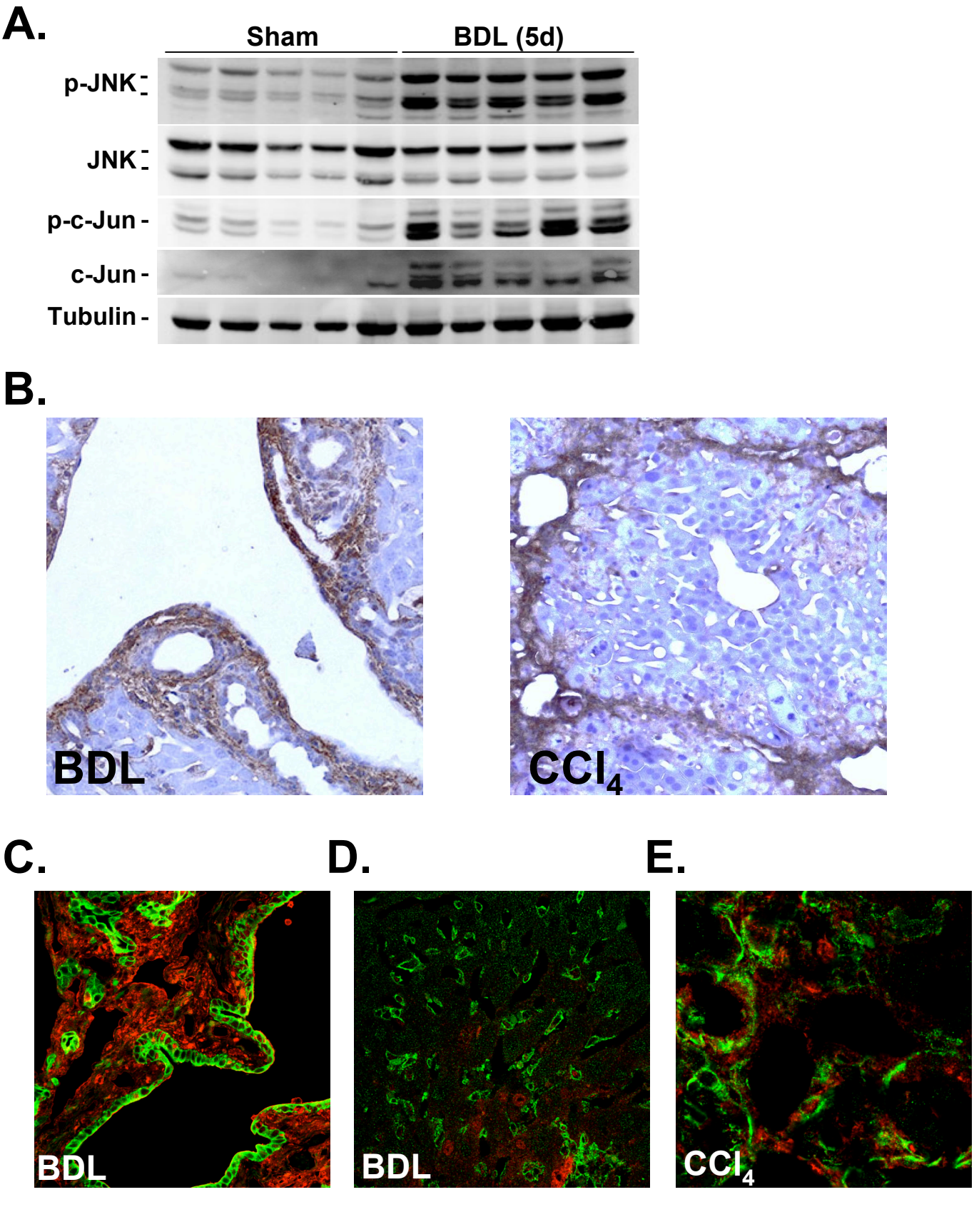
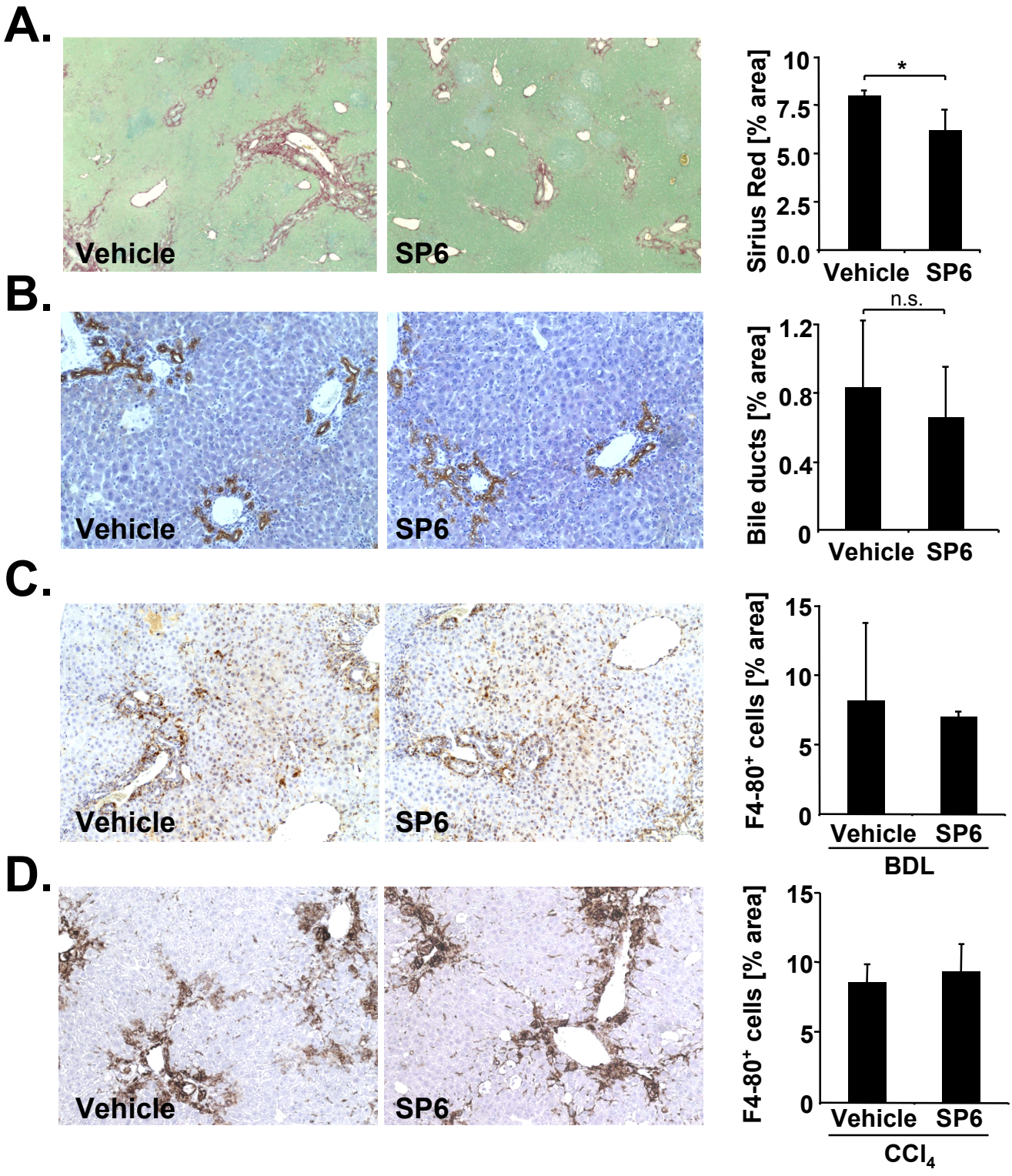
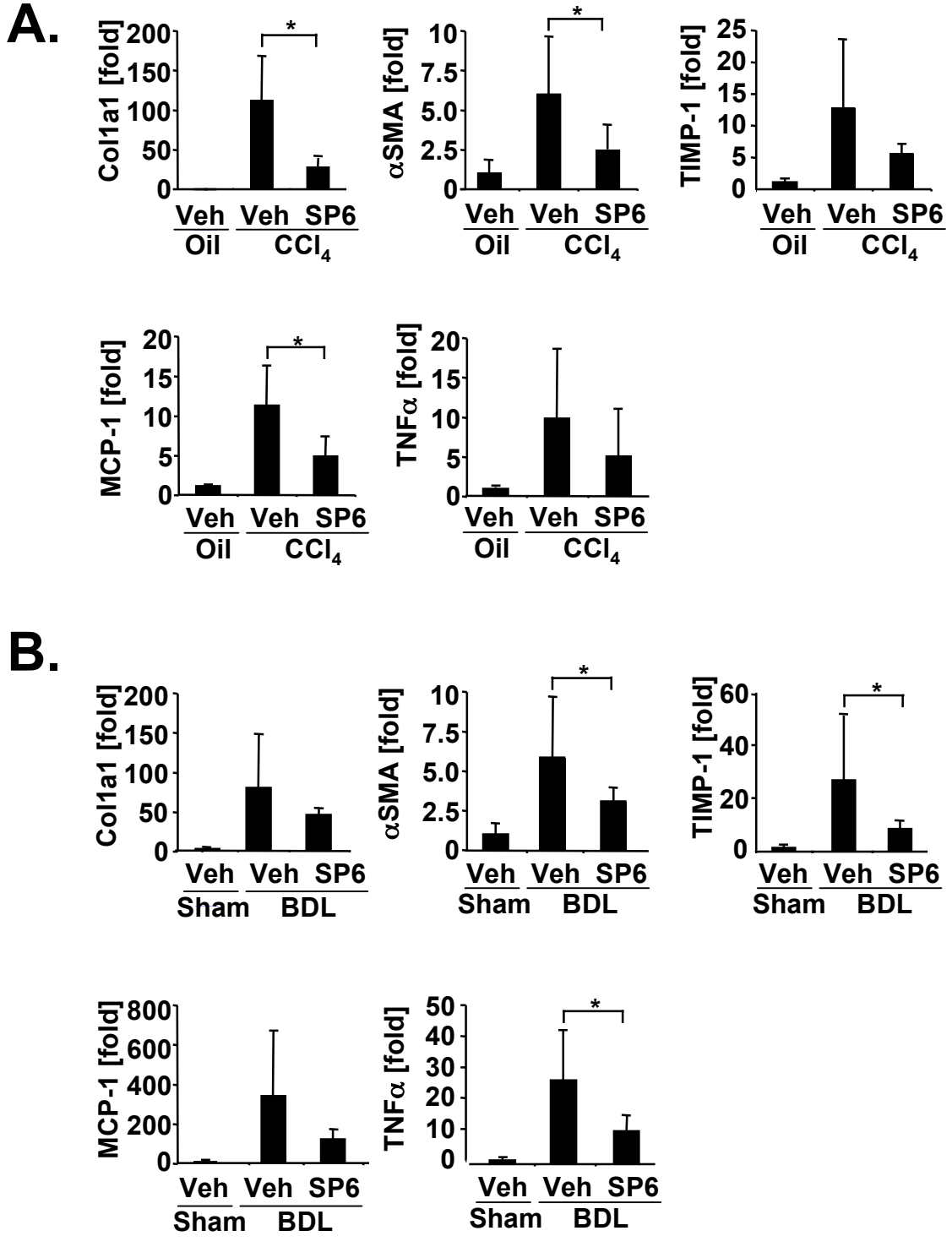

A.
Sham
BDL (5d)
p-JNK
JNK
p-c-Jun
c-Jun
Tubulin
B.
BDL
CCl4
C.
D.
E.
BDL
BDL
CCl4

Suppl. Fig.1

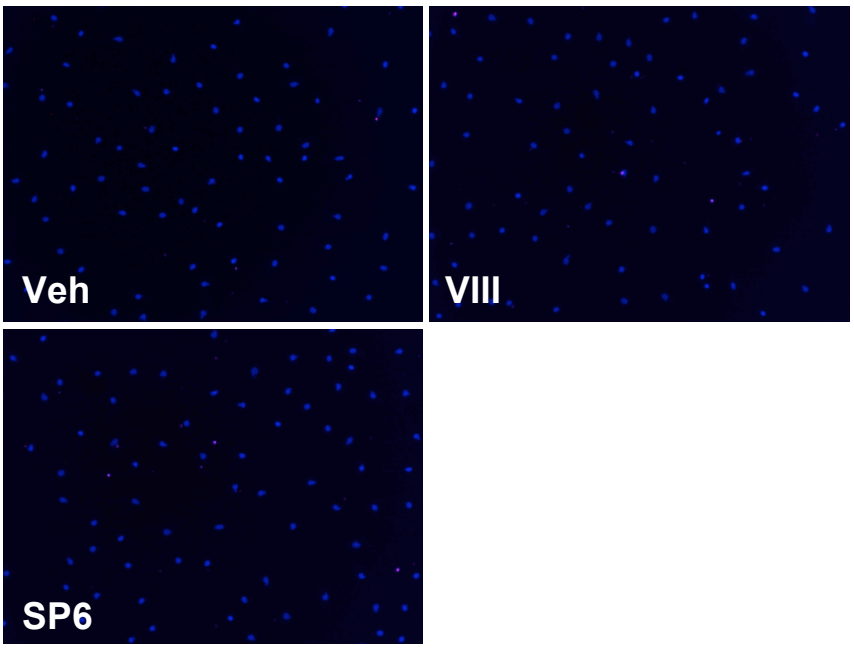

A.
Vehicle
SP6
Sirius Red [% area]
10
7.5
5.0
2.5
0.0
*
Vehicle
SP6
B.
Vehicle
SP6
Bile ducts [% area]
1.2
0.8
0.4
0
n.s.
Vehicle
SP6
C.
Vehicle
SP6
F4-80+ cells [% area]
15
10
5
0
Vehicle
SP6
BDL
D.
Vehicle
SP6
F4-80+ cells [% area]
15
10
5
0
Vehicle
SP6
CCl4

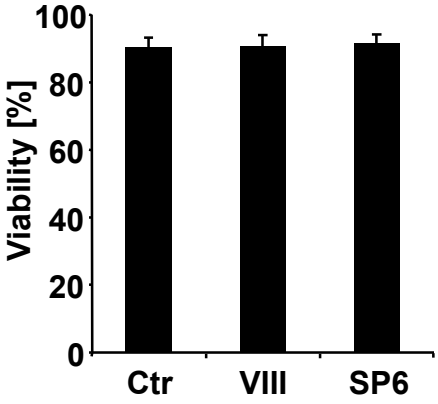
Suppl. Fig.2


A.
Col1a1 [fold]
200
150
100
50
0
*
Veh
Oil
Veh
SP6
CCl4
αSMA [fold]
10
7.5
5.0
2.5
0
TIMP-1 [fold]
25
20
15
10
5
0
MCP-1 [fold]
20
15
10
5
0
TNFα [fold]
B.
Sham
BDL
60
40
20
800
600
400
200
50
40
30
20
10

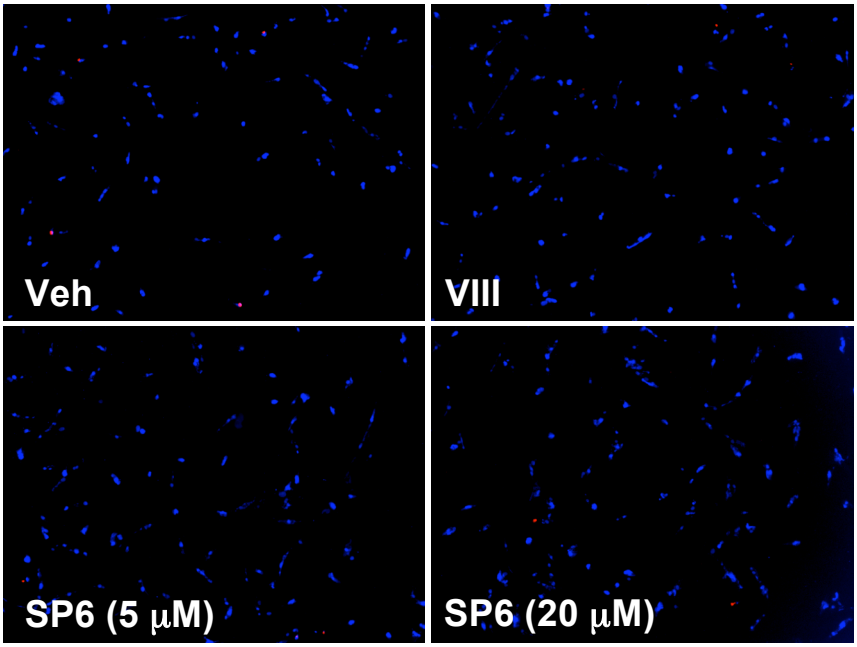
Suppl. Fig.3

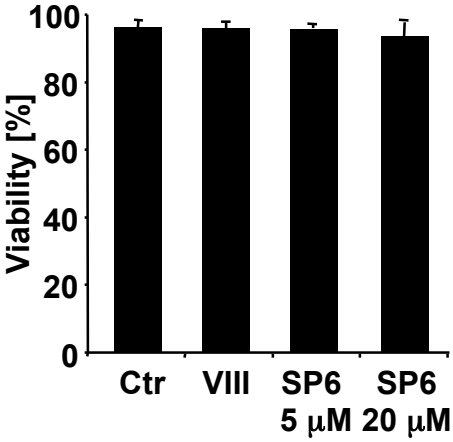
**A.**



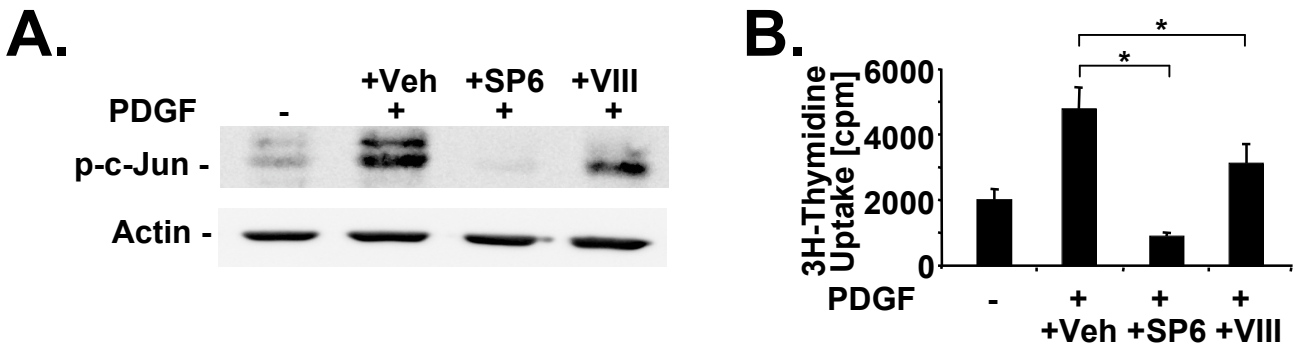




**B.**



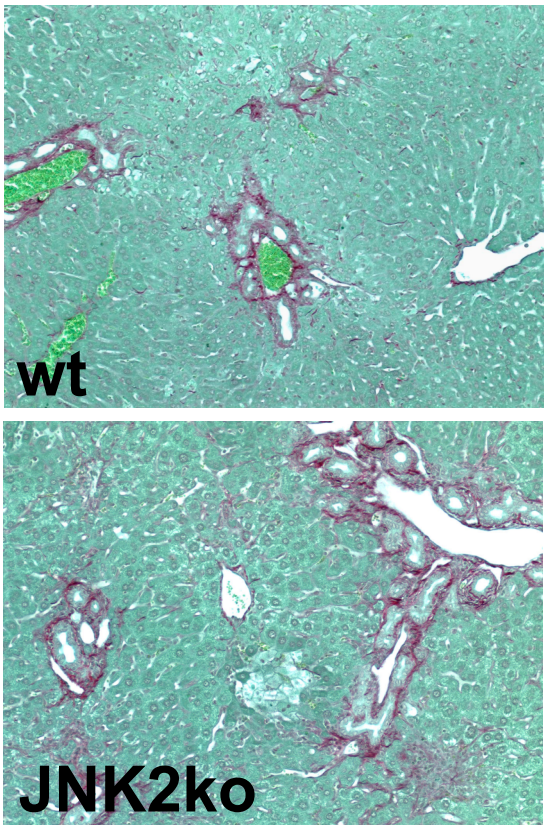



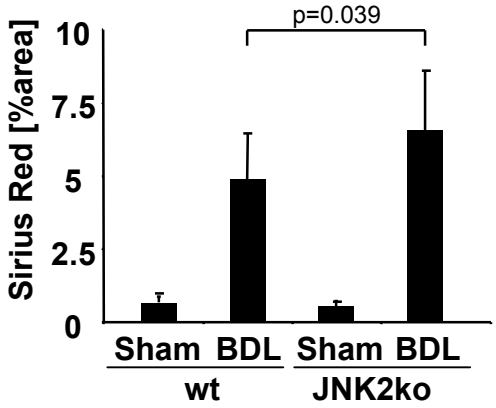

Suppl. Fig.4


A.
PDGF
-
+Veh
+
+SP6
+
+VIII
+
p-c-Jun -
Actin -
B.
3H-Thymidine Uptake [cpm]
6000
4000
2000
0
*
*
PDGF
-
+
+
+
+Veh
+SP6
+VIII

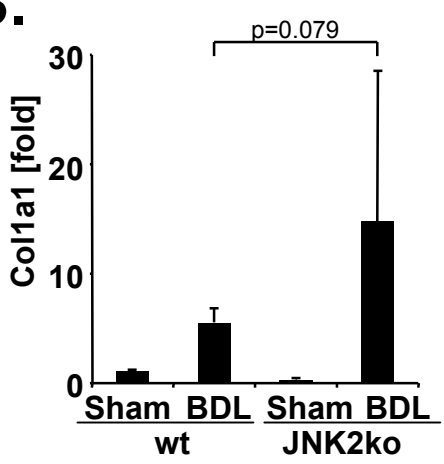
Suppl. Fig.5

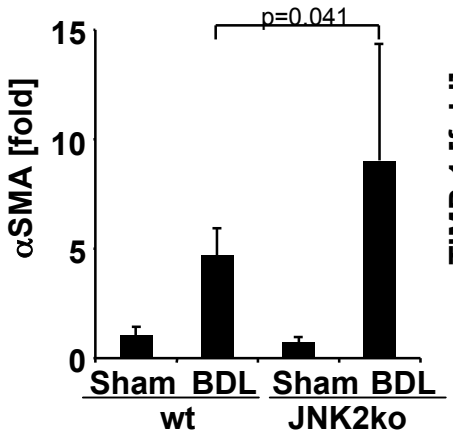
A.

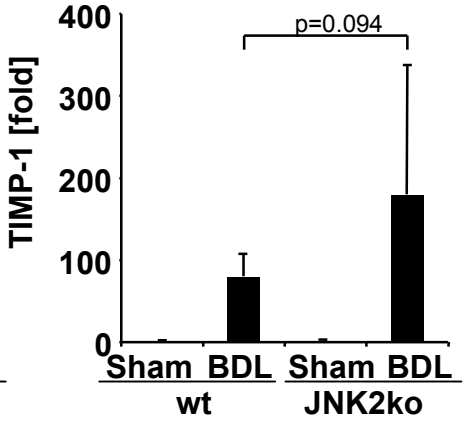


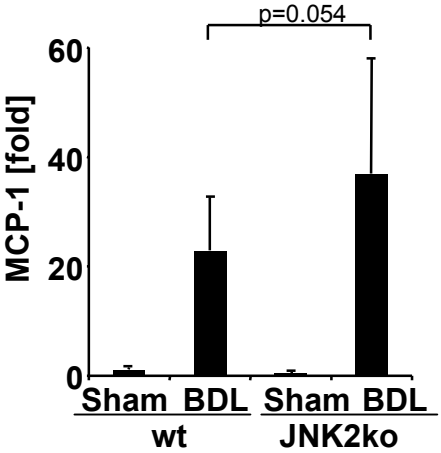



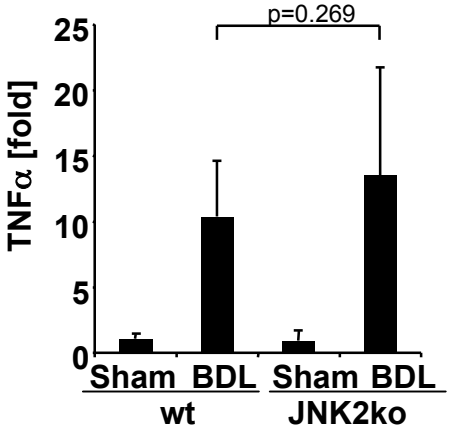

B.



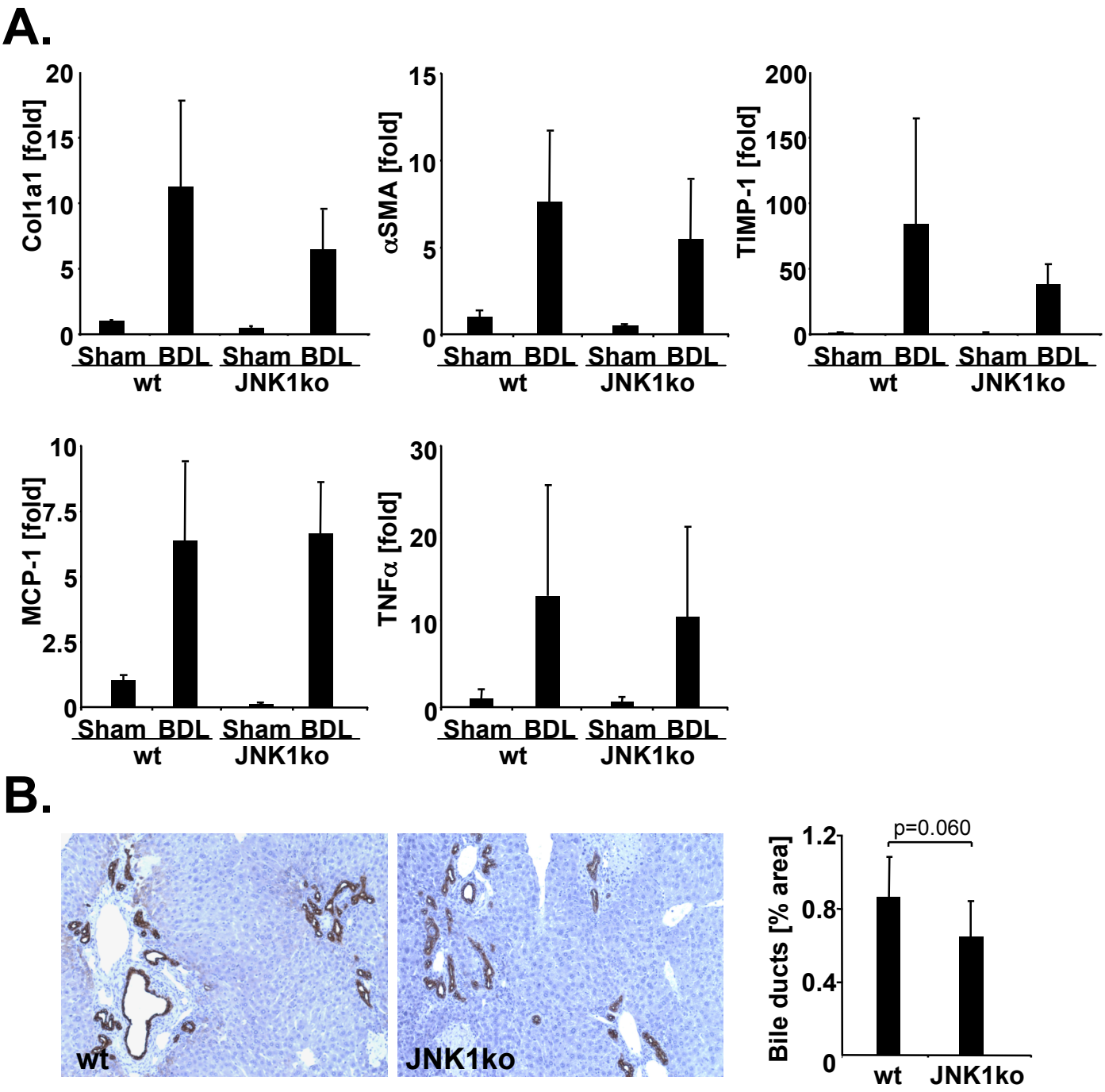



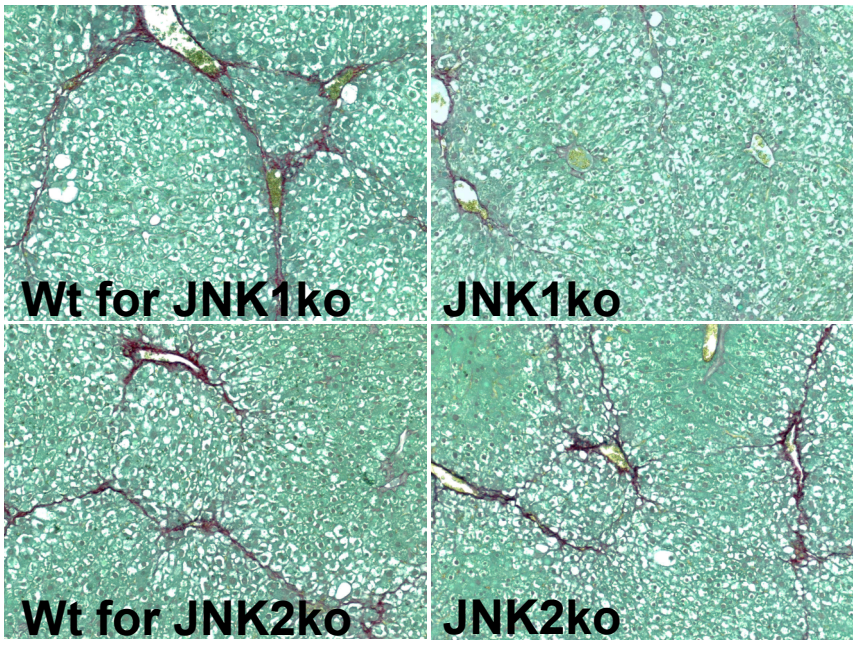



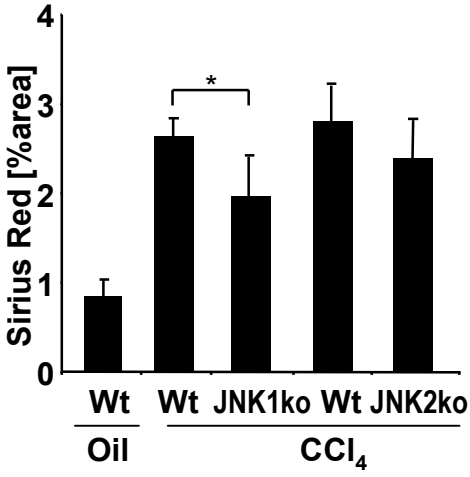

Suppl. Fig.6


A.
Col1a1 [fold]
20
15
10
5
0
Sham BDL Sham BDL
wt JNK1ko
αSMA [fold]
15
10
5
0
Sham BDL Sham BDL
wt JNK1ko
TIMP-1 [fold]
200
150
100
50
0
Sham BDL Sham BDL
wt JNK1ko
MCP-1 [fold]
10
7.5
5
2.5
0
Sham BDL Sham BDL
wt JNK1ko
TNFα [fold]
30
20
10
0
Sham BDL Sham BDL
wt JNK1ko
B.
wt
JNK1ko
Bile ducts [% area]
1.2
0.8
0.4
0
p=0.060
wt JNK1ko

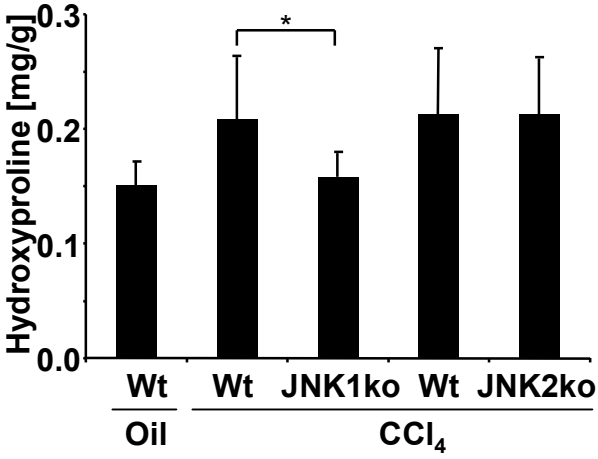
Suppl. Fig.7

**A.**

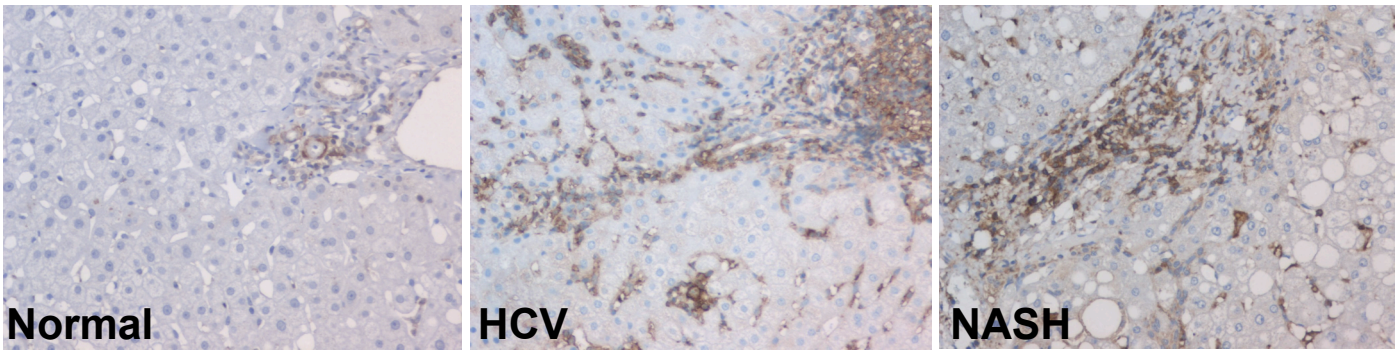


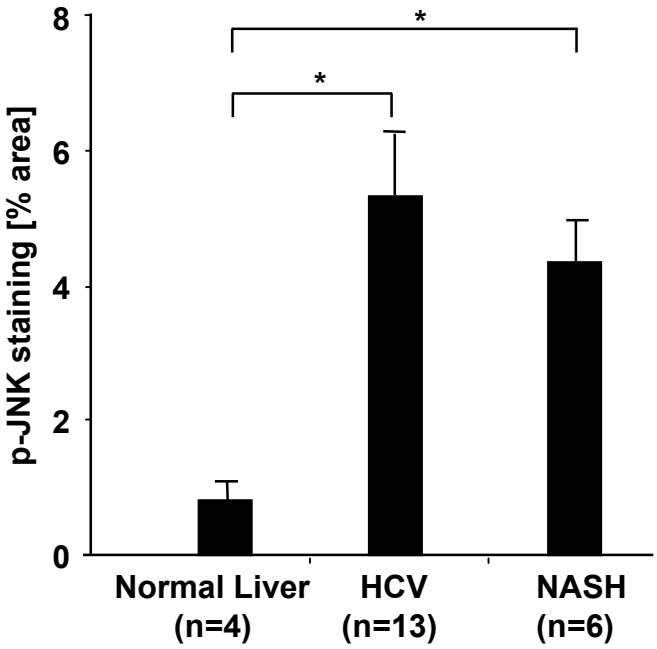




**B.**



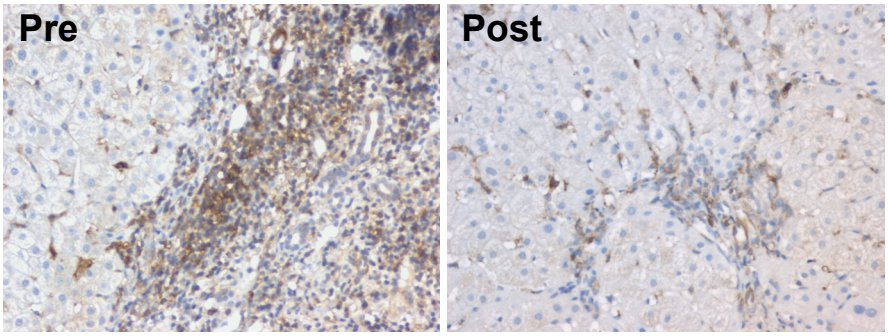

Suppl. Fig.8


Normal
HCV
NASH

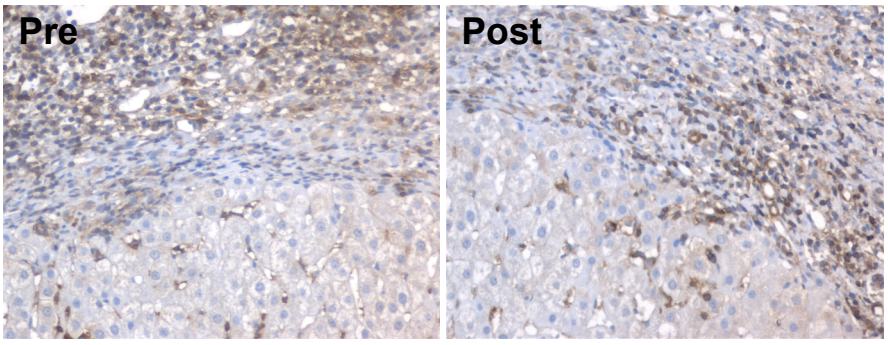

p-JNK staining [% area]
8
6
4
2
0
*
*
Normal Liver
(n=4)
HCV
(n=13)
NASH
(n=6)

Suppl. Fig.9

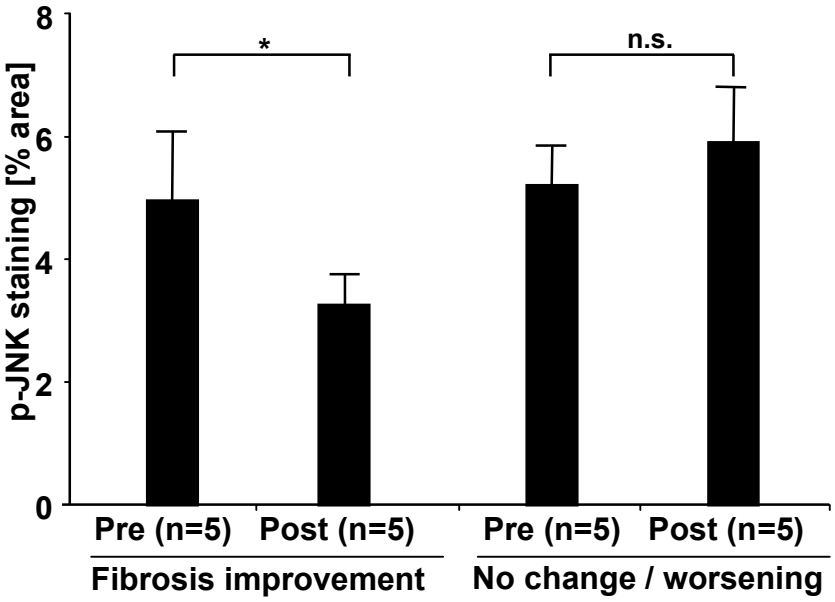
A.




B.




C.




Suppl. Fig.10

## Suppl. Table: Characteristics of the patients included in the study.

| | HCV | NASH |
|---|---|---|
| n | 13 | 8 |
| Age (years) | 55 ( 47 – 59 ) | 49 ( 39 – 64 ) |
| Male (%) | 56 | 37 |
| Body Mass Index ($kg/m^2$) | 25 ( 23 – 27 ) | 30 ( 26 – 38 ) |
| Serum AST (UI/L) | 63 ( 50 – 94 ) | 45 ( 32 – 102 ) |
| Serum ALT (U/L) | 98 ( 70 – 133 ) | 79 ( 58 – 123 ) |
| Serum $\gamma$-GT (U/L) | 62 ( 38 –187 ) | 80 ( 39 –157 ) |
| Serum Bilirubin (mg/dL) | 1.35 ( 0.87 – 2.24 ) | 1.55 ( 0.64 – 1.94 ) |
| INR | 1.25 ( 1.00 –1.58 ) | 1.13 ( 1.00 – 1.34 ) |
| Platelets ($*10^9/L$) | 155 ( 127 – 208 ) | 176 ( 137 – 268 ) |
| Metavir scale (1-2/3-4) | 54/46 | 63/37 |
| Serum HCV-RNA ($UI/mL*10^6$) | 2.48 (1.67 – 5.15) | n.a. |

**Supplementary Fig. 1. JNK activation in murine liver fibrosis. A-B.** Balb/c mice underwent BDL or sham operation for 5 days, JNK activation was determined by immunoblotting of phospho-c-Jun or p-JNK. **B.** Balb/c mice underwent BDL for 3 weeks (left panel) or 8 injections of $CCl_4$ (right panel). pJNK expression was determined by immunohistochemistry (B). **C-E.** Balb/c mice underwent BDL for 3 weeks (C, D) or 8 injections of $CCl_4$ (E). Livers were stained for keratin 8 (green) and p-JNK (red) (C.), or F4/80 (green) and p-JNK (red) (D-E).

**Supplementary Fig. 2. Effect of pharmacologic JNK inhibition of hepatic fibrosis, bile duct proliferation and macrophage infiltration. A.** Male Balb/c mice underwent BDL for 15 days, and were treated with either 0.5 mg SP600125 twice daily (n=8) or DMSO vehicle (n=7). Hepatic fibrosis was evaluated by Sirius Red staining and subsequent quantification of the Sirius red positive area. **B.** Bile duct proliferation was assessed in SP600125-treated and control mice (1 mg SP600125 (n=7) or vehicle (n=10) twice daily) two weeks after BDL. **C-D.** Infiltration of F4/80-positive macrophages was determined by immunohistochemistry in SP600125-treated (n=7) or vehicle-treated mice (n=10) after BDL (C), and in SP600125-treated (n=9) and vehicle-treated mice (n=9) after 4 gavages of $CCl_4$ (D). * $p<0.05$, n.s. = non-significant

**Supplementary Fig. 3 Effect of pharmacologic JNK inhibition on liver fibrosis and inflammation. A.** Balb/c mice treated with SP600125 (1mg i.p. twice daily, n=9) or

vehicle (n=9) received 4 gavages of $CCl_4$. Expression of Col1a1, αSMA, TIMP-1, MCP-1 and TNFα was determined by qPCR. **B.** Balb/c mice treated with SP600125 (1 mg twice daily, n=7) or vehicle (n=10) twice daily) underwent after BDL for two weeks. Expression of Col1a1, αSMA, TIMP-1, MCP-1 and TNFα was determined by qPCR. * $p<0.05$

**Supplementary Fig. 4. Pharmacologic JNK inhibition does not reduce HSC viability. A.** Mouse HSCs (d1 activated) were serum starved for 24h followed by treatment with JNK inhibitor VIII (16 μM), SP600125 (5 μM) or vehicle (0.1% DMSO) for additional 48h. Cells were incubated with Hoechst 33342 to stain all cells and propidium iodide to identify dead cells. Similar results were obtained with mouse d5 activated HSCs (data not shown). **B.** Human HSCs (passage 3) were serum starved for 24h followed by treatment with JNK inhibitor VIII (16 μM), SP600125 (5 μM or 20 μM) or vehicle (0.1% DMSO) for additional 24h. Cell viability was determined as described above.

**Supplementary Fig. 5. Pharmacologic JNK inhibition decreases PDGF-induced proliferation. A-B.** HSCs were treated with JNK inhibitor as described above followed by PDGF (20 ng/ml) treatment. c-Jun phosphorylation (A) and [$^3$H]-thymidine incorporation (B) were determined 15 minutes and 26h after PDGF treatment, respectively. * $p<0.05$

**Supplementary Fig.6. Effect of JNK2 deficiency on BDL-induced liver fibrosis and inflammation. A-B.** JNK2-deficient mice (n=11) and wild-type mice (n=8) underwent

BDL for 3 weeks followed by quantification of Sirius Red (A), and qPCR for Col1a1, αSMA, TIMP-1, MCP-1 and TNFα (B.).

**Supplementary Fig. 7. Effect of JNK1 deficiency on BDL-induced liver fibrosis , inflammation and bile duct proliferation.** JNK1-deficient mice (n=10) and wild-type mice (n=7) underwent BDL for 3 weeks. **A.** Col1a1, αSMA, TIMP-1, MCP-1 and TNFα were determined by qPCR. **B.** Bile duct proliferation was assessed by keratin staining.

**Supplementary Fig. 8. JNK1 deficiency reduces $CCl_4$-induced liver fibrosis. A-B.** JNK1-deficient mice (n=7) and matching wild-type control mice (n=5) as well as JNK2-deficient mice (n=10) and matching wild-type control mice received 6 gavages of $CCl_4$ (0.5 μl/g). Hepatic fibrosis was evaluated by Sirius Red staining (A) and hydroxyproline measurement (B). * $p<0.05$

**Supplementary Fig. 9. Phospho-JNK expression is increased in human liver fibrosis.** **S**ections of fibrotic human liver samples from healthy individuals (n=4), patients with chronic HCV (n=13) or NASH (n=6) were stained for p-JNK. Shown are representative images, and morphometric analysis of p-JNK staining. * $p<0.05$

**Supplementary Fig. 10. Reduced hepatic p-JNK expression in chronic hepatitis C patients responding to losartan. A-B.** Patients with chronic hepatitis C were treated

with the angiotensin type 1 receptor blocker losartan (50 mg/day for 18 months). p-JNK expression was assessed before and after losartan treatement by immunohistochemistry in patients that either responded to losartan with a decrease in fibrosis (n=5) or did not respond (n=5). Shown are representative images of p-JNK in the liver from a losartan responder before ("pre") and after ("post") losartan treatment (A), and respective images from a non-responder (B), as well as morphometric analysis of p-JNK staining. * $p<0.05$, n.s. = non-significant.

**Supplementary Table 1. Characteristics of patients with chronic hepatitis and NASH.** Described are the patient samples used for p-JNK immunohistochemistry and confocal immunofluorescence. Results are expressed as median (95% CI) or percentage. Patients for the losartan intervention study have been described previously (Colmenero et al. 2009).