Recent advances in functional genomics afford the opportunity to interrogate the expression profiles of thousands of genes simultaneously and examine the function of these genes in a high-throughput manner. In this study, we describe a rational and efficient approach to identifying novel regulators of insulin secretion by the pancreatic β-cell. Computational analysis of expression profiles of several mouse and cellular models of impaired insulin secretion identified 373 candidate genes involved in regulation of insulin secretion. Using RNA interference, we assessed the requirements of 10 of these candidates and identified four genes (40%) as being essential for normal insulin secretion. Among the genes identified was Hadhsc, which encodes short-chain 3-hydroxyacyl-coenzyme A dehydrogenase (SCHAD), an enzyme of mitochondrial β-oxidation of fatty acids whose mutation results in congenital hyperinsulinism. RNA interference-mediated gene suppression of Hadhsc in insulinoma cells and primary rodent islets revealed enhanced basal but normal glucose-stimulated insulin secretion. This increase in basal insulin secretion was not attenuated by the opening of the KATP channel with diazoxide, suggesting that SCHAD regulates insulin secretion through a KATP channel-independent mechanism. Our results suggest a molecular explanation for the hyperinsulinemia hypoglycemic seen in patients with SCHAD deficiency. (Molecular Endocrinology 21: 765–773, 2007)
In this study, we describe a rational and efficient functional genomics approach to identifying novel regulators of insulin secretion. We began by determining the expression profiles of multiple paradigms of abnormal insulin secretion, including several mouse models of impaired β-cell function, as well as cell culture models of robust or impaired glucose-stimulated insulin release. Computational analysis of these expression profiles identified genes likely to play an important role in insulin secretion. The functional relevance to β-cell function of 10 potential targets identified in this manner were evaluated using RNA interference (RNAi), and several were found to be required for normal insulin secretion. Most notably, the gene encoding short-chain 3-hydroxyacyl-coenzyme A dehydrogenase (SCHAD) was shown to play a crucial role in a KATP channel-independent mechanism of insulin secretion (lines 832/13 and 833/15) vs. INS1-derived cells with weak glucose-stimulated insulin secretion (lines 832/1 and 832/2) (16, 17). Finally, we examined the expression profiles of 832/13 cells cultured in the presence or absence of a 0.5-mM olate: palmitate/albumin, using RPMI 1640 medium that also contains a relatively high glucose concentration (11 mM) to simulate glucolipotoxicity encountered in type 2 diabetes, as we have previously demonstrated that culture of 832/13 cells in the presence of elevated fatty acids and glucose for 48 h causes a striking impairment in glucose-stimulated insulin secretion (Table 1) (18). Through computational analysis of the differentially expressed genes in these five models (see Materials and Methods, Fig. 1), we derived a paradigm list of 373 candidate genes that may play a role in regulating insulin secretion (supplemental Table 1, published as supplemental data on The Endocrine Society’s Journals Online web site at http://mend.endojournals.org).

To assess the potential contribution of these genes to β-cell function, we employed RNAi for loss of function analysis in insulinoma cells (Table 2, supplemental Table 2). Of 373 genes in our paradigm list, we focused on 59 that changed greater than 1.2-fold in at least two of the paradigms. Of these 59, only 29 corresponded to identifiable genes with characterized reference sequences. For 21 of these genes, siRNA duplexes were commercially available, and we chose 10 at random for further evaluation. Transfection of 832/13 cells with siRNAs against individual genes led to a minimal reduction of expression of 43%, with several genes inhibited by more than 70% (Fig. 2A). Strikingly, glucose-stimulated insulin secretion was affected in four out of 10 genes analyzed (40%), confirming the utility of the functional genomics approach in narrowing the field of targets to be screened (Fig. 2B). Down-regulation of reticulon 4, argininosuccinate synthetase 1, and baculoviral inhibitors of apoptosis (IAP) repeat-containing 5 resulted in a striking decrease in glucose-

### Table 1. Models of Impaired β-Cell Function

<table>
<thead>
<tr>
<th>Models</th>
<th>Description</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foxa1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Hypoglycemic, impaired glucagon, and insulin secretion</td>
<td>Kaestner et al. (11); Shih et al. (13); Vatamanuk et al. (15)</td>
</tr>
<tr>
<td>Foxa2&lt;sup&gt;loxP/loxP&lt;/sup&gt;, InsCre</td>
<td>Hyperinsulinemic hypoglycemia, abnormal insulin secretion, reduced expression of K&lt;sub&gt;ATP&lt;/sub&gt; channel</td>
<td>Sund et al. (14); Lantz et al. (9)</td>
</tr>
<tr>
<td>Hnf-4&lt;sub&gt;α&lt;/sub&gt; &lt;sup&gt;loxP/loxP&lt;/sup&gt;, InsCre</td>
<td>Impaired glucose tolerance due to abnormal glucose-stimulated insulin secretion by β-cell</td>
<td>Gupta et al. (8); Miura et al. (12)</td>
</tr>
<tr>
<td>INS1 model 1</td>
<td>Glucose-responsive vs. glucose unresponsive INS1 subclones</td>
<td>Chen et al. (16); Hohmeier et al. (17)</td>
</tr>
<tr>
<td>INS1 model 2: lipotoxicity</td>
<td>Time course of lipid-induced β-cell toxicity</td>
<td>Boucher et al. (18)</td>
</tr>
</tbody>
</table>
stimulated insulin secretion. Conversely, reducing the expression of \( \text{Hadhsc} \) encoding SCHAD caused a marked increase in basal insulin secretion. Together, these results confirm that the functional genomics approach described here provides a rational and efficient method for identifying novel regulators of insulin secretion.

SCHAD Regulates Basal Insulin Secretion Independent of K\(_{\text{ATP}}\) Channels

Among the genes validated from the paradigm list is \( \text{Hadhsc} \), encoding SCHAD, the mitochondrial enzyme that catalyzes the conversion of 3-hydroxyacyl-coenzyme A to 3-ketoacyl-coenzyme A, the penultimate reaction in the \( \beta \)-oxidation of fatty acids (19). Notably, patients with mutations in \( \text{HADHSC} \) leading to functional SCHAD deficiency develop congenital hyperinsulinism (19–22). However, it remains unclear whether hypersulinism in these patients is due to a primary defect in \( \beta \)-cell function or is a secondary consequence of SCHAD deficiency in other tissues. In addition, there is much controversy regarding whether or not reduced \( \beta \)-oxidation of fatty acids regulates insulin secretion through \( K_{\text{ATP}} \) dependent or independent pathways. Thus, we sought to investigate the role of Hadhsc in insulin secretion in further detail.

Table 2. Expression Changes of Candidate Genes in Various Paradigms

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Locus symbol</th>
<th>Foxa1(^{-/-})</th>
<th>Foxa2(^{loxP/loxP}), InsCre</th>
<th>HNF-4(^{loxP/loxP}), InsCre</th>
<th>INS1 model 1</th>
<th>INS1 model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromogranin B</td>
<td>Chgb</td>
<td>−N.C.</td>
<td>3.2</td>
<td>2.1</td>
<td>2.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Transforming growth factor ( \alpha )</td>
<td>Tgra</td>
<td>1.2</td>
<td>−N.C.</td>
<td>7.8</td>
<td>−N.C.</td>
<td>1.2</td>
</tr>
<tr>
<td>Sulphotransferase family 1D</td>
<td>Sult1d1</td>
<td>1.8</td>
<td>−N.C.</td>
<td>−3.7</td>
<td>−N.C.</td>
<td>−N.C.</td>
</tr>
<tr>
<td>( L )-3-hydroxyacyl-coenzyme A dehydrogenase, short chain</td>
<td>Hadhsc</td>
<td>−1.3</td>
<td>−2.8</td>
<td>−N.C.</td>
<td>−N.C.</td>
<td>−N.C.</td>
</tr>
<tr>
<td>Pyruvate kinase, muscle</td>
<td>Pkm2</td>
<td>−1.2</td>
<td>1.2</td>
<td>−N.C.</td>
<td>−N.C.</td>
<td>−N.C.</td>
</tr>
<tr>
<td>Aromatic L-amino acid decarboxylase</td>
<td>Aacdc</td>
<td>−N.C.</td>
<td>−2.1</td>
<td>−2.5</td>
<td>−N.C.</td>
<td>−N.C.</td>
</tr>
<tr>
<td>Baculoviral IAP repeat-containing 5</td>
<td>Birc5</td>
<td>−1.6</td>
<td>−1.2</td>
<td>−N.C.</td>
<td>−N.C.</td>
<td>−N.C.</td>
</tr>
<tr>
<td>Arginosuccinate synthetase 1</td>
<td>Ass1</td>
<td>1.3</td>
<td>1.3</td>
<td>−N.C.</td>
<td>−N.C.</td>
<td>−N.C.</td>
</tr>
<tr>
<td>FK506 binding protein 11</td>
<td>Fkbp11</td>
<td>−N.C.</td>
<td>−N.C.</td>
<td>1.4</td>
<td>−N.C.</td>
<td>1.2</td>
</tr>
<tr>
<td>Reticulon 4</td>
<td>Rtn4</td>
<td>−1.2</td>
<td>−1.7</td>
<td>−1.2</td>
<td>−3.8</td>
<td>N.C.</td>
</tr>
</tbody>
</table>

Fold changes listed above are relative to littermate control groups used for each individual experiment. N.C., No significant difference compared with controls.
Although nucleofection of RNAi oligonucleotides allows for rapid screening of multiple candidate genes in insulinoma cell lines, we have not found this method to be efficient for gene transfer into primary pancreatic islets. For further analysis of Hadhsc function in β9252-cell, we employed adenoviral-mediated gene transduction in primary islets in addition to insulinoma cells (23, 24). We constructed a recombinant adenovirus containing a short hairpin RNA (shRNA) sequence specific to this gene [adenovirus expressing siRNA against Hadhsc (Ad-siHadhsc)]. To test the ability of Ad-siHadhsc to suppress Hadhsc expression, we assayed Hadhsc mRNA levels in INS1 cells after transduction with varying multiplicities of infection of Ad-siHadhsc for 24 or 48 h. Treatment of 832/13 cells with Ad-siHadhsc caused decreases in Hadhsc transcript levels in as little as 24 h after transduction, with maximal suppression (86%) at 48 h (Fig. 3A). Increasing the multiplicity of infection had no further effect on the efficiency of the shRNA approach (Fig. 3A).

Next we measured glucose-stimulated insulin secretion in insulinoma cells with reduced levels of Hadhsc. We used Ad-siHadhsc at a dose of 100 plaque-forming units (pfu) per cell for 48 h to suppress Hadhsc mRNA levels by 83% in 832/13 cells. Suppression of Hadhsc expression caused a significant increase in basal insulin secretion compared with untreated cells and cells transduced with an adenovirus expressing a scramble sequence (Ad-siScramble) (Fig. 3B and C).
3B). To determine if this increase in basal insulin secretion is mediated through a $K_{ATP}$ channel-dependent mechanism, we performed glucose-stimulated insulin secretion assays in the presence of diazoxide, which functions to keep $K_{ATP}$ channels open, thus suppressing the $K_{ATP}$-dependent pathway to insulin release. Treatment of the cells with diazoxide did not alter the enhanced basal insulin secretion caused by suppression of Hadhsc, although the same dose of diazoxide completely abrogated glucose-stimulated insulin secretion in Ad-siScramble-treated cells (Fig. 3C). Together these results indicate that SCHAD functions directly in $\beta$-cells to regulate a $K_{ATP}$-independent pathway to insulin secretion.

**SCHAD Is Required for Basal Insulin Secretion in Primary Mouse Islets**

Next, we examined the impact of SCHAD deficiency on basal insulin secretion in primary islets because insulinoma cells do not always replicate all aspects of normal $\beta$-cell biology. To test the efficiency of recombinant adenovirus to transduce islets, we assayed green fluorescent protein (GFP) expression in islets after transduction with an adenovirus expressing GFP (Ad-GFP). Treatment of mouse islets with Ad-GFP at a viral dose of $1.3 \times 10^6$ pfu per islet for 24 h followed by culture for 2 more days resulted in efficient GFP expression within the islets. In addition, when we treated islets with Ad-siHadhsc, Hadhsc transcript levels were reduced by 80% 4 d after transduction (Fig. 4A), consistent with our experiences in other studies (24, 25) employing adenovirus vectors for knock-down of gene expression in primary rodent islets.

Insulin secretion from isolated islets was determined at 3 or 16.7 mM glucose in a 2-h static incubation assay. Adenovirus-mediated silencing of Hadhsc increased basal insulin secretion in islets, confirming our findings in insulinoma cells; however, there was no difference in glucose-stimulated insulin secretion (Fig. 4B). Thus, we conclude that Hadhsc has a primary function in pancreatic islets for the regulation of basal insulin secretion.

**DISCUSSION**

Although there has been substantial progress in understanding $\beta$-cell biology and the contributions made by $\beta$-cell dysfunction to the development of diabetes, treatment options remain less than optimal. Thus, it is important to search for genes that regulate $\beta$-cell function, growth, and survival. Such genes could be targets for development of more effective drugs for the treatment of diabetes. Previous large-scale screens to identify regulators of signaling cascades and physiological processes have used large siRNA libraries containing over 20,000 siRNA duplexes. Although these studies have identified hundreds of regulators, these approaches have a relatively low success rate, with as little as 2% of the siRNAs producing a measurable phenotype (3, 7).

In this study, we describe a rational and efficient approach to identifying regulators of insulin secretion. By combining computational analysis with expression profiling, we have derived a list of potential new target genes that affect the function of the $\beta$-cell. Screening of 10 of these candidate genes identified four targets (40%) that are involved in regulating insulin secretion. Blindly screening genes without using the guidance of the expression data would have likely required an order of magnitude more genes to reach the same number of positive results. In addition, the paradigm gene list of 373 genes provides a valuable resource to be exploited by $\beta$-cell researchers in the future.

Among the genes identified as essential regulators of glucose-stimulated insulin release are Argininosuc-
argininosuccinate synthetase 1, Baculoviral IAP repeat containing 5, and Reticulon 4. Argininosuccinate synthetase catalyzes the synthesis of argininosuccinate, the immediate precursor of arginine, from citrulline and aspartate. First identified in the liver, argininosuccinate synthetase is now recognized as a ubiquitous enzyme in mammalian tissues whose regulation is dependent on arginine utilization in the tissue of interest (26). Previous studies have shown that L-arginine stimulates insulin release from pancreatic β-cells. One hypothesis is that the L-arginine potentiation of glucose-induced insulin secretion is mediated by L-arginine-derived nitrogen oxides (27), whereas another group proposes that it occurs via membrane depolarization, which stimulates insulin secretion through protein kinase A- and C-sensitive mechanisms (28). In this study, down-regulation of argininosuccinate synthetase expression by RNAi results in reduced insulin secretion, indicating that argininosuccinate synthetase is a potential activator of insulin secretion.

Baculoviral IAP repeat containing 5 (survivin) is a member of the mammalian IAP family, along with baculoviral IAP repeat-containing 4, which encodes negative regulatory proteins that prevent apoptotic cell death. Previous studies have shown that overexpression of baculoviral IAP repeat-containing 4 in β-cell lines and human islets enhances β-cell survival, possibly by inhibiting TNF-related apoptosis-inducing ligand mediated pathways (29–31). In this study, down-regulation of baculoviral IAP repeat containing 5 expression by RNAi results in reduced basal and glucose-stimulated insulin release. Further studies are needed to determine how proteins in the IAP family regulate insulin secretion.

Likewise, very little is known about the function of reticulons in insulin secretion. In neuroendocrine cells, reticulons are localized primarily to the endoplasmic reticulum and can immunoprecipitate with soluble N-ethylmaleimide-sensitive factor attachment protein receptors, which are essential for secretory granule release from β-cells (32). Therefore, it is tempting to speculate that reticulin 4 functions to control the priming or release of insulin secretory granules. Indeed, down-regulation of reticulin 4 results in a dramatic reduction of basal and glucose-stimulated insulin secretion. The INS1 cells with reduced reticulin 4 expression described in this study provide novel tools to examine the exocytotic machinery involved in regulating insulin secretion.

Most notable among the genes validated from the paradigm list is Hadhsc. Hadhsc encodes SCHAD, the mitochondrial enzyme that catalyzes the conversion of 3-hydroxyacyl-coenzyme A to 3-ketoacyl-coenzyme A, the penultimate reaction in the β-oxidation of fatty acids. Several cases of hyperinsulinism in children associated with mutations in HADHSC have been described (19–22). However, until now it has remained unclear whether hyperinsulinism in these patients is due directly to the loss of SCHAD in pancreatic β-cells, or occurs secondary to the metabolic stress initiated by altered lipid metabolism in other tissues. Our in vitro model of reduced SCHAD expression demonstrates for the first time that SCHAD is required directly in β-cells for the regulation of basal insulin release.

There is much speculation regarding the mechanism of increased insulin release from SCHAD-deficient β-cells. Molven et al. (22) proposed that the L-form of 3-hydroxybutyryl-carnitine that accumulates in these patients may interfere with potassium channel function or with the ATP-independent and lipid-sensitive mechanism of insulin secretion. We observed that increased basal insulin secretion from cells with suppressed SCHAD expression is sustained in the presence of diazoxide, supporting a potassium channel-independent pathway, thus making it unlikely that human SCHAD deficiency manifests in impaired regulation of insulin secretion via effects on K_ATP channels. Clayton et al. (19) suggested that the accumulation of short-chain acyl-coenzyme A esters in the mitochondrion causes insulin secretion by inhibition of carnitine palmitoyltransferase I. Fatty acids are also known to increase insulin secretion by stimulation of G-protein-coupled receptors and by activation of L-type Ca2+ channels (21, 33, 34). Future studies with this model of SCHAD deficiency will be used to identify mechanisms by which fatty acid and fatty acid metabolites modulate insulin release.

In summary, by combining expression profiling with RNAi, we provide a rational and efficient approach to identifying novel regulators of insulin secretion by the β-cell. This functional genomics approach can be applied to other mammalian systems and may someday lead to the development of novel therapeutic regimens for the treatment of diseases such as diabetes mellitus.

**MATERIALS AND METHODS**

**Expression Profiling**

Gene expression profiling was performed on multiple two-state models of islet function/dysfunction. This included a comparison of gene expression in isolated islets of three mouse models of perturbed β-cell function [Foxa1<sup>−/−</sup>, Foxa2<sup>-<sub>loxP/loxP</sub></sup> insulin promoter-driven Cre-recombinase (InsCre); Hnf4α<sub>loxP/loxP</sub>, InsCre] (8, 11, 14), compared in each case to littermate controls. We also included a comparison of INS1-derived cell lines with robust glucose-stimulated insulin secretion (lines 832/13 and 833/15) vs. INS1-derived cells with weak glucose-stimulated insulin secretion (lines 832/1 and 832/2) (16, 17). Finally, we cultured a robustly glucose-responsive INS1-derived cell line (832/13) in the presence or absence of a 0.5 mM oleate:palmitate/albunin, using RPMI medium that also contains a relatively high glucose concentration (11 mM) to simulate conditions encountered in type 2 diabetes. Boucher et al. (18) have previously demonstrated that culture of 832/13 cells in the presence of elevated fatty acids and glucose for 48 h causes a striking impairment in glucose-stimulated insulin secretion. Replicate RNA samples (three to five per condition) were collected from all of these two state models and used for microarray analysis on PancChip cDNA microarrays (version 4.0 for the
Hardy log2(C1) values were computed for each spot considered, where the 13,008 for PancChip 5.0 experiments. For each assay, the number of spots analyzed was 11,400 for PancChip 4.0 and PCR controls were removed from the analyses (thus, the total number of spots analyzed was 11,400 for PancChip 4.0 and 13,008 for PancChip 5.0 experiments). For each assay, M values were computed for each spot considered, where the M value for two conditions, C1 and C2, is defined as log2(C2). The M values were normalized on each assay with the print-tip loess approach (38) using the implementation provided by the R (http://cran.r-project.org) mar- rayNorm package from Bioconductor (http://www.biocon- ductor.org), with default parameter settings (R version 1.8.1, mar-rayNorm version 1.1.6). After normalization, the M values for each available pair of dye-swap were combined 

\[
|M1 - M2|^{2}
\]

For each of the five paradigms described above, differentially expressed transcripts between the conditions of interest were identified using a combination of approaches. For the three mouse models of impaired β-cell function, the conditions of interest were mutant vs. littermate controls. For each model we ranked all spots according to LOD scores (39) and performed statistical analysis of microarrays [SAM, version 2.0 (http://www-stat.stanford.edu/~tibs/SAM)]. The same tools were used for the comparison of the robustly versus poorly glucose responsive INS1-derived cell lines. Finally, for the study of lipid-induced impairment of glucose-stimulated insulin secretion, we analyzed a time course of exposure to oleate/palmitate (0, 12, 24, and 48 h), which required a different computational approach. First, we compared treated to untreated cells at each time point separately in a pair-wise (by biological sample) mode. Secondly, we compared all of the treated vs. all of the control assays using SAM with an unpaired block design with three blocks, one per time point.

Computational Analysis of Expression Profiles

After analyzing each study individually, the results were combined to generate a master list of candidate targets to be considered for further validation. This paradigm list was compiled using multiple criteria. First, we computed a "top" list (based on LOD scores) for each study, after removing spots with PCR failure flags and spots flagged by GenePix in greater than 40% of the arrays. The number of “top” spots for each paradigm was established according to a suitable weight given to each of the five studies and to each of the comparisons within such studies. The weight of each para- digm took into account the number of replicates available in that study and the number of differentially expressed genes identified by the SAM analyses. The union of these lists yielded 373 distinct transcripts, which were annotated ac- cording to their additional lines of evidence for differential expression coming from the SAM analyses. The complete paradigm list is available at http://mend.endojournals.org/ (supplemental Table 1).

Gene Silencing in Insulinoma Cells with siRNA Duplexes

The rat insulinoma line 832/13 was cultured as described (17). Transfection of siRNA duplexes (Ambion, Austin, TX) (supplemental Table 2) was performed using the Amaxa nucleofection system (Amaxa, Gaithersburg, MD) as described (42). Briefly, 2 μg of siRNA duplexes was transfected into 1.5 × 106 832/13 cells using program no. T-27 of the nucleofector device. After 48 h, cell RNA was extracted for quantitative RT-PCR analysis. Primers used for analysis of gene expression are available upon request.

Construction of an Adenoviral Vector Producing a shRNA against Hadhsc

Two shRNA adenoviruses were constructed to target rat Hadhsc and mouse Hadhsc mRNAs. The 5’ end of the target corresponds to rat Hadhsc (accession no. NM_057186) nucleo- tidies 840–858 (AGT TCA TCT TAG ACG GGT G) and mouse Hadhsc (accession no. NM_008212) nucleotides 401–419 (GAA CGA GCT GTT CCA GAG G). Ad-GFP and Ad-siScramble (GAG ACC CTA TCC GTG ATT A) were used as controls (23). Sense and antisense oligonucleotides were designed and synthesized as described (40). Oligonucleotides were annealed in STE buffer (10 mmol/liter Tris, 1 mmol/liter EDTA, 50 mmol/liter NaCl, pH 8.0) and ligated into BglII and HindIII-linearized pSUPER (Oligo- Engine, Seattle, WA). The shRNA expression cassette was ex- cised from the pSUPER-based plasmid using EcoRI-HindIII and ligated into EcoRI-HindIII linearized adenoviral shuttle vector EH006 (23). Adenoviruses were created by homologous recom- bination as previously described (23). Titers of tertiary viral lysates were 2.0 × 10^8 – 1.7 × 10^9 pfu/ml. For adenoviral transduction, cells were cultured in six-well plates at 60,000 cells/cm² and the following day transduced with Ad-siHadhsc or Ad-siScramble at a viral dose of 100 pfu per cell for 24 h. Virus was removed, fresh medium was added, and cells were cul- tured for an additional 24 h. Hadhsc transcript levels were assayed by real-time PCR analysis with the following primers: (5’-CGTGGGCTTGGGAAACTTGTGA-3’ and 5’-AAGATGG- GCCAAGAACCAAG-3’).

Glucose-Stimulated Insulin Secretion in Insulinoma Cells

Insulin secretion was assayed in HEPES balanced salt solu- tion (HBSS) (114 mmol/liter NaCl, 4.7 mmol/liter KCl, 1.2 mmol/liter KH₂PO₄, 1.16 mmol/liter MgSO₄, 20 mmol/liter HEPES, 2.5 mmol/liter CaCl₂, 25.5 mmol/liter NaHCO₃, and 0.2% BSA, pH 7.2). Cells were washed in 1 ml HBSS with 3 mmol/liter glucose followed by a 2-hr preincubation in 2 ml of the same buffer. Insulin secretion was then measured by static incubation for a 2-h period in 1 ml HBSS containing various glucose concentrations. For studies of KATP channel-independent insulin secretion, assays were performed in the presence of 250 μM diazoxide. Insulin levels were determined by ELISA using Crystal Chem Ultra Sensitive Rat Insulin ELISA Kit (Crystal Chem Inc., Downers Grove, IL).

Gene Silencing in Mouse Islets of Langerhans

Under a protocol approved by the University of Pennsylvania Institutional Animal Care and Use Committee (IACUC), pan- creatic islets of Langerhans were isolated from 6-wk-old female CD1 mice using standard collagenase digestion fol- lowed by purification through a Ficoll gradient (41). RPMI culture medium was supplemented with penicillin, strepto-
Glucose-Stimulated Insulin Secretion in Mouse Islets

Four days after viral treatment, glucose-stimulated insulin secretion was assessed. Insulin secretion was assayed in HBSS (114 mmol/liter NaCl, 4.7 mmol/liter KCl, 1.2 mmol/liter glucose) and was pre-incubated for 1 h in 1 ml HBSS with 2.8 mmol/liter glucose. Insulin secretion was then measured by static incubation for 2 h at pH 7.4. Ilets were assayed in 4 d before measurement of Hadhsc transcript levels as described above with the following primers (5'-AATGCCACACCAAGAAGAAG and 5'-CGTGTGTTTG-ATGACCTCCA).

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