Supplemental Materials

Expanded Materials and Methods

Optical mapping: Mice were injected with heparin sulfate (550 U/100 g) and anesthetized with ketamine and xylazine (35 mg/kg and 5 mg/kg, respectively). After a thoracotomy was performed, the heart was quickly removed and immersed in cardioplegic solution (in mM: glucose 280, KCl 13.44, $NaHCO_3$ 12.6, mannitol 34). The aorta was quickly cannulated and retrogradely perfused with warm (37±1°C) oxygenated Tyrode's solution (in mM: NaCl 130, $CaCl_2$ 1.8, KCl 4, $MgCl_2$ 1.0, NaH_2PO_4 1.2, $NaHCO_3$ 24, glucose 5.5, pH 7.4) under constant pressure. The heart was immersed in a chamber and superfused with the Tyrode's solution. Blebbistatin (10 μmol/L) was added to the Tyrode's solution to reduce motion artifacts. Hearts were periodically paced at progressively reduced basic cycle length (BCL), ranging from 150 ms to 30 ms in steps of 10 ms. Burst pacing (10 attempts per heart) was applied to initiate arrhythmias. Optical movies were recorded at each BCL as well as during burst pacing. At each BCL, action potential duration (APD) were measured at 80% repolarization (APD_{80}) (1). Two-dimensional (2D) APD maps were constructed for all BCLs. The heterogeneity was calculated as the difference of APD in the left ventricle (LV) MI zone with respect to the LV remote zone (RZ): heterogeneity = (APD_{RZ} – APD_{MI})/APD_{RZ}. Local conduction velocity (CV) was calculated as described previously.(1) Specifically, the distributions of activation times (measured at dV/dt_{max}) for the spatial regions of 3×3 pixels were fitted with the plane, and gradients of activation times g_x and g_y were calculated for the each plane along the x- and y-axes, respectively. The magnitude of the local CV was calculated for each pixel as $(g_x^2 + g_y^2)^{-1/2}$.

Genotyping of cardiac-specific PERK knockout (PERKKO) mice: Confirmation of the cardiac-specific knockout of PERK was confirmed with genotyping of every mouse by following the

protocols #23633 and #26338 provided by the Jackson Laboratory website. Briefly, we first screened for Myh6-Cre with two bands at ~300 bp for the transgene and at 200 bp as internal positive control, respectively. We then screened the positive mice with the 300 bp band for Eif2ak3 with bands at ~480 bp as the homogenous mutant. Representative images of genotyping are shown in Supplemental Figure VIII.

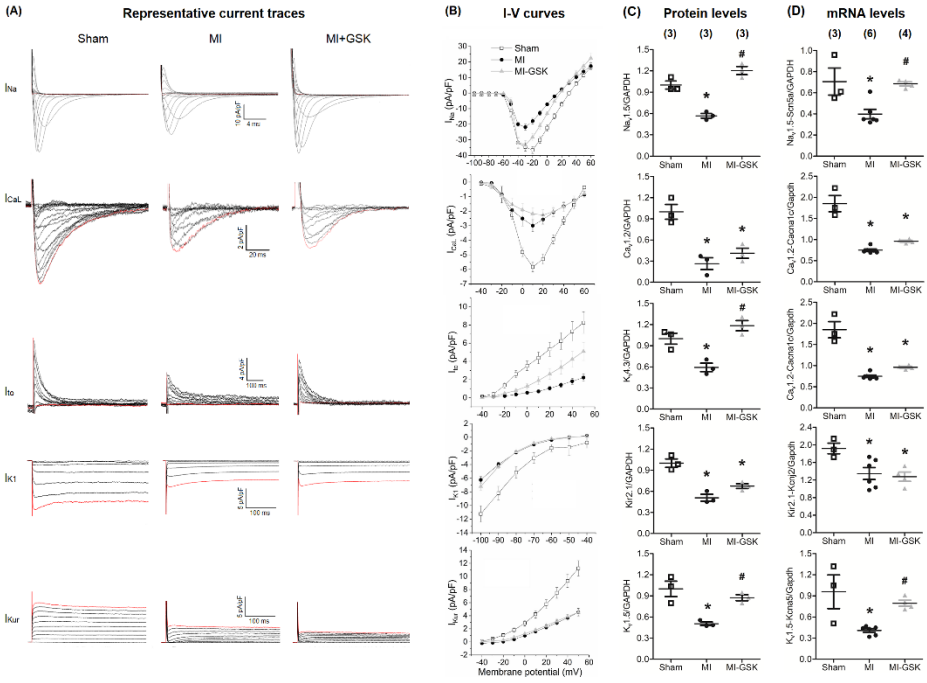
Telemetry**:** As we have done before (2, 3), mice were implanted with ETA-F10 telemetry monitors (Data Sciences International, St. Paul, Minnesota) under the anesthesia of isoflurane. The two leads were placed in the area of the right upper chest and the left lower chest respectively. Continuous ECG signals between 1-2 am and between 1-2 pm were recorded and evaluated using Dataquest ART (Data Sciences International, St. Paul, Minnesota) to obtain ventricular premature beats.

Histological evaluation of the infarct size of MI mouse hearts**:** Hearts were excised under 2% isoflurane anesthesia. KCl (10%) was injected into the LV chamber before the hearts were harvested to arrest them in diastole (4). The left and right atria and large vessels were resected, and the heart was washed with PBS (ice-cold) and fixed in 10% formalin overnight. Then, heart tissues were embedded in paraffin using a Tissue-TeK VIP processor (Sakura Finetek USA, Inc., Torrance, CA). Tissue blocks were cut at 5 µm thickness with 100 µm between each section though the block. Sections were stained with routine Masson Trichrome (Rowley Biochemical Inc., Danvers, MA) by following the manufacturer's instructions. Five wild type MI hearts (2 male and 3 female) and seven MI-PERKKO hearts (3 male and 4 female) were used to evaluate the scar size after 3 weeks post MI surgery. The images were taken with NIKON Eclips E800M microscope (Nikon Instruments Inc., Melville, NY) and NIS-Elements D 5.02.00 64-bit imaging software (Nikon Instruments Inc.). The scar size was analyzed with ImageJ-FIJI software. The scar size was

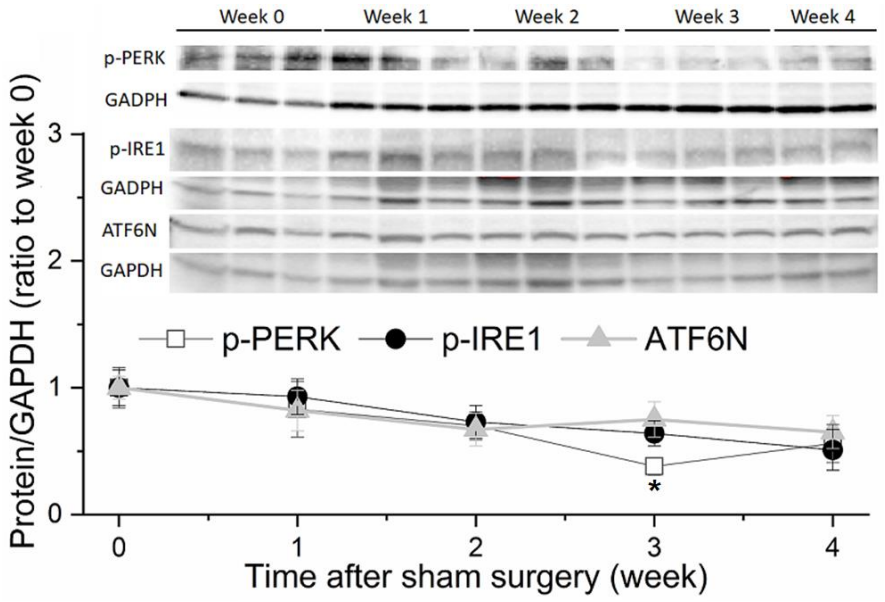
evaluated by the endocardial and epicardial infarct ratio technique (4). The endocardial infarct ratio was obtained by dividing the sum of endocardial infarct lengths from all sections by the sum of endocardial circumferences from all sections. The epicardial infarct ratio was obtained by dividing the sum of epicardial infarct lengths from all sections by the sum of epicardial circumferences from all sections.

Supplemental Figures:

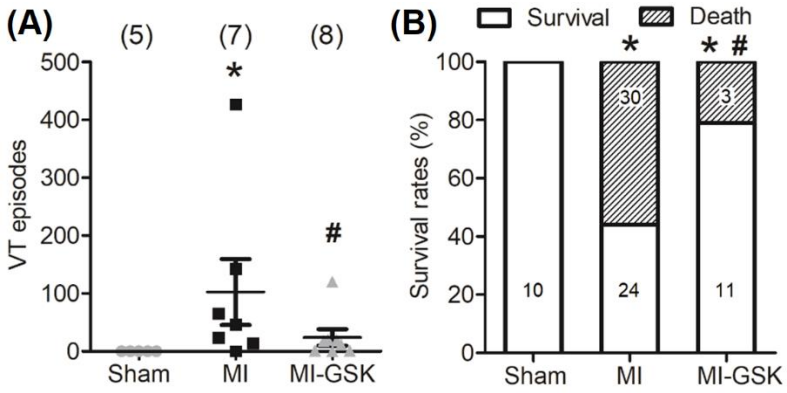
Supplemental Figure I. Cardiac ion channels are altered in MI and MI-GSK mice. (**A**) Representative current traces. (**B**) current-voltage (I-V) relationship curves. Fifteen to 36 cardiomyocytes isolated from the remote zone of three to five mouse left ventricles were tested for each group. The cardiomyocytes of the Sham and MI group were isolated from Sham and MI mice at the end of week 3 after sham and MI surgery, respectively. The cardiomyocytes of MI-GSK group were isolated at the end of 3 weeks of MI surgery and 3 weeks of GSK treatment. (**C**) Channel protein levels. Three ventricles were tested for each group. (**D**) Channel mRNA levels. Three to seven ventricles were tested for each group. Statistics was analyzed with one-way ANOVA analysis of variance with post hoc tests of significance corrected; *P<0.05 vs. sham and #P<0.05 vs. MI. Abbreviations: I_{Na}, cardiac Na^+ current conducted by $Na_v1.5$; I_{CaL}, Ca^{2+} current conducted by $Ca_v1.2$; I_{to}, the transient outward K^+ current conducted by $K_v4.3$; I_{K1}, the inward rectifier K^+ current conducted by Kir2.1; I_{Kur}, the ultrarapid delayed rectifier K^+ current conducted by $K_v1.5$.



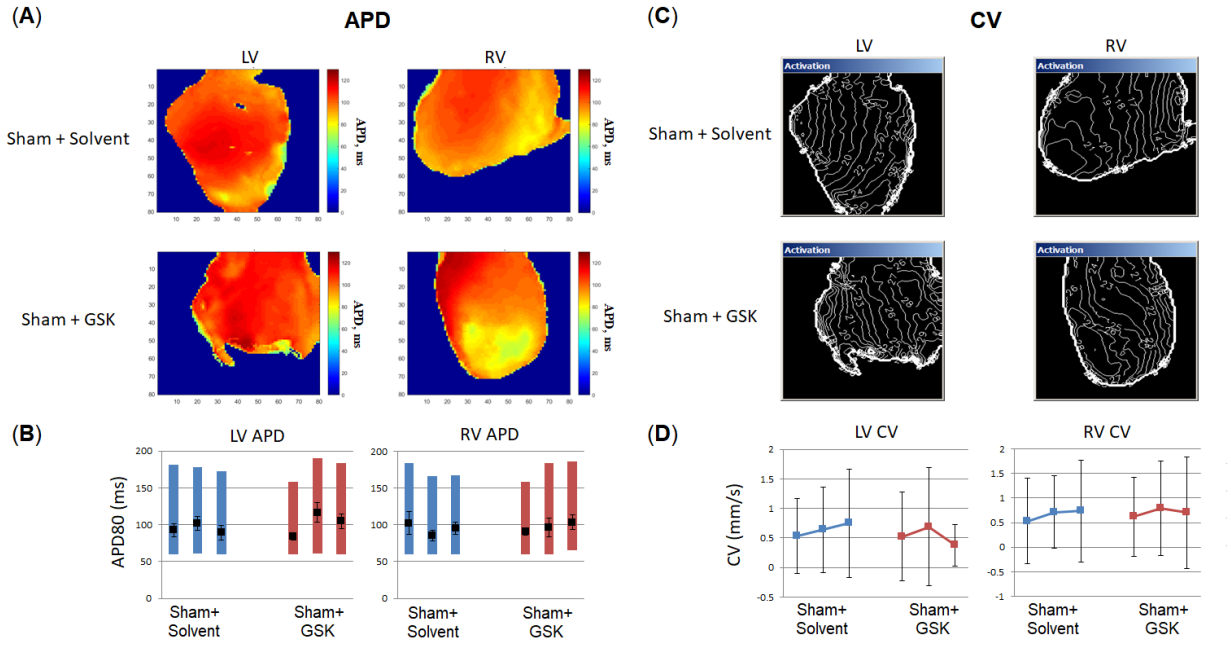
Supplemental Figure II. The time line of UPR activation in sham mouse heart tissues before (week 0) and after the sham operation (week 1-4). The inset was protein bands obtained from Western blot. Two to three mouse left ventricles were tested for each data point. Abbreviations: ATF6N, the cleaved N-terminus of ATF6α; p-PERK, phosphorylated PERK; p-IRE1, phosphorylated inositol-requiring ER-to-nucleus signal kinase 1. * P<0.05 for p-PERK week 3 vs. p-PERK week 0 with one-way ANOVA analysis of variance with post hoc tests of significance corrected.



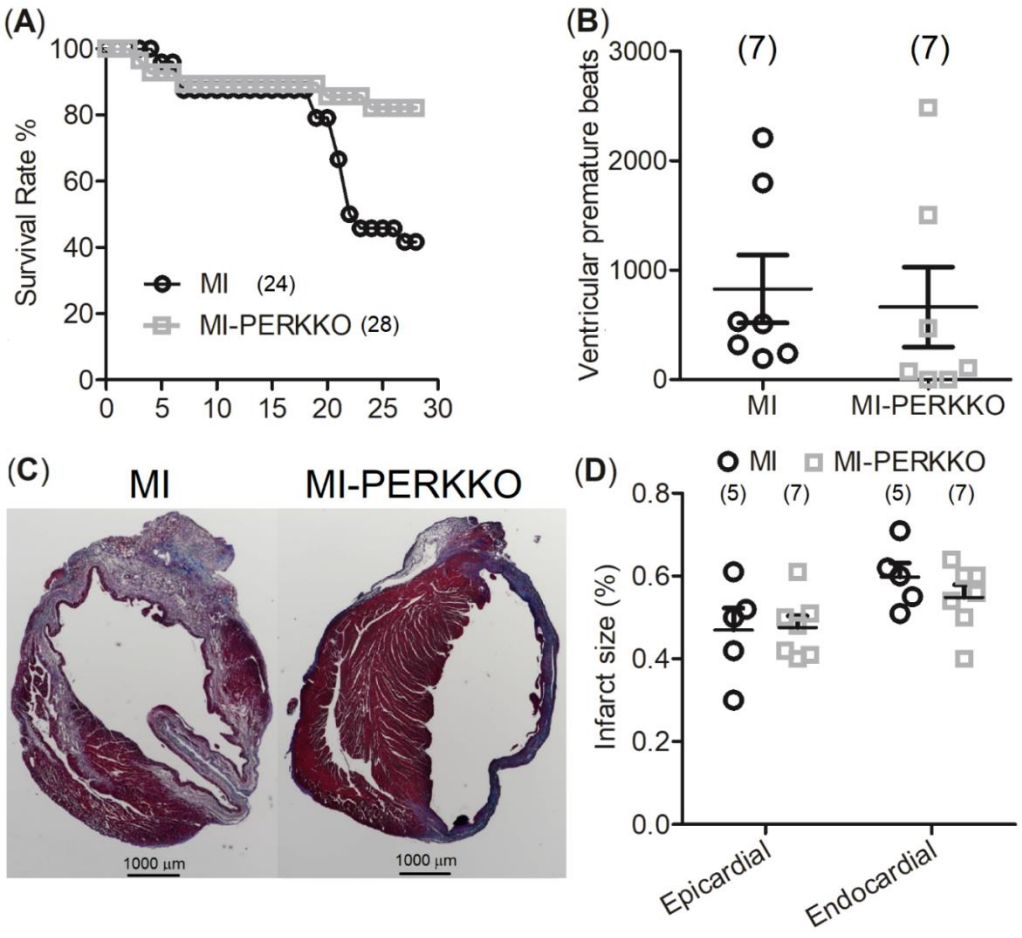
Supplemental Figure III. PERK inhibition by GSK improved VT episodes and survival in MI mice. (**A**) GSK (100 mg/kg/day for 3 weeks) prevented an increase in VT episodes seen with MI. *, P<0.05 vs Sham; #, P<0.05 vs. MI. Statistical significance was determined by one-way ANOVA analysis of variance with post hoc tests of significance corrected for multiple comparisons. (**B**) GSK treatment was associated with increased survival after MI. Statistical significance was evaluated using the Fisher's exact test, *P<0.01 vs. sham and #P<0.01 for MI. The numbers of tested mice are shown in parentheses or in the figure bars.



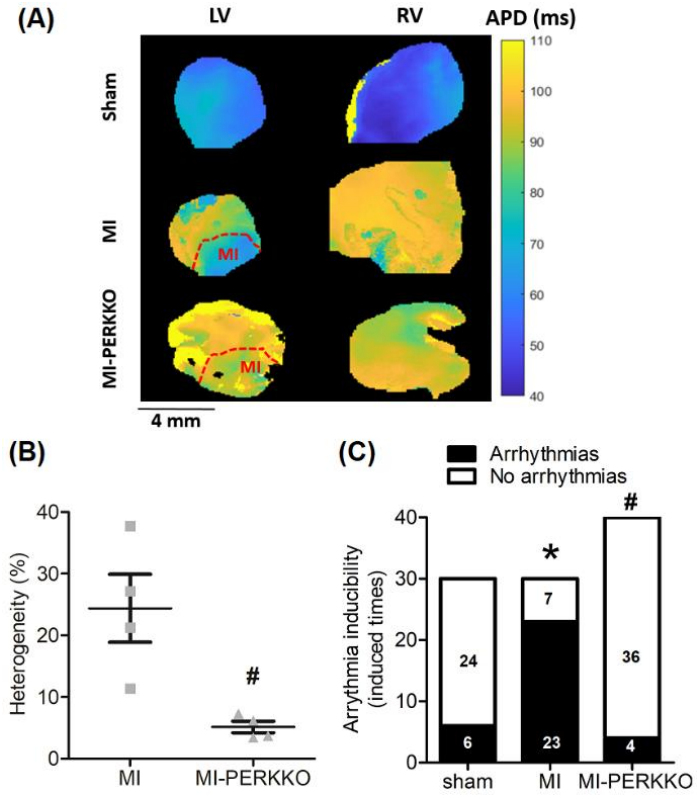
Supplemental Figure IV. GSK treatment on sham mice showed no impact on the action potential duration (APD) and conduction velocity (CV) measured with optical mapping. (**A** and **C**) Representative optical maps of the APD and CV. (**B** and **D**) The averaged APD and CV obtained from left (LV) and right ventricles (RV). Three mice were tested for each group. GSK (100 mg/kg/day) or the solvent (0.5% hydroxypropylmethyl cellulose and 0.1% Tween-80) used to solve GSK was gavage fed to mice for 3 weeks after sham surgery. Abbreviations: APD, the action potential duration; CV, conduction velocity; GSK, GSK2606414; LV, left ventricle; RV, right ventricle.



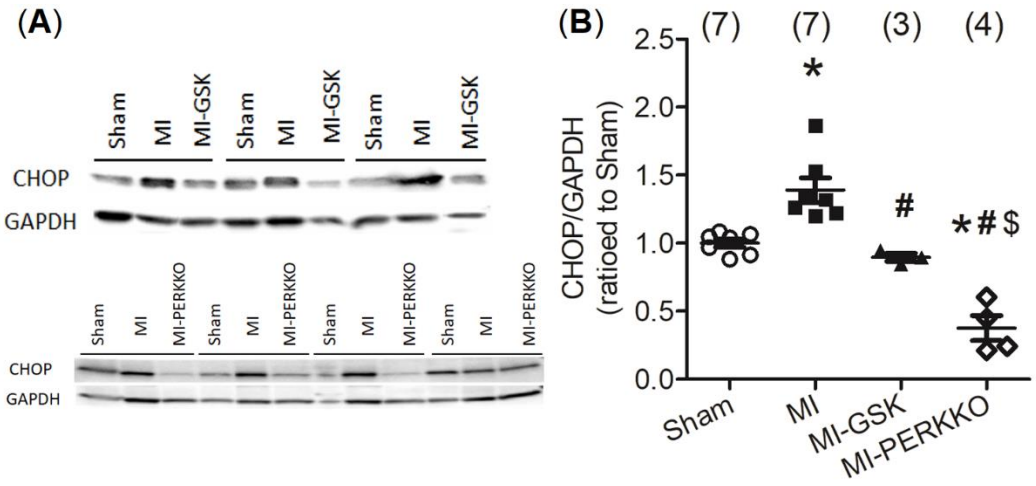
Supplemental Figure V. PERK knockout improved survival after MI without changes in infarct size. (**A**) The survival curves of the MI and MI-PERKKO mice showed improved survival in MI-PERKKO mice in 4 weeks post MI surgery (The Log-rank test gave a P=0.0049). Cardiac-specific PERK knockout showed no effects on (**B**) ventricular premature beats or (**C** and **D**) the epicardial and endocardial infarct scar size. The numbers of tested mice are shown in parentheses. The ventricular premature beats were obtained with 7 mice for both group from 2-hour telemetry data (1-2 am and 1-2 pm). The infarct scar size data were obtained with 5 wild type MI mice and 7 MI-PERKKO mice.



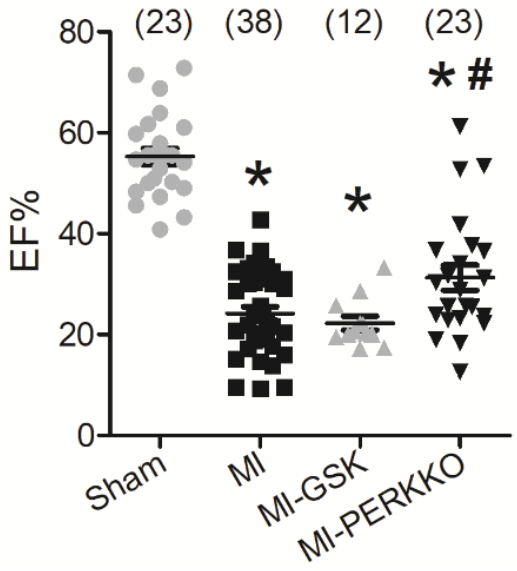
Supplemental Figure VI. Optical mapping measurements showed improved arrhythmia inducibility and reduced APD heterogeneity in MI-PERKKO mouse hearts as compared to MI alone. (**A**) Representative 2-dimensional APD maps of sham, wild type MI and MI-PERKKO for the left ventricular (LV) and right ventricular (RV) epicardial surfaces of mouse hearts. Hearts were paced at cycle length = 120 ms. Red dashed lines indicate approximate MI regions. (**B**) APD heterogeneity, calculated as the difference of APD in the LV MI region with respect to the LV remote zone, was reduced when comparing MI (n=4) and MI-PERKKO (n=4) (# P=0.0138 vs. MI). Statistical significance was tested using a Student's t-test. (**C**) PERK inhibition during MI resulted in decreased arrhythmia inducibility as indicated by the number of ventricular arrhythmias induced via burst pacing (10 bursts in each heart) in sham (n=3), MI (n=3) and MI-PERKKO (n=4) mouse hearts. Total number of inducible and non-inducible episodes are shown in the bars. Statistical significance was evaluated using the Fisher's exact test (*P<0.0001 for sham vs. MI and #P<0.0001 for MI vs. MI-PERKKO).



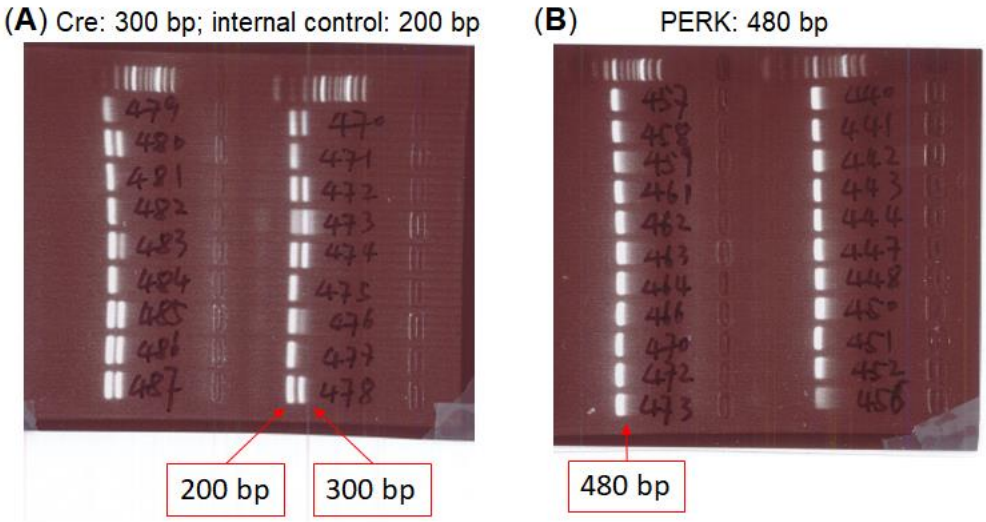
Supplemental Figure VII. Pharmacological and genetic inhibition of PERK in MI mouse hearts resulted in decreased apoptosis with decreased protein levels of C/EBP homologous protein (CHOP) as shown in (**A**) the Western blot protein bands and (**B**) the scattered-dot plot with CHOP protein levels ratioed to GAPDH and the sham group. The ventricles were collected at the end of week 3 after sham and MI surgery, respectively, and at the end of GSK treatment for the MI-GSK group. Three to seven mouse ventricles were tested for each group. Statistics was analyzed with one-way ANOVA analysis of variance with post hoc tests of significance corrected. *, P<0.05 vs. sham; # P<0.05 vs. MI; $, P<0.05 vs. MI-GSK.



Supplemental Figure VIII. PERK inhibition by cardiac-specific PERK knockout improved systolic function after MI (ejection fraction=EF%) but GSK treatment did not (100 mg/kg/day for 3 weeks). The numbers of tested mice are shown in parentheses. EF% was analyzed with one-way ANOVA analysis of variance with post hoc tests of significance corrected. *, P<0.05 vs Sham; #, P<0.05 vs. MI.



Supplemental Figure IX. Representative images of genotyping results to confirm cardiac-specific PERK knockout: (**A**) Cre (transgene = ~300 bp and internal positive control = 200 bp) and (**B**) PERK (mutant = ~480 bp), by following the protocols provided by the Jackson Laboratory website (#23633 and #26338).



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