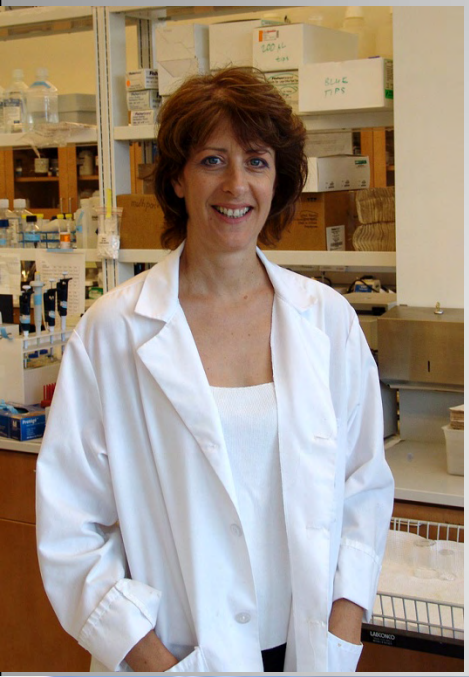
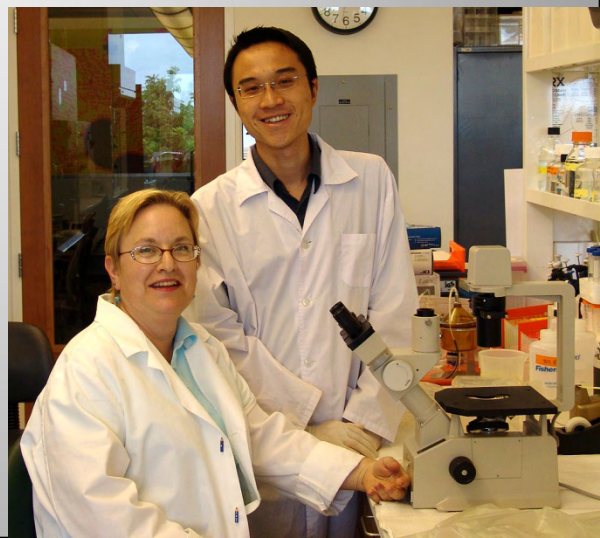
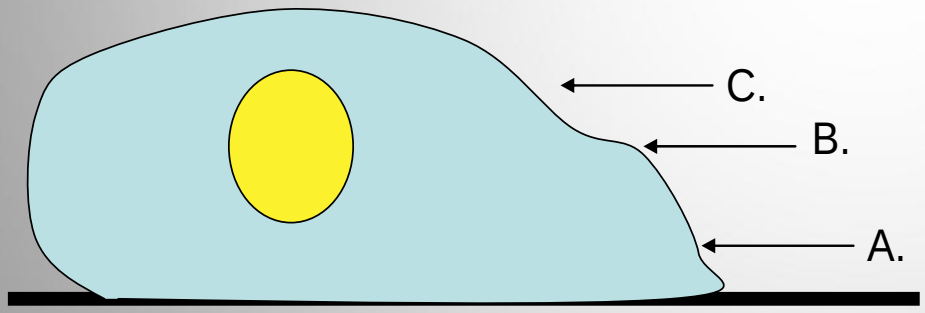
Impact of Islet Size on Graft Function

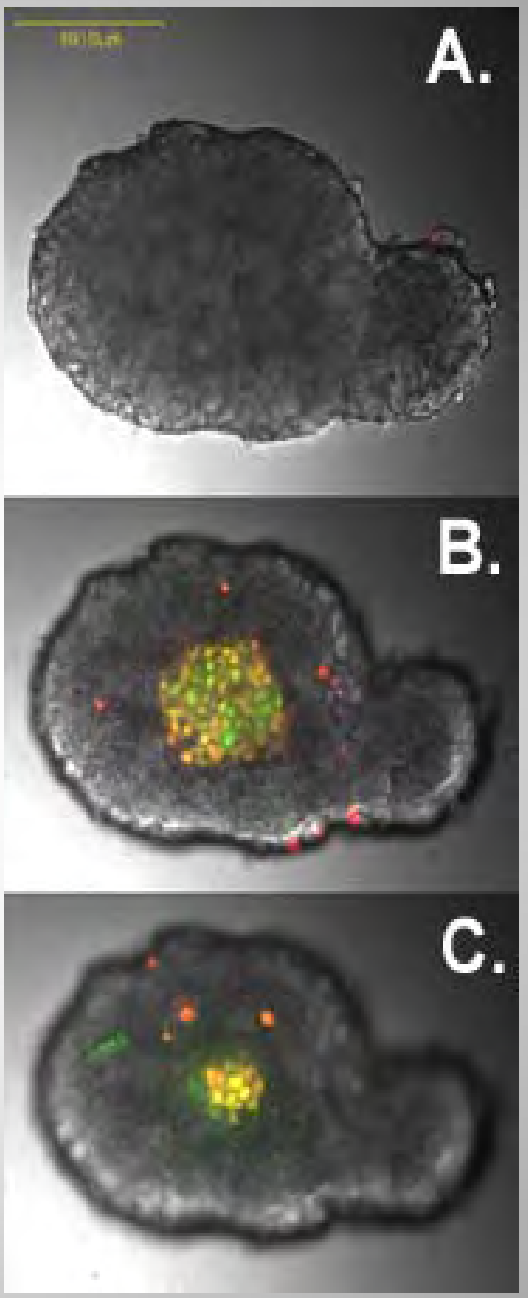


Lisa Stehno-Bittel, PhD, PT
University of Kansas Medical Center
Great Plain Diabetes Institute



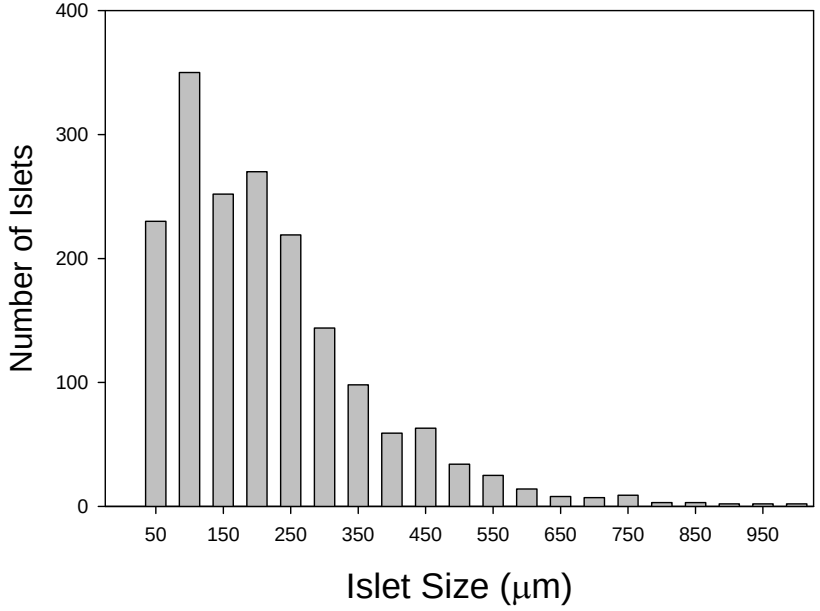
Core Cell Death in Low-Insulin Secreting Islets



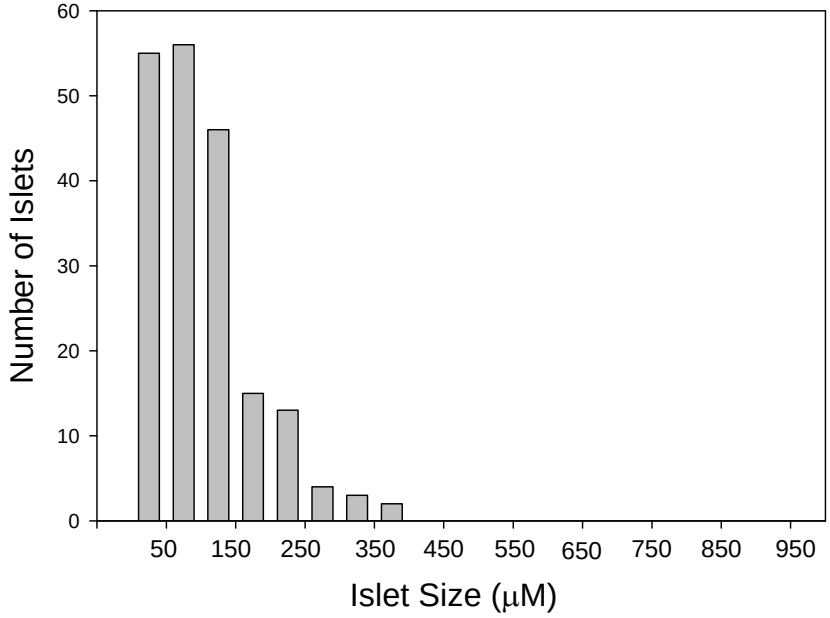


MacGregor et al, 2006

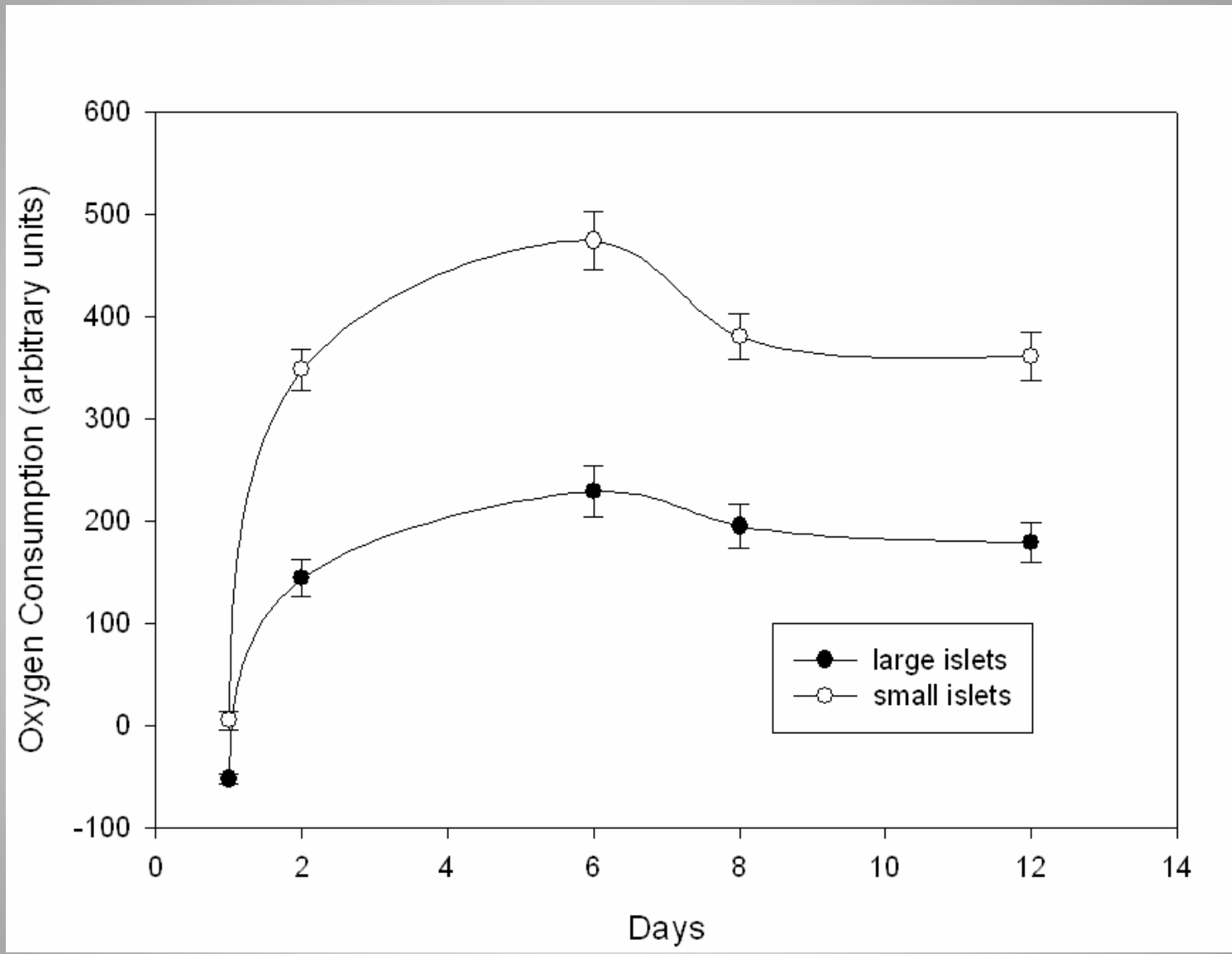
Human Islet Survival

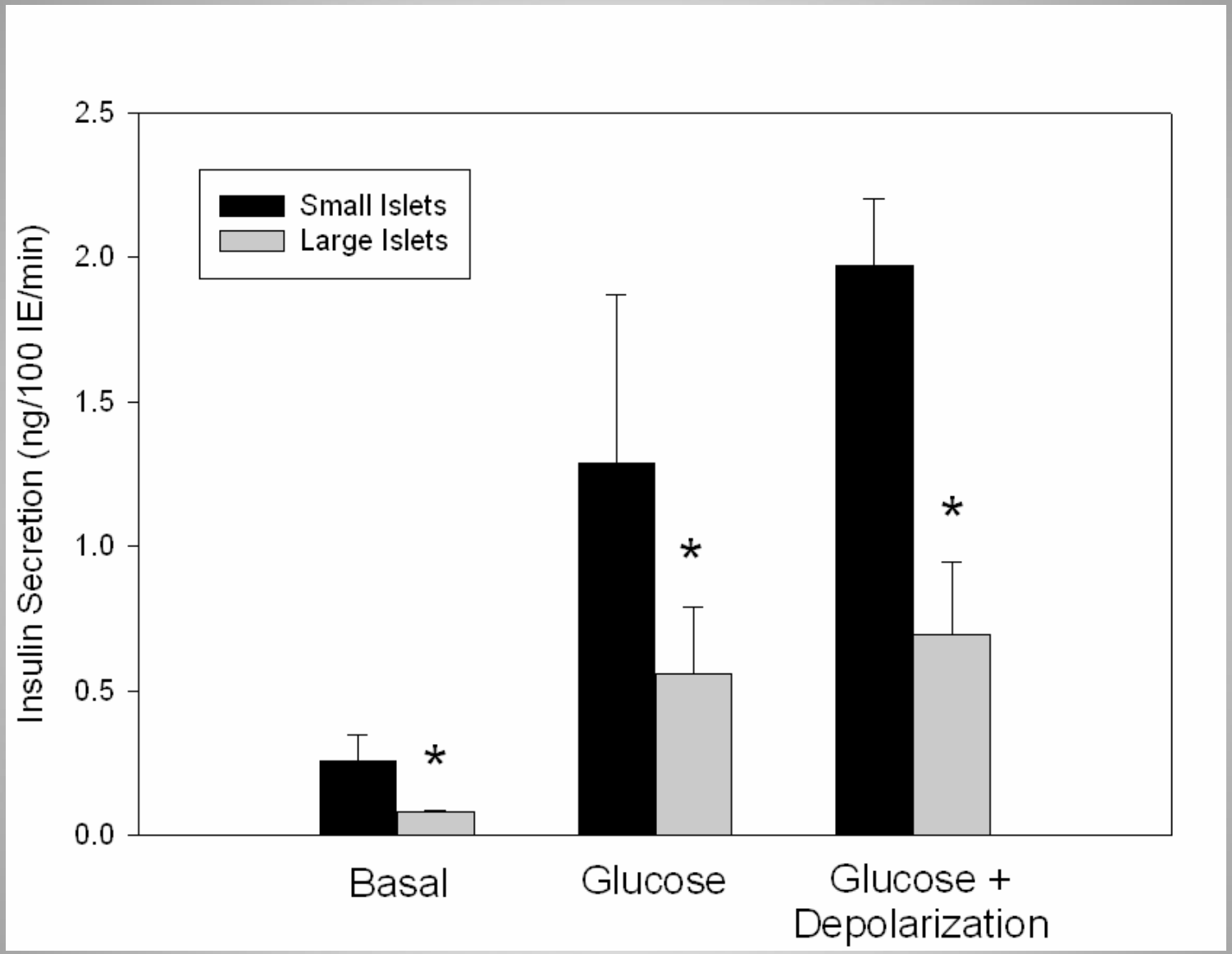


Day 1

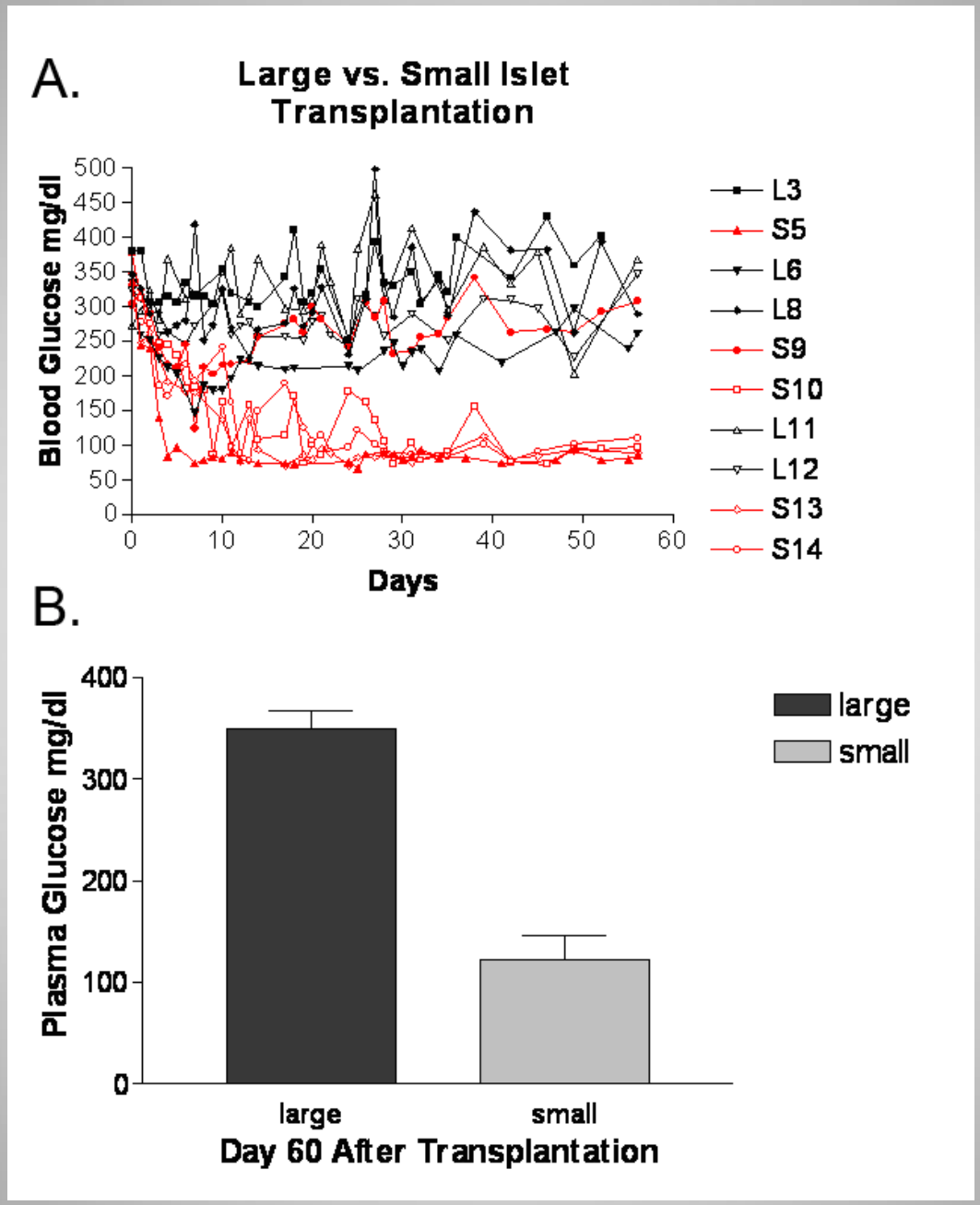


Day 12

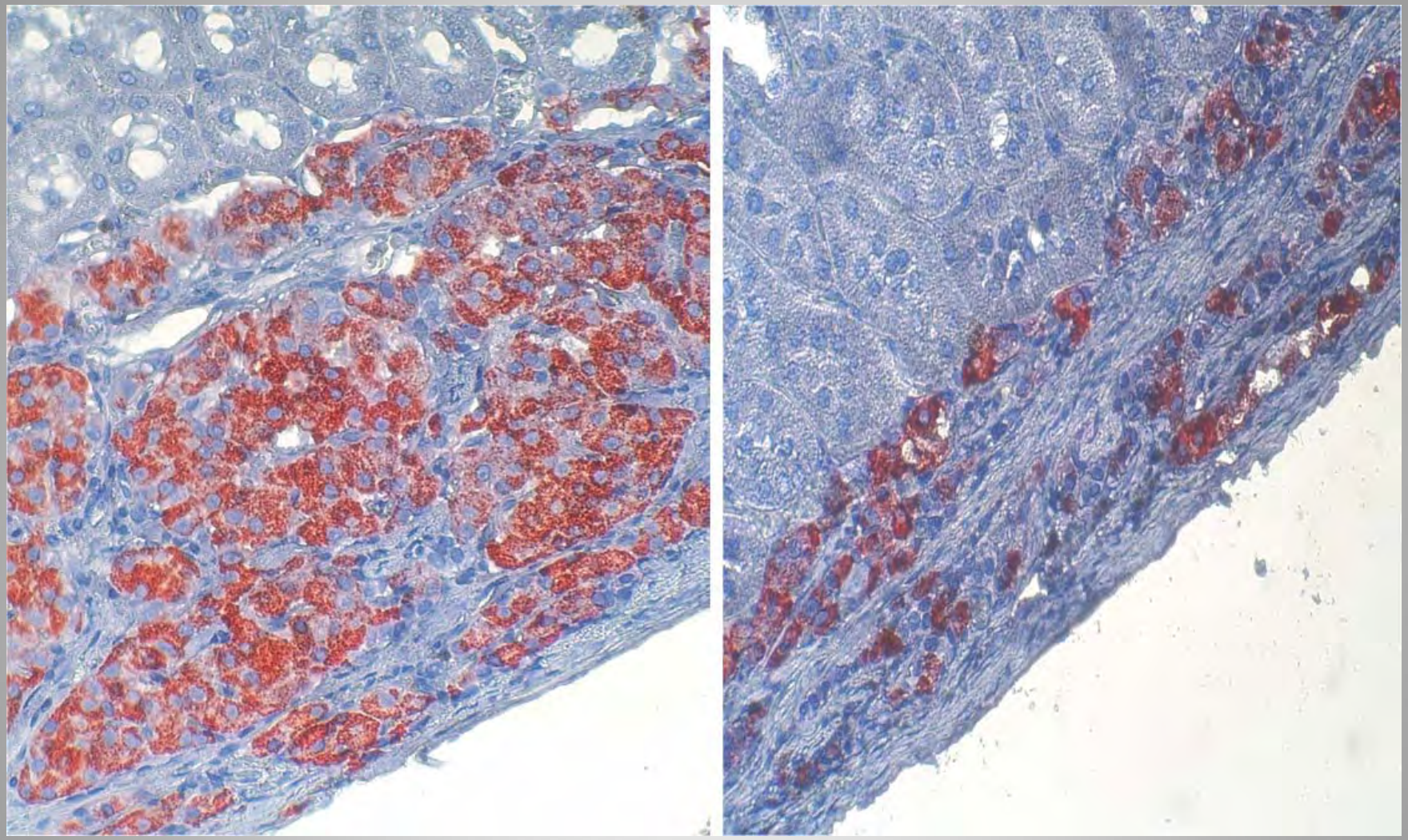

600
500
400
300
200
100
0
-100
Oxygen Consumption (arbitrary units)
0
2
4
6
8
10
12
14
Days
large islets
small islets



MacGregor et al, 2006

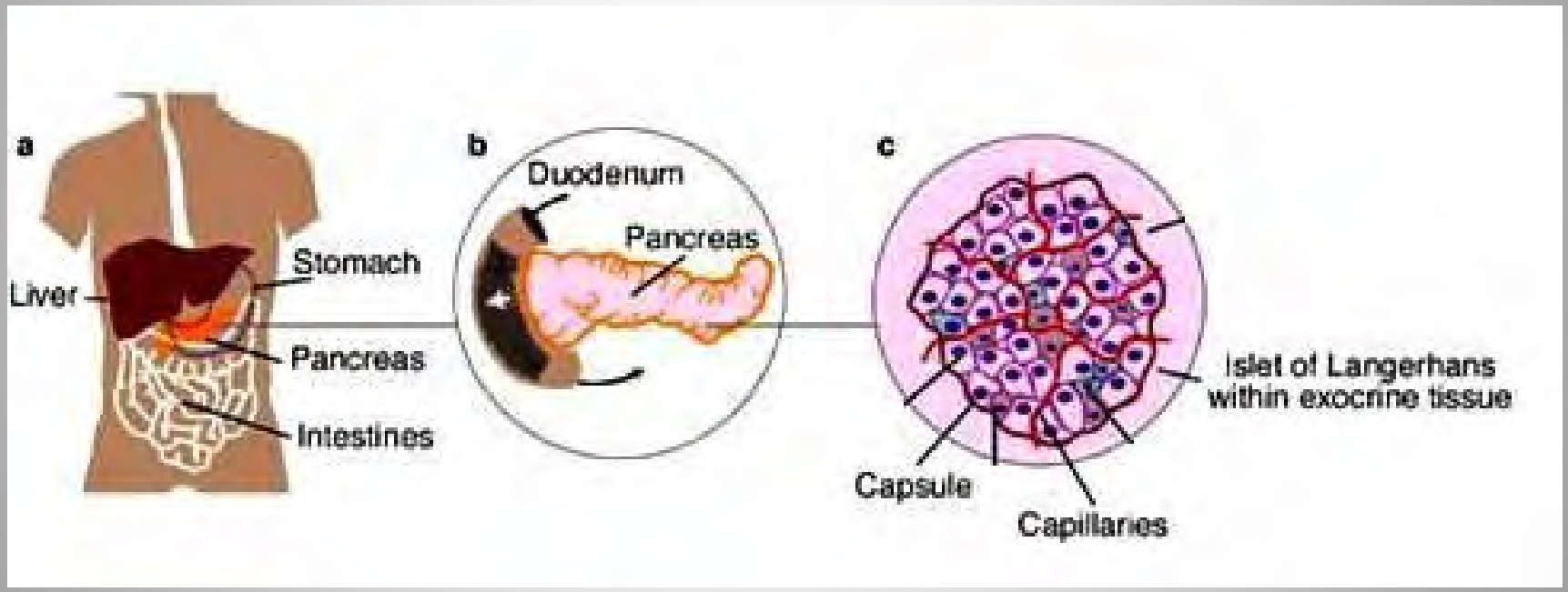


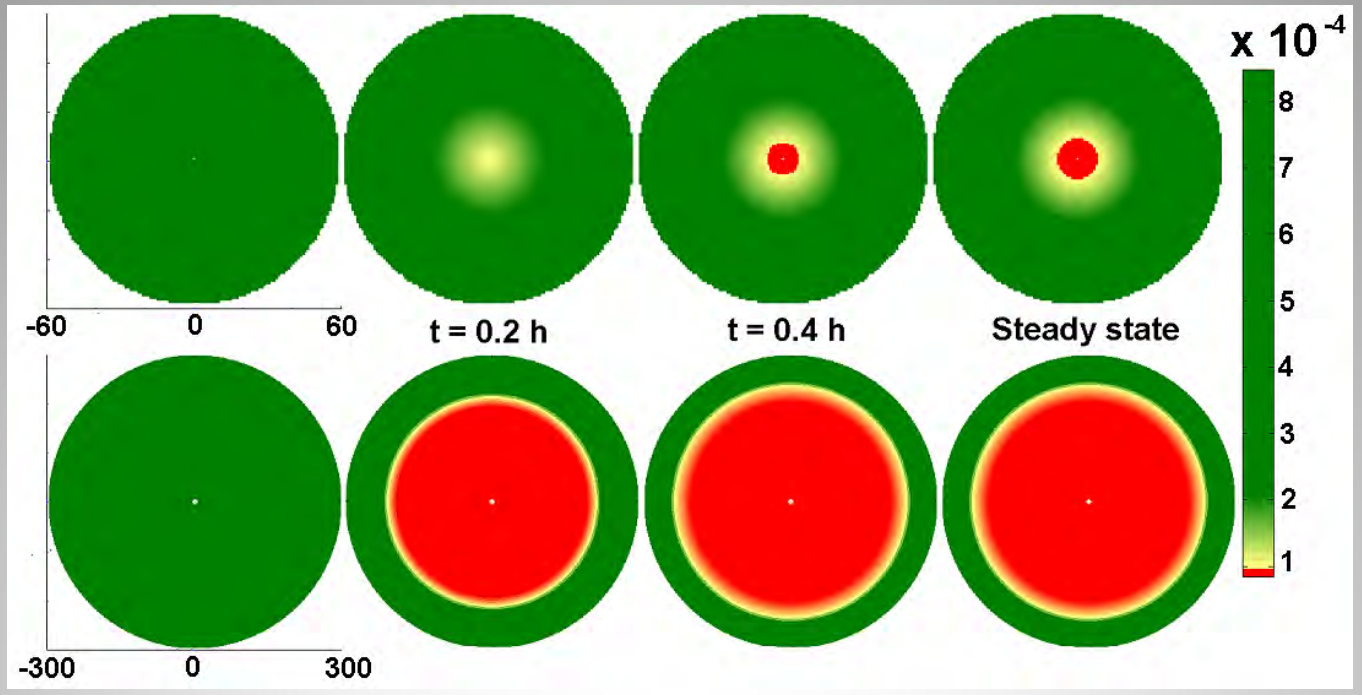
MacGregor et al, 2006



MacGregor et al, 2006

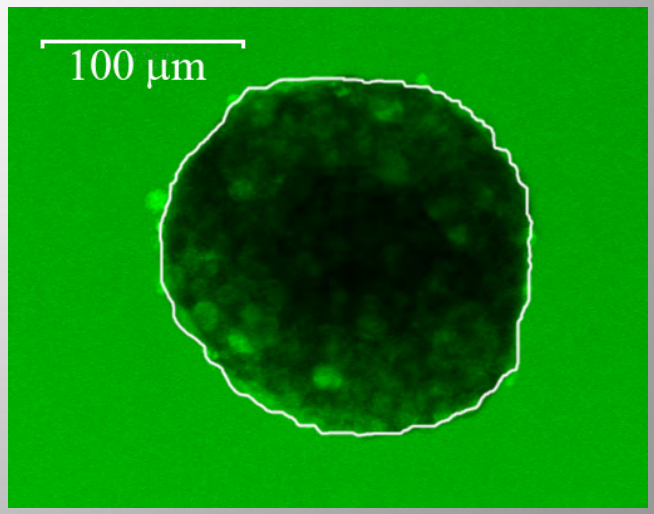
Are Decreased Functional Outcomes for Large LIS Islets Due to Diffusion Barrier?





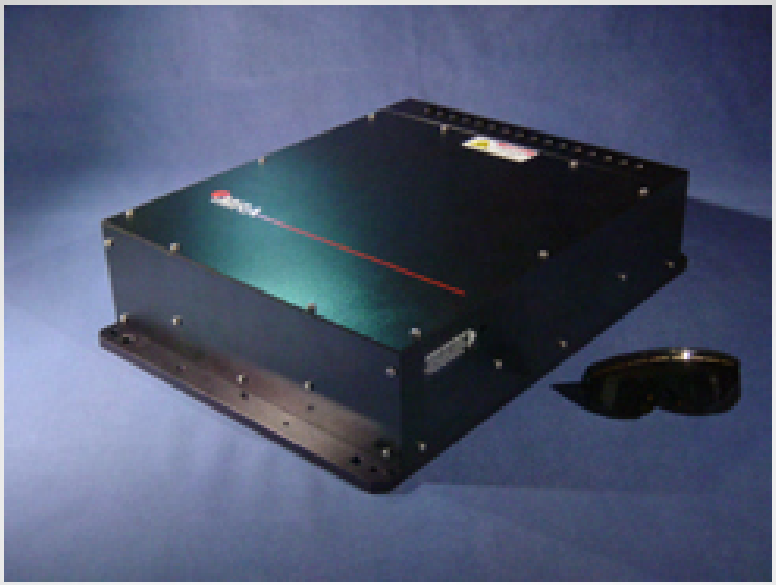
Dynamic partial differential equations for a spherical islet were solved numerically using finite-difference method in spherical coordinates

Confirmed with 2 NBDG diffusion experiments

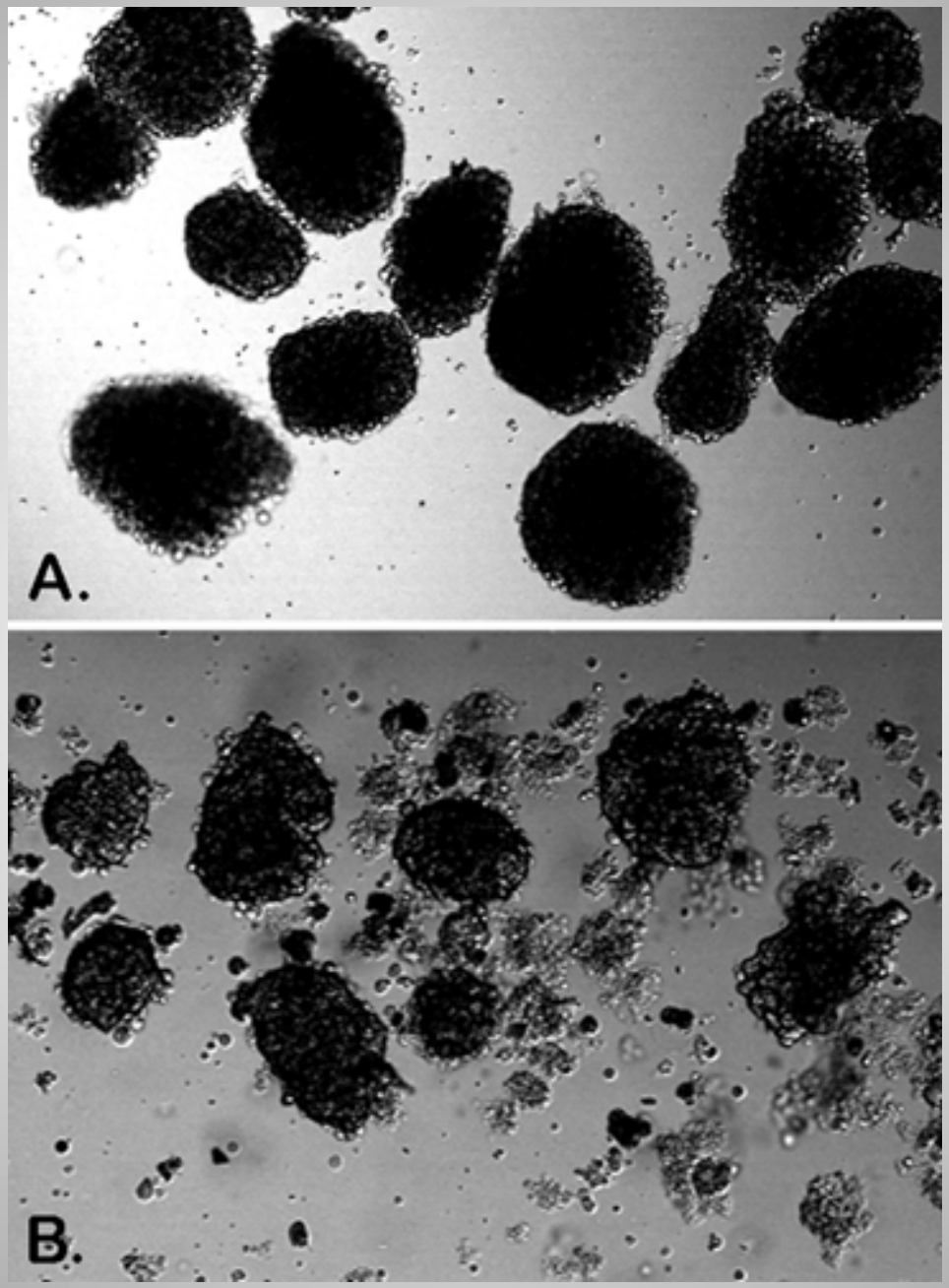


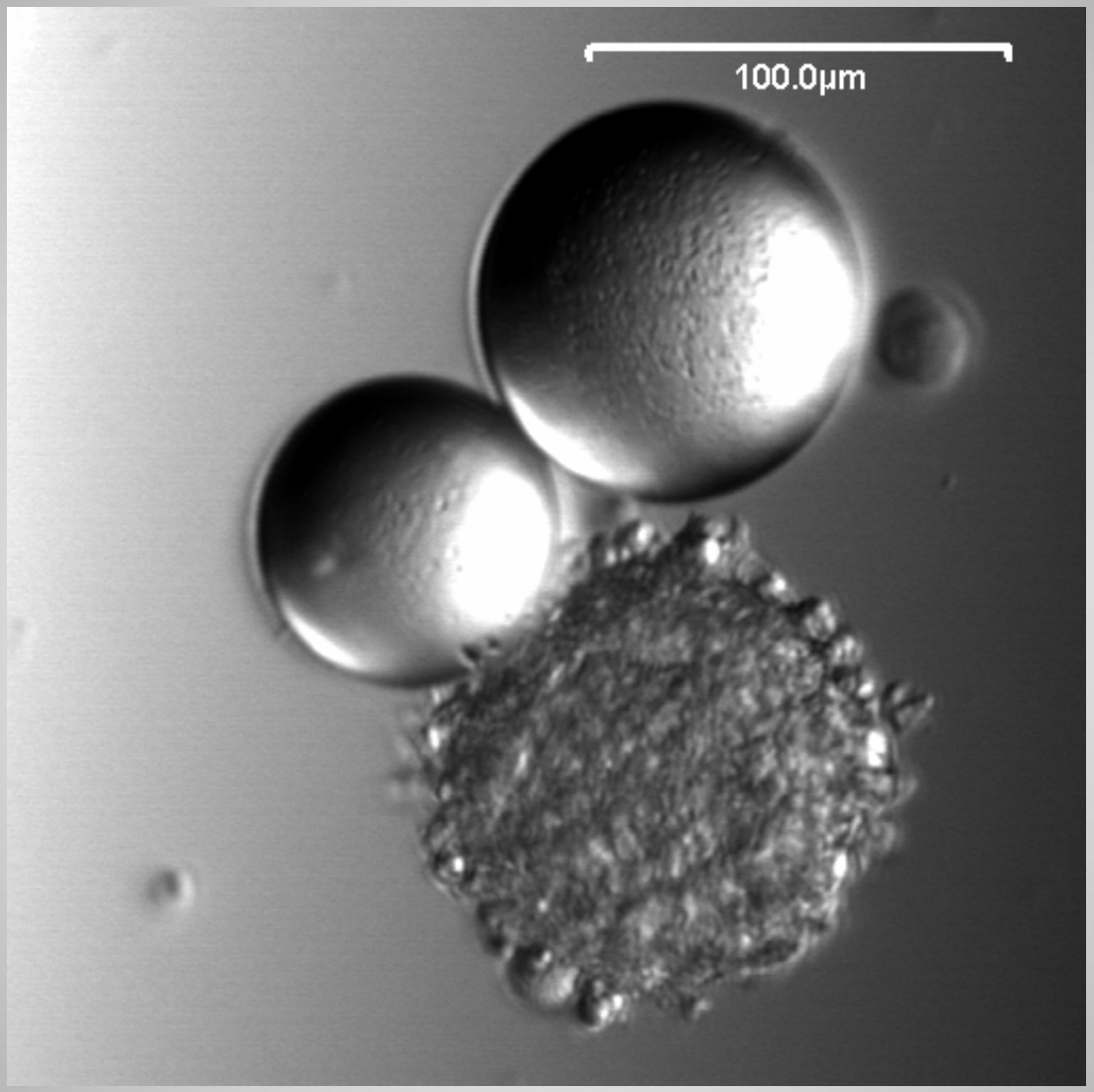
Will reducing the diffusion barrier in large islets improve their function?

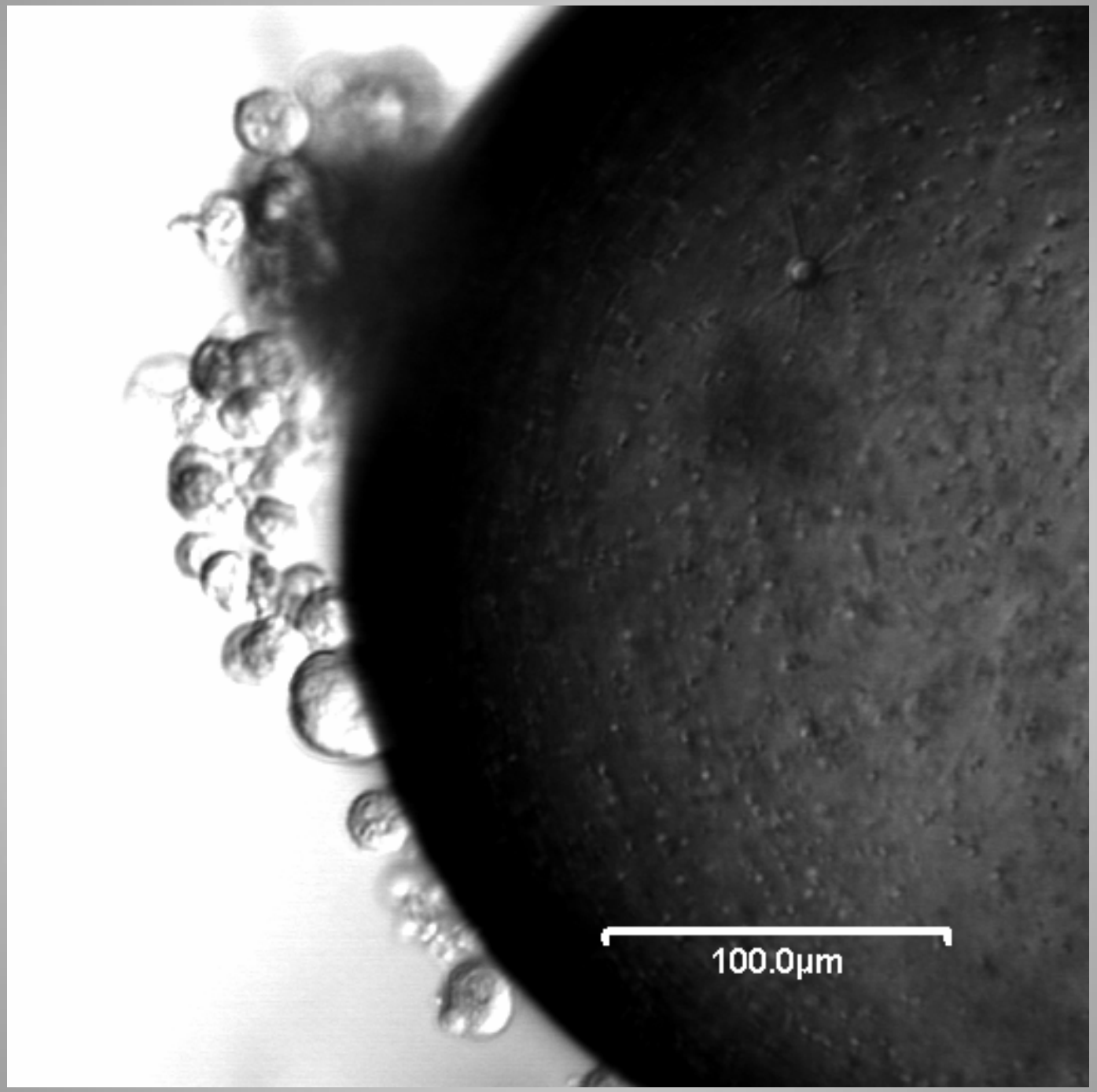
1) Cut islet into segments
2) Engineer islets
3) Make islets porous

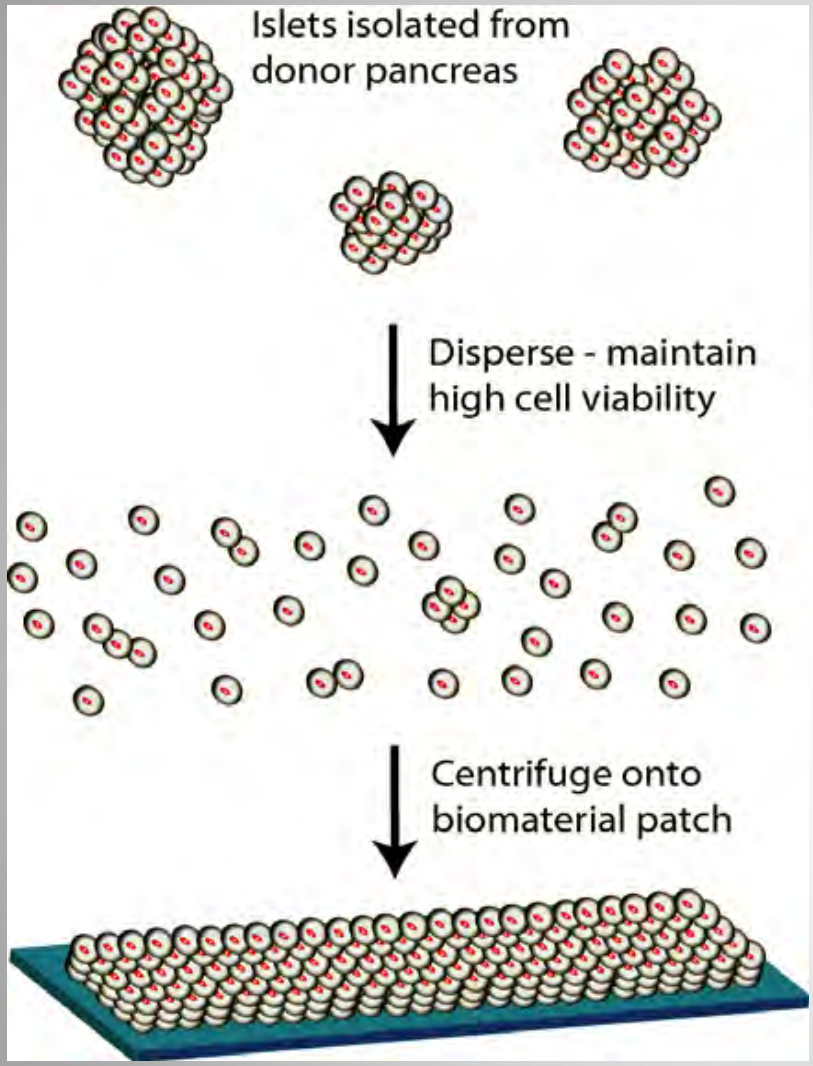


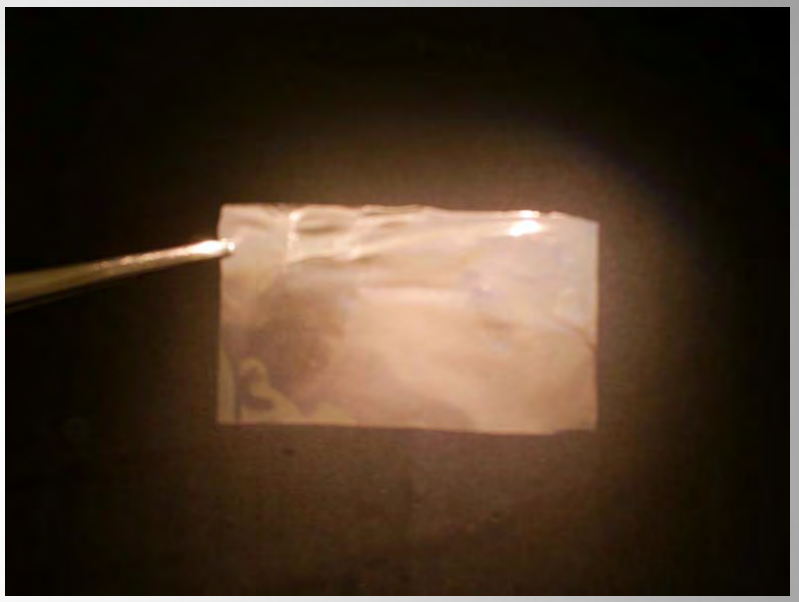
- IMRA FCPA µJ laser
- Yb-fiber oscillator / amplifier system 1 µm wavelength output.
- Pulse widths to < 500 fs

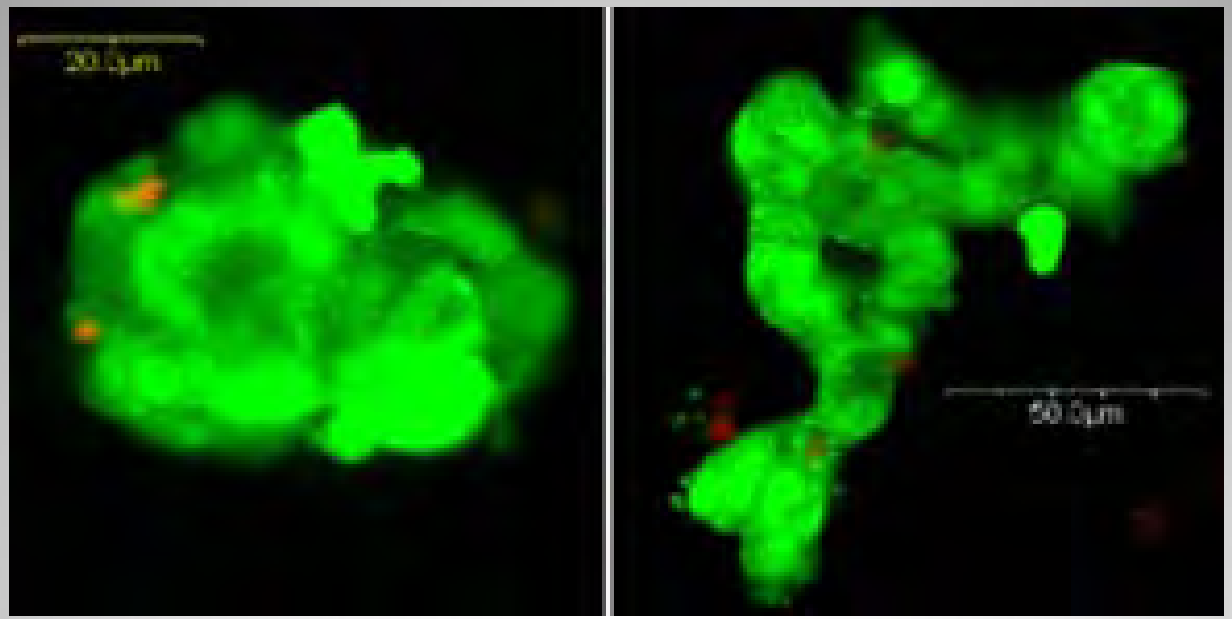

A.
B.

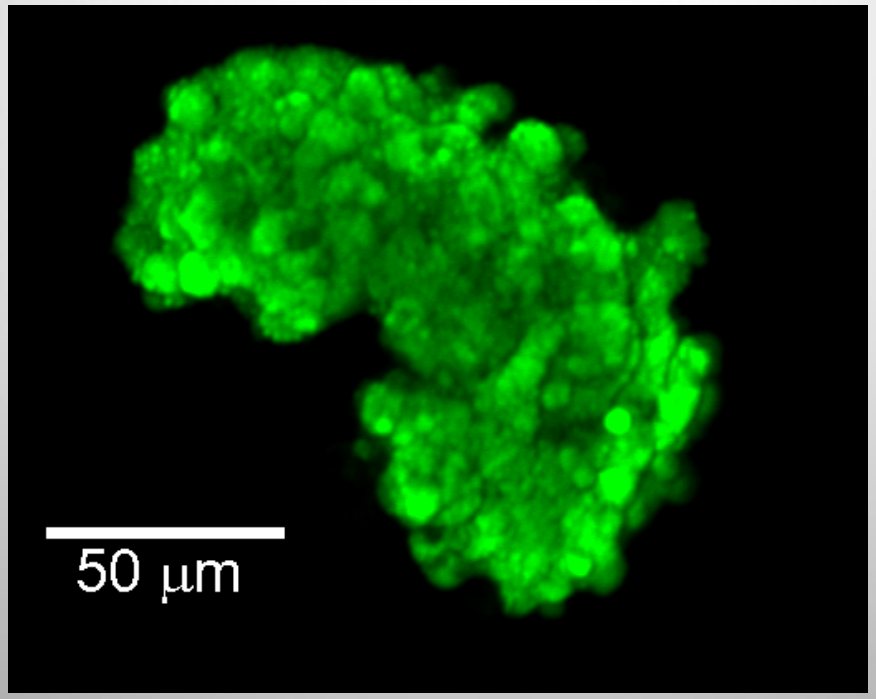

100.0µm


100.0µm


Islets isolated from
donor pancreas
Disperse - maintain
high cell viability
Centrifuge onto
biomaterial patch

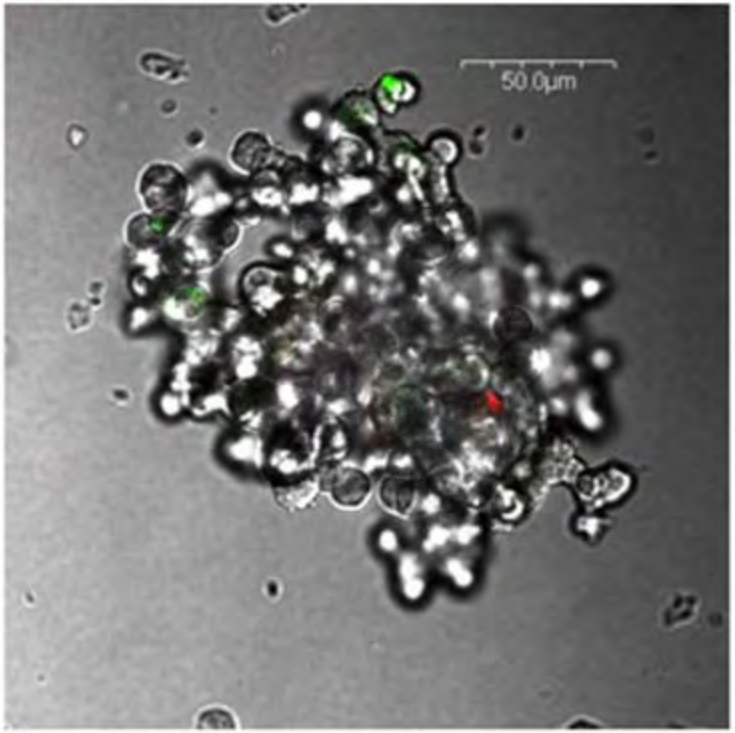




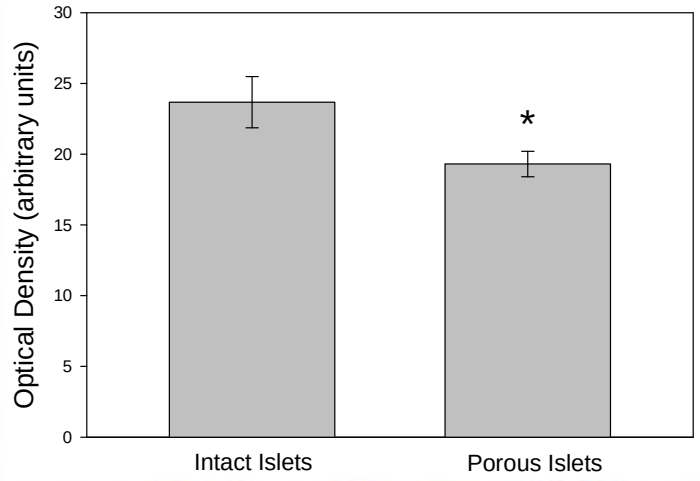


How can we prove that islets are porous ?

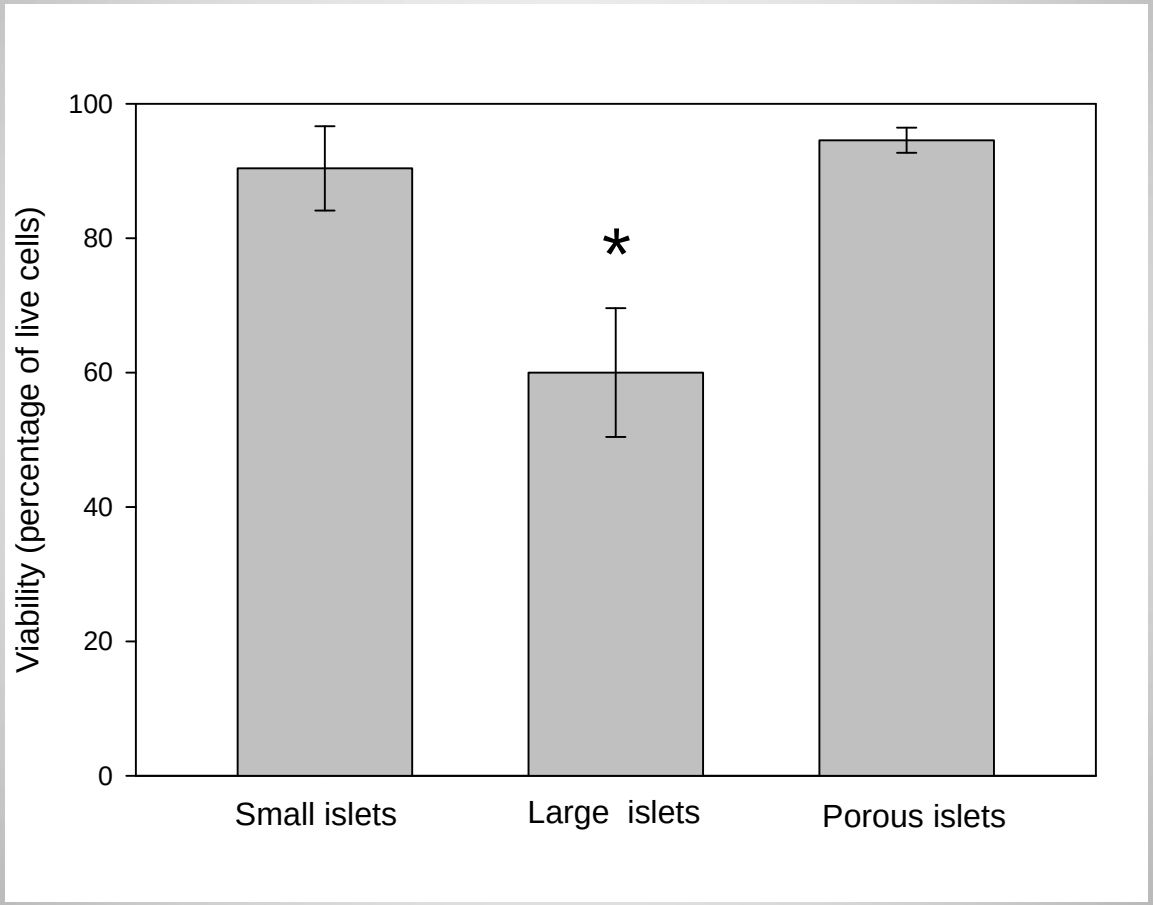
A.

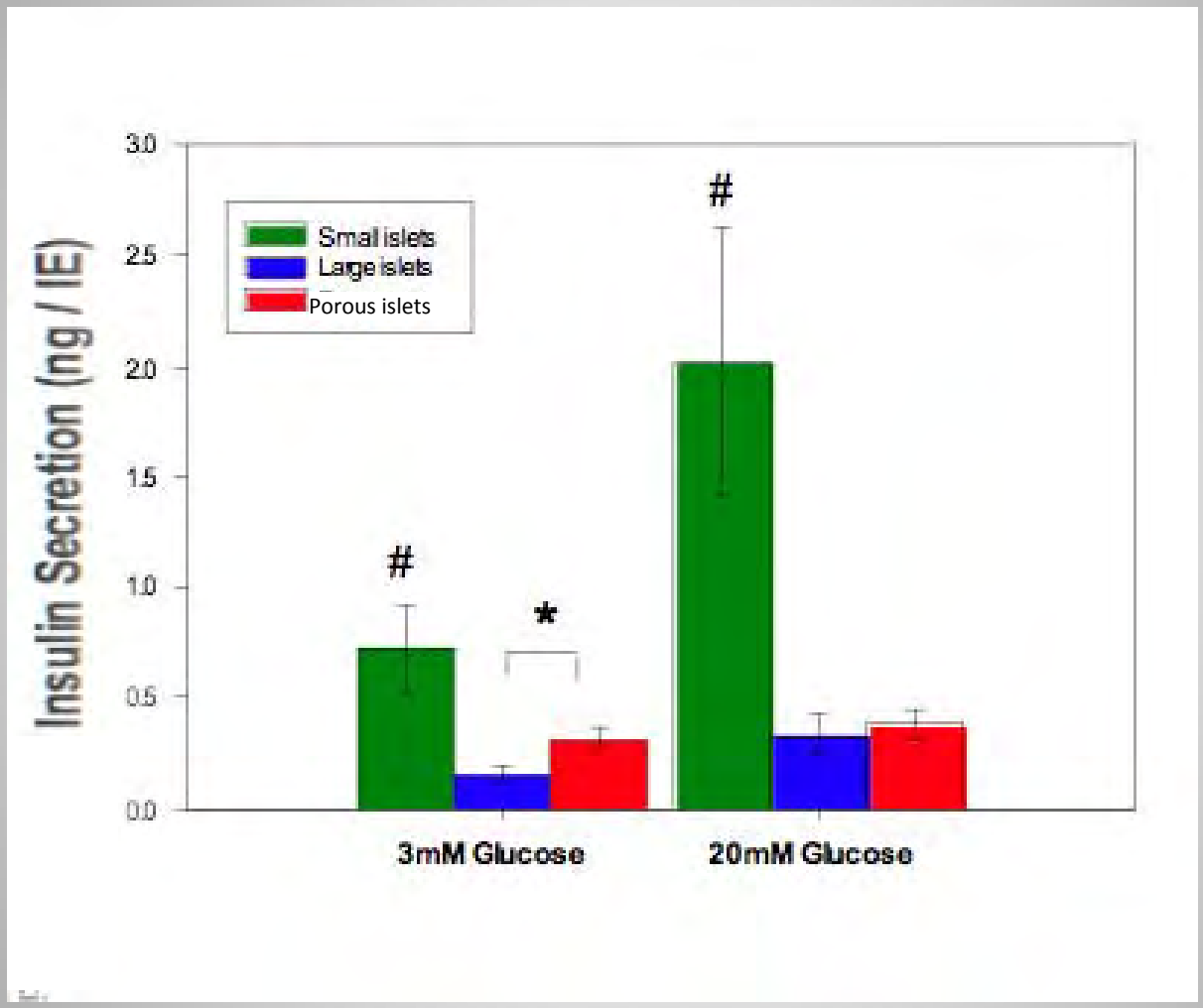

50.0µm

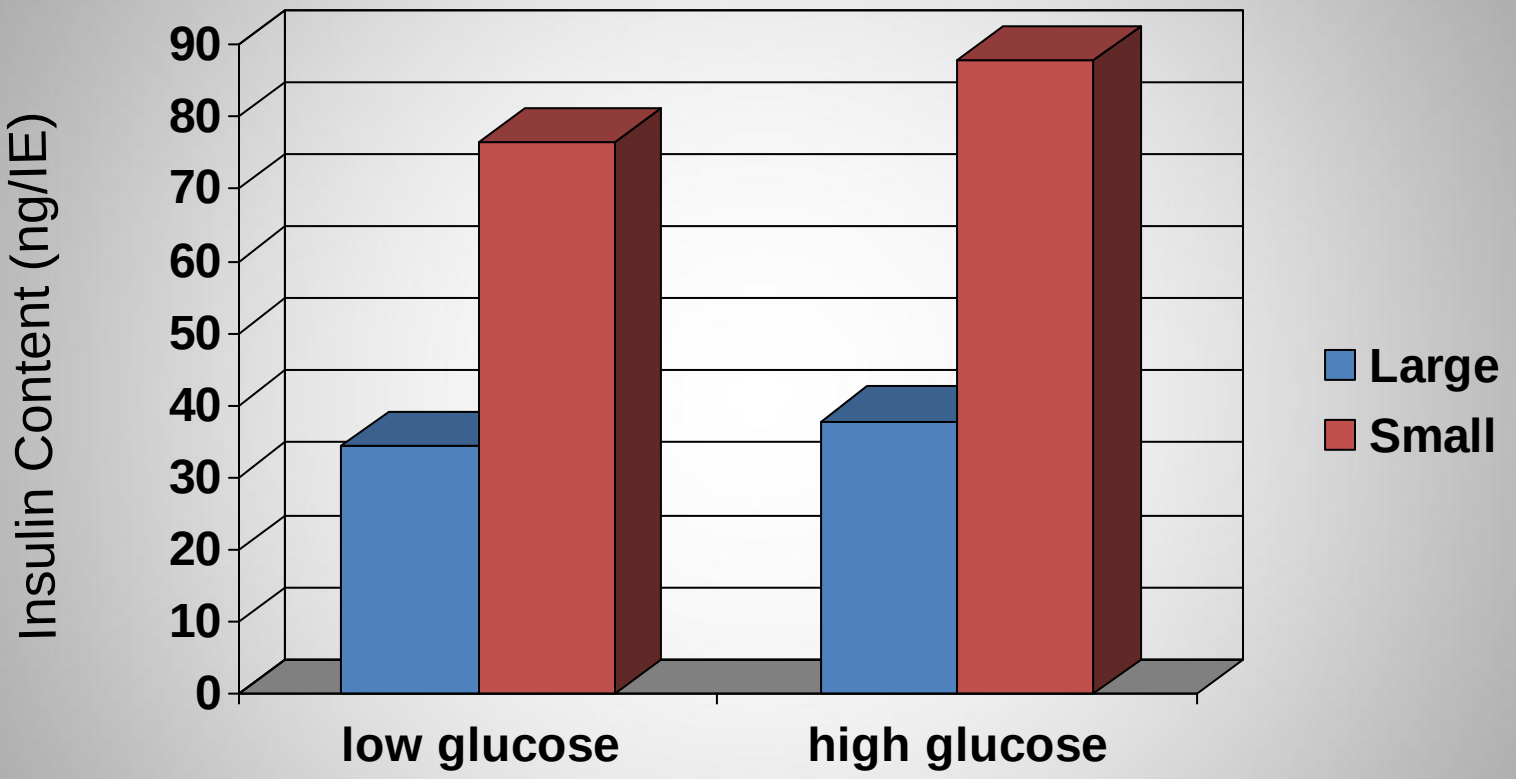
B.


Optical Density (arbitrary units)
30
25
20
15
10
5
0
*
Intact Islets
Porous Islets

Increasing Surface Area Increased Viability




Insulin Secretion (ng / IE)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Small islets
Large islets
Porous islets
#
*
#
3mM Glucose
20mM Glucose


Insulin Content (ng/IE)
90
80
70
60
50
40
30
20
10
0
low glucose
high glucose
Large
Small

Is there something inherently different between small and large islets?

Are these different sub-populations of islets?

Evidence of Islet Subpopulations

Morphological analysis, first reported differences in size distribution, number and volume in several species (Haist & Pugh, *Am. J. Physiol*. 1947)

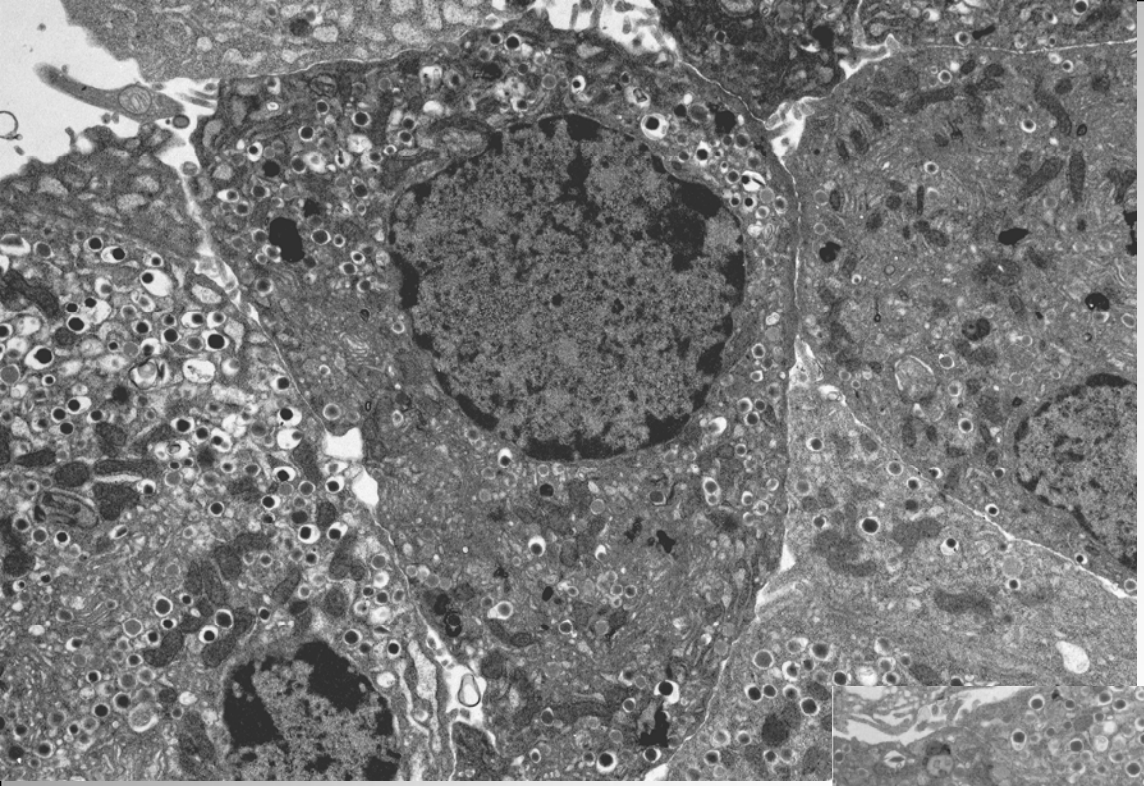
Morphological differences in human islets (Saito et al., *J. Exp. Med.* 1978)

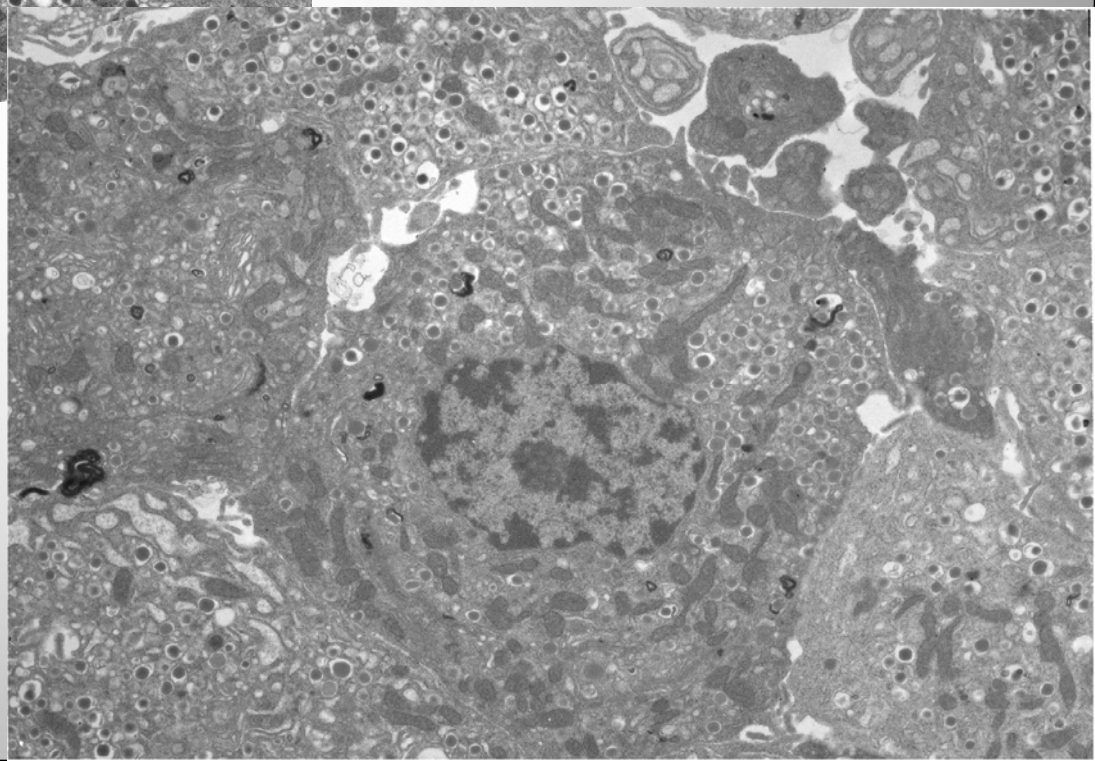
3-D reconstruction demonstrates 2 types of islets (Baetens et al, *Science*, 1979)

Histochemical differences is sub-populations in rat islets (Elayat et al, *J. Anat*. 1995)

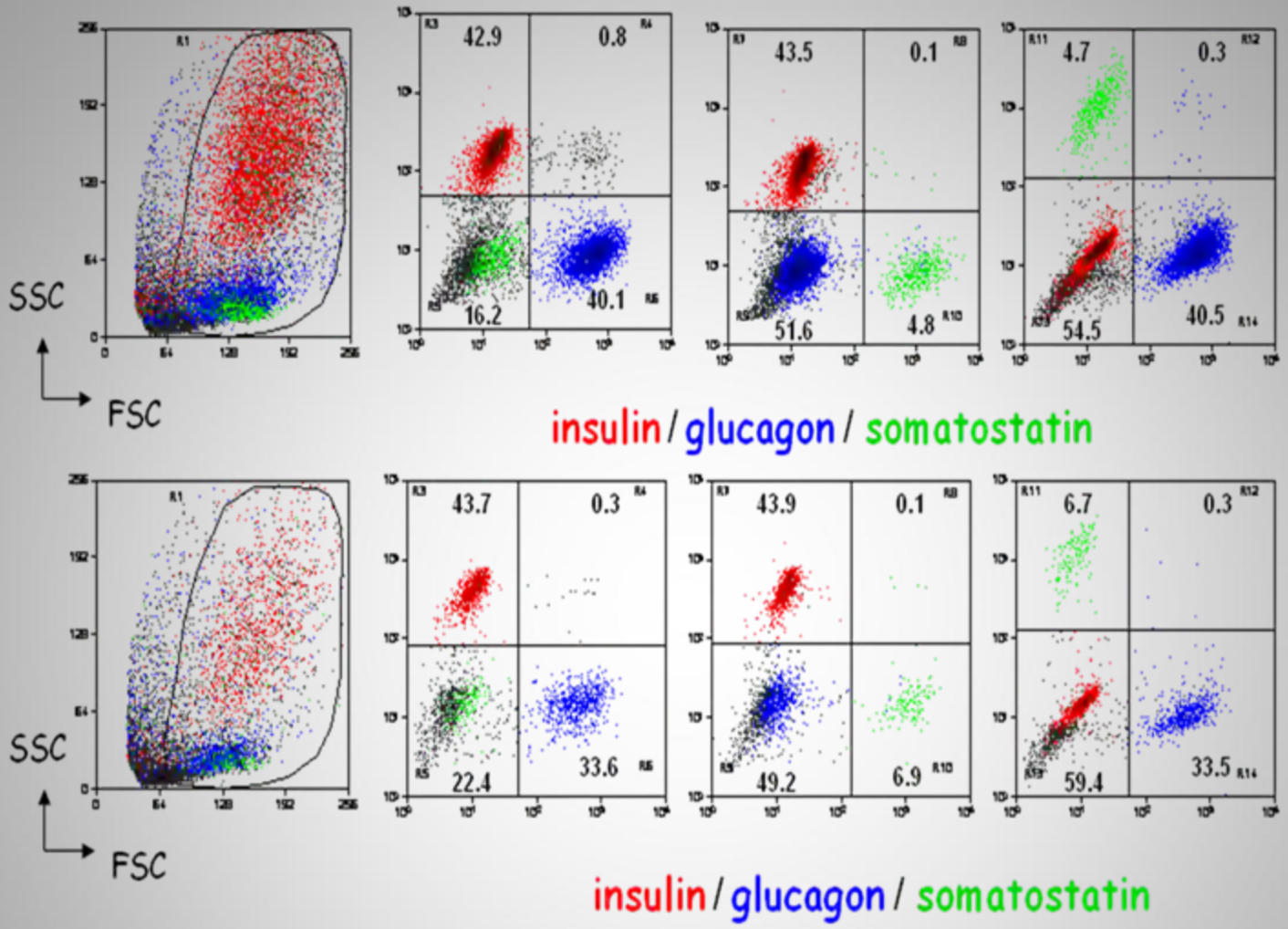
Functional differences in islet populations in healthy animals (Aizawa et al, *Life Sci*. 2001)

Cell from small islet





Cell from large islet

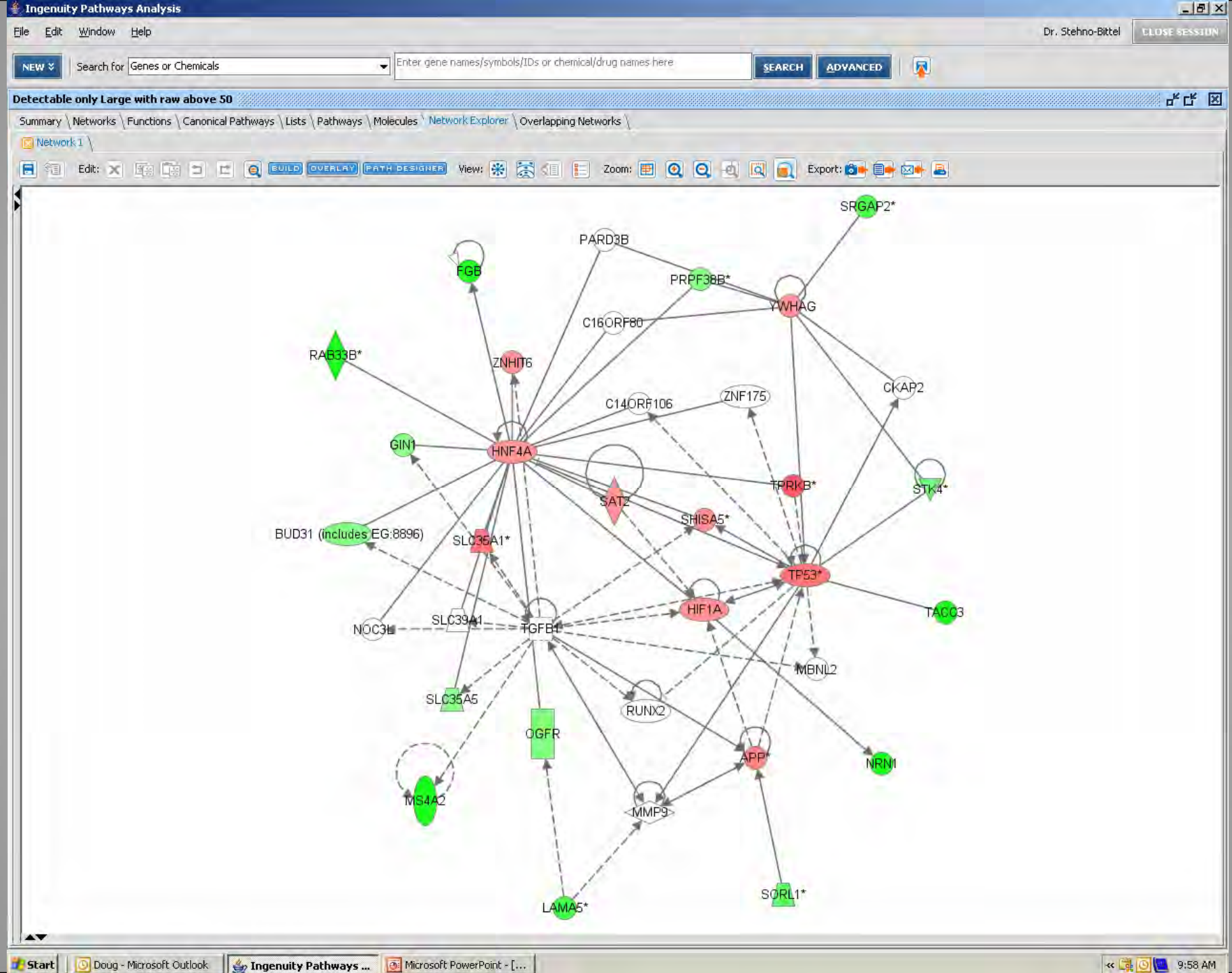


Type of cell	Large Islets	Small Islets
Beta (insulin)	52%	57%
Alpha (glucagon)	38%	30%
Delta (somatostatin)	11%	6%

Total DNA content was 3 times higher in small islets than large when normalized for EI (rats).

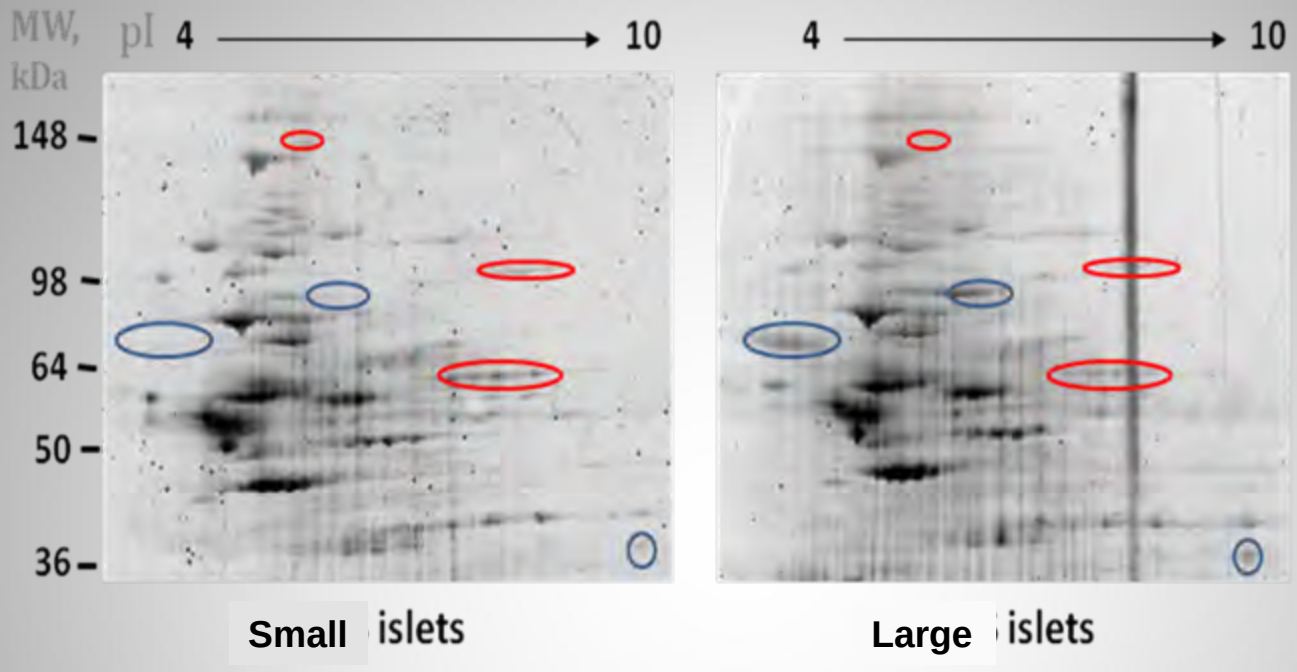
Is there a difference in genetic regulation in large and small islets?

Gene (protein product)	Fold difference in expression
Reg 1 Regenerating Gene	7.11 greater in small islets
Atk1 Protein kinase B	6.10 greater in small islets
Pdx-1 Pancreatic Duodenal Homeobox-1	2.41 greater in large islets
VEGF Vascular endothelial growth factor	12.04 greater in large islets
IAPP Islet Amyloid Polypeptide	2.55 greater in small islets
LDLR Low Density Lipoprotein Receptor	4.26 greater in large islets
Ins2 Pre-insulin	2.97 greater in large islets
Irs2 Insulin receptor substrate	15.27 greater in large islets
Ddit3 (part of CHOP 10) (differentiation and apoptosis)	4.56 greater in large islets
HNF4a Hepatocyte Nuclear Factor (beta cell development)	2.38 greater in small islets
GLUT 2/Slc2a2 Glucose transporter	8.88 greater in large islets
NOS2 Nitric Oxide Synthase	3.71 greater in small islets


Ingenuity Pathways Analysis
File Edit Window Help
Dr. Stehno-Bittel
CLOSE SESSION
NEW
Search for Genes or Chemicals
Enter gene names/symbols/IDs or chemical/drug names here
SEARCH
ADVANCED
Detectable only Large with raw above 50
Summary Networks Functions Canonical Pathways Lists Pathways Molecules Network Explorer Overlapping Networks
Network 1
Edit: BUILD OVERLAY PATH DESIGNER View: Zoom: Export:
SRGAP2*
PARD3B
FGB
PRPF38B*
YWHAG
C16ORF80
RAB33B*
ZNHIT6
C14ORF106
ZNF175
CKAP2
GIN1
HNF4A
TPRKB*
STK4*
SAT2
SHISA5*
BUD31 (includes EG:8896)
SLC35A1*
TP53*
HIF1A
TACC3
NOC3L
SLC39A1
TGFB1*
MBNL2
SLC35A5
RUNX2
OGFR
APP*
NRN1
MS4A2
MMP9
LAMA5*
SORL1*
Start
Doug - Microsoft Outlook
Ingenuity Pathways ...
Microsoft PowerPoint - [...
9:58 AM

Total Protein Content in an islet	μg/islet
Small	0.04
Large	0.35

Total Protein Content per volume	μg/IE
Small	0.58
Large	0.10

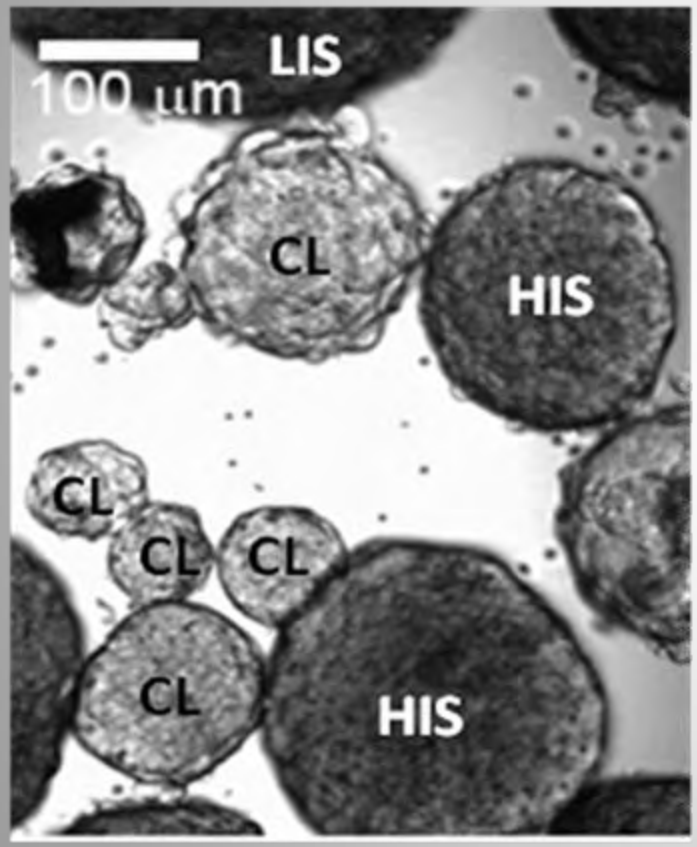


2D PAGE

Small > Large Red

Large > Small Blue

Islet Subpopulations



Small = High insulin secreting islets
Under 125 μm diameter

Large = Low insulin secreting islets
Over 150 μm diameter

CL = Clear islets
Range from 5 – 200 μm

Summary

- Islet sub-populations are associated with different functions.
- Small islets are more viable in vitro
- Small islets secrete more insulin in vitro
- Transplantation with small islets reverse diabetes
- Reducing the diffusion barrier in large islets increases viability, but does not improve in vitro insulin secretion or islet transplantation outcome
- Small islets have greater insulin content
- Ultrastructural differences exist between large and small islets
- Small islets have a higher % of beta cells
- Small islets have more total protein content
- Selective genes are expressed in detectible levels in large islets that are not detected in small islets
- Selective proteins are found in either small or large islets

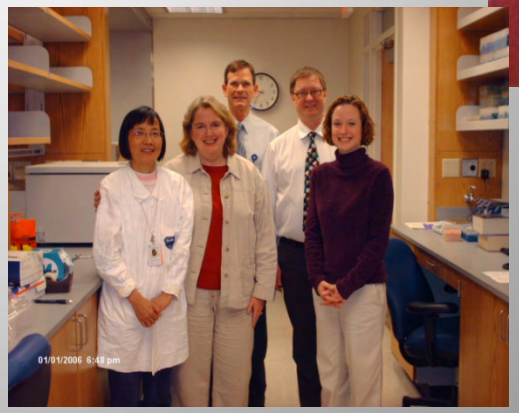
Summary

Why do we care?

Acknowledgements

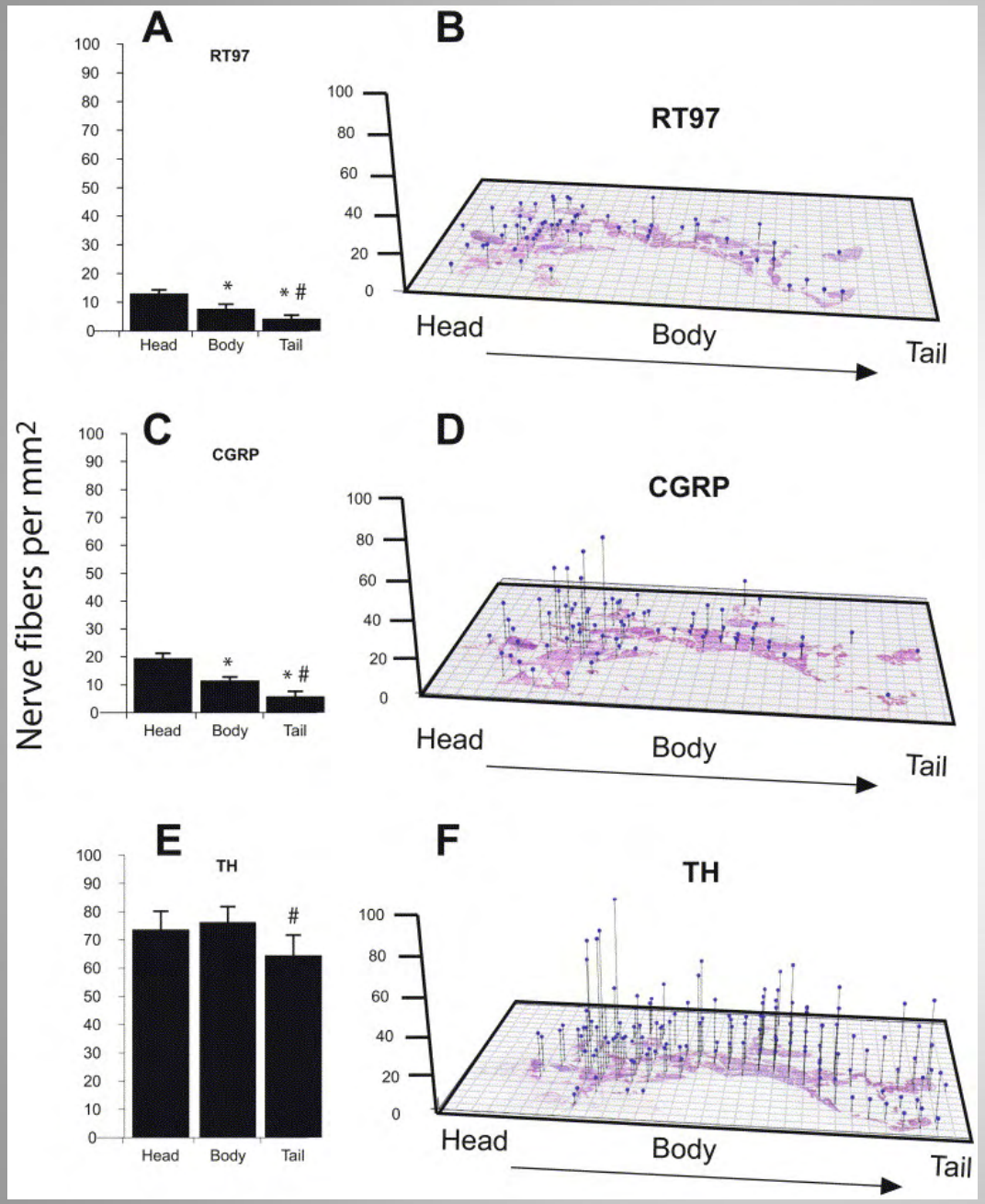
- KU Diabetes Research Lab
  - Dr. Irina Smirnova
  - Dr. Lesya Novikova
  - S. Janette Williams
  - Floyd Huang
  - Maheswari Mukherjee
  - Katie Holztman
  - Dr. Rajprasad Loganathan

- KU, Chemical & Petroleum Engineering
  - Dr. Cory Berkland
  - Dr. Kristin Lindsey Woo
- KU, Anatomy and Cell Biology
  - Dr. Ronal MacGregor
- Children's Mercy Hospital
  - Dr. Wayne Moore
  - Dr. Karen Kover
  - Pei Tong



Acknowledgements

- Juvenile Diabetes Research Foundation
- Emilie Rosebud Diabetes Research Foundation
- NIH (NIDDK, NCRR)
- Union Biometrica

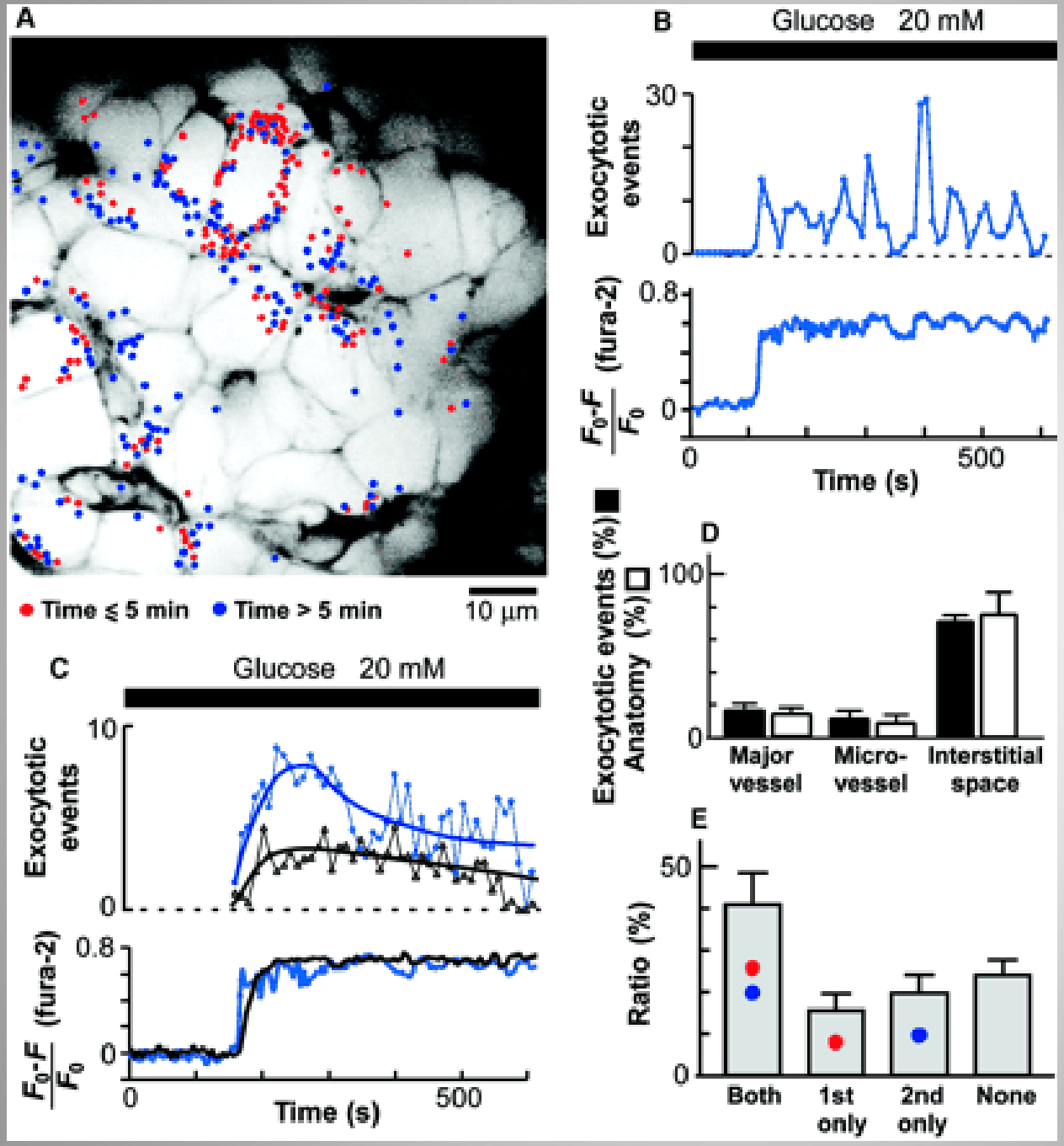


Large diameter, myelinated

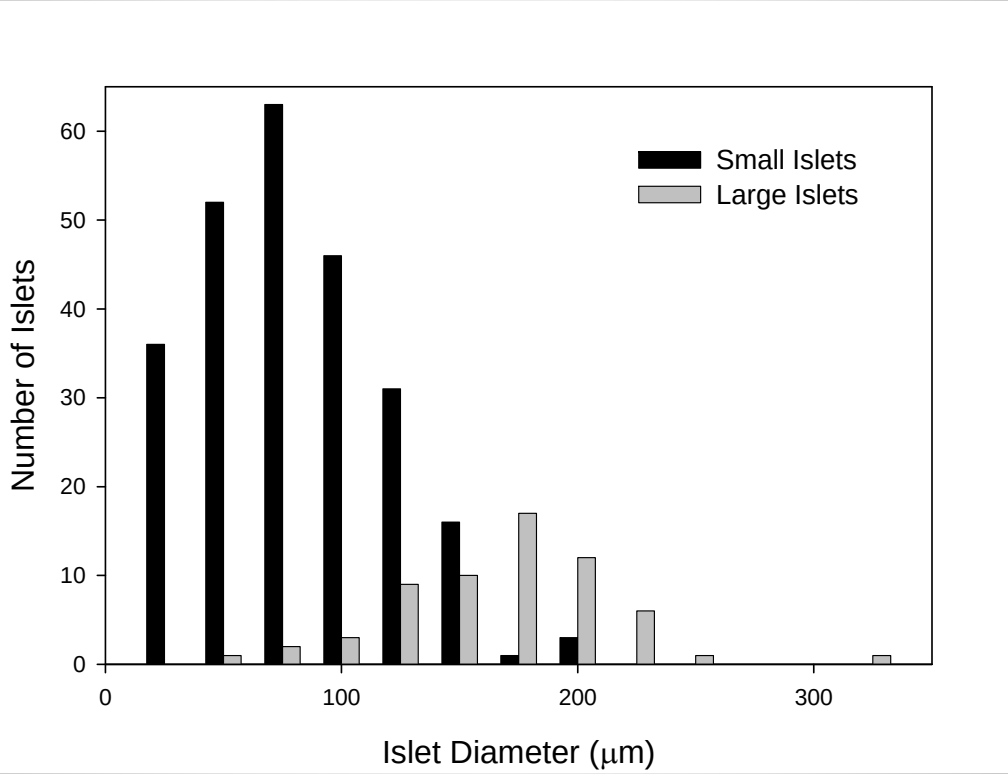
Small diameter thinly myelinated and medium diameter unmyelinated peptidergic

Sympathetic nerve fibers

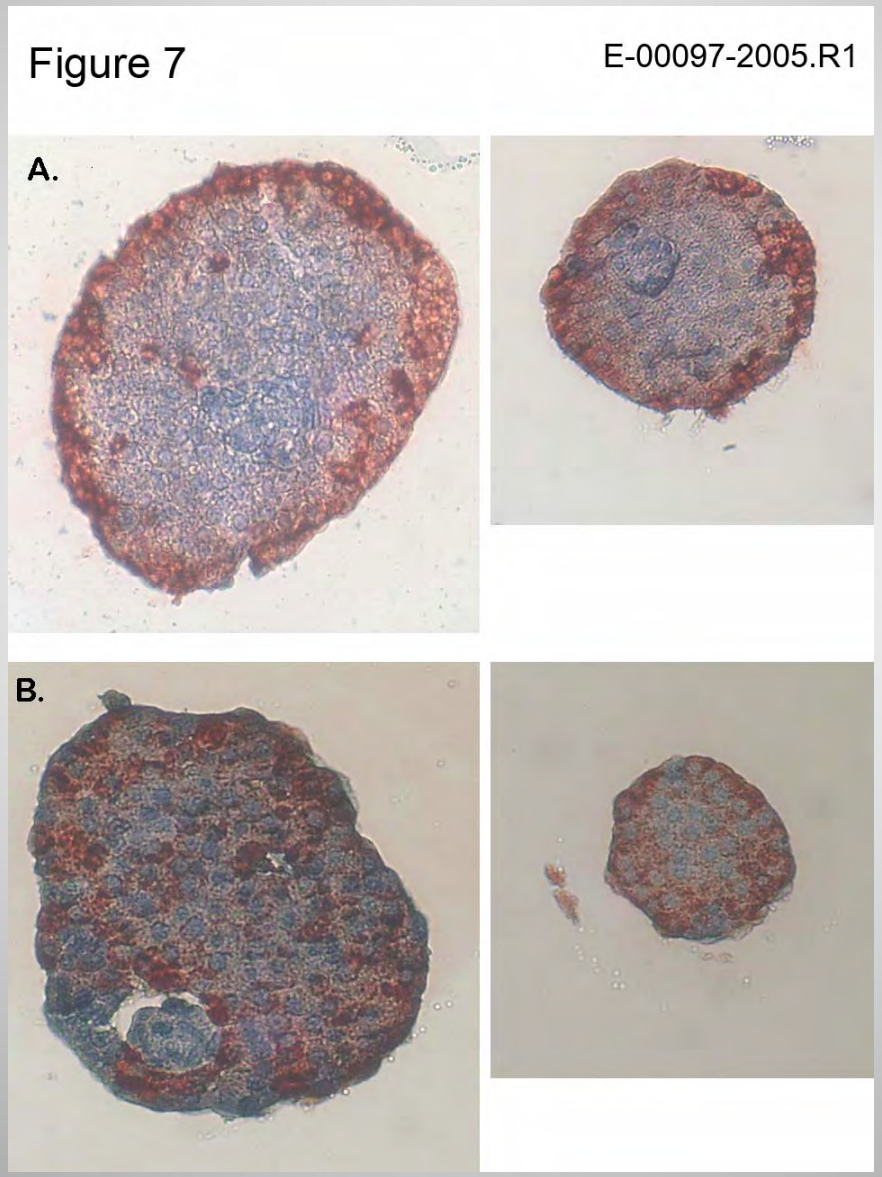
Lindsay et al., 2006



Takahashi et al., Science 2002


Small Islets
Large Islets
Number of Islets
0
10
20
30
40
50
60
0
100
200
300
Islet Diameter (μm)

Distribution of Cells the Same in Both



MacGregor et al, 2006