Supplementary Table 1. Body/Liver/Fat Weight and Lipid Level in WT, TLR9$^{-/-}$, IL-1R$^{-/-}$, and MyD88$^{-/-}$ Mice at 22 Weeks After Standard, CSAA and CDAA Diet Feeding

	WT			TLR9$^{-/-}$		
	Standard chow (n = 6)	CSAA diet (n = 5)	CDAA diet (n = 8)	Standard chow (n = 4)	CSAA diet (n = 4)	CDAA diet (n = 8)
Body weight at week 0 (*g*)	24.2 ± 0.75	22.4 ± 0.89	23.2 ± 1.60	23.8 ± 0.96	25.0 ± 0.82[a]	24.4 ± 0.98[a]
Body weight at week 22 (*g*)	30.8 ± 0.5	41.5 ± 2.22[b]	40.1 ± 2.76[b]	26.8 ± 1.18	31.5 ± 2.07[a,b]	28.2 ± 3.34[a]
Liver weight (*g*)	1.34 ± 0.16	2.40 ± 0.40[b]	2.48 ± 0.41[b]	1.11 ± 0.09[a]	1.31 ± 0.33[a]	1.23 ± 0.29[a]
Liver weight (%)	4.38 ± 0.49	5.75 ± 0.69[b]	6.17 ± 0.68[b]	4.13 ± 0.19	4.14 ± 0.79[a]	4.31 ± 0.58[a]
Epididymal fat (*g*)	0.74 ± 0.36	2.59 ± 0.35[b]	2.47 ± 0.33[b]	0.51 ± 0.09	1.08 ± 0.16[a,b]	0.89 ± 0.37[a,b]
Epididymal fat (%)	2.29 ± 0.84	6.21 ± 0.58[b]	6.17 ± 0.81[b]	1.90 ± 0.26	3.43 ± 0.33[a,b]	3.05 ± 1.00[a,b]
Plasma						
T-Bil (*mg/mL*)	0.85 ± 0.66	0.90 ± 0.22	1.19 ± 1.23	0.77 ± 0.21	0.58 ± 0.34	0.76 ± 0.40
ALT (*U/L*)	24.7 ± 6.25	44.0 ± 10.7[b]	122.0 ± 29.0[b,c]	13.0 ± 4.36[a]	32.0 ± 13.3	41.1 ± 16.2[a,b]
ALP (*U/L*)	118.2 ± 8.78	133.3 ± 12.2	165.9 ± 57.9	125.4 ± 16.6	126.0 ± 13.6	162.9 ± 30.8[c]
Triglyceride (*mg/dL*)	30.8 ± 0.92	43.1 ± 13.4	67.3 ± 5.85[b,c]	22.4 ± 0.69[a]	24.0 ± 3.15	23.2 ± 9.33[a]
Total cholesterol (*mg/dL*)	66.4 ± 13.5	161.2 ± 22.9[b]	107.2 ± 25.0[b,c]	54.2 ± 4.26	151.5 ± 12.3[a,b]	70.3 ± 32.0[c]
Free fatty acid (*mmol/L*)	0.29 ± 0.07	0.29 ± 0.12	0.30 ± 0.06	0.42 ± 0.05	0.35 ± 0.22[a]	0.43 ± 0.26
Liver						
Triglyceride (*mg/g liver*)	31.0 ± 10.3	87.3 ± 19.9[b]	163 ± 12.2[b,c]	28.0 ± 1.42	63.2 ± 9.52[b]	83.1 ± 28.5[a]
Total cholesterol (*mg/g liver*)	4.13 ± 2.40	25.5 ± 2.22[b]	25.9 ± 3.29[b]	2.11 ± 1.00	8.39 ± 1.46[b]	9.84 ± 4.67[a]
Free fatty acid (*μmol/g liver*)	0.56 ± 0.07	2.61 ± 0.17[b]	2.70 ± 0.20[b]	0.36 ± 0.33	0.69 ± 0.10	0.76 ± 0.11[a]

Note: Values are mean ± SD.
[a]Significantly different from WT, $P < .05$.
[b]Significantly different from standard chow, $P < .05$.
[c]Significantly different from CSAA, $P < .05$.

Supplementary Table 1. Continued

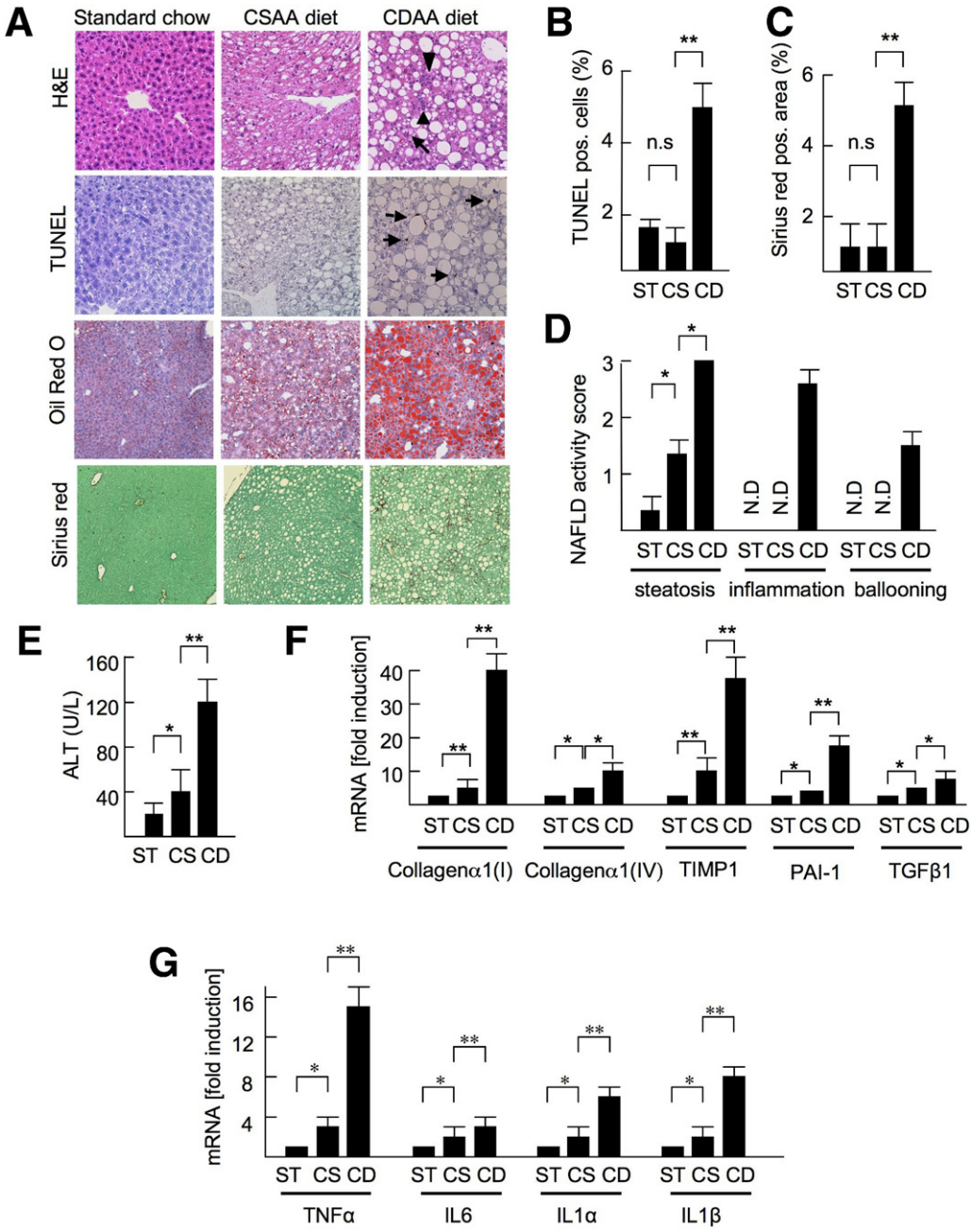
IL-1R$^{-/-}$			MyD88$^{-/-}$		
Standard chow (n = 4)	CSAA diet (n = 4)	CDAA diet (n = 8)	Standard chow (n = 4)	CSAA diet (n = 4)	CDAA diet (n = 7)
25.0 ± 1.00	25.0 ± 0.01[a]	24.9 ± 0.90	25.5 ± 0.71	25.0 ± 0.01[a]	24.6 ± 1.94
26.0 ± 0.77	32.0 ± 0.57[a,b]	38.4 ± 5.83[b,c]	25.7 ± 0.94	30.2 ± 5.42[a]	36.8 ± 4.20[b]
1.18 ± 0.07	1.50 ± 0.15[a,b]	2.23 ± 0.61[b,c]	0.96 ± 0.09[a]	1.35 ± 0.14[a,b]	2.03 ± 0.58[b,c]
4.53 ± 0.22	4.67 ± 0.46[a]	5.72 ± 0.84[b,c]	3.75 ± 0.20[a]	4.51 ± 0.34[a,b]	5.44 ± 1.02[b,c]
0.29 ± 0.16[a]	1.30 ± 0.06[a,b]	1.80 ± 0.71[b]	0.20 ± 0.07[a]	1.05 ± 0.72[a,b]	1.59 ± 0.65[a,b]
1.10 ± 0.61[a]	4.06 ± 0.18[a,b]	4.55 ± 1.41[a,b]	0.80 ± 0.04[a]	3.32 ± 1.79[a]	4.19 ± 1.36[a,b]
0.86 ± 0.20	0.90 ± 0.17	1.05 ± 0.56	1.01 ± 0.03	1.04 ± 0.42	1.16 ± 0.51
28.0 ± 7.21	39.3 ± 8.74	59.9 ± 14.5[a,b,c]	23.0 ± 2.83	28.7 ± 7.37[a]	41.6 ± 6.21[a,b,c]
103.0 ± 8.90[a]	157.5 ± 66.2	214.8 ± 96.2[b]	170.2 ± 53.2	180.1 ± 63.7	219.2 ± 27.9[a]
22.9 ± 9.15	21.3 ± 4.08[a]	21.8 ± 9.24[a]	29.3 ± 3.84	17.7 ± 6.82[a,b]	41.4 ± 8.86[a,b,c]
55.2 ± 12.6	102.9 ± 0.59[a,b]	68.6 ± 17.9[a,c]	59.7 ± 11.4	113.5 ± 4.32[a,b]	74.6 ± 14.9[a,c]
0.32 ± 0.06	0.59 ± 0.01[a,b]	0.27 ± 0.08[c]	0.35 ± 0.07	0.23 ± 0.02[b]	0.20 ± 0.06[a,b]
35.6 ± 3.43	55.0 ± 14.0[a,b]	84.4 ± 10.9[a,b,c]	34.9 ± 4.05	46.7 ± 1.58[a,b]	60.7 ± 39.2[a]
3.07 ± 0.36	5.31 ± 0.23[a,b]	6.74 ± 1.40[a,b]	1.97 ± 0.11	5.69 ± 0.22[a,b]	5.12 ± 2.73[a]
0.60 ± 0.04	0.92 ± 0.56	1.07 ± 0.56[a]	0.35 ± 0.02[a]	0.56 ± 0.19	0.69 ± 0.33[a]

Supplementary Table 2. Energy Composition of Standard, CSAA, and CDAA Diets

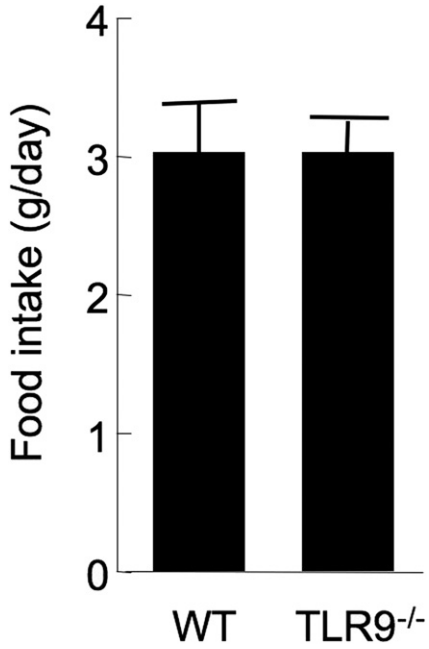
	Standard chow	CSAA diet	CDAA diet
Total calorie (*kcal/g*)	3.41	4.27	4.33
Carbohydrates (%)	62.1	63.2	68.5
Protein (%)	24.6	20.1	17.4
Fat (%)	13.2	16.2	14.0

Supplementary Table 3. Sequence of Primers Used for Real-Time Quantitative PCR

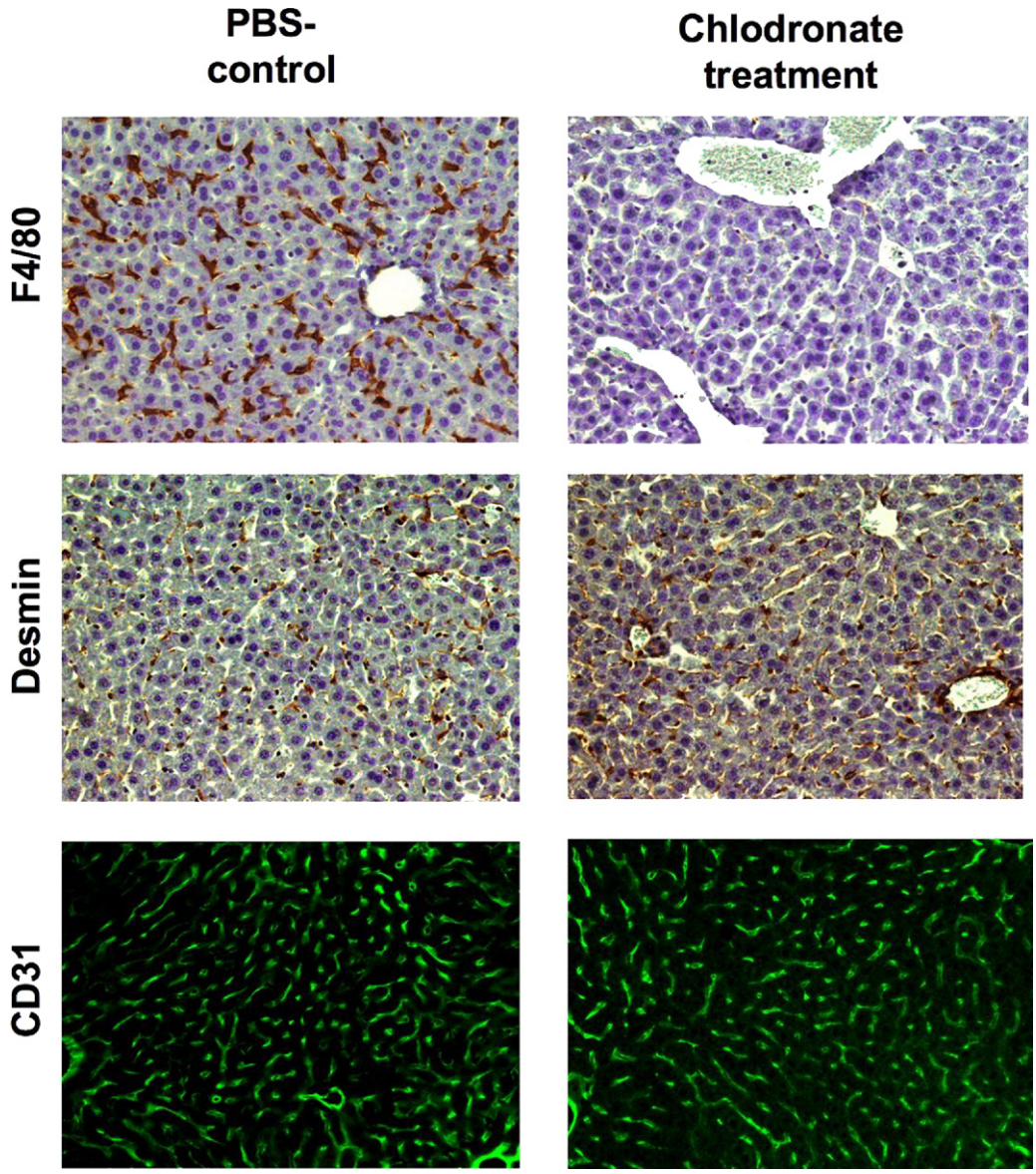
Gene	Forward	Reverse
18S	AGTCCCTGCCCTTTGTACACA	CGATCCGAGGGCCTCACTA
Bambi	TGAGCAGCATCACAGTAGCA	CGCCACTCCAGCTACTTCTT
Bcl2	CTTTCTGCTTTTTATTTCATGAGG	CAGAAGATCATGCCGTCCTT
Bax	GATCAGCTCGGGCACTTTAG	TTGCTGATGGCAACTTCAAC
DGAT1	TCACCACACACCAATTCAGG	GACGGCTACTGGGATCTGA
DGAT2	GAAGATGTCTTGGAGGGCTG	CGCAGCGAAAACAAGAATAA
Collagen α1(I)	TAGGCCATTGTGTATGCAGC	ACATGTTCAGCTTTGTGGACC
Collagen α1(IV)	CACATTTTCCACAGCCAGAG	GTCTGGCTTCTGCTGCTCTT
IL-1α	CCAGAAGAAAATGAGGTCGG	AGCGCTCAAGGAGAAGACC
IL-1β	GGTCAAAGGTTTGGAAGCAG	TGTGAAATGCCACCTTTTGA
IL-6	ACCAGAGGAAATTTTCAATAGGC	TGATGCACTTGCAGAAAACA
PAI-1	GCCAGGGTTGCACTAAACAT	GCCTCCTCATCCTGCCTAA
TGFβ1	GTGGAAATCAACGGGATCAG	ACTTCCAACCCAGGTCCTTC
TIMP1	AGGTGGTCTCGTTGATTTCT	GTAAGGCCTGTAGCTGTGCC
TNFα	AGGGTCTGGGCCATAGAACT	CCACCACGCTCTTCTGTCTAC



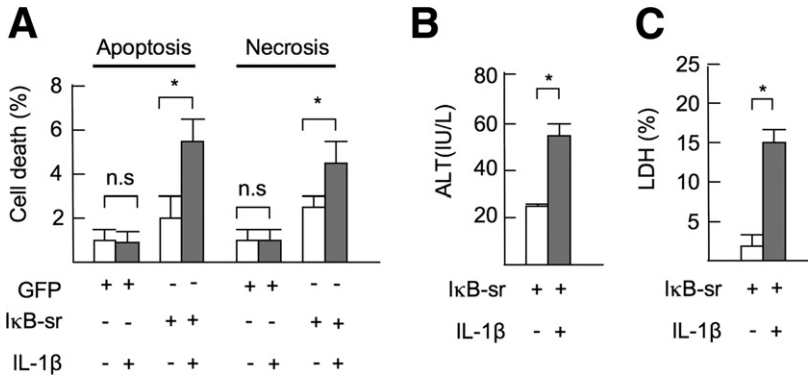
Supplementary Figure 1. CDAA diet-induced steatohepatitis mimics human NASH. WT mice were fed standard chow (ST, n = 6), CSAA (CS, n = 4), and CDAA (CD, n = 5) diets for 22 weeks. (*A*) Liver sections were stained with H&E, Oil Red O, TUNEL, and Sirius Red. Inflammatory cell infiltration (*arrowheads*) and ballooning hepatocytes (*arrow*) are seen in the CDAA diet group by H&E staining. *Arrows* indicate apoptotic cells in TUNEL staining. Perisinusoidal fibrosis is seen in the CDAA group by Sirius red staining. Original magnification, ×200 for H&E and TUNEL, ×100 for Oil Red O and Sirius red staining. (*B*) Number of TUNEL-positive cells, (*C*) Sirius red–positive area, and (*D*) NAFLD activity score were calculated. N.D, not detected. (*E*) Serum ALT level. (*F*) Hepatic mRNA levels of profibrogenic markers were determined by quantitative real-time PCR. Genes were normalized to 18S RNA as an internal control. Data represent mean ± SD; **P* < .05, ***P* < .01; n.s., not significant.



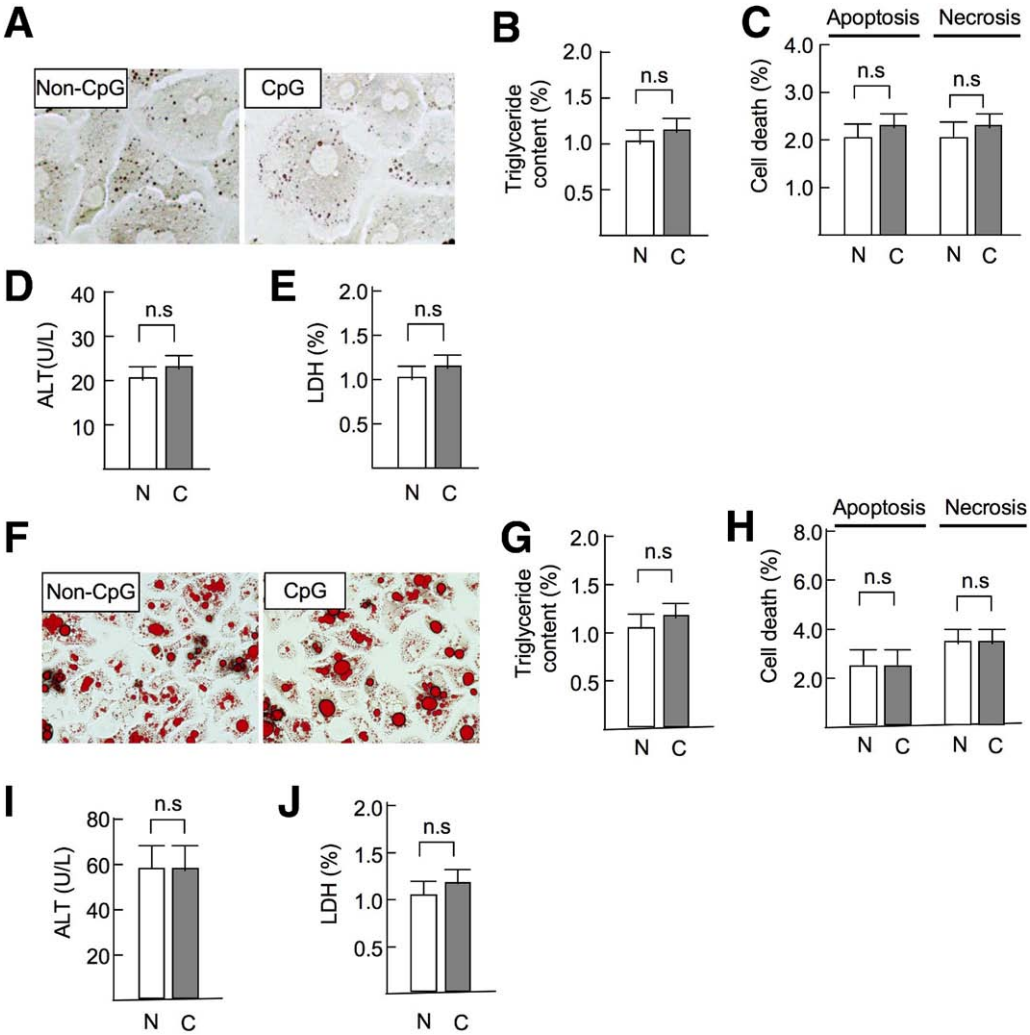
Supplementary Figure 2. Comparable food intake in WT and TLR9$^{-/-}$ mice. A CDAA diet intake was measured on WT and TLR9$^{-/-}$ mice.



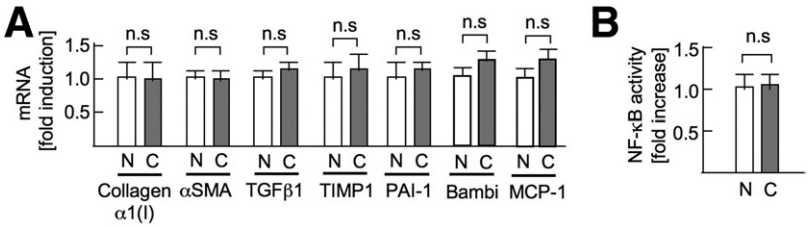
Supplementary Figure 3. Specific deletion of Kupffer cells, but not HSCs and sinusoidal endothelial cells, by liposomal clodronate treatment. (*A* and *B*) Liver samples were harvested 24 hours after the injection of liposomal clodronate. Kupffer cells, HSCs, sinusoidal endothelial cells were determined by immunohistochemistry for F4/80 (*A*), desmin (*B*), and CD31 (*C*). Liposomal clodronate injection completely depleted Kupffer cells, but it did not influence the number of HSCs and sinusoidal endothelial cells.



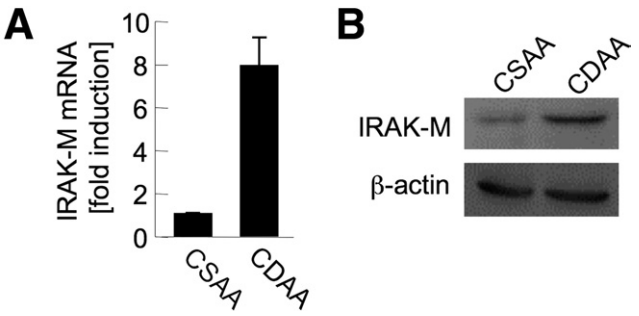
Supplementary Figure 4. Inhibition of NF-κB activation is essential for IL-1β–induced cell death. Inactivation of NF-κB increased the susceptibility to IL-1β–induced hepatocyte cell death. NF-κB activation in hepatocytes was inhibited by adenovirus expressing IB-super-repressor (IκB-sr). Cell death (*A*), ALT (*B*), and LDH (*C*) levels were examined (n = 4, each group).



Supplementary Figure 5. An effect of CpG-ODN on hepatocytes. A CpG-ODN induced neither lipid accumulation nor cell death in both normal hepatocyte (*A–E*) and lipid-accumulated hepatocytes (*F–J*). Hepatocytes were treated with 5 μg/mL CpG-ODN (C) or non–CpG-ODN (N) for 24 hours. (*A* and *F*) Oil Red O staining, (*B* and *G*) triglyceride content in hepatocytes, (*C* and *H*) cell death, (*D* and *I*) ALT, and (*E* and *J*) LDH levels in supernatant are shown (n = 4, each group). Original magnification, ×400 (A), ×200 (F).



Supplementary Figure 6. HSCs are not activated by TLR9 ligand. (*A* and *B*) WT stellate cells were treated with 5 μg/mL CpG-ODN (C) or non–CpG-ODN (N) for 8 hours followed by measurement of mRNA expression of profibrogenic markers by quantitative real-time PCR. (*B*) NF-κB activity in response to IL-1β was examined by the infection of adenovirus expressing NF-κB–driven luciferase. n.s., not significant.



Supplementary Figure 7. Strong IRAK-M expression in hepatocytes isolated from the CDAA diet. (*A* and *B*) Hepatocytes were isolated from WT mice fed a CSAA or CDAA diet for 22 weeks. (*A*) IRAK-M mRNA expression was determined by quantitative real-time PCR. (B) Immunoblots of IRAK-M are shown. Significant up-regulation of IRAK-M expression in hepatocytes from mice fed the CDAA diet was observed.