Supplementary Methods

TG and apoB secretion*:* 4-hour fasted mice were injected with triton WR-1339 (Tyloxapol; 5µl/g body weight of 15% tyloxapol in saline; T8761, Sigma-Aldrich) and [^{35}S] methionine (200µCi) into the femoral vein. Blood samples were taken prior to the Tyloxapol/[^{35}S] methionine injection and 30, 60, 90, and 120 minutes post injection. The blood was immediately placed on ice and spun for 6 minutes at 12,000xG within 15 minutes. TG concentration was measured by colorimetric assay. TG secretion rate is calculated as the rate of increased plasma TG concentration between 30 and 120 minutes (mg/dl/hr).

Newly synthesized and secreted apoB100 was measured by the incorporation of [^{35}S] methionine into apoB100 that was secreted into the systemic circulation during the same 2 hour period. ApoB100 was separated from plasma via discontinuous SDS polyacrylamide gel electrophoresis (3% stacking gel, 4% resolving gel). The apoB100 bands were cut and analyzed for [^{35}S] activity by liquid scintillation counting. To mitigate differences in hepatic protein synthesis, loading volumes were adjusted based on the incorporation of [^{35}S] methionine into all proteins in plasma (plasma TCA precipitated protein counts). To calculate TCA precipitated protein counts, 5µl of plasma from each 60 and 120 minute sample was blotted onto a 1x1cm square of filter paper. Plasma proteins were precipitated by incubating the filter paper in 20% trichloroacetic acid (TCA) on ice followed by 10% TCA heated to 100˚C. The filter paper was subsequently rinsed with 100% ethanol, dried, and [^{35}S] activity calculated by liquid scintillation counter. TCA adjusted plasma volumes varied less than 30%. In one experiment, hepatic secretion of apoA1 and albumin was also measured.

Histology: Liver tissue was immediately excised and blotted dry. The tissue was fixed in 10% formalin for 24 hours. Liver tissue for oil red-O staining was transferred to a 30% sucrose solution and then prepared for frozen sections by the Columbia Histology Core. 5 micron slices were stained with hematoxylin and eosin (H&E) as well as oil red-O. Liver tissue for immuno-staining was transferred to 70% ethanol for 24 hours. It was then transferred to the Histology core lab for paraffin block preparation and sectioning into 5 micron slices.

Primary hepatocyte isolation*:* Primary hepatocytes were isolated from apobec-1 KO mice or C57BL/6J mice treated with apoB ASO or Control ASO for 6 weeks. We were unable to get viable cells from mice treated with ASO directed to MTP because of the severity of the steatosis in these mice. Livers were perfused with Hanks Balanced Salt Solution without calcium (HBSS Invitrogen,) and 10mM HEPES (Invitrogen) through the vena cava (portal vein was severed immediately before perfusion) for 8 min (4ml/min) at a temperature of 37º C. This was followed by a perfusion of DMEM with collagenase type I (80mg/100ml; Worthington,) for 6min (4ml/min) warmed to 37º C. The liver was removed and transferred to a Petri dish containing 4ml of warm DMEM with collagenase for an additional 2-4 minutes while the tissue was minced with scissors. Cold DMEM (40ml) was added and the digested liver was filtered through nylon mesh and collected in a 50ml conical tube. The cells were centrifuged for 5 minutes at 500rpm. The floating dead cells were aspirated and the pellet was washed 3 times with 30ml of cold DMEM. Viable cells were counted after staining with trypan blue. Cell viability was greater than 90 percent. Hepatocytes isolated from MTP-ASO treated mice did not pellet and appeared to lyse during the isolation protocol. Cells were plated into collagen coated 6 well plates at a density of 500,000 cells/well in 4 ml of DMEM+10% fetal bovine serum (FBS) for at least 2 hrs. The cells were then washed 2 times with PBS.

ER Tracker: Primary hepatocytes were plated on collagen glass bottom 35mm plates. After 24 hrs, cells were incubated with ER Tracker Red (Invitrogen) for 30 minutes in DMEM at 37º C after which the media was removed and fresh growth media was added. Images were visualized using Zeiss Axiovert 200M microscope at 200x magnification.

Triglyceride turnover*:* Isolated primary hepatocytes were plated onto collagen coated 6 well plates in DMEM and 10% (v/v) fetal bovine serum. After 2 hours, the media was changed to DMEM with 1.5% BSA and [^{3}H] glycerol (20uCi/ml). After 2 hours later the media was changed to DMEM with 1.5% BSA for either 0, 1, 2, 4, 8, 12, or 24 hrs of chase. At the completion of the chase period, lipid was extracted from cells and separated by TLC. Separate wells were analyzed for cellular ^{3}H activity in TG and PL. Turnover of TG is defined as the amount of ^{3}H TG relative to amount of total 3H TG present at the end of the 2 hr label (0hr). ^{3}H TG counts were normalized to total cell protein.

Electron microscopy: The livers of 6 weeks and 3 week ASO treated mice were perfused with 10ml of Sorensen's phosphate buffer (0.1M phosphate, pH 7.2) followed by 20ml of 2.5% glutaraldehyde in 0.1% Sorensen's phosphate buffer. The perfused liver was excised and several small (0.1mm x 2mm x 2mm) pieces were sliced and stored in the 2.5% glutaraldehyde solution until processed. The samples were subsequently processed by the Columbia Electron Microscopy Core Facility.

Gene expression: Gene expression from liver and small intestines was measured by quantitative real time PCR (qRT-PCR). mRNA was isolated and reverse transcribed as previously described. In brief RNA was extracted from small intestine and liver samples with Trizol and quantified by NanoDrop ND-1000 Spectrophotometer (Thermo Scientific). 5ug of RNA was reversed

transcribed to cDNA. The cDNA was diluted 40 times and used for qRT-PCR analysis. All samples are analyzed relative to a standard curve made from pooled cDNA and expressed relative to actin gene expression.

Western Blot: 100mg of liver was homogenized in 2ml of buffer reagent containing protease inhibitor (Roche). Protein concentration was measured by BCA protein reagent. Liver homogenates were subject to electrophoresis on appropriate percentage SDS PAGE gels and transferred to polyvinylidene-fluoride membranes. LC3B (cat#2775), , phos eIF2α, Apoptosis sample kit (cat#9915), Atg7 (cat#2631), phos-mTOR(Ser2448) (cat # 2971), total MTOR (cat # 2983), phospho-AMPkalfa(Thr172) (cat # 2535), total AMPKalpha (cat # 5831) were all purchased from Cell Signaling. Grp78 (Stressgen; SPA826), p62(Abnova; H00008878-M01), apoA1 antibody(ThermoScientific;cat#PA5-19784), ABCA1 (Novus Biologicals cat #400-105) and SR-BI(Novus Biologicals cat #400-101) CaMKII alpha/beta[p Thr286, p Thr287] (Novus Biologicals cat # NB110-96869)and actin (1:10,000; A5441, Sigma) were also used. All primary antibodies were incubated overnight at 4˚C. Species specific secondary IgG antibodies conjugated with peroxidase (1:10,000-1:15,000) were incubated for 1 hour at room temperature. Protein bands were visualized using SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific).

Measurement of LC3-I and LC3-II protein*:* Primary hepatocytes from Control and apoB ASO mice were cultured for 24 hrs after plating in DMEM+ 1.5%BSA alone (NT) or DMEM+ 1.5%BSA+ 20mM Ammonium Chloride+ 100μM Leupeptin (lysosomal inhibitors). Cells were collected in 200μl of lysis buffer (62.5 mM sucrose, 0.5% sodium deoxycholate, 0.5% Triton X-100, 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM benzamidine, 5 mM EDTA, 100 U/ml aprotinin, 50 μg/ml leupeptin, 50 μg/ml pepstatin A, and 10 mM HEPES, pH 8.0) and boiled

with sample buffer for 5 min. Equal amounts of protein were run in a 12% SDS-PAGE gel for 1.5 hrs and then transferred to a PVDF membrane for western blotting for total LC3B and actin. The LC3-II and actin bands were quantified using ImageJ and the ratio of LC3-II to actin was calculated and is presented as a percentage of Control ASO.

XBP1 splicing: mRNA from Control, apoB and MTP ASO-treated livers was reversed transcribed to cDNA. XBP1 cDNA encompassing the region of restriction site was amplified by PCR using previously described primers (Ota et al). The PCR product was incubated with the Pst1 restriction enzyme for 5 hrs at 37ºC, followed by separation of the restriction digests on a 2% agarose gel with ethidium bromide. The gels were photographed under UV transillumination. The amount of 601-bp material (top band) is indicative of XBP1 activation and thus, of ER stress.

Chloroquine treatment: 9-13 week old Apobec-1 KO mice were placed on HFD for 6 weeks and then injected biweekly with 50mg/kg either Control, MTP, or apoB ASO for 6 weeks. The mice were then injected i.p. with either saline or 60mg/kg chloroquine (in saline) for 10 days.

De novo lipogenesis*:* Non-fasting mice were injected IP with 1mCi of tritiated water (^3H_2O). After 1 hour, the mice were anesthetized with Ketamine / Xylazine and 250µl of blood was collected from the retro-orbital plexus. The plasma was used to calculate the specific activity of tritium in total body water. Mice were quickly euthanized; livers were quickly excised and flash frozen. Lipogenesis was calculated based on the tritium incorporated into FAs. 500mg of liver was incubated with 2.5ml of 2.5M KOH at 75˚C for 2 hours or until liver was completely dissolved. Saponified lipids were isolated by the addition of 7ml of 80% ethanol and 10ml of hexane. The bottom phases, containing the saponified lipids (fatty acid salts), were acidified with

1.5ml of 3M sulfuric acid. The liberated FAs were isolated with the addition of 10ml of hexane. The hexane layer was rinsed 2 times with 7ml of 50mM sulfuric acid. The hexane was blown to dryness and reconstituted in 500µl hexane. FAs were isolated via TLC and the resulting spot was analyzed for tritium activity. Tritium incorporated into FAs was calculated relative to the specific activity of tritium in plasma.

Fatty acid uptake in vivo: Anesthetized mice were injected with 100µl of 10µCi/mL BSA bound ^{14}C oleic acid (OA) in saline (6% BSA) into the femoral vein. Blood samples were drawn at 30 seconds, 1, 2, 3.5, and 5 minutes. Mice were immediately euthanized after the last blood draw. The livers were quickly excised, blotted dry, and flash frozen in pre-weighed tubes. 100mg of liver tissue was homogenized in 2ml of PBS. 20µl of the homogenate and 10µl of plasma from each time point were analyzed for ^{14}C by liquid scintillation counting. Percentage of ^{14}C OA uptake is calculated relative to the inferred peak plasma enrichment defined as the 30 second plasma specific activity.

Total plasma ketones: Blood was collected from mice after 6 weeks of ASO treatment. The blood was spun for 6 minutes at 12,000 x *g*, plasma transferred, and frozen. Total plasma ketones were measured using a colormetric assay from Wako.

Fatty Acid Secretion: Two hours after isolation, primary hepatocytes plated in 6 well plates were incubated with DMEM with 1.5% BSA for 1 hr. The media was changed to DMEM with 1.5% BSA, 0.2mM OA and ^{14}C OA (1uCi/ml). 16 hours later the media was changed to DMEM with 1.5% BSA. The media was collected after 4 hours. The lipids were isolated from the media via the Folch method (chloroform/methanol/water = 8:4:3). TG and FAs were separated based on relative mobility on silica 60 TLC plates. The FA spots were scraped into a scintillation vial and

analyzed for ^{14}C activity. ^{14}C appearance in FA in the media is represented relative to cellular protein (CPM/mg protein).

Plasma lipid and liver enzymes measurements: Blood was collected from mice fed after 6 weeks of ASO treatment. The blood was spun for 6 minutes at 12,000xG and plasma transferred and frozen. Triglyceride, total cholesterol, and free fatty acids were measured by colorimetric assays (WAKO Chemicals, Richmond, Virginia). Plasma Asparatate Aminotransferase and Alanine Aminotransferase levels were measured using an assay kit from Diagnostic Chemicals Limited.

Atg7 ASO experiments: Apobec-1 KO mice were placed on HFD for 6 weeks and then injected i.p. biweekly with original dose of 50mg/kg of Control or apoB ASO for 3 weeks. After 3 weeks, mice were injected i.p. biweekly with either 75mg/kg Control ASO, 37.5mg/kg Control ASO+ 37.5mg/kg Atg7 ASO, 37.5mg/kg apoB ASO+ 37.5mg/kg Control ASO, or 37.5mg/kg apoB ASO+ 37.5mg/kg Atg7 ASO for an additional 3 weeks. We reduced the amount of total apoB ASO from 50mg.kg to 37.5mg/kg biweekly to reduce the total amount of ASO given in combination with the Atg7 ASO from a potential 100mg/kg to 75mg/kg. Based on data published from ISIS pharmaceuticals, doses as low as 25mg/kg biweekly for only 2 weeks were effective in decreasing liver apoB protein levels.

Subcellular fractionation and lipids extraction. To isolate ER fraction, livers of mice treated with Control, apoB or MTP ASO for 3 and 6 weeks were homogenized (1:2 wt/vol) in 0.25M sucrose buffer. The homogenate was subjected to centrifugation at 2000g for 5 minutes and then pre-cleared at 17,000g. To collect ER fraction, the supernatant was centrifuged at 100,000g for 1

hr at 4°C. The collected material was processed further for the lipid extraction by Folch extraction.

Mass spectrometric analysis of isolated lipids from ER fractions. Lipids extracted from isolated ER were dried and then re-suspended in water, and spiked with a cocktail of internal standards for quantitative measurements of subclasses of lipids. The lipids extracts from ER fractions were analyzed in multiple reactions monitoring (MRM) mode using Agilent 6490 Triple Quad LC-MS. Reverse-phase HPLC was used to separate sterols and glycerolipids, while polar phospholipids and sphingolipids were separated via normal-phase HPLC. Raw data, with more than 500 lipid species, was analyzed using Agilent software; statistical analysis was conducted using SigmaPlot software.

Supplementary Data

Figure S1: **ApoB and MTP ASO do not affect intestinal mRNA levels. A**pobec1 KO mice were fed for 6 weeks with HFD and then injected with Control, apoB, or MTP ASO for 6 weeks while still being fed HFD. mRNA of apoB and MTP from intestine was measured by qRT-PCR. Data is normalized to B-actin and expressed relative to the Control ASO group; N=4-6/group. Values are means +/- SD.

Figure S2: Plasma cholesterol levels are reduced in ApoB ASO-treated mice at both 3 and 6 weeks. Plasma apoA-I levels are not different between MTP ASO- and apoB ASO-treated mice at either time. (A) Plasma cholesterol was measured enzymatically from 4 hour fasting plasma. N=3/group. (B) Plasma (1 microliter) from 3 and 6 weeks ASO treated 4 hour fasted mice was separated by 12% SDS-PAGE and immunoblotted for apoA1 with antibody from Thermo Scientific; N=3/group Immunoblots for apoA1 were quantified using ImageJ densitometry. Results are displayed as Arbitrary Units. Values are means +/- SD.

Figure S3: ApoB ASO-treated mice have reduced levels of LDL and HDL at both 3 and 6 weeks of treatment. HDL is also smaller at 3 and 6 weeks of apoB ASO treatment. Pooled plasma samples from 3 mice (90ul) fasted from 8 a.m. to 12 pm were subjected to FPLC analysis using one Tricorn Superose 6 10/300 GL column (GE Healthcare). Forty 0.5-ml fractions were collected. Cholesterol was determined in each fraction using a commercial kit from Wako Diagnostics.

Figure S4: Secretion of newly synthesized proteins, including albumin and apoA-I, are not affected by ER autophagy at 6 weeks of ASO treatments. Albumin and apoA1 were isolated by 12% SDS PAGE from the 120 min plasma samples in the Triton studies described in Figure 1. The 12 % gel was soaked in 20% polyethylene glycol (PEG-400) and 50% methanol for 15 minutes prior to drying on filter paper to prevent cracking. Bands were cut and radioactivity quantitated by scintillation counting. Results show CPMs. Shown is one representative sample per group in duplicate. Densitometry was performed using Image J. N =4 mice/ group.

Figure S5: Neither SR-B1 nor ABCA1 protein in liver was affected by ASO treatments for 3 or 6 weeks: Liver homogenates from Control ASO-, apoB ASO-, and MTP ASO-treated mice for 3 weeks (A) or 6 weeks (B) were separated by SDS-PAGE and immunoblotted for SR-B1 and ABCA1 antibodies from Novus Biologicals. Densitometry analysis relative to actin control is shown below blots; N=3/group.

Figure S6: MTP ASO treatment increases expression of lipid droplet and lipogenic genes. Livers were obtained after 6 weeks of treatment with ASO's and mRNAs measured by qRT-PCR, normalized to B-actin, and expressed relative to the Control ASO group; N=4-5/group. Values are means +/- SD. Groups with different superscript symbols are significantly different as shown on the figure.

Figure S7: **(A) Increased staining for LAMP2, a lysosomal membrane protein, is present in apoB ASO treated liver sections**. 5μm liver sections from Control, MTP or apoB ASO-treated mice were incubated with anti-LAMP2 Ab (Cell Signaling) overnight and then with anti-rabbit IgG A conjugated with Alexafluor647 (green). Sections were then stained with DAPI (blue). Pictures were taken by a NikonA1RMP confocal microscope at 600x magnification. Representative images are shown for N=3 livers per group; 5 images/liver. Bar in left image represents 40 μm.

(B) There was no evidence for co-localization of LC3 and VDAC3, a mitochondrial protein, or LC3 and Plin2, a lipid droplet protein, but there was increased co-localization of LC3 and LAMP2 in apoB ASO-treated mice. Liver sections were incubated with anti-LC3 Ab (red) and either anti-VDAC3 Ab, Anti-Plin2 Ab, or Anti-Lamp2 Ab (green) and stained with DAPI (blue). Pictures were taken with Nikon A1RPM microscope at 600x magnification. Representative images are shown for N=3 livers/ group; 5 images per liver. Bars in top row of images represent 20 μm.

(C) There was no co-localization of LC3 and Plin2 in primary hepatocytes from control or apoB ASO-treated mice. Primary hepatocytes from Control ASO- and ApoB ASO-treated for 6 weeks mice were plated on cover slips and after 16 hrs of incubation were fixed in 4% PFA. Then cells were stained with LC3 and Plin2 antibodies for 16 hrs. Images were taken using Nikon Ti Eclipse inverted confocal microscope with 60x/1.49 NA oil lens. Scale bar, 15 μm. N=3 hepatocyte isolations per group; 3 images per isolation. Bar equals 10um.

Figure S8: p62 levels did not differ between control, MTP, or apoB ASO treatment groups at either 3 or 6 weeks: Liver homogenates from Control ASO- and ApoB ASO-treated mice were analyzed on SDS-PAGE gels; immunoblots were incubated with anti-p62 and anti-actin antibodies. The densities of the bands were determined with ImageJ. N=3/group.

Figure S9: Densitometry of ER stress data in Figure 4A. Liver homogenates from Control ASO-, MTP ASO-, and ApoB ASO-treated mice after 6 weeks were analyzed on SDS-PAGE gels; immunoblots were incubated with (A)anti- Grp78 or (B) anti-phos eIF2α and anti-actin antibodies and quantified using Image J densitometry (C) XBP1 splicing quantification, data are presented as percentage of total XBP1 that is spliced ; N=5-12/group. Values are means +/- SD.

Figure S10: β OH Butryate Levels were not different across the three treatment groups at either 3 or 6 weeks. Plasma 3-hydroxybutyrate was measured in plasma taken from mice treated for 3 or 6 weeks with ASOs using an enzymatic assay from Wako Diagnostics; N=3/group

Figure S11: There were no differences in acidification status of lysosomes at 6 weeks of treatment with control or apoB ASO treatment. Primary hepatocytes from Control ASO- and ApoB ASO-treated mice were cultured on the coverslips for 24hrs; the media was changed and cells were loaded with LysoSensor Yellow/Blue DND-160 (5μM) for 20 min. Images were taken

using Nikon Ti Eclipse inverted confocal microscope with 60x/1.49 NA oil lens. Scale bar, 10 μm.

Figure S12: The combination of NH_4 and leupeptin significantly reduce levels of FA oxidation in apoB ASO-treated mice. Primary hepatocytes from Control ASO- and ApoB ASO-treated mice were labeled with ^{14}C OA for 16 hrs and then chased for 4 hrs with unlabeled media in the presence or absence of 20mM Ammonium Chloride and 100μM Leupeptin, after which the amounts of $^{14}CO_2$ and ^{14}C ASM were measured and combined and the normalized to cell protein . NT = no treatment. N=6 wells from 2 mice/group; Values are means +/- SD. Groups with different superscript letters are significantly different from the others at $p < 0.05$.

Figure S13: Etomoxir inhibits FA oxidation in apoB ASO-treated mice at 6 weeks. Primary hepatocytes isolated from Control ASO- and ApoB ASO-treated mice were labelled with [^{14}C] OA for 2 hrs and then chased in unlabeled media for 16 hrs in the presence or absence of 200 μM Etomoxir. The media was collected between 14 and 16 hrs incubation and used to determine FA oxidation. The amount of $^{14}CO_2$ and ^{14}C ASM was summed and presented as total ^{14}C OA oxidized. N=3 wells from one control ASO- and one apoB ASO-treated mouse;Values are means +/- SD. * $p<0.05$ apoB ASO-treated vs. control ASO-treated hepatocytes by ANOVA.

Figure S14: ApoB ASO reduced secretion of TG and newly synthesized apoB in WT C57BL/6J mice similarly to what was observed in apobec1 knockout mice. Triton WR1339 and ^{35}S-methionine were injected IV into C57BL/6J mice treated with Control ASO or apoB ASO for 6 weeks and bloods were obtained over the next 120 min. **(A)** TG in plasma was measured enzymatically.

(B) ApoB was isolated by 4% SDS PAGE from the 120 min. plasma sample, and the bands were cut and counted; N=3mice/group. Values are means +/- SD. *$p<0.05$ in apoB ASO-treated mice vs Control ASO-treated mice.

(C) Liver TG was not different in the apoB ASO-treated C57BL/6J mice compared to control ASO-treated C57BL/6J mice. Liver lipids were extracted from mice treated with either Control or apoB ASO for 6 weeks. Liver TG was measured enzymatically and normalized to liver protein; N=7-8 livers/group. Values are means +/- SD.

(D) LC3 is increased with perinuclear localization in apoB ASO-treated C57BL/6J mice. Livers from C57BL/6J mice treated with Control or apoB ASO for 6 weeks were fixed with formalin, paraffin embedded, sliced into 5μm sections, and then incubated with Anti-LC3 Ab (Cell Signaling) overnight followed by anti-rabbit IgG conjugated with Alexa fluor555 (red). Sections were then stained with DAPI (blue). Pictures were taken by a NikonA1RMP confocal microscope at 600x magnification. Representative images are shown for N=3 livers/ group;5 images per liver. Bar in left image represents 20 μm.

(E) LC3 and calnexin are increased and co-localize in apoB ASO-treated C57BL/6J mice. Liver sections from ASO-treated C57BL/6J mice were incubated with anti-LC3 Ab (red) and

anti-calnexin Ab (green) and thenstained with DAPI (blue). Pictures were taken with Nikon A1RPM microscope at 600x magnification. Increased LC3 is evident in a perinuclear position only in apoB-ASO-treated liver (lower left panel). Representative images are shown for N=3 livers/ group; 5 images per liver. Bar in left image represents 20 μm.

(F) ApoB ASO-treated C57BL/6J mice have increased hepatic fatty acid oxidation. Primary hepatocytes from apoB and Control ASO-treated were labeled with ^{14}C OA for 16 hrs and then chased for 4 hrs with unlabeled media which the amount of $^{14}CO_2$ and ^{14}C acid soluble materials was measured and normalized to cell protein; N =6 wells from 2 mice/group. Values are means +/- SD. *$p<0.05$ in apoB ASO-treated mice vs control ASO-treated mice.

(G) Increased fatty acid oxidation in apoB ASO-treated C57BL/6J mice is inhibited by chloroquine. The 16 hr ^{14}C OA label and 4 hr chase was also performed in the presence or absence of 50μM chloroquine and oxidation was measured; NT = no treatment. N=6 wells from 2 mice/group. Values are means +/- SD. # $p<0.05$ NT apoB ASO vs NT Control ASO; *$p<0.05$ chloroquine treated apoB ASO vs NT apoB ASO.

Figure S15: Densitometry of data in Figure 6E and XBP1 splicing. Liver homogenates from Control ASO- and ApoB ASO-treated mice after 3 weeks were analyzed on SDS-PAGE gels; immunoblots were incubated with (A)anti- Grp78 or (B) anti-phos eIF2α and anti-actin antibodies and quantified using Image J densitometry (C) XBP1 splicing quantification, data are presented as percentage of total XBP1 that is spliced ; N=5-12/group. Values are means +/- SD.

Figure S16: Calnexin and Lipid Droplets, as identified by Bodipy staining, do not overlap in mice treated for three weeks with either control or apoB ASO. Primary hepatocytes isolated from Control ASO- and ApoB ASO-treated mice were plated on cover slips and incubated for 16 hrs in full media. Then cells were incubated with BODIPY for 30 min, washed in PBS and fixed in 4% PFA; stained with Calnexin antibody for 16 hrs. Nuclei were stained with DAPI (blue). Images were taken using Nikon Ti Eclipse inverted confocal microscope with 60x/1.49 NA oil lens. Scale bar, 10 μm.

Figure S17: EMs from livers of apoB ASO mice treated for 3 weeks show expanded ER. Representative EMs images, at 6,000X of livers from mice treated for 3 weeks with Control ASO (left), MTP ASO (middle) or apoB ASO (right). Bar equals 2um.

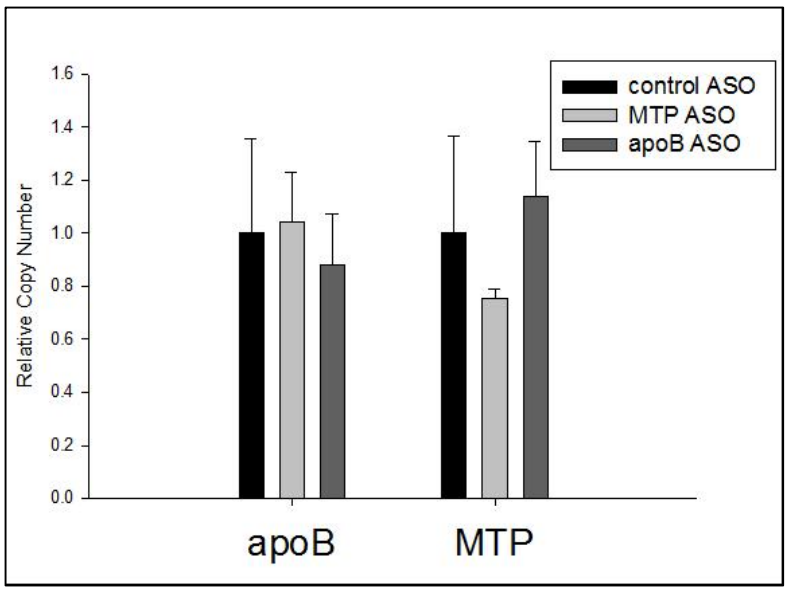
Figure S18: Mass spectrometric analysis of ER lipids showed dramatic decrease of TG and DG, and increase of PE levels between 3 and 6 weeks of apoB ASO treatment. Livers from mice treated with Control, apoB and MTP ASO for 3 and 6 weeks were homogenized in 0.25 M sucrose and pre-cleared supernatant was subjected to centrifugation to collect ER. Lipids extracted using Folch method were spiked with a cocktail of internal standards and analyzed on Triple Quad LC-MS. Lipids of particular relevance are shown from an analysis of 33 lipid species. The results were plotted as mol percent of total lipids and normalized to the control. N=3

ER fractions per group. Values are means +/- SE. *p<0.05 in apoB ASO-treated mice vs control ASO-treated mice.

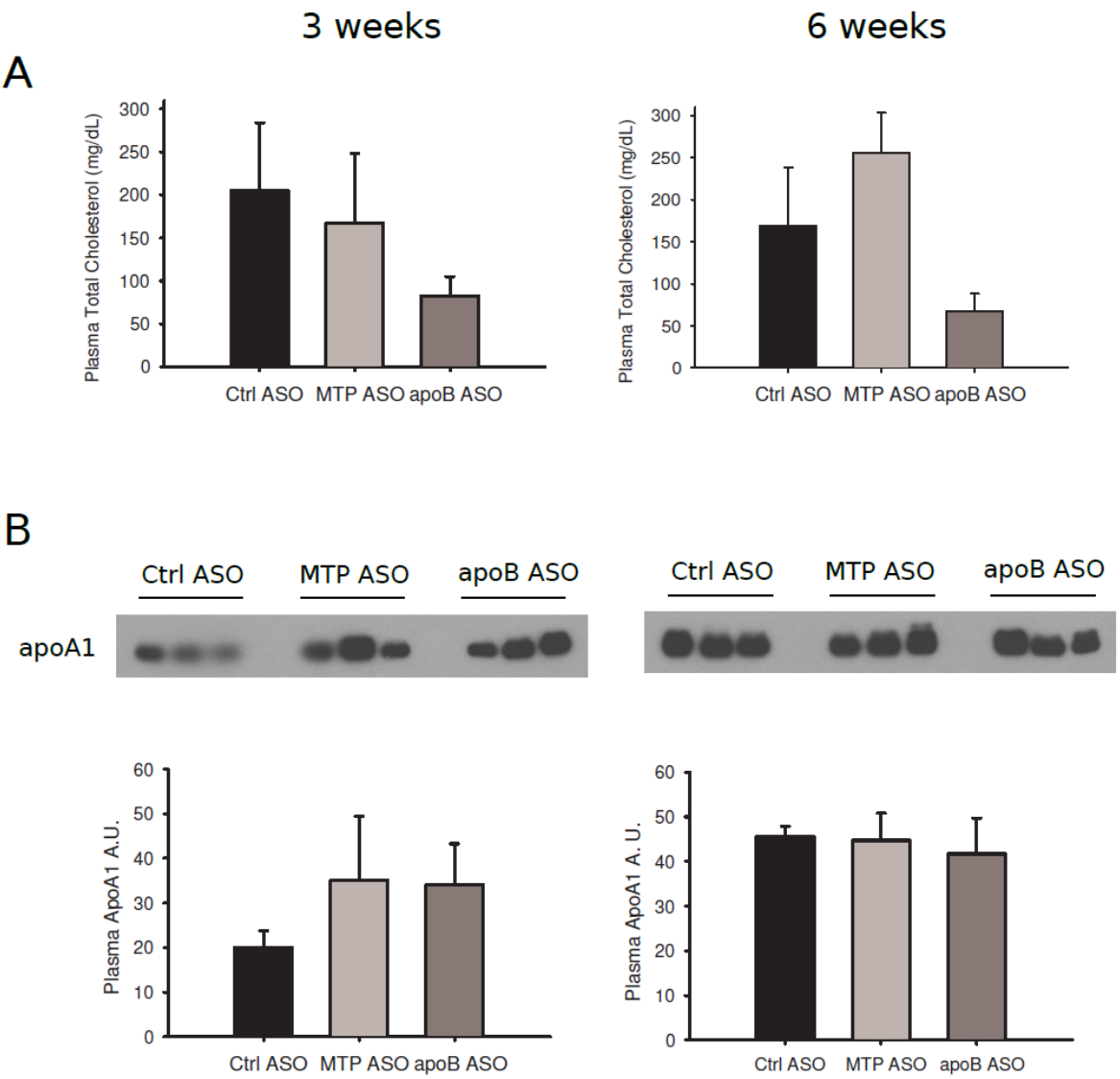
Figure S19: MTP and apoB ASO treatments for either 3 or 6 weeks do not affect liver PEMT mRNA levels. Apobec1 KO mice were fed for 6 weeks with HFD and then injected with Control, MTP, or apoB ASO for either 3 weeks or 6 weeks while still being fed HFD. mRNA of PEMT from liver was measured by qRTPCR. Data are normalized to actin and expressed relative to the 3 or 6 week Control ASO groups; N=3/group. Values are means +/- SD.

Figure S20: ApoB ASO treated mice have altered AMPK and mTOR activation at 3 and 6 weeks of treatment. Liver homogenates from either 3 or 6 week Control ASO-, apoB ASO-, and MTP ASO-treated mice were separated by SDS-PAGE and immunoblotted for phospho-mTOR (Ser2448), total mTOR, phospho-AMPKa (Thr172), and total AMPKa with antibodies from Cell Signaling. Densitometry analysis, adjusting phophorylated protein for total protein is shown below each blot; N=3/group.

Figure S21: There were no differences in CAMKII phosphorylation between the three groups of mice at either 3 or 6 weeks of ASO-treatment. Liver homogenates from mice treated for 3 or 6 weeks with Control ASO, MTP ASO, and ApoB ASO were analyzed on SDS-PAGE gels; immunoblots were incubated with anti-CAMK-p and anti-actin antibodies. The densities of the bands were determined with ImageJ. N=3 per group. Upper gel and bar graph depict 3 week results; lower gel and bar graph depict 6 week data.



Supplemental Figure S1



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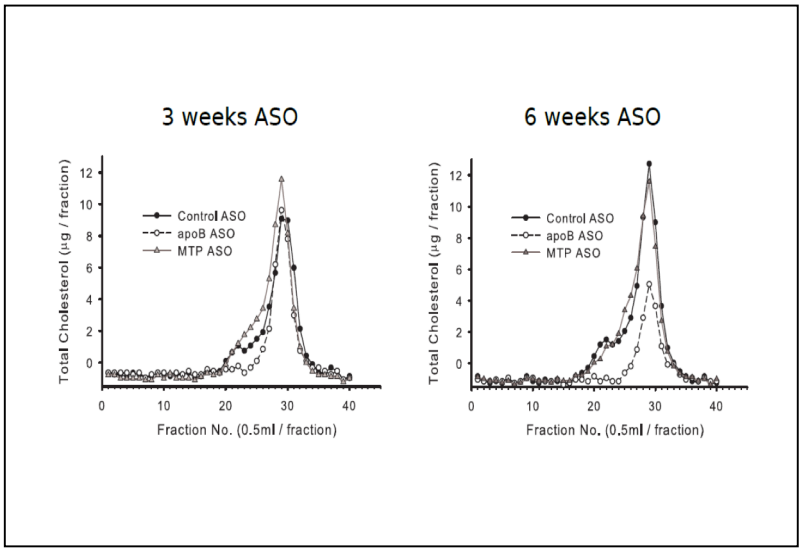

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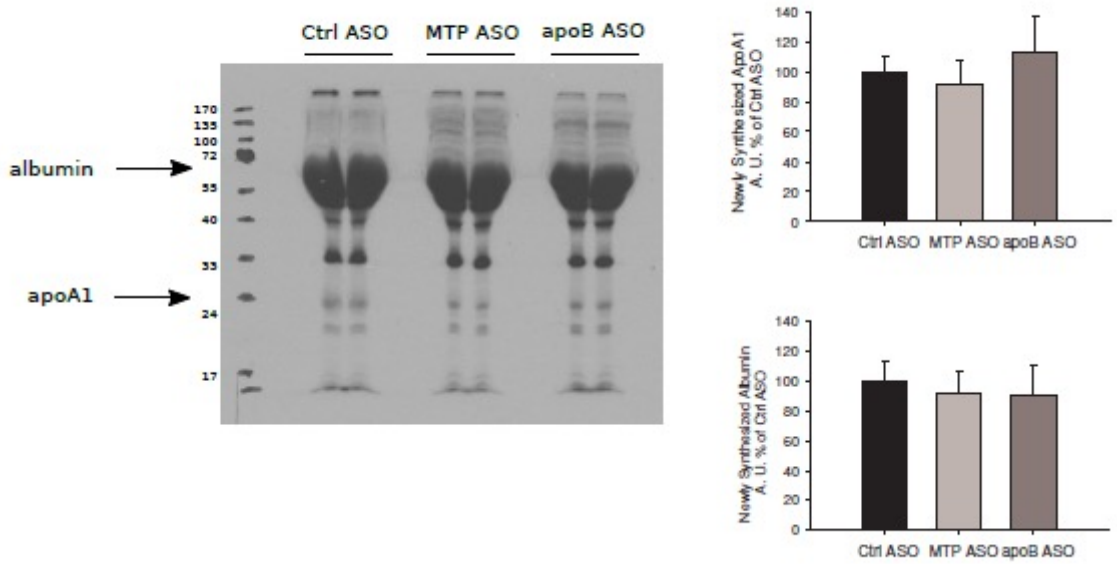

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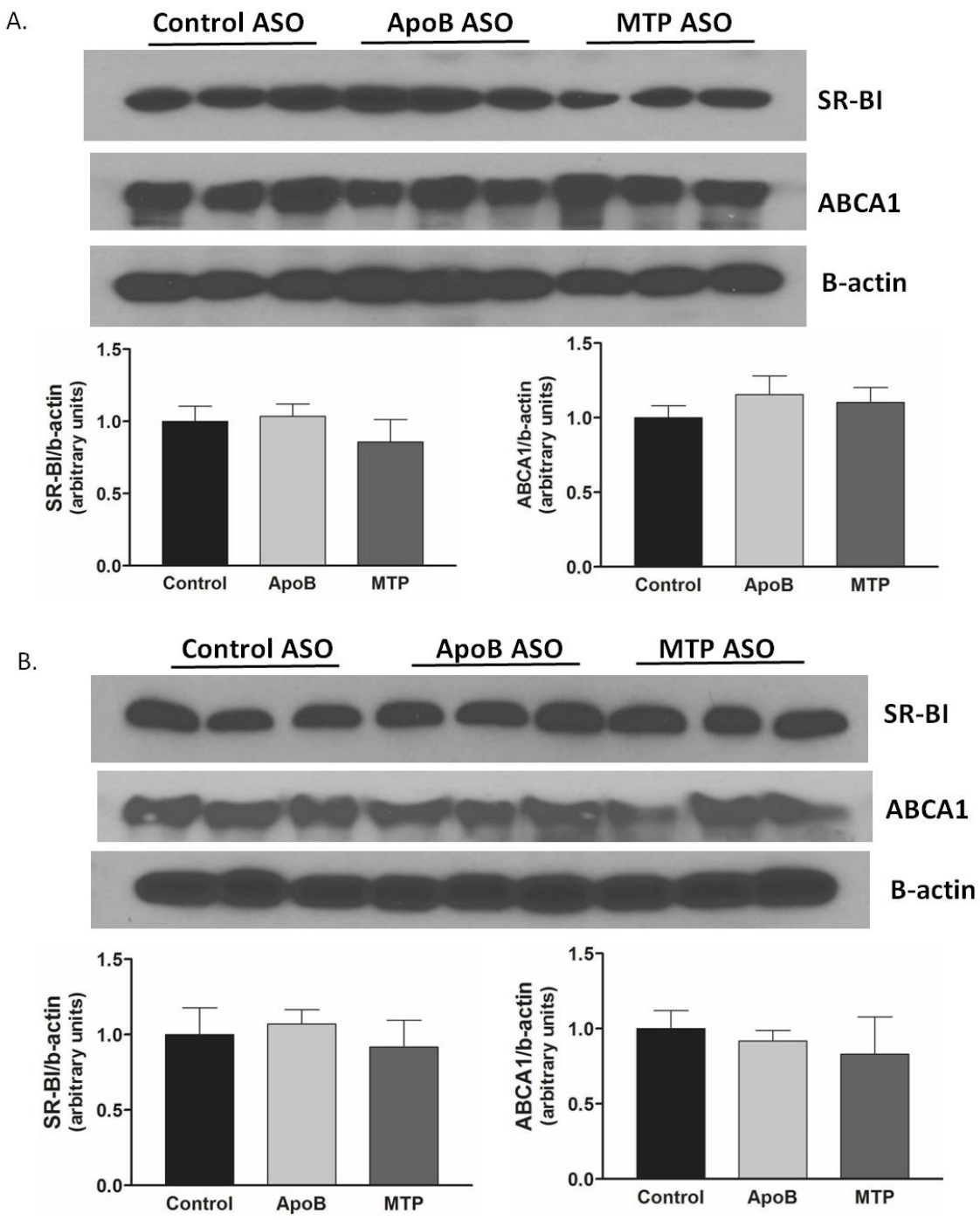

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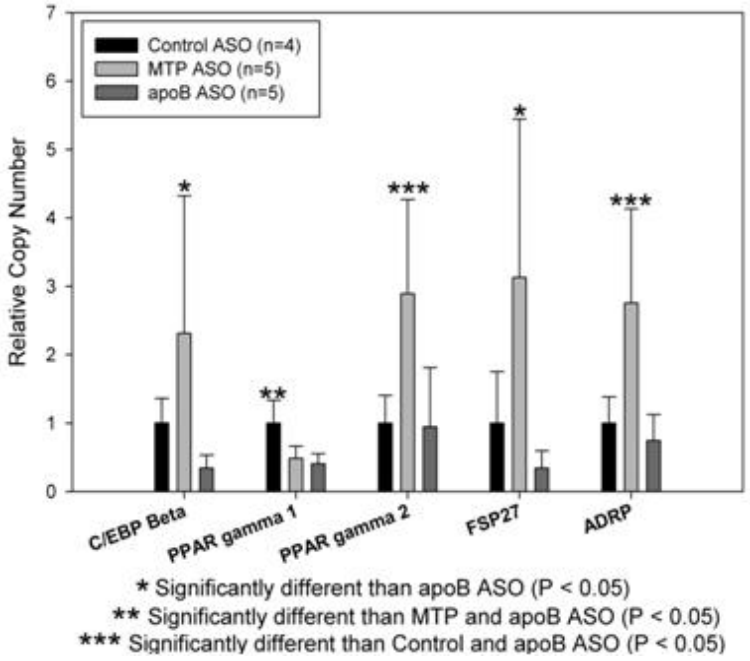

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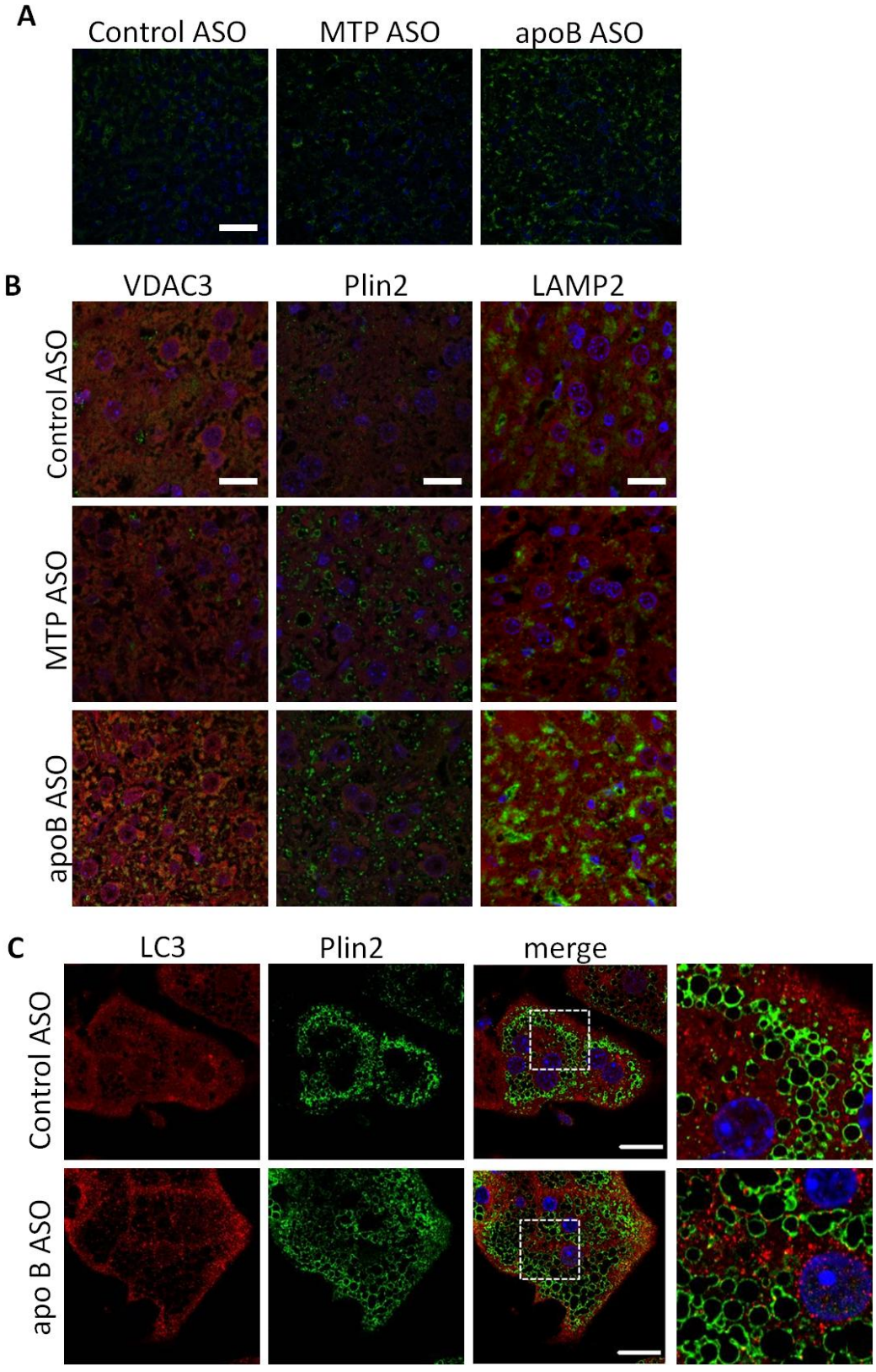
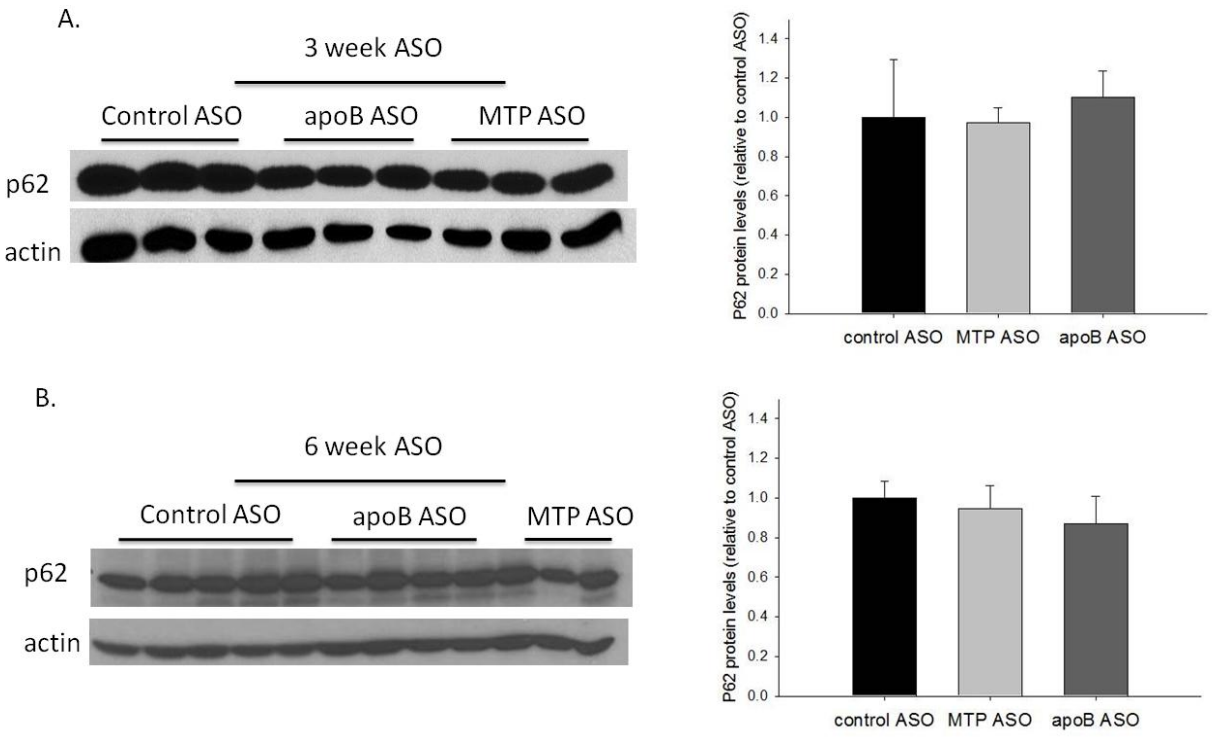
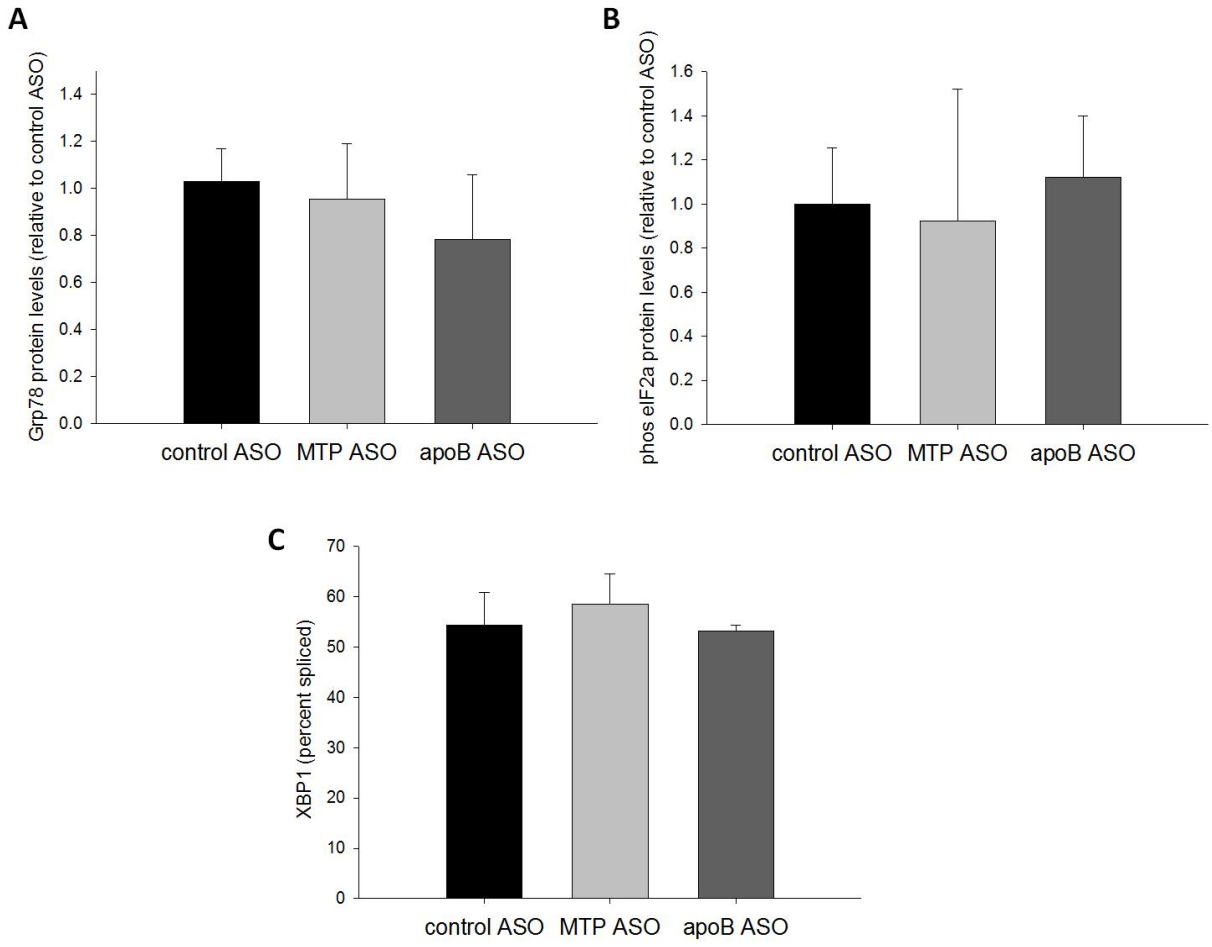


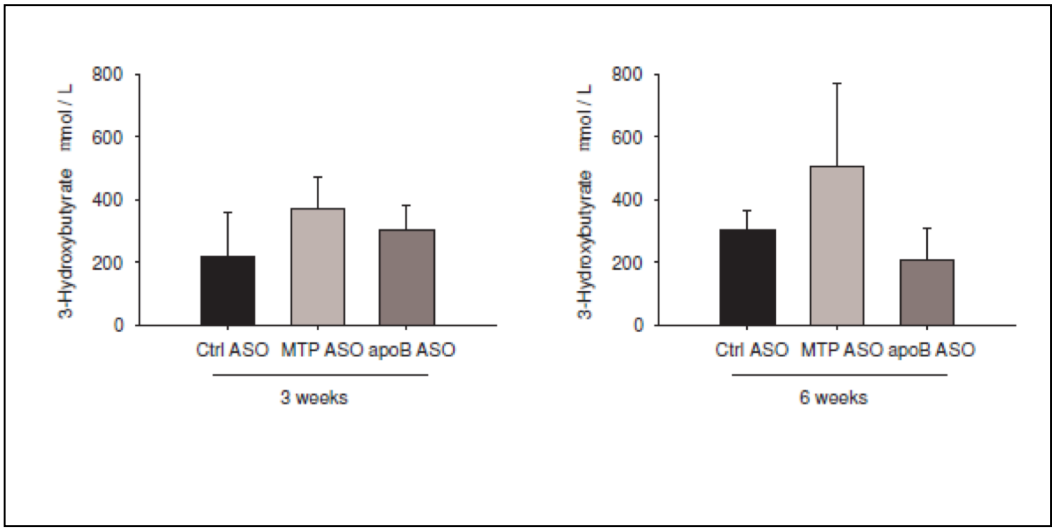
Supplemental Figure S7



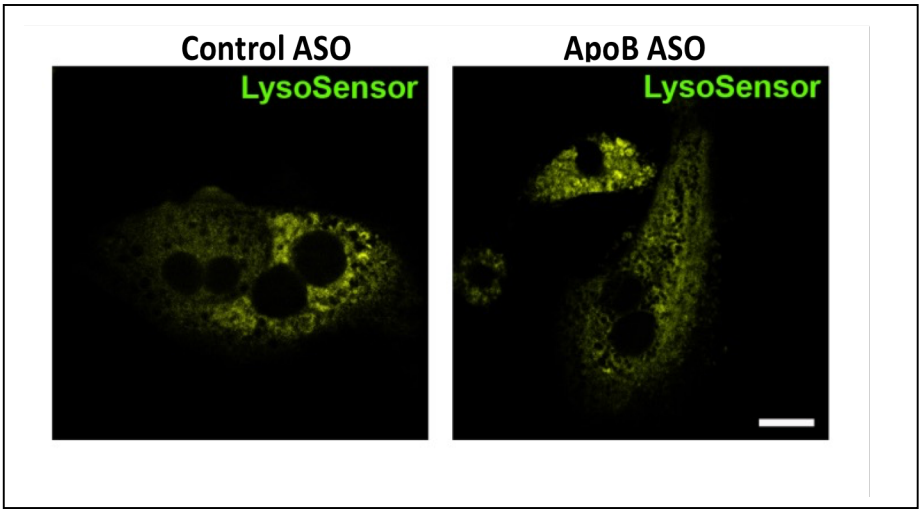
Supplemental Figure S8



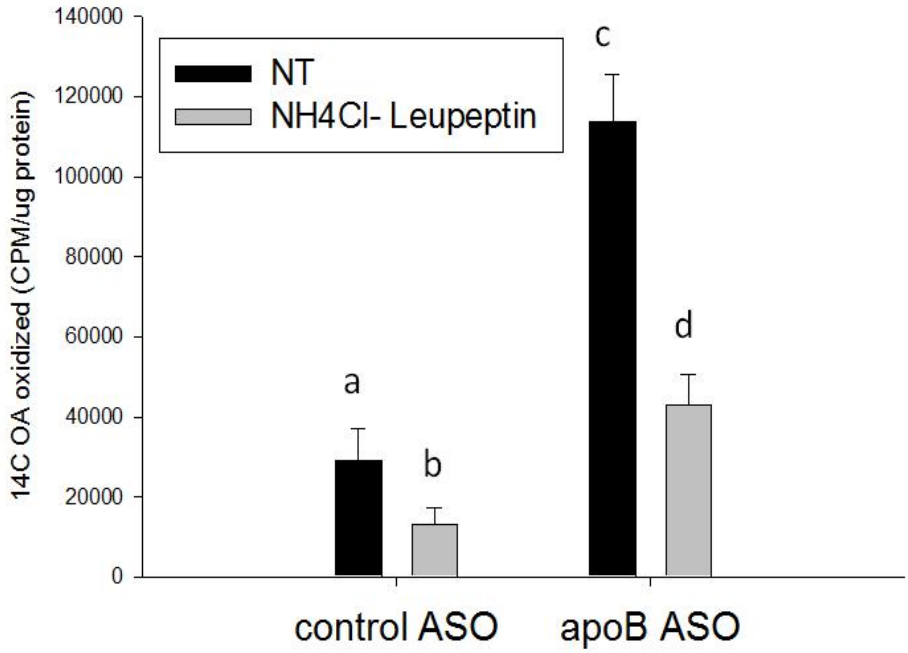
Supplemental Figure S9



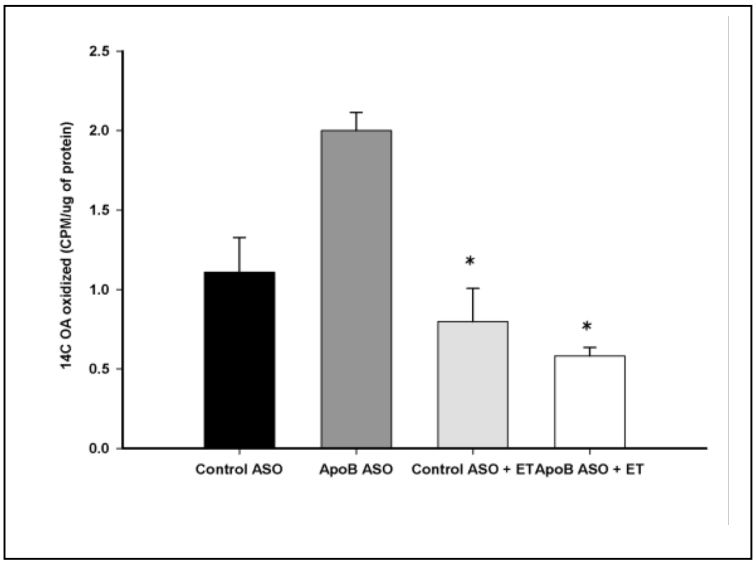
Supplemental Figure S10



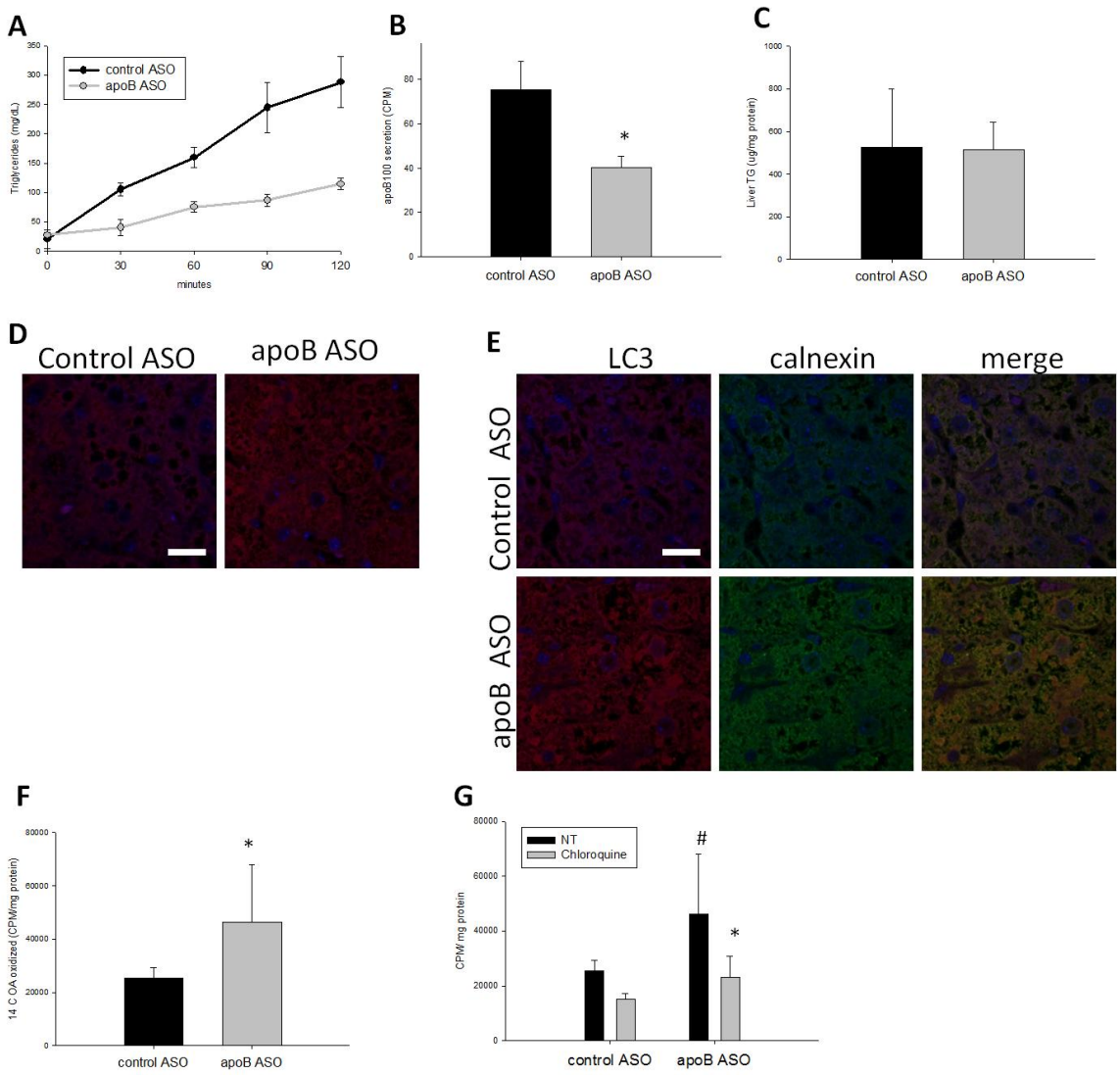
Supplemental Figure S11



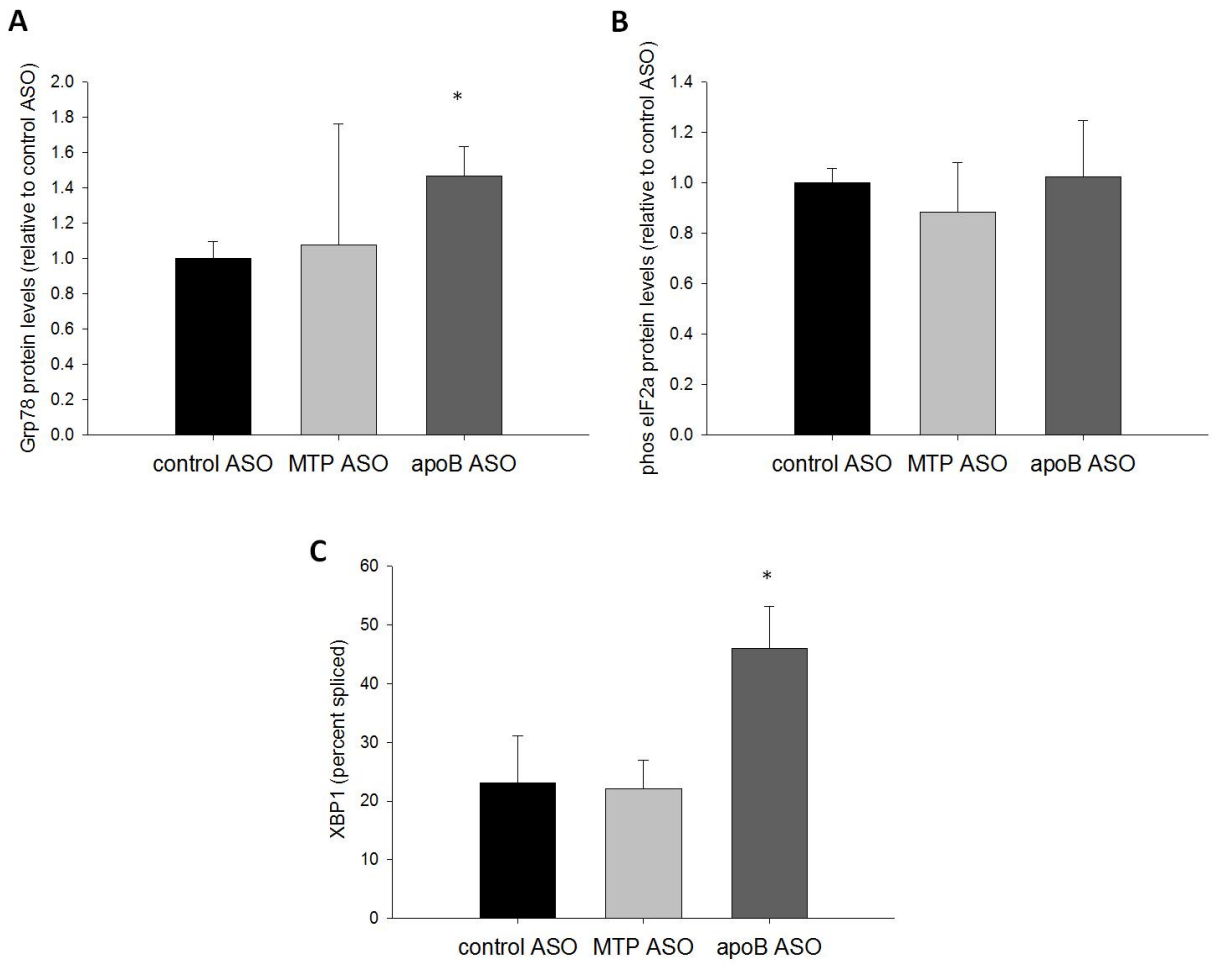
Supplemental Figure S12



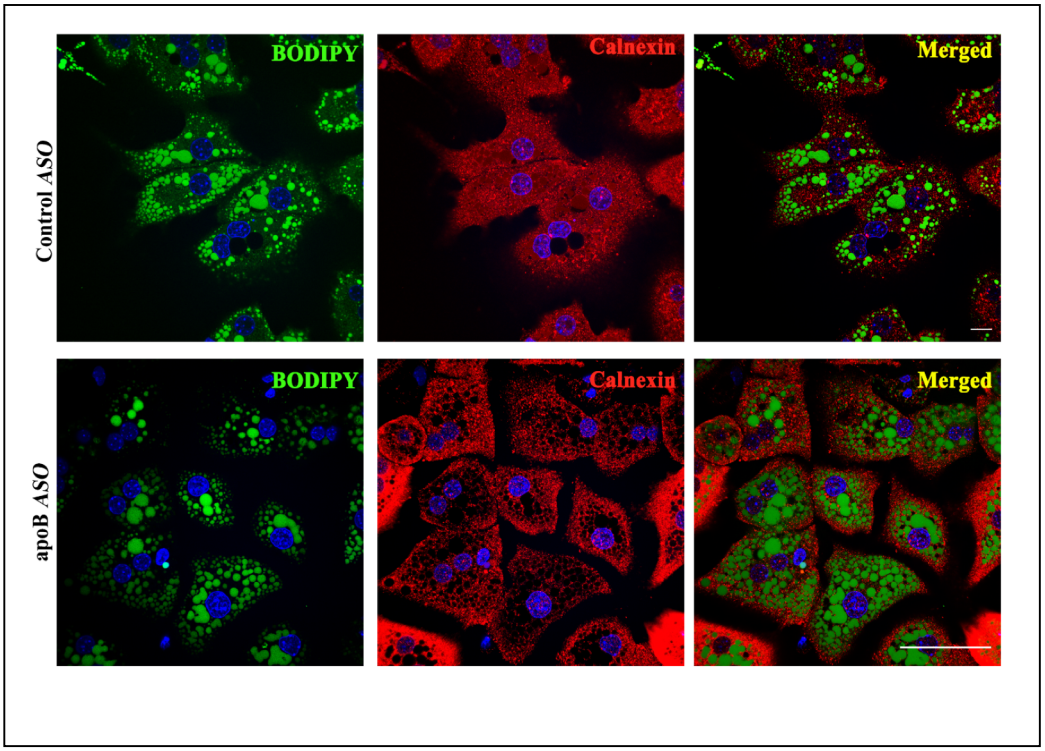
Supplemental Figure S13



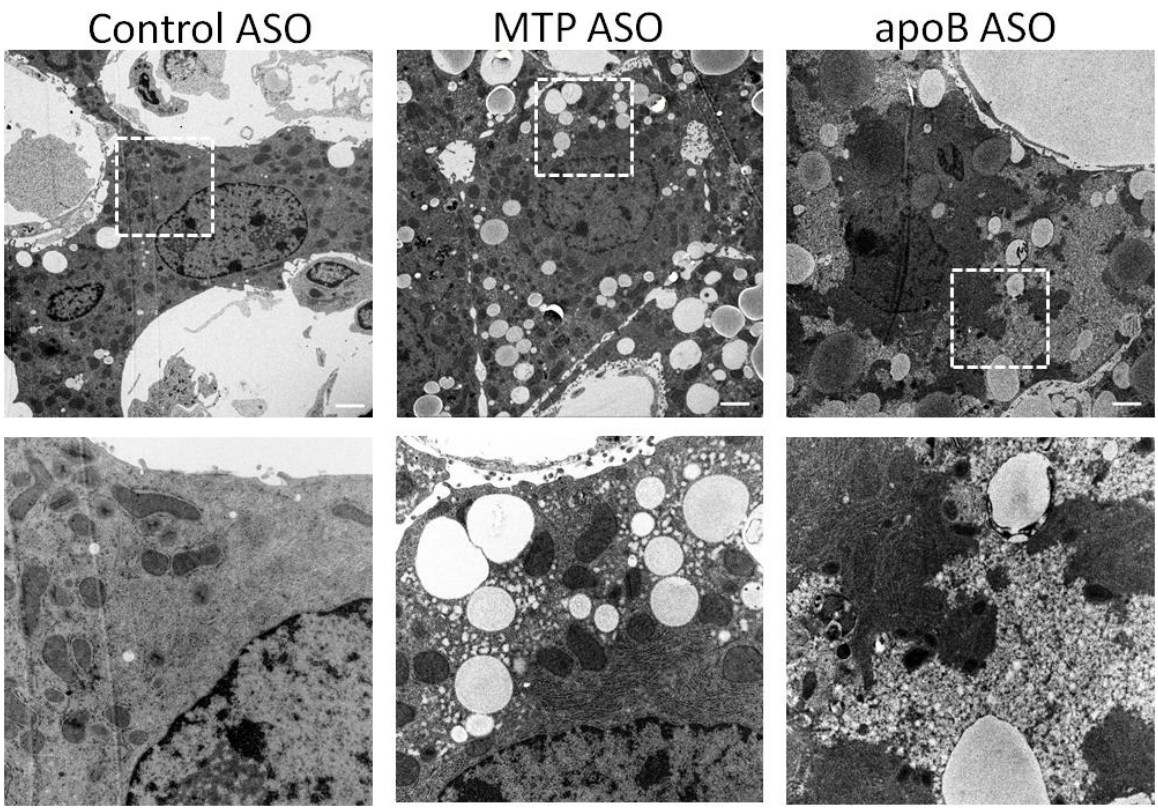
Supplemental Figure S14



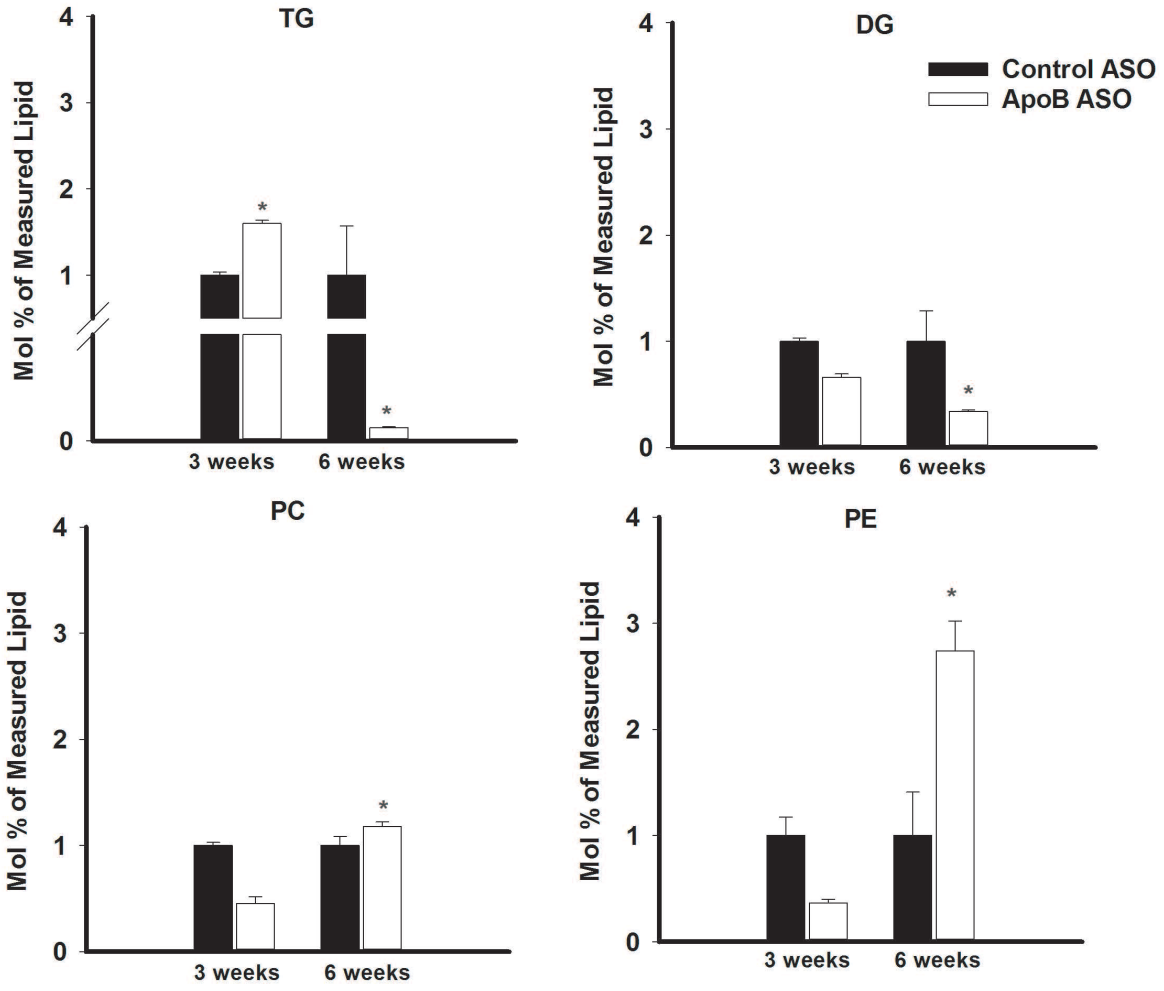
Supplemental Figure S15



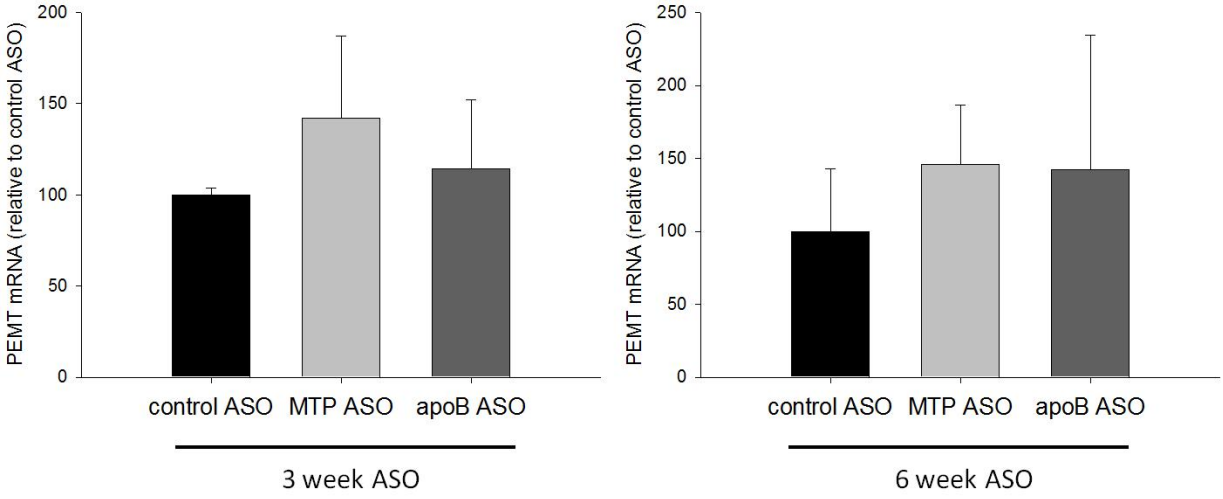
Supplemental Figure S16



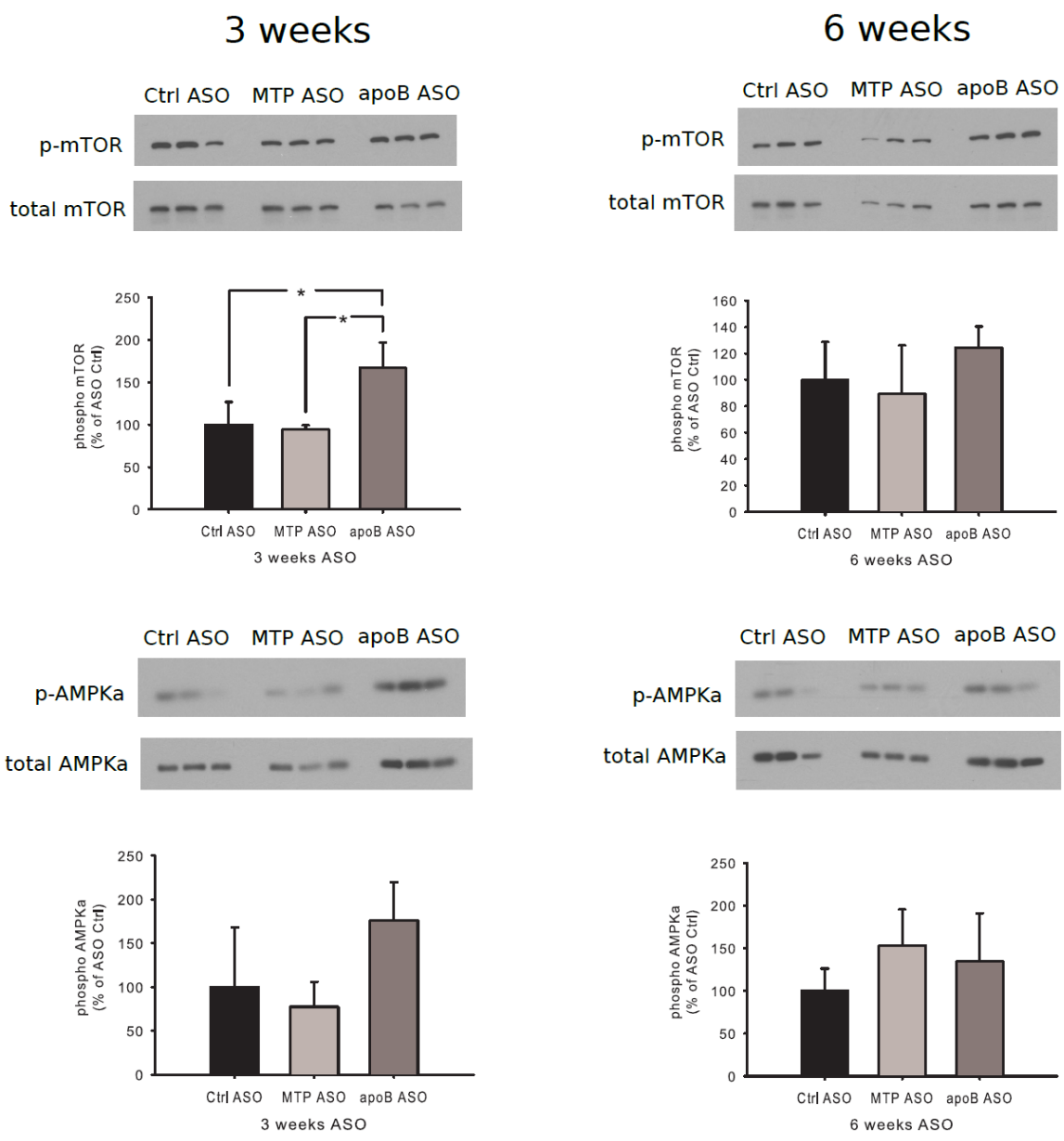
Supplemental Figure S17



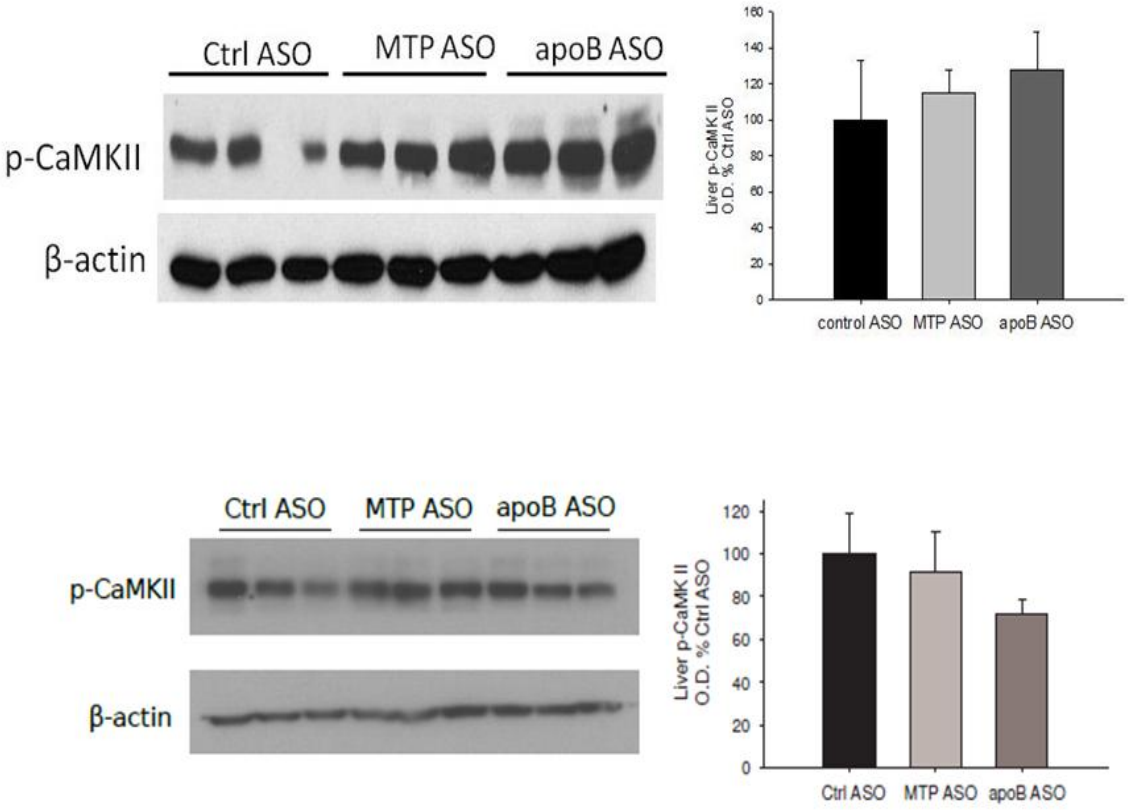
Supplemental Figure S18



Supplemental Figure S19



Supplemental Figure S20



Supplemental Figure 21