Adult Human Primary Cardiomyocytes:
Predicting Inotropic Mechanisms of Action

HESI Cardiac Safety Committee Workshop
May 15-16, 2018

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Vice President R&D

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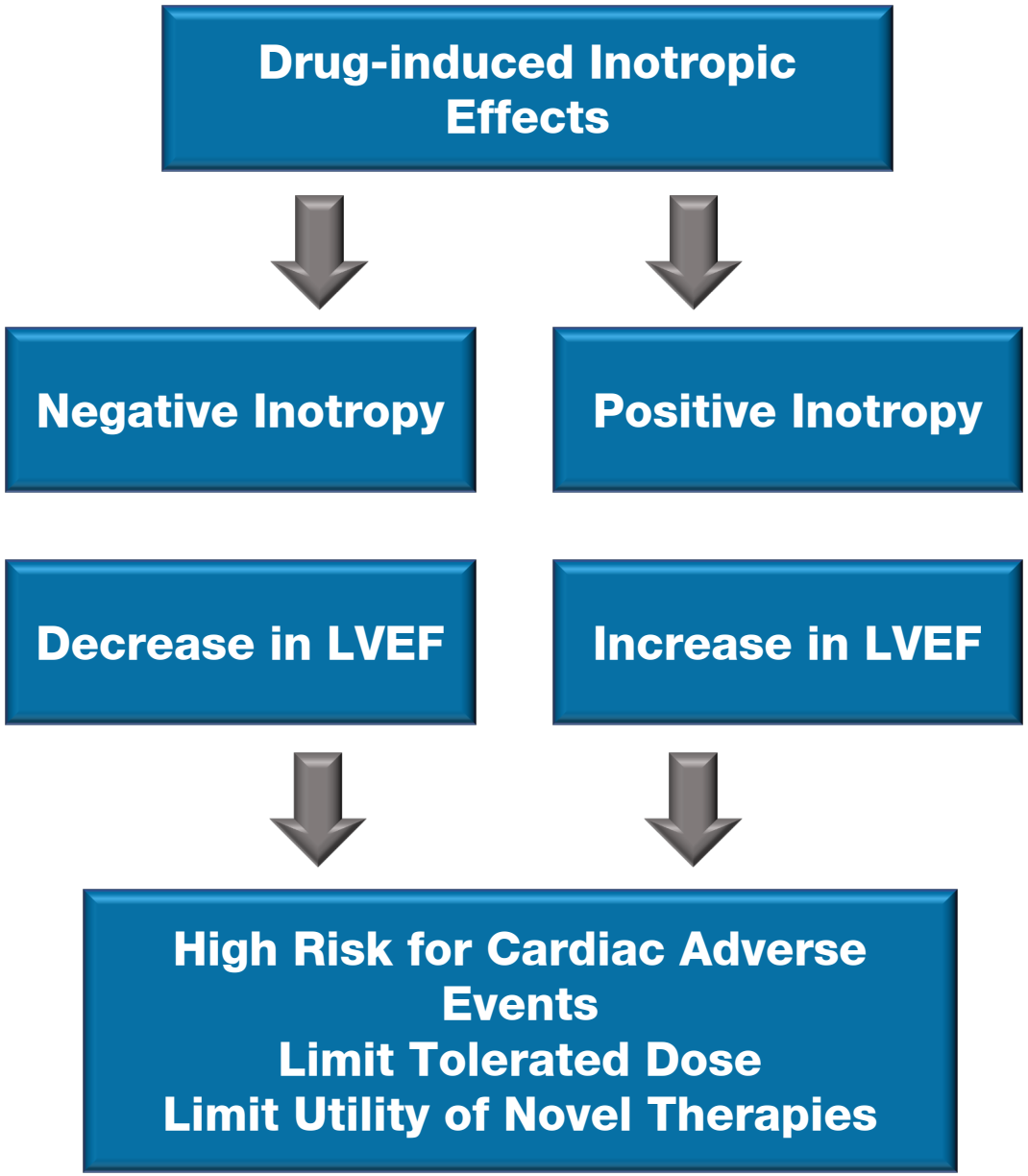
- Need for Preclinical Contractility Safety Testing
- Human Cardiomyocyte Excitation-Contraction Coupling
- Identification of Drug-induced Inotropic Effects
- Predicting Inotropic Mechanisms of Action
- Summary & Questions

Overview



Need for Preclinical Human Contractility Safety Testing

Early stages of drug discovery

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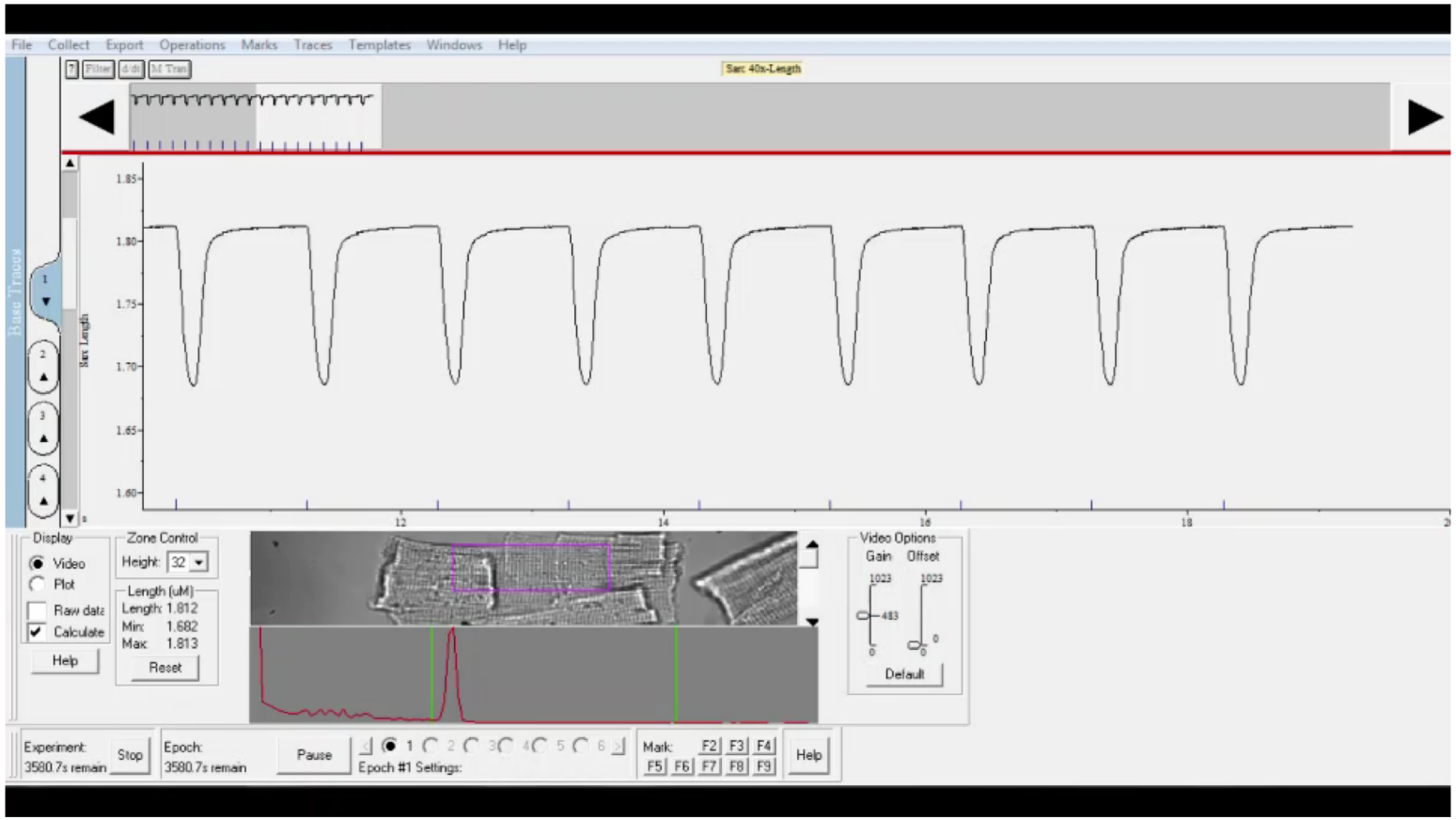
- Address both positive and negative inotropic risks
- Predictive of clinical outcome
- Scalable to medium- / high-throughput

Required features for an Adult Human Primary Cardiomyocyte Inotropic Contractility Assay



Non-Invasive measurement of contraction using Bright-Field Imaging

Low Technical Complexity
No Cytotoxic Reagents
High Information Content

IonOptix: Sarcomere shortening measured by digital cell geometry tracking

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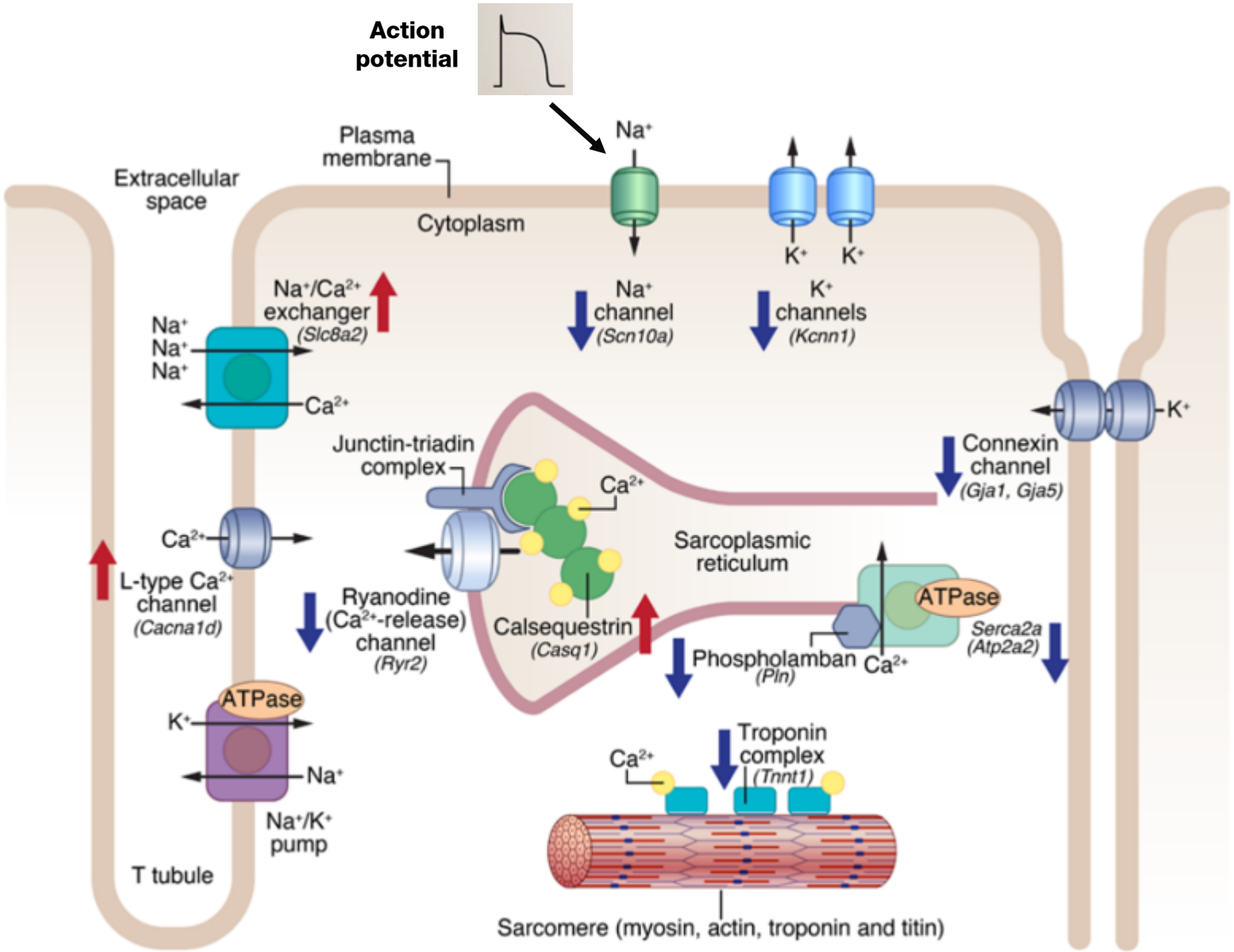


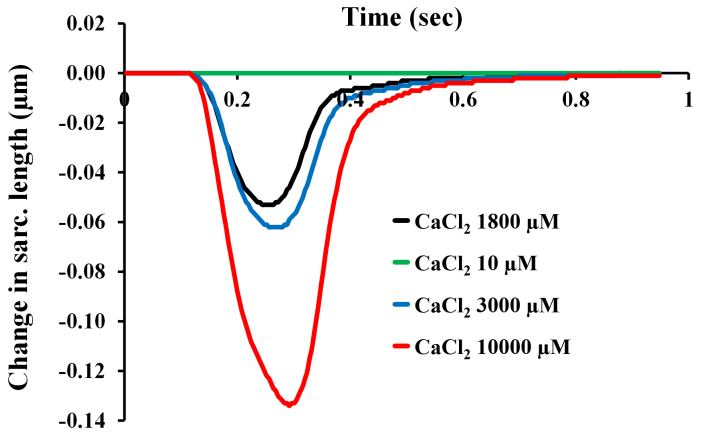
Baskin et al., 2016 JCI

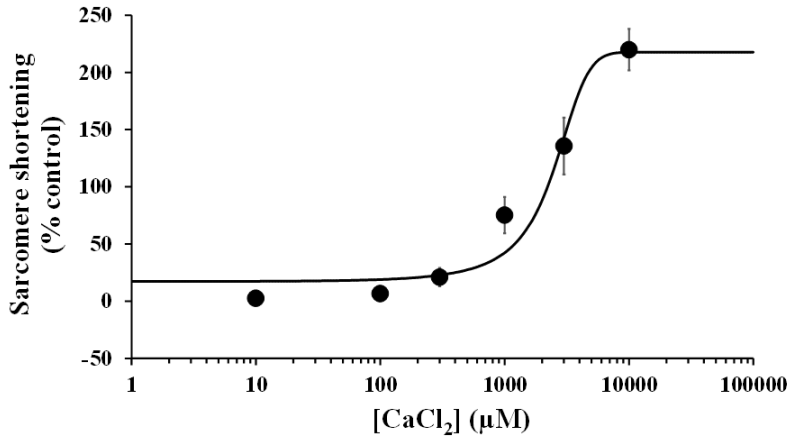
Excitation-Contraction Coupling

Converting an electrical stimulus to a mechanical response

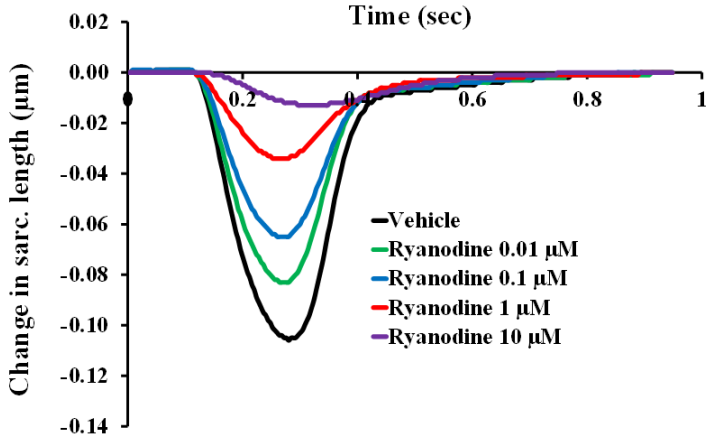
Ca^{2+}-induced Ca^{2+} release (CICR)

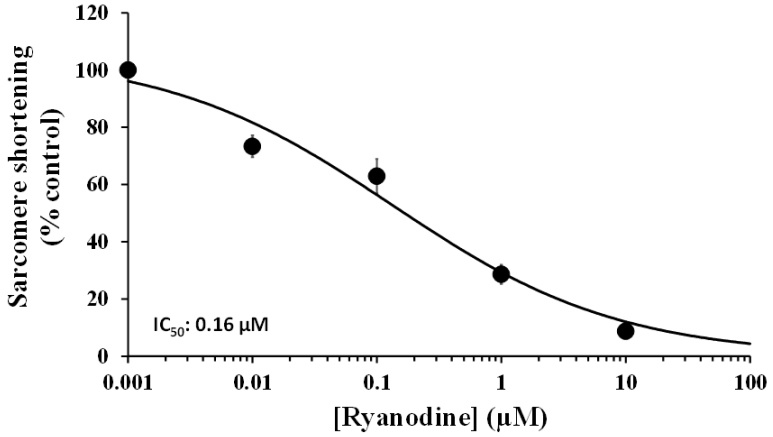
Sarcolemmal Ca^{2+} Influx





SR Ca^{2+} Release





Sarcolemmal Ca^{2+} Influx and CICR Regulate Systolic Ca^{2+}

Ryanodine: Ryanodine receptor (RyR) inhibitor

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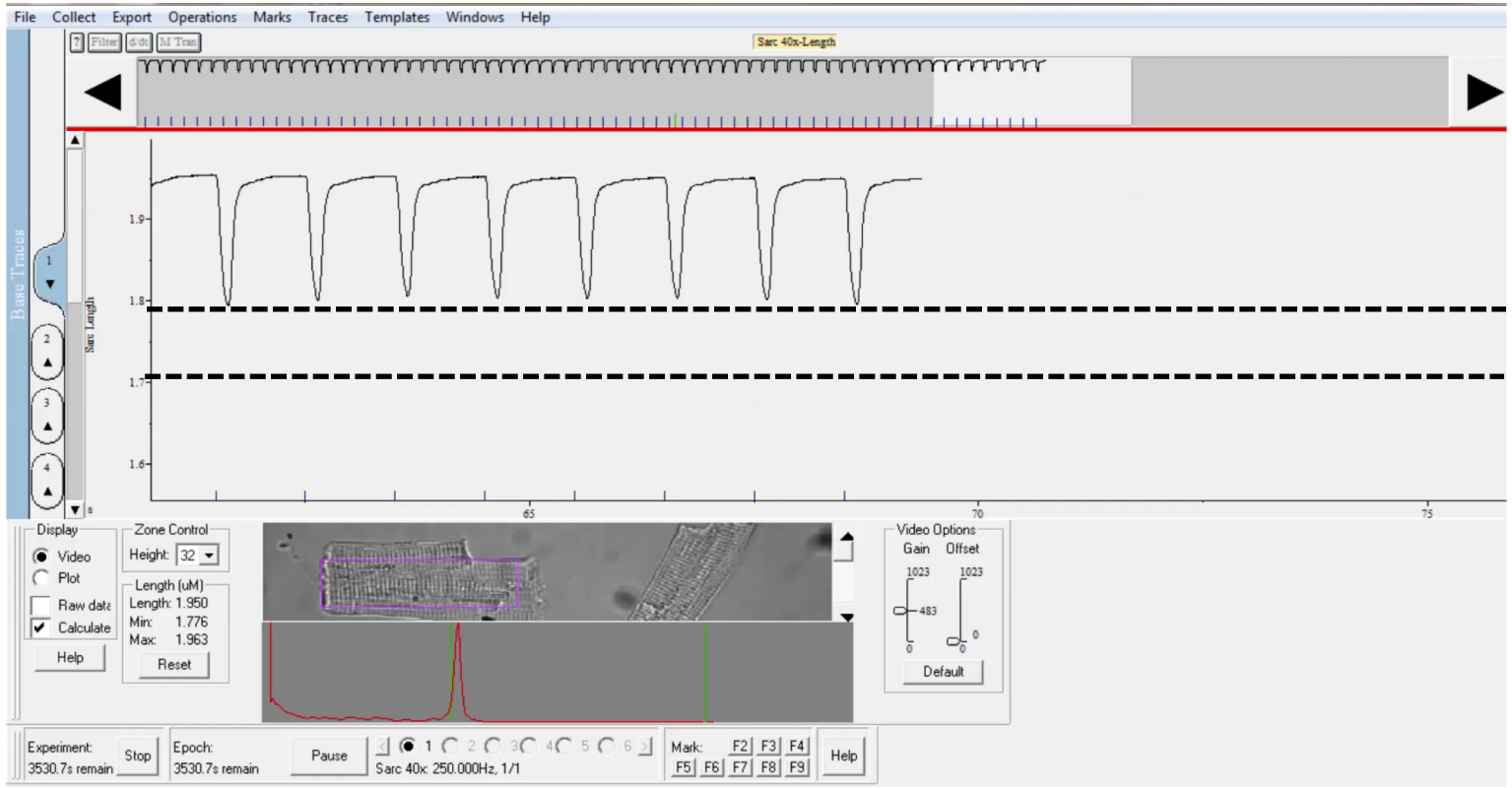
Inotropic Effect	Mechanism of Action	Drug	Investigation
Positive	Na^+/K^+ pump inhibition	Digoxin	This study
Positive	Na^+/K^+ pump inhibition	Ouabain	This study
Positive	Na^+/Ca^{2+} exchanger inhibition	SEA-0400	This study
Positive	Myosin activation	Omecamtiv Mecarbil	This study
Positive	Ca^{2+} sensitization	Levosimendan	This study
Positive	Non-selective β-adrenoceptor activation	Isoproterenol	This study
Positive	Non-selective β-adrenoceptor activation	Epinephrine	This study
Positive	β1-adrenoceptor activation	Dobutamine	This study
Positive	PDE3 inhibition	Milrinone	This study
Positive	PDE inhibition	IBMX	This study
Positive	Ca^{2+} channel activation	Bay-K 8644	This study
Positive	Adenylyl cyclase activation	Forskolin	This study
Positive	Hypercalcemia	CaCl2	This study
Positive	SERCA activation	N106	This study
Positive	RyR activation	Caffeine	This study
Negative	SERCA inhibition	Thapsigargin	This study
Negative	RyR inhibition	Ryanodine	This study
Negative	Ca^{2+} channel inhibition	Nitrendipine	Nguyen et al., 2017
Negative	Ca^{2+} channel inhibition	Nifedipine	Nguyen et al., 2017
Negative	Ca^{2+} channel inhibition	Diltiazem	Nguyen et al., 2017
Negative	Ca^{2+} channel inhibition	Mibefradil	Nguyen et al., 2017
Negative	Ca^{2+} channel inhibition	Verapamil	Nguyen et al., 2017
Negative	Na+ channel inhibition	Mexiletine	Nguyen et al., 2017
Negative	Na+ channel inhibition	Flecainide	Nguyen et al., 2017

Excitation-Contraction Coupling Modulation with Well Characterized Controls



Isoproterenol-induced Positive Inotropic Effect

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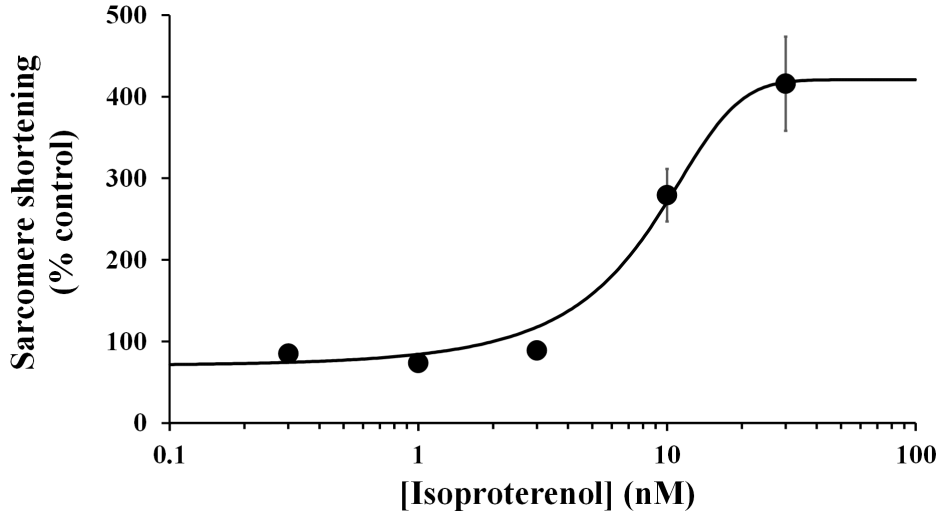

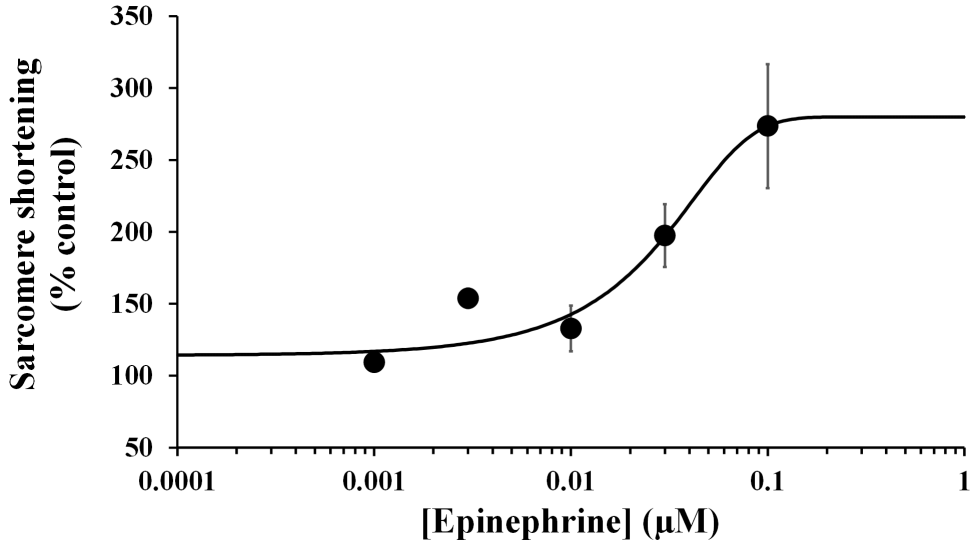

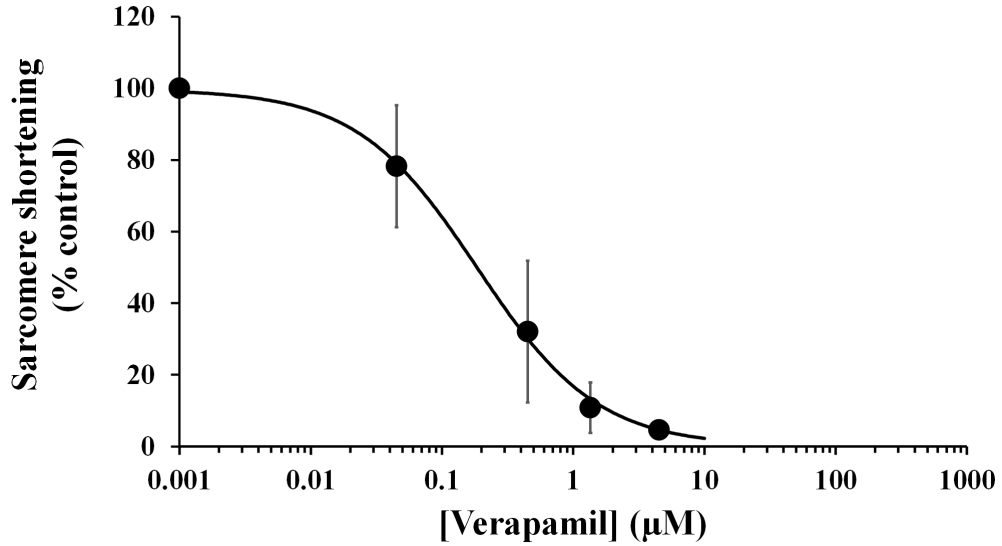

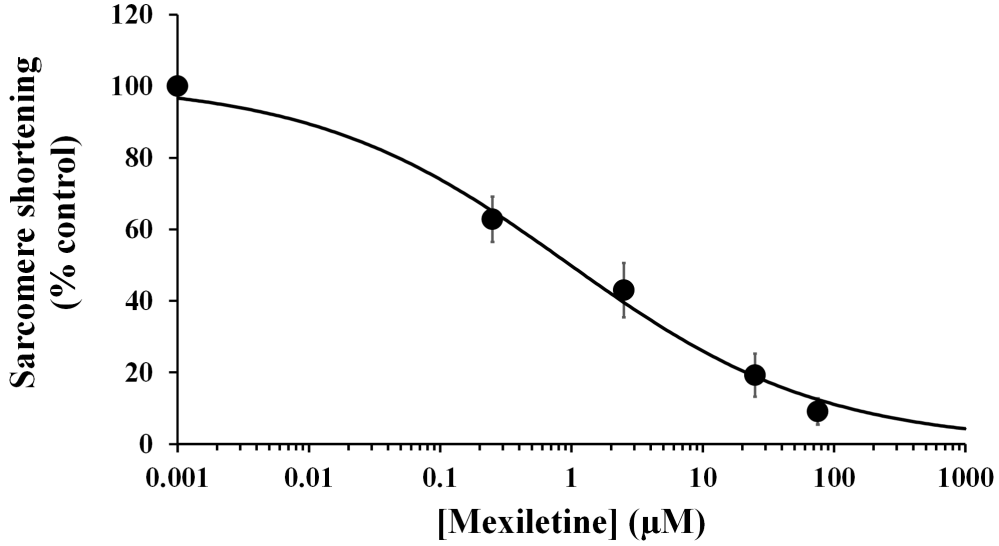

- Compounds associated with either positive or negative inotropic effect can be identified
- Assessment of drug effects on sarcomere shortening provides phenotypic outcome only

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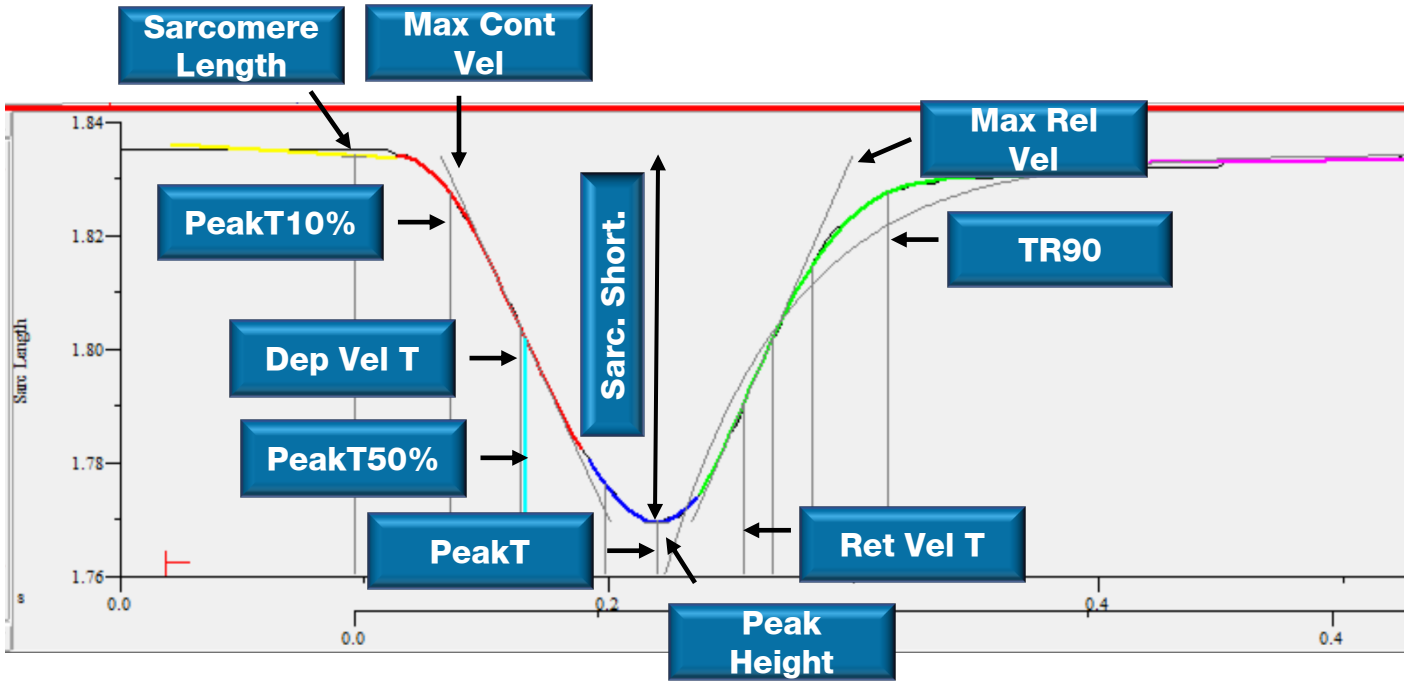
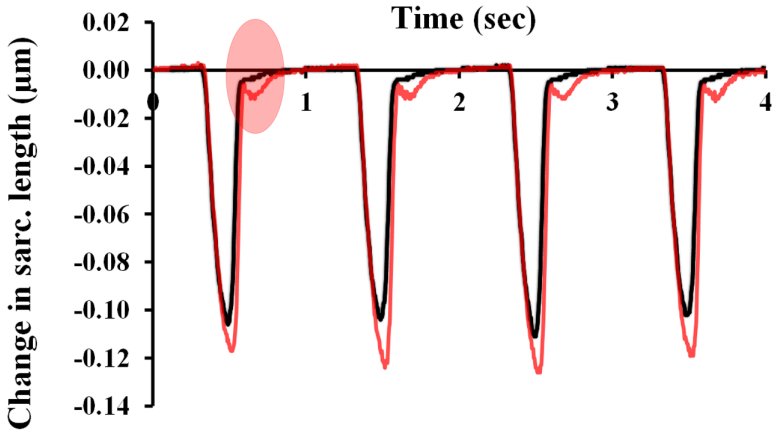
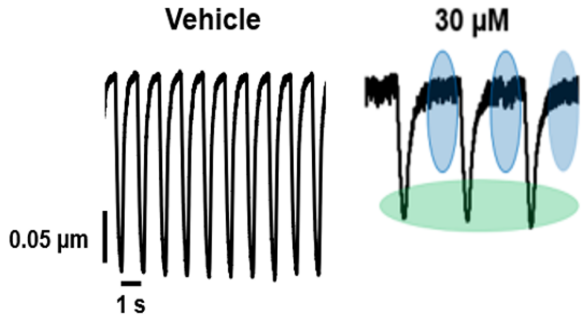
- Effects of drugs on additional contractility transient parameters were also evaluated:





Aftercontraction (AC)



Contractility Escape (CE)

Can Mechanistic Activity Be Derived from Phenotypic Data?

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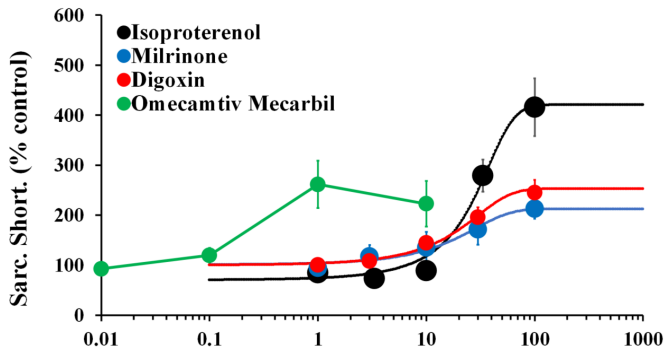
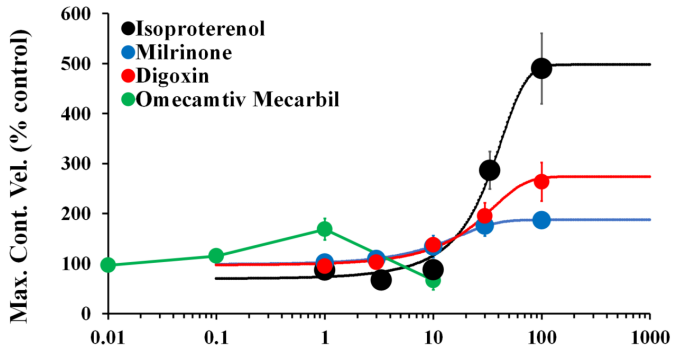
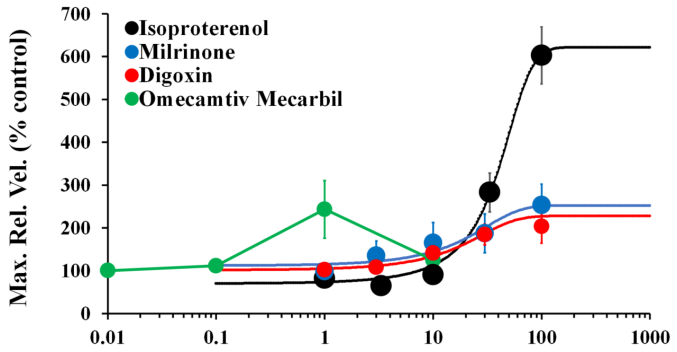
Sarcomere Shortening



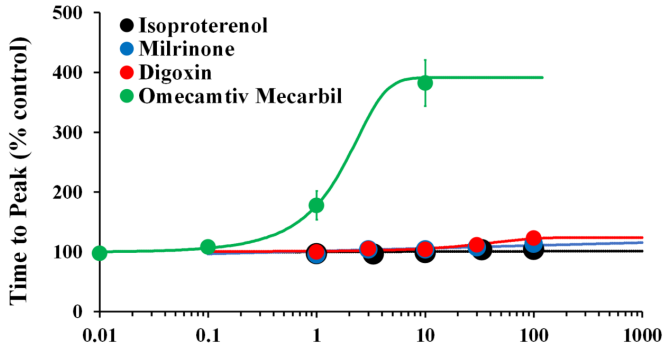
Maximum Contraction Velocity



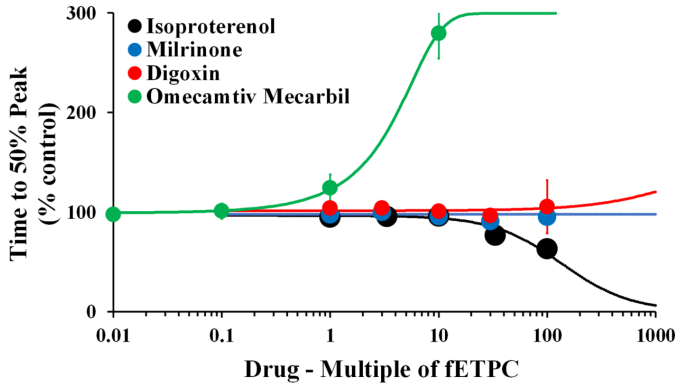
Maximum Relaxtion Velocity



Time to Peak



Time to 50% Peak



Finger-Printing Different Positive Inotropic Mechanisms of Action

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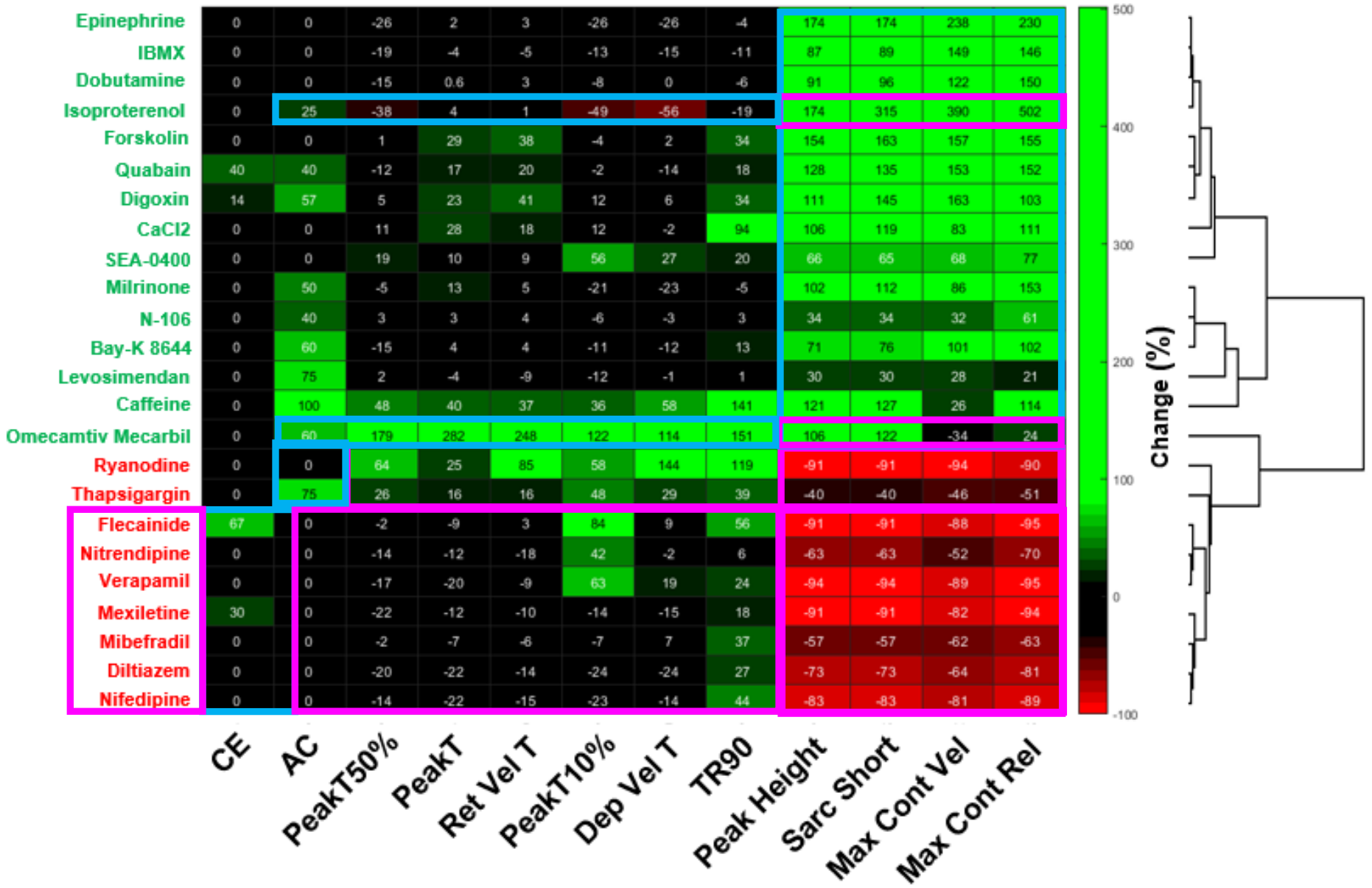

- Hierarchical Clustering Analysis at top test concentration
- Drug-induced effects on contractility transient parameters may allow identification of mechanisms of action (MoA)

Red and green colors indicate decrease and increase of >25% and 10% change, respectively. Black colors indicate no effect (<-25% < % change < 10%). Numbers in boxes indicate means % change relative to vehicle.

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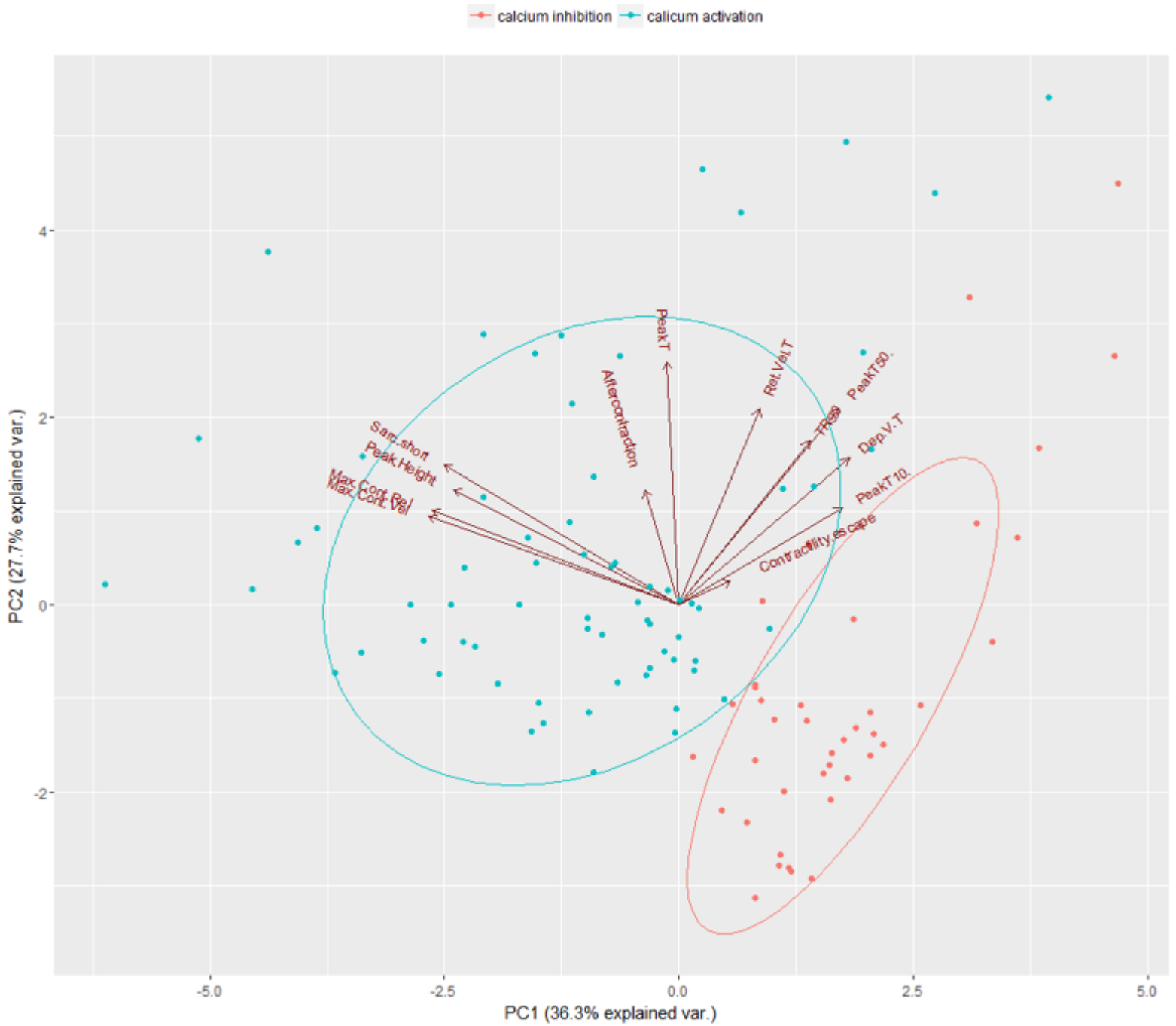

- Separating compounds between those increasing and decreasing Ca^{2+}, clouds represent the 68% confidence interval of predicting if a novel unknown compound increases or decreases Ca^{2+}

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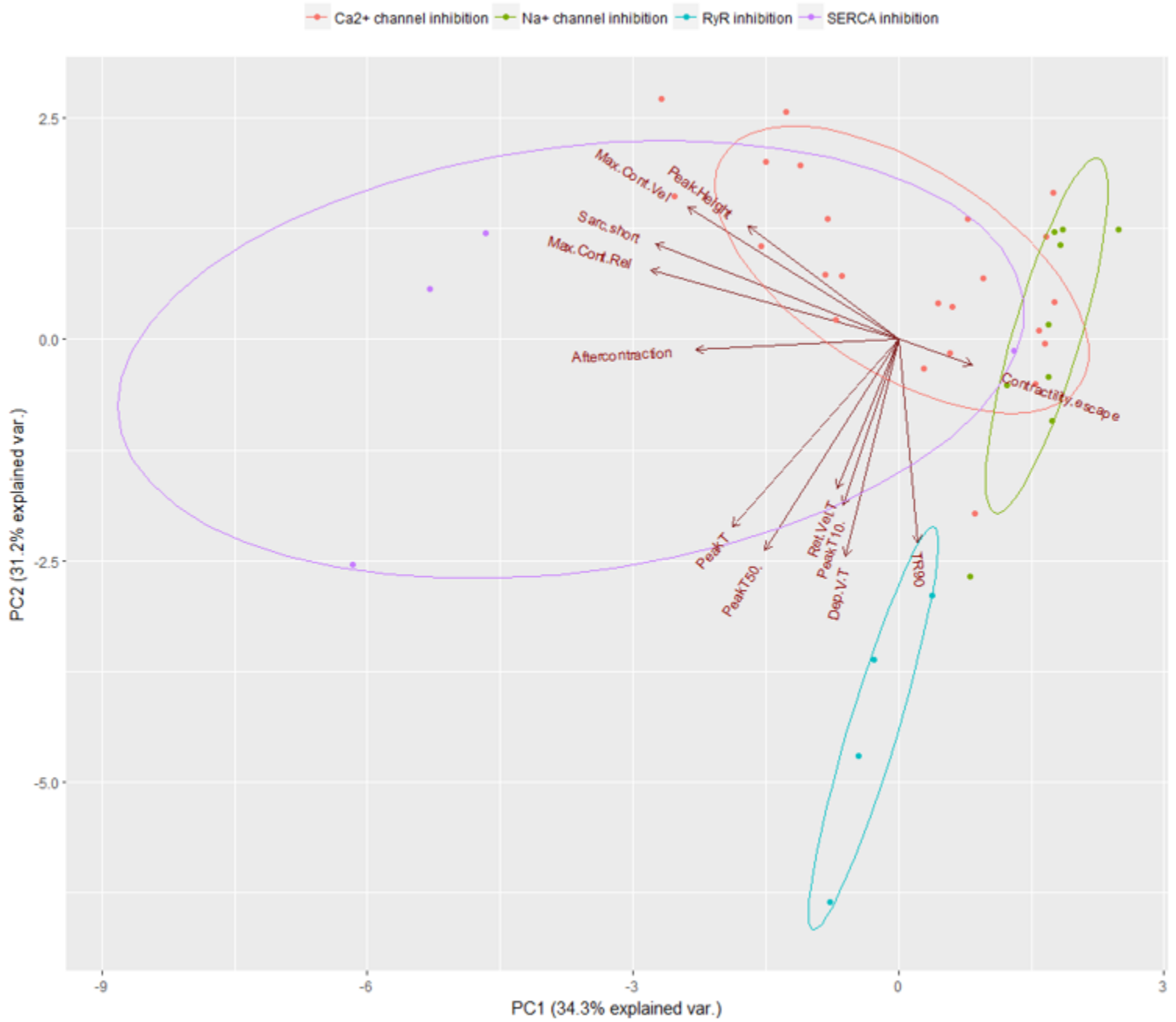

- When considering four negative inotropic MoA, clouds represent the 68% confidence interval of predicting if a novel unknown compound inhibits Ca^{2+} channel, Na^{+} channel, RyR or SERCA

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Summary

- Adult human primary cardiomyocyte-based model:

    1) Most human relevant model for identifying the potential of drugs to modulate contractility

    2) Scalable to medium- / high-throughput

    3) Efficient and cost effective

    4) More predictive for contractility assessment than models based on stem-cell derived cardiomyocytes

- Integrating drug-induced effects on contractility parameters may allow prediction of mechanistic activity

- Thank you very much for your attention!

Questions

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