

Supplementary methods

Table S1. Primers used in RT-qPCR

Gene	Primer sequence (5'-3')	Product size (bp)
SOX2	Forward TGGACAGTTACGCGCACAT	215
	Reverse CGAGTAGGACATGCTGTAGGT	
REX1	Forward GCCTTATGTGATGGCTATGTGT	96
	Reverse ACCCCTTATGACGCATTCTATGT	
OCT4	Forward CGAGAAGGATGTGGTCCGAG	136
	Reverse CAGAGGAAAGGACACTGGTC	
NANOG	Forward AGATGCCTCACACGGAGACT	96
	Reverse GTTTGCCTTTGGGACTGGTG	
TNNT2	Forward CATGGAGAAGGACCTGAATGA	108
	Reverse CGTCTCTCGATCCTGTCTTTG	
MYH6	Forward CCACCCAAGTTCGACAAGAT	127
	Reverse CACAGAAGAGGCCCGAGTAG	
NKX2-5	Forward CAAGTGTGCGTCTGCCTTT	105
	Reverse GCGCACAGCTCTTTCTTTTC	
GATA4	Forward TGGCCTGTCATCTCACTACG	126
	Reverse TAGCCTTGTGGGGAGAGCTT	
TBX5	Forward CAGACTCGGCACAGCGGCAA	237
	Reverse GTGGGGAGCCATGGTTGGCC	
DES	Forward CTGAGCAAAGGGGTCTGAG	109
	Reverse ACTTCATGCTGCTGCTGTGT	
GAPDH	Forward AGTCCCTGCCCACTCAG	123
	Reverse TACTTTATTGATGGTACATGACAAGG	
SCN5A	Forward TCATCGTAGACGTCTCTCTGGT	318
	Reverse GGCTCTTGTTGTTACCGATGGT	
CACNA1D	Forward GGGCAATGGGACCTCATAAATAA	141
	Reverse TTACCTGGTTGCGAGTGCATTA	
KCNJ12	Forward GCCAGCTAGGCTCTGTTTGTG	152
	Reverse CTGAGACACATCTCTAAGGTAC	
KCND3	Forward AGAGAGCTGATAAACGCAGGG	209
	Reverse CAGGCAGTGCAGCAGGTGAT	
HCN2	Forward CGCCTGATCCGCTACATCCAT	230
	Reverse AGTGCGAAGGAGTACAGTTCACT	
PPARGC1A	Forward GCAATTGAAGAGCGCCGTGTGA	134
	Reverse CTGTCTCCATCATCCCGCAGAT	
NRF1	Forward AGGCTGGGGGAAAGAAAG	303
	Reverse CCAACCTGGATAAGTGAGAC	
HIF1A	Forward CCAACAGTAACCAACCTCAG	302
	Reverse TCCTGTGGTGACTTGCCTT	

Extracellular Flux analysis

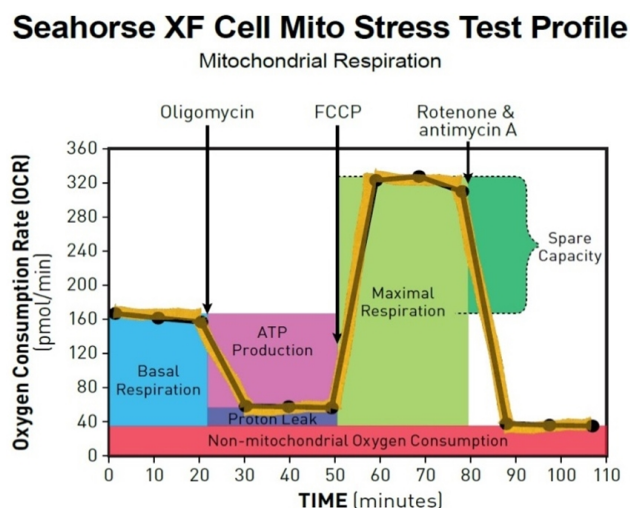
Extracellular Flux analysis was done using Seahorse XFp Extracellular Flux analyzer, Cell Mito Stress Test Kit and Cell Energy Phenotype Test Kit (Agilent Technologies, USA). The device simultaneously detects changes in oxygen concentration and in proton concentration (or pH) in the assay medium.

Cell Energy Phenotype Test Kit measures the basal oxygen consumption rate (OCR) and then OCR after the injection of the stressor mix (1 μ M of Oligomycin and 1 μ M of FCCP). Oligomycin inhibits ATP production by the mitochondria and causes a compensatory increase in the rate of glycolysis as the cells attempt to meet their energy demands via the glycolytic pathway. FCCP depolarizes the mitochondrial membrane and drives OCR higher as the mitochondria attempt to restore the mitochondrial membrane potential. The main parameters are as follows:

- Oxygen consumption rate (OCR): The rate of decrease of oxygen concentration in the assay medium. OCR is a measure of the rate of mitochondrial respiration of the cells.
- Extracellular acidification rate (ECAR): The rate of increase in proton concentration (or decrease in pH) in the assay medium. ECAR is a measure of the rate of glycolysis of the cells.
- Baseline phenotype: OCR and ECAR of cells at starting assay conditions.
- Stressed phenotype: OCR and ECAR of cells in the presence of stressor compounds.
- Metabolic potential: Percentage increase of stressed OCR over baseline OCR and stressed ECAR over baseline ECAR. Metabolic potential is the measure of cells' ability to meet the energy demand via respiration and glycolysis.

Cell Mito Stress Test Kit measures mitochondrial function in the cell by detecting OCR at the baseline level and after the addition of electron transfer chain (ETC) modulators: Oligomycin inhibits ATP synthase (complex V) and the decrease in OCR following injection of Oligomycin correlates to the mitochondrial respiration associated with cellular ATP production. Carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP) is an uncoupling agent that collapses the proton gradient and disrupts the mitochondrial membrane potential. As a result, electron flow through the ETC is uninhibited and oxygen is maximally consumed by complex IV. The third injection is a mix of Rotenone, a complex I inhibitor, and Antimycin A, a complex III inhibitor. This combination shuts down mitochondrial respiration and enables the calculation of non-mitochondrial respiration driven by processes outside the mitochondria.

The exemplary profile of Cell Mito Stress Test (from the user manual):



The main parameters are as follows:

- Basal respiration: Oxygen consumption used to meet cellular ATP demand and resulting from mitochondrial proton leak. Calculated as (Last measurement before the first injection) - (Non-mitochondrial respiration rate).
- ATP Production: The decrease in oxygen consumption rate upon injection of Oligomycin represents the portion of basal respiration that was being used to drive ATP production. Shows ATP produced by the mitochondria that contributes to meeting the energy needs of the cell. Calculated as (Last rate measurement before Oligomycin in-

jection) – (Minimum rate measurement after Oligomycin injection).

- H⁺ (Proton) leak: Remaining basal respiration not coupled to ATP production. Proton leak can be a sign of mitochondrial damage or can be used as a mechanism to regulate the mitochondrial ATP production. Calculated as (Minimum rate measurement after Oligomycin injection) – (Non-mitochondrial respiration).
- Maximal respiration: The maximal oxygen consumption rate attained by adding the uncoupler FCCP. FCCP mimics a physiological “energy demand” by stimulating the respiratory chain to operate at maximum capacity, which causes rapid oxidation of substrates to meet this metabolic challenge. Shows the maximum rate of respiration that the cell can achieve. Calculated as (Maximum rate measurement after FCCP injection) – (Non-mitochondrial respiration).
- Spare respiratory capacity: This measurement indicates the capability of the cell to respond to an energetic demand as well as how closely the cell is to respire to its theoretical maximum. The cell's ability to respond to demand can be an indicator of cell fitness or flexibility. Calculated as (Maximal respiration) – (Basal respiration).
- Nonmitochondrial respiration: Oxygen consumption that persists due to a subset of cellular enzymes continuing to consume oxygen after Rotenone and Antimycin A addition. This is important for getting an accurate measure of mitochondrial respiration. Calculated as the minimum rate measurement after Rotenone/Antimycin A injection.

Enrichment.Process_STRING database

#Pathway ID	Pathway description	Observed gene count	False discovery rate	Matching proteins in your network (IDs)	Matching proteins in your network (labels)
GO.0003209	cardiac atrium morphogenesis	5	1.97e-10	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000356286	GATA4,MYH6,NKX2-5,TBX5,TNNT2
GO.0003230	cardiac atrium development	5	1.97e-10	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000356286	GATA4,MYH6,NKX2-5,TBX5,TNNT2
GO.0003229	ventricular cardiac muscle tissue development	5	1.42e-09	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000356286	GATA4,MYH6,NKX2-5,TBX5,TNNT2
GO.0003208	cardiac ventricle morphogenesis	5	4.48e-09	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000356286	GATA4,MYH6,NKX2-5,TBX5,TNNT2
GO.0003231	cardiac ventricle development	5	3.38e-08	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000356286	GATA4,MYH6,NKX2-5,TBX5,TNNT2
GO.0051891	positive regulation of cardioblast differentiation	3	2.15e-07	ENSP00000309913,ENSP00000327758,ENSP00000334458	GATA4,NKX2-5,TBX5
GO.0008016	regulation of heart contraction	5	2.26e-07	ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000356286,ENSP00000363071	DES,GATA4,MYH6,NKX2-5,TNNT2
GO.0051146	striated muscle cell differentiation	5	3.05e-07	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000356286	GATA4,MYH6,NKX2-5,TBX5,TNNT2
GO.0003007	heart morphogenesis	5	6.7e-07	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000356286	GATA4,MYH6,NKX2-5,TBX5,TNNT2
GO.0055007	cardiac muscle cell differentiation	4	1.9e-06	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634	GATA4,MYH6,NKX2-5,TBX5
GO.0060413	atrial septum morphogenesis	3	2.91e-06	ENSP00000309913,ENSP00000327758,ENSP00000334458	GATA4,NKX2-5,TBX5
GO.0003283	atrial septum development	3	5.15e-06	ENSP00000309913,ENSP00000327758,ENSP00000334458	GATA4,NKX2-5,TBX5
GO.0055002	striated muscle cell development	4	7.11e-06	ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000356286	GATA4,MYH6,NKX2-5,TNNT2
GO.0007507	heart development	5	1.24e-05	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000356286	GATA4,MYH6,NKX2-5,TBX5,TNNT2
GO.0061061	muscle structure development	5	1.3e-05	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000356286	GATA4,MYH6,NKX2-5,TBX5,TNNT2
GO.0045214	sarcomere organization	3	2.71e-05	ENSP00000327758,ENSP00000348634,ENSP00000356286	MYH6,NKX2-5,TNNT2
GO.0048729	tissue morphogenesis	5	3.84e-05	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000356286	GATA4,MYH6,NKX2-5,TBX5,TNNT2
GO.0030049	muscle filament sliding	3	5.16e-05	ENSP00000348634,ENSP00000356286,ENSP00000363071	DES,MYH6,TNNT2
GO.0055010	ventricular cardiac muscle tissue morphogenesis	3	6.34e-05	ENSP00000327758,ENSP00000348634,ENSP00000356286	MYH6,NKX2-5,TNNT2
GO.0060420	regulation of heart growth	3	6.62e-05	ENSP00000327758,ENSP00000334458,ENSP00000348634	GATA4,MYH6,NKX2-5

Enrichment.Process_STRING database

#Pathway ID	Pathway description	Observed gene count	False discovery rate	Matching proteins in your network (IDs)	Matching proteins in your network (labels)
GO.0055013	cardiac muscle cell development	3	7.42e-05	ENSP00000327758,ENSP00000334458,ENSP00000348634	GATA4,MYH6,NKX2-5
GO.0003281	ventricular septum development	3	7.72e-05	ENSP00000309913,ENSP00000327758,ENSP00000334458	GATA4,NKX2-5,TBX5
GO.0060048	cardiac muscle contraction	3	7.8e-05	ENSP00000327758,ENSP00000348634,ENSP00000356286	MYH6,NKX2-5,TNNT2
GO.0003285	septum secundum development	2	8.02e-05	ENSP00000327758,ENSP00000334458	GATA4,NKX2-5
GO.0030239	myofibril assembly	3	9.09e-05	ENSP00000327758,ENSP00000348634,ENSP00000356286	MYH6,NKX2-5,TNNT2
GO.0003012	muscle system process	4	9.7e-05	ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000363071	DES,GATA4,MYH6, NKX2-5
GO.0072358	cardiovascular system development	5	0.000109	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000356286	GATA4,MYH6,NKX2-5,TBX5,TNNT2
GO.0072359	circulatory system development	5	0.000109	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000356286	GATA4,MYH6,NKX2-5,TBX5,TNNT2
GO.0060047	heart contraction	3	0.000112	ENSP00000327758,ENSP00000348634,ENSP00000356286	MYH6,NKX2-5,TNNT2
GO.0003166	bundle of His development	2	0.000129	ENSP00000309913,ENSP00000327758	NKX2-5,TBX5
GO.0003164	His-Purkinje system development	2	0.00019	ENSP00000309913,ENSP00000327758	NKX2-5,TBX5
GO.0048644	muscle organ morphogenesis	3	0.000207	ENSP00000327758,ENSP00000348634,ENSP00000356286	MYH6,NKX2-5,TNNT2
GO.0048646	anatomical structure formation involved in morphogenesis	5	0.000246	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000356286	GATA4,MYH6,NKX2-5,TBX5,TNNT2
GO.0003228	atrial cardiac muscle tissue development	2	0.000247	ENSP00000348634,ENSP00000356286	MYH6,TNNT2
GO.0030509	BMP signaling pathway	3	0.000247	ENSP00000327758,ENSP00000334458,ENSP00000348634	GATA4,MYH6,NKX2-5
GO.0055009	atrial cardiac muscle tissue morphogenesis	2	0.000247	ENSP00000348634,ENSP00000356286	MYH6,TNNT2
GO.0071773	cellular response to BMP stimulus	3	0.000294	ENSP00000327758,ENSP00000334458,ENSP00000348634	GATA4,MYH6,NKX2-5
GO.0060070	canonical Wnt signaling pathway	3	0.0003	ENSP00000327758,ENSP00000334458,ENSP00000348634	GATA4,MYH6,NKX2-5
GO.0060290	transdifferentiation	2	0.000432	ENSP00000309913,ENSP00000334458	GATA4,TBX5
GO.0044057	regulation of system process	4	0.000449	ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000363071	DES,GATA4,MYH6, NKX2-5
GO.0003211	cardiac ventricle formation	2	0.000663	ENSP00000309913,ENSP00000327758	NKX2-5,TBX5
GO.0007512	adult heart development	2	0.0013	ENSP00000327758,ENSP00000348634	MYH6,NKX2-5
GO.0003181	atrioventricular valve morphogenesis	2	0.00148	ENSP00000309913,ENSP00000334458	GATA4,TBX5
GO.0006937	regulation of muscle contraction	3	0.00154	ENSP00000327758,ENSP00000334458,ENSP00000356286	GATA4,NKX2-5,TNNT2
GO.0003215	cardiac right ventricle morphogenesis	2	0.00164	ENSP00000327758,ENSP00000334458	GATA4,NKX2-5
GO.0003171	atrioventricular valve development	2	0.00204	ENSP00000309913,ENSP00000334458	GATA4,TBX5
GO.0060485	mesenchyme development	3	0.00204	ENSP00000309913,ENSP00000327758,ENSP00000334458	GATA4,NKX2-5,TBX5

Enrichment.Process_STRING database

#Pathway ID	Pathway description	Observed gene count	False discovery rate	Matching proteins in your network (IDs)	Matching proteins in your network (labels)
GO.0060045	positive regulation of cardiac muscle cell proliferation	2	0.00225	ENSP00000309913,ENSP00000334458	GATA4,TBX5
GO.0055017	cardiac muscle tissue growth	2	0.00292	ENSP00000327758,ENSP00000334458	GATA4,NKX2-5
GO.0006936	muscle contraction	3	0.00315	ENSP00000327758,ENSP00000348634,ENSP00000363071	DES,MYH6,NKX2-5
GO.0060419	heart growth	2	0.00315	ENSP00000327758,ENSP00000334458	GATA4,NKX2-5
GO.0007010	cytoskeleton organization	4	0.00422	ENSP00000327758,ENSP00000348634,ENSP00000356286,ENSP00000363071	DES,MYH6,NKX2-5, TNNT2
GO.0003197	endocardial cushion development	2	0.00423	ENSP00000309913,ENSP00000334458	GATA4,TBX5
GO.0060043	regulation of cardiac muscle cell proliferation	2	0.00449	ENSP00000327758,ENSP00000334458	GATA4,NKX2-5
GO.0007517	muscle organ development	3	0.00634	ENSP00000327758,ENSP00000348634,ENSP00000356286	MYH6,NKX2-5,TNNT2
GO.0008015	blood circulation	3	0.00641	ENSP00000327758,ENSP00000348634,ENSP00000356286	MYH6,NKX2-5,TNNT2
GO.0009790	embryo development	4	0.00679	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634	GATA4,MYH6,NKX2-5, TBX5
GO.0051239	regulation of multicellular organismal process	5	0.00935	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000363071	DES,GATA4,MYH6, NKX2-5,TBX5
GO.1903115	regulation of actin filament-based movement	2	0.0102	ENSP00000334458,ENSP00000356286	GATA4,TNNT2
GO.0043462	regulation of ATPase activity	2	0.0115	ENSP00000348634,ENSP00000356286	MYH6,TNNT2
GO.0001947	heart looping	2	0.0128	ENSP00000327758,ENSP00000334458	GATA4,NKX2-5
GO.0061371	determination of heart left/right asymmetry	2	0.014	ENSP00000327758,ENSP00000334458	GATA4,NKX2-5
GO.0030029	actin filament-based process	3	0.0169	ENSP00000327758,ENSP00000348634,ENSP00000363071	DES,MYH6,NKX2-5
GO.0007389	pattern specification process	3	0.0171	ENSP00000309913,ENSP00000327758,ENSP00000334458	GATA4,NKX2-5,TBX5
GO.0001570	vasculogenesis	2	0.0179	ENSP00000309913,ENSP00000327758	NKX2-5,TBX5
GO.0002009	morphogenesis of an epithelium	3	0.0193	ENSP00000309913,ENSP00000327758,ENSP00000334458	GATA4,NKX2-5,TBX5
GO.0006928	movement of cell or subcellular component	4	0.0197	ENSP00000309913,ENSP00000348634,ENSP00000356286,ENSP00000363071	DES,MYH6,TBX5, TNNT2
GO.0044707	single-multicellular organism process	6	0.0208	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000356286,ENSP00000363071	DES,GATA4,MYH6, NKX2-5, TBX5,TNNT2
GO.0035050	embryonic heart tube development	2	0.021	ENSP00000327758,ENSP00000334458	GATA4,NKX2-5
GO.0055117	regulation of cardiac muscle contraction	2	0.0251	ENSP00000327758,ENSP00000334458	GATA4,NKX2-5
GO.2000026	regulation of multicellular organismal development	4	0.0328	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634	GATA4,MYH6,NKX2-5, TBX5
GO.0009888	tissue development	4	0.0341	ENSP00000327758,ENSP00000334458,ENSP00000348634,ENSP00000356286	GATA4,MYH6,NKX2-5, TNNT2
GO.0048598	embryonic morphogenesis	3	0.0356	ENSP00000309913,ENSP00000327758,ENSP00000334458	GATA4,NKX2-5,TBX5
GO.0035295	tube development	3	0.0376	ENSP00000309913,ENSP00000327758,ENSP00000334458	GATA4,NKX2-5,TBX5
GO.0006357	regulation of transcription from RNA polymerase II promoter	4	0.0418	ENSP00000309913,ENSP00000327758,ENSP00000334458,ENSP00000348634	GATA4,MYH6,NKX2-5, TBX5
GO.0043009	chordate embryonic development	3	0.044	ENSP00000327758,ENSP00000334458,ENSP00000348634	GATA4,MYH6,NKX2-5

Enrichment.KEGG_STRING database

#Pathway ID	Pathway description	Observed gene count	False discovery rate	Matching proteins in your network (IDs)	Matching proteins in your network (labels)
5410	Hypertrophic cardiomyopathy (HCM)	3	0.000219	ENSP00000348634,ENSP00000356286,ENSP0000363071	DES,MYH6,TNNT2
5414	Dilated cardiomyopathy	3	0.000219	ENSP00000348634,ENSP00000356286,ENSP0000363071	DES,MYH6,TNNT2
4260	Cardiac muscle contraction	2	0.0189	ENSP00000348634,ENSP00000356286	MYH6,TNNT2
4919	Thyroid hormone signaling pathway	2	0.0332	ENSP00000334458,ENSP00000348634	GATA4,MYH6
4261	Adrenergic signaling in cardiomyocytes	2	0.0399	ENSP00000348634,ENSP00000356286	MYH6,TNNT2

Enrichment.Component_STRING database

#Pathway ID	Pathway description	Observed gene count	False discovery rate	Matching proteins in your network (IDs)	Matching proteins in your network (labels)
GO.0030016	myofibril	3	0.0166	ENSP00000348634,ENSP00000356286,ENSP00000363071	DES,MYH6,TNNT2