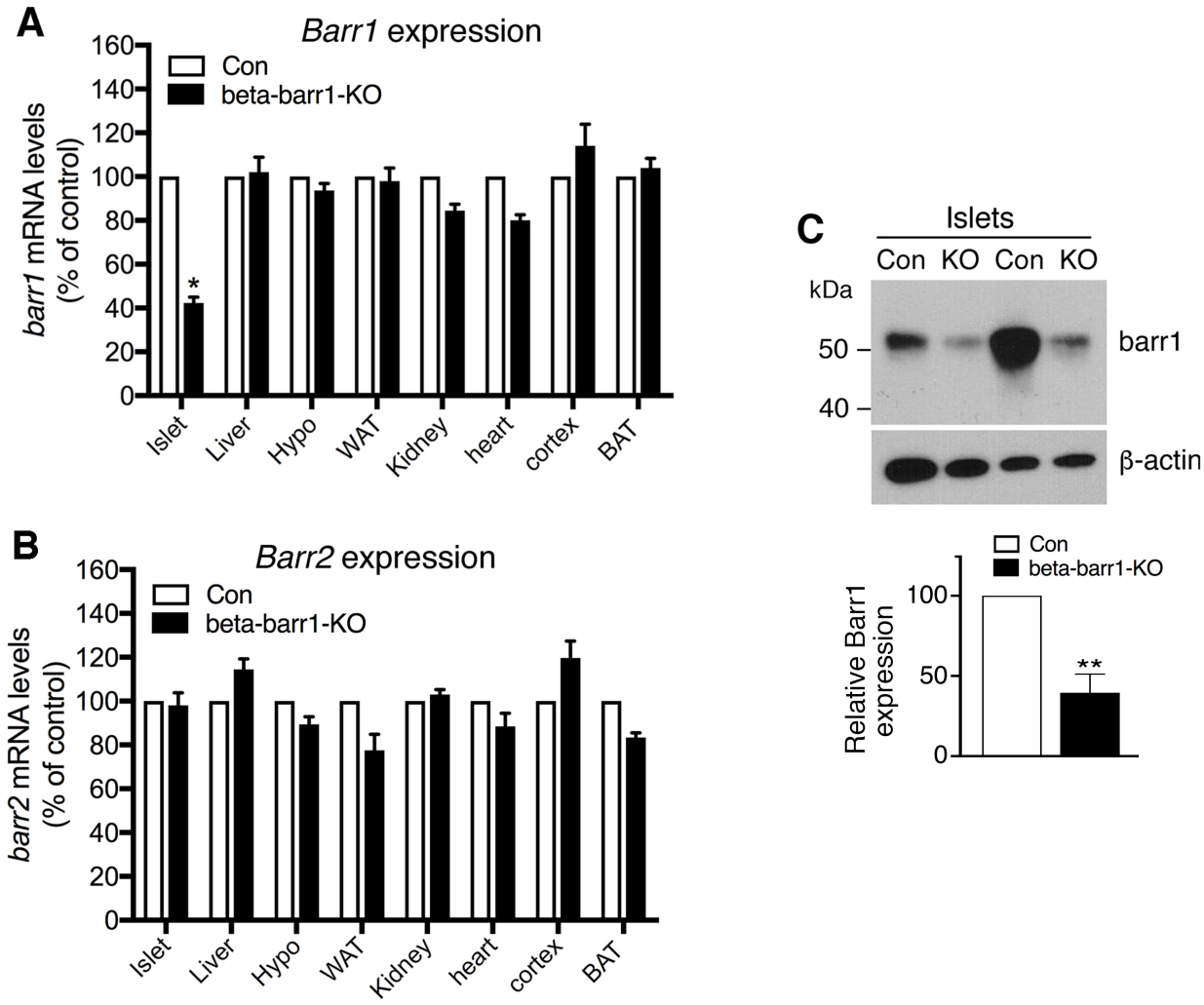
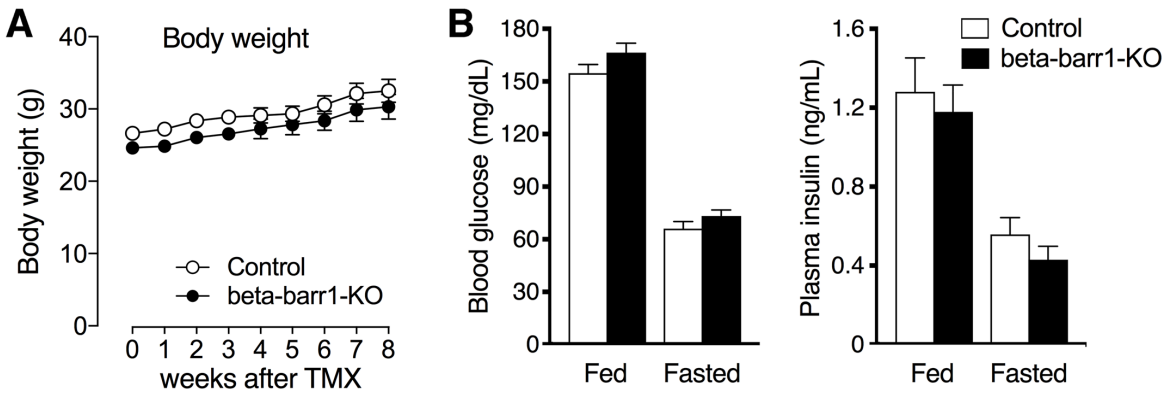
Supplemental Data

β-Cell-intrinsic β-arrestin 1 signaling enhances sulfonylurea-induced insulin secretion

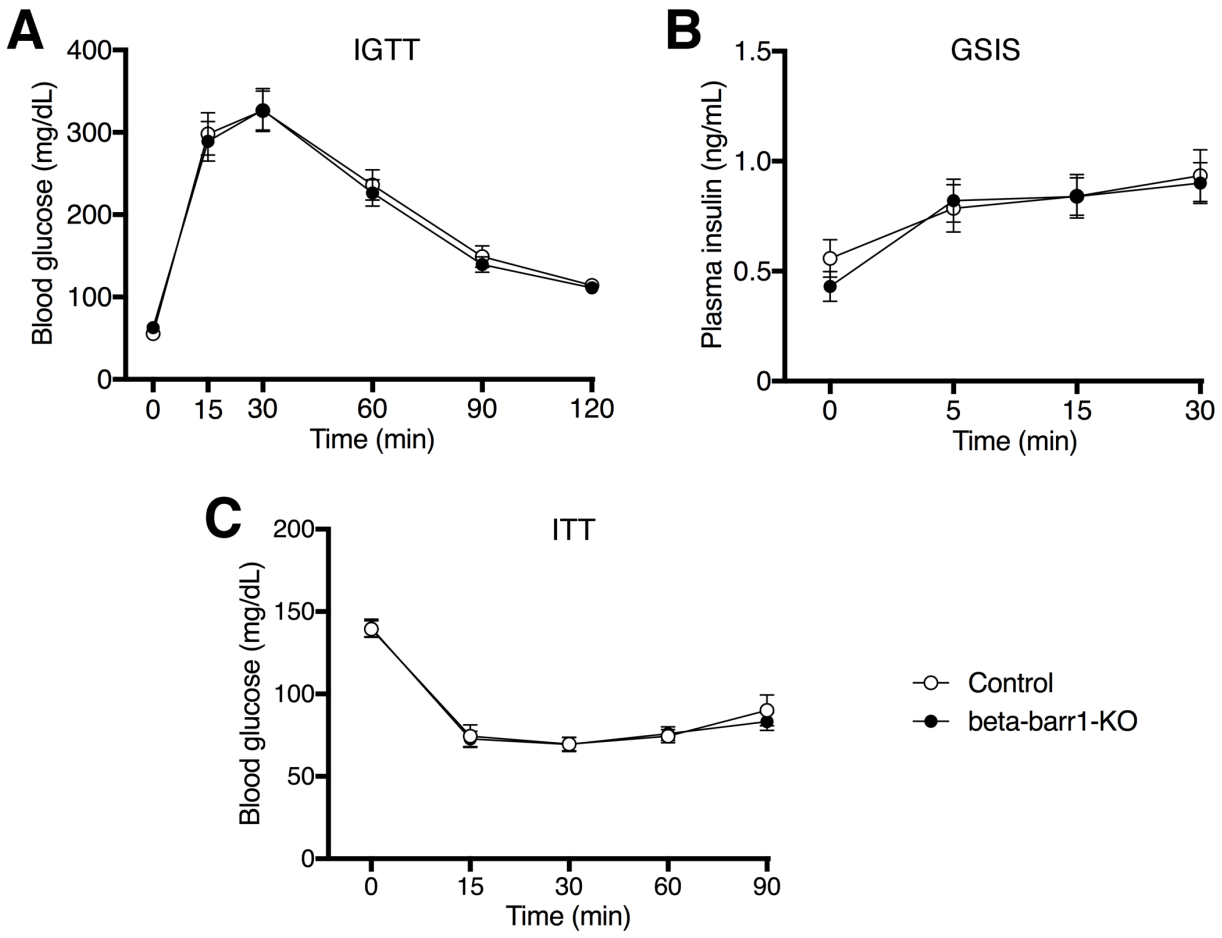
Luiz F. Barella, Mario Rossi, Lu Zhu, Yinghong Cui, Fang C. Mei, Xiaodong Cheng, Wei Chen, Vsevolod V. Gurevich, and Jürgen Wess



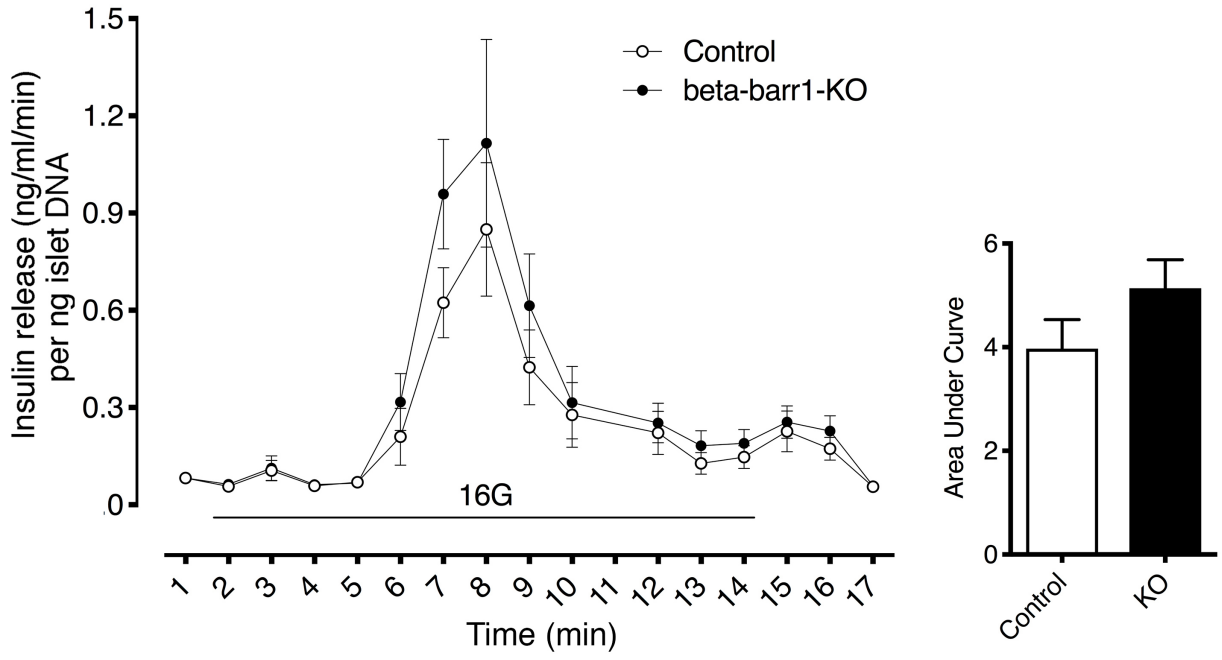
Supplemental Figure 1. Selective reduction of *Barr1* gene and Barr1 protein expression in pancreatic islets of beta-barr1-KO mice. (A, B) qRT-PCR studies. Tissues were harvested from control and beta-barr1-KO mice, and total RNA was isolated as described in Supplemental Methods. Only RNA extracted from the mutant islets showed a significant decrease in *Barr1* mRNA levels. *Barr2* mRNA levels were similar in tissues from control and mutant mice. Con, control; Hypo, hypothalamus; WAT, white adipose tissue; BAT, brown adipose tissue. **(C)** Western blotting studies. Lysates from pancreatic islets derived from control (Con) and beta-barr1-KO (KO) mice were subjected to Western blotting studies as described in Supplemental Methods. Blots were probed with anti-Barr1 and anti-β-actin antibodies. A representative blot is shown. The bar diagram shows relative Barr1 expression levels normalized to β-actin expression (control=100%; 3 mice per group). For all experiments, adult male mice were used. Data shown in (A, B) are means ± SEM of three independent experiments. **P*<0.05, ***P*<0.01, as compared to control (two-tailed Student's t-test).



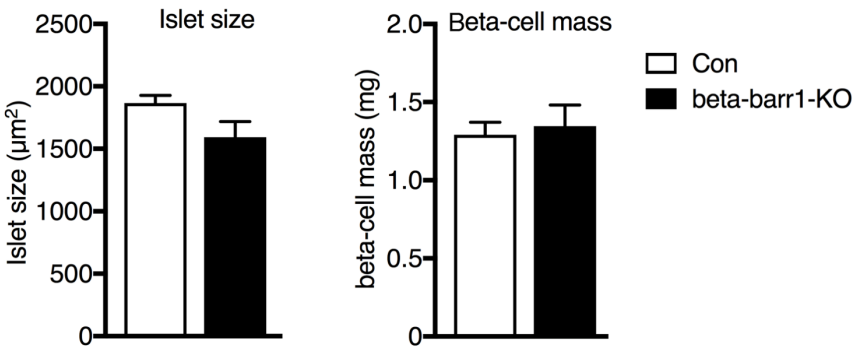
Supplemental Figure 2. Body weight and blood glucose and plasma insulin levels are unchanged in beta-barr1-KO mice. (A) Body weight gain of control and beta-barr1-KO mice (males). TMX, tamoxifen. **(B)** Blood glucose and plasma insulin levels in freely fed or fasted (14-16 overnight fast) mice. Measurements were performed with male littermates that were at least 10 weeks old. Data are means ± SEM (8-13 mice per group).



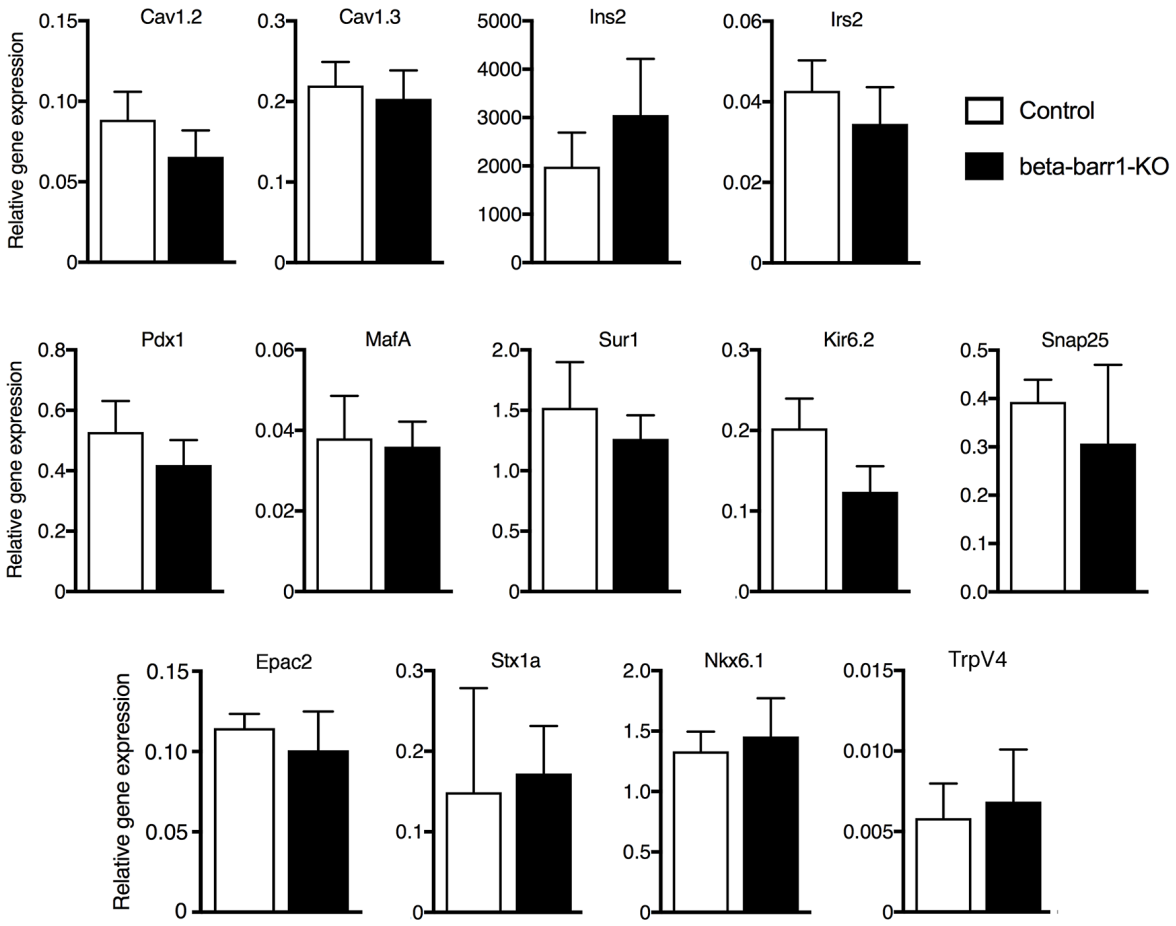
Supplemental Figure 3. ***Barr1*** **deletion in beta-cells has no significant effect on glucose tolerance, glucose-stimulated insulin secretion, and insulin tolerance** ***in vivo*****. (A)** I.p. glucose tolerance test (IGTT). After an overnight fast, mice were injected with glucose (2 g/kg i.p.). **(B)** Glucose-stimulated insulin secretion (GSIS). Mice that had been fasted overnight were injected with glucose (2 g/kg i.p.), followed by the measurement of plasma insulin levels at the indicated time points. **(C)** Insulin tolerance test (ITT). Mice were fasted for 4 hr and then injected with insulin (1 U/kg i.p.). Blood glucose levels were measured at the indicated time points. All experiments were carried out with male littermates (age: 10-14 weeks). Data are means ± SEM (8-14 mice per group).



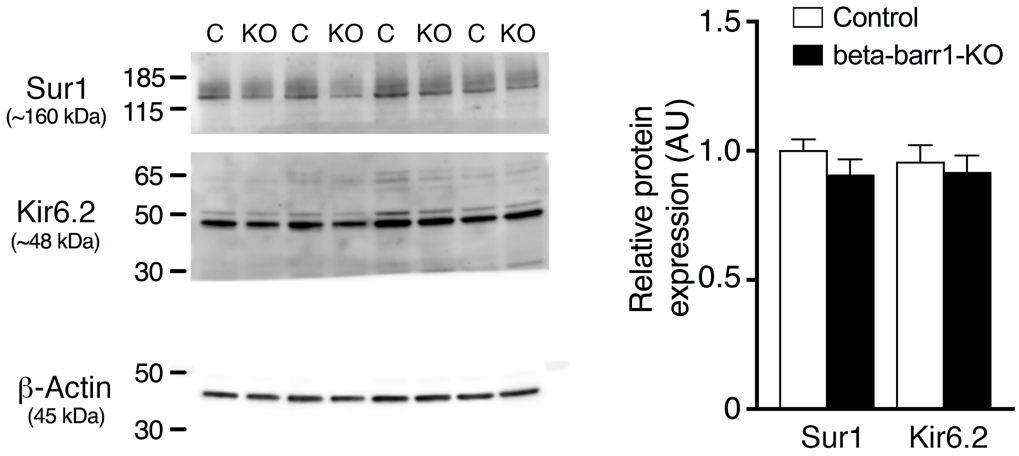
Supplemental Figure 4. Glucose triggers similar insulin responses in beta-barr1-KO and control islets. Following a 1 hr incubation period with low glucose (3 mM), islets were perifused with a high concentration of glucose (16 mM; 16G). The amount of secreted insulin was normalized to islet DNA content. All experiments were carried out with islets prepared from male littermates that were 12-15 weeks old. Data are means ± SEM (n=5 perifusions with 100 islets per perifusion chamber; islets were isolated from 5 mice per genotype).



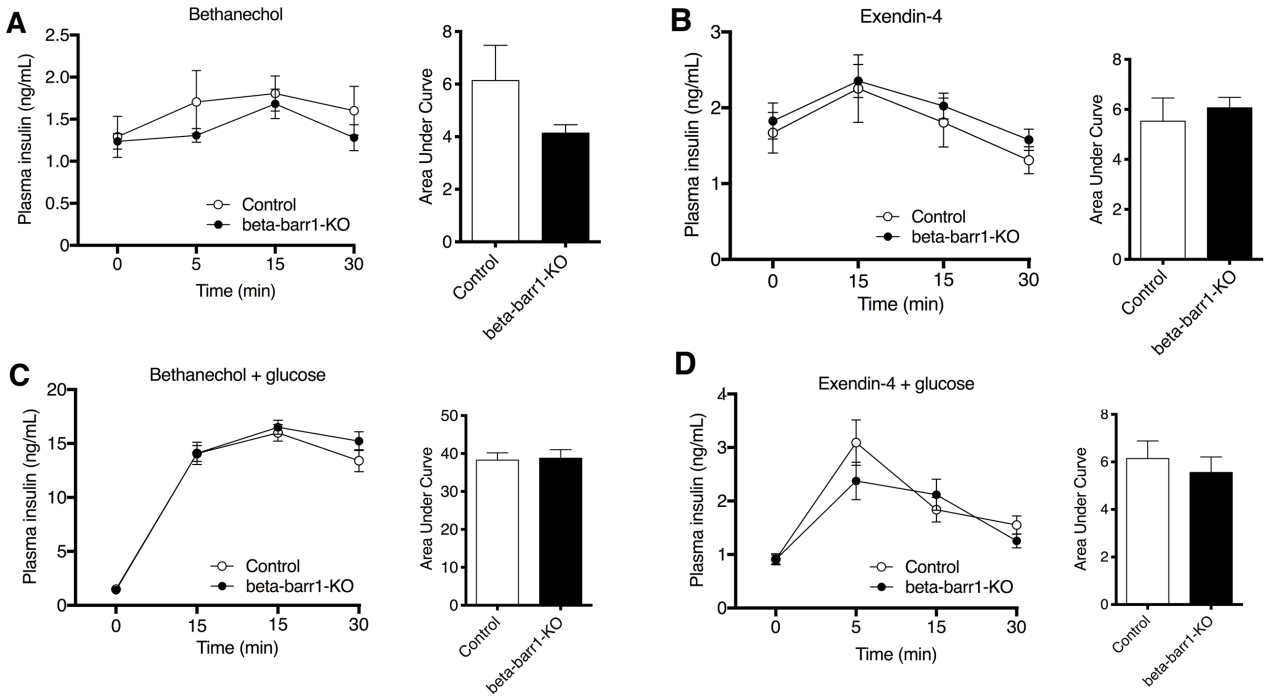
Supplemental Figure 5. Islet size and beta-cell mass are not affected by beta-cell Barr1 deficiency. Islet size and beta-cell mass were similar in control and beta-barr1-KO mice. For details, see Supplemental Methods. All data were collected from male littermates that were 13-14 weeks old. Data are means ± SEM (3 mice per group).



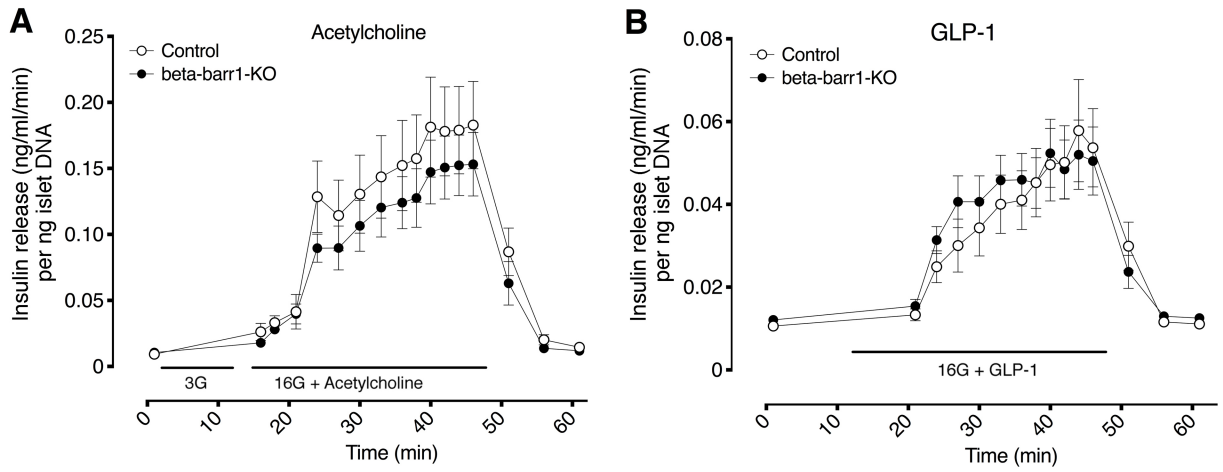
Supplemental Figure 6. Beta-cell gene expression profile is unaltered by beta-cell *Barr1* deletion. The expression levels of several genes involved in the maintenance and function of beta-cells were measured via qRT-PCR. All data were collected from male littermates that were 13-14 weeks old. Data are means ± SEM (3-5 mice per group).



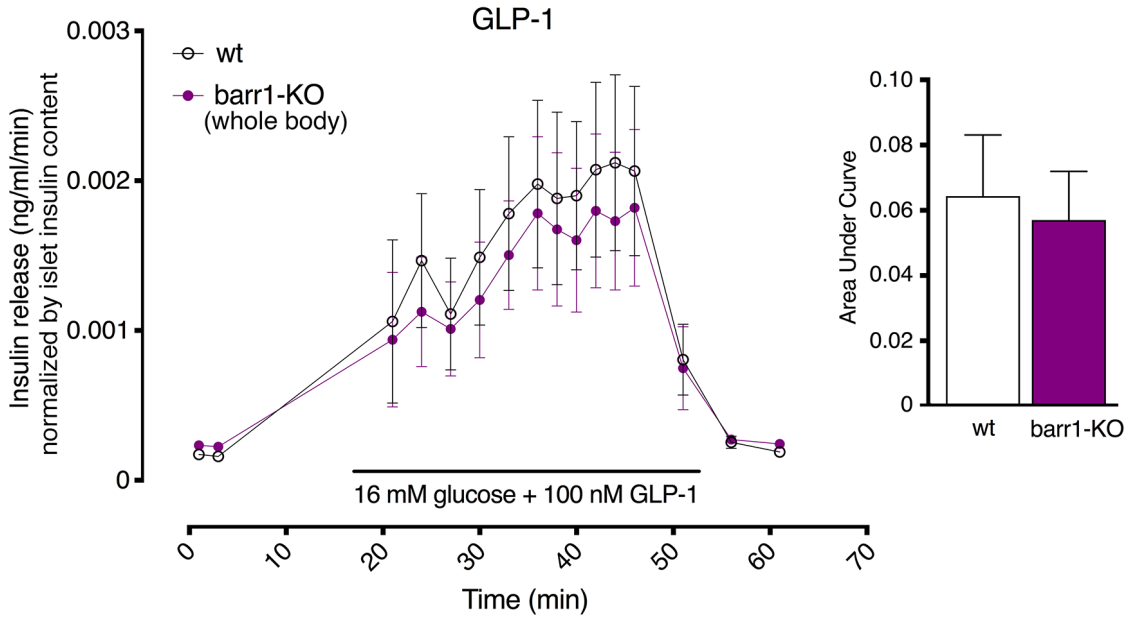
Supplemental Figure 7. Sur1 and Kir6.2 protein expression levels are similar in beta-barr1-KO and control islets. Lysates from pancreatic islets derived from control ('C') and beta-barr1-KO ('KO') mice were subjected to Western blotting studies as described in Supplemental Methods. Blots were probed with anti-Sur1, anti-Kir6.2, and anti-β-actin antibodies. The bar diagram (right panel) shows a quantification of the immunoreactive bands shown in the left panel. Sur1 and Kir6.2 expression levels were normalized to β-actin expression. For all experiments, adult male mice were used. Data shown are means ± SEM of 4 mice per genotype. AU, arbitrary units.



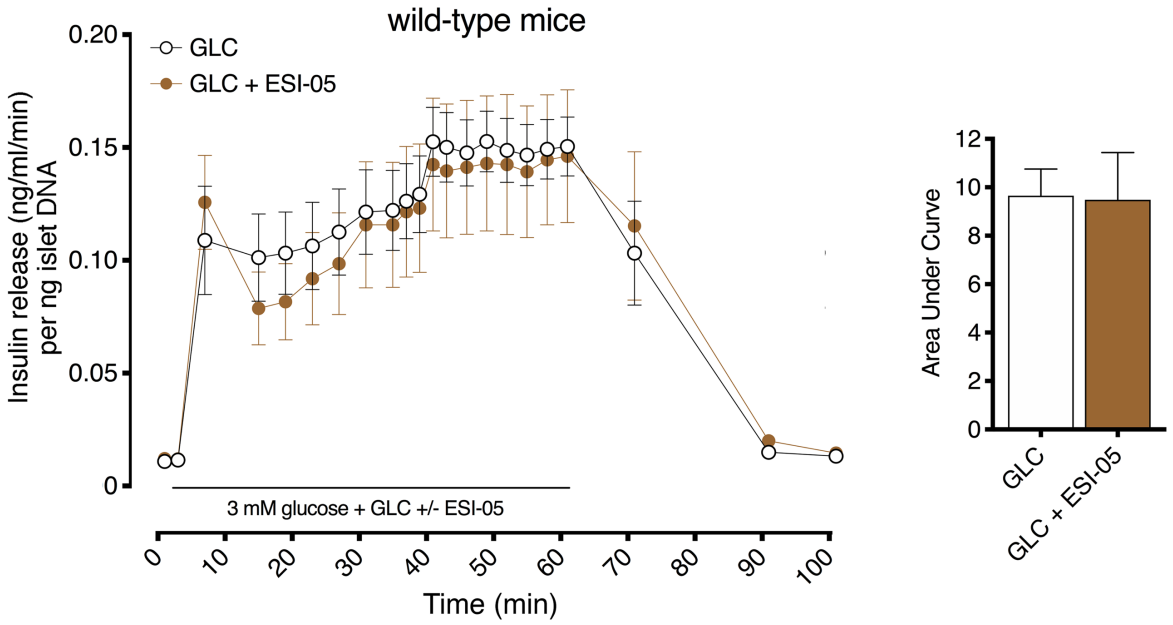
Supplemental Figure 8. Beta-barr1-KO and control mice show similar insulin responses *in vivo* when treated with a muscarinic agonist (bethanechol) or a GLP-1 receptor agonist (exendin-4). Freely fed mice were injected with bethanechol (2 mg/kg s.c.) **(A)** or exendin-4 (12 nmoles/kg i.p.) **(B)**. For i.p. co-injection experiments, mice were fasted for 4 hr and then injected with bethanechol (10 mg/kg i.p.) **(C**) or exendin-4 (12 nmoles/kg i.p.) **(D)**, together with 2 g/kg glucose. Plasma insulin levels were measured at the indicated time points. All experiments were carried out with male littermates that were 10-14 weeks old. Data are given as means ± SEM (A-C: n=7 or 8 per group; D: n=16 per group).



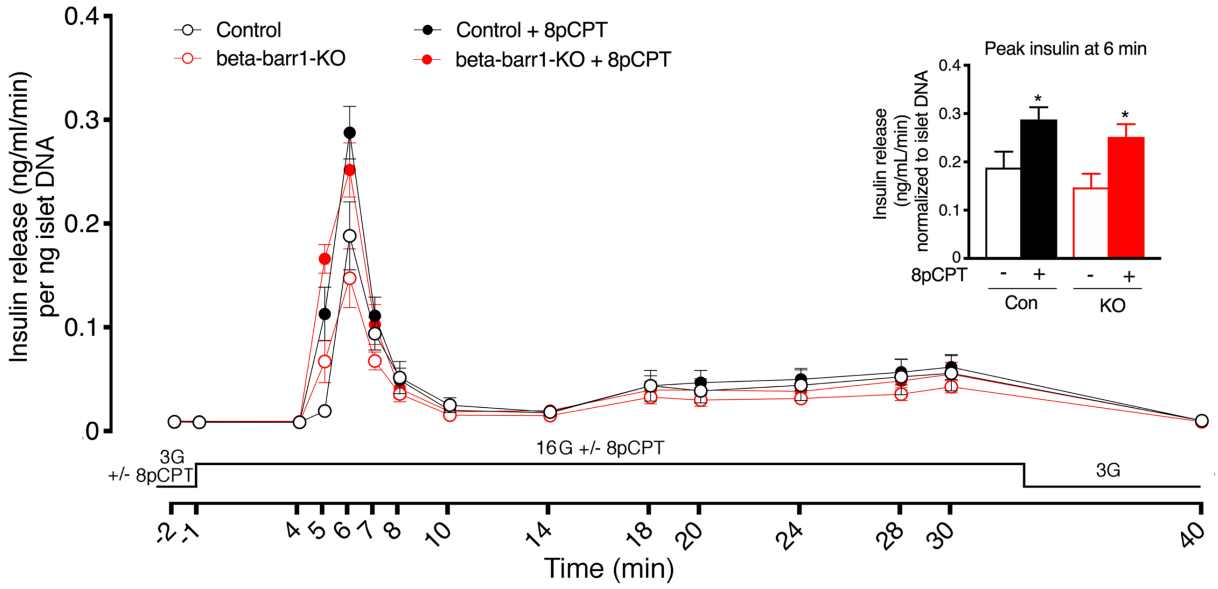
Supplemental Figure 9. Acetylcholine and GLP-1 stimulate insulin secretion in a similar fashion in beta-barr1-KO and control islets. (A, B) Treatment of perifused islets with two different GPCR agonists, acetylcholine and GLP-1. After a 1 hr incubation period with low glucose (3 mM), islets were perifused with high glucose (16 mM; 16G) plus acetylcholine (0.5 μM) (A) or GLP-1 (0.1 μM) (B). To prevent enzymatic degradation of acetylcholine and GLP-1, neostigmine (10 μM) and a DPP-4 inhibitor (1 μM of vildagliptin), respectively, were added to the perfusion fluid. The amount of secreted insulin was normalized to islet DNA content. All experiments were carried out with islets from male littermates that were 12-15 weeks old. Data are presented as means ± SEM (5-9 perifusions with 50 islets per perifusion chamber; islets were isolated from 6 or 7 mice per genotype).



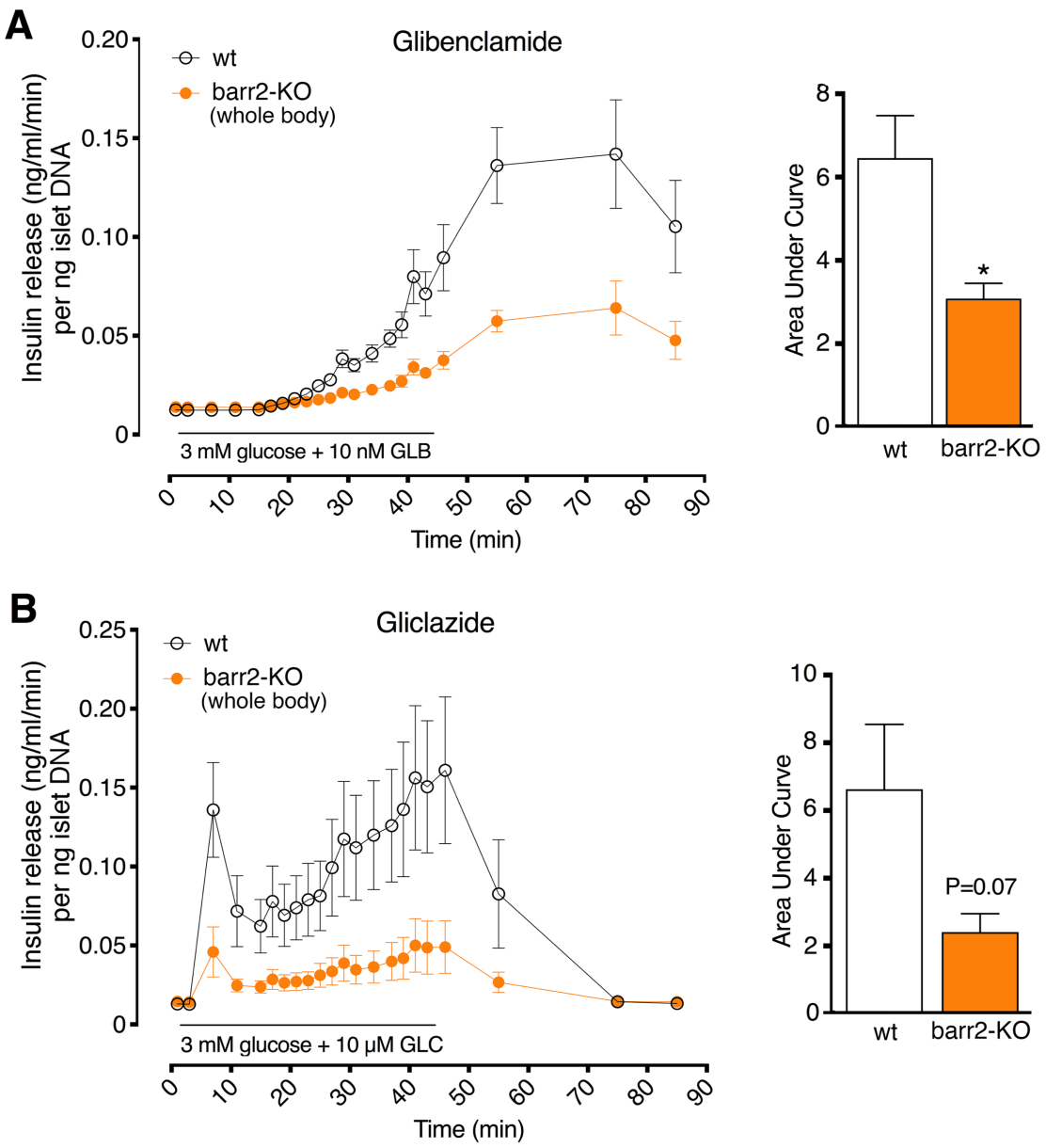
Supplemental Figure 10. GLP-1-stimulated insulin secretion remains unaffected by the lack of Barr1 in islets from whole body Barr1-KO mice. Treatment of perifused islets prepared from wild-type (wt) or whole body Barr1-KO mice with GLP-1. After a 1 hr incubation period with low glucose (3 mM), islets were perifused with high glucose (16 mM) plus GLP-1 (100 nM). To prevent enzymatic degradation of GLP-1, a DPP-4 inhibitor (1 μM vildagliptin) was added to the perifusion fluid. Since the whole body Barr1-KO islets show decreased total insulin content, the amount of secreted insulin was normalized to islet insulin content. The bar diagram to the right provides a summary of all perifusion experiments. All experiments were carried out with islets from male littermates that were 12-15 weeks old. Data are presented as means ± SEM (8 or 9 perifusions with 50 islets per perifusion chamber; islets were isolated from 6 mice per genotype).



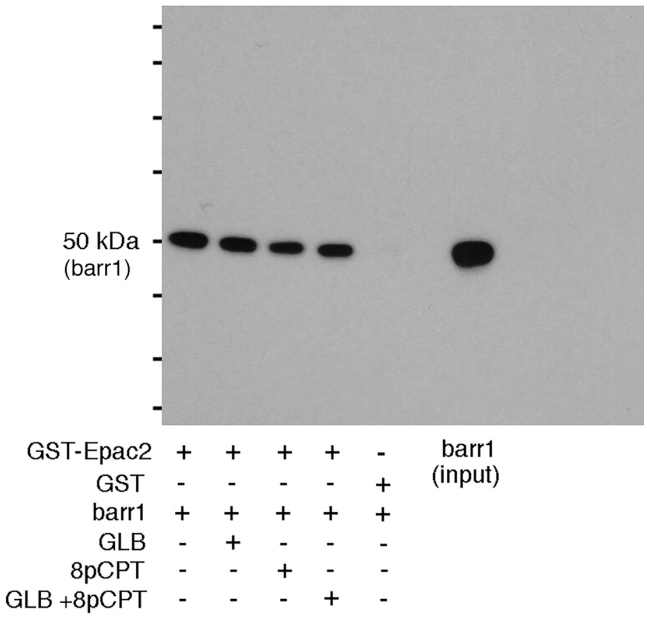
Supplemental Figure 11. Gliclazide-induced insulin secretion remains unaffected by ESI-05 in wild-type islets. Islets from wild-type mice were perifused with 3 mM glucose and 10 μM gliclazide (GLC), either in the absence or presence of ESI-05 (10 μM), a selective Epac2 inhibitor. The amount of secreted insulin was normalized to islet DNA content. The bar diagram to the right provides a summary of all perifusion experiments. All experiments were carried out with male littermates that were 14-17 weeks old. Data are means ± SEM (8 perifusions with 50 islets per perifusion chamber; islets were isolated from 6 wild-type mice).



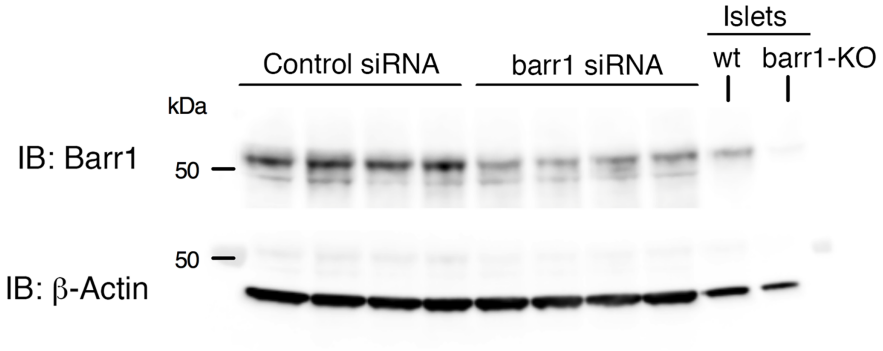
Supplemental Figure 12. The Epac agonist 8pCPT causes similar insulin secretion responses in beta-barr1-KO and control islets. Following a 1 hr incubation period with low glucose (3 mM), islets were perifused with a high concentration of glucose (16 mM; 16G), either in the absence or presence of 8pCPT (5 μM). The inset shows changes in peak insulin secretion measured 6 min after the start of the 16G perifusion period. The amount of secreted insulin was normalized to islet DNA content. All experiments were carried out with islets prepared from male littermates that were 12-15 weeks old. Data are means ± SEM (n=3 perifusions with 50 islets per perifusion chamber; islets were isolated from 4 mice per genotype). **P*<0.05, as compared to no drug (two-tailed Student's t-test).



Supplemental Figure 13. Pancreatic islets from whole body Barr2-KO show impaired insulin secretion in response to glibenclamide and gliclazide. (A, B) Islets from wild-type (wt) and whole body Barr2-KO mice were perifused with 3 mM glucose in the presence of glibenclamide (10 nM; GLB) (A) or gliclazide (10 µM; GLC) (B). The amount of secreted insulin was normalized to islet DNA content. The bar diagrams to the right provide summaries of all perifusion experiments. All islets were prepared from male littermates that were 12-15 weeks old. Data are given as means ± SEM (4 perifusions with 50 islets per perifusion chamber; islets were isolated from 6 mice per genotype). **P*<0.05 (two-tailed Student's t-test).



Supplemental Figure 14. Purified Barr1 interacts with purified Epac2 in a pulldown assay. A GST-Epac2 fusion protein (5 μg) or GST alone (negative control; 5 μg) were immobilized to a glutathione affinity resin. The immobilized proteins were then incubated with purified Barr1 (5 μg), followed by the elution of bound proteins with glutathione buffer. Eluates were subjected to Western blotting using an anti-Barr1 antibody. Note that purified Barr1 was able to bind to GST-Epac2 but not to GST. The addition of glibenclamide (GLB; 100 nM), 8-pCPT (1 μM), or a mixture of GLB (100 nM) and 8-pCPT (1 μM) had no significant effect on the intensity of the Barr1 immunoreactive bands. The blot shown is representative of three independent experiments.



Supplemental Figure 15. Barr1 protein expression levels are reduced after treatment of MIN6-K8 cells with *Barr1* siRNA. Lysates from MIN6-K8 cells treated with either scrambled control siRNA or *Barr1* siRNA were subjected to Western blotting studies as described in Supplemental Methods. Blots were probed with anti-Barr1 and anti-β-actin antibodies. Please note that transfection with *Barr1* siRNA leads to a pronounced decrease in Barr1 protein levels. Pancreatic islets isolated from wild-type (wt) and whole body Barr1-KO mice were used as positive and negative controls, respectively. Data are given means ± SEM (n=4).

Supplemental Methods

Reagents

Sources of common chemicals, media, and commercial kits are provided in the text below or were from Sigma-Aldrich (St. Louis, MO). Gliclazide was from LKT Labs (St. Paul, MN). 8-pCPT-2'-O-Me-cAMP-AM (8-pCPT; selective Epac agonist) was acquired from Axxora (Farmingdale, NY).

Mouse maintenance

All animal work was approved by the National Institute of Diabetes and Digestive and Kidney Diseases/NIH Animal Care and Use Committee. Mice were fed ad libitum, kept on a 12-hr light/dark cycle, and maintained at room temperature (23 ºC). Mice consumed a standard chow (7022 NIH-07 diet, 15% kcal fat, energy density 3.1 kcal/g, Envigo Inc.).

Culture of MIN6-K8 cells

MIN6-K8 mouse insulinoma cells (1) were kindly provided by Dr. Susumu Seino (Kobe University, Japan). Cell were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum and maintained in a humidified incubator with 95% air and 5% CO_2 at 37 ºC. For *Barr1* knockdown studies, ~1.5 x 10^6 cells were electroporated using a Nucleofector™ kit (VCA-1002, Lonza) and 100 pmoles of mouse *Barr1* siRNA or scrambled (control) siRNA (SMARTpool siRNA; Dharmacon), according to the manufacturer's instructions. Assays were conducted about 48 hr after electroporation. In order to overexpress Barr1 and Epac2 in MIN6-K8 cells, we infected cells for 24 hr with adenoviruses (50 MOI each) coding for human Barr1 (Vector Biolabs, cat. no. 1529) and a FLAG-tagged version of mouse Epac2 (2).

Intraperitoneal glucose tolerance test (IGTT)

After an overnight (14-16 hr) fast, mice were injected i.p. with normal saline containing 2 g/kg glucose. Blood glucose levels were measured with a Bayer Contour glucometer using blood collected from the tail vein before and at the indicated time points after glucose injection.

Insulin tolerance test (ITT)

Starting at 10 a.m., mice were first fasted for 4 hr and then injected i.p. with human insulin (1 U/kg; Humulin R, Eli Lilly). Blood glucose levels were measured before and at the indicated time points after insulin injection.

In vivo insulin secretion tests

Following an overnight (14-16 hr) fast, mice were injected i.p. with normal saline containing 2 g/kg glucose. In other *in vivo* insulin secretion tests, freely fed mice were injected at 9 a.m. with glibenclamide (5 mg/kg i.p.), tolbutamide (25 mg/kg i.p.), gliclazide 10 mg/kg i.p.), bethanechol (2 mg/kg s.c.), or exendin-4 (12 nmoles/kg i.p.). Moreover, mice that had been fasted for 4 hr were injected i.p. with bethanechol (10 mg/kg) or exendin-4 (12 nmoles/kg) in presence of 2 g/kg glucose. Plasma insulin levels were determined using a commercial ELISA kit (Crystal Chem).

Perifusion studies with isolated islets

Islets were isolated as previously described (3). An automated perifusion system was utilized to dynamically measure insulin secretion from pancreatic islets (Biorep Perifusion System, Miami, FL). A peristaltic pump pushed HEPES-buffered solution (composition in mM: 125 NaCl, 5.9 KCl, 2.56 $CaCl_2$, 1.2 $MgCl_2$, 25 HEPES, and 0.1% BSA [pH 7.4]; perifusion rate: 100 μl/min) through a sample container with the islets immobilized in Bio-Gel P-4 Gel (Bio-Rad, Hercules, CA). The concentrations of the various agents used to induce insulin secretion are given in the text and/or figure legends. Stimuli were applied with the HEPES-buffered solution, and the perfusate was collected every min in a 96-well plate for further analysis. The chambers containing the islets were kept at 37 ^{o}C, and the perfusate in the collecting plate was kept at <4 ^{o}C. Insulin concentrations in the perfusates were determined with an ultrasensitive mouse insulin ELISA kit (Mercodia, Winston Salem, NC).

Western blotting studies

Standard methods were used to carry out immunoblotting studies, as described in detail elsewhere (3). Primary and secondary antibodies were diluted 1:1,000 and 1:3,000, respectively (for antibody details, see Supplemental Table S1). Bands were detected using an Azure C600 gel imaging system (Azure Biosciences; Dublin, CA) and quantified using ImageJ software (National Institutes of Health, Bethesda, MD).

GST pull-down assay using purified Barr1 and GST-tagged Epac2

We used purified bovine Barr1 (4), GST-tagged Epac2 (murine) (5), and GST (Pierce, Thermo Scientific, Grand Island, NY) along with a GST protein interaction pull-down kit (Thermo Scientific), following the manufacturer's instructions. In brief, the GST-Epac2 fusion protein (5 μg) or GST alone (5 μg) were immobilized to a glutathione affinity resin, following the manufacturer's protocol. The immobilized proteins were then incubated with purified Barr1 (5 μg) for 1 hr at 4 °C. Bound proteins were eluted with glutathione (10 mM) elution buffer. Eluates were analyzed by SDS-PAGE/Western blotting.

Co-immunoprecipitation of Barr1/Epac2 complexes in cultured beta-cells

Co-immunoprecipitation (coIP) assays were performed with a Dynabeads™ co-immunoprecipitation kit (Thermo Scientific) using MIN6-K8 cells infected with adenoviruses coding for human Barr1 (Vector Biolabs) and mouse Epac2-FLAG (2) (50 MOI each), following the manufacturer's instructions. MIN6-K8 cells treated with glibenclamide (1 μM) for 30 and 60 min at 37 °C or left untreated were lysed in coIP lysis buffer supplemented with 100 mM NaCl and protease inhibitors (Roche). Briefly, anti-FLAG antibody or IgG was covalently coupled to the epoxy beads provided by the kit. Following this step, beads were incubated for 30 min at 4 °C with MIN6-K8 cell lysates. Protein complexes were eluted in elution buffer containing LDS sample buffer (Thermo Scientific). Samples were analyzed by Western blotting using anti-Barr1 and anti-Epac2 antibodies.

Pull-down assay monitoring the formation of Rap1-GTP

After preincubation with 2.8 mM glucose for 30 min, ~1 x 10^6 MIN6-K8 cells transfected with scrambled control siRNA or *Barr1* siRNA were treated in 6-well plates for 30 min with HEPES-KRB buffer containing 2.8 mM glucose in the absence or presence of 100 nM glibenclamide. Subsequently, cell lysates were incubated with GST-RalGDS-RID fusion protein that is able to bind Rap1-GTP, the activated form or Rap1. The GST fusion protein was then immobilized to a glutathione resin, using the active Rap1 detection kit from Cell Signaling. All steps were carried out according to the manufacturer's instructions. After incubation of the lysates with GST-RalGDS-RID at 4 °C for 60 min, reducing sample buffer was added to each pull-down reaction to elute the affinity-purified protein complexes. The eluted proteins were subjected to SDS-

PAGE, followed by immunoblotting with an anti-Rap1-GTP antibody (Cell Signaling). Bands were detected using an Azure C600 gel imaging system (Azure Biosciences) and quantified using ImageJ software (National Institutes of Health, Bethesda, MD).

Morphometric studies of pancreatic islets

The methods used for islet morphometric studies have been described in detail previously (6). Briefly, pancreata were fixed overnight with 4% paraformaldehyde/phosphate-buffered saline, and embedded in paraffin. Five μm thick pancreatic sections from three distinct levels, 100 μm apart, were mounted on slides and subjected to standard hematoxylin/eosin staining. To determine beta-cell mass and islet size, three distinct sections per pancreas (100 μm apart) were blocked with normal goat serum for 1 hr and incubated overnight at 4 ^{o}C with a guinea pig anti-insulin antibody. The primary antibody was detected with an Alexa Fluor 555 goat anti-guinea secondary antibody. All sections were counterstained with DAPI (Vectashield mounting medium with DAPI, Vector Laboratories) to visualize the nuclei (blue color). Slides were imaged on a Keyence digital microscope (BZ-9000) with a CFI Plan Apo λ 4x lens. Image acquisition and merging were performed using BZ-II Viewer and BZ-II Analyzer software (Keyence). ImageJ was used for the measurement of beta-cell mass, which was calculated as the ratio of islet cross-sectional area to total pancreatic area multiplied by pancreatic weight. Islet size was calculated by dividing islet area by the total number of islets.

Quantification of RNA expression via qRT-PCR

RNA was extracted from different mouse tissues or MIN6-K8 cells using the RNeasy Mini Kit (Qiagen, Germantown, MD), following the manufacturer's instructions. cDNA was prepared using Superscript III first-strand synthesis SuperMix for qRT-PCR (Thermo Scientific). PCR reactions were run in triplicate using SYBR Green Master Mix (Applied Biosystems, Foster City, CA) in a real-time PCR detection system (Bio-Rad, Hercules, CA). Primer sequences are given in Supplemental Table S2. Relative quantification of gene expression was done by using the $2^{-\Delta\Delta Ct}$ method (difference between the threshold cycle (Ct) value of the target gene and the Ct of the *beta-actin* housekeeping gene). The precise experimental conditions used for the qRT-PCR studies were similar to those described by Jain et al. (6).

Supplemental Table S1. Antibodies Used for Immunoblotting (IB), Immunoprecipitation (IP), and Immunofluorescence (IF) Studies

Antibody target	Source	Catalog #	Dilution	Usage
FLAG tag	Sigma-Aldrich	F1804	5 μg/mg of beads	IP
Normal rabbit IgG	Millipore Sigma	12-370	5 μg/mg of beads	IP
Epac2	Cell Signaling	43239	1:1,000	IB
Rap1	Cell Signaling	8825	1:1,000	IB
Beta-actin (D6A8)	Cell Signaling	8457	1:1,000	IB
Beta-arrestin-1 (D803J)	Cell Signaling	12697	1:1,000	IB
Beta-arrestin-1/2 (D24H9)	Cell Signaling	4674	1:1,000	IB
GST	Abcam	Ab19256	1:2,000	IB
Sur1	Novus Biologicals	NBP2-59320	1:200	IB
Kir6.2	Santa Cruz	Sc-390104	1:250	IB
Anti-rabbit IgG, HRP-linked secondary antibody	Cell Signaling	7074	1:3,000	IB
Insulin (guinea pig)	Abcam	Ab7842	1:100	IF
Alexa Fluor 555 goat anti-guinea pig	Invitrogen	A21435	1:500	IF

Supplemental Table S2. Primers Used for qRT-PCR Studies

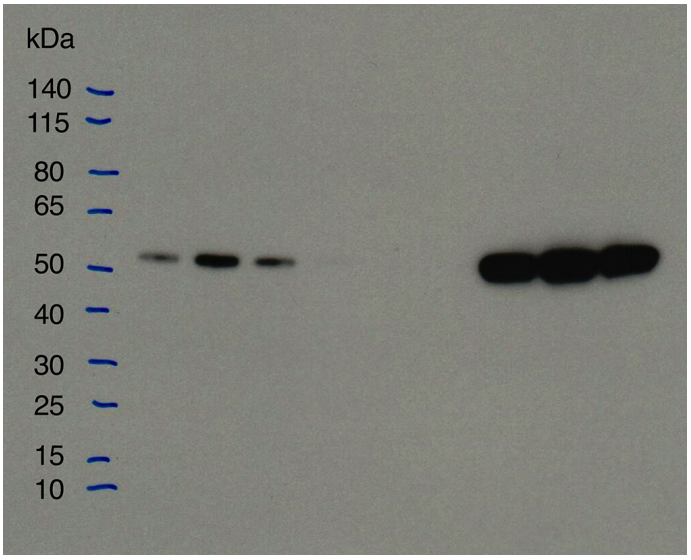
Mouse gene	Primer sequence	Amplicon (bp)
Beta-actin	QuantiTect Primer (Qiagen) Catalog #: QT01136772	77
Barr1 (Arrb1)	Forward: 5' AAGAAGGCAAGCCCCAAT Reverse: 5' CGCAGGTCAGTGTCACGTAG	151
Barr2 (Arrb2)	Forward: 5' GTCTTCAAGAAGTCGAGCCCT Reverse: 5' CACGAACACTTTCCGGTCCT	144
Cav1.2	QuantiTect Primer (Qiagen) Catalog #: QT00150752	115
Cav1.3	QuantiTect Primer (Qiagen) Catalog #: QT00112238	96
Ins2	Forward: 5' CTGGCCCTGCTCTTCCTCTGG Reverse: 5' CTGAAGGTCACCTGCTCCCGG	204
Irs2	Forward: 5' CTGCGTCCTCTCCCAAAGTG Reverse: 5' GGGGTCATGGGCATGTAGC	124
Pdx1	Forward: 5' CCCCAGTTTACAAGCTCGCT Reverse: 5' CTCGGTTCCATTCGGGAAAGG	177
MafA	Forward: 5' AGGAGGAGGTCATCCGACTG Reverse: 5' CTTCTCGCTCTCCAGAATGTG	113
Sur1 (Abcc8)	QuantiTect Primer (Qiagen) Catalog #: QT01042300	77
Kir6.2 (Kcnj11)	QuantiTect Primer (Qiagen) Catalog #: QT00305319	87
Snap25	Forward: 5' CAACTGGAACGCATTGAGGAA Reverse: 5' GGCCACTACTCCATCCTGATTAT	177
Stx1a	Forward: 5' GAGCCAGGGGGAGATGATTGA Reverse: 5' ATCCAAAGATGCCCCCGATG	194
Nkx6.1	Forward: 5' CAGCAAATCTTCGCCCTGGA Reverse: 5' AACCAGACCTTGACCTGACT	116
Trpv4	Forward: 5' ACTGGAACCAGAACTTGGGC Reverse: 5' GAGGACCAACGATCCCTACGA	112
Epac2	Forward: 5' CAAGATGTCTTGGTACTGGAGAAG Reverse: 5' CAGGTGTTCCTGACATCACAGTAT	103

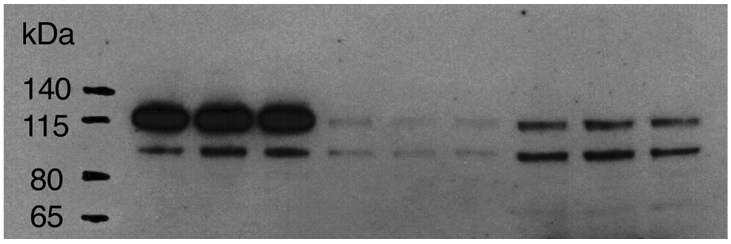
References

1. Iwasaki M, Minami K, Shibasaki T, Miki T, Miyazaki J, and Seino S. Establishment of new clonal pancreatic beta-cell lines (MIN6-K) useful for study of incretin/cyclic adenosine monophosphate signaling. *Journal of diabetes investigation.* 2010;1(4):137-42.
2. Chepurny OG, Bertinetti D, Diskar M, Leech CA, Afshari P, Tsalkova T, et al. Stimulation of proglucagon gene expression by human GPR119 in enteroendocrine L-cell line GLUTag. *Molecular endocrinology (Baltimore, Md).* 2013;27(8):1267-82.
3. Rossi M, Ruiz de Azua I, Barella LF, Sakamoto W, Zhu L, Cui Y, et al. CK2 acts as a potent negative regulator of receptor-mediated insulin release in vitro and in vivo. *Proc Natl Acad Sci U S A.* 2015;112(49):E6818-24.
4. Vishnivetskiy SA, Zhan X, Chen Q, Iverson TM, and Gurevich VV. Arrestin expression in E. coli and purification. *Curr Protoc Pharmacol.* 2014;67:Unit 2.11.1-9.
5. Tsalkova T, Blumenthal DK, Mei FC, White MA, and Cheng X. Mechanism of Epac activation: structural and functional analyses of Epac2 hinge mutants with constitutive and reduced activities. *J Biol Chem.* 2009;284(35):23644-51.
6. Jain S, Ruiz de Azua I, Lu H, White MF, Guettier JM, and Wess J. Chronic activation of a designer G(q)-coupled receptor improves beta cell function. *J Clin Invest.* 2013;123(4):1750-62.

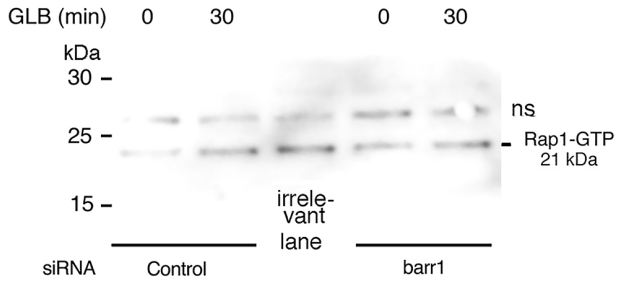
Uncropped blots for Figure 4A

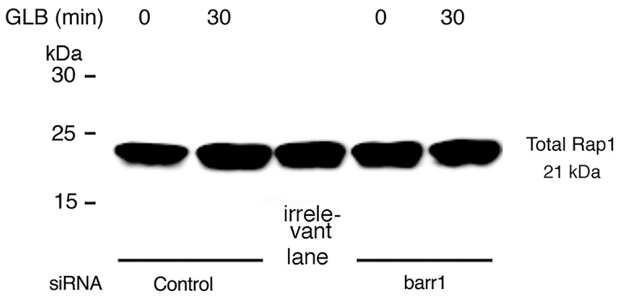
IB: Anti-Barr1





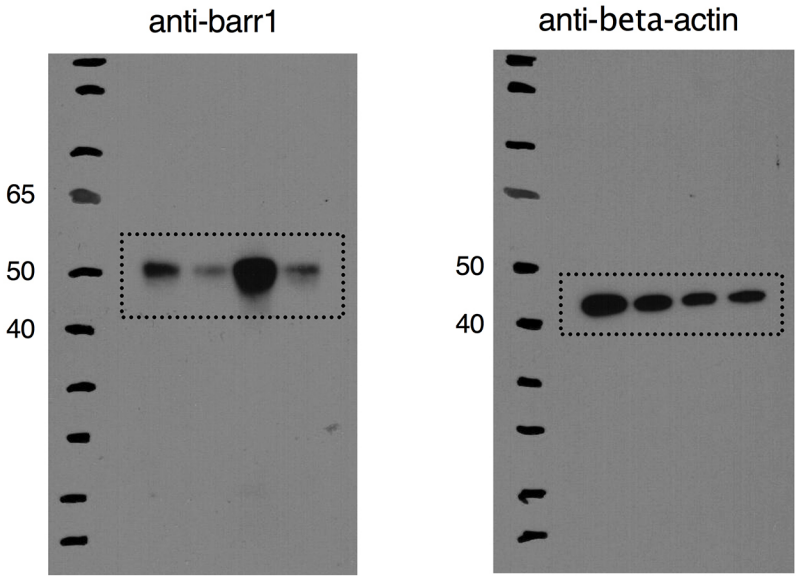
Uncropped blots for Figure 4D



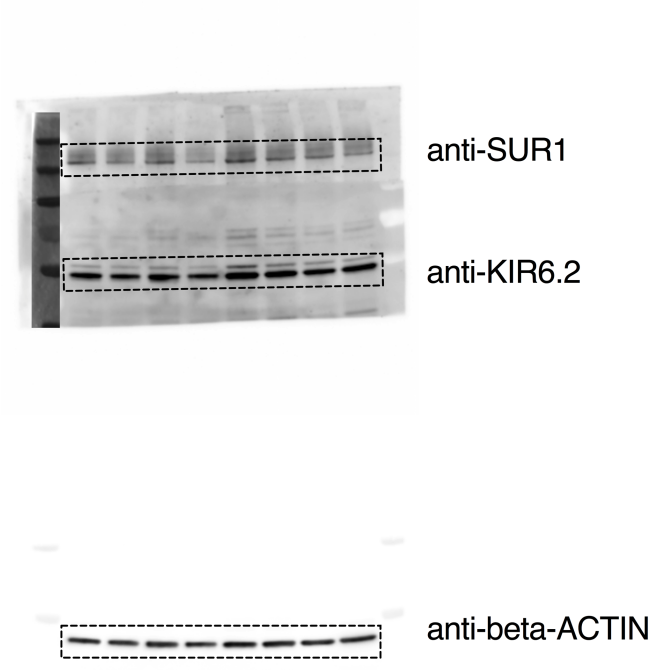


ns = non-specific band

Uncropped blot for Figure S1B

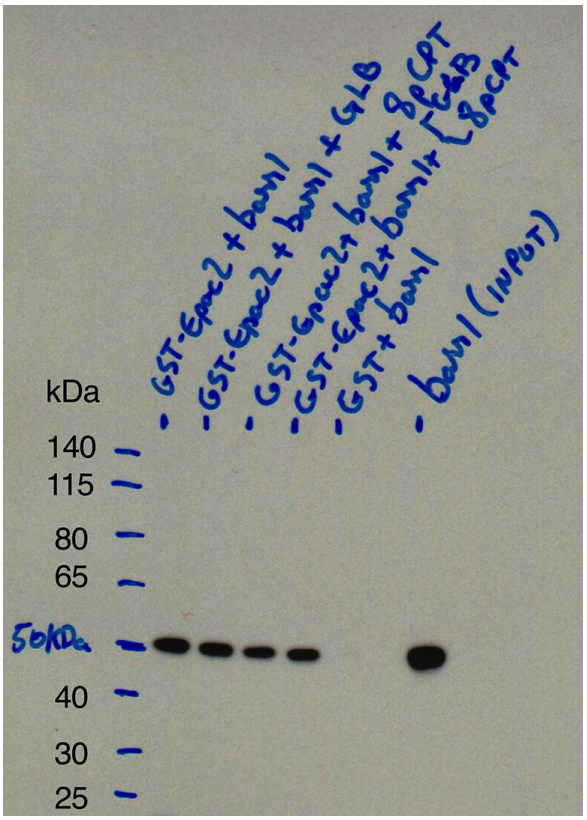


Uncropped blots for Figure S11



Uncropped blots for Figure S14

IB: Anti-Barr1



Uncropped blots for Figure S15