SUPPLEMENTAL MATERIAL

Apolipoprotein A-I Promotes Atherosclerosis Regression in Diabetic Mice by Suppressing Myelopoiesis and Plaque Inflammation

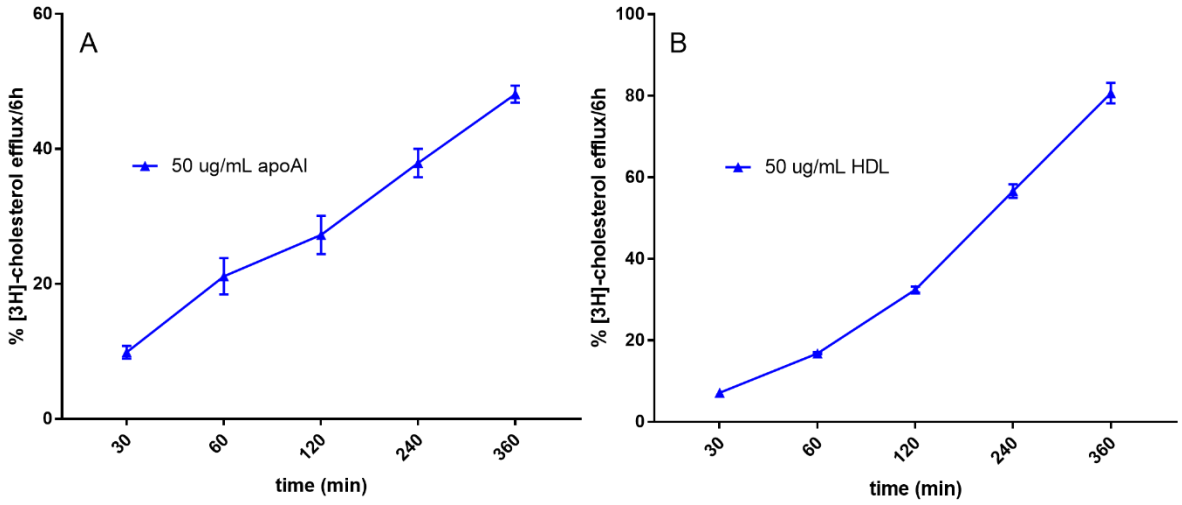
Tessa J. Barrett, PhD, Emilie Distel, PhD, Andrew J. Murphy, PhD, Jiyuan Hu, PhD, Michael S. Garshick, MD, Yoscar Ogando, BS, Jianhua Liu, MD, Tomas Vaisar, PhD, Jay W. Heinecke, MD, Jeffrey S. Berger, MD, Ira J. Goldberg, MD, Edward A. Fisher, MD, PhD

Supplementary Table 1.
Association between HbA1c (%) and WBCs in diabetic and control humans

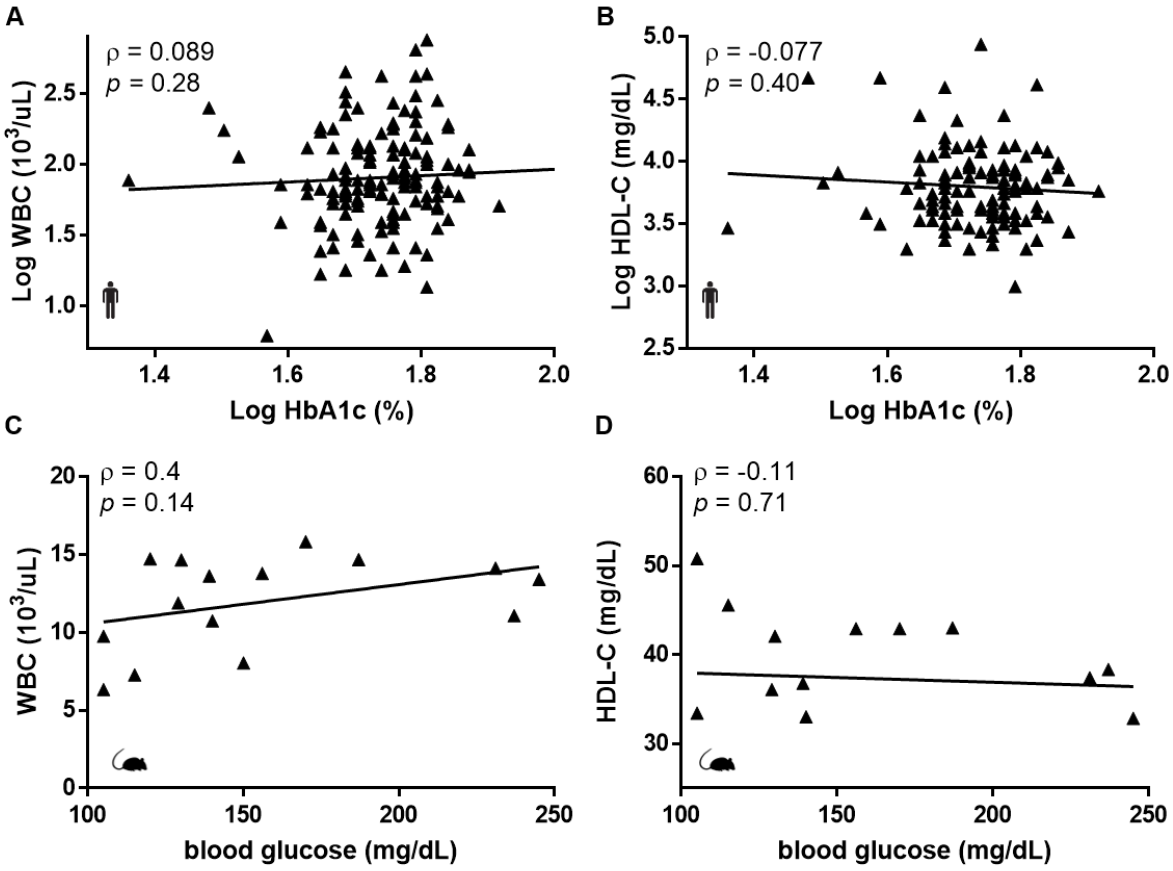
Variable	**Diabetic**		**Control**	
	Beta (95% CI)	*p-value*	*Beta* (95% CI)	*p*-value
Log HbA1c (%)	0.457 (0.103,0.811)	0.013	0.300 (-0.478,1.078)	0.452
Sex (Male)	0.011 (-0.139,0.162)	0.883	0.015 (-0.149,0.178)	0.859
Log Age	-0.229 (-0.610,0.153)	0.244	-0.202 (-0.499,0.094)	0.183
Log BMI	0.061 (-0.391,0.512)	0.793	0.064 (-0.299,0.427)	0.731
Log Cholesterol	0.029 (-0.244,0.302)	0.837	0.003 (-0.137,0.144)	0.961
Log LDL-C	0.056 (-0.116,0.228)	0.524	0.088 (-0.061,0.238)	0.250
Log Triglycerides	-0.017	0.804	0.124	0.056
(Yes)	(-0.148,0.115)		(-0.002,0.250)	
Hypertension (Yes)	0.090 (-0.137,0.317)	0.440	0.100 (-0.041,0.241)	0.166

Supplementary Table 2.
Association between HbA1c (%) and HDL-C in diabetic and control humans.

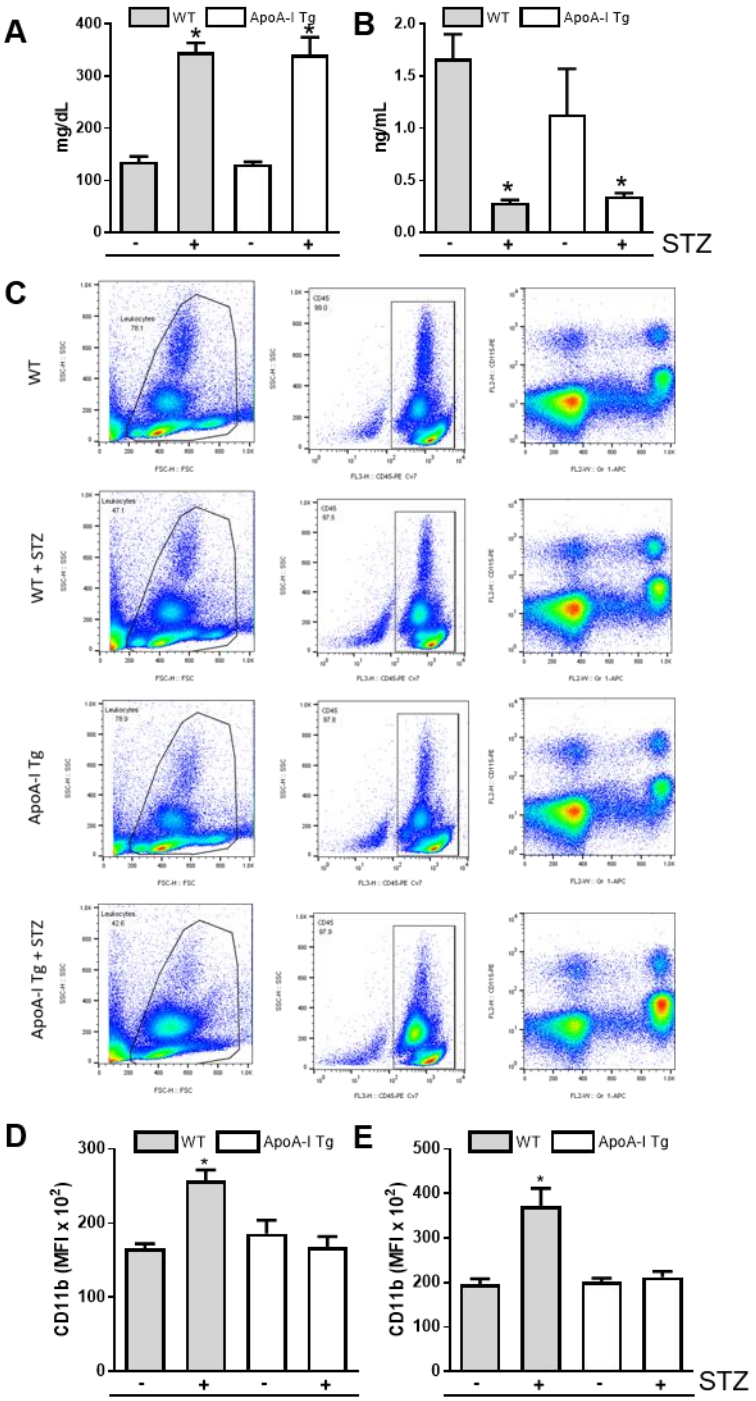
Variable	**Diabetic**		**Control**	
	Beta (95% CI)	*p-value*	*Beta* (95% CI)	*p*-value
Log HbA1c (%)	-0.417 (-0.780,-0.054)	0.030	0.500 (-3.132,4.132)	0.792
Sex (Male)	-0.197 (-0.358,-0.036)	0.022	-0.188 (-0.836,0.461)	0.581
Log Age	0.301 (-0.187,0.789)	0.234	0.689 (-0.873,2.251)	0.403
Log BMI	0.039 (-0.392,0.470)	0.859	1.118 (-0.248,2.484)	0.133
Log Cholesterol	0.364 (0.134,0.594)	0.004	0.001 (-0.184,0.186)	0.990
Log LDL-C	0.026 (-0.139,0.192)	0.757	0.295 (-0.137,0.727)	0.203
Log Triglycerides (Yes)	-0.180 (-0.367,0.006)	0.065	-0.025 (-0.449,0.400)	0.911
Hypertension (Yes)	-4.6E-04 (-0.011,0.010)	0.932	0.010 (-0.025,0.044)	0.595



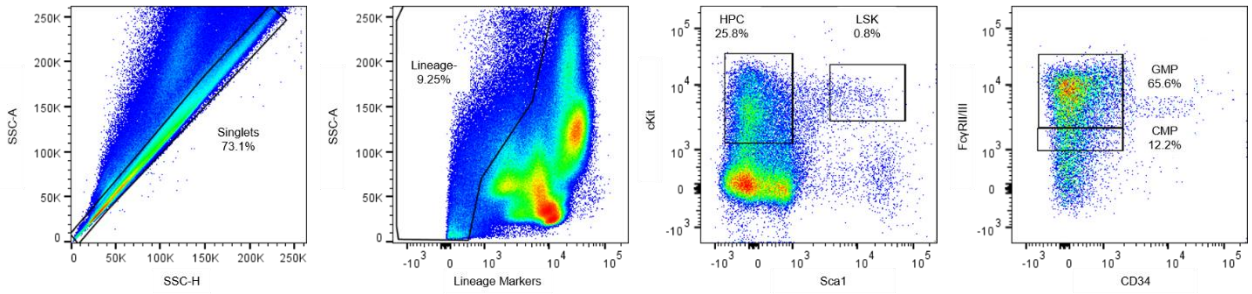
Supplemental Figure 1. Cholesterol efflux over time from monocyte progenitors in the presence of (A) apoA-I (50 ug/mL) and (B) HDL (50 ug/mL). Data are expressed as mean ± SEM at each time point.



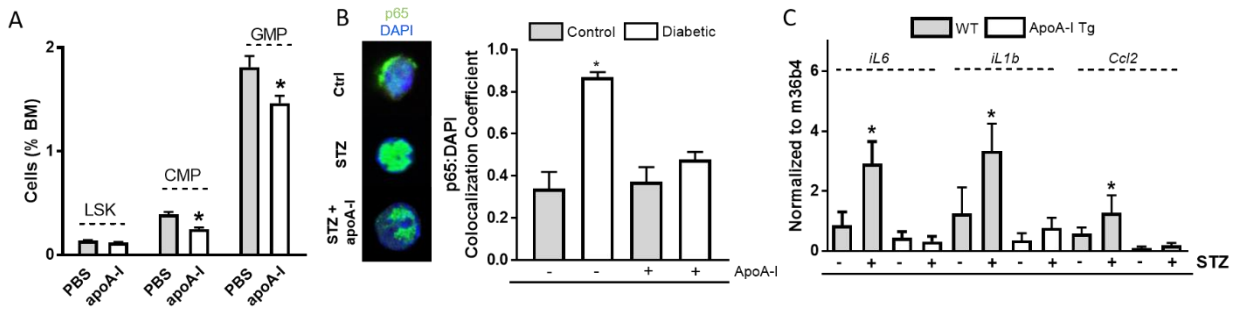
Supplemental Figure 2. Hyperglycemia is not associated with white blood cell count or HDL-C in control humans and mice. (A) White blood cells and (B) HDL-C do not associate with HbA1c in control humans (n=148, n=122). (C) White blood cells and (D) HDL-C do not associate with blood glucose in control mice (n=15). Pearson's correlation coefficient (ρ) and *p* values were assessed in R. Black triangles and line represent observations and regression line.



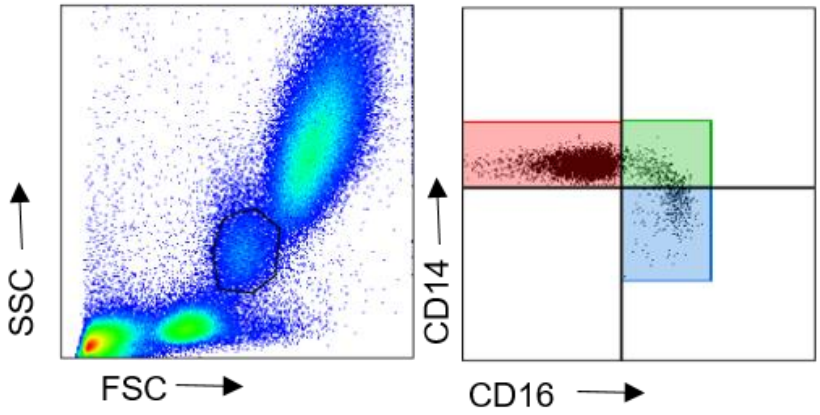
Supplemental Figure 3. (A) Blood glucose and (B) insulin levels in WT and apoA-I Tg control and diabetic mice. (C) Gating strategy for the identification of blood monocytes, plots correspond to data shown in Figure 2F. (D) Total monocyte and (E) Ly6C^{hi} monocyte CD11b expression as determined by flow cytometry. In panels A, B, D &E, data are expressed as mean ± SEM. (n=8-10/grp). * p<0.01 compared to WT control group as determined via 2-way ANOVA and Tukey's post-hoc test.



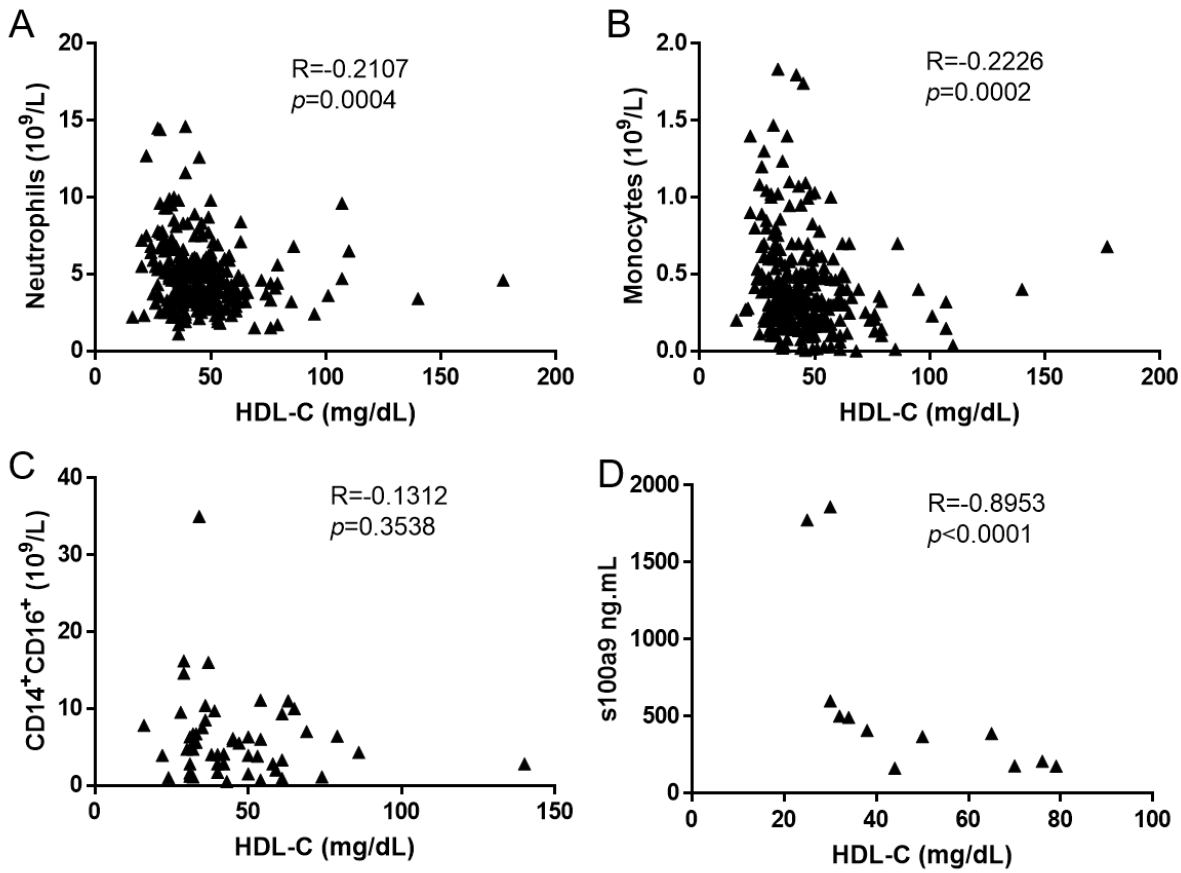
Supplemental Figure 4. Gating strategy for the identification of monocyte precursors within the bone marrow.



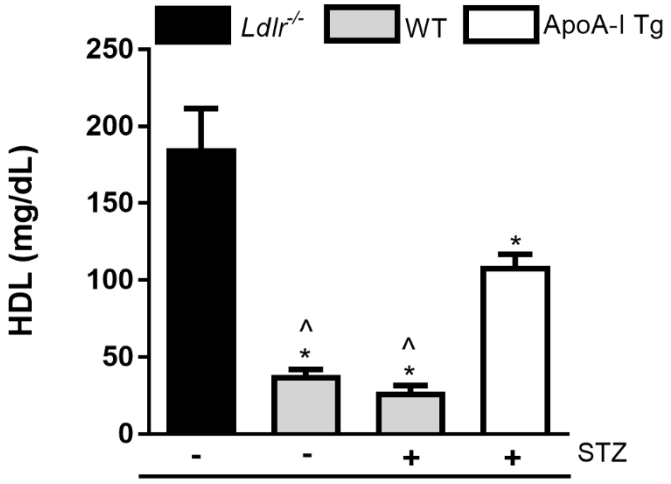
Supplemental Figure 5. (A) Quantification of LSKs, CMPs and GMPs in the bone marrow of diabetic WT mice who received saline or apoA-I injections (n=6 mice/grp). Data are expressed as mean ± SEM. * p<0.0001 determined by independent two-sample t-tests (two-tailed). (B) p65 staining of monocyte progenitors isolated from WT or STZ-treated mice and incubated with 50 ug/mL of apoA-I for 2 h. Cells were fixed and then stained for p65. Data represent progenitors pooled from 3 mice. The percentage co-localization of p65 and DAPI was calculated using the JACoP plug-in of ImageJ (NIH) after an appropriate threshold was established. (C) Transcript expression of NFκB targets in CMPs isolated from WT, diabetic WT, apoA-I Tg and apoA-I Tg diabetic mice (n=6 mice/grp). Data are expressed as mean ± SEM. * p<0.01 compared to WT control group as determined via 2-way ANOVA and Tukey's post-hoc test.



Supplemental Figure 6. Gating Strategy for the Identification of Human Monocyte Populations.



Supplemental Figure 7. HDL-C is negatively associated with leukocyte subsets and inflammation. (A) Neutrophil, and (B) monocyte counts correlated with HDL-C (n=336, n=335) and (C) $CD14^{+}CD16^{+}$ monocyte populations correlated with HDL-C (n=52). (D) Plasma S100A9 concentration as determined via ELISA (n=14). Spearman's rank correlation coefficient (R) and *p* values were assessed and depicted in each panel.



Supplemental Figure 8. HDL levels of mice in the regression study (n= 7-11 mice/grp). Data are expressed as mean ± SEM. * $p<0.01$ compared to the *Ldlr*$^{-/-}$ recipient group or ^ $p<0.01$ compared to ApoA-I Tg diabetic recipient group as determined via 1-way ANOVA and Tukey's post-hoc test.