SI Appendix

Supplementary methods

Animals

Both male *db/db* and *db/m*$^{+}$ mice were supplied by the Laboratory Animal Center, The Chinese University of Hong Kong (CUHK). Twelve-week old male *db/db* and *db/m*$^{+}$ mice were used. The body weights for all *db/m*$^{+}$ mice and *db/db* mice were similar. Obese mice were induced by 12-week feeding of male C57BL/6J mice (6 weeks old) with a high-fat rodent diet containing 45% kcal% fat (D12451; Research Diets Inc., New Brunswick, NJ). Obese mice had the similar body weight at the time of sacrifice. All animal protocols were approved by the CUHK Animal Experimentation Ethics Committee and in compliance with the Guide for the Care and Use of Laboratory Animals (NIH Publication Eighth Edition, updated 2011). For *in vivo* studies, endothelial function was measured 2 days after tail intravenous injection of *db/m*$^{+}$ SExo or *db/db* SExos to *db/m*$^{+}$ mice. For the *ex vivo* studies, after mice were sacrificed by CO_2 inhalation, aortas and mesenteric arteries were dissected out for functional and molecular assays while serum was used for preparation of exosomes.

Endothelial cell culture

Primary mouse endothelial cells (MAECs) and primary rat endothelial cells (RAECs) were cultured as reported (1, 2). Porcine coronary artery endothelial cells (PCECs) were cultured from pig left ascending coronary arteries as previously described (3). H5V mouse endothelial cell line, human umbilical vein endothelial cell (HUVEC), and bovine aortic endothelial cell (BAEC) were purchased from American Type Culture Collection. H5Vcells were cultured in Dulbecco's Modified Eagle's Media (DMEM, Gibco, USA), HUVEC and BAEC were cultured in 20% FBS-containing endothelial cell growth medium (EGM, Lonza, USA).

Human blood samples

The study design was approved by the Chinese University of Hong Kona-New Territories East Cluster Clinical Research Ethics Committee (CREC Ref. No: 2013.304) and by the Beijing Anzhen Hospital Medical Ethics Committee (No: 2017005). Written informed consent was obtained from all participants. All clinical samples and clinical information were collected from Wales Hospital, the teaching hospital affiliated to the Chinese University of Hong Kong and Beijing Anzhen Hospital, the teaching hospital affiliated to Capital Medical University (Table S2). All the participants underwent baseline anthropometric and blood glucose assessments using standard procedures. All fasting blood samples for serum extracellular microvesicles (SExos) purification were collected using standard serum draw without anticoagulation. Individuals invited to participate as the diabetes group (n=6) were according to the criteria: a known diagnosis of diabetes, with fasting blood glucose ≥ 7 mmol/L, or 2-hour plasma glucose during 75 g oral glucose tolerance test of≥ 11.1mmol/ L, or being on glucose-lowering treatment for diabetes. The inclusion criteria of the non-diabetes group: the control subjects had fasting blood glucose <6 mmol/L (n=6). All the participants were male. The exclusion criteria for diabetes or non-diabetes group: the subjects with hypertension, hyperlipidemia, cancer and infection, stroke and coronary heart disease, and those who could not give a complete medical history were excluded.

Serum exosome isolation and identification

The mouse or human blood was collected in 1.5-mL tubes and allowed to clot for 1 hour at 37 ˚C without anticoagulation. Thereafter, it was centrifuged at 2,000×g for 10 minutes to obtain serum. The serum was next centrifuged at 3,000×g for 10 minutes. The supernatant was diluted in sterile PBS in a 1:1 ratio and centrifuged again at 10,000×g for 30 minutes followed by 2-hour ultracentrifugation at 200,000×g (Hitachi CS-150GXII Micro Ultracentrifuge). The pellet was washed in a large volume of PBS, filtered through a 0.2-μm syringe filter and centrifuged at 200,000×g for 1 hour (4). The pellet was then collected and re-suspended in PBS or culture medium for later functional or biochemical assay. All centrifugations were performed at 4 ˚C. After isolation, mouse SExos were assessed by transmission

electron microscopy (TEM) and Delsa Nano C particle analyzer (Beckman-Coulter). Negative staining was carried out after SExos were isolated. The exosomes were placed on a Formvar carbon-coated copper grid and stained for 60 seconds by adding an equal volume of 2% (w/v) uranyl acetate. The grid was then viewed under a FEI Tecnai 20 lectron microscope. Electron microscope analysis of whole-mounted exosomes was carried out according to the reported protocol (5). Briefly, the SExo pellets were fixed with 2% PFA and deposited onto EM grids. The grids were separately transferred into 1% glutaraldehyde and methyl cellulose-UA for 10 mins. After air dry, the grid was viewed under FEI Tecnai lectron microscope. The sizes of the isolated exosomes were determined by Delsa Nano C particle analyzer. The distribution of exosome intensity according to diameters was calculated. The concentration of SEMV was analyzed by NanoSight NS300 (Malvern Instruments, UK).

Western blotting and silver staining

Exosomes or exosome-free serum was suspended in 1×RIPA butter and protein concentrations were determined by BCA method. 5×SDS loading buffer was added to each sample and the mixture was denatured for 5 minutes at 95˚C. 5 μg of protein from each sample was first separated on SDS-PAGE and then transferred to a PVDF membrane. The membrane was incubated with antibodies and detected by ECL system. Primary antibodies of CD63 and CD81 (exosome markers) were purchased from System Biosciences (California, USA); primary antibody against Arg1 was from Abcam (Cambridge, UK) and primary antibodies against Apo-A2, Apo-C3 and Clusterin were from Santa Cruz (Texas, USA). Secondary antibodies were from Dako (California, USA). Silver staining was used as loading control. Briefly, the gel was fixed for 20 minutes in 50% methanol plus 50% acetic acid followed by sensitizing for 20 minutes in 0.02% sodium thiosulfate. Thereafter, the gel was immersed for 20 minutes in silver reaction buffer (0.1% silver nitrate in 0.08% formalin) and finally developed with 2% sodium carbonate in 0.04% formalin.

Immunofluorescence staining, fluorescent labeling of SExos and confocal microscopy

Mouse aortas or MAECs were fixed, blocked and incubated with the indicated primary antibodies (Anti-HA, anti-myc (1:1000,Sigma, USA)); Anti-Arg1, anti-Rab5, and anti-Caveolin-1 (1:100, Abcam, Cambridge, UK) at 4°C overnight, then the samples were incubated with fluorescein-conjugated secondary antibodies (1:100 dilutions) for 1 hour at room temperature. The slides then were washed and covered with mounting medium. SExos were labeled with fluorescent dye PKH67 (Sigma, USA, Cat# MINI67) according to the manufacturer's instruction with minor modifications. Briefly, the exosome pellet was gently re-suspended in 300 μl Diluent C and then in 2 μl PKH67 (10 μM). After 2-minute incubation at room temperature, 300 μl exosome-free serum was added to stop the reaction. After dilution with 1 mL PBS, the sample was centrifuged at 100,000g for 1 hour at 4˚C. The pellet was washed in 300-μl PBS and again centrifuged at 100,000g for 1 hour. Finally, the pellet was re-suspended in serum free-culture medium in which mouse aortas were submerged. After the incubation, aortas were cut open, washed, fixed, and visualized under confocal microscope. The nuclei of endothelial cells were stained in blue with DAPI (excitation wavelength: 405 nm) and exosomes were stained in green with PKH67 (excitation wavelength: 488 nm). Confocal microscopic images were captured with Olympus Fluoview 1000 (FV1000, Olympus, Tokyo, Japan) and analyzed with Olympus Fluoview Version 1.5 (FV1000, Olympus, Tokyo, Japan).

Vascular functional study

SExos from 1mL mouse blood were suspended in 1 mL DMEM (Gibco, Gaithersberg, MD, USA) supplemented with 10% exosome-free FBS (Exo-FBS™ Exosome-depleted FBS, System Biosciences) together with 100 IU/mL penicillin and 100 μg/mL streptomycin during culture of mouse aortas. The volume of serum is the same in all the experiments using SExo-free serum as a negative control or SExo-containing serum. After incubation for 24-48 hours, aortas were removed and suspended in wire myograph (Danish Myo Technology, Aarhus, Denmark) for recording of changes in isometric force. Once phenylephrine (Phe, 1 μmol/L) induced a stable tension, acetylcholine (ACh) (3 nmol/L-10 μmol/L) was added cumulatively to evoke endothelium-dependent relaxations.

For flow-mediated dilatation (FMD) assay, segments of second-order resistance mesenteric arteries were dissected in sterilized PBS and cultured in DMEM for 48 hours. Thereafter, each vessel was cannulated between two glass cannulas onto pressure myograph. The vessel diameter was recorded using Zeiss Axiovert 40 microscope (model 110P) aided with video camera (Danish Myo Technology, Aarhus, Denmark)(6). Phe (10 μmol/L) was used to produce steady constriction in the artery stabilized at 80 mmHg intraluminal pressure; and FMD was triggered by pressure change that equals ~15 dynes/cm^2 shear stress. By the end of each experiment, perfusion solution was switched to a Ca^{2+}-free, EGTA (2 mmol/L)-containing Krebs solution to induce maximum passive dilatation. FMD was presented as % of diameter changes: (flow-mediated dilatation - Phe tone)/(passive dilatation - Phe tone).

Elimination assay for exosomal RNAs or proteins

SExos were re-suspended in exosome-free medium for the following processing. (1) To remove RNAs from SExos, the medium underwent five freeze-thaw cycles (-170˚C ~37˚C) and was then treated for 1 hour with RNase A (Takara, 10 μg/mL, 37˚C), followed by 1-hour incubation with RNase A inhibitor (Takara, 2,000 units/mL, 37˚C) to inactiv ate RNase A (the procedure marked in purple arrow, Figure 3A). A portion of this medium was additionally exposed for 2 hours to proteinase (Sigma, 0.5 mg/mL, 37˚C) to degrade proteins to yield a medium free of exosomal RNAs and proteins (the procedure marked in red arrow, Figure 3A). (2) To remove proteins, the medium underwent five freeze-thaw cycles (-170˚C ~37˚C) and was then treated for 2 hours with proteinase to obtain a medium containing only exosomal RNAs (the procedure marked in green arrow, Figure 3A). (3) To inactivate proteins, the medium underwent ten freeze-thaw cycles (-170˚C ~100˚C) to harvest a medium free of active proteins (the procedure marked in blue arrow). A portion of this medium was additionally treated for 1 hour with RNase A (Takara, 10 μg/mL, 37˚C) to remove RNAs followed by 1-hour incubation with RNase A inhibitor (Takara, 2,000 units/mL, 37˚C) to obtain a medium free of active proteins and RNAs (the procedure marked in gray arrow).

qPCR detection of mRNAs in endothelial cells or microRNAs in SExos

After dissection, mouse aortas were cleaned of adventitial adipose tissue and the lumen was quicklyflushed with 150 μL QIAzol lysis reagent (QIAGEN) twice using a 23g syringe connected to a microfuge tube. The intimal RNAs were isolated from the eluate using RNeasy mini kit (QIAGEN) as instructed by the manufacturer. The remaining aortas (media plus adventitia) after luminal flushing were homogenized and RNAs were isolated. The RNAs were reverse transcribed and amplified using SuperScrip III one-step RT-PCR kit (Invitrogen). qPCR was performed to detect the mRNA expression for VE-cadherin, α-SMA, Arg1, Arg2 and GAPDH (internal control) using SYBR® Green Real-Time PCR master Mixes (Life Technology, USA). Each reaction was performed in triplicate. Primers for mouse Arg1 were sense: 5'ATGGGCAACCTGTGTCCTTT3', antisense: 5' TCTACGTCTCGCAAGCCAAT3'; primers for mouse Arg2 were sense: 5' TCCTTGCGTCCTGACGAG3', antisense:5'AGGGATCATCTTGTGGGACA3'; primers for mouse α-SMA were sense: 5' AGACTCTCTTCCAGCCATCT 3', antisense: 5' CCTGACAGGACGTTGTTAGC3'; primers for mouse VE-cadherin were sense: 5' ATTGGCCTGTGTTTTCGCAC3', antisense: 5'CACAGTGG GGTCATCTGCAT3'; and primers for mouse GAPDH were sense: 5'GATGCCCCCATGTTTGTGAT3', antisense: 5'GGTCATGAGCCCTTCCACAAT 3'. Primers for human Arg1 were sense: 5'GGCAGAAGTCAAGAAGAACGG3', antisense: 5' CCAGAGATGCTTCCAATTGCC 3'. Primers for human GAPDH were sense: 5' TGAAGGTCGGAGTCAACGG3', antisense: 5' CCTGGAAGATGGTGATGGG3'. SExo miRNA in conditional medium was extracted using mirVana™ miRNA Isolation Kit (Ambion, USA). MiRNA expression levels were determined by Applied Biosystems Taqman miRNA Assay system as described previously (7).Reactions were carried out in ABI ViiA7 system (Applied Biosystems Carlsbad, CA, USA). Primer identification catalog numbers were: 002245 for mmu-miR-122-5p; 000397 formmu-miR-21a-3p; 000430mmu-miR-92a-3p; 002228 for mmu-miR-126a-3p and 002295for mmu-miR-223-3p (Applied Biosystems, Carlsbad, CA, USA). Each miRNA level was compared to the one in R&P fractions.

Arginine and ornithine detection by LC-MS/MS

H5V cells (endothelial cell line) were treated with *db/db* SExos or *db/m*$^+$ SExos for 48 hours and then washed with PBS twice before measurement of arginine by

HPLC. The samples were extracted with 1 mL 80% of methanol (methanol/H2O (v/v) = 80/20), and followed by repeated freeze-thaw for 5 cycles. After centrifugation at 14,200g for 10 min (4 °C), the supernatant was transferred to a new 1.5 mL micro-centrifuge tube. The sample alone with appropriate concentrations of internal standards (4-Chlorophenylalanine) were evaporated to dryness, and were then reconstituted in 200 µL 40% of methanol /water (v/v = 40:60). Arginine and ornithine were analyzed on Ultimate 3000 rapid separation liquid chromatography coupled with TSQ Quantiva triple quadrupole mass spectrometry (MS). The chromatographic separation was performed on UPLC BEH amide column (Bridged ethylene hybrid, 2.1 mm × 100 mm, 1.7µm, Waters) with a BEH amide guard column (2.1 mm × 20 mm, 1.7µm, Waters) . The mobile phases were 0.1% formic acid (FA) in water (A) with 10 mm ammonium acetate and 0.2% FA in 95 % acetonitrile (B) (acetonitrile/H_2O (v/v) = 95/5) with 10mm ammonium acetate. The flow rate was 300 µL min^{-1}. The gradient program began at 15 % A, increased to 55 % A at 6 min, ramped to 90 % A at 7 min, and kept at 90 % A for 2 min. After each run the column was equilibrated for 5 min. The injection volume was 10 µL. The MS parameters of Quantiva were described previously(8).

***In situ* hybridization**

In situ hybridization was performed as previously described(9). Briefly, an Arg1 fragment was cloned into pGEM-T Easy vector using primers, FW: 5'aaaactgcagcagaggtccagaagaatgg3'; RE: 5'acatgcatgcgtaagataggcctcccagaac3'. Sense and antisense probes were then generated by *in vitro* transcription with Dig RNA labeling kit (Roche, 11175025910). After post-fixation with 4% PFA, cryostat sections at 5 µm were acetylated, permeated, pre-hybridized and then incubated with hybridization buffer containing 4 µg/mL sense or antisense probes at 55°C overnight. After stringent washes and RNase treatment, the sections were blocked and incubated with anti-DIG-alkaline phosphatase antibody (1:2000) (Abcam, ab119345) in blocking buffer at 4℃ overnight. The sections then were incubated with alkaline phosphatase substrates NBT/BCIP (Roche, 1383221/1383213) for appropriate time to develop the Arg1 mRNA signal. The images were captured by Spot digital camera and Leica AS LMD microscope.

Protein digestion, iTRAQ labeling, LC-MS/MS analysis, protein identification and quantification analysis.

Quantitative proteomic analysis was performed after purifying serum exosomes (SExos) from *db/m*$^+$ and *db/db* mice. The proteins extracted from SExos were digested with trypsin, labeled by iTRAQ, and then analyzed by nano LC-MS/MS. To obtain reliable results, two biological replicates and two technical replicates were carried out. Proteins from *db/m*$^+$ SExos were labeled with iTRAQ reagents, 114 or 116, and proteins from *db/db* SExos were labeled with iTRAQ reagents, 115 or 117. The samples for iTRAQ quantitative analysis was prepared according to iTRAQ™ Reagents Protocol (Applied Biosystems, USA) with little modification. In brief, 10 µg SExo protein precipitated by acetone was dissolved in 10 µL 8 mol/L urea (pH 7.5) plus 10 µL Dissolution Buffer. Then, 2 µL Reducing Reagent was added and incubated at 37°C for 1 hour, and subsequently 1 µL Cysteine Blocking Reagent was added to each sample at room temperature for 10 minutes. Protein digestion by trypsin (Invitrogen, USA) (0.3 µg trypsin to digest 10 µg protein) was carried out after adding Dissolution Buffer (75 µl). After incubation at 37℃ overnight, the trypsin digestion process was terminated at -20°C for 30 minutes. The protein digestions were dried in a centrifugal vacuum concentrator, reconstituted in 20 µl Dissolution Buffer plus 70 µl ethanol, then transferred to one iTRAQ™ Reagent vial and incubated at room temperature for 2 hours. After that, all the iTRAQ™ Reagent-labeled tryptic peptides were combined into one tube, dried in a centrifugal vacuum concentrator, and diluted in 200 µl 0.5% formic acid (FA). 50 µl FA-diluted peptides (about 10 ug) were desalted using C18 ZipTip cleanup (Millipore, USA) according to the manufacturer's instruction. Biefly, ZipTip was equilibrated in 100% acetonitrile and 0.1% formic acid 3 times. Thereafter, the peptide sample was loaded on the ZipTip by pipetting the protein digest up and down for 10 times. Then, ZipTip was washed five times in 0.1% formic acid, and the peptides were eluted with 40% /0.1% formic acid and 60% acetonitrile/0.1% formic acid for 3 times. Finally, the sample was dried by a centrifugal vacuum concentrator and diluted in 15 µl 0.5% FA for the LC-MS/MS analysis.

All nano LC-MS/MS experiments were performed on a Q Exactive (Thermo Scientific, USA) equipped with an Easy n-LC 1,000 HPLC system (Thermo Scientific, USA). The labeled peptides were loaded onto a 100 µm id×2 cm fused silica trap column packed in -house with reversed phase silica (Reprosil-Pur C18 AQ, 5 µm, Dr. Maisch GmbH) and then separated on an a 75 µm id×20 cm C18 column packed with reversed phase silica (Reprosil -Pur C18 AQ, 3 µm, Dr. Maisch

GmbH). The peptides bounded on the column were eluted with a 78-minute linear gradient. The solvent A consisted of 0.1% FA in water and the solvent B consisted of 0.1% FA in acetonitrile solution. The segmented gradient was 5–8% B, 8 minutes; 8–22% B, 50 minutes; 22–32% B, 12 minutes; 32-95% B, 1 minute; 95% B, 7 minutes at a flow rate of 280 nl/min.

The MS analysis was performed with Q Exactive mass spectrometer (Thermo Scientific). In a data-dependent acquisition mode, the MS data were acquired at a high resolution 70,000 (m/z 200) across the mass range of 300–1600 m/z. The target value was 3.00E+06 with a maximum injection time of 60 ms. The top 20 precursor ions were selected from each MS full scan with isolation width of 2 m/z for fragmentation in the HCD collision cell with normalized collision energy of 27%. Subsequently, MS/MS spectra were acquired at resolution 17,500 at m/z 200. The target value was 5.00E+04 with a maximum injection time of 80 ms. The dynamic exclusion time was 40 s. For nano electrospray ion source setting, the spray voltage was 2.0 kV; no sheath gas flow; the heated capillary temperature was 320°C. For each analysis, 2 μg of peptides was injected and each sample was measured in duplicate.

The raw data from Q Exactive were analyzed with Proteome Discovery version 1.4 using Sequest HT search engine for protein identification and Percolator for FDR (false discovery rate) analysis. The Uniprot mouse database (updated on 05 - 2015) was individually used for searching the data from mouse sample. Some important searching parameters were set as followings: trypsin was selected as enzyme and one missed cleavages were allowed for searching; the mass tolerance of precursor was set as 10 ppm and the product ions tolerance was 0.02 Da.; MMTS was set as a fixed modification of cysteine and methionine oxidation and iTRAQ 4 plex labeled lysine and N-terminus of peptides were specified as variable modifications. FDR analysis was performed with Percolator and FDR < 1% was set for protein identification. The high peptides confidence was set for peptides filtering.

Proteins quantification was also performed by Proteome Discovery (version 1.4) using the ratio of the intensity of reporter ions from the MS/MS spectra. Only unique peptides of proteins or protein groups were selected for protein relative quantification. The *db/m*$^{+}$ SExos from two groups labeled with tag 113 and 115 were taken as control reference for calculating the ratios of 114:113 and 116:115, in which the *db/db* SExos from the two groups were labeled with tags 115 and 116, respectively.

All ratios were transformed to base 2 logarithm values. A 95% confidence intervals (z score = 1.96) were used to determine the cutoff values for significant changes. Normalization to the protein median of each sample was used to correct experimental bias and the number of minimum protein count must be greater than twenty. The fold change threshold for up- or down-regulation was set as mean±1.960σ.

The mass spectrometry proteomics raw data have been deposited to the ProteomeXchange Consortium via the PRIDE (10) partner repository with the dataset identifier PXD009221.

Adeno-associated virus (AAV) construction

To constructArg1 over-expressing AAV (Arg1 OE), Mouse Arg1 was PCR amplified from the pcDNA3.1-mArg1-Flag (a gift from Peter Murray, Addgene plasmid # 34574). A myc sequence was appended to the reverse primer to replace the flag sequence. The PCR product was then subcloned into pAAV-MCS (Clonetech) to generate pAAV-mArg1. For AAV mediated gene silencing, shRNA sequence targeting mouse Arg1 was adopted from Sigma-Aldrich Mission RNAi (TRCN0000101796). The oligo 5'GATCCGCCTTTGTTGATGTCCCTAATCTCGAGATTAGGGACATCAACAAAGGCTTTTTA3' and 3'AGCTTAAAAAG CCTTTGTTGATGTCCCTAATCTCGAGATTAGGGACATCAACAAAGGCG5' were synthesized, annealed and ligated to the pAAV-ZsGreen -shRNA (YRGene) shuttle vector to construct pAAV-Arg1 shRNA plasmid. AAV was packaged with a standard protocol. Briefly, HEK-293T cell was seeded to 15cm^2 dish at 70% confluence. The next day, pAAV-mArg1, pAAV-Arg1 shRNA or pAAV-control shRNA (4 μg) was co-transfected together with endothelial enhanced RGDLRVS-AAV9 plasmid (4 μg) (a gift from Dr. O.J. Müller, University Hospital Heidelberg, Heidelberg, Germany) and pHelper (6 μg) (Stratagene) respectively. Adeno-associated viral particles were harvested as reported before with modification(11). Briefly, the cells were re-suspended in hypertonic buffer (10 mM HEPES, 1.5 mM $MgCl_2$, 10 mM KCl, 175 mg of spermine) and the nuclei were extracted by homogenization. Viral particles were released by nuclear lysis buffer (0.5% sodium deoxycholate). The viral particles were purified by PEG concentration, followed by dialysis against saline with 100K MWCO dialysis tubing (Spectrum

Labs) to remove impurities, and then concentrated. The viral titration was determined by quantitative realtime PCR and adjusted to 10^{10}/mL in PBS containing 4% sucrose.

Measurement of nitrite and NO

After mouse aortas were exposed to ACh (10 μmol/L for 10 minutes) to stimulate NO generation in the presence of nitrate reductase, aortic tissues were homogenized and nitrite level in the supernatants was measured using a Griess reagent kit (Molecular Probes, Eugene, OR). The results were presented relative to protein content.

For NO measurement, HUVECs (Lonza, San Diego, CA) were incubated in NO-sensitive fluorescent dye 4-amino-5-methylamino-2',7'-difluorofluorescein diacetate (1 μmol/L for 15 minutes, Invitrogen) at 37ºC and then washed for 15 minutes in NPSS (mmol/L:140 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 5 Hepes and 10 D-glucose). Fluorescent signal was measured by FV1000 confocal microscope (Olympus, Tokyo, Japan) at excitation wavelength of 488 nm and emission filter of 505-525 nm. Changes in $[NO]_i$ were displayed as a ratio of fluorescence relative to the intensity before addition of 100 nmol/L A23187 (F1/F0) analyzed by the Fluoview software (Olympus).

Arginase activity assay

Arginase activity in the exosomes was measured by a commercially available kit (Cat. #ab180877; Abcam, Cambridge, UK) in accordance with the manufacturer's instructions. Exosome was isolated from 200 μl serum and dissolved in 100 μl Arginase Assay Buffer provided in the kit, followed by seven cycles of freeze/thaw in liquid nitrogen and 37°C water bath. Exosome lysate was then centrifuged at 10,000xg for 5 minutes. The supernatant was used for measuring arginase activity. All samples were assessed in duplicate and measured at 570 nm every two minutes for 30 min at 37°C using xMARK Microplate Absorbance Spectrophotometer (Bio-Rad, CA, USA).

Fasting blood glucose and intra-peritoneal insulin tolerance test

After 8-h fasting, mouse blood was drawn from the tail and blood glucose was measured using a commercial glucometer (Ascensia ELITE®, Bayer,Mishawaka, IN). For insulin tolerance test, mice were first fasted for 2 h and then received an injection of insulin (0.75 U/kg body weight). Blood glucose was determined at 0, 15, 30, 60, 90 and 120 minutes after insulin administration.

Statistics

Results represent means±SEM of n separate experiments. Concentration-response curves were analyzed by nonlinear regression curve fitting using GraphPad Prism software (Version 4.0). Student's *t*-test (two-tailed) was used when two groups were compared. One-way ANOVA followed by the Bonferroni post hoc test was used when more than two treatments were compared. Protein expression was quantified by Quantity One software (Bio-Rad) and normalized to GAPDH. MicroRNA expression was normalized to snRU6. $P<0.05$ indicates statistical difference between groups.

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Supplementary Tables

Table S1. Exosome proteins from *db/m*$^{+}$ or *db/db* serum identified and quantified by quantitative mass spectrometry†

Accession	Description	Gene symbol	Score	Coverage	Unique Peptides	PSMs	AAs	MW [kDa]	PAF	pI	ratio 1	ratio 2	ratio 3	ratio 4	mean	SD	reported
A2A997	Complement component C8 alpha chain	C8a	178.30	49.48	26	59	580	65.4	0.903	6.24	0.399	0.444	0.407	0.483	0.433	0.038	
A2ATR8	Plasma protease C1 inhibitor	Serping1	10.57	8.65	3	5	347	37.6	0.133	6.04	0.482		0.458		0.470		√
A2BIN0	Major urinary proteins 11 and 8	Mup11	7.58	29.79	2	3	94	10.8	0.278	4.70	0.608		0.585		0.597		
A6X935	Inter alpha-trypsin inhibitor, heavy chain 4	Itih4	159.35	34.08	25	52	942	104.6	0.497	6.40	1.001		0.980		0.990		
A8DUK4	Beta-globin	Hbb-b1	105.16	78.91	11	32	147	15.7	2.033	7.69	0.804	0.805	0.805	0.831	0.811	0.013	√
B2RPV6	Multimerin-1	Mmrn1	66.33	17.93	19	24	1210	136.0	0.176	7.71	1.073	1.163	1.129	1.238	1.151	0.069	√
B7FAV1	Filamin, alpha (Fragment)	Flna	38.64	5.96	13	15	2583	274.5	0.055	5.97	0.686	0.648	0.643	0.679	0.664	0.021	√
D3YW52	Protein Pzp	Pzp	411.10	41.14	59	136	1507	167.2	0.814	6.83	1.000		1.000		1.000		
D3YXF5	Protein C7	C7	212.83	41.89	33	78	845	93.3	0.836	6.38	0.455	0.480	0.503	0.491	0.483	0.020	
D3YY36	Protein 1300017J02Rik	1300017J02Rik	10.98	4.50	2	4	622	68.6	0.058	7.88	1.172		1.254		1.213		
E0CXN0	Hepatocyte growth factor-like protein	Mst1	7.82	4.81	3	3	707	79.7	0.038	7.49	1.012	1.087	0.948	1.078	1.031	0.065	
E0CZ58	Proteoglycan 4	Prg4	5.86	26.78	3	3	1221	134.5	0.022	6.86	0.892		1.105		0.998		√
E9PVS1	Inter-alpha-trypsin inhibitor heavy chain H3	Itih3	34.20	15.31	11	12	699	78.0	0.154	6.84	0.922		0.931		0.927		√
E9PX70	Collagen alpha-1(XII) chain	Col12a1	12.61	1.40	4	4	3064	333.5	0.012	5.80	0.630	0.773	0.685	0.835	0.731	0.091	√
E9Q414	Apolipoprotein B-100	Apob	1230.14	39.80	170	404	4505	509.1	0.794	6.81	0.922	0.895	0.902	0.899	0.905	0.012	√
E9Q5L2	Inter alpha-trypsin inhibitor, heavy chain 4	Itih4	155.03	40.43	30	53	925	102.8	0.516	6.37	1.032		1.026		1.029		√
E9Q6C2	Protein C1s	C1s	238.97	53.17	17	72	694	77.5	0.930	5.12	0.689		0.772		0.731		
E9Q748	Antileukoproteinase	Slpi	10.50	23.36	3	5	107	11.9	0.419	8.85	1.248	1.052	1.231	1.149	1.170	0.089	
E9Q8B5	Protein Gm4788	Gm4788	244.94	36.06	24	84	879	99.4	0.845	7.44	0.596	0.598	0.611	0.584	0.597	0.011	
F6TQW2	Protein Ighg2c	Ighg2c	286.75	35.48	10	79	403	44.1	1.793	6.90	1.446	1.511	1.456	1.470	1.471	0.028	
G3X8T9	Serine (Or cysteine) peptidase inhibitor, clade A, member 3N, isoform CRA_a	Serpina3n	24.17	19.62	4	7	418	46.7	0.150	5.82	1.055	1.106	1.289	1.110	1.140	0.103	√
G3X9T8	Ceruloplasmin	Cp	94.61	28.11	25	32	1060	121.0	0.264	5.85	1.206	1.214	1.219	1.193	1.208	0.011	√
H3BLB8	Paraoxonase 1, isoform CRA_c	Pon1	3.06	9.39	2	2	181	20.2	0.099	5.80	1.242		1.281		1.262		√
H7BX99	Prothrombin	F2	141.50	42.63	25	50	617	70.2	0.713	6.43	1.064		1.181		1.122		√
J3QNZ9	Uncharacterized protein	Igkv4-62	11.17	35.42	2	3	96	10.4	0.288	6.44	1.781	2.406	1.280	2.253	1.930	0.509	

O08538	Angiopoietin-1	Angpt1	38.28	22.09	11	13	498	57.5	0.226	6.76	0.796	0.900	0.871	0.879	0.861	0.045	√
O08677	Kininogen-1	Kng1	88.03	30.56	15	29	661	73.1	0.397	6.54	0.866	0.663	0.908	0.707	0.786	0.119	√
O09173	Homogentisate 1,2-dioxygenase	Hgd	17.01	16.40	7	7	445	49.9	0.140	7.24	1.545	1.898	2.180	1.718	1.835	0.271	√
O54890	Integrin beta-3	Itgb3	9.21	4.32	3	3	787	86.7	0.035	5.24	0.698		0.727		0.712		√
O70165	Ficolin-1	Fcn1	75.67	35.63	12	28	334	36.3	0.772	6.52	0.853	0.804	0.853	0.852	0.841	0.024	√
O70362	Phosphatidylinositol-glycan-specific phospholipase D	Gpld1	6.53	2.27	2	2	837	93.2	0.021	7.12	1.052		0.989		1.021		
O70570	Polymeric immunoglobulin receptor	Pigr	103.45	24.25	19	33	771	84.9	0.388	5.40	2.555	2.409	2.259	2.430	2.413	0.122	√
O88200	C-type lectin domain family 11 member A	Clec11a	7.68	7.93	3	3	328	36.4	0.082	5.00	0.683	0.923	0.668	0.934	0.802	0.146	√
O88783	Coagulation factor V	F5	161.71	16.90	33	53	2183	247.1	0.215	6.05	1.256	1.299	1.271	1.227	1.263	0.030	√
O88947	Coagulation factor X	F10	18.40	10.19	5	6	481	54.0	0.111	5.66	1.100	1.081	1.058	1.110	1.087	0.023	
P00687	Alpha-amylase 1	Amy1	13.29	12.13	6	6	511	57.6	0.104	6.96	1.473		1.461		1.467		√
P01027	Complement C3	C3	2571.98	80.40	133	856	1663	186.4	4.593	6.73	1.059	1.054	1.087	1.064	1.066	0.015	√
P01029	Complement C4-B	C4b	680.30	54.55	88	237	1738	192.8	1.229	7.53	0.885	0.837	0.841	0.888	0.863	0.027	√
P01592	Immunoglobulin J chain	Igj	59.02	64.78	8	24	159	18.0	1.333	4.89	1.089	1.050	0.944	1.107	1.048	0.073	√
P01630	Ig kappa chain V-II region 7S34.1		12.86	21.24	2	4	113	12.5	0.320	8.65	1.446	0.843	1.726	1.222	1.309	0.373	√
P01631	Ig kappa chain V-II region 26-10		32.75	38.94	3	12	113	12.3	0.978	8.88	1.563	1.442	1.706	1.460	1.543	0.121	√
P01633	Ig kappa chain V19-17	Igk-V19-17	12.18	24.83	3	8	149	16.4	0.487	6.92	1.133	1.112	1.208	1.035	1.122	0.071	√
P01634	Ig kappa chain V-V region MOPC 21		8.62	22.79	2	5	136	14.9	0.336	6.76	1.357		1.394		1.376		√
P01635	Ig kappa chain V-V region K2 (Fragment)		30.07	40.87	4	9	115	12.6	0.716	8.31	1.330	1.315	1.195	1.315	1.289	0.063	√
P01636	Ig kappa chain V-V region MOPC 149		34.00	35.19	3	9	108	12.0	0.749	7.28	1.353	1.175	1.606	1.345	1.370	0.177	√
P01638	Ig kappa chain V-V region L6 (Fragment)		49.74	40.87	4	13	115	13.0	1.002	7.81	1.251	0.975	1.144	1.013	1.096	0.126	√
P01639	Ig kappa chain V-V region MOPC 41	Gm5571	21.54	31.54	3	7	130	14.3	0.489	5.48	0.628	0.788	0.808	0.662	0.722	0.090	√
P01642	Ig kappa chain V-V region L7 (Fragment)	Gm10881	9.76	28.70	3	9	115	12.6	0.714	5.94	0.942	1.247	1.162	1.308	1.165	0.160	√
P01644	Ig kappa chain V-V region HP R16.7		41.21	66.67	2	13	108	11.9	1.092	7.97	1.114	1.069	1.132	1.010	1.081	0.054	√
P01649	Ig kappa chain V-V regions		12.35	32.41	2	5	108	12.0	0.415	7.96	0.664	0.592	0.679	0.580	0.629	0.050	√
P01654	Ig kappa chain V-III region PC 2880/PC 1229		44.92	96.40	4	11	111	12.0	0.919	5.34	1.538	1.773	1.270	1.397	1.495	0.215	√
P01665	Ig kappa chain V-III region PC 7043		53.01	53.15	3	12	111	12.0	1.000	4.61	0.798	0.781	0.790	0.831	0.800	0.022	√
P01670	Ig kappa chain V-III region PC 6684		38.67	93.69	2	11	111	12.0	0.914	8.00	1.132		1.295		1.214		√

P01675	Ig kappa chain V-VI region XRPC 44		34.48	37.38	3	10	107	11.6	0.861	8.44	0.752	0.802	0.809	0.832	0.799	0.034	√
P01680	Ig kappa chain V-IV region S107B		5.88	13.18	2	3	129	13.8	0.217	8.47	0.697		0.780		0.738		√
P01723	Ig lambda-1 chain V region		13.21	35.90	2	3	117	12.2	0.246	5.21	1.404		1.126		1.265		√
P01747	Ig heavy chain V region 36-65		16.32	17.50	2	6	120	13.3	0.451	9.14	1.139	1.206	1.137	1.041	1.131	0.068	√
P01749	Ig heavy chain V region 3	Igh-VJ558	20.26	49.57	3	6	117	13.0	0.461	7.87	1.432	1.556	1.507	1.467	1.490	0.053	√
P01750	Ig heavy chain V region 102		27.70	40.17	2	11	117	12.9	0.855	8.60	0.805	0.813	0.785	0.895	0.824	0.048	√
P01790	Ig heavy chain V region M511		26.20	31.97	3	7	122	13.6	0.513	7.93	1.494	1.234	1.294	1.310	1.333	0.112	√
P01796	Ig heavy chain V-III region A4		20.22	34.51	2	6	113	12.7	0.474	7.28	1.476	1.278	1.593	1.558	1.476	0.141	√
P01806	Ig heavy chain V region 441		20.68	31.03	5	9	116	12.9	0.698	8.27	1.318	1.400	1.312	1.447	1.369	0.065	√
P01837	Ig kappa chain C region		208.52	54.72	7	83	106	11.8	7.051	5.41	1.223	1.206	1.168	1.177	1.193	0.025	√
P01843	Ig lambda-1 chain C region		81.49	61.90	4	23	105	11.6	1.988	6.27	1.349	1.398	1.578	1.383	1.427	0.103	√
P01844	Ig lambda-2 chain C region	Iglc2	48.34	63.46	4	13	104	11.2	1.156	6.27	0.811	0.770	0.771	0.912	0.816	0.067	√
P01867-2	Isoform 2 of Ig gamma-2B chain C region	Igh-3	155.17	42.69	12	47	335	36.6	1.285	7.36	1.227	1.089	1.081	1.119	1.129	0.067	
P01868	Ig gamma-1 chain C region secreted form	Ighg1	88.78	44.75	10	23	324	35.7	0.645	7.40	1.096	0.970	1.088	1.024	1.044	0.059	√
P01872	Ig mu chain C region secreted form	Igh-6	300.79	52.42	29	113	454	49.9	2.263	7.01	1.172	1.157	1.175	1.143	1.162	0.015	√
P01878	Ig alpha chain C region		150.59	40.41	13	45	344	36.9	1.221	5.06	1.039	1.128	1.142	1.092	1.100	0.046	
P01887	Beta-2-microglobulin	B2m	7.22	23.53	4	5	119	13.8	0.363	8.44	1.426	0.886	1.247	0.996	1.139	0.244	√
P01898	H-2 class I histocompatibility antigen, Q10 alpha chain	H2-Q10	89.17	43.69	14	32	325	37.2	0.860	5.25	1.254	1.403	1.360	1.427	1.361	0.076	
P03987-2	Isoform 2 of Ig gamma-3 chain C region		157.04	39.51	11	49	329	36.2	1.353	8.27	1.077	1.091	1.078	1.105	1.088	0.013	
P04186	Complement factor B	Cfb	105.99	29.57	26	46	761	85.0	0.541	7.37	1.104	1.083	1.099	1.103	1.097	0.010	√
P04202	Transforming growth factor beta-1	Tgfb1	7.74	6.92	3	3	390	44.3	0.068	8.62	0.951	0.851	0.837	0.892	0.883	0.051	√
P04940	Ig kappa chain V-VI region NQ2-17.4.1		7.81	14.95	2	4	107	11.6	0.346	9.36	0.913		0.868		0.890		√
P05366	Serum amyloid A-1 protein	Saa1	7.15	18.03	2	2	122	13.8	0.145	7.03	0.756	0.811	0.826	0.812	0.801	0.031	√
P06330	Ig heavy chain V region AC38 205.12		91.02	72.03	7	27	118	12.9	2.089	7.11	0.821	0.785	0.862	0.762	0.807	0.044	√
P06683	Complement component C9	C9	91.89	34.31	19	36	548	62.0	0.581	5.78	0.626	0.577	0.602	0.579	0.596	0.023	√
P06684	Complement C5	C5	818.99	57.98	87	274	1680	188.8	1.452	6.81	0.661	0.629	0.664	0.671	0.656	0.019	√
P06728	Apolipoprotein A-IV	Apoa4	65.90	37.22	13	19	395	45.0	0.422	5.47	0.553	0.596	0.546	0.612	0.577	0.032	√
P06909	Complement factor H	Cfh	1224.35	65.32	67	405	1234	139.0	2.913	6.99	0.870	0.865	0.889	0.885	0.877	0.011	√
P07309	Transthyretin	Ttr	36.46	38.78	6	12	147	15.8	0.761	6.16	1.014	1.168	1.103	1.140	1.106	0.067	√

P07724	Serum albumin	Alb	340.43	72.86	40	118	608	68.6	1.719	6.07	1.214	1.143	1.154	1.129	1.160	0.037	√
P07758	Alpha-1-antitrypsin 1-1	Serpina1a	67.98	29.78	3	25	413	46.0	0.544	5.72	0.747	0.618	0.780	0.633	0.695	0.081	√
P07759	Serine protease inhibitor A3K	Serpina3k	75.06	28.47	6	22	418	46.8	0.470	5.16	0.614	0.593	0.614	0.580	0.600	0.017	√
P08226	Apolipoprotein E	Apoe	163.26	55.31	23	63	311	35.8	1.758	5.68	1.325	1.336	1.295	1.293	1.312	0.022	√
P08607	C4b-binding protein	C4bpa	176.20	33.05	15	61	469	51.5	1.185	7.15	0.991	0.948	0.985	0.958	0.970	0.021	√
P09813	Apolipoprotein A-II	Apoa2	22.17	41.18	3	9	102	11.3	0.796	7.18	1.796	2.534	1.525	2.563	2.105	0.525	√
P10404	MLV-related proviral Env polyprotein		14.09	9.36	5	5	641	69.6	0.072	7.96	0.504	0.790	0.494	0.715	0.626	0.150	
P11276	Fibronectin	Fn1	1633.03	61.97	118	524	2477	272.4	1.924	5.59	1.358	1.395	1.343	1.420	1.379	0.035	√
P11680	Properdin	Cfp	179.73	37.28	16	64	464	50.3	1.273	7.84	0.639	0.630	0.615	0.630	0.629	0.010	
P12246	Serum amyloid P-component	Apcs	18.69	16.96	4	7	224	26.2	0.267	6.35	1.405	1.657	1.370	1.831	1.566	0.218	√
P12399	Protein CTLA-2-alpha	Ctla2a	4.53	11.68	2	2	137	15.9	0.126	5.34	0.519		0.817		0.668		
P13020	Gelsolin	Gsn	161.04	42.56	31	52	780	85.9	0.605	6.18	1.095	1.074	1.070	1.100	1.085	0.015	√
P14106	Complement C1q subcomponent subunit B	C1qb	59.80	40.32	8	18	253	26.7	0.674	8.15	0.760	0.781	0.865	0.827	0.808	0.047	√
P14847	C-reactive protein	Crp	39.28	41.78	8	14	225	25.3	0.552	6.20	1.334	1.094	1.321	1.254	1.251	0.110	
P16301	Phosphatidylcholine-sterol acyltransferase	Lcat	22.18	13.70	5	7	438	49.7	0.141	6.43	0.874	0.780	0.919	0.925	0.874	0.067	
P18531	Ig heavy chain V region 3-6	Ighv3-6	12.99	22.41	2	3	116	13.1	0.229	8.78	1.028	1.130	1.046	1.199	1.101	0.079	√
P19096	Fatty acid synthase	Fasn	21.81	4.55	10	10	2504	272.3	0.037	6.58	1.418	1.637	1.659	1.566	1.570	0.109	√
P19221	Prothrombin	F2	119.58	46.60	26	44	618	70.2	0.627	6.43	1.084		1.103		1.093		
P20918	Plasminogen	Plg	509.49	72.78	59	172	812	90.7	1.895	6.60	0.831	0.814	0.838	0.811	0.823	0.013	√
P21614	Vitamin D-binding protein	Gc	26.94	18.49	8	10	476	53.6	0.187	5.50	1.168	1.276	1.309	1.233	1.247	0.061	√
P23953	Carboxylesterase 1C	Ces1c	10.97	5.23	2	3	554	61.0	0.049	5.06	0.936	0.842	1.062	0.882	0.930	0.096	√
P26039	Talin-1	Tln1	64.45	8.74	20	22	2541	269.7	0.082	6.18	0.549	0.517	0.580	0.601	0.562	0.037	√
P26262	Plasma kallikrein	Klkb1	51.77	25.39	16	19	638	71.3	0.266	8.02	0.801	0.915	0.808	0.890	0.854	0.057	√
P28665	Murinoglobulin-1	Mug1	329.75	32.99	45	106	1476	165.2	0.642	6.42	0.776	0.781	0.795	0.777	0.782	0.009	√
P29416	Beta-hexosaminidase subunit alpha	Hexa	7.11	4.55	2	2	528	60.6	0.033	6.54	0.877		1.070		0.973		
P29699	Alpha-2-HS-glycoprotein	Ahsg	23.37	17.97	6	7	345	37.3	0.188	6.51	0.806	0.762	0.695	0.792	0.764	0.049	√
P29788	Vitronectin	Vtn	71.91	32.85	12	26	478	54.8	0.474	5.88	0.481	0.556	0.514	0.528	0.520	0.031	√
P32261	Antithrombin-III	Serpinc1	33.00	18.71	8	10	465	52.0	0.192	6.46	1.003	0.994	0.927	0.962	0.972	0.034	
P33622	Apolipoprotein C-III	Apoc3	55.40	50.51	4	16	99	11.0	1.458	4.75	1.759	1.680	1.865	1.868	1.793	0.091	√
P34928	Apolipoprotein C-I	Apoc1	19.65	38.64	5	7	88	9.7	0.722	9.09	2.795	1.653	2.632	1.925	2.251	0.549	
P39039	Mannose-binding protein A	Mbl1	63.71	42.68	9	24	239	25.4	0.946	7.61	1.150	1.010	1.166	1.078	1.101	0.072	

P39876	Metalloproteinase inhibitor 3	Timp3	4.89	7.58	2	2	211	24.2	0.083	8.76	1.037	0.787	0.954	0.764	0.886	0.132	
P40240	CD9 antigen	Cd9	2.54	6.19	2	2	226	25.2	0.079	7.23	0.642		0.659		0.650		√
P41317	Mannose-binding protein C	Mbl2	79.60	37.70	9	29	244	25.9	1.118	5.06	1.170	1.300	1.319	1.273	1.266	0.067	√
P42703-2	Isoform 2 of Leukemia inhibitory factor receptor	Lifr	6.56	4.17	3	3	719	81.2	0.037	7.05	0.515		0.532		0.524		
P46412	Glutathione peroxidase 3	Gpx3	25.43	36.73	8	10	226	25.4	0.394	8.22	0.879	1.087	1.076	1.095	1.034	0.104	√
P51910	Apolipoprotein D	Apod	10.57	23.28	4	4	189	21.5	0.186	4.91	0.897	1.004	0.962	1.045	0.977	0.063	√
P52430	Serum paraoxonase/arylesterase 1	Pon1	8.17	9.30	4	4	355	39.5	0.101	5.22	1.207		1.330		1.268		√
P55065	Phospholipid transfer protein	Pltp	22.73	17.65	7	8	493	54.4	0.147	6.62	1.664	1.471	1.651	1.384	1.543	0.138	√
P60710	Actin, cytoplasmic 1	Actb	31.62	24.00	2	10	375	41.7	0.240	5.48	0.918		0.814		0.866		√
P70194	C-type lectin domain family 4 member F	Clec4f	12.66	7.48	4	4	548	61.2	0.065	7.15	1.445	1.481	1.606	1.472	1.501	0.072	√
P70195	Proteasome subunit beta type-7	Psmb7	5.29	6.86	2	2	277	29.9	0.067	7.99	1.031		1.133		1.082		√
P70274	Selenoprotein P	Sepp1	11.23	5.79	2	4	380	42.7	0.094	7.1	1.138		0.949		1.043		√
P70375	Coagulation factor VII	F7	10.72	4.48	2	3	446	50.2	0.060	6.6	0.893	0.896	0.955	0.858	0.901	0.040	√
P70389	Insulin-like growth factor-binding protein complex acid labile subunit	Igfals	8.47	7.46	3	3	603	66.9	0.045	6.6	0.666	0.583	0.673	0.702	0.656	0.051	√
P82198	Transforming growth factor-beta-induced protein ig-h3	Tgfbi	5.15	2.64	2	2	683	74.5	0.027	7.1	0.934		1.055		0.995		
P84750	Ig kappa chain V region Mem5 (Fragment)		7.74	15.70	2	4	121	13.2	0.302	8.8	1.710		1.707		1.709		
P97290	Plasma protease C1 inhibitor	Serping1	14.12	10.71	5	5	504	55.5	0.090	6.3	0.655		0.597		0.626		√
P97298	Pigment epithelium-derived factor	Serpinf1	9.23	8.15	3	4	417	46.2	0.087	7.0	1.357	0.876	1.329	0.986	1.137	0.242	
P97333	Neuropilin-1	Nrp1	9.76	4.12	3	3	923	102.9	0.029	5.9	0.477	0.625	0.451	0.661	0.554	0.105	√
P98064	Mannan-binding lectin serine protease 1	Masp1	158.34	43.04	8	48	704	79.9	0.601	5.6	1.081	1.104	0.953	1.042	1.045	0.067	√
P98064-2	Isoform 2 of Mannan-binding lectin serine protease 1	Masp1	103.79	31.92	4	33	733	82.4	0.401	5.4	1.022	0.861	1.022	0.850	0.939	0.096	√
P98086	Complement C1q subcomponent subunit A	C1qa	60.82	26.12	8	20	245	26.0	0.770	9.1	0.838	0.848	0.795	0.905	0.846	0.045	√
Q00623	Apolipoprotein A-I	Apoa1	113.68	56.06	19	48	264	30.6	1.569	5.7	0.661	0.702	0.753	0.681	0.699	0.039	√
Q00724	Retinol-binding protein 4	Rbp4	4.56	8.96	2	2	201	23.2	0.086	6.0	0.928		0.931		0.930		√
Q00897	Alpha-1-antitrypsin 1-4	Serpina1d	53.06	27.85	3	19	413	46.0	0.413	5.4	0.865	0.879	0.862	0.982	0.897	0.057	
Q00898	Alpha-1-antitrypsin 1-5	Serpina1e	76.11	30.51	3	25	413	45.9	0.545	5.7	0.455	0.303	0.484	0.302	0.386	0.097	
Q01339	Beta-2-glycoprotein 1	Apoh	133.33	54.49	19	51	345	38.6	1.321	8.2	1.813	1.629	1.685	1.732	1.715	0.078	√
Q01853	Transitional endoplasmic reticulum ATPase	Vcp	48.92	23.20	15	18	806	89.3	0.202	5.3	1.175	1.133	1.223	1.092	1.156	0.056	√

Q02105	Complement C1q subcomponent subunit C	C1qc	62.22	34.55	7	22	246	26.0	0.847	8.5	0.947	0.791	0.960	0.924	0.905	0.078	√
Q03734	Serine protease inhibitor A3M	Serpina3m	37.77	17.94	2	10	418	47.0	0.213	6.1	1.387		1.288		1.338		√
Q06890	Clusterin	Clu	133.81	36.61	17	42	448	51.6	0.814	5.7	0.605	0.609	0.616	0.589	0.605	0.011	√
Q07235	Glia-derived nexin	Serpine2	8.76	9.82	4	4	397	44.2	0.091	9.8	0.891	1.316	1.091	1.179	1.119	0.178	
Q07456	Protein AMBP	Ambp	21.79	10.03	3	7	349	39.0	0.179	6.3	1.063	1.060	1.103	1.094	1.080	0.022	√
Q07797	Galectin-3-binding protein	Lgals3bp	22.44	12.65	5	6	577	64.4	0.093	5.1	1.043	0.996	1.061	1.234	1.083	0.104	√
Q07968	Coagulation factor XIII B chain	F13b	106.06	37.07	23	40	669	76.1	0.525	6.9	0.885	0.870	0.910	0.909	0.893	0.020	√
Q08761	Vitamin K-dependent protein S	Pros1	6.35	3.26	3	3	675	74.9	0.040	5.8	0.877	0.858	0.935	0.835	0.876	0.043	
Q08879	Fibulin-1	Fbln1	94.26	29.93	7	31	705	78.0	0.398	5.2	0.634	0.619	0.717	0.626	0.649	0.046	√
Q08879-2	Isoform C of Fibulin-1	Fbln1	57.54	21.46	2	19	685	75.2	0.253	5.3	0.573	0.449	0.429	0.522	0.493	0.067	
Q3SXB8	Collectin-11	Colec11	24.98	22.43	6	9	272	29.0	0.311	5.1	1.022	1.127	1.223	1.106	1.120	0.083	√
Q4LDF6	Protein Cfhr2 (Precursor)	Cfhr2	220.14	55.42	4	80	332	37.9	2.110	7.7	0.878	0.942	1.021	1.029	0.968	0.072	
Q60605	Myosin light polypeptide 6	Myl6	6.04	13.91	2	2	151	16.9	0.118	4.7	0.594	0.466	0.634	0.618	0.578	0.076	
Q60841-3	Isoform 3 of Reelin	Reln	4.61	0.44	2	2	3428	383.0	0.005	5.6	0.803	1.055	0.857	0.969	0.921	0.113	√
Q60994	Adiponectin	Adipoq	30.00	24.70	5	9	247	26.8	0.336	5.6	0.696	1.011	0.797	0.777	0.820	0.134	
Q61129	Complement factor I	Cfi	104.62	38.64	24	40	603	67.2	0.595	7.5	1.290	1.195	1.371	1.269	1.281	0.072	
Q61176	Arginase-1	Arg1	21.25	22.29	5	7	323	34.8	0.201	7.0	1.866	1.983	1.936	1.939	1.931	0.048	√
Q61247	Alpha-2-antiplasmin	Serpinf2	40.87	23.63	13	16	491	54.9	0.291	6.3	0.768	0.613	0.763	0.706	0.713	0.072	√
Q61268	Apolipoprotein C-IV	Apoc4	13.12	21.77	3	5	124	14.3	0.350	9.5	2.600	2.429	2.244	2.354	2.407	0.149	√
Q61406	Complement factor H-related 1	Cfhr1	50.83	17.78	7	19	343	38.4	0.495	7.9	0.828	0.883	0.920	0.936	0.892	0.048	√
Q61508	Extracellular matrix protein 1	Ecm1	34.21	19.32	9	12	559	62.8	0.191	6.8	1.163	1.061	1.056	1.026	1.077	0.060	√
Q61646	Haptoglobin	Hp	30.90	26.22	10	14	347	38.7	0.362	6.3	1.420	1.851	1.664	1.748	1.671	0.184	√
Q61702	Inter-alpha-trypsin inhibitor heavy chain H1	Itih1	102.88	24.70	17	28	907	101.0	0.277	7.0	1.077	1.015	1.041	1.085	1.054	0.033	
Q61703	Inter-alpha-trypsin inhibitor heavy chain H2	Itih2	115.92	23.36	22	39	946	105.9	0.368	7.3	1.017	1.008	1.050	1.064	1.035	0.026	
Q61704	Inter-alpha-trypsin inhibitor heavy chain H3	Itih3	35.85	11.25	10	12	889	99.3	0.121	6.0	0.835		0.826		0.831		
Q61805	Lipopolysaccharide-binding protein	Lbp	7.87	4.99	3	3	481	53.0	0.057	8.5	1.250		1.168		1.209		√
Q61838	Alpha-2-macroglobulin	A2m	376.54	38.33	55	120	1495	165.7	0.724	6.7	1.094		1.061		1.077		√
Q62009-5	Isoform 5 of Periostin	Postn	6.29	4.21	3	3	783	87.0	0.034	7.7	0.933	0.783	0.981	0.886	0.896	0.084	√
Q62351	Transferrin receptor protein 1	Tfrc	6.47	3.41	3	3	763	85.7	0.035	6.6	0.631	0.406	0.673	0.445	0.539	0.133	√
Q6S9I0	Kng2 protein	Kng2	14.59	10.39	2	7	433	47.9	0.146	6.2	0.911		1.166		1.038		√

Q80YQ1	Thrombospondin 1	Thbs1	581.41	57.13	30	187	1171	129.6	1.443	4.9	0.821	0.907	0.814	0.848	0.847	0.042	√
Q80YX1-2	Isoform 2 of Tenascin	Tnc	58.06	9.41	17	23	2019	221.7	0.104	4.9	0.771		0.863		0.817		√
Q80YX1-3	Isoform 3 of Tenascin	Tnc	49.98	8.59	14	20	1747	191.9	0.104	4.9	0.782		0.785		0.783		√
Q8BH35	Complement component C8 beta chain	C8b	143.59	44.99	24	52	589	66.2	0.786	7.8	0.385	0.370	0.407	0.404	0.391	0.018	√
Q8BH61	Coagulation factor XIII A chain	F13a1	100.14	24.73	21	40	732	83.2	0.481	5.9	0.886	0.887	0.905	0.865	0.886	0.017	√
Q8BPB5	EGF-containing fibulin-like extracellular matrix protein 1	Efemp1	36.92	16.84	7	11	493	54.9	0.200	5.1	1.157	1.258	1.147	1.079	1.160	0.074	√
Q8CF98	Collectin-10	Colec10	11.89	14.80	4	4	277	30.5	0.131	7.3	1.107		1.343		1.225		√
Q8CG14	Complement C1s-A subcomponent	C1sa	190.28	46.80	15	61	688	76.8	0.794	5.1	0.737		0.730		0.733		√
Q8CG16	Complement C1r-A subcomponent	C1ra	219.73	53.89	36	75	707	80.0	0.937	5.7	0.723	0.759	0.764	0.753	0.750	0.019	√
Q8CG19-3	Isoform 3 of Latent-transforming growth factor beta-binding protein 1	Ltbp1	5.06	1.34	2	2	1341	147.3	0.014	5.0	1.002	1.359	1.194	1.210	1.191	0.147	√
Q8CIZ8	von Willebrand factor	Vwf	7.64	1.60	4	4	2813	309.1	0.013	5.5	0.636	0.680	0.703	0.664	0.671	0.028	√
Q8K0E8	Fibrinogen beta chain	Fgb	141.04	54.26	24	51	481	54.7	0.932	7.1	0.778	0.793	0.853	0.813	0.809	0.032	√
Q8R0Y6	Cytosolic 10-formyltetrahydrofolate dehydrogenase	Aldh1l1	4.64	2.33	2	2	902	98.6	0.020	5.9	1.162		1.229		1.196		
Q8R0Z6	Angiopoietin-related protein 6	Angptl6	8.17	7.66	3	3	457	51.1	0.059	9.0	1.365		1.333		1.349		√
Q8R121-2	Isoform 2 of Protein Z-dependent protease inhibitor	Serpina10	7.98	7.87	3	4	394	45.3	0.088	5.2	0.794	0.842	0.930	0.820	0.847	0.059	√
Q8VCM7	Fibrinogen gamma chain	Fgg	103.40	43.58	19	33	436	49.4	0.669	5.9	0.761	0.737	0.823	0.758	0.770	0.037	√
Q8VDD5	Myosin-9	Myh9	24.22	4.85	10	10	1960	226.2	0.044	5.7	0.589	0.597	0.671	0.652	0.627	0.040	√
Q91VB8	Alpha globin 1	Hba-a1	75.64	63.38	7	23	142	15.1	1.523	8.2	0.712	0.796	0.763	0.780	0.763	0.036	
Q91WP0	Mannan-binding lectin serine protease 2	Masp2	161.92	33.43	22	60	685	75.5	0.795	6.1	1.243	1.309	1.205	1.203	1.240	0.050	√
Q91X70	Complement component 6	C6	187.16	50.59	30	61	769	86.6	0.705	6.1	0.507	0.489	0.533	0.462	0.498	0.030	√
Q91X72	Hemopexin	Hpx	58.20	36.09	13	19	460	51.3	0.370	7.8	1.032	1.018	1.096	1.006	1.038	0.040	√
Q91Y47	Coagulation factor XI	F11	9.00	7.69	5	6	624	69.7	0.086	8.3	0.464		0.507		0.486		√
Q91ZX7	Prolow-density lipoprotein receptor-related protein 1	Lrp1	9.49	0.70	3	4	4545	504.4	0.008	5.4	0.846	0.859	0.882	0.853	0.860	0.015	√
Q921I1	Serotransferrin	Tf	179.17	47.92	33	67	697	76.7	0.874	7.2	1.162	1.151	1.199	1.158	1.168	0.021	√
Q99K47	Fibrinogen, alpha polypeptide	Fga	99.07	46.68	22	32	557	61.3	0.522	7.5	0.766	0.802	0.787	0.769	0.781	0.017	√
Q9CQW3	Vitamin K-dependent protein Z	Proz	16.25	12.78	6	9	399	44.3	0.203	5.9	0.691	0.697	0.689	0.708	0.696	0.008	√
Q9DAC2	Complement component 8, gamma subunit, isoform CRA_b	C8g	45.80	50.00	7	13	168	18.9	0.687	8.3	0.456	0.517	0.486	0.473	0.483	0.026	√
Q9DBB9	Carboxypeptidase N subunit 2	Cpn2	25.89	11.33	6	8	547	60.4	0.132	5.9	1.117	1.094	1.201	1.014	1.106	0.077	√
Q9EQI5	Chemokine (C-X-C motif) ligand 7,	Ppbp	14.34	32.74	3	5	113	12.2	0.408	8.7	0.964	0.673	0.979	0.813	0.857	0.144	

	isoform CRA_b															
Q9ES30	Complement C1q tumor necrosis factor-related protein 3	C1qtnf3	11.20	10.98	3	4	246	26.8	0.149	6.7	0.805	0.769	0.796	0.674	0.761	0.060	√
Q9ESB3	Histidine-rich glycoprotein	Hrg	74.47	32.57	17	29	525	59.1	0.490	7.7	0.657	0.716	0.696	0.665	0.684	0.028	√
Q9JHH6	Carboxypeptidase B2	Cpb2	5.71	5.69	2	3	422	48.8	0.061	8.0	0.873		1.011		0.942		√
Q9JJN5	Carboxypeptidase N catalytic chain	Cpn1	13.55	11.38	5	5	457	51.8	0.097	8.3	1.245	1.156	1.175	1.195	1.193	0.038	√
Q9JM99-4	Isoform D of Proteoglycan 4	Prg4	4.82	2.49	2	2	925	102.0	0.020	8.6	1.071		1.234		1.152		√
Q9QWK4	CD5 antigen-like	Cd5l	150.10	65.06	25	57	352	38.8	1.468	5.2	1.010	0.926	0.957	1.000	0.973	0.039	√
Q9R0E2	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	Plod1	6.41	3.71	2	2	728	83.5	0.024	6.5	0.851		1.004		0.928		√
Q9R0G6	Cartilage oligomeric matrix protein	Comp	8.10	4.11	2	4	755	82.3	0.049	4.6	1.020		1.118		1.069		√
Q9R1P4	Proteasome subunit alpha type-1	Psma1	5.93	6.84	2	2	263	29.5	0.068	6.5	1.216		1.270		1.243		√
Q9WVF5	Epidermal growth factor receptor	Egfr	5.57	3.82	2	2	655	72.9	0.027	7.0	0.445		0.500		0.473		√
Q9Z126	Platelet factor 4	Pf4	38.57	28.57	3	14	105	11.2	1.246	9.3	1.000	1.013	0.953	1.028	0.999	0.032	√
Q9Z1R3	Apolipoprotein M	Apom	13.52	19.47	4	5	190	21.3	0.235	6.5	0.346	0.377	0.340	0.447	0.377	0.049	√
Q9Z1T2	Thrombospondin-4	Thbs4	91.63	24.30	16	36	963	106.3	0.339	4.7	0.865	0.985	0.818	0.904	0.893	0.071	√

†Exosome proteins were extracted from *db/m*$^{+}$ or *db/db* serum. The whole serum exosome proteins were subjected to LC-MS/MS analysis after iTRAQ labeling.
Note: PSMs, peptide-spectrum matches; AAs, Atomic Absorption Spectrometry; MW, molecular weight; pI, isoelectricpoint; PAF, protein abundance factor ratio, diabetes sample/normal sample

Table S2. Blood metabolic indexes of participants with or without diabetes

	Control (n=6)	diabetes (n=6)	P value
Age (years)	51.2±11.2	53.5±16.1	0.76
Gender (male, %)	100	100	-
Total cholesterol (mmol/L)	4.6±0.7	4.1±0.7	0.13
HDL cholesterol (mmol/L)	1.3±0.4	1.1±0.2	0.24
LDL cholesterol (mmol/L)	2.7±0.4	2.3±0.7	0.20
Triglycerides (mmol/L)	1.4±0.5	1.4±0.5	0.84
Blood glucose (mmol/L)	5.5±0.4	12.4±6.5	0.05
Body mass index (BMI)	22.7±2.3	26.8±4.1	0.15

Table S3. The different SExo proteins in normal and diabetic mouse blood identified by quantitative mass spectrometry.

Accession	Description	Gene Symbol	NCBI Entry	ratio 1	ratio 2	ratio 3	ratio 4	iTRAQ ratio (diabetes /normal, mean±SD)	p value
A2A997	Complement component C8 alpha chain	C8a		0.399	0.444	0.407	0.483	0.43±0.04	0.016
Q9Z1R3	Apolipoprotein M	Apom	AAH21597.1	0.346	0.377	0.340	0.447	0.38±0.05	0.022
Q8BH35	Complement component C8 beta chain	C8b	NP_598643.1	0.385	0.370	0.407	0.404	0.39±0.02	0.026
D3YXF5	Protein C7	C7	Q4KMM3.3	0.455	0.480	0.503	0.491	0.48±0.02	0.023
Q00898	Alpha-1-antitrypsin 1-5	Serpina1e	NP_033273.1	0.455	0.303	0.484	0.302	0.39±0.1	0.008
Q9DAC2	Complement component 8, gamma subunit, isoform CRA_b	C8g	EDL08254.1	0.456	0.517	0.486	0.473	0.48±0.03	0.024
P29788	Vitronectin	Vtn	NP_035837.1	0.481	0.556	0.514	0.528	0.52±0.03	0.035
Q91X70	Complement component 6	C6	AAF14577.1	0.507	0.489	0.533	0.462	0.5±0.03	0.028
P26039	Talin-1	Tln1	NP_035732.2	0.549	0.517	0.580	0.601	0.56±0.04	0.019
P06728	Apolipoprotein A-IV	Apoa4	AAA37253.1	0.553	0.596	0.546	0.612	0.58±0.03	0.024
Q08879-2	Isoform C of Fibulin-1	Fbln1		0.573	0.449	0.429	0.522	0.49±0.07	0.047
Q60605	Myosin light polypeptide 6	Myl6	Q60605.3	0.594	0.466	0.634	0.618	0.58±0.08	0.026
E9Q8B5	Protein Gm4788	Gm4788		0.598	0.596	0.584	0.611	0.6±0.01	0.026
Q06890	Clusterin	Igk	NP_001128599.1	0.605	0.609	0.616	0.589	0.6±0.01	0.009
P07759	Serine protease inhibitor A3K	Serpina3k	NP_035588.2	0.614	0.593	0.614	0.580	0.6±0.02	0.044
P06683	Complement component C9	C9	NP_038513.1	0.626	0.577	0.602	0.579	0.6±0.02	0.026
P11680	Properdin	Cfp		0.639	0.630	0.615	0.602	0.62±0.02	0.034
P12246	Serum amyloid P-component	Apcs	AAA40093.1	1.405	1.657	1.370	1.831	1.57±0.22	0.048
P19096	Fatty acid synthase	Fasn	NP_032014.3	1.418	1.637	1.659	1.566	1.57±0.11	0.047
Q61646	Haptoglobin	Hp	NP_059066.1	1.420	1.851	1.664	1.748	1.67±0.18	0.049
P01749	Ig heavy chain V region 3	Igh-VJ558	P01749.1	1.432	1.556	1.507	1.467	1.49±0.05	0.037
P70194	C-type lectin domain family 4 member F	Clec4f		1.445	1.481	1.606	1.472	1.5±0.07	0.028
O09173	Homogentisate 1,2-dioxygenase	Hgd	AAH37628.2	1.545	1.898	2.180	1.718	1.84±0.27	0.025
Q61176	Arginase-1	Arg1	NP_031508.1	1.866	1.983	1.936	1.939	1.93±0.05	0.018
P33622	Apolipoprotein C-III	Apoc3	NP_075603.1	1.759	1.680	1.865	1.868	1.79±0.09	0.044
Q01339	Beta-2-glycoprotein 1	Apoh	NP_038503	1.813	1.629	1.685	1.732	1.71±0.08	0.044
O70570	Polymeric immunoglobulin receptor	Pigr	NP_035212.2	2.555	2.409	2.259	2.430	2.41±0.12	0.033
Q61268	Apolipoprotein C-IV	Apoc4	NP_031411.1	2.600	2.429	2.244	2.354	2.41±0.15	0.024

iTRAQ ratios was presented with mean±SD. 95% confidence intervals (z score = 1.96) were used to determine the cutoff values for proteins with changes. Significant test for the intergroup variables were analyzed with the Student's t-test with $p \leq 0.05$ considered statistically significant.

Supplementary Figures

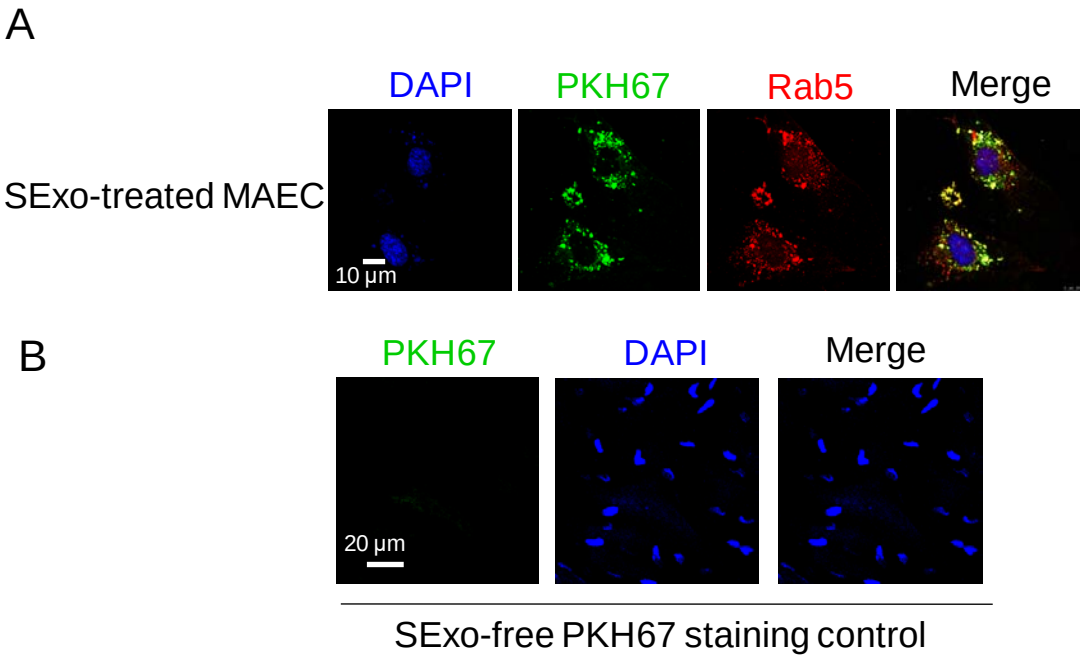

Fig. S1. The cellular location of PKH67-stained SExo in mouse endothelial cells. ***(A)*** Immunofluorescence showed that most of the PKH67-labeled SExo signals (green) were co-localized with the intracellular endosome marker Rab5 (red, TRITC) in primary cultured mouse aortic endothelial cells (MAECs). Nuclei were stained with DAPI in blue, bar=10 μm. ***(B)*** The *en face* confocal microscopic images showed that PKH67 signal (in green) was invisible in aortic endothelial cells treated with SExo-free PKH67 (2 μL) for 48 hours.

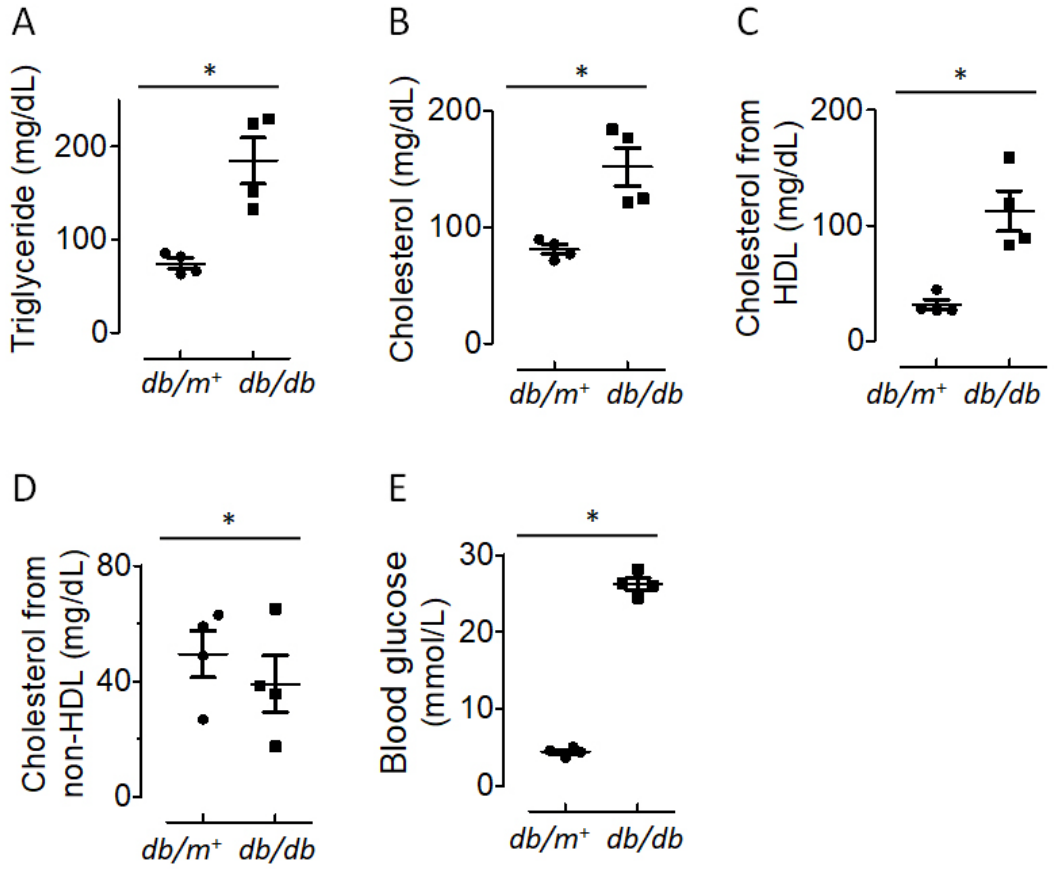

Fig. S2. **The metabolic indexes of *db/db* and *db/m*$^+$ mice.** The blood concentration of triglyceride **(*A*)**, cholesterol **(*B*)**, cholesterol from HDL **(*C*)**, cholesterol from non-HDL **(*D*)** and glucose **(*E*)** were measured in *db/db* and *db/m*$^+$ mice. Results are means±SEM (n=4). **P* < 0.05 vs. *db/m*$^+$.

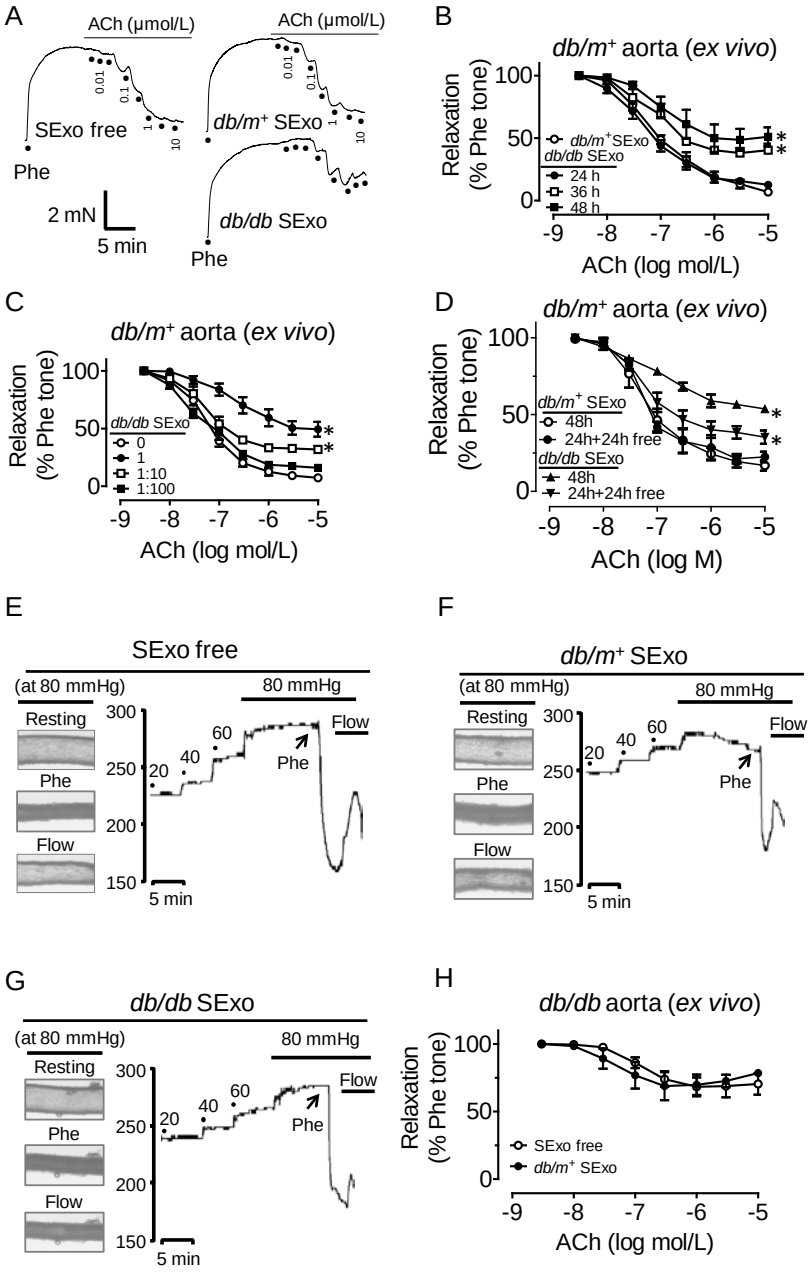

Fig. S3. *db/db* mouse SExos impair endothelial function. ***(A)*** Representative traces showing the impaired EDRs in *db/m*$^+$ mouse aortas treated for 48 h with *db/m*$^+$ or *db/db* SExos (exosomes from 1 mL of blood re-suspended in 1 mL exosome-free culture medium). Treatment with *db/db* SExos impaired acetylcholine (ACh)-induced endothelium-dependent relaxations (EDRs) in *db/m*$^+$ mouse aortas in time- **(*B*)** and dose- **(*C*)** dependent manners. 0: SExos-free; 1:n: SExos from 1 mL of blood was suspended in n mL of SExo-free medium. ***(D)*** The impaired EDRs in mouse aortas after 24-h treatment with *db/db* SExos followed by washout of *db/db* SExos and then 24-hour incubation in SExo-free medium was significantly less than EDRs in aortas treated for 48 hours with *db/db* SExos. Representative traces showing the impact on flow-mediated dilatation in *db/m*$^+$ mouse resistance mesenteric arteries exposed to SExos-free medium **(*E*)**, *db/m*$^+$ mouse SExos **(*F*)**, and *db/db* SExos **(*G*)** for 48 hours. **(*H*)** The unaffected EDRs in *db/db* mouse aortas treated with *db/m*$^+$ SExos for 48 hours. Results are means±SEM (n=4). $^*P < 0.05$ vs. *db/m*$^+$ SExo (*B* and *D*) or 0 (*C*).

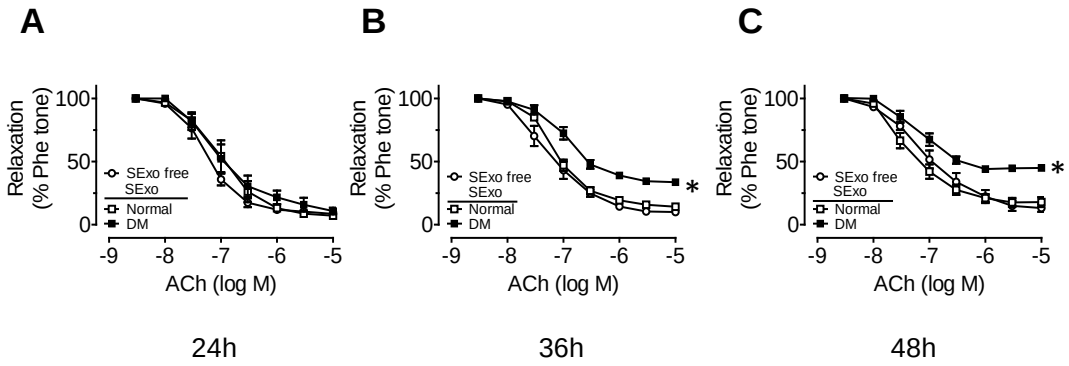

Fig. S4. SExos from diabetic patients impair mouse endothelial functions in a time-dependent manner. Twenty-four-hour treatment with diabetic (DM) patient SExos had no effect on mouse endothelial function (***A***), while 36- or 48-hour treatment with diabetic patient SExos significantly impaired mouse endothelial function when compared to the SExos from normal subjects (Normal) (***B-C***). Results are means±SEM (n=4), $^{*}P < 0.05$ vs. Normal.

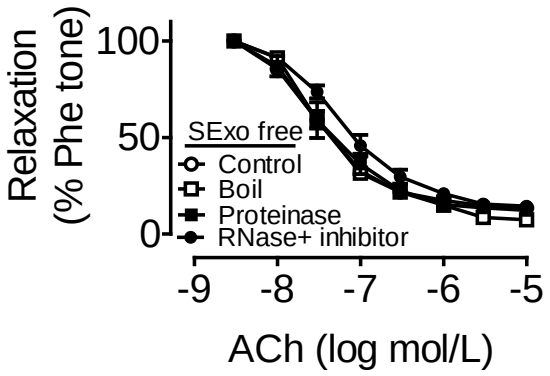

Fig. S5. All control treatments used in separating *db/db* SExo components do not affect acetylcholine-induced relaxations (EDRs). The conditioned medium treated with RNase A (10 μg/ml, 37 ºC, 60 minutes) plus RNase A inhibitor (2000 units/ml, 37 ºC, 60 minutes), proteinase (0.5 mg/ml，37 ºC, 120 minutes) or heating (at 100 ºC followed returning to room temperature) did not affect EDRs in *db/m*$^{+}$ mouse aortas after 48-hour incubation. Results are means±SEM (n=4).

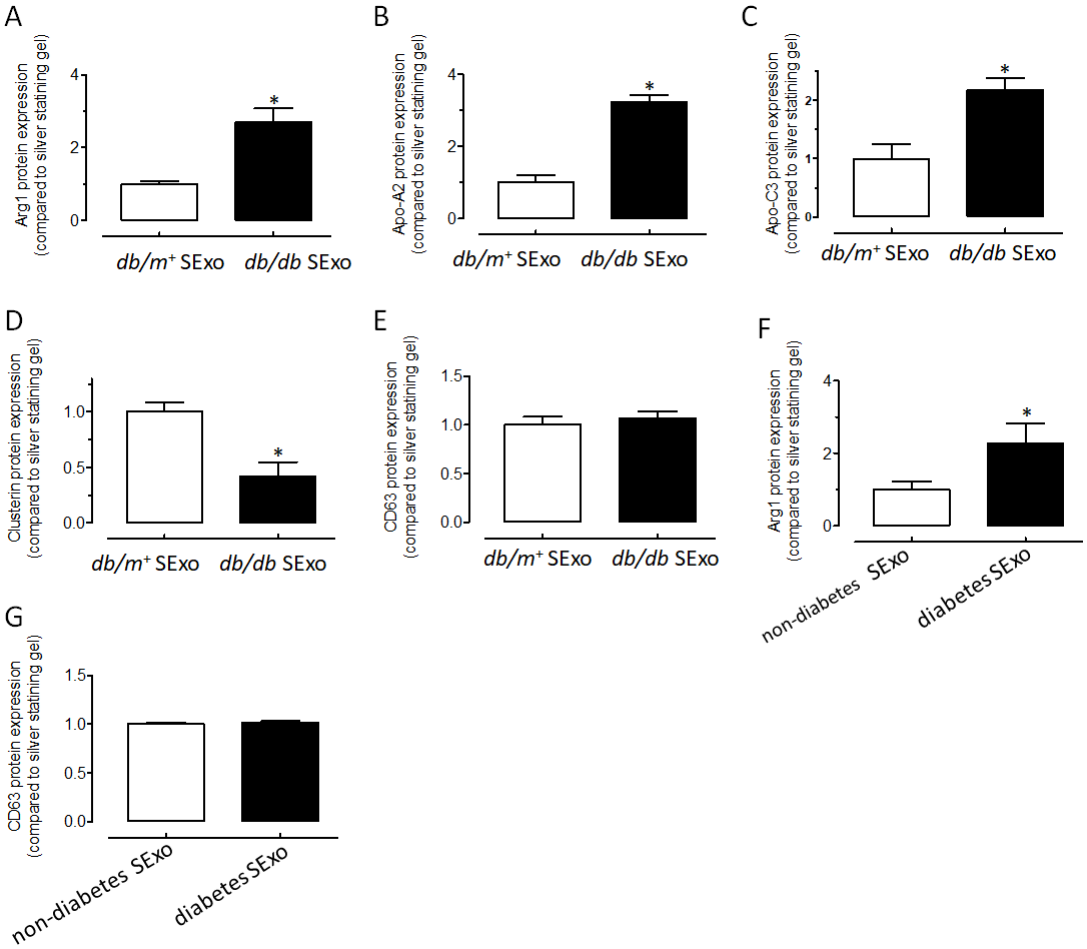

Fig. S6. Protein levels of Arg1, Apo-A2, Apo-C3, clusterin and CD63 in SExos from diabetic *db/db* and non-diabetic *db/m*$^+$ mice or in diabetic patients and non-diabetic subjects. (*A*-*E*) The levels of mouse serum exosomal Arg1, Apo-A2, Apo-C3, Clusterin and CD63 were analyzed by Quantity One software with the intensity of silver staining gel as internal control. **(*F*-*G*)** The levels of human serum exosomal Arg1 and CD63 were analyzed by Quantity One software with the intensity of silver staining as internal control. Results are means±SEM (n=3). *$P < 0.05$ vs. *db/m*$^+$ SExo or non-diabetes SExos.

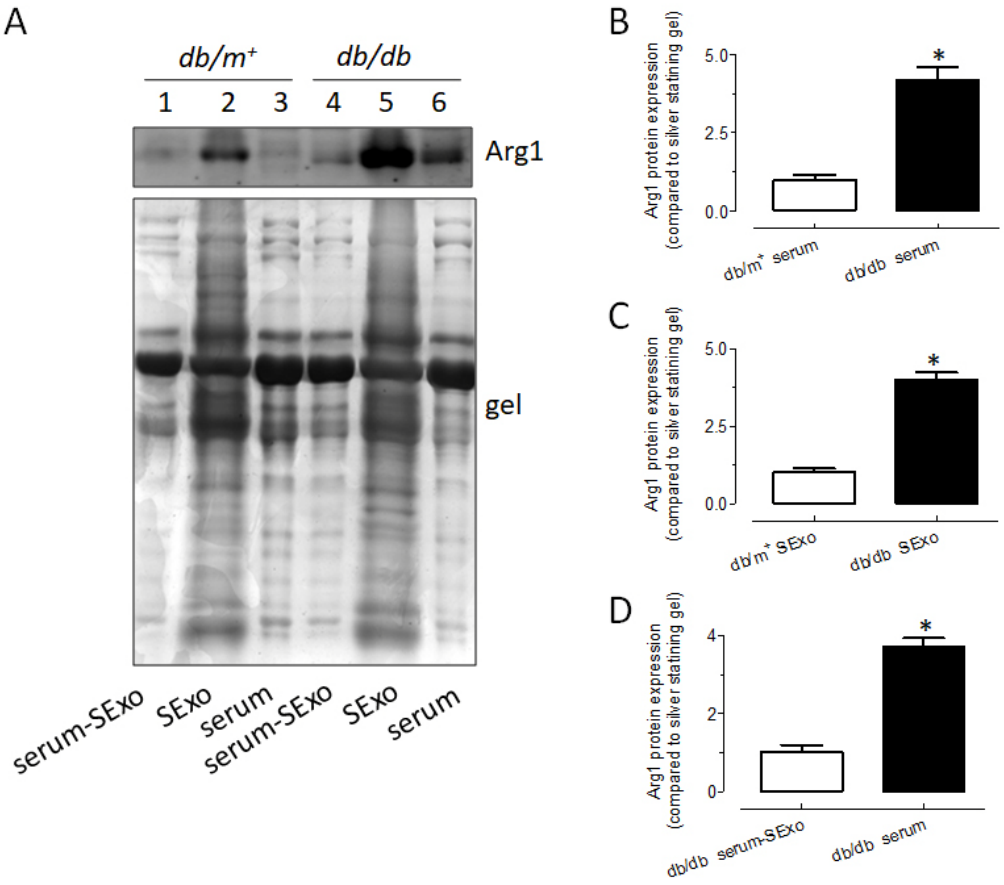

Fig. S7. **Arg1 content in SExos and in serum with or without SExos from *db/db* and *db/m*$^+$ mice. (*A*)** The upper panel: Western blotting showed that Arg1 content was increased in *db/db* SExos and in *db/db* serum with or without SExos compared with samples from *db/m*$^+$ mice. The lower panel: silver staining gel showed the amounts of protein loading for each sample. (***B-D***) Quantification of Arg1 in different preparations. Serum-SExo indicates SExo-depeleted serum. Results are means±SEM (n=4). $^*P < 0.05$ vs. *db/m*$^+$ serum **(*B*)**, or *db/m*$^+$ SExo **(*C*)** or *db/db* serum-SExo **(*D*).**

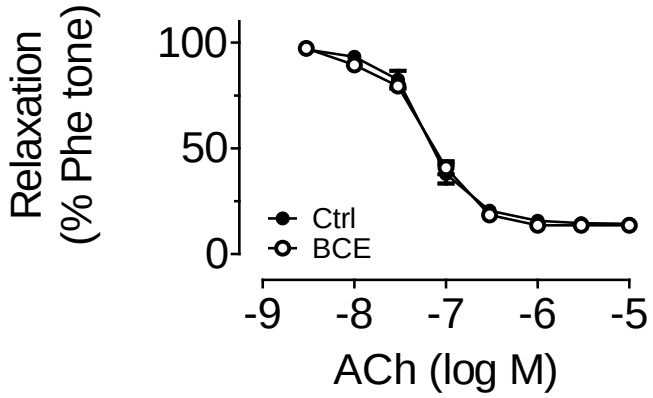

Fig. S8. The unaffected ACh-induced relaxations in *db/m*$^{+}$ mouse aortas treated with the arginase inhibitor S-(2-boronoethyl)-l-cysteine (BCE, 100 μM, 24 hours). Results are means±SEM (n=4).

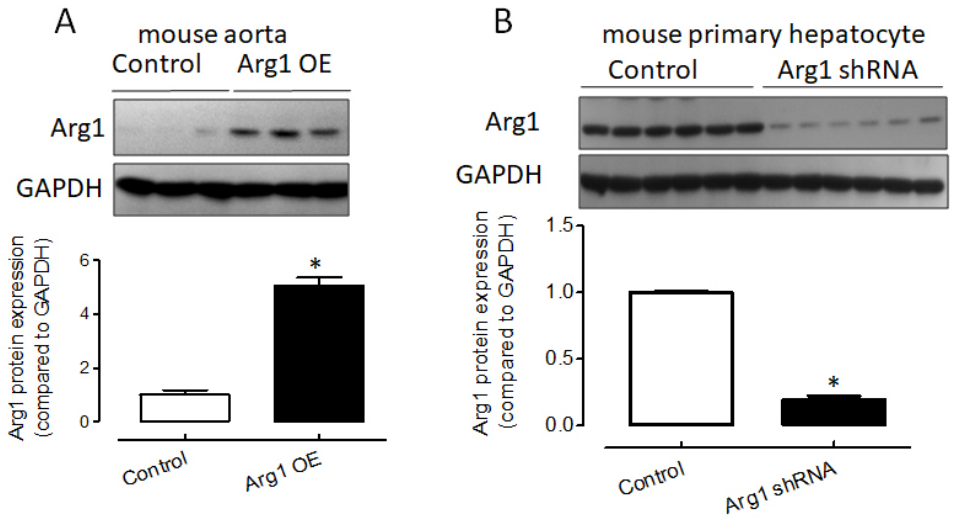

Fig. S9. Protein expression of Arg1 in Arg1 overexpressing virus (Arg1 OE)-treated C57BL/6 aortas or in AAV-Arg1 shRNA (Arg1shRNA)-treated mouse hepatocytes. (*A*) Arg1 protein levels in Arg1 overexpressing virus (Arg1 OE)-treated aortas. **(*B*)** Arg1 protein levels in AAV-Arg1 shRNA (Arg1 shRNA)-treated mouse hepatocytes. Results are means±SEM (n=3-6). $^{*}P < 0.05$ vs. Control.

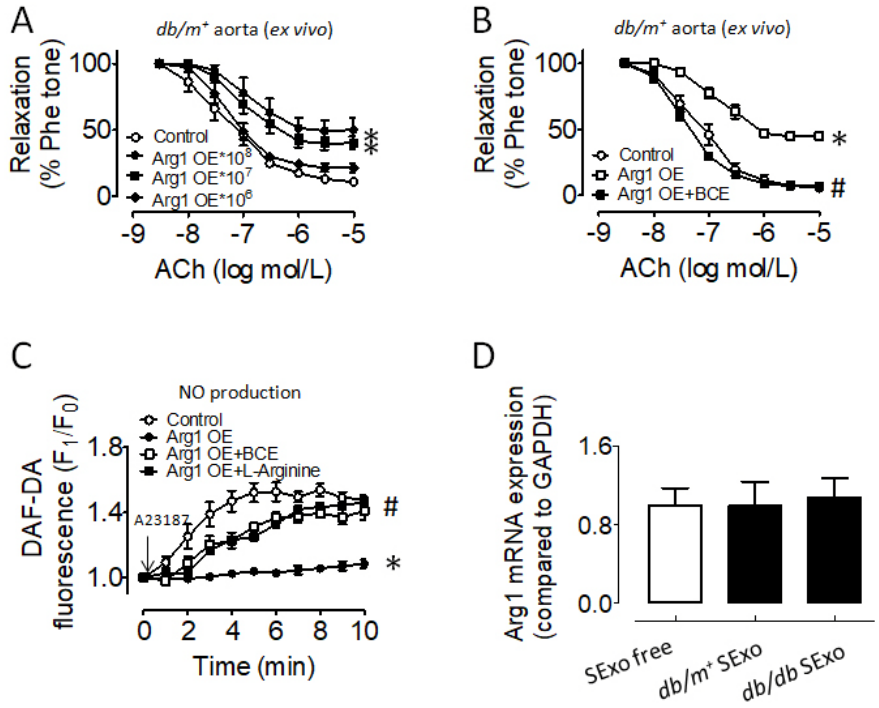

Fig. S10. Arg1 overexpression leads to endothelial dysfunction and reduces NO producion. (*A*) EDRs in *db/m*$^+$ mouse aortas after incubation with different dosages of Arg1 OE for 48 h. **(*B*)** The arginase inhibitor BCE (100 μmol/L, 24 h) improved EDRs in Arg1 OE-treated *db/m*$^+$ mouse aortas. **(*C*)** The reduced A23187-stimulated NO production detected by confocal fluorescence microscopy in Arg1 OE-treated HUVECs (48 h) was rescued by co-treatment of BCE (100 μmol/L, 24 h) or L-arginine (300 μmol/L, 24 h). **(*D*)** qPCR showed the Arg1 mRNA expression in HUVECs after 24-hour incubation with *db/m*$^+$ SExos or *db/db* SExos**.** Results are means±SEM (n=4-5). $^*P< 0.05$ vs. Control; $^\#P <0.05$ vs. Arg1 OE.

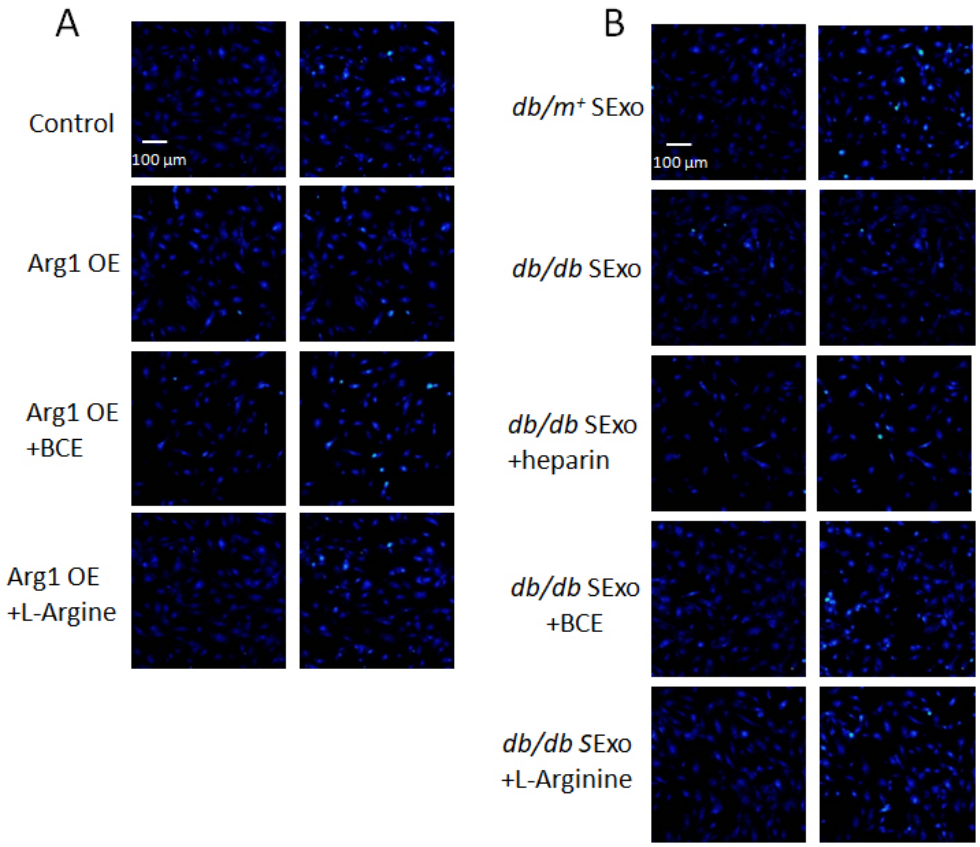

Fig. S11. Representative confocal images showing the NO generation in HUVECs subjected to different treatments. (*A*) A23187-stimulated NO production was measured by confocal fluorescent microscopy in arginase 1-overexpressing HUVECs in control and in the presence of BCE (100 μmol/L, 24 hours) or L-Arginine (300 μmol/L, 24 hours). **(*B*)** NO production in *db/db* SExos-treated HUVECs (48 hours) with or without the co-treatment of heparin (0.3 μg/ml, 48 hours), BCE (100 μmol/L, 24 hours) or L-Arginine (300 μmol/L, 24 hours). Bar: 100 μm.

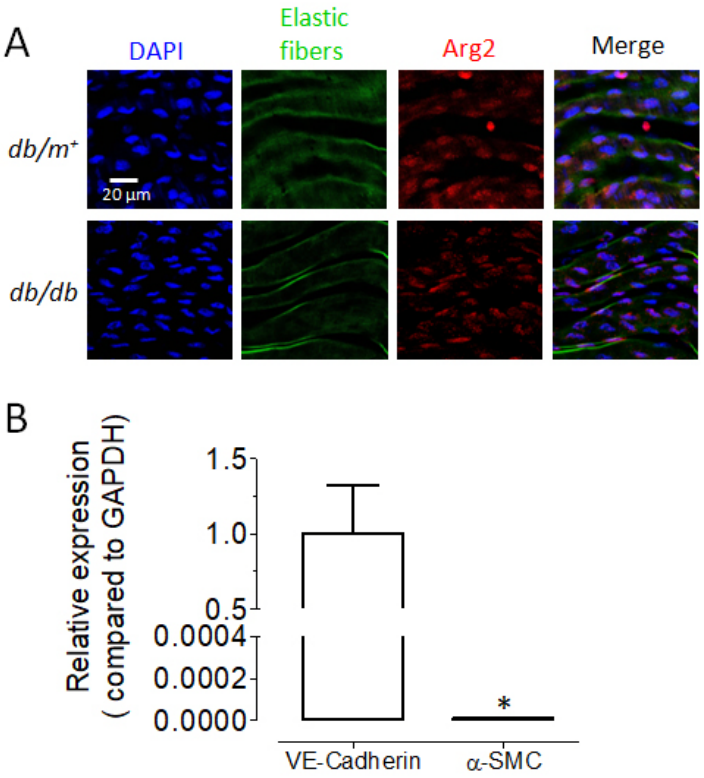

Fig. S12. Arginase 2 (Arg2) expression in aortic endothelial cells from *db/m*$^+$ and *db/db* mice and verification of the endothelial cells harvested from *db/m*$^+$ mouse aortas. (*A*) Immunofluorescence showed the Arg2 protein expression in *db/m*$^+$ and *db/db* mouse aortic *en face* endothelial cells. Nuclei stained with DAPI in blue; Arg2 stained with TRITC in red; auto-fluorescence of elastic fibers in green; bar: 20 µm. (***B***) qPCR analysis showed that the harvested cells from *db/m*$^+$ mouse aortas were VE-Cadherin positive. Results are means±SEM (n=4). $^*P < 0.05$ vs. VE-Cadherin.

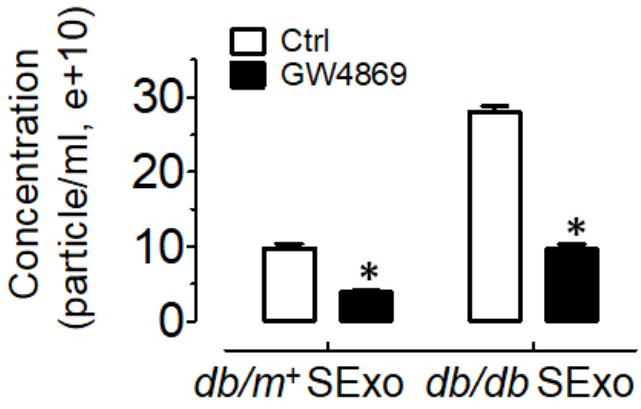

Fig. S13. ***In vivo*** **GW4869 treatment reduces the number of SExos.** GW4869 (1 mg/kg/day) was intraperitoneally injected to *db/m*$^{+}$ and *db/db* mice. Two weeks later, serum was collected and, the number of SExo was calculated by NanoSight NS300 (Malvern Instruments, UK). Results are means±SEM (n=4). $^{*}P < 0.05$ vs.Ctrl.

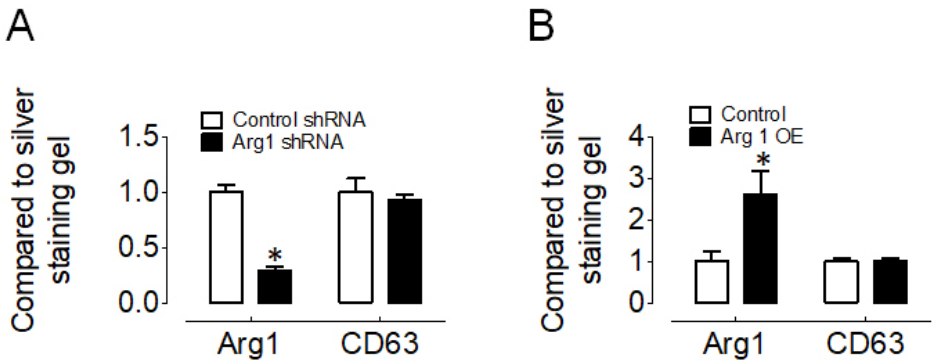

Fig. S14. The summarized data showing Arg1 expression in SExos after adenovirus treatment. ***(A)*** SExo Arg 1 protein expression in Arg 1 shRNA adenovirus-treated *db/db* mice. ***(B)*** SExo Arg 1 protein expression in Arg 1 OE adenovirus-treated *db/m*$^{+}$ mice. The silver staining was used to normalize the protein expression of Arg1 and CD63. Results are means±SEM (n=3). $^{*}P < 0.05$ vs. Control shRNA (A) or Control (B).

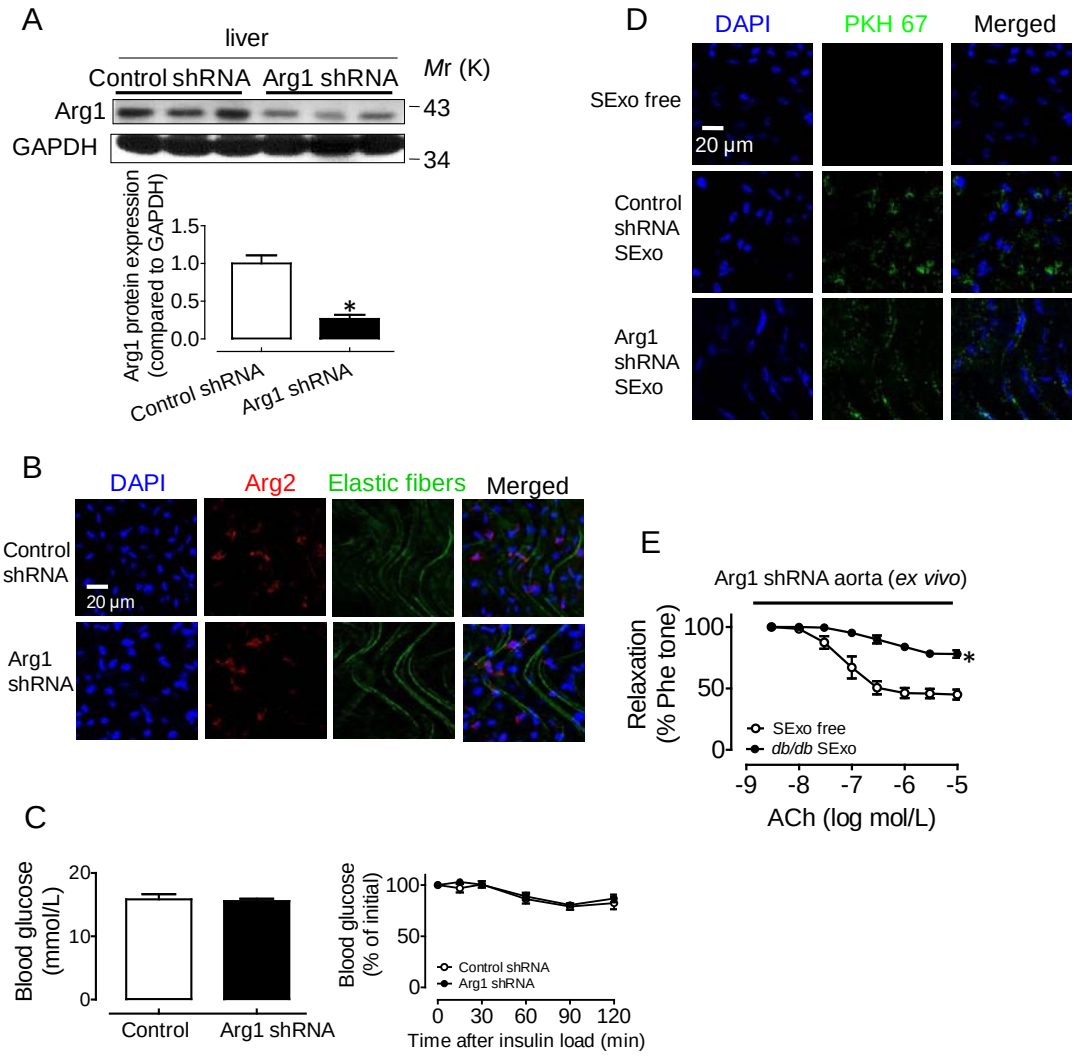

Fig. S15. Aortas isolated from arginase 1 knockdown mice are still responsive to *db/db* SExos. (*A*) Western blot analysis showed the markedly decreased arginase 1 level in *db/db* liver 7 days after intravenous administration of Arg1 shRNA. The lower panel presented the summarized data. **(*B*)** The *en face* immunofluorescence showed no change of arginase 2 protein levels in aortic endothelial cells from *db/db* mice 7 days after intravenous administration of Arg1 shRNA. Nuclei stained with DAPI in blue; arginase 2 stained with TRITC in red; auto-fluorescence of elastic fibers in green; bar: 20 μm. **(*C*)** Arg1 shRNA *in vivo* treatment did not affect the fasting blood glucose level (left panel) or insulin sensitivity (right panel) in *db/db* mice. **(*D*)** SExos isolated from control shRNA- or Arg1 shRNA-treated *db/db* mice were absorbed by *db/m*$^{+}$ mouse aortic endothelial cells. Nuclei stained with DAPI in blue; SExo stained with PKH67 in green; bar: 20μm. **(*E*)** *db/db* SExos were still able to impair EDRs in aortas from Arg1 shRNA-treated *db/db* mice. Results are means±SEM (n=4-5). $^{*}P < 0.05$ vs. Control ShRNA (*A*) or free (*E*).

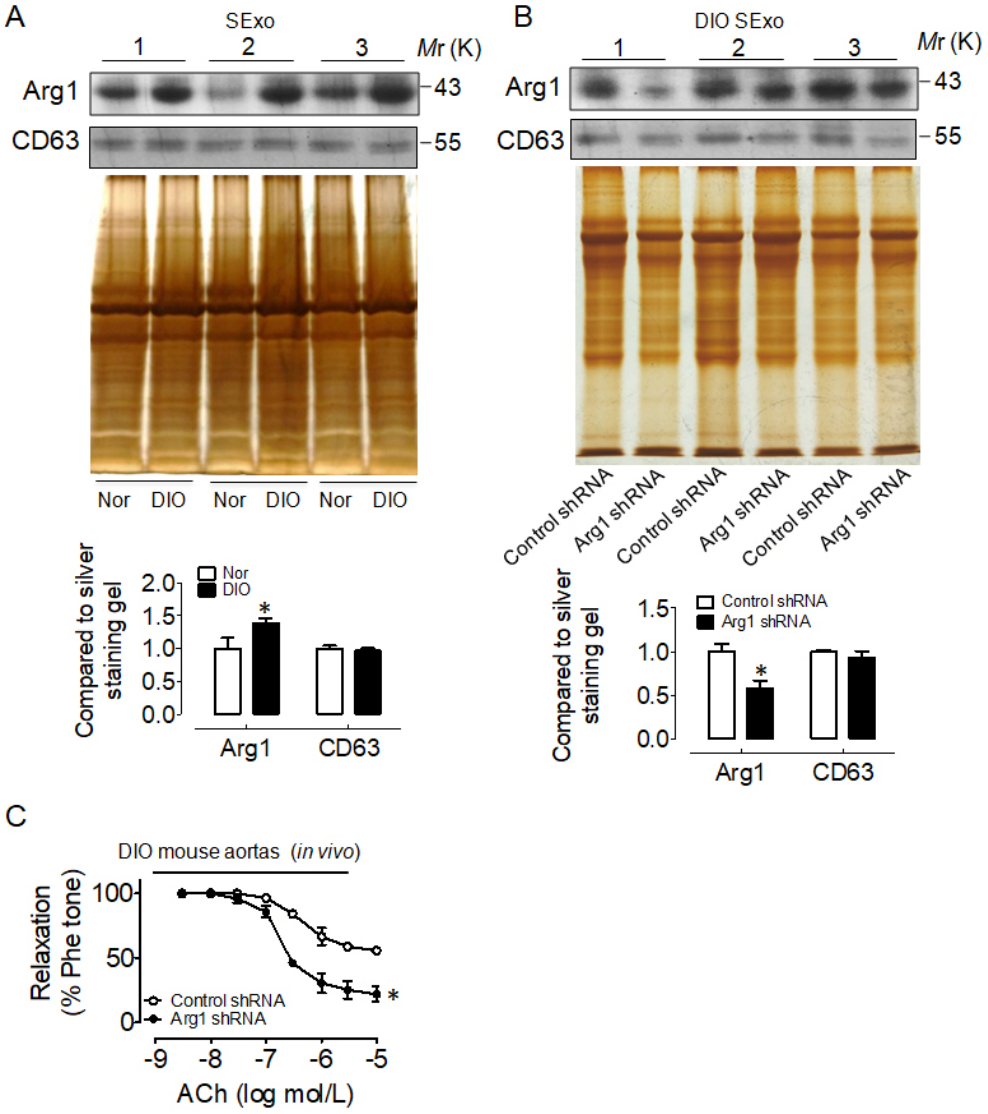

Fig. S16. Arginase 1 protein levels in SExos from diet-induced obese (DIO) mice and the EDRs in aortas from DIO mice treated with Arg1 shRNA. (*A*) Elevated level of arginase 1 was detected by Western blotting in SExos isolated from normal C57BL/6 and DIO mice. Silver staining indicated the protein loading amounts. The lower panel presented the summarized data. **(*B*)** Arginase 1 protein levels in SExos from DIO mice 7 days after intravenous administration of control shRNA or Arg1 shRNA. Silver staining indicated the protein loading amounts. The lower panel presented the summarized data. **(*C*)** Arg1 shRNA *in vivo* treatment improved EDRs in DIO mouse aortas. Results are means±SEM (n=4). $^{*}P <$ 0.05 vs. Nor (*A*) or Control shRNA (*B*).

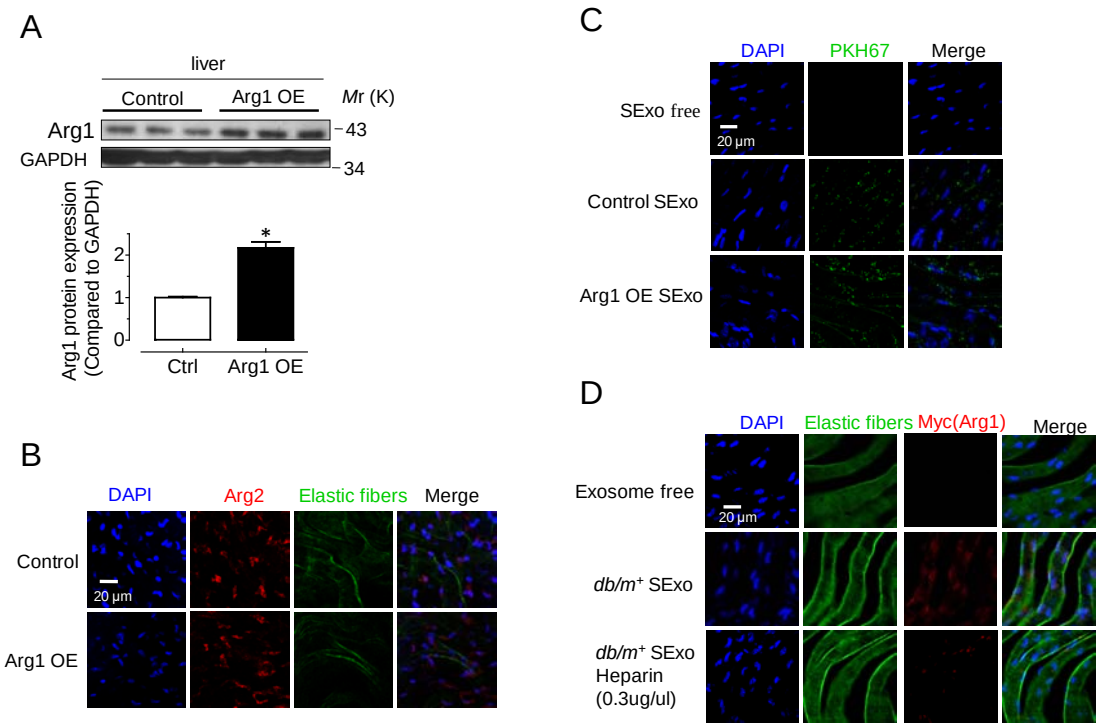

Fig. S17. Characterization of arginase 1 expression in arginase 1 overexpressing *db/db* mice. (*A*) Western blotting analysis showed the increased arginase 1 expression in *db/db* mouse liver 7 days after intravenous administration of arginase 1-overexpressing virus Arg1-Myc OE. The lower panel presented the summarized data. **(*B*)** Immunofluorescence showed no change in the arginase 2 signal in aortic endothelial cells from *db/db* mice 7 days after intravenous administration of Arg1-Myc OE. Nuclei stained with DAPI in blue; arginase 2 stained with TRITC in red; auto-fluorescence of elastic fibers in green; bar: 20 μm. **(*C*)** SExos isolated from control or Arg1 OE-treated *db/db* mice were absorbed by *db/m*$^{+}$ mouse aorta endothelial cells. Nuclei stained with DAPI in blue; SExos stained with PKH67 in green; bar: 20 μm. **(*D*)** Immunofluorescence showed the Myc signal in aortic endothelial cells from *db/db* mice 7 days after intravenous administration of Arg1-Myc OE. Nuclei stained with DAPI in blue; Myc stained with TRITC in red; auto-fluorescence of elastic fibers in green; bar: 20 μm. Results are means±SEM (n=4). $^{*}P < 0.05$ vs.Ctrl (A).

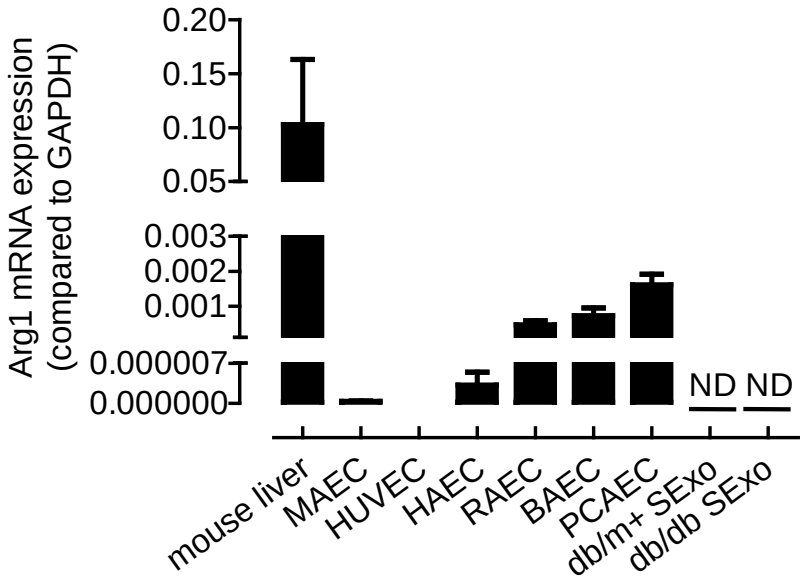

Fig. S18. The expression of Arginase 1 mRNA is very low in MAECs and HUVECs and is undetectable in *db/m*$^+$ and *db/db* SExos. Arg1 mRNA expression in mouse liver (positive control), mouse aortic endothelial cells (MAECs), HUVECs, HAECs (human aortic endothelial cells), rat aortic endothelial cells (RAECs), bovine aortic endothelial cells (BAECs), and porcine coronary artery endothelial cells (PCAECs). SExos from *db/m*$^+$ and *db/db* mice was detected by qPCR assay. ND, Not detectable. Results are means±SEM (n=3-5).

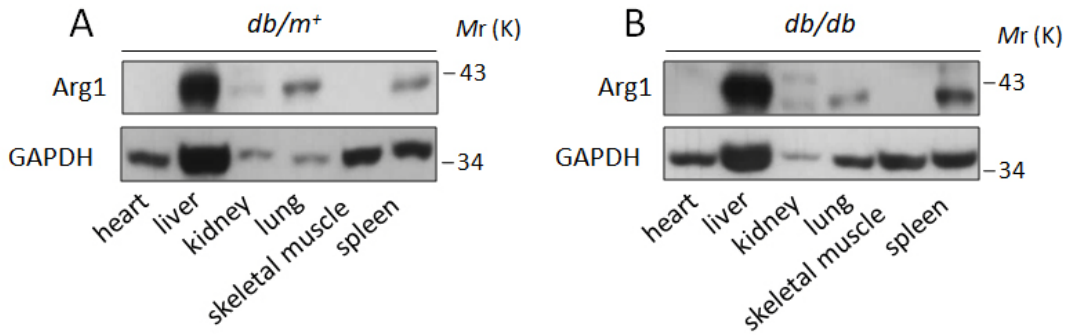

Fig. S19. Arginase 1 expression profile in different organs from *db/m*$^+$ and *db/db* mice. Western blot analysis showed the arginase 1 expression levels in heart, liver, kidney, lung, skeletal muscle and spleen from *db/m*$^+$ **(*A*)** and *db/db* mice **(*B*)**.