**Targeting SUR1/Abcc8-Type Neuroendocrine K\textsubscript{ATP} Channels in Pancreatic Islet Cells**

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**Abstract**

ATP-sensitive K\textsuperscript{+} (K\textsubscript{ATP}) channels play a regulatory role in hormone-secreting pancreatic islet \(\alpha\)-, \(\beta\)- and \(\delta\)-cells. Targeted channel deletion would assist analysis and dissection of the intraislet regulatory network. Toward this end Abcc8/Sur1 flox mice were generated and tested by crossing with glucagon-(GCG)-cre mice to target \(\alpha\)-cell K\textsubscript{ATP} channels selectively. Agonist resistance was used to quantify the percent of \(\alpha\)-cells lacking channels. 41% of Sur1\textsuperscript{loxP/loxP}/GCG-cre\(-\) and \textasciitilde64% of Sur1\textsuperscript{loxP/loxP}/GCG-cre\(-\) \(\alpha\)-cells lacked K\textsubscript{ATP} channels, while \textasciitilde65% of \(\alpha\)-cells expressed enhanced yellow fluorescent protein (EYFP) in ROSA-EYFP/GCG-cre matings. The results are consistent with a stochastic two-recombination event mechanism and a requirement that both floxed alleles are deleted.

**Introduction**

Diabetes mellitus is a major worldwide health problem increasingly understood to be a bihormonal disease characterized by dysregulation of insulin secretion from pancreatic \(\beta\)-cells and glucagon secretion from \(\alpha\)-cells [1,2]. Failure to adequately suppress glucagon secretion from \(\alpha\)-cells following a meal contributes to the pathogenesis of type 2 diabetes mellitus. Impaired glucagon counter-regulation and the fear of hypoglycemia is a major deterrent to maintaining tight glucose control in type 1 diabetes mellitus. The control of insulin and glucagon release in response to varying blood sugar is complex, hierarchical and redundant. Regulation involves inputs from the central nervous system and local control via a network of interactions between islet cells, including \(\alpha\)-, \(\beta\)- and \(\delta\)-cells [1,3,4]. Our understanding of the local control network is inadequate at the cellular and molecular levels and there are few tools or mouse models available to dissect network interactions. K\textsubscript{ATP} channels can act as metabolic sensors in \(\alpha\)-, \(\beta\)- and \(\delta\)-cells. Closure of K\textsubscript{ATP} channels in \(\beta\)- and \(\delta\)-cells, secondary to increased glucose metabolism, potentiates insulin and somatostatin release, respectively. The role of these channels in \(\alpha\)-cells is more controversial. Opening of channels during hypoglycemia has been proposed to be necessary for glucagon secretion [5] reviewed in [6]. Alternatively the intra-islet insulin hypothesis proposes that the paracrine actions of \(\beta\)-cell secretion suppress glucagon secretion [7,8] potentially via a K\textsubscript{ATP}-dependent mechanism [9], reviewed in [10]. Glucagon is reported to have small local stimulatory effects on release of somatostatin [reviewed in [11]]. Somatostatin and somatostatin analogs are used clinically to inhibit multiple functions including insulin release. Recent studies show local somatostatin release attenuates the secretion of both insulin and glucagon (for example [12,13,14,15]).

The availability of mouse models selectively targeting islet cell K\textsubscript{ATP} channels should aid the dissection of network interactions by uncoupling hormone release from glucose metabolism. Thus SUR1 flox mice, in which exon 2 of the Abcc8/Sur1 (ATP binding cassette C3/Sulfonflyurea receptor type 1) gene is flanked by flox sites, were generated with the intention of targeting K\textsubscript{ATP} channels in select islet cell types. Exon 2 was targeted to complement Sur1\textsuperscript{loxP/loxP} mice in which exon 2 deletion globally eliminates SUR1 neuroendocrine type K\textsubscript{ATP} channels [16]. In comparison with the severe hypoglycemia characteristic of patients with congenital hyperinsulinism (CHI) secondary to loss of K\textsubscript{ATP} channel function [reviewed in [17]], Sur1\textsuperscript{loxP/loxP} mice, with the equivalent channel deficit, show near normal glucose homeostasis unless stressed [16,18,19,20]. The mechanism(s) of compensation in the mouse model are unclear, but may reflect differences in human versus mouse islet architecture and thus differences in network feedback loops in addition to differences in the glucose-dependent amplification pathway [21].

The successful generation of these mouse models requires that sufficient, ideally all, of the targeted islet cells lack SUR1. Unlike inactivation of a gene where haploinsufficiency produces loss of function, studies of Sur1\textsuperscript{loxP/loxP} and Sur1\textsuperscript{loxP/loxP} islet cells show that deletion of both exons 2 alleles is required to eliminate K\textsubscript{ATP} channels. Previous studies showed the number of channels in Sur1\textsuperscript{loxP/loxP} \(\beta\)-cells was indistinguishable from wildtype (WT), while Sur1\textsuperscript{loxP/loxP} \(\beta\)-cells showed a complete loss [16]. Similarly, CHI is a recessive genetic disorder. Therefore we tested the ability of cre-recombinase to produce K\textsubscript{ATP} channel deficient \(\alpha\)-cells in Sur1\textsuperscript{loxP/loxP} and Sur1\textsuperscript{loxP/loxP} animals in which one or two recombination events are needed to delete channel function, respectively.

In animal models, the frequency of single recombination events is often determined by crossing cre-recombinase into a cre-
reporter mouse strain, for example ROSA26-stop-lacZ [22] or ROSA26-stop-EYFP [23], then assessing what fraction of a specific cell type expresses the reporter. Reported frequencies are often >0.8 for a single event which, assuming a random process, would give a frequency of >0.64 of targeted islet cells lacking KATP channels. To test this idea Sur1loxP/loxP and Sur1loxP/- animals GCG-cre mice expressing cre-recombinase under control of the glucagon promoter [24] were used to generate Sur1loxP/loxP;GCG-cre and Sur1loxP/-;GCG-cre mice. The frequency of channel-deficient \( \alpha \)-cells was compared with the single event frequency for expression of EYFP in \( \alpha \)-cells from ROSA-stop-EYFP GCG-cre crosses. EYFP was expressed in ~65% of \( \alpha \)-cells, while ~41% of Sur1loxP/loxP GCG-cre \( \alpha \)-cells showed complete loss of KATP channels versus 64% in Sur1loxP/-;GCG-cre \( \alpha \)-cells. The results are consistent with a stochastic two-hit mechanism and provide two animal models with varying levels of KATP channel deficient \( \alpha \)-cells.

Materials and Methods

All of the animal studies were approved by the Institutional Animal Care and Use Committee of the Pacific Northwest Diabetes Research Institute. The Pacific Northwest Diabetes Research Institute has an approved Animal Welfare Assurance on file with the Office for Laboratory Animal Welfare (A3357-01). Animals were maintained with a 12-h light-dark cycle at constant temperature (22±2°C) and were given free access to food and water.

Generation of Sur1loxP/loxP mice. A targeting vector (Figure 1A) was constructed using a 10.63 kb region subcloned from a C57BL/6 BAC clone (RPCI23: 301A13). The construct was designed with a long homology arm extending approximately 7.1 kb 5’ of exon 2 including exon 1 and a short homology arm extending approximately 2.59 kb 3’ of exon 2. A single \( loxP \) site was inserted 5’ of exon 2 and a \( loxP/FRT \) flanked Neo cassette was inserted on the 3’ side of exon 2. The targeted region is 928 bp including exon 2. The targeting vector was confirmed by restriction digests and by sequencing. The targeting vector was assembled and transfected into C57BL/6N x 129SvEv hybrid embryonic stem cells by inGenious Targeting Laboratory, Inc (Stony Brook, New York). G418, an aminoglycoside antibiotic, was used to select cells carrying the Neomycin resistance cassette. Cells were selected and correctly targeted recombinant ES cells were identified by PCR analysis. Retention of the upstream \( loxP \) site was confirmed by PCR analysis and by sequencing. Sur1loxP/neo mice were crossed with an FLP deleter mouse strain (B6.Cg-Tg(ActFLPe)9205Dym/J; Jackson Laboratories, Inc.) to eliminate the Neo cassette. The knockout allele was identified using forward (5’-TGA GAT CGC TGA GGG TAT CC-3’) and reverse (5’-AAG ACC GCG AAG AGT TTG AAA GTC GCT CTG AGT-3’) primers.

Figure 1. Conditional targeting strategy to create Sur1 flox mice. (A) Illustration of the targeting construct and possible recombinant event to produce founder mice carrying the neomycin resistance cassette. (B) Examples of PCR products from a total of 10 mice are shown; WT (lane 8), homozygous Sur1loxP/loxP (lanes 1, 4 and 9) and heterozygous Sur1loxP/- (lanes 2, 3, 5, 6, 7 and 10). The arrows show the position of 500 and 1000 base-pair markers.

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Generation of Sur1loxP/-;GCG-cre mice. Crossing Sur1loxP/loxP; GCG-cre and Sur1/- [16] generated Sur1loxP/-;GCG-cre mice. The knockout allele was identified using forward (5’-AGG TTC TTG GTG GAG GTC AGC-3’) and reverse (5’-GCT ACT TCC ATT TGT CAG G-3’) primers.

Generation of GCG-cre-ROSA26-stop-EYFP mice. GCG-cre-ROSA26-stop-EYFP mice were generated by crossing GCG-cre and ROSA26-stop-EYFP (B6.129X1-Gt(ROSA)26Sortm1(EYFP)Cos/J; Jackson Laboratories, Inc.) animals. The Gt(ROSA)26Sortm1(EYFP)Cos allele was identified using forward (5’-AAA GTC CTG CTG AGT TGT CAT -3’) and reverse (5’-AAG ACC GCG AAG AGT TTG TC-3’) primers.

Islet isolation. On the day of pancreas removal, animals were anesthetized with a ketamine (600 mg/Kg)-xylazine (50 mg/Kg) mixture and then killed by removing blood from the heart. Pancreata were cannulated for infusion of collagenase and then removed from the animal for processing as described [9] using 1 mg/ml collagenase. Islets were dissociated by mechanical dispersion in a Ca\(^{2+}\)-free medium with 0.1 mM EGTA. The mix of isolated islet cells, primarily \( \alpha \), \( \beta \), and \( \delta \)-cells, and small clusters were plated on glass cover slips and cultured overnight in RPMI medium 1640 containing 10% FBS, 11.1 mM glucose, and 100 units/ml of penicillin, 100 mg/ml of streptomycin, and 0.25 mg/ml of amphotericin B (Gibco/Life technologies, Inc).

Calcium Imaging. The cytoplasmic free Ca\(^{2+}\) ([Ca\(^{2+}\)\(_{i}\)]\(_{c}\)) concentration was measured by dual excitation-emission spectro-fluorimetry using fura-2 [25] (Molecular Probes, Inc., Eugene,
OR). Cells were loaded with 0.2 μM fura-2/AM for 30 min and perfused in Krebs-Ringer bicarbonate HEPES buffer (KRB-HEPES) containing (mM) 129 NaCl, 4.7 KCl, 1.2 KH2PO4, 1.2 MgCl2, 2 CaCl2, 5 NaHCO3, 10 HEPES (equilibrated pH 7.4) supplemented with 0.1% BSA. Measurements were carried out using a Leica DM6000B microscope. Excitation was at 340 and 380 nm; emission was recorded at 510 nm at intervals of 3 seconds. Relative \([\text{Ca}^{2+}]_{c}\) is defined as the 340/380 ratio.

### Results

**Insertion of loxP sites does not affect the general phenotype of Sur1loxP/loxP animals**

The Sur1loxP/+ and Sur1loxP/loxP animals are viable, fertile, appear phenotypically normal and have a normal lifespan. Table I compares the body weight and fed blood glucose values of WT versus Sur1loxP/+ and Sur1loxP/loxP animals at 4 weeks of age. The results indicate insertion of loxP sites around exon 2 of the Abcc8/Sur1 gene does not have deleterious effects on development or glucose homeostasis.

### Table I. Mouse body weight and blood glucose values.

| Strain   | BW (gram) | BG (mg/dl) |
|----------|-----------|------------|
| **Males** |           |            |
| WT       | 18.6±0.8  | 135.7±10.7 |
| Sur1loxP/+ | 18.7±0.4  | 140.7±5.2  |
| Sur1loxP/loxP | 18.0±0.7 | 135±4.5   |
| **Females** |         |            |
| WT       | 15.9±0.2  | 1192±5.7   |
| Sur1loxP/+ | 15.9±0.3  | 1199±4.8   |
| Sur1loxP/loxP | 16.0±0.5 | 120.6±6.4  |

Mean ± SEM, n = 6–10.

To discriminate whether KATP channels are present, a rapid \(\text{Ca}^{2+}\)-imaging assay was developed based on the arginine stimulation test used to assess the secretion capacities of \(\alpha\)- and \(\beta\)-cells in patients [29,30]. Changes in \([\text{Ca}^{2+}]_{c}\), were assessed in isolated islet cells in response to a pulse of arginine at three glucose concentrations. The effect of KATP channel loss was assessed by comparing the responses of isolated WT and Sur1−/− islet cells. As indicated in Materials and Methods islet cell types were discriminated by their response to a 5 minute pulse of epinephrine (5 μM) in 2.8 mM glucose; \(\alpha\)-cells exhibit the reverse response. This method was motivated in part by understanding of the glucagon counter-regulatory response to hypoglycemia where epinephrine hyperpolarizes \(\beta\)-cells and \(\delta\)-cells [26] thus inhibiting their hormone release, while stimulating \(\alpha\)-cells to secrete glucagon [27].

**Assay for KATP channel deletion.** The absence of channels was determined by assessing the response of isolated \(\alpha\)-cells to the KATP channel agonist, diazoxide. Fura2-loaded \(\alpha\)-cells, in KRB-HEPES with 2.8 mM glucose; identified functionally by their response to epinephrine, were perfused with 1 mM arginine ± 100 μM diazoxide. In WT \(\alpha\)-cells arginine elevates \([\text{Ca}^{2+}]_{c}\) and will stimulate glucagon release; opening KATP channels with diazoxide completely suppresses the arginine-induced \([\text{Ca}^{2+}]_{c}\) increase. In \(\alpha\)-cells lacking KATP channels, e.g., isolated from Sur1−/− mice, the agonist has no effect. Diazoxide half-maximally stimulates SUR1/Kir6.2 KATP channels at ~60 μM [28] therefore concentrations (10, 30 and 100 μM) bracketing this value were used.

**Blood glucose measurements.** Glucose was measured on blood from a tail vein using a Freestyle glucometer (Abbott Laboratories, Abbott Park, Illinois, U.S.A.). Measurements were made in the morning on fed animals, i.e., animals given free access to food and water.

Ca\(^{2+}\)-imaging assay for presence of K\(_{\text{ATP}}\) channels in islet cells

To discriminate whether K\(_{\text{ATP}}\) channels are present, a rapid Ca\(^{2+}\)-imaging assay was developed based on the arginine stimulation test used to assess the secretion capacities of \(\alpha\)- and \(\beta\)-cells in patients [29,30]. Changes in \([\text{Ca}^{2+}]_{c}\), were assessed in isolated islet cells in response to a pulse of arginine at three glucose concentrations. The effect of K\(_{\text{ATP}}\) channel loss was assessed by comparing the responses of isolated WT and Sur1−/− islet cells. As indicated in Materials and Methods islet cell types were discriminated by their response to a 5 minute pulse of epinephrine (5 μM) in 2.8 mM glucose; \(\alpha\)-cells exhibit a robust increase in \([\text{Ca}^{2+}]_{c}\), while \(\beta\)-cells show a robust increase in \([\text{Ca}^{2+}]_{c}\). In WT \(\beta\)-cells, under hypoglycemic conditions (2.8 mM glucose), the openings of K\(_{\text{ATP}}\) channels are sufficient to inhibit the depolarizing effect of arginine (to 30 mM; Figure 2C). When K\(_{\text{ATP}}\) channels in WT \(\beta\)-cells are closed by increasing the glucose concentration arginine readily increases \([\text{Ca}^{2+}]_{c}\), (Figure 2C). Similarly, Sur1−/− \(\beta\)-cells lacking K\(_{\text{ATP}}\) channels are readily depolarized by arginine even in 2.8 mM glucose (Figure 2D). As expected, the initial \(\beta\)-cell \([\text{Ca}^{2+}]_{c}\) values are elevated by increasing concentrations of glucose in both WT and Sur1−/− \(\beta\)-cells [21,31,32].

We assume that arginine induces depolarization of \(\alpha\)-cells and activates voltage-gated L-type Ca\(^{2+}\)-channels since the rise in \([\text{Ca}^{2+}]_{c}\), is blocked by the L-type channel antagonist, nifedipine (10 μM; Figure 2B). Tolbutamide (200 μM) had no significant effect on arginine activation of \(\alpha\)-cells in 2.8 mM glucose (data not shown). The results imply that under these conditions, K\(_{\text{ATP}}\) channels do not make a significant contribution to determining the membrane potential of isolated \(\alpha\)-cells, in contrast to their role in \(\beta\)-cells. Therefore opening \(\alpha\)-cell K\(_{\text{ATP}}\) channels with a channel agonist like diazoxide should reduce the stimulatory effect of arginine. Figure 3 shows that diazoxide blocks the stimulatory action of arginine in WT (Figure 3A, B), but not Sur1−/− \(\alpha\)-cells (Figure 3C). The results show that when K\(_{\text{ATP}}\) channels are present opening them with diazoxide blocks the stimulatory action...
Figure 2. Stimulation of isolated pancreatic islet cells by arginine. (A) α- and β-cells were distinguished by their response to an epinephrine pulse in 2.8 mM glucose. (B) Arginine stimulation was blocked by nifedipine (10 μM). The solid traces and shaded areas are the means ± SEM, respectively, for the indicated number of cells. These experiments were repeated four times with similar results using different islet preparations derived from 1 or 2 mice. (C and D) Responses of WT and Sur1−/− α- and β-cells to increasing concentrations of arginine at three concentrations of glucose. Each trace is an average of Ca²⁺ values from 4–10 cells; the experiments were repeated 4 times with similar results using different islet preparations. Islet preparations were from 1 or 2 mice. The pulse lengths are 5 minutes. The 5.6 and 16.7 mM glucose α-cell traces are offset 0.1 and 0.2 units respectively, for clarity.

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Analysis of $K_{ATP}$ channels in Sur1$^{\text{loxp/loxp}}$;GCG-cre$^+$ and Sur1$^{\text{loxp/loxp}}$;GCG-cre$^+$ islet cells

Figure 4A shows an analysis of islet cells isolated from a Sur1$^{\text{loxp/loxp}}$;GCG-cre$^+$ mouse. Islet cells in 2.8 mM glucose were stimulated with arginine (1 mM) in the presence or absence of diazoxide (100 μM). The individual traces for six epinephrine-stimulated α-cells are shown. Two types of α-cells were found, those resistant and those sensitive to diazoxide (gray and black traces respectively). In this example, 4 of 6 α-cells are resistant to diazoxide, i.e., the fraction of diazoxide resistant cells was 0.67. The assumption is that both exon 2 alleles have been deleted in the diazoxide resistant cells, whereas the diazoxide sensitive cells could have one or both alleles intact. In a separate experiment epinephrine suppressed non-α-cells, primarily β-cells, which were stimulated with high glucose (16.7 mM). Figure 4B shows that in 20 of 20 cells a pulse of diazoxide (100 μM) reduced [Ca$^{2+}$]$^c$. The mean ± SEM is shown in the upper trace, which is offset for clarity. The result shows that cre-recombinase is expressed selectively in α-cells.

Table II compares the fraction of diazoxide resistant α-cells in islets from Sur1$^{\text{loxP/loxP}}$;GCG-cre$^+$ and Sur1$^{\text{loxP/loxP}}$;GCG-cre$^+$ mice with the fraction of EYFP positive α-cells in ROSA-EYFP;GCG-cre$^+$ mice (all 12–15 weeks of age). The results are consistent with the need to delete both floxed exon 2 alleles in an animal expressing cre-recombinase driven by the glucagon promoter. There is a small age dependence of cre-recombinase activity in this model. Sur1$^{\text{loxP/loxP}}$;GCG-cre$^+$ mice had a frequency of recombination $= 0.68 (42/62)$ at 24 weeks of age vs 0.64 at 12–15 weeks.

Discussion

The objective was to develop an assay to assess the presence or absence of SUR1/Kir6.2 neuroendocrine-type $K_{ATP}$ channels in single islet cells and use this assay to determine the efficiency of channel deletion when Abcc8/Sur1 flox mice are crossed with an animal expressing cre-recombinase driven by a promoter selective for pancreatic islet α-cells. In Sur1 flox mice exon 2 of the Abcc8/Sur1 gene is flanked by loxP sites. Deletion of both copies of exon 2 in Sur1 global knockout mice, Sur1$^{-/-}$, eliminates their neuroendocrine-type $K_{ATP}$ channels, while islet cells isolated from heterozygous animals have channel densities indistinguishable from WT [16]. Therefore assessing the efficiency of $K_{ATP}$ channel deletion requires looking at individual cells. An IV bolus of arginine is used clinically to assess the secretory capacities of both α- and β-cells [29,30], thus the effect of pulses of arginine on isolated islet cells was determined using standard Ca$^{2+}$-imaging techniques. α- and β-cells were identified by their response to epinephrine. Under hypoglycemic conditions, 2.8 mM glucose, arginine (≤30 mM) failed to stimulate a rise in [Ca$^{2+}$]$^c$, in WT β-cells implying that under these conditions open $K_{ATP}$ channels are sufficient to prevent arginine-induced membrane depolarization and activation of voltage-dependent Ca$^{2+}$ channels. Closing channels, either by increasing the glucose concentration or using Sur1$^{-/-}$ β-cells, produced a concentration-dependent response to added arginine. Arginine induced a rise in α-cell [Ca$^{2+}$]$^c$, at the three concentrations of glucose tested, thus the application of 1 mM arginine in 2.8 mM glucose was used to stimulate α-cells selectively. Tolbutamide did not affect the α-cell Ca$^{2+}$ response to arginine significantly implying $K_{ATP}$ channels are mainly closed in these cells even in 2.8 mM glucose. This is consistent with the observation that ATP levels as determined by NAD/P+H fluorescence are nearly unchanged in mouse α-cells at even lower glucose concentrations.
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The GCG-cre mice have been used in several studies including lineage tracing studies [24], to reduce the number of insulin receptors in α-cells [34], to generate animals with fluorescent α-cells by crossing with ROSA reporter mice [35,36,37] and to reduce UCP2 in α-cells [38]. The glucagon promoter sequence in the GCG-cre mouse is an ~890 basepair SacI fragment that ends approximately 75 basepairs upstream of the start site in glucagon [24]. The frequency of single recombination events using the GCG-cre mouse has been estimated by crossing with ROSA-lacZ or ROSA-EYFP mice. A single recombination event is required to delete the STOP codon from a loxP-STOP-loxP cassette and allow expression of the marker in these animals. The frequency estimates range from ~0.85 [34], 0.76 [36] to 0.72±0.1 [39] determined as marker positive cells versus glucagon positive cells identified immunohistochemically. Using a functional assay, simulation by epinephrine, to identify α-cells we determined a value of 0.65.

This was approximately equivalent to the frequency of α-cells lacking KATP channels in Sur1loxP/-;GCG-cre+ islets where excision of a single exon 2 allele should result in channel loss. The frequency, ~0.41, of α-cells without KATP channels in Sur1loxP/-;GCG-cre+ islets is consistent with a need for two recombination events, i.e., (0.65×0.65~0.42).

Diazoxide resistance can also be used to assess loss of channels in other islet cells. As shown in Figure 4B, we have not observed loss of channels in β-cells in crosses of SUR1 flox and GCG-cre animals.

The single recombination event frequency (0.64) and the observation that Abcc8 is haplosufficient, i.e., both exon 2 alleles need to be eliminated, limits the percentage of Sur1loxP/-;GCG-cre+ α-cells lacking KATP channels to about 41%. This is improved about two-fold in Sur1loxP/-;GCG-cre+ α-cells with a single exon 2 allele. It is not clear what limits the frequency of recombination in these animals. Araki et al [39] have reported a positive correlation between the level of cre-recombinase expression and frequency of recombination in a transient expression system. We have attempted, unsuccessfully, to detect cre-recombinase expression using a sensitive double immunofluorescence assay [40]. Thus a low level of enzyme expression may be a factor. It is worth noting that while excision by cre-recombinase is usually thought to be irreversible, it is an enzymatic reaction and cre technology is used in transgenesis experiments to insert selective markers at engineered loxP sites in genomic DNA (reviewed in [41,42]). Studies are in progress to determine if higher level expression of cre-recombinase will increase the frequency of recombination and thus the percent of KATP channel deficient islet cells.

This project validates the functionality of Sur1 flox mice and provides two animal models to analyze the role of KATP channels in α-cell function in vivo. Based on in vitro studies using isolated islets opening KATP channels have been argued either to be necessary for glucagon secretion during hypoglycemia or to be stimulated and suppress glucagon release during hyperglycemia. Comparison

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**Figure 4. Analysis of Sur1loxP/loxP;GCG-cre+ islet cells.** (A) α-Cells in 2.8 mM glucose were pulsed for five minutes with arginine (1 mM) or arginine plus diazoxide (100 μM) as indicated. In this experiment diazoxide had no effect in four cells (gray traces), while in two cells (black traces) the channel agonist blocked stimulation by arginine. All six cells were stimulated by epinephrine (5 μM). (B) In a separate experiment β-cells were stimulated with 16.7 mM glucose and pulsed for five minutes with diazoxide (100 μM). The individual traces show [Ca2+]i was reduced to baseline values in all of the β-cells. The upper trace, offset by 0.3 units, shows the mean ± SEM values (n = 20).

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**Table II. Frequency of recombination in Cre mouse strains.**

| Fraction of α-cells lacking KATP channels | Fraction of EYFP positive α-cells |
|------------------------------------------|----------------------------------|
| Sur1loxP/loxP;GCG-cre+                    | ROSA-EYFP/SCR87;GCG-cre+         |
| # Dz resistant / epi positive            | # EYFP positive / epi positive   |
| 35/86 ~ 0.41                             | 45/70 ~ 0.64                     |
| 67/103 ~ 0.65                            |                                  |

epi = epinephrine; male mice 12-15 weeks old.

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of glucose homeostasis in these two models versus WT mice should aid in discriminating between these two hypotheses.

Author Contributions
Conceived and designed the experiments: YN JB. Performed the experiments: YN JB. Analyzed the data: YN JB. Contributed reagents/materials/analysis tools: YN JB. Wrote the paper: YN JB.

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