Islet β MPS: background & future applications

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Postdoctoral scholar
Healy lab

Contents

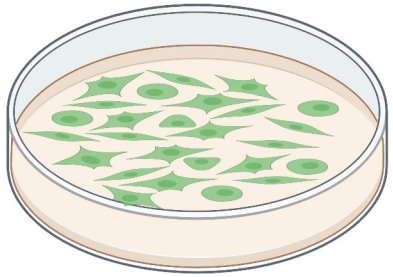
- Microphysiological systems (MPS) in the context of metabolism
- Diabetes-on-chip
  - What goes wrong in diabetes?
  - Interactions of tissues in MPS
  - Challenges in integration of MPS-based tissues
- Islets
- Traditional tools & techniques
- Islet β MPS
- Future application

Why microphysiological systems?

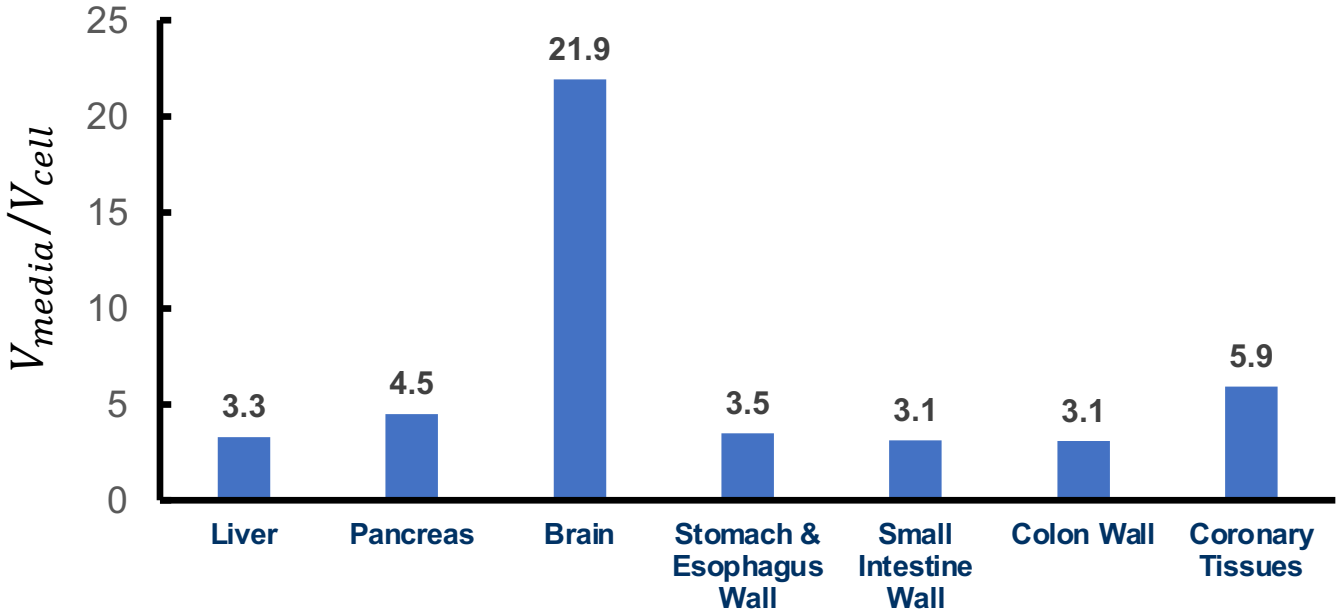
Approximate calculation of culture media volume to cell/tissue volume

In vitro

$$\frac{V_{media}}{V_{cell}} \approx 1000$$

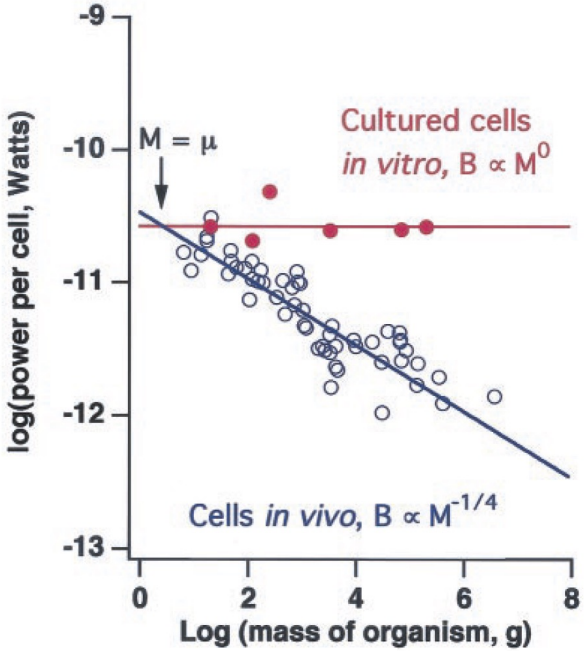


In vivo

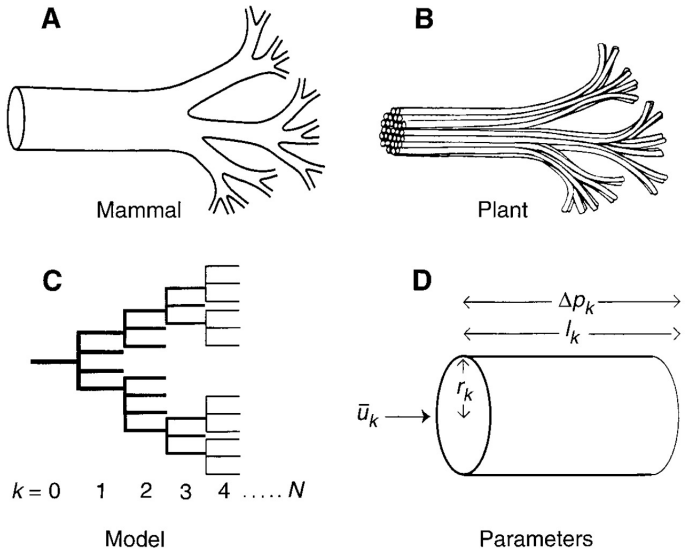


Data source: Wayson et al. Phys Med Biol 2019

Why microphysiological systems?



West et al. PNAS 2002

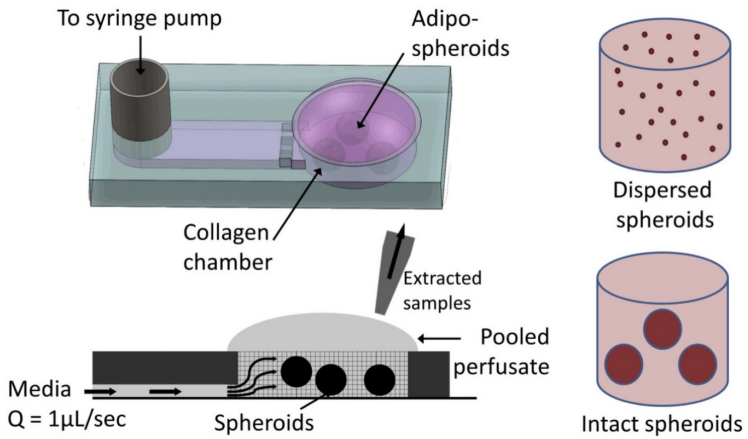


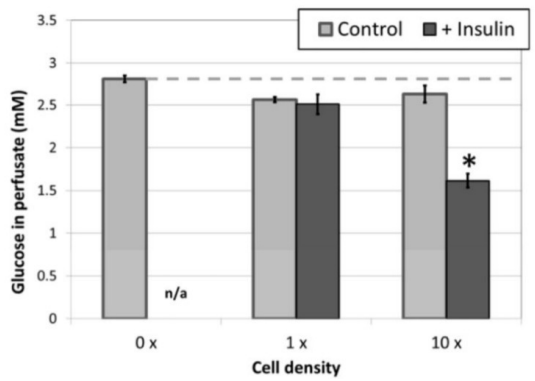
Cross over point

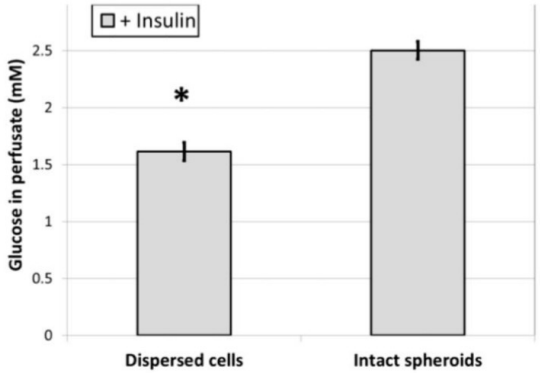
$$r^2/l \approx 8\nu/\rho c_0$$

West et al. Science 1997

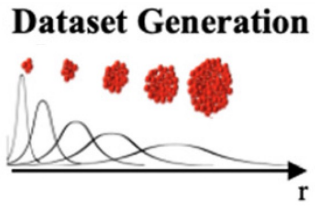
Why microphysiological systems?

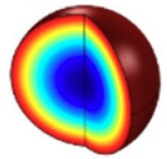


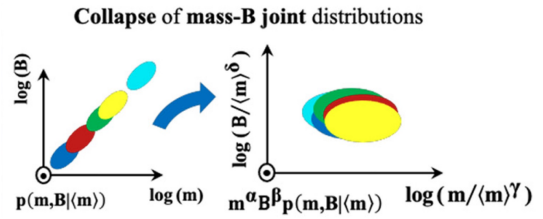


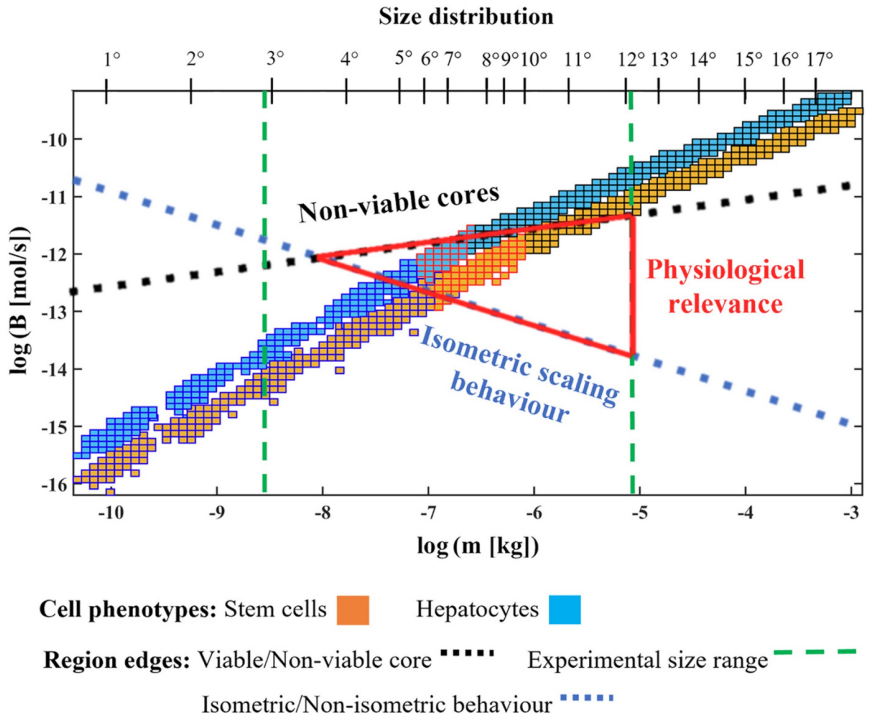


Moraes et al. Intrg. Biol. 2013





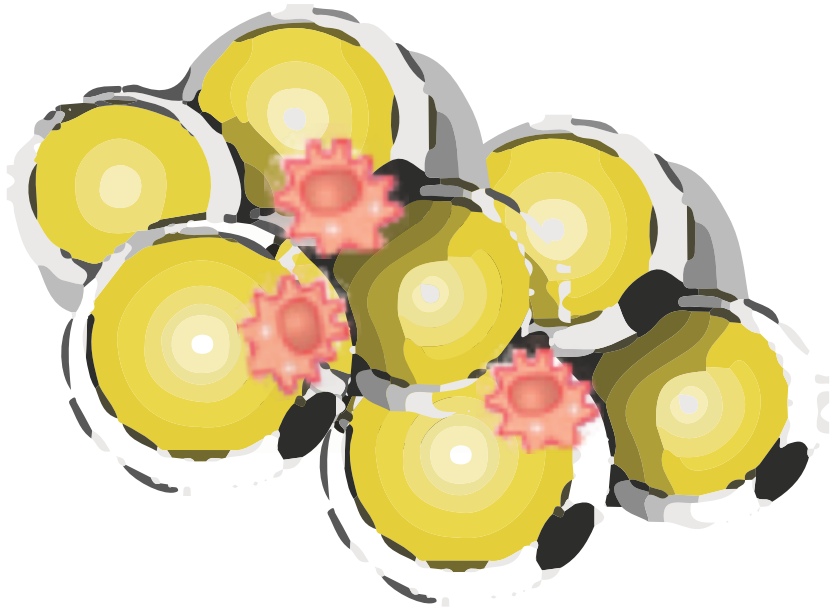




Botte et al. PNAS 2021

Obesity/Diabetes: What goes wrong

Adipocyte Hypertrophy

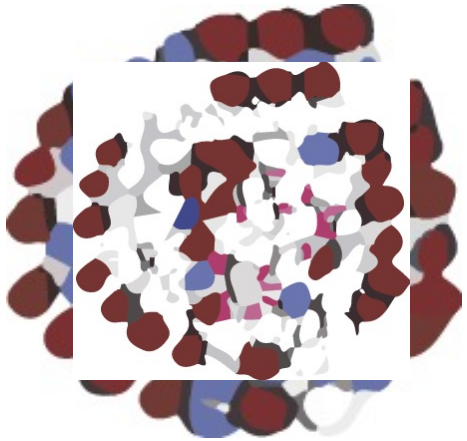


Macrophage Recruitment
Change in Polarization
Insulin Resistance
Enhanced Fatty Acid Release

Hepatic Steatosis

Kupffer Cell Activation
Steatohepatitis
Insulin Resistance
Enhanced Glucose Release

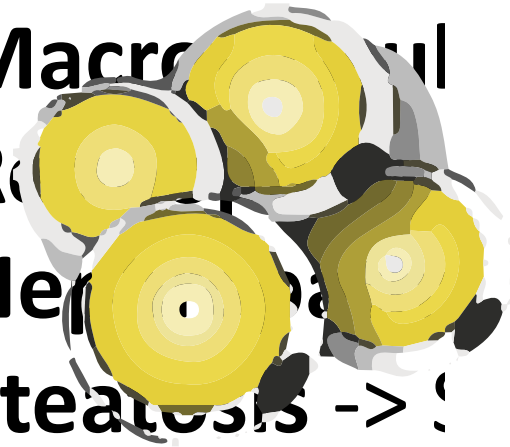
ß-Cell Death
ß-Cell Expansion

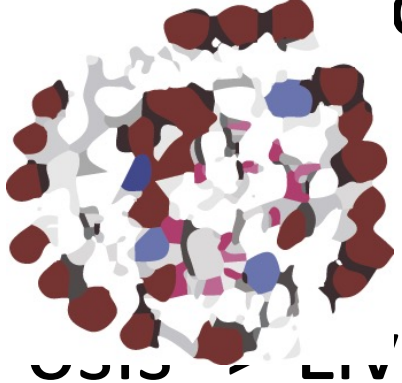


Loss of Glycemic Control
Overt Diabetes

Obesity/Diabetes: What goes wrong

Fat *Liver* *Islets*





Macr[illegible]**ul**

R[illegible]

Ne[illegible]

Steatosis -> [illegible]

Failure/Hepatocarcinoma

[illegible]ttacks/Strokes

[illegible]Liver

Peripheral Neuropath -> Necrosis/Gang Green -> Amputations

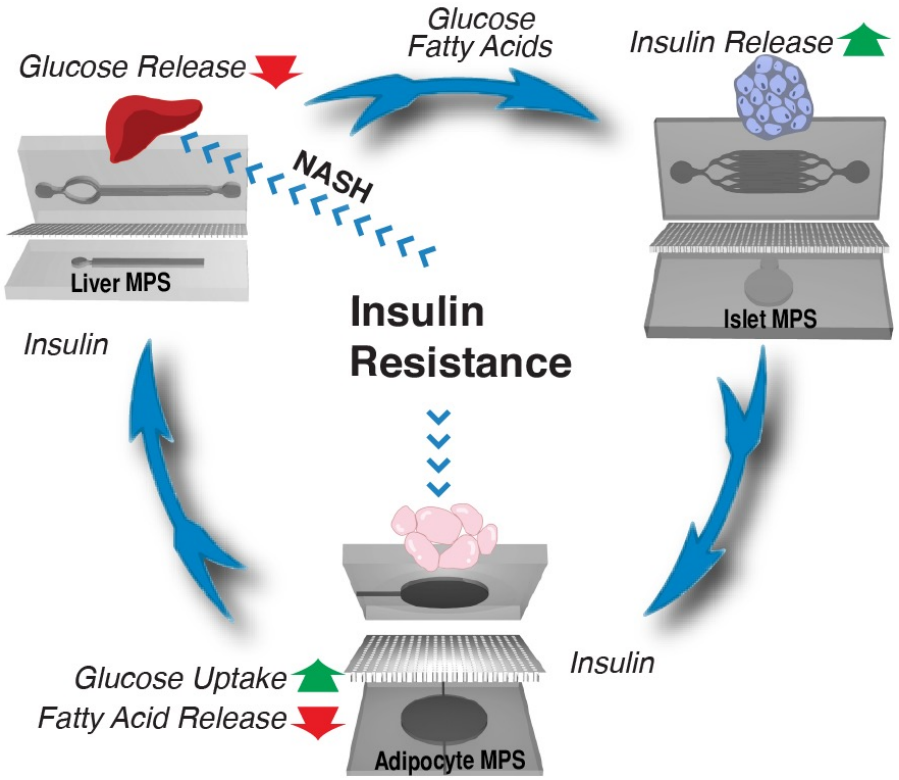
Cognitive Dysfunction -> Alzheimer's Disease/Neurodegeneration

+ + = Diabetes

Hyperglycemia + Hyperlipidemia = Chronic disease -> Mortality

Diabetes-on-Chip

Interrogation of tissue interaction in MPS



Team

Principal Investigators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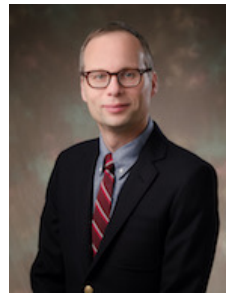
Kevin E. Healy


Matthias Hebrok


Edward Hsiao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Trainees

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Marko Groeger (Willenbring)

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Aditi Sharma (Hsiao)

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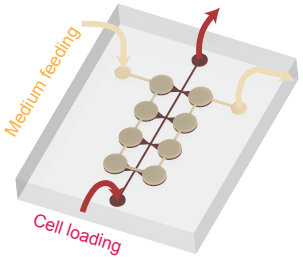
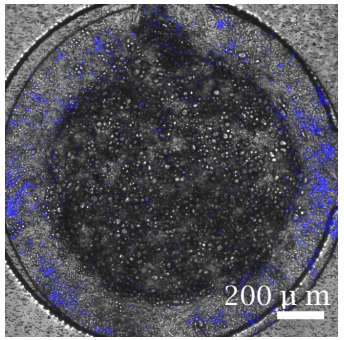
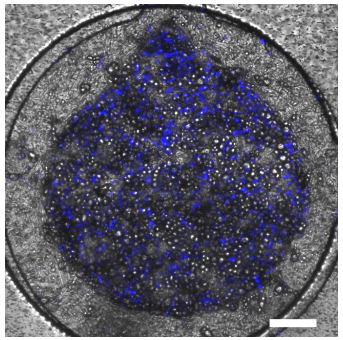
Koji Matsuo (Hsiao)

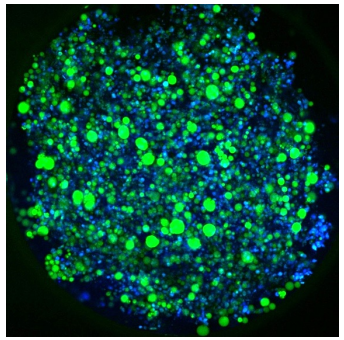
Interconnection of MPS

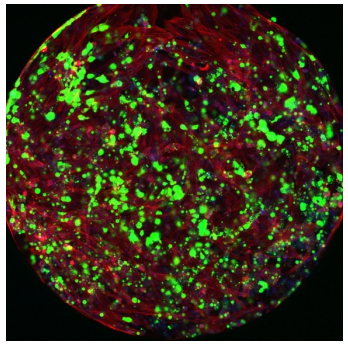
Fat MPS







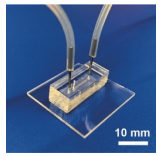


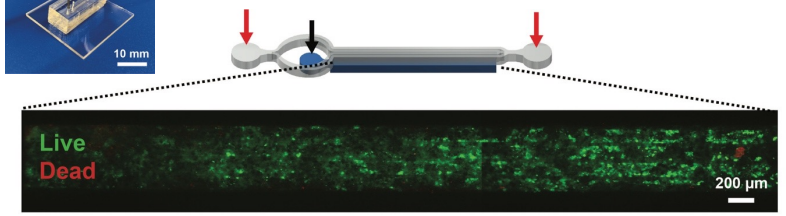


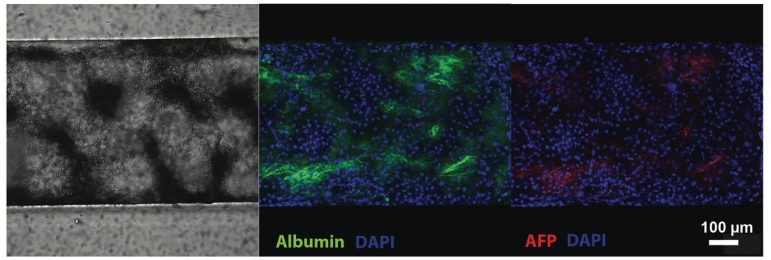
Qi et al., *Small 2022*

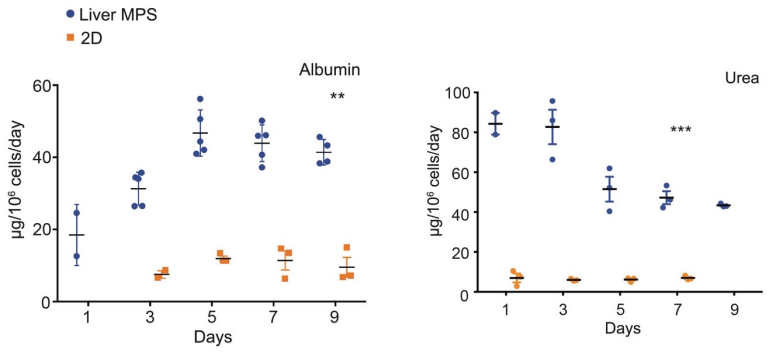
Loskill et al., *Lab Chip.*, 2017

Liver MPS



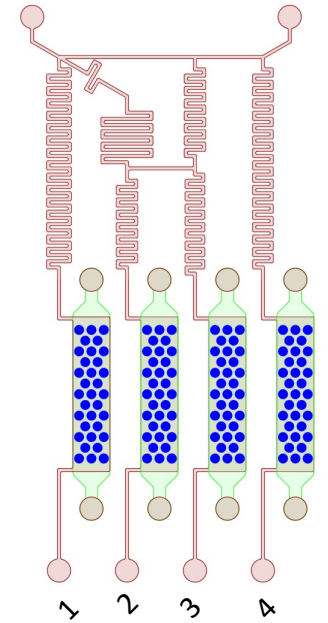


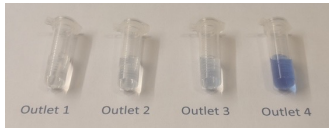


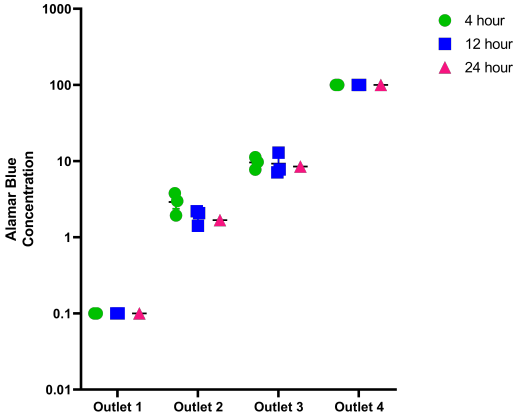


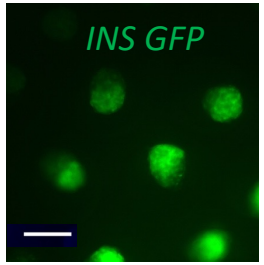
Lee-Montiel et al., *Front. Pharmacol.*, 2021

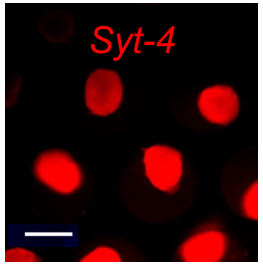
Islet MPS

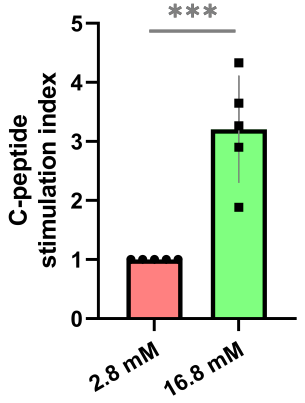






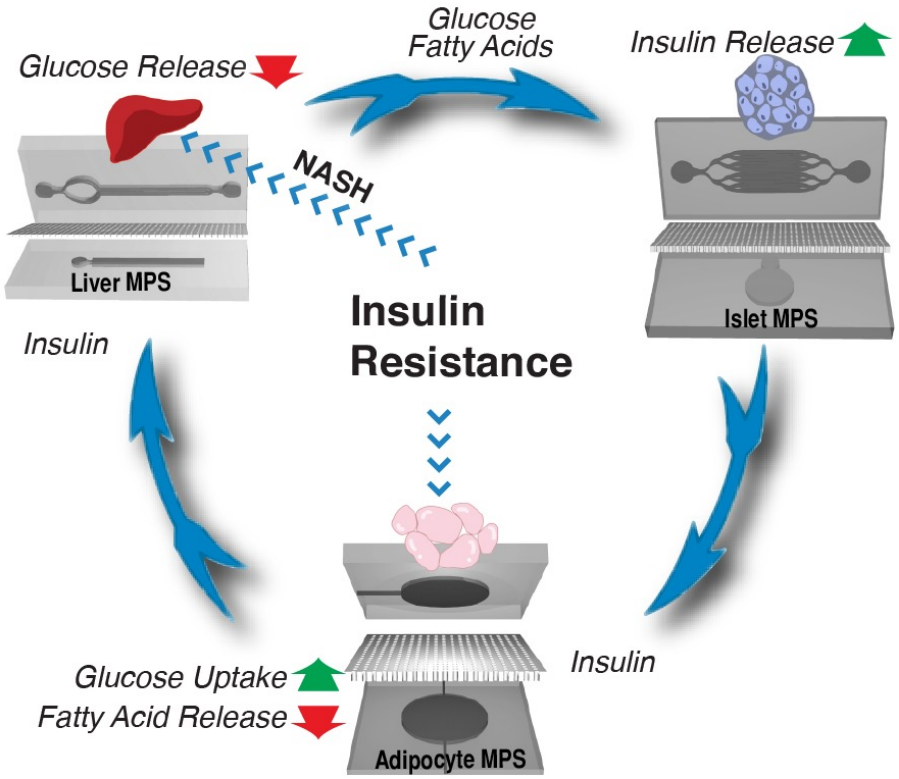


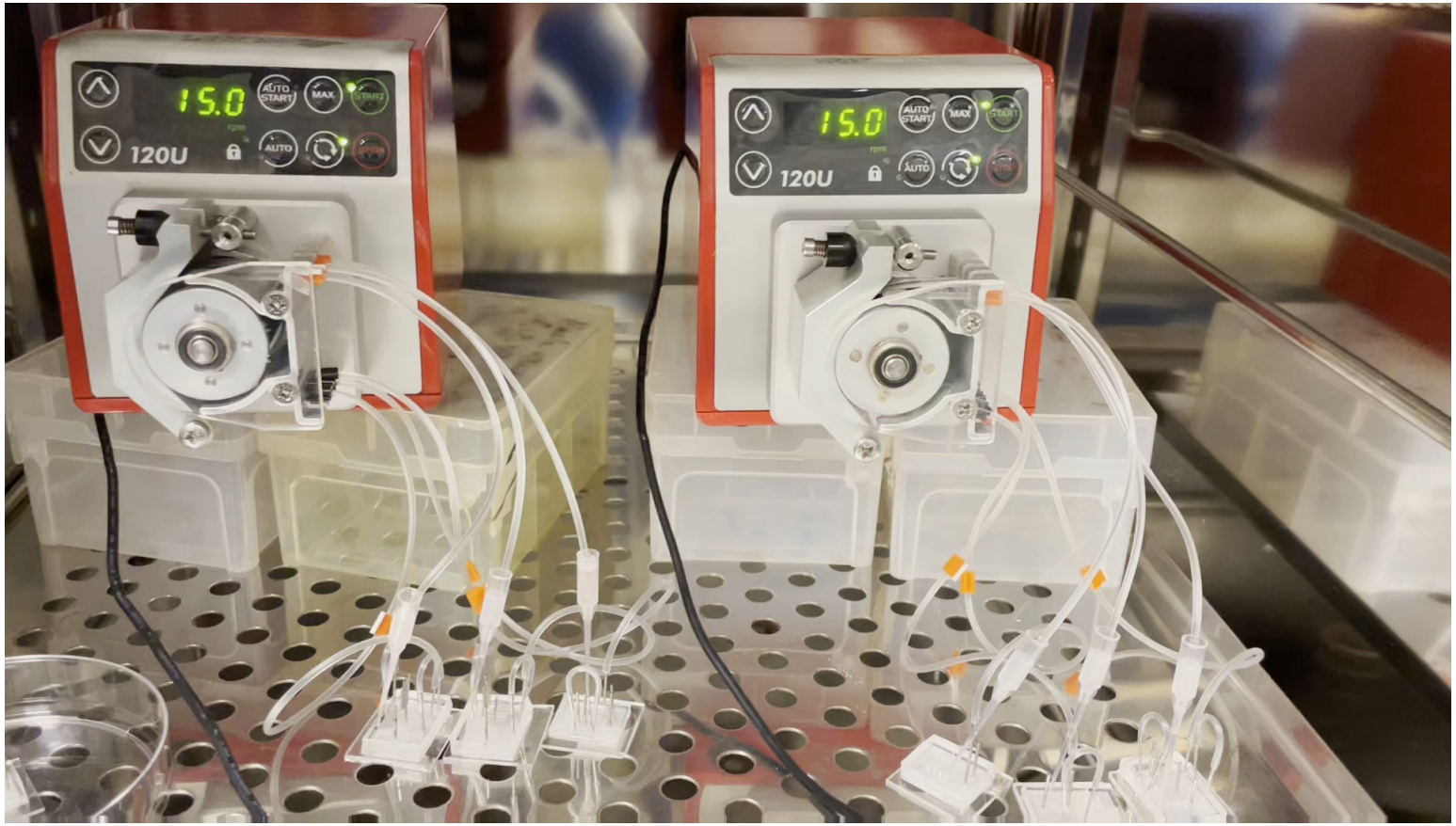




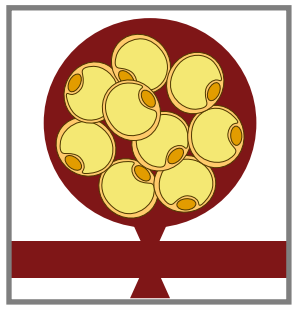
Goswami et al., *In Review*

Interrogation of tissue interaction in MPS

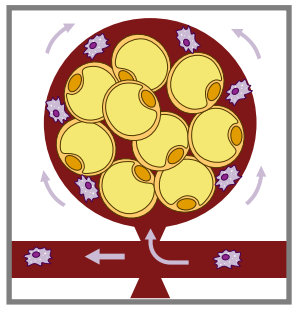




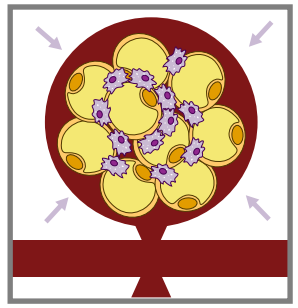
Interconnection of MPS: sample outputs



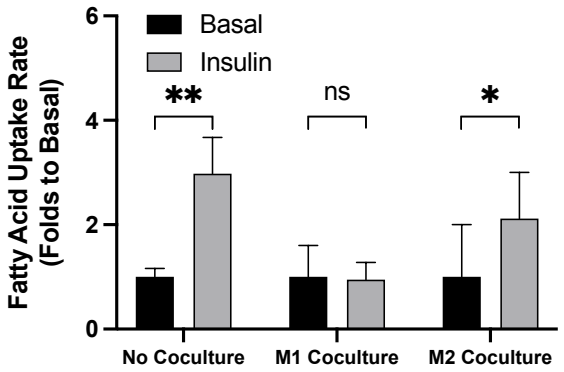
Mature A-MPS

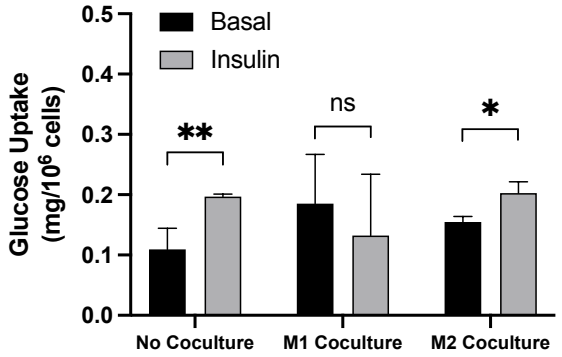


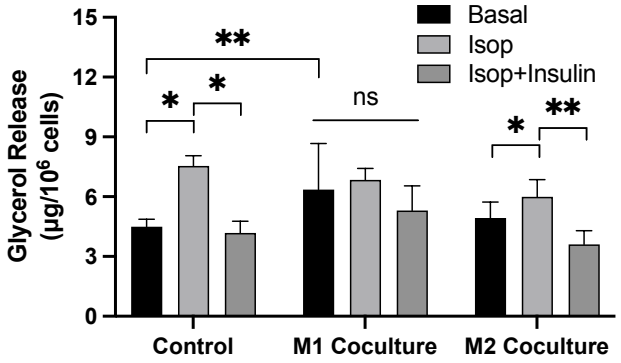
Load M in A-MPS

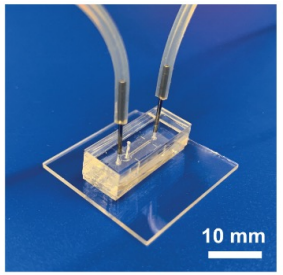


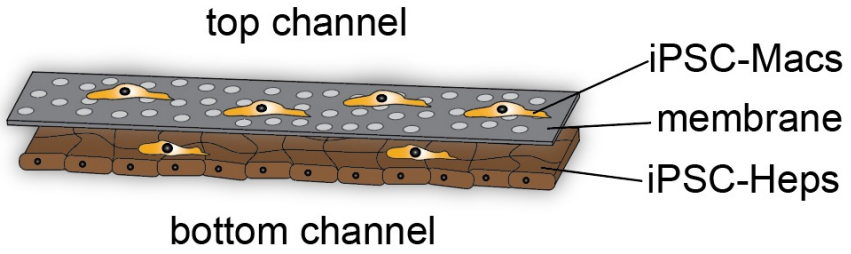
M migrate, form CLSs, interact with A in MPS

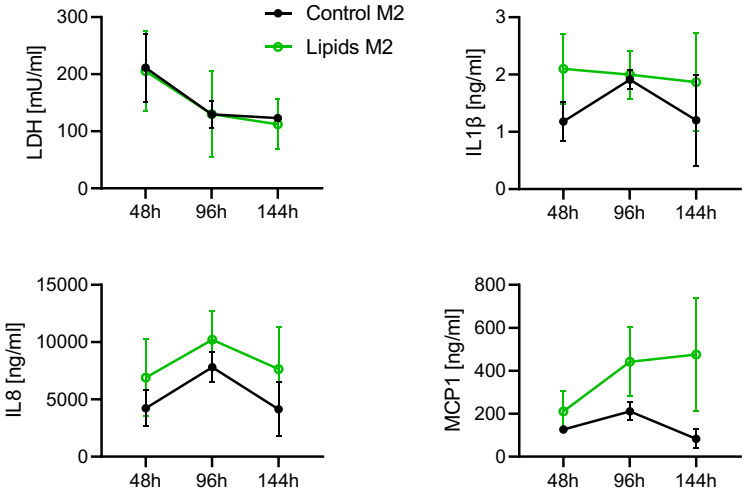






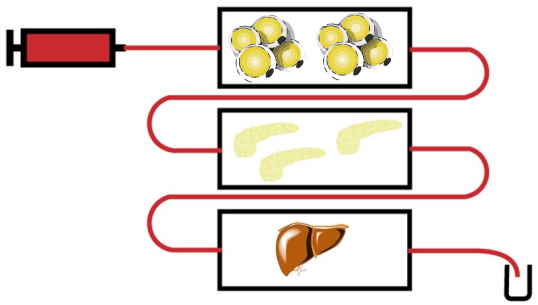


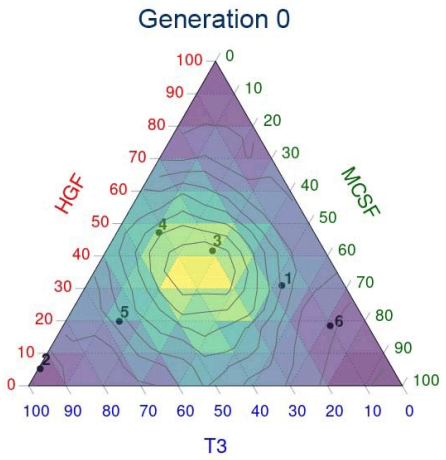




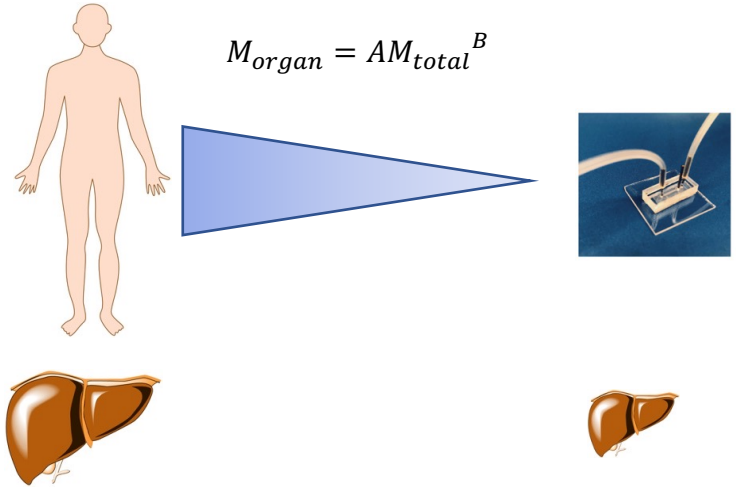
Challenges in interconnection

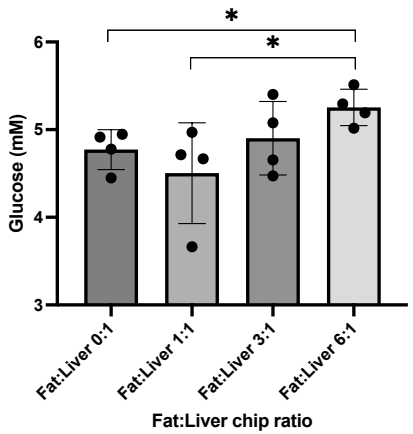
Common media





Scaling of tissues

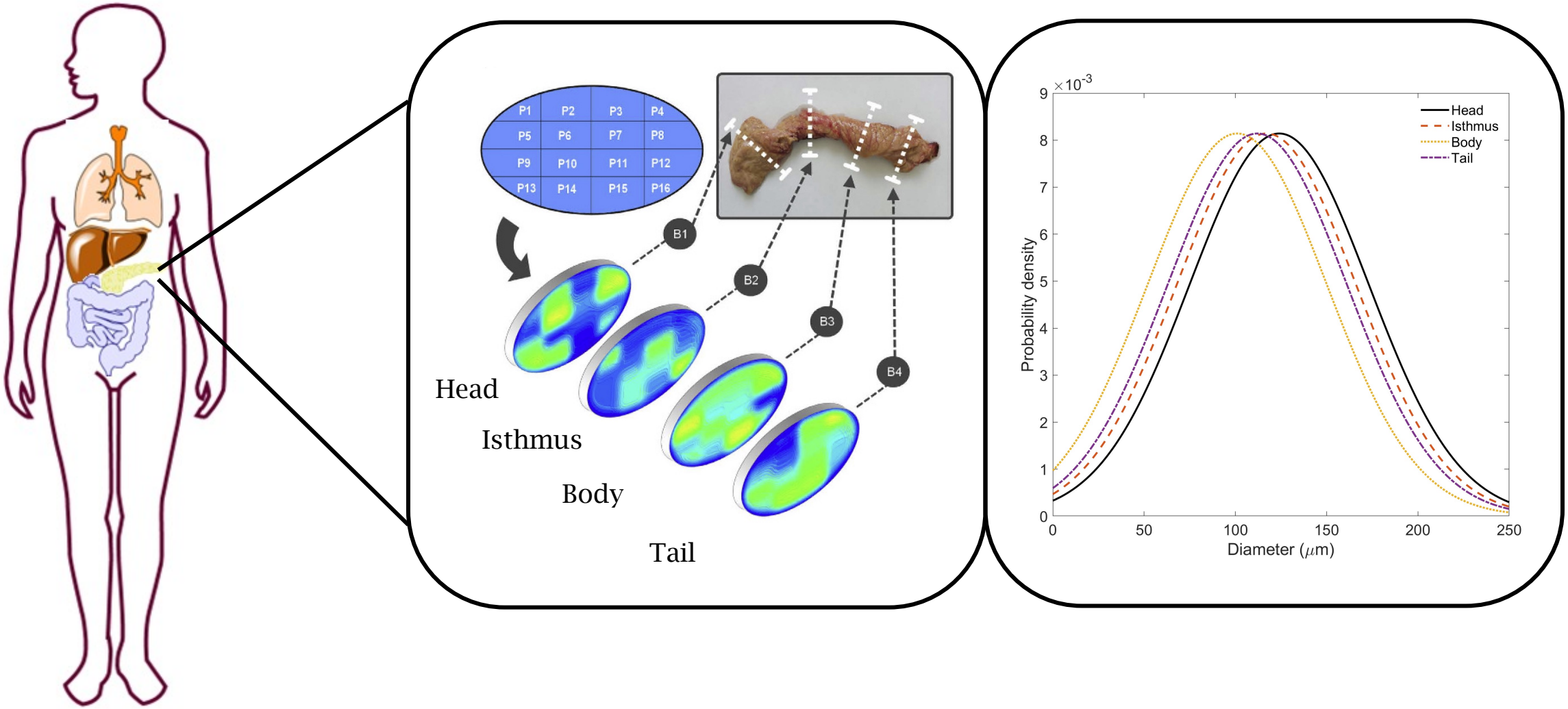




Reliability of system

Fat MPS	Liver MPS	Islet MPS
Fat MPS	Liver MPS	Islet MPS
Fat MPS	Liver MPS	Islet MPS
Fat MPS	Liver MPS	Islet MPS
Fat MPS	Liver MPS	Islet MPS
Fat MPS	Liver MPS	Islet MPS

Islet



Adapted from: Ionescu-Tirgoviste et al. Sci. Rep. 2015

Interspecies differences in islet features

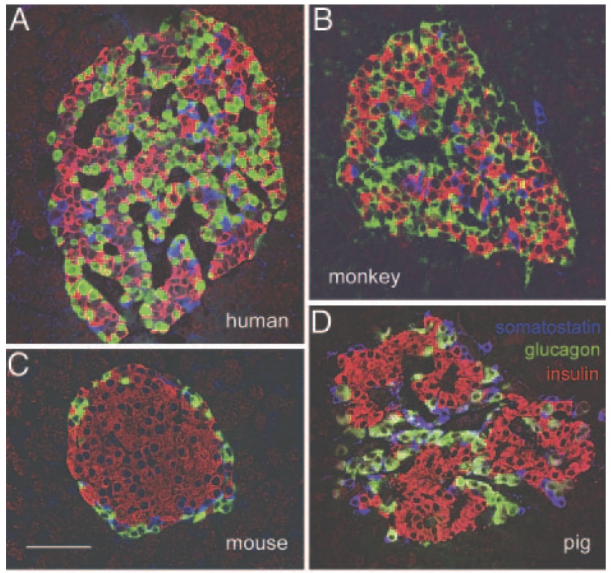
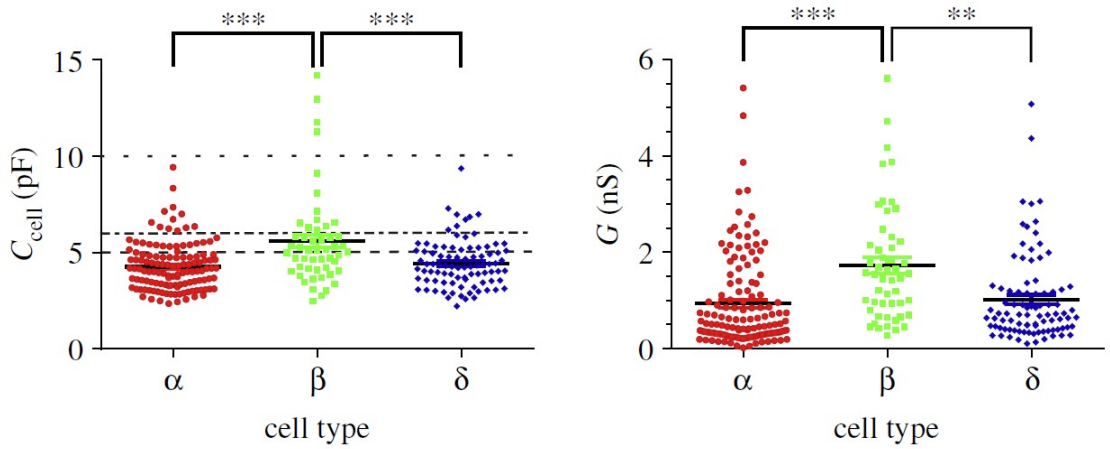
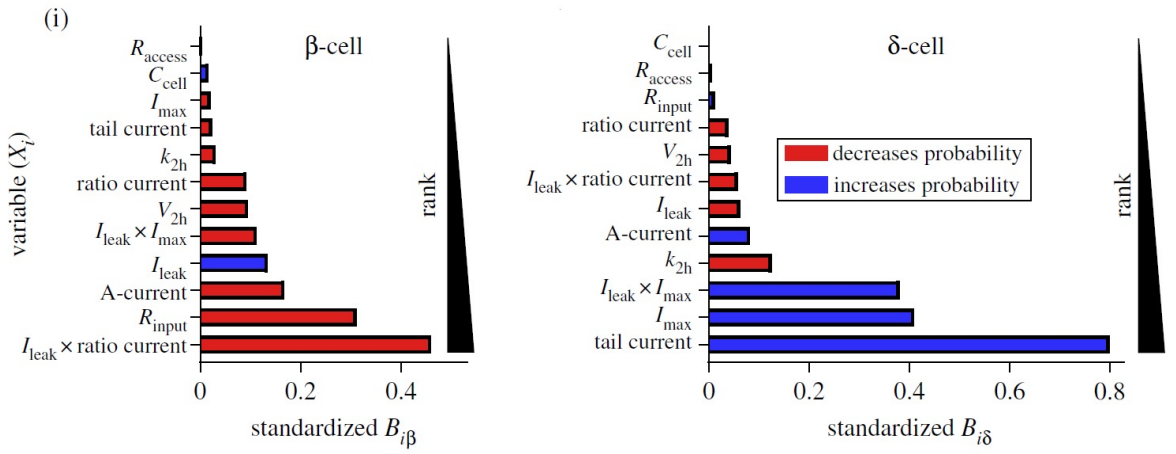


Table 1. Homotypic and heterotypic contacts of cells within the islet

	Mouse		Human	
Cell type	Heterotypic, %	Homotypic, %	Heterotypic, %	Homotypic, %
β cells	28 ± 6	71 ± 4	71 ± 5	29 ± 5
α cells	98 ± 1	2 ± 1	90 ± 3	10 ± 3

Carbera et al. PNAS 2006





Briant et al. J. R. Soc. Interface 2017

Exocytosis of insulin granules

Glucose

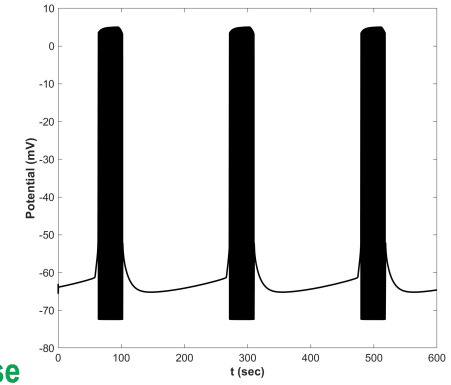
K^+

Na^+

K^+

Ca^{2+}

Glucokinase



Glucose-6-Phosphate

Phosphoglucose isomerase

Fructose-6-Phosphate

Phosphofructo kinase

Fructose-1,6-Bisphosphate

Glyceraldehyde 3-P dehydrogenase

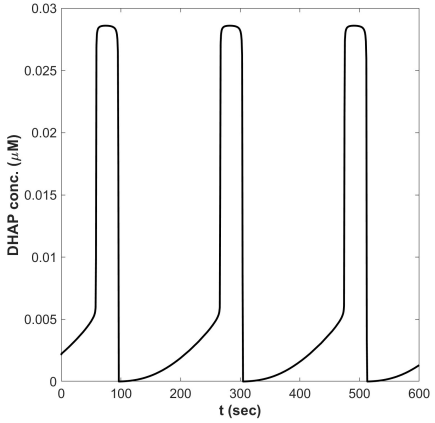
Pyruvate

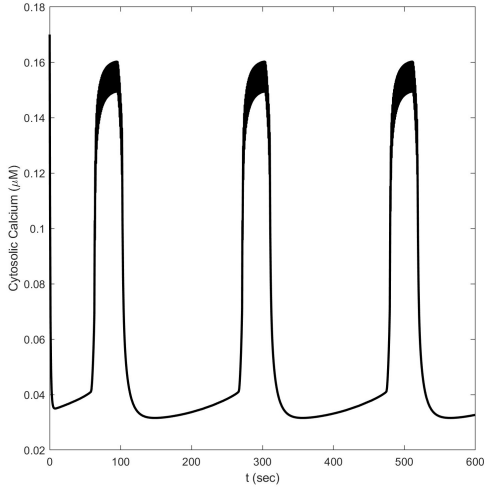
ATP:ADP

Ca^{2+}

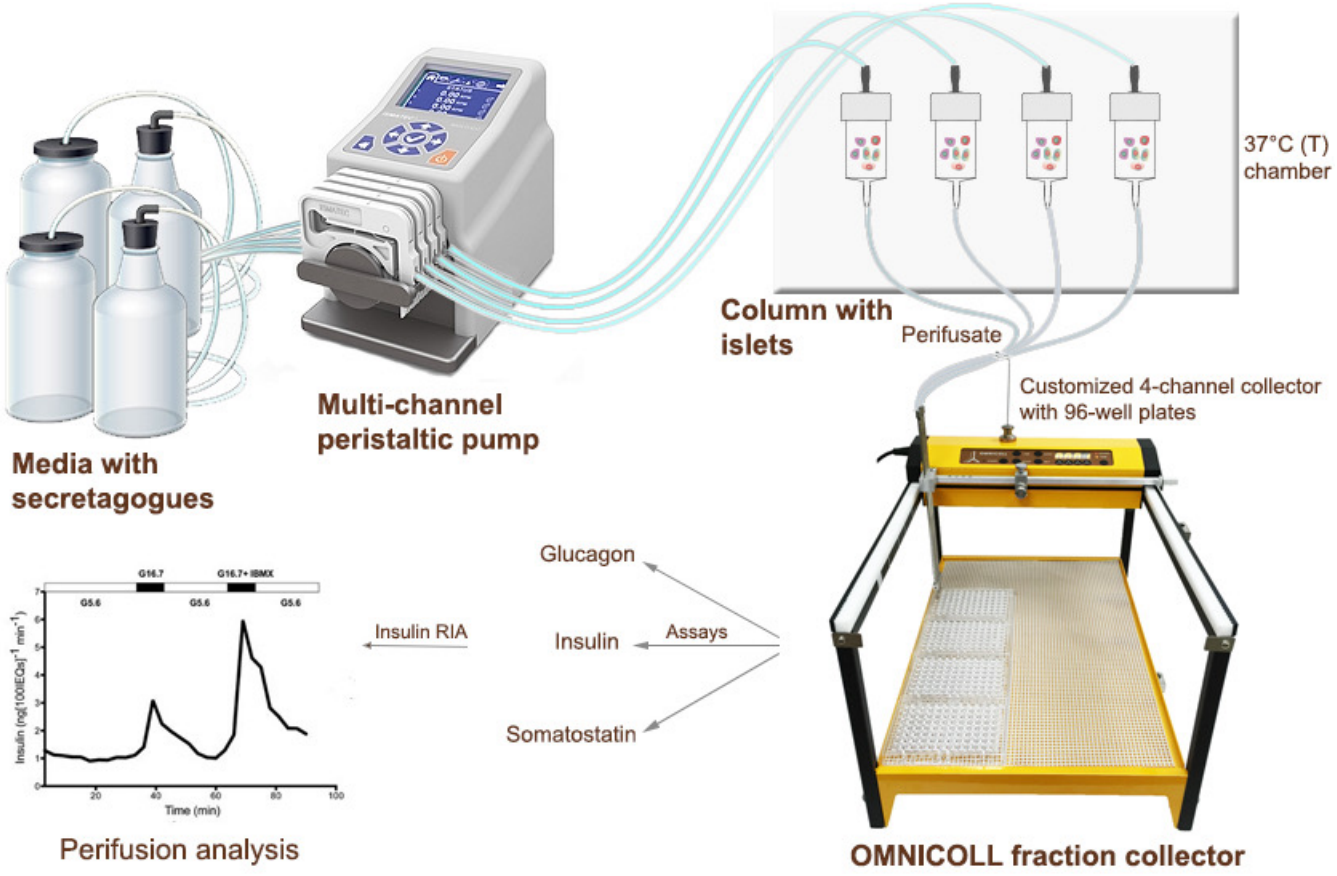
ATP:ADP

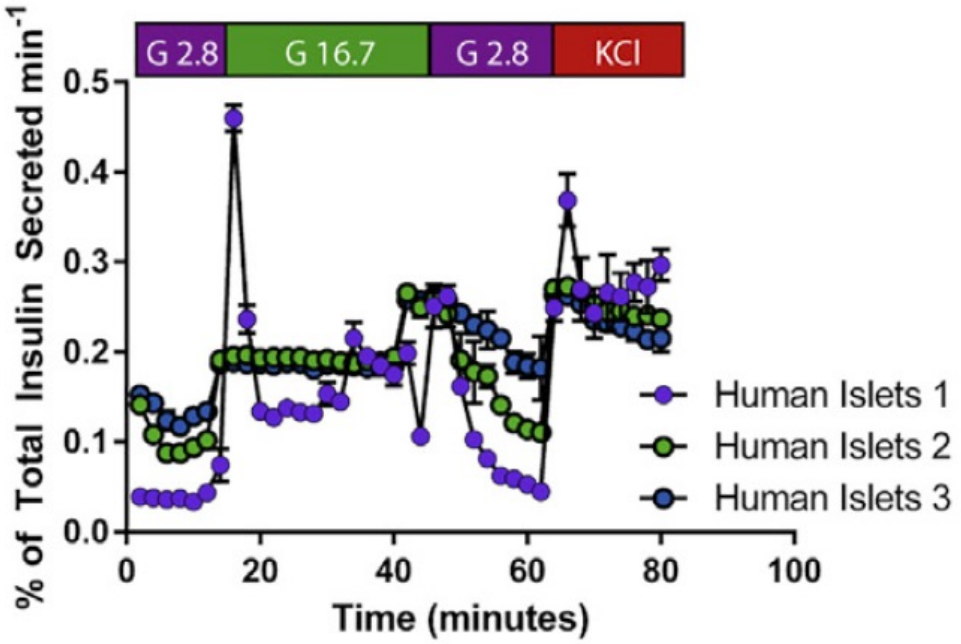
Vesicles with insulin granules





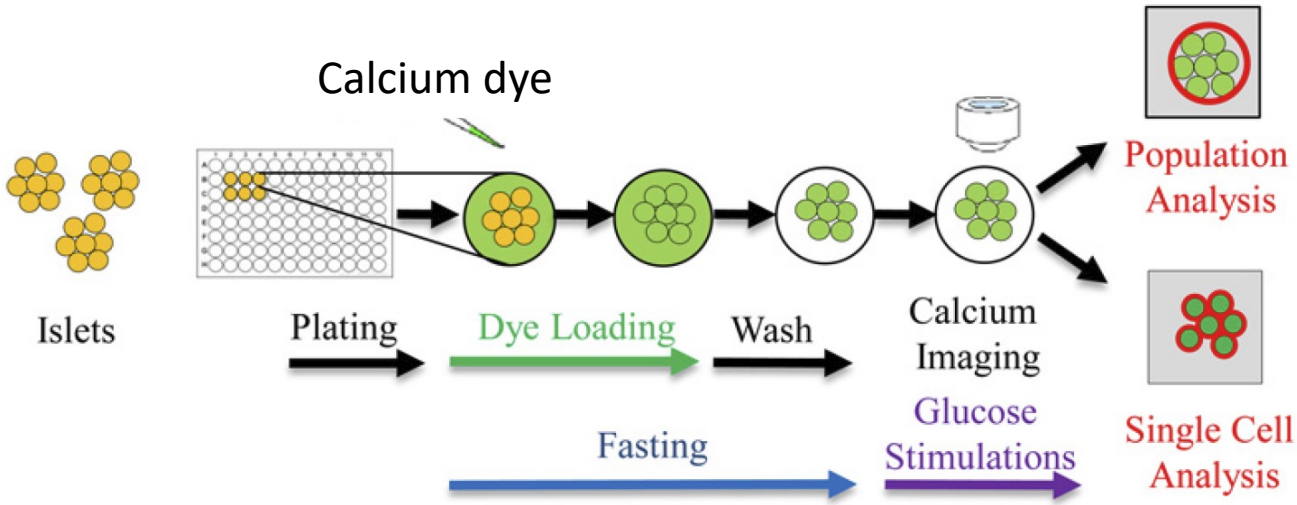
Gold standard protocols for islet functionality

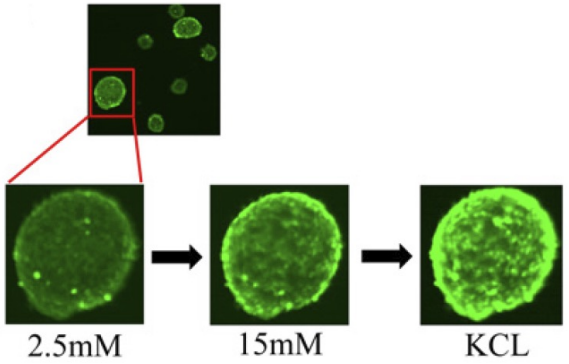


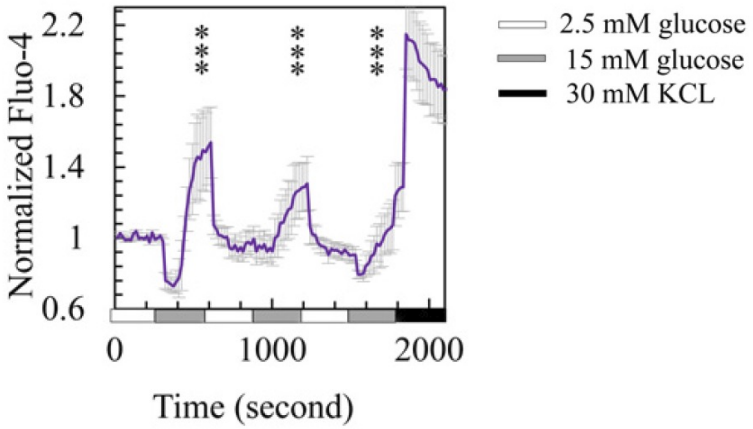


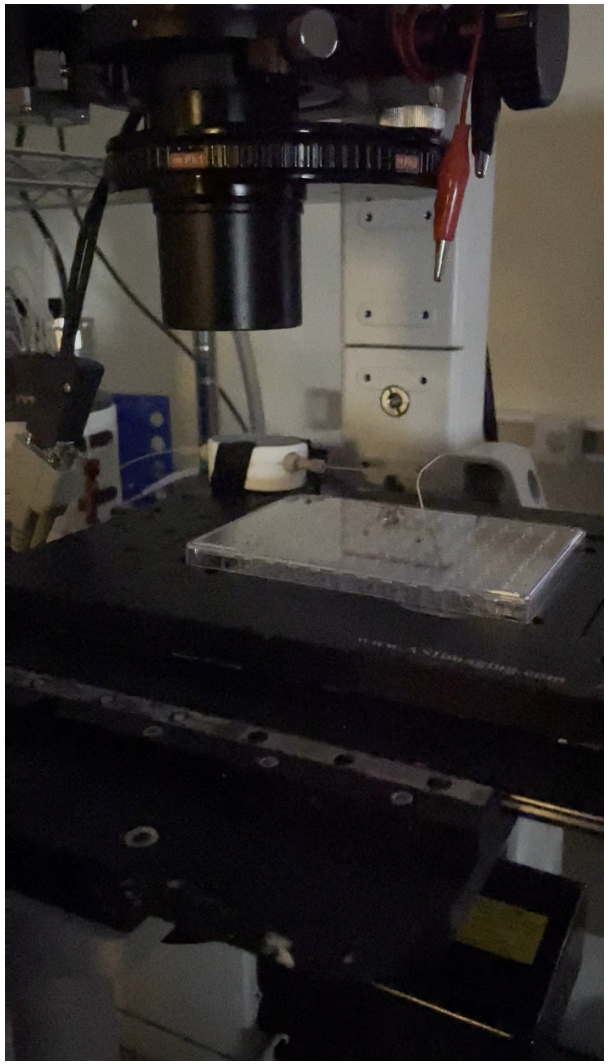
Adapted from: Davis et al. Cell Rep. 2020

Gold standard protocols for islet functionality



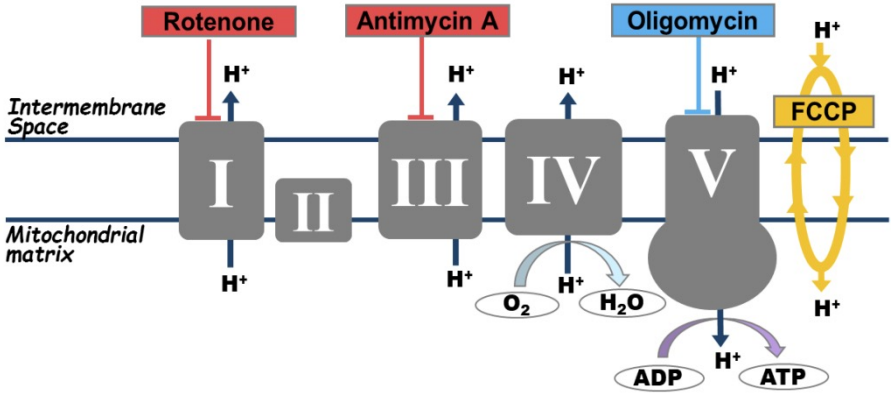


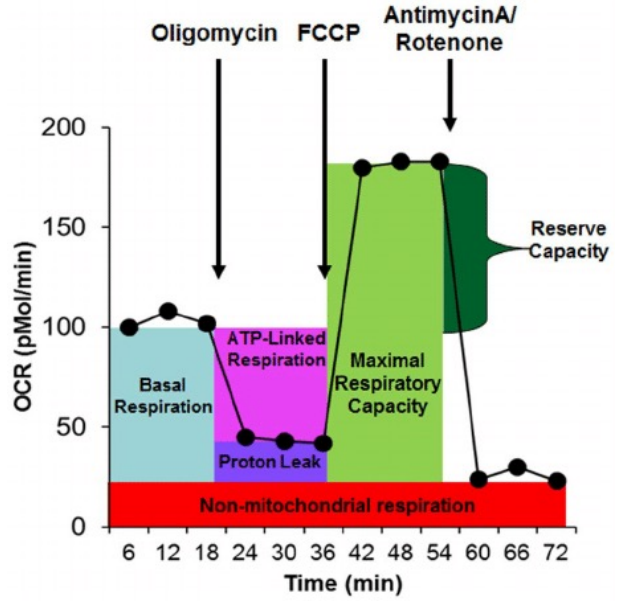


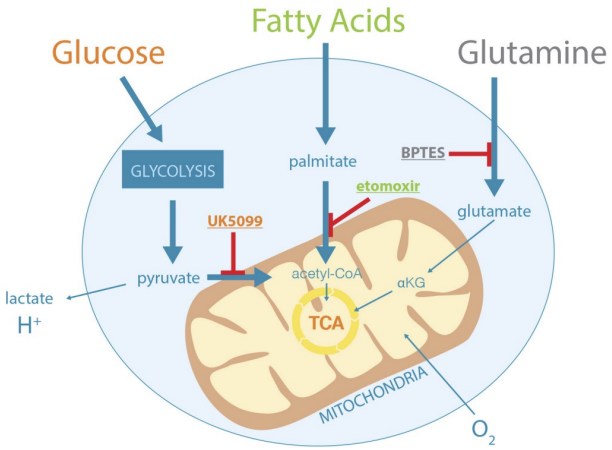


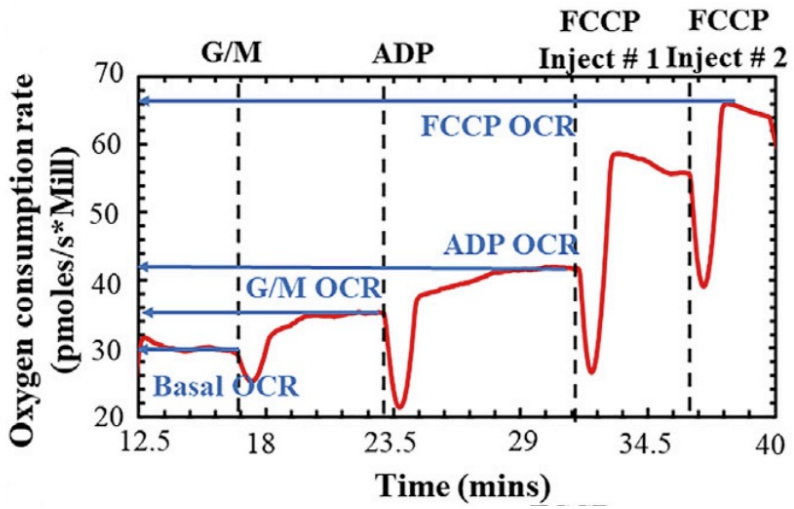
Adapted from: Kenty et al. PLOS One 2015

Gold standard protocols for islet functionality





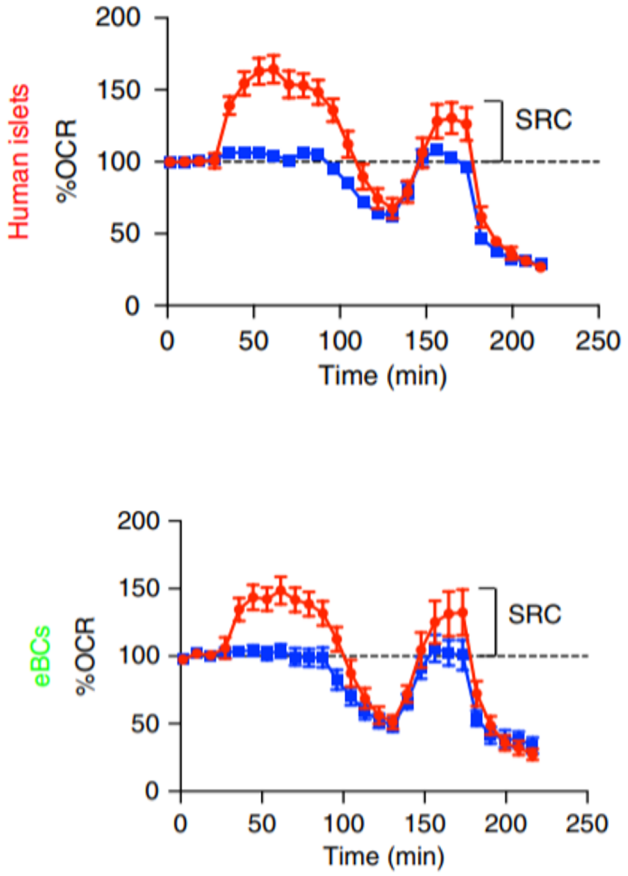


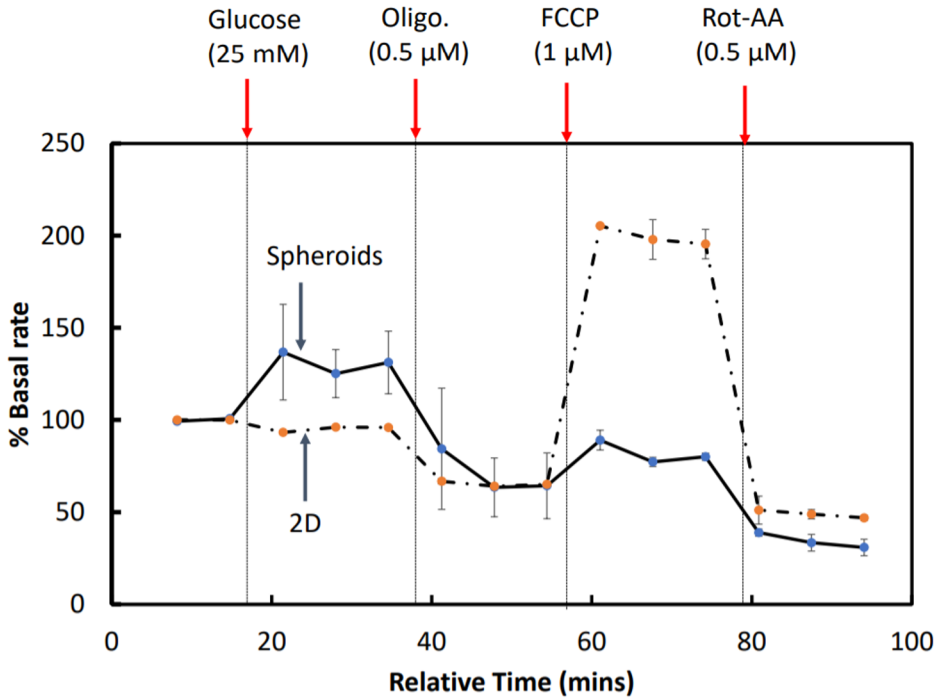


Source: Agilent manual

Goswami et al. Biophysical J. 2018

Gold standard protocols for islet functionality

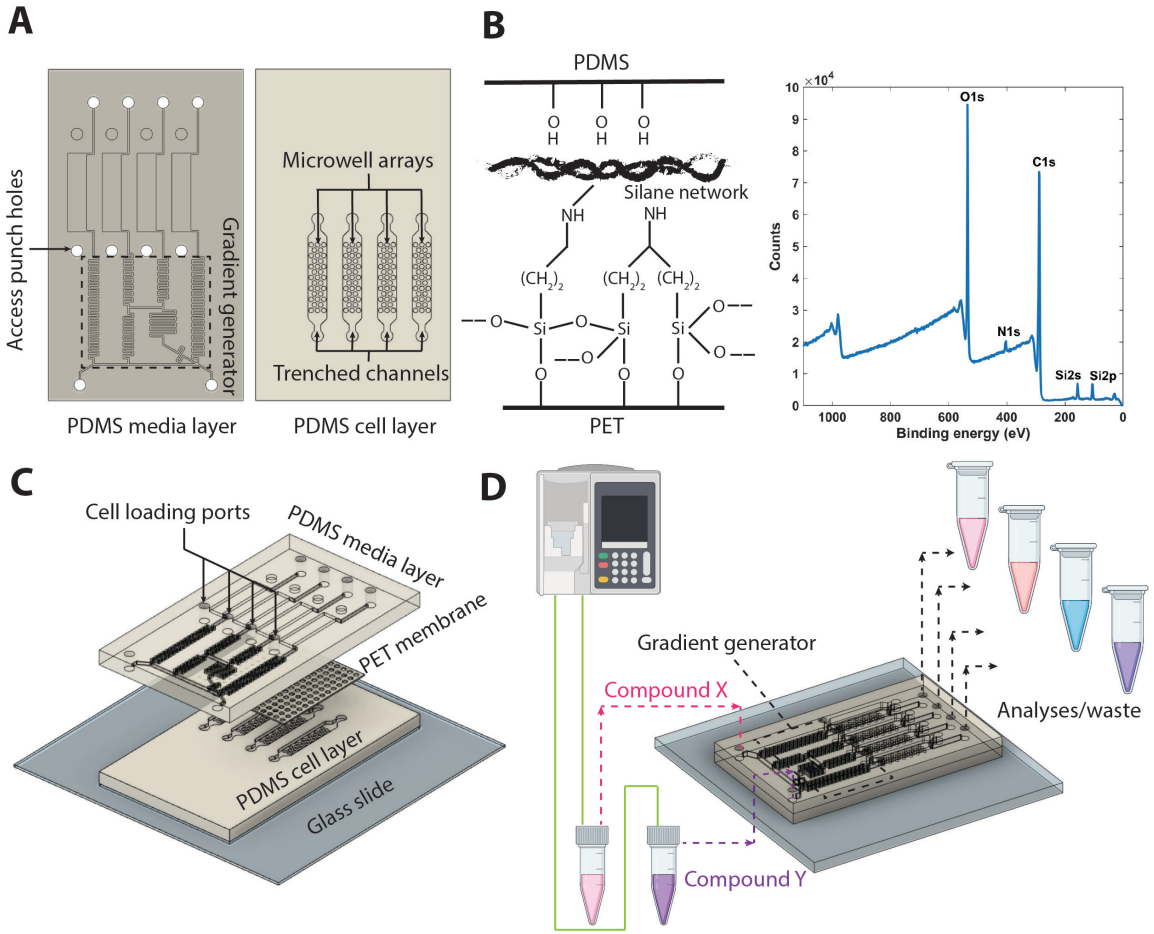


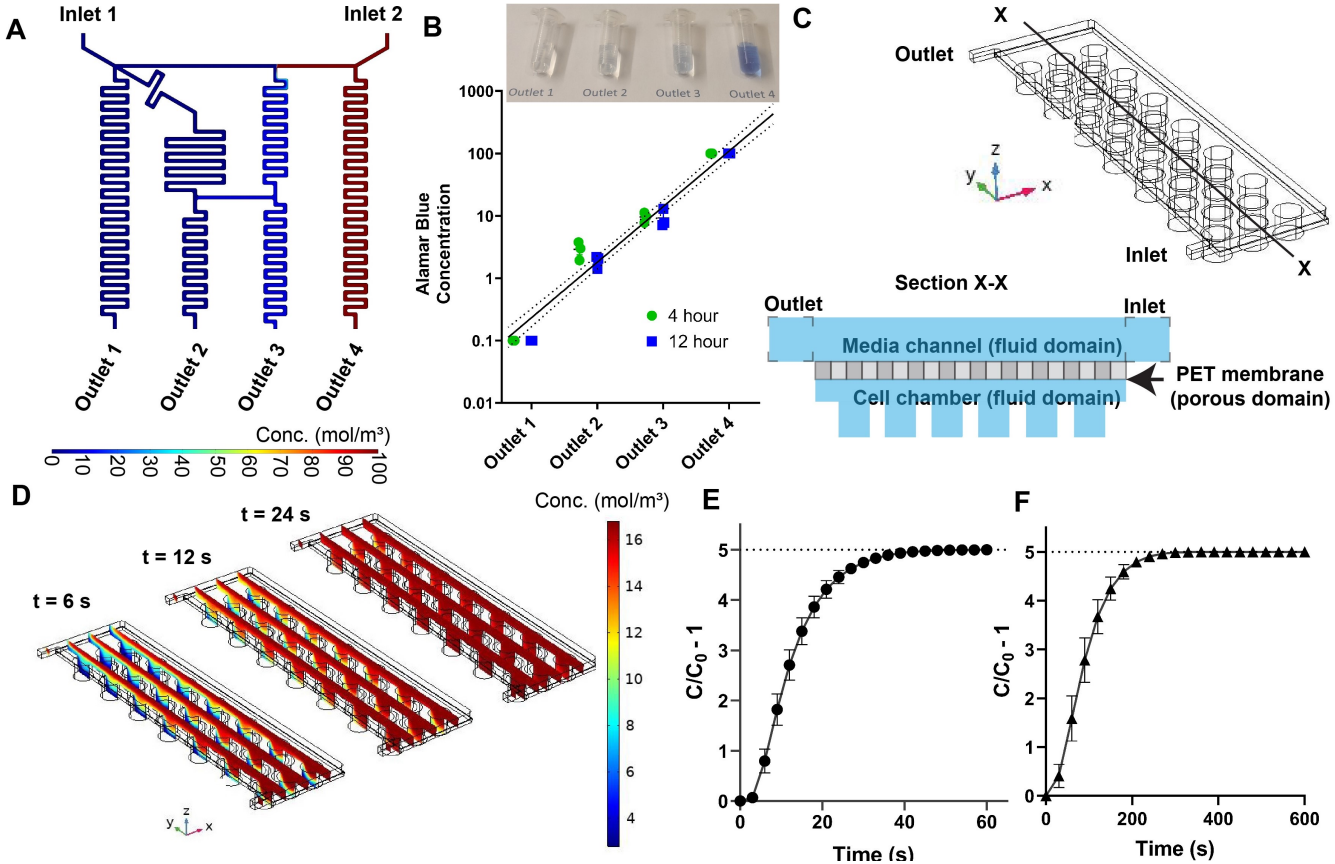


Seahorse Mitochondrial respiration test (n=3 for spheroids, n=6 for 2D monolayer)

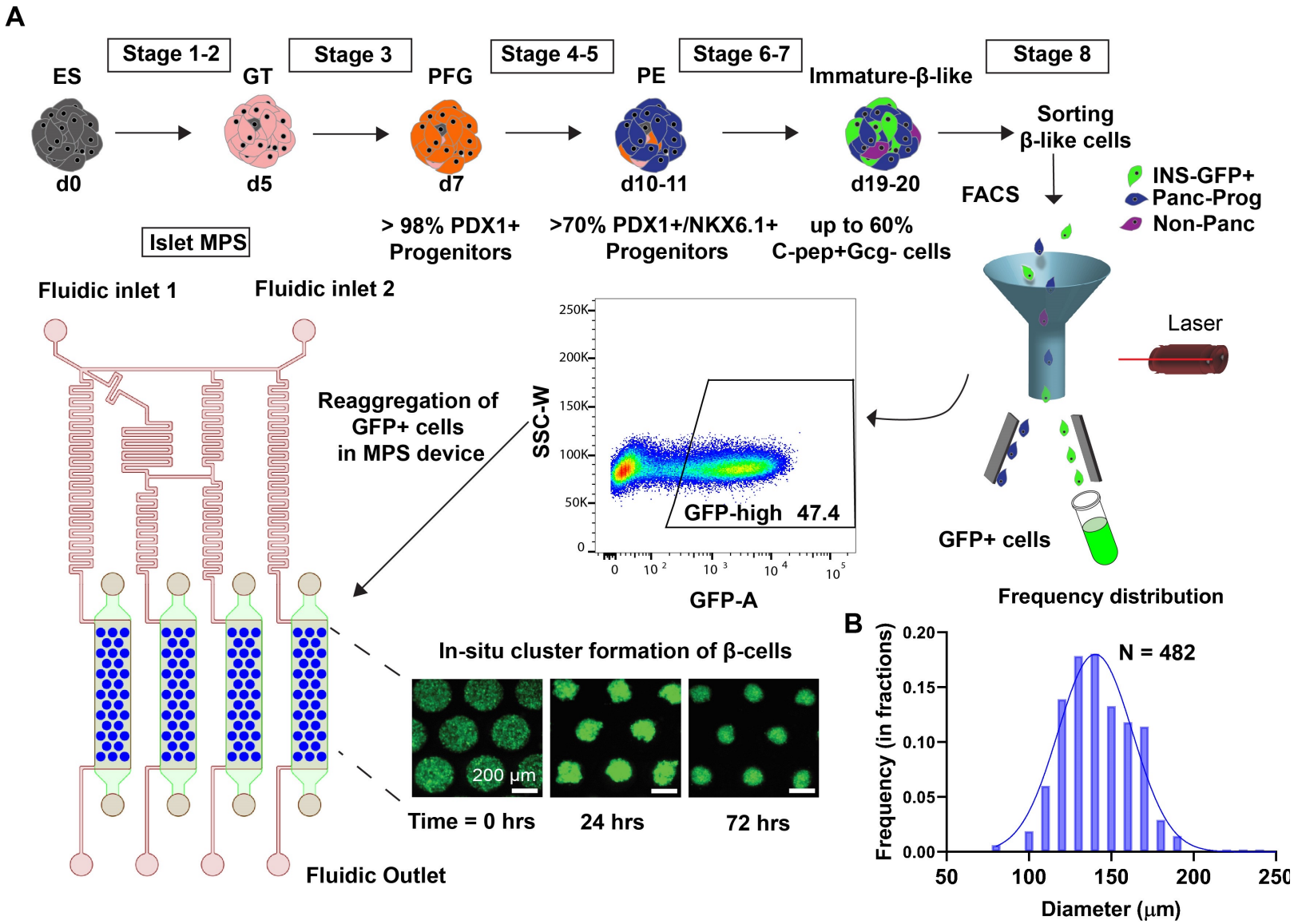
Source: Nair et al. Nat. Cell Biol. 2018

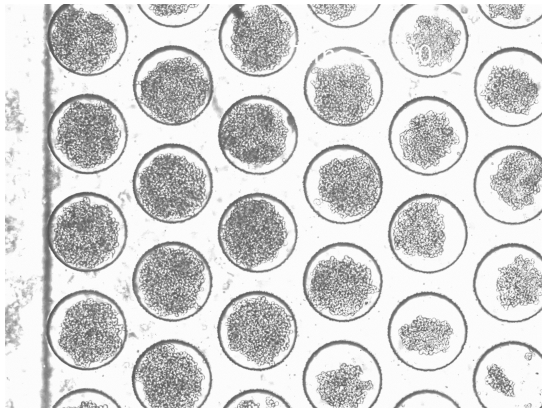
Islet β MPS

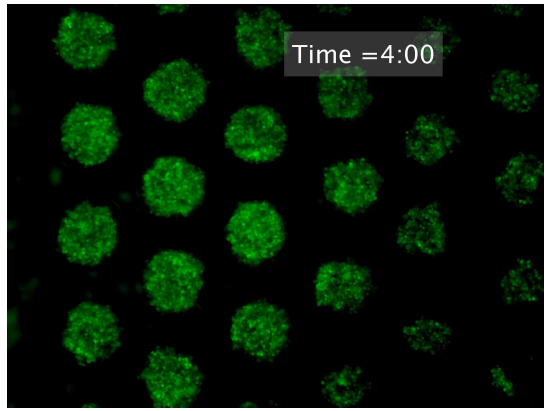

A
Access punch holes
Gradient generator
Microwell arrays
Trenched channels
PDMS media layer
PDMS cell layer
B
PDMS
Silane network
PET
O1s
C1s
N1s
Si2s
Si2p
Counts
Binding energy (eV)
C
Cell loading ports
PDMS media layer
PET membrane
PDMS cell layer
Glass slide
D
Gradient generator
Compound X
Compound Y
Analyses/waste


A
Inlet 1
Inlet 2
Outlet 1
Outlet 2
Outlet 3
Outlet 4
Conc. (mol/m³)
B
Alamar Blue Concentration
4 hour
12 hour
C
Outlet
Inlet
Section X-X
Media channel (fluid domain)
Cell chamber (fluid domain)
PET membrane (porous domain)
D
t = 6 s
t = 12 s
t = 24 s
Conc. (mol/m³)
E
C/C0 - 1
Time (s)
F
C/C0 - 1
Time (s)

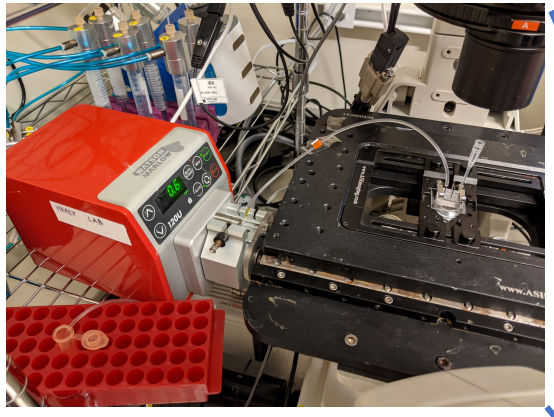
Islet β MPS

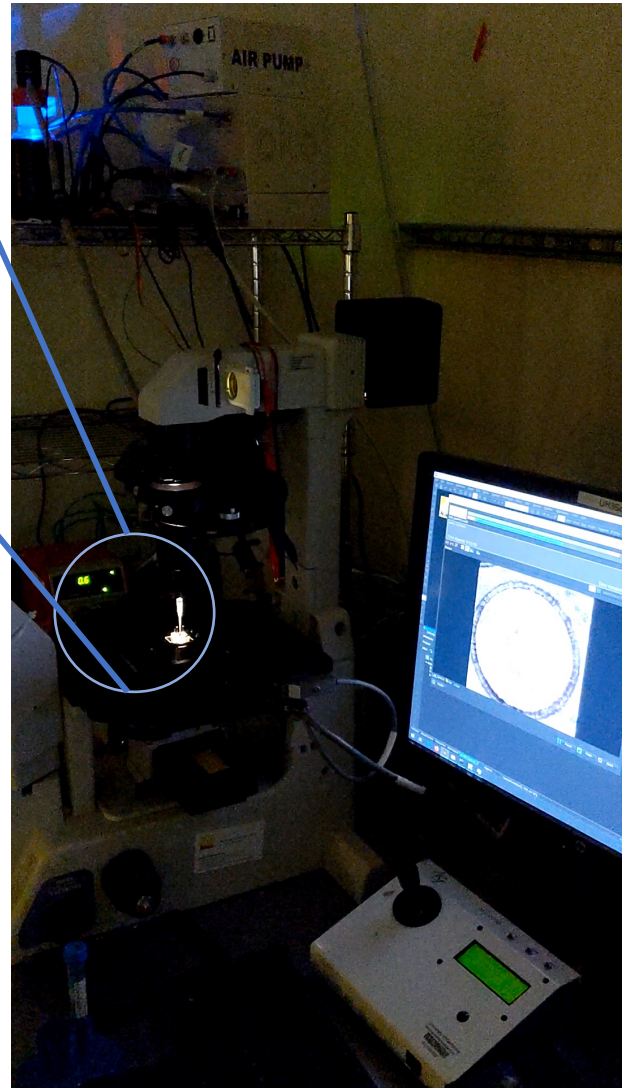

A
Stage 1-2
Stage 3
Stage 4-5
Stage 6-7
Stage 8
ES
GT
PFG
PE
Immature-β-like
d0
d5
d7
d10-11
d19-20
Sorting
β-like cells
FACS
INS-GFP+
Panc-Prog
Non-Panc
Islet MPS
> 98% PDX1+
Progenitors
>70% PDX1+/NKX6.1+
Progenitors
up to 60%
C-pep+Gcg- cells
Fluidic inlet 1
Fluidic inlet 2
Laser
Reaggregation of
GFP+ cells
in MPS device
SSC-W
GFP-high 47.4
GFP-A
GFP+ cells
Frequency distribution
In-situ cluster formation of β-cells
200 µm
Time = 0 hrs
24 hrs
72 hrs
Fluidic Outlet
B
Frequency (in fractions)
N = 482
Diameter (µm)

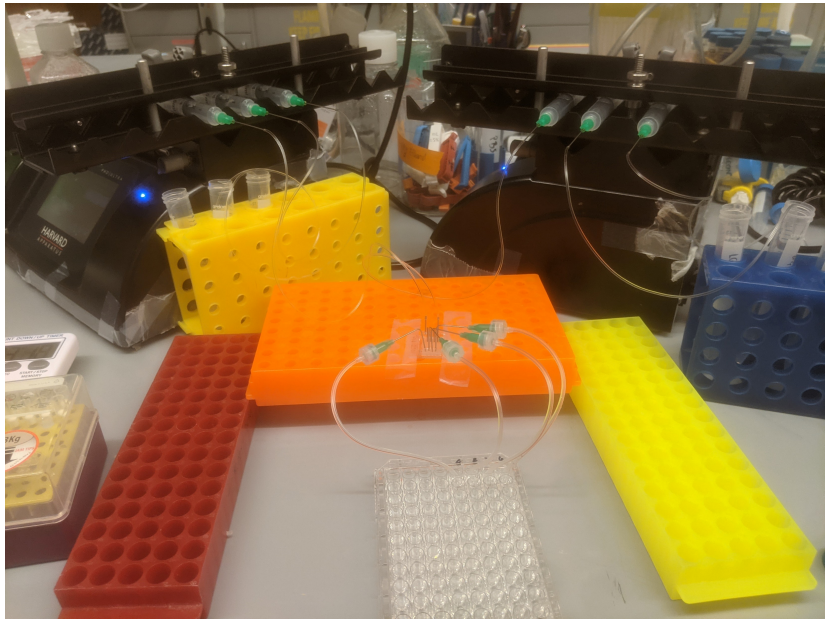


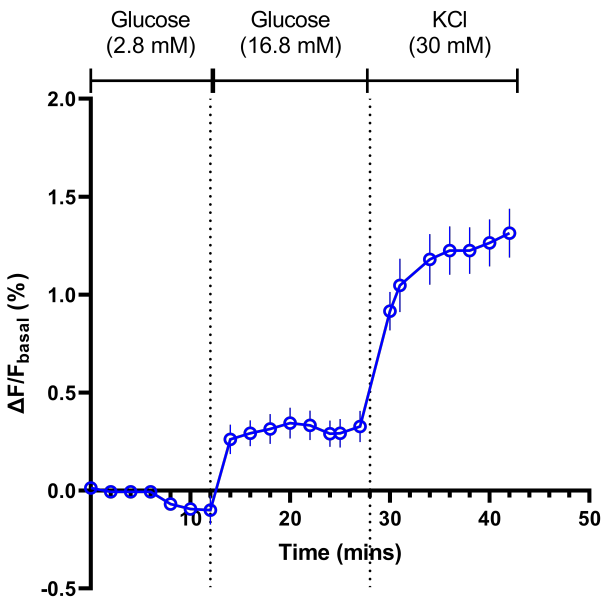

Time =4:00

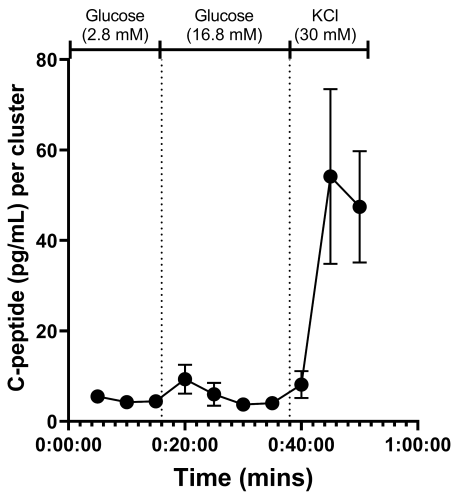
Functionality measurements in device

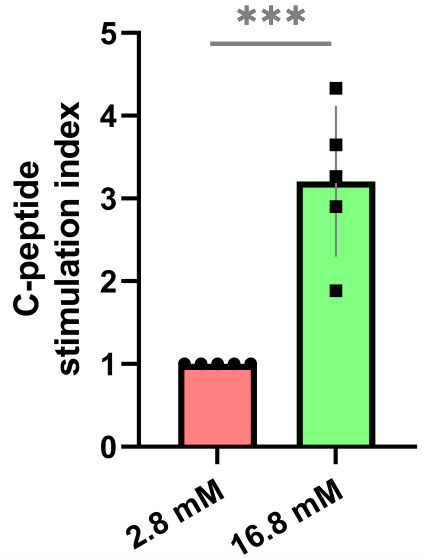




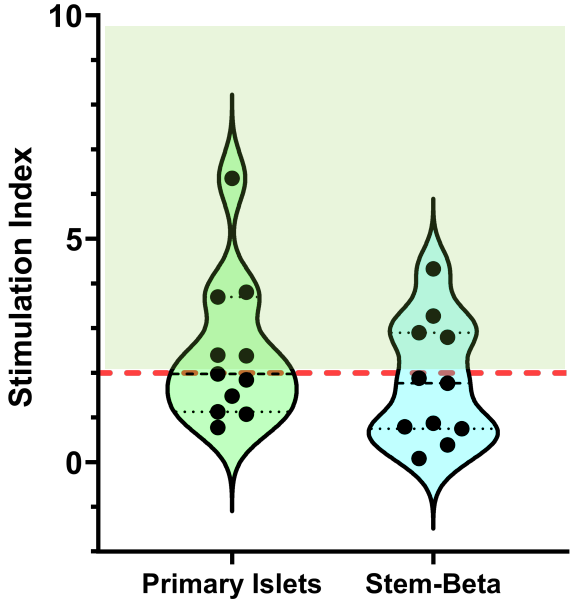


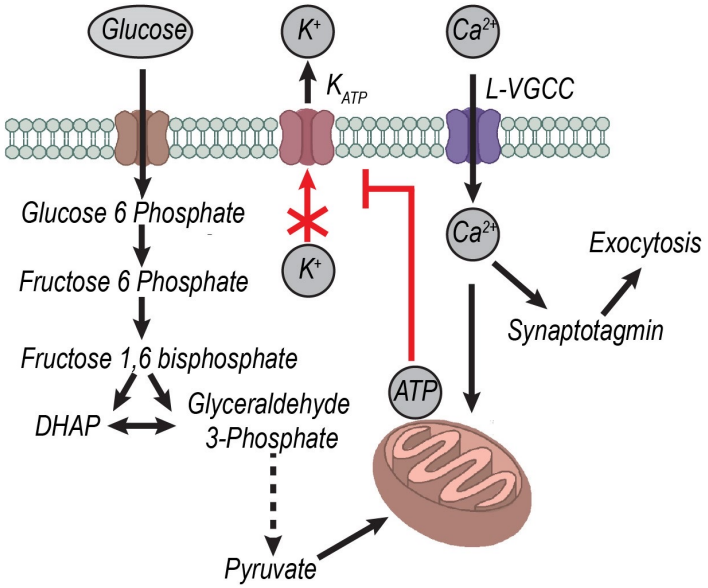


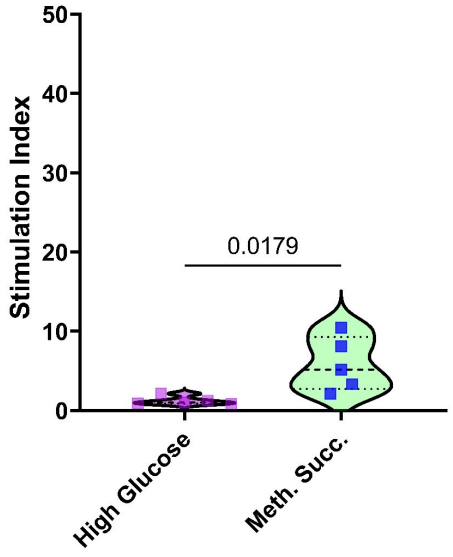


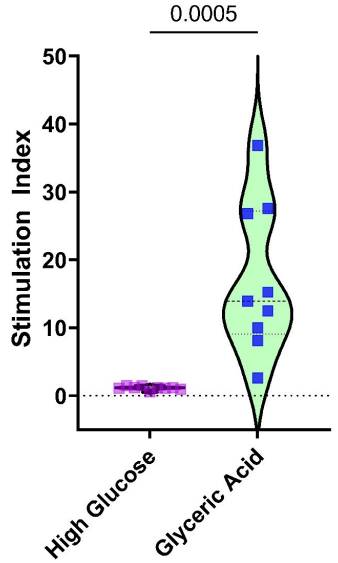


Islet β MPS

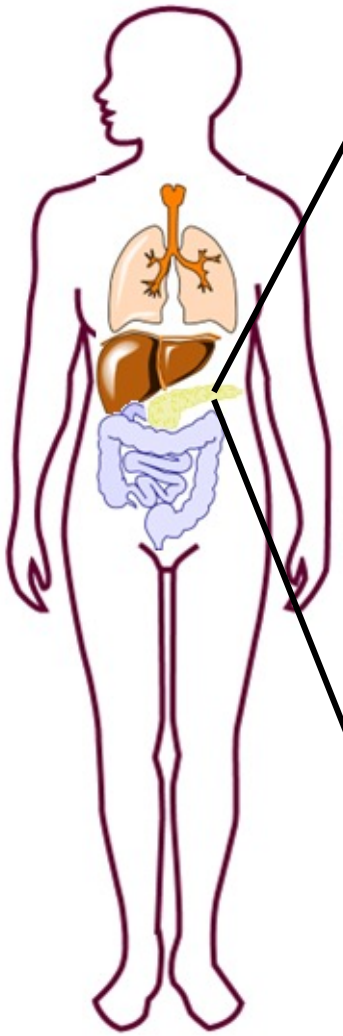

10
5
0
Stimulation Index
Primary Islets
Stem-Beta


Glucose
K+
Ca2+
KATP
L-VGCC
Glucose 6 Phosphate
Fructose 6 Phosphate
Fructose 1,6 bisphosphate
DHAP
Glyceraldehyde 3-Phosphate
Pyruvate
ATP
Exocytosis
Synaptotagmin

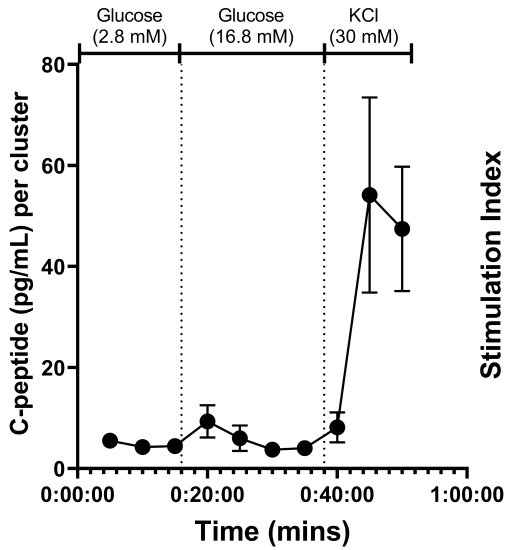

50
40
30
20
10
0
Stimulation Index
0.0179
High Glucose
Meth. Succ.

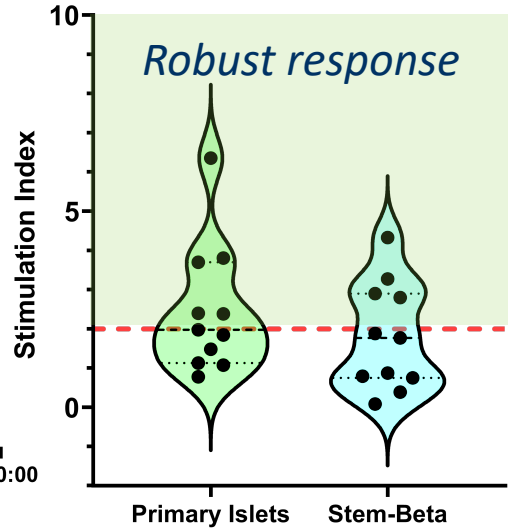

0.0005
50
40
30
20
10
0
Stimulation Index
High Glucose
Glyceric Acid

Engineering robust tissues in MPS

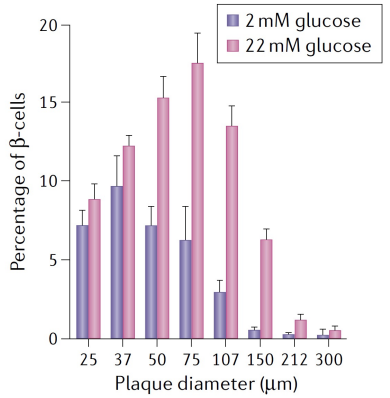


Dynamic range of functionality



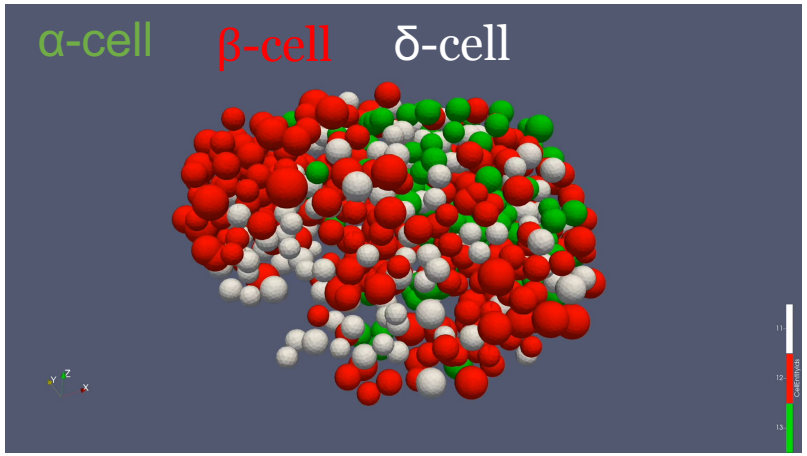


Source: Goswami et al. 2022

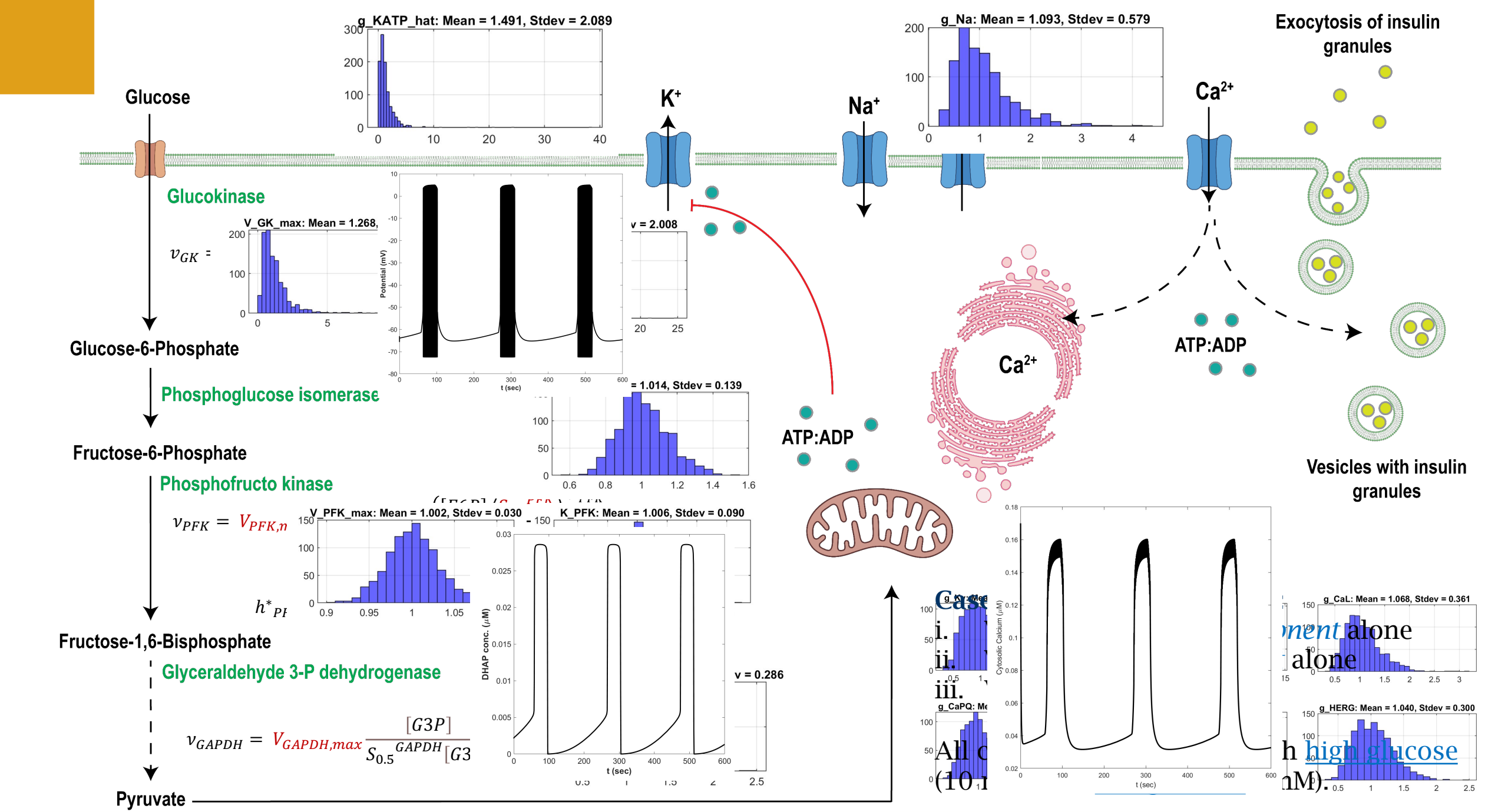


Adapted from: Benninger et al. 2022 & Wojtusciszyn et al. 2008

Computational model of functional range



Source: Andy Edwards



$v_{GK} =$

$v_{PFK} = V_{PFK,n}$

h^*_{PI}

$v_{GAPDH} = V_{GAPDH,max} \frac{[G3P]}{S_{0.5}^{GAPDH}[G3}$

Cast

i.

ii.

iii.

ment alone

alone

All c

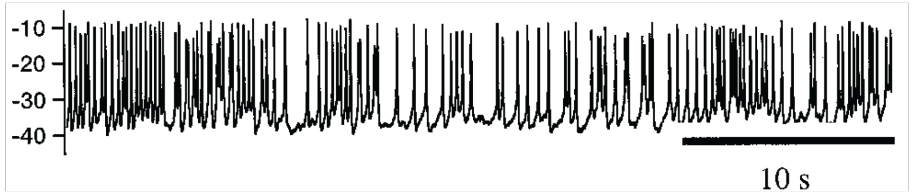
(10 r

h high glucose

nM).

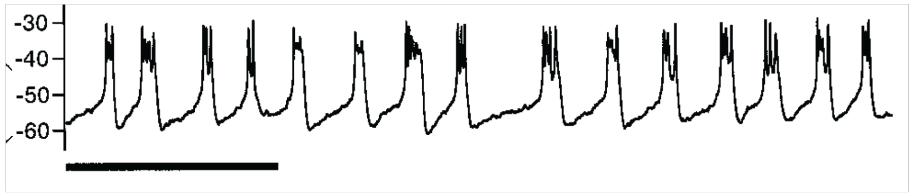
Constraining criterion

Class I: Spiking cells

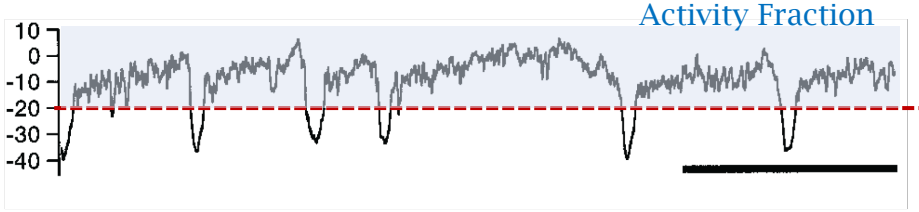


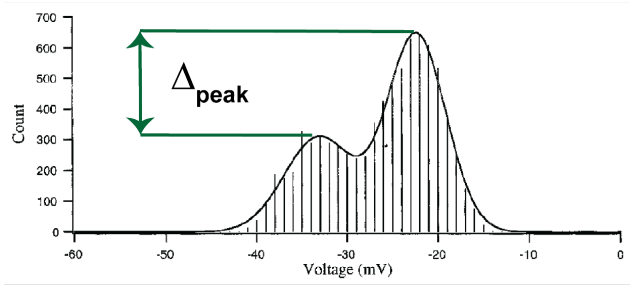
Class II: Bursting cells

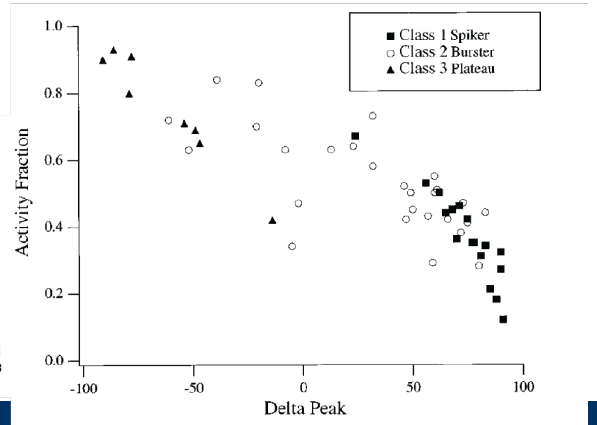
Potential (mV)



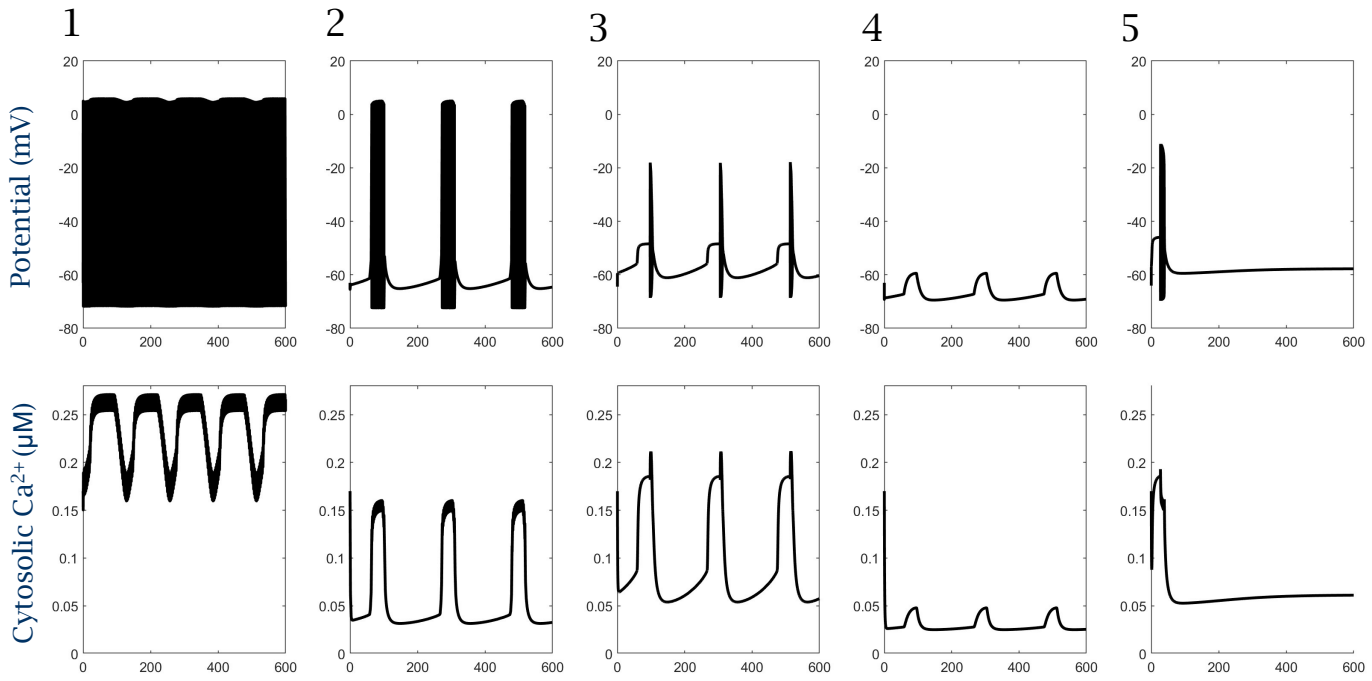
Class III: Plateau cells







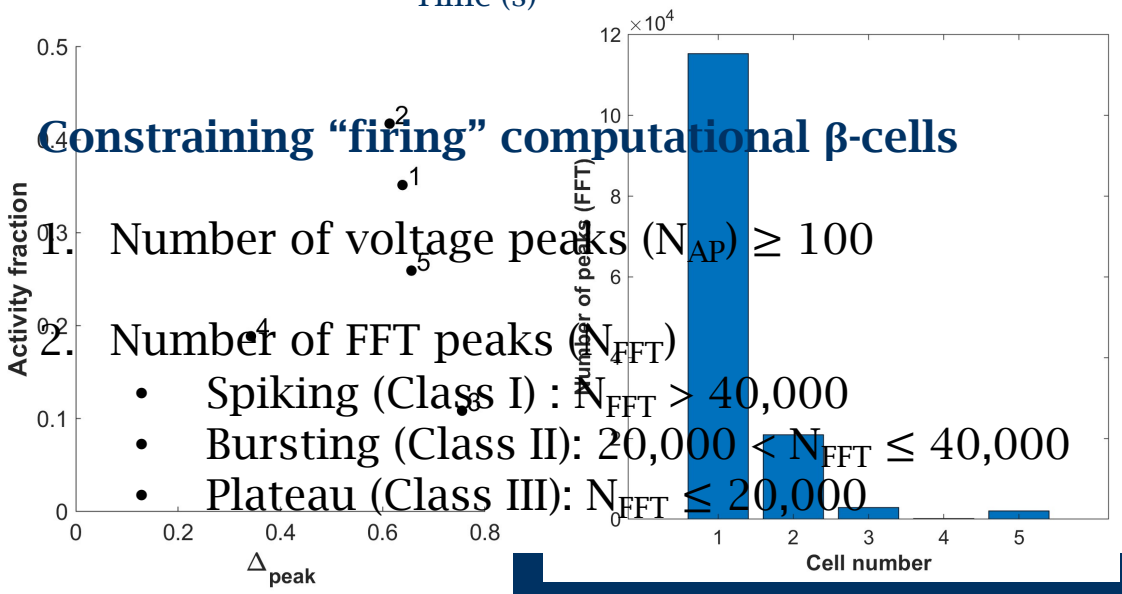
Select simulation output variables

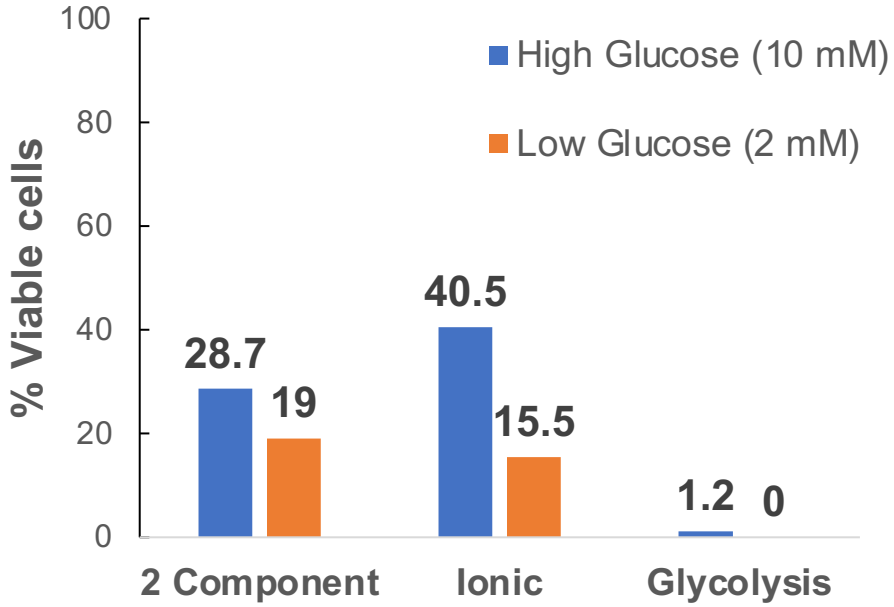


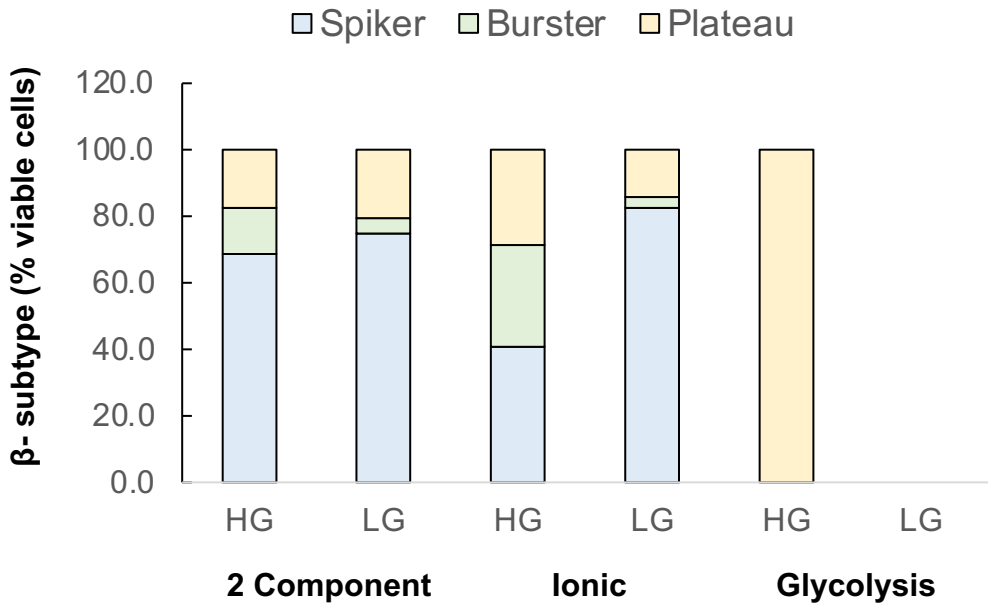
Constraining "firing" computational β-cells

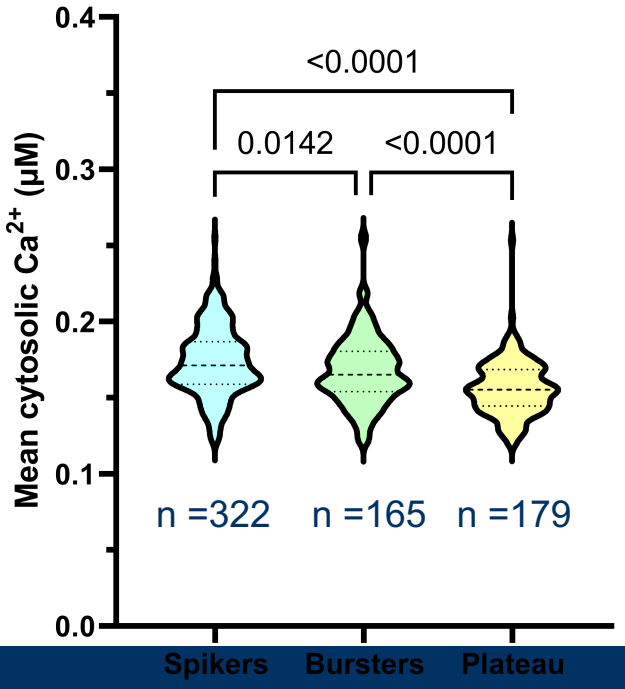
1. Number of voltage peaks (N_{AP}) ≥ 100

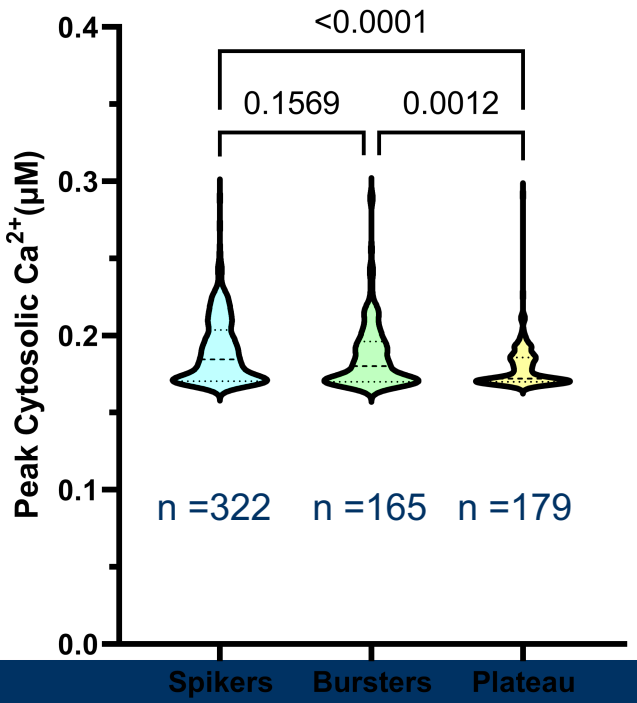
2. Number of FFT peaks (N_{FFT})
   - Spiking (Class I) : $N_{FFT} > 40{,}000$
   - Bursting (Class II): $20{,}000 < N_{FFT} \leq 40{,}000$
   - Plateau (Class III): $N_{FFT} \leq 20{,}000$

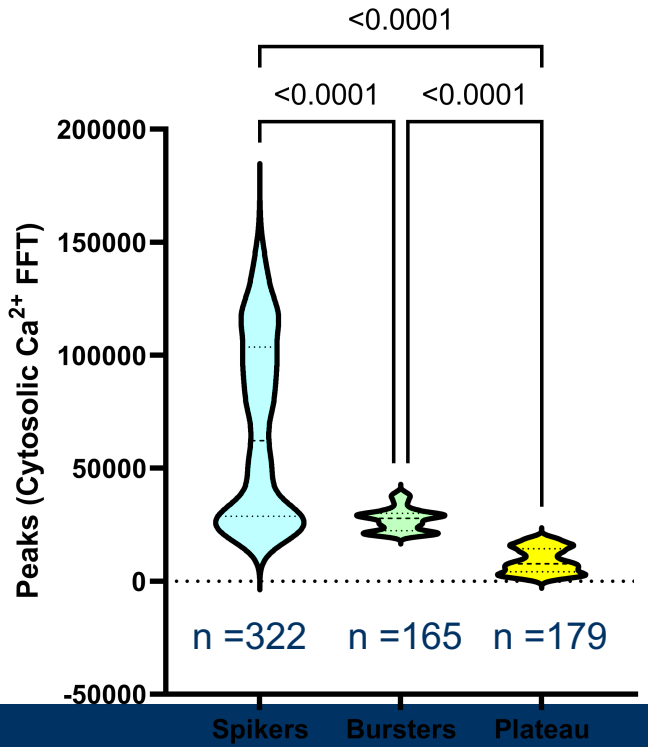



High Glucose (10 mM)
Low Glucose (2 mM)
% Viable cells
100
80
60
40
20
0
28.7
19
40.5
15.5
1.2
0
2 Component
Ionic
Glycolysis


Spiker
Burster
Plateau
β- subtype (% viable cells)
120.0
100.0
80.0
60.0
40.0
20.0
0.0
HG
LG
HG
LG
HG
LG
2 Component
Ionic
Glycolysis


Mean cytosolic Ca2+ (μM)
0.4
0.3
0.2
0.1
0.0
<0.0001
0.0142
<0.0001
n =322
n =165
n =179
Spikers
Bursters
Plateau


Peak Cytosolic Ca2+(μM)
0.4
0.3
0.2
0.1
0.0
<0.0001
0.1569
0.0012
n =322
n =165
n =179
Spikers
Bursters
Plateau


Peaks (Cytosolic Ca2+ FFT)
200000
150000
100000
50000
0
-50000
<0.0001
<0.0001
<0.0001
n =322
n =165
n =179
Spikers
Bursters
Plateau

Conclusions

- Microphysiological systems (MPS) are better in-vitro platform to recapitulate key metabolic features of tissues.
- MPS tissue integration will allow studying pertinent hallmarks & cross-talks associated with human metabolic disease.
- Integration is technically challenging that requires multi-disciplinary approach.
- Synergistic coupling of computational tools with experiments can help many of these challenges associated with integration.