

SUPPLEMENTAL DATA

Supplemental Figures

Figure S1, Related to Figure 2: 27HC increases atherosclerotic lesion formation in the aortic root within 6 months of age. Cholesterol (A) and 27HC concentrations (B) were measured (n=6-12) and lesion areas were quantified in the aortic roots of wild-type (WT), *cyp7b1*^{-/-}, *apoe*^{-/-}, and *apoe*^{-/-};*cyp7b1*^{-/-} intact male and female mice. Summary data for lesion areas in males and females are provided in scatter plots in C and D, respectively. Mean values are indicated by the long horizontal line, and SEM by the short horizontal line. Related data are provided in Table S2. *p<0.05 vs WT, †p<0.05 vs *apoe*^{-/-}.

Figure S2, Related to Figure 3: Effect of 27HC and E₂ on lipid profiles in ovariectomized female *apoe*^{-/-} or *apoe*^{-/-};*cyp7b1*^{-/-} mice. Analyses were performed on samples pooled from 3 mice treated with vehicle or E₂ (6 ug/d).

Figure S3, Related to Figure 4: 27HC promotes macrophage accumulation in atherosclerotic lesions. Immunohistochemistry was performed on aortic root sections of 12 month-old male *apoe*^{-/-} or *apoe*^{-/-};*cyp7b1*^{-/-} mice using antibody to Mac-3. A. Representative images. B. Summary data for area of macrophage staining. * p<0.05 vs *apoe*^{-/-}. C. Ratio of lesion size to area of macrophage staining, normalized to value for *apoe*^{-/-} mice. Values are mean±SEM.

Figure S4, Related to Figures 4 and 5: 27HC has proinflammatory actions that are LXR-independent. A. Plasma TNF- α levels were measured in 12 month-old male wild-type (WT), *cyp7b1*^{-/-}, *apoe*^{-/-}, and *apoe*^{-/-};*cyp7b1*^{-/-} mice. Values are mean \pm SEM, n=6-7, *p<0.05 vs WT, †p<0.05 vs *apoe*^{-/-}. B-D. Peritoneal macrophages from wild-type or *lxr*^{-/-} mice devoid of both LXR α and LXR β were treated with vehicle or 27HC for 20h, and transcript abundance for TNF- α (B), IL-1 β (C) or IL-6 (D) was evaluated. Values are mean \pm SEM expressed relative to vehicle treatment, n= 3, *p<0.05 vs vehicle. E-G. Peritoneal macrophages from wild-type male mice were treated with vehicle (Veh) or 27HC for 20h, and the abundance of TNF- α (E), IL-1 β (F) or IL-6 (G) in the cell supernatant was quantified by ELISA. Values are mean \pm SEM, n=4-5, *p<0.05 vs vehicle.

Figure S5, Related to Figure 6: Estrogen blunts NF-kB activation in endothelial cells. A. The subcellular distribution of the NF-kB subunit p65 was evaluated by immunofluorescence in bovine aortic endothelial cells treated with vehicle, LPS (100nM), or LPS plus E₂ (1 nM) for 60 min. p65 shown in green, nuclei stained with Dapi. B. Summary data for n=4, values are mean \pm SEM expressed relative to vehicle treatment, *p<0.05 vs. vehicle, †p<0.05 vs no E₂.

Figure S6, Related to Figure 7: Role of estrogen receptors in 27HC modulation of leukocyte-endothelial cell adhesion, and lipid profiles of 27HC-treated *apoe*^{-/-} and *apoe*^{-/-};*era*^{-/-} mice. A. Male C57BL/76 mice were treated with varying doses of ICI 182,780 and intravital microscopy was then performed to evaluate leukocyte-endothelial cell

adhesion in the mesenteric microcirculation. Values are mean±SEM, n=4-9, *p<0.05 vs no ICI 182,780. B,C. Lipid profiles in vehicle- and 27HC-treated *apoe*^{-/-} (B) or *apoe*^{-/-}; *era*^{-/-} male mice (C) administered the agents for 6 weeks. Analyses were performed on samples pooled from 2 mice.

Supplemental Tables

Table S1, Related to Figure 2: Body weight and triglyceride status of 12 month-old mice.

Table S2, Related to Figure 2: Body weight and triglyceride status of 6 month-old mice.

Table S3, Related to Figure 3: Cholesterol and 27HC status of E₂-treated *apoe*^{-/-} and *apoe*^{-/-}; *cyp7b1*^{-/-} mice.

Supplemental Movies

Movie S1, Related to Figure 4E: Intravital microscopy displaying leukocyte-endothelial cell adhesion in the mesenteric microcirculation of a vehicle-treated mouse.

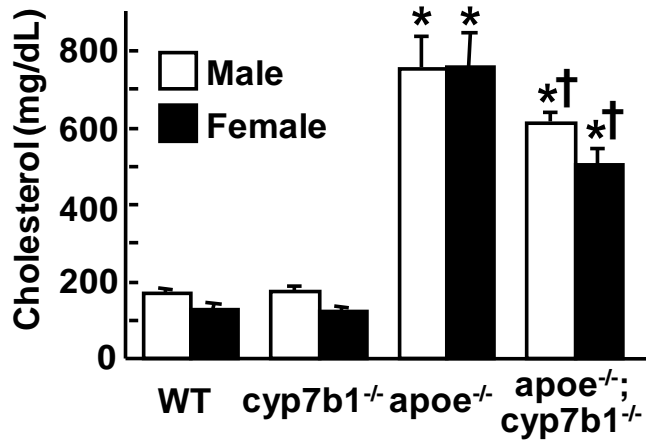
Movie S2, Related to Figure 4F: Intravital microscopy displaying leukocyte-endothelial cell adhesion in the mesenteric microcirculation of a 27HC-treated mouse.

Movie S3, Related to Figure 4G: Intravital microscopy displaying leukocyte-endothelial cell adhesion in the mesenteric microcirculation of a TNF-α-treated mouse.

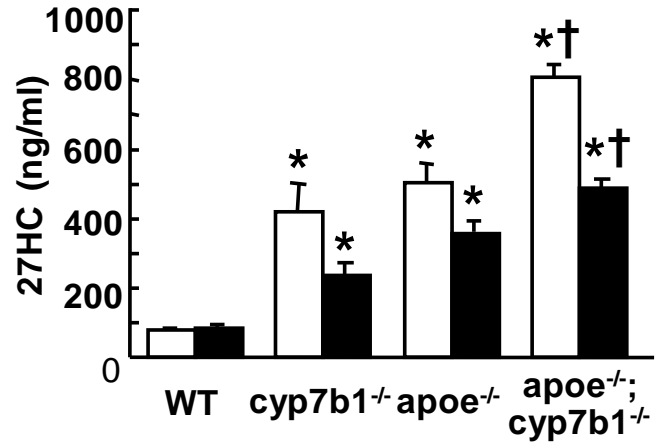
Movie S4, Related to Figure 4H: Intravital microscopy displaying leukocyte-endothelial cell adhesion in the mesenteric microcirculation of a 27HC- and TNF- α -treated mouse.

Figure S1

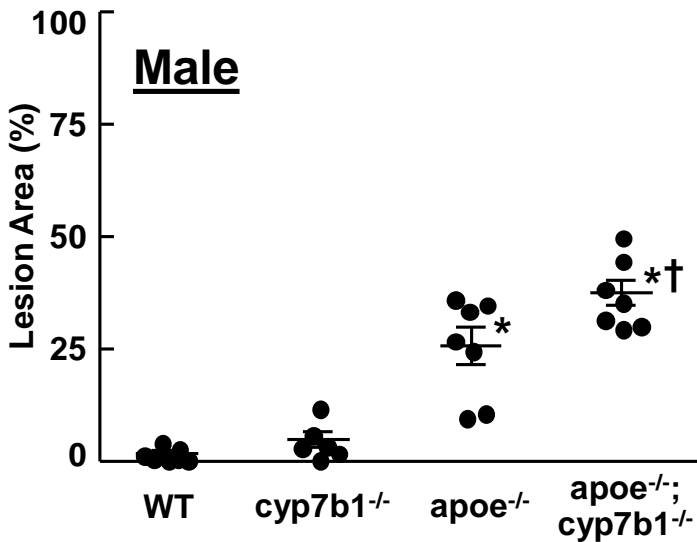
A



B



C



D

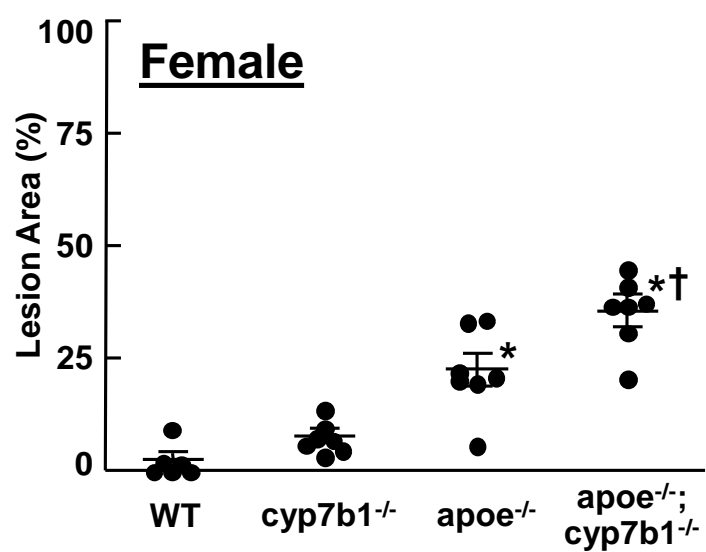


Figure S2

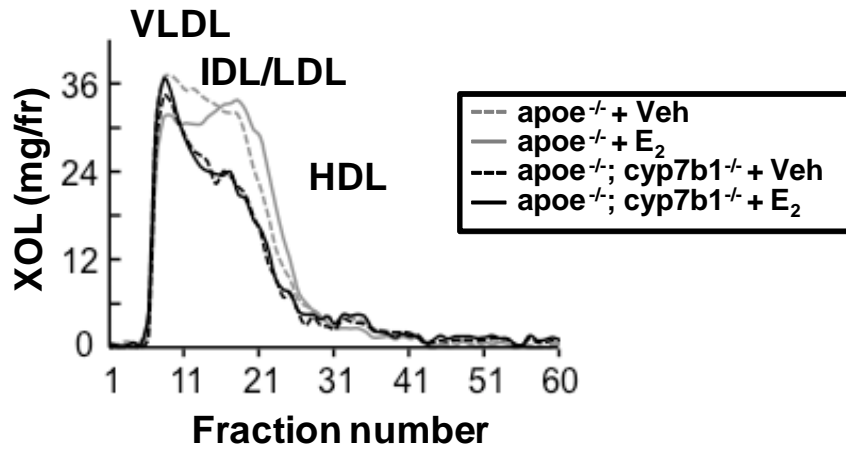


Figure S3

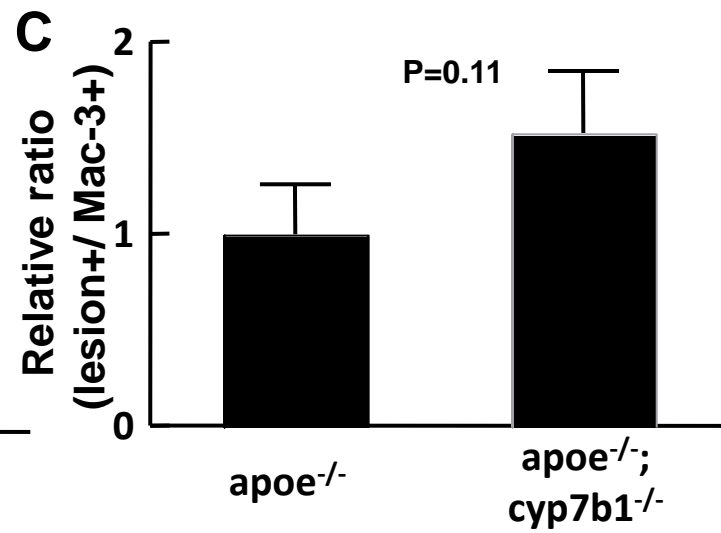
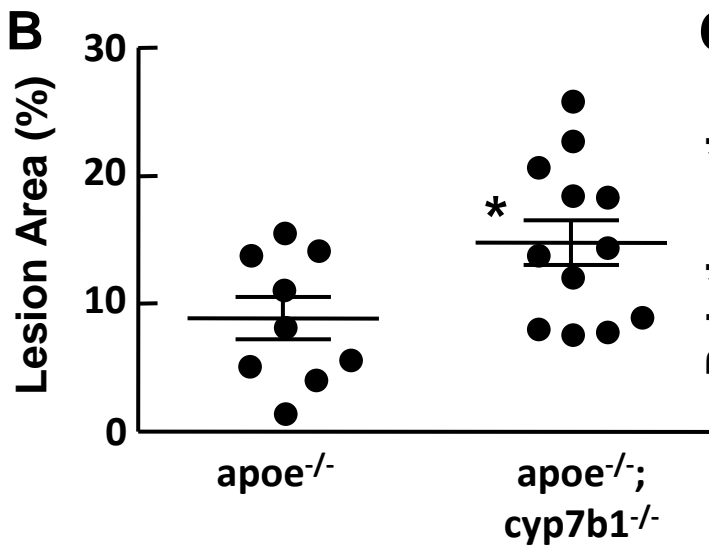
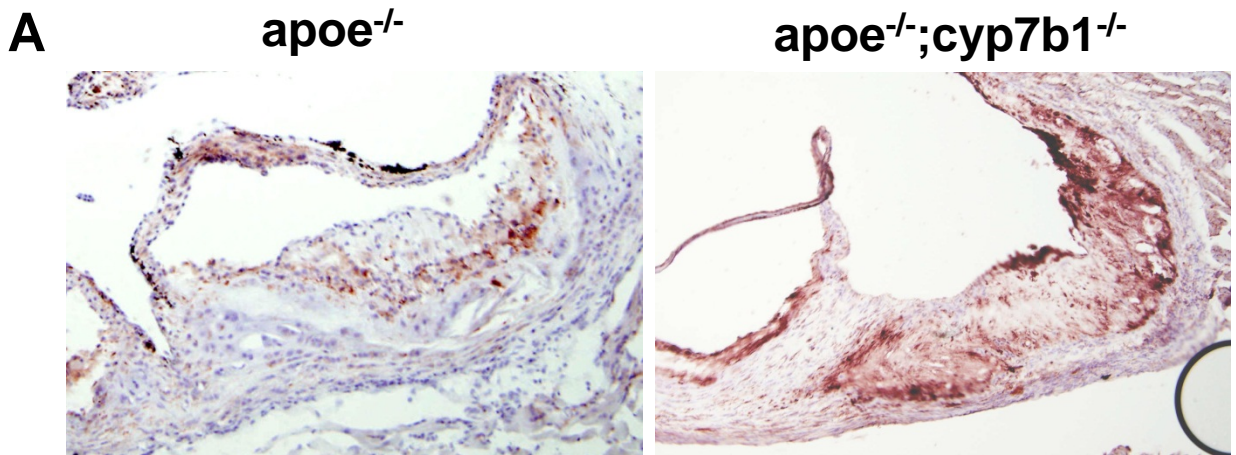


Figure S4

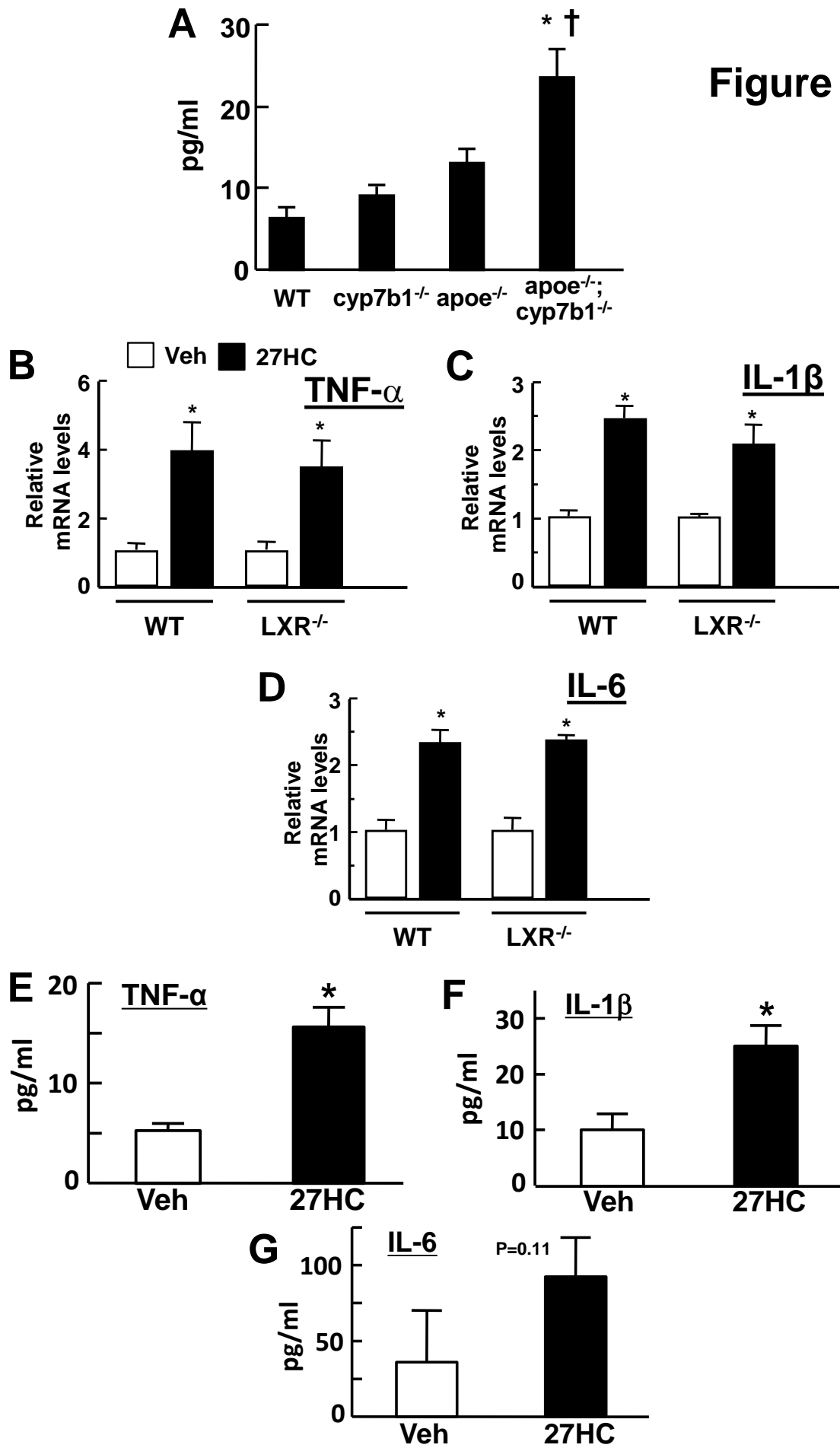


Figure S5

