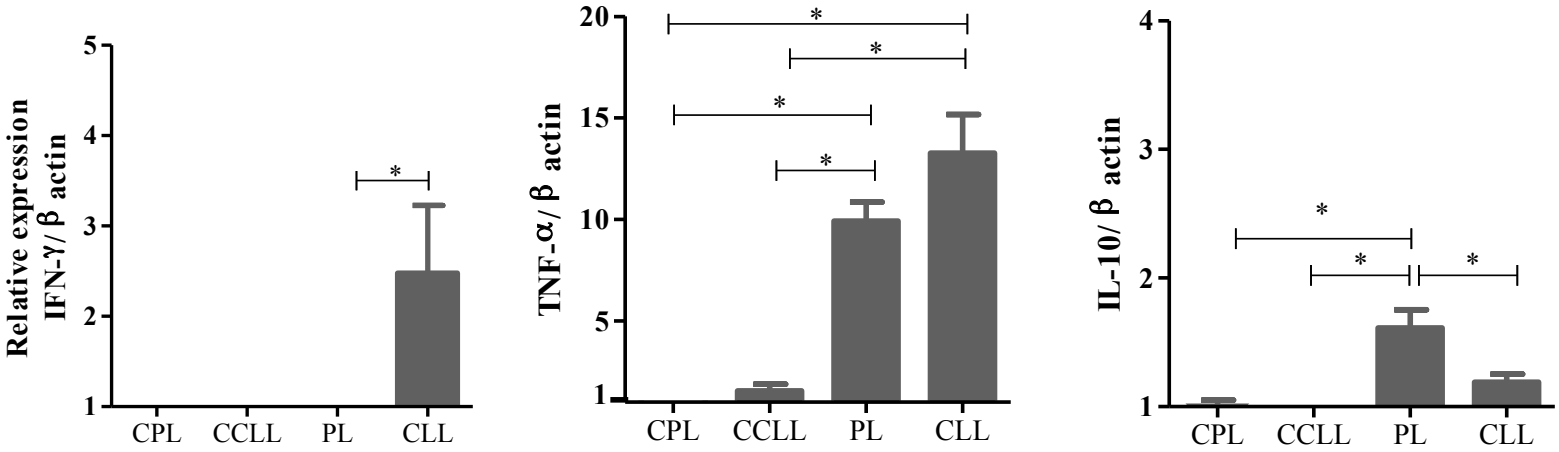
Relative expression
IFN-γ/ β actin
5
4
3
2
1
*
CPL
CCLL
PL
CLL
TNF-α/ β actin
20
15
10
5
1
*
*
*
*
CPL
CCLL
PL
CLL
IL-10/ β actin
4
3
2
1
*
*
*
CPL
CCLL
PL
CLL

**Figure S5.**

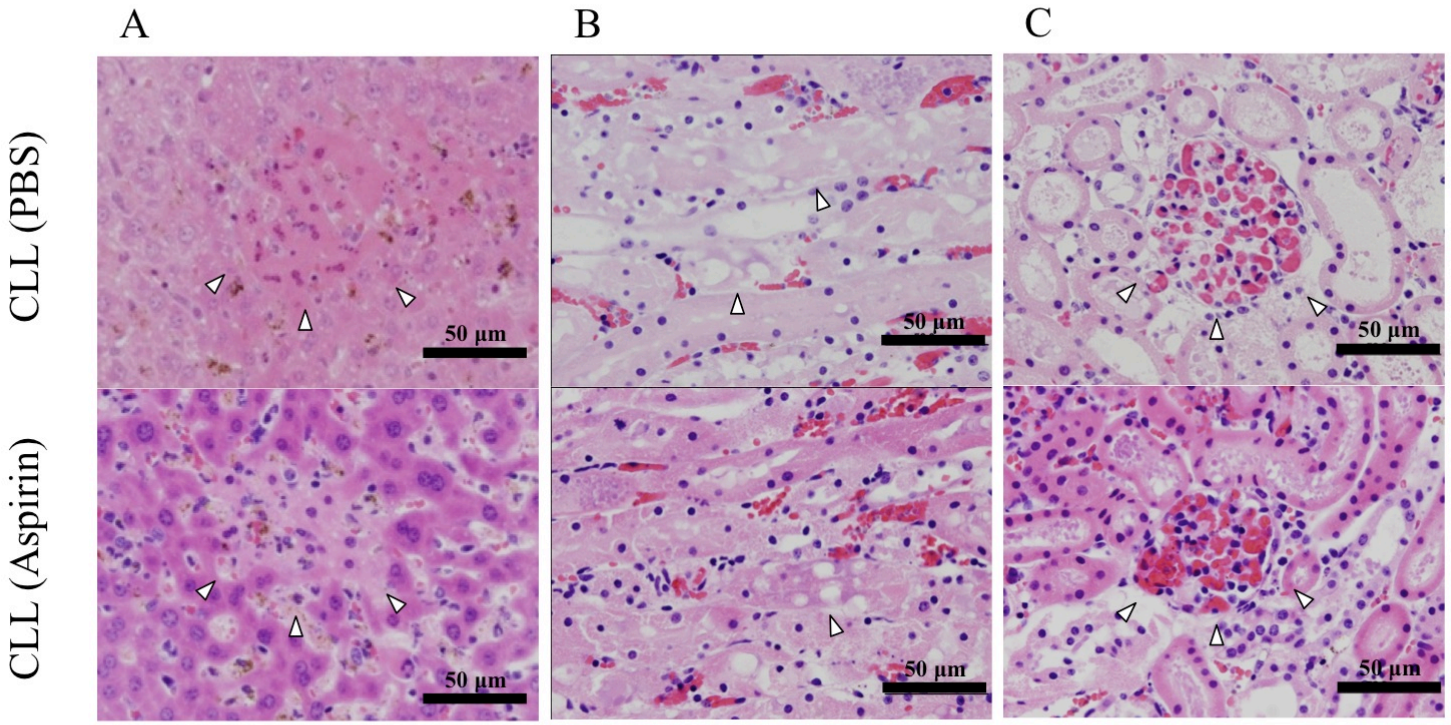


**Figure S6.**

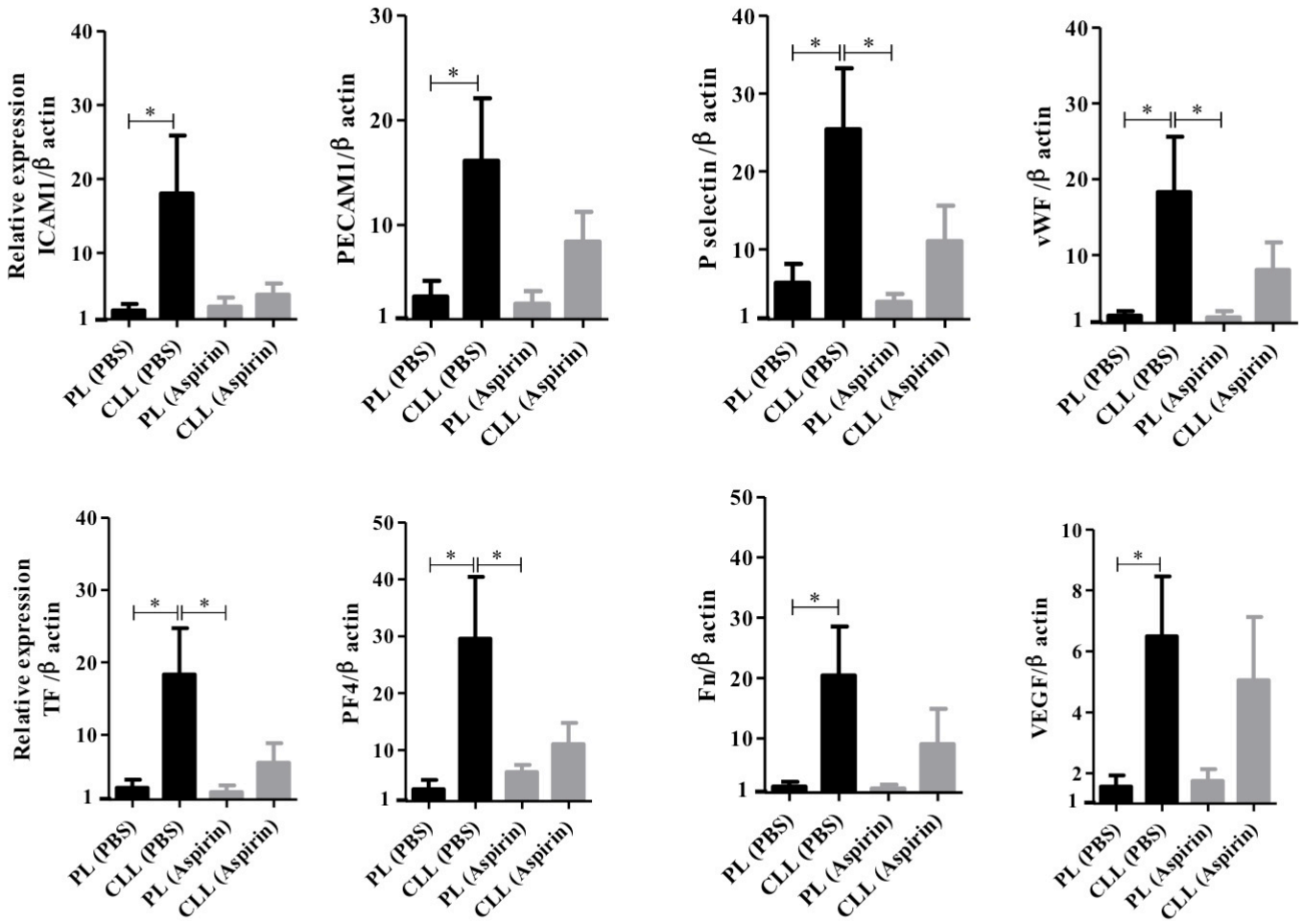
Relative expression ICAM1/β actin
PECAM1/β actin
P selectin /β actin
vWF /β actin
Relative expression TF /β actin
PF4/β actin
Fn/β actin
VEGF/β actin
PL (PBS)
CLL (PBS)
PL (Aspirin)
CLL (Aspirin)

**Figure S7.**