# CD226 promotes renal fibrosis by regulating macrophage activation and migration

Yun Song[1], Yazhen Wang[1], Juan Li[2], Yuting Shen[1], Yongli Hou[1], Zhaoyue Fu[1], Liang Fang[1], Boquan Jin[1], and Lihua Chen[1]

[1]Fourth Military Medical University
[2]Northwest University

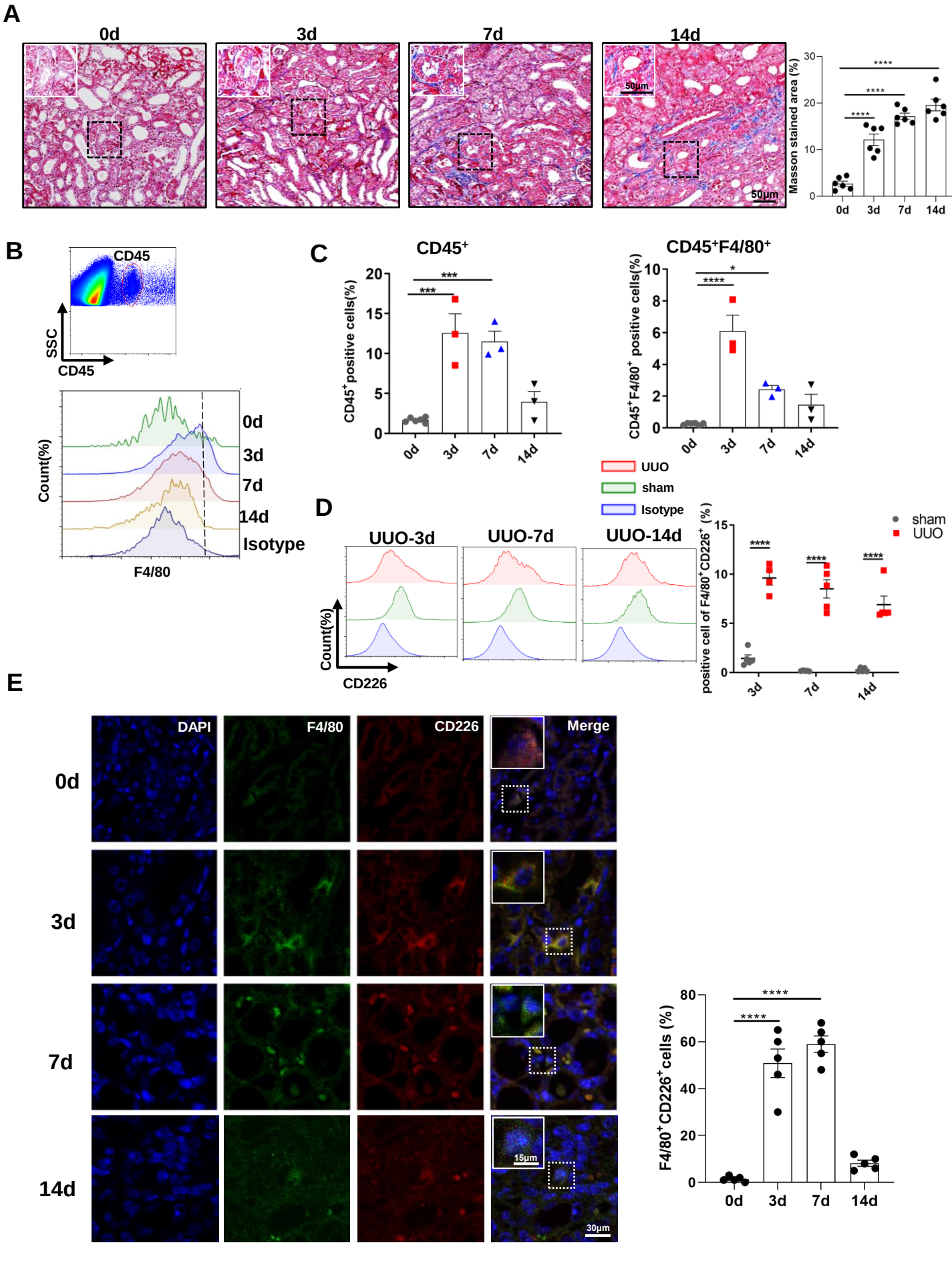
May 20, 2023

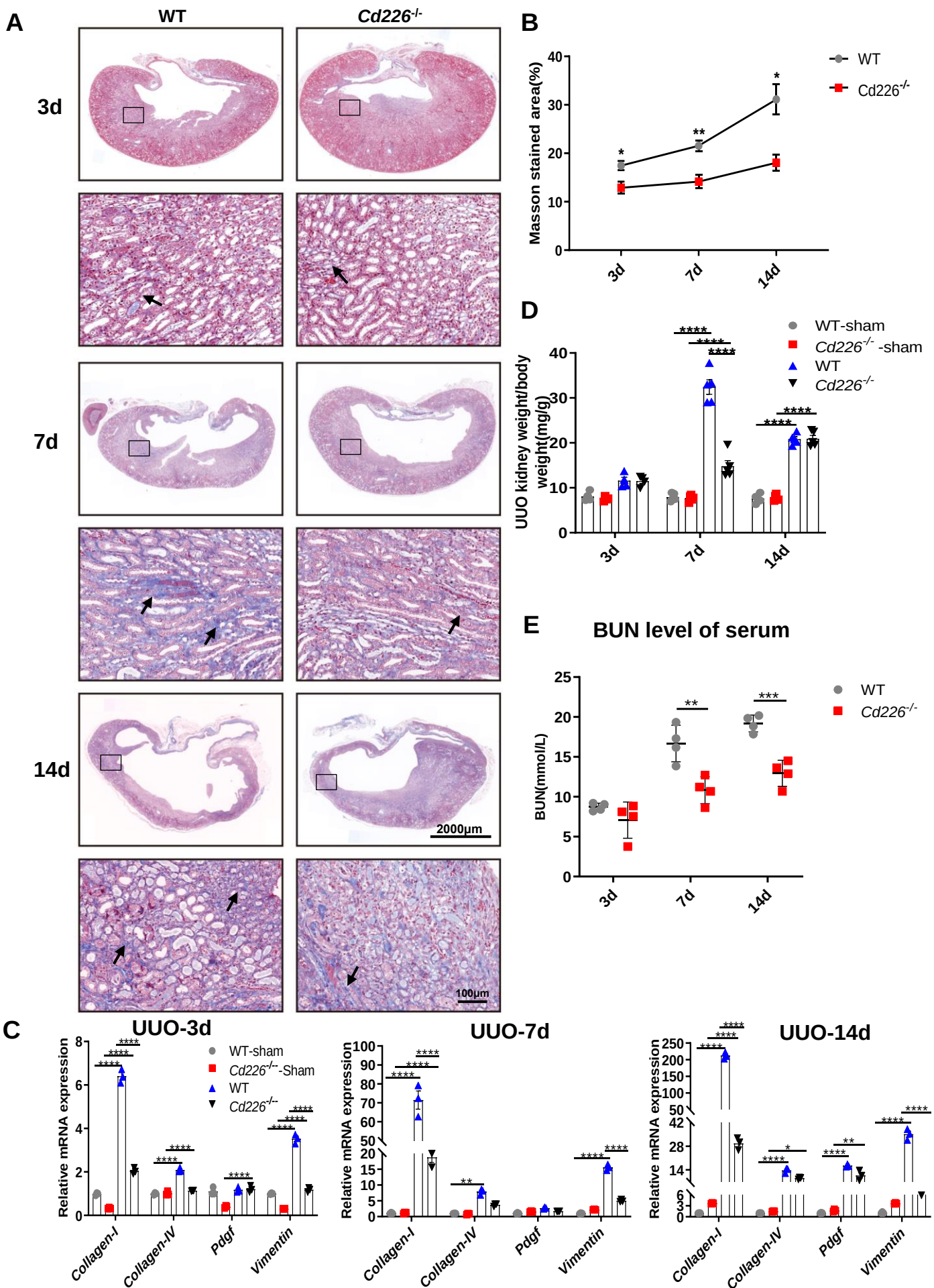
## Abstract

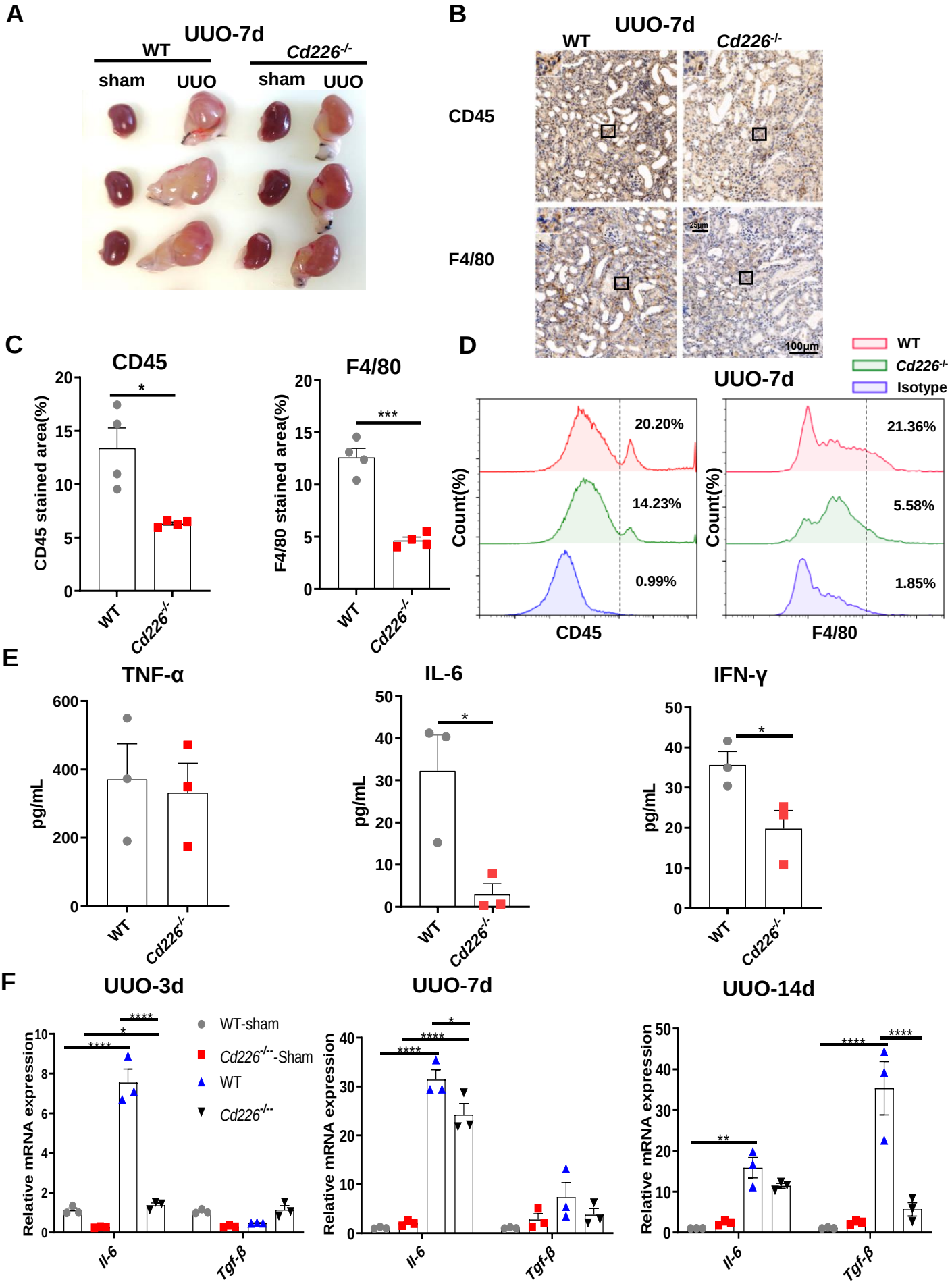
It has been found that CD226 plays an important role in regulating macrophage function, but its expression and function on macrophage during renal fibrogenesis have not been studied. Our data demonstrated that CD226 expression in macrophages was obviously upregulated in the UUO model, while CD226 deficiency attenuated collagen deposition in renal inerestitum along with fewer number of M1 within renal cortex and renal medulla and a lower level of proinflammatory factors compared to control littermates. Further studies demonstrated that Cd226-/–BMDMs transferring to Cd226+/+ mice could significantly reduce the tubular injury, collagen deposition and proinflammatory cytokines secretion compared with WT-BMDMs group and WT-PBS group in adoptively transferring assay. Mechanistic investigations revealed that CD226 could suppress KLF4 expression in macrophages, which subsequently promoted more proinflammatory M1 accumulation in the kidney of WT mice than that of CD226 deficient mice. In vitro, we silenced KLF4 expression in BMDMs deriving from WT or CD226 deficient mice and the trend that CD226 promting more numbers of M1 disappeared. Therefore, our results uncover a pathogenic role of CD226 during the development of CKD by promoting monocyte infiltration from peripheral blood into the kidney and enhancing macrophage activation towards to the inflammatory phenotype by suppressing KLF4 expression.

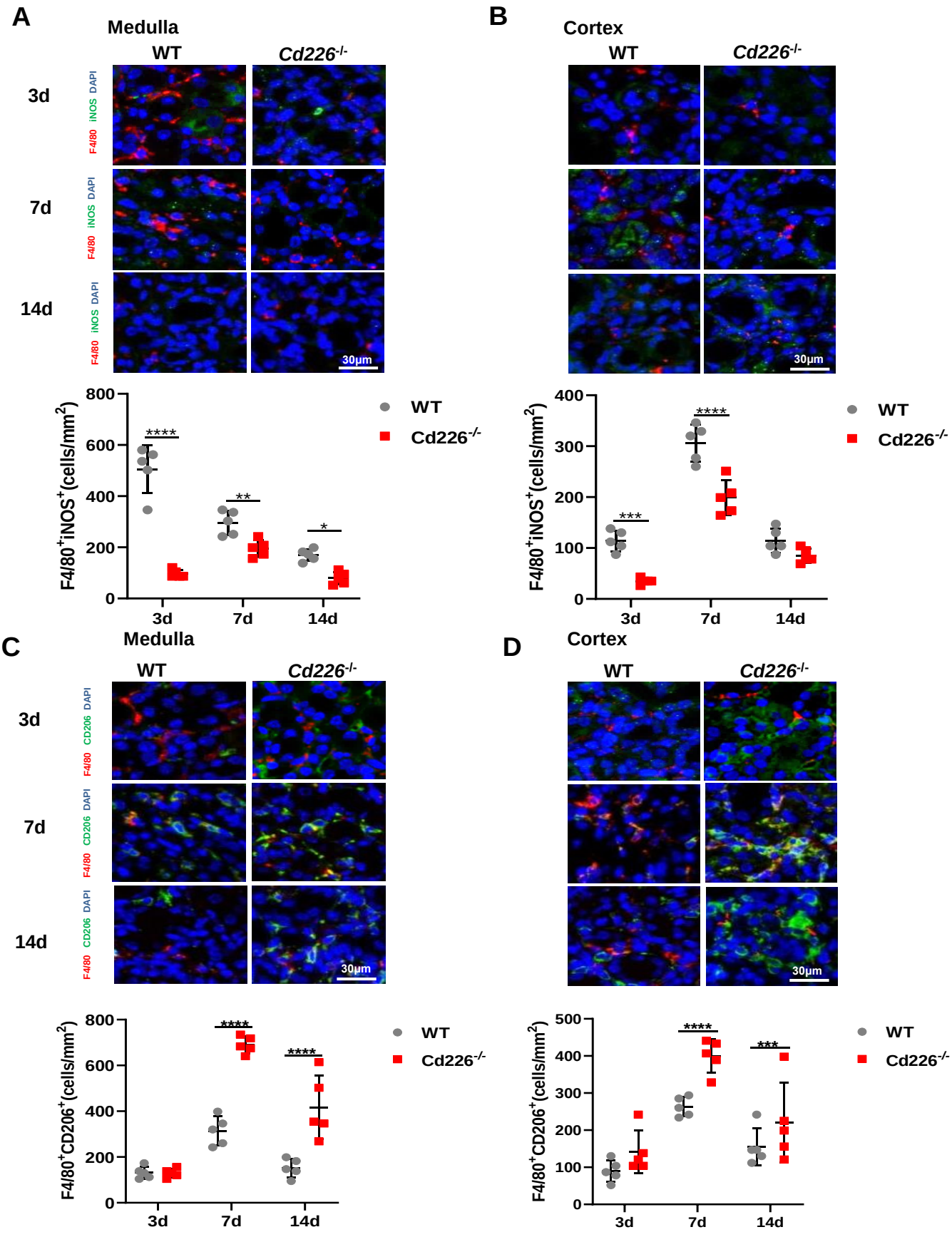
## Hosted file

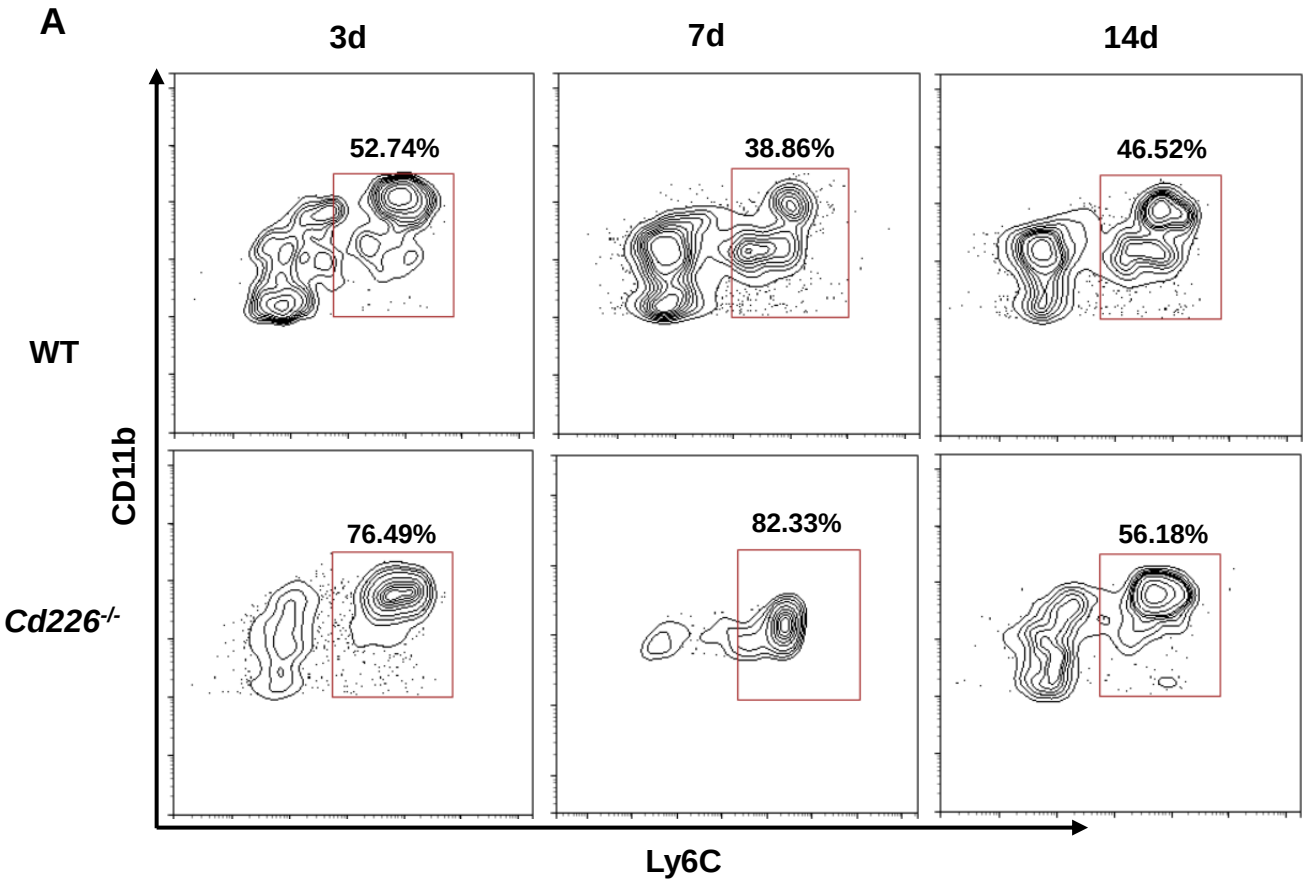
`Maunscript.docx` available at https://authorea.com/users/620373/articles/644429-cd226-promotes-renal-fibrosis-by-regulating-macrophage-activation-and-migration

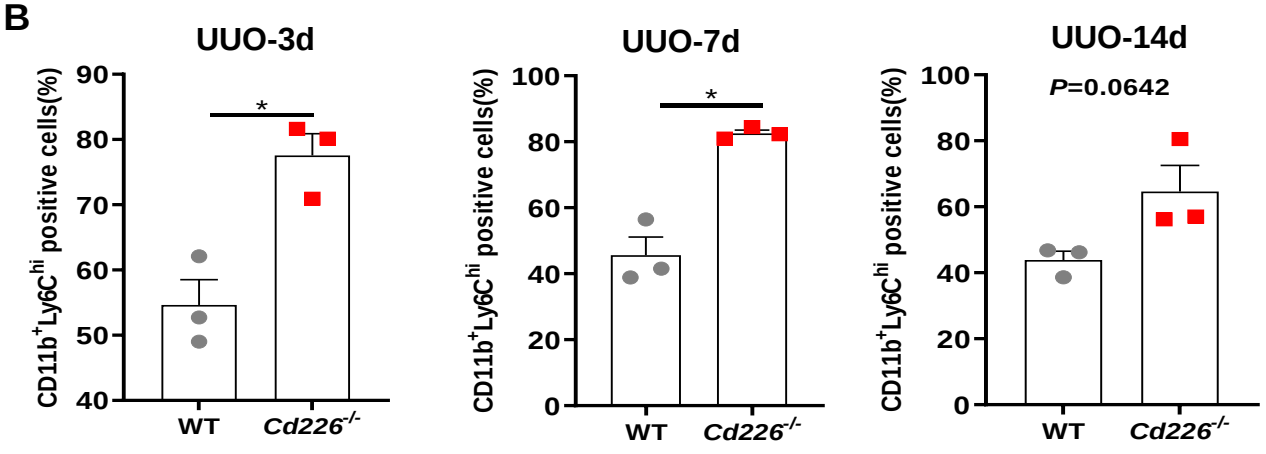

A
0d
3d
7d
14d
50μm
Masson stained area (%)
B
CD45
SSC
CD45
Count(%)
0d
3d
7d
14d
Isotype
F4/80
C
CD45+
CD45+positive cells(%)
CD45+F4/80+
CD45+F4/80+ positive cells(%)
UUO
sham
Isotype
D
UUO-3d
UUO-7d
UUO-14d
Count(%)
CD226
positive cell of F4/80+CD226+ (%)
sham
UUO
E
DAPI
F4/80
CD226
Merge
15μm
30μm
F4/80+CD226+cells (%)

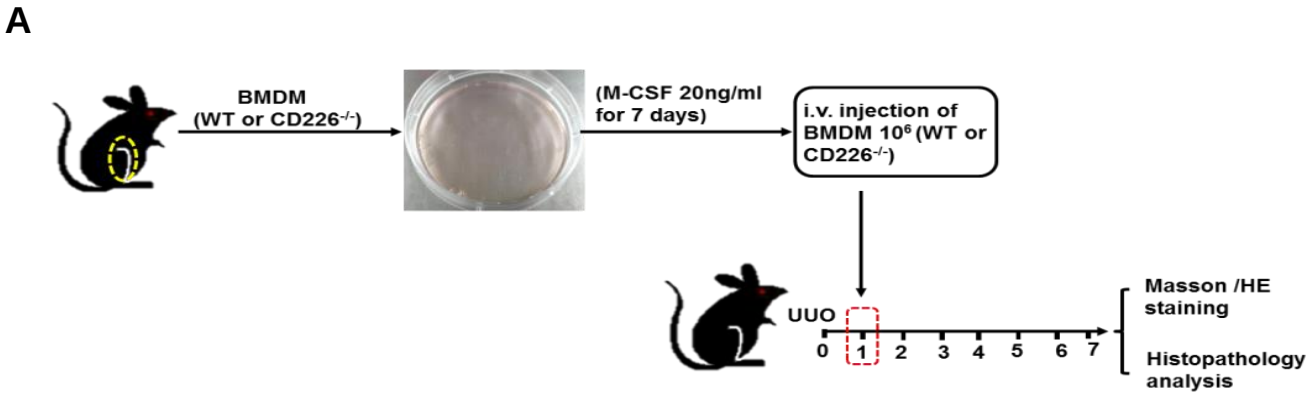

A
WT
Cd226-/-
3d
7d
14d
2000μm
100μm
B
Masson stained area(%)
WT
Cd226-/-
3d
7d
14d
D
UUO kidney weight/body weight(mg/g)
WT-sham
Cd226-/- -sham
WT
Cd226-/-
E
BUN level of serum
BUN(mmol/L)
WT
Cd226-/-
C
UUO-3d
UUO-7d
UUO-14d
Relative mRNA expression
WT-sham
Cd226-/--Sham
WT
Cd226-/-
Collagen-I
Collagen-IV
Pdgf
Vimentin

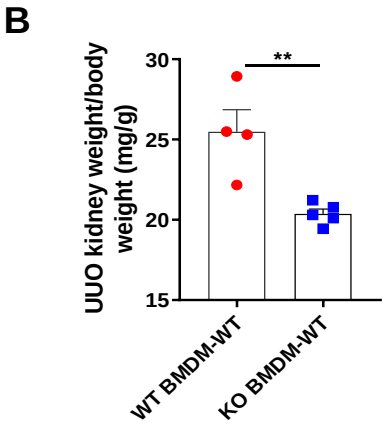

A
UUO-7d
WT
Cd226-/-
sham
UUO
sham
UUO
B
UUO-7d
WT
Cd226-/-
CD45
F4/80
25μm
100μm
C
CD45
CD45 stained area(%)
*
WT
Cd226-/-
F4/80
F4/80 stained area(%)
***
WT
Cd226-/-
D
WT
Cd226-/-
Isotype
UUO-7d
Count(%)
20.20%
14.23%
0.99%
CD45
Count(%)
21.36%
5.58%
1.85%
F4/80
E
TNF-α
pg/mL
WT
Cd226-/-
IL-6
pg/mL
*
WT
Cd226-/-
IFN-γ
pg/mL
*
WT
Cd226-/-
F
UUO-3d
UUO-7d
UUO-14d
WT-sham
Cd226-/--Sham
WT
Cd226-/-
Relative mRNA expression
Il-6
Tgf-β
Relative mRNA expression
Il-6
Tgf-β
Relative mRNA expression
Il-6
Tgf-β

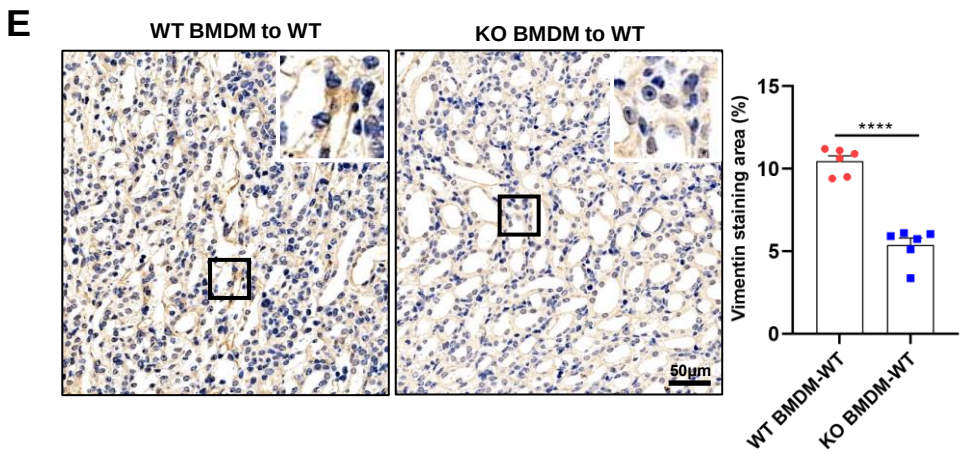

A
Medulla
WT
Cd226-/-
3d
7d
14d
F4/80 iNOS DAPI
30μm
F4/80+iNOS+(cells/mm2)
WT
Cd226-/-
****
**
*
B
Cortex
***
C
Medulla
F4/80 CD206 DAPI
F4/80+CD206+(cells/mm2)
D
Cortex

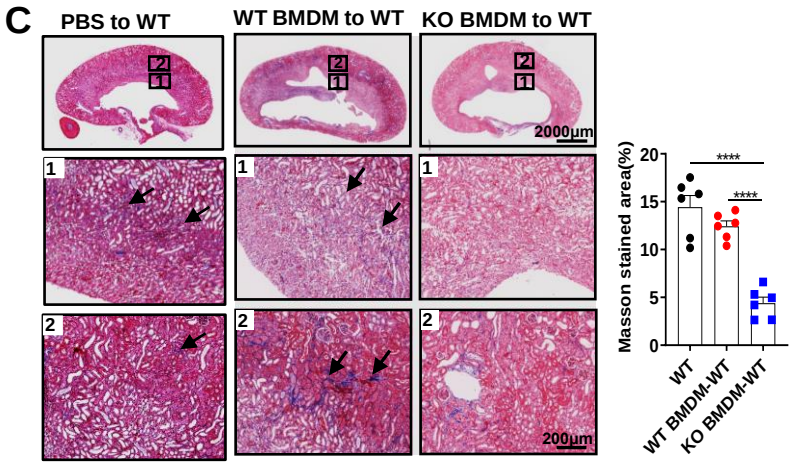

A
3d
7d
14d
52.74%
38.86%
46.52%
WT
CD11b
76.49%
82.33%
56.18%
Cd226-/-
Ly6C

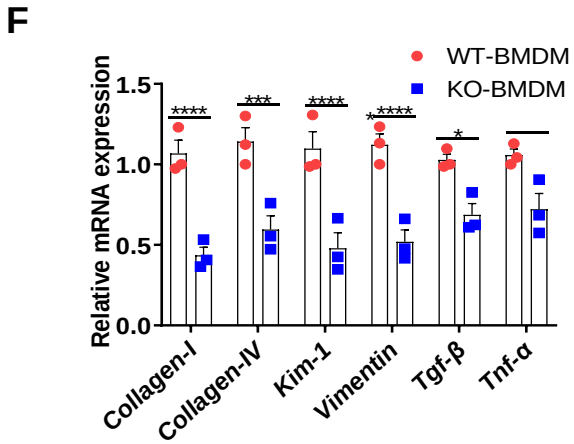

B
UUO-3d
UUO-7d
UUO-14d
CD11b+Ly6Chi positive cells(%)
90
80
70
60
50
40
100
20
0
*
P=0.0642
WT
Cd226-/-

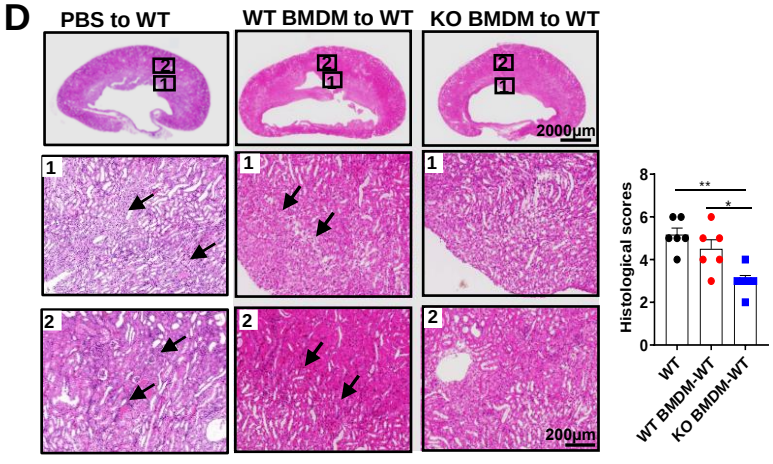

A
BMDM
(WT or CD226-/-)
(M-CSF 20ng/ml
for 7 days)
i.v. injection of
BMDM 10⁶ (WT or
CD226-/-)
UUO
0 1 2 3 4 5 6 7
Masson /HE
staining
Histopathology
analysis

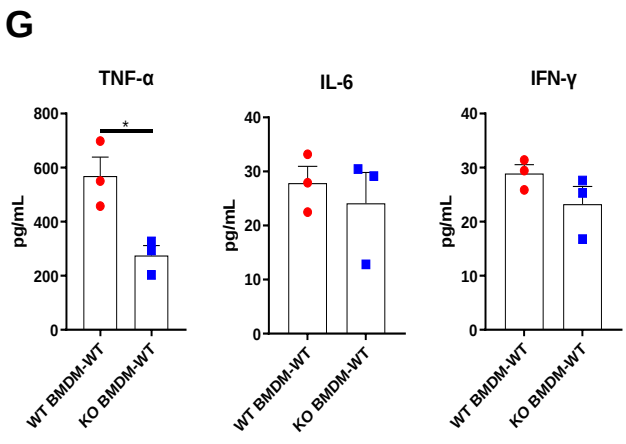

B
UUO kidney weight/body
weight (mg/g)
30
25
20
15
**
WT BMDM-WT
KO BMDM-WT


E
WT BMDM to WT
KO BMDM to WT
50μm
Vimentin staining area (%)
15
10
5
0
****
WT BMDM-WT
KO BMDM-WT

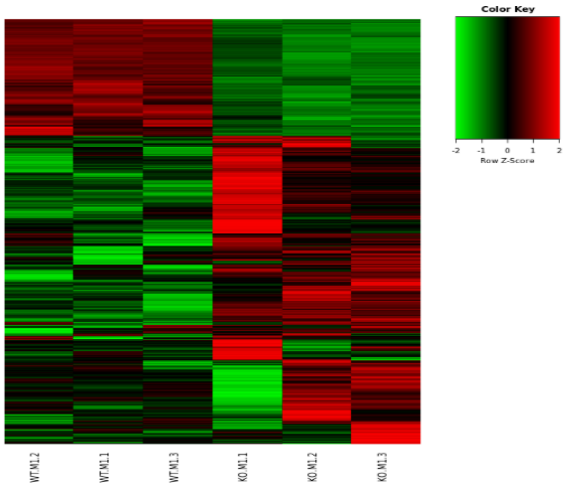

C
PBS to WT
WT BMDM to WT
KO BMDM to WT
2000μm
200μm
Masson stained area(%)
20
15
10
5
0
****
****
WT
WT BMDM-WT
KO BMDM-WT


F
WT-BMDM
KO-BMDM
Relative mRNA expression
1.5
1.0
0.5
0.0
****
***
****
****
*
Collagen-I
Collagen-IV
Kim-1
Vimentin
Tgf-β
Tnf-α

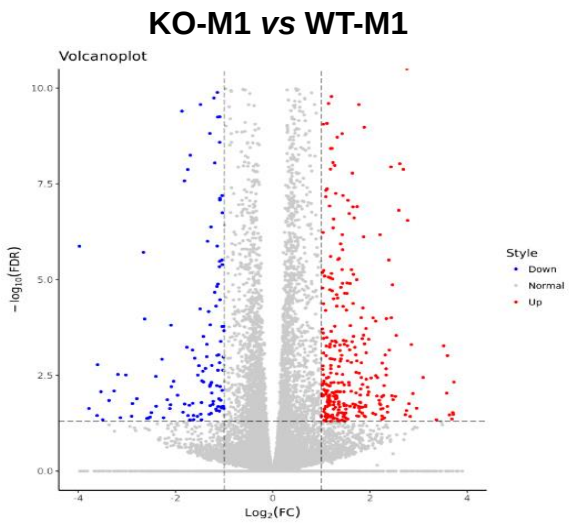

D
PBS to WT
WT BMDM to WT
KO BMDM to WT
2000μm
200μm
Histological scores
8
6
4
2
0
**
*
WT
WT BMDM-WT
KO BMDM-WT


G
TNF-α
IL-6
IFN-γ
pg/mL
800
600
400
200
0
40
30
20
10
0
*
WT BMDM-WT
KO BMDM-WT

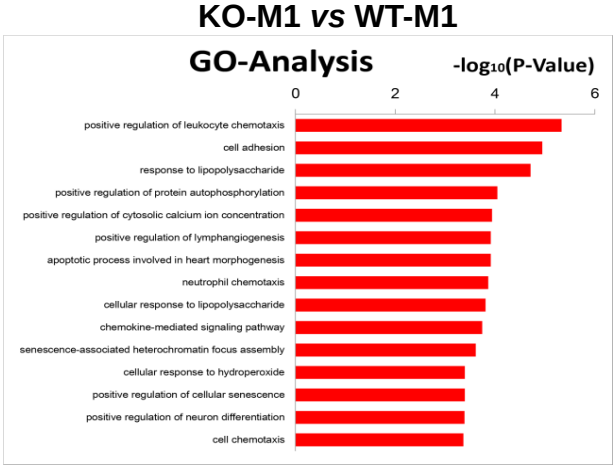
A


Color Key
-2
-1
0
1
2
Row Z-Score
WT.M1.2
WT.M1.1
WT.M1.3
KO.M1.1
KO.M1.2
KO.M1.3

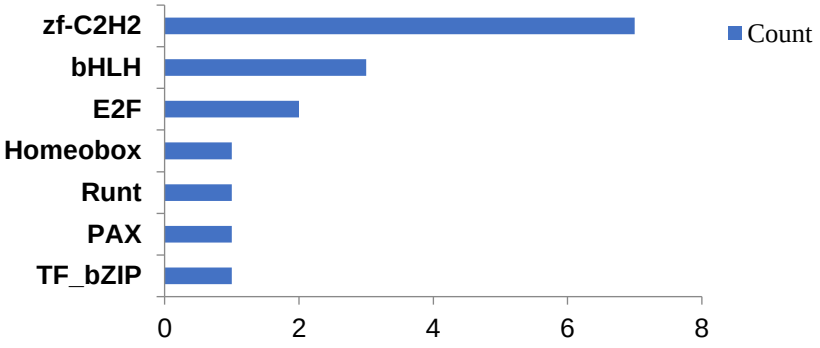
B


KO-M1 vs WT-M1
Volcanoplot
10.0
7.5
5.0
2.5
0.0
−log10(FDR)
-4
-2
0
2
4
Log2(FC)
Style
Down
Normal
Up

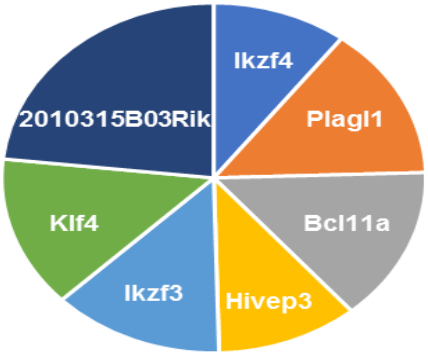
C

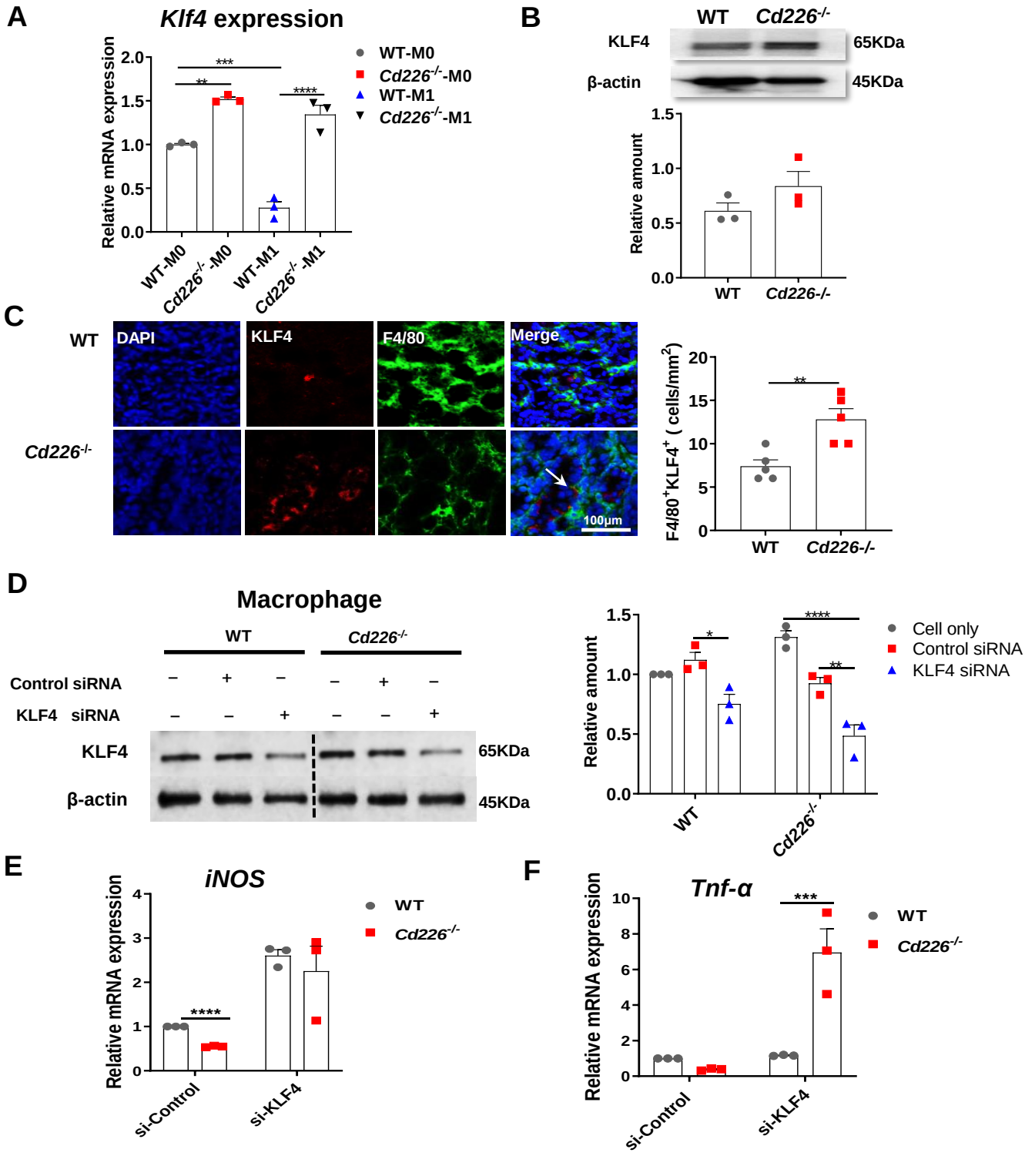

KO-M1 vs WT-M1
GO-Analysis
-log10(P-Value)
0
2
4
6
positive regulation of leukocyte chemotaxis
cell adhesion
response to lipopolysaccharide
positive regulation of protein autophosphorylation
positive regulation of cytosolic calcium ion concentration
positive regulation of lymphangiogenesis
apoptotic process involved in heart morphogenesis
neutrophil chemotaxis
cellular response to lipopolysaccharide
chemokine-mediated signaling pathway
senescence-associated heterochromatin focus assembly
cellular response to hydroperoxide
positive regulation of cellular senescence
positive regulation of neuron differentiation
cell chemotaxis

D


TF(Transcription factors) family
zf-C2H2
bHLH
E2F
Homeobox
Runt
PAX
TF_bZIP
Count
0
2
4
6
8

E


Log2 Fold Chang
Ikzf4
Plagl1
2010315B03Rik
Klf4
Bcl11a
Ikzf3
Hivep3


A
Klf4 expression
Relative mRNA expression
WT-M0
Cd226-/--M0
WT-M1
Cd226-/--M1
B
WT
Cd226-/-
KLF4
65KDa
β-actin
45KDa
Relative amount
C
WT
Cd226-/-
DAPI
KLF4
F4/80
Merge
100μm
F4/80+KLF4+ ( cells/mm2)
D
Macrophage
Control siRNA
KLF4 siRNA
Cell only
Control siRNA
KLF4 siRNA
E
iNOS
si-Control
si-KLF4
F
Tnf-α