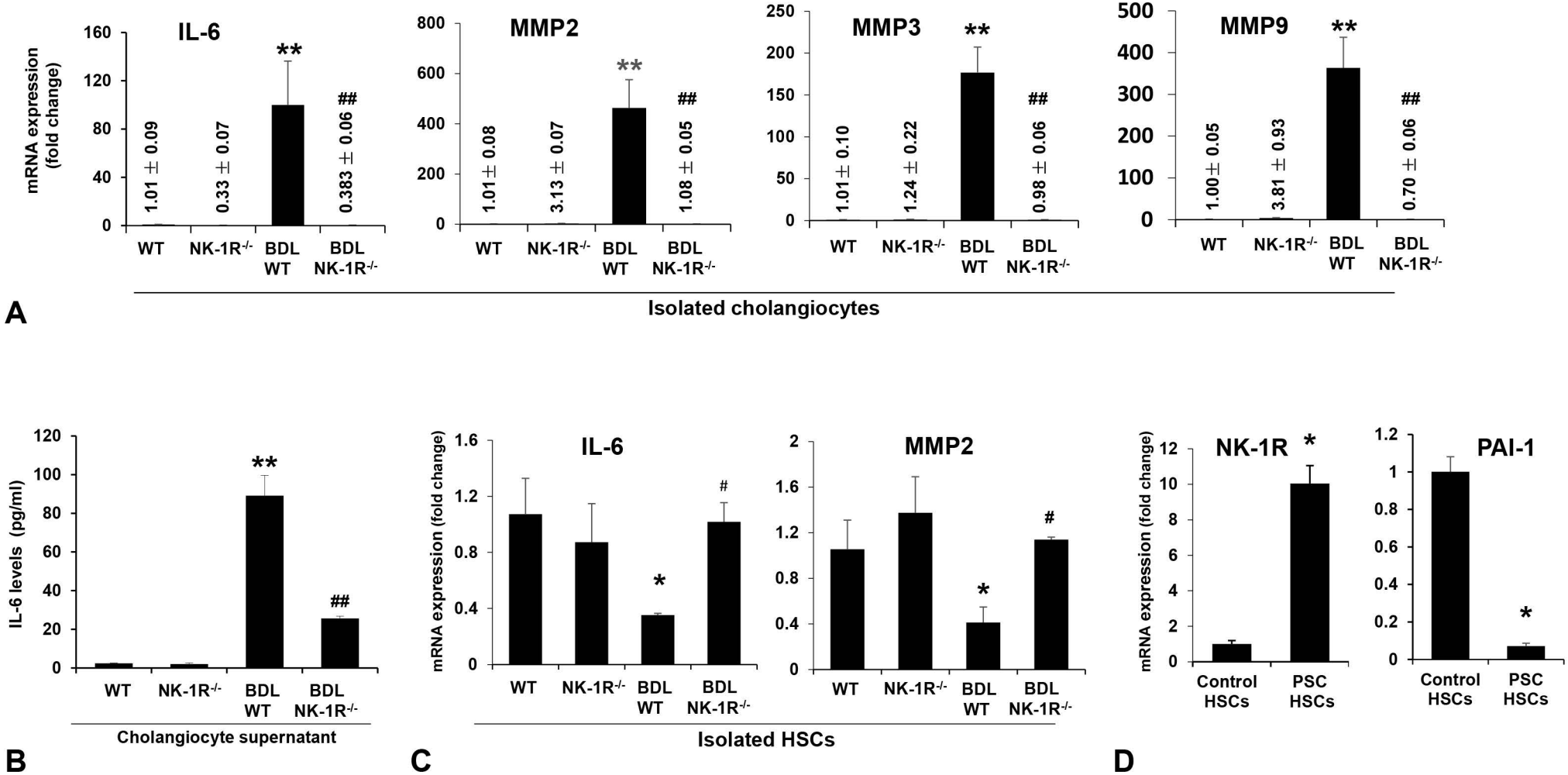
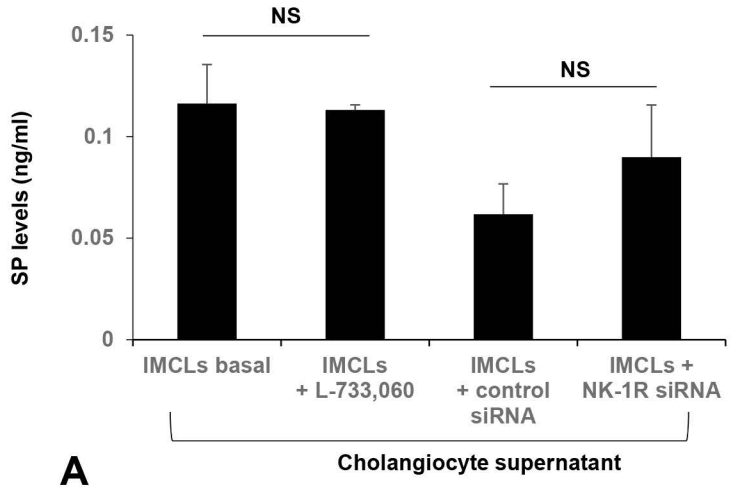
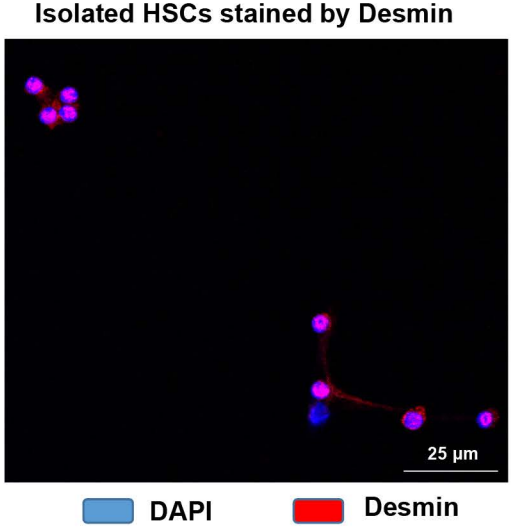
# Supplemental figure 1



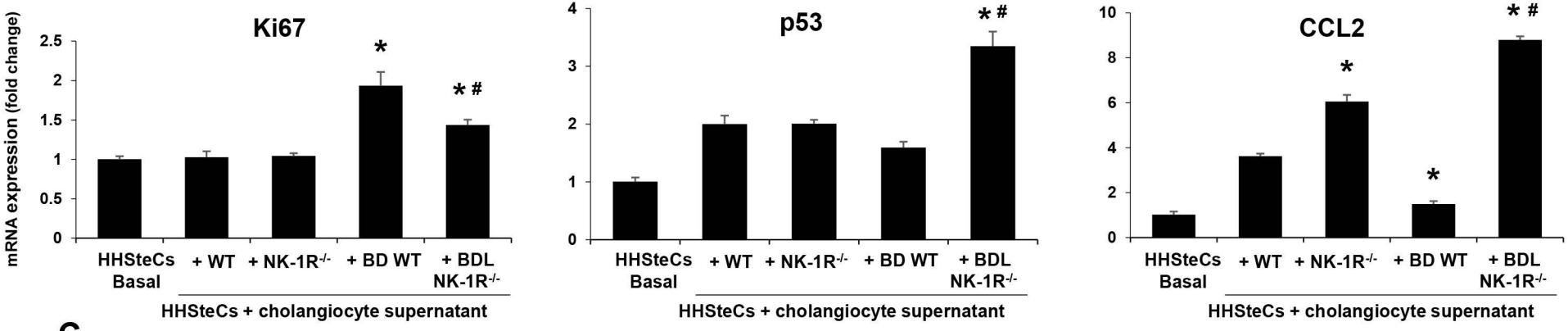
# Supplemental figure 2




A




B




C

**Supplementary information**

**Supplementary Figure legends**

**Supplementary Figure 1** **[A-C]** There was reduced expression of IL-6, MMP2, MMP3 and MMP9 in cholangiocytes as well as IL-6 levels in cholangiocyte supernatant (n=6) but increased expression of IL-6 and MMP2 in HSCs (n=3) from BDL NK-1R$^{-/-}$ mice compared to BDL WT mice. **[D]** There was enhanced expression of NK-1R and decrease expression of PAI-1 in LCM-isolated HSCs from late stage PSC patients compared to control samples (n = 3). *$P$<0.05, **$P$<0.01 vs. normal WT mice or control HSCs. $^{\#}P$＜0.05, $^{\#\#}P$＜0.01 vs. BDL WT mice.

**Supplementary Figure 2** **[A]** No significant changes in SP levels were observed in the supernatant of IMCLs treated with L-733,060 compared to basal and in IMCLs transfected with NK-1R siRNA compared to IMCLs transfected with control siRNA. **[B]** The isolated HSCs from FVB/NJ normal WT mice was confirmed by staining with α-SMA. (Magnification, ×63). **[C]** There was decreased expression of Ki67 but increased expression of p53 and CCL2 in HHSteCs treated with cholangiocyte supernatant from BDL NK-1R$^{-/-}$ mice compared to HHSteCs treated with cholangiocyte supernatant from BDL WT mice. *$P$<0.05 vs. HHSteCs treated with cholangiocyte supernatant from normal WT mice. $^{\#}P$＜0.05 vs. HHSteCs treated with cholangiocyte supernatant from BDL WT mice.

**Supplementary Table 1. Characteristics of Healthy Controls and PSC Patients**

| Groups | Patient | Diagnosis | Sample | Gender | Cirrhosis | Therapy | Origin |
|---|---|---|---|---|---|---|---|
| **Control** | 1 | Normal Liver | RNA | Male | | Untreated | BioChain |
| | 2 | Normal Liver | RNA | Male | | Untreated | BioChain |
| | 3 | Normal Liver | RNA | Male | | Untreated | BioChain |
| **PSC** | 1 | Late stage PSC | RNA from paraffin sections | Male | Yes | Untreated | Humanitas Research Hospital |
| | 2 | Late stage PSC | RNA from paraffin sections | Male | No | Untreated | Humanitas Research Hospital |
| | 3 | Late stage PSC | RNA from paraffin sections | Male | No | Untreated | Humanitas Research Hospital |
| **Control** | 1 | Normal Liver | Serum | Male | | | Humanitas Research Hospital |
| | 2 | Normal Liver | Serum | Male | | | Humanitas Research Hospital |
| | 3 | Normal Liver | Serum | Male | | | Humanitas Research Hospital |
| **Early stage PSC** | 1 | Early stage PSC | Serum | Male | No | Untreated | Humanitas Research Hospital |
| | 2 | Early stage PSC | Serum | Male | No | Untreated | Humanitas Research Hospital |
| | 3 | Early stage PSC | Serum | Male | No | Untreated | Humanitas Research Hospital |
| **Late stage PSC** | 1 | Late stage PSC | Serum | Male | No | Untreated | Humanitas Research Hospital |
| | 2 | Late stage PSC | Serum | Male | No | Untreated | Humanitas Research Hospital |
| | 3 | Late stage PSC | Serum | Male | No | Untreated | Humanitas Research Hospital |

PSC = Primary Sclerosing Cholangitis. Unidentified human samples were obtained from Dr. P. Invernizzi (Humanitas Research Hospital, Rozzano, Italy).

**Supplementary Table 2. Evaluation of serum levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and bilirubin**

| Groups | Alanine Aminotransferase, units/L | Aspartate Aminotransferase, Units/L | Alkaline Phosphatase, Units/L | Total Bilirubin, mg/dL |
|---|---|---|---|---|
| FVB/NJ WT (n=14) | 144.00±24.61 | 379.00±71.81 | 86.29±7.53 | 0.16±0.02 |
| $Mdr2^{-/-}$ (n=28) | 454.43±31.36* | 608.00±51.37* | 305.00±21.63* | 0.23±0.02* |
| $Mdr2^{-/-}$ + L-733,060 (n=6) | 142.33±14.19# | 307.50±47.51# | 199.00±22.30*# | 0.13±0.03# |

* $P<0.05$ vs. FVB/NJ WT; # $P<0.05$ vs. $Mdr2^{-/-}$.

**Supplementary Table 3. List of commercially available real-time PCR primers used**

| Gene | Species | Detected transcript | Source |
|---|---|---|---|
| ACTA2(α-SMA) | Mouse | NM_007392 | QIAGEN |
| CCL2 | Mouse | NM_011333 | QIAGEN |
| Cdkn1a (p21) | Mouse | NM_001111099 | QIAGEN |
| Cdkn2a (p16) | Mouse | NM_001040654 | QIAGEN |
| Col1α1 | Mouse | NM_007742 | QIAGEN |
| Fn-1 | Mouse | NM_010233 | QIAGEN |
| GAPDH | Mouse | NM_008084 | QIAGEN |
| IL-6 | Mouse | NM_031168 | QIAGEN |
| MMP2 | Mouse | NM_008610 | QIAGEN |
| MMP3 | Mouse | NM_010809 | QIAGEN |
| MMP9 | Mouse | NM_013599 | QIAGEN |
| TAC1 | Mouse | NM_009311 | QIAGEN |
| TACR1 (NK-1R) | Mouse | NM_009313 | QIAGEN |
| TGF-β1 | Mouse | NM_011577 | QIAGEN |
| ACTA2(α-SMA) | Human | NM_001141945 | QIAGEN |
| CCL2 | Human | NM_002982 | QIAGEN |
| Cdkn1a (p21) | Human | NM_000389 | QIAGEN |
| Cdkn2a (p16) | Human | NM_000077 | QIAGEN |
| Col1α1 | Human | NM_000088 | QIAGEN |
| GAPDH | Human | NM_001256799 | QIAGEN |
| Ki67 | Human | NM_001145966 | QIAGEN |
| p53 | Human | NM_000546 | QIAGEN |
| Serpine 1(PAI-1) | Human | NM_000602 | QIAGEN |
| Smad2 | Human | NM_001003652 | QIAGEN |
| TAC1 | Human | NM_003182 | QIAGEN |
| TACR1 (NK-1R) | Human | NM_001058 | QIAGEN |
| TGF-β1 | Human | NM_000660 | QIAGEN |

**Supplementary Materials and methods**

***Materials***

Reagents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) unless otherwise indicated. The rabbit polyclonal antibody against NK-1R was purchased from Thermo Fisher Scientific (Rockford, IL). The following were purchased from Abcam (Burlingame, CA): (i) rabbit polyclonal antibodies against monocyte chemoattractant protein-1 (MCP-1/CCL2), a-SMA and fibronectin-1 (Fn-1); and (ii) rabbit monoclonal antibodies against desmin (a marker of HSCs) [(1)] and cytokeratin-19 (CK-19). Mouse antibodies against p16 and p21 were purchased from Santa Cruz Biotechnology (Dallas, TX). ELISA kits to measure transforming growth factor-b1 (TGF-b1) levels were purchased from Affymetrix Inc. (Santa Clara, CA). Mouse IL-6 ELISA kit was purchased from BD Bioscience (San Jose, CA). Synthetic SP and EIA kits (for detecting SP levels) were purchased from Phoenix Pharmaceuticals (Belmont, CA). The NK-1R antagonist (L-733,060) [(2)] was purchased from Tocris Bioscience (Minneapolis, MN). The RNeasy Mini Kit for RNA purification and all selected primers were purchased from Qiagen (Valencia, CA). The iScript cDNA Synthesis Kit (170-8891) and iTaq Universal SYBR Green Supermix (172-5124) were purchased from Bio-Rad (Hercules, CA). All of primers for real-time PCR were purchased from Qiagen (Valencia, CA) and specific information about the primers is listed in Supplementary Table 3.

**Supplementary references**

1. Puche JE, Lee YA, Jiao J, Aloman C, Fiel MI, Munoz U, Kraus T, et al. A novel murine model to deplete hepatic stellate cells uncovers their role in amplifying liver damage in mice. Hepatology 2013;57:339-350.

2. **Meng F, DeMorrow S,** Venter J, Frampton G, Han Y, Francis H, Standeford H, et al. Overexpression of membrane metalloendopeptidase inhibits substance P stimulation of cholangiocarcinoma growth. Am J Physiol Gastrointest Liver Physiol 2014;306:G759-768.