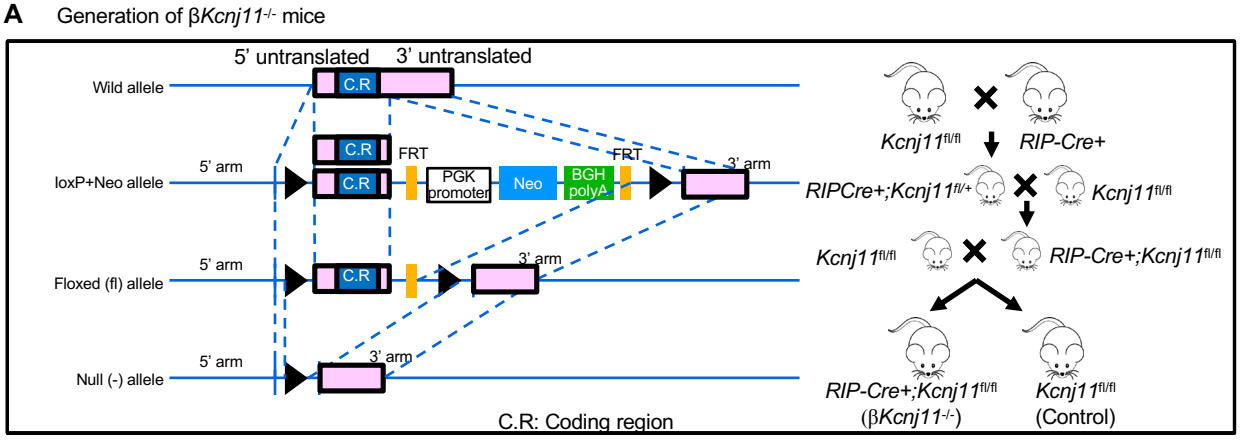
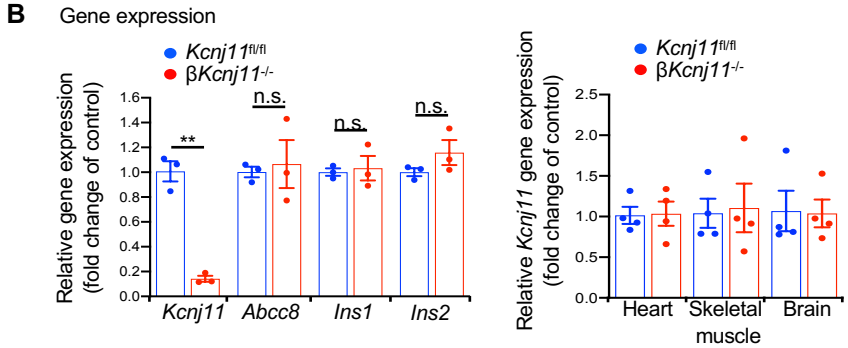
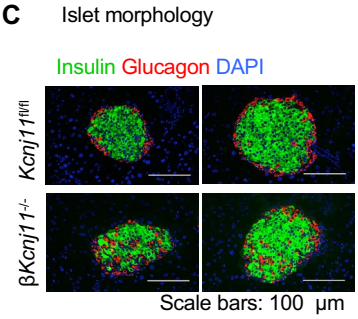
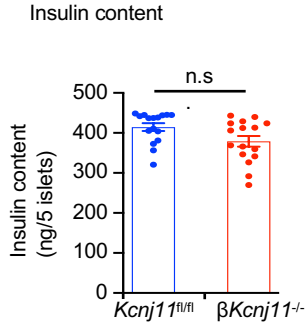
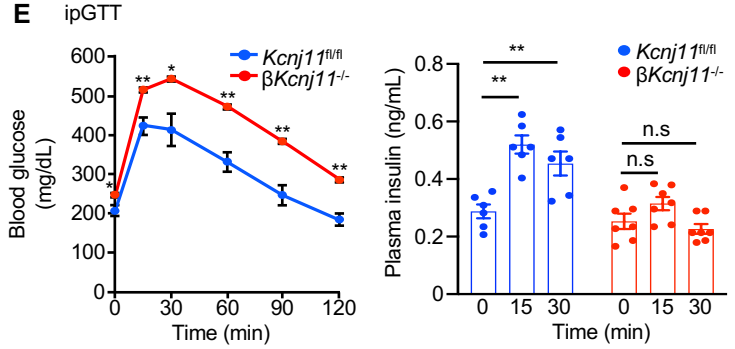
Supplementary Information

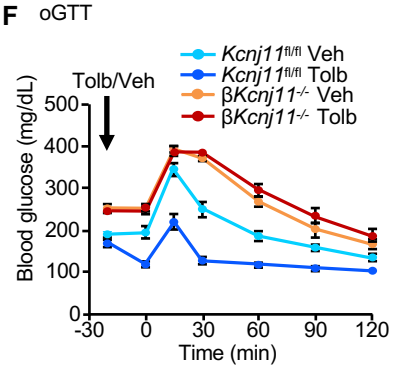


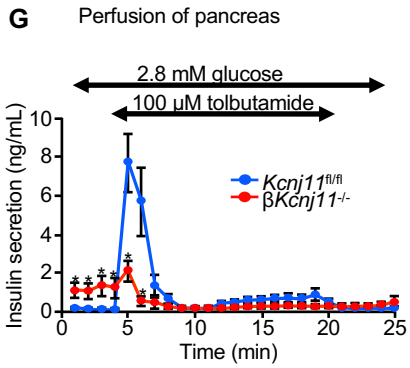


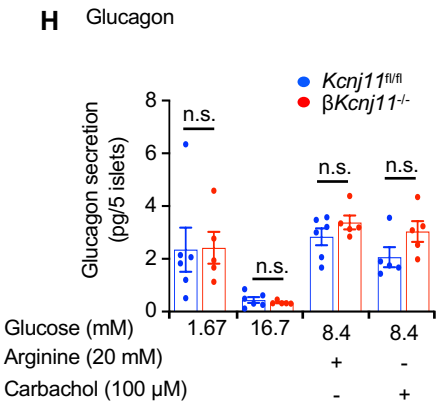












Supplementary Figure 1.

(**A**) Generation of β*Kcnj11*$^{-/-}$ mice. *Left*, Schematic diagram of *Kcnj11* gene targeting. The targeting vector contained phosphoglycerol kinase (PGK) promoter/a neomycin (Neo) resistant selection cassette flanked by flippase recognition target (FRT) sites and loxP sites on either side of the exon of the *Kcnj11* gene. The neomycin resistant (Neo^r) cassette (flanked by FRT sites) was added to the targeting vector for positive selection, and was deleted in mice using FLP-FRT recombination system. Targeted allele, floxed allele after flippase recombination and final allele with the targeted region deleted are shown. *Right*, Production of β*Kcnj11*$^{-/-}$ mice by crossing *Kcnj11*$^{fl/fl}$ mice with RIP-Cre+; *Kcnj11*$^{fl/fl}$ mice.

(**B**) Expressions of *Kcnj11*, *Abcc8*, *Ins1*, and *Ins2* genes in the islets and *Kcnj11* in the brain, heart, and skeletal muscles assessed by qPCR (n = 3 for each). Expression of each gene was normalized by that of *Gapdh* and presented as fold change of control.

(**C**) Immunohistochemistry of the islets. Green, insulin; Red, glucagon.

(**D**) Insulin content of the islets (n = 16 for both groups).

(**E**) Intraperitoneal(ip) glucose tolerance test. *Left*, blood glucose. *Right*, plasma insulin (n = 7 for both groups).

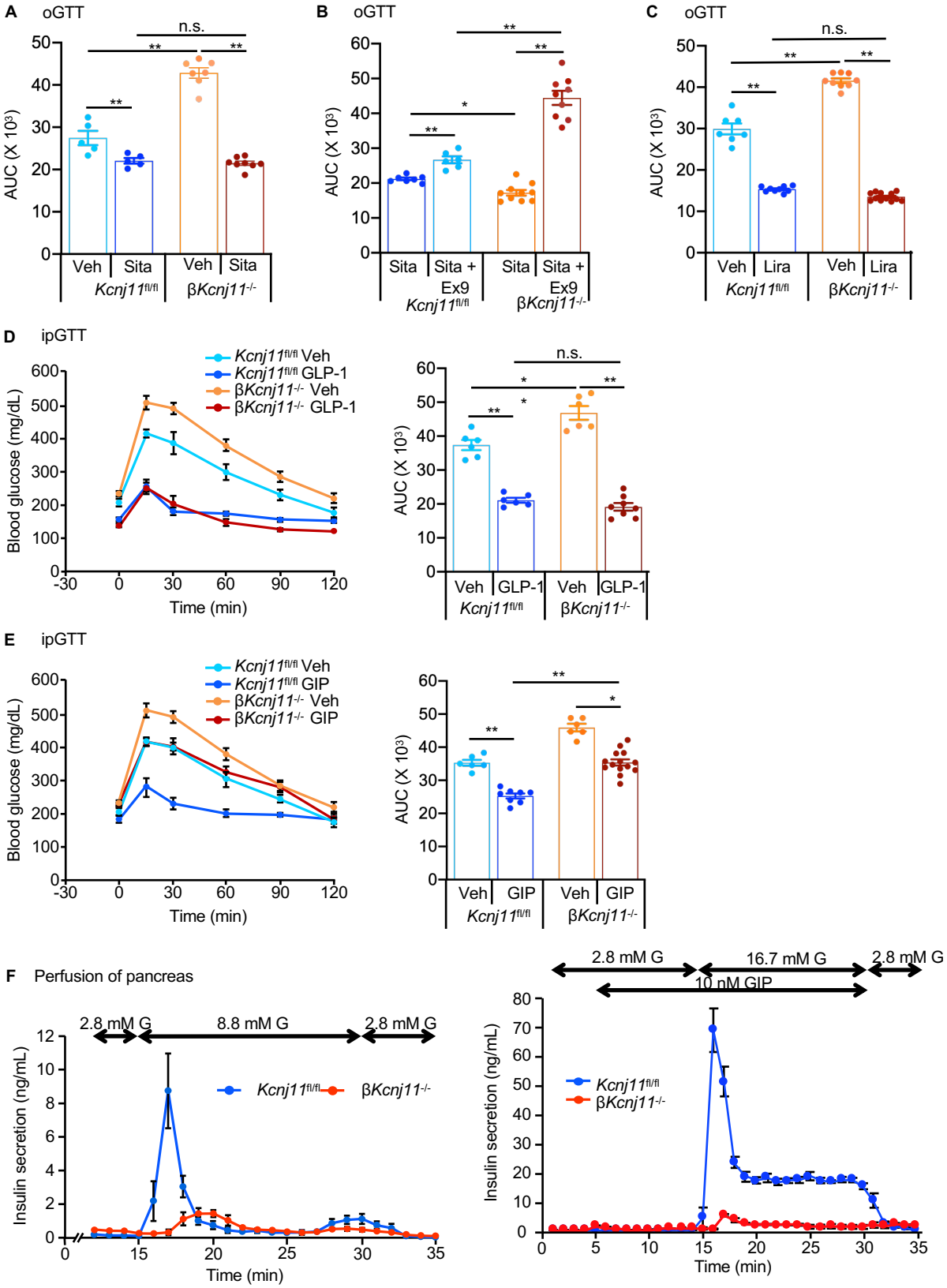
(**F**) oGTT following tolbutamide (Tolb) administration. Tolb (50 mg/kg) was orally administered to 6 h-fasted mice 20 min prior to glucose challenge (1.5 g/kg) (n = 6 per group). Veh, vehicle (0.5% carboxymethyl cellulose).

(**G**) Effects of Tolb on insulin secretion from perfused pancreases in the presence of 2.8 mM glucose. Basal insulin secretion in β*Kcnj11*$^{-/-}$ mice was significantly elevated compared to control (n = 4 for both groups).

(**H**) Glucagon secretion from the islets at high glucose (16.7 mM), low glucose (1.67 mM), and arginine or carbachol at 8.4 mM glucose (n = 5-6 for each condition).

Statistical analyses were performed by 2-tailed Student's unpaired t-test for (**B**), (**D**), and (**H**) or by 2-way ANOVA followed by Dunnett's post hoc test for (**E**), (**F**), and (**G**).

Data represent mean ±SEM. *$p < 0.05$, **$p < 0.01$


A oGTT
AUC (X 10³)
n.s.
Veh
Sita
Kcnj11fl/fl
βKcnj11-/-
B oGTT
Sita
Sita + Ex9
C oGTT
Lira
D ipGTT
Kcnj11fl/fl Veh
Kcnj11fl/fl GLP-1
βKcnj11-/- Veh
βKcnj11-/- GLP-1
Blood glucose (mg/dL)
Time (min)
GLP-1
E ipGTT
Kcnj11fl/fl GIP
βKcnj11-/- GIP
GIP
F Perfusion of pancreas
2.8 mM G
8.8 mM G
16.7 mM G
10 nM GIP
Insulin secretion (ng/mL)

Supplementary Figure 2.

(**A**) AUC during oGTT following sitagliptin (Sita) administration in Figure 2A (n = 5-8 per group).

(**B**) AUC during oGTT following sitagliptin (Sita) and exendin-9 (Ex9) administration in Figure 2D (n = 6-10 per group).

(**C**) AUC during oGTT following liraglutide (Lira) administration in Figure 2E (n = 7-12 per group).

(**D**) ipGTT following exogenous administration of GLP-1 (left) and AUC (right) (n = 6-8 per group).

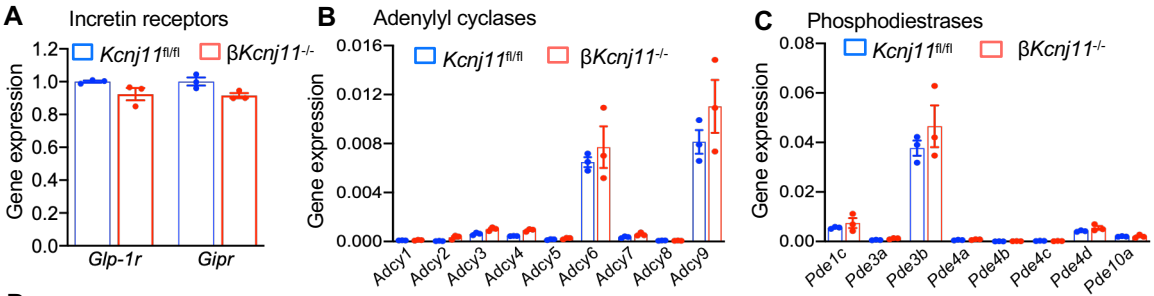
(**E**) ipGTT following exogenous administration of GIP (left) and AUC (right) (n = 6-14 per group).

(**F**) Effect of glucose alone on insulin secretion from perfused pancreases (n = 4 for both groups).

(**G**) Effect of high GIP concentration (10 nM) on insulin secretion from perfused pancreases (n = 4 for both groups).

GLP-1 or GIP (100 μg/kg, each) was intraperitoneally administered to mice 20 min before glucose challenge in (**D**) and (**E**). Veh, vehicle (water); AUC, area under the curve. Mice were fasted overnight before perfusion experiments commenced in (**F**) and (**G**).

Statistical analyses were performed by 2-way ANOVA followed by Tukey's post hoc test. Data represent mean ±SEM. $^{*}p < 0.05$, $^{**}p < 0.01$



D *Kcnj11* gene sequence

Wild-type sequence

TATCCTGAGGAATATGTGCTGACCCGGCTGGCAGAGGACCCTGCAGAGCCCAGGTACCGTACTCGAGAGAGGAGGGCCCGCTTCGTG

Kcnj11$^{-/-}$βCL1 allele 1: -13

TATCCTGAGAAATA ---------- CTGGCAGAGGACCCTGCAGAGCCCAGGTACCGTACTCGAGAGAGGAGGGCCCGCTTCGTG

Kcnj11$^{-/-}$βCL1 allele 2: +37

TATCCTGAGGAATATGTGCTGACCCGGCTGGCAGAGGACCCTGCAGAGCCCAGGTACCGTACTCGAGAGAGGAGGGCCCGCTTCGTG

GTGCTGACCCGGCTGGCAGAGGACCCTGCAGAGCCAG

Kcnj11$^{-/-}$βCL2 allele 1: +80 (-5, +90)

TATCCTGAGGAATATGTGCTGACCCGGCTGGCAGAGGACCCTGCAGAGCCCAGGTACCGT- - - - - CGAGAGGAGGGCCCGCTTCGTG

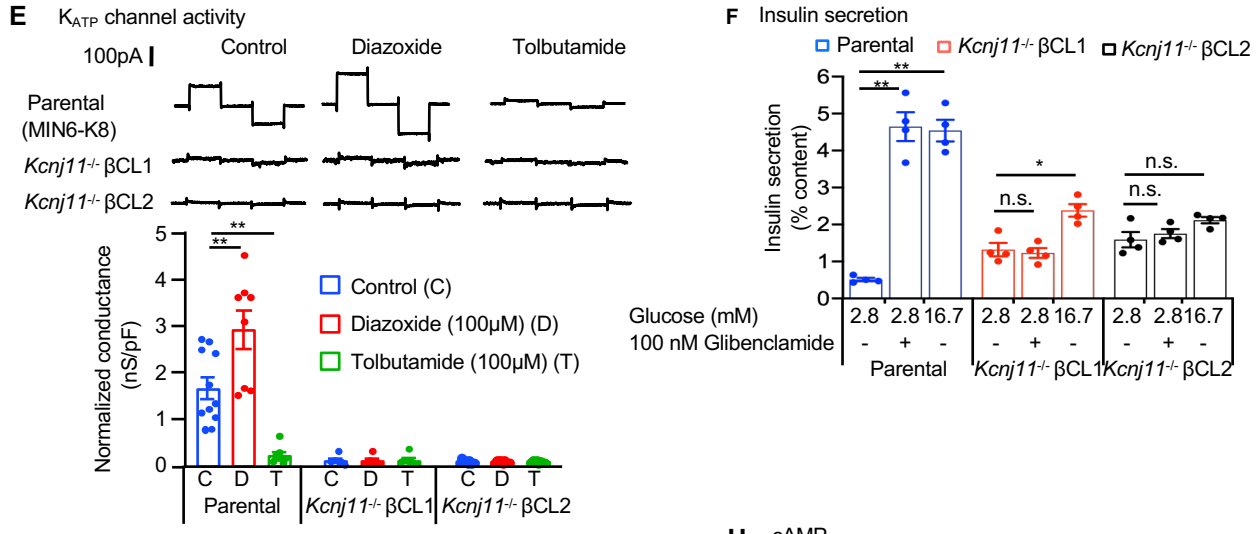
GCTGACCCGGCTGGCAGACCCGGCTGGCAGAGGACCCTGCAGAGCCCAGGTGCTGACCCGGCTGGCAGAGGACCCTGCAGAGCCCAGGT

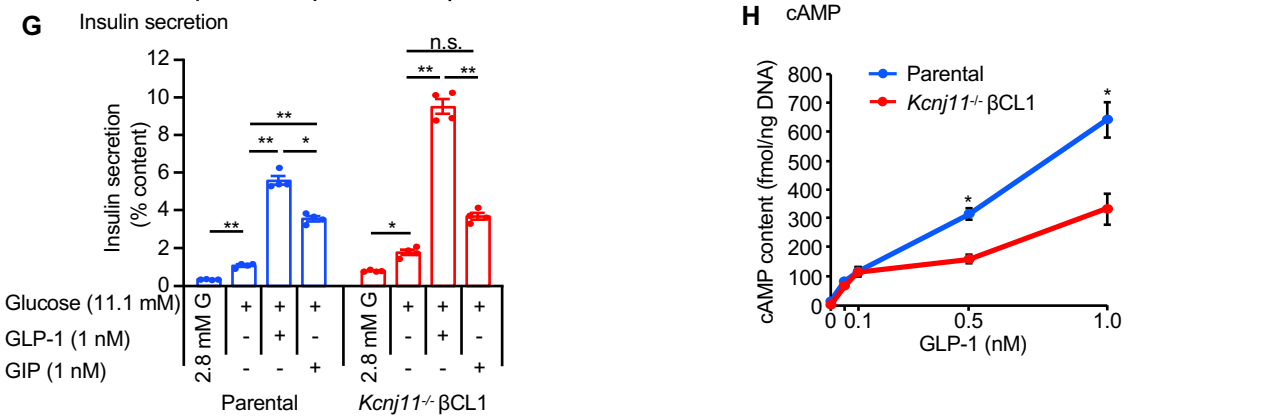
Kcnj11$^{-/-}$βCL2 allele 2: +124 (+168, -44)

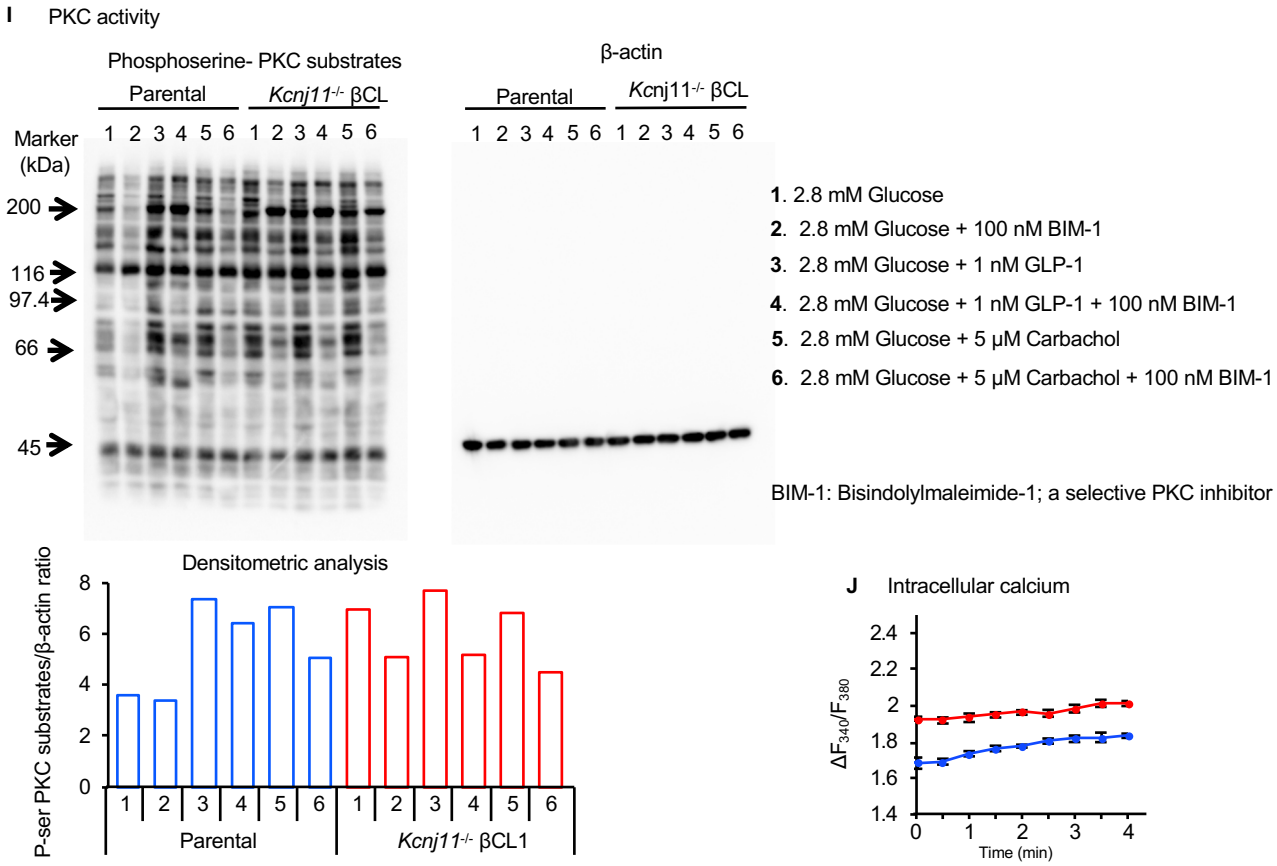
TATCCTGAGGAATATGTGCTGACCCGGCTGGCAGAGGACCCTGCAGAGCCCAGGTACCGTACTCGAGAGAGGAGGGCCCGCTTCGTG

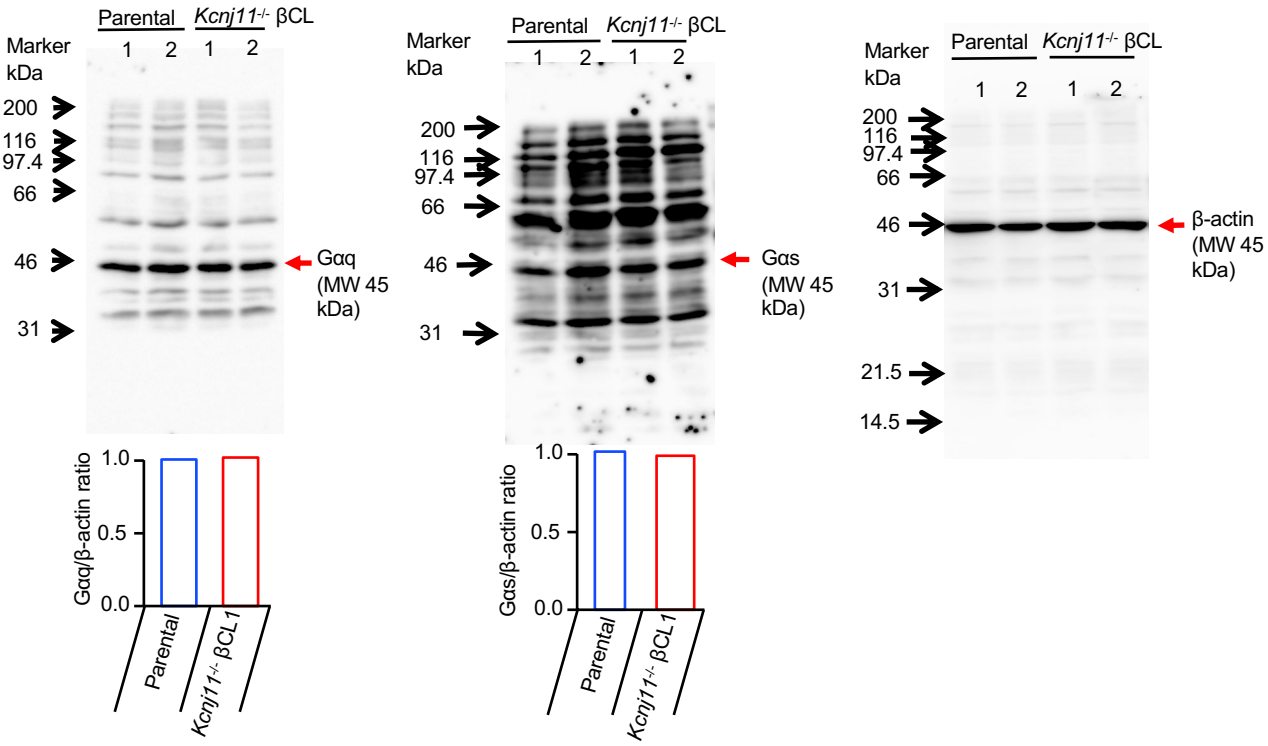
GAAAATGGATATACAAGCTCCCGGGAGCTTTTTGCAAAAGCCTAGGCCTCCAAAAAAGCCTCCCCACTACTTCTGGAATAGCTCAGAGGCAGA

GGCGGCCTCGGCCTCTGCATAAATAAAAAAAATTAGTCAGCCATGGGGCGGAGAATGGGCGGAACTGGGCGGAG






I PKC activity
Phosphoserine- PKC substrates
Parental
Kcnj11-/- βCL
Marker (kDa)
1 2 3 4 5 6 1 2 3 4 5 6
200
116
97.4
66
45
β-actin
Parental
Kcnj11-/- βCL
1 2 3 4 5 6 1 2 3 4 5 6
1. 2.8 mM Glucose
2. 2.8 mM Glucose + 100 nM BIM-1
3. 2.8 mM Glucose + 1 nM GLP-1
4. 2.8 mM Glucose + 1 nM GLP-1 + 100 nM BIM-1
5. 2.8 mM Glucose + 5 μM Carbachol
6. 2.8 mM Glucose + 5 μM Carbachol + 100 nM BIM-1
BIM-1: Bisindolylmaleimide-1; a selective PKC inhibitor
Densitometric analysis
P-ser PKC substrates/β-actin ratio
0 2 4 6 8
1 2 3 4 5 6 1 2 3 4 5 6
Parental
Kcnj11-/- βCL1
J Intracellular calcium
ΔF340/F380
1.4 1.6 1.8 2 2.2 2.4
0 1 2 3 4
Time (min)


K Gs and Gq protein expression
Parental
Kcnj11-/- βCL
Marker kDa
1 2 1 2
200
116
97.4
66
46
31
Gαq (MW 45 kDa)
Gαq/β-actin ratio
0.0 0.5 1.0
Parental
Kcnj11-/- βCL1
Gαs (MW 45 kDa)
Gαs/β-actin ratio
21.5
14.5
β-actin (MW 45 kDa)

Supplementary Figure 3. Expressions of *Glp-1r* and *Gipr* (**A**), adenylyl cyclases (Adcy) (**B**), and phosphodiesterases (Pde) (**C**) in pancreatic islets assessed by qPCR (n = 3 for both mouse groups for each gene expression). Expression of gene was normalized by that of *Gapdh* and presented as fold change of control (**A**). Expression of gene was normalized by that of *Gapdh* (**B, C**).

(**D**) Schematic DNA sequencing of two isogenic mutant *Kcnj11* in cell lines (designated *Kcnj11*$^{-/-}$ βCL1 and 2) derived from parental MIN6-K8. Blue letters, dashed line, and red letters indicate guide RNAs, deleted bases, and inserted bases, respectively. Protospacer adjacent motif (PAM) is indicated by green letters. The mutations are therefore indels generating frame shifts.

(**E**) Whole-cell K_{ATP} channel activity of parental cell line (MIN6-K8), *Kcnj11*$^{-/-}$ βCL1, and *Kcnj11*$^{-/-}$ βCL2. *Top*, Representative trace recorded under control conditions and after addition of the K_{ATP} channel activator diazoxide and the inhibitor tolbutamide, respectively. *Lower*, Histograms summarizing conductance under control conditions (blue) and in the presence of diazoxide (red) and tolbutamide (green) in parent control cells and the two K_{ATP} channel-deficient cell lines (n = 6-11 for each condition).

(**F**) Glibenclamide-induced insulin secretion from parental cell line (MIN6-K8), *Kcnj11*$^{-/-}$ βCL1, and *Kcnj11*$^{-/-}$ βCL2 (n = 4 for each).

(**G**) Incretin-induced insulin secretion from *Kcnj11*$^{-/-}$ βCL1 (n = 4 for each).

(**H**) GLP-1-induced cAMP production. GLP-1 concentrations: 0, 0.5, 0.1, 0.5, and 1 nM (n = 6-7 for each point).

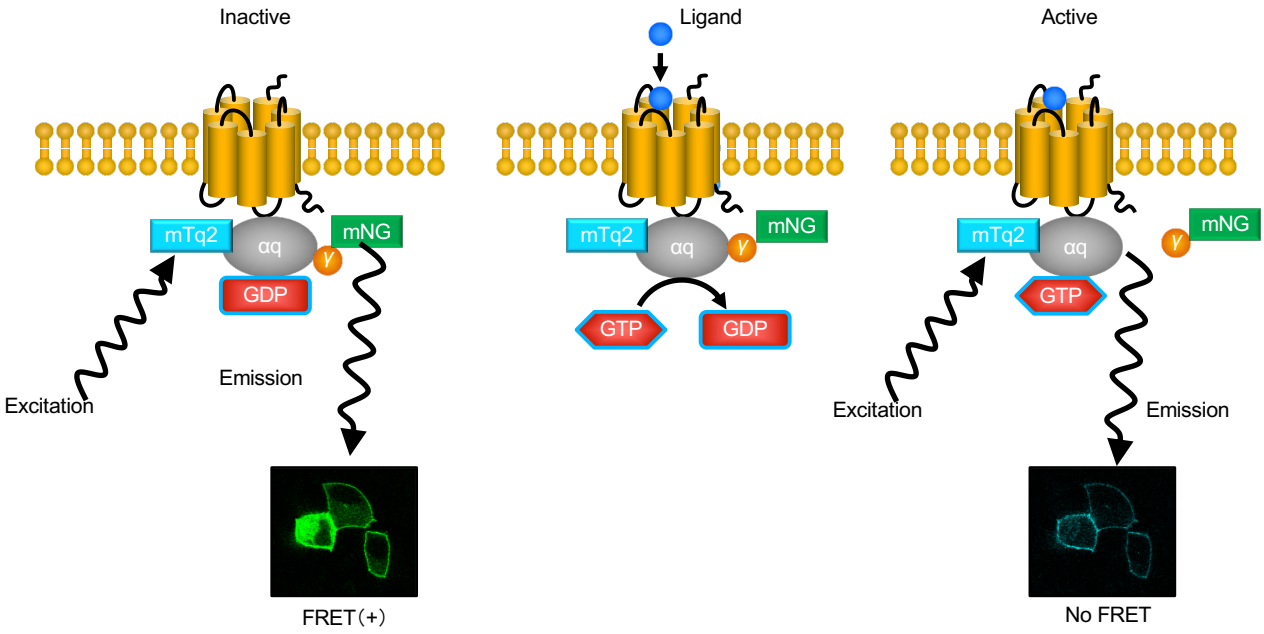
(**I**) Western blot analysis of phosphoserine PKC substrates in β-cell lines. *Upper*, Representative blots. *Lower*, Densitometric analysis of the relative abundance of phospho-serine PKC substrates. Mean of the results from two independent experiment is shown. Samples (lane 1-6) for each cell line were prepared from whole cell lysates. 7.5% polyacrylamide gel was used.

(**J**) Basal $[Ca^{2+}]_i$ in the presence of 2.8 mM glucose (n = 6 for each).

(**K**) Western blot analysis of Gαq or Gαs in β-cell lines. *Upper*, Representative blots. *Lower*, Densitometric analysis of the relative abundance of Gαq or Gαs proteins. Mean of the results from two independent experiment is shown. Samples (1 and 2) for each cell line were prepared from whole cell lysates in two independent experiments. Mean of the two results (1 and 2) is shown. 10% polyacrylamide gel was used for Gαq and Gαs and while 12% gel was used for β-actin. Antibody dilution: Gαq and Gαs (1:250), β-actin (1:1000).

Statistical analyses were performed by 2-tailed student's unpaired t-test for (**A**), (**B**), (**C**) or by 2-way ANOVA followed by Dunnett's for (**E**), (**F**), and (**H**) or Tukey's post hoc test for (**G**). Data represent mean ±SEM. *$p < 0.05$, **$p < 0.01$

A mNG-Gγ-IRES-Gαq-mTq2 FRET sensor



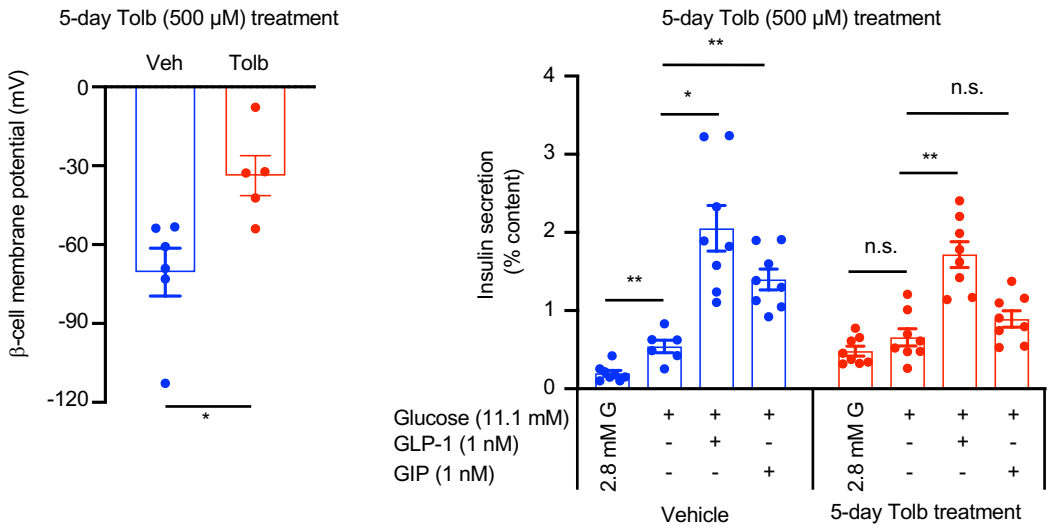
Donor:
Monomeric Turquoise2
(mTq2)

Acceptor:
Monomeric NeonGreen
(mNG)

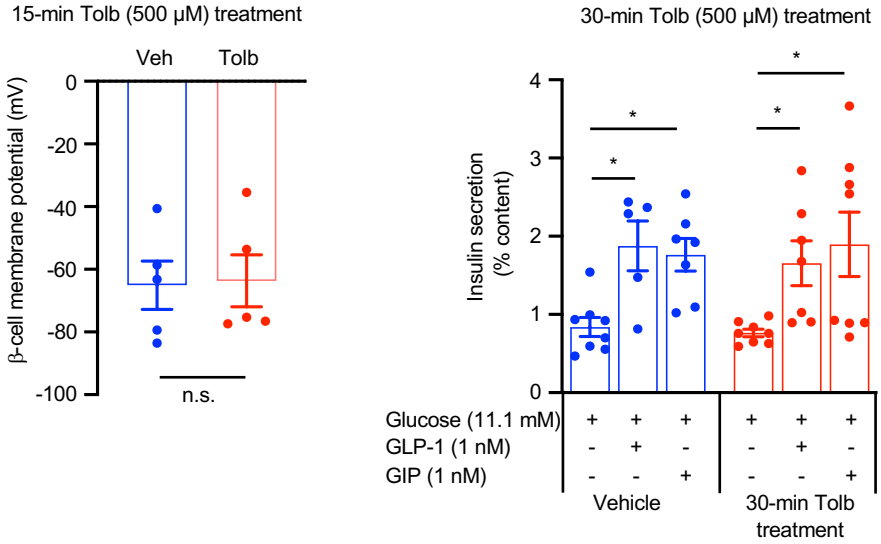
Supplementary Figure 4.

Schematic presentation of the principle of the Gq FRET sensor. The plasmid containing the Gq-specific FRET sensor mNG-Gγ-IRES-Gαq-mTq2 (48) in which the CFP variant Turquiose 2 (mTq2) as the donor and mNeonGreen (mNG) as the acceptor are attached to Gαq and Gγ, respectively, was transfected into parental MIN6-K8 and *Kcnj11*$^{-/-}$ βCL1. In the absence of ligands, the GDP-form of Gαq and Gγ associate with each other in the inactive state of Gq that causes FRET (left). Upon ligand binding to the respective GqPCR, GDP bound to Gαq is replaced with GTP (middle), and then the GTP-form of Gαq dissociates from Gγ in the active state of Gq that does not cause FRET due to lack of transfer of resonance energy from mTq2 to mNG (right). Imaging was performed as described previously (74). Note: the β-subunit (Gβ) is not required to monitor Gq activation status in this FRET system (49).

A Chronic Tolb treatment of wild-type mouse islets



B Acute Tolb treatment of wild-type mouse islets



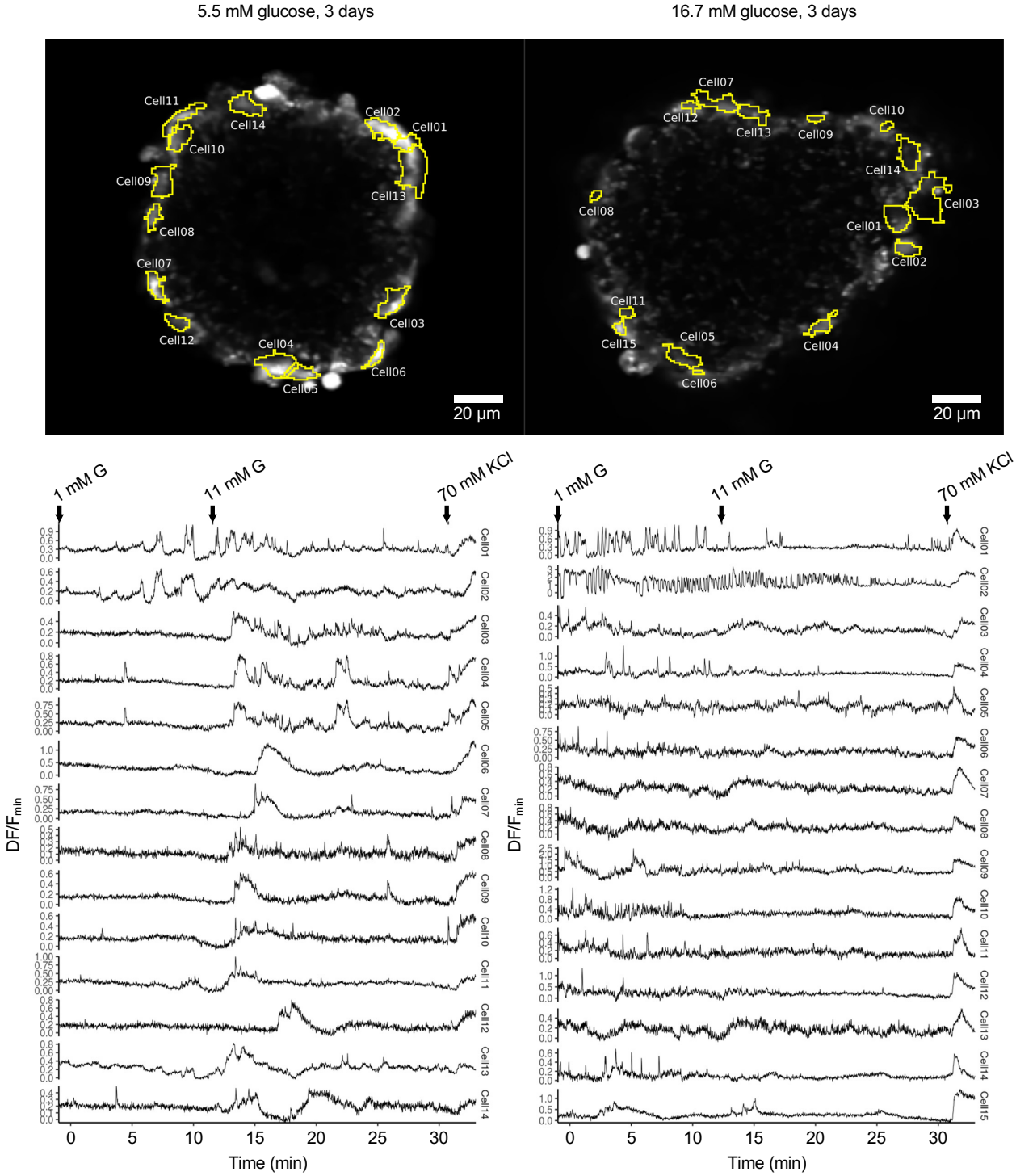
Supplementary Figure 5.

(**A**) Effects of chronic tolbutamide (500 μM) treatment on membrane potential of primary β-cells (left) and incretin-induced insulin secretion (IIIS) from islets (right) of wild-type mice. The islets were cultured in tolbutamide (500 μM) for 5 days (n = 5 for each condition for membrane potential; n = 6-8 for each condition for insulin secretion). G, glucose; Tolb, tolbutamide.

(**B**) Effects of acute tolbutamide (500 μM) treatment on membrane potential of primary β-cells (left) and IIIS from islets (right) of wild-type (B6) mice. Primary β-cells were incubated with tolbutamide (500 μM) for 15 min before measurement of the membrane potential, and the islets were incubated with tolbutamide (500 μM) for 30 min before measurement of insulin secretion (n = 5 for each condition for membrane potential; n = 6-8 for each condition for insulin secretion). Tolb, tolbutamide.

Statistical analyses were performed by 2-tailed Student's unpaired t-test for (**A**, left) and (**B**, left) or by 2-way ANOVA followed by Tukey's post hoc test for (**A**, right) and (**B**, right). Data represent mean ±SEM. *$p < 0.05$, **$p < 0.01$

A Calcium imaging of non-diabetic human islets



Supplementary Figure 6.

Ca^{2+} imaging of human islets incubated at either 5.5 mM glucose or 16.7 mM glucose for 3 days. A representative of 4 different preparations is shown. G, glucose.

Gene	*Kcnj11*$^{fl/fl}$ Mean expression ± SEM		β*Kcnj11*$^{-/-}$ Mean expression ± SEM	
GPCR related				
Gnas	749.031758	14.7104021	750.307795	28.37916
Gna11	38.0001667	0.72078354	39.2537	0.34449399
Gna12	14.8660333	1.10121255	18.8792667	0.39277684
Gna13	15.1619333	0.16589239	17.0882667	0.21151676
Gna14	4.78411	1.08896818	5.35930333	0.20421953
Gna15	0.27455967	0.08582774	0.218103	0.04618666
Gnao1	59.1486667	2.7108598	61.8515333	4.67327937
Gnaq	20.4897333	0.07836879	21.0624333	0.50064865
Gnal	1.676353	0.18865026	1.66360463	0.08618024
Plcb1	3.81703667	0.18337697	3.60878767	0.2119466
Plcb4	6.81506	0.40195147	6.31416	0.31968427
Plcd3	1.026049	0.2813006	1.42669667	0.18913105
Plcd4	0.06440788	0.00816251	0.0696659	0.01465823
Plce1	0.52324867	0.05981629	0.74805067	0.03355272
Plch1	3.60709502	0.09929683	2.80030882	0.11247062
Plch2	0.03176255	0.00787383	0.04808869	0.00642488
Plcg1	11.4427667	0.1214957	11.2961333	0.14786193
Plcg2	4.57236333	0.88218329	4.81139667	0.48607586
Plcz1	0	0	0.02360743	0.01189371
Arrb1	2.46785967	0.31857379	3.848573	0.44926491
Arrb2	14.0173465	0.17072458	14.934537	0.60017795
Ramp1	3.35396974	0.32160288	3.34732836	0.03844908
Ramp2	14.8932667	0.79842671	12.7177333	0.31823338
Ramp3	6.45109	0.4947469	9.70433333	0.01427167
Prkca	9.70501	0.30529096	8.24053667	0.34553016
Prkcb	16.7486333	0.87029874	12.1067	0.92261114
Prkcd	11.0045667	0.83834872	13.5210333	0.79920683
Prkcdbp	10.21931	0.6400495	14.4586667	1.55415152
Prkce	9.64198	0.45964908	9.93367333	0.28777377
Prkch	2.49266	0.04564839	3.47503667	0.07478078
Prkci	9.78932	0.67494858	10.7620667	0.48037556
Prkcq	0.03880747	0.01970633	0.04209333	0.01096537
Prkcz	6.61598645	0.54347562	6.8318917	0.13523535
Prkd1	5.32628333	0.27746296	5.03886	0.16310457
Prkd2	5.872121	0.32754099	6.77944667	0.27649392
Prkd3	3.80609782	0.1622822	4.82579645	0.46191065
Marcks	35.2647	1.84315582	39.2662	3.27668547
Marcksl1	21.5779667	1.58049294	30.7245	4.04168941

Supplementary Table 1. Gene expression of select genes from RNA sequencing of isolated islets. Data presented as Fragments Per Kilobase Million (FPKM) values.

Gene	*Kcnj11*$^{fl/fl}$ Mean expression ± SEM		β*Kcnj11*$^{-/-}$ Mean expression ± SEM	
Calcium signalling related				
Cacna1c	6.30037034	0.11882735	6.41105567	0.33203118
Cacna1d	13.546383	0.59922269	12.8841347	0.98549134
Cacna1b	0.969014	0.09338965	0.857772	0.07374755
Cacna1a	7.15704677	0.22081247	8.24843512	0.16008255
Cacna1e	0.056299	0.0113669	0.04189657	0.00645374
Cacna1g	1.06794192	0.2827902	1.62180844	0.03188473
Cacna1h	1.18034874	0.08142341	1.37067903	0.0776692
Cacna1f	0.144563	0.03034	0.0881116	0.00878618
Cacna1i	0.21841867	0.00263237	0.11480267	0.01104132
Cacna1s	0	0	0.00889776	0.00889776
Itpr2	2.09077043	0.12167004	2.02408853	0.10904849
Itpr3	20.2312667	2.31749487	20.8033333	1.9515953
Ryr1	0.00266587	0.00163106	0.0072401	0.00050369
Ryr2	0.0311145	0.00376435	0.02969913	0.00409268
Ryr3	0.28168467	0.04622473	0.312295	0.01355465
Insulin exocytosis related				
Syt1	1.1260048	0.08466525	1.00243656	0.10286738
Syt2	1.37352333	0.1410292	1.05450533	0.06892947
Syt3	3.75955396	0.39076042	2.84502821	0.30424691
Syt4	66.6418333	2.26609592	44.3242667	2.52355767
Syt5	65.0657667	1.84032724	51.9170667	4.19214083
Syt6	0.61380824	0.09533097	0.75593706	0.0478121
Syt7	44.1628833	3.07022645	51.44944	4.05803605
Syt8	0.37011427	0.02822546	0.45736633	0.07128555
Syt9	5.55106667	0.57890178	6.12555667	0.13802355
Syt11	17.8345	1.20915414	18.3806333	0.7469996
Syt12	1.69628333	0.31557571	2.22527	0.28605027
Syt13	277.286	10.7433908	269.107	16.1853407
Syt15	0.31281649	0.11903333	0.44735849	0.05162005
Syt16	0.959064	0.0575638	1.33115	0.1402433
Syt17	0.59392167	0.07426157	1.12580333	0.04126824
Syt14	7.87552954	0.18311097	7.0400608	0.32147694
Sytl1	11.68446	1.52864463	13.0959667	0.5838311
Sytl3	1.14828471	0.19443652	1.26691992	0.12123522
Sytl2	1.57551874	0.23357828	1.71138303	0.08188202
Sytl4	46.78565	3.52021007	36.9639667	4.61228365
Sytl5	8.78606	0.38846215	9.68246333	0.4133551

Supplementary Table 1. Continued.

Gene	*Kcnj11*$^{fl/fl}$ Mean expression ± SEM		βKcnj11$^{-/-}$ Mean expression ± SEM	
Calcium signalling related				
Snap23	23.6595315	1.13094684	24.1327189	0.44192978
Snap25	64.3516267	2.73829682	58.0115567	4.49762581
Snap29	13.8738	0.06454218	13.7824	0.08427718
Snap91	5.88476765	0.40659606	4.52530957	0.33326221
Snap47	47.4445	2.10184045	45.9771667	1.98716519
Syncrip	27.60384	1.77039921	29.2430333	0.25178385
Stx11	1.36381614	0.11014383	1.95773807	0.18345193
Stx12	45.5340667	0.64579042	45.2485667	0.60917636
Stx16	17.2164367	1.27847085	16.9516833	0.2283938
Stx17	5.03813333	0.10587266	5.22482	0.06965942
Stx19	0.706988	0.1217035	0.524813	0.02548468
Stx1a	6.06028	0.89269757	6.37682333	0.56827027
Stx1b	0.18394933	0.02928766	0.38449833	0.09937136
Stx2	5.6896945	0.37461595	6.261027	0.08755054
Stx3	18.6467859	0.29741893	17.8529243	0.55815181
Stx4a	34.0596	1.29609709	34.6238667	1.37854661
Stx5a	39.3301333	0.60237342	39.8474	0.71439962
Stx6	23.4911667	0.13902561	24.1856	0.15499595
Stx7	51.5431667	2.57896419	47.8514333	1.21082165
Stx8	6.0993	0.28002652	5.91382667	0.12649572
Stxbp1	63.5885767	0.33321993	54.1309667	2.98807181
Stxbp2	26.9809	1.13356556	30.1321667	0.57051692
Stxbp3a	15.9626333	0.07143567	15.9374333	0.53531134
Stxbp4	2.37924	0.15190442	2.37472333	0.04549559
Stxbp5	7.65513667	0.28543223	7.06949667	0.20628953
Stxbp5l	1.0967041	0.05158436	1.05299796	0.09057104
Stxbp6	7.60512667	0.1827278	7.43650667	0.43178006
Stx18	20.1471567	0.38424876	22.4075823	1.06562496
Stxbp3b	0.33493967	0.08146236	0.19298267	0.01311735
Vamp1	2.74093067	0.12933485	2.56272	0.13907504
Vamp2	72.5185333	2.61568687	66.7171667	5.33477436
Vamp3	41.4207667	0.55911009	43.7489	0.75560626
Vamp4	11.2842867	1.32317972	9.20977667	0.26492122
Vamp5	2.42319	0.27548372	2.763446	0.14954144
Vamp8	126.016	4.35416402	134.148	1.26169344
Vapa	65.5585667	1.77361254	73.0442	1.37499639
Vapb	25.8023	0.28712524	23.7327667	0.80458609

Supplementary Table 1. Continued.

Antibody	Company	Catalogue number
Guinea pig anti-insulin	Dako	AO564
Mouse anti-glucagon	Zymed	A0565
ECL anti-rabbit IgG	GE Healthcare	NA934V
ECL anti-mouse IgG	GE Healthcare	NA931V
Phospho-(Ser) PKC Substrate	Cell signalling	2261
Anti $G\alpha_{s/olf}$ (A-5)	Santa Cruz	SC-55545
Anti $G\alpha_{q}$ (10)	Santa Cruz	SC-136181
β-Actin	Cell signalling	4967
Bacterial Strains		
DH5α Competent Cells	Invitrogen	18258012
Chemical reagents, Peptides, and Recombinant Proteins		
GLP-1(Human)	Peptide institute	4344-V
GIP (Human)	Peptide institute	4178-V
YM-254890	Wako	257-00631
Sitagliptin phosphate	Sigma-Aldrich	654671-77-9
Liraglutide (Victoza)	Novo Nordisk	204656-20-2
MK-2305	Merck	N/A
Carbamylcholine chloride (Carbachol)	Nacalai Tesque	51-83-2
Palmitate	Wako	165-00102
IBMX	Sigma	28822-58-4
Tolbutamide	Sigma	64-77-7
Glibenclamide	Sigma	10238-21-8
Fura-2-AM	Dojindo	343-05401
MDL-12, 330A hydrochloride	Sigma	M182-59
Exendin-(9-39)	Abcam	Ab141101
Bisindolylmaleimide-1 (BIM-1)	Millipore	133052-90-1
DAPI	Dojindo	28718-90-3
Lipofectamine 2000	Invitrogen	11668-019
Effectene Transfection Reagent	Qiagen	301427
High glucose Dulbecco's Modified Eagles Medium (DMEM)	Sigma	D5796
Low glucose DMEM	Sigma	D5030
Rosewell Park Memorial Institute (RPMI)-1640	Sigma	R8758
Glucose free RPMI medium	Gibco	11879-020
Fetal Bovine Serum	Bio west	S1400-500
Penicillin and Streptomycin	Wako	168-23191
Protease inhibitor cocktail	Sigma-Aldrich	11697498001
Phosphatase inhibitor cocktail	Roche Diagnostics	04906837001
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	24893881
Collagenase P	Roche Diagnostics	11249002001
Aprotinin	Wako	010-11834
KR-62436 hydrate (DPP4 inhibitor)	Sigma-Aldrich	761414-79-3
Commercial assays		
Insulin Ultra Sensitive HTRF Assay kit	Cisbio	62IN2PEH
cAMP Gs dynamic kit	Cisbio	62AM4PEC
IP-One HTRF kit	Cisbio	62IPAPEB
Morinaga Ultra Sensitive Mouse Insulin ELISA Kit	MORINAGA	M1104
GLP-1 High Sensitive ELISA Kit	Wako	299-75501

Supplementary Table 2. Reagents and resources.

GLP-1, Active form (High Sensitive) Assay Kit	IBL	27700
Rat/Mouse (Total) GIP ELISA Kit	Millipore	EZRMGIP-55K
Mouse GIP (Active) ELISA Kit	Wako	299-73801
Mercodia Glucagon ELISA	Mercodia	10-1271-01
BCA protein assay kit	Thermo Fisher Scientific	23227
RNA extraction kit	Qiagen	74106
DNA SimplePrep reagent	Takara Bio	9180
ReverTra Ace qPCR RT kit	Toyobo	FSQ-101
Experimental mouse models		
C57BL/6JJcl mice	Japan CLEA	C57BL/6JJcl
KK-Ay/TaJcl mice	Japan CLEA	KK-Ay/TaJcl
KK/TaJcl mice	Japan CLEA	KK/TaJcl

Supplementary Table 2. Continued.

Islet Preparation	1	2	3	4	5	6	7	8	9
Unique Identifier	HP1930	HP1933	HP1935	HP2001	HP2002	H2366	R344	HP-19311-01	H2491
Donor age (years)	44	41	42	49	51	72	59	29	71
Donor sex (M/F)	M	M	F	M	M	M	M	F	M
Donor BMI (kg/m²)	26.1	24	37	31	25	32.7	27.5	26.2	22.4
Donor HBA1c or other measure of blood glucose control	5.4	5.5	5.8	nd	5.5			5.1	5.9
Islet isolation centre	Oxford	Oxford	Oxford	Oxford	Oxford	Uppsala	Alberta ADI	Prodo labs	Uppsala
Donor history of diabetes	no	no	no	no	no	no	no	no	
Donor cause of death	Road-traffic accident	intracranial hemorrhage	intracranial hemorrhage	intracranial hemorrhage	cardiac arrest			stroke	Brain death
Warm ischaemia time						36h		72h	
Cold islchaemia time	7	5.75	9.75	6.5	5.5	6h	24h	36h	22h
Estimated purity (%)	80	50	85	60	70	97	95	90-95	96
Estimated viability (%)	72	81	76	71	85	50		95	

Supplementary Table 3. Information on human donors for islet studies.

Gene name	Source	Identifier
Abcc8	Thermo Fisher Scientific	Mm00803450-_m1
Adcy1	Thermo Fisher Scientific	Mm01187829_m1
Adcy2	Thermo Fisher Scientific	Mm00467874_m1
Adcy3	Thermo Fisher Scientific	Mm00460371_m1
Adcy4	Thermo Fisher Scientific	Mm00475491_m1
Adcy5	Thermo Fisher Scientific	Mm00674122_m1
Adcy6	Thermo Fisher Scientific	Mm00475772_m1
Adcy7	Thermo Fisher Scientific	Mm00545780_m1
Adcy8	Thermo Fisher Scientific	Mm00507722_m1
Adcy9	Thermo Fisher Scientific	Mm00507743_m1
Gapdh	Thermo Fisher Scientific	Mm99999915_g1
Glp-1r	Thermo Fisher Scientific	Mm00445292_m1
Gipr	Thermo Fisher Scientific	Mm01316351_g1
Ins1	Thermo Fisher Scientific	Mm01259683_g1
Ins2	Thermo Fisher Scientific	Mm00731595_gH
Kcnj11	Thermo Fisher Scientific	Mm00440050_s1
Pde1c	Thermo Fisher Scientific	Mm00478051_m1
Pde3a	Thermo Fisher Scientific	Mm00479581_m1
Pde3b	Thermo Fisher Scientific	Mm01271639_m1
Pdce4a	Thermo Fisher Scientific	Mm00480071_m1
Pde4b	Thermo Fisher Scientific	Mm00480166_m1
Pde4c	Thermo Fisher Scientific	Mm01343237_m1
Pde4d	Thermo Fisher Scientific	Mm00456879_m1
Pde10a	Thermo Fisher Scientific	Mm00449329_m1
Kcnj11$^{fl/fl}$ and *Kcnj11*$^{-/-}$ mice genotyping primers		
Gene	Forward	Reverse
Kcnj11	ATGTAGAGTGGTGGGGTGCG	CCAAGACGGGCTTTGAGACC
Rat insulin II promoter Cre	CCGCAGAACCTGAAGATGTTCGC	CAGATTACGTATATCCTGGCAGGG
Genotyping primers for CRISPR/Cas9-mediated knock out β-cell lines		
Gene	Forward	Reverse
Kcnj11	TAACCTGAGGAGAGGGCTCA	GGCAGATGAAAAGGAGTGGA

Supplementary Table 4. PCR primers.

Supplementary Methods.

RNA sequencing. RNA samples were processed as fragments per kilobase of transcript per million mapped reads (FPKM), and determined by the RNA-sequencing (RNA-seq) service provider Macrogen (Japan), who also mapped trimmed reads to the Mus musculus reference genome using AnnoOnly(-G) Transcriptome Resequencing NGS Advanced and then performed transcript assembly using Cufflinks-G. Data were log transformed and quantile normalization was performed with Pre-process Core's R library by Macrogen. Samples with at least one FPKM value of 0 were removed from consideration.