GCN5L1 regulates glucose and ketone body metabolism during cardiac ischemia-reperfusion injury

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Abstract

Ischemic heart diseases are the leading cause of death among cardiovascular diseases. Reperfusion of the ischemic myocardium is the only way to treat IHD. However, reperfusion can damage the ischemic myocardium if it does not happen at the right time. Therefore, understanding the pathophysiology of cardiac ischemic reperfusion injury is essential to identifying new therapeutic targets. We reported the cardioprotective role of GCN5L1 in ex-vivo cardiac ischemic reperfusion injury through the regulation of cytoprotective signaling by reducing oxidative stress. The current study investigated the role of GCN5L1 in in-vivo cardiac ischemic reperfusion injury and in-vitro H/R injury in AC16 cells. The cardiac-specific GCN5L1 KO did not further deteriorate the cardiac function and infarct size. We measured the glucose metabolism-related protein expression among all experimental groups and found that GCN5L1 KO significantly decreases glucose metabolism by PDH phosphorylation. We further evaluated the role of GCN5L1 in in-vitro H/R injury in genetically modified AC16 cells (GCN5L1 KD and overexpressed and control). GCN5L1 KD in AC16 cells promotes phosphorylation of PDH; similarly, overexpression reduces the p-PDH levels. We identified that the regulation of PDH phosphorylation via PDK4/PDP1/PDPR and GCN5L1 regulates the PDK4 expression. We measured the ketone body metabolism related to protein expression in control and KD cells after H/R injury and found the upregulation of ketone body metabolism-related protein expression in KD cells compared to control AC16 cells. Altogether, our in vivo and in vitro studies findings conclude that GCN5L1 is required for glucose metabolism in ischemic reperfusion injury, and in the absence of GCN5L1, ketone body metabolism accomplishes the cardiac energy requirements and prevents I/R induced cardiac damage and infarct size.

1. Introduction

Coronary artery disease (CAD) is one of the leading causes of morbidity globally. According to WHO reports 2021 17.9 million have lost their lives because of cardiovascular disease. Ischemic heart disease is the leading cause of death among cardiovascular disease (1). In ischemic heart disease conditions, cardiomyocytes are deprived of oxygen and nutrients which ultimately leads to cardiomyocyte death and ultimately leads to heart failure. To prevent heart failure timely restoration of blood flow to an ischemic area of the myocardium in the early stage effectively reduces the infarct size which ultimately reduces the mortality associated with heart failure (2, 3). Reperfusion does affect the ischemic myocardium instead of protecting and, and it was first reported by Jennings et al in 1960 (4), Ischemia has four phases myocardial stunning(5), second is a no-reflow phenomenon (6), 3rd is a reperfusion-induced arrhythmia(7), and the lethal refusion injury (8). The first and second phases can be reversed, while the 3rd and 4th phases of ischemic reperfusion injuries cannot be reversible (9). Currently, physicians are following various strategies to tackle the ischemic-reperfusion-induced cardiac damage/mortality like ischemic preconditioning, postconditioning, and administration of Glucose and insulin (10). The latter two phases of cardiac ischemic reperfusion injury are tackled by treating with antiplatelet and anticoagulant therapy (11, 12). Still, there is an urgent need to understand the pathophysiology of cardiac ischemic reperfusion injury to identify new therapeutic avenues to prevent cardiac ischemic reperfusion injury-associated morbidity. Cardiac ischemic reperfusion injury produces excessive ROS production, metabolic imbalance, mitochondrial dysfunction, activation of apoptotic and inflammatory signaling pathways, DAMPs Damage associated molecular patterns, etc (13-15).

GCN5L1 is a mitochondrial acetyltransferase, and it can regulate various cellular functions like mitochondrial function (16), oxidative stress, and metabolism, and cytoprotective signaling (17-21). GCN5L1 plays a crucial role in a variety of disease conditions like neurodegenerative diseases, nonalcoholic fatty liver diseases (NAFLD)(22), diabetic cardiomyopathy (20), etc. GCN5L1 is crucial in regulating cytoprotective signaling in hypoxia conditions in AC16 cells (19) and in ex-vivo cardiac ischemic reperfusion injury (23). In the current manuscript, we are interested in evaluating the role of GCN5L1 in in-vivo cardiac ischemic reperfusion injury in a mouse model of LAD ligation for about 45 minutes followed by 24 h reperfusion.

2. Materials and methods

2.1.1 Generation of cardiac-specific GCN5L1 mice

All mice were propagated and housed according to the University of Pittsburgh's IACUC protocols. At our request, EUCOMM created cardiomyocyte-specific conditional knockouts on a C57BL/6J background by crossing αMHC-Cre (Jax B6.FVB (129)-Tg (Myh6-cre/Esr1*)1JMK/J) mice (i.e. αMHC-MerCreMer) to mice with LoxP sites introduced around exon3 of BLOCK1S1 ($GCN5L1^{FL}$). Cardiomyocyte-restricted knockout mice were produced by a single 40 mg/kg i.p. tamoxifen injection and confirmed with qPCR and western blotting 4 weeks post-tamoxifen injection(24).

2.1.2 Cardiac ischemic-reperfusion injury surgery

Mice were anesthetized with 3 % Isoflurane in an induction chamber; then, mice were intubated. Mice's dorsal surface hair was removed by applying Nair; thoracotomy was performed between the 3rd and 4th intercoastal ribs, and the heart was exposed by using homemade retractors. LAD was identified below (~2mm) to the left atrium, and LAD was blocked with 8-0 suture for 45 min. reperfusion was done by removing the knot around the LAD, and reperfusion was allowed for 24 h. After reperfusion, intercoastal muscles and ribs were sutured back with 6-0 silk sutures, and skin was also sutured back with 6-0 silk sutures by interrupted pattern. Cardiac function and myocardial strain were measured after 24 h of reperfusion. For sham-operated groups, we followed a similar procedure except for coronary artery blockage. The LAD surgery was adopted from Xu et al. 2014 and Xing et al. 2021 (25, 26).

2.1.3 Echocardiography

To evaluate the cardiac function and structure, we performed transthoracic echocardiography using a Visual Sonics Vevo 3100 system (Visual Sonics, Toronto, Ontario, Canada) with an MS400 (30-MHz centerline frequency) probe before the surgery and after ischemia and reperfusion injury surgery to explore the role of GCN5L1 on cardiac function at baseline and after I/R stress. Mice were anesthetized with isoflurane (induction 3% and maintenance 1.5–2%), and hair was removed. Then, mice were placed in a supine position on a heated table (core temperature was maintained at 37°C) with embedded ECG leads. B- and M-mode images were acquired from a parasternal short-axis view to evaluate LV function EF and FS, LVIDd, and LVIDs by M-mode images. The myocardial strain was assessed by B-mode scanning using the parasternal long-axis view for the

longitudinal stress and the parasternal short-axis view for the radial strain. As previously described, echocardiographic speckle-tracking analyses were performed on parasternal long-axis B-mode loops using a VisualSonics Vevo 3100 system (VisualSonics, Toronto, Ontario, Canada) (27, 28).

2.1.4 Measurement of Myocardial Infarct Size

TTC and Evans blue double staining were performed to determine the role of cardiac-specific GCN5L1 KO on infarct size in acute myocardial ischemic reperfusion injury. The mice were anesthetized and ventilated as described previously. After 45 min of ischemia and 24 h of reperfusion, the suture thread around the LAD artery was retied and Evans blue dye (2 % in PBS) was injected into the left ventricular cavity. The heart was quickly excised and incubated for a few seconds in the liquid nitrogen. The heart was cut into 5 slices (about 2 mm thickness) and stained with 1% TTC for 30 min at 37 °C in the dark. After three washings, the tissue was fixed in 4% paraformaldehyde. The non-infarcted myocardium was stained deep blue with Evans blue. The viable myocardium was stained red with Evans blue-TTC double staining. The necrotic myocardium appeared white in color after Evans blue-TTC double staining. ImageJ software was used to quantify the infarcted area(29).

2.1.5 Cardiac Markers in Ischemic-reperfusion Injury

Cardiac ischemic reperfusion injury releases the cardiac proteins troponin-T and LDH into the circulation. We assessed lactate dehydrogenase (LDH) serum content, and ELISA evaluated troponin-T (27, 30).

2.2 Cell culture

Ac16 cells were purchased from Sigma-Millipore (Cat No: SCC109). They were created by the fusion of primary adult ventricular cardiomyocytes with SV40 fibroblasts to mimic protein expression and metabolic properties like primary human cardiomyocytes (31-33). AC16 cells were propagated under standard conditions (i.e. 5% CO2, and 37^0 C, in presence of high-glucose DMEM (25mM), 10% FBS (ThermoFisher, USA), antibiotic-antimycotic (ThermoFisher, USA)).

2.2.1 Generation of GCN5L1 KD and overexpressed stable cell lines

AC16 cells were transduced with GCN5L1 shRNA and ORF lentiviral particles at a multiplicity of infection (MOI) of 10 with scrambled control or GCN5L1 shRNA (Sigma-Aldrich, USA) and Control and GCN5L1 ORF lentiviral particles (Origene, USA), followed by puromycin selection. GCN5L1 KD and overexpression were confirmed by RT-qPCR and western blotting.

2.2.2 Hypoxia/reoxygenation experiments

After 24 hrs of propagation in the 10 cm cell culture plates, hypoxia studies were performed on control and GCN5L1 KD AC16 cells. Cells were washed three times with 1X PBS, then cells were incubated with Esumi Buffer (12 mM KCl, 0.9 mM CaCl2, 0.5 mM MgCl2, 137 mM NaCl, 20 mM HEPES, 20 mM 2-deoxy-D-glucose (2-DG), and Lactic acid,20 mM pH 6.2) 3 hrs (34, 35). After completion of hypoxia treatment, cells were washed with 1X PBS and lysed in CHAPS lysis buffer, added protease inhibitor, phosphatase inhibitor, and Deacetylase inhibitors.

2.2.3 RT-qPCR:

RNA was collected from the cells without H/R and after H/R stress with the RNEasy kit (Qiagen) the RNA quality and quantity was evaluated using a µLITE analyzer (BioDrop, USA). C-DNA was synthesized with 1 ug of RNA using Maxima Reverse Transcriptase (ThermoFisher, USA), and gene expression studies were performed with specific primers for GCN5L1(BOC1S1) (Cat.No. QT00016002), PDK4(Cat.No. QT00157248), and GAPDH (Cat. No. QT00079247).

2.2.4 Immunoblotting

Cells and cardiac tissues were lysed in 1% CHAPS lysis buffer. Protein samples were quantified using a µLITE analyzer (BioDrop, USA) and 20 ug of proteins were separated on an SDS-PAGE gel. Protein bands were transferred onto 0.2/0.45µm nitrocellulose membrane, which was blocked using Odyssey blocking buffer (Li-Cor, USA). Membranes were incubated with primary antibodies (GAPDH, Cell Signaling, Cat. 97166 [D4C6R], mouse mAb, 1:1000; (A-Tubulin, Cell Signaling, Cat. 3873 [DM1A], mouse mAb, 1:1000; PDH, Cell Signaling, Cat. 3205S [C54G1], rabbit mAb, 1:1000; p-PDH[Ser293], Novo-Bioscience, Cat. NB110-93477, PDK4, Thermoscientific, Cat. PA5-102685, rabbit mAb 1:1000; BDH1, ThermoScientific, Cat.no. 15417-1-AP rabbit mAb 1:1000, SCOT1/OXCT1 Thermoscientific, Cat.no. 12175-1-AP, rabbit mAb 1:1000, GCN5L1 1:500, rabbit pAb) overnight at 4 ^{0}C, membranes were washed with PBST 3 times, and membranes were developed with secondary antibodies followed by incubation at room temperature with fluorescent secondary antibodies for 1 h (800 nm anti-rabbit, 700 nm anti-mouse) at room temperature. Protein bands were visualized by an Odyssey Imager and analyzed using Image-J software.

2.3 **Statistical analysis:** Statistical analyses were performed with GraphPad Prism 7. One-way ANOVA followed by Tukey's multiple comparison tests was used for more than two experimental groups, and for two experimental groups, data was analyzed using a student's T-test. A *p*-value < 0.05 was considered statistically significant. Data were represented as Mean ±SEM.

3 Results and discussion

Cardiovascular diseases (CVDs) are the leading cause of death globally, and ischemic heart disease is the leading cause among the CVDs. Ischemia is nothing but a lack of nutrients and oxygen supply to the myocardium due to the occlusion in the coronary microcirculation. Reperfusion of the ischemic myocardium at the right time is the only way to save the myocardium from ischemic damage. If reperfusion does not happen at the right time, it causes severe damage due to reperfusion itself. In this current research study, we wanted to understand the pathophysiology of cardiac ischemic reperfusion injury and the role of GCN5L1 in the cardiac ischemic reperfusion context. For this, we generated cardiac-specific GCN5L1 KO mice and confirmed the same by measuring the protein and gene expression of GCN5L1 in cardiac tissues (Figure S1 A, and B). We assessed the cardiac function of WT and KO male animals before performing the I/R surgery, and we did not notice any significant changes in cardiac function i.e. Ejection Fraction, Fractional Shortening, Left ventricular lumen volume during the diastole (LVDd), Left ventricular lumen volume during the systole (LVDs), and Global longitudinal strain (GLS) because of cardiac-specific GCN5L1 deletion (Figure S2 A-E). These observations conclude that cardiac-specific GCN5L1 KO is not affecting cardiac function at the basal level. These observations support our earlier report by Manning et al. 2019 (23).

Next, we performed cardiac ischemic reperfusion (i.e., 45-minute ischemia followed by 24 h reperfusion) injury surgery on these animals and assessed their cardiac function. We observed a significant decrease in cardiac function (i.e., Declined LV function is represented by a diminution in ejection fraction and fractional shortening, an increase in LVDs, and no change in LVDd) after I/R injury in both WT and KO animals. Interestingly, the cardiac function (i.e. Ejection fraction and Fractional shortening, LVDs, and GLS) of KO animals after IR injury was like that of WT animals. We anticipated that cardiac-specific GCN51 KO further worsens the cardiac function after I/R stress compared to WT animals (Fig 1 A-D). Next, we assessed the myocardial strain by speckle-tracking analysis, and we noticed a significant reduction of the myocardial strain, i.e., Global longitudinal strain (GLS) (Figure 1 E) after I/R injury, which is similar in both WT and KO animals.

We also measured the cardiac myocardial infarct size using Evans Blue and TTC double staining (i.e., The deep blue color tissue represents the un-infarcted region/remote area of the

myocardium, and the Deep red color region represents the border zone of the myocardium, the lastly, pale red/white color region represents an infarcted area of the myocardium) and the cardiac tissue damage markers Troponin-I and LDH in the circulation. We observed a significant increase in the myocardial infarct size, Troponin-I, and LDH after I/R injury in both WT and KO animals, but we could not see a significant difference between WT-IR and KO-IR of infarct size and cardiac Troponin-I and LDH (Figure 2 A-D). In our previous study, i.e., Ex-vivo cardiac ischemic reperfusion injury by Manning et al. 2019, we could see severe cardiac dysfunction and increased infarct size after ischemic reperfusion injury in ex-vivo(23). However, we could not see the same in our *in-vivo* cardiac ischemic reperfusion injury. We were interested in understanding the possible reason for this interesting observation, and we could identify one possible potential reason for this that is GCN5L1 is essential for glucose metabolism, and ablation of GCN5L1 limits glucose metabolism, which is essential for recovering cardiomyocytes from Ischemic reperfusion injury(36). Like our hypothesis, multiple reports support the idea that glucose/ketone bodies are essential for cardiac function or recovery from ischemic reperfusion injury (37-39). Our previous report by Manning et al. 2019 was done on ex-vivo cardiac ischemic reperfusion injury where we can provide glucose as a substrate/source of energy, and our GCN5L1-KO-mice myocardium cannot use glucose as an energy source, and it showed great cardiac dysfunction and infarct size than the WT-mice up on ex-vivo cardiac ischemic reperfusion injury. In our current study (in-vivo cardiac ischemic reperfusion injury), the myocardium will have access to other nutrients (i.e., Ketone bodies, fatty acids, branched-chain amino acids, etc.) through systemic circulation, which is not like ex-vivo cardiac I/R injury. To confirm the same, we measured the glucose metabolism-related protein expression in cardiac tissues by western blotting, and we noticed a significant increase of PDH phosphorylation in KO and after cardiac ischemic reperfusion injury, p-PDH levels were even more than in the KO animals. This supports that GCN5L1 KO and I/R injury impair glucose metabolism (Figure 2 A-E).

Pyruvate dehydrogenase (PDH) is a key metabolic enzyme in glucose metabolism and regulates the overall cellular glucose metabolism. PDH enzyme activity was monitored by a key posttranslational modification, which is phosphorylation. PDK4 (Pyruvate dikinase-4) is a kinase that phosphorylates PDH, suppresses PDH activity, and prohibits glucose oxidation. To counteract this, there is another class of enzyme phosphatases (i.e., PDP: Pyruvate dehydrogenase

phosphatases), PDP1, which removes the phosphate from the PDH. To decipher the role of GCN5L1 and cardiac ischemic reperfusion injury on glucose metabolism, we generated stable GCN5L1 Knocked down and overexpressed AC16 cells (Figure 3 A, C) and measured the PDH and p-PDH levels in control and GCN5L1 KD and overexpressed cells at basal level and found a significant increase in p-PDH levels in KD cells and which was significantly decreased by GCN5L1 overexpression (Figure 3 C-F). We quantified the mRNA levels of PDK4 in control and GCN5L1 KD and overexpressed cells and found a significant increase in PDK4 expression (~10-fold increase) in GCN5L1 KD cells, but no change was observed in the overexpression condition. We did not notice any significant changes in PDP1 protein levels due to GCN5L1 genetic manipulation. We measured the PDK4 m-RNA levels in control and KD and Overexpressed cells and found there was a significant increase in PDK4 mRNA levels in KD cells, and there was no change after GCN5L1 overexpression. We further confirmed the role of H/R injury on PDK4 expression in control and KD and overexpressed cells and found that GCN5L1 KD cells represented a 20-fold increase in PDK4 mRNA expression, whereas control and overexpressed cells showed a similar 10-fold increase in PDK4 mRNA expression after H/R injury. This observation makes it very clear that GCN5L1 controls glucose metabolism at baseline through PDH phosphorylation via PDK4, and it is essential for cardiomyocytes to recover from the H/R injury. Next, we subjected this control and GCN51 KD cells to H/R stress to test the role of GCN5L1 after H/R stress on cardiomyocyte glucose metabolism. We witnessed a significant increase in p-PDH and PDK4 protein levels after H/R stress in control and GCN5L1 KD cells, and GCN5L1 KD also showed increased PDK4 protein levels at basal levels as well. We also measured the PDP1, and no change in PDP1 was observed across the experimental groups. If glucose metabolism is impaired in the absence of GCN51, how can GCN5L1 KD cardiomyocytes (AC16) cells and GCN5L1 KO heart tissue meet their energy requirements? We wanted to look at alternate energy sources (i.e., Ketone body metabolism) metabolism after H/R stress. SCOT1(Succinyl-CoA:3-ketoacid CoA transferase) and BDH1 (β-hydroxybutyrate dehydrogenase) are the key proteins involved in ketone body metabolism (40). Several reports claim increased ketone body metabolism after cardiac I/R injury to recover from Ischemic reperfusion injury (41). In our study, we measured the expression of key ketone body metabolism-related (i.e., SCOT1 and BDH1) proteins. We observed a significant increase in the ketone body metabolism-related enzyme

SCOT1 and BDH1 protein levels in GCN5L1 KD cells at the basal level, and it was further increased up on H/R injury.

4 Conclusion

The current study deciphered the cardioprotective role OF GCN5L1 after *in-vivo* cardiac ischemic reperfusion injury and *in-vitro* H/R stress. Our results conclude that GCN5L1 plays an important role in regulating glucose metabolism after I/R injury. To rescue from I/R injury, GCN5L1 KO cardiomyocytes or GCN5L1 KD AC16 cells utilize the ketone body as an energy source. Our findings support that targeting glucose and ketone body metabolism can be a potential therapeutic target for treating cardiac ischemic reperfusion injury.

Figure legends:

Figure 1 Cardiomyocyte-specific ablation of GCN5L1 does not further aggravate cardiac dysfunction after *in-vivo* I/R injury. A. Ejection fraction after Cardiac I/R injury. **B**. Fractional Shortening after I/R injury. **C.** Left ventricular lumen volume during systole (LVDs) after in-vivo I/R injury. **D.** Left ventricular volume during diastole (LVDd) after in-vivo I/R injury. **E.** Global longitudinal strain (GLS) after in-vivo I/R strain. Data were represented as Mean ± SEM. N=8. ****p>0.0001 vs *In-vivo* Cardiac ischemic reperfusion injury.

Figure 2 Cardiomyocyte-specific ablation of GCN5L1 does not further aggravate cardiac damage after in-vivo I/R injury. A-B. In-vivo cardiac ischemic reperfusion injury significantly increased infarct size. **C**. In-vivo cardiac ischemic reperfusion injury significantly increased circulating cardiac troponin. **D**. *In-vivo* cardiac ischemic reperfusion injury significantly increased serum LDH levels. Data were represented as Mean ± SEM. N=4 for myocardial infarct size analysis. N=5 for serum cardiac damage markers (serum cardiac troponin-I and LDH), **p>0.01vs, ***p>0.001 vs *In-vivo* Cardiac ischemic reperfusion injury.

Figure 3 Cardiomyocyte-specific GCN5L1 KO and *In-vivo* cardiac ischemic reperfusion injury prohibits cardiac glucose oxidation. A-B. Confirmation of GCN5L1 KO in heart tissue and effect of I/R injury on GCN5L1 protein levels. **C.** Representative western blot images of PDH and p-PDH proteins. **D.** Effect of GCN5L1 KO and in-vivo cardiac ischemic reperfusion injury on PDH levels. **E.** Effect of cardiac-specific GCN5L1 KO and cardiac ischemic reperfusion injury on

PDH phosphorylation. **F.** Ration of p-PDH/PDH. Data were represented as Mean ± SEM. N=4, **p>0.01vs, ***p>0.001 vs *In-vivo* Cardiac ischemic reperfusion injury.

Figure 4 Genetic manipulation of GCN5L1 in AC16 cells significantly affects glucose metabolism. A. Western blot images of proteins from control and GCN5L1 overexpression and knockdown. **B.** Confirmation of GCN5L1 overexpression and knockdown. **C.** Effect of GCN5L1 genetic manipulation on PDH expression. **D.** Effect of GCN5L1 genetic manipulation on PDH phosphorylation. **E.** Effect of GCN5L1 genetic manipulation on the ratio of p-PDH/PDH. **F.** Effect of GCN5L1 genetic manipulation on PDP1 expression. Data were represented as Mean ± SEM. *p>0.05, **p>0.01, ***p>0.001, ****p>0.0001 vs Control AC16 cell.

Figure 5 Effect of GCN5L1 overexpression and knockdown on PDK4 expression at basal level and after hypoxia-reoxygenation stress. A. Effect of GCN5L1 overexpression and knockdown on PDK4 mRNA expression at basal level. B. Effect of H/R stress on PDK4 mRNA expression in control AC16 cells. C. Effect of H/R stress on PDK4 mRNA expression in GCN5L1 overexpressed cells. D. Effect of H/R stress on PDK4 mRNA expression in GCN5L1 knockdown AC16 cells. Data were represented as Mean ± SEM. n=6, ***p>0.001, ****p>0.0001 vs Control AC16 cell and vs H/R stress.

Figure 6 GCN5L1 knockdown in Human cardiomyocyte (AC16) cells decreases glucose oxidation after hypoxia re-oxygenation stress. A. Representative western blot images. **B**. Effect of GCN5L1 KD and H/R stress on PDH protein expression. **C.** Effect of GCN5L1 KD and H/R stress on PDH protein phosphorylation expression. **D.** Effect of GCN5L1 KD and H/R stress on PDK4 protein expression. **E.** Effect of GCN5L1 KD and H/R stress on PDP1 protein expression. Data were represented as Mean ± SEM. n=3, ***p>0.001, ****p>0.0001 vs Control AC16 cell and vs H/R stress.

Figure 7 GCN5L1 knockdown in AC16 cells increased ketone body metabolism at basal level and after H/R stress. **A.** Representative western blot images. **B**. Effect of GCN5L1 KD and H/R stress on fatty acid transporter CPT1A protein expression. **C.** Effect of GCN5L1 KD and H/R stress on SCOT1 protein expression. **D.** Effect of GCN5L1 KD and H/R stress on BDH1 protein expression. Data were represented as Mean ± SEM. n=3, ***p>0.001, ****p>0.0001 vs H/R stress.

Figure S1 Confirmation of Cardiac GCN5L1 KO in mice by western blot and RT-qPCR. A. GCCN5L1 KO confirmation by western blot. **B.** GCN5L1 KO confirmation by RT-qPCR.

Figure S2 Cardiomyocyte-specific GCN5L1 KO does not affect the cardiac function at the basal level. A. Effect of Cardiomyocyte-specific GCN5L1 KO on Ejection fraction before I/R surgery. **B.** Effect of Cardiomyocyte-specific GCN5L1 KO on Fractional shortening before I/R surgery. **C.** Effect of Cardiomyocyte-specific GCN5L1 KO on LVDd before I/R surgery. **D.** Effect of Cardiomyocyte-specific GCN5L1 KO on LVDs before I/R surgery. **E.** Effect of Cardiomyocyte-specific GCN5L1 KO on global longitudinal strain before I/R surgery.

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Figure 1

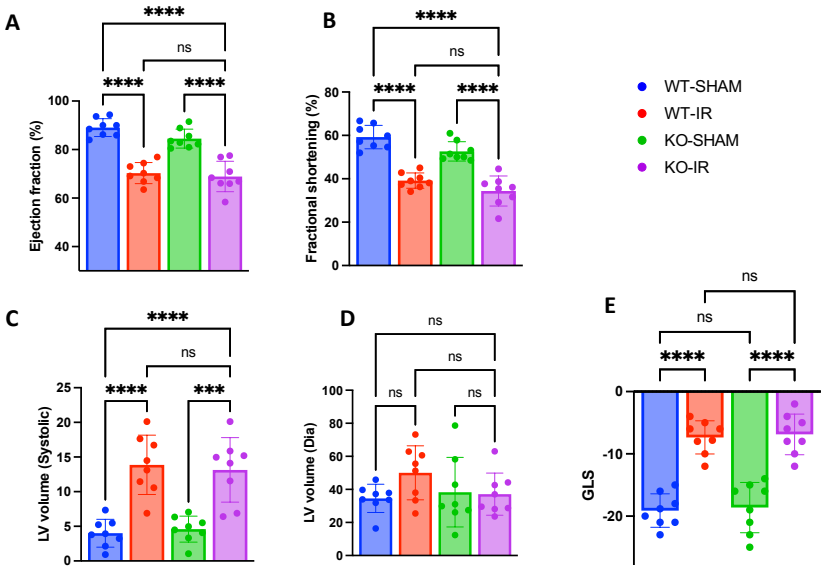


Figure 2

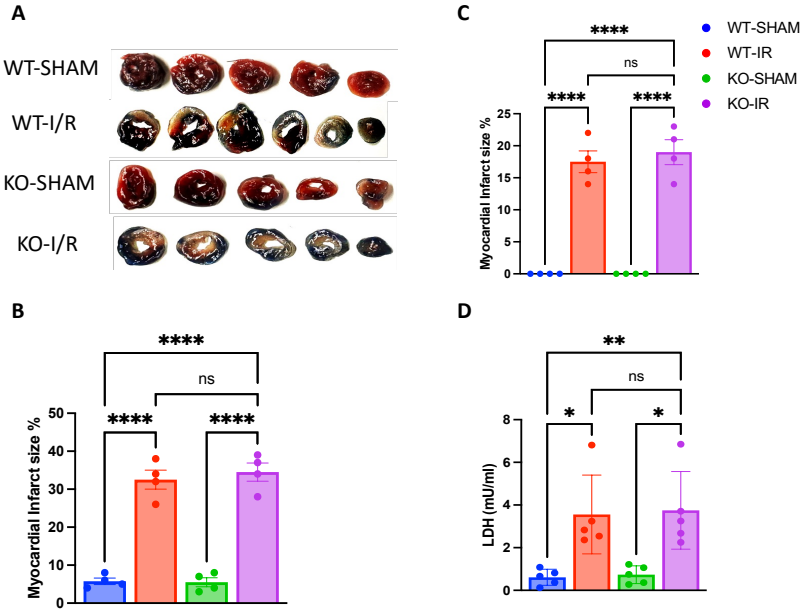
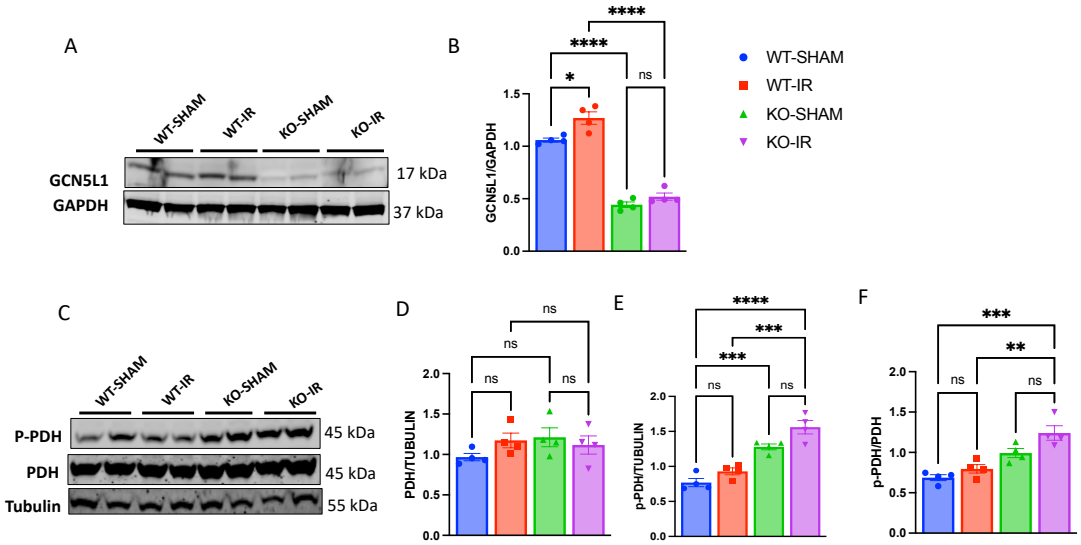


Figure 3



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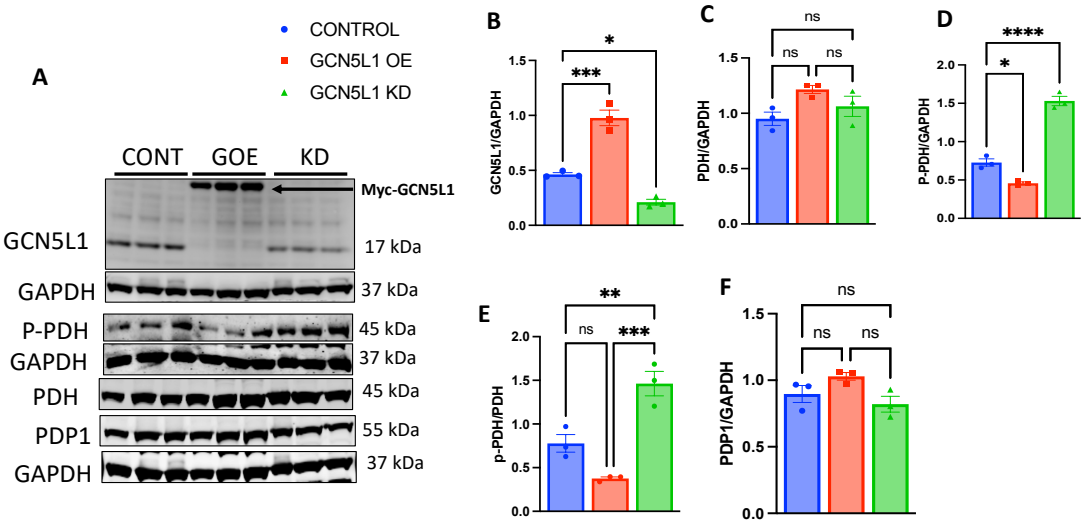
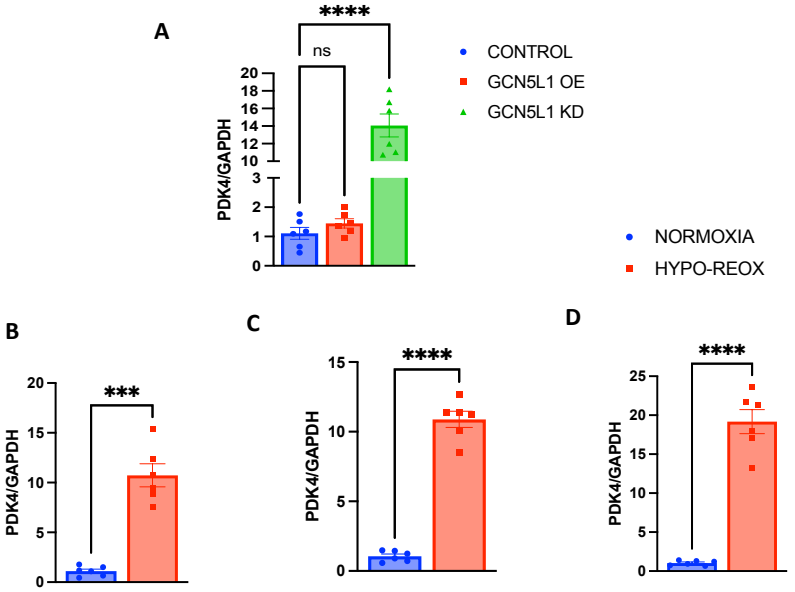


Figure 5



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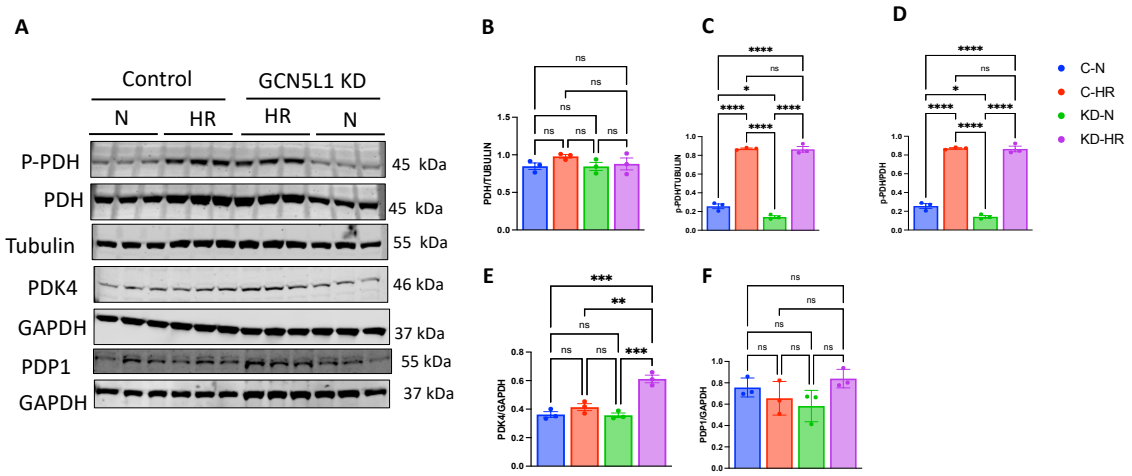

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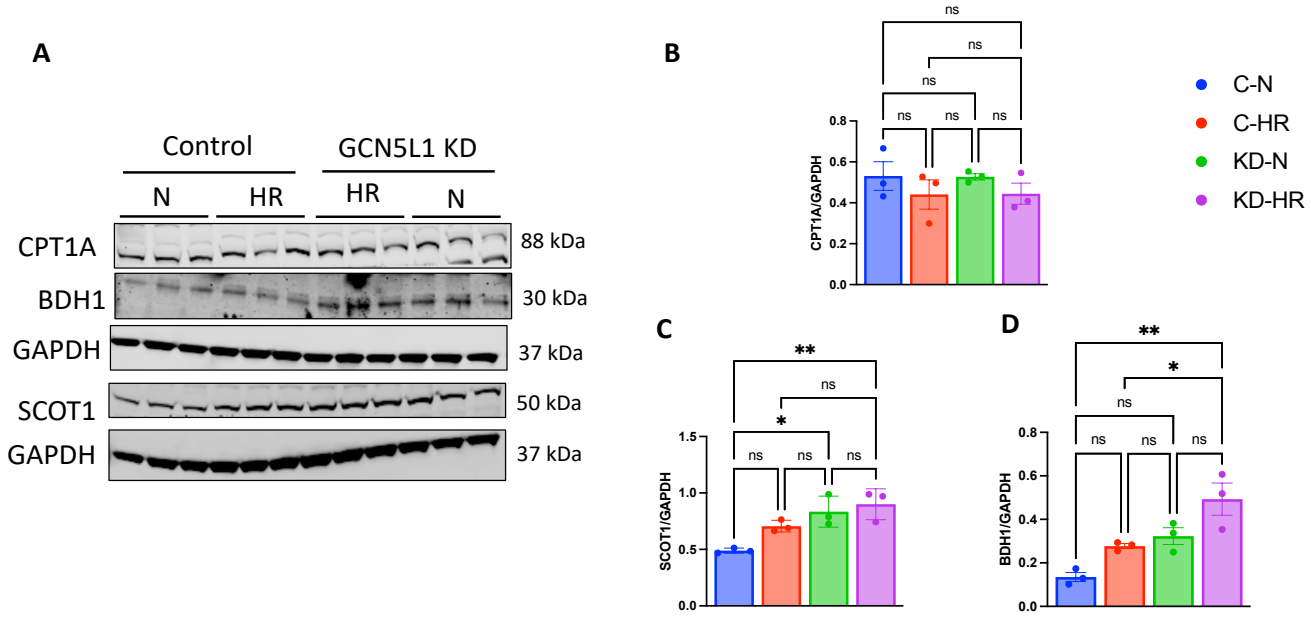


Figure S1

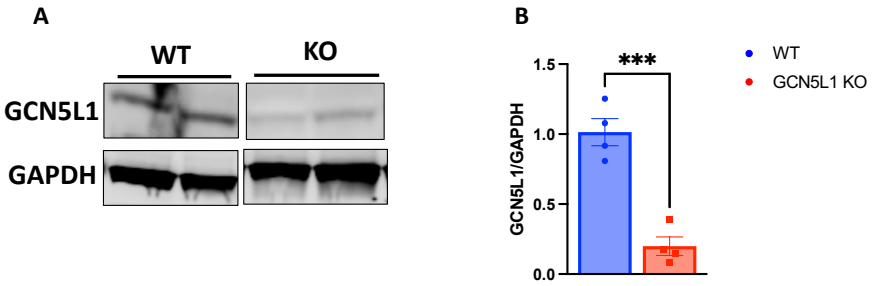
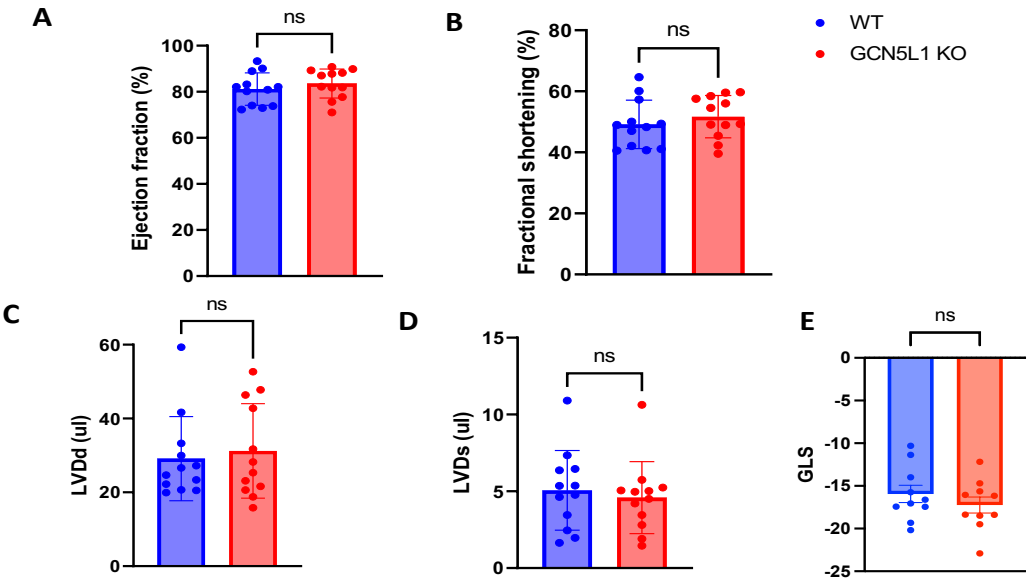


Figure S2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C
ns
LVDd (ul)
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40
20
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D
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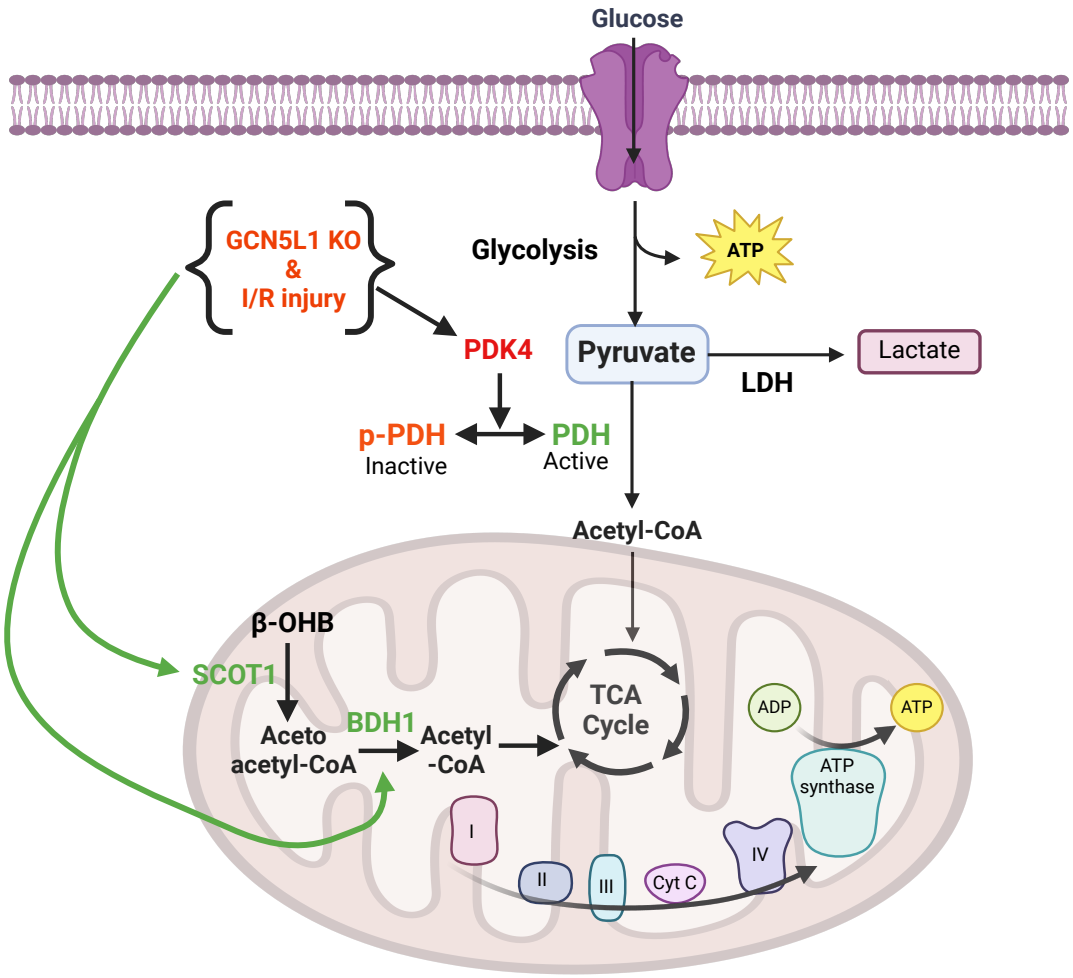
Graphical Abstract



Graphical abstract of the manuscript

Altogether, our results conclude that Cardiomyocyte-specific GCN5L1 KO in-vivo cardiac ischemic reperfusion injury and in-vitro Hypoxia-reoxygenation stress significantly affect glucose metabolism by suppressing glucose metabolism. Cardiac-specific GCN5L1 KO mice in-vivo and knockdown in AC16 cells invitro upregulates the expression of enzymes that are involved in the ketone body metabolism to rescue from in-vivo cardiac ischemic reperfusion injury and invitro H/R stress.