

Presenter Disclosure Information

Alberto Pugliese, MD

Nothing to disclose

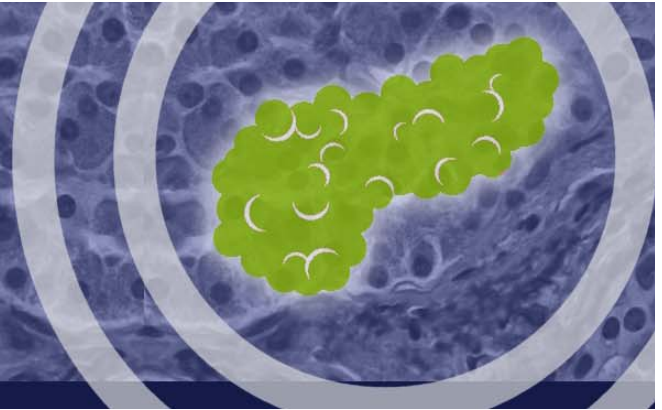
New Insights to Human Type 1 Diabetes from nPOD



- A tissue bank
- A data cloud resource
- An open, collaborative research project focused on key questions about human diabetes

nPOD

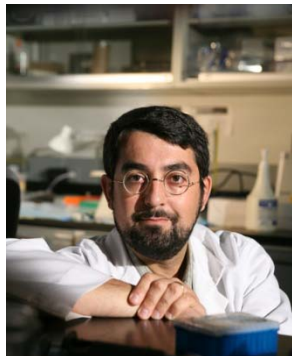
Network for Pancreatic Organ Donors with Diabetes



www.JDRFnPOD.org



Mark Atkinson, PhD
University of Florida
Executive Director

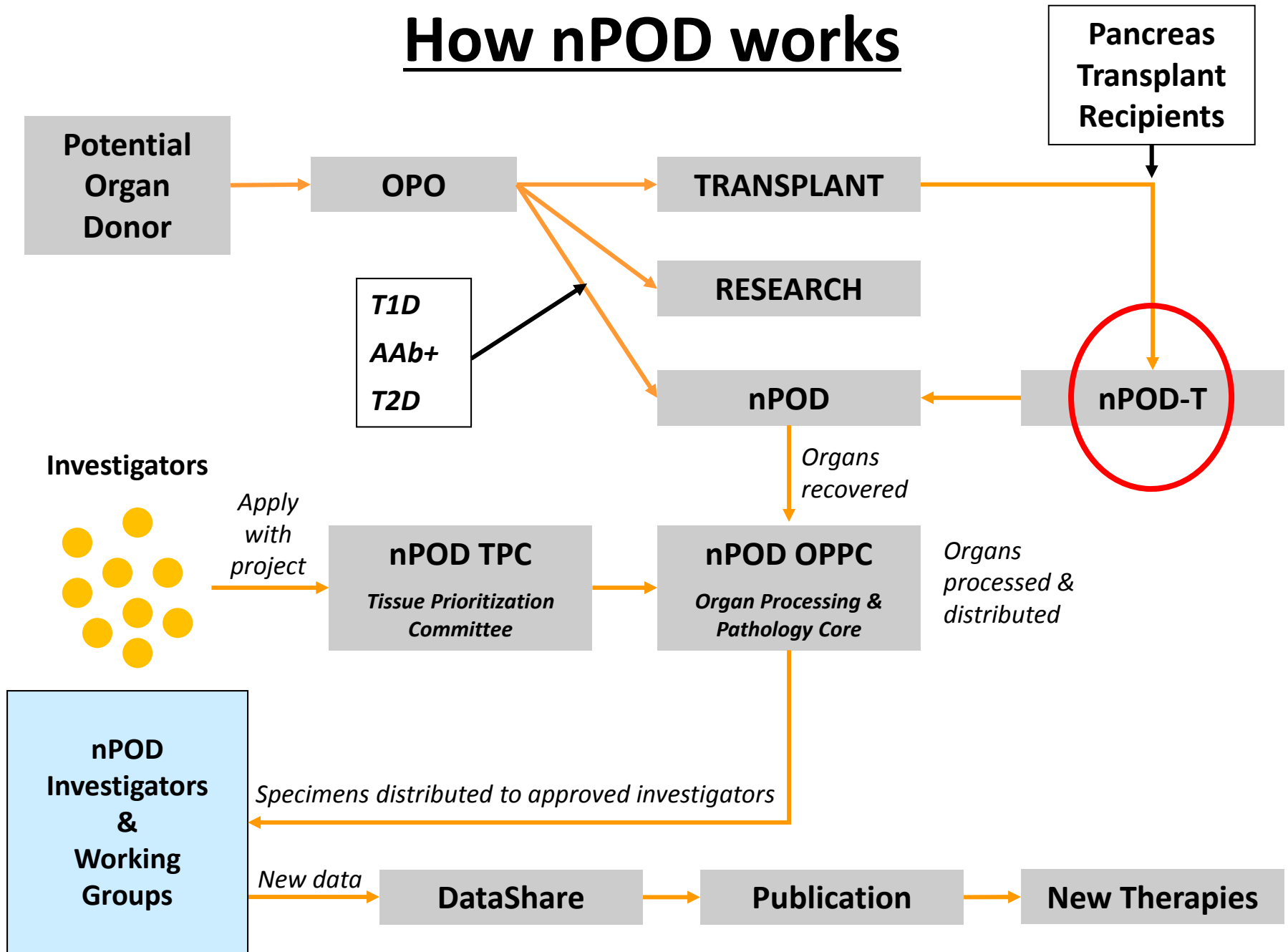


Alberto Pugliese, MD
University of Miami
Executive Co-Director

Mission

- Obtain tissues from organ donors with T1D (diagnosed or sub-clinical)
- Distribute tissues to research projects (>100 since 2007)
- Promote tissue and data sharing, collaboration, manage project interactions
- Promote a comprehensive understanding of human T1D and identify new therapeutic targets

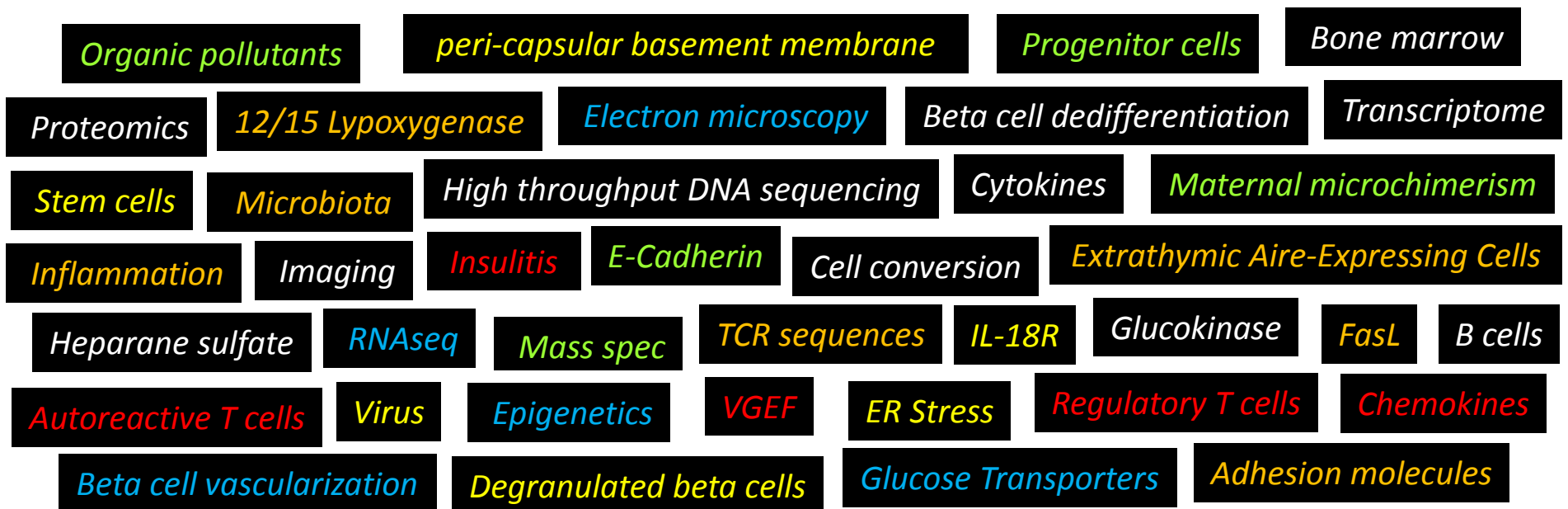
How nPOD works



nPOD supported projects by main research areas

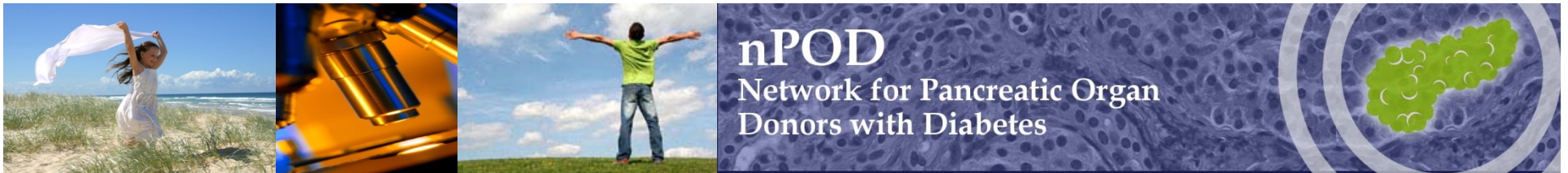
88 active as of June 2013 (from >100 since 2007)

RESEARCH AREA	NUMBER OF PROJECTS
Beta Cell Development, Differentiation & Regeneration	14
Immunology	30
Beta Cell Physiology and Dysfunction	18
Pathology	16
Type 1 Diabetes Etiology & Environment	10



nPOD Recoveries as of June 2013

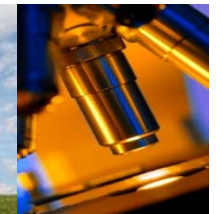
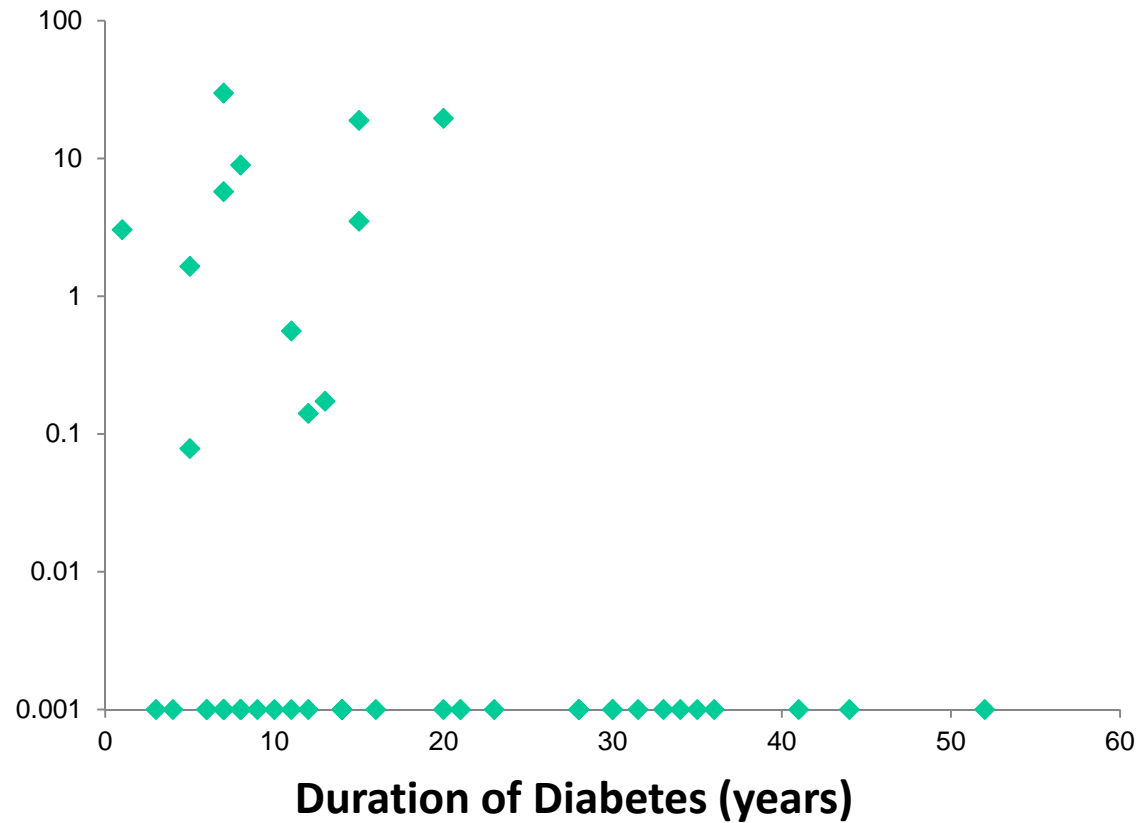
Donor Type	Number	Age (Mean)	Age (StDev)	Minimum	Maximum
No diabetes	82	22.21	20.60	0	75
Autoab Pos	19	32.38	18.46	0.17	66
T1D	67	28.12	11.74	4.4	61.0
T1D Medalist	10	74.30	9.43	59	89
T2D	17	44.5	15.18	18.8	76.3
T2D with Incretin Therapy	9	58.4	10.3	46.0	74.2
Gestational diabetes	2	33.35	0.21	33.2	33.5
Pregnancy	1	38		38	38
Cystic fibrosis	3	31.13	1.85	29.3	33
Other	6	30.6	16.37	15.5	62
Pending	4	28	10.94	17	42.9
Fulminant	1	14.00		14	14
Transplant	3	43.00	6.24	38	50
Total	224				



Beta cell mass versus T1D duration

Roberto Gianani

Percentage of control mean BCM in gms



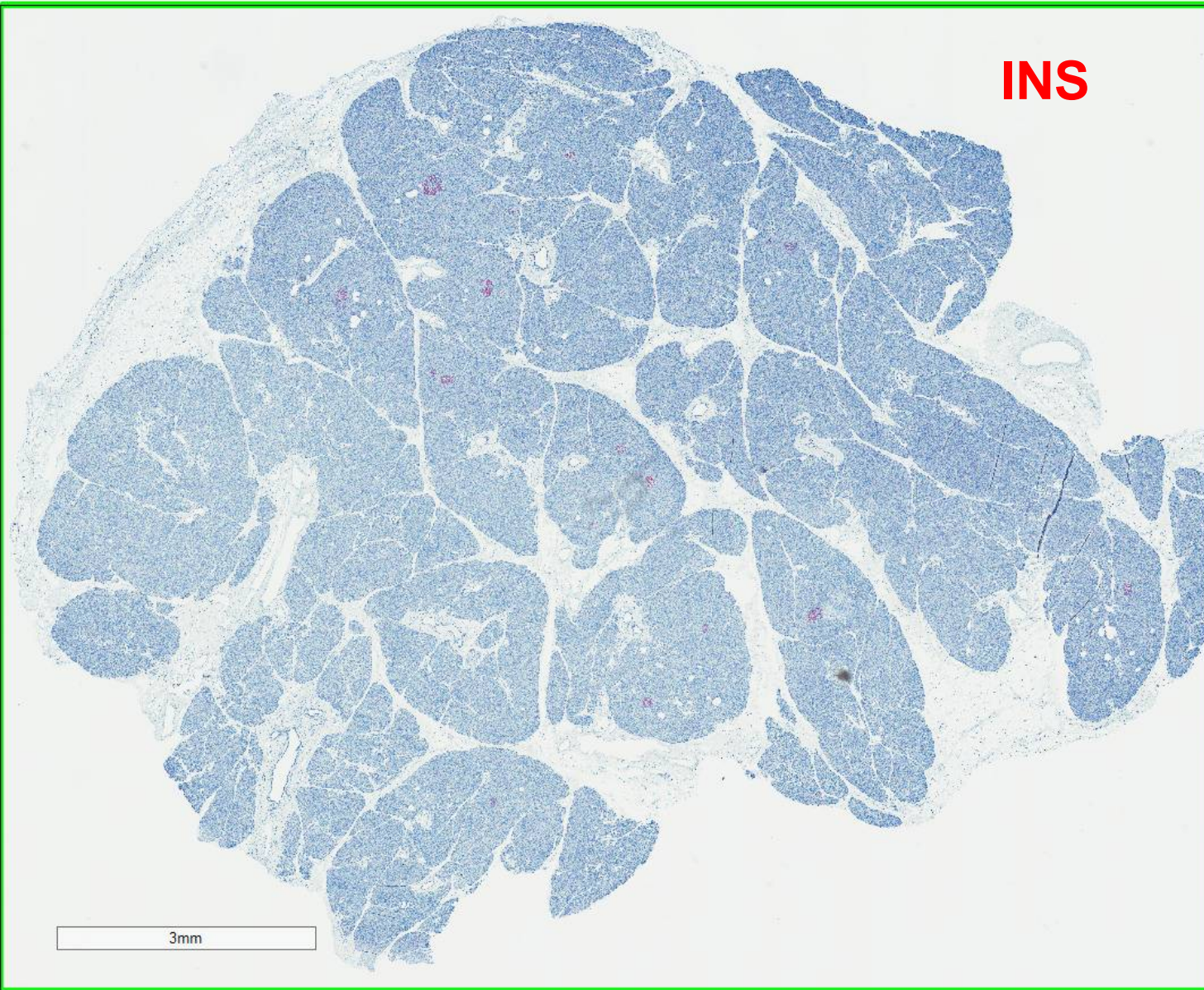
T1D donors with residual beta cell mass

Roberto Gianani

Case #	Age of onset	DM Duration in years	Antibody status	Insulinitis	HLA-DR	Residual BCM (% of control mean)
6069	15.00	7.00	na	no	DR3/DR12	29.86
6039	16.70	12	P	yes	DR4/DR3	0.14
6046	10.80	8.00	P	no	DR1/DR4	8.97
6052	11.00	1.00	P	yes	DR9/DR2	3.04
6070	15.60	7.00	P	yes	DR10/DR2	5.75
6088	26.20	5.00	P	yes	DR1/DR3	1.65
6180	16.00	11.00	P	no	DR1/DR3	0.56
6196	11.00	15.00	P	no	DR12/DR13	18.87
6195	14.20	5.00	P	no	DR4/DR2	0.08
6038	17.20	20.00	N	no	DR1/DR3	19.58
6051	7.30	13.00	N	no	DR2/DR3	0.17
6081	16.40	15.00	N	no	DR4/DR8	3.50

Longer Duration T1D – 8 years

INS



6046

18 years old

8 year duration

Caucasian Female

AutoAb: IA2A+ZnT8+

C peptide: <0.05 ng/ml

BMI: 25.2

Histopathology : Ins+ islets in some lobules, other lobules/entire blocks ins-/gluc+ islets. Insulitis +. CD3+ or CD45+ used. Also infiltrates are mainly acinar/extra-acinar. Mild acinar atrophy and adipose infiltration.

HLA: A*0201/0301

B*1501/3901

DRB1*0101/0401

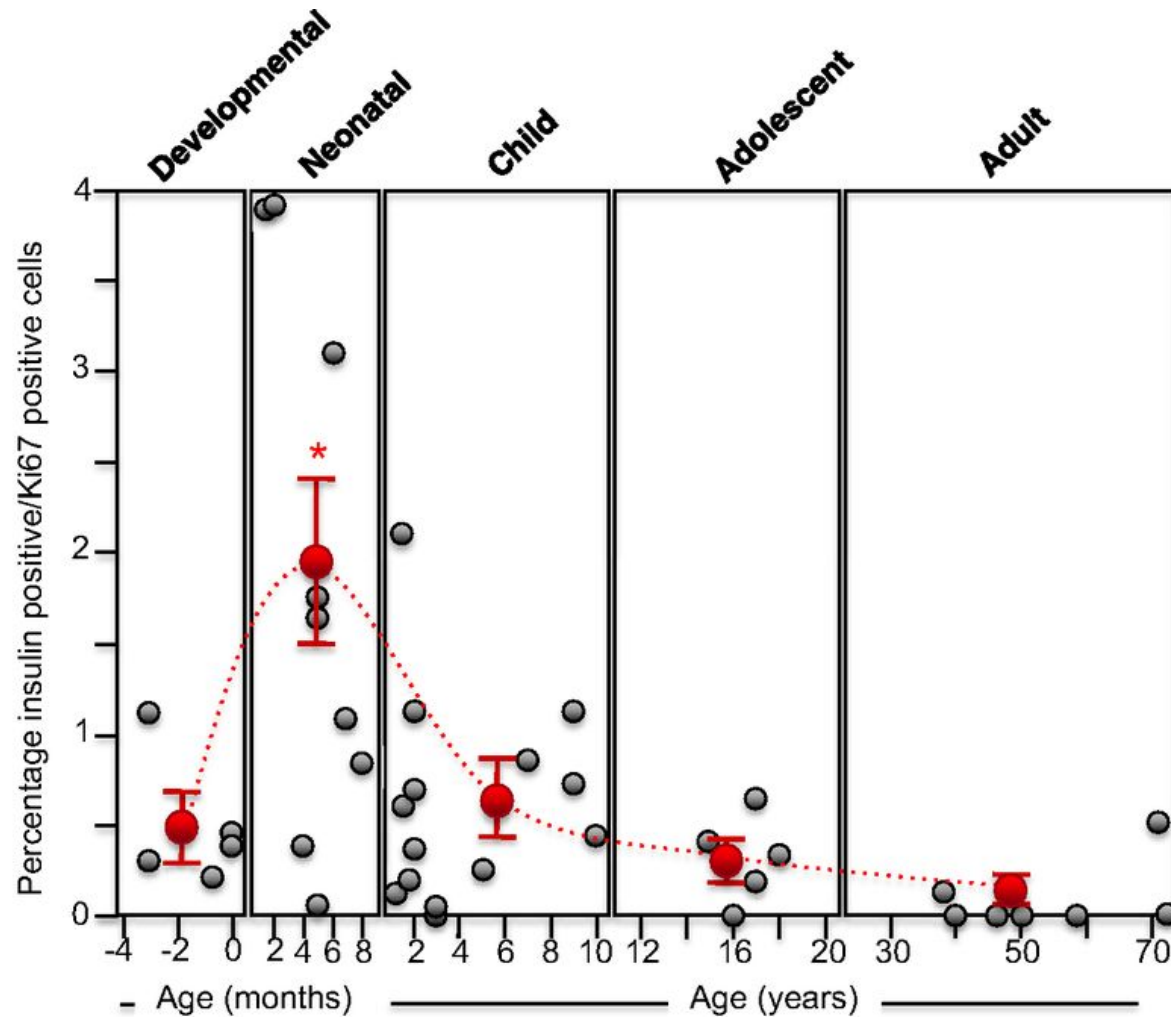
DQA1*0101/0301

DQB1*0302/0501

Formation of a Human β -Cell Population within Pancreatic Islets Is Set Early in Life

Brigid E. Gregg, Patrick C. Moore, Damien Demozay, Ben A. Hall, Mei Li, Aliya Husain, Amy J. Wright, Mark A. Atkinson, and Christopher J. Rhodes

THE JOURNAL OF
CLINICAL
ENDOCRINOLOGY
& METABOLISM
2012;97:3197-3206

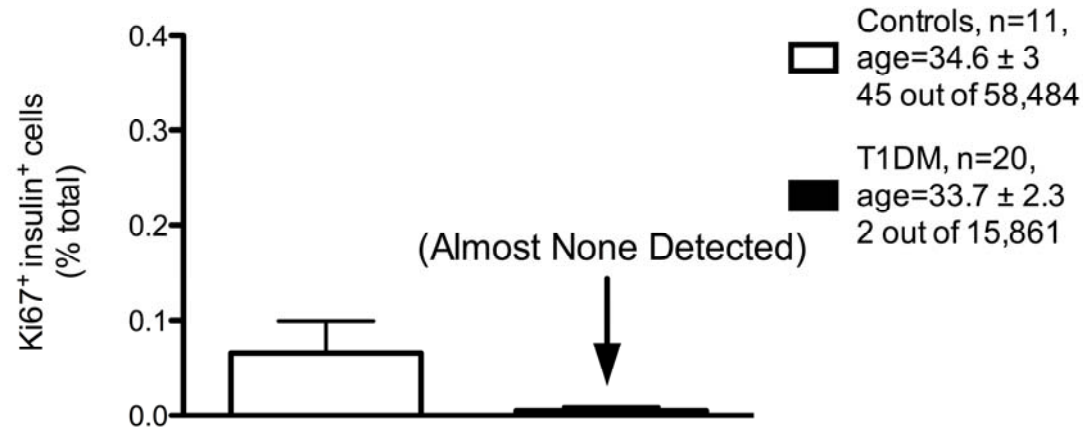


β -cell persist in some T1D pancreata without evidence of replication nor insulin-glucagon co-expression

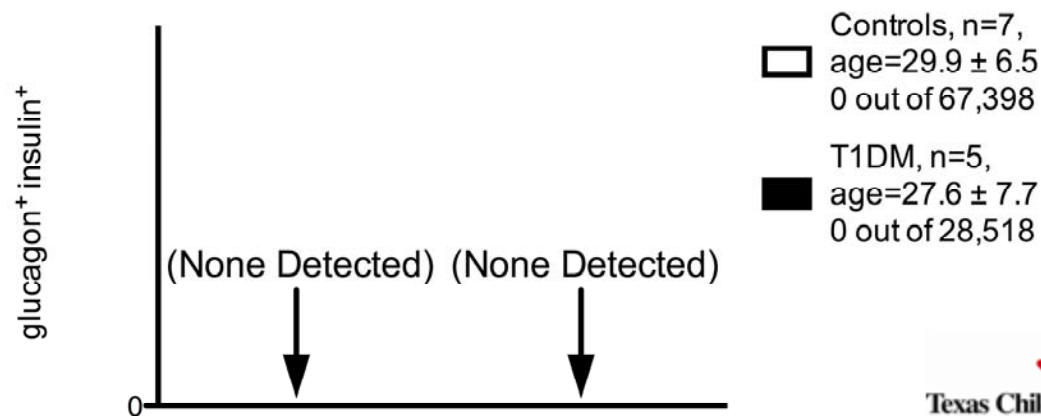
Replication is actually reduced in adult T1D

Matthew Rankin and Jake Kushner

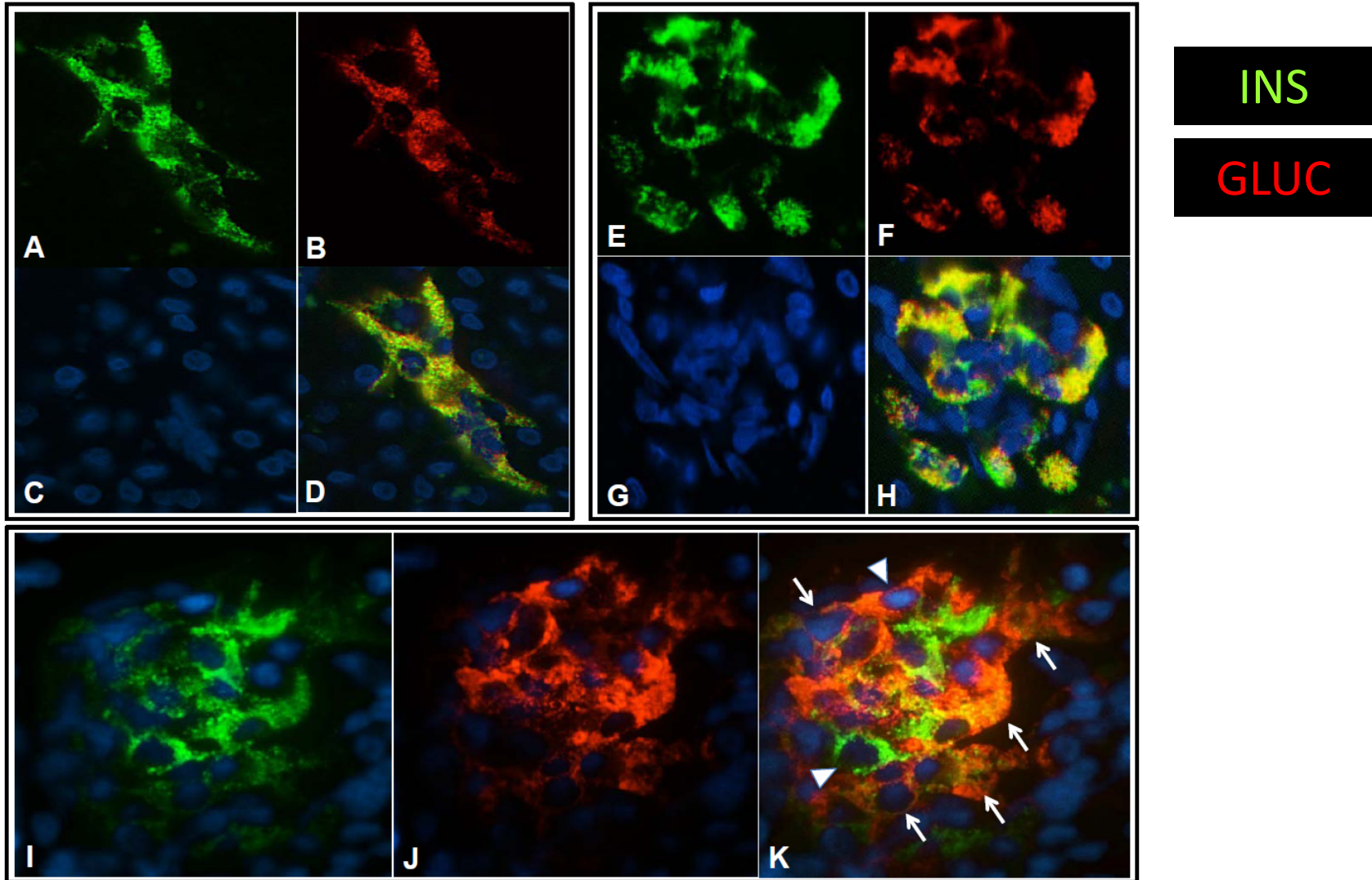
Beta Cell Replication in Adults (19-50 yrs)



Copositives

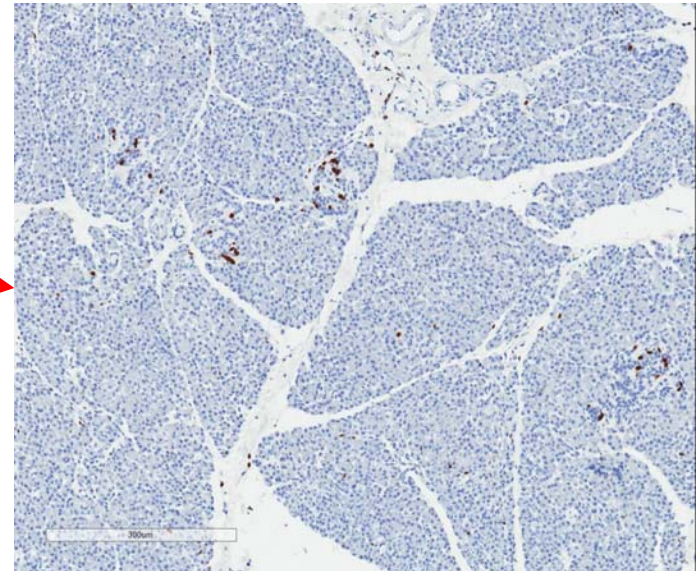
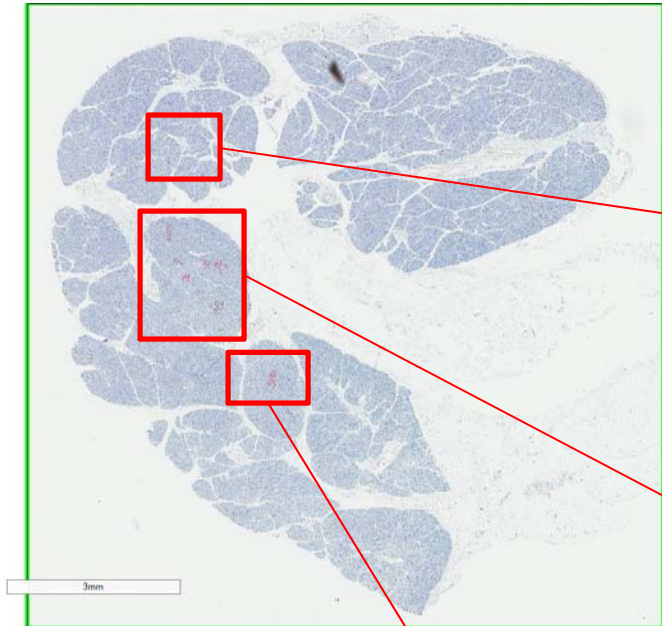


Double-hormone positive islet cells found in T2D donors treated with incretin therapies



Butler et al. Diabetes 2013

INS Ki67



6052
12 years old (**1 year duration**) Male AA
AutoAb: IA2A+mIAA+
C peptide: 0.18 ng/ml

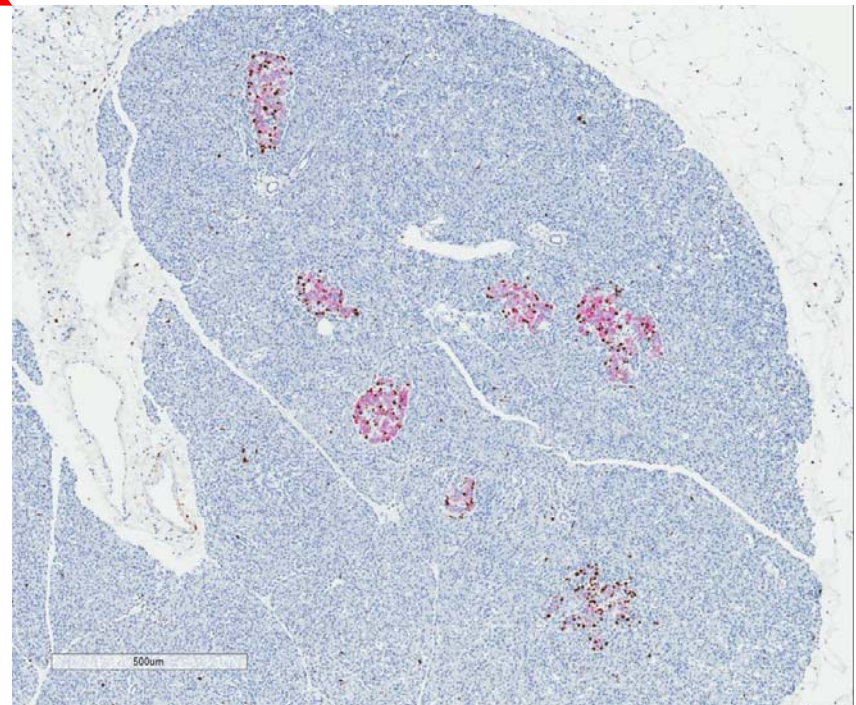
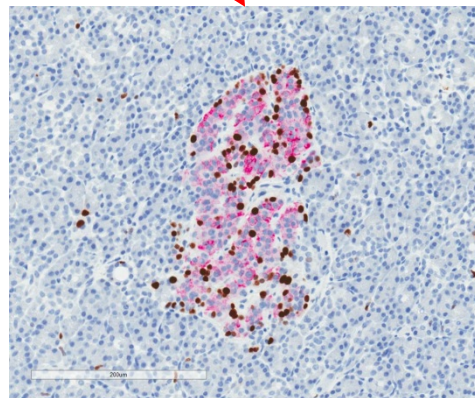
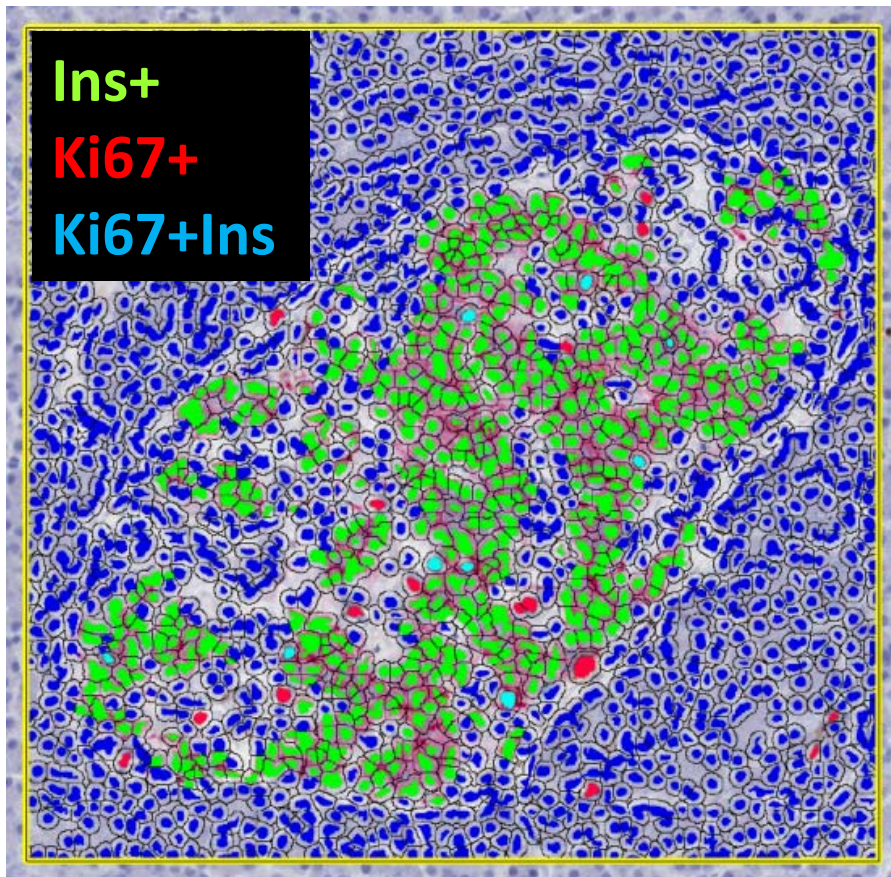
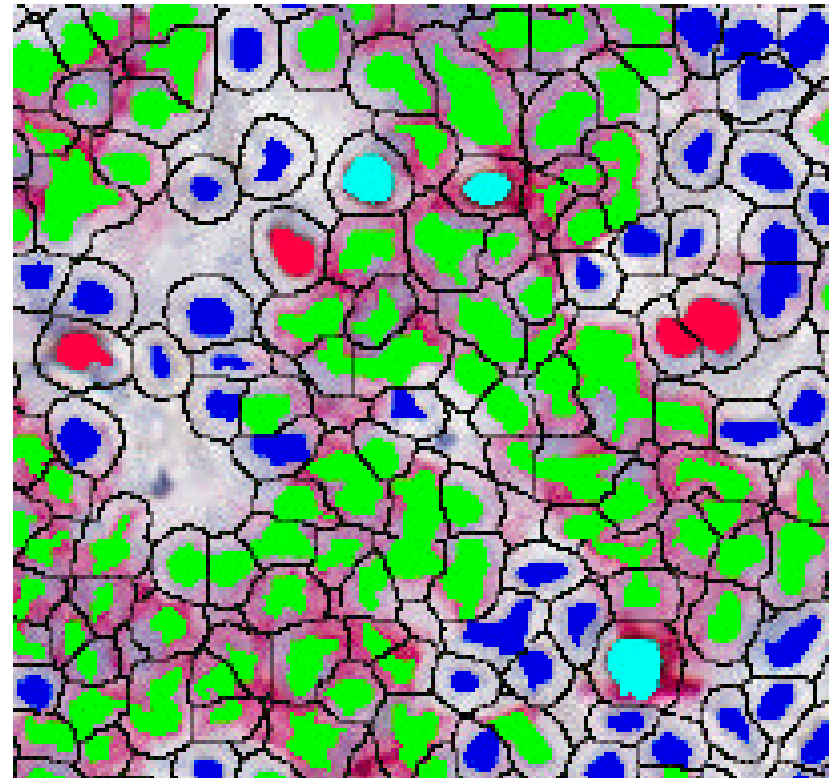


Image Analysis- β cell Quantification
Cell numbers, Ki67+ colocalization (replication)
Martha Campbell-Thompson



6052
12 years old (1 year duration) Male AA
2 days on ventilator!

Indica Labs Cytonuclear Software

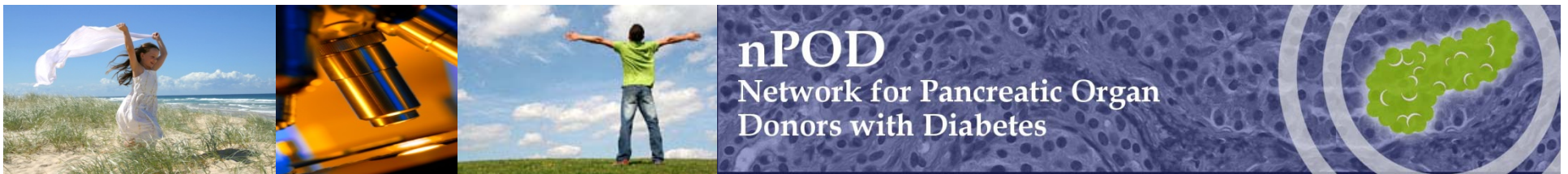


~30% of Ki67+ cells were beta cells
Unusual but it can occur!

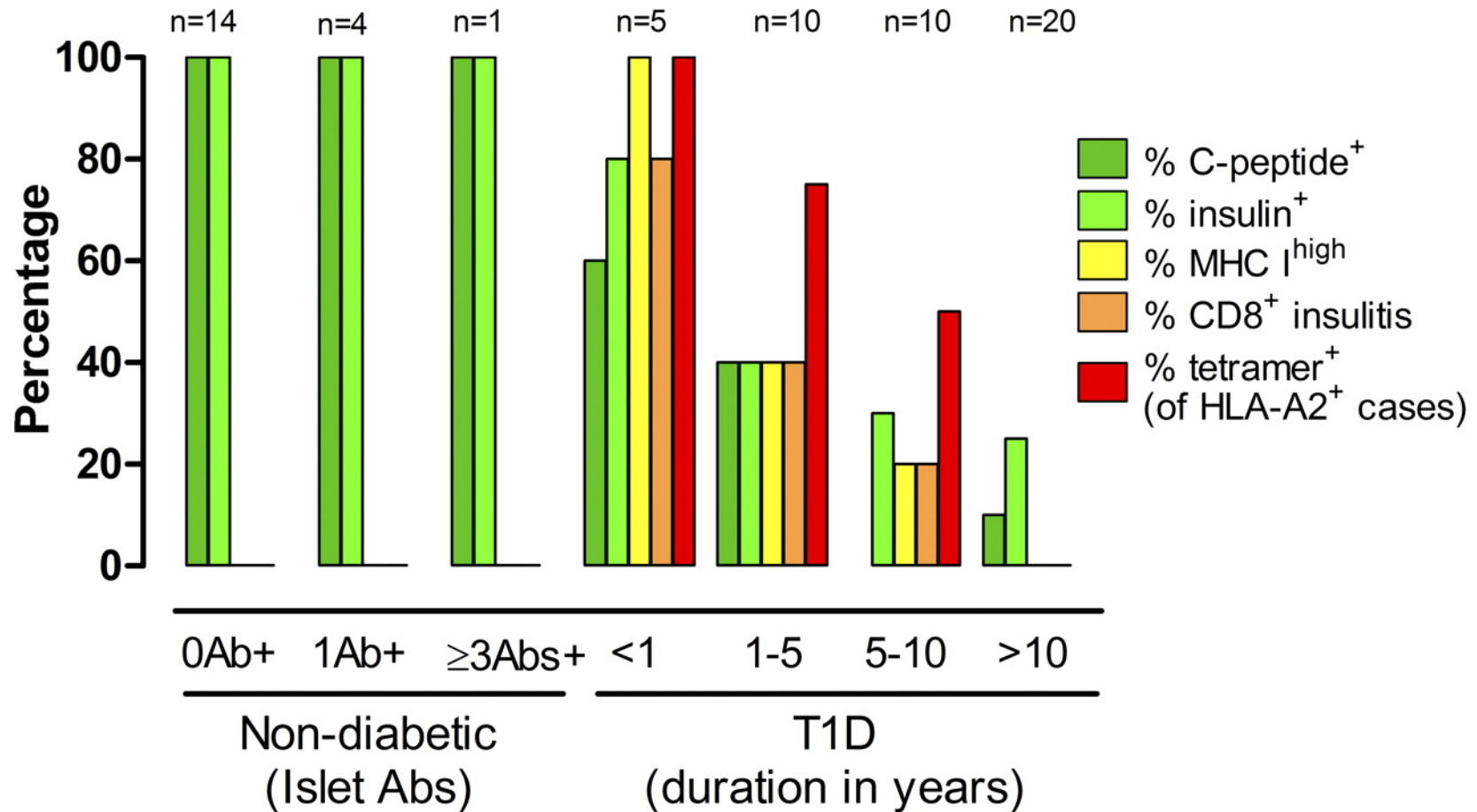
nPOD Donors with Insulinitis (15 as of June 2013)

Case	Group	AutoAb Status	Age (years)	Duration (years)	Gender	Ethnicity	C-peptide (ng/ml)	Insulinitis (%)
6197	AAb+	GADA+ IA2A+	22	N/A	Male	AA	17.48	pending
6228	T1D	GADA+ IA2A+ ZnT8+	13	0	Male	C	0.1	pending
6209	T1D	IA2A+ZnT8+mIAA+	5	0.25	Female	UND	0.1	pending
6052	T1D	IA-2A+ mIAA+	12	1	Male	AA	0.18	9
6113	T1D	mIAA+	13.1	1	Female	C	<0.05	3.8
6063	T1D	mIAA+	4.4	3	Male	C	<0.05	1
6198	T1D	GADA+ IA2A+ ZnT8+ mIAA+	22	3	Female	H	<0.05	pending
6088	T1D	GADA+ IA-2A+ ZnT8A+ mIAA+	31.2	5	Male	C	<0.05	0.5
6195	T1D	GADA+ IA2A+ ZnT8+ mIAA+	19.2	5	Male	C	<0.05	3.6
6212	T1D	mIAA+	20	5	Male	C	<0.05	pending
6062	T1D	No serum available	10.7	6	Male	AA	n/a	0.7
6070	T1D	IA-2A+ mIAA+	22.6	7	Female	C	<0.05	4.5
6046	T1D	IA-2A+ ZnT8A+	18.8	8	Female	C	<0.05	2.6
6039	T1D	GADA+ IA-2A+ ZnT8A+ mIAA+	28.7	12	Female	C	<0.05	1
6076	T1D	GADA+ mIAA+	25.8	14	Male	C	<0.05	0.9

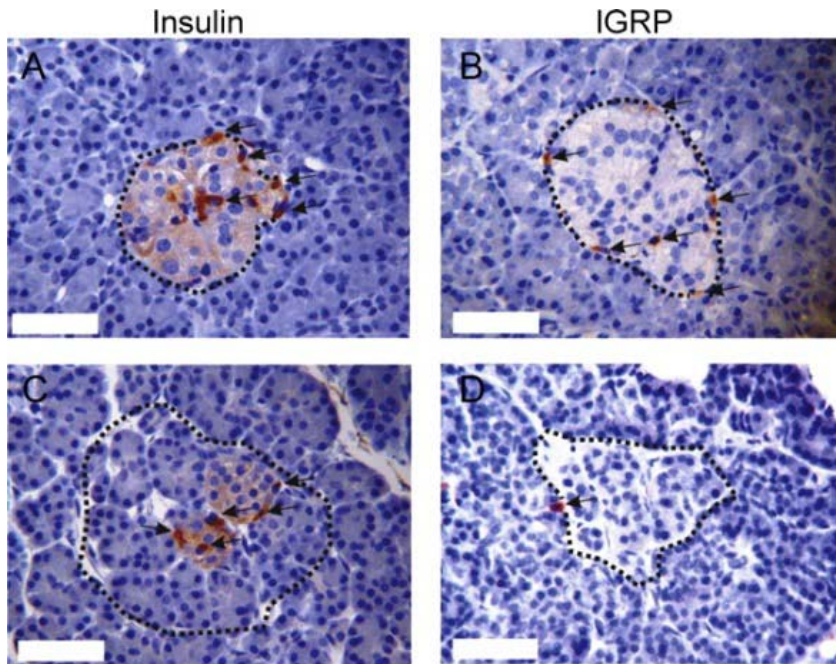
Insulinitis: ≥ 6 CD3 T cells adjacent or within islets ($n \geq 3$ islets/section) with pseudoatrophic (insulin deficient) islets within pancreas (M. Campbell-Thompson)



Overview of results from systematic staining and in situ tetramer assay



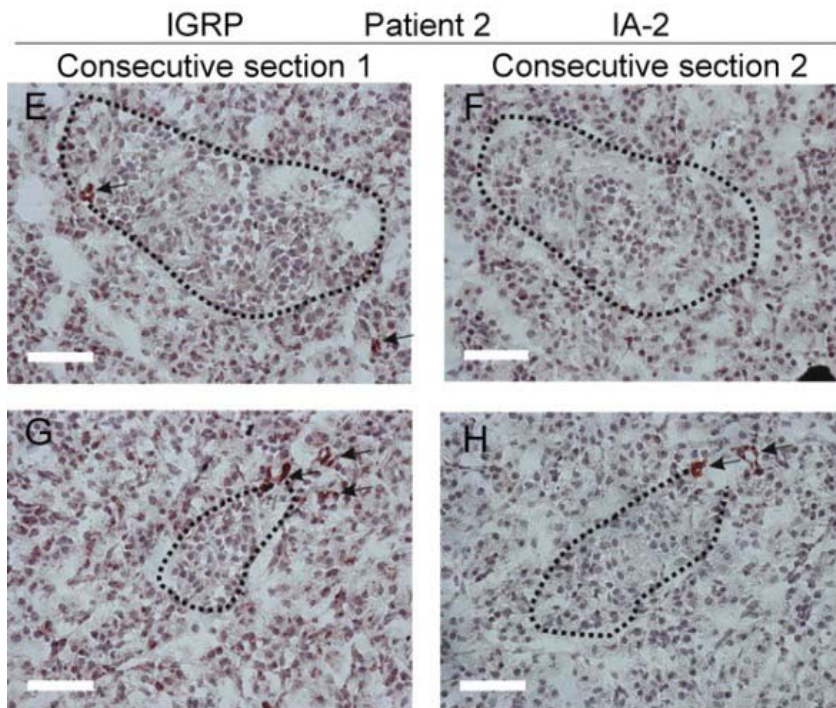
Coppieters K T et al. J Exp Med 2012;209:51-60



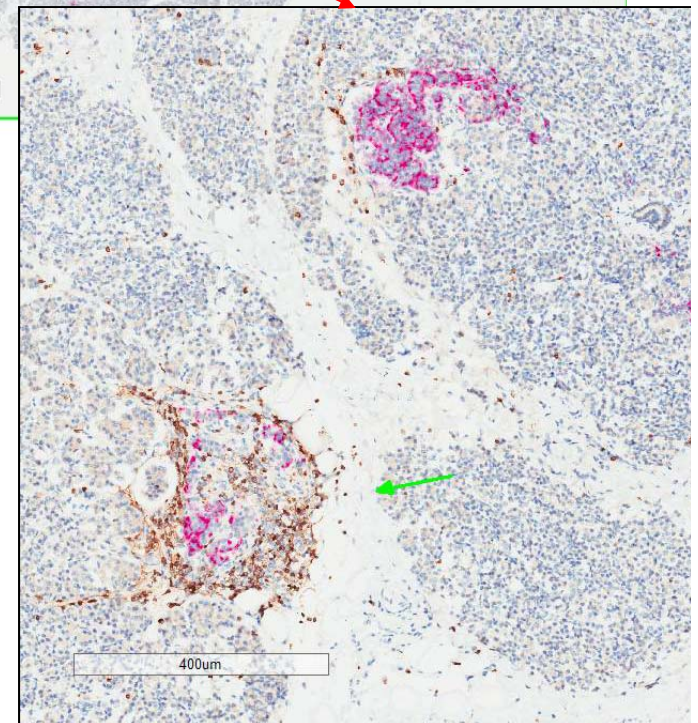
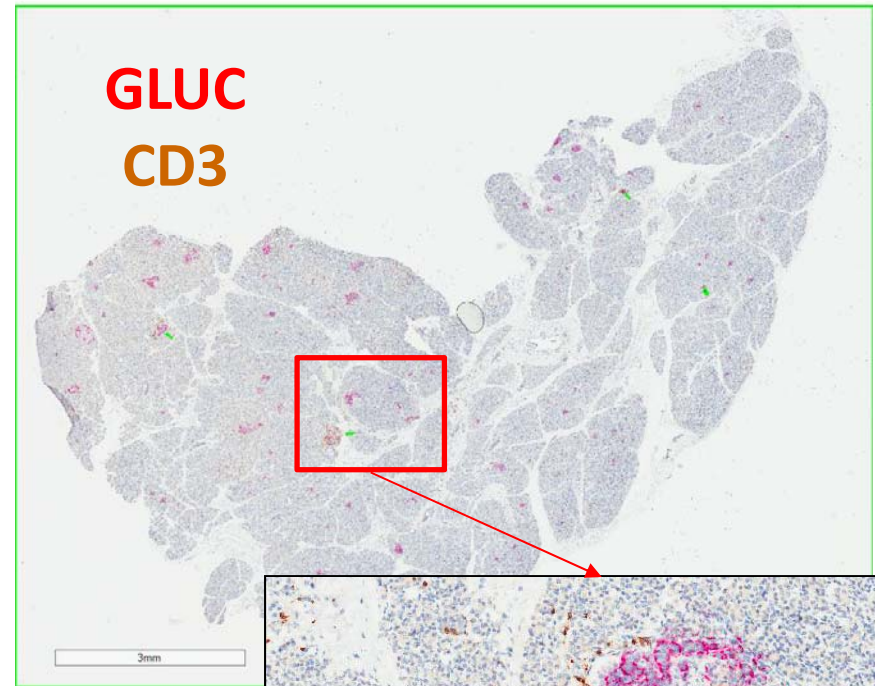
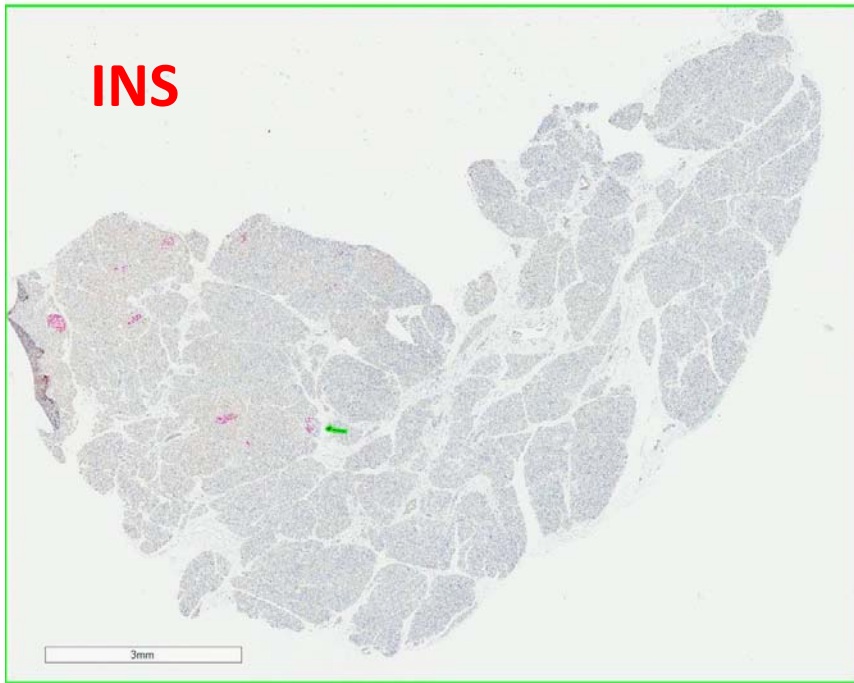
Immunohistochemical detection of islet antigen-reactive CD8 T cells within insulitic lesions of T1D patients

Coppieters K T et al. J Exp Med 2012;209:51-60

Both in recent onset and long standing diabetes



Young Donor with Recent Onset T1D – 0.25 years



6209

5 years old (**0.25 year duration**) Female

AutoAb: IA2A+ZnT8A+mIAA+

C peptide: 0.1ng/ml

HighRes HLA: A*0101,0201; DRB1*0401,0301;

DQA1*0301,0501, DQB1*0302,0201

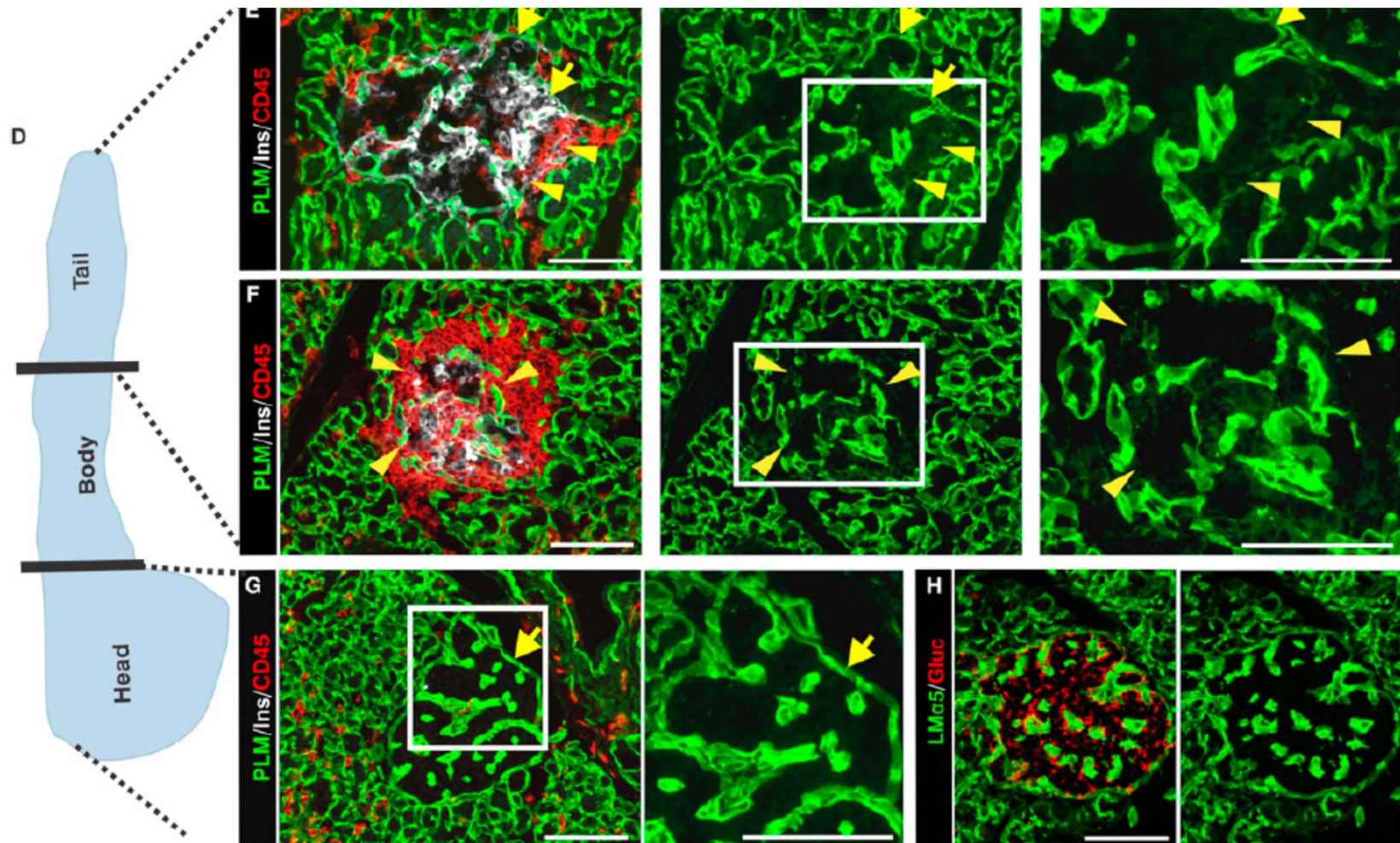
Histopathology: Ins+ (reduced)/Gluc+ islets with insulinitis.

Low Ki67 in all cell types. No pancreatitis.

The Peri-islet Basement Membrane, a Barrier to Infiltrating Leukocytes in Type 1 Diabetes in Mouse and Human

Diabetes 62:531–542, 2013

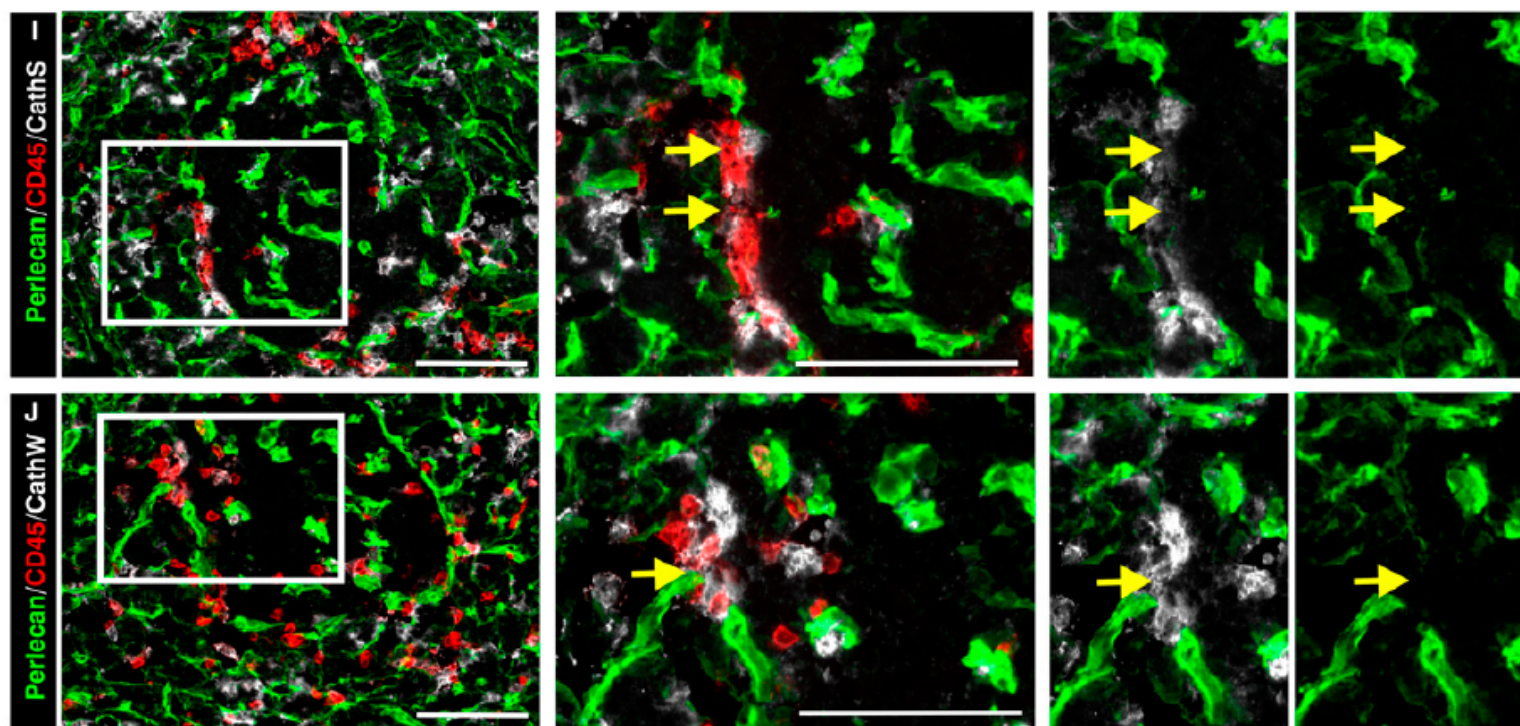
Éva Korpos,¹ Nadir Kadri,^{2,3} Reinhild Kappelhoff,⁴ Jeannine Wegner,¹ Christopher M. Overall,^{4,5} Ekkehard Weber,⁶ Dan Holmberg,⁷ Susanna Cardell,² and Lydia Sorokin¹



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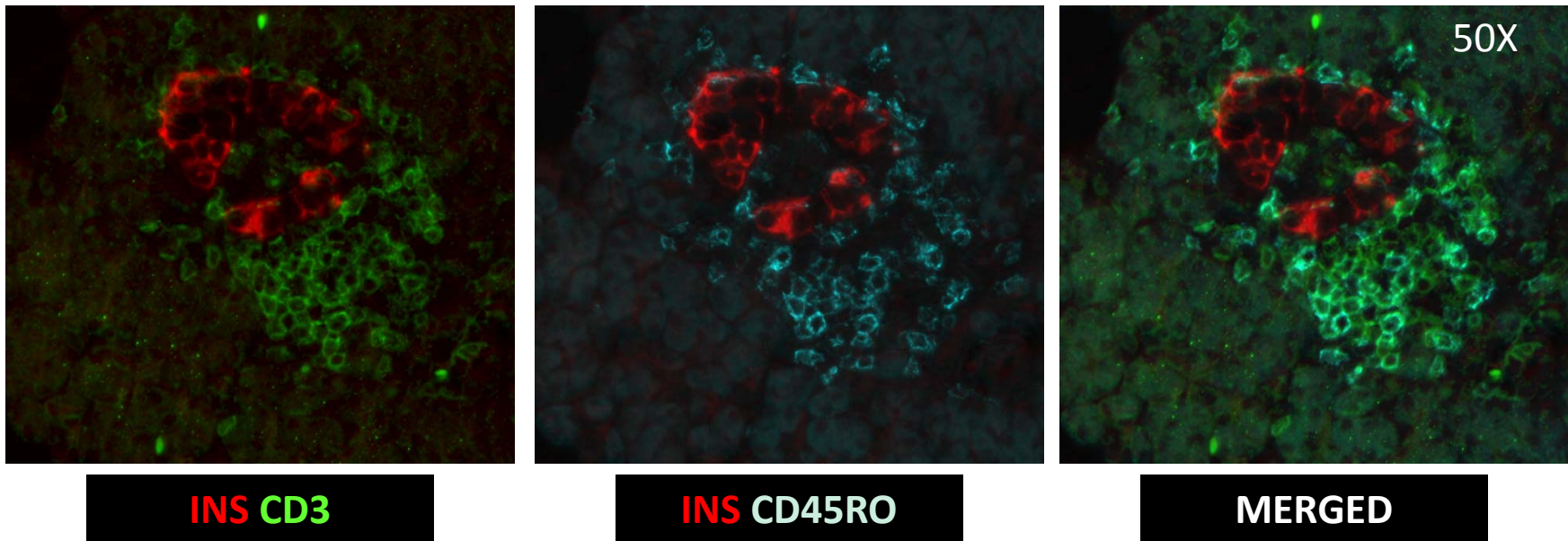


Co-localization of cathepsin S and W near CD45+ infiltrating cells and areas of basement membrane interruption

nPOD-3678 – Pancreas Transplant Recipient with Recurrent T1D

Memory T cells are present in the insulitis

Francesco Vendrame



Insulitis: Frequency of memory T cells

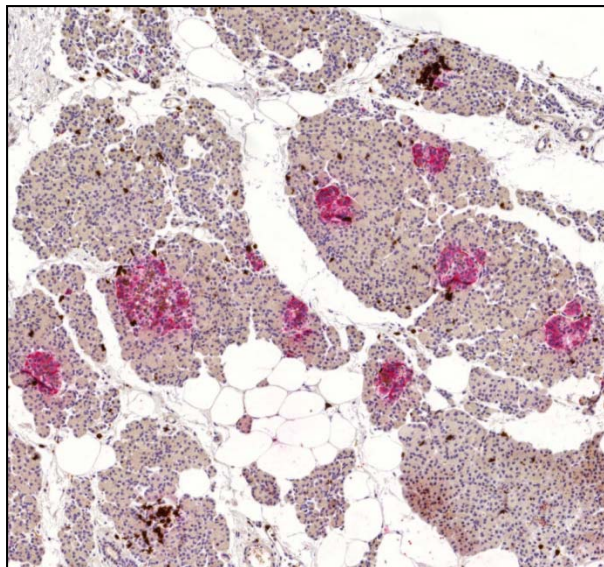
	Block 1		Block 2		Block 3			
	n	%	n	%	n	%	Mean	SD
CD3+ CD45RO+	234	73.8	149	92.0	298	71.3	79.0	11.3
CD3+ CD45RO-	83	26.2	13	8.0	120	28.7	21.0	11.3

nPOD-3678 – Pancreas Transplant Recipient with Recurrent T1D

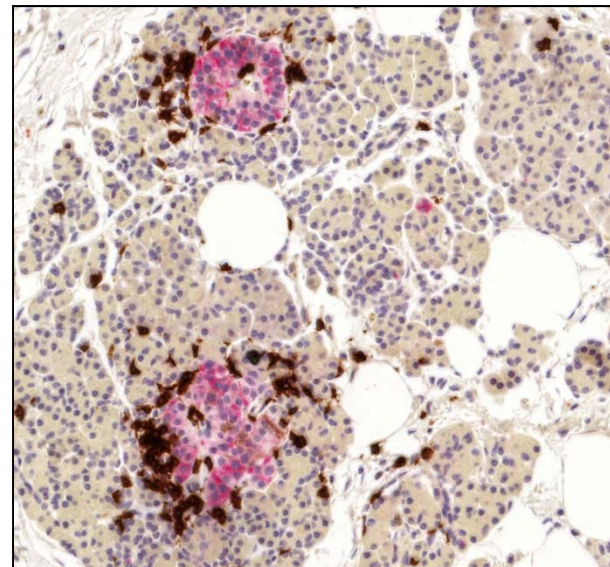
Only a proportion of islets are affected near diagnosis

Francesco Vendrame

	Block 1		Block 2		Block 3		Mean	SD
	n	%	n	%	n	%		
ins+ gluc+ CD3-	73	84.9	31	59.6	41	67.2	70.6	13.0
ins+ gluc+ CD3+	11	12.8	11	21.2	8	13.1	15.7	4.7
ins- gluc+ CD3+	0	0.0	1	1.9	0	0.0	0.6	1.1
ins- gluc+ CD3-	2	2.3	9	17.3	12	19.7	13.1	9.4
Total	86		52		61			

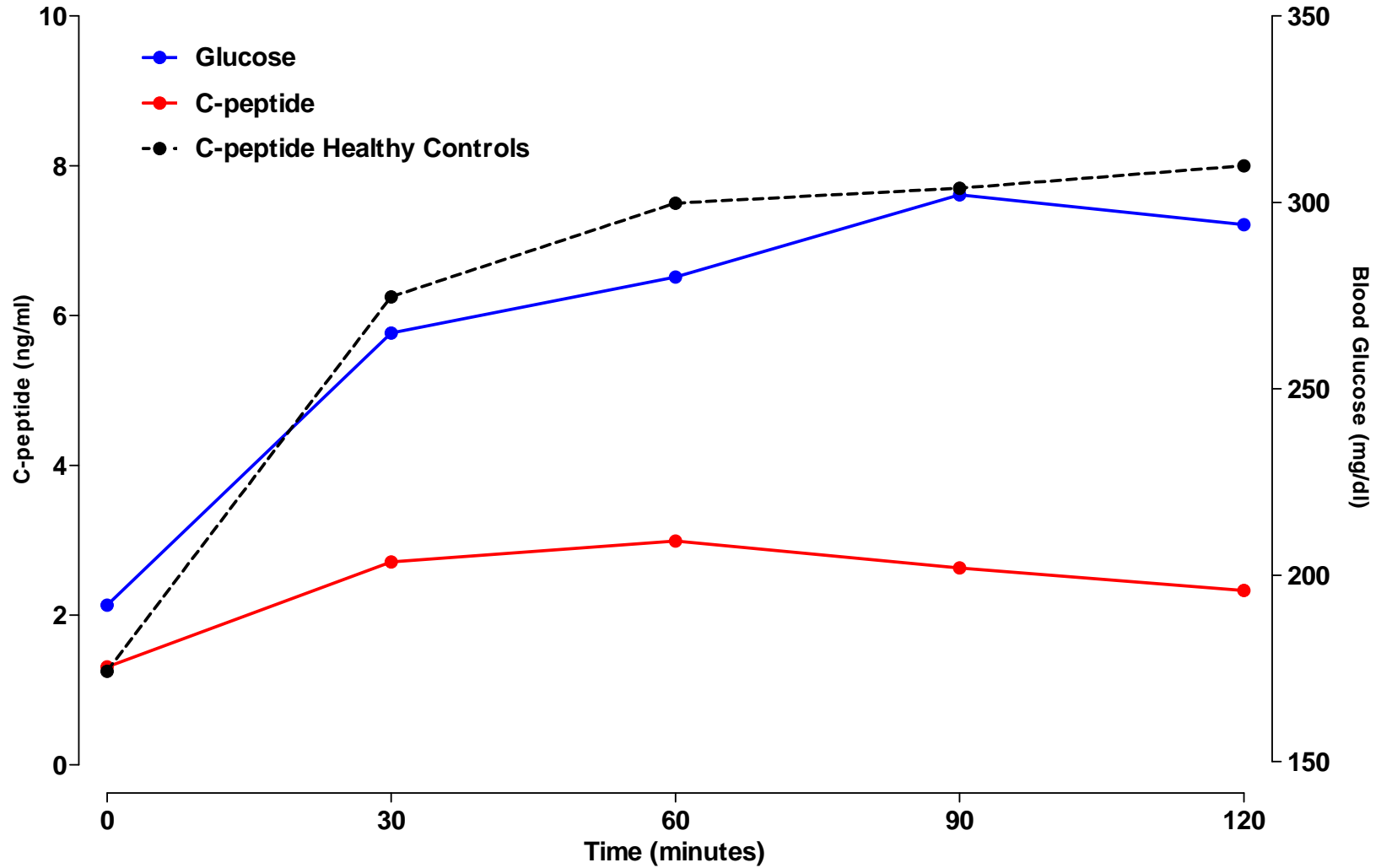


INS
CD8
40X



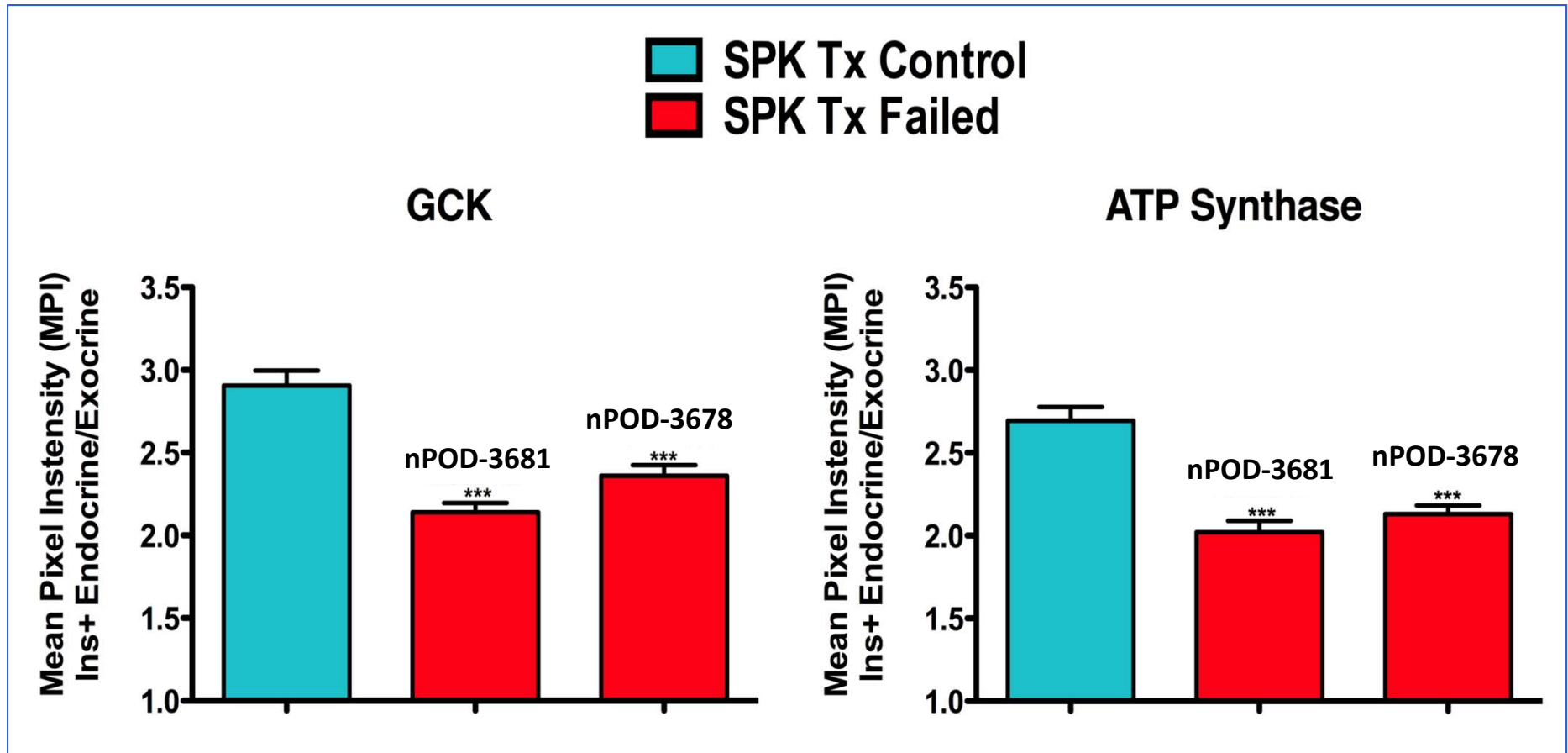
SPK Patient nPOD-3678

MMTT

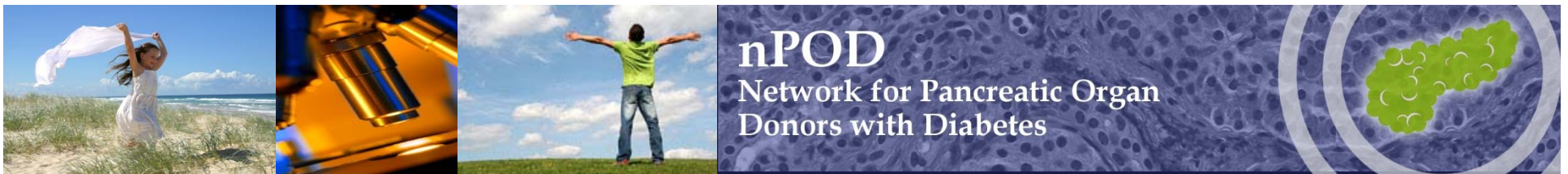


MMTT WAS PERFORMED 11 DAYS PRIOR TO BIOPSY; HbA1c WAS 10.7%

Lower Expression of GCK & ATP Synthase in the Insulin Positive Cells of SPK Patients with T1D Recurrence



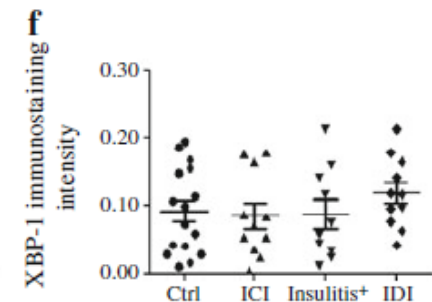
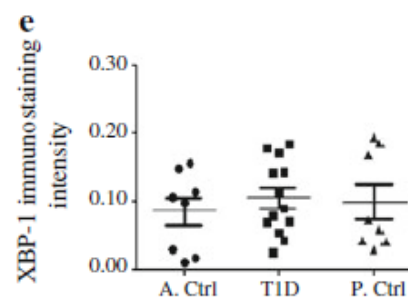
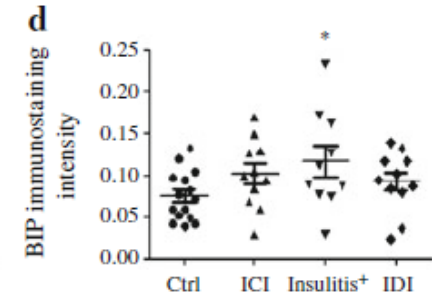
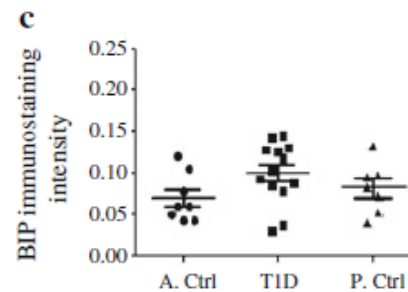
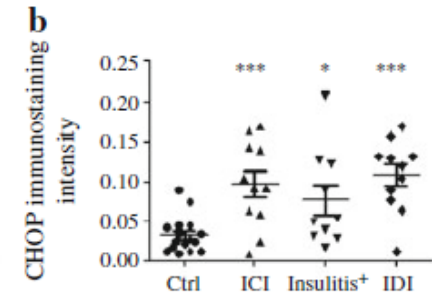
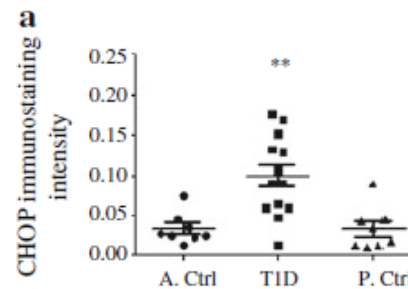
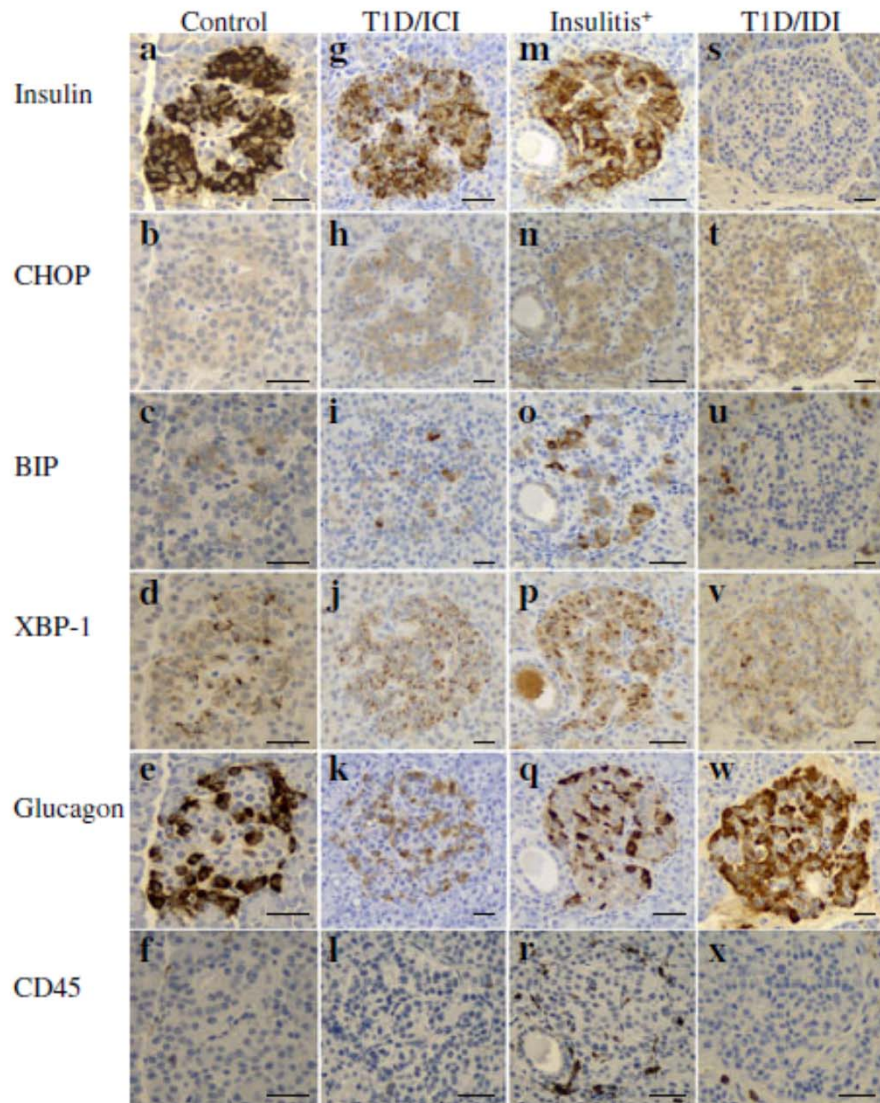
Through nPOD collaboration: Clayton Mathews, University of Florida



Expression of endoplasmic reticulum stress markers in the islets of patients with type 1 diabetes

Diabetologia (2012) 55:2417–2420

I. Marhfour • X. M. Lopez • D. Lefkaditis • I. Salmon •
 F. Allagnat • S. J. Richardson • N. G. Morgan •
 D. L. Eizirik



Expression of the enteroviral capsid protein VP1 in the islet cells of patients with type 1 diabetes is associated with induction of protein kinase R and downregulation of Mcl-1

S. J. Richardson • P. Leete • A. J. Bone • A. K. Foulis • N. G. Morgan

Diabetologia (2013) 56:185–193

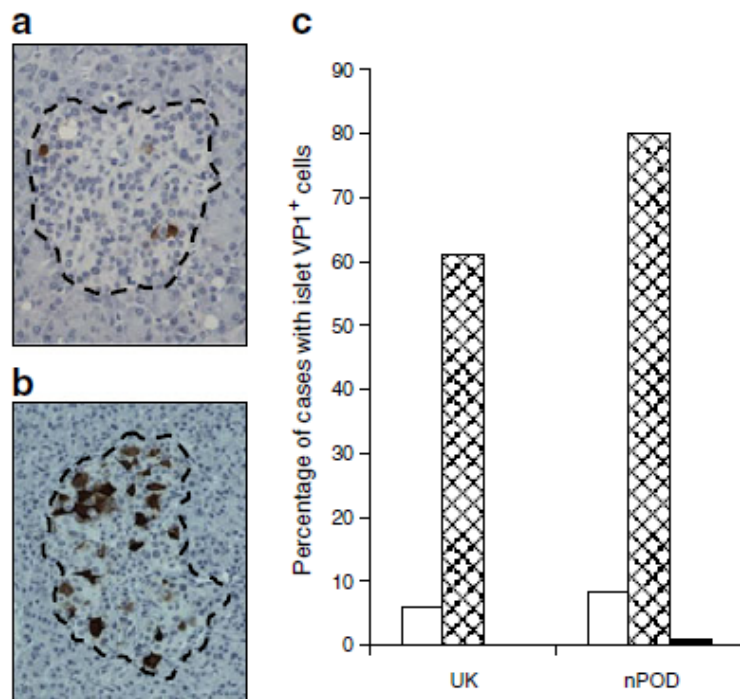


Fig. 1 Representative immunohistochemical images of enteroviral VP1 levels in the UK (donor D4) (a) and nPOD (donor 6052-01) cohorts (b). (c) The proportion of cases with islets staining intensely positive for VP1. White bars, non-diabetic controls (UK $n=50$ [7]; nPOD $n=12$); hatched bars, type 1 diabetes cases with ICIs (UK $n=72$ [7], nPOD $n=10$); black bars, type 1 diabetes cases containing only IDIs (nPOD $n=7$)

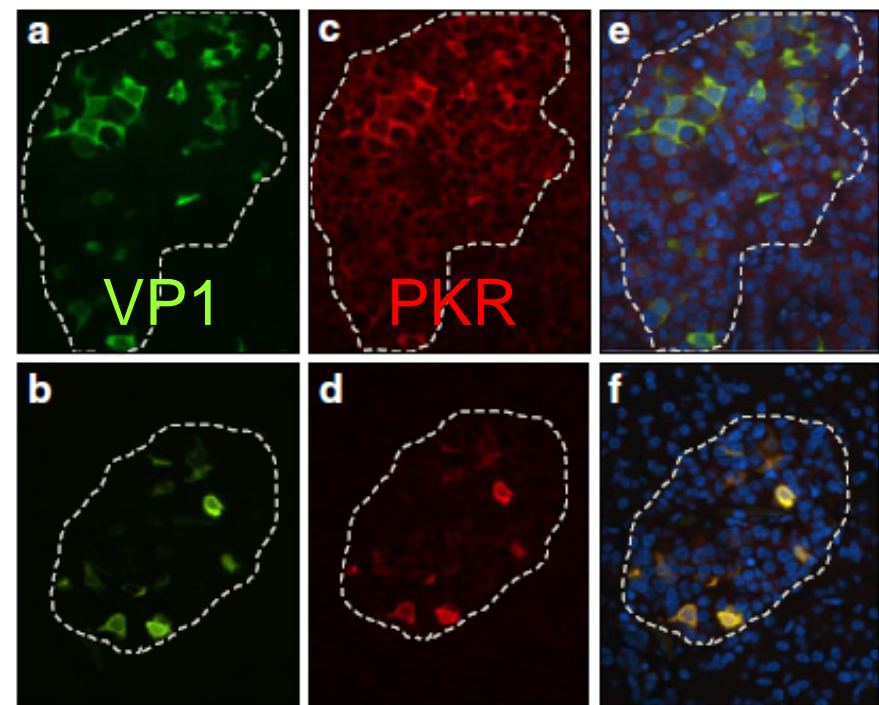
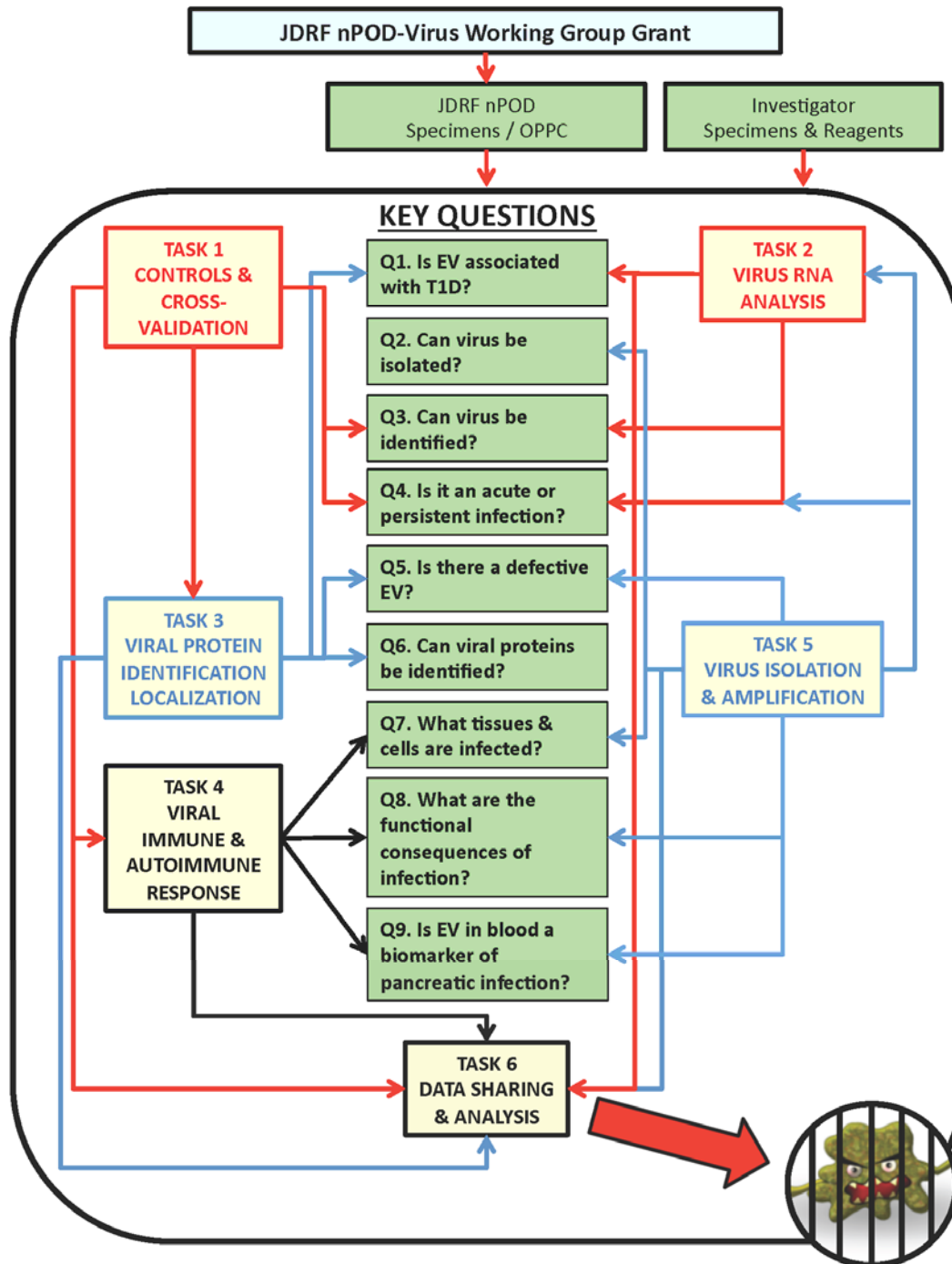


Fig. 4 Photomicrographs of representative islets from the nPOD (donor 6084-01; a, c, e) and UK (donor D4; b, d, f) cohorts reveals that VP1 (green; a, b) co-localises with PKR (red; c, d). Double-positive cells are stained yellow and are visible in (e) and (f). Nuclear DAPI staining is shown in blue in the merged images (e, f)



nPOD-Virus Group

KEY QUESTIONS & TASKS

Overall aim is to develop a pipeline for comprehensive and integrated understanding of the role of enteroviruses in disease pathogenesis



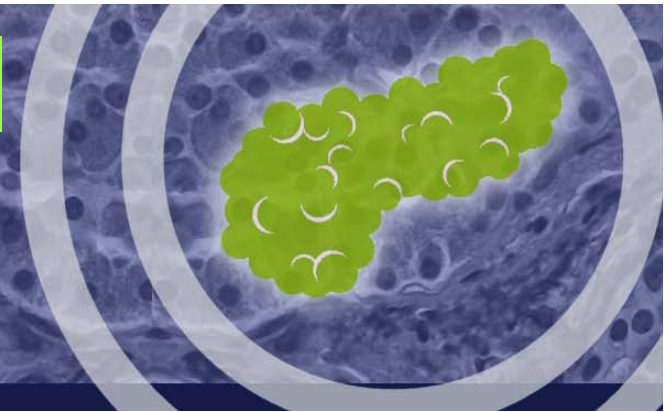
Is there a wider therapeutic window & multiple therapeutic targets?

- The T1D disease process does not end at diagnosis!
- Insulinitis may be found for years after onset
- Autoreactive T cells were identified in insulinitis
- Insulinitis largely consists of memory cells in pancreas transplant patients with T1D recurrence
- Insulin-positive islets, detected for years after onset
- Beta cell replication appears rare, and dependent on age, but exceptions exist – triggers?
- Discrepancy between beta cell loss and impairment of insulin secretion – possible roles of inflammation and epigenetics on beta cell dysfunction
- Relevance of nPOD studies for clinical trials

nPOD

Network for Pancreatic Organ Donors with Diabetes

www.JDRFnPOD.org



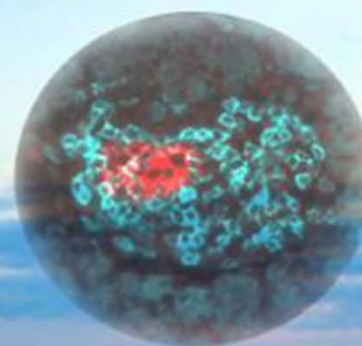
- Mark Atkinson
- Martha Campbell-Thompson
- Suzy Ball
- Jayne Moraski
- Mingder Yang
- Irina Kusmarteva
- Clive Wasserfall
- Teresa Miller
- John Kaddis
- Roberto Gianani
- Patrick Rowe
- Des Schatz
- Les Jebson
- Tiffany Heiple
- Sandra Lawson
- Kim Young
- Francesco Vendrame

nPOD Meeting, Atlantic Beach, February 2013



FEBRUARY 23-26, 2014

ONE OCEAN RESORT Atlantic Beach, Florida



2014 JDRF nPOD ANNUAL SCIENTIFIC SYMPOSIUM

Dedicated to Collaborative Human Type 1 Diabetes Research



nPOD
Network for Pancreatic Organ
Donors with Diabetes

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