MATERIALS AND METHODS

Microvessels isolation:
The MVs were isolated as previously described. Briefly, rat cortical tissue free of large superficial blood vessels was homogenized in ice cold Dulbecco’s phosphate buffer saline (DPBS) (Life Technologies Corporation, NY, USA), centrifuged at 3300 × g for 15 min, and the pellet was resuspended in 17.5% dextran (Therma Fisher Scientific, Waltham, MA). The suspension was passed through a 300 µm filter (pluriSelect Life Science, CA, USA), and the filtrate was centrifuged at 7900 × g for 15 min. The contaminated myelin was removed, the MV pellet was resuspended in 2% bovine serum albumin (BSA) (Sigma-Aldrich, St Louis, MO) and filtered through a 70 µm filter (Corning Incorporated, NY, USA). To obtain the contamination-free MVs, the resulting sample was initially centrifuged at 13000 × g for 15 min, with a final clean-up with 17.5% dextran followed by 2% BSA. Finally, the MV pellet was resuspended in DPBS and stored at -80°C. The purity of the MV preparation has been verified by light and electron microscopy, immunostaining, and western blotting, and our studies of mitochondrial respiration have shown that the MVs are viable for ex vivo studies.

Switching Mechanism at 5′ end of RNA Template (SMART)-Seq bulk RNA-Seq:
RNA isolation: Total RNA from the male and female rat MVs were isolated using the RNeasy Micro kit (QIAGEN, Germantown, MD), according to the manufacturer’s instructions. The quality and the quantity of the RNA was assessed using the Agilent 2100 Bioanalyzer.

RNA sequencing: Total RNA samples were quantitated using Qubit RNA HS Assay kit (Invitrogen #Q32855). A RNA integrity number (RIN) for each sample was determined by Agilent 2100 Bioanalyzer using an RNA Pico Chip (Manual #G2938-90046 Rev. C). Ten nanogram (ng) of each sample was used to make SMART-Seq libraries following the SMART-Seq Stranded Kit User Manual (Takara Bio, Mountain View, CA). The first step in the cDNA synthesis protocol is fragmentation of the total RNA. Fragmentations at 85°C were determined by RIN: RINs > 7 were fragmented 6 min, while RINs < 7 were fragmented for 4 min. cDNA was generated using random primers. Cytoplasmic rRNA was removed enzymatically using mammalian ribosomal specific probes. Final SMART cDNA libraries were quantitated using
Qubit dsDNA HS assay kit (Thermo Fisher Scientific). The quality of each library was determined by running each on an Agilent 2100 Bioanalyzer using the DNA HS kit (Agilent: Guide G2938-90321 Rev. B, Kit #5067-4626). Smear analysis was performed using Agilent 2100 Expert Software (Version B.02.09.SI725 [SR1]) with a range of 200-600 bp to determine the average library. Size and concentration were then used to calculate the molarity of each library. Each library was pooled and denatured following the standard normalization method in the Illumina Denature and Dilute Libraries Guide for the NextSeq System (Illumina Part #15048776). Finally, denatured libraries were loaded onto an Illumina NextSeq 550 v2 High Output Reagent cartridge (Illumina #20024907) at a final concentration of 1.8 pM in HT1 buffer. Denatured PhiX control library v3 (Illumina #FC-110-3001) was also included at 1% concentration. Paired end 75 bp single index sequencing was performed on an Illumina High flow cell v2.5 (#20024907) yielding approximately 66 M PE reads per sample. For RNA-Seq expression analysis, samples were aligned to Rat-rn6 (Rattus norvegicus version 6) using Kallisto v0.46.0. Abundance values (counts) from Kallisto were then used as input for Sleuth v0.30.0 for transcript/isoform-level analysis. Abundance values for transcripts/isoforms were collapsed for each gene and supplied to Sleuth for gene-level analysis. With Kallisto, the abundance values are normalized by the length of the transcript. Normalized count values are reported as TPMs (Transcript Per Million). The TPM values for genes in each sample were presented in columns B-G in the Supplementary Data-1. Using Sleuth, the likelihood ratio (LRT) and Wald tests (WT) were applied. The LRT models the likelihood of the data for the full (transcript abundance affected on one or more dependent variables) and reduced (transcript abundance unaffected by the treatment) models. For each transcript, the LRT then estimates the ratio of the 2 likelihoods and produces a q-value (FDR) which was used to measure significance. Unlike the LRT, which does not produce any metric equivalent to fold change between 2 conditions, the Wald test (WT) generates a q-value and a beta value. The beta value approximates the log2 fold change in expression between the 2 conditions tested. The LRT is considered a better test that the WT, so significance filtering in our analysis Core is based on LRT q-values, where genes/transcripts with q-values less than or equal to 0.05 called significant.

**Discovery-based quantitative proteomics, data analyses, and bioinformatics:**
The samples were prepared for discovery-based proteomic analysis, as described previously. Briefly, each sample was prepared by reduction and alkylation of cysteines before trypsin digestion. Tryptic peptides were labeled using a tandem mass tags (TMT) 6-plex reagent kit (Thermo Scientific, Waltham, MA), according to the manufacturer’s protocol. Off-line fractionation was used to reduce the sample complexity using basic pH reverse-phase separation. This created 12 fractions for the pooled TMT-labelled sample. Each of the 12 fractions were then run on a Dionex U3000 nano flow system coupled to a Thermo Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific). Each fraction was subjected to on-line 90 min chromatography using a gradient from 2 to 25% acetonitrile (ACN) in 0.1% formic acid (FA; ACN/FA) over the course of 65 min, a gradient to 50% ACN/FA for an additional 10 min, a step to 90% ACN/FA for 5 min and a 10 min reequilibration to 2% ACN/FA. The separation column was PicoChip REPROSIL-Pur C18-AQ, 3 mm, 120 A, 105 mm. The entire run had a 0.3 µL/min flow rate. Electrospray was achieved at 2.6 kV.

TMT data acquisition utilized an MS3 approach for data collection. Survey scans (MS1) were performed in the orbitrap. Data dependent MS2 scans were performed in the linear ion trap. TMT reporter ions were fragmented and detected in the orbitrap at a resolution of 30,000 (m/z 400). Three technical replicates were acquired. TMT data analysis was performed using Proteome Discoverer 2.2 and searched using the SEQUEST algorithm. The protein FASTA database was Rattus norvegicus SwissProt tax ID=10116, version 2017-10-25. Static modifications included TMT reagents on lysine and N-terminus (+229.163), carbamidomethyl on cysteines (+57.021), and dynamic modifications included phosphorylation of serine, threonine, and tyrosine (+79.966 Da), and dynamic modification of oxidation of methionine (+15.9949). Parent ion tolerance was 10 ppm, fragment mass tolerance was 0.6 Da, and the maximum number of missed cleavages was set to 2. High ranking of peptides were determined using an FDR of 1%.

The subsequent bioinformatic analyses were performed using GeneGo (PANTHER) and STRING prior to loading into Qiagen’s Ingenuity Pathway Analysis (IPA). IPA content information: Report Date: 31 May 2019, Report Version: 485480, Content Version: 47547484 (Release Date: 2 August 2019). Those proteins with a minimum abundance ratio > 1.5 and a
statistically significant p-value < 0.05 were considered in the high confidence analysis. Proteome Discoverer 2.4 inspects data for total number of identifications when using t-test in conjunction with Pairwise Ratio protein ratio calculation method. If there are enough identifications, as with our data, studies have shown that the normality of the data is guaranteed (https://www.mcponline.org/content/8/5/1130.short). IPA organism settings included human, mouse, and rat. The initial dataset of 1969 proteins was uploaded, filtered to the top 20% of statistical confidence, and all proteins were differentially expressed by at least 1.5 fold change, which yielded 554 “analysis ready” molecules, which were used for protein enrichment analysis of the dataset.

**Western blot analysis:**

Western blot analysis from MVs were prepared as described previously. In brief, 20 µg of samples were separated by gel electrophoresis on an SDS-PAGE gradient gel (4–20%), transferred onto a PVDF membrane, blocked with blocking buffer (Li-cor, Lincoln, NE), and incubated overnight with primary antibodies. The following primary antibodies are used: anti-lysine-specific demethylase 5D (KDM5D) (cat#ab194288), anti-eukaryotic translation initiation factor 2 subunit 3, Y-linked (EIF2S3Y) (cat#ab33207), anti-NADH:ubiquinone oxidoreductase subunit A9 (NDUFA9) (cat#ab14713), and anti-Collagen Type XXIII Alpha 1 Chain (COL23A1) (cat#ab168356) were from Abcam, Cambridge, MA; anti-DEAD-box RNA helicase 3 (DDX3) (cat#8192), anti-Minichromosome Maintenance Complex Component 2 (MCM2) (cat#3619), anti-Cyclin Dependent Kinase 2 (CDK2) (cat#2546) from Cell Signaling, Danvers MA; anti-ATP Synthase alpha (complex V) (COX5) (cat#459240) from Thermo Fisher Scientific, Waltham, MA; β-actin (cat#A5441) were from Sigma-Aldrich, St. Louis, MO. After incubation with the primary antibody, the membranes were washed and incubated with secondary antibody at room temperature for 90 min. Immunoblots were visualized using chemiluminescence (LumiGLO, Gaithersburg, MD) and autoradiography. The optical density of the specific bands was quantified, normalized to the intensity of the corresponding β-actin band using ImageJ software (version 1.50), and compared.

**Real-time RT-qPCR:**
Total RNA from the rat MVs were isolated using the RNeasy Micro kit (QIAGEN, Germantown, MD), according to the manufacturer’s instructions. The quality the RNA was assessed using the NANODROP 2000 spectrophotometer (Thermo Scientific). We used iTaq™ Universal SYBR Green One-Step kit (Bio-Rad Laboratories, Hercules, CA), and followed the manufacturer’s instructions to amplify the selected genes. The list of forward and reverse primers of the selected genes were synthesized from IDT (Coralville, Iowa) and they were listed in the Supplementary Figure 5. The housekeeping gene β-actin served as a control for quantifying the real-time PCR results. PCR product quantification was calculated by applying the SYBR-Green method. Reactions were performed in a Bio-Rad® CFX96™ machine using the following program: reverse transcription reaction at 50°C for 10 min, polymerase activation and DNA denaturation at 95°C for 1 min. Followed by 40 cycles of 95°C for 15 s, 60°C for 15 s, 72°C for 15 s. The delta Ct (ΔCT) was calculated relative to the β-actin gene. The ΔΔCT was calculated with respect average ΔCT of female MVs. The relative gene expression was represented the value of $2^{\Delta\Delta CT}$. 