# Supplementary Materials for

## Siglec-F-expressing neutrophils are essential for creating a pro-fibrotic microenvironment in the obstructed kidney

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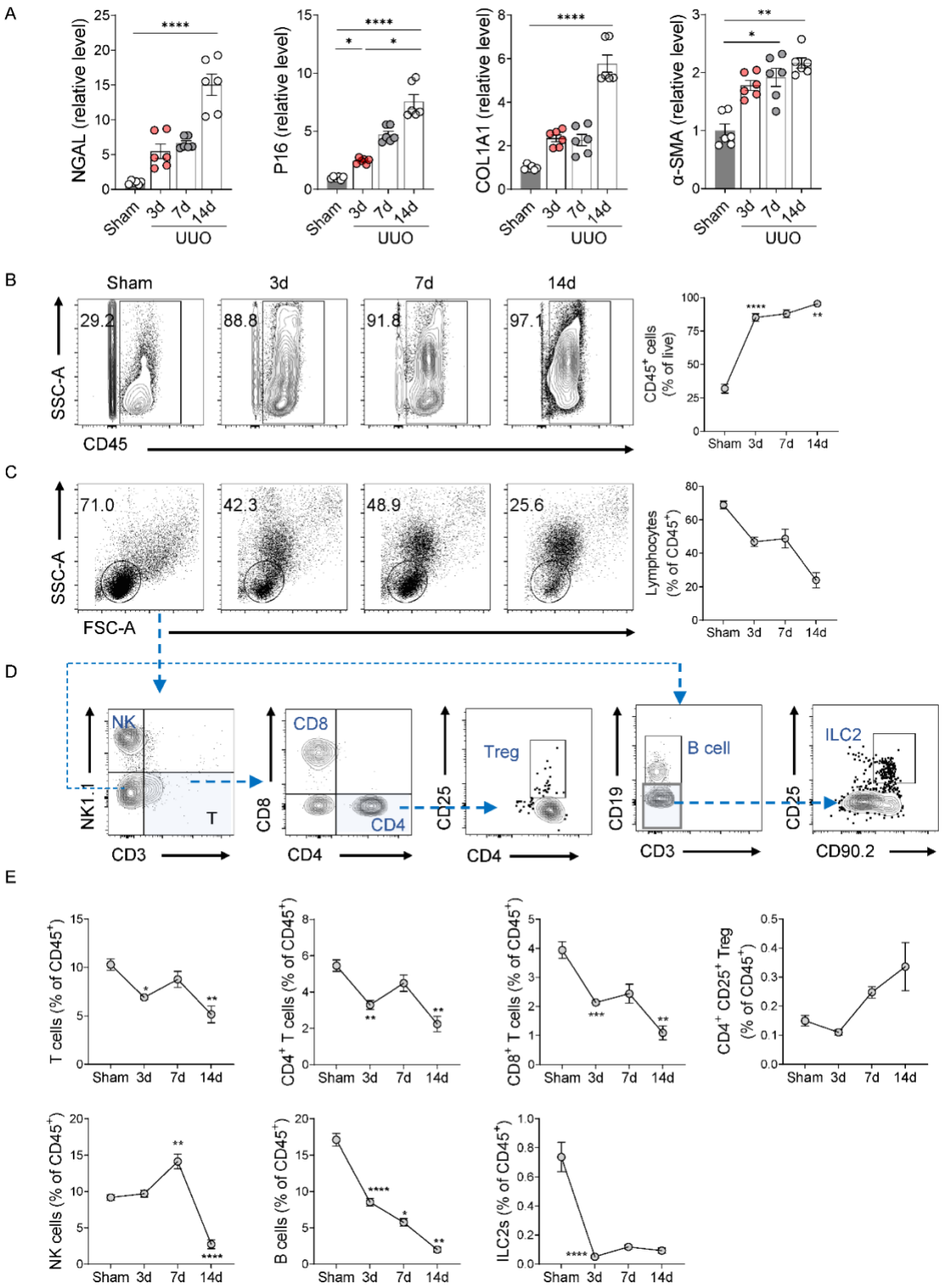
A
NGAL (relative level)
P16 (relative level)
COL1A1 (relative level)
α-SMA (relative level)
Sham
3d
7d
14d
UUO
B
Sham
3d
7d
14d
29.2
88.8
91.8
97.1
SSC-A
CD45
CD45+ cells (% of live)
C
71.0
42.3
48.9
25.6
SSC-A
FSC-A
Lymphocytes (% of CD45+)
D
NK
T
NK1.1
CD3
CD8
CD4
CD25
Treg
CD19
B cell
ILC2
CD90.2
E
T cells (% of CD45+)
CD4+ T cells (% of CD45+)
CD8+ T cells (% of CD45+)
CD4+ CD25+ Treg (% of CD45+)
NK cells (% of CD45+)
B cells (% of CD45+)
ILC2s (% of CD45+)

**Figure S1. Inflammation and fibrosis evaluation in UUO-induced kidney injury.** UUO induced kidney injury was induced and evaluated on days 0, 3, 7, 14 from the UUO surgery. **(A)** Relative expression of NGAL, P16, COL1A1, and α-SMA evaluated by Western blot. **(B-C)** Frequency of $CD45^+$ leukocytes **(B)** and lymphocytes **(C)** analyzed with flow cytometry. **(D-E)** Immune cell profiling for total T cells, $CD4^+$, $CD8^+$ T cells, regulatory T cells ($CD4^+$ $CD25^+$), NK cells, B cells, and ILC2s ($CD3^-$ $CD19^-$ $NK1.1^-$ $CD90.2^+$ $CD25^+$). All results are shown as mean ± SEM and statistical analysis was performed using one-way ANOVA test. **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001; n = 5-7 mice in each group.

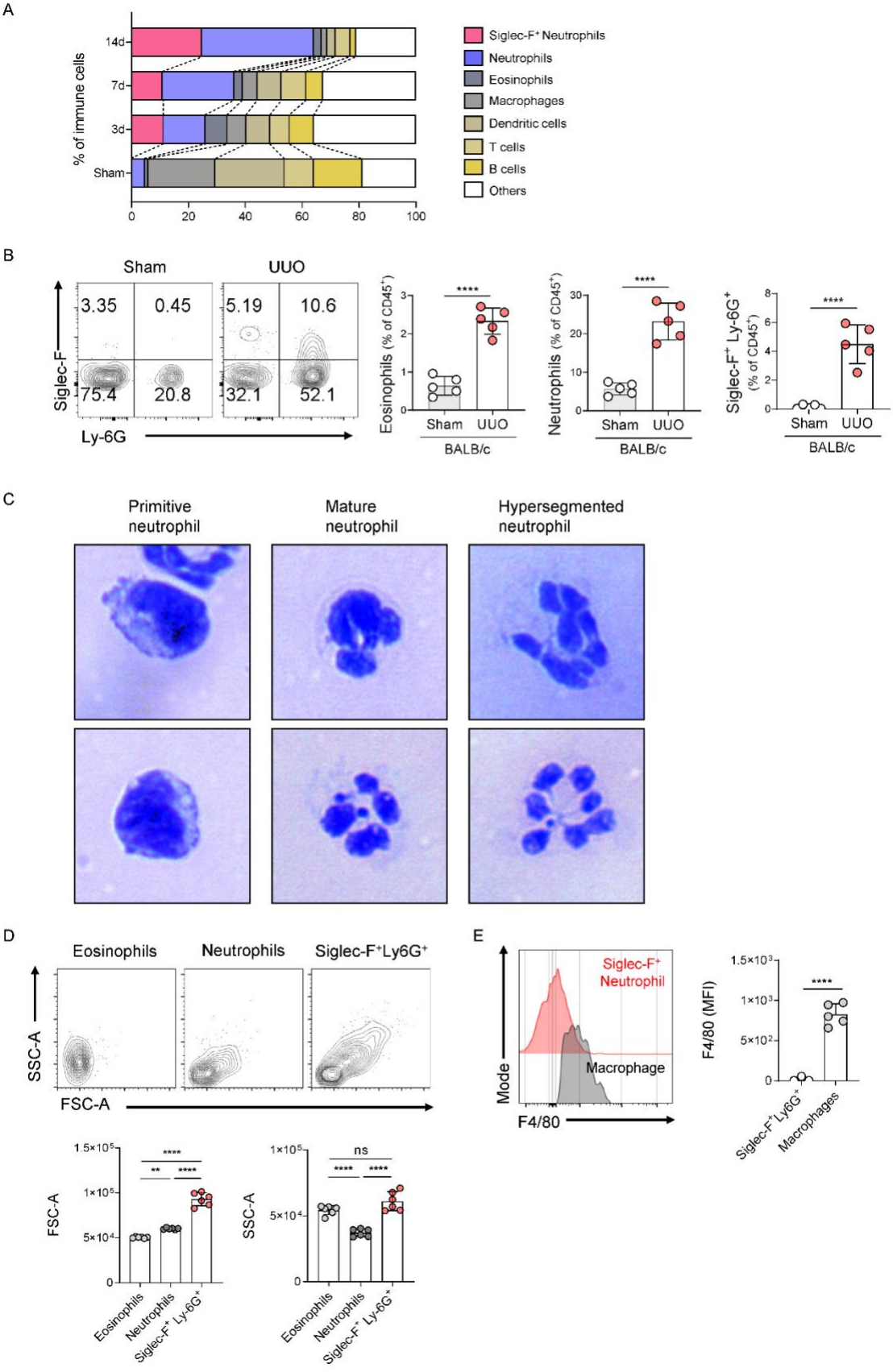
A
14d
7d
3d
Sham
% of immune cells
0
20
40
60
80
100
Siglec-F+ Neutrophils
Neutrophils
Eosinophils
Macrophages
Dendritic cells
T cells
B cells
Others
B
Sham
UUO
3.35
0.45
5.19
10.6
75.4
20.8
32.1
52.1
Siglec-F
Ly-6G
Eosinophils (% of CD45+)
Neutrophils (% of CD45+)
Siglec-F+ Ly-6G+ (% of CD45+)
****
Sham
UUO
BALB/c
C
Primitive neutrophil
Mature neutrophil
Hypersegmented neutrophil
D
Eosinophils
Neutrophils
Siglec-F+Ly6G+
SSC-A
FSC-A
1.5×10^5
1×10^5
5×10^4
0
**
****
ns
Eosinophils
Neutrophils
Siglec-F+ Ly-6G+
E
Siglec-F+ Neutrophil
Macrophage
Mode
F4/80
F4/80 (MFI)
1.5×10^3
1×10^3
5×10^2
Siglec-F+Ly6G+
Macrophages

**Figure S2. Characteristics of Siglec-F expressing neutrophils. (A)** Kinetic changes of immune cells during UUO-induced renal fibrosis. **(B)** Siglec-$F^+$ Ly-$6G^+$ cells emerged in UUO-induced kidney on BALB/c mice. **(C)** Morphologies of primitive, mature, and hypersegmented neutrophils sorted on day 14. **(D)** Forward scatter (FSC) and side scatter (SSC) comparison between eosinophils, neutrophils, and Siglec-$F^+$ Ly-$6G^+$ cells. **(E)** Comparison of F4/80 expression between Siglec-$F^+$ Ly-$6G^+$ cells and macrophages from day-7 UUO kidney All results are shown as mean ± SEM and statistical analysis was performed using Mann-Whitney test **(B, E)** or one-way ANOVA test **(D)**. **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001; n = 5-7 mice in each group.

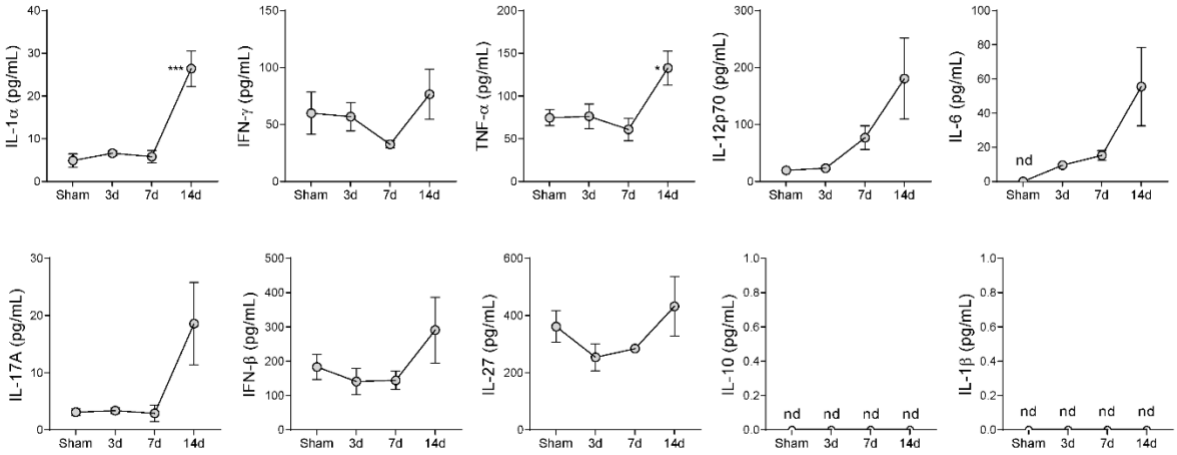


**Figure S3. Proinflammatory cytokine analysis of injured renal tissue.** Cytokine bead arrays were used to analyze the proinflammatory cytokines increased in the UUO induced renal injury. All results are shown as mean ± SEM and statistical analysis was performed using one-way ANOVA test. **P*<0.05; ****P*<0.001; n = 4-5 mice in each group. ns, not significant.

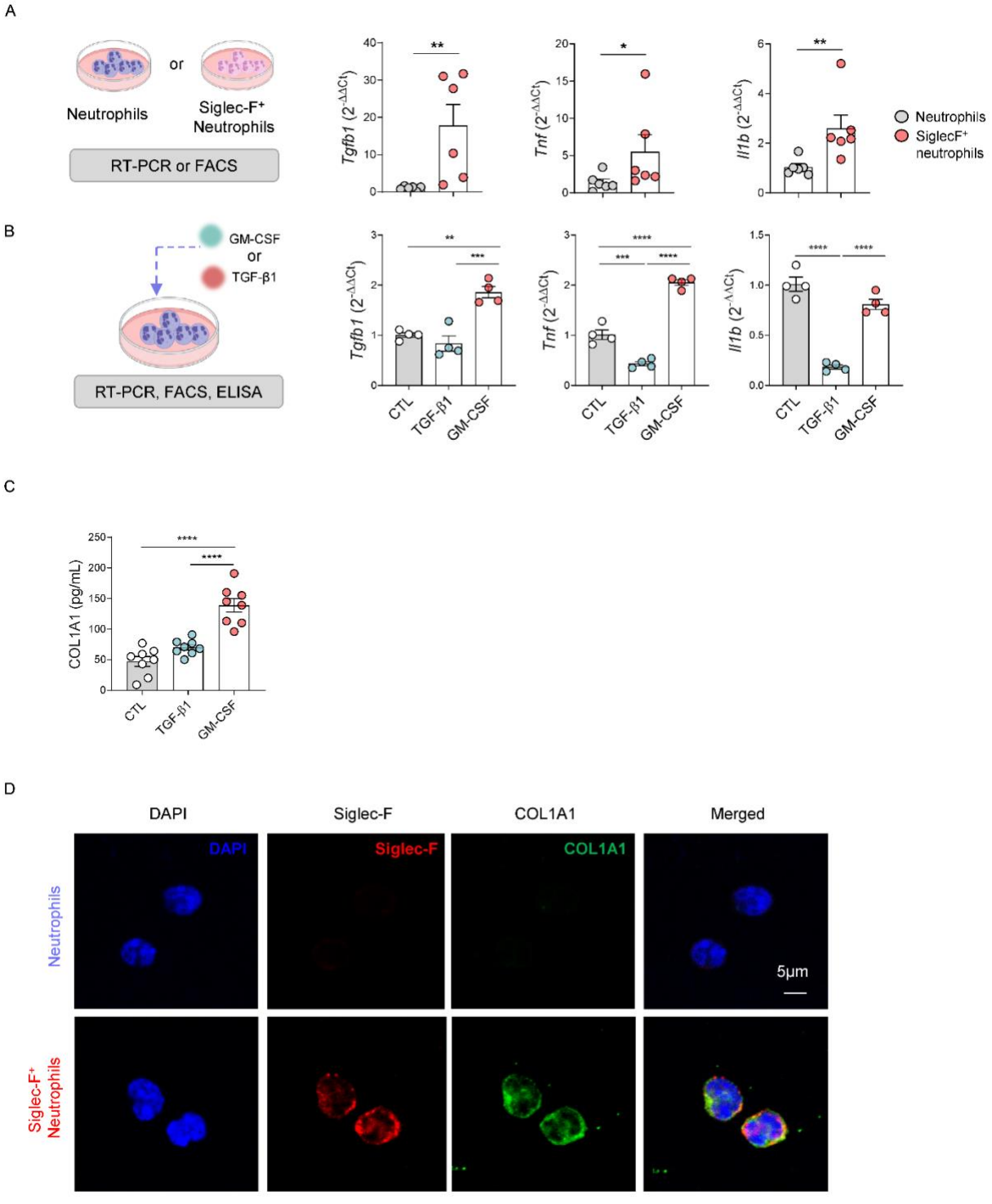
A
Neutrophils
or
Siglec-F+ Neutrophils
RT-PCR or FACS
Tgfb1 (2-ΔΔCt)
Tnf (2-ΔΔCt)
Il1b (2-ΔΔCt)
Neutrophils
SiglecF+ neutrophils
B
GM-CSF
or
TGF-β1
RT-PCR, FACS, ELISA
CTL
TGF-β1
GM-CSF
C
COL1A1 (pg/mL)
D
DAPI
Siglec-F
COL1A1
Merged
Neutrophils
Siglec-F+ Neutrophils
5μm

**Figure S4. Cytokine and collagen 1 production by Siglec-F$^+$ neutrophils generated in vivo and in vitro. (A)** RT-qPCR analysis of sorted neutrophils and Siglec-F$^+$ neutrophils from UUO-induced kidney (n = 6 in each group). **(B)** RT-qPCR analysis of TGF-β1 or GM-CSF primed neutrophils (n = 4 in each group). **(C-D)** COL1A1 expression of TGF-β1 or GM-CSF primed neutrophils evaluated by ELISA (n = 8 in each group) **(C)** and immunofluorescence **(D)**. All results are shown as mean ± SEM and statistical analysis was performed using Mann-Whitney test (A) or one-way ANOVA test (B-C). **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001.

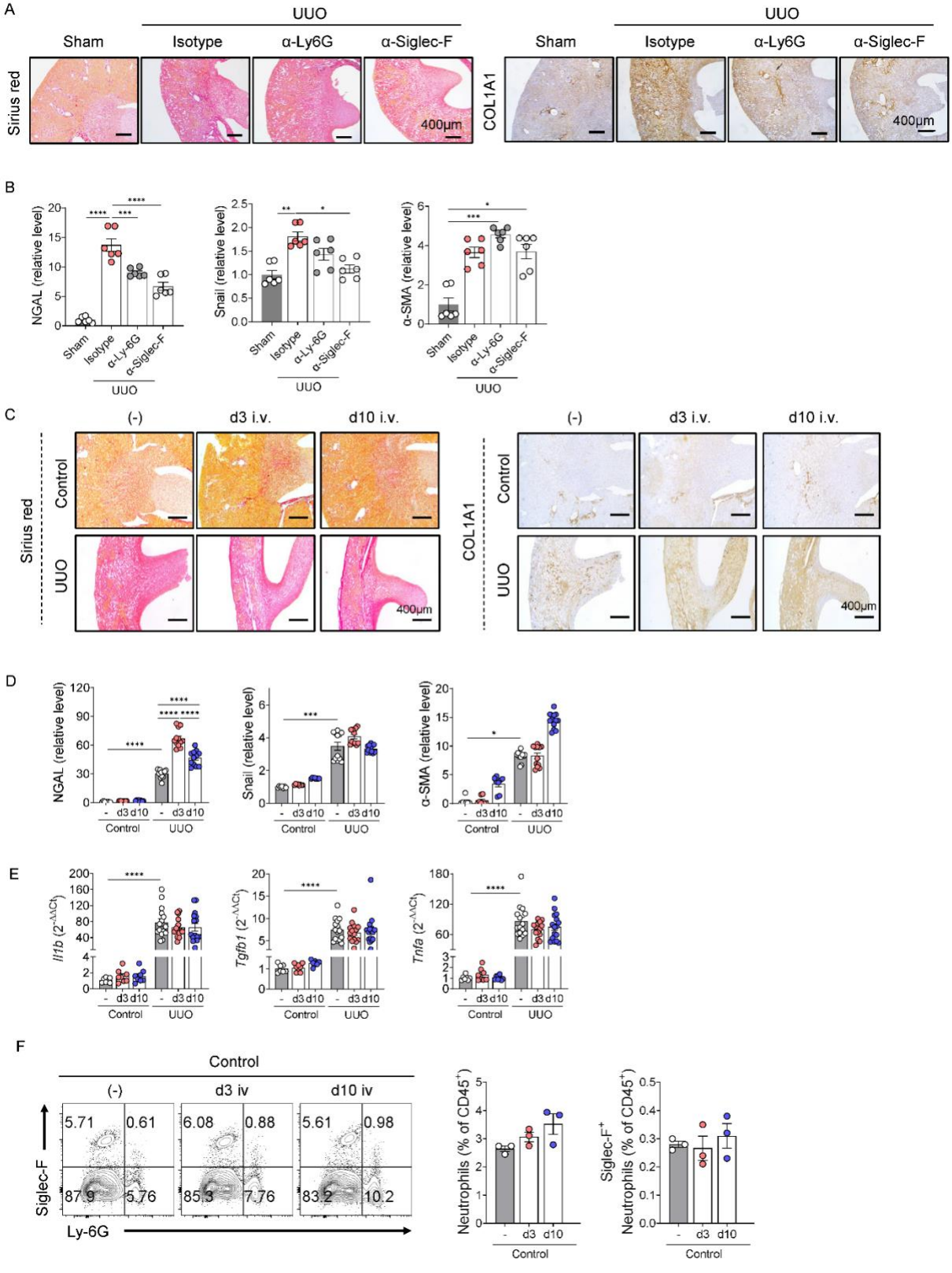
A
UUO
Sham
Isotype
α-Ly6G
α-Siglec-F
Sirius red
COL1A1
400μm
B
NGAL (relative level)
Snail (relative level)
α-SMA (relative level)
C
(-)
d3 i.v.
d10 i.v.
Control
UUO
D
E
Il1b (2-ΔΔCt)
Tgfb1 (2-ΔΔCt)
Tnfa (2-ΔΔCt)
F
Control
d3 iv
d10 iv
5.71
0.61
87.9
5.76
6.08
0.88
85.3
7.76
5.61
0.98
83.2
10.2
Siglec-F
Ly-6G
Neutrophils (% of CD45+)
Siglec-F+ Neutrophils (% of CD45+)

**Figure S5. Evaluation of renal injury and fibrosis by the depletion and adoptive transfer of Siglec-F+ neutrophils. (A-B)** Sirius red and COL1A1 staining **(A)**, and NGAL, Snail, and α-SMA expression **(B)** of Siglec-$F^+$ neutrophil depleted UUO mice. **(C-D)** Sirius red and COL1A1 staining **(C)**, and NGAL, Snail, and α-SMA expression **(D)** of control or UUO mice adoptively transferred with Siglec-$F^+$ neutrophils. **(E)** Proinflammatory cytokines expressed on renal tissues of Siglec-$F^+$ neutrophil-transferred control or UUO mouse kidneys. **(F)** Conventional and Siglec-F+ neutrophils in Siglec-F+ neutrophil transferred control mouse kidneys. All results are shown as mean ± SEM and statistical analysis was performed using one-way ANOVA test. **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001; n = 6-7 mice in each group for A-B, n=7-16 mice in each group for C-E. and n=3 mice in each group for F.

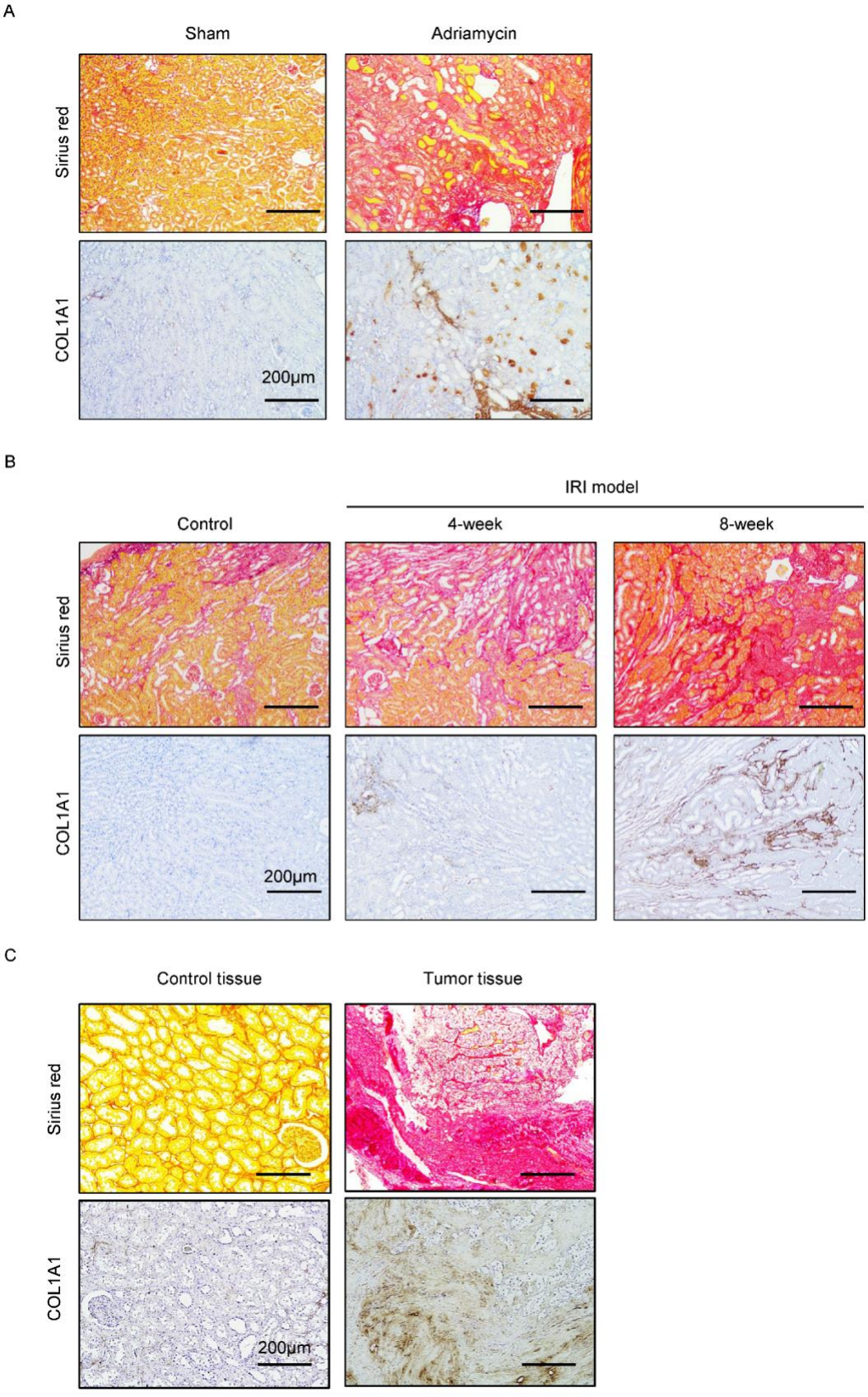
A
Sham
Adriamycin
Sirius red
COL1A1
200μm
B
IRI model
Control
4-week
8-week
Sirius red
COL1A1
200μm
C
Control tissue
Tumor tissue
Sirius red
COL1A1
200μm

**Figure S6. Fibrotic changes of other murine CKD models and tumor tissues from nephrectomy specimen of RCC patients.** Sirus-red and COL1A1 immunohistochemistry of **(A)** Adriamycin model, **(B)** renal IRI model, and **(C)** tumor tissues from RCC patients.