

## Supplemental Information Appendix

### SI Appendix Methods

#### *In vitro* Glucose Stimulated Insulin secretion (GSIS)

For *in vitro* GSIS studies, mouse islets were harvested at 4wks of age. 15 IEQs were hand-picked and cultured in 24-well plates with 1mL KREBS buffer + low glucose (2.2mM) for 30min, followed by 1mL KREBS + high glucose (22.2mM) for 30min, and finally 1mL 5%v/v acid-ethanol (to assess total insulin content) and frozen at -20C overnight. After each step, media/acid-ethanol was harvested for insulin assay.

#### *In vivo* $\beta$ -cell proliferation

$\beta$ -cell proliferation was quantified as percentage of Ki67-insulin to total insulin-positive cells on pancreatic sections stained with antibodies against insulin and Ki67 (ThermoFisher Scientific, Waltham MA; #RM-9106-S1), using Alexa Fluor 488 anti-guinea pig and Alexa Fluor 594 anti-rabbit (Life Technologies, Carlsbad CA; #A11073 and # A11037, respectively) as secondary antibodies.

### SI Appendix Figure Legends

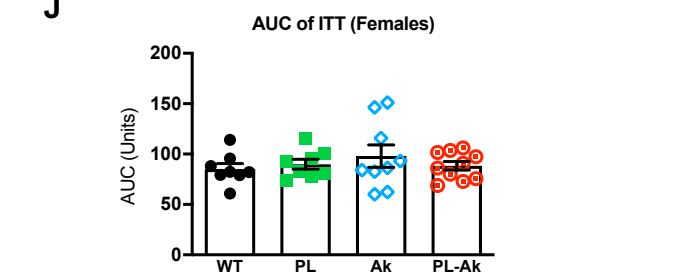
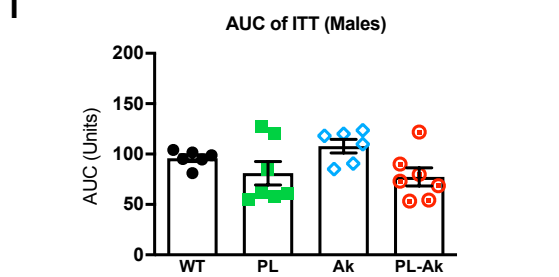
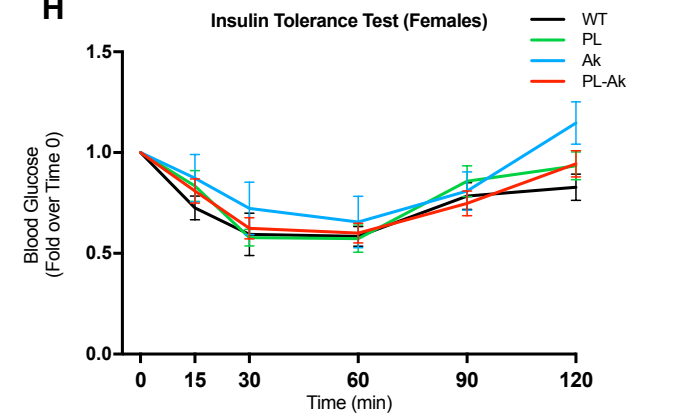
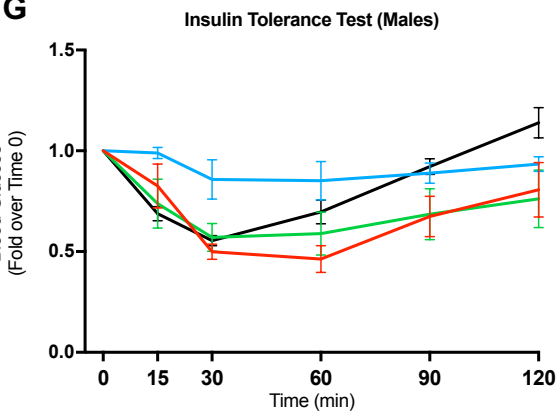
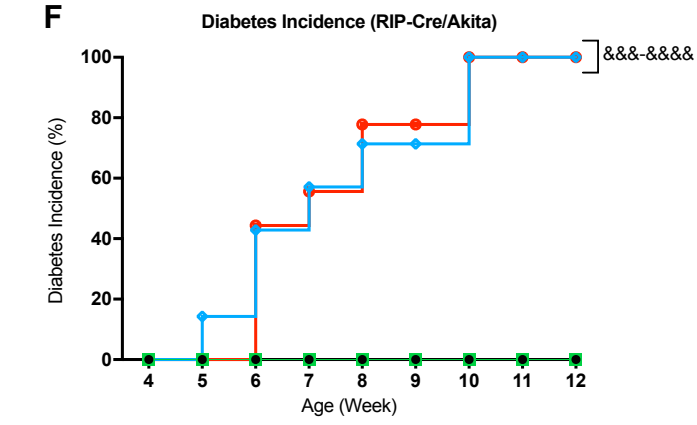
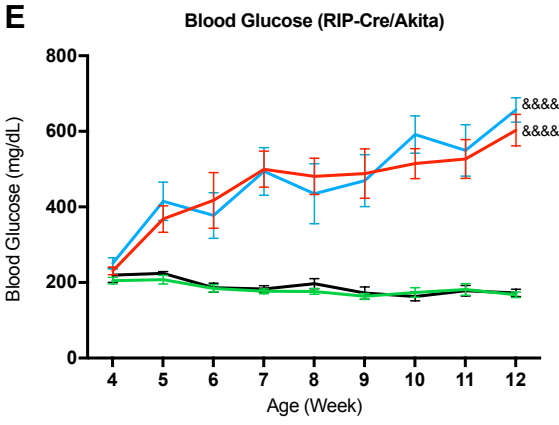
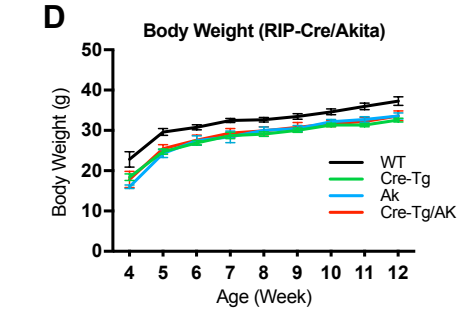
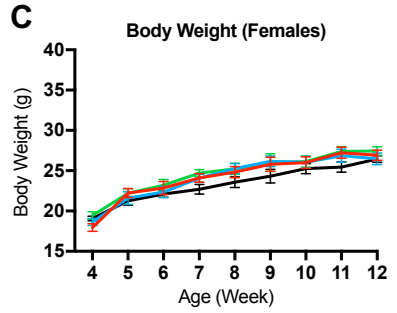
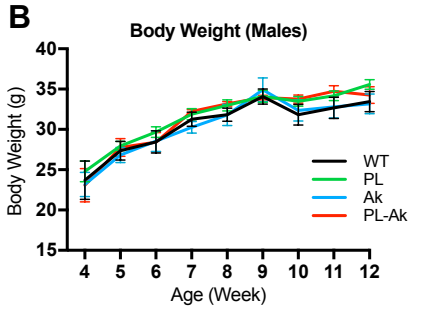
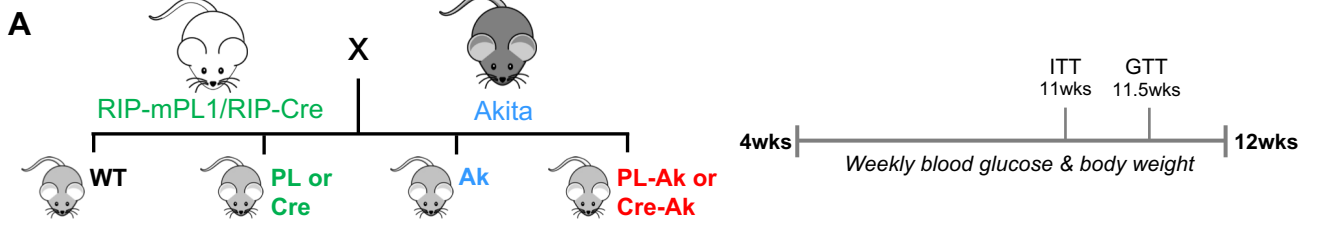
**Supplemental Figure 1. Experimental design and characterization of F1 progeny from RIP-mPL1/Akita and RIP-Cre/Akita breeding.** **A)** Genotypes and experimental design of the RIP-mPL1/Akita and RIP-Cre/Akita mouse models. Weekly body weight of WT (black), PL (green), Ak (blue), and PL-Ak (red) mice in **B)** males (n=8-13 mice/group) and **C)** females (n=8-14 mice/group). Weekly analysis of WT (black), Cre (green), Ak (blue), and Cre-Ak (red) male mice from the Akita/RIP-Cre F1 progeny, of: **D)** body weight (n=6-9 mice/group); **E)** non-fasting blood glucose (n=6-9 mice/group), using the Mixed Model Analysis for statistical significance; and **F)** diabetes incidence (defined as two consecutive weekly non-fasting blood glucose readings  $\geq 300$  mg/dL), with pairwise comparisons using the Log-Rank (Mantel-Cox) Test for statistical analysis. ITT depicted as fold over time 0, in WT (black), PL (green), Ak (blue), and PL-Ak (red) mice from the Akita/RIP-mPL1 F1 progeny, in **G)** males (n=6-7 mice/group) and **H)** females (n=8-10 mice/group); and AUC for the ITT in **I)** males and **J)** females, using one-way ANOVA for

statistical analysis.  $p < 0.001$ ,  $p < 0.0001$  versus WT and Cre. All error bars represent mean  $\pm$  SEM.

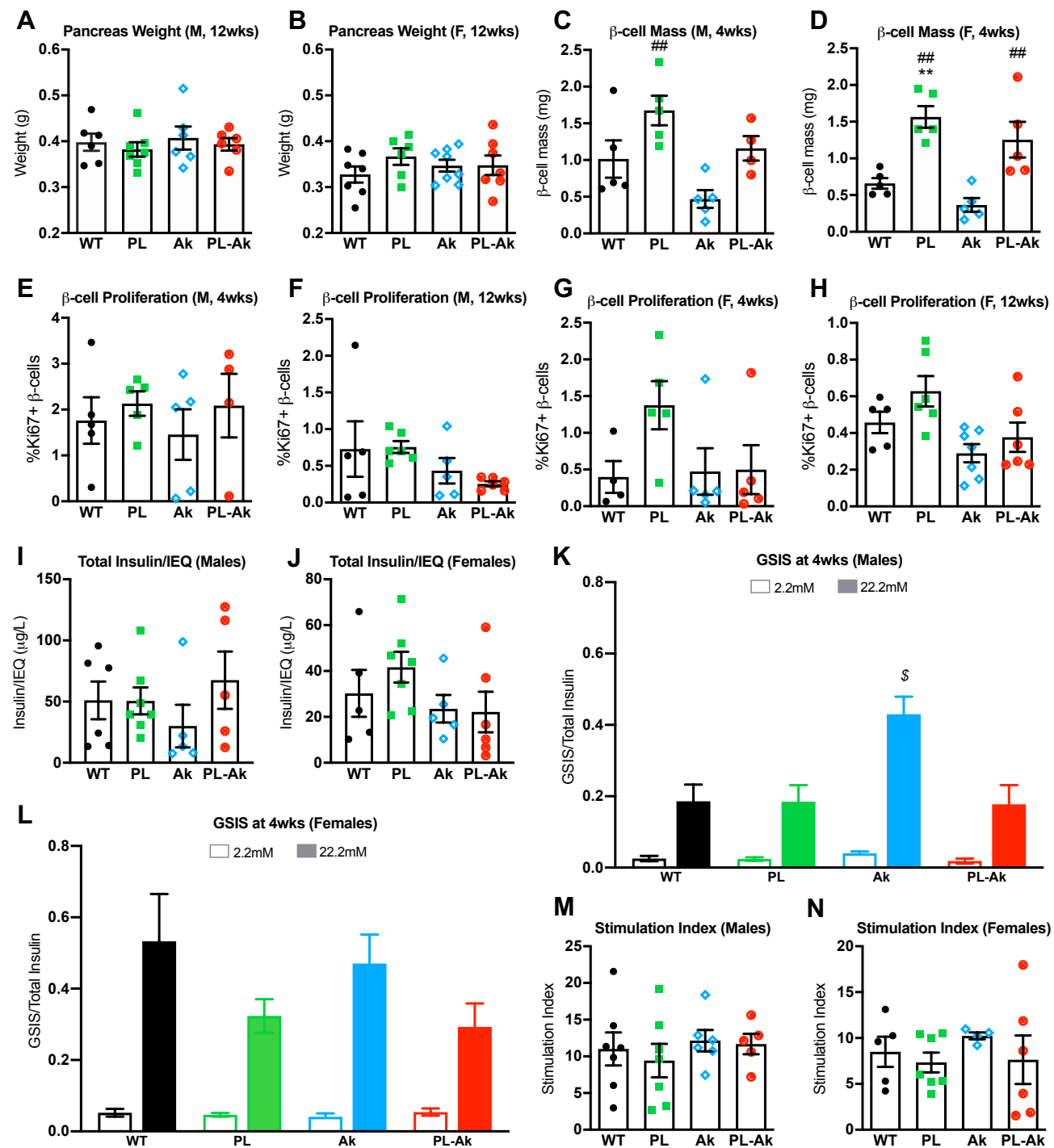
**Supplemental Figure 2.  $\beta$ -cell homeostasis, insulin content, and in vitro GSIS of islets from RIP-mPL1/Akita progeny.** Analysis of WT (black), PL (green), Ak (blue), and PL-Ak (red) mice for: Pancreas weight at 12 weeks of age in **A**) males (n=5-6 mice/group) and **B**) females (n=5-7 mice/group);  $\beta$ -cell mass at 4 weeks of age in **C**) males (n=4-5/mice group) and **D**) females (n=5 mice/group);  $\beta$ -cell proliferation in males **E**) at 4 (n=4-5 mice/group) and **F**) 12 (n=5-6 mice/group) weeks of age, and females **G**) at 4 (n=4-5 mice/group) and **H**) 12 (n=5-7 mice/group) weeks of age; total insulin content per islet equivalent (IEQ) at 4 weeks of age in **I**) males (n=5-7 mice/group) and **J**) females (n=5-7 mice/group); GSIS at 4 weeks of age in **K**) males (n=5-7 mice/group) and **L**) females (n=5-7 mice/group); insulin stimulation index at 4 weeks of age in **M**) males and **N**) females calculated as the ratio of insulin secretion at 22.2mM glucose/insulin secretion at 2.2mM glucose, from GSIS in (K) and (L), respectively.  $**p < 0.01$  versus WT;  $##p < 0.01$  versus Ak;  $\$p < 0.05$  versus other three groups; by one-way ANOVA. All error bars represent mean  $\pm$  SEM.

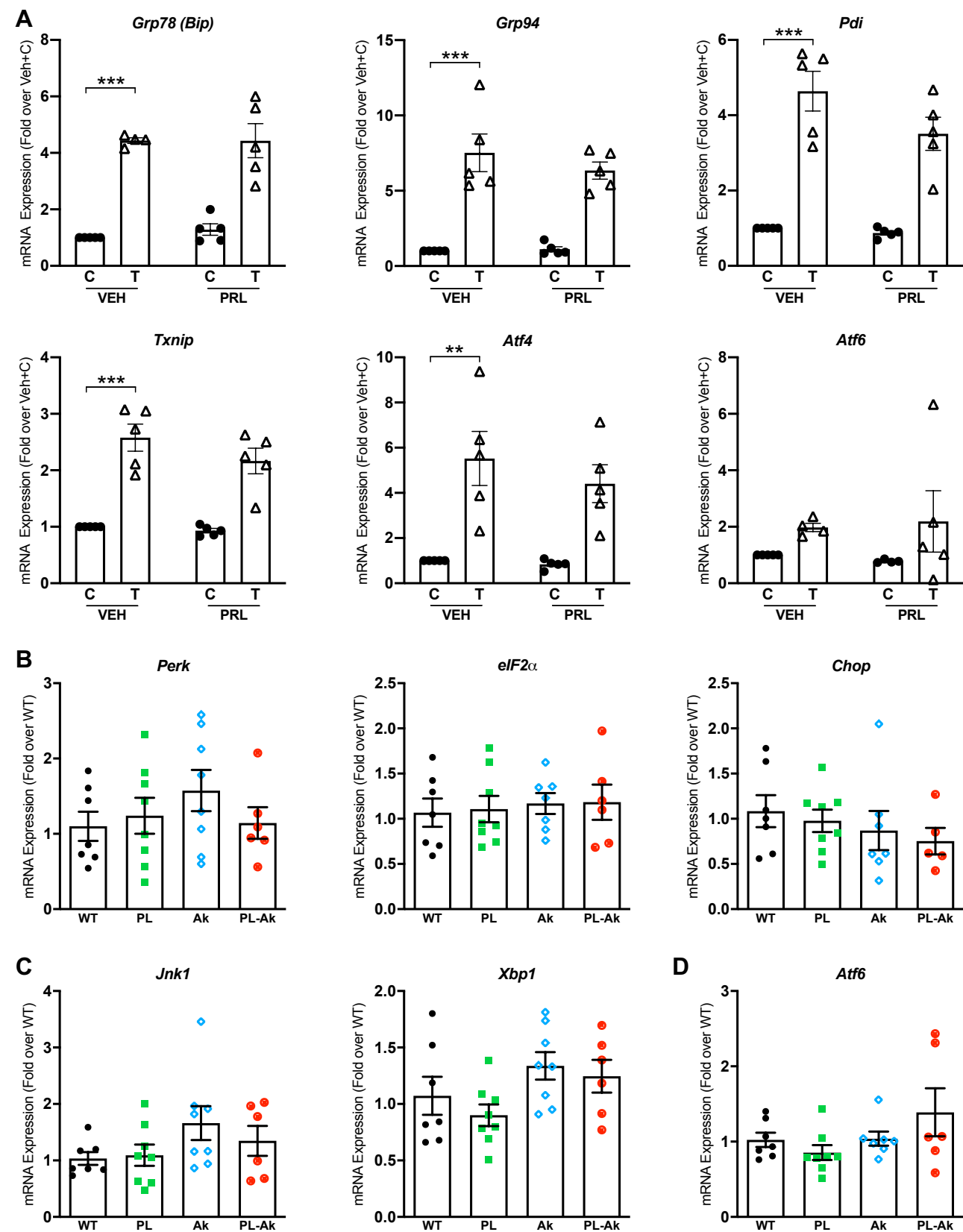
**Supplemental Figure 3. ER stress pathway gene expression in INS1 cells and islets from RIP-mPL1/Akita progeny.** mRNA expression of ER stress genes measured by qRT-PCR in **A**) INS1 cells treated for 24h + TM (T) or DMSO (C), + PRL or vehicle (Veh), analyzing for protein folding chaperones (*BiP*, *Grp94*, *protein disulfide isomerase*) and components of the three ER-stress pathways (*Txnip*, *Atf4*, *Atf6*), relative to actin expression, and expressed as fold over control (Veh+C) (n=4-5); in **B-D**) islets from 4wk old WT (black), PL (green), Ak (blue), and PL-Ak (red) female mice analyzing for the **B**) Perk (*Perk*, *eIF2a*, *Chop*), **C**) Ire1 $\alpha$  (*Jnk1*, *Xbp1*), and **D**) Atf6 pathways, relative to *Nkx6.1* expression, and expressed as fold over WT (n=5-7/group).  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$  versus Veh+C in INS1 cells by one-way ANOVA. All error bars represent mean  $\pm$  SEM.

# Supplementary Figure 1



## Supplementary Figure 2





**Table 1. Human islet donor information**

<b>Islet Preparation</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
Unique identifier	SAMN08769130	SAMN08769390	SAMN08769824	SAMN08769830	SAMN08930666
Donor Age (years)	37	33	24	43	45
Donor Sex (M/F)	M	F	F	M	F
Donor BMI (kg/m <sup>2</sup> )	27.6	34.2	40.2	24.4	26.6
Donor HbA1c	5.6	5.5	5.3	5.4	4.5
Origin/source of islets	IIDP	IIDP	IIDP	IIDP	IIDP
Islet isolation center <sup>a</sup>	University of Wisconsin	University of Wisconsin	University of Pennsylvania	The Scharp-Lacy Research Institute	University of Illinois
Donor history of diabetes? Yes/No	No	No	No	No	No
<b>If Yes, complete the next two lines if this information is available</b>					
Diabetes duration (years)	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
Glucose-lowering therapy at time of death <sup>b</sup>	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
Cause of death	Cerebrovascular /stroke	Cerebrovascular /stroke	Anoxia	Anoxia	Cerebrovascular /stroke
Warm ischemia time (h)	Not Reported	Not Reported	Not Reported	0.8	Not Reported
Cold ischemia time (h)	11.3	14.3	12.3	11.2	7.8
Estimated purity (%) By islet isolation center at time of shipment with Dithizone (DTZ) staining per IIDP SOP	90	95	90	95	90
Estimated viability (%) By islet isolation center at time of broadcast with Fluorescence Diacetate/Propidium Iodide (FDA/PI) staining per IIDP SOP	98	98	92	95	97
Total culture time(h) <sup>c</sup> Calculate[start date/time of experiment]-[date/ time islet culture began (below)]	64	90	56	75	89
Date/Time Islet Culture Began at islet isolation center (Pacific Standard Time, 24-hour format)	2016-09-18 16:00	2016-05-29 18:22	2016-02-25 13:15	2016-02-04 06:20	2016-01-04 04:00
Glucose-stimulated insulin secretion or other functional measurement <sup>d</sup> Stimulation Index (SI) by static incubation at islet isolation center pre-shipment per IIDP SOP	SI (G2.8mM-G28mM)= 2.2	SI (G2.8mM-G28mM)= 1.6	SI (G2.8mM-G28mM)= 2.3	SI (G2.8mM-G28mM)= 3.6	SI (G2.8mM-G28mM)= 1.2
Glucose-stimulated insulin secretion or other functional measurement Area Under the Curve (AUC) by perfusion at HIPP post shipment sample per HIPP SOP	AUC (G5.6mM-G16.7mM)= Not Available	AUC (G5.6mM-G16.7mM)= Not Available	AUC (G5.6mM-G16.7mM)= Not Available	AUC (G5.6mM-G16.7mM)= Not Available	AUC (G5.6mM-G16.7mM)= Not Available
Handpicked to purity? Yes/No	Yes	Yes	Yes	Yes	Yes
Additional notes	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable

**Table 1. Human islet donor information**

<b>Islet Preparation</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>
Unique identifier	SAMN13067487	SAMN13175912	SAMN13254972	SAMN13515839	SAMN13938639
Donor Age (years)	57	34	60	64	50
Donor Sex (M/F)	M	F	F	M	F
Donor BMI (kg/m <sup>2</sup> )	21.3	30.3	44.4	20.9	39.2
Donor HbA1c	4.9	4.8	5.6	5.5	5.0
Origin/source of islets	IIDP	IIDP	IIDP	IIDP	IIDP
Islet isolation center <sup>a</sup>	University of Miami	University of Pennsylvania	University of Wisconsin	The Scharp-Lacy Research Institute	Southern California Islet Cell Resource Center
Donor history of diabetes? Yes/No	No	No	No	No	No
<b>If Yes, complete the next two lines if this information is available</b>					
Diabetes duration (years)	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
Glucose-lowering therapy at time of death <sup>b</sup>	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
Cause of death	Cerebrovascular /stroke	Anoxia	Head trauma	Cerebrovascular /stroke	Cerebrovascular /stroke
Warm ischemia time (h)	Not Reported	0.5	0.3	Not Reported	Not Reported
Cold ischemia time (h)	9.0	14.3	6.2	8.6	11.6
Estimated purity (%) By islet isolation center at time of shipment with Dithizone (DTZ) staining per IIDP SOP	90	95	97	95	80
Estimated viability (%) By islet isolation center at time of broadcast with Fluorescence Diacetate/Propidium Iodide (FDA/PI) staining per IIDP SOP	90	93	98	95	95
Total culture time(h) <sup>c</sup> Calculate[start date/time of experiment]-[date/ time islet culture began (below)]	72	24	24	24	24
Date/Time Islet Culture Began at islet isolation center (Pacific Standard Time, 24-hour format)	2019-10-17 11:30	2019-10-30 19:00	2019-11-10 17:00	2019-12-08 14:00	2020-01-28 01:30
Glucose-stimulated insulin secretion or other functional measurement <sup>d</sup> Stimulation Index (SI) by static incubation at islet isolation center pre-shipment per IIDP SOP	SI (G2.8mM-G28mM)= 2.2	SI (G2.8mM-G28mM)= 1.4	SI (G2.8mM-G28mM)= 2.3	SI (G2.8mM-G28mM)= 3.6	SI (G2.8mM-G28mM)= 1.2
Glucose-stimulated insulin secretion or other functional measurement Area Under the Curve (AUC) by perfusion at HIPP post shipment sample per HIPP SOP	AUC (G5.6mM-G16.7mM)= 21.2 (ng/100 IEQs)	AUC (G5.6mM-G16.7mM)= Not Available	AUC (G5.6mM-G16.7mM)= 10.0 (ng/100 IEQs)	AUC (G5.6mM-G16.7mM)= 24.8 (ng/100 IEQs)	AUC (G5.6mM-G16.7mM)= 0.0 (ng/100 IEQs)
Handpicked to purity? Yes/No	Yes	Yes	Yes	Yes	Yes
Additional notes	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable

**Table 1. Human islet donor information**

<b>Islet Preparation</b>	<b>11</b>	<b>12</b>	<b>13</b>
Unique identifier	HP-19305-01	HP-20043	HP-20052
Donor Age (years)	44	64	34
Donor Sex (M/F)	F	F	F
Donor BMI (kg/m <sup>2</sup> )	24.37	17.24	34.56
Donor HbA1c	5.8	5.3	5.5
Origin/source of islets	Prodo Laboratories Inc	Prodo Laboratories Inc	Prodo Laboratories Inc
Islet isolation center <sup>a</sup>			
Donor history of diabetes? Yes/No	No	No	No
<b>If Yes, complete the next two lines if this information is available</b>			
Diabetes duration (years)	Not Applicable	Not Applicable	Not Applicable
Glucose-lowering therapy at time of death <sup>b</sup>	Not Applicable	Not Applicable	Not Applicable
Cause of death	Anoxia	Cerebrovascular /stroke	Anoxia
Warm ischemia time (h)			
Cold ischemia time (h)			
Estimated purity (%)			
By islet isolation center at time of shipment with Dithizone (DTZ) staining per IIDP SOP			
Estimated viability (%)			
By islet isolation center at time of broadcast with Fluorescence Diacetate/Propidium Iodide (FDA/PI) staining per IIDP SOP			
Total culture time(h) <sup>c</sup> Calculate[start date/time of experiment]-[date/ time islet culture began (below)]	24	36	18
Date/Time Islet Culture Began at islet isolation center (Pacific Standard Time, 24-hour format)	11/1/2019	2/12/2020	2/21/2020
Glucose-stimulated insulin secretion or other functional measurement <sup>d</sup> Stimulation Index (SI) by static incubation at islet isolation center pre-shipment per IIDP SOP			
Glucose-stimulated insulin secretion or other functional measurement Area Under the Curve (AUC) by perfusion at HIPP post shipment sample per HIPP SOP			
Handpicked to purity? Yes/No	Yes	Yes	Yes
Additional notes	Not Applicable	Not Applicable	Not Applicable



**Table 2. Antibodies and GSIS reagents**

Reagent		Company	Catalog #	Dilution
Rabbit $\alpha$ -Cleaved Caspase-3		Cell Signaling Technology	9661	1:200
Guinea Pig $\alpha$ -Insulin		DAKO (Agilent)	A0564	1:1000
Rabbit $\alpha$ -Ki67		ThermoFisher Scientific	RM-9106-S1	1:200
Rabbit $\alpha$ - $\beta$ -actin		Sigma-Millipore	SAB5500001	1:2000
Mouse $\alpha$ -PRLR		Invitrogen	35-9200	1:1000
Mouse $\alpha$ -CHOP (L63F7)		Cell Signaling Technology	2895	1:100
Alexa Fluor 488 Goat $\alpha$ -Rabbit		Life Technologies	A11034	1:200
Alexa Fluor 594 Goat $\alpha$ -Guinea Pig		Life Technologies	A11076	1:200
Alex Fluor 594 Goat $\alpha$ -Rabbit		Life Technologies	A11037	1:200
Alexa Fluor 488 Goat $\alpha$ -Guinea Pig		Life Technologies	A11073	1:200
Alexa Fluor 488 Goat $\alpha$ -Mouse		Life Technologies	A11029	1:250
HRP Donkey $\alpha$ -Guinea Pig		Jackson ImmunoResearch	706-035-148	1:500
HRP Goat $\alpha$ -Rabbit		Abcam	ab7090	1:5000
HRP Goat $\alpha$ -Mouse		Abcam	ab97040	1:5000
DAPI (4',6-Diamidino-2-Phenylindole, Dilactate)		Life Technologies	D3571	1:1000
DAB Peroxidase Substrate Kit		Vector Labs	SK 4100	
DeadEnd Fluorometric TUNEL Kit		Promega	G3250	
KREBS Buffer	Sodium chloride (NaCl 129mM)	Sigma	S7653	
	Potassium chloride (KCl 4.8mM)	Sigma	P9333	
	Calcium chloride dihydrous (CaCl <sub>2</sub> 2H <sub>2</sub> O 2mM)	Sigma	C1016	
	Sodium bicarbonate (NaHCO <sub>3</sub> 24mM)	Sigma	S5761	
	Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> 1.2mM)	Sigma	10487	
	Magnesium sulfate heptahydrate (Mg <sub>2</sub> SO <sub>4</sub> 7H <sub>2</sub> O 1.2mM)	Sigma	1374361	
	HEPES (6mM)	Corning	25-060-CI	
	BSA (0.2%)	Gemini	700-100P	
5% acid-ethanol	Acetic acid	Sigma	A6283	
	Ethanol	Decon	2701	

**Table 3. Primer sequences for genotyping and qRT-PCR**

<b>Mouse Primers</b>		
<b>Gene</b>	<b>Sequence</b>	<b>Expected Size</b>
mPL1	F: 5' CTA CGG GCT GCT CTA CTG CTT CAG G 3'	Between 100 & 200bp
	R: 5' GGC ACT GGA GTG GCA ACT TCC AAG G 3'	
GAPDH	F: 5' CGT GGA GTC TAC TGG TGT CTT CAC C 3'	259bp
	R: 5' GAT GGC ATG GAC TGT GGT CAT GAG C 3'	
Akita	F: 5'TGC TGA TGC CCT GGC CTG CT 3'	WT 140bp HT 280bp
	R: 5' TGG TCC CAC ATA TGC ACA TG 3'	
<b>Rat Primers (Mouse Compatible)</b>		
$\beta$ -actin	F: 5' CAA CCT TCT TGC AGC TCC TC 3'	
	R: 5' TTC TGA CCC ATA CCC ACC AT 3'	
Cyclophilin A	F: 5' AGC CAC TCA GTC TTG GCA GT 3'	
	R: 5' AGC ACT GGG GAG AAA GGA TT 3'	
Atf4	F: 5' GTT GGT CAG TGC CTC AGA GA 3'	
	R: 5' CAT TGC AAA CAG AGC ATC GA 3'	
Atf6	F: 5' GGT TGT CAC CAT CTG ACG TG 3'	
	R: 5' CAG AGG CTC AAA GTC CCA AG 3'	
BiP (Grp78)	F: 5' CCA CCA GGA TGC AGA CAT TG 3'	
	R: 5' AGG GCC TCC ACT TCC ATA GA 3'	
Chop	F: 5' TCT CAT CCC CAG GAA ACG AA 3'	
	R: 5' ATC TGG AGA GCG AGG GCT TT 3'	
eIF2a	F: 5' AAT GCC CTC CTT CAT GTC AG 3'	
	R: 5' TTG TGT TTG GAG TCC CTG GT 3'	
Grp94	F: 5' TCG GGA AGC AAC AGA GAA G 3'	
	R: 5' TCA TCT TCC TTA ACC CTC CG 3'	
Ire1a	F: 5' GTT TGC TCT TGG CCT CTG TC 3'	
	R: 5' GCG ATG GAC TGG TGG TAA CT 3'	
Jnk1	F: 5' CGG AAC ACC TTG TCC TGA AT 3'	
	R: 5' TCG CCT GAC TGG CTT TAA GT 3'	
Perk	F: 5' TTG GGC TAG TGA CTG CAA TG 3'	
	R: 5' TTG TCC CGT GTG TGT AGC AT 3'	
Pdi	F: 5' AAG AAC CTG GAA CCC AAG TA 3'	
	R: 5' TGG TAG GAA AAC CCT TGA CT 3'	
Txnip	F: 5' CTG ATG GAG GCA CAG TGA GA 3'	
	R: 5' CTC GGG TGG AGT GCT TAG AG 3'	
Xbp1	F: 5' AAA GGG AGG CTG GTA AGG AA 3'	
	R: 5' ATT CTG ACG CTG TTG CCT CT 3'	
Jak2	F: 5' GCATTCCTGATTTGCT 3'	
	R: 5' CCCTGCAGTCTGCCTTCTAC 3'	
<b>Human Primers</b>		
$\beta$ -actin	F: 5' CAT GTA CGT TGC TAT CCA GGC 3'	
	R: 5' 3'CTC CTT AAT GTC ACG CAC GAT 3'	
hPRLR	F: 5' ACCAGCAGGTGAATGTTTCC 3'	
	R: 5' TCCTATTCATGGAGGCGAAC 3'	