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CK-4021586 is a new class of cardiac myosin inhibitor for potential treatment of the hyper-contractile subgroup of HFpEF

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Emerging Cardiovascular Translational Technologies

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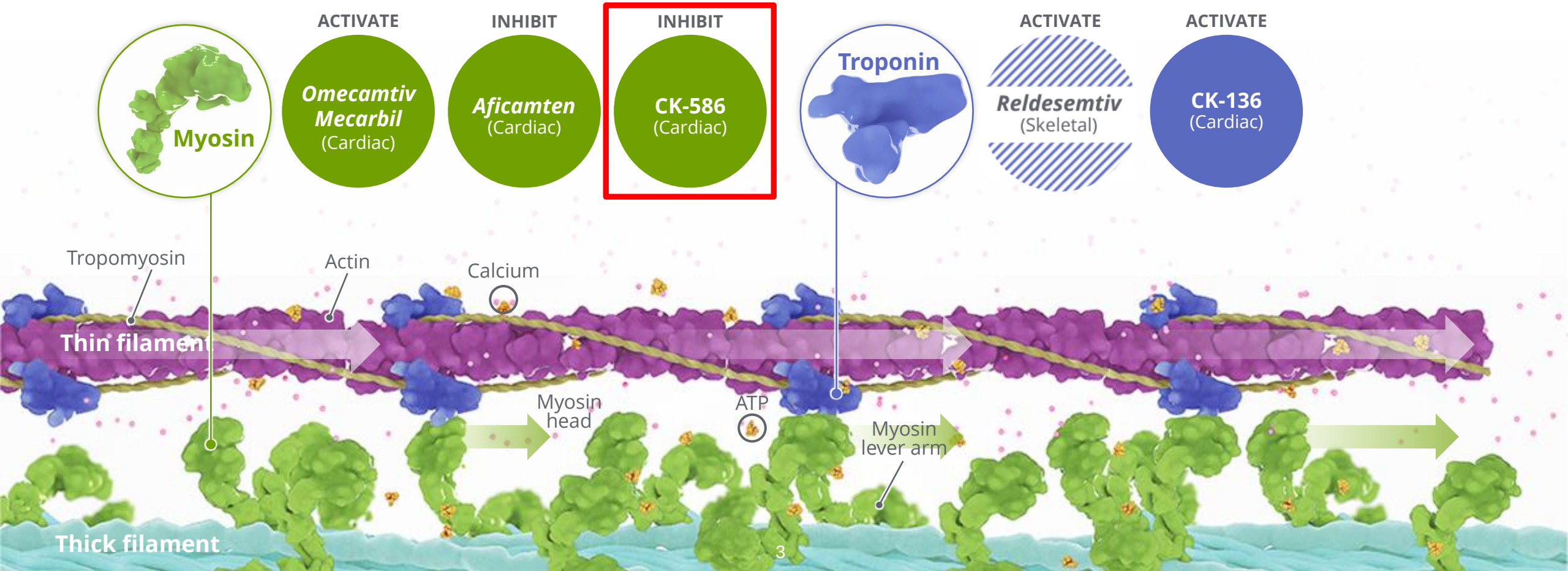
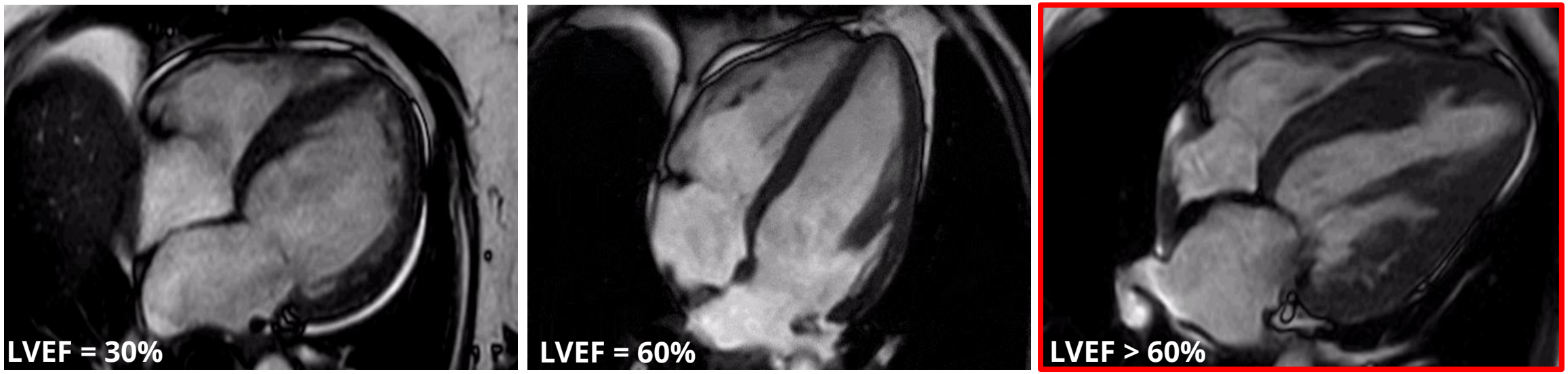
Sarcomere directed drug discovery & development

The sarcomere is a molecular structure found in skeletal and cardiac muscle that enables muscle to contract and generate force



Diseases associated with reduced and increased cardiac contractility

HFrEF (Hypocontractility)	Normal Contractility	HCM & HFpEF (subset) (Hypercontractility)



HFrEF = Heart Failure with Reduced Ejection Fraction
HFpEF = Heart Failure with Preserved Ejection Fraction
HCM = Hypertrophic Cardiomyopathy

Hypothesis: Normalization of Contractility May Treat the Root Cause of the Disease

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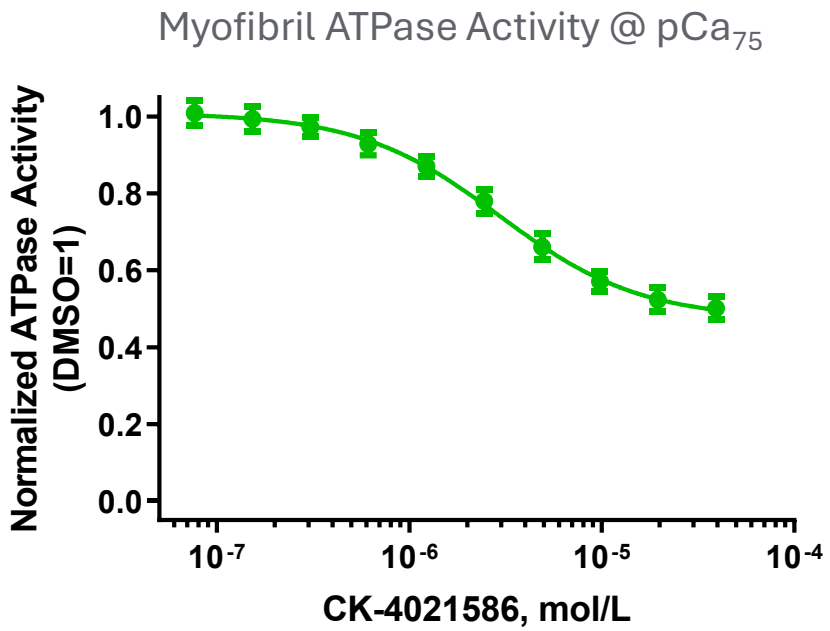
Opportunities exist for another cardiac myosin inhibitor

- Shallow concentration-effect profile:
  - Compounds with shallow *in vivo* concentration-response profiles, due either to intrinsic mechanism of action or drug properties, can facilitate titration of muscle modulation
- Ease of use:
  - Lessening the safety requirements for echo-based titration in HFpEF
- Different mechanisms-of-action may provide different opportunities
  - Enlarging the toolbox of muscle modulators can both inform our understanding of muscle mechanics as well as provide additional opportunities for therapeutic benefit

CK-586 is a sarcomere inhibitor with a unique mechanism of action

CK-4021586

- small molecule (<500 Da)
- Initial hit identified in high throughput screen for inhibitors of cardiac myofibril ATPase activity
- Optimized by iterative medicinal chemistry (>2000 compounds synthesized in series)
- Favorable predicted human pharmacokinetics (predicted $t_{1/2}$ ~ 15 hours)
- Desirable off-target profile
- Unique biochemical phenotype: partial inhibition of myofibril ATPase activity



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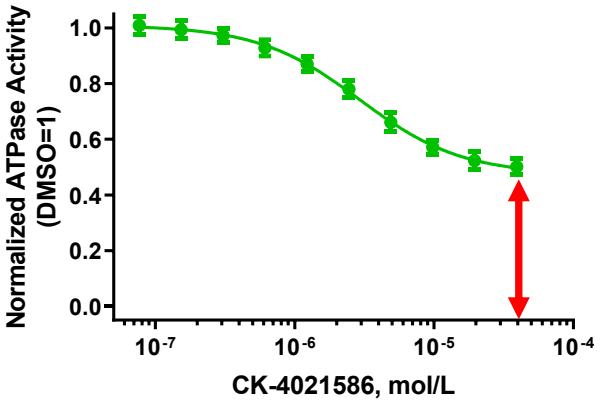
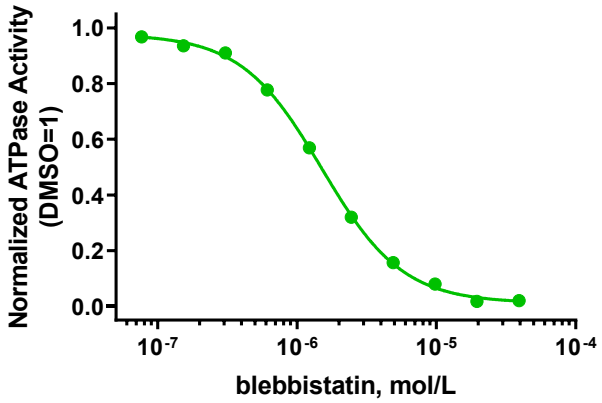
CK-586 Inhibition Profile Differs from Other Cardiac Myosin Inhibitors

Partial inhibition of cardiac myofibril ATPase is observed within the chemical series

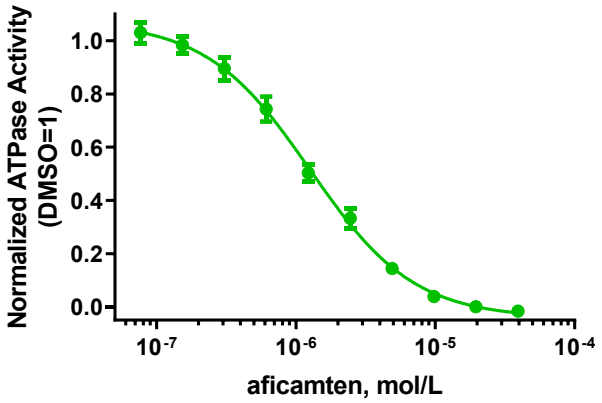
CK-586



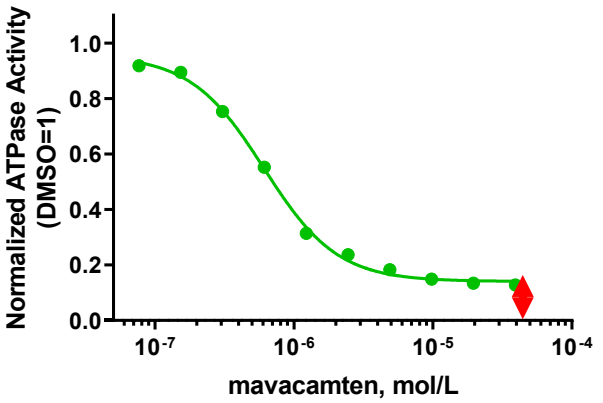
Blebbistatin



Aficamten



Mavacamten



Bovine Cardiac Myofibril ATPase Activity @ pCa_{75}

	CK-586 (n=31)	Blebbistatin (n=404)	*Aficamten* (n=17)	*Mavacamten* (n=302)
EC_{50} (μM)	2.9	1.5	1.3	0.6
Min. Normalized ATPase Activity*	0.47	0.02	-0.02	0.12

* Non-myosin ATPase activity subtracted from cardiac myofibril ATPase assays using saturating blebbistatin to improve assay reproducibility

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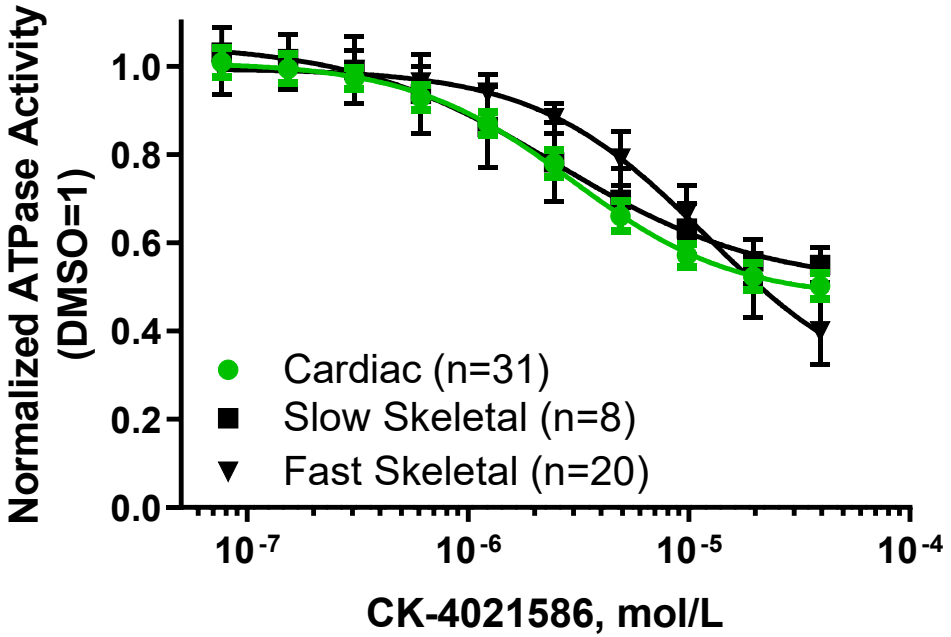
CK-586 Selectively Inhibits Cardiac and Slow Skeletal Myofibrils

Similar selectivity observed across the chemical series

	IC_{15} (μM)	EC_{50} (μM)
Cardiac	1.4	2.9
Slow skeletal	1.4	2.5
Fast skeletal	3.3	12

Myofibril ATPase Activity @ pCa_{75}



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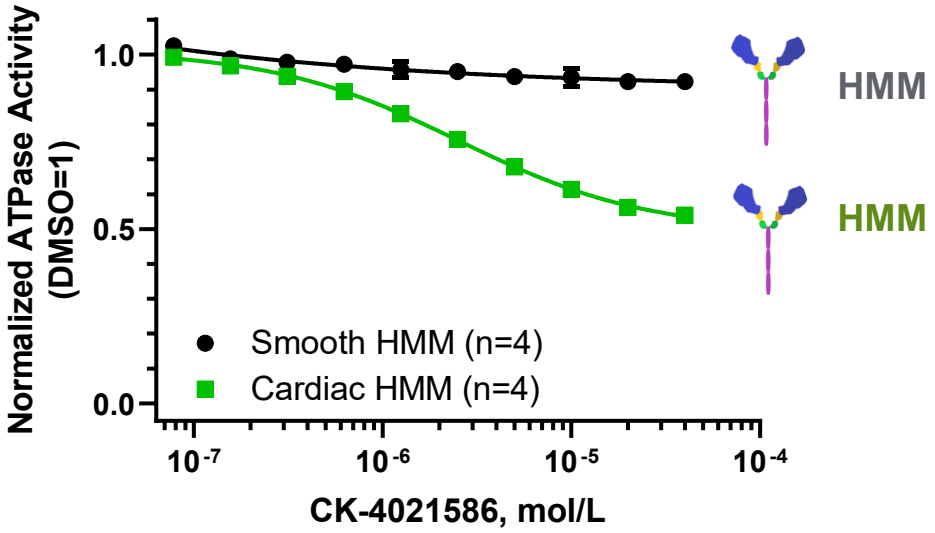
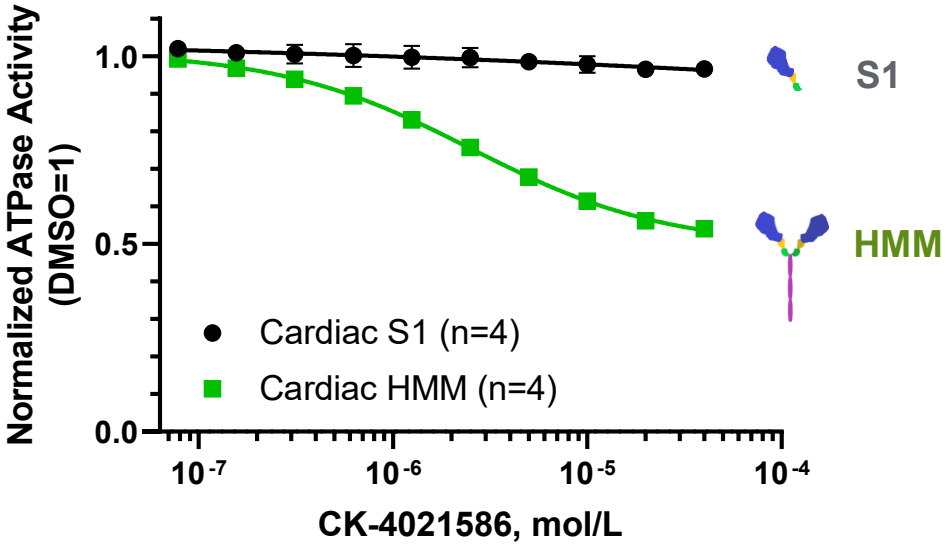
CK-586 Inhibits HMM but not S1 Preparations of Cardiac Myosin

Establishes cardiac myosin as target of CK-586

S1 = Subfragment-1, single-headed myosin fragment
HMM = Heavy meromyosin, two-headed myosin fragment

Actin-activated ATPase Activity





	EC_{50} (μM)
Bovine cardiac S1	>39
Bovine cardiac HMM	2.5
Chicken Gizzard Smooth HMM	>39

Potency in cardiac HMM actin-activated ATPase assay is similar to cardiac myofibrils (EC_{50} = 2.9 μM)

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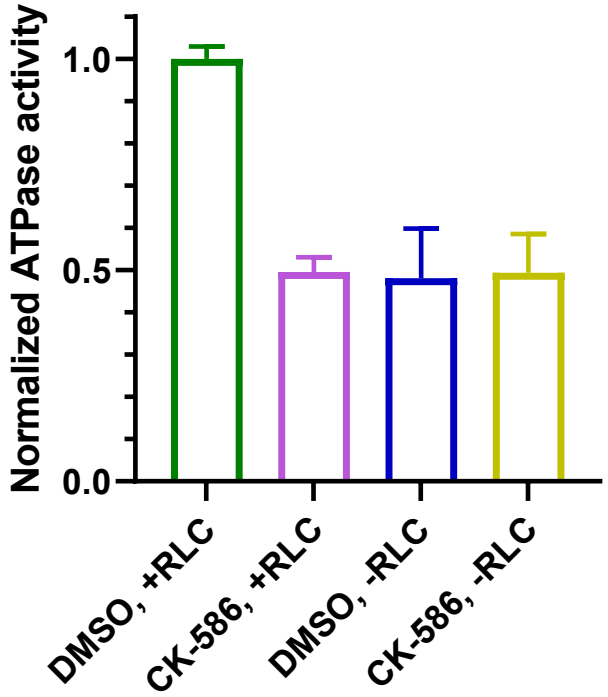
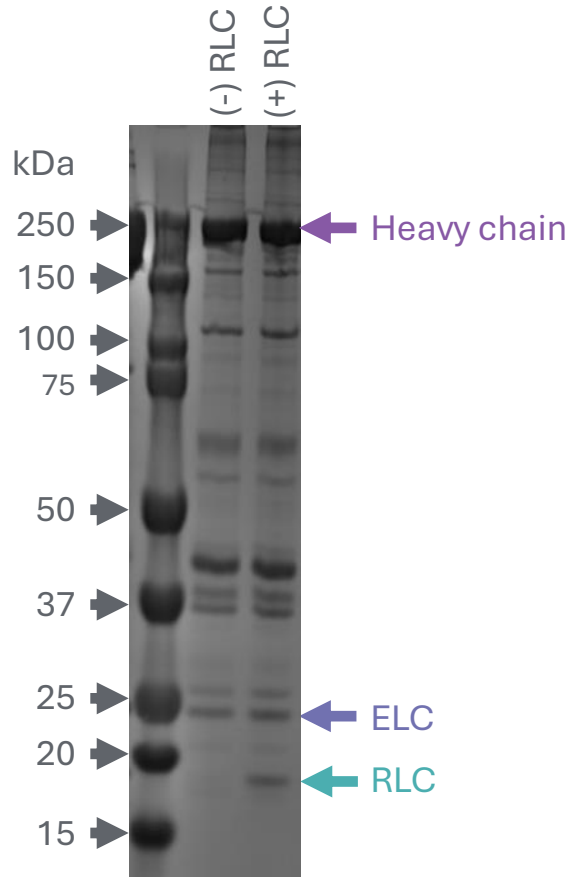
Inhibitory Activity of CK-586 Requires the Regulatory Light Chain (RLC)

CK-586 is unable to further inhibit bovine cardiac myofibrils after RLC depletion

Myofibril ATPase Activity @ 1 mM $CaCl_2$



Depletion of RLC from Bovine Cardiac Myofibrils by Triton/CDTA Treatment



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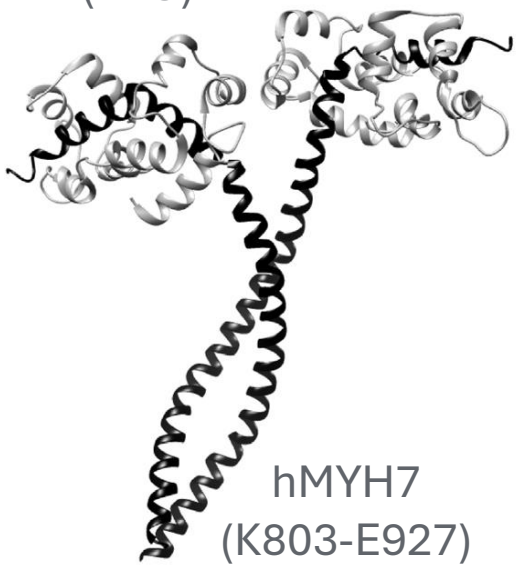
CK-586 Binds to the RLC + Myosin Heavy Chain Fragment

Isothermal titration calorimetry demonstrates direct, stereoselective interaction

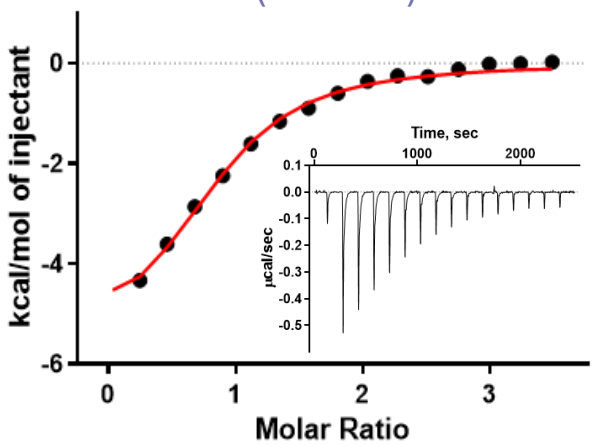
hMYL2
(RLC)



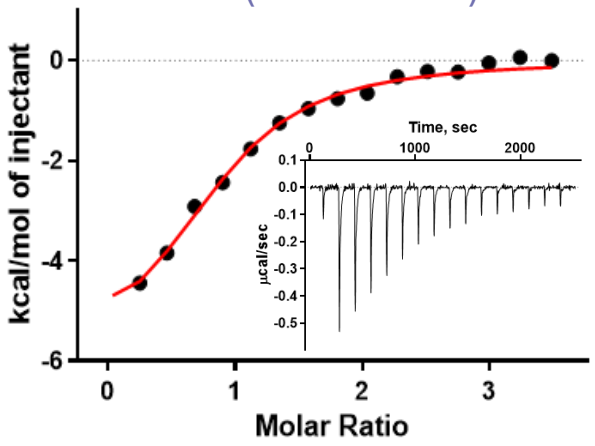
hMYH7
(K803-E927)

Human cardiac version of recombinant RLC + heavy chain fragment

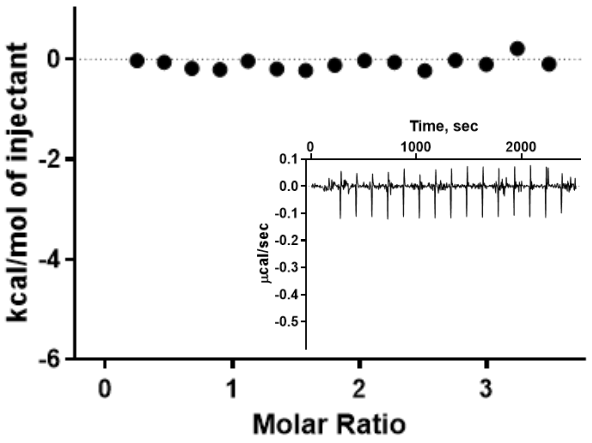
RLC + 12-hep HCF
(dimeric)



RLC + 2-hep HCF
(monomeric)



RLC alone



	RLC + 12-hep HCF	RLC + 2-hep HCF
Stoichiometry (N) (ligand/target)	0.78 ± 0.1	0.94 ± 0.084
Affinity (K_D, µmol/L)	7.3 ± 1.3	6.6 ± 1.5
Enthalpy (ΔH, kcal/mol)	-6.1 ± 1.2	-5.3 ± 0.65
Entropy (ΔS, e.u.)	3.0 ± 4.1	6.0 ± 2.6

~ 1 / monomer

Tolkatchev et al. (2018)

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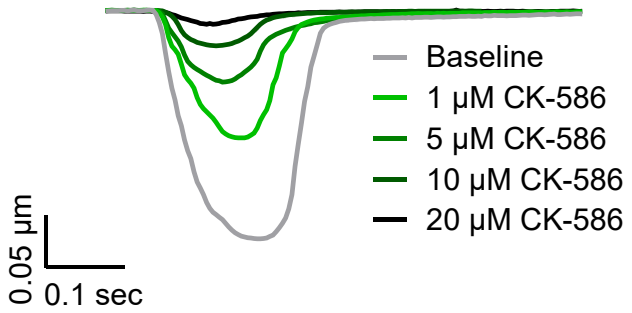
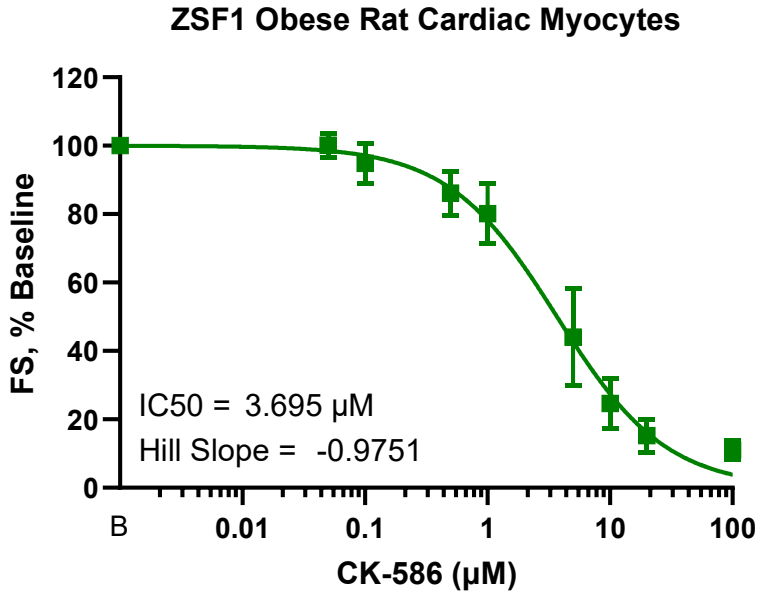
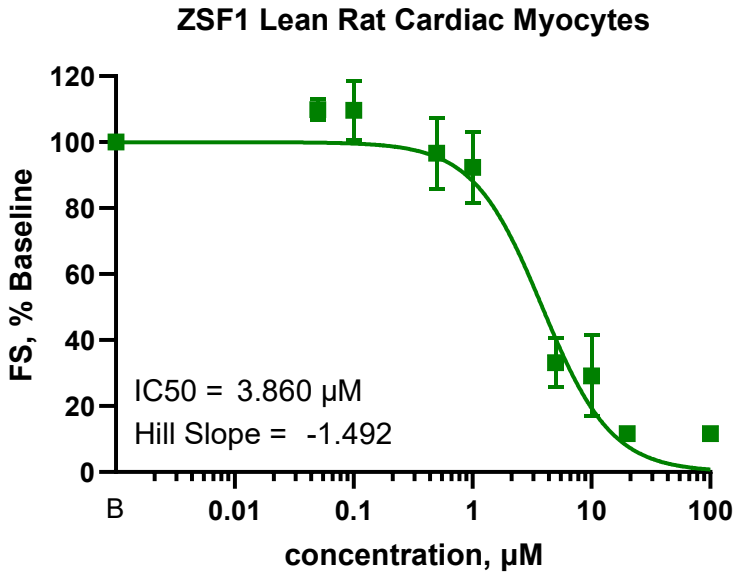
CK-586 decreases contractility of ZSF1 obese rat ventricular myocytes

IC50 values are consistent across genotypes and comparable to myofibril assay (EC50 = 2.9 µM)

Contractility is inhibited to a much greater extent than myofibril ATPase activity







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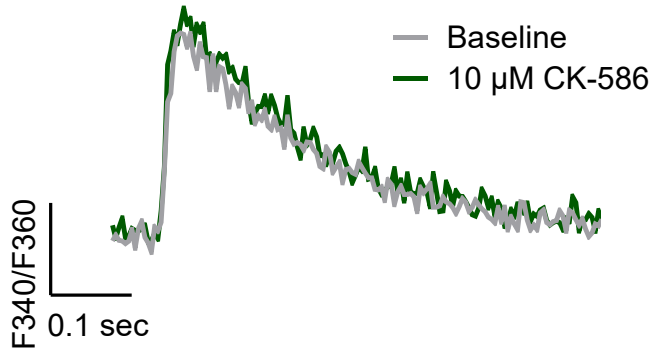
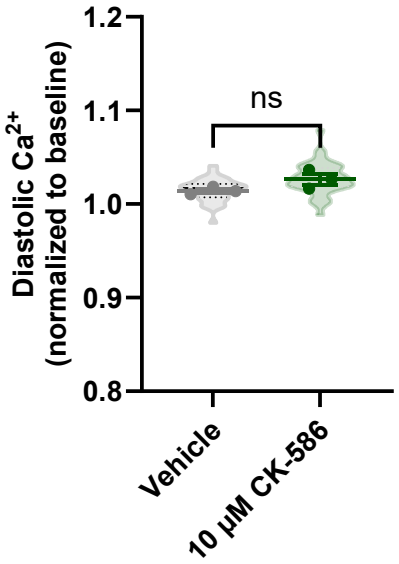
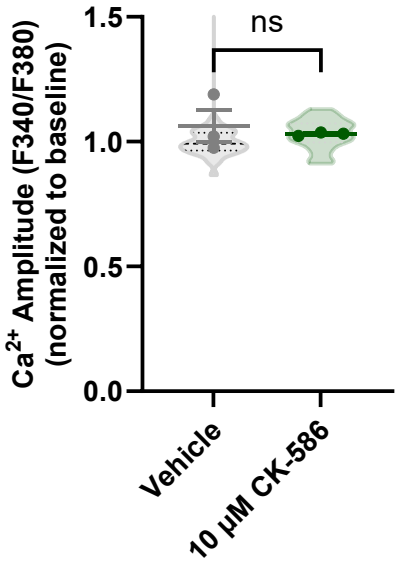
CK-586 decreases contractility of ZSF1 obese rat ventricular myocytes

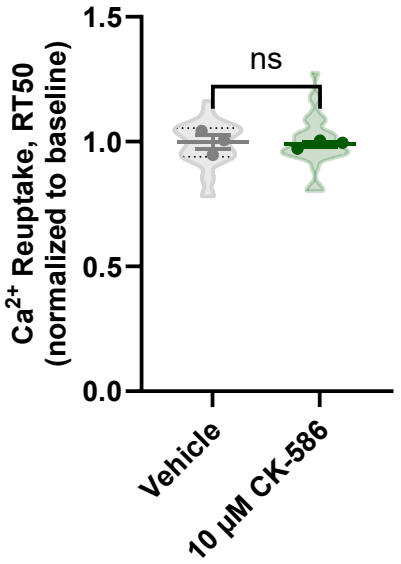
Acute treatment with CK-586 does not alter calcium transients

Results are consistent with direct inhibition of the cardiac sarcomere









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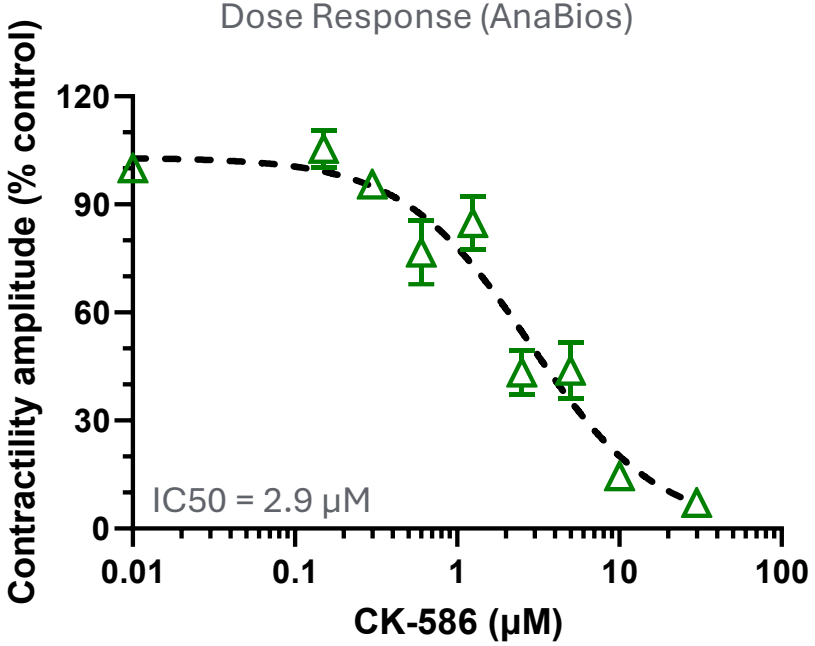
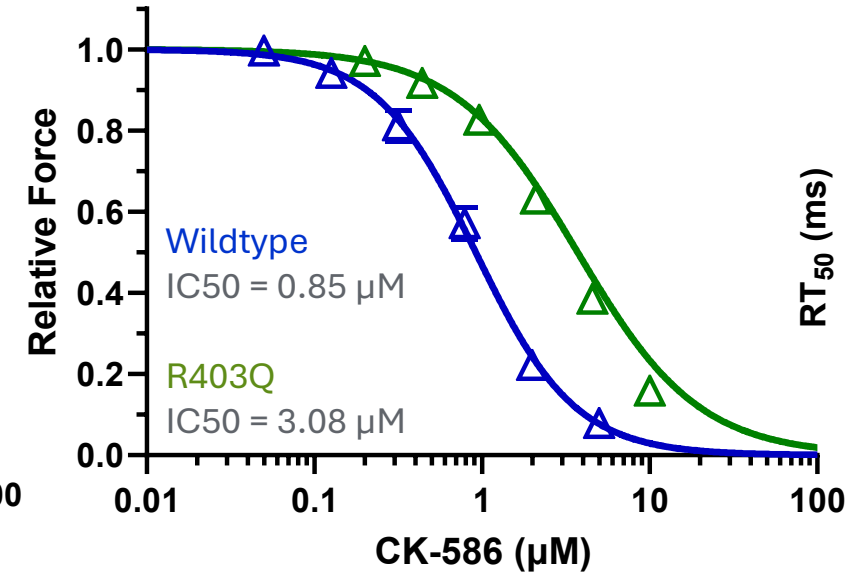
CK-586 inhibits contractility of human cardiomyocytes with similar IC50

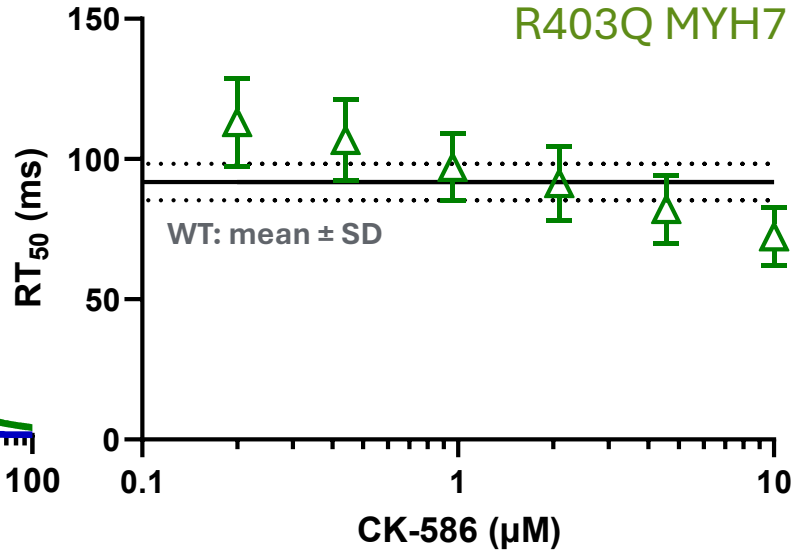
Data supportive of rodent to human translation

Human Adult Cardiomyocyte
Dose Response (AnaBios)



Engineered Heart Tissues with iPSC-derived
Cardiomyocytes (Propria)





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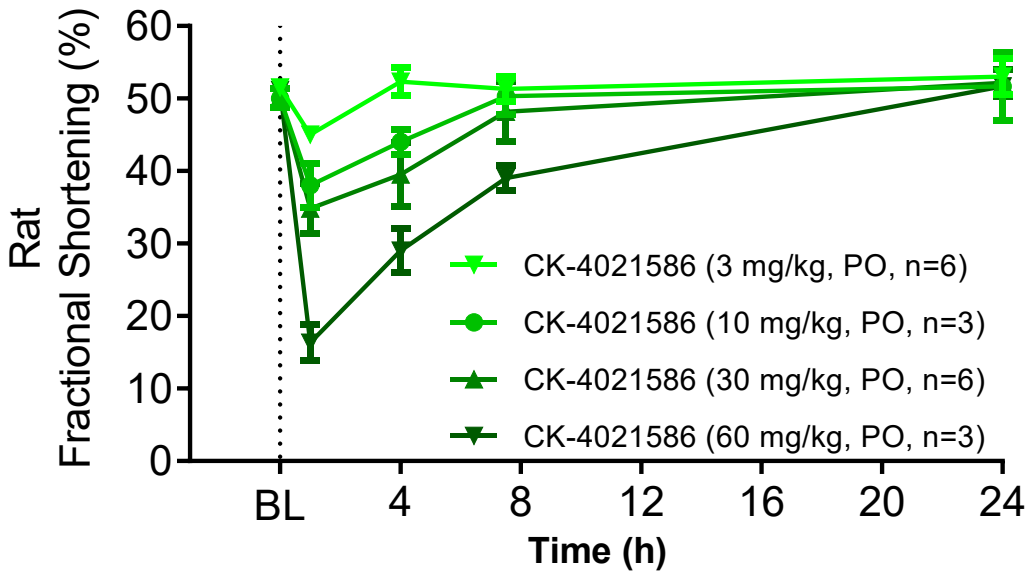
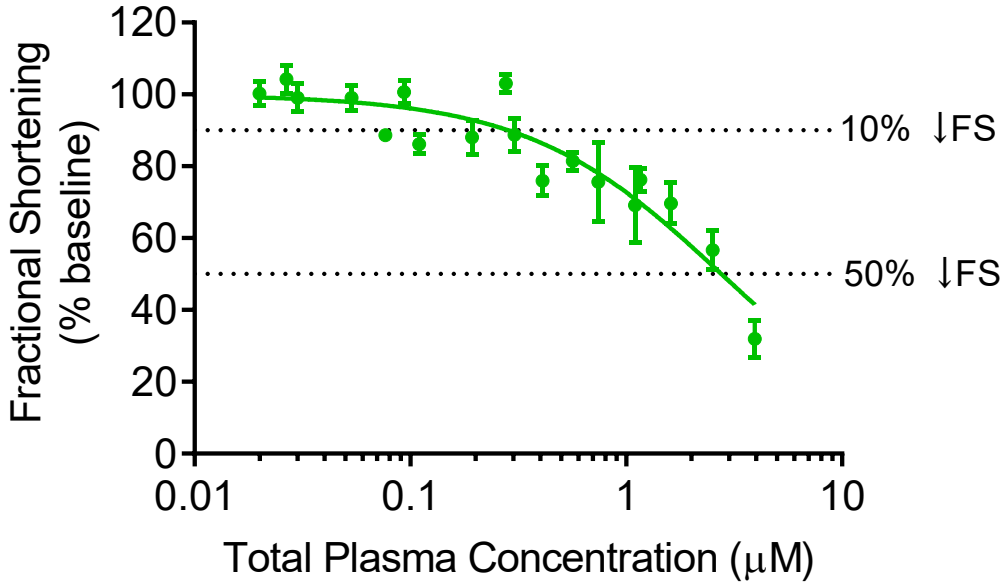
CK-586 has a shallow *in vivo d*ose-response in normal Sprague Dawley rats

Shallow concentration-effect profile optimized empirically to maximize titratability

Serial echocardiography following single bolus oral dosing





Fractional Shortening	
IC_{10}	0.28 μM
IC_{50}	2.76 μM

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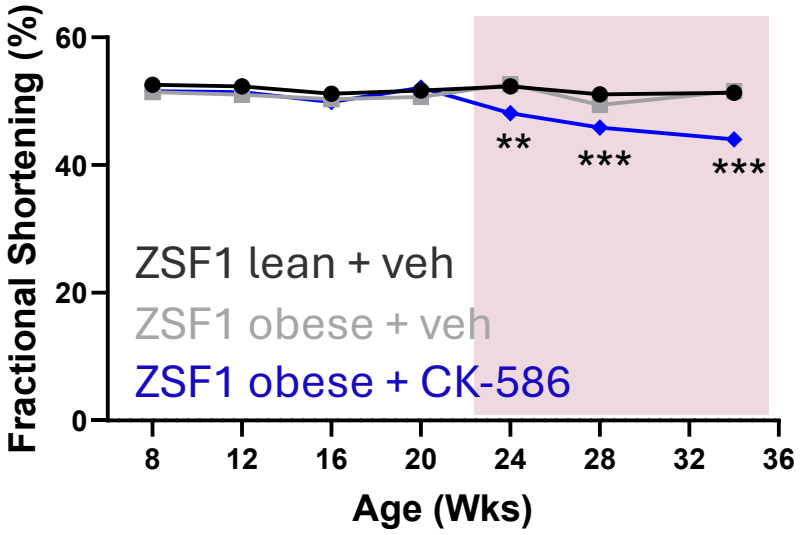
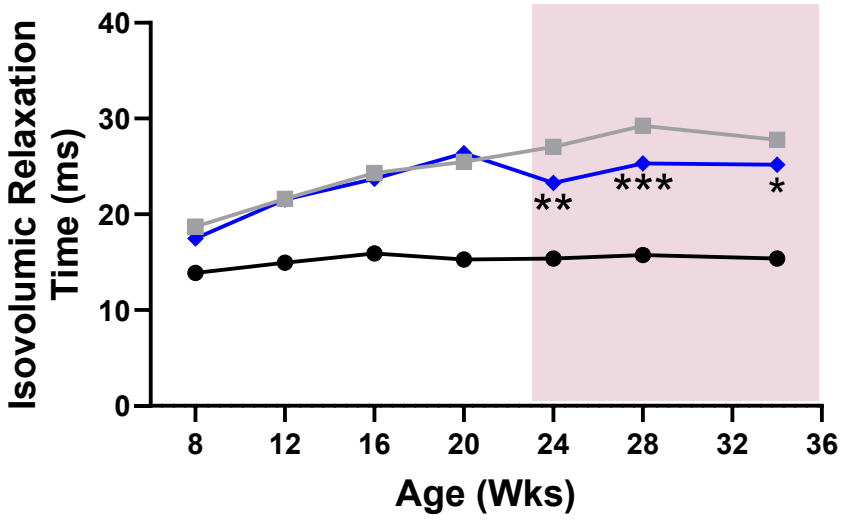
CK-586 is Efficacious in ZSF1 Obese Rat Model of HFpEF

12 weeks of oral treatment (10mg/kg) improved diastolic function and reduced cardiac fibrosis

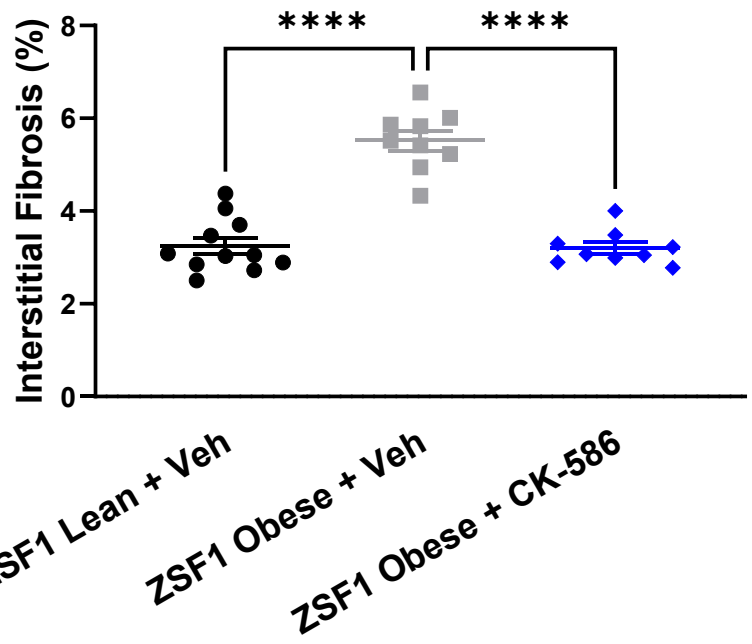
Reduced Fractional Shortening



Improved Diastolic Function



Reduced Fibrosis



Model is representative of hypertensive, diabetic, hyperphagic aspects of HFpEF

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Conclusions

- CK-586 is a selective, small molecule allosteric inhibitor of cardiac myosin with a different mechanism-of-action than either mavacamten or aficamten:
  - Inhibits HMM but not S1 preparations of cardiac myosin
  - Requires the presence of the regulatory light chain (RLC) for activity
  - Likely binds on or near the RLC in a complex with myosin heavy chain
- CK-586 inhibits cardiomyocyte contractility across species and disease phenotypes without significant effects on calcium
- In normal rats, CK-586 reduces fractional shortening with a shallow in vivo concentration-response (similar to aficamten), supporting titratability of sarcomere modulation
- In obese ZSF1 rats, CK-586 reduces fractional shortening, improves relaxation, and reduces fibrosis supporting exploration of therapeutic benefit in HFpEF, particularly where ejection fractions are elevated

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Thank You

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