

Supplementary Information for

Human Species-Specific Loss of CMP-*N*-acetylneuraminic Acid Hydroxylase Enhances Atherosclerosis via Intrinsic and Extrinsic Mechanisms

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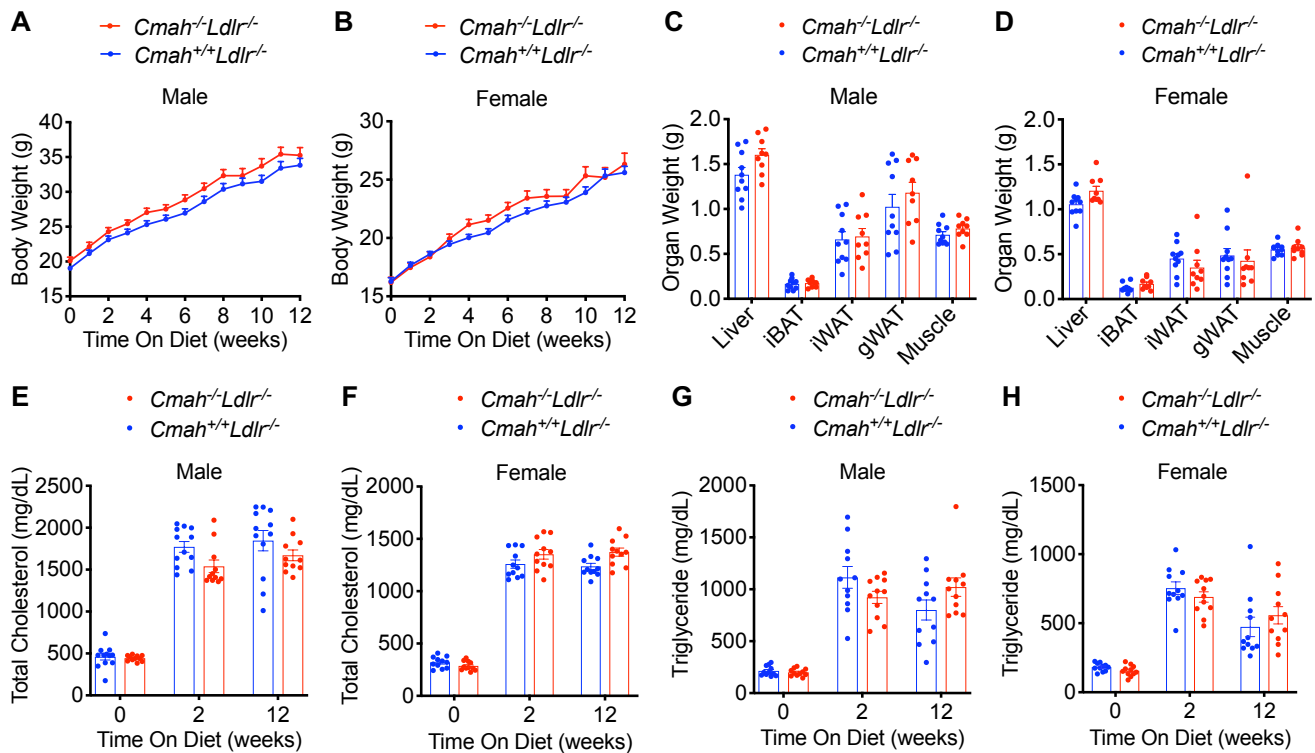


Fig. S1 *Cmah*^{-/-}*Ldlr*^{-/-} does not show any differences in body weight, organ weight, and hyperlipidemia after HFD feeding in comparison with *Cmah*^{+/+}*Ldlr*^{-/-}.

6 week old *Cmah*^{+/+}*Ldlr*^{-/-} and *Cmah*^{-/-}*Ldlr*^{-/-} male and female mice were fed a soy-based (free of Sialic acids, Sias) high fat diet (HFD) for 12 weeks. **(A), (B)** Graphical representation of body weight changes (male and female, n = 14 - 16). **(C), (D)** Organs weight at 12 weeks: liver; interscapular brown adipose tissue, iBAT; inguinal subcutaneous white adipose tissue, iWAT; gonadal white adipose tissue, gWAT; biceps femoris muscle (male and female, n = 9 - 10). **(E), (F)** Plasma total cholesterol and **(G), (H)** triglyceride level (male and female, n = 10 - 12). Mean values ± SEM.

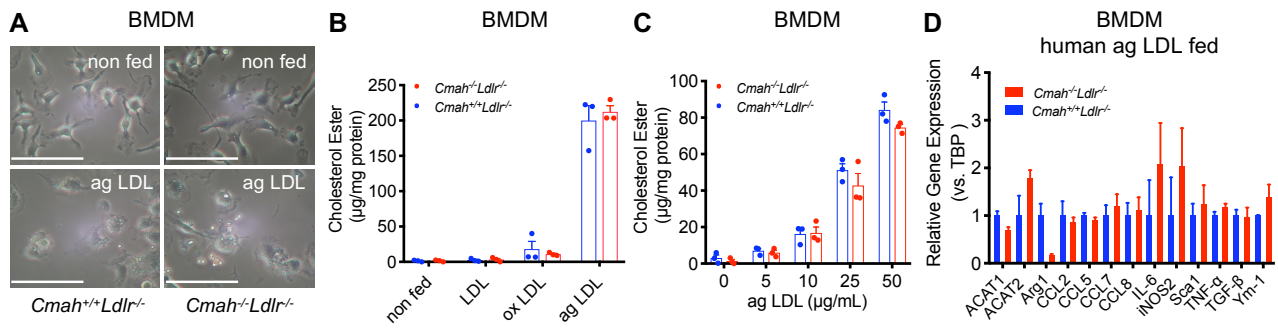


Fig. S2 In vitro conversion to foam cells using mice BMDM and human LDL.

(A) Bone marrow derived macrophage (BMDM) and BMDM fed with aggregated human LDL (ag LDL) from *Cmah^{-/-}Ldlr^{-/-}* and *Cmah^{+/+}Ldlr^{-/-}* female mice (8-12 weeks age, soy-based control diet fed). (Scale bars, 100 µm.) **(B)** Cholesterol ester levels in BMDM after a 24 hr incubation with 0 or 50 mg/ml of human LDL, oxidized LDL (ox LDL), and ag LDL (female, n = 3 each). **(C)** Cholesterol ester levels in BMDM after a 24 hr incubation with different concentrations of human ag LDL (female, n = 3 each). **(D)** Expression of cytokines in BMDM after a 24 hr incubation with 50 mg/ml ag LDL (female, n = 4 each). Mean values ± SEM.

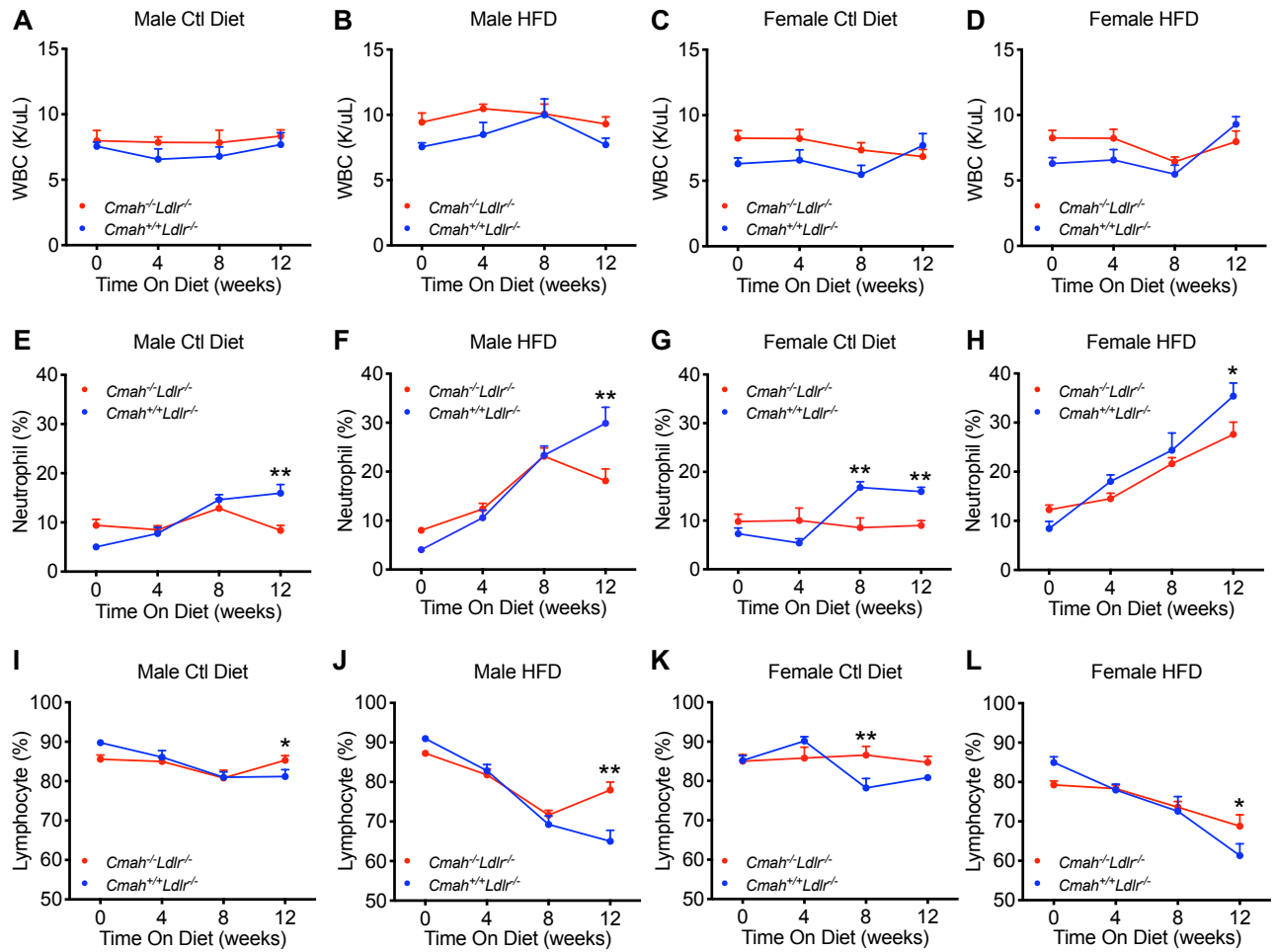


Fig. S3 Inflammatory cell population in peripheral blood.

6 week old $Cmah^{+/+}Ldlr^{-/-}$ and $Cmah^{-/-}Ldlr^{-/-}$ male and female mice were fed a soy-based (Sias free) control diet or soy-based high fat diet (HFD) for 12 weeks. (A) - (D) Peripheral white blood cell population in blood, (E) - (H) neutrophil population, (I) - (L) lymphocyte population during control diet or HFD feeding for 12 weeks in (male and female, n = 10 each). Mean values \pm SEM, * $P < 0.05$ and ** $P < 0.01$.

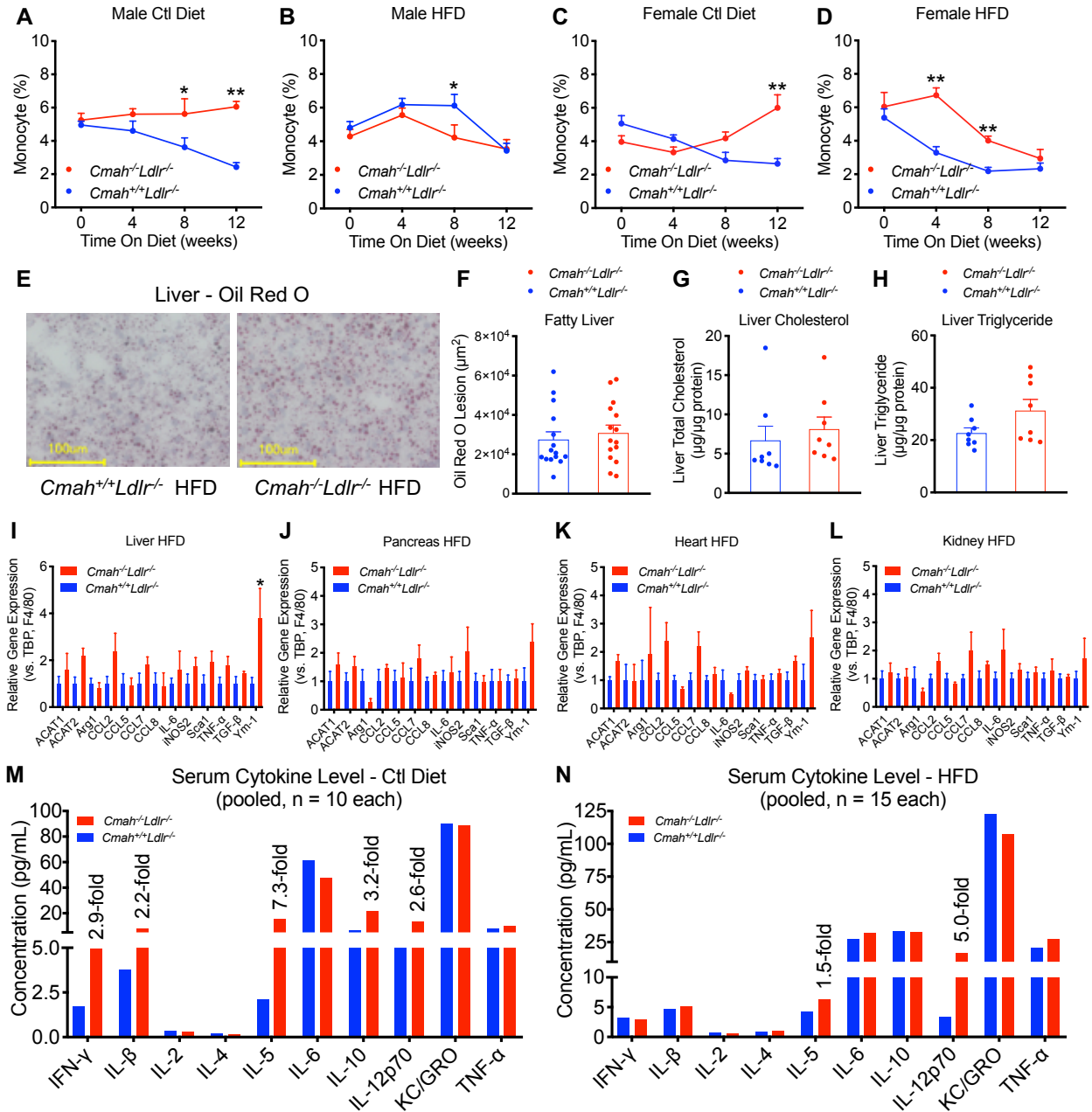


Fig. S4 Peripheral monocyte population in blood in *Cmah^{-/-}Ldlr^{-/-}* mice does not affect hepatic steatosis. (A) - (D) Peripheral monocyte cell population in blood of *Cmah^{-/-}Ldlr^{-/-}* and *Cmah^{+/+}Ldlr^{-/-}* mice during control diet or HFD feeding for 12 weeks (male and female, n = 10 each). (E) Representative Images for Oil red O stained liver, (Scale bars, 100 μm.) (F) Oil red O stain positive lesion size, (G) homogenized liver total cholesterol and (H) triglyceride level (normalized with protein concentration) after 12 weeks of HFD feeding (*Cmah^{-/-}Ldlr^{-/-}* and *Cmah^{+/+}Ldlr^{-/-}* female mice, n = 8 each). (I) Liver, (J) pancreas, (K) heart, and (L) kidney cytokine expressions after 12 weeks of HFD feeding (female, n = 4 each). (M) Multiplex serum cytokine analysis and concentration after 12 weeks control diet or (N) HFD (pooled female samples from n = 10 or 15 each). Mean values ± SEM, **P* < 0.05 and ***P* < 0.01.

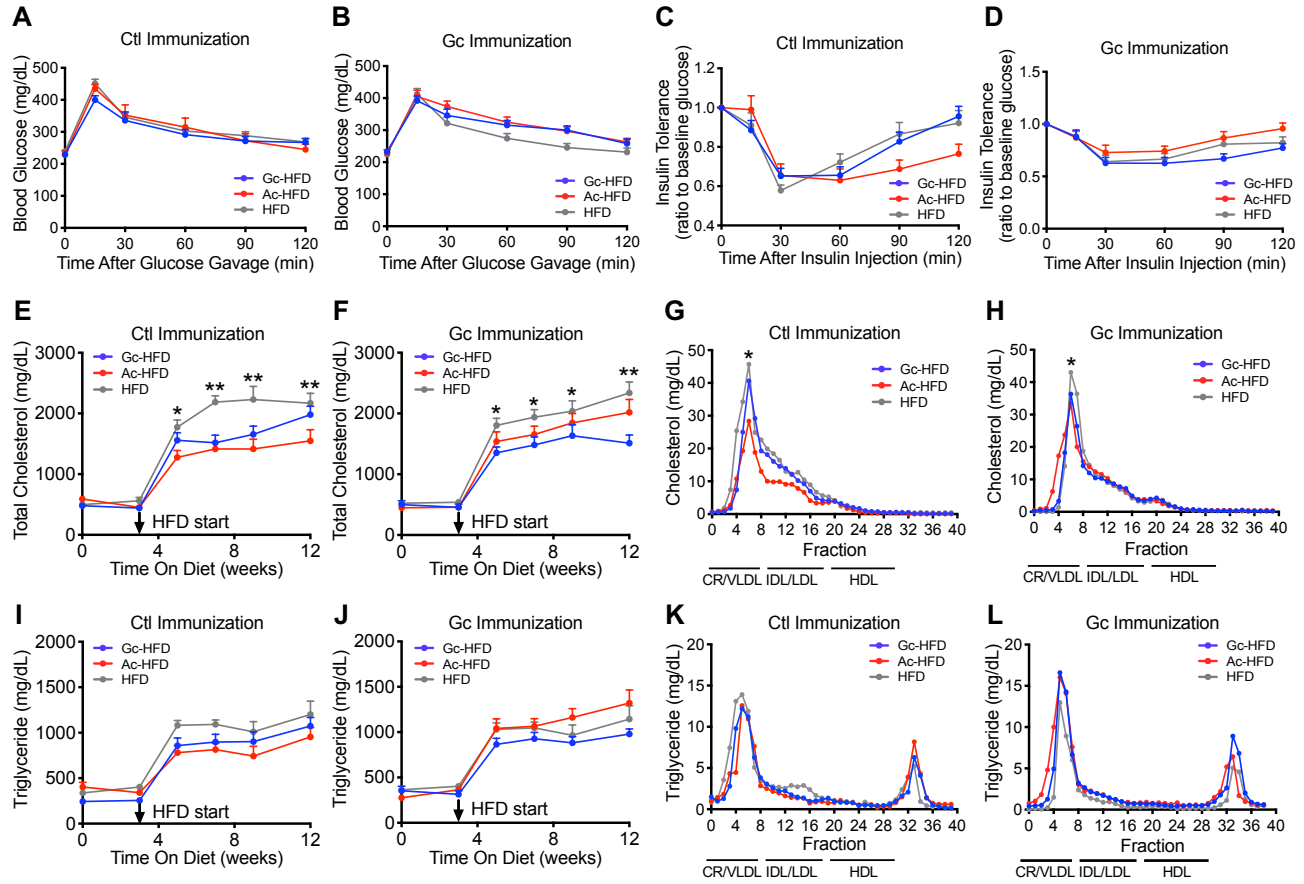


Fig. S5 Diabetic phenotype and lipid analysis in a xenialitis model using human-like *Cmah^{-/-}Ldlr^{-/-}* mice.

Cmah^{-/-}Ldlr^{-/-} mice that were immunized with control or Neu5Gc antigen (Ctl Immunization or Gc Immunization), then fed with Neu5Ac or Neu5Gc or non-Sias high fat diet (Ac-HFD or Gc-HFD or HFD) for 9 weeks (male, $n = 14 - 16$). (A), (B) Glucose tolerance tests, and (C), (D) insulin tolerance tests were performed after 8 weeks of Sias or non-Sias HFD feeding (male, $n = 10$ each). (E) - (H) Total or lipoprotein analysis on plasma cholesterol or (I) - (L) plasma triglyceride after 9 weeks feeding (pooled from male, $n = 14 - 16$), chylomicron, CR; very-low-density lipoprotein, VLDL; intermediate density lipoprotein, IDL; low-density lipoprotein, LDL; high-density lipoprotein, HDL. Mean values \pm SEM, * $P < 0.05$ and ** $P < 0.01$.

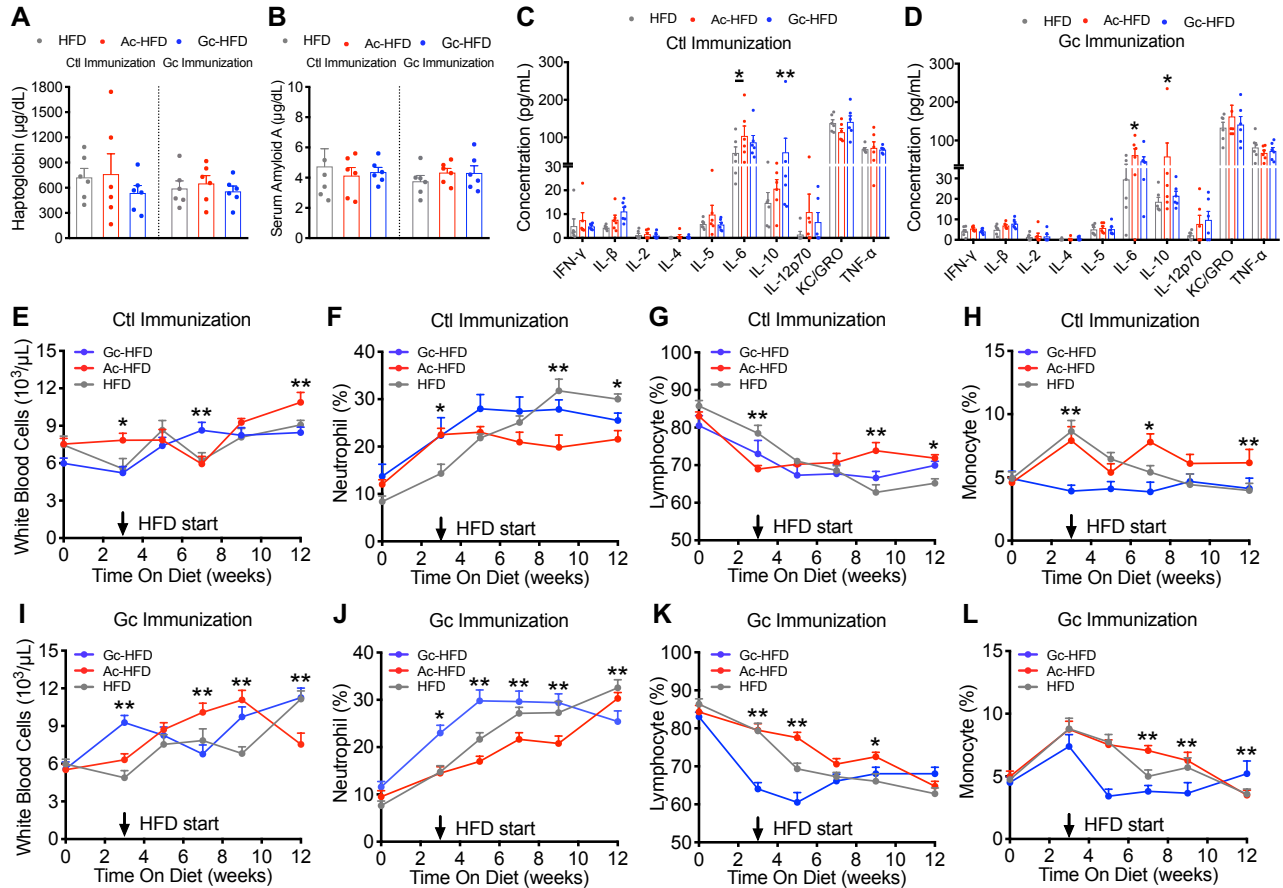


Fig. S6 Serum cytokine levels and inflammatory cell population in a xenosialitis model.

(A) Serum haptoglobin, and (B) amyloid A in serum of *Cmah^{-/-}Ldlr^{-/-}* mice that were immunized with control or Neu5Gc antigen (Ctl Immunization or Gc Immunization), then fed with Neu5Ac or Neu5Gc or non-Sias high fat diet (Ac-HFD or Gc-HFD or HFD) for 9 weeks (male, n = 6 each). (C), (D) Multiplex cytokine profiling in serum samples after 9 weeks HFD feeding (male, n = 6 each). (E) - (L) Peripheral white blood cell population in blood during immunization (0 - 3weeks) and HFD feeding (3 - 12weeks) (male, n = 10 each). Mean values ± SEM, **P* < 0.05 and ***P* < 0.01.

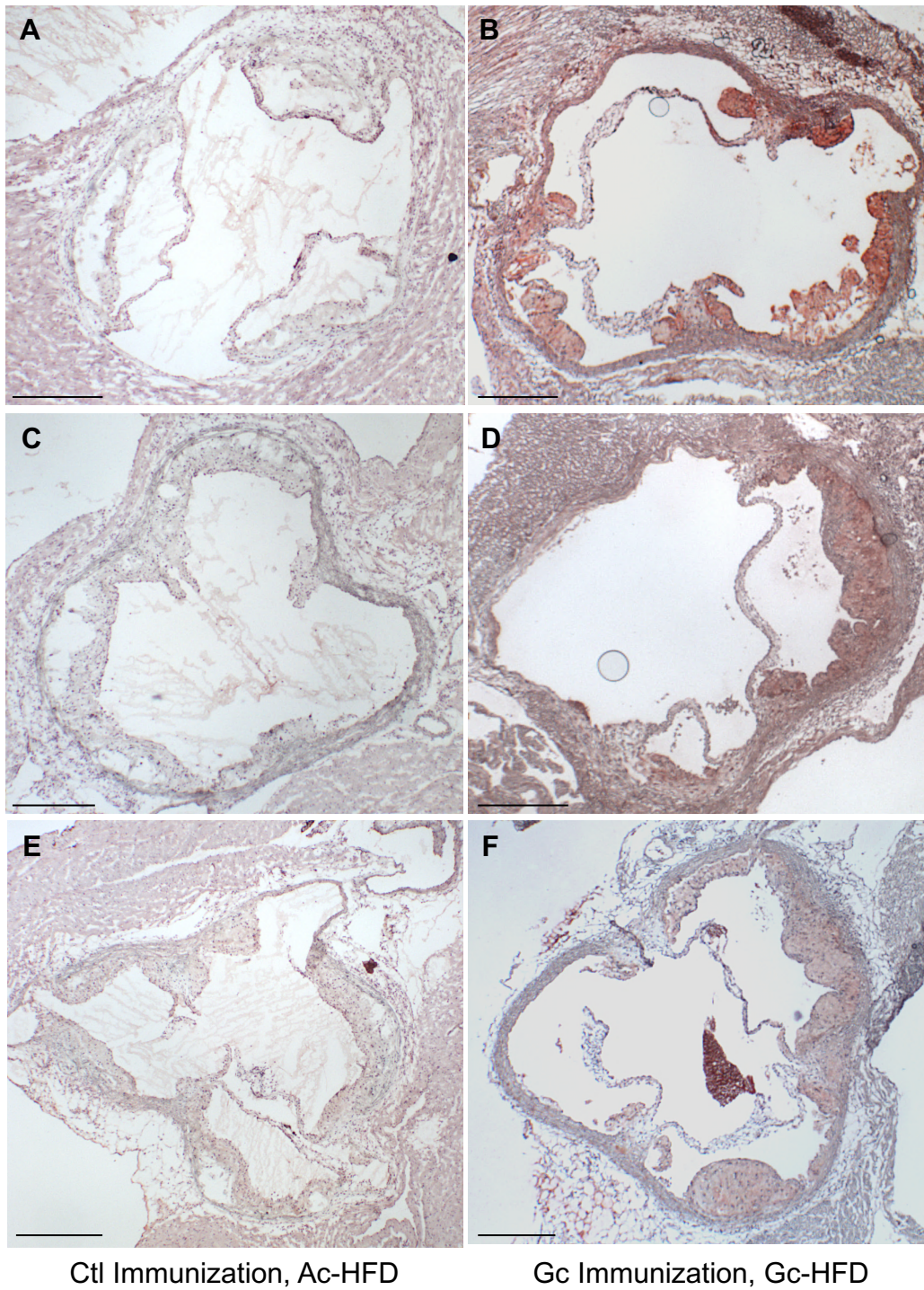


Fig. S7 Neu5Gc accumulation occurs in atheroma lesion in a xenosialitis model as human atheroma. *Cmah^{-/-}Ldlr^{-/-}* male mice that were immunized with control or Neu5Gc antigen (Ctl or Gc Immunization), then fed with Neu5Ac or Neu5Gc high fat diet (Ac-HFD or Gc-HFD) for 9 weeks (n = 3 each). Immunohistochemistry images for Neu5Gc: (A), (C), (E) Ctl immunized then Ac-HFD fed mice; (B), (D), (F) Gc Immunized then Gc-HFD fed mice. (Scale bars, 300 μ m.)

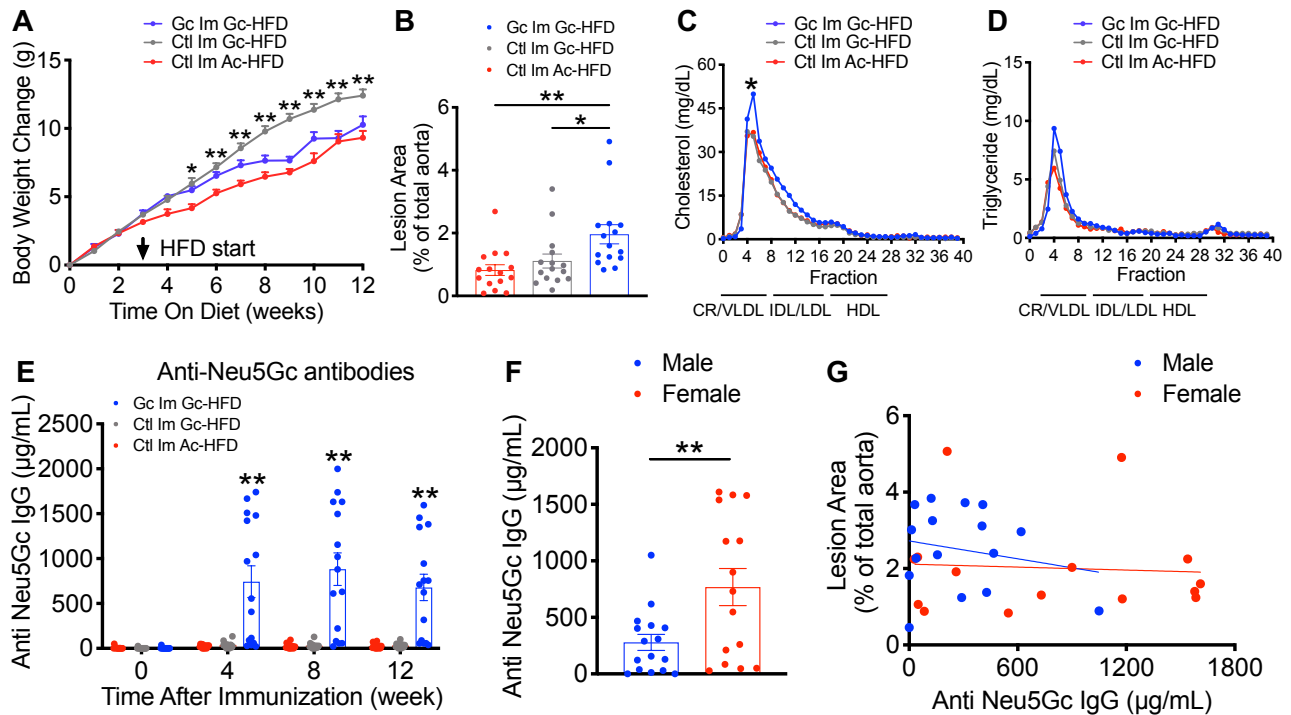


Fig. S8 Combination of human-like anti-Neu5Gc antibodies and Neu5Gc-HFD increases atherosclerosis in female as well as male.

Cmah^{-/-}Ldlr^{-/-} female mice that were immunized with control or Neu5Gc antigen (Ctl Im or Gc Im), then fed with Neu5Ac or Neu5Gc high fat diet (Ac-HFD or Gc-HFD) for 9 weeks ($n = 15$ each). **(A)** Body weight change for 12 weeks (HFD started 3rd weeks, arrow) in three groups. **(B)** En face analysis of atherosclerosis in 12 weeks HFD feeding, **(C)**, **(D)** FPLC analysis of lipoproteins after 12 weeks of HFD diet feeding (pooled serum from $n = 15$), chylomicron, CR; very-low-density lipoprotein, VLDL; intermediate density lipoprotein, IDL; low-density lipoprotein, LDL; high-density lipoprotein, HDL. **(E)** Anti-Neu5Gc antibodies titer measured with bovine submaxillary mucin coated ELISA in *Cmah^{-/-}Ldlr^{-/-}* female mice ($n = 15$ each). **(F)** Average titer of anti-Neu5Gc antibodies for *Cmah^{-/-}Ldlr^{-/-}* male ($n = 16$) and female ($n = 15$) mice that were immunized with Neu5Gc antigen (Gc Im), then fed with Neu5Gc high fat diet (Gc-HFD) for 9 weeks. Average titer was calculated from anti-Neu5Gc IgG ($\mu\text{g/mL}$) in 4, 8, and 12 weeks after Neu5Gc immunization. **(G)** Association between average titer of anti-Neu5Gc IgG (x-axis) and atheroma lesion size measured by en face analysis (y-axis). Mean values \pm SEM, $*P < 0.05$ and $**P < 0.01$.

Table. S1 qPCR Primers

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Acat1</i>	AGCCCAGAAAAATTCATGGACACATACAG	CCCTTGTTCTGGAGGTGCTCTCAGATCTTT
<i>Acat2</i>	GACTTGGTGCAATGGACTCG	GGTCTTGCTTGTAGAATCTGG
<i>Arg1</i>	GGAATCTGCATGGGCAACCTGTGT	AGGGTCTACGTCTCGCAAGCCA
<i>Ccl2 (Mcp--1)</i>	AGGTCCCTGTCATGCTTCTG	GCTGCTGGTGATCCTCTTGT
<i>Ccl5 (Rantes)</i>	CATATGGCTCGGACACCA	ACACACTTGGCGGTTCT
<i>Ccl7 (Mcp-3)</i>	CCTGGGAAGCTGTTATCTTCAA	TGGAGTTGGGGTTTTTCATGTC
<i>Ccl8 (Mcp-2)</i>	GCTGTGGTTTTCCAGACCAA	GAAGGTTCAAGGCTGCAGAA
<i>F4/80</i>	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
<i>Il-6</i>	CCAGAGATACAAAGAAATGATGG	ACTCCAGAAGACCAGAGGAAAT
<i>iNos2</i>	GTTCTCAGCCCAACAATACAAGA	GTGGACGGGTCGATGTCAC
<i>Sca1 (Ly6A)</i>	ATGGACACTTCTCACACTACAAAG	TCAGAGCAAGGTCTGCAGGAGGACTG
<i>Tbp</i>	GAAGCTGCGGTACAATTCCAG	CCCCTGTACCCTTCACCAAT
<i>TgFβ</i>	GGAGAGCCCTGGATACCAAC	AAGTTGGCATGGTAGCCCTT
<i>Tnfa</i>	CCAGACCCTCACACTCAGATC	CACTTGGTGGTTTGCTACGAC