Supplementary Figures

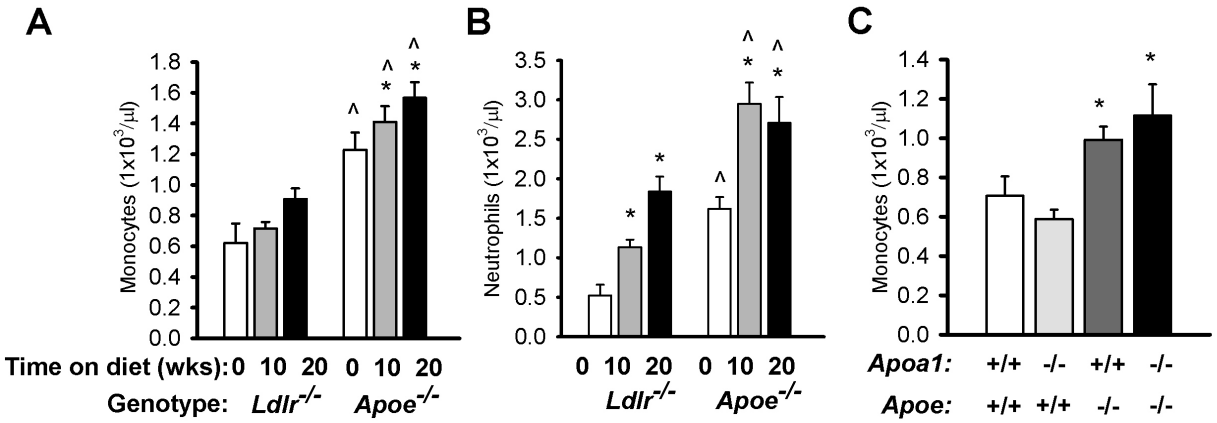

Figure S1. Absolute leukocyte numbers.

A,B) 8 week old mice were fed a WTD for time periods shown Monocytes and neutrophils were analyzed by flow cytometry and *converted to cells/µL using counts from the CBCs* **p*<0.05, diet effect over time for *Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$, ^*p*<0.05 genotype effect *Apoe*$^{-/-}$ vs *Ldlr*$^{-/-}$ at each respective time point. Data is presented as mean ± SEM, n=6-8. ***C)*** *WT, Apoa1*$^{-/-}$*, Apoe*$^{-/-}$ *and Apoa1*$^{-/-}$*Apoe*$^{-/-}$ *mice were fed a chow diet until 20wks of age. The population of blood Monocytes was identified by flow cytometry and converted into total numbers from CBCs.* **p*<0.05 vs WT. Data is presented as mean ± SEM, n=5-8.

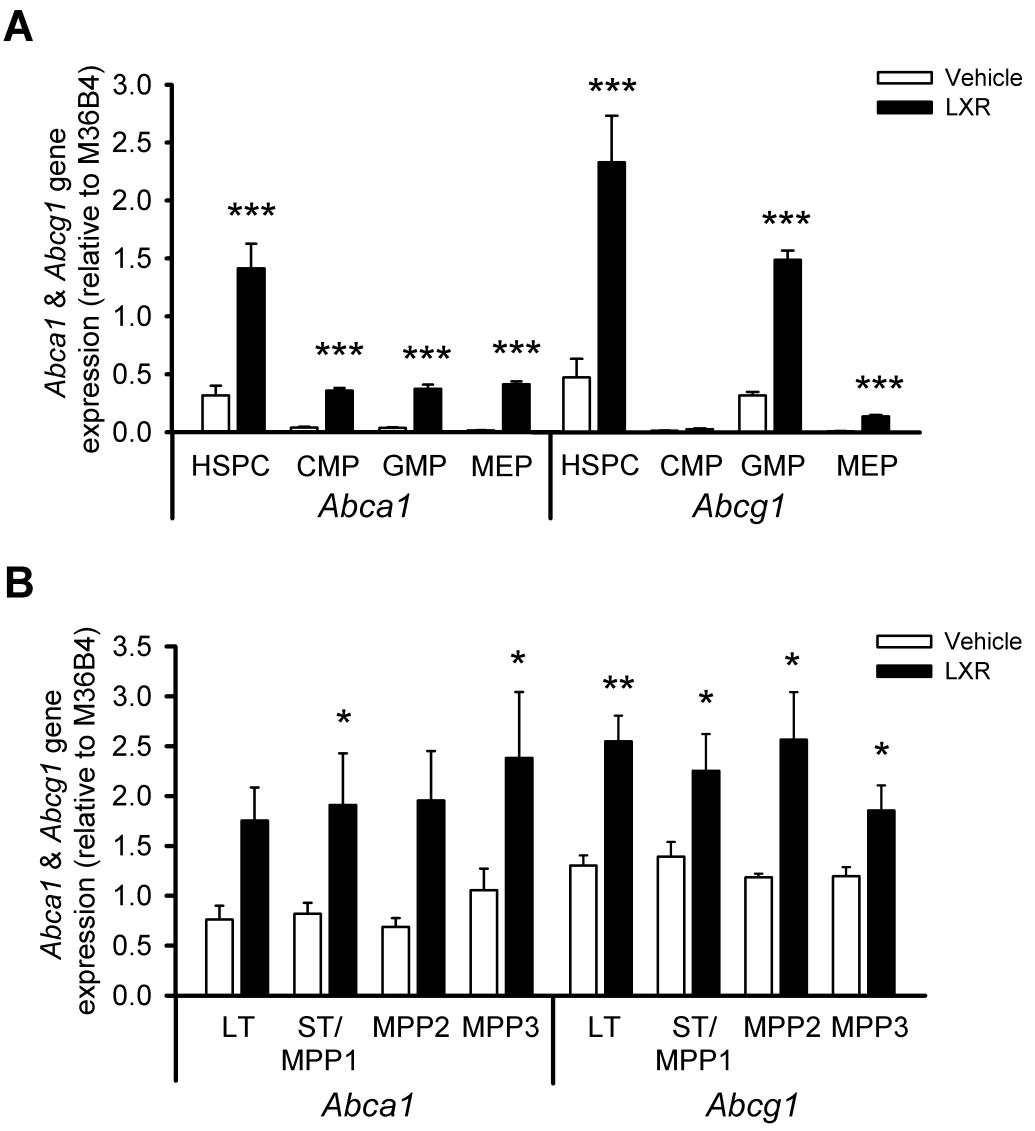

Figure S2. *Abca1 and Abcg1 gene expression in BM stem cells. WT mice injected with saline or LXR activator (T0901317, 25mg/kg body weight).* ***A&B)*** *HSPC and progenitor populations were isolated from the BM, cDNA prepared and different mRNAs quantified by real time PCR.* **A)** Expression in stem and progenitor cell subsets. Values represent mean of each group (n=5) ± SEM. * - ****p*<0.05 - 0.001 vs control for each respective cell population. **B)** HSPCs were sorted via flow cytometry to obtain LT-repopulating, ST-repopulating and MPP1, MPP2 and MPP3 subsets. * - ****p*<0.05 - 0.001 vs control (Vehicle) for each respective cell population. Data presented as mean ± SEM, n=5.

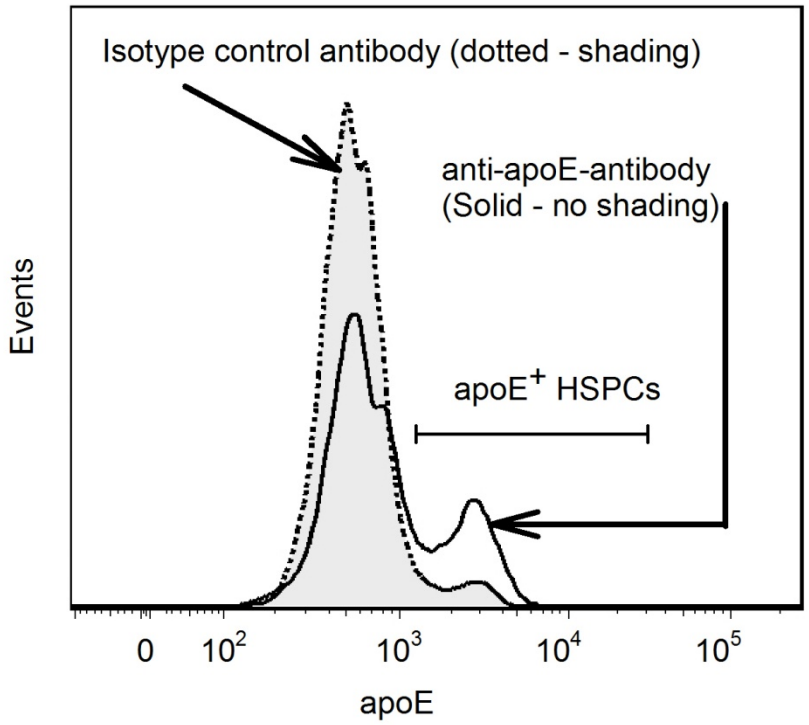

Figure S3. *Isoytpe control for apoE cell surface expression*. BM was isolated from WT mice and incubated with antibodies to identify HSPCs and an isotype control antibody (dotted line - shading) or an anti-apoE-antibody (solid line - no shading). Specific anti-apoE-antibody signal can be seen above.

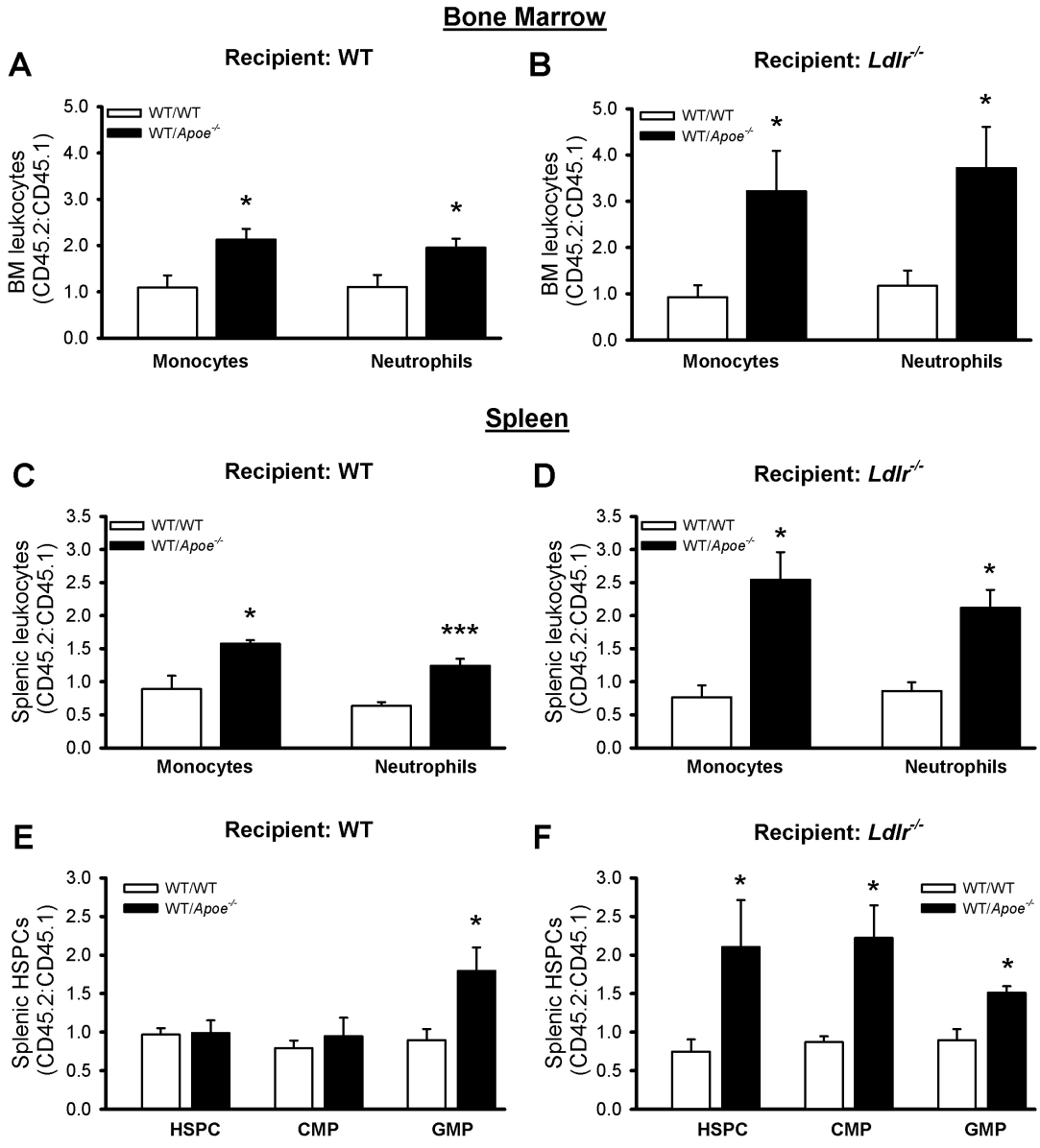

Figure S4. *Expansion of Leukocytes and HSPCs in the BM and spleen is cell autonomous and enhanced in settings of hypercholesterolemia. WT or Ldlr$^{-/-}$ mice received a BMT as explained in Fig 3A. Data is presented as a ratio of CD45.2:CD45.1.* ***A&B)*** *Ratio of monocytes and neutrophils in the BM of WT (A) and Ldlr$^{-/-}$ (B) mice.* ***C&D)*** *Ratio of monocytes and neutrophils in the spleen of WT (C) and Ldlr$^{-/-}$ (D) mice.* ***E&F)*** *HSPCs, CMPs and GMPs in the spleen of WT (E) and Ldlr$^{-/-}$ (F) recipient mice. Cell populations were quantified via flow cytometry. *p<0.05, **p<0.01. Data is expressed as mean* ± SEM, n=*6.*

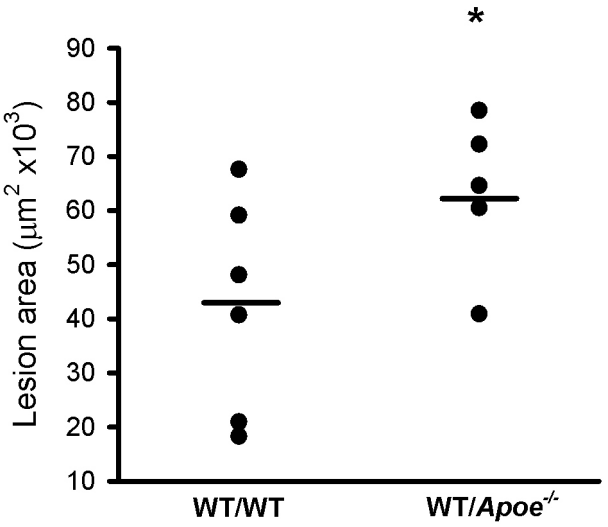

Figure S5. *Ldlr$^{-/-}$ mice transplanted with Apoe$^{-/-}$ CD45.2 competing BM have larger lesions than mice transplanted with WT CD45.2 competing BM.* Mean lesion areas from the competitive BM transplantation study. *p<0.05 WT/WT vs. WT/*Apoe*$^{-/-}$. Each dot represents mean lesion size per mouse.

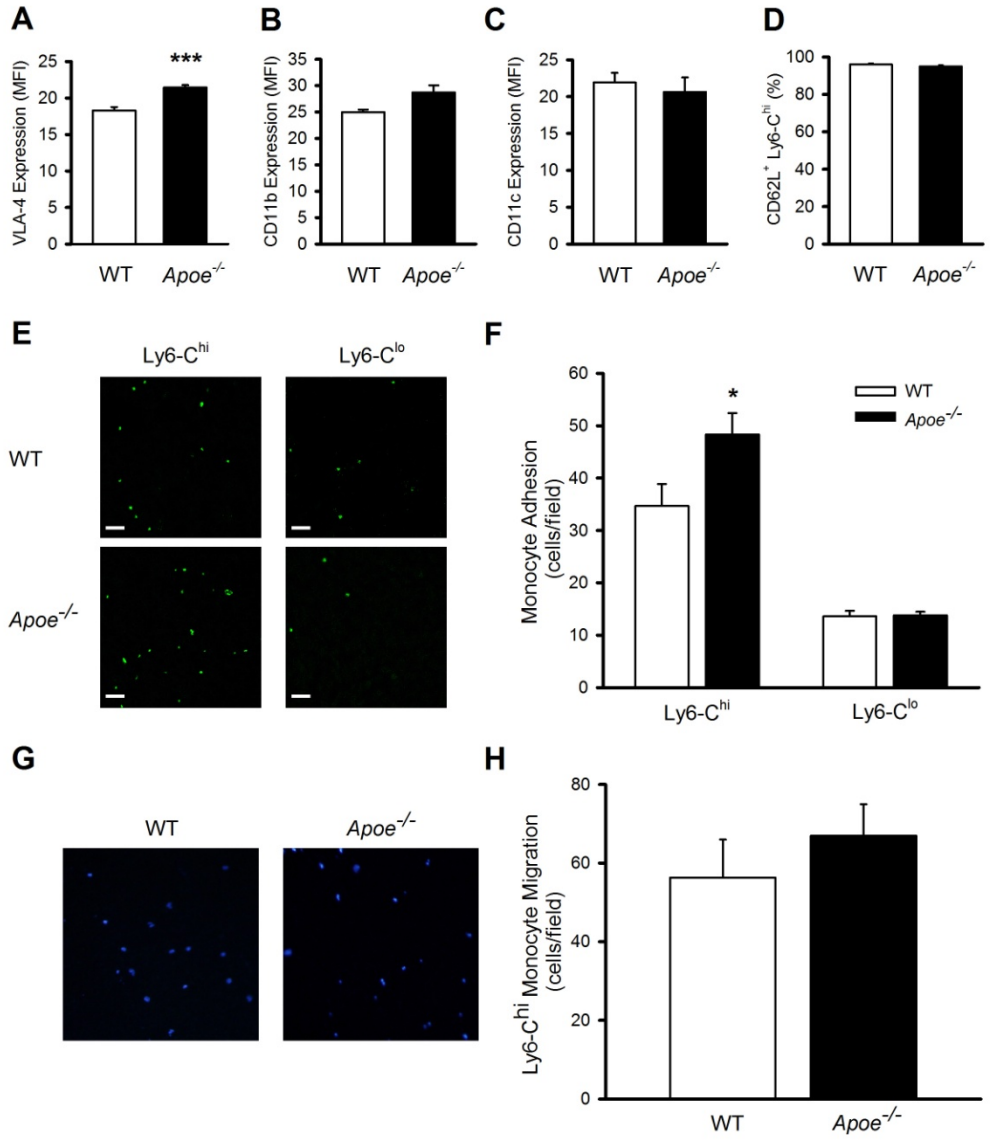

Figure S6. *Apoe$^{-/-}$ Ly6-C^{hi} monocytes from hypercholesterolemic mice are slightly primed for adhesion and but do not exhibit enhanced migratory responses.* WT and *Apoe$^{-/-}$* Ly6-C^{hi} monocytes were isolated from hypercholesterolemic mice by FACS. Ly6-C^{hi} monocyte activation was determined by **A)** VLA-4, **B)** CD11b, **C)** CD11c expression and **D)** percentage of CD62L^{+} cells. ***p<0.001 vs WT, n=6. **E&F)** Ly6-C^{hi} and Ly6-C^{lo} monocyte adhesion to HAECs. *p<0.05 *vs.* WT, n=5. Bar=100μM. **G&H)** Ly6-C^{hi} monocyte migration to MCP-1. 20X objective. n=5. P=*NS*. *Data is expressed as mean* ± SEM.

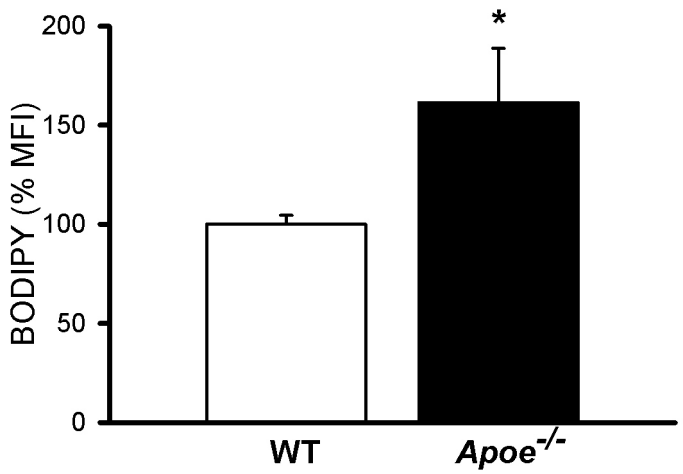

Figure S7. *Neutral lipid is increased in Apoe$^{-/-}$ HSPCs. WT and Apoe$^{-/-}$ mice were fed a WTD for 4 weeks. Bone marrow was isolated and HSPCs were identified using cell surface markers. BODIPY 493/503 was used to quantify neutral lipid content. Data presented as mean ± SEM, n=6.*

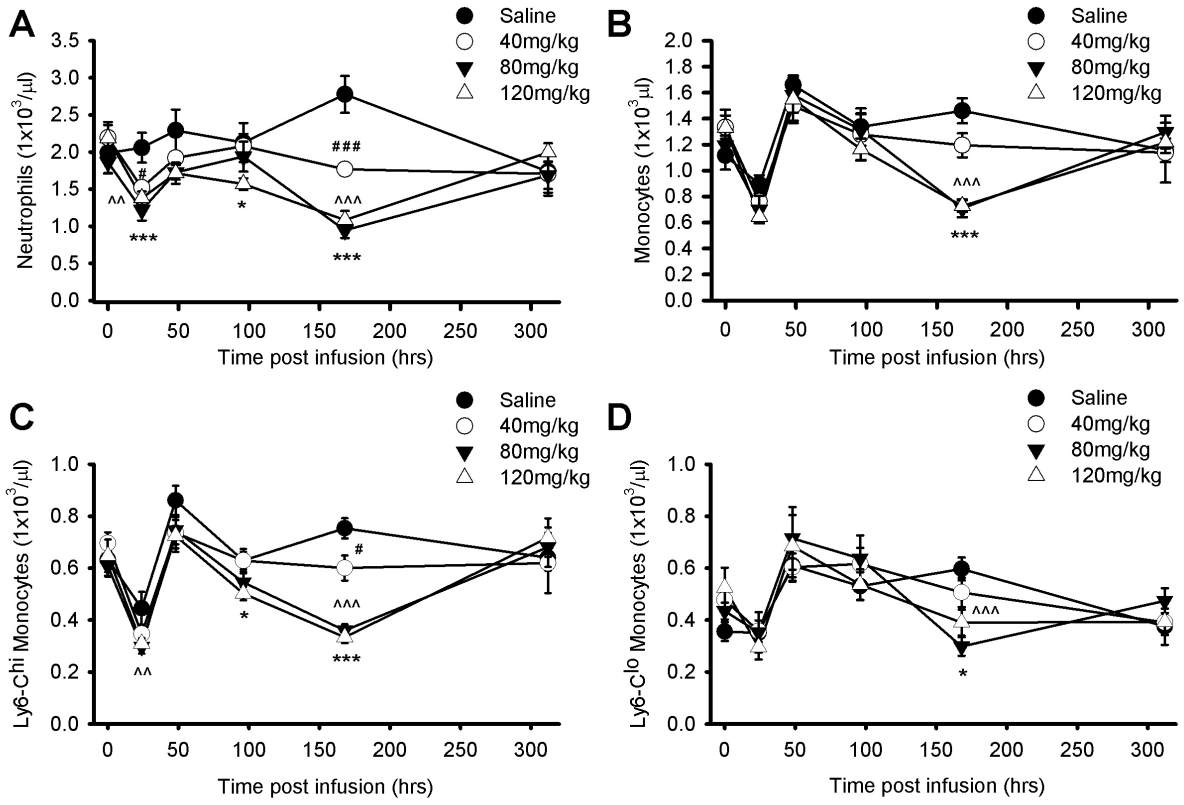

Figure S8. *Infusion of rHDL dose-dependently attenuates monocytosis and neutrophilia in WTD-fed Apoe$^{-/-}$ mice. Apoe$^{-/-}$ mice were placed on a WTD for 4 weeks prior to infusion with either saline or increasing doses of rHDL (CSL-111, at 40, 80 and 120 mg/kg).* ***A)*** *Neutrophils,* ***B)*** *Monocytes and* ***C)*** *Ly6-C^{hi}and* ***D)*** *Ly6-C^{lo} were assessed via flow cytometry and converted to cells/μL using counts from the CBCs. $^{\#\text{ - }\#\#\#}$p<0.05 - 0.001, 40 mg; ^ - ^^^p<0.05 - 0.001, 80 mg and * - ***p<0.05 - 0.001, 120 mg vs saline. Data presented as mean ± SEM, n=8.*

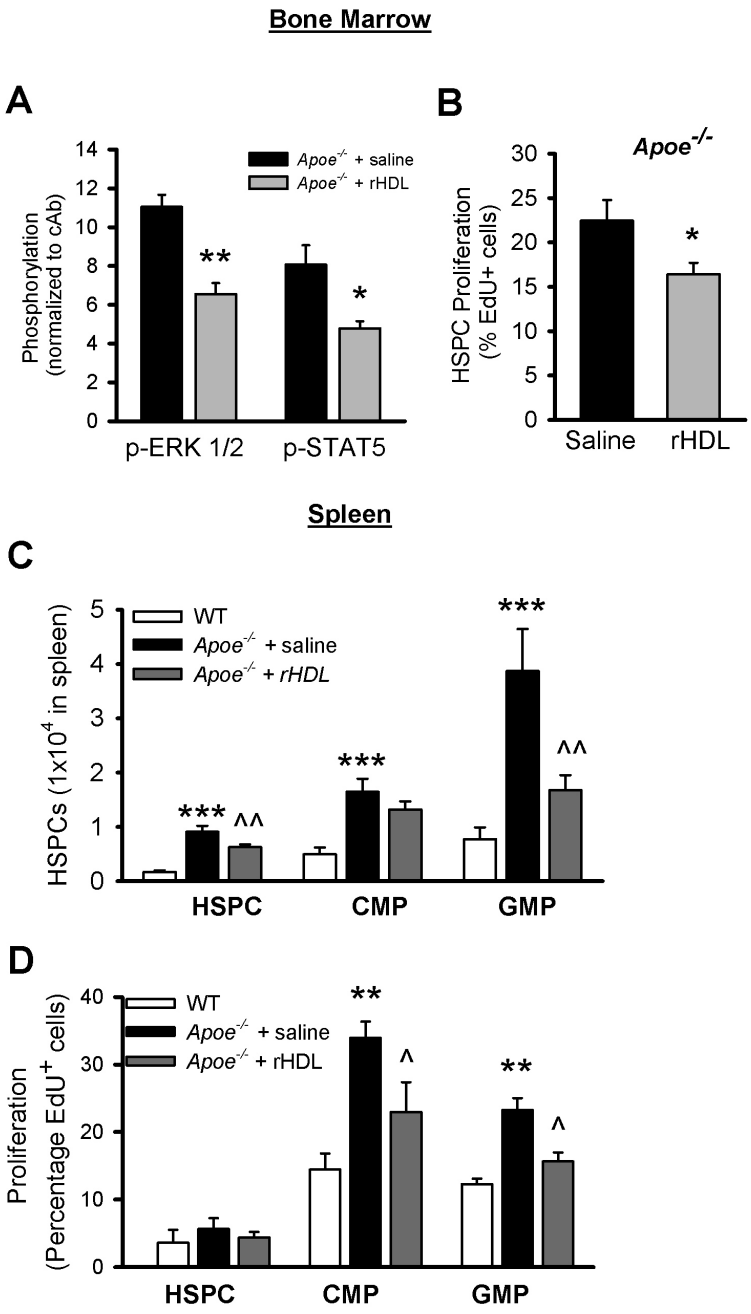

Figure S9. *Infusion of rHDL attenuates BM and Splenic HSPCs and proliferation in WTD-fed Apoe$^{-/-}$ mice. Apoe$^{-/-}$ mice were placed on a WTD for 4 weeks prior to infusion with either saline or rHDL (CSL-111; 80 mg/kg) and injected with EdU 18hrs prior to* sacrifice. ***Bone Marrow: A)*** Phosphorylated ERK1/2 and STAT5 was quantified by phospho-flow. ***B)*** *BM HSPCs proliferation.* **Spleen: C)** Number of HSPCs and progenitors in the spleen was quantified by flow cytometry and applied to the total number of cells in the spleen. **D)** In vivo proliferation of splenic HSPCs, CMPs and GMPs as determined by EdU incorporation. ***-*** p<0.01 - p<0.001 vs WT saline and ^-^^p<0.05 - p<0.01 vs. Apoe$^{-/-}$ Saline. Data presented as mean ± SEM, n=8.*

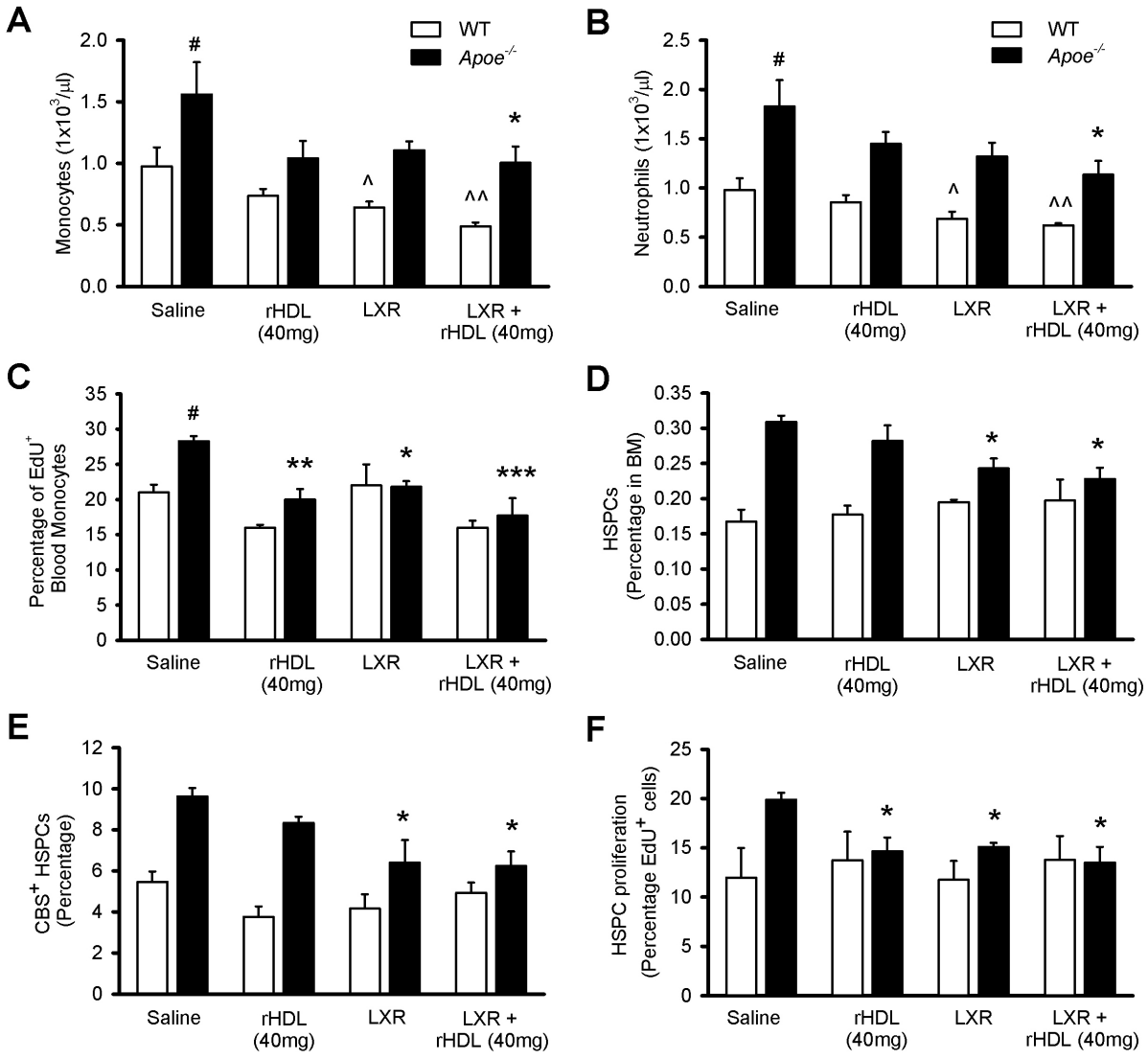

Figure S10. *Administration of rHDL and/or LXR agonist to 4 week WTD-fed WT or Apoe$^{-/-}$ mice: effects on suppressing leukocytosis at the level of the HSPCs.* WT (white bars) and *Apoe*$^{-/-}$ (black bars) mice were fed a WTD for 4 weeks prior to treatment with 3 daily injections of the LXR agonist TO901317 or vehicle (the day before, the day of and the day after infusion of rHDL/saline) and infused with either rHDL or saline. **A)** monocytes and **B)** neutrophils from peripheral blood 48 hrs post infusion. ^p<0.05, ^^p<0.01 vs WT saline and *p<0.05 vs *Apoe*$^{-/-}$ saline. **C)** In vivo monocyte release/proliferation over an 18 h period in WT and *Apoe* $^{-/-}$ mice 96 hrs post infusion. *p<0.05, **p<0.01, ***p<0.001 vs *Apoe*$^{-/-}$ saline. Data presented as mean ± SEM, n=6. **D-F)** 96hrs post infusion the mice were sacrificed and bone marrow stem cell populations were quantified via flow cytometry. **D)** The HSPCs and **E)** the population of *CBS*$^{+}$ HSPCs. *p<0.05 vs *Apoe*$^{-/-}$ saline. **F)** In vivo proliferation of the HSPCs as determined by EdU incorporation. *p<0.05, vs *Apoe*$^{-/-}$ saline. Data presented as mean ± SEM, n=6.

Table S1. *Plasma lipid and leukocyte levels*.

	Control (n=11)	LCAT (n=5)	Tangier (n=4)
Total Cholesterol (mg/dL)	**173±10**	**130±15**	**87±31***
HDL-C (mg/dL)	**69±7**	**9±7***	**2±2***
Apolipoprotein A-I (g/L)	**165±7.6**	**19±8***	**30±30***
Leukocytes (10^9/L)	**6.2±0.5**	**6.9±1**	**6.5±1.3**
Monocytes (10^9/L)	**0.52±0.05**	**0.51±0.06**	**0.53±0.07**
Neutrophils (10^9/L)	**3.39±0.34**	**4.12±1**	**4.11±0.99**

Results expressed as mean ± SEM. *p< 0.05 *vs*. control.

Table S2. *Genetic Mutations in LCAT and Tangier Patients.*

	Mutation	Type of defect
LCAT-1	**W99S/T147I**	**COMP**
LCAT-2	**T147I/V333M**	**COMP**
LCAT-3	**T147I**	**HOM**
LCAT-4	**T147I**	**HOM**
LCAT-5	**T147I**	**HOM**
Tangier-1	**Q1038X**	**HOM**
Tangier-2	**C1477R/IVS24+ G>C**	**COMP**
Tangier-3	**p. T929I/p. GG5277**	**COMP**
Tangier-4	**L1056P**	**HOM**

HOM=Homozygous COMP=Compound Heterozygous