**Supplemental Information**

Identification of *Vps13c* as a key regulator of mitochondrial retrograde signaling and diastolic function in the heart

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**Materials and Methods**

**Animal studies**

All animal experiments were approved by the University of Science and Technology of China (USTC) Ethics Committee. All mice were purchased from the GemPharmatech Co., Ltd. and maintained on a 12-h light/dark cycle from 8 am to 8 pm. Body composition was determined using nuclear magnetic resonance (NMR, Bruker Minispec). Echocardiography was performed 7 weeks after fed with the indicated diet to assess cardiac function. The experiments included in this study were performed on both male and female mice. Mito-TEMPO (1 mg/kg body weight) were obtained from Sigma-Aldrich (Cat# SML0737) and intraperitoneally injected every day during the feeding of HFD + l-NAME. 8 weeks old male C57BL/6J mice (The Jackson Labotory, Cat# 000664) were utilized in the studies unless specifically mentioned. B6.FVB-Tg(*Myh6*-cre)2182Mds/J mice were brought from The Jackson Labotory (Cat# 011038). *Vps13c*-flox strain was created and maintained on a C57BL/6 background. *Vps13c*-flox mice were further crossed with *Myh6*-Cre mice to generate the cardiac-specific knockout (*Vps13c*-cKO) mice.Animals were randomly assigned to each group, and experiments were performed under standard laboratory procedures of randomization and blinding. The sample size was determined empirically based on results obtained from similar experimental measurements. No samples were intentionally excluded from the analyses.

**HMDP Models**

HFpEF was induced by HFD + l-NAME feeding for 7 weeks. 8-week-old male and female mice were fed with HFD (Research Diets, Cat# D12492). Nω-Nitro-L-arginine methyl ester hydrochloride (l-NAME) (Sigma, Cat# HY-18729A) was dissolved in drinking water (0.5 g/L, pH = 7.4). For ISO-HMDP, 9-week-old female mice from 105 inbred strains were treated with isoproterenol (30 mg/kg/day) via an intra-abdominally implanted osmotic pump for 21 days. Mice were maintained on a chow diet. For HS/HS-HMDP, 8-week-old male and female mice from 100 inbred strains were treated with high-fat/high-sucrose diet (Research Diets, Cat# D12266B) for 8 weeks. Upon sacrifice, tissues were weighed and instantly frozen in liquid nitrogen.

**Statistical Analysis**

Statistical analysis was performed using GraphPad Prism software version 10.4.0 Computational procedures including heatmaps and principal component analysis were carried out using R (v.4.4.3). Spearman's rank correlation was used to examine correlations. Single comparisons between two groups were performed using two-tailed Student’s *t*-tests with 95% confidence intervals. All analysis was performed with R or GraphPad Prism and p-value < 0.05 was considered statistically significant. Multiple comparisons were assessed using an ordinary 1-way ANOVA or using a 2-way ANOVA with Sidak's multiple comparison correction. All data presented as mean ± SEM. Values were considered significant at p < 0.05. \*p<0.05, \*\*p<0.01, and \*\*\*p<0.001.

**Expression Quantitative Trait Loci (eQTL) Analysis and Systems Genetics Approach**

Genome-wide association study (GWAS) for gene expression wasperformed with FaST-LMM (fast spectrally transformed linear mixed models), associating log‑transformed transcript levels of annotated genes with high‑quality nucleotide polymorphisms (SNPs) across ~100 inbred mouse strains of the HMDP. To delineate trans‑eQTLs, we considered SNPs > 1 Mb from their target gene or located on different chromosomes, applying a permutation‑based threshold p value < 4.1e-6. To delineate cis‑eQTLs, we considered SNPs < 1 Mb from their target gene on the same chromosome, applying a permutation‑based threshold p value < 1e-3. To focus on mitochondrial genomic regulation, we first curated a list of nuclear‑encoded mitochondrial genes from MitoCarta3.0, resulting in ~1,100 transcripts consistently expressed in heart tissue. We then extracted cis- and trans-eQTL associations for this mitochondrial gene set and mapping all the genes on the same plot with at least one significant eQTL effect exceeded permutation‑based expectation.

**Transthoracic Echocardiography**

Trans-thoracic echocardiography was conducted with Vevo 3100 high-frequency, high-resolution digital imaging system (FUJIFILM VisualSonics, Vevo F2, Canada). The mice were anesthetized and maintained with 1-2% isoflurane in 95% oxygen. The heart rate was maintained at 450-500 bpm. A parasternal short-axis view as indicated by the presence of papillary muscles was used to obtain M-mode images for analysis of fractional shortening, ejection fraction, and other cardiac functional parameters. An apical four-chamber view was used to obtain tissue Doppler imaging (TDI) mode and pulse-wave Doppler (PWD) mode for analysis of myocardial velocity and blood flow velocity, respectively. All parameters were measured at least three times. After the test all mice recovered from anesthesia without difficulties and were returned to the original cages immediately. Echocardiographic results in the different groups of mice were listed in Table 1.

**Exercise Exhaustion Test**

After three days of acclimatization to treadmill, an exercise exhaustion test was performed to determine exercise tolerance. Mice ran on the treadmill (20°) at a warming-up speed of 5 m/min, and the speed was increased gradually to 18 m/min. The speed was kept at 18 m/min until the mice were exhausted. Exhaustion was defined as the inability of the mice to return to running within 10 s of direct contact with an electric-stimulus grid. Running time was recorded and running distance was calculated. Anesthesia was not required, and mice were returned to original cages immediately after the test.

**ATP Measurement**

ATP levels in heart tissue were measured using a ATP assay kit (Beyotime, Cat# S0026) according to the manufacturer’s instructions. Briefly, samples were lysed and homogenized. Following lysis, samples were centrifuged at 12,000 × g for 5 minutes at 4°C. The supernatant was mixed with the ATP detection reagent, and luminescence was measured using a microplate reader (Molecular Devices, SpectraMax iD5). ATP concentrations were calculated based on a standard curve generated with known ATP standards.

**Isolated** **Heart Perfusion and 13C Nuclear Magnetic Resonance (NMR) Spectroscopy**

Isolated mouse hearts were perfused in Langendorff mode as previously described. In brief, mice were heparinized (100 U intraperitoneally) and anesthetized with sodium pentobarbital (150 mg/kg intraperitoneally). The hearts were quickly mounted on a Langendorff apparatus and perfused at a constant perfusion pressure of 80 mmHg with Krebs-Henseleit (KH) buffer containing (mM): 118 NaCl, 25 NaHCO3, 5.3 KCl, 2 CaCl2, 1.2 MgSO4, 0.5 EDTA and 1 of the following 2 different combinations of substrates: “glucose/pyruvate buffer” (containing 10 mM glucose and 0.5 mM pyruvate) or “mixed-substrate buffer” (containing 5.5 mM glucose, 0.4 mM mixed long-chain fatty acids bound to 1% albumin, 1 mM lactate, and 50 μU/mL insulin). The perfusate was equilibrated with 95% O2 and 5% CO2 (pH = 7.4) and maintained at 37.5°C throughout the experimental protocol.

Isolated mouse hearts were perfused with unlabeled mixed-substrate buffer for 15 minutes and 13C-labeled mixed-substrate buffer containing [1,6-13C] glucose (5.5 mM; Cambridge Isotope Laboratories) and [U-13C] mixed long-chain fatty acids ﻿(0.4 mM; Cambridge Isotope Laboratories) for 40 minutes. At the end of the perfusion protocol, hearts were freeze-clamped with Wollenberger tongs precooled in liquid nitrogen. Tissues were extracted with perchloric acid and neutralized by KOH. After lyophilization, dry extracts of each heart were dissolved in deuterium oxide (D2O) and loaded into a 3-mm NMR tube. Proton-decoupled 13C NMR spectra of tissue extracts were obtained using a vertical wide bore Bruker Spectrometer (AV III-800 MHz). Spectra were generated by Fourier transformation following multiplication of the free-induction decays (FIDs) by an expobnential function. The peak areas of the C3 and C4 of glutamate were quantified using ACD/Labs 1D NMR Manager (ACD/Labs, Toronto, Canada). The contributions of each labeled substrate and the total of the unlabeled exogenous and endogenous substrates to oxidative metabolism were determined by modeling the tricarboxylic acid (TCA) cycle flux using the peak areas of the C3 and C4 of glutamate (TCACALC, Dallas, TX). To track the intracellular fate of glucose, the enrichment patterns of C3-13C alanine and C3-13C lactate were analyzed using Lorentzian line fitting and normalized by the peak areas of 13C4-glutamate (S, D34). Ratios were then referenced and normalized to the myocardial ATP concentration.

**Transmission Electron Microscopy (TEM)**

Hearts were collected from mice, immediately after euthanasia by isoflurane overdose, and the left ventricular free wall was dissected as ~1 mm³ tissue blocks. Tissues were fixed at 4℃ in a buffer containing 2.5% glutaraldehyde for 6-12h. After primary fixation, samples were rinsed in PBS for 1–6 h and post-fixed in 1% osmium tetroxide (TED PELLA INC, Cat# 18456) for 1–2 h at room temperature. Samples were dehydrated in a graded ethanol series (Sinopharm Chemical Reagent Co., Ltd., Cat# GB/T 678‑2002): 30% ethanol for 10 min, 50% ethanol for 10 min, 70% ethanol containing uranyl acetate for 3 h, 80% ethanol for 10 min, 95% ethanol for 15 min, and absolute ethanol twice for 50 min each. This was followed by treatment with propylene oxide (Meryer Chemical Technology Co., Ltd., Cat# M25514) for 30 min. For resin infiltration, tissues were incubated in a 1:1 mixture of propylene oxide and Eponate® 12 Resin (TED Pella Inc., Cat# 18005) for 1–2 h, followed by pure Eponate® 12 Resin for 2–3 h. The resin was prepared with DDSA (TED Pella Inc., Cat# 18022), NMA (TED Pella Inc., Cat# 18032), and DMP-30 (TED Pella Inc., Cat# 18042) according to the manufacturer’s instructions. Samples were polymerized at 40 °C for 12 h, then at 60 °C for 48 h. Ultrathin sections (~70 nm) were cut using an ultramicrotome (Leica UC-7), mounted on copper grids, and stained with uranyl acetate and lead citrate trihydrate (TED Pella Inc., Cat# 19312). Sections were imaged using a JEOL 1400, operated at 120–200 kV. Mitochondrial area, density, and cristae density were quantified using ImageJ software with electron-dense thresholding. Mitochondria-associated membrane (MAM) contact length was measured as the total length of ER-mitochondria apposition within 25 nm distance.

**RNA Extraction and Reverse Transcription-quantative PCR (RT-qPCR)**

Upon euthanasia, tissues were instantly frozen in liquid nitrogen. Frozen tissue was homogenized in trizol (1 mL/20 mg tissue weight; Invitrogen, Cat# 15596026) using Tissuelyser-24L (Shanghai Jingxin). Cells were homogenized in trizol. Then the homogenate was mixed with chloroform (200 µL per 1 mL trizol) for phase separation. Samples were incubated at room temperature for 2–3 minutes before centrifugation at 10,000 g for 15 min at 4°C. The upper aqueous phase was carefully transferred to an RNase-free microcentrifuge tube, mixed with 500 µL isopropanol (equal to the aqueous phase volume), inverted 5–6 times, and incubated at room temperature for 10 min. After centrifugation at 10,000 rpm for 15 minutes at 4°C, the RNA pellet was washed with 750 µL 70% ethanol by gentle pipetting, followed by centrifugation at 10,000 rpm for 15 min at 4°C. The ethanol was discarded, and the pellet was air-dried at room temperature until nearly transparent before downstream use. Total RNA was dissolved in approximately 50 µL RNase-free water and RNA concentration was examined using a Multiskan SkyHigh microplate spectrophotometer (Thermo Scientific). 1 µg of total RNA per sample was reverse transcribed using a HiScript III RT SuperMix (Vazyme, Cat# R323-01-AC) with random primers. Reverse-transcribed cDNA was then diluted in water (10x dilution) for quantitative PCR analysis.

Quantitative PCR was carried out using a AceQ qPCR SYBR Green Master Mix (Vazyme, Cat# Q111). Samples were run on a LightCycler® 96 (Roche) and analyzed with the Roche LightCycler 1.5.0 Software. qPCR targets were normalized to *Actin* and quantified using the delta Ct method. All qPCR primer sequences were obtained from PrimerBank (<https://pga.mgh.harvard.edu/primerbank/>). Sequences of qPCR primers are listed in Table 2.

**Subcellular Fractionation of Heart Tissue**

Freshly excised mouse left ventricular tissues were homogenized in ice-cold buffer (220 mM mannitol, 70 mM sucrose, 10 mM HEPES, pH=7.4, 1 mM EGTA) with protease/phosphatase inhibitors using a Potter-Elvehjem homogenizer on ice. The homogenate was centrifuged at 700g for 10 min to pellet nuclei. To obtain cytosol: centrifuge supernatant at 100,000 g for 1 h. Collect supernatant for cytosol. The post-nuclear supernatant prior to ultracentrifugation was centrifuged at 9,000 g for 10 min to pellet crude mitochondria/MAM. This pellet was processed with the Mitochondria Isolation Kit (Beyotime, Cat# C3606) following manufacturer’s instructions to yield purified mitochondria/MAM. The resulting post-mitochondrial supernatant was retained for subsequent ER isolation using the ER Isolation Kit (Bestbio, Cat# BB-36051) according to the manufacturer’s protocol.

To isolate MAM, prepare a 30% Percoll solution: 225 mM mannitol, 25 mM HEPES pH = 7.4, 1 mM EGTA, 30% Percoll. Layer 0.5 mL crude mitochondrial/MAM resuspension under 6 mL Percoll cushion in ultracentrifuge tube and top it with 0.5 mL homogenization buffer. Centrifuge at 95,000 g for 30 min (SW55Ti rotor, approx. 40 K rpm) at 4 °C. Pure mitochondria as dense pellet or lower darker band; MAM resets as lighter band above mitochondria. Western blot each fraction.

**Mitochondrial Function Analysis**

Real-time measurement of oxygen consumption rate (OCR) in isolated mitochondria was carried out using the XF24 Extracellular Flux Analyzer (Agilent). Upon sacrifice, the heart was instantly washed in cold PBS and homogenized in MSHE buffer. Mitochondria were isolated by dual centrifugation (800 g and 8000 g) and resuspended in MAS buffer. One to three micrograms mitochondria were transferred to a XFe24 microplate and centrifuged at 2000 g for 10 min at 4°C. After centrifugation, all wells in the microplate were filled with MAS to a final amount of 500 μl containing pyruvate/malate (20 mM/4 mM, for complex I respiration) or succinate/rotenone (20 mM/4 μM, for complex II respiration). The microplate was placed in a CO2-free incubator at 37°C for 10 min and then transferred to the XFe24 Analyzer. The OCR was measured in response to sequential injection of ADP (4 mM), oligomycin (2.5 μg/ml), trifluorocarbonyl cyanide phenylhydrazone (FCCP, 4 μM), and antimycin A (4 μM), at 37°C. OCR was normalized per protein content using a Bradford assay.

**UPLC-MS/MS**After 4 hours of fasting, mice were euthanized with isoflurane and blood was collected immediately by retroorbital bleeding in a BD Microtainer. Plasma was collected after 10,000 rpm for 5 min at 4°C. Heart tissues were frozen in liquid nitrogen until analysis. All the standards were obtained from Sigma-Aldrich (St. Louis, MO, USA), Steraloids Inc. (Newport, RI, USA) and TRC Chemicals (Toronto, ON, Canada). All the standards were accurately weighed and prepared in water, methanol, sodium hydroxide solution, or hydrochloric acid solution to obtain individual stock solution at a concentration of 5.0 mg/mL. Appropriate amount of each stock solution was mixed to create stock calibration solutions. Formic acid was of Optima grade and obtained from Sigma-Aldrich (St. Louis, MO, USA). Methanol (Optima LC-MS), acetonitrile (Optima LC-MS), and isopropanol (Optima LC-MS) were purchased from Thermo-Fisher Scientific (FairLawn, NJ, USA). Ultrapure water was produced by a Mill-Q Reference system equipped with a LC-MS Pak filter (Millipore, Billerica, MA, USA).

Sample Preparation and Instrumentation

Each tissue sample (~10 mg) that was harvested and stored in an Eppendorf Safelock microcentrifuge tube, was mixed with 10 pre-chilled zirconium oxide beads and 20 μL of deionized water. The sample was homogenated for 3 minutes and 120ìL of Methanol containing internal standard was added to extract the metabolites. The sample was homogenated for another 3 minutes and then centrifuged at 18000 g for 20 minutes. Then the supernatant was transferred to a 96-well plate. The following procedures were performed on a Eppendorf epMotion Workstation (Eppendorf Inc., Humburg, Germany). 20 μL of freshly prepared derivative reagents was added to each well. The plate was sealed and the derivatization was carried out at 30 °C for 60 mins. After derivatization, the sample was evaporated for 2h. 330 μL of ice-cold 50% methanol solution was added to reconstitute the sample. Then the plate was stored at -20 °C for 20 minutes and followed by 4000 g centrifugation at 4 °C for 30 minutes. 135 μL of supernatant was transferred to a new 96-well plate with 10 μL internal standards in each well. Serial dilutions of derivatized stock standards were added to the left wells. Finally the plate was sealed for LC-MS analysis.

A ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) system (ACQUITY UPLC-Xevo TQ-S, Waters Corp., Milford, MA, USA) was used to quantitate all targeted metabolites in this project. The optimized instrument settings are briefly described in Supplementary Information Table 3. The instrument performance optimization and routine maintenance were performed every week.

Analytical Quality Control Procedures

The rapid turnover of many intracellular metabolites makes immediate metabolism quenching necessary. The extraction solvents are stored in -20 °C freezer overnight and added to the samples immediately after the samples were thawed. We use ice-salt bath to keep the samples at a low temperature and minimize sample degradation during sample preparation. All the prepared samples should be analyzed within 48 hours after sample extraction and derivatization. A comprehensive set of rigorous quality control/assurance procedures is employed to ensure a consistently high quality of analytical results, throughout controlling every single step from sample receipt at laboratory to final deliverables. The ultimate goal of QA/QC is to provide the reliable data for biomarker discovery study and/ or to aid molecular biology research. To achieve this, three types of quality control samples i.e., test mixtures, internal standards, and pooled biological samples are routinely used in our metabolomics platform. In addition to the quality controls, conditioning samples, and solvent blank samples are also required for obtaining optimal instrument performance. Internal standards were added to the test samples in order to monitor analytical variations during the entire sample preparation and analysis processes. The Pooled QC samples were prepared by mixing aliquots of the study samples such that the pooled samples broadly represent the biological average of the whole sample set. The QC samples for this project were prepared with the test samples and injected at regular intervals (after every 14 test samples for LC-MS) throughout the analytical run.

Reagent blank samples are a mixture of solvents used for sample preparation and are commonly processed using the same procedures as the samples to be analyzed. The reagent blanks serve as a useful alert to systematic contamination. As the reagent blanks consist of high purity solvents and are analyzed using the same methods as the study samples, they are also used to wash the column and remove cumulative matrix effects throughout the study. The calibrators consist of a blank sample (matrix sample processed without internal standard), a zero sample (matrix sample processed with internal standard), and a series of seven concentrations covering the expected range for the metabolites present in the specific biological samples. LLOQ and ULOQ are the lowest and highest concentration of the standard curve that can be measured with acceptable accuracy and precision.

Sample Run Order and Sample Control Procedure (ISO9001, QAIC/CN/170149)

To diminish analytical bias within the entire analytical process, the samples were analyzed in group pairs but the groups were analyzed randomly. The QC samples, calibrators, and blank samples were analyzed across the entire sample set. Each sample received was accessioned into Metabo-Profile LIMS system and was assigned by the LIMS a unique identifier, which was associated with the original source identifier only. This identifier was used to track all sample handling, tasks, results etc. The samples and aliquots were bar-coded and tracked by the LIMS system. All portions of any sample were automatically assigned their own unique identifiers by the LIMS when a new task was created; the relationship of these samples was also tracked. All samples were maintained at -80 °C until processed. Metabo -Profile will help customers store the remaining samples for up to six months before return or disposal. A formal disposal form authorized by the customer should be acquired.

Data Control Procedure (ISO9001, QAIC/CN/170149)

The data retained on instrument control computers are immediately removed and transferred to a local data server (Metabo-Profile) for further data analysis located in a locked room. Metabo-Profile will not share any information with other customers until permitted. The data will be retained on Metabo-Profile server for up to 6 months.

Data Analysis

The The raw data files generated by UPLC-MS/MS were processed using the TMBQ software (v1.0, Metabo-Profile, Shanghai, China) to perform peak integration, calibration, and quantitation for each metabolite. The self-developed platform iMAP (v1.0, Metabo-Profile, Shanghai, China) was used for statistical analyses, including PCA, univariate analysis and pathway analysis, et al.. Mass spectrometry-based quantitative metabolomics refers to the determination of the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration (i.e., calibration curve). The calibration curve is a plot of how the analytical signal changes with the concentration of the analyte (the substance to be measured). For most analyses a plot of instrument response vs. concentration will show a linear relationship. This yields a model described by the equation y = ax + b, where y is the instrument response e.g., peak height or area, a represents the slope/sensitivity, and b is a constant that describes the background. The analyte concentration (x) of unknown samples may be calculated from this equation1.

Detailed information about chemicals and reagents, sample preparation, instrumentation, analytical quality control procedures, sample run order, sample control procedure (ISO9001, QAIC/CN/170149) and data control procedure (ISO9001, QAIC/CN/170149) are provided in Table 3.

**RNA-seq Library Preparation, Sequencing, and Data Processing**

Total RNA was isolated using the Trizol Reagent (Invitrogen), after which the concentration, quality and integrity were determined using a NanoDrop spectrophotometer (Thermo Scientific). Three micrograms of RNA were used as input material for the RNA sample preparations. Sequencing libraries were generated according to the following steps. Firstly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in an Illumina proprietary fragmentation buffer. First strand cDNA was synthesized using random oligonucleotides and Super Script II. Second strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities and the enzymes were removed. After adenylation of the 3’ ends of the DNA fragments, Illumina PE adapter oligonucleotides were ligated to prepare for hybridization. To select cDNA fragments of the preferred 400-500 bp in length, the library fragments were purified using the AMPure XP system (Beckman Coulter, Beverly, CA, USA). DNA fragments with ligated adaptor molecules on both ends were selectively enriched using Illumina PCR Primer Cocktail in a 15 cycle PCR reaction. Products were purified (AMPure XP system) and quantified using the Agilent high sensitivity DNA assay on a Bioanalyzer 2100 system (Agilent). The sequencing library was then sequenced on NovaSeq 6000 platform (Illumina) Shanghai Personal Biotechnology Co. Ltd. Detailed information about transcriptome analysis flow are provided in Supplementary Materials and Methods.

**Histology**

After mice euthanasia, hearts and intestines were harvested and fixed in 4% paraformaldehyde (Solarbio, Cat# P1110) overnight and processed for routine paraffin histology. 5-μm sections were stained with hematoxylin and eosin (H&E), wheat germ agglutinin (WGA, Vector Laboratories, Cat# AS-2024), and Masson’s trichrome (MT) staining. For immunofluorescence staining, sections were incubated with first antibody against VPS13C (1:100; Proteintech, Cat# 28676-1-AP) and PDI (1:500; Proteintech, Cat# 11245-1-AP) overnight and then incubated with Fluorescein (FITC)–conjugated anti-rabbit secondary antibody (1:200; Proteintech, Cat# SA00003-2), Fluorescein (FITC)–conjugated anti-mouse secondary antibody (1:200; Proteintech, Cat# SA00003-1), and DAPI (1:500; proteintech, Cat# CM07245) for 30 min. For mitochondria staining, cells were incubated with 100 nM MitoTracker (CST, Cat# 9082S) in pre-warmed medium for 30 min at 37 °C in the dark, washed twice with PBS. For mitochondrial ROS staining, cells were incubated with 5 µM MitoSOX™ reagent (Beyotime Biotechnology, Cat# S0061S) in pre-warmed medium for 30 min at 37 °C in the dark, washed twice with PBS. Images were obtained using Zeiss confocal LSM 980 and were assessed with ImageJ software.

**Western Blotting**

Heart tissues were lysed in whole cell extraction buffer containing protease inhibitor (Thermo scientific, Cat# A32965) and homogenized using Tissuelyser-24L (Shanghai Jingxin). Protein content was measured using BCA protein assay kit (Thermo Scientific, Cat# 23225) and samples were denatured in 5x SDS-PAGE loading buffer at 95°C for 5–10 min. Samples were loaded into SDS-PAGE and separated out at 80 volts. Protein was then transferred to NC membrane (Pall, Cat# P-N66485) for 1.5–2 hours at 100 volts. Following transfer, membranes were blocked in 5% milk in TBST for 1 hour at room temperature. Membranes were then placed in primary antibodies on a shaker overnight at 4°C. Primary antibodies were used as follows (1:1000 dilution): GAPDH (Proteintech, Cat# 60004-1-Ig), TOM20 (Proteintech, Cat# 66777-1-Ig), PDI (Proteintech, Cat# 11245-1-AP), IP3R3 (Proteintech, Cat# 20729-1-AP) and Lamin A/C (Proteintech, Cat#10298-1-AP). The following day, membranes were washed in TBST then incubated with secondary antibodies (Proteintech, anti-rabbit: Cat# RGRA001, anti-mouse: Cat# SA00001-1, 1:10000 dilution) for 1.5 hours at room temperature. Blots were then washed in TBST and placed in Sensitive ECL detection solution (Proteintech, Cat# PK10012). Blots were imaged using Tanon 5200 and bands were quantified using ImageJ software. Full scans of cropped representative images were shown in Table 4.

Table 1. Echocardiographic results in the different groups of mice

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Condition | MV E/A | MV E/e’ | EF | FS | LV Mass |
| Male mice |  |  |  |  |  |
| Chow\_WT\_baseline | 1.27±0.03 | 20.62±0.71 | 54.30±1.99 | 27.70±1.30 | 102.39±4.06 |
| Chow\_*Vps13c*-cKO\_baseline | 2.43±0.21 | 34.48±1.39 | 56.70±1.59 | 29.18±1.02 | 123.22±5.40 |
| HFpEF\_WT\_7w | 1.66±0.08 | 30.28±1.71 | 58.23±1.92 | 30.13±1.27 | 118.09±6.84 |
| HFpEF\_*Vps13c*-cKO\_7w | 2.60±0.28 | 38.49±2.24 | 58.47±1.90 | 30.36±1.21 | 133.85±4.59 |
| Female mice |  |  |  |  |  |
| Chow\_WT\_baseline | 1.24±0.05 | 21.54±1.17 | 58.01±2.33 | 29.96±1.64 | 94.73±2.17 |
| Chow\_*Vps13c*-cKO\_baseline | 1.95±0.19 | 30.93±1.16 | 58.10±2.03 | 30.03±1.37 | 106.05±1.62 |
| HFpEF\_WT\_7w | 1.74±0.06 | 29.66±0.55 | 57.60±2.08 | 29.55±1.37 | 103.92±3.00 |
| HFpEF\_*Vps13c*-cKO\_7w | 2.78±0.23 | 35.42±1.18 | 58.84±2.99 | 30.60±2.10 | 114.47±3.72 |
| Male mice with MTE |  |  |  |  |  |
| HFpEF\_WT\_7w | 2.11±0.09 | 35.06±1.01 | 60.37±4.33 | 32.03±3.02 | 107.91±7.58 |
| HFpEF\_*Vps13c*-cKO\_7w | 2.83±0.18 | 40.59±1 | 61.61±2.76 | 32.81±1.92 | 134.62±5.65 |
| HFpEF\_*Vps13c*-cKO\_MTE\_7w | 1.84±0.24 | 31.7±2.16 | 55.6±3.15 | 28.69±2 | 116.38±4.61 |

Table 2. List of qPCR primers utilized.

|  |  |  |
| --- | --- | --- |
| Species | Gene | Sequence (5’-3’) |
| Mouse | *Vps13c*-F | GAAGCTAAAGTAAAAGCCCACGA |
| Mouse | *Vps13c*-R | ACACATCAGAGGTGTTGACAATG |
| Mouse | *Nppa*-F | CTTCTTCCTCGTCTTGGCCT |
| Mouse | *Nppa*-R | CTGCTTCCTCAGTCTGCTCA |
| Mouse | *Nppb*-F | CATGGATCTCCTGAAGGTGC |
| Mouse | *Nppb*-R | CCTTCAAGAGCTGTCTCTGG |
| Mouse | *Tnnt1*-F | CCTGTGGTGCCTCCTTTGATT |
| Mouse | *Tnnt1*-R | TGCGGTCTTTTAGTGCAATGAG |
| Mouse | *Tnf*-F | CCTGTAGCCCACGTCGTAG |
| Mouse | *Tnf*-R | GGGAGTAGACAAGGTACAACCC |
| Mouse | *Ndufs1*-F | AGGATATGTTCGCACAACTGG |
| Mouse | *Ndufs1*-R | TCATGGTAACAGAATCGAGGGA |
| Mouse | *Idh2*-F | GGAGAAGCCGGTAGTGGAGAT |
| Mouse | *Idh2*-R | GGTCTGGTCACGGTTTGGAA |
| Mouse | *Tgfb2*-F | TCGACATGGATCAGTTTATGCG |
| Mouse | *Tgfb2*-R | CCCTGGTACTGTTGTAGATGGA |
| Mouse | *Tnni2*-F | AGAGTGTGATGCTCCAGATAGC |
| Mouse | *Tnni2*-R | AGCAACGTCGATCTTCGCA |
| Mouse | *Col1a1*-F | GCTCCTCTTAGGGGCCACT |
| Mouse | *Col1a1*-R | CCACGTCTCACCATTGGGG |
| Mouse | *Col1a2*-F | GTAACTTCGTGCCTAGCAACA |
| Mouse | *Col1a2*-R | CCTTTGTCAGAATACTGAGCAGC |
| Mouse | *Col3a1*-F | CTGTAACATGGAAACTGGGGAAA |
| Mouse | *Col3a1*-R | CCATAGCTGAACTGAAAACCACC |
| Mouse | *Gdf15*-F | CTGGCAATGCCTGAACAACG |
| Mouse | *Gdf15*-R | GGTCGGGACTTGGTTCTGAG |
| Mouse | *Ccn4*-F | CAGCACCACTAGAGGAAACGA |
| Mouse | *Ccn4*-R | CTGGGCACATATCTTACAGCATT |
| Mouse | *Actin*-F | AGTTCGCCATGGATGACGATAT |
| Mouse | *Actin*-R | TCCAAGGCCACTTATCACCAG |

Table 3. UPLC-MS/MS instrument settings

|  |  |
| --- | --- |
| UPLC | |
| Column | ACQUITY UPLC BEH C18 1.7 μM VanGuard pre-column (2.1×5 mm) and ACQUITY UPLC BEH C18 1.7 μM  analytical column (2.1 × 100 mm) |
| Column Temp. (°C) | 40 |
| Sample Manager Temp. (°C) | 10 |
| Mobile Phases | A=water with 0.1% formic acid; and B=acetonitrile / IPA (70:30) |
| Gradient Conditions | 0-1 min (5% B), 1-11min (5-78% B), 11-13.5 min (78-95%  B), 13.5-14 min (95-100% B), 14-16 min (100% B)，16-16.1  min (100-5% B)，16．1-18 min (5% B). |
| Flow Rate (mL/min) | 0.40 |
| Injection Vol. (μl) | 5.0 |
| MASS SPECTROMETER | |
| Capillary (Kv) | 1.5 (ESI+), 2.0 (ESI-) |
| Source Temp (°C) | 150 |
| Desolvation Temp (°C) | 550 |
| Desolvation Gas Flow (L/Hr) | 1000 |

Table 4. Full scans of cropped representative images of Western Blot

图示

AI 生成的内容可能不正确。

**Supplementary** **References**

1. Xie G, Wang L, Chen T, Zhou K, Zhang Z, Li J, Sun B, Guo Y, Wang X, Wang Y, et al. A Metabolite Array Technology for Precision Medicine. *Anal Chem*. 2021;93:5709–5717. doi: 10.1021/acs.analchem.0c04686