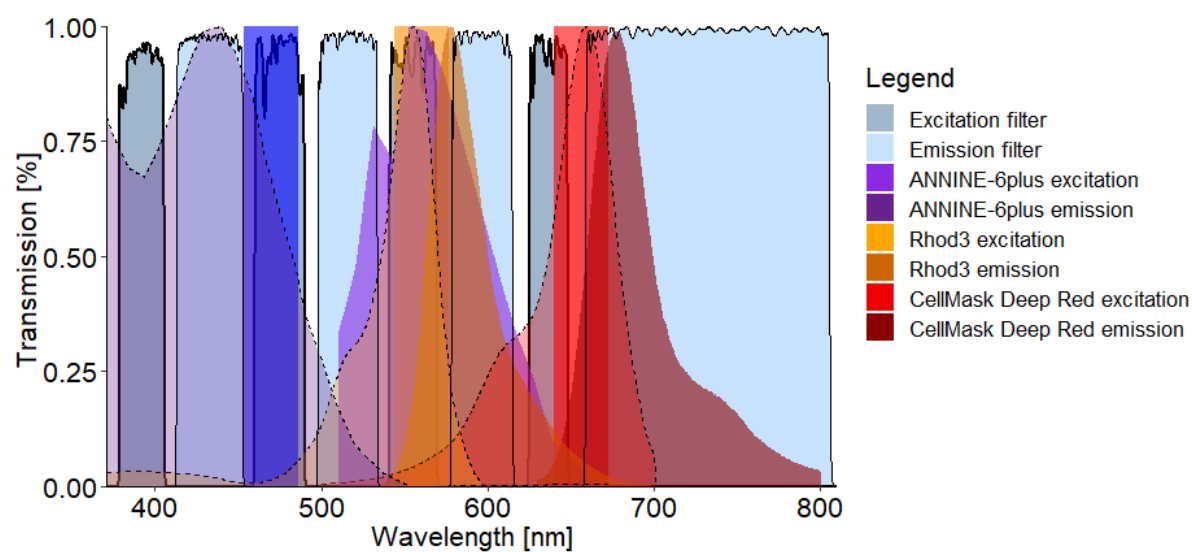


Simultaneous measurement of excitation-contraction coupling parameters identifies mechanisms underlying contractile responses of hiPSC-derived cardiomyocytes

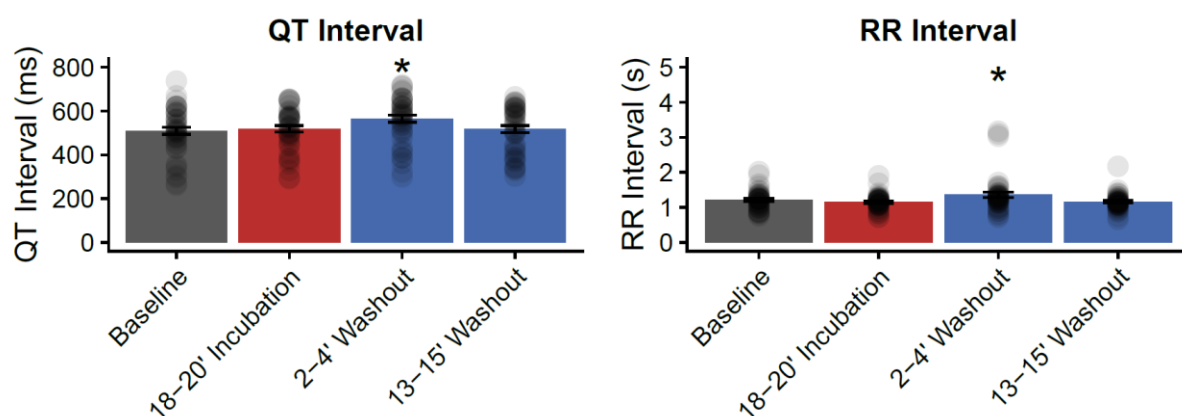
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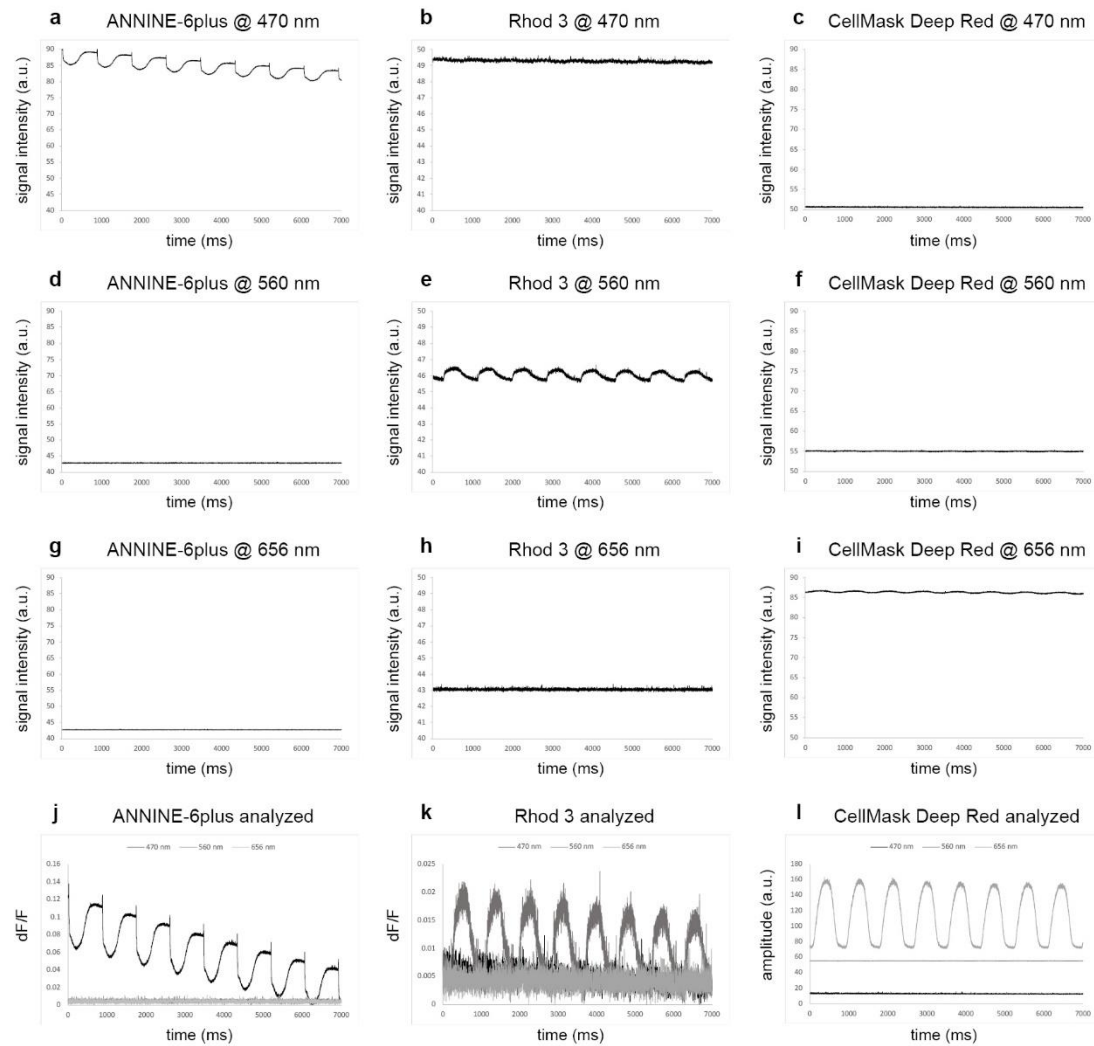
SUPPLEMENTARY FIGURES



Supplementary Figure 1: Spectral overview of TTM system and dyes used.



Supplementary Figure 2: Effect on the electrophysiological properties of hiPSC-CMs by combined loading of ANNINE-6plus, Rhod-3 and CellMask Deep Red. QT and RR-interval are shown before addition (grey), after 18-20 minutes incubation (red) and 2-4 and 13-15 minutes after washout (blue). N=46 measurements. One-way ANOVA was used to test for differences in means between conditions but all p-values were larger than 0.05. The bars in the bar graph indicate the mean and the error bars indicate SEM. Source data are provided as a Source Data file.



Supplementary Figure 3: Absence of crosstalk between the different channels. Different wells of hiPSC-CMs were loaded with either ANNINE-6plus, Rhod 3 or CellMask Deep Red. These wells were then exposed to the three different LEDs of the system individually (470nm, 560 nm and 656 nm) to investigate whether a measurable signal occurred in the different channels. Measurable signals were exclusively detected at 470 nm, 560 nm and 656 nm for ANNINE-6plus (a), Rhod 3 (e) and CellMask Deep Red (i), respectively. In contrast, no signals were detected for ANNINE-6plus at 560 nm (d) or 656 nm (g), for Rhod 3 at 470 nm (b) or 656 nm (h), and for CellMask Deep Red at 470 nm (c) or 560 nm (f). To confirm whether the raw signals were indeed not contributing to the analysis of the transients we have performed the first steps of analysis for the dyes: dF/F calculation (ANNINE-6plus and Rhod 3) and MUSCLEMOTION analysis (CellMask Deep Red). Again, the only relevant contribution to the signal was originating from the 470 nm, 560 nm and 656 nm wavelength for ANNINE-6plus (j), Rhod 3 (k) and CellMask Deep Red (l), respectively.

Supplementary Table 1: Overview of all probability scores in the hypothesis table per drug per concentration.							
	Concentration (µM)	Contractility+ (cAMP)	Contractility+ (calcium)	Contractility+ (myosin)	Contractility- (calcium)	Contractility- (toxicity)	No change
levosimendan	Vehicle	2.38	3.13	4.64	2.19	3.01	5.85
	0.01	1.86	3.31	3.73	1.9	3.18	4.51
	0.03	1.94	3.31	4.79	1.8	3.34	5.47
	0.1	2.82	3.59	4.53	2.12	2.85	5.21
	0.3	2.28	2.73	4	2.82	3.34	4.9
	1	1.97	3.29	3.48	2.44	3.38	4.95
omecamtiv mecarbil	Vehicle	2.88	3.04	3.9	2.52	2.64	4.78
	0.01	2.1	2.6	3.94	2.24	3.24	4.4
	0.03	2.4	2.76	3.73	2.18	2.49	3.68
	0.1	2.48	2.71	3.1	2.14	2.22	3.4
	0.3	4.12	3.46	4.54	3.27	2.63	4.18
	1	2.84	3.18	4.25	2.01	2.19	3.32
doxorubicin	Vehicle	2.54	3.15	4.29	2.48	3.12	5.69
	1	4.07	3.34	4.37	3.47	3.17	5.38
	3	3.42	3.37	3.9	2.3	2.52	4.74
	10	3.06	2.34	3.66	3.35	3.59	4.94
	30	2.09	2.17	2.31	3.62	4.47	3.76
	100	0.22	0.18	0.17	0.58	0.87	0.19
verapamil	Vehicle	2.74	3.45	4.55	2.46	3.17	5.69
	0.01	3.06	2.99	4.16	4.09	4.01	4.83
	0.03	3.87	2.27	3.18	5.66	4.33	3.27
	0.1	3.63	1.77	2.41	5.8	3.7	1.82
	0.3	2.84	1.75	1.52	4.56	2.8	1.22
	1	1.42	1.07	0.67	2.23	1.17	0.67
aspirin	Vehicle	2.16	2.98	4.25	1.99	2.8	5.67
	10	2.09	3.17	4.78	2.35	3.3	5.5
	30	2.61	2.76	4.81	2.66	2.86	5.3
	100	2.57	3.03	4.95	2.09	2.62	5.53
	300	2.22	2.65	4.22	2.79	3.13	5.07
	1000	2.95	3.08	4.64	3.1	3.08	5.27
captopril	Vehicle	2.62	3.35	3.79	2.66	3.41	4.86
	1	2.1	3.26	3.44	1.57	2.82	4.73
	3	2.28	3.71	4.85	1.25	2.82	5.84
	10	2.3	3.25	3.55	1.83	3.11	4.81
	30	2.83	3.61	4	2.22	3.41	4.77
	100	2.29	3.31	4.48	1.67	2.93	5.55
sunitinib	Vehicle	2.56	3.15	4.18	2.3	3.03	5.67
	0.1	1.06	2.72	4.45	1.95	3.3	4.72
	0.3	1.15	2.49	3.81	1.87	3.01	4.13
	1	1.69	2.28	3.04	2.45	3.74	3.65
	3	1.88	1.99	2.62	3.17	4.38	3.29
	10	2.69	1.46	2.16	5.02	5.43	2.35
atenolol	Vehicle	3	3.45	4.66	2.64	3.1	5.39
	0.1	2.98	3.05	5.12	2.42	2.84	5.03
	0.3	2.86	2.77	3.97	2.99	3.5	4.13
	1	3.03	3.24	4.84	2.89	3.69	5.06
	3	3.61	3.31	3.84	3.07	3.31	4.24
	10	3.46	3.53	4.96	2.71	3.23	5.07
pimobendan	Vehicle	1.94	2.96	4.95	1.83	2.97	7.01
	1	2.16	2.89	4.2	1.45	2.68	5.57
	3	2.34	3.24	4.52	1.92	2.54	6.35
	10	1.95	3.1	4.31	1.48	2.54	6.06
	30	2.14	2.91	4.54	1.85	2.36	4.7
	100	1.92	2.98	3.29	1.26	1.96	5.05
forskolin	Vehicle	2.22	2.89	4.39	2.33	3.08	5.74
	0.1	5.04	3.74	4.3	3.43	2.77	4.79
	0.3	6.05	3.95	3.11	4.33	2.69	3.15
	1	6.61	3.87	2.13	5.28	2.86	2.06
	3	6.63	3.77	2.45	4.8	2.89	2.51
	10	6.61	3.97	2.35	4.45	2.43	1.98
epinephrine	Vehicle	2.11	3.23	5.04	1.88	2.95	6.7
	0.01	3.45	3.51	3.3	2.85	3.29	4.4
	0.03	4.48	3.84	4.88	3.16	2.66	5.12
	0.1	5.36	4.17	3.99	4.2	2.74	4.42
	0.3	4.56	3.13	2.83	3.42	2.11	3
	1	5.7	3.98	3.85	4.48	2.86	4.21
ouabain	Vehicle	2.46	3.05	4.08	2.32	2.98	5.19
	0.03	2.66	3.34	4.3	3	2.81	4.53
	0.1	2.89	3.65	3.9	2.08	2.43	4.65
	0.3	2.45	3.13	4.01	2.34	3.25	4.88
	1	2.48	3.07	2.33	1.87	2.58	2.56
	3	1.63	1.7	0.88	1.4	1.47	0.83

Supplementary Table 2: Effects assigned by the hypothesis based model.

Drug	Epinephrine	MOA: Co ⁺ _{cAMP}		
	ALL	AP only	Ca only	Co only
Vehicle	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼
0.01 μM	Co ⁼	Co ⁺ _{cAMP}	Co ⁼	Co ⁼
0.03 μM	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼
0.1 μM	Co ⁺ _{cAMP}	Co ⁺ _{cAMP}	Co ⁺ _{Myosin}	Co ⁼
0.3 μM	Co ⁺ _{cAMP}	Co ⁺ _{cAMP}	Co ⁺ _{cAMP}	Co ⁼
1 μM	Co ⁺ _{cAMP}	Co ⁺ _{cAMP}	Co ⁺ _{cAMP}	Co ⁼

Drug	Pimobendan	MOA: Co ⁺ _{cAMP}		
		ALL	AP only	Ca only
Vehicle	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼
1 μM	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼
3 μM	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼
10 μM	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼
30 μM	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁺ _{Myosin} / Co ⁺ _{Ca}
100 μM	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼

Drug	Forskolin	MOA: Co ⁺ _{cAMP}		
	ALL	AP only	Ca only	Co only
Vehicle	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼
0.1 μM	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁺ _{cAMP}
0.3 μM	Co ⁺ _{cAMP}	Co ⁺ _{cAMP}	Co ⁺ _{cAMP}	Co ⁺ _{cAMP}
1 μM	Co ⁺ _{cAMP}	Co ⁺ _{cAMP}	Co ⁺ _{cAMP}	Co ⁺ _{cAMP}
3 μM	Co ⁺ _{cAMP}	Co ⁺ _{cAMP}	Co ⁺ _{cAMP}	Co ⁺ _{cAMP}
10 μM	Co ⁺ _{cAMP}	Co ⁺ _{cAMP}	Co ⁺ _{cAMP}	Co ⁺ _{cAMP}

Drug	Aspirin	MOA:	MOA: $\text{Co}^=$	
	ALL	AP only	Ca only	Co only
Vehicle	$\text{Co}^=$	$\text{Co}^= / \text{Co}^+$ Myosin	$\text{Co}^=$	$\text{Co}^=$
10 μM	$\text{Co}^=$	$\text{Co}^= / \text{Co}^+$ Myosin	$\text{Co}^=$	$\text{Co}^=$
30 μM	$\text{Co}^=$	$\text{Co}^= / \text{Co}^+$ Myosin	$\text{Co}^=$	$\text{Co}^=$
100 μM	$\text{Co}^=$	$\text{Co}^= / \text{Co}^+$ Myosin	$\text{Co}^=$	$\text{Co}^=$
300 μM	$\text{Co}^=$	$\text{Co}^= / \text{Co}^+$ Myosin	Co^-_{Ca}	$\text{Co}^=$
1000 μM	$\text{Co}^=$	$\text{Co}^= / \text{Co}^+$ Myosin	$\text{Co}^+_{\text{Myosin}}$	$\text{Co}^=$

Drug	Levosimendan	MOA: Co ⁺ _{CAMP}		
	ALL	AP only	Ca only	Co only
Vehicle	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼
0.01 μM	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁺ _{Ca}	Co ⁼
0.03 μM	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼
0.1 μM	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼
0.3 μM	Co ⁼	Co ⁼ _{Tox}	Co ⁼	Co ⁼
1 μM	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼

Drug	Atenolol	MOA: Co ⁼		
	ALL	AP only	Ca only	Co only
Vehicle	Co ⁼	Co ⁼ / Co ⁺ Myosin	Co ⁼	Co ⁼
0.1 μM	Co ⁼	Co ⁼ / Co ⁺ Myosin	Co ⁼	Co ⁺ Myosin / Co ⁺ Ca
0.3 μM	Co ⁺ Myosin	Co ⁼ / Co ⁺ Myosin	Co ⁼	Co ⁺ Myosin / Co ⁺ Ca
1 μM	Co ⁼	Co ⁼ / Co ⁺ Myosin	Co ⁼	Co ⁺ Myosin / Co ⁺ Ca
3 μM	Co ⁼	Co ⁼ / Co ⁺ Myosin	Co ⁺ cAMP	Co ⁺ cAMP
10 μM	Co ⁼	Co ⁼ / Co ⁺ Myosin	Co ⁼	Co ⁼

Drug	Verapamil	MOA: Co^-_{Ca}		
	ALL	AP only	Ca only	Co only
Vehicle	Co^-	$\text{Co}^- / \text{Co}^+_{\text{Myosin}}$	Co^-	Co^-
0.01 μM	Co^-	$\text{Co}^- / \text{Co}^+_{\text{Myosin}}$	Co^-_{Ca}	Co^-_{Tox}
0.03 μM	Co^-_{Ca}	Co^-_{Ca}	Co^-_{Ca}	Co^-_{Ca}
0.1 μM	Co^-_{Ca}	Co^-_{Ca}	Co^-_{Ca}	Co^-_{Ca}
0.3 μM	Co^-_{Ca}	Co^-_{Ca}	Co^-_{Ca}	-
1 μM	Co^-_{Ca}	Co^-_{Ca}	Co^-_{Ca}	-

Drug	Captopril	MOA: Co ⁼		
	ALL	AP only	Ca only	Co only
Vehicle	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼
1 μM	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼
3 μM	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼
10 μM	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼
30 μM	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼
100 μM	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼

Drug	Doxorubicin	MOA: Co ⁻ _{Tox}		
	ALL	AP only	Ca only	Co only
Vehicle	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁼	Co ⁼
1 μM	Co ⁼	Co ⁼ / Co ⁺ _{Myosin}	Co ⁺ _{Myosin}	Co ⁼
3 μM	Co ⁼	Co ⁺ _{cAMP}	Co ⁼	Co ⁼
10 μM	Co ⁼	Co ⁻ _{Ca}	Co ⁺ _{Myosin}	Co ⁼
30 μM	Co ⁻ _{Tox}	Co ⁻ _{Tox}	Co ⁻ _{Tox}	Co ⁼
100 μM	Co ⁻ _{Tox}	Co ⁻ _{Tox}	-	Co ⁻ _{Ca}

Drug	Omecamtiv Mecarbil	MOA: Co^+ Myosin		
	ALL	AP only	Ca only	Co only
Vehicle	Co^-	$\text{Co}^- / \text{Co}^+$ Myosin	Co^-	Co^-
0.01 μM	Co^-	$\text{Co}^- / \text{Co}^+$ Myosin	Co^-	Co^-
0.03 μM	Co^+ Myosin	$\text{Co}^- / \text{Co}^+$ Myosin	Co^+ Myosin	Co^+ Myosin / Co^+ Ca
0.1 μM	Co^-	$\text{Co}^- / \text{Co}^+$ Myosin	Co^+ Myosin	Co^-
0.3 μM	Co^+ Myosin	Co^+ cAMP	Co^+ Myosin	Co^+ Myosin / Co^+ Ca
1 μM	Co^+ Myosin	$\text{Co}^- / \text{Co}^+$ Myosin	Co^+ Myosin	Co^+ Myosin / Co^+ Ca

Drug	Sunitinib	MOA: Co^-_{Tox}		
	ALL	AP only	Ca only	Co only
Vehicle	Co^-	$\text{Co}^- / \text{Co}^+_{\text{Myosin}}$	Co^-	Co^-
0.1 μM	Co^-	Co^-_{Tox}	Co^-	$\text{Co}^+_{\text{Myosin}} / \text{Co}^+_{\text{Ca}}$
0.3 μM	Co^-	Co^-_{Tox}	Co^-	$\text{Co}^+_{\text{Myosin}} / \text{Co}^+_{\text{Ca}}$
1 μM	Co^-	Co^-_{Tox}	Co^-	Co^-
3 μM	Co^-_{Tox}	Co^-_{Tox}	Co^-	Co^-_{Ca}
10 μM	Co^-_{Tox}	Co^-_{Tox}	Co^-_{Ca}	Co^-_{Ca}

Drug	Ouabain	MOA: Co^+_{Ca}		
	ALL	AP only	Ca only	Co only
Vehicle	Co^-	$\text{Co}^- / \text{Co}^+_{\text{Myosin}}$	Co^-	Co^-
0.03 μM	Co^-	$\text{Co}^- / \text{Co}^+_{\text{Myosin}}$	Co^-	Co^-
0.1 μM	Co^-	$\text{Co}^- / \text{Co}^+_{\text{Myosin}}$	Co^-	Co^-
0.3 μM	Co^-	Co^-_{Tox}	Co^-	Co^-
1 μM	Co^+_{Ca}	Co^+_{Ca}	Co^+_{Ca}	Co^-
3 μM	Co^+_{Ca}	Co^+_{Ca}	Co^-_{Tox}	Co^+_{Ca}

Supplementary Table 3: A comparison of the outcome of the hypothesis-based algorithm and traditional statistics. P-values were calculated using a Dunnett’s test after one-way ANOVA. Source data are provided as a Source Data file.

	Concentration (μM)	Algorithm output	Contraction amplitude	Calcium amplitude	Contraction time	Relaxation time	Calcium time to peak	Calcium decay time	APD90	Action potential amplitude	Triangulation	AP[Trise]
levosimendan	Vehicle	No change	--	--	--	--	--	--	--	--	--	--
	0.01	No change	p<0.05									
	0.03	No change										
	0.1	No change	p<0.01									
	0.3	No change										
	1	No change										
omecamtiv mecarbil	Vehicle	No change	--	--	--	--	--	--	--	--		--
	0.01	No change										p<0.01
	0.03	Contractility+ (myosin)										
	0.1	No change										
	0.3	Contractility+ (myosin)										
	1	Contractility+ (myosin)	p<0.01		p<0.01							
doxorubicin	Vehicle	No change	--	--	--	--	--	--	--	--	--	--
	1	No change					p<0.05					
	3	No change					p<0.05					
	10	No change							p<0.001			
	30	Contractility- (toxicity)				p<0.01			p<0.0001	p<0.001		
	100	Contractility- (toxicity)				NA	NA	NA	p<0.0001	p<0.0001		
verapamil	Vehicle	No change	--	--	--	--	--	--	--	--	--	--
	0.01	No change	p<0.05								p<0.05	
	0.03	Contractility- (calcium)	p<0.01			p<0.0001			p<0.05	p<0.01	p<0.0001	
	0.1	Contractility- (calcium)	p<0.01		p<0.01	p<0.0001		p<0.0001	p<0.0001	p<0.0001	p<0.0001	
	0.3	Contractility- (calcium)	NA	NA	NA	p<0.0001		p<0.0001	p<0.0001	p<0.0001	p<0.0001	
	1	Contractility- (calcium)	NA	NA	NA	p<0.0001		p<0.001	p<0.0001	p<0.0001	p<0.0001	
aspirin	Vehicle	No change	--	--	--	--	--	--	--	--	--	--
	10	No change										
	30	No change										
	100	No change										
	300	No change										
	1000	No change										
captopril	Vehicle	No change	--	--	--	--	--	--	--	--	--	--
	1	No change	p<0.05			p<0.01			p<0.05			
	3	No change										
	10	No change										
	30	No change										
	100	No change										
sunitinib	Vehicle	No change	--	--	--	--	--	--	--	--	--	--
	0.1	No change										
	0.3	No change										
	1	Contractility- (toxicity)								p<0.01	p<0.05	
	3	Contractility- (toxicity)				p<0.01				p<0.0001	p<0.05	
	10	Contractility- (toxicity)				p<0.0001		p<0.0001	p<0.0001	p<0.01		

atenolol	Vehicle		No change	--	--	--	--	--	--	--	--	--
	0.1		Contractility+ (myosin)							p<0.05		
	0.3		No change							p<0.05		p<0.01
	1		No change	p<0.01								p<0.05
	3		No change									
	10		No change									
pimobendan	Vehicle		No change	--	--	--	--	--	--	--	--	--
	1		No change									
	3		No change									
	10		No change									
	30		No change									
	100		No change									
forskolin	Vehicle		No change	--	--	--	--	--	--	--	--	--
	0.1		Contractility+ (cAMP)			p<0.01			p<0.01		p<0.01	
	0.3		Contractility+ (cAMP)			p<0.05	p<0.05		p<0.001		p<0.0001	p<0.01
	1		Contractility+ (cAMP)			p<0.001			p<0.0001	p<0.001	p<0.0001	p<0.001
	3		Contractility+ (cAMP)			p<0.0001			p<0.0001		p<0.0001	p<0.001
	10		Contractility+ (cAMP)	p<0.05		p<0.001	p<0.05	p<0.05	p<0.0001	p<0.01	p<0.0001	p<0.0001
epinephrine	Vehicle		No change	--	--	--	--	--	--	--	--	--
	0.01		No change									
	0.03		No change									
	0.1		Contractility+ (cAMP)			p<0.05			p<0.01	p<0.05	p<0.0001	
	0.3		Contractility+ (cAMP)			p<0.01			p<0.01		p<0.0001	
	1		Contractility+ (cAMP)			p<0.001			p<0.0001		p<0.0001	
ouabain	Vehicle		No change	--	--	--	--	--	--	--	--	--
	0.03		No change									
	0.1		No change									
	0.3		No change		p<0.05				p<0.05			
	1		Contractility+ (calcium)							p<0.05		p<0.01
	3		Contractility+ (calcium)		p<0.0001					p<0.001		p<0.01