SUPPLEMENTAL MATERIALS

Part One: Supplemental Methods

Canine Pacing Models

All studies were approved by the Johns Hopkins Animal Care and Use Committee, and performed by trained personnel.

Details of the canine model have been previously reported[1, 2]. All models except control received a pacemaker implant (Medtronic, Minneapolis, MN). HF_{dys} and CRT models received left bundle branch block (LBBB) by radio-frequency ablation at the time of pacemaker implant, while the HF_{sync},V3A3, AVA groups did not. Animals were given a 1-week recovery period before pacing began. All pacing models were tachypaced at 200 bpm for 6 weeks, which induced heart failure. The location of pacing varied by model: HF_{dys} and HF_{sync} were atrially tachypaced for the entire 6 weeks, while CRT animals were subjected first to three weeks of atrial tachypacing, followed by three weeks of biventricular tachypacing (RV apex and LV lateral wall), the V3A3 model was RV paced for the first 3 weeks, and then received 3 weeks of atrial tachypacing, while the AVA model was atrially paced atrially for the entire period excepting a two week period in the middle when it received right ventricular pacing. At the end of the study, animals were anesthetized with pentobarbital, intubated, and the heart excised under ice-cold cardioplegia.

Membrane Permeabilized Myocytes

Tissue from the endocardium layer of the left ventricular lateral wall was flash frozen in liquid nitrogen and stored at -80 °C. Subsequently, the tissue was homogenized in isolation solution, in the presence of 0.3% Triton X-100, and protease and phosphatase inhibitors. Isolation solution contained (in mM): 5.5 Na_2ATP, 7.11 $MgCl_2$, 2 EGTA, 108.01 KCl, 8.91 KOH, 10 imidazol, 10 DTT. Myocytes were then washed in the absence of Triton X-100 to remove the detergent. Myocytes were glued, using silicone, to the tips of 150 µm diameter minutia pins attached to a force transducer and motor arm (Aurora Scientific Inc., Aurora, ON, Canada). Sarcomere length was monitored by video camera (Imperx, Boca Raton, FL) and calculated by the High-speed Video Sarcomere Length Program (Aurora Scientific Inc.). Myocytes were maintained at a sarcomere length of 2.1µm. Myocytes were kept in relaxing buffer containing (in mM): 5.95 Na_2ATP, 6.41 $MgCl_2$, 10 EGTA, 100 BES, 10 phosphocreatine, 50.25 potassium propionate, 10 DTT, protease and phosphatase inhibitors. Force was measured as the myocyte was exposed to increasing calcium, by combining relaxing and activating buffers in set rations. Activating buffer contained (in mM): 5.95 Na_2ATP, 6.2 $MgCl_2$, 10 Ca^{2+}EGTA, 100 BES, 10 phosphocreatine, 29.98 potassium propionate, 10 DTT, protease and phosphatase inhibitors. All buffers were adjusted to a pH of 7.0. A complete activation of the myocyte occurred at the beginning and end of the experiment, and the myocyte discarded if there was >10% rundown. Force-Ca^{2+} data were fit to the Hill Equation: $F = F_{max} * Ca^h/(EC^h_{50} + Ca^h)$.

Subsets of myocytes were exposed to either glycogen synthase kinase-3β (GSK-3β, 10 µg/mL, 20 min, Sigma-Aldrich, St. Louis, MO), PKA (0.125 units/mL, 20 minutes,

Sigma-Aldrich), or Akt (10 μg/mL, 20 min, Sigma-Aldrich). Force-calcium data was collected before and after the treatment.

Multicellular Fragments

Tissue samples were minced into 2-4 mm pieces then mechanically homogenized three times at low speed (1,000 rpm, 3 sec), and re-suspended in relaxing solution containing Triton X-100 overnight at 4 ^{o}C. All solutions contained protease inhibitors (Sigma-Aldrich).

Multicellular skinned fragments (0.1-0.25 mm wide; 1-2.5 mm long) were attached to aluminum T-clips and mounted to arms extending from a force transducer (World Precision Instruments) and a high-speed length controller (Aurora Scientific Inc). Sarcomere length was set to 2.3 μm by laser diffraction. The isolated muscle was then exposed to a range of calcium solutions while generated tension and consumed ATP were measured simultaneously during the contraction. ATP consumption was measured as previously described using a UV coupled optical absorbance method[3]. Following each contraction, calibration steps were performed by step-wise injection of 250 pmol of ADP. Activating solution contained (in mM): 20 Ca^{2+}EGTA, 1.55 potassium propionate, 6.59 magnesium chloride, 100 BES, 5 sodium azide, 1 DTT, 10 phosphoenolpyruvate, 0.01 oligomycin, 0.01 PMSF, and 0.01 A_2P_5, as well as protease inhibitor cocktail. Relaxing solution was identical except it contained (in mM) 20 EGTA, potassium proprionate 21.2, and magnesium chloride 7.11. The pre-activating solution contained (in mM) 0.5 EGTA, 19.5 HDTA (Fluka), and 21.8 potassium propionate. Furthermore, all solutions used in the ATPase assay contained 0.5 mg/mL pyruvate kinase and 0.05 mg/mL lactate dehydrogenase (Sigma).

High frequency muscle length perturbations (1%; 500 Hz) were applied continuously to measure stiffness (the relative number of attached cycling cross-bridges during tension development). The rate of force redevelopment following a release-restretch maneuver, k_{tr}, was measured during a final contraction at maximum Ca^{2+}. Only muscles that maintained greater than 80% maximal tension throughout the protocol were included for analysis.

Trabeculae Experiments

Trabeculae were isolated from the right ventricular free wall and skinned overnight in the presence of 1% Triton X-100 (to remove all membranous structures), and protease (Sigma-Aldrich, St. Louis, MO) and phosphatase inhibitors (Roche Diagnostics, Indianapolis, IN) at 4 ^{o}C. Trabeculae were attached via a “basket and hook” technique[4], to a force transducer (SI Heidelberg) and stationary hook. The muscle was stretched to produce 5 mN•mm^{-2} of passive tension (corresponding to maximum twitch force). Force was measured as bath solution Ca^{2+} was increased from 0 to saturating levels (46.8 μM), obtained by proportional mixing of activating and relaxing solutions. Relaxing solution contained (in mM): 10 K_2H_2EGTA, 5.45 $MgCl_2$, 5.5 Na_2ATP, 15 Na_2CrP, 80 KCl, 25 HEPES, 5 DTT, protease and phosphatase inhibitors. Activating solution contained (in mM): 10 Ca^{2+}EGTA, 5.15 $MgCl_2$, 5.5 Na_2ATP, 15 Na_2CrP, 80 KCl, 25 HEPES, 5 DTT, protease and phosphatase inhibitors. All buffers were adjusted to a pH of 7.2. Trabeculae were completely activated at the beginning and end of each experiment, and if there was greater than 10% rundown, the experiment was discarded.

In a subset of trabeculae, a second force-Ca^{2+} relation was determined after exposure to protein phosphatase 1 (PP1, 25,000 mU/mL for 60 minutes, New England Biolabs, Ipswich, MA).

Phos-tag gel

Tissue samples from Control, HF_{dys}, and CRT hearts are treated to obtain a myofilament-enriched protein sample as previously described[5] in the presence of protease and phosphatase inhibitors. Briefly, tissue was homogenized, using a glass dounce homogenizer, in ice-cold standard rigor buffer (SRB) and 1% Triton X-100. SRB contained (in mM): 75 KCl, 10 imidazole, 2 $MgCl_2$, 2 EGTA, 1 NaN_3, plus protease and phosphatase inhibitors. The sample was then centrifuged at 16,000 g for 1 minute at 4 °C. The supernatant was discarded, and the pellet was re-suspended in SRB without Triton X-100. This was repeated three times. The pellet was then solubilized in 8M urea and 4% CHAPS. The sample was centrifuged at maximum speed, room temperature, for 15 minutes, and the pellet was discarded.

Myofilament-enriched samples were run on phos-Tag gels (Wako Pure Chemical Industries, Ltd., Chuo-ku, Osaka, Japan), transferred to a membrane and cardiac troponin I (TnI) was detected with the 8I-7 TnI antibody (Spectral Diagnostics, Toronto, ON, Canada).

Western Blots

Tissue samples were prepared as above, biopsies were prepared using a 0.1 mL dounce micro-homogenizer (Radnoti LLC, Monrovia, CA), in 40 µL cell lysis buffer (Cell Signaling, Danvers, MA), and further solubilized by adding 1% SDS. Samples were run on 4-12% pre-cast Bis-Tris gels (Invitrogen), transferred to nitrocellulose membranes, and blotted using custom MyBPC antibodies against phospho Ser-273, Ser-282, Ser-302[6] and total (a kind gift from Dr. Sakthivel Sadayappan), custom MLC2 phospho-antibodies against phospho Ser-15 (a kind gift from Dr. Neal Epstein), phospho TnI (Cell Signaling), total TnI (Spectral Diagnostics), phospho-serine motif (Zymed Laboratories, San Francisco, CA), phospho-threonine motif (Zymed), Troponin T (Cell Signaling), total MLC2 (Novus Biologicals, Littleton, CO), P-Ser9 GSK-3β (Sigma-Aldrich), and/or total GSK-3β (Santa Cruz, Dallas, TX). Blots were scanned using an Odyssey Infrared Imager (Li-Cor Biosciences, Lincoln, NE), and analyzed using Odyssey Application Software (v3.0.30, Li-Cor Biosciences).

In some cases, blots were stripped with NewBlot Nitro stripping buffer (Licor), and reprobed. Blots were scanned before re-probed to ensure efficient stripping. In the case of the phospho MyBPC blots, loading was normalized using Direct Blue 71 (Sigma) total protein stain

Silver Stain

After running, gels were fixed in 40% ethanol, 10% acetic acid for 1 hour, and then washed in H_2O overnight. The gel was the sensitized in 0.02% sodium thiosulfate for 1 minute, washed, and then incubated in cold 0.1% silver nitrate, 0.02% formaldehyde for 20 minutes. The gel was then washed and developed in 3% sodium carbonate, 0.05% formaldehyde for two minutes. The reaction was terminated with 5% acetic acid.

2D-DiGE Gels

Myofilament-enriched HF_{dys} and CRT samples were labeled with cyanine (Cy) 3 or Cy5, and a mixture of the two (as an internal control) labeled with Cy2 (0.4 µL dye/ 50 µg protein). All three samples were then isoelectrically focused on an IEF strip (GE Healthcare Biosciences, Piscataway, NJ). The IEF instrument was run with the following steps: 10 hours active rehydration at 50V, 1 hour rapid ramp to 250V, 1 hour rapid ramp to 500V, 1 hour rapid ramp to 1000V, 4 hours normal ramp to 10,000V, 50,000 V•hours at 10,000V. The strips were alkylated and reduced, and then separated by weight on a large-format (18 cm) 10% Bis-Tris resolving gel in MES buffer. After fixing and washing the gel, it was scanned on a Typhoon scanner (GE Healthcare). Image analysis was performed by REDFIN (Ludesi, Malmö, Sweden).

Mass Spectrometry

Myofilament-enriched HF_{dys} and CRT samples (1 mg protein in 8M urea, 1% SDS) were reduced, alkylated, then digested overnight in solution using trypsin (Promega, Madison, WI) (100 µg/1.0 mg protein). The reaction was stopped with 10% TFA, and the samples were desalted with Oasis HLB cartridges (Waters, Milford, MA). Strong cation exchange (SCX) columns were prepared using SCX bulk media (Nest Group, Southborough, MA). The sample was split into two parts: 60% was run through the SCX columns (1/3 collected as flow through, 1/3 eluted with SCX buffer plus 40 mM KCl, and 1/3 eluted with SCX buffer plus 150 mM KCl). These three fractions, along with the 40% not run through the SCX column, were then individually desalted.

To phospho-enrich the samples, 50 µl of titanium oxide (TiO_2) beads (Glycen Corp., Columbia, MD) was added to each fraction (4 per sample, 6 samples per group) and incubated at room temperature on a shaker (1400 rpms) overnight. The TiO_2 beads were then washed, and the sample eluted in 10% ACN/3% NH_4OH. Samples were then dried down and resuspended in 70% ACN/1% FA for LC-MS/MS analysis.

Phospho-peptides were analyzed on an Agilent 1200 nano-LC system (Agilent, Santa Clara, CA) connected to an LTQ-Orbitrap mass spectrometer (Thermo Scientific, Waltham, MA), equipped with a nanoelectrospray ion source. The peptides were separated on a C_{18} RP-HPLC column (75 µm x 10 cm self-packed with 5 µm, 200 Å Magic C18; Michrom Bio Resources, Auburn, CA) at a flow rate of 300 nL/min. Each fraction (4 per sample) was run separately to maximize the number of peptides acquired. Each MS1 scan was followed by collision induced dissociation of the 5 most abundant precursor ions. Mono-isotopic precursor selection was enabled and dynamic exclusion was enabled with a repeat count of 2, repeat duration of 30 seconds and exclusion duration of 60 seconds. Only MS1 signals exceeding 1000 counts triggered the MS2 scans and +1 and unassigned charge states were not selected for MS2 analysis.

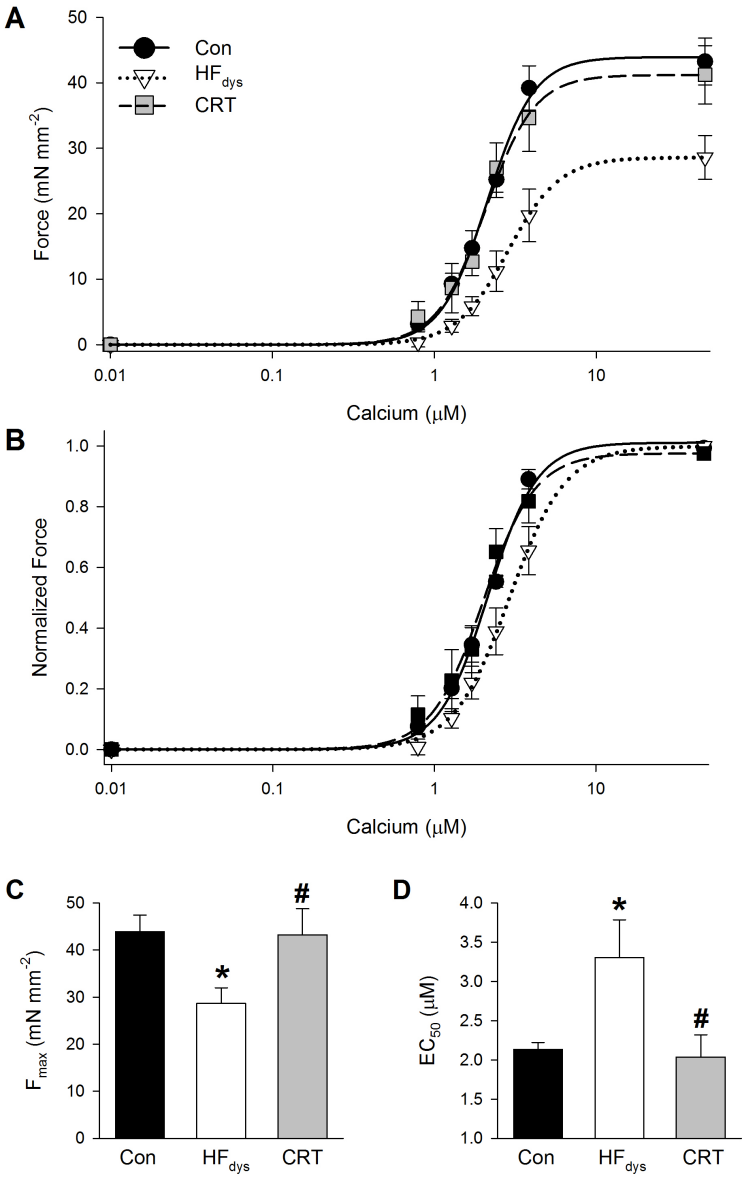
All raw MS/MS data was searched using the Sorcerer 2™-SEQUEST® algorithm (Sage-N Research, Milpitas, CA). Data was searched against a canine database allowing for: carbamidomethyl (fixed modification), oxidation (variable modification), and phosphorylation (variable modification). Tolerances were: 1.00 Da for fragments and 0.037-0.170 Da for parent ions, and allowing for 2 missed cleavages. Analysis was

done in Scaffold 3 (v3.4.9, Proteome Software, Portland, OR) with protein and peptide probability thresholds of 95% and 90%, respectively (providing a protein false discovery rate of 0.5% and a peptide FDR of 0.4%). Additional analysis was done using Scaffold PTM (v1.1.3, Proteome Software). For analysis, spectral counts were summed over all fractions per sample.

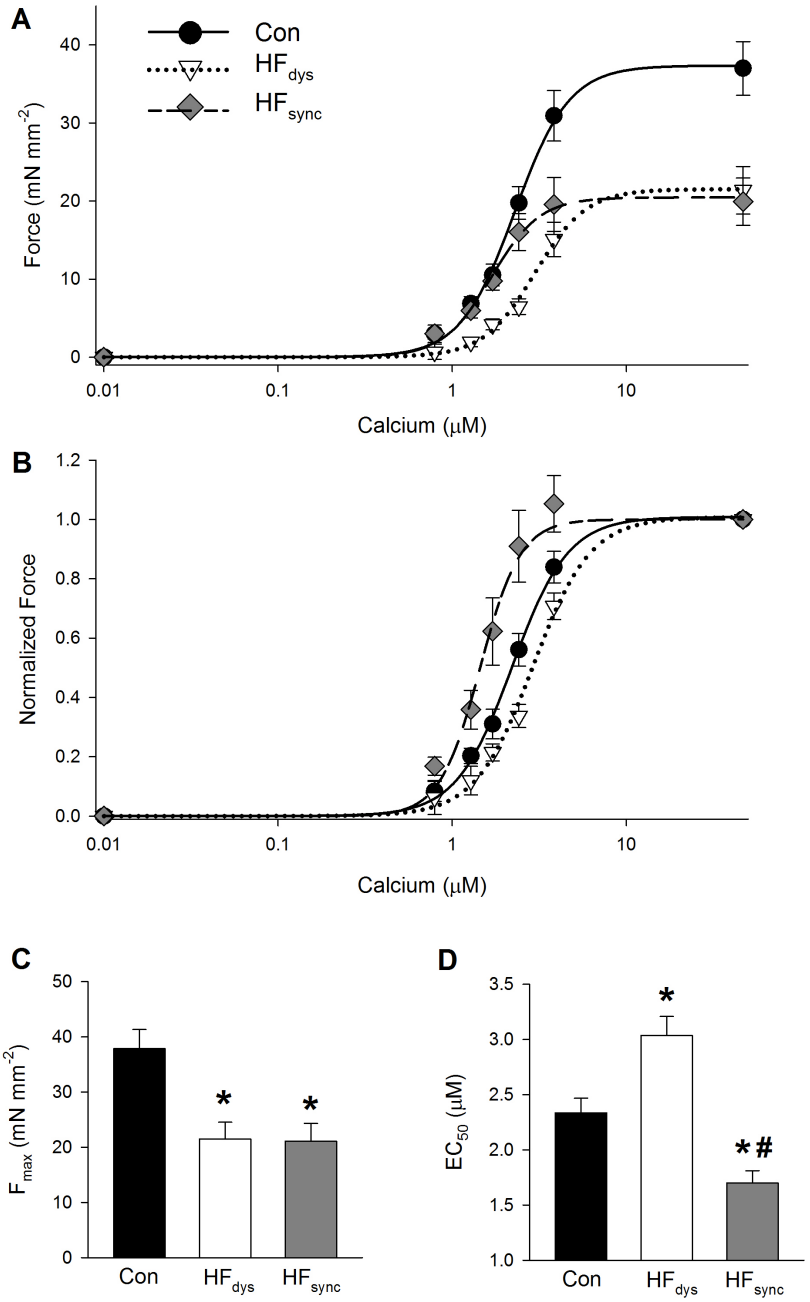
Kinase consensus amino acid sequences were predicted using NetPhosK (cbs.dtu.dk/services/NetPhosK/) and using the Human Protein Reference Database (hprd.org) PhosphoMotif finder.

A second set of samples was prepared for the GSK-3β treatment. Myofilament enriched samples (isolated myofibrils) were prepared from HF_{dys} tissue as before. The sample from each dog was split into two equal parts, one incubated in labeled relax buffer, and the second incubated in labeled relax buffer plus 4 μg of GSK-3β. Labeled relax buffer was prepared by combining relax buffer with ATP γ-$P^{18}O$ (Cambridge Isotope Laboratories, Inc., Andover, MA). The labeled relax buffer contained a 1:1 ratio of unlabeled and ^{18}O labeled ATP (20 μmols of each, per sample). The samples were incubated at 30 °C for 30 minutes. Post treatment, samples were prepared, run on the mass spectrometer, and analyzed in the same manner as above, with one exception: searching also included an additional variable PTM: labeled phosphorylation that includes an additional 6.01 Da shift due to the ^{18}O-labeled phosphate group.

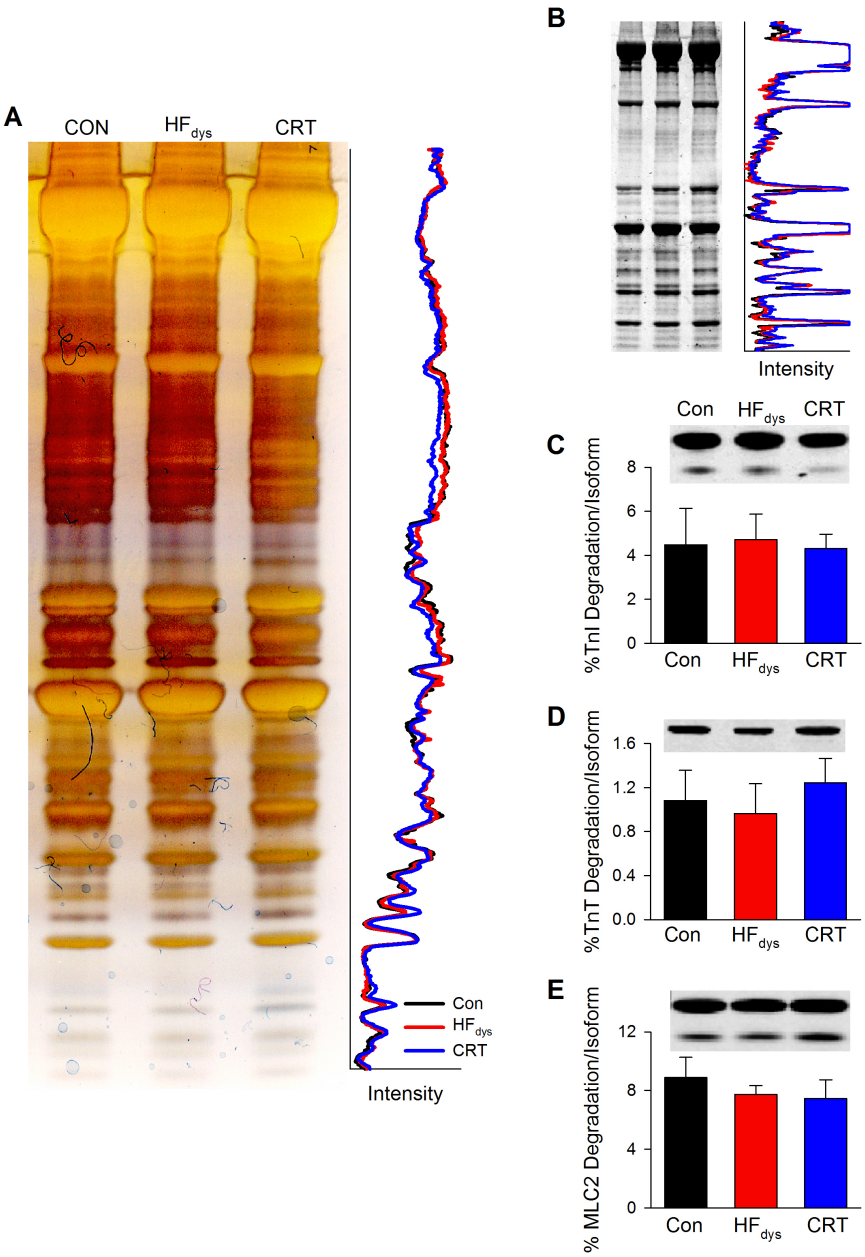
Part Two: Supplemental Figures



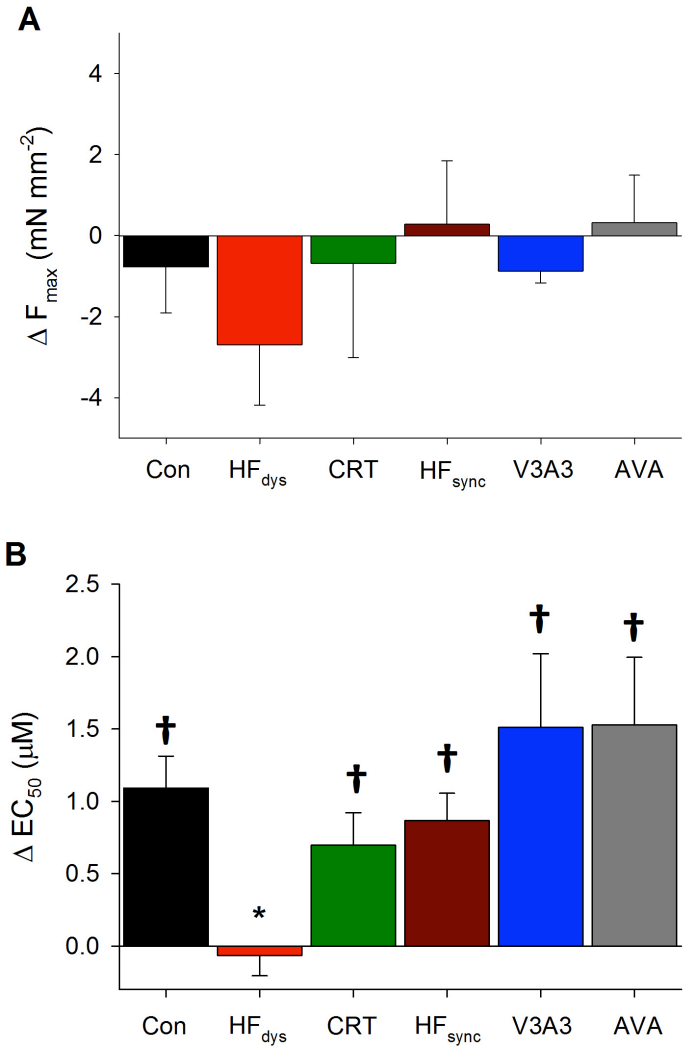
Supplemental Figure 1. Skinned myocytes from the left anterior septum exhibited the same functional changes as those from the LV lateral wall. (**A, B**) Force-calcium data and curves, showing (**A**) actual force and (**B**) normalized force. (**C**) Compared to Con (*n* = 8 myocytes from 3 dogs), maximum force was reduced in HF_{dys} (*n* = 7 myocytes from 3 dogs) and restored by CRT (*n* = 8 myocytes from 3 dogs). (**D**) HF_{dys} was desensitized to calcium, but CRT restored the value to near the Con value. *, $P < 0.05$ vs Con. #, $P < 0.05$ vs HF_{dys}.



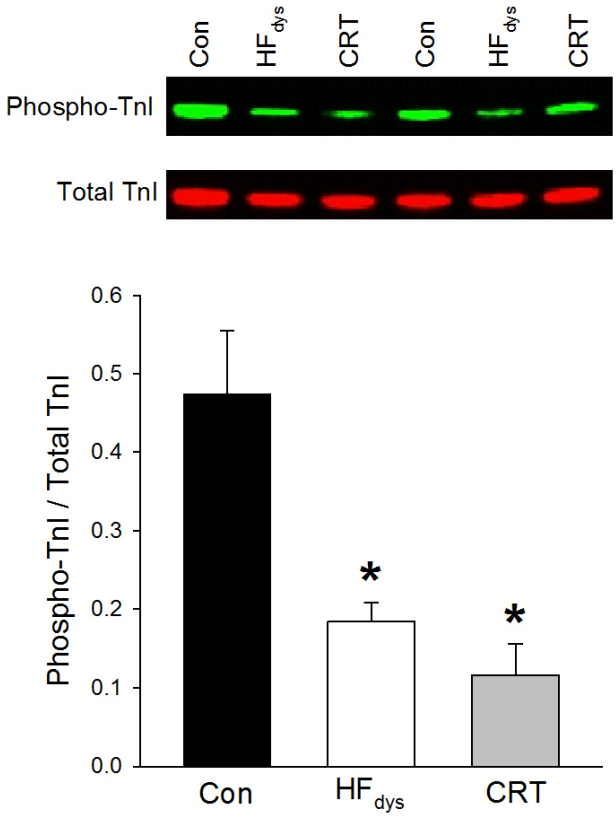
Supplemental Figure 2. Always synchronous heart failure (HF_{sync}) exhibited reduced myofilament function. (**A, B**) Force-calcium data and curves, showing (**A**) actual force and (**B**) normalized force. Con and HF_{dys} skinned myocytes shown for comparison to HF_{sync} (n = 15 myocytes from 5 dogs). (**C**) Maximum force was reduced similarly in both HF_{dys} and HF_{sync}. (**D**) HF_{sync} exhibited a hyper-sensitization to calcium. *, $P < 0.05$ vs Con. #, $P < 0.05$ vs HF_{dys}.



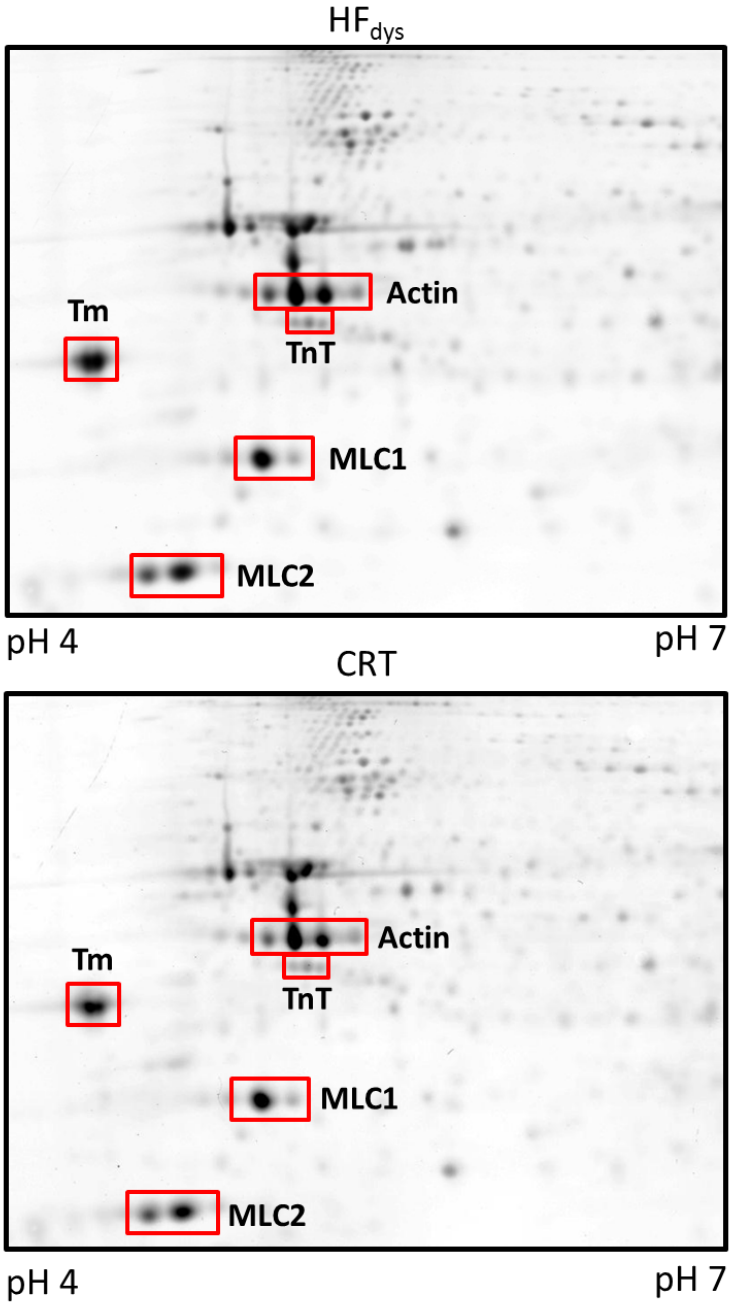
Supplemental Figure 3. There were no changes in protein degradation or isoforms between Control, HF_{dys}, and CRT. (**A**) High sensitivity silver stain of myofilament enriched samples and the gray-intensity for the each group. There were no differences in any bands between the three groups (*n* = 3 per group). (**B**) Lower sensitivity coomassie stain was also used to more accurately quantify the higher abundant proteins. The lack of changes was also confirmed with antibodies specific to (**C**) TnI, (**D**) TnT, and (**E**) MLC2, all of which showed secondary bands (representing 1-10% of the total) were was unchanged in HF_{dys} or CRT (*n* = 3 per group).



Supplemental Figure 4. The effect of PP1 in all dog models. (**A**) The change in F_{max} in each of the dog models in response to PP1 treatment. None of the groups showed any change in F_{max} post-treatment. (**B**) As presented in **Figure 5B**, Con and CRT each experienced desensitization to calcium in response to PP1 treatment, while there was no effect in HF_{dys}. Here, the other groups (HF_{sync}, V3A3, and AVA) also exhibited calcium desensitization in response to PP1 treatment. This suggests that the benefits of CRT are reversed similarly in the CRT variants (V3A3 and AVA). (†, $P < 0.05$ vs. 0. *, $P < 0.05$ vs. Con).

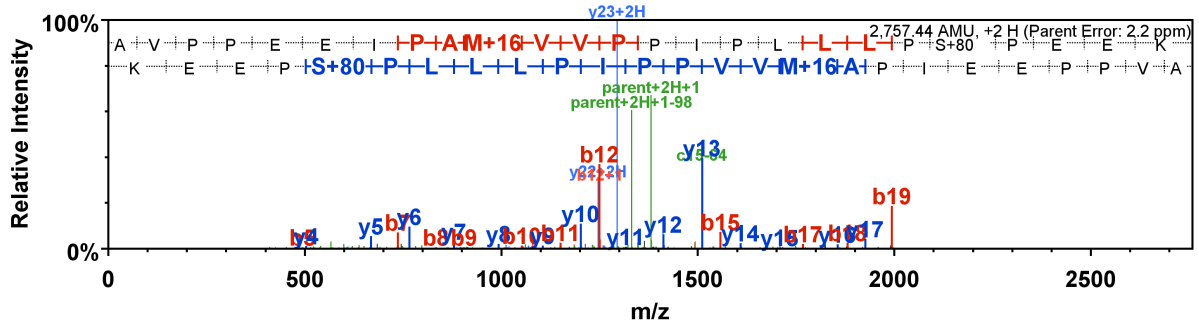


Supplemental Figure 5. The phos-Tag gel (**Figure 5C**) showed that overall TnI phosphorylation was decreased in HF_{dys} and CRT, but could not show whether the PKA or PKC sites specifically were decreasing. Antibodies specific to the S22/S23 PKA sites indicate that the PKA-phosphorylated form of TnI decreased in both HF_{dys} and CRT (n = 4 per group), confirming the data from the Phos-Tag gel. As PKA phosphorylation was unchanged between HF_{dys} and CRT, as was total phosphorylation, it suggests that there was also no change in any of the other highly phosphorylated sites. *, $P < 0.05$ vs. Con by one-way ANOVA.



Supplemental Figure 6. To confirm there were no phosphorylation changes among the most abundant myofilament proteins between HF_{dys} and CRT, 2D-DiGE gels were performed. The two images represent the HF_{dys} (top) and CRT (bottom) channels that were differentially labeled with Cy5 and Cy3, respectively, and run simultaneously on the same gel. The two channels have been independently presented here for clarity. Analysis by Ludesi REDFIN confirmed that there were no phosphorylation changes (*n* = 4 per group). The red boxes indicate a selection of myofilament proteins. Note: troponin I is not represented on these 2D gels because it has a very high pI.

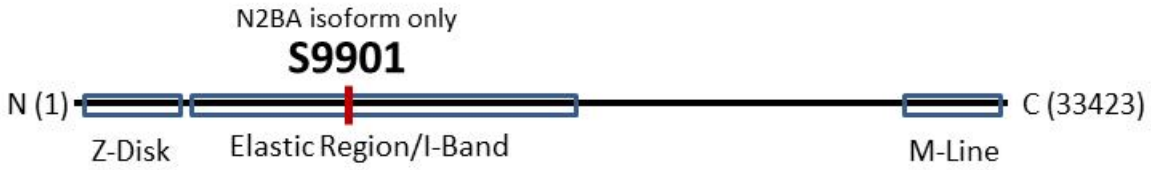
A



B

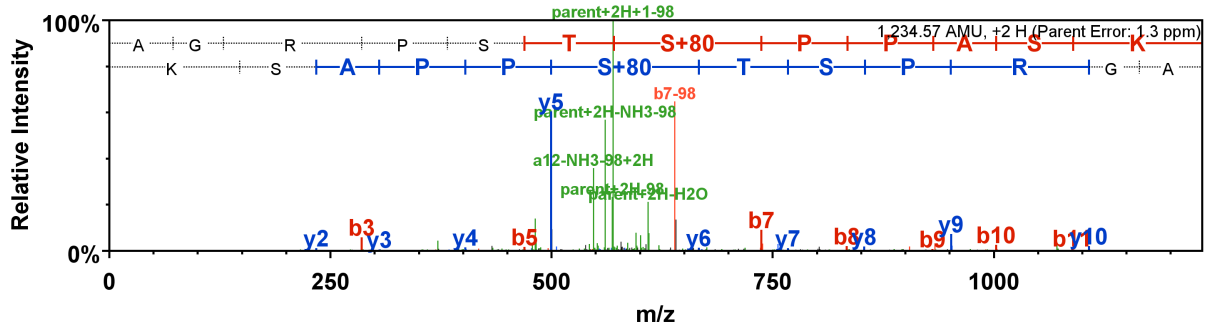
B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	72.04				A	2,758.44	1,379.72	2,741.41	2,740.43	25
2	171.11				V	2,687.40	1,344.20	2,670.38	2,669.39	24
3	268.17				P	2,588.33	1,294.67	2,571.31	2,570.32	23
4	365.22				P	2,491.28	1,246.14	2,474.25	2,473.27	22
5	494.26			476.25	E	2,394.23	1,197.62	2,377.20	2,376.22	21
6	623.3	312.16		605.29	E	2,265.19	1,133.10	2,248.16	2,247.18	20
7	736.39	368.7		718.38	I	2,136.14	1,068.58	2,119.12	2,118.13	19
8	833.44	417.22		815.43	P	2,023.06	1,012.03	2,006.03	2,005.05	18
9	904.48	452.74		886.47	A	1,926.01	963.51	1,908.98	1,908.00	17
10	1,051.51	526.26		1,033.50	M+16	1,854.97	927.99	1,837.94	1,836.96	16
11	1,150.58	575.79		1,132.57	V	1,707.93	854.47	1,690.91	1,689.92	15
12	1,249.65	625.33		1,231.64	V	1,608.87	804.94	1,591.84	1,590.85	14
13	1,346.70	673.85		1,328.69	P	1,509.80	755.4	1,492.77	1,491.79	13
14	1,443.76	722.38		1,425.74	P	1,412.74	706.88	1,395.72	1,394.73	12
15	1,556.84	778.92		1,538.83	I	1,315.69	658.35	1,298.66	1,297.68	11
16	1,653.89	827.45		1,635.88	P	1,202.61	601.81	1,185.58	1,184.60	10
17	1,766.98	883.99		1,748.97	L	1,105.55	553.28	1,088.53	1,087.54	9
18	1,880.06	940.53		1,862.05	L	992.47	496.74	975.44	974.46	8
19	1,993.14	997.08		1,975.13	L	879.39	440.2	862.36	861.38	7
20	2,090.20	1,045.60		2,072.19	P	766.3	383.65	749.28	748.29	6
21	2,257.20	1,129.10		2,239.19	S+80	669.25		652.22	651.24	5
22	2,354.25	1,177.63		2,336.24	P	502.25		485.22	484.24	4
23	2,483.29	1,242.15		2,465.28	E	405.2		388.17	387.19	3
24	2,612.33	1,306.67		2,594.32	E	276.16		259.13	258.14	2
25	2,758.44	1,379.72	2,741.41	2,740.43	K	147.11		130.09		1

C



Supplemental Figure 7. Mass spectrometry (MS) data for phosphorylation at Titin (N2BA isoform) S9901. (**A**) MS spectra for the peptide containing S9901. (**B**) Fragmentation Table. (**C**) Known domains and protein binding regions shown with location of the identified phosphorylation site.

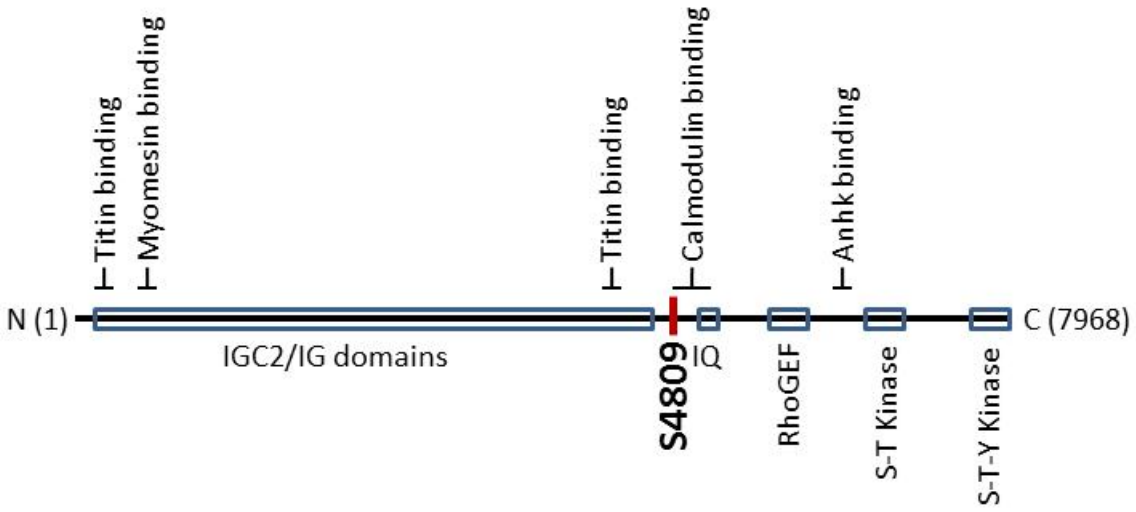
A



B

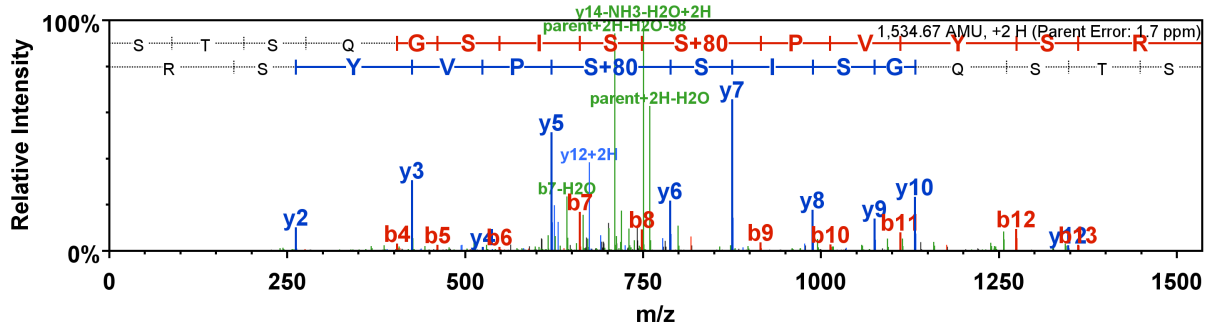
B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	72.04				A	1,235.58	618.29	1,218.55	1,217.57	12
2	129.07				G	1,164.54	582.77	1,147.51	1,146.53	11
3	285.17	143.09	268.14		R	1,107.52	554.26	1,090.49	1,089.51	10
4	382.22	191.61	365.19		P	951.42	476.21	934.39	933.41	9
5	469.25	235.13	452.23	451.24	S	854.37	427.69	837.34	836.36	8
6	570.3	285.65	553.27	552.29	T	767.33	384.17	750.31	749.32	7
7	737.3	369.15	720.27	719.29	S+80	666.29	333.65	649.26	648.28	6
8	834.35	417.68	817.32	816.34	P	499.29		482.26	481.28	5
9	931.4	466.21	914.38	913.39	P	402.23		385.21	384.22	4
10	1,002.44	501.72	985.41	984.43	A	305.18		288.16	287.17	3
11	1,089.47	545.24	1,072.45	1,071.46	S	234.14		217.12	216.13	2
12	1,235.58	618.29	1,218.55	1,217.57	K	147.11		130.09		1

C



Supplemental Figure 8. Mass spectrometry (MS) data for phosphorylation at Obscurin S4809. (**A**) MS spectra for the peptide containing S4809. (**B**) Fragmentation Table. (**C**) Known domains and protein binding regions shown with location of the identified phosphorylation site.

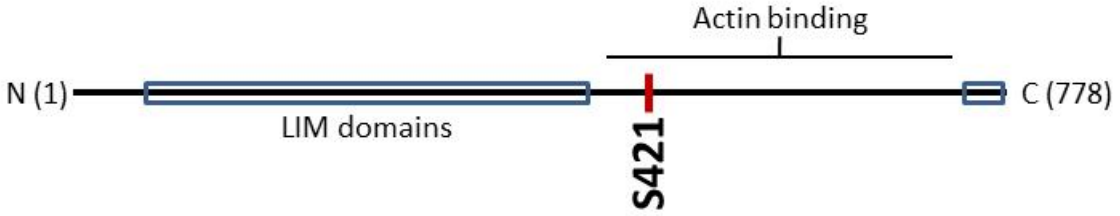
A



B

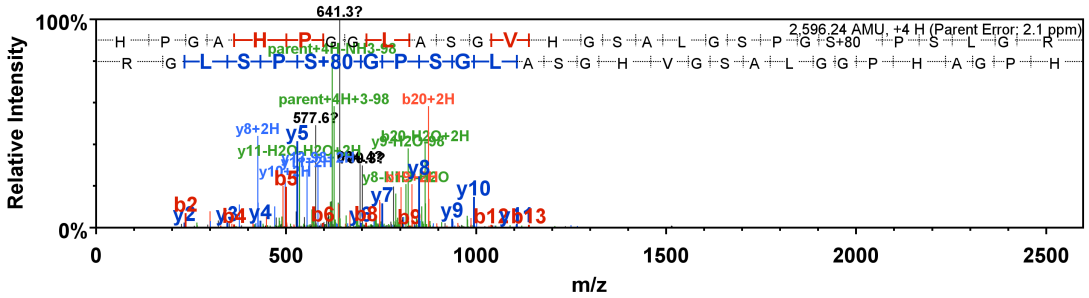
B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	88.04			70.03	S	1,535.67	768.34	1,518.65	1,517.66	14
2	189.09			171.08	T	1,448.64	724.82	1,431.61	1,430.63	13
3	276.12			258.11	S	1,347.59	674.3	1,330.57	1,329.58	12
4	404.18		387.15	386.17	Q	1,260.56	630.78	1,243.54	1,242.55	11
5	461.2		444.17	443.19	G	1,132.50	566.76	1,115.48	1,114.49	10
6	548.23	274.62	531.2	530.22	S	1,075.48	538.24	1,058.46	1,057.47	9
7	661.32	331.16	644.29	643.3	I	988.45	494.73	971.42	970.44	8
8	748.35	374.68	731.32	730.34	S	875.37	438.19	858.34	857.36	7
9	915.35	458.18	898.32	897.33	S+80	788.33	394.67	771.31	770.32	6
10	1,012.40	506.7	995.37	994.39	P	621.34		604.31	603.32	5
11	1,111.47	556.24	1,094.44	1,093.46	V	524.28		507.26	506.27	4
12	1,274.53	637.77	1,257.50	1,256.52	Y	425.21		408.19	407.2	3
13	1,361.56	681.28	1,344.54	1,343.55	S	262.15		245.12	244.14	2
14	1,535.67	768.34	1,518.65	1,517.66	R	175.12		158.09		1

C



Supplemental Figure 9. Mass spectrometry (MS) data for phosphorylation at ABLIM1 S421. (**A**) MS spectra for the peptide containing S421. (**B**) Fragmentation Table. (**C**) Known domains and protein binding regions shown with location of the identified phosphorylation site.

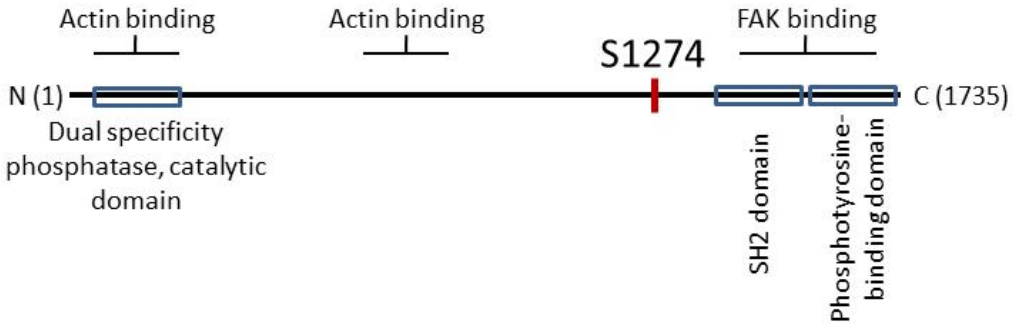
A



B

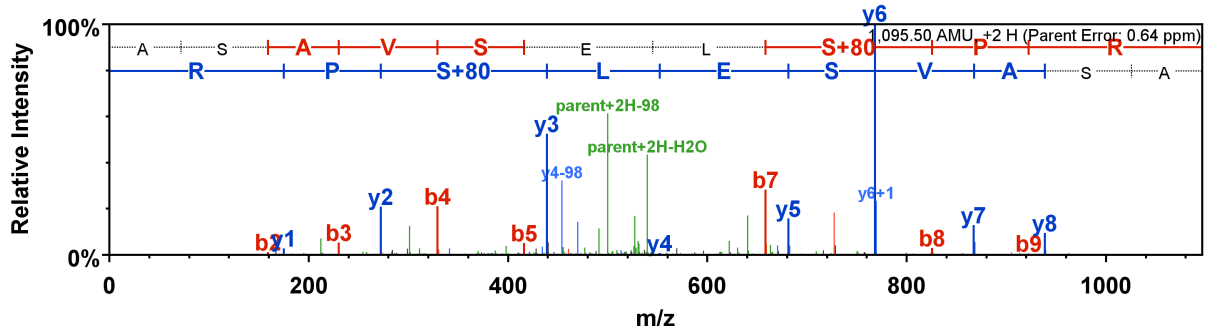
B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	138.07	69.54			H	2,597.24	1,299.12	2,580.21	2,579.23	28
2	235.12	118.06			P	2,460.18	1,230.59	2,443.15	2,442.17	27
3	292.14	146.57			G	2,363.13	1,182.07	2,346.10	2,345.11	26
4	363.18	182.09			A	2,306.10	1,153.56	2,289.08	2,288.09	25
5	500.24	250.62			H	2,235.07	1,118.04	2,218.04	2,217.06	24
6	597.29	299.15			P	2,098.01	1,049.51	2,080.98	2,080.00	23
7	654.31	327.66			G	2,000.95	1,000.98	1,983.93	1,982.94	22
8	711.33	356.17			G	1,943.93	972.47	1,926.91	1,925.92	21
9	824.42	412.71			L	1,886.91	943.96	1,869.89	1,868.90	20
10	895.45	448.23			A	1,773.83	887.42	1,756.80	1,755.82	19
11	982.49	491.75		964.47	S	1,702.79	851.9	1,685.76	1,684.78	18
12	1,039.51	520.26		1,021.50	G	1,615.76	808.38	1,598.73	1,597.75	17
13	1,138.58	569.79		1,120.56	V	1,558.74	779.87	1,541.71	1,540.73	16
14	1,275.63	638.32		1,257.62	H	1,459.67	730.34	1,442.64	1,441.66	15
15	1,332.66	666.83		1,314.65	G	1,322.61	661.81	1,305.58	1,304.60	14
16	1,419.69	710.35		1,401.68	S	1,265.59	633.3	1,248.56	1,247.58	13
17	1,490.72	745.87		1,472.71	A	1,178.56	589.78	1,161.53	1,160.55	12
18	1,603.81	802.41		1,585.80	L	1,107.52	554.26	1,090.49	1,089.51	11
19	1,660.83	830.92		1,642.82	G	994.44	497.72	977.41	976.42	10
20	1,747.86	874.43		1,729.85	S	937.41	469.21	920.39	919.4	9
21	1,844.92	922.96		1,826.90	P	850.38	425.69	833.36	832.37	8
22	1,901.94	951.47		1,883.93	G	753.33	377.17	736.3	735.32	7
23	2,068.94	1,034.97		2,050.92	S+80	696.31	348.66	679.28	678.3	6
24	2,165.99	1,083.50		2,147.98	P	529.31		512.28	511.3	5
25	2,253.02	1,127.01		2,235.01	S	432.26		415.23	414.25	4
26	2,366.10	1,183.56		2,348.09	L	345.22		328.2		3
27	2,423.13	1,212.07		2,405.11	G	232.14		215.11		2
28	2,597.24	1,299.12	2,580.21	2,579.23	R	175.12		158.09		1

C



Supplemental Figure 10. Mass spectrometry (MS) data for phosphorylation at Tensin-1 S1274. (**A**) MS spectra for the peptide containing S1274. (**B**) Fragmentation Table. (**C**) Known domains and protein binding regions shown with location of the identified phosphorylation site.

A



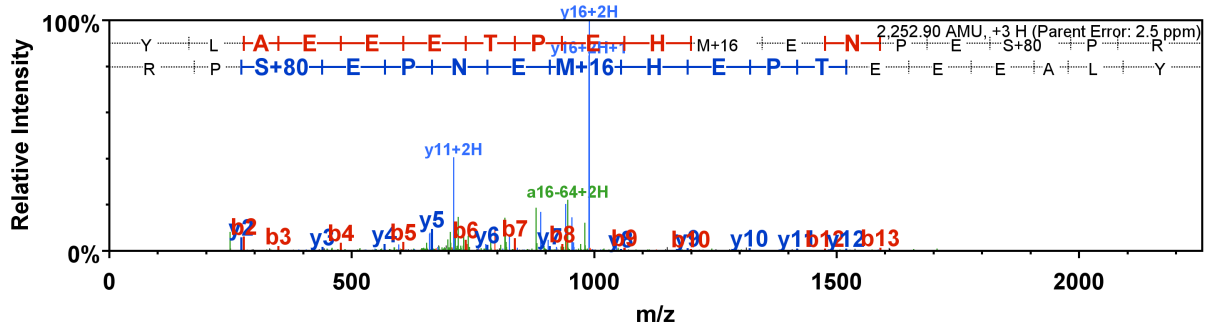
B

B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	72.04				A	1,096.50	548.76	1,079.48	1,078.49	10
2	159.08			141.07	S	1,025.47	513.24	1,008.44	1,007.46	9
3	230.11			212.1	A	938.43	469.72	921.41	920.42	8
4	329.18			311.17	V	867.4	434.2	850.37	849.39	7
5	416.21			398.2	S	768.33	384.67	751.3	750.32	6
6	545.26	273.13		527.25	E	681.3		664.27	663.29	5
7	658.34	329.67		640.33	L	552.25		535.23	534.24	4
8	825.34	413.17		807.33	S+80	439.17		422.14	421.16	3
9	922.39	461.7		904.38	P	272.17		255.15		2
10	1,096.50	548.76	1,079.48	1,078.49	R	175.12		158.09		1

C

Supplemental Figure 11. Mass spectrometry (MS) data for phosphorylation at TRAP3 S244. (**A**) MS spectra for the peptide containing S244. (**B**) Fragmentation Table. (**C**) Known domains and protein binding regions shown with location of the identified phosphorylation site.

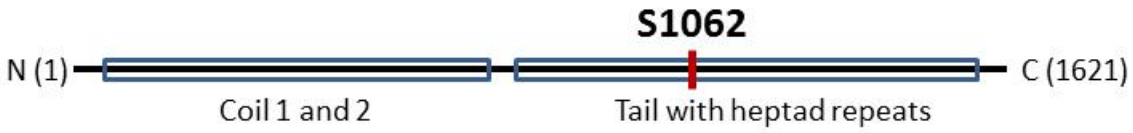
A



B

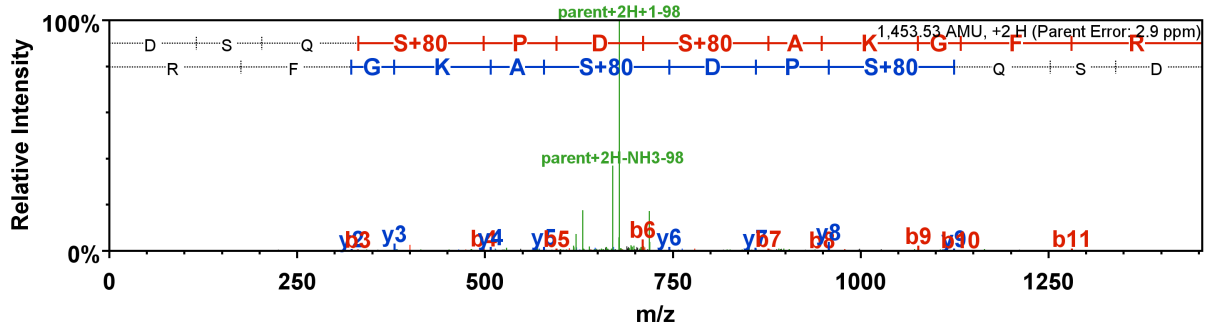
B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	164.07				Y	2,253.90	1,127.45	2,236.87	2,235.89	18
2	277.15				L	2,090.84	1,045.92	2,073.81	2,072.83	17
3	348.19				A	1,977.75	989.38	1,960.73	1,959.74	16
4	477.23			459.22	E	1,906.72	953.86	1,889.69	1,888.71	15
5	606.28			588.27	E	1,777.67	889.34	1,760.65	1,759.66	14
6	735.32	368.16		717.31	E	1,648.63	824.82	1,631.60	1,630.62	13
7	836.37	418.69		818.36	T	1,519.59	760.3	1,502.56	1,501.58	12
8	933.42	467.21		915.41	P	1,418.54	709.77	1,401.51	1,400.53	11
9	1,062.46	531.73		1,044.45	E	1,321.49	661.25	1,304.46	1,303.48	10
10	1,199.52	600.26		1,181.51	H	1,192.45	596.73	1,175.42	1,174.43	9
11	1,346.56	673.78		1,328.55	M+16	1,055.39	528.2	1,038.36	1,037.38	8
12	1,475.60	738.3		1,457.59	E	908.35	454.68	891.32	890.34	7
13	1,589.64	795.32	1,572.62	1,571.63	N	779.31	390.16	762.28	761.3	6
14	1,686.70	843.85	1,669.67	1,668.68	P	665.27		648.24	647.25	5
15	1,815.74	908.37	1,798.71	1,797.73	E	568.21		551.19	550.2	4
16	1,982.74	991.87	1,965.71	1,964.73	S+80	439.17		422.14	421.16	3
17	2,079.79	1,040.40	2,062.76	2,061.78	P	272.17		255.15		2
18	2,253.90	1,127.45	2,236.87	2,235.89	R	175.12		158.09		1

C



Supplemental Figure 12. Mass spectrometry (MS) data for phosphorylation at Nestin S1062. (**A**) MS spectra for the peptide containing S1062. (**B**) Fragmentation Table. (**C**) Known domains and protein binding regions shown with location of the identified phosphorylation site.

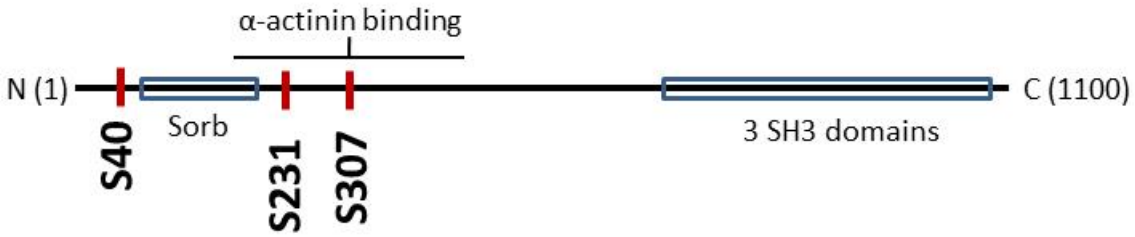
A



B

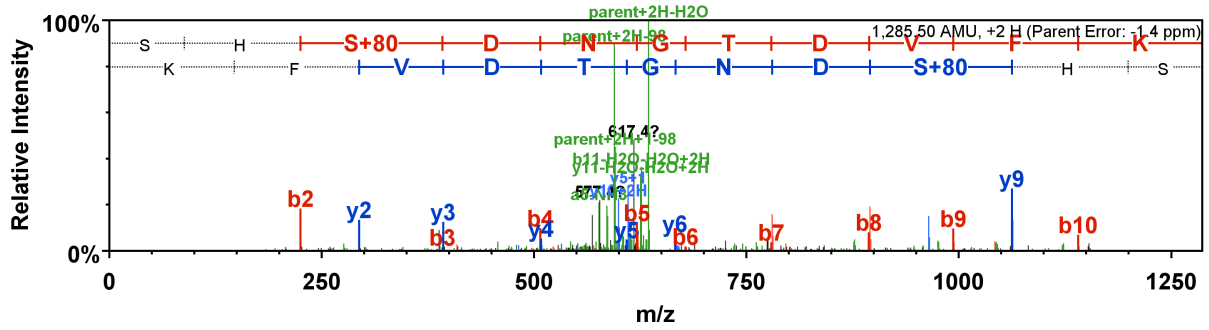
B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	116.03			98.02	D	1,454.53	727.77	1,437.51	1,436.52	12
2	203.07			185.06	S	1,339.51	670.26	1,322.48	1,321.50	11
3	331.12		314.1	313.11	Q	1,252.48	626.74	1,235.45	1,234.47	10
4	498.12		481.1	480.11	S+80	1,124.42	562.71	1,107.39	1,106.41	9
5	595.18		578.15	577.17	P	957.42	479.21	940.39	939.41	8
6	710.2	355.61	693.18	692.19	D	860.37	430.69	843.34	842.36	7
7	877.2	439.1	860.17	859.19	S+80	745.34	373.17	728.31	727.33	6
8	948.24	474.62	931.21	930.23	A	578.34	289.67	561.31		5
9	1,076.33	538.67	1,059.31	1,058.32	K	507.3	254.16	490.28		4
10	1,133.35	567.18	1,116.33	1,115.34	G	379.21		362.18		3
11	1,280.42	640.72	1,263.40	1,262.41	F	322.19		305.16		2
12	1,454.53	727.77	1,437.51	1,436.52	R	175.12		158.09		1

C



Supplemental Figure 13. Mass spectrometry (MS) data for phosphorylation at SORB2 S40. (**A**) MS spectra for the peptide containing S40. (**B**) Fragmentation Table. (**C**) Known domains and protein binding regions shown with location of the identified phosphorylation site.

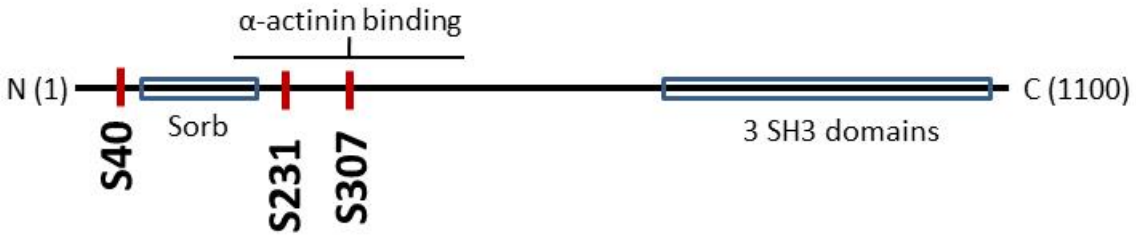
A



B

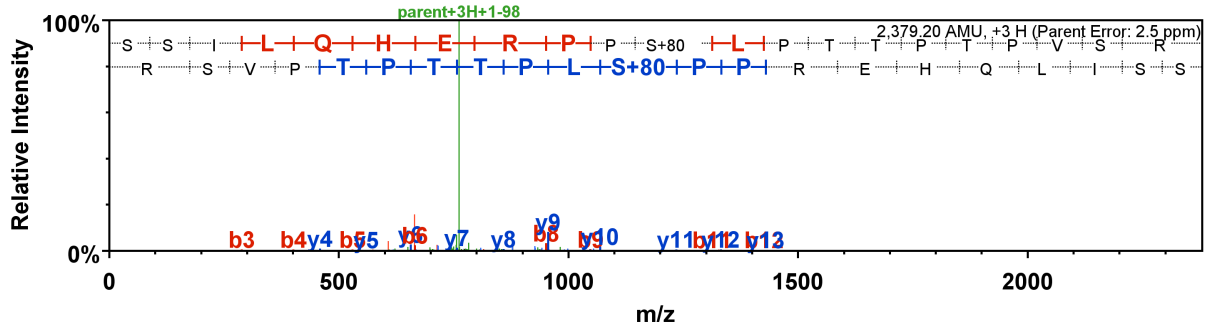
B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	88.04			70.03	S	1,286.50	643.76	1,269.48	1,268.49	11
2	225.1	113.05		207.09	H	1,199.47	600.24	1,182.45	1,181.46	10
3	392.1	196.55		374.09	S+80	1,062.41	531.71	1,045.39	1,044.40	9
4	507.12	254.07		489.11	D	895.42	448.21	878.39	877.41	8
5	621.17	311.09	604.14	603.16	N	780.39	390.7	763.36	762.38	7
6	678.19	339.6	661.16	660.18	G	666.35	333.68	649.32	648.34	6
7	779.24	390.12	762.21	761.23	T	609.32		592.3	591.31	5
8	894.26	447.63	877.24	876.25	D	508.28		491.25	490.27	4
9	993.33	497.17	976.3	975.32	V	393.25		376.22		3
10	1,140.40	570.7	1,123.37	1,122.39	F	294.18		277.15		2
11	1,286.51	643.76	1,269.48	1,268.49	K	147.11		130.09		1

C



Supplemental Figure 14. Mass spectrometry (MS) data for phosphorylation at SORB2 S231. (**A**) MS spectra for the peptide containing S231. (**B**) Fragmentation Table. (**C**) Known domains and protein binding regions shown with location of the identified phosphorylation site.

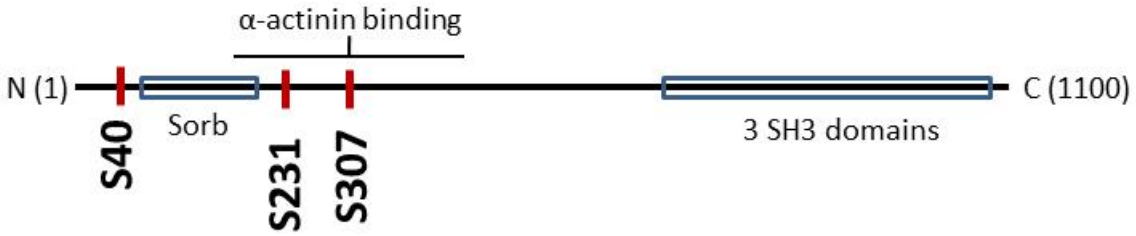
A



B

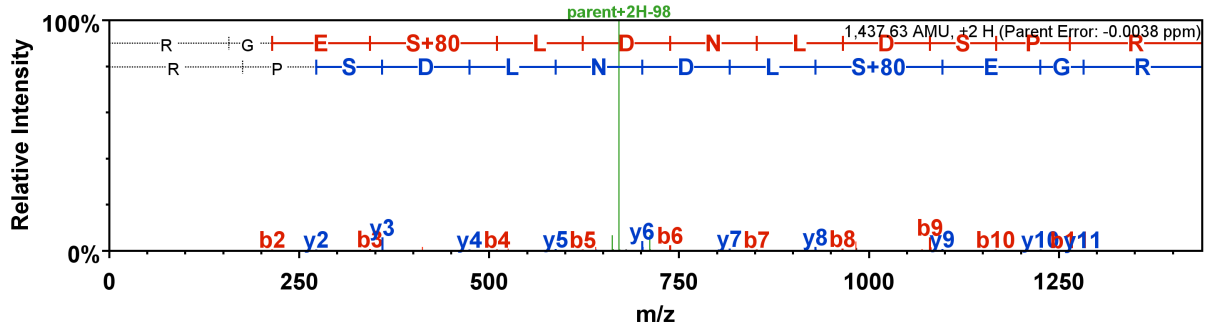
B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	88.04			70.03	S	2,380.20	1,190.60	2,363.18	2,362.19	21
2	175.07			157.06	S	2,293.17	1,147.09	2,276.14	2,275.16	20
3	288.16			270.14	I	2,206.14	1,103.57	2,189.11	2,188.13	19
4	401.24			383.23	L	2,093.05	1,047.03	2,076.03	2,075.04	18
5	529.3		512.27	511.29	Q	1,979.97	990.49	1,962.94	1,961.96	17
6	666.36	333.68	649.33	648.35	H	1,851.91	926.46	1,834.88	1,833.90	16
7	795.4	398.2	778.37	777.39	E	1,714.85	857.93	1,697.83	1,696.84	15
8	951.5	476.25	934.47	933.49	R	1,585.81	793.41	1,568.78	1,567.80	14
9	1,048.55	524.78	1,031.53	1,030.54	P	1,429.71	715.36	1,412.68	1,411.70	13
10	1,145.61	573.31	1,128.58	1,127.60	P	1,332.66	666.83	1,315.63	1,314.65	12
11	1,312.60	656.81	1,295.58	1,294.59	S+80	1,235.60	618.31	1,218.58	1,217.59	11
12	1,425.69	713.35	1,408.66	1,407.68	L	1,068.60	534.81	1,051.58	1,050.59	10
13	1,522.74	761.87	1,505.72	1,504.73	P	955.52	478.26	938.49	937.51	9
14	1,623.79	812.4	1,606.76	1,605.78	T	858.47	429.74	841.44	840.46	8
15	1,724.84	862.92	1,707.81	1,706.83	T	757.42	379.21	740.39	739.41	7
16	1,821.89	911.45	1,804.86	1,803.88	P	656.37	328.69	639.35	638.36	6
17	1,922.94	961.97	1,905.91	1,904.93	T	559.32		542.29	541.31	5
18	2,019.99	1,010.50	2,002.96	2,001.98	P	458.27		441.25	440.26	4
19	2,119.06	1,060.03	2,102.03	2,101.05	V	361.22		344.19	343.21	3
20	2,206.09	1,103.55	2,189.06	2,188.08	S	262.15		245.12	244.14	2
21	2,380.20	1,190.60	2,363.18	2,362.19	R	175.12		158.09		1

C



Supplemental Figure 15. Mass spectrometry (MS) data for phosphorylation at SORB2 S307. (**A**) MS spectra for the peptide containing S307. (**B**) Fragmentation Table. (**C**) Known domains and protein binding regions shown with location of the identified phosphorylation site.

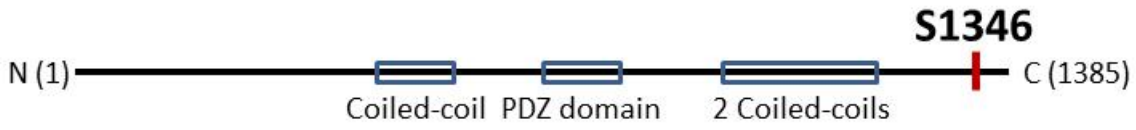
A



B

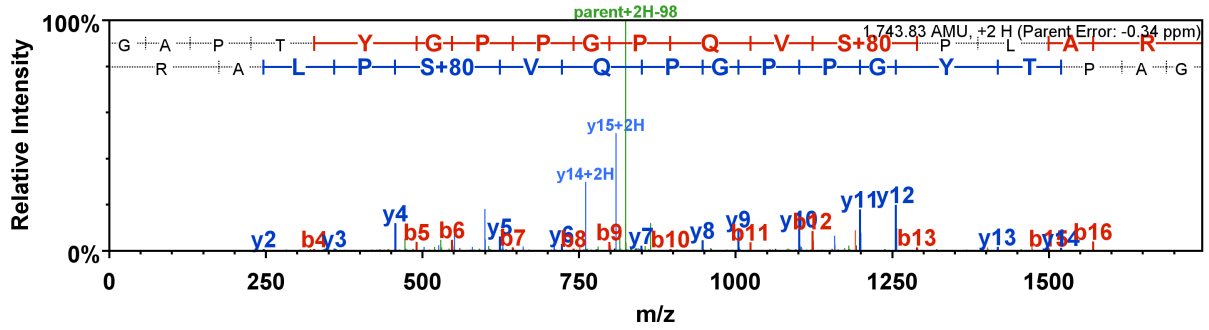
B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	157.11	79.06	140.08		R	1,438.63	719.82	1,421.61	1,420.62	12
2	214.13	107.57	197.1		G	1,282.53	641.77	1,265.50	1,264.52	11
3	343.17	172.09	326.15	325.16	E	1,225.51	613.26	1,208.48	1,207.50	10
4	510.17	255.59	493.14	492.16	S+80	1,096.47	548.74	1,079.44	1,078.46	9
5	623.25	312.13	606.23	605.24	L	929.47	465.24	912.44	911.46	8
6	738.28	369.64	721.26	720.27	D	816.38	408.7	799.36	798.37	7
7	852.32	426.67	835.3	834.31	N	701.36	351.18	684.33	683.35	6
8	965.41	483.21	948.38	947.4	L	587.31		570.29	569.3	5
9	1,080.44	540.72	1,063.41	1,062.43	D	474.23		457.2	456.22	4
10	1,167.47	584.24	1,150.44	1,149.46	S	359.2		342.18	341.19	3
11	1,264.52	632.76	1,247.49	1,246.51	P	272.17		255.15		2
12	1,438.63	719.82	1,421.61	1,420.62	R	175.12		158.09		1

C



Supplemental Figure 16. Mass spectrometry (MS) data for phosphorylation at LMO7 S1346. (**A**) MS spectra for the peptide containing S1346. (**B**) Fragmentation Table. (**C**) Known domains and protein binding regions shown with location of the identified phosphorylation site.

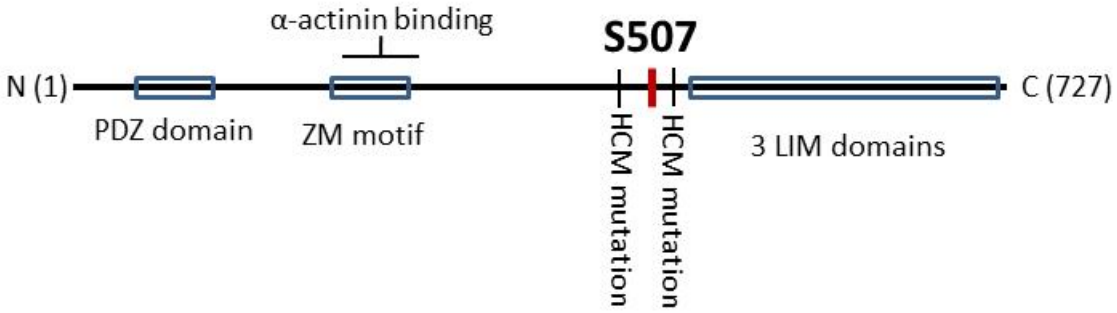
A



B

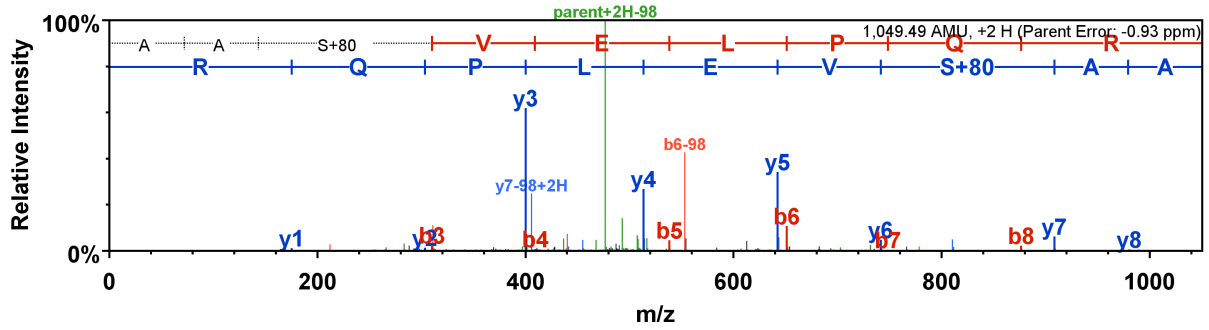
B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	58.03				G	1,744.84	872.92	1,727.82	1,726.83	17
2	129.07				A	1,687.82	844.41	1,670.79	1,669.81	16
3	226.12				P	1,616.78	808.9	1,599.76	1,598.77	15
4	327.17			309.16	T	1,519.73	760.37	1,502.70	1,501.72	14
5	490.23			472.22	Y	1,418.68	709.85	1,401.66	1,400.67	13
6	547.25	274.13		529.24	G	1,255.62	628.31	1,238.59	1,237.61	12
7	644.3	322.66		626.29	P	1,198.60	599.8	1,181.57	1,180.59	11
8	741.36	371.18		723.35	P	1,101.55	551.28	1,084.52	1,083.53	10
9	798.38	399.69		780.37	G	1,004.49	502.75	987.47	986.48	9
10	895.43	448.22		877.42	P	947.47	474.24	930.44	929.46	8
11	1,023.49	512.25	1,006.46	1,005.48	Q	850.42	425.71	833.39	832.41	7
12	1,122.56	561.78	1,105.53	1,104.55	V	722.36	361.68	705.33	704.35	6
13	1,289.56	645.28	1,272.53	1,271.55	S+80	623.29		606.26	605.28	5
14	1,386.61	693.81	1,369.58	1,368.60	P	456.29		439.27		4
15	1,499.69	750.35	1,482.67	1,481.68	L	359.24		342.21		3
16	1,570.73	785.87	1,553.70	1,552.72	A	246.16		229.13		2
17	1,744.84	872.92	1,727.82	1,726.83	R	175.12		158.09		1

C



Supplemental Figure 17. Mass spectrometry (MS) data for phosphorylation at LDB3 (Cypher/Zasp) S507. (**A**) MS spectra for the peptide containing S507. (**B**) Fragmentation Table. (**C**) Known domains and protein binding regions shown with location of the identified phosphorylation site.

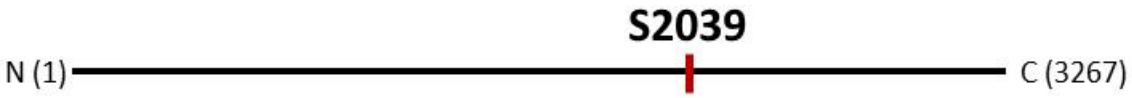
A



B

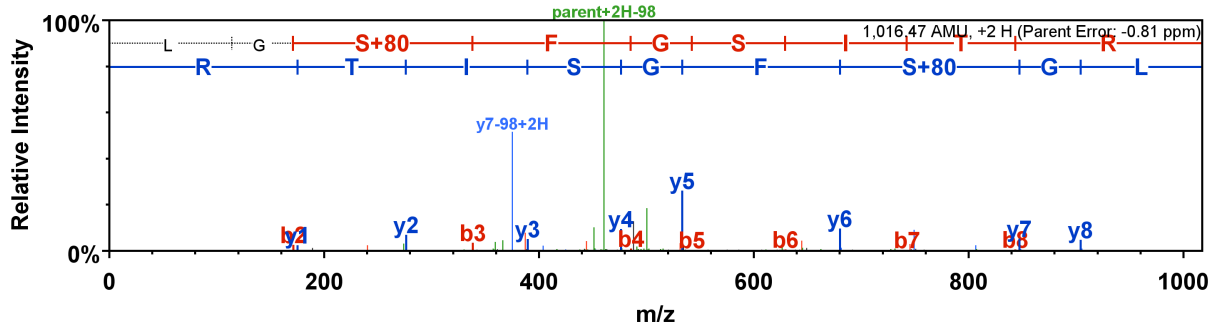
B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	72.04				A	1,050.50	525.75	1,033.47	1,032.49	9
2	143.08				A	979.46	490.23	962.43	961.45	8
3	310.08			292.07	S+80	908.42	454.72	891.4	890.41	7
4	409.15			391.14	V	741.43	371.22	724.4	723.41	6
5	538.19			520.18	E	642.36		625.33	624.35	5
6	651.27	326.14		633.26	L	513.31		496.29		4
7	748.33	374.67		730.32	P	400.23		383.2		3
8	876.39	438.7	859.36	858.38	Q	303.18		286.15		2
9	1,050.50	525.75	1,033.47	1,032.49	R	175.12		158.09		1

C



Supplemental Figure 18. Mass spectrometry (MS) data for phosphorylation at SPEG S2039. (**A**) MS spectra for the peptide containing SS2039. (**B**) Fragmentation Table. (**C**) Known domains and protein binding regions shown with location of the identified phosphorylation site.

A



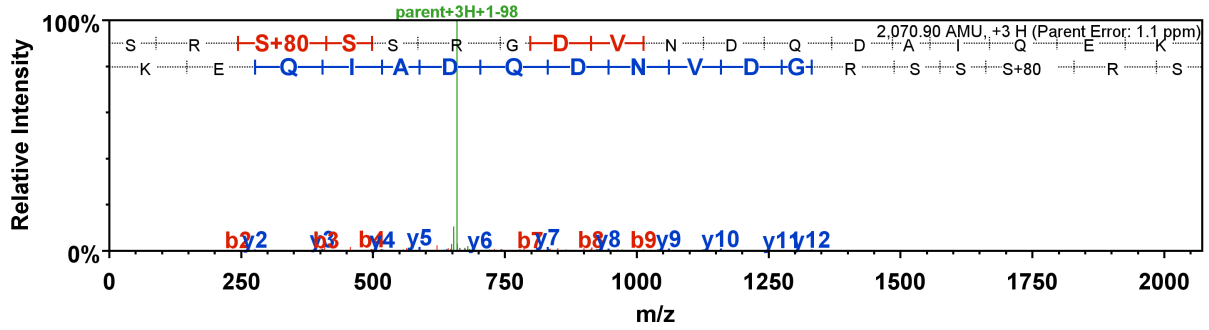
B

B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	114.09				L	1,017.48	509.24	1,000.45	999.47	9
2	171.11				G	904.39	452.7	887.37	886.38	8
3	338.11			320.1	S+80	847.37	424.19	830.34	829.36	7
4	485.18			467.17	F	680.37	340.69	663.35	662.36	6
5	542.2			524.19	G	533.3		516.28	515.29	5
6	629.23	315.12		611.22	S	476.28		459.26	458.27	4
7	742.32	371.66		724.31	I	389.25		372.22	371.24	3
8	843.36	422.19		825.35	T	276.17		259.14	258.16	2
9	1,017.48	509.24	1,000.45	999.47	R	175.12		158.09		1

C

Supplemental Figure 19. Mass spectrometry (MS) data for phosphorylation at Filamin-C S2228. (**A**) MS spectra for the peptide containing S2228. (**B**) Fragmentation Table. (**C**) Known domains and protein binding regions shown with location of the identified phosphorylation site.

A



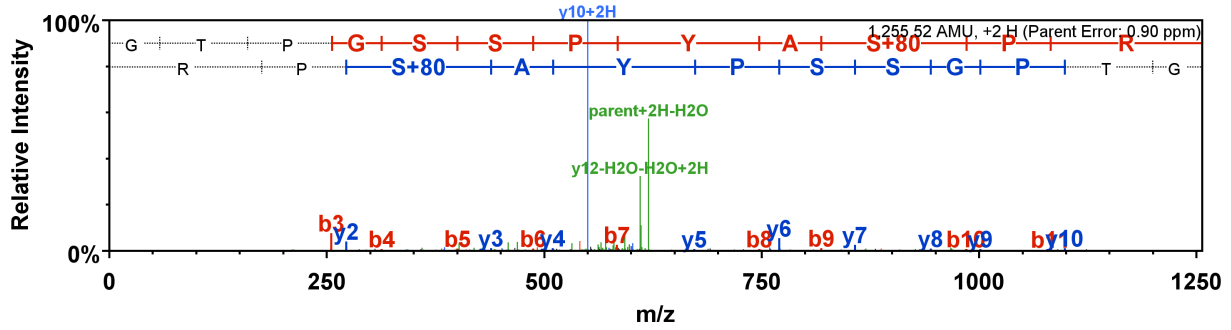
B

B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	88.04			70.03	S	2,071.90	1,036.46	2,054.88	2,053.89	18
2	244.14	122.57	227.11	226.13	R	1,984.87	992.94	1,967.85	1,966.86	17
3	411.14	206.07	394.11	393.13	S+80	1,828.77	914.89	1,811.74	1,810.76	16
4	498.17	249.59	481.14	480.16	S	1,661.77	831.39	1,644.75	1,643.76	15
5	585.2	293.1	568.18	567.19	S	1,574.74	787.87	1,557.71	1,556.73	14
6	741.3	371.16	724.28	723.29	R	1,487.71	744.36	1,470.68	1,469.70	13
7	798.33	399.67	781.3	780.31	G	1,331.61	666.31	1,314.58	1,313.60	12
8	913.35	457.18	896.33	895.34	D	1,274.59	637.8	1,257.56	1,256.58	11
9	1,012.42	506.71	995.39	994.41	V	1,159.56	580.28	1,142.53	1,141.55	10
10	1,126.46	563.74	1,109.44	1,108.45	N	1,060.49	530.75	1,043.46	1,042.48	9
11	1,241.49	621.25	1,224.46	1,223.48	D	946.45	473.73	929.42	928.44	8
12	1,369.55	685.28	1,352.52	1,351.54	Q	831.42	416.21	814.39	813.41	7
13	1,484.58	742.79	1,467.55	1,466.57	D	703.36	352.18	686.34	685.35	6
14	1,555.61	778.31	1,538.59	1,537.60	A	588.34		571.31	570.32	5
15	1,668.70	834.85	1,651.67	1,650.69	I	517.3		500.27	499.29	4
16	1,796.76	898.88	1,779.73	1,778.75	Q	404.21		387.19	386.2	3
17	1,925.80	963.4	1,908.77	1,907.79	E	276.16		259.13	258.14	2
18	2,071.90	1,036.46	2,054.88	2,053.89	K	147.11		130.09		1

C

Supplemental Figure 20. Mass spectrometry (MS) data for phosphorylation at Myotilin S231. (**A**) MS spectra for the peptide containing S231. (**B**) Fragmentation Table. (**C**) Known domains and protein binding regions shown with location of the identified phosphorylation site.

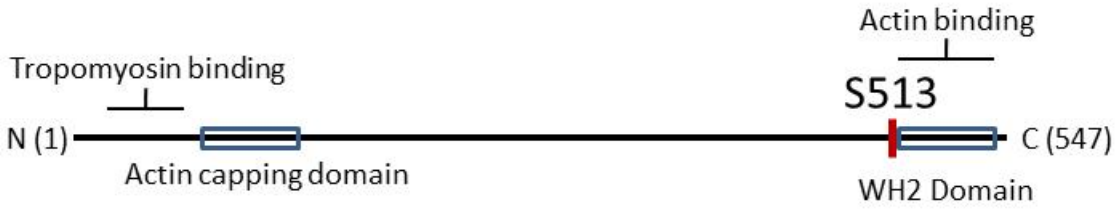
A



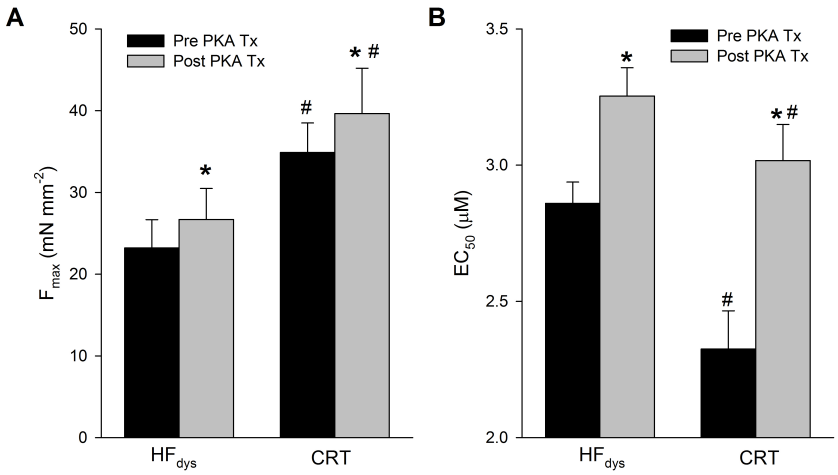
B

B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	58.03				G	1,256.53	628.77	1,239.50	1,238.52	12
2	159.08			141.07	T	1,199.51	600.26	1,182.48	1,181.50	11
3	256.13			238.12	P	1,098.46	549.73	1,081.44	1,080.45	10
4	313.15			295.14	G	1,001.41	501.21	984.38	983.4	9
5	400.18			382.17	S	944.39	472.7	927.36	926.38	8
6	487.21	244.11		469.2	S	857.36	429.18	840.33	839.34	7
7	584.27	292.64		566.26	P	770.32	385.67	753.3	752.31	6
8	747.33	374.17		729.32	Y	673.27		656.24	655.26	5
9	818.37	409.69		800.36	A	510.21		493.18	492.2	4
10	985.37	493.19		967.36	S+80	439.17		422.14	421.16	3
11	1,082.42	541.71		1,064.41	P	272.17		255.15		2
12	1,256.53	628.77	1,239.50	1,238.52	R	175.12		158.09		1

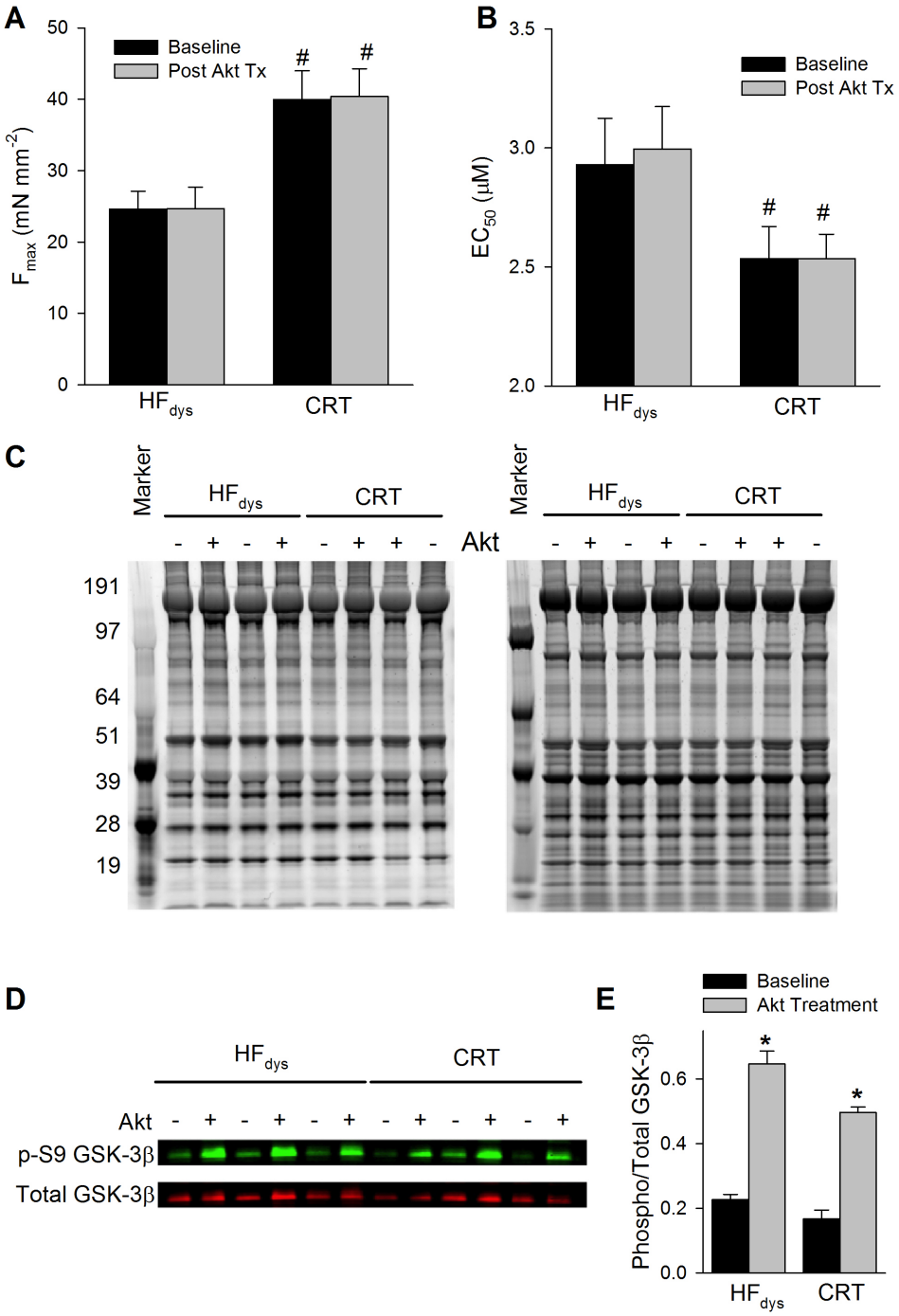
C



Supplemental Figure 21. Mass spectrometry (MS) data for phosphorylation at Leiomodin-2 S513. (**A**) MS spectra for the peptide containing S513. (**B**) Fragmentation Table. (**C**) Known domains and protein binding regions shown with location of the identified phosphorylation site.



Supplemental Figure 22. Affect of PKA treatment on HF_{dys} and CRT myocytes. (**A**) There was a small, but significant, increase in F_{max} in response to PKA treatment, in both HF_{dys} and CRT. (**B**) It is known that PKA has a desensitizing affect on the myofilament, and this was observed equally in both models. *, $P < 0.05$ vs. pre-treatment; #, $P < 0.05$ vs. HF_{dys} with corresponding treatment; via two-way ANOVA. The interaction term was not significant for either panel.



Supplemental Figure 23. Affect of Akt treatment on HF_{dys} and CRT myocytes. (**A and B**) Akt treatment had no affect on either F_{max} or EC_{50} in either group. (**C**) Akt did not induce any detectable phosphorylation of myofilament targets, by Pro-Q Diamond phospho-stain. (**D**) Akt treatment did induce phosphorylation of known targets in both groups, as shown by phosphorylation of serine 9 on GSK-3β (cytosolic fraction). (**E**) There was a statistically significant increase in GSK-3β serine 9 phosphorylation after Akt treatment. There was a borderline significant higher phosphorylation in HF_{dys} compared to CRT ($P = 0.07$). *, $P < 0.05$ vs. pre-treatment; #, $P < 0.05$ vs. HF_{dys} with corresponding treatment; via two-way ANOVA.

Part Three: Supplemental Tables

	Con/BL	HF_{dys}		CRT		HF_{sync}		V3A3	
		3 weeks	6 weeks	3 weeks	6 weeks	3 weeks	6 weeks	3 weeks	6 weeks
Echocardiography									
n	14	16	14	11	10	11	13	11	9
DI (ms)	28 ± 3	*70 ± 4*	*71 ± 6*	*77 ± 4*	34 ± 8 †#	23 ± 3	24 ± 3	*53 ± 8 #*	22 ± 4 †#
LVEDV (mL)	58 ± 4	69 ± 4	*88 ± 6* †	*76 ± 4*	*93 ± 8*	59 ± 7	64 ± 6	66 ± 5	*75 ± 6*
LVESV (mL)	24 ± 2	*52 ± 4*	*70 ± 5* †	*58 ± 4*	*72 ± 6*	*42 ± 5*	*48 ± 4*	*50 ± 4*	*56 ± 4*
LVEF (%)	59 ± 2	*25 ± 2*	*20 ± 1*	*24 ± 2*	*22 ± 1*	*30 ± 2*	*24 ± 1* †	*25 ± 2*	*24 ± 3*
Hemodynamic									
n	15		16		8		20		13
HR (bpm)	117 ± 10		137 ± 8		161 ± 6		140 ± 6		123 ± 5
LVPes (mmHg)	143 ± 6		*113 ± 6*		*104 ± 7*		*123 ± 4*		*113 ± 5*
LVPed (mmHg)	7 ± 1		*31 ± 3*		*11 ± 3* *		*23 ± 3*		*19 ± 4* *
SBP (mmHg)	145 ± 7		*115 ± 7*		*104 ± 7*		*123 ± 4*		*113 ± 5*
DBP (mmHg)	58 ± 4		50 ± 5		*38 ± 5*		*46 ± 3*		*45 ± 3*
MAP (mmHg)	87 ± 5		*72 ± 6*		*60 ± 6*		*73 ± 2*		*68 ± 3*
dP/dt_{max} ($mmHg \cdot s^{-1}$)	2310 ± 127		*993 ± 75*		*1105 ± 98*		*1237 ± 67* *		*1141 ± 87*
dP/dt_{min} ($mmHg \cdot s^{-1}$)	-2227 ± 144		*-1204 ± 84*		*-1104 ± 53*		*-1340 ± 67*		*-1261 ± 86*
$(dP/dt)/(IP)$ (s^{-1})	31 ± 1		*14 ± 1*		*19 ± 1* *		*17 ± 1* *		*17 ± 1* *

Supplemental Table 1. Echocardiographic (at 3 and 6 weeks) and Hemodynamic (at sacrifice) indices in the dog models. DI, dyssynchrony index; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; SV, stroke volume; LVEF, left ventricular ejection fraction; HR, heart rate; LVPes, left ventricular end-systolic pressure; LVPed, left ventricular end-diastolic pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; dP/dt_{max}, maximum rate of change of the pressure waveform; dP/dt_{min} minimum rate of change of the pressure waveform; IP, isovolumic pressure. *Italicized* values indicates $P < 0.05$ vs. Control/Baseline value. *, $P < 0.05$ vs. HF_{dys},†, $P < 0.05$ vs. the 3 week value in the same model.

Protein	Site	Loc Prob	Total Spectra	*P*-value
MLC2	S15	100%	22	N.S.
cMyBP-C	S279	100%	132	N.S.
cMyBP-C	S288	100%	1800	N.S.
cMyBP-C	S290	92%	43	N.S.
cTnI	S23	100%	312	N.S.
cTnI	S24	100%	342	N.S.
cTnI	S167	100%	4	N.S.
cTnI	S200	100%	7	N.S.
α-Tropomyosin	T282	98%	736	N.S.
α-Tropomyosin	S283	100%	3577	N.S.

Supplemental Table 2. A selection of phosphorylation sites identified by mass spectrometry, which were not significantly different between HF_{dys} and CRT. As stated in the online methods, the protein probability threshold was set at 95%; however, MLC2, included in this table, had a 93% protein probability. Loc. Prob., localization probability as calculated by Scaffold PTM (v1.1.3, Proteome Software) using the A-score algorithm.

Part Four: Supplemental References

1. Chakir K, Daya SK, Aiba T, Tunin RS, Dimaano VL, Abraham TP, Jaques-Robinson KM, Lai EW, Pacak K, Zhu WZ, Xiao RP, Tomaselli GF, Kass DA. Mechanisms of enhanced beta-adrenergic reserve from cardiac resynchronization therapy. *Circulation*. 2009;119:1231-1240
2. Chakir K, Daya SK, Tunin RS, Helm RH, Byrne MJ, Dimaano VL, Lardo AC, Abraham TP, Tomaselli GF, Kass DA. Reversal of global apoptosis and regional stress kinase activation by cardiac resynchronization. *Circulation*. 2008;117:1369-1377
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4. Gao WD, Backx PH, Azan-Backx M, Marban E. Myofilament ca2+ sensitivity in intact versus skinned rat ventricular muscle. *Circ Res*. 1994;74:408-415
5. Arrell DK, Neverova I, Fraser H, Marban E, Van Eyk JE. Proteomic analysis of pharmacologically preconditioned cardiomyocytes reveals novel phosphorylation of myosin light chain 1. *Circ Res*. 2001;89:480-487
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