Supplemental Material

Generation of Human MYBPC3 *Expressing Adenovirus (adWT)*:

Full length human *MYBPC3* cDNA in pCMV-SPORT6 (pCMV-*MYBPC3*^{WT}) was obtained from Open Biosystems™. The full-length cMyBP-C open reading frame was subsequently PCR-amplified using *Pfu*Ultra (Agilent Technologies) according to the manufacturer's protocol using the following primer sequences: h*MYBPC3* C0 Myc F - CACCATGGAACAAAAACTTATTTCTGAAGAAGATCTGATGCCTGAGCCGGGGAAG and h*MYBPC3* C10R – TCACTGAGGCACTCGCACCTCCAGG. During amplification, a Myc epitope tag was added inframe with the *MYBPC3* coding region, allowing for subsequent production of N-terminally Myc-tagged wild type cMyBP-C. The 3,855 bp PCR product thus produced was subsequently cloned into the pENTR™/D-TOPO entry vector (Invitrogen) according to the manufacturer's protocol. Following characterization by DNA sequencing, the Myc-tagged human cMyBP-C encoding inserts were subcloned into the pAd/CMV/V5-DEST adenoviral shuttle vector (component of the ViraPower Adenoviral Expression System, Invitrogen), using the Gateway LR Clonase recombination system (Invitrogen) to form the pAdWT plasmid. adWT adenoviral particles were produced using the ViraPower™ Adenoviral Expression System (Invitrogen), according to the manufacturer's protocol. Briefly, the pAdWT plasmid was digested with *Pac*I (New England BioLabs) to expose the left and right viral inverted terminal repeat sequences prior to transfection into the 293A cells using Lipofectamine™2000 (Invitrogen) according to the manufacturers' protocol. Viral particles were harvested from 293A cells by repeated rapid freeze-thaw, 18 days post-transfection. The crude, low titer adWT-containing lysates were subsequently used to re-infect fresh 293A cells, from which adWT was harvested, purified and titred according to the manufacturer's protocol (Invitrogen).

Generation of adC^{-} Adenovirus

A control adenovirus (adC^{-}) was generated from a pENTR™/D-TOPO entry vector (Invitrogen) into which the full length cMyBP-C open reading frame was inadvertently cloned in the opposite orientation. adC^{-} is predicted to lack any significant open reading frame and was generated and tittered using protocols identical to that used to generated adWT. The effect of adenoviral transduction on contractile kinetics was subsequently assessed in ECT generated from cMyBP-C$^{-/-}$ cells transduced with adC^{-} at an MOI of 20.

Adenoviral Transduction of Cardiac Cells in Two-Dimensional (2D) Primary Culture:

In order to assess adenoviral transduction efficiency, WT and cMyBP-C$^{-/-}$ neonatal mouse ventricular cardiac cells suspended in mouse media (60.3% high glucose DMEM (Gibco); 20% F12 nutrient mix (Gibco) supplemented with 1 mg/mL gentamicin (Sigma), 8.75% fetal bovine serum (HyClone®), 6.25% horse serum (HyClone®), 1% HEPES (Sigma), 1x non-essential amino acid cocktail (Gibco), 3 mmol/L sodium pyruvate (Gibco), 0.00384% (w/v) $NaHCO_3$ (Gibco), 1 µg/mL insulin (Sigma)) were plated in 12-well flat bottom tissue culture plates (Falcon) at a density of 2 x 10^5 CMs per well. CMs were allowed to adhere to the surface of the dish for 48 hours prior to transduction with adWT at a multiplicity of infection (MOI) of 0, 5, 10, 20, 50 and 100. The adenovirus-containing media was removed and replaced with mouse

culture media 24 hours post-transduction and cells cultured for an additional 48 hours prior to harvesting of RNA or protein.

RNA Extraction and qRT-PCR:

Total RNA was extracted from WT and cMyBP-C$^{-/-}$ hearts, WT, cMyBP-C$^{-/-}$ and cMyBP-C$^{-/-}$ adWT ECT, as well as WT CM transduced with adWT at MOI of 0, 5, 10, 20, 50 and 100 in 2D culture using standard protocols. Briefly, tissue/cells were homogenized in TriZol reagent (Invitrogen) according to the manufacturer's protocol. Following addition of an appropriate amount of chloroform (Sigma), mixing, incubation and centrifugation according to the TriZol reagent protocol, the RNA containing aqueous phase was collected and treated with *DNase*I (RNase-free DNase set; Qiagen) for one hour. RNA was subsequently purified using the RNeasy® mini kit (Qiagen), according to the manufacturer's protocol. First strand cDNA synthesis was performed using the SuperScript III cDNA synthesis kit (Invitrogen) with minor modifications to the manufacturer's protocol. In order to facilitate full length first strand cDNA synthesis of most transcripts, samples were incubated for 90 minutes at 55^{o}C prior to heat inactivation of the reverse transcriptase at 70^{o}C for 15 minutes, oligo dT priming and 200 to 500 ng of total RNA as template.

Expression levels of human *MYBPC3*, mouse *Mybpc3*, mouse *Nppa*, mouse *Nppb*, mouse *Myh6*, mouse *Myh7,* mouse *Atp2a2,* and mouse *Actb* were subsequently assessed using TaqMan® gene expression assays (Hs00165232_m1 detecting human *MYBPC3*; Mm00435104_m1 detecting mouse *Mybpc3*; Mm 01255748_g1 detecting mouse *Nppa*; Mm01255770_g1 detecting mouse *Nppb*; Mm00440359_m1 detecting mouse *Myh6*; Mm00600555_m1 detecting mouse *Myh7;* Mm01201431_m1 detecting mouse *Atp2a2*, Mm00465917_m1 detecting mouse *Ryr2*, Mm00441524_m1 detecting mouse *Slc8a1*, Mm00502426_m1 detecting mouse *Tnni1*, Mm00437164_m1 detecting mouse *Tnni3*, Mm01290252_g1 detecting mouse *Tnnt2*, Mm00600378_m1 detecting mouse *Tpm1*, Mm01333821_m1 detecting mouse *Actc1*, Mm00473657_m1 detecting mouse *Actn2* and 4352933E detecting mouse *Actb*; all obtained from Applied Biosystems™). The equivalent of 10 ng reverse transcribed RNA and 2x TaqMan® Gene Expression Master Mix (Applied Biosystems™) was used as template in each TaqMan® qPCR reaction. Thermal cycling and fluorescence measurement was performed in an Mx3005P qPCR system (Stratagene). Data was analyzed using MxPro software (Stratagene) and expression levels calculated using the Δ_{CT}-method and expressed as a percentage of the *Actb* expression level. Additionally, qPCR was performed on appropriate molar amounts of pCMV-*MYBPC3*^{WT} (encoding full length human cMyBP-C) and pET-*Mybpc3*^{WT} (encoding full length mouse cMyBP-C), allowing quantification and comparison of human *MYBPC3* and mouse *Mybpc3* transcript levels using the standard curve method.

Protein Analysis:

Total protein lysates were prepared from WT and cMyBP-C$^{-/-}$ cardiac cells transduced with adWT at MOI of 0, 5, 10, 20, 50 and 100 in 2D culture, as well as WT, cMyBP-C$^{-/-}$ and cMyBP-C$^{-/-}$ adWT ECT. Protein lysates were electrophoresed using pre-cast 10% Criterion Gels (Bio-Rad) (10 µg for lysates from cells in 2D culture and 35 µg for lysates from ECT). Following electrophoresis, proteins were transferred to 0.2 µm nitrocellulose membranes (Bio-Rad) prior to being washed and equilibrated using standard techniques. Membranes were subsequently

incubated in blocking buffer for 1 hour (Odyssey® Infrared Imaging Systems), prior to overnight hybridization with primary specific antibodies. Primary antibodies used were, a goat anti-cMyBP-C polyclonal antibody diluted at 1:200 (Santa Cruz Biotechnology; sc-50115) that was able to detect both human and mouse cMyBP-C, and a rabbit anti-myosin heavy chain polyclonal antibody diluted at 1:500 (Sigma/Atlas HPA001239), a mouse anti-beta myosin heavy chain monoclonal antibody diluted at 1:200 (Millipore; MAB1552), a mouse anti-alpha cardiac actinin monoclonal antibody diluted at 1:5000 (Sigma; A7811), a mouse anti-cardiac troponin T monoclonal antibody diluted at 1:100 (University of Iowa Developmental Studies Hybridoma Bank; CT3) and a mouse anti-alpha tropomyosin monoclonal antibody diluted at 1:1000 (University of Iowa Developmental Studies Hybridoma Bank; CH1). Primary antibodies were subsequently visualized using secondary donkey anti-goat (Li-Cor, IRDye 800CW), goat anti-mouse (Li-Cor, IRDye 800CW) and goat anti-rabbit (Li-Cor, IRDye 680LT) immunofluorescent secondary antibodies diluted at 1:10,000 using an Odyssey imaging system (Li-Cor Biosciences) according to the manufacturer's protocols.

Beta Galactosidase Staining of CM

In order to establish transduction efficiency, mouse cardiac cells in 2D culture were transduced with the ad*Lac*Z control virus (in which expression of the *Lac*Z reporter gene is driven by the same CMV promoter as is used to drive expression of human cMyBP-C in cells/ECT transduced with adWT) at MOI of 0, 5, 10, 20, 50 and 100. The virus was removed and the media changed after 24 hours and beta-galactosidase staining performed 48 hours post transduction. Fixing and beta-galactosidase staining of CMs were performed using the β-Gal staining kit (Invitrogen) according to the manufacturer's protocol. CMs were visualized and photographed on a Zeiss Stemi 2000 Stereomicroscope.

MTT Assay

In order to establish whether transduction of mouse cardiac cells adversely affected cell survival, WT and cMyBP-C$^{-/-}$ were transduced with adWT and the ad*Lac*Z control virus in 2D culture at MOI of 0, 5, 10, 20, 50 and 100. The virus was removed and the media changed after 24 hours. Following an additional 48 hours in culture, the media was changed and an equal volume of 2 μg/mL 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Sigma) diluted in 1x PBS was added to cells. Cells were subsequently incubated for 30 minutes at 37^{o}C, where after an equal volume of cell lysis buffer (20% (w/v) SDS (Sigma); 50% (v/v) dimethyl formamide (Fischer Scientific); pH 4.7) was added. Cell lysates were incubated at room temperature for 3 - 4 hours where after optical densities at 550 nm were measured.

Routine Histology and Light Microscopy

Murine ECT were fixed with 4% paraformaldehyde (Sigma) in 1x PBS (pH = 7.2) (Mediatech) at 4^{o}C overnight. The ECT were subsequently dehydrated and embedded with paraffin using standard protocols in an automated tissue vacuum infiltration processor (Tissue-Tek V.I P; Sakura). All tissue blocks are embedded in paraffin using a Sakura embedding center, prior to sectioning at a thickness of 5 – 6 μm using a microtome (Leica). Sections used for routine light microscopy were placed on non-charged glass microscope slides and incubated at 60^{o}C for 15 minutes prior to hematoxylin and eosin (H&E) staining in a Tissue-Tek DRS automatic slide

stainer (Sakura). Following staining, sections were covered by a drop of Permount slide mounting fluid (The Science Company) prior to coverslipping. Sections were visualized and photographed on a Zeiss model T635b photomicroscope.

Immunohistochemistry

For immunohistochemistry, ECT constructs were rinsed 1 x 1 min in 0.1 mol/L KCl to relax the sarcomeres and then 1 x 1 min in 1x PBS (pH 7.0) before being fixed in Dent's fixative (80% Methanol:20% DMSO) for 2 hours at 4 °C. Constructs were then dehydrated in methanol series and stored at -20 °C for 24 hours. For sectioning, fixed constructs were transferred directly to xylenes and allowed to equilibrate for 5 minutes prior to being placed in paraffin and maintained at 60 °C for 90 minutes. They were subsequently embedded in fresh paraffin in disposable plastic moulds and allowed to cool before being sectioned at 8 µm on a Leica RM2165 microtome, mounted on glass sides, and dried overnight on a flattening plate at 37 °C. After drying, slides were heated to 60 °C, placed in xylenes for 5 minutes, rehydrated through ethanol series to water and rinsed twice in 1x PBS. Blocking was performed in 2 steps to minimize non-specific binding of mouse- and goat-derived antibodies. First, slides were blocked for 1 hour in 1x PBS containing 5 % [vol/vol] sheep serum, 2 mg/ml bovine serum albumin and 0.1 % [vol/vol] Tween20, then blocked secondarily with the Vectastain Mouse-on-Mouse (M.O.M) basic kit, following manufacturer's instructions (Vector Laboratories, Burlingame, CA). Sections were incubated with 1:50 anti-Desmin monoclonal antibody (NCL-L-DES-DERII from Novocastra Laboratories, Buffalo Grove, NV) and 1:100 anti-cMyBPC rabbit polyclonal antibody for 1h at room temperature in M.O.M protein solution (Vector Laboratories) in a humidity chamber. After rinsing 2 x 2 min in PBS sections were incubated for 1 hour at room temperature in the dark with 1:200 AlexaFluor 568 goat anti-mouse IgG1, 1:200 AlexaFluor 488 goat anti-rabbit IgG(H+L) and/or 1:250 AlexaFluor 647 goat anti-rabbit IgG(H+L) secondary antibodies (Molecular Probes, Eugene, OR) in M.O.M protein solution as before. After rinsing 2 x 2 min in PBS, sections were coverslipped using warmed ProLong Gold Antifade Reagent with 4′,6-diamidino-2-phenylindole (DAPI). Imaging was performed using the Nikon A1R inverted confocal microscope using a 60x oil-immersion objective and 405, 488, 568 and 647 nm to excite DAPI, AlexaFluor 488 and AlexaFluor 568 AlexaFluor 647, respectively. Images were acquired using a built-in automated tile scan routine and NIS-Elements software suite.

Transmission Electron Microscopy

Tissues were immersion fixed in a solution 2.5% glutaraldehyde, 2.0% paraformaldehyde in 0.1 mol/L sodium phosphate buffer (pH 7.4) overnight at 4^{o}C. The tissue was then post fixed in 1% OsO_4 in the same buffer for 2 hours at room temperature. Following OsO_4 post-fixation, the samples were dehydrated in a graded ethanol series, then further dehydrated in propylene oxide and embedded in Epon epoxy resin. Samples were sectioned for transmission electron microscopy using an ultramicrotome (Reichert-Jung Ultracut-E) and contrasted with Reynolds lead citrate and 8% uranyl acetate in 50% ethanol. Ultrathin sections were observed with a Philips CM120 electron microscope and images were captured with a MegaView III side mounted digital camera.

Mechanical Testing of ECT:

Isometric force generated by ECT was measured using protocols similar to those described by Tobita et al. (2006)[2]. In brief, each ECT construct was transferred from the culture dish (figure 1A) to a model 801B small intact fiber test apparatus (Aurora Scientific) in Krebs-Henseleit buffer (119 mmol/L NaCl; 12 mmol/L Glucose; 4.6 mmol/L KCl; 25 mmol/L $NaHCO_3$; 1.2 mmol/L KH_2PO_4; 1.2 mmol/L $MgCl_2$; 1.8 mmol/L $CaCl_2$, gassed with 95% O_2 / 5% CO_2 (pH 7.4)). ECT constructs were attached with sutures between a model 403A force transducer (Aurora Scientific) and model 322C high speed length controller (Aurora Scientific) (figure 1B). ECT were perfused with 24^{o}C Krebs-Henseleit buffer at a rate of 1 mL/min and field-stimulation initiated at 2 Hz (2.5 ms, 8 - 12.5 V). The longitudinal length of each construct was increased stepwise until maximal twitch force was achieved. ECT were then equilibrated for 10 – 20 minutes or until a stable level of passive tension was achieved while being paced at 2 Hz. The pacing frequency was increased to 4 Hz and the temperature of the perfusate in the chamber adjusted to 37^{o}C prior to force measurements being made at stimulation frequencies of 4 Hz, 5 Hz, 6 Hz, 7 Hz and 8 Hz. Data from force measurements were analyzed and averaged using IonWizard 6.0 software (IonOptix). For each stimulation frequency, force of 80 to 160 successive contractions were collected and averaged. Contraction data was exported to Microsoft Excel and the magnitude and kinetics of force generation calculated.

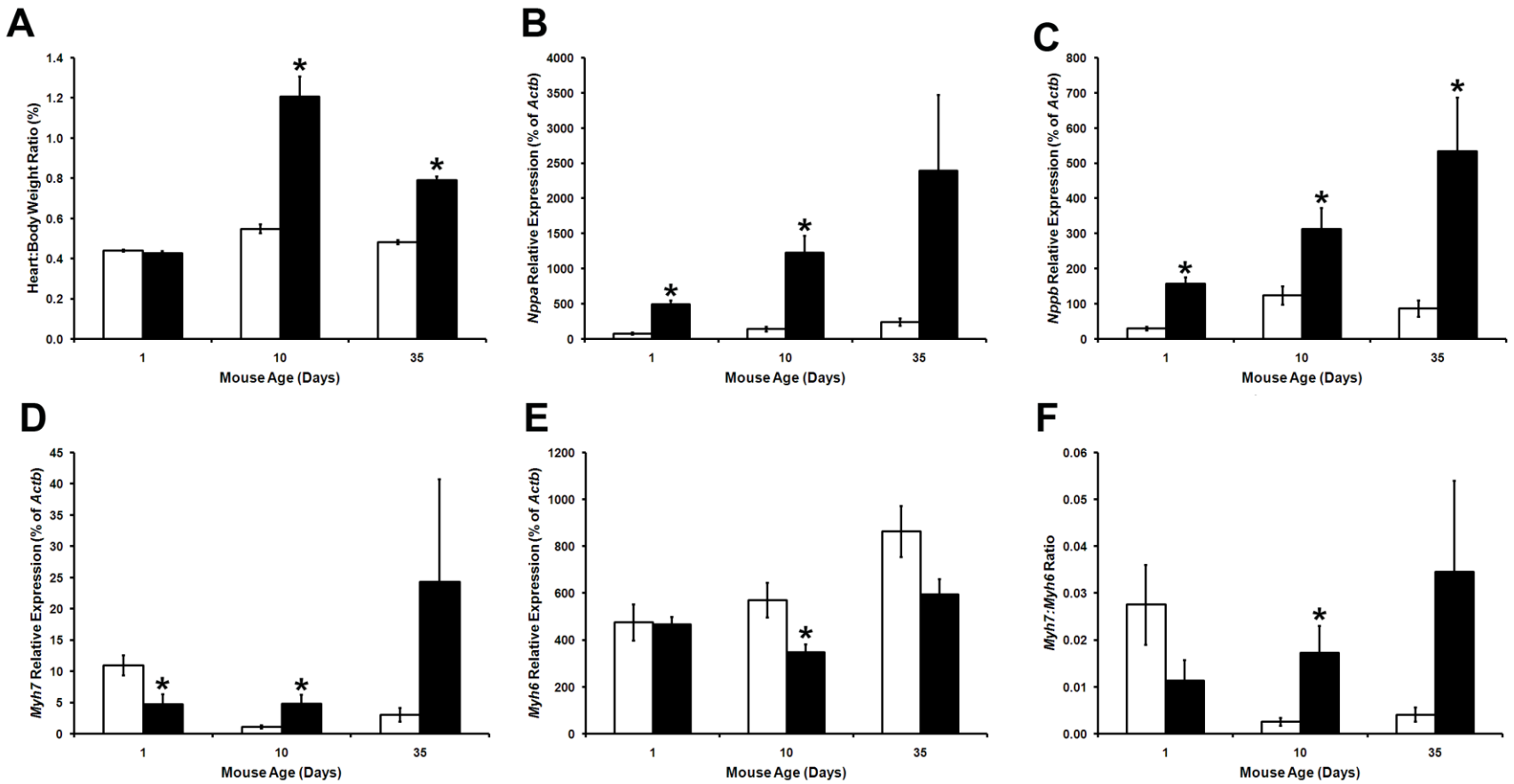
Statistical Analysis

SPSS software was used to perform statistical analysis. Student's t-tests were used for two way comparisons, while one way ANOVA with Bonferroni's correction for post-hoc analysis was used for multiple comparisons. All error bars are standard error of the mean (SEM). Statistical significance was set at $p < 0.05$.

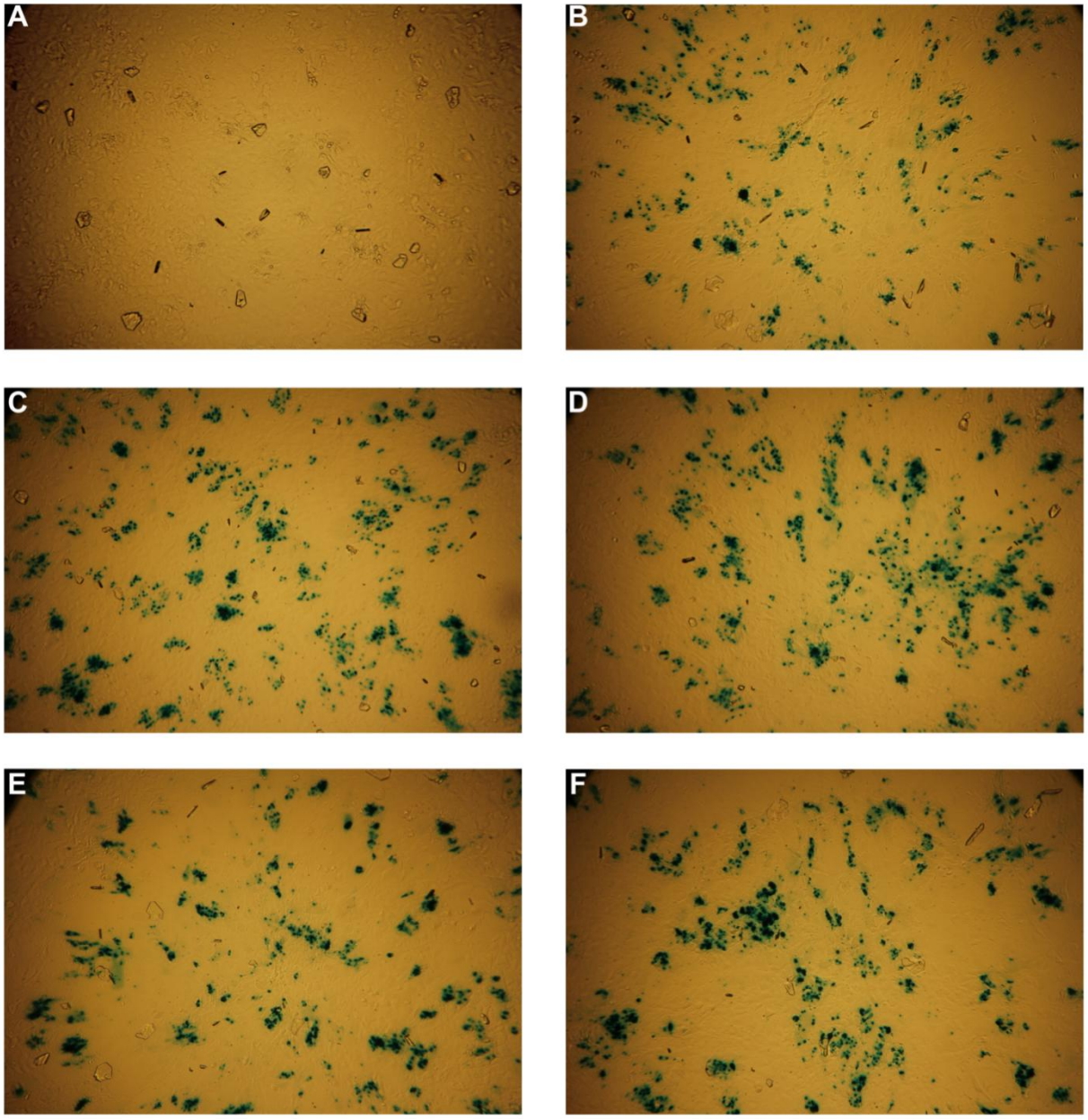
Supplemental Reference List:

1. Tong CW, Stelzer JE, Greaser ML, Powers PA, Moss RL. Acceleration of crossbridge kinetics by protein kinase a phosphorylation of cardiac myosin binding protein c modulates cardiac function. *Circ Res*. 2008;103:974-982
2. Tobita K, Liu LJ, Janczewski AM, Tinney JP, Nonemaker JM, Augustine S, Stolz DB, Shroff SG, Keller BB. Engineered early embryonic cardiac tissue retains proliferative and contractile properties of developing embryonic myocardium. *Am J Physiol Heart Circ Physiol*. 2006;291:H1829-1837

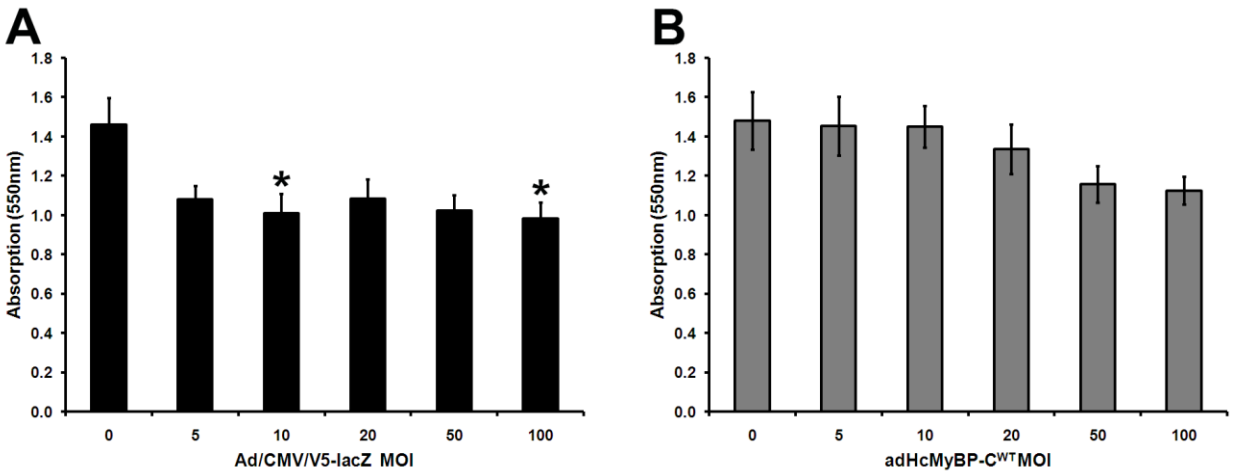
ONLINE FIGURES



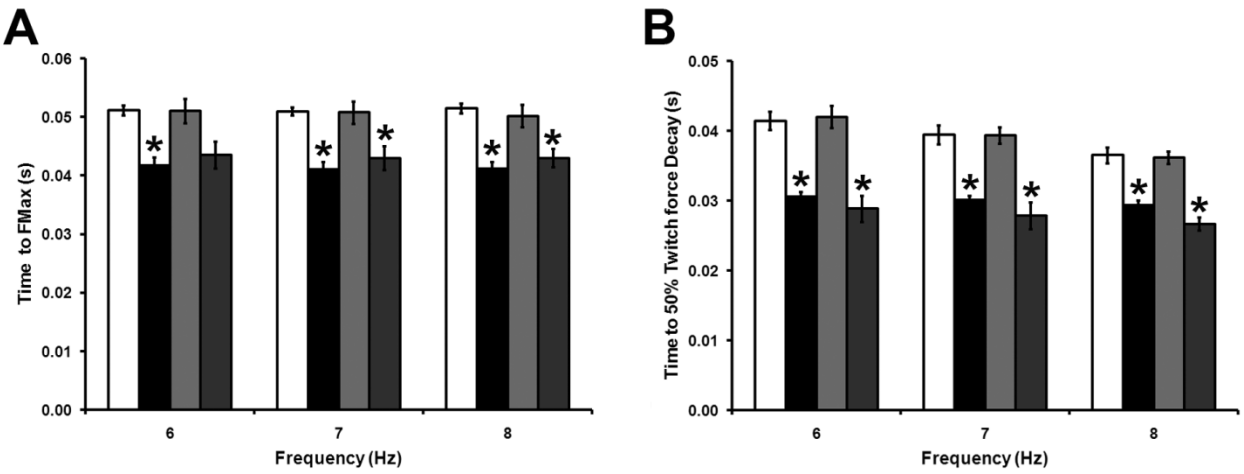
Online Figure I. Hypertrophic Response in WT and cMyBP-C$^{-/-}$ ECT Hearts. Heart to body weight ratios **(A)** in 1 day, 10 day and 35 day old WT (open bars) and cMyBP-C$^{-/-}$ (filled bars) mice. mRNA expression levels of *Nppa* **(B)**, *Nppb* **(C)**, *Myh7* **(D)**, *Myh6* **(E)** in WT and cMyBP-C$^{-/-}$ mouse hearts collected from 1 day, 10 day and 35 day old mice. The *Myh7:MyH6* ratio is shown in F. *P < 0.05 vs. WT (Student's T-test; n ≥ 12 for each group in A; n ≥ 6 for each group in B-E). Elevated heart to body weight ratios and increased expression levels of the late hypertrophic response-genes (*Myh6* and *Myh7*) in day 10 and day 35 cMyBP-C$^{-/-}$ hearts indicate that hypertrophic remodeling takes place in the first 10 days of life. Elevated expression of the early hypertrophic marker genes (*Nppa* and *Nppb*) in neonatal cMyBP-C-/- hearts indicates that early hypertrophic signaling precedes overt hypertrophy.



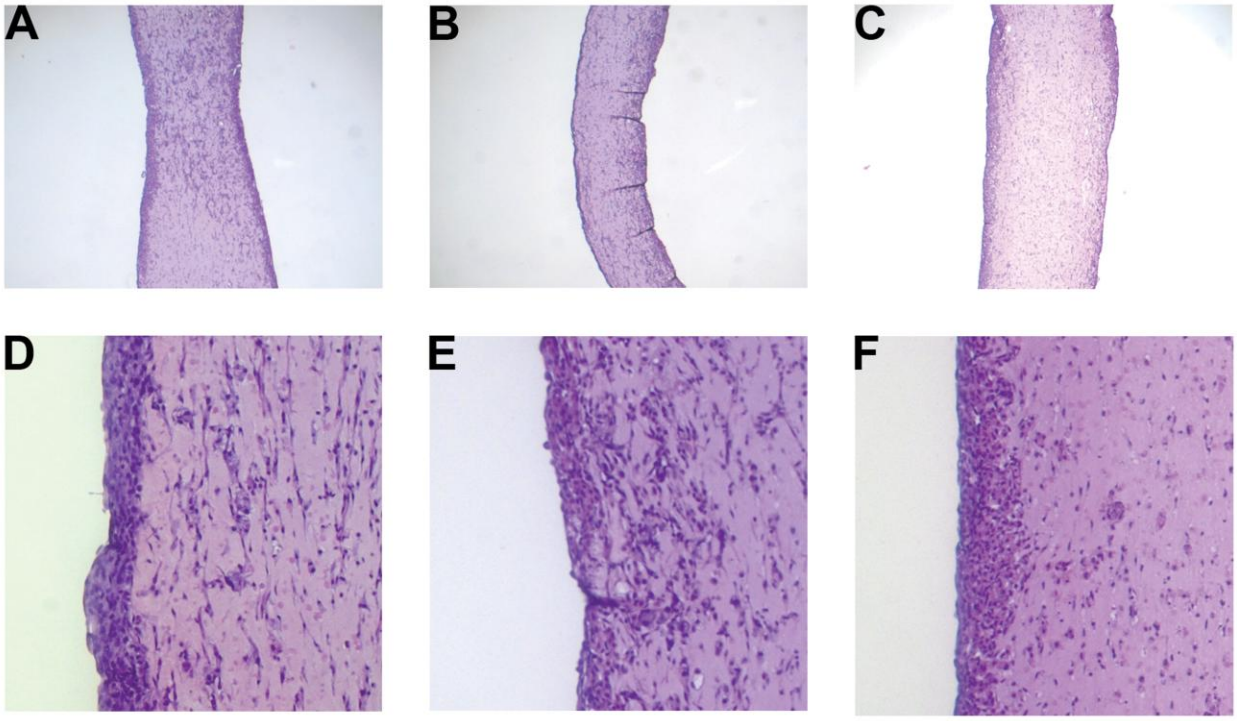
Online Figure II. Beta-Galactosidase Staining of Neonatal Cardiac Cells. WT neonatal cardiac cells in 2D culture transduced with adLacZ at 0 MOI **(A)**; 5 MOI **(B)**; 10MOI **(C)**; 20 MOI **(D)**; 50 MOI **(E)** and 100MOI **(F)**. This data suggest that most cardiomyocytes are transduced with adLacZ at MOI of 10 and above.



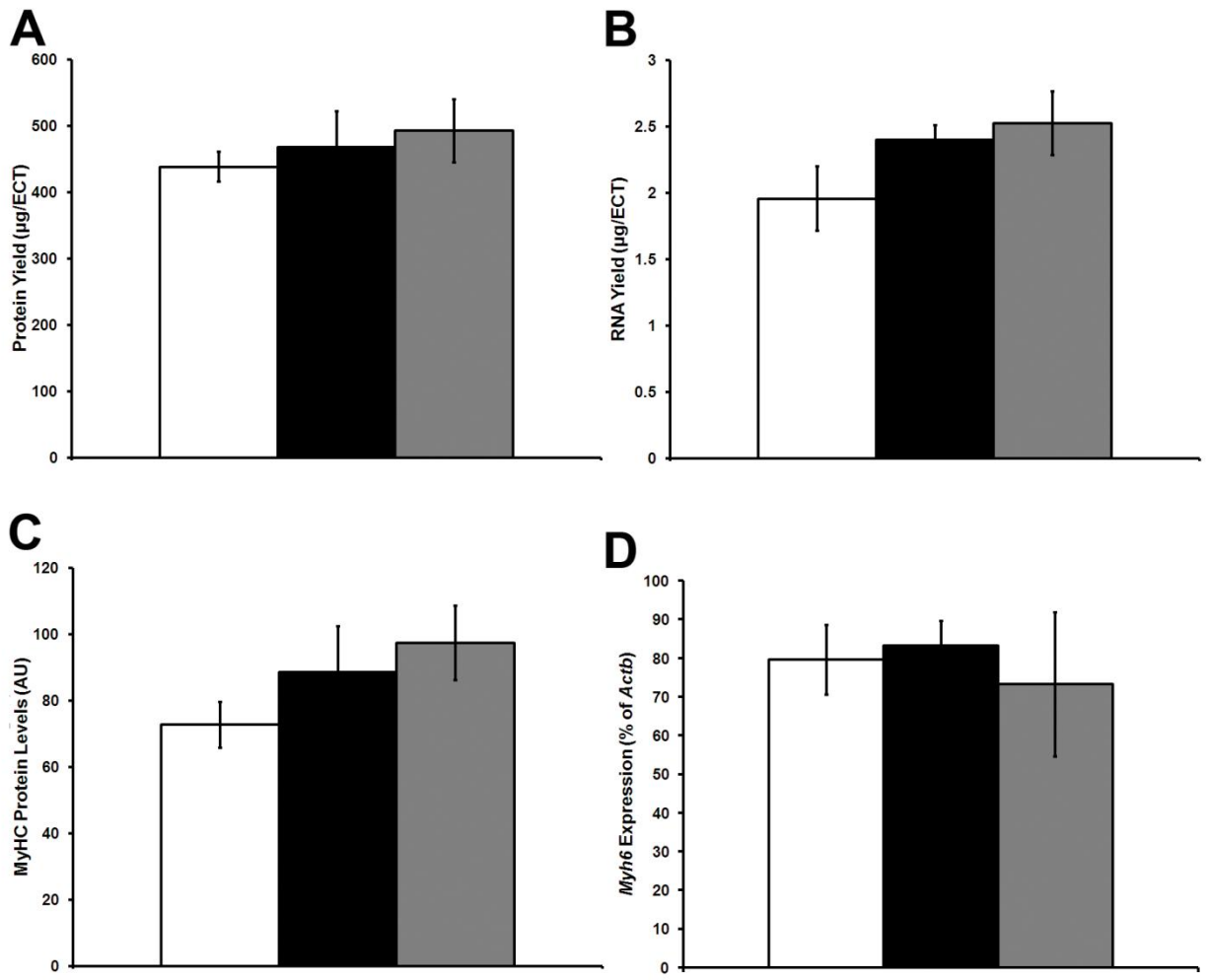
Online Figure III. Effect of Adenoviral Transduction on Neonatal Cardiac Cell Survival. MTT assays performed on neonatal mouse cardiac cells transduced with adLacZ **(A)** and adWT **(B)** at MOI of 0, 5, 10, 20, 50 and 100. *P<0.05 vs. MOI 0 (one way ANOVA, Bonferroni's post-hoc analysis; n = 5 for each group). This data indicates that while transduction of cardiomyocytes with adLacZ had a modestly cytotoxic effect, transduction with adWT did not significantly affect cell survival.



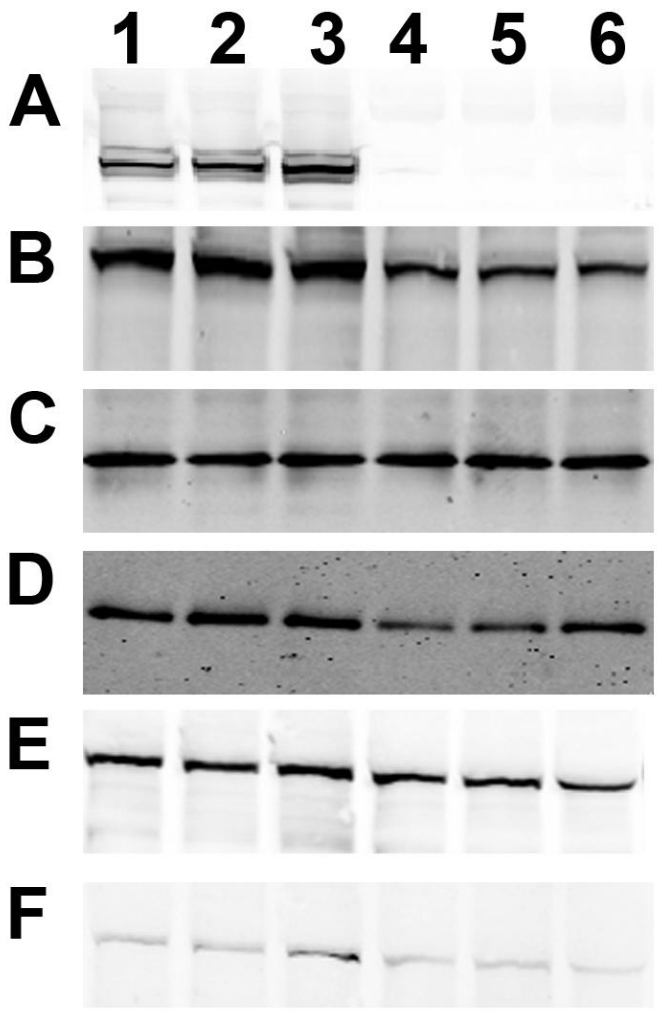
Online Figure IV. Effect of Expression of adC^{-} in cMyBP-C$^{-/-}$ ECT on Contractile Kinetics. **(A)** Time to peak developed twitch force in WT, cMyBP-C$^{-/-}$, cMyBP-C$^{-/-}$ adWT and cMyBP-C$^{-/-}$ adC^{-} ECT paced at 6Hz, 7Hz and 8Hz. **(B)** Time from peak developed twitch force to 50% force decay in WT, cMyBP-C$^{-/-}$, cMyBP-C$^{-/-}$ adWT and cMyBP-C$^{-/-}$ adC^{-} ECT paced at 6Hz, 7Hz and 8Hz. WT ECT- open bars; cMyBP-C$^{-/-}$ ECT- filled bars; cMyBP-C$^{-/-}$ adWT ECT- light grey bars; cMyBP-C$^{-/-}$ adC^{-} ECT- dark grey bars. * $P < 0.05$ vs. WT and cMyBP-C$^{-/-}$ adWT (one way ANOVA, Bonferroni's post-hoc analysis; WT n = 7, cMyBP-C$^{-/-}$ n = 6; cMyBP-C$^{-/-}$ adWT n = 6; cMyBP-C$^{-/-}$ adC^{-} n = 3). This data confirms that restoration of contractile function in cMyBP-C$^{-/-}$ adWT ECT is the result of expression of exogenous human cMyBP-C, rather than a direct effect of adenoviral transduction.



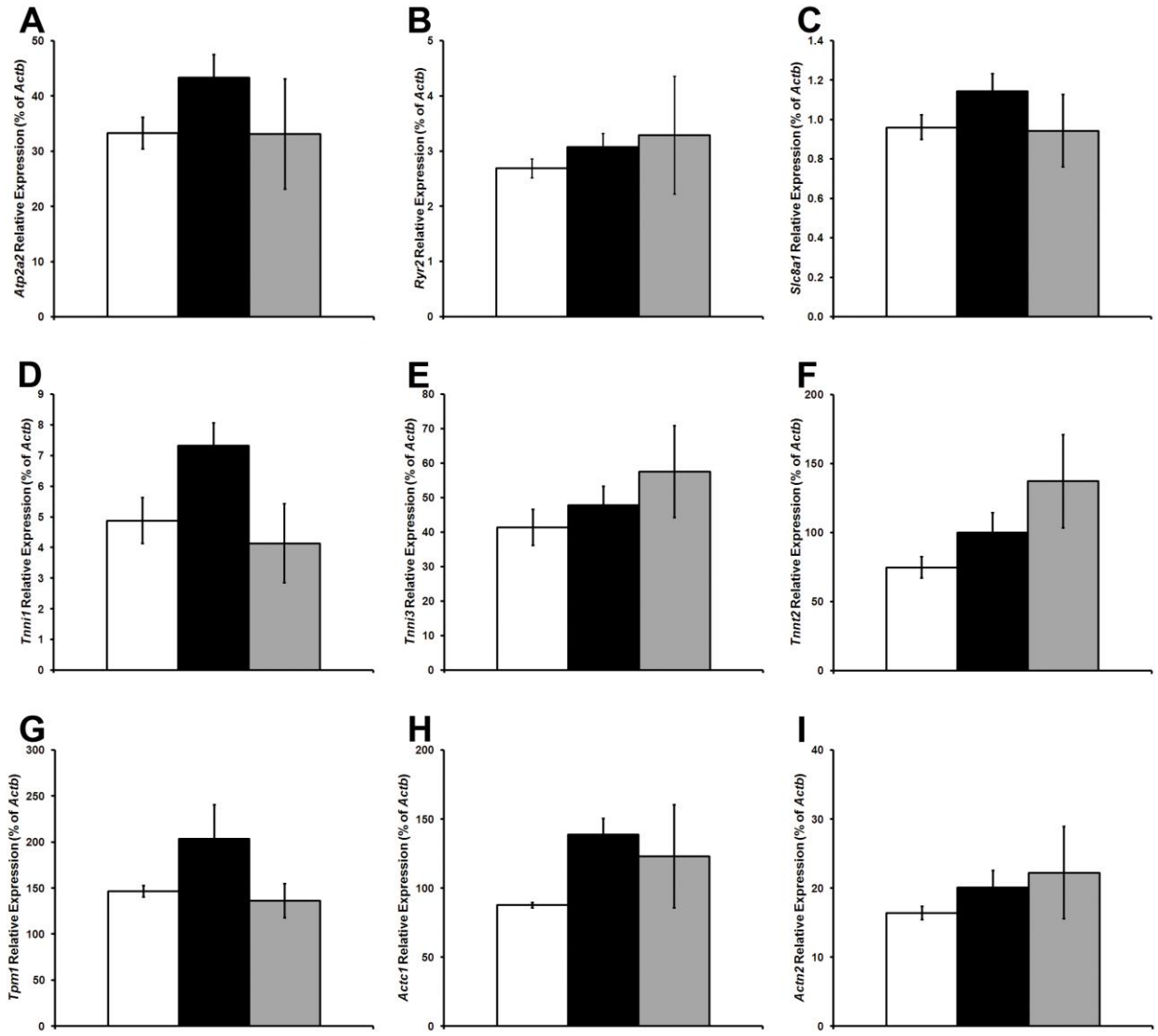
Online Figure V. Histological Comparison of WT, cMyBP-C$^{-/-}$ and cMyBP-C$^{-/-}$ adWT ECT. H&E-stained WT (A and D); cMyBP-C$^{-/-}$ (B and E) and cMyBP-C$^{-/-}$ adWT (C and F) ECT. 2.5x objective (A - C) and 10x objective (D - F). The morphology of WT, cMyBP-C$^{-/-}$ and cMyBP-C$^{-/-}$ adWT ECT were similar with well-organized, well-aligned cardiomyocytes found predominantly at, or close to, the outer surface of the ECT.



Online Figure VI. Protein and RNA content in ECT. Total protein (A) and RNA (B) yield from WT, cMyBP-C$^{-/-}$ and cMyBP-C$^{-/-}$ ECT. Total myosin heavy chain protein (C) and alpha myosin heavy chain mRNA (D) levels were assessed by Western blotting and qRT-PCR respectively. WT ECT- open bars; cMyBP-C$^{-/-}$ ECT- filled bars; cMyBP-C$^{-/-}$ adWT ECT- light grey bars. No significant differences were detected between any groups for any parameters analyzed (one way ANOVA, Bonferroni's post-hoc analysis; n ≥ 7). Similar protein and RNA yields from WT, cMyBP-C$^{-/-}$ and cMyBP-C$^{-/-}$ adWT ECT indicate that total cell number is not influenced by ECT genotype. Furthermore, similar total myosin heavy chain protein and *Myh6* transcript levels indicate that the cardiomyocyte number were similar in ECT of all three genotypes.



Online Figure VII. Expression of Sarcomeric Proteins in WT and cMyBP-C$^{-/-}$ ECT. A- cardiac myosin binding protein-C; B- alpha actinin; C- cardiac troponin T; D- alpha tropomyosin; E- total myosin heavy chain; F- beta myosin heavy chain. Lanes 1-3 – protein from WT ECT; lanes 4-6 – protein from cMyBP-C$^{-/-}$ ECT (35µg of total protein were loaded per lane). While cMyBP-C protein was absent in cMyBP-C$^{-/-}$ ECT, expression of other sarcomeric proteins was not increased by its ablation. These findings indicate that cardiomyocyte levels are similar in WT and cMyBP-C$^{-/-}$ ECT and that cMyBP-C$^{-/-}$ ECT has not undergone hypertrophic remodeling.



Online Figure VIII. Expression of Genes Encoding Sarcomeric and Ca^{2+} Handling/Sensing Proteins. Relative expression of *Atpa2,* encoding Serca2A (A); *Ryr2*, encoding cardiac ryanodine receptor (B); *Slc8a1*, encoding sodium-calcium exchanger (C); *Tnni1*, encoding slow skeletal troponin I (D); *Tnni3*, encoding cardiac troponin I (E); *Tnnt2*, encoding cardiac troponin T (F); *Tpm1*, encoding alpha tropomyosin (G); *Actc1*, encoding cardiac alpha actin (H) and *Actn2*, encoding alpha actinin (I). WT ECT- open bars; cMyBP-$C^{-/-}$ ECT- filled bars; cMyBP-$C^{-/-}$ adWT ECT- light grey bars. Relative expression levels are represented as a percentage of *Actb* (beta actin) expression levels. No significant differences were detected between any groups for any gene analyzed (one way ANOVA, Bonferroni's post-hoc analysis; n = 4). These findings indicate that expression levels of sarcomeric protein encoding genes, as well as genes encoding proteins involved in Ca^{2+} sensing/handling are similar in WT, cMyBP-$C^{-/-}$ and cMyBP-$C^{-/-}$ ad WT ECT.

Online TABLE I

Contractile function of WT, cMyBP-C$^{-/-}$ and cMyBP-C$^{-/-}$ adWT ECT

* P < 0.05 (one way ANOVA, Bonferroni's post-hoc analysis)

	Hz	WT (N=7)		cMyBP-C$^{-/-}$ (N=6)		cMyBP-C$^{-/-}$ adWT (N=6)		P = WT vs. cMyBP-C$^{-/-}$	P = WT vs. cMyBP-C$^{-/-}$ adWT	P = cMyBP-C$^{-/-}$ vs. cMyBP-C$^{-/-}$ adWT
		Value	SEM	Value	SEM	Value	SEM			
F_{Max} (mN)	6	0.530	0.084	1.049	0.160	0.639	0.031	0.008*	1.000	0.045*
	7	0.495	0.079	0.972	0.150	0.585	0.033	0.009*	1.000	0.001*
	8	0.447	0.073	0.872	0.135	0.507	0.035	0.011*	1.000	<0.001*
t to F_{Max} (s)	6	0.051	0.001	0.042	0.001	0.051	0.002	0.001*	1.000	0.045*
	7	0.051	0.001	0.041	0.001	0.051	0.002	<0.001*	1.000	<0.001*
	8	0.052	0.001	0.041	0.001	0.050	0.002	<0.001*	1.000	<0.001*
t F_{Max} to 50%F_{Max} (s)	6	0.041	0.001	0.031	0.001	0.042	0.002	<0.001*	1.000	0.011*
	7	0.039	0.001	0.030	0.001	0.039	0.001	<0.001*	1.000	0.001*
	8	0.037	0.001	0.029	0.001	0.036	0.001	<0.001*	1.000	0.001*