Supplemental Figure legends

Supplemental Figure 1. Macrophage depletion affected the mortality in LPS-induced sepsis. (A) The ratio of $F4/80^{+}CD11b^{+}$ splenic macrophages after i.v. injection of clodronate liposome (CLOP, 0.1ml/10g, the left of A) and PBS-control liposome (CONT, 0.1ml/10g, the right of A) for 24 hours. (B) The C57BL/6J mice were pretreated with i.v. injection of CONT or CLOP for 24 hours before treated with i.p. injection of LPS (40 mg/kg) or LPS (40 mg/kg) plus 3×10^{7} peritoneal macrophages (n=8, *P<0.05, **P<0.01, CLOP vs. CONT; #P<0.05, CLOP vs. CLOP+Mφ). Survival analysis utilized Log-rank (Mantel-Cox) test.

Supplemental Figure 2. Swiprosin-1 deletion decreased macrophages recruitment after CLP treatment. Swiprosin-1 knockout mice showed impaired macrophages recruitment in the lung and kidney after CLP treatment for 18 hours (green fluorescence: F4/80+ macrophage, blue fluorescence: nucleus, scan bar=200μm).

Supplemental Figure 3. Effect of swiprosin-1 on macrophages migration after LPS treatment. (A) Cell viability of macrophages after LPS treatment for 6 hours (n=6), results depicted as mean ± SEM. (B) Wound healing analysis in macrophages after LPS treatment.

Supplemental Figure 4. Effect of swiprosin-1 on mRNA levels of pro-inflammatory cytokines in macrophages after LPS treatment. The mRNA

levels of IL-1β, IL-6, and TNF-α in macrophages after LPS treatment (n=3, *P<0.05, ***P<0.001, WT-LPS vs. WT-0 hour; ##P<0.01, ###P<0.001, KO-LPS vs. KO-0 hour; ┼┼┼P<0.001, WT-LPS vs. KO-LPS at the same time point), ANOVA (LSD test), results depicted as mean ± SEM.

Supplemental Figure 5. Effect of swiprosin-1 on IL-10 expression in macrophages after LPS treatment. IL-10 mRNA and protein expression in macrophages after LPS treatment (n=3-6, *P<0.05, **P<0.01, ***P<0.001 WT-LPS vs. WT-0 hour; #P<0.05, ###P<0.001, KO-LPS vs. KO-0 hour; ┼P<0.05, ┼┼P<0.01, WT-LPS vs. KO-LPS at the same time point), ANOVA (LSD test), results depicted as mean ± SEM.

Supplemental Figure 6. Activation of NF-κB and MAPKs pathways in macrophages after LPS treatment. (A) Phosphorylation of IκBα, IKKα/β, and p65 in macrophages after LPS stimulation (n=3, *P<0.05,**P<0.01 WT-LPS vs. WT-0 hour; #P<0.05, ##P<0.01, KO-LPS vs. KO-0 hour), ANOVA (LSD test), results depicted as mean ± SEM. (B) Phosphorylation of p38, JNK, and ERK in macrophages after LPS stimulation (n=3, *P<0.05,**P<0.01 WT-LPS vs. WT-0 hour; #P<0.05, ##P<0.01, KO-LPS vs. KO-0 hour), ANOVA (LSD test), results depicted as mean ± SEM.

Supplemental Figure 7. Swiprosin-1 deletion attenuated IFN-γ expression in macrophages and T cells after LPS treatment. (A) mRNA and peotein expression of IFN-γ in macrophages after LPS treatment for 12 hours (n=3-6, **P<0.01, WT LPS compared with WT 0h; ┼┼P<0.01, ┼┼┼P<0.001, KO LPS compared with WT LPS at the same time point), ANOVA (LSD test), results depicted as mean ± SEM. (B) IFN-γ level in T cells after LPS treatment for 12 hours (n=6, ***P<0.001, WT-LPS vs. WT-CON; ┼┼┼P<0.001, WT-LPS vs. KO-LPS), ANOVA (LSD test), results depicted as mean ± SEM.

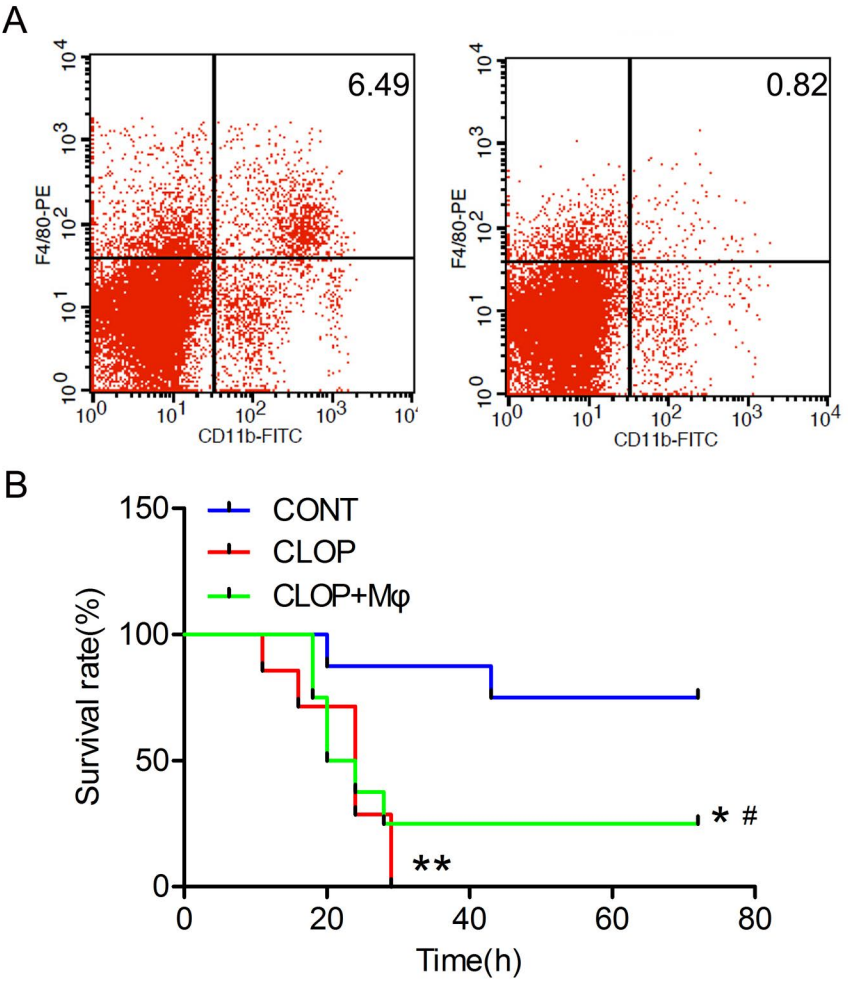
Supplemental Figure 8. Effect of swiprosin-1 on mRNA levels of TLR4/CD14 in macrophages after LPS treatment. TLR4 and CD14 gene expression in macrophages after LPS treatment for 12 hours (n=3, **P<0.01, WT-LPS vs. WT-CON; ##P<0.01, KO-LPS vs. KO-CON), ANOVA (LSD test), results depicted as mean ± SEM.

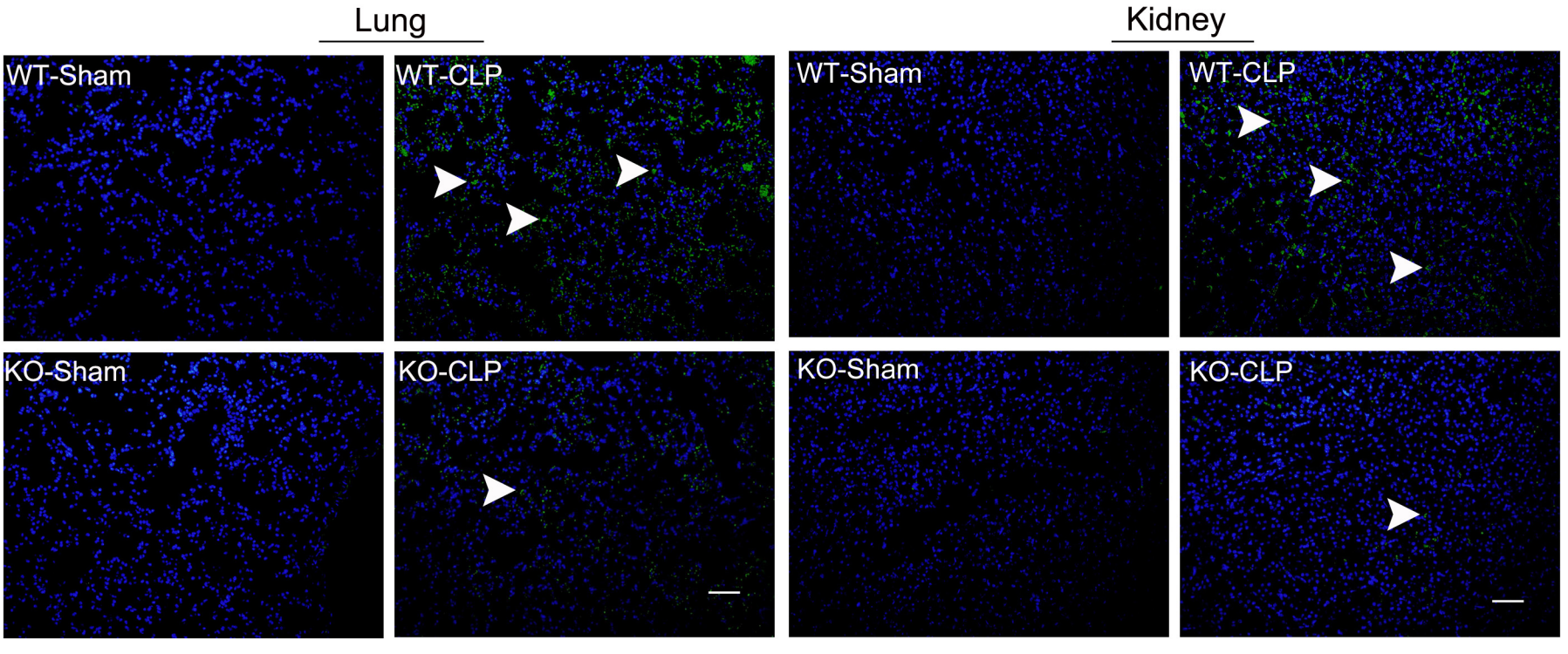
Supplemental Figure 9. Effect of swiprosin-1 on pro-inflammatory cytokines and JAK-STAT pathway in THP-1 cells after LPS treatment. (A) Expression of swiprosin-1 in swiprosin-1-siRNA-treated THP-1 cells after LPS treatment for 12 hours (n=3, ***P<0.001, NC-LPS vs. NC-CON; ###P<0.001, siRNA-LPS vs. siRNA-CON; ┼┼┼P<0.001, siRNA-LPS vs. NC-LPS), ANOVA (LSD test), results depicted as mean ± SEM. (B) Expression of pro-inflammatory cytokines in swiprosin-1-siRNA-treated THP-1 cells after LPS treatment for 12 hours (n=6,

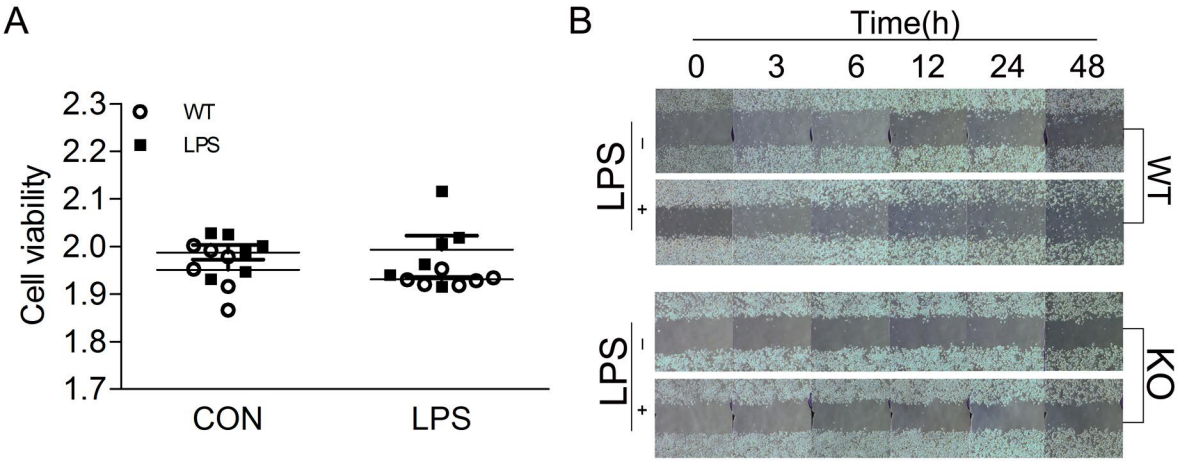
**P<0.01, ***P<0.001, NC-LPS vs. NC-CON; ##P<0.01, ###P<0.001, siRNA-LPS vs. siRNA-CON; ┼┼P<0.01, ┼┼┼P<0.001, siRNA-LPS vs. NC-LPS), ANOVA (LSD test), results depicted as mean ± SEM. (C) Activation of JAK-STAT pathway in swiprosin-1-siRNA-treated THP-1 cells after LPS treatment for 12 hours (n=3, **P<0.01, ***P<0.001, NC-LPS vs. NC-CON; #P<0.05, ###P<0.001, siRNA-LPS vs. siRNA-CON; ┼P<0.05, ┼┼P<0.01, ┼┼┼P<0.001, siRNA-LPS vs. NC-LPS), ANOVA (LSD test), results depicted as mean ± SEM.

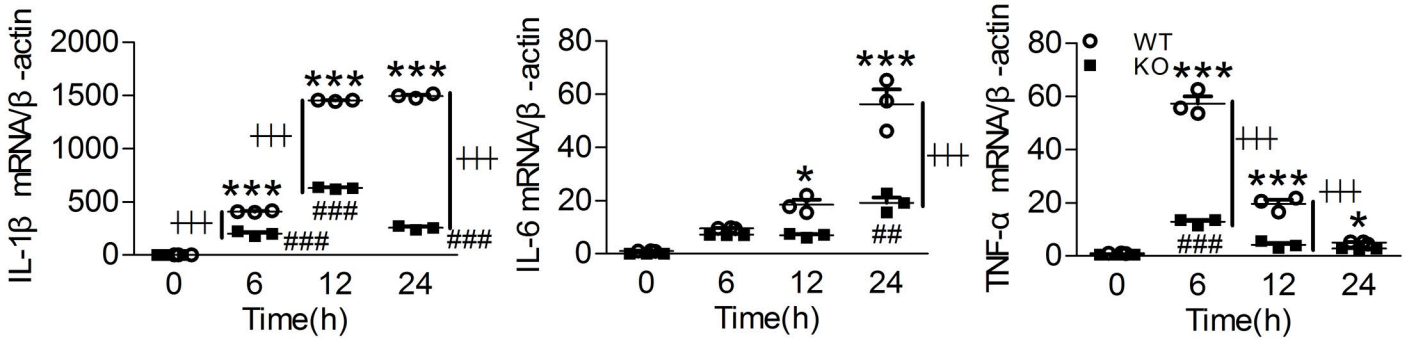
Supplemental Methods

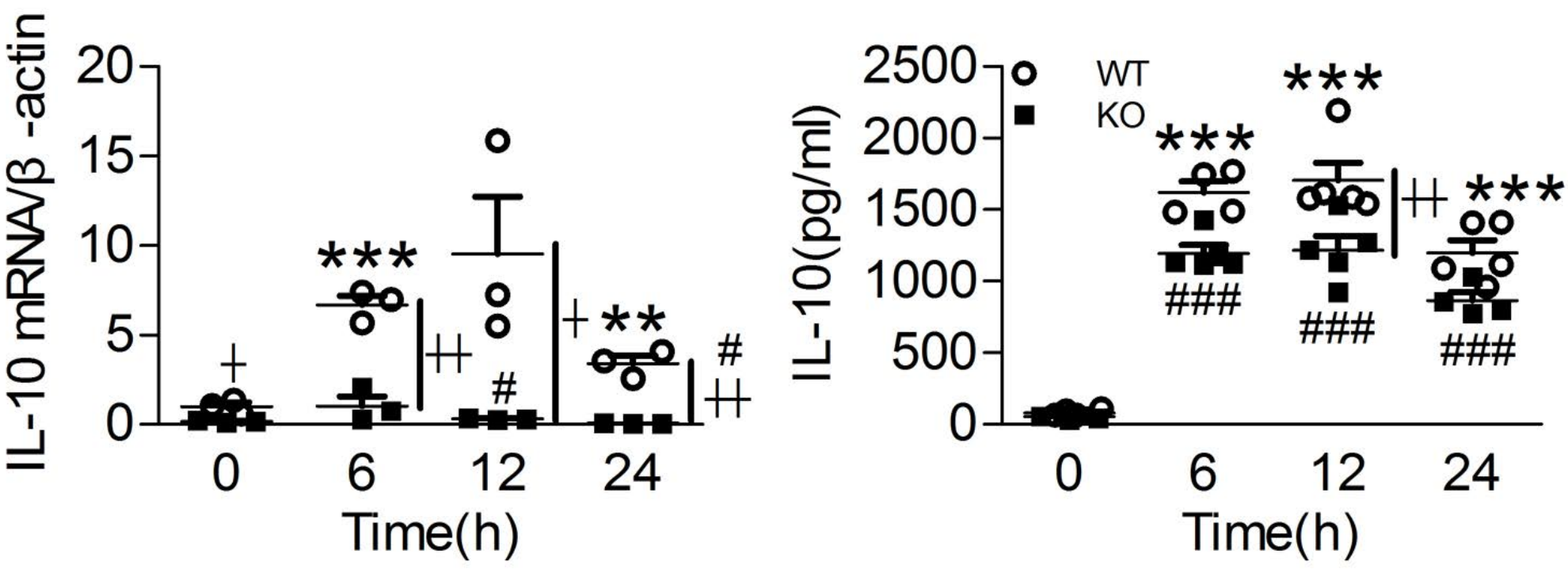
Swiprosin-1 siRNA Transfection. Plate 10^6 THP-1 cells in 500μl RPMI-1640 medium without antibiotics. Dilute 2μl swiprosin-1 siRNA (sequence: 3'-UCAAGGAGUUCUCCAGGAATT-5', 3'-UUCCUGGAGAACUCCUUGATT-5') oligomer in 50μl RPMI medium without serum and 1μl Lipofectamine 2000 in 50μl RPMI medium. Mix gently and incubate for 5 minutes at room temperature. After the 5-minute incubation, combine the diluted oligomer with the diluted Lipofectamine 2000. Mix gently and incubate for 20 minutes at room temperature. Add the oligomer-Lipofectamine 2000 complexes to each well containing cells and medium. Change the medium after 6 hours and incubate the cells with 100ng/ml PMA (Beyotime Biotechnology, Jiangsu, China) for 24 hours, and then detect the expression of RNA and protein.

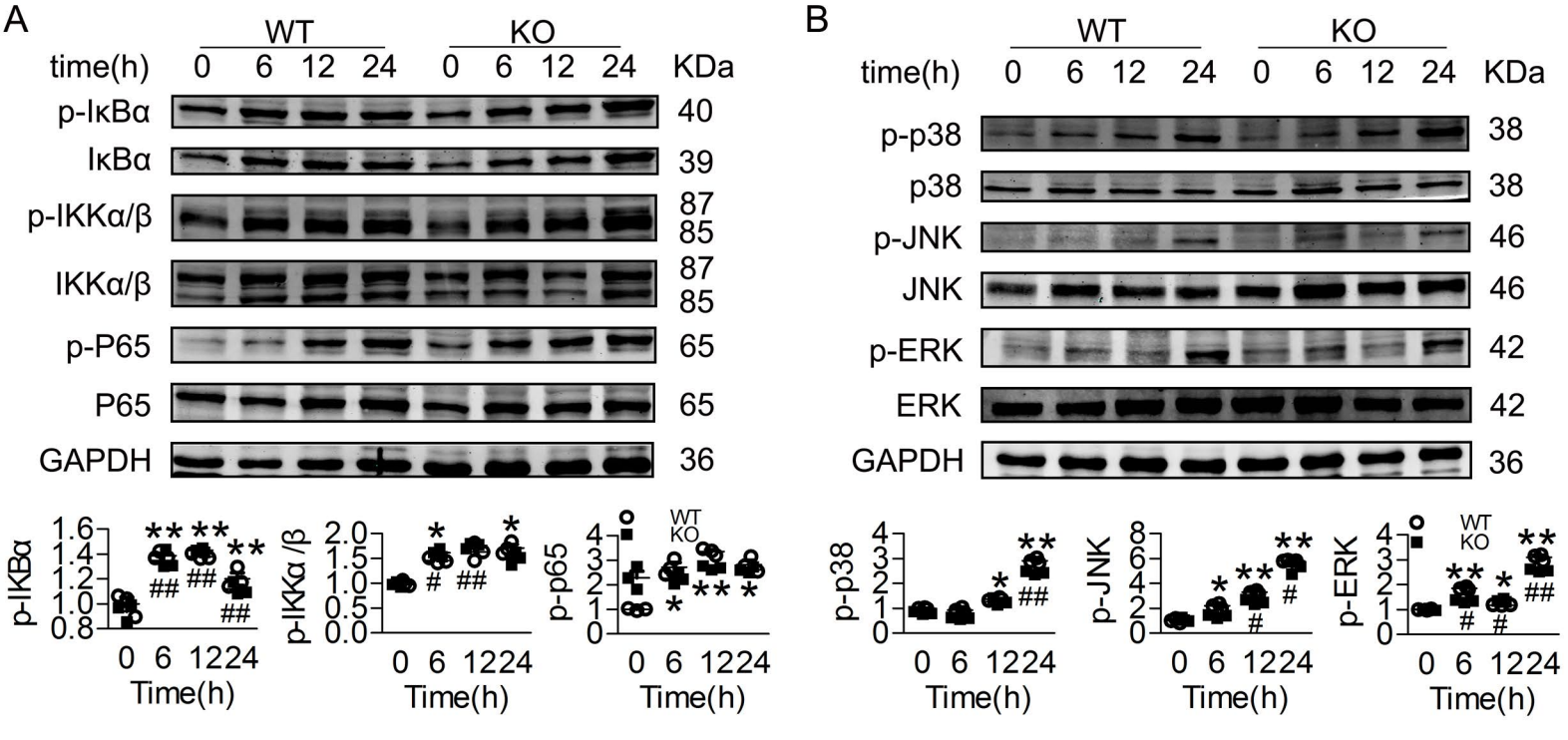

A
6.49
0.82
F4/80-PE
CD11b-FITC
B
CONT
CLOP
CLOP+Mφ
Survival rate(%)
Time(h)
* #
**

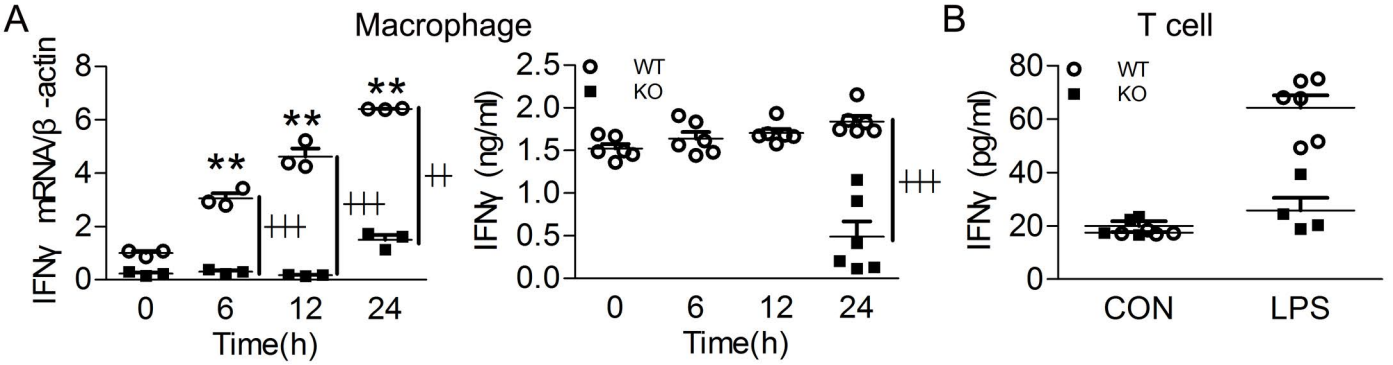

Lung
Kidney
WT-Sham
WT-CLP
WT-Sham
WT-CLP
KO-Sham
KO-CLP
KO-Sham
KO-CLP

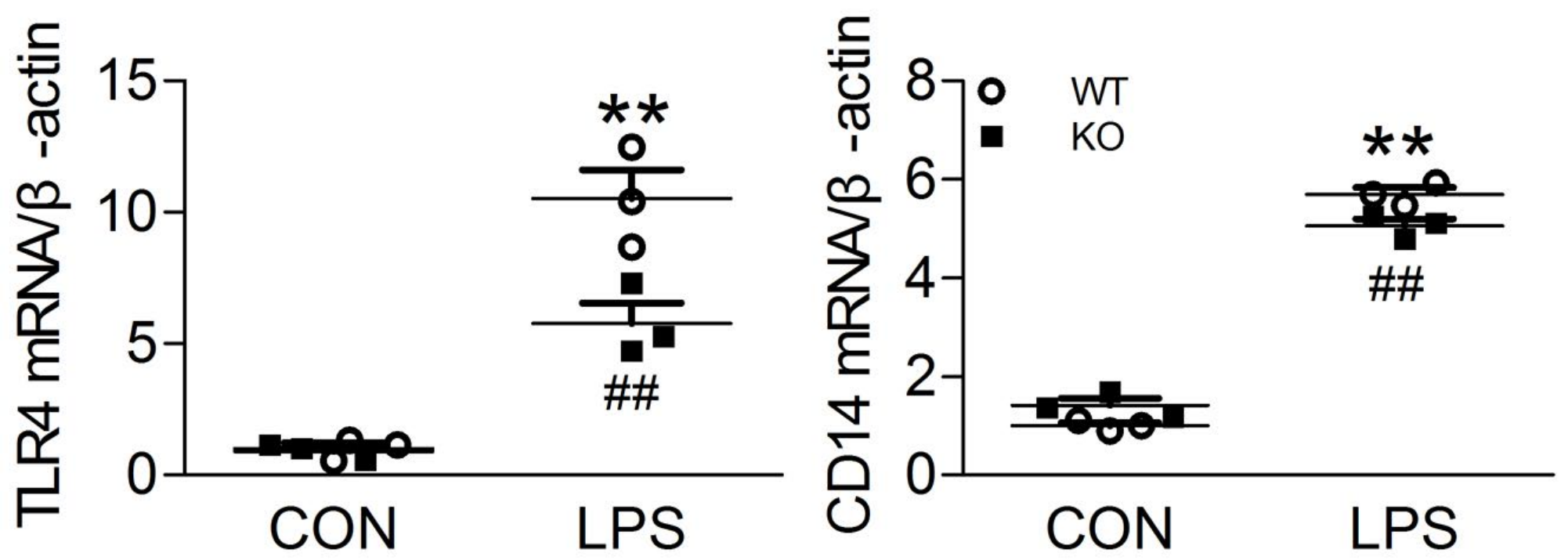

A
Cell viability
2.3
2.2
2.1
2.0
1.9
1.8
1.7
WT
LPS
CON
LPS
B
Time(h)
0
3
6
12
24
48
LPS
-
+
WT
LPS
-
+
KO

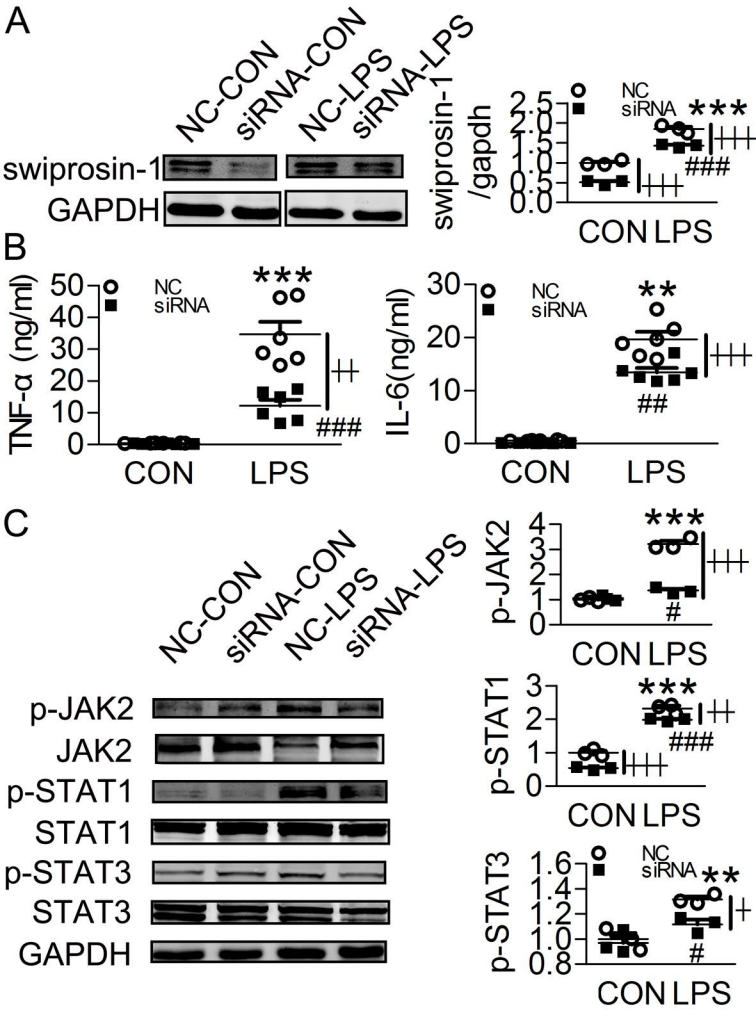

IL-1β mRNA/β -actin
2000
1500
1000
500
0
0
6
12
24
Time(h)
***
***
***
+++
+++
+++
###
###
###
IL-6 mRNA/β -actin
80
60
40
20
0
0
6
12
24
Time(h)
*
***
+++
##
TNF-α mRNA/β -actin
80
60
40
20
0
0
6
12
24
Time(h)
WT
KO
***
***
*
+++
+++
###


IL-10 mRNA/β -actin
20
15
10
5
0
***
**
+
++
#
0
6
12
24
Time(h)
IL-10(pg/ml)
2500
2000
1500
1000
500
0
WT
KO
***
###
Time(h)


A
WT
KO
time(h)
0 6 12 24 0 6 12 24
KDa
p-IκBα
IκBα
p-IKKα/β
IKKα/β
p-P65
P65
GAPDH
40
39
87
85
87
85
65
65
36
p-IKBα
p-IKKα /β
p-p65
WT
KO
Time(h)
B
WT
KO
time(h)
0 6 12 24 0 6 12 24
KDa
p-p38
p38
p-JNK
JNK
p-ERK
ERK
GAPDH
38
38
46
46
42
42
36
p-p38
p-JNK
p-ERK
WT
KO
Time(h)


A
Macrophage
IFNγ mRNA/β -actin
8
6
4
2
0
**
**
**
0
6
12
24
Time(h)
IFNγ (ng/ml)
2.5
2.0
1.5
1.0
0.5
0.0
WT
KO
0
6
12
24
Time(h)
B
T cell
IFNγ (pg/ml)
80
60
40
20
0
WT
KO
CON
LPS


TLR4 mRNA/β -actin
15
10
5
0
**
##
CON
LPS
CD14 mRNA/β -actin
8
6
4
2
0
WT
KO
**
##
CON
LPS


A
NC-CON
siRNA-CON
NC-LPS
siRNA-LPS
swiprosin-1
GAPDH
swiprosin-1 /gapdh
NC siRNA
CON LPS
B
TNF-α (ng/ml)
IL-6(ng/ml)
C
p-JAK2
JAK2
p-STAT1
STAT1
p-STAT3
STAT3
GAPDH