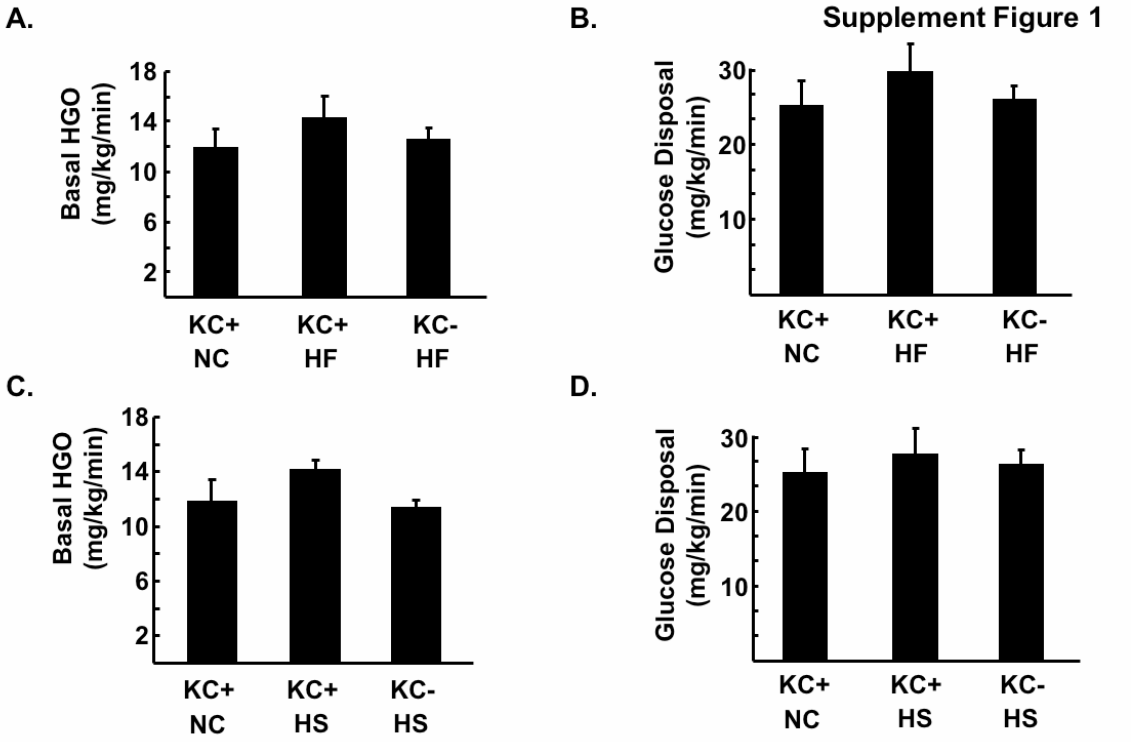
ONLINE APPENDIX – Supplemental Table and Figures

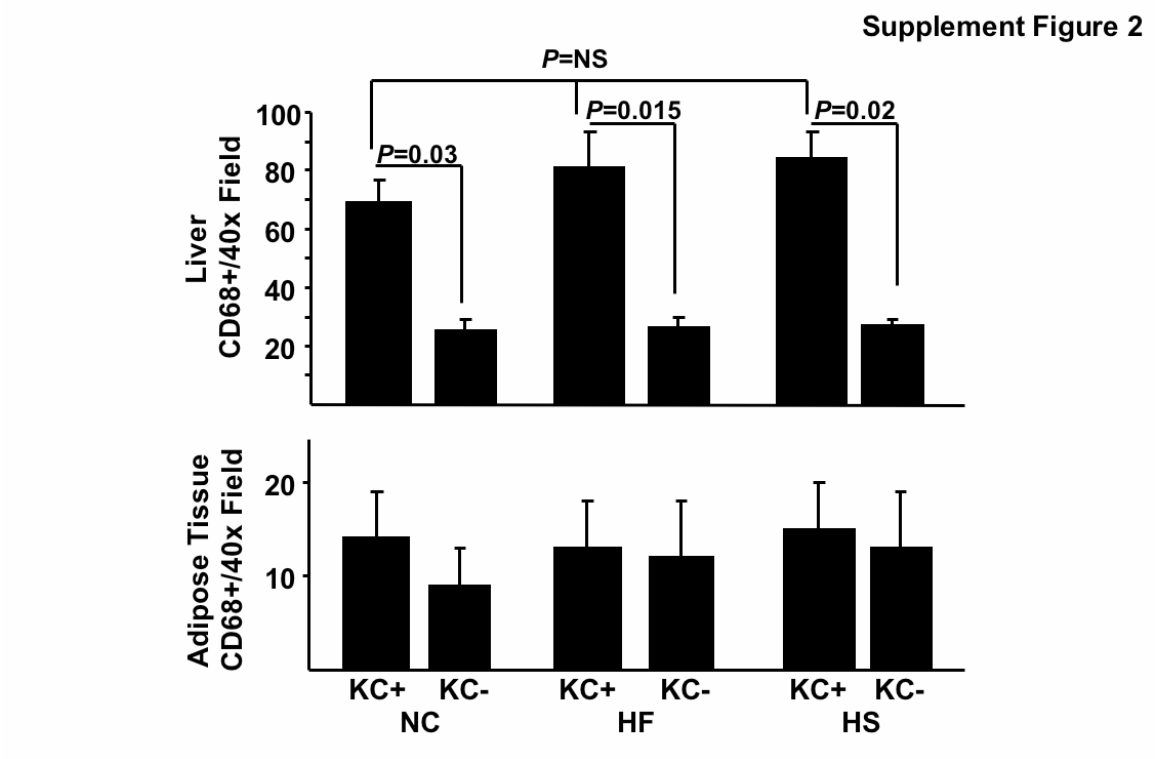
Supplemental Table 1.

qRT-PCR primer sequences used for the assessment of liver gene expression
F4/80
for-ATGACCACACTTGCCATCCT
rev-GGCGAGTCGCTTCTAAGACA
CD68
for-TGGACTCAGCAGCTCTACCA
rev-CCTGTGGGTGGTCGTAGG
PPARa,
for-AAGCCATCTTCACGATGCTG
rev-TCAGAGGTCCCTGAACAGTG
CPT1,
for-TATGTGAGGATGCTGCTTCC
rev-CTCGGAGAGCTAAGCTTGTC
MTTP
for-GAACCTGAGAACCTGTCCAACG
rev-TGAACTTGCTAAGGAGGGCTTG
ACC1
for-TGTCTATTCGGGGTGACTTTCG
rev-CCGTTCATTATCACCACGTAGG
LCAD
for-AAGGATTTAAGGGCAAGAAGC
rev-GGAAGCGGAGGCGGAGTC
DGAT2
for-ATCTTCTCTGTCACCTGGCT
rev-CCTTTCTTGGGCGTGTTCC

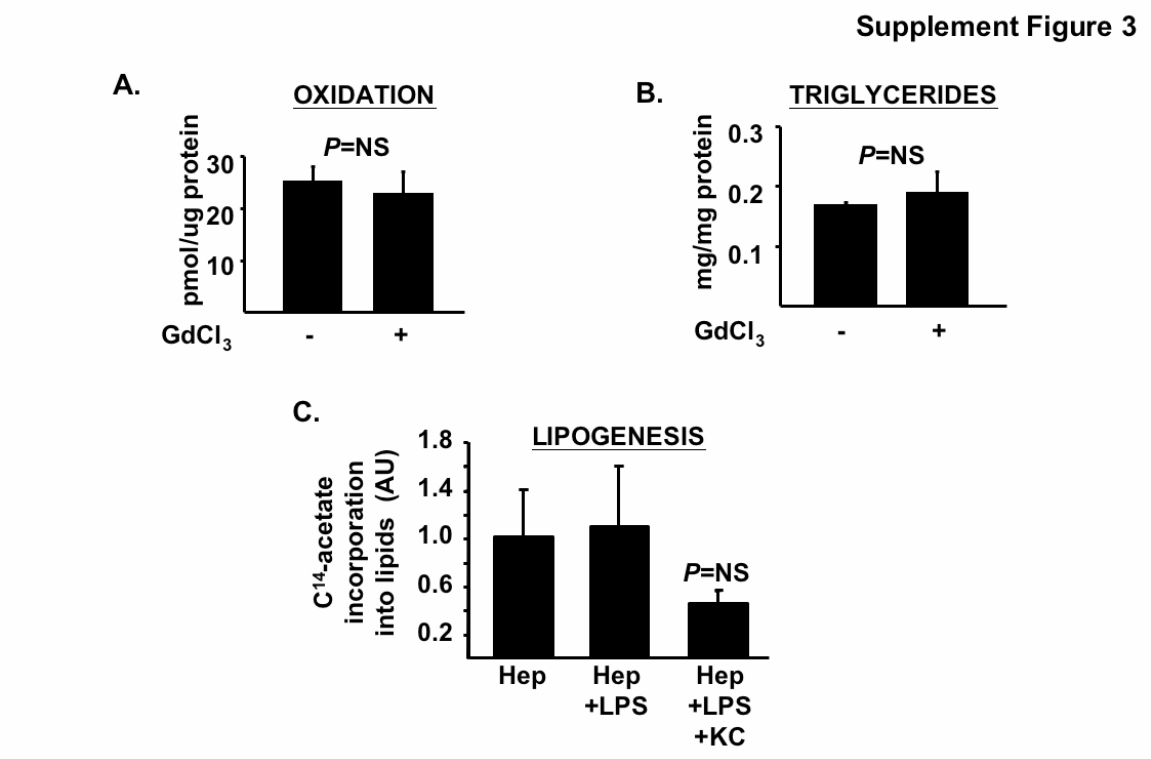
Supplement Figure 1. The effects of Kupffer cell depletion on basal hepatic glucose output (HGO) and insulin-stimulated glucose disposal rate (GDR) on a high fat or high sucrose diet. Male Wistar rats depleted of Kupffer cells or control rats were exposed to a standard chow, high fat or high sucrose diet for two weeks. Subsequently, animals were fasted overnight and then underwent an infusion of [3-H^3]-glucose and a subsequent hyperinsulinemic glucose clamp for the measurement of basal HGO (Panels A and C) and GDR (Panels B and D) as described in Research Design and Methods. Data are presented as mean±SE. n=a minimum of 4 in each group.



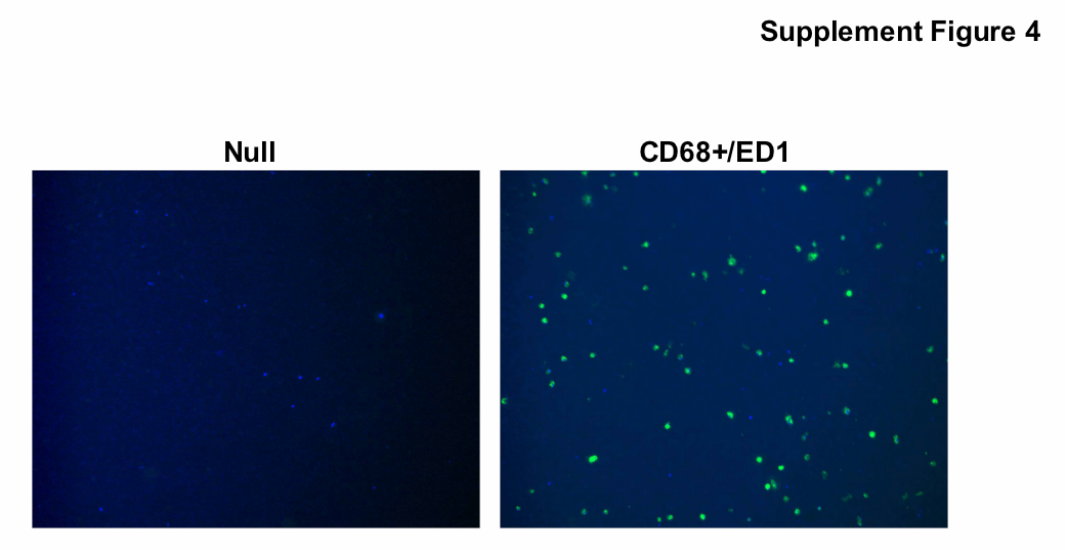
Supplement Figure 2. Effects of diet on KC numbers and KC depletion in response to $GdCl_3$ in liver and adipose tissue. Male Wistar rats were depleted of Kupffer cells by administration of $GdCl_3$ as described and then placed on a NC, HF, or HS diet. Subsequently, liver and adipose tissue were isolated and the presence of macrophages was determined by counting of CD68/ED1 positive cells after immunohistochemical staining of liver and adipose tissue sections. Statistical significance is indicated. n=a minimum of 4 in each group.



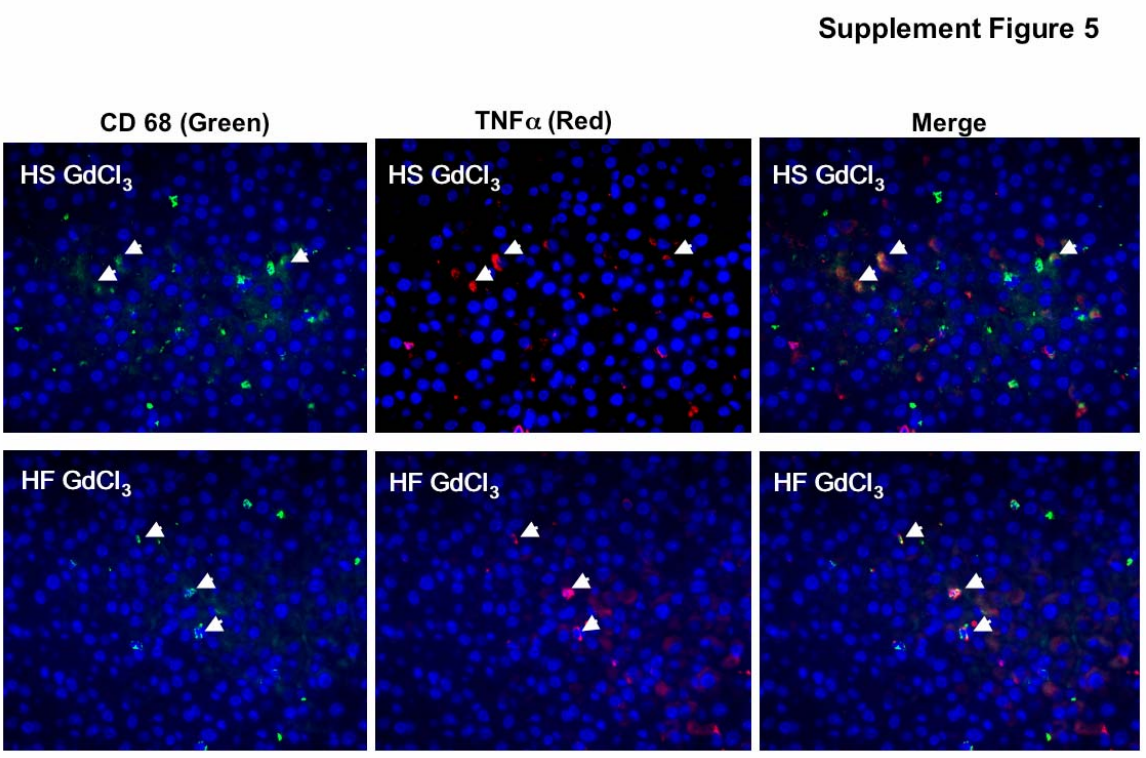
Supplement Figure 3. The effects of $GdCl_3$ on lipid metabolism and the effects of KC on lipogenesis in primary hepatocytes. Panels A and B: Primary hepatocytes exposed to 500ug/ml GdCl for 24h. The effects of this exposure on fatty acid oxidation and triglyceride levels was then assessed. n=a minimum of 3 in triplicate. **Panel C:** Primary heptocytes were exposed to C^{14}-acetate in the absence or presence of LPS and KC. After 24h incorporation of label into lipids was determined. n=a minimum of 3 in triplicate.



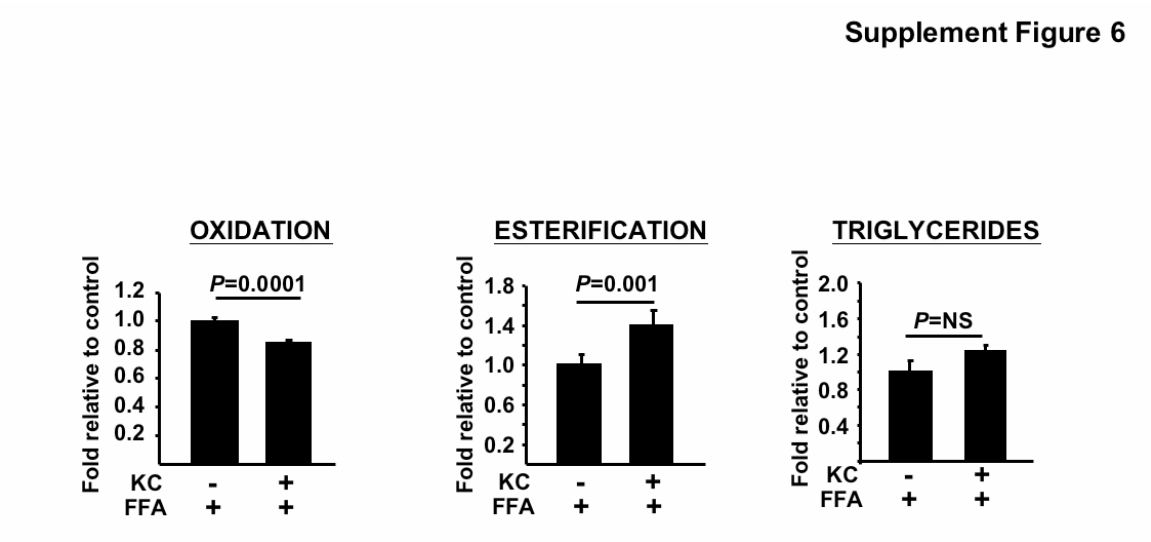
Supplement Figure 4. Isolation and plating of Kupffer cells. Kupffer cells were isolated from rat liver as described in Research Design and Methods. Subsequently, the cells were attached to a transwell insert. Attachment of Kupffer cells was confirmed by the assessment of CD68/ED1 positive cells after immunohistochemical staining as described in Research Design and Methods.



Supplement Figure 5. KC TNFα expression in the liver of $GdCl_3$ treated rats on a on a high fat or high sucrose diet. . Male Wistar rats depleted of Kupffer cells or control rats were exposed to a high sucrose (Top) or high fat (Bottom) diet for two weeks. Subsequently, the livers were isolated, sections were prepared, and immunofluorescent staining for Kupffer cells and TNFα was performed. The left panel shows CD68/ED1 positive cells (green), the center panels show TNFα positive cells (red), and the right panels show the merged images. In all panels Hoescht staining was used to visualize cell nuclei (blue). n=a minimum of 3.



Supplement Figure 6. The effects of saturated free fatty acids on Kupffer cell-induced alterations in heptatocyte lipid metabolism. Hepatocytes and Kupffer cells were isolated from rat livers, plated and cultured in the presence of 0.4mM palmitate. Subsequently, the effects of the indicated exposures on fatty acid oxidation, fatty acid esterification and triglyceride levels (Panels A-C) were assessed. Data are presented as mean±SE. Statistical significance is indicated. n=a minimum of 6 in triplicate for each metabolic measurement.



Supplement Figure 7. The effects of depletion of a panel of cytokines on hepatocyte fatty acid oxidation. Hepatocytes and Kupffer cells were isolated from rat livers, plated and cultured as described in Research Design and Methods. Subsequently, the effects of the indicated exposures in the absence or presence of a panel of cytokine neutralizing antibodies on fatty acid oxidation were assessed as described in Research Design and Methods. n=a minimum of 3 in triplicate for each experimental condition.

Supplement Figure 7