Legends to Supplementary Figures

Suppl. Figure 1 *Impaired anti-bacterial immune responses in fibrotic liver*

(A, B) Liver immunohistochemistry two weeks after sham- or BDL-operation (A) or after 12 weeks of CCl_4 treatment (B). Top H&E, Bottom sirius red staining for collagen fibers (F3B). **(C) (F)** Time kinetic of ALT and AST in the serum of mice treated as in (A)**. (D-F)** ELISA for IL-6 and TNF (D), CRP (E) and albumin (F) in the serum of oil or CCl_4 treated mice at the indicated time points after infection.

Suppl. Figure 2 *Innate immune functions of myeloid cells against bacterial infection during liver fibrosis*

(A) Flow cytometric analysis of macrophages and dendritic cells in livers of sham or BDL-operated mice on day 3 p.i. with *L.m*. **(B)** Total numbers of monocytes, granulocytes, macrophages and dendritic cells in livers of oil or CCl_4 treated mice on day 3 p.i. with *L.m*. **(C) (C)** Phagocytosis of viable *L.m*. and **(F)** killing of intracellular *L.m*. *in vitro* by Kupffer cells and monocytes isolated from oil or CCl_4 treated mice. **(E-H)** IL-12p35 and IFNγ mRNA expression in the spleen **(E,G)** and protein levels in the blood **(F,H)** of sham or BDL-operated mice at the indicated time points p.i. with *L.m*.

Suppl. Figure 3 *Elevated type I interferon during liver fibrosis induces IL-10 expression after infection with L. monocytogenes*

(A) Liver immunofluorescence staining showing nuclear Mx1 expression and quantification of Mx1 positive cells two weeks after sham (left) or BDL-operation (right). (**B**) Quantification of (A).

Suppl. Figure 4 *Translocation of gut microbiota triggers hepatic IFN expression during liver fibrosis*

(A, B) Bacterial counts (colony forming units -CFU) in portal venous blood (A) or liver tissue (B) at d10 after sham or BDL-operation. **(C, D)** Immunofluorescence staining for F4/80 and collagen **(C)** and quantification **(D)** of collagen type IV fluorescence in the liver two or three weeks after sham or BDL-operation of SPF or GF mice, respectively. **(E)** *In vitro* killing assay of phagocytosed *L.m*. by $CD11b^+$ cells isolated from the liver of sham or BDL-operated SPF or GF mice.

Suppl. Figure 5 *Enhanced IL-10 expression after infection with pathogenic intracellular bacteria in myeloid cells from fibrotic mice or humans with liver cirrhosis*

(A) Time kinetics of IFNβ mRNA expression in the spleen of sham or BDL-operated mice. **(B)** IL-10 mRNA expression relative to GAPDH in spleen of the mice in (A). **(C)** IL-10 protein ELISA in supernatants of liver $CD11b^+$ myeloid cells from sham or BDL-operated mice treated for 48 hrs with 200 U/ml of recombinant IFNβ before. *in vitro* infection with *L.m*.

Suppl. Figure 6 *Mice deficient for IFNAR signaling are protected from infection-associated mortality during liver fibrosis.*

(A, B) IL-12p35 and IFNγ mRNA expression in the liver of sham or BDL-operated wild type and $IFNAR^{-/-}$ mice at the indicated time points after *L.m*. infection. **(C)** *In vitro* killing assay of *L.m.* by $CD11b^+$ cells isolated from the liver of sham or BDL-operated wild type and $IFNAR^{-/-}$ mice. **(D)** Kaplan-Meier survival curve for sham or BDL-operated LysMcre-$IFNAR^{flox/flox}$ (LysM-Cre^+) mice or littermates (LysM-Cre^-) after *L.m*. infection ($5x10^3$); n = 10 mice per group. **(E)** *L.m.* burden in the spleen of sham or BDL-operated LysMcre-$IFNAR^{flox/flox}$ (LysM-Cre^+) mice or littermates (LysM-Cre^-) after *L.m*. infection ($5x10^3$). **(D)** Time kinetic of granulocytes number in the blood of sham or BDL-operated wild type and $IFNAR^{-/-}$ mice at the indicated time points. **(G)** ELISA of GM-CSF in the blood of sham or BDL-operated wild type mice at the indicated time points after *L.m*. infection. **(H)** CCL5, CCL2 and CXCL2 mRNA expression in the live of mice in (G). **(I)** Number of $Ly6G^+$ granulocytes in the bone marrow of mice in (D).

Suppl. Figure 7 *No change in liver damage in the absence of IFNAR or IL-10R signaling*

(A) Liver immunohistochemistry of BDL-operated mice treated with anti-IFNAR1, anti-IL-10R blocking antibody or isotype. Top α-SMA, Bottom sirius red staining for collagen fibers (F3B). **(B)** Western Blot analysis of liver tissue of mice in (A). **(C)** ALT levels in the serum of BDL-operated mice anti-IFNAR1, anti-IL-10R blocking antibody or isotype at the indicated time points after *L.m*. infection.

Supplementary material and methods

Isolation of liver cell populations

Murine livers were perfused via the portal vein with a 0.05% collagenase solution, mechanically disrupted and digested for 20 min at 37 °C in Gey's balanced salt solution (GBSS) with 0.04% collagenase, then filtered through a 250-µm cell strainer. Liver cells were resuspended in 10 ml PBS and separated by 25% and 50% Percoll gradient centrifugation for 30 min at 1,350 x *g* at 4°C. After centrifugation, cells were collected from the interface and subjected to flow cytometric analysis or cell sorting.

Isolation of human monocytes from healthy donors or patients with liver cirrhosis

Blood mononuclear cells were prepared from the peripheral blood of healthy donors and cirrhosis patients using Ficoll (Amersham Biosciences). Monocytes were isolated by autoMACS separation using the Pan Monocyte Isolation Kit (Miltenyi).

Reagents and flow cytometry

Antibody staining was performed in presence of an Fc-block (10 µg/ml, 2.4G2) in flow cytometry buffer. Data were acquired on a CantoII or LSRFortessa (BD Biosciences) and analyzed with FlowJo sofware (TreeStar Inc.). To exclude dead cells, Hoechst 33258 (10 µg/ml, Sigma-Aldrich) or LIVE/DEAD Fixable Dead Cell Stain Kit (Invitrogen) was used. All antibodies were obtained from eBioscience if not otherwise indicated: PerCp-Cy5.5 or phycoerythrin (PE)-conjugated anti-CD11b (M1/70), PE-conjugated anti-NKp46 (29A1.4), Alexa Fluor 647-, 488- or 405-conjugated anti-mouse CD146 (ME-9F1), allophycocyanin-conjugated anti-Ly6C (HK1.4), APC-eFluor 780- or PE-Cy5-conjugated anti-F4/80 (BM8), phycoerythrin-conjugated anti-Ly6G (1A8; BioLegend), allophycocyanin- or Alexa Fluor 488-conjugated anti-CD11C (N418). For flow-sorting $CD11b^-$ $CD146^+$ (LSEC), $CD11b^+$ $Ly6G^+$ (granulocytes), $CD11b^+$ $Ly6G^-Ly6C^+$ (monocytes) or $CD11b^+$ $Ly6G^-$ $Ly6C^-$ $F4/80^+$ (macrophages) liver cells were isolated as described above, stained and filtered through a 100-µm mesh after adding Hoechst 33258 to exclude dead cells and subjected to cell sorting using a FACSAria III sorter (BD Biosciences). Anti-Mx1

polyclonal antibody was kindly provided by Dr. Peter Staehli (Freiburg; Germany). Production of IL-1β, IL-10, IL-6, TNF, CRP and albumin in cell culture supernatants or serum was analyzed using ELISA kits from eBiosciences and R&D Systems, respectively.

Serum alanine and aspartate aminotransferase (ALT, AST) determination

Serum ALT and AST were analyzed from whole blood using ALT, AST strips from Roche according to the manufacturer's instructions. Measurement was performed in a Reflovet machine from SCIL animal care. Values above assay range were diluted prior to measurement.

Measurement of reactive oxygen species (ROS)

ROS production was assessed by luminol-enhanced chemiluminescence as previously described [1] 40,000 cells per well were placed in a 96-well luminometer plate (Costar). Subsequently, 100 µl medium containing 100 µM luminol (Fluka) with or without heat-killed *L. monocytogenes* (HKLM) and laminarin at the indicated concentrations was added. Chemiluminescence was measured at 5-min intervals. Luminescence is expressed as relative luciferase units/minute/1000 cells.

Endotoxin quantification

Blood from healthy donors or cirrhosis patients was collected in pyrogen-free tubes. Plasma was stored at -80°C until analysis and endotoxin concentration was measured using an Endpoint Chromogenic LAL Assay according to the manufacturer's manual (Lonza).

Blockade of IL-10R and IFNAR signaling *in vivo*

To block signaling downstream of IL-10 receptors, mice were injected i.v. with 250µg of anti-IL-10R monoclonal antibody (clone 1B1.3; BioXcell, USA) or isotype-specific immunoglobulin as control each other day over a period of 6 days before *L. monocytogenes* infection. To block signaling downstream of IFNAR, mice were injected i.v. with 250µg of anti-IFNAR1 antibody (clone MAR1-5A3; Leinco Technologies, St. Louis MO) or isotype-specific immunoglobulin as

control each other day starting from day 4 post BDL and continued during *L. monocytogenes* infection.

Immunohistochemistry and Immunofluorescence staining

Preparation of liver sections for immunohistochemistry was described previously [2]. Paraffin-embedded sections (2–3µm) were stained with hematoxylin-eosin (H&E) to detect collagen fibers paraffin-embedded sections (2–3µm) were treated with 0.1% Sirius-red F3B in saturated picric acid (Chroma, Münster, Germany). Histological stainings were digitalized using Panoramic MIDI (3DHistech, Budapest, Hungary). For immunofluorescence staining, liver tissue was harvested, fixed and frozen as described previously [2]. Frozen sections were permeabilized, blocked and stained in 0.1M Tris (AppliChem) containing 0.3% Triton X-100 (GERBU Biotechnik), 1% FCS (Biochrom AG), 1% GCWFS (Sigma Aldrich) and normal mouse serum (Life Technologies). Analysis was performed on a 710 confocal microscope (Carl Zeiss Microimaging). The following antibodies were used for staining: anti-CD11b (M1/70), anti-Ly6C (HK1.4), anti-F4/80 (BM8), anti-CD11C (N418), anti-Mx1 polyclonal rabbit antibody and chicken anti rabbit-594 antibody.

Human liver and blood samples

Liver samples were taken during liver transplantation from patients with alcoholic cirrhosis (n=5) and liver samples from non-cirrhotic patients undergoing liver resection for metastasis served as controls (n=5). Blood samples were drawn from seven patients with alcoholic liver cirrhosis and severe portal hypertension selected for transjugular intrahepatic portosystemic shunt (TIPS) insertion for complication of portal hypertension (ascites in six patients and bleeding in one patient). During the TIPS procedure, blood samples from the cubital and hepatic vein as well as from the right atrium were collected. Additionally, samples from the portal vein were collected as soon as the right branch of the portal vein had been cannulated. The Human Ethics Committee of the University of Bonn (029/13) approved the use of human liver and blood samples.

Quantitative real-time PCR

Total RNA from tissue or from flow-sorted cells was purified by TRIzol reagent (Life technologies) and reversely transcribed into cDNA using a SuperScript® VILO™ cDNA synthesis

kit (Invitrogen). Quantitative Real Time PCR (TaqMann) for the expression IFNγ, IL-12p35, IL-10 and GAPDH was performed using pre-designed primers and probes (Gen Expression Assay) from Applied Biosystems on an ABiPrism 7900 HT cycler (Applied Biosystems).

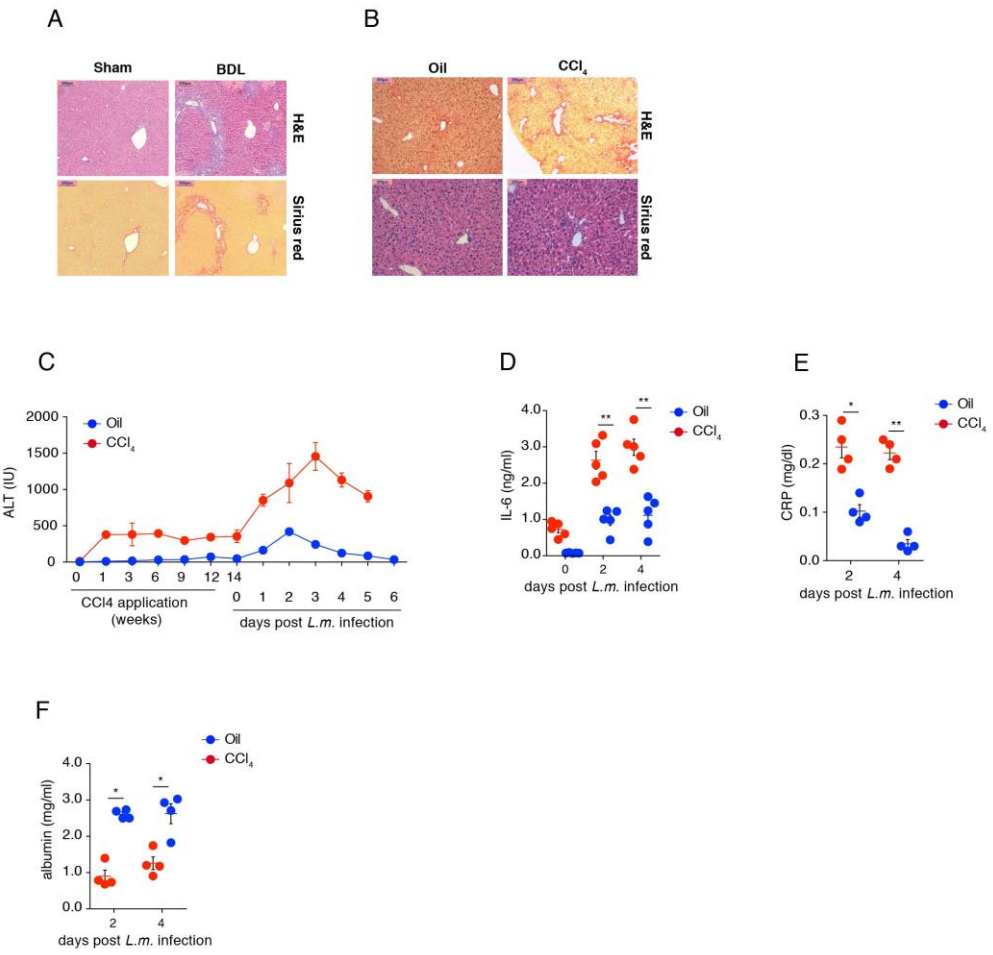
Multiplex gene expression analysis

The expression level of murine *Oas1a*, *Oas2*, *Oasl1*, *Oas1g*, Mx1, *Ptgs2*, *Hprt* and *Gapdh* mRNA and human *OAS1*, *OAS2*, *OAS3*, *OASL* , MX2, *PTGS2*, *HPRT* and *GAPDH* was detected using QuantiGene 2.0 Plex Assay (Panomics, Affymetrix, Santa Clara, CA, USA) using pre-designed probe sets. Liver tissue samples were lysed and hybridized with the probe set according to the manufacturer's protocol. Samples were read on a Luminex flow cytometer (Luminex Corp., Austin, TX, USA).

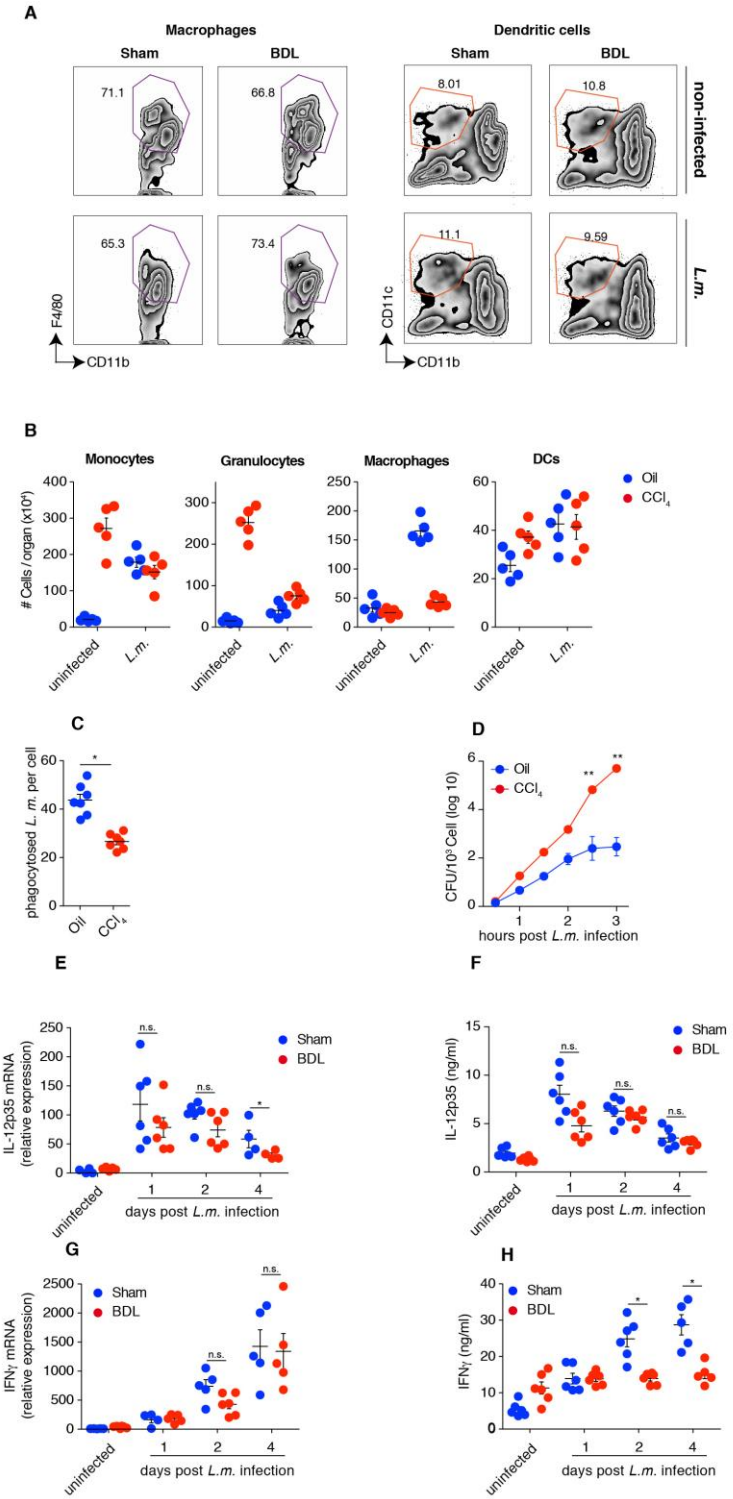
References

1 Coteur G, Danis B, Dubois P. Echinoderm reactive oxygen species (ROS) production measured by peroxidase, luminol-enhanced chemiluminescence (PLCL) as an immunotoxicological tool. Prog Mol Subcell Biol 2005;**39**:71-83.
2 Kastenmuller W, Brandes M, Wang Z, Herz J, Egen JG, Germain RN. Peripheral prepositioning and local CXCL9 chemokine-mediated guidance orchestrate rapid memory CD8+ T cell responses in the lymph node. Immunity 2013;**38**:502-13.

Supplemental Figure 1



Supplemental Figure 2



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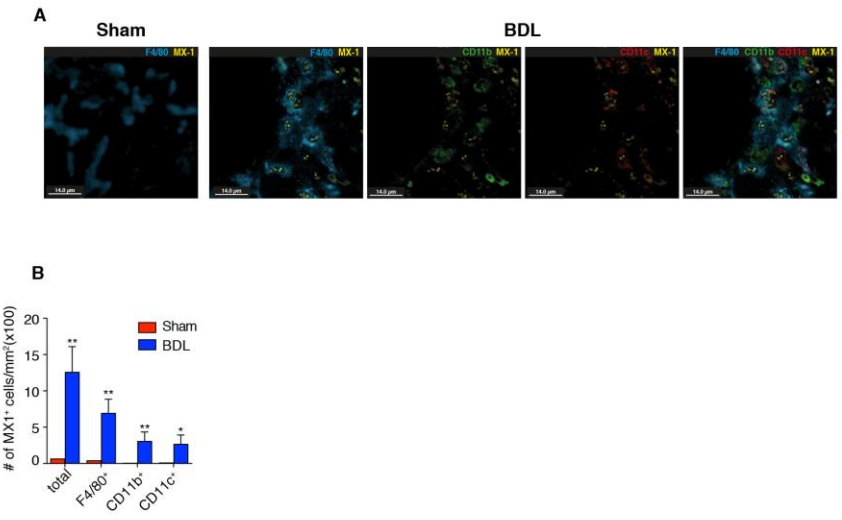

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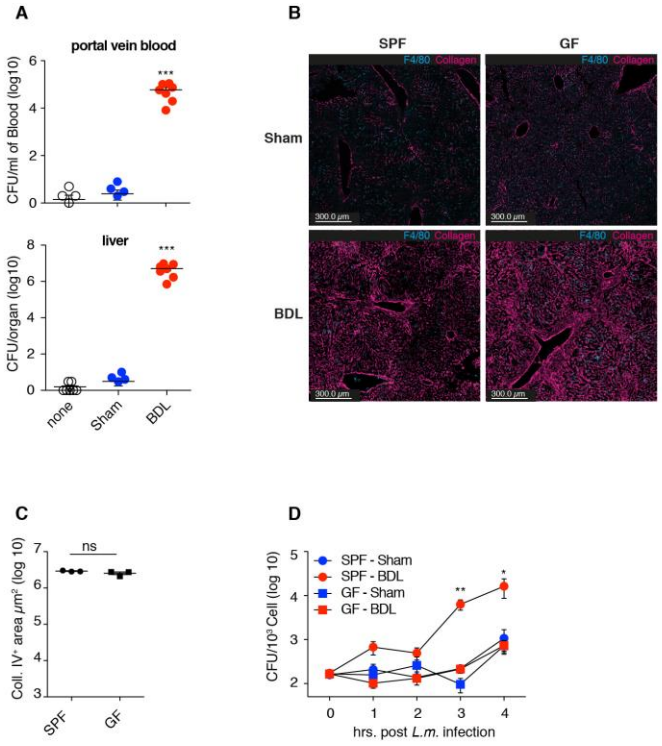

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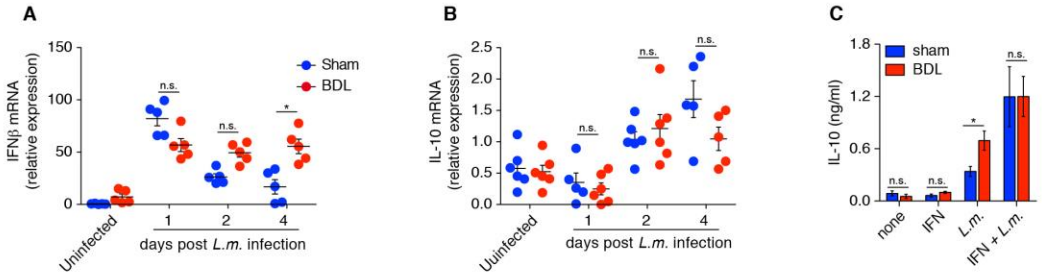

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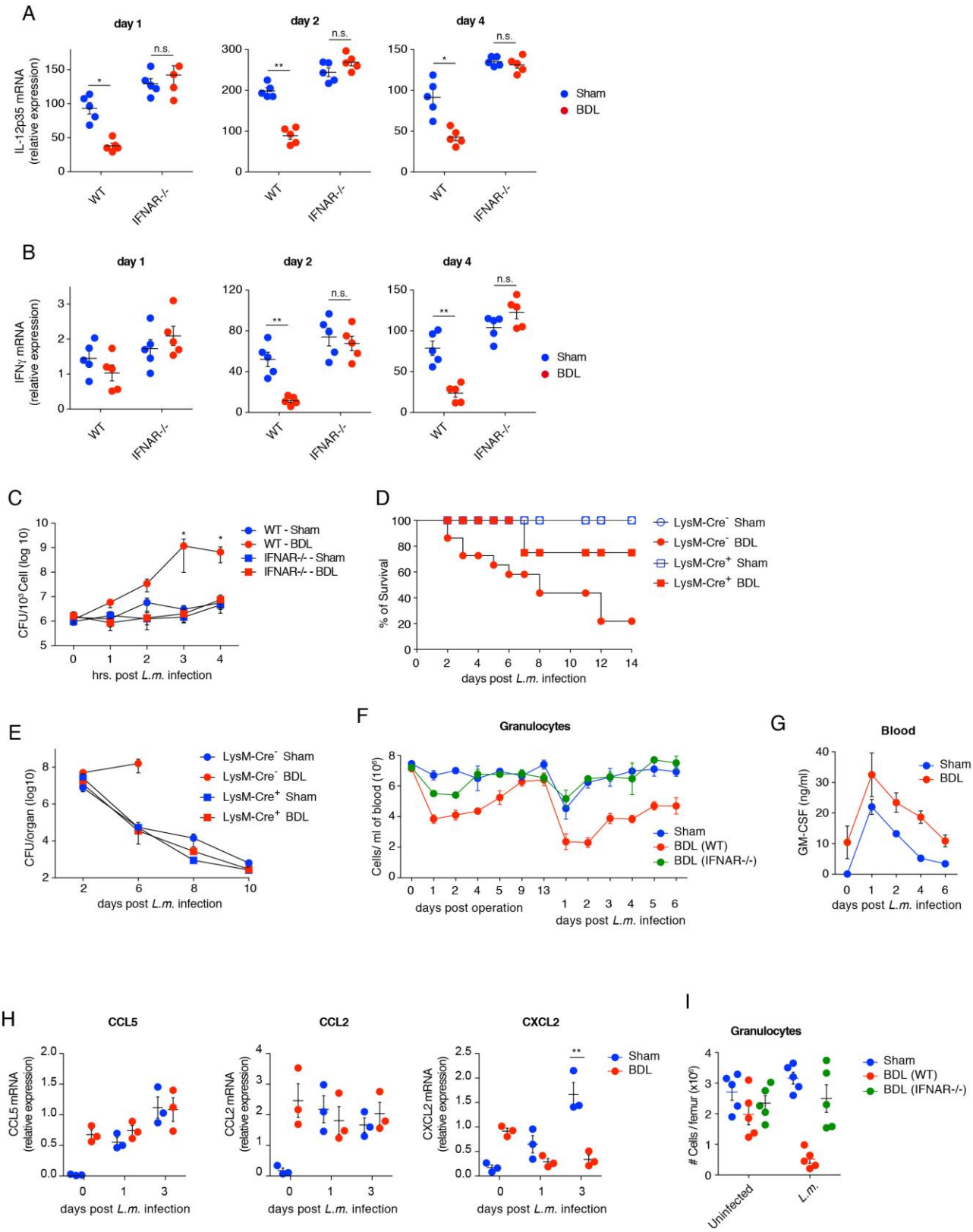
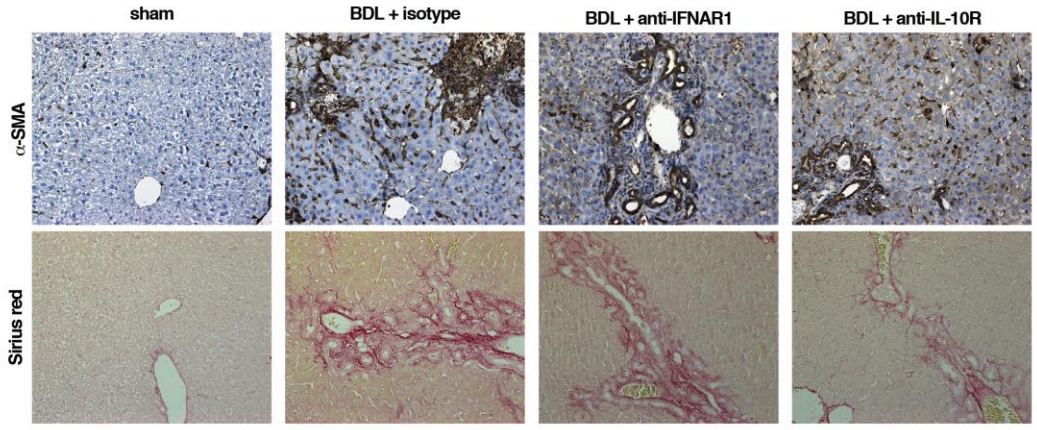


Rev. Figure 5

A



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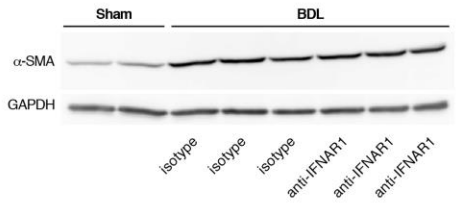

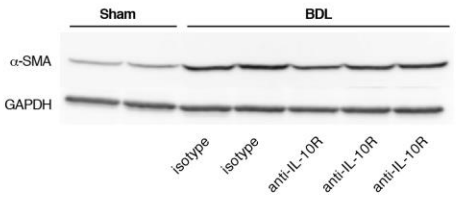

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