Supplementary Information

Methods:

Patient sera and microarray analysis

Patients enrolled in the “Renoprotection in early diabetic nephropathy in Pima Indians” study (NCT00340678) were stratified based on the loss of measured glomerular filtration rate (mGFR, iothalamate) over a 10-year period. Two groups of 15 and 16 patients each representing the two tails of the normal distribution of mGFR were studied and assigned to the non-progressor (NP) group when part of the group with the least change in mGFR, or to the progressor (P) group characterized by the greatest change in mGFR over the 10-year period. NP and P were matched for age, blood pressure, weight, duration of diabetes and circulating lipids (Supplementary table 1). Sera collected at enrollment prior to the development of DKD and seven years later at time of a research kidney biopsy were utilized for in vitro experiments. Treatment of podocytes with patient sera was carried out as described in supplemental methods. Data were normalized and quantified using custom CDF annotations on an Affymetrix U133 platform on a probe set level, as previously described(70).

Podocyte cell culture

Human podocytes gifted from Dr. Moin Saleem (University of Bristol, Bristol, England) were cultured and grown at 33°C under permissive conditions in RPMI culture medium containing 10% FBS and 1% penicillin/streptomycin and 0.01 mg/ml recombinant human insulin, 0.0055 mg/ml human transferrin (substantially iron-free), and 0.005 µg/ml sodium selenite(71). Human podocytes were then

thermoshifted and differentiated for 14 days at 37°C in RPMI medium 10% FBS and 1% penicillin/streptomycin. ABCA1 siRNA knockdown (siABCA1) and non-targeting siRNA control (siCO), ABCA1 overexpression (ABCA1OE) and empty vector (EV) podocytes were generated as previously described(14). On day 12 of differentiation, podocytes were serum-starved for 24 hours, followed by treatment with patient serum (4% sera in FBS free RPMI) for 24 hours. Podocytes were analyzed in various assays as described below.

Quantitative real-time PCR

mRNA was extracted and purified from human differentiated podocytes or mouse glomeruli using the RNAeasy mini kit (Qiagen). cDNA was synthesized for individual samples by reverse transcription using qScript cDNA supermix (Quanta). All quantitative real-time PCR (RT-PCR) assays were carried out using the StepOnePlus system (Applied biosystems) with either PerfeCTA SYBR Green FastMix (Quanta) using primers as indicated for genes of interest, or with Taqman Fast Advanced Master Mix (Applied biosystems) and the respective probes (Invitrogen). Delta-delta cycle threshold (ddCT=dCT(Gene of interest-dCT(housekeeping gene); $FC=2^{-ddCT}$)) values were obtained and fold change values determined for all gene expression data.

Cholesterol Efflux

Cholesterol efflux was measured from differentiated human podocytes as previously described with some modifications(14, 72). Briefly, differentiated podocytes were loaded with radiolabeled [^{3}H] cholesterol at a concentration of 1µCi/mL in 1%FBS RPMI for 24 hours at 37°C, then medium was removed,

followed by two PBS washes. Equilibration medium (RPMI with 0.2% BSA) was added and cells were incubated overnight at 37°C. The next day, an aliquot of equilibration medium (time 0) was collected prior to adding 20µg/mL of ApoAI in RPMI for 6 hours. The supernatant collected at time 0 (Cpm0) was measured for radioactivity scintillation counting. Cells were then incubated with 20µg/mL of recombinant ApoAI (Millipore #APL10) in equilibration media for 6 hours at 37°C. Following incubation, the supernatant at time 6 hours (Cpm6) was collected and the amount of radioactivity present was determined via scintillation counting. Cells were washed twice with PBS and then lysed with cell lysis solution (0.1M NaOH, 0.1%SDS). Cell lysate (CpmLysate) was recovered and radioactivity was measured. Cholesterol efflux to ABCA1 (via ApoAI) was expressed as fold change of the percentage of efflux calculated as: Efflux (%) = 100 x [Cpm6/(Total Cpm(Cpm0+Cpm6))-CpmLysate)].

Lipid droplet quantification

As previously published, human podocytes were fixed with 4%PFA, 2% sucrose following differentiation and/or treatment. Following fixation, cells were stained with either HCS LipidTox Red or Bodipy 493/503 (Invitrogen) and HCS CellMask Blue (Invitrogen) using the manufacturers protocols. Lipid droplet images were acquired using the Opera high content screening system (20x confocal lens) and analyzed using the Acapella high content image analysis software (Perkin Elmer). Lipid droplet intensity per cell was determined.

ROS and cytotoxicity assays

Reactive oxygen species were assessed in human differentiated podocytes following patient sera treatment using Cell ROX green reagent (Invitrogen) according to manufacturer's instructions. Cells were fixed with 4%PFA, 2% sucrose and analyzed for cytoplasmic intensity of cell ROX using the Opera high content screening system (20x confocal lens) and Acapella high content image analysis software (Perkin Elmer). Cytotoxicity assays were carried out in differentiated podocytes using the ApoTox-Glo triplex assay (Promega) according to manufacturer's protocol. Fluorescence and luminescence were measured on a SpectraMax i3x multi-mode microplate reader (Molecular devices).

Oxygen consumption rate assays

Oxygen consumption rates were measured as previously described(73), using a Clark oxygen electrode in a micro water-jacketed, magnetically stirred chamber set at 37°C (Hansatech Instruments Limited, Norfolk, UK). Briefly, approximately $2x10^6$ differentiated podocytes were collected in 1 mL of permeabilized-cell respiration buffer (PRB) (0.3 M mannitol, 10 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM EGTA, 1 mg/mL BSA, 10 mM KH_3PO_4, pH 7.4) and 500 µL were used for the assay. Endogenous cell respiration was recorded for 1 minute. Cells were then permeabilized with digitonin (60 µg/10^6 cells). After permeabilization, complex I substrates (5 mM glutamate and 5 mM malate) or complex II substrate (5 mM succinate) were added to the chamber using a Hamilton microsyringe, and substrate driven respiration was measured for an additional 1 minute. Respiration was inhibited by addition of 700 µM potassium cyanide (KCN), a

specific CIV inhibitor. Oxygen consumption rate values were determined as nmol of oxygen consumed per minute normalized to protein content.

BN-PAGE analysis of OXPHOS complexes

Blue native-polyacrylamide gel electrophoresis (BN-PAGE) was performed as previously described(73). Briefly, $2.5x10^6$ differentiated podocytes were permeabilized in 280 μL of PBS with 2.3 mg/mL digitonin. Permeabilized cell were pelleted and resuspended in 100 μL of 1.5 M aminocaproic acid, 50 mM Bis-Tris pH 7.0 and proteins were extracted with either 1% lauryl maltoside (for analysis of individual OXPHOS complex steady state levels) or 1.2% digitonin (for analysis of mitochondrial supercomplexes). Following clarification spin at 22.000g for 30 min at 4°C, extracts were analyzed by BN-PAGE on a linear 3-12% native polyacrylamide gel. OXPHOS compexes and supercomplexes were detected by Western blot analysis using specific antibodies for OXPHOS subunits as indicated.

In cell and in gel OXPHOS complex enzymatic activity assays

Enzymatic activity assays were carried out as previously described(73). Briefly, differentiated podocytes were harvested and resuspended in Hanks salt solution and pelleted. Cells were permeabilized by mechanical breakage (4-5 freeze thaw cycles) and resuspended in mannitol buffer (225mM mannitol, 75mM sucrose, 10mM Tris pH 7.2, 0.1mM EDTA) at a protein concentration of 3mg/mL. 10μL of cell homogenate were used to record: i. Rotenone-sensitive NADH oxidation in presence of decylubiquinone (complex I activity). ii. Antimycin A-sensitive

cytochrome *c* reduction in presence of decylubiquinol (complex III activity). iii. KCN-sensitive cytochrome *c* oxidation (complex IV activity).

In gel complex I activity was determined by BN-PAGE analysis of lauryl maltoside mitochondrial extracts followed by incubation of the gel in 2 mM Tris-HCl pH 7.4, 0.1 mg/mL NADH (Roche) and 2.5 mg/mL iodonitrozolium (Sigma)(74) at room temperature for 30 minutes. Signal intensity of the respective band was analyzed via densitometry using ImageJ software.

Mitochondrial membrane potential assays

Mitochondrial membrane potential was determined using the JC-9 probe (Invitrogen) according to the manufacturer's protocol with some modifications. Briefly, 0.1µg/mL of JC-9 was added to differentiated podocytes for 30 minutes. CCCP was utilized as a control as it is readily known to collapse membrane potential. 10µM of CCCP was added concomitantly with the JC-9 probe. The JC-9 fluorescence signal was detected at both 514 and 585 (excitation) on a SpectraMax i3x multi-mode microplate reader (Molecular devices) in order to quantify the monomer versus the aggregate signal, respectively. Membrane potential was calculated as the 585-signal intensity divided by the 514-signal intensity.

Mitochondrial network

Mitochondrial network was observed using mitotracker deep red (Invitrogen) on differentiated podocytes grown on glass cover slips. Cells were then fixed with 4% PFA/sucrose, stained and mounted with Prolong GOLD DAPI mounting media (Invitrogen). Image acquisition was carried out using laser scanning

confocal microscopy using a Leica SP5 inverted microscope with 63x objective (Leica microsystems CMS GmbH).

Mitochondrial isolation and lipid extraction for mass spectrometry analysis

Mitochondria were isolated from differentiated podocytes as previously described(75). Briefly, cells were broken by swelling and homogenization with a glass/Teflon homogenizer in ice-cold TKMg buffer (10mM Tris HCl, 10mM KCl, 0.15mM $MgSO_4$). Mitochondria were then isolated by a serial centrifugation in TKMg sucrose buffer (TKMg + 0.25M sucrose). For lipidomics analysis, mitochondria were resuspended in TE buffer (0.01M Tris pH 7, 0.1mM EDTA), then pelleted again at 14,000g for 5 minutes and lyophilized in a speed vacuum overnight at 37°C. The next day, the mitochondrial pellets were resuspended in chloroform:methanol (2:1) and lipids were extracted. Total lipid extracts were purified with two sequential salt (0.9% NaCl) washes. Purified lipid extracts were analyzed using mass spectrometry. Briefly, lipids were eluted using reverse phase chromatography with an Acclaim C30 column utilized for high performance liquid chromatography (HPLC). The HPLC system (Thermo fisher scientific Accela system), connected to a heated electrospray ionization probe, was coupled to a Qexactive mass spectrometer (Thermo fisher scientific). Lipid files acquired were run in triplicate and then analyzed and identified using Lipidsearch software (Dr. Ryo Taguchi and Mitsui Knowledge Industry Co.). Content of a specific lipid class was normalized to total lipids and represented as a percentage.

Cardiolipin oxidation analysis

The Thermo RAW files obtained from the LC-MS/MS analysis were converted to centroid mzXML format using the MSconvertGUI software. The mzXML files were imported into MZmine2 software, normalized and baseline-corrected. Chromatograms were generated from these files and deconvoluted. Identification of regular and oxidized CLs was done using the built-in lipid database, or online using lipid maps. In either case, the search was restricted to the 4 cardiolipin classes, with a m/z tolerance of 0.001 or 5ppm.

Animal Studies

Abca1$^{fl/fl}$ were kindly provided by Dr. John Parks (Wake Forest, Winston-Salem, NC). Podocyte-specific *Abca1*$^{fl/fl}$ (Abca1 fl/fl) mice were generated by breeding of Abca1 fl/fl mice to Podocin-Cre mice as previously described(14). *Soat1*$^{-/-}$ (Soat1 KO) mice were gifted from Dr. Ta-Yuan Chang (Dartmouth, Hanover, NH). Abca1 fl/fl, Soat1-/- (DKO) mice were generated and analyzed at 1 year of age. Abca1 fl/fl mice were backcrossed to BTBR for 7 generations prior to breeding with *BTBR*$^{ob/+}$ (Jackson Laboratory, stock# 004824)(ob/+) mice in order to generate Abca1 fl/fl; *ob/ob* mice. Abca1 fl/fl; *ob/ob*, *ob/ob*, and *ob/+* mice were generated and analyzed at 16 weeks of age. Abca1 fl/fl mice were injected with 50 mg/kg of streptozotocin (STZ, Sigma catalog# S-0130) daily for 5 consecutive days. Ob/ob mice were aged for 16 weeks and then treated with daily intraperitoneal injections of Elamipretide (3mg/kg/day; BOC sciences) or saline for 4 weeks. Mice were sacrificed at 20 weeks. Abca1 fl/fl; ob/ob mice were aged for 12 weeks and then treated with daily intraperitoneal injections of Elamipretide (3mg/kg/day; BOC sciences) or saline for 4 weeks. Mice were sacrificed at 16 weeks.

B6.BKS$^{db/db}$ and B6.BKS$^{db/+}$ mice were (Jackson laboratory, stock# 000697) aged to 14 weeks. Daily treatment with vehicle (1.25% Hydroxypropyl methyl cellulose, 0.10% docusate sodium salt, 0.18% Methyl t-hydroxybenzoate sodium, 0.18% propyl paraben sodium, 0.02% citric acid monohydrate, pH 6) or an Abca1 inducer (30mg/Kg/day, Roche, A30)(34) by oral gavage was started at 14 weeks of age. Mice were sacrificed at 18 weeks of age and tissues were processed and analyzed as described below.

Phenotypic analysis of mice

Spot urine samples were collected at baseline and time of sacrifice. Urinary albumin to creatinine ratios were determined using an albumin ELISA kit (Bethyl laboratories) and a colorimetric assay for creatinine determination (Stanbio). Albuminuria was analyzed and expressed as µg of albumin divided by mg of creatinine. Weight and glycemia were measured on a bi-weekly basis. At time of sacrifice mice were perfused via left ventricle perfusion with isotonic saline solution. The right kidney was harvested for glomeruli isolation for mRNA analysis using the sieving method and mRNA was extracted as mentioned above(9). The left kidney was further sectioned for various analyses as described next. Kidney cortex sections were embedded in OCT for further analysis of immunofluorescent staining and lipid droplet analysis. The podocyte number per glomerular cross-section was determined using 4µm-thick tissue sections that were cut and stained with WT1 antibody (1:200, Santa Cruz) and prolong GOLD DAPI mounting media. Images were acquired with confocal microscopy using a

Leica SP5 inverted microscope with the 40x wet objective. 20 glomeruli per mouse were quantified(76).

For transmission electron microscopy kidney cortex sections were placed in 4% paraformaldehyde, 1% gluteraldehyde in 0.1M phosphate buffer (pH 7.4). Foot processes were quantified per 1µm of the glomerular basement membrane. Kidney cortex sections were snap frozen and utilized for lipid extraction and cholesterol content determination(14). Total lipids were extracted from kidney cortex by placing samples in homogenization buffer (2mM potassium phosphate) and homogenized via sonication. Lipids were extracted using hexane: isopropanol (3:2) for two sequential 30-minute extractions. The solvent was then dried using a speed vacuum at 37°C for approximately 2 hours. Lipids were reconstituted using isopropanol: NP-40 (9:1) and cholesterol content was quantified using the Amplex red cholesterol assay kit (Invitrogen) according to the manufacturers protocol or sent for mass spectrometry lipid analysis (as described above). Kidney cortex sections were fixed in 10% formalin and then paraffin-embedded and cut at 4µm thick for periodic acid-Schiff (PAS). Mesangial expansion was scored based on semi-quantitative analysis (scale 0-5) or percent of glomeruli with mesangial expansion (%), performed in a double-blinded manner(9).

Statistics

All values are presented as means with standard deviations. Animals were grouped unblinded, but randomized, and investigators were blinded for the quantification experiments. Significant outliers were excluded from further

statistical analysis. Prism GraphPad 7 software was used to perform all statistical analysis. When comparing between two groups a two-tailed student's t-test was performed, otherwise results were analyzed using one-way ANOVA followed by Tukey's post-test of multiple comparisons. P values <0.05 were considered statistically significant.

Study Approval

All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Miami. The University of Miami (UM) has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare, NIH (A-3224-01, effective November 24, 2015). Additionally, UM is registered with the US Department of Agriculture Animal and Plant Health Inspection Service, effective December 2014, registration 58-R-007. As of October 22, 2013, the Council on Accreditation of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International) has continued UM's full accreditation. All study protocols involving human samples were granted IRB exemption since the samples were collected under an IRB-approved research study for which informed consent was obtained and there was no access to identifiable information (Human Subjects Research Office, Miami, FL, 2012, and Human Research Protection Program, Ann Arbor, MI, 2015). Appropriate safeguards were in place to protect subject confidentiality and privacy.

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Supplemental tables:

Supplemental table 1: Characteristics of Pima Indians with T2D by whether they were non-progressors or progressors

	Non-progressor (n=16)	Progressor (n=15)
Age	48±9.0	51±11.5
Sex	M/F(5/11)	M/F(6/9)
SBP (mmHg)	120±10.2	133±17.7
DBP (mmHg)	78±7.7	83±13.5
Weight (kg)	101.4±31.7	89.9±23.5
HbA1C (%)	9.3±2.0	10.6±1.5
Diabetes duration	6.6±2.9	8.8±5.1
GFR (mL/min)	157±50	192±7.3*
ACR (mg/g)	24±3.7	57±13.9*
Total cholesterol (mg/dL)	172±8.8	161±10.8
Triglyceride (mg/dL)	176±16.7	198±62.1

a. Two tailed t-test, *p<0.05.

Supplemental table 2: Correlation analysis between ABCA1 expression and clinical parameters of Pima Indians with T2D

Parameter 1	Parameter 2	P value	r^2 value
Serum Creatinine	Glomerular ABCA1 expression	<0.05	0.24
BUN	Glomerular ABCA1 expression	<0.05	0.26
ACR	Glomerular ABCA1 expression	0.09	0.26
mGFR at baseline	Glomerular ABCA1 expression	0.50	0.08
mGFR at 10 year follow-up	Glomerular ABCA1 expression	0.54	0.08
ACR	Sera treated podocytes ABCA1 expression	<0.01	0.44
Mean BMT	Sera treated podocytes ABCA1 expression	<0.05	0.13

a. Linear regression analysis

Supplemental table 3: Serological analysis of diabetic ob/ob and Abca1 fl/fl; ob/ob mice

Serum parameter	ob/ob	Abca1 fl/fl; ob/ob	P-value
BUN (mg/dL)	23±0.5774	29±1.08	0.0071**
Serum creatinine (mg/dL)	0.82±0.0663	0.8±0.0633	0.8327
LDL (mg/dL)	17±3.189	38.67±6.064	0.0187*
Triglycerides (mg/dL)	166±10.43	154.8±3.967	0.345
Cholesterol (mg/dL)	190.3±6.434	214±13.83	0.1704

a. Two-tailed t-test. *p<0.05, **p<0.01.

Supplemental table 4: Mitochondrial lipid analysis of siCO versus siABCA1 podocytes

Lipid Class	siCO	siABCA1	P-value
Cholesterol Ester	1	0.14 ± 0.12	0.0003***
Diglyceride	1	0.90 ± 0.05	0.1332
Lysophosphatidylcholine	1	1.26 ± 0.24	0.3529
Lysophosphatidylethanolamine	1	1.05 ± 0.43	0.9219
Lysophosphatidylinositol	1	0.99 ± 0.18	0.964
Lysophosphatidylserine	1	0.56 ± 0.15	0.027*
Phosphatidic acid	1	1.57 ± 0.64	0.4272
Phosphadtidylcholine	1	1.20 ± 0.03	0.0006***
Phosphaditylethanolamine	1	0.79 ± 0.07	0.0381*
Phosphatidylinositol	1	0.99 ± 0.09	0.9217
Phosphatidylserine	1	0.97 ± 0.03	0.319

a. Values are presented as fold change (± standard deviation) compared to siCO of relative quantity of specific lipid class to total lipids. Two-tailed t-test.

Supplemental figure legends:

Supplemental figure 1: Increased cytotoxicity but not ROS in podocytes treated with the sera from patients collected at time of biopsy.

(A-B) Bar graph analysis showing ROS production via CellRox cytoplasmatic intensity (n=5 pooled sera per group) **(A)** and cytotoxicity normalized to viability (n: NP=16, P=15) **(B)** in human podocytes treated with time of biopsy P patient sera compared to NP sera treated podocytes. Two-tailed t-test. *p<0.05.

Supplemental figure 2: Fission and fusion genes are significantly downregulated in Abca1 deficient diabetic mice.

Quantitative real-time PCR quantification of *Fis1* (mitochondrial fission) and *Mfn1* (mitochondrial fusion) normalized to *Actb*, comparing ob/+, Abca1 fl/fl, ob/ob and Abca1 fl/fl; ob/ob mice (n=3-5 per group). One-way ANOVA followed by Tukey's test *p<0.05, **p<0.01.

Supplemental figure 3: Weight, serological and histological parameters of Abca1 fl/fl; ob/ob and Abca1 fl/fl STZ mice.

(A) Body weight measurement (g) of Abca1 fl/fl;ob/ob mice (n=8) compared to ob/ob (n=7) controls. **(B)** Kidney weight to body weight ratio (mg/g) of Abca1 fl/fl; ob/ob (n=8) mice compared to ob/ob (n=7). **(C)** Glycemic index (mg/dL) of wild-type (WT), Abca1 fl/+ (Het) and Abca1 fl/fl (KO) with STZ injections compared to vehicle injections (n=5 per group). **(D)** Body weight measurements (g) of WT, Het

and KO mice with STZ injections compared to vehicle injections (n=5 per group). **(E)** PAS stain of kidney cortex sections showing KO STZ injected mice compared to WT STZ (25μm scale bars). Two-tailed t-test. ***p<0.001, ****p<0.0001.

Supplemental figure 4: siABCA1 podocytes experience alterations in mitochondrial network distribution with no changes in membrane potential. **(A)** Bar graph analysis of ATP content normalized to protein comparing siCO and siABCA1 podocytes (n=4 per group). **(B)**Representative image and quantification of In-gel complex I (CI) enzymatic activity (n=3 per group). Western blot for ACTB was used as loading control. **(C)** Representative Western blot image of BN-PAGE analysis at low exposure (same blot shown at higher exposure in Figure 4C) of mitochondrial extracts obtained with digitonin sequentially probed for core 2 antibody (CIII), COX I antibody (CIV) and SDHA (CII), used for the quantification of CIV (shown in Figure 4D). **(D)** Mitochondrial membrane potential quantification by JC-9 probe comparing siCO and siABCA1 podocytes at baseline (NT) as well as treatment with an uncoupler agent (CCCP) (n=3 per group). **(E)** Representative images of mitotracker red and DAPI of siCO and siABCA1 podocytes (original magnification 60x). Two-tailed t-test. *p<0.05.

Supplemental figure 5: SOD2 expression is increased in siABCA1 podocytes.

(A) Representative Western blot image of SDS-PAGE analysis of cell lysates from siCO and siABCA1 podocytes probed with SOD2 antibody. **(B)**

Densiometric quantification of Western blot analysis (n=3 per group). Two-tailed t-test. *p<0.05.

Supplemental figure 6: Abca1 deficiency is associated with a significant reduction in fatty-acid β oxidation genes.

(A-B) Quantification of qRT-PCR of *PPARA*, *ACADM*, *ACOX1* and *ACOX2* normalized to *ACTB* comparing: non-progressor versus progressor at baseline and time of biopsy (n=4-15 per group) **(A)**; and Abca1 fl/fl to WT mice (n=3-5 per group) **(B)**. **(C)** Bar graph analysis of qRT-PCR of *ACADM* normalized to *ACTB* in siCO and siABCA1 podocytes (n=5-6 per group). Two-tailed t-test. *p<0.05, **p<0.01.

Supplemental figure 7: ABCA1 overexpression results in decreased lipid droplet content and increased expression of fatty-acid β oxidation genes.

(A) Lipid droplet quantification via bodipy between empty vector (EV) and ABCA1 overepxression (ABCA1 OE) podocytes after non-progressor or progressor sera treatment (n=10-15 per group). **(B)** Quantification of qRT-PCR of *PPARA*, *ACADM*, and *ACOX2* normalized to *ACTB* comparing EV versus ABCA1 OE after non-progressor or progressor sera exposure (n=4-6 per group). Two-tailed t-test (panel A), One-way ANOVA followed by Tukey's test (panel B). *p<0.05, **p<0.01, ****p<0.0001.

Supplemental figure 8: Abca1 inducer treatment does not alter glycemic index nor body weight in db/db mice.

(A) Bar graph of glycemic index (mg/dL) measurements taken from db/db mice treated with vehicle (n=5) or Abca1 inducer (A30, n=6). **(B)** Bar graph of body weight measurements (g) obtained from db/+ (n=6), db/db vehicle (n=5) and db/db A30 (n=6). Two-tailed t-test, ****p<0.0001.

Supplemental figure 9: ABCA1 expression is increased in kidney cortexes of db/db mice treated with A30.

(A) Representative Western blot image of SDS-PAGE analysis of kidney cortex lysates from non-diabetic (db/+), diabetic vehicle treated (db/db + V) and diabetic ABCA1 inducer (db/db + A30) treated mice probed with ABCA1 antibody. **(B)** Densiometric quantification of Western blot analysis shown in A (n=3 per group). **(C)** Correlation analysis between BUN and CE (r-squared and p-value shown on graph). Two-tailed t-test (panel B) and linear regression used for correlation analyses with r^2 and p values shown (panel C). *p<0.05, **p<0.01.

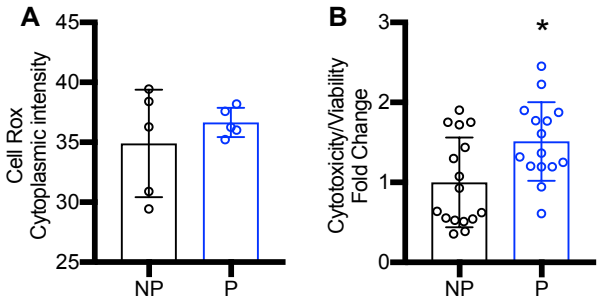
Supplemental figure 10: Parent base peak for oxidized cardiolipin species represented in Figure 6. Representative image of parent chromatogram of a db/+ mouse sample showing peaks between m/z values of 790-800.

Supplemental figure 11: Elamipretide treatment improves podocyte number and mitochondrial morphology in Abca1 fl/fl, ob/ob mice.

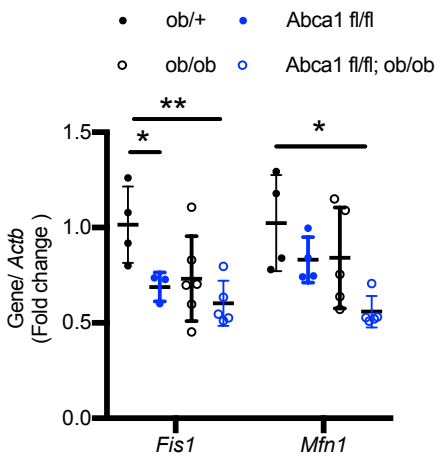
(A) The mesangial expansion score was determined in saline treated ob/ob (n=7), Abca1 fl/fl; ob/ob (n=3) and elamipretide treated Abca1 fl/fl; ob/ob (n=3) mice using PAS staining. **(B)** Representative images of PAS staining are shown (25μm scale bars). **(C)** Bar graph analysis of podocyte number per glomerular cross section (n=4-6 per group) determined by WT1 staining in saline treated ob/+, ob/ob, Abca1 fl/fl; ob/ob and elamipretide treated Abca1 fl/fl; ob/ob mice. **(D)** Representative TEM images of Abca1 fl/fl; ob/ob and elamipretide treated Abca1 fl/fl; ob/ob mice showing mitochondria (yellow asterisk) in podocyte cell body (PC) (original magnification 80,000X, 200nm scale bar). One-way ANOVA followed by Tukey's test *$p<0.05$, ***$p<0.001$, ****$p<0.0001$.

Supplemental figure 12: Parent base peak for oxidized cardiolipin species represented in Figure 7K. Representative image of parent chromatogram of db/+ mouse sample showing peaks between m/z values of 790-800.

Supplemental Figure 1



Supplemental Figure 2



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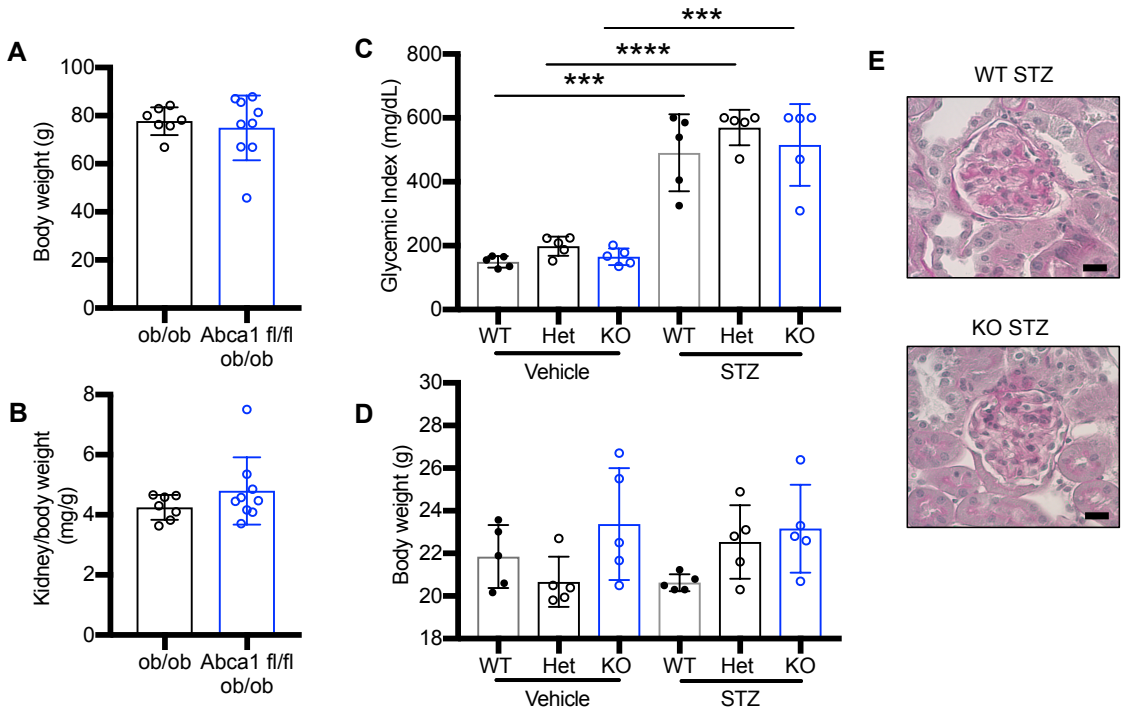

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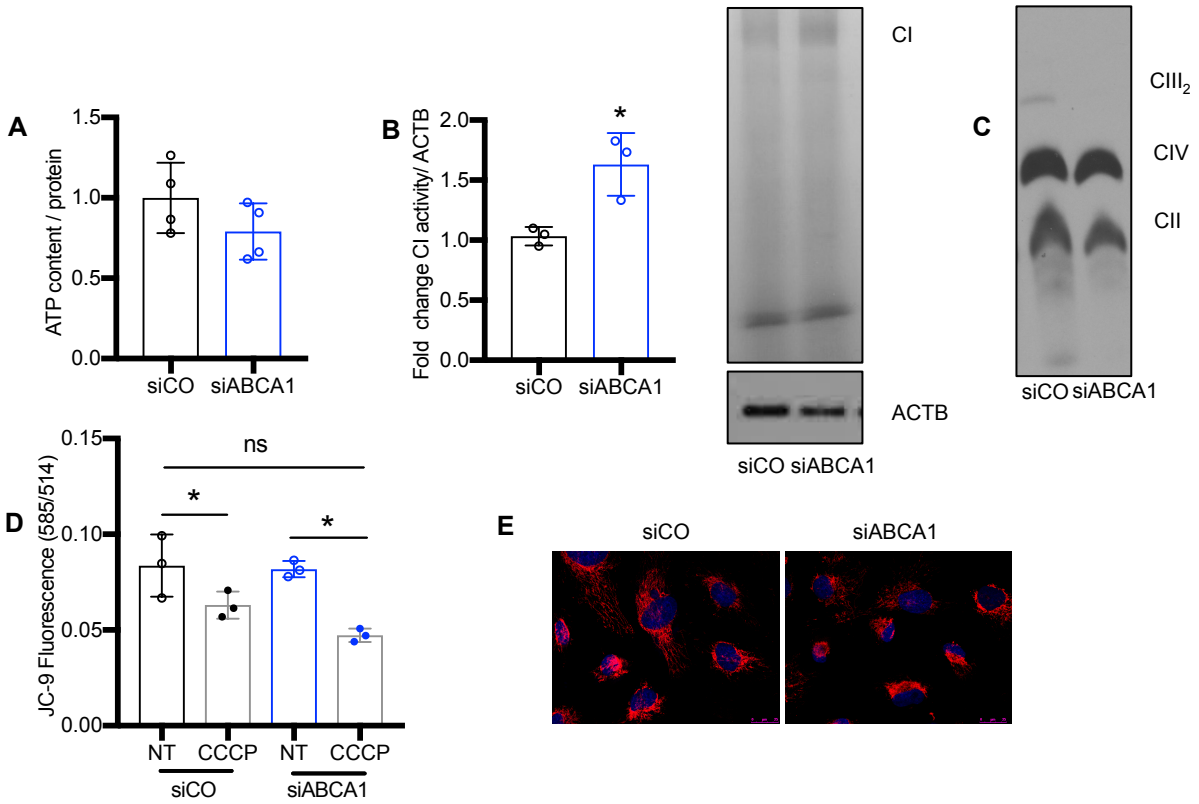
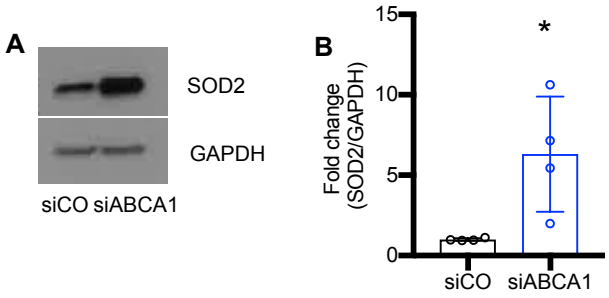
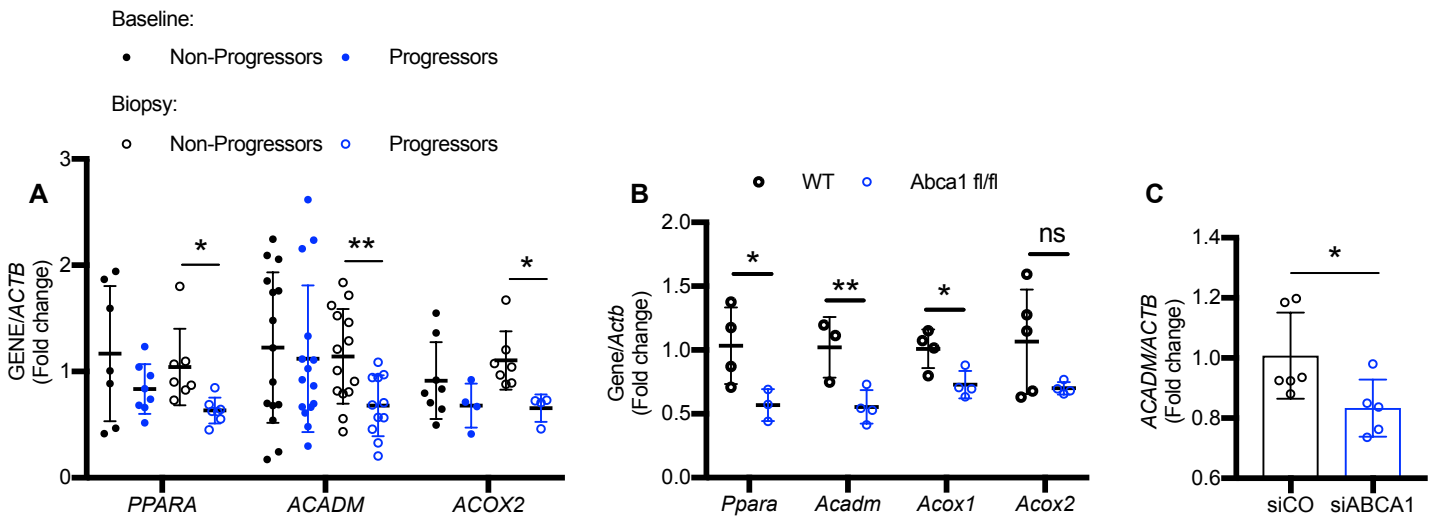


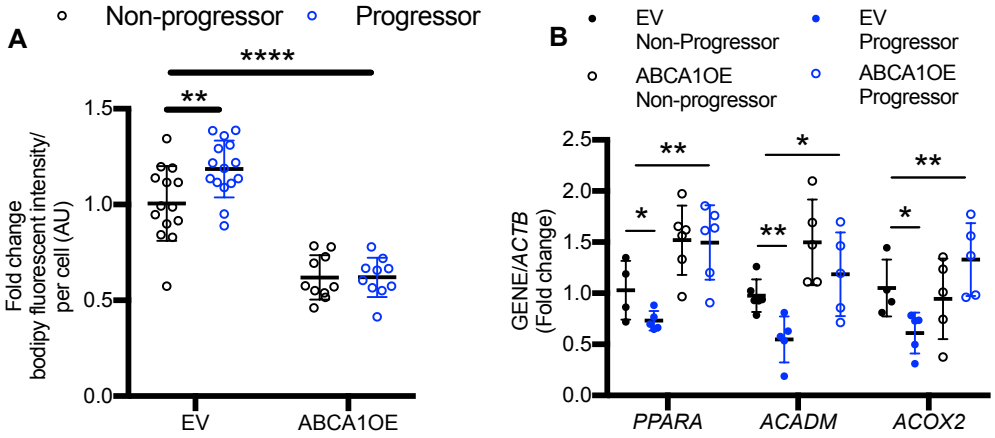
Supplemental Figure 5



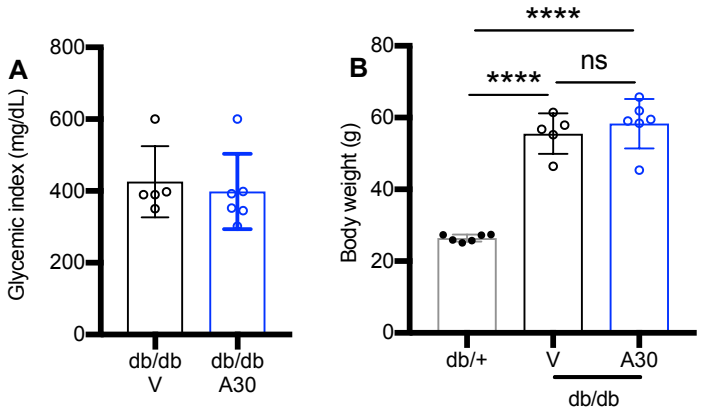
Supplemental Figure 6



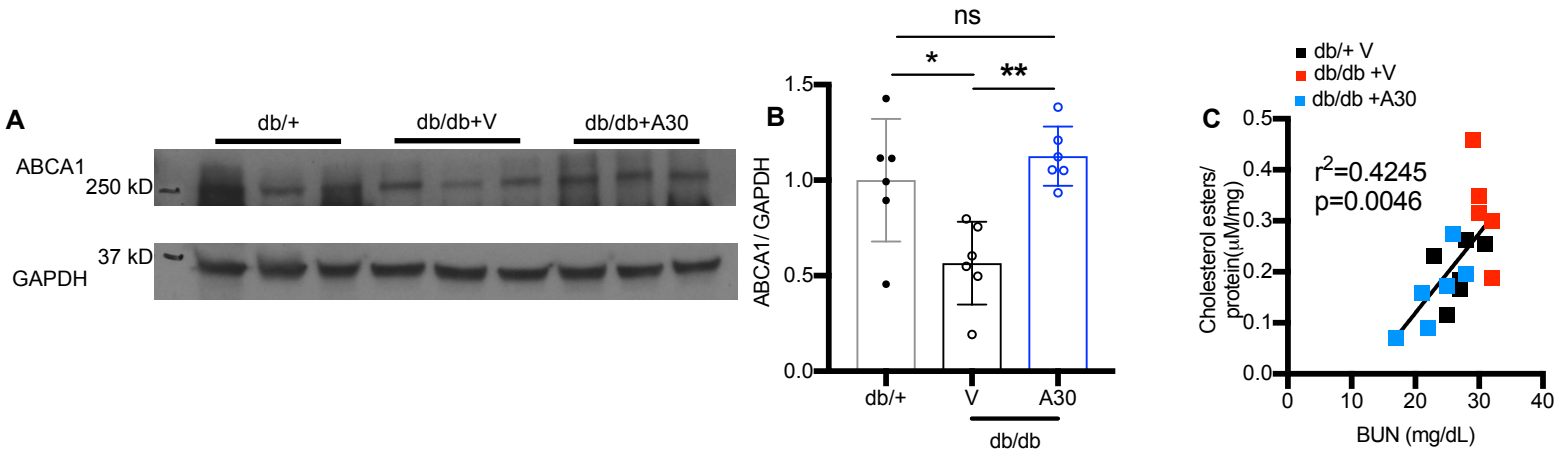
Supplemental Figure 7



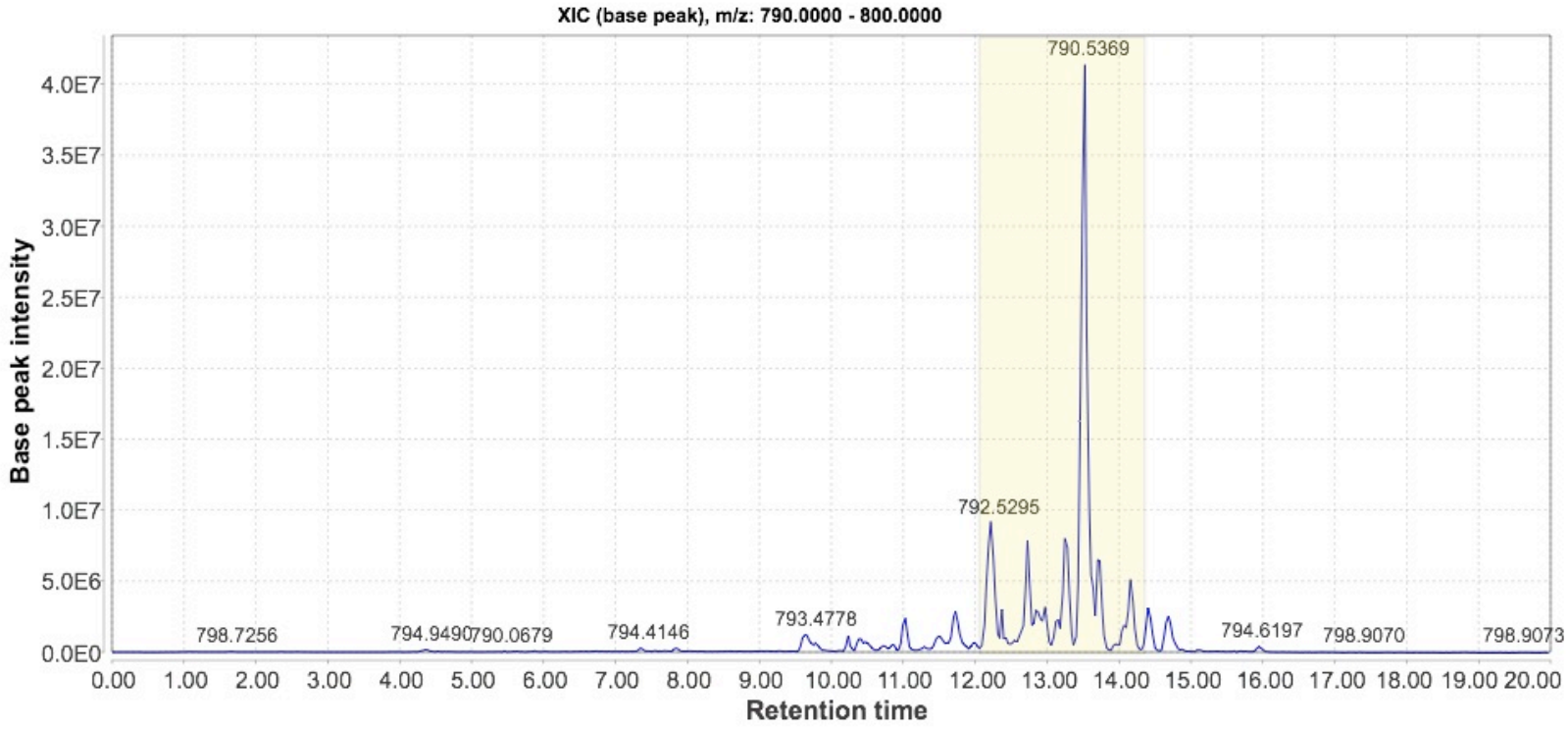
Supplemental Figure 8



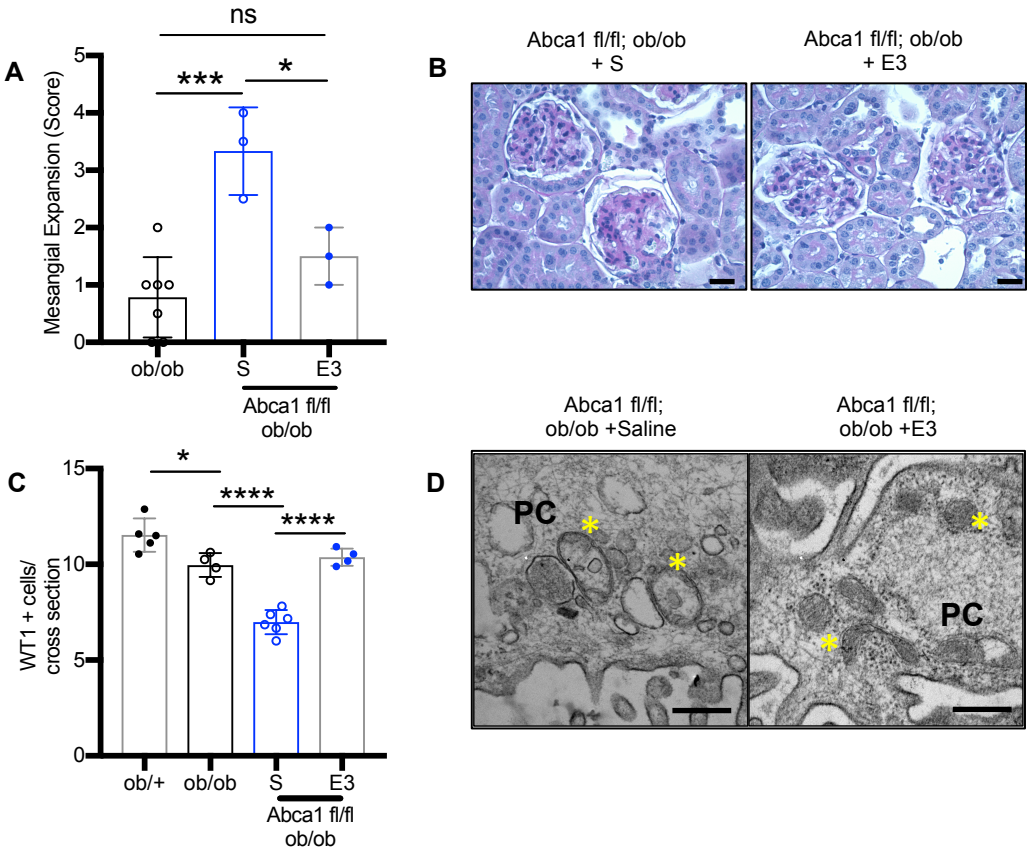
Supplemental Figure 9



Supplemental Figure 10



Supplemental Figure 11



Supplemental Figure 12