# Supporting Information

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### SI Experimental Procedures

**Animals.** Mice were housed on a 12-h/12-h light/dark cycle in controlled-climate rooms (21.5–22.5 °C). All mouse lines were maintained on a background of C57BL/6J mice obtained from Jackson Laboratories-West. All procedures used were approved by the UCSF Institutional Animal Care and Use Committee and were in accordance with regulations of UCSF. The RIP-rtTA (1), MIP-GFP (2), ePet1-cre (3), Ro1-RASSL (4), ROSA-PTX (5), and *Adra2a*$^{+/-}$ (6) mouse lines are described elsewhere.

Heterozygous mice from the Ins2-cre [formal name: Tg(Ins2-cre)25Mgn] (7), Neurog3-cre [formal name: Tg(Neurog3-cre) 1Herr] (8), and Ptf1a-cre [formal name: Ptf1a$^{tm1.1(cre)Cvw}$] (9) lines were crossed with homozygous mice from the LacZ reporter line Gt(ROSA)26Sor$^{tm1Sor}$ (10). Double heterozygotes and single transgenics (negative control) were analyzed.

RIP-rtTA and tetO transgenic mice were given food containing 200 mg/kg doxycycline (BioServ) during the indicated time periods. Animals identified as sick by veterinary staff were not used for experiments.

**Generation of ePet1-*Htr1a* Transgenic Mice.** The plasmid pFLAG-Htr1a+iresGFP was generated by linking a signal sequence and the coding sequence for the FLAG epitope tag to the 5′ end of the coding sequence for the mouse Htr1a receptor followed by the IRES-eGFP sequence from pIRES-EGFP2 (Clontech Laboratories). The 4-kb insert from pFLAG-5-HT1A+iresGFP was inserted downstream of a minimal β-globin promoter element and the mouse *Pet1* enhancer in pBAC-ePet (3) to generate the ePet1-*Htr1a* transgene shown in Fig. S1*A*. The final construct was confirmed by both restriction digests and DNA sequencing. After removal from the plasmid backbone, the transgene was injected into mouse oocyte pronuclei, and the resulting pups were screened by PCR for the transgene as previously described (3). Transgene copy number was estimated by real-time PCR quantification performed on purified tail DNA from ePet1-*Htr1a*-H and ePet1-*Htr1a*-L mice for the transgene and was compared with the *Actb* gene sequence.

**Generation of tetO-PTX Mice.** The plasmid ptetO-S1-PTX was generated by inserting the cDNA sequence encoding S1-PTX [generously provided by Eitan Reuveny, the Weizmann Institute of Science, Rehovot, Israel (11)] downstream of the β-globin intron in the tTA-inducible pUHG 10-3 plasmid. The construct was confirmed by both restriction digests and DNA sequencing. After removal from the plasmid backbone, the transgene was injected into mouse oocyte pronuclei. Eight founder mice were identified by PCR analysis of genomic DNA isolated from tail tips (primers 5′-CCA TAG AAG ACA CCG GGA CCG-3′ and 5′-GGA ACG TCC GGT CAG ATG GTC GA-3′ resulting in a 288-bp fragment). Mice were available through the Mutant Mouse Regional Resource Centers (https://www.mmrrc.org/catalog/sds.php?mmrrc_id=14241).

**Glucose-Tolerance Tests.** All mice were fasted overnight and placed in fresh cages to ensure that no food was available on the cage floor. The following morning, each mouse was injected with 2 mg/kg of glucose dissolved in saline via i.p. injection. Glucose measurements were taken from tail blood at the indicated times, using a OneTouch Ultra Glucometer and test strips (LifeScan, Inc.). Insulin in plasma from blood obtained from the tail vein at 0 and 15 min after glucose injection was assayed by ELISA kit (Apco Diagnostics).

**Insulin-Tolerance Tests.** All mice were fasted overnight and placed in fresh cages to ensure that no food was available on the cage floor. The following morning, each mouse was injected with 0.75 units of insulin/kg of body weight, and glucose measurements were taken from tail blood as described above.

**Pancreatic Histology.** Mice were anesthetized with Avertin, and unconsciousness was verified by lack of response to tail pinch. Then animals were transcardiac perfused with 10 $cm^3$ of 4 °C PBS, followed by 10 $cm^3$ of 4% PFA. The pancreas was isolated using a dissection scope and was postfixed in 4% PFA at 4 °C for 4 h. The pancreas then was taken through an ethanol dehydration series, mounted in paraffin, cut into 6-μm-thick sections on a microtome at 4 °C, and mounted on Superfrost Plus glass slides (Fisher Scientific). Sections were incubated with primary antibodies against insulin (rabbit, 1:1,000; EMD Millipore Corporation), glucagon (guinea pig, 1:2,000; EMD Millipore Corporation), pdx1 [guinea pig, 1:2,000; (12)], and tyrosine hydroxylase (rabbit, 1:500; EMD Millipore Corporation) diluted in PBS with 5% normal goat serum overnight at 4 °C in a humidified chamber, followed by five washes for 5 min each in 4 °C PBS. Slides then were stained with secondary antibodies: Cy3-conjugated goat anti-guinea pig or goat anti-rabbit and FITC-conjugated goat-anti rabbit or goat anti-guinea pig (all 1:500; Jackson ImmunoResearch Laboratories) in PBS with 5% normal goat serum for 30 min. Then slides were washed in 4 °C PBS five times for 5 min each. Stained slides were coverslipped with Vectastain mounting medium containing DAPI and were sealed with clear nail polish. Images were obtained using a Zeiss Axio Scope widefield fluorescence microscope with Zeiss AxioVision software.

For measurement of β-cell area, every 30th pancreatic section was examined on a Zeiss Axio Scope widefield fluorescence microscope using a 10× objective, and images were saved with the Zeiss Axiovision program. The area of fluorescent staining in β cells was quantified by circling stained cells using AxioVision. The total pancreatic section area was measured similarly using AxioVision on images taken using a 1.25× microscope objective. The percent of β-cell area was defined as the β-cell area divided by pancreatic section area, multiplied by 100.

**Brain Histology.** Brain histology has performed as described (13) Mice were deeply anesthetized and then perfused with PBS followed by 4% PFA in PBS; brains were removed, cryoprotected in 30% sucrose in PBS, and sectioned (50-μm thickness, coronal) on a freezing microtome and collected into PBS. For β-galactosidase staining, sections were incubated overnight at 37 °C in 10 mM Tris·HCl (pH 7.4), 5 mM K4FeCN6, 5 mM K3FeCN6, 2 mM MgCl2, and 0.8 mg/mL X-Gal (Invitrogen) and then were mounted on slides. Slides were imaged using a brightfield dissection microscope.

**Pancreatic RNA Isolation and Real-Time PCR.** Mice at the ages indicated were anesthetized with 4% Avertin, and lack of consciousness was verified by lack of response to tail pinch. Subsequently, the pancreas was dissected and was placed immediately in RNAlater (Ambion) on ice. The pancreas was minced in the RNAlater solution using microscissors and then was incubated in RNAlater overnight at 4 °C. Subsequently, the pancreatic pieces were pelleted by centrifugation for 2 min at 15,000 rpm at 4 °C in a microcentrifuge, the supernatant was removed, and the pancreatic pieces were suspended in 5 mL of TRIzol and homogenized with a pellet pestle motor using

an Rnase-free pestle (Fisher Scientific). RNA then was isolated as previously described (14).

TaqMan real-time PCR was performed with an Applied Biosystems 7300 Real-Time PCR System using 50 ng of cDNA per reaction in 96-well plates or 384-sample microfluidic plates for ABI low-density arrays. Results were normalized to levels of *Gapdh* mRNA for *Htr1a* expression, to *Gusb* in Fig. 4, *Gapdh* in Fig. S4, and to the average of *Actb*, *Gapdh*, and *Ppia* in Tables S1 and S2. The sequences of the primers and probes used are available on request.

**GPCR Gene-Expression Analysis.** E18.5 and P4 MIP-GFP mice were killed, and their pancreata were dissected and digested as described above. $GFP^+$ β cells were separated by FACS as previously described (15). RNA was isolated from these cells using the TRIzol method as described above. cDNA was obtained by reverse transcription from these RNA samples and then was run on a 384-well TaqMan Low Density Mouse GPCR array card (Applied Biosystems) in an Applied Biosystems 7300 Real-Time PCR System. Data from four independent isolations were used for Fig. 4*A*. Adult islet and β-cell data were derived from massively parallel sequencing of cDNA purified from isolated 4-mo-old adult mouse islets and sorted β cells (16) and were expressed as RPKM.

**β-Cell Replication Rates by Flow Cytometry.** β-Cell replication in MIP-GFP mice was measured as previously described (14). In brief, pups were killed at day P1, and their pancreata were dissected out and placed in ice-cold PBS. Pancreata were placed in 1.5 mL trypsin-EDTA at 37 °C for 5 min and then in 1 mL ice-cold FBS, and 2 $cm^3$ of ice-cold PBS (with divalent cations) were added to quench the trypsin. The tube then was spun at 2,000 rpm at 4 °C for 5 min, and the pellet was resuspended in 1 mL of DMEM containing 5 μg/mL Hoechst 33342 dye. The solution was incubated for 1 h at 37 °C with light vortexing every 10 min to keep the cells in suspension. Three milliliters of ice-cold PBS was added to the cells, and the solution was centrifuged at 2,000 rpm at 4 °C for 5 min. The pellet was resuspended in 5 mL of PBS with 1% FBS containing a 1:100,000 dilution of TOPRO3 dye to identify dead cells. This solution was incubated on ice for several minutes; then the cells were analyzed by flow cytometry with gating and parameters as previously described (14, 15).

**Islet Culture and Proliferation Assessment.** Islets were isolated and cultured for 5 d as previously described (17), followed by 6 d of treatment with the drugs shown at 1 μm with 0.1% DMSO. On day 6 of treatment, islets were treated with 10 mM EdU for 3 h and then were fixed immediately in 4% PFA/10 mM PBS solution for 25 min. Fixed islets were washed three times with 10 mM PBS for 20 min, permeabilized with 0.3% Triton X-100 in 10 mM PBS for 3 h, blocked overnight at 4 °C in 5% goat serum/0.15% Triton X-100/10 mM PBS, and then washed twice with antibody dilution buffer for 15 min at room temperature. Islets were stained with the primary antibody, rabbit anti-human NKX6.1 (1:500; Sigma-Aldrich), and the secondary antibody, Cy3-conjugated goat anti-rabbit (1:500; Sigma-Aldrich), diluted in 1% BSA/0.2% Triton X-100/10 mM PBS for 24 h at 4 °C. After immunostaining, replicating cells were labeled with the Click-iT EdU Alexa Fluor Imaging Kit (Invitrogen). Islets were mounted with Fluoromount (Sigma-Aldrich) and imaged using a Leica SP5 confocal laser scanning microscope (Leica). The Volocity software (PerkinElmer) colocalization macro was used to count nuclei costaining for EdU and the unique β-cell nuclear marker Nkx6.1 (Fig. S3) (18). The percent of proliferating β cells was calculated by dividing the number of costaining nuclei by the total number of $Nkx6.1^+$ nuclei and multiplying by 100.


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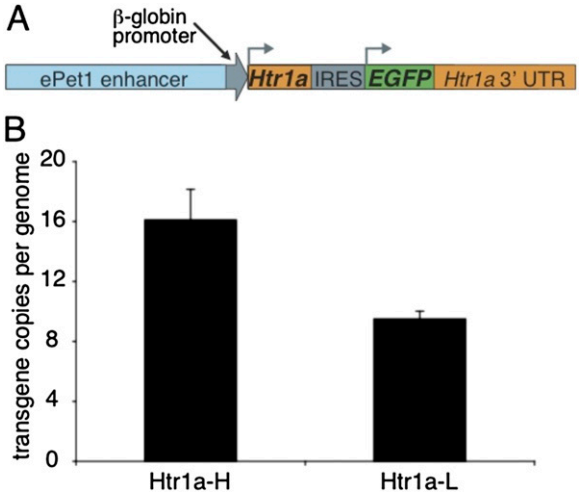


**Fig. S1.** Transgene design in ePet1-*Htr1a* mice. (*A*) The diagram shows the linear ePet1-*Htr1a* transgene construct. (*B*) Transgene copy numbers for the ePet1-*Htr1a* transgenic mouse lines were estimated using real-time PCR quantification compared with the *Actb* gene sequence.

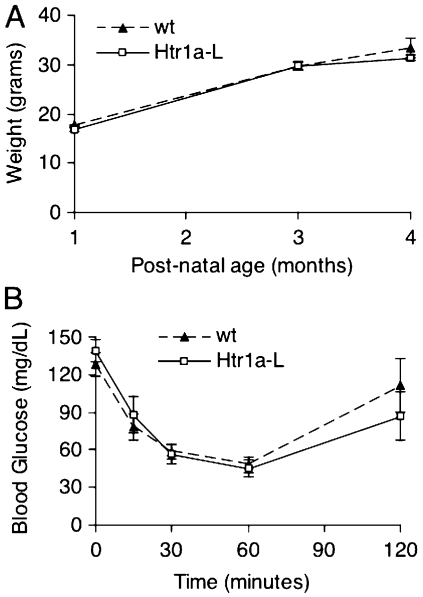


**Fig. S2.** Phenotype of ePet1-*Htr1a*-L transgenic mice. (*A*) Weights of *Htr1a*-L mice and wild-type littermates are shown as a function of postnatal age. (*B*) An insulin-tolerance test was performed in ePet1-*Htr1a*-L mice and nontransgenic control littermates. Blood glucose levels were measured at the indicated time points following i.p. injection of insulin (0.75 units/kg body weight).

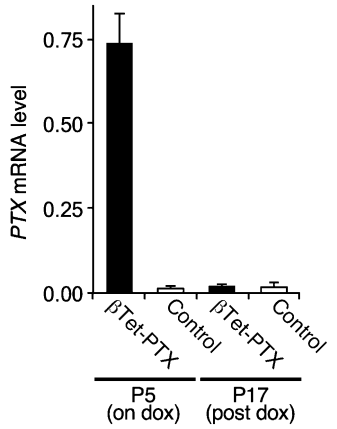


**Fig. S3.** PTX expression in Tet-inducible transgenic mice. Levels of PTX mRNA were measured by real-time RT-PCR and expressed relative to the levels of *Gapdh* mRNA in pancreata from βTet-PTX mice and littermate controls. All animals were treated with doxycycline as in Fig. 3 *C–F*, and pancreata were harvested at day P5 (during doxycycline treatment) or at P17 (after termination of doxycycline treatment). All data points represent the mean ± SEM of seven (βTet-PTX at P5) or three (all others) pups. $P < 0.0005$ for βTet-PTX at P5 vs. each of the other groups by two-tailed Student's *t* test.

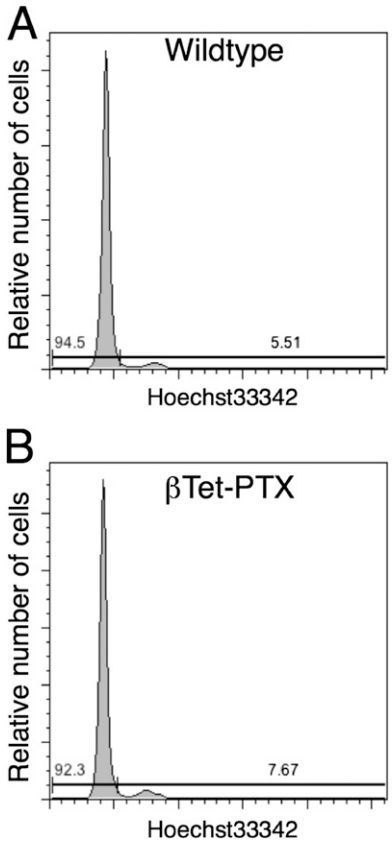


**Fig. S4.** PTX increases replication in mouse β-cells as assessed by flow cytometry. Cells were dissociated from mouse pancreata, stained with the DNA dye Hoechst 33342, and analyzed by flow cytometry. The percentage of $eGFP^+$ cells with increased DNA content proliferating in S/G2/M phases is shown. Representative examples from wild-type (*A*) and βTet-PTX (*B*) littermates are shown.

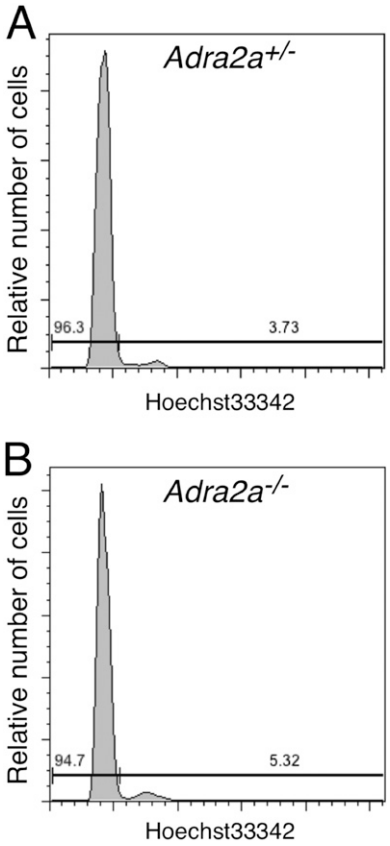


**Fig. S5.** Adra2a decreases replication in mouse β cells as assessed by flow cytometry. Cells were dissociated from mouse pancreata, stained with the DNA dye Hoechst 33342, and analyzed by flow cytometry. The percentage of $eGFP^{+}$ cells with increased DNA content proliferating in S/G2/M phases is shown. Representative examples from *Adra2a*$^{+/-}$ (*A*) and *Adra2a*$^{-/-}$ (*B*) littermates are shown.

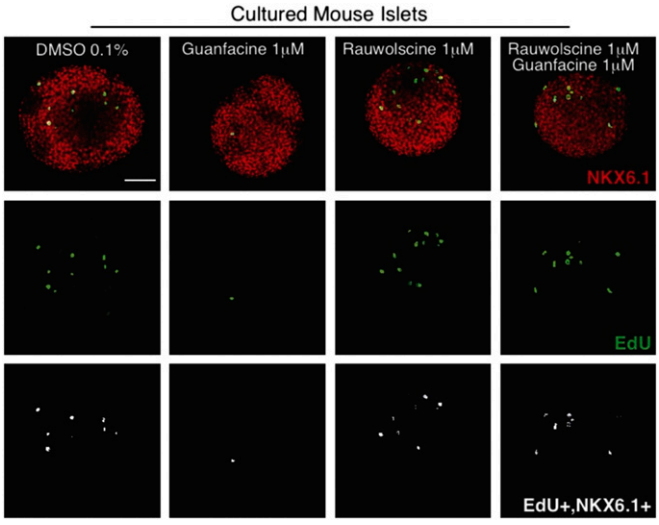


**Fig. S6.** Replication in mouse islets treated with Adra1a agonist guanfacine and antagonist rauwolscine. Examples are shown of the islets used for the quantification shown in Fig. 4*C*. Islets were treated with the drugs shown and stained for the β-cell nuclear marker Nkx6.1 (red, *Top Row*) and deoxythymidine analog EdU (green, *Middle Row*). (*Bottom Row*) Nuclei costaining for Nkx6.1 and EdU are shown in white. (Scale bar, 50 μm.)

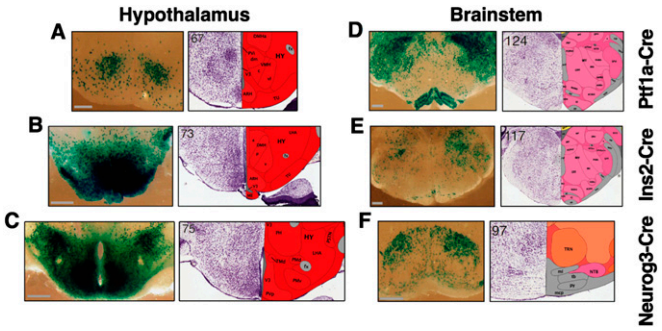


**Fig. S7.** CNS recombination patterns for widely used transgenic pancreatic Cre lines. Double transgenic adult mice were generated using Cre transgenes inserted into the mouse *Ptf1a* gene (*A* and *D*) or driven by the promoters from the rat *Ins2* (*B* and *E*) or human *NEUROG3* (*C* and *F*) genes and a β-galactosidase Cre reporter transgene (for genotype details, see *SI Experimental Procedures*). Brain sections (40-μm) were prepared, stained for β-galactosidase activity, and imaged using wide-field brightfield microscopy. Sections were matched to anatomical reference images from the Allen Mouse Brain Atlas shown to the right of each panel (1). (©2014 Allen Institute for Brain Science. Allen Mouse Brain Atlas [Internet]. Available from mouse.brain-map.org.) Numbers at top left of reference images correspond to the image number from the Allen adult mouse brain reference image set. For anatomical location and abbreviations for brain regions, refer to the online resource. (Scale bars, 400 μm.)

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## Other Supporting Information Files

Table S1 (DOCX)
Table S2 (DOCX)
Table S3 (DOCX)
Table S4 (DOCX)
Table S5 (DOCX)
Table S6 (DOCX)
Table S7 (DOCX)
Table S8 (DOCX)
Table S9 (DOCX)
Table S10 (DOCX)