Serotonin Regulates Pancreatic β-Cell Mass during Pregnancy

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Supplementary Information

Supplementary Table S1. Islet mRNA expression by RNA sequencing during pregnancy*

name	Name	Pregnant	Non-pregnant	Ratio	p-value	Category
Lrrc55	Leucine rich repeat containing 8D	15.9 ± 0.55	0.23 ± 0.12	68.17	< 0.01	Highly upregulated
Tph1	Tryptophan hydroxylase 1	47.6 ± 2.21	0.72 ± 0.72	65.91	< 0.01	Highly upregulated
Cldn8	claudin-8	49.0 ± 3.06	1.08 ± 0.61	45.46	< 0.01	Highly upregulated
Rps23	Ribosomal protein S23	343 ± 37.34	15.4 ± 5.39	22.21	< 0.01	Highly upregulated
Fmo1	Flavin containing monooxigenase-1	57.0 ± 4.14	3.54 ± 0.15	16.09	< 0.01	Highly upregulated
Tph2	Tryptophan hydroxylase 2	6.04 ± 0.89	0.46 ± 0.11	13.18	< 0.01	Highly upregulated
Sftpd	Surfactant associated protein D	17.5 ± 1.5	1.39 ± 0.51	12.67	< 0.01	Highly upregulated
Tnfrsf11b	Tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	11.2 ± 1.56	1.24 ± 0.43	9.03	< 0.01	Highly upregulated
Ehhadh	Enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	27.5 ± 0.44	4.53 ± 0.41	6.09	< 0.01	Highly upregulated
Cish	Cytokine inducible SH2-containing protein	134 ± 10.10	25.2 ± 2.85	5.32	< 0.01	Highly upregulated
Tph1	Tryptophan hydroxylase 1	47.6 ± 2.21	0.72 ± 0.72	65.91	< 0.01	5-HT related
Tph2	Tryptophan hydroxylase 2	6.04 ± 0.89	0.46 ± 0.11	13.18	< 0.01	5-HT related
Htr2b	5-HT receptor 2B	0.29 ± 0.20	0.08 ± 0.08	3.68	0.369	5-HT related
Slc18a1	Vesicular amine transporter 1	7.61 ± 0.35	4.57 ± 0.93	1.67	0.017	5-HT related
Ddc	Aromatic-L-amino-acid decarboxylase	178 ± 4.51	120 ± 7.29	1.48	< 0.01	5-HT related
Lrp5	Low density lipoprotein receptor-related protein 5	18.15 ± 0.85	17.75 ± 2.8	1.02	0.897	5-HT related
Htr1b	5-HT receptor 1B	0.21 ± 0.16	0.23 ± 0.23	0.93	0.952	5-HT related
Htr3b	5-HT receptor 3B	0.22 ± 0.21	0.24 ± 0.22	0.91	0.910	5-HT related
Slc18a2	Vesicular amine transporter 2	0.96 ± 0.18	1.15 ± 0.45	0.84	0.673	5-HT related
Slc18a3	Vesicular amine transporter 3	5.13 ± 0.23	6.42 ± 1.11	0.80	0.238	5-HT related
Htr3a	5-HT receptor 3A	2.98 ± 0.65	4.05 ± 0.29	0.74	0.249	5-HT related
Slc6a4	5-HT transporter	0.17 ± 0.05	0.23 ± 0.23	0.72	0.759	5-HT related
Htr1d	5-HT receptor 1D	0.07 ± 0.05	0.15 ± 0.08	0.51	0.442	5-HT related
Chgb	Chromogranin B	1733 ± 130	365 ± 35	4.75	< 0.01	Islet related
Ins1	Insulin-1	99856 ± 7484	70844 ± 10752	1.41	0.031	Islet related
Ins2	Insulin-2	410047 ± 12055	357880 ± 38514	1.15	0.321	Islet related
Gck	Glucokinase	55.6 ± 2.71	51.0 ± 4.45	1.09	0.370	Islet related
Iapp	Islet amyloid polypeptide	12238 ± 944	11637 ± 562	1.05	0.600	Islet related
Slc2a2	GLUT2	145 ± 6.08	144 ± 14.4	1.01	0.900	Islet related
Sst	Somatostatin	6185 ± 97.3	6648 ± 1108	0.93	0.641	Islet related
Ppy	Pancreatic polypeptide	5773 ± 14.9	7652 ± 846	0.75	0.045	Islet related
Gcg	Glucagon	12340 ± 1236	18025 ± 1035	0.68	0.014	Islet related

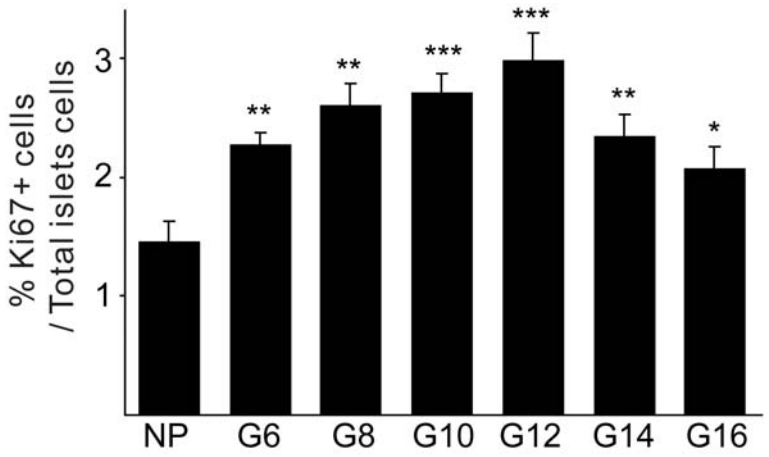
Ghrl	Ghrelin	16.2 ± 1.38	24.9 ± 3.75	0.65	0.052	Islet related
Prlr	Prolactin receptor	99.2 ± 2.97	32.9 ± 7.58	3.02	< 0.01	PRL signaling
Stat5b	Signal transducer and activator of transcription 5b	18.8 ± 1.51	22.2 ± 2.19	0.85	0.214	PRL signaling
Stat5a	Signal transducer and activator of transcription 5a	5.8 ± 0.75	9.44 ± 1.77	0.61	0.082	PRL signaling
Foxm1	Forkhead box M1	2.41± 0.30	1.48 ± 0.17	1.63	0.052	Transcription factor
Mafa	MAFa	204 ± 49.2	136 ± 37.9	1.50	0.268	Transcription factor
Pdx1	Pancreatic and duodenal homeobox 1	92 ± 13.6	71.9 ± 12.4	1.28	0.301	Transcription factor
Neurod1	NeuroD1	53.6 ± 0.96	46.2 ± 3.53	1.16	0.063	Transcription factor
Hnf4a	Hepatic nuclear factor 4, alpha	9.3 ± 0.67	8.9 ± 2.8	1.05	0.887	Transcription factor
Nkx2-2	NK2 homeobox 2	73 ± 6.69	71.6 ± 13.1	1.02	0.917	Transcription factor
Rfx6	Regulatory factor X, 6	23 ± 0.48	23.9 ± 4.02	0.96	0.790	Transcription factor
Hnf4g	Hepatic nuclear factor 4, gamma	2.7 ± 0.46	3.0 ± 1.7	0.90	0.810	Transcription factor
Nkx6-1	NK6 homeobox 1	117 ± 14.3	144 ± 21.2	0.81	0.295	Transcription factor
Ccnf	G2/mitotic-specific cyclin-F	2.03 ± 0.41	0.66 ± 0.07	3.07	0.024	Cell cycle related
Cdkl2	Cyclin-dependent kinase-like 2	5.69 ± 0.45	3.81 ± 0.21	1.49	0.013	Cell cycle related
Cdk8	Cyclin-dependent kinase 8	35.0 ± 1.73	23.9 ± 0.53	1.46	< 0.01	Cell cycle related
Ccnd1	G1/S-specific cyclin-D1	45.5 ± 1.69	39.1 ± 1.49	1.16	0.031	Cell cycle related
Ccnk	Cyclin-K	13.3 ± 0.68	16.1 ± 0.50	0.82	0.017	Cell cycle related
Ccnl2	Cyclin-L2	42.3 ± 3.57	53.2 ± 0.87	0.79	0.033	Cell cycle related
Cdk9	Cyclin-dependent kinase 9	21.3 ± 1.95	27.0 ± 0.71	0.79	0.042	Cell cycle related
Ccnl1	Cyclin-L1	20.3 ± 1.08	26.1 ± 1.78	0.78	0.027	Cell cycle related
Cdkn1b	Cyclin-dependent kinase inhibitor 1B (p27/Kip1)	20.7 ± 0.95	29.8 ± 1.86	0.69	< 0.01	Cell cycle related
Cdkn1a	cyclin-dependent kinase inhibitor 1A (p21/Cip1)	18.8 ± 1.92	28.0 ± 3.59	0.67	0.040	Cell cycle related
Cdk5r1	Cyclin-dependent kinase 5 activator 1	2.21 ± 0.44	3.71 ± 0.36	0.59	0.039	Cell cycle related
Mdm1	Nuclear protein MDM1	16.0 ± 0.89	33.5 ± 3.50	0.48	< 0.01	Cell cycle related

* Pancreatic islets were isolated from adult non-pregnant and pregnant (G13−15) C57BL/6J mice, and quantification of gene expression was assessed by RNA sequencing (n = 3 per group). The quantified transcription levels were expressed as reads per kilobase of exon model per million mapped reads (RPKM). Statistical data are presented as mean ± standard error and p values were calculated by Student's t test.

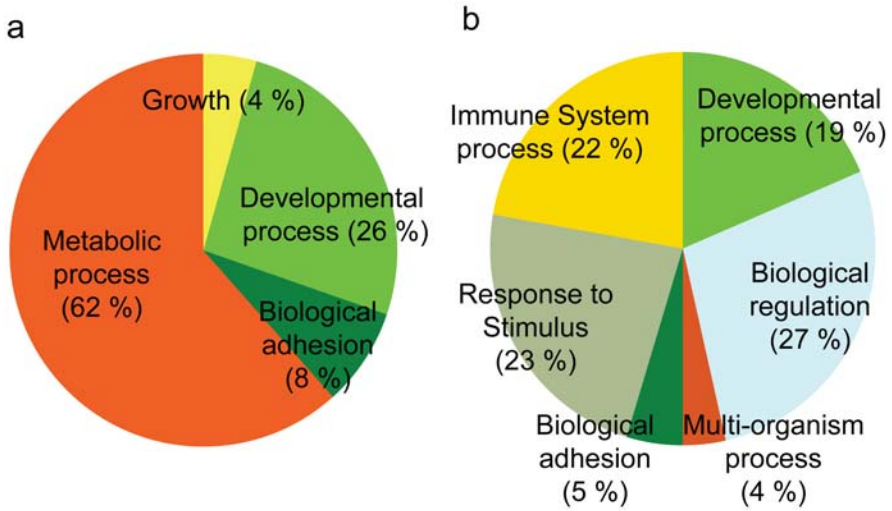
Supplementary Table S2. Islet mRNA expression by microarray hybridization during pregnancy*

name	Name	Fold	Category
Tph1	Tryptophan hydroxylase 1	112.9	Upregulated
Cldn8	Claudin 8	69.3	Upregulated
Sftpd	Surfactant associated protein D	26.3	Upregulated
Slc26a3	Solute carrier family 26, member 3	19.7	Upregulated
Gbp8	Guanylate-binding protein 8	18.6	Upregulated
4833427G06Rik	RIKEN cDNA 4833427G06	14.5	Upregulated
Neb	Nebulin	13.6	Upregulated
Tph2	Tryptophan hydroxylase 2	12.5	Upregulated
Fmo1	Flavin containing monooxygenase 1	10.5	Upregulated
Ncapg	non-SMC condensin II complex, subunit G2	10.0	Upregulated
Cdca8	Cell division cycle associated 8	7.9	Upregulated
Car8	Carbonic anhydrase 8	7.5	Upregulated
Gm131	Gene model 131, similar to prochymosin	7.4	Upregulated
Matn2	Matrilin 2	7.3	Upregulated
Crispld2	Cysteine-rich secretory protein LCCL domain containing 2	6.2	Upregulated
Ivd	Isovaleryl coenzyme A dehydrogenase	4.5	Upregulated
Mctp	Multiple C2 domains, transmembrane 1	4.5	Upregulated
Uhrf1	Ubiquitin-like, containing PHD and RING finger domains, 1	4.4	Upregulated
Dscc1	Defective in sister chromatid cohesion 1 homolog	4.3	Upregulated
Ehhadh	enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	4.3	Upregulated
Cdc6	Cell division cycle 6 homolog	4.2	Upregulated
Sntg2	Syntrophin, gamma 2	4.1	Upregulated
Hsp8	Heat shock protein 8	0.63	Down regulated
Pcdh15	Protocadherin 15	0.59	Down regulated
Il1r1	Interleukin 1 receptor, type I	0.59	Down regulated
Upp2	Uridine phosphorylase 2	0.48	Down regulated
Arl4d	ADP-ribosylation factor-like 4D	0.48	Down regulated
Sult1d1	Sulfotransferase family 1D, member 1	0.48	Down regulated
Rgs2	Regulator of G-protein signaling 2	0.40	Down regulated

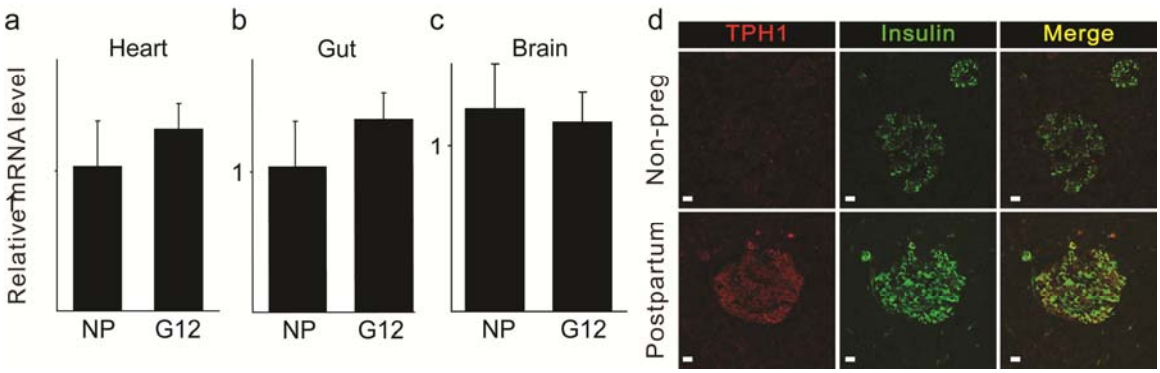
* Pancreatic islets were isolated from adult non-pregnant and pregnant (G12) C57BL/6J mice, and quantification of gene expression was assessed by hybridization with the Affymetrix mouse genome 430 2.0 Array (n = 3 per group). Data presented as the ratio of expression in islets from pregnant mice to expression in islets from non-pregnant mice.



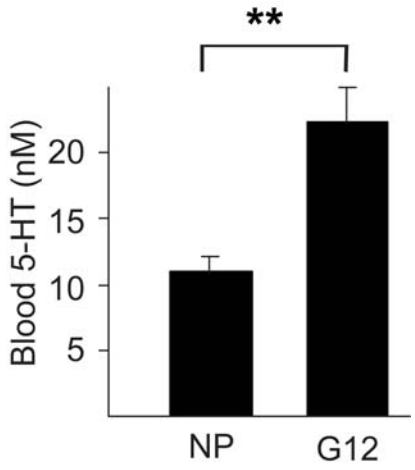
Supplementary Figure S1. Islet cell proliferation during pregnancy. The percent of islet cells staining for proliferation marker Ki67 was determined in pancreatic sections from non-pregnant and pregnant mice at the dates shown. n = 5−6 mice per time point. All data are presented as mean ± standard error. Statistical significance *vs.* non-pregnant control was analyzed by Student's t test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.



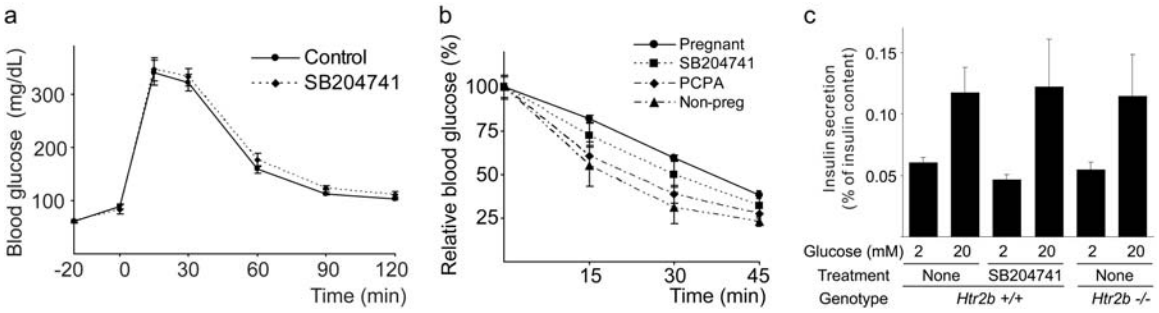
Supplementary Figure S2. 1000 islet genes most affected during pregnancy. Gene ontology charts were generated using the 500 most up-regulated (a) and 500 most down-regulated genes (b) in mouse islets during pregnancy (G13−G15) as determined by RNA sequencing. The slices in the pies represent percent of genes in each category. Data were categorized using the gene ontology database (www.geneontology.org).



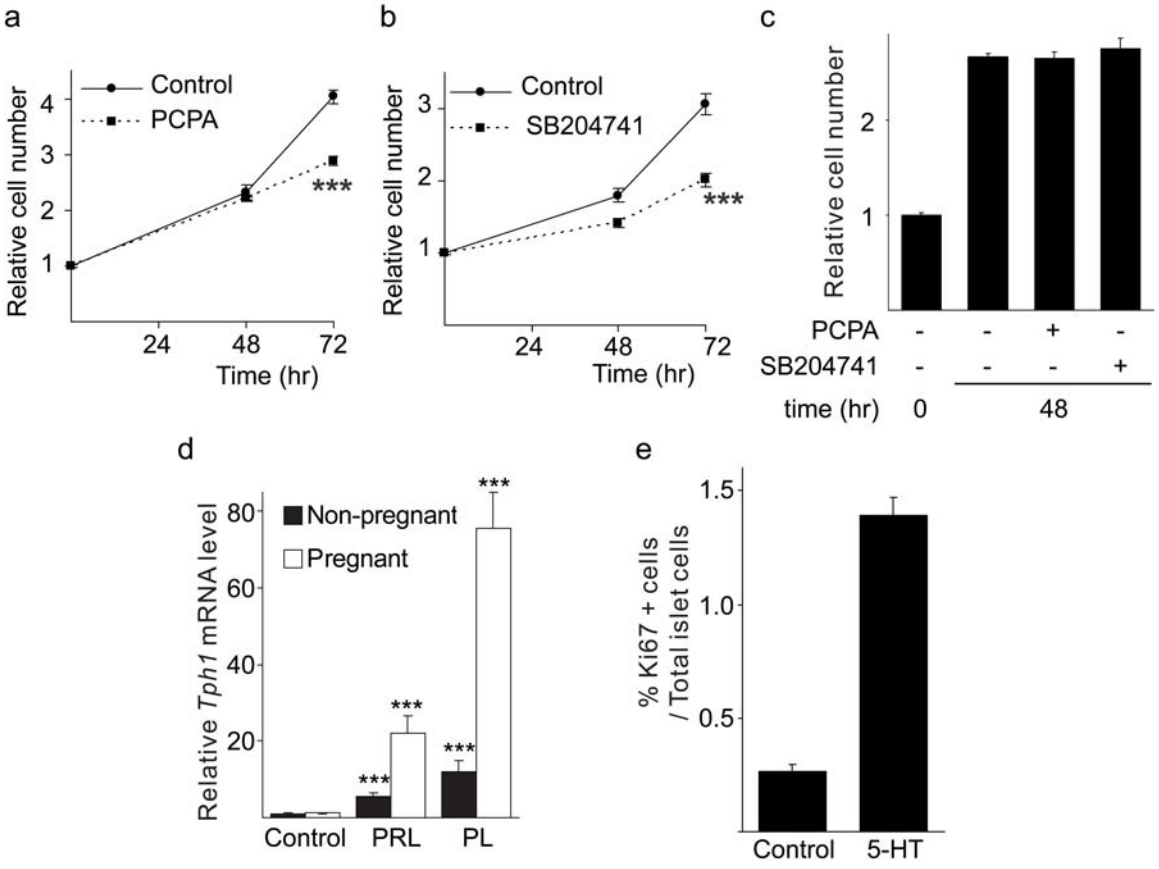
Supplementary Figure S3. *Tph1* expression in non-pancreatic tissue during pregnancy and TPH1 expression in post-partum human pancreas. *Tph1* mRNA levels were measured by real-time RT-PCR in RNAs from heart (a), gut (stomach + duodenum) (b) and brain (c) in non-pregnant female mice (NP) and G12 pregnant mice and expressed relative to levels in the non-pregnant mice. n = 3−4 mice per data point. All data are presented as mean ± standard error. (d) Immunofluorescent staining labels TPH1 (red) and insulin (green) in pancreata from non-pregnant (Non-preg) and postpartum day 3 human autopsies. Scale bar indicates 20 μm.



Supplementary Figure S4. HPLC analysis of blood 5-HT concentration. Whole blood was collected from normal non-pregnant (NP) and pregnant (G12) female mice, and blood 5-HT levels were measured using HPLC. n = 4−7 mice per sample. Data are presented as mean ± standard error. Statistical significance *vs.* non-pregnant control was analyzed by Student's t test: **, $P < 0.01$



Supplementary Figure S5. Effects of Htr2b signaling on insulin secretion. (a) 20 minutes after intraperitoneal injection of selective Htr2b inhibitor SB204741 (1 mg kg^{-1}) in pregnant mice (G13), glucose (2 g kg^{-1}) was injected intraperitoneally followed by measurement of blood glucose levels at the times shown. n = 4−8 mice per group. (b) Insulin tolerance was assessed by measuring blood glucose levels at the times shown after injection of insulin (0.75 units kg^{-1}) in non-pregnant female mice (Non-preg), G13 pregnant mice without treatment (Pregnant), or pregnant mice treated with SB204741 or PCPA as outlined in Fig 2a. n = 3−4 mice per group. (c) Secreted insulin was assayed by ELISA following a 10 minutes incubation of islets isolated from pregnant (G13) wild type (*Htr2b*$^{+/+}$) or Htr2b null mice (*Htr2b*$^{-/-}$) mice in 2 mM or 20 mM glucose in the presence or absence of SB204741 (35 μM). n = 3−6 groups of 40 islets. Data are presented as mean ± standard error.



Supplementary Figure S6. Impact of serotonin signaling on β-cell proliferation. Growth of MIN6 β-cells (a, b) and NIH3T3 cells (c) treated with PCPA (480 μM, a, c) or SB204741 (35 μM, b, c) was determined by cell counting at the indicated time points, and shown relative to the starting cell number. $n = 3-6$ samples per data point. (d) mRNA levels for *Tph1* were measured by real time RT-PCR in islets isolated from non-pregnant and pregnant (G3) female mice cultured with mouse prolactin (PRL, 200 ng ml^{-1}) or human placental lactogen (PL, 2 μg ml^{-1}) for 48 hours and are shown relative to the levels in control islets cultured without added hormones. $n = 4-8$ per data point. (e) Proliferation was quantified by counting the percent of $Ki67^{+}$ cells in mouse islets cultured with 5-HT (10 μM) for 2 days. $n = 3$ sets of islets per data point. Data are presented as mean ± standard error. Statistical significance *vs.* vehicle-treated control was analyzed by Student's *t* test: ***, $P < 0.001$

Supplementary Methods

Mice.

C57BL/6J mice were housed on a 12-h light/dark cycle in climate-controlled, pathogen-free barrier facilities. The Institutional Animal Care and Use Committees at UCSF or Juntendo University approved all studies involving mice. *Htr2b* targeted mice were provided by L. Maroteaux[14] and backcrossed with C57BL/6J mice for more than 7 generations. C57BL/6J mice were purchased from Jackson laboratory. Mating was confirmed by the presence of a vaginal plug the next morning, designated day 0 of gestation (G0). 4-Chloro-DL-phenylalanine methyl ester hydrochloride (PCPA, Sigma-Aldrich, 300 mg kg^{-1} day^{-1}), SB204741 (Sigma-Aldrich, 1 mg kg^{-1} day^{-1}), Methysergide (Sigma-Aldrich, 3 mg kg^{-1} day^{-1}) and Ketanserin (Sigma-Aldrich, 1 mg kg^{-1} day^{-1}) were administered daily for 7 days from G6 to G12 by intraperitoneal injection. Tryptophan-free diet, histidine-free diet, low tryptophan diet and the equivalent control diet (Baker amino acid diet) were purchased from Labdiet. The control diet contained 0.2% tryptophan and 0.4% histidine, and the low tryptophan diet contained 0.04% tryptophan.

Glucose and insulin tolerance and insulin secretion tests were performed on non-pregnant female or pregnant mice at G13. Mice were fasted for 15 hours from 6 P.M. to 9 A.M. and injected intraperitoneally with glucose (2 g kg^{-1}) or insulin (0.75 U kg^{-1}), and blood glucose levels were measured at the times shown from tail vein blood using a portable glucometer (FreeStyle flash blood glucose monitoring system, Abbott Diabetes Care Inc., Alameda, CA). For insulin secretion tests, mice were fasted for 6 hours and injected intraperitoneally with glucose (2 g kg^{-1}), and blood was sampled from retroorbital plexus using heparinized microvette (Sarstedt AG & Co.) at 0 and 30 min after glucose injection. Plasma insulin was determined using the Linco Mouse Insulin ELISA kit (Millipore).

5-bromo-2'-deoxyuridine (BrdU, Sigma-Aldrich) was administered in drinking water bottles at 1 mg ml^{-1} for 4 days for non-pregnant mice and for pregnant mice from G9 to G13.

Cell culture.

Mouse pancreatic islets were isolated by collagenase digestion and hand-picked [31]. Isolated islets were incubated in RPMI-1640 supplemented with 10% horse serum, 100 units ml^{-1} penicillin and 100 g ml^{-1} streptomycin and treated with mouse recombinant prolactin (R & D Biosystems, 200 ng ml^{-1}, 1000 ng ml^{-1}), human placental lactogen (National Hormone and Peptide Program, 2 μg/ml), or 5-HT (Sigma-Aldrich, 10 μM) or glucose (for the indicated times. Media was changed every 24 hours. For BrdU incorporation assays, islets were cultured for 4 days in the presence of prolactin (1,000 ng ml^{-1}) or 5-HT (10 μM) and BrdU (10 μM) was added to the medium during the last 48 hours. For Ki67 staining, islets were cultured for 2 days in the presence 5-HT (10 μM).

The MIN6 beta-cell line[32] and NIH3T3 cells were maintained in DMEM supplemented with 15% FBS, 100 units ml^{-1} penicillin, 100 g ml^{-1} streptomycin and 71.5 μM β-mercaptoethanol. During prolactin treatment, FBS was replaced with 10% horse serum. Cells were plated in 12 well plates (2×10^5 cells/well) and cultured overnight. The following morning, cells were treated with SB204741 (35 μM), PCPA (480 μM) or vehicle. Media was changed every 12 hours. Viable cells were counted with a

hemocytometer after trypan blue staining.

Human pancreas.

Human pancreatic tissue was obtained at autopsy at the UCSF Medical Center and Hirosaki University affiliated teaching hospitals. The cause of death in case 1 (Fig. 1g and h), age 35, was pulmonary embolism of amniotic fluid at 37 weeks of pregnancy, in case 2 (Fig. 1g), age 25, was dissecting aneurysm at 2 days postpartum, and in case 3 (Supplementary Fig. S3d), age 25, was acute respiratory distress syndrome secondary to pulmonary hypertension at 3 days postpartum. Nine age-matched non-pregnant female and 5 male control pancreata were used for comparison. These cases had no history of diabetes. Human pancreas tissues were all fixed in formalin and stored in paraffin blocks from which sections were obtained for conventional hematoxylin-eosin (HE) staining and immunohistochemistry.

Immunohistochemistry

Immunohistochemical staining for human cases 1 and 2 was conducted by avidin-biotin-peroxidase complex method (for 5-HT) and biotin free polymer detection system for TPH1 (MACH3 kit, Biocare Medical LLC). Briefly, 4 μm-thick deparaffinized sections were immersed in Tris buffered saline (TBS) and placed in a pressure chamber (Pascal, DAKO Cytomation) for antigen retrieval at 121 ^{o}C for 1 minute in Tris-EDTA buffer. To eliminate endogenous peroxidase activity, the sections were pretreated with background sniper (Biocare Medical) for 10 minutes at room temperature. Then the sections were incubated with the primary antibody against serotonin (clone 5HT-H209, 1:100, monoclonal, Dako Cytomation) or TPH1[9] (1:800). The immunoreaction products were colorized with diaminobenzidine. Specificity was confirmed by lack of staining after omission of the first antibody or replacement with non-immune serum. Double immunochemistry with immunofluorescence was applied to confirm co-expression for insulin and TPH1. First, TPH1 was stained with MACH3 kit as described above. After washing with TBS and blocking with normal goat serum, insulin antibody (guinea pig polyclonal ,1:200, Dako Cytomation) was applied to the same section for 1 hour at room temperature followed by incubation with Alexa-fluor 594-conjugated secondary antibody (1:10,000, Invitrogen) for 30 minutes at room temperature. They were colorized with specific chromogens, yielding TPH1-positive cells as dense black reactions of peroxidase with diaminobenzidine and nickel enhancer, and insulin-positive cells red on a fluorescent microscope (Axioimager M1, Carl Zeiss Co.), respectively.

Mouse tissue harvesting, fixation, sectioning and staining was performed as previously described[33]. Cultured mouse islets were fixed in 10% neutral formalin solution. Islets were resuspended in liquefied Histogel (Richard-Allan Scientific) and solidified at room temperature. Fixed islets in Histogel were processed in the same manner as fixed tissues and embedded in paraffin. Immunofluorescent staining for human case 3 was conducted in the same manner as mouse tissues. The following primary antisera were used: 1:3,000 guinea pig anti-glucagon (Millipore), 1:3,000 guinea pig anti-insulin (Millipore), 1:2,000 rabbit anti-serotonin (Immunostar), 1:200 rat anti-BrdU (AbD Serotec), 1:1,000 rabbit anti-TPH1[9], and 1:200 rabbit anti-Ki67 (BD Bioscience, San Jose, CA). Secondary antibodies (Jackson Immunoresearch) were used at 1:200 (fluorescein isothiocyanate) and 1:800 (Cy3) dilutions, and biotinylated anti-guinea pig

IgG was used at 1:200 dilution (Vector Laboratories). Slides were visualized with a Zeiss AxiosKope microscope.

Counting of BrdU positive β-cells in pancreas, kidney and cultured islets and morphometric analyses with pancreas were performed by serially sectioning the entire tissues followed by staining and counting every 20th section. A minimum of 3,000 and 200 insulin positive cells were counted per pancreas and kidney, respectively. The pancreas and β-cell areas were measured with the Zeiss AxioImager Brightfield Microscope and software.

RNA assays.

RNA extraction, RT-PCR and real time RT-PCR were performed as previously described[33]. Primer sequences are available on request.

For sequencing, total RNA from pooled islets from 4 non-pregnant or pregnant (G13−G15) mice was converted into a library of template molecules using mRNA-Seq Sample Prep Kit (Illumina) according to manufacturer's protocol. $Poly(A)^+$ mRNA purified using poly-dT oligo-attached Dynal magnetic beads (Invitrogen) was fragmented in RNA fragmentation buffer (Ambion) at 94 ˚C for 5 minutes. First strand cDNA synthesis from the RNA fragments using Superscript III (Invitrogen) and random hexamers was followed by second strand synthesis using DNA Polymerase I and RNaseH, addition of a 3'deoxyadenosine, and ligation of adaptors. Double stranded cDNAs of 200 ± 25 bp were purified by electrophoresis through a 2% low melting-point agarose gel, extracted using the QIAquick Gel Extraction Kit (Qiagen) and further purified using a QiaQuick PCR purification kit (Qiagen). Libraries were expanded with the Illumina Cluster generation protocol and sequenced at the UCSF Genomics Core Facility using the 36 cycle sequencing kit v3 (Illumina). 31-bp reads were mapped to the Refseq collection of mouse transcripts generated from mouse genome build MM9 using Seqmap[34]. Mapped reads were quantified using the reads per kilobase exon model metric[35]. Sequencing was performed with 3 independent libraries from 3 separate islet preparations per group.

For microarray expression studies, 3 sets of 300 islets each were subjected to analysis. Total RNA isolated from islets was reverse transcribed to cDNA with T7 oligo d(T) primer (Affymetrix). The cDNA synthesis product was used for *in vitro* transcription with T7 RNA polymerase and biotinylated nucleotide analog (pheudouridine base). The labeled cRNA products were fragmented, loaded on to mouse genome 430 2.0 Array (Affymetrix) and hybridized according to the protocol provided by the manufacturer. The GeneChip array data were analyzed by DNA Microarray Viewer (Kurabo Industries), provided by Kurabo Industries, which is the authorized service provider of Affymetrix Japan.

Protein and hormone assays.

Protein measurement by western blot was performed as previously described[36] with antisera against Tph1 (1:2,000) [9] and β-actin (1:5,000, Sigma-Aldrich). High pressure liquid chromatography (HPLC) coupled to electrochemical detection was used to measure 5-HT concentration in tissue, whole blood, and tissue culture media by the Neurochemistry Core Laboratory at Vanderbilt University's Center for Molecular Neuroscience Research[37]. Insulin assays were performed on plasma and culture media by ELISA **(**Millipore**)** per the manufacturer's protocol.

To assess insulin and 5-HT secretion in response to glucose from mouse pregnant islets, islets were isolated from pregnant (G13−G15) mice and pooled. Islets were handpicked and preincubated in modified Krebs-Ringer bicarbonate buffer (KRBB) containing 2 mM glucose for 1 hour. After preincubation, islets were washed with KRBB containing 2 mM glucose and 40 islets were handpicked into each well and incubated for 1 hour in KRBB containing 2 mM or 20 mM glucose. After incubation, genomic DNA was extracted and its concentrations were measured using Nanodrop. Insulin and 5-HT secretion was expressed as secreted 5-HT or insulin/genomic DNA concentration.

To assess the effect of Htr2b signaling on glucose stimulated insulin secretion, pancreatic islets were isolated from pregnant (G13) wild type (*Htr2b*$^{+/+}$) or Htr2b KO (*Htr2b*$^{-/-}$) mice and pooled. Islets were handpicked and preincubated in KRBB containing 2 mM glucose in the presence or absence of SB204741 (35 μM) for 1 hour. After preincubation, islets were washed with KRBB containing 2 mM glucose and 40 islets were handpicked into each well and incubated for 10 minutes consecutively in KRBB containing 2 mM and 20 mM of glucose in the presence or absence of SB204741 (35 μM). After insulin secretion, islets were collected and tissue insulin was extracted using acid ethanol. Insulin secretion was expressed as % secreted insulin/insulin content.

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