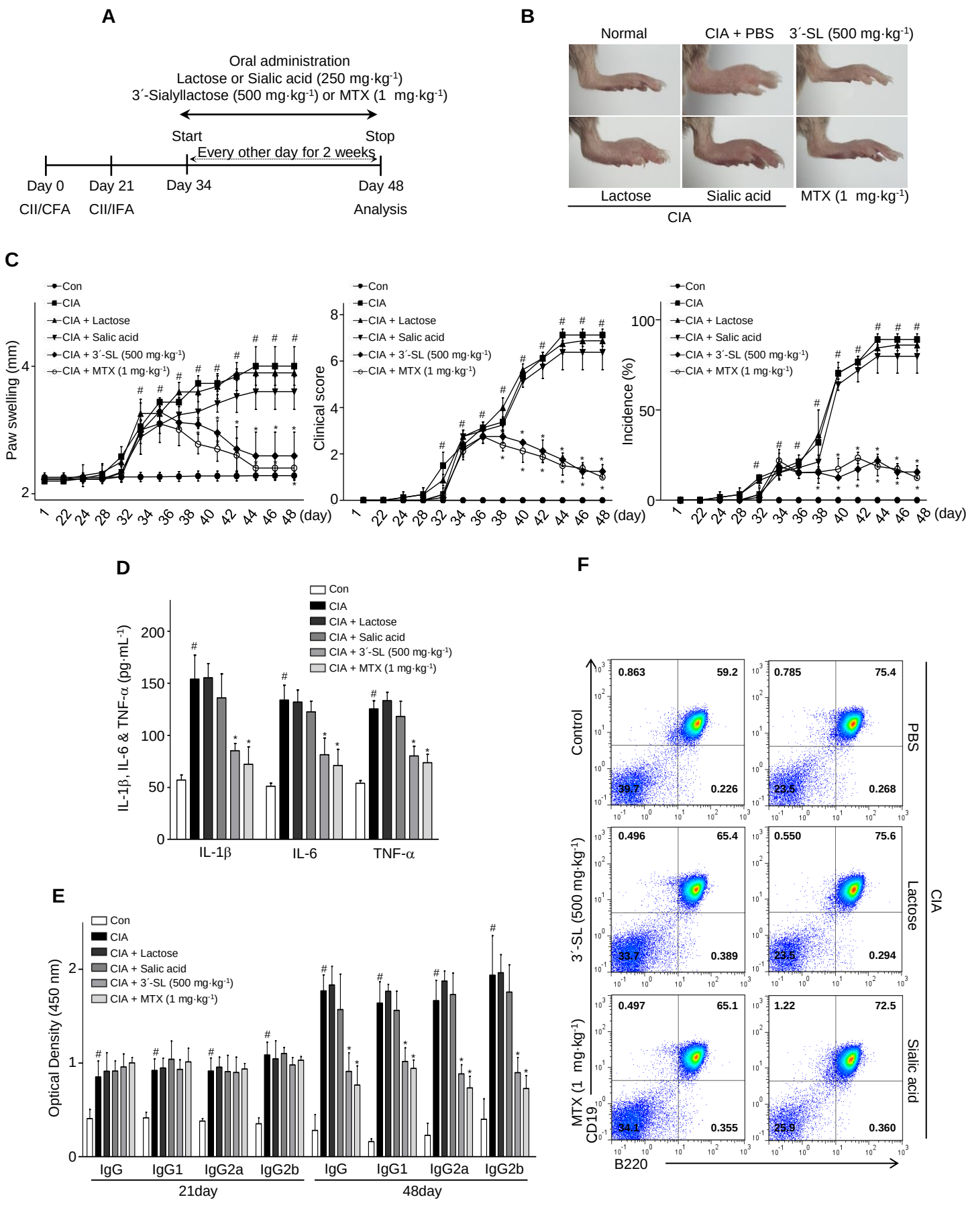

A
Oral administration
Lactose or Sialic acid (250 mg·kg-1)
3´-Sialyllactose (500 mg·kg-1) or MTX (1 mg·kg-1)
Start
Stop
Every other day for 2 weeks
Day 0
CII/CFA
Day 21
CII/IFA
Day 34
Day 48
Analysis
B
Normal
CIA + PBS
3´-SL (500 mg·kg-1)
Lactose
Sialic acid
MTX (1 mg·kg-1)
CIA
C
Con
CIA
CIA + Lactose
CIA + Salic acid
CIA + 3´-SL (500 mg·kg-1)
CIA + MTX (1 mg·kg-1)
Paw swelling (mm)
Clinical score
Incidence (%)
(day)
D
IL-1β, IL-6 & TNF-α (pg·mL-1)
IL-1β
IL-6
TNF-α
E
Optical Density (450 nm)
IgG
IgG1
IgG2a
IgG2b
21day
48day
F
Control
3´-SL (500 mg·kg-1)
MTX (1 mg·kg-1)
CD19
B220
PBS
Lactose
Sialic acid
CIA
0.863
59.2
39.7
0.226
0.785
75.4
23.5
0.268
0.496
65.4
33.7
0.389
0.550
75.6
23.5
0.294
0.497
65.1
34.1
0.355
1.22
72.5
25.9
0.360

Figure S1. Therapeutic effect of 3′-SL after onset of CIA. (A) Experimental scheme for CIA analysis. Eight-week-old DBA/1 mice were immunised on days 0 and 21. Oral administration of 3′-SL, lactose, sialic acid, and MTX occurred at 2-day intervals for 2 weeks after onset of CIA. (B) Representative hind paws from each treatment group are shown (2 weeks after induction of arthritis). (C) Paw swelling (left), clinical score (middle), and incidence (right) in mice with CIA that were administered lactose, sialic acid, MTX, or 3′-SL. (D, E) Production of pro-inflammatory cytokines (D) and type II collagen-specific autoantibodies (E) in sera of mice with CIA that were treated as indicated. Serum samples were collected on day 21 or 48. Production of autoantibodies and cytokines was measured by ELISA. (F) Percentage of $CD19^{+}B220^{+}$ B cells in spleens from CIA mice administered lactose or 3′-SL. Values are presented as means ± SEM (n = 8 mice per group). $^{\#}$ $P < 0.05$ compared with control group; * $P < 0.05$ compared with CIA group (Lactose administration).

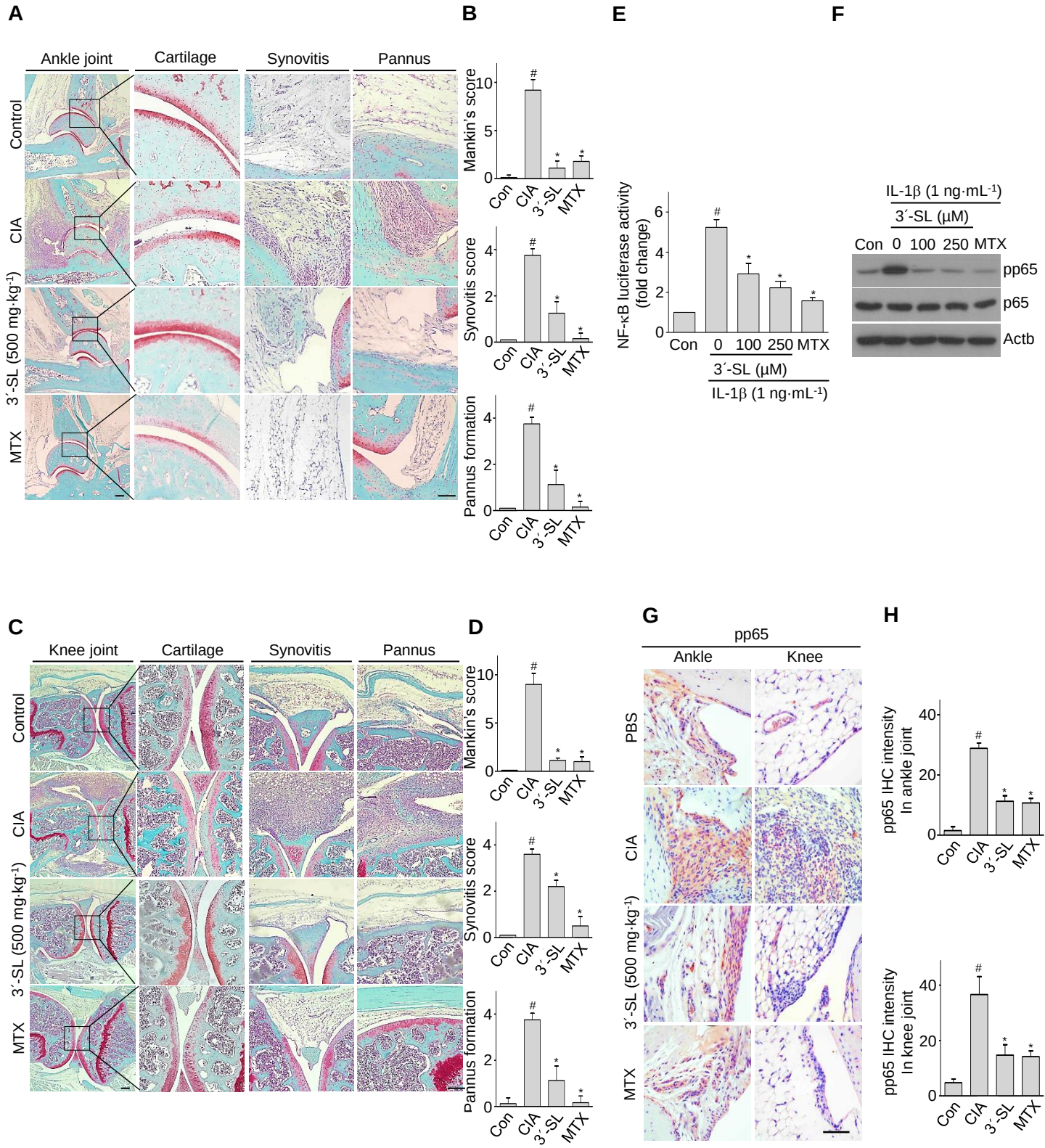

A
Ankle joint
Cartilage
Synovitis
Pannus
Control
CIA
3´-SL (500 mg·kg-1)
MTX
B
Mankin's score
Synovitis score
Pannus formation
Con
CIA
3´-SL
MTX
E
NF-κB luciferase activity (fold change)
Con
0
100
250
MTX
3´-SL (μM)
IL-1β (1 ng·mL-1)
F
IL-1β (1 ng·mL-1)
3´-SL (μM)
Con 0 100 250 MTX
pp65
p65
Actb
C
Knee joint
Cartilage
Synovitis
Pannus
Control
CIA
3´-SL (500 mg·kg-1)
MTX
D
Mankin's score
Synovitis score
Pannus formation
Con
CIA
3´-SL
MTX
G
pp65
Ankle
Knee
PBS
CIA
3´-SL (500 mg·kg-1)
MTX
H
pp65 IHC intensity In ankle joint
pp65 IHC intensity In knee joint
Con
CIA
3´-SL
MTX

Figure S2. Attenuation of CIA progression after onset of CIA by 3′-SL and MTX via blocking NF-κB activation. (A-D) Immune cell infiltrations into synovium of ankle (A) and knee (C) joints were analysed by haematoxylin and safranin-O staining. Quantification of synovitis and pannus formation in ankle (B) and knee (C) joints was assessed by clinical scores. (E) Mouse CD90.2^{+} FLS transfected with NF-κB reporter gene plasmid were treated with IL-1β (1 ng·mL^{-1}) in absence or presence of 3′-SL and MTX. NF-κB transcriptional activity was measured by reporter gene assay. (F) Mouse CD90.2^{+} FLS were co-treated with different concentrations of 3′-SL and MTX for 24 h and IL-1β (1 ng·mL^{-1}) for 10 min. Phosphorylation of p65 was detected by western blotting. (G) Immunohistochemical staining of pp65 in synovial tissues of ankle and knee joints after oral administration of 3′-SL or MTX to mice with CIA. Scale bar = 50 μm. (H) Quantification of pp65 expression in ankle (upper panel) and knee (lower panel) joints. $^{\#}$ $P < 0.05$ compared with control; * $P < 0.05$ compared with IL-1β-treated or CIA group (Lactose administration).

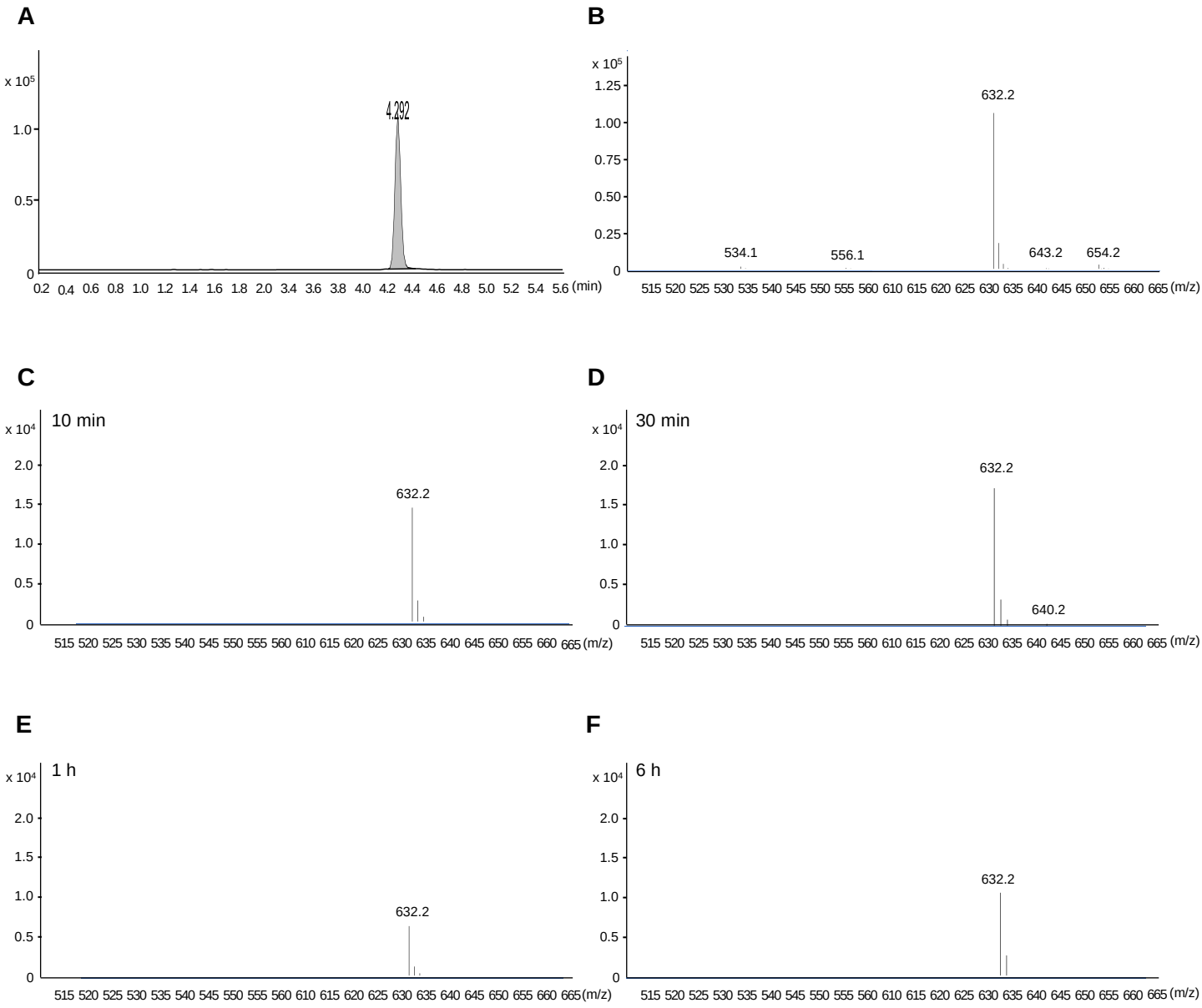


Figure S3. (A) Extracted-ion chromatogram (EIC) of 3′-SL standard in negative ion mode ($[M-H]^-$); (B) MS spectra of 3′-SL standard in negative ion mode ($[M-H]^-$); (C) – (F) MS spectra of 3′-SL for 10 min to 6 h in treated serum.

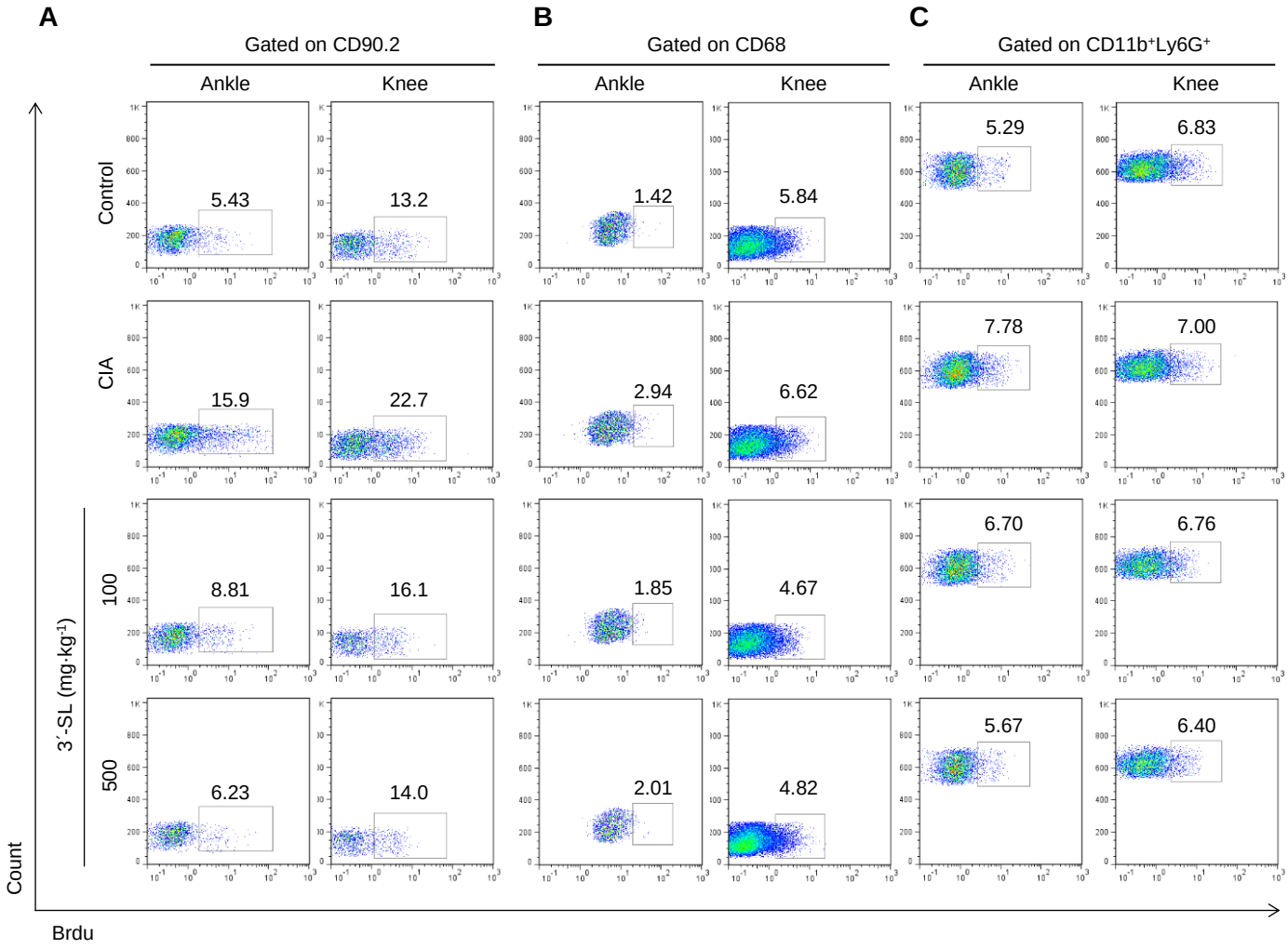
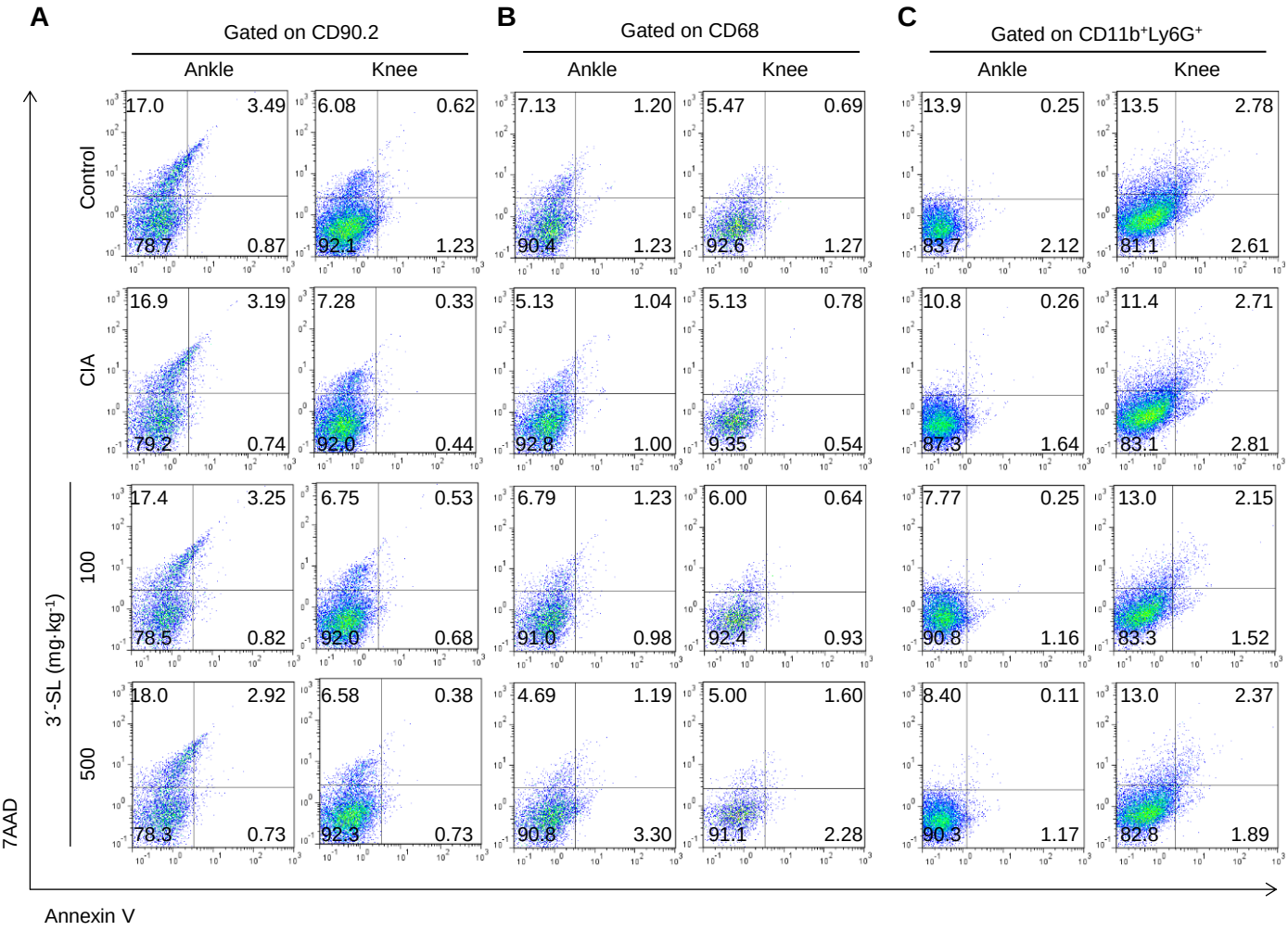
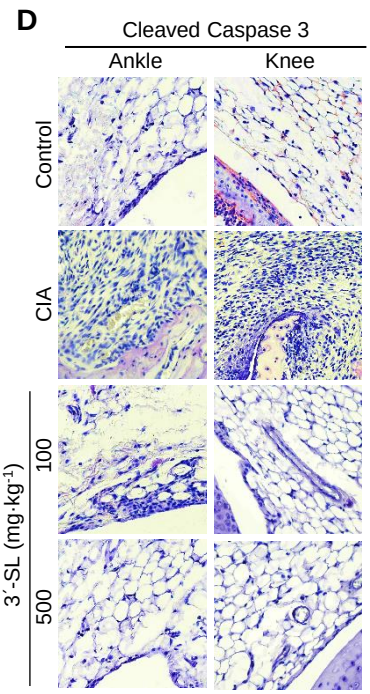


Figure S4. Mice were administered BrdU for 2 days. Isolated cells from the ankle and knee were labelled with anti-CD90.2 (A), anti-CD68 (B), or anti-CD11b^{+}Ly6G^{+} (C) for FLS, macrophages, and neutrophils, respectively, and analysed by FACS.


A
Gated on CD90.2
Ankle
Knee
B
Gated on CD68
Ankle
Knee
C
Gated on CD11b+Ly6G+
Ankle
Knee
Control
CIA
3´-SL (mg·kg-1)
100
500
7AAD
Annexin V
17.0 3.49 78.7 0.87
6.08 0.62 92.1 1.23
7.13 1.20 90.4 1.23
5.47 0.69 92.6 1.27
13.9 0.25 83.7 2.12
13.5 2.78 81.1 2.61
16.9 3.19 79.2 0.74
7.28 0.33 92.0 0.44
5.13 1.04 92.8 1.00
5.13 0.78 9.35 0.54
10.8 0.26 87.3 1.64
11.4 2.71 83.1 2.81
17.4 3.25 78.5 0.82
6.75 0.53 92.0 0.68
6.79 1.23 91.0 0.98
6.00 0.64 92.4 0.93
7.77 0.25 90.8 1.16
13.0 2.15 83.3 1.52
18.0 2.92 78.3 0.73
6.58 0.38 92.3 0.73
4.69 1.19 90.8 3.30
5.00 1.60 91.1 2.28
8.40 0.11 90.3 1.17
13.0 2.37 82.8 1.89


D
Cleaved Caspase 3
Ankle
Knee
Control
CIA
3´-SL (mg·kg-1)
100
500

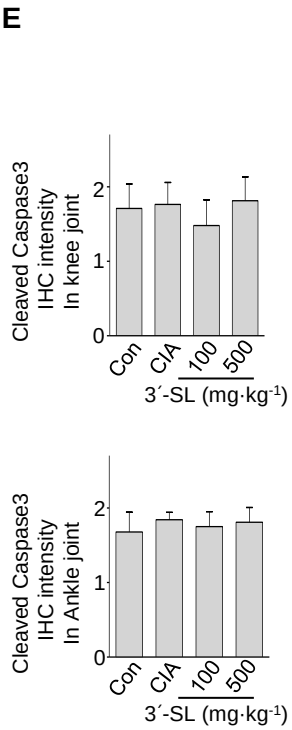

E
Cleaved Caspase3
IHC intensity
In knee joint
0
1
2
Con
CIA
100
500
3´-SL (mg·kg-1)
Cleaved Caspase3
IHC intensity
In Ankle joint
0
1
2
Con
CIA
100
500
3´-SL (mg·kg-1)

Figure S5. Annexin V and 7-AAD double-positive staining show the proportion of apoptotic cells in ankle and knee joints. Cells were isolated from ankle and knee joints and labelled with anti-CD90.2 (A), anti-CD68 (B), or anti-$CD11b^{+}Ly6G^{+}$ (C). (D) Immunohistochemical staining of representative cleaved caspase 3 in the synovial tissues of ankle (left) and knee (right) joints. (E) Quantification of cleaved caspase 3 expression in ankle (left panel) and knee (right panel) joints.

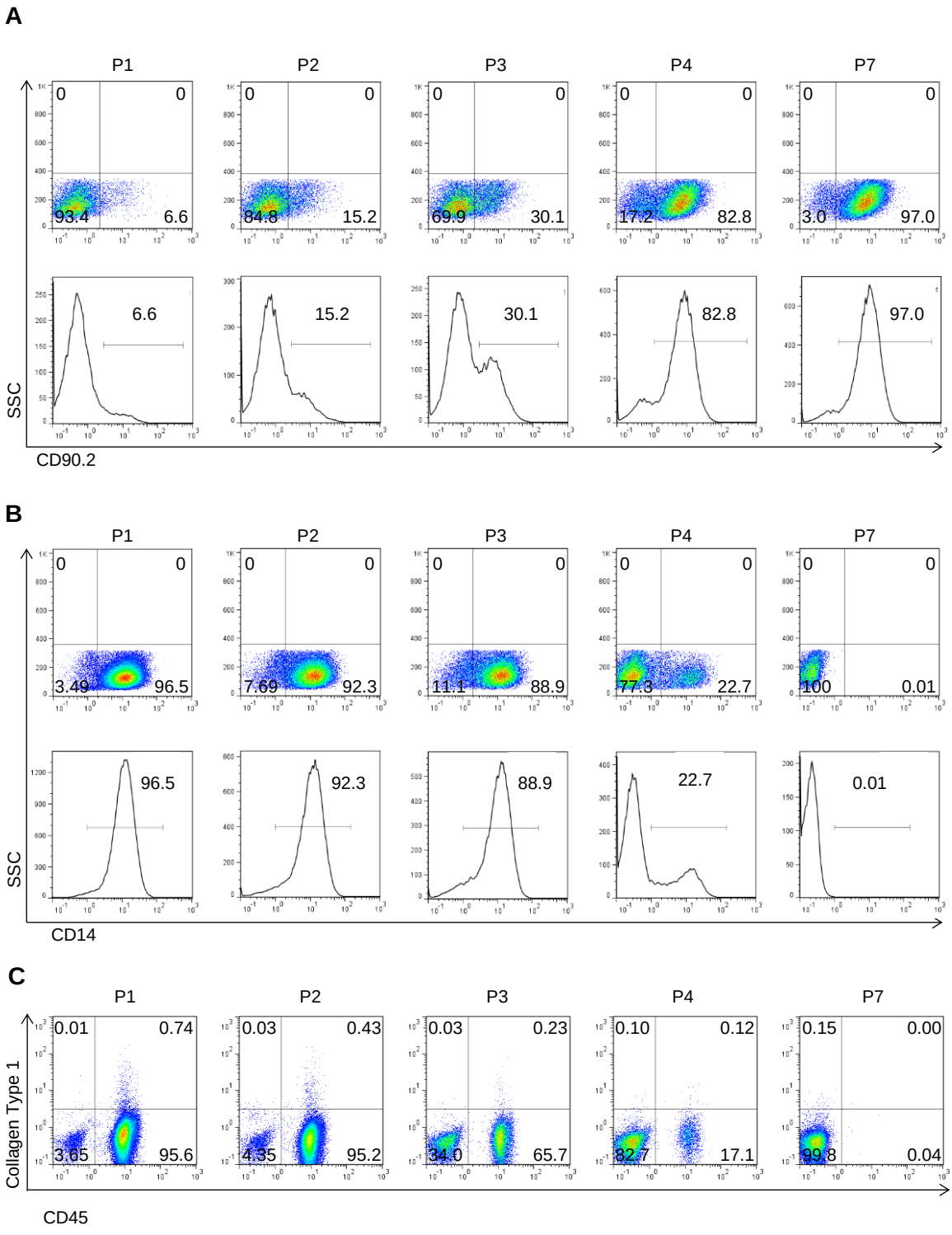


Figure S6. FACS analysis of mouse CD90.2$^+$ FLS surface marker expression with increasing passages. Mouse CD90.2$^+$ FLS were maintained as described in the Methods section. (A, B) Mouse CD90.2$^+$ FLS were harvested and stained with anti-CD90.2, anti-CD14, anti-CD45, and anti-collagen type 1 antibodies in different passage conditions. Percentages of CD90.2$^+$ (A), CD14$^+$ (B), and collagen type 1$^+$CD45$^+$ (C) were analysed by FACS analysis.

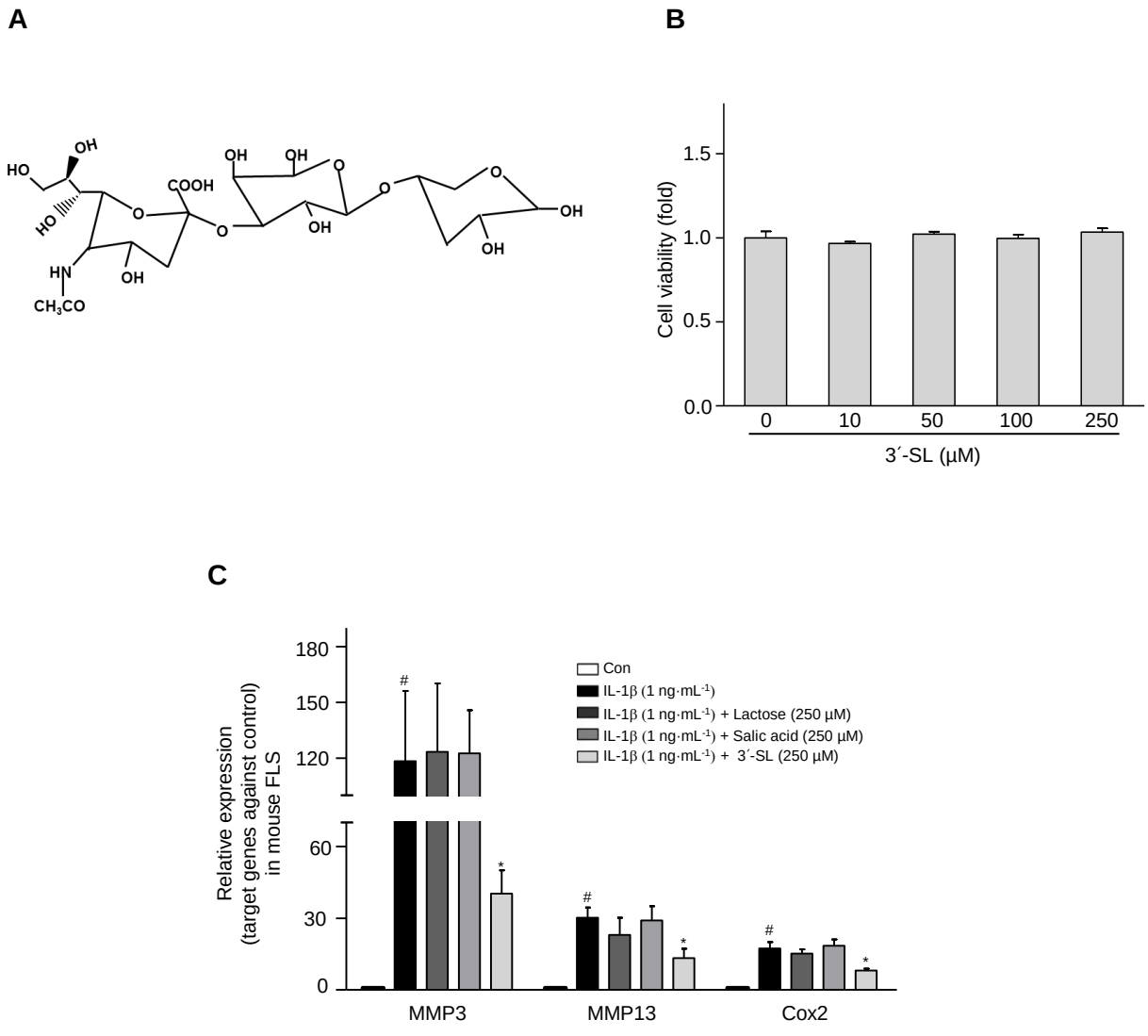


Figure S7. Effect of 3′-SL on CD90.2$^+$ FLS viability. (A) Chemical structure of 3′-SL. (B) Effect of 3′-SL on CD90.2$^+$ FLS viability and proliferation was determined by WST-1 assay. CD90.2$^+$ FLS were cultured with various concentrations of 3′-SL for 24 h. Cell viability was then determined by WST-1 assay. (C) Effect of lactose, sialic acid, and 3′-SL in IL-1β-treated mouse CD90.2$^+$ FLS. Values are presented as means ± SEM (n = 5). $^{\#}$ $P < 0.05$ compared with control; * $P < 0.05$ compared with IL-1β-treated group.

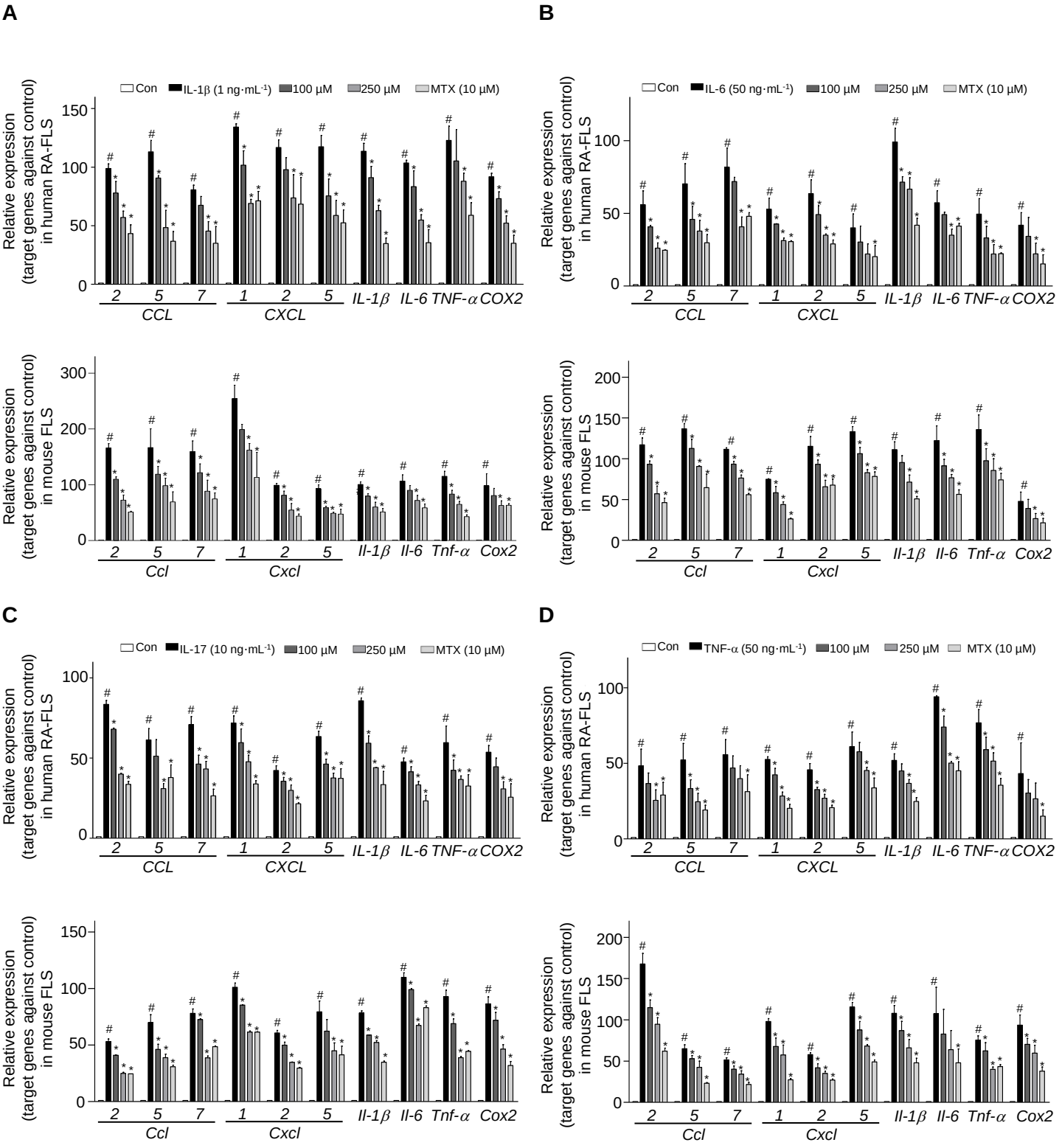

A
B
C
D
Con
IL-1β (1 ng·mL⁻¹)
IL-6 (50 ng·mL⁻¹)
IL-17 (10 ng·mL⁻¹)
TNF-α (50 ng·mL⁻¹)
100 μM
250 μM
MTX (10 μM)
Relative expression (target genes against control) in human RA-FLS
Relative expression (target genes against control) in mouse FLS
2 5 7 1 2 5
CCL
CXCL
IL-1β IL-6 TNF-α COX2
Ccl
Cxcl
Il-1β Il-6 Tnf-α Cox2

Figure S8. Chemokines, pro-inflammatory cytokines, and COX2 expression induced by IL-1β, IL-6, IL-17, and TNF-α are suppressed by 3′-SL in human RA-FLS and mouse CD90.2^{+} FLS. Human RA-FLS and mouse CD90.2^{+} FLS treated with IL-1β (1 ng·mL^{-1}) (A), IL-6 (50 ng·mL^{-1}) (B), IL-17 (10 ng·mL^{-1}) (C), and TNF-α (50 ng·mL^{-1}) (D) were co-treated with 3′-SL or MTX for 24 h at the indicated concentrations. Expression of chemokines, pro-inflammatory cytokines, and COX2 was determined by qRT-PCR. Values are presented as means ± SEM (n = 5). $^{\#}$ $P < 0.05$ compared with control group; * $P < 0.05$ compared with IL-1β-, IL-6-, IL-17-, and TNF-a-treated groups.

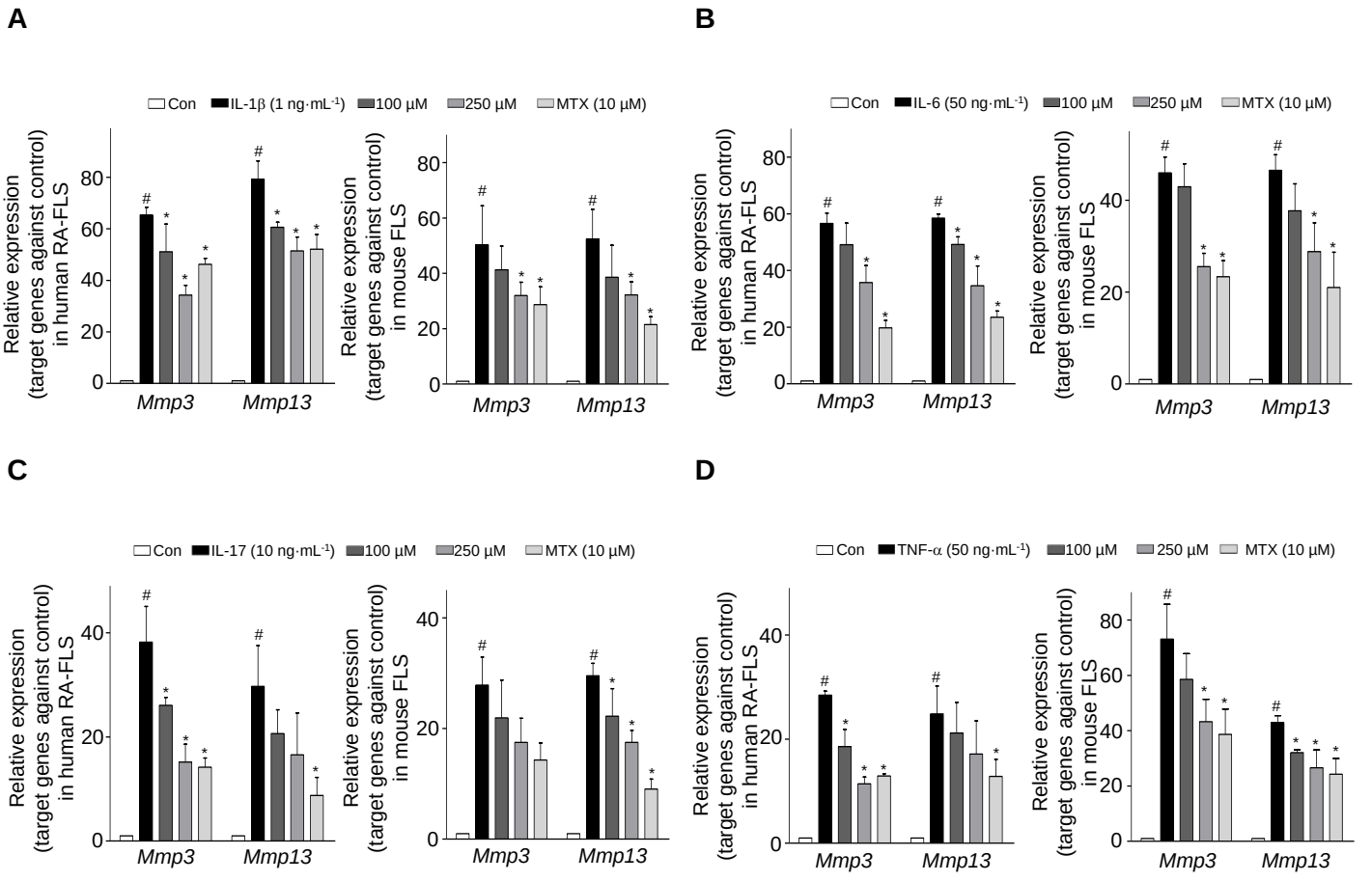
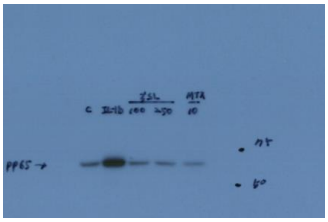


Figure S9. MMP expression induced by IL-1β, IL-6, IL-17, and TNF-α was inhibited by 3′-SL in human RA-FLS and mouse CD90.2$^+$ FLS. Human RA-FLS and mouse CD90.2$^+$ FLS treated with IL-1β (1 ng·mL$^-$1) (A), IL-6 (50 ng·mL$^-$1) (B), IL-17 (10 ng·mL$^-$1) (C), and TNF-α (50 ng·mL$^-$1) (D) were co-treated with 3′-SL or MTX for 24 h at the indicated concentrations. Expression of MMP3 and MMP13 was determined by qRT-PCR. Values are presented as means ± SEM (n = 5). # $P < 0.05$ compared with control group; * $P < 0.05$ compared with IL-1β-, IL-6-, IL-17-, and TNF-α-treated groups.

Supporting information figure S2F

pp65



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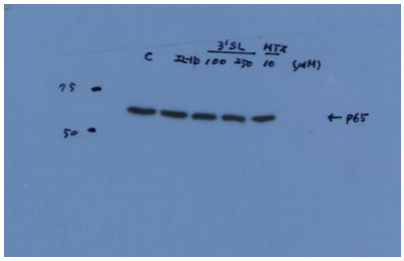

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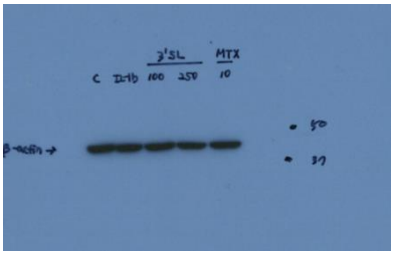

Figure 6B (Left; Human RA-FLS)

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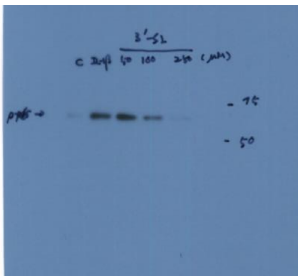

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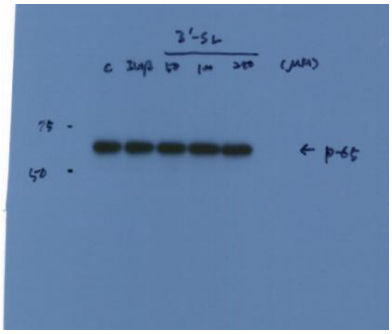

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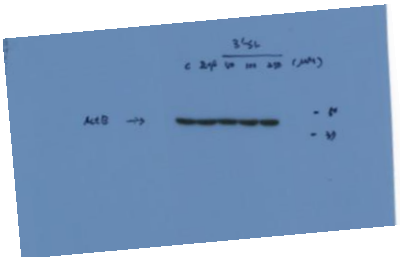

Figure 6B (Right; mouse FLS)

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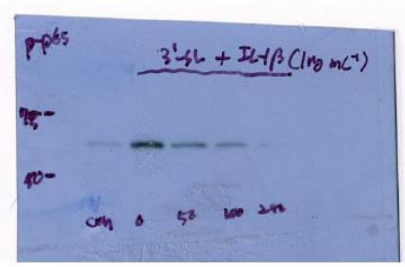

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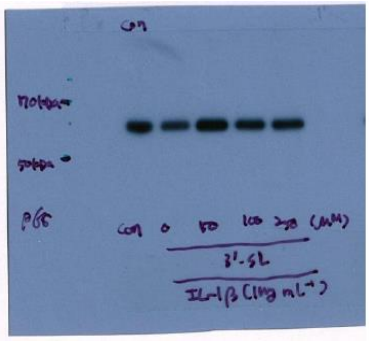

ACTB