Altered contractility in mutation-specific hypertrophic cardiomyopathy: a mechano-energetic in silico study with pharmacological insights

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Abstract

Introduction: Mavacamten (MAVA), Blebbistatin (BLEB), and Omecamtiv mecarbil (OM) are promising drugs directly targeting sarcomere dynamics, with demonstrated efficacy against hypertrophic cardiomyopathy (HCM) in clinical trials. However, the molecular mechanism affecting cardiac contractility regulation, and the diseased cell mechano-energetics are not fully understood yet. Methods: We present a new metabolic-sensitive computational model of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) electromechanics to investigate the pathology of R403Q HCM mutation and the effect of MAVA, BLEB, and OM on the cell mechano-energetics. Results: We offer a mechano-energetic HCM calibration of the model, capturing the prolonged contractile relaxation due to R403Q mutation (~33%), without assuming any further modifications such as an additional Ca$^{2+}$ flux to the thin filaments. The HCM model variant correctly predicts the negligible alteration in ATPase activity in R403Q HCM condition compared to normal hiPSC-CMs. The simulated inotropic effects of MAVA, OM, and BLEB, along with the ATPase activities in the control and HCM model variant agree with in vitro results from different labs. The proposed model recapitulates the tension-Ca$^{2+}$ relationship and action potential duration change due to 1uM OM and 5uM BLEB, consistently with in vitro data. Finally, our model replicates the experimental dose-dependent effect of OM and BLEB on the normalized isometric tension. Conclusion: This work is a step toward deep-phenotyping the mutation-specific HCM pathophysiology, manifesting as altered interfilament kinetics. Accordingly, the modeling efforts lend original insights into the MAVA, BLEB, and OM contributions to a new interfilament balance resulting in a cardioprotective effect.
Altered contractility in mutation-specific hypertrophic cardiomyopathy: a mechano-energetic in silico study with pharmacological insights

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Abstract

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Keywords: In silico modeling, Human stem cell-derived cardiomyocyte, Action potential, Immature Cardiomyocytes, Cardiac Metabolism, Hypertrophic Cardiomyopathy, Pharmacology

1. Introduction

With a prevalence ranging from of 1/500 to 1/200 [1,2], HCM represents the most prevalent genetic cardiac disorder mainly associated with pathogenic variants in sarcomere protein genes [3]. Pathologies such as myocardium hypercontractility [4], impaired relaxation [5], elevated cardiac energy consumption and arrhythmogenesis, diastolic dysfunction, and heart failure [4] manifest due to such variants. The driver of the cyclic interactions between thin and thick filaments is the ATP hydrolysis by myosin—the enzymatic motor of sarcomere [6]. In HCM, myosin binding protein C and adult cardiac myosin isoforms (mainly programmed by
Mavacamten (MAVA), Blebbistatin (BLEB), and Omecamtiv mecarbil (OM) are compounds directly modulating myofilament dynamics with promising effectiveness in treatment of sarcomeric cardiomyopathies. MAVA is an allosteric inhibitor of cardiac myosin ATPase with a negative inotropic effect and demonstrating efficacy in R403Q HCM clinical trials [3,10]. BLEB, a well characterized ATPase inhibitor, alters the Ca$^{2+}$ sensitivity of the myofilament and has been widely used in trials [11–14]. OM is a recently developed myosin ATPase activator with a positive inotropic effect enhancing cardiac contractility [15].

The mechanisms of action of these drugs and their effects on cardiomyocyte electromechano-energetics are still to be fully known and under active research [3,15]. Accordingly, computational studies on the effects of these drugs are mostly lacking. Focusing on R403Q HCM mutation, Margara et al. investigated the efficacy of MAVA, simulating the tension-Ca$^{2+}$ relationship and active tension curves [16]. They hypothesized that the impaired tension relaxation phase is caused by feedback from crossbridge (XB) cycling to the thin filament. Although this assumption results in consistent simulated impaired tension relaxation, the proposed MAVA mechanism of action coupled with a lack of a metabolic-sensitive mechanism in the contractile element (CE) urged us to investigate further the cause of this impairment.

On the other hand, in silico models have been employed to probe the effect of BLEB beside animal muscle fibre experiments [13], highlighting the role of BLEB in shifting the rate-limiting momentum from weakly to strongly bound states in XB cycling [13]. Finally, as an ion channel study, the effect of OM has been simulated using an in silico model of human ventricular action potential (AP) focusing on pro-arrhythmic assessments [17]. Of note, Qu et al. report an IC$_{50}$ of 125.5 µM highlighting the significance of OM influence on the CE in 1-10 µM of OM compared with channel blocking formalism [17]. In summary, to the best of our knowledge, no computational study has reported experimentally validated drug-induced Ca$^{2+}$ sensitivity, ATPase dynamics, and dose-dependent effect of MAVA, BLEB, and OM on the tension-Ca$^{2+}$ relationship, regarding the HCM pathophysiology. This coupled with the facts that these compounds are essentially myosin ATPase activators/inhibitors necessitate studying the drug effects using advanced metabolic-sensitive models. In addition, HCM mutations misregulate sarcomere function and the cardiac energy consumption [18] further highlighting the need for a model capturing electro-mechano-energetics in pathophysiological investigations.

MYBPS3 and MYH7 genes, respectively) host most of these pathogenic variants in sarcomere [7–9].

Here, we investigate the pathophysiology of HCM R403Q myosin mutation using a computational metabolic-sensitive model of hiPSC-CMs electromechanics developed based on ordinary differential equations (ODEs). This model, named hiMCE, is an update of our previous model of hiPSC-CM electromechanics [19] capturing ATPase activity and accounting for metabolic-sensitive kinetics (Fig. 1) in the XB cycling and extending the capacity of models of the molecular mechanism of contraction and the drug effect predictions. The drugs studied here are modulators of myofilament dynamics, we reparametrized the CE of the model using available experimental data and presented novel mechanistic methods to simulate the effect of MAVA, OM, and BLEB on the Ca$^{2+}$ sensitivity, contractility, and energetics of the hiPSC-CMs.
2. Methods

2.1. Extension to a metabolic-sensitive contractile element

We previously integrated a reparametrized mathematical model of the CE by Rice et al. [20] with a new passive force handling into the hiPSC-CM model of electrophysiology by Paci et al. (Paci2020) [21] and studied the inotropic effect of different compounds [19]. Based on Rice et al. CE [20], Tran et al. introduced a mathematical model of a CE that incorporated metabolic-sensitivity [22]. This was achieved by extending the model by Rice et al. [20], with new parameters that account for the competitive binding of metabolic protons (H+) to the binding sites of Ca$^{2+}$ on troponin C, and incorporates the binding kinetics of MgADP in the XB cycling (Fig. 1). The extended mechanistic description divides the strongly bound state post isomerized rotation (XB$_{psR}$) into two substrates in rapid equilibrium, AM1 and AM2, to capture MgADP binding kinetics in the XBs (Fig. 1).

The thermodynamically constrained model of XB kinetics and Ca$^{2+}$ activation is divided into four states including a non-permissive (N$_{xb}$), a permissive (P$_{xb}$), a pre power stroke state (XB$_{pre}$), and a post power stroke state (XB$_{ps}$) (Fig.1) [22]. XB$_{pre}$ and XB$_{ps}$ States both denote the state of strongly bound myosin heads to the actins [20,22]. In diastole, XBs are in N$_{xb}$ state and when activated by Ca$^{2+}$ XBs enter P$_{xb}$ state where they can participate in the binding and unbinding of myosin heads and processes of tension generation (Fig. 1) [22]. The active tension produced as the result of the process is equal to the product of myosin head strain and strongly bound states fractional occupancy [22].

In the present work, we have taken this metabolic-sensitive CE model proposed by Tran et al. [22], and modified it to incorporate active contraction mechanisms integrated with the Paci2020 model of hiPSC-CMs electrophysiology [21]. This extended CE model was manually tuned using the information from a previous sensitivity analysis of the contractile machinery [19] (Table 1). We calibrated the model to capture the AP, CaT, and contractile experimental biomarkers measured in hiPSC-CMs in control conditions, listed in Table 6.

| # | Parameter | hiPSC-CM-CE | hiMCE (control mode) |
|---|-----------|-------------|-----------------------|
| 1 | F1        | 1           | 1                     |
| 2 | F2        | 1           | 1                     |
| 3 | K$_{on}$ (s$^{-1}$ mM$^{-2}$) | 62.5×10$^3$ | 62.5×10$^3$ |
| 4 | K$_{off}$ (s$^{-1}$) | 200 | 200 |
| 5 | K$_{onf}$ (s$^{-1}$) | 25 | 25 |
| 6 | perm$_{50}$ | 0.6 | 0.6 |
| 7 | $n_{perm}$ | 11.28 | 11.55 |

Table 1 The values of the contractile element parameters for hiPSC-CM-CE [19] and hiMCE. F1 and F2 represent DRX:SXR/(DRX:SXR)$_{control}$ ratio in the XB cycling. K$_{on}$, K$_{off}$ and K$_{onf}$ are rate constants for Ca$^{2+}$ binding to troponin, the forward and backward transition rates between Pxb and Nxb states, respectively [20]. $n_{perm}$ and perm$_{50}$ denote the Hill coefficient and the half-activation constant, respectively, describing the nonlinearity of the cooperativity in Ca$^{2+}$ activation of XBs [20]. K$_{off}$ and K$_{onf}$ represent the rate constants affecting Ca$^{2+}$ unbinding from low and high affinity sites on troponin, respectively [20]. m denotes the mass term in the model of sarcomere by Rice et al. [20]. kxb represents the tension scaler detailed in [19]. xbmmodsp is a species-dependent XB cycling rate scaler [20]. $h_f$ denotes the rate constant in the forward transition between XB$_{pre}$ and XB$_{psR}$ [20].
This calibrated control/healthy model variant was then modified to describe an HCM mutation and the effect of pharmaceutical mechanical modulators, and in sections 2.2 and 2.3, we explain the tuning of the relevant CE parameters for each scenario. To enlighten the role of these parameters, we describe the XB kinetics [23] as follows in relation to Fig. 1:

\[ \frac{d}{dt} P_{XB} = k_{npt} \times N_{XB} + ap3 \times XB_{pstr} + am1 \times XB_{preh} - \left( k_{npt} + ap1 + am3 \right) \times P_{XB} \] (1)

\[ \frac{d}{dt} XB_{preR} = ap1 \times F1 \times P_{XB} - am1 \times F2 \times XB_{preR} - ap2 \times XB_{preh} + am2 \times XB_{pstr} \] (2)

\[ \frac{d}{dt} XB_{pstr} = am3 \times P_{XB} + ap2 \times XB_{preR} - \left( am2 + ap3 \right) \times XB_{pstr} - \] (3)

\[ N_{XB} = 1 \times P_{XB} + XB_{preR} + XB_{pstr} \] (4)

where \( k_{npt} \) and \( k_{npt} \) are transition rates between \( N_{XB} \) and \( P_{XB} \) and responsible for XB cycling activation [20]. \( am1 \) takes into account the Pi-dependent transition rate in the XB and has been defined in [23]. \( ap1 \) is equal to the attachment rate to \( XB_{preR} \) [23] which is given as:

\[ f_{ap} \times xbmodesp \times Q_f^{\left(T_{mpc}^{-37}/10\right)} \] (5)

\( f_{ap} \) value is set to 500 s\(^{-1}\) and \( Q_f^{\left(T_{mpc}^{-37}/10\right)} \) is the temperature-dependence set to 6.25 [20], in this work. \( xbmodesp \) (species-dependent XB cycling rate scalar [20]) value is given in Table 1. \( am3 \) denotes a thermodynamically constrained transition rate from \( P_{XB} \) to \( XB_{pstr} \) state accounting for MgATP release when the myosin heads transit from weakly to strongly attached XB states detailed in [23]. \( ap3 \) is the MgADP and MgATP-sensitive transition rate from \( XB_{pstr} \) to \( P_{XB} \) [23] and is calculated as:

\[ ap3 = \left[ MgATP \right] \times g_{xsb} \times \left( k_{dADP} + [MgADP]^+ \right) \] (6)

Where \( MgADP^+ \) denotes the reference (physiological) concentration of MgADP of 36 µM and \( k_{dADP} \) is the MgADP dissociation constant detailed in [23]. \( g_{xsb} \) denotes a first order rate constant tuned by Tran et al. to maintain the validity of original Rice CE model [20] under physiological metabolic conditions regarding MgADP kinetics detailed in [23]. \( am2 \) is equal to the transition from \( XB_{pstr} \) to \( XB_{preR} \) which is proton and MgADP-sensitive [23]:

\[ am2 = h_{ht} \times [H^+] \times \left( k_{dADP} + [MgADP]^+ \right) \times \left( [MgATP] \right) \] (7)

Again, \( h_{ht} \) represents the adjustment of the rate constant affecting the transition between \( XB_{preR} \) and \( XB_{pstr} \) states to include physiological proton and MgADP dependent effects.

| 8 | \( K_{np} \) (s\(^{-1}\)) | 550 | 550 |
|---|----------------|-----|-----|
| 9 | \( K_{pn} \) (s\(^{-1}\)) | 50  | 50  |
| 10| \( K_{offmod} \) | 0.5 | 0.5 |
| 11| m (s\(^{-1}\) µm\(^{-3}\)) | 2×10\(^5\) | 2×10\(^5\) |
| 12| kxb | 12  | 13.1 |
| 13| xbmodesp | 0.2 | 0.2 |
| 14| hi (s\(^{-1}\)) | 2000 | 2000 |
further detailed in [23]. Following the method proposed in [16], we defined F1 and F2 (Eq. 2) as modulators of the transition between permissive binding state on actin (PXB) and strongly bound XBs before isomerized rotation (XPBpref). In our model, F1 and F2 represent the (DRX:SRX)/(DRX:SRX)control ratio (=1 in control mode) and indirectly affect the ATPase dynamics (Fig. 1). ap2 is equal to the forward transition rate between XPBpref and XPBpstR, which is defined as [20]:

\[ h_f \times h_{fmd} \times \text{xbmodsp} \times Q_{hf}^{(Tmpc-37)/10} \] (8)

\[ h_{fmd} = e^{(-\text{sign}(xXB_{pref}) \times h_{fmdc} \times (\frac{xXB_{pref}}{x_0})^2)} \] (9)

The value for hf is given in Table 1. Qhf represents the temperature dependence, here set to 6.25. h_{fmd} incorporates strain dependence into the forward transition rate, h_{f}. h_{fmdc} is set to 5 and specifies the extent to which the isomerization rate is influenced by the mean strain of XPBpref (xXB_{pref}). x_0 is the mean strain (distortion) of XPBpstR state when the net motion between actin and myosin filaments is absent. Here, x_0 is set to 0.007 μm.

Furthermore, ap1 to ap3 and am1 to am3 also affect the time rate of change in the mean strains, Eqs. 10 and 11, and the steady-state population of strongly bound XB states Eqs. 12-14 [23]:

\[ \frac{d}{dt}XB_{pref} = 0.5 \times \frac{dSL}{dt} + \frac{\varphi}{XB_{Dfract}} \left[ -(ap1 \times xXB_{pref}) + am2 \times (XB_{pstR} - x_0 - xXB_{pref}) \right] \] (10)

\[ \frac{d}{dt}XB_{pstR} = 0.5 \times \frac{dSL}{dt} + \frac{\varphi}{XB_{Dfract}} \left[ ap2 \times (xXB_{pref} + x_0 - xXB_{pstR}) \right] \] (11)

\[ XB_{Dfract}^{pref} = \frac{am3 \times am2 + ap3 \times ap1 + am2 \times ap1}{\sum XB_{Dfract}} \] (12)

\[ XB_{Dfract}^{pstR} = \frac{ap1 \times ap2 + am3 \times am1 + am3 \times ap2 + am3 \times am2 + ap3 \times ap1 + am2 \times ap1 + ap2 \times ap3 + am3 \times am1 + ap3 \times am1}{\sum XB_{Dfract}} \] (13)

\[ \sum XB_{Dfract} = ap1 \times ap2 + am3 \times am1 + am3 \times ap2 + am3 \times am2 + ap3 \times ap1 + am2 \times ap1 + ap2 \times ap3 + am3 \times am1 + ap3 \times am1 \] (14)

Where \( \frac{dSL}{dt} \) denotes the sarcomere length velocity and \( \varphi \) represents an empirical scaler equal to 2 [20]. For the detailed explanation of the metabolic-sensitive XB cycling we refer the readers to [23].

2.2. The contractile element calibration for HCM model variant

Our baseline CE inherits the main effects of contractile metabolic products such as MgATP, MgADP, inorganic Phosphate (Pi), and H+ on the tension development mechanism from the original Tran et al. model [22,23]. We used this baseline model to develop an HCM mutant variant (R403Q) model, with altered myofilament kinetics. This model variant was created by modifying specific metabolic parameters to achieve a simulated state consistent with experimental reports of R403Q HCM (Table 2).
To obtain the HCM model variant, we changed F1 and F2 values following [16] and in line with the sensitivity test given in Fig. S4. We also increased the value of MgADP concentration and the reference value of Pi. Finally, we changed the ap2 coefficient regarding the model sensitivity given in Fig. S2.

| #  | Parameter          | Control value | Value in HCM model variant |
|----|--------------------|---------------|-----------------------------|
| 1  | Pi_ref (mM)        | 2             | 18.9                        |
| 2  | MgADP (mM)         | 36×10^{-3}    | 72×10^{-3}                  |
| 3  | ap2 coef.          | 1             | 0.315                       |
| 4  | F1                 | 1             | 1.3                         |
| 5  | F2                 | 1             | 1.3                         |

Fig. 1 Schematics of the interfilament coupling in cardiac force generation (A) the modelled crossbridge cycling used in hiMCE model (B), and the schematic of hiPSC-CM cell main functional components (C). DRX: Disturbed relax state. SRX: Super relaxed state. T: troponin, TCa: Ca^{2+} bound troponin, N XB: non-permissive state preventing XB formation, P XB: permissive state of XB formation, XB preR: strongly bound XB before isomerised rotation, XB pstR: XB in strongly bound post isomerised rotation state, AM1 and AM2 are strongly-bound rapid equilibrium substates contributing equally to the force generation and we assumed MgADP binds to am1 [23].

2.3. The contractile element calibration for drug-induced effects

Previously, Margara et al. assumed in their computational study [16] that MAVA mainly influences the transitions between XB preR and P XB states following the DRX:SRX disturbing theory reported in [5]. This was implemented by introducing F1 and F2 (with default values of 1) coefficients (Eq.2) representing DRX:SRX ratios to the time-dependent description of XB preR state.
In contrast, we simulated the effect of MAVA not only by altering the values of F1 and F2 but also modifying the parameters listed in Table 1 to obtain a comprehensive and accurate simulation of the effect of 0.5 µM MAVA on our HCM R403Q model variant as given in Table 3. These further modifications, done as a manual parameter tuning, are based on the reported effect of MAVA on Ca$^{2+}$ activation and binding process [24], Pi [25], and ATPase activity [10]. Specifically, changes in $K_{on}$ and $n_{perm}$ values have been made regarding the sensitivity analyses given in [19]. The F1 and F2 values were changed according to model sensitivity behavior given in Fig. S4. Further, $ap1$ and $ap3$ coefficients and A-E values were obtained by trial and error.

Table 3 Modifications to the model parameters to simulate the effect of Mavacamten. A, B, C, D, and E are coefficients in Eqs. 15-19 affecting $P_{ XBXB_{preR}}$ regulation, Pi-dependent transition in $P_{ XBXB_{preR}}, XB_{preR}-XB_{postR}$ regulation, Proton-dependent transition in $XB_{postR}-XB_{preR}$, and MgATP-dependent transition from $XB_{postR}-P_{ XB}$, respectively. BL is the baseline value given in Table 1. Default values of A-E, $ap1$ and $ap3$ coefs., F1 and F2 are equal to 1.

| #  | Parameter          | Values in 0.5 µM MAVA |
|----|--------------------|-----------------------|
| 1  | $K_{on}$(mM$^{-1}$s$^{-1}$) | BL × 1.048            |
| 2  | $n_{perm}$         | BL × 0.688            |
| 3  | A                  | 0.26                  |
| 4  | B                  | 0.4                   |
| 5  | C                  | 5.4                   |
| 6  | D                  | 0.4                   |
| 7  | E                  | 2.39                  |
| 8  | $ap1$ coef.        | 1.45                  |
| 9  | $ap3$ coef.        | 0.28                  |
| 10 | F1                 | 0.1                   |
| 11 | F2                 | 0.1                   |

To indicate, A modulates $P_{XB}$ to $XB_{preR}$ transition, B influences Pi-dependent $XB_{preR}$ to $P_{XB}$ transition, C takes effect on $XB_{preR}$ to $XB_{postR}$ transition, D affects proton-dependent $XB_{postR}$ to $XB_{preR}$ transition, and E controls MgATP-dependent transition from $XB_{postR}$ to $P_{XB}$ states. Correspondingly, the proposed modulations (A to E in Table 3) to Eqs. 15-19, are in line with a disturbed interfilament signaling that affects the force-producing states of XB suggested in the etiology of R403Q [26]. Our motivation for the A-E coefficient modifications was the role of $xbmodsp$ parameter in contraction relaxation time observed in the sensitivity study before [19]. As changing $xbmodsp$ solely could not lead to an accurate simulation of impaired relaxation restored by 0.5 µM MAVA, we used A-E values to optimize the distribution of $xbmodsp$ effect on the XB cycling.

$$f_{{apt}} = f_{{ap}} \times A \times xbm_{{modsp}} \times Q_{{f_{{ap}}}}^{\left(\left(\frac{T_{mpc}-37}{10}\right)\right)}$$  \hspace{1cm} (15)

$$g_{{apt}} = g_{{ap}} \times g_{{apstim}} \times B \times xbm_{{modsp}} \times Q_{{g_{{ap}}}}^{\left(\left(\frac{T_{mpc}-37}{10}\right)\right)}$$  \hspace{1cm} (16)

$$h_{{ft}} = h_{{f}} \times h_{{fmd}} \times C \times xbm_{{modsp}} \times Q_{{h_{{f}}}}^{\left(\left(\frac{T_{mpc}-37}{10}\right)\right)}$$  \hspace{1cm} (17)

$$h_{{bt}} = h_{{b}} \times D \times xbm_{{modsp}} \times Q_{{h_{{b}}}}^{\left(\left(\frac{T_{mpc}-37}{10}\right)\right)}$$  \hspace{1cm} (18)
\[ g_{xbt} = g_{xb} \times \max (g_{xbmd}, 1) \times E \times xbmosp \times Q_{g_{xb}}^{(T_{mpc} - 37)/10} \]  \hspace{1cm} (19)

Values of thin filament regulation and XB cycling parameters \( h_b \), \( g_{ap} \), \( g_{apstm} \) and temperature dependences \( Q_{h_b}, Q_{g_{xb}}, Q_{g_{ap}} \) were directly taken from [20]. Also, \( g_{xbmd} \) is a strain-dependent rate modifier defined in [20].

The values of parameters changed in the model to simulate the effects of 5 \( \mu \)M BLEB and 1 \( \mu \)M OM are given in Table 4. F1 and F2 were obtained with attention to sensitivity plots given in Fig. S4. Similarly, \( ap2 \) and \( am2 \) coefficients were found regarding the model behavior shown in Figs. S2, S3 and S5. Lastly, values of parameters listed in Table 4 rows 7 to 15 were obtained manually according to the sensitivity reports given in [19].

Moreover, to capture the dose-dependent effect of BLEB and OM on the normalized tension, based on model sensitivity tests (Figs. S2-S5) and previous sensitivity analyses [19], we identified the main variable of the CE governing the maximum developed tension, \( ap2 \), and identified coefficient values accordingly (Tables S1 and S2). Figs. S2-S5 show the sensitivity of tension-\( Ca^{2+} \) relationships, active tensions, and ATPase rates to \( ap2 \), \( am2 \), and \( R (=F1=F2) \) coefficients. Overall, these sensitivity analyses combined with our previous sensitivity investigations on the CE of our previous model [19] were the basis of the informed parameter tunings done to obtain HCM- and drug-induced calibrations in this work.

Table 4 The modifications to the parameters to simulate the effects of BLEB and OM. BL: Baseline values of hiMCE model given in Table 1.

| # | Parameter   | Modifications for 5 \( \mu \)M BLEB | Modifications for 1 \( \mu \)M OM |
|---|-------------|--------------------------------------|----------------------------------|
| 1 | F1          | 5.015                                | 4.1                              |
| 2 | F2          | 0.1                                  | 0.1                              |
| 3 | Tropreg coef.| 0.2                                  | 0.2                              |
| 4 | ap2 coef.   | 0.012                                | 0.02                             |
| 5 | ap3 coef.   | 0.03                                 | 0.03                             |
| 6 | am2 coef.   | 0.25                                 | 0.15                             |
| 7 | \( K_{on} \) (mM\(^{-1}\) s\(^{-1}\)) | -                                    | BL \times 1.28                   |
| 8 | \( n_{perm} \) | -                                    | BL \times 1.182                   |
| 9 | \( perm_{50} \) | BL \times 1.33                      | BL \times 1.33                   |
| 10 | \( K_{on} \) (s\(^{-1}\)) | -                                    | BL \times 0.2                    |
| 11 | \( K_{np} \) (s\(^{-1}\)) | -                                    | BL \times 1.182                   |
| 12 | \( K_{offmod} \) | -                                    | BL \times 0.52                    |
| 13 | \( K_{offL} \) (s\(^{-1}\)) | -                                    | BL \times 1.75                    |
| 14 | \( K_{offH} \) (s\(^{-1}\)) | -                                    | BL \times 0.6                     |
| 15 | \( h_{f} \) (s\(^{-1}\)) | -                                    | BL \times 2                      |

2.4. The experimental data for calibrations and validations

Table 5 gives the experimental data using which the results of this work have been calibrated and validated.

Table 5 The experimental data used for calibration of the model and validation of the simulated results.
| Calibration | [5] | hiPSC-CMs | 33% increase of tension relaxation in R403Q | Fig. 3C |
|---|---|---|---|---|
| | | | Corrected tension relaxation due to MAVA in R403Q | |
| | | | The fractional cell shortening in R403Q | Fig. 3F |
| [5,10] | hiPSC-CMs, Murine | Reduction in fractional cell shortening due to MAVA | Fig. 3F |
| [10,24] | Human, Murine, and Bovine | Reduction in maximum tension due to MAVA | Fig. 3C |
| [11] | Rat | Change in pCa50, Hill coefficient, and maximum tension due to OM and BLEB | Table 7, Fig. 4A |
| | | OM and BLEB dose-dependent contractile response | Fig. 4D&E |
| Validation | [27] | hiPSC-CMs | The unaffected CaT in R403Q | Fig. 3B |
| | [4,26] | Rabbit & Bovine, hiPSC-CMs | Negligible change in ATPase in R403Q | Fig. 3D |
| | [10] | Murine | Unchanged CaT and pCa50 in Tension-pCa curve due to MAVA | Fig. 3B, Fig. S1 |
| | [28] [29] [10] [30] | Human, Bovine, and Murine | Reduction in ATPase rate due to MAVA | Fig. 3D |
| | [29] | Human & Bovine | Slowed relaxation in ATPase rate due to MAVA | Fig. 3D |
| | [31] | Canine | Change in APD due to 1 µM OM | Fig. 5A |
| | [32,33] | Murine Pigs | ATPase basal value increase due to OM | Fig. 5B |
3. Results

3.1. The metabolic-sensitive model of hiPSC-CMs

First, we show that our computational model can correctly simulate the main AP, Ca\(^{2+}\) transients (CaTs), and active tension (AT) biomarkers as had been simulated by our previous electromechanical hiPSC-CM-CE model [19]. As Table 6 shows, our new metabolic-sensitive hiMCE model is able to simulate the main biomarkers within the experimental ranges in the validation datasets. The increased thermodynamic detail of the CE did not significantly alter the biomarker values compared to our previous reparameterization [19] and the original Paci et al. hiPSC-CM model [21]. Also, Fig. 2 shows the contractility characteristics simulated using the hiMCE model are consistent with the previously validated results, while also illustrating selected fundamental outputs [34,35].

Table 6 Action potential (AP), Ca\(^{2+}\) transients (CaT), and active tension (AT) calculated biomarkers in spontaneous condition and their comparison with Paci2020 and hiPSC-CM-CE model (i.e. no metabolic-sensitive CE) and the experimental values [21,36]. APA: AP amplitude, MDP: maximum diastolic potential, CL: cycle length, dV/dt max: maximum upstroke velocity, APD\(_{10}\) and APD\(_{30}\) and APD\(_{90}\): AP duration at 10, 30, 90% of repolarization, respectively, AP Tri: AP triangulation index. The simulated biomarkers of CaT are DURATION: duration of the transient, tRise\(_{10,\text{peak}}\) time to peak, tRise\(_{10,50}\) and tRise\(_{10,90}\) rise time from 10 to 50% and 90% of maximum threshold, respectively, and tDecay\(_{90,10}\): decay time from 90 to 10%. AT: Active tension, RT\(_{50}\): time from peak contraction to 50% of relaxation, %FS: percent of fractional shortening. The experimental ranges for contraction biomarkers are from [19]. The third column is taken directly from the original Paci2020 publication [21].

| No. | Biomarker          | Paci2020 | hiPSC-CM-CE | hiMCE  | Exp. Value (Mean±SD) |
|-----|--------------------|----------|-------------|--------|----------------------|
| 1   | APA (mV)           | 102      | 103         | 103    | 104±6                |
| 2   | MDP (mV)           | -74.9    | -75.0       | -75.0  | -75.6±6.6            |
| 3   | AP CL (ms)         | 1712     | 1644        | 1644   | 1700±548             |
| 4   | dV/dt max (V/s)    | 20.5     | 23.9        | 24.0   | 27.8±26.3            |
| 5   | APD\(_{10}\) (ms)  | 87.0     | 95.0        | 95.1   | 74.1±26.3            |
| 6   | APD\(_{30}\) (ms)  | 224      | 238         | 238    | 180±59               |
| 7   | APD\(_{90}\) (ms)  | 390      | 403         | 403    | 415±119              |
| 8   | AP Tri             | 2.8      | 2.9         | 3      | 2.5±1.1              |
| 9   | CaT DURATION (ms)  | 691      | 693         | 693    | 805±188              |
| 10  | CaT tRise\(_{10,\text{peak}}\) (ms) | 184 | 163 | 163 | 270±108 |
| 11  | CaT tRise\(_{10,50}\) (ms) | 54.9 | 46.2 | 45.9 | 82.9±50.5 |
| 12  | CaT tRise\(_{10,90}\) (ms) | 118 | 102 | 102 | 167±70 |
| 13  | CaT tDecay\(_{90,10}\) (ms) | 341 | 343 | 343 | 410±100 |
| 14  | AT magnitude (kPa) | -        | 0.055       | 0.055  | 0.055±0.009          |
| 15  | RT\(_{50}\) (ms)   | -        | 161         | 158    | 158±12.1             |
| 16  | %FS                | -        | 3.45        | 3.23   | 3.27±0.37            |
Fig. 2 Standard results of the model: Action Potentials (A), Ca$^{2+}$ Transients (B), Active Tensions (C), ATPase rate (D), Flux of Ca$^{2+}$ towards the contractile element (E), Fractional cell shortening at 1 Hz pacing (F). Cited works: [19,34,35].

3.2. Hypercontractility in R403Q HCM and Mavacamten

In order to simulate the abnormal prolonged relaxation in the developed active tension due to R403Q HCM mutation, Margara et al. hypothesized a feedback from XB cycling to the thin filament activation [16]. To investigate further, using the parameter values in Table 2 and consistent with the metabolic data detailed in section 2.2, we simulated the active tension and ATPase rate in R403Q HCM model variant. The CaT morphology remains unchanged in the HCM R403Q mode (Fig. 3B), consistently with experimental data reported for hiPSC-CMs [27]. Interestingly, the results in Fig. 3C suggest that including energetics in the CE reacts to the pathological changes due to HCM and can correctly predict the prolonged relaxation in the developed active tension (~33%), consistently with in vitro hiPSC-CMs data [5]. Moreover, the increased fractional cell shortening (~40%) due to the R403Q mutation is consistent with experimental measurements in [5]. Of note, the model also correctly predicts the negligible change in the ATPase activity (Fig. 3D), consistently with the experimental data (Table 5) [4,26].

To simulate the electro-mechano-energetic effect of 0.5 µM MAVA, we used the model calibration values listed in Table 3. We have assumed that MAVA would shift the elevated metabolites in the HCM model variant, Pi and MgADP, towards their baseline values. Our model could accurately predict the unaffected CaTs due to MAVA as reported experimentally earlier [10]. Further, the order of reduction in the simulated ATPase rate (19.3%) due to 0.5
µM MAVA, Fig. 3D, is within the reduction range, 17.9 to 28.5%, reported in previous experimental ATPase activity measurements [30]. Also, the model consistently predicts the reduction in the relaxation phase in the ATPase rate (Fig. 3D) [29]. Notably, our simulations quantitatively capture the reduction in the fractional cell shortening and prolonged tension relaxation due to R403Q mutated hiPSC-CMs after 0.5 µM MAVA (14.6% and 20.9%, respectively), consistently with recent experimental measurements [5]. Finally as shown in Fig. S1, the CE model accurately predicts the unchanged pCa50 in the tension-Ca²⁺ relationship consistent with the experimental data for 0.5 µM MAVA [10].

![Fig. 3 Simulated action potential (A), Calcium transients (B), active tensions (C), ATPase rate (D), Flux of Ca²⁺ towards the myofilament (E), and fractional cell shortenings (F) in R403Q hypertrophic cardiomyopathy and Mavacamten modes (All simulations were done at 1Hz pacing). The percents of prolonged tension relaxation in R403Q mode (C), the reduction in tension relaxation due to MAVA (C), the reduction in fractional shortening in R403Q mode due to MAVA (F), and the reduction in ATPase rate due to MAVA (D) agree with the experimental data [5,30].](image)

**3.3. Simulated effects of Omecamtiv mecarbil and Blebbistatin**

Using the CE parameter values listed in Table 4, we simulated the effect of 5 µM BLEB and 1 µM OM. As Table 7 shows, the drug-induced calibration of the hiMCE model results in accurate predictions of Ca²⁺ sensitive effects of 5 µM BLEB and 1 µM OM consistent with experimental data [11] as also Fig. 4A qualitatively confirms. Additionally, the selected values for the coefficients of the tension governing variables in the CE, ap2S (Fig. 4B and C and Tables S1 and S2), leads to the correct dose-dependent prediction for BLEB and OM (Fig. 4D and E) and the expected inverse Hill curves reported experimentally [11].

**Table 7 Experimental data [11] and hiMCE results due to the effect of 1 uM OM and 5 uM BLEB. pCa50 represents the -log of the Ca²⁺ concentration associated with 50% of maximum tension. cTnC-E: data from**
cardiac troponin C (cTnC) E-helix obtained by a rhodamine probe. cRLC-E: Data from a probe connected to the myosin regulatory light chain (RLC).

| #  | Item                                                                 | Kampourakis et al. 2018                        | hi-MCE Model |
|----|----------------------------------------------------------------------|-----------------------------------------------|--------------|
| 1  | Increase in pCa_{50} due to 1 µM OM                                   | 5.4-6.9% (cRLC-E) 3.8-6.4% (cTnC-E)           | 5.8%         |
| 2  | Decrease in pCa_{50} due to 5 µM BLEB                                  | 2.1-5.5% (cRLC-E) 2.6-5.7% (cTnC-E)           | 4.8%         |
| 3  | Reduction of Hill coef. due to 1 µM OM                                | 53.2-64.5% (cRLC-E) 57.1-68.1% (cTnC-E)       | 58.9%        |
| 4  | Reduction of Hill coef. due to 5 µM BLEB                              | 59.5-73.5% (cRLC-E) 44.8-58.3% (cTnC-E)       | 49.9%        |
| 5  | Reduction in max tension due to 1 µM OM                               | 0-29% (cRLC-E) 18-47% (cTnC-E)                | 25.3%        |
| 6  | Reduction in max tension due to 5 µM BLEB                              | 66-90% (cRLC-E) 64-80% (cTnC-E)               | 76.4%        |

Moreover, our model predicts an insignificant reduction in AP duration (3.4%) due to 1 µM OM (Fig. 5A), evaluated by calculating APD_{90} values (Fig. 5A). This translates to 17 ms reduction in APD_{90} (502 to 485 ms) which is consistent with the order of APD_{90} reduction due...
to 1 µM OM, 12.2 ms, reported for canine cardiomyocytes at 1 Hz pacing [31]. Also, the simulated increase in the basal ATPase rate due to 1 µM OM (Fig. 5H) is qualitatively consistent with the experimental data reported before [32,33].

Our model predicts a 23% increase in the amplitude of the Ca\(^{2+}\) flux towards the myofilament, JCB (Fig. 5B). The subsequent accumulation of intracellular Ca\(^{2+}\) is seen as a 4.5% increase in CaT peak (Fig. 5E). Interestingly, the steady-state alterations in sarcolemmal Ca\(^{2+}\) transport are very subtle: virtually unchanged I\(_{\text{CaL}}\) (Fig. 5C) and only very slightly increased I\(_{\text{pCa}}\) (Fig. 5F). Whereas there is a more substantial 17% increase in the amplitude of the I\(_{\text{NCX}}\) (Fig. 5D). The enhanced reverse mode of I\(_{\text{NCX}}\) causes accumulation of intracellular Na\(^{+}\) (7.04 vs 7.43 mM) that promotes a stronger repolarizing I\(_{\text{NaK}}\) (Fig. 5G). This appears to be the mechanism that causes the subtle yet visible 3.4% decrease in the AP duration (Fig. 5A), consistently with the reported experiments suggesting OM as a safe compound on cardiac electrophysiology in clinically tolerated doses [31]. These predictions can be insightful regarding the consequences of the disturbed interfilament signaling that OM elicits in the XB.

Fig. 5 Predicted effect of 1 µM OM by hiMCE on action potentials (A), Ca\(^{2+}\) flux towards the myofilament (B), L-type Ca\(^{2+}\) current (C), Na\(^{+}/Ca\(^{2+}\) exchanger I\(_{\text{NCX}}\) (D) Ca\(^{2+}\) transients (E), sarcolemmal Ca\(^{2+}\) pump current (F), Na\(^{+}/K\(^{+}\) pump (G), and ATPase rates (H). The change of APD has been considered calculating APD\(_{90}\) in agreement with experimental data reported in [31]. OM: Omecamtiv mecarbil.
4. Discussion

4.1. HCM and energetics of contraction

Cardiomyocytes, with no self-renewal capacity, must provide two billion beats during an average lifetime for which the cardiac muscle requires a significant amount of energy, six kg of ATP per day \[37\]. This energy consumption is predominantly due to the function of sarcomeres in contraction. Therefore, the pathological conditions directly caused by sarcomeric mutations, such as R403Q HCM, necessitate studying contractile function of cardiomyocytes regarding cardiac metabolism. Our analysis demonstrates that the incorporated scheme of the metabolic-sensitive CE is able to capture the impaired (prolonged) tension relaxation, ~33%, due to the R403Q mutation. Interestingly, with the energetics included, the additional feedback from XB cycling to the thin filaments, proposed previously by \[16\], was not necessary to replicate the altered relaxation. This further highlights the importance of considering (patho)physiologically constrained metabolic-sensitive computational models in the investigation of sarcomeric cardiomyopathies.

4.2. HCM and drug-induced model calibrations

Contractile energetics become highly important when studying promising drugs reported in HCM clinical trials such as MAVA, BLEB, and OM. As our results show, the quantitatively valid simulation of the effect of MAVA, BLEB, and OM, in single dose or dose-dependently, could not be done without the calibration of parameters in the CE that directly or indirectly affect the energetics (Tables 3, 4, S1, and S2). Markedly, one of the important insights of this study stems from the parameters involved in the calibration of the model for the simulation of 0.5 µM MAVA. We took the MAVA modeling one step further by calibrating the CE altering parameters affecting Pi-dependent transition between permissive binding state on actin (PXB) and the strongly bound XBS before isomerized rotation (XB\text{preR}) state. Also, we modulated Ca\textsuperscript{2+} binding and sensitivity of the CE, and MgATP-dependent transitions between PXB and XBS in strongly bound post isomerized rotation state (XB\text{postR}) in accord with experimental metabolic reports \[10,24,25\]. Towards decoding the precise drug mechanism of action, the modulations proposed here to explain the effect of MAVA (Table 3) implies that, alongside altering the disturbed DRX:SRX ratio, MAVA might also induce a new interfilament equilibrium, modulating tension-producing and energetic terms that explicitly affect the reverse transition at play between XB\text{preR} and XB\text{postR} affecting the strain-dependent isomerization of myosin heads. This possible pharmacological insight emerging from our model is interesting as MAVA mechanism of action inherently shifts the R403Q impaired metabolism towards normal regulation and this involves the impaired proton-dependent transition in R403Q mode \[5\].

The drug- and HCM-related calibrations presented in this work are in line with the proposed OM and BLEB structure-function relationships detailed in \[11\]. To enumerate, Kampourakis et al. have implied that OM-bound myosin heads relocate the tropomyosin to its on state when binding to actin in the absence of Ca\textsuperscript{2+} bound to troponin. Importantly, the XB activation, which is due to the effect of OM, has been significantly attributed to the stabilizing
of the ON state of thick filaments. Further, these stabilized ON positions in thick filaments have been considered to promote an ON thin filament state through preventing tropomyosin returning to their off positions [11]. This has been translated in our model by modifying $k_{\text{off}}$ constants which are the rate constants affecting Ca$^{2+}$ unbinding from low and high affinity sites on troponin. These transition rates affect regulatory sites on cardiac troponin leading to activation of XB cycle [23].

On the other hand, at intermediate Ca$^{2+}$ in the physiological range (pCa 9 to 4.3), OM activates acts along with the thick filaments. In our model, this has been translated by modifying $K_{\text{on}}$, $K_{\text{np}}$ and $K_{\text{pn}}$ rate constants for Ca$^{2+}$ binding to troponin, forward and backward transition rates between $P_{\text{xb}}$ and $N_{\text{xb}}$ states, respectively (Table 4). Further, the constant rates directly affecting Ca$^{2+}$ bound troponin thin filaments regulation induced by Ca$^{2+}$ bound troponin ($K_{\text{on}}$, $n_{\text{perm}}$, $k_{\text{offH}}$, $k_{\text{offL}}$, $k_{\text{offK}}$, $k_{\text{offmode}}$) have only been calibrated towards activation for OM and they are missing in BLEB calibration as BLEB does not switch both filaments to their ON states [11]. Further, modification of coefficient of Tropreg variable, the fraction of actins with Ca$^{2+}$ bound, detailed in [20], in both OM and BLEB calibrations is consistent with the reported effect of these drugs as both OM and BLEB generally greatly decrease the Ca$^{2+}$ activation cooperativity [11].

In the HCM variant model, the significant increase in Pi concentration is consistent with the experimental metabolic reports of HCM R403Q and R92Q mutations in animal mouse models [38,39]. Moreover, the impaired coronary perfusion due to HCM has been related to an abnormal energy reproduction that contributes to elevation of ADP and Pi [40], which is also consistent with conservation of phosphate and creatin reaction [23]. Congruently, the HCM mutation-induced alterations in myofilament kinetics lead to increase in ADP-mediated products [18]. Therefore, we increased the MgADP concentration within physiological ranges [23]. Notably, the Ca$^{2+}$ dependence in the activation of myofilaments for HCM and dilated cardiomyopathy has been shown to be altered in a similar fashion due to OM and BLEB influence [6], implying that the etiology of HCM includes a disturbed actomyosin signaling. In addition, the underlying mechanism of HCM-induced hypercontractility, including R403Q HCM, has been explained in light of thick filament structural alterations and the tension generation [41–43]. Granted that, since with change in $\alpha p$ coefficient (Table 2) the XB cycling machinery could closely capture the HCM behavior (Fig. 3), our model potentially attributes the HCM-induced disturbed interfilament signaling to a distortion-dependent forward transition from $X_{\text{B,preR}}$ to $X_{\text{B,postR}}$ state and the subsequent effect due to population of strongly bound XB states in the steady-state condition. This could imply that the HCM-induced disturbed actomyosin signaling might stem from a misregulated isomerization of myosin heads from pre-rotated to post-rotated force generating state affecting the strain induced in the myosin neck region. Analogically, the same process might explain how OM, BLEB, and Mava contribute to a new interfilament balance, restoring normal XB cycling tension generation. This hypothesis based on our model predictions could be insightful for accelerating the future drug development for sarcomere cardiomyopathies and mutation-specific HCM.
We have introduced a mechanistic solution to incorporate the dose-dependent effect of Blebbistatin and Omecamtiv mecarbil (Fig. 4) consistent with experiments [11] focusing on ap2. Explicitly, the inverse Hill function trend observed in dose-dependent effects of OM and BLEB [11] also reflects in the values of ap2 coefficients obtained for the studied concentrations (Fig 4B-E). Moreover, changes in F2, hf, and ap2 (Table 4) suggest that OM also favors the rapid detachment of XBs as another contributor to the disturbed actomyosin coupling involved in OM mechanism of action. Furthermore, the accurate simulation of shortened APD due to 1 μM OM combined with elevated basal ATPase rate values further signifies that inclusion of metabolic-sensitive transitions in the XB cycling of the CE cannot be ignored in precision medicine, especially when simulating the effects of drugs whose main mechanism of action impacts ATPases.

4.3. Limitations and future works

The developed mathematical model naturally has some limitations and potentials for advancements in the future studies. Firstly, as we use ODEs instead of computationally expensive PDEs, the cooperative spatial interactions between regulatory proteins and XB action have been approximated with a mean-field technique [20]. Secondly, we assumed that 0.5 μM MAVA restores the elevated MgADP and Pi in HCM model variant to their basal values (listed in Table 2). Although this assumption is consistent with MAVA mechanism of action [10], it is still a simplification. Thirdly, one fundamental limitation of hiPSC-CMs is that they rely on glycolytic metabolism, in contrast to the fatty acid-based metabolism of native human adult ventricular cardiomyocytes. A further source of energetic dissimilarities is the differences between surrounding medium in vitro vs in vivo conditions. Given these and as our model does not include energy production process, we consider the metabolic differences out of the scope of this work. As detailed in vitro data on hiPSC-CM metabolism emerges, our modeling efforts serve as a solid basis for the next phase of cardiomyocyte models with energy production included. Finally, the model can benefit from the inclusion of a metabolic-sensitive formulation of the intracellular SERCA pump, as a key ATP-dependent transporter. However, as the focus of this work was sarcomeric cardiomyopathies we have considered it out of scope here.

5. Conclusion

As cardiac precision medicine arises [44–46], the demand for comprehensive computational models capable of performing high throughput pharmacological investigations heightens. This works proposes a novel metabolic-sensitive computational model of hiPSC-CMs electromechanics with demonstrated capacity to simulate sarcomeric cardiomyopathies and the compounds directly affecting the myosin dynamics considering the metabolic pathways. The mechanistic method offered for simulating the effects of HCM and drugs in this work lends insights upon the molecular interactions in contractile function and advance our pathophysiological understanding of the development of future therapeutics for HCM.
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7. Conflicts of Interest

The authors declare no conflicts of interest.

8. References

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9. Appendix

Table S1 ap2 coefficients at different Blebbistatin (BLEB) concentrations and simulated normalised tensions. T is the isometric force and T₀ is the isometric force in the absence of drugs.

| #  | Bleb Concentration (M) | ap2 Coefficient | Simulated T/T₀ |
|----|------------------------|-----------------|----------------|
| 1  | 1×10⁻⁸                 | 0.1100          | 0.9964         |
| 2  | 1×10⁻⁷                 | 0.0930          | 0.9407         |
| 3  | 5×10⁻⁷                 | 0.0780          | 0.8796         |
| 4  | 1×10⁻⁶                 | 0.0626          | 0.8003         |
| 5  | 2×10⁻⁶                 | 0.0390          | 0.6270         |
| 6  | 5×10⁻⁶                 | 0.0130          | 0.2735         |
| 7  | 1×10⁻⁵                 | 0.0074          | 0.1816         |
| 8  | 5×10⁻⁵                 | 0.0010          | 0.0288         |

Table S2 ap2 coefficients at different Omecamtiv mecaribib (OM) concentrations and simulated normalised tensions. T is the isometric force and T₀ is the isometric force in the absence of drugs.

| #  | OM Concentration (M) | ap2 Coefficient | Simulated T/T₀ |
|----|-----------------------|-----------------|----------------|
| 1  | 1×10⁻⁸                | 0.0470          | 0.9996         |
| 2  | 5×10⁻⁸                | 0.0440          | 0.9827         |
| 3  | 1×10⁻⁷                | 0.0370          | 0.9360         |
| 4  | 5×10⁻⁷                | 0.0286          | 0.8604         |
| 5  | 1×10⁻⁶                | 0.0200          | 0.7462         |
| 6  | 2.53×10⁻⁶             | 0.0090          | 0.4848         |
| 7  | 5×10⁻⁶                | 0.0051          | 0.3260         |
| 8  | 1×10⁻⁵                | 0.0034          | 0.2366         |
| 9  | 5×10⁻⁵                | 0.0014          | 0.1088         |
| 10 | 1×10⁻⁴                | 0.0011          | 0.0884         |
Fig. S1 Tension-Ca\(^{2+}\) relationships and the effect of 0.5 \(\mu\)M Mavacamten in isometric conditions.
Fig. S2 Tension-pCa relationships in response to \( ap2 \) coefficient modulation in isometric condition.

Fig. S3 Tension-pCa relationships in response to \( am2 \) coefficient modulation in isometric condition.
Fig. S4 Active tension (A) and ATPase rate (B) in response to $R (=F_1=F_2)$ modulation.

Fig. S5 Active tension (A) and ATPase rate (B) in response to $am_2$ coefficient modulation.