Supplemental Methods

Inducible NASH model using wildtype mice

Eight-week-old wildtype mice were kept under standard diet (SD) for 4 weeks, or fed Western diet (WD) for 16 weeks, and were received a single injection of CCl_4 (WAKO, Osaka, Japan) intraperitoneally at a dose of 0.1 ml/kg diluted 1:40 in olive oil. Then, the mice were kept on SD or WD and were sacrificed at each time point.

Induction of hepatocyte death with anti-Fas antibody

After 4-week WD feeding, MC4R-KO mice received a single injection of anti-Fas antibody (Jo2, BD Bioscience, San Jose, CA) intravenously at a dose of 10 μg/kg. Then, the mice were kept on WD and were sacrificed at each time point.

Supplemental Table 1. Body and tissue weights in MC4R-KO mice reconstituted with bone marrow cells from CCR2-KO mice.

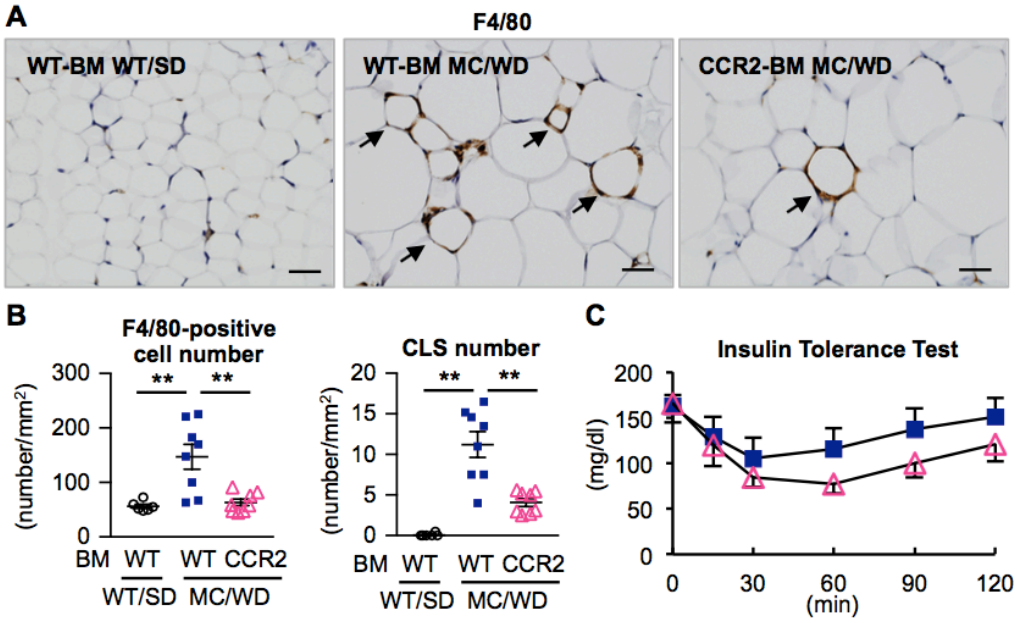
	WT/SD	MC4R/WD	
	WT-BM	WT-BM	CCR2 KO-BM
Body weight (g)	25.8 ± 0.3	46.8 ± 0.6**	44.0 ± 0.5
Liver (g)	1.28 ± 0.03	4.73 ± 0.28**	3.90 ± 0.22
Epididymal fat (g)	0.33 ± 0.01	1.45 ± 0.12**	1.65 ± 0.09
Blood glucose (mg/dl, *ad lib*)	129.6 ± 0.2	170.8 ± 6.9**	163.1 ± 14.0

WT, wildtype mice; MC4R, melanocortin 4 receptor-deficient mice; CCR2-KO, C-C chemokine receptor 2-deficient mice; SD, standard diet; WD, Western diet; BM, bone marrow. Data represent mean ± SEM. **$P < 0.01$ versus WT-BM WT/SD (Tukey-Kramer test). n = 5-9.

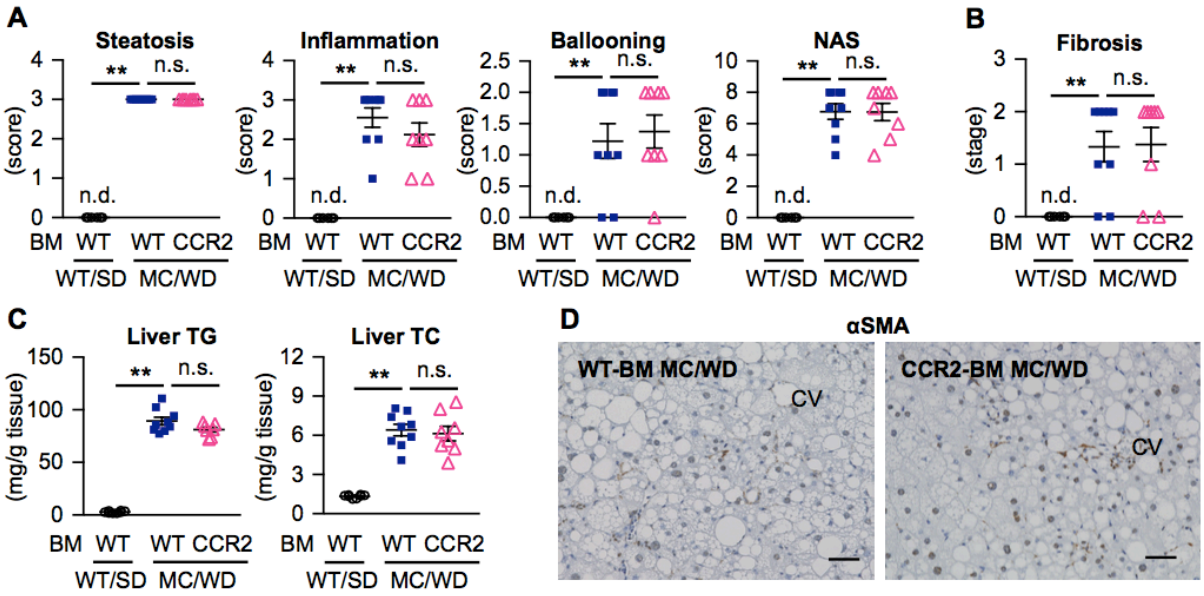
Supplemental Table 2. Primers used in this study

Genes	Primers
Ccr2	Fw: ACAAATCAAAGGAAATGGAAGACAAT
	Rv: TGCCGTGGATGAACTGAGG
Clec4f	Fw: GATGGGACACCATTCAACAATG
	Rv: CTCTCCGTTCCTATGTCTCCAGTT
Col1a1	Fw: CCTCAGGGTATTGCTGGACAAC
	Rv: ACCACTTGATCCAGAAGGACCTT
Emr1	Fw: CTTTGGCTATGGGCTTCCAGT
	Rv: GCAAGGAGGACAGAGTTTATCGTG
Itgam	Fw: TTACCTGGGTTATGCTTCTGCAG
	Rv: AAGCTTTGGACACGGTTCCTC
Itgax	Fw: GCCATTGAGGGCACAGAGA
	Rv: GAAGCCCTCCTGGGACATCT
Ly6c1	Fw: GCAGTGCTACGAGTGCTATGG
	Rv: ACTGACGGGTCTTTAGTTTCCTT
Siglec1	Fw: GCAGCCTCTTTCAATGCTAAGG
	Rv: TGTATTTGACGGTGTGATGACCA
Tgfb1	Fw: CCTGAGTGGCTGTCTTTTGACG
	Rv: AGTGAGCGCTGAATCGAAAGC
Timp1	Fw: CATCACGGGCCGCCTA
	Rv: AAGCTGCAGGCACTGATGTG
Tnfa	Fw: ACCCTCACACTCAGATCATCTTC
	Rv: TGGTGGTTTGCTACGACGT
18S	Fw: GTAACCCGTTGAACCCCATT

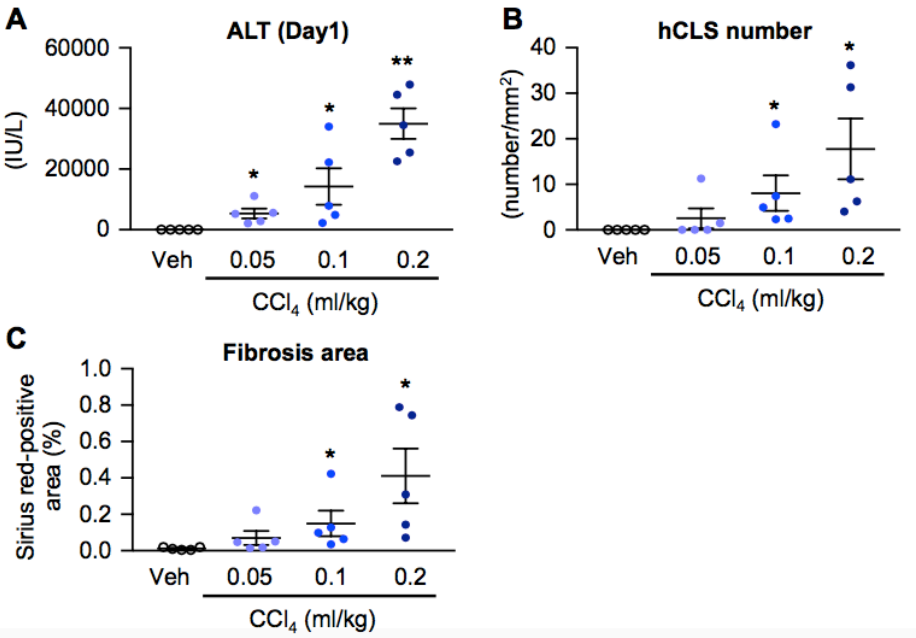
		Rv: CCATCCAATCGGTAGTAGCG
	36B4	Fw: GGCCCTGCACTCTCGCTTTC
		Rv: TGCCAGGACGCGCTTGT



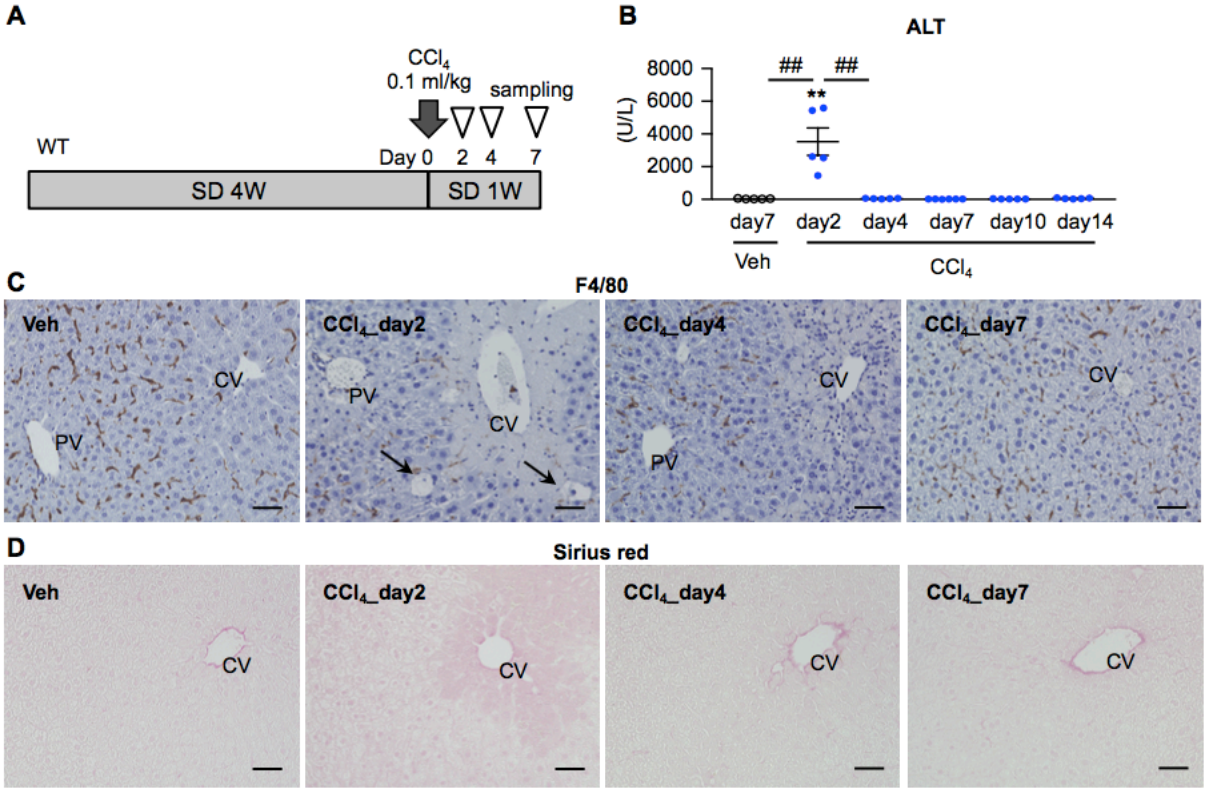
Supplemental Figure 1. Effect of CCR2 deficiency on inflammatory changes in the adipose tissue.
(A) F4/80 immunostaining in the epididymal fat from wildtype mice on standard diet (SD) and MC4R-KO mice reconstituted with CCR2-KO or wildtype mice-derived bone marrow cells on Western diet (WD) for 20 weeks. Scale bars, 50 μm. (B) The number of F4/80-positive cells and crown-like structure (CLS). (C) The insulin tolerance test at 11 weeks of WD feeding. MC4R-KO mice were challenged with 1 U/kg insulin after 1 hour fasting, and blood glucose was measured at indicated time point. Data represent mean ± SEM. ** $P < 0.01$ (Tukey-Kramer test). n = 5-9.



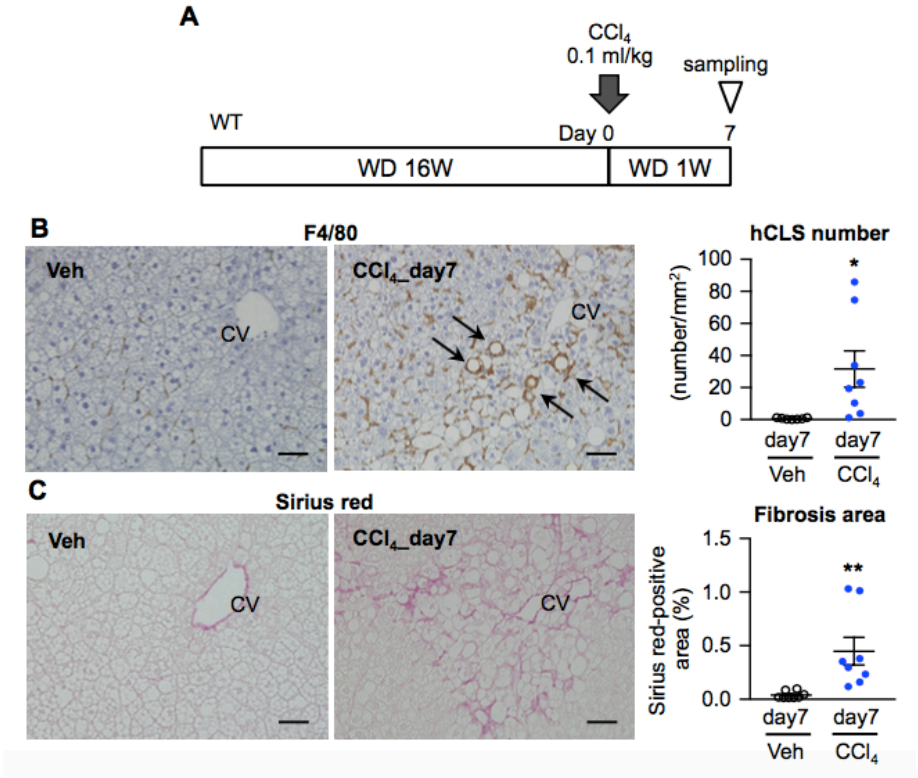
Supplemental Figure 2. Histological analysis and lipid content of the livers from bone marrow-specific CCR2-deficient MC4R-KO mice.
Histological scores for hepatic steatosis, lobular inflammation, ballooning degeneration, NAFLD activity score (NAS) (A), and fibrosis stage (B). (C) Hepatic content of triglyceride and total cholesterol. (D) Representative images of immunostaining for αSMA. CV, central veins. Scale bars, 50 μm. Data represent mean ± SEM. ** $P < 0.01$ (Tukey-Kramer test); n.s., not significant. $n = 5$-9.



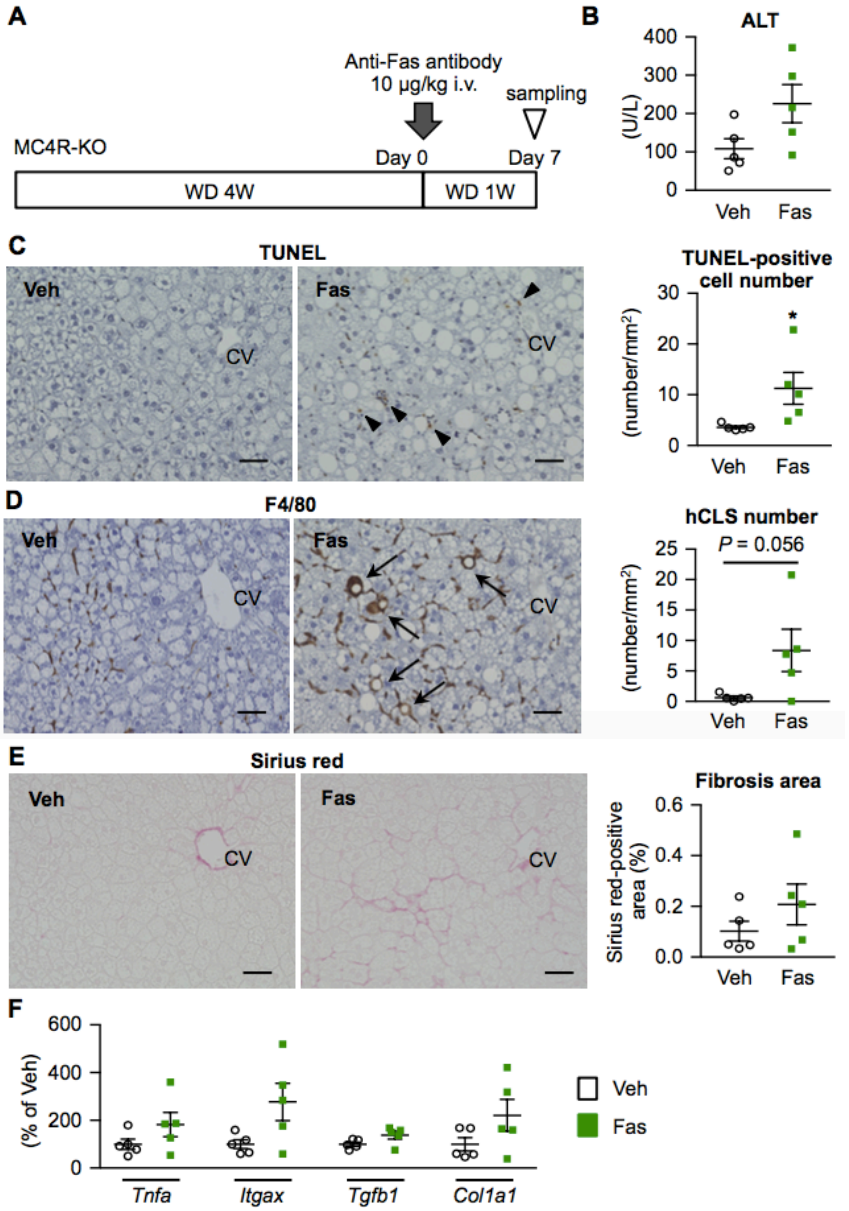
Supplemental Figure 3. Dose-dependent effect of CCl_4 on hCLS formation and liver fibrosis.
(A) Serum alanin aminotransferase (ALT) concentrations 1 day after CCl_4 injection. (B) hCLS number evaluated by F4/80 immunostaining. (C) Fibrosis area evaluated by Sirius red staining 7 days after CCl_4 injection. Data represent mean ± SEM. * $P < 0.05$, ** $P < 0.01$ vs. veh (day 7) (2-tailed unpaired Student's *t* test). $n = 5$.



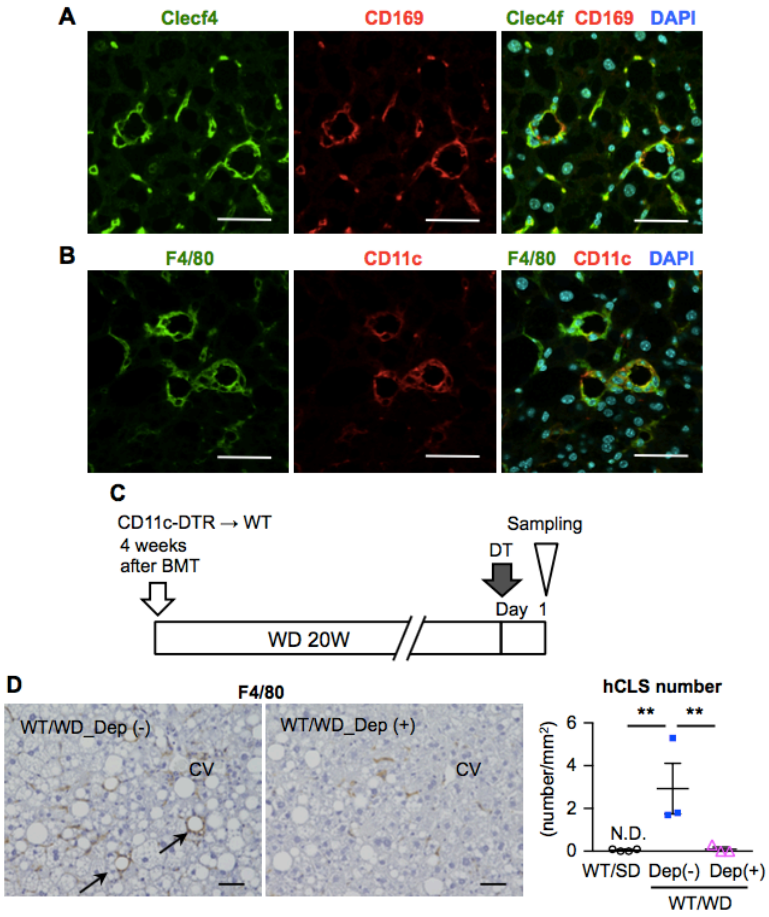
Supplemental Figure 4. Effect of low-dose CCl_4 on wildtype mice fed standard diet.
(A) Experimental protocol of CCl_4 injection to wildtype mice fed SD. Eight-week-old wildtype mice were kept under SD for 4 weeks, and received a single injection of CCl_4 at a dose of 0.1ml/kg or olive oil as vehicle (Veh) intraperitoneally. (B) Time course of serum ALT concentrations after CCl_4 administration. Data represent mean ± SEM. ** $P < 0.01$ vs. veh (day 7); ## $P < 0.01$ (Tukey-Kramer test). Representative images of F4/80 immunostaining (C) and Sirius red staining (D). Arrows, swollen hapatocytes. CV, central veins; PV, portal veins. Scale bars, 50 μm. n = 5-6.



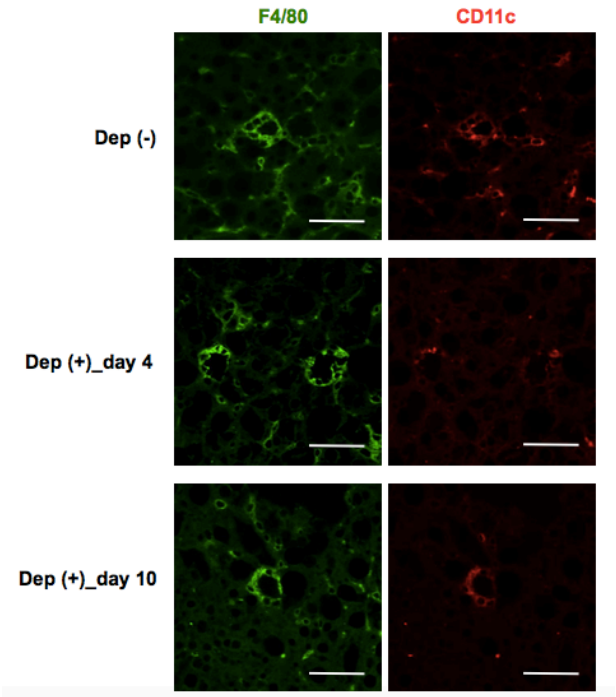
Supplemental Figure 5. Effect of low-dose CCl_4 on wildtype mice fed Western diet.
(A) Experimental protocol of CCl_4 injection to wildtype mice fed WD. Eight-week-old wildtype mice were fed WD for 16 weeks, and received a single injection of CCl_4 at a dose of 0.1ml/kg or olive oil as vehicle (Veh) intraperitoneally. (B) F4/80 immunostaining, and (C) Sirius red staining 7 days after CCl_4 injection. Arrows, hCLS. CV, central veins. Scale bars, 50 μm. Data represent mean ± SEM. * $P < 0.05$, ** $P < 0.01$ (2-tailed unpaired Student's *t* test). *n* = 7-8.



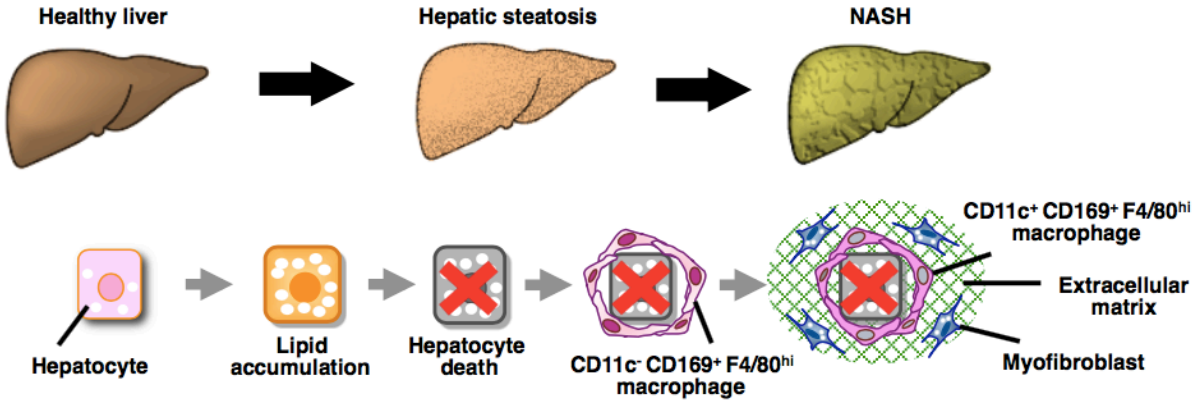
Supplemental Figure 6. Anti-Fas antibody-induced hepatocyte death and hCLS formation.
(A) Experimental protocol for induction of hepatocyte death by anti-Fas antibody. Eight-week-old MC4R-KO mice were fed WD for 4 weeks, and received injection of anti-Fas antibody at a dose of 10 μg /kg (Fas) or normal saline (Veh) intravenously. *n*= 5. (B) Changes in serum ALT levels 7 days after antibody injection. (C) TUNEL staining, (D) F4/80 immunostaining, and (E) Sirius red staining 7 days after antibody injection. Arrowheads, TUNEL-positive cells; Arrows, hCLS. Scale bars, 50 μm. (F) Hepatic mRNA expression of genes related to inflammation (*Emr1* (F4/80), *Tnfa*, and *Itgax* (CD11c)) and fibrogenesis (*Tgfb1* and *Col1a1*). Data represent mean ± SEM. * $P < 0.05$, ** $P < 0.01$ (2-tailed unpaired Student's *t* test).



Supplemental Figure 7. Role of CD11c-positive macrophages in hCLS formation in wildtype mice fed WD.
Representative images of immunofluorescent staining for Clec4f and CD169 (A), and F4/80 and CD11c (B) of the livers from wildtype fed WD for 8 months. (C) Experimental protocol for depletion of CD11c-positive cells in CD11c-DTR bone marrow-chimeric wildtype (CD11c-DTR-BM WT) mice fed WD for 20 weeks. (D) Representative images of F4/80 immunostaining and the number of hCLS. WT/SD, SD-fed WT-BM wildtype mice with DT treatment; Dep (-), WT-BM wildtype mice fed WD for 20 weeks with DT treatment; Dep (+), CD11c-DTR-BM wildtype mice fed WD for 20 weeks with DT treatment. Arrows, hCLS. scale bars, 50 μm. N.D., not detected. ** $P < 0.01$ (Tukey-Kramer test). $n = 3$-4.



Supplemental Figure 8. Expression of CD11c in the restored macrophages after diphtheria toxin treatment in the conventional NASH model using MC4R-KO mice. Representative images of immunofluorescent staining for F4/80 and CD11c of the livers from CD11c-DTR-BM MC4R-KO mice with or without macrophage depletion (4 and 10 days after DT treatment). Dep (-), WT-BM MC4R-KO mice fed WD for 20 weeks with DT treatment; Dep (+)_day4 or 10, CD11c-DTR-BM MC4R-KO mice fed WD for 20 weeks at each time point after DT treatment. scale bars, 50 μm.



Supplemental Figure 9. Potential role of CD11c-positive resident macrophages in hepatocyte death-triggered liver fibrosis in NASH.
During the development of NASH, resident ($CD169^{+}$ $F4/80^{hi}$) macrophages aggregate arround dying or dead hepatocytes to constitute hCLS, where CD11c-positive resident macrophages promote liver fibrosis. Our data suggests that these macrophages in hCLS become CD11c-positive in response to hepatocyte death, with unique polarization profiles. Therefore, CD11c-positive resident macrophages in hCLS would be a novel macrophage subset that drives hepatocyte death-induced liver fibrosis.