SUPPLEMENTARY DATA

Mitochondrial proton leak regulated by Cyclophilin D elevates insulin secretion in islets at non-stimulatory glucose levels

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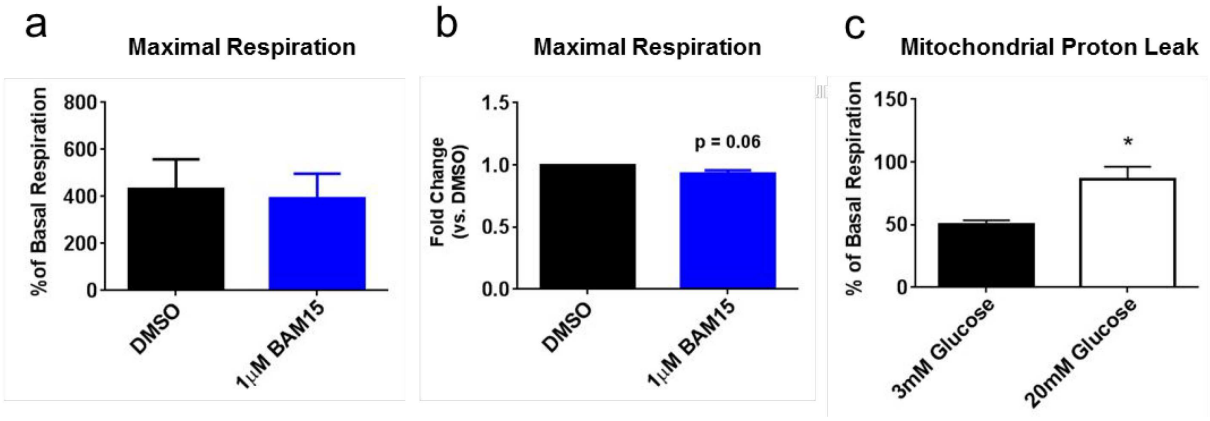
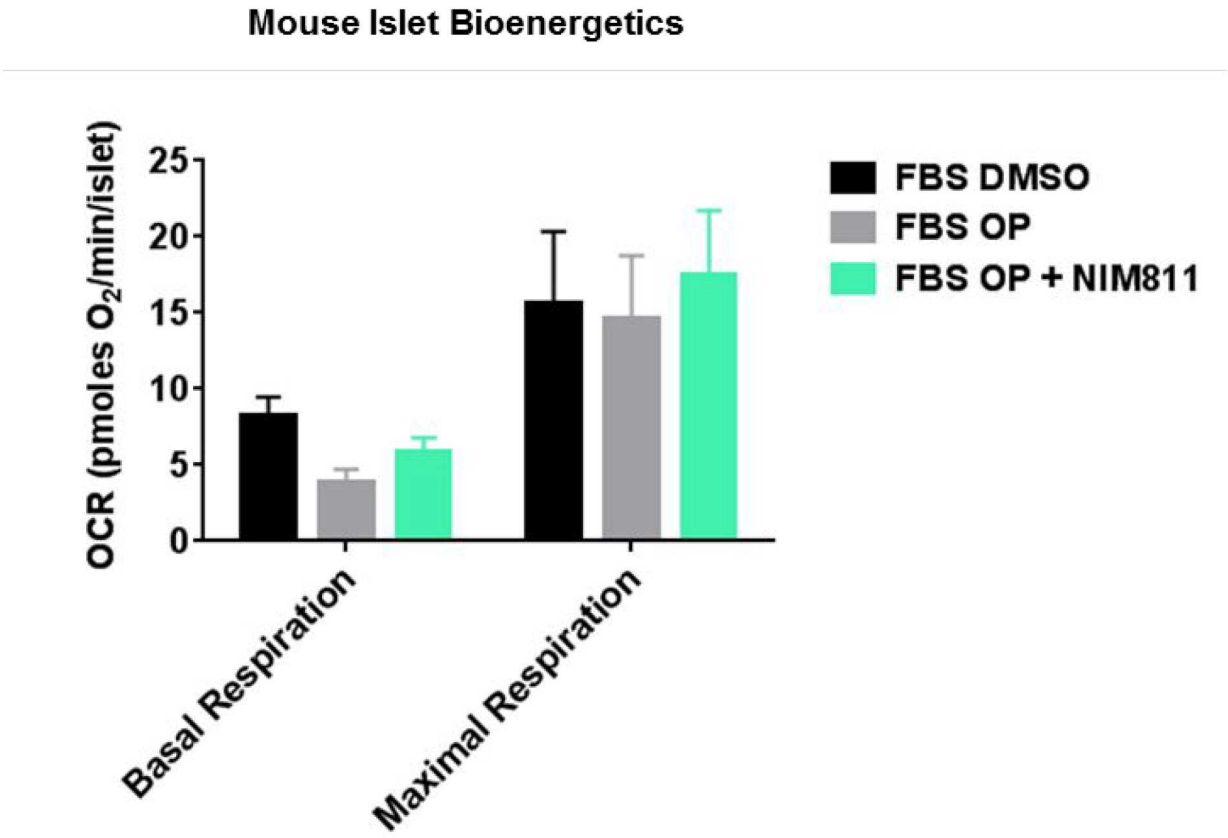
Supplementary Figure 1. Effects of pharmacological induction of proton leak on maximal respiration in pancreatic islets. (a) FCCP-induced maximal respiration of C57BL/6J mouse islets treated acutely with either 1µM BAM15 or DMSO vehicle. Data are normalized to basal OCR. n = 3 independent experiments. (b) Maximal respiration of BAM15-treated mouse islets, normalized to DMSO control. n = 3 independent experiments. Data analyzed by two-tailed, unpaired t-test. (c) Mitochondrial proton leak of mouse islets acutely exposed to 3mM or 20mM glucose Seahorse media. n = 10 experiments. *p < 0.05 by two-tailed, unpaired t-test. Data are means ± SEM.



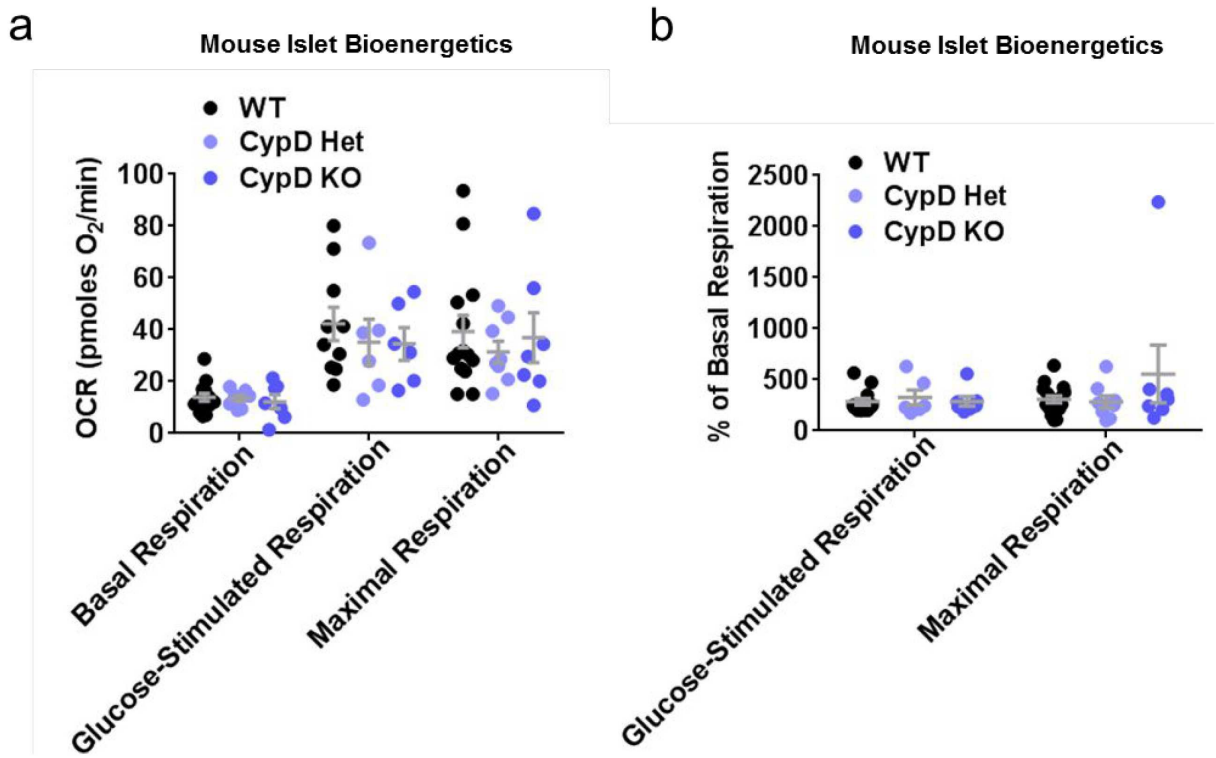
SUPPLEMENTARY DATA

Supplementary Figure 2. Effect of NIM811 on basal and maximal respiration of mouse islets. Basal and FCCP-stimulated maximal respiration from DMSO or NIM811-treated islets in the absence or presence of NEFA. Islets were pretreated with DMSO vehicle or 1µM NIM811 for 1hr in 3mM glucose Seahorse media, as noted in Figure 2. Islets were then acutely exposed to either FBS DMSO or FBS OP, followed by Oligo, FCCP and Ant A. Respiration (pmoles O_2/min) was normalized to islet number per well. n = 4 independent experiments. Data are means ± SEM.



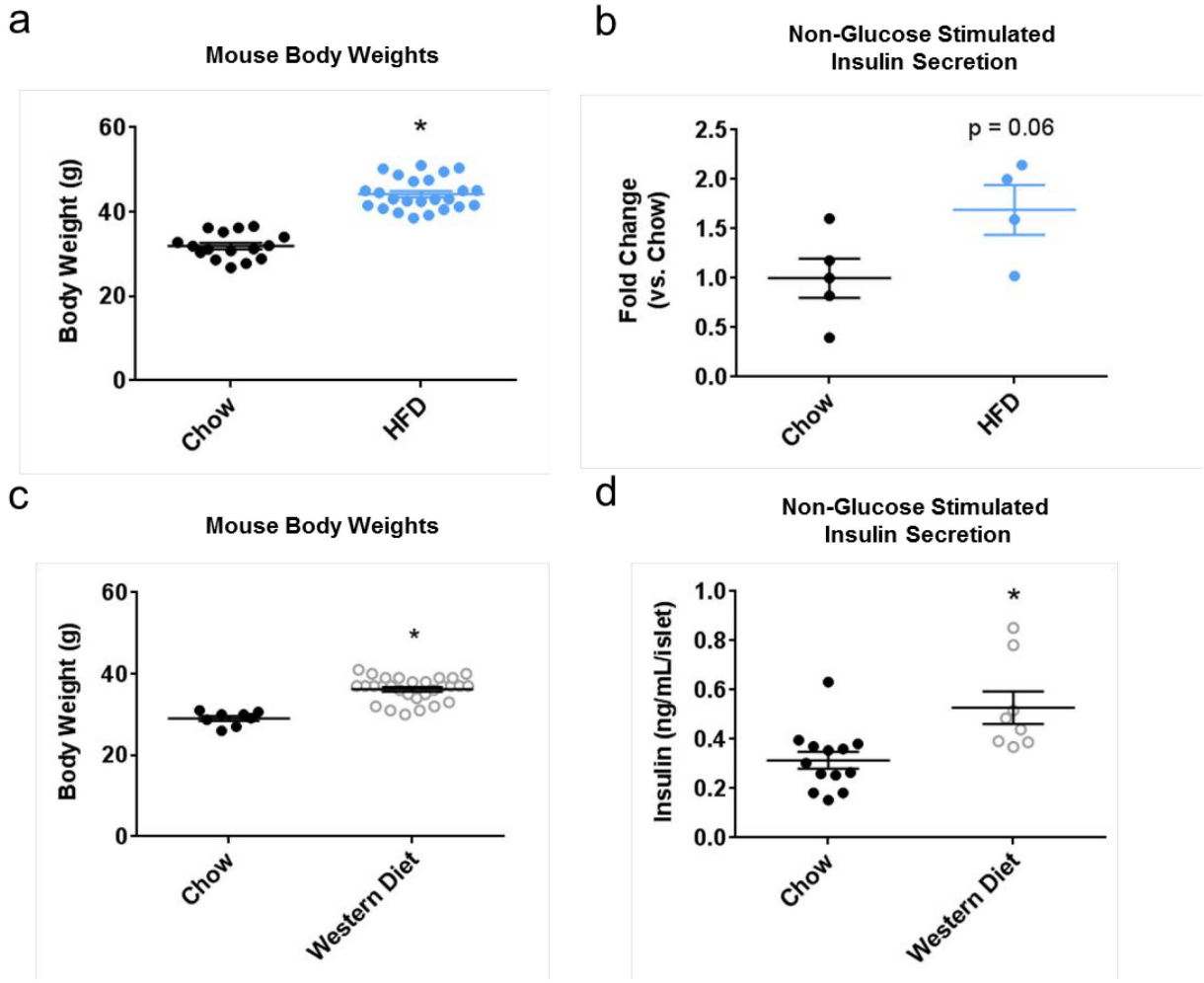
SUPPLEMENTARY DATA

Supplementary Figure 3. Bioenergetics of islets from mice with genetic reduction of CypD expression. (a) Basal, glucose-stimulated and maximal respiration of islets isolated from WT, CypD Het and CypD KO mice expressed in pmoles O_2/min. n = 10-14 WT, 6-8 Het and 6-7 KO mice. (b) Glucose-stimulated and maximal respiration of WT, CypD Het and CypD KO mouse islets normalized to basal respiration. n = 12-16 WT, 6-8 Het and 7 KO mice. Data are means ± SEM.



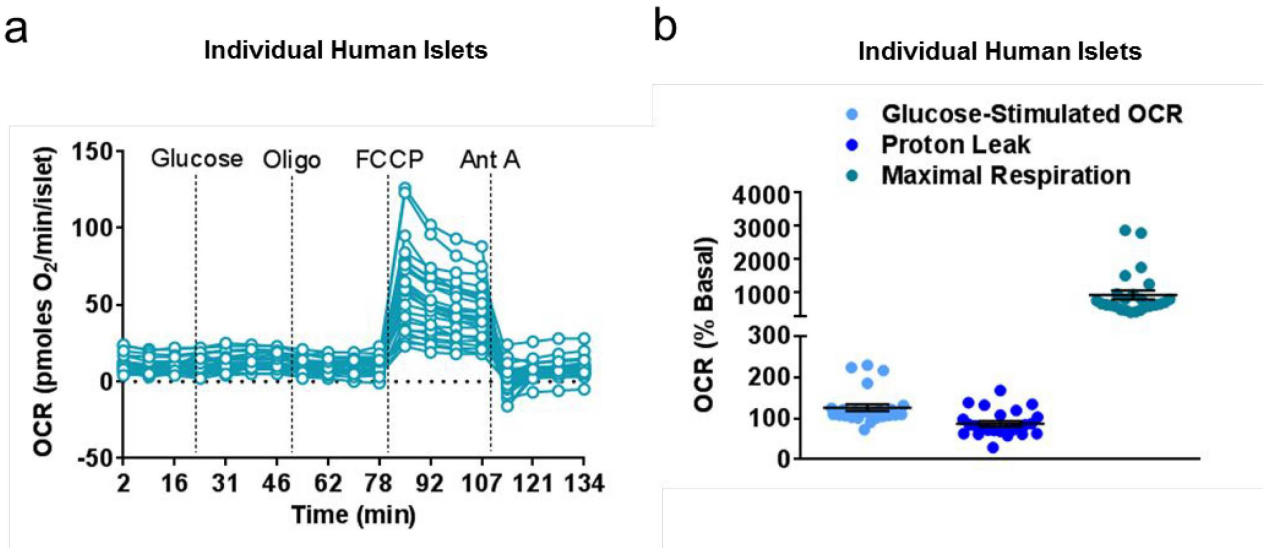
SUPPLEMENTARY DATA

Supplementary Figure 4. Body weights and NGSIS of lean and obese mice. (a) Body weights of mice fed chow or HFD for 12-28 weeks. n = 16 chow and 24 HFD mice. *p < 0.05 by two-tailed, unpaired t-test. (b) NGSIS from individual islets of chow or HFD mouse islets. Data are expressed as fold change compared to 3mM glucose chow control values. n = 4 mice per diet. Data analyzed by two-tailed, unpaired t-test. (c) Body weights of mice fed normal chow or a Western diet for 7 weeks. For chow, n = 8 mice, and for western diet, n = 29 mice. (d) NGSIS from islets of age-matched mice fed chow or western diet for 7-15 weeks. For chow, n = 10 experiments, with islets pooled from 2-4 mice per experiment. For western diet, n = 8 individual mice, derived from 4 experiments. *p < 0.05 by two-tailed, unpaired t-test. Data are means ± SEM.



SUPPLEMENTARY DATA

Supplementary Figure 5. High-throughput individual human islet respirometry with the Seahorse XF96 Matrigel-embedding platform. (a) Representative OCR traces of individual Matrigel-embedded human islets measured via high-throughput islet respirometry with the Seahorse XF96 cell culture plate. Islets were sequentially exposed to stimulatory glucose (20mM final concentration), Oligo, FCCP and Ant A. Each trace represents an individual islet. n = 26 individual islets from one donor. (b) Glucose-stimulated respiration, mitochondrial proton leak and maximal respiration of individual human islets. OCR data are normalized to basal respiration (% Basal). n = 25 islets from one donor. Bioenergetics are calculated from islets of the same donor as shown in (a). Islets were obtained from living donors with pancreatitis undergoing total pancreatectomy with islet auto-transplantation at the University of Minnesota Schulze Diabetes Institute. Data in (b) are means ± SEM.



SUPPLEMENTARY DATA

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Checklist for Reporting Human Islet Preparations Used in Research

Adapted from Hart NJ, Powers AC (2018) Progress, challenges, and suggestions for using human islets to understand islet biology and human diabetes. Diabetologia https://doi.org/10.1007/s00125-018-4772-2.

Manuscript DOI: https://doi.org/10.2337/**[insert manuscript submission number]** **(**Example, https://doi.org/10.2337/db18-1234)

Title: Mitochondrial proton leak regulated by Cyclophilin D elevates insulin secretion in islets at non-stimulatory glucose levels

Author list: Evan P. Taddeo, Nour Alsabeeh, Siyouneh Baghdasarian, Jakob D. Wikstrom, Eleni Ritou, Samuel Sereda, Karel Erion, Jin Li, Linsey Stiles, Muhamad Abdulla, Zachary Swanson, Josh Wilhelm, Melena D. Bellin, Richard G. Kibbey, Marc Liesa, and Orian Shirihai

Corresponding author: Orian Shirihai, Marc Liesa (shared) | **Email address:** oshirihai@mednet.ucla.edu, mliesa@mednet.ucla.edu

Islet preparation	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**[a]
MANDATORY INFORMATION								
Unique identifier	H1196	H1197	H1204	H1212	H1217	H1223	H1231	H1235
Donor age (years)	62	50	51	57	7	36	47	42
Donor sex (M/F)	F	F	M	M	M	M	F	F

SUPPLEMENTARY DATA

Donor BMI (kg/m^2)	29.55	28.3	31	24.49	18.33	27.68	19.11	27.3
Donor HbA_{1c} or other measure of blood glucose control	HbA1c = 6.9	HbA1c = 5.6	HbA1c = 7.6	HbA1c = 5.8	HbA1c = 5.4	HbA1c = 5.8	HbA1c = 4.8	HbA1c = 4.6
Origin/source of islets[b]	TPIAT procedure (University of Minnesota)	TPIAT procedure (University of Minnesota)	TPIAT procedure (University of Minnesota)	TPIAT procedure (University of Minnesota)	TPIAT procedure (University of Minnesota)	TPIAT procedure (University of Minnesota)	TPIAT procedure (University of Minnesota)	TPIAT procedure (University of Minnesota)
Islet isolation centre	Schulze Diabetes Institute (University of Minnesota)	Schulze Diabetes Institute (University of Minnesota)	Schulze Diabetes Institute (University of Minnesota)	Schulze Diabetes Institute (University of Minnesota)	Schulze Diabetes Institute (University of Minnesota)	Schulze Diabetes Institute (University of Minnesota)	Schulze Diabetes Institute (University of Minnesota)	Schulze Diabetes Institute (University of Minnesota)
Donor history of diabetes? Yes/No	Yes	No	Yes	No	No	Yes	No	No
If Yes, complete the next two lines if this information is available								
Diabetes duration (years)								
Glucose-lowering therapy at time of death[c]			Insulin			Insulin		

RECOMMENDED INFORMATION								
Donor cause of death	NA (Donor alive, TPIAT procedure)	NA (Donor alive, TPIAT procedure)	NA (Donor alive, TPIAT procedure)	NA (Donor alive, TPIAT procedure)	NA (Donor alive, TPIAT procedure)	NA (Donor alive, TPIAT procedure)	NA (Donor alive, TPIAT procedure)	NA (Donor alive, TPIAT procedure)
Warm ischaemia time (h)								
Cold ischaemia time (h)								

SUPPLEMENTARY DATA

Estimated purity (%)								
Estimated viability (%)								
Total culture time (h)[d]								
Glucose-stimulated insulin secretion or other functional measurement[e]	GSIS (8.5x fold over basal)	FCCP-Induced Maximal Respiration (229.3% of basal respiration)	GSIS (2.15x fold over basal)	Glucose-stimulated respiration (125.8% of basal respiration)	GSIS (1.52x fold over basal)	GSIS (1.46x fold over basal)	FCCP-Induced Maximal Respiration (172% of basal respiration)	ATP-linked respiration (57.28% of basal respiration)
Handpicked to purity? Yes/No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Additional notes	Hyperlipidemia, heavy fat infiltration in pancreas		Hypertriglyceridemia			Hypertriglyceridemia, type 3c/2 diabetes with insulin resistance		

[a]If you have used more than eight islet preparations, please complete additional forms as necessary
[b]For example, IIDP, ECIT, Alberta IsletCore
[c]Please specify the therapy/therapies
[d]Time of islet culture at the isolation centre, during shipment and at the receiving laboratory
[e]Please specify the test and the results

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Corresponding author: Orian Shirihai, Marc Liesa (shared) **Email address:** oshirihai@mednet.ucla.edu, mliesa@mednet.ucla.edu

Islet preparation	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**[a]
MANDATORY INFORMATION								
Unique identifier	H1236	H1239	H1240	H1245	H1247	H1248	H1249	H1258
Donor age (years)	38	34	9	23	41	15	42	21

SUPPLEMENTARY DATA

Donor sex (M/F)	M	F	F	M	M	F	F	F
Donor BMI (kg/m^2)	24.65	23.32	20.3	22.1	22.7	24.5	31.3	25.1
Donor HbA_{1c} or other measure of blood glucose control	HbA1c = 5.2	HbA1c = 4.5	HbA1c = 6.1	HbA1c = 5.2	HbA1c = 5.7	HbA1c = 5.2	HbA1c = 4.7	HbA1c = 5.4
Origin/source of islets[b]	TPIAT procedure (University of Minnesota)	TPIAT procedure (University of Minnesota)	TPIAT procedure (University of Minnesota)	TPIAT procedure (University of Minnesota)	TPIAT procedure (University of Minnesota)	TPIAT procedure (University of Minnesota)	TPIAT procedure (University of Minnesota)	TPIAT procedure (University of Minnesota)
Islet isolation centre	Schulze Diabetes Institute (University of Minnesota)	Schulze Diabetes Institute (University of Minnesota)	Schulze Diabetes Institute (University of Minnesota)	Schulze Diabetes Institute (University of Minnesota)	Schulze Diabetes Institute (University of Minnesota)	Schulze Diabetes Institute (University of Minnesota)	Schulze Diabetes Institute (University of Minnesota)	Schulze Diabetes Institute (University of Minnesota)
Donor history of diabetes? Yes/No	No	No	No	No	No	No	No	No
If Yes, complete the next two lines if this information is available								
Diabetes duration (years)								
Glucose-lowering therapy at time of death[c]								

RECOMMENDED INFORMATION								
Donor cause of death	NA (Donor alive, TPIAT procedure)	NA (Donor alive, TPIAT procedure)	NA (Donor alive, TPIAT procedure)	NA (Donor alive, TPIAT procedure)	NA (Donor alive, TPIAT procedure)	NA (Donor alive, TPIAT procedure)	NA (Donor alive, TPIAT procedure)	NA (Donor alive, TPIAT procedure)
Warm ischaemia time (h)								

SUPPLEMENTARY DATA

Cold ischaemia time (h)								
Estimated purity (%)								
Estimated viability (%)								
Total culture time (h)[d]								
Glucose-stimulated insulin secretion or other functional measurement[e]	ATP-linked respiration (48.1% of basal respiration)	GSIS (2.28x fold over basal)	GSIS (1.1x fold over basal)	GSIS (15.9x fold over basal)	GSIS (1.86x fold over basal); Glucose-stimulated respiration (102.25% of basal respiration)	GSIS (3.03x fold over basal)	GSIS (3.24x fold over basal); Glucose-stimulated respiration (195.72% of basal respiration)	GSIS (1.125x fold over basal); GSIS w/ FFAs (1.5x fold over basal)
Handpicked to purity? Yes/No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Additional notes								Very high fasting C-peptide (7.1ng/mL)

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Corresponding author: Orian Shirihai, Marc Liesa (shared)	**Email address:** oshirihai@mednet.ucla.edu, mliesa@mednet.ucla.edu

Islet preparation	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8[a]**
MANDATORY INFORMATION								
Unique identifier	H1260							
Donor age (years)	30							
Donor sex (M/F)	M							

Donor BMI (kg/m^2)	23.1							
Donor HbA_{1c} or other measure of blood glucose control	HbA1c = 5.4							
Origin/source of islets[b]	TPIAT procedure (University of Minnesota)							
Islet isolation centre	Schulze Diabetes Institute (University of Minnesota)							
Donor history of diabetes? Yes/No	No							
If Yes, complete the next two lines if this information is available								
Diabetes duration (years)								
Glucose-lowering therapy at time of death[c]								

RECOMMENDED INFORMATION								
Donor cause of death	NA (Donor alive, TPIAT procedure)							
Warm ischaemia time (h)								
Cold ischaemia time (h)								

SUPPLEMENTARY DATA

Estimated purity (%)								
Estimated viability (%)								
Total culture time (h)[d]								
Glucose-stimulated insulin secretion or other functional measurement[e]	Maximal respiration (388.4% of basal OCR); GSIS (3.44x fold over basal)							
Handpicked to purity? Yes/No	Yes							
Additional notes	Pre-diabetic; impaired fasting glucose (120mg/dL), elevated fasting c-peptide (3.3 ng/mL)							

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