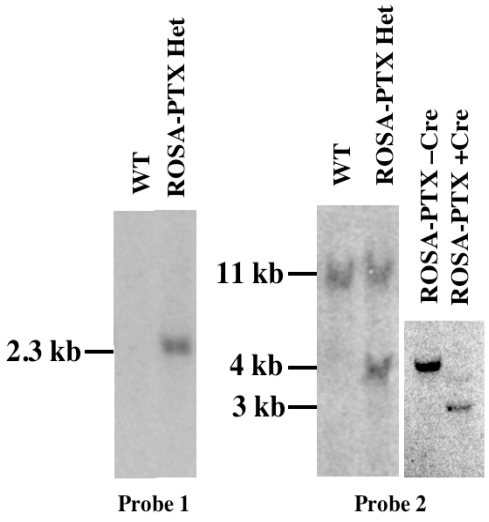
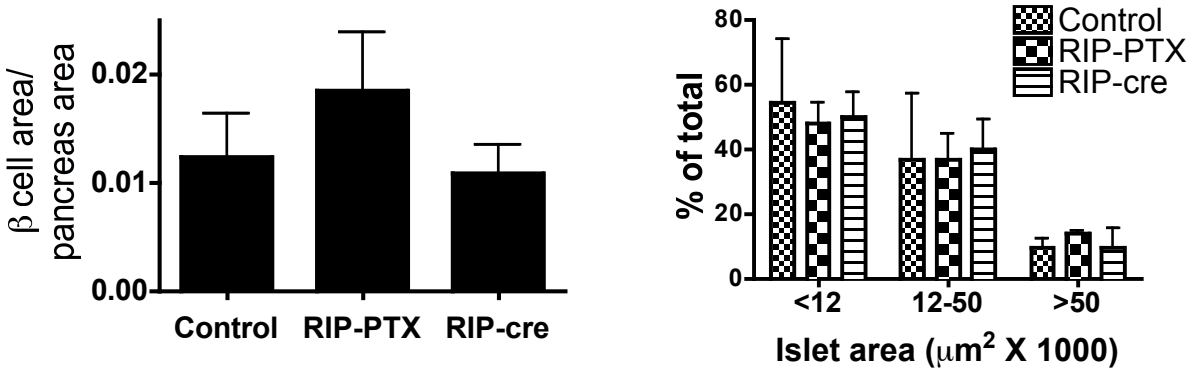
**Supplemental Figure S1: Correct targeting and Cre-dependent recombination of the *ROSA$^{PTX}$* allele.**




Southern blot hybridization was performed to confirm correct integration and Cre-dependent recombination of *ROSA26$^{PTX}$*. In all cases DNA was digested to completion with EcoRV. See Fig. 1A in manuscript for allele diagram with restriction map and probe location.

**Supplemental Figure S2: RIP-PTX and RIP-cre mice have grossly normal β cell mass and islet size.**

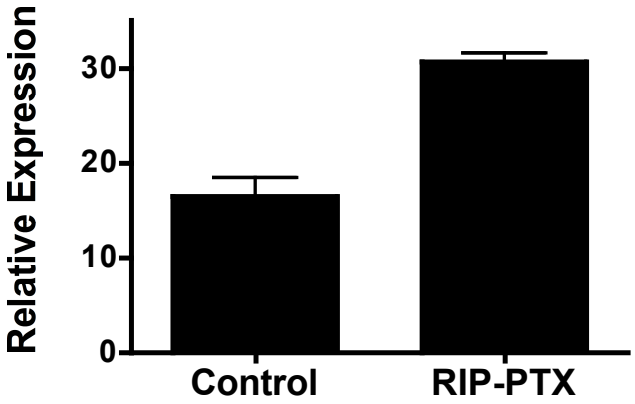



β cell mass (left) was quantified in 8-10 week old RIP-PTX, littermate control and age-matched, background-matched RIP-cre positive mice (n=3 each group). Multiple insulin- and glucagon-stained pancreas sections, separated by at least 60μm, were imaged using a Zeiss Axiophot2 plus microscope. Pancreatic and islet areas were quantified using Open Lab software (Improvision, Lexington, MA) and are presented as a ratio (AVG±SD). Islet areas (right) were outlined and quantified with the OpenLab software. A total of 116 control, 139 RIP-PTX and 181 RIP-cre islets were quantified.

Compared to age-matched wild-type controls from the same colony, we did note a trend toward glucose intolerance in 8-10 week-old male mice of mixed 129/C57BL6 background bearing only the RIP-cre transgene, and glucose intolerance in these mice seemed to increase with age. Note that such a β cell loss-of-function phenotype is opposite the insulin hypersecretion β cell gain-of-function phenotype noted in mice with both RIP-cre transgene

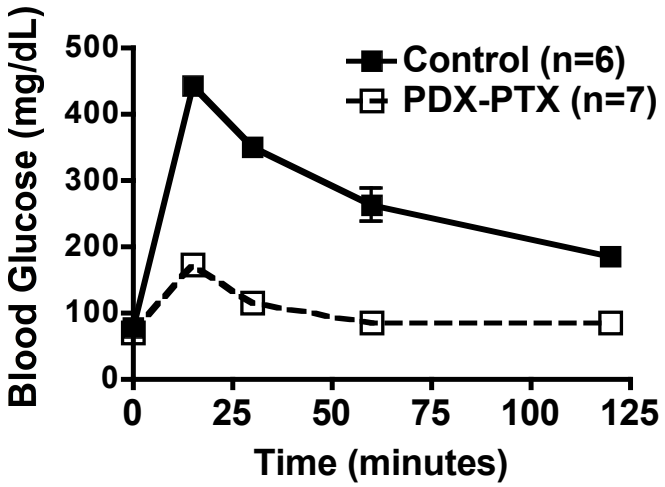
and ROSA26 conditional PTX allele (and no change in glucose tolerance was seen in mice with only the ROSA26 conditional PTX allele), strengthening the conclusion that both the cre transgene and the conditional allele are necessary for the RIP-PTX phenotype.

**Supplemental Figure S3: Insulin mRNA levels are elevated in RIP-PTX mice.**



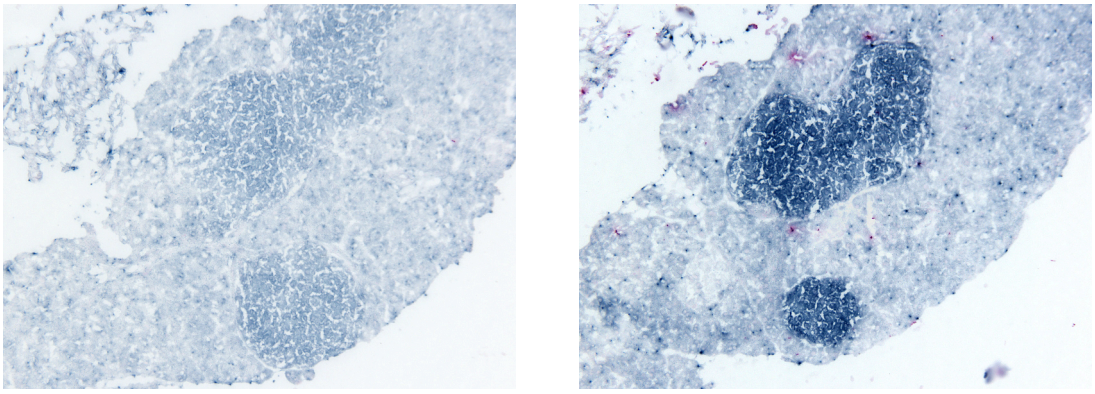

Whole pancreata were dissected from mice at 10-12 weeks of age and total RNA was isolated. qPCR was performed to quantify mRNA levels of insulin and cyclophilin. Insulin mRNA levels relative to cyclophilin mRNA levels are expressed as $2^{-\Delta Ct}$ X 100. (AVG±SEM; n=3; p=0.0024) Insulin mRNA is upregulated in RIP-PTX mice 2-fold relative to littermate controls.

**Supplemental Figure S4: PDX-PTX mice show improved glucose clearance.**



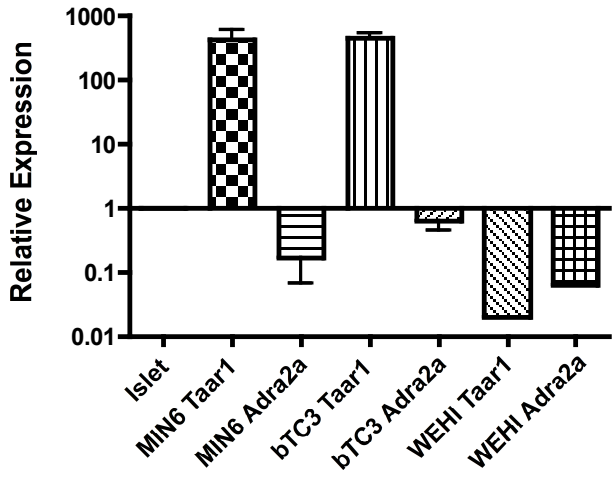

PDX-PTX mice and cre negative littermate controls were fasted overnight and administered 2g/kg glucose (i.p.). Blood glucose levels were quantified at the indicated time point by glucometer (AVG±SEM). Similar to RIP-PTX, PDX-PTX mice show improved clearance of glucose.

**Supplemental Figure S5: Taar1 mRNA is expressed in mouse β cells in vivo.**



In situ hybridization was performed on serial sections of mouse pancreas with sense probe (left) and anti-sense *Taar1* probe (right). Note strong islet-specific staining in a distribution consistent with expression in β cells (i.e., pattern was same as that of insulin immunostaining and different from glucagon staining; the latter was concentrated at the periphery of islets).

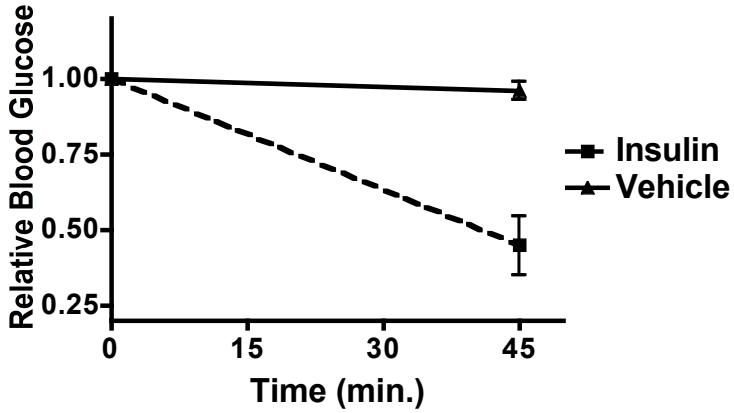
**Supplemental Figure S6: Quantification of select GPCR expression in isolated islets and β cell lines.**




Cycle threshold values from TaqMan analysis for expression of the indicated GPCRs and internal controls (AVG±SD; n=2-5). ND=not done. Note that Taar1, Adra2a and Par2 mRNA are present in human and mouse islets and two mouse β cell lines (table). Using the $2^{-\Delta\Delta Ct}$ method, Taar1 is highly upregulated in two β cell lines (MIN6 and bTC3) but not in a hematopoietic cell line (WEHI) relative to whole islet tissue (graph).

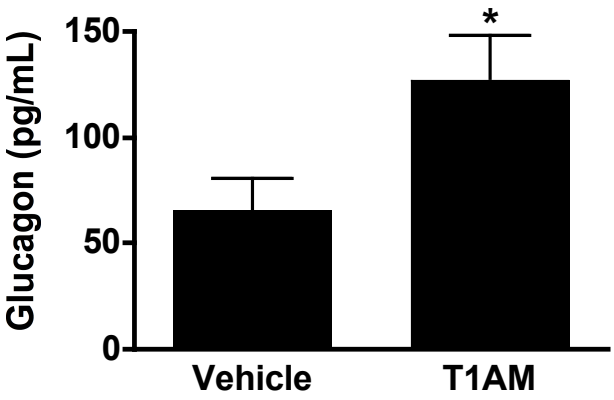
| | β-actin | GAPDH | cyclophilin | Taar1 | Adra2a | Par2 |
|---|---|---|---|---|---|---|
| **Mouse Islet** | 21.615±0.262 | 24.109±0.319 | 22.166±0.154 | 31.491±0.046 | 29.996±0.550 | 31.189±0.311 |
| **Human Islet** | ND | 22.253±0.167 | ND | 32.389±0.275 | | |
| **Human Islet** | ND | 22.565±0.403 | ND | | 27.409±0.183 | 26.496±0.406 |
| **MIN6** | 20.721±0.102 | 21.344±0.579 | 18.419±0.030 | 18.744±0.132 | 28.602±0.223 | 28.238±0.078 |
| **βTC3** | 18.753±0.226 | 20.715±0.579 | 16.997±0.072 | 18.789±0.565 | 26.695±0.345 | 29.677±0.121 |

**Supplemental Figure S7: Mice treated with T1AM remain sensitive to insulin.**



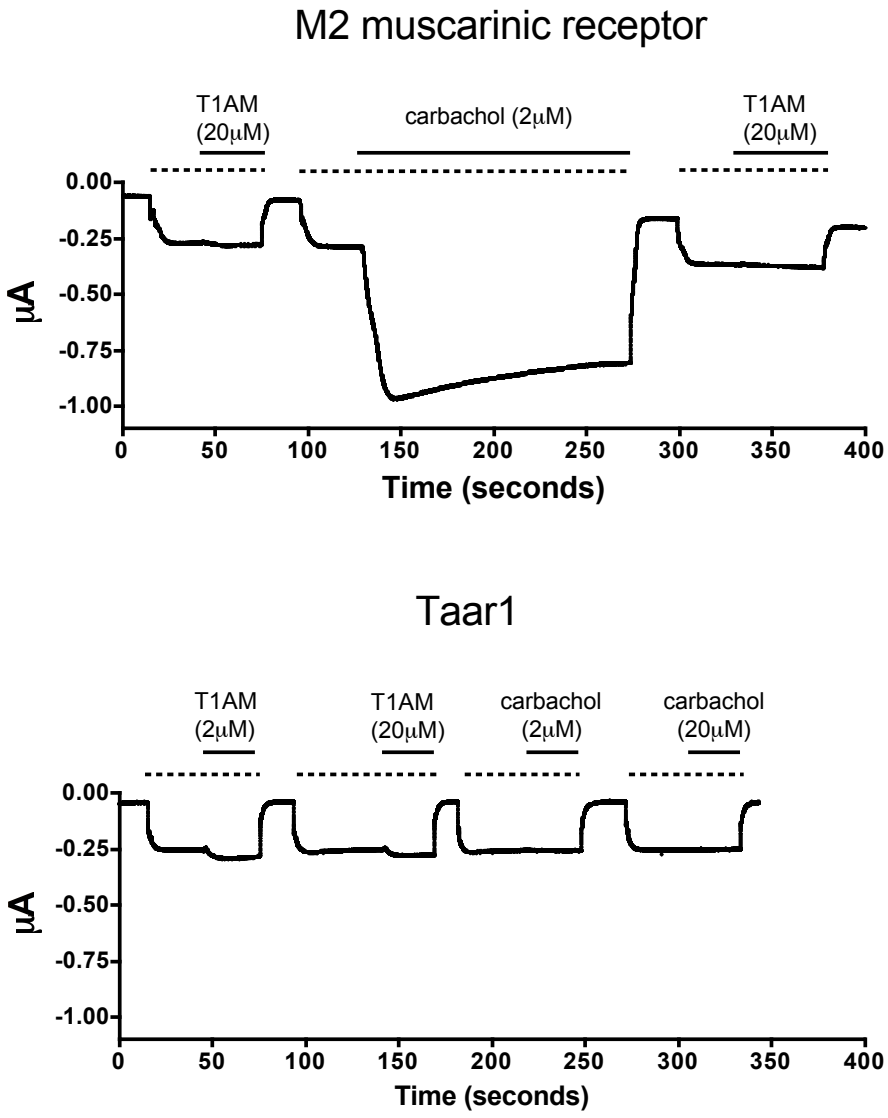

Two hours following T1AM administration mice were hyperglycemic. Injection of 0.75U/kg hr-insulin, but not vehicle, quickly reversed this hyperglycemia, suggesting T1AM administration did not inhibit insulin sensitivity. Data is presented as fractional change in blood glucose following insulin (AVG±SEM; n=6).

**Supplemental Figure S8: T1AM administration increases plasma glucagon concentrations.**



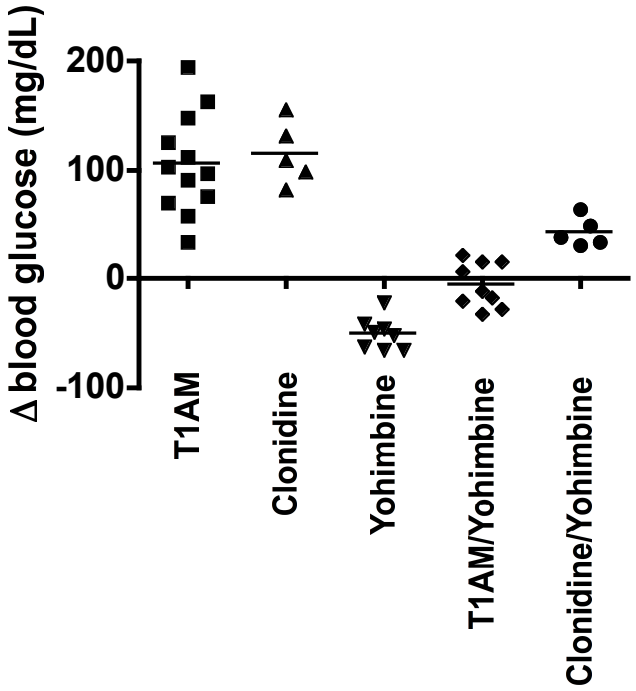

10-week old C57Bl/6 mice were fasted for 2 hours than injected with either T1AM (50mg/kg) or vehicle. Blood was collected 2 hours following injection, plasma isolated and glucagon levels were quantified by radio-immuno assay (Glucagon RIA; Linco research). Glucagon levels in T1AM-treated mice were approximately 2-fold great than vehicle-treated [vehicle 65.2±14.9 pg/mL vs. T1AM 126.8±20.9 pg/mL; AVG±SEM; n=10; *p=0.027).

**Supplemental Figure S9: Taar1 does not couple to $G\alpha_i$ in Xenopus oocytes.**



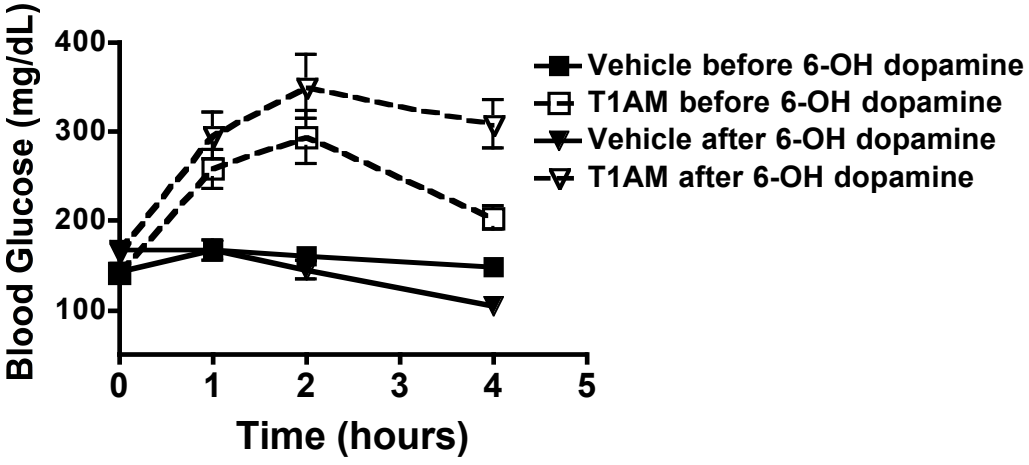

Experiments were performed as described [1]. Xenopus oocytes were microinjected with GIRK channel mRNAs plus M2 muscarinic receptor (top) or Taar1 mRNA (bottom). After 24-96 hours, currents were recorded by two-electrode voltage clamp during constant perfusion. Injected oocytes were first perfused with recording solution containing 90mM KCl (dashed line), current was allowed to stabilize, then oocytes were perfused with recording solution containing the indicated agonist (solid line). The acetylcholine receptor agonist carbachol, but not T1AM, triggered inward potassium currents in M2 muscarinic receptor mRNA-injected but not Taar1 mRNA-injected oocytes, indicative of GPCR-mediated GIRK channel activation via $G\alpha_i$. T1AM, a known Taar1 agonist, failed to trigger significant potassium channel activity in Taar1 mRNA-injected oocytes, suggesting Taar1 does not couple to $G\alpha_i$ in this sensitive system. Varying durations of oocyte incubation to allow for GPCR expression and varying amounts of receptor mRNAs were tried with similar results.

**Supplemental Figure S10: Yohimbine inhibits the hyperglycemic effects of T1AM.**



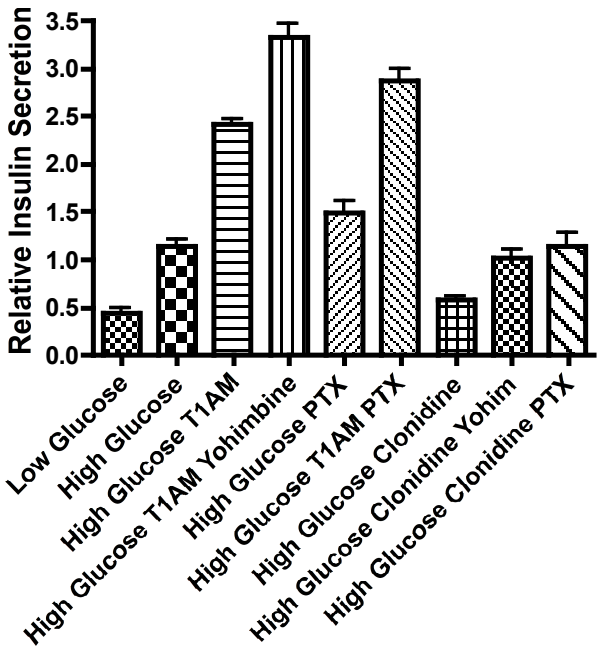

C57/Bl6 mice were administered T1AM (50mg/kg), the Adra2 agonist clonidine (1mg/kg), the Adra2 antagonist yohimibine (5mg/kg), or the indicated combination. Blood glucose levels were quantified at time zero and 1 hour following treatment and changes in glucose levels are shown. Yohimbine inhibited the hyperglycemic effects of both clonidine and T1AM, suggesting T1AM may be acting through an Adra2 receptor.

**Supplemental Figure S11: Ablation of adrenergic neurons by 6-OH dopamine treatment does not inhibit T1AM-induced hyperglycemia.**




C57/Bl6 mice were administered 50mg/kg T1AM or vehicle and blood glucose levels were measured. 3 days following T1AM treatment mice were administered 100mg/kg 6-OH dopamine (i.p.) and allowed to recover 10 days. Mice were given either vehicle or T1AM and blood glucose levels quantified. Destruction of adrenergic neurons by 6-OH dopamine did not diminish T1AM's hyperglycemic effect, suggesting T1AM is not acting via sympathetic innervation of islets. [AVG±SEM, n=6-8].

**Supplemental Figure S12: Effects of T1AM on insulin secretion in MIN6 cells.**




Glucose stimulated insulin secretion was examined in MIN6 cells as in Fig. 6A. Consistent with T1AM regulating insulin secretion through 2 GPCRs (Taar1 and Adra2a), T1AM-treatment led to increased insulin secretion that was further augmented by co-treatment with the Adra2a antagonist yohimbine. Clonidine, a specific Adra2 agonist, inhibited insulin release in a manner that was yohimbine and PTX sensitive. Note that Taar1 is upregulated >200 fold in MIN6 cells relative to mouse islets (see supplemental figure S6), which likely explains the predominance of the stimulatory effect of Taar1 in vitro and the inhibitory effect of Adra2a in vivo. Data are presented as AVG±SEM (n=4). Three independent experiments yielded similar results. (T1AM = 10μM, yohimbine = 10μM, clonidine = 1μM)

**References:**

1. Minor, D.L., Jr., et al., *Transmembrane structure of an inwardly rectifying potassium channel.* Cell, 1999. **96**(6): p. 879-91.