

– Supplemental Material –
Long-term cultivation of human atrial myocardium

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1 Materials and Equipment

1.1 Buffers and Media

Name	Solvent	Composition / Supplements	Concentration
Storage solution	ddH ₂ O	NaCl NaH ₂ PO ₄ butadiene-monoxide (BDM) Glucose HEPES KCl CaCl ₂	138 mmol/l 0.33 mmol/l 30 mmol/l 10 mmol/l 10 mmol/l 5.4 mmol/l 0.5 mmol/l
MTT buffer	ddH ₂ O	CaCl ₂ butadiene-monoxide (BDM) Glucose HEPES KCl MgCl ₂ NaCl NaH ₂ PO ₄	1.8 mmol/l 30 mmol/l 10 mmol/l 10 mmol/l 5.4 mmol/l 1 mmol/l 136 mmol/l 0.33 mmol/l
Ca ²⁺ imaging buffer	ddH ₂ O	NaCl KCl CaCl ₂ MgCl ₂ NaH ₂ PO ₄ HEPES glucose	138 mmol/l 4 mmol/l 2 mmol/l 1 mmol/l 0.33 mmol/l 10 mmol/l 10 mmol/l
Culture medium	M199 (Sigma, M4530)	Penicillin Streptomycin Insulin Transferrin Selenium 2-Mercaptoethanol Cortisol	10 U/ml 10 µg/ml 10 µg/ml 5.5 µg/ml 6.7 ng/ml 50 µmol/l 20 nmol/l

1.2 Chemicals and reagents

Name	Manufacturer	REF number
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)	Roth	9105.3
Bovine serum albumine fraction V (BSA)	Roth	163.2
Butanedione monoxime (BDM)	AlfaAesar	114339
Bradford protein assay reagent	BioRad	5000006
Calcium chloride	Roth	5239.2
Cortisol	Sigma	H4001
Dofetilide	Sigma	PZ0016
Glucose	Merck	50997
Isoprenaline	Sigma	I5627
Magnesium chloride	Roth	A537.1
Normal goat serum	Sigma Aldrich	G9023
Paraformaldehyde	Sigma Aldrich	158127
Polyethylene glycol p-(1,1,3,3-tetramethylbutyl)-phenyl ether (Triton X-100)	Roth	3051.3
Potassium hydroxide	Merck	105032
Sodium chloride	Roth	9265.1
Sodium dihydrogenphosphate	Sigma Aldrich	S5011
Thiazolyl blue tetrazolium bromide (MTT)	Sigma	M2128
Penicillin/Streptomycin	Biochrom	A2213
Insulin Transferrin Selenium (100x)	Gibco	41400045
Pluronic Acid F127	Biotium	59005

1.3 Antibodies, fluorophores and dyes

Antibody/Reagent	Manufacturer	REF number	Dilution / concentration
Mouse anti α -actinin IgG1	Sigma	A7811	1:200
Rabbit anti connexin-43	Sigma	C6219	1:400
Goat anti-mouse IgG1-AlexaFluor 488	Invitrogen	A21121	1:200
Goat-anti-rabbit IgG-AlexaFluor 555	Invitrogen	A21428	1:400
4',6-diamidino-2-phenylindole (DAPI)	Roth	63351	2 μ g/ml
Wheat germ agglutinin (WGA) - AlexaFluor 647	Invitrogen	W32466	40 μ g/ml
Calbryte 520-AM Ca ²⁺ indicator	AAT Bioquest	20651	10 μ mol/l

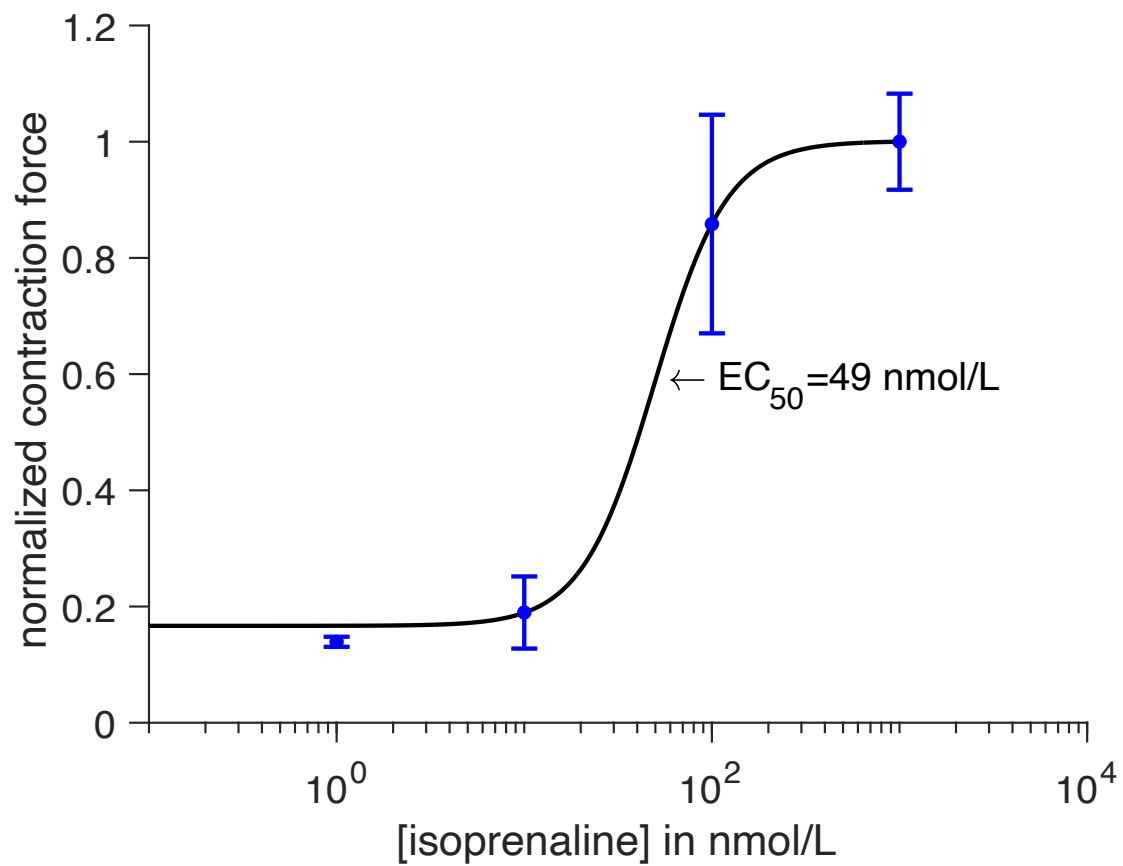
1.4 PCR primers

Target gene (protein)	Fwd (5'-3')	Rev (5'-3')
<i>CACNA1C</i> (LTCC)	TGTCCGGAGTCCCAAGTCTC	CATGGCCTTGATGATGGAA
<i>ATPA2</i> (SERCA)	ACAATGGCGCTCTCTGTTCT	ATCCTCAGCAAGGACTGGTTT
<i>GJA1</i> (Cx43)	TACCAAACAGCAGCGGAGTT	TGGGCACCACTCTTTTGCTT
<i>KCNJ2</i> (Kir2.1)	CTTACATGCCTCTGTACCCCC	TCTCTGGGAGCCTTGTGGTT
<i>KCNJ4</i> (Kir2.3)	CTCTCGTCGGACCCTCC	GTGTCCGTGCATGTCCTGAA
<i>EEF2</i>	GGATGGGACACCCAACACTT	CTTTCTGGGGCAAAAGCCAC
<i>HPRT1</i>	GGCAGTATAATCCAAAGATGGTC	TTCAAATCCAACAAAGTCTGGC

1.5 Devices, tools and other materials

Device/tool/material	Model	Manufacturer (REF)
Confocal microscope	LSM780	Zeiss
Cryotome	CM3050S	Leica Biosystems
Culture Chambers	MyoDish Culture chamber, spring stiffness #2	InVitroSys
Embedding compound	O.C.T. Tissue Tek	Sakura (4583)
Fluorescence microplate reader	GENios	Tecan
Incubator	ICO240	Memmert
Mounting Media	Fluoromount G Sigma	Sigma (F4680)
Real-Time Thermocycler	StepOnePlus	Applied Biosystems
Tissue Adhesive	Histoacryl glue	Surgibond (ZGS)
Tissue Culture System	MyoDish 1	InVitroSys
Tissue homogenizer	T10 basic	IKA (0003737000)
Spectrophotometer	DS 11+	Denovix (31DS-11PLUS-B)
RNA isolation kit	NucleoSpin RNA-Kit	Macherey-Nagel (740955.50)
Reverse transcriptase kit	QuantiTect RT-Kit	Qiagen (205311)
PCR reagent	SYBR™ Select assay	Thermo Fisher (4472903)
Inverted microscope	DM IRB	Leica (090134010000)
Fluorescence imaging system	FSI800, MyoCamS3, PMT400	IonOptix

2 Supplemental Figure 1



Supplemental Figure 1. Isoprenaline dose-response curve from 3 trabeculae immediately after installation in culture.