Methods:

Tissue procurement: Explanted human ventricular tissue from Nonfailing (NF) human hearts were obtained from unused organ donors not implanted for technical reasons (Adult: n=25, Age: 49±3 yrs, EF: 62±2%; Pediatric: n=12, Age: 6±1 yrs, EF: 49±6%). Failing hearts were obtained from cardiac transplant recipients with advanced nonischemic IDC (Adult: n=22, Age: 48±2 yrs, EF: 13±2%; Pediatric: n=31, Age: 4±1 yrs, EF: 24±2%). The EFs were significantly lower in each age-matched disease group (both $p<0.0001$). As this is an ongoing tissue bank and some pediatric hearts are quite small, not all assays were performed on all samples.

At the time of cardiac transplantation, the explanted hearts were immediately cooled in ice cold oxygenated Tyrodes in the operating room. The LV was rapidly dissected, flash frozen and stored at -80^0C until further use. For all subsequent studies adult and pediatric samples were handled in an identical fashion. The study was approved by an institutional review committee and the subjects gave informed consent.

Antibodies: PLB Ser16 (A010-12) and Thr17 (A010-13) antibodies were purchased from Badrilla. PLB total (05-205) antibody was purchased from Millipore. αMyHC antibody was a gift from Dr. Eva Van Rooij (Miragen, Inc.). P-CaMK, Sp1 and GAPDH antibodies were purchased from SCBT and CaMKIIδ antibody was a gift from Dr. Ginnan Roman (Albany Medical College). Calnexin antibody was purchased from Abcam (AB 13504). The HRP (115-035-146) anti-mouse and anti-rabbit were purchased from Jackson Laboratories.

β-adrenergic receptor density: All membrane preparations were subjected to three wash steps during isolation to remove any bound therapeutic agents. The membrane protein concentration was between 100 and 300 μg/ml and the buffer for [^{125}I]CYP binding was 150mM NaCl, 20mM Tris pH 7.5 with 1mM ascorbic acid. Binding displacable by 1μM (-)-propranolol

was considered specific binding. Experiments were conducted at 30^0C for 120 minutes. The percent of β_1-AR was determined using 1 μM CGP20712A which binds exclusively to β_1-AR and subtracting the percentage of β_2-AR binding from total specific binding. To determine the levels of β_1-AR and β_2-AR subtypes, betaxolol–ICYP competition curves were performed.

Real Time PCR (RT-PCR): Total RNA was extracted by mirVana^{TM} kit (Ambion). 0.5 μg of RNA were reverse transcribed into cDNA using I-script (Bio-Rad). Typically, 0.1 ng of cDNA, 12.5 nM of each primer and Power Syber Green PCR Master Mix (ABI) were used in the RT-PCR reactions. Reactions were performed using the ABI7300 system. The primers are listed below.

Primers for RT-PCR:

18S F- 5' GCCGCTAGAGGTGAAATTCTTG

18S R- 5' CTTTCGCTCTGGTCCGTCTT

Connexin43 (Cx43) F-5'AGTTCAATCACTTGGCGTGACTTCACTA

Cx43 R-5'CCTGGGCACCACTCTTTTGCTTA

BNP F-5'ATGGTGCAAGGGTCTGGCT

BNP R-5'TCTTAATGCCGCCTCAGCA

αMyHC F-5'CCTCGGGAACCTCACTCTT

αMyHC R-5'GGCACTCATATTTATTACAGGTTGG

βMyHC F-5'GCCACATCTTGATCTGCTCA

βMyHC R-5'CCTCCCAAGGAGCTGTTACA

SERCA F-5'CCAGTGGCTGATGGTGCT

SERCA R-5'ACTTGAGCGTCTCATCCATG

ANP F-5'AATCCCATGTACAATGCCGTG

ANP R-5'TCTTCCAAATGGTCCAGCAAA

β_1-AR F-5'GGGCATCATCATGGGCGTCTT

β_1-AR R-5'TTCACCACGTTGGCCAGGAAG

β_2-AR F-5'CAAGTACCAGAGCCTGCTGACCAA

β_2-AR R-5'GGAGGTAAGGCCTGACACAATCCA

PP2A F-5'GCC TTG GTG GAT GGG CAG ATC TT

PP2A R-5'ATG GCG AGA GAC CAC CAT GTA GAC

PP1β F-5' GGA GGT TTC CCA CCA GAA GCC AA

PP1β R-GCT TTC CTC TGT CCA CAT AAT CTC CT

Western blots: Antibodies were diluted 1:15000 (PLB), 1:1000 (p-CaMK, Sp-1 and CaMKIIδ), 1:5000 (GAPDH) and 1:50 (αMyHC) in 1X TBS (20mM Tris 500mM NaCl pH 7.5) containing 3% BSA and 0.1% tween and incubated with the blot overnight at 4^0 C.

cAMP assay: Left ventricle tissue was homogenized by Polytron (Model PT 1200E) on ice for 2 x 10 seconds in 10 volumes of 1X Cell Lysis 5 Buffer from the Parameter Cyclic AMP Assay Kit (KGE002) from R&D Systems. The homogenate was centrifuged at 4^0C for five minutes at 20,800 x g. The supernatants were extracted and snap frozen in liquid nitrogen and stored at -80ºC. Protein assays were performed on the supernatants using the Pierce BCA Protein Assay (23225). Results are presented as a relative comparison of pediatric or adult non-failing to failing patients. cAMP levels in non-failing subjects were arbitrarily defined as 100%.

Statistical Analysis: All continuous data were reviewed for normality using the Shapiro-Wilk method [1]. Data not meeting criteria for normal distribution were log transformed and retested prior to analysis. Disease and age comparisons were performed by Student's t-Test (two-sided) for all experimental outcomes. 2-way Analysis of Variance (ANOVA) was performed on all outcomes except for mRNA including all groups to evaluate for interactions.

1. Shapiro SS, Wilk, M.B. An analysis of variance test for normality *Biometrika*. 1965;52:591-611

Table S1: Pediatric Subject Characteristics and Analyses

ID	Sex	Race	Ethnicity	Group	Age at onset of HF	Age at tissue collection	EF/FS	mRNA	PPRT	βAR	cAMP	CAMK WB	PLB WB	αMHC WB	Inotrope	Digoxin	ACEI	Beta-Blocker	Diuretic	Amiodarone
NF																				
1	F	NA	NA	NF		1	EF 49%	X	X	X	X	X	X	X	X					
2	M	NA	NA	NF		1	NA	X			X		X	X	NA					
3	F	NA	NA	NF		2	EF 27%	X		X			X	X	X					
4	F	NA	NA	NF		3	EF 57%		X	X	X	X	X	X	X					
5	M	NA	NA	NF		7	EF 63%	X	X	X	X	X	X	X	X					
6	M	NA	NA	NF		7	NA	X	X	X	X	X	X	X	NA					
7	F	NA	NA	NF		8	NA		X	X	X	X	X	X	NA					
8	F	NA	NA	NF		9	EF 61%	X	X	X	X	X	X	X	X				X	
9	M	NA	NA	NF		9	NA				X		X	X	X			X		
10	M	NA	NA	NF		10	NA	X					X	X	NA					
11	F	NA	NA	NF		11	NA	X	X	X	X	X	X	X	X					
IDC																				
13	M	White	NH	IDC	Birth	0.1	EF 20%	X					X	X		X			X	
14	F	NA	NA	IDC	Birth	0.1	EF 10%	X							X					
15	F	White	NH	IDC	Birth	0.2	EF 9%				X		X	X	X		X		X	
16	F	White	NH	IDC	Birth	0.3	EF 21%	X			X		X	X	X		X		X	
17	F	NA	NA	IDC	0.5	0.8	EF 8%	X								X	X		X	
18	F	NA	NA	IDC	NA	0.8	EF 35%	X	X	X	X		X	X		X	X		X	
19	F	NA	NA	IDC	0.6	0.9	EF 30%	X			X				X	X	X		X	
20	M	White	NH	IDC	0.6	1	EF 16%	X			X		X	X	X	X	X		X	
21	M	White	H	IDC	Birth	1	EF 15%	X								X	X		X	X
22	F	White	H	IDC	1	1	FS 14%	X							X				X	
23	F	White	H	IDC	1	1	FS 16%	X							X	X	X		X	
24	M	White	H	IDC	0.7	1	EF 17%				X		X	X	X		X		X	
25	M	White	H	IDC	1	2	EF 28%	X							X	X			X	
26	F	White	H	IDC	0.3	2	EF 41%	X	X		X	X	X	X	X	X	X		X	
27	M	White	NH	IDC	2	3	EF 15%	X	X	X	X	X	X	X			X		X	X
28	M	Asian	NH	IDC	2	3	EF 14%	X	X	X	X	X	X	X	X	X	X		X	
29	M	Asian	NH	IDC	1	3	EF 13%	X			X		X	X	X		X		X	
30	F	White	H	IDC	1	3	FS 16%	X	X	X	X	X	X	X	X	X	X	X	X	
31	F	Asian	NH	IDC	4	4	EF 28%	X		X	X	X	X	X	X		X		X	
32	F	White	NH	IDC	1	4	EF 32%	X	X							X	X		X	
33	F	Asian	NH	IDC	2	4	EF 13%	X	X	X	X	X	X	X			X		X	
34	F	White	NH	IDC	3	4	EF 12%	X		X	X	X	X	X	X		X			
35	M	White	H	IDC	3	4	EF 15%	X			X		X	X	X	X	X	X	X	
36	M	White	NH	IDC	2	5	FS 16%	X	X	X	X	X	X	X			X			
37	F	White	NH	IDC	Birth	9	EF 34%	X					X	X	X		X	X	X	X
38	F	Asian	NH	IDC	NA	9	NA	X	X	X			X	X	X					
39	F	White	NH	IDC	Birth	10	EF 30%	X		X			X	X	X		X		X	
40	F	White	H	IDC	3	10	FS 22%	X		X					X	X	X	X	X	X
41	F	White	H	IDC	3	11	EF 15%	X		X	X		X	X			X		X	X
42	F	White	NH	IDC	11	12	EF 25%	X							X				X	
43	M	White	NH	IDC	12	12	FS 11%	X			X		X	X	X	X	X	X	X	
44	M	White	NH	IDC	12	12	FS 11%	X			X		X	X		X	X		X	
45	F	White	H	IDC	12	12	EF 25%	X		X			X	X	X		X		X	
NF Mean	**50% Male**					**7±4**														
IDC Mean	**38% Male**					**5±4**									**70%**	**48%**	**82%**	**15%**	**88%**	**15%**

NH – Non-hispanic, H – Hispanic, EF – Ejection Fraction, FS – Fractional Shortening, NA – Not Available

Table S2: Adult Summary Table

Adult	Sex	Age at tissue collection	EF/FS	Beta-Blocker	Inotropes	ACEI
NF Mean	50% Male	49	EF 70%	33%	85%	23%
IDC Mean	65% Male	48	EF 13%	25%	35%	70%