Supplemental material Table of Contents

Figure S1 Gating strategy for flow cytometric analysis of dissociated kidney cells. Live leukocytes were identified by CD45 expression, LD marker exclusion and scatter propeties. Among CD11b^{+} myeloid cells, subsets were defined according to MHCII, F4/80 and CD206 expression.

Figure S2 (A) Representative image of immunofluorescent staining for Ki67 and γH2AX in control versus senescent primary tubular epithelial cells (PTEC). (B) Quantification of γH2AX^{+} and Ki67^{-} PTEC as shown in (A), (n=3). (C) Representative immunohistochemical staining for SerpinB2 in young (8 weeks) versus old (2 years) mouse kidney. (D, E) Representative immunofluorescent staining for p21 and SerpinB2 and quantification (E) in young (8 weeks) versus old (2 years) mouse kidney, (n=6). (F, G) Representative immunofluorescent staining for p21 and SerpinB2 and quantification (G) in kidney of control versus unilateral ureteral obstruction (UUO), (n=5) (H, I) Representative immunofluorescent staining for Ki67

and SerpinB2 and quantification (I) in control versus UUO kidney, (n=5). Values are given as means ±SEMs. Unpaired t-test two tailed. ***P*<0.05, ****P*<0.01, ***** *P*<0.001.

Figure S3 (A) Quantitative RT-PCR to quantify tPA, uPA, uPAR, Pai-1 and Mmp2 in kidneys from old WT and KO mice, (n=6). (B) uPA activity in kidney lysates from old WT and KO mice, (n=4). (C-E) Quantitative RT-PCR of neutrophil gelatinase-associated lipocalin (Ngal), fibronectin (Fn1) and α smooth muscle actin (αSma) mRNA in kidneys from WT and KO mice at 14 days (C), 3 days (D) and 7days (E) post UUO, (n=5). (F) Quantitative RT PCR to quantify tPA, uPA, uPAR , Pai-1 and Mmp2 in kidney from WT and KO mice at 14 days post UUO (n=6). (G) uPA activity in kidney lysates from old WT and KO mice at 14 days post UUO, (n=4). Wildtype = WT, SerpinB2$^{-/-}$ = KO. Values are given as means ±SEMs. Unpaired t-test two tailed. ***P*<0.05, ****P*<0.01, ***** *P*<0.001.

Figure S4 (A-C) Quantitative RT-PCR of neutrophil gelatinase-associated lipocalin (Ngal), fibronectin (Fn1) and α smooth muscle actin (αSma) mRNA in kidneys from WT and KO mice at 3 (A), 7 (B) and 14 days (C) post unilateral IR, (n=5). (D, E) Representative images of picrosirius red (PRed) and immunofluorescent staining for FN1 and αSMA and quantification (E) in kidney sections from WT and KO mice at 14 days post unilateral IR, (n=5). Original magnification X 200 in D. Wildtype = WT, SerpinB2$^{-/-}$ = KO. Values are given as means ±SEMs. Unpaired t-test two tailed. ***P*<0.05, ****P*<0.01, ***** *P*<0.001.

Figure S5 (A, B) Serum creatinine and serum urea measurements in WT and KO mice at indicated time points post bilateral ischemia reperfusion (IR) (n=4). (C-F) Flow cytometry for total CD45^{+} live leucocytes (C), total macrophages (CD11b^{+}-F4/80^{high}) (D), M1-type (F4/80^{+}-MHCII^{high}) (E) and M2-type macrophages (CD11b^{+}-CD206^{high}) (F) in kidneys from WT and KO mice at indicated time points post IR,

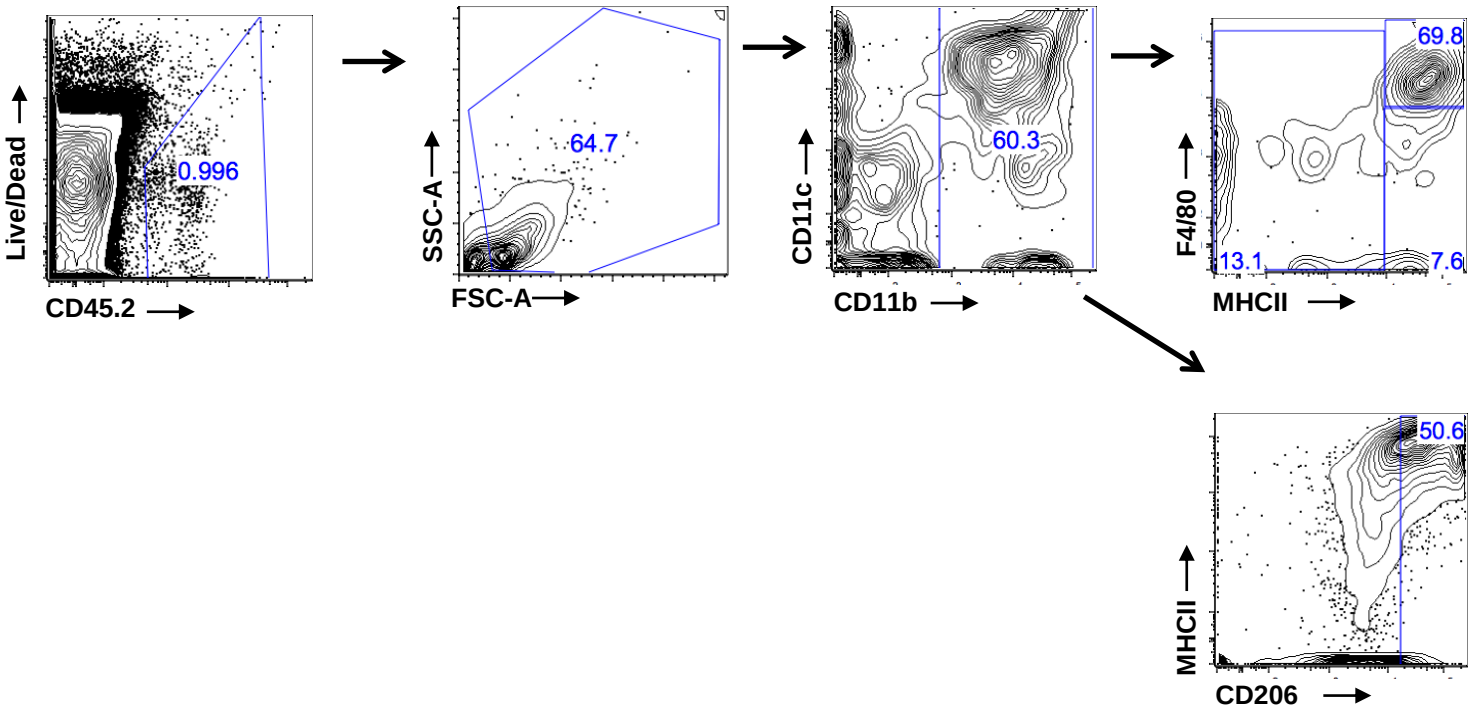
(n=5). (G) Quantitative RT PCR to quantify tPA, uPA, uPAR, Pai-1 and Mmp2 in kidneys from old WT and KO mice post IR (n=3). Unpaired t-test two tailed. Wildtype = WT, SerpinB2$^{-/-}$ = KO. Values are given as means ±SEMs. *P<0.05, **P<0.01, ***P<0.001.

Figure S6 Quantitative RT-PCR of Ccl2 mRNA in phorbol 12-myristate 13-acetate (PMA) treated primary tubular epithelial cells (PTEC) from WT and KO (n=3). (B) ELISA quantification of CCL2 in PMA treated PTEC from WT and KO mice. (C) Quantitative RT-PCR of Ccl2 in control or Ccl2 siRNA treated PTEC (n=3). (D) Quantification of invading bone marrow derived macrophages (BMDM) in co-culture experiment with WT BMDM in upper chamber and control or Ccl2 siRNA treated PTEC in lower chamber, (n=4). (E, F) ELISA quantification of CCL2 in kidney homogenates from WT and KO mice post UUO or IR at 3, 7 and 14 days. Wildtype = WT, SerpinB2$^{-/-}$ = KO. Values are given as means ±SEMs (n=4 for each data point). Two-way ANOVA with Bonferroni‘s multiple comparison test. *P<0.05, **P<0.01, ***P<0.001.

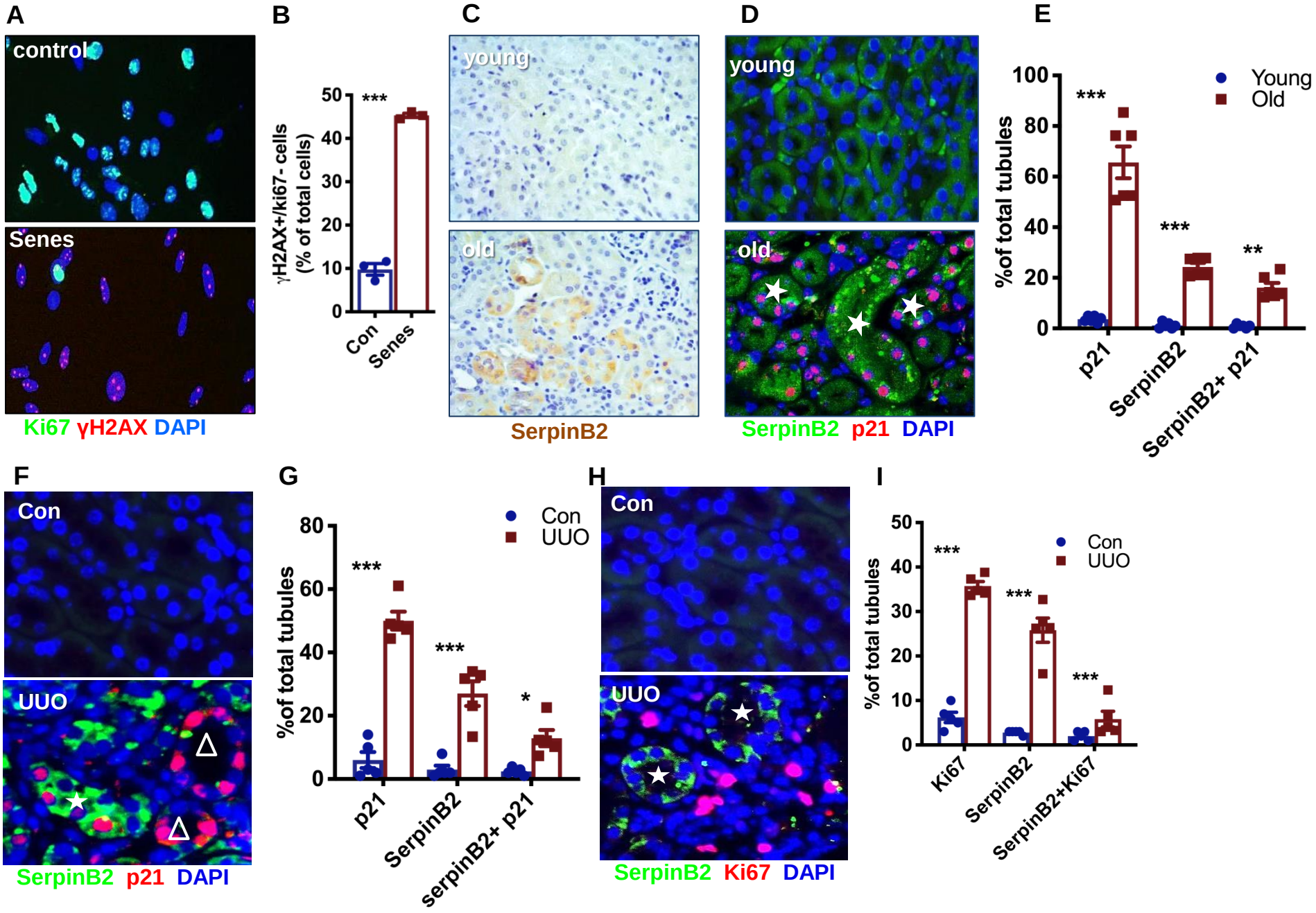
Figure S7 (A) Quantitative RT-PCR of Ccl2 and its receptor Ccr2 mRNA in bone marrow derived macrophages (BMDM) from WT and KO mice. (B, C) Flow cytometry for total intrarenal live leucocytes and M1-type -F4/80^{+}MHCII^{high} macrophages, normalized to the mean of WT unilateral ureteral obstruction (UUO) kidney from vehicle injected mice. Kidneys from WT and KO mice injected with liposomal clodronate (LC) are compared to kidneys from vehicle (Veh) injected mice. Wildtype = WT, SerpinB2$^{-/-}$ = KO. Values are given as means ±SEMs (n=4 for each data point). Two-way ANOVA with Bonferroni‘s multiple comparison test. *P<0.05, **P<0.01, ***P<0.001.

Figure S8 (A, B) Quantification and representative images for picrosirius red (PRed) and immunofluorescence for fibronectin (FN1) and α smooth muscle actin (αSMA) in kidneys from bone marrow transplantation experiment from 4 separate groups (WT Kid-WT BM), (KO Kid-WT BM), (WT Kid-KO BM) and (KO Kid-KO BM). Original magnifications X 200. Wildtype = WT, SerpinB2$^{-/-}$ = KO. Values are given as means ±SEMs. Two-way ANOVA with Bonferroni's multiple comparison test. ***P*<0.05, ****P*<0.01, ***** *P*<0.001

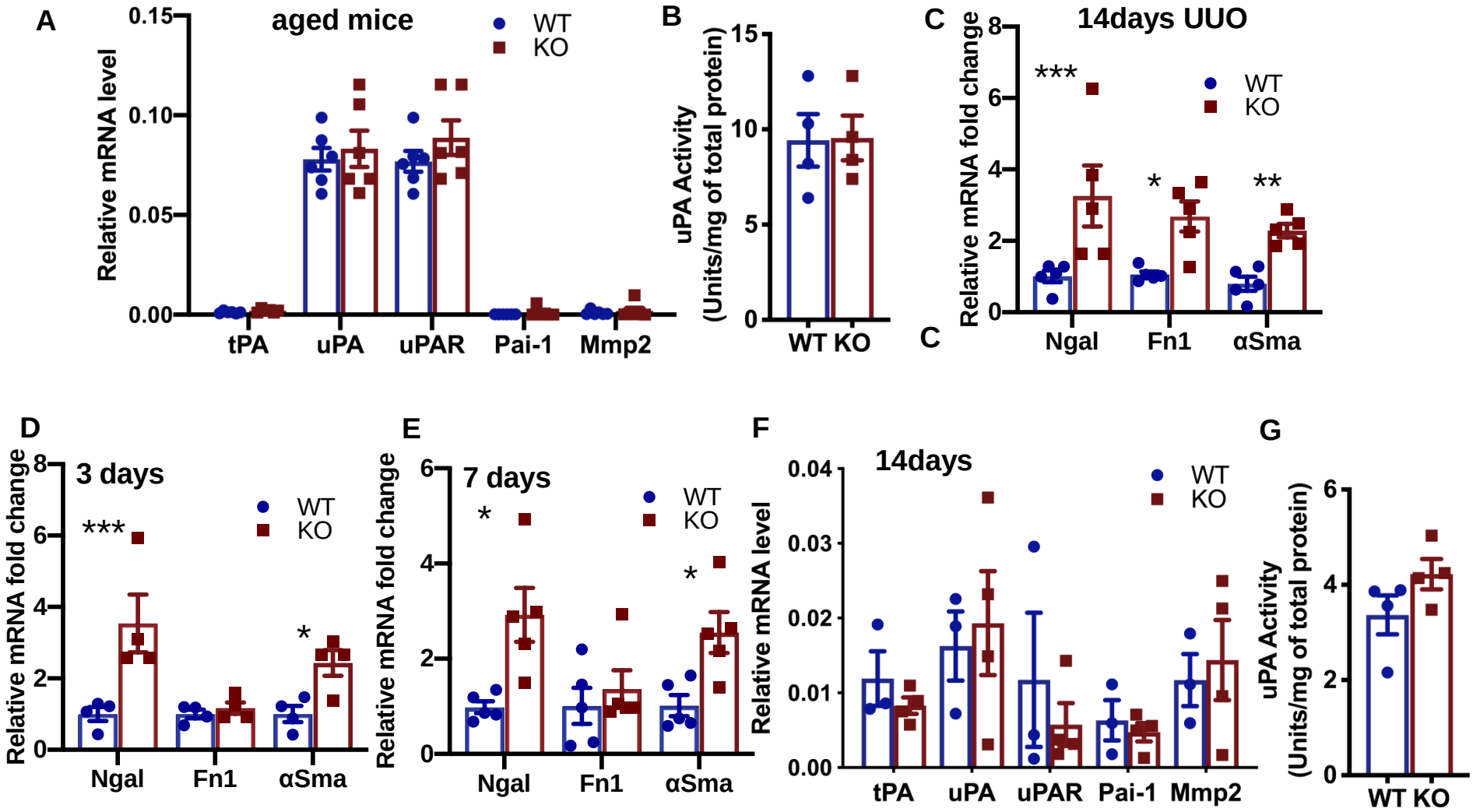
FigS1


Live/Dead
CD45.2
0.996
SSC-A
FSC-A
64.7
CD11c
CD11b
60.3
F4/80
MHCII
69.8
13.1
7.6
MHCII
CD206
50.6

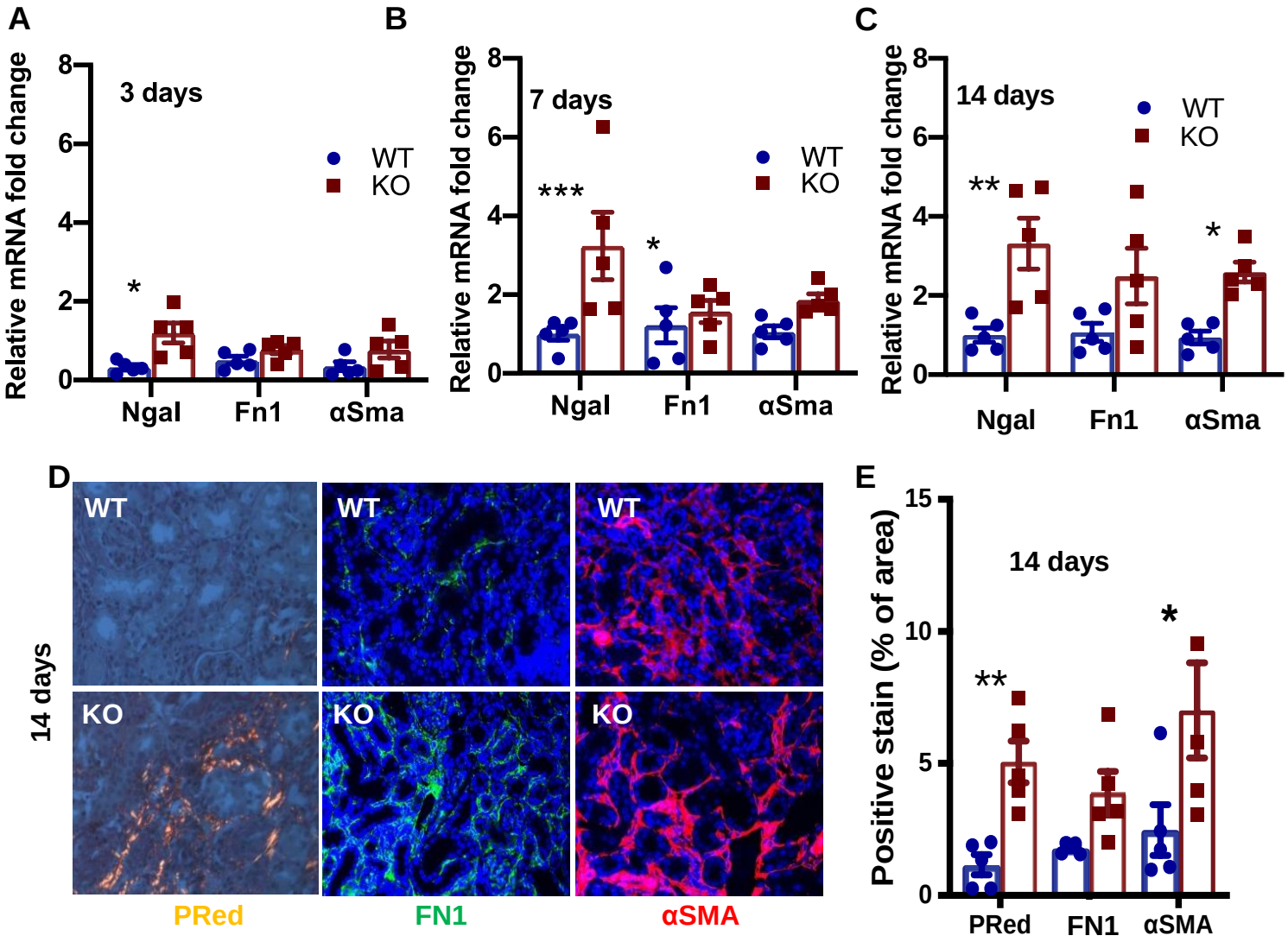
FigS2


A
control
Senes
Ki67 γH2AX DAPI
B
γH2AX+/ki67- cells (% of total cells)
***
Con
Senes
C
young
old
SerpinB2
D
young
old
SerpinB2 p21 DAPI
E
%of total tubules
Young
Old
***
***
**
p21
SerpinB2
SerpinB2+ p21
F
Con
UUO
SerpinB2 p21 DAPI
G
%of total tubules
Con
UUO
***
***
*
p21
SerpinB2
serpinB2+ p21
H
Con
UUO
SerpinB2 Ki67 DAPI
I
%of total tubules
Con
UUO
***
***
***
Ki67
SerpinB2
SerpinB2+Ki67

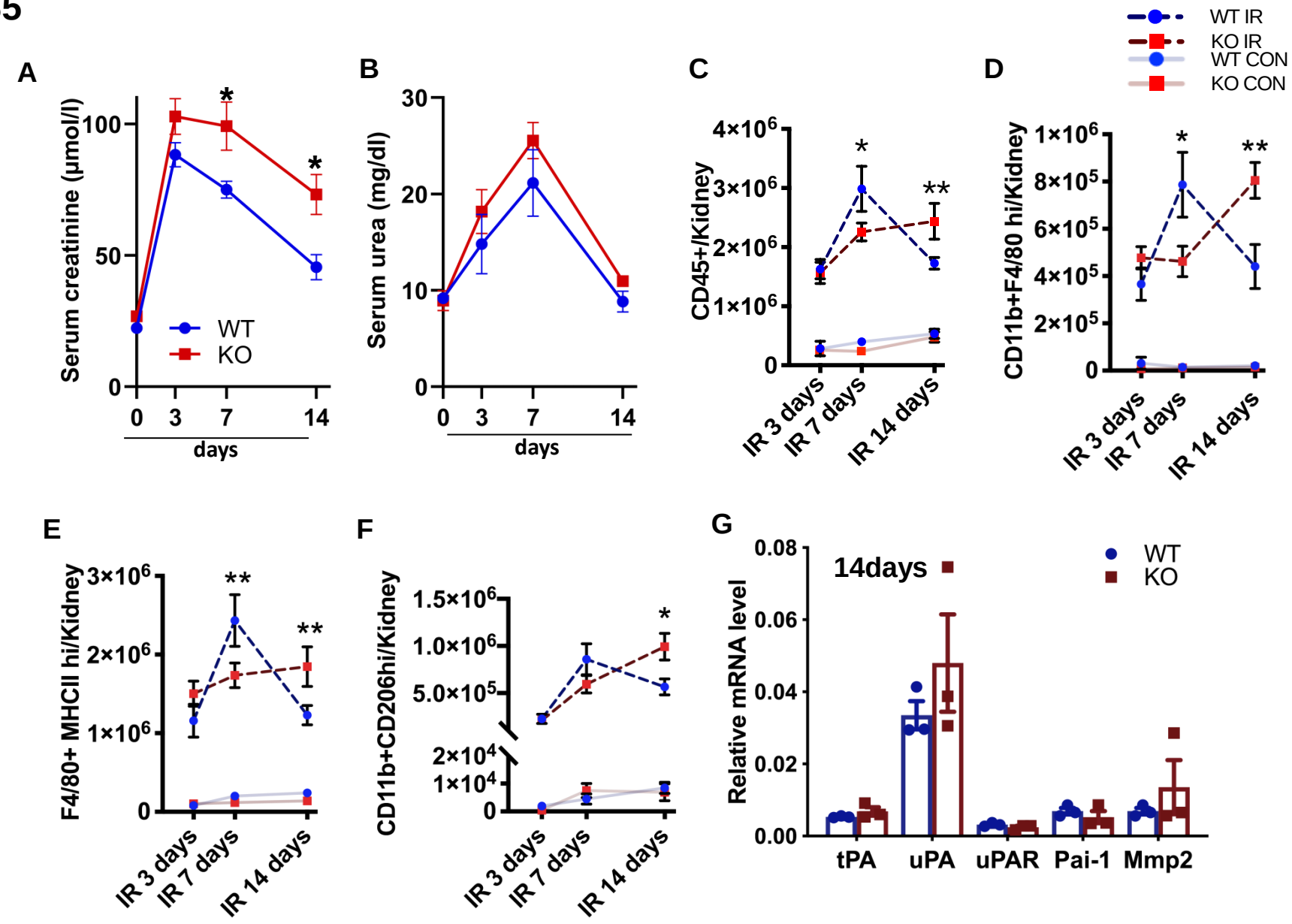
FigS3


A
aged mice
WT
KO
Relative mRNA level
0.15
0.10
0.05
0.00
tPA
uPA
uPAR
Pai-1
Mmp2
B
uPA Activity
(Units/mg of total protein)
15
10
5
0
WT KO
C
14days UUO
Relative mRNA fold change
8
6
4
2
0
***
*
**
WT
KO
Ngal
Fn1
αSma
C
D
3 days
Relative mRNA fold change
8
6
4
2
0
***
*
WT
KO
Ngal
Fn1
αSma
E
7 days
Relative mRNA fold change
6
4
2
0
*
*
WT
KO
Ngal
Fn1
αSma
F
14days
Relative mRNA level
0.04
0.03
0.02
0.01
0.00
WT
KO
tPA
uPA
uPAR
Pai-1
Mmp2
G
uPA Activity
(Units/mg of total protein)
6
4
2
0
WT KO

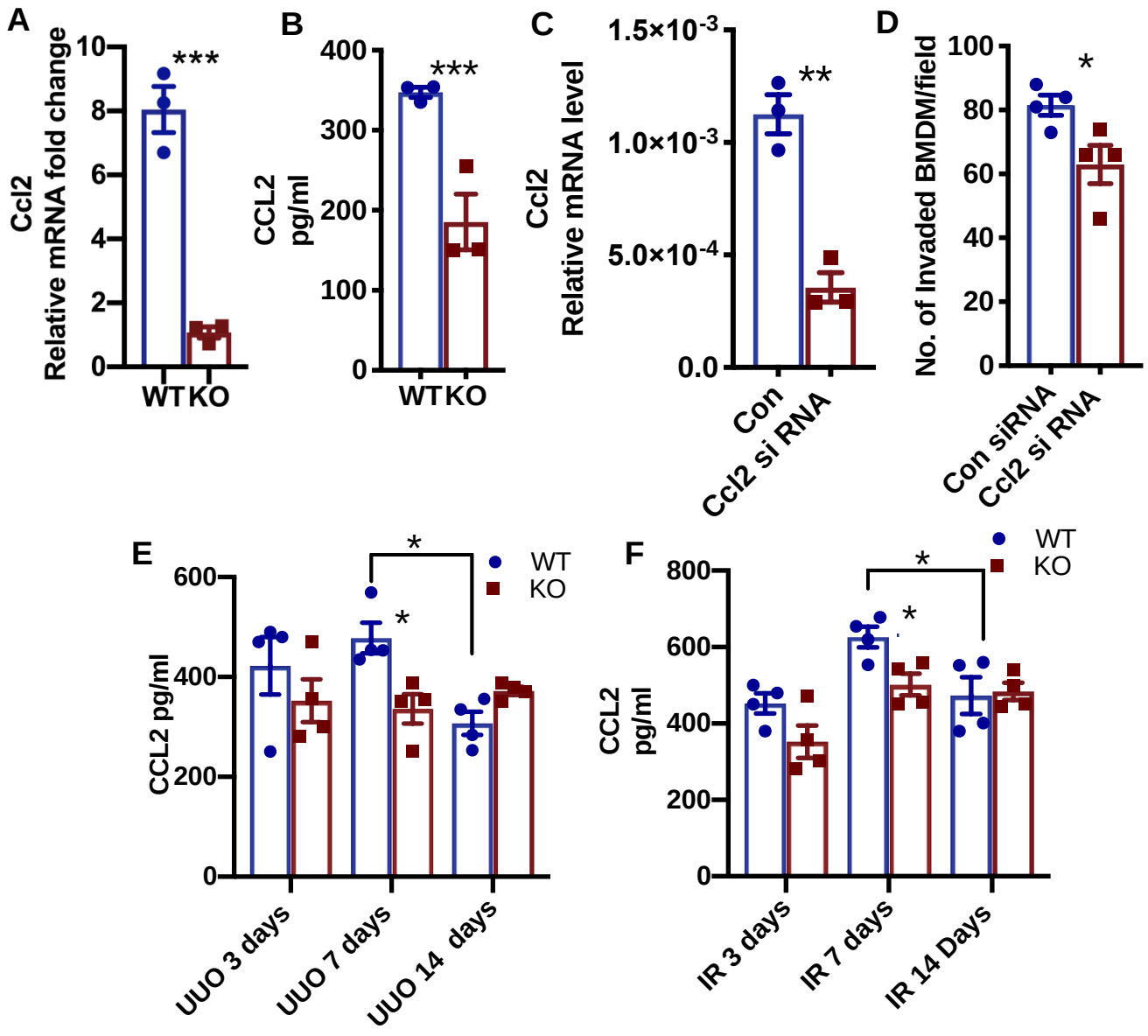
FigS4


A
B
C
3 days
7 days
14 days
Relative mRNA fold change
WT
KO
Ngal
Fn1
αSma
D
14 days
WT
KO
PRed
FN1
αSMA
E
Positive stain (% of area)

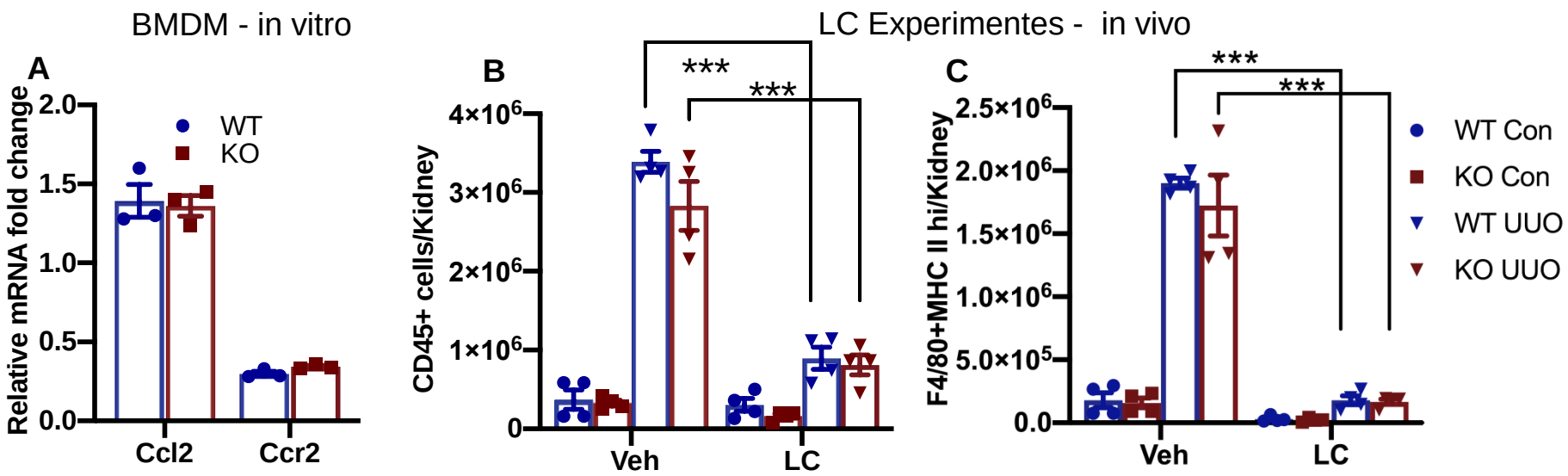
FigS5


A
Serum creatinine (μmol/l)
100
50
0
*
*
WT
KO
0 3 7 14
days
B
Serum urea (mg/dl)
30
20
10
0
0 3 7 14
days
C
CD45+/Kidney
4×10^6
3×10^6
2×10^6
1×10^6
0
*
**
IR 3 days
IR 7 days
IR 14 days
D
CD11b+F4/80 hi/Kidney
1×10^6
8×10^5
6×10^5
4×10^5
2×10^5
0
*
**
IR 3 days
IR 7 days
IR 14 days
WT IR
KO IR
WT CON
KO CON
E
F4/80+ MHCII hi/Kidney
3×10^6
2×10^6
1×10^6
0
**
**
IR 3 days
IR 7 days
IR 14 days
F
CD11b+CD206hi/Kidney
1.5×10^6
1.0×10^6
5.0×10^5
2×10^4
1×10^4
0
*
IR 3 days
IR 7 days
IR 14 days
G
14days
WT
KO
Relative mRNA level
0.08
0.06
0.04
0.02
0.00
tPA
uPA
uPAR
Pai-1
Mmp2

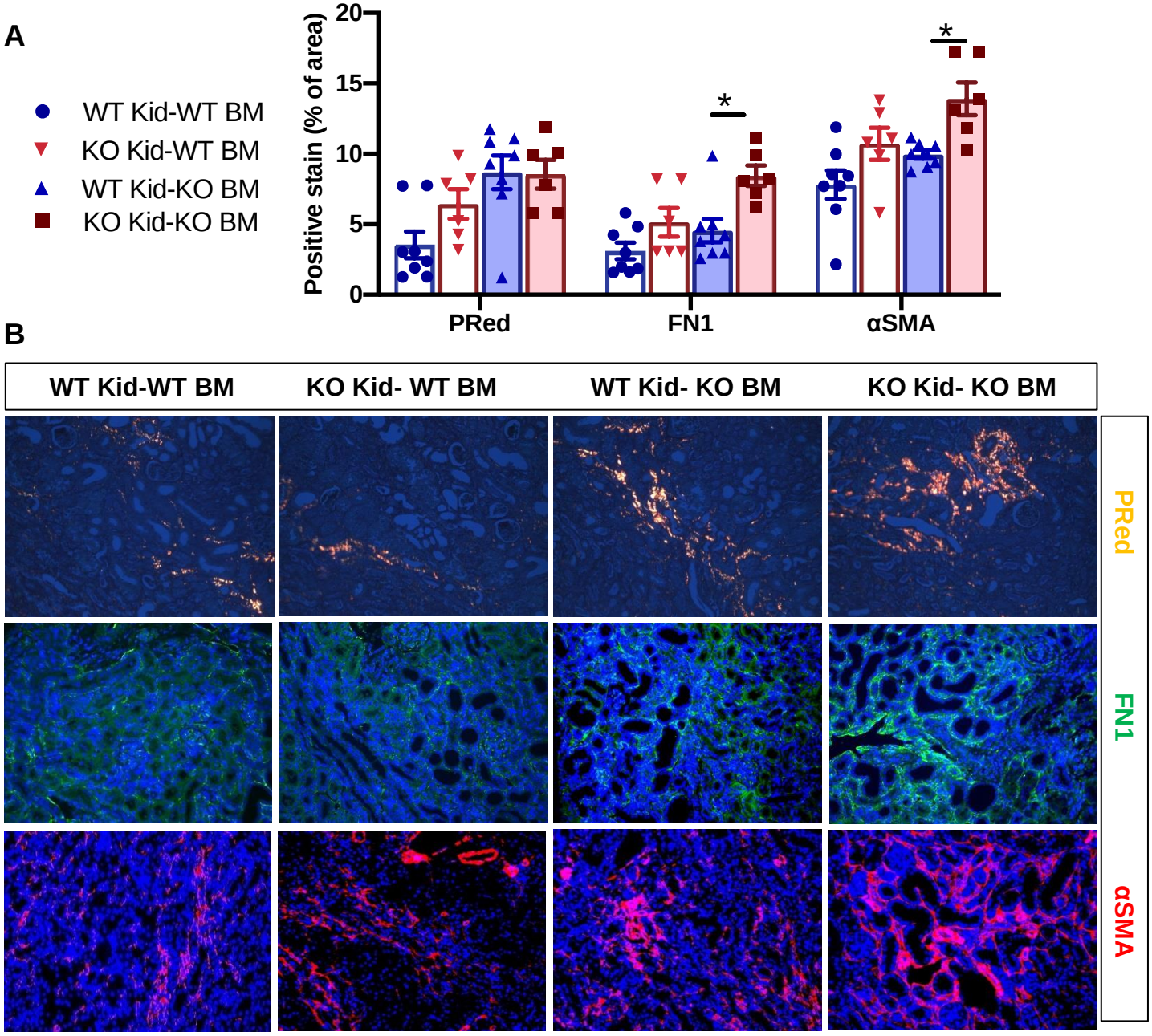
FigS6


A
Ccl2
Relative mRNA fold change
10
8
6
4
2
0
***
WT
KO
B
CCL2
pg/ml
400
300
200
100
0
***
WT
KO
C
Ccl2
Relative mRNA level
1.5×10-3
1.0×10-3
5.0×10-4
0.0
**
Con
Ccl2 si RNA
D
No. of Invaded BMDM/field
100
80
60
40
20
0
*
Con siRNA
Ccl2 si RNA
E
CCL2 pg/ml
600
400
200
0
*
*
WT
KO
UUO 3 days
UUO 7 days
UUO 14 days
F
CCL2
pg/ml
800
600
400
200
0
*
*
WT
KO
IR 3 days
IR 7 days
IR 14 Days

FigS7


BMDM - in vitro
A
Relative mRNA fold change
2.0
1.5
1.0
0.5
0.0
WT
KO
Ccl2
Ccr2
LC Experimentes - in vivo
B
CD45+ cells/Kidney
4×10^6
3×10^6
2×10^6
1×10^6
0
***
***
Veh
LC
C
F4/80+MHC II hi/Kidney
2.5×10^6
2.0×10^6
1.5×10^6
1.0×10^6
5.0×10^5
0.0
***
***
Veh
LC
WT Con
KO Con
WT UUO
KO UUO

FigS8


A
WT Kid-WT BM
KO Kid-WT BM
WT Kid-KO BM
KO Kid-KO BM
Positive stain (% of area)
20
15
10
5
0
*
*
PRed
FN1
αSMA
B
WT Kid-WT BM
KO Kid- WT BM
WT Kid- KO BM
KO Kid- KO BM
PRed
FN1
αSMA