Liver fibrosis and CD206^{+} macrophage accumulation are suppressed by anti-GM-CSF therapy

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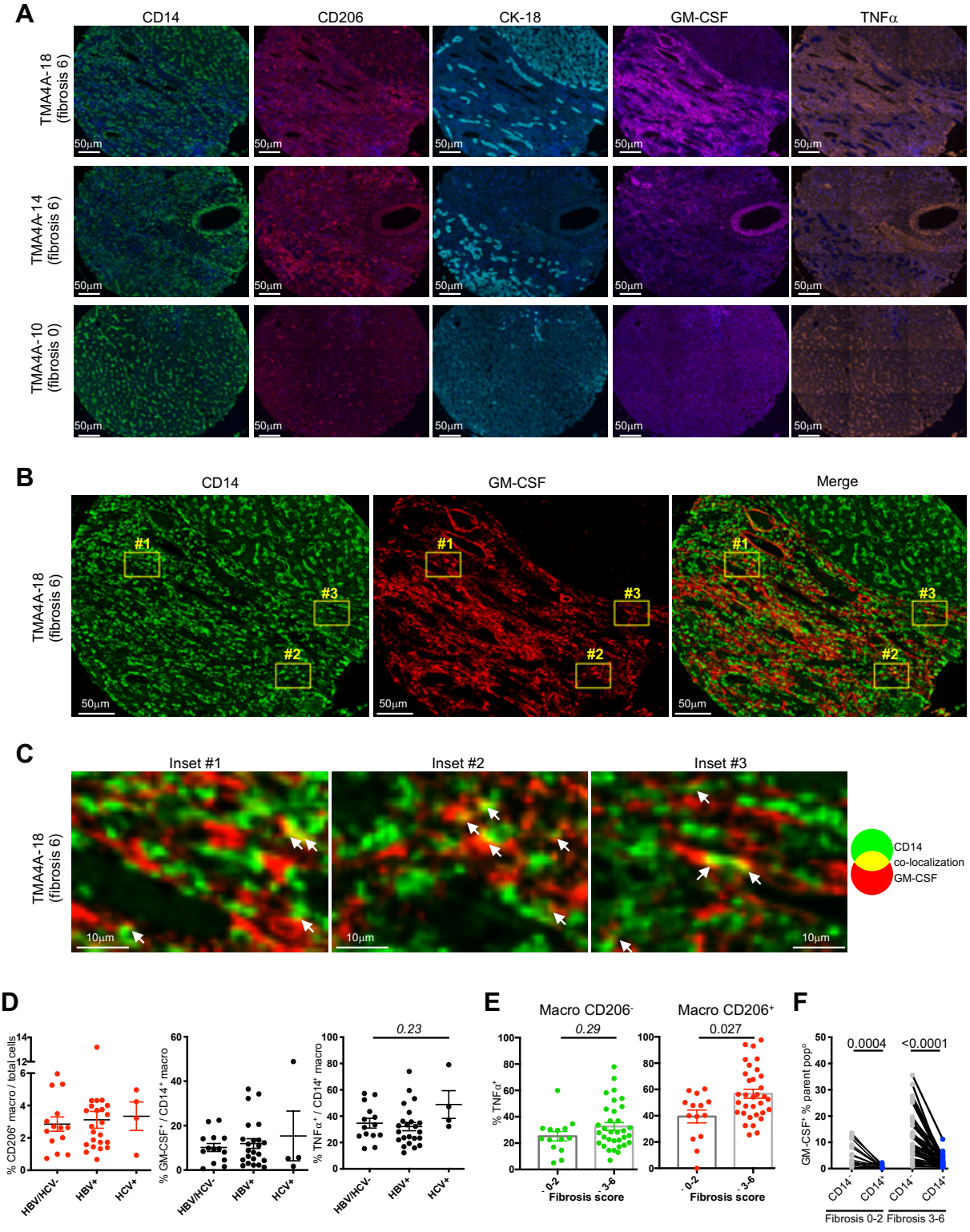

Fig. S1. (**A**) Images of single stainings each with Dapi from immunofluorescence images shown

in **Fig. 1C-E**. Dapi is shown in each single staining image. (**B**) Single stainings images (left and middle panels) and merged image (right panel) of CD14 (green) and GM-CSF (red; displayed in this colour here to obtain yellow when co-localized with green) for TMAA4-18. (**C**) Higher magnifications of insets defined in the merged CD14/GM-CSF image in (**B**) are shown. (**D**) Quantifications of histo-cytometry data showing the proportion of $CD206^+$ macrophages among total cells (left panel), of $GM\text{-}CSF^+$ cells (middle panel) and of $TNF\alpha^+$ cells (right panel) among $CD14^+$ macrophages in HBV^-/HCV^-(n=14, HBV^+ (n=23) and HCV^+ (n=4) HCC patients. (**E**) Proportion of $TNF\alpha^+$ cells among $CD206^-$ or $CD206^+$ macrophages in HCC patients split based on fibrosis scores of 0-2 and 3-6. Data displayed as mean +/-SEM, p values calculated by Mann-Whitney test. (**F**) Proportion of $GM\text{-}CSF^+$ cells among $CD14^-$ or $CD14^+$ cells in normal adjacent liver of HCC patients split based on fibrosis scores of 0-2 and 3-6. P values were calculated by Wilcoxon test.

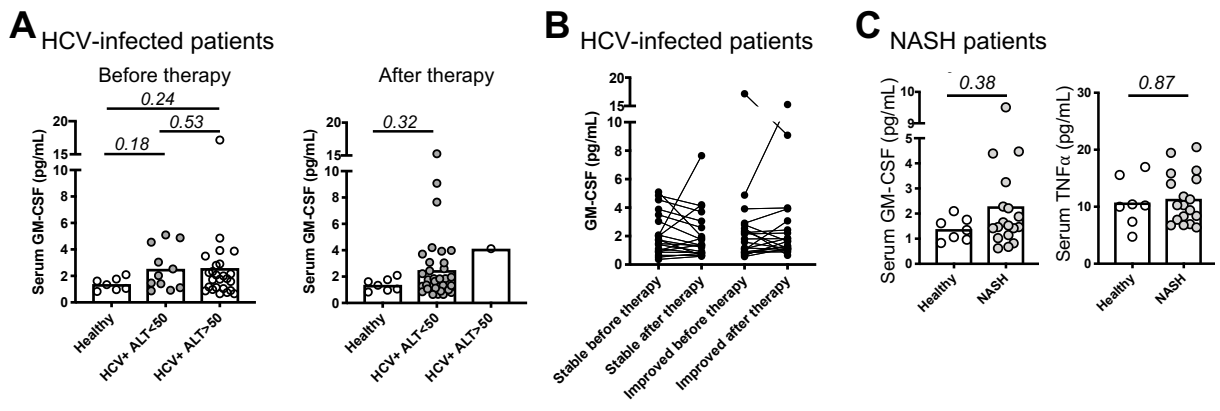

Fig. S2. (**A-B**) Serum GM-CSF concentrations were measured by Luminex in (**A)** healthy human donors (n=7) or CHC patients before (left panel) or after (right panel) DAA therapy with low (<50 IU/L), or high (>50 IU/L) serum ALT. Values are shown as mean concentration (pg/mL). (**B**) Serum GM-CSF concentrations are displayed before and after therapy and patients were separated based on a stable or an improved disease post-therapy. (**C**) Serum GM-CSF concentrations were measured by Luminex in (**A)** healthy human donors (n=7) or NASH patients (n=18).

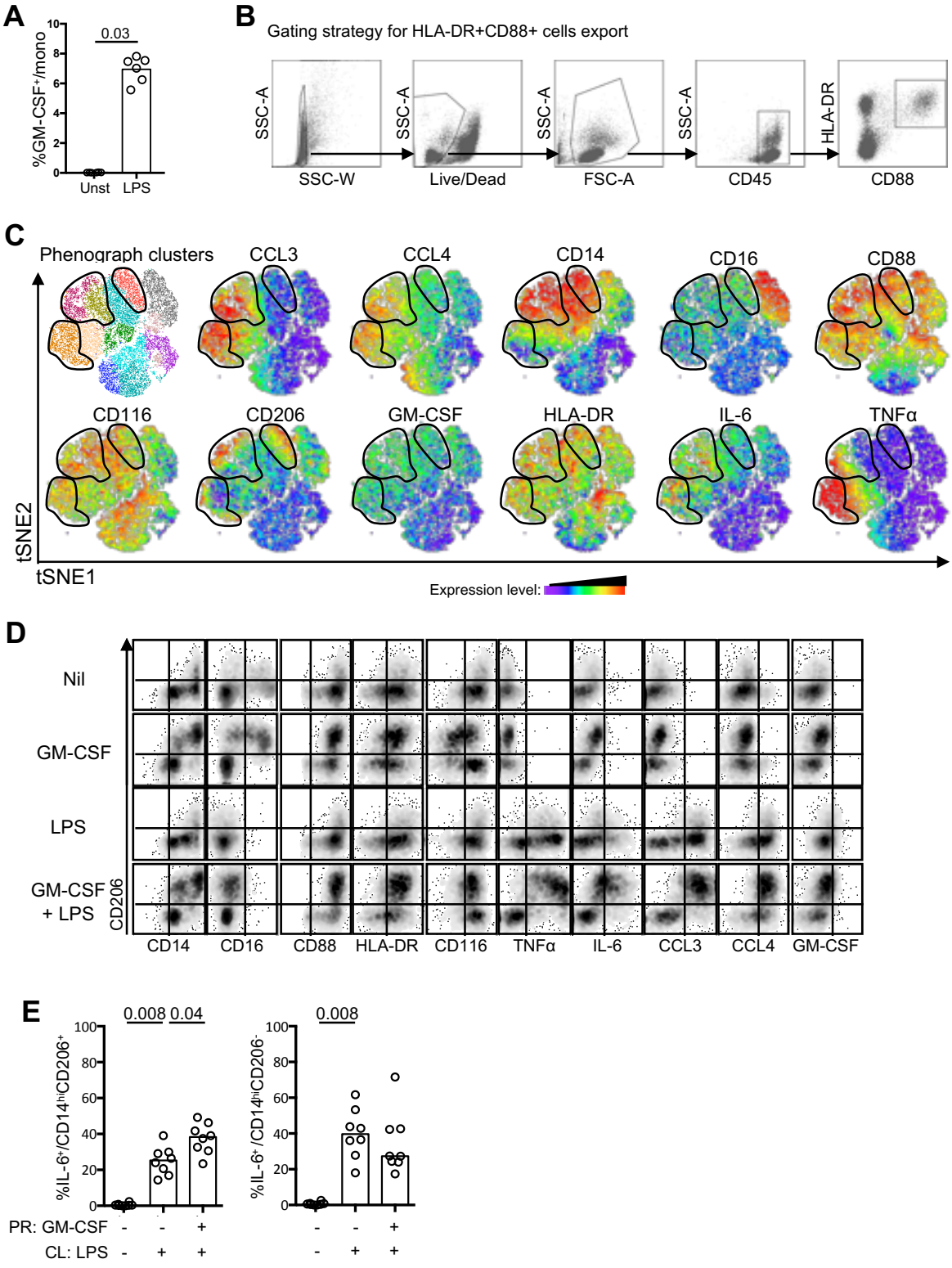

Fig. S3. (**A**) Intracellular cytokine staining of GM-CSF in human peripheral blood monocytes stimulated or not with LPS. (**B**) Gating strategy to define single live $CD45^{+}HLA\text{-}DR^{+}CD88^{+}$ cells.

(**C**) Concatenated tSNE dot plot with PhenoGraph-defined cell clusters overlaid (upper left) and expression heatmaps of various myeloid markers, cytokines and chemokines of single live $CD45^{+}HLA\text{-}DR^{+}CD88^{+}$ cells from healthy human peripheral blood. (**D**) Density plots of $CD45^{+}HLA\text{-}DR^{+}CD88^{+}$ cells from healthy human peripheral blood following 24hr priming with or without GM-CSF and subsequent challenge with LPS. The expression of CD206 (y-axis) and various myeloid markers, cytokines and chemokines (x-axis) are shown. (**E**) Frequency of $IL\text{-}6^{+}$ cells among $CD14^{hi}CD206^{+}$ (left panel) or $CD14^{hi}CD206^{-}$ (right panel) cells from healthy human PBMCs (n=8) primed (PR) with or without GM-CSF for 24hrs and subsequently challenged (CL) with LPS (10ng/mL) for 6hrs.

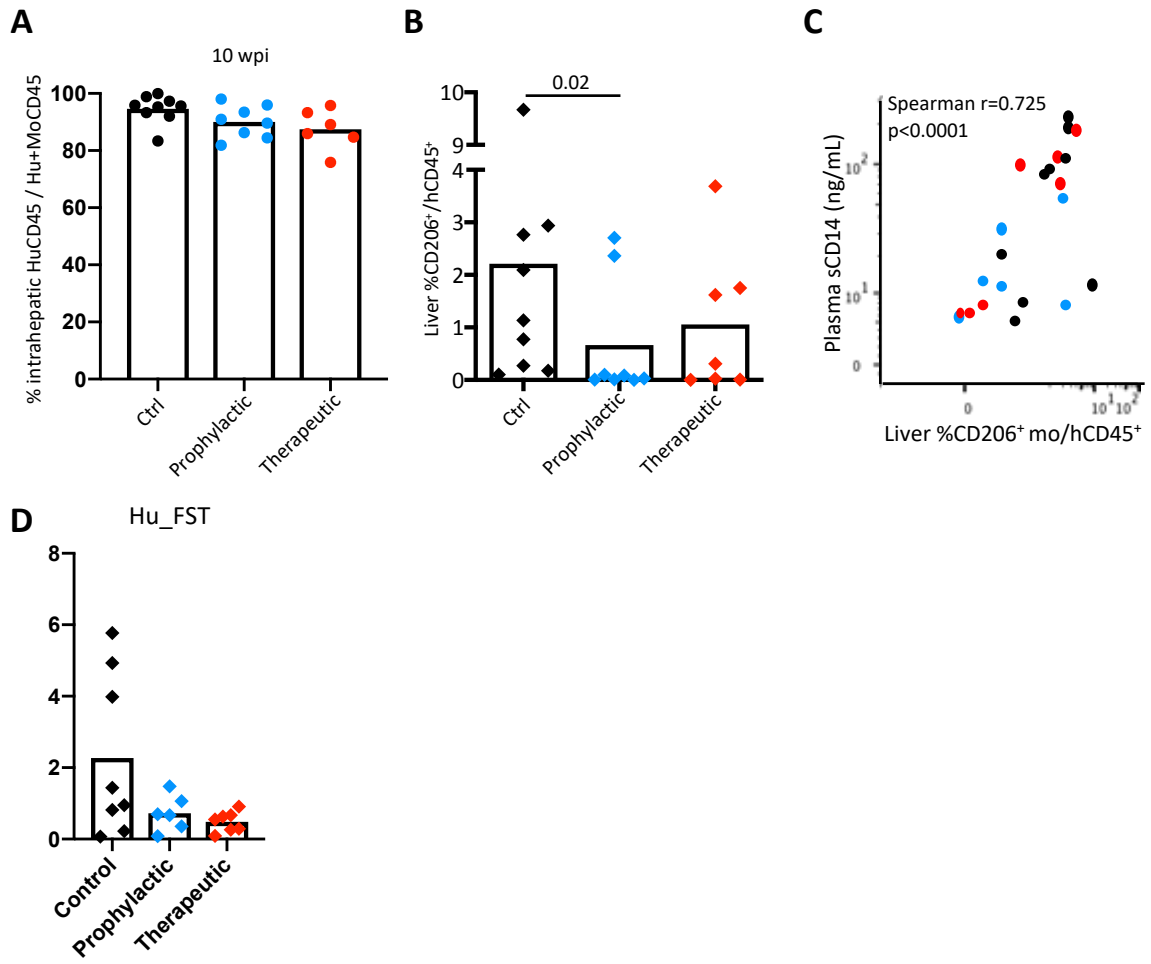

Fig. S4. (**A**) Frequencies of chimerism [% huCD45$^+$ cells among total blood mononuclear cells (Human + mouse CD45$^+$ cells)], in the liver of HIL mice at the end of the experiment [10 weeks post-infection (wpi)] (n=23 mice). (**B**) Frequency among total human CD45$^+$ cells of intrahepatic CD14$^+$HLA-DR^{hi}CD206$^+$ macrophages in HBV-infected mice at 10 wpi that were untreated (n=9) or that received anti-GM-CSF antagonistic antibody at 0 wpi (prophylactic group, n=8) or at 6 wpi (therapeutic group, n=7). (**C**) Correlative analysis of serum sCD14 concentrations and intrahepatic CD14$^+$HLA-DR^{hi}CD206$^+$ cell frequency in HBV-infected humanised mice untreated or treated with anti-GM-CSF antibody (total n=24) by Spearman's rank correlation coefficient (black, Ctrl; blue, prophylactic; red, therapeutic). (**D**) Expression of the human pro-fibrotic gene FST within HIL mouse livers at 10 wpi relative to the human albumin (ALB) gene.

Supplementary tables

Table S1. Antibodies used for immunohistochemical (IHC) labelling of liver tissue sections

Antibody	Clone	Cat. Number	Provider	dilution	Source	Labelling cell compartment
Cytokeratin	EPR1626	ab133263	Abcam	1:100	Rabbit	Cytoplasm and Membrane
CD206	43146	MCA2155T	AbD Serotec	1:200	Mouse	Cytoplasm and Membrane
CD14	EPR3653	Ab133335	Abcam	1:500	Rabbit	Cytoplasm and Membrane
TNFα	polyclonal	ab6671	Abcam	1:50	Rabbit	Cytoplasm and Membrane
GM-CSF	OTI8G5	NBP2-46364	Novus Biological	1:50	Mouse	Cytoplasm and Membrane

Table S2. List of primer sequences used for real time PCR

Targets	Forward Sequence	Reverse sequence
hIGFBP-5	GAAGCAGTGAAGAAGGACCG	GAATCCTTTGCGGTCACAAT
hα-SMA	AGGCACCCCTGAACCCCAA	CAGCACCGCCTGGATAGCC
hCOL1α1	GGCTTCCCTGGTCTTCCTGG	CCAGGGGGTCCAGCCAAT
hTGFβ1	CGCTAAGGCGAAAGCCCTCAATTT	ACAATTCCTGGCGATACCTCAGCA
hFST	TGTGGTGGACCAGACCAATA	CCGAAATGGAGTTGCAAGAT
hALB	GCACAGAATCCTTGGTGAACAG	ATGGAAGGTGAATGTTTTCAGCA