SUPPLEMENTAL INFORMATION

Experimental procedures

We obtained human pancreatic islets from the Integrated Islet Distribution Program (IIDP) of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health, and the Juvenile Diabetes Research Foundation (JDRF). Human pancreatic tissue samples (from the head of the pancreas) from healthy and type 2 diabetic donors were obtained from the Human Islet Cell Processing Facility at the Diabetes Research Institute, University of Miami. A summary of the characteristics (age, gender, BMI, and cause of death) of the human donors used in the study is provided as Supplementary Material (Table S1). Animals were maintained under conditions approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Miami and procedures were monitored by the Division of Veterinary Resources of the University of Miami and approved by the IACUC.

Biosensor cells

Real time measurements of serotonin secretion were performed using Chinese hamster ovary (CHO) cells expressing 5-HT_{2C} receptors (Huang et al., 2005). After loading them with 4 μM fura-2 AM for 1 h at room temperature, an aliquot of biosensor cells was transferred to a recording chamber and islets (human or mouse) were placed on the top of the biosensors. Cells were excited at 340 nm, followed by 380 nm, and the ratios were calculated (ratio 340/380 shown in Figure 2). Real time measurements of acetylcholine secretion were performed in a similar way but using instead CHO cells stably expressing muscarinic M3 receptors (Rodriguez-Diaz et al., 2012). The non-selective 5-HT_2

antagonist mianserin (10 μM, Tocris) and the muscarinic receptor antagonist atropine (10 μM, Tocris) completely eliminated responses generated in the respective biosensor cells by islet stimulation. To eliminate the contribution of purinergic P2Y receptors that could be expressed in biosensor cells, biosensor cells were incubated prior to the experiment for 60 minutes in 500 μM ATP to desensitize receptors. Pharmacological agents used in the study did not themselves elicit biosensor responses or alter the ability of biosensors to respond to their designated transmitter.

Reserpine (500 nM, Tocris) was used to deplete endogenous serotonin and fluvoxamine (500 nM) to increase serotonin levels (500 nM, Tocris). Fluoxetine, the most common inhibitor of serotonin reuptake, could not be used in these studies because it inhibited the 5-HT_2 receptors expressed in biosensor cells. Representative traces of biosensor responses show the average response (black line) and SEM (in grey) (Figures 2, S2 and S4, $n > 5$ cells).

Insulin and glucagon secretion

Human islets were received and kept in culture medium at 37°C. Perifusion experiments were performed 24h-72h after islet arrival. Hormone secretion from isolated human islets (100 islets per column) was measured with an automated perifusion system, where stimuli were applied to the perifusion solution and the perifusate collected every minute. One hundred islet equivalents were not always sufficient to detect glucagon secretion from human islets. We therefore used 200 islet equivalents to be able to detect glucagon secretion with a success rate of about 90%. The flow rate in the perifusion setup was 50 μL/min. To avoid protease digestion of the hormones, aprotinin (5 μg/mL) was added to

the perifusate and collection plates were continuously kept at 4°C. Hormone secretion from human islets was stimulated by raising glucose concentration from 3 mM to 11 mM (to elicit insulin and somatostatin secretion) and by decreasing it from 11 mM to 1 mM (to elicit glucagon secretion), in the absence or presence of exogenous serotonin (different concentrations; Sigma), fluvoxamine (500 nM), or the 5-HT_{1F}-specific agonist LY344864 (100 nM, Tocris). Drugs were applied for 5 min before raising glucose concentration and kept for 10 min after decreasing it. To deplete serotonin irreversibly, islets were incubated for 1 h at 37°C in reserpine (100 nM) before placing the islets in perifusion columns. Hormone concentration in the perifusate was then determined with insulin or glucagon Elisa Kits (Mercodia). DNA extracted from islets was quantified using PicoGreen kit (Invitrogen) and responses to KCl (25 mM) were used for normalization of islet size and number.

We observed that the quality of the human islets varied greatly between different preparations. This could affect the magnitude of the responses to changes in glucose concentrations. Even within the same preparation, different replicates responded differently. To be able to compare the data between different experiments, we normalized the responses of drug-treated islets to those obtained under control conditions (vehicle treated) and presented the data always relative to the control response. Only islet preparations where the alpha cell response (calculated as the change (delta) in glucagon concentration when glucose was switched from 11 mM to 1 mM divided by glucagon concentration at 11 mM) was greater than 10% were included in the analyses (10% of the data did not reach this criterion and were excluded). We did not see any significant correlation of the magnitude of the alpha cell response with donor age or BMI.

Immunohistochemistry

Human pancreatic tissues from healthy and type 2 diabetic donors (at least 3 different donors in each group, samples taken from the head of the pancreas) were fixed overnight in 4% PFA, cryoprotected in a sucrose gradient (10, 20 and 30% w/w sucrose), and frozen in Tissue-Tek Optimal Cutting Temperature (OCT) compound before cryosectioning (-20°C). Mouse pancreatic tissue was perfused with 4% PFA and processed similarly. Human islets were incubated with culture medium plus p-Chlorophenylalanine (PCPA, Tocris, Cat. Nr. 0938) (10 μM) for 2 h at 37°C and then placed on a coverslip and fixed with 4% PFA for 1 h.

After a rinse with PBS-Triton X-100 (0.3%), coverslips with islets or sections (10 μm) were incubated in blocking solution (PBS-Triton X-100 0.3% and Universal Blocker Reagent; Biogenex, San Ramon, CA). Thereafter, sections were incubated 24 h (20º C) with primary antibodies diluted in blocking solution. We immunostained beta cells (guinea pig anti-insulin antibody, Accurate Chemical & Scientific, Wesbury, NY), alpha cells (mouse anti-glucagon antibody, Sigma, St. Louis, MO), serotonin receptors (see supplementary material information, Novus Biologicals), Tph1 (sheep anti-Tph1, Millipore, AB1541), and serotonin (rabbit anti-serotonin antibody, Sigma). Immunostaining was visualized by using Alexa Fluor conjugated secondary antibodies (1:500 in PBS; 16 h at 20ºC; Invitrogen, Carlsbad, CA). Cell nuclei were stained with DAPI. Slides were mounted with Vectashield mounting medium (Vector Laboratories). We used ImageJ software (http://imagej.nih.gov/ij/) to estimate the total number of cells that contained serotonin. For this quantification, we first subtracted the background signal

and considered only "positive" cells that showed a clear dapi-labeled nucleus. We divided the total number of serotonin-positive cells by the islet area and multiplied these values by an estimated average islet area of 15,000 μm^2. Similarly, we estimated the number of alpha, beta or delta cells that expressed Tph1 or serotonin receptors. Quantifications were performed in at least 3 confocal planes per islet, a minimum of 3 islets per section, and a minimum of 3 pancreatic sections from healthy (6 donors) and T2D (3 donors).

cAMP measurements

To determine changes in cyclic AMP (cAMP) levels in real time, we infected human islets with a green upward cADDis cAMP sensor (Catalog # U0200G, Montana Molecular, Bozeman, MT). The BacMam vector carrying the sensor is a modified baculovirus, which can be used for delivery to, and expression in, a wide variety of mammalian cells. cADDis cAMP sensor is a mNeon green fluorescence protein and its expression is under a CMV promoter. Increases in intracellular cAMP enhance fluorescence intensities in infected cells. Human islets were infected with $2x10^9$ VG in culture medium in the presence of sodium butyrate (5 mM). The medium was changed after 24h and the islets imaged 48-72h later on a confocal microscope. The islets were placed on a coverslip in an imaging chamber (Warner instruments, Hamden, CT, USA) for imaging on a Leica TCS SP5 upright laser-scanning confocal microscope (Leica Microsystems, Wetzlar, Germany). Islets were continuously perfused with extracellular solution (in mmol/l: 125 NaCl, 5.9 KCl, 2.56 CaCl2, 1 $MgCl_2$, 25 HEPES, 0.1% BSA, 3 mmol/l glucose, pH 7.4, 37°C) and confocal images were acquired with LAS AF

software (Leica Microsystems) using a 20× water immersion objective. We used a resonance scanner for fast image acquisition to produce time-lapse recordings spanning 50 μm of the islet (z-step: 5 μm, stack of ten confocal images with a size of 512 × 512 pixels) at 5s resolution (XYZT imaging). cAMP sensor fluorescence was excited at 488 nm and emission detected at 510–550 nm. We recorded the changes in cAMP induced by Forskolin (10 μM) + IBMX (100 μM), LY344864 (100 nM), adrenaline (10 μM) or 5HT (10 μM), all in 3 mM glucose.

Quantitative real-time RT-PCR

RNA from approximately 400 human islets from 4 different donors was extracted using RNAeasy mini kit (Quiagen) one day after receiving the islets, and stored at -80° C. RNA quality and purity was assessed using a Nanodrop (ND-1000 Spectrophotometer) and only RNA with a RNA integrity number (RIN) >7 was used further for reverse transcription. For cDNA synthesis, we used a high-capacity cDNA reverse transcription kit (Applied Biosystems) and 200 ng of RNA in each reaction (final volume 20 mL), following the manufacturer's protocols. Quantitative real-time PCR was performed using TaqMan fast universal PCR master mix and 1 ng of cDNA were used per reaction. We used TaqMan primers (FAM dye labeled) to determine expression of serotonin receptors, insulin and beta actin using a StepOnePlus real-time PCR system (Applied Biosystems). Expression of each gene was normalized to that of 18S ribosomal RNA (*Rn18s*) as a reference gene. The relative quantification (RQ) of the levels of each gene was measured based on the equation RQ = 2^(-ΔCt) x 10,000, where ΔCt is the difference between the cycle threshold (Ct) value of the target gene and the Ct value of the reference *Rn18s*. The

experiments were performed in duplicate and the results averaged. Quantitative real-time PCR experiments were performed according to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) (Bustin et al., 2009).

In situ RNA hybridization

In situ RNA hybridization was performed using RNAscope technology (Advanced Cell Diagnostics, Newark, California) following the manufacturer's protocol. 10 μm sections of PFA-fixed frozen human pancreatic samples from three different donors (Table S1) were used. After dehydration, the slides were subjected to RNAscope Multiplex Fluorescent Assay. To demonstrate that the signal comes from hybridization of probes (*Htr1F* and *Glucagon*) with mRNA, after pretreatment 3 (protease) step some slides were treated with RNase A (5 mg/ml) for 30 min at 37°C. At the end of the RNase treatment the slides were washed 5x with water, hybridized with RNAscope probes for 2 h at 40°C and the remainder of the assay protocol was implemented. To identify islets after the RNase treatment, sections were stained with an antibody against insulin. The sections were incubated with a blocking solution (50% fetal bovine serum, 400 u/ml rRNasin in PBS) for 1 h at room temperature, and then with an anti-insulin antibody (guinea pig) in the blocking solution with 0.1% Tween 20 overnight at 4°C. The slides were washed 3x for 5 min with PBS/0.1% Tween 20 (PBST) and incubated with Alexa Fluor 568 conjugated anti-guinea pig secondary antibody (1:100 dilution) in PBST for 2 h at room temperature. The slides were then washed 3x for 5 min with PBST and mounted. The fluorescent signal was visualized and captured using an open-field Nikon Eclipse TE2000-U microscope. According Advanced Cell Diagnostics, each mRNA molecule

hybridized to a probe appears as a separate small fluorescent dot (Wang et al., 2012), see Fig 4D below. Since glucagon mRNA is very abundant, the individual dots are hard to resolve and they appears in the images as continuous stain. We quantified in confocal sections the mean fluorescence intensity of 5-HT_{1F} hybridization signal (green channel) on regions of interest corresponding to alpha cells (glucagon positive, red channel), non-alpha cells (islet cells negative for glucagon) and acinar cells.

***In vivo* experiments**

C57BL6 mice (male, 8 weeks old, body weight 17 – 23 g) were fasted for 4h prior to drug administration and insulin tolerance tests. Water was freely available throughout the study. LY344864 (Tocris, cat. nr. 2451), a 5-HT_{1F} specific agonist, was initially dissolved in DMSO (100 mM stock solution). LY344864 was further dissolved in saline solution and administered at 1 mg/kg with an intravenous injection (Phebus et al., 1997). Control mice received an i.v. injection of saline + 0.65% DMSO (vehicle). Mice were randomly allocated in each experimental group and measurements were conducted in a blinded fashion. One hour after LY344864 administration, insulin was injected (i.p., 1 U/kg) and glycemia was measured at 0, 15 and 30 min after insulin injection from tail blood. Blood was collected before, 1h after LY344864 administration, and 30-45 min after insulin injection, and glucagon concentration in the plasma was determined using a glucagon ELISA kit (Mercodia). C57BL6 mice were rendered diabetic with an injection of streptozotocin (i.v., 200 mg/kg body weight). Mice that did not turn diabetic after streptozotocin injection were excluded. Six days after diabetes induction, diabetic mice (4 glycemic readouts > 350 mg/dL) were treated with LY344864 (i.v., 1 mg/kg) and

changes in glycemia were determined. Food was restored approximately 3h after drug administration.

Statistical analyses

All analyses were performed blindly. For statistical comparisons we used GraphPad Prism 5.0 and performed Student's *t* test, one-sample *t* test to compare the actual mean to a theoretical mean, or one-way analysis of variance (ANOVA) followed by a Tukey's Multiple Comparison Test. We considered statistical significance when *p* values were lower than 0.05. All data were assessed to ensure normal distribution and equal variance between groups. Throughout the manuscript we present data as mean ± SEM. For all animal experiments, the sample size required to achieve adequate power (80%) was estimated on the basis of pilot work (expected changes) and previous experience in the lab (standard deviation).

Table S1 Human donors (islets and pancreatic tissue) used in the study. Related to Figure 1.

A summary of the characteristics (age, sex, BMI, and cause of death) of the human donors used in the study is provided below.

Human Islets

UNOS ID	Date received	Age	Sex	BMI	Cause of death	Experiments performed
ABI1081	30.09.2014	29	female	21	Head trauma	Perifusion 5HT exogenous
ACCZ042A	31.03.2015	44	female	23.8	Cerebrovascular/stroke	Perifusion 5HT exogenous
ACDL087	17.04.2015	42	male	36.8	Cerebrovascular/stroke	Perifusion 5HT exogenous, reserpine
ACD1098	05.05.2015	57	male	29.4	Cerebrovascular/stroke	Perifusion 5HT endogenous (reserpine/fluvoxamine); RNA extraction
ACEC298	12.05.2015	27	male	31.3	Head trauma	Perifusion 5HT endogenous (reserpine/fluvoxamine); 5HT biosensors
ACEQ383	21.05.2015	41	male	29.3	Head trauma	Perifusion 5HT endogenous (reserpine/fluvoxamine); 5HT biosensors
ACFC227	09.06.2015	59	female	23.1	Cerebrovascular/stroke	Perifusion 5HT endogenous (reserpine/fluvoxamine)
ACFO053A	24.06.2015	29	male	30.9	Head trauma	Perifusion 5HT endogenous (reserpine/fluvoxamine) with cyclosomatostatin
ACGG001A	16.07.2015	28	male	18.6	CNS tumor	Perifusion 5HT exogenous; 5HT biosensors; RNA extraction
ACGW350	29.07.2015	33	female	32.9	Cerebrovascular/stroke	Perifusion 5HT endogenous (reserpine/fluvoxamine); RNA extraction
ACJN493	21.10.2015	52	female	39.1	Cerebrovascular/stroke	Perifusion 5HT1F agonist
ACJV399	28.10.2015	41	female	43.1	Cerebrovascular/stroke	Perifusion 5HT1F agonist; RNA extraction
ACJV169A	30.10.2015	26	male	44.8	Head trauma	Perifusion 5HT1F agonist
ACJ4169	04.11.2015	45	male	40.4	Cerebrovascular/stroke	Perifusion 5HT1F agonist
ACKD092B	11.11.2015	23	female	25.3	Head trauma	Perifusion 5HT1F agonist
ADEB181	05.05.2016	62	male	27.5	Cerebrovascular/stroke	cAMP measurements
ADEH311	12.05.2016	37	male	30.5	Head trauma	cAMP measurements
ADE1444	01.06.2016	33	female	34.2	Cerebrovascular/stroke	cAMP measurements; perifusion Tph1 inhibitor pCPA
ADFR455	22.06.2016	53	male	31.1	Anoxia	Perifusion Tph1 inhibitor pCPA
ADFZ083	30.06.2016	24	male	34.8	Head trauma	Perifusion Tph1 inhibitor pCPA

Human Pancreas

Healthy individuals	History of T2D	Age	Sex	BMI	Cause of death	Experiments performed
HP2125	none	44	male	22.2	Motorvehicle accident	Immunostaining (5HT, Tph1, 5HT receptors), in situ
HP2126	none	64	female	29.4	Cardiovascular accident	Immunostaining (5HT, Tph1, 5HT receptors), in situ
HP2127	none	41	male	29	Cardiovascular accident	Immunostaining (5HT, Tph1, 5HT receptors), in situ
HP2050	none	22	female	28.2	Suicide	Immunostaining (5HT, Tph1, 5HT1F, VMAT2, SERT)
HP2052	none	52	female	29	Anoxia	Immunostaining (5HT, Tph1, 5HT1F)
HP2129	none	54	male	23.4	Cardiovascular accident	Immunostaining (5HT, Tph1, 5HT1F, VMAT2, SERT)
HP1987	none	37	male	37.97	head trauma	Immunostaining (5HT, Tph1, 5HT1F, VMAT2, SERT)
Type 2 diabetics						
HP2021	T2D > 5 years	54	female	48.5	Anoxia	Immunostaining (5HT, 5HT1F)
HP2036	T2D ~ 10 years	45	female	28.4	Cardiovascular accident	Immunostaining (5HT, 5HT1F)
HP2047	T2D > 4 years	43	female	33.3	Anoxia	Immunostaining (5HT, 5HT1F)

List of antibodies used to label serotonin receptors:

Anti-5-HT_{1E}: NBP1-02660, raised in rabbit, Novus Biologicals

Anti-5-HT_{1D}: NLS590, raised in rabbit, Novus Biologicals

Anti-5-HT_{3E}: NBP2-33578, raised in rabbit, Novus Biologicals

Anti-5-HT_{5A}: NB100-58983, raised in rabbit, Novus Biologicals

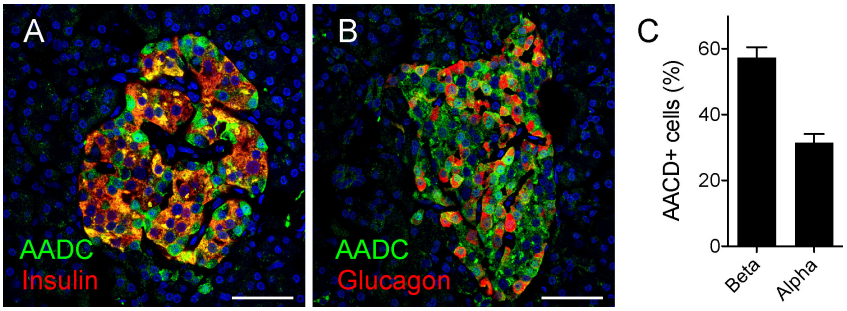
Anti-5-HT_{1B}: NLS598, raised in rabbit, Novus Biologicals

Anti-5-HT_{1F}: NBP1-02371, raised in rabbit, Novus Biologicals

Anti-5-HT_{1A}: LS-B970, raised in rabbit, LSBio

SUPPLEMENTARY FIGURES

Figure S1 Expression of aromatic amino acid decarboxylase (AADC) in human islets. Related to Figure 1.

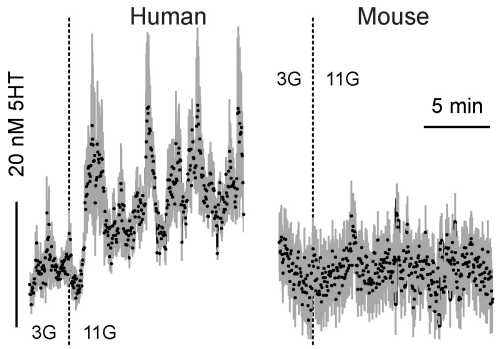


(A) Confocal image of a human pancreatic section showing islets immunostained for aromatic amino acid decarboxylase (AADC) (green) and insulin (red).

(B) Confocal image of a human pancreatic section showing islets immunostained for aromatic amino acid decarboxylase (AADC) (green) and glucagon (red). DNA (dapi) is shown in blue.

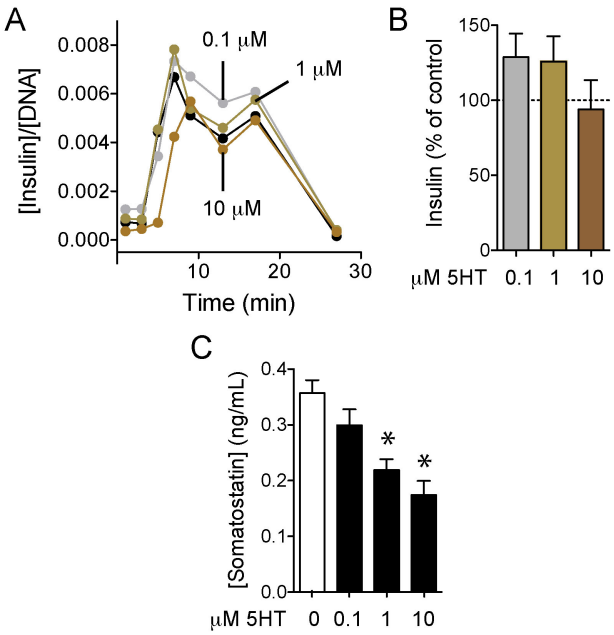
(C) Quantification of the number of AADC-positive cells that are beta (insulin positive) or alpha (glucagon positive) cells. Scale bars represent 50 µm.

Figure S2 High glucose-stimulated serotonin secretion from mouse and human islets. Related to Figure 2.



Glucose changed from 3 mM (3G) to 11 mM (11G). Increases in glucose concentration did not result in a detectable release of serotonin from mouse islets. $[Ca^{2+}]_i$ levels were normalized to the maximum level achieved upon direct application of serotonin (100 nM).

Figure S3 Effect of exogenous serotonin on insulin and somatostatin secretion from human islets. Related to Figure 3.



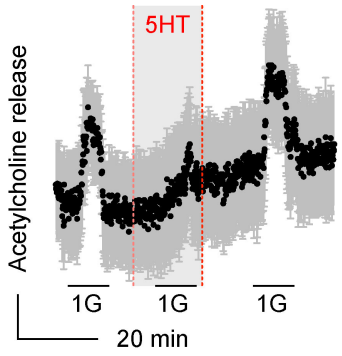
(A) To determine the effect of serotonin on insulin secretion, different concentrations of serotonin (0.1- 10 μM 5HT) were applied 5 min before increasing glucose concentration from 3 mM to 11 mM. A representative tracing of this response is shown for each concentration.

(B) Quantification of the total amount of insulin secreted during 15 min in high glucose (11 mM) (area under the curve, AUC) in the presence of different concentrations of exogenous serotonin (in μM) (n = 6 experiments from 3 islet preparations; normalized AUC values are not significantly different from a theoretical mean of 100% (one sample *t* tests).

(C) Quantification of the amount of somatostatin secreted by human islets in 3 mM glucose before and 5 min after application of exogenous serotonin (0.1- 10 μM 5HT). *p

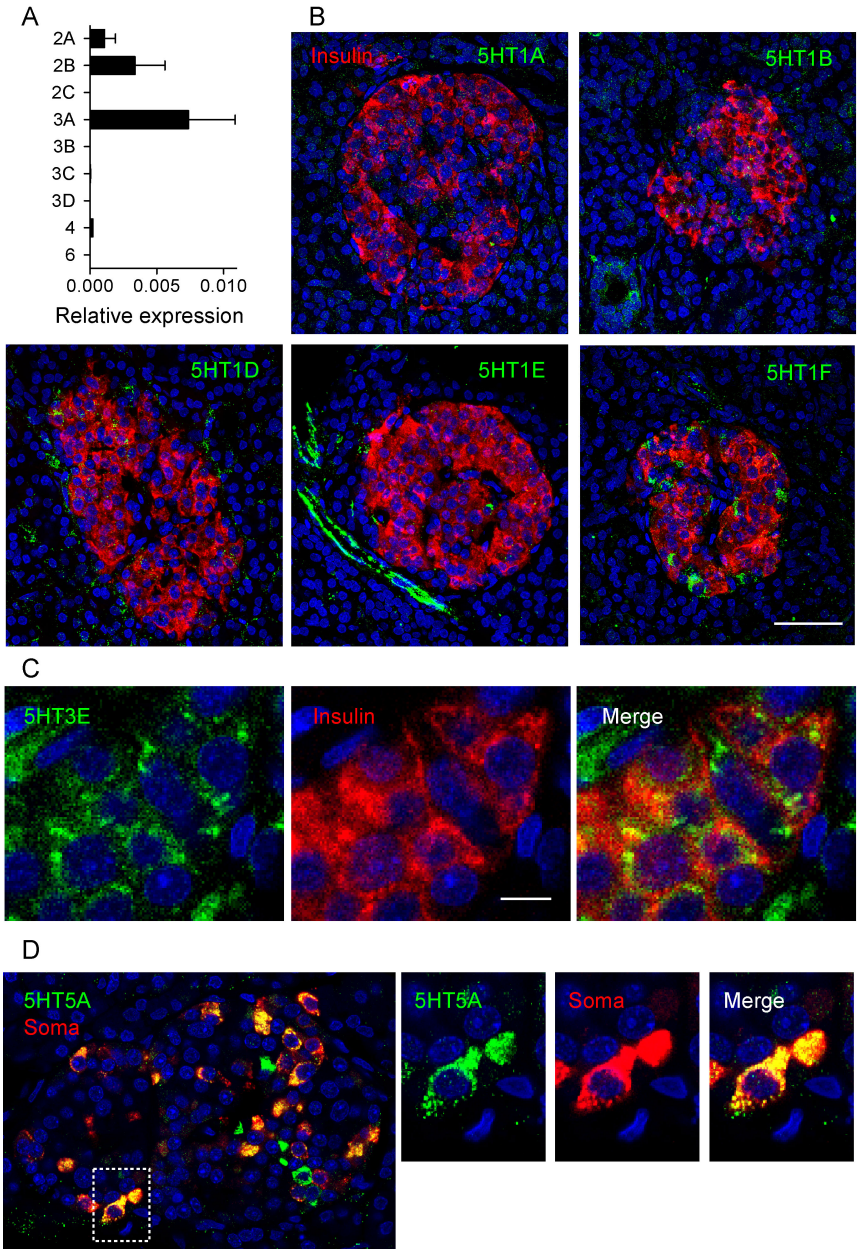
value < 0.05 (one-way analysis of variance (ANOVA) followed by a Tukey's Multiple Comparison Test, n = 4 experiments, 2 islet preparations).

Figure S4 Serotonin inhibits acetylcholine release from alpha cells. Related to Figure 3.



Acetylcholine secretion was measured with acetylcholine biosensor cells. Acetylcholine is released from human islets in response to lowering glucose concentration (11 mM to 1 mM (1G), horizontal bars denote time in 1G) is reduced in the presence of serotonin (5-HT 10 μM; mean ± SEM. of 6 biosensor cells; see Experimental Procedures).

Figure S5 Expression of serotonin receptors in human islets. Related to Figure 4.



(A) Relative mRNA levels for serotonin receptors 5-HT_2 family, 5-$HT3_{A-D}$ subunits, 5-HT_4 and 5-HT_6.

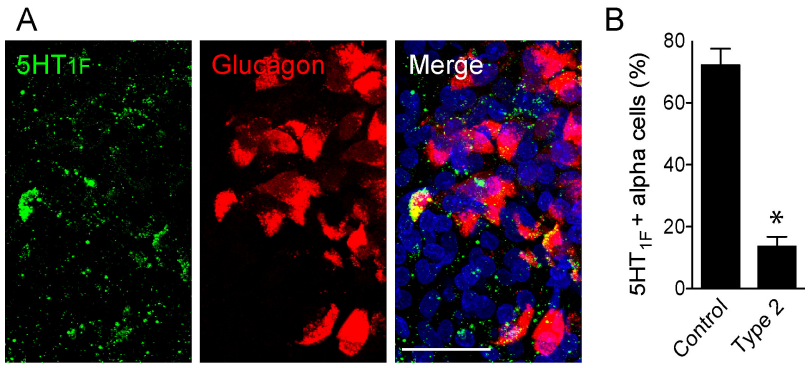
(B) Z-stack of confocal images of a human pancreatic section showing islets immunostained for serotonin receptors (5-$HT_{1A,}$ 5-$HT_{1B,}$ 5-$HT_{1D,}$ 5-$HT_{1E,}$ 5-$HT_{1F,}$ shown in green) and insulin (red). DNA (dapi) is shown in blue.

(C) Confocal image of a human pancreatic section showing an islet region immunostained for the serotonin receptor 5-HT_{3E} (green) and insulin (red).

(D) Confocal image of a human pancreatic section showing an islet immunostained for the serotonin receptor 5-HT_{5A} (green) and somatostatin (red). Panels on the right show zoomed images of islet region delimited in (D). 5-HT_{5A} expression is specific for delta cells and double-labeled cells appear yellow.

Scale bars represent 50 μm (B), 5 μm (C).

Figure S6 Expression of 5-HT$_{1F}$ is decreased in islets from T2D donors. Related to Figure 4.



(A) Maximal projection of confocal images of a pancreatic section from a type 2 diabetic patient showing an islet immunostained for the serotonin receptor 5-HT$_{1F}$ (green, left panel), for glucagon (red, middle panel), or both (merge, right panel). Scale bar represent 20 µm.

(B) Quantification of the percentage of alpha cells that express 5-HT$_{1F}$ receptor in islets from healthy donors and type 2 diabetic patients (n = 3 donors each group). * p value < 0.05 (Unpaired Student's t test).