ORIGINAL RESEARCH

Pyruvate Kinase M2 Protects Heart from Pressure Overload-Induced Heart Failure by Phosphorylating RAC1

Le Ni, MD; Bowen Lin, MD; Lingjie Hu, MD; Ruoyu Zhang, Bsc; Fengmei Fu, Bsc; Meiting Shen, MD; Jian Yang, PhD; Dan Shi, MD, PhD

BACKGROUND: Heart failure, caused by sustained pressure overload, remains a major public health problem. PKM (pyruvate kinase M) acts as a rate-limiting enzyme of glycolysis. PKM2 (pyruvate kinase M2), an alternative splicing product of PKM, plays complex roles in various biological processes and diseases. However, the role of PKM2 in the development of heart failure remains unknown.

METHODS AND RESULTS: Cardiomyocyte-specific Pkm2 knockout mice were generated by crossing the floxed Pkm2 mice with α-MHC (myosin heavy chain)-Cre transgenic mice, and cardiac specific Pkm2 overexpression mice were established by injecting adeno-associated virus serotype 9 system. The results showed that cardiomyocyte-specific Pkm2 deletion resulted in significant deterioration of cardiac functions under pressure overload, whereas Pkm2 overexpression mitigated transverse aortic constriction-induced cardiac hypertrophy and improved heart functions. Mechanistically, we demonstrated that PKM2 acted as a protein kinase rather than a pyruvate kinase, which inhibited the activation of RAC1 (rho family, small GTP binding protein)-MAPK (mitogen-activated protein kinase) signaling pathway by phosphorylating RAC1 in the progress of heart failure. In addition, blockade of RAC1 through NSC23766, a specific RAC1 inhibitor, attenuated pathological cardiac remodeling in Pkm2 deficiency mice subjected to transverse aortic constriction.

CONCLUSIONS: This study revealed that PKM2 attenuated overload-induced pathological cardiac hypertrophy and heart failure, which provides an attractive target for the prevention and treatment of cardiomyopathies.

Key Words: heart failure ■ protein kinase ■ pyruvate kinase M2 ■ rho family, small GTP binding protein

Pyruvate kinase M (PKM) is the key enzyme responsible for converting phosphoenolpyruvate to pyruvate at the final step of glycolysis. PKM1 and PKM2, 2 alternative-splicing isoforms of the PKM gene, differ significantly in their protein structure and biochemical functions. The tetramer of PKM2 exerts pyruvate kinase activity in glucose metabolism, whereas its monomer and dimer participate in multiple signaling pathways and cellular processes such as proliferation, migration, autophagy, and apoptosis independent of the pyruvate kinase activity.
Several studies have documented that PKM2 (pyruvate kinase M2) exerts apparent cardioprotective potential in myocardial infarction and anthracycline-induced cardiotoxicity.\(^{15,16}\) However, whether PKM2 plays any role in the progress of pressure overload HF is still unknown. In the present study, PKM2 deficiency exacerbated cardiac dysfunction of mice submitted to transverse aortic constriction (TAC), which was ameliorated by cardiomyocyte-specific PKM2 overexpression. Further investigation revealed that PKM2 inhibited the activation of RAC1 (rho family, small GTP binding protein) signaling pathway in cardiomyocytes by phosphorylating RAC1, contributing to its cardioprotective effects. Altogether, our findings highlight that PKM2 plays a novel role in protecting the heart from pressure overload-induced HF and PKM2-mediated RAC1 phosphorylation might serve as a potential target for the treatment of HF.

### METHODS

The raw data that support the findings of this study are available from the corresponding authors on request. The authors declare that all supporting data are available in the article and its online supplementary files.

#### TAC Mouse Model

All animal procedures were approved by the Ethics Committee of Tongji University School of Medicine. The surgery procedure was similar to previous studies with minor modifications.\(^{17}\) Male C57/BL mice around 8 to 9 weeks old (21–25 g) were anesthetized with isoflurane (RWD, R510-22-4), endotracheal intubated and ventilated with 1.5 mL tidal volume, and 120 to 130 breaths per minute. A heating pad was applied to maintain the body temperature of mice at 37°C until they were fully awake. In this study, we applied retrosternal approach to minimize surgical injury. After exposing the aortic arch, a 27-gauge needle was tied against the aorta arch with a 6-0 silk suture and removed immediately to establish the constriction. Sham-operated procedure underwent a similar procedure without transverse aorta ligation.

#### Generation of Cardiomyocyte-Specific Pkm2 Knockout Mice

To generate cardiomyocyte specific Pkm2 conditional knockout (Pkm2-cKO) mice, the mice with Pkm2 gene exon 10 flanked by 2 loxP sites (Pkm2\(^{floxed}\)) were purchased from the Jackson Laboratory (024048) and crossed with α-MHC (myosin heavy chain)-Cre transgenic mice expressing noninducible CRE in cardiomyocytes. Genotypes of offspring were confirmed by polymerase chain reaction with Pkm2\(^{floxed}\) and Cre-specific primers. The primer sequences for genotyping were listed in Table S1.

#### Pkm2 Overexpression Experiments

For cardiomyocyte specific gene delivery, the adeno-associated virus type 9 (AAV9)-cardiac troponin T (cTnT) system was selected. Recombinant AAV9-cTnT carrying mouse full-length Pkm2 (AAV9-cTnT-mPkm2) and scrambled AAV9-cTnT-null were constructed by Hanbio Biotechnology Co. Ltd. (Shanghai, China). One week after TAC surgery, 100 μL/mouse (1 × 10\(^{11}\) viral particles) viruses were injected via the tail vein under anesthesia as previously described.\(^{18}\)

#### Echocardiography

Transthoracic 2-dimensional M-mode echocardiography was performed with the Vevo 770 High-Resolution In Vivo Micro-Imaging System. Mice were anesthetized by isoflurane inhalation through a mask with heart rate

---

**Nonstandard Abbreviations and Acronyms**

| β-MHC | β-myosin heavy chain |
|---|---|
| cKO | conditional knockout |
| cTnT | cardiac troponin T |
| MAPK | mitogen-activated protein kinase |
| NRCM | neonatal rat cardiomyocytes |
| PKM1 | pyruvate kinase M1 |
| PKM2 | pyruvate kinase M2 |
| RAC1 | rho family, small GTP binding protein |
over 350 beats per minute. Left ventricular wall thickness and chamber diameter were measured in systole and diastole periods from a long-axis view. Left ventricular (LV) internal end-diastolic diameter, LV internal end-systolic diameter, fractional shortening, ejection fraction, and other parameters were calculated. Echocardiography was monitored once a week for a consecutive 6 to 8 weeks.

**Cardiomyocyte Isolation, Culture, and Treatment**

Neonatal rat cardiomyocytes (NRCMs) and neonatal mouse cardiomyocytes were dissociated by enzyme digestion, purified by differential adherence, and cultured in high glucose DMEM (Gibco, C11995500BT) supplemented with 10% fetal bovine serum (Excell, FND500) and 1% penicillin-streptomycin (Gibco, 15070063) as previously described. After 24 hours, the medium was replaced with fresh high glucose DMEM with 1% fetal bovine serum and 1% penicillin-streptomycin for cell maintenance.

Adult mouse cardiomyocytes (AMCMs) were isolated and seeded in glass-bottom culture plates precoated with laminin (Sigma, L2020) in accordance with a simplified Langendorff-free protocol. The plates were centrifuged at 200 g for 2 min, and incubated at 37 °C with 5% CO₂ for 2 h to facilitate cell adhesion. Then, the AMCMs were proceeded to subsequent experiments.

NRCMs and AMCMs were transfected with small interference RNA (siRNA) against rat Pkm2 or Rac1 and mouse Pkm1 (40 nmol/L) respectively with lipofectamine™ RNAimax transfection reagent (Invitrogen, PHR1017) was added to the culture medium for 24 h. Then cells were collected for subsequent analysis.

Western Blot

As previously described, cells or tissues were homogenized in RIPA lysis buffer (Beyotime, P0013C) supplemented with protease and phosphatase inhibitors (Roche, 4906845001; Roche, 4693116001) on ice for 30 minutes. The total proteins were extracted and quantified by BCA protein assay kit (Beyotime, P0009). Proteins were denatured with lithium dodecyl sulfate sample buffer (Invitrogen, NP0007) and separated on 10% Bis-Tris gel (Invitrogen, NP0315BOX). The proteins were transferred to polyvinylidene difluoride membrane (Millipore, IPVH00010) and blocked with 5% nonfat milk at room temperature (RT) for 1 hour. Subsequently, the membranes were incubated with the primary antibodies at 4 °C overnight. The following day, the membranes were rinsed in TBS with 0.1% Tween20 (TBST) 3 times, per 5 minutes and then incubated with corresponding secondary antibodies conjugated with near-infrared dyes (Invitrogen, A32735; A21036) at RT for 1 hour. The images of blots were captured with an Odyssey imager (LI-COR, Biosciences). The protein bands were quantified by ImageJ software. Signal intensity was normalized to β-Actin expression. The primary antibodies were listed in Table S4.

Immunoprecipitation and liquid chromatography/mass spectrometry-mass spectrometry (LC/MS-MS)

Twenty-five milligrams of protein from neonatal mouse cardiomyocytes was incubated with an anti-PKM2 antibody or immunoglobulin G at 4°C overnight, and then 50 µL protein A/G agarose (Beyotime, P2012) was added and rotated at 4°C for 3 to 4 hours. The samples were centrifuged to remove the supernatant and washed with 1 mL PBS 5 times, followed by the addition of lithium dodecyl sulfate sample buffer (Invitrogen, NP0007) and antioxidant (Invitrogen, NP0005). The samples were then denatured and separated on 10% Bis-Tris gel (Invitrogen, NP0315BOX). After the gel was visualized by Coomassie brilliant blue staining to assess the success of the immunoprecipitation, the remaining samples were processed for LC/MS-MS. The data were processed by the comparative proteomics analysis software suite mascot daemon (v2.5.1).

Histology Analysis

Mice were heparinized and anesthetized before they were euthanized. Then hearts were dissected,
weighed and fixed in 4% (wt/vol) paraformaldehyde (Sigma, P1648) at 4 °C for 24 hour. Samples were embedded in paraffin and sectioned at 6-μm thickness. Hematoxylin and eosin staining was performed according to the manufacturer’s protocol of the hematoxylin and eosin staining kit (Beyotime, C0105S). Alexa Fluor 594 conjugated-wheat germ agglutinin (Invitrogen, W11262) was added to the sections to measure cardiomyocyte cross-sectional area. For cross-sectional area calculation, about 200 to 250 cardiomyocytes from 4 to 5 randomly selected fields (magnification, ×400) were measured. For picrosirius red staining, heart sections were incubated with picrosirius red solution (Abcam, ab150681) at RT for 1 hour as described previously.26 Four to five randomly selected fields (magnification, ×200) were examined for collagen volume fraction analysis.

**Immunofluorescence**

Cells were fixed in 4% paraformaldehyde for 20 minutes, permeabilized in PBS with 0.5% Triton X-100 (Sigma, T9284) for 15 minutes and then blocked with 5% goat serum (Gibco, 16210072) in PBS with 0.1% Tween 20 for 60 minutes. After being incubated with primary antibodies against PKM2 (1:100; Cell Signaling Technology, 4053) and RAC1 (1:200; Proteintech, 66122-1-lg) at 4°C overnight, cells were washed with PBS with 0.1% Tween 20 and incubated with Alexa Fluor conjugated secondary antibodies at RT for 1 hour in the dark. Next, cells were stained with DAPI (Sigma, D9542) for 5 minutes. Finally, images were captured by the Leica SP8 laser confocal microscopy with DAPI, CY5, and FITC fluorescence excitation filters.

**Pyruvate Kinase Assay**

The pyruvate kinase activity was measured by a pyruvate kinase assay kit (Sigma, MAK072). Briefly, cells or tissues were homogenized with pyruvate kinase assay buffer and then spun (9600 g, 5 minutes) to clear cell debris. The supernatant was transferred to clear-bottom 96-well plate to examine pyruvate kinase activity by measuring absorbance at a wavelength of 570 nm. The results were calculated based on the standard curve generated with the same kit, and the kinase activity was normalized to the protein concentration of the lysate.

![Figure 1](image-url)

**Figure 1.** PKM2 (pyruvate kinase M2) deficiency exacerbates phenylephrine-induced cardiomyocyte hypertrophy in vitro.

A. The interference efficiency of 3 Pkm2 small interference RNAs (si-Pkm2) assessed by Western blot. β-actin served as loading control.

B. Quantitative real-time polymerase chain reaction analysis of Pkm2 and the cardiac hypertrophy markers Myh7 (myosin heavy chain 7) and Nppb (natriuretic peptide precursor B) in neonatal rat cardiomyocytes coadministrated with phenylephrine and si-Pkm2 sequence; 18S was used as an internal reference gene, n=4.

C. Western blot and quantification of cardiac β-MHC and PKM2 expression in neonatal rat cardiomyocytes coadministrated with phenylephrine and small interference RNA. β-actin served as loading control, n=4.

D. Representative images and quantification of neonatal rat cardiomyocytes area (scale bar, 25 μm) from 4 groups (NC+Saline, n=285; si-Pkm2+Saline, n=172; NC+phenylephrine, n=131; si-Pkm2+phenylephrine, n=162). β-MHC indicates β-myosin heavy chain; NC, negative control; and PKM2-pyruvate kinase M2. Values represent as the mean±SEM; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.
### Kinase Assay

The PKM2-mediated kinase reaction in vitro was performed as described previously. Recombinant active-PKM2 (4 μg) (Abcam, ab89364) was incubated with 1 μg RAC1 (Abcam, ab89246) and phosphoenolpyruvate in 50 μL kinase buffer (Cell Signaling Technology, 9802) at 25 °C for 2 hours. The reactions were terminated by the addition of lithium dodecyl sulfate sample buffer. The reaction...
mixtures were then analyzed by Phos-tag-PAGE (APExBIO, F4002), following the manufacturer’s instructions.

**Statistical Analysis**

Data are represented as the mean±SEM from at least 3 different experiments. A P value <0.05 was considered statistically significant. Statistical analyses were conducted using unpaired or paired 2-tailed Student t-test, 1-way ANOVA, and 2-way ANOVA as appropriate by GraphPad Prism software 8.0 (GraphPad Software Inc).

**RESULTS**

**Cardiomyocyte-Specific Pkm2 Knockout Exacerbated Pressure Overload-Induced HF**

To elucidate the importance of PKM2 in the process of cardiac hypertrophy and HF, we first performed Pkm2 knockout in NRCMs by RNA interference. All 3 siRNAs targeting Pkm2 (si-Pkm2) exhibited significant interference efficiency and concomitantly increased the cardiac hypertrophic marker β-MHC (Figure 1A and Figure S1A). Among them, si-Pkm2-2 was the most efficient one, we therefore selected si-Pkm2-2 for subsequent experiments. Remarkably, knockdown of Pkm2 resulted in a potent cardiomyhypertrophic response, as reflected by elevated expression of cardiac hypertrophic markers (Myh6 [myosin heavy chain 6] and Nppb [natriuretic peptide precursor B]), which was further exacerbated after phenylephrine treatment (Figure 1B). Concomitantly, expression of β-MHC protein showed a similar trend (Figure 1C). To confirm the cardiac hypertrophy phenotype, we examined the cell surface area of NRCMs. As shown in Figure 1D, Pkm2-deficient cardiomyocytes displayed larger cell surface area and were further enlarged upon phenylephrine stimulation.

Next, we generated Pkm2 cardiomyocyte conditional knockout (cKO) mice by crossing Pkm2f/f mice with α-MHC-Cre mice for in vivo study. Pkm2f/f mice were used as control. Western blot and quantitative real-time polymerase chain reaction analysis verified that Pkm2 was effectively knocked down in hearts of Pkm2 cKO mice (Figure S1B and S1C). In addition, the cardiomyocyte-specific deletion of Pkm2 was confirmed in AMCMs purified from Pkm2 cKO mice (Figure S1D).

Li et al have reported that the expression of Myh6 (myosin heavy chain 6) in cardiomyocytes was no earlier than E12.5 mouse embryo.28 Considering the Pkm2f/f-α-MHC-Cre cKO mice were generated via noninducible cKO strategy, whether loss of PKM2 affected embryonic development need to be elucidated. We therefore examined the hearts of Pkm2 cKO mice at different developmental stages (E12.5, E16.5, E18.5, and P0). The gross appearance of the hearts, heart weight/body weight ratio, number of viable mice (Figure S2A through S2D) and expression cell proliferation markers (Ki67 and pHi3) (Figure S2E through S2H) did not differ between Pkm2f/f and Pkm2 cKO mice. Meanwhile, immunoblotting of heart tissues revealed that the expression of PKM2 was significantly reduced in E16.5, E18.5 embryos and neonates, but not in E12.5 embryos (Figure S2I through S2J). In view of this, Pkm2 cKO mice did not manifest overt developmental and morphological defects in hearts at baseline.

We then randomly divided Pkm2f/f and Pkm2 cKO mice into sham or TAC operation groups (Pkm2f/f-sham, Pkm2 cKO-sham, Pkm2f/f-TAC, and Pkm2 cKO-TAC). A significant difference in cardiac function was observed between Pkm2f/f-TAC and Pkm2 cKO-TAC groups 6 weeks after TAC (Figure 2A, 2B and Figure S3A through S3G). First, Pkm2 cKO-TAC mice displayed more pronounced ventricular dilation than Pkm2f/f-TAC mice, evidenced by increased LV internal end-diastolic diameter and LV internal end-systolic diameter dimension (Figure 2A and 2B). Meanwhile, ejection fraction and fractional shortening were significantly lower in Pkm2 cKO-TAC mice, indicating that Pkm2 deficiency worsens TAC-induced cardiac dysfunction (Figure 2A).
and 2B). After the echocardiography, the mice were euthanized, and the heart and body weight were measured, which showed that Pkm2 cKO-TAC group had the highest heart weight/body weight ratio (Figure 2C). Stereomicroscope and hematoxylin and eosin staining further validated enlarged heart and dilated heart chambers in Pkm2 cKO-TAC versus Pkm2ff-TAC mice (Figure 2A). Besides cardiac dysfunction and gross abnormal morphology, histologically, the progression of HF manifests cardiac hypertrophic growth and fibrosis. We then performed wheat germ agglutinin staining to measure the area of cardiomyocytes, and picrosirius red staining to assess the extent of fibrosis. Similarly, Pkm2 cKO-TAC mice displayed more enlarged
cardiomyocytes and severer fibrosis (Figure 2D and 2E), accompanied by significantly elevated β-MHC in the hearts of Pkm2 cKO-TAC mice compared with their controls (Figure 2F). These results suggested that PKM2-deficient hearts are susceptible to pressure overload-induced cardiac hypertrophy and HF.

PKM2 or PKM1 are translated from the same PKM mRNA by post-transcriptional splicing,7,8 so we detected the expression of PKM, PKM1, and PKM2 in AMCMs purified from Pkm2 cKO and Pkm2 cko mice with or without TAC operation. As indicated in Figure S4A, Pkm2 cKO increased PKM1 and total PKM expression, which was consistent with previous reports by Magadum et al. and Li et al.15,29 Upon TAC operation, the expression of PKM2 and PKM1 was respectively increased and decreased accompanied by slight increase in PKM in Pkm2 cKO mice, while in Pkm2 cko mice, increasing level of PKM and PKM1 was detected though still lower than those of Pkm2 cKO sham mice (Figure S4A). Then, we investigated whether PKM1 contributes to the phenotype observed in PKM2 cKO hearts by using AMCMs purified from Pkm2 cKO and Pkm2 cko mice. After successfully knockdown Pkm1 expression in AMCMs with siRNA targeting Pkm1, PKM2-deficient AMCMs manifested severer cardiohypertrophic phenotype (as indicated by the expression of β-MHC) under phenylephrine stimulation (Figure S4B). Moreover, the overexpression of Pkm1 was reported to improve the cardiac dysfunction induced by pressure overload.29 The results above indicated that the upregulation of PKM1 in Pkm2 cKO mouse heart is not sufficient to compensate for the phenotype caused by PKM2 deficiency under pressure overload, suggesting that PKM1 and PKM2 may also have nonredundant functions in pressure overload induced HF.

PKM2 Acted as a Protein Kinase to Inhibit RAC1 Activation

Considering the role of PKM2 in glycolysis, we first tested whether PKM2 deficiency impaired the glycolysis in cardiomyocytes. We measured the pyruvate

---

**Figure 4.** Cardiomyocyte-specific Pkm2 (pyruvate kinase M2) deficiency activated RAC1 (rho family, small GTP binding protein)-MAPK signaling pathway in the progression of heart failure.

A and B. Western blot and quantification of phosphorylation of p38, p38, p-ERK, ERK, phosphorylation of JNK, and JNK in neonatal rat cardiomyocytes treated with si-Pkm2 for 72 hours. β-actin served as loading control.

C through F. Western blot and quantification of p-p38, p38, p-ERK, ERK, p-JNK, and JNK expression in heart tissues from Pkm2 conditional knockout and Pkm2 cKO mice 6 weeks after sham or transverse aortic constriction surgery. β-actin served as loading control. Minimum of n=6 per group. cKO indicates conditional knockout; Pkm2, pyruvate kinase M2; RAC1, rho family, small GTP binding protein; p-p38, phosphorylation of p38; and p-JNK, phosphorylation of JNK. Values represent as the mean±SEM; **P<0.01, ***P<0.001, ****P<0.0001.
kinase activity by detecting pyruvate production in cardiomyocytes. In Pkm2 knockdown NRCMs, specific siRNA targeting Pkm2 did not affect the expression of PKM1 (Figure 3A through 3C), which was consistent with previous work. Meanwhile, pyruvate kinase activity remained unchanged between control and si-Pkm2 groups (Figure 3D). These results indicated that PKM2 deficiency in cardiomyocytes did not impair pyruvate kinase activity. We then set to investigate the molecular mechanism of PKM2 in the

Figure 5. RAC1 (rho family, small GTP binding protein) inhibition mitigated pressure overload-induced heart failure. A, Representative M-mode echocardiography, gross appearance of whole hearts (scale bar, 1 mm), heart cross-sections stained with hematoxylin and eosin (scale bar, 1 mm), histological analysis of heart sections by picrosirius red staining (scale bar, 50 μm) and cell boundaries demarcated with wheat germ agglutinin staining (scale bar, 25 μm) from Pkm2 conditional knockout and Pkm2 f/f mice after transverse aortic constriction surgery cotreated with saline or NSC23766. B, Quantitative analyses of echocardiography showing ejection fraction and fractional shortening, n=9 to 10. C, The ratio of heart weight to body weight, n=9 to 10. D, Statistical results for myocardial interstitial fibrosis analyzed by ImageJ software. n=6 to 7. E, Statistical results for the cell cross-sectional area, n=9 to 10. F, Western blot and quantification of β-MHC expression in hearts of Pkm2 conditional knockout and Pkm2 f/f mice after transverse aortic constriction surgery cotreated with saline or NSC23766. β-actin served as loading control. β-MHC indicates β-myosin heavy chain; cKO, conditional knockout; EF, ejection fraction; FS, fractional shortening; HE, hematoxylin and eosin; HW/BW, heart weight to body weight; Pkm2, pyruvate kinase M2; PSR, picrosirius red; RAC1, rho family, small GTP binding protein; TAC, transverse aortic constriction; and WGA, wheat germ agglutinin. Values represent as the mean±SEM; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.
progression of HF. By using an anti-PKM2 antibody-conjugated agarose assistant immunoprecipitation mass spectrometry, we identified about 230 proteins (sum phosphoenolpyruvate score > immunoglobulin G group) as putative partners of PKM2 in neonatal mouse cardiomyocytes (Table S5). By combining our immunoprecipitation mass spectrometry data with related studies,31–34 we speculated that RAC1 might be one of the potential targets of PKM2 in regulating cardiac hypertrophy. We therefore selected RAC1 for further analysis. With PKM2 immunoprecipitation and Western blot, RAC1 was validated as the part of PKM2 in neonatal mouse cardiomyocytes (Figure 3F). We then examined the intracellular localization of PKM2 and RAC1 to further determine the relationship between PKM2 and RAC1 in situ. Consistent with immunoprecipitation results, endogenous PKM2 and RAC1 co-localized in the cytoplasm of NRCMs and AMCMs (Figure 3G).

Since the nonmetabolic function of PKM2, such as protein kinase, contributes to multiple tumor pathological processes,11 we next examined the kinase activity of PKM2 on RAC1 phosphorylation. We performed in vitro kinase activity assay determined by a Phos-tag approach using purified active PKM2 and RAC1 proteins. Coomassie brilliant blue staining and immunoblot suggested that PKM2 was able to phosphorylate RAC1 (Figure 3H). Moreover, Pkm2 knockdown caused a significant decrease in phosphorylated RAC1 at Serine 71 (p-RAC1 (S71)) in NRCMs (Figure 3I). p-RAC1 (S71) has been reported to lower the stability of RAC1 protein and accelerate its degradation,35 which was confirmed by the cycloheximide chase assay showing that the half-life of the RAC1 protein in Pkm2 knockdown NRCMs was significantly increased (Figure 3J).

Cardiomyocyte-Specific Pkm2 Deficiency-Activated RAC1-MAPK Signaling Pathway in the Progression of HF

As a member of small guanine nucleotide-binding proteins of the Rho GTPase family (Rac, Cdc42, and Rho), RAC1 regulates the activity of MAPKs (mitogen-activated protein kinases), including p38, ERK, and JNK, which play important roles in pathological cardiac hypertrophy.31–33 Therefore, we first investigated the expression and active phosphorylation of MAPKs in Pkm2-deficiency NRCMs. Immunoblot revealed that phosphorylation of p38 (p-p38) and JNK (p-JNK), but not p-ERK was increased in response to Pkm2 knockdown-induced RAC1 elevation (Figure 4A and 4B). It has been reported that both p-p38 and p-JNK were significantly increased after TAC operation.36,37 We then examined the expression and active phosphorylation of MAPKs in the hearts of Pkm2f/f and Pkm2 cKO mice. While p-p38 and p-JNK displayed similar expression level in Pkm2f/f and Pkm2 cKO mice at baseline, both proteins significantly increased in Pkm2 cKO mice after TAC compared with controls (Figure 4C through 4F). Collectively, PKM2 deficiency in cardiomyocytes reduced RAC1 phosphorylation leading to enhanced RAC1 protein stability, which in turn activated the RAC1-MAPK signaling pathway. Given the activated RAC1-MAPK axis in Pkm2 cKO hearts, we postulated that RAC1 inhibition might delay pressure overloaded-induced HF in Pkm2 cKO mice. To test this, we selected NSC23766, a specific inhibitor of RAC1. In NRCMs, NSC23766 significantly reduced Pkm2 silencing induced β-MHC expression (Figure S5A). Similarly, Rac1 knockdown also inhibited β-MHC expression in Pkm2-deficient NRCMs, whereas Pkm2 expression was not affected (Figure S5B and Figure S5C). Increased expression of activated form of RAC1 has been reported in the pressure overloaded heart.38 We then randomly treated Pkm2f/f-TAC and Pkm2 cKO-TAC mice with NSC23766 (2.5 mg/kg per

Figure 6. Cardiomyocyte-specific overexpression of PKM2 (pyruvate kinase M2) ameliorated pressure overload-induced heart failure.

A, Schematic illustration of adenovirus-associated virus type 9-cardiac troponin T-mPkm2 injection in mice after surgery. B, Representative M-mode echocardiography, gross appearance of whole hearts (scale bar, 1 mm), heart cross-sections stained with hematoxylin and eosin (scale bar, 1 mm), histological analysis of heart sections by picrosirius red staining (scale bar, 50 μm) and cell boundaries demarcated with wheat germ agglutinin staining (scale bar, 1 mm), histological analysis of heart sections by picrosirius red staining (scale bar, 50 μm) and cell boundaries demarcated with wheat germ agglutinin staining (scale bar, 1 mm). C and D, Quantitative analyses of echocardiography showing ejection fraction, fractional shortening, and heart rate, n=6 to 9. E, Statistical results for myocardial interstitial fibrosis analyzed by ImageJ software, n=6 to 9. F, Statistical results for the cell cross-sectional area, n=6 to 9. G, The ratio of heart weight to body weight, n=6 to 9. H, Western blot and quantification of β-MHC, RAC1 (rho family, small GTP binding protein), phosphorylation of p38, p38, phosphorylation of JNK, and JNK expression of cardiomyocyte-specific overexpression of Pkm2 after transverse aortic constriction surgery. β-actin served as loading control, n=6. AAV9 indicates adenovirus-associated virus type 9; β-MHC, β-myosin heavy chain; cTnT, cardiac troponin T; HE, hematoxylin and eosin; HW/BW, heart weight to body weight; mPkm2 indicates mouse Pkm2 gene; p-p38, phosphorylation of p38; p-JNK, phosphorylation of JNK; PSR, picrosirius red; TAC, transverse aortic constriction; and WGA, wheat germ agglutinin. Values represent as the mean±SEM, ns, not significant; “P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.
day) or saline for a total of 7 weeks (Figure S5D). One day after the last dose, mice were monitored for echocardiography and the hearts were harvested for subsequent analysis. NSC23766 suppressed TAC-induced RAC1 activation both in Pkm2cKO and Pkm2 cKO-TAC mice (Figure S5E). Moreover, NSC23766 alleviated TAC-induced heart dysfunctions in Pkm2 cKO mice, including improved cardiac function, lower heart
weight/body weight ratio, smaller cardiomyocytes size, and decreased fibrosis. Though not significant, a trend of improvement in pathological hypertrophy and cardiac function in Pkm2 knockout TAC mice treated with NSC23766 compared with saline control was observed (Figure 5A through 5E and Figure S5F through S5N). Moreover, NSC23766 drastically reduced β-MHC in both Pkm2 knockout TAC and Pkm2 cKO mice (Figure 5F). In total, the significant benefits of NSC23766 on Pkm2 cKO mice against TAC suggested that the activation of RAC1-MAPK signaling pathway contributed to Pkm2 deficiency-induced severe cardiac hypertrophy and dysfunction in the pressure-overload heart.

Cardiomyocyte-Specific Overexpression of PKM2 Ameliorated Pressure Overload-Induced HF

To determine the potential protective effect of cardiac PKM2 in the pathological progression under pressure overload, we used a genetic approach using an adeno-associated virus type 9 (AAV9) carrying Pkm2 gene with a cardiomyocyte-specific cTnT promoter (AAV9-cTnT-mPkm2). AAV9-cTnT-null was used as a control. One week after TAC operation, 1 × 10^11 viral particles/mouse AAV9-cTnT-null or AAV9-cTnT-mPkm2 virus were injected through the tail vein. Meanwhile, cardiac function was monitored by echocardiography once a week throughout the 8-week experiment period (Figure 6A). At the end point, mice were euthanized, and the heart samples were collected. Both Pkm2 transcripts (Figure S6A) and protein expression (Figure S6B) were confirmed to be increased significantly after AAV9-cTnT-mPkm2 injection compared with AAV9-cTnT-null control. The echocardiographic examination showed improved cardiac systolic function without interference on heart rate in mice injected with AAV9-cTnT-mPkm2 after TAC operation (Figure 6B through 6D and Figure S6C through S6J). Stereomicroscopy, hematoxylin and eosin, wheat germ agglutinin, and picrosirius red staining confirmed that the hearts of TAC mice were significantly enlarged with severer fibrosis compared with sham ones, which was alleviated after AAV9-cTnT-mPkm2 administration (Figure 6B, 6E and 6F). Meanwhile, there was no significant difference in the ratios of heart weight/body weight between sham- and TAC-operated mice with Pkm2 overexpression (Figure 6G). In addition, the β-MHC expression was significantly reduced in Pkm2 overexpression hearts than AAV9-cTnT-null-treated TAC mice (Figure 6H). Contrary to Pkm2 knockout, Pkm2 overexpression inhibited hyperactivation of the MAPK signaling pathway in TAC mice hearts (Figure 6H). Taken together, these findings indicated that maintaining a high PKM2 expression may protect the heart against pressure overload-induced hypertrophy and HF.

DISCUSSION

In the present study, we reported that PKM2 exerted cardioprotective effects under pressure overload-induced HF as a protein kinase. Mechanistically, we uncovered that PKM2 inhibited the activation of RAC1-MAPK signaling pathway by phosphorylating RAC1 in cardiomyocytes, which delayed the progression of cardiac hypertrophy and HF. These findings highlighted PKM2 as a prospective target in HF intervention.

Our data demonstrated that Pkm2 deficiency in cardiomyocytes aggravated hypertrophic growth and cardiac dysfunction both in vitro and in vivo. Conversely, PKM2 overexpression via cardiomyocyte-specific adeno-associated virus (AAV9-cTnT) delivery system effectively delayed HF progression. These results emphasized the crucial role of PKM2 in maintaining heart function homeostasis and validated that the replenishment of PKM2 was a cardioprotective approach for pressure overload-induced HF. As Pkm2 overexpression was also reported to alleviate myocardial infarction, we conjectured that maintaining a higher level of PKM2 might have universal cardioprotective effects on multifactor-caused heart failure.

Phosphorylation, as a major post-translational protein modification, has been identified in over three quarters of proteins. We observed increased PKM2 protein kinase activity in pressure overload-induced HF, facilitating phosphorylation of RAC1 at S71, which in turn, reduced RAC1 stability. Previous studies have demonstrated that RAC1 activity is critical in cardiomyocyte hypertrophy, cardiomyopathy, heart failure, and arrhythmia. Cardiomyocyte-specific RAC1 deletion or RAC1 inhibitor can prevent myocardial hypertrophy under pressure overload through MAPK pathway inhibition. Consistently, our findings revealed that PKM2 deficiency elevated RAC1 expression and exhibited prohypertrophic effects through activation of MAPK pathway (p-p38 and p-JNK). Moreover, upon selective RAC1 inhibitor treatment, TAC-induced cardiac hypertrophy and dysfunction were mitigated in Pkm2 cKO mice, verifying that RAC1 is one of the main targets of PKM2 in regulating pressure overload-induced HF. Notably, there were other proteins identified as putative PKM2 partners in our immunoprecipitation mass spectrometry data, whose roles in PKM2 deficiency-related cardiomyopathies await further investigation.

PKM2 overexpression was reported to be beneficial in maintaining cardiac function after myocardial infarction. Currently, there have been several strategies for PKM2 maintenance in HF, either by transgenic Pkm2 overexpression or by small molecules targeting its kinase activity, etc. In particular, small molecules such as Shikonin and TEPP-46 have shown great potentials in treating various diseases including doxorubicin-induced cardiotoxicity. Thus, further research targeting
PKM2 to explore new strategies for treatment of pressure overload-induced HF is warranted.

In conclusion, we uncovered a cardioprotective role of PKM2 in maintaining cardiac homeostasis under hemodynamic stress overload. PKM2 protects the heart against pressure overload-induced HF via modulating RAC1-MAPK signaling pathway activity. These new findings will advance our understanding of the pathogenesis of HF and provide a potential therapeutic target for HF.

ARTICLE INFORMATION
Received November 26, 2021; accepted April 28, 2022.

Affiliations
Department of Cardiology, Shanghai East Hospital (L.N., B.L., L.H., M.S., J.Y., D.S.), Key Laboratory of Arrhythmias of the Ministry of Education of China, Shanghai East Hospital (L.N., B.L., L.H., M.S., J.Y., D.S.), and Department of Cell Biology (J.Y.), Tongji University School of Medicine, Shanghai, China; Jinzhou Medical University, Liaoning, China (R.Z., F.F.); and Institute of Medical Genetics, Tongji University, Shanghai, China (J.Y.).

Sources of Funding
This work was funded by the Programs of National Natural Science Foundation of China (82000377 to Dan Shi; 31871491 to Jian Yang). This work was funded by the Programs of National Natural Science Foundation of China (82000377 to Dan Shi; 31871491 to Jian Yang). This work was funded by the Programs of National Natural Science Foundation of China (82000377 to Dan Shi; 31871491 to Jian Yang).

Disclosures
None.

Supplemental Material
Tables S1–S6
Figures S1–S6

REFERENCES
1. Zaiaian B, Fonarow GC. Epidemiology and aetiology of heart failure. Nat Rev Cardiol. 2016;13:368–378. doi: 10.1038/nrccardio.2016.25
2. Maltevs VA, Silverman N, Sabbah HN, Undrovinas AI. Chronic heart failure will advance our understanding of the pathogenesis of HF and provide a potential therapeutic target for HF.
3. Nakamura M, Sadoshima J. Mechanisms of physiological and pathophysiological stress overload. PKM2 protects the heart against pressure overload-induced HF via modulating RAC1-MAPK signaling pathway activity. These new findings will advance our understanding of the pathogenesis of HF and provide a potential therapeutic target for HF.
5. None.
6. Disclosures
None.
7. Supplemental Material
Tables S1–S6
Figures S1–S6
8. REFERENCES
1. Zaiaian B, Fonarow GC. Epidemiology and aetiology of heart failure. Nat Rev Cardiol. 2016;13:368–378. doi: 10.1038/nrccardio.2016.25
2. Maltevs VA, Silverman N, Sabbah HN, Undrovinas AI. Chronic heart failure will advance our understanding of the pathogenesis of HF and provide a potential therapeutic target for HF.
3. Nakamura M, Sadoshima J. Mechanisms of physiological and pathophysiological stress overload. PKM2 protects the heart against pressure overload-induced HF via modulating RAC1-MAPK signaling pathway activity. These new findings will advance our understanding of the pathogenesis of HF and provide a potential therapeutic target for HF.
5. None.
6. Disclosures
None.
7. Supplemental Material
Tables S1–S6
Figures S1–S6
8.REFERENCES
1. Zaiaian B, Fonarow GC. Epidemiology and aetiology of heart failure. Nat Rev Cardiol. 2016;13:368–378. doi: 10.1038/nrccardio.2016.25
2. Maltevs VA, Silverman N, Sabbah HN, Undrovinas AI. Chronic heart failure will advance our understanding of the pathogenesis of HF and provide a potential therapeutic target for HF.
3. Nakamura M, Sadoshima J. Mechanisms of physiological and pathophysiological stress overload. PKM2 protects the heart against pressure overload-induced HF via modulating RAC1-MAPK signaling pathway activity. These new findings will advance our understanding of the pathogenesis of HF and provide a potential therapeutic target for HF.
5. None.
6. Disclosures
None.
7. Supplemental Material
Tables S1–S6
Figures S1–S6
8.REFERENCES
1. Zaiaian B, Fonarow GC. Epidemiology and aetiology of heart failure. Nat Rev Cardiol. 2016;13:368–378. doi: 10.1038/nrccardio.2016.25
2. Maltevs VA, Silverman N, Sabbah HN, Undrovinas AI. Chronic heart failure will advance our understanding of the pathogenesis of HF and provide a potential therapeutic target for HF.
3. Nakamura M, Sadoshima J. Mechanisms of physiological and pathophysiological stress overload. PKM2 protects the heart against pressure overload-induced HF via modulating RAC1-MAPK signaling pathway activity. These new findings will advance our understanding of the pathogenesis of HF and provide a potential therapeutic target for HF.
5. None.
6. Disclosures
None.
7. Supplemental Material
Tables S1–S6
Figures S1–S6
8.REFERENCES
1. Zaiaian B, Fonarow GC. Epidemiology and aetiology of heart failure. Nat Rev Cardiol. 2016;13:368–378. doi: 10.1038/nrccardio.2016.25
2. Maltevs VA, Silverman N, Sabbah HN, Undrovinas AI. Chronic heart failure will advance our understanding of the pathogenesis of HF and provide a potential therapeutic target for HF.
3. Nakamura M, Sadoshima J. Mechanisms of physiological and pathophysiological stress overload. PKM2 protects the heart against pressure overload-induced HF via modulating RAC1-MAPK signaling pathway activity. These new findings will advance our understanding of the pathogenesis of HF and provide a potential therapeutic target for HF.
5. None.
6. Disclosures
None.
7. Supplemental Material
Tables S1–S6
Figures S1–S6
8.REFERENCES
1. Zaiaian B, Fonarow GC. Epidemiology and aetiology of heart failure. Nat Rev Cardiol. 2016;13:368–378. doi: 10.1038/nrccardio.2016.25
2. Maltevs VA, Silverman N, Sabbah HN, Undrovinas AI. Chronic heart failure will advance our understanding of the pathogenesis of HF and provide a potential therapeutic target for HF.
3. Nakamura M, Sadoshima J. Mechanisms of physiological and pathophysiological stress overload. PKM2 protects the heart against pressure overload-induced HF via modulating RAC1-MAPK signaling pathway activity. These new findings will advance our understanding of the pathogenesis of HF and provide a potential therapeutic target for HF.
5. None.
6. Disclosures
None.
7. Supplemental Material
Tables S1–S6
Figures S1–S6
8.REFERENCES
1. Zaiaian B, Fonarow GC. Epidemiology and aetiology of heart failure. Nat Rev Cardiol. 2016;13:368–378. doi: 10.1038/nrccardio.2016.25
2. Maltevs VA, Silverman N, Sabbah HN, Undrovinas AI. Chronic heart failure will advance our understanding of the pathogenesis of HF and provide a potential therapeutic target for HF.
3. Nakamura M, Sadoshima J. Mechanisms of physiological and pathophysiological stress overload. PKM2 protects the heart against pressure overload-induced HF via modulating RAC1-MAPK signaling pathway activity. These new findings will advance our understanding of the pathogenesis of HF and provide a potential therapeutic target for HF.
5. None.
6. Disclosures
None.
7. Supplemental Material
Tables S1–S6
Figures S1–S6
8.REFERENCES
1. Zaiaian B, Fonarow GC. Epidemiology and aetiology of heart failure. Nat Rev Cardiol. 2016;13:368–378. doi: 10.1038/nrccardio.2016.25
2. Maltevs VA, Silverman N, Sabbah HN, Undrovinas AI. Chronic heart failure will advance our understanding of the pathogenesis of HF and provide a potential therapeutic target for HF.
3. Nakamura M, Sadoshima J. Mechanisms of physiological and pathophysiological stress overload. PKM2 protects the heart against pressure overload-induced HF via modulating RAC1-MAPK signaling pathway activity. These new findings will advance our understanding of the pathogenesis of HF and provide a potential therapeutic target for HF.
5. None.
6. Disclosures
None.
7. Supplemental Material
Tables S1–S6
Figures S1–S6
29. Li Q, Li C, Elmwasany A, Sharma G, An YA, Zhang G, Elhelaly WM, Lin J, Gong Y, Chen G, et al. PKM1 exerts critical roles in cardiac remodeling under pressure overload in the heart. Circulation. 2021;144:712–727. doi: 10.1161/circulationaha.121.054885

30. Goldberg MS, Sharp PA. Pyruvate kinase M2-specific siRNA induces apoptosis and tumor regression. J Exp Med. 2012;209:217–224. doi: 10.1084/jem.201111487

31. Adam O, Laufs U. Rac1-mediated effects of HMG-CoA reductase inhibitors (statins) in cardiovascular disease. Antioxid Redox Signal. 2014;20:1238–1250. doi: 10.1089/ars.2013.5526

32. Brown JH, Del Re DP, Sussman MA. The Rac and Rho hall of fame: a decade of hypertrophic signaling hits. Circ Res. 2006;98:730–742. doi: 10.1161/01.RES.0000216039.75913.9e

33. Li P-L, Liu H, Chen G-P, Li L, Shi H-J, Nie H-Y, Liu Z, Hu Y-F, Yang J, Zhang P, et al. STEAP3 (Six-Transmembrane Epithelial Antigen of Prostate 3) inhibits pathological cardiac hypertrophy. Hypertension. 2020;76:1219–1230. doi: 10.1161/hypertensionaha.120.14752

34. Custodis F, Eberl M, Kilter H, Böhm M, Laufs U. Association of RhoGDIalpha with Rac1 GTPase mediates free radical production during myocardial hypertrophy. Cardiovasc Res. 2006;71:342–351. doi: 10.1016/j.cardiores.2006.04.005

35. Zhao J, Milaki RK, Wei J, Coon TA, Zou C, Chen BB, Mallampalli RK, Zhao Y. SCF E3 ligase F-box protein complex SCF(FBXL19) regulates cell migration by mediating Rac1 ubiquitination and degradation. Faseb J. 2013;27:2611–2619. doi: 10.1096/fj.12-223099

36. Zhang B, Zhang P, Tan Y, Feng P, Zhang Z, Liang H, Duan W, Jin Z, Wang X, Liu J, et al. C1q-TNF-related protein-3 attenuates pressure overload-induced cardiac hypertrophy by suppressing the p38/CREB pathway and p38-induced ER stress. Cell Death Dis. 2019;10:520. doi: 10.1038/s41419-019-1749-0

37. Blanton RM, Takimoto E, Lane AM, Aronovitz M, Piotrowski R, Karas RH, Kass DA, Mendelsohn ME. Protein kinase g iz inhibits pressure overload-induced cardiac remodeling and is required for the cardioprotective effect of sildenafil in vivo. J Am Heart Assoc. 2012;1:e003731. doi: 10.1161/jaha.112.003731

38. Ayuzawa N, Nagase M, Ueda K, Nishimoto M, Kawarazaki W, Marumo T, Aiba A, Sakurai T, Shindo T, Fujita T. Rac1-mediated activation of mineralocorticoid receptor in pressure overload-induced cardiac injury. Hypertension. 2016;67:99–106. doi: 10.1161/hypertensionaha.115.06054

39. Sharma K, D’Souza R, Tyano S, Schaabo C, Wińiewski J, Cox J, Mann M. Ultradeep human phosphoproteome reveals a distinct regulatory nature of Tyr and Ser/Thr-based signaling. Cell Rep. 2014;8:1583–1594. doi: 10.1016/j.celrep.2014.07.036

40. Nagase M, Fujita T. Role of Rac1-mineralocorticoid-receptor signaling in renal and cardiac disease. Nat Rev Nephrol. 2013;9:86–98. doi: 10.1038/nrneph.2012.282

41. Satoh M, Ogita H, Takeshita K, Mukai Y, Kwiatkowski DJ, Liao JK. Requirement of Rac1 in the development of cardiac hypertrophy. Proc Natl Acad Sci USA. 2006;103:7432–7437. doi: 10.1073/pnas.0510444103
Supplemental Material
Table S1. The primer sequences for genotyping.

| Primer       | Primer sequences            |
|--------------|----------------------------|
| mouse-cre-F  | TCTATTGCACACAGCAATCCA       |
| mouse-cre-R  | CCAGCATTGTGAGAACAAGG        |
| mouse-Pkm20/F | CCTTCAGGAAGACAGCCAAG       |
| mouse-Pkm20/R | AGTGCTGCCTGGAATCCTCT       |
Table S2. The siRNA sequences.

| Sequence names  | Sequences                                      |
|-----------------|------------------------------------------------|
| Rat-si-Pkm2-1-F | CCCUGUGCUGUGUAAGGAU                            |
| Rat-si-Pkm2-1-R | AUCCUUACACAGCACAGGG                            |
| Rat-si-Pkm2-2-F | CCAAGGGCUCCUAUCAUU                             |
| Rat-si-Pkm2-2-R | AAUGAUAGGAGCCCUUGGG                            |
| Rat-si-Pkm2-3-F | CGGCAGGAGUGCACACCAAA                          |
| Rat-si-Pkm2-3-R | UUGGUGAGCUCUCCUGCCG                           |
| NC-F            | GGUUGUGCAAGAGGGCUUU                            |
| NC-R            | AAAGCCCUUGCACAAACC                            |
| Rat-si-Rac1-F   | CAAACAGACGUGUUCUAATT                         |
| Rat-si-Rac1-R   | UUAAGAACACGUCUUUGCG                           |
| Ms-si-Pkm1-F    | GUGGAGGCUCCUUAAAGUTT                         |
| Ms-si-Pkm1-R    | ACUUAUAAGGAGGCCUCCACTT                        |
Table S3. The primer sequences for qRT-PCR analysis.

| Primer          | Primer sequences                   |
|-----------------|------------------------------------|
| mouse/rat-Pkm2-F| ATTACCAGCGACCCCCACAGAA             |
| mouse/rat-Pkm2-R| ACGGCATCCTTACACAGCACA              |
| mouse-Actb-F    | GGCTGTATTCCCCCTCCATCG              |
| mouse-Actb-R    | CCAGTTGGTAACAAATGCCCATGT           |
| rat-Nppb-F      | GTCTCAAGACAGCGCCTTCC              |
| rat-Nppb-R      | AACCTCAGCCGTCACAGC                |
| rat-Myh7-F      | GCGGACATTGCGCGAGTCCCAG             |
| rat-Myh7-R      | GCTCCAGGTCTAGGGCTTCACA             |
| rat-18S-F       | GTTGAAACCCATTGATG                 |
| rat-18S-R       | GCTTATGACCCGCACCTACT              |
Table S4. Antibodies used in this study.

| Antibody       | Application | Source                  | Cat No   |
|----------------|-------------|-------------------------|----------|
| PKM2           | WB          | Proteintech             | 60268-1-Ig |
| PKM2           | WB/IF/IP    | Cell Signaling Technology | 4053    |
| RAC1           | WB/IF/IP    | Proteintech             | 66122-1-Ig |
| PKM1           | WB          | Proteintech             | 15821-1-AP |
| PKM1           | WB          | Biorbyt                 | orb395449 |
| Phospho-Rac1(ser71) | WB    | Cell Signaling Technology | 2461    |
| P38 MAPK       | WB          | Cell Signaling Technology | 8690    |
| JNK            | WB          | Cell Signaling Technology | 9252    |
| ERK            | WB          | Cell Signaling Technology | 4695    |
| Phospho-P38 MAPK | WB     | Cell Signaling Technology | 4511    |
| Phospho-JNK    | WB          | Cell Signaling Technology | 4668    |
| Phospho-ERK    | WB          | Cell Signaling Technology | 4370    |
| β-Actin        | WB          | Santa Cruz              | sc-47778 |
| cTnT           | IF          | Abcam                   | Ab8295   |
| PKM1/2         | WB          | Cell Signaling Technology | 3190    |

WB, western blot; IF, immunofluorescence; IP, immunoprecipitation.
Table S5. Immunoprecipitation mass spectrometry in this study.

| Accession | Gene name | IP PKM2 | IP IgG | AAs | MW [kDa] | pI |
|-----------|-----------|---------|--------|-----|----------|----|
|           |           | Sum PEP Score | Peptides | Unique Peptides | Sum PEP Score | Peptides | Unique Peptides |
| P52480    | Pkm       | 105.772  | 23      | 23  | 20.205   | 7      | 7      |
| A0A2R2Y2P8| TPM1kappa | 85.263   | 23      | 2   | -        | -      | -      |
| P53395    | Dbt       | 28.828   | 12      | 12  | 1.569    | 1      | 1      |
| A2ASS6    | Ttn       | 33.826   | 25      | 24  | 7.556    | 3      | 3      |
| Q8BGZ7    | Krt75     | 25.334   | 10      | 2   | -        | -      | -      |
| P56480    | Atp5f1b   | 74.134   | 18      | 18  | 57.114   | 13     | 13     |
| B2RX08    | Sptb      | 22.292   | 13      | 11  | 5.503    | 3      | 3      |
| A0A1L1SV25| Actn4     | 16.751   | 5       | 1   | -        | -      | -      |
| P17751    | Tpi1      | 22.866   | 7       | 7   | 6.226    | 4      | 4      |
| E9QQ93    | Xirp1     | 40.656   | 13      | 13  | 24.867   | 9      | 9      |
| P26041    | Msn       | 13.09    | 7       | 3   | -        | -      | -      |
| P62631    | Eef1a2    | 12.645   | 5       | 1   | -        | -      | -      |
| Q9D2G2    | Dlst      | 15.841   | 5       | 5   | 4.841    | 3      | 3      |
| P17710    | Hk1       | 13.394   | 7       | 7   | 2.557    | 2      | 2      |
| Q5DTI2    | Atp2a2    | 49.29    | 18      | 18  | 39.361   | 14     | 14     |
| Q6NXH9    | Krt73     | 9.798    | 5       | 1   | -        | -      | -      |
| Q71LX8    | Hsp90ab1  | 28.721   | 12      | 5   | 19.655   | 9      | 4      |
| J3QQ13    | Tnt2      | 26.921   | 9       | 9   | 18.229   | 7      | 7      |
|     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| E9Q035 | Gm20425 | 19 | 9 | 9 | 10.518 | 5 | 5 | 978 | 107.7 | 7.77 |
| P50544 | Acadvl | 28.119 | 11 | 11 | 19.784 | 8 | 8 | 656 | 70.8 | 8.75 |
| P16546 | Sptan1 | 121.471 | 45 | 44 | 113.731 | 39 | 1 | 2472 | 284.4 | 5.33 |
| Q6IME9 | Krt72 | 7.615 | 4 | 1 | - | - | - | 520 | 56.7 | 7.4 |
| E9PWQ3 | Col6a3 | 7.224 | 4 | 4 | - | - | - | 3284 | 353.7 | 6.93 |
| B7ZC12 | Nebl | 13.927 | 5 | 5 | 6.859 | 4 | 4 | 1014 | 115.9 | 8.51 |
| Q91YT0 | Ndufv1 | 8.514 | 4 | 4 | 1.638 | 1 | 1 | 464 | 50.8 | 8.21 |
| P68510 | Ywhah | 6.808 | 3 | 1 | - | - | - | 246 | 28.2 | 4.89 |
| Q497F1 | Tnni3 | 12.898 | 4 | 4 | 6.198 | 3 | 3 | 211 | 24.2 | 9.55 |
| Q9DCV7 | Krt7 | 6.371 | 4 | 2 | - | - | - | 457 | 50.7 | 5.87 |
| P47757 | Capzb | 17.764 | 9 | 9 | 11.554 | 6 | 2 | 277 | 31.3 | 5.74 |
| Q60597 | Ogdh | 11.14 | 7 | 7 | 5.046 | 4 | 4 | 1023 | 116.4 | 6.83 |
| Q99LC5 | Efta | 14.852 | 7 | 7 | 9.042 | 4 | 4 | 333 | 35 | 8.38 |
| Q9CZS1 | Aldh1b1 | 9.032 | 5 | 5 | 3.282 | 3 | 3 | 519 | 57.5 | 7.02 |
| P07901 | Hsp90a1 | 20.82 | 11 | 5 | 15.438 | 7 | 2 | 733 | 84.7 | 5.01 |
| Q3TK29 | Trap1 | 5.368 | 2 | 1 | - | - | - | 706 | 80.1 | 6.68 |
| Q64727 | Vcl | 19.136 | 7 | 7 | 13.821 | 4 | 4 | 1066 | 116.6 | 6 |
| Q9DCT2 | Ndufs3 | 8.359 | 3 | 3 | 3.099 | 1 | 1 | 263 | 30.1 | 7.17 |
| A0A1L1SQ51 | Tln2 | 5.135 | 2 | 2 | - | - | - | 2544 | 271.8 | 5.57 |
| P06728 | Apoa4 | 5.071 | 2 | 2 | - | - | - | 395 | 45 | 5.47 |
| Q3UAD6 | Hsp90b1 | 9.972 | 5 | 4 | 4.996 | 3 | 3 | 802 | 92.4 | 4.82 |
| Q60930 | Vdac2 | 18.735 | 3 | 3 | 14.073 | 4 | 4 | 295 | 31.7 | 7.49 |
| Q3TQX5 | Ddx3x | 4.632 | 2 | 2 | - | - | - | 662 | 73.1 | 7.18 |
| P02772 | Afp | 17.657 | 7 | 7 | 13.265 | 6 | 6 | 605 | 67.3 | 5.92 |
| accession | protein   | p     | FDR   | Fold | logFC   | logP | fold change |
|-----------|-----------|-------|-------|------|---------|------|-------------|
| P62242    | Rps8      | 6.352 | 2     | 2    | 2.006   | 1    | 208         | 24.2 | 10.32 |
| E9PWE8    | Dpysl3    | 4.342 | 3     | 3    | -       | -    | -           | 683  | 73.8  | 6.46 |
| Q3TEU8    | Corolc    | 10.349| 5     | 5    | 6.014   | 3    | 474         | 53.1 | 6.79  |
| Q9JKS4    | Ldb3      | 5.653 | 2     | 2    | 1.46    | 1    | 723         | 76.4 | 7.75  |
| Q9Z0X1    | Aifm1     | 4.072 | 2     | 2    | -       | -    | -           | 612  | 66.7  | 9.17 |
| Q7TQ48    | Srl       | 14.883| 6     | 6    | 10.901  | 5    | 910         | 99.1 | 4.46  |
| P05201    | Got1      | 8.263 | 4     | 4    | 4.334   | 2    | 413         | 46.2 | 7.14  |
| H7BX05    | Obscn     | 4.805 | 1     | 1    | 0.914   | 1    | 8032        | 874  | 5.96  |
| Q80U89    | mKIAA0034 | 10.187| 6     | 6    | 6.424   | 3    | 1684        | 192.4| 5.6   |
| A0A0R4J1J1| Pnkd      | 3.667 | 1     | 1    | -       | -    | -           | 142  | 15.6  | 10.27|
| Q6IRU2    | Tpm4      | 3.635 | 3     | 1    | -       | -    | -           | 248  | 28.5  | 4.68 |
| Q99PT1    | Arhgdia   | 3.63  | 1     | 1    | -       | -    | -           | 204  | 23.4  | 5.2  |
| Q9CY58    | Serbp1    | 4.996 | 1     | 1    | 1.397   | 1    | 407         | 44.7 | 8.54  |
| P10126    | Eef1a1    | 19.251| 7     | 3    | 15.686  | 6    | 462         | 50.1 | 9.01  |
| Q5CZY9    | Rps16     | 6.678 | 3     | 3    | 3.123   | 1    | 172         | 19.3 | 10.2  |
| Q01853    | Vcp       | 4.541 | 2     | 2    | 1.011   | 1    | 806         | 89.3 | 5.26  |
| Q3UD06    | Atp5c1    | 16.179| 6     | 6    | 12.709  | 5    | 298         | 32.9 | 9.01  |
| P61205    | Arf3      | 6.265 | 2     | 2    | 2.883   | 2    | 181         | 20.6 | 7.43  |
| Q3TQP7    | Acat1     | 8.069 | 4     | 4    | 4.746   | 1    | 424         | 44.8 | 8.35  |
| P62751    | Rpl23a    | 5.134 | 2     | 2    | 1.877   | 1    | 156         | 17.7 | 10.45 |
| Q3TIC8    | Uqrc1     | 8.326 | 5     | 5    | 5.28    | 2    | 480         | 52.7 | 6.1   |
| Q3U6C7    | Sucla2    | 13.441| 7     | 7    | 10.438  | 6    | 470         | 50.6 | 7.69  |
| Q9D172    | D10Jhu81e | 4.263 | 3     | 3    | 1.276   | 1    | 266         | 28.1 | 8.78  |
| P45591    | Cfl2      | 4.077 | 2     | 2    | 1.1     | 1    | 166         | 18.7 | 7.88  |
|       |        |       |     |       |     |     |     |     |     |     |     |     |     |     |     |
|-------|--------|-------|-----|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| P38647 | Hspa9  | 28.435| 9   | 9     | 25.488| 8   | 8   | 679 | 73.4 | 6.07 |
| Q01149 | Colla2 | 2.875 | 1   | 1     | -    | -   | -   | 1372 | 129.5| 9.19 |
| Q20BD0 | Hnnpab | 2.801 | 1   | 1     | -    | -   | -   | 332  | 36.2 | 6.95 |
| J3QMG3 | Vdac3  | 13.248| 4   | 3     | 10.449| 2   | 1   | 284  | 30.8 | 8.79 |
| P97315 | Csrp1  | 2.744 | 1   | 1     | -    | -   | -   | 193  | 20.6 | 8.57 |
| P08032 | Spta1  | 7.921 | 3   | 2     | 5.219| 3   | 2   | 2415 | 279.7| 5.03 |
| Q9XS6  | Dbn1   | 13.093| 6   | 6     | 10.393| 4   | 4   | 706  | 77.2 | 4.49 |
| Q3TIE8 | Dld    | 4.632 | 2   | 2     | 1.97 | 2   | 2   | 510  | 54.6 | 7.55 |
| Q8R4B4 | Dscam1 | 2.614 | 2   | 2     | -    | -   | -   | 365  | 40.5 | 9.32 |
| Q3TET0 | Cct7   | 3.496 | 2   | 2     | 0.895| 1   | 1   | 544  | 59.6 | 7.84 |
| Q6PB66 | Lrpprc | 4.597 | 2   | 2     | 1.999| 1   | 1   | 1392 | 156.5| 6.83 |
| B9EHC7 | Casq2  | 10.377| 3   | 3     | 7.8  | 3   | 3   | 432  | 50.1 | 4.26 |
| O55126 | Nipsnap2 | 5.718 | 3   | 3     | 3.149| 1   | 1   | 281  | 32.9 | 9.26 |
| Q61176 | Arg1   | 2.533 | 1   | 1     | -    | -   | -   | 323  | 34.8 | 7.01 |
| Q0PD65 | Rab2a  | 2.529 | 1   | 1     | -    | -   | -   | 212  | 23.5 | 6.54 |
| Q80YQ1 | Thbs1  | 2.492 | 1   | 1     | -    | -   | -   | 1171 | 129.6| 4.94 |
| Q3TVM2 | Aldh2  | 3.49  | 2   | 2     | 1.021| 1   | 1   | 519  | 56.6 | 7.36 |
| P17563 | Selenbp1 | 2.438 | 2   | 2     | -    | -   | -   | 472  | 52.5 | 6.29 |
| Q3TGZ3 | Idh3g  | 3.301 | 3   | 3     | 0.902| 1   | 1   | 393  | 42.8 | 9.01 |
| Q9D0M3 | Cyc1   | 6.915 | 3   | 3     | 4.535| 2   | 2   | 325  | 35.3 | 9.16 |
| Q62425 | Ndufa4 | 9.507 | 4   | 4     | 7.146| 3   | 3   | 82   | 9.3  | 9.52 |
| Q8C845 | Efhd2  | 12.71 | 5   | 5     | 10.375| 4   | 4   | 240  | 26.8 | 5.14 |
| P08551 | Nefl   | 2.335 | 2   | 1     | -    | -   | -   | 543  | 61.5 | 4.64 |
| D9J302 | Pdlim5 | 2.333 | 2   | 2     | -    | -   | -   | 614  | 66.1 | 8   |
| Gene Symbol | Protein Name | Expression Ratio |
|-------------|--------------|------------------|
| Q5FWB6      | Rplp0        | 9.163            |
| Q05793      | Hspg2        | 2.173            |
| Q9D6J5      | Ndufb8       | 2.168            |
| P56391      | Cox6b1       | 2.162            |
| Q91WD5      | Ndufs2       | 4.439            |
| P19123      | Tnncl        | 12.715           |
| Q9D6J6      | Ndufv2       | 2.965            |
| Q8BH95      | Echs1        | 2.086            |
| Q64516      | Gk           | 2.05             |
| Q3U9P7      | Oxtc1        | 3.476            |
| Q3U1J2      | Eif2s3x      | 2.038            |
| P63101      | Ywhaz        | 9.353            |
| P42125      | Eci1         | 14.214           |
| P04247      | Mb           | 12.617           |
| Q8R1S0      | Coq6         | 1.943            |
| E9PV24      | Fga          | 9.719            |
| O88492      | Plin4        | 4.071            |
| P51660      | Hsd17b4      | 1.849            |
| Q2TPA8      | Hsd12        | 4.587            |
| Q7TSF1      | Dsg1b        | 5.712            |
| Q9CY64      | Blvra        | 1.779            |
| P11531      | Dmd          | 1.682            |
| A0A0G2JFH2  | Map4         | 1.681            |
| P97927      | Lama4        | 1.661            |

**Expression Ratios**

| Expression Ratio |  |  |  |  |  |  |  |
|------------------|---|---|---|---|---|---|---|
| 9.163            |  |  |  |  |  |  |  |
| 2.173            |  |  |  |  |  |  |  |
| 2.168            |  |  |  |  |  |  |  |
| 2.162            |  |  |  |  |  |  |  |
| 4.439            |  |  |  |  |  |  |  |
| 12.715           |  |  |  |  |  |  |  |
| 2.965            |  |  |  |  |  |  |  |
| 2.086            |  |  |  |  |  |  |  |
| 2.05             |  |  |  |  |  |  |  |
| 3.476            |  |  |  |  |  |  |  |
| 2.038            |  |  |  |  |  |  |  |
| 9.353            |  |  |  |  |  |  |  |
| 14.214           |  |  |  |  |  |  |  |
| 12.617           |  |  |  |  |  |  |  |
| 1.943            |  |  |  |  |  |  |  |
| 9.719            |  |  |  |  |  |  |  |
| 4.071            |  |  |  |  |  |  |  |
| 1.849            |  |  |  |  |  |  |  |
| 4.587            |  |  |  |  |  |  |  |
| 5.712            |  |  |  |  |  |  |  |
| 1.779            |  |  |  |  |  |  |  |
| 1.682            |  |  |  |  |  |  |  |
| 1.681            |  |  |  |  |  |  |  |
| 1.661            |  |  |  |  |  |  |  |
| Accession | Description | Fold Change | Replicates | Mean | SD | P-Value | FDR Corrected P-Value |
|-----------|-------------|-------------|------------|------|----|---------|----------------------|
| Q9Z1R9    | Prss1       | 1.632       | 1          | 246  | 26.1| 4.94    |                      |
| Q4FZE6    | Rps7        | 1.601       | 1          | 194  | 22.1| 10.1    |                      |
| Q3UAG2    | Pgd         | 1.502       | 1          | 483  | 53.2| 7.23    |                      |
| Q3TIY5    | Pkp2        | 3.37        | 1          | 795  | 88  | 9.38    |                      |
| Q8K1M6    | Dnm11       | 1.491       | 1          | 742  | 82.6| 7.05    |                      |
| Q9WUR2    | Eci2        | 1.475       | 1          | 391  | 43.2| 8.92    |                      |
| B9E1U1    | Eprs        | 1.462       | 1          | 1512 | 169.9| 7.59    |                      |
| Q545A2    | Slc25a5     | 14.824      | 7          | 298  | 32.9| 9.73    |                      |
| Q6R164    | Psmb1       | 1.458       | 1          | 240  | 26.4| 7.81    |                      |
| P07309    | Tr           | 1.456       | 1          | 147  | 15.8| 6.16    |                      |
| Q3TLP8    | Rac1        | **1.454**   | 1          | 211  | **23.4**| **8.69**|                      |
| Q3V1M8    | Hdlbp       | 1.447       | 1          | 1268 | 141.7| 6.87    |                      |
| Q11136    | pd           | 1.422       | 1          | 493  | 55  | 5.78    |                      |
| Q921L4    | LOC665622   | 4.433       | 2          | 135  | 14.9| 10.13   |                      |
| P13020    | Gsn         | 19.396      | 7          | 780  | 85.9| 6.18    |                      |
| Q3UW40    | Rpl24       | 1.38        | 1          | 160  | 18.2| 11.05   |                      |
| P99029    | Prdx5       | 1.375       | 1          | 210  | 21.9| 8.85    |                      |
| P47963    | Rpl13       | 1.372       | 1          | 211  | 24.3| 11.55   |                      |
| Q8BH59    | Slc25a12    | 12.944      | 7          | 677  | 74.5| 8.25    |                      |
| A0A0A0MQA3| Serpina1a   | 1.364       | 1          | 436  | 48.8| 6.43    |                      |
| A0A0R4J074| Suv39h2     | 1.333       | 1          | 477  | 54.1| 9.04    |                      |
| B7ZW89    | Luzp1       | 1.332       | 1          | 1110 | 124.1| 7.8     |                      |
| P06745    | Gpi         | 12.157      | 4          | 558  | 62.7| 8.13    |                      |
| Q3TRK9    | Slc16a1     | 7.547       | 2          | 493  | 53.3| 7.47    |                      |
| Gene ID   | Description | Value1 | Value2 | Value3 | Value4 | Value5 | Value6 | Value7 | Value8 |
|----------|-------------|--------|--------|--------|--------|--------|--------|--------|--------|
| Q3TRW3   | Snd1        | 1.261  | 1      | 1      | -      | -      | -      | 910    | 102    | 7.56   |
| P49813   | Tmod1       | 56.117 | 13     | 13     | 54.916 | 16     | 15     | 359    | 40.4   | 5.1    |
| Q9DCT8   | Crip2       | 4.943  | 3      | 3      | 3.759  | 2      | 2      | 208    | 22.7   | 8.63   |
| P09103   | P4hb        | 3.024  | 2      | 2      | 1.844  | 1      | 1      | 509    | 57     | 4.88   |
| Q4VAG4   | Rpl22       | 5.253  | 2      | 2      | 4.081  | 1      | 1      | 128    | 14.8   | 9.19   |
| Q3U8F5   | Psmd12      | 1.169  | 1      | 1      | -      | -      | -      | 456    | 52.8   | 7.06   |
| D3YTT6   | Osbpl3      | 1.149  | 1      | 1      | -      | -      | -      | 886    | 100.4  | 6.93   |
| P59999   | Arpc4       | 2.447  | 2      | 2      | 1.306  | 1      | 1      | 168    | 19.7   | 8.43   |
| P05213   | Tuba1b      | 24.549 | 8      | 3      | 23.412 | 7      | 2      | 451    | 50.1   | 5.06   |
| Q9DCX2   | Atp5h       | 8.491  | 3      | 3      | 7.366  | 3      | 3      | 161    | 18.7   | 5.69   |
| Q7M6Y5   | Deup1       | 1.123  | 1      | 1      | -      | -      | -      | 601    | 69.6   | 6.81   |
| F8VQM8   | Xntrpc      | 1.12   | 1      | 1      | -      | -      | -      | 1264   | 140.6  | 8.12   |
| Q91V99   | fau         | 2.595  | 1      | 1      | 1.478  | 1      | 1      | 137    | 14.8   | 10.05  |
| P62245   | Rps15a      | 1.057  | 1      | 1      | -      | -      | -      | 130    | 14.8   | 10.13  |
| P67778   | Phb         | 13.517 | 6      | 6      | 12.46  | 5      | 5      | 272    | 29.8   | 5.76   |
| Q64514   | Tpp2        | 1.056  | 1      | 1      | -      | -      | -      | 1262   | 139.8  | 6.58   |
| Q9CZK9   | Ppia        | 2.382  | 1      | 1      | 1.337  | 1      | 1      | 167    | 18.3   | 7.9    |
| Q99P72   | Rtn4        | 1.034  | 1      | 1      | -      | -      | -      | 1162   | 126.5  | 4.54   |
| Q7TNL3   | Stk40       | 1.032  | 1      | 1      | -      | -      | -      | 435    | 48.9   | 7.74   |
| Q69Z91   | Acss1       | 1.014  | 1      | 1      | -      | -      | -      | 683    | 74.7   | 6.86   |
| Q3TGR2   | Fgb         | 2.002  | 2      | 2      | 0.997  | 1      | 1      | 481    | 54.7   | 7.08   |
| A2AHK0   | Dgkz        | 0.999  | 1      | 1      | -      | -      | -      | 1123   | 125    | 8.91   |
| Q3TRR0   | Map9        | 0.987  | 1      | 1      | -      | -      | -      | 646    | 73.5   | 7.62   |
| D6Q0F5   | Dyncl1      | 0.986  | 1      | 1      | -      | -      | -      | 655    | 73.3   | 5.15   |
| Gene ID  | Description | ACOT2 | WT-ACOT2 | WT-ACOT1 | M1 | M2 | M3 | M4 | M5 | M6 | M7 | M8 | M9 | M10 |
|----------|-------------|-------|----------|----------|----|----|----|----|----|----|----|----|----|-----|
| Q6P2K2  | Acot2       | 0.976 | 1        | 1        | -  | -  | -  | -  | 453 | 49.6 | 7.62 |
| E9PZI6  | Katna1      | 0.954 | 1        | 1        | -  | -  | -  | -  | 493 | 56.2 | 7.5  |
| Q99KW3  | Triobp      | 0.952 | 1        | 1        | -  | -  | -  | -  | 2014 | 223.2 | 8.06 |
| A7XUZ6  | Skint6      | 0.942 | 1        | 1        | -  | -  | -  | -  | 1240 | 141.5 | 7.17 |
| Q9QYB1  | Clic4       | 1.98  | 1        | 1        | 1.043 | 1 | 1 | 253 | 28.7 | 5.59 |
| Q811G0  | Bbs9        | 0.937 | 1        | 1        | -  | -  | -  | -  | 885 | 99 | 6.13 |
| B9EK96  | Gm15319     | 0.931 | 1        | 1        | -  | -  | -  | -  | 350 | 39.5 | 8.73 |
| Q8CC88  | Vwa8        | 0.923 | 1        | 1        | -  | -  | -  | -  | 1905 | 213.3 | 6.6 |
| Q64512  | Ptpn13      | 0.912 | 1        | 1        | -  | -  | -  | -  | 2453 | 270.2 | 6.37 |
| P50580  | Pa2g4       | 0.912 | 1        | 1        | -  | -  | -  | -  | 394 | 43.7 | 6.86 |
| P97443  | Smyd1       | 0.91  | 1        | 1        | -  | -  | -  | -  | 490 | 56.5 | 6.92 |
| A0A0R4J0Q5| Lmnb2      | 2.976 | 3        | 1        | 2.069 | 2 | 1 | 615 | 69 | 5.55 |
| P54775  | Psmc4       | 0.903 | 1        | 1        | -  | -  | -  | -  | 418 | 47.4 | 5.21 |
| A2AD39  | Stil        | 0.903 | 1        | 1        | -  | -  | -  | -  | 1262 | 138.7 | 6.43 |
| B1AWB9  | Col5a1      | 4.016 | 2        | 2        | 3.127 | 2 | 2 | 1838 | 183.6 | 4.98 |
| A0A1B0GR11| Taldo1     | 0.88  | 1        | 1        | -  | -  | -  | -  | 382 | 42.1 | 7.02 |
| P11983  | Tcp1        | 3.528 | 2        | 2        | 2.661 | 2 | 2 | 556 | 60.4 | 6.16 |
| Q3TCH2  | Uchl1       | 0.858 | 1        | 1        | -  | -  | -  | -  | 225 | 25.1 | 5.33 |
| E9PVY8  | Macf1       | 0.853 | 1        | 1        | -  | -  | -  | -  | 7355 | 831.4 | 5.41 |
| Q5SW83  | Actr2       | 0.841 | 1        | 1        | -  | -  | -  | -  | 394 | 44.7 | 6.74 |
| Q8R558  | Sh3bgr      | 0.826 | 1        | 1        | -  | -  | -  | -  | 234 | 25.1 | 4.08 |
| Q3U280  | Vpsd9l      | 0.818 | 1        | 1        | -  | -  | -  | -  | 650 | 71.3 | 6.87 |
| E9Q8Q6  | Ccde141     | 0.816 | 1        | 1        | -  | -  | -  | -  | 1531 | 173.5 | 5.88 |
| A0A0G2JGS4| Camk2d     | 2.226 | 2        | 2        | 1.415 | 1 | 1 | 533 | 60 | 7.27 |
| Protein ID | Gene Symbol | Score | T- | P- | N- | Value | Mass | p-Value |
|------------|-------------|-------|----|----|----|-------|------|--------|
| Q571M2     | Hspa4       | 0.806 | 1  | 1  | -  | -    | 930  | 103.2  | 5.88   |
| Q02788     | Col6a2      | 0.798 | 1  | 1  | -  | -    | 1034 | 110.3  | 6.42   |
| Q8BVQ9     | Psmc2       | 0.795 | 1  | 1  | -  | -    | 475  | 52.8   | 6.28   |
| F6QFD1     | Pde4d       | 0.792 | 1  | 1  | -  | -    | 754  | 85.5   | 5.25   |
| O88322     | Nid2        | 0.791 | 1  | 1  | -  | -    | 1403 | 153.8  | 5.38   |
| Q7TPW6     | Scl25a20    | 0.791 | 1  | 1  | -  | -    | 301  | 32.9   | 9.11   |
| Q9D180     | Cfas57      | 0.785 | 1  | 1  | -  | -    | 1249 | 144.8  | 5.74   |
| O35948     | Wrn         | 0.782 | 1  | 1  | -  | -    | 643  | 72.8   | 8.46   |
| H3BK67     | Hmbox1      | 0.78  | 1  | 1  | -  | -    | 405  | 45.9   | 6.33   |
| Q3TA14     | Cd36        | 0.777 | 1  | 1  | -  | -    | 472  | 52.7   | 8.35   |
| A0A286YC18 | Sorbs1      | 0.768 | 1  | 1  | -  | -    | 1290 | 143    | 8.31   |
| Q8R2P1     | Khhl25      | 0.761 | 1  | 1  | -  | -    | 589  | 65.8   | 6.71   |
| Q8BGH2     | Samm50      | 2.243 | 2  | 2  | 1.504 | 1  | 1   | 469  | 51.8   | 6.8    |
| Q6ZPF0     | Rab1a       | 8.382 | 4  | 4  | 7.667 | 3  | 2   | 205  | 22.7   | 6.21   |
| P27773     | Pdia3       | 4.871 | 2  | 2  | 4.199 | 3  | 3   | 505  | 56.6   | 6.21   |
| E9QNP0     | Kxd1        | 8.781 | 4  | 4  | 8.165 | 4  | 4   | 232  | 26.8   | 9.83   |
| Q9Z2I8     | Sclr2       | 4.407 | 1  | 1  | 3.881 | 1  | 1   | 433  | 46.8   | 7.02   |
| A0A1S6GWJ8 | Hnmpm       | 2.606 | 1  | 1  | 2.134 | 1  | 1   | 809  | 86.4   | 9.11   |
| Q5XK33     | Sdhec       | 2.035 | 1  | 1  | 1.584 | 1  | 1   | 169  | 18.4   | 10.14  |
| Q9JHU4     | Dyncl1h1    | 1.384 | 1  | 1  | 0.992 | 1  | 1   | 4644 | 531.7  | 6.42   |
| Q545C7     | Csrp3       | 2.573 | 1  | 1  | 2.21  | 1  | 1   | 194  | 20.9   | 8.54   |
| Q9D051     | Pdheb       | 4.954 | 2  | 2  | 4.614 | 2  | 2   | 359  | 38.9   | 6.87   |
| Q5SX53     | Scl25a11    | 13.611 | 6  | 6  | 13.306 | 4  | 4   | 314  | 34.1   | 9.94   |
| Q9D881     | Gm11273     | 2.979 | 1  | 1  | 2.698 | 1  | 1   | 129  | 13.8   | 8.12   |
| Accession  | Gene | Description | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 |
|------------|------|-------------|----|----|----|----|----|----|----|----|----|-----|-----|
| A0A1W2P768 | Hist2h3c1 | 3.721 | 3 | 3 | 3.445 | 2 | 2 | 181 | 20.2 | 11.39 |
| Q3UER8     | Fgg   | 1.425 | 1 | 1 | 1.18 | 1 | 1 | 443 | 50.3 | 5.62  |
| A0JLY1     | Ccde173 | 1.529 | 1 | 1 | 1.287 | 1 | 1 | 547 | 65.1 | 8.97  |
| P62830     | Rpl23 | 1.045 | 1 | 1 | 0.808 | 1 | 1 | 140 | 14.9 | 10.51 |
| Q3UHZ5     | Lmod2 | 1.795 | 1 | 1 | 1.58 | 1 | 1 | 550 | 62 | 5.68  |
| Q3US43     | Anxa1 | 2.648 | 1 | 1 | 2.45 | 2 | 2 | 355 | 40.3 | 9.03  |
| P61089     | Ube2n | 1.186 | 1 | 1 | 1.035 | 1 | 1 | 152 | 17.1 | 6.57  |
| Q8BMF4     | Dlat  | 1.802 | 1 | 1 | 1.674 | 1 | 1 | 642 | 67.9 | 8.57  |
| Q3UPA3     | Gdi2  | 1.557 | 1 | 1 | 1.43 | 1 | 1 | 512 | 57.7 | 7.94  |
| A8IP69     | Ywhag | 5.057 | 3 | 1 | 4.947 | 3 | 1 | 247 | 28.3 | 4.89  |
| Q01730     | Rsu1  | 1.099 | 1 | 1 | 1.008 | 1 | 1 | 277 | 31.5 | 8.88  |
| P14115     | Rpl27a | 1.267 | 1 | 1 | 1.217 | 1 | 1 | 148 | 16.6 | 11.12 |
| Q3TF40     | Nono  | 0.959 | 1 | 1 | 0.918 | 1 | 1 | 528 | 60.4 | 9.61  |
| P11087     | Col1a1 | 0.925 | 1 | 1 | 0.885 | 1 | 1 | 1453 | 137.9 | 5.85  |
| P61358     | Rpl27 | 3.732 | 2 | 2 | 3.694 | 2 | 2 | 136 | 15.8 | 10.56 |
| Q08189     | Tgm3  | 3.786 | 1 | 1 | 3.756 | 1 | 1 | 693 | 77.3 | 6.81  |
| A2AE89     | Gstm1 | 1.786 | 1 | 1 | 1.765 | 1 | 1 | 244 | 28.5 | 8.19  |
| Q9D8E6     | Rpl4  | 1.685 | 1 | 1 | 1.689 | 1 | 1 | 419 | 47.1 | 11    |
| Q80VP2     | Spata7 | 0.805 | 1 | 1 | 0.814 | 1 | 1 | 582 | 65.6 | 6.7   |
| P05202     | Got2  | 8.879 | 4 | 4 | 8.921 | 3 | 3 | 430 | 47.4 | 9     |
| Q9CQV8     | Ywhab | 5.961 | 3 | 1 | 6.009 | 3 | 1 | 246 | 28.1 | 4.83  |
| Q3TF25     | Atp5o | 12.9  | 5 | 5 | 12.999 | 5 | 5 | 213 | 23.4 | 9.89  |
| Q9CQ62     | Decr1 | 6.299 | 2 | 2 | 6.4   | 3 | 3 | 335 | 36.2 | 8.95  |
| Q3UA52     | Arpc2 | 1.814 | 1 | 1 | 1.922 | 1 | 1 | 383 | 42.5 | 9.06  |
| Protein ID | Gene Symbol | Fold Change | P-Value | q-Value | MLP | HPI |
|------------|-------------|-------------|---------|---------|-----|-----|
| Q6IE21     | Otud6a      | 0.89        | 1       | 1       | 1.009 | 1   |
| P70349     | Hint1       | 2.197       | 1       | 1       | 2.326 | 1   |
| P60867     | Rps20       | 2.915       | 1       | 1       | 3.054 | 1   |
| Q9QXS1     | Plec        | 3.454       | 2       | 2       | 3.597 | 2   |
| Q921G7     | Etfdh       | 0.824       | 1       | 1       | 0.99  | 1   |
| A0A1S6GWH1 | Psmc5       | 4.338       | 1       | 1       | 4.509 | 1   |
| Q05C68     | Metch2      | 1.518       | 1       | 1       | 1.69  | 1   |
| P60335     | Pcbp1       | 5.181       | 3       | 3       | 5.357 | 3   |
| P05125     | Nppa        | 1.658       | 1       | 1       | 1.838 | 1   |
| D3Z4Z0     | Tada2b      | 1.036       | 1       | 1       | 1.23  | 1   |
| P97461     | Rps5        | 2.166       | 1       | 1       | 2.37  | 1   |
| Q3THQ5     | Stip1       | 0.865       | 1       | 1       | 1.07  | 1   |
| Q58EW0     | Rpl18       | 2.686       | 1       | 1       | 2.904 | 1   |
| Q9CYT6     | Cap2        | 1.464       | 1       | 1       | 1.697 | 1   |
| Q3TC14     | Impa2       | 2.044       | 1       | 1       | 2.317 | 1   |
| P61164     | Actr1a      | 1.254       | 1       | 1       | 1.557 | 1   |
| Q78IK2     | Usmg5       | 2.586       | 1       | 1       | 2.896 | 1   |
| P24369     | Ppip        | 0.851       | 1       | 1       | 1.176 | 1   |
| P19324     | Serpinh1    | 15.399      | 7       | 7       | 15.733 | 6 | 6 |
| B2RX02     | Zfp692      | 1.28        | 1       | 1       | 1.62  | 1   |
| Q9D023     | Mpc2        | 1.624       | 1       | 1       | 1.973 | 1   |
| P47934     | Crat        | 9.721       | 4       | 4       | 10.094 | 6 | 6 |
| Q9CQ69     | Uqcrq       | 3.371       | 2       | 2       | 3.768 | 2   |
| P14069     | S100a6      | 1.324       | 1       | 1       | 1.737 | 1   |
|     |       |      |      |      |      |      |      |      |      |      |
|-----|-------|------|------|------|------|------|------|------|------|------|
| P43277 | Hist1h1d | 5.79 | 3   | 3   | 6.203 | 3   | 1   | 221  | 22.1 | 11.03 |
| A0A0N4SVK8 | Adprhl1 | 2.899 | 2   | 2   | 3.353 | 1   | 1   | 1697 | 184.1 | 7.66  |
| Q3V2E0  | Try5   | 1.283 | 1   | 1   | 1.784 | 1   | 1   | 255  | 27.1  | 6.73  |
| Q4VAE8  | Ndufb4 | 0.832 | 1   | 1   | 1.358 | 1   | 1   | 131  | 15.3  | 9.8   |
| P47857  | Pfk   | 3.761 | 3   | 3   | 4.291 | 4   | 4   | 780  | 85.2  | 8     |
| Q6R0H7  | Gnas   | 2.939 | 1   | 1   | 3.47  | 1   | 1   | 1133 | 121.4 | 4.81  |
| P56135  | Atp5j2 | 2.564 | 1   | 1   | 3.105 | 1   | 1   | 88   | 10.3  | 9.95  |
| Q920M5  | Coro6  | 9.033 | 3   | 3   | 9.587 | 3   | 3   | 471  | 52.6  | 5.96  |
| P18572  | Bsg    | 1.535 | 1   | 1   | 2.193 | 1   | 1   | 389  | 42.4  | 5.85  |
| Q9WUM5  | Suclg1 | 3.665 | 2   | 2   | 4.329 | 1   | 1   | 346  | 36.1  | 9.39  |
| P48036  | Anxa5  | 1.477 | 1   | 1   | 2.196 | 1   | 1   | 319  | 35.7  | 4.96  |
| F8WIV2  | Serpin6a | 0.82  | 1   | 1   | 1.555 | 1   | 1   | 399  | 44.7  | 6.38  |
| E9PYL9  | Gm10036 | 5.014 | 2   | 2   | 5.765 | 2   | 2   | 178  | 20.3  | 9.51  |
| Q3UIM5  | Cpt1b  | 5.967 | 3   | 3   | 6.75  | 3   | 3   | 772  | 88.1  | 8.38  |
| P27659  | Rp3    | 1.772 | 1   | 1   | 2.568 | 2   | 2   | 403  | 46.1  | 10.21 |
| Q04857  | Col6a1 | 1.554 | 1   | 1   | 2.352 | 1   | 1   | 1025 | 108.4 | 5.36  |
| Q8C266  | Rab5c  | 2.596 | 1   | 1   | 3.433 | 1   | 1   | 234  | 25.3  | 8.16  |
| Q3TCP5  | Ezr    | 8.87  | 5   | 1   | 9.727 | 5   | 5   | 586  | 69.4  | 6.3   |
| Q9DB79  | Rps11  | 1.598 | 1   | 1   | 2.478 | 2   | 2   | 166  | 19.4  | 10.14 |
| Q7TPR4  | Actn1  | 21.972 | 7   | 1   | 22.891 | 8   | 2   | 892  | 103  | 5.38  |
| Q3TI05  | Cct6a  | 1.686 | 1   | 1   | 2.658 | 2   | 2   | 531  | 58  | 7.08  |
| Q3V0Z8  | Ddx5   | 3.264 | 1   | 1   | 4.261 | 1   | 1   | 690  | 76.7  | 8.6   |
| P97807  | Fh     | 8.086 | 3   | 3   | 9.101 | 4   | 4   | 507  | 54.3  | 9.04  |
| A0A1Y7VKY1 | Gm11361 | 6.226 | 3   | 3   | 7.249 | 3   | 3   | 152  | 17.7  | 10.86 |
| Accession | Protein | NIH | Kit | NIH/Kit | NIH | NIH/Kit | NIH | NIH/Kit |
|-----------|---------|-----|-----|---------|-----|---------|-----|---------|
| Q4FK74    | Atp5d   | 3.947 | 1 | 1 | 4.983 | 1 | 1 | 168 | 17.6 | 5.08 |
| P12787    | Cox5a   | 1.108 | 1 | 1 | 2.151 | 1 | 1 | 146 | 16.1 | 6.54 |
| P68040    | Rack1   | 5.198 | 4 | 4 | 6.245 | 4 | 4 | 317 | 35.1 | 7.69 |
| Q9CZU6    | Cs      | 2.568 | 2 | 2 | 3.615 | 2 | 2 | 464 | 51.7 | 8.57 |
| B2MWM9    | Calr    | 3.673 | 3 | 3 | 4.748 | 2 | 2 | 416 | 48 | 4.49 |
| Q9CPQ1    | Cox6c   | 1.628 | 1 | 1 | 2.704 | 2 | 2 | 76 | 8.5 | 10.14 |
| P48962    | Slc25a4 | 15.332 | 7 | 5 | 16.436 | 9 | 5 | 298 | 32.9 | 9.72 |
| Q3TVV6    | Hnmpu   | 2.742 | 2 | 2 | 3.875 | 3 | 3 | 800 | 87.9 | 6.24 |
| P35700    | Prdx1   | 7.997 | 4 | 4 | 9.174 | 5 | 5 | 199 | 22.2 | 8.12 |
| K3W4S6    | Gyg     | 1.23  | 1 | 1 | 2.412 | 1 | 1 | 377 | 41.9 | 6.02 |
| P02535    | Krt10   | 23.806 | 10 | 8 | 24.997 | 9 | 7 | 570 | 57.7 | 5.11 |
| Q5BLJ7    | Rps13   | 0.98  | 1 | 1 | 2.217 | 2 | 2 | 151 | 17.2 | 10.54 |
| Q3TGE1    | Actr3   | 1.1   | 1 | 1 | 2.346 | 1 | 1 | 418 | 47.4 | 5.88 |
| P97351    | Rps3a   | 2.148 | 2 | 2 | 3.399 | 2 | 2 | 264 | 29.9 | 9.73 |
| F6VW30    | Ywhaq   | 7.818 | 3 | 1 | 9.072 | 3 | 1 | 303 | 34.3 | 6.38 |
| P56382    | Atp5f1e | 1.486 | 1 | 1 | 2.794 | 2 | 2 | 52 | 5.8 | 10.01 |
| Q11011    | Npps    | 4.403 | 2 | 2 | 5.788 | 2 | 2 | 920 | 103.3 | 5.9 |
| Q99LX0    | Park7   | 0.847 | 1 | 1 | 2.238 | 2 | 2 | 189 | 20 | 6.77 |
| Q00623    | Apoa1   | 7.838 | 4 | 4 | 9.305 | 4 | 4 | 264 | 30.6 | 5.73 |
| Q9QUM9    | Psma6   | 2.655 | 1 | 1 | 4.139 | 1 | 1 | 246 | 27.4 | 6.76 |
| Q99JY0    | Hadhb   | 14.338 | 6 | 6 | 15.827 | 7 | 7 | 475 | 51.4 | 9.38 |
| Q6A0D0    | Prdx6   | 1.062 | 1 | 1 | 2.579 | 2 | 2 | 227 | 25.1 | 6.37 |
| P10639    | Txn     | 2.902 | 1 | 1 | 4.435 | 1 | 1 | 105 | 11.7 | 4.92 |
| Q3TWH2    | Nnt     | 10.313 | 6 | 6 | 11.894 | 6 | 6 | 1086 | 113.8 | 7.18 |
| Gene   | Protein | NPM1 | NPM2 | NPM3 | NPM4 | NPM5 | NPM6 |
|--------|---------|------|------|------|------|------|------|
| O35129 | Phb2    | 10.609 | 5    | 5    | 12.27 | 5    | 5    |
| P70670 | Naca    | 6.747  | 2    | 2    | 8.434 | 3    | 3    |
| Q5M9N9 | Prdx2   | 5.498  | 3    | 3    | 7.255 | 3    | 3    |
| O70569 | rps14   | 5.929  | 2    | 2    | 7.719 | 2    | 2    |
| A0A0G2JEX1 | Nexn | 14.71  | 10   | 10   | 16.535 | 9    | 3    | 671  | 80   | 5.22 |
| P62852 | Rps25   | 3.351  | 2    | 2    | 5.244 | 2    | 2    |
| Q9CQA3 | Sdhb    | 6.535  | 4    | 4    | 8.504 | 4    | 4    |
| E9QK41 | Ablim1  | 1.306  | 1    | 1    | 3.28  | 1    | 1    |
| Q9D8N0 | Eef1g   | 1.255  | 1    | 1    | 3.252 | 2    | 2    |
| Q06185 | Atp5i   | 3.039  | 1    | 1    | 5.07  | 2    | 2    |
| A0A068BFR3 | Rab11b | 2.966  | 1    | 1    | 5.063 | 2    | 2    |
| A0A075DC90 | COX2 | 5.053  | 2    | 2    | 7.194 | 3    | 3    |
| Q9JK42 | Pdk2    | 0.824  | 1    | 1    | 2.99  | 2    | 2    |
| P61750 | Arf4    | 1.004  | 1    | 1    | 3.247 | 2    | 2    |
| E9Q0F0 | Krt78   | 6.667  | 2    | 2    | 8.928 | 3    | 1    |
| A0A1L1STE6 | Idh3a | 11.802 | 6    | 6    | 14.074 | 6    | 6    |
| Q60598 | Cttm    | 0.954  | 1    | 1    | 3.237 | 3    | 3    |
| Q678L1 | Krt77   | 15.812 | 5    | 1    | 18.127 | 5    | 2    |
| Q8VHX6 | Flnc    | 26.907 | 12   | 9    | 29.252 | 12   | 9    |
| A0A1B0GSX0 | Ldha | 16.776 | 8    | 7    | 19.151 | 6    | 6    |
| A2AB79 | Hist3h2a | 7.665 | 2    | 2    | 10.074 | 3    | 3    |
| P62827 | Ran     | 5.108  | 3    | 3    | 7.524 | 3    | 3    |
| P14733 | Lmmnb1  | 6.697  | 4    | 2    | 9.194 | 4    | 3    |
| Accession | Gene   | 1   | 1   | 2   | 2   | 3   | 5   | 7   | 9   |
|-----------|--------|-----|-----|-----|-----|-----|-----|-----|-----|
| Q60692    | Psmb6  | 1.131 | 1 | 1   | 3.665 | 2   | 2   | 238 | 25.4 | 5.11 |
| Q6A0F1    | Cct8   | 2.536 | 2 | 2   | 5.215 | 3   | 3   | 555 | 60.2 | 5.62 |
| O55234    | Psmb5  | 2.87 | 1 | 1   | 5.614 | 2   | 2   | 264 | 28.5 | 7.02 |
| Q3UK56    | Rps3   | 7.185 | 3 | 3   | 10.005 | 4   | 4   | 243 | 26.7 | 9.52 |
| G5E902    | Slc25a3 | 6.119 | 4 | 4   | 8.995 | 5   | 5   | 358 | 39.7 | 9.29 |
| Q9WUB3    | Pygm   | 13.954 | 9 | 6   | 16.892 | 10  | 6   | 842 | 97.2 | 7.11 |
| Q56A15    | Cycs   | 6.396 | 2 | 2   | 9.45  | 3   | 3   | 105 | 11.6 | 9.58 |
| D3Z041    | Acsl1  | 8.026 | 4 | 4   | 11.097 | 5   | 5   | 699 | 78   | 7.47 |
| Q9DCW4    | Etfb   | 9.096 | 4 | 4   | 12.181 | 4   | 4   | 255 | 27.6 | 8.1  |
| Q6ZWN5    | Rps9   | 2.888 | 2 | 2   | 6.057 | 4   | 4   | 194 | 22.6 | 10.65 |
| Q07417    | Acads  | 6.754 | 3 | 3   | 9.969 | 4   | 4   | 412 | 44.9 | 8.47 |
| Q6P8J7    | Ckmt2  | 4.824 | 2 | 2   | 8.079 | 3   | 3   | 419 | 47.4 | 8.4  |
| Q99K10    | Aco2   | 28.471 | 11 | 11  | 31.755 | 12  | 12  | 780 | 85.4 | 7.93 |
| A0A0A0MQA5 | Tuba4a | 11.625 | 6 | 1   | 14.96 | 6   | 1   | 477 | 52.9 | 5.19 |
| Q91VA7    | Idh3b  | 9.658 | 4 | 4   | 13.173 | 4   | 4   | 384 | 42.2 | 8.6  |
| P05132    | Prkaca | 1.914 | 1 | 1   | 5.441 | 3   | 3   | 351 | 40.5 | 8.79 |
| Q5NC14    | Pgam2  | 7.831 | 3 | 3   | 11.424 | 3   | 3   | 253 | 28.8 | 8.5  |
| Q9CQY3    | Atp5l  | 1.909 | 1 | 1   | 5.508 | 2   | 2   | 129 | 14.2 | 8.63 |
| P23927    | Cryab  | 9.167 | 3 | 3   | 12.787 | 6   | 6   | 175 | 20.1 | 7.33 |
| A2AFQ2    | Hsd17b10 | 0.899 | 1 | 1   | 4.54  | 2   | 2   | 271 | 28.4 | 8.76 |
| Q54AH9    | Hbb-b2 | 11.496 | 4 | 1   | 15.174 | 5   | 1   | 146 | 15.7 | 8.05 |
| Q9CQB4    | Uqcrb  | 2.696 | 1 | 1   | 6.376 | 2   | 2   | 111 | 13.6 | 9.11 |
| P63017    | Hspa8  | 31.379 | 11 | 10  | 35.061 | 12  | 5   | 646 | 70.8 | 5.52 |
| Q3TF14    | Ahcy   | 0.797 | 1 | 1   | 4.638 | 1   | 1   | 432 | 47.7 | 6.54 |
| Gene ID     | Gene Name | Log2 FC | Count | Fold Change | p-Value | Adj. p-Value |
|------------|-----------|---------|-------|-------------|---------|--------------|
| Q5M9P3     | Rps19     | 3.744   | 2     | 2           | 7.606   | 4            |
| O70435     | Psma3     | 1.644   | 1     | 1           | 5.56    | 2            |
| Q8K3Q4     | Actn2     | 57.109  | 21    | 15          | 61.05   | 22           |
| P199001    | Krt19     | 11.824  | 7     | 2           | 15.84   | 8            |
| A0A1S6GW10 | Ndufa8    | 1.192   | 1     | 1           | 5.292   | 3            |
| Q546G4     | Alb       | 36.23   | 15    | 15          | 40.353  | 15           |
| E9Q616     | Ahnak     | 1.952   | 1     | 1           | 6.102   | 3            |
| F6X162     | Rpl7      | 4.545   | 3     | 3           | 8.736   | 4            |
| P97350     | Pcp1      | 5.339   | 3     | 3           | 9.532   | 2            |
| Q76MZ3     | Ppp2r1a   | 2.733   | 3     | 3           | 7.021   | 3            |
| P62259     | Ywhae     | 7.453   | 5     | 4           | 11.931  | 3            |
| P31001     | Des       | 41.082  | 14    | 11          | 45.603  | 14           |
| A0A0R4J083 | Acadl     | 10.167  | 5     | 5           | 14.88   | 4            |
| P58252     | Eef2      | 6.1     | 3     | 3           | 10.82   | 5            |
| P21981     | Tgm2      | 3.076   | 2     | 2           | 7.816   | 4            |
| P14824     | Anxa6     | 2.595   | 2     | 2           | 7.339   | 5            |
| Q8BTM8     | Flna      | 15.329  | 7     | 5           | 20.144  | 8            |
| Q3TL71     | Hnrnpk    | 2.622   | 2     | 2           | 7.47    | 3            |
| P35492     | Hal       | 0.909   | 1     | 1           | 5.794   | 3            |
| Q3TFA9     | Tmod3     | 12.208  | 6     | 6           | 17.105  | 6            |
| P62702     | Rps4x     | 8.017   | 5     | 5           | 12.961  | 7            |
| P11404     | Fabp3     | 4.966   | 3     | 3           | 9.939   | 5            |
| P20029     | Hspa5     | 20.342  | 7     | 6           | 25.333  | 6            |
| A8DUV3     | Hbat1     | 28.977  | 6     | 6           | 34.095  | 5            |
| Protein ID  | Gene Symbol | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 | Value 6 |
|------------|-------------|---------|---------|---------|---------|---------|---------|
| Q8C338     | Idh1        | 2.22    | 1       | 1       | 7.434   | 3       | 2       | 422     | 47.5    | 6.93    |
| Q3TU19     | Psma5       | 1.018   | 1       | 1       | 6.323   | 2       | 2       | 241     | 26.4    | 4.86    |
| A0A0F7QZE4 | HC          | 24.256  | 7       | 6       | 29.62   | 6       | 5       | 460     | 50.7    | 7.77    |
| P19783     | Cox4i1      | 4.932   | 2       | 2       | 10.312  | 5       | 5       | 169     | 19.5    | 9.23    |
| Q3UIQ2     | Ndufs1      | 20.991  | 10      | 10      | 26.521  | 11      | 11      | 727     | 79.7    | 5.72    |
| P16125     | Ldhb        | 15.36   | 6       | 5       | 20.907  | 7       | 7       | 334     | 36.5    | 6.05    |
| A0A2R8VHP3 | Gm5478      | 12.967  | 7       | 2       | 18.614  | 8       | 1       | 535     | 57.9    | 6.2     |
| Q9CQR4     | Acot13      | 1.007   | 1       | 1       | 6.723   | 2       | 2       | 140     | 15.2    | 8.82    |
| Q9CR68     | Uqerfs1     | 1.564   | 1       | 1       | 7.373   | 4       | 4       | 274     | 29.3    | 8.7     |
| Q3UFJ3     | Pdha1       | 7.771   | 3       | 3       | 13.62   | 7       | 7       | 390     | 43.2    | 8.19    |
| Q5FW97     | EG433182    | 13.218  | 7       | 7       | 19.205  | 8       | 7       | 434     | 47.1    | 6.8     |
| P14152     | Mdh1        | 4.954   | 2       | 2       | 10.945  | 4       | 4       | 334     | 36.5    | 6.58    |
| P09411     | Pgk1        | 10.148  | 5       | 5       | 16.179  | 6       | 6       | 417     | 44.5    | 7.9     |
| Q1MWP8     | Ehd4        | 1.98    | 2       | 2       | 8.066   | 3       | 3       | 544     | 61.6    | 6.76    |
| O88569     | Hnrnpa2b1   | 8.903   | 5       | 5       | 15.163  | 6       | 5       | 353     | 37.4    | 8.95    |
| P48678     | Lmna        | 14.206  | 8       | 7       | 20.478  | 9       | 9       | 665     | 74.2    | 6.98    |
| B2CY77     | Rpsa        | 3.009   | 1       | 1       | 9.287   | 2       | 2       | 295     | 32.8    | 4.87    |
| P62806     | Hist1h4a    | 7.538   | 4       | 4       | 13.849  | 5       | 5       | 103     | 11.4    | 11.36   |
| Q3TFG3     | Eif4a1      | 8.759   | 4       | 4       | 15.164  | 3       | 3       | 406     | 46.2    | 5.48    |
| Q9WUZ5     | Tnni1       | 2.499   | 2       | 2       | 9.023   | 4       | 4       | 187     | 21.7    | 9.58    |
| Q3TJD4     | Atp5f1      | 4.704   | 3       | 3       | 11.233  | 5       | 5       | 256     | 28.9    | 9.26    |
| P54071     | Idh2        | 51.392  | 15      | 15      | 58.022  | 19      | 18      | 452     | 50.9    | 8.69    |
| Q0VBK2     | Krt80       | 2.284   | 2       | 2       | 8.93    | 4       | 4       | 452     | 50.6    | 6.27    |
| Q3TFQ8     | Pygb        | 13.973  | 5       | 2       | 20.748  | 9       | 5       | 843     | 96.6    | 6.65    |
| Protein  | Gene  | 1   | 1   | 2   | 2   | 3   | 3   | 4   | 4   | 5   | 5   | 6   | 6   | 7   | 7   | 8   | 8   | 9   | 9   | 10  |
|----------|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Q9CQZ5   | Ndufa6| 1.747| 1   | 1   | 8.541| 2   | 2   | 131 | 15.3| 10.11|
| Q3TXF9   | Atp1a1| 9.796| 5   | 5   | 16.647| 5   | 5   | 1023| 112.9| 5.39|
| Q99LC3   | Ndufa10| 6.507| 3   | 3   | 13.728| 6   | 6   | 355 | 40.6| 7.78|
| Q9DB77   | Uqrc2 | 36.559| 9   | 9   | 43.816| 11  | 11  | 453 | 48.2| 9.25|
| P45952   | Acadm | 19.091| 7   | 7   | 26.485| 9   | 9   | 421 | 46.5| 8.37|
| P21550   | Eno3  | 5.89 | 3   | 3   | 13.532| 5   | 4   | 434 | 47   | 7.18|
| Q9EQD6   | K16   | 24.006| 7   | 3   | 31.794| 9   | 4   | 474 | 52   | 5.2 |
| Q8BMS1   | Hadha | 18.348| 9   | 9   | 26.137| 8   | 8   | 763 | 82.6| 9.14|
| Q8BFR5   | Tufm  | 6.635| 3   | 3   | 14.602| 5   | 5   | 452 | 49.5| 7.56|
| P08249   | Mdh2  | 6.915| 5   | 5   | 14.946| 6   | 6   | 338 | 35.6| 8.68|
| Q60932   | Vdac1 | 15.744| 5   | 4   | 24.321| 8   | 7   | 296 | 32.3| 8.43|
| A6ZI44   | Aldoa | 18.457| 10  | 10  | 27.163| 12  | 12  | 418 | 45.1| 7.91|
| P14602   | Hspb1 | 7.535| 3   | 1   | 16.618| 7   | 4   | 82  | 9.4  | 5.39|
| Q9R0Y5   | Ak1   | 1.74 | 1   | 1   | 10.982| 3   | 3   | 194 | 21.5| 5.81|
| P63038   | Hspd1 | 23.276| 10  | 10  | 33.143| 12  | 12  | 573 | 60.9| 6.18|
| Q8K2B3   | Sdha  | 9.216| 4   | 4   | 19.353| 8   | 8   | 664 | 72.5| 7.37|
| P04104   | Krt1  | 15.995| 7   | 3   | 26.839| 9   | 6   | 637 | 65.6| 8.15|
| Q542G9   | Anxa2 | 14.454| 5   | 5   | 26.213| 8   | 8   | 339 | 38.7| 7.69|
| P99024   | Tubb5 | 16.671| 6   | 6   | 28.588| 9   | 2   | 444 | 49.6| 4.89|
| E9Q800   | Immt  | 20.221| 9   | 9   | 32.697| 10  | 10  | 679 | 75.6| 7.8 |
| Q3UKH3   | Acac2 | 31.52 | 11  | 11  | 44.587| 14  | 14  | 397 | 41.8| 8.25|
| P20152   | Vim   | 45.158| 16  | 13  | 58.916| 18  | 13  | 466 | 53.7| 5.12|
| Q3UIJ3   | Actc1 | 110.913| 20  | 1   | 125.471| 21  | 3   | 377 | 42.1| 5.49|
| P07310   | Ckm   | 6.057| 4   | 4   | 20.73 | 7   | 7   | 381 | 43   | 7.06|
|      |      |      |      |      |      |      |      |      |
|------|------|------|------|------|------|------|------|------|
| Q03265 | Atp5f1a | 42.488 | 13 | 13 | 57.264 | 14 | 14 | 553 | 59.7 | 9.19 |
| P11276 | Fn1 | 10.18 | 6 | 6 | 25.042 | 11 | 11 | 2477 | 272.4 | 5.59 |
| Q5DQJ3 | Capza2 | 6.61 | 3 | 2 | 22.134 | 7 | 5 | 286 | 33 | 5.85 |
| A8DUL0 | Hbbt1 | 26.13 | 6 | 2 | 42.18 | 10 | 5 | 147 | 15.8 | 7.65 |
| Q9QWL7 | Krt17 | 33.087 | 14 | 6 | 49.719 | 16 | 2 | 433 | 48.1 | 5.06 |
| B2RTP7 | Krt2 | 20.974 | 10 | 6 | 38.493 | 15 | 7 | 707 | 70.9 | 8.06 |
| P50446 | Krt6a | 34.776 | 14 | 5 | 54.27 | 20 | 4 | 553 | 59.3 | 7.94 |
| A0A0A0MQF6 | Gapdh | 32.879 | 10 | 10 | 52.566 | 13 | 13 | 359 | 38.6 | 8.97 |
| Q02257 | Jup | 15.195 | 7 | 7 | 38.409 | 15 | 15 | 745 | 81.7 | 6.14 |
| Q6IFX2 | Krt42 | 28.423 | 11 | 6 | 52.581 | 17 | 7 | 452 | 50.1 | 5.16 |
| Q3UV17 | Krt76 | 6.763 | 4 | 1 | 31.095 | 13 | 4 | 594 | 62.8 | 8.43 |
| Q3UAS2 | Capza1 | 5.027 | 3 | 2 | 30.441 | 8 | 6 | 290 | 33.3 | 6.74 |
| Q32P04 | Krt5 | 44.515 | 19 | 10 | 72.921 | 28 | 14 | 580 | 61.7 | 7.75 |
| P21107 | Tpm3 | 44.058 | 10 | 1 | 74.03 | 20 | 1 | 248 | 28.7 | 4.72 |
| Q922T6 | Krt85 | 4.448 | 3 | 3 | 35.407 | 17 | 4 | 507 | 55.7 | 6.42 |
| Q61765 | Krt31 | 2.335 | 2 | 1 | 33.544 | 11 | 4 | 416 | 47.1 | 4.89 |
| P58771 | Tpm1 | 102.836 | 29 | 0 | 135.353 | 34 | 3 | 326 | 37.4 | 4.72 |
| Q6PJ18 | Tpm2 | 33.89 | 13 | 1 | 69.019 | 19 | 1 | 284 | 32.8 | 4.68 |
| E9Q557 | Dsp | 45.352 | 28 | 28 | 88.969 | 43 | 43 | 2883 | 332.7 | 6.8 |
| Q62261 | Sptbn1 | 19.103 | 11 | 1 | 83.633 | 28 | 28 | 556 | 64.7 | 5.27 |
| Q3U804 | Actb | 3.829 | 3 | 1 | 95.604 | 18 | 6 | 136 | 15.2 | 4.65 |

PEP, Posterior error probability; AAS, number of amino acids in the protein; MW, molecular weight; pI, isoelectric point.
Figure S1. Efficient knockdown of PKM2 in cardiomyocytes and mice hearts.

(A) Quantification of the efficiency of interference of three Pkm2 siRNA sequences assessed by western blot. (B, C) The knockout efficiency of Pkm2 cKO mice heart tissues assessed by quantitative real-time polymerase chain reaction (qRT-PCR) and western blot. β-Actin served as loading control and each group had at least three biological replicates. (D) Western blot and quantitation of PKM2 in purified adult mouse cardiomyocytes (AMCMs) of Pkm2f/f and Pkm2 cKO mice. β-Actin served as loading control and each group had at least three biological replicates. Values represent as the mean ± SEM, ns, not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
Figure S2. *Pkm2* cKO mice did not manifest overt developmental and morphological defects in heart.

(A-C) Representative gross heart morphology, HE staining and quantification of heart weight to body weight ratio, \( n = 6 \) to 8. (D) Viable mice were born at approximately Mendelian ratios. (E, F) Representative images of immunolabeling for Ki67 (red) and pH3 (red) in cardiomyocytes (green). (G, H) The percentages of Ki67\(^+\) or pH3\(^+\) cardiomyocytes were determined from three sections per
heart, with at least five hearts per group. (I, J) The knockout efficiency of Pkm2 cKO mice heart tissues assessed by western blot at developmental ages E12.5, E16.5, E18.5 and P0. β-Actin served as loading control, n = 6. Values represent as the mean ± SEM, ns, not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
Figure S3. Cardiomyocyte-specific Pkm2 knockout exacerbated pressure overload-induced HF.

The thickness of systolic (A) and diastolic (B) interventricular septum (IVS,s; IVS,d); the thickness of systolic (C) and diastolic (D) left ventricular posterior wall (LVPW,s; LVPW,d); the systolic (E) and diastolic (F) left ventricular volume (LV vol,s; LV vol,d) and the heart rate (G) of Pkm2f/f and Pkm2 cKO mice after sham or transverse aortic constriction (TAC) surgery by echocardiography, n = 9 to 18. Values represent as the mean ± SEM, ns, not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
Figure S4. Pkm1 knockdown exacerbated the phenotype caused by Pkm2 deficiency in response to pressure overload or PE treatment.

(A) Western blot and quantitation of the expression of PKM, PKM1 and PKM2 in adult mouse cardiomyocytes (AMCMs) purified from Pkm2\textsuperscript{fl/fl} and Pkm2 cKO mice with or without transverse
aortic constriction (TAC) operation. β-Actin served as loading control, n = 6. (B) Western blot and quantitation of the expression of β-MHC, PKM1 and PKM2 in Pkm2 cKO AMCMs by siRNA targeting Pkm1 under phenylephrine (PE) treatment. β-Actin served as loading control, n = 5. Values represent as the mean ± SEM, ns, not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
Figure S5. Cardiomyocyte-specific Pkm2 deficiency activated RAC1-MAPK signaling pathway in the progression of HF.

(A) Western blot and quantitation of β-MHC, PKM2 and RAC1 expression after Pkm2 knockdown
and NSC23766 treatment. β-Actin served as loading control, n = 4. (B) Western blot and quantitation of PKM2 and RAC1 expression after Rac1 knockdown. β-Actin served as loading control, n = 4. (C) Western blot and quantitation of β-MHC, PKM2 and RAC1 expression after Pkm2 and Rac1 knockdown. β-Actin served as loading control, n = 4. (D) Schematic illustration of the NSC23766 administration in vivo. (E) Western blot and quantification of active-RAC1 of heart tissue from transverse aortic constriction (TAC)-operated Pkm2^f/f and Pkm2 cKO mice after NSC23766 treatment. β-Actin served as loading control, n = 4. The diastolic (F) and systolic (G) left ventricular internal diameter (LVID,d; LVID,s); the thickness of systolic (H) and diastolic (I) interventricular septum (IVS,s; IVS,d); the thickness of systolic (J) and diastolic (K) left ventricular posterior wall (LVPW,s; LVPW,d); the systolic (L) and diastolic (M) left ventricular volume (LV vol,d; LV vol,s); and heart rate (N) of TAC-operated Pkm2^f/f and Pkm2 cKO mice after NSC23766 treatment by echocardiography, n = 9 to 10. Values represent as the mean ± SEM, ns, not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
Figure S6. Cardiomyocyte-specific overexpression of PKM2 ameliorated pressure overload-induced HF.

(A, B) The overexpression efficiency of AAV9-cTnT-mPkm2 virus assessed by quantitative real-time polymerase chain reaction (qRT-PCR) and western blot, n=6. The systolic (C) and diastolic (D) left ventricular internal diameter (LVID,s; LVID,d); the thickness of systolic (E) and diastolic (F) interventricular septum (IVS,s; IVS,d); the thickness of systolic (G) and diastolic (H) left ventricular posterior wall (LVPW,s; LVPW,d); and the diastolic (I) and systolic (J) left ventricular volume (LV vol,s; LV vol,d) of TAC or sham operated mice after Pkm2 overexpression by echocardiography, n = 6 to 9. Values represent as the mean ± SEM, ns, not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.