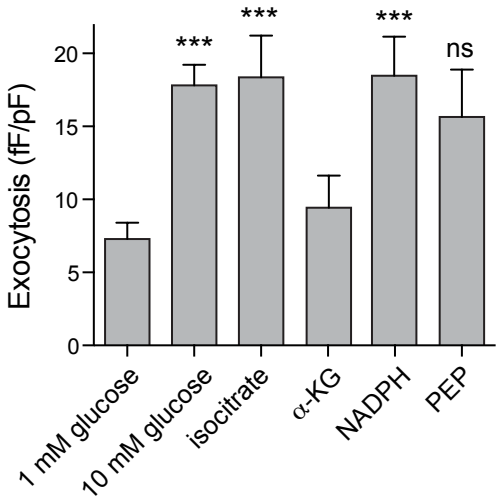
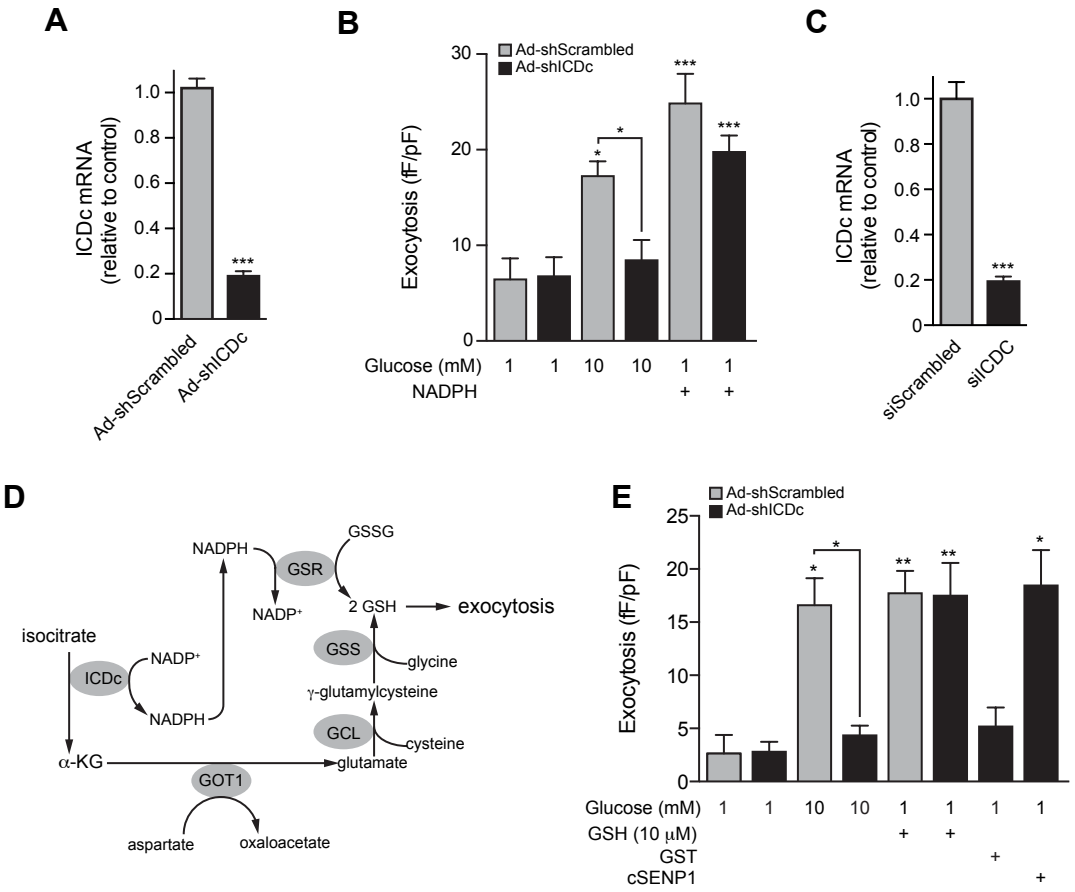
Figure S1



In INS 832/13 insulinoma cells, the total exocytotic resposne to a train of 10 membrane depolarizations following acute glucose (10 mM) pre-treatment, or upon intracellular infusion of isocitrate (400 μM), α-ketoglutarate (α-KG; 400 μM), NADPH (at a 10:1 molar ratio with $NADP^+$), or phosphoenolpyruvate (PEP; 400 μM) (n=50, 41, 20, 24, 13, 17 cells).

Data are shown as average +/- standard error and were compared with the nonparametric Kruskal-Wallis one-way ANOVA followed by Dunn's post-test. ****P*<0.001 compared with the 1 mM glucose control.

Figure S2



A) Knockdown of ICDc expression, measured by quantitative PCR, in INS 832/13 cells transduced with Ad-shICDc compared with control (Ad-shScrambled).

B) Knockdown of ICDc prevents the glucose-dependent (10 mM) but not hte NADPH-dependent (10:1 with $NADP^+$) amplification of exocytosis in INS 832/13 cells (n=13, 32, 13, 21, 27, 22 cells).

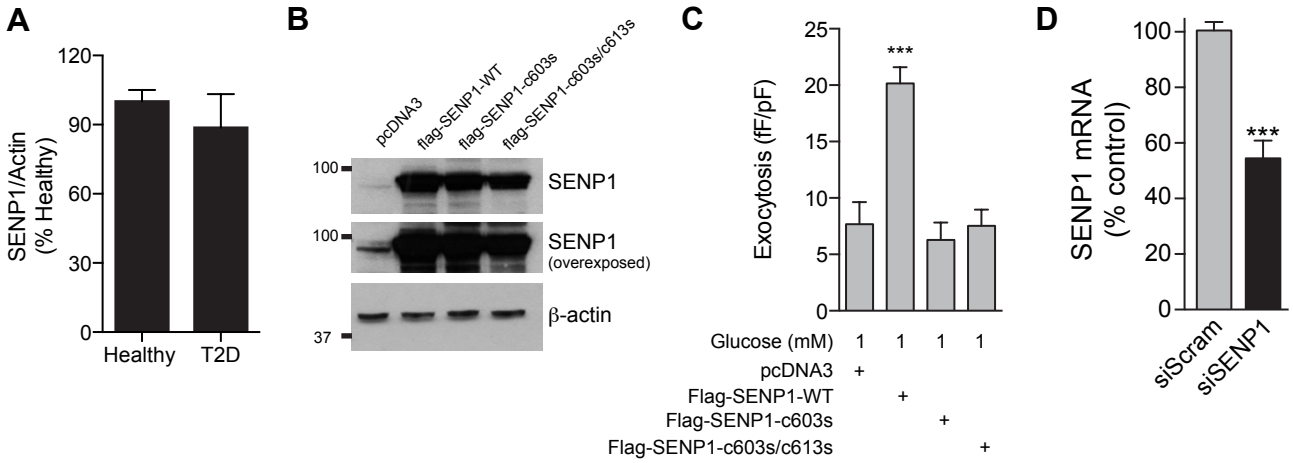
C) Knockdown of ICDc expression in dispersed human islet cells, measured by quantitative PCR, following transfection with siRNA duplexes targeting ICDc (siICDc) or control duplexes (siScrambled).

D) Proposed scheme coupling isocitrate to glutathione biosynthesis and reduction, and the amplification of insulin exocytosis. ICDc – cytosolic isocitrate dehydrogenase; α-KG – α-ketoglutarate; GOT1 - glutamic-oxaloacetic transaminase; GCL – glutamate-cysteine ligase; GSS – glutathione synthetase; GSR – glutathione reductase; GSSG – oxidized glutathione; GSH – reduced glutathione.

E) Knockdown of ICDc in INS 832/13 cells prevents the glucose-dependent amplification of insulin exocytosis, and this is rescued by intracellular provision of 10 μM GSH or the catalytic domain of SENP1 (cSENP1; 4 μg/ml; n=10, 12, 10, 11, 16, 20, 8, 14 cells).

Data are shown as average +/- standard error and were compared with the nonparametric Kruskal-Wallis one-way ANOVA followed by Dunn's post-test or, or by two-tailed student's t-test (panels A and C). *P<0.05, **P<0.01, ***P<0.001 compared with the control condition, or as indicated

Figure S3



A) Expression of SENP1 mRNA in islets from healthy donors (n=11 donors) and donors with T2D (n=13 donors) assessed by quantitative PCR.

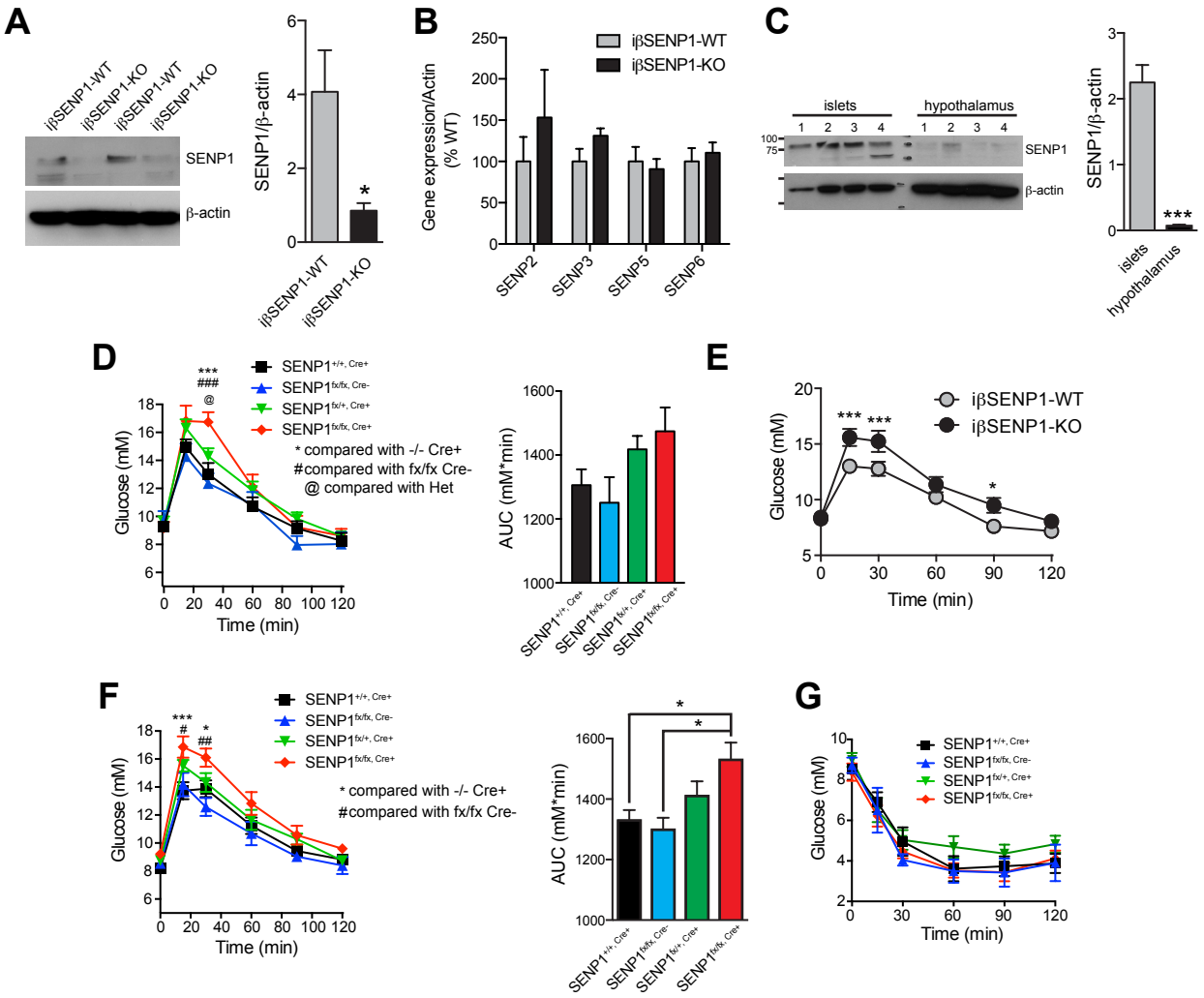
B) Western blotting showing the over-expression of flag-tagged wild type (WT) SENP1, or catalytically inactive mutants (c603s; c603s/c613s) in INS 832/13 cells. Over-exposure of the membrane (middle panel) demonstrates expression of the native SENP1 in these cells.

C) The total exocytotic response of INS 832/13 insulinoma cells was measured at low (1 mM) glucose as the capacitance response to a series of ten 500 ms membrane depolarizations from -70 to 0 mV. Cells were transfected with plasmid expressing pcDNA 3 alone (n=16), or the wild type (WT) SENP1 (n=38) or catalytically inactive SENP1 mutants (c603s, n=14; c603s/c613s, n=18).

D) SENP1 mRNA was measured by quantitative PCR following transfection of human islet cells (n=6 separate donors) with control siRNA (siScram) or siRNA targeting SENP1 (siSENP1).

Data are shown as average +/- standard error and were compared with the nonparametric Kruskal-Wallis one-way ANOVA followed by Dunn's post-test, or by two-tailed student's t-test (panels A and D). ***P<0.001 compared with control.

Figure S4



A) SENP1 was detected by western blotting of lysates from islets isolated from iβSENP1-WT and iβSENP1-KO mice (2 separate mice of each genotype shown) following treatment with tamoxafen as outlined in the Materials and methods. Densitometry confirms the reduction in SENP1 protein (n=4 mice of each genotype).

B) Measurement of SENP2, 3, 5 and 6 expression by quantitative PCR demonstrated no compensatory changes at the level of mRNA expression following loss of SENP1 in islets from iβSENP1-KO mice (n=6 mice of each genotype).

C) SENP1, detected by western blotting in isolated mouse islets, is expressed at low levels in mouse hypothalamus. Densitometry confirms the difference in SENP1 protein expression (n=4 separate mice).

D) Oral glucose tolerance was impared in the *p*SENP1-KO mice ($SENP1^{fx/fx\ Cre+}$) compared with two different littermate control groups ($SENP1^{+/+\ Cre+}$; $SENP1^{fx/fx\ Cre-}$) at 6 (**e**; n=9, 6, 17 and 12 mice of each genotype) weeks of age.

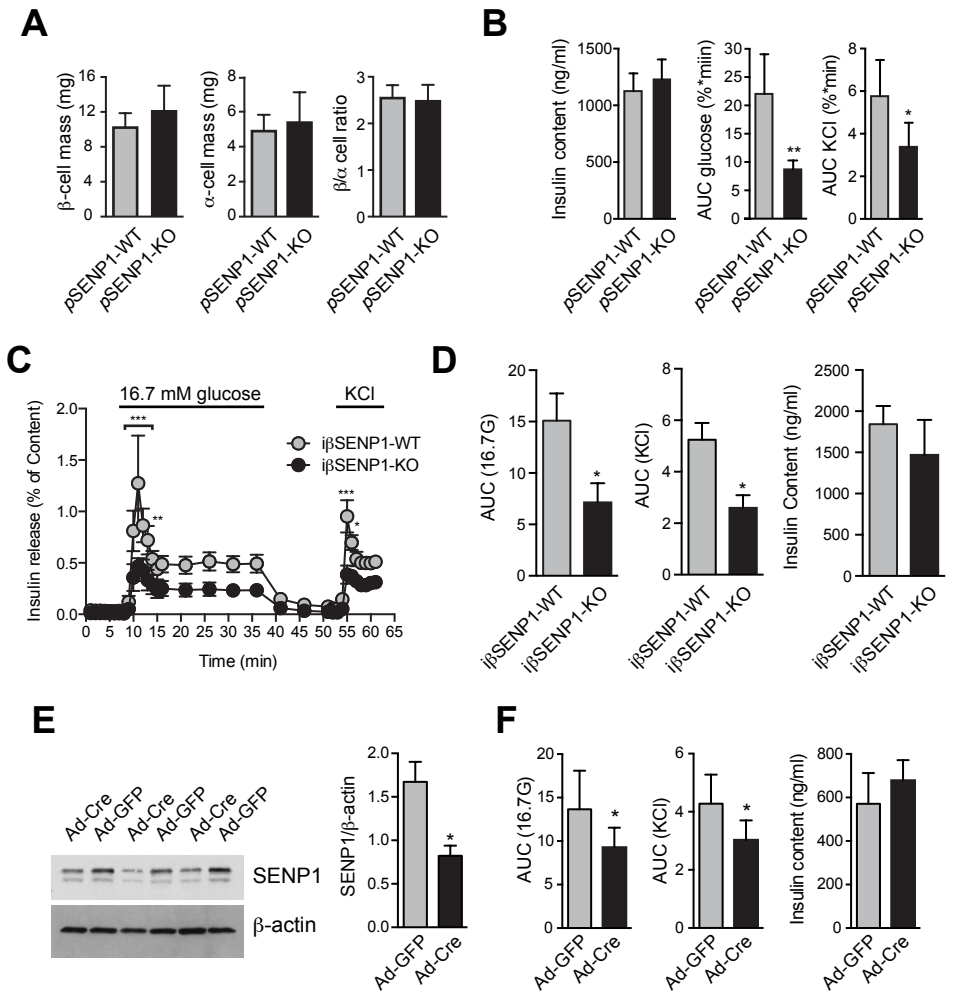
E) Compared with iβSENP1-WT littermate controls (n=21), iβSENP1-KO mice (n=11) displayed an exaggerated glucose excursion in response to an oral glucose challenge.

F) Oral glucose tolerance was impared in the *p*SENP1-KO mice ($SENP1^{fx/fx\ Cre+}$) compared with two different littermate control groups ($SENP1^{+/+\ Cre+}$; $SENP1^{fx/fx\ Cre-}$) at 12 (**f**; n=11, 7, 15 and 9 mice of each genotype) weeks of age.

G) IP insulin tolerance in littermate mice (n=13, 4, 14 and 10 mice of each genotype).

Data are shown as average +/- standard error and were compared with ANOVA followed by Bonferroni post-test, or by two-tailed student's t-test (panels A and C). **P*<0.05, ***P*<0.01, and ****P*<0.001 compared with control.

Figure S5



A) Quantification of α- and β-cell mass demonstrated no difference between *p*SENP1-WT and *p*SENP1-KO mice at 14 weeks of age (from **Fig. 8a**, n=4 mice of each genotype).

B) Insulin content of isolated was not different between groups, while the area under the curve (AUC) of the secretory response to glucose and KCl from pSENP1-KO islets was reduced (from **Fig. 8b**, n=4 mice of each genotype).

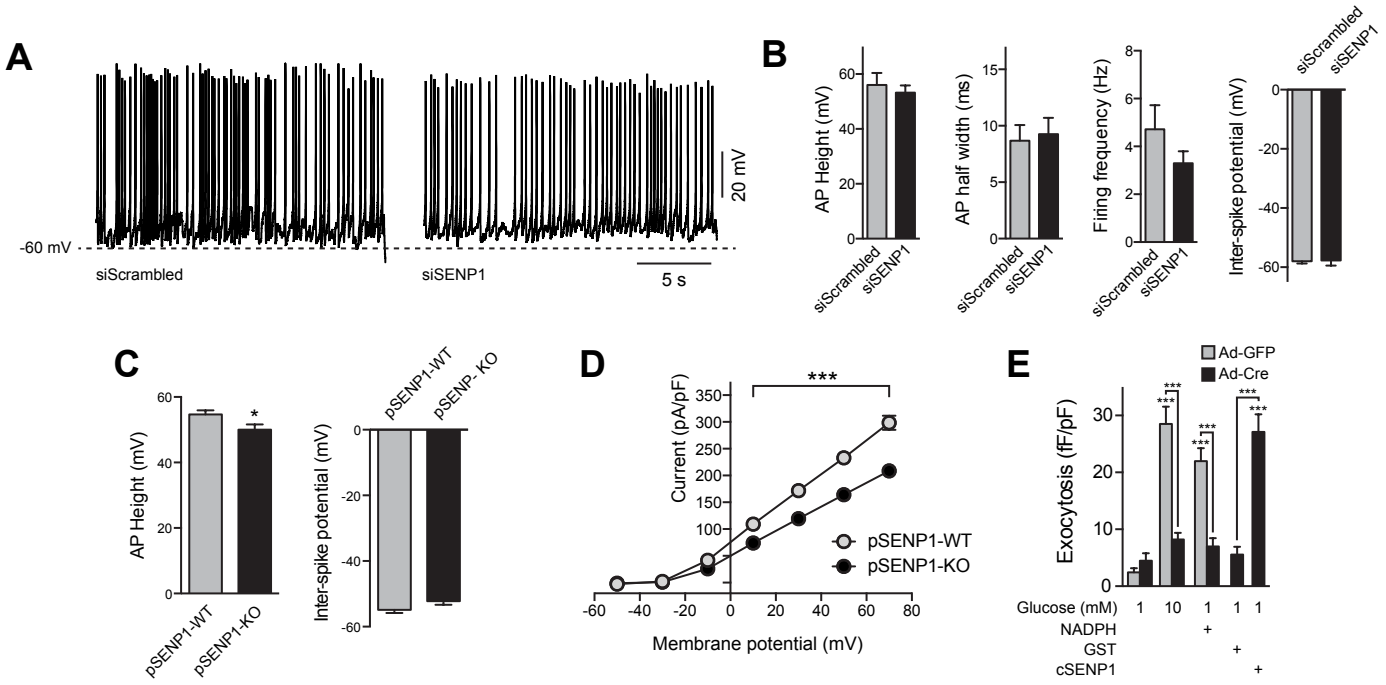
C-D) In vitro, islets from iβSENP1-WT (n=4) and iβSENP1-KO (n=4) mice displayed an impaired glucose and KCl-induced insulin secretory response in a perifusion assay, also shown as a reduced area under the curve (AUC) of the responses to glucose and KCl. Insulin content was not different between iβSENP1-WT and iβSENP1-KO mice.

E) SENP1 expression was also reduced in islets isolated from $SNEP1^{fx/fx}$ mice infected with adenovirus expressing Cre (Ad-Cre) compared to a GFP-only control (Ad-GFP). Knockdown of SENP1 was partial in this case, likely due to the limitations of virus penetration in intact islets (n=4 mice for each treatment).

F) Insulin content and the AUC of insulin secretory responses to glucose and KCl measured by perifusion (as in **panel C**) of $SENP1^{fx/fx}$ islets infected with Ad-GFP (n=8) or Ad-Cre (n=8).

Data are mean +/- standard error and compared bythe two-tailed student's t-test or with ANOVA followed by Bonferroni post-test (panel C). **P*<0.05, ***P*<0.01, and ****P*<0.001 compared with control or as indicated.

Figure S6



A) Representative action potentials recorded via perforated patch in human β-cells transfected with control siRNA (siScrambled) or siRNA targeting SENP1 (siSENP1). Representative of 6 and 7 cells from 2 donors.

B) Quantification of action potential height, half-width, firing frequency, and inter-spike potential in human β-cells transfected with either siScrambled or siSENP1.

C) Quantification of action potential height and inter-spike membrane potential, measured using perforated patch-clamp, in β-cells from pSENP1-WT and pSENP1-KO mice (from Fig. 8d; n=17, 19 cells, 3 and 4 mice of each genotype).

D) Current-voltage relationship of voltage-dependent K^+ currents measured in β-cells from pSENP1-WT (grey; n=41 cells, 3 mice) and pSENP1-KO (black, n=41 cells, 4 mice).

E) The total exocytotic response of single $SENP1^{fx/fx}$ β-cells infected with Ad-GFP or Ad-Cre confirm that reduction of SENP1 in vitro blunts both the glucose- and NADPH-dependent amplification of exocytosis, which can be rescued by direct re-introduction of cSENP1 (4 μg/ml; n=41, 38, 63, 55, 54, 54, 31, 60 cells, 4 separate mice).

Data are mean +/- standard error and were compared by the two-tailed student's t-test or with ANOVA followed by Bonferroni post-test (panels D and E). **P*<0.05, ***P*<0.01, and ****P*<0.001 compared with control or as indicated.

Donor	Age (years)	Sex	BMI	HbA1c (%)
R022	48	F	18	
R045	27	F	19	5.2
R047	56	F	32	
R049	79	M	21	
R050	72	F	40	5.8
R051	72	M	24	5.7
R055	67	F	26	6.0
R058	79	F	24	5.8
R062	41	M	20	5.2
R065	38	M	30	
R066	44	M	32	
R067	60	M	26	5.5
R072	54	F	31	6.2
R073	74	F	28	5.4
R074	55	M	26	5.5
R075	27	M	26	5.4
R076	63	F	26	
R077	54	F	25	5.2
R081	68	M	24	5.9
R082	65	F	25	5.4
R084	63	F	30	5.5
R085	61	M	30	5.6
R088	20	M	41	5.8
R089	63	F	20	
R090	46	M	22	
R091	65	F	26	5.6
R092	77	M	21	5.7
R094	46	F	28	5.8
R096	53	F	19	5.0
R097	56	F	26	5.0
R098	62	F	21	6.3
R099	34	F	39	5.5
R100	80	M	20	5.2
R101	59	F	26	5.5
R102	54	M	41	5.4
R104	42	M	27	5.4
R105	59	F	35	5.5
R106	52	M	20	5.6
R108	59	F	25	5.4
R109	60	F	25	6.1
R112	54	F	33	5.5
R113	62	M	31	5.7

Donor	Age (years)	Sex	BMI	HbA1c (%)
R114	65	F	33	6.0
R115	62	F	19	5.5
R116	43	M	28	
R118	63	M	23	5.3
R120	60	M	32	5.8
R121	18	M	19	
R122	55	M	29	5.9
R123	43	F	21	
R124	43	F	25	5.2
R126	38	M	24	5.2
R130	52	M	26	6.8
R133	53	M	34	5.6
H1440	49	M	27	5.3
H1442	44	M	23	5.9
H1577	58	M	22	5.8
H1621	27	M	35	5.6
H1648	67	F	30	5.3
H1657	49	F	29	6.3
H1669	21	M	35	5.7
H1674	57	F	20	5.1
H1675	53	M	24	5.7
H1687	21	M	23	
H1732	61	M	28	5.5
H1743	73	F	22	5.4
H1745	47	M	26	6.4
H1758	69	M	23	6.1
H1759	48	M	26	5.2
H1769	66	M	27	5.4
H1773	48	M	20	5.4
H1781	50	F	25	5.8
H1799	35	M	26	5.8
H1807	55	M	24	5.9
HI1823	54	F	27	5.9
H1851	14	M	20	5.9
H1852	58	F	33	6.5
H1858	57	M	31	5.5
H1876	54	M	31	5.6
AVG	53.2		26.5	5.63
SEM	1.7		0.6	0.04

Table S1. Non-diabetic Human Islet Donors. Summary characteristics of non-diabetic donors studied. BMI – Body Mass Index, HbA1c – glycated hemoglobin. Donors indicated with 'R' isolated at the Alberta Diabetes Institute IsletCore (www.bcell.org/IsletCore.html). Donors starting with 'H' isolated at the Alberta Clinical Islet Laboratory.

Donor	Age (years)	Sex	BMI	HbA1c (%)	Diabetes Duration (years, if known)	Diabetes Medication (if known)
R023	65	M	29	8.3		
R024	52	F	29			
R031	54	M	30	7.2		
R046	65	F	18			Metformin/Insulin
R054	73	F	22	6.1	5	
R057	53	F	36	10.3	20	Metformin
R059	68	F	28			
R063	57	F	23	6.1	2	
R064	36	M	28	10.9	1.5	
R070	33	F	32	7.1		None
R071	28	M	28	6.6	5	Insulin
R078	47	F	35	5.9	6	
R080	57	M	28	5.8	5	Metformin
R083	71	F	27	6.6	20	
R086	45	M	24	7.4	2.5	
R093	75	M	26	6.3		Metformin
R107	56	F	28	9.3	20	
R110	41	M	27	9.3	2	Metformin
R125	70	M	29	7.7	20	Metformin
R131	52	M	26	6.8		Metformin
AVG	54.9		27.7	7.51***	9.1	
SEM	3.1		0.9	0.38	2.4	

****P*<0.001 compared with the non-diabetic donors in Table S1.

Table S2. Human Islet Donors with Type 2 Diabetes (T2D). Summary characteristics of T2D donors studied. BMI – Body Mass Index, HbA1c – glycated hemoglobin. Islets were isolated at the Alberta Diabetes Institute IsletCore (www.bcell.org/IsletCore.html).

qPCR	Human SENP1	F	5'-TTACCTCGAAACCCGAAAGA -3'
		R	5'-TCCAAGATGGACTTGGAACAG-3'
	Human beta actin	F	5'-GGACTTCGAGCAAGAGATGG-3'
		R	5'-AGCACTGTGTTGGCGTACAG-3'
	Mouse SENP1	F	5'-CGTTCTTCCAGGCAGAGCTA-3'
		R	5'-TGAAGCTGTAGTGCCAATGC-3'
	Mouse SENP2	F	5'-TGAATGGGAGTGACTGTGGA -3'
		R	5'-TGAAGGATCTCCCACACCAT -3'
	Mouse SENP3	F	5'-ACCCCATCATAACCCTTGGT -3'
		R	5'-TGGACAGAACTGGCTCAATG -3'
	Mouse SENP5	F	5'-TCCATTATCAGCTCGCCATA -3'
		R	5'-GGGCCAGACATCAAAAGTGT -3'
	Mouse SENP6	F	5'-ACCCAAAAGTCCCACAACAG -3'
		R	5'-GGGAGGAAACCAGTTCATCA -3'
	Mouse beta actin	F	5'-TGTTACCAACTGGGACGACA-3'
		R	5'-GGGGTGTTGAAGGTCTCAAA-3'
	Human ICDc	F	5'- TTTGGCCTGAGCTAGTTTGA-3'
		R	5'- CTGGCTTCATGACCAAGGAC-3'
	Rat ICDc	F	5'- AAAATATCCCCCGGCTAGTGA-3'
		R	5'-TCATCATTGGCCGACACG-3'
Genotyping	Flox	F	5'-AGAGTGAGACCCTGTCTCAACCCAAGC-3'
		R	5'-CACACAACTAAGTTAACTGCTGGAAACCAGAGC-3'
	Cre	F	5'-TCTCACGTACTGACGGTGG-3'
		R	5'-ACCAGCTTGCATGATCTCC-3'
	Positive control	F	5'- CTAGGCCACAGAATTGAAAGATCT-3'
		R	5'- GTAGGTGGAAATTCTAGCATCATCC -3'
Muta-genesis	SENP1 C603S	F	5'-TCAGCAGATGAATGGAAGTGACTCTGGGATGTTTGCCTGCAAATATG-3'
		R	5'-CATATTTGCAGGCAAACATCCCAGAGTCACTTCCATTCATCTGCTGA- 3'
	SENP1 C613S	F	5'-GTTTGCCTGCAAATATGCTGACTCTATTACCAAAGACAGACCAATCAACTTC-3'
		R	5'-GAAGTTGATTGGTCTGTCTTTGGTAATAGAGTCAGCATATTTGCAGGCAAA- 3'
siRNA	siScrambled (Integrated DNA Tech.)		5'-GAGACCCTATCCGTGATTA-3'
	siICDc rat (Integrated DNA Tech.)		5'- GTATGATGGACGCTTCAAAGA-3'
	siICDc human (Ambion)	1	5'- CAUUAAAGGUUUACCCAAU(tt)-3'
		2	5'- CGAAUCAUUUGGGAAUUGA(tt)-3'
	siScrambled (Qiagen)		Not known - Allstars negative control, catalog #, SI03650318
	siSENP1 human (Life Technol.)		5'- CACAGUGUAUAUUCCCUAUtt- 3'

Table S3. Primers. List of primers used in the study, along with supplier name, if available.