

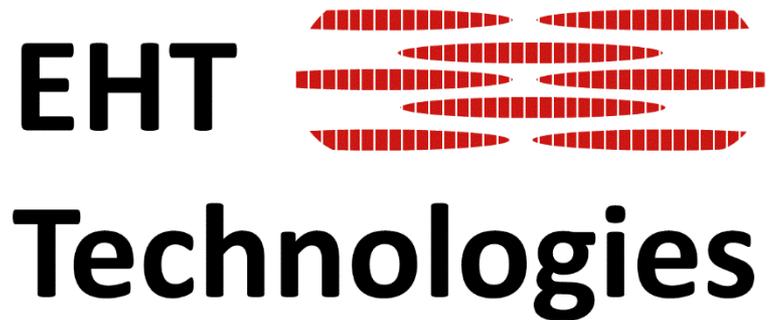
Engineered heart tissues: analysis of contractile force in hiPSC-CM

Public workshop, March 29th, 2019

FDA Headquarters, White Oak, Silver Spring

Arne Hansen

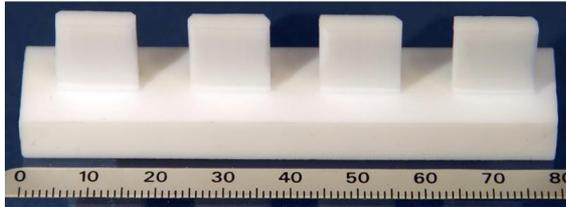
Conflict of interest



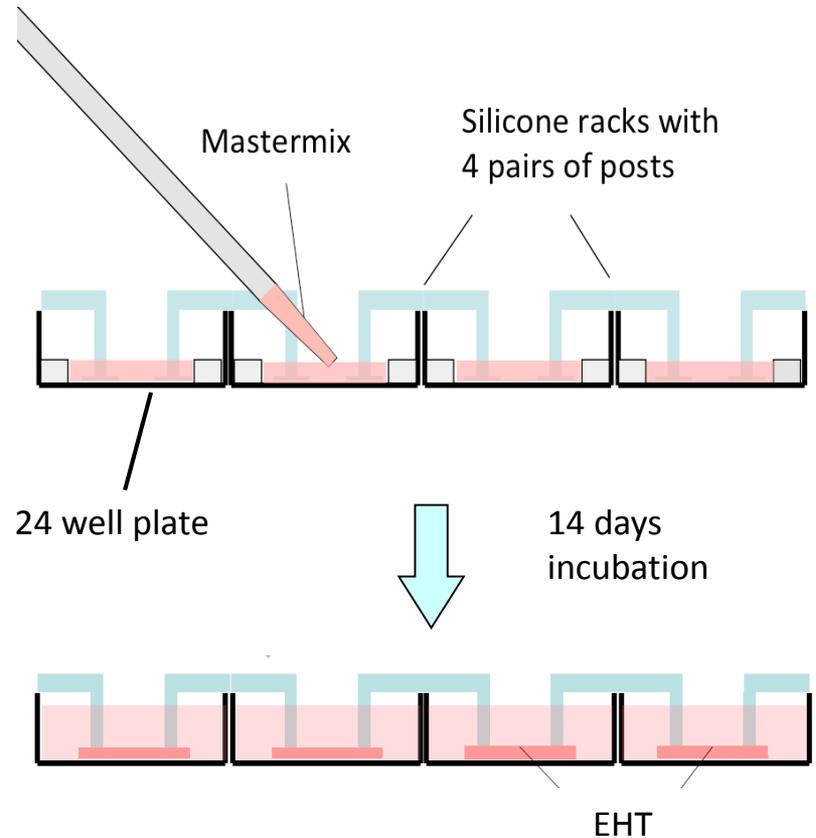
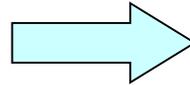
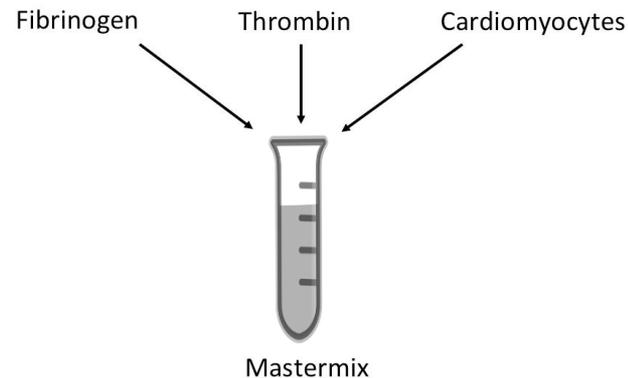
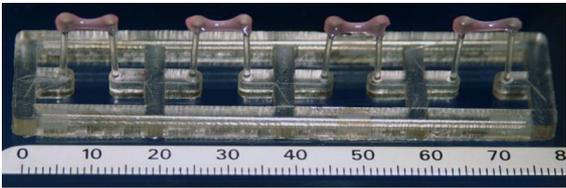
EHT Technologies GmbH Hamburg
www.eht-technologies.com

Generation of engineered heart tissue (EHT)

PTFE spacer



PDMS rack with EHT



Hansen et al, Circ Res, 2010 (NRCM)
 Schaaf et al. PLoS ONE, 2011 (hESC-CM)
 Mannhardt et al, SCR, 2016 (hiPSC-CM)

EHT development

Day 0

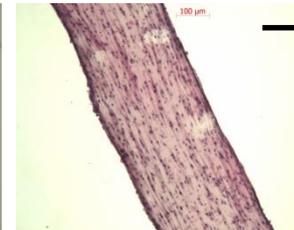
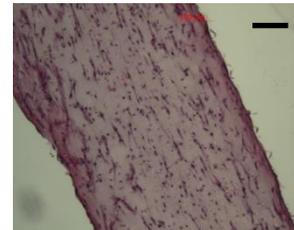
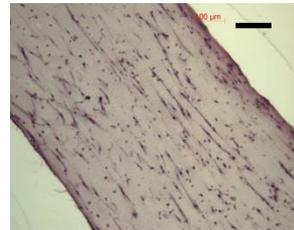
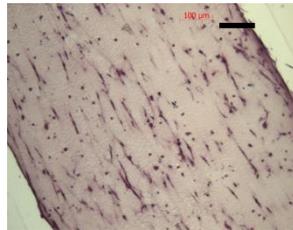
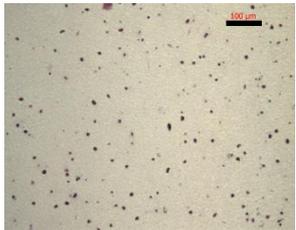
Day 3

Day 6

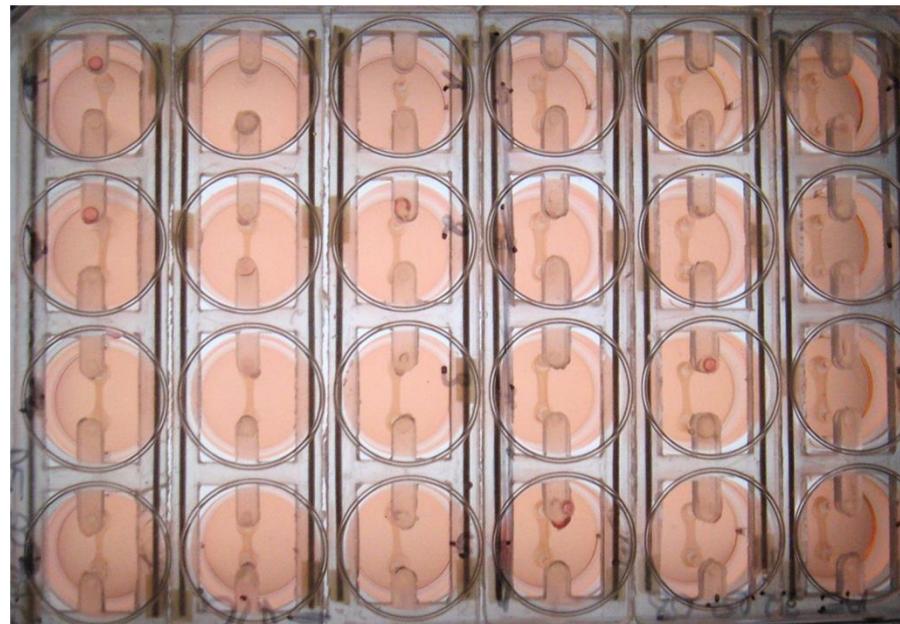
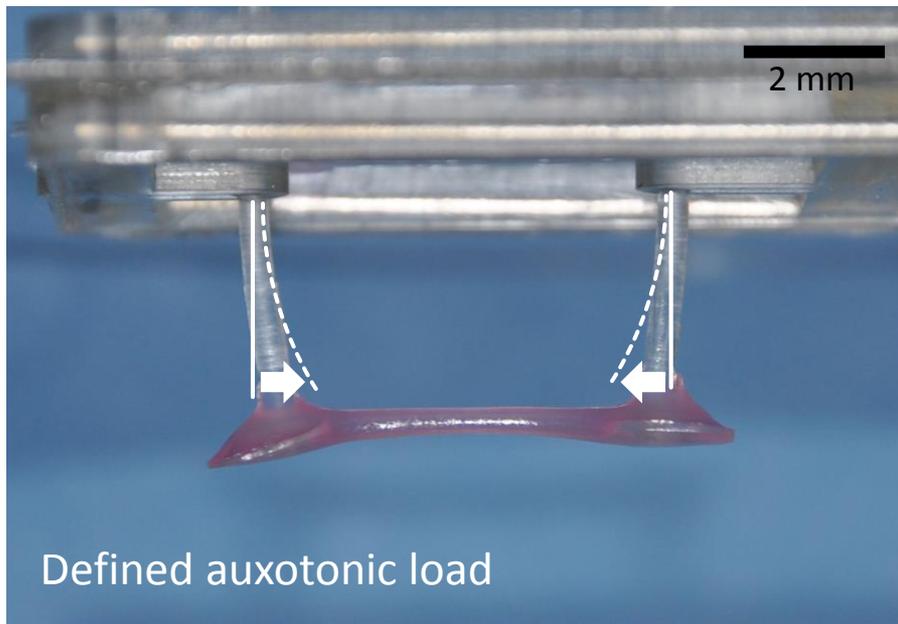
Day 9

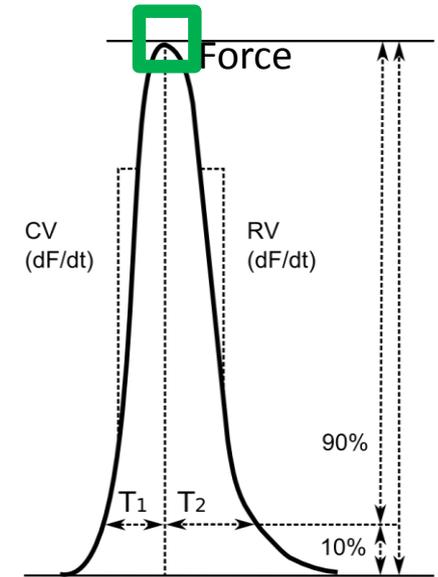
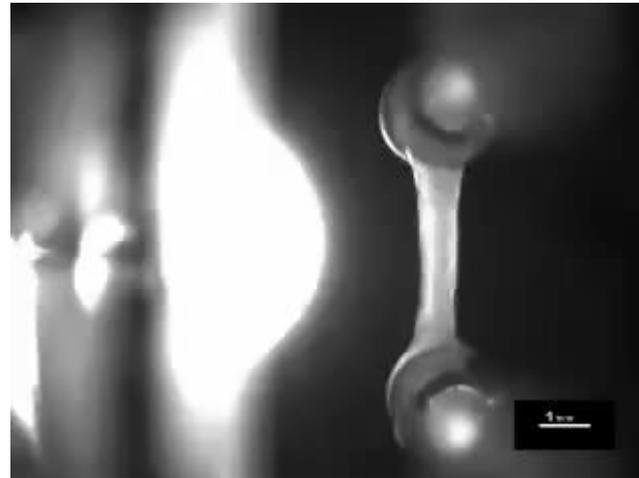
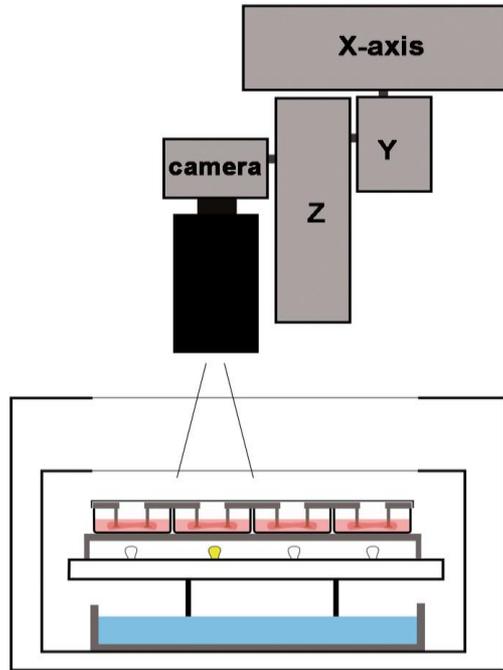
Day 12

Day 15



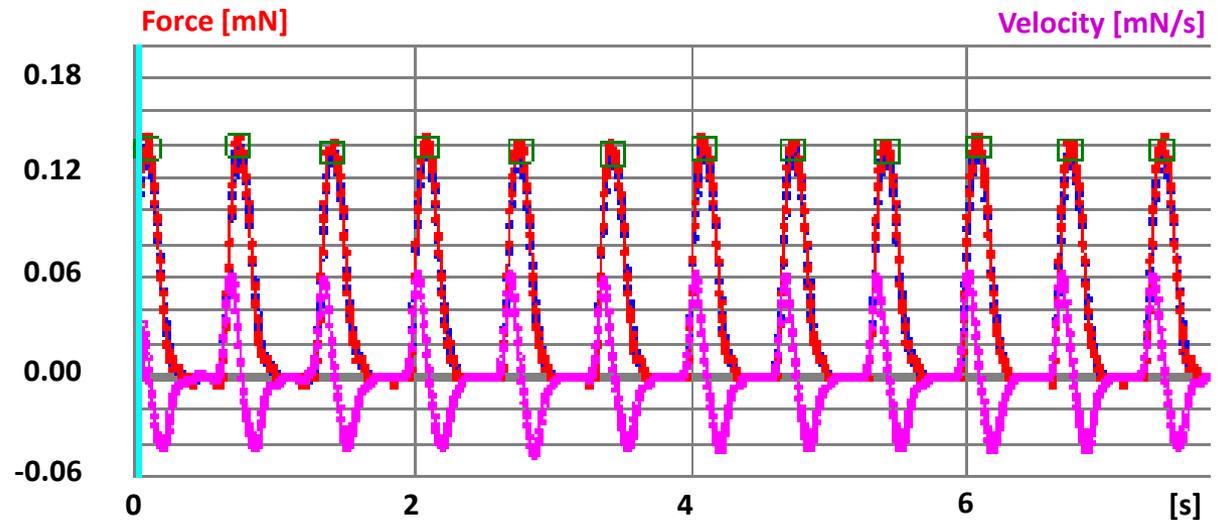
Scale bar 100 μm



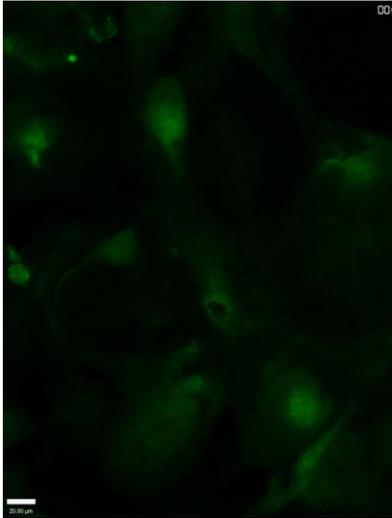


Automation

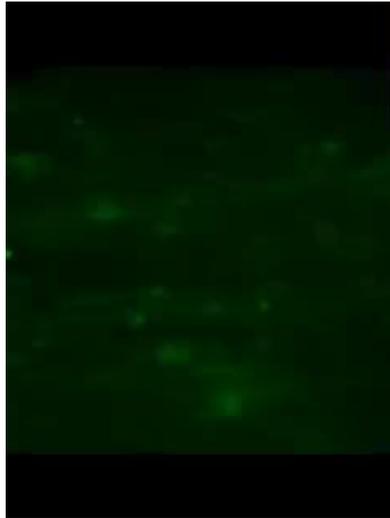
- EHT contour recognition
- Contractility report
- Sterile procedure
- Repeated measurements



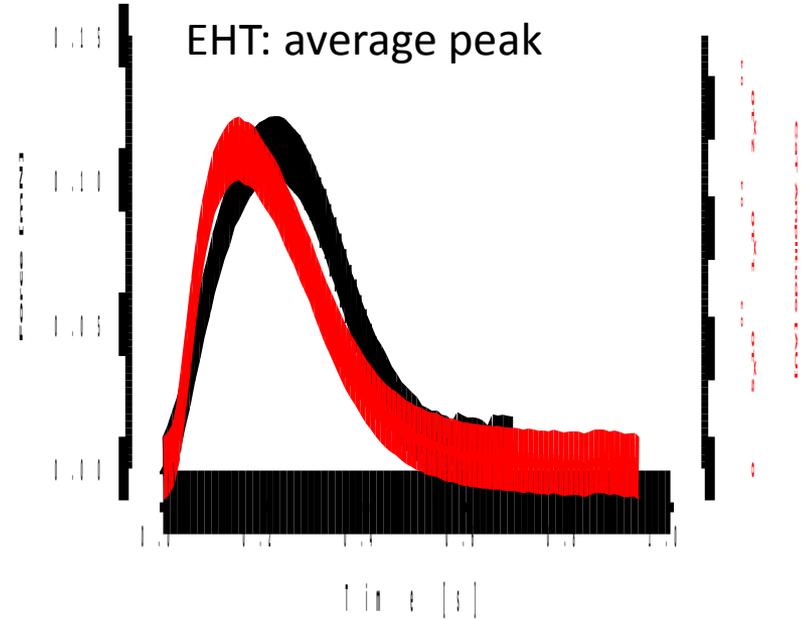
2D hiPSC CM



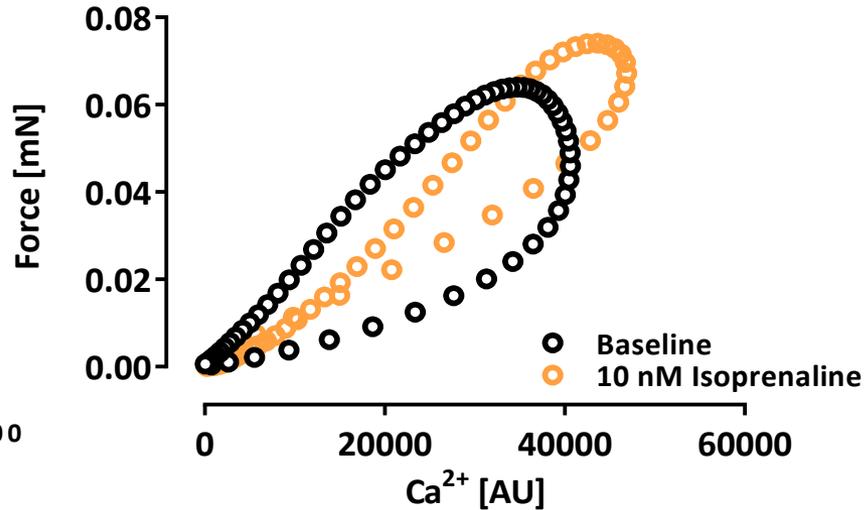
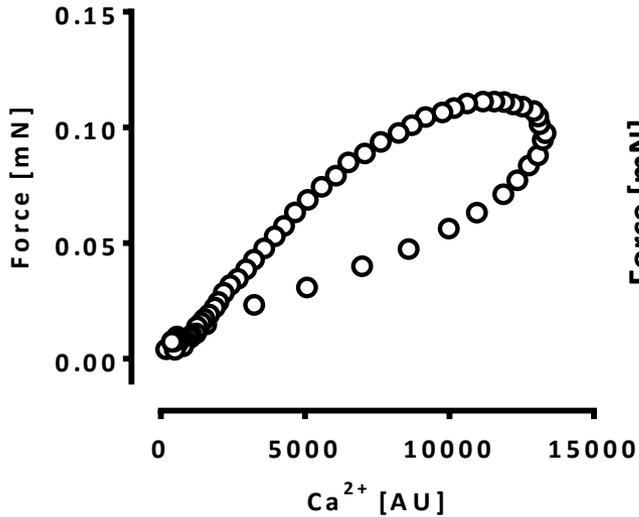
3D hiPSC CM



EHT: average peak



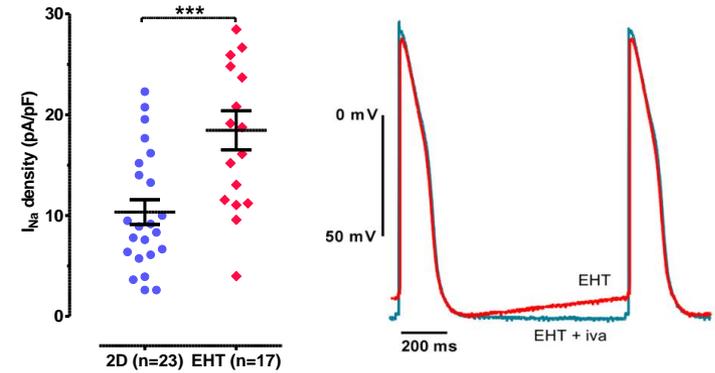
EHT: force /CaT loop



HiPSC-CM EHT

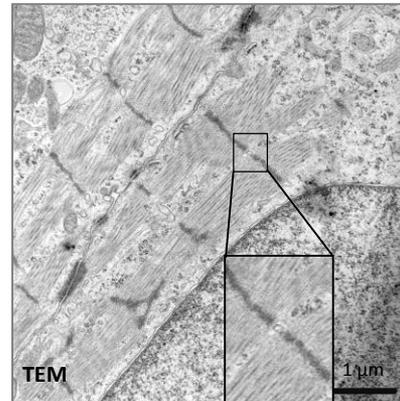
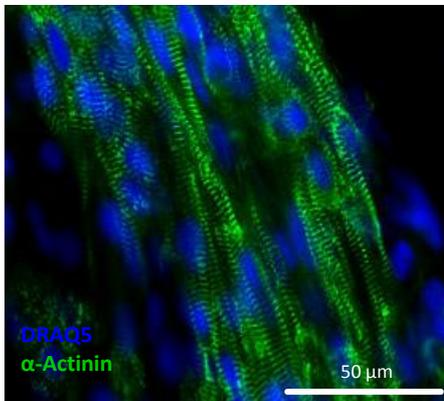


Electrophysiological maturation



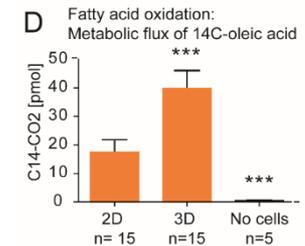
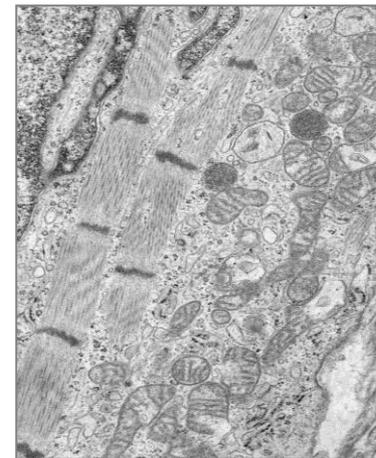
Lemoine et al. Sci Rep. 2017,
Lemoine et al. Circ Arrhythm Electrophysiol. 2018

Morphological maturation

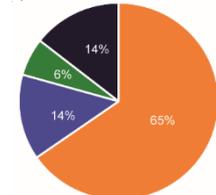


Mannhardt et al, Stem Cell Reports, 2016

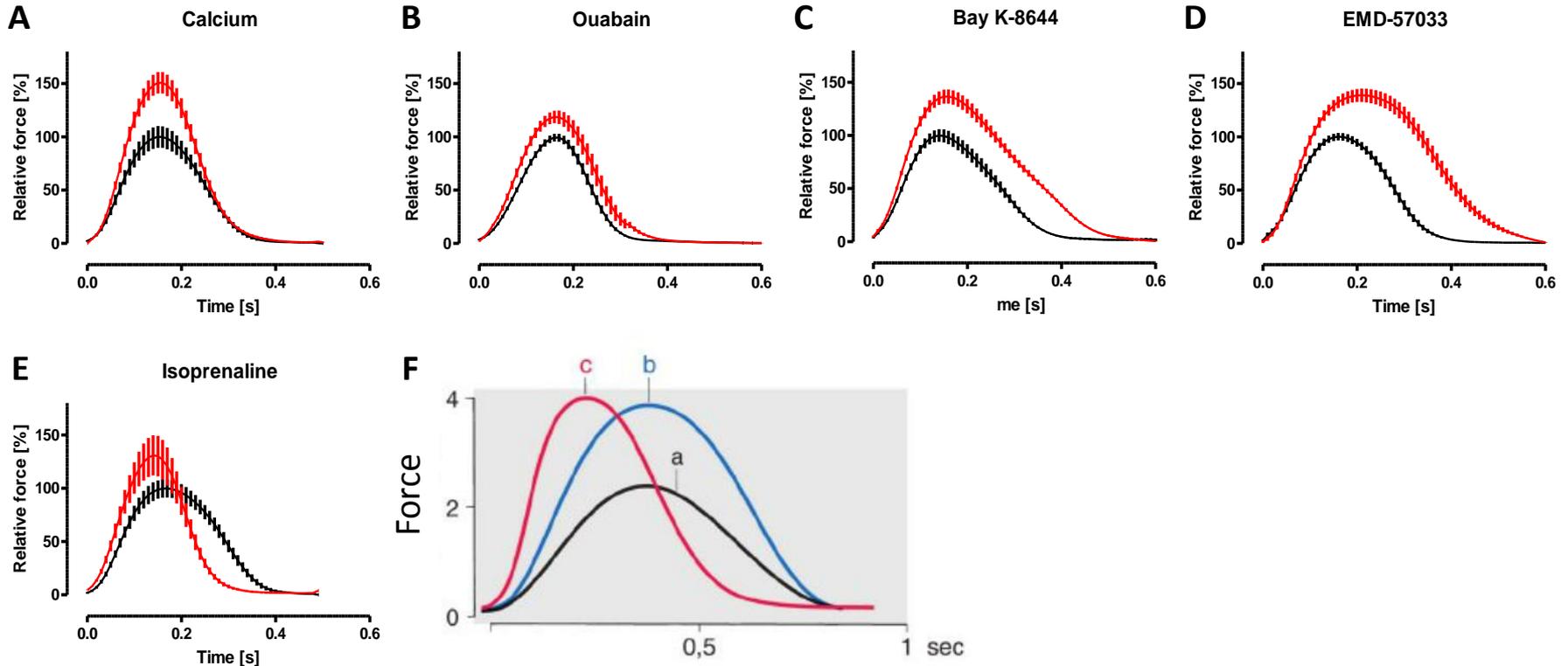
Metabolic maturation



ATP production



Ulmer et al, Stem Cell Reports, 2018



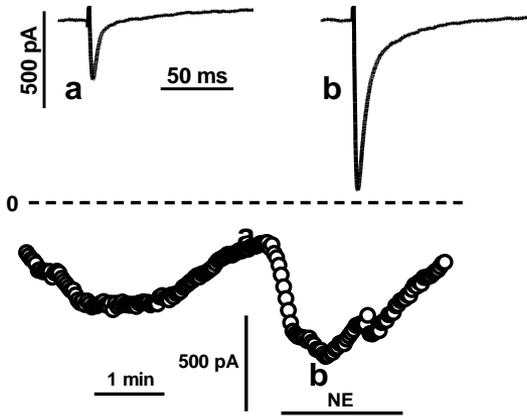
Papillary muscle contraction

- Control
- Noradrenaline
- Frank-Starling

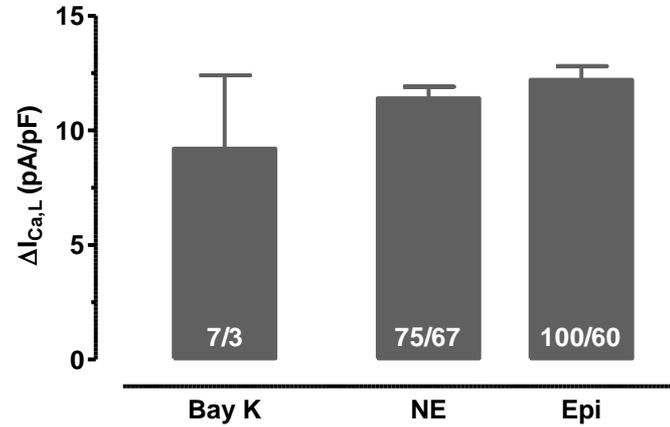
Forth, Henschler, Rummel
12. Edition, 2017

Mannhardt et al,
Stem Cell Reports, 2016

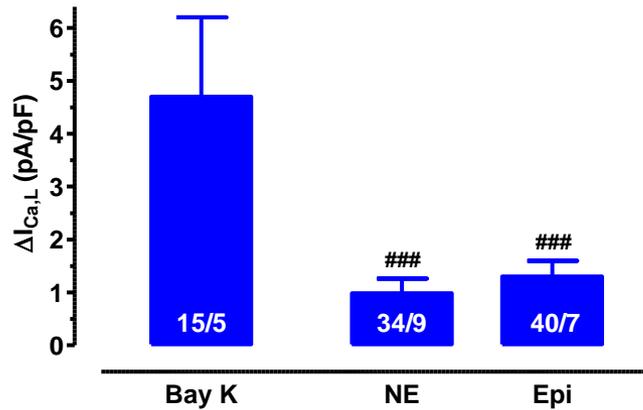
$I_{Ca,L}$: effect of inotropes



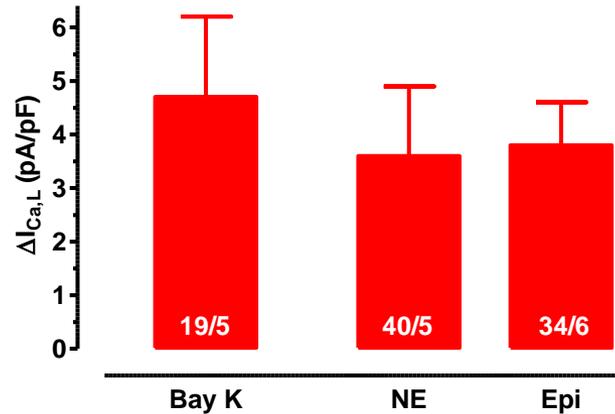
Human atrial CM



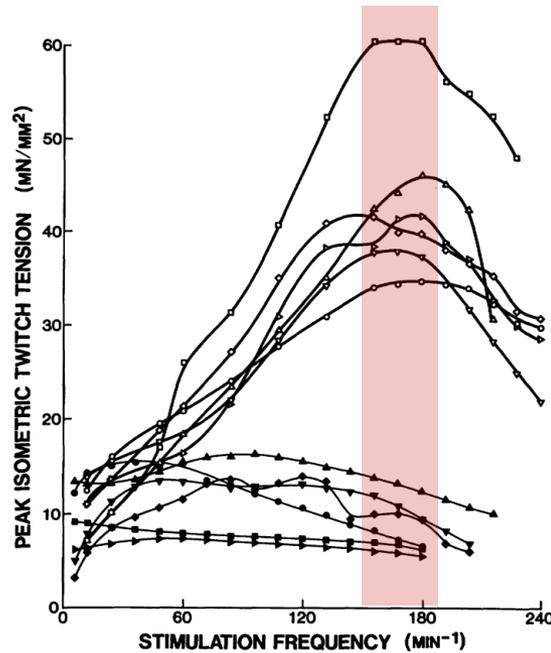
HiPSC-CM 2D



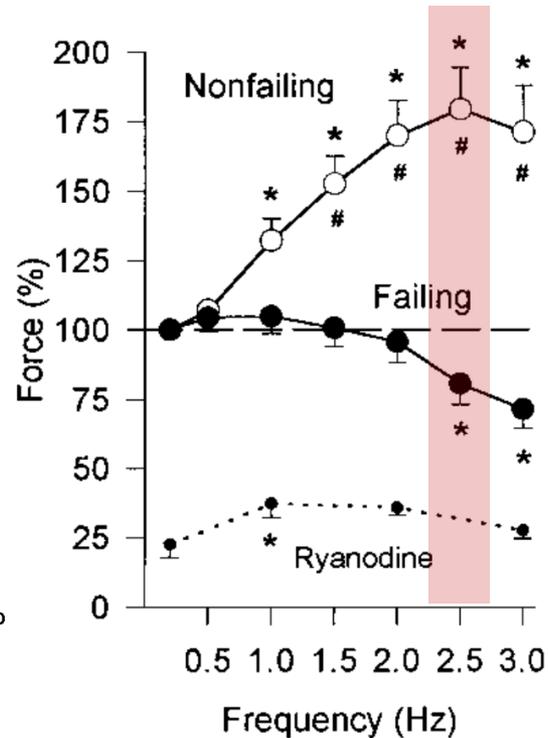
HiPSC-CM EHT



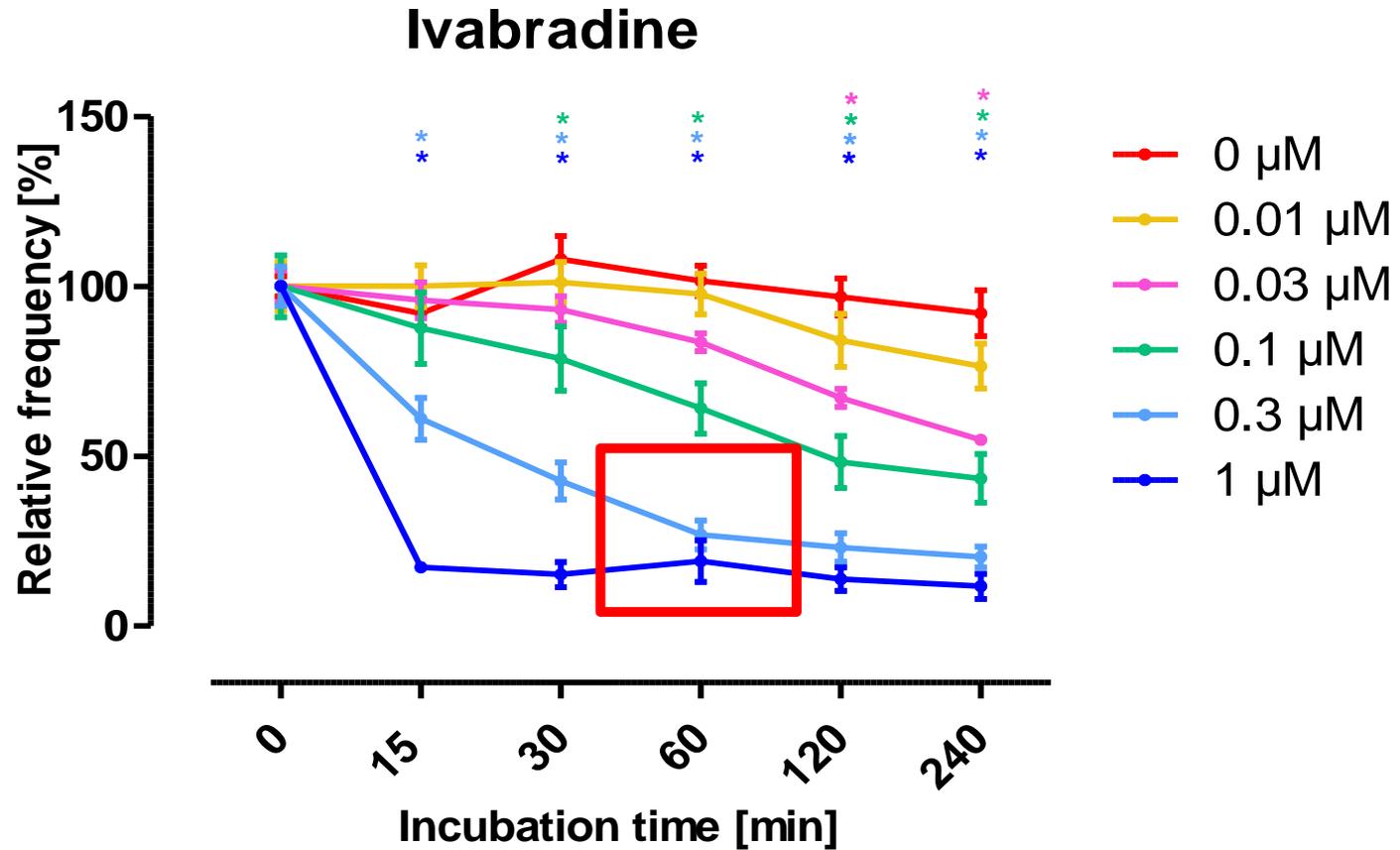
Force frequency relationship in non-failing human heart



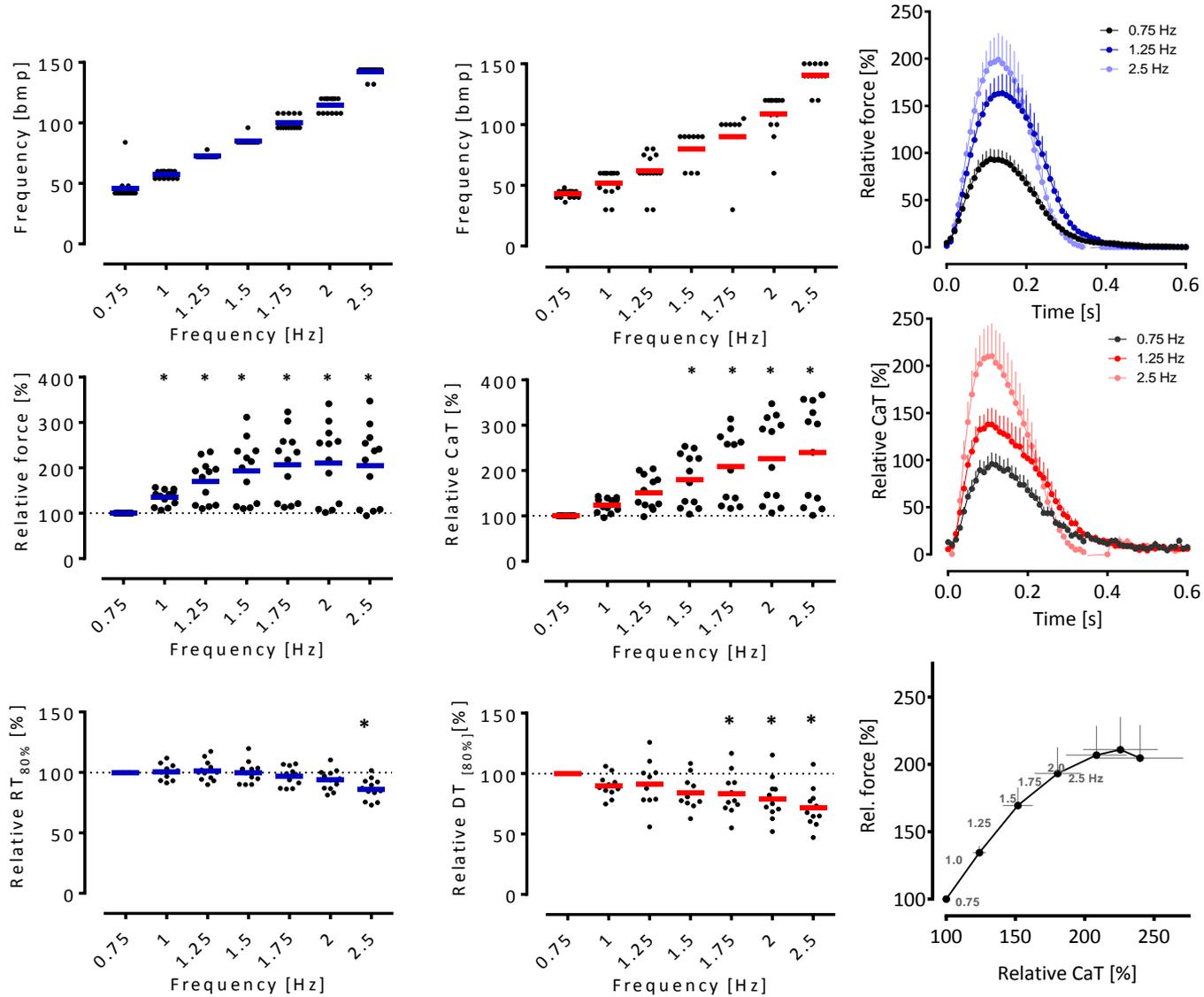
Mulieri et al., 1992
Circulation
0.5-2.9 Hz



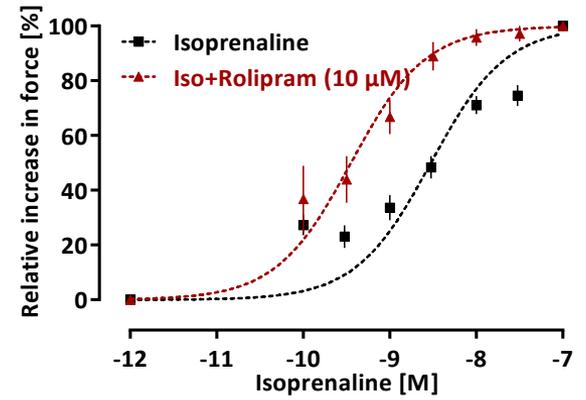
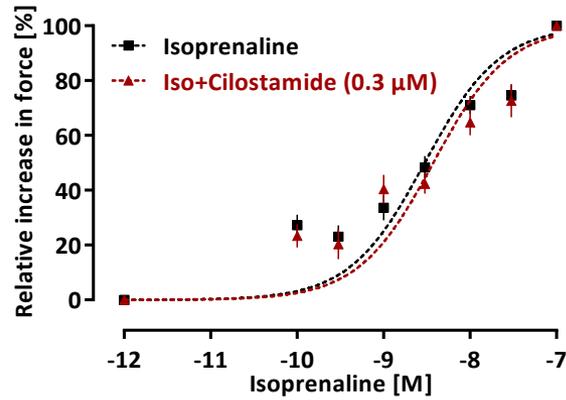
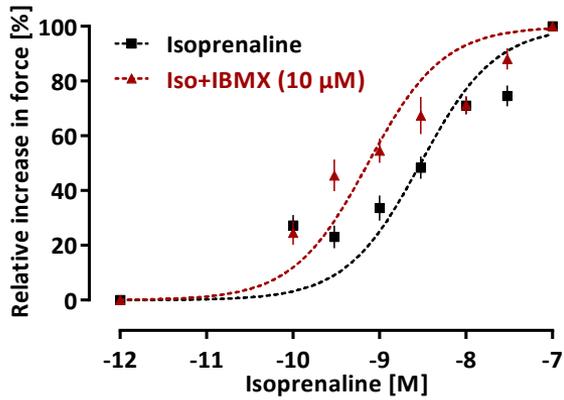
Pieske, Hasenfuss, 1999
Circ Res
0.3-2.5 Hz



FFR in hiPSC-CM EHT (+ ivabradine 300 nM)



Effect of PDE inhibitor on ISO potency to induce PI



n = 40; 6 diff. batches (Iso)
n = 12-16; 2-3 diff. batches (PDEI)



National Centre
for the Replacement
Refinement & Reduction
of Animals in Research



Challenge 13: InPulse

Objective

To generate a **physiologically-relevant contractility platform** with cells that are phenotypically '**mature**', possess a **robust contractile apparatus**, move calcium between intracellular and extracellular spaces and metabolically generate substantive amounts of energy.

Nottingham – Chris Denning

Leiden – Christine Mummery

Glasgow – Godfrey Smith

Hamburg – Arne Hansen, Thomas Eschenhagen

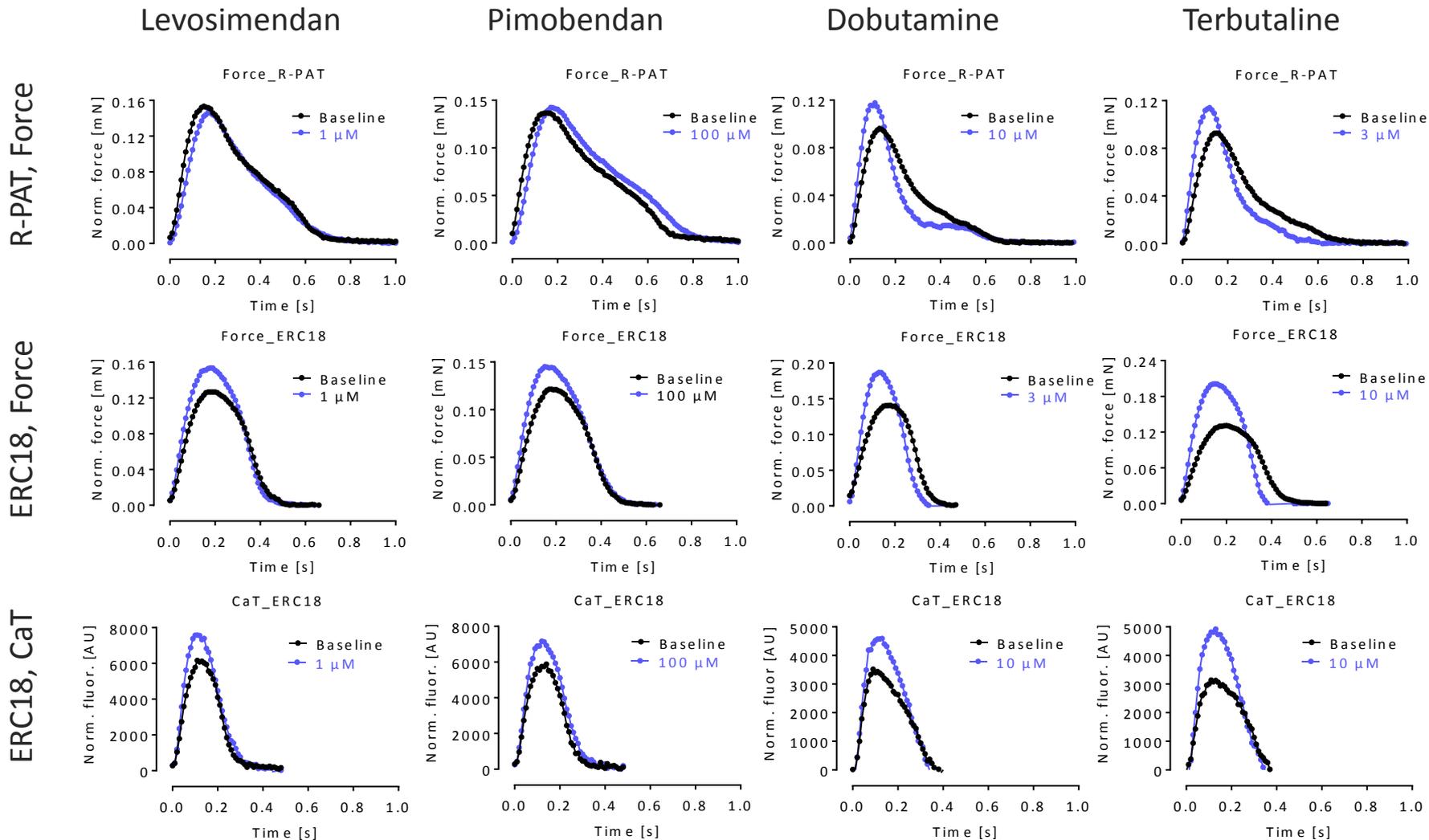
GSK – Peter Clemens

Blinded screen of 28 positive (PI), negative (NI) and no effect (NE) compounds

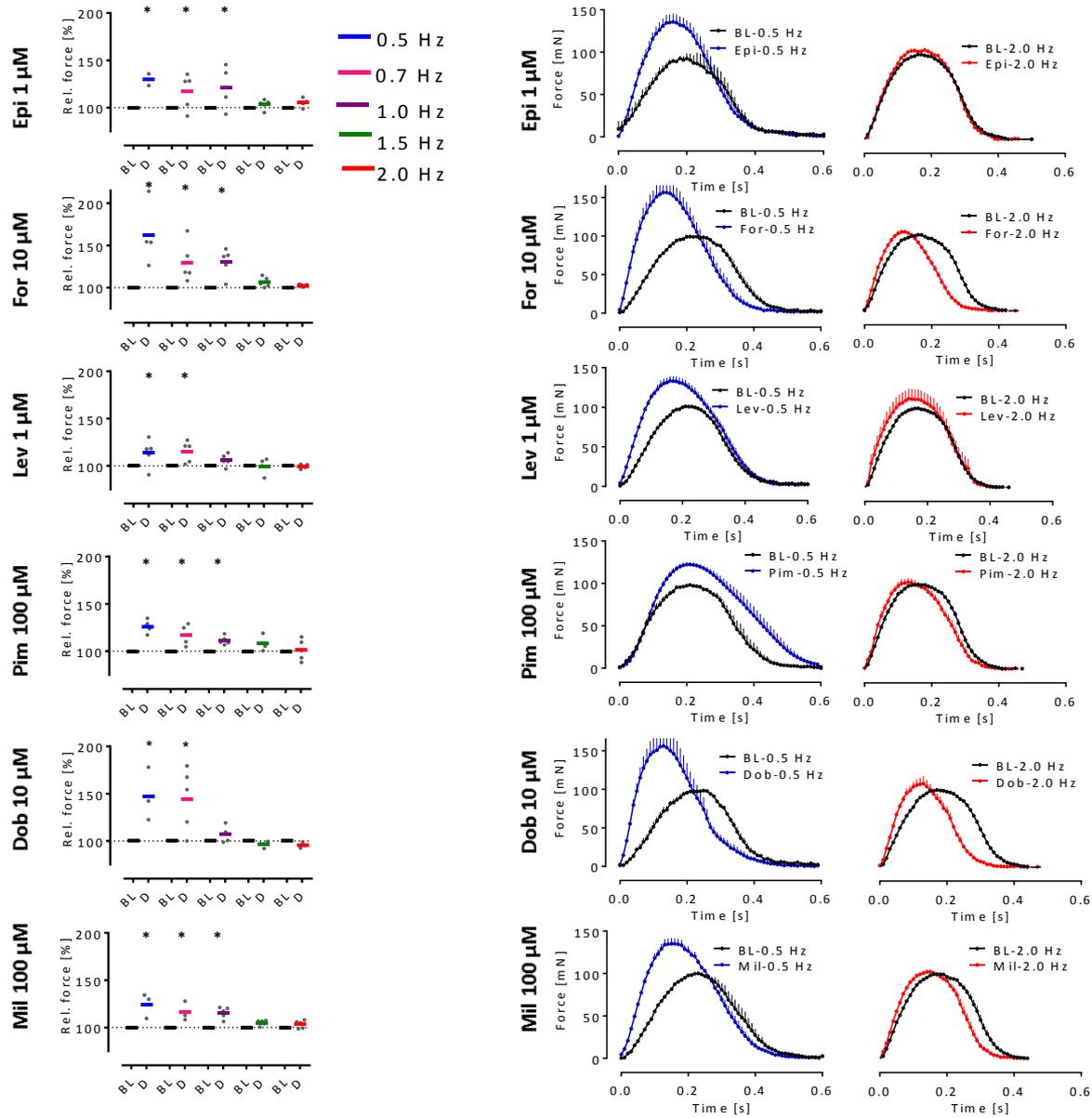
Predictivity

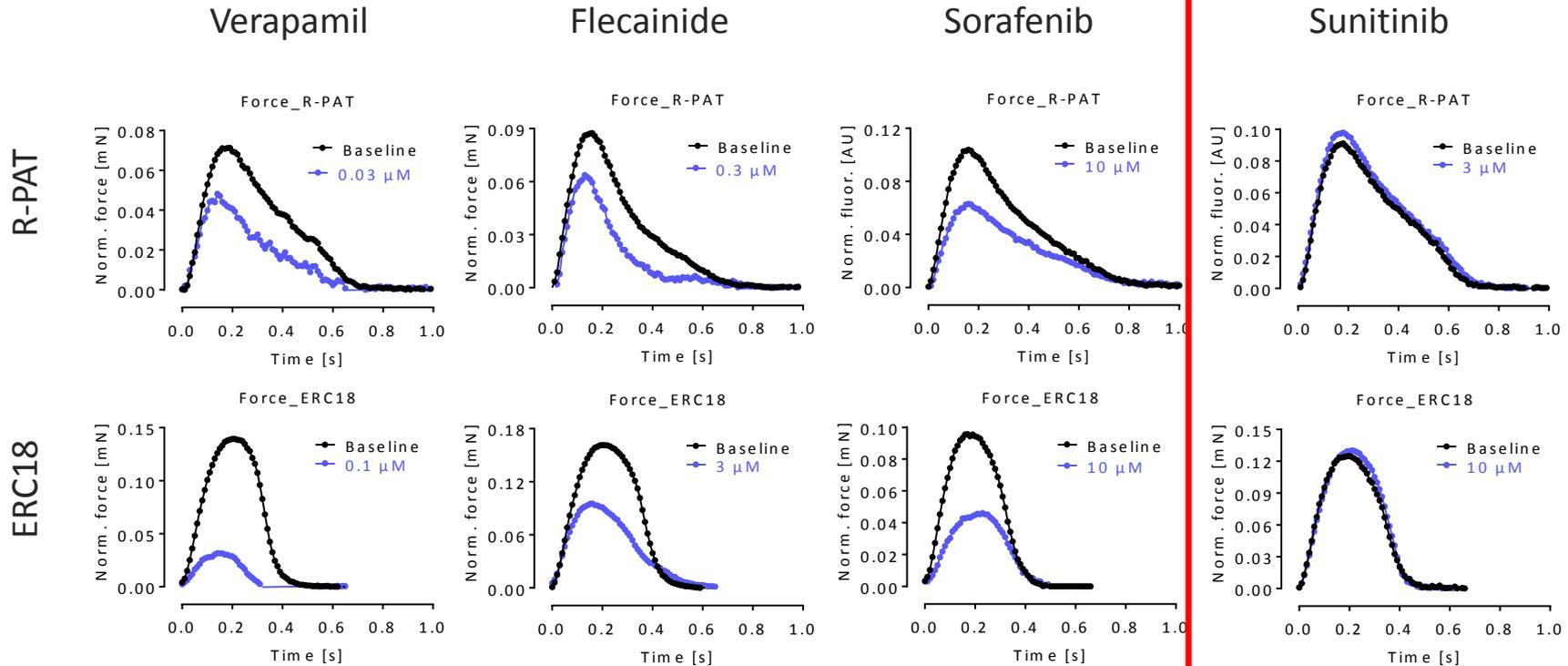
Platform	Format	PI	NI	NE	Total
CelloPTIQ (GP-CM)	2D	2/4 (50%)	2/2 (100%)	3/4 (75%)	(70%)
CelloPTIQ (hiPSC-CM)	2D	0/4, 1/4, 0/8 (8%)	2/3, 2/3, 4/8 (61%)	3/3, 3/3, 10/11 (97%)	(54%)
TTM (hiPSC-CM)	2D	2/4 (50%)	2/3 (67%)	3/3 (100%)	(70%)
EHT (hiPSC-CM)	3D	6/8, 6/8 (75%)	7/8, 7/8 (88%)	9/11, 8/11 (78%)	(80%)

Analysis of positive inotropic compounds

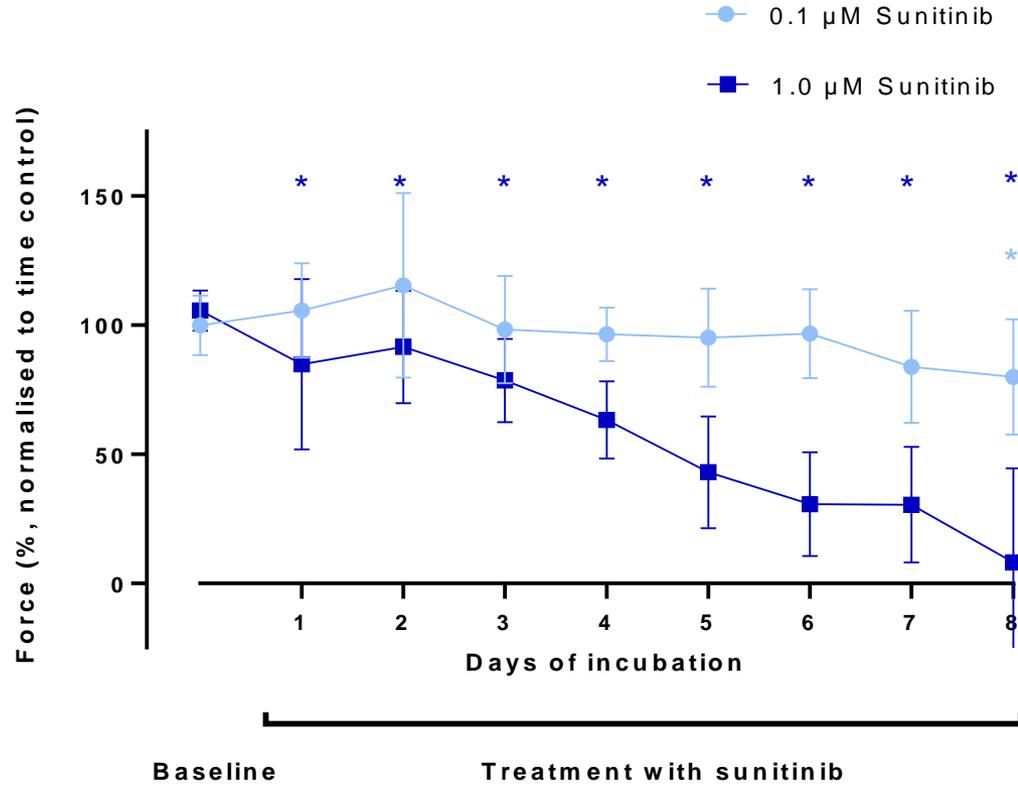


Analysis of positive inotropic compounds (single concentration, ivabradine, 300 nM)





Sunitinib, long term exposure



n = 14 (sunitinib 0.1 μM), n = 14 (sunitinib 1.0 μM), n = 14 (time control, not shown).

* p<0.05 TwoWay ANOVA

- 3D EHT model, standardized generation, automated, sterile contractility analysis
- Morphological, electrophysiological, metabolic maturation
- FFR, PDE: experimental conditions and limitations need to be carefully defined
- Inotropic assessment: CRC curve + single high [Con] at low freq (PI) + long term incubation (NI)

Acknowledgement

Thomas Eschenhagen

Team IEPT UKE

Umber Saleem

Jessica Nebel

Ingra Mannhardt

Torsten Christ

Chris Denning lab

Christine Mummery lab

Godfrey Smith lab

Peter Clemens, GSK



Department of Experimental Pharmacology and Toxicology
Cardiovascular Research Center Hamburg
German Centre for Cardiovascular Research
University Medical Center Hamburg-Eppendorf

Support

Deutsches Zentrum für Herz-Kreislauf-Forschung e.V.

Deutsche Forschungsgemeinschaft (DFG)

EU-ERARE

British Heart Foundation

CRACK-IT Consortium

Deutsche Stiftung für Herzforschung

Freie und Hansestadt Hamburg

