Suppl. Figure Legends

Suppl. Fig. 1. **A**. Heparanase induction. Human MSTO-211H and NCI-H2052 mesothelioma cells and SGC-7901 gastric carcinoma cells were left untreated (0) as control (Con) or were stimulated with the indicated concentration of cisplatin (Cis), doxorubicin (Doxo), or PCT for 24 hours. Total RNA was then extracted and subjected to qPCR analyses applying primers specific for human heparanase. Data are presented as fold-increase related to control cells set arbitrarily to a value of 1. **B**. Chemical structure of H1001 (MW=499). **C, D**. Heparanase activity. **C**. Intracellular heparanase. U87 glioma cells were left untreated (Con) or were treated with H1001 (2 µg/ml) for 24 hours. Cells were then washed with PBS, lysed by three freeze/thaw cycles, and heparanase activity was determined as described under 'Materials and Methods'. **D**. Purified active heparanase (200 ng) was applied on S^{35}-labelled ECM without (Con) or with the indicated concentration of H1001 for 5 hours and heparanase activity was then evaluated as above. **E**. Cell invasion. B16 melanoma cells ($2x10^5$) were plated on Matrigel-coated 8-µm trans-well filters in the absence or presence of H1001 (1 µg/ml). After 24 hours cells invading and adhering to the lower side of the filter membrane were visualized and photographed. **F**. Heparanase processing. Recombinant latent heparanase (1 µg/ml) was incubated without (Hepa) or with H1001 (50 µg/ml) for 30 minutes and was then added to HEK 293 cells. After 4 hours cells were washed and cell lysates were subjected to immunoblotting applying anti-heparanase (upper panel) and anti-actin (lower panel) antibodies. Note that H1001 inhibits not only heparanase activity but also it's processing into an active enzyme.

Suppl. Fig. 2. **A**. Cell migration. Macrophages were isolated from WT and Hpa-KO mice and were left untreated (Con) or were treated with PCT (15 µg/ml) for 24 hours. Conditioned medium (CM) was then collected and added to the lower compartment of a Boyden chamber apparatus as chemoattractant. WT macrophages ($2x10^5$) were plated in the upper compartment and cells were allowed to migrate for 24 hours. Cells migrating and adhering to the lower side of the trans-well (8-µm) filters were then photographed (upper panels) and quantified by counting (lower panels). **B**. Matrigel plugs. Macrophages were isolated from WT mice and were left untreated (Con) or were treated with PCT. After 24 hours, macrophages were detached and suspended in Matrigel ($3x10^6$/ml). Matrigel containing untreated (Mat+Con) or PCT-treated (Mat+PCT) macrophages were injected subcutaneously

(0.5 ml/mouse), and Matrigel plugs were collected 12 days later. Total RNA was then extracted and subjected to qPCR applying primers specific for F4/80.

Suppl. Fig. 3. **A**. Matrigel plug. Matrigel (0.5 ml/mouse) was injected subcutaneously in C57/BL mice. After two weeks the resulting plugs were collected, fixed in formalin and embedded in paraffin. Five-micron sections were stained with hematoxylin and eosin. Host cells seem not to be recruited into the plug in the absence of macrophages embedded in the implanted Matrigel. **B**. FACS analyses. Peritoneal macrophages were isolated from WT and Hpa-KO mice and were left untreated or were treated with PCT (15 μg/ml) or cisplatin (11 μg/ml) for 24 hours. Macrophages were then detached and subjected to FACS analysis applying anti-CD11c (APC-Cy7; Biolegend, clone N418) and anti-CD206 (BV421; Biolegend, clone C068C2) (upper panels). The gating strategy is shown in the lower panels: first we gated the total population (FSC vs. SSC; left) and then the macrophages (F4/80- PE, Biolegend clone BM8; right). **C**. Tumor growth. LLC cells ($4x10^5$) were inoculated subcutaneously to WT C57BL/6 mice without (LLC) or with an equal number of untreated (+Con) or PCT-treated (+PCT) macrophages. At termination, tumors were excised, weighed (upper panel) and photographed (lower panel).

Suppl. Fig. 4. **A.** TLR. Peritoneal macrophages were isolated from WT and Hpa-KO mice and were left untreated (0) or were treated with PCT for the time indicated. Total RNA was then extracted and subjected to qPCR applying primers specific for TLR2 (upper panel) and TLR4 (second panel). WT and Hpa-KO macrophages were similarly isolated and were left untreated (Con) or were treated with PCT in the absence (PCT) or presence of anti-Myd88 (PCT+Myd) or control scrambled (PCT+Scr) peptide. After 24 hours total RNA was extracted and subjected to qPCR applying primers specific for MIP2 (lower panel). **B**-**C**. COMPASS components. Peritoneal macrophages were isolated from WT and Hpa-KO mice and were left untreated (0) or were treated with PCT for the time indicated. Total RNA was then extracted and subjected to qPCR applying primers specific for SET1A (**B**, upper panel), MLL1 (**B**, second panel), MLL2 (**B**, third panel), MLL3 (**B**, fourth panel), MLL4 (**B**, lower panel), and RBBP5 (**C**).

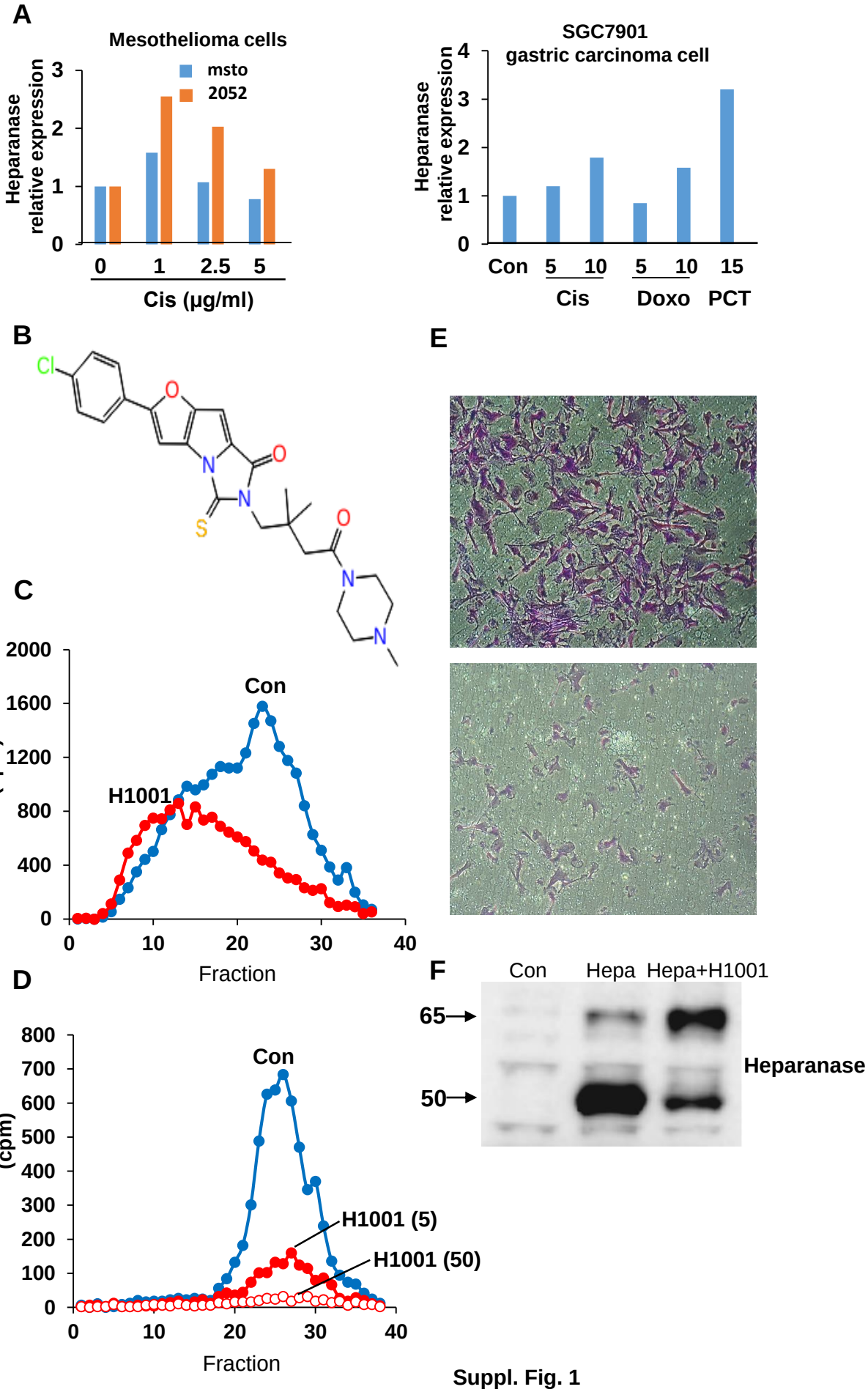
Suppl. Figure 5. **A**. WDR5 inhibitor. Peritoneal macrophages were isolated from WT mice and were left untreated (Con) or were treated with PCT (15 μg/ml) in the absence (0) or the indicated concentrations of OICR-9429, a WDR5 inhibitor. DMSO was added as vehicle

control (0). Total RNA was extracted after 24 hours and was subjected to qPCR applying primers specific for TNFα (upper panel) and IL6 (lower panel). **B**. Conditioned medium. Macrophages were isolated from WT mice and were left untreated (Con) or were treated with PCT (15 µg/ml) for 24 hours. Conditioned medium (CM) was then collected and was added to macrophages isolated from Hpa-KO mice for the time indicated. Whereas medium conditioned by control, untreated WT macrophages, failed to stimulate MIP2 in KO macrophages (Con CM), PCT CM efficiently stimulated MIP2 expression in these cells (PCT CM), comparable in magnitude to MIP2 induction by PCT in WT macrophages (WT, PCT). NT = non-treated (untreated) KO macrophages. **C**. CXCL1 expression. Total RNA was extracted from WT and Hpa-KO macrophages and was subjected to qPCR applying primers specific for CXCL1 (left). CXCL1 expression was similarly quantified (qPCR) in untreated (Con) and PCT-treated WT macrophages (right). Macrophages were isolated from WT and Hpa-KO mice and were left untreated (Con) or were treated with PCT (15 µg/ml) for the time indicated. Total RNA was then extracted and subjected to qPCR applying primers specific for CXCL5 (**D**), LEDGF (**E**, upper panel), and Ash2L (**E**, lower panel).

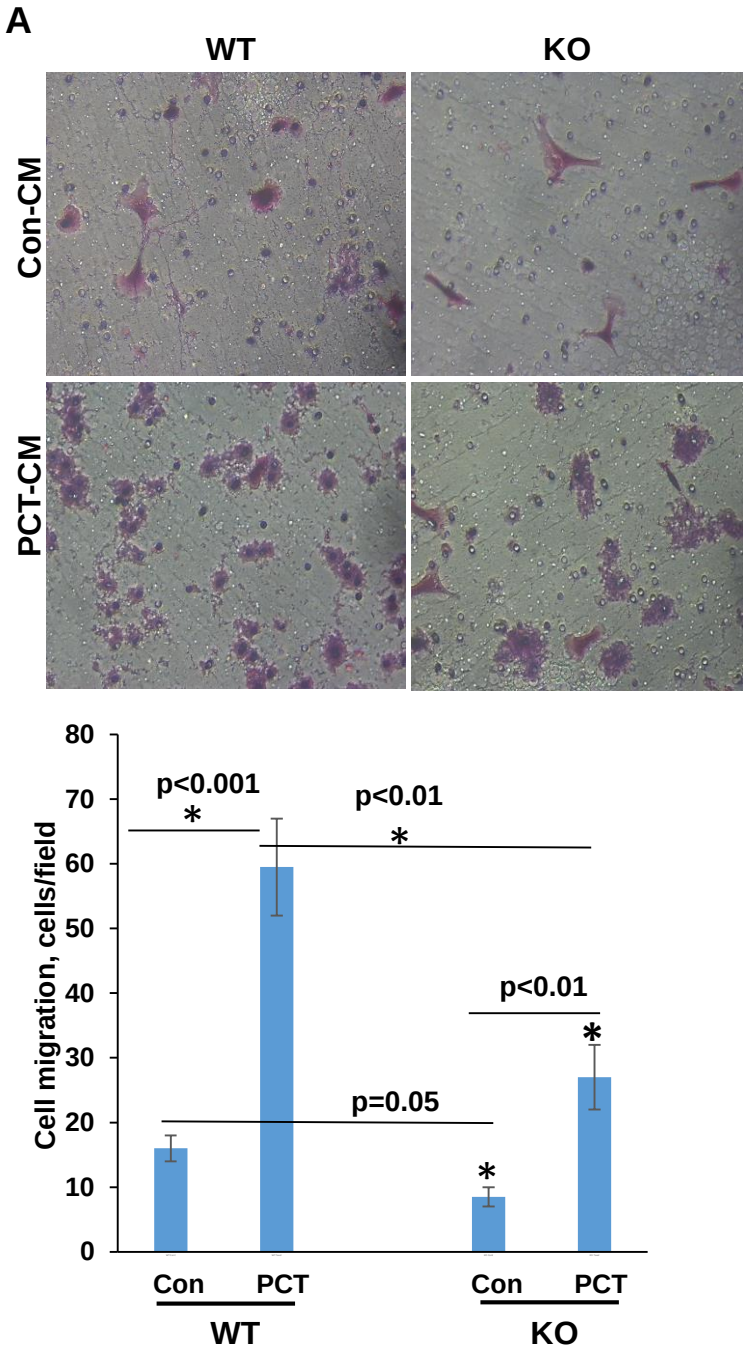
Suppl. Figure 6. MKL1. Macrophages were isolated from WT and Hpa-KO mice and were left untreated (Con) or were treated with PCT (15 µg/ml) for the time indicated. Total RNA was then extracted and subjected to qPCR applying primers specific for MKL1 (**A**). Peritoneal macrophages were collected from WT mice and were left untreated (0) or were treated with the indicated concentrations of CCG-203971 (MKL1 inhibitor) in the absence (Con) or presence of PCT. Total RNA was extracted after 24 h and was subjected to qPCR analyses applying primers specific for TNFα (**B**, upper panel), IL6 (**B**, middle), and MIP2 (**B**, lower panel). DMSO was added to untreated cells (0) as vehicle control. **C**-**D**. WDR5 overexpression/gene silencing. WT macrophages were transfected with control (anti-GFP) and anti-WDR5 oligonucleotides. Total RNA was extracted 48 hours thereafter and subjected to qPCR applying primers specific for WDR5 (**C**, left) and MIP2 (**C**, right). WT macrophages were transfected with control plasmid (Vo) and WDR5 expression vector (Addgene; Watertown, MA). Total RNA was extracted 48 hours thereafter and subjected to qPCR applying primers specific for WDR5 (**D**, left) and MIP2 (**D**, right).

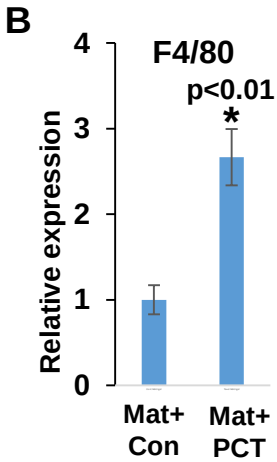
Suppl. Figure 7. Signaling. **A**-**B**. JNK activation by PCT. Peritoneal macrophages were isolated from WT and Hpa-KO mice and were left untreated (0) or were treated with PCT for the time indicated. Protein extracts were then prepared and subjected to immunoblotting

applying antibodies directed against phospho-JNK (pJNK; **A**, upper panel), JNK (**A**, second panel), BiP (**A**, third panel) and actin (**A**, lower panel). Densitometry analyses of JNK phosphorylation and BiP expression are shown in the right panels. Corresponding cell cultures were treated with PCT for 6 h, fixed with cold methanol and subjected to immunofluorescent staining applying anti phospho-JNK antibody (**B**, middle panels). Nuclear staining (ToPro) is shown in blue (**B**, left panels). Merged images are shown in the right panels. **C**. Cytokine expression. WT macrophages were left untreated (0) or were treated with the indicated concentrations of sp600125 (JNK inhibitor) in the absence (Con) or presence of PCT. Total RNA was extracted after 24 h and was subjected to qPCR analyses applying primers specific for TNFα (left panel) and IL6 (right panel). DMSO was added to untreated cells (0) as vehicle control. **D**. Histone methylation.WT macrophages were left untreated (0) or were treated with the indicated concentrations of sp600125 (left panel) or SB208530 (p38 inhibitor, right) in the absence (Con) or presence of PCT. Protein extracts were prepared after 24 hours and were subjected to immunoblotting applying anti tri-methylated histone 3 (H3K4Me3, upper panels) and anti-histone H3 (second panels) antibodies. DMSO was added as vehicle control.

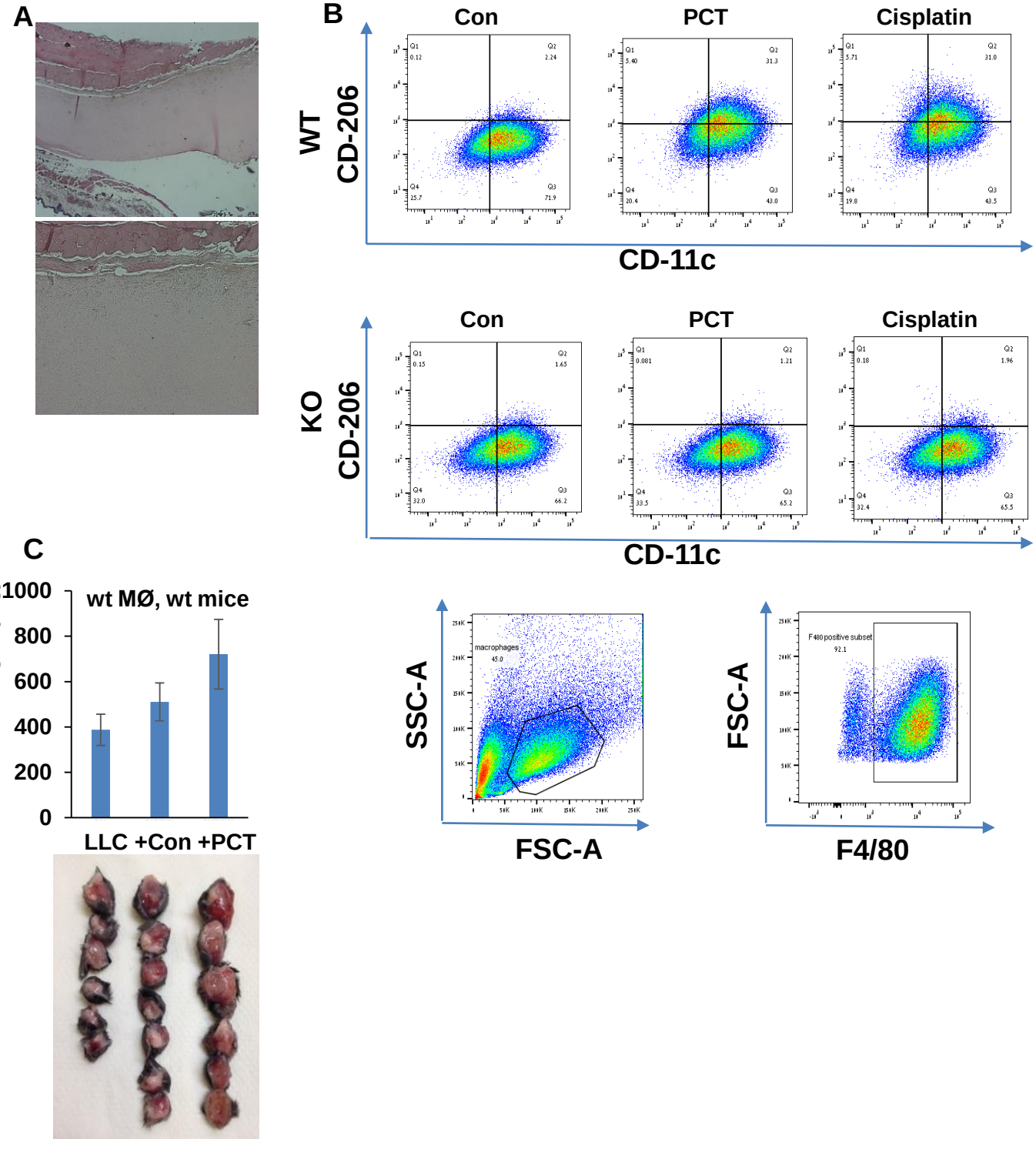

A
Mesothelioma cells
msto
2052
Heparanase relative expression
0
1
2
3
0
1
2.5
5
Cis (μg/ml)
SGC7901
gastric carcinoma cell
Heparanase relative expression
0
1
2
3
4
Con
5
10
5
10
15
Cis
Doxo
PCT
B
Cl
O
N
O
N
S
O
N
N
C
Sulfate labeled material (cpm)
2000
1600
1200
800
400
0
Con
H1001
0
10
20
30
40
Fraction
D
Sulfate labeled material (cpm)
800
700
600
500
400
300
200
100
0
Con
H1001 (5)
H1001 (50)
0
10
20
30
40
Fraction
E
F
Con
Hepa
Hepa+H1001
65
50
Heparanase

Suppl. Fig. 1

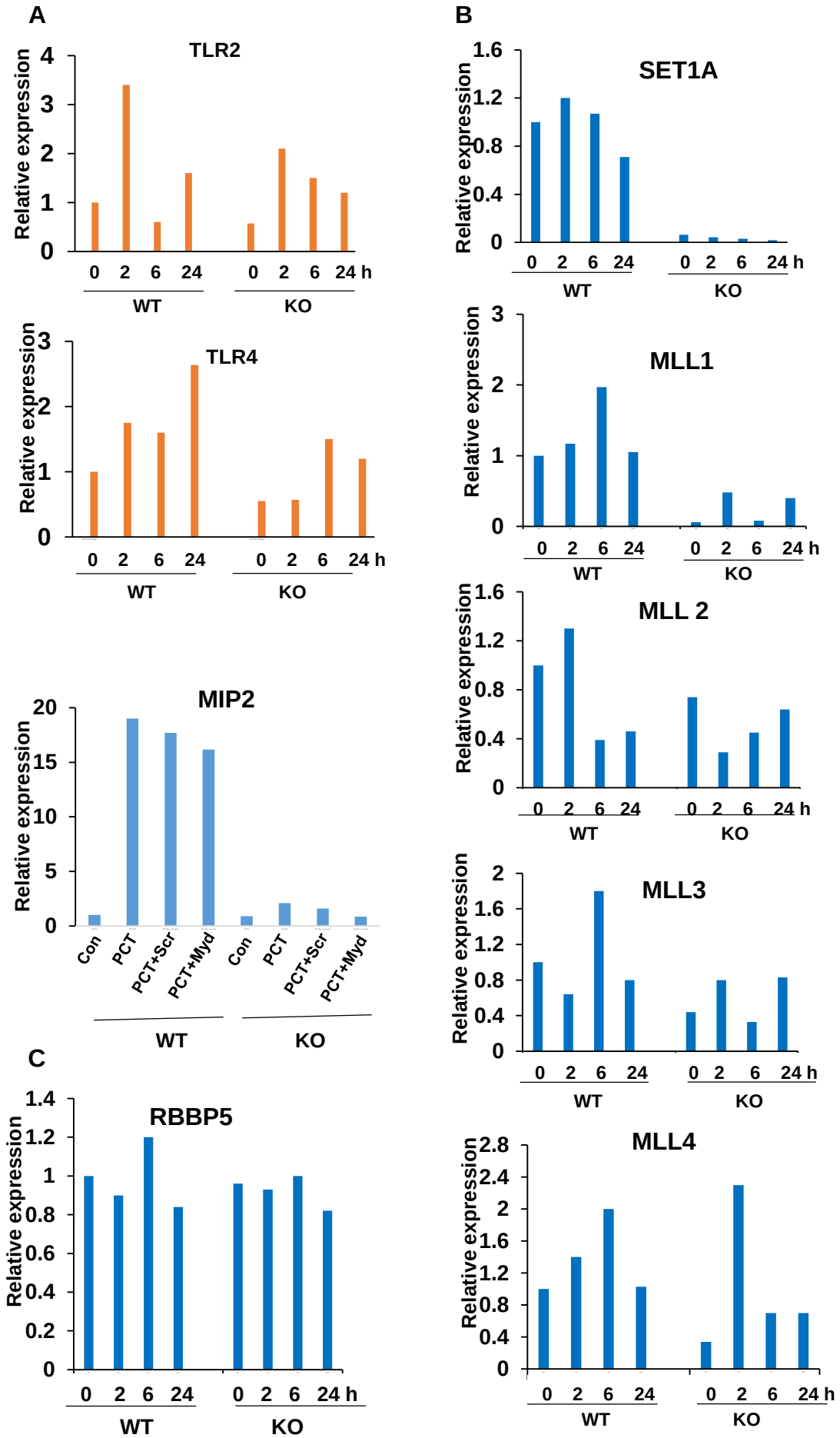

A
WT
KO
Con-CM
PCT-CM
Cell migration, cells/field
80
70
60
50
40
30
20
10
0
p<0.001
*
p<0.01
*
p<0.01
*
p=0.05
*
Con
PCT
Con
PCT
WT
KO


B
F4/80
p<0.01
*
Relative expression
4
3
2
1
0
Mat+ Con
Mat+ PCT

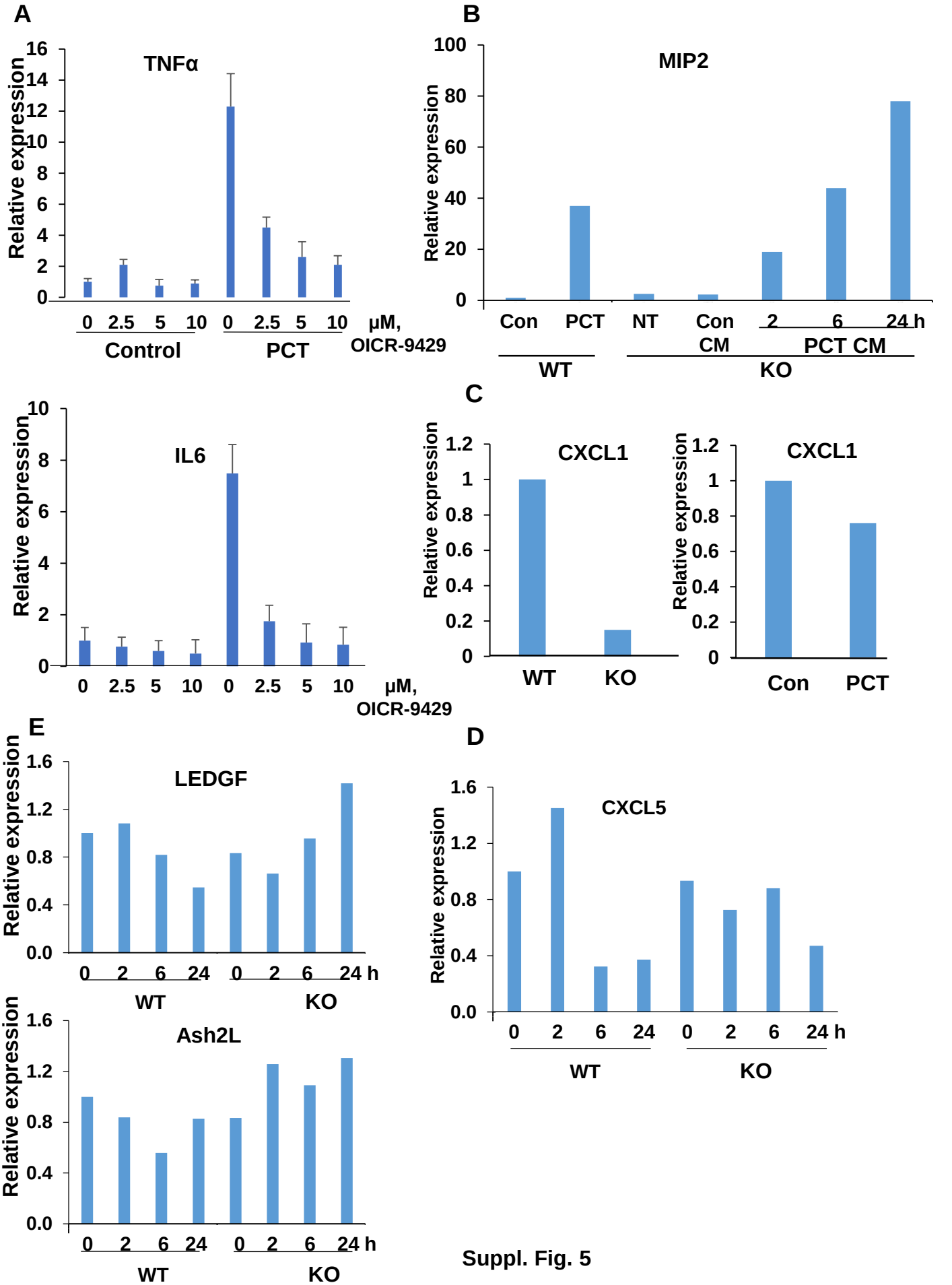
Suppl. Fig. 2


A
B
Con
PCT
Cisplatin
WT
CD-206
CD-11c
KO
C
wt MØ, wt mice
Tumor weight (mg)
1000
800
600
400
200
0
LLC +Con +PCT
SSC-A
FSC-A
macrophages
45.0
F480 positive subset
92.1
F4/80

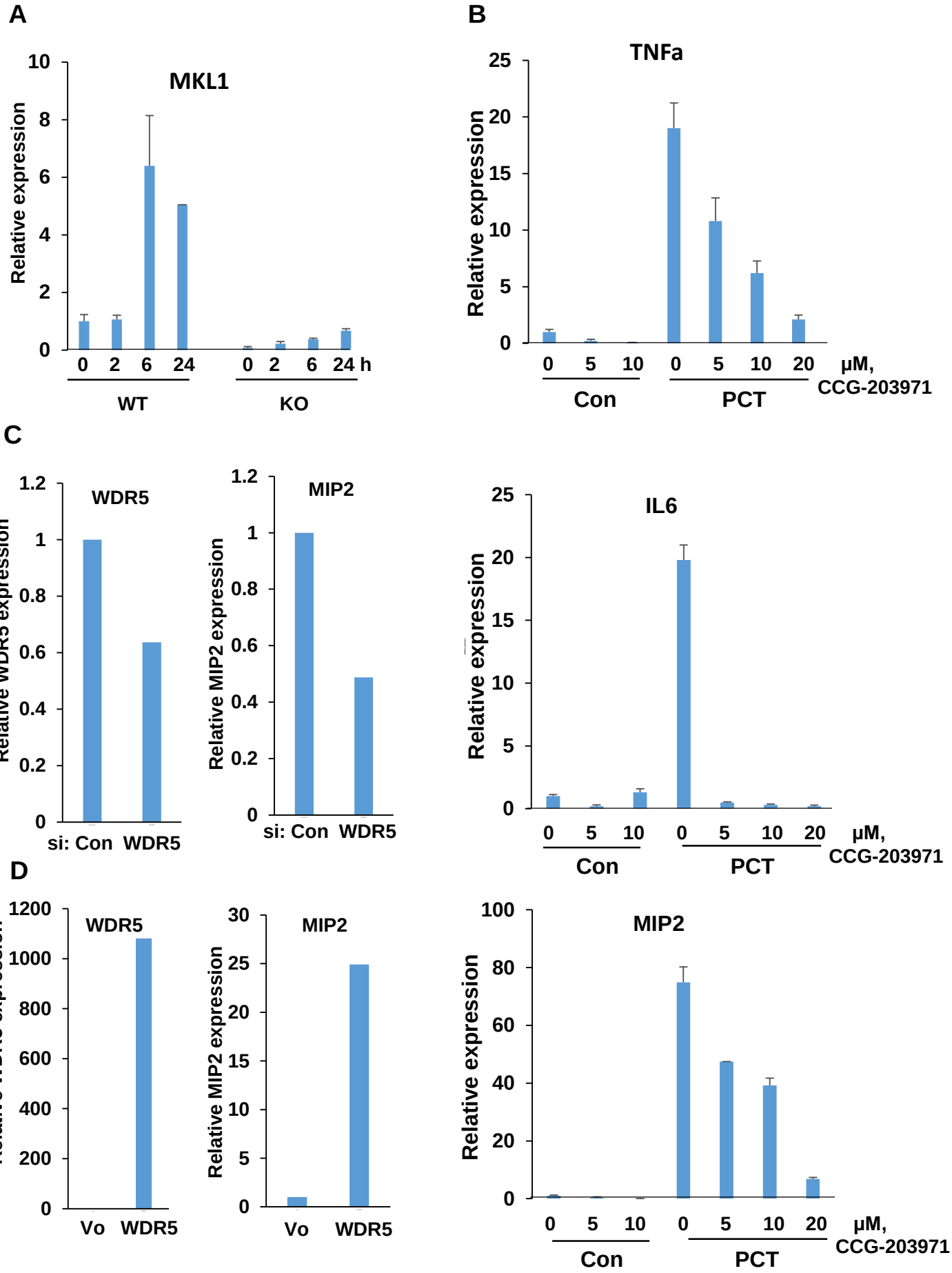
Suppl. Fig. 3

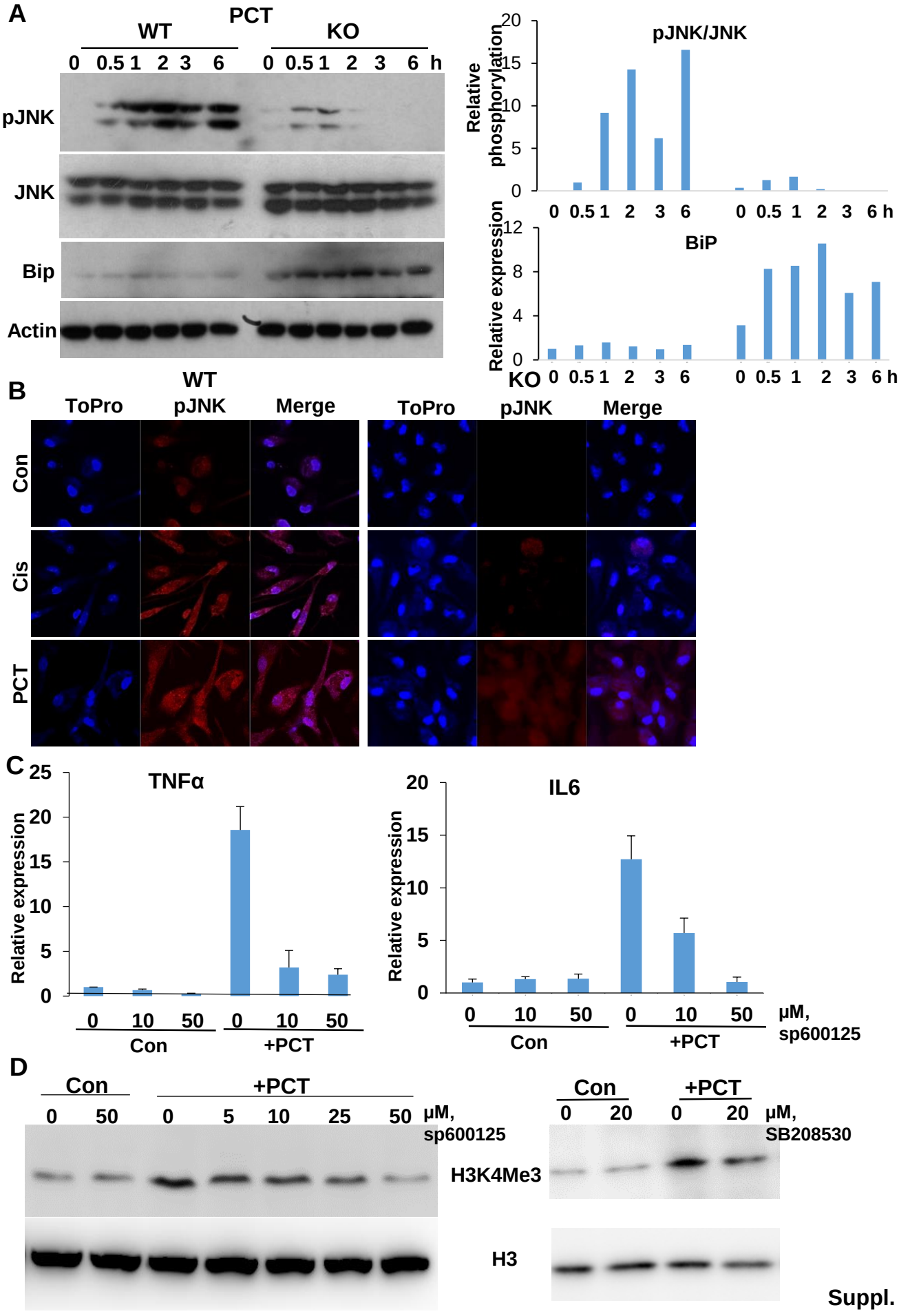

A
TLR2
Relative expression
0 1 2 3 4
0 2 6 24 0 2 6 24 h
WT
KO
TLR4
Relative expression
0 1 2 3
0 2 6 24 0 2 6 24 h
WT
KO
MIP2
Relative expression
0 5 10 15 20
Con PCT PCT+Scr PCT+Myd Con PCT PCT+Scr PCT+Myd
WT
KO
B
SET1A
Relative expression
0 0.4 0.8 1.2 1.6
0 2 6 24 0 2 6 24 h
WT
KO
MLL1
Relative expression
0 1 2 3
0 2 6 24 0 2 6 24 h
WT
KO
MLL 2
Relative expression
0 0.4 0.8 1.2 1.6
0 2 6 24 0 2 6 24 h
WT
KO
MLL3
Relative expression
0 0.4 0.8 1.2 1.6 2
0 2 6 24 0 2 6 24 h
WT
KO
MLL4
Relative expression
0 0.4 0.8 1.2 1.6 2 2.4 2.8
0 2 6 24 0 2 6 24 h
WT
KO
C
RBBP5
Relative expression
0 0.2 0.4 0.6 0.8 1 1.2 1.4
0 2 6 24 0 2 6 24 h
WT
KO

Suppl. Fig. 4


A
TNFα
Relative expression
16
14
12
10
8
6
4
2
0
0 2.5 5 10 0 2.5 5 10 μM, OICR-9429
Control
PCT
B
MIP2
Relative expression
100
80
60
40
20
0
Con PCT NT Con 2 6 24 h
CM PCT CM
WT
KO
C
IL6
Relative expression
10
8
6
4
2
0
0 2.5 5 10 0 2.5 5 10 μM, OICR-9429
CXCL1
Relative expression
1.2
1
0.8
0.6
0.4
0.2
0
WT KO
CXCL1
Relative expression
Con PCT
D
CXCL5
Relative expression
1.6
1.2
0.8
0.4
0.0
0 2 6 24 0 2 6 24 h
WT KO
E
LEDGF
Relative expression
1.6
1.2
0.8
0.4
0.0
0 2 6 24 0 2 6 24 h
WT KO
Ash2L
Relative expression
1.6
1.2
0.8
0.4
0.0
0 2 6 24 0 2 6 24 h
WT KO

Suppl. Fig. 5


A
MKL1
Relative expression
10
8
6
4
2
0
0 2 6 24 0 2 6 24 h
WT
KO
B
TNFa
Relative expression
25
20
15
10
5
0
0 5 10 0 5 10 20 μM, CCG-203971
Con
PCT
C
WDR5
Relative WDR5 expression
1.2
1
0.8
0.6
0.4
0.2
0
si: Con WDR5
MIP2
Relative MIP2 expression
1.2
1
0.8
0.6
0.4
0.2
0
si: Con WDR5
IL6
Relative expression
25
20
15
10
5
0
0 5 10 0 5 10 20 μM, CCG-203971
Con
PCT
D
WDR5
Relative WDR5 expression
1200
1000
800
600
400
200
0
Vo WDR5
MIP2
Relative MIP2 expression
30
25
20
15
10
5
0
Vo WDR5
MIP2
Relative expression
100
80
60
40
20
0
0 5 10 0 5 10 20 μM, CCG-203971
Con
PCT


A
PCT
WT
KO
0 0.5 1 2 3 6 0 0.5 1 2 3 6 h
pJNK
JNK
Bip
Actin
pJNK/JNK
Relative phosphorylation
BiP
Relative expression
B
WT
KO
ToPro
pJNK
Merge
Con
Cis
PCT
C
TNFα
IL6
Relative expression
Con
+PCT
μM, sp600125
D
μM, sp600125
μM, SB208530
H3K4Me3
H3

Suppl. Fig. 7

Table S1. Mouse primers sets utilized in this study.

	F (5'-3')	R
Heparanase	ACCGACGACGTGGTAGACTT	TGCAGGAGATAAGCCTCTAGCC
MIP2	CCAACCACCAGGCTACAGG	GCGTCACACTCAAGCTCTG
VEGF-A	GTACCTCCACCATGCCAAGT	GCATTCACATCTGCTGTGCT
TNF alpha	TCAGCCTCTTCTCATTCCTG	TGAAGAGAACCTGGGAGTAG
IL-10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
IL-6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
Actin	ATGCTCCCCGGGCTGTAT	CATAGGAGTCCTTCTGACCCATT
F4/80	GATACAGCAATGCCAAGCAGT	TTGTGAAGGTAGCATTCACAAGTGTA
CD4	GAAGATTCTGGGGCAGCATGGCAAAG	TTTGGAATCAAAACGATCAA
CD8	CTGCGTGGCCCTTCTGCTGTCCT	GGGACATTTGCAAACACGCT
CXCL1	CTGGGATTCACCTCAAGAACATC	CAGGGTCAAGGCAAGCCTC
MKL1	AGGACCGAGGACTATTTGAAACG	CCACAATGATAGCCTCCTTCAG
Ly6g	TGCCCCTTCTCTGATGGATT	TGCTCTTGACTTTGCTTCTGTGA
Set 1a	AGAAGAGGTCAGAATTGATCGCC	TGGTTGACAAATGTTGTTACCCA
MLL1	GCAGATTGTAAGACGGCGAG	GAGAGGGGGTGTTCCTTCCTT
MLL2	GTGGCTGTTCCACACCCAG	AGCTTGAGCTTCTCAGCATCG
MLL3	TGTTCACAGTGTGGTCAATGTT	GAGGGTCTAGGCAGTAGGTATG
MLL4	GTGGCTGTTCCACACCCAG	AGCTTGAGCTTCTCAGCATCG
WDR5	TTTGAAGATTTGGGACGTGAGTT	ATGGGCAGGCAAAGTCTTGAG
TLR2	GCAAACGCTGTTCTGCTCAG	AGGCGTCTCCCTCTATTGTATT
TLR4	AGCTCCTGACCTTGGTCTTG	CGCAGGGGAACTCAATGAGG
IFN-gamma	ATGAACGCTACACACTGCATC	CCATCCTTTTGCCAGTTCCTC
FAP	GTCACCTGATCGGCAATTTGT	CCCCATTCTGAAGGTCGTAGAT
RbBP5	TGGACAGAACTACCCAGAGGA	CCATCGTTACAGCCAACAGC
CXCL5	TGCGTTGTGTTTGCTTAACCG	CTTCCACCGTAGGGCACTG
LEDGF	CGCCAAGATGAAAGGTTATCCT	GTTTGTTTGTGGGTGGTTTTACA
Ash2L	TATGAACGAGTCTTGTTAGCCCT	CCCTTTGCGTACCCCATGAG

Table S2. Antibodies utilized for FACS analyses

Antigen	**Company**	**Clone**
CD8-a	Biolegend	53-6.7
CD4	Biolegend	GK1.5
CD3-e	Biolegend	145-2c11
CD11B	BD	N1/70
NK1.1	Biolegend	PK136
Ly6C	Biolegend	HK1.4
Ly6G	BD	1A8
Ly6G+Ly6C (GR.1)	BD	RB-8C5
F4/80	Biolegend	BM8
CD206	Biolegend	C068C2
CD11C	Biolegend	N418