Antibodies and plasmids
The following antibodies were used in this study: Anti-Cx43 (Chemicon, MAB3068), Anti-ZO-1 (Zymed, 40-2200), Anti-Pan-Cadherin (Sigma, C1821), Anti-Caveolin-3 (BD-Transduction Loboratories, 610421), Anti-LC3 (Sigma, L8918), HRP conjugated Cholera Toxin B (Molecular Probes, C-34780). Human Cx43 was PCR amplified from plasmid DNA (a gift from Steven Taffet at SUNY Upstate, Syracuse, NY) and subcloned into the mammalian expression pcDNA3.1 vector (Invitrogen, V790-20). The EGFP-LC3 plasmid was created by Karla Kirkegaard (Stanford University) [1] and obtained through the Addgene plasmid repository (Addgene plasmid 11546, www.addgene.org).

Cell Culture
HeLa cells were maintained in DMEM supplemented with 10% FBS in a humidified incubator at 37°C and 5% CO_2. Cells plated on coverslips were transfected with plasmids using Lipofectamine 2000 (Invitrogen) according to manufacturer's specifications.

Canine Heart Failure Model
Mongrel dogs were rapidly paced into heart failure as previously described [2]. All procedures were approved by the Johns Hopkins Animal Care and Use Committee.

Neonatal rat ventricular myocyte (NRVM) isolation and culture
NRVMs were enzymatically dissociated from the ventricles of 2-day-old Sprague-Dawley rats (Harlan) with trypsin and collagenase (Worthington) as previously described[3]. NRVMs were maintained in a humidified incubator at 37°C and 5% CO_2 and transfected with plasmids using Lipofectamine 2000 (Invitrogen) according to manufacturer's specifications.

Immunofluorescent confocal microscopy
For immunohistochemistry the tissue was cut into 5-6 mm^3 cubes and fixed with 4% formaldehyde in PBS for 1 hour at room temperature. The tissue was infiltrated with 15% sucrose in PBS until it sank, followed by infiltration with 30% sucrose in PBS overnight at 4°C. The tissue was placed in cryomolds with OCT freezing medium and floated on isopentane cooled with dry ice. Ten micron sections were cut with a cryostat and adhered to microscope slides. For immunocytochemistry, and confocal microscopy HeLa cells or NRVMs were grown on coverslips, then fixed with 4% formaldehyde in PBS for 30 minutes. Fixed samples were washed with TBS followed by permeabilization with TBS and 0.1% Triton X-100 for 30 minutes. The samples were blocked with 5% non-fat dry milk in TBS -T for 2 hours at RT, incubated with primary antibodies, then washed and incubated with fluorescent secondary antibodies. The samples were washed extensively with TBS-T, and mounted with Pro-Long Gold Fluorescent Mounting Medium (Invitrogen), sealed with nail polish and imaged with an LSM-510 META confocal microscope system (Carl Zeiss). All confocal image analysis was carried out using ImageJ software. Control experiments were performed with secondary antibody only to confirm absence of non-specific secondary antibody binding (not shown).

Immuno-gold Transmission Electron Microscopy (TEM)
In preparation for immunogold TEM the tissue was minced into 1-2mm^3 cubes and fixed in 4% formaldehyde and 0.1% glutaraldehyde in PBS at room temperature for 1 hour. Fixed tissue was then infiltrated with10% bovine gelatin and placed at 4°C to solidify. The tissue was then cryo-preserved in 2.3M sucrose with 20% polyvinylpyrrolidone and frozen in liquid nitrogen. Thin cryosections were cut with a Leica Ultracut UCT ultramicrotome and placed on formvar film coated, glow discharged nickel grids. The cryosections were labeled with anti-Cx43 primary antibody followed by goat anti-mouse colloidal gold conjugated secondary antibody. The grids were then contrasted and stained by the PVA-embedding method. Grids were imaged with a

Hitachi 7600 transmission electron microscope operated at 80kV. Control experiments were performed with secondary antibody only to confirm absence of non-specific secondary antibody binding (not shown).

Conventional TEM

Conventional TEM was performed with tissue that was minced into 1-2 mm^3 cubes and fixed with 2% glutaraldehyde in 0.1M cacodylate buffer for 1 hour at RT. The tissue was postfixed with 2% osmium tetroxide (OsO_4) for 1 hour at 4°C then stained with 2% uranyl acetate for 30 minutes at RT. The tissue was progressively dehydrated with a series of ethanol washes (50%, 70%, 90%, and 100% EtOH) followed by dehydration with propylene oxide followed by infiltration with 1:1 propylene oxide:Epon with 1.5% DMP-30 for 1 hour. The tissue was then infiltrated with Epon overnight, followed by 3 more incubations with fresh Epon for 2 hours each, then placed in fresh Epon and baked at 60°C for 2 days. The tissue was cut into 70 nm sections with an ultramicrotome, placed on TEM grids, stained with lead citrate, and imaged with either a Hitachi 7600 or Philips CM120 transmission electron microscope operated at 80kV.

Lipid raft fractionation

Frozen cardiac tissue was homogenized in 10 volumes (vol/wt) of ice cold MES buffered saline (MBS) with 1% Triton X-100 plus phosphatase, kinase, and protease inhibitors. Homogenates were maintained on ice for one hour then mixed 1:1 (vol:vol) with 80% sucrose in MBS (final sucrose concentration is 40%) and 1 mL was placed at the bottom of an ultracentrifuge tube with 6 mL of 38% sucrose in MBS, followed by 4 mL of 5% sucrose in MBS layered on top. The samples were centrifuged at 39,000 rpm in an SW41 rotor for 18 hours with no braking. Serial 1 mL fractions were collected.

Western and dot blotting

For dot blotting, aliquots of sucrose fractions were mixed 1:1 (vol:vol) with 2X TBS-Tween20 and incubated at 37°C for 30 min. Equal volumes of each fraction were blotted onto nitrocellulose membranes using a BioRad BioDot Filtration System (Bio-Rad). For western blotting, aliquots of sucrose fractions were precipitated by TCA/acetone precipitation. Precipitates were pelleted and resuspended in 2% SDS and protein concentrations were determined by the BCA assay (Pierce). Dephosphorylation was performed by resuspending the precipitate in RIPA buffer (50mM Tris pH8, 150mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) and incubating with or without alkaline phosphatase (AP) (New England Biolabs) overnight at 37°C. Samples were prepared in Laemmeli buffer for SDS-PAGE. Proteins were transferred onto 0.45 µm nitrocellulose membranes using an iBLOT apparatus (Invitrogen). Equal protein loads were confirmed by Ponceau-S stain. Western blotting was performed using standard procedures and visualized with chemiluminescent substrate and X-ray film. Signals were quantified with Progenesis software (Non-Linear Dynamics). Control experiments were performed with secondary antibody only to confirm absence of non-specific secondary antibody binding (not shown).

Statistical analysis

For comparison of the frequency of lateralized gap junctions between normal and failing hearts, statistical significance was determined by a proportion test of the mean frequencies. Statistical significance of Western blot data was determined using unpaired, two-tailed Student's T-tests. Error bars represent standard deviation of the mean.

1. Jackson WT, Giddings TH, Jr., Taylor MP, Mulinyawe S, Rabinovitch M, Kopito RR, Kirkegaard K. Subversion of cellular autophagosomal machinery by RNA viruses. *PLoS Biol*. 2005;3:e156.

2. Akar FG, Nass RD, Hahn S, Cingolani E, Shah M, Hesketh GG, DiSilvestre D, Tunin RS, Kass DA, Tomaselli GF. Dynamic changes in conduction velocity and gap junction properties during development of pacing-induced heart failure. *Am J Physiol Heart Circ Physiol*. 2007;293:H1223-30.
3. Iravanian S, Nabutovsky Y, Kong CR, Saha S, Bursac N, Tung L. Functional reentry in cultured monolayers of neonatal rat cardiac cells. *Am J Physiol Heart Circ Physiol*. 2003;285:H449-56.