Supplementary Material

Methods:

Animal resources and genotype analysis

Cx43/KO and K258stop/KO littermates were obtained by crossing the previously described Cx43/KO (Theis *et al.,* 2001) and Cx43/K258stop mice (Maass *et al*., 2004), both backcrossed onto a 94% C57BL/6 background. Cx43/KO mice were initially obtained by breeding mice harboring a conditional, floxed *connexin43* allele with mice expressing the *Cre*-recombinase under control of the ubiquitous phosphoglycerate-kinase promoter (PGK-Cre). Genotypes were confirmed by polymerase chain reaction (PCR) analysis. To detect the *connexin43* wild-type or the *cx43K258stop* allele, forward primer delCT-HO (5'-GCATCCTCTTCAAGTCTGTCTTCG) and reverse primer RO-delCT (5'-CAAAACACCCCCCAAGGAACCTAG) were used, resulting in an 851-base pair amplicon for the *connexin4*3 allele and a 452-base pair amplicon for the *cx43K258stop* allele. The *cx43knockout* allele was detected by using the primer 43del-forward (5'-GGCATACAGACCCTTGGACTCC) and the primer 43del-reverse (5' TGCGGGCCTCTTCGCTATTACG), resulting in a 700-base pair amplicon.

Acute myocardial infarction and reperfusion of isolated perfused mouse hearts

Adult animals (3-6 months of age) were heperanized (9 units/mg) for 30 min and then brought under deep anesthesia (ketamin (116 mg/kg), acepromazine (11 mg/kg), xylazine (23 mg/kg)); hearts were rapidly excised and placed in oxygenated modified Krebs-Henseleit buffer (118.3mM NaCl, 2.7mM KCl, 1mM MgSO4 · 7H20, 1.4mM KH2PO4, 29mMNaHCO3, 3.4mM CaCl2, 10mM glucose, 70mU/L insulin and 2.8% BSA). Hearts were perfused retogradely by aortic flow at a preload of 55mmHg in an Isolated Heart Perfusion System (model IH-1, Type 844, IH-SR Isolated Heart for small rodents, Hugo Sachs Elektronik – Harvard Apparatus GmbH, March-Hugstetten, Germany) with oxygenated buffer at

37°C using a fiber oxygenator (Fiber Oxgenator Type 100HG, HSE, Harvard Apparatus, Holliston, MA).. Aortic flow rate and perfusion pressure were recorded (electromagnetic flowmeter head, Isotec pressure transducers), amplified and digitalized through the Digi Med System Intergrator software (DMSI Version 1.12, Micro-Med, Inc., Louisville, KY); data were monitored and recorded as waveforms using an Analog Signal Analyzer ^{TM} 400 (ASA, Micro-Med, Inc.) and a Blood Pressure Analyzer ^{TM} 400 unit (BPA, Micro-Med, Inc.). Surface of the hearts was kept moist with 37C perfusate throughout the experiment, additionallyt, temperature within the perfusion chamber was monitored; electrogram recordings were only inbcluded for timepoints were the chamber temperature was within +/- 0.5 degree centigrade of 37°C. After initial stabilization of 30 min, the LAD was occluded for one hour. In order for this occlusion to be reversible, a piece of PE-10 tubing was fixed to the end of 10-0 proplene suture. Occlusion knots were made on top of the PE-tubing, preventing injury to the epicard. Successful LAD occlusion was initially monitored by a rise in perfusion pressure and a drop in aortic perfusion flow. Subsequently, epicardial tissue in the occlusion area turned pale, verifying the ischemic insult. After one hour of occlusion, the knots were opened and reperfusion was allowed for 4 hours. Hearts were arrested in diastole by briefly perfusing with cardiopledgic solution (25 mM KCl, 5% dextrose in phosphate buffered saline). To distinguish between viable and infarcted tissue, hearts were perfused and incubated in 1% Triphenyltetrazolium chloride (TTC, in phosphate buffer, pH 7.4) for 10min at 37°C. Hereafter, the LAD was retied in the same place and the heart perfused with 1% Evans Blue (in dH20, filtered) to distinguish between areas perfused or not perfused during the LAD occlusion (Area at risk, AAR). The base and the right ventricular free wall were dissected off, the left ventricle was frozen at -20C for 2hrs and subsequently cut into coronal sections. Sections were fixed over night in 4% PFA. The next day, sections were weighed and the base-near sides were photographed.

Hearts in which the Evans Blue perfusion was not successful were omitted from the study.

Quantification of infarct size

After incubation with TTC, viable tissue was stained red, while necrotic (infarcted) tissue turned white. Additionally, the area perfused during the ischemic insult was stained blue by Evan's Blue dye. Accordingly, the area at risk (AAR) was defined as the area not stained by Evan's Blue dye. Left ventricular mass, mass of the AAR and mass of the infarct were quantified using Image J sofware. In brief, total mass of the left ventricle was measured by adding the weights of individual slices. Mass of the total AAR was measured as follows: Σ (area AAR_{i}/area LV_{i} * $weight_{i}$ + area AAR_{ii}/area LV_{ii} *$weight_{ii}$ …) with $_{i}$, $_{ii}$ … standing for slices of an individual left ventricle. The mass of the infarct was calculated accordingly; Σ (area $infarct_{i}$/area LV_{i} * $weight_{i}$ + area $infarct_{ii}$/area LV_{ii} + $weight_{ii}$ …). Infarct size was then expressed as percent of the mass of the left ventricle or the AAR.

Isolation of myocytes:

Adult mouse ventricular myocytes were obtained by enzymatic dissociation [17]. Briefly, mice were injected with 1 ml heparin (100 IU/ml i.p.) 20 min before heart excision and anesthetized by Carbondioxyd Inhalation. Hearts were quickly removed from the chest and retrogradely perfused through the aorta at a constant flow (3 ml/min) and 37°C for 4 min with a Ca2+-free buffer containing (in mmol/L): 113 NaCl, 4.7 KCl, 1.2 MgSO4, 0.6 Na2HPO4, 0.6 KH2PO4, 10 KHCO3, 12 NaHCO3, 10 HEPES, 10 2.3-butanedione monoxime (BDM, Sigma), 30 taurine, and 5.5 glucose. All solutions were filtered (0.2-µm filter) and equilibrated with 100% O2 for at least 20 min before use. Enzymatic digestion was initiated by adding Collagenase type II (Worthington) (773.4 u/ml), trypsin (0.14 mg/ml), and CaCl2 (12.5µmol/L) to the perfusion solution. After 4-5 min of digestion, the hearts were removed into a stopping buffer (perfusion buffer plus 10%fetal bovine serum 12.5 µmol/L and CaCl2). Ventricles

were cut into small pieces, and gently triturated with Pasteur pipettes. Ca2+ concentration was increased gradually to 1 mmol/L. Cells were used for electrophysiological recording within 8 hours after isolation.

Electrophysiology

Electrogram Recordings: An 8-polar electrophysiology catheter (CIB'ER MOUSE, NuMED, Inc., Hopkinton, NY) was introduced into the cavity of the left ventricle through the left atrial appendage. The two most distal electrodes were connected to a Pulsar 6i stimulator (FHC Inc, Bowdoinham, ME) and used for pacing; intra-cavity electrograms were recorded by two more proximal electrodes. Signals were digitalized through the Digi Med System Intergrator software (DMSI Version 1.12), monitored and recorded as waveforms with a Sinus Rhythm Analyzer ^{TM} 400 unit (SRA, Micro-Med, Inc.). For each heart, the respective electrodes were chosen in such a way that distinct deflections for atrial and ventricular activations could be recorded. After a stabilization period of 15 min, the pacing threshold for capture of ventricular activation was determined. A burst pacing series consisted of 18 S1 stimuli applied with an interpulse interval of 80, 60, 40 and 20 ms. Stimuli amplitude was set at 2.5x threshold. Each train of S1 stimuli was carried out 3 consecutive times for each cycle length (inter-train interval was 5 s). This protocol was also carried out at 15 (n=6, Cx43/KO hearts; n=5, K258stop/KO hearts), 30, 45 and 60min after LAD occlusion (n=12, Cx43/KO hearts; n=11, K258stop/KO hearts). Spontaneous and burst-pacing induced ventricular tachyarrhythmia (VT) were quantified and qualitatively analyzed regarding the morphology (mono- or polymorphic VT), the number of ectopic beats and the duration of individual episodes. VT was defined as a minimum of three consecutive ectopic beats. Electrophysiological data were analyzed offline using the Digi-Med Data Review software (DMSI Version 1.12). Cycle length (RR intervals) and QRS duration (from onset of ventricular activation to peak of second deflection, "S;") were measured from intra-ventricular electrograms. Cycle length was

averaged as the mean of 10 consecutive beats at 30 min intervals throughout the experiment. QRS duration was averaged from 5 clearly defined complexes within an one-second interval.

Dual Whole Cell Voltage Clamp Analysis: The dual whole-cell voltage clamp technique was used to record ventricular gap junctional currents, as previously described [18, 19]. Patch pipettes were filled with a solution containing cesium (in mmol/L: 130 CsCl; 0.5 $CaCl_2$; 10 HEPES; 10 EGTA; 2.0 Na_2ATP; 3.0 MgATP; pH 7.2). To acidify the intracellular space, the pipette solution was prepared as described above but HEPES was replaced by MES (10 mmol/L). The final pH of the internal pipette solution was 6.2. Junctional current was measured immediately after patch break and every 20 seconds thereafter. Whole cell junctional conductance (G_j)was calculated as described previously. [20] In brief, series resistance voltage drop across the patch electrode resistance is accounted for as a function of the respective whole cell current for each cell relative to the command potential.

Statistical Analysis

The ischemia-reperfusin study was conducted on 12 hearts per genotype. Smaller sample sizes for a subset of analyses were due to experimental limitations (infart study: Evan's Blue staining of perfused area successful in 8 of 12 hearts per genotype; spontaneous arrhythmia analysis: clear identification of atrial deflections in electrograms throughout entire experiment only possible in 7 of 12 hearts per genotype) or subsequent introduction of measurment timepoint (Burst pacing series at 15min of LAD occlusion). Statistical significance of incidences was assessed by Fishers's Exact Test. Statistical analysis of sample populations was performed using ANOVA with subsequent post hoc Tukey test (Origin Version 7.0; Origin Lab Corporation, Northampton, MA), if necessary, corrected for multiple measurements. Values are shown as mean ± SEM. Statistical significance was set at $p < 0.05$.

Significant diffrences ($p<0.05$) within the same genotype group (i.e.,compared to initial value) are indicated by the symbol#*;* significance between experimental groups is indicated by *. .

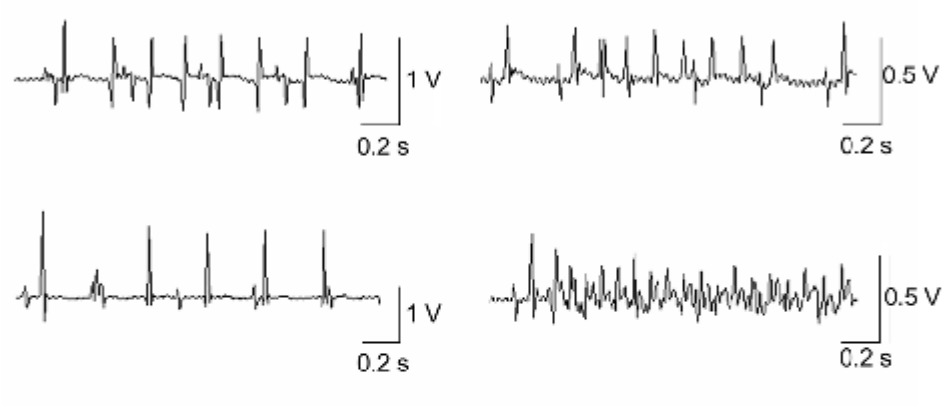
Supp Figure 1:

Spontaneous VT during reperfusion: Examples of short- and long-lived spontaneous VTs in Cx43/KO (left) and K258stop/KO hearts (right) during reperfusion. Horizontal bars: 200ms, vertical bars: 500mV, respectively.

Supp Figure 2:

Premature ventricular complexes (PVC). Premature ventricular complexes of representative Cx43/KO (left) and K258stop/KO hearts (right). Traces of one second duration are shown for early (first 10 min, top) and late reperfusion (forth hour, bottom). Horizontal bars: 200 ms, vertical bars: 1 V, respectively.

Supp. Figure 1



Supp. Figure 2