Supplemental Figure Legends

Supplemental Figure 1. Western blot analysis indicated that MIF was detected in the fractions of plasma membrane and cytosol but not in nuclear fraction isolated from *Pkd1* null MEK cells. The isolation (fractionation) of plasma membrane, cytosol and nuclear was described in Methods section. Na/K-ATPase, α-tubulin and lamin A/C were used as markers for membrane, cytosol and nuclear, respectively.

Supplemental Figure 2. Treatment with ISO-1 delayed cyst growth in *Pkd1*$^{flox/flox}$*:Ksp-Cre* neonates. (**A**) Histologic examination of PN7 kidneys from *Pkd1*$^{flox/flox}$*:Ksp-Cre* neonates treated with vehicle (DMSO) (n = 10) or ISO-1 (n = 6), respectively. Scale bar, 1 mm. (**B**) Quantification of the percentage of cystic areas over total kidney section areas of PN7 kidney sections from *Pkd1*$^{flox/flox}$*:Ksp-Cre* neonates treated as in **A**. Shown is mean ± s.e.m. of all sections quantified for each condition. $p < 0.01$. (**C** and **D**) KW/BW ratios (**C**) and BUN levels (**D**) were decreased in *Pkd1*$^{flox/flox}$*:Ksp-Cre* PN7 neonates treated with ISO-1 compared to that treated with DMSO (control). $p < 0.001$. (**E**) ISO-1 treatment reduced cyst lining epithelial cell proliferation in *Pkd1*$^{flox/flox}$*:Ksp-Cre* PN7 kidneys as detected by Ki67 staining. $p < 0.001$. Scale bar, 100 µm. (**F**) ISO-1 treatment induced cyst lining epithelial cell apoptosis in *Pkd1*$^{flox/flox}$*:Ksp-Cre* PN7 kidneys as detected by TUNEL assay. $p < 0.001$. Scale bar, 100 µm.

Supplemental Figure 3. Statistical analysis of the body weight of *Pkd1*$^{flox/flox}$*:Ksp-Cre* mice at postnatal day 7, *Pkd1*$^{flox/flox}$*:Pkhd1-Cre* mice at postnatal day 25 and *Pkd1*$^{nl/nl}$ mice at postnatal day 28 treated with DMSO and ISO-1 respectively.

Supplemental Figure 4. Western blot analysis of MIF in two kidneys from *Pkd1*$^{flox/flox}$*:Ksp-Cre:MIF*$^{-/-}$, *Pkd1*$^{+/+}$*:Ksp-Cre*:*MIF*$^{+/+}$ and *Pkd1*$^{flox/flox}$*:Ksp-Cre*:*MIF*$^{+/+}$ neonates, respectively.

Supplemental Figure 5. Knockout of MIF or inhibition of MIF with ISO-1 leads to diminished levels of macrophages in pericystic and interstitial regions of *Pkd1* conditional knockout mouse kidneys. (**A)** The accumulation/recruitment of macrophages to pericystic sites and interstitium in kidneys from *Pkd1*$^{flox/flox}$*:Ksp-Cre*:*MIF*$^{-/-}$ mice was dramatically reduced at postnatal day 7 compared to that from age matched kidneys of *Pkd1*$^{flox/flox}$*:Ksp-Cre*:*MIF*$^{+/+}$ mice. $p < 0.001$. Scale bar, 100 µm. (**B-D**) Treatment with ISO-1 dramatically reduced the accumulation/recruitment of macrophages to pericystic sites and interstitium in kidneys from *Pkd1*$^{flox/flox}$*:Ksp-Cre* mice at postnatal day 7 (**B**), *Pkd1*$^{flox/flox}$:*Pkhd1-Cre* mice at postnatal day 25 (**C**) and *Pkd1*$^{nl/nl}$ mice at postnatal day 28 (**D**) compared to that from age matched kidneys of *Pkd1*$^{flox/flox}$*:Ksp-Cre* mice, *Pkd1*$^{flox/flox}$:*Pkhd1-Cre* mice and *Pkd1*$^{nl/nl}$ mice treated with DMSO, respectively. $p < 0.001$. Scale bar, 100 µm.

Supplemental Figure 6. Treatment with ISO-1 decreased the expression of MIF in *Pkd1* mutant kidneys from *Pkd1*$^{flox/flox}$*:Ksp-Cre* mice and *Pkd1*$^{flox/flox}$:*Pkhd1*-Cre mice compared to that from age matched control mice treated with DMSO. (**A** and **B**) Western blot analysis of the expression of MIF in kidneys from three different *Pkd1*$^{flox/flox}$*:Ksp-Cre* mice (**A**) and *Pkd1*$^{flox/flox}$:*Pkhd1-Cre* mice (**B**) treated with DMSO or ISO-1, respectively. (**C** and **D**) Immunohistochemistry staining of MIF in kidneys from *Pkd1*$^{flox/flox}$*:Ksp-Cre* mice (**C**) and *Pkd1*$^{flox/flox}$*:Pkhd1-Cre* mice (**D**) treated with DMSO or ISO-1, respectively. Scale bar, 100 µm.

Supplemental Figure 7. MIF can be secreted in cell culture media of renal epithelial cells and is highly enriched in cyst fluids and urines derived from two distinct orthologous mouse models of ADPKD. (**A**) Western blot analysis of the levels of MIF in cell culture media of *Pkd1* wild type (WT) and mutant (Null) MEK cells standardized to the same cell numbers of different cell types. $p < 0.001$. (**B**) Western blot analysis of the levels of MIF in cyst fluids from four different *Pkd1*$^{flox/flox}$*:Ksp-Cre* mice and

Pkd1$^{flox/flox}$:*Pkhd1-Cre* mice, respectively. (**C**) Western blot analysis of the levels of MIF in urines from *Pkd1*$^{flox/flox}$*:Ksp-Cre* mice at PN7 and PN14 as well as from *Pkd1*$^{flox/flox}$:*Pkhd1-Cre* mice at PN14 and PN25, respectively.

Supplemental Figure 8. (**A**) Western blot analysis of the levels of MIF in cyst fluids from five male and five female ADPKD patients, respectively. Our results indicated that MIF might form a dimer in human cyst fluids. (**B**) Western blot analysis of the levels of MIF in urines from four male ADPKD patients and four normal males (*top panel*) as well as from four female patients and four normal females (*bottom panel*), respectively.

Supplemental Figure 9. MIF promotes macrophage migration. Addition of ISO-1 (100 μm) blocked ADPKD CM-induced migration of human THP-1 monocytes. $p < 0.0001$; ANOVA post hoc test.

Supplemental Figure 10. (**A**) MCP-1 expression was increased in *Pkd1* mutant mouse embryonic kidney (MEK) cells and postnatal *Pkd1* homozygous PN24 cells compared to *Pkd1* wild-type MEK cells and postnatal *Pkd1* heterzygous PH2 cells as analyzed with western blot. (**B-D**) MCP-1 was elevated in kidneys from *Pkd1*$^{flox/flox}$*:Ksp-Cre* mice (**B**) and *Pkd1*$^{flox/flox}$:*Pkhd1*-Cre mice (**C**) as well as in kidneys from ADPKD patients (**D**) compared to that in age matched wild type mouse kidneys and normal human kidneys as analyzed with immunohistochemistry staining, respectively. Treatment with ISO-1 decreased the expression of MCP-1 in *Pkd1* mutant kidneys from *Pkd1*$^{flox/flox}$*:Ksp-Cre* mice (**B**) and *Pkd1*$^{flox/flox}$:*Pkhd1*-Cre mice (**C**) compared to that in age matched control kidneys treated with DMSO, respectively.

Supplemental Figure 11. Treatment with ISO-1 decreased the levels of MCP-1 mRNA (**A**) and protein (**B**) in kidneys from *Pkd1*$^{flox/flox}$*:Pkhd1*-Cre mice compared with DMSO treated control mice as examined by qRT-PCR and western blot, respectively. n = 3, $p < 0.05$; ANOVA post hoc test.

Supplemental Figure 12. MIF promotes the expression of MCP-1 and TNF-α whereas TNF-α also induces the expression of MIF in renal epithelial cells. (**A and B**) Western blot analysis of the expression of MCP-1 and TNF-α from whole cell lysates of *Pkd1* mutant PN24 cells (**A**) and *Pkd1* mutant MEK cells (**B**) induced with MIF (10 ng/ml) at indicated time points. **C**. Western blot analysis of the expression of MIF from whole cell lysates of *Pkd1* wild type and null MEK cells induced with TNF-α (50 ng/ml) at indicated time points.

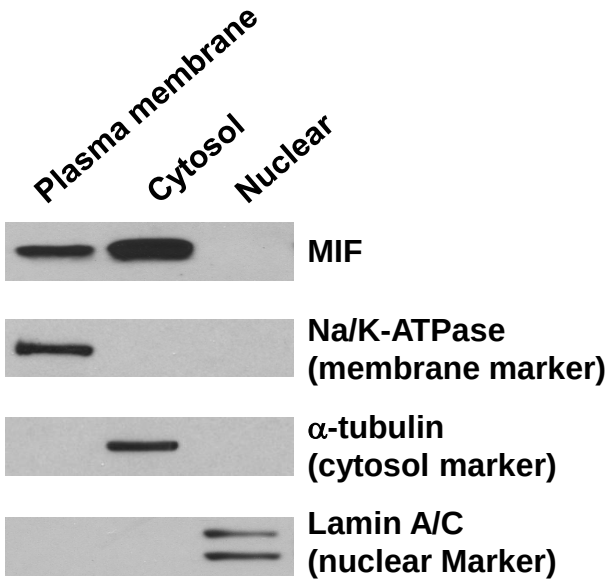
Supplemental Figure 13. (**A and B**) qRT-PCR analysis of the expression of *Hk1* (**A**) and *Ldha* (**B**) mRNA in *Pkd1* wild type (WT) and mutant (Null) MEK cells untreated or treated with ISO-1 or MIF siRNA. The transcription of the key glycolytic enzymes, *Hk1* and *Ldha* ($p < 0.001$; ANOVA post hoc test.) was significantly increased in *Pkd1* mutant (Null) MEK cells compared to *Pkd1* wild type MEK cells. Treatment with ISO-1 or MIF siRNA did not affect the transcription of the key glycolytic enzymes, *Hk1* and *Ldha*, in *Pkd1* wild type MEK cells. However, treatment with ISO-1 or MIF siRNA significantly decreased the transcription of *Hk1* and *Ldha* ($p < 0.001$; ANOVA post hoc test.) in *Pkd1* mutant (Null) MEK cells compared to untreated *Pkd1* mutant (Null) MEK cells, respectively.

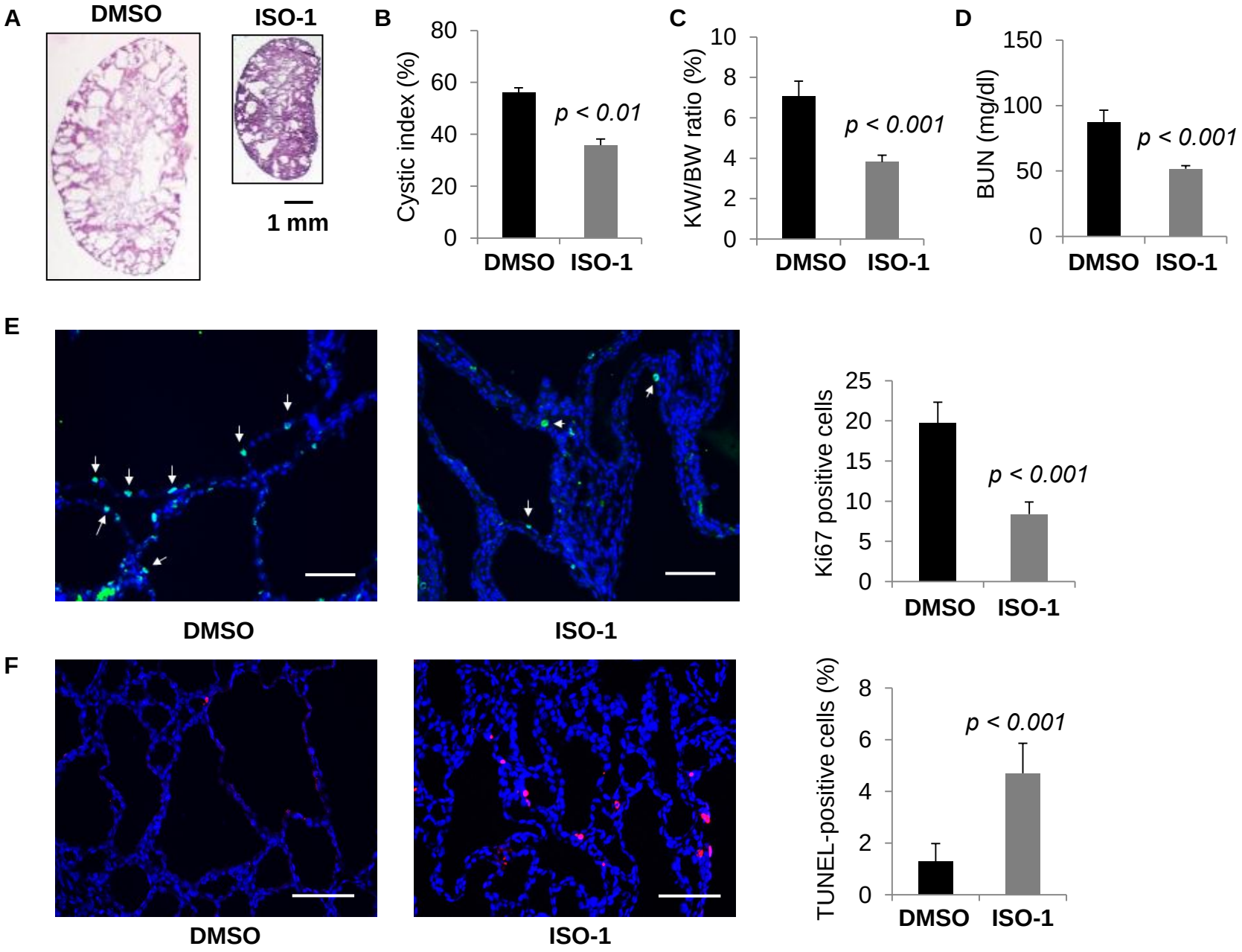
Supplemental Figure 14. (**A**) Western blot analysis of the expression of ERK and phospho-ERK from whole cell lysates of *Pkd1* mutant PN24 cells treated with MIF (10 ng/ml) or ISO-1 (100 μM) at indicated time points. (**B**) Western blot analysis of the levels of phospho-ERK in *Pkd1* null cells and PN24 cells treated with conditional media collected from *Pkd1* null MEK cells transfected with control siRNA or MIF siRNA for 72 hours. The phosphorylation of ERK could be induced in *Pkd1* null cells and PN24 cells treated with conditional media collected from cystic renal epithelial cells transfected with control siRNA at indicated time points but could not be induced in these cells treated with conditional media collected from MIF knockdown cystic renal epithelial cells.

Supplemental Figure 15. Western blot analysis of the expression of MIF from whole cell lysates of *Pkd1* mutant (Null) MEK cells treated with Hsp90 inhibitors, STA9090 (100 nM) or 17-AAG (1 μM), at indicated time points.

Supplemental Figure 16. (**A-B**) Analysis of the expression of CD74 protein (**A**) and mRNA (**B**) of *Pkd1* wild-type (WT) and *Pkd1*$^{null/null}$ (Null) MEK cells as well as postnatal *Pkd1* heterozygous PH2 (PH2) cells and *Pkd1* homozygous PN24 (PN24) cells by Western blot and qRT-PCR, respectively. (**C**) CD74 was elevated in kidney sections from normal and ADPKD kidneys as examined by immunohistochemistry staining with anti-CD74 antibody. Scale bar, 100 μm. (**D**) Western blot analysis of the expression of phospho-ERK, total ERK and MCP-1 in PN24 cells that were pretreated with normal IgG or CD74 antibody for 2 hours and then were stimulated with recombinant MIF (10 ng/ml) at indicated time points. Treatment with CD74 antibody blocked MIF induced the phosphorylation of ERK but did not affect MIF induced MCP-1 expression.

Supplemental Figure 1

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DMSO
ISO-1
1 mm
B
Cystic index (%)
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60
40
20
0
p < 0.01
DMSO
ISO-1
C
KW/BW ratio (%)
10
8
6
4
2
0
p < 0.001
DMSO
ISO-1
D
BUN (mg/dl)
150
100
50
0
p < 0.001
DMSO
ISO-1
E
DMSO
ISO-1
Ki67 positive cells
25
20
15
10
5
0
p < 0.001
DMSO
ISO-1
F
DMSO
ISO-1
TUNEL-positive cells (%)
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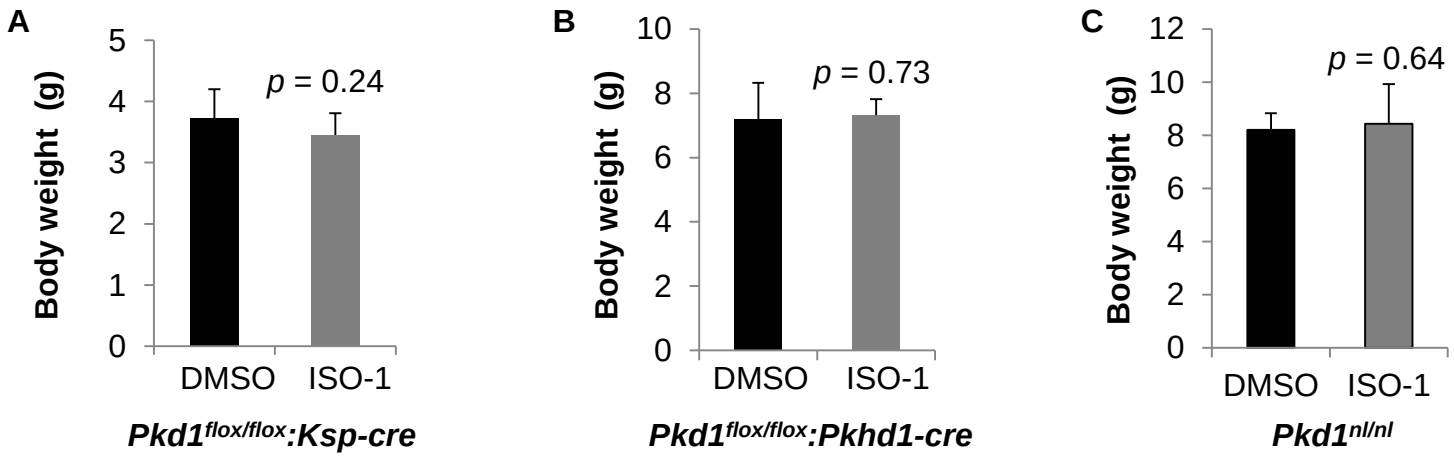
6
4
2
0
p < 0.001
DMSO
ISO-1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4
3
2
1
0
p = 0.24
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ISO-1
Pkd1flox/flox:Ksp-cre
B
Body weight (g)
10
8
6
4
2
0
p = 0.73
DMSO
ISO-1
Pkd1flox/flox:Pkhd1-cre
C
Body weight (g)
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10
8
6
4
2
0
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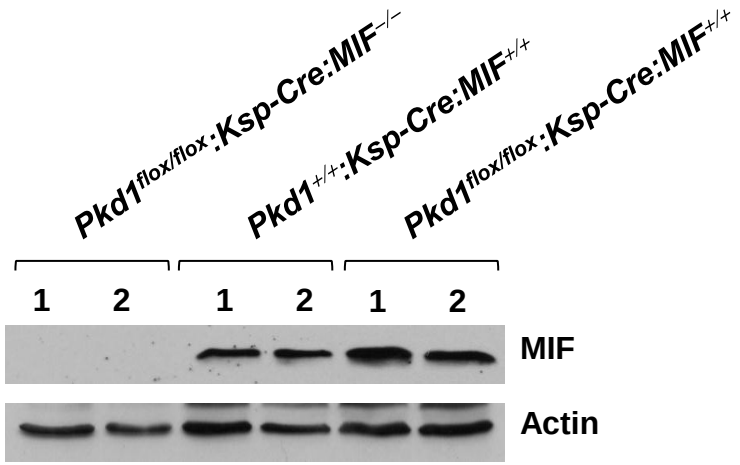
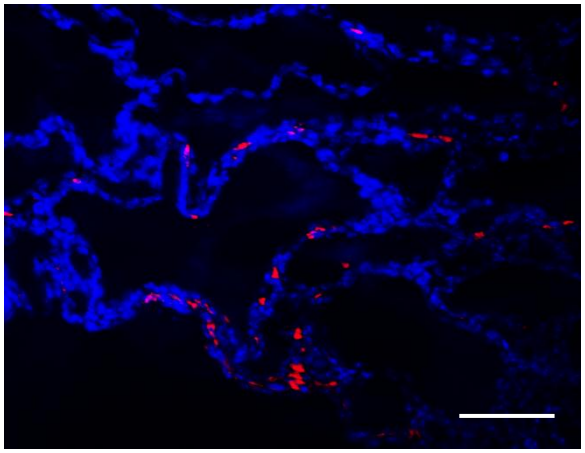
DMSO
ISO-1
Pkd1nl/nl

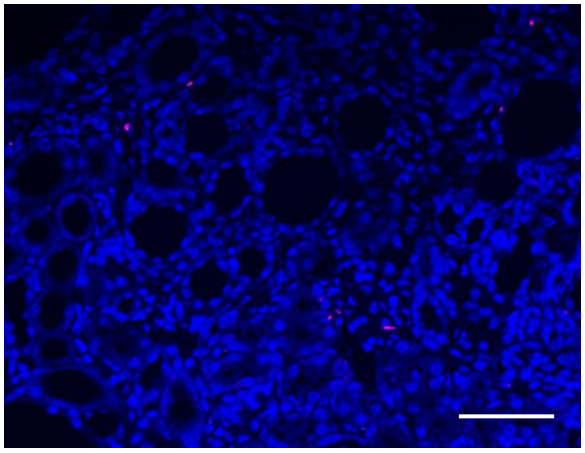
Supplemental Figure 4



A



***Pkd1*$^{flox/flox}$*:Ksp-Cre:MIF*$^{+/+}$



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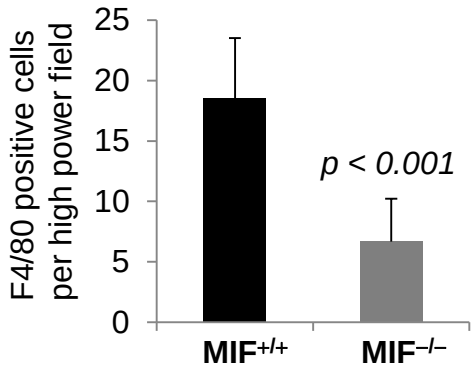

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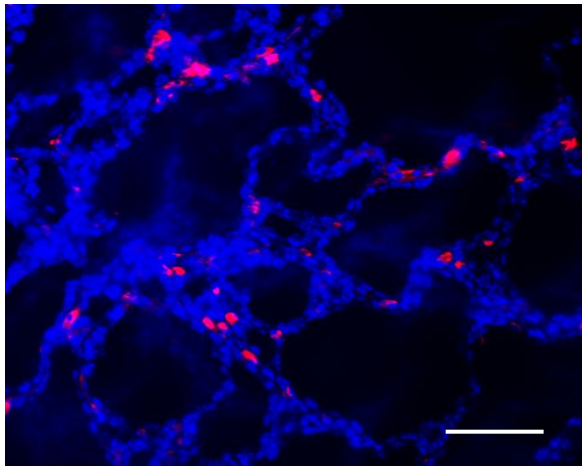
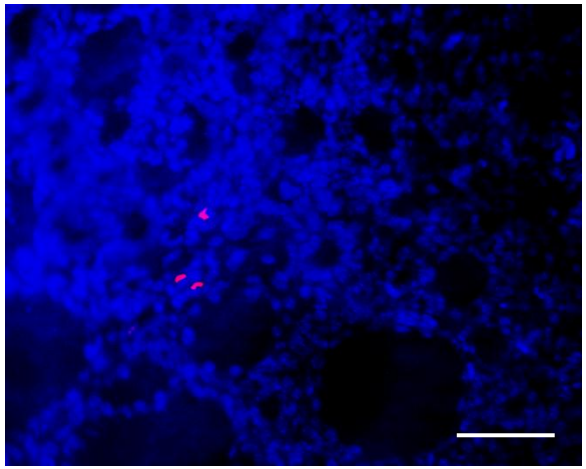
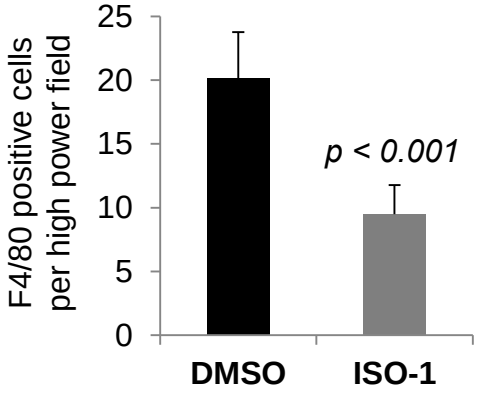


***Pkd1*$^{flox/flox}$*:Ksp-cre* + DMSO**



***Pkd1*$^{flox/flox}$*:Ksp-cre* + ISO-1**



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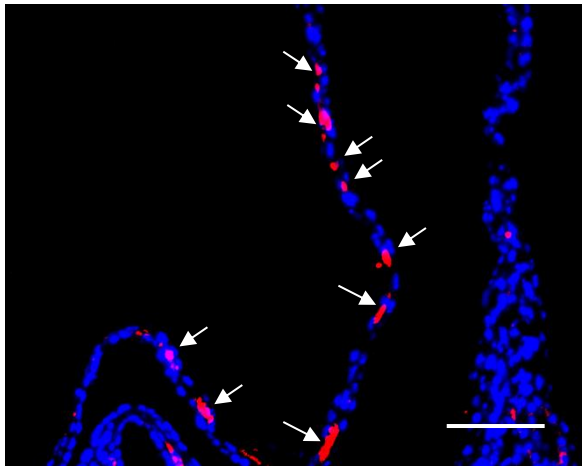
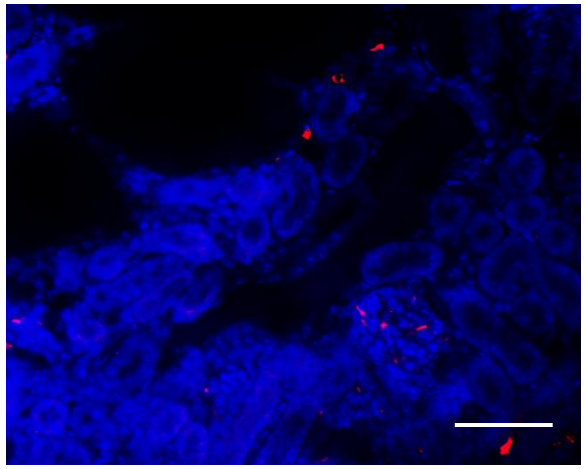
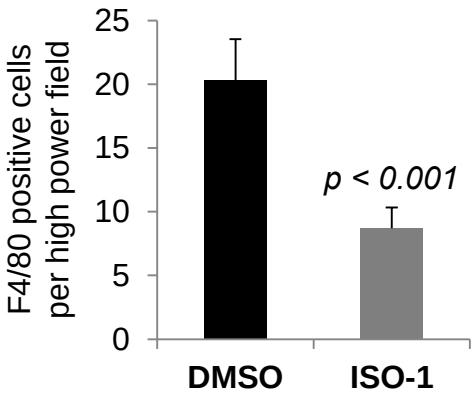


***Pkd1*$^{flox/flox}$*:Pkhd1-cre* + DMSO**



***Pkd1*$^{flox/flox}$*:Pkhd1-cre* + ISO-1**



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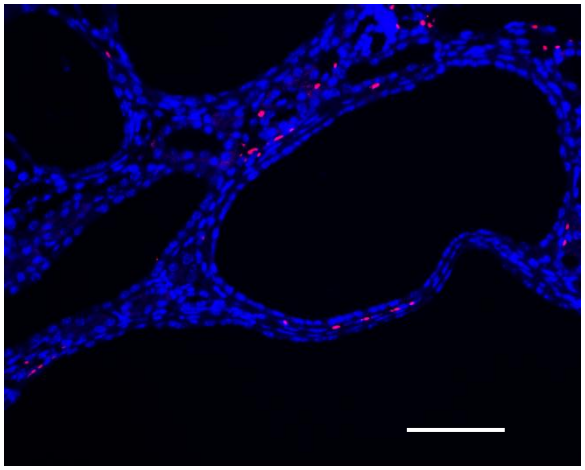
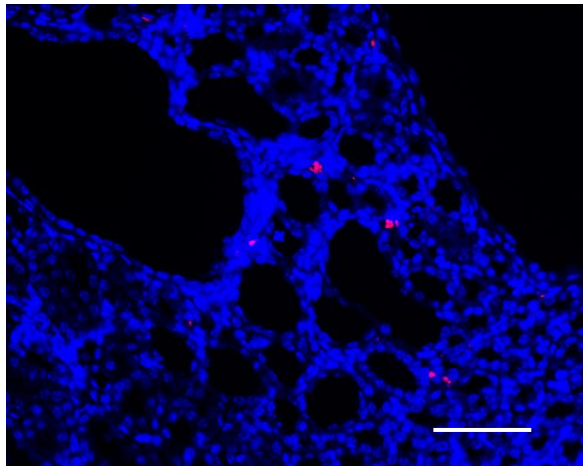
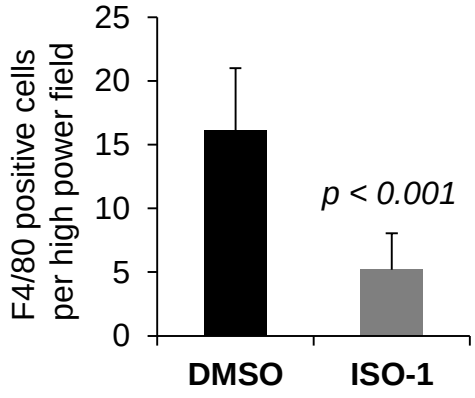
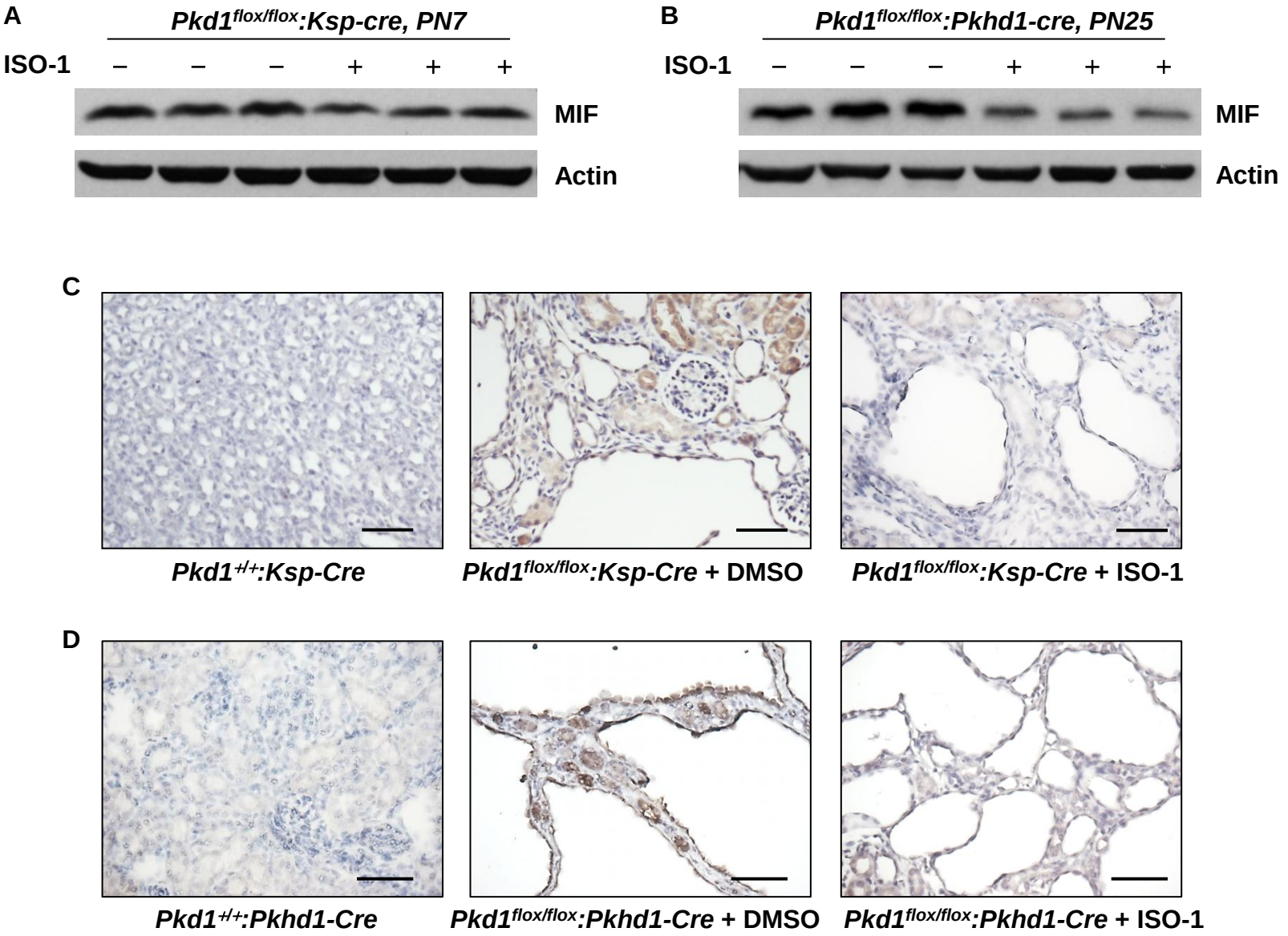


***Pkd1*$^{nl/nl}$ + DMSO**



***Pkd1*$^{nl/nl}$ + ISO-1**

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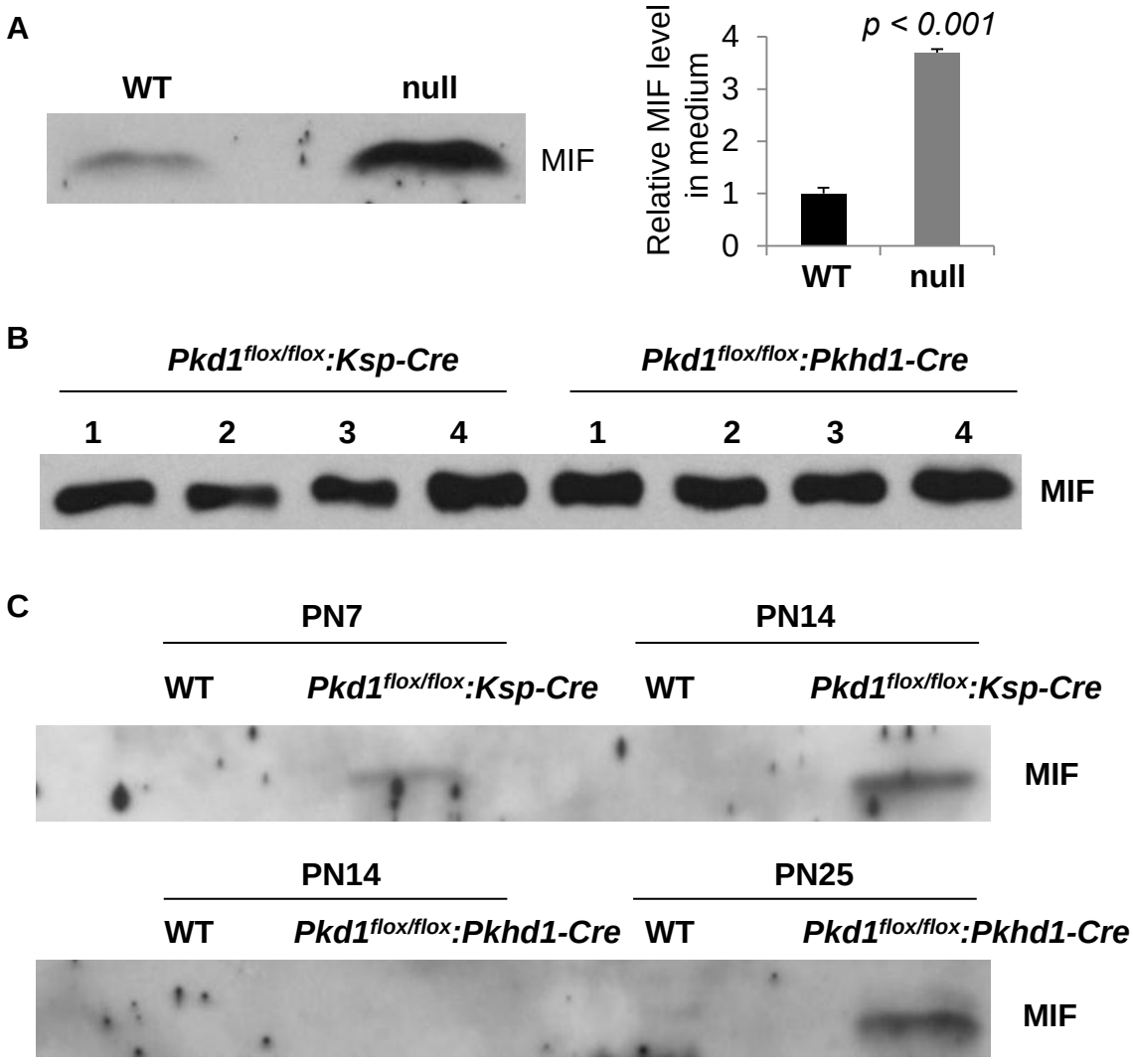
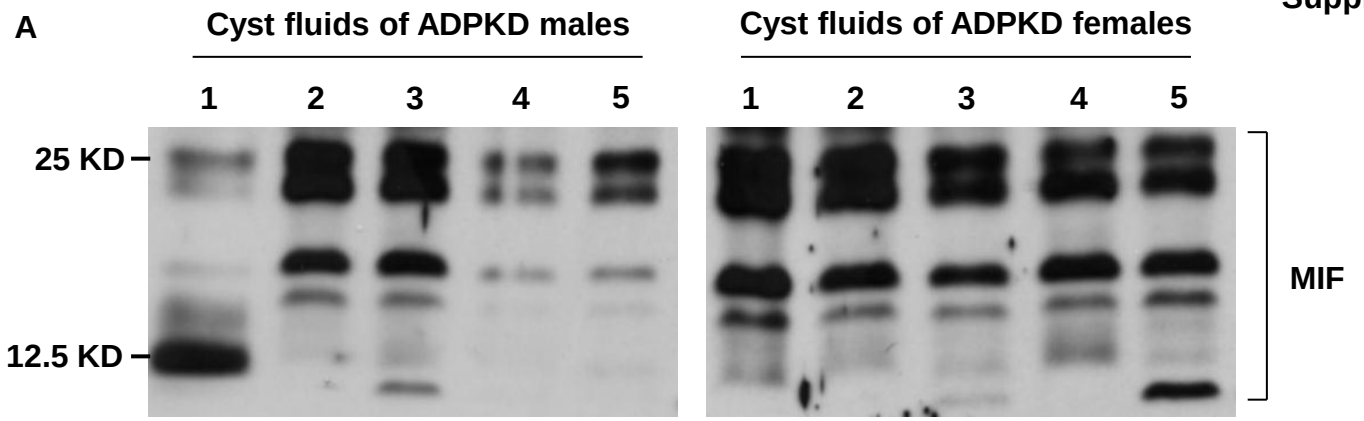
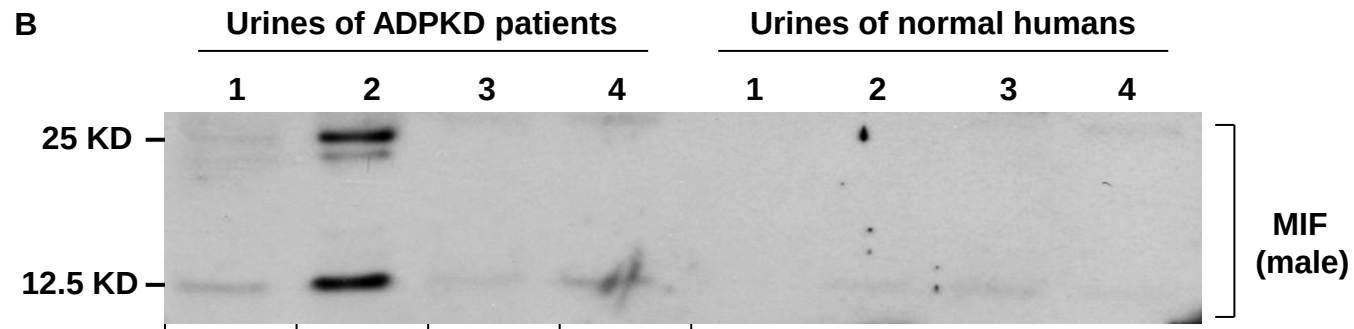
Pkd1flox/flox:Ksp-cre, PN7
ISO-1 – – – + + +
MIF
Actin
B
Pkd1flox/flox:Pkhd1-cre, PN25
ISO-1 – – – + + +
MIF
Actin
C
Pkd1+/+:Ksp-Cre
Pkd1flox/flox:Ksp-Cre + DMSO
Pkd1flox/flox:Ksp-Cre + ISO-1
D
Pkd1+/+:Pkhd1-Cre
Pkd1flox/flox:Pkhd1-Cre + DMSO
Pkd1flox/flox:Pkhd1-Cre + ISO-1

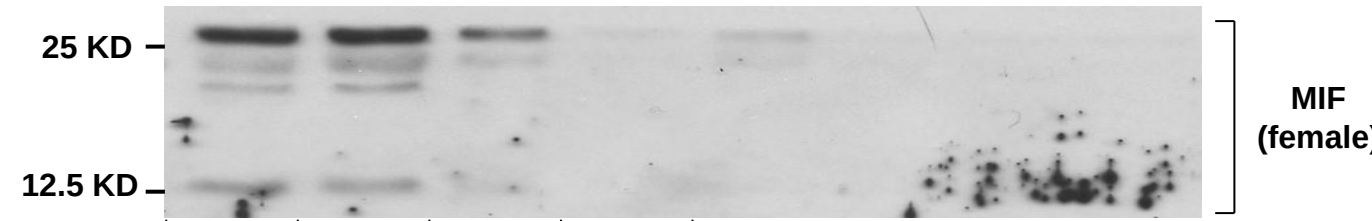

A
WT
null
MIF
Relative MIF level in medium
p < 0.001
4
3
2
1
0
WT
null
B
Pkd1flox/flox:Ksp-Cre
Pkd1flox/flox:Pkhd1-Cre
1
2
3
4
1
2
3
4
MIF
C
PN7
PN14
WT
Pkd1flox/flox:Ksp-Cre
WT
Pkd1flox/flox:Ksp-Cre
MIF
PN14
PN25
WT
Pkd1flox/flox:Pkhd1-Cre
WT
Pkd1flox/flox:Pkhd1-Cre
MIF

Supplemental Figure 8



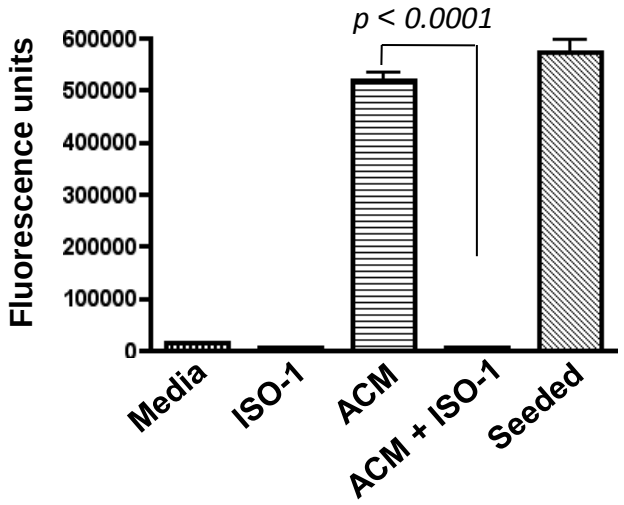


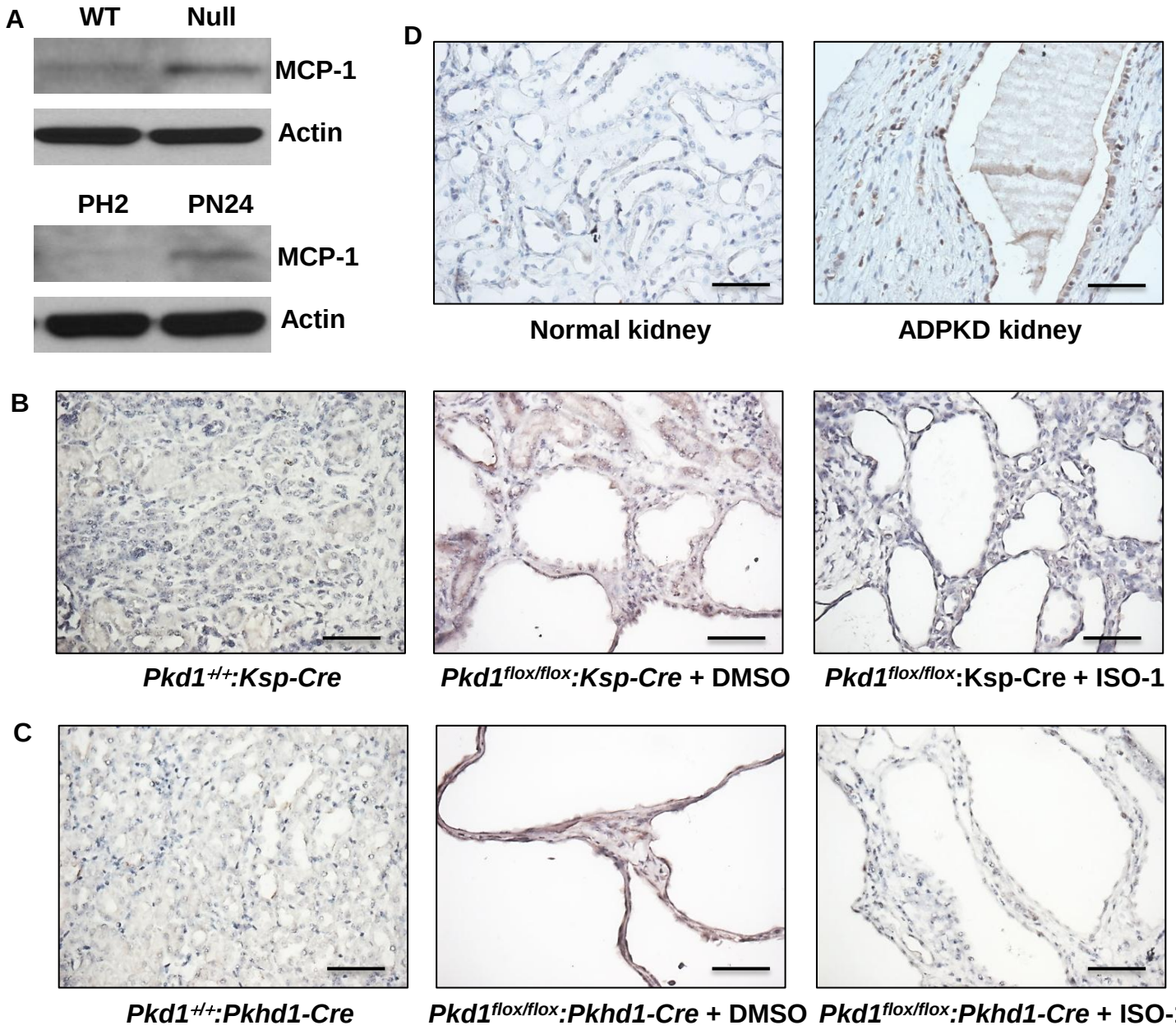
GFR	16	35	77	77
BUN	47	30	17	11
Age	51	44	46	41

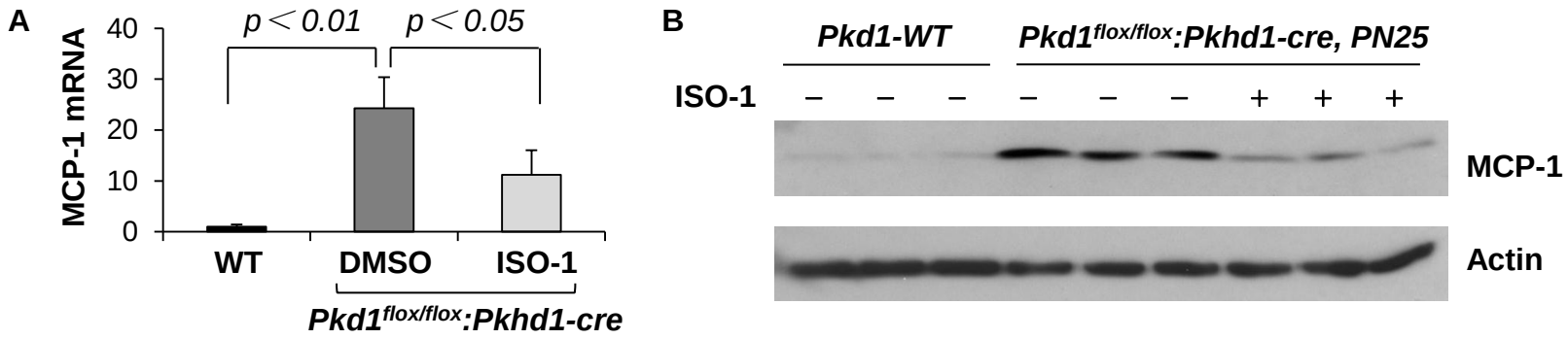


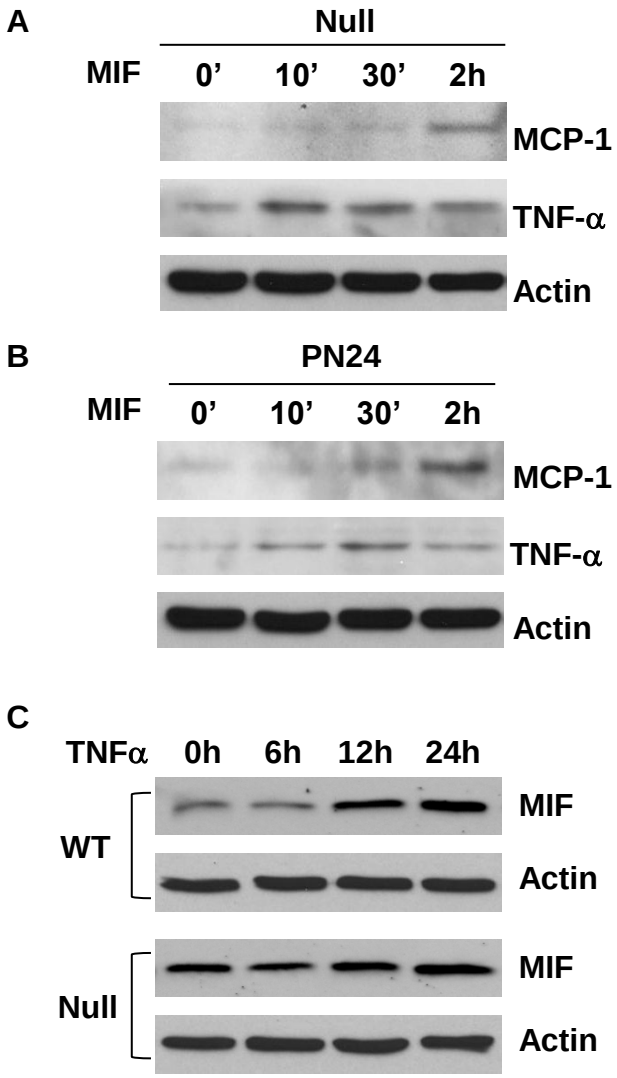
GFR	12	24	40	> 60
BUN	49	36	22	12
Age	57	48	62	40

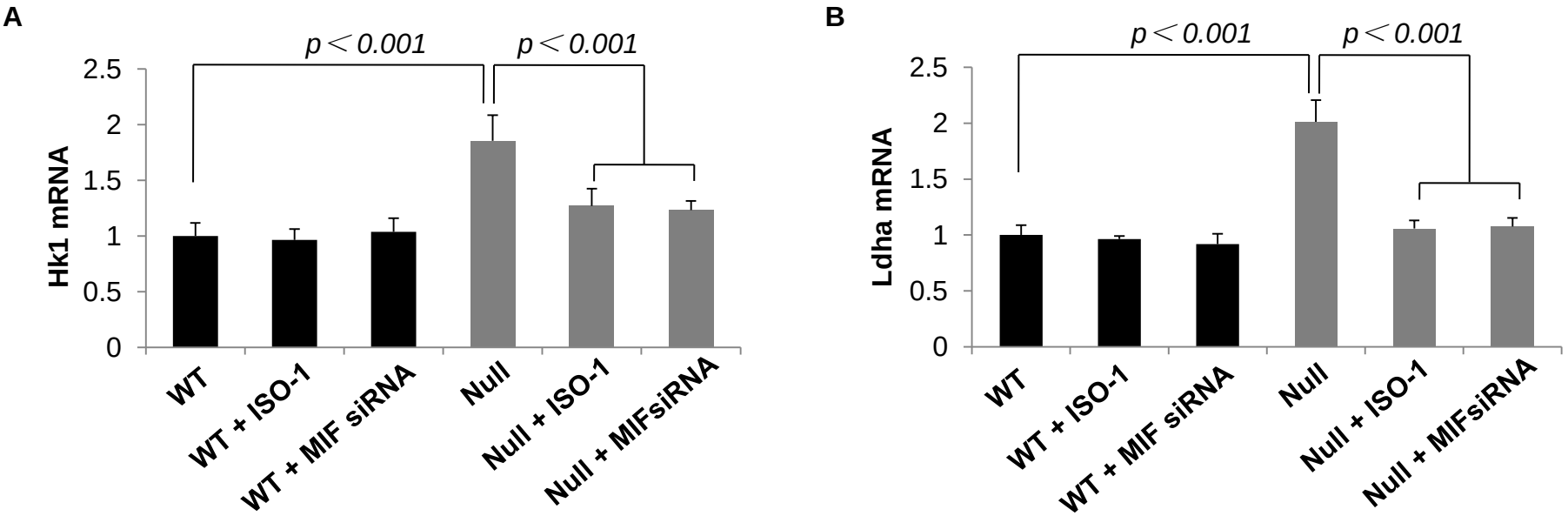
Supplemental Figure 9

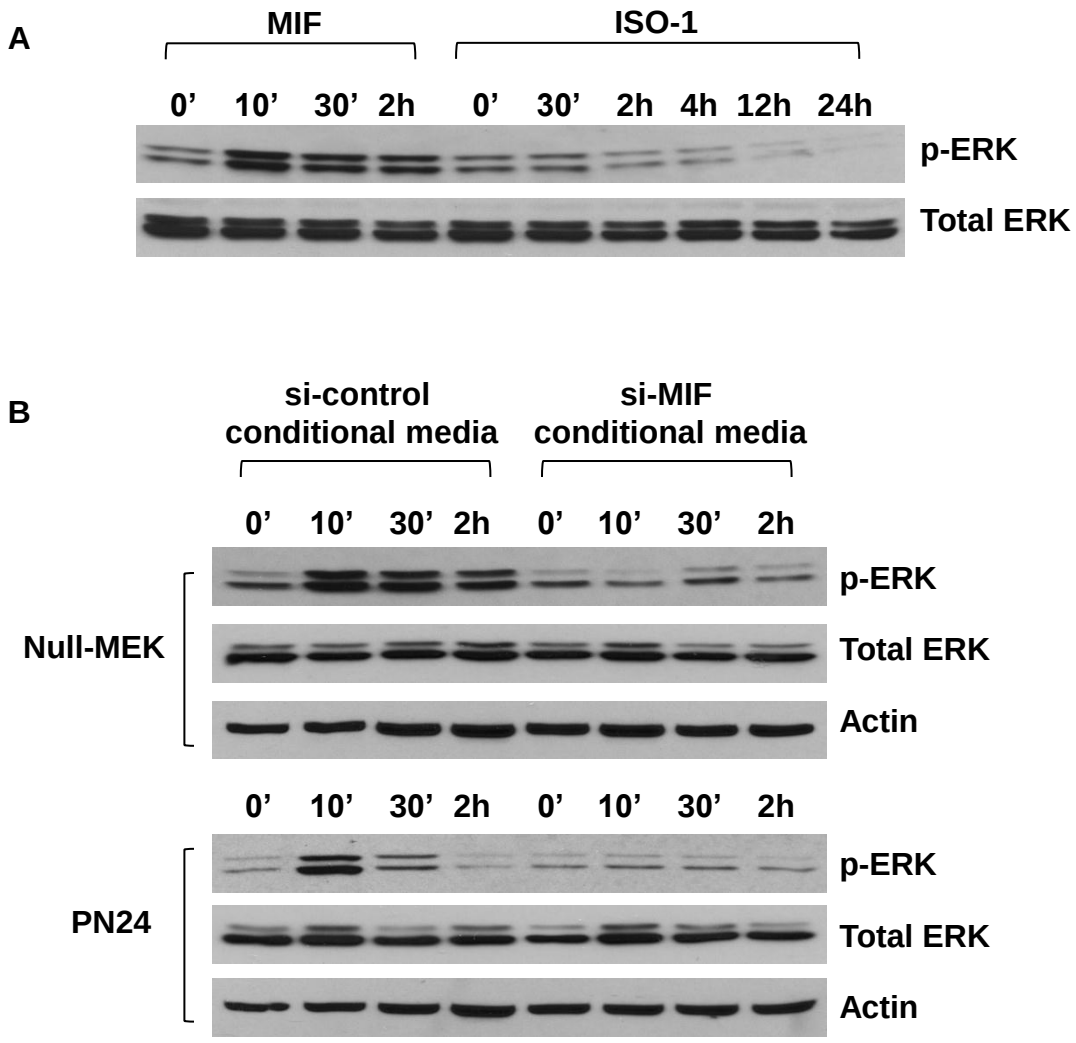



A
WT
Null
MCP-1
Actin
PH2
PN24
MCP-1
Actin
D
Normal kidney
ADPKD kidney
B
Pkd1+/+:Ksp-Cre
Pkd1flox/flox:Ksp-Cre + DMSO
Pkd1flox/flox:Ksp-Cre + ISO-1
C
Pkd1+/+:Pkhd1-Cre
Pkd1flox/flox:Pkhd1-Cre + DMSO
Pkd1flox/flox:Pkhd1-Cre + ISO-1


A
MCP-1 mRNA
40
30
20
10
0
p < 0.01
p < 0.05
WT
DMSO
ISO-1
Pkd1flox/flox:Pkhd1-cre
B
Pkd1-WT
Pkd1flox/flox:Pkhd1-cre, PN25
ISO-1
– – – – – – + + +
MCP-1
Actin


A
Null
MIF
0’
10’
30’
2h
MCP-1
TNF-α
Actin
B
PN24
MIF
0’
10’
30’
2h
MCP-1
TNF-α
Actin
C
TNFα
0h
6h
12h
24h
WT
MIF
Actin
Null
MIF
Actin


A
p < 0.001
p < 0.001
Hk1 mRNA
2.5
2
1.5
1
0.5
0
WT
WT + ISO-1
WT + MIF siRNA
Null
Null + ISO-1
Null + MIFsiRNA
B
p < 0.001
p < 0.001
Ldha mRNA
2.5
2
1.5
1
0.5
0
WT
WT + ISO-1
WT + MIF siRNA
Null
Null + ISO-1
Null + MIFsiRNA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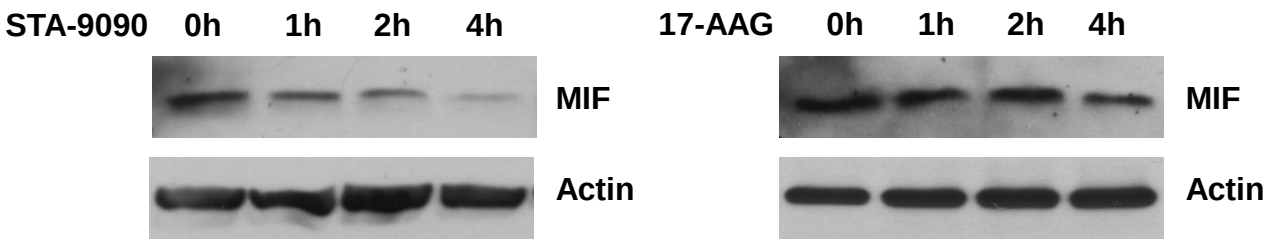
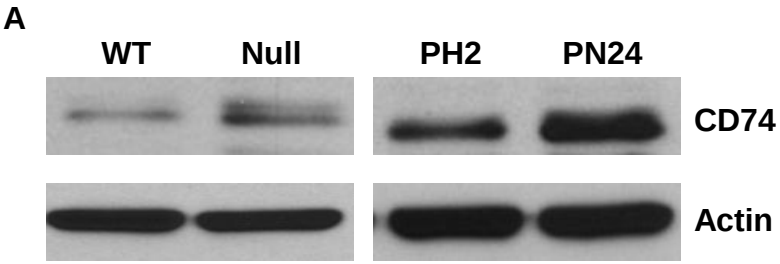
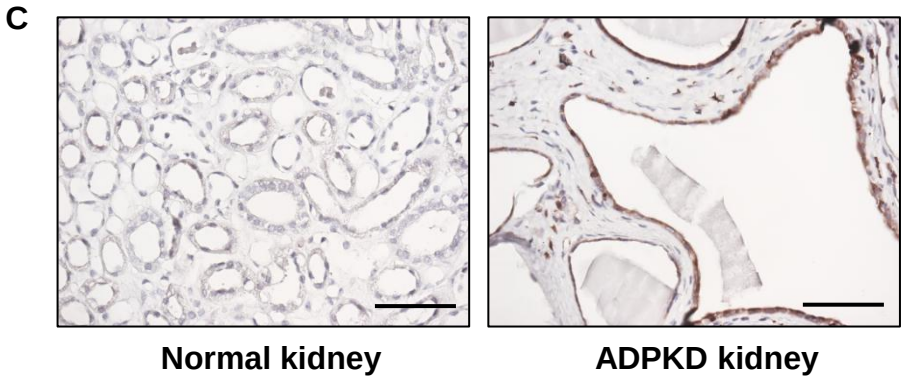
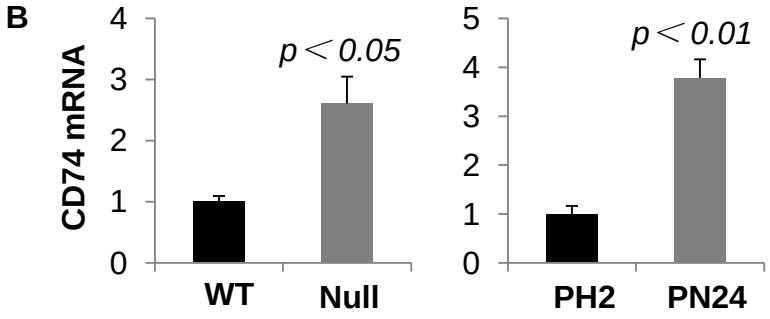
MIF
ISO-1
0’ 10’ 30’ 2h 0’ 30’ 2h 4h 12h 24h
p-ERK
Total ERK
B
si-control conditional media
si-MIF conditional media
0’ 10’ 30’ 2h 0’ 10’ 30’ 2h
Null-MEK
p-ERK
Total ERK
Actin
0’ 10’ 30’ 2h 0’ 10’ 30’ 2h
PN24
p-ERK
Total ERK
Actin

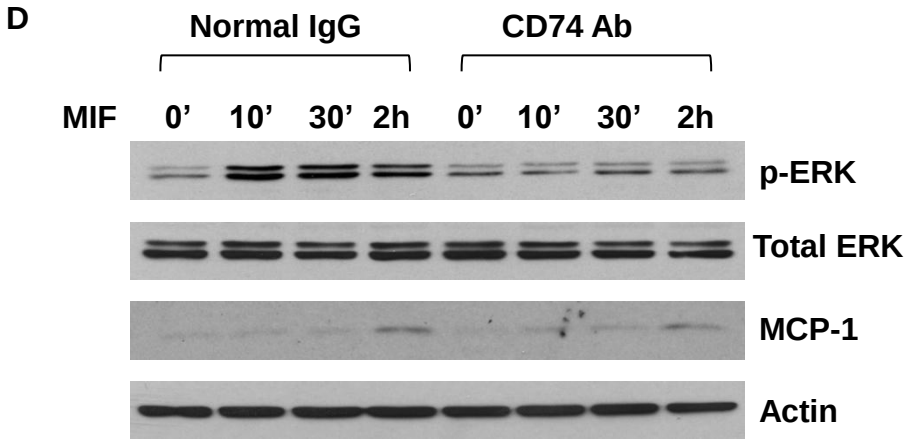
Supplemental Figure 15




A
WT
Null
PH2
PN24
CD74
Actin


C
Normal kidney
ADPKD kidney


B
CD74 mRNA
p < 0.05
p < 0.01
WT
Null
PH2
PN24


D
Normal IgG
CD74 Ab
MIF
0'
10'
30'
2h
0'
10'
30'
2h
p-ERK
Total ERK
MCP-1
Actin