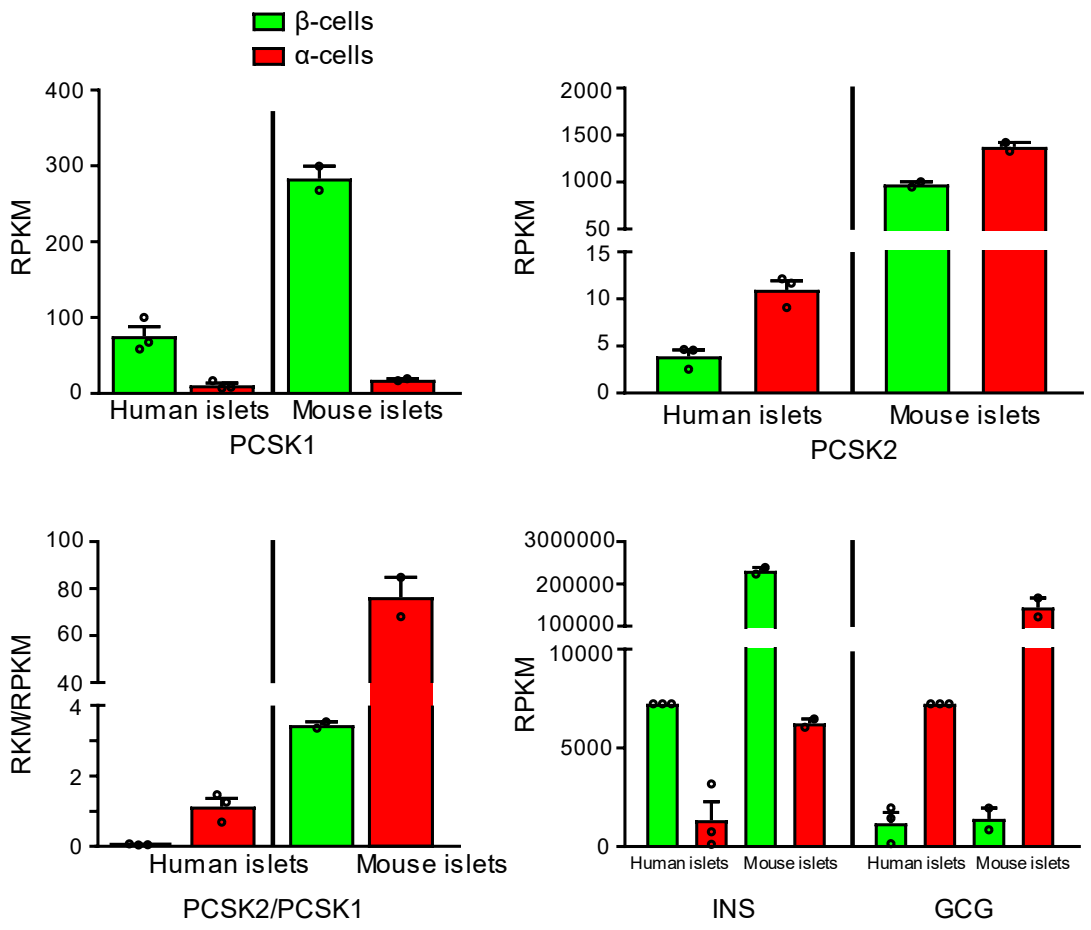


Figure S1 *Human β-cells have less PCSK2 than PCSK1 in published RNAseq databases on sorted human and mouse islet cells.*

Reanalysis of publicly available RNAseq databases (Bramswig et al., 2013; Benner et al., 2014). Relative RPKM for *PCSK2* and *PCSK1* compared in human β-cells and α-cells as well as mouse β-cells and α-cells. There is likely α-cell impurity contributing to *PCSK2* in sorted β-cells population given high *GCG* RPKM in β-cell sample.

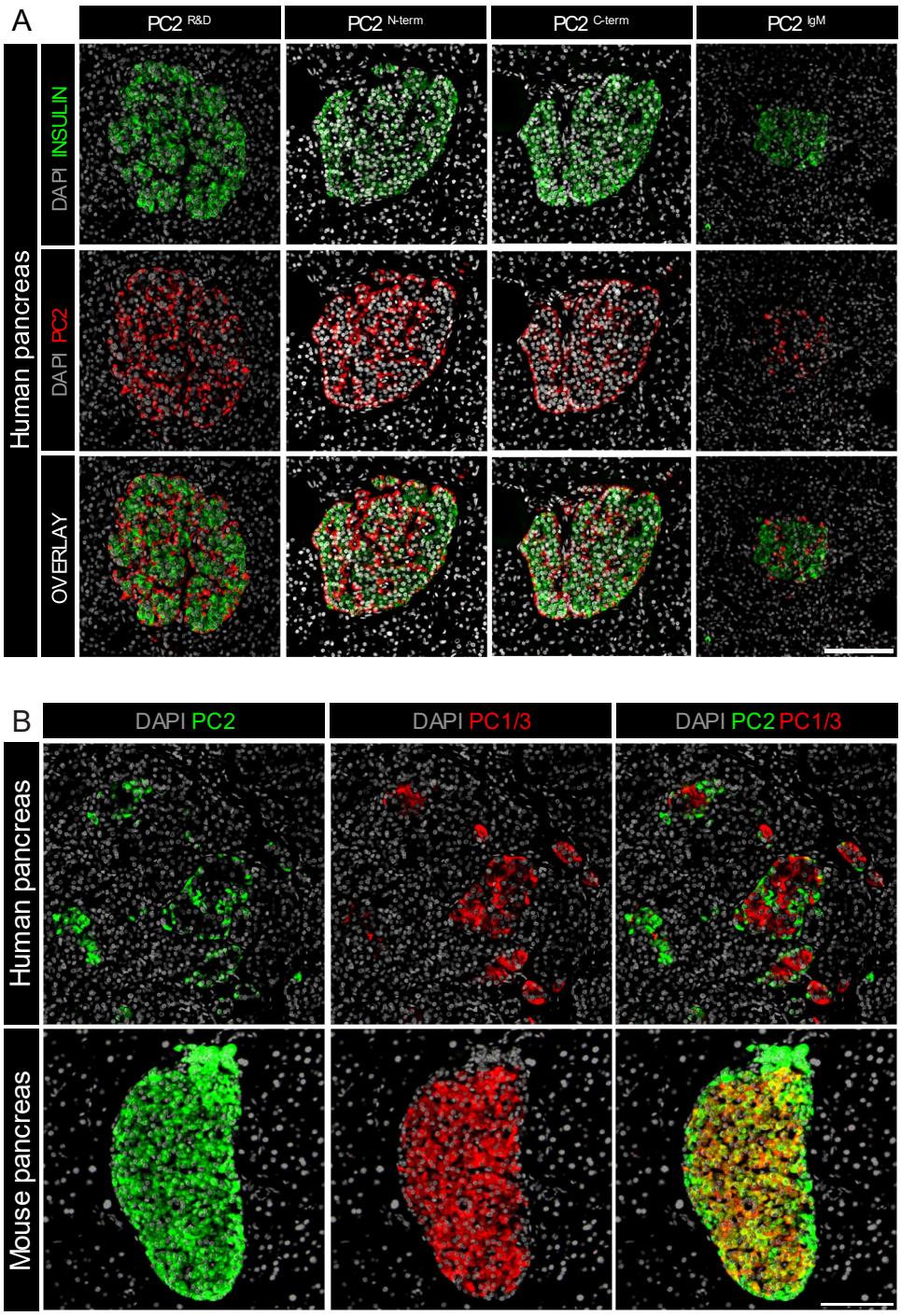


Figure S2 *Single channel immunostaining for insulin and PC2 using multiple antibodies and a lack of colocalization of PC1/3 and PC2 in human pancreas* (*related to Figure 1).*

(A) Individual channels from Figure 1D. Immunostaining for insulin (green) and PC2 (red) using four antibodies (see key resources table in supplemental methods for antibody details) in adult human pancreas with nuclei labelled with DAPI (grey). Scale bar is 100 µm.

(B) Immunostaining for PC2 (green) and PC1/3 (red) in adult human and mouse pancreas with nuclei labelled with DAPI (grey). Scale bar is 100 µm.

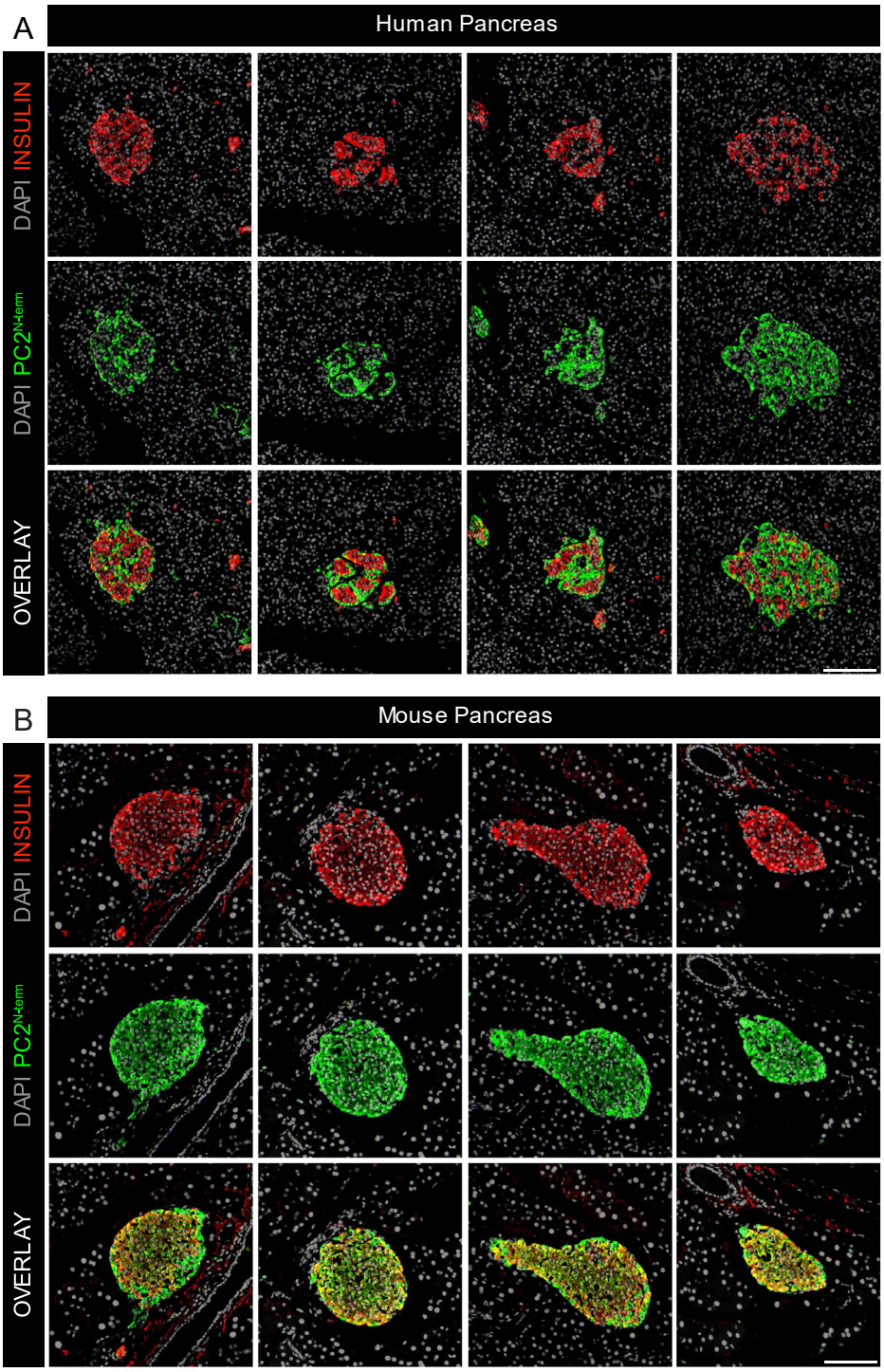


Figure S3 *In multiple human pancreas donors PC2 does not colocalize with insulin and in multiple mouse pancreata PC2 colocalizes with insulin (related to Figure 1).*

Immunostaining for insulin (red) and PC2 (green) in adult human (A) and mouse (B) pancreas with nuclei labelled with DAPI (grey). Scale bars are 100 µm.

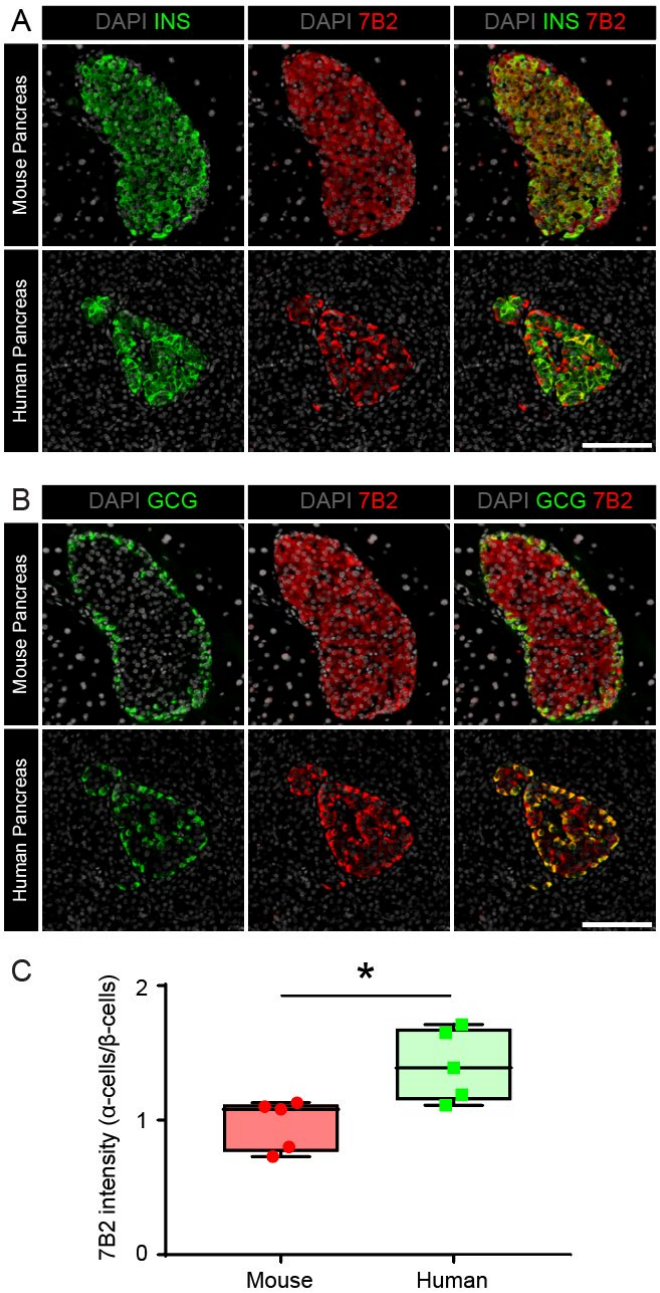


Figure S4 *Human β-cells have less immunoreactivity for 7B2 than α-cells whereas mouse β-cells have comparable immunoreactivity to α-cells* (*related to Figure 1).*

(A) Immunostaining for 7B2 (red) and insulin (green) in human and mouse pancreas. Overlays shown with nuclei labelled with DAPI (grey). Representative image shown (n=4-5). Scale bar is 100μm.

(B) Immunostaining for 7B2 (red) and glucagon (green) in human and mouse pancreas. Overlays shown with nuclei labelled with DAPI (grey). Representative image shown (n=4-5). Scale bar is 100μm.

(C) Quantification of relative mean fluorescent intensity of 7B2 in Ins+ or Gcg+ cells. Data shown on box and whisker plots. *$p < 0.05$ (two-tailed t-test).

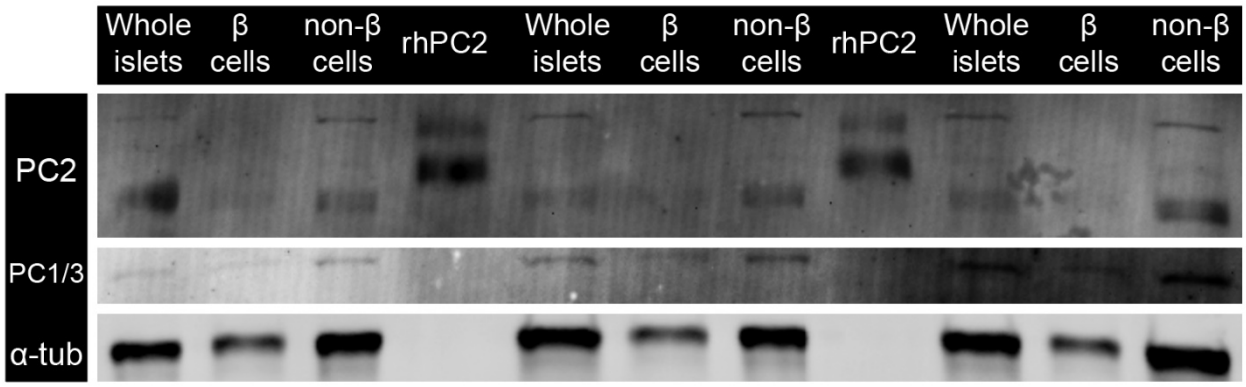


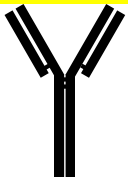
Figure S5 *Sorted human β-cells do not have detectable PC2 immunoreactivity unlike intact islets from the same donors.*
β-cells from human islets from organ donors (R317, R318, R341 from left to right) were purified by magnetic bead approach and cell lysates or intact islets or purified β-cells were used for western blot. The β-cell fractions did not have detectable *Gcg* mRNA and had 4-8x increase *Ins* mRNA by qPCR compared to intact islets (data not shown).

Table S1 *Alignment of PC2 sequences for human, mouse, rat, pig, and dog (related to Figure 2).*

Residues 94-116 and 622-638 from PC2 corresponding to the published epitopes of two commercially available PC2 antibodies. Sequences highlighted in yellow produced no immunoreactivity in pancreas. Residues in purple are conservative and residues in red are non-conservative. Proposed epitopes are shown.

N-terminal PC2 antibody (Thermo Fisher Scientific, PA5-14595, RRID#: AB_2299126):

Human	R	V	K	M	A	L	Q	Q	E	G	F	D	R	K	K	R	G	Y	R	D	I	N	E
Mouse	R	I	K	M	A	L	Q	Q	E	G	F	D	R	K	K	R	G	Y	R	D	I	N	E
Rat	R	V	K	M	A	L	Q	Q	E	G	F	D	R	K	K	R	G	Y	R	D	I	N	E
Pig	R	V	K	R	A	L	Q	Q	E	G	F	D	R	K	K	R	G	Y	R	D	I	N	E
Dog	R	V	K	M	A	L	Q	Q	E	G	F	N	R	K	K	R	G	Y	R	D	I	N	E



C-terminal PC2 antibody (Thermo Fisher Scientific, PA1-058, RRID#: AB_2158593):

Human	L	D	E	A	V	E	R	S	L	K	S	I	L	N	K	N
Mouse	L	D	E	A	V	E	R	S	L	Q	S	I	L	R	K	N
Rat	L	D	E	A	V	E	R	S	L	Q	S	I	L	R	K	N
Pig	L	D	E	A	V	E	R	S	L	K	S	I	L	G	K	H
Dog	L	D	E	A	V	E	R	S	L	K	S	I	L	R	K	N

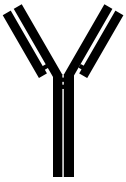


Table S2 *List of pancreatic organ donors* for histological experiments

Code	Age	Sex	BMI or bodyweight	HbA1c (%, mmol/mol)	Diabetes status	Diabetes medications	Corresponding figures
588	41	M	68.2kg	Unknown	None	None	3, S5
594	40	M	94.9kg	Unknown	None	None	3, S5
634	23	F	105kg	Unknown	None	None	3
R265	64	F	23.7	6.6, 49	None	None	2
R267	64	M	37.7	6.3, 45	None	None	2
R271	60	F	26	5.5, 37	None	None	2
R278	57	M	27.6	5.7, 39	None	None	4
R282	57	M	26.4	6.0, 42	None	None	4
R283	22	M	22.5	Unknown	None	None	4
R286	69	F	27.7	5.2, 33	None	None	4
R145	55	F	24.1	5.5, 37	None	None	1, S2, S5
R146	52	M	25.3	5.5, 37	None	None	1, S2, S5
R147	62	M	26	5.4, 36	None	None	1, S2, S5
R148	40	M	29.1	Unknown	None	None	1, S2
R151	46	F	26.7	5.4, 36	None	None	1, S2
R155	56	M	34	6.3, 45	None	None	1
R156	69	F	28.8	6.2, 44	None	None	1
R317	54	M	26.4	5.1, 32	None	None	S5
R318	54	M	20.5	5.0, 31	None	None	S5
R316	52	M	26	5.7, 39	None	None	5
R294	32	M	27.8	5.7, 39	None	None	5
R313	41	M	29	5.5, 37	None	None	5
R311	35	F	24	5.5, 37	None	None	5
R309	47	F	27.4	5.5, 37	None	None	5
R305	60	M	21	5.6, 38	None	None	5
R299	44	F	25.4	4.7, 28	None	None	5
R314	31	F	30.3	5, 31	None	None	5
R277	47	M	33.7	5.6, 38	None	None	5
R290	74	F	35.7	5.9, 41	None	None	5
R296	50	F	23.9	6.3, 45	T1DM	Not stated	5
R289	44	M	29.4	7.1, 54	T1DM	Not stated	5
R132	39	F	24.6	5.9, 41	T1DM	Not stated	5

R119	27	M	18.6	Unknown	T1DM	Not stated	5
R095	44	M	23.7	14.6, 137	T1DM	Not stated	5
R079	32	M	21.9	9.3, 78	T1DM	Not stated	5
R035	38	F	Unknown	10.9, 96	T1DM	Not stated	5
R312	52	F	27.5	9.3, 78	T2DM	None	5
R240	55	F	30.9	6.9, 52	T2DM	None	5
R198	47	F	29.7	5.4, 36	T2DM	None	5
R191	54	M	30.6	6.6, 49	T2DM	None	5
R171	72	F	27.5	7.4, 57	T2DM	None	5
R236	51	M	35.2	8.6, 70	T2DM	None	5
R231	41	F	37.1	6.8, 51	T2DM	None	5
R307	43	M	25.4	6.5, 48	T2DM	Metformin	5
R263	71	M	38.2	7.6, 60	T2DM	Metformin	5
R244	48	F	30.4	7.5, 58	T2DM	Metformin	5
R222	62	M	25.9	10, 86	T2DM	Metformin	5
R213	57	M	23.1	6.7, 50	T2DM	Metformin	5
R206	54	M	36	7, 53	T2DM	Metformin	5
R173	77	M	35.7	6.7, 50	T2DM	Metformin	5
R170	49	M	38.6	6.5, 48	T2DM	Metformin	5
R154	57	F	40.8	7.2, 55	T2DM	Metformin	5
R152	54	F	42.6	8.3, 67	T2DM	Metformin	5
R137	56	F	36.7	5.5, 37	T2DM	Metformin	5
R133	53	M	34.1	5.6, 38	T2DM	Metformin	5
R125	70	M	29.4	7.7, 61	T2DM	Metformin	5
R110	41	M	26.7	9.3, 78	T2DM	Metformin	5
R057	53	F	35.5	10.3, 89	T2DM	Metformin	5

Table S3 *List of human islet preparations used for pulse-chase experiments.*

Islet preparation	**1**	**2**	**3**	**4**
Unique identifier	R278	R282	R283	R286
Donor age (years)	57	57	22	69
Donor sex (M/F)	M	M	M	F
Donor BMI (kg/m^2)	27.6	26.4	22.5	27.7
Donor HbA_{1c}	5.7% (39 mmol/mol)	6.0% (42 mmol/mol)	Unknown	5.3% (33 mmol/mol)
Origin/source of islets[b]	ADI IsletCore	ADI IsletCore	ADI IsletCore	ADI IsletCore
Islet isolation centre	ADI IsletCore	ADI IsletCore	ADI IsletCore	ADI IsletCore
Donor history of diabetes?	No	No	No	No
Donor cause of death	NDD (neurological)	NDD (neurological	NDD (neurological	NDD (neurological
Warm ischaemia time (h)	Unknown	Unknown	Unknown	Unknown
Cold ischaemia time (h)	13	9.5	8.5	13.5
Estimated purity (%)	75	90	90	95
Estimated viability (%)	Unknown	Unknown	Unknown	Unknown
Total culture time (h)[d]	Unknown	Unknown	Unknown	Unknown
Glucose-stimulated insulin secretion or other functional measurement[e]	Stim index: 35 (16.7mM/1mM)	Stim index: 4 (16.7mM/1mM)	Stim index: 4.1 (16.7mM/1mM)	Stim index: 5.3 (16.7mM/1mM)
Handpicked to purity?	No	No	No	No

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit α-Insulin (C27C9)	Cell Signaling	Cat#: 3014; AB_2126503
Mouse α-Insulin (L6B10)	Cell Signaling	Cat#: 8138; AB_10949314
Guinea pig α-Insulin	Linco	Cat#: 4011-01F; AB_433703
Rabbit α-PC1/3	Lakshmi Devi	AB_2665530
Mouse α-PC1/3	Gunilla Westermark	(10), AB_2665529
Rabbit α-PC2$^{C\text{-term}}$ Epitope at C-terminus of mouse PC2 (residues 622-638)	Thermo Fisher Scientific	Cat#: PA1-058; AB_2158593
Rabbit α-PC2$^{N\text{-term}}$ Epitope near N-terminus of human PC2 (residues 87-116)	Thermo Fisher Scientific	Cat#: PA5-14595; AB_2299126
Mouse α-PC2$^{R\&D}$ Unknown epitope within human PC2 (residues 110-638)	R&D Systems	Cat#: MAB6018; AB_10718108
Rabbit α-PC2^{IgM} Polyclonal IgM antibody shared by Dr. Westermark	Gunilla Westermark	(10), AB_2737594
Mouse α-α-tubulin	Sigma-Aldrich	T9026, AB_477593
Mouse α-glucagon antibody	Sigma-Aldrich	Cat#: G2654; AB_259852
Rabbit α-glucagon antibody	AbCam	Cat#: EP3070; Ab10561971
Rabbit α-7B2 antibody	Nabil Seidah	AB_2737595
Mouse α-digoxigenin antibody	Novus Biologicals	Cat#: NB100-1879; AB_530922
Goat α-mouse IgG, alkaline phosphatase conjugated antibody	Sigma-Aldrich	Cat#: A3562; AB_258091
IRdye 680RD goat α-mouse IgG	LI-COR Biosciences	Cat#: 925-68070; AB_2651128
Biological Samples		
Isolated human islets	University of Alberta IsletCore	https://www.ualberta.ca/alberta-diabetes/facilities/core-services/isletcore
Human pancreas biopsies	Ike Barber Human Islet Laboratory	http://surgery.med.ubc.ca/research/labs/ike-barber-human-islet-transplant-laboratory/
Chemicals, Peptides, and Recombinant Proteins		
Recombinant human PC2	R&D Systems	6018-SE-010
3H-Leucine	Perkin Elmer	NET460005MC
Brefeldin-A	Sigma-Aldrich	B5936
1,3-Cyclohexanediamine, N1,N3-bis(4,5-dihydro-1H-imidazol-2-yl)-4,6-bis[4-[(4,5-dihydro-1H-imidazol-2-yl) amino]phenoxy]-, (1R,3S,4S,6R)-	MedChem Shortcut LLC	166830, (4)

Guanidine, N,N'''-[(1R,3S,4S,6R)-4-[[4-(aminoiminomethyl)amino]-1-naphthalenyl] oxy]-6-[4[(aminoiminomethyl)amino]phenoxy]-1,3-cyclohexanediyl]bis-, rel-	MedChem Shortcut LLC	166811, (4)
1-(1,3-thiazolan-2-yl)pentane-1,2,3,4,5-pentaol	ChemDiv	5408-0471, (6)
Proprotein convertase inhibitor - Calbiochem	Millipore	537076, (5)
Critical Commercial Assays		
In Situ Hybridization Kit	BioChain	K2191050
Experimental Models: Cell Lines		
Human: EndoC-βH1 (passage 70)	Dr. Raphael Scharfmann	(11)
Mouse: αTC-1 (passage 28)	American Type Culture Collection	(12)
Mouse: αTCΔPC2 (passage 12)	Dr. Don Steiner	(13)
Mouse: βTC-3 (passage 12)	Dr. Shimon Efrat	(14)
Experimental Models: Organisms/Strains		
PC2$^{-/-}$ mouse pancreas (18-week-old)	The Jackson Laboratory	B6;129-Pcsk2tm1Dfs/J
Dog pancreas (adult male)	Dr. Alan Charrington	Vanderbilt University
Pig pancreas (4-year-old)	Sinclair Bio Resources	Yucatan™ miniature swine
Mouse pancreas (12-week-old)	The Jackson Laboratory	C57Bl/6j
Rat Pancreas (8-week-old)	UBC Center for Disease Modelling	Wistar
Software and Algorithms		
MetaXpress® High-Content Image Acquisition & Analysis Software	Molecular Devices Corporation	https://www.moleculardevices.com/products/cellular-imaging-systems/acquisition-and-analysis-software/metaxpress
Odyssey Infrared Imaging System Application Software Version 3.0.16	LI-COR Biosciences	https://www.licor.com/
GraphPad Prism V7	GraphPad Software	https://www.graphpad.com/scientific-software/prism/
Oligonucleotides		
5'/DIG/GTG GTC ATG AAA GGC CAC TTG TCA AAG CCC ACC TTG GAG TCG TC -3'	Integrated DNA Technologies	*PCSK2* probe A
5'/DIG/CTT TAT GCA ACT CCA CAA GAA AAT GAT TCG TGA AGA CSG GTC -3'	Integrated DNA Technologies	*PCSK2* probe B
5'/DIG/CAC CAT GGC ACC TGC ATC AAG GAC CCC GTA GCC AAA GAG GTG ATT AAA TTC CAG GCC -3'	Integrated DNA Technologies	*PCSK2* probe C
5'/AAG ATG GCT TTG CAG CAG GAA GGA	Integrated DNA Technologies	PCSK2 forward primer
5'/AGC CAC ATT CAA ATC AAG GCC AGG	Integrated DNA Technologies	PCSK2 reverse primer

5'/AAG CAA ACC CAA ATC TCA CCT GGC	Integrated DNA Technologies	PCSK1 forward primer
5'/TCA CCA TCA AGC CTG CTC CAT TCT	Integrated DNA Technologies	PCSK1 reverse primer
5'/GCT CTC TAC CTG GTG TGT GGG GAG C	Integrated DNA Technologies	*Ins* forward primer
5'/GGA GCA GAT GCT GGT GCA GCA CTG	Integrated DNA Technologies	*Ins* reverse primer
5'/G(W)G T(S)A ACG GAT TTG G(Y)C GT	Integrated DNA Technologies	GAPDH forward primer
5'/GAC AAG CTT CCC (R)TT CTC (R)G	Integrated DNA Technologies	GAPDH reverse primer
5'/GGA CTT CGA GCA AGA GAT GG	Integrated DNA Technologies	ACTB forward primer
5'/AGC ACT GTG TTG GCG TAC AG	Integrated DNA Technologies	ACTB reverse primer