Supplemental Methods

Excision of mouse hearts

Hearts were rapidly excised via thoracotomy and immediately rinsed in an oxygenated (95% O_2, 5% CO_2) modified Krebs buffer containing (in mM) 118.5 NaCl, 5 KCl, 1.2 $MgSo_4$, 2 NaH_2PO_4, 25 $NaHCO_3$, 1.8 $CaCl_2$, and 10 glucose. Hearts were then perfused, and ventricles splayed open in oxygenated modified Krebs with 0.1 $CaCl_2$ and 20 2,3-butanedione 2-monoxime (BDM) to minimize damage.

Isometric force and rate of tension redevelopment

Frozen porcine left ventricular tissue or freshly excised mouse hearts were permeabilized in 50:50 glycerol relaxing solution containing (in mM) 100 KCl, 10 MOPS, 5 K_2EGTA, 9 $MgCl_2$ and 5 Na_2ATP (adjusted to pH = 7 with KOH), 1% (by vol) Triton X-100, 1% protease inhibitor (sigma P8340), and 50% (by vol) glycerol at 4°C overnight. The solution was changed to the same 50:50 glycerol relaxing solution without Triton X-100 for storage up to one week at -20°C.

F-pCa data was fit to the Hill equation to calculate the pCa_{50}, the pCa at half-maximal force, and the Hill coefficient (n_H), a measure of the cooperativity of force. The rate of tension redevelopment (k_{tr}; following a 15% rapid release-restretch transient) was calculated from the half time of force recovery. High frequency stiffness (HFS) was measured by applying a 1000 Hz sinusoidal length change (±0.5% muscle length (ML)) and was calculated from the ratio of peaks of the Fourier transforms of the force and ML signals.

Quick stretch

Solution recipes were calculated and prepared as follows.[1] Relaxing solution (in mM): pCa 8.0, 5 EGTA, 5 MgATP, 1 Mg^{2+}, 0.3 Pi, 35 phosphocreatine, 300 U/mL creatine kinase, 200 ionic strength, 3% dextran T-500 (w/v), pH 7.0. The activating solution was the same as relaxing solution but adjusted to a free [Ca^{2+}] of pCa 4.8. Dissecting solution (in mM): 50 BES, 30.83 K propionate, 10 Na azide, 20 EGTA, 6.29 $MgCl_2$, 6.09 ATP, 1 DTT, 20 BDM, 50 Leupeptin, 275 Pefabloc, and 1 E-64, pH 7.0.

Stress (force per cross-sectional area) was recorded following a step-length change of 0.5% muscle length to assess cross-bridge kinetics as a function of [MgATP]. The stress response was fit to a dual exponential function to characterize the rate of stress release (k_{rel}) associated with the cross-bridge detachment rate as [MgATP] varied (Figure S6). Given that strongly bound cross-bridge attachment events occupy the myosin-MgADP state and rigor state of the cross-bridge cycle as [MgATP] was titrated from 5 mM towards 0 mM, k_{rel} as a function of [MgATP] can be described by Eq. 1. As [MgATP] decreases, cross-bridge detachment slows due to slower MgATP binding, which increases the amount of time a cross-bridge spends in the rigor state.

$$k_{rel}(MgATP) = \frac{k_{-ADP}[MgATP]}{\frac{k_{-ADP}}{k_{+ATP}}+[MgATP]} \qquad \text{Eq. (1)}$$

This k_{rel} vs. MgATP relationship was fit to Eq. 1 for each individual fiber to estimate the cross-bridge rates of MgADP release (k_{-ADP}) and MgATP binding (k_{+ATP}).

Sinusoidal length-perturbation analysis of demembranated pig tissue

Sinusoidal length perturbations of 0.125% myocardial strip length (clip-to-clip) were applied at 48 discreet frequencies from 0.125 to 250 Hz to measure the complex modulus as a function of frequency. The complex modulus represents viscoelastic myocardial stiffness, which arises from the change in stress divided by the change in muscle length that is in-phase (elastic modulus) and out-of-phase (viscous modulus) with the sinusoidal length change at each frequency.

The characteristics of the elastic and viscous moduli responses over the measured frequency range provide a signature of cross bridge binding and cycling kinetics. Vertical shifts in the elastic and viscous moduli are useful for assessing changes in the number of bound cross-bridges between experimental conditions. Frequency-dependent (horizontal) shifts in the moduli are useful for assessing changes in the work-producing and work absorbing characteristics of the myocardium that arise from differences in force generating cross-bridge kinetics.

Phosphate inhibition of demembranated pig tissue

Experiments assessing the effects of inorganic phosphate (Pi) were done at 21°C in equivalent physiological solutions as above made at pCa 9 and pCa 4, where Pi was incorporated into the recipe for a final [Pi] of 0, 1, 3, and 10 mM Pi.

Myofibril mechanics

Myofibril preparations were isolated from demembranated frozen pig cardiac tissue as previously described.[22] Briefly, permeabilized cardiac tissues were rinsed in Rigor Buffer containing (in mM), 50 Tris, 100 KCl, 2 $MgCl_2$, 1 EGTA, pH 7.0, and 1x protease inhibitor (Sigma-Aldrich, St. Louis, MO). Homogenized tissue generated myofibrils using 2 x 30 sec pulses of (VWR Radnor, PA) at low speed and stored at 4 °C.

X-ray diffraction

Permeabilized tissues were prepared as described previously.[30,31] Briefly, frozen left ventricle wall tissues (about 0.5 -1 cm^3) was defrosted at room temperature in skinning solution (in mM: 91 K+-propionate, 3.5 $MgCl_2$, 0.16 $CaCl_2$, 7 EGTA, 2.5 Na_2ATP, 15 Creatine phosphate, 20 Imidazole, 30 BDM, 1% Triton-X100 and 3% Dextran at pH 7) before dissecting into smaller strips (~5 -10 mm long and 1-2 mm wide). The tissues were permeabilized at room temperature for 3 hours before further dissected into preparations 4 mm long with a diameter of ~200 µm before attaching aluminum T-clips to both ends and stored in pCa8 solution with 3% dextran on ice.

X-ray diffraction experiments were performed at the BioCAT beamline 18ID at the Advanced Photon Source, Argonne National Laboratory. The X-ray beam energy was set to 12 keV (0.1033 nm wavelength) at an incident flux of ~5 $\times 10^{12}$ photons per second. The specimen to detector distance was ~ 3 m. X-ray fiber diffraction patterns were collected in pCa 8 solution in the absence or presence of 50 µM of Dani on a MarCCD 165 detector (Rayonix Inc., Evanston IL) with a 1 s exposure time. To minimize radiation damage, the muscle samples were oscillated along their horizontal axes at a velocity of 1 - 2 mm/s. The irradiated areas were moved vertically after each exposure to avoid overlapping X-ray exposures.

X-ray diffraction patterns were analyzed using the MuscleX software package developed at BioCAT.[31] The equatorial reflections were analyzed by the Equator module in the MuscleX software package as described previously.[30,31] X-ray patterns were subsequently quadrant folded and background subtracted to improve signal to noise ratio for further analysis using the Quadrant Fold module of the MuscleX program suite. The

meridional and layer line reflections were measured using the Projection Traces module of MuscleX program suite as described.[25]

Stopped flow assay

Stopped-flow experiments were conducted on a HiTech TgK Scientific DX stopped-flow spectrometer. All experiments were excited with 365 nm wavelength light and emission was measured by a PMT through a DD400 long pass filter, unless otherwise stated. HMM was prepared as described in the in vitro motility section, above. All experiments were run in Rigor Buffer as described in the myofibril section, above.

ATP binding: HMM and mantATP (fluorescently-labeled ATP; Jena Bioscience) were rapidly mixed inside the stopped flow observation chamber to a final concentration of 100 nM HMM and mantATP ranging from 0.5 – 4 μM. Change in fluorescence was fit to a single exponential growth curve to derive the k_{obs} at each [mantATP]. This yielded a plot of k_{obs} vs [mantATP], with the slope defining the apparent 2nd order rate constant of ATP binding. This was calculated for 0, 1, 3, and 10 μM danicamtiv.

ATP turnover: HMM was incubated with mantATP for 1 minute to allow binding then rapidly mixed with unlabeled ATP in the stopped flow observation chamber to final concentrations of 250 nM HMM, 2 μM mantATP, and 125 μM unlabeled ATP. Change in fluorescence was fit to a single exponential decay and the rate of decay was defined as the rate constant for ATP turnover. This was calculated for 0, 1, 3, 10, and 15 μM danicamtiv.

In vivo mouse treatment studies

Dani was dissolved in dimethyl sulfoxide (DMSO) to final concentration of 10 mM and stored at -80°C freezer until day of experiments. Dosing of 2 mg/kg for each mouse was

formulated in a solution of N,N-dimethylacetamide (DMA): polyethylene glycol 400 (PEG-400): 30% 2-hydroxypropyl-β-cyclodextrin (2-HPβ-CD) (5:25:70) per previous pharmacology studies.[44] Control and I61Q cTnC mice were lightly anesthetized and held under anesthesia via inhalation of 1-2% isoflurane in 95% oxygen. Transthoracic echocardiography was performed using Vevo 3100 high-frequency, high-resolution imaging system (VisualSonics) equipped with MS400 MicroScan Transducer. The transducer was placed on the animal's chest to obtain the parasternal short axis view at the mid-papillary level. M-mode images for measurements of left ventricular inner diameters at the end of diastole and end of systole, fractional shortening, and ejection fraction. After initial measurements, a 27G butterfly needles was inserted into the tail vein and total 125 μL of DMA:PEG-400: 2-HPβ-CD (5:25:70) containing 2 mg/kg of Dani or DMSO (vehicle) was delivered. This was followed by a 500 μL of normal saline and removal of the catheter. The mouse was continued under sedation and repeat echocardiogram was performed 10 minutes after the injection.

Supplemental Tables

Table S1. Numerical values of the mechanical measurements from permeabilized porcine cardiac tissue and myofibrils treated with Danicamtiv. Data is from 19 paired demembranated tissue preparations and 18 DMSO (no drug) or 21 Dani treated myofibrils from 3 biological replicates. Data is reported as mean ± SEM. P values are for comparison of no drug vs. Dani for each parameter using paired two-tailed t-test (demembranated mechanics), Welch's unpaired two-tailed t-test (myofibrils, pCa 4.0), or two-way ANOVA followed by Tukey's multiple comparisons test (myofibrils, pCa 5.8).

Demembranated Mechanics	**No drug**	**1 μm Dani**	**P value**
Max | Force (mN/mm^2)	31.80 ± 1.03	35.09 ± 1.43	0.024
pCa$_{50}$	5.72 ± 0.02	5.91 ± 0.05	0.033
Hill Coefficient	3.96 ± 0.31	2.27 ± 0.29	7.80e-3
Max | k_{tr}	1.91 ± 0.14	0.83 ± 0.08	7.80e-3
Myofibrils	**Maximal Calcium (pCa 4.0)**		
Force (mN/mm^2)	86.07 ± 1.63	110.60 ± 2.88	0.0042
k_{ACT} (s^{-1})	1.24 ± 0.01	0.86 ± 0.06	0.0243
$k_{REL(slow)}$ (s^{-1})	0.77 ± 0.03	0.25 ± 0.05	0.0020
$t_{REL(slow)}$ (ms)	56.5 ± 1.9	103.7 ± 9.4	0.0326
$k_{REL(fast)}$ (s^{-1})	10.64 ± 0.20	6.34 ± 0.22	1.49e-4
	Submaximal Calcium (pCa 5.8)		
Force (mN/mm^2)	56.56 ± 3.17	93.62 ± 2.17	3.66e-9
% maximal force	65.10 ± 1.47	84.66 ± 1.45	0.0007
k_{ACT} (s^{-1})	0.70 ± 0.01	0.53 ± 0.03	2.08e-3
$k_{REL(slow)}$ (s^{-1})	1.34 ± 0.10	0.39 ± 0.06	1.03e-3
$t_{REL(slow)}$ (ms)	49.1 ± 1.7	95.9 ± 12.2	0.032
$k_{REL(fast)}$ (s^{-1})	12.81 ± 1.05	7.61 ± 0.53	1.49e-3
k_{ACT} (s^{-1}) with 50% ADP	0.46 ± 0.02	0.43 ± 0.02	0.781 (ns)
$k_{REL,slow}$ (s^{-1}) with 50% ADP	0.29 ± 0.01	0.16 ± 0.03	0.030
$t_{REL(slow)}$ (ms) with 50% ADP	155.2 ± 3.0	222.5 ± 13.6	0.170(ns)
$k_{REL(fast)}$ (s^{-1}) with 50% ADP	4.34 ± 0.32	2.39 ± 0.19	0.190 (ns)

Table S2. Numerical values of X-ray diffraction reflections of relaxing muscle (pCa 8.0). Values represent mean ± S.E.M. for n= 11 preparations using paired two-tailed t-test analysis.

X-ray reflection	No drug	1 μm Dani	P value
$d_{1,0}$ (nm)	36.62 ± 0.30	36.88 ± 0.3	0.003
$I_{1,1}/I_{1,0}$	0.35 ± 0.02	0.47 ± 0.02	0.0001
I_{M3} (a.u.)	15.6 ± 1.5	11.4 ± 1.1	0.0101
I_{MLL1} (a.u.)	23.2 ± 2.2	15.0 ± 1.7	0.0002
I_{M6} (a.u.)	2.93 ± 0.29	2.55 ± 0.31	0.114 (ns)
S_{M6} (nm)	7.209 ± 0.002	7.224 ± 0.002	0.0008

Table S3. Numerical values of mechanical measurements of demembranated cardiac tissue from DCM mice in absence (ND) or presence of 1 μm Danicamtiv (Dani). Values represent mean ± S.E.M. for N = 6-8 animals and <12 preparations per group. * P<0.05 vs. ND, † P<0.005 vs. ND, # P<0.0001 vs. ND using a two-way ANOVA analysis with Šídák's multiple comparisons test.

	Control		
Mechanics Parameter	**ND**	**Dani**	**P value**
Max | Force (mN/mm^2)	64.6 ± 5.8	63.1 ± 5.3	0.970
pCa_{50}	5.61 ± 0.01	5.76 ± 0.02	0.008
Hill Coefficient	3.11 ± 0.09	2.15 ± 0.13	5.15e-7
Max | k_{tr}	28.1 ± 1.4	10.8 ± 1.0	2.90e-8
	I61Q cTnC		
	ND	**Dani**	**P value**
Max | Force (mN/mm^2)	36.7 ± 4.9	41.0 ± 4.2	0.838
pCa_{50}	5.32 ± 0.05	5.45 ± 0.03	0.030
Hill Coefficient	2.45 ± 0.07	1.66 ± 0.07	7.21e-5
Max | k_{tr}	28.2 ± 2.3	11.6 ± 1.7	6.81e-7

Table S4: Numerical values of intact trabecula twitch measurements of DCM mice in absence (ND) or presence of 1 or 3 μm Danicamtiv (Dani). Values represent mean ±S.E.M. for 15 control mice and 11 I61Q mice. *All P-values vs. ND using a mixed-effect analysis with Dunnett's multiple comparisons test.

	Control				
Twitch Parameter	**ND**	**Dani 1 μM**	**P-value**	**Dani 3 μM**	**P-value**
T_P (kPa)	30.9 ± 4.3	43.8 ± 4.3	0.004	52.0 ± 8.0	0.021
Tension Index (x10^3)	-8.6e^{-007} ± 1.6	7.15 ± 2.01	0.007	15.68 ± 4.53	0.015
Time to peak (ms)	106.5 ± 5.9	114.8 ± 7.5	0.052	132.0 ± 8.6	0.016
RT_{50} (ms)	60.1 ± 4.2	73.6 ± 4.2	0.006	95.6 ± 7.2	0.001
RT_{90} (ms)	102.6 ± 7.6	125.0 ± 7.5	0.014	175.6 ± 13.6	0.001
Max $+dT/dt$ (kPa/ms)	0.54 ± 0.09	0.71 ± 0.08	0.001	0.72 ± 0.12	0.140
Max $-dT/dt$ (kPa/ms)	0.40 ± 0.07	0.44 ± 0.05	0.476	0.35 ± 0.06	0.735
	I61Q cTnC				
	ND	**Dani 1 μM**	**P-value**	**Dani 3 μM**	**P-value**
T_P (kPa)	18.7 ± 4.1	29.8 ± 4.5	0.006	38.9 ± 7.9	0.009
Tension Index (x10^3)	-4.14 ± 1.67	0.39 ± 1.91	0.004	2.169 ± 2.24	0.013
Time to peak (ms)	110.7 ± 4.6	112.6 ± 3.3	0.938	115.6 ± 5.1	0.625
RT_{50} (ms)	56.3 ± 3.2	63.3 ± 3.8	0.022	74.5 ± 3.4	0.008
RT_{90} (ms)	96.1 ± 6.0	110.2 ± 6.3	0.026	132.8 ± 7.8	0.011
Max $+dT/dt$ (kPa/ms)	0.29 ± 0.06	0.46 ± 0.07	0.007	0.63 ± 0.15	0.020
Max $-dT/dt$ (kPa/ms)	0.23 ± 0.05	0.33 ± 0.05	0.029	0.38 ± 0.09	0.031

Table S5: Numerical values of cross bridge detatchment rate constants (k_{rel}) at various concentrations of [MgATP] for untreated, 1 μM Dani, and 1 μM OM in porcine cardiac muscle. Data is from 9 tissue preparations for ND and Dani and 6 for OM. P-value vs. ND.

[MgATP] (mM)	ND	Dani	P-value	OM	P-value
0.025	6.40 ± 0.41	5.38 ± 0.21	0.459	4.19 ± 0.72	0.073
0.05	7.79 ± 0.46	5.72 ± 0.26	0.059	4.73 ± 0.61	0.009
0.1	8.35 ± 0.47	6.53 ± 0.32	0.105	5.52 ± 0.59	0.017
0.25	9.00 ± 0.40	7.91 ± 0.33	0.421	7.04 ± 0.96	0.124
0.5	10.33 ± 0.39	8.14 ± 0.17	0.042	6.84 ± 0.64	0.003
1	10.81 ± 0.78	9.34 ± 0.28	0.221	7.19 ± 0.67	0.002
2.5	14.65 ± 0.97	10.71 ± 0.40	0.0001	8.15 ± 1.02	6.45e-6
5	22.90 ± 1.69	15.12 ± 0.70	2.46e-9	11.56 ± 1.87	4.59e-13

Table S6: Numerical values of *in vivo* echocardiography of control and I61Q mice pre and 10 minutes post-injection with 2 mg/kg Dani. Values represent mean ±S.E.M. for 7 each control and I61Q mice with Dani. P-values vs. pre-injection using a two-way ANOVA analysis with Šídák's multiple comparisons test.

2mg/kg Dani	**Control**		
Echo Parameter	**Pre**	**10 min post**	**P-value**
HR (bpm)	410 ± 20	388 ± 24	0.731
Ejection Fraction (%)	57.5 ± 2.2	73.1 ± 2.3	1.56e-2
Fractional Shortening (%)	29.8 ± 1.6	41.6 ± 1.9	1.56e-2
LVIDs (mm)	2.64 ± 0.05	2.12 ± 0.10	0.002
LVIDd (mm)	3.76 ± 0.09	3.63 ± 0.11	0.449
LVAWs (mm)	1.51 ± 0.10	1.56 ± 0.09	0.964
LVAWd (mm)	1.05 ± 0.06	1.05 ± 0.07	>0.999
LVPWs (mm)	1.35 ± 0.08	1.53 ± 0.09	0.104
LVPWd (mm)	1.12 ± 0.07	1.19 ± 0.09	0.315
	I61Q cTnC		
	Pre	**10 min post**	**P-value**
HR (bpm)	451 ± 23	396 ± 17	0.032
Ejection Fraction (%)	36.6 ± 2.9	46.4 ± 2.0	1.56e-2
Fractional Shortening (%)	17.7 ± 1.5	23.2 ± 1.2	1.56e-2
LVIDs (mm)	3.80 ± 0.08	3.58 ± 0.14	0.290
LVIDd (mm)	4.62 ± 0.10	4.66 ± 0.16	0.979
LVAWs (mm)	1.14 ± 0.05	1.26 ± 0.08	0.577
LVAWd (mm)	0.87 ± 0.02	0.96 ± 0.04	0.606
LVPWs (mm)	1.07 ± 0.07	1.27 ± 0.07	0.065
LVPWd (mm)	0.93 ± 0.05	0.97 ± 0.05	0.757

Table S7: Numerical values of *in vivo* echocardiography of control and I61Q mice pre and 10 minutes post-injection with vehicle (DMSO). Values represent mean ±S.E.M for 3 control and 4 I61Q mice. *P-values vs. pre-injection using a two-way ANOVA analysis with Šídák's multiple comparisons test.

Vehicle	**Control**		
Echo Parameter	**Pre**	**10 min post**	**P-value**
HR (bpm)	452 ± 28	438 ± 10	0.869
Ejection Fraction (%)	57.6 ± 2.5	62.4 ± 2.4	0.528
Fractional Shortening (%)	30.0 ± 1.6	33.2 ± 1.6	0.670
LVIDs (mm)	2.84 ± 0.12	2.66 ± 0.21	0.781
LVIDd (mm)	4.06 ± 0.16	3.97 ± 0.21	0.934
LVAWs (mm)	1.25 ± 0.07	1.54 ± 0.11	0.250
LVAWd (mm)	0.92 ± 0.06	1.13 ± 0.10	0.222
LVPWs (mm)	1.28 ± 0.18	1.22 ± 0.09	0.978
LVPWd (mm)	0.96 ± 0.20	1.01 ± 0.18	0.156
	I61Q cTnC		
	Pre	**10 min post**	**P-value**
HR (bpm)	386 ± 35	391 ± 14	0.975
Ejection Fraction (%)	47.6 ± 2.9	49.7 ± 2.3	0.788
Fractional Shortening (%)	23.7 ± 2.0	24.9 ± 1.3	0.975
LVIDs (mm)	3.14 ± 0.32	3.07 ± 0.21	0.985
LVIDd (mm)	4.09 ± 0.33	4.08 ± 0.25	>0.999
LVAWs (mm)	1.13 ± 0.04	1.11 ± 0.05	>0.999
LVAWd (mm)	0.85 ± 0.01	0.80 ± 0.02	0.977
LVPWs (mm)	1.14 ± 0.14	1.20 ± 0.12	0.968
LVPWd (mm)	0.96 ± 0.13	0.93 ± 0.13	0.469

Supplemental Figures

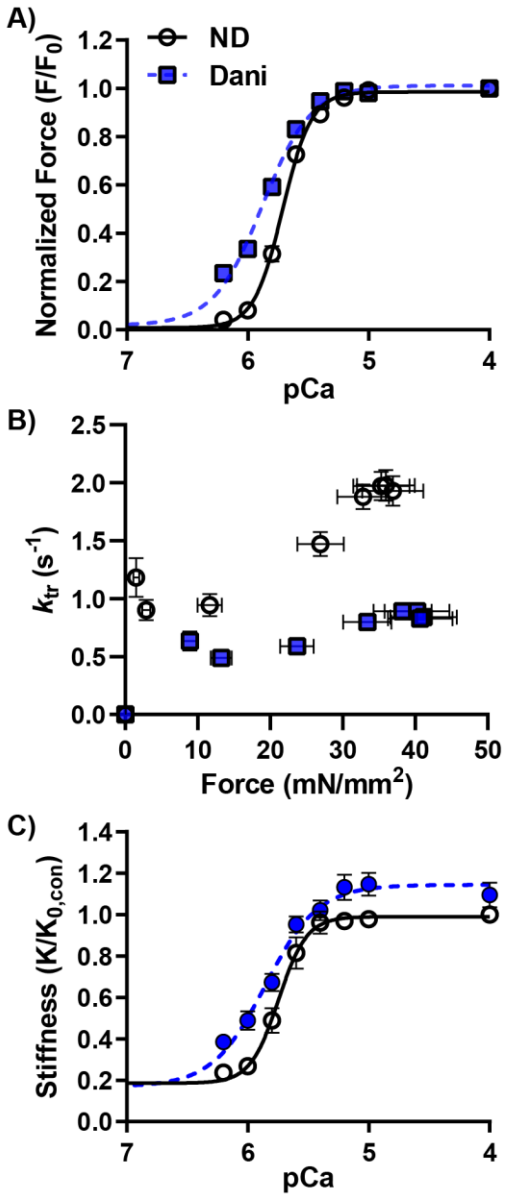

Figure S1. **Permeabilized mechanics in porcine cardiac tissue with 1μM Danicamtiv.** Force, normalized to maximal force in each condition, versus pCa showed a left shift of the curve with a decrease in the hill efficient (A). Dani reduced force redevelopment rate constants (k_{tr}) at comparable force measurements. Stiffness, normalized to maximal stiffness without drug, increased proportionally to force(C), suggesting an increase in the number of cross bridges bound with no change in force per cross bridge.

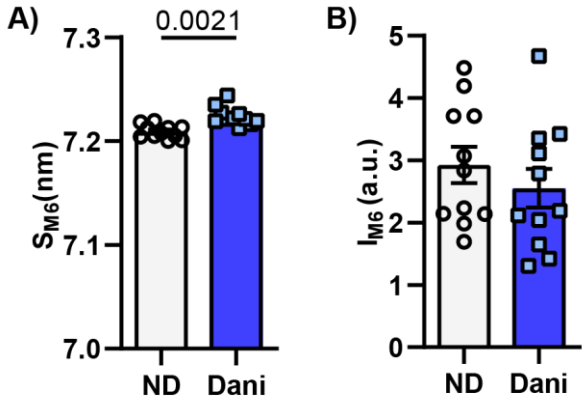

Figure S2. Dani altered the thick filament backbone structure. 50 μM Dani increased spacing of the M6 reflections (A) without changing its intensity (B).

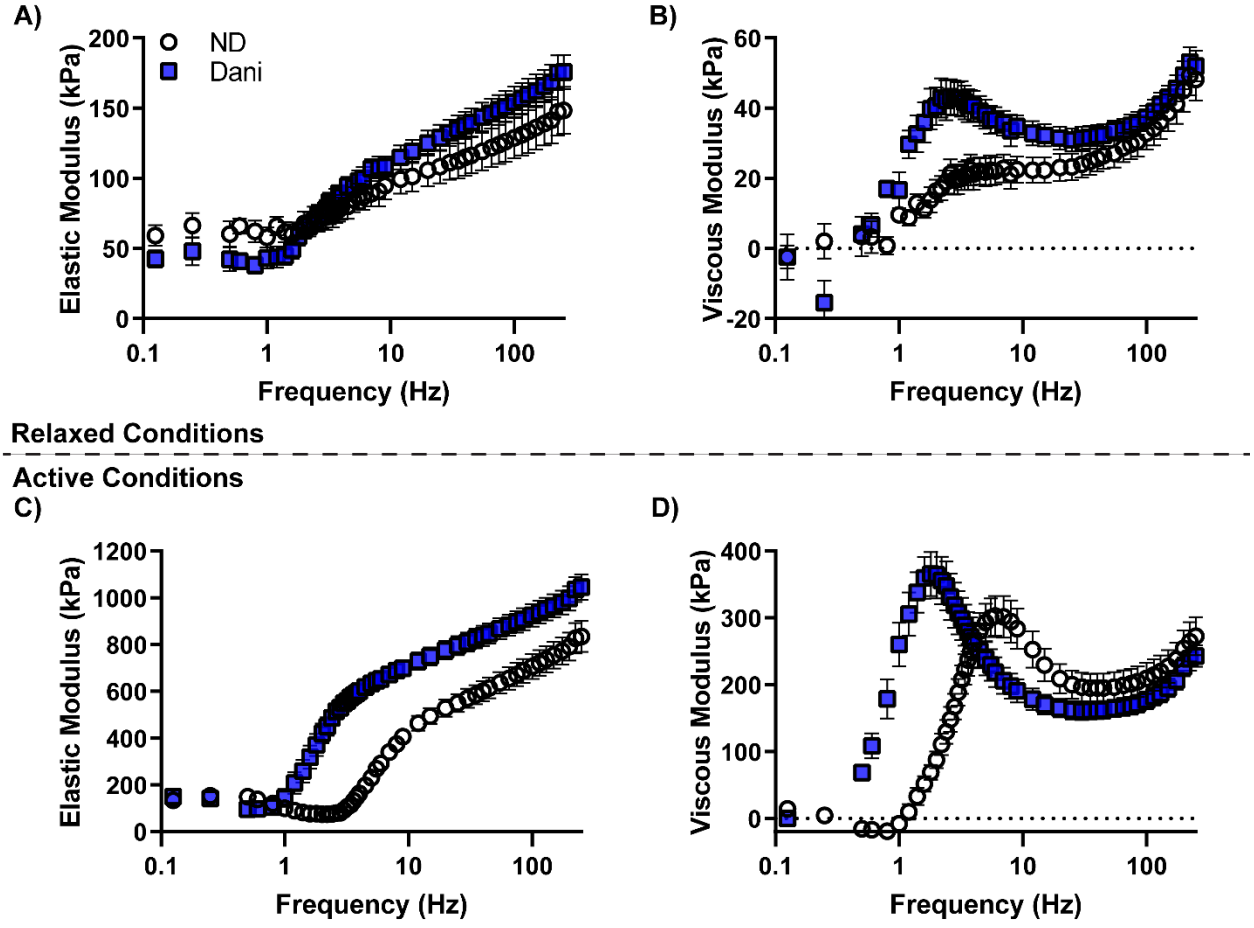

Figure S3. **Passive and active elastic and viscous moduli were modified in the presence of 1 μM Danicamtiv.** In relaxed muscle, Dani did not alter the elastic moduli (A). Under similar conditions, Dani increased the viscous moduli at a sub-set of oscillatory frequencies, suggesting greater cross bridge binding or cross bridge activity(B). In activated muscle, both elastic (C) and viscous (D) moduli values were greater in the

Danicamtiv-treated preparations across a wide range of frequencies, suggested greater cross bridge binding.

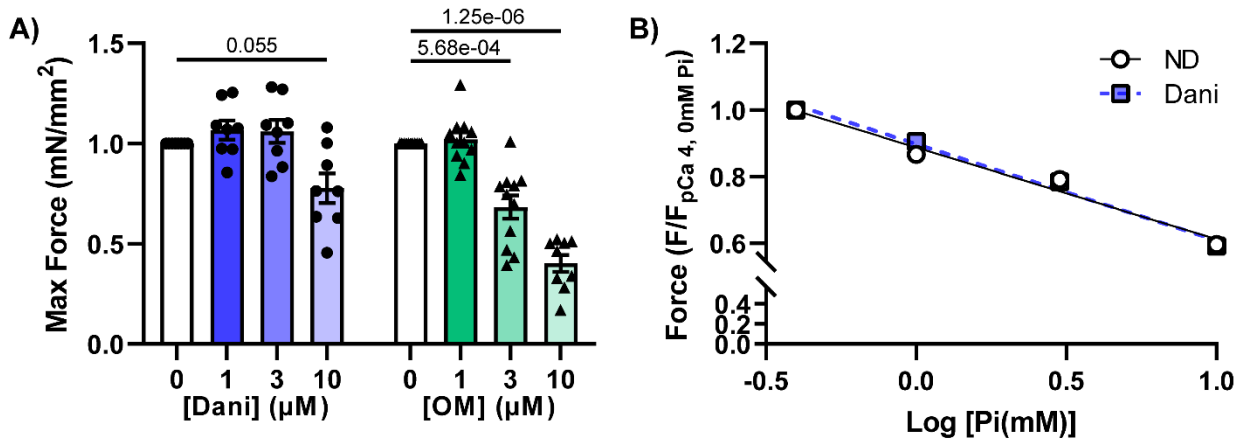

Figure S4. OM inhibited maximal force more than Dani without Dani altering Pi release. (A) Increasing dose of Dani or OM inhibited maximal force with OM inhibiting at lower concentrations and to a greater extent (B). Treatment with 1 μM Dani did not alter the linear relationship between relative force and Log [Pi] in permeabilized mechanics in porcine cardiac tissue.

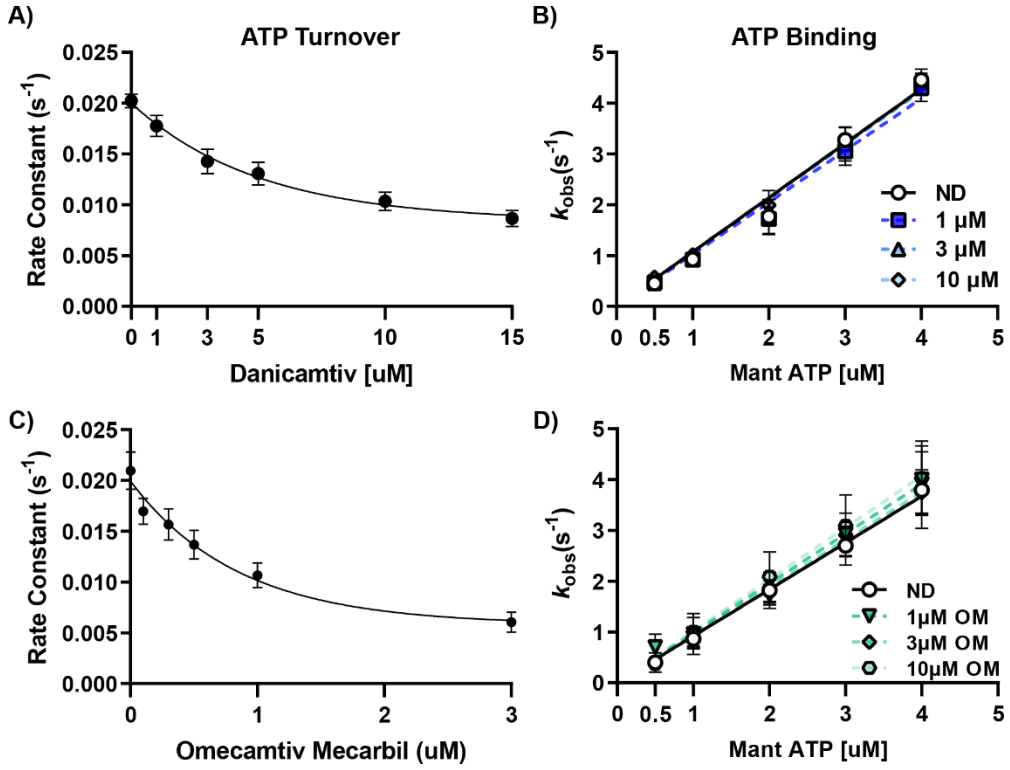

Figure S5. Danicamtiv slowed cross bridge turnover without altering ATP binding in isolated HMM. Increasing dose of Dani (A) or OM (C) inhibited the rate constant for

ATP turnover in porcine cardiac heavy meromyosin. Slope of k_{obs} vs [mantATP], which is the 2nd order rate constant of ATP binding, was unchanged after treatment with 1, 3, and 10 μM Dani (B) or OM (D).

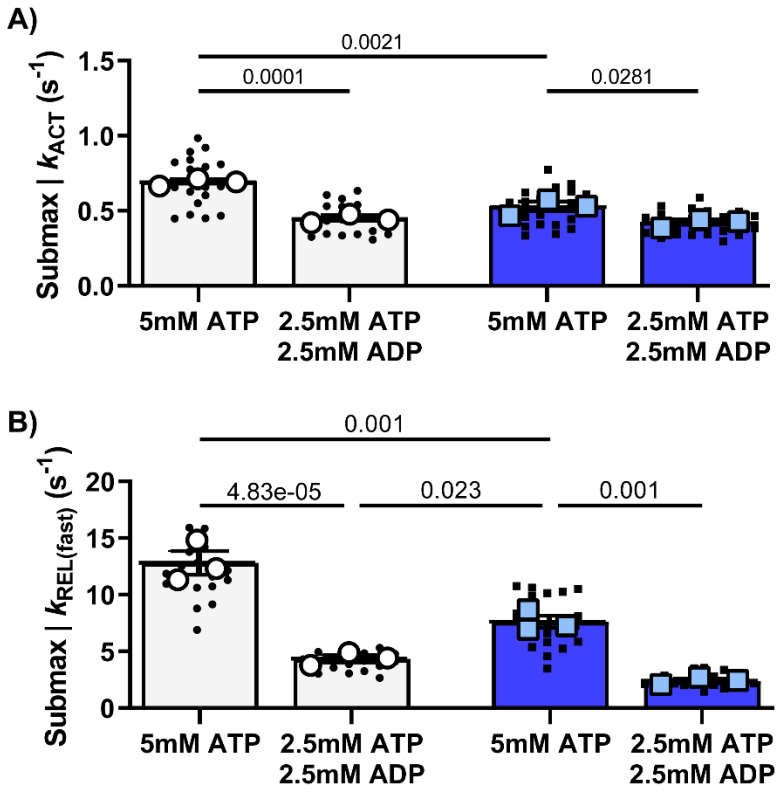

Figure S6. 1 μM Danicamtiv and ADP altered myofibril activation and relaxation rates. Dani and ADP decreased exponential activation (A) and relaxation (B) kinetics in porcine myofibrils. The combination of ADP and Dani decreased these rates further.

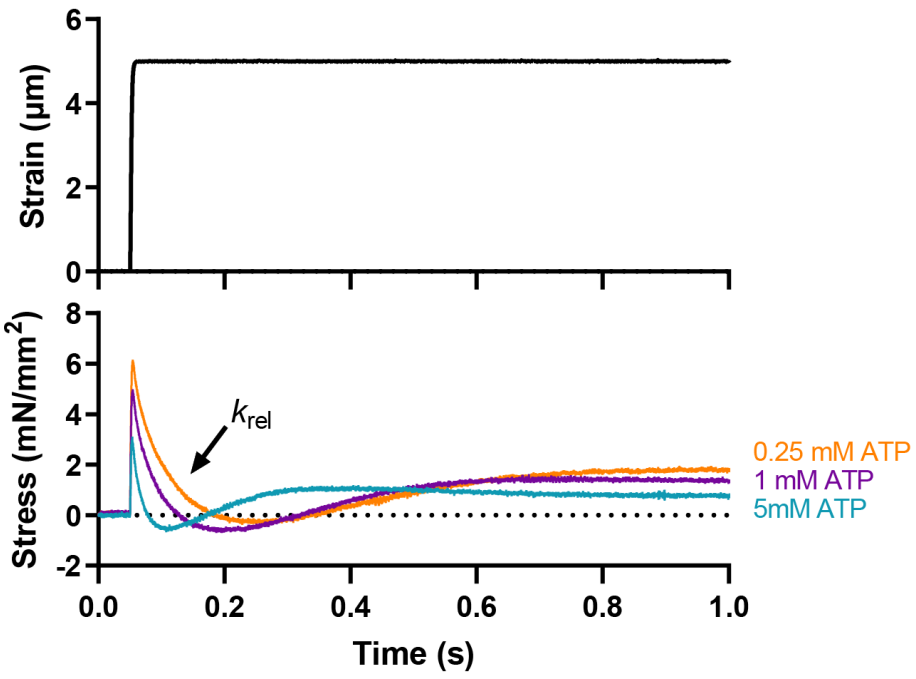

Figure S7. **Quick stretch protocol used for calculating nucleotide binding and release rates.** Stress (=force per cross-sectional area) responses were recorded following a step-length change of 0.5% muscle length (above) to assess cross bridge kinetics with increasing [MgATP]. The stress response was fit to a dual exponential function to calculate the rate of stress release (k_{rel}) associated with the cross-bridge detachment rate.

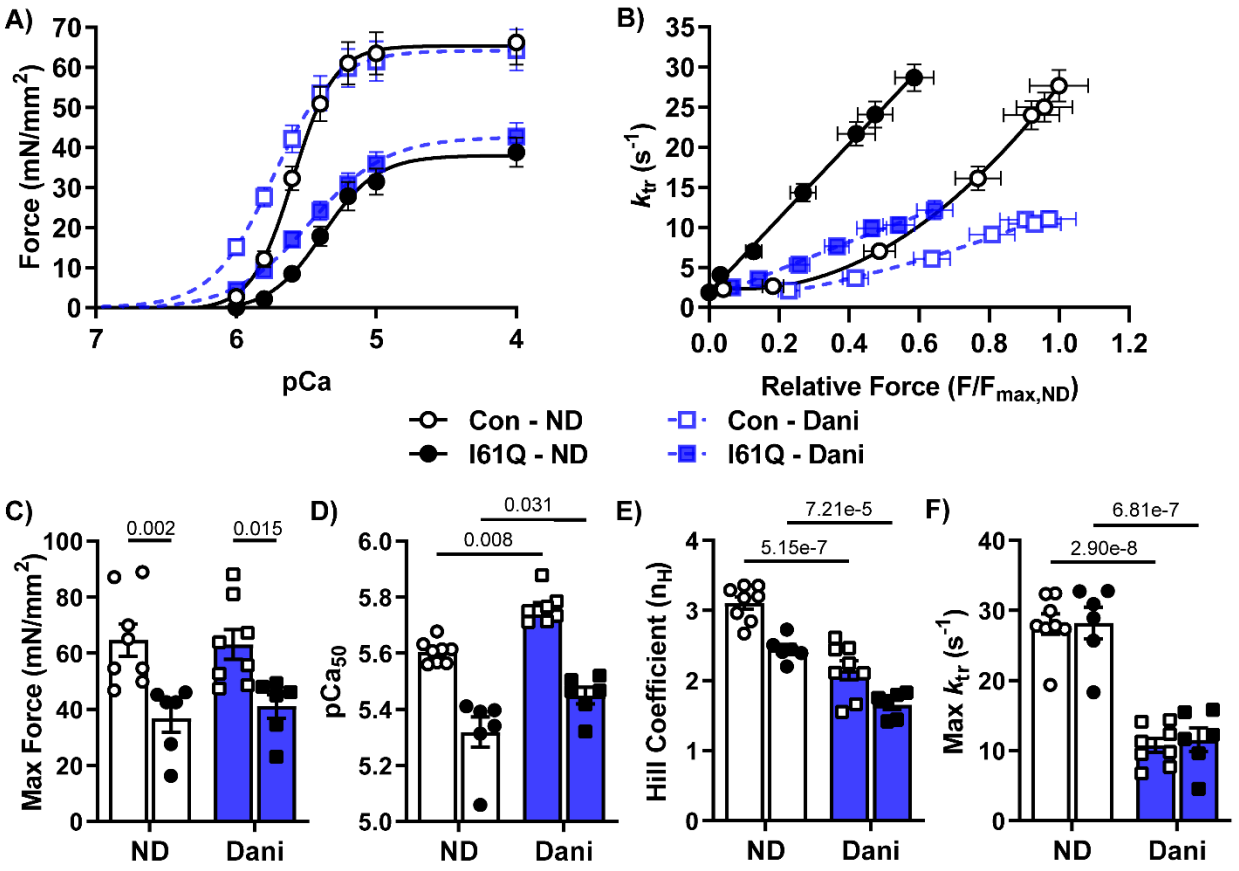

Figure S8. Permeabilized mechanics in control and I61Q cTnC mouse cardiac tissue treated with 1 μM Danicamtiv show increased force and calcium sensitivity. Force vs. pCa curves (A) show a right shift in I61Q cTnC compared to control mice. Treatment with 1 μm Dani results in a left shift in both control and I61Q cTnC hearts. Force redevelopment rate constants (k_{tr}) plotted against relative force (B) was steeper in I61Q cTnC hearts and flattened in both genotypes after treatment with Dani. Maximum force was reduced in I61Q cTnC compared to control and not increased in either group after treatment with Dani (C). Treatment with Dani increased pCa_{50} in both genotypes(C) with a significant decrease in Hill coefficient (D) and max k_{TR} (E).

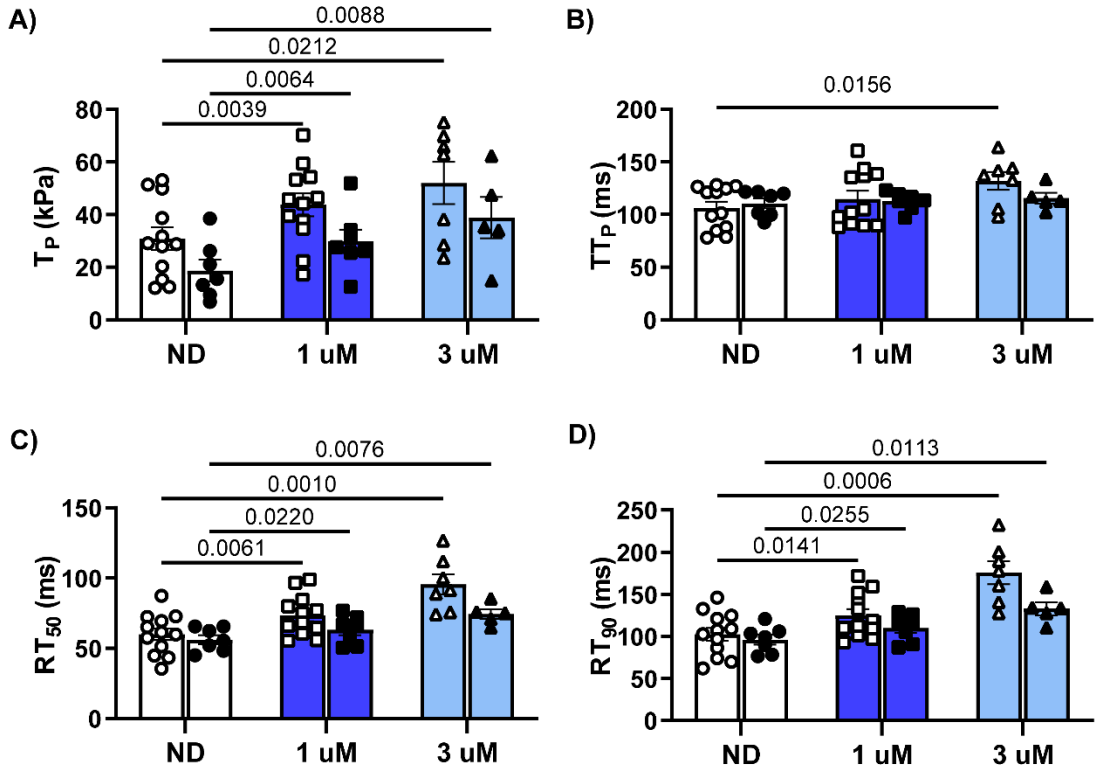

Figure S9. Danicamtiv increased peak tension (T_p) and prolonged relaxation in both control and I61Q cTnC cardiac twitches. T_p is reduced in I61Q cTnC compared to control mice and both increased after treatment with Dani in dose dependent manner (A). Time to peak (TT_P) was not affected at baseline with slight increase at 3 μm Dani in control mice only (B). Time to 50% (RT_{50}) and 90% (RT_{90}) relaxation time were similar in both genotypes at baseline. Treatment with 1 and 3 μm Dani increased both relaxation times with a larger effect in control hearts.

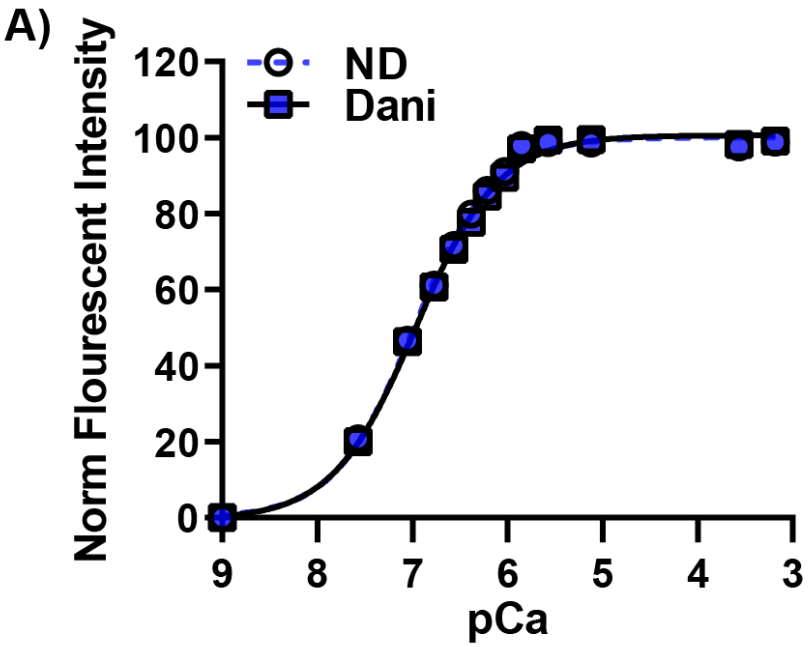

Figure S10. 1 μM Danicamtiv did not alter calcium binding to cTnC. Dani did not alter Ca^{2+}-dependent changes in the fluorescence of recombinant human $cTnC_{IANBD}^{C35S}$. Excitation was at 490 nm, and emission was monitored at 530 nm. Data is represented as fluorescence normalized to the maximum for each experiment.