

## SUPPLEMENTAL MATERIAL

Replacing saturated fat with unsaturated fat in western diet reduces foamy monocytes and atherosclerosis in male *Ldlr*<sup>-/-</sup> mice

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## Supplemental Tables

Table I. Diet composition

Diet components (% by weight)	ND	WD	EVOND
Saturated fat	1.4	13.3	2.6
MUFA	1.54	5.9	13.4
PUFA	2.36	0.9	4.3
Cholesterol	undetectable	0.2	0.2
Carbohydrates	58	49	49
Protein	25	19	19

Fat source in Western diet (WD) is milkfat (21% w/w) and in extra-virgin olive oil and nuts diet (EVOND) is extra-virgin olive oil (15% w/w), walnuts (3% w/w), almonds (1.5% w/w), and hazelnuts (1.5% w/w).

Table II. Major fatty acid composition in the diets

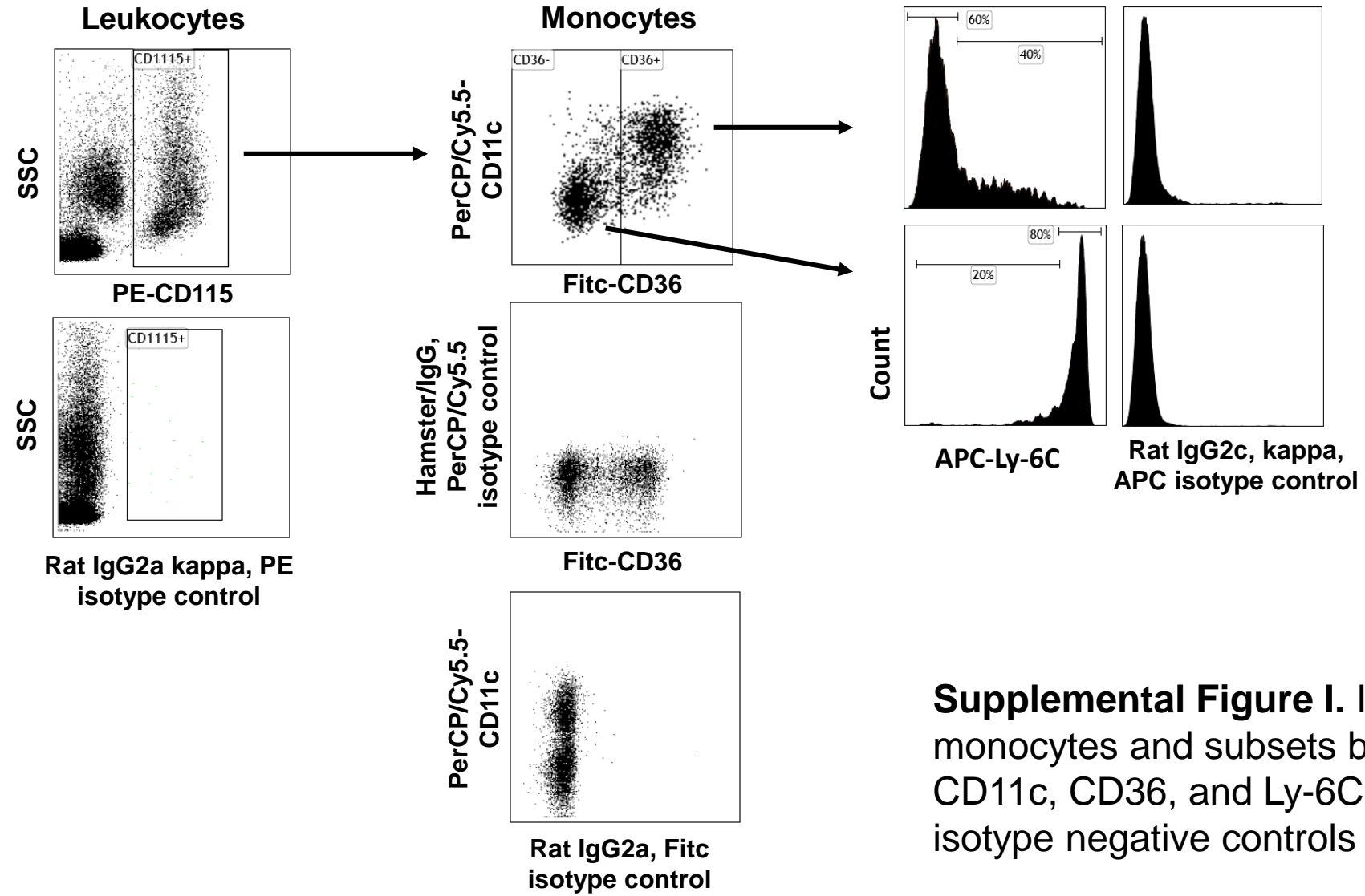
Fatty acid (% of total measured)	Nomenclature	ND	WD	EVOND
<b>Saturated fat</b>				
Capric acid	C10:0	0.1	3.58	0.14
Lauric acid	C12:0	0.25	2.47	0.11
Myristic acid	C14:0	2.27	10.93	0.32
Palmitic acid	C16:0	29.52	52.71	12.95
Stearic acid	C18:0	5.06	9.58	1.65
<b>Monounsaturated fat</b>				
Palmitoleic acid	C16:1	1.93	0.7	0.31
Oleic acid	C18:1	22.39	18.56	72.52
<b>Polyunsaturated fat</b>				
Linoleic acid	C18:2	34.63	0.61	11.75
$\alpha$ -linolenic acid	C18:3	0.9	0.34	0.12

Arachidonic acid	C20:4	1.01	0.25	0.09
Eicosapentaenoic acid (EPA)	C20:5	1.82	0.02	0.01
Docosahexaenoic acid (DHA)	C22:6	0.14	0.24	0.002

Table III. Primer sequences used in qPCR

Mouse genes	Primers	Sequences
GAPDH	Forward	CGACTTCAACAGCAACTCCCCTCTTCC
	Reverse	TGGGTGGTCCAGGGTTTCTTACTCCTT
Srebf1	Forward	GATGTGCGAACTGGACACAG
	Reverse	CATAGGGGGCGTCAAACAG
SCD1	Forward	TTCTTGCGATACTCTGGTGC
	Reverse	CGGGATTGAATGTTCTTGTCGT
FAS	Forward	AAGTGTCTGGACTGTGTCATTTTTACA
	Reverse	TTAATTGTGGGATCAGGAGAGCAT
CD36	Forward	TCCAGCCAATGCCTTTGC
	Reverse	TCAGTGCAGAAACAATGGTTGTC

# Supplemental Figure I



**Supplemental Figure I.** Identification of blood monocytes and subsets by staining for CD115, CD11c, CD36, and Ly-6C or with corresponding isotype negative controls in *Ldlr*<sup>-/-</sup> mice.

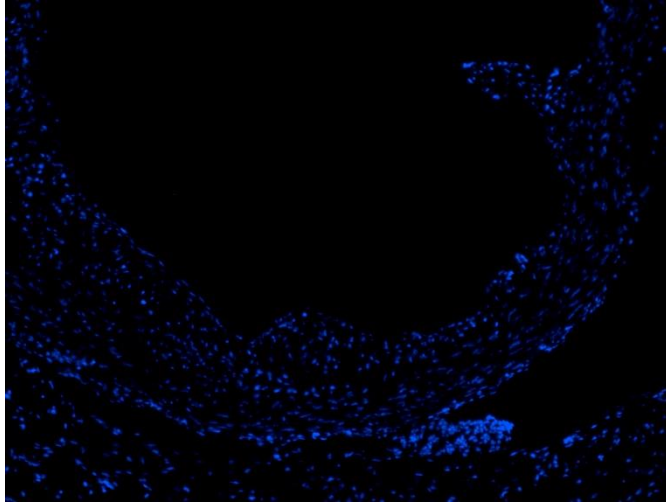
## Supplemental Figure II



**Supplemental Figure II.** Fluorescent isotype control staining of on-chip adhesion assay of monocytes (20x original magnification). PE, rat IgG2a  $\kappa$  isotype control; FITC, Alexa Fluor® 488 Armenian hamster IgG isotype control. See Figure 5B for positive staining.

## Supplemental Figure III

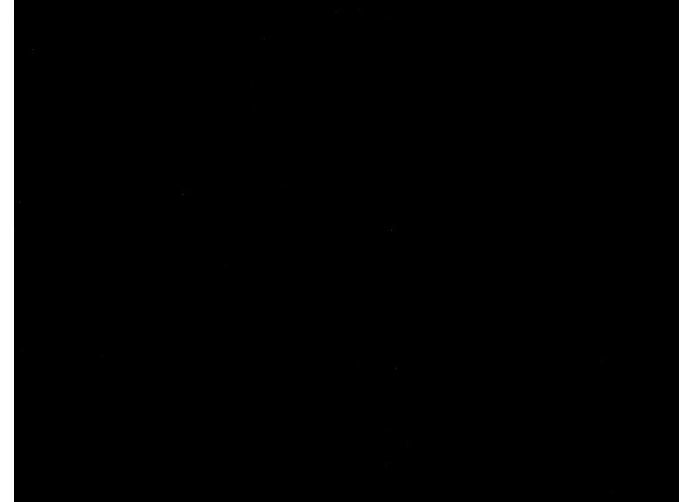
DAPI



FITC

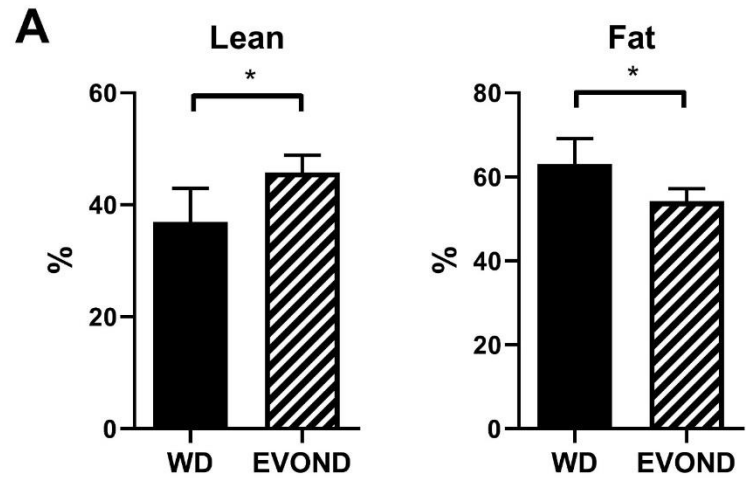


PE

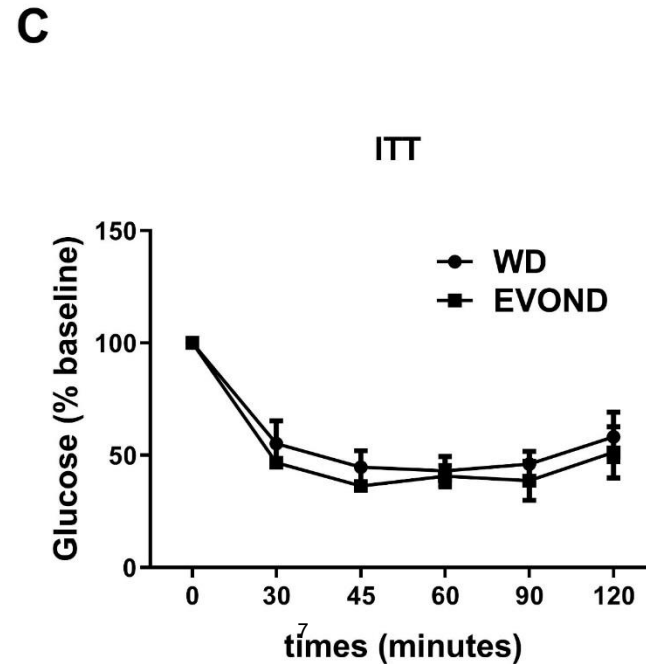
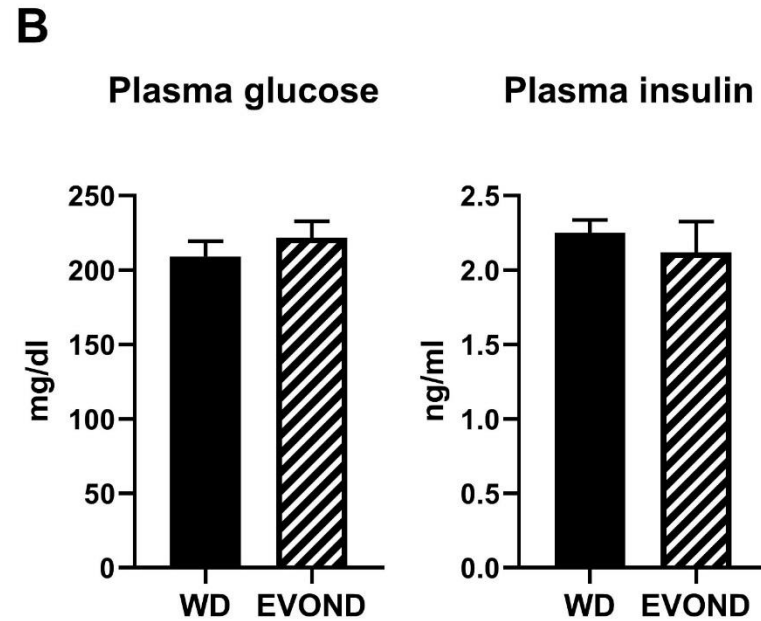


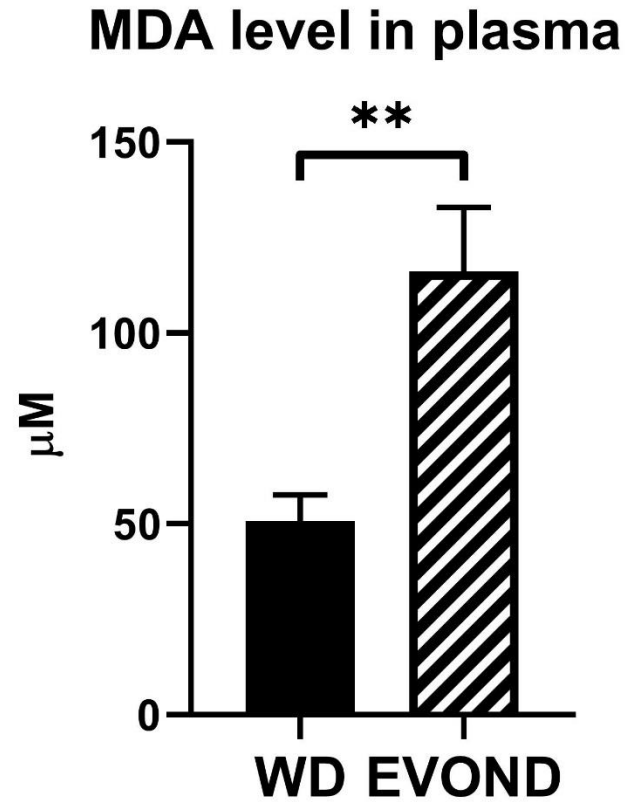
**Supplemental Figure III.** Fluorescent isotype control staining of aortic sinus lesions (10x original magnification). PE, hamster IgG1  $\kappa$  isotype control; FITC, rat IgG2b  $\kappa$  isotype Ccontrol. See Figure 6C for positive staining.

# Supplemental Figure IV



**Supplemental Figure IV.** Body composition, plasma glucose and insulin levels, and insulin tolerance test (ITT) in mice on WD or EVOND for 3 months. A, Percentages of lean mass and fat mass were measured using Lunar scanner. B, Plasma glucose and insulin levels. C, Insulin tolerance test. Data are shown as mean  $\pm$  SEM. n=8/group, \*p<0.05.

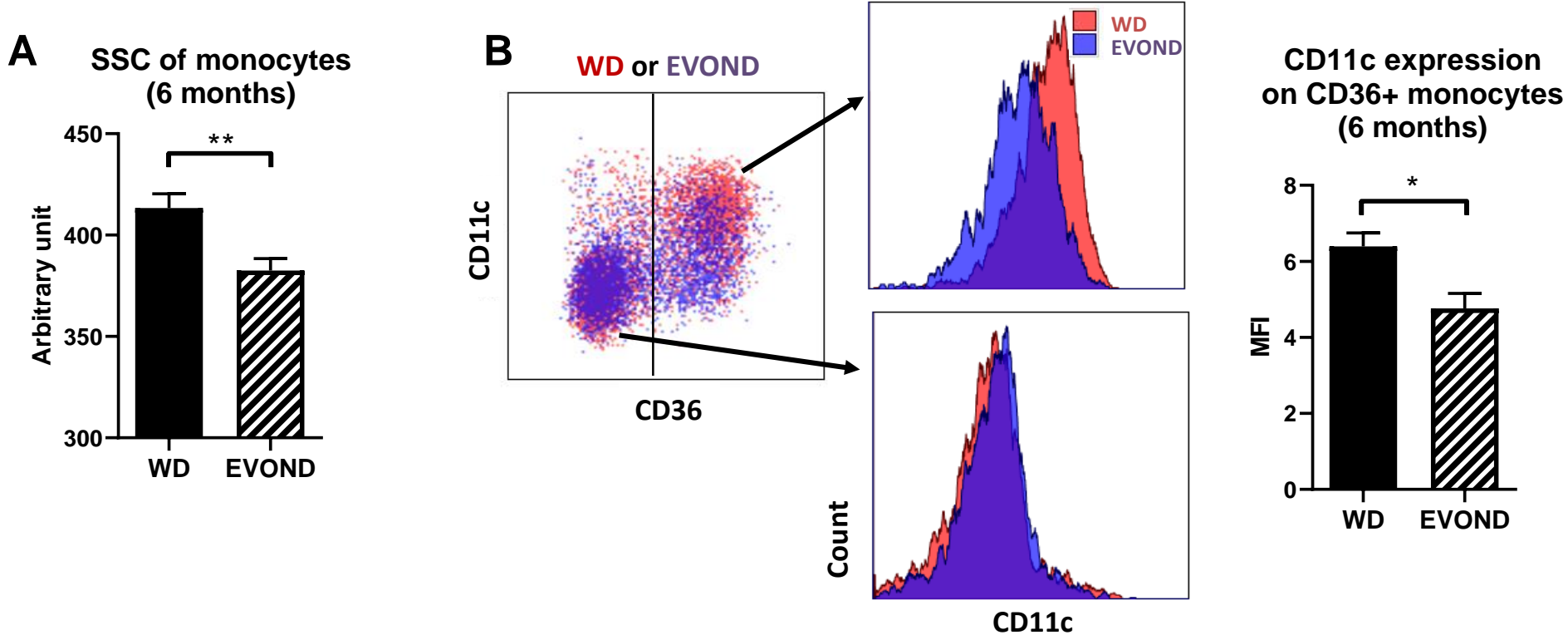




**Supplemental Figure V.** Malondialdehyde (MDA) levels measured by the thiobarbituric acid–reactive substances (TBARS) assay in plasma from mice on WD and EVOND. Data are expressed as mean  $\pm$  SEM. n=7/group, \*\*p<0.01.

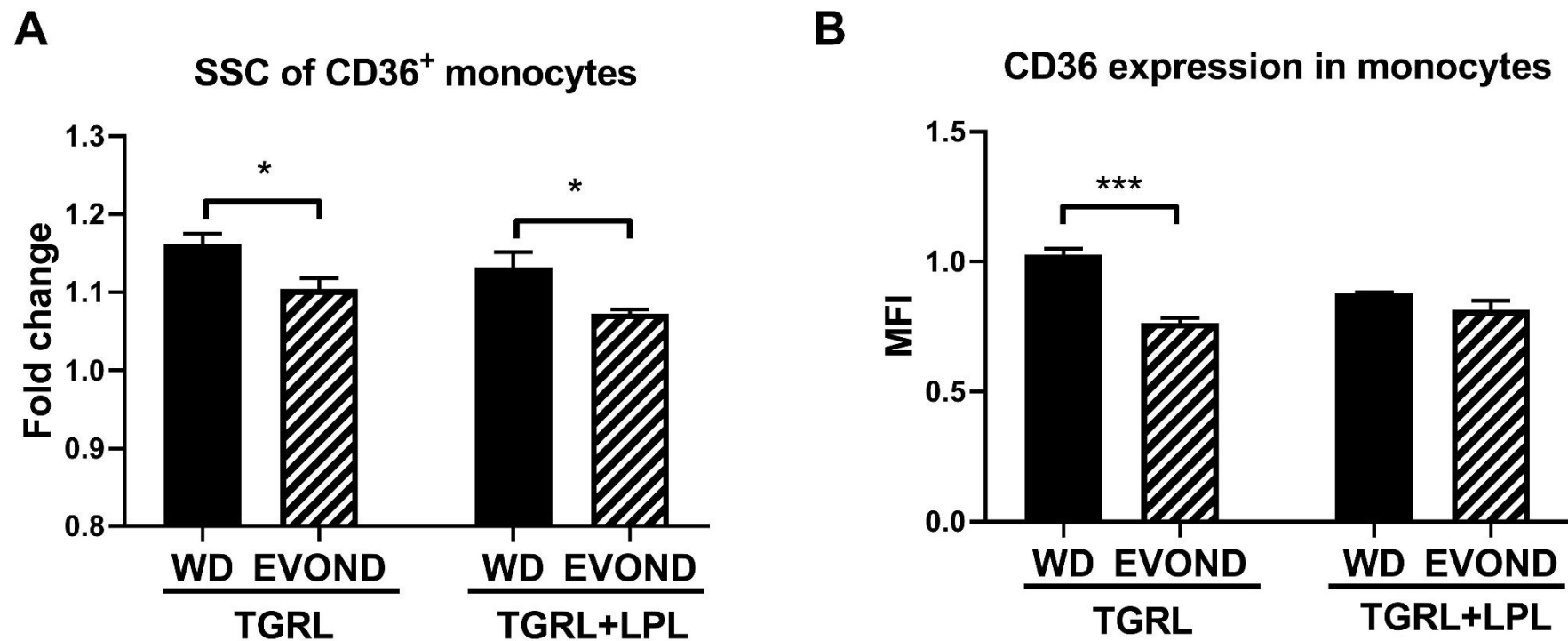


# Supplemental Figure VI



**Supplemental Figure VI.** Mice on EVOND for 6 months had improved monocyte phenotypes. A, SSC value of circulating monocytes of mice on different diets. B, EVOND reduced CD11c expression on CD36<sup>+</sup> monocytes compared to WD. Data are shown as mean  $\pm$  SEM. n=7/group, \*p<0.05, \*\*p<0.01.

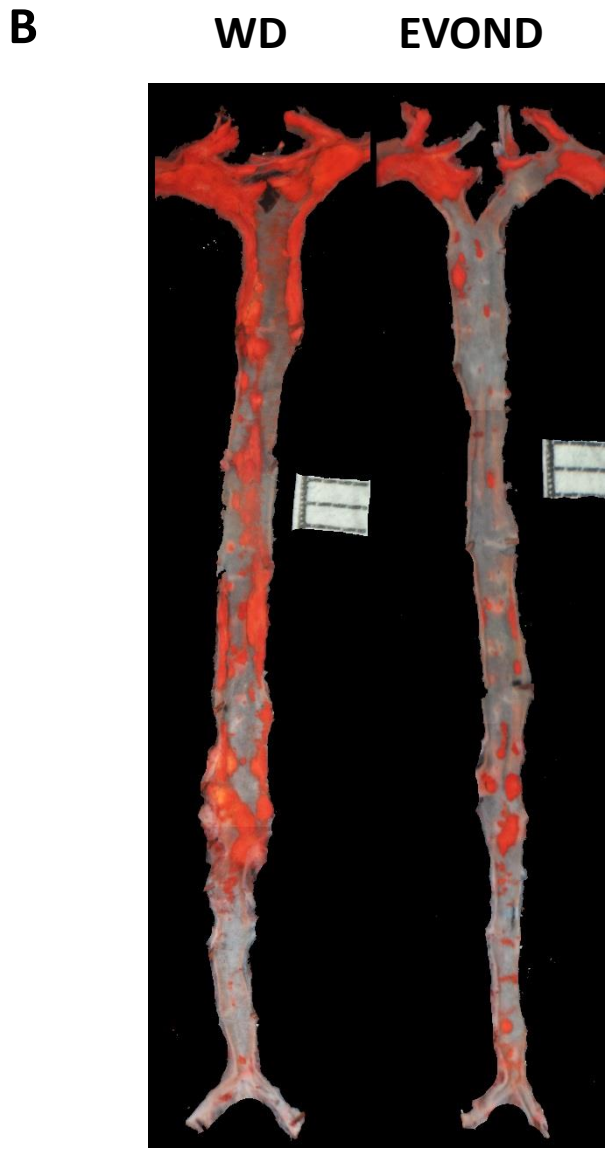
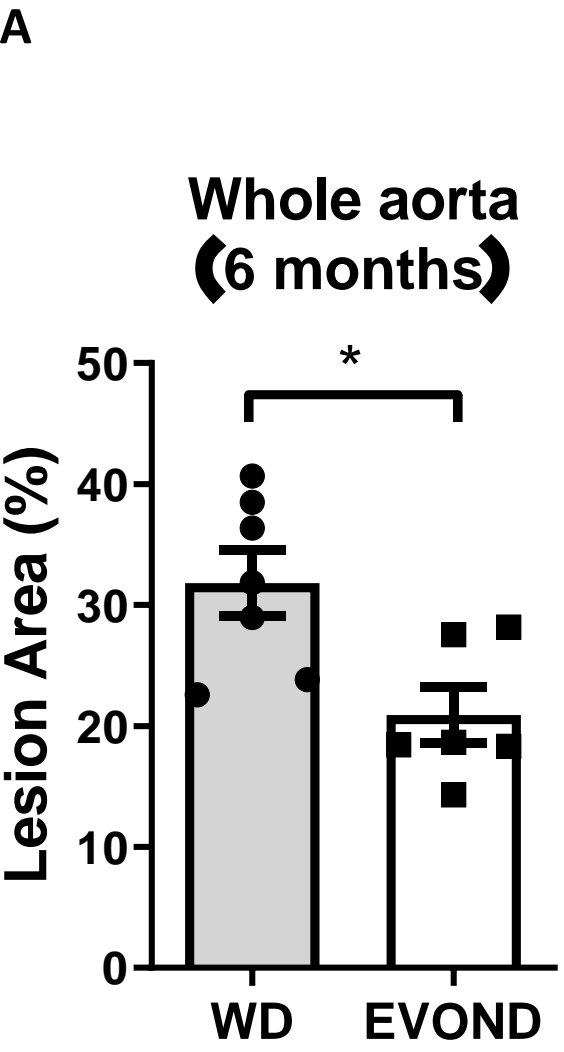
## Supplemental Figure VII



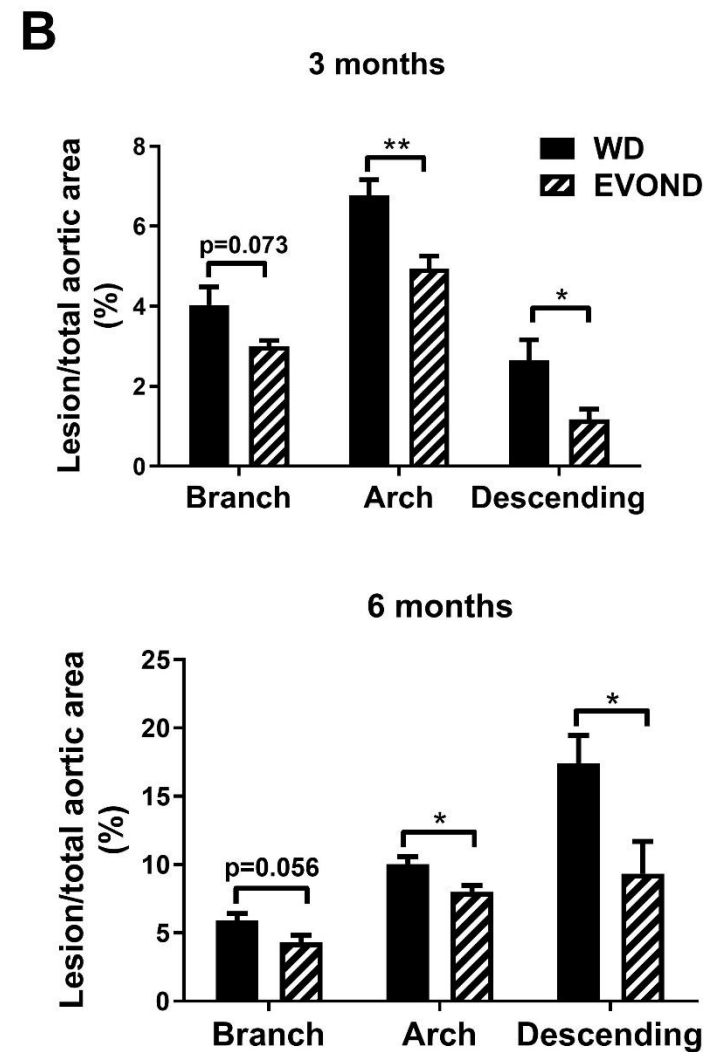
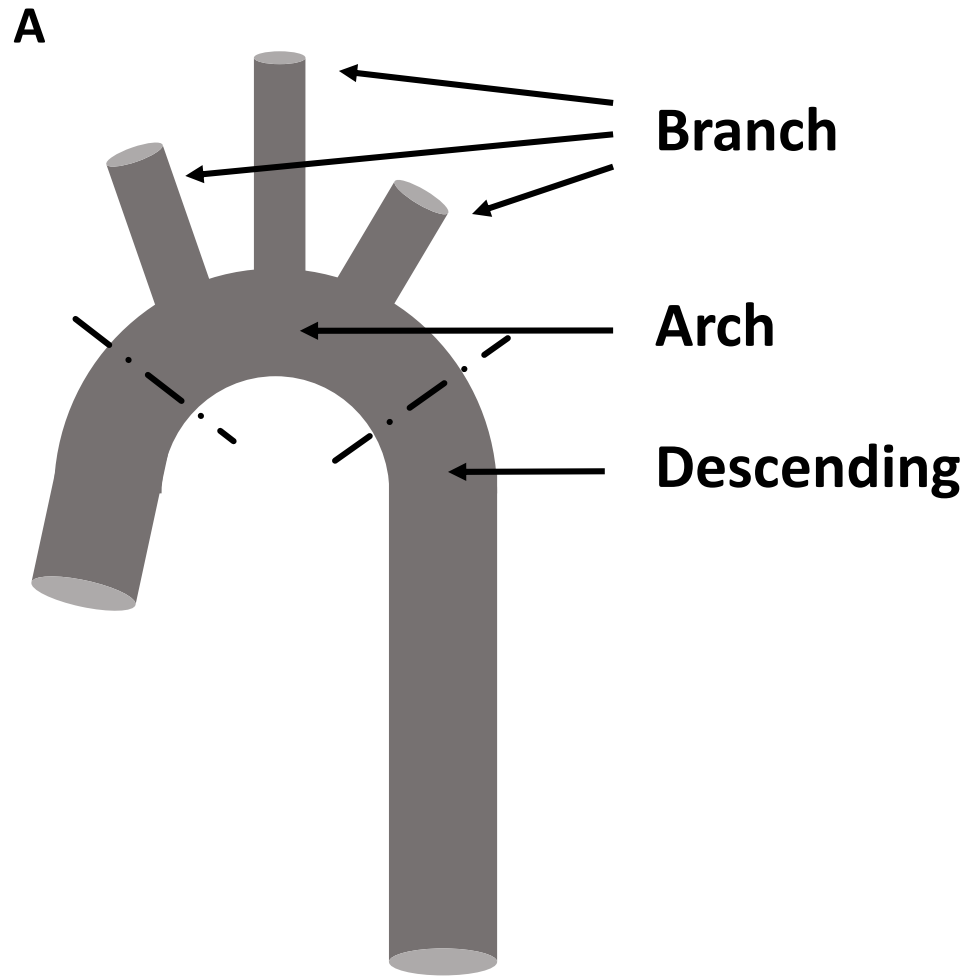
**Supplemental Figure VII.** EVOND reduced monocyte uptake of triglyceride-rich lipoprotein (TGRL) with or without lipoprotein lipase (LPL) pretreatment in vitro. A, SSC value of CD36<sup>+</sup> monocytes (normalized fold change to no TRGL treatment control) and B, MFI level of CD36 on monocytes (from ND-fed *Ldlr*<sup>-/-</sup> mice), after incubation with TGRL alone or TGRL plus LPL from mice on WD or EVOND (n=3/group). Data are shown as mean  $\pm$  SEM. \*p<0.05, \*\*\*p<0.001.

# Supplemental Figure VIII

**Supplemental Figure VIII.** Mice on EVOND vs. WD for 6 months had greater reductions in atherosclerosis than those on diets for 3 months. A, Quantification of oil red O-positive lesion size of the whole aorta from mice on WD or EVOND. B, Representative en face oil red O staining of whole aorta. Data are shown as mean  $\pm$  SEM. n=7/group, \*p<0.05.



# Supplemental Figure IX



**Supplemental Figure IX.** Quantification of atherosclerotic lesions at aortic branch and arch areas and descending aorta from mice on WD or EVOND. A, Schematic view of aorta areas for lesion size quantification. B, Lesion size as percentage of the whole aorta area from mice on WD or EVOND for 3 months (n=8/group) or 6 months (n=7/group). Data are shown as mean  $\pm$  SEM. \*p<0.05, \*\*p<0.01.

## Major Resources Tables

### Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex
B6.129S7-Ldlr <sup>tm1Her</sup> /J	The Jackson Laboratories	C57BL/6J	male

### Animal breeding

	Species	Vendor or Source	Background Strain	Other Information
Parent - Male	B6.129S7-Ldlr <sup>tm1Her</sup> /J	The Jackson Laboratories	C57BL/6J	
Parent - Female	B6.129S7-Ldlr <sup>tm1Her</sup> /J	The Jackson Laboratories	C57BL/6J	

### Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)
PE anti-mouse CD115	eBioscience	12-1152-83	0.06µg/100µL	
FITC anti-mouse CD204	Bio-Rad Laboratories	MCA1322F	0.1µg/100µL	
FITC anti-mouse CD36	Bio-Rad Laboratories	MCA2748F	1µg/100µL	
PerCP/cy5.5 anti-mouse CD11c	eBioscience	45-0114-82	0.25µg/100µL	
PE anti-mouse TNFα	eBioscience	12-7321-41	0.125µg/100µL	
PE anti-mouse IL-1β	eBioscience	12-7114-82	0.06µg/100µL	
FITC anti-mouse Mac3	BD Biosciences	553323	5µg/mL	
PE anti-mouse CD11c	eBioscience	12-0114-82	2µg/mL	
Alexa Fluor 488 anti-mouse CD11c	Biolegend	117313	5µg/mL	
APC anti-mouse Ly-6C	eBioscience	17-5932-82	0.125µg/100µL	

PerCP-Cyanine5.5 anti-mouse F4/80	eBioscience	45-4801-82	0.5µg/100µL	
PE Hamster IgG1 κ Isotype Control	eBioscience	12-4888-81	2µg/mL	
FITC Rat IgG2b, κ Isotype Control	BD Biosciences	556923	5µg/mL	
PE Rat IgG2a kappa Isotype Control	eBioscience	12-4321-80	0.06µg/100µL	
Alexa Fluor® 488 Armenian Hamster IgG Isotype Ctrl	Biolegend	400923	5µg/mL	
APC Rat IgG2c, κ Isotype Ctrl Antibody	Biolegend	400713	0.125µg/100µL	
PerCP-Cyanine5.5, Armenian Hamster IgG Isotype Control	eBioscience	45-4888-80	0.25µg/100µL	
PerCP-Cyanine5.5, Rat IgG2a, κ Isotype Control	BD Biosciences	550765	5µg/mL	
FITC Rat IgG2a, κ Isotype Control	BD Biosciences	554688	1µg/100µL	