

Supplementary material

GDF10 attenuates MASH progression by restoring quiescent hepatic stellate cells via competitive inhibition of TGF- β /SMAD2 signaling

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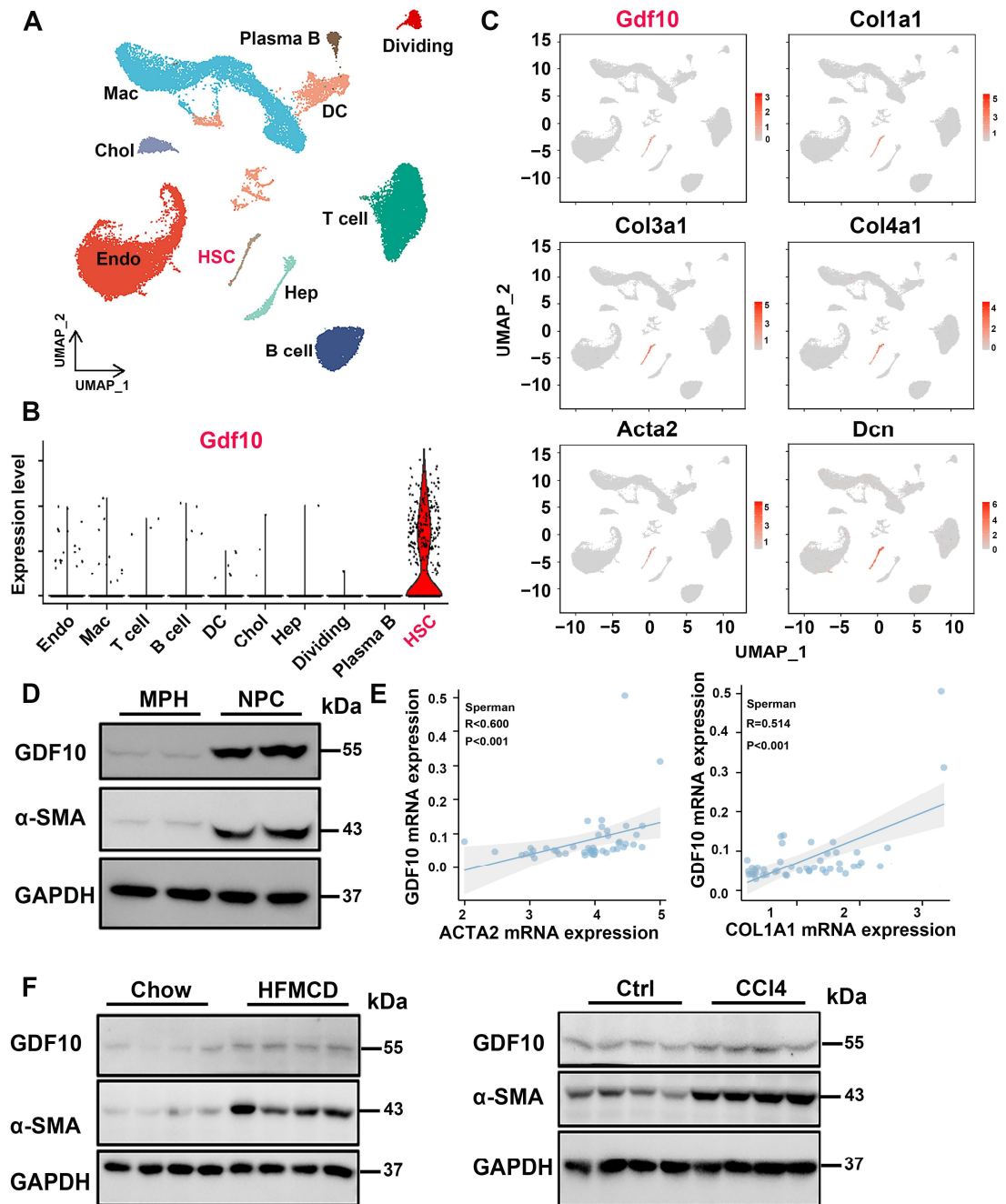


Figure S1. GDF10 is predominantly secreted by HSCs in MASH and correlates with disease progression, related to Figure 1. A. UMAP visualization of liver cell clusters (GSE129516). B. Violin plots showing *Gdf10* gene expression for each cluster. C. UMAP visualization *Gdf10* and fibrosis-related gene mRNA levels in the liver. D. Immunoblot analysis of GDF10 and α -SMA in MPH or NPCs, respectively. E. Linear regression for *GDF10* with *ACTA2* or *COL1A1* mRNA expression as a covariate in the human liver cirrhosis (GSE25097). F. Immunoblots analysis of GDF10 and α -SMA in

liver from HFMCD- or CCl₄-induced fibrosis mice, respectively (n = 4 for chow diet group and n = 4 for HFMCD diet group; n = 4 for control and n = 4 for CCl₄ group).

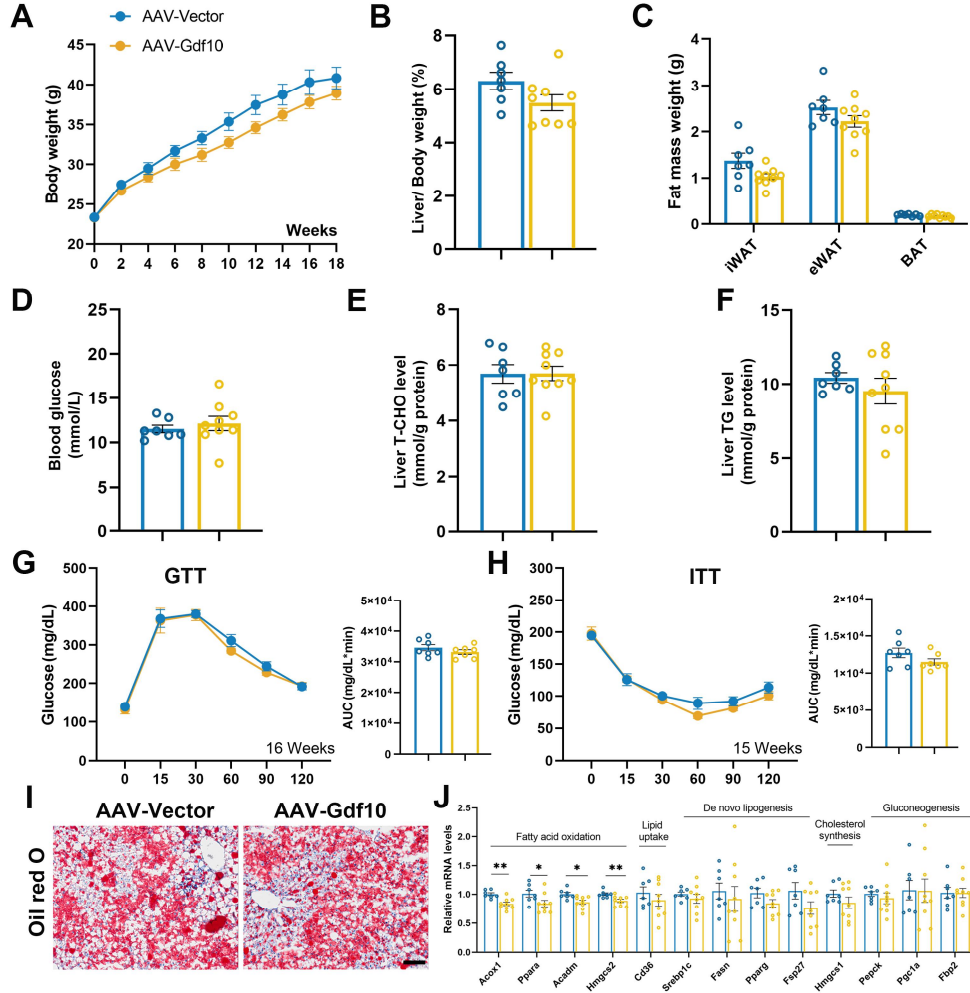


Figure S2. *Gdf10* overexpression had no effect on glucose or lipid metabolism parameters, related to figure 2. A-C. Measurement of body weight (A), liver/body weight, fat mass levels in HFFC-diet MASH mice treated with *Gdf10-OE* virus (n = 7 for control and n = 9 for *Gdf10-OE* group). D-F. Blood glucose levels (D), liver T-CHO levels (E), and the concentrations of liver TG (F) were evaluated in HFFC-diet MASH mice treated with *Gdf10-OE* virus (n = 7 for control and n = 9 for *Gdf10-OE* group). G, H. GTT and ITT were performed in mice after 16- or 15-weeks intervention, respectively (n = 6 for control and n = 7 for *Gdf10-OE* group). The area under the curve of GTT (G) and ITT (H) were measured. I. Representative images of oil red O staining of liver tissue in mice, scale bars, 100 μ m. J. qPCR analysis of indicated genes of the liver in Ctrl and *Gdf10-OE* mice (n = 7 for control and n = 9 for *Gdf10-OE* group). Data are presented as mean $\pm$ SEM. *P < 0.05, **P < 0.01.

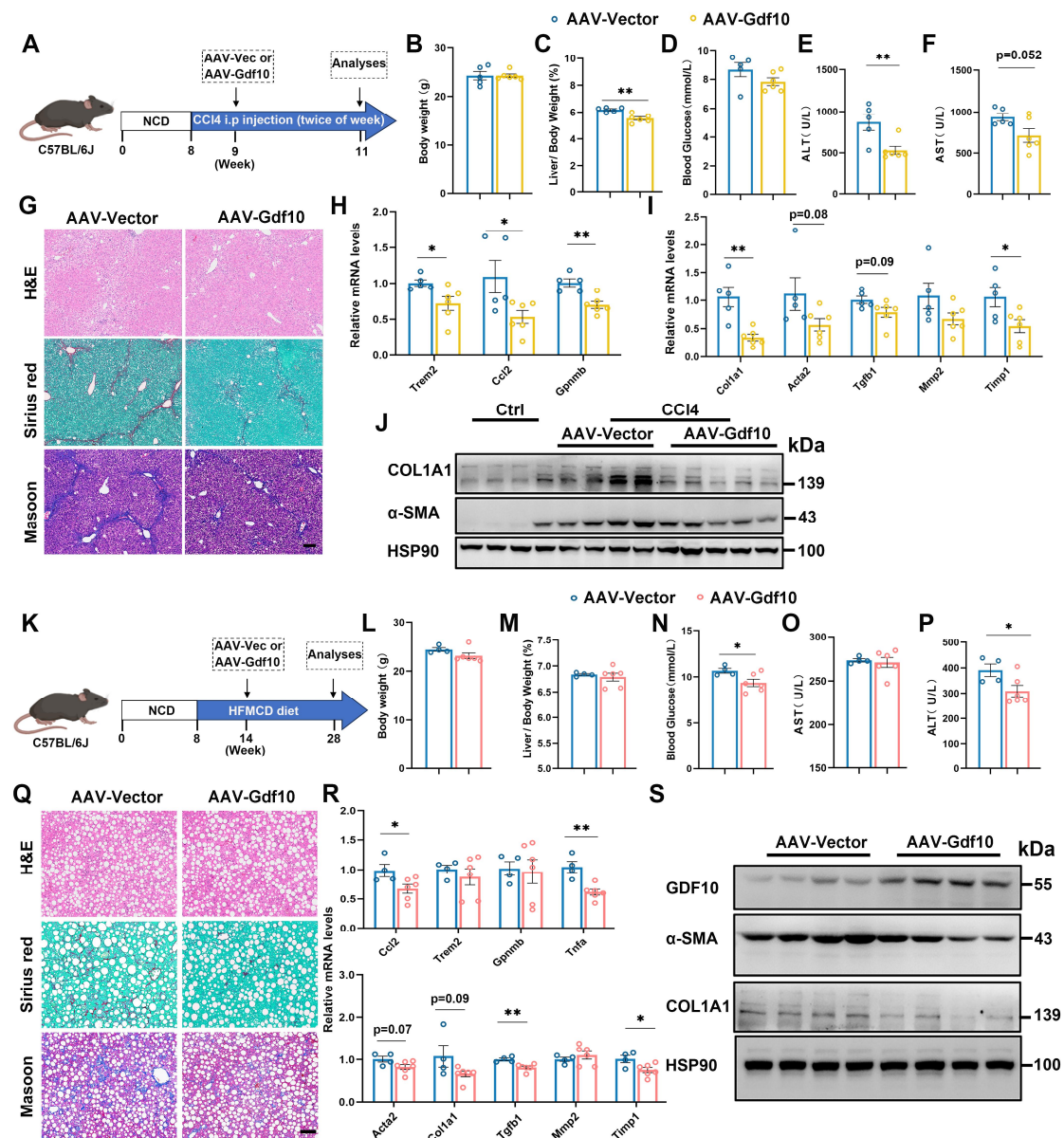


Figure S3. *Gdf10* overexpression attenuates liver fibrosis in CCl4-induced fibrosis mice and diet-induced MASH models, related to figure 2. A. Experimental design for B-J. B-F. Measurement of body weight (B), liver/body weight (C), blood glucose levels (D), serum ALT (E) and AST (F) in the CCl4-induced fibrosis mice treated with *Gdf10*-OE virus (n = 5 for control and n = 5 for *Gdf10*-OE group). G. Representative images and qualification of H&E, Sirius Red, and Masson staining in CCl4-induced fibrosis mice treated with *Gdf10*-OE virus. scale bars, 100 μm. H-J. qPCR and analysis of indicated genes in fibrosis-related genes mRNA (H, I) and protein (J) levels of the liver in CCl4-induced fibrosis mice treated with *Gdf10*-OE virus. K. Experimental design for L-S. L-P. Measurement of body weight (L), liver/body weight (M), blood

glucose levels (N), serum AST (O) and ALT (P) in the HFMCD-diet MASH mice treated with *Gdf10-OE* virus (n = 4 for control and n = 6 for *Gdf10-OE* group). Q. Representative images and qualification of H&E, Sirius Red, and Masson staining in the HFMCD-diet MASH mice treated with *Gdf10-OE* virus. scale bars, 100 μ m. R, S. qPCR (R) and immunoblot (S) analysis of indicated genes of the liver in CCl₄-induced fibrosis mice treated with *Gdf10-OE* virus. Data are presented as mean $\pm$ SEM. *P < 0.05, **P < 0.01.

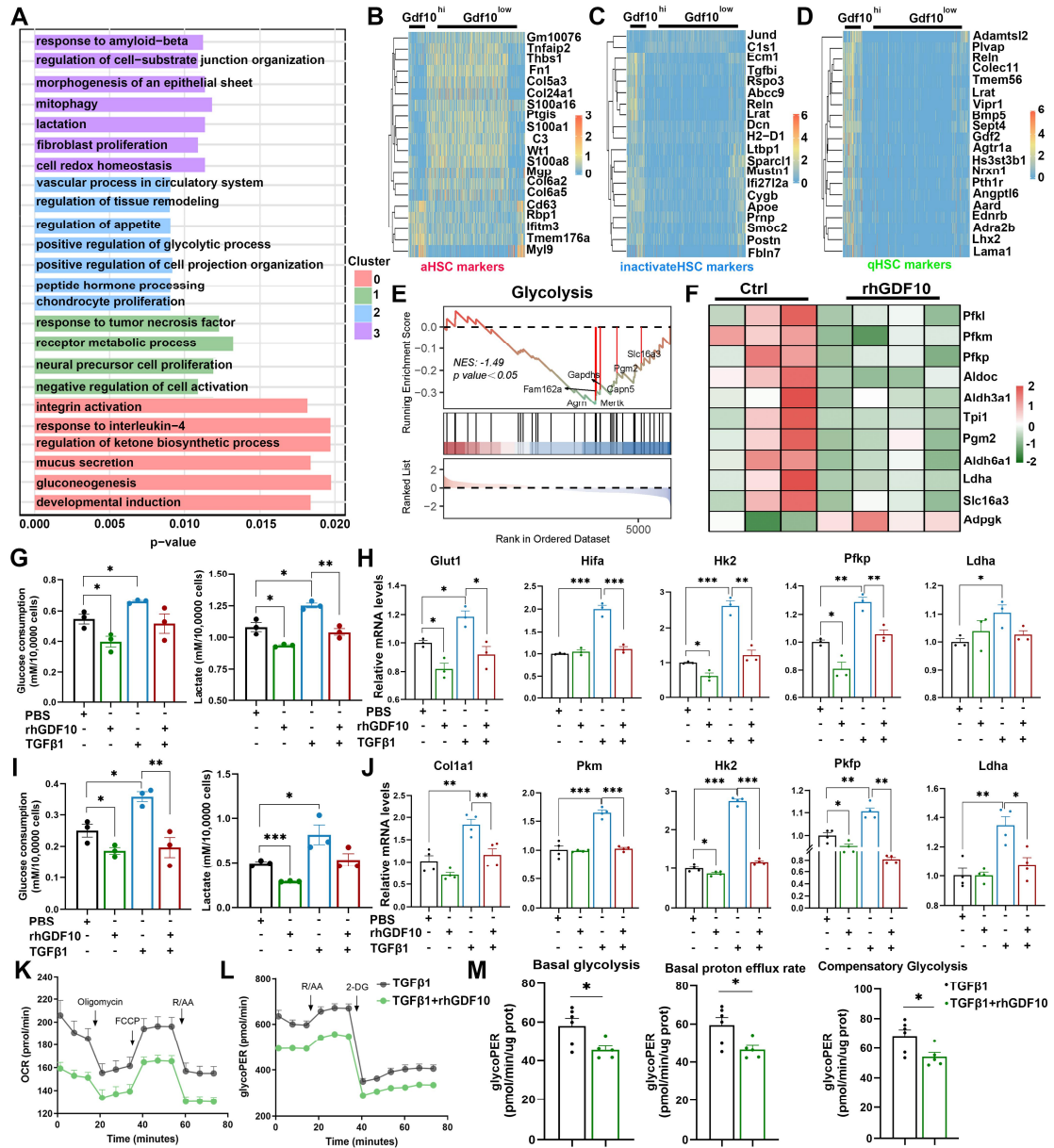


Figure S4. GDF10 functionally shifts activated HSCs into a quiescent state, related to figure 5. A. Representative GO terms enriched with DEGs in four subclusters of HSCs (CRA007803). B-D. Heatmap showing the aHSC, inactivate HSC and qHSC

marker genes of DEGs from *Gdf10^{low}* versus *Gdf10^{hi}* HSC. E. GSEA analysis of the pathway between Ctrl versus rhGDF10 treated immortalized HSCs. F. Heatmap showing the glycolysis pathway genes of DEGs from Ctrl or rhGDF10 treated immortalized HSCs. G. The concentration of glucose uptake and lactate secretion levels in the supernatant in Ctrl or rhGDF10 treated immortalized HSCs after culture for 24 h. H. qPCR analysis of glycolysis related genes in TGFβ1 or rhGDF10 treated immortalized HSCs. I. The concentration of glucose uptake and lactate secretion levels in the supernatant in Ctrl or rhGDF10 treated LX2 after culture for 24 h. J. qPCR analysis of glycolysis related genes in TGFβ1 or rhGDF10 treated LX2. K-M. OCR (K), GlycoPER (L) and qualification (M) in LX2 with rhGDF10 or TGFβ1 treatment in OCR or GlycoPER process, respectively. Data are representative of three independent experiments. Data are presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001.

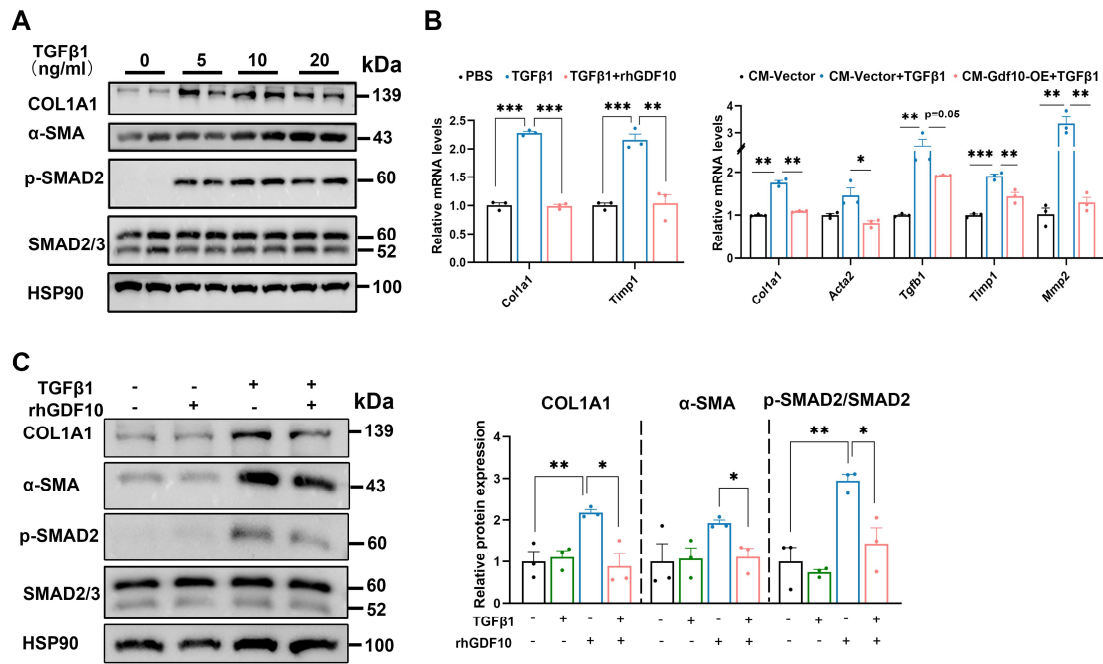


Figure S5. GDF10 exerts anti-fibrotic effects by competitively inhibiting TGF-β-dependent SMAD2/3 signaling in HSCs, related to figure 6. A. Immunoblot analysis of COL1A1, α-SMA and phosphorylation of SMAD2 of the HSCs with difference dose of TGFβ1 treatment. B, C. qPCR analysis of indicated genes in TGFβ1 or rhGDF10 (B), and TGFβ1 or CM-*Gdf10-OE* treated HSCs (C). D. Immunoblot analysis of COL1A1, α-SMA and phosphorylation of SMAD2 of the LX2 with TGFβ1 or

rhGDF10 treatment. Data are representative of three independent experiments and are presented as mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

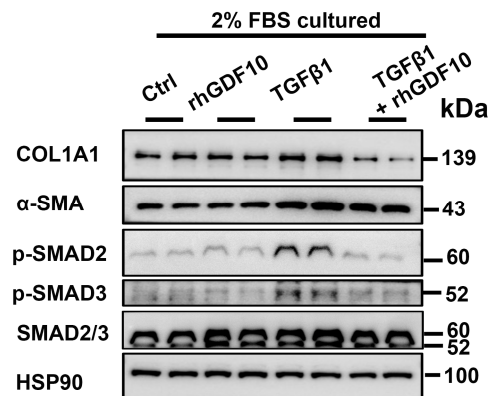


Figure S6. Immunoblot analysis of COL1A1, α -SMA and phosphorylation of SMAD2 and SMAD3 in HSCs cultured in 2% FBS treated with TGF β 1 or rhGDF10.

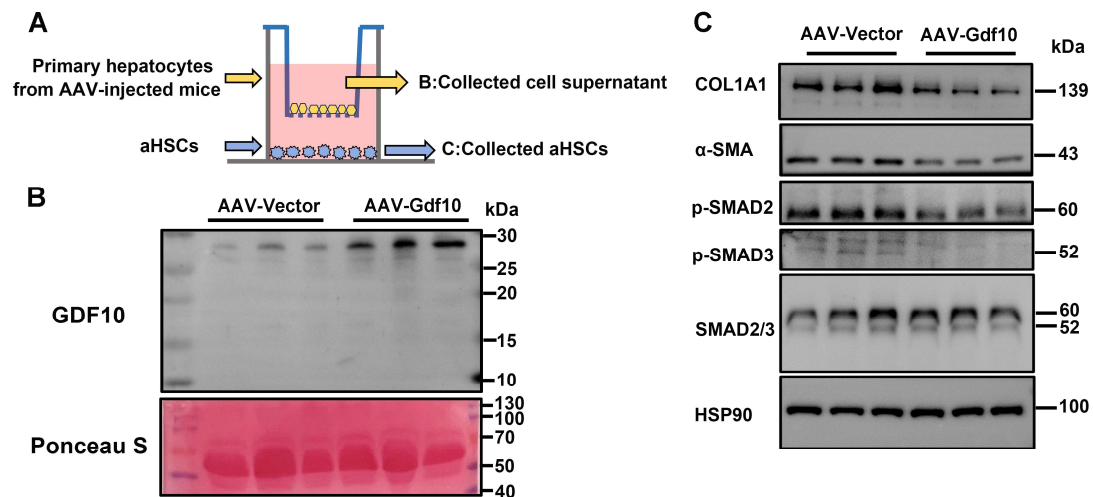


Figure S7. Analysis of hepatocytes-HSCs co-culture system. A. Schematic of co-cultured primary hepatocytes and aHSCs. B. Immunoblot analysis of GDF10 protein levels in the conditioned medium collected from primary hepatocytes. Ponceau S staining is shown as an internal loading control. C. Immunoblot analysis of COL1A1, α -SMA and phosphorylation of SMAD2 and SMAD3 in aHSCs. Data shown are representative of three independent experiments.

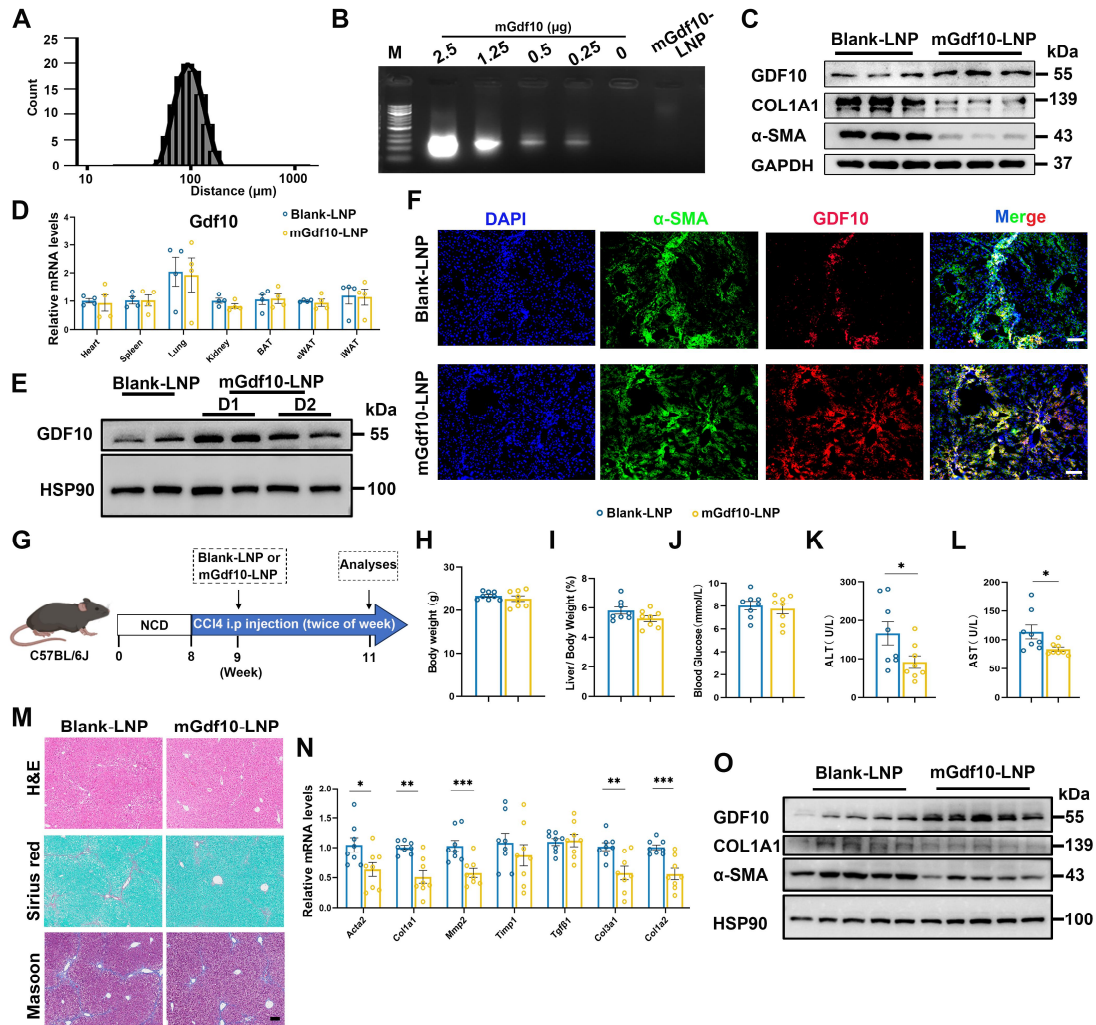


Figure S8. LNP-encapsulated *mGdf10* exhibits anti-fibrotic effects, related to figure 7. A. Nanoparticle tracking analysis of the *mGdf10*-LNP. B. mRNA with different total amount was analyzed by 1% agarose gel electrophoresis. 1.25 ug *mGdf10*-LNP was loaded into the last lane to detect encapsulation efficiency. C. Immunoblot analysis of COL1A1, α-SMA and phosphorylation of SMAD2 of the cultured aHSCs with LNP or *mGdf10*-LNP treatment. D, E. qPCR (D) and immunoblot (E) analysis of *Gdf10* mRNA levels of multiple tissues in LNP (n = 4) or *mGdf10*-LNP (n = 4) treated mice. F. IF analysis of GDF10 and α-SMA co-expression of the liver in LNP or *mGdf10*-LNP treated mice. scale bars, 100 μm. G. Experimental design for H-O. H-L. Measurement of body weight (H), liver/body weight (I), blood glucose levels (J), serum ALT (K) and AST (L) in the CCl4-induced fibrosis mice treated with *mGdf10*-LNP (n = 8 for control and n = 8 for *mGdf10*-LNP group). M. Representative images of H&E, Sirius Red, and Masson staining in the CCl4-induced fibrosis mice treated with *mGdf10*-LNP. scale bars, 50 μm. N, O. qPCR (N) and immunoblot (O)

analysis of indicated genes of the liver in the CCl₄-induced fibrosis mice treated with *mGdf10*-LNP. Data are presented as mean $\pm$ SEM. *P < 0.05, **P < 0.01, ***P < 0.001.

Table S1. Primer sequences for qPCR of specific genes.

Gene	Species	Forward Primers	Reverse Primers
<i>36b4</i>	Mouse	GAAACTGCTGCCTCACATCCG	GCTGGCACAGTGACCTCACACG
<i>Mmp13</i>	Mouse	TGTTTGCAGAGCACTACTTGAA	CAGTCACCTCTAAGCCAAAGAAA
<i>Timp1</i>	Mouse	CGAGACCACCTTATACCAGCG	ATGACTGGGGTGTAGGCGTA
<i>Mmp2</i>	Mouse	CAACGGTCGGGAATACAGCAGC	TGGAAGCGGAACGGGAACCTTG
<i>Tgfb1</i>	Mouse	ACCATGCCAACTTCTGTCTGGGAC	ACAACTGCTCCACCTTGGGCTTG
<i>Acta2</i>	Mouse	CTGACAGAGGCACCACTGAA	CATCTCCAGAGTCCAGCACA
<i>Col3a1</i>	Mouse	CTGTAACATGGAACTGGGGAAA	CCATAGCTGAACTGAAAACCAACC
<i>Col1a2</i>	Mouse	AGGTCCTAATGGAGATGCCG	CACAGGGCCTTCTTTACCAG
<i>Col1a1</i>	Mouse	AAGAGGCGAGAGAGGTTTCC	AGAACCATCAGCACCTTTGG
<i>Gdf10</i>	Mouse	GCAAGCCCCGAGCTAAGAA	GATTGAGGAGATGTCCTTGGC
<i>Gfap</i>	Mouse	GGGGCAAAGCACCAAGAAG	GGGACAACTTGTATTGTGAGCC
<i>Bambi</i>	Mouse	CATTGCTGGCGGACTGATCTT	CTTGCCCCTTCTTGAATGGT
<i>Insig1</i>	Mouse	CTGTATTGCCGTGTTCTGTTG	CTTCGGGAACGATCAAATGT
<i>Pparg</i>	Mouse	TATGGAGTGACATAGAGTGTGCT	GTCGCTACACCACTTCAATCC
<i>Acox1</i>	Mouse	GCCTGCTGTGTGGGTATGTCATT	GTCATGGGCGGGTGAT
<i>Ppara</i>	Mouse	GCAGTGCCCTGAACATCGA	CGCCGAAAGAAGCCCTTAC
<i>Acadm</i>	Mouse	GCTGGAGACATTGCCAATCA	GGCGTCCCTCATCAGCTTCT
<i>Hmgcs2</i>	Mouse	GACATCAACTCCCTGTGCCTG	GATGTCAGTGTTGCCTGAATC
<i>Cd36</i>	Mouse	TTAGATGTGGAACCCATAACTGGA	TTGACCAATATGTTGACCTGCAG
<i>Srebp1c</i>	Mouse	CACCAGCATAGGCGAAGGA	ATGCCGACCAGATTCCCTAA
<i>Fasn</i>	Mouse	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
<i>Fsp27</i>	Mouse	TCGACCTGTACAAGCTGAACCCT	AGGTGCCAAGCAGCATGTGACC
<i>Hmgcs1</i>	Mouse	AAAGATGTGGGAATCGTTGC	GGCCGATGGTATACTTTCCA
<i>Pepck</i>	Mouse	CTGCATAACGGTCTGGACTTC	CAGCAACTGCCCCGTACTCC
<i>Pgc1a</i>	Mouse	TATGGAGTGACATAGAGTGTGCT	GTCGCTACACCACTTCAATCC
<i>Fbp2</i>	Mouse	GGTTCCATGGTGGCTGATGT	GGCCACAGGATTGCATTCAT
<i>Trem2</i>	Mouse	CAGCACCTCCAGGAATCAAGA	AGGATCTGAAGTTGGTGCCC
<i>Ccl2</i>	Mouse	AGGTCCCTGTCATGCTTCTG	TCTGGACCCATTCTTCTTG
<i>Gpnmb</i>	Mouse	GAGCACAACCAATTACGTGGCT	GGTGATATTGGAACCCACCAGA
<i>Tnfa</i>	Mouse	AGCCCCCAGTCTGTATCCTT	CTCCCTTTGCAGAACTCAGG
<i>Glut1</i>	Mouse	CCTGTCTCTTCTACCCAACC	GCAGGAGTGTCCGTGTCTTC
<i>Hifa</i>	Mouse	ACCTTCATCGGAACTCCAAAG	CTGTTAGGCTGGGAAAAGTTAGG
<i>Hk2</i>	Mouse	CCGCCGTGGTGGACAAGATA	AGCAGTGATGAGAGCCGCTC
<i>Pfkip</i>	Mouse	CGCCTATCCGAAGTACCTGGA	CCCCGTGTAGATTCCCATGC
<i>Ldha</i>	Mouse	GGTGATCCCATTTCCAC	GTCTGCGCTCTTCTTCAGG
<i>36B4</i>	Human	AGGCGTCCTCGTGGAAGTGA	GCGGATCTGCTGCATCTGCT

<i>COL1A1</i>	Human	AACATGACCAAAAACCAAAAGTG	CATTGTTTCCTGTGTCTTCTG
<i>PKM</i>	Human	ATGTCGAAGCCCCATAGTGAA	TGGGTGGTGAATCAATGTCCA
<i>HK2</i>	Human	GAGCCACCACTCACCTACT	CCAGGCATTCTGGCAATGTG
<i>PFKP</i>	Human	GCATGGGTATCTACGTGGGG	CTCTGCGATGTTTGAGCCTC
<i>LDHA</i>	Human	ATGGCAACTCTAAAGGATCAGC	CCAACCCCAACAACCTGTAATCT
