Supplemental material for:
Elevated Apolipoprotein C3 augments diabetic kidney disease and associated atherosclerosis in type 2 diabetes

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Supplemental Methods:

Glucose tolerance test

Mice were fasted for 4-6 hours before injecting either 1 mg/body weight or 0.75 mg/g of glucose intraperioneally. Blood glucose was monitored at 0, 15, 30, 60 and 120 minutes after glucose administration.

Insulin tolerance test

Mice were fasted for 4-6 hours before injecting 2 mU/g body weight of human recomniant insulin intraperioneally. To avoid stress-induced hyperglycemia, mice were first subjected to a sham injection and blood collection prior to the actual injection of insulin (1). Blood glucose was monitored at 0, 15, 30, 60 and 120 minutes after glucose administration.

Pyruvate tolerance test

Mice were fasted for 6 hours before injecting 1.5 mg/g body weight of sodium pyruvate intraperioneally. Blood glucose was monitored at 0, 15, 30, 60 and 120 minutes after glucose administration.

Supplemental Table 1: Characteristics of people with diabetic nephropathy and non-diabetic controls

	Controls (n=11)	Diabetic (n=19)
Age (years)	52.1 ± 3.2	58.1 ± 2.9
Male gender (%)	9 (81.8 %)	10 (52.6 %)
Glomerular size (μm^2)	12759 ± 2058	18664 ± 1738**
Diabetic kidney disease stage 1	N/A	4
Diabetic kidney disease stage 2 (a and b)	N/A	8
Diabetic kidney disease stage 3	N/A	7

Data are means ± SEM or n (%), *p<0.05, Mann-Whitney tests.

Supplemental Table 2: Blood glucose, plasma triglycerides and cholesterol over time (14-week study)

	cASO				LDLR ASO			
	WT	WT APOC3 ASO	OB	OB APOC3 ASO	WT	WT APOC3 ASO	OB	OB APOC3 ASO
N	13	14	10	11	19	15	13	13
Sex (Male)	7	8	7	4	8	8	8	5
Baseline								
Body weight (g)	23.2±1.0	22.6±0.8	30.5±1.3*	27.3±1.9*	30.5±1.3	27.3±1.9	30.0±1.5*	28.4±1.4*
Blood glucose (mg/dl)	193.8±13.0	180.8±7.1	273.5±43.4	257.2±36.3	181.1± 5.42	171.5.2±6.1	266.9±23.4	319.0±39.4*
Cholesterol (mg/dl)	77.5±2.7	75.6±2.9	121.4±3.0*	124.0±6.6*	121.4±3.0	124.0±6.6	139.1±8.0*	110.1±7.7*#
Triglycerides (mg/dl)	112.7±9.1	102.6±8.3	133.3±29.8	132.4±11.2	133.3±29.8	132.4±11.2	197.4±25.0	208.8±37.4
2 weeks								
Body weight (g)	29.8±0.9	30.2±0.8	50.7±1.2*	47.9±1.3*	29.2±1.0	29.1±1.3	44.8±1.0*	44.0±1.7*
Blood glucose (mg/dl)	173.2±6.3	176.9±6.4	391.5±45.6*	330.1±29.4*	157.3±3.3	160.3±4.7	428.9±23.3*	431.5±30.7*$
Cholesterol (mg/dl)	139.8±9.0	130.4±7.7	283.7±12.3	282.1±16.1	428.8±22.1$	325.7±29.9$	804.7±85.5*$	780.8±51.7*$
Triglycerides (mg/dl)	111.3±10.5	54.9±3.2	173.1±22.3	114.5±11.5	165.5±12.3	153.7±38.8	500.1±53.9*$	340.5±39.2*#$
4 weeks								
Body weight (g)	32.6±1.1	32.8±1.2	61.3±1.1*	59.4±1.2*	32.2±0.9	31.7±1.3	51.3±1.3*	52.2±1.8*
Blood glucose (mg/dl)	169.5±4.8	164.0±4.8	299.3±32.8*	235.3±12.8	163.9±5.7	166.4±4.8	447.6±27.0*$	321.7±29.9*#$
Cholesterol (mg/dl)	139.8±9.0	113.6±6.5	293.5±11.3*	257.1±18.0*	401.7±13.6$	375.7±16.3$	829.2±46.8*$	971.3±46.0*#$
Triglycerides (mg/dl)	89.1±8.2	55.9±5.7	135.7±12.4*	109.9±17.9	161.8±12.9	106.0±10.7	467.6±22.2*$	407.3±41.7*$
8 weeks								
Body weight (g)	36.7±1.3	36.1±1.2	74.1±1.4*	69.2±0.9*	35.9±0.9	34.8±1.1	55.8±1.4*	57.9±1.9*
Blood glucose (mg/dl)	161.4±6.1	143.2±4.7	220.7±24.0	194.5±16.9	156.5±4.3	162.4±6.1	372.3±32.6*$	203.1±30.5#
Cholesterol (mg/dl)	135.4±8.1	115.0±5.4	327±22.32*	274.6±18.5	350.9±13.2$	337.5±18.7$	946.2±68.8*$	798.0±52.0*#$
Triglycerides (mg/dl)	84.4±7.2	38.3±2.9	115.1±15.5	64.7±3.1	100.5±7.2	63.8±3.8	473.5±31.4*$	263.0±18.7*#$
14 weeks								
Body weight (g)	41.2±1.3	39.3±1.0	83.7±1.5*#	74.7±1.7*	39.5±1.2	38.0±1.1	71.6±1.4*	65.4±2.8*
Blood glucose (mg/dl)	169.5±7.2	152.7±4.0	242.9±23.8	203.1±17.7	160.7±4.4	159.9±5.1	331.8±35.7*	205.9±32.4#
Cholesterol (mg/dl)	140.6±7.8	116.2±9.1	274.2±30.1	242.8±23.0	319.8±14.2$	264.0±19.5$	722.6±66.5*$	625.8±64.3*$
Triglycerides (mg/dl)	81.8±12.4	27.7±4.1	108.5±16.0	61.7±5.2	93.2±12.0	66.6±11.6	462.7±31.7*$	164.9±18.7#$

Data are means ± SEM, p<0.05 * vs WT (same treatment, e.g. WT vs OB), # vs APOC3 (same treatment), $ vs LDLR ASO within the same treatment.

Supplemental Table 3: Urine markers (14-week study)

	cASO				LDLR ASO			
	WT	WT APOC3 ASO	OB	OB APOC3 ASO	WT	WT APOC3 ASO	OB	OB APOC3 ASO
Urine creatinine, 8 weeks (mg/ml)	0.45±0.09 (9)	0.47±0.03 (13)	0.19±0.01* (10)	0.20±0.02* (13)	0.39±0.05 (10)	0.37±0.08 (9)	0.19±0.03 (9)	0.22±0.02 (13)
Urine volume, 8 weeks (µl)	160±32	157±25	337±60	447±62	129±26	142±24	299±59	347±71
Urine albumin, 8 weeks (µg/ml)	219±110	239±23	784±103*	574±69*	204±50	184±44	711±68*	445±88
Urine creatinine, 14 weeks (mg/ml)	0.69 ±0.17 (9)	0.29±0.05 (11)	0.29±9.08 (9)	0.46±0.08 (13)	0.54±0.19 (15)	0.29±0.04 (13)	0.15±0.02 (14)	0.42±0.1 (12)
Urine volume, 14 weeks (µl)	110±37	145±27	330±100	380±80	180±30	140±30	690±110*#	150±40
Urine albumin, 14 weeks (µg/ml)	104±27	130±29	892±111*	660±70*	136±17	113±24	511±69*	729±99*
Plasma BUN, 14 weeks (mg/dl)	24.7±1.0 (11)	21.8±1.4 (13)	35.0±4.4 (9)	28.9±9 (13)	25.5±1.5 (20)	24.1±1.7 (15)	43.3±2.5* (13)	40.2±2.8* (12)

Data are means ± SEM, $p<0.05$ * vs WT (same treatment), # vs APOC3 (same treatment).

Supplemental Table 4: Plasma inflammatory markers (14-week study)

	cASO				LDLR ASO			
	WT	WT APOC3 ASO	OB	OB APOC3 ASO	WT	WT APOC3 ASO	OB	OB APOC3 ASO
N	7	6	10	13	13	6	13	12
14 weeks								
Plasma IL-18 (pg/ml)	49.3±18	83.7±24.5	151.2±49	98.5±25	161.9±32	118.1±53	423.4±38*#	226.9±57
Plasma IL-6 (pg/ml)	15.0±6.3	9.6±3.1	15.9±5.2	9.2±1.9	17.5±4.1	16.6±9.8	6.8±1.7	17.0±5.4
Plasma TNFα (pg/ml)	8.1±1.0	7.7±0.5	6.8±0.4	6.1±0.3	7.6±0.6	8.0±1.0	7.0±0.4	8.3±1.0
Plasma TGFβ1 (pg/ml)	4256±636	3681±469	5091±1183	5415±1113	3688±343	2816±515	8197±1294*	8354±1694*
Plasma IL-10 (pg/ml)	25.2±8.6	33.7±19.8	34.2±7.1	41.3±2.9	21.6±4.1	33.9±3.5	52.4±6.6*	57.4±4.2
Plasma SAA (µg/ml)	67.0±47 (4)	83.0±26 (3)	198.2±74* (4)	136.5±17 (4)	43.8±14 (4)	26.4.0±6 (4)	115.2±20 (7)	154.2±17* (10)

Data are means ± SEM, $p<0.05$ * vs WT (same treatment), # vs APOC3 (same treatment).

Supplemental Table 5: Blood glucose, triglycerides, and cholesterol (4-week study)

	LDLR ASO			
	WT	WT APOC3 ASO	OB	OB APOC3 ASO
N	11	6	7	8
Sex (Male)	7	4	5	5
Body weight (g)	35.2±1.2	33.5±0.9	47.2±0.6*	50.1±1.8*
Blood glucose (mg/dl)	158±6	159±5	536±33*#	409±33*
Cholesterol (mg/dl)	463±33	425±44	850±141*	765±57*
Triglycerides (mg/dl)	143±13	94±8	385±39*#	267±29*
Monocytes (% of CD45+)	9.0±0.7	10.0±0.9	23.8±1.2*	21.8±1.4*
$Ly6C^{hi}$ monocytes (% of CD45+)	4.0±0.3	5.7±0.7	12.9±1.1*	13.1±0.9*
$Ly6C^{hi}$ LFA1 (MFI)	1226±108	1361±202	1225±81	1389±152
$Ly6C^{lo}$ monocytes (% of CD45+)	4.6±0.5	3.9±0.4	9.2±1.2*	7.5±1.3
$Ly6C^{lo}$ CD49D (MFI)	8010±242	8188±397	8551±352	9003±461
$Ly6C^{lo}$ CX3CR1 (MFI)	4301±245	4050±323	3380±311	3175±132

Data are means ± SEM, $p<0.05$ * vs WT (same treatment), # vs APOC3 (same treatment).

Supplemental Table 6: Blood glucose, triglycerides, and cholesterol (LFA1 study)

	LDLR ASO			
	WT Cont Ab	WT LFA1 Ab	OB Cont Ab	OB LFA1 Ab
N	5	5	5	6
Sex (male)	3	4	3	2
Blood glucose (mg/dl)	165.0±13.20	163.4±8.346	525.6±34.67*	504.7±38.81*
Cholesterol (mg/dl)	354.5±50.75	319.2±63.41	787.8±25.85*	743.8±57.20*
Triglyceride (mg/dl)	135.7±15.98	123.2±18.11	845.2±150.2*	595.4±70.67*

Data are means ± SEM, $p<0.05$ * vs WT (same treatment), # vs LFA1 ab (same treatment).

Supplemental Table 7: Blood glucose, triglycerides, and cholesterol (12-week GalNAc APOC3)

	LDLR ASO			
	WT cASO	WT APOC3 ASO	OB cASO	OB APOC3 ASO
N	4	4	4	5
Sex (Male)	4	4	4	5
Baseline				
Body weight (g)	26.7±1.3	25.8±0.9	31.4.±3.2	30.5±1.0
Blood glucose (mg/dl)	152.0±8.4	158.5±8.5	370.8±72*	313±41*
4 weeks				
Body weight (g)	34.0±0.4	33.8±0.9	41.8.±1.2*	40.1±1.8*
Blood glucose (mg/dl)	156.8±6	156.8±14	480.3±36*	353.2±31*
Cholesterol (mg/dl)	206.8±20	176.2±26	606.2±13*#	434.0±51*
Triglyceride (mg/dl)	121.3±16	60.0±10	232.4±22*#	107.8±21
8 weeks				
Body weight (g)	33.5±2.6	36.6±1.4	45.9.±1.9*	48.1±1.0*
Blood glucose (mg/dl)	153.8±8	158.3±12	478.5±63*	398.6±60*
Cholesterol (mg/dl)	145. 4±11	115.0±21	532.0±52*#	297.9±68*
Triglyceride (mg/dl)	88.0±11	43.9±8	339.2±47*#	93.9±26
12 weeks				
Body weight (g)	36.9±0.5	37.8±1.4	52.0.±1.1*	53.3±2.0*
Blood glucose (mg/dl)	125.3±10	137.0±3.0	425.5±58*#	254.8±41
Cholesterol (mg/dl)	159.8±13	158.5± 26	685.2±63*	523.4±90*
Triglyceride (mg/dl)	56.4±7.6	33.9±3.0	252.1±14*#	100.2±21*

Data are means ± SEM, $p<0.05$ * vs WT (same treatment), # vs APOC3 (same treatment).

Supplemental Table 8: Antibodies and key reagents

Antibody/reagent	Clone/product ID	Manufacturer	Dilution/Dose
CD45-FITC	30-F11	eBioscience	1:500 (0.1 µg/100 µl)
CD115-APC	AFS98	eBioscience	1:100 (0.2 µg/100 µl)
GR1-PE-Cy7	RB6-8C5	eBioscience	1:1000 (0.05µg/100 µl)
CD49D-PE (α4 integrin)	PS/2	Southern Biotech	1:1000 (0.01 µg/100 µl)
CD11B-PE	M1/70	eBioscience	1:500 (0.1 µg/100 µl)
F4/80-PE-Cy7	BM8	eBioscience	1:200 (0.2 µg/100 µl)
Viability dye e450	65-0863-14	eBioscience	1:1000
Mac-2	CL8942AP	Cedarlane	1 µg/ml
Fluoresbrite yellow green microspheres (latex particles)	1715210	Polysciences, Inc.	250 µl/mouse (diluted 1:4)
Alpha Smooth muscle actin	Ab5694	Abcam	0.2 µg/ml
APOB (biotinylated) Control (BAF108)	BAF3556	RnD systems	1:50
Tyramide-AlexaFluor488 (100x stock) used for APOB staining	B40953	ThermoFisher	1x
APOE	AB183597	Abcam	1:2000
Perilipin 2	NB110-40877	Novus	1:400
ICAM1	14-0542-82	eBioscience	1:100
biotinylated-*Lycopersicon esculentum-derived* lectin	B-1175	Vector Laboratories	0.1 mg/mouse
EasySep Biotin Positive selection kit II	17683	Stem cell technologies	NA
APOC3 ELISA	Ab217777	Abcam	1:250,000 (plasma)
Albumin ELISA	1011	Ethos Bioscience	1:50 (urine)
The Creatinine Companion (Creatinine Assay)	1012	Ethos Bioscience	1:20 (urine)
IL-18 ELISA	88-50618	Invitrogen	1:2 (plasma)
IL-6 Mouse ProQuantum Immunoassay Kit	A43656	Invitrogen	1:5 (plasma)
TNF alpha Mouse ProQuantum Immunoassay Kit	A43658	Invitrogen	1:10 (plasma)
TGF-β1 ELISA	BMS608-4	Invitrogen	1:20 (plasma)
IL-10 ELISA	88-7105-22	Invitrogen	1:2 (plasma)
ICAM1 blocking ab	clone M17/4; (BE0006)	BioXcell	300 µg/mouse x3 times per week
Control ab (against trinitrophenol, not expressed in mammals)	clone 2A3 (BE0089)	BioXcell	300 µg/mouse x3 times per week
Mouse Insulin	clone K36AC10 (I2018)	Sigma Aldrich	1:20,000
APOC3 ASO (Gen 2)	CCAGCTTTATTAGGGACAGC	Ionis	50 mg/kg/week
Control for APOC3 ASO (Gen 2)	CCTTCCCTGAAGGTTCCTCC	Ionis	50 mg/kg/week
LDLR ASO	CTTTATCTTTAACCTC	Ionis	5 mg/kg/week
Control for LDLR ASO	GGCCAATACGCCGTCA	Ionis	5 mg/kg/week
APOC3 GalNAc	CCAGCTTTATTAGGGACAGC	Ionis	10 mg/kg/week
GalNAc control	CCTTCCCTGAAGGTTCCTCC	Ionis	10 mg/kg/week

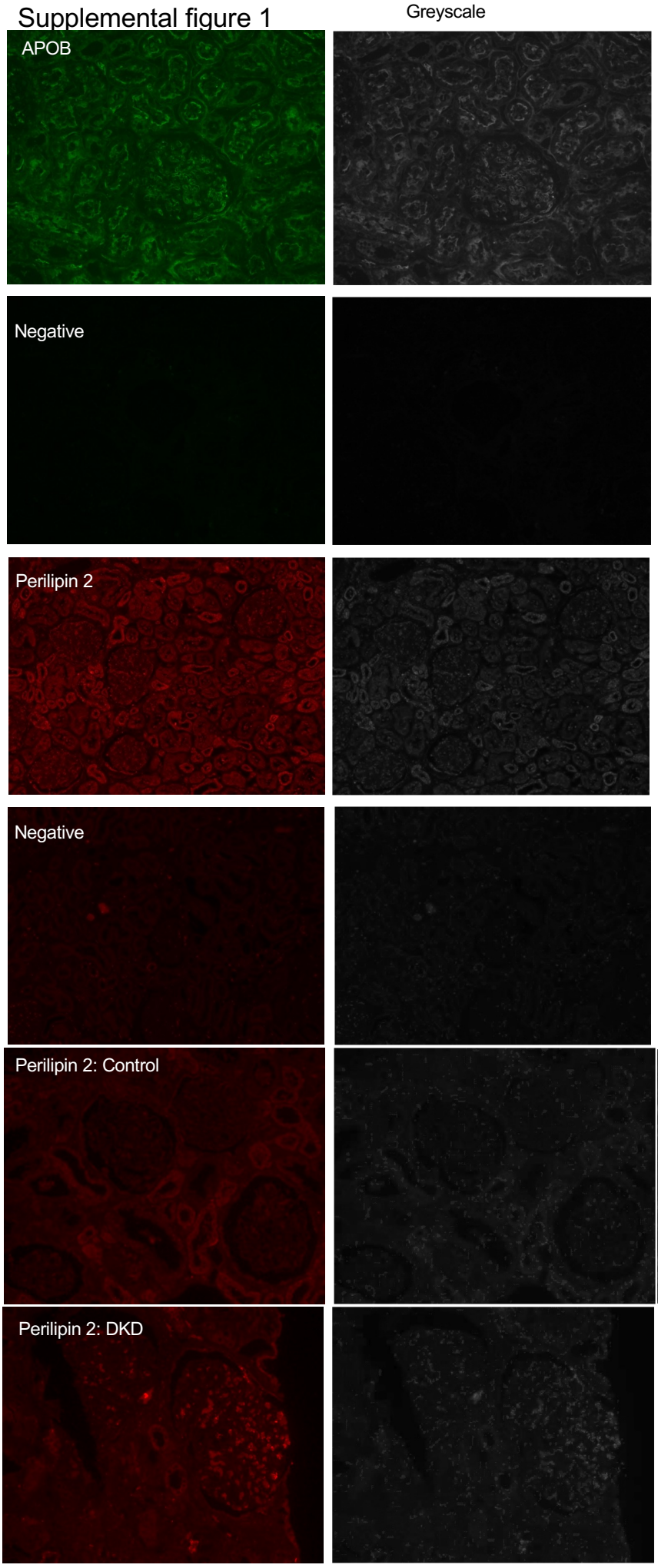
Supplemental table 9: Primer sequences

Gene	Forward primer	Reverse primer
Rn18s	CATTAAATCAGTTATGGTTCCTTTGG	CCCGTCGGCATGTATTAGCT
Ccl2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
Il1b	GGGCTGCTTCCAAACCTTTG	TGATACTGCCTGCCTGAAGCTC
Tnfa	CCTGTAGCCCACGTCGTAG	GGGAGTAGACAAGGTACAACCC
Il6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
Icam1	GGCATTGTTCTCTAATGTCTCC	GCTCCAGGTATATCCGAGCTTC
Vcam1	TGCACAGTCCCTAATGTGTATCC	GACTTTATGCCCATTTCCTCCA
Apoc3	TACAGGGCTACATGGAACAAGC	CAGGGATCTGAAGTGATTGTCC
Perilipin 2	CTTGTGTCCTCCGCTTATGTC	GCAGAGGTCACGGTCTTCAC

Supplemental references

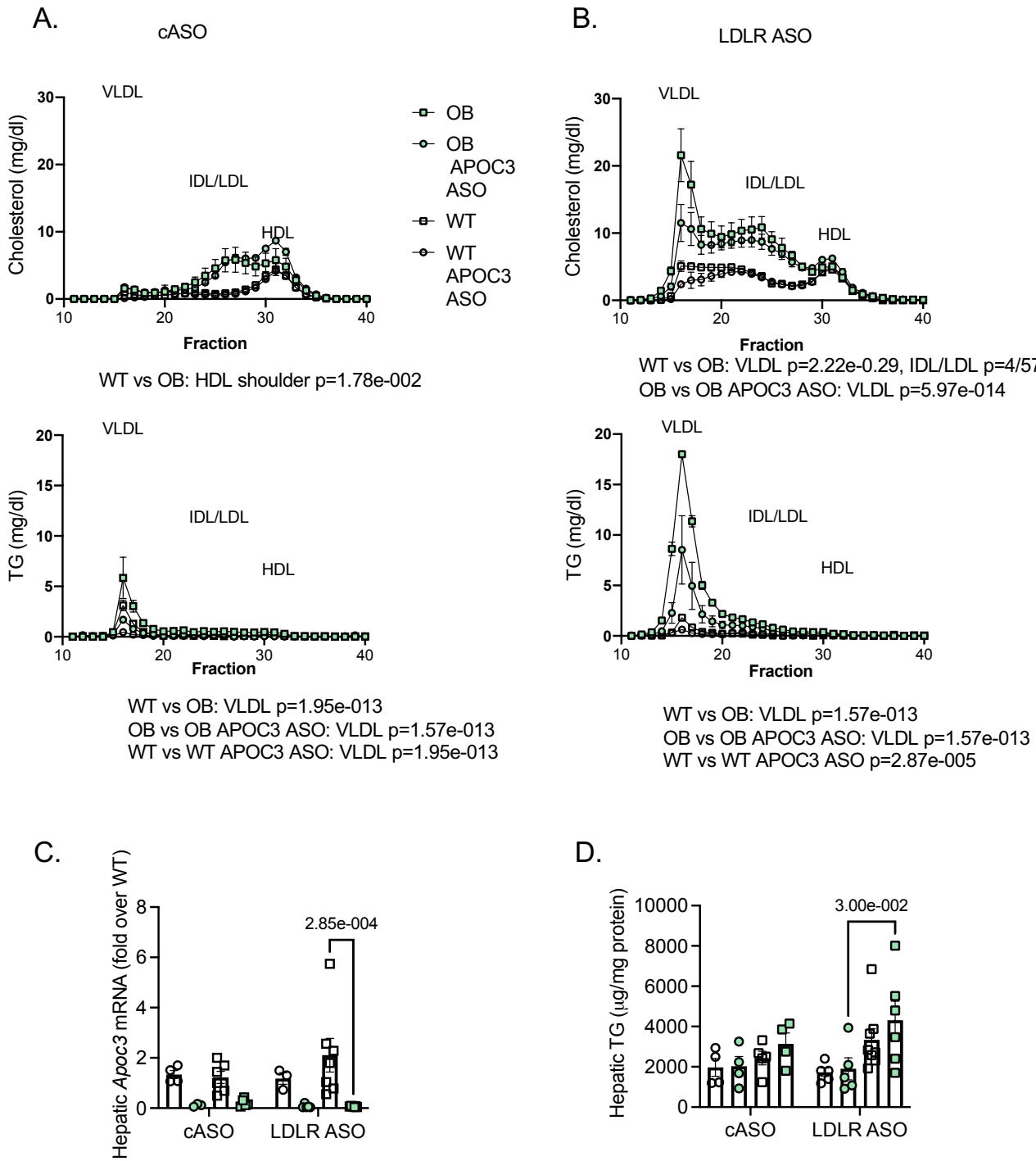
1. Hull RL, Hackney DJ, Giering EL, and Zraika S. Acclimation Prior to an Intraperitoneal Insulin Tolerance Test to Mitigate Stress-Induced Hyperglycemia in Conscious Mice. *J Vis Exp.* 2020(159).

Supplemental figure 1



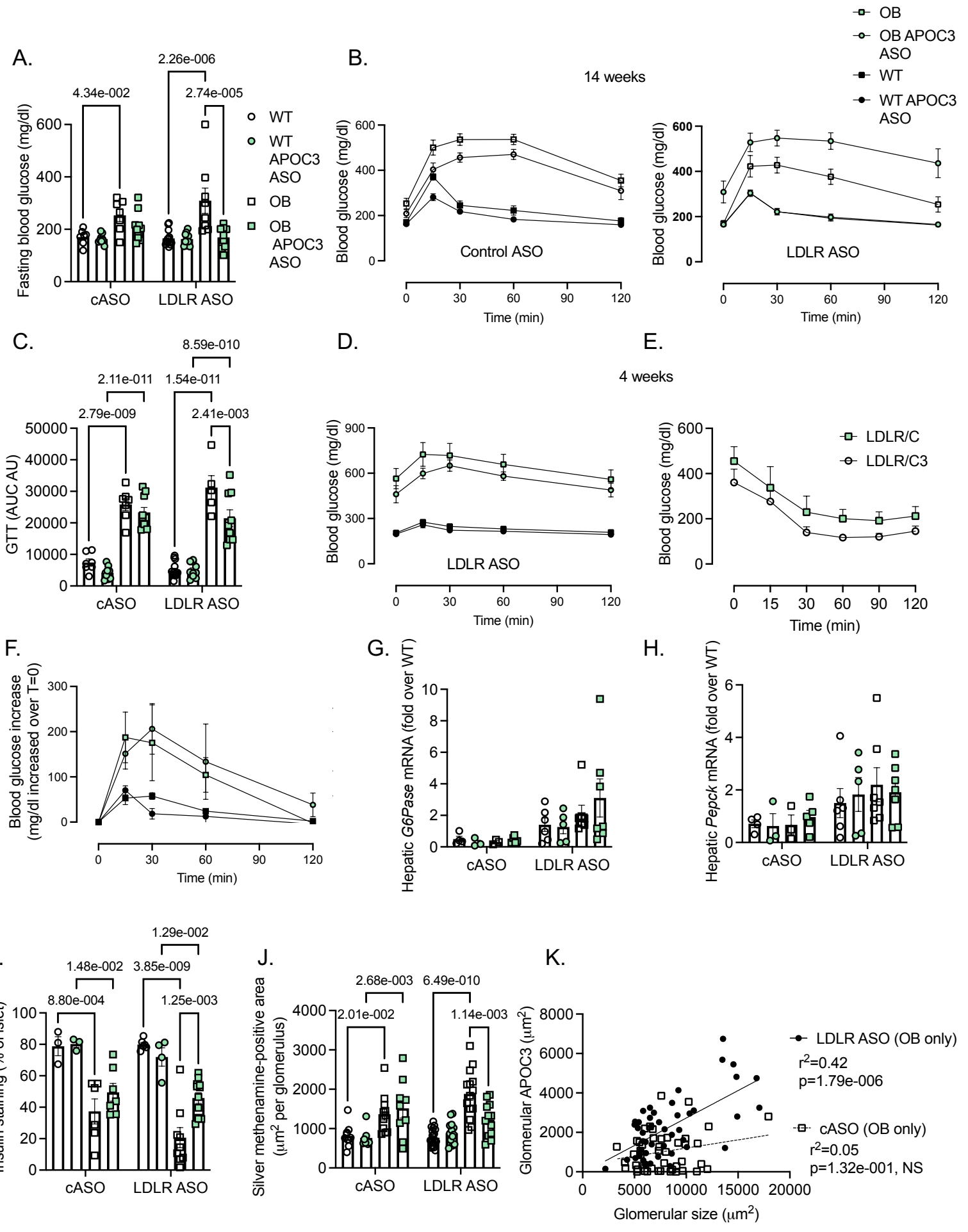
Supplemental figure 1. Examples of APOB and Perilipin 2 staining. Representative images for APOB and Perilipin 2 staining with negative controls (no primary antibody) and representative images of Perilipin 2 and APOB staining from control and DKD in the original color and in greyscale.

Supplemental figure 2



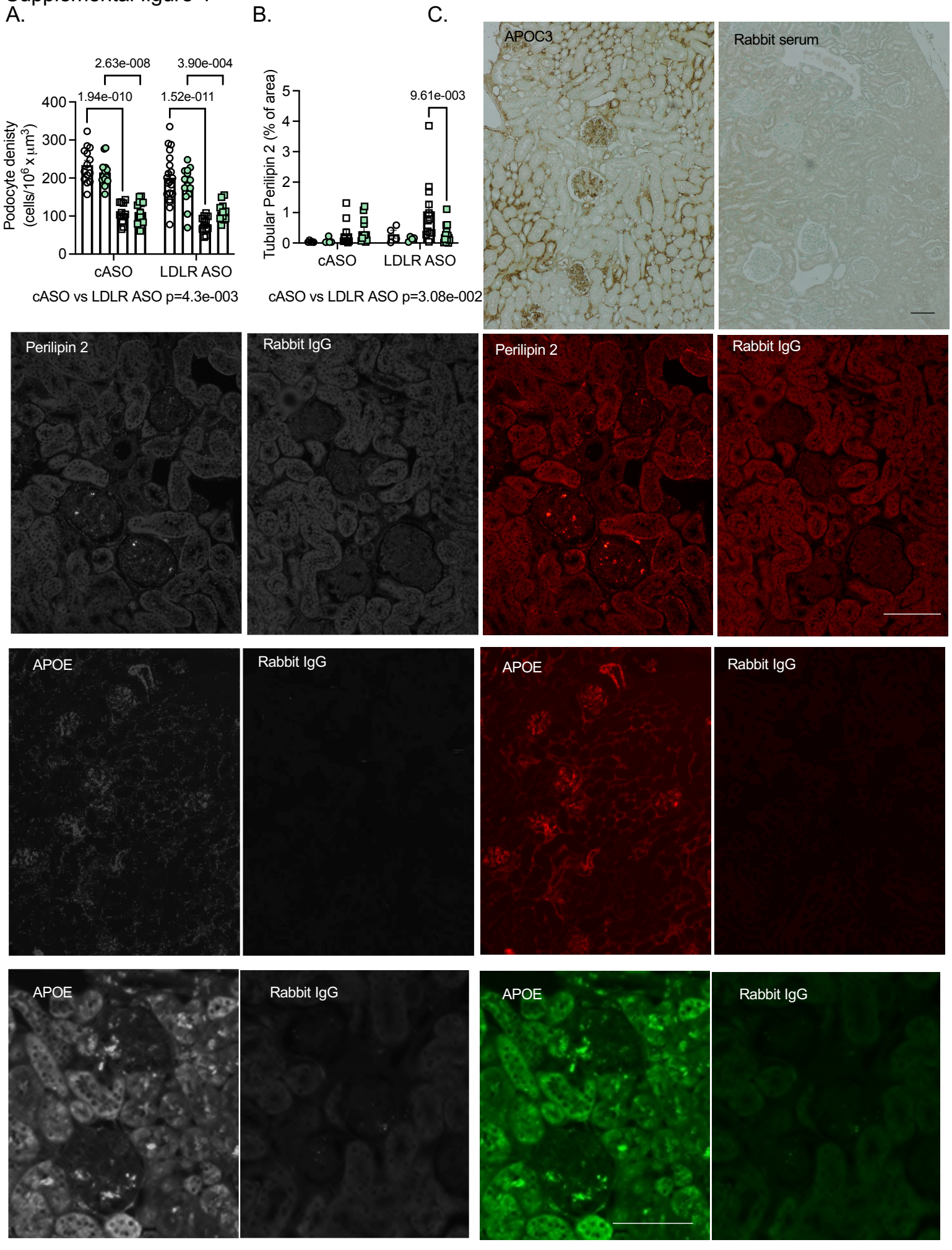
Supplemental figure 2. APOC3 ASO treatment reduces VLDL. Briefly, WT and leptin-deficient OB mice were treated with either a control antisense oligonucleotide (cASO) or LDLR ASO. Within each group, a subset was treated with either a cASO or an ASO to APOC3. Mice were then placed on a high-fat diet for 14 weeks. FPLC separation of plasma and analysis of cholesterol and triglycerides (TG) in cASO-treated mice (A) and LDLR ASO treated mice (B) (N=4). C. Hepatic expression of *Apoc3* mRNA (N=3-7). D. Hepatic triglycerides (TG; N=4-8). Data expressed as mean ± SEM. Data was analyzed by 2-WAY ANOVA followed by Tukey's multiple comparisons test. Text under the graph indicates the overall significance.

Supplemental figure 3



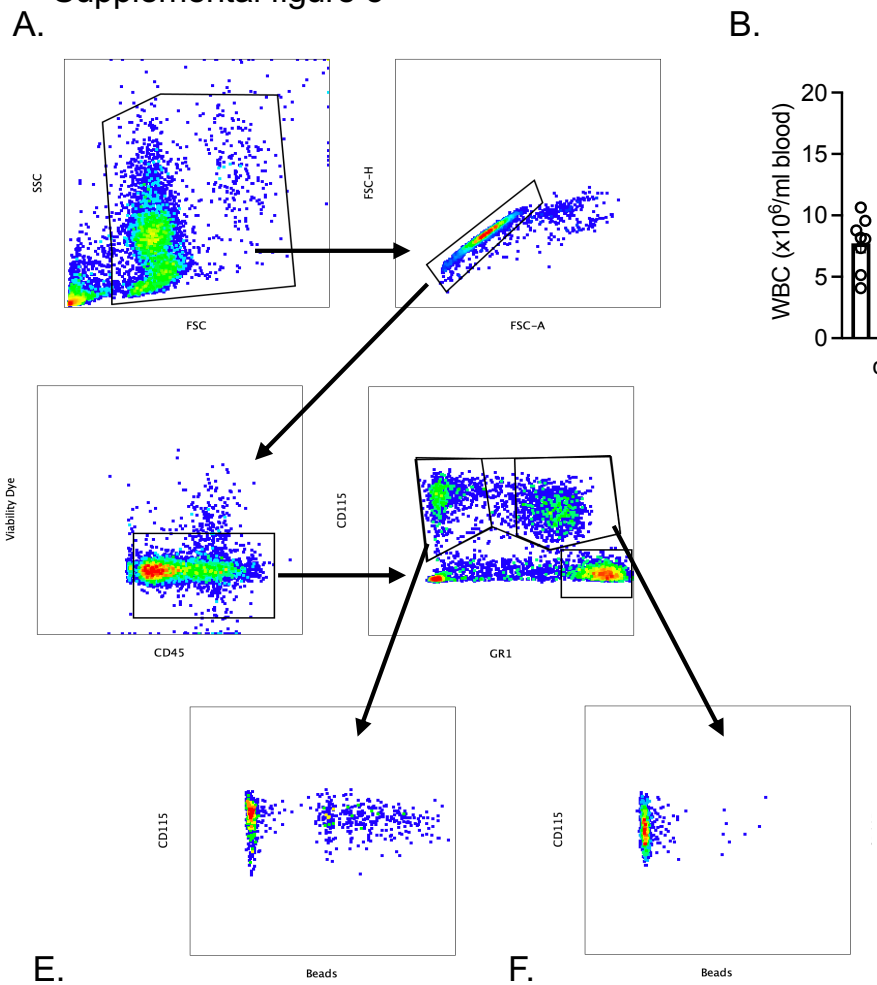
Supplemental Figure 3. APOC3 ASO treatment results in preservation of islet insulin. Mice were treated as in Figure S2. A. Fasting glucose. B. Intraperiontal (IP) glucose tolerance test (GTT) with 1 mg glucose/g body weight. C. Area under each individual mouse's curve from the GTT. D-F. GTT, insulin tolerance test (ITT) and pyruvate tolerance test (PTT) in mice treated for 4 weeks with LDLR ASO and APOC3 ASO or cASO (4-week study). D. IP-GTT with 0.75 mg/g. E. IP-ITT using 2mU of insulin/g of body weight F. Pyruvate tolerance test with 1.5 mg pyruvate/g body weight. Data expressed increment in blood glucose over time point 0. G. Hepatic mRNA expression of *G6pase* from the 14-week study (N=3-7). H. Hepatic mRNA expression of *Pepck* from the 14-week study (N=3-7). I. Pancreas insulin staining from the 14-week study (N=3-12). J. Glomerular silver methenamine stain in the 14-week study (N=8-21). K. Correlation between APOC3 and glomerular size on a per glomerulus size in OB mice only (n=48-92, from 5-7 mice). Data expressed as mean ± SEM. Data was analyzed by 2-WAY ANOVA followed by Tukey's multiple comparisons test. Text under the graph indicates the overall significance. N as indicated in Figure 5A unless otherwise noted.

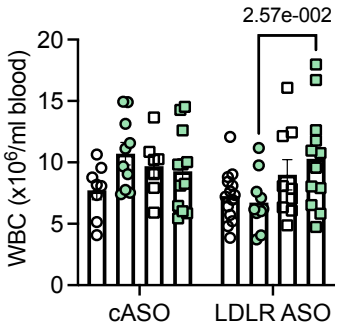
Supplemental figure 4

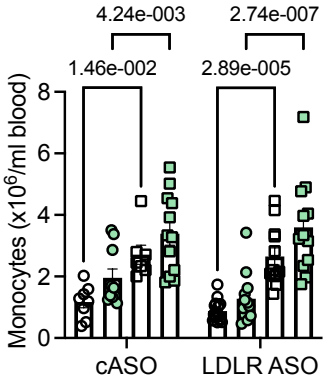


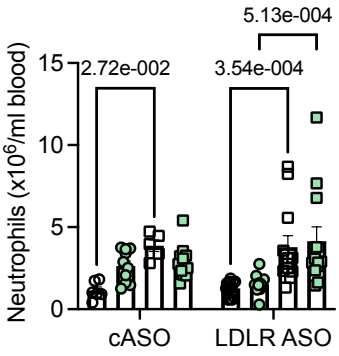
Supplemental figure 4. Examples of APOC3, Perilipin 2 staining, APOE and APOB. A. Glomerular WT-1 (podocytes) staining in the 14-week study (N=11-20). B. Quantification of non-glomerular perilipin 2 staining in 14-week study (N=4-12). C. Representative images for APOC3, Perilipin 2 staining, APOE and APOB with negative controls (corresponding IgG control) in the original color and in greyscale. Data expressed as mean ± SEM. Data was analyzed by 2-WAY ANOVA followed by Tukey's multiple comparisons test. 2 statistical outlier were removed from the perilipin 2 analysis.

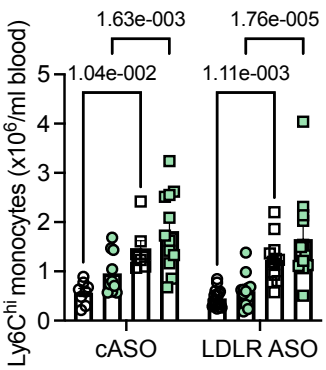
Supplemental figure 5

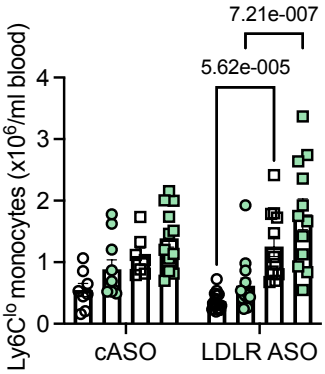


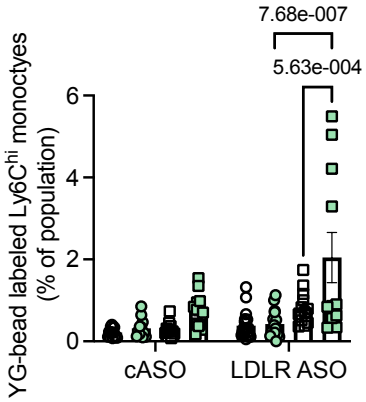


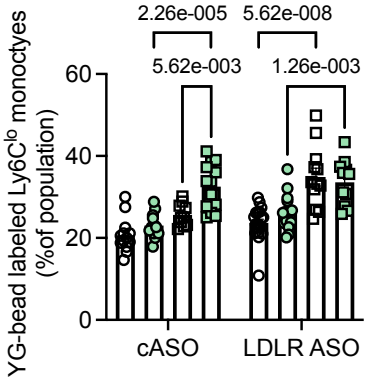


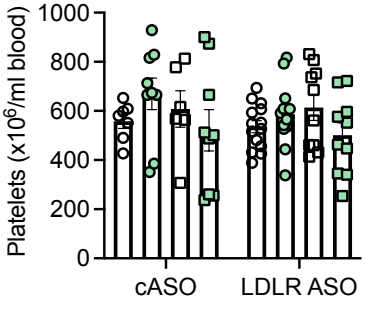


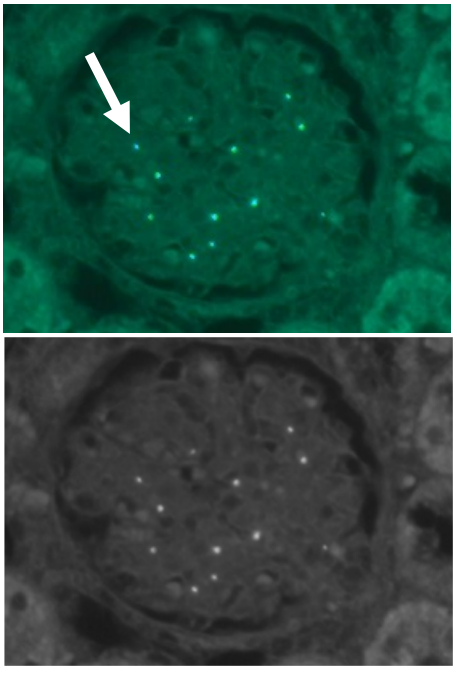






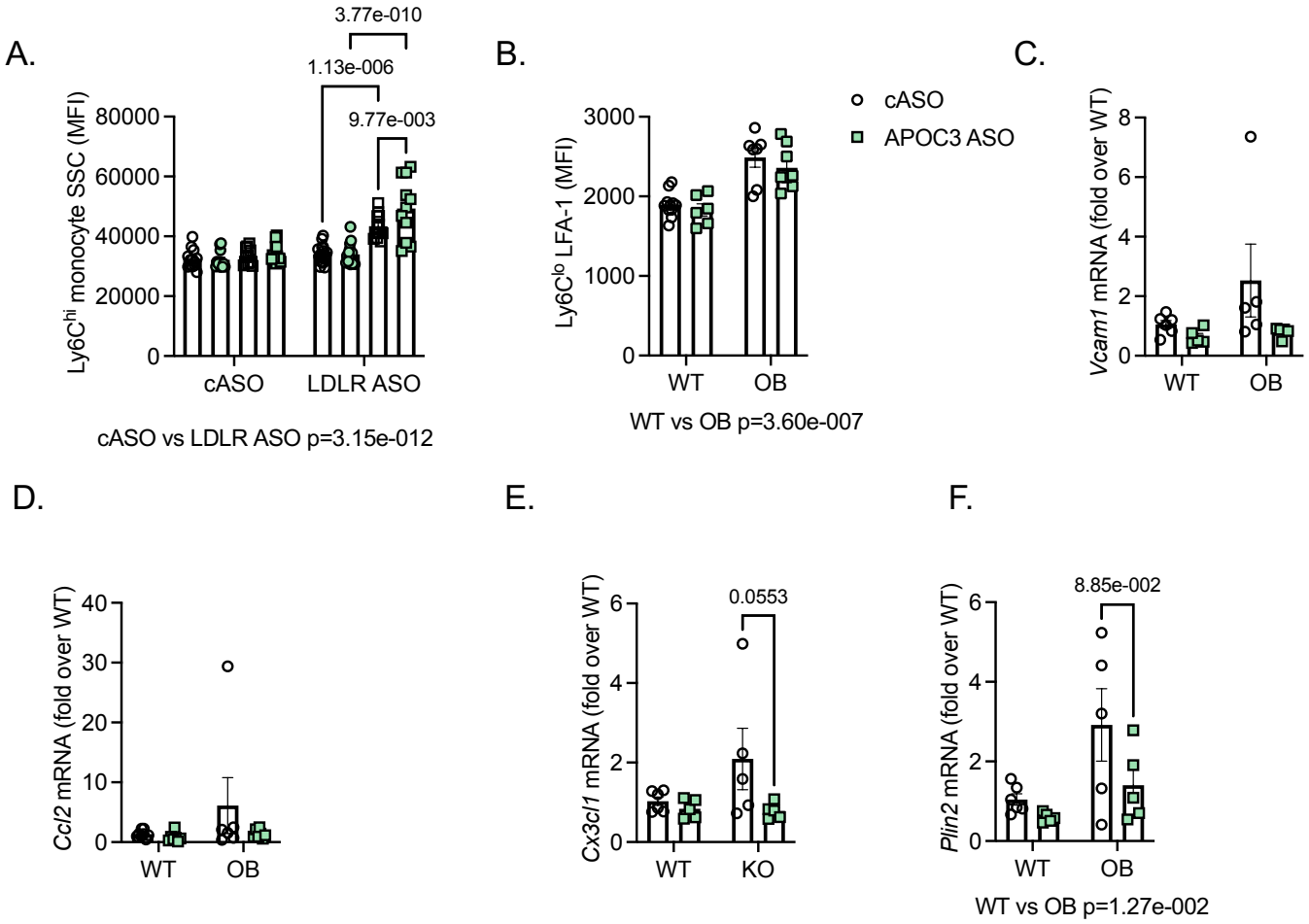






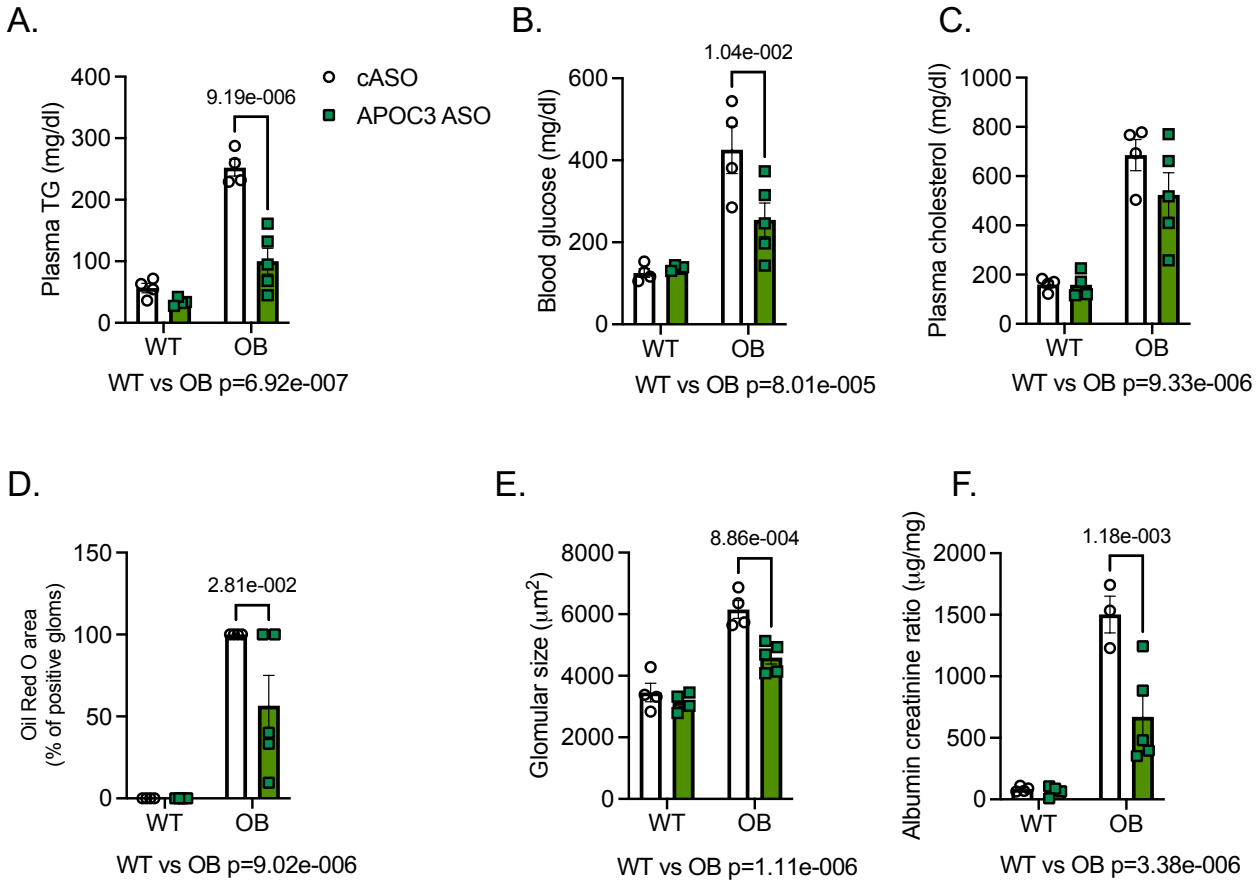
Supplemental figure 5. Flow cytometry of circulating leukocytes. Mice were treated as in Supplemental figure 2. A. Representative gating strategy to identify bead-positive monocyte populations in blood. B. White blood cells (WBC) from whole blood measured using a hemavet. C-H, cell populations based on flow cytometry combined with hemavet analysis. C. Blood monocytes. D. Blood neutrophils. E. Blood $Ly6C^{hi}$ monocytes. F. Blood $Ly6C^{lo}$ monocytes. G. Yellow-green (YG) positive blood $Ly6C^{hi}$ monocytes. H. YG-positive blood $Ly6C^{lo}$ monocytes. I. Platelets, based on hematvet analysis. J. Representative image of YG beads in glomerulus. Data expressed as mean ± SEM. Data was analyzed by 2-WAY ANOVA followed by Tukey's multiple comparisons test. N as indicated in Figure 2D.

Supplemental figure 6



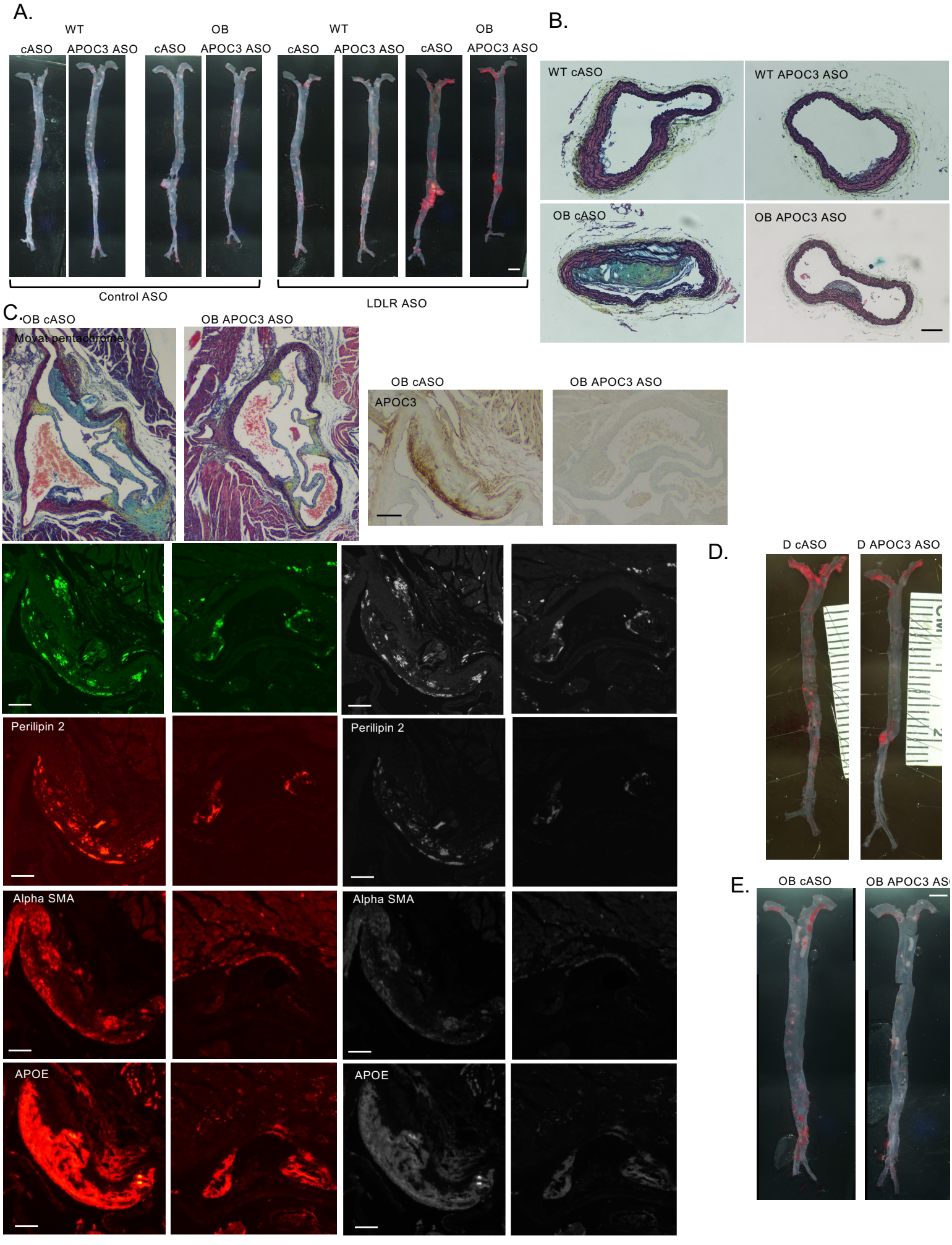
Supplemental figure 6. Leptin-deficiency increases Ly6C^{lo} monocyte LFA1. A. Side scatter (SSC) in Ly6C^{hi} monocytes, which is indicative of lipid loading from the mice in the 14-week study. B. LFA1 expression in Ly6C^{lo} monocytes from BTBR WT and OB mice were treated for 4 weeks with LDLR ASO and APOC3 ASO or cASO (4-week study). C. *Vcam1* mRNA in isolated kidney cortex endothelial cells. D. *Ccl2* mRNA in isolated kidney cortex endothelial cells. E. *Cx3Ccr1* mRNA in isolated kidney cortex endothelial cells. F. *Plin2* mRNA in isolated kidney cortex endothelial cells. Data expressed as mean ± SEM. Data was analyzed by 2-WAY ANOVA followed by Tukey's multiple comparisons test. N as indicated in Figure 5A.

Supplemental figure 7



Supplemental figure 7. APOC3 silencing using GalNAc-modified APOC3 ASO. BTBR WT and OB mice were treated for 12 weeks with LDLR ASO and APOC3 ASO or cASO while maintained on a low-fat diet. A. Plasma triglycerides (TG) at 12 weeks. B. *Ad lib* fed blood glucose at 12 weeks. C. Plasma cholesterol at 12 weeks. For additional time points, see **Supplemental Table 8**. D. Glomerular oil red o-staining expressed as the overall % of positive glomeruli (independent of extent of staining within a positive glomerulus). E. Glomerular size. F. Urine albumin to creatinine ratio at 12 weeks. Data expressed as mean ± SEM. Data was analyzed by 2-WAY ANOVA followed by Tukey's multiple comparisons test. N=4-5.

Supplemental figure 8



Supplemental figure 8. APOC3 silencing reduces atherosclerosis. Representative images of *en face* aortic lesions as assessed by Sudan IV staining from the 14-week study. B. Representative images of Movat pentachrome stained brachiocephalic artery lesions from the 14-week study. C. Representative images of Movat pentachrome stained aortic sinus lesions, APOC3, Mac-2, Perilipin 2, alpha-smooth muscle actin (SMA) and APOE from OB (LDLR ASO) treated with cASO and APOC3 ASO (14-week study) in the original color and in greyscale. D. Representative images of *en face* aortic lesions as assessed by Sudan IV staining from the type 1 diabetes study. Representative images of *en face* aortic lesions as assessed by Sudan IV staining from the 12-week study using the GalNAc-ASO. Scale bar in A and E is 2 mm, 100 μm in B and C.