ONLINE SUPPLEMENT METHODS AND FIGURES

Tissue Harvest and Grid Mapping

In anesthetized dogs, the heart was excised and perfused through the coronary arteries with cold oxygenated cardioplegic solution (in mmol/L: NaCl 110, KCl 16, $MgCl_2$ 16, $CaCl_2$ 1.2, and $NaHCO_3$ 10). Epicardial (Epi) tissue sections (~1.5×1.5×0.1cm) were harvested from the posterior left ventricular (LV) free wall in a region devoid of coronary arteries and stored in cold cardioplegic solution for grid mapping followed by flash freezing for Western blotting, or formalin fixation for histologic & IHC analysis. High-density grid mapping on LV Epi was performed in oxygenized Krebs buffer (in mmol/L, NaCl 127, KCl 4, $CaCl_2$ 1.2, $MgCl_2$ 1.0, $NaHCO_3$ 22, NaH_2PO_4 0.9, and glucose 20 at pH 7.35, 37°C) to measure longitudinal CV (CV_L) and transverse CV (CV_T) *in vitro* with a specially-designed 14x16 (224-site) high-density grid-electrode array (4.2×5.6 mm, inter-electrode distance of 350μm; as described.[1]) At the end of the experiment (~10 min total recording time), mapped epicardial tissue sections were labeled with sutures to mark orientation and then formalin-fixed, paraffin-embedded, and sectioned (5 μm per slide) for immunohistochemical (IHC) and histology studies.

In each mapping procedure, one piece of tissue was superfused in a 37°C tissue bath for a total of approximately 30 min. To validate tissue viability of grid mapped LV tissue pieces over the course of electrical pacing and recording, isochronal maps of the electrical recordings obtained 15 min apart from the same tissue piece were compared. CV_L and CV_T measured at different time points (within 15 min) were calculated. We found CV_L and CV_T in both Ctl and HF LV tissue pieces were consistent throughout the recording period (Supplemental **Fig.S1**). Moreover, Western blot data demonstrated an

unchanged expression and dephosphorylation of Cx43 with up to 60min superfusion of grid-mapped tissue pieces (Supplemental **Fig.S2**).

Western blot and immunoprecipitation (IP)

Western blotting and IP were performed as described.[2, 3] Flash-frozen mapped LV tissues were homogenized in RIPA buffer (in mmol/L, Tris 25, NaCl 150, SDS 0.1%, sodium deoxycholate 0.5%, NP-40 1%, pH 8). After clearing by centrifugation, lysates (20μg) were subjected to Western blotting using specific antibodies. Primary antibodies were used for Cx43-T (BD Science), Cx43-NP (Invitrogen), PP1 (Abcam), PP2A (BD), and GAPDH (Chemicon). The proteins were visualized with ECL reagent (Thermo Scientific) and scanned with a Bio-Rad gel imaging system. The protein levels were quantified with the gel imager software and normalized with internal loading control GAPDH. Cx43-NP was probed first, and Cx43T was re-probed on the same membrane after stripping. For Cx43-T immunoblots, the lower faint band corresponded to the NP form. IP was done with specific antibody to Cx43-T (total Cx43 proteins, Chemicon). In brief, canine ventricular myocytes (1mg protein) were incubated with a monoclonal total Cx43 specific antibody overnight at 4°C (IgG alone was used as a negative control). Pulled-down total Cx43 proteins with co-immunoprecipitated PP2A were detected using PP2A specific antibodies.

Myocytes isolation

Canine LV myocytes were isolated as previously described.[3, 4] In brief, canine LV wedges were cannulated through LAD or circumflex artery and perfused with Ca-free normal Tyrorde's solution (in mmol/L: NaCl 140, KCl 4, $MgCl_2$ 1, HEPES 5, glucose 10,

pH 7.25 with NaOH) in a Langendorff perfusion apparatus (37°C) for 5 mins followed by collagenase digestion (0.75 mg/mL collagenase and 0.05% albumin) until the LV wedge became flaccid. LV tissue was finely dissected into 2 x 2 mm pieces for further incubation at 37°C if necessary. Incubations were stopped at 1-4 minutes by adding 40mL of 200 µmol/L normal Tyrode's solution with 0.05% albumin, and the tissue was filtered through gauze. Dissociated myocytes were then washed three times with 200 µmol/L Ca normal Tyrode's solution (with albumin) before using for experiments.

Action potential (AP) recordings and cellular data

Intracellular recording of AP's (CL=600 ms) from the LV posterior wall epicardial surface of Krebs-perfused Ctl and HF canine hearts was performed using a conventional microelectrode as described.[5] Cardiac myocytes were isolated from Ctl and HF hearts by enzymatic digestion of cannulated LV wedges[2] for microscopic measurement of resting cell length.

References

[1] M.L. Cohen, R.H. Hoyt, J.E. Saffitz, P.B. Corr, A high density in vitro extracellular electrode array: description and implementation, Am J Physiol 257(2 Pt 2) (1989) H681-9.
[2] X. Ai, S.M. Pogwizd, Connexin 43 downregulation and dephosphorylation in nonischemic heart failure is associated with enhanced colocalized protein phosphatase type 2A, Circulation research 96(1) (2005) 54-63.
[3] X. Ai, W. Zhao, S.M. Pogwizd, Connexin43 knockdown or overexpression modulates cell coupling in control and failing rabbit left ventricular myocytes, Cardiovasc Res 85(4) (2010) 751-62.
[4] X. Ai, S.M. Pogwizd, Connexin 43 downregulation and dephosphorylation in nonischemic heart failure is associated with enhanced colocalized protein phosphatase type 2A, Circ Res 96(1) (2005) 54-63.
[5] J. Huang, X. Zhou, W.M. Smith, R.E. Ideker, Restitution properties during ventricular fibrillation in the in situ swine heart, Circulation 110(20) (2004) 3161-7.

Supplemental Table 1

	Ctl	HF	*p*-value
N	7	10	
PR (ms)	92.5 +/- 3.6	109.9 +/- 4.4	0.01 (**)
QRS (ms)	46.2 +/- 2.7	70.6 +/- 3.7	0.0002 (***)
QT (ms)	187.7 +/- 5.9	199.0 +/- 2.2	0.06 (NS)
QTc (ms)	229.6 +/- 5.3	245.1 +/- 2.1	0.01 (**)

Table1. Summarized data of PR interval, QRS width, QT interval and QTc in 7 Ctl and 10 HF dogs. Abbreviation: control (Ctl), heart failure (HF).

Supplemental Fig. S1

Fig. S1 Validation of tissue viability during grid mapping study

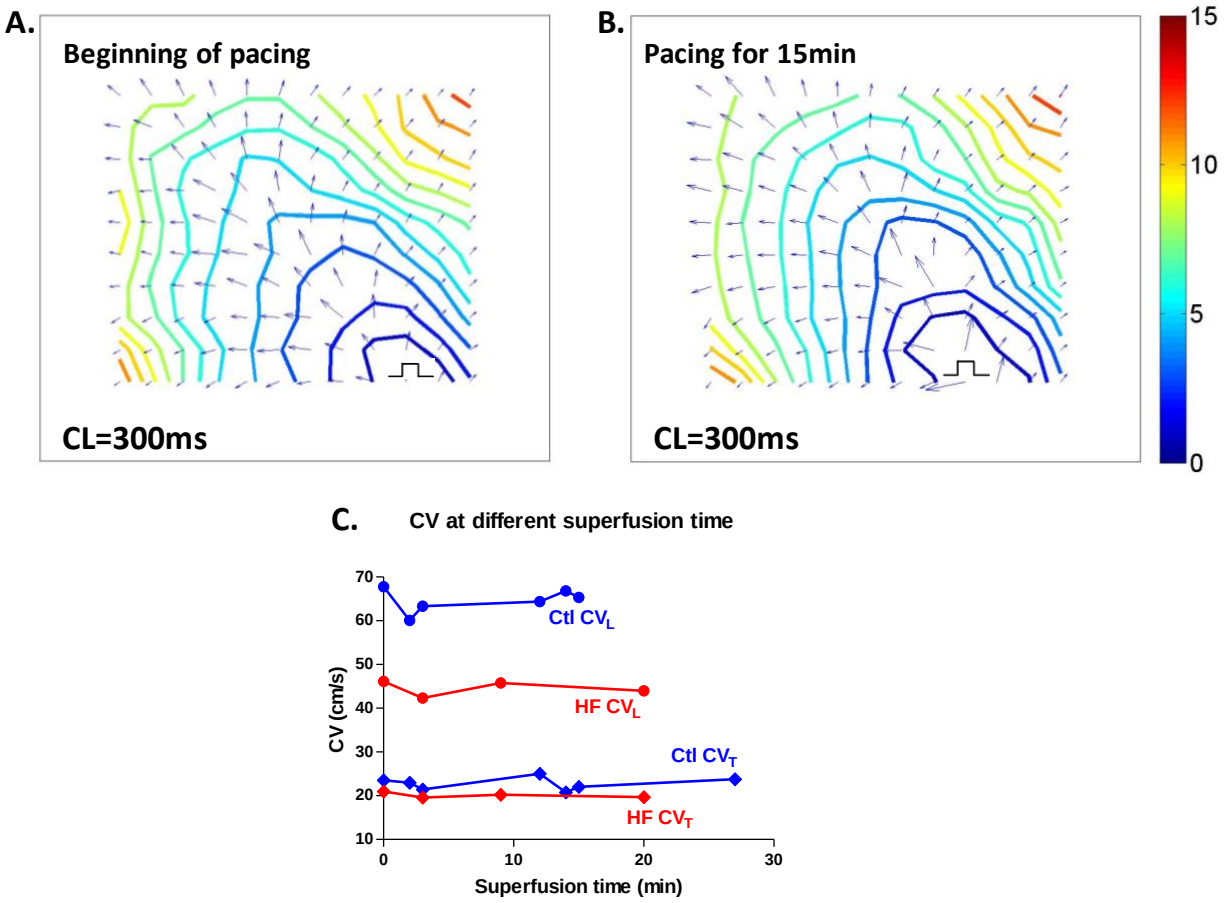

Fig.S1: Validation of tissue viability over the course of grid mapping. Isochronal maps generated from a representative LV Epi section at the beginning of the pacing (A), and 15 min after pacing started (B). Repeated CV measurements in representative LV Epi sections from Ctl and HF hearts demonstrating consistent CV_T and CV_L at different superfusion times (C).

Supplemental Fig. S2

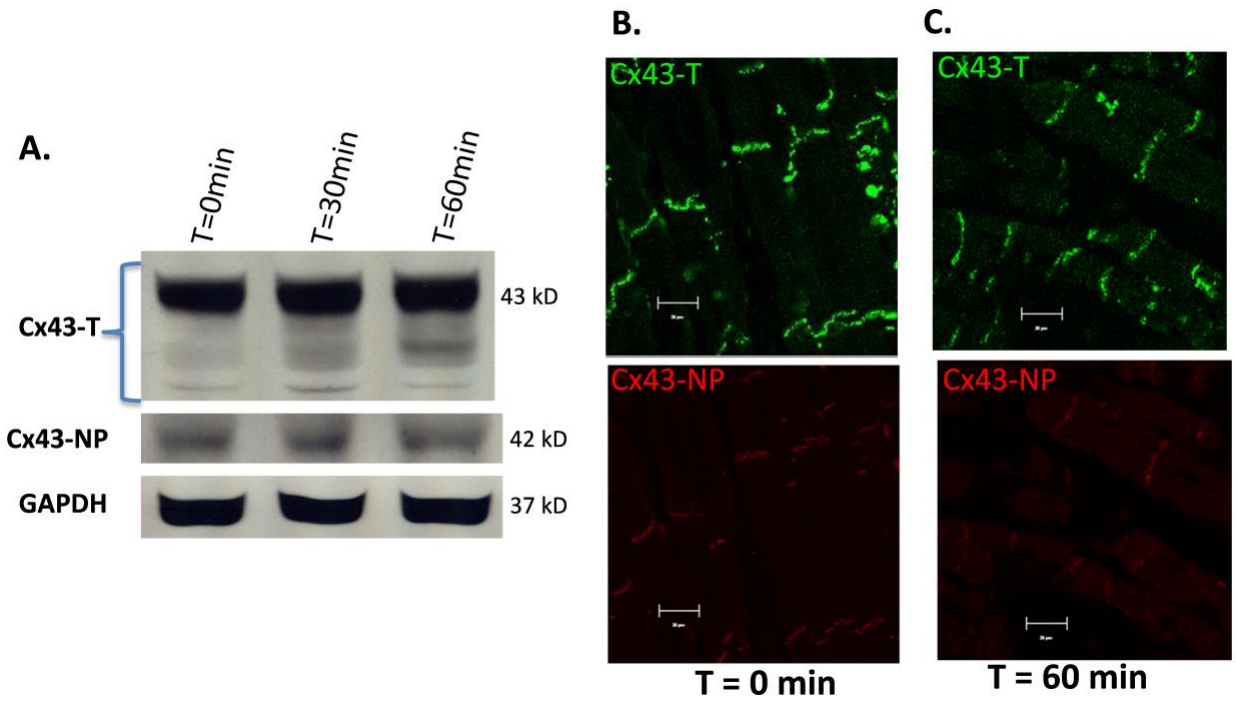

Fig. S2: Validation of Cx43-T and Cx43-NP expression in superfused LV tissue. (A). Western blot showing comparable levels of Cx43-T and Cx43-NP in mapped LV Epi tissue sections with 0 min, 30min, and 60min superfusion. Immunohistochemistry showed unchanged amount of Cx43-T and Cx43-NP in mapped LV tissue sections with 0 min (B) or 60 min (C) superfusion.

Supplemental Fig. S3

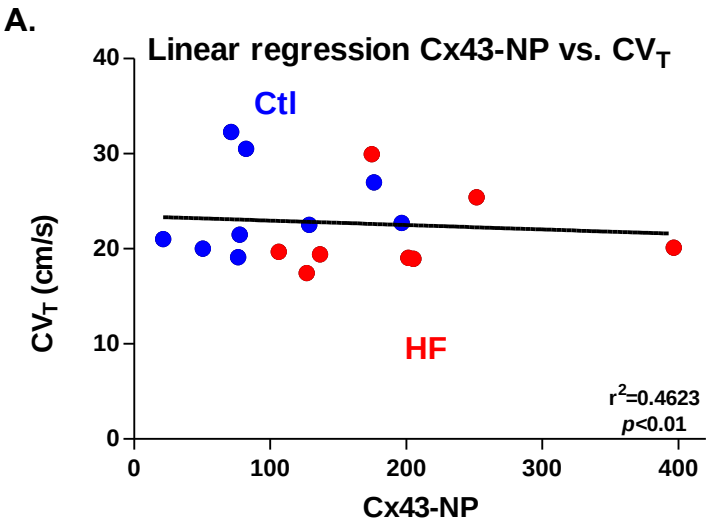

Fig.S3 Enhanced Cx43-NP is negatively correlated with slowed CVL by IHC in HF. A, Linear regression of Cx43-NP and CV_T. N_{animal}=9,8. p=NS