# Acute treatment with aficamten rescues contractile function in a MyoPod® engineered heart tissue model of hypertrophic cardiomyopathy

**Cassady Rupert**[1], Manmeet Raval[2], James J Hartman[2], Anne N Murphy[2], Bradley P Morgan[2], Stuart G Campbell[1,3], Yale Univ, New Haven, CT

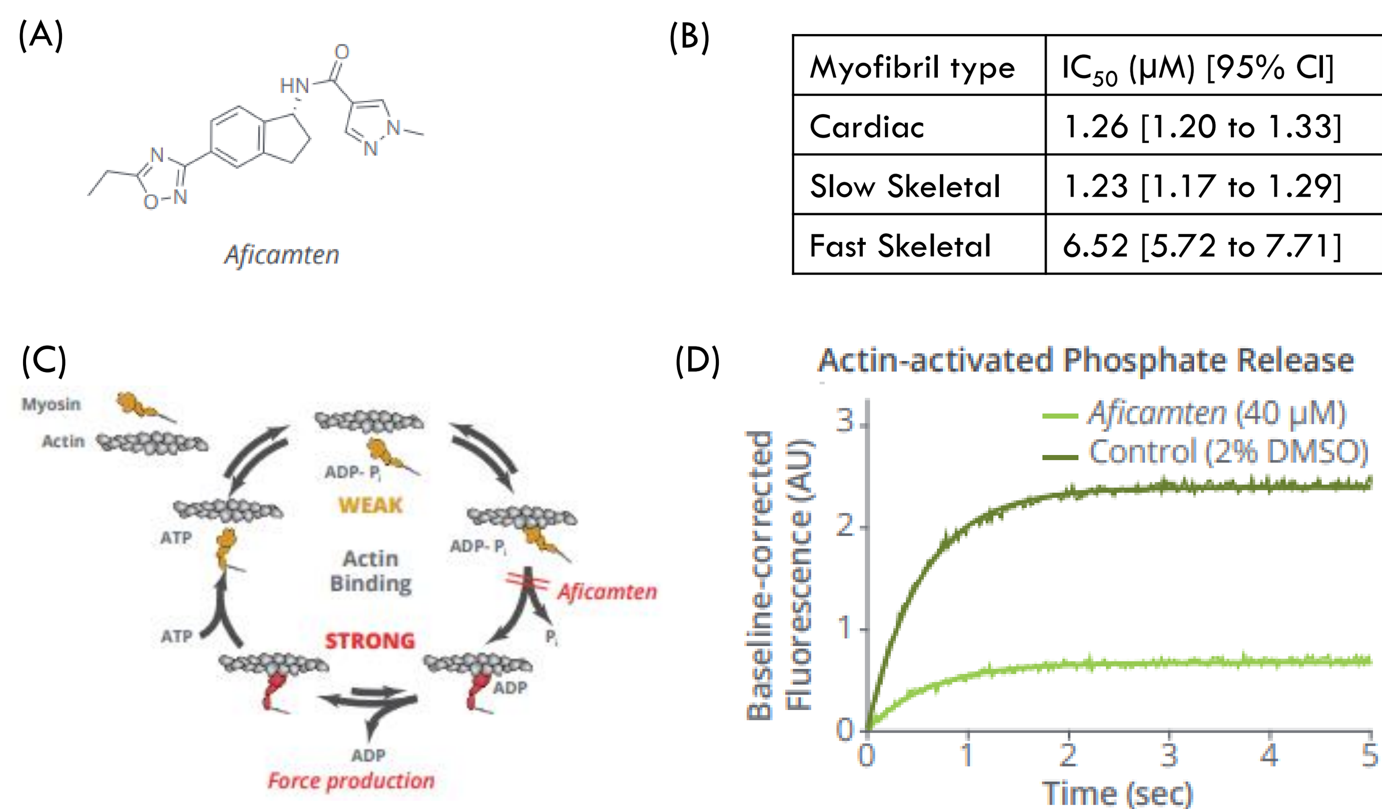
[1]Propria LLC, Branford, CT, USA; [2]Cytokinetics, South San Francisco, CA, USA; [3]Yale University, New Haven, CT, USA

## Abstract

*Aficamten* is a cardiac myosin inhibitor currently in Phase III clinical trials to assess safety and efficacy in treating hypertrophic cardiomyopathy (HCM). Characterizing compound modulation of contractility in an in vitro system of human engineered heart tissue (EHT) is valuable to demonstrate efficacy, characterize mechanics, and to elucidate how *aficamten* performs in different etiologies of HCM. In these studies, MyoPod® EHTs[1] were seeded with human induced pluripotent stem cell-derived (hiPSC)-cardiomyocytes, either wild-type isogenic control (WT) or carrying the HCM-associated[2] myosin mutation MYH7 R403Q$^{+/-}$, and treated acutely with *aficamten* to assess the genotype-specific effects of the compound, chronic response to acute exposure, and the ability to rescue mutant EHT contractile function.

## Background

*Aficamten* is a cardiac myosin inhibitor that acts by slowing actin-activated phosphate release and stabilizing a weak actin-binding state of myosin.




| Myofibril type | $IC_{50}$ (µM) [95% CI] |
|---|---|
| Cardiac | 1.26 [1.20 to 1.33] |
| Slow Skeletal | 1.23 [1.17 to 1.29] |
| Fast Skeletal | 6.52 [5.72 to 7.71] |

**Figure 1.** (A) The chemical structure of *aficamten*. (B) Dose–response analysis was performed with cardiac (bovine, n=17), slow skeletal (bovine, n=6), and fast skeletal (rabbit, n=6) detergent-skinned myofibrils.[3] Free Ca2+ concentrations were fixed at approximately $pCa_{75}$ for each myofibril type. Raw ATPase rates were normalized to reactions containing an equivalent DMSO, and for cardiac and slow skeletal reactions, ATPase rates in the presence of the nonselective myosin inhibitor blebbistatin were subtracted to eliminate the effects of non-myosin ATPases (C) Myosin chemomechanical cycle. (D) Actin-activated phosphate release was measured by rapidly mixing bovine cardiac myosin subfragment-1 (2 µM) with ATP (1 µM), aging the reaction for 30 seconds, followed by mixing with bovine cardiac actin (28 µM) and MDCC-PBP (10 µM). The graph shows the average of 4-5 fluorescence transients, averaged and fit to a single exponential.

## Methods

EHTs were formed with hiPSC-cardiomyocytes in MyoPod® scaffolds and tested on the MyoLab® tissue analyzer.

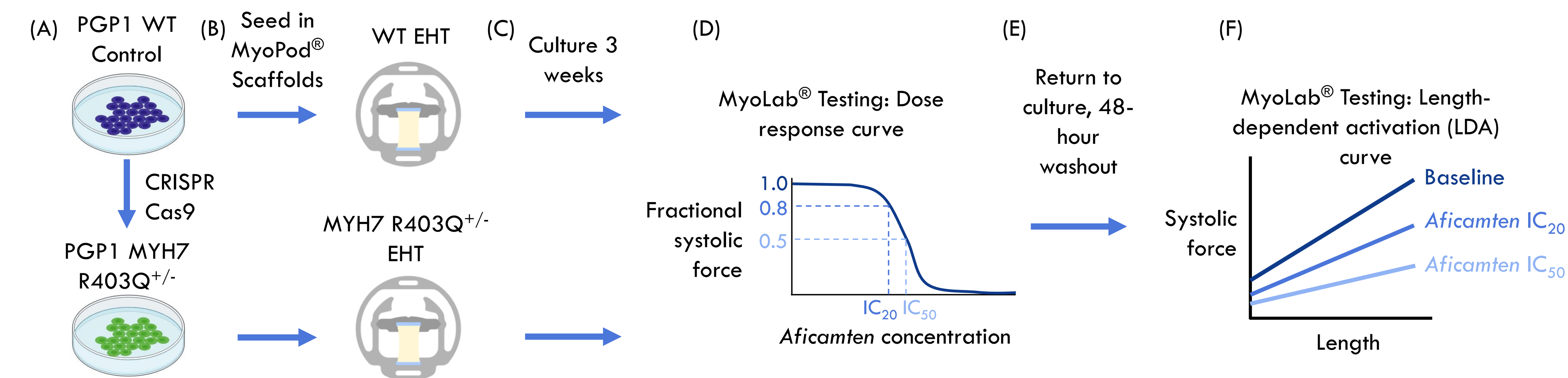
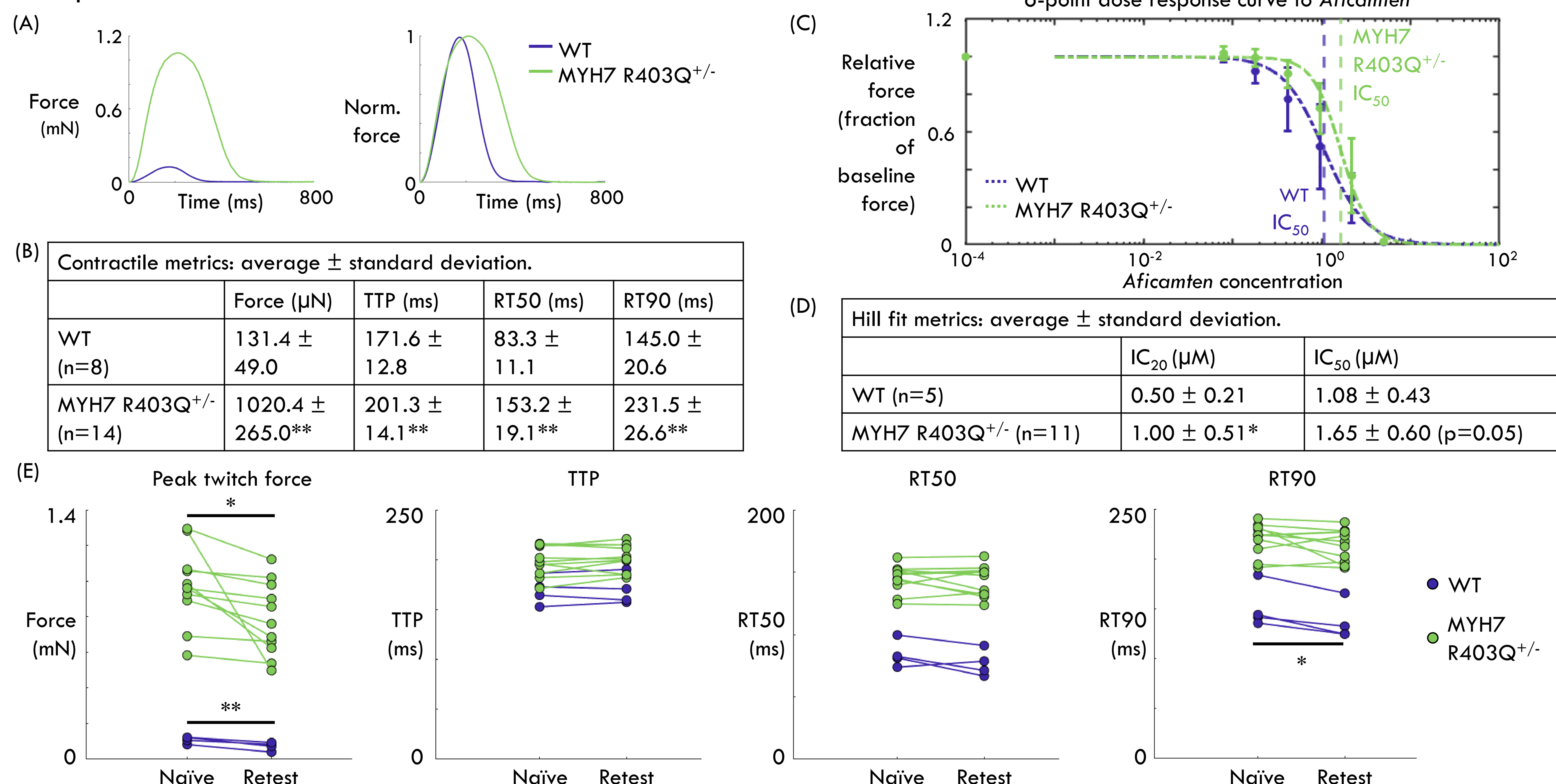



**Figure 2.** EHTs were formed by seeding WT or MYH7 R403Q+/- hiPSC-cardiomyocytes (A) and human cardiac fibroblasts on MyoPod® scaffolds of decellularized porcine ventricular myocardium (B) and were cultured for 3 weeks (C). Mechanical testing was then performed on a MyoLab® instrument to obtain the contractile phenotype and six-point dose response to *aficamten* (D). EHTs were returned to culture for 48 hours (E) and then retested, undergoing length sweeps at baseline and $IC_{20}$ and $IC_{50}$ of *aficamten*, determined from dose response curves (F).

## Results

MYH7 R403Q$^{+/-}$ EHTs exhibit an impaired contractile phenotype and differential acute and chronic response to *aficamten*, compared to WT EHTs.




(B)

| Contractile metrics: average ± standard deviation. | Force (µN) | TTP (ms) | RT50 (ms) | RT90 (ms) |
|---|---|---|---|---|
| WT (n=8) | 131.4 ± 49.0 | 171.6 ± 12.8 | 83.3 ± 11.1 | 145.0 ± 20.6 |
| MYH7 R403Q$^{+/-}$ (n=14) | 1020.4 ± 265.0** | 201.3 ± 14.1** | 153.2 ± 19.1** | 231.5 ± 26.6** |

(D)

| Hill fit metrics: average ± standard deviation. | $IC_{20}$ (µM) | $IC_{50}$ (µM) |
|---|---|---|
| WT (n=5) | 0.50 ± 0.21 | 1.08 ± 0.43 |
| MYH7 R403Q$^{+/-}$ (n=11) | 1.00 ± 0.51* | 1.65 ± 0.60 (p=0.05) |

**Figure 3.** (A) Representative raw (left) and normalized (right) traces of WT and MYH7 R403Q$^{+/-}$ EHTs at naïve testing. (B) Baseline contractile metrics of EHTs. **P<0.01 vs. WT by Student's unpaired *t*-test. (C) Hill fit of relative peak force as a function of *aficamten* concentration in WT (n=5) and MYH7 R403Q$^{+/-}$ (n=11). Markers are average relative force at each concentration, error bars are standard deviation. (D) Hill fit metrics of EHTs. *P<0.05 vs WT by Student's unpaired *t*-test. (E). Contractile metrics of WT (n=4) and MYH7 R403Q$^{+/-}$ (n=11) at naïve testing and re-testing. Circular markers represent average for a single EHT. Solid lines connect measures made in the same EHT. *P<0.05, **P<0.01 by Student's paired *t*-test. TTP: time to peak force, RT50 & RT90: time from peak force to 50% and 90% relaxation, respectively.

## Results

MYH7 R403Q$^{+/-}$ EHTs exhibit an impaired length-dependent activation (LDA) response that is rescued in the presence of *aficamten*.

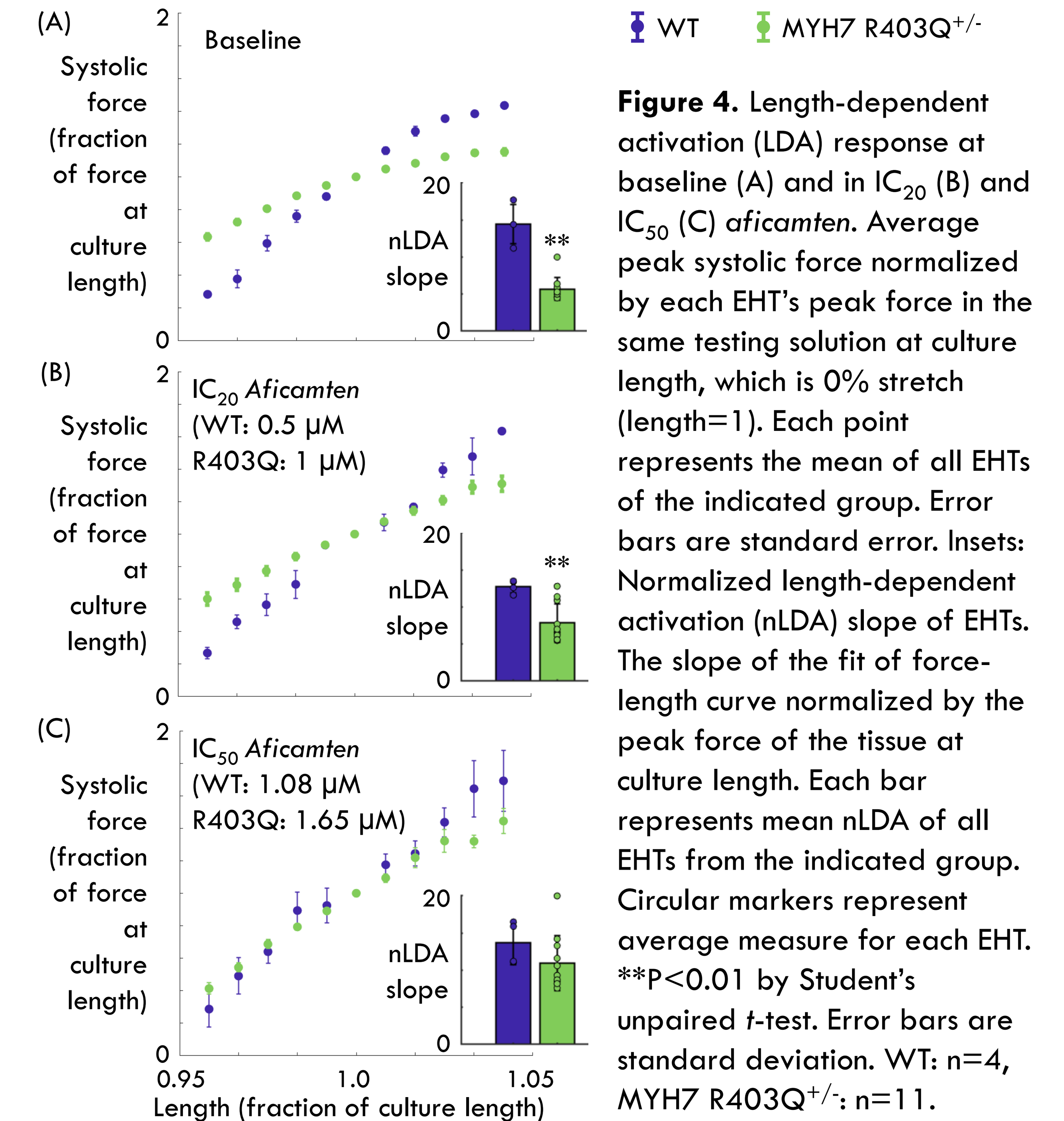



**Figure 4.** Length-dependent activation (LDA) response at baseline (A) and in $IC_{20}$ (B) and $IC_{50}$ (C) *aficamten*. Average peak systolic force normalized by each EHT's peak force in the same testing solution at culture length, which is 0% stretch (length=1). Each point represents the mean of all EHTs of the indicated group. Error bars are standard error. Insets: Normalized length-dependent activation (nLDA) slope of EHTs. The slope of the fit of force-length curve normalized by the peak force of the tissue at culture length. Each bar represents mean nLDA of all EHTs from the indicated group. Circular markers represent average measure for each EHT. **P<0.01 by Student's unpaired *t*-test. Error bars are standard deviation. WT: n=4, MYH7 R403Q$^{+/-}$: n=11.

## Summary

- MYH7 R403Q$^{+/-}$ EHTs possess an HCM-like phenotype distinct from WT isogenic control EHTs.
- Acute exposure to *aficamten* reduces force acutely and chronically in MYH7 R403Q$^{+/-}$ EHTs.
- *Aficamten* shifts the length-dependent response of MYH7 R403Q$^{+/-}$ EHTs toward WT levels in this instance.

## References & Disclosures

[1]Schwan et al., Sci. Rep., 2016. [2]Geisterfer-Lowrance et al., Cell, 1990. [3]Hwee et al., J Pharmacol Exp Ther., 2015

Disclosures: C.R. and S.G.C. are equity shareholders and/or employees of Propria LLC. S.G.C. is faculty at Yale University. MR, JJH, AHM, and BPM are equity shareholders and employees of Cytokinetics, Inc.

©2023 CYTOKINETICS, All Rights Reserved.

CYTOKINETICS® and the CYTOKINETICS and C-shaped logo are registered trademarks of Cytokinetics in the U.S. and certain other countries.

More info: cassady.rupert@propriabio.com | Propriabio.com