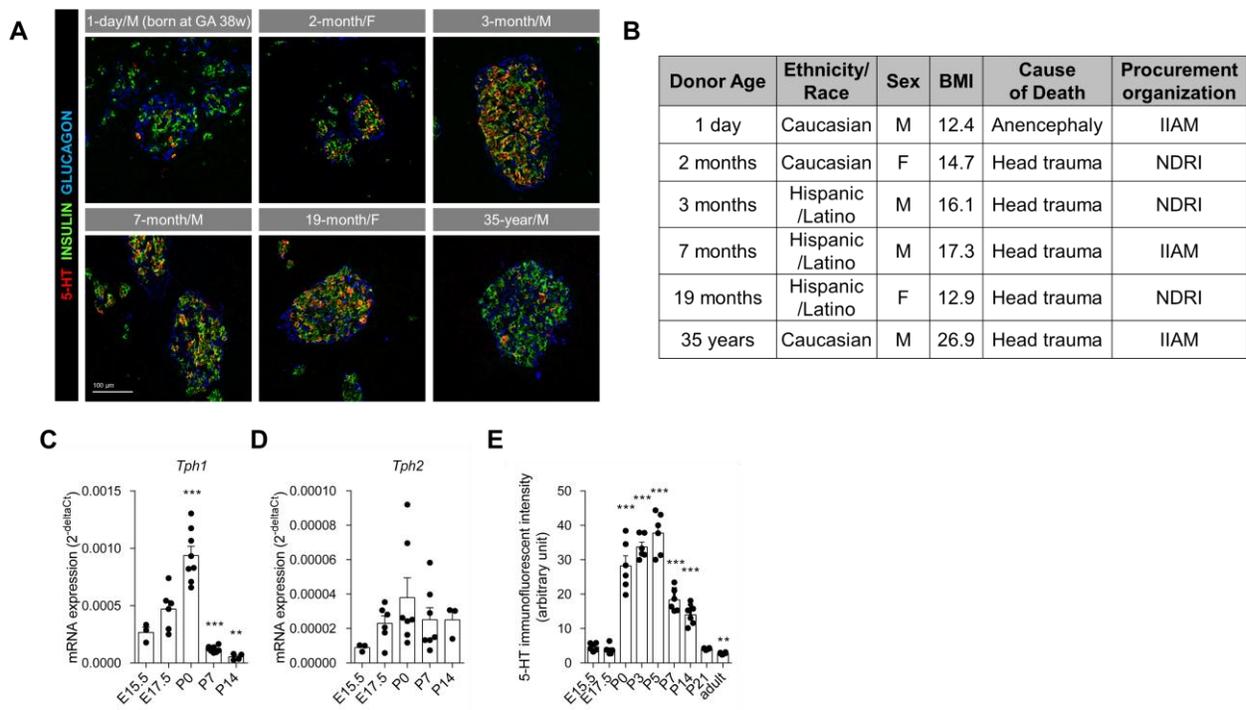


SUPPLEMENTARY DATA

Supplementary Figure 1. 5-HT is produced from human and mouse β cell during the perinatal period. (A) Immunofluorescent staining of human pancreas labels insulin (green) and 5-HT (red). Scale bar indicates 100 μ m. (B) Demographic information of human pancreas donors. (C, D) Expression levels of (C) *Tph1* and (D) *Tph2* were measured by real-time RT-PCR in RNAs from mouse pancreas during the perinatal period and expressed relative to levels of β -actin mRNA. n = 3-8 mice per each time point. (E) The immunofluorescence intensity of 5-HT was measured using the Nikon NIS software (reference range: 0 – 256). The insulin-positive area was manually drawn as a region of interest. The background (exocrine) intensity ranged from 2.16 to 2.91. n = 6 per group. All data are presented as mean \pm standard error. * P <0.05, ** P <0.01 and *** P <0.001.

Pancreas from deceased human organ donors in Supplementary Figure 1A were obtained within 18 hours of cold clamp through partnerships with the International Institute for Advancement of Medicine (IIAM) and National Disease Research Interchange (NDRI). Detailed donor demographic information is summarized in Supplementary Figure 1B; the Vanderbilt University Institutional Review Board does not classify de-identified human pancreatic specimens as human subject research. Tissues were lightly fixed and immunofluorescent staining was performed on 5 μ m cryosections as described (ref. S1).

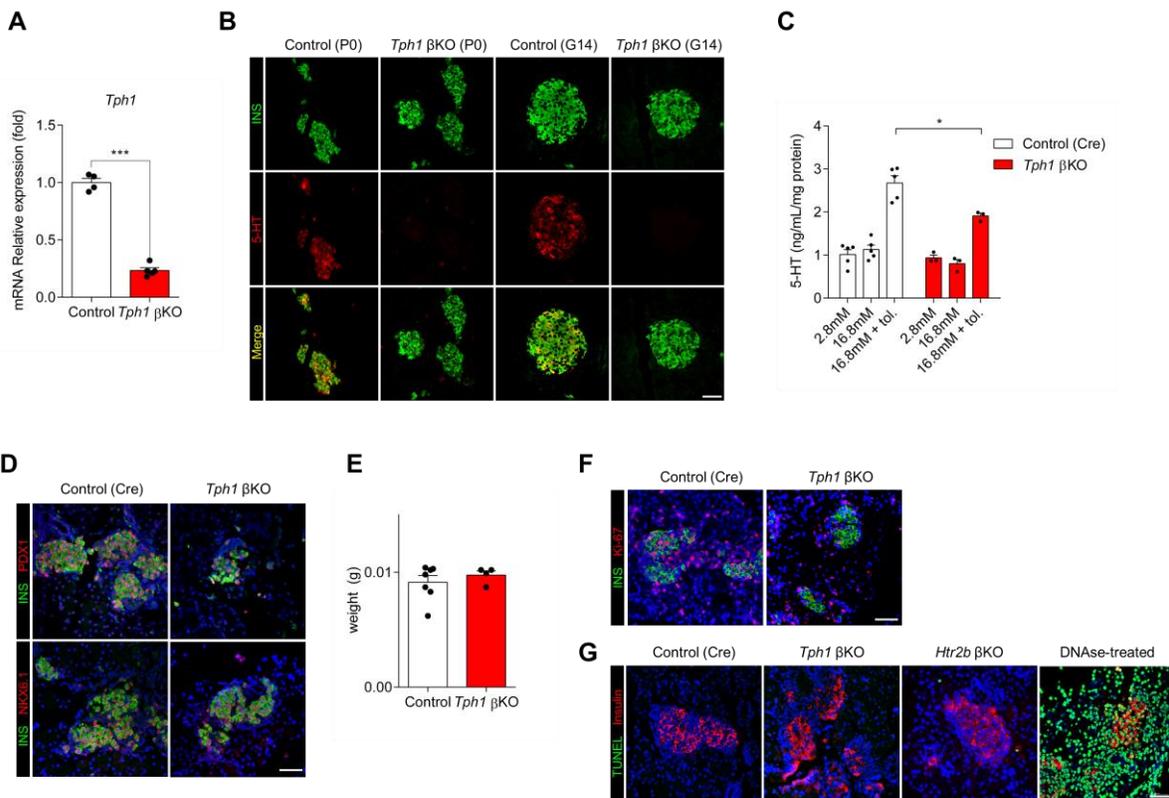
(S1) Brissova, M., Haliyur, R., Saunders, D., Shrestha, S., Dai, C., Blodgett, D.M., Bottino, R., Campbell-Thompson, M., Aramandla, R., Poffenberger, G., et al. (2018). α Cell Function and Gene Expression Are Compromised in Type 1 Diabetes. Cell Reports 22, 2667-2676.



SUPPLEMENTARY DATA

Supplementary Figure 2. *Tph1* β KO in the perinatal period

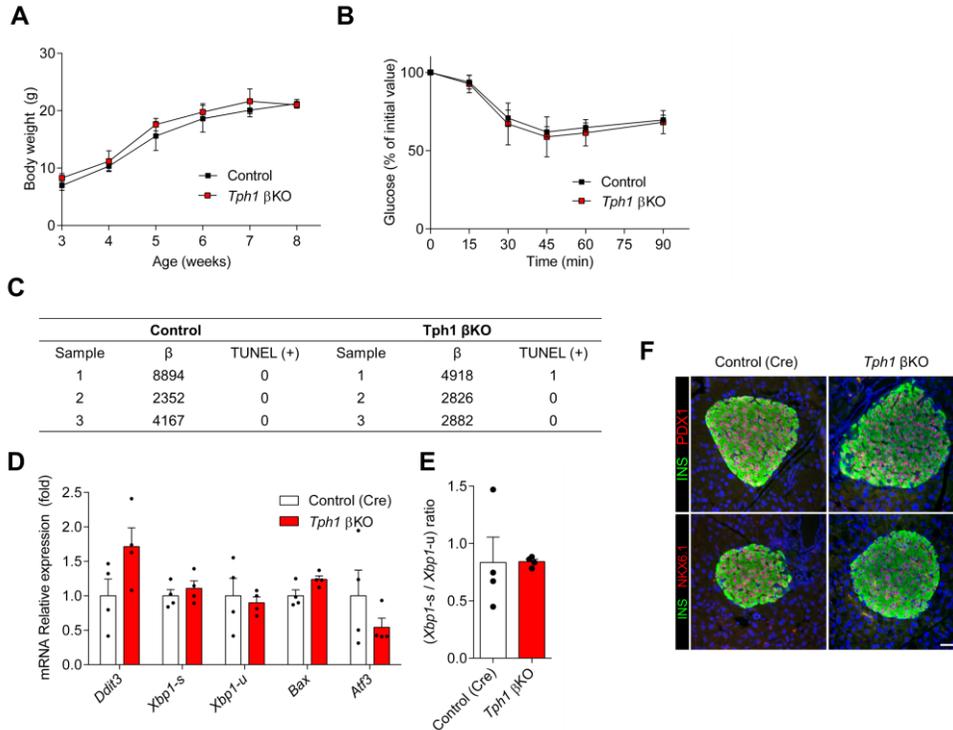
(A) *Tph1* mRNA levels were measured by real-time RT-PCR in RNAs from pancreas in control and *Tph1* β KO mice at postnatal day 0 (P0) and expressed relative to levels of β -actin mRNA. n = 4 mice per group. (B) Representative immunofluorescent (IF) staining labels 5-HT (red) and insulin (green) in pancreas from control and *Tph1* β KO mice at P0 and during pregnancy (day 14). (C) Pancreas from P0 control and *Tph1* β KO mice were incubated for 10 minutes with 2.8 mM glucose, 16.8 mM glucose, or 16.8 mM glucose with 500 mM tolbutamide, and secreted 5-HT was measured with ELISA. n = 3-5 per group. (D) IF images staining β cell markers (PDX1 and NKX6.1) in control and *Tph1* β KO at P0. (E) Pancreas weight at P0. n = 4-6 mice per group. (F) IF staining of β cell proliferation labels insulin (green) and Ki-67 (red) at P0. (G) TUNEL IF staining of *Tph1* β KO and *Htr2b* β KO at P0. Apoptosis was rare in all samples. DNase-treated section was used as a positive control. n \geq 3 mice. Scale bar indicates 50 μ m. All data are presented as mean \pm standard error. * P <0.05, ** P <0.01 and *** P <0.001.



SUPPLEMENTARY DATA

Supplementary Figure 3. *Tph1* βKO in adulthood

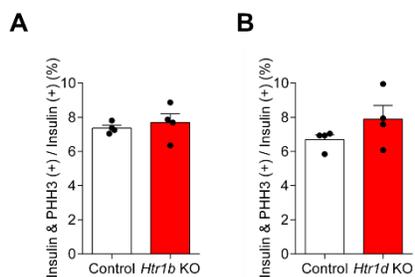
(A-B) Metabolic phenotypes of 9-week-old male *Tph1* βKO mice. n = 4 mice per group; (A) body weight, (B) intraperitoneal insulin tolerance test (0.75U/kg) after a 6-hour fasting. (C) The numbers of TUNEL-positive cells and β cells in 9-week-old control and *Tph1* βKO mice. Apoptosis was rare in all samples. n = 3 per group. (D) mRNA expression levels of different ER stress markers and (E) *Xbp1-s* to *Xbp1-u* ratio in islets of 9-week-old control and *Tph1* βKO mice. n = 4 per group. (F) Representative immunofluorescent staining labels β cell markers (PDX1 and NKX6.1, red) and insulin (green) in pancreata from 9-week-old control (Cre) and *Tph1* βKO mice. Scale bar indicates 50 μm. All data are presented as mean ± standard error.



SUPPLEMENTARY DATA

Supplementary Figure 4. HTRs and β cell proliferation

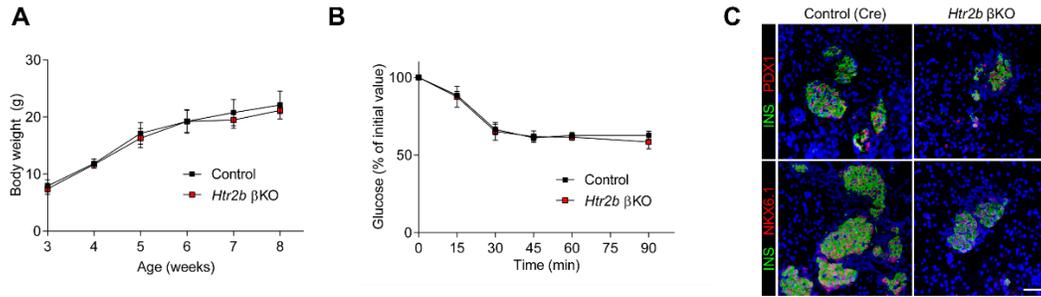
β cell proliferation of (A) *Htr1b* and (B) *Htr1d* KO mice at P0 was quantified as the percentage of insulin and pHH3 co-positive cells in all β cells. n = 4 mice per group. All data are presented as mean \pm standard error.



SUPPLEMENTARY DATA

Supplementary Figure 5. *Htr2b* β KO in adulthood

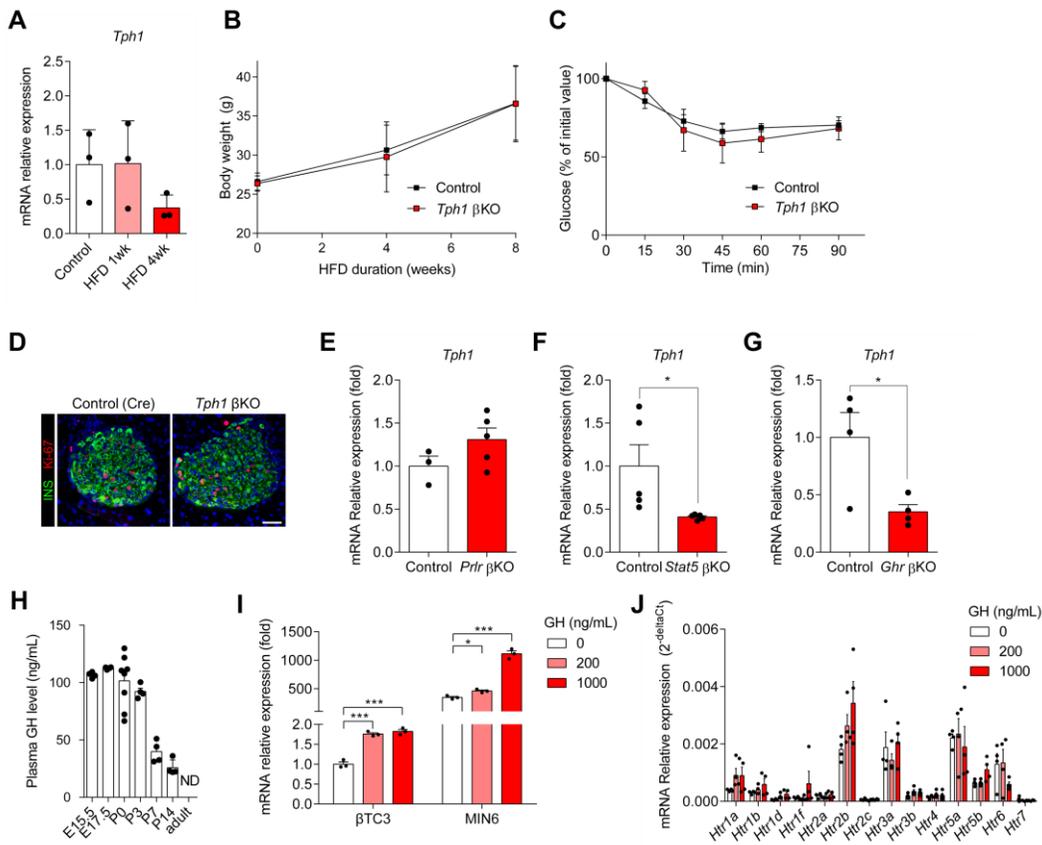
(A-C) Metabolic phenotypes of 9-week-old male *Htr2b* β KO mice. n = 4 mice per group; (A) body weight, (B) intraperitoneal insulin tolerance test (0.75U/kg) after a 6-hour fasting. (C) Representative immunofluorescent staining labels β cell markers (PDX1 and NKX6.1, red) and insulin (green) in pancreata from 9-week-old control (Cre) and *Htr2b* β KO mice. Scale bar indicates 50 μ m. All data are presented as mean \pm standard error.



SUPPLEMENTARY DATA

Supplementary Figure 6. *Tph1* β KO upon metabolic stress

(A) *Tph1* mRNA levels were measured by real-time RT-PCR in RNAs from pancreas in C57BL6/J mice fed high-fat-diet for 1 to 4 weeks and expressed relative to levels of β -actin mRNA. n = 3 mice per group. (B-C) High-fat-diet was fed to 12-week-old control and *Tph1* β KO mice for 8 weeks. n = 4 mice per group; (B) body weight, (C) intraperitoneal insulin tolerance test (0.75U/kg) after a 6-hour fasting. (D) Representative immunofluorescent staining labels β cell proliferation (Ki-67, red) and insulin (green) in pancreata from 9-week-old male mice administered S-961 (100 nmol/kg mouse/twice a day) for 7 days. Scale bar indicates 50 μ m. (E-G) mRNA expression levels of *Tph1* in (E) *Prlr* β KO, (F) *Stat5* β KO, and (G) *Ghr* β KO mice at P0. n = 3-5 per group. (H) Plasma GH levels (ng/mL) during and after the perinatal period. n = 4-8 per group. (I) mRNA expression levels of *Tph1* in β TC3 and MIN6 cells treated with recombinant mouse GH (ng/mL) for 24 hours after serum fasting for 12 hours. n = 3 per group. (J) mRNA expression levels of 5-HT receptors in islets obtained from 3-week-old C57BL6/J mice and treated with mouse GH (ng/mL) for 24 hours. n = 4 per group. All data are presented as mean \pm standard error. * P <0.05, ** P <0.01 and *** P <0.001.



SUPPLEMENTARY DATA

Supplementary Table 1. Primer sequences for qRT-PCR

		Primer sequence
<i>Tph1</i>	Forward	TTCTGACCTGGACTTCTGCG
	Reverse	GGGGTCCCATGTTTGTAGT
<i>Tph2</i>	Forward	GCCATGCAGCCCGCAATGATGATG
	Reverse	CTACCGCTGTCTTGCTGCTC
<i>Mki67</i>	Forward	GACTGCGAGCTTCACCGAGA
	Reverse	CACTACAGGCAGCTGGATACGAA
<i>Ccna2</i>	Forward	GGCTGACACTCTTTCCG
	Reverse	CTGGTAGCAAGAATTAGAGCAT
<i>Ccnb1</i>	Forward	AGCAAATATGAGGAGATGTACC
	Reverse	CGACTTTAGATGCTCTACGGA
<i>Ccnb2</i>	Forward	GAAACCCACAGCCTCTGTGAA
	Reverse	TTCATGGAGACATCCTCAGGG
<i>Ccnd1</i>	Forward	GTGCTGCGAGCCATGCTCAA
	Reverse	TTCATGGCCAGCGGGAAGA
<i>Ccnd2</i>	Forward	GCGTTCTGCAGAACCTGTTGACCA
	Reverse	GGTAATTCATGGCCAGAGGAAAGAC
<i>Ccnd3</i>	Forward	ATGGAGCTGCTGTGTTGCGA
	Reverse	TTGATCTCCTTTTGCACGCAC
<i>Ccne1</i>	Forward	TGCACCAGTTTGCTTATGTT
	Reverse	CCGTGTCGTTGACATAGG
<i>Htr1a</i>	Forward	TCAGCTACCAAGTGATCACCTCT
	Reverse	GTCCACTTGTTGAGCACCTG
<i>Htr1b</i>	Forward	TGCTCCTCATCGCCCTCTATG
	Reverse	CTAGCGGCCATGAGTTTCTTCTT
<i>Htr1d</i>	Forward	CCTCCAACAGATCCCTGAATG
	Reverse	CAGAGCAATGACACAGAGATGCA
<i>Htr1f</i>	Forward	TGTGAGAGAGAGCTGGATTATGG
	Reverse	TAGTTCCTTGGTGCCTCCAGAA
<i>Htr2a</i>	Forward	AGCTGCAGAATGCCACCAACTAT
	Reverse	GGGATTGGCATGGATATACCTAC
<i>Htr2b</i>	Forward	AAATAAGCCACCTCAACGCCT
	Reverse	TCCCGAAATGTCTTATTGAAGAG
<i>Htr2c</i>	Forward	TTCTTAATGTCCCTAGCCATTGC
	Reverse	GCAATCTTCATGATGGCCTTAGT
<i>Htr3a</i>	Forward	AAATCAGGGCGAGTGGGAGCTG
	Reverse	GACACGATGATGAGGAAGACTG
<i>Htr3b</i>	Forward	CGTGTGGTACCGAGAGGTTT
	Reverse	GGATGGGCTTGTGGTTTCTA
<i>Htr4</i>	Forward	ATGGACAAACTTGATGCTAATGTGA
	Reverse	TCACCAGCACCGAAACCAGCA
<i>Htr5a</i>	Forward	GATTGACTTCAGTGGGCTCG
	Reverse	AAAGTCAGGACTAGCACTCG
<i>Htr5b</i>	Forward	GGAGCCTTCTACCTGCCTCT
	Reverse	ATGAGCTCCGTCAGGAAGAA
<i>Htr6</i>	Forward	CCTCACATGGCTGGGATACT
	Reverse	ATCTGAGTTGGGTGGCAGAG
<i>Htr7</i>	Forward	CTCGGTGTGCTTTGTCAAGA
	Reverse	TTGGCCATACATTTCCATT
<i>Actb</i>	Forward	GGTACCACCATGTACCCAGG
	Reverse	GAAAGGGTGTAAAACGCAGC

SUPPLEMENTARY DATA

Supplementary Table 2. 5-HT related transcript levels of human and mouse β cells

(A) Comparison of mRNA transcript levels (TPM, Transcripts Per Million) of human fetal and adult purified β cells (Blodgett et al. (ref. 20)) (B) *Tph1* mRNA transcript levels (TPM) of purified mouse β cells during the perinatal period (Qiu et al. (ref. 30))

A

HUMAN	fetal β cell	adult β cell
<i>TPH1</i>	2.226667	1.248571
<i>TPH2</i>	0.148333	0.358571
<i>HTR1B</i>	0	0.122857
<i>HTR1D</i>	0.471667	0.377143
<i>HTR1E</i>	0	0
<i>HTR1F</i>	8.615	6.698571
<i>HTR2B</i>	5.776667	17.62429
<i>HTR2C</i>	0	0
<i>HTR3A</i>	0	0
<i>HTR3B</i>	0.736667	0
<i>HTR3C</i>	0	0
<i>HTR3D</i>	0.021667	0.068571
<i>HTR3E</i>	0.105	0.382857
<i>HTR4</i>	0.033333	0.022857
<i>HTR6</i>	0	0.191429
<i>HTR7</i>	0.075	0
<i>GHR</i>	4.73	0.475714
<i>PRLR</i>	1.138333	1.128571

B

MOUSE β cell	E17.5	P0	P3	P9	P15	P18	P60
<i>Tph1</i>	1.43	3.04	0.49	0.42	0	0.03	0.03