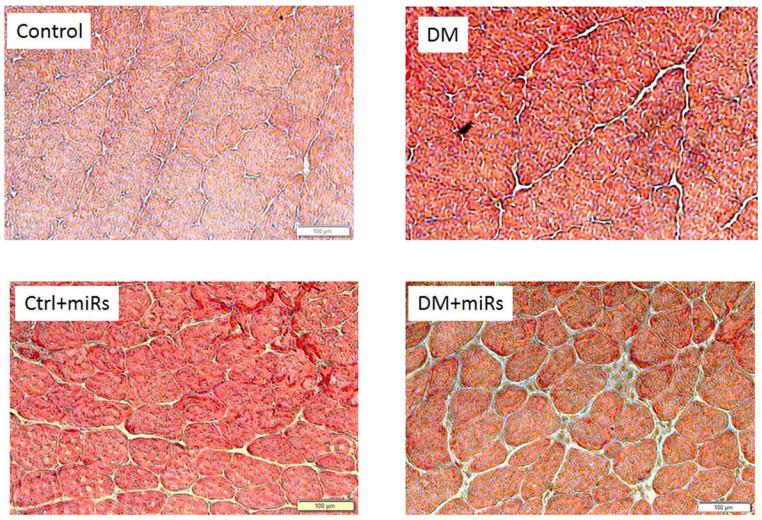
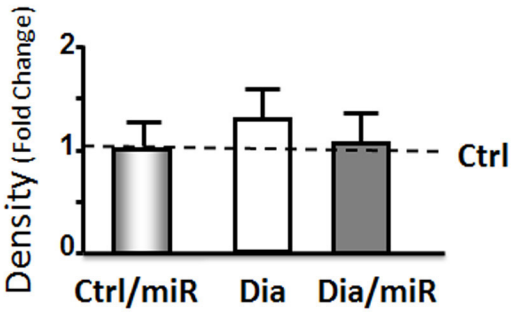
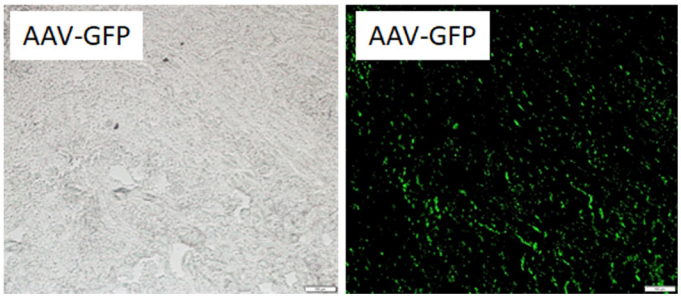


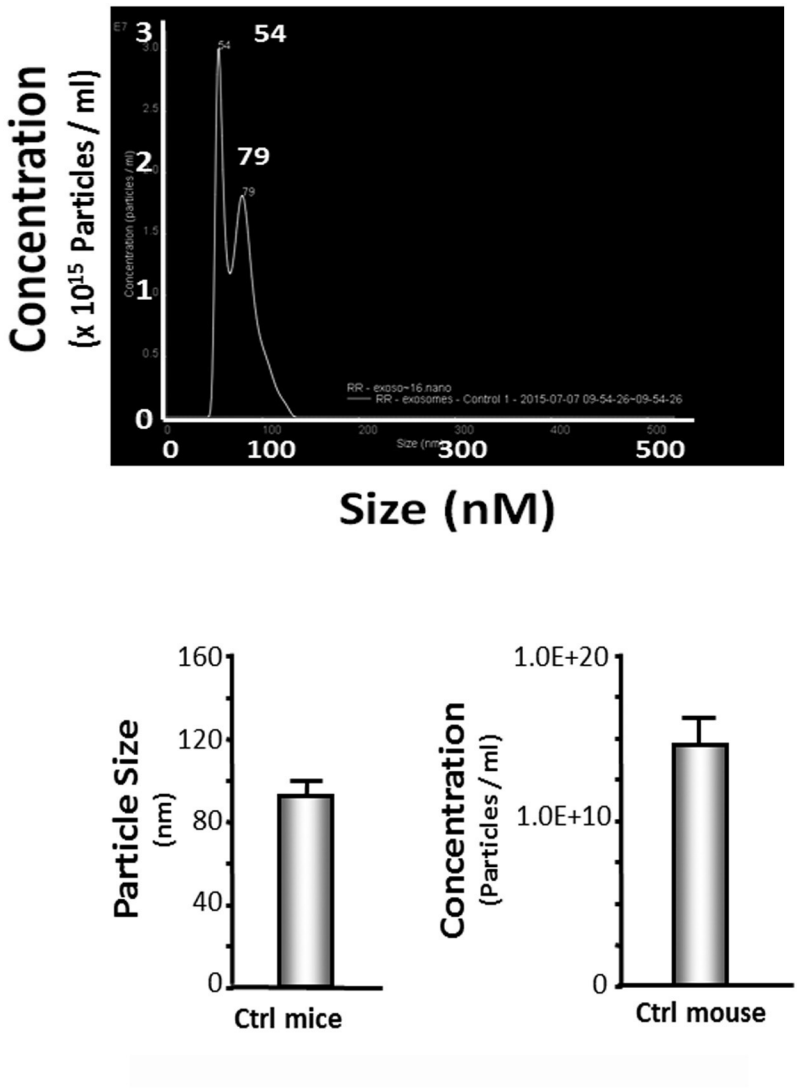
Masson staining



Supplement Figure 1: Shown are representative Masson's Trichrome staining of paraffin-sections from TA muscle from control plus AAV-GFP, control plus AAV-miR23a/27a, diabetes plus AAV-GFP, and diabetes plus AAV-miR23a/27a mice. The bar graph shows collagen amount (blue color), measured by the CellSens Dimension 1.9 with count & measure Software, from the muscle of each group of mice as a fold change relative to controls as 1-fold (Bars: mean ± s.e.; n=6/group; *=$p<0.05$ vs. control).



Supplementary Figure 2: Shown are representative frozen sections of kidney from normal control mice that had muscles transduced with AAV-GFP. The right panel shows fluorescence microscopy to detect GFP expression in the kidney section; the left panel is a bright-field microscopic image of the same field (bar: 100 um).



Supplement Figure 3: Exosomes were isolated from the serum of control mouse. The exosome size and concentration was measured by NanoSight Instruments. The bar graphs shows the size (nanometer diameter) of exosomes from serum of 3 of mice (Bars: mean ± s.e.; n=3/group).

METHODS:

Animals and diabetic mice model: The mice (C57BLKS/J) were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and bred in the Department of Animal Resources at Emory University (23). The experiments were approved by the Institutional Animal Care and Use Committee of Emory University (protocol 2002853). Diabetic mice were produced by STZ (Pfanstiehl Lab, Waukegan IL, USA) injection using a 2-day protocol in which 150 mg/kg (prepared fresh in 0.1 M citrate buffer, pH 4.0) was given by intra-peritoneal injection for 2 consecutive days. The control mice were injected with vehicle only. The experiments are performed in male mice since, in our hands, female mice resist diabetes after injection of STZ. Mice (C57BL/6J, 6-8 weeks) were randomly assigned into four groups: control/AAV-GFP (control), control/AAV-miR-23a~27a~24-2, diabetes/AAV-GFP and diabetes/AAV-miR-23a~27a~24-2 (n = 12/group). The muscle and kidney were harvested 12 weeks after the second STZ injection. Muscle grip function was measured using a mouse grip strength meter with dual computerized sensors to detect and record the grip force (Columbus Instruments, Columbus, OH). Mice are allowed to grip a metal grid connected to a force transducer and gently pulled by the tail for 5 seconds. The computerized sensors determine what force was needed to counter-balance the grip of the mice (24). Muscle function was tested in mice before and 12-weeks after the STZ injection. The grip strength of each mouse was tested 5 times on each testing occasion, with 10 minutes rest between each test. The average of the 5 determinations was reported. The technicians were blinded to the grip-strength measurements. Blood urea nitrogen (BUN) was measured with a BUN Kinetic Procedure Kit (Thermo Electron, Louisville, CO.).

Production of high titer adeno-associated virus (AAV): The miR-23a~27a~24-2 was cloned into cis-plasmid pTR-UF5 vector (pTR-miR-23a~27a~24-2) with CMV promoter by Emory Integrated Genomics Core. Two controls were prepared. The miR-mimic-control or GFP gene were each cloned into the pTR-UF5 vector to produce the two AAV-controls. Cells transfected with either control showed the same miR expression, so to facilitate tracking we used AAV-GFP for the experiment control virus. The AAV was produced and tittered by the Viral Vector Core of the Emory using three plasmids system: pTR-miR-23a~27a~24-2 or pTR-GFP, pAAV2-9 (p5E18-VD2-9, AAV

trans-plasmid containing AAV rep and cap genes), and pDF6 (adenovirus helper plasmid). The stock virus titer for AAV-miR23 is $3.4x10^{14}$ and $6.5x10^{14}$ for AAV-control. 15 μl of viral preparation (10^{10}) was injected into TA muscle over 5 min using a Hamilton syringe with 30 gauge needle (23). Only left legs of the mouse were injected with rAAV.

Western blot and antibodies: Equal amounts of protein from TA muscle or whole kidney were used for Western blot (25, 26). Protein bands were scanned and quantified using the Li-cor Odyssey infrared scanning system (Li-COR Biosciences, Lincoin, Nebraska). Primary antibodies (1:1000 dilution except indicated): Akt (C67E7), p-Akt Ser473 (D9E), FoxO1 (75D8), p-FoxO1 (Thr24, #9464), pSmad2/3 (D27F4) and Smad2/3 (D7G7) are from Cell Signaling (Danvers, MA). Type I collagen (131001; Southern Biotech, Birmingham, AL), GFP and PTEN (FL-403) are from Santa Cruz (Santa Cruz, CA); TGFβ (AB-100-NA) is from RD (R&D Systems, Inc. Minneapolis, MN), GAPDH is from Millipore (Burlington, MA). COL4A1(ab6586), TRIM63/MuRF1 (ab77577), FBXO32/atrogin-1 (ab168372) and Myostatin (ab71808) are from Abcam (Cambridge, MA); αSMA (A2547), fibronectin (F3648), vimentin (V2258) and actin (A2066) are from Sigma-Aldrich (St. Louis, MO). Actin cleavage was detected by an antibody against the C-terminal actin fragment (recognizes the c-terminal 11 amino acids of actin; 1:500 dilution) (27).

Exosome isolation, NanoSight measurement and in vivo imaging: serum was 5X diluted with PBS and Cell debris and organelles were eliminated by centrifugation at 1,000 g for 10 min, 4°C. The supernatant fraction was further centrifuged at 16,000 g for 30 min. The second supernatant was sterile filtered through a 0.22 μm filter. Exosomes were pelleted at 120,000 g for 90 min at 4°C (L8-70M ultracentrifuge, Beckman-Coulter, Indianapolis IN). Finally, the exosome pellets were re-suspended in 100-400 μl PBS. Protein content was quantitated using a Bradford protein assay. The exosomal protein marker, TSG101, was assessed by Western blot (Figure 6D). Exosome concentration and size (Supplement Figure 2) were measured with NanoSight instruments which performs nanoparticle tracking analysis (NanoSight NS300, Malvern Instruments, Inc. Westborough, MA). *In vivo* exosome distribution imaged was taken with the Bruker Small Animal Optical Imaging System (In-Vivo Xtreme II; Billerica, MA).

Quantitative PCR (qPCR): Total RNA from muscle and kidney were extracted using Tri-Reagent (Molecular Research Inc., Cincinnati, OH). Exosomal RNA was isolated using miRNAeasy kit (217004, Qiagen Sciences, Germantown, MD) and quantified using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE). For mRNA expression, total RNA (1-2 μg) was reverse transcribed using a Thermoscript RT-PCR kit (Invitrogen Carlsbad, CA). Real-time qPCR was performed with the SYBR Green PCR reagent (Bio-Rad, Hercules, CA) and the following PCR parameters: 94°C for 2 minutes and 40 cycles at 94°C for 15 seconds, 55°C for 30 seconds, 72°C for 30 seconds with final extension at 72°C for 10 minutes (24, 28). The Cq (threshold cycle) was defined as the number of cycles required for the fluorescence signal to exceed the detection threshold. Individual mRNA expression was standardized to 18S gene and expression was calculated as the different between the threshold values of the two genes (ΔΔcq). Melting curve analysis was always performed during real-time qPCR to analyze and verify the specificity of the reaction. Primers for mRNA were listed in Table 2. For microRNA, RNA was reverse transcribed using a universal cDNA synthesis kit II (Exiqon 203301, Wobum, MA), the primers were purchased from Exiqon. Real-time qPCR was performed with the ExiLENT SYBR green master mix (Exiqon 203421). Expression of individual miR-23a-3p, miR-27a-3p and miR-24-3p were standardized to the mouse U6 mRNA and calculated as the difference between the threshold values of the two genes (ΔΔcq). The expression of individual miRs in serum exosome was normalized to miR-103a.

Muscle and kidney histology: Tissues were fixed in 3.7 % formaldehyde/PBS (pH 7.4), and dehydration, paraffinization and section at Histology Lab of Department of Medicine. Masson trichromatic staining was performed with a Masson modified IMEB stain kit (K7298, IMEB inc. San Marcos, CA). Images were visualized with an Olympus 1X 51 inverted microscopes and captured by DP73-1-51-17MP color camera. Collagen area (blue color) in kidney was measured using the CellSens Dimension 1.9 with count & measure full Software (Olympus, Melville, NY, USA) and calculated from 10 individual field.

For Immunohistology: muscles were embedded under Tissue Freezing Media (TBS; Fisher, Pittsburgh, PA, USA) in isopentane cooled in dry ice. Cross sections (10

mm) from the mid-belly of different muscles were mounted on gelatin-coated slides were fixed in 4% paraformaldehyde for 10 min. Tissue was permeabilized in 0.05% Triton X-100 (in PBS) for 10 min, and quench-fixed in 50 mM NH4Cl for another 10 min. Samples were blocked with 5% bovine serum albumin for 1 h, followed by incubation overnight with primary antibody. Sections were subsequently washed as incubated for 60 min with FITC-labeled anti-rabbit IgG (111-095-144; diluted 1:100; Jackson Immuno Research Lab, West Grove, PA). Nuclei were stained by DAPI. Images were visualized with an Olympus 1X51 inverted fluorescence microscope and captured by DP73-1-51-17MP color camera. Muscle fiber cross-sectional area was determined in TA muscles using an anti-laminin antibody (1:50 dilution; Sigma-Aldrich) and at least 500 individual myofibers per muscle were measured.

Primary muscle satellite cell culture: Satellite cells were isolated from the hindlimb muscles of 4 month old mice. A Skeletal Muscle Dissociation Kit (130-098-305; MACS, Miltenyi Biotec, Inc. Auburn, CA) was used to dissociate mouse skeletal muscle tissue into cell suspensions and a Satellite Cell Isolation Kit (130-104-267, MACS) was used to isolate satellite cells. Isolated satellite cells were passaged a maximum of 5 times. Cells were cultured in Ham's F-10 Nutrient Mixture medium (Invitrogen) with 20% fetal bovine serum, 100 u/ml penicillin, 100 µg/ml streptomycin (growth medium). Myotube differentiation was inhibited by the addition of 5ng/ml human β-fibroblast growth factor (FGF, Atlanta Biologicals, Atlanta, GA) to the media. FGF was removed for 2 days before the experiments. Immunohistochemistry was used to assess the cell purity. Satellites were stained with anti-eMyHC or anti-α-smooth muscle actin (Sigma) to identify purification and contaminating fibroblasts which were minimal (18).

Luciferase reporter assay and transfection (29): Effectene transfection reagent was used for transfection (Qiagen, Valencia, CA). Firefly and Renilla luciferase activities were measured by dual-luciferase assays (Promega) using TD-20/20 Luminometer (Turner designs, Sunnyvale, CA). The luciferase report vectors (pMIR-REPORT Luciferase) were purchase from Applied BIOSYSTEMS (Waltham, MA) and constructs were made by Emory Integrated Genomics Core. miR-23a/27a mimic (RIDIAN mmu-miR-23a/27aa-3p) and miR-23a/27a inhibitor (sh-miR-23a/27a, RIDIAN

mmu-miR-23a/27ab-3p–hairpin inhibitor) were purchased from GE Healthcare Dharmacon Inc (Chicago, IL).

Statistical analysis: Data were presented as mean ± se. To identify significant differences between two groups, comparisons were made by using the paired t-test. When multiple treatments were compared, ANOVA was performed with a post hoc analysis by the Student-Newman-Keuls test. The relationship between muscle and kidney GFP intensity was calculated by linear regression modeling. Differences with P values < 0.05 were considered significant. *Acknowledgments*

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Disclosure

All the authors declared no competing interests.