Material and Methods

Animals and Transverse Aortic Constriction (TAC)

Adult (12-15 weeks) high-expressing Line 720 SUR1-tg mice [1] and their WT littermates were used in this study. Kir6.2 KO mice [2] backcrossed for five generations into a C57BL/6 background were further crossed into a C57BL/6 background 2 additional times to generate heterozygous Kir6.2 mice as breeders. The homozygous Kir6.2 KO and wild type littermates produced were used for the study. PGC1α KO mice were generated as previously described [3]. All animal studies were performed according to a protocol approved by the University of Minnesota Institutional Animal Care and Use Committee. Severe TAC (using a 27G needle to calibrate the degree of aortic constriction) was performed in SUR1-tg mice as described [4, 5]. Previous studies demonstrated that severe TAC caused a high mortality rate in both Kir6.2 KO [6] and PGC1α KO mice; consequently, to allow a 6 week observation period in these strains, moderate TAC (using a 26G needle to calibrate the degree of aortic constriction) was performed. Echocardiography was performed while the mice were anesthetized with 1.5% isoflurane by inhalation.

Western Blots and Quantitative Real-time PCR

Primary antibody against ANP was from Peninsula Lab., Bachem AG; antibodies against PGC-1α, COXI, COXIII, UCP3, and SUR2A were from Santa Cruz Biotech; antibodies against cytochrome C, phos-Akt^{Ser472}, total-Akt, phos-FOXO1^{Thr24}, total-FOXO1 were from Cell Signaling, and antibody against CPT-1b was from Alpha

Diagnostics. HRP conjugated secondary antibodies were from BioRad Laboratories and Sigma.

Total RNA was reverse-transcribed using random hexamers and Moloney murine leukemia virus (MMLV) reverse transcriptase (Clontech). The real-time PCR reaction was carried out using the Light Cycler Thermocycler (Roche Diagnostics Corp). Primers are listed in Online Table II. Results were normalized to α-sarcomeric actin.

Cell Culture, Transfection, Reporter Gene Assay

Rat neonatal cardiomyocytes were isolated and cultured as described previously [7]. Reporter genes were transfected with Lipofectamine 2000 (Invitrogen) and SUR2 specific siRNA or ON-TARGETplus siCONTROL. Non-Targeting siRNA (Dharmacon) were transfected with DharmaFECT 3 reagent (Dharmacon) into neonatal cardiomyocytes. Forty-eight hours after transfection, cells were treated with 50μM glibenclamide or vehicle (DMSO) for 45 minutes and then subjected to 24 hours of hypoxia (1% O_2) followed by 7-8 hours of reoxygenation. After hypoxia/reoxygenation (H/R), cells were harvested for reporter assay or for real-time PCR and Western blot analysis.

Reporter constructs were generated by inserting 3.1Kb PGC-1α promoter [8] into pGL3B vector (Promega). The mutations were introduced into the wild type reporter using QuickChange Multi Site-Directed Mutagenesis Kit (Stratagene). LacZ reporter driven by CMV promoter was used as an internal control for transfection, and relative luciferase activity was determined by normalizing luciferase activity to the corresponding LacZ activity.

Preparation of Nuclear Extract

Frozen heart tissue was ground in liquid nitrogen into fine power and homogenized in hypotonic buffer. Rat neonatal cardiomyocytes were washed in DPBS (Invitrogen) and resuspended in hypotonic buffer. Then nuclear extract was prepared as described [9].

ChIP Assay

ChIP assay was performed as described [10]. DNA was purified from the immunocomplex precipitated with anti-FOXO1 antibody and subjected to real-time PCR. Chromatin solutions with non-specfic IgG were used as control for total input.

Statistical Analysis

Results are expressed as mean ± standard error of the mean (SEM). The number of samples in each group for each experiment was at least six or as indicated in the legends. An unpaired two-tailed Student's t-test was used to determine *p* values. A *p* value of ≤ 0.05 was considered significant.

References

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Online Table I. Disruption of K_{ATP} channel activity exacerbated LV dysfunction in response to TAC

	WT sham (n=10)	**WT TAC (n=10)**	**SUR1-tg sham (n=10)**	**SUR1-tg TAC (n=9)**
Body weight (g)	20.0±0.6	21.5±0.6	20.3±0.7	20.8±0.4
Heart weight (mg)	86.2±2.3	142.9±7.6	89.6±1.7	154.6±4.3
Lung weight (mg)	128.5±3.3	235.3±25.4	126.9±3.7	366.9±31.7*#
Heart rate (min^{-1})	516±12	468±19*	481±11#	449±20*
LV diameter systole (mm)	2.11±0.08	2.42±0.20	1.97±0.11	3.18±0.18*#
LV diameter diastole (mm)	3.33±0.06	3.78±0.11*	3.22±0.12	4.02±0.15*
Wall thickness systole (mm)	0.98±0.02	1.24±0.03*	0.99±0.01	1.23±0.03*
Wall thickness diastole (mm)	0.65±0.01	0.93±0.02*	0.70±0.01	0.97±0.02*
LV ejection fraction (%)	74.1±1.9	71.9±5.0	76.8±1.9	49.9±5.1*#
%FS	36.7±1.7	36.6±3.8	39.0±1.7	21.3±2.7*#

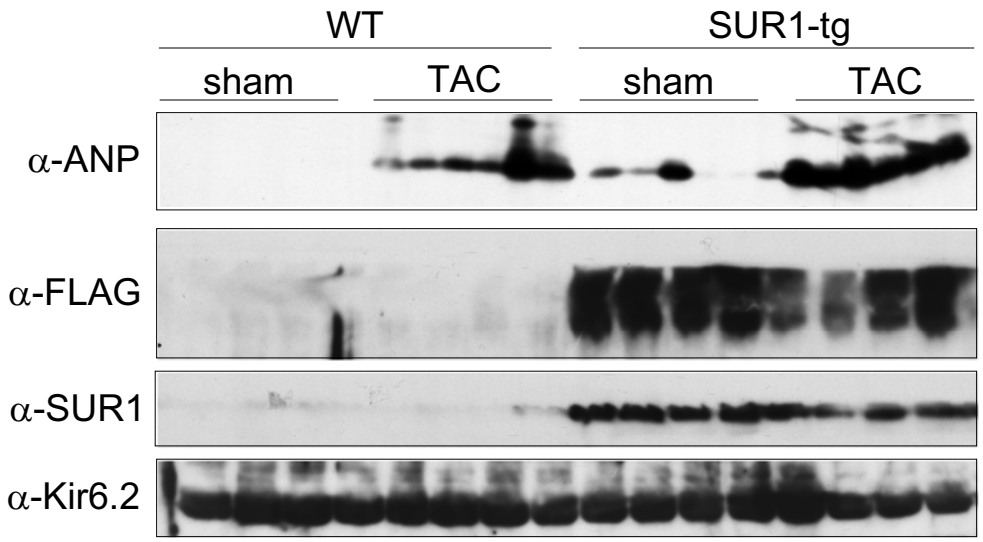
* $p<0.05$ versus sham, # $p<0.05$ versus WT littermate

Online Table II. Primers used in real-time PCR, ChIP assay and point mutation

	sense	antisense
Real-time PCR		
ANP	5'-TCGTCTTGGCCTTTTGGCT-3'	5'-TCCAGGTGGTCTAGCAGGTTCT-3'
BNP	5'-AGGGAGAACACGGCATCATT-3'	5'-GACAGCACCTTCAGGAGAT-3'
VLCAD	5'-ATGCAGTCGGCTCGGATGACCC-3'	5'-TGAGAAATTGTGCCTGTTCTTC-3'
VLCAD (rat)	5'-TCTTGTGCTTCATCAGCTTCG-3'	5'-TGAGTCGCAGTGGTGAACTGG-3'
MCAD	5'-CTAACCCAGATCCTAAAGTACCCG-3'	5'-GGTGTCGGCTTCCA AATGA-3'
CPT1b	5'-GAGTTCTCGATGGCTTTCCG-3'	5'-GACAGGACACTGTGTGGGTGAG-3'
CPT1b(rat)	5'-GCAGTTCCAGAGAATCCTCG-3'	5'-GCCCACTCTACCCTTCCTCCTG-3'
COX I	5'-TGAAACCCCCAGCCATAAC-3'	5'-CCAGCGGGATCAAAGAAAG-3'
COXIII	5'-CATCGTCTCGGAAGTATTTTT-3'	5'-ATTAGTAGGGCTTGATTTATGTGG-3'
PGC-1α	5'-AACTCAGCAAGTCCTCAGGGCT-3'	5'-TTAGTTCACTGGTCTTGTCTGA-3'
PGC-1α(rat)	5'-GTTCAAGGTCACCCTACAGCCG-3'	5'-CTTTCGTGCTCATTGGCTTCAT-3'
PGC-1β	5'-CACGGTTTTATCACCTTCCG-3'	5'-GCTCATTGCGCTTTCTCAGG-3'
ERRα	5'-TGGAGCGGGAGGAGTACGTC-3'	5'-CAGCCTCAGCATCTTCAATGTG-3'
ERRγ	5-CTCTGTGACTTGGCTGACCG-3'	5'-CCAGGGACAGTGTGGAGAAGC-3'
PPARα	5'-TTGTGGCCAAGATGGTGGCCAA-3'	5'-CAGTTCTAAGGCATTGAACTTC-3'
PPARγ	5'-CAAAGTAGAACCTGCATCTCC-3'	5'-CCTTCACAAGCATGAACTCC-3'
Tfam	5'-GTCCATAGGCACCGTATTGC-3'	5'-CCCATGCTGGAAAAACACTT-3'
NRF1	5'-GGCGGGAGGATCTTTTATATGCTTTTGA-3'	5'-GGCCTCTGATGCTTGCGTCGTCT-3'
ChIP Assay		
Site 1	5'-TCCCCATTGACTCAGGTACGAC-3'	5'-GTGCCCTGGGTTGTACAGTTTGTT-3'
Site 2	5'-ACTGCCAAGGAGACAGCTGATTTG-3'	5'-CCCTATTCCTTCCTTTCTTTCTTCCTTCC-3'
Site 3	5'-GGCAAGGGTGTAGCTACTGTGTCA-3'	5'-CCAGCTTTGAATGCCACCAACTCT-3'
Point Mutations		
5'-GATACCATTTCAGTGT*GGA*TCCTTCATTCCCTGGA-3' (sense)		
5'-AAACAAATGTGGCGGT*ACC*GTTGACTAAACATGGA-3' (sense)		
5'-TTGCTATTTGCCTGTT*CC*GGATGGAAAATAAATT-3' (sense)		

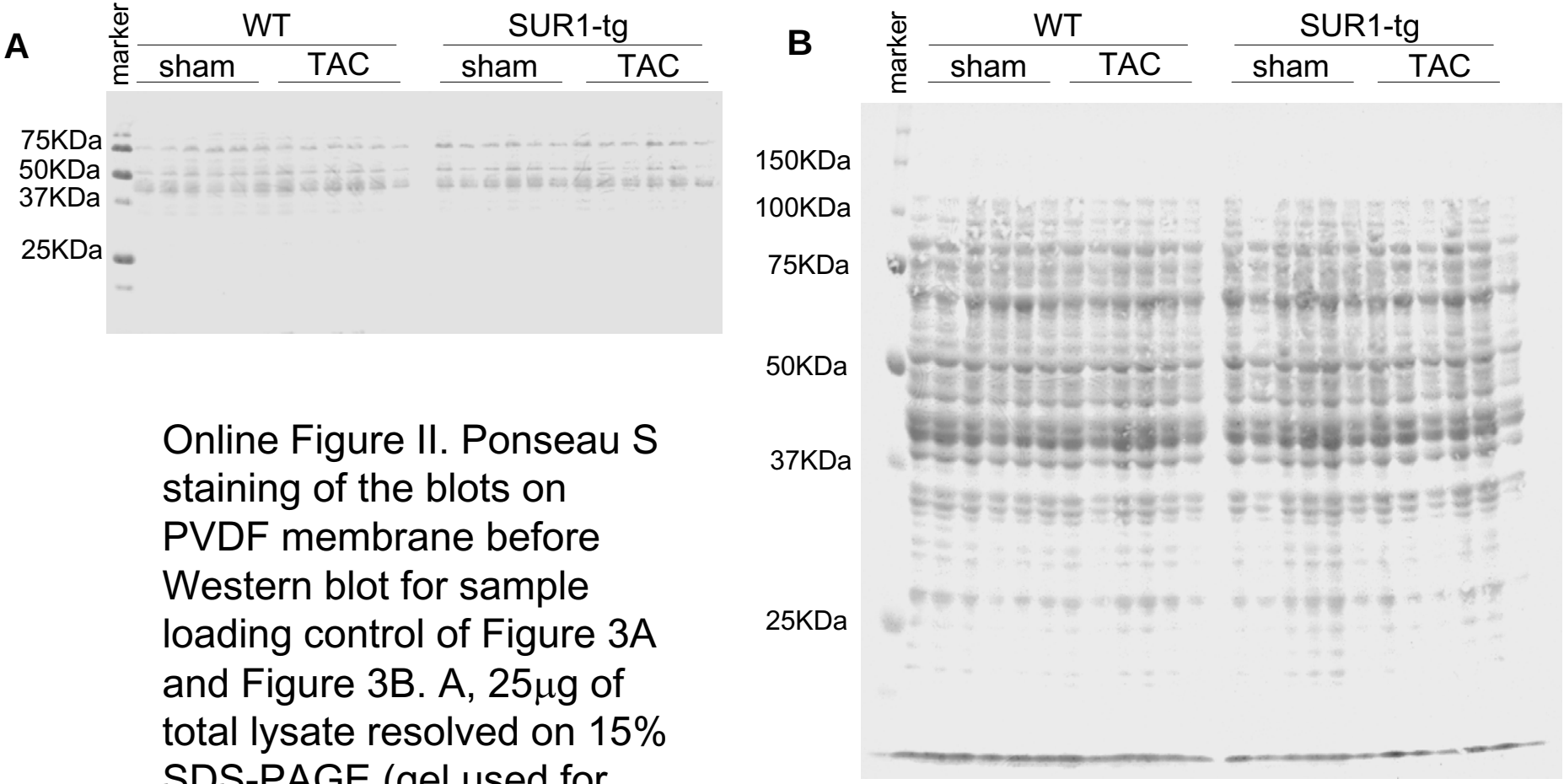
Primers were designed according to mouse cDNA or genomic DNA sequences if not specifically labeled. For point mutations, only sense primers were used for mutagensis of the reporter gene. The mutated nucleotides were in italic and underlined.

Online Figure I



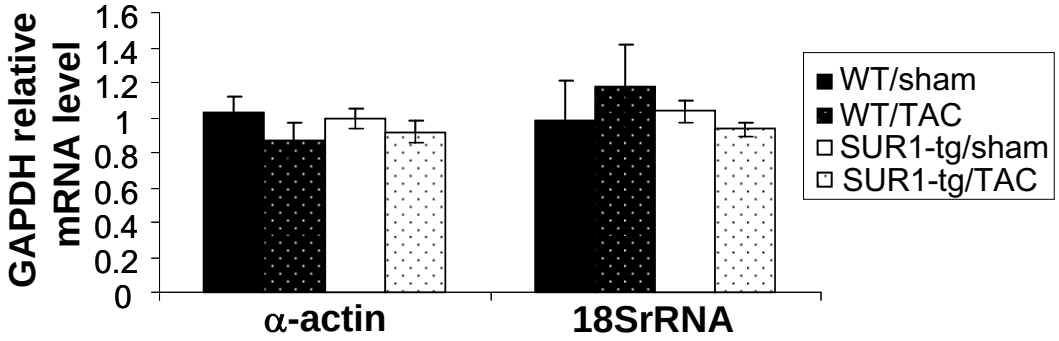
Online Figure I. Myocardial ANP expression was significantly increased in the SUR1-tg mice. The protein level of the pore forming subunit Kir6.2 was not changed in the SUR1-tg mice or in response to TAC.

Online Figure II



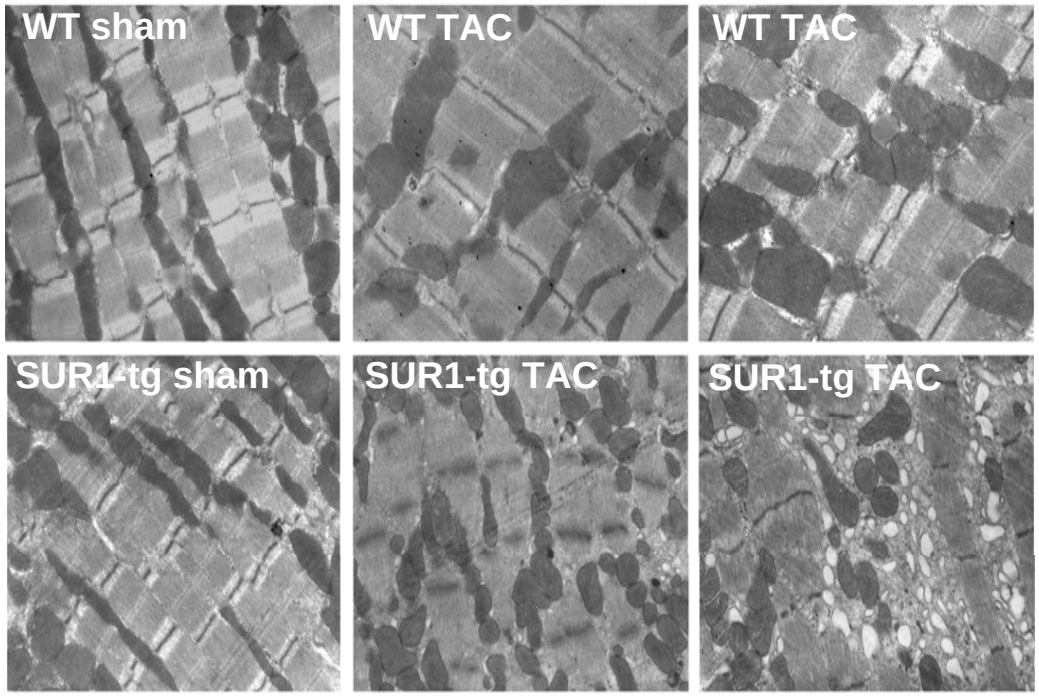
Online Figure II. Ponseau S staining of the blots on PVDF membrane before Western blot for sample loading control of Figure 3A and Figure 3B. A, 25μg of total lysate resolved on 15% SDS-PAGE (gel used for Figure 3A); B, 75μg of total lysate resolved on 10% SDS-PAGE (gel used for Figure 3B).

Online Figure III



Online Figure III. The mRNA levels of GAPDH in the SUR1-tg and WT mice after normalized to α-actin and 18SrRNA. The RT and real-time PCR results in this study were normalized to α-actin.

Online Figure IV



Online Figure IV. Following TAC the mitochondria volume density is not significantly different between the SUR1-tg and WT mice.