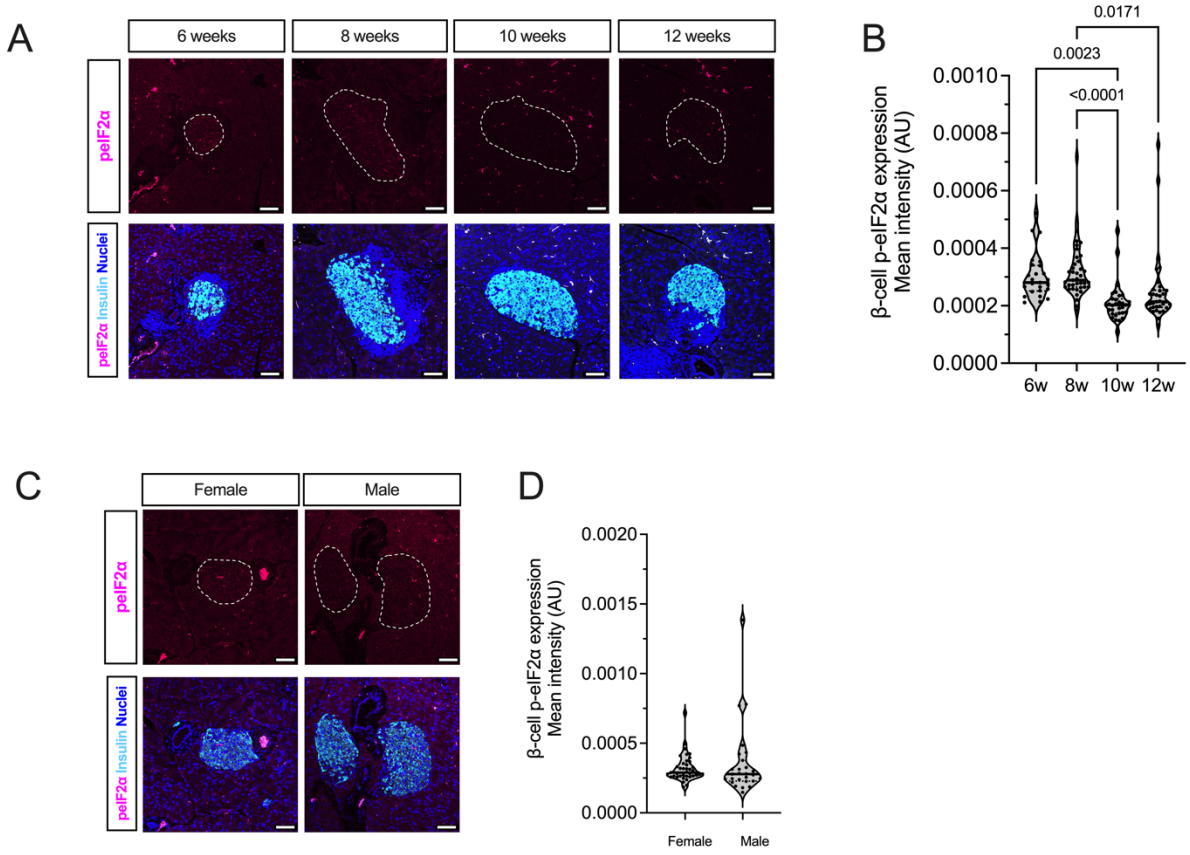
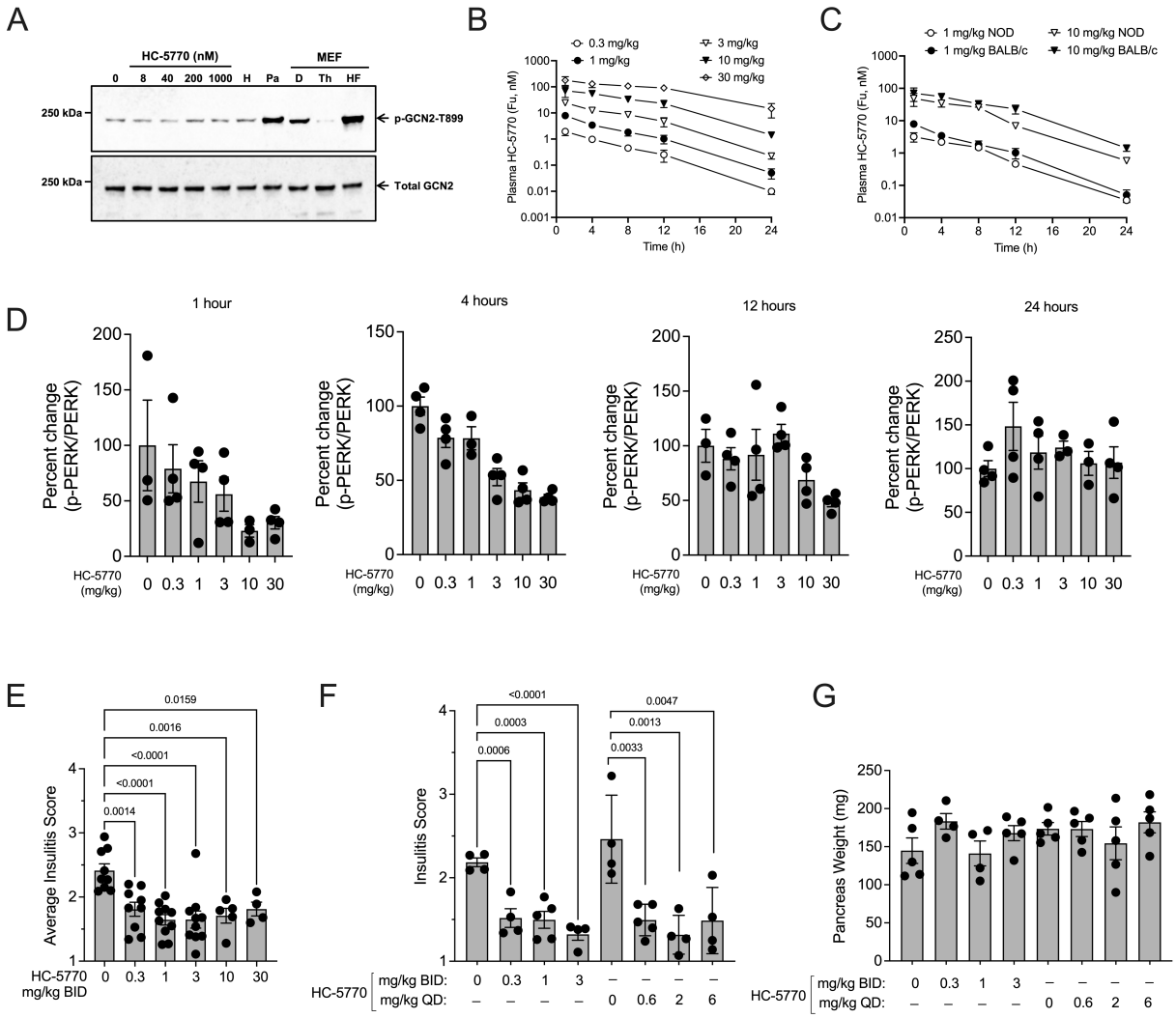
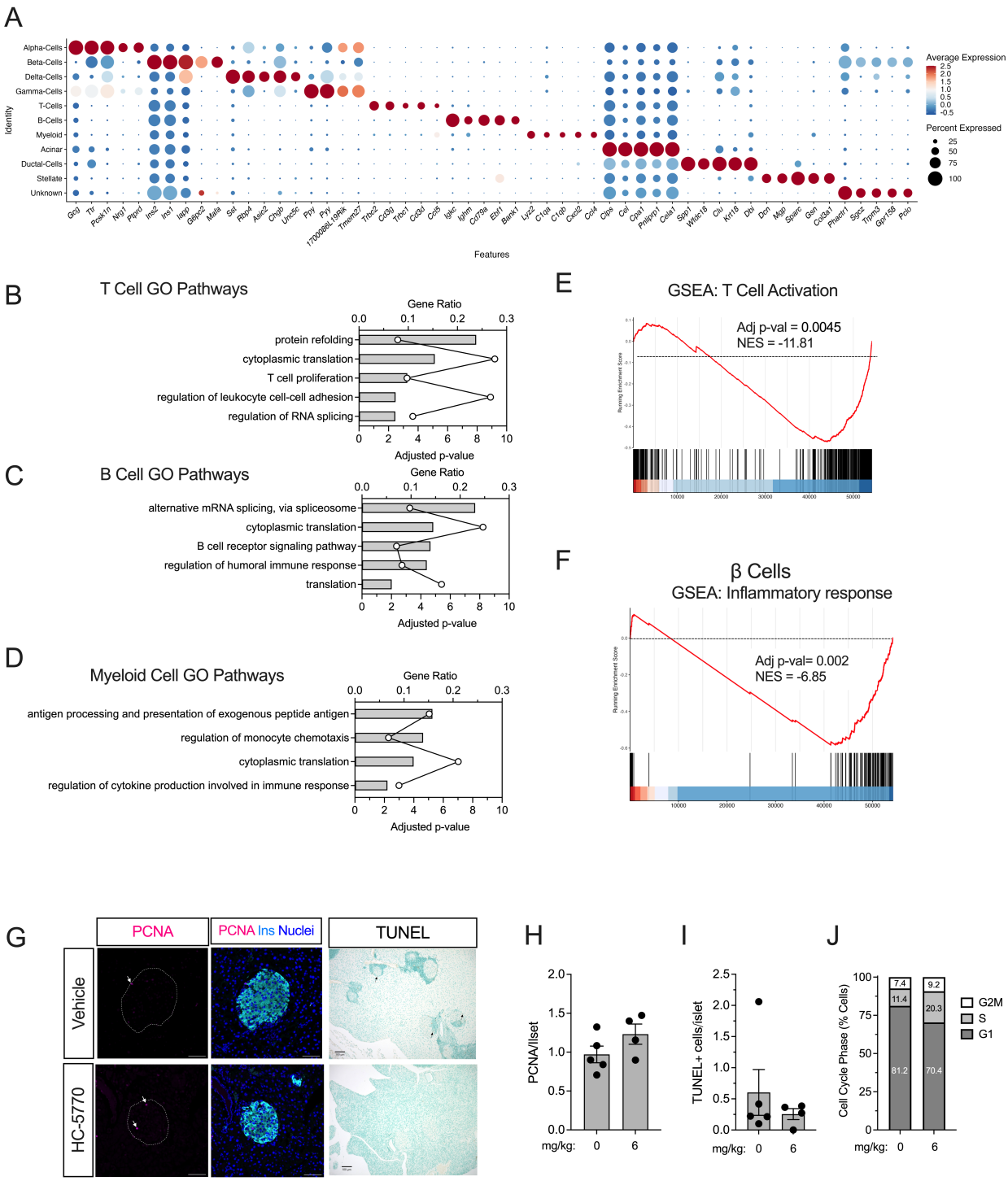


Supplemental Figure 1: Phospho-eIF2α levels in NOD mice islets. (***A***) Representative images of pancreata from female NOD mice at the indicated ages immunostained for phospho-eIF2α (p-eIF2α) (*magenta*), insulin (*cyan*), and nuclei (*blue*), scale bar = 50 μm; *dotted lines* indicate islet*s.* (***B***) Quantification of the p-eIF2α fluorescence intensity from data in *panel (A)*; each dot represents an islet, N=4-5 mice, and N>5 islets per mouse (ANOVA). (***C***) Representative images of pancreata from 8-week-old male and female NOD mice immunostained for p-eIF2α (*magenta*), insulin (*cyan*), and nuclei (*blue*), scale bar = 50 μm; *dotted lines* indicate islet*s.* (***D***) Quantification of the p-eIF2α fluorescence intensity from data in *panel (C)*; each dot represents an islet, N=4-5 mice, and N>5 islets per mouse (T-test).

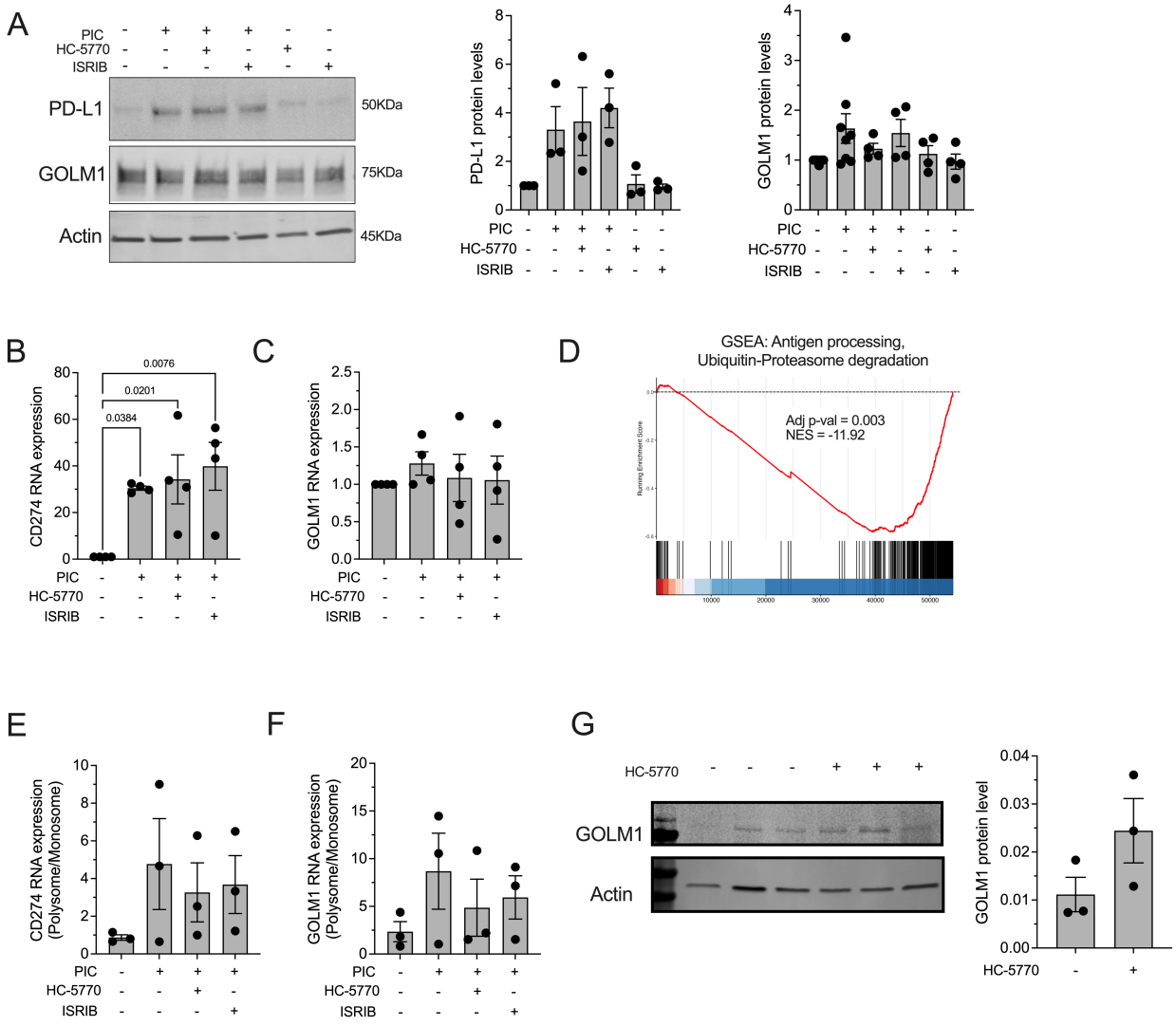


Supplemental Figure 2: Pharmacokinetics and pharmacodynamics of HC-5770. (***A***) Immunoblot analysis for phospho-GCN2 (p-GCN2) and total GCN2 of CD1 mouse islets, whole mouse pancreas (Pa), and mouse embryonic fibroblasts (MEF) under different treatment conditions: HC-5770, harmine (H), DMSO vehicle (D), thapsigargin (Th), or halofuginone (HF). (***B***) Free unbound concentration of HC-5770 in plasma following a single oral administration in BALB/c mice. HC-5770 quantified by LC-MS/MS (n=5 mice/group/time point). (***C***) Comparison of free drug concentrations in BALB/c and NOD mice plasma. HC-5770 quantified by LC-MS/MS following a single oral administration at 1 or 10 mg/kg. NB: BALB/c plasma data replicated from *panel (B)* for comparative purposes. (***D***) SimpleWestern® quantification of pPERK/PERK levels in BALB/c mouse pancreas following a single oral administration of HC-5770. (***E***) Insulitis scores in NOD mice following two weeks of twice daily (BID) treatment with HC-5770 (ANOVA). (***F***) Insulitis scores of NOD mice following two weeks of treatment with HC-5770 on a twice-daily (BID) or once-daily (QD) dosing schedule (performed contemporaneously). NB: QD dosing data are replicated in the data presented in Figure 2H, but they are shown here for comparative purposes (ANOVA). (***G***) Gross pancreas weight of NOD mice following two weeks of treatment with HC-5770 on both a QD and a BID dosing schedule (performed contemporaneously) (ANOVA). Data are presented as mean ±SEM.



Supplemental Figure 3. Single-cell RNA sequencing of islets from NOD mice treated with HC-5770. 6-week-old female NOD mice were treated with Vehicle or HC-5770 (6 mg/kg) for 2 weeks, and isolated islets were subjected to scRNA-seq. (***A***) Dot plots of the top 5 genes used to identify cell type clusters. The size of the dots indicates the percentage of cells that express the indicated gene. The color scale shows the change in normalized average gene expression within the different groups. N=3 biological replicates for scRNA-seq. (***B***) Gene ontology (GO) analysis of pathways observed in islet-resident T cells with HC-5770 treatment. (***C***) GO analysis of pathways observed in islet-resident B cells with HC-5770

treatment. (***D***) GO analysis of pathways observed in islet-resident myeloid-derived cells with HC-5770 treatment. (***E***) Gene set enrichment analysis (GSEA) of T cells showing HALLMARK: T cell activation. (***F***) GSEA of β cell clusters showing HALLMARK: Inflammatory response pathway. (***G***) Representative images of pancreata stained for PCNA (*magenta*, *arrows* indicate PCNA+ cells), insulin (*cyan*), and nuclei (*blue*); scale bar = 50 μm (*left and middle panels*) or TUNEL (*brown*, *arrows* indicate TUNEL+ cells), scale bar = 100 μm (*right panel*). (***H***) Quantification of PCNA+ cells in the islet; each data point represents the average of PCNA+ cells/islet from 3 separate pancreas sections from one mouse; N=4-5 mice (T-test). (***I***) Quantification of TUNEL+ cells in the islet; each data point represents the average of PCNA+ cells/islet from 3 separate pancreas sections from one mouse; N=4-5 mice (T-test). (***J***) Percentage of β cells in G1, S, and G2M cell cycle phases from scRNA-seq data. Data are presented as mean ±SEM.



Supplemental Figure 4. Coordinate increases in PD-L1 and GOLM1 in human islets and β cells and mouse islets. (***A***) Representative immunoblot analysis of PD-L1 and GOLM1 of human islets treated with or without proinflammatory cytokines (PIC), HC-5770, or ISRIB (*left panel*); immunoblot quantification of PD-L1 levels (*middle panel*) and GOLM1 levels (*right panel*). N=3-8 independent experiments from different islet donors (ANOVA). (***B, C***) Relative *CD274* or *GOLM1* mRNA levels in human islets treated ±PIC, HC-5770, or ISRIB; N=4 independent experiments from different islet donors (ANOVA). (***D***) Gene set enrichment analysis (GSEA) of β cell clusters from scRNA-Seq following treatment of NOD mice with HC-5770 (Figure 3) showing REACTOME: antigen processing, ubiquitin-proteasome degradation. (***E, F***) Relative *CD274* and *GOLM1* levels in polyribosome to monoribosome fractions in human islets treated ±PIC, HC-5770, or ISRIB. N=3 independent experiments from different islet donors (ANOVA). (G) Immunoblot for GOLM1 and actin (loading control) from islets of 8-week-old female NOD mice treated with Vehicle or HC-5770 (6 mg/kg) for two weeks (*left panel*) and quantitation of the data from islets from N=3 mice per group (T-test). Data are presented as mean ±SEM.

Supplemental Table 1: Pathway analysis for specific β cell clusters

Clusters	GO term	GO #	Gene ratio	-log10(adj p-val)
β0	chaperone-mediated protein folding	GO:0061077	0.077	1.921
	digestion	GO:0007586	0.056	1.521
	cellular response to calcium ion	GO:0071277	0.055	1.526
	translation	GO:0006412	0.029	2.500
	regulation of apoptotic process	GO:0042981	0.013	1.467
β1	insulin secretion	GO:0030073	0.117	4.003
	peptide hormone secretion	GO:0030072	0.094	4.068
	antigen processing and presentation	GO:0019882	0.056	2.660
	protein localization to extracellular region	GO:0071692	0.051	2.492
	regulation of response to stress	GO:0080134	0.013	1.420
β2	cytoplasmic translation	GO:0002181	0.115	9.355
	antigen processing and presentation of peptide antigen via MHC class I	GO:0002474	0.085	2.095
	digestion	GO:0007586	0.067	2.357
	response to endoplasmic reticulum stress	GO:0034976	0.035	2.032
	regulation of apoptotic process	GO:0042981	0.013	2.040
β3	antigen processing and presentation of exogenous peptide antigen	GO:0002478	0.089	1.907
	digestion	GO:0007586	0.078	2.592
	secretion	GO:0046903	0.020	1.435
	proteolysis	GO:0006508	0.014	1.839
β4	chaperone cofactor-dependent protein refolding	GO:0051085	0.152	3.264
	digestion	GO:0007586	0.067	3.116
	proteolysis	GO:0006508	0.014	3.827
	response to stress	GO:0006950	0.007	1.678
β5	digestion	GO:0007586	0.089	4.678
	response to hormone	GO:0009725	0.017	1.466
	proteolysis	GO:0006508	0.013	1.613
β6	cytoplasmic translation	GO:0002181	0.044	2.212
	proteolysis	GO:0006508	0.009	1.742
β7	response to endoplasmic reticulum stress	GO:0034976	0.026	1.759
	protein processing	GO:0016485	0.024	1.313
	secretion	GO:0046903	0.014	1.597
	proteolysis	GO:0006508	0.014	5.539
β8	digestion	GO:0007586	0.044	1.757
	proteolysis	GO:0006508	0.009	3.110

Supplemental Table 2: Non-diabetic human donor islet characteristics

Donor ID	Islet source	Age (yrs)	Sex	BMI	HbA1c (%)
RRID: SAMN19470079	Integrated Islet Distribution Program (IIDP)	39	M	27.6	5.5
RRID: SAMN19591106		61	M	29.3	5.9
RRID: SAMN19897466		28	F	24.7	5
RRID: SAMN28867622		36	M	29.6	5.4
RRID: SAMN29657580		40	M	23	5.2
RRID: SAMN30648329		54	M	21.5	5.2
RRID: SAMN30986138		67	M	34.4	5.3
RRID: SAMN32537360		51	M	25	5.3
RRID: SAMN19796386	University of Alberta	40	M	31.7	5.4
RRID: SAMN19859645		58	M	27.4	5.5
RRID: SAMN29094695		61	F	36.1	5.8
RRID: SAMN33456515		48	M	24.9	5.8
RRID: SAMN34997905		40	M	30.4	5.4
RRID: SAMN35795155		50	M	33.8	5.8
NDRI 2304-02252	National Disease Research Interchange	27	M	31.22	N/A

Supplemental Table 3: Human Pancreas Analysis Program (HPAP) donor characteristics

Donor ID	Donor type	Age (yrs)	Sex	BMI	HbA1C
RRID: SAMN19776470	Non-diabetic	5	F	16.3	6.8
RRID: SAMN19776475		3	F	12	5.3
RRID: SAMN22562815		4	M	20.63	4.9
RRID: SAMN19776478		8	M	16.82	N/A
RRID: SAMN19776465		13	M	18.6	5.2
RRID: SAMN19776467		23	F	16	5.2
RRID: SAMN19776457		24	M	20.8	4.9
RRID: SAMN19842611		25	M	23.96	5.6
RRID: SAMN22562810		28	F	24.7	5
RRID: SAMN19776458		31	F	32.71	4.4
RRID: SAMN19776466		35	M	26.91	5.2
RRID: SAMN19842585		33	M	32.89	5.6
RRID: SAMN19776468		35	F	21.9	5.3
RRID: SAMN19776471		M	35	23.98	5.4
RRID: SAMN19776453		F	39	34.7	4.7
RRID: SAMN19776455	Donors with single islet-specific autoantibody (AAb1+)	M	18	24.3	5.5
RRID: SAMN19776460		M	23	28.6	5.3
RRID: SAMN19776469		M	13	18.34	5.7
RRID: SAMN19776476		F	27	26.2	5.2
RRID: SAMN19776480		M	29	37.2	5.4
RRID: SAMN19776481		F	21	28.99	5.1
RRID: SAMN19842601		M	19	23.1	5.6
RRID: SAMN19842621		M	21	25.59	5.6
RRID: SAMN19776474	Donors with single islet-specific autoantibody (AAb2+)	M	15	24.07	5.9
RRID: SAMN25600003		M	15	23.59	5.3
RRID: SAMN19776451	Donors with T1D	M	14	13.2	N/A
RRID: SAMN19776452		F	13	21.4	N/A
RRID: SAMN19776454		F	17	21.35	8.9
RRID: SAMN19776463		F	10	16.3	9
RRID: SAMN19776485		M	24	27.9	10.4
RRID: SAMN19842593		M	24	16.98	13
RRID: SAMN19842600		F	12	15.42	9.8
RRID: SAMN19842613		F	12	18.5	13.3
RRID: SAMN19842616		F	15	19.3	10.4