Carboxymethyl chitosan (CMC) prolongs adenovirus-mediated expression of IL-10 and ameliorates hepatic fibrosis in a mouse model

Running Title: CMC encapsulated Ad-IL10 mitigates mouse hepatic fibrosis

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Supporting Materials

Table S1. List of TqPCR Primers

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Gene	Forward primer	Reverse primer	Accession No.
Mouse Gapdh	ATGCCATCACTGCCACCC	GCCAGTGAGCTTCCCGTT	NM_001289726.1
Mouse IL-10	GCAGAGAAGCATGGCCCA	TGCTCCACTGCCTTGCTC	NM_010548.2
Mouse Collagen type I	GAACAGCTGGCCTCCCTG	GGCCAGGAGCTCCGTTTT	NM_007742.4
Mouse α-Sma	CAGGCTGTGCTGTCCCTC	CAGCCAAGTCCAGACGCA	NM_007392.3
Mouse Timp1	TTGGGGCATGTGCAGAGG	CGCACAGCCTGGTTACCA	NM_001044384.1

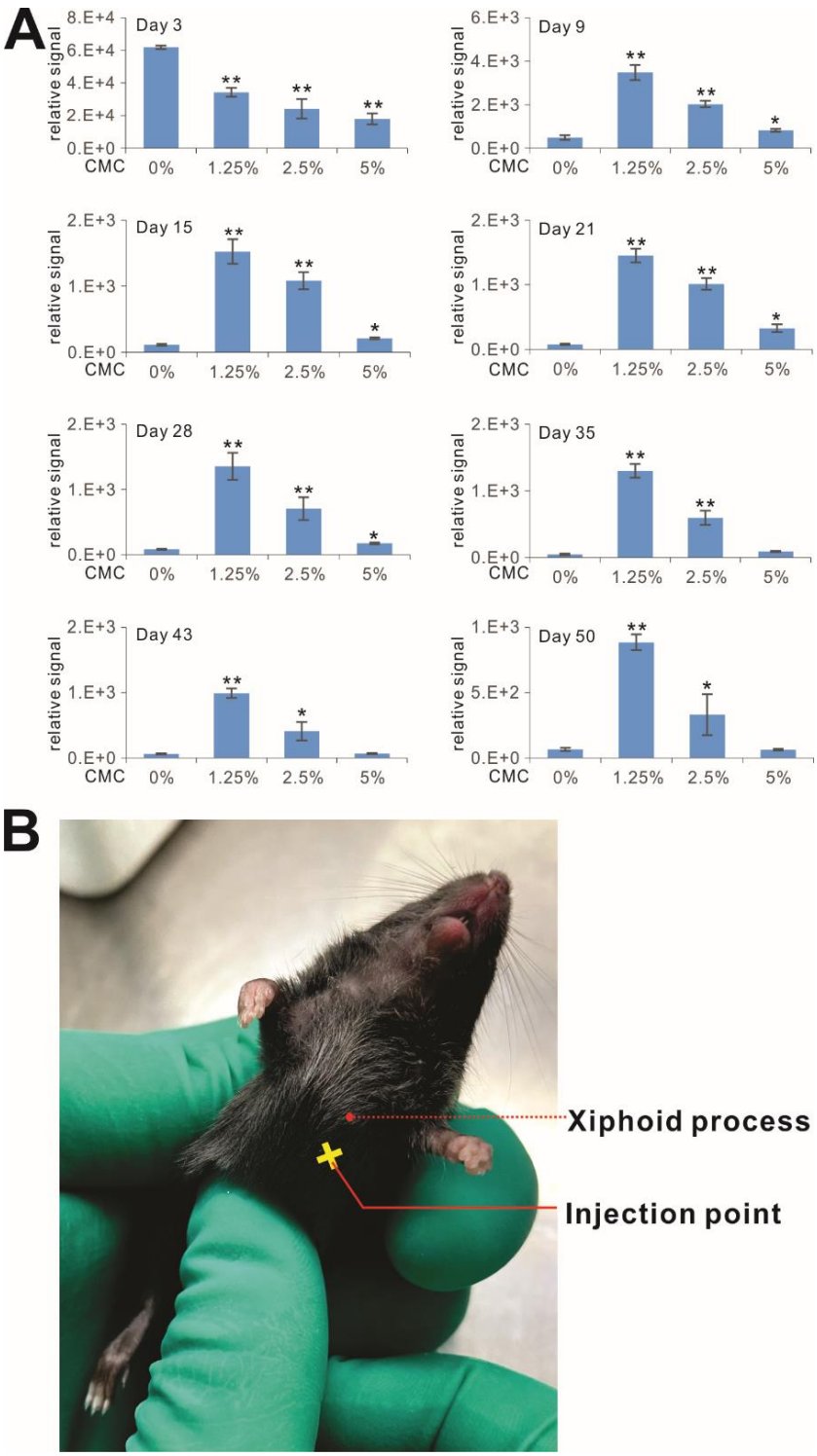

Figure S1. (A) Quantitative analysis of the Xenogen bioluminescence imaging data. The average relative signals (in photons/sec/cm2/steradian) were calculated for each time point by using Xenogen Living Image software. "*" $p<0.05$, "**" $p<0.01$, compared with that of the 0% CMC group. **(B) Location for intrahepatic adenovirus injection into mice**. The mouse xiphoid process served as a reference point. A 25G needle was inserted 5mm vertically in depth at 5~8mm below the xiphoid process reference point.

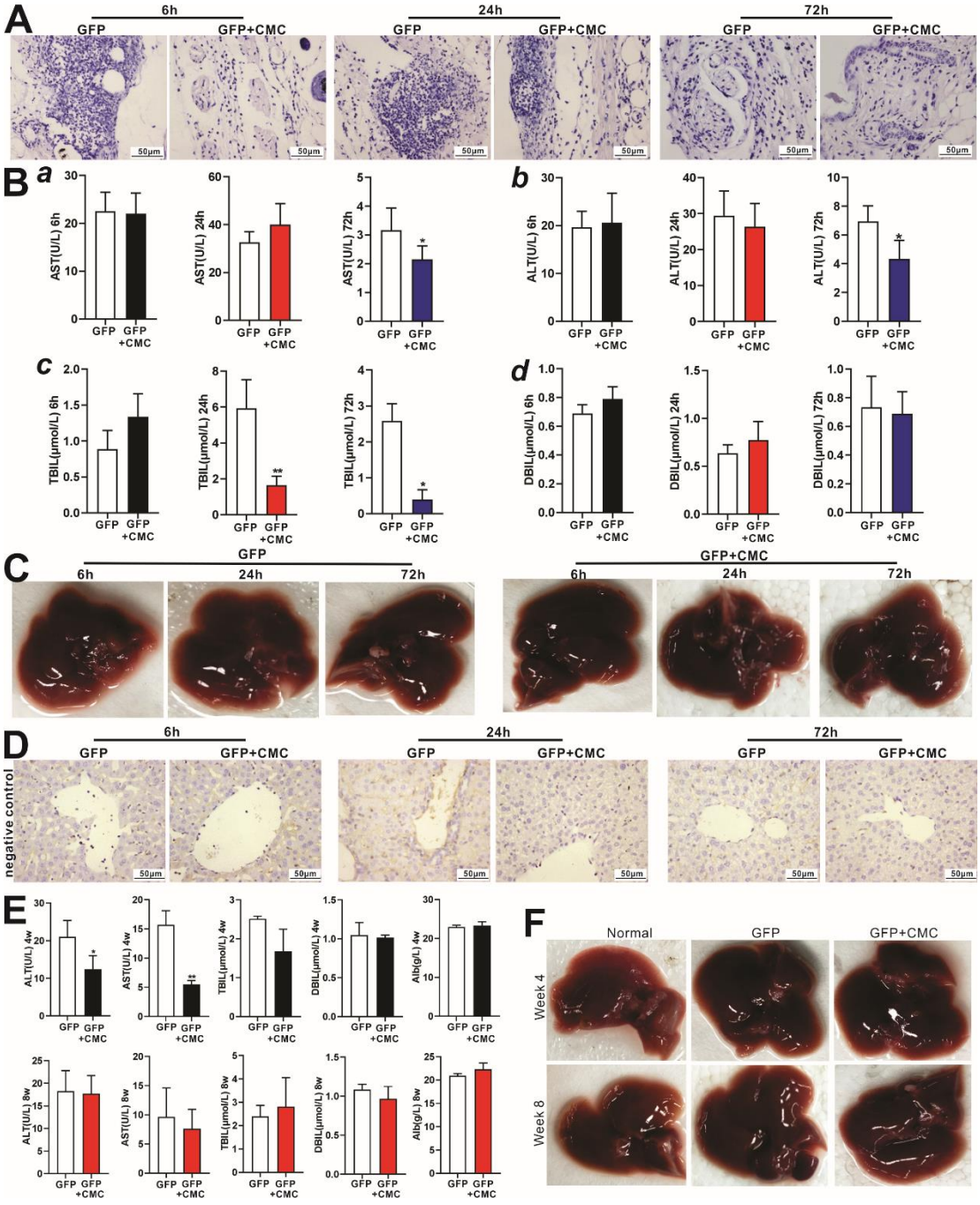

Figure S2. (A) IHC negative controls for **Fig. 2B-b**. The paraffin sections of skin samples at 6h, 24h and 72h were subjected to IHC staining without any primary antibody (x400). **(B)** The levels of serum AST (***a***), ALT (***b***), TBDL (***c***) and DBIL (***d***) at 6h, 24h and 72h for the samples in the **Fig. 3** were measured. "**" $p < 0.01$, "*" $p < 0.05$, GFP group vs. GFP+CMC group. **(C)** Representative gross images of liver samples in **Fig. 3** were obtained at 6h, 24h and 72h, respectively. **(D)** Paraffin sections of liver samples in **Fig. 3** were subjected to IHC staining without any primary antibody (x400). **(E)** The levels of serum ALT, AST, TBDL, DBIL and Alb in **Fig. 4** were measured at week 4 and 8, respectively. "**" $p < 0.01$, "*" $p < 0.05$, GFP group vs. GFP+CMC group. **(F)** Representative gross images of liver samples in **Fig. 4** were obtained at weeks 4 and 8, respectively.

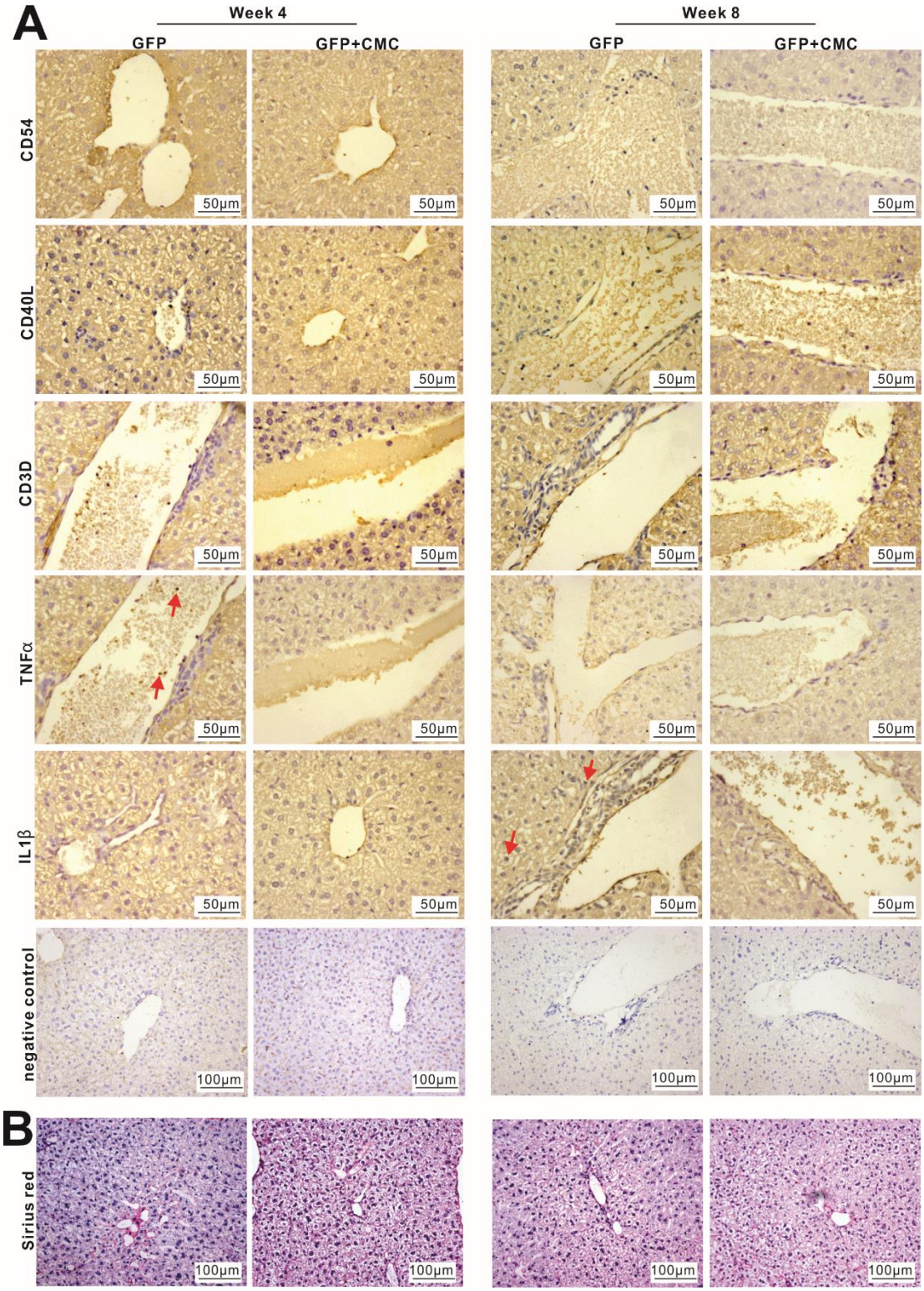

Figure S3. (A) The retrieved liver tissues in **Fig. 4** were subjected to IHC staining with primary antibodies against CD54, CD40L, CD3D, TNFα, and IL1β, or negative control (minus antibody), and representative positive stains are indicated with red arrows. Representative images are shown (x400). **(B)** The retrieved liver samples from weeks 4 and 8, respectively, in **Fig. 4** were subjected to Sirius red staining (x200).

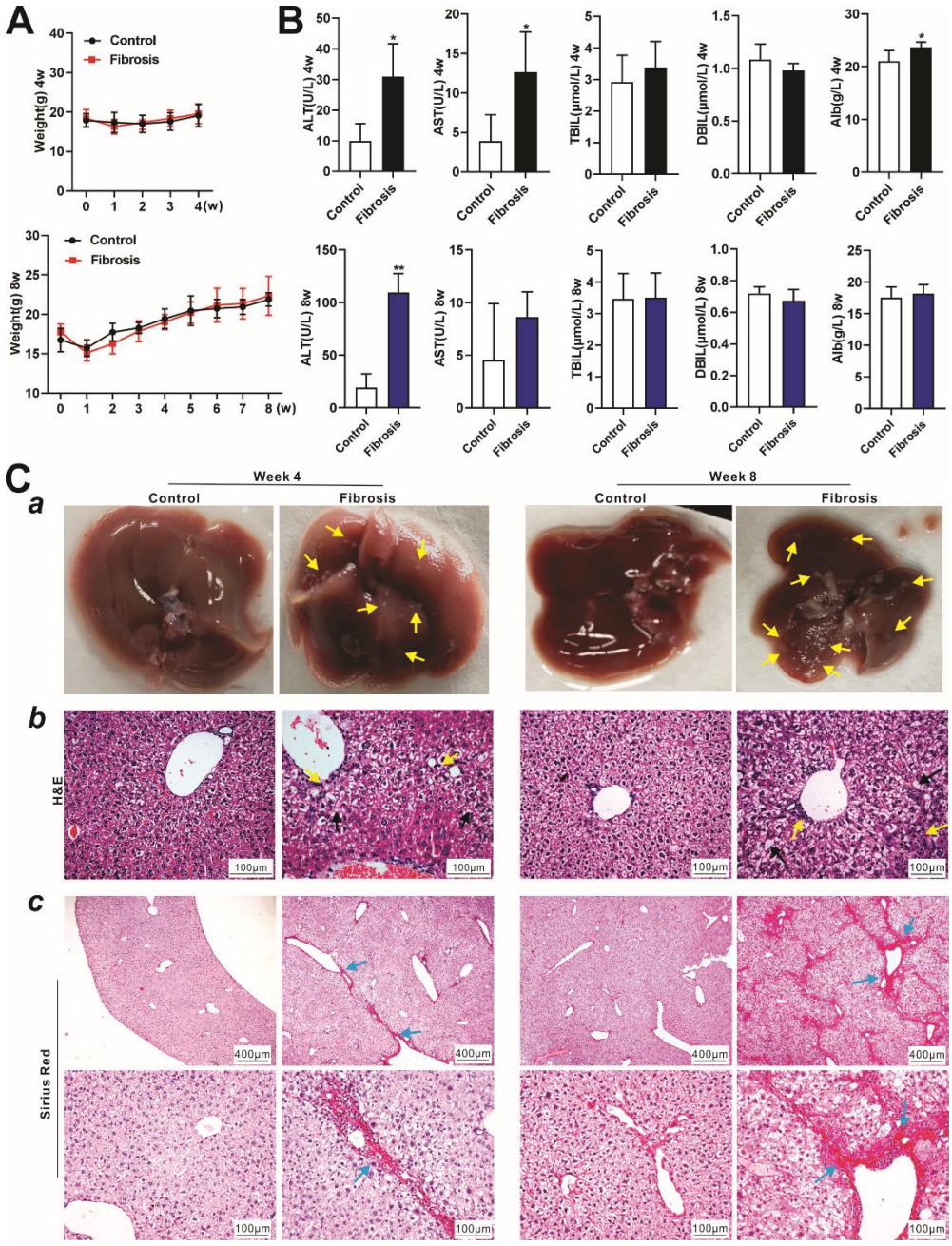

Figure S4. Characterization of the CCl_4-induced liver fibrosis mouse model. Intraperitoneal injection of CCl_4 was used to induce mouse hepatic fibrosis. Mice were sacrificed at weeks 4 and 8, respectively. **(A)** The dynamic weight changes during the course of the experiment. **(B)** The levels of serum ALT, AST, TBIL, DBIL and Alb were measured. "**" $p < 0.01$, "*" $p < 0.05$, Control group vs. Fibrosis group. **(C)** The gross appearance of the retrieved livers was observed, and representative fibrotic nodules are indicated with yellow arrows (***a***). The retrieved liver tissues were subjected to H & E staining (***b***). Representative hepatic fibrotic damage on hepatocytes and necrosis are indicated with black arrows, while representative infiltrating inflammatory cells are indicated by yellow arrows (x200). The retrieved liver tissues were also subjected to Sirius red staining, and the positively stained collagen fibers are indicated with blue arrows (x50 and x200) (***c***). Representative results are shown.

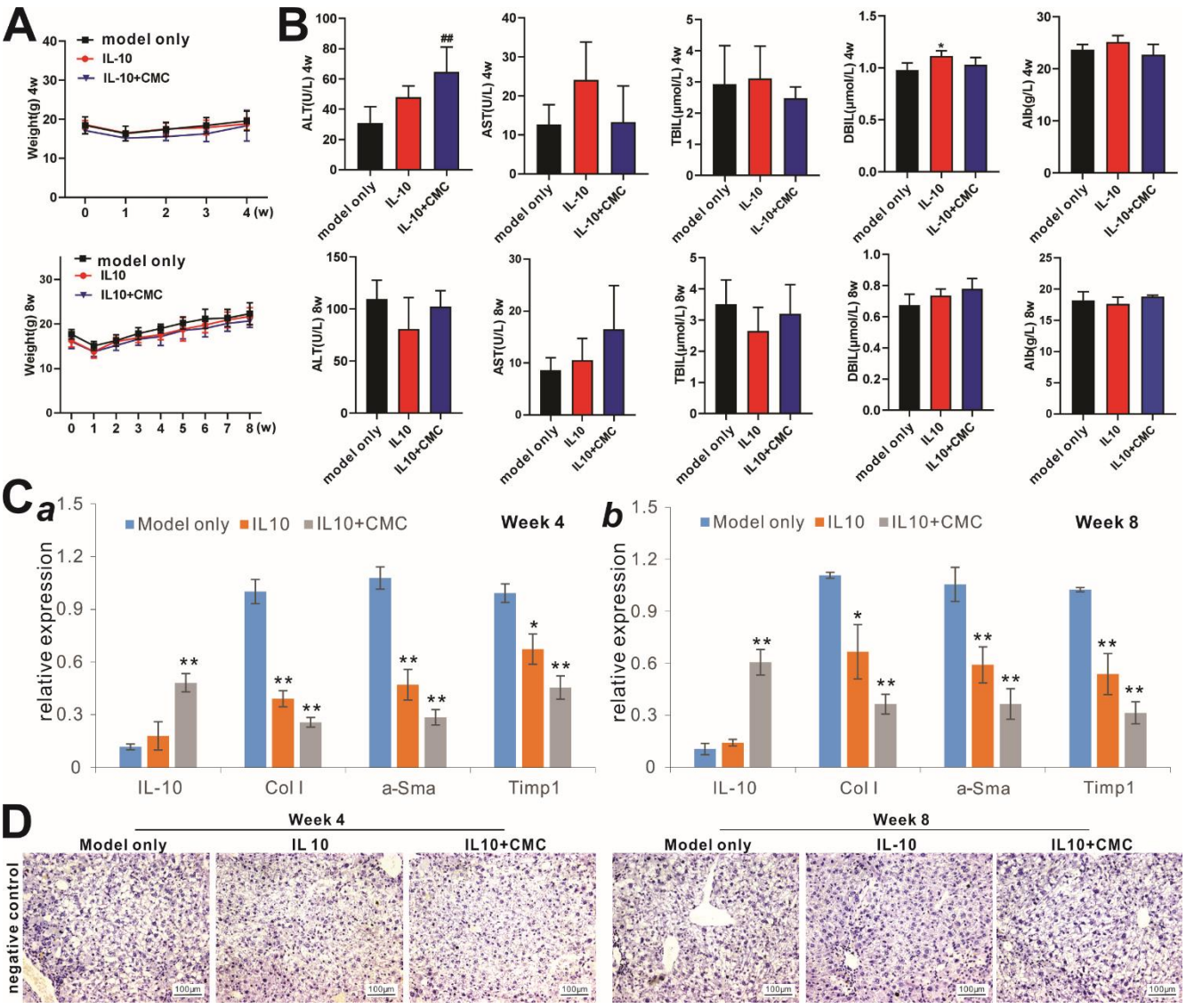

Figure S5. (A) The weekly dynamic weight changes of mice in **Fig. 5** were recorded for week 4 and week 8 groups, respectively. **(B)** The serum levels of ALT, AST, TBIL, DBIL and Alb were determined for week 4 and week 8 groups, respectively. "*" $p < 0.05$, Fibrosis "model only" group vs. IL-10 group. "##" $p < 0.01$, Fibrosis "model only" group vs. IL-10+CMC group. **(C)** Total RNA was extracted from mouse liver tissues. The expressions of IL10 and liver fibrosis related genes was detected by qPCR at week 4 and week 8 respectively. "*" $p < 0.05$, "**" $p < 0.01$, compared with the fibrosis "model only" group. **(D)** IHC negative controls for **Fig. 6**. Paraffin sections of liver samples from week 4 and week 8 groups in **Fig. 5** were subjected to IHC staining without primary antibody (x200). Representative images are shown.