Supplementary Tables and Figures

CCR2 regulates the hepatic recruitment of myeloid cells that promote obesity-induced hepatic steatosis

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Supplemental Table 1 Chemokine receptor correlation with body mass

Genbank	Name	Gene	r	P Value
NM_009915	chemokine (C-C motif) receptor 2	*Ccr2*	0.4626356	0.01
NM_009911	chemokine (C-X-C motif) receptor 4	*Cxcr4*	0.2187263	>.05
---	chemokine-like receptor 1	*Cmklr1*	0.2065287	>.05
NM_017466	chemokine (C-C motif) receptor-like 2	*Ccrl2*	0.100153	>.05
NM_009914	chemokine (C-C motif) receptor 3	*Ccr3*	0.0947937	>.05
NM_011798	chemokine (C motif) receptor 1	*Xcr1*	0.0903048	>.05
NM_021609	chemokine binding protein 2	*Ccbp2*	0.0633085	>.05
NM_009917	chemokine (C-C motif) receptor 5	*Ccr5*	0.0550358	>.05
NM_009913	chemokine (C-C motif) receptor 9	*Ccr9*	0.050005	>.05
NM_009917	chemokine (C-C motif) receptor 5	*Ccr5*	0.0496397	>.05
NM_007718	chemokine (C-C motif) receptor 1-like 1	*Ccr1l1*	0.048858	>.05
NM_009912	chemokine (C-C motif) receptor 1	*Ccr1*	0.0175567	>.05
NM_007720	chemokine (C-C motif) receptor 8	*Ccr8*	0.0049005	>.05
NM_009835	chemokine (C-C motif) receptor 6	*Ccr6*	-0.0046136	>.05
NM_009916	chemokine (C-C motif) receptor 4	*Ccr4*	-0.0414812	>.05
NM_007719	chemokine (C-C motif) receptor 7	*Ccr7*	-0.113356	>.05
NM_009917	chemokine (C-C motif) receptor 5	*Ccr5*	-0.2096656	>.05
NM_145700	chemokine (C-C motif) receptor-like 1	*Ccrl1*	-0.2375284	>.05
NM_007722	chemokine orphan receptor 1	*Cmkor1*	-0.2576402	>.05
NM_009987	chemokine (C-X3-C) receptor 1	*Cx3cr1*	-0.2967323	0.05
NM_009910	chemokine (C-X-C motif) receptor 3	*Cxcr3*	-0.5354881	0.001

Supplemental Table 2: Chemokine correlation with body mass

Genbank	Name	Gene	r	P Value
NM_011333	chemokine (C-C motif) ligand 2	*Ccl2*	0.3946412	0.01
NM_027294	chemokine-like factor super family 8	*Cklfsf8*	0.3001117	0.05
NM_009142	chemokine (C-X3-C motif) ligand 1	*Cx3cl1*	0.2887943	>.05
NM_013652	chemokine (C-C motif) ligand 4	*Ccl4*	0.2840962	>.05
NM_009137	chemokine (C-C motif) ligand 22	*Ccl22*	0.2171073	>.05
NM_013655	chemokine (C-X-C motif) ligand 12	*Cxcl12*	0.1629795	>.05
NM_013653	chemokine (C-C motif) ligand 5	*Ccl5*	0.1471806	>.05
NM_021443	chemokine (C-C motif) ligand 8	*Ccl8*	0.1407212	>.05
NM_008176	chemokine (C-X-C motif) ligand 1	*Cxcl1*	0.138592	>.05
NM_009140	chemokine (C-X-C motif) ligand 2	*Cxcl2*	0.1288327	>.05
NM_008176	chemokine (C-X-C motif) ligand 1	*Cxcl1*	0.0761071	>.05
NM_019568	chemokine (C-X-C motif) ligand 14	*Cxcl14*	0.0714309	>.05
NM_011337	chemokine (C-C motif) ligand 3	*Ccl3*	0.0590564	>.05
NM_013655	chemokine (C-X-C motif) ligand 12	*Cxcl12*	0.0286748	>.05
NM_021274	chemokine (C-X-C motif) ligand 10	*Cxcl10*	-0.0072687	>.05
NM_009141	chemokine (C-X-C motif) ligand 5	*Cxcl5*	-0.0195215	>.05
NM_011329	chemokine (C-C motif) ligand 1	*Ccl1*	-0.0243389	>.05
NM_011331	chemokine (C-C motif) ligand 12	*Ccl12*	-0.0277421	>.05
NM_011330	small chemokine (C-C motif) ligand 11	*Ccl11*	-0.0407867	>.05
NM_153582	Chemokine-like factor super family 4	*Cklfsf4*	-0.0437703	>.05
NM_013654	chemokine (C-C motif) ligand 7	*Ccl7*	-0.1000884	>.05
NM_008599	chemokine (C-X-C motif) ligand 9	*Cxcl9*	-0.1288588	>.05
NM_026036	chemokine-like factor super family 6	*Cklfsf6*	-0.1479583	>.05
NM_018866	chemokine (C-X-C motif) ligand 13	*Cxcl13*	-0.1526731	>.05
NM_009142	chemokine (C-X3-C motif) ligand 1	*Cx3cl1*	-0.1589876	>.05
NM_011329	chemokine (C-C motif) ligand 1	*Ccl1*	-0.1852904	>.05
NM_011338	chemokine (C-C motif) ligand 9	*Ccl9*	-0.2296025	>.05
NM_024217	chemokine-like factor super family 3	*Cklfsf3*	-0.2374132	>.05
NM_011332	chemokine (C-C motif) ligand 17	*Ccl17*	-0.2383486	>.05
NM_009139	chemokine (C-C motif) ligand 6	*Ccl6*	-0.2572483	>.05
NM_009138	chemokine (C-C motif) ligand 25	*Ccl25*	-0.2602967	>.05
NM_019932	chemokine (C-X-C motif) ligand 4	*Cxcl4*	-0.2614796	>.05
NM_011888	chemokine (C-C motif) ligand 19	*Ccl19*	-0.2828253	>.05
NM_011336	chemokine (C-C motif) ligand 27	*Ccl27*	-0.2905451	>.05
NM_008510	chemokine (C motif) ligand 1	*Xcl1*	-0.3000183	0.05
NM_013655	chemokine (C-X-C motif) ligand 12	*Cxcl12*	-0.3684182	0.05
NM_133978	chemokine-like factor super family 7	*Cklfsf7*	-0.3690552	0.05
NM_011336	chemokine (C-C motif) ligand 27	*Ccl27*	-0.4151852	0.01
---	chemokine (C-C motif) ligand 21b /// chemokine (C-C motif) ligand 21a /// chemokine (C-C motif) ligand 21c (leucine)	*Ccl21b /// Ccl21a /// Ccl21c*	-0.5687912	0.001

Table 1-2: Correlation of expression of chemokine receptors and ligands with body mass. Microarray data derived from livers of 45 mice demonstrate correlation of *Ccr2* and *Ccl2* with body mass. Correlations are measured by pearson correlation.

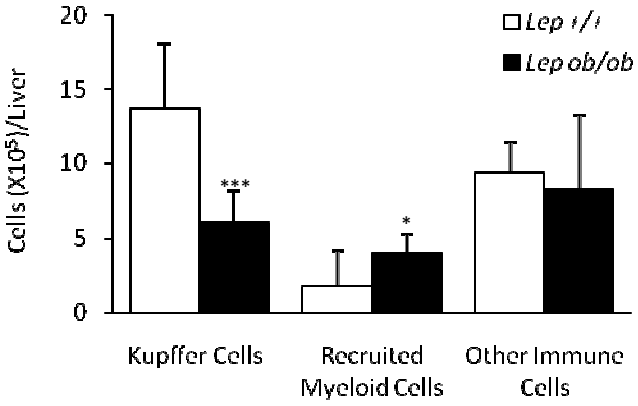
Supplemental Table 3: Acute phase genes correlation with *Ccr2* expression

Genbank	Name	Gene	r	P Value
NM_031167	interleukin 1 receptor antagonist	*Il1rn*	0.5014487	0.001
NM_023196	phospholipase A2, group XIIA	*Pla2g12a*	0.485602	0.001
NM_008458	serine (or cysteine) peptidase inhibitor, clade A, member 3C	*Serpina3c*	0.4842194	0.001
NM_011016	orosomucoid 2	*Orm2*	0.4616396	0.01
NM_011314	serum amyloid A 2	*Saa2*	0.395184	0.01
NM_008489	lipopolysaccharide binding protein	*Lbp*	0.3805643	0.01
NM_007576	complement component 4 binding protein	*C4bp*	0.3687146	0.05
NM_008198	complement factor B	*Cfb*	0.3641171	0.05
NM_023196	phospholipase A2, group XIIA	*Pla2g12a*	0.3389597	0.05
NM_011314	serum amyloid A 2	*Saa2*	0.3374719	0.05
NM_177091.3	fibronectin type III domain containing 7	*Fndc7*	0.3185578	0.05
NM_013737	phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	*Pla2g7*	0.3163691	0.05
NM_011316	serum amyloid A 4	*Saa4*	0.312971	0.05
NM_010554	interleukin 1 alpha	*Il1a*	0.4504065	0.01
NM_013693	tumor necrosis factor alpha	*Tnf*	0.305358	0.05
NM_031168	interleukin 6	*Il6*	0.3601626	0.05
NM_008361	interleukin 1, beta	*Il1b*	0.3131237	0.05

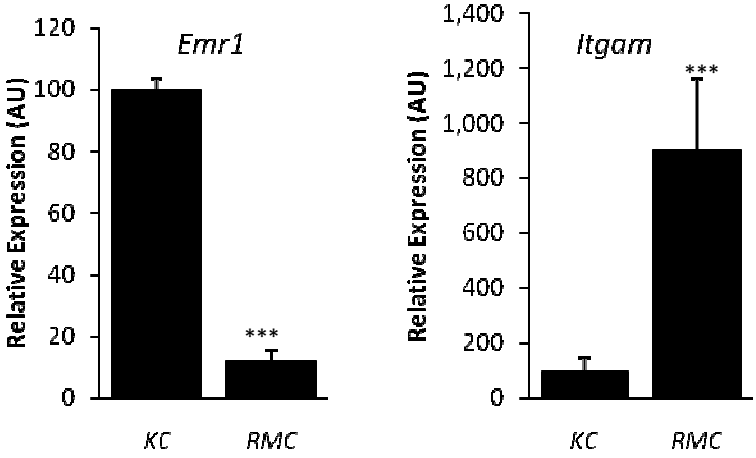
Table 3: Correlation of expression of Ccr2 and various acute phase response genes and cytokines in liver.

Supplemental Table 4 - PCR Primers

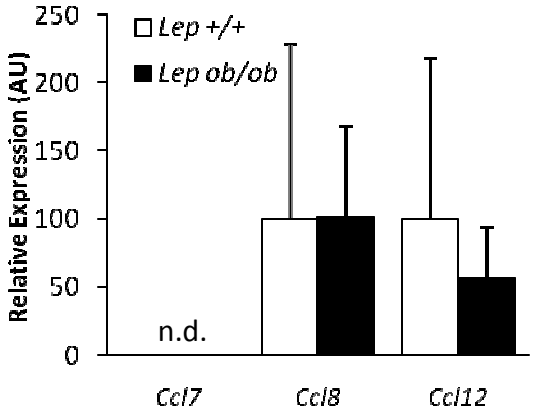
Gene	Primers
	5'-TGCCATCATAAAGGAGCCA -3'
Ccr2	5'-AGCACATGTGGTGAATCCAA -3'
	5'-AGGTCCCTGTCATGCTTCTGG-3'
Ccl2	5'-CTGCTGCTTGGTGATCCTCTTG-3'
	5'-CCAGACCCTCACACTCAGATCA-3'
Tnf	5'-CACTTGGTGGTTTGCTACGAC-3'
	5'-CTTTGGCTATGGGCTTCCAGTC-3'
Emr1	5'-GCAAGGAGGACAGAGTTTATCGTG-3'
	5'-ATTCGGTGATCCCTTGGATT -3'
Itgam	5'-GTTTGTTGAAGGCATTTCCC -3'
	5'-AAG GGC TGG CAT TGT CTC TAA-3'
Icam1	5'-CTC CTC AGT CAC CTC TAC CAA GG-3'
	5'-GCA AAG TGG AAT CAA ACC GTA-3'
Vcam1	5'-GAA GGG GAG TCA CAG CCA ATA G-3'
	5'-TCT GGG TAC AAG ATC CCT GAA -3'
Chi3l3	5'-TTT CTC CAG TGT AGC CAT CCT T -3'
	5'-GCTTCGGCTCAGGGTCTAC -3'
Jag1	5'-GGCGAAACTGAAAGGCAGTA -3'
	5'-TACAGCTCCACGCTATGGATT-3'
Mrc2	5'-CACTCTCCCAGTTGAGGTACT-3'
	5'-CTCCAAGCCAAAGTCCTTAGAG-3'
Arg1	5'-AGGAGCTGTCATTAGGGACATC-3'
	5'-GCTCTCGTCCTTCTTCCTCA -3'
Ppargc1b	5'-GAGGTCAAGCTCTGGCAAGT -3'
	5'-GCGACATGATTAATGGCACA -3'
Cd36	5'-CCTGCAAATGTCAGAGGAAA -3'
	5'-TTTGGCTCCAGAGTTTGACC -3'
Lpl	5'-TGTGTCTTCAGGGGTCCTTAG -3'
	5'-GGTACATGTGGGAGTACCCG -3'
Acadl	5'-TCTTGCGATCAGCTCTTTCA -3'
	5'-GCTCGTGAGCACATTGAAAA-3'
Acadm	5'-CATTGTCCAAAAGCCAAACC-3'
	5'-CCCGTATTTGAGATCCGTGTT-3'
Adfp	5'-TAGGTATTGGCAACCGCAAT-3'
	5'-CTA CAC GGA GGA AGA AGC CAA G -3'
Cyc1	5'-AGC CAG TGA GCA GGG AAA ATA C -3'
	5'-GCATTCCTTCCCATTTGACAC-3'
Cpt1a	5'-TACAGGTGCTGGTGCTTTTCAC-3'
	5'-TGCCTGGATGAAGTTTGATG -3'
Pck1	5'-TCTGGATGGTTTTAATGGCA -3'
	5'-ACTGTGGGCATCAATCTCCT -3'
G6pc	5'-GTGTCCAGGACCCACCAATA -3'
	5'-TCTTTCTAACAACCACCCTCTGG-3'
Fasn	5'-CTTCACGACTCCATCACGAATG-3'
	5'-GGTGGAAGTAGGCACAGAAGTG-3'
Acaca	5'-TGTCAGGCGAATGTTGATTTTC-3'
	5'-CCCAAGCTTGTGAATCAAGG -3'
Gpam	5'-CCTCTTCTGCCACAACATCA-3'
	5'-CGCAGCGAAAACAAGAATAA -3'
Dgat2	5'-GAAGATGTCTTGGAGGGCTG -3'
	5'-GGCACTAAGTGCCCTCAACCT-3'
Srebf1	5'-GCCACATAGATCTCTGCCAGTGT-3'
	5'-GCC CTT TGG TGA CTT TAT GGA G-3'
Pparg	5'-GCA GCA GGT TGT CTT GGA TG-3'
	5'-GAAGGGGGATCTTCAGCTTT-3'
Ccl8	5'-TCTTTGCCTGCTGCTCATAG-3'
	5'-TTCCTCTTGGGGATCTTTTG-3'
Ccl7	5'-CTGCTTTCAGCATCCAAGTG-3'
	5'-CTTCCGGACGTGAATCTTCT-3'
Ccl12	5'-AGTCCTCAGGTATTGGCTGG-3'
	5'-CAGCAAGTTCCATCGTGTCATC-3'
Ppib	5'-CTCTTTCCTCCTGTGCCATCTC-3'
	5'-AAACGGCTACCACATCCAAG-3'
18S	5'-CCTCCAATGGATCCTCGTTA-3'



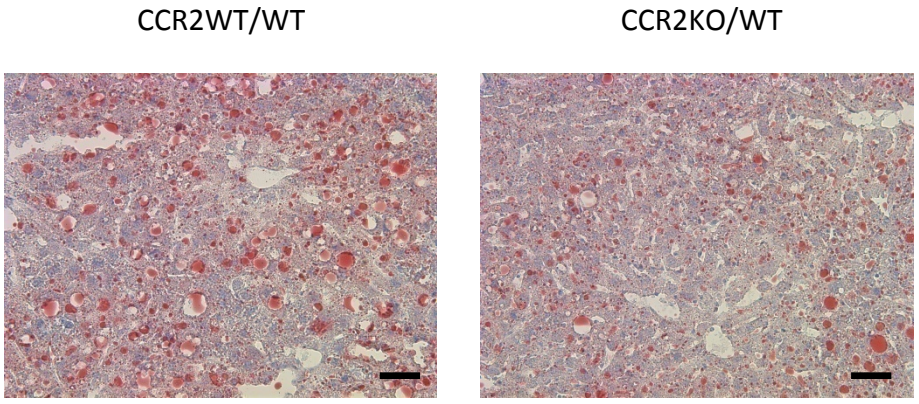
Supplemental Figure 1: The absolute number of Kupffer cells in livers is decreased and CD11b+ F4/80dim is increased in obese (Lepob/ob) compared to lean (Lep+/+) mice, while the total of other immune cells is unaltered by obesity.



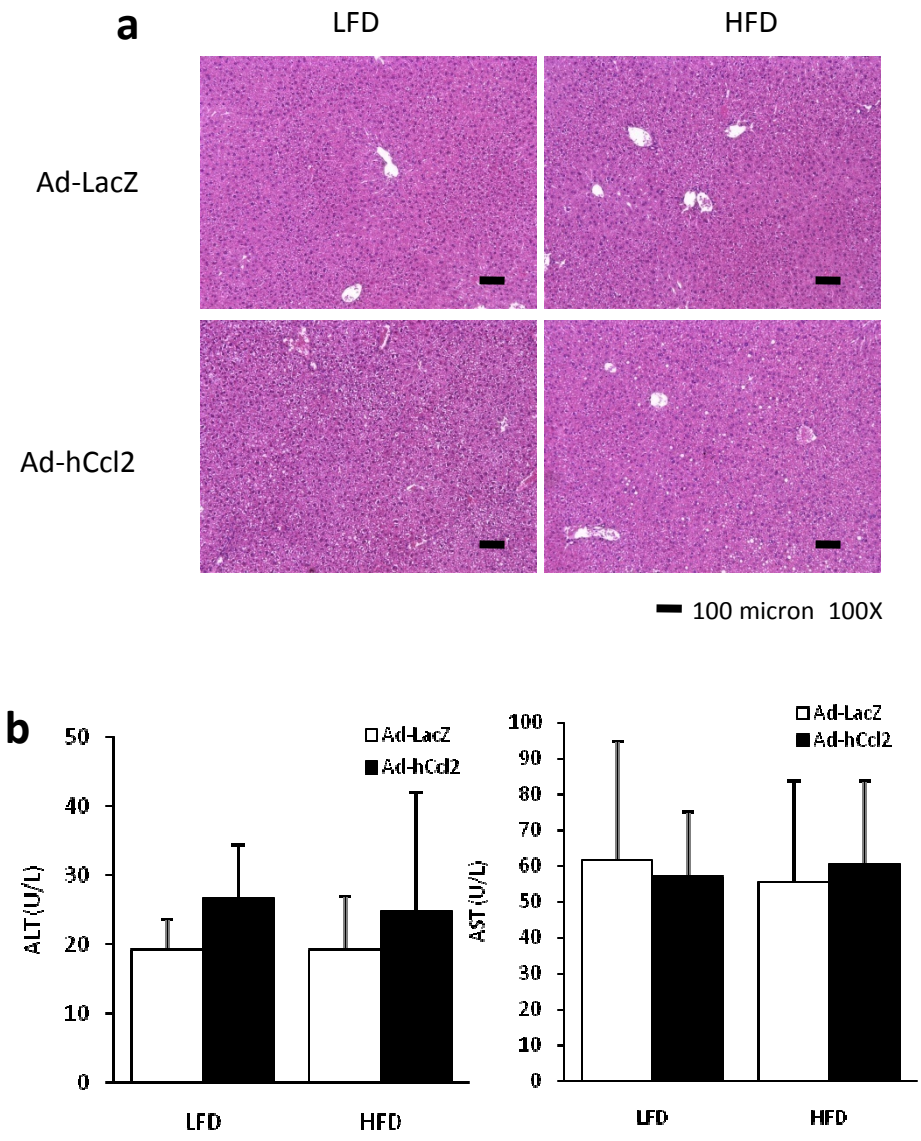
Supplemental Figure 2: Liver nonparenchymal cells from C57BL/6J obese Lepob/ob mice and lean Lep+/+ controls were stained with antibodies as depicted in Figure 2a, and were FACS sorted into FACS buffer. In addition to identifying cells as depicted in Figure 2b, neutrophils were removed by staining for Ly6g and gating positive cells out. RNA was extracted and quantitative PCR performed on cDNA for expression of genes that were part of the sorting criteria.



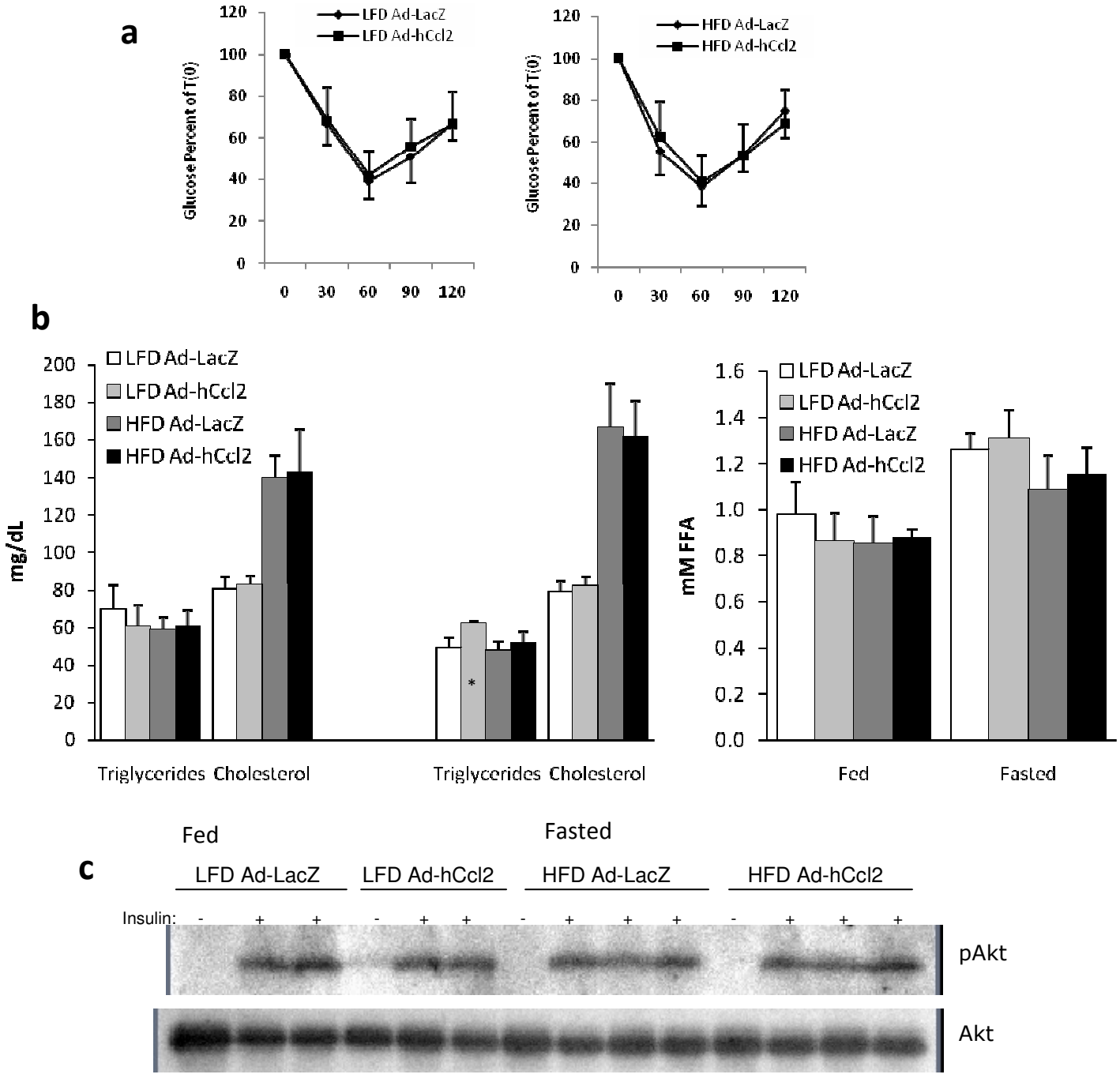
Supplemental Figure 3: RNA from livers used in Figure.1b were assessed for relative expression of CCR2 ligands other than Ccl2 by quantitative PCR. Ccl7 could not be detected.



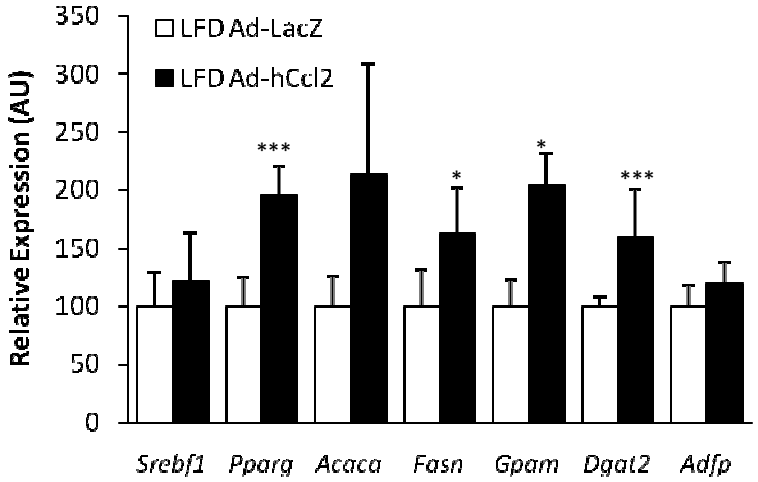
Supplement Figure 4: Hematopoietic deficiency of CCR2 ameliorates HFD induced hepatosteatosis. Frozen liver sections from mice fed HFD for 36 weeks were stained with Oil red O. Bar is equivalent to 50 microns.



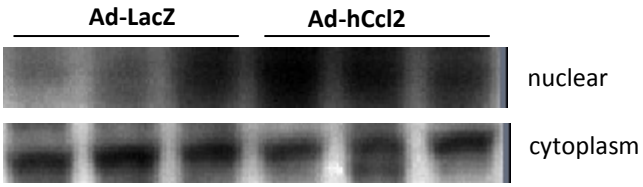
Supplement Figure 5: Mice injected with Ad-hCcl2 or Ad-LacZ were assessed for frank hepatitis by assessment of liver histology (a) and measurement of serum transaminases (b). Bar is equivalent to 100 microns (100X magnification). For transaminases no differences were detectable between Ad-hCcl2 and Ad-LacZ treated animals ($p > 0.1$)



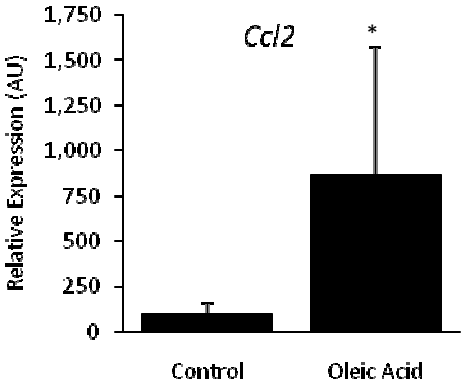
Supplemental Figure 6: Insulin sensitivity was not different between mice expressing h*Ccl2* (Ad-hCcl2) or control (Ad-LacZ) virus (a).No difference was found in serum concentrations of TG, total cholesterol and free fatty acids as well (b). For total serum triglycerides, cholesterol, and free fatty acids $p > 0.2$ (comparisons are within diet groups and between Ad-LacZ and Ad-hCcl2 groups), except fasted TG ($p = 0.04$) was higher in low fat fed mice expressing hCcl2 compared to low fat fed control mice. Figure (c) depicts biochemical assessment of liver specific insulin sensitivity by western blotting for insulin stimulated (5 U/kg) phosphorylation of Akt (S473) and a duplicate membrane simultaneously loaded with identical amounts of total protein probed for total Akt.



Supplemental Figure 7: RMC recruitment induced expression of genes involved in TG formation without changing hepatic TG mass. Mice sacrificed two weeks after adenoviral injection. Data represent mean S.D., *- $p < 0.05$; **- $p < 0.01$; ***- $p < 0.005$.



Supplemental Figure 8: CCL2 mediated RMC recruitment induced nuclear localization of mature form of SREBP1c in HFD mice. Nuclear and cytoplasmic fractions were isolated from whole liver, protein extracted and equal amounts of protein were probed using SREBP1c specific antibody. Mice were sacrificed three weeks after adenoviral injection.



Supplemental Figure 9:. Intravenously infused oleic acid (6 mM) or saline for 6 hours induced hepatic expression of Ccl2 expression in lean C57BL/6J mice (n = 4-6)