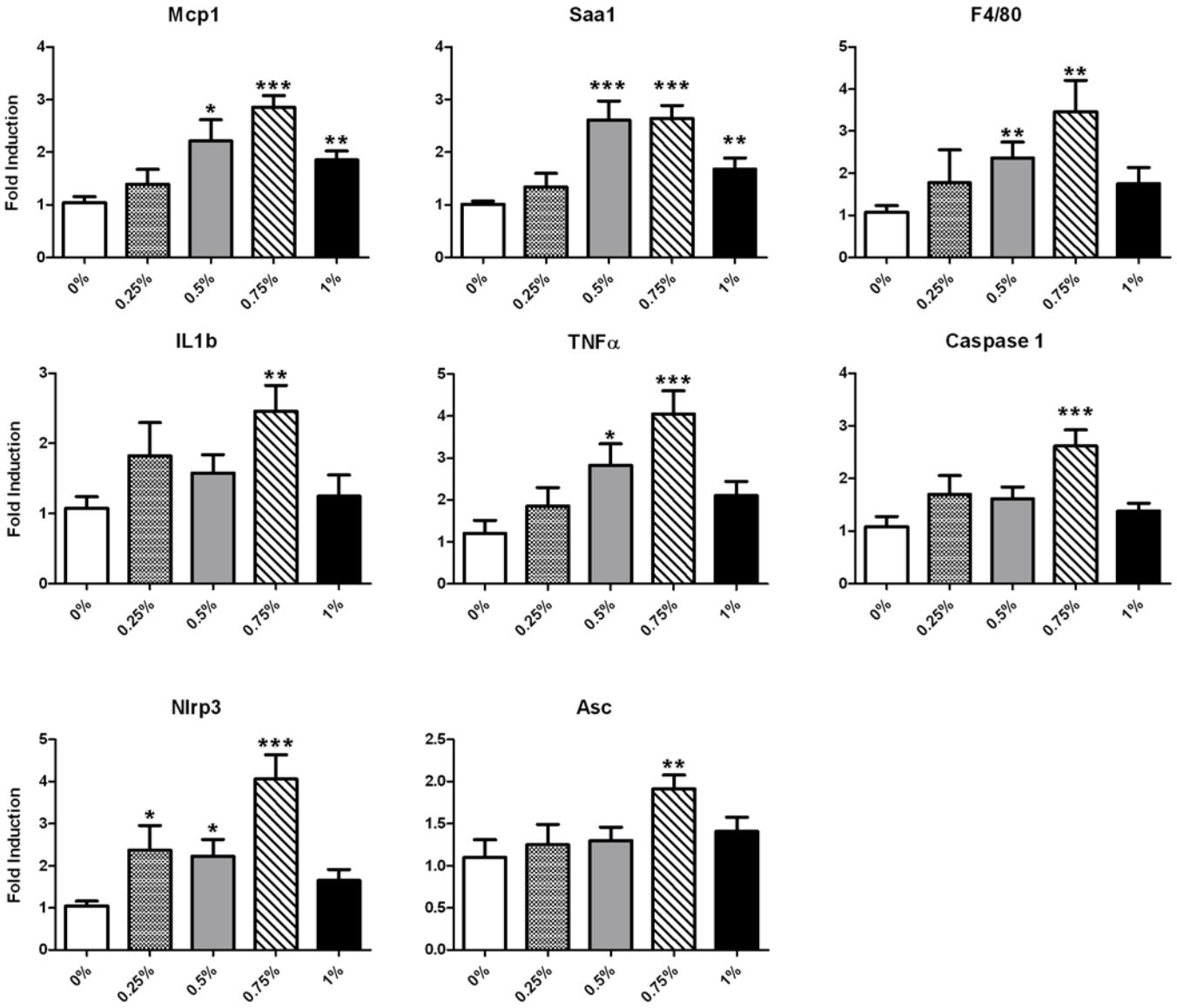
Supplemental Table 1. Composition of the high-fat, high-cholesterol diet(6, 22)

Protein, g/100g	13
Carbohydrate, g/100g	56
Fat, g/100g	15
Cholesterol, g/100g	0, 0.25, 0.5, 0.75 or 1
Fatty Acids, g/100g	
C14:0	0.013
C16:0	3.6
C16:1	0.03
C18:0	4.5
C18:1	4.8
C18:2	1.4
C18:3	0.1
Total Saturated	8.2
Total Monounsaturated	4.8
Total Polyunsaturated	1.5
Protein, kcal/gm	0.5
Carbohydrate, kcal/gm	2.3
Fat, kcal/gm	1.4
Total kcal/gm	4.2

Supplemental Figure 1. Hepatic gene expression (mRNA) studies. Dietary cholesterol causes increased chemotactic (*Mcp1*) and proinflammatory (Saa1, F4/80, Il1b, TNFa) gene expression. There is also increased expression of genes related to the NLRP3 inflammasome (Caspase 1, Nlrp3 and Asc). Interestingly, maximum expression occurred with 0.75% dietary cholesterol rather than 1% cholesterol.

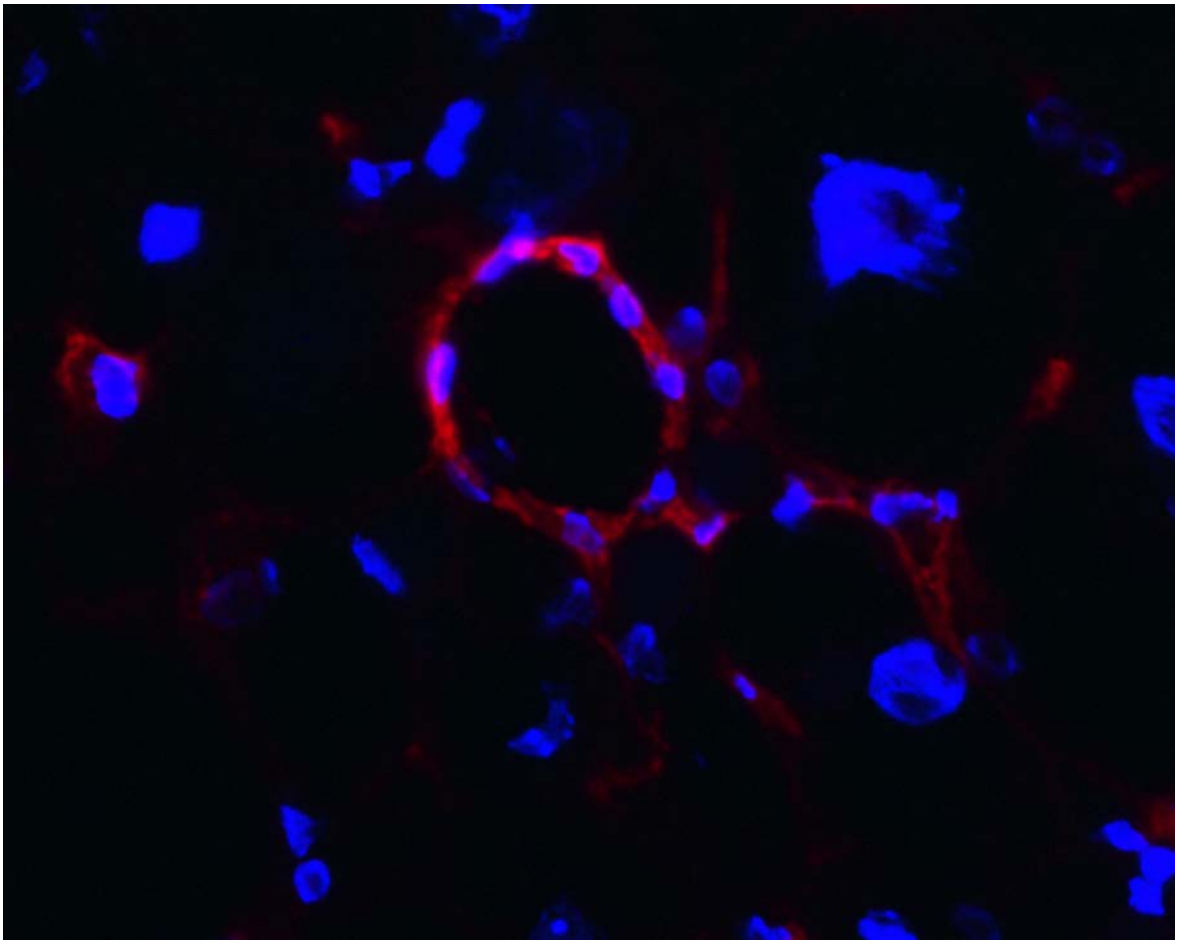
* p<0.05 vs 0% cholesterol
** p<0.01 vs 0% cholesterol
*** p<0.001 vs 0% cholesterol



Supplemental Figure 2.

A. Staining with anti-CLEC4F antibody confirms that the cells making up the crown-like structures are resident macrophages/Kupffer cells as opposed to recruited myeloid cells. The figure shows Kupffer cells staining red forming a ring (crown-like structure) around a hepatocyte remnant lipid droplet.

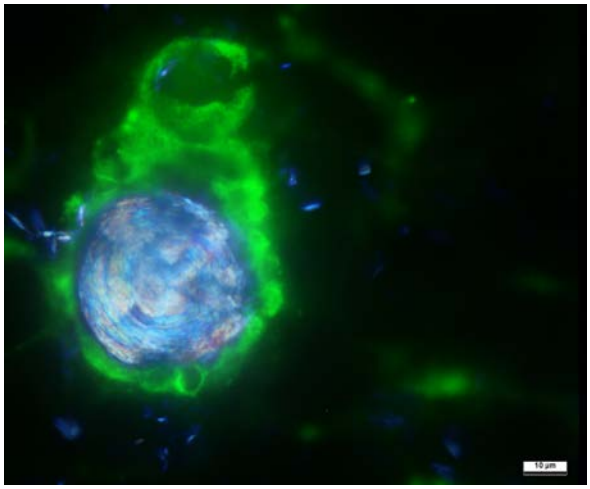
Red = CLEC4F
Blue = nuclei



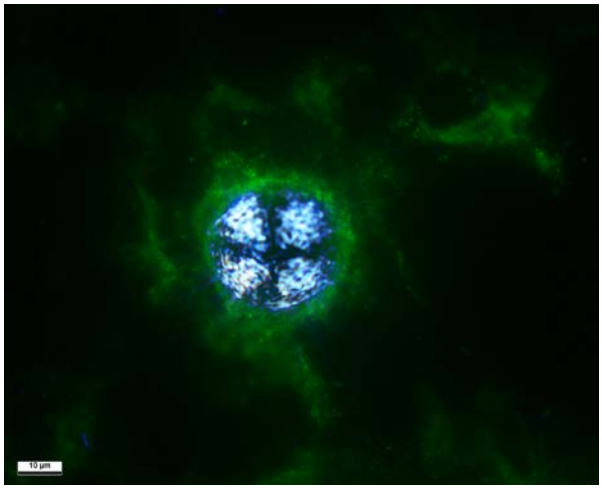
Supplemental Figure 2.

B. Overlay of CD68 or TNFa immunostains and polarized field showing highly birefringent lipid droplets containing cholesterol crystals surrounded by CD68-positive and TNFa-positive Kupffer cells forming crown-like structures.

Overlay of CD68 (green) and polarized field.

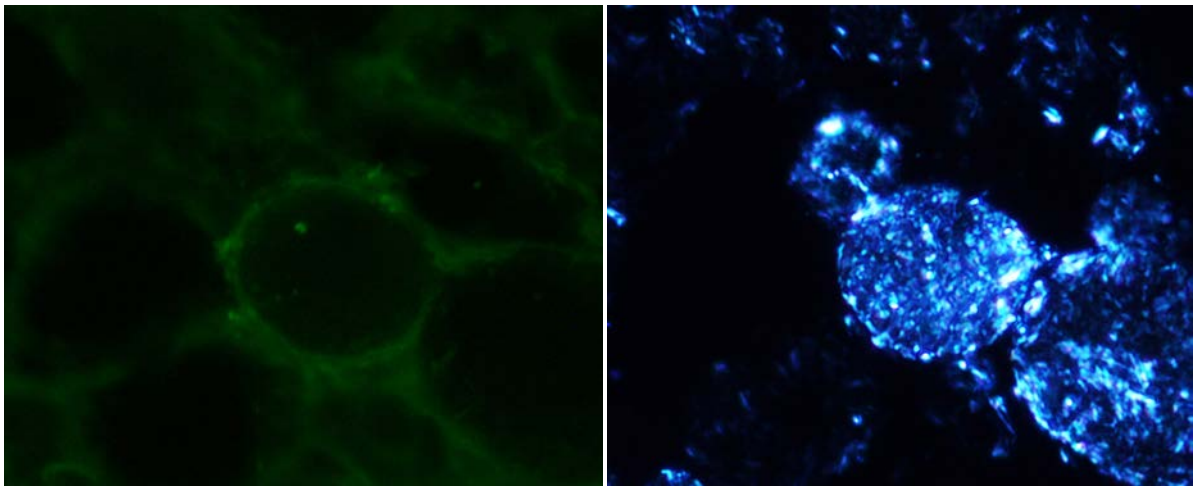


Overlay of TNFa (green) and polarized field.



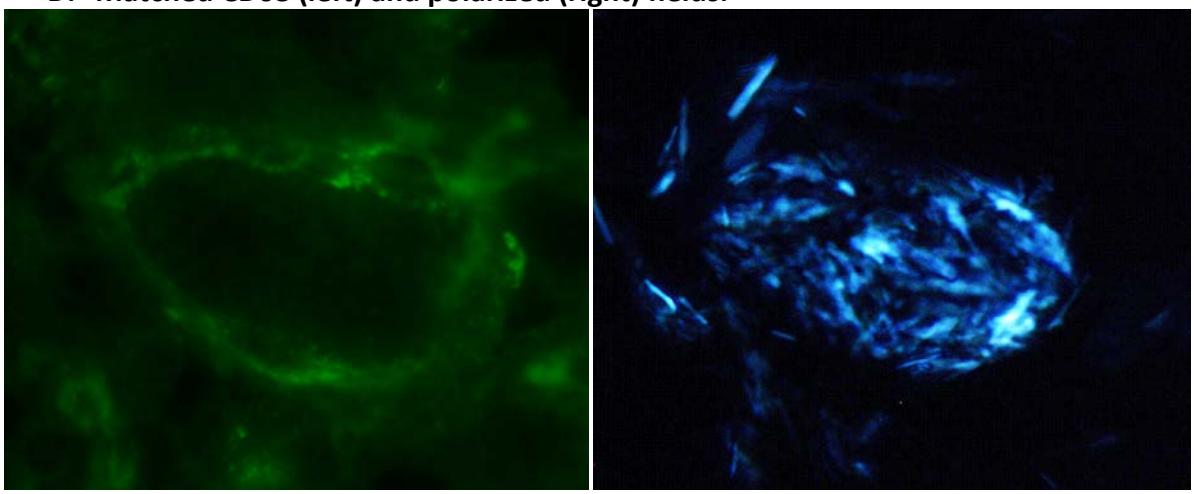
Supplemental Figure 2.

C. Matched Caspase 1 (left) and polarized (right) fields



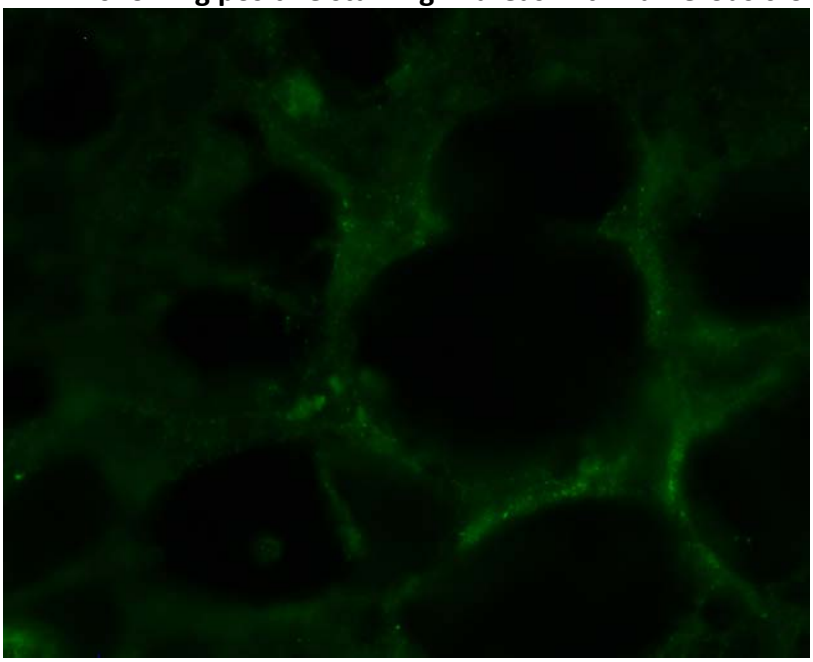
Supplemental Figure 2.

D. Matched CD68 (left) and polarized (right) fields.



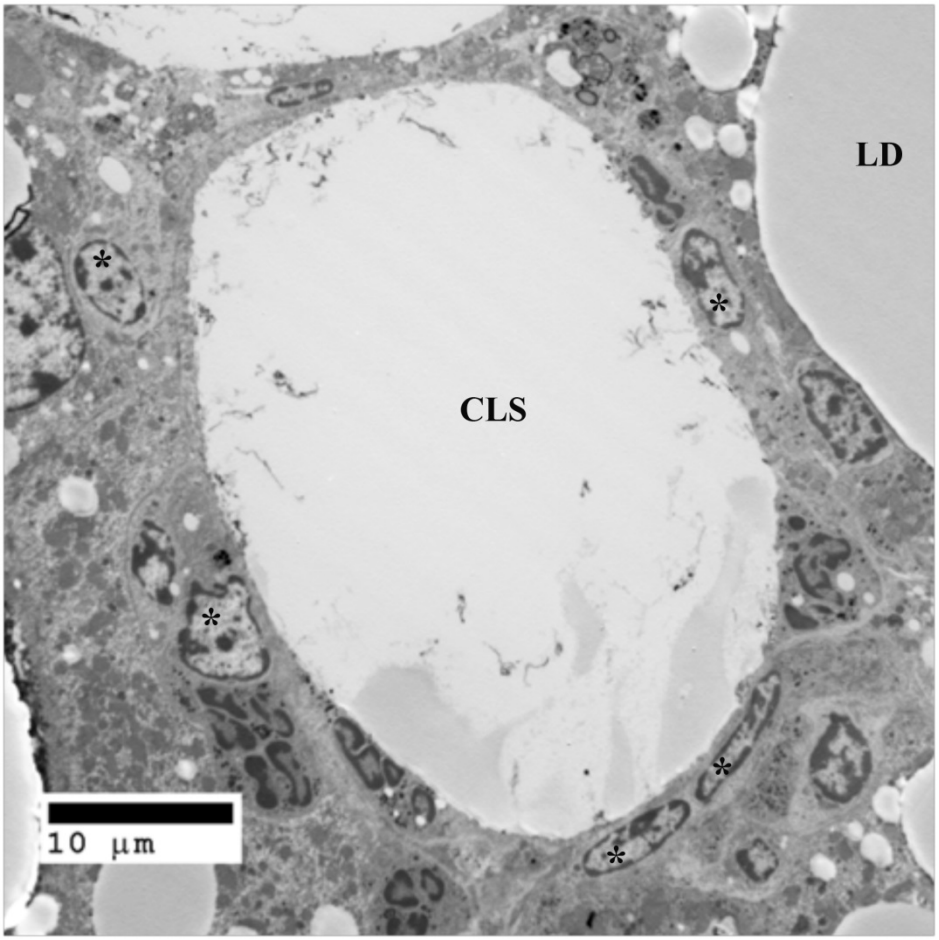
Supplemental Figure 2.

E. Immunohistochemical staining for alpha smooth muscle actin, a marker of stellate cell activation, showing positive staining in areas with numerous crown-like structures

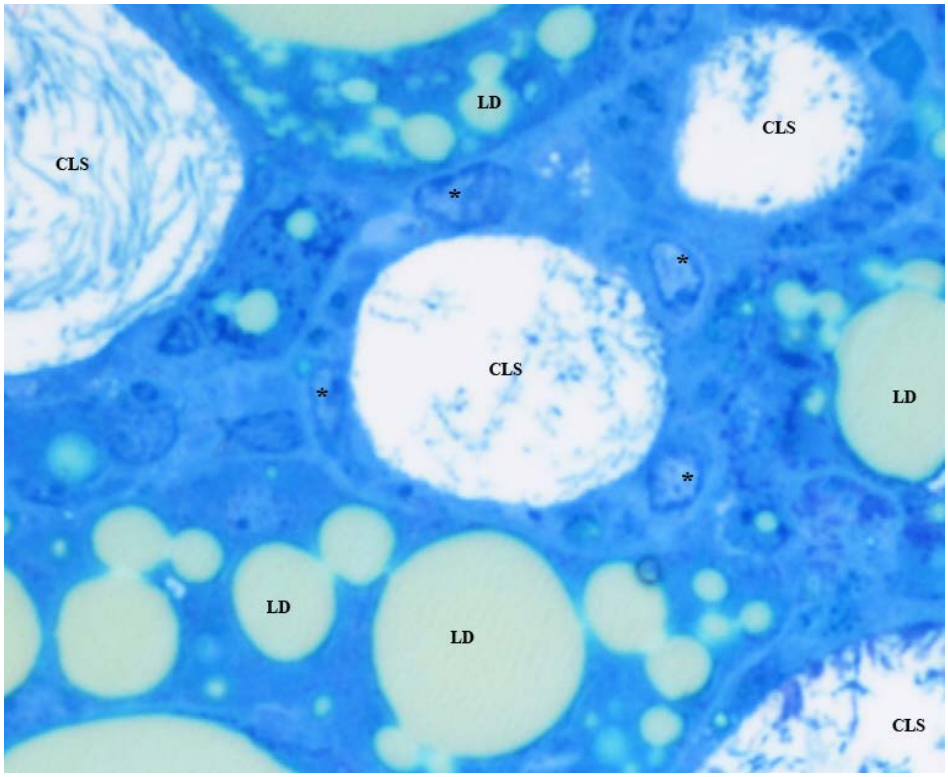


Supplemental Figure 3. Electron micrograph (A) and photomicrograph of osmium and methylene blue stained liver sections (B) showing large (30-40 µm) remnant lipid droplets of dead hepatocytes being processed by a ring of Kupffer cells (marked by asterisks *) forming a crown like structure (CLS). Due to their large size, these lipid droplets could only have arisen in hepatocytes. There is direct apposition of the Kupffer cells onto the lipid droplet demonstrating that the lipid droplet is no longer part of a living hepatocyte. Furthermore, the lipid in these remnant LDs being processed by Kupffer cells does not stain grey with osmium as other normal lipid droplets in normal hepatocytes do (marked LD) because it is already being hydrolyzed by the lysosomal enzymes of the Kupffer cells (osmium stains carbon to carbon double bonds)

A.



B.



* = macrophage nucleus LD = Lipid Droplet CLS = Center of crown-like structure