SUPPLEMENTAL MATERIAL

Methods

Mouse Experiments. The mice were housed in the Medical College of Wisconsin Biological Resource Center, and all experiments were performed in accordance with an IACUC approved animal procedure protocol 1022. The *Snrk* cmcKO adult mice were generated from MYH6CRE positive *Snrk* LoxP/wild type (WT) males mated to *Snrk* LoxP/LoxP females. When possible age and litter matched male and female mice were used for each animal experiment. Mixed backcrossed animals were used for all of the mouse experiments. Primer information for mouse genotyping were provided previously[1].

Human Cardiomyocyte System. Directed differentiation of CMs from human embryonic stem cells (hESCs) is described in previous studies[2, 3]. Briefly, hESCs maintained on matrigel in mTeSR1 were dissociated into single cells using Accutase (Life Technologies) at 37°C for 5 min and then were seeded onto a matrigel-coated 12-well plate at 100,000-200,000 cell/cm^2 in mTeSR1 supplemented with 5 μ M Y-27632 (Tocris) for 24 h. After 24 h, fresh mTeSR1 medium was used every day to expand stem cells. When cells achieved a density of 500,000 cell/cm^2, cells were treated with 12 μ M CHIR99021 (Selleckchem) in RPMI/B27 without insulin for 24 h (day 0 to day 1). The medium was changed to RPMI/B27 without insulin on day 1, followed by 5 μ M IPW2 (Tocris) treatment on day 3. During the day 5 medium change IWP2 was removed. Cells were maintained in the RPMI/B27 starting from day 7, with the medium changed every 3 days.

Lentiviral Transduction of CMs and Drug Treatments. Day six CMs differentiated from hESCs were dissociated into single cells with Accutase at 37°C for 5-8 mins, and then were spun down into aliquots of 1.5×10^6 cells. Cells were re-suspended in 30 μ L concentrated lentivirus medium in the presence of 6 μ g/mL polybrene (Sigma), and then incubated at room temperature for 10 mins. At the end of

incubation, 2 mL of DMEM/10% FBS + 5 μ M Y-27632 (Tocris) was added and the suspension was transferred into 2 wells of a 12-well plate pre-coated with Matrigel, and incubated at 37°C, 5% CO_2 overnight. The medium was replaced with fresh DMEM/10% FBS on the second day of lentiviral infection, and afterwards cells were treated with water (control), and 5 μ M Y27632 for 9 days. Two days later, infected cells were selected and enriched for 3 days based on resistance to 1 μ g/mL puromycin (Sigma). 48 h before sample collection, cells were switched to RPMI containing B27 (no insulin) supplement. On day 15, the cells were rinsed with PBS and the proteins were collected for western blot analysis as previously described[1, 4, 5].

Echocardiography and Image Analysis. Transthoracic echocardiography was performed in anesthetized (2% isoflurane) six months old litter matched males. An investigator (L.M.H) who was blinded to the study groups performed the measurements and data analyses. A comprehensive 2D transthoracic echocardiogram with Doppler was performed on animals using Animals were studied with a commercially available echocardiographic system (Vivid 7, General Electric, Wauwatosa, WI), with an 11-MHz M12-L linear array transducer. A comprehensive 2D transthoracic echocardiogram with Doppler was performed from the cardiac short axis of the left ventricle at the papillary muscle level. Using the anatomical M-mode feature of the Vivid 7 echo, an M-mode display was generated from raw data 2D images of the short-axis of the left ventricle at the level of the papillary muscle with the line selected passing through the anterior and inferior segments. Ejection fraction % (EF) was measured using left ventricle end diastolic volume (LVEDV) and left ventricle end systolic volume (LVESV) using the formula EF= LVEDV - LVESV/ LVEDV x 100. Fractional shortening % (FS) was calculated with the formula: left ventricle end diastolic dimension (LVEDD)-left ventricle end systolic dimension (LVESD)/LVEDD x 100. To assess diastolic function, pulse wave Doppler was used to assess transmitral inflow velocities in the apical 4-chamber view to obtain E velocities and isovolumic relaxation times (IVRT). Tissue Doppler was

used to assess tissue motion velocity from the mitral annulus to obtain e' velocities. E/e' is a calculated ratio. Pulse wave Dopplers was also used to sample velocities at the right ventricular outflow tract to assess for pulmonary acceleration time (PAT) and ejection time (ET). For two-dimensional strain analysis, the images were processed with EchoPac Q analysis software, (General Electric, Wauwatosa, WI). A cardiac cycle was defined from the peak of one R wave to the peak of the following R wave. Unless stated otherwise, for every measurement, three consecutive cardiac cycles were measured, and the average used for reporting. The method has been previously described[6]. Briefly, for radial and circumferential strain, the endocardial border was traced in an end-systolic frame in the short-axis view at the mid ventricle as identified by the prominent papillary muscles. The software automatically selected 6 equidistant tissue-tracking regions of interest in the myocardium. The outer border was adjusted to approximate the epicardial border. The computer then provided a profile of radial (myocardial deformation toward the center) and circumferential (myocardial deformation along the curvature) strain (%) with time.

Purification of SNRK. Cloned full length human SNRK sequence was transformed into BL21(DE3) competent cells (New England Biolabs). Bacterial cells were cultured overnight at 37°C in LB and induced with 1mM IPTG and incubated at 25°C for 6 h. The bacteria were then pelleted and the supernatant was removed. The pelleted bacteria were lysed using a bacterial lysis buffer containing 13 mL BPER (Pierce Technologies), 50 μ L DNAse (2 U/μ L), 41 μ L lysozyme (50 mg/mL) and 40 μ L 1M $MgCl_2$, protease inhibitor (Roche). The pellet was incubated with the lysis buffer at room temperature for 15 mins. One tablet of complete Mini EDTA-free protease inhibitor cocktail (Sigma) was add to the mixture and centrifuged at 7,600 x g for 15 mins. The supernatant was removed and dialyzed in a Slide-A-Lyzer with 10K molecular cutoff (ThermoFisher) overnight at room temperature in 1X binding buffer (57mM NaH2PO4 pH8.0, 300mM NaCl). The dialyzed supernatant was subjected to Nickel-NTA column

purification, and pure protein eluted in 10 mM, 25 mM and 250 mM Imidazole elution buffer. Samples of the fractions were resolved on a 10% Mini-PROTEAN TGX precast gel (BioRad), subjected to SDS-PAGE and subsequently stained with coomassie blue. Fractions eluted with 250 mM Imidazole were concentrated using Amicon Ultra 15 mL 50 MWL, kDa centrifugal filter units (EMD Millipore). Purified protein was subjected to Mass Spectometry analysis for identity confirmation. SNRK kinase activity was confirmed using the ADP-Glo Kinase Assay (Promega). In short, purified SNRK protein was incubated with 10mM ATP, different amounts Histone H3 (0, 500ng, 1000ng, 2000ng, 4000ng) and kinase reaction buffer. ADP-Glo reagent was then added to stop the kinase reaction and deplete the unconsumed ATP. After 40 mins, the kinase detection reagent was added to convert the ADP to ATP for luciferase detection of the ATP. The luciferase was measured using a plate-reading luminometer (SpectraMaxGeminiEM, Molecular Devices) with SoftMax Pro software.

Identification of SNRK Candidate Substrates. All possible kinase substrates at each concentration of SNRK were evaluated by the Z-Factor rank within the array and were compared to the negative control assay. A protein was defined as being a candidate substrate for SNRK if it met the following conditions: 1) The Z-Factor, or signal-to-noise ratio, was greater than 0.35 on at least one assay, indicating a signal greater than 1.5-fold above the noise. 2) The Signal Used value was greater than 2,000 relative unites on at least one array probed with SNRK and was greater than 2-fold higher than the Signal Used value for the corresponding protein in the negative control assay. 3) The replicate spot coefficient of variation (CV) was less than 50% on the corresponding array. 4) The inter-assay CV was less than 50%.

Mass Spectral Analysis. Immunoprecipitated ROCK1 protein was incubated with or without 50 nM purified SNRK protein in the presence of 4 μ M ATP (Cell Biolabs) in kinase buffer [20 mM HEPES (pH 7.7), 20 mM $MgCl_2$, 2 mM DTT, 1X protease inhibitor (Roche) and 1X phosphatase inhibitor (Roche)]. The

samples were allowed to incubate for 15 mins at 30°C. After incubation the samples were washed 2-3 times with kinase buffer and re-suspended in Lamelli sample buffer. The samples were resolved using gel electrophoresis and then subjected to in-gel tryptic digestion followed by Mass Spectral analysis.

Mass spectral analysis was performed on an LTQ-Orbitrap Velos mass spectrometer (Thermo Scientific, Waltham, MA, USA) coupled with a nanoAQUITY ultraperformance liquid chromatographer (UPLC) (Waters Corp.) interfaced with a nanoelectrospray ion source. Peptide separation was carried out on a capillary column built in-house with 5µm C18 nanoparticles to 10 cm long column with 75 µm inner diameter tubing. The fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) for the columns were pulled by a micropipette puller P-2000 (Sutter Instrument Company, CA, USA) and packed with C18 resin using a bomb-loader. The solvents A and B used for chromatographic separation of peptides were 2% acetonitrile in 0.1% formic acid and 98% acetonitrile in 0.1% formic acid, respectively. The peptides injected onto the microcapillary column were resolved at the rate of 200 nl/min, using the following gradient conditions: 0 to 10 min, 2 to 20% B; 120 min, 40% B; 135 min, 60% B; 150 min, 70% B; 158 min, 98%B, which was held for 4 min and then switched to 98% A and held for another 18 min. The ions eluted from the column were electrosprayed at a voltage of 1.70 kV. The ion transfer temperature was kept at 250°C. No auxiliary or sheath gas was used.

Survey full-scan mass spectrometry (MS) spectra (*m/z*, 300 to 2,000) were recorded in the Orbitrap analyzer at a resolution of 30,000 followed by tandem mass spectrometry (MS/MS) of the 10 most intense peptide ions in the linear ion trap analyzer. Neutral loss-triggered multistage activation for simultaneous fragmentation of neutral loss product and precursor was enabled at *m/z* of -98, -49 and -32.6 relative to the precursor ion, corresponding to a neutral loss of phosphate moiety from +1, +2, and +3 charged ions. The chromatographic and mass spectral functions were controlled using the Xcalibur data system (ThermoFinnigan, Palo Alto, CA, USA). Peptides were identified using the Mascot search

algorithm for human protein database at a global false-discovery rate of 5%. The resulting files were evaluated using Visualize tool[7].

Tissue Sectioning, Staining and Image Analysis. Adult mouse hearts were fixed in 4% paraformaldehyde/PBS for 1 to 2 days at 4°C. After fixation tissues were washed extensively in PBS and embedded in paraffin or sucrose/OCT as previously described[1]. Seven micron thick sections were stained with Gomori's Trichrome staining and Hematoxylin counter staining. The microscopic images of the entire tissue section were scanned at 40x magnification using the Nanozoomer 2.0-HT (Hamamatsu, Japan) digital slide scanning system (Children's Research Institute's Imaging Core). The amount of fibrosis was quantified using software assisted, unbiased microimage quantification method. The scanned images were imported into Visiopharm software (Denmark) and using the imager module, three 20x ROI images were extracted from left, center and right regions of the heart. Collagen positive regions (navy blue stains) were identified by thresholding the specific image channel filter and the rest of the tissue (muscle - stained red and nucleus - stained deep blue) classified as tissue region with total tissue region defined as sum of muscle, nuclear and collagen region without any interstitial space. All original images were processed with this preset thresholds and linear Bayesian classification to generate a processed image. Total collagen positive area per ROI was measured in microns and represented as a percentage of the total tissue area.

References

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Supplemental Figures and Tables

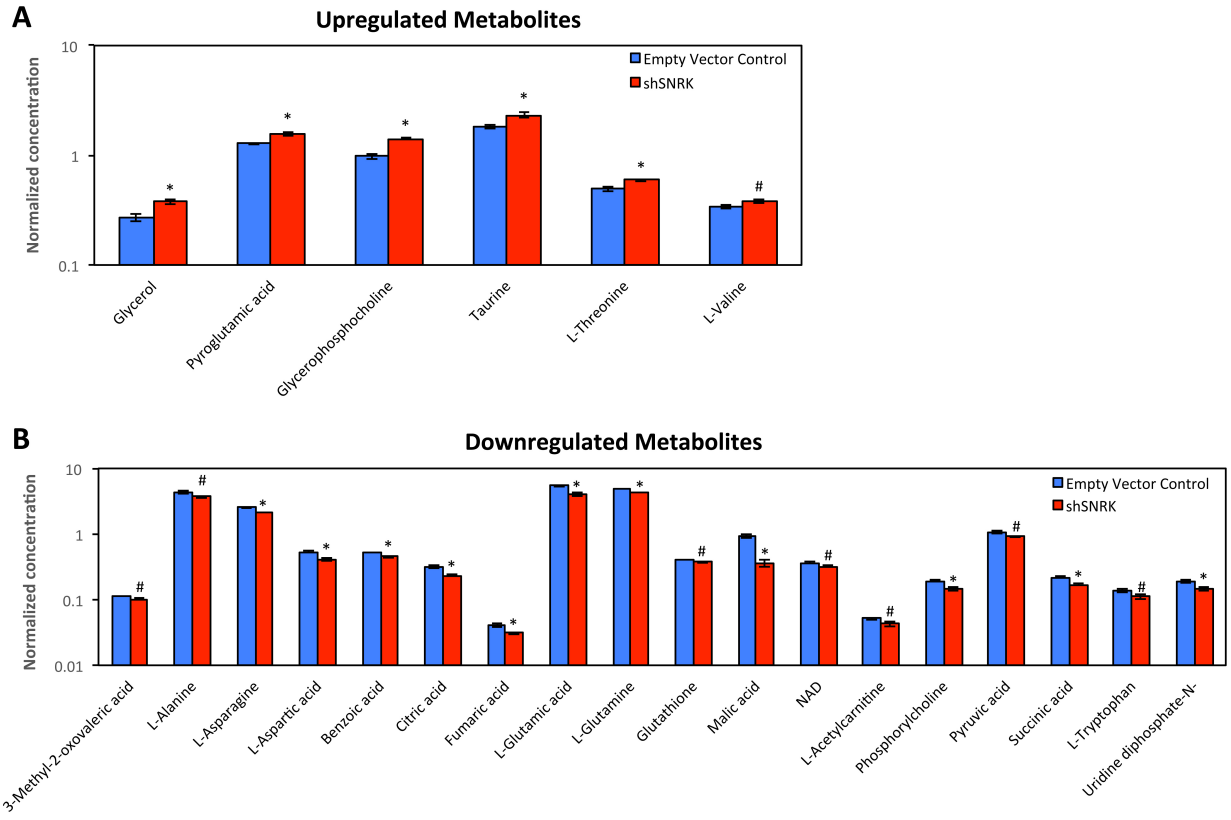
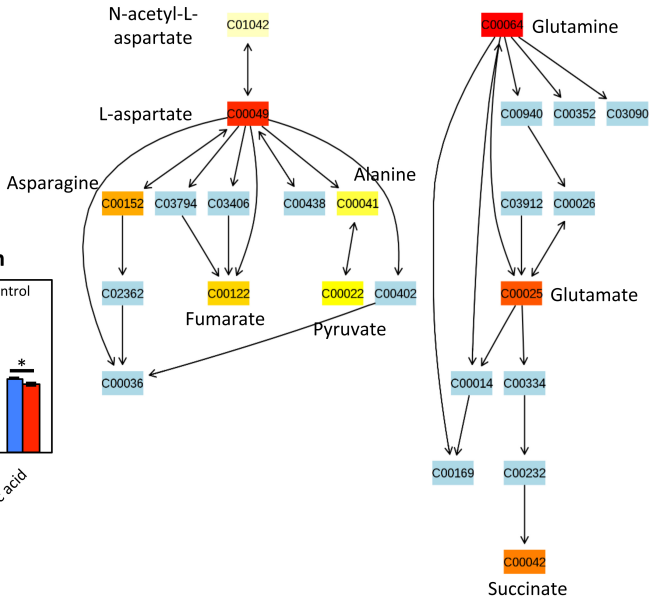


Figure S1. Loss of SNRK in cardiomyocytes results in altered metabolic profiles. Panel A shows the upregulated metabolites and B shows the downregulated metabolites. *p<0.05, # p<0.1.

A

Pathway	Impact	P value	FDR
Alanine, aspartate and glutamate metabolism	0.68571	0.0026	0.0095

B



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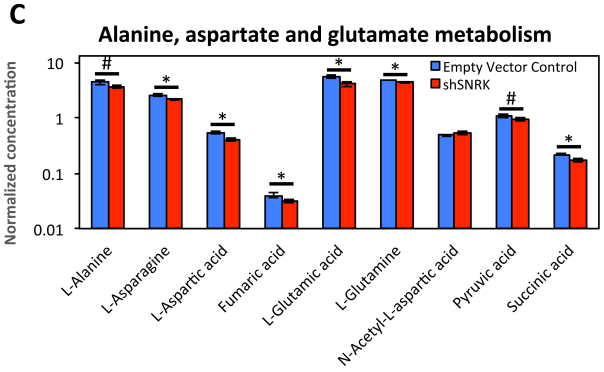


Figure S2. Loss of SNRK in cardiomyocytes results in altered alanine, aspartate, and glutamate metabolism. Panel A is the Pathway topology analysis impact score, p value and FDR. Panel B is the pathway view showing the corresponding Alanine, aspartate and glutamate metabolic pathways. The matched metabolites are highlighted according to their *P* values. Panel C is the normalized concentration fro the individual metabolites. *p<0.05, # p<0.1.

A

Pathway	Impact	P value	FDR
Citric acid cycle	0.1875	3.47e-6	1.8e-4

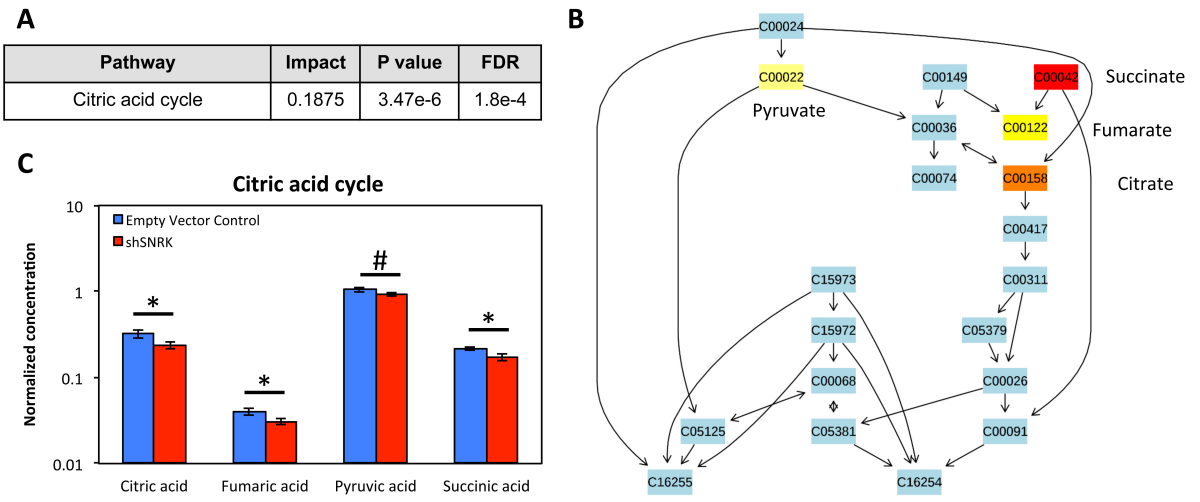


Figure S3. Loss of SNRK in cardiomyocytes results in altered Citric acid cycle metabolites. Panel A is the Pathway topology analysis impact score, p value and FDR. Panel B is the pathway view showing the altered metabolites in the citric acid cycle. The matched metabolites are highlighted according to their *P* values. Panel C is the normalized concentration from the individual metabolites. *p<0.05, # p<0.1.

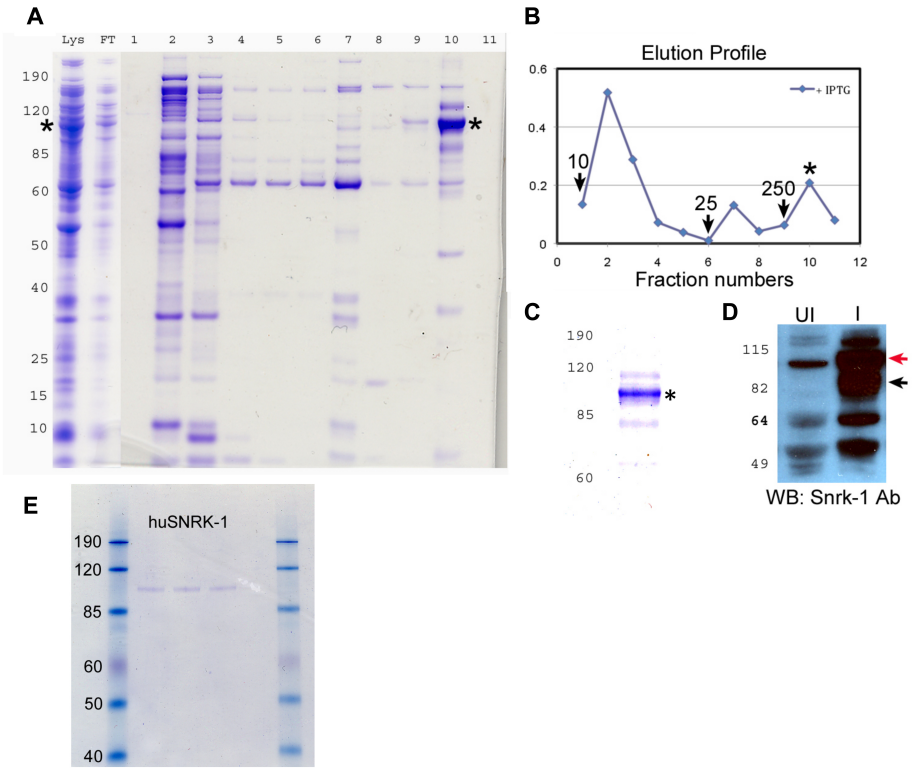
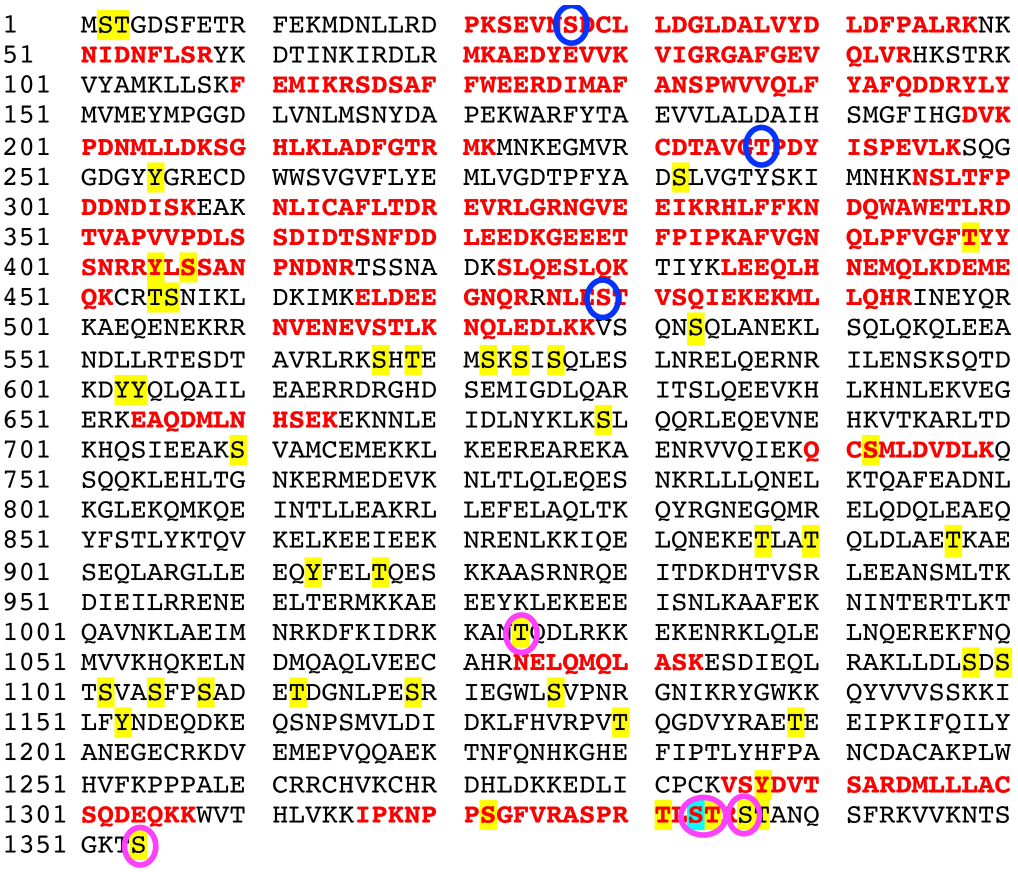


Figure S4. Human histidine tagged SNRK protein generation and purification. Panel **A** indicates a SDS-PAGE gel resolving the different fractions. Lys: lysate, FT: flow through. Numbers indicate the fraction number post 10 mM (1-5), 25 mM (6-8) and 250 mM (9-11) imidazole elution. The numbers on top of each lane corresponds to fraction elution profile in panel **B**. Panel **B** indicates elution profile and arrows indicate imidazole elution points with each number corresponding to concentration of imidazole. Only IPTG induced bacterial profile is shown. Panel **C** shows fraction #10 (black asterisks), re-purified a second round on Nickel NTA column and eluted with 250 mM imidazole. Panel **D** shows a western blot with SNRK antibody in uninduced (UI) and induced (I) lysate. Red arrow indicates the full-length protein and black arrow indicates degraded products. Numbers on left of each gel indicate protein size markers in kDa. Panel **E** indicates the three fractions from 250 mM imidazole purification that were subsequently concentrated and run on Coomassie-stained SDS-PAGE gel before the final product was sent for array experiment.



Match to ROCK1_HUMAN (Q13464 UniProtKB) Score: 1535
Sequence Coverage: 29%

- PhosphoID in current study
- Phosphorylation confirmed by Site Directed Mutagenesis
- Reported in literature to induce enzymatic activity in rat and mouse (**S1333**)
- Previously identified by Proteomics

Matched peptides shown in Bold Red

Figure S5. Mass spectral (MS) analysis of the SNRK mediated phosphorylation sites on ROCK1. MS analysis was used to identify the residues on ROCK which are phosphorylated by SNRK in vitro. Full length ROCK was incubated with or without SNRK. The SNRK dependent phosphorylated residues are depicted with a blue circle and the previously published phosphorylated residues are depicted with a magenta circle. MS identified amino acids are shown in bold red (current study), and the yellow highlighted residues were previously identified but not yet confirmed by proteomic analysis on PhosphoSitePlus.

Tricrhome Staining

Genotype	Average % Collagen
CRE Negative 1	6.60
CRE Negative 2	7.24
CRE Negative 3	12.25
CRE Negative 4	15.45
Average	**10.38**
SEM	**2.07**
MYH6CRE SNRK L/L 1	17.62
MYH6CRE SNRK L/L 2	21.70
MYH6CRE SNRK L/L 3	18.63
Average	**19.32**
SEM	**1.23**

Adult Heart Lysate Western Blot Quantification

% Relative Intensity	Ratio					
Genotype	**ROCK1/Tubulin**	**ROCK2/Tubulin**	**pMBS/MBS**	**pERK/ERK**	**pAKT/AKT**	**pIKK/IKK**
CRE Negative 1	100.00	100.00	100.00	100.00	100.00	100.00
CRE Negative 2	105.91	129.55	105.39	118.63	100.26	101.15
CRE Negative 3	94.09	70.45	94.61	81.37	99.74	98.85
Average	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
SEM	**3.41**	**17.06**	**3.11**	**10.75**	**0.15**	**0.66**
MYH6CRE SNRK L/L 1	68.89	96.79	121.84	168.36	41.77	23.39
MYH6CRE SNRK L/L 2	123.78	107.34	112.14	105.87	67.13	51.21
MYH6CRE SNRK L/L 3	76.34	55.27	134.66	276.17	29.53	59.24
Average	**89.67**	**86.47**	**122.88**	**183.47**	**46.15**	**44.62**
SEM	**17.19**	**15.89**	**6.52**	**49.74**	**11.07**	**10.86**

Cardiomyocyte Lysate Western Blot Quantification

% Relative Intensity	Ratio			
Genotype	**pMBS/MBS**	**pAKT/AKT**	**pIKK/IKK**	**pERK/ERK**
Non-Sliencing-Control 1	100.00	100.00	100.00	100.00
Non-Sliencing-Control 2	100.00	100.00	100.00	100.00
Non-Sliencing-Control 3	100.00	100.00	100.00	100.00
Non-Sliencing-Control 4	No Sample	100.00	No Sample	100.00
Average	**100.00**	**100.00**	**100.00**	**100.00**
SEM	**0.00**	**0.00**	**0.00**	**0.00**
Non-Sliencing-Rock Inh 1	35.48	744.30	78.44	88.78
Non-Sliencing-Rock Inh 2	99.99	337.96	268.41	193.19
Non-Sliencing-Rock Inh 3	64.57	16.79	244.95	61.88
Non-Sliencing-Rock Inh 4	No Sample	No Sample	No Sample	27.17
Average	**66.68**	**366.35**	**197.27**	**92.75**
SEM	**18.65**	**210.49**	**59.80**	**35.78**
shSNRK-Control 1	83.02	168.95	249.50	545.89
shSNRK-Control 2	161.12	280.99	318.23	335.43
shSNRK-Control 3	125.92	81.92	195.43	70.86
shSNRK-Control 4	No Sample	161.20	No Sample	46.28
Average	**123.35**	**173.26**	**254.39**	**249.62**
SEM	**22.58**	**40.94**	**35.54**	**118.48**
shSNRK-Rock Inh 1	8.24	301.99	344.53	19.71
shSNRK-Rock Inh 2	56.12	118.15	653.82	80.37
shSNRK-Rock Inh 3	57.90	9.26	176.38	32.75
shSNRK-Rock Inh 4	No Sample	No Sample	No Sample	8.58
Average	**40.75**	**143.13**	**391.58**	**35.36**
SEM	**16.26**	**85.42**	**139.82**	**15.80**

Table S1. Individual data values for the Trichome staining and Western blot analysis.

Sample ID	3-Methyl-2-oxovaleric acid	Acetic acid	L-Alanine	L-Arginine	L-Asparagine	L-Aspartic acid	Benzoic acid	Choline	Citric acid	Creatine
CNT-1	0.11	4.66	4.48	1.83	2.62	0.52	0.54	0.13	0.36	0.08
CNT-2	0.12	4.39	3.90	2.01	2.41	0.52	0.51	0.12	0.29	0.11
CNT-3	0.11	4.50	4.75	1.88	2.70	0.56	0.54	0.14	0.32	0.12
Average	**0.11**	**4.52**	**4.38**	**1.91**	**2.58**	**0.54**	**0.53**	**0.13**	**0.32**	**0.10**
SEM	**0.00**	**0.08**	**0.25**	**0.05**	**0.09**	**0.01**	**0.01**	**0.01**	**0.02**	**0.01**
shSNRK-1	0.11	4.64	3.87	1.93	2.23	0.44	0.48	0.15	0.24	0.17
shSNRK-2	0.10	3.90	3.80	1.84	2.16	0.40	0.47	0.14	0.21	0.16
shSNRK-3	0.10	4.48	3.37	1.65	2.06	0.38	0.42	0.14	0.25	0.08
Average	**0.10**	**4.34**	**3.68**	**1.80**	**2.15**	**0.41**	**0.46**	**0.14**	**0.23**	**0.14**
SEM	**0.00**	**0.22**	**0.16**	**0.08**	**0.05**	**0.02**	**0.02**	**0.00**	**0.01**	**0.03**

Sample ID	Phosphocreatine	Formic acid	Fumaric acid	D-Glucose	L-Glutamic acid	L-Glutamine	Glutathione	Glycerol	Glycine	Isobutyric acid
CNT-1	0.12	2.78	0.04	1.28	5.88	4.74	0.41	0.31	3.26	0.03
CNT-2	0.13	2.48	0.04	0.73	5.15	4.87	0.42	0.27	3.14	0.03
CNT-3	0.19	2.53	0.04	0.65	5.48	4.88	0.41	0.24	3.39	0.04
Average	**0.15**	**2.60**	**0.04**	**0.89**	**5.50**	**4.83**	**0.41**	**0.27**	**3.26**	**0.03**
SEM	**0.02**	**0.09**	**0.00**	**0.20**	**0.21**	**0.04**	**0.00**	**0.02**	**0.07**	**0.00**
shSNRK-1	0.17	2.95	0.03	0.64	4.37	4.34	0.40	0.35	3.56	0.06
shSNRK-2	0.19	2.57	0.03	0.48	4.04	4.42	0.36	0.41	3.32	0.04
shSNRK-3	0.11	2.75	0.03	0.75	3.62	4.22	0.38	0.37	3.16	0.04
Average	**0.16**	**2.76**	**0.03**	**0.62**	**4.01**	**4.33**	**0.38**	**0.38**	**3.35**	**0.05**
SEM	**0.02**	**0.11**	**0.00**	**0.08**	**0.22**	**0.06**	**0.01**	**0.02**	**0.11**	**0.01**

Sample ID	L-Isoleucine	L-Lactic acid	L-Leucine	L-Lysine	Malic acid	L-Methionine	Myoinositol	N-Acetyl-L-aspartic acid	NAD	L-Acetylcarnitine
CNT-1	0.81	42.69	0.56	0.38	0.92	0.26	3.76	0.51	0.35	0.05
CNT-2	0.85	46.56	0.63	0.46	0.83	0.25	2.97	0.46	0.38	0.05
CNT-3	0.84	42.72	0.62	0.40	1.07	0.28	3.75	0.48	0.36	0.05
Average	**0.83**	**43.99**	**0.60**	**0.41**	**0.94**	**0.26**	**3.49**	**0.48**	**0.36**	**0.05**
SEM	**0.01**	**1.29**	**0.02**	**0.02**	**0.07**	**0.01**	**0.26**	**0.01**	**0.01**	**0.00**
shSNRK-1	0.79	44.02	0.61	0.42	0.30	0.26	4.29	0.55	0.34	0.05
shSNRK-2	0.84	47.45	0.67	0.40	0.43	0.29	3.80	0.48	0.30	0.04
shSNRK-3	0.92	47.92	0.72	0.44	0.33	0.27	4.05	0.57	0.33	0.04
Average	**0.85**	**46.47**	**0.67**	**0.42**	**0.36**	**0.27**	**4.05**	**0.53**	**0.32**	**0.04**
SEM	**0.04**	**1.23**	**0.03**	**0.01**	**0.04**	**0.01**	**0.14**	**0.03**	**0.01**	**0.00**

Sample ID	Phosphorylcholine	O-Phosphoethanolamine	Pantothenic acid	L-Phenylalanine	L-Proline	Pyroglutamic acid	Pyruvic acid	L-Serine	Glycerophosphocholine	Succinic acid
CNT-1	0.20	4.11	0.09	0.25	1.66	1.25	1.07	1.13	0.88	0.21
CNT-2	0.19	3.92	0.10	0.29	1.61	1.30	1.13	1.00	1.01	0.23
CNT-3	0.18	4.28	0.08	0.29	1.73	1.27	0.99	1.08	1.03	0.22
Average	**0.19**	**4.10**	**0.09**	**0.27**	**1.67**	**1.27**	**1.06**	**1.07**	**0.97**	**0.22**
SEM	**0.01**	**0.11**	**0.00**	**0.01**	**0.04**	**0.02**	**0.04**	**0.04**	**0.05**	**0.01**
shSNRK-1	0.16	4.53	0.08	0.24	1.85	1.56	0.99	1.01	1.45	0.17
shSNRK-2	0.15	4.34	0.09	0.27	1.77	1.44	0.90	0.94	1.41	0.19
shSNRK-3	0.12	4.18	0.08	0.26	1.63	1.67	0.89	1.06	1.37	0.16
Average	**0.14**	**4.35**	**0.08**	**0.26**	**1.75**	**1.56**	**0.93**	**1.01**	**1.41**	**0.17**
SEM	**0.01**	**0.10**	**0.00**	**0.01**	**0.06**	**0.07**	**0.03**	**0.03**	**0.02**	**0.01**

Sample ID	Taurine	L-Threonine	Trans-4-Hydroxy-L-proline	L-Tryptophan	L-Tyrosine	Uridine diphosphate-N-acetylglucosamine	L-Valine
CNT-1	1.86	0.55	1.27	0.13	0.25	0.19	0.31
CNT-2	1.64	0.47	0.95	0.15	0.25	0.17	0.35
CNT-3	1.98	0.48	1.20	0.13	0.24	0.21	0.36
Average	1.83	0.50	1.14	0.14	0.25	0.19	0.34
SEM	0.10	0.03	0.10	0.01	0.00	0.01	0.01
shSNRK-1	2.55	0.55	1.09	0.12	0.26	0.16	0.36
shSNRK-2	2.32	0.61	0.85	0.10	0.28	0.13	0.37
shSNRK-3	2.11	0.61	0.87	0.11	0.23	0.15	0.40
Average	2.33	0.59	0.94	0.11	0.26	0.14	0.38
SEM	0.13	0.02	0.08	0.01	0.01	0.01	0.01

Table S2. Individual data values for the Metabolomics data analysis.

Compound Name	HMDB	Fold change	pvalue	Compound Name	HMDB	Fold change	pvalue
3-Methyl-2-oxovaleric acid	HMDB00491	-1.1045	0.0579	L-Leucine	HMDB00687	1.1034	0.1765
4-Hydroxyproline	HMDB00725	-1.2171	0.1779	L-Lysine	HMDB00182	1.0209	0.7521
Acetic acid	HMDB00042	-1.0403	0.5029	L-Methionine	HMDB00696	1.0318	0.5483
Benzoic acid	HMDB01870	-1.1618	0.0293	L-Phenylalanine	HMDB00159	-1.0657	0.3195
Choline	HMDB00097	1.0970	0.1850	L-Proline	HMDB00162	1.0506	0.3155
Citric acid	HMDB00094	-1.3709	0.0187	L-Serine	HMDB00187	-1.0653	0.2615
Creatine	HMDB00064	1.3478	0.2904	L-Threonine	HMDB00167	1.1904	0.0452
D-Glucose	HMDB00122	-1.4290	0.2758	L-Tryptophan	HMDB00929	-1.2443	0.0691
Formic acid	HMDB00142	1.0608	0.3383	L-Tyrosine	HMDB00158	1.0252	0.6969
Fumaric acid	HMDB00134	-1.2891	0.0320	L-Valine	HMDB00883	1.1174	0.1000
Glutathione	HMDB00125	-1.0884	0.0604	Malic acid	HMDB00744	-2.6446	0.0018
Glycerol	HMDB00131	1.3832	0.0170	Myoinositol	HMDB00211	1.1585	0.1364
Glycerophosphocholine	HMDB00086	1.4451	0.0012	N-Acetyl-L-aspartic acid	HMDB00812	1.1035	0.1920
Glycine	HMDB00123	1.0256	0.5675	NAD	HMDB00902	-1.1205	0.0677
Isobutyric acid	HMDB01873	1.3424	0.1492	O-Phosphoethanolamine	HMDB00224	1.0601	0.1671
L-Acetylcarnitine	HMDB00201	-1.2026	0.0890	Pantothenic acid	HMDB00210	-1.0767	0.3511
L-Alanine	HMDB00161	-1.1898	0.0777	Phosphocreatine	HMDB01511	1.0772	0.7406
L-Arginine	HMDB00517	-1.0563	0.3586	Phosphorylcholine	HMDB01565	-1.3449	0.0126
L-Asparagine	HMDB00168	-1.1999	0.0135	Pyroglutamic acid	HMDB00267	1.2219	0.0139
L-Aspartic acid	HMDB00191	-1.3150	0.0044	Pyruvic acid	HMDB00243	-1.1466	0.0589
L-Glutamic acid	HMDB00148	-1.3717	0.0079	Succinic acid	HMDB00254	-1.2730	0.0104
L-Glutamine	HMDB00641	-1.1167	0.0024	Taurine	HMDB00251	1.2745	0.0350
L-Isoleucine	HMDB00172	1.0221	0.6772	Uridine diphosphate-N-acetylglucosamine	HMDB00290	-1.3140	0.0225
L-Lactic acid	HMDB00190	1.0563	0.2365				

Table S3. Full metabolomic data set. HMDB: human metabolic database ID number.

Database ID	Ultimate ORF ID	Signal Used	Z-Factor	Z-Score	CI P-Value	Replicate Spot CV	Interassay CV	Assay	Signal Used Neg	Ratio to Neg	Description
BC036089.1	IOH27267	2714	0.71	0.92	5.27E-02	5%	33%	50 nM	31	87.7	MLLT3
BC000250.1	IOH4515	4650	0.79	1.76	1.75E-02	5%	3%	50 nM	1423	3.3	NME3
BC000633.1		37106	0.54	12.01	7.75E-04	11%	0%	5 nM	6431	5.8	TTK
BC000633.1		10465	0.84	4.31	2.98E-03	4%	2%	50 nM	4132	2.5	TTK
BC005403.1	IOH61712	2200	0.65	0.70	7.47E-02	6%	10%	50 nM	887	2.5	C11orf57
BC008668.1	IOH9808	15077	0.86	4.71	4.25E-03	3%	9%	5 nM	4132	3.6	GAK
BC008668.1	IOH9808	2758	0.78	0.94	4.37E-02	2%	4%	50 nM	1181	2.3	GAK
BC010357.1	IOH14260	3147	0.58	1.11	3.72E-02	9%	5%	50 nM	842	3.7	RSRC1
BC012576.1	IOH12217	3144	0.62	1.11	4.10E-02	8%	26%	50 nM	1134	2.8	BAZ2B
BC014298.1	IOH13449	2232	0.70	0.71	8.14E-02	7%	0%	50 nM	620	3.6	PRKRIP1
BC016330.1	IOH14709	3175	0.82	1.12	3.60E-02	1%	37%	50 nM	606	5.2	RAD51AP1
BC017070.1	IOH11214	2106	0.85	0.66	7.54E-02	1%	14%	50 nM	521	4.0	CLUAP1
BC020726.1	IOH12969	2249	0.60	0.45	2.13E-01	2%	3%	5 nM	753	3.0	SCEL
BC020810.1	IOH27927	17729	0.97	7.46	1.09E-03	1%	3%	50 nM	8713	2.0	CHD2
BC024208.1	IOH12635	3825	0.85	1.41	2.24E-02	2%	5%	50 nM	1788	2.1	RBM23
BC036434.1	IOH62212	4915	0.88	1.34	4.02E-02	0%	4%	5 nM	1446	3.4	VRK2
BC053878.1	IOH28978	19734	0.60	6.25	2.69E-03	9%	7%	5 nM	3803	5.2	ZAP70
BC057783.1	IOH29271	6275	0.77	2.48	8.68E-03	6%	6%	50 nM	2768	2.3	SFRS2B
NM_001005158.1	IOH57124	16956	0.96	7.14	1.07E-03	1%	2%	50 nM	8020	2.1	SFMBT1
NM_001204.3		3430	0.42	0.81	4.17E-02	1%	12%	5 nM	841	4.1	BMPR2
NM_001396.2		7538	0.93	2.21	1.68E-02	1%	6%	5 nM	3478	2.2	DYRK1A
NM_002005.2	IOH21035	5454	0.48	1.52	4.08E-02	15%	9%	5 nM	1402	3.9	FES
NM_002031.2	IOH56137	9494	0.70	2.85	1.18E-02	9%	0%	5 nM	2768	3.4	FRK
NM_002031.2	IOH56137	3096	0.68	1.09	3.69E-02	6%	2%	50 nM	1315	2.4	FRK
NM_002904.4	IOH14621	2048	0.46	0.39	2.93E-01	9%	17%	5 nM	1003	2.0	RDBP
NM_003609.2	IOH4232	3524	0.47	1.27	3.02E-02	4%	2%	50 nM	984	3.6	HIRIP3
NM_004103.2		3888	0.81	1.00	6.82E-02	3%	11%	5 nM	664	5.9	PTK2B
NM_005197.2	IOH56874	7269	0.77	2.91	6.37E-03	6%	8%	50 nM	3354	2.2	FOXN3
NM_005902.1	IOH27044	5846	0.58	2.28	1.05E-02	2%	9%	50 nM	1420	4.1	SMAD3
NM_005902.1	IOH27044	4092	0.58	1.06	6.83E-02	10%	1%	5 nM	1181	3.5	SMAD3
NM_006399.2	IOH21626	6765	0.49	2.69	9.05E-03	12%	4%	50 nM	2400	2.8	BATF
NM_007026.1	IOH21130	5090	0.65	1.40	4.22E-02	8%	1%	5 nM	842	6.0	DUSP14
NM_007026.1	IOH21130	2328	0.39	0.75	7.20E-02	4%	1%	50 nM	673	3.5	DUSP14
NM_007067.3	IOH21942	7049	0.86	2.82	6.54E-03	3%	1%	50 nM	3298	2.1	MYST2
NM_014840.2	IOH36839	17020	0.68	5.35	3.45E-03	6%	10%	5 nM	4972	3.4	NUAK1
NM_015191.1	IOH45349	20216	0.83	6.09	1.17E-03	3%	2%	5 nM	3835	5.3	SNF1LK2
NM_015191.1	IOH45349	14831	0.84	6.21	1.49E-03	5%	4%	50 nM	3204	4.6	SNF1LK2
NM_015640.1	IOH22934	3931	0.74	0.96	3.38E-02	5%	16%	5 nM	1792	2.2	SERBP1 transcript variant 4
NM_015978.1		3943	0.53	1.46	2.19E-02	5%	11%	50 nM	1180	3.3	TNNI3K
NM_015978.1		4746	0.58	1.22	2.52E-02	11%	19%	5 nM	1788	2.7	TNNI3K

NM_016072.2	IOH10546	2213	0.40	0.70	7.53E-02	0%	2%	50 nM	986	2.2	GOLT1B
NM_016440.1		2296	0.51	0.47	2.14E-01	5%	4%	5 nM	1117	2.1	VRK3
NM_016542.2	IOH39650	13193	0.46	4.08	6.85E-03	17%	10%	5 nM	5936	2.2	MST4 transcript variant 1
NM_017588.1	IOH4895	2681	0.64	0.60	1.54E-01	5%	2%	5 nM	933	2.9	WDR5 transcript variant 1
NM_018328.1	IOH12893	2155	0.66	0.42	2.49E-01	5%	6%	5 nM	289	7.4	MBD5
NM_022073.1	IOH13327	6798	0.67	1.86	1.17E-02	9%	0%	5 nM	1423	4.8	EGLN3
NM_022073.1	IOH13327	23848	0.78	10.15	5.61E-04	3%	23%	50 nM	6431	3.7	EGLN3
NM_024039.1	IOH4379	3950	0.64	1.46	2.21E-02	6%	3%	50 nM	1310	3.0	MIS12
NM_078630.1	IOH37755	2307	0.49	0.74	7.78E-02	8%	11%	50 nM	930	2.5	MSL3L1 transcript variant 2
NM_152789.1	IOH21683	11585	0.80	4.80	2.47E-03	5%	3%	50 nM	940	12.3	FAM133B transcript variant 1
P3049		37904	0.81	12.27	6.91E-04	6%	6%	5 nM	17278	2.2	ABL1 transcript variant a
PV3500		14551	0.89	4.53	4.54E-03	3%	14%	5 nM	5279	2.8	CSNK1E transcript variant 2
PV3504		25678	0.71	8.22	1.59E-03	9%	0%	5 nM	8713	2.9	TBK1
PV3504		3472	0.84	1.25	3.08E-02	3%	45%	50 nM	1626	2.1	TBK1
PV3691		20367	0.69	6.13	1.22E-03	6%	21%	5 nM	940	21.7	ROCK1
PV3691		3144	0.88	1.11	3.27E-02	0%	1%	50 nM	1120	2.8	ROCK1
PV3810		9817	0.72	2.96	9.80E-03	1%	27%	5 nM	3634	2.7	MINK1 transcript variant 4
PV3824		4272	0.63	1.07	3.00E-02	9%	14%	5 nM	1922	2.2	GRK5
PV3836		15152	0.68	4.73	4.65E-03	10%	11%	5 nM	3204	4.7	IKBKB
PV3836		2695	0.89	0.91	4.53E-02	1%	15%	5 nM	990	2.7	IKBKB
PV3864		17281	0.91	5.16	1.60E-03	2%	9%	50 nM	8020	2.2	ABL1 transcript variant a; mutant E255K
PV3864		7745	0.65	3.12	5.81E-03	8%	8%	50 nM	3755	2.1	ABL1 transcript variant a
PV3869		4017	0.79	1.04	6.30E-02	2%	5%	5 nM	1654	2.4	KIT transcript

											variant 1; mutant T670I
›V4211		9708	0.47	2.93	1.13E-02	9%	5%	5 nM	2400	4.0	MAP4K2
›V4211		2293	0.78	0.74	6.62E-02	4%	23%	50 nM	647	3.5	MAP4K2
›V4792		26922	0.84	8.20	6.80E-04	5%	17%	5 nM	8002	3.4	SNF1LK2
›V4877		6157	0.71	1.75	2.83E-02	8%	20%	5 nM	1608	3.8	ACVR1
›V4883		2994	0.61	0.67	6.19E-02	8%	10%	5 nM	1079	2.8	ACVRL1
‹M_378350.2	IOH43457	4789	0.85	1.30	4.34E-02	2%	46%	5 nM	2344	2.0	LOC400027

Table S4. Putative SNRK targets identified in the human ProtoArray microarray.

		IVSd/ BW cm/g	LVIDd/BW cm/g	LVPWd/ BW cm/g	LVIDs/ BW cm/g	EDV/ BW ml/g	EDV/ BW ml/g	EF %	% FS	SVI ml/g
Baseline	WT-Fasudil	0.0038	0.0162	0.0035	0.0113	0.0061	0.0028	65.1820	30.8120	0.0038
	SEM	0.0001	0.0008	0.0004	0.0008	0.0004	0.0004	2.5973	1.7259	0.0003
	WT-Saline	0.0036	0.0152	0.0031	0.0102	0.0055	0.0017	67.8775	32.6525	0.0038
	SEM	0.0003	0.0007	0.0004	0.0007	0.0004	0.0001	2.1391	1.5991	0.0004
	cmcKO-Fasudil	0.0035	0.0175	0.0036	0.0131	0.0070	0.0031	55.5460	24.7720	0.0038
	SEM	0.0003	0.0009	0.0003	0.0006	0.0004	0.0003	3.4404	1.9683	0.0004
	cmcKO-Saline	0.0032	0.0158	0.0031	0.0118	0.0066	0.0029	50.6606	22.7046	0.0036
	SEM	0.0006	0.0026	0.0005	0.0019	0.0011	0.0006	8.7211	4.1137	0.0007
	p-value WT vs cKO	0.4390	0.0647	0.9133	0.0069	0.0029	0.0346	0.0044	0.0041	0.6616
4 Weeks	WT-Fasudil	0.0034	0.0173	0.0032	0.0120	0.0068	0.0026	65.5200	30.9580	0.0045
	SEM	0.0003	0.0010	0.0003	0.0007	0.0005	0.0003	1.2654	0.8764	0.0003
	p-value Baseline vs 4W	0.1731	0.0419	0.6962	0.2277	0.1285	0.7612	0.9258	0.9522	0.0977
	WT-Saline	0.0032	0.0150	0.0029	0.0104	0.0050	0.0017	64.7750	30.3450	0.0032
	SEM	0.0005	0.0008	0.0002	0.0006	0.0002	0.0002	1.0070	0.6997	0.0001
	p-value Baseline vs 4W	0.6045	0.7238	0.5379	0.4949	0.4890	0.8590	0.2120	0.2117	0.2558
	cmcKO-Fasudil	0.0036	0.0173	0.0031	0.0133	0.0064	0.0030	53.0000	23.2280	0.0035
	SEM	0.0003	0.0008	0.0002	0.0005	0.0005	0.0003	3.1926	1.7103	0.0004
	p-value Baseline vs 4W	0.7446	0.3887	0.3435	0.3304	0.0090	0.3337	0.3549	0.3536	0.3204
	cmcKO-Saline	0.0031	0.0176	0.0029	0.0142	0.0082	0.0046	44.5500	18.8950	0.0036
	SEM	0.0003	0.0005	0.0002	0.0004	0.0009	0.0009	5.6200	2.8531	0.0001
	p-value Baseline vs 4W	0.2214	0.0842	0.6275	0.0639	0.4938	0.1240	0.1827	0.1957	0.3698
	p-value WT Saline vs Fasudil	0.9247	0.0387	0.8162	0.0024	0.0116	0.0233	0.0122	0.0080	0.0672
	p-value cKO Saline vs Fasudil	0.7807	0.9981	0.8366	0.1761	0.5866	0.3612	0.0065	0.0038	0.0827

		LVD Mass ASE/ BW	IVRT msec	E m/s	e' m/s	E/e'	PAT msec	ET msec	PAT/ET	TVI cm
Baseline	WT-Fasudil	0.0297	10.6680	0.8400	0.0460	19.6333	20.1080	57.4940	35.3786	1.8700
	SEM	0.0017	0.3683	0.0475	0.0060	2.8415	1.2891	3.7072	2.5844	0.1727
	WT-Saline	0.0276	9.8875	0.8925	0.0450	19.9625	22.2700	58.3625	38.0623	2.1325
	SEM	0.0020	0.4827	0.0614	0.0029	1.3057	1.8250	2.3427	2.1493	0.1944
	cmcKO-Fasudil	0.0308	14.4460	0.9200	0.0300	32.9833	17.1400	52.9360	32.4109	1.3960
	SEM	0.0016	0.5700	0.0680	0.0045	4.2813	1.8103	2.7460	3.1585	0.1426
	cmcKO-Saline	0.0271	12.4015	0.8680	0.0286	29.4483	15.6255	47.9093	29.6675	1.3508
	SEM	0.0045	2.1918	0.1530	0.0053	5.3304	2.8885	7.9683	5.0607	0.2373
	p-value WT vs cKO	0.6408	0.0009	0.2123	0.0038	0.0008	0.0288	0.0827	0.1098	0.0060
4 Weeks	WT-Fasudil	0.0301	9.8840	0.8220	0.0380	22.0767	15.5560	50.8800	30.9007	1.9720
	SEM	0.0018	0.6332	0.0449	0.0037	1.2939	1.2965	2.1687	3.1973	0.1388
	p-value Baseline vs 4W	0.2628	0.4603	0.6198	0.3375	0.4641	0.1386	0.2669	0.4262	0.7531
	WT-Saline	0.0278	9.0025	0.7800	0.0325	23.8958	16.7650	50.4775	34.1109	1.5900
	SEM	0.0017	0.6718	0.0842	0.0025	1.2423	0.9554	3.9011	4.1712	0.1165
	p-value Baseline vs 4W	0.6799	0.2206	0.0272	0.0154	0.0648	0.1234	0.1105	0.5707	0.0221

cmcKO-Fasudil	**0.0306**	**12.4140**	**0.9600**	**0.0280**	**36.4500**	**16.1680**	**44.2920**	**36.6407**	**1.1560**
SEM	0.0016	1.2858	0.0674	0.0037	5.2192	1.0374	1.6690	2.5284	0.0604
p-value Baseline vs 4W	**0.3034**	**0.2376**	**0.6077**	**0.7040**	**0.6906**	**0.7079**	**0.0858**	**0.3898**	**0.1666**
cmcKO-Saline	**0.0285**	**13.2200**	**0.9600**	**0.0275**	**36.0833**	**17.5275**	**48.0825**	**36.6870**	**1.4175**
SEM	0.0018	1.0904	0.0280	0.0025	4.3999	0.8889	1.5983	2.7263	0.1521
p-value Baseline vs 4W	**0.3448**	**0.6803**	**0.7601**	**0.1817**	**0.3725**	**0.7515**	**0.4933**	**0.1937**	**0.4379**
p-value WT Saline vs Fasudil	**0.7930**	**0.0165**	**0.0887**	**0.2070**	**0.0372**	**0.5803**	**0.5906**	**0.6237**	**0.4026**
p-value cKO Saline vs Fasudil	**0.8538**	**0.1155**	**0.1267**	**0.0955**	**0.0282**	**0.7220**	**0.0427**	**0.1967**	**0.0007**

Table S5. Comparison of the Fasudil treatment between *Snrk* WT and Cardiomyocyte-conditional SNRK null mice (Snrk cmcKO) – ECHO Analysis. ECHO was performed on 6-4 month adult male and female *Snrk* WT and cmcKO mice. The mice received daily injections of saline or fasudil as described in the materials and methods. Left ventricular dimensions were normalized to body weight (BW) g, interventricular septal end diastole (IVSd) cm, left ventricular internal diameter end diastole and end systole (LVIDd, LVIDs) cm, left ventricular posterior wall end diastole (LVPWd) cm, end diastolic volume (EDV), end systolic volume (ESV) parameters, ejection fraction (EF) %, fractional shortening (FS) %, left ventricular mass in diastole (LVD Mass ASE) g, stroke volume index (SVI) ml/g, isovolumic relaxation time (IVRT) msec, early mitral inflow velocity (E) m/s / early diastolic mitral annular velocity (e') m/s, pulmonary acceleration time (PAT) msec, ejection time (ET) msec and time-velocity integral (TVI) cm. There were 2 males and 2-3 females per experimental group.