Data S1. Supplemental Methods

**Echocardiography and Hemodynamics measurements**

Mice subjected to T2DM or control with or without DL treatment were anesthetized by inhalation of 1.5-2% isoflurane59, 60. Afterward, the cardiac structure and function were monitored using a MyLab 30CV system (Biosound Esaote, Inc.) equipped with a 15 MHz probe in a small animal ultrasound instrument. Parameters were obtained from more than three beats and then averaged. Left ventricular internal diameter at end-diastole (LVIDd), left ventricular internal diameter at end-systole (LVIDs) and E/A ratio (E/A) were tested. Hemodynamic parameters were obtained using a 1.4-French catheter-tip micromanometer catheter (SPR-839; Millar Instruments, Houston, TX, USA), which was inserted into the left ventricle (LV) through the right carotid artery. Subsequently, pressure-volume parameters were recorded using an ARIA pressure-volume conductance system (MPVS-300 Signal Conditioner, Millar Instruments, Houston, TX, USA) coupled to a Power Lab/4SPA/D converter, and then analyzed by Lab Chart 8.0 software.

**Intraperitoneal glucose and insulin tolerance test**

Intraperitoneal glucose tolerance test (GTT) was performed after 12 hours fasting. During each experiment, rodents were housed in fresh cages without food, water or nesting material. For intraperitoneal GTT, the mice received glucose injections of 1.0 mg/kg. Blood glucose concentrations were determined after 0-, 30-, 60-, and 120-min. Likewise, insulin tolerance test (ITT) was performed after 2 hours fasting. For intraperitoneal ITT, the mice received insulin injections of 0.75 U/kg. For the insulin resistance (IR) test, mice were injected with 2 g/kg of glucose and blood was collected by submaxillary puncture at 30 min after injection. Blood droplets collected from the tail vein were used for glucose measurements (Sano care, China). Insulin (Jiancheng, Nanjing) in serum were quantified using ELISA following the manufacturer’s instructions.

**Enzyme linked immunosorbent assay (ELISA)**

Blood samples were obtained from orbital vein. Serum was obtained after blood centrifugation at 1,000g for 10 min and then frozen at −80 ºC. Urine was collected from mice in the bladder. Each urine sample represented a pool of three independent mice. Urinary albumin was measured using an immunoturbidimetric method, and urinary creatinine by an enzymatic method, both on an automated analyzer. Total ketone body content was assessed using Wako Diagnostics in vitro assay following the
manufacturer’s instructions. The content of insulin, C-peptide, glycated hemoglobin (HbA1c), TC, TG, LDL-c, HDL-c in peripheral blood were analyzed by using the insulin assay kit (Jiancheng, Nanjing; H203-1-1), C peptide assay Kit (Jiancheng, Nanjing; H391-1), glycosylated hemoglobin assay kit (Jiancheng, Nanjing; A056-1-1), total cholesterol assay kit (Jiancheng, Nanjing; A111-1-1), triglyceride assay kit (Jiancheng, Nanjing; A110-1-1), LDL-c assay kit (Jiancheng, Nanjing; A113-1-1) and HDL-c assay kit (Jiancheng, Nanjing; A112-1-1). And the content of myocardial enzyme profile plasma concentrations of troponin T (TnT) was detected by TnT assay kit (Jiancheng, Nanjing; H149-3), according to the manufacturer's instructions.

**Histological preparation**

Hearts were harvested and fixed with 4% PFA overnight. Samples were de-hydrated, paraffin embedded and sectioned into 5 μm thick slices on a sliding microtome (Leica, Nussloch, Germany) and mounted on Super-frost Plus slides for H&E, Masson's trichrome and IHC staining.

**Oil Red O Staining**

For Oil Red O staining, frozen liver sections were prepared in Tissue-Tek OCT compound. Serial sections (8 μM were made and stained with 0.5% Oil Red O (G1015, Servivebio) for 30min, counterstained with hematoxylin for 5 min. The red lipid droplets were visualized by microscopy (Olympus, CX31).

**Metabolite analysis by high-performance liquid chromatography (HPLC) and mass spectrometry (MS)**

Cardiac biopsies from mice were subjected to mass spectrometric metabolomics extract a maximum of information on a wide spectrum of metabolites as described previously. The standard and reagents included: acetonitrile, isopropanol, methanol, chloroform, acetic acid, formic acid, methoxyamine hydrochloride, MSTFA - N-methyl-N-(trimethylsilyl) trifluoroacetamide, pyridine, 3-nitrophenylhydrazine, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), sulfosalicylic acid. All chemicals were purchased from Sigma-Aldrich unless otherwise noted.

To analyze cardiac tissue metabolites, deep-frozen rat LV tissue samples were weighed and solubilized in tubes with ceramic beads using 1 ml of ice-cold lysate buffer (methanol/water, 9:1 v/v). Subsequently, the samples were homogenized three times for 20 s at 5500 rpm using a Precellys 24 tissue homogenizer (Bertin Technologies, Paris, France). The mixture was centrifuged at 15000g, for 10 min at 4°C to precipitate proteins and tissue residues. The upper phase of the supernatant (300 μl) was used for
the UHPLC–MS, evaporated at 40°C in a pneumatically assisted concentrator (Techne DB3). Dried extracts were then solubilized with 200 μl deionized/MilliQ water, aliquoted or transferred in LC vials and injected into UHPLC–MS or kept at -80°C until injection.

**High-energy phosphate analysis**

Adenine nucleotides and creatine compounds were quantified by reverse-phase ion-pair liquid chromatography as previously described in detail62. Briefly, left ventricle and gastrocnemius samples were quickly excised, snap-frozen in liquid nitrogen and homogenized in perchloric acid (HClO4) solution (10% w/v) containing the internal standard, 2-chloroadenosine (2-ClAd; 1 mg/ml).

Samples were then centrifuged (16000g, 20 min, 0°C) and the pellet was resuspended in 1M NaOH for protein quantification (Bradford method), while the supernatant was collected and neutralized with potassium dihydrogen phosphate solution (1M KH2PO4, pH of 12) for analysis. After salt precipitation, samples were again centrifuged (16000g, 10 min, 0°C) and the supernatant was analyzed with a photodiode array detector (Flexar FX-10 UHPLC, PerkinElmer). The optical densities of creatine (Cr) and phosphocreatine (PCr) were recorded at 214 nm whereas adenine nucleotides were recorded at 256 nm. Samples were run in duplicate and chromatograms were analyzed using Chromera 3.2.0 software (Perkin Elmer). Free ADP concentrations were calculated as previously described from the creatinine kinase (CK) equilibrium (60, 61), using the formula: ADP = (ATP * Cr) / (PCr * H+ * KCK), assuming an equilibrium constant of (KCK) of 1.66*109 M⁻¹, and an intracellular pH of 7.2, in addition to spectrophotometrically determined concentrations of Cr, PCr rand ATP. All chemicals were purchased from Sigma-Aldrich unless otherwise stated.

**Transmission electron microscopy**

Transmission electron microscopy analysis was used to access the mitochondrial damage in heart, as previously reported63. Hearts were harvested from WT mice, in control and T2DM with or without DL treatment. 1 mm³ heart pieces from the left ventricular wall were fixed in 1.25% glutaraldehyde (v:v in 0.1 M sodium cacodylate, pH 7.2) overnight at 4°C. Heart samples were washed in 0.1 M sodium cacodylate (3 times for 30 minutes), post-fixed and thin sections were imaged on a Tecnai-20 electron microscopy (Philips-FEI, Hillsboro, Oregon). EM image showing disarrayed cristae was regarded as damaged mitochondria with loss of cristae and quantitation of damaged mitochondria in heart section. In quantification of the lipid droplet number, 10-15
random fields (10000x) per heart sample (6 mice per group) were selected and counted the number of lipid droplet in each field.

**Seahorse extracellular flux analyzer assays**

NRVMs were measured using a Seahorse XFe24 extracellular flux analyzer in intact caSMMC and caEC. We performed glycolysis stress tests and energy substrate (glucose and palmitate) oxidation with mito stress test as per the manufacturer’s instructions14. Mito stress test drugs (Oligomycin-A and FCCP) working concentrations required for caSMMC and caEC were determined with dose-titration assays. 20,000 caSMMC and 25,000 caEC per well were cultured in V-7 Seahorse plates. NRVMs were incubated in glucose-free Seahorse assay media supplemented with 1mM pyruvate at 37°C in incubator without CO2 for 1-h prior to the assay. Injectors were loaded to add 20mM glucose, 1μM Oligomycin, 1μM FCCP, 1μM Rotenone and 2μM Antimycin A during mito stress test. Exogenous glucose oxidation and others mito stress test parameters such as basal respiration, maximal respiration, spare capacity, ATP production, proton leak and non-mitochondrial respiration were calculated as oxygen consumption rate (OCR).

**cAMP assays**

As described in previous study64, isolated mouse hearts were perfused with buffer containing IBMX (100 μM) for 10-min, then switched to buffer mixed with peptide of interest. Coronary effluents were collected at timed intervals and cAMP levels measured using a cAMP enzymatic immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI). For in vitro measurements of cytoplasmic cAMP, cells (~2x10^5) were lysed with 0.1N HCL, followed by acetylation of lysates with a mixture of KOH and acetic anhydride, and cAMP concentrations were determined using the Cyclic AMP ELISA kit (Cayman Chemicals, Ann Arbor, MI). Specific inhibitors KH7 (20 μM), Ddox (50 μM) were added for 3-h, while probenecid (2 mM) was added only 15-min prior to peptide treatments.

**Isolation of Cardiac Mitochondria**

Isolation of cardiac mitochondria used Tissue Mitochondria Isolation Kit (Beyotime, C3606)65. Briefly, mouse heart ventricles were collected, minced, and incubated with trypsin before homogenization with a glass/teflon Potter Elvehjem homogenizer on ice. Ventricle homogenates were centrifuged at 800 g at 4°C for 10 min; the supernatant was collected and centrifuged at 16,000 g at 4°C for 10 min; the resulting pellet containing normal- and small-size mitochondria were washed once and centrifuged at 16, 000 g at
4°C for 10 min. After re-suspension, mitochondrial protein concentrations were colorimetrically measured using protein assay dye reagent concentrate (Bio-Rad, 500-0006) and subsequent mitochondrial functional assays were performed.

**Isolation of neonatal rat cardiomyocytes**

Neonatal rat ventricular myocytes (NRVMs) were obtained from the hearts of 1-2 days old Sprague-Dawley rats as previously described. Hearts of 1- to 3-day-old pups were excised and the atria removed. The ventricles were digested 3-4 times at 37°C for 5 minutes with 0.125% trypsin freshly suspended in calcium-free HBSS solution, pH 7.4. The supernatants containing isolated cells were collected from each digestion, and an equal volume of serum-rich medium was added to stop the digestion. Isolated cells were collected and centrifuged at 800 rpm for 5 minutes to separate non-myocardial cells. The resuspended cells were pre-plated twice for 30 minutes to further reduce fibroblast contamination. NRVMs were finally plated in 3 cm dishes or 24-well plates at a density of 0.3-1 × 10^6 cells/well, and 15% fetal bovine serum was added into the medium and cultured at 37°C and 5% CO₂. Accordingly, dulaglutide (50 nM) was incubated with NRVMs cultured in high (20 mmol/L glucose) or low (5.6 mmol/L glucose) glucose medium. And Palmitic acid (PA) (at a final concentration of 250 mmol/L in culture medium).

**siRNA transfection**

Small siRNA oligos (RiboBio, Guangzhou, China) were transfected with Lip6000 reagent (Beyotime Biotechnology, China) following manufacturer’s protocol. Briefly, 1~5×10^5 cells were inoculated into a 6-well plate or 0.5~1×10^4 cells into a 24-well plate containing appropriate amount of complete medium to achieve a cell density of 30% to 50% during transfection. After the siRNA was diluted, we added lip6000-DMEM for 5 min at room temperature, then added the mixed liquid into each well, and changed medium after transfected for 6 h, and continued to be cultured for 48-72 hours, as previous study described. Western blot was used to detect the transfection efficiency of siRNA.

**Western blot analysis**

Isolated cardiomyocytes and myocardial tissue in indicated groups were lysed in radioimmunoprecipitation assay (RIPA) buffer (Servicebio, China). The composition of the RIPA buffer (in mmol/L) was: Tris (20), NaCl (150), EDTA (5), EGTA (5), DTT (1) plus 1.0% Triton X-100 and 0.5% deoxycholate. Myocardial tissue was disrupted.
in extraction buffer (0.1 M Tris–HCl, 0.01 M EDTA, 0.04 M DTT, 10% SDS, pH 8.0) by an ultrasonic device. The composition of protease inhibitors (in mmol/L) were: Na⁺ pyrophosphate (2.5), β-glycerophosphate (1.0), Na3VO4 (1.0), PMSF (1.0), NaF (2.0), and 10μg leupeptin. One tablet of a protease inhibitor cocktail (Complete Mini, Roche, Germany) and one tablet of phosphatase inhibitor (Phos-stop, Roche, Germany) were added into the mixture with per 10 mL buffer. The buffer was adjusted to pH 7.4. Lysates to assay for protein concentration by the bicinchoninic acid assay. BSA was used to produce a standard curve. In each group, 20μg protein lysate was prepared for SDS-PAGE, and then the proteins were electrophoresed and transferred to PVDF membrane (Millipore, USA). After blocked for an hour, the primary antibody (provided in Table S1) was incubated at 4°C overnight. Next day, the chemical secondary antibody was incubated at room temperature for an hour, and the chemiluminescent protein detection developer (ECL, Broad, USA) was utilized with a chemiluminescence scanning membrane apparatus to obtain protein bands. Image J software was used to analysis western blot strips.

**RNA purification and qPCR**

Total RNA was purified from tissue or cultured cell with Trizol (Invitrogen) following manufacturer’s protocol. A part of total RNA was used for reverse transcription with SSTIII kit (Invitrogen) to generate cDNA. The cDNA is used in SYBR-based real-time qPCR on the following cycling conditions: hold for 2 min at 50°C followed by 10 min at 95°C to heat-start the Taq polymerase enzyme, then 40 cycles of 95°C for 10 min, 60°C for 1 min. Relative mRNA levels were analyzed using comparative Ct calculations normalized to GAPDH. The sequences of the primers for each gene detected are listed in Table S2.

**Statistical analysis**

Data are expressed as mean ± SEM. Statistical analyses were performed with GraphPad Prism (version 8.0, GraphPad Software, San Diego, CA). Normality assumption of the data distribution was assessed using Kolmogorov-Smirnov test. Comparisons between two groups were performed using two-tailed Student’s t test for normally distributed data and Mann-Whitney rank sum test for non-normally distributed data. Differences between multiple groups with one variable were determined using one-way ANOVA followed by post hoc Tukey’s test. To compare multiple groups with more than one variable, two-way ANOVA followed by post hoc Tukey’s test was used. All statistical
details regarding P-value and n are available in the figure legends and supplemental figure legends. In the figures, each point represents a biological replicate, and the assays are performed once if no other indication is made.
Table S1. Primary antibodies used in the study

| Antibody       | Customer | Product number | Dilution | Application |
|----------------|----------|---------------|----------|-------------|
| VDAC1          | Abcam    | Ab14734       | 1:1000   | WB          |
| GAPDH          | CST      | 2118          | 1:1000   | WB          |
| Drp1           | SANTA    | SC-32898      | 1:500    | WB          |
| pS637-Drp1     | Abcam    | ab193216      | 1:1000   | WB          |
| pSer491-AMPKα2 | Abcam    | Ab109402      | 1:500    | WB          |
| T-AMPKα2       | Abcam    | Ab3760        | 1:1000   | WB          |
| pSer485-AMPKα1 | CST      | 2537S         | 1:500    | WB          |
| T-AMPKα1       | CST      | 2603P         | 1:1000   | WB          |
| Gene   | Species | Forward primer (5’→3’)      | Reverse primer (5’→3’)          |
|--------|---------|-----------------------------|---------------------------------|
| Anp    | Mouse   | ACCTGCTAGACCACCTGGAG        | CCTTGGCTGTATCTTCGGGTACCGG       |
| Bnp    | Mouse   | GAGGTCACTCTTATCTCTCTGG      | GCCATTTCCTCCGACTTTTCTC          |
| β-MHC  | Mouse   | CCGAGTCCCAGGTCAACAA         | CTTCACGGGCACCCTTGGGA            |
| Collagen I | Mouse | AGGCTTCAGTGGTTGGATG        | CACCAACAGCACCATCGTGA            |
| Collagen III | Mouse | GTCAGCTGGATAAGCGACA        | GAAGCACAGGAGCAAGGTGTA          |
| Ctgf   | Mouse   | AGGGCCTCTTCTCGGATTTCC      | CTTTGAAGGACTCACCCTGCT          |
| Gapdh  | Mouse   | CCCTTAAGAGGGATGGCTGCC      | ACTGTGCCGTTGAATTTGCC           |
Table S3. LV echocardiography and hemodynamic parameters in T2DM or Control mice with or without Dulaglutide treatment

| Parameter  | Control-Vehicle (N=10) | Control-DL (N=10) | T2DM-Vehicle (N=12) | T2DM-DL (N=11) | P values (Post-hoc analysis (vs. T2DM-Vehicle)) | Effect size (ES) (vs. T2DM-Vehicle) |
|------------|------------------------|-------------------|---------------------|-----------------|-----------------------------------------------|-----------------------------------|
| HR (BMP)  | 462 ± 14               | 460 ± 17          | 472 ± 19            | 467 ± 12        | 0.1764                                        | -0.61                             |
| SV (μL)   | 33.9 ± 1.5             | 32.5 ± 1.3        | 25.8 ± 1.8          | 30.2 ± 1.6      | 0.0018                                        | 0.73                              |
| LVEF (%)  | 78 ± 3.8               | 79 ± 2.9          | 51 ± 2.4            | 64 ± 3.3        | 0.0095                                        | -0.61                             |
| LVFS (%)  | 39 ± 2.1               | 41 ± 1.7          | 31 ± 2.3            | 37 ± 1.8        | 0.0016                                        | 0.74                              |
| LV Mass (mg) | 111.5 ± 6.4         | 109.1 ± 5.8       | 134.2 ± 6.2         | 125 ± 5.1       | 0.0049                                        | -0.68                             |
| ESV (μL/cm²) | 41.6 ± 2.3          | 42.1 ± 2.5        | 31.1 ± 1.9          | 37.4 ± 2.2      | <0.0001                                       | 0.77                              |
| EDV (μL/cm²) | 38.9 ± 2.8          | 41.5 ± 2.2        | 27.9 ± 2.9          | 34.2 ± 1.7      | <0.0001                                       | -0.62                             |
| ESP (mmHg) | 144 ± 4.1             | 141 ± 3.9         | 157 ± 4.7           | 146 ± 3.5       | <0.0001                                       | 0.61                              |
| EDP (mmHg) | 8.1 ± 0.5             | 7.8 ± 0.7         | 12 ± 1.1            | 8.9 ± 0.7       | 0.0002                                        | -0.73                             |
| dP/dt max (mmHg/s) | 9954 ± 365       | 9748 ± 317        | 4922 ± 264          | 6961 ± 257      | 0.0026                                        | 0.72                              |
| dP/dt min (mmHg/s) | -9841 ± 276       | -9911 ± 291       | -5249 ± 173         | -7721 ± 246     | 0.0047                                        | -0.68                             |
| E/A       | 1.7 ± 0.02            | 1.8 ± 1.1         | 1.1 ± 0.03          | 1.3 ± 0.3       | 0.0020                                        | 0.66                              |
Abbreviations: dP/dt max, peak rate of pressure rise; dP/dt min, peak rate of pressure decay; ESP, end-systolic pressure; EDP, end-diastolic pressure; ESV, indexed end-systolic volume; EDV, indexed end-diastolic volume; LVEF, Left Ventricular Ejection Fractions; LVFS, Left Ventricular Fraction Shortening; SV, Stroke Volume. Data show means ± SEM of the indicated number of mice per group.
Table S4. LV echocardiography and hemodynamic parameters in T2DM or Control mice with or without Dulaglutide treatment in AMPKα2 KO mice

| Parameter            | Control-Vehicle (N=10) | Control-DL (N=10) | T2DM-Vehicle (N=12) | T2DM-DL (N=11) | Control-Vehicle | T2DM-DL | Control-DL | T2DM-DL |
|----------------------|------------------------|-------------------|---------------------|----------------|----------------|---------|------------|---------|
| HR (BMP)             | 466±12                 | 464±13            | 459±17              | 461±14        | 0.2149         | 0.3611  | 0.48       | -0.17   |
| SV (µL)              | 32.8±1.4               | 33.1±1.5          | 26.6±1.7            | 26.2±1.4      | 0.0028         | 0.5831  | 0.56       | -0.26   |
| LVEF (%)             | 79±3.1                 | 78±2.8            | 49±2.8              | 52±3.1        | 0.0086         | 0.0841  | 0.73       | -0.52   |
| LVFS (%)             | 40±1.8                 | 41±2.2            | 31±1.7              | 32±2.3        | 0.0041         | 0.6782  | 0.76       | -0.39   |
| LV Mass (mg)         | 110.3±5.5              | 109.4±4.6         | 136.5±6.9           | 134±5.7       | 0.0008         | 0.3155  | 0.77       | 0.27    |
| ESV (µL/cm²)         | 42.3±2.8               | 41.5±2.1          | 28.5±1.9            | 27.5±2.9      | <0.0001        | 0.1671  | 0.79       | -0.23   |
| EDV (µL/cm²)         | 39.2±2.3               | 40.6±2.7          | 26.5±2.1            | 25.9±2.5      | 0.0007         | 0.1834  | 0.73       | -0.19   |
| ESP (mmHg)           | 146±3.3                | 144±4.2           | 156±3.9             | 157±2.8       | <0.0001        | 0.1659  | -0.79      | 0.17    |
| EDP (mmHg)           | 7.6±0.7                | 8.1±0.9           | 14.8±1.3            | 15.1±1.2      | 0.0024         | 0.2743  | -0.69      | 0.27    |
| dP/dt max (mmHg/s)   | 10212±385              | 9828±344          | 4766±253            | 5136±241      | 0.0025         | 0.2614  | 0.73       | -0.25   |
| dP/dt min (mmHg/s)   | -9942±221              | -10316±354        | -5188±122           | -4925±148     | 0.0007         | 0.1668  | -0.72      | 0.31    |
| E/A                  | 1.6±0.05               | 1.7±0.03          | 0.9±0.07            | 0.9±0.06      | <0.0001        | 0.8028  | 0.64       | -0.08   |

Abbreviations: dP/dt max, peak rate of pressure rise; dP/dt min, peak rate of pressure decay; ESP, end-systolic pressure; EDP, end-diastolic pressure; ESV, indexed end-systolic volume; EDV, indexed end-diastolic volume; LVEF, Left Ventricular Ejection Fractions; LVFS, Left Ventricular Fraction Shortening; SV, Stroke Volume. Data show means ± SEM of the indicated number of mice per group.
Figure S1. Effects of DL dose gradient on LV systolic function in T2DM mice.

(A). Representative M-model echocardiography in T2DM mice received 8 weeks of DL dose gradient or vehicle treatment. (B). Left ventricular ejection fraction was analyzed in T2DM mice. n=6 per group, significance was assessed by one-way ANOVA and Tukey’s post hoc test. Data are shown as the mean±SEM.
Figure S2. Efficacy and Safety of Dulaglutide in Control Mice (related to Figure 1).

(A). Glucose levels in serum samples following a glucose tolerance test (GTT) in mice fed with standard chow following vehicle or DL for 8 weeks (n=8 in chow-veh group and n=11 in chow-DL group). Significance was assessed by two-way ANOVA and Tukey’s post hoc test. (B). HbA1c levels in serum samples in mice fed with standard chow following vehicle or DL for 8 weeks (n=6 per group). Significance was assessed by student’s t test. (C). Glucose levels in serum samples following an insulin tolerance test (ITT) in mice fed with standard chow following vehicle or DL for 8 weeks (n=8 in chow-veh group and n=11 in chow-DL group). Significance was assessed by two-way ANOVA and Tukey’s post hoc test. (D-E). C-peptide and ketone body levels in serum samples in mice fed standard chow following vehicle or DL for 8 weeks (n=6 per group). Significance was assessed by student’s t test. (F-G). Mice fed with standard chow or T2DM following vehicle or DL for 8 weeks, triglycerides (TG) and high-density lipoprotein cholesterol (HDL-c) levels in serum were detected. (n=4 per group), significance was assessed by two-way ANOVA and Tukey’s post hoc test. Data are shown as the mean±SEM.
Figure S3. DL improves cardiac remodeling in Mice (related to Figure 2).

(A-C). Relative mRNA level of heart growth markers in heart tissues from mice fed with standard chow or T2DM following vehicle or DL (n=4 per group). Significance was assessed by two-way ANOVA and Tukey’s post hoc test.

(D-F). Relative mRNA level of fibrosis markers in heart tissues from mice fed with standard chow or T2DM following vehicle or DL (n=4 per group). Significance was assessed by two-way ANOVA and Tukey’s post hoc test. Each point represents a heart or a mouse.

Data are shown as the mean±SEM.
Figure S4. DL impacts upon cardiac metabolism in mice (related to Figure 3).

(A). Principle component analysis (PCA) of heart metabolome in control, T2DM and T2DM-DL, (n=3/6/6 mice in control/T2DM/T2DM-DL).

(B). Differential Abundance Score Analysis upon metabolites in T2DM-DL vs T2DM mice.

(C). mRNA expression related to cardiac metabolism and transcription factors in the indicated hearts (n=4 per group). Significance was assessed by two-way ANOVA and Tukey’s post hoc test. Data are shown as the mean±SE
Figure S5. DL impacts upon redox metabolism in NRVMs (related to Figure 3).

(A). Representative mitoROS images in indicated NRVMs received MitoSOX Red Mitochondrial Superoxide Indicator. n=3 independent experiment. Scan bar 80μm.

(B). Representative images of immunoblot analysis of SOD2 in isolated mitochondrial proteins of indicated NRVMs. n=6 per group.

(C). Flow cytometric analysis showing number of mito ROS positive NRVMs after HG+PA or DL treatment. n=3 independent experiment.
Figure S6. Effects of DL on cAMP-AMPKα and phosphorylation in hearts (related to Figure 4).

(A). cAMP activity in heart tissues was detected by ELISA assay (n=6 per group).

(B). Schematic diagram of AMPKα structure.

(C-D). Representative images of western blot and quantitative immunoblot analysis of AMPKα1 in indicated mice (n=6 per group). Significance was assessed by two-way ANOVA and Tukey’s post hoc test. Each point represents a sample. Data are shown as the mean±SEM.
Figure S7. DL regulates blood glucose and triglycerides in AMPKα2 KO mice (related to Figure 5).

(A). Verification of deletion of AMPKα2 in mice by western blot analysis of heart tissues from AMPKα2 KO mice (n=3 per group).

(B-C). TC and TG were examined in plasma isolated from AMPKα2 KO mice fed with standard chow or T2DM following vehicle or DL for 8 weeks (n=6 per group). Significance was assessed by one-way ANOVA and Tukey’s post hoc test. Each point represents a mouse. Data are shown as the mean±SEM.
Figure S8. DL preserves AMPKα2-dependent mitochondrial homeostasis in NRVMs (related to Figure 5).

(A). A schematic diagram showing the procedure of NRVMs. (B). Verification of knockdown of AMPKα2 in NRVMs by western blot analysis of cell lysates from AMPKα2 knockdown mice (n=3 per group). (C-D). Mitochondrial length of cardiomyocytes was analyzed in NRVMs from indicated group (n=16/17/47/39/38/42). Yellow arrow indicated the length of mitochondria. (E-F). Oxygen consumption rate (OCR) and quantitative statistical analysis of maximal respiration in NRVMs (n=6 per group). Significance was assessed by one-way ANOVA and Tukey’s post hoc test. Each point represents an independent experiment.

Data are shown as the mean±SEM.